IMIDAZOL-4-ONES: THEIR APPLICATION IN NEURODEGENERATION AND THE FIRST TOTAL SYNTHESIS OF NORTOPSENTIN D

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Chemistry-Doctor of Philosophy

2021

ABSTRACT

IMIDAZOL-4-ONES: THEIR APPLICATION IN NEURODEGENERATION AND THE FIRST TOTAL SYNTHESIS OF NORTOPSENTIN D

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Herein, imidazol-4-ones are applied to the areas of methodology development, total synthesis, and small molecule design for proteasome activation. The first total synthesis of nortopsentin D is described, which was completed in 7 linear steps with an overall yield of 1.6%. This convergent synthesis involved the condensation of amidine and dione, followed by a cyclization via a pinacol-like rearrangement to produce the central imidazol-4-one ring. Additionally, a range of imidazol-4-ones were explored for their use as proteasome activators. Out of this research, a new scaffold, *N*-acylated fluspirilene (**5-12**), was discovered. This small molecule represents the Tepe lab's first proteasome activator to dock within the α2-3 intersubunit pocket and produce a significant increase in IDP degradation *in vitro* (AC_{200} 1.9 μM, max fold enhancement >2000%) as well as in cells.

ACKNOWLEDGEMENTS

I have a lot of people to thank for supporting me throughout this PhD journey, and I am truly grateful for everyone in my life.

Firstly, I would like to thank Dr. Jetze Tepe. Thank you for supporting my interests and allowing me to the do the research I am most passionate about. Additionally, thank you for taking a chance on a student who had no prior organic chemistry experience; your patience and willingness to teach me yet grant me so much independence in lab is what made me the chemist I am today.

To my committee, Dr. Huang, Dr. Wulff, and Dr. Borhan, I want to thank you for your feedback and willingness to answer all my questions, whether it be about my research, classes, or seminar. Your support is greatly appreciated. I also want to thank some other faculty at MSU for their assistance. Specifically, I want to thank Dr. Draths, Dr. Vasileiou, Dr. Hunt, and Dr. Severin for being awesome advisors for MSU's Women in Chemistry (WiC) chapter. Some of my fondest memories of graduate school are from WiC, and it would not have been possible without their backing. Additionally, I want to thank Dr. Dan Holmes for answering all my NMR questions, especially regarding my natural product, and Dr. Tony Schilmiller for his endless help with mass spectroscopy. Lastly, I would like to thank Dr. Richard Staples for determining the crystal structure of my natural product.

To my family, thank you for supporting me during this time, even if you never fully understood what I was doing or why. As a first-generation college student and while my life path has been very different than the rest of my siblings, I have always felt supported in my decision to pursue a PhD and encouraged to keep going, even when things got rough. Thank you for doing all you could to help me achieve my dreams.

To my friends, Morgan, Sarah, Hunter, Yuting, Pepe, Abby, Shannon, Ciera, thank you for always being there to celebrate wins and drown losses. I appreciate the game nights, weekly phone calls, girls' weekends, Thai and Hopcat dinners, mid-day walks and Sparty's coffee runs more than you could ever know.

To my lab mates, new and old, thank you for your constant support within lab and outside of lab. To Corey, Travis, Evert, and Matt- thank you for helping me when I first joined the Tepe lab. I had no synthetic experience and without your willingness to train me and answer my questions (no matter how stupid they were), I would not be the scientist I am today. A big thank you to Shafaat- it was always comforting to know there was someone else in lab to talk to about seminar, orals, interviews, etc. who was living through it at the same time as me. Thank you to Grace, Sophie, Allison, and Taylor- it has been a true blessing working in a lab where we are not just lab mates, but also friends. Lastly, to the younger students, Konika, Charles, Dare, Daniel, Evan, and Kyra- thank you all for challenging me to be a better chemist through your questions and our conversations. I never thought I could be the lab member who younger students went to discuss their work and troubleshoot. That means so much to me and has helped me kick imposter syndrome in the pants!

A special thanks goes out to my emotional support cats- Kyra and Benji. While they will never be able to read this, I could not have gotten through graduate school without their presence, especially during the 2020 coronavirus pandemic. Their love kept me going during quarantine, and their entertaining spirits made Zoom meetings a lot lighter.

Lastly, to my loved ones who are no longer with us today, Grandpa Bob, Grandma Kay, and Grandpa Keel- thank you for showing me what determination and a bit of moxie can do for your life. I love you, and I hope I have made you proud.

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KEY TO ABBREVIATIONS

A53T	Alpha-synuclein protein
AC_{200}	200% activity concentration
AcOH	Acetic acid
AGE	Advanced glycation end products
ALS	Amyotrophic lateral sclerosis
AMC	7-amino-4-methylcoumarin
Aq	Aqueous
Ar	Aryl-
ASN	Asparagine
Atm	Atmospheric pressure
ATP	Adenosine triphosphate
BACE	Beta-site APP cleaving enzyme
BBB	Blood brain barrier
BEMP	2-Tert-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine
Bn	Benzyl-
Boc	Tert-butyloxycarbonyl
BSA	N,O-bistrimethylsilylacetamide
BTZ	Bortezomib
°C	Celsius
Casp-L	Caspase-like
Cbz	Benzyl formate
CC_{50}	50% cytotoxic concentration

CD	Circular dichroism
CDI	Carbonyldiimidazole
CDK	Cyclin-dependent kinase
CLK	CDC2-like kinase
c-MYC	c-myelocytomatosis oncogene
CSI	Chlorosulfonyl isocyanate
CT-L	Chymotrypsin-like
CuTC	Copper (I) thiophene-2-carboxylate
D2R	Dopamine receptor D2
DBDMH	1,3-Dibromo-5,5-dimethylhydantoin
DCC	N,N′-dicyclohexylcarbodiimide
DCM	Dichloromethane
DIPEA	N,N-diisopropylethylamine
DMA	Dimethylacetamide
DMAP	4-Dimethylaminopyridine
DMDO	Dimethyldioxirane
DME	Dimethoxyethane
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DYRK	Dual specificity YAK1-related kinase
EC_{50}	50% effective concentration
EDC/EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Equiv.	Equivalents
Et	Ethyl-
FASN	Fatty acid synthase

FDA	U.S. Food and Drug Administration
FTIR	Fourier transform infrared
GLN	Glycine
H	Hours
HATU	Hexafluorophosphate azabenzotriazole tetramethyl uronium
HCT116	Human colorectal carcinoma cell line
HD	Huntington's disease
HeLa cells	Henrietta Lacks cells
hPTHR1	Human parathyroid hormone receptor 1
hv	Light
*i*Bu	Iso-butyl-
IC_{50}	Half-maximal inhibitory concentration
IDPs	Intrinsically disordered proteins
ILE	Isoleucine
IPA	Isopropyl alcohol
*i*Pr	Isopropyl-
KHMDS	Potassium bis(trimethylsilyl)amide
LAH	Lithium aluminum hydride
LiHMDS	Lithium bis(trimethylsilyl)amide
LYS	Lysine
MAO	Monoaminooxidase
MCF-7	Michigan Cancer Foundation-7 breast cancer cell line
mCPBA	m-chloroperbenzoic acid
Me	Methyl-
MeCN	Acetonitrile

MIO	3,5-dihydro-5-methylidene-4*H*-imidazol-4-one
MOM	Methoxymethyl ether
mp	Melting point
MS	Mass spectroscopy
MS	Molecular sieves
MsCl	Methanesulfonyl chloride
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide
MW	Microwave
NBS	*N*-bromosuccinimide
*n*Bu	n-butyl-
NEt_3/TEA	Triethylamine
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
*n*Hex	Hexanes
NHS	*N*-hydroxysuccinimide
NIH	National Institutes of Health
NIS	*N*-iodosuccinimide
NMO	*N*-methylmorpholine-*N*-oxide
NMR	Nuclear magnetic resonance
NOS	Nitric oxide synthase
Nu	Nucleophile
ORAC	Oxygen radical absorbance capacity
OTf	Trifluoromethanesulfonate
PAI	Pyrrole-aminoimidazole
Ph	Phenyl-

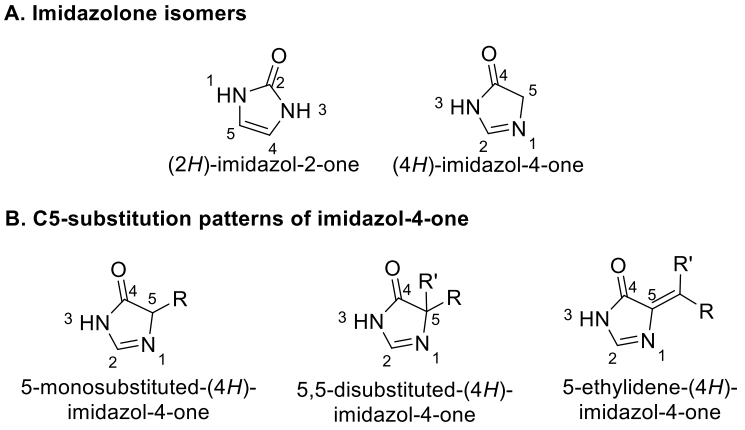
PHE	Phenylalanine
PPA	Phenylpropanolamine
Pr/*n*Pr	Propyl-
RNA	Ribonucleic acid
RT	Room temperature
Sat.	Saturated
SEM	Scanning electron microscope
Soln.	Solution
TBAB	Tetrabutylammonium bromide
TBAF	Tetrabutylammonium fluoride
TBHP	Tert-butylhydroperoxide
*t*Bu	Tert-butyl-
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
T-L	Trypsin-like
TLC	Thin layer chromatography
TMS	Trimethylsilyl-
ToF	Time of flight
Ts	Tosyl-
TYR	Tyrosine

1 Chapter 1: Known preparative methods of (4*H*)-imidazol-4-ones*

1.1 Introduction

Imidazolones are five-membered heterocyclic rings containing two non-adjacent nitrogens and a carbonyl group. There are two isomers of imidazolones, depending on the placement of the carbonyl: imidazol-2-ones and imidazol-4-ones (**Figure 1.1A**).

Figure 1.1 Isomers of imidazolones



Imidazol-4-ones are an important heterocycle utilized for a large range of applications, including medicinal chemistry[1–5], fluorescent protein chromophores[6–8], agrochemicals[9], and natural products. This heterocyclic structural motif is also found naturally occurring in the body. Imidazol-4-ones are found as advanced glycation end products (AGE)[10–12], post-translational modifications of several amino acids- aka 3,5-dihydro-5-methylidene-4*H*-imidazol-4-one (MIO)[13], and creatinine, a waste product used to indicate kidney health.[14] Despite being found in a vast assortment of fields, the first review written on the efforts made towards preparing imidazol-4-ones was published in 2020. This chapter of my dissertation is an excerpt from our recently published review

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article titled “The preparation of (4*H*)-imidazol-4-ones and their application in the total synthesis of natural products“.[15]

Preparation of (4*H*)-imidazol-4-ones goes back as far as 1907, when H. Finger first reported the synthesis of a (4*H*)-imidazol-4-one.[16] Since then, a number of unique methodologies have been developed for the production of imidazol-4-ones. These methodologies can be used to produce three *C*5-substitution patterns, as shown in **Figure 1.1B**. In an effort to centralize this information, section 1.2 summarizes the methodologies developed for the synthesis of imidazol-4-ones. Details of each reaction are discussed, as well as any advantages or disadvantages to the method.

1.2 Preparation of (4*H*)-imidazol-4-ones

Since the first report of the synthesis of imidazol-4-ones, there have been numerous reported methods of preparation. In this section, many preparative methods of imidazol-4-ones will be discussed. The methods have been categorized into three main transformations: condensation reactions, aza-Wittig reactions, and heterocyclic rearrangements.

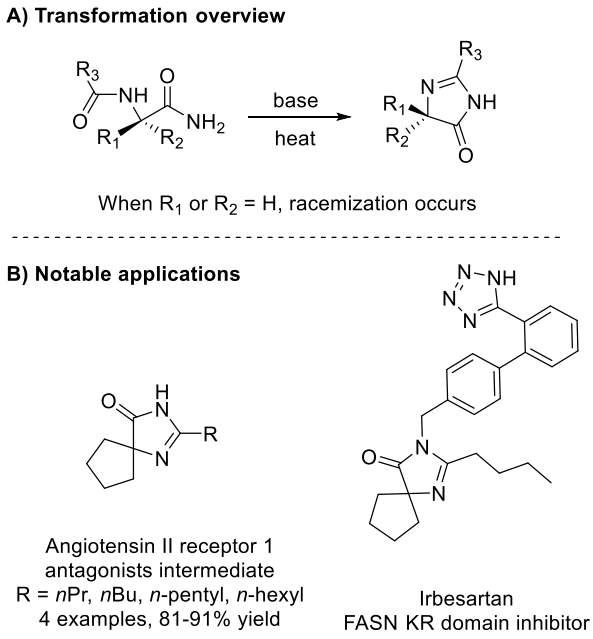
1.2.1 Condensation reactions

1.2.1.1 Cyclization of diamides

Under basic conditions, with or without heat, a diamide will cyclize to produce a range of substituted imidazol-4-ones. Some notable examples of this reaction being used within the last 5 years include the synthesis of derivatives of irbesartan for fatty acid synthase (FASN) KR domain inhibition[5,17] and angiotensin II receptor 1 antagonists (**Scheme 1.1**).[1] This method has also been used to produce enantiopure 5,5-disubstituted-4-imidazolones from enantiopure diamides, but does not work well for the production of enantiopure 5-monosubstituted-4-imidazolones, as heat and base can cause the

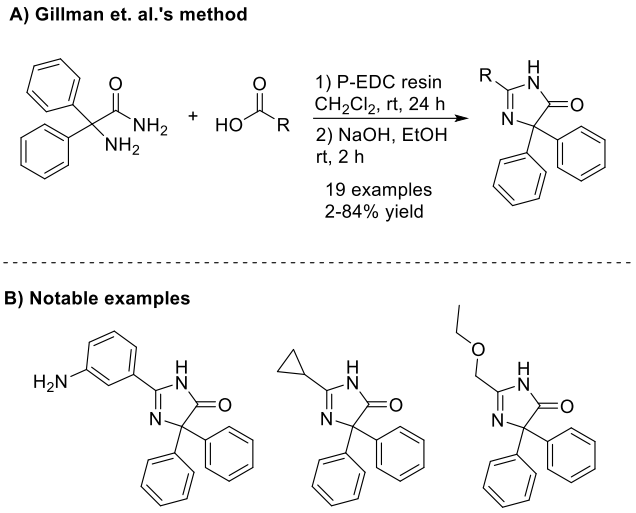
stereocenter to tautomerize.[18] Additionally, this reaction has been used to produce a number of 5-ethylidene-4-imidazolones, with the alkene's conformation being driven by steric interactions with the imidazolone's carbonyl.

Scheme 1.1 General transformation from diamide to imidazol-4-one



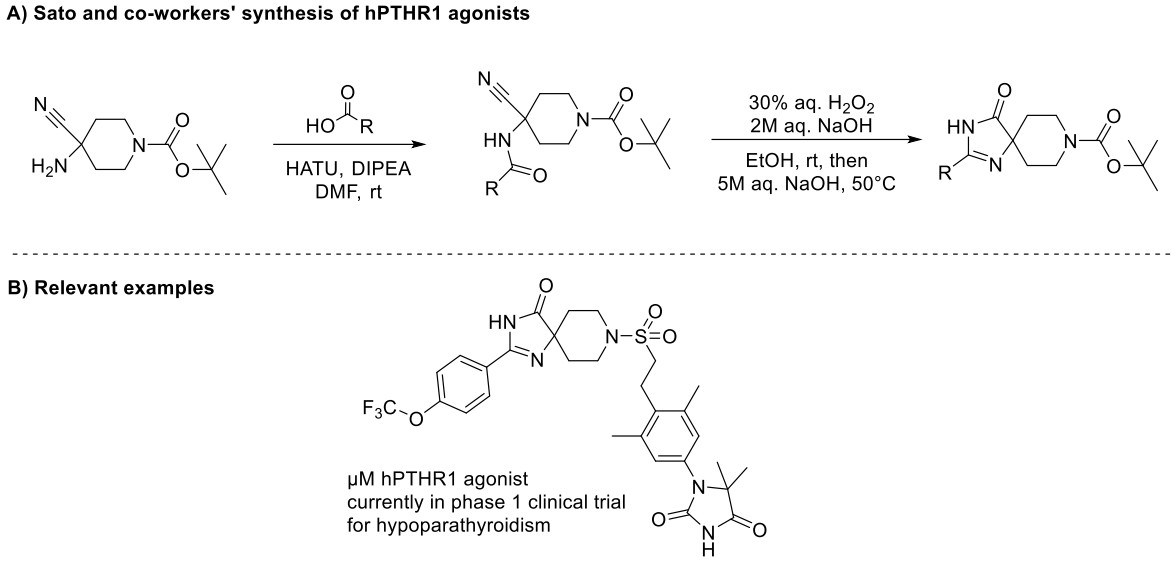
The diamide is a common intermediate in several preparative methods of imidazol-4-ones. One example is the condensation of an α-amino amide and acid derivative. Gillman et. al. synthesized a number of imidazol-4-ones using an α-amino amide and carboxylic acid (see **Scheme 1.2**).[19] Here, the amine and carboxylic acid were coupled together using a polymer-supported carbodiimide reagent, producing diamides in relatively low yields (10-50%), due to the steric bulk surrounding the amine. The diamide was then cyclized upon the addition of sodium hydroxide in ethanol.

Scheme 1.2 Gillman et. al.'s synthesis of imidazolones as neuropeptide Y5 receptor antagonists



Diamides can also be produced through the oxidation of α-amino nitriles using hydrogen peroxide. α-amino nitriles were first used to synthesize imidazol-4-ones in 1981 by Marinus Los.[20] In the first step of this synthesis, an amine reacts with a carboxylic acid or acid chloride to produce an amide. The second step involves oxidation of the nitrile to an amide using hydrogen peroxide, which leads to the formation of an imidazol-4-one through cyclization of the diamide intermediate. Sato and co-workers used this methodology to produce a range of imidazol-4-ones as human parathyroid hormone receptor 1 (hPTHR1) agonists for treatment of hypoparathyroidism, as shown in **Scheme 1.3**.[21,22] One of their analogues, shown in **Scheme 1.3B**, is currently in a phase 1 clinical trial for the treatment of hypoparathyroidism.

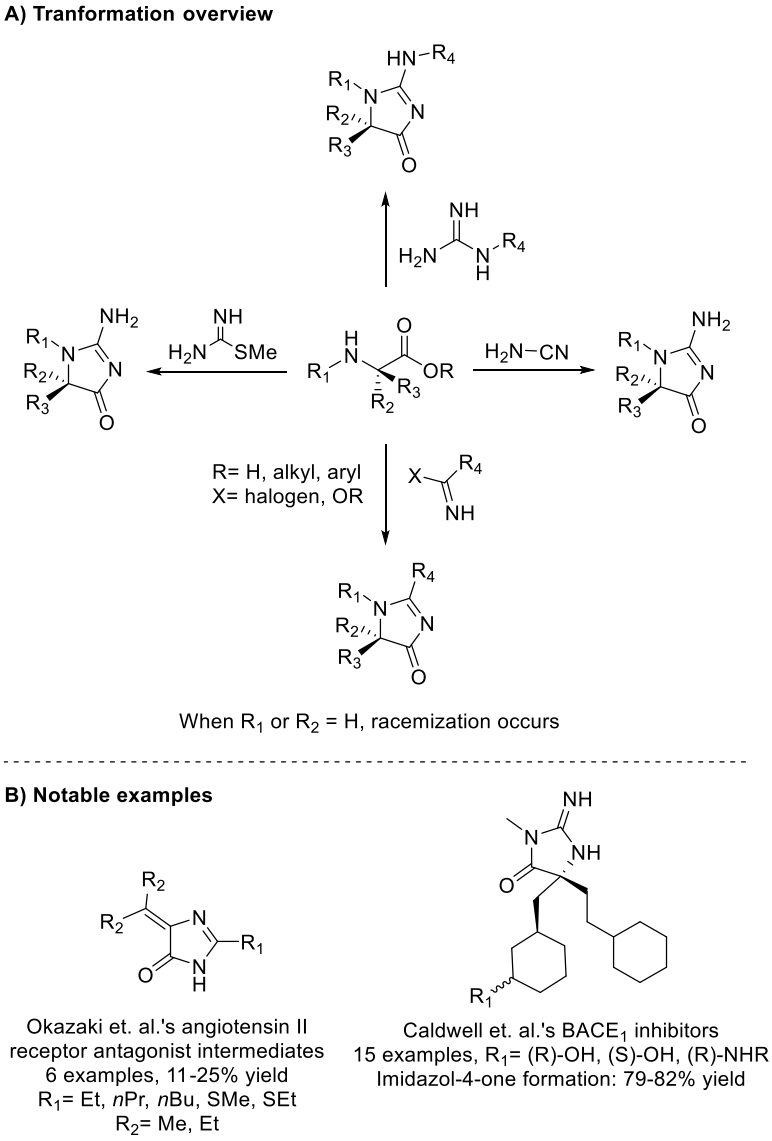
Scheme 1.3 The synthesis of piroimidazol-4-ones for treatment of hypoparathyroidism



1.2.1.2 Amino ester and cyanamide/guanidine

Amino esters are versatile building blocks used to synthesize a range of heterocycles, including imidazol-4-ones. The condensation of amino esters with cyanamides, guanidines, imino halides, imidates, or methyl carbamimidothioates has been used to produce a range of 5-monosubstituted, 5,5-disubstituted, and 5-ethylidene imidazol-4-ones for biological applications and natural product syntheses.[23–26] **Scheme 1.4** summarizes the different nucleophiles that react with amino esters to produce imidazol-4-ones. Also demonstrated in **Scheme 1.4**, this method can be used to produce enantiopure 5,5-disubstituted imidazol-4-ones, as this reaction is considered stereospecific when the 5-position is disubstituted, similar to the cyclization of diamides.[25]

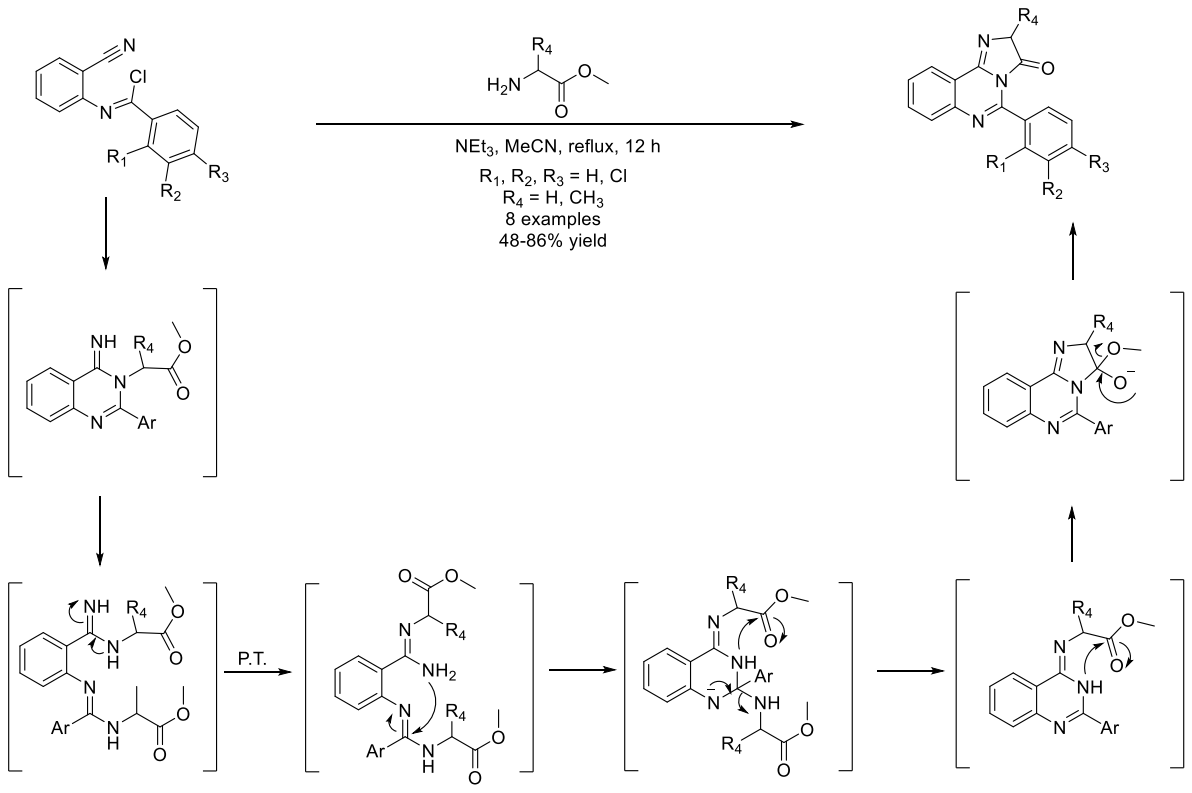
Scheme 1.4 Transformation of amino ester to imidazolone using a variety of nucleophiles



In 2020, Fathalla et. al. utilized an intramolecular cyclization of amidine and amino ester to produce imidazoquinazolinones through a domino synthesis (**Scheme 1.5**).[27] Here, a range of benzyimidoyl chlorides were reacted with glycine or L-alanine methyl ester hydrochloride to produce a few quinazolin-4(3*H*)-imines. The quinazolin-4(3*H*)-imine then undergoes a series of ring deconstructions and formations, ultimately producing some complex imidazoquinazolinones in 48-86% yield. This one-pot

methodology was also used to construct pyrimidoquinazolinones by reacting benzymidoyl chloride with a β-alanine methyl ester.[27]

Scheme 1.5 Domino synthesis of imidazoquinazolinones

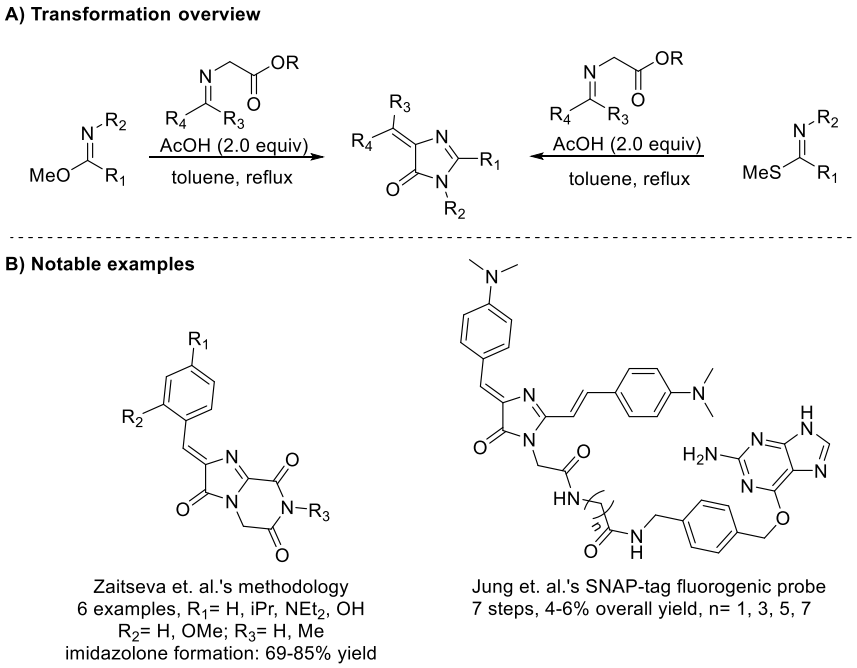


1.2.1.3 Imidate and α-imino ester

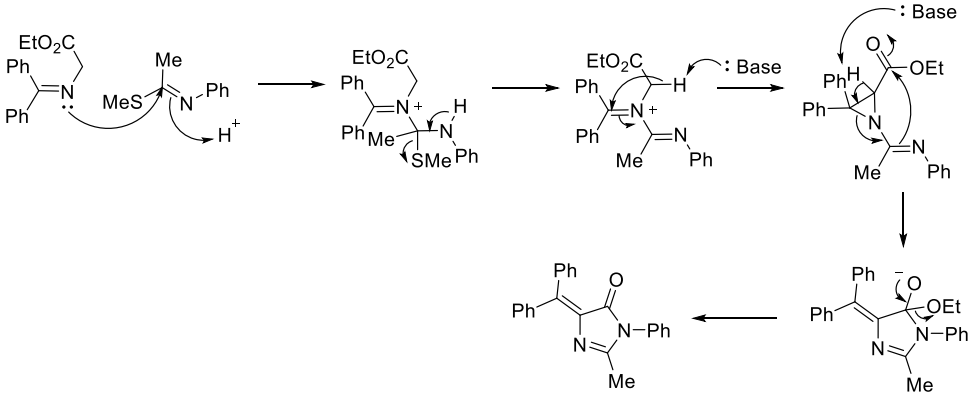
Imidates and thioimidates can also be used to produce 5-ethylidene-4*H*-imidazol-4-ones when reacted with α-imino esters (**Scheme 1.6**).[28,29] The first report of the cyclization of thioimidate and α-imino ester was by Miyashita and coworkers in 2012, where they synthesized five fused-ring 5-ethylidene-4*H*-imidazol-4-ones in 43-85% yield.[29] **Scheme 1.7** displays the proposed mechanism for this reaction. The key step to this mechanism is the formation of an aziridine, which then ring opens to the imidazol-4-one product. In the past two years alone, this reaction has been reported for the production of a number of fluorescent protein chromophores, which require the ethylidene moiety for

their function.[30–33] While this method is used for the production of 5-ethylidene-4*H*-imidazol-4-ones, there are no reported cases of it being used to produce 5-monsubstituted or disubstituted imidazol-4-ones.

Scheme 1.6. General condensation of iminoester and α-imino or α-amino ester



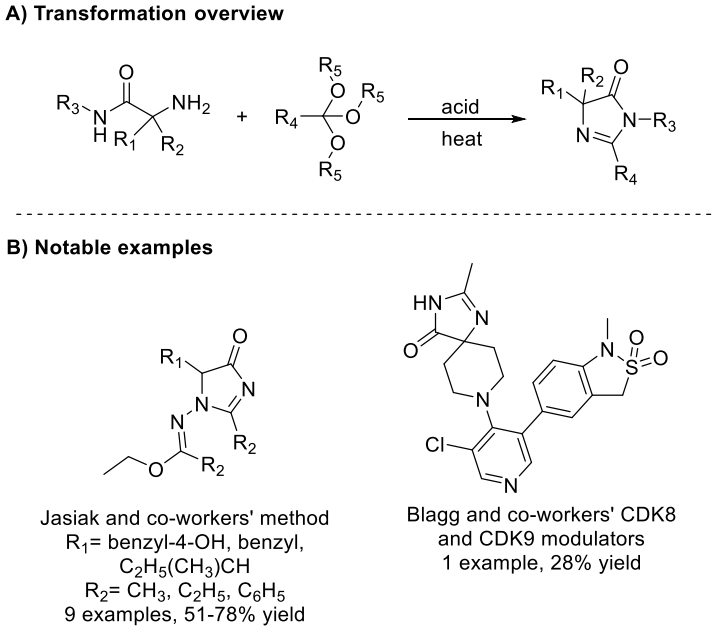
Scheme 1.7 Miyashita and coworker's proposed mechanism for the formation of 5-ethylidene-4*H*-imidazol-4-ones



1.2.1.4 Orthoester and α-amino amide

Another way of synthesizing (4*H*)-imidazol-4-ones is through the condensation of orthoesters and amino amides (**Scheme 1.8**). One of the first reports of this reaction was in 1956 by Brunken and Bach.[34,35] In this reaction, the amine from the amino amide reacts with an orthoester, creating an α-imino amide in situ which then cyclizes to form an imidazol-4-one. Typically, this reaction needs to be activated by acid and/or heat to get good conversion to cyclized product. Since the first report of this reaction, it has been used a number of times in the production of di- and tri-substituted imidazolones.[36–38]

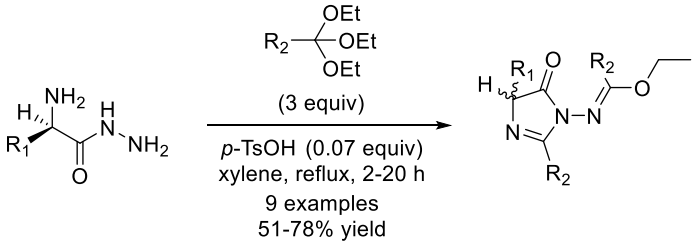
Scheme 1.8 General condensation of orthoester and α-amino amide



A study done by Jasiak and co-workers in 2013 demonstrates the synthesis of imidazol-4-ones from optically active α-amino carboxylic acid hydrazides (**Scheme 1.9**).[36] Nine examples were synthesized in good yields (51-78%). Additionally, several triazines were produced using almost the exact same conditions. It is known that excess orthoester is required to produce an imidazol-4-one as the major product. When

equimolar amounts of orthoester and amino amide are used, the major product isolated is the triazine (**Scheme 1.9**). Interestingly, even though optically pure amino amides were used in this reaction, racemates were produced. While the chiral carbon is not directly affected by the transformation to imidazol-4-one, the carbon can tautomerize through hydrogen migration from the stereogenic carbon to the imino or carbonyl groups, causing a shift of the double bond, leading to loss of optical activity.

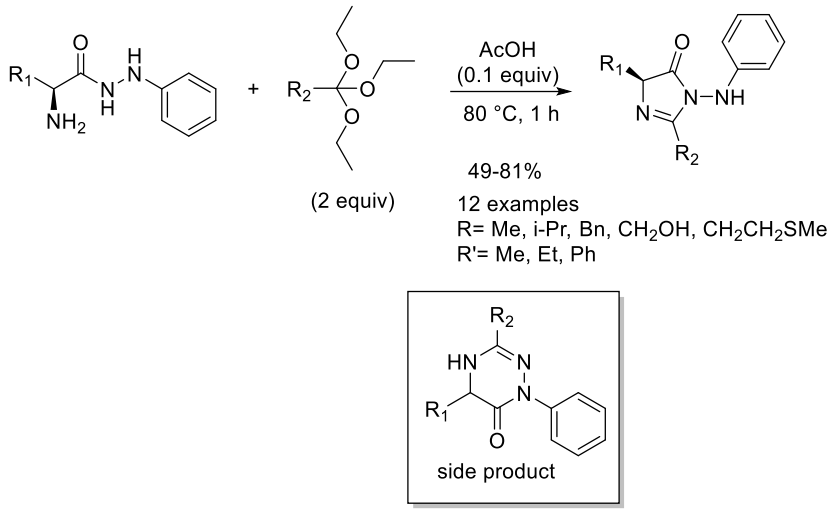
Scheme 1.9 Jasiak and co-workers' synthesis of imidazol-4-ones from optically active α-aminocarboxylic acid hydrazides



In 2014, Kacem and Hassine published an interesting modification to this reaction, leading to the synthesis of enantioselective, 5-monosubstituted imidazol-4-ones.[9] This methodology involved a solvent-free condensation between chiral α-amino acid phenylhydrazides and triethyl orthoesters with catalytic dry acetic acid (**Scheme 1.10**). While solvent-free conditions produced the highest yields in the shortest amount of time, this reaction was also performed under a variety of solvents. Under solvent free conditions, all the compounds were prepared enantioselectively within an hour. While

they do not mention why this reaction was enantioselective when previous reports were not, it may have to do with the milder conditions and shortened reaction time the solvent-free synthesis allowed for.

Scheme 1.10 Kacem and Hassine's solvent-free synthesis of imidazolones

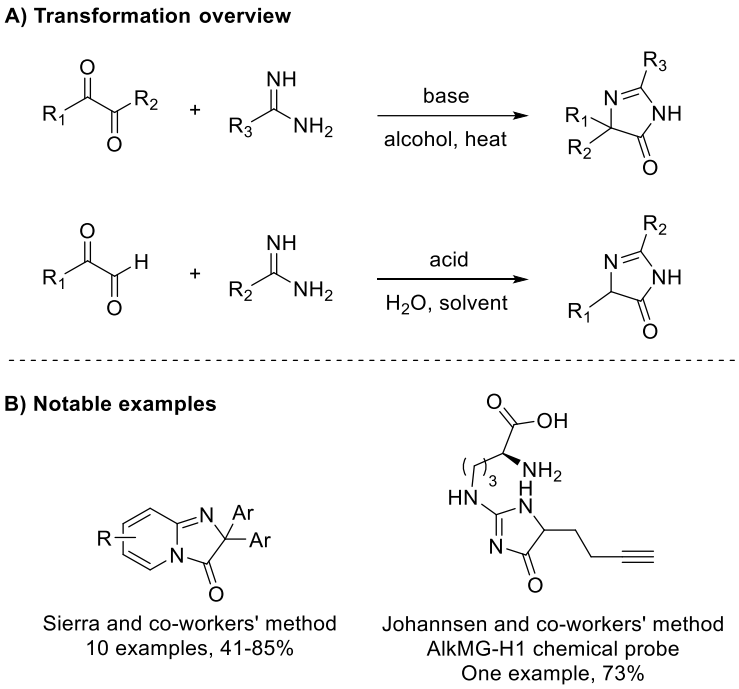


1.2.1.5 Diketone and amidine/guanidine

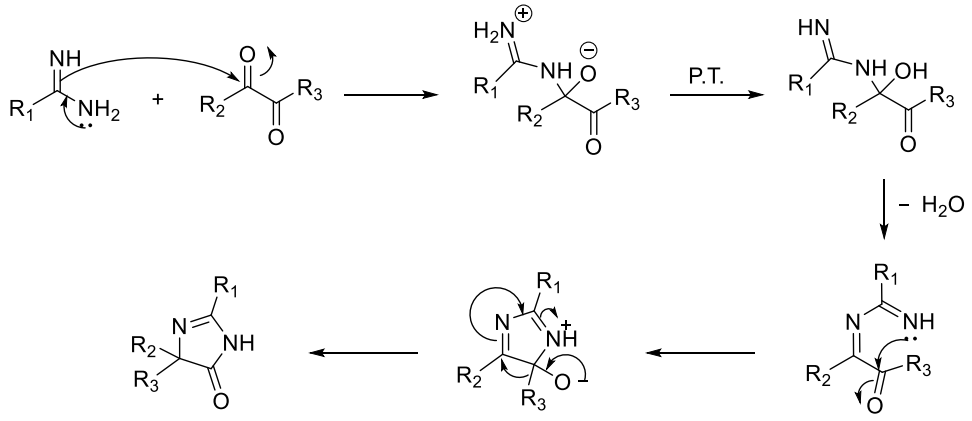
Condensation of a diketone and an amidine or guanidine is a common way to produce 5,5-disubstituted imidazol-4-ones (**Scheme 1.11**). One of the first reports of this reaction was in 1950.[39] In this reaction, the diketone and amidine react under basic conditions, creating a diimine intermediate, which then cyclizes to a 5-hydroimidazole. The 5-hydroimidazole then undergoes a pinacol-like rearrangement to produce the desired imidazol-4-one product. This mechanism is displayed in **Scheme 1.12**.[40] Furthermore, 5-monosubstituted imidazol-4-ones can be produced starting with an α-keto aldehyde under acidic conditions (**Scheme 1.11**). Over the past 10 years, this reaction has been used to produce imidazol-4-ones and 2-aminoimidazol-4-ones for an assortment of medicinal applications.[41–43] Additionally, this cyclization can be used to produce bicyclic

imidazolones through the condensation of a diketone and 2-aminopyridine or 2-aminopyrimidine.[44,45]

Scheme 1.11 General condensation of diketone and amidine to produce di- and tri-substituted imidazol-4-ones

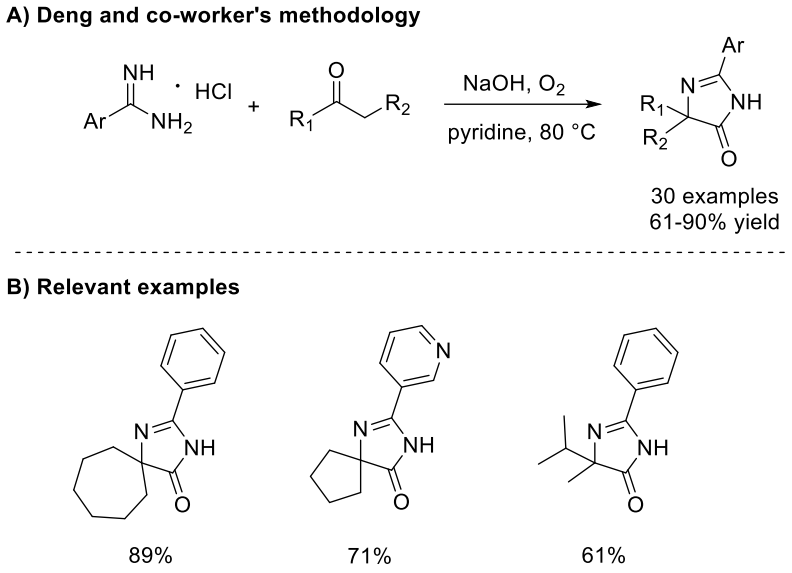


Scheme 1.12 Reported mechanims for the cyclization of amidine and diketone to produce 5,5-disubstituted imidazol-4-ones



In 2015, Deng and coworkers reported a one-pot oxidative condensation of ketones and amidines (**Scheme 1.13**).[40] Here, molecular oxygen is used to oxidize the α-keto carbon to a diketone in situ, which then cyclizes under basic conditions to produce tri-substituted imidazol-4-ones, including spiroimidazol-4-ones, in good yields (30 examples, 61-90% yield). This reaction has not been used to produce any enantioselective imidazolones, mainly due to racemization which occurs during the rearrangement.

Scheme 1.13 Deng and co-workers' synthesis of imidazol-4-ones from ketone and amidine containing starting materials

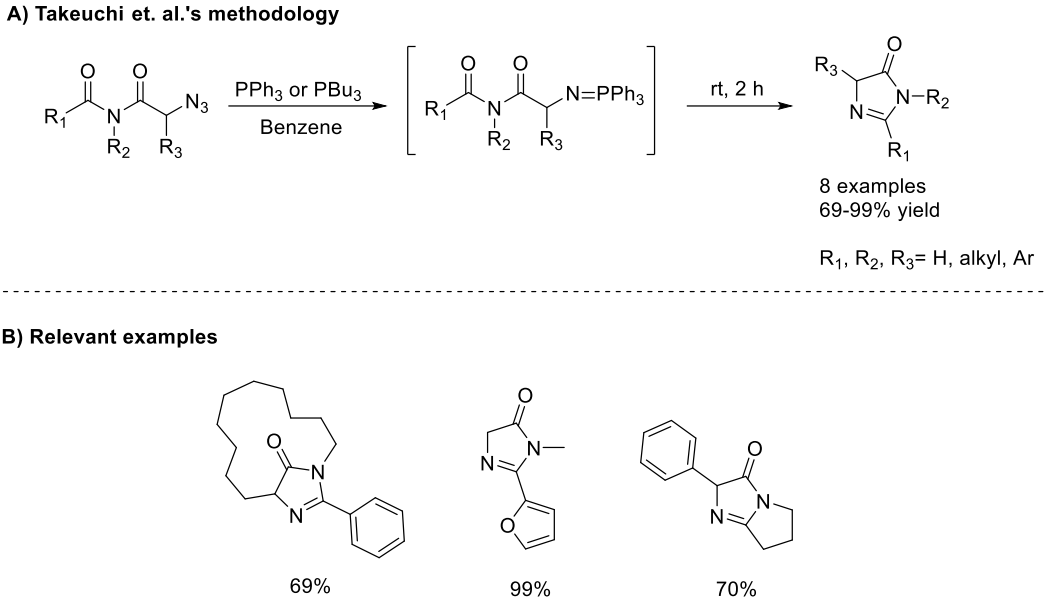


1.2.2 Aza-Wittig reaction

1.2.2.1 Intramolecular aza-Wittig reaction

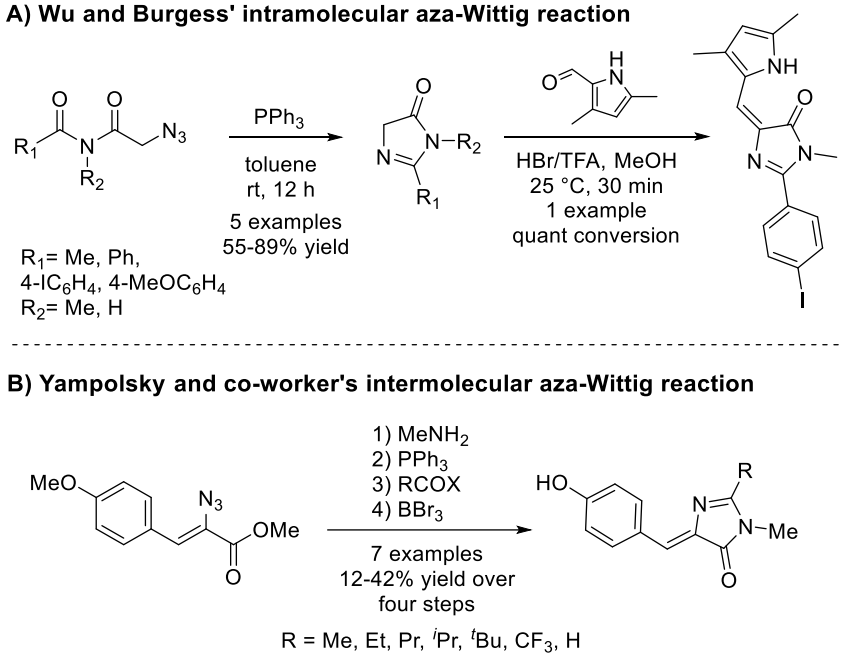
The intramolecular aza-Wittig reaction is another common way to produce imidazol-4-ones. Takeuchi et. al. was one of the first to report this reaction in 1989, using azido-substituted imides.[46] These imides reacted with triphenylphosphine to afford some 5-monosubstituted-4-imidazolones in good yields (69-99%) via a Staudinger reaction, followed by an intramolecular aza-Wittig reaction (**Scheme 1.14**).

Scheme 1.14 Takeuchi et. al.'s intramolecular aza-Wittig reaction to produce imidazolones



Thus far, this reaction appears to be used mainly to produce a range of 5-ethylidene-4-imidazolones.[47–50] There are two different pathways this reaction can be implemented to produce 5-ethylidene-4-imidazolones; both are shown in **Scheme 1.15**. In method A, the more popular method, the imidazol-4-one ring is formed from a terminal azidoimide.[51] Then, the 5-ethylidene substituent is added via a Knoevenagel condensation reaction. Method B first condenses the azidoamide and aldehyde to produce an internal azide.[50] This then reacts with triphenylphosphine, followed by an acid halide to produce an iminoyl halide, which will cyclize upon condensation with the present amide. Alkene conformation is driven by steric interactions as well as the potential to hydrogen bond. Variations of this reaction have been used a couple times to produce 5,5-disubstituted-4-imidazolones.[52,53]

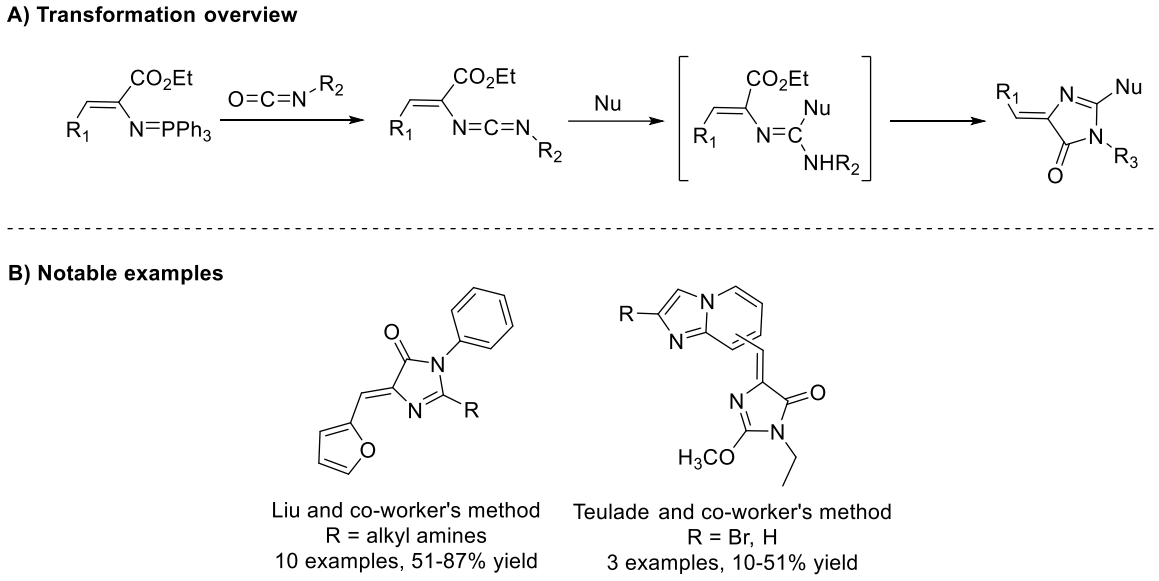
Scheme 1.15 Aza-Wittig reaction to produce 5-ethylidene-4-imidazolones



1.2.2.2 Tandem aza-Wittig/heterocumulene-mediated annulation

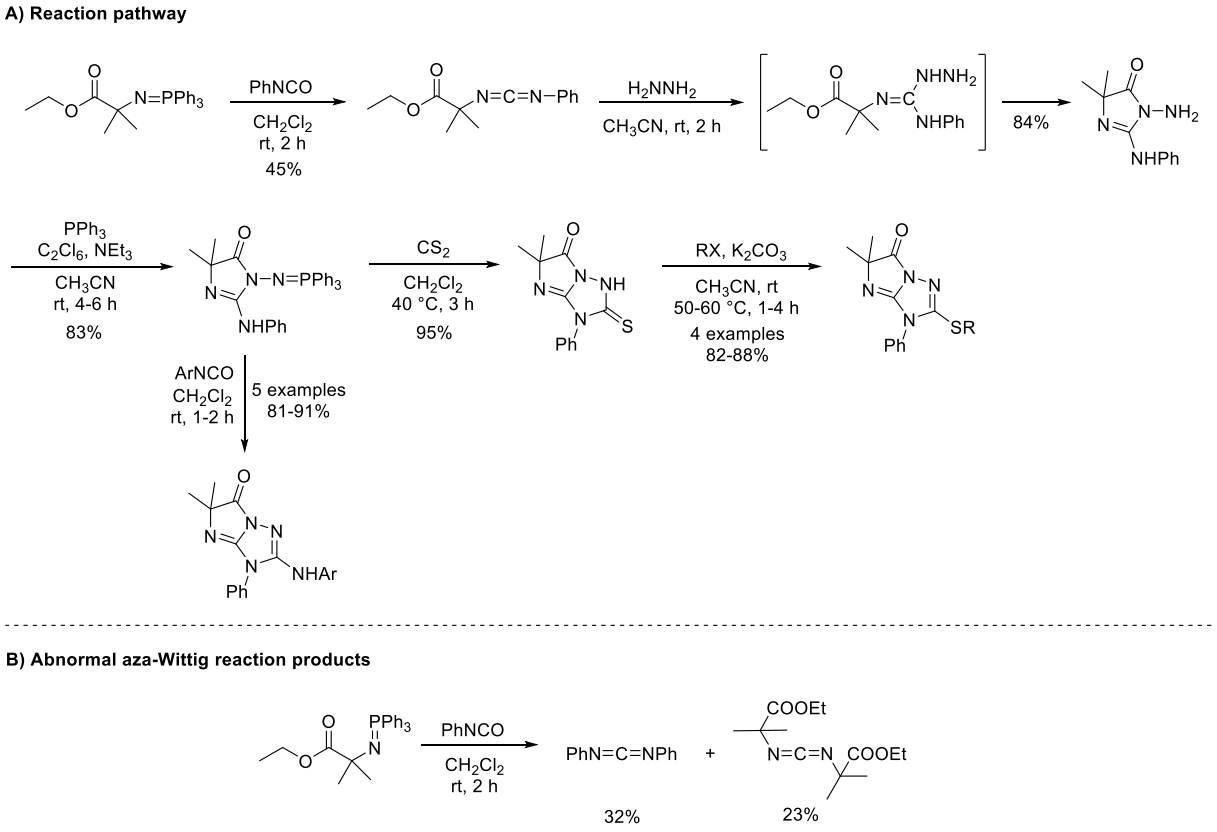
The aza-Wittig reaction can also be used as part of a heterocumulene-mediated annulation reaction to produce imidazol-4-ones. The first report of this reaction was in 1992 by Molina et. al. for the synthesis of aplisinopsin-like alkaloids.[54] In this reaction, an aza-Wittig reagent reacts with isocyanate to produce a heterocumulene intermediate. Upon addition of a nucleophile, the intermediate will cyclize to produce a variety of 5-ethylidene imidazol-4-ones. The mechanism and notable examples of this reaction are shown in **Scheme 1.16**. Some examples of nucleophiles (Nu) include alcohols, amines, thiols, and heterocycles.[4,55–57]

Scheme 1.16 General heterocumulene-mediated annulation initiated by an aza-Wittig reagent



While more uncommon, the aza-Wittig/heterocumulene-mediated annulation can also be used to produce 5,5-disubstituted imidazol-4-ones. Yang and co-workers reported an interesting cascade of reactions to produce 3,5-dihydro-6*H*-imidazo[1,2-b]-1,2,4-triazol-6-ones (**Scheme 1.17**).[58] In this report they highlighted some abnormal aza-Wittig reactions, which led to unanticipated side products, reducing the yield of the isocyanate intermediate (**Scheme 1.17B**). It was noted that only the main aza-Wittig reaction product was produced when a similar but less sterically hindered iminophosphorane was used. Therefore, one limitation to this method is sterics, which can hinder formation of the isocyanate intermediate. This may explain why more 5,5-disubstituted imidazol-4-ones are not made via the aza-Wittig/heterocumulene mediated annulation methodology. There is also one report of this reaction being used to synthesize 5-monosubstituted imidazol-4-ones.[59] Interestingly, this reaction was performed stereospecifically, using enantiopure amino acids to produce enantiopure 5-monosubstituted imidazolones.

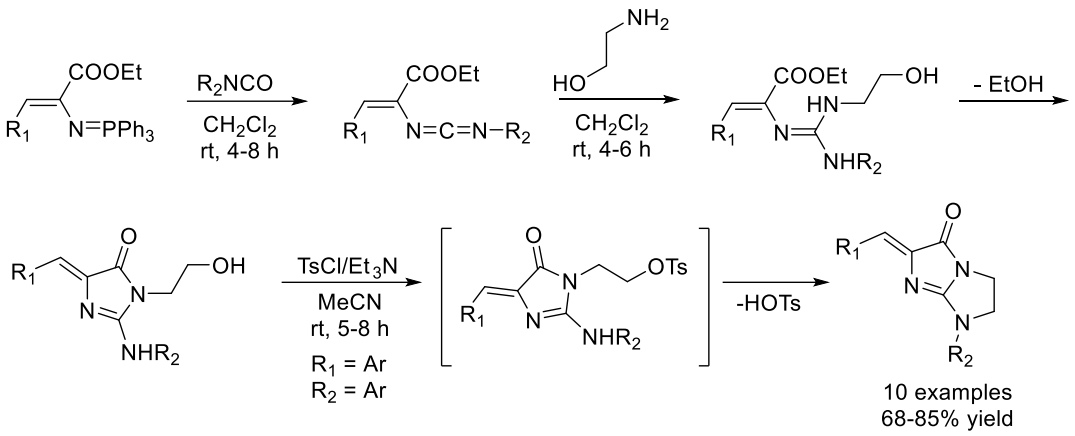
Scheme 1.17 Yang and co-worker's synthesis of 3,5-dihydro-6*H*-imidazo[1,2-b]-1,2,4-triazol-6-ones



In 2019, a one-pot, three step reaction was reported by Ding and co-workers using an aza-Wittig/heterocumulene mediated annulation to produce a variety of bicycloimidazol-4-ones.[60] **Scheme 1.18** describes their one-pot, three-step synthesis of a variety of bicycloimidazol-4-ones in good yields (68-85%). In this reaction, a (vinylimino)phosphorane is treated with a variety of aromatic isocyanates to produce a carbodiimide. 2-aminoethanol is then added to the reaction, which produces a guanidine intermediate that cyclizes upon the loss of ethanol. Then, upon addition of tosyl chloride (TsCl) and triethylamine (NEt_3), the primary alcohol is converted to a tosyl ether, which

leaves upon the formation of the second ring. These three steps were performed in sequence, without isolation.[60]

Scheme 1.18 One pot, multicomponent synthesis of bicycloimidazolones reported by Ding and co-workers



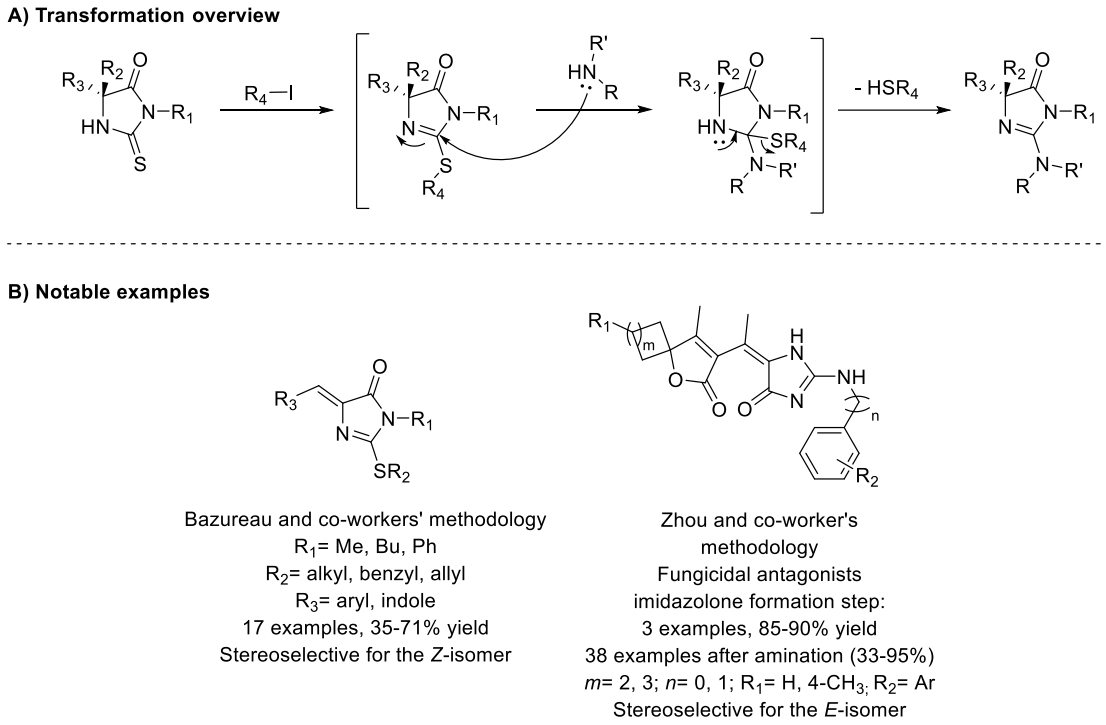
1.2.3 Heterocyclic conversion/rearrangements

1.2.3.1 Thiohydantoin conversion to imidazolone

One of the most commonly used methodologies for the production of imidazol-4-ones is the conversion of thiohydantoins using alkyl halides. This was first reported by Daboun and Ibrahim in 1981.[61] Since then, there have been numerous reports of this transformation being used to synthesize various imidazol-4-ones for medicinal applications[62,63] and the total synthesis of natural products.[64–68] **Scheme 1.19** describes the basic transformation from thiohydantoin to 2-aminoimidazol-4-one. This reaction usually takes two steps. In the first step, the thiohydantoin is converted to 2-(alkylthio)imidazol-4-one by reacting the thiohydantoin with an alkyl halide and some base. The second step is then addition of a nucleophile (anime, hydrazine, boronic acid, ether, etc.) with heat, which converts the 2-thioether to a variety of functional groups. Substitution at the 5 position of the ring is also versatile, withstanding mono- and di-substitution as well as an

ethylidene functional group. Moreover, the 5-position substitution is not affected during transformation from thiohydantoin to imidazol-4-one, which allows this method to be used for the production of stereoselective compounds.[69] As with many of these methods, the stereochemistry of the 5-ethylidene group is driven by sterics, to avoid interactions with the imidazolone's carbonyl. This typically leads to the *Z*-isomer being favored.[63] However, in some instances, the *E*-isomer may be favored, like in Zhou and coworker's work, mentioned in **Scheme 1.19B**.[62] In this scenario, the E-isomer was identified from a X-ray structure.

Scheme 1.19 Conversion of thiohydantoin into 2-aminoimidazolone

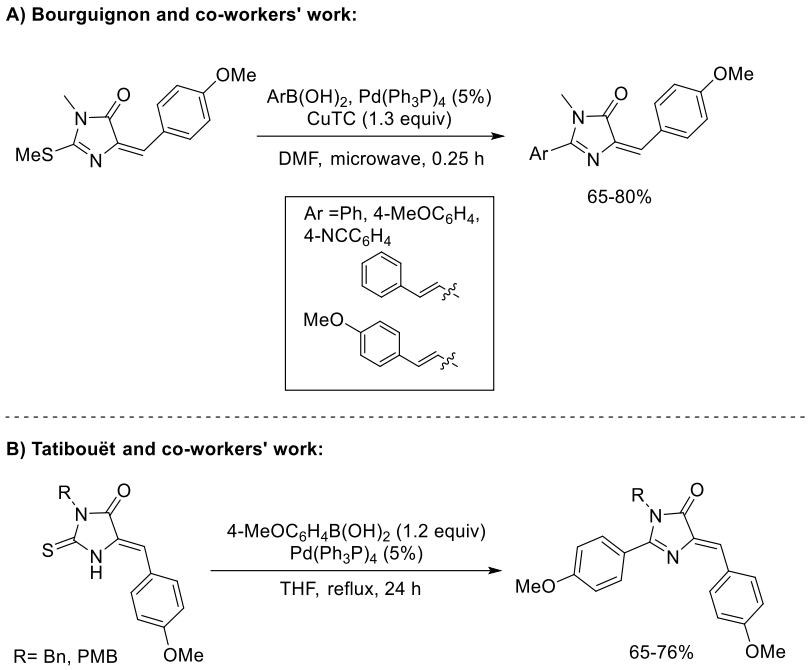


Ease of alteration of the 2-position of the thiohydantoin ring can lead to a multitude of unique imidazol-4-ones. Amination of the 2-position is performed with a desired amine and heat; this is the same procedure for substitution to a hydrazine. Etherfication can be accomplished using an alcohol and some base, along with heat.[70]

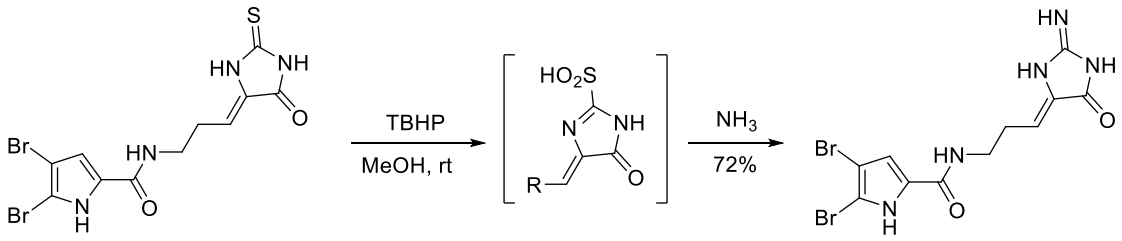
Arylation of the 2-position of the imidazol-4-one is also possible, following the Liebeskind-Srogl reaction. This method was first reported by Bourguignon and co-workers in 2004 (**Scheme 1.20**).[71] The Liebeskind-Srogl reaction creates a C-C bond through the cross coupling of a boronic acid and thioether in the presence of copper(I) thiophene-2-carboxylate (CuTC) and tetrakis(triphenylphosphine)palladium ($Pd(Ph_3P)_4$).[72] Surprisingly, the authors report the *E*-isomer was isolated from the Knoevenagel condensation of 4-methoxy benzaldehyde and thiohydantoin. This led to isolation of *E*-imidazol-4-one. In another study, Tatibouet and co-workers reported the use of boronic acids and $Pd(PPh_3)_4$ to directly convert thiohydantoins into 2-arylimidazolones (see **Scheme 1.20**).[72] In their work, the Knoevenagel condensation of 4-methoxy benzaldehyde and thiohydantoin led to the isolation of the *Z*-isomer of thiohydantoin and subsequentially, *Z*-imidazol-4-one.

The conversion of thiohydantoin to 2-aminoimidazol-4-one can be performed using a one-pot, two step procedure with tert-butylhydroperoxide (TBHP) and aqueous ammonia in methanol at room temperature. This reaction was first mentioned in the total synthesis of dispacamide, reported by Lindel and Hoffmann in 1997 (**Scheme 1.21**).[64] In this reaction, TBHP is used to oxidize the sulfur to a sulfinic acid in situ, which is then easily removed by nucleophilic attack of ammonia. Since this reaction was first reported, it has been used to synthesize a range of (4*H*)-imidazol-4-ones.[68,73,74]

Scheme 1.20 Conversion of thiohydantoin to 2-aryl-imidazol-4-one



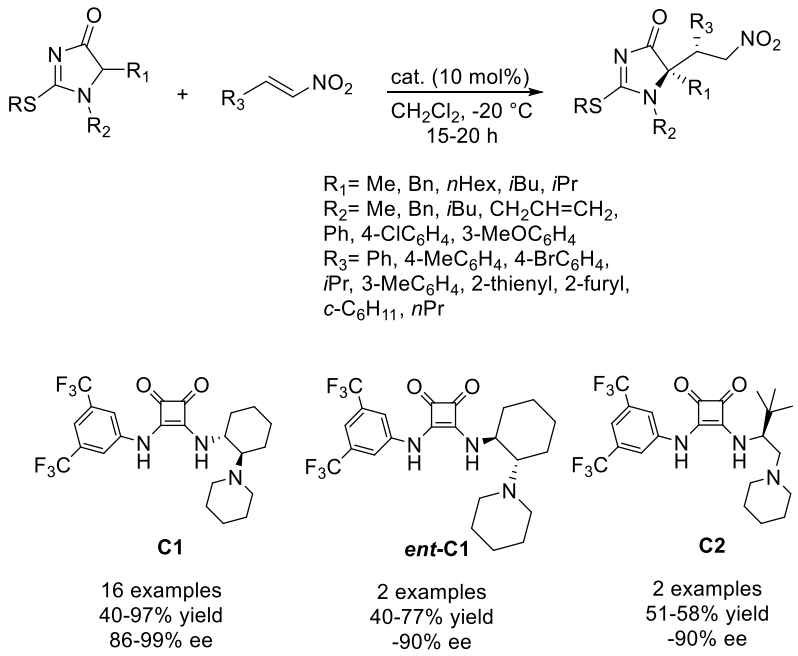
Scheme 1.21 First reported conversion of thiohydantoin to imidazolone using TBHP



Palomo and co-workers have been working on a method to produce enantiopure 5,5-disubstituted imidazolones from 5-monosubstituted imidazolones.[75–77] In their reaction, a 5-monosubstituted imidazolone reacts with an electrophile under the influence of a bifunctional Bronsted base/H-bond catalyst to create a stereoselective 5,5-disubstituted product (**Scheme 1.22**). Additionally, they used this method to produce some enantioselective bi- or tricyclic imidazol-4-ones. Since this first report, they have elaborated on the variety of groups used to enantioselectively alkylate the 5-position of

the ring, including aldol products and enols. A variety of catalysts were also used, with varying success.[75–77]

Scheme 1.22 Enantioselective reaction between imidazol-4-ones and nitroolefins



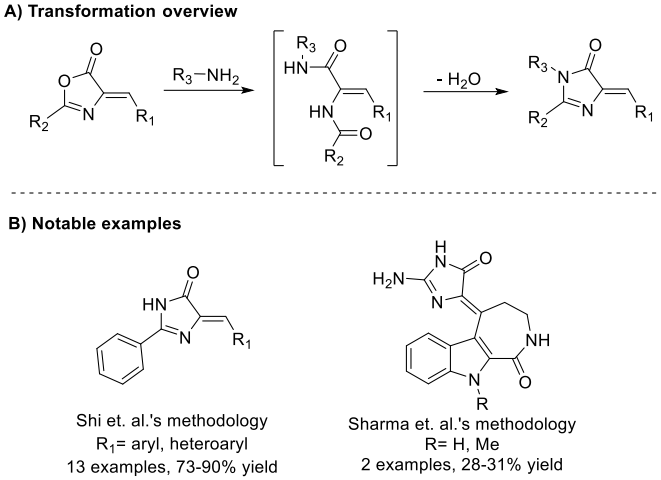
1.2.3.2 Oxazolone rearrangement

Rearrangement of an oxazolone ring upon addition of an amine can be used to produce di-substituted and tri-substituted imidazol-4-ones for a wide range of biological applications,[2,78–81] total syntheses,[82] and other applications.[83] **Scheme 1.23** displays the basic mechanism for this reaction. The reaction is initiated by nucleophilic attack of a primary amine, which opens the oxazolone ring into a diamide. The diamide will then cyclize to form an imidazol-4-one upon loss of water. Reagents used to promote this reaction can vary, and the reaction can be run under acidic, neutral, or basic conditions; however most reactions require heat. Some popular conditions include refluxing the amine in pyridine or ethanol, or refluxing with sodium acetate in acetic acid. A wide

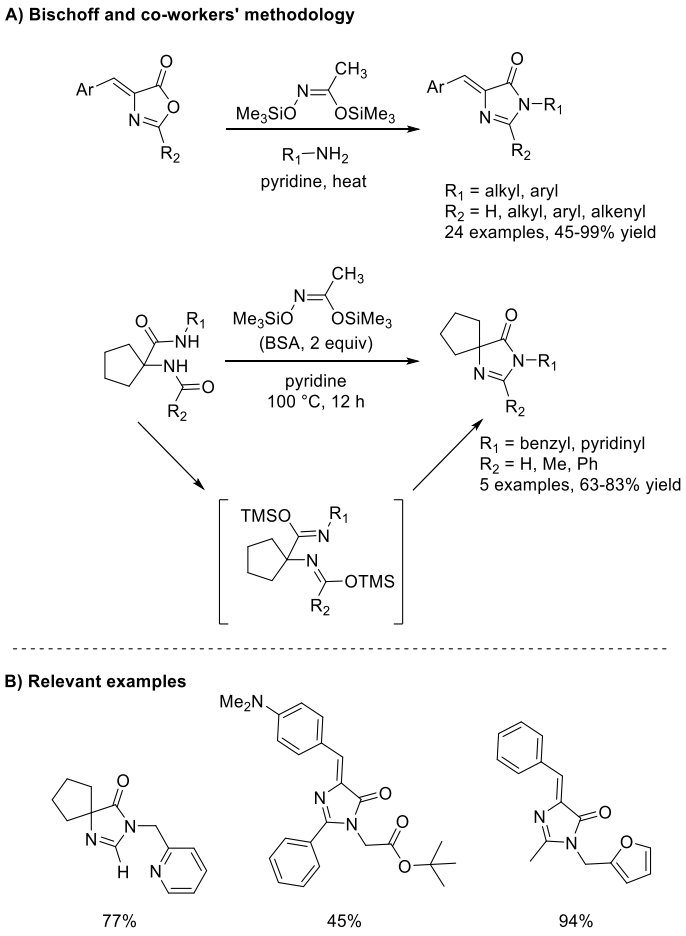
range of amines can be used as the nucleophile for the ring opening amination, including primary aryl, alkyl, benzyl, silyl and alkenyl amines, amino acids, ammonium acetate, and hydrazines.[2,3,78] Additionally, the use of benzyl carbamimidothioate has been reported to produce 2-amino-4*H*-imidazol-4-ones.[84,85] This reaction is mainly used to produce 5-ethylidene-4-imidazolones, but has been used several times to produce 5,5-disubstituted-4-imidazolones.[86,87] There have been no reports of 5-monosubstituted imidazol-4-ones being produced via this method. A slight variation of the oxazolone rearrangement is the use of a thiazol-4(5*H*)-one, which ring opens upon addition of a secondary amine. The intermediate will then recyclize, releasing sulfur in the process. This reaction has been reported only a few times, beginning in 2007.[88–90]

One of the downfalls of this reaction is the harsh conditions needed to convert from diamide to imidazol-4-ones, which limits this reaction, avoiding the use of sterically bulky amines. An interesting alteration to this reaction is described in an article by Bischoff and co-workers, where they used *N*,*O*-bistrimethylsilylacetamide (BSA) to promote the transformation of oxazolone to imidazol-4-one.[91] In this reaction, BSA is a dehydration reagent, used to speed up the dehydration of diamide under mild conditions. This reaction proved to be a mild, one-pot method to produce a range of imidazolones (**Scheme 1.24**). BSA provided them with a large tolerance towards a variety of functional groups, including 5,5-dialkyl and 5-benzylidene imidazolones as well as 2*H*- and 2-substituted imidazolones, in mild to good yields (45-99%).[91] Some of the highlights from this procedure include its compatibility with tert-butyl groups, formamides, activated double bonds, and *N*-arylamides, which are poorly reactive.

Scheme 1.23 General transformation of oxazolone to imidazolone



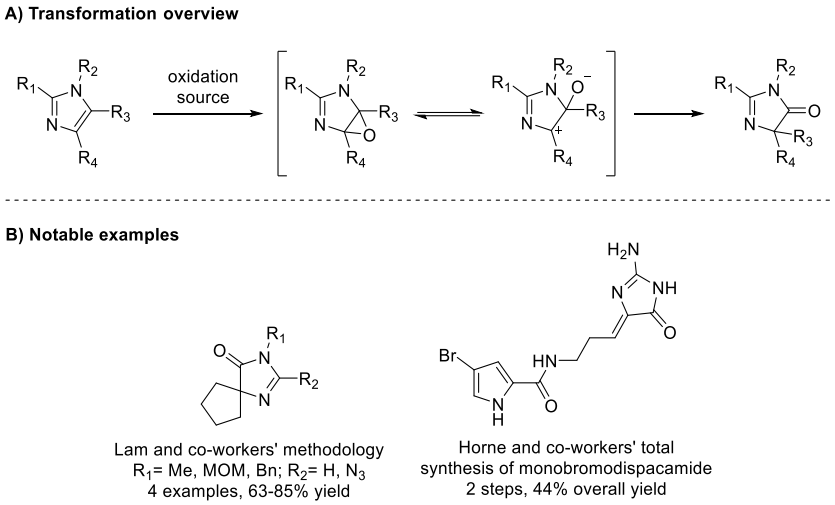
Scheme 1.24 Bischoff and co-workers' work on a BSA-mediated formation of imidazolones from oxazolones



1.2.3.3 Oxidative pinacol-like rearrangement of imidazole

The oxidative rearrangement of imidazoles is an alternative method of producing imidazol-4-ones. In this reaction, an oxidation source, like singlet oxygen or dimethyldioxirane (DMDO), is used to epoxidate the imidazole, which can subsequently undergo a pinacol-like rearrangement to form an imidazol-4-one (**Scheme 1.25**). One of the first reports of this reaction was by Guy Rio and Bernard Serkiz in 1975, where they used molecular oxygen as an oxygen radical source.[92] Since then, this reaction has been performed using a variety of oxidants, such as DMDO,[93] Davis reagent,[94,95] m-chloroperbenzoic acid (mCPBA),[96] and other peroxides.[97] This reaction has been used to produce a wide range of imidazolones for natural product total synthesis, including calcaridine A, hymenialdisine, oxysceptrin and monobromodispacamide.[98–102] Additionally, there is some evidence to support the formation of enantioselective products from the pinacol-like rearrangement. Kimura and co-workers report a stereoselective 1,5-phenyl migration on several imidazolols upon addition of base in DMSO, producing 5,5-diarylated imidazol-4-ones (ee > 90%).[103] In nature, the pinacol rearrangement is used to convert 2'-deoxyguanosine to a spiroimidazolone.[104] This reaction was found to occur naturally through single-electron oxidation under basic conditions (pH > 8) and can also take place in acetic acid with m-chloroperbenzoic acid (mCPBA) or dimethyldioxirane (DMDO).[105]

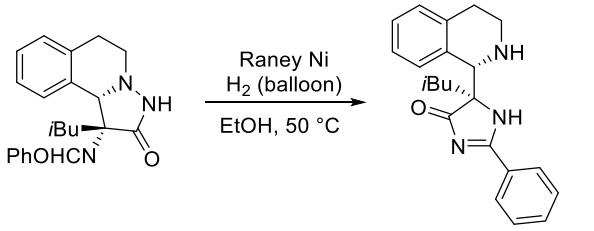
Scheme 1.25 General mechanism for the pinacol-like rearrangement of imidazole to imidazolone



1.2.3.4 Rearrangement of pyrazolidin-3-one

In 2016, Su and co-workers reported the rearrangement of pyrazolidin-3-one using Raney nickel and hydrogen (**Scheme 1.26**).[106] Raney nickel and atmospheric hydrogen converts the pyrazolidine-3-one to imidazol-4-one through a reductive cyclization via cleavage of the nitrogen-nitrogen bond. Not much is reported on this reaction, as it has only been used once for the purpose of synthesizing an imidazolone, however, it has been used previously to produce an indolizidine from *N*-amino-3,4-dihydroisoquinoline.[107,108]

Scheme 1.26 Reductive rearrangement of pyrazolidine-3-one to imidazol-4-one

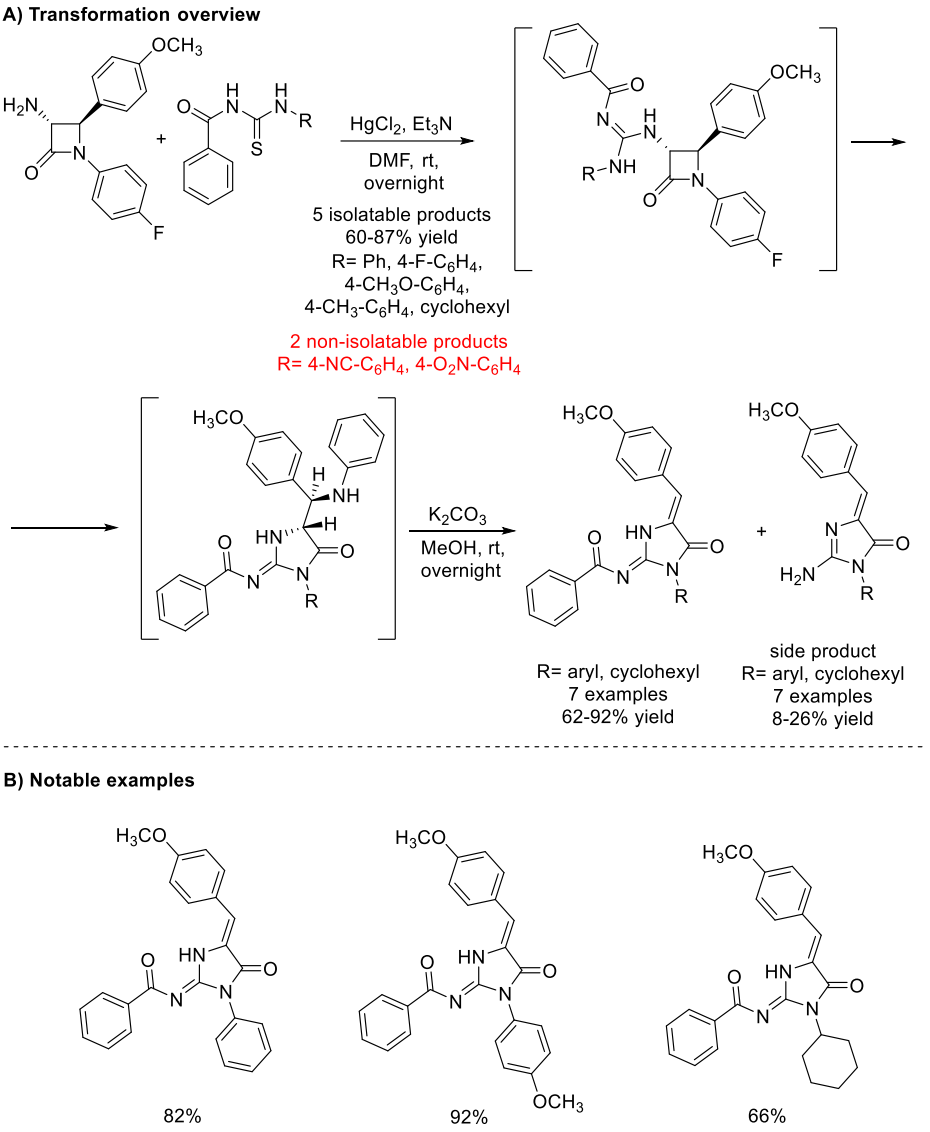


1.2.3.5 Ring expansion of amino-β-lactam

In 2015, Habuš and coworkers reported a base-promoted amino-β-lactam ring expansion that produced 2-amino-4-imidazolones (**Scheme 1.27**).[109] In this reaction, the amino-β-lactam reacts first with thiourea. Then, upon treatment with potassium carbonate, there is an amidolysis via the N1-C2 bond, which leads to a rearrangement which provides a 5-membered imidazol-4-one ring. It was found that they could not isolate 2-guanidine β-lactams that contained an electron withdrawing group on the guanidine nitrogen. Instead, those compounds rearranged to a 5-membered ring without addition of potassium carbonate. Presumably, the electron withdrawing groups (i.e. NO_2, CN) allow for easier deprotonation, which makes triethylamine a strong enough base to deprotonate the guanidine, allowing it to react with the C2 carbon. The stereochemistry around the the double bond was determined to be *Z* based on a crystal structure.

This rearrangement has been used previously to produce other heterocycles, like thiohydantoins, hydantoins, and imidazolines. [110,111] In 2017, this methodology was used to produce leucettamine B and C.[112]

Scheme 1.27 Habuš and co-workers' beta-lactam ring expansion to produce 2-amino-4-imidazolones

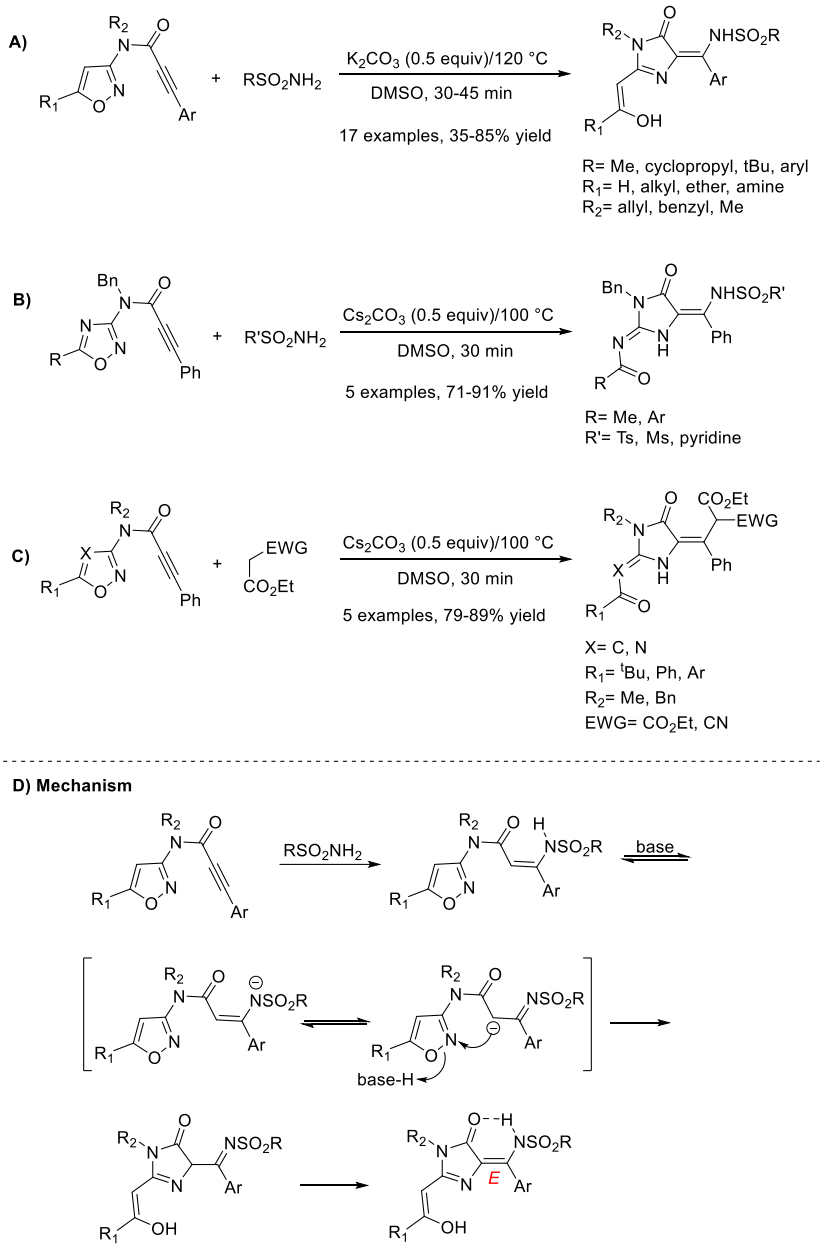


1.2.3.6 Boulton-Katritzky rearrangement of isoxazole

In 2019, Cai and co-workers reported a stereoselective synthesis of (*E*)-5-tetrasubstituted-ylidene-3,5-dihydro-4*H*-imidazol-4-ones starting from isoxazoles.[113] This reaction is a combination of a Michael addition reaction followed by a Boulton-Katritzky rearrangement, using a nucleophilic carbon during the rearrangement process. Several

conditions were screened, including changes in the base, base equivalents, solvent, temperature, and time. Ultimately, the best conditions were reported as using potassium carbonate (0.5 equivalents) in DMSO at 120 °C for 45 minutes (81% yield). These conditions were used to produce a range of imidazol-4-ones, varying at three different positions (see **Scheme 1.28A**) in moderate to good yields (35-85%). Additionally, this reaction produced some 2-amino-4-imidazolones in good yields (71-91%) by altering the starting material (see **Scheme 1.28B** and **Scheme 1.28C**). The mechanism of this reaction is displayed in Scheme 28D. The *E* stereoselectivity is due to a hydrogen bond formation between the NH of the alkene and oxygen atom of the carbonyl group. Notably, this reaction has only been used to produce 5-ethylidene-4-imidazolones.

Scheme 1.28 Cai and co-workers' synthesis of imidazol-4-ones via rearrangement of isoxazoles



In summary, this section of the dissertation aimed to highlight the known efforts towards the development of novel methodologies for the preparation of imidazol-4-ones. A number of methods were described, with examples of their usability for medicinal and other applications. Advantages and disadvantages of each method were highlighted,

including the scope of the reaction, limitations to substitution patterns of the imidazol-4-ones, and abilities to produce enantioselective products. **Table 1.1** highlights the methods discussed in this section and summarizes the substitution patterns which can be produced from each method. There are six methods which can be used to produce all three substitution patterns: cyclization of diamides, cyclization of amino ester and cyanamide/guanidine, tandem aza-Wittig/heterocumulene-mediated annulation, thiohydantoin conversion to imidazolone, oxazolone rearrangement, and oxidative pinacol-like rearrangement of imidazole. Several of the other methods were specifically designed for the synthesis of 5-ethylidene-4-imidazolones, including the cyclization of imidate and α-imino esters, ring expansion of amino-β-lactam, and the Boulton-Katritzky rearrangement of isoxazole. There are several reported methods to produce of 5-monosubstituted and 5,5-disubstituted imidazol-4-ones, however, there is a lack of methods that produce enantiopure 5-substituted imidazol-4-ones. In fact, there is only one reaction reported that produces enantiopure 5,5-disubstitued imidazol-4-ones stereo-selectively from a starting material where the stereocenter was not already in place.[75–77] Overall, this chapter highlights the importance of imidazol-4-ones within a range of different applications and displays the known preparative methods for this heterocyclic scaffold.

Table 1.1. Substitution patterns of imidazol-4-ones produced from referenced reactions, where green symbolizes produced scaffolds and red symbolizes scaffolds which have not been produced using that methodology.

Reaction	**5-monosubstituted**	**5,5-disubstituted**	**5-ethylidene**
Cyclization of diamides			
Amino ester and cyanamide/guanidine			
Imidate and α-imino ester			
Orthoester and α-amino amide			
Diketone and amidine/guanidine			
Intramolecular aza-Wittig reaction			
Tandem aza-Wittig/heterocumulene-mediated annulation			
Thiohydantoin conversion to imidazolone			
Oxazolone rearrangement			
Oxidative pinacol-like rearrangement of imidazole			
Rearrangement of pyrazolidin-3-one			
Ring expansion of amino-β-lactam			
Boulton-Katritzky rearrangement of isoxazole			

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2 Chapter 2: Preparation of (4*H*)-imidazol-4-ones for the treatment of neurodegeneration

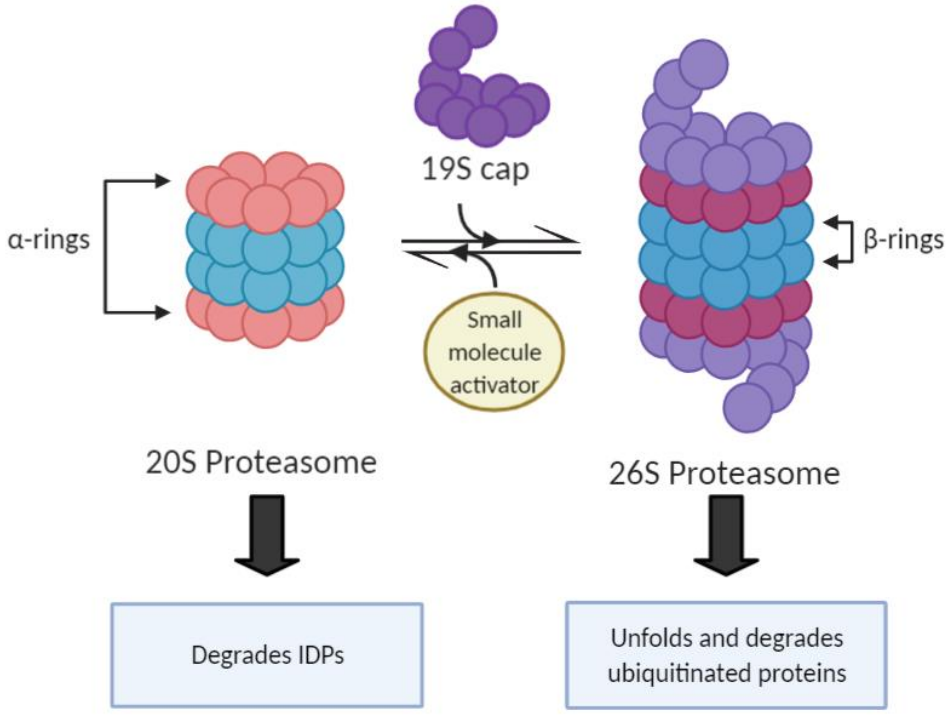
2.1 Introduction

Neurodegeneration is defined as a progressive atrophy and decline in the function of neurons within your body.[1] It can lead to several known neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS).[2–4] In these neurodegenerative diseases, disruption of proteostasis and toxic signaling is caused by the accumulation of intrinsically disordered proteins (IDPs) which leads to unmanageable aggregation and oligomerization.[5–7] Therapeutic methods for inhibition of IDPs are difficult, as their disordered structure prevents drug-design for small-molecule binding pockets on the target protein. Neurodegeneration is a prevalent area of research due to a lack of treatment options for neurodegenerative diseases. In fact, up until 2021 and the FDA's accelerated approval of Aduhelm for the treatment of Alzheimer's disease, there has not been an approved treatment for neurodegenerative diseases that treats the underlying cause of the disease.[8,9] In the Tepe lab, we are targeting the proteasome as a possible therapeutic target for the increased degradation of these toxic IDPs.[10–14]

The proteasome is a protein complex in the body that is responsible for the proteolysis of redundant, misfolded, or damaged proteins down into recyclable peptide fragments.[15–17] While there are many forms of the proteasome, we will focus on the two most predominate forms, the 26S and 20S proteasomes (**Figure 2.1**). The 26S proteasome is composed of two main subcomplexes; the 20S proteasome, also known as the catalytic core, and two 19S regulatory particles/caps. The 20S catalytic core particle is composed of four stacked rings: two outer alpha-rings and two inner beta rings. The beta rings contain three catalytic sites for the proteolysis of

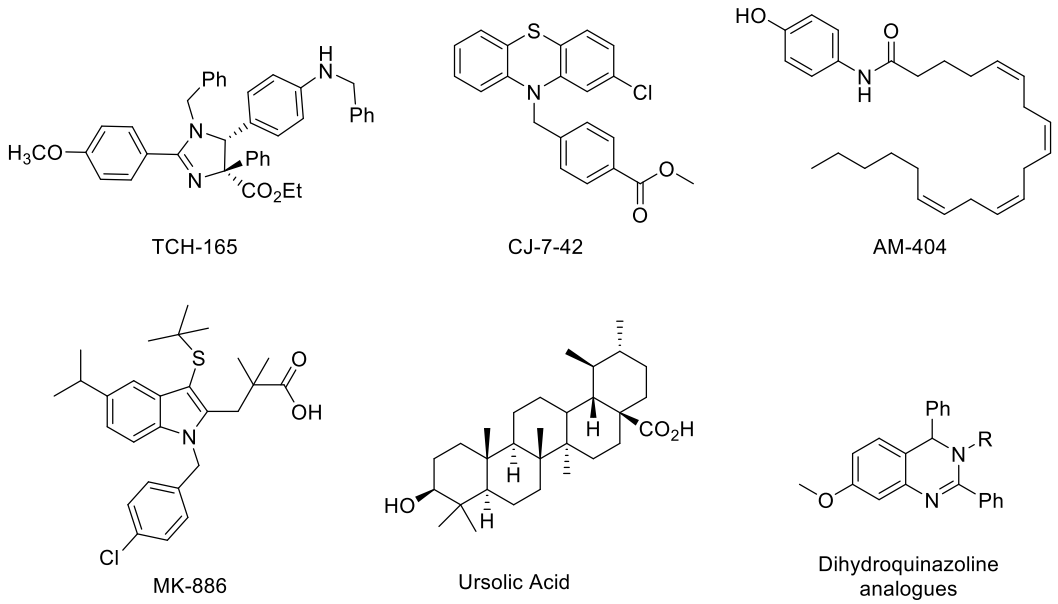
proteins, while the alpha rings act as a gate, which opens and closes upon activation by the 19S regulatory particles. The regulatory particles are responsible for "activating" the proteasome, allowing for the unfolding and degradation of "flagged" (ubiquitinated) proteins. Without the 19S caps present, folded proteins cannot be degraded by the 20S core particle, however, it has been shown that the 20S proteasome has the ability to degrade IDPs.[18,19] Unfortunately, without the 19S caps present, the 20S proteasome is considered a latent state, with the gates being predominantly closed, not allowing for IDP proteolysis. This is why the Tepe lab as well as others have been seeking small molecules proficient in enhancing the proteolytic activity of the 20S proteasome, with several 20S activators already reported (see **Figure 2.2** for some examples).[11,14,16,20–25] It is believed that these activators are enhancing IDP degradation by allowing increased access to the catalytic core via alpha-ring gate regulation.

Figure 2.1 Diagram of the 26S and 20S proteasomes



TCH-165 (**Figure 2.2**) is the Tepe lab's most successful proteasome "activator" to date. It has been shown to increase proteasome activity 8-fold *in vitro* and in cell culture.[11,13,14] While TCH-165 displays good activity towards the proteasome, it lacks the typical drug-like characteristics[26–29] one would expect of such an active compound. TCH-165 is very large (595 g/mol) and lipophilic (Log P 8.42).

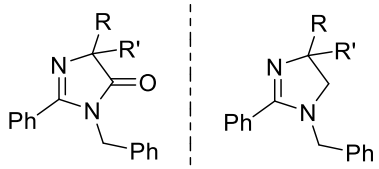
Figure 2.2 Some recent examples of small moelcule 20S proteasome activators



To reduce lipophilicity and molecular weight, we proposed imidazol-4-ones be explored as potential proteasome activators, due to their similarity to the imidazoline scaffold of TCH-165. To the best of our knowledge, the imidazol-4-one scaffold has never been tested for proteasome modulation, however, imidazol-4-ones do have precedence in nature as biologically useful and active scaffolds.[30–35] The proposed imidazol-4-one scaffold is displayed in **Figure 2.3**. Additionally, we recognize a second, imidazoline scaffold can easily be synthesized through a simple reduction of imidazol-4-one. Overall, this chapter will highlight several synthetic methods explored for the synthesis of imidazol-4-ones. Furthermore, results of proteasome

activation studies from a small library of synthesized 5,5-disubstituted imidazol-4-ones and imidazolines will be discussed.

Figure 2.3 Proposed imidazolone scaffolds for methodology development and proteasomal activity testing



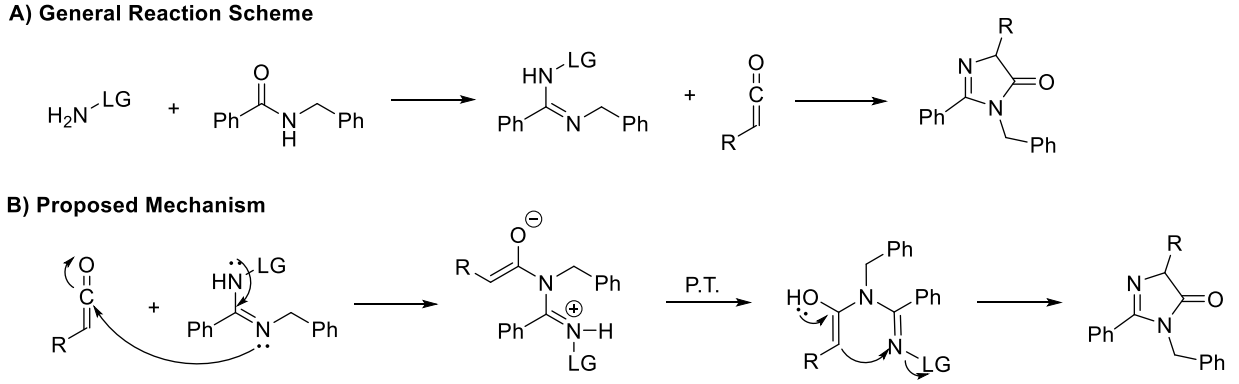
Scaffold A Scaffold B

2.2 Results and discussion

2.2.1 Staudinger-like ketene-imine inspired cyclization

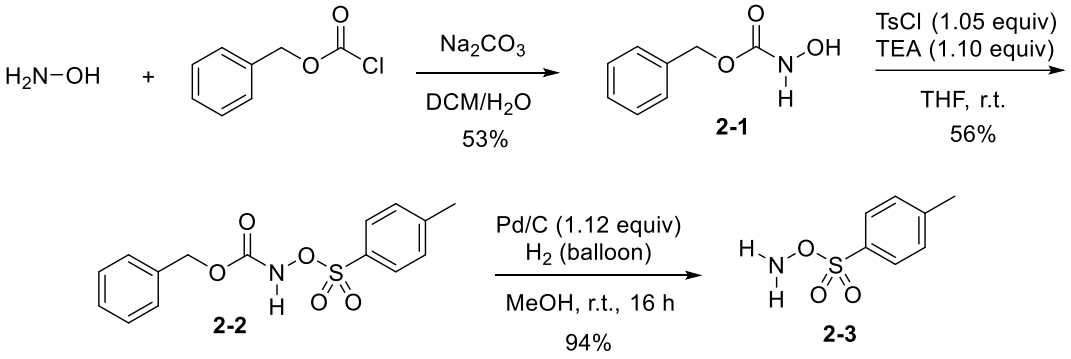
Ketenes and imines typically undergo a [2+2] cycloaddition reaction in the presence of base or heat to produce beta-lactams.[36,37] The product of this [2+2] cycloaddition can ring expand upon the addition of heat. One example of this was reported by Barr and Storr, where the [2+2] cycloaddition of ketene and imidoylazimines led to the formation of a β-lactam, which ring expanded upon the addition of heat to give a range of products, including benzocinnoline, 5,5-disubstituted imidazol-4-ones and triazolines.[38] Since then, several papers have used a similar method for the formation of pyrimidinones.[39-42] While Barr and Storr described how an imidazol-4-one could be synthesized through the ring expansion of a β-lactam, there have been no follow up reports exploring the scope and overall potential of this method to produce imidazol-4-ones. It can be envisioned that a similar pathway may be used to synthesize mono-substituted imidazol-4-ones via a Staudinger-like ketene-imine cyclization of amidine and ketene (**Scheme 2.1A**). The proposed mechanism of this reaction is shown in **Scheme 2.1B**, where using an appropriate leaving group such as tosylate, methoxy, or acetate could facilitate a 5-exo-trig cyclization or the formation of a nitrene intermediate.

Scheme 2.1 Staudinger-like ketene-imine cyclization proposed reaction and mechanism to make imidazol-4-ones

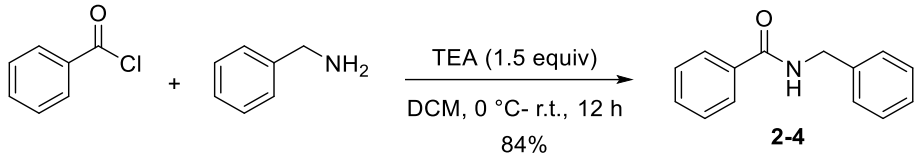


To begin exploring this methodology, the starting materials were prepared: namely *O*-tosylhydroxylamine and *N*-benzylbenzamide. **Scheme 2.2** describes the synthetic pathway to produce *O*-tosylhydroxylamine, adapted from Wen et. al's 2010 report.[43] Here, hydroxylamine was first *N*-protected using benzyl chloroformate and sodium carbonate in dichloromethane, providing 53% yield of the *N*-protected product. Then, p-toluenesulfonyl chloride was used to *O*-tosylate the hydroxyl in 56% yield. The last step of this pathway was the deprotection of the benzyloxycarbonyl using palladium on carbon and hydrogen gas, synthesizing the desired *O*-tosylhydroxylamine in 94% yield. *N*-benzylbenzamide was produced as described in **Scheme 2.3**. Here, benzyl chloride and benzylamine were reacted under basic conditions, using triethyl amine, to produce *N*-benzylbenzamide in 84% yield.[44]

Scheme 2.2 Synthesis of *O*-tosylhydroxylamine

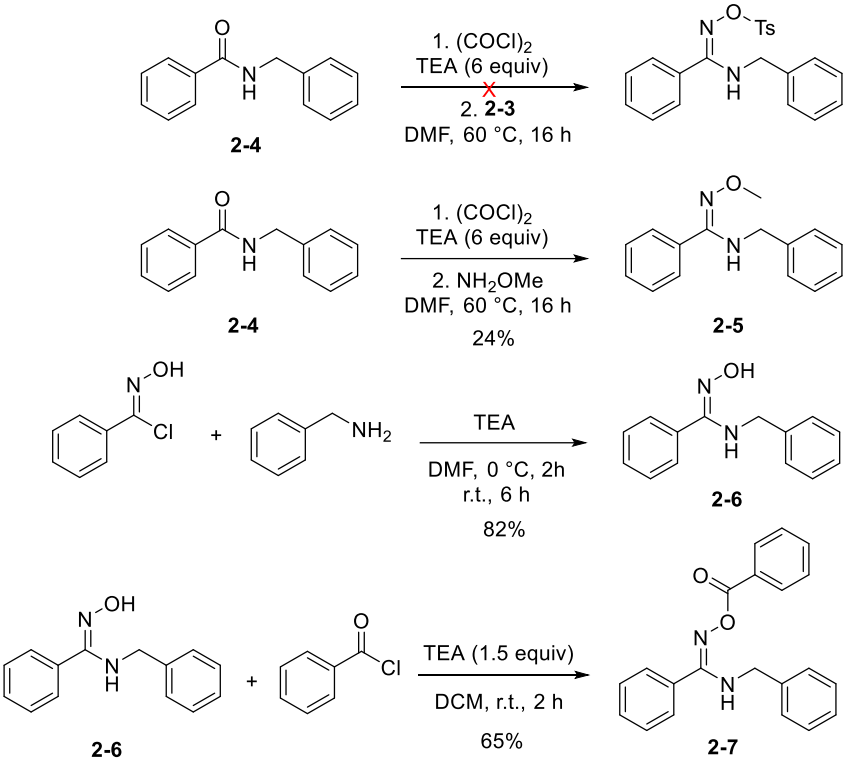


Scheme 2.3 Synthesis of *N*-benzylbenzamide



Once both starting materials were produced, the synthesis of *N*-benzyl-*N*'-tosylbenzimidamide was attempted (**Scheme 2.4**). Following a known method for the formation of amidine from an amide,[44] *N*-benzylbenzamide (**2-4**) was reacted with oxalyl chloride and triethylamine, followed by the addition of *O*-tosylhydroxylamine. Unfortunately, this reaction did not work as expected: it was observed that both an acid/base extraction and the purification of this amidine on a silica or alumina column led to the decomposition of any isolated product. However, this method was repeated using methoxyamine hydrochloride and afforded 24% yield of *N*-benzyl-*N*'-methoxybenzimidamide. Additionally, an alternative method was utilized in the production of *N*-benzyl-*N*'-hydroxybenzimidamide in 82% yield.[45-47] From there, the hydroxyl group could be protected using a range of different groups. One example of this was an *O*-benzoyl protection using benzoyl chloride, which led to 65% of the desired product.

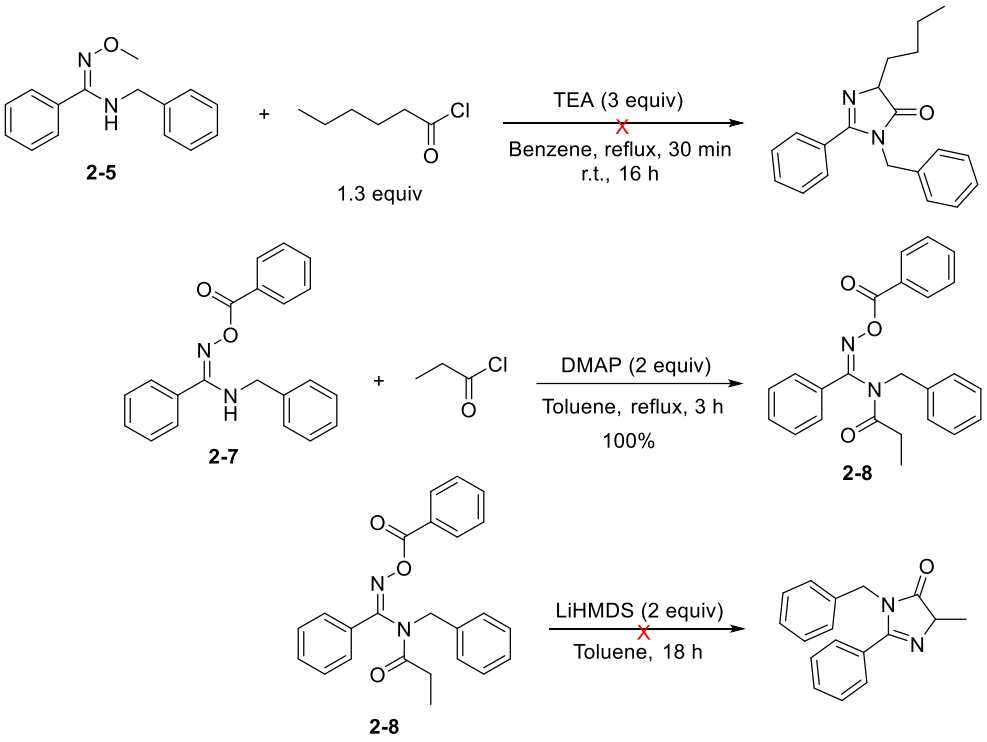
Scheme 2.4 Synthesis of *N*-benzyl-benzimidamide derivatives



Scheme 2.5 describes efforts made towards the cyclization of amidine and ketene, in the presence of a base. First, hexanoyl chloride and an excess of triethylamine were heated in benzene to form the desired ketene *in situ*,[48-50] then *N*-benzyl-*N*'-methoxybenzimidamide (**2-5**) was added and the reaction was stirred overnight at room temperature. Unfortunately, there was no evidence of product formation from this reaction. The next reaction took propionyl chloride and 4-DMAP, refluxing in toluene under basic conditions to again produce a ketene *in situ*, then *N*'-(benzoyloxy)-*N*-benzylbenzimidamide was added, and the reaction was refluxed for some time. Here, the only isolated product was the tertiary amine **2-8**. Unfortunately, none of the anticipated cyclized product was formed under these conditions. To facilitate cyclization, LiHMDS was added to the tertiary amine, but unfortunately no cyclized product was observed

here either. It is important to note that the ketene formation was never confirmed but was based on previously reported methods for the formation of a ketene.

Scheme 2.5 Attempted synthesis of imidazolone through reaction of activated amidine and ketene

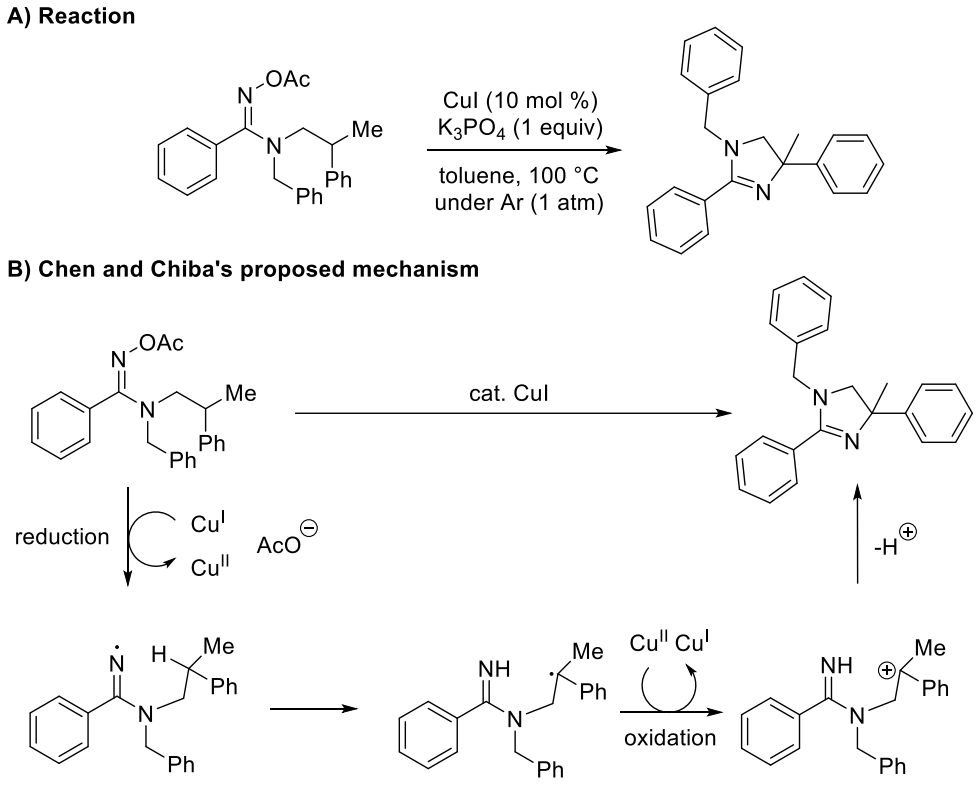


At this time, efforts towards the above methodology were stopped, as the main goal of this project was to quickly synthesize a library of imidazol-4-ones for biological testing. I do believe more exploration could be done on this method, focusing more towards a formal [3+2] cycloaddition. There are several recent literature examples of Staudinger-like [3+2] cycloadditions to form pyrazolidinones and γ-lactams[51, 52] as well as a [2+2+1] cycloaddition to form β-pyrrolinones.[53] Additionally, ring opening of β-lactams has been utilized in the production of a range of heterocycles including thiohydantoins, hydantoins, imidazolines and several 2-amino-5-ethylidene-imidazol-4-ones as well as the synthesis of leucettamine B and C.[54-57]

2.2.2 Copper-catalyzed redox-neutral imidazol-4-one formation

Inspired by the already synthesized complex amidine **2-8**, a copper-catalyzed redox-neutral cyclization to produce imidazol-4-ones was explored. This method was inspired by a report from Chen and Chiba[45], who synthesized imidazolines from activated amidines. In this reaction, as shown in **Scheme 2.6**, Chen and Chiba reacted fully functionalized amidines with a copper catalyst to facilitate a radical, redox-neutral C-H amination, cyclizing to form an imidazoline. Additionally, they used this same method to produce imidazoles and quinazolinones.[10] It was envisioned this method may also be used for the synthesis of imidazol-4-ones, starting from the complex amidine **2-8**.

Scheme 2.6 Chen and Chiba's copper-catalyzed redox-neutral imidazoline synthesis



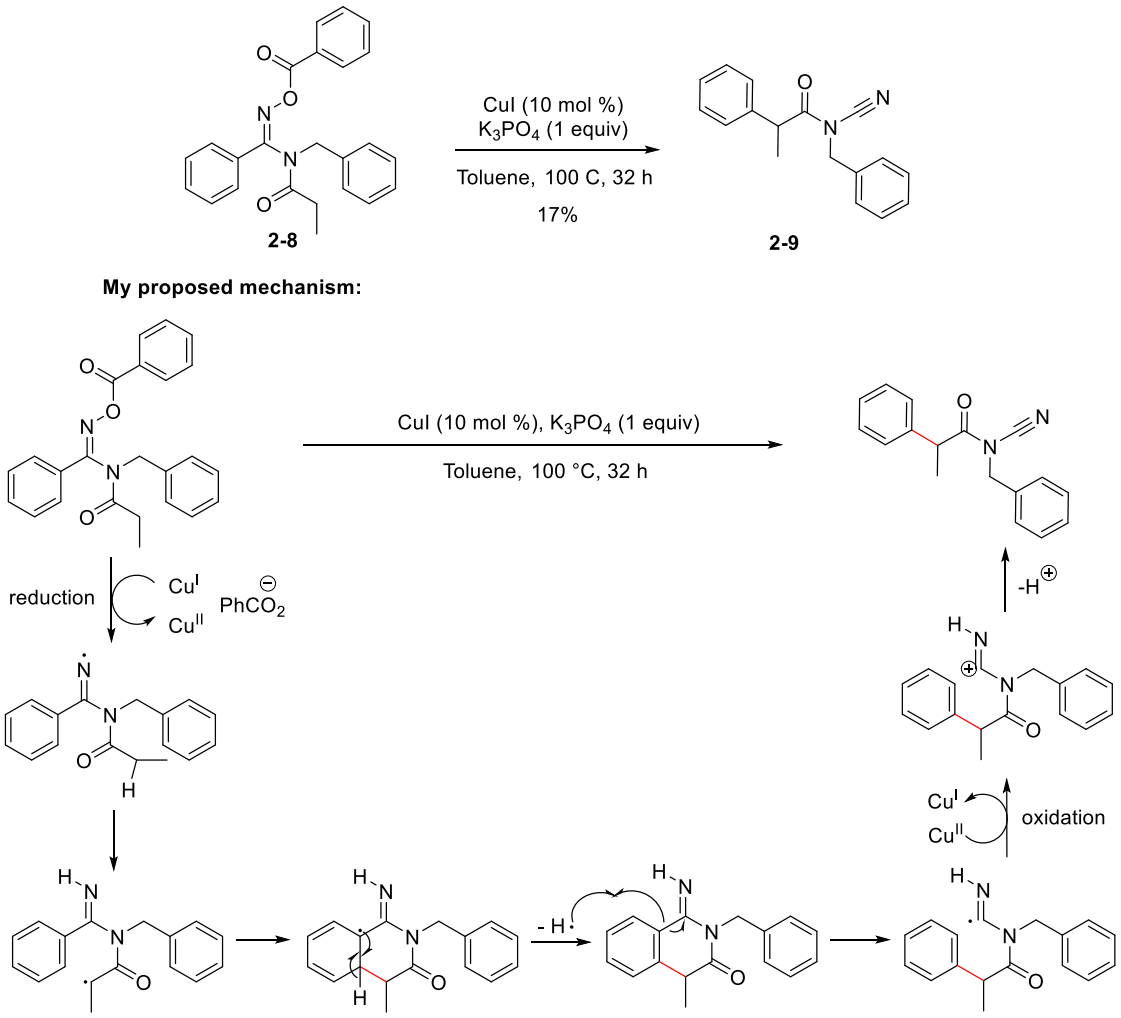
Scheme 2.7 shows my attempt at imidazol-4-one formation via the above-mentioned method. Here, the reported reaction conditions were mirrored, running for a total of 32 hours.

Interestingly, no imidazol-4-one was produced from this reaction. Instead, it was determined through characterization that a cyanamide had been made (**2-9**). The reaction did in fact go through a redox neutral process, as observed by a color change during the reaction: brown to blue and then back to brown, signifying a change from Cu^{I} to Cu^{II} then back to Cu^{I}. Additionally, it is evident from the product that the phenyl group has shifted carbons. Based on these observations, a mechanism for this reaction was proposed (**Scheme 2.7**). First, as with Chen and Chiba's reaction mechanism, there is a 1,5-*H* radical shift of the amidinyl radical, generating a C-radical. Interestingly, it appears the radical favoured the α-keto carbon over the benzyl methylene. From there, it was proposed propagation could have led to the formation of a carbon-carbon bond between the α-keto carbon and the phenyl ring, producing a new 6-membered ring. Re-aromatization of the phenyl ring led to an imino-radical, which upon oxidation created an imino-cation. Loss of a proton could then lead to the formation of a cyanamide.

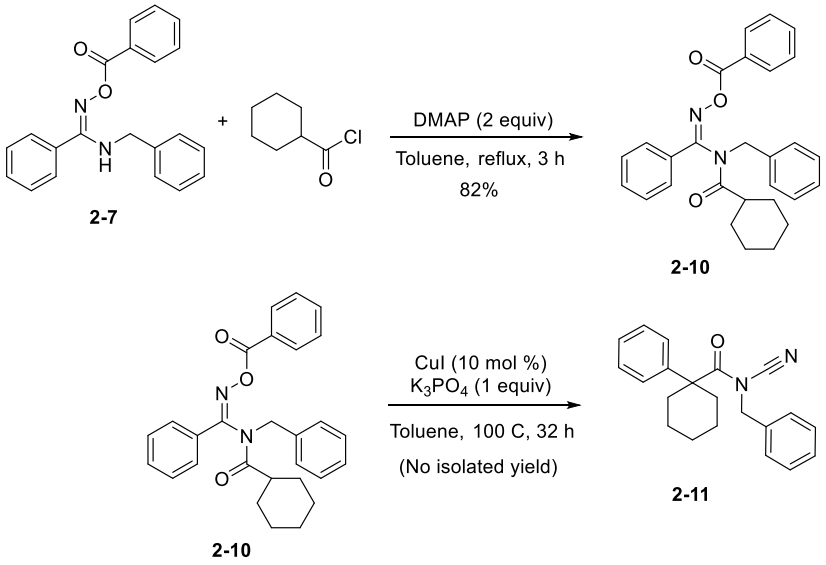
It is important to clarify that this is my own interpretation of the mechanism, and no literature was found that directly supports this theory. Additionally, no mechanistic studies were performed on this reaction. However, based on the proposed mechanism, it was believed that a bulkier group α to the carbonyl may prevent the formation of the 6-membered ring. To test this theory, cyclohexanecarbonyl chloride was used to functionalize the amidine in 82% yield (**Scheme 2.8**). Then, the copper-catalysed cyclization was attempted. Interestingly, while the reaction was very messy and a yield was not determined for the reaction, characterization supports the formation of a *N*-acyl cyanamide, as previously seen with compound **2-9**. At this stage in development, it was determined that this reaction was not able to produce the desired imidazol-4-one product, and thus the methodology was no longer pursued. However, there is potential for this reaction to be used to produce functionalized *N*-acyl cyanamides. Literature

suggests this functional group is used in several different applications, such as fungicides,[58] cysteine protease inhibitors,[59] and in the preparation of different complex heterocycles such as quinazolinones.[60-63] Further exploration of this reaction could lead to a new methodology to produce *N*-acyl cyanamides.

Scheme 2.7 Chen and Chiba's copper-catalyzed redox-neutral method on compound **2-8** and the proposed mechanism for nitrile formation



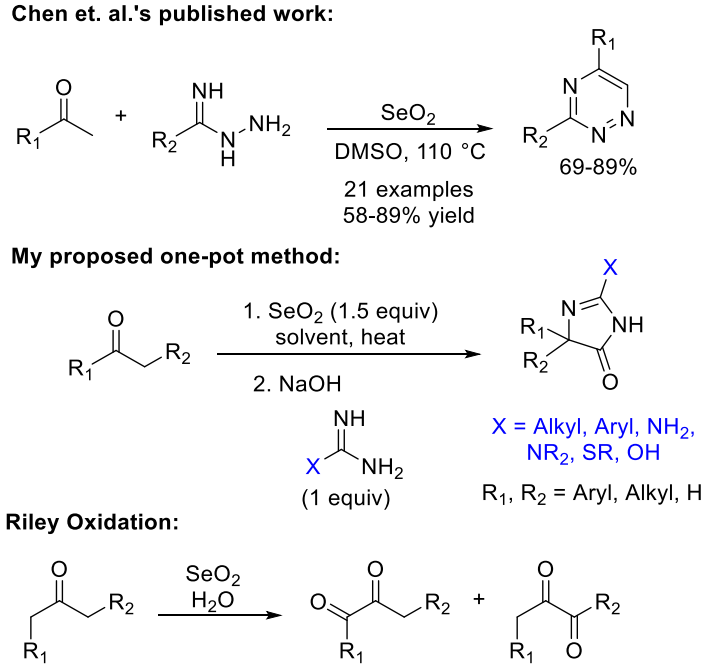
Scheme 2.8 Attempted synthesis of imidazol-4-one from a cyclohexanecarbonyl functionalized amidine



2.2.3 Oxidative cyclization of ketones and amidines via Riley oxidation

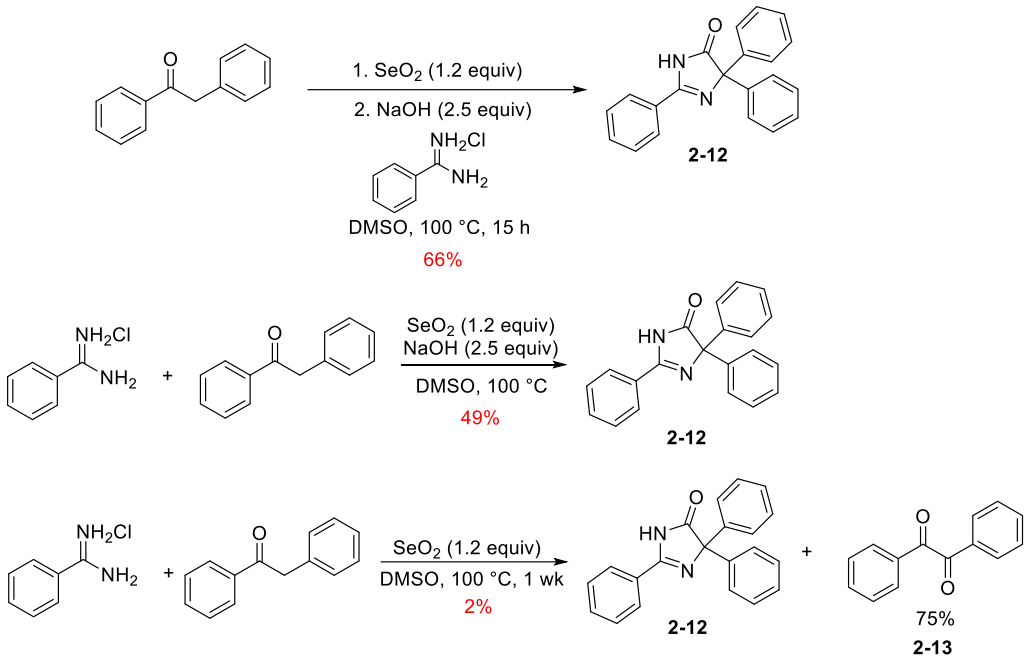
Another proposed method for the synthesis of imidazol-4-ones was based on a paper published by Chen et. al. in 2016.[64] Here they synthesized 1,2,4-triazines from a [4 + 2] annulation reaction of ketones and amidrazones, with initial oxidation of the ketone via selenium dioxide (Riley oxidation). This paper inspired an idea for the synthesis of imidazol-4-ones via the oxidative cyclization of ketones and amidines using selenium dioxide as the oxygen source (**Scheme 2.9**). The novelty of this method lies solely in the ability to produce imidazol-4-ones in one-pot from a variety of ketones and amidines. The oxidation of ketone to diketone using selenium dioxide is known, and the cyclization of diketone and amidine under basic conditions to produce imidazol-4-ones is also known.

Scheme 2.9 Proposed imidazol-4-one preparation via oxidative cyclization, inspired by Chen et. al.'s work on the formation of 1,2,4-triazines



The first reaction I ran was a one-pot, two-step reaction in which the first step was oxidation of deoxybenzoin using selenium dioxide. Once the oxidation was complete, as monitored via TLC, benzamidine hydrochloride and sodium hydroxide were added to the reaction, and it was heated overnight. This reaction produced the desired imidazol-4-one in 66% yield (**2-12**, **Scheme 2.10**). Next the reaction was run as a one-pot, one-step reaction, which provided 49% yield of **2-12**. The last experiment shown in **Scheme 2.10** is a control experiment where no base was added to the reaction pot. Without the addition of base, only 2% of imidazol-4-one was produced after a week of stirring, but 75% yield of benzil was isolated. The base proved necessary for the reaction, as it is used to desalt benzamidine hydrochloride and facilitates the cyclization as a proton transfer source. Unfortunately, I never explored whether the cyclization would occur without base if the amidine was neutral, as I had difficulties isolating the neutral amidine. This would have been the best comparison to Chen et. al.'s original work.[64]

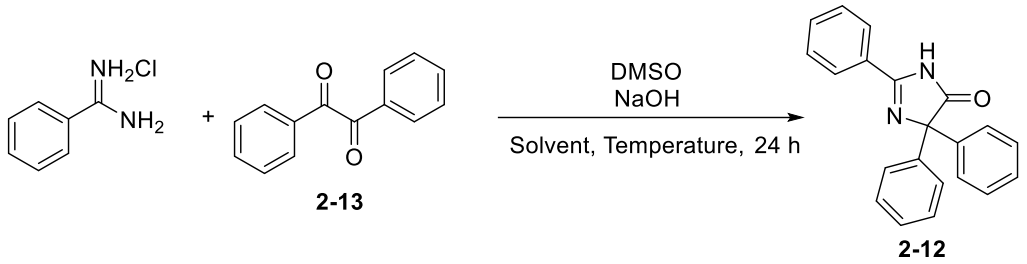
Scheme 2.10 Oxidative rearrangement of imidazolones via Riley oxidation



Several reactions were performed in order to optimize the one-pot, one-step process, and the results are described in **Table 2.1**. This table specifically highlights results from varying the conditions for the cyclization of benzil and benzamidine hydrochloride, including temperature, base, and reaction time. First, the reaction was attempted without the addition of any sodium hydroxide- questioning whether DMSO was required for the cyclization of benzamidine hydrochloride and benzil. Results indicate DMSO is required to solvate the benzamidine hydrochloride, especially if no base is added to the reaction. Entry 1 provided a 59% recovery of benzamidine hydrochloride upon workup but displayed no product formation. In entry 2 and after the addition of 4 equivalents of DMSO, 23% of imidazol-4-one was produced in 24 hours at 100 °C. While additional time had little impact in isolated yield of **2-12**, results showed that refluxing the reaction provided almost double the isolated yield (44%) compared to the 100 °C reaction (23%).

Moving forward with this reaction under reflux for 24 hours in pyridine with 4 equivalents of DMSO, the addition of sodium hydroxide (2 equiv.) encouraged further conversion to **2-12**. With 2 equivalents of sodium hydroxide added, 72% yield of **2-12** was isolated after 24 hours. Heartened by these promising results, a solvent screen was run to identify the best solvent for this reaction. The solvent screen included DMSO, pyridine, DMF, and ethanol. DMF provided similar yields (66%) to pyridine (72%), while DMSO provided much lower yields when used on its own (35%). Ethanol provided the best yield at 85% of imidazol-4-one isolated after 24 hours of reacting.

Table 2.1 Optimization of the conditions for condensation of benzamidine hydrochloride and benzil[a]

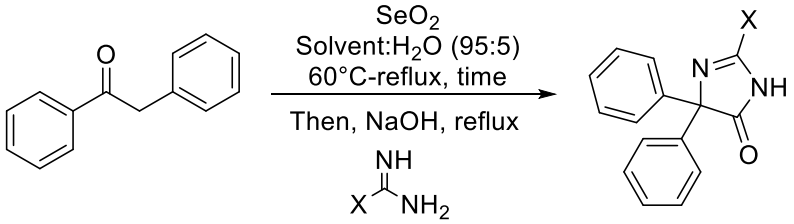


Entry #	NaOH (equiv.)	DMSO (equiv.)	Solvent	Temperature (°C)	Time reacted (h)	Yield (%)
1[b]	0	0	Pyridine	100	24	0
2	0	4	Pyridine	100	24	23
3	0	4	Pyridine	100	48	33
4	0	4	Pyridine	Reflux	24	44
5	2	4	Pyridine	Reflux	24	72
6	2	4	DMF	Reflux	24	66
7	2	NA	DMSO	Reflux	24	35
8	2	4	Ethanol	Reflux	24	85

[a]Reaction conditions: Benzamidine hydrochloride (1 equiv.), **2-13** (1 equiv.), DMSO, and NaOH in designated solvent, heated for the designated period of time at the designated temperature or at the solvent's boiling point (reflux), stirred with a magnetic stir bar and monitored for completion via TLC. Yields reported are isolated yields, which were determined after workup and purification via silica column. [b]There was a 59% recovery of benzil from this reaction.

Once the cyclization step was explored, I also investigated the oxidative cyclization as a one-pot, two-step reaction to produce imidazol-4-ones and similar heterocycles. **Table 2.2** describes efforts in exploring the scope of the reaction, altering X to produce a variety of nitrogen-containing heterocycles as well as reaction optimization, varying the solvent system and reaction time. DMF appeared superior in product conversion, as compared to 1,4-dioxane. Additionally, it was observed that DMF required less time for oxidation than 1,4-dioxane (3 hours versus 20 hours using 1,4-dioxane). More than likely, this is in correlation with DMF's higher boiling point (153 °C) and thus an increase in reaction temperature as compared to 1,4-dioxane (boiling point = 101 °C). From this method, an imidazol-4-one, 2-amino-imidazol-4-one, and thiohydantoin were produced.

Table 2.2 Scope exploration at C2 of the imidazol-4-one

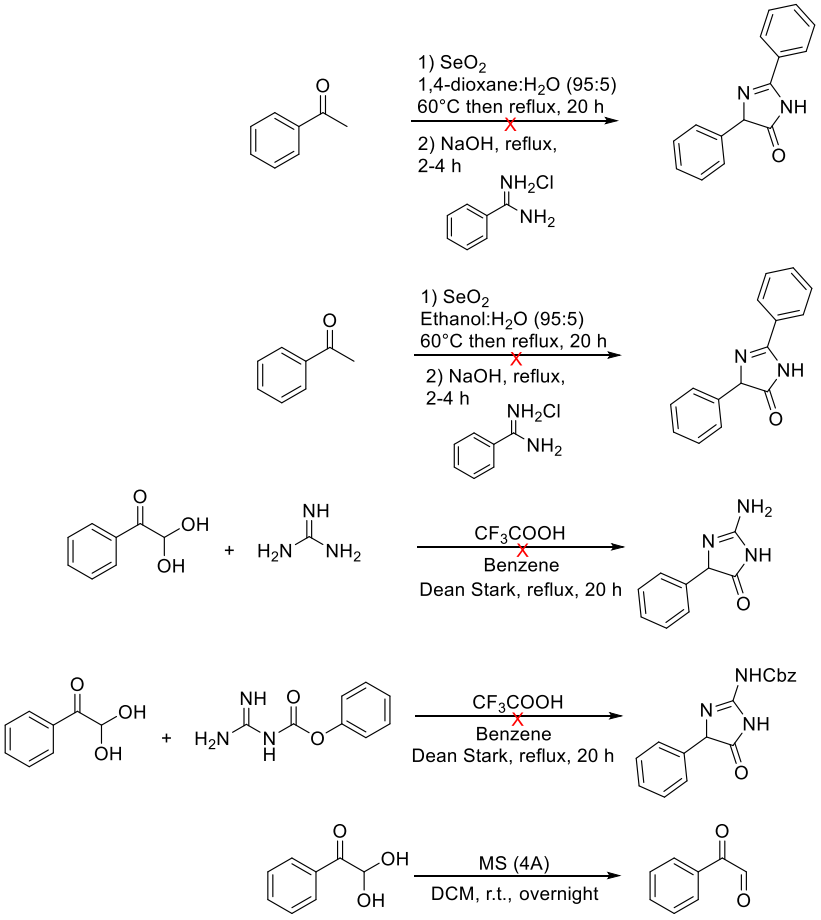


Solvent	Time (h)	X	Yield (%)	Compound produced
1,4-dioxane	1	Phenyl[b]	14	**2-12**
1,4-dioxane	20	Phenyl[b]	62	**2-12**
1,4-dioxane	20	S[c]	72	**2-14**
1,4-dioxane	20	NH_2[d]	23	**2-15**
DMF	1	Phenyl[b]	68	**2-12**
DMF	2	Phenyl[b]	76	**2-12**
DMF	3	Phenyl[b]	84	**2-12**
DMF	3	NH_2[d]	70	**2-15**

[a]Reaction conditions: Selenium dioxide (1.5 equiv) was stirred in 1,4-dioxane or DMF and water (95:5) for 15 minutes at 60 °C. Then deoxybenzoin (1 equiv) was added and the reaction was refluxed until oxidation was complete, as monitored by TLC. When complete, the appropriate amidine (1 equiv) was added to the reaction along with one NaOH pellet. The reaction was refluxed and stirred until complete, as monitored by TLC. Reported yields are isolated yields after their respective workups. [b]Used workup method A. [c]Used workup method C. [d]Used workup method B.

To explore how large of a scope this method could attain, I next attempted to alter the C5 position, focusing on synthesizing 5-monosubstituted-imidazol-4-ones. **Scheme 2.11** describes the efforts towards production of a 5-monosubstituted-imidazol-4-one. To begin, phenyl methyl ketone and selenium dioxide were heated in a mixture of 1,4-dioxane and water (95:5) overnight. Once phenyl glyoxal had been made, as visible by TLC, benzamidine hydrochloride and sodium hydroxide were added to the reaction, which was refluxed for an additional 2-4 hours. This reaction failed, even when changing the solvent system from 1,4-dioxane to ethanol. Unfortunately, it was discovered that basic conditions were not conducive for this cyclization due to the formation of phenyl glyoxal hydrate. Acidic conditions were also attempted, heating phenyl glyoxal hydrate and guanidine in benzene with trifluoroacetic acid, using a Dean Stark trap to drive off any water. However, under these conditions with both guanidine and Cbz-protected guanidine, there was no evidence of product formation. Lastly, molecular sieves (MS) were used in an effort to trap the phenyl glyoxal as an aldehyde by removing excess water. The aldehyde was observed by NMR. Unfortunately, the exploration of this methodology was put on hold before imidazol-4-one formation could be attempted with molecular sieves added. At this time, it was determined the library of imidazol-4-ones I wanted to synthesize could be accomplished through other means, using an already reported methodology.

Scheme 2.11 Attempts at formation of a mono-substituted imidazolone

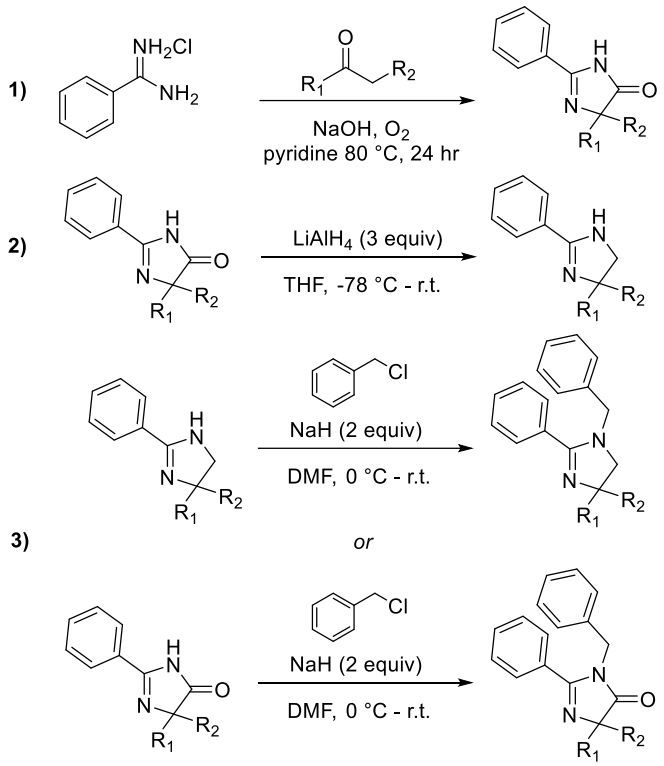


2.2.4 Library of 5,5-disubstituted-(4*H*)-imidazol-4-ones and 5,5-disubstituted imidazolines for 20S proteasome activation

Scheme 2.12 describes the three-step process used to produce 5,5-disubstituted imidazol-4-ones, imidazolines and their *N*-benzylated derivatives for proteasome activation studies. The first step is a cyclization of benzamidine hydrochloride and a range of ketones to form 5,5-disubstituted-imidazol-4-ones, following a previously reported method for the oxidative cyclization of imidazolones (see Section 1.2.1.5 for more details).[65] The imidazol-4-one was then reduced to an imidazoline using lithium aluminum hydride ($LiAlH_4$) in THF. The last step of this three-step process was a *N*-benzylation of both the imidazolines and imidazol-4-ones using

benzyl chloride and sodium hydride. This method was used to synthesize a small library of 27 analogues for proteasome modulation studies.

Scheme 2.12 The three step synthesis of imidazolines and imidazol-4-ones for proteasome activation studies



From this three-step synthetic process, a small library of 27 imidazol-4-ones and imidazolines was synthesized for testing in several proteasome activation assays. This library contained compounds with more "drug-like" properties as compared to TCH-165, ranging in values of molecular weight, log P, and log S. One assay used to explore the proteasome activation potential of these small molecules was a fluorogenic peptide assay, performed by Dr. Corey Jones. In this assay, purified human 20S proteasome is dosed with 10 μM of small molecule and the aminomethylcoumarin labelled fluorogenic chymotryptic-like peptide substrate (Suc-LLVY-AMC). Proteolytic activity was quantified by measuring the release of AMC from a fluorogenic substrate (Suc-LLVY-AMC). Compounds capable of enhancing 20S proteasome

mediated proteolytic activity should show a significant increase in fluorescence, as compared to the vehicle proteasome. Unfortunately, the results suggest none of the compounds were significantly active in proteasome activation, especially compared to TCH-165, which displays a 6-fold increase over the proteasomal activity as compared to vehicle.

To further confirm these results, the small molecules were also tested using two luciferase reporter assays (i.e. the MYC-luc and NF-κB-luc reporter assays), performed by Christi Harris. In these luciferase reporter assays the measured bioluminescence is used to determine whether MYC-luc or NF-kB-luc mediated gene transcription is activated or repressed by the addition of a small molecule. For instance, in the MYC and NF-κB reporter assays, we were specifically looking for a decrease in luminescence, which would suggest our small molecules are active for proteasome modulation. Unfortunately, none of my synthesized small molecules showed a significant decrease in % luminescence within either reporter assay, especially when compared with TCH-165 which, at 10 μM, displays a 81% decrease in luminescence in the MYC-luc reporter assay and a 70% decrease in luminescence in the NF-κB-luc reporter assay. **Table 2.3** summarizes the results of the *in vitro* proteasomal activity, some of the compounds' important physiochemical properties, as well as their cellular effects on MYC and NF-kB mediated gene transcription,

Table 2.3 Characterization and proteasomal modulation *in vitro* assay results of synthesized imidazolones and imidazolines

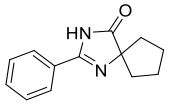
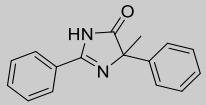
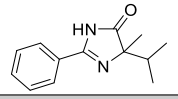
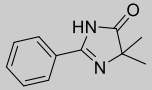
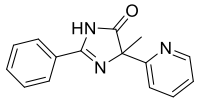
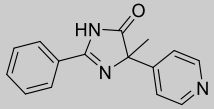
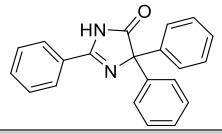
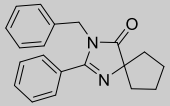
Compound number[a]	Structure	Molecular weight (g/mol)[b]	Predicted Log P[b]	Predicted Log S[b]	Lab book number	Proteasomal Fold Activity (10 μM)[c]	MYC Reporter Assay (% Luminescence)[d]	NF-κB Reporter Assay (% Luminescence)[e]
TCH-165	See **Figure 2.2**	595.74	8.87	-10.5	NA	6	19	30
2-16		214.27	2.28	-2.9	KK-01-96	1	98	95
2-17		250.30	3.20	-4.0	KK-01-116	1	99	95
2-18		216.28	2.69	-2.9	KK-01-139	1	91	93
2-19		188.23	1.80	-2.4	KK-01-137	1	93	97
2-20[f]		251.29	2.28	-3.2	KK-02-22	ND	ND	ND
2-21		251.29	1.86	-3.2	KK-02-28	1	90	102
2-12		312.27	4.59	-5.6	KK-02-84	ND	77	88
2-22		304.39	4.25	-4.86	KK-01-109	1	88	116

Table 2.3 (cont'd)

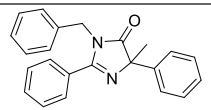
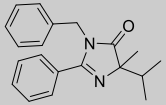
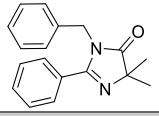
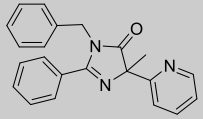
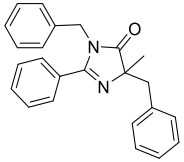
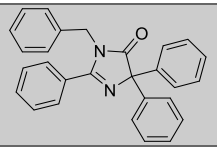
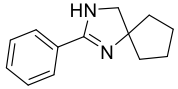
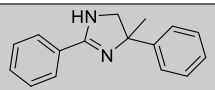
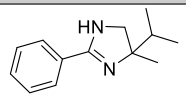
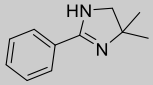
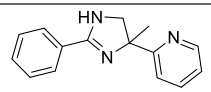
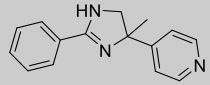
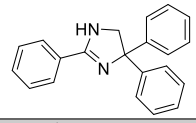
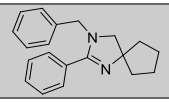
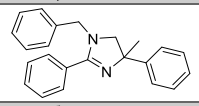
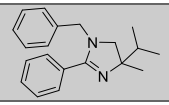
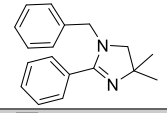
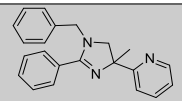
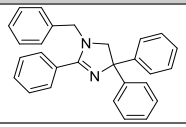
2-23		**340.36**	**5.17**	**-6.0**	**KK-01-119**	**1**	**93**	**88**
2-24		306.41	4.66	-4.9	KK-02-61	ND	73	102
2-25		278.36	3.77	-4.4	KK-02-37	1	84	97
2-26		341.41	4.25	-5.2	KK-02-51	1	84	93
2-27		354.45	5.45	-6.0	KK-02-54	0.8	77	80
2-28		402.50	6.56	-7.7	KK-02-90	ND	71	79
2-29		200.29	2.70	-3.2	KK-01-121	1	101	96
2-30		236.32	3.62	-4.3	KK-01-118	1	75	136
2-31		202.30	3.11	-3.2	KK-01-142	1	93	89
2-32		174.25	2.23	-2.7	KK-01-138	ND	90	91

Table 2.3 (cont'd)

2-33[f]		**237.31**	**2.71**	**-3.5**	**KK-02-24**	**ND**	**ND**	**ND**
2-34		237.31	2.28	-3.4	KK-02-29	1	85	106
2-35		298.39	5.01	-5.9	KK-02-89	ND	69	121
2-36[f]		290.41	4.81	-5.4	KK-01-129	ND	ND	ND
2-37		326.44	5.73	-6.6	KK-01-128	1.5	71	121
2-38		292.43	5.22	-5.5	KK-02-17	1	80	110
2-39		264.37	4.34	-4.9	KK-01-140	1	ND	ND
2-40		327.43	4.82	-5.8	KK-02-26	3.4	ND	ND
2-41		388.51	7.12	-8.2	KK-02-95	ND	49	125

[a]Compounds were synthesized via the three-step process reported in Scheme 2.12. [b]Molecular weight, Log P, and Log S were predicted using ChemDraw software. [c]Proteasomal fold activation was determined using a fluorogenic peptide assay upon the addition of 10 μM of compound, performed by Dr. Corey Jones. [d]The MYC reporter assay was performed by Christi Harris. HCT-116 cells stable transfected Myc-Luc reported cells were exposed to various concentrations of the compounds (0.1-20 mM) for 16 hours after which luminescence was measured and compared to vehicle control. [e]The NF-κB reporter assay was performed by Christi Harris. HCT-116 cells stable transfected NF-kappaB-Luc reported cells were exposed to various concentrations of the compounds (0.1-20 mM) for 16 hours after which luminescence was measured and compared to vehicle control. Data reported as a n=3 and the IC_{50} values were calculated using the equation for the sigmoidal curve for variable slope. [f]Several of the compounds were synthesized, but their proteasomal activity was never assessed. "ND" stands for "not determined" and reflects when a compound was not tested within a particular assay.

2.3 Conclusions

While they were considered more drug-like in structure, the synthesized imidazol-4-one and imidazoline small molecules proved inactive for proteasome activation. It was later discovered that these compounds showed poor preference for the α-binding pocket of the proteasome, as viewed using docking studies described in chapter 5. Additionally, three new methodologies to produce imidazol-4-ones were discussed in this chapter, however, the projects were stopped once we discovered the scaffold's lack of proteasomal activity and poor preference for the proteasome's α-binding pockets. While a new preparative method was not discovered, it was determined that the oxidative cyclization used to produce imidazol-4-ones for proteasome activation could have another application within the total synthesis of 5,5-disubstituted imidazol-4-one containing natural products, which will be discussed in depth in chapter 4 of this dissertation.

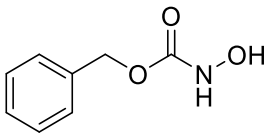
2.4 Experimental

General information

Reactions were carried out under a nitrogen atmosphere in flame-dried glassware. Solvents and reagents were purchased from commercial suppliers and used without further purification. Anhydrous THF was distilled over sodium and benzophenone directly before use. Magnetic stirring was used for all reactions. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. Infrared spectra were recorded on a Jasco Series 6600 FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus-500 or 600 spectrometer. Chemical shifts are reported relative to the residue peaks of the solvent ($CDCl_3$: 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C) (DMSO-*d6*: 2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C). The following abbreviations are used to denote the multiplicities: s = singlet, d =

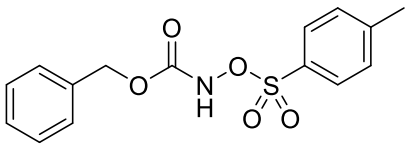
doublet, dd = doublet of doublets, t = triplet, and m = multiplet. HRMS were obtained at the Mass Spectrometry Facility of Michigan State University with a Micromass Q-ToF Ultima API LC-MS/MS mass spectrometer.

Synthetic methods



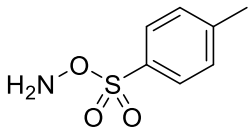
benzyl hydroxycarbamate (2-1). Sodium carbonate (6.5 g, 61 mmol) was dissolved in 20 mL of water, and the solution was cooled in an ice bath. Hydroxylamine (3.2 g, 46 mmol) was added to the stirring solution. Benzyl chloroformate (6.8 g, 40 mmol, 5.7 mL) was then diluted in 15 mL of dichloromethane, and the solution was added to the stirring reaction dropwise via an addition funnel. The solution was stirred 16 hours at room temperature. When complete, as monitored by TLC, the solution was acidified using a 10% aqueous HCl solution. The organics were then extracted into DCM (3 x 20 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was recrystallized from ether and hexanes to provide the pure product as a white solid (3.55 g, 53% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.36 (m, 5H), 5.17 (s, 2H) (Note: the exchangeable protons are not visible in the ^{1}H NMR spectra). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 159.23, 135.32, 128.64, 128.58, 128.38, 67.93. IR (neat): 3359 (br.), 3295, 1699, 1496, cm^{-1}. m.p.: 64˚C. Known compound, data matches literature values (L. Qin, Z. Zhou, J. Wei, T. Yan and H. Wen, *Synth. Commun.*, 2010, **40**, 642–646).



benzyl (tosyloxy)carbamate (2-2). 2-1 (3.2 g, 19.1 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 60 mL of anhydrous THF. Triethylamine (2.1 g, 21 mmol, 2.9 mL) was added to the stirring solution. The reaction was then cooled to 0°C using an ice bath. Tosyl chloride (3.8 g, 20 mmol) was added to the solution slowly as a solid. The reaction stirred at room temperature until complete, as monitored by TLC. When complete, the reaction was washed with 50 mL of a saturated aqueous ammonium chloride solution x 2. The organic layer was collected and dried over Na_2SO_4. The organics were then concentrated to half of its volume and a 100 mL solution of 1:4 DCM:hexanes was added. The product was then allowed to precipitate out overnight. The crystals were collected via filtration (do not wash with hexanes) and the pure product was a white solid. (3.47 g, 56% yield).

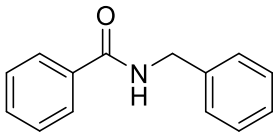
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (s, br., 1H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.35 (m, 3H), 7.27 (d, *J* = 8.5 Hz, 2H), 7.23 – 7.18 (m, 2H), 5.04 (s, 2H), 2.43 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.32, 146.11, 134.49, 130.12, 129.71, 129.55, 128.70, 128.57, 128.35, 68.62, 21.84. IR (neat): 3378, 3299, 1702, 1495, 1362, 1181, cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+Na)^+$] calc for ($C_{15}H_{15}NO_5SNa^+$): 344.0569. Found: 344.0561. mp: 122˚C. Known compound, data matches literature values (L. Qin, Z. Zhou, J. Wei, T. Yan and H. Wen, *Synth. Commun.*, 2010, **40**, 642–646).



***O*-tosylhydroxylamine (2-3)**. **2-2** (2.7 g, 8.4 mmol) was added to a clean, dry round bottom flask under nitrogen at room temperature and was dissolved in 100 mL of methanol. 10 wt% Pd/C (1 g, 9.4 mmol) was then added to the reaction. The reaction was then stirred under H_2 gas until complete, as monitored by TLC (20 hours). Once complete, the solution was filtered

through celite to remove excess Pd/C. The filtrate was concentrated to dryness to give pure product as an oily solid. (1.49 g, 94% yield).

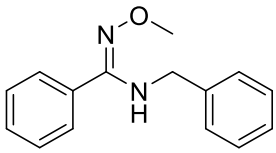
^{1}H NMR (500 MHz, DMSO-d_6) δ 7.49 (d, J = 7.8 Hz, 2H), 7.15 (s, br., 2H), 7.13 (d, J = 7.7 Hz, 2H), 2.29 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 145.78, 138.34, 128.61, 125.93, 21.25. IR (neat): 3046 (br.), 1455, 1423, 1119 cm^{-1}. Known compound, data matches literature values (L. Qin, Z. Zhou, J. Wei, T. Yan and H. Wen, *Synth. Commun.*, 2010, **40**, 642–646).



***N*-benzylbenzamide (2-4)**. Benzylamine (4.9 g, 46 mmol, 5.0 mL) was added to a clean, dry round bottom flask under nitrogen and was dissolved in 100 mL of dichloromethane. The solution was cooled to 0°C in an ice bath. Triethylamine (6.1 g, 60 mmol, 8.4 mL) was added to the solution. Benzoyl chloride (5.6 g, 40 mmol, 4.7 mL) was then added to the solution dropwise. The solution was stirred 18 hours, monitored for completion via TLC. Once the reaction was complete, the solution was washed with 50 mL of water. The organics were then washed with 50 mL of a 0.5 M aqueous HCl solution. The organic layer was collected, dried over Na_2SO_4, and concentrated *in vacuo* until the solution turned cloudy. Hexanes was then added to the solution until a solid started to fall out. The solution was then put in the refrigerator overnight to allow solids to crash out. The solids were filtered off, washed with hexanes, and dried *in vacuo* to provide the pure product as a white solid. (8.45 g, 84% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, J = 7.1 Hz, 2H), 7.51 (t, J = 7.4 Hz, 1H), 7.43 (t, J = 7.6 Hz, 2H), 7.37 (m, 4H), 7.31 (m, 1H), 6.48 (s, br., 1H), 4.66 (d, J = 5.6 Hz, 2H, assumed to be split by N-H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.35, 138.18, 134.38, 131.57, 128.81, 128.61,

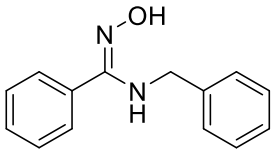
127.94, 127.65, 126.96, 44.15. IR (neat): 3276, 1635, 1548 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{14}H_{14}NO^+$): 212.1075 found: 212.1052. mp: 103˚C.



***N*-benzyl-*N*'-methoxybenzimidamide (2-5). 2-4** (0.26 g, 1.2 mmol) was added to a clean, dry round bottom flask stirring under nitrogen and was dissolved in 4 mL of DCM. Triethylamine (0.62 g, 6.1 mmol, 0.86 mL) was added to the solution. The solution was then cooled to 0˚C using an ice bath. Oxalyl chloride (0.16 g, 1.2 mmol, 0.11 mL) was then added to the solution slowly (about 0.04 mL every five minutes) until all was added. The solution was stirred at 0°C for 1.5 hours. Then, the dichloromethane was evaporated from the solution *in vacuo*, and 4 mL of DMF was added to the round bottom flask. Methoxyamine hydrochloride (0.086 g, 1.2 mmol) was added to the solution as a solid, and the solution was heated to 60˚C using an oil bath and stirred for 16 hours. Once complete, the reaction was cooled to room temperature and poured into a separatory funnel that contained 40 mL of ethyl acetate. The organics were washed three times with 20 mL of a 10% aqueous lithium bromide solution. The product was then extracted into a 1.0 M aqueous solution of HCl (20 mL). The aqueous layer was basified to a pH of 12 using NaOH pellets, and the product was extracted into ethyl acetate (30 mL x 3). The organic layer was dried in Na_2SO_4 and concentrated *in vacuo* to provide the product as a clear oil. (0.056 g, 24% yield).

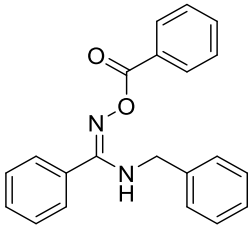
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (m, 2H), 7.42 – 7.38 (m, 3H), 7.36 – 7.31 (m, 2H), 7.30 – 7.25 (m, 1H), 7.23 (d, *J* = 7.1 Hz, 2H), 5.63 (s, br., 1H), 4.23 (d, *J* = 6.6 Hz, 2H), 3.91 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.17, 139.55, 131.12, 129.64, 128.65, 128.53, 127.42, 127.31, 126.89, 61.33, 47.57. IR (neat): 2858, 1591, 1565, 1454 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$]

calc. for ($C_{15}H_{17}N_2O^+$): 241.1341 found: 241.1342. Known compound, data matches literature values (F.-L. Zhang, Y.-F. Wang and S. Chiba, *Org. Biomol. Chem.*, 2013, **11**, 6003–6007.)

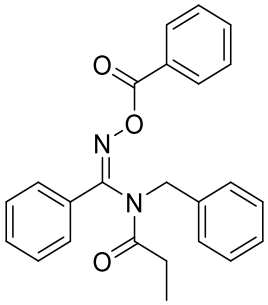


***N*-benzyl-*N*'-hydroxybenzimidamide (2-6)**. Benzylamine (0.26 g, 2.4 mmol) was added to a clean, dry round bottom flask under nitrogen and was dissolved in 5-10 mL of DMF. Triethylamine (0.40 g, 4.0 mmol, 0.56 mL) was added to the solution dropwise. The solution was stirred and cooled to 0˚C in an ice bath. (*Z*)-*N*-hydroxybenzimidoyl chloride (0.31 g, 2.0 mmol) was added to the solution. The reaction was stirred for two hours at 0˚C, then 4 hours at room temperature. Then, the reaction was diluted with water (10 mL), and the organics were extracted in ethyl acetate (3 x 10 mL). The organic layer was washed with brine (20 mL) and dried using Na_2SO_4. The organics were concentrated *in vacuo* to give the pure product as a white solid (0.37 g, 82% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 – 7.44 (m, 2H), 7.43 – 7.34 (m, 3H), 7.33 – 7.28 (m, 2H), 7.27 – 7.18 (m, 3H), 5.71 (s, br., 1H), 4.24 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.67, 139.50, 131.11, 129.69, 128.62, 128.53, 128.47, 127.26, 126.80, 47.48. IR (neat): 3411, 3075 (br.), 1642, 1493, cm^{-1}. HRMS (TOF MS AP+) calc. for ($C_{14}H_{15}N_2O$): 227.1184. Found: 227.1161. mp: 103˚C. Known compound, data matches literature values (F.-L. Zhang, Y.-F. Wang and S. Chiba, *Org. Biomol. Chem.*, 2013, **11**, 6003–6007.)



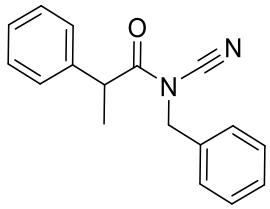
***N*'-(benzoyloxy)-*N*-benzylbenzimidamide (2-7). 2-6** (0.25 g, 1.1 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 10 mL of dichloromethane. Triethylamine (0.17 g, 1.7 mmol) was added to the solution dropwise via a syringe. Benzoyl chloride (0.16 g, 1.2 mmol) was then added dropwise to the stirring solution, and the reaction was stirred for 2 hours. Once complete, the reaction was quenched with 15 mL of water. The organics were extracted into DCM (3 x 10 mL). The organics were combined and washed with water (10 mL) and dried with Na_2SO_4. The organics were then concentrated, and the crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). The pure product was a white solid (0.24 g, 65% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 – 8.02 (m, 2H), 7.57 (m, 3H), 7.48 – 7.29 (m, 8H), 7.23 (d, *J* = 7.0 Hz, 2H), 5.69 (s, br., 1H), 4.34 (d, *J* = 6.4 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.17, 160.86, 138.71, 133.08, 130.63, 129.82, 129.69, 129.60, 129.25, 129.00, 128.73, 128.64, 127.83, 126.84, 48.02. IR (neat): 3407, 3363, 1725, 1714 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc. for ($C_{21}H_{19}N_2O_2^+$): 331.1447 Found: 331.1449. mp: 114 °C.



***N*-(((benzoyloxy)imino)(phenyl)methyl)-*N*-benzylpropionamide (2-8).** 4-DMAP (0.1 g, 0.83 mol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 5 mL of anhydrous toluene. Propanoyl chloride (0.07 mL, 0.76 mmol) was added to the solution, and the reaction was stirred for 20 minutes. Then, **2-7** (0.25 g, 0.76 mmol) was dissolved in 5 mL of anhydrous toluene and introduced to the reaction via syringe. The reaction was stirred and

refluxed for 1 hour. After the reaction cooled, a precipitate formed, was filtered off and washed with diethyl ether. The filtrate was concentrated *in vacuo* to provide the product as a yellow oil. (0.27 g, 92% yield).

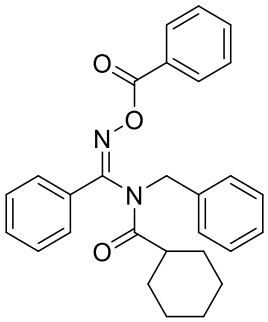
^{1}H NMR (500 MHz, DMSO-d_6) δ 7.82 – 7.76 (m, 2H), 7.69 – 7.56 (m, 6H), 7.53 – 7.47 (m, 2H), 7.36 – 7.28 (m, 2H), 7.28 – 7.23 (m, 1H), 7.23 – 7.19 (m, 2H), 4.63 (s, 2H), 2.64 (q, *J* = 7.4 Hz, 2H), 1.08 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.86, 163.04, 161.95, 137.29, 134.42, 132.28, 129.68, 129.63, 129.47, 129.38, 128.97, 128.87, 128.38, 128.11, 127.80, 49.54, 28.50, 10.37. IR (neat): 1741, 1680, 1601 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+H)$^{+}$] calc. for ($C_{24}H_{23}N_2O_3^{+}$): 387.1709. Found: 387.1750.



***N*-benzyl-*N*-cyano-2-phenylpropanamide (2-9). 2-8** (0.32 g, 0.84 mmol) was added to a clean, dry 3-neck round-bottom flask under argon and dissolved in 5 mL of anhydrous toluene. Potassium phosphate (0.18 g, 0.84 mmol) was added to the solution while it stirred. Copper iodide (CuI, 0.016 g, 0.084 mmol, 10 mol%) was then added to the reaction carefully and while dark (CuI is light sensitive). The solution was stirred at 100°C and monitored via TLC. The reaction was stirred overnight, and unfortunately the solvent completely evaporated. Toluene was then re-added to the residue, and the reaction was stirred another 12 hours. The reaction was quenched with 7 mL of 9 pH ammonium buffer solution. The organics were extracted into ethyl acetate (3 x 10 mL). The organics were combined, dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40

microns, ethyl acetate/hexanes gradient 0-100%) to provide the pure product as a yellow oil (0.038 g, 17% yield).

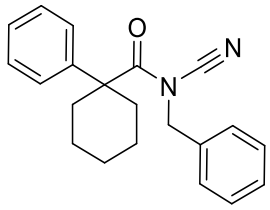
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.21 (m, 10H), 4.74 (d, *J* = 14.3 Hz, 1H), 4.63 (d, *J* = 14.3 Hz, 1H), 4.29 (q, *J* = 6.8 Hz, 1H), 1.55 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.14, 138.45, 133.73, 129.04, 128.98, 128.87, 128.59, 127.89, 127.81, 110.41, 50.23, 45.00, 19.44. IR (neat): 2233, 1725 (the signal is more blue shifted than a typical amide because it is a cyanamide) cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc. for ($C_{17}H_{17}N_2O^+$): 265.1341. Found: 265.1339.



***N*-(((benzoyloxy)imino)(phenyl)methyl)-*N*-benzylcyclohexanecarboxamide (2-10).** 4-DMAP (0.081 g, 0.67 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 5 mL of anhydrous toluene. Cyclohexanecarbonyl chloride (0.089 g, 0.60 mmol) was added to the solution and the reaction was stirred for 20 minutes. **2-7** (0.2 g, 0.6 mmol) was dissolved in 5 mL of anhydrous toluene, and this solution was introduced to the reaction via syringe. The reaction was stirred while refluxing for 1 hour. Once complete, the reaction was cooled, and the precipitate was filtered off and washed with ether. The filtrated was concentrated *in vacuo* to provide the product as a yellow oil. (0.22 g, 82% yield).

^{1}H NMR (500 MHz, $CDCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (m, 2H), 7.60 – 7.33 (m, 8H), 7.29 – 7.23 (m, 5H), 4.80 (s, 2H), 2.81 (tt, *J* = 11.5, 3.3 Hz, 1H), 1.90 – 1.83 (m, 2H), 1.74 (m,

2H), 1.64 – 1.55 (m, 3H), 1.26 – 1.10 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.13, 163.26, 161.44, 136.88, 133.54, 131.62, 129.94, 129.82, 129.40, 128.86, 128.83, 128.68, 128.59, 128.49, 127.63, 50.28, 44.05, 30.28, 25.84, 25.77. IR (neat): 1752, 1677, 1602 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc. for ($C_{28}H_{29}N_2O_3^+$): 441.2178. Found: 441.2174.

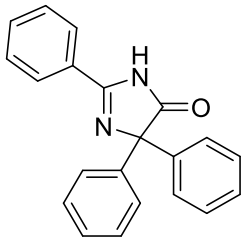


***N*-benzyl-*N*-cyano-1-phenylcyclohexane-1-carboxamide (2-11)**: **2-10** (0.22 g, 0.5 mmol) was added to a clean, dry 3-neck round-bottom flask under argon and was dissolved in 5 mL of anhydrous toluene. Potassium phosphate (0.18 g, 0.84 mmol) was added to the solution, and it was stirred for 20 minutes. Copper iodide (CuI, 0.0095 g, 0.050 mmol, 10 mol%) was added to the reaction carefully and while dark (CuI is light sensitive). The solution was stirred at 100°C and monitored via TLC. The reaction was stirred overnight, and unfortunately the solvent completely evaporated. Toluene was then re-added to the residue, and the reaction was stirred another 12 hours. The reaction was quenched with 7 mL of 9 pH ammonium buffer solution. The organics were extracted into ethyl acetate (3 x 10 mL). The organics were combined, dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%) to provide the pure product as a tan solid. (yield was not determined).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.18 (m, 10H), 4.68 (s, 2H), 2.59 (d, J = 13.9 Hz, 2H), 1.84 (m, 2H), 1.73 – 1.67 (m, 2H), 1.59 – 1.50 (m, 2H), 1.31 (m, 2H).

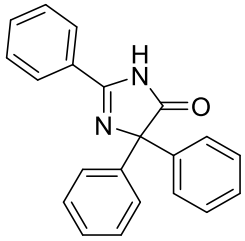
^{13}C NMR (126 MHz, $CDCl_3$) δ 174.22, 141.74, 134.33, 128.92, 128.88, 128.79, 128.64, 127.68, 126.55, 110.40, 53.29, 52.15, 35.42, 25.69, 23.38. IR (neat): 2229, 1708 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc. for ($C_{21}H_{23}N_2O^+$): 319.1810. Found: 319.1804. mp: 73 °C.

Methods used to make the following compound:

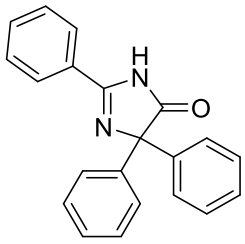


1. **2,5,5-triphenyl-3,5-dihydro-4*H*-imidazol-4-one (2-12)**: Deoxybenzoin (0.255 g, 1.3 mmol) and selenium dioxide (0.17 g, 1.53 mmol) were added to a clean, dry round bottom flask under nitrogen, and they were dissolved in DMSO (8 mL). The solution was stirred at 100 °C for 6 hours, or until all starting material had disappeared by TLC. Benzamidine hydrochloride (0.2 g, 1.3 mmol) and sodium hydroxide pellets (0.13 g, 3.25 mmol) were then added and stirred at 100 °C for 15 more hours. After cooling down, the reaction was carefully quenched with water, to a pH of 4. Organics were extracted into EtOAc (10 mL, x3). Organic layers were combined and washed once with aq. NaCl solution (30 mL). Crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%) to produce off white solid (0.27g , 66% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.87 (s, 1H), 8.24 (d, *J* = 7.1 Hz, 2H), 7.81 (d, *J* = 7.2 Hz, 4H), 7.75 (t, *J* = 7.4 Hz, 1H), 7.70 (t, *J* = 7.6 Hz, 2H), 7.53 (t, *J* = 7.5 Hz, 4H), 7.47 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 186.41, 158.43, 140.32, 132.26, 129.06, 128.61, 128.34, 127.96, 127.48, 127.32, 79.86. IR (neat): 1715, 1626 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc. for ($C_{21}H_{17}N_2O^+$): 313.1341 Found: 313.1345. m.p.: 236 °C



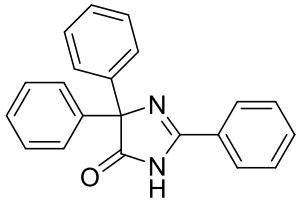
2. Benzamidine hydrochloride (0.2 g, 1.3 mmol), deoxybenzoin (0.255 g, 1.3 mmol), selenium dioxide (0.17 g, 1.53 mmol), and sodium hydroxide pellets (0.13 g, 3.25 mmol) were added to a clean, dry round bottom flask under nitrogen. DMSO (8 mL) was added to dissolve the solids. The solution was stirred at 100 °C for 24 hours. After cooling down, the reaction was carefully quenched with water, to a pH of 4. The organics were extracted into EtOAc (3 x 10 mL), combined and washed once with an aqueous NaCl solution (30 mL). The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%) to produce an off white solid (0.2 g, 49% yield).



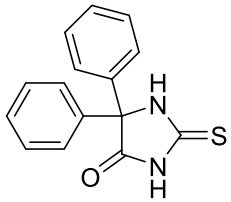
3. Deoxybenzoin (0.255 g, 1.3 mmol) and selenium dioxide (0.17 g, 1.53 mmol) were added to a clean, dry round bottom flask under nitrogen. DMSO (8 mL) was added to dissolve solids. The solution stirred at 100 °C for 6 hours, or until all starting material disappeared by TLC. Benzamidine hydrochloride (0.2 g, 1.3 mmol) was then added and stirred at 100 °C. The reaction was monitored for one week via TLC, with no indication of product formation. After cooling down, the reaction was carefully quenched with water, to a pH of 4. Organics were extracted into EtOAc (10 mL, x3). Organic layers were combined and washed once with aq. NaCl solution (30

mL). The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%) to produce an off-white solid (8 mg, 2% yield). The major product was benzil (0.20 g, 75% yield).

Benzil (2-13): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 8.4, 1.3 Hz, 4H), 7.66 (tt, *J* = 7.1, 1.3 Hz, 2H), 7.55 – 7.48 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.71, 135.04, 133.08, 130.03, 129.15. IR (neat): 1678, 1594, 1577 cm^{-1}. m.p.: 134 °C.



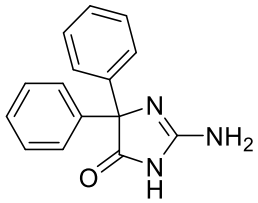
4. **2,5,5-triphenyl-3,5-dihydro-4*H*-imidazol-4-one (2-12):** Selenium dioxide was added to a clean, dry round bottom flask under nitrogen and was dissolved in 3.8 mL of solvent and 0.2 mL of water. The reaction was stirred for 15 minutes at 60°C to allow for the selenium dioxide to dissolve. Deoxybenzoin (0.15 g, 0.76 mmol) was then added to the solution. The reaction was stirred on reflux and monitored for completion via TLC. Once complete, benzamidine hydrochloride (0.12 g, 0.76 mmol) and sodium hydroxide (excess, one pellet) were added. The reaction was stirred on reflux for 4 hours. Once complete, as monitored by TLC, the appropriate workup method was performed (see Workup Method A) to provide the pure product as a solid.



5,5-diphenyl-2-thioxoimidazolidin-4-one (2-14): Selenium dioxide (0.085 g, 0.76 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 3.8 mL of solvent and 0.2 mL of water. The reaction was stirred for 15 minutes at 60 °C to allow for the selenium

dioxide to dissolve. Deoxybenzoin (0.1 g, 0.51 mmol) was then added to the reaction. The reaction was stirred on reflux and monitored for completion via TLC. Once complete, thiourea (0.039 g, 0.51 mmol) and sodium hydroxide (excess, one pellet) were added. The reaction was stirred on reflux for 4 hours. Once the reaction was complete, the appropriate workup method was performed (see Workup Method C).

^{1}H NMR (500 MHz, Acetone-d_6) δ 10.92 (s, br., 1H), 10.10 (s, br., 1H), 7.48 – 7.36 (m, 10H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 181.56, 174.58, 138.75, 128.74, 128.54, 126.92, 73.74. IR (neat): 3744, 3687, 3156 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+Na)$^{+}$] calc for ($C_{13}H_{14}N_2OSNa^{+}$): 269.0724. Found: 269.0752. m.p.: 243 °C



2-amino-5,5-diphenyl-3,5-dihydro-4*H*-imidazol-4-one (2-15): Selenium dioxide (0.085 g, 0.76 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 3.8 mL of solvent and 0.2 mL of water. The reaction was stirred for 15 minutes at 60 °C to allow for the selenium dioxide to dissolve. Deoxybenzoin (0.1 g, 0.51 mmol) was then added to the reaction. The reaction was stirred on reflux and monitored for completion via TLC. Once complete, guanidine hydrochloride (0.049 g, 0.51 mmol) and sodium hydroxide (excess, one pellet) were added. The reaction was stirred on reflux for 4 hours. Once the reaction was complete, the appropriate workup method was performed (see Workup Method B).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.09 (s, br., 1H), 7.33 (m, 10H), 7.27 (s, br., 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 187.00, 170.73, 141.54, 128.11, 127.24, 126.85, 72.54. IR (neat): 3709,

3626, 3354, 1702, 1659 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+Na)^+$] calc for ($C_{13}H_{15}N_3ONa^+$): 252.1113. Found: 252.1138. m.p.: >250 °C.

Workup Method A

The reaction was poured into 50 mL of ethyl acetate, and a red solid fell out. The precipitate was filtered off. Then, the filtrate was washed with 50 mL of an aqueous. LiBr solution twice. The organics were collected, dried with Na_2SO_4, and concentrated in vacuo. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%) to afford a white solid.

Workup Method B

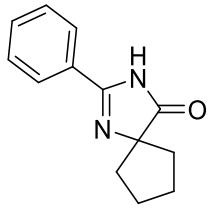
The reaction was poured into ethanol (20 mL), and the precipitate was filtered off. The filtrate was concentrated in vacuo to about 5 mL and 50 mL of water was added. The solution was left in the fridge until a precipitate fell out. The precipitate was filtered off and recrystallized from ethanol to afford a white solid.

Workup Method C

Ethanol was added to the reaction to dissolve the product (very insoluble) and whatever precipitate was left over was filtered off (selenium biproducts). Water (10-20 mL) was then added to the filtrate, which was acidified using a 10% HCl aqueous solution. Ethyl acetate was used to extract the organics from the water. The organics were dried with Na_2SO_4 and concentrated in vacuo to afford a white solid.

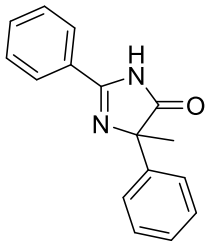
General procedure for the formation of imidazol-4-one:

Sodium hydroxide (4.5 equiv.) and benzamidine hydrochloride (1 equiv.) were added to a clean, dry Schlenk flask as solids. The sealed flask was purged with oxygen three times. Pyridine (20 mL) was then added to the flask using a syringe. Ketone (1.5 equiv.) was added to the solution using a syringe. The solution was stirred using a magnetic stir rod for 24 hours at 80 °C. Once complete, the reaction was concentrated *in vacuo*. Dichloromethane was added to the residue until everything dissolved. Upon addition of diethyl ether, a salt precipitated out. The precipitate was filtered off. The salt was dissolved in water and adjusted to a pH of 6 using 10% aq. HCl sol'n. The organics were extracted into DCM (30 mL x 3), dried using Na_2SO_4 and concentrated *in vacuo* to provide the product. Any changes to the procedure are mentioned under the specific compound.



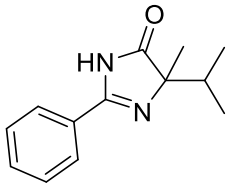
2-phenyl-1,3-diazaspiro[4.4]non-1-en-4-one (2-16). This reaction was conducted with benzamidine hydrochloride (0.78 g, 5.0 mmol) and cyclohexanone (0.78 mL, 7.5 mmol). The pure product was a white solid. (0.46 g, 43% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.51 (s, br., 1H), 7.88 (d, J = 7.0 Hz, 2H), 7.58 – 7.46 (m, 3H), 2.10-1.94 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 201.94, 173.73, 136.54, 128.64, 127.53, 127.42, 78.87, 37.05, 25.92. IR (neat): 2944, 2866, 1593, 1568 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{13}H_{15}N_2O^+$): 215.1184. Found: 215.1188. mp: 196 °C.



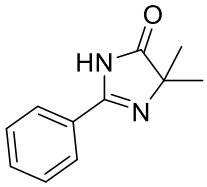
5-methyl-2,5-diphenyl-3,5-dihydro-4*H*-imidazol-4-one (2-17). This reaction was conducted with benzamidine hydrochloride (0.78 g, 5.0 mmol) and propiophenone (1 mL, 7.5 mmol). The pure product was a white solid. (0.61 g, 49% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.70 (s, br., 1H), 8.06 – 8.01 (m, 2H), 7.66 (d, *J* = 7.4 Hz, 2H), 7.60 – 7.50 (m, 3H), 7.39 – 7.33 (m, 2H), 7.32 – 7.27 (m, 1H), 1.85 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 188.69, 158.48, 139.80, 132.18, 129.08, 128.62, 128.45, 127.88, 127.24, 125.94, 73.82, 25.74. IR (neat): 1716, 1624 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{16}H_{15}N_2O^+$): 251.1184. Found: 251.1207. mp: 146 °C.



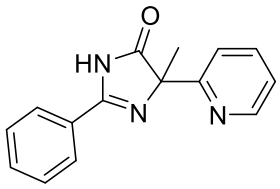
5-isopropyl-5-methyl-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one (2-18). This reaction was conducted with benzamidine hydrochloride (0.78 g, 5.0 mmol) and 4-methylpentan-2-one (0.94 mL, 7.5 mmol). The pure product was a white solid. (0.59 g, 55 % yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.10 (s, br.,1H), 7.93 (d, *J* = 7.2 Hz, 2H), 7.53 (m, 3H), 2.13 (hept, *J* = 6.8 Hz, 1H), 1.45 (s, 3H), 1.08 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 189.63, 157.77, 131.90, 129.09, 128.73, 126.95, 75.32, 35.11, 21.41, 17.10, 17.02. IR (neat): 3141, 1713, 1619 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{13}H_{17}N_2O^+$): 217.1341. Found: 217.1369. mp: 118 °C.



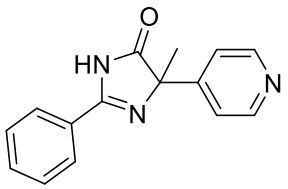
5,5-dimethyl-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one (2-19). This reaction was conducted with benzamidine hydrochloride (0.78 g, 5.0 mmol) and ethyl methyl ketone (0.13 mL, 7.5 mmol). The pure product was a white solid. (0.18 g, 19% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 – 7.94 (m, 2H), 7.58 – 7.47 (m, 3H), 1.48 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.81, 158.44, 132.03, 129.08, 128.60, 127.08, 69.31, 24.14. IR (neat): 1734, 1708 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{11}H_{13}N_2O^+$): 189.1028. Found: 189.1045. mp: 198 °C.



5-methyl-2-phenyl-5-(pyridin-2-yl)-3,5-dihydro-4*H*-imidazol-4-one (2-20). This reaction was conducted with benzamidine hydrochloride (0.62 g, 4.0 mmol) and 1-(pyridin-2-yl)propan-1-one (0.80 g, 5.9 mmol). The pure product was a white solid. (0.14 g, 14% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (s, br., 1H), 8.03 (d, *J* = 7.7 Hz, 2H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.48 (m, 2H), 7.40 (m, 2H), 7.16 (m, 1H), 1.89 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.05, 157.90, 149.50, 137.08, 132.18, 129.05, 128.74, 127.49, 123.92, 123.07, 120.91, 67.94, 29.83. IR (neat): 3162, 1715, 1623 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{15}H_{14}N_3O^+$): 252.1137. Found: 252.1167. m.p.: 184 °C.

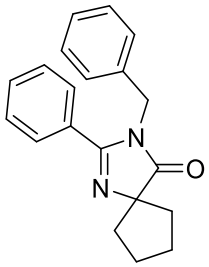


5-methyl-2-phenyl-5-(pyridin-4-yl)-3,5-dihydro-4*H*-imidazol-4-one (2-21). The reaction was conducted with benzamidine hydrochloride (0.47 g, 3.3 mmol) and 1-(pyridin-4-yl)propan-1-one (0.49 g, 3.6 mmol). The pure product was a yellow solid. (0.24 g, 32 % yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.60 – 8.56 (m, 2H), 8.02 (d, *J* = 7.3 Hz, 2H), 7.63 – 7.60 (m, 2H), 7.59 (m, 1H), 7.54 (t, *J* = 7.5 Hz, 2H), 1.80 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 186.77, 149.65, 132.31, 129.26, 128.99, 128.64, 127.37, 127.24, 121.09, 72.45, 25.88. IR (neat): 2975, 1733, 1597 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{15}H_{14}N_3O^+$): 252.1137. Found: 252.1173. m.p.: 58 °C.

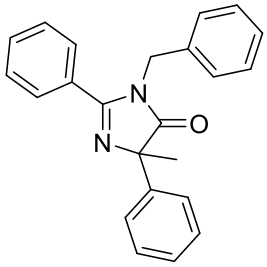
General procedure for the benzylation of imidazol-4-one: Imidazol-4-one (1 equiv.) was added to a clean, dry pressure tube at room temperature. Benzyl chloride (2 mL) was then added to the tube all at once. This was put in an oil bath at 80 °C and stirred with a magnetic stir bar for several minutes. Sodium hydride (2 equiv.) was then added to the solution. Then, some dichloromethane (1-2 mL) was added to help the imidazol-4-one dissolve. The reaction was stirred at 80 °C with the cap loosened to allow for slow evaporation of the dichloromethane. The reaction was stirred for 18 hours total. Once complete, the solution was quenched with 2 mL of water, and it was stirred for 5 minutes. Organics were then extracted into ethyl acetate (5 mL x 2). The organics were concentrated *in vacuo*, and the crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%)

to provide the pure product. Any changes to the procedure are mentioned under the specific compound.



3-benzyl-2-phenyl-1,3-diazaspiro[4.4]non-1-en-4-one (2-22). The reaction was conducted with 2-phenyl-1,3-diazaspiro[4.4]non-1-en-4-one (0.10 g, 4.7 mmol). The pure product was a yellow oil. (0.17 g, 40% yield).

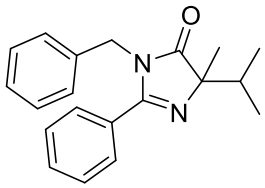
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 – 7.42 (m, 1H), 7.41 – 7.34 (m, 4H), 7.26 – 7.21 (m, 3H), 7.00 – 6.94 (m, 2H), 4.71 (s, 2H), 2.16 – 1.90 (m, 8H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 187.21, 161.13, 136.80, 130.88, 130.07, 128.86, 128.75, 128.31, 127.68, 126.97, 44.99, 37.87, 31.73, 26.27, 22.80, 14.27. IR (neat): 2950, 2866, 1723, 1623 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{20}H_{21}N_2O^+$): 305.1654. Found: 305.1673.



3-benzyl-5-methyl-2,5-diphenyl-3,5-dihydro-4*H*-imidazol-4-one (2-23). The reaction was conducted with 5-methyl-2,5-diphenyl-3,5-dihydro-4*H*-imidazol-4-one (0.10 g, 4.0 mmol). The pure product was a clear oil. (0.059 g, 43% yield).

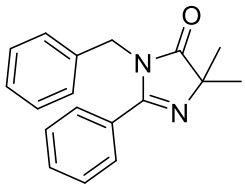
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 – 7.69 (m, 2H), 7.52 – 7.48 (m, 3H), 7.44 – 7.36 (m, 4H), 7.34 – 7.29 (m, 1H), 7.23 (m, 3H), 6.97 (m, 2H), 4.73 (q, *J* = 15.8 Hz, 2H), 1.85 (s, 3H). ^{13}C

NMR (126 MHz, $CDCl_3$) δ 184.91, 162.13, 139.94, 136.50, 131.15, 129.89, 128.86, 128.80, 128.64, 128.34, 127.79, 127.73, 126.94, 125.93, 72.16, 45.16, 26.62. IR (neat): 1725, 1622 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{23}H_{21}N_2O^+$): 341.1654. Found: 341.1656.



3-benzyl-5-isopropyl-5-methyl-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one (2-24). The reaction was conducted with 5-isopropyl-5-methyl-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one (0.20 g, 0.92 mmol). The pure product was a clear oil. (0.15 g, 53% yield).

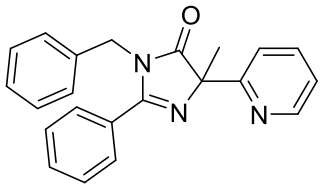
^1H NMR (500 MHz, $CDCl_3$) δ 7.54 – 7.49 (m, 1H), 7.48 – 7.40 (m, 4H), 7.32 – 7.25 (m, 3H), 7.04 (m, 2H), 4.78 – 4.67 (m, 2H), 2.19 (hept., *J* = 6.8 Hz, 1H), 1.48 (s, 3H), 1.11 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 186.74, 161.90, 136.76, 130.90, 130.19, 128.77, 128.29, 127.71, 127.33, 127.08, 73.62, 44.89, 34.99, 21.73, 17.14, 17.03. IR (neat): 2959, 1721, 1629 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{20}H_{23}N_2O^+$): 307.1810. Found: 307.1859.



3-benzyl-5,5-dimethyl-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one (2-25). The reaction was conducted with 5,5-dimethyl-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one (0.11 g, 0.60 mmol). The pure product was a white solid. (51 mg, 31%).

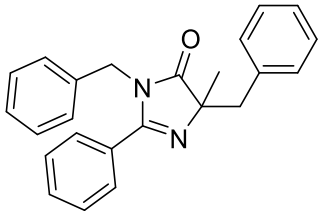
^1H NMR (500 MHz, $CDCl_3$) δ 7.48-7.36 (m, 5H), 7.23 (m, 3H), 6.98 (d, *J* = 7.4 Hz, 2H), 4.72 (s, 2H), 1.47 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 187.00, 161.20, 136.66, 130.97, 129.92,

128.84, 128.71, 128.21, 127.70, 126.88, 67.71, 44.92, 24.25. IR (neat): 2976, 1719, 1614 cm^{-1}. HRMS (ESI-TOF) m/z: $[(M+H)^+]$ calc for ($C_{18}H_{19}N_2O^+$): 279.1497. Found: 279.1546. m.p.: 64 °C.



3-benzyl-5-methyl-2-phenyl-5-(pyridin-2-yl)-3,5-dihydro-4*H*-imidazol-4-one (2-26). The reaction was conducted with 5-methyl-2-phenyl-5-(pyridin-2-yl)-3,5-dihydro-4*H*-imidazol-4-one (60 mg, 0.24 mmol). The pure product was a clear oil. (33 mg, 40% yield).

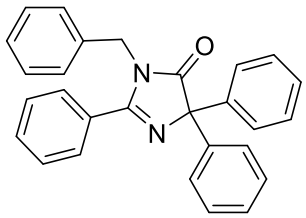
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (m, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 7.53 – 7.46 (m, 4H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.28 – 7.21 (m, 4H), 7.09 (d, *J* = 7.4 Hz, 2H), 4.82 (s, 2H), 1.96 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 184.31, 163.18, 158.08, 149.90, 136.90, 136.61, 131.17, 129.85, 128.83, 128.73, 128.40, 127.68, 127.05, 122.96, 120.69, 74.73, 45.28, 22.98. IR (neat): 1789, 1624 cm^{-1}. HRMS (ESI-TOF) m/z: $[(M+H)^+]$ calc for ($C_{22}H_{20}N_3O^+$): 342.1606. Found: 342.1607.



3,5-dibenzyl-5-methyl-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one (2-27). The reaction was conducted with 5-methyl-2-phenyl-5-(pyridin-4-yl)-3,5-dihydro-4*H*-imidazol-4-one (60 mg, 0.24 mmol). The pure product was a clear oil. (23 mg, 27% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (t, *J* = 7.5 Hz, 1H), 7.19 – 7.13 (m, 2H), 7.11 (m, 5H), 7.03 – 6.99 (m, 3H), 6.96 (t, *J* = 7.2 Hz, 2H), 6.36 (d, *J* = 7.3 Hz, 2H), 4.36 (d, *J* = 16.0 Hz, 1H), 4.22

(d, *J* = 16.0 Hz, 1H), 3.11 (d, *J* = 2.9 Hz, 2H), 1.49 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 185.58, 161.97, 136.36, 136.04, 131.00, 130.70, 130.14, 128.87, 128.82, 128.29, 128.19, 127.50, 127.24, 126.86, 72.32, 45.00, 44.25, 24.16. IR (neat): 1731, 1628 cm^{-1}. HRMS (ESI-TOF) m/z: $[(M+H)^+]$ calc for ($C_{24}H_{23}N_2O^+$): 355.1810. Found: 355.1820.

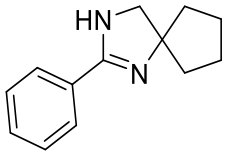


3-benzyl-2,5,5-triphenyl-3,5-dihydro-4*H*-imidazol-4-one (2-28). The reaction was conducted with 2,5,5-triphenyl-3,5-dihydro-4*H*-imidazol-4-one. The pure product was a clear oil. (44 mg, 34% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 – 7.61 (m, 4H), 7.58 – 7.47 (m, 3H), 7.45 – 7.28 (m, 8H), 7.25 – 7.20 (m, 3H), 7.02 – 6.95 (m, 2H), 4.79 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.70, 162.49, 140.49, 136.37, 131.21, 129.79, 128.85, 128.80, 128.64, 128.46, 127.85, 127.75, 127.37, 127.00, 78.22, 45.37. IR (neat): 1731, 1633 cm^{-1}. HRMS (ESI-TOF) m/z: $[(M+H)^+]$ calc for ($C_{28}H_{23}N_2O^+$): 403.1810, Found: 403.1810.

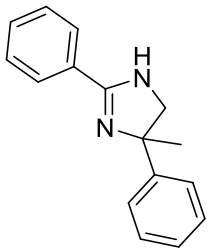
General procedure for the reduction of imidazole-4-one to imidazoline: Lithium aluminum hydride (3.5 equiv.) was added to a clean, dry round bottom flask under nitrogen, and it was dissolved in 5 mL of anhydrous THF. The solution was stirred and cooled to –78 °C. Once cool, imidazol-4-one (1 equiv.) in 5 mL of THF was slowly added to the reaction over several minutes. The solution was stirred, as temperature slowly increased to room temperature. When a TLC indicated complete consumption of starting material, the reaction was cooled to –78 °C, and

water was slowly added to quench the reaction. The pH was adjusted to 8 using an aqueous 10% HCl solution. The organics were extracted into chloroform (3 x 20 mL). The organics were dried with $NaSO_4$ and concentrated *in vacuo* to provide the pure product. Any changes to the procedure are mentioned under the specific compound.



2-phenyl-1,3-diazaspiro[4.4]non-1-ene (2-29). The reaction was conducted with 2-phenyl-1,3-diazaspiro[4.4]non-1-en-4-one (0.1 g, 0.00040 mol). The pure product was a white solid. (73% yield).

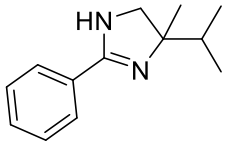
^{1}H NMR (500 MHz, CD_3CN) δ 7.79 – 7.75 (m, 2H), 7.48 – 7.38 (m, 3H), 3.53 (s, 2H), 1.78 (m, 4H), 1.69 – 1.60 (m, 4H). ^{13}C NMR (126 MHz, CD_3CN) δ 162.47, 131.92, 131.23, 129.26, 127.92, 75.12, 62.17, 40.71, 24.64. IR (neat): 3250, 1614 cm^{-1}. HRMS (ESI-TOF) m/z: $[(M+H)^+]$ calc for ($C_{13}H_{17}N_2^+$): 201.1392. Found: 201.1399. mp: 77 °C.



4-methyl-2,4-diphenyl-4,5-dihydro-1*H*-imidazole (2-30). The reaction was conducted with **5**-methyl-2,5-diphenyl-3,5-dihydro-4*H*-imidazol-4-one (0.10 g, 0.40 mmol). The pure product was an off-white solid. (0.060 g, 63% yield).

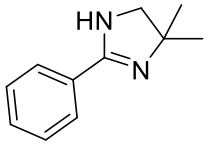
^{1}H NMR (500 MHz, CD_3CN) δ 7.91 – 7.85 (m, 2H), 7.51 – 7.42 (m, 5H), 7.34 (t, *J* = 7.8 Hz, 2H), 7.25 – 7.20 (m, 1H), 3.80 (d, *J* = 11.4 Hz, 1H), 3.65 (d, *J* = 11.4 Hz, 1H), 1.56 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.40, 148.21, 130.81, 130.68, 128.58, 128.51, 127.25, 126.72,

125.38, 29.80, 28.84, 1.13. IR (neat): 1733, 1595 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{16}H_{17}N_2^+$): 237.1392. Found: 237.1389. mp: 94 °C.



4-isopropyl-4-methyl-2-phenyl-4,5-dihydro-1*H*-imidazole (2-31). The reaction was conducted with 5-isopropyl-5-methyl-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one (0.15 g, 0.69 mmol) The pure product was an off-white solid. (0.13 g, 90% yield).

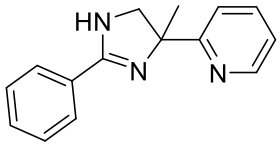
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 – 7.71 (m, 2H), 7.40 – 7.30 (m, 3H), 4.84 (s, br., 1H), 3.62 (d, *J* = 12.5 Hz, 1H), 3.39 (d, *J* = 12.5 Hz, 1H), 1.81 (hept, *J* = 6.8 Hz, 1H), 1.20 (s, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.95, 130.74, 130.47, 128.36, 127.09, 68.58, 60.52, 37.12, 24.10, 17.77, 17.59. IR (neat): 3153, 1596 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{13}H_{19}N_2^+$): 203.1548. Found: 203.1639. mp: 70 °C.



5,5-dimethyl-2-phenyl-4,5-dihydro-1*H*-imidazole (2-32). The reaction was conducted with 5,5-dimethyl-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one (0.15 g, 0.80 mmol). The pure product was an off-white solid. (0.12 g, 89% yield).

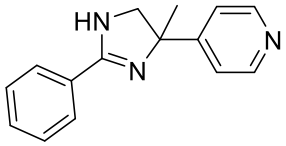
^{1}H NMR (500 MHz, CD_3CN) δ 7.78 (d, *J* = 8.0 Hz, 2H), 7.51 – 7.39 (m, 3H), 3.44 (s, 2H), 1.28 (s, 6H). ^{13}C NMR (126 MHz, CD_3CN) δ 161.27, 131.21, 130.19, 128.29, 126.90, 63.06, 62.81, 27.83.

IR (neat): 3177, 1567 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{11}H_{15}N_2^+$): 175.1235. Found: 175.1263. mp: 86 °C.



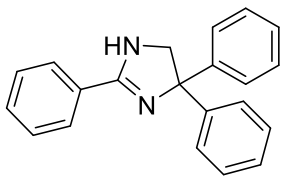
2-(4-methyl-2-phenyl-4,5-dihydro-1*H*-imidazol-4-yl)pyridine (2-33). The reaction was conducted with 5-methyl-2-phenyl-5-(pyridin-2-yl)-3,5-dihydro-4*H*-imidazol-4-one (0.14 g, 0.56 mmol). The pure product was a yellow solid. (0.090 g, 67% yield).

^{1}H NMR (500 MHz, CD_3CN) δ 8.51 (m, 1H), 7.91 – 7.84 (m, 2H), 7.70 (m, 1H), 7.62 (m, 1H), 7.50 – 7.41 (m, 3H), 7.18 (m, 1H), 3.93 (d, *J* = 11.6 Hz, 1H), 3.74 (d, *J* = 11.6 Hz, 1H), 1.59 (s, 3H). ^{13}C NMR (126 MHz, CD_3CN) δ 167.88, 163.36, 149.65, 137.54, 131.85, 131.46, 129.34, 128.09, 122.64, 120.88, 62.49, 30.35, 28.98. IR (neat): 3170, 1614 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{15}H_{16}N_3^+$): 238.1349. Found: 238.1349.



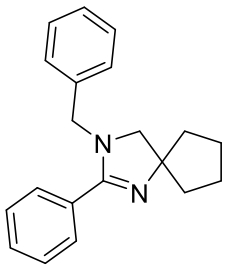
4-(4-methyl-2-phenyl-4,5-dihydro-1*H*-imidazol-4-yl)pyridine (2-34). The reaction was conducted with 5-methyl-2-phenyl-5-(pyridin-4-yl)-3,5-dihydro-4*H*-imidazol-4-one (0.16 g, 0.64 mmol). The pure product was a yellow solid. (0.094 g, 62% yield from 18).

^{1}H NMR (500 MHz, CD_3CN) δ 8.54 – 8.45 (m, 2H), 7.90 – 7.85 (m, 2H), 7.53 – 7.40 (m, 5H), 3.79 (d, *J* = 11.0 Hz, 1H), 3.62 (d, *J* = 11.2 Hz, 1H), 1.55 (s, 3H). ^{13}C NMR (126 MHz, CD_3CN) δ 163.34, 158.66, 150.71, 131.63, 129.58, 129.39, 128.14, 121.64, 76.87, 55.32, 28.80. IR (neat): 3060, 1587, 1570 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{15}H_{16}N_3^+$): 238.1344. Found: 238.1333. m.p.: 55 °C.



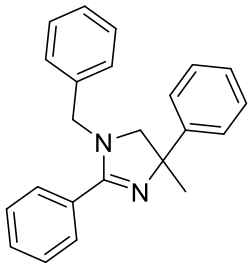
2,4,4-triphenyl-4,5-dihydro-1*H*-imidazole (2-35). The reaction was conducted with 2,5,5-triphenyl-3,5-dihydro-4*H*-imidazol-4-one. The pure product was a solid. (0.13 g, 66% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 – 7.87 (m, 2H), 7.52 – 7.38 (m, 7H), 7.34 (m, 4H), 7.29 – 7.23 (m, 2H), 4.41 (s, br., 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.27, 147.25, 130.83, 130.48, 128.50, 128.41, 127.30, 126.95, 126.66, 29.73. IR (neat): 1593, 1557 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{21}H_{19}N_2^+$): 299.1548. Found: 299.1580. m.p.: 164 °C.

General procedure for the benzylation of imidazolines: Imidazoline (1 equiv.) was added to a clean, dry round bottom flask under nitrogen, and it was dissolved in 10 mL of anhydrous DMF. The solution was stirred with a magnetic stir bar and cooled to 0 °C in an ice bath. Sodium hydride (2 equiv.) was addedto the solution all at once. Then, benzyl chloride (1.2 equiv.) was added slowly via a syringe. The stirring reaction was monitored via TLC for 16 hours. When complete, the solution was added to 40 mL of ethyl acetate. The organics were washed three times with 40 mL of an aqueous LiBr solution to remove the DMF. The organic solution was dried with $NaSO_4$, concentrated *in vacuo,* and purified using automated CombiFlash chromatography (silica loader, alumina column, 20-40 microns, ethyl acetate/hexanes gradient 0-100%) to provide the pure product. Any changes to the procedure are mentioned under the specific compound.



3-benzyl-2-phenyl-1,3-diazaspiro[4.4]non-1-ene (2-36). The reaction was conducted with 2-phenyl-1,3-diazaspiro[4.4]non-1-ene (0.16 g, 0.8 mmol). The pure product was a white solid. (0.011 g, 5% yield).

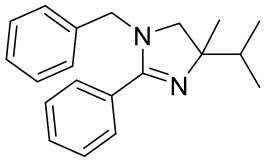
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 – 7.56 (m, 2H), 7.42 – 7.33 (m, 5H), 7.29 - 7.24 (m, 3H), 4.28 (s, 2H), 3.24 (s, 2H), 2.02 – 1.90 (m, 2H), 1.90 – 1.79 (m, 2H), 1.66 – 1.55 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.39, 138.39, 131.63, 129.81, 128.75, 128.56, 128.50, 127.35, 127.20, 75.57, 63.14, 52.86, 41.10, 24.42. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{20}H_{23}N_2^+$): 291.1861. Found: 291.1859.



1-benzyl-4-methyl-2,4-diphenyl-4,5-dihydro-1*H*-imidazole (2-37). The reaction was conducted with 5-methyl-2,5-diphenyl-3,5-dihydro-4*H*-imidazol-4-one (0.30 g, 1.3 mmol). A plug column was used to purify the product, since it sits on baseline until TEA is added. The plug column was first run with 100% ethyl acetate to remove all impurities. Then 99% ethyl acetate and 1% trimethylamine was used to collect pure product. The product was collected and dried (off white solid, 0.090 g, 22% yield).

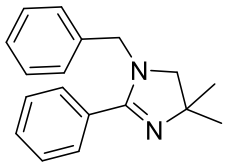
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (m, 2H), 7.54 (d, *J* = 7.7 Hz, 2H), 7.48 (m, 3H), 7.39 (m, 4H), 7.31 (m, 4H), 4.58 (d, *J* = 15.7 Hz, 1H), 4.16 (d, *J* = 15.7 Hz, 1H), 3.67 (d, *J* = 9.3 Hz, 1H), 3.49 (d, *J* = 9.3 Hz, 1H), 1.71 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.85, 149.20, 137.91, 131.21, 130.20, 128.84, 128.70, 128.55, 128.42, 127.51, 127.17, 126.49, 125.51, 70.18, 64.51,

52.81, 30.58. IR (neat): 3063, 1613 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{23}H_{23}N_2^+$): 327.1861. Found: 327.1858. mp: 80 °C.



1-benzyl-4-isopropyl-4-methyl-2-phenyl-4,5-dihydro-1*H*-imidazole (2-38). The reaction was conducted with 4-isopropyl-4-methyl-2-phenyl-4,5-dihydro-1*H*-imidazole (0.16 g, 0.81 mmol). The pure product was an oil. (37 mg, 16% yield).

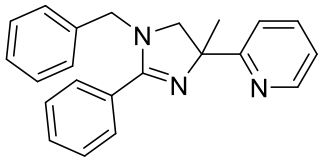
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 – 7.55 (m, 2H), 7.44 – 7.22 (m, 8H), 4.36 (d, *J* = 15.7 Hz, 1H), 4.18 (d, *J* = 15.7 Hz, 1H), 3.17 (d, *J* = 9.5 Hz, 1H), 2.99 (d, *J* = 9.5 Hz, 1H), 1.86 (hept, *J* = 6.8 Hz, 1H), 1.26 (s, 3H), 0.90 (dd, *J* = 17.5, 6.8 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.62, 138.33, 131.69, 129.84, 128.78, 128.58, 128.46, 127.39, 127.20, 71.20, 58.50, 52.87, 37.13, 26.17, 17.88, 17.47. IR (neat): 2958, 1614, 1595 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{20}H_{25}N_2^+$): 293.2018. Found: 293.1978.



1-benzyl-4,4-dimethyl-2-phenyl-4,5-dihydro-1*H*-imidazole (2-39). The reaction was conducted with 5,5-dimethyl-2-phenyl-4,5-dihydro-1*H*-imidazole (0.10 g, 0.57 mmol). The pure product was an oil. (24 mg, 16% yield).

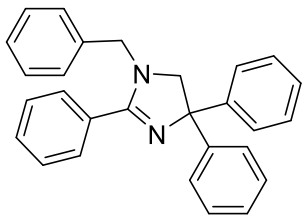
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 – 7.57 (m, 2H), 7.42 – 7.33 (m, 5H), 7.30 – 7.26 (m, 1H), 7.24 (d, *J* = 6.7 Hz, 2H), 4.30 (s, 2H), 3.14 (s, 2H), 1.32 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.09, 138.30, 131.52, 129.92, 128.80, 128.58, 128.47, 127.42, 127.21, 64.87, 63.31, 52.78,

29.54. IR (neat): 2962, 1594 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{18}H_{21}N_2^+$): 265.1705. Found: 265.1741.



2-(1-benzyl-4-methyl-2-phenyl-4,5-dihydro-1*H*-imidazol-4-yl)pyridine (2-40). The reaction was conducted with 2-(4-methyl-2-phenyl-4,5-dihydro-1*H*-imidazol-4-yl)pyridine (0.090 g, 0.38 mmol). The pure product was an oil. (28 mg, 22% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, *J* = 4.7 Hz, 1H), 7.79 (d, *J* = 7.9 Hz, 1H), 7.70 – 7.63 (m, 3H), 7.48 – 7.38 (m, 3H), 7.31 (t, *J* = 7.3 Hz, 2H), 7.28 – 7.18 (m, 3H), 7.12 (m, 1H), 4.48 (d, *J* = 15.6 Hz, 1H), 4.16 (d, *J* = 15.6 Hz, 1H), 3.81 (d, *J* = 9.8 Hz, 1H), 3.63 (d, *J* = 9.8 Hz, 1H), 1.67 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.17, 165.38, 148.88, 137.83, 136.64, 131.26, 130.20, 128.77, 128.71, 128.55, 127.46, 127.29, 121.70, 120.69, 72.29, 62.39, 52.60, 30.19. IR (neat): 3060, 1697, 1570 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{22}H_{22}N_3^+$): 328.1814. Found: 328.1806.



1-benzyl-2,4,4-triphenyl-4,5-dihydro-1*H*-imidazole (2-41). The reaction was conducted with 2,4,4-triphenyl-4,5-dihydro-1*H*-imidazole. The pure product was an oil. (65 mg, 50% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 – 7.70 (m, 2H), 7.53 – 7.20 (m, 18H), 4.39 (s, 2H), 4.06 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.91, 148.17, 137.83, 131.26, 130.14, 128.85, 128.67, 128.60, 128.32, 127.53, 127.20, 126.82, 126.63, 76.57, 64.00, 53.07. IR (neat): 1614, 1595,

1568, 1496, 1441 cm^{-1}. HRMS (ESI-TOF) m/z: $[(M+H)^+]$ calc for ($C_{28}H_{25}N_2^+$): 389.2018. Found: 389.2013. m.p.: 145 °C

APPENDIX

APPENDIX

Figure 2.4 ^{1}H and ^{13}C NMR spectra of compound **2-1**

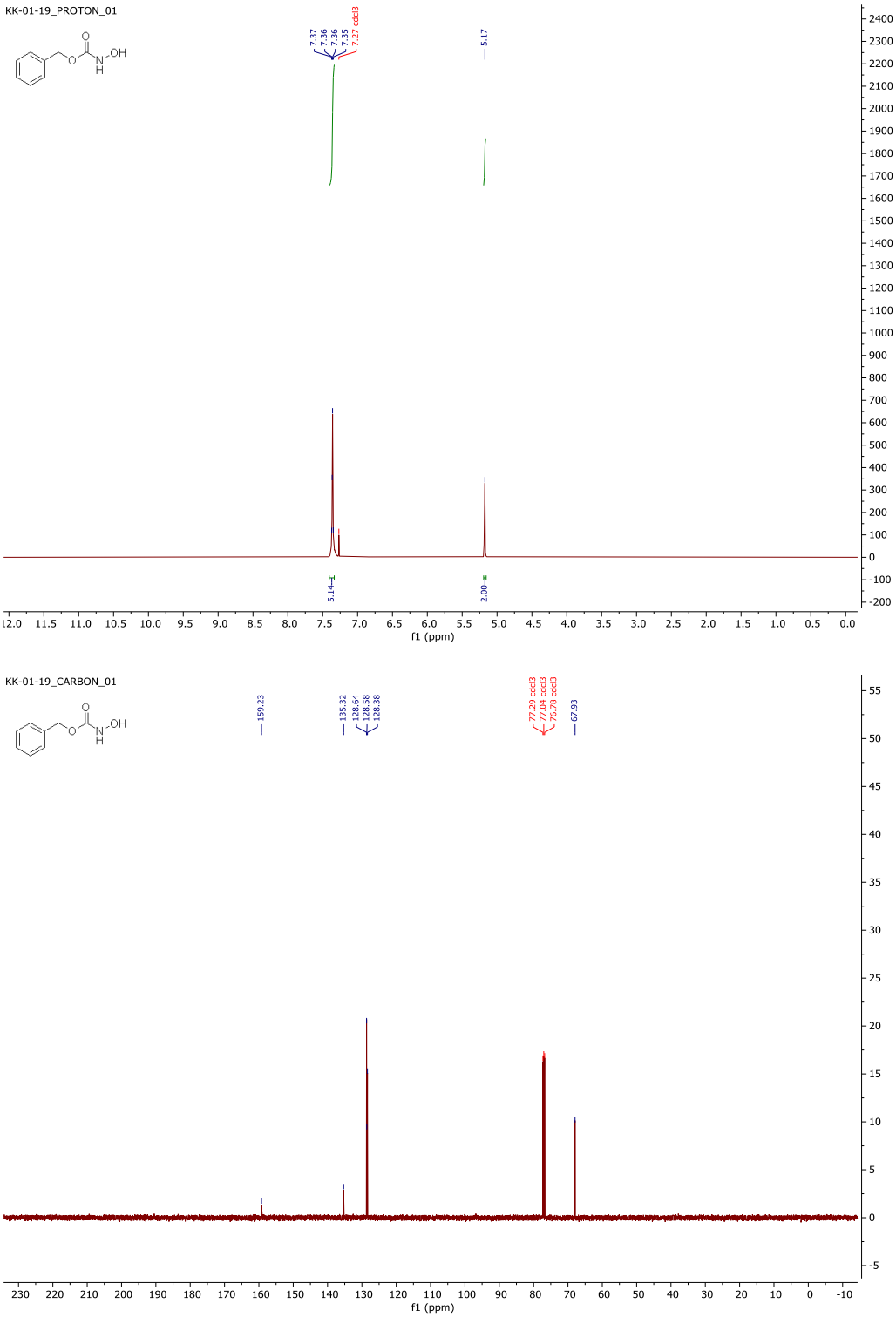


Figure 2.5 ^{1}H and ^{13}C NMR spectra of compound **2-2**

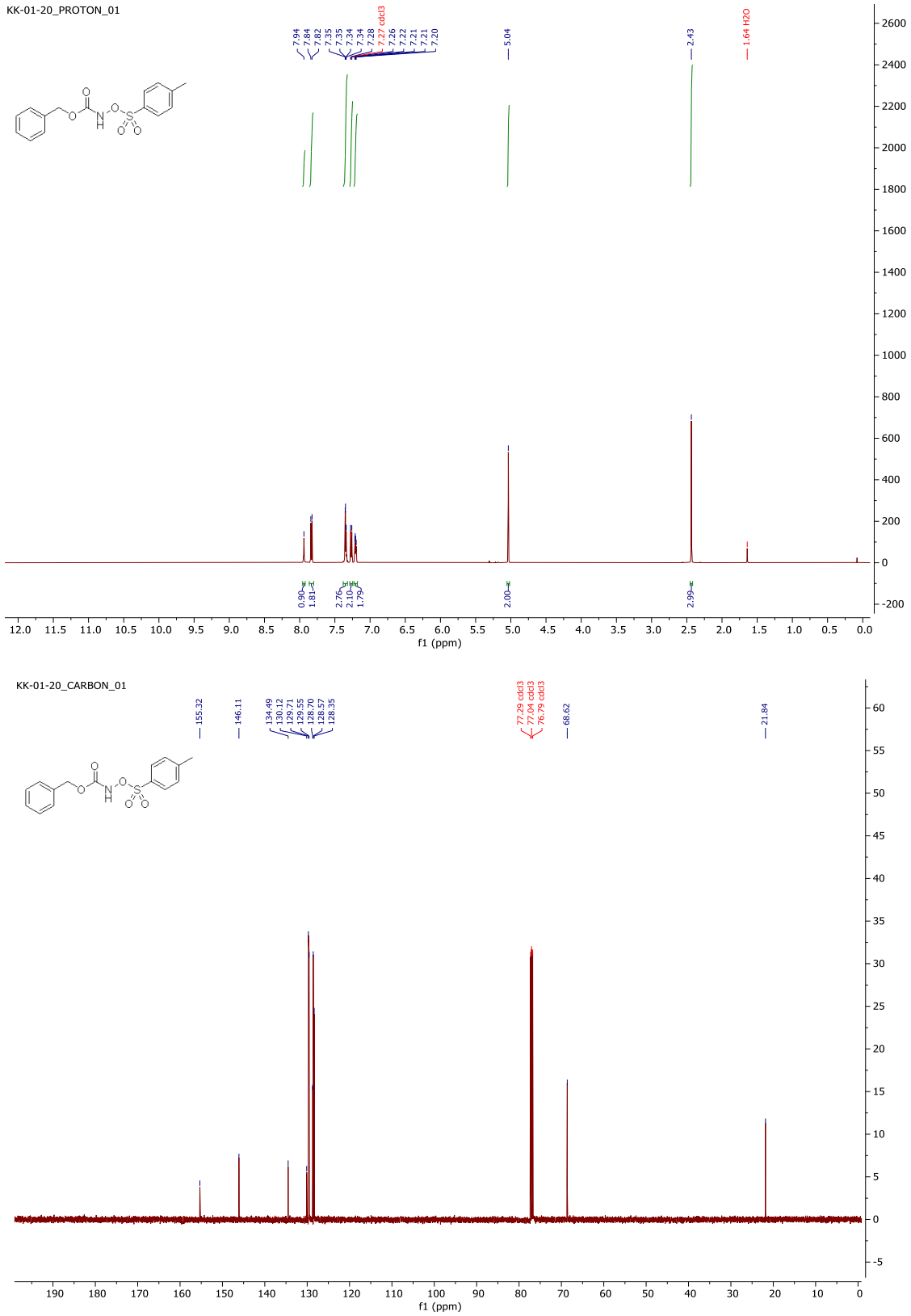


Figure 2.6 ^{1}H and ^{13}C NMR spectra of compound **2-3**

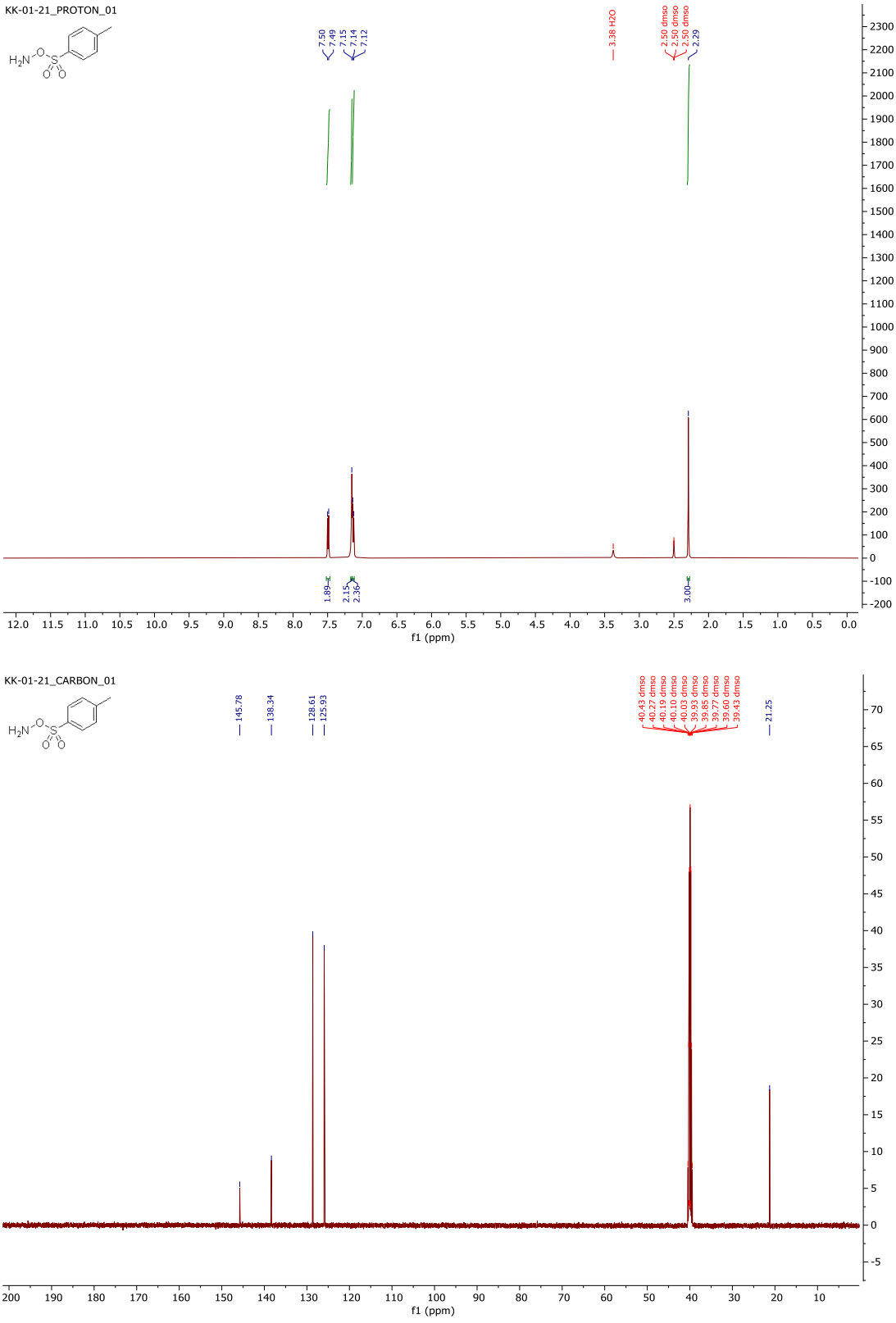


Figure 2.7 ^{1}H and ^{13}C NMR spectra of compound **2-4**

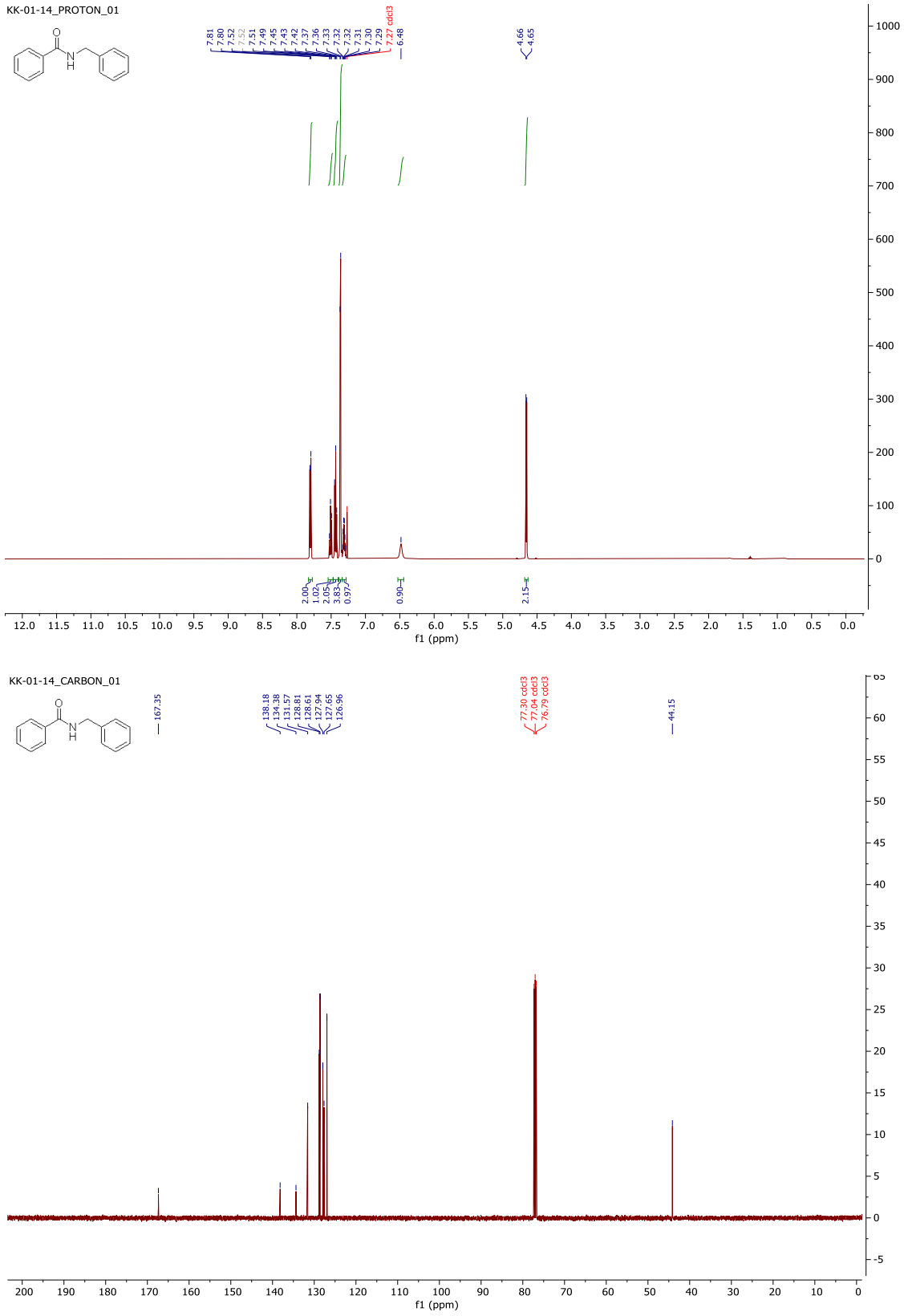


Figure 2.8 ^{1}H and ^{13}C NMR spectra of compound **2-5**

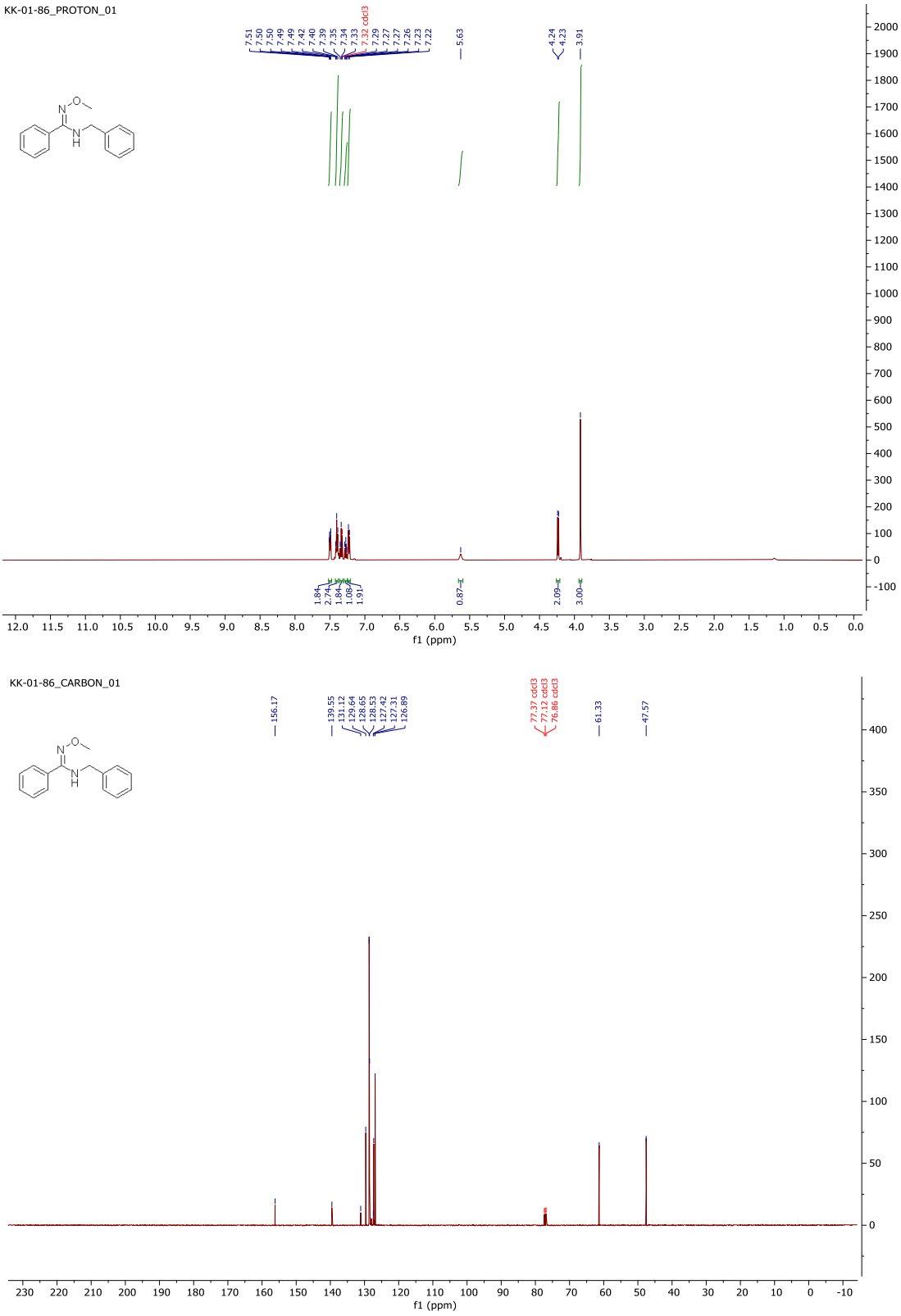
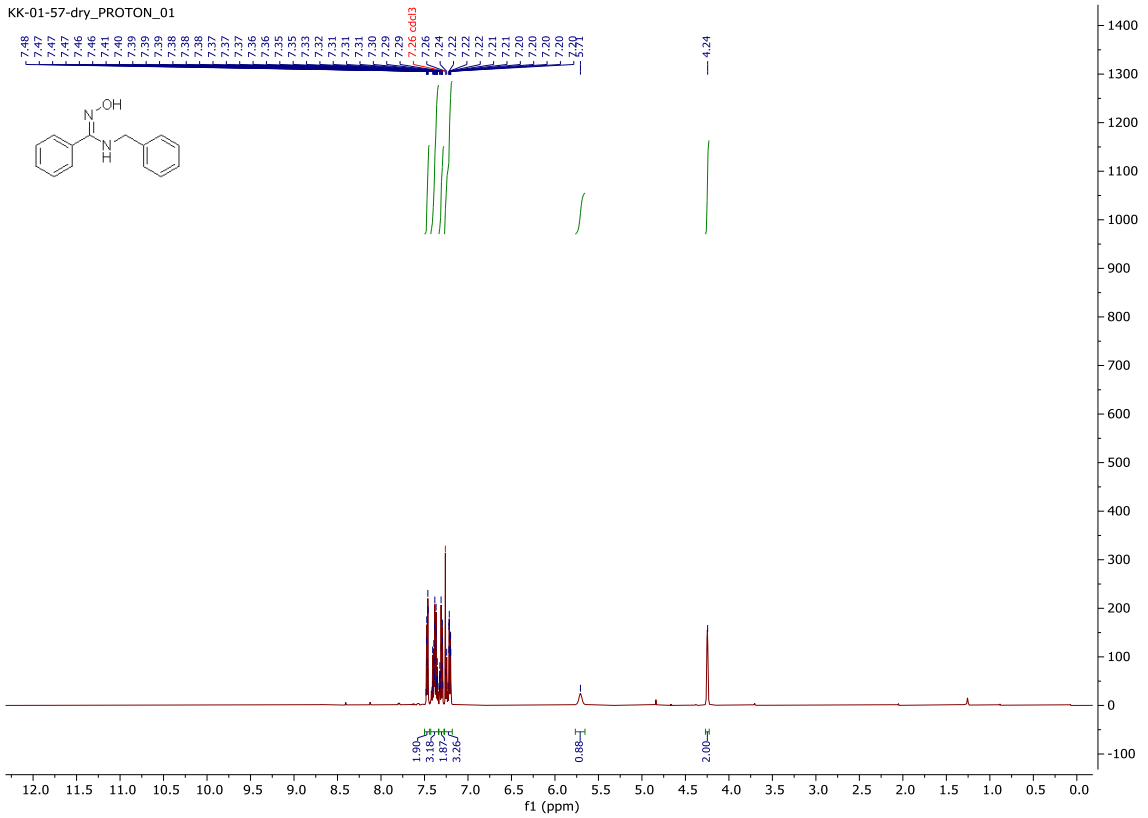


Figure 2.9 ^{1}H and ^{13}C NMR spectra of compound **2-6**



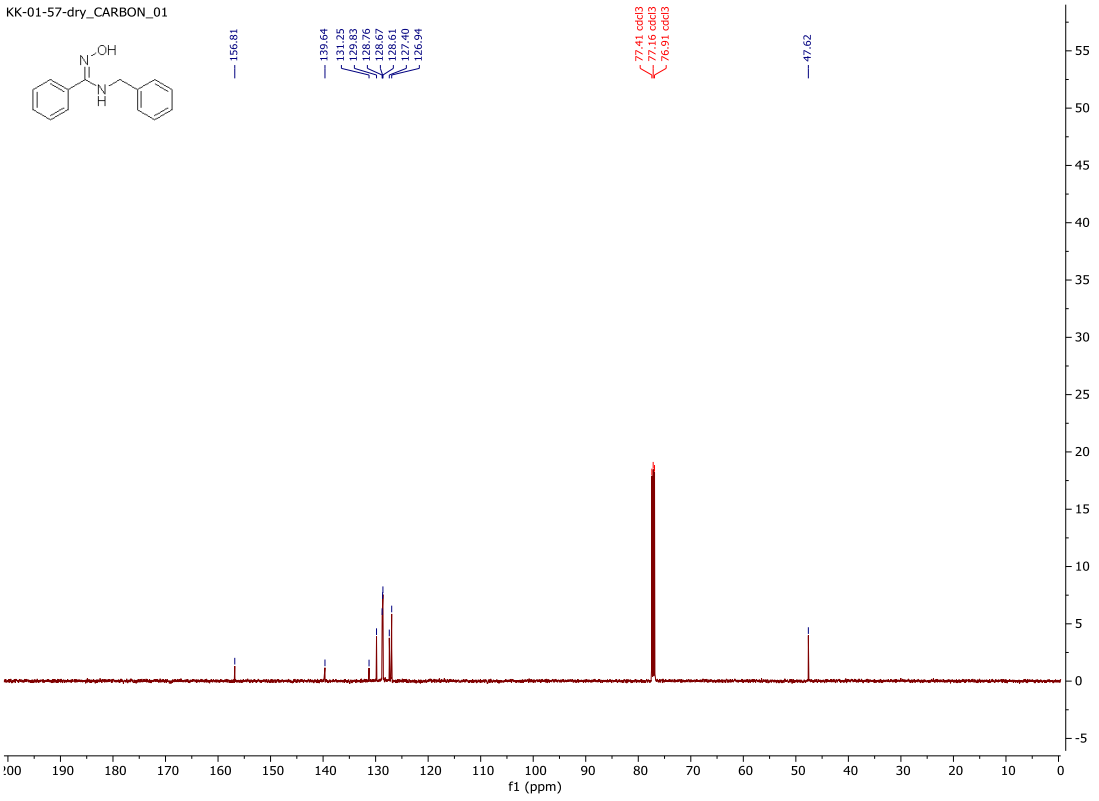


Figure 2.10 ^{1}H and ^{13}C NMR spectra of compound **2-7**

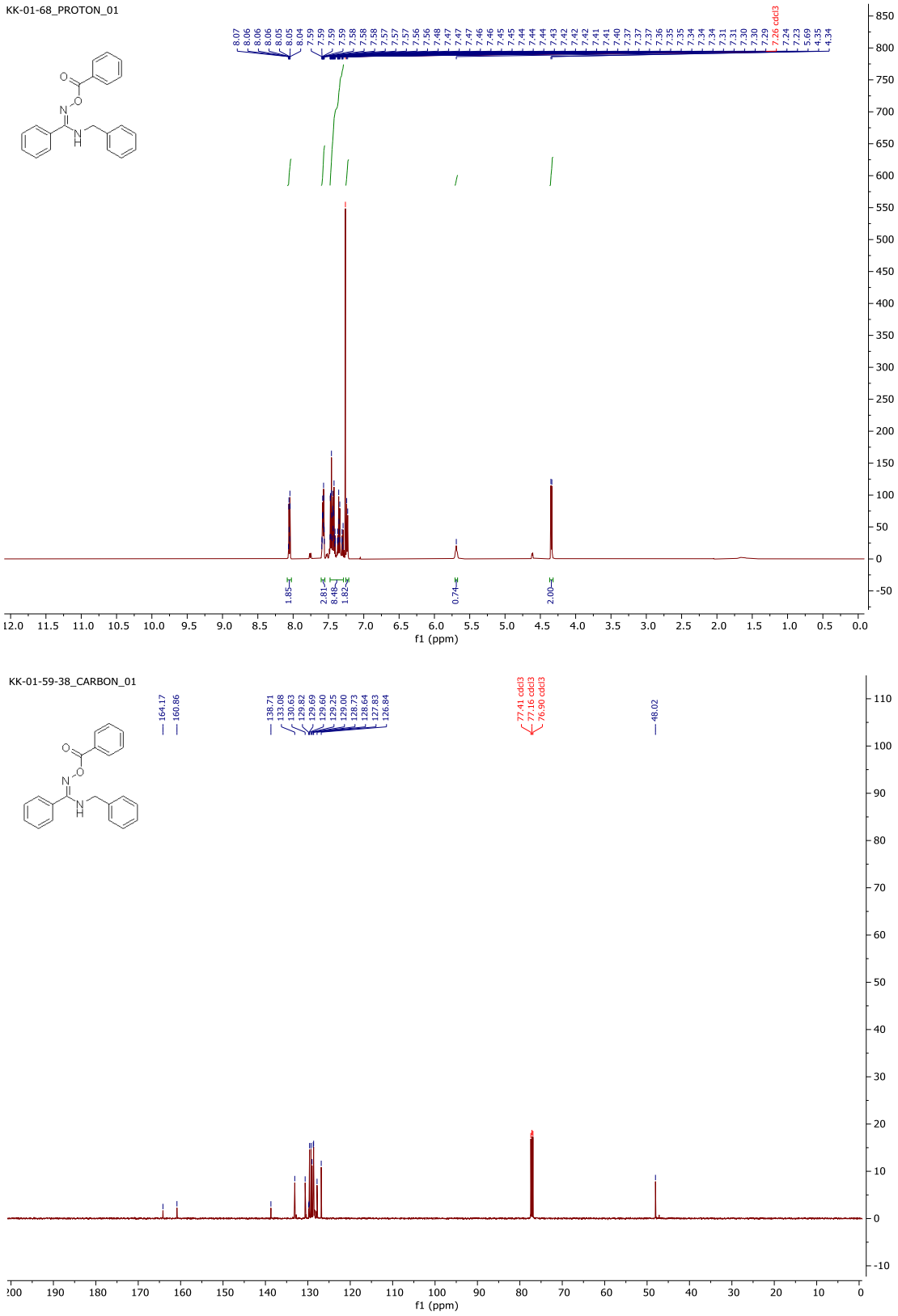
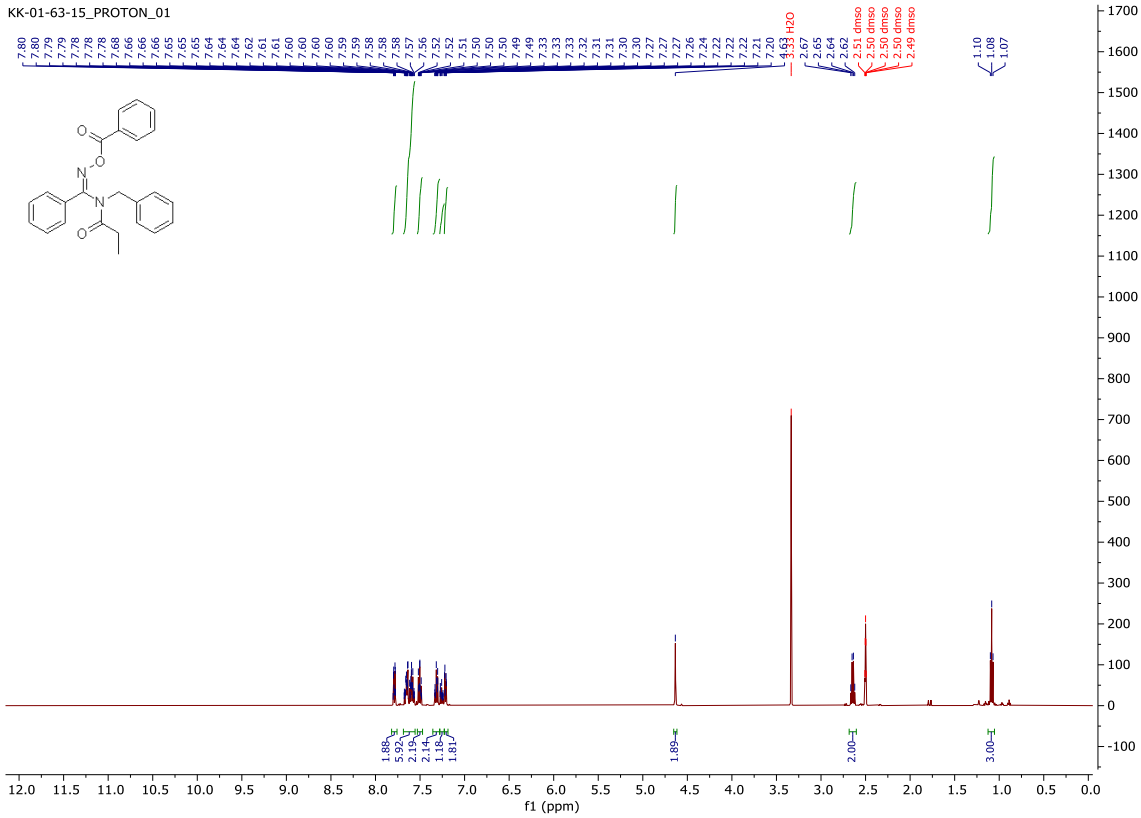


Figure 2.11 ^{1}H and ^{13}C NMR spectra of compound **2-8**



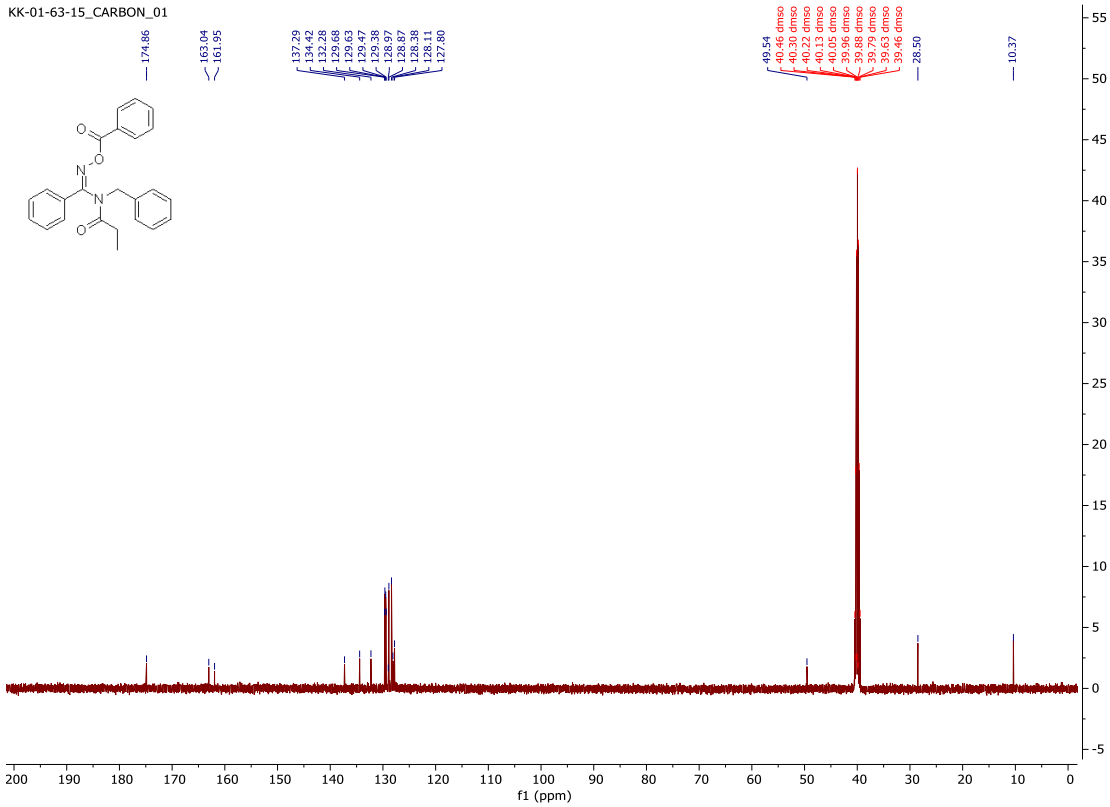
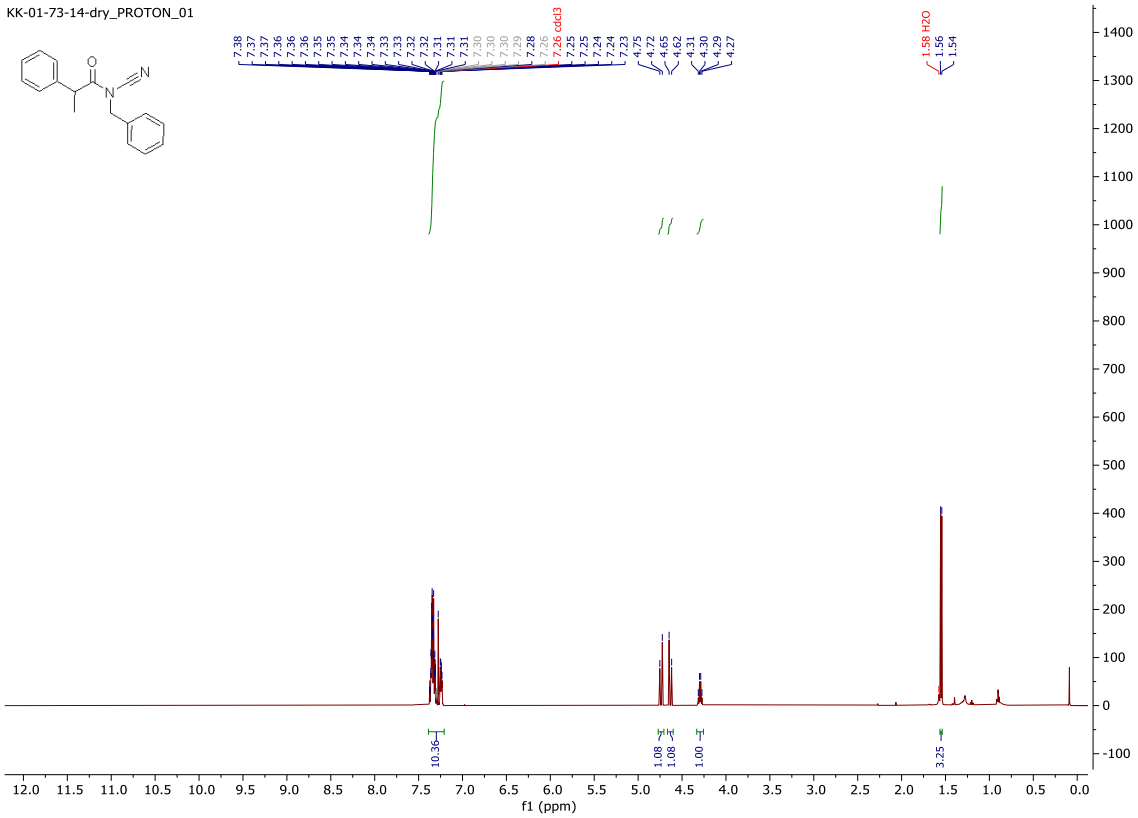


Figure 2.12 ^{1}H and ^{13}C NMR spectra of compound **2-9**



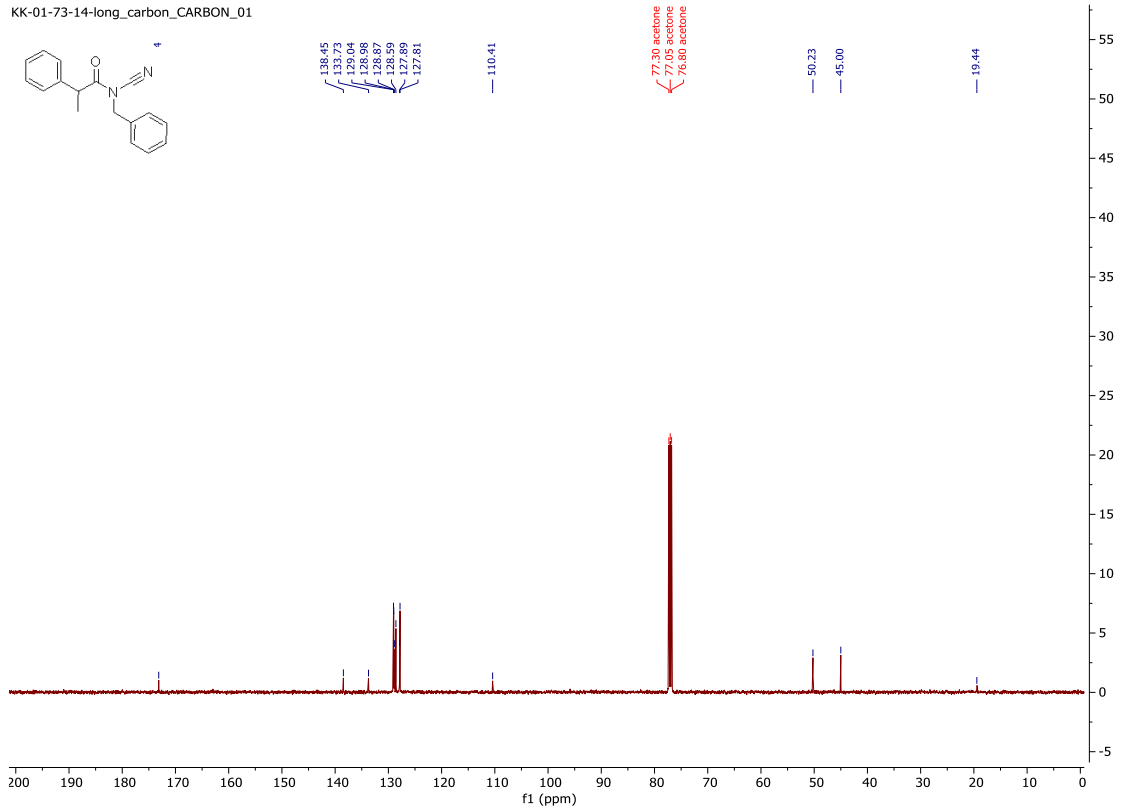


Figure 2.13 ^{1}H and ^{13}C NMR spectra of compound **2-10**

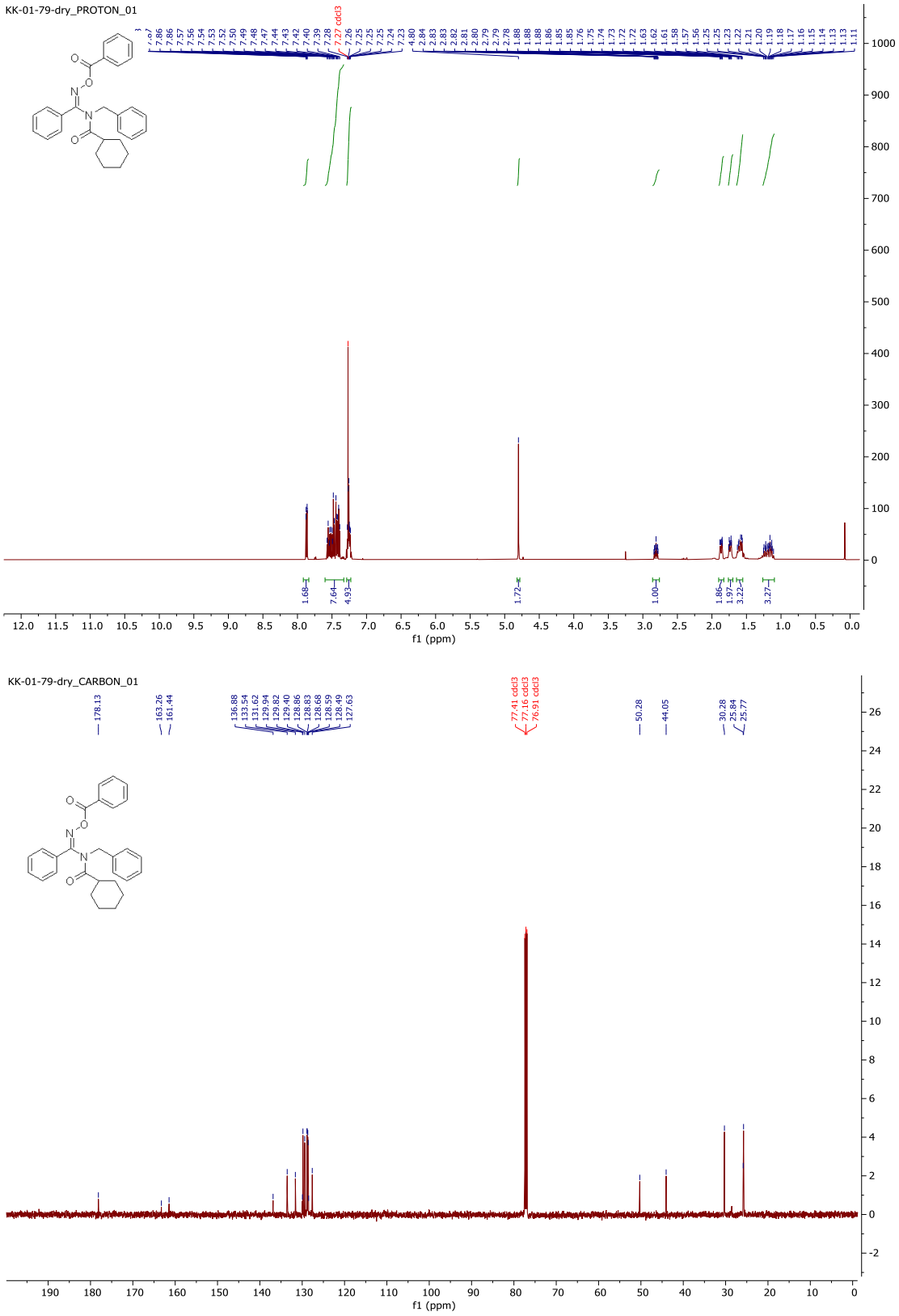
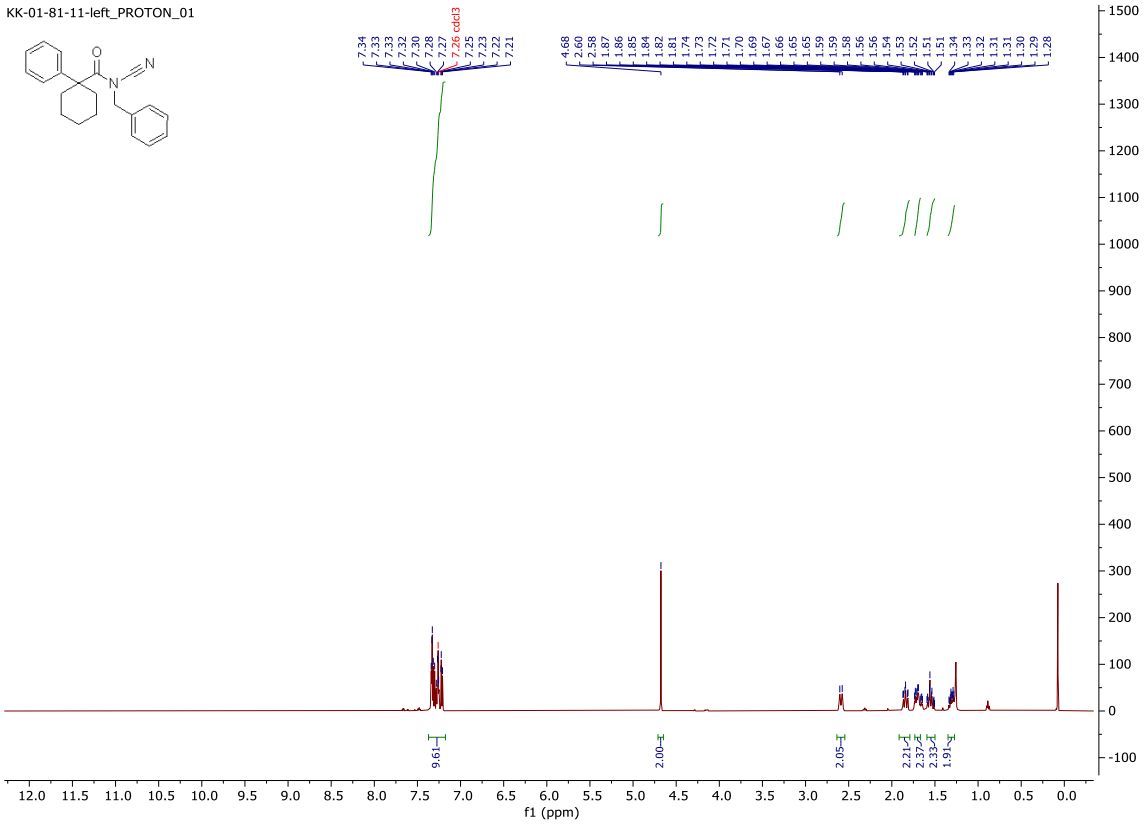


Figure 2.14 ^{1}H and ^{13}C NMR spectra of compound **2-11**



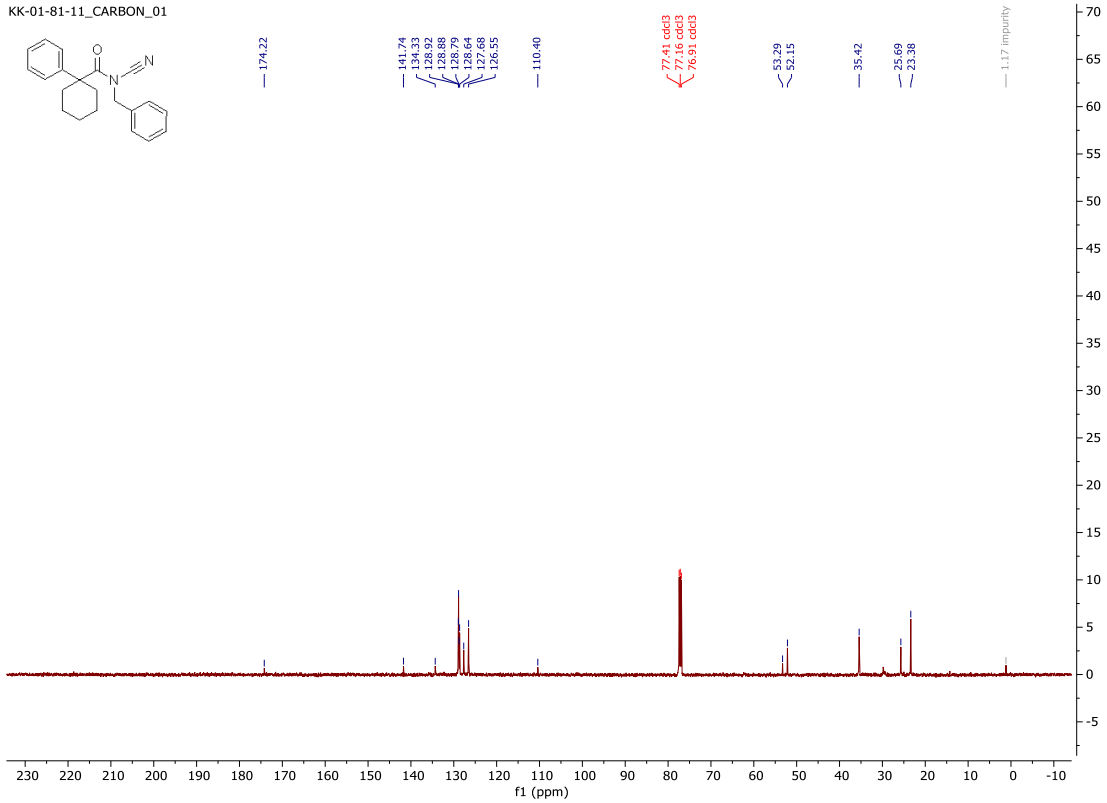


Figure 2.15 ^{1}H and ^{13}C NMR spectra of compound **2-12**

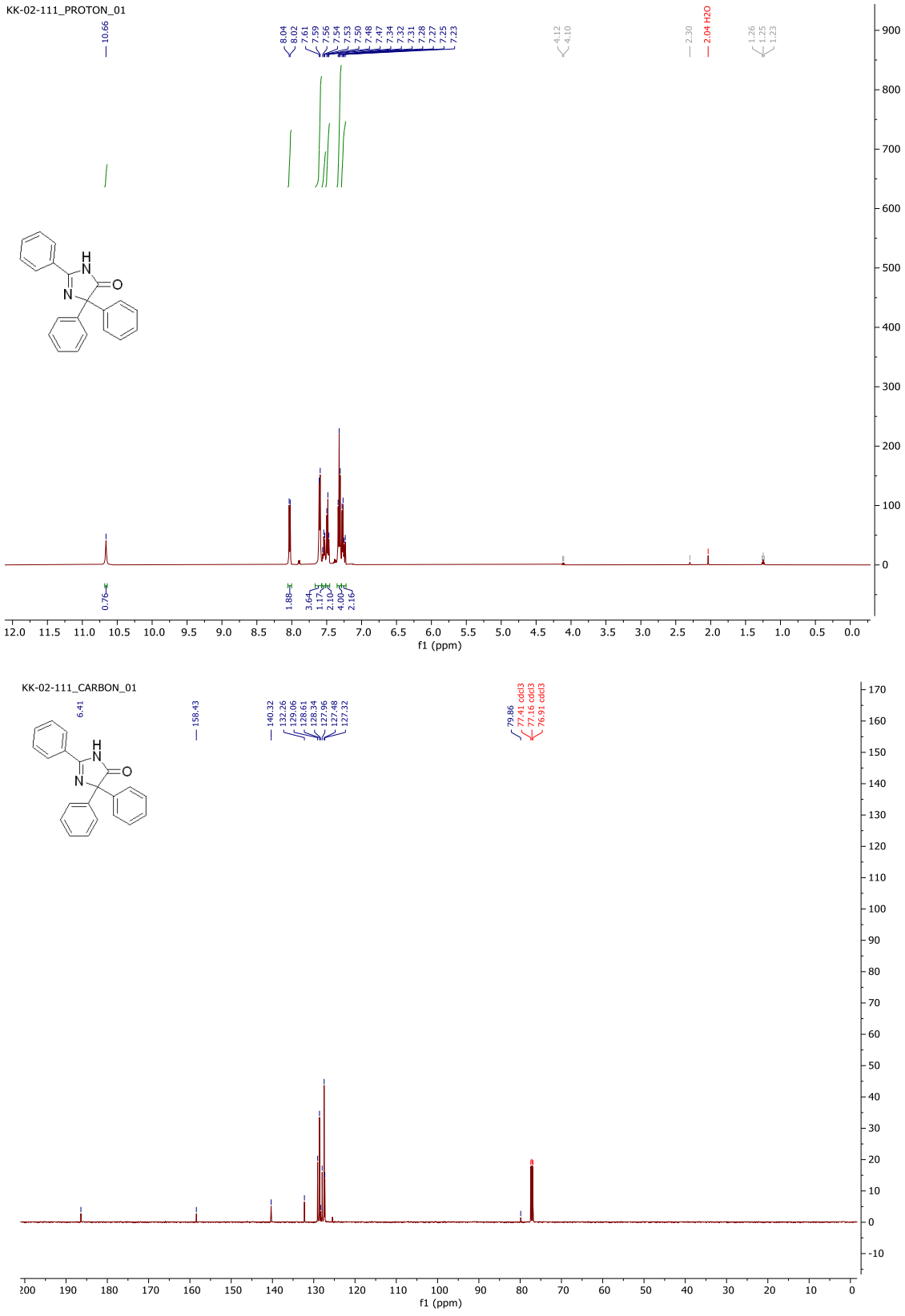


Figure 2.16 ^{1}H and ^{13}C NMR spectra of compound **2-13**

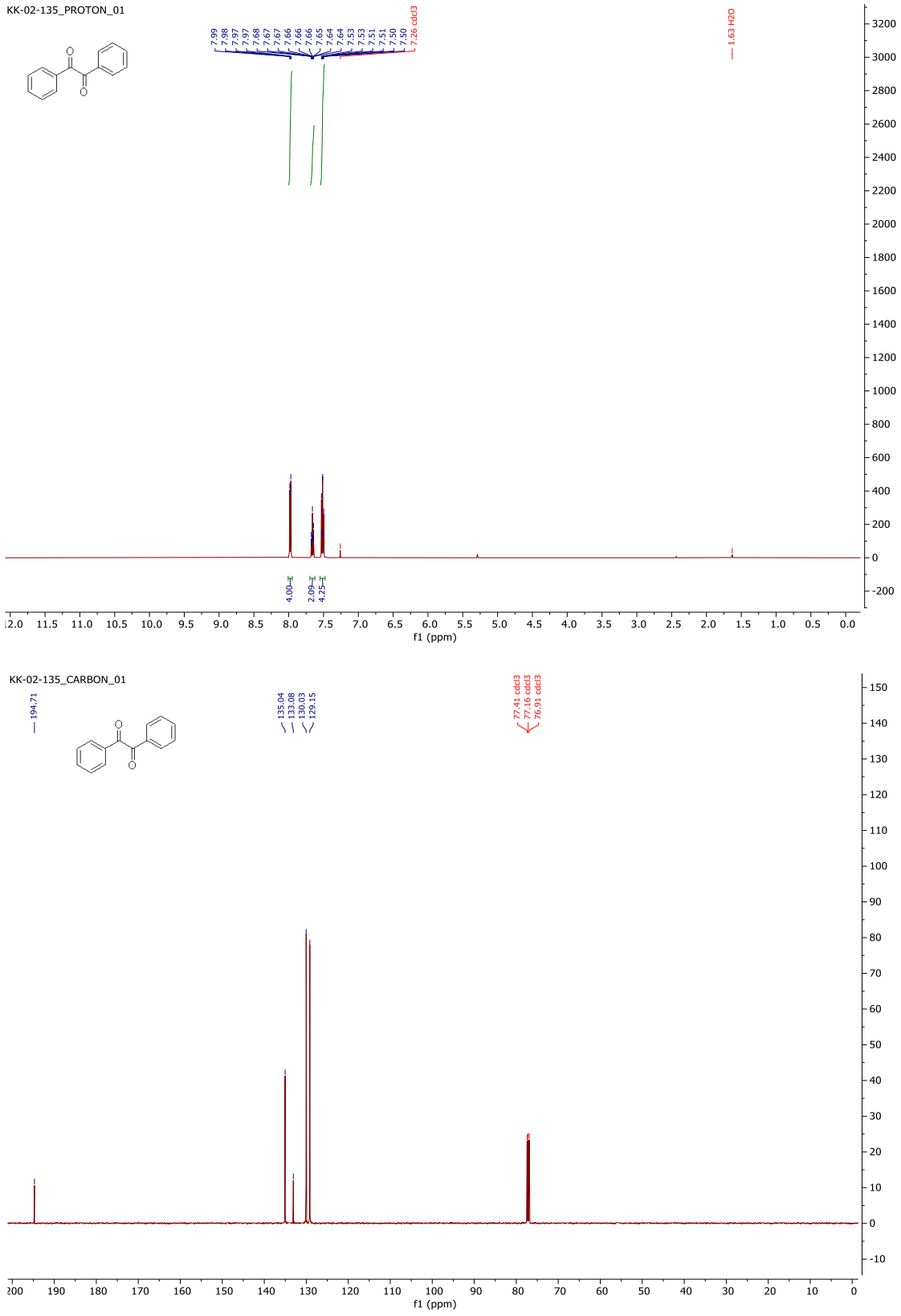
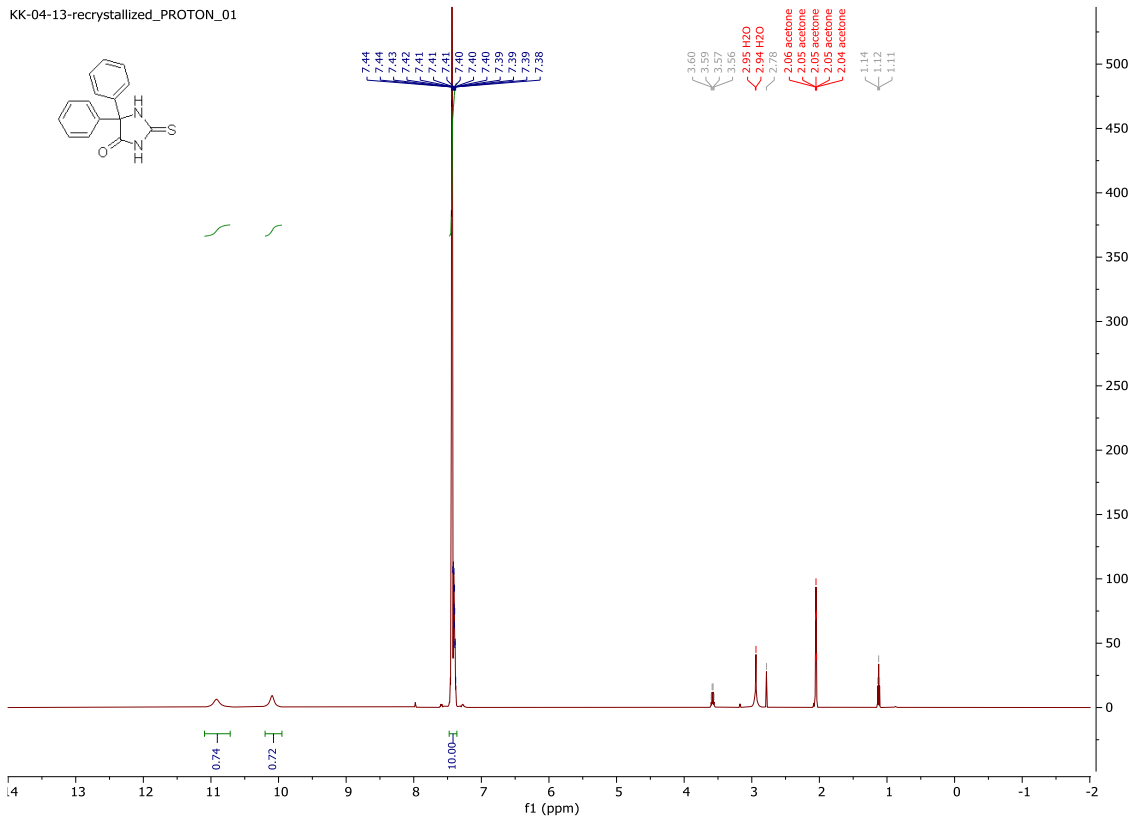


Figure 2.17 ^{1}H and ^{13}C NMR spectra of compound **2-14**



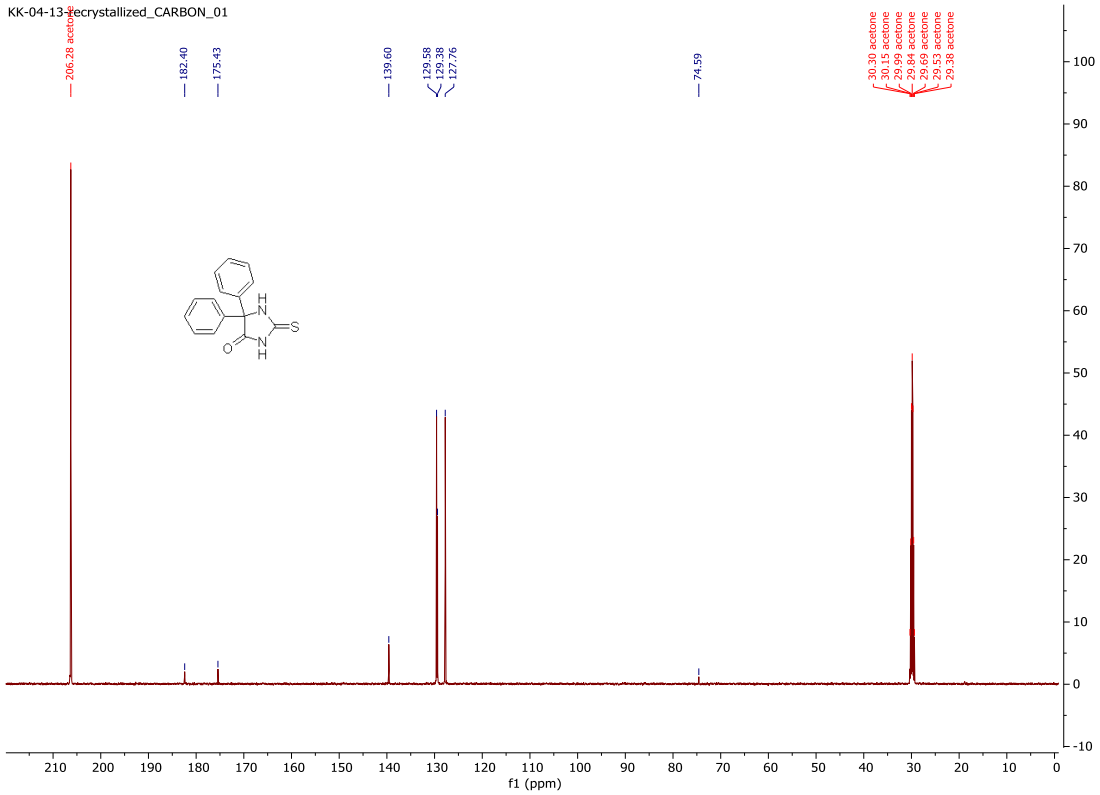
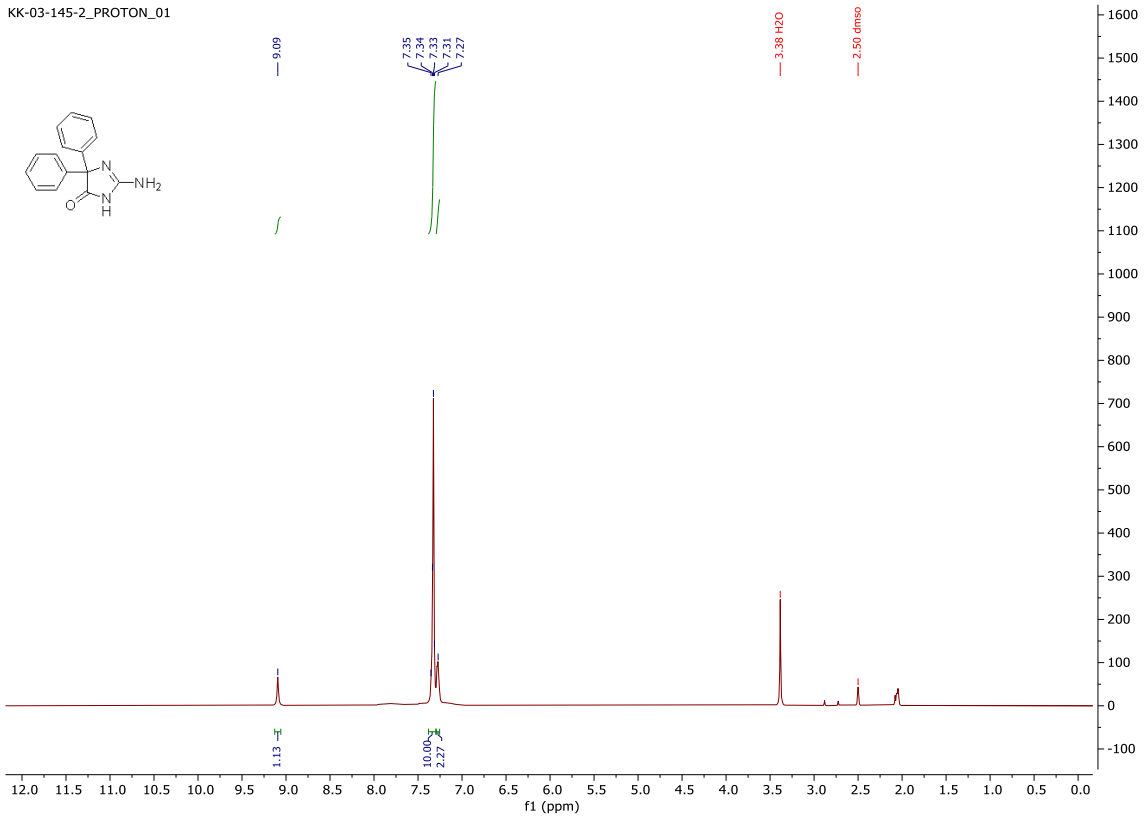


Figure 2.18 ^{1}H and ^{13}C NMR spectra of compound **2-15**



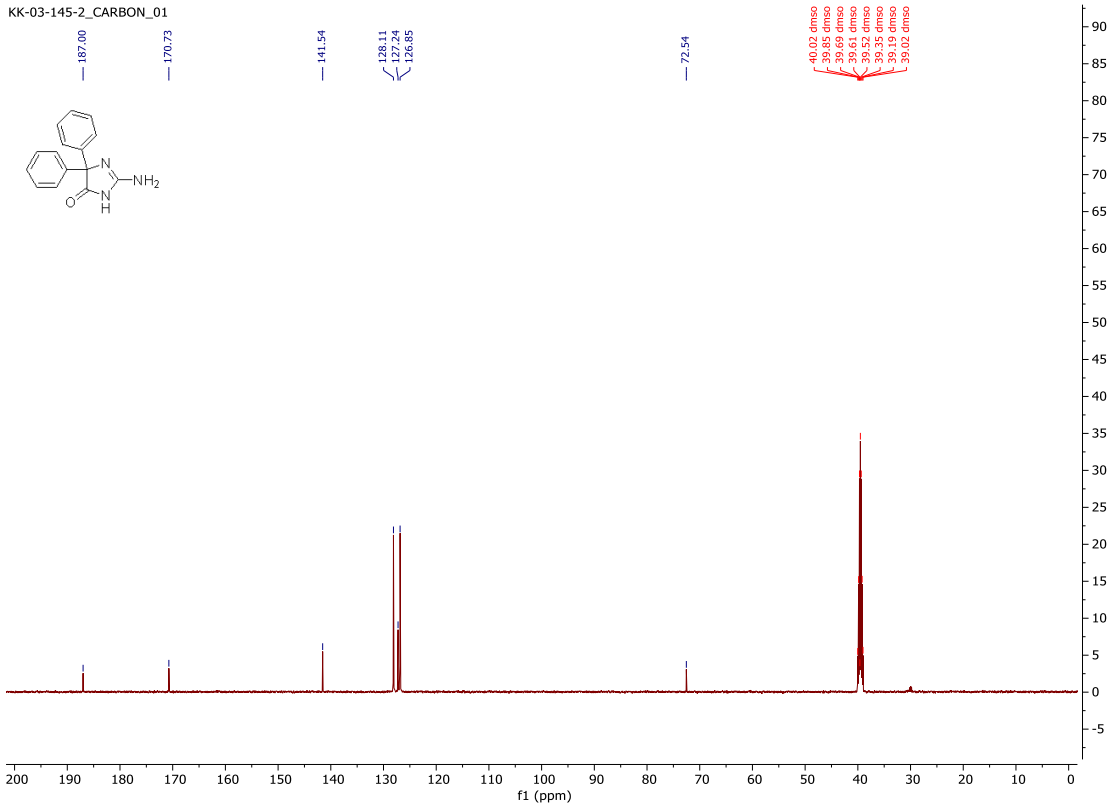
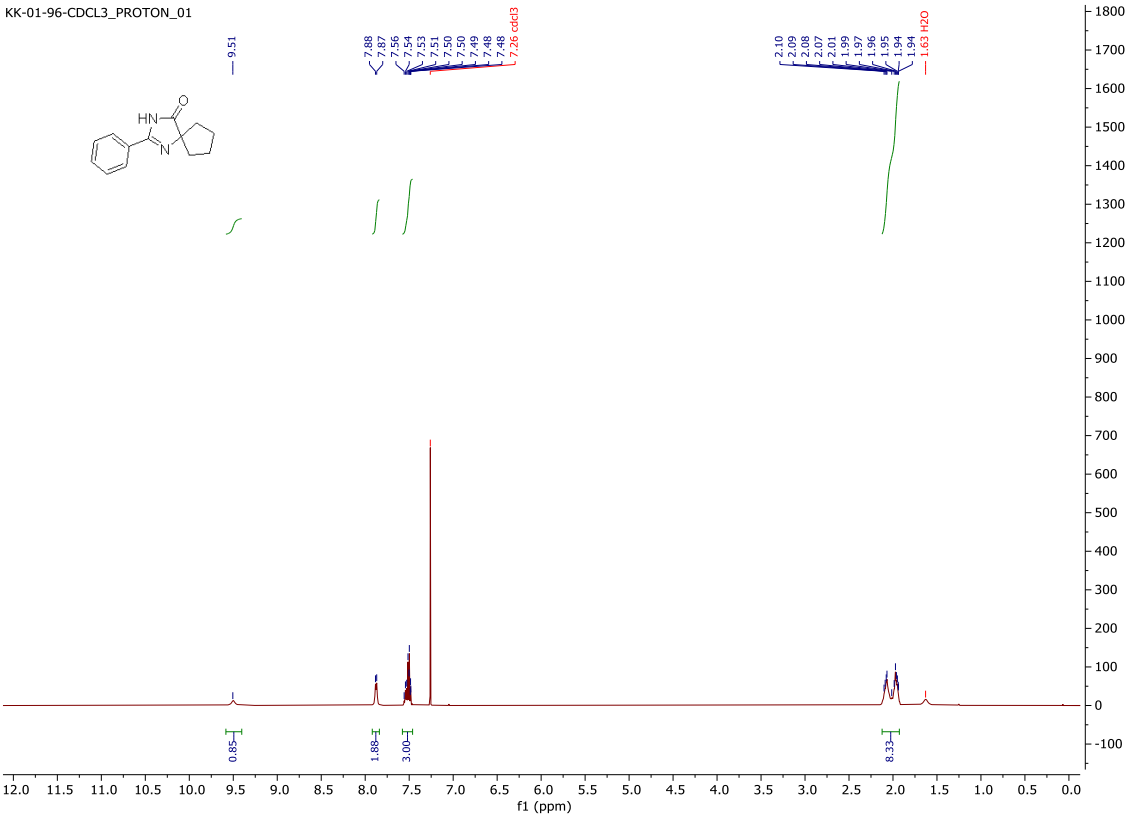


Figure 2.19 ^{1}H and ^{13}C NMR spectra of compound **2-16**



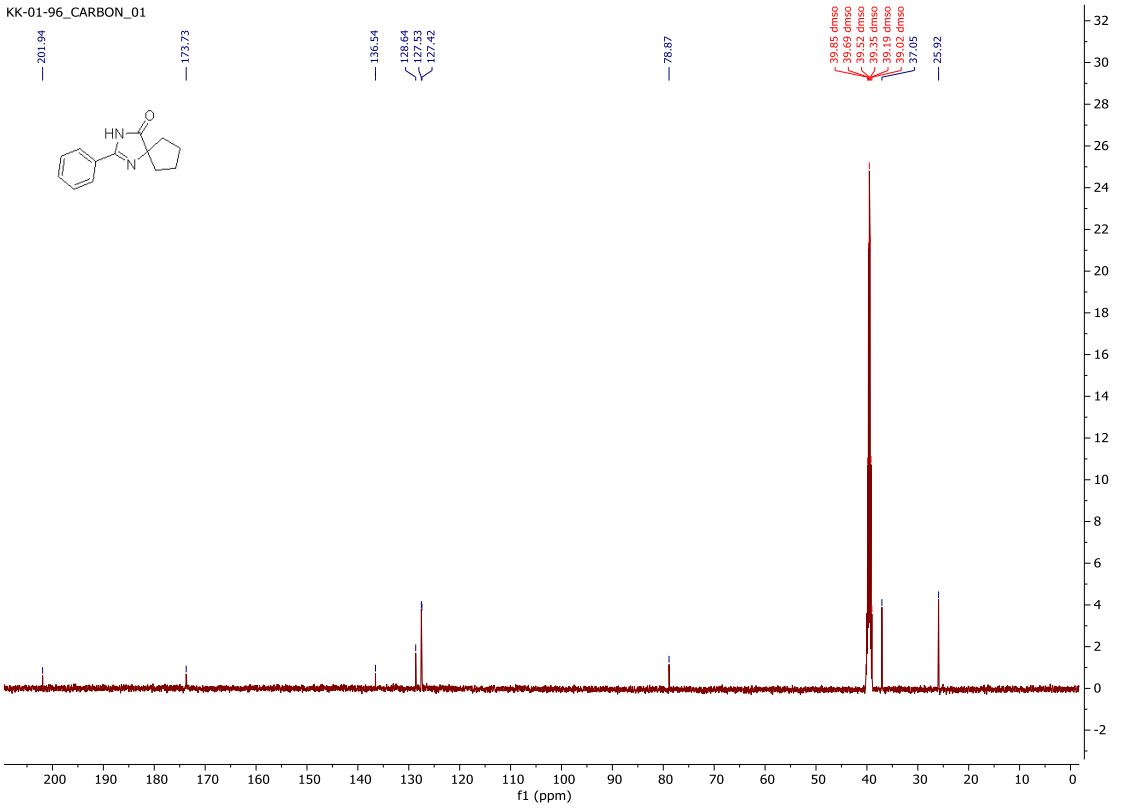


Figure 2.20 ^{1}H and ^{13}C NMR spectra of compound **2-17**

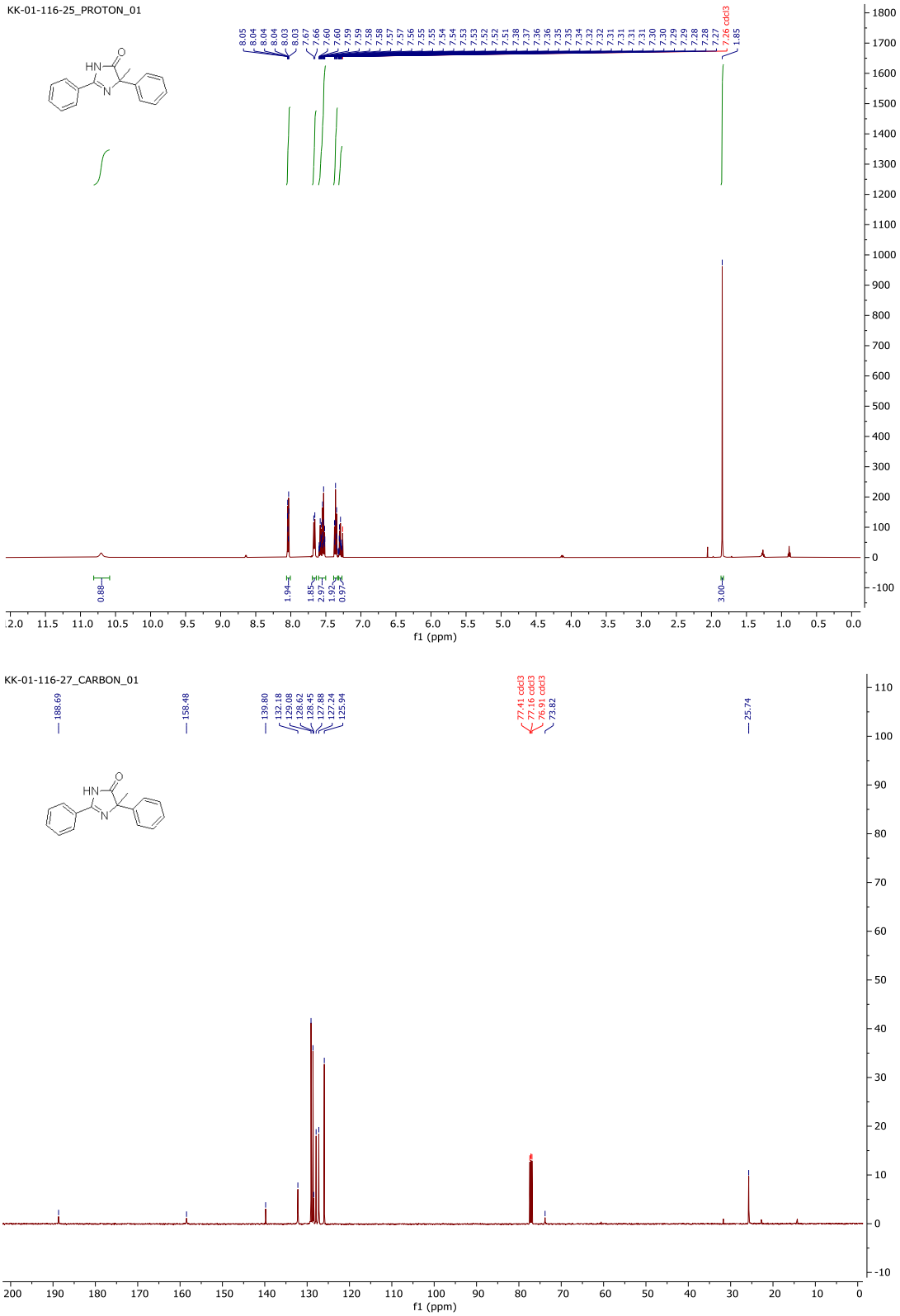
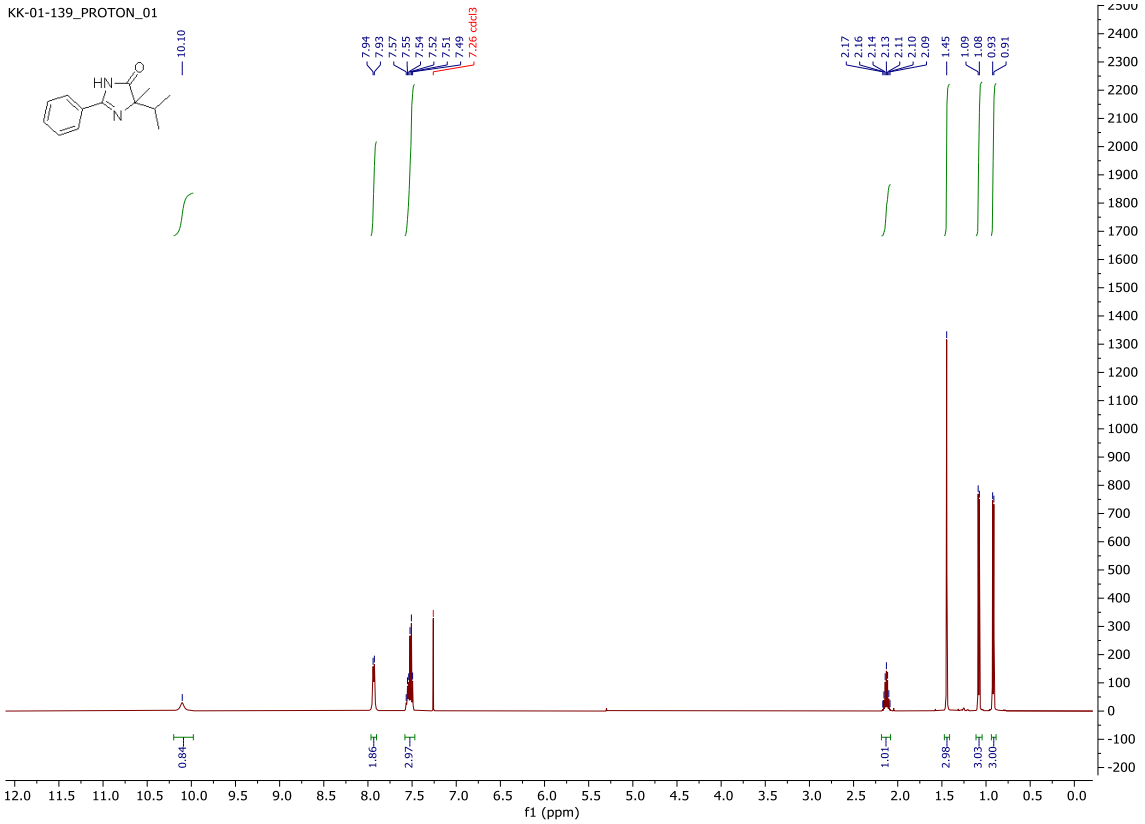


Figure 2.21 ^{1}H and ^{13}C NMR spectra of compound **2-18**



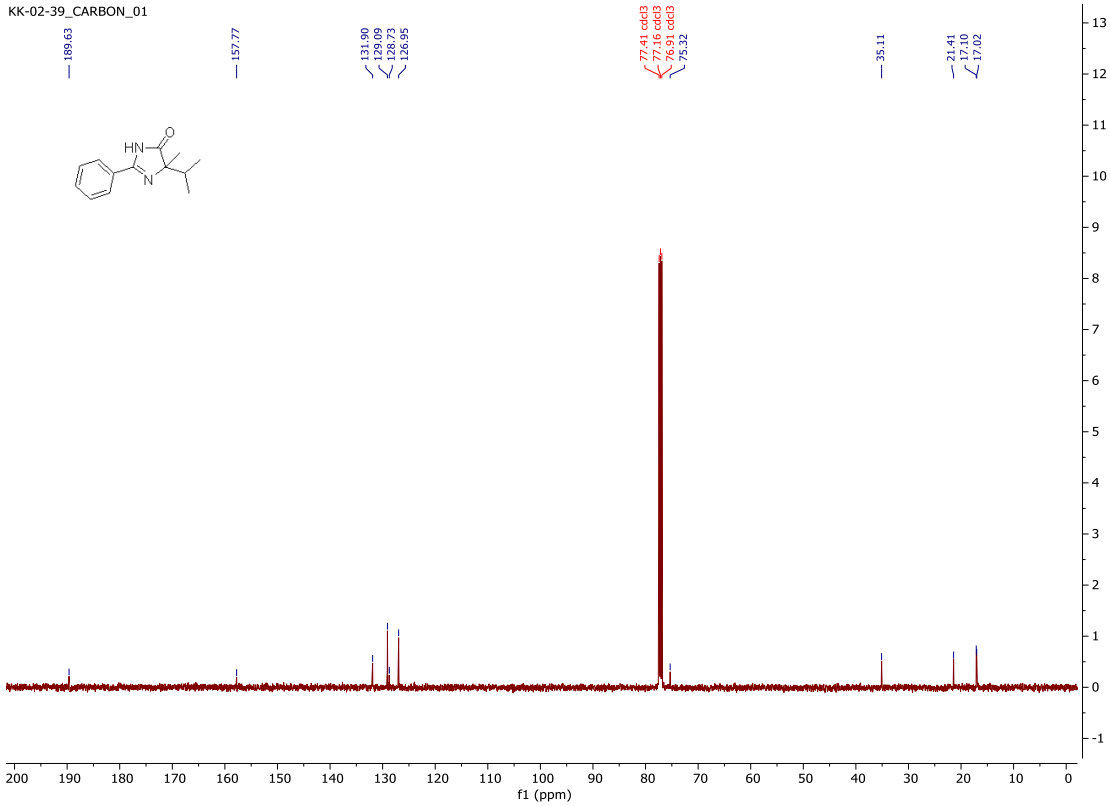
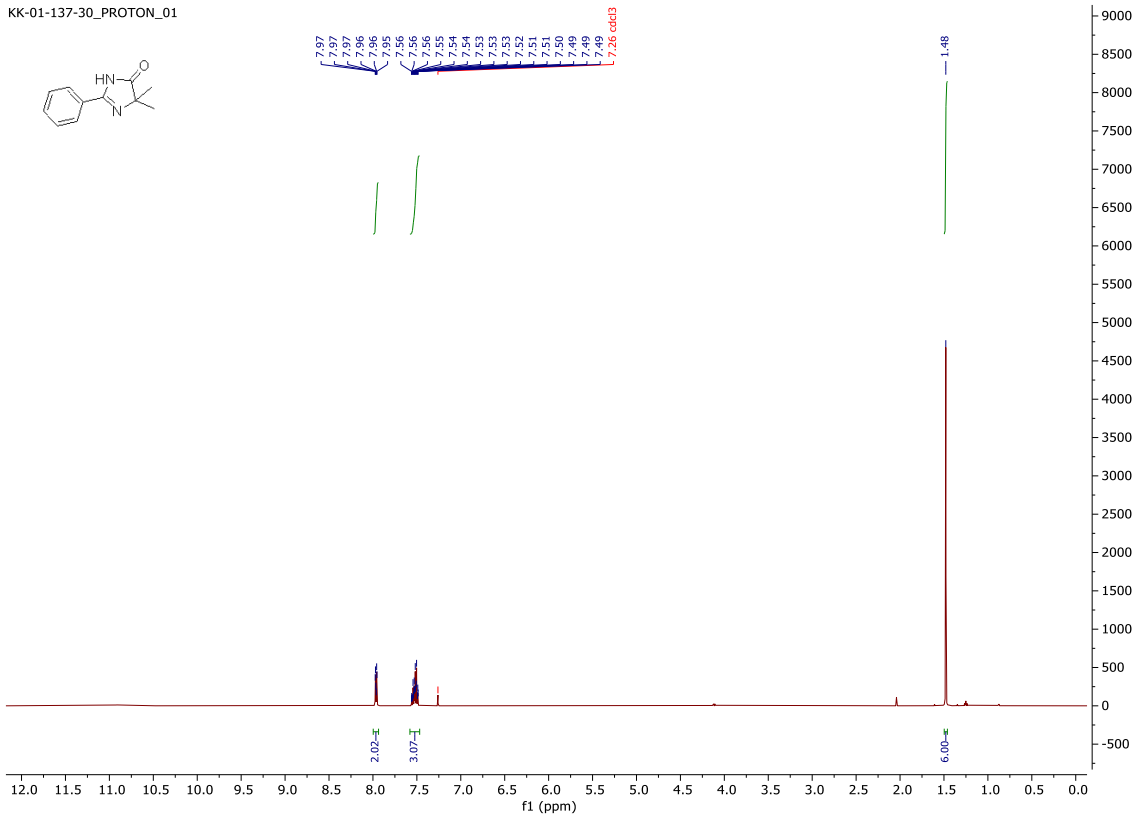


Figure 2.22 ^{1}H and ^{13}C NMR spectra of compound **2-19**



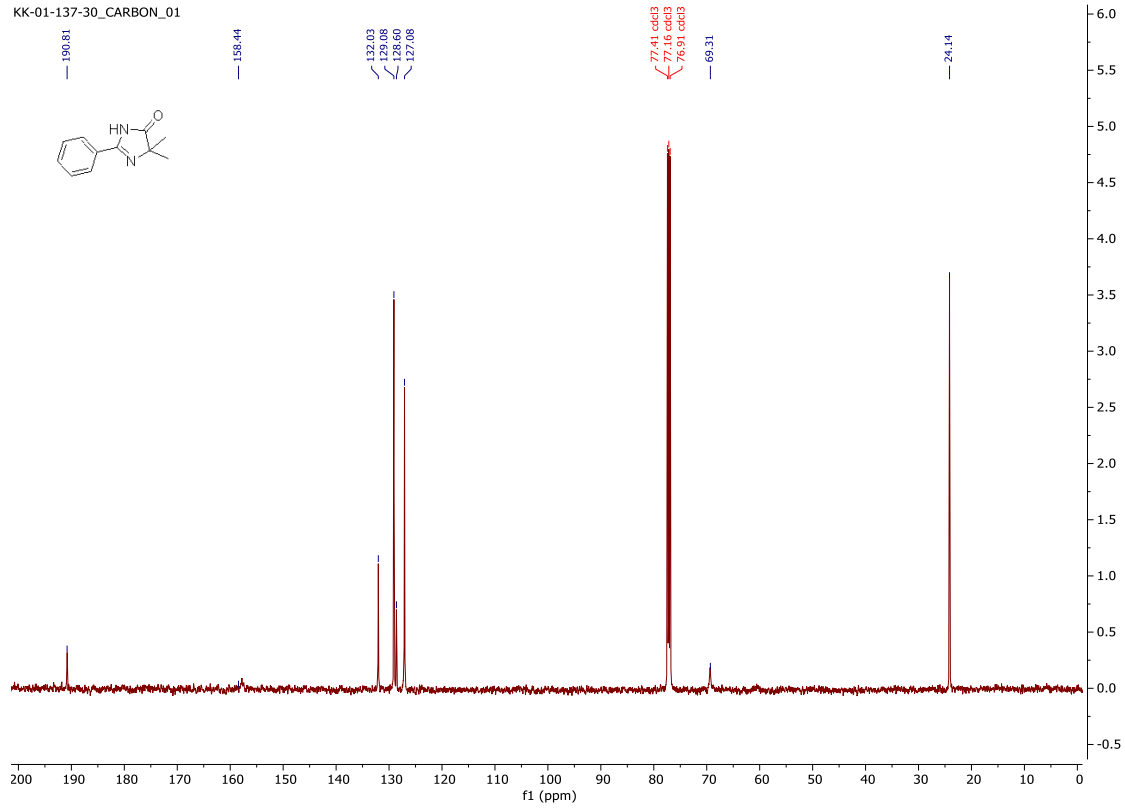


Figure 2.23 ^{1}H and ^{13}C NMR spectra of compound **2-20**

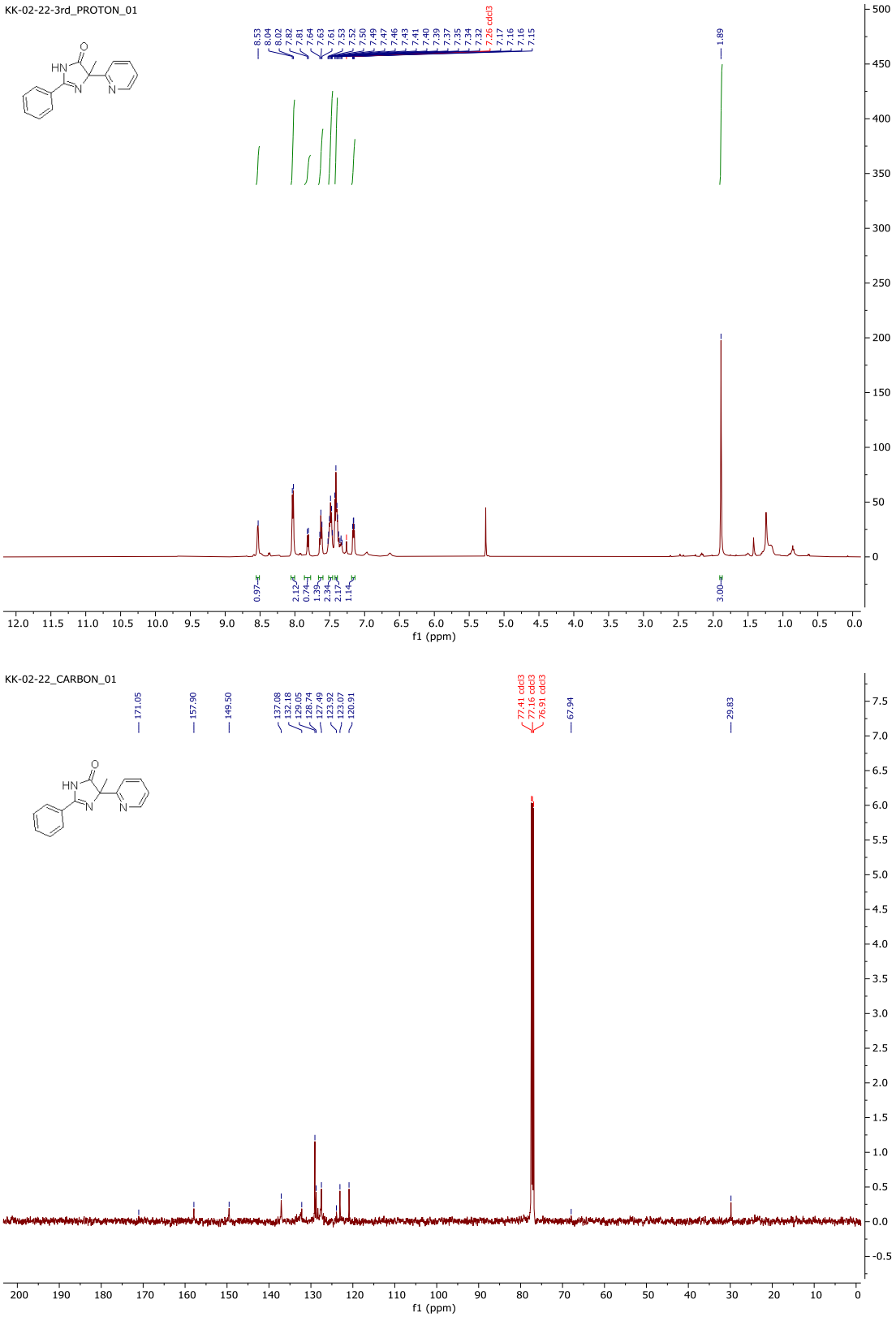
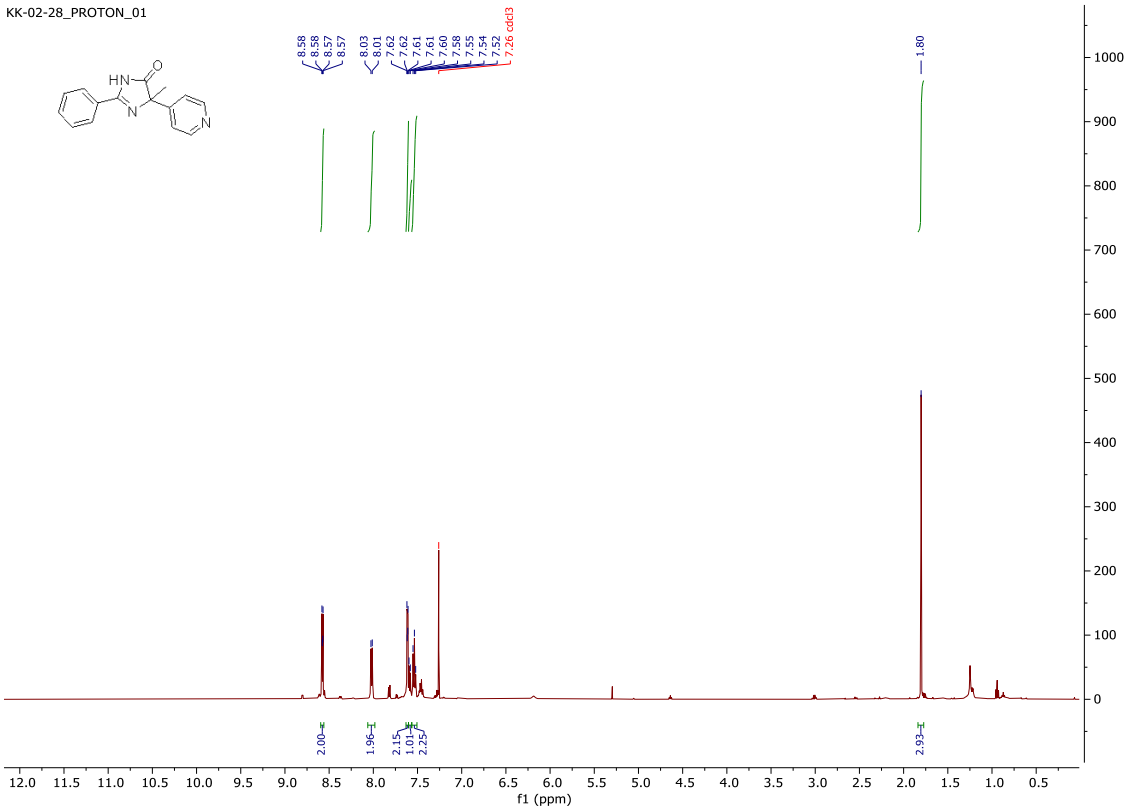


Figure 2.24 ^{1}H and ^{13}C NMR spectra of compound **2-21**



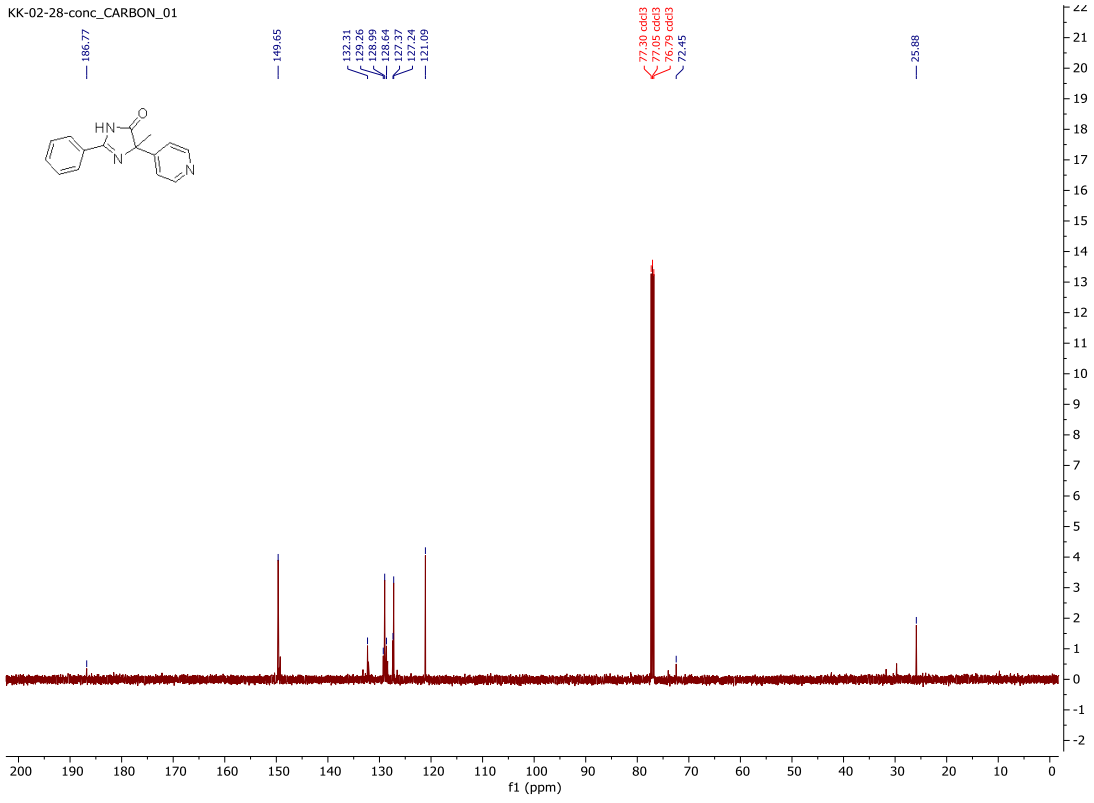
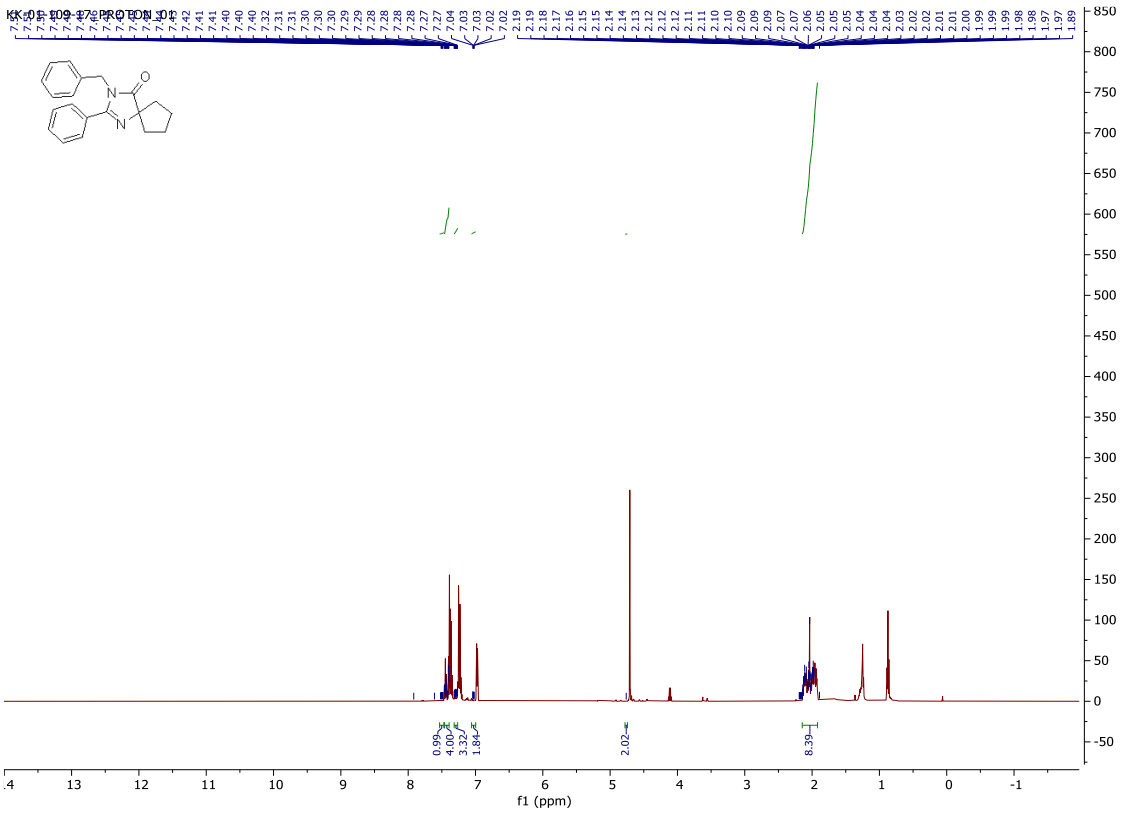


Figure 2.25 ^{1}H and ^{13}C NMR spectra of compound **2-22**



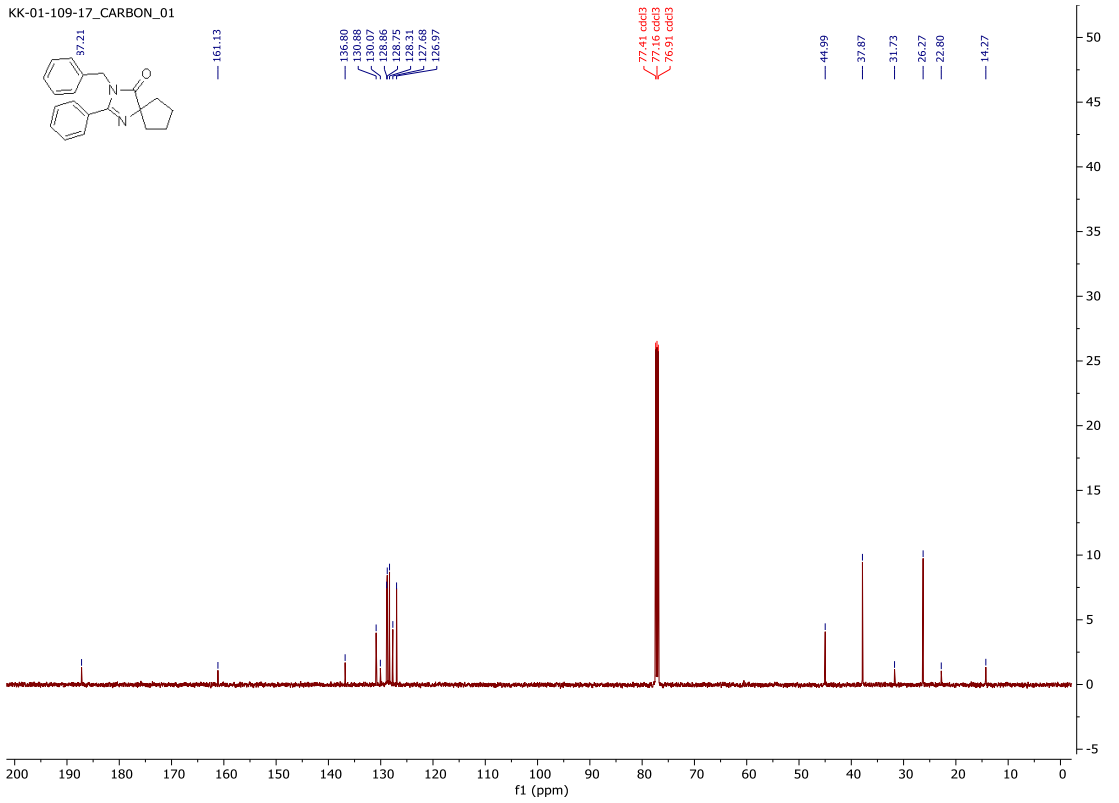
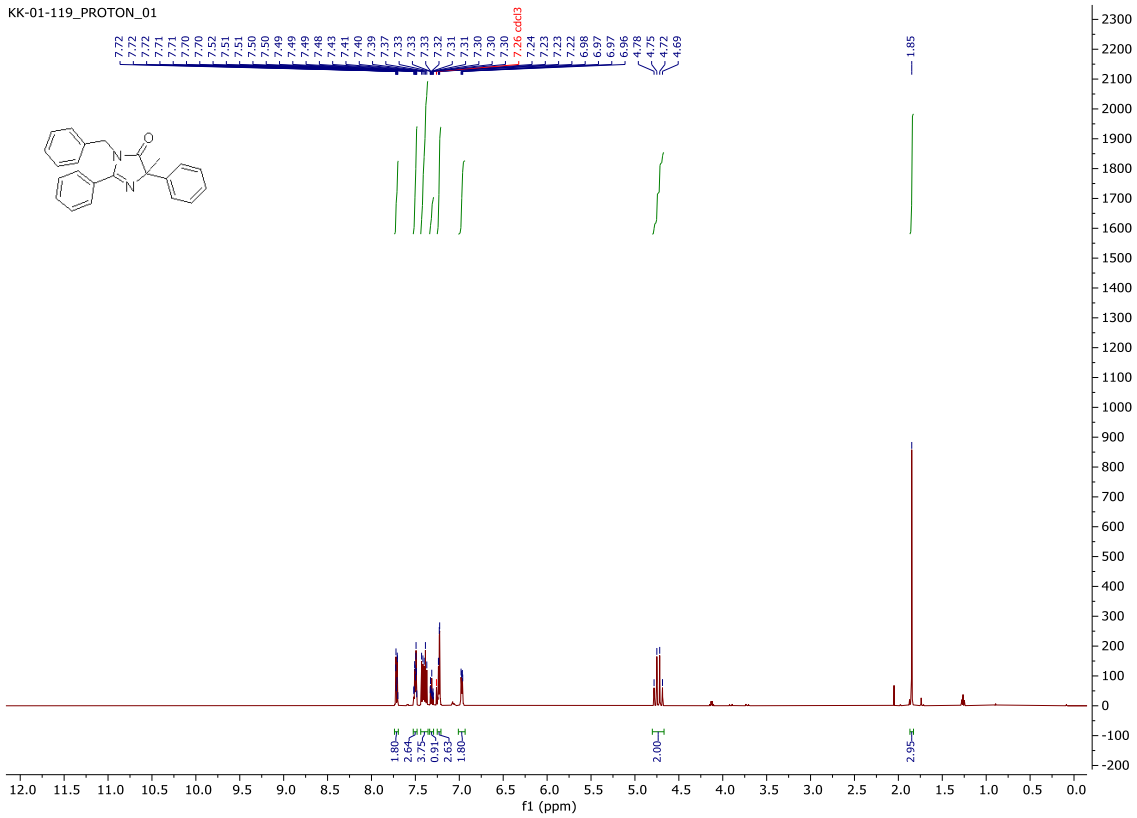


Figure 2.26 ^{1}H and ^{13}C NMR spectra of compound **2-23**



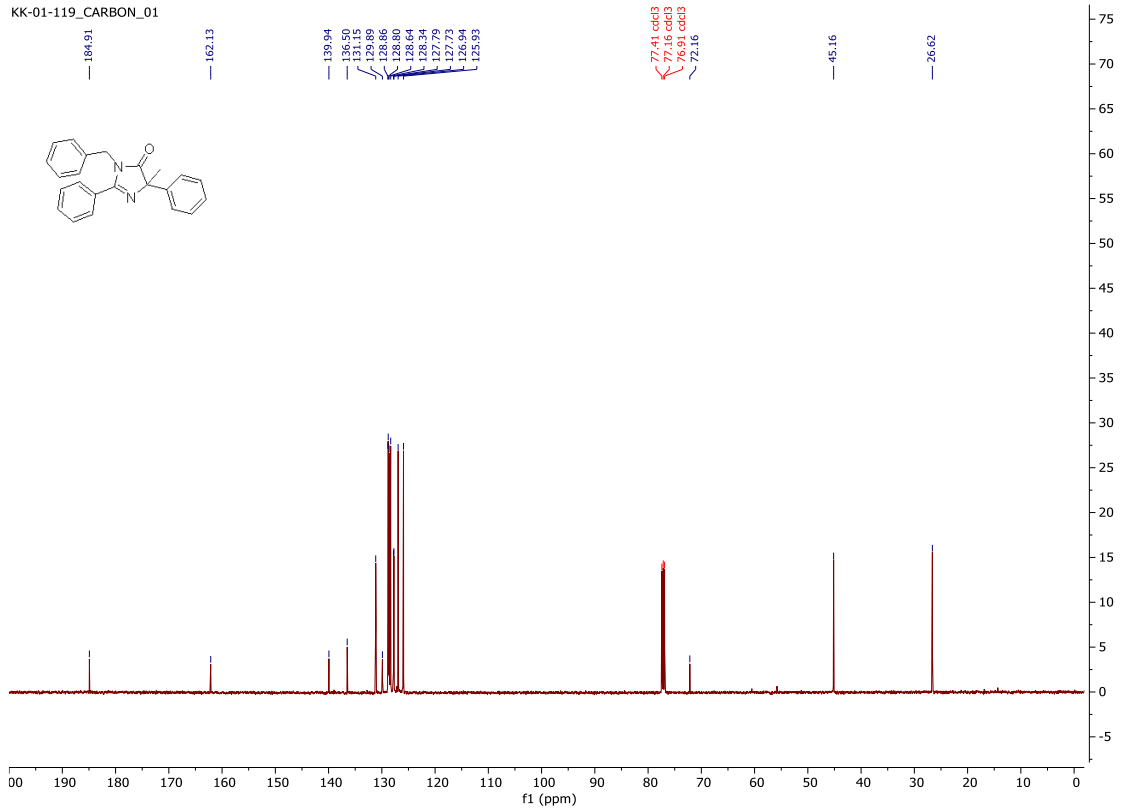


Figure 2.27 ^{1}H and ^{13}C NMR spectra of compound **2-24**

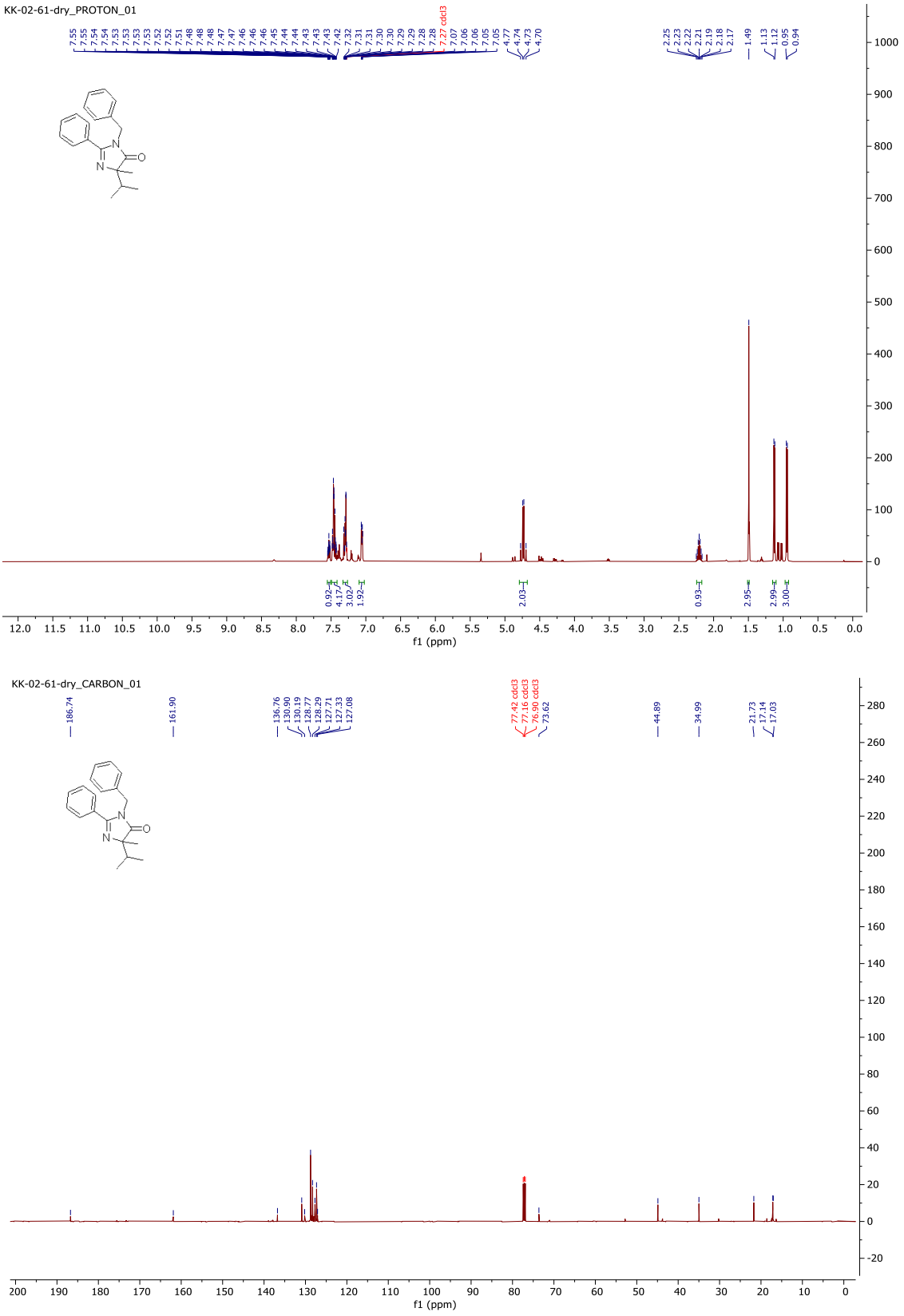


Figure 2.28 ^{1}H and ^{13}C NMR spectra of compound **2-25**

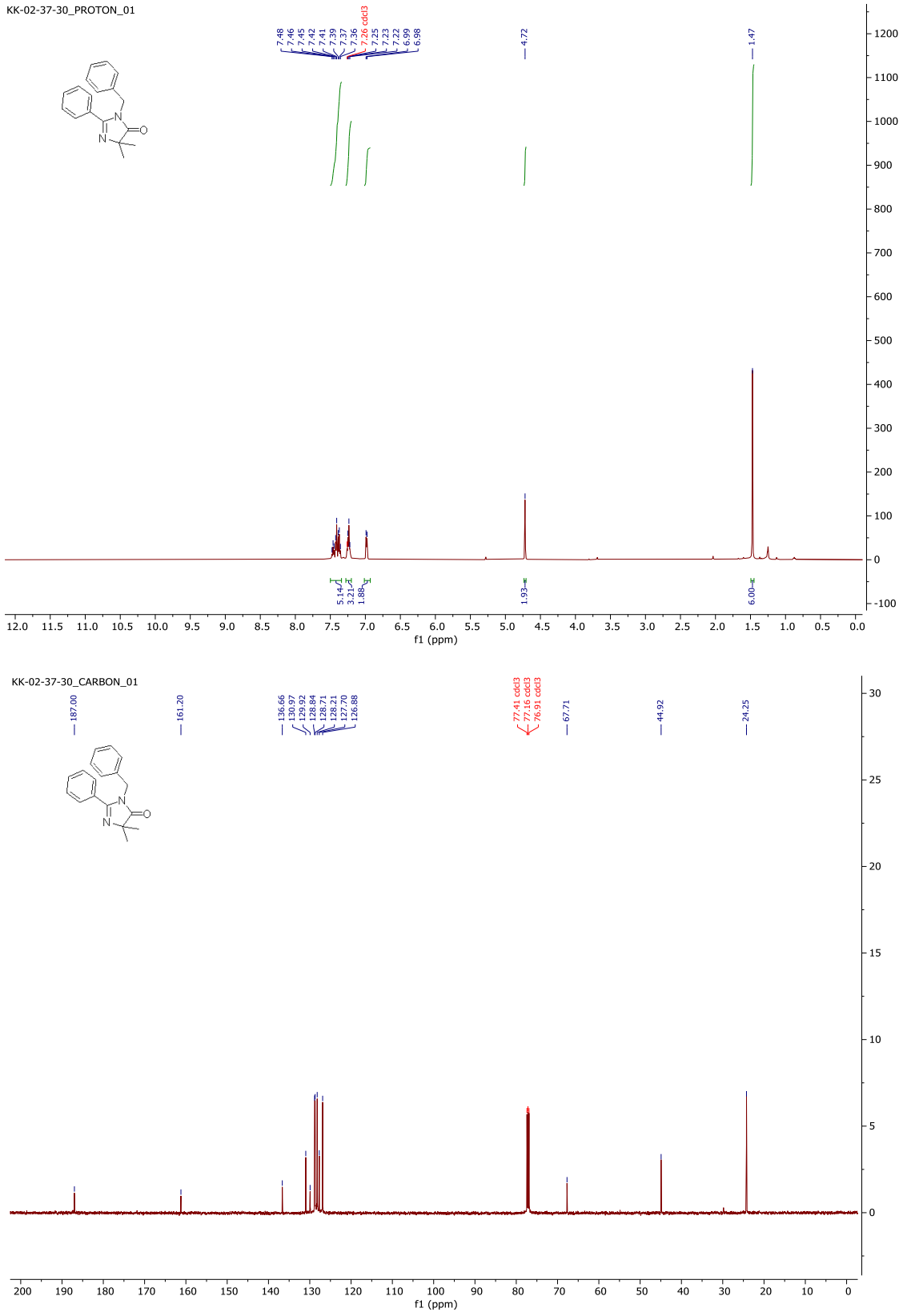


Figure 2.29 ^{1}H and ^{13}C NMR spectra of compound **2-26**

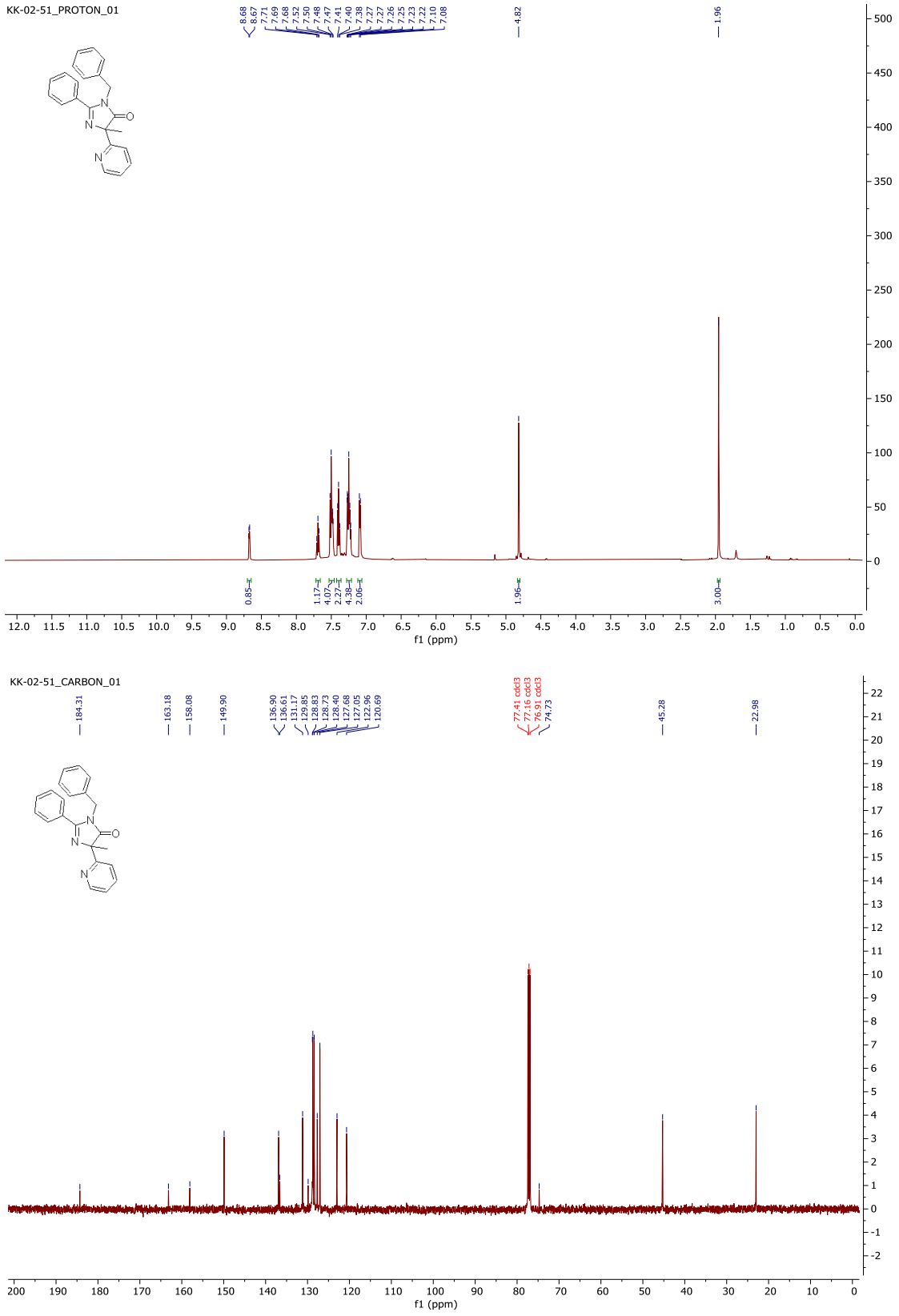


Figure 2.30 ^{1}H and ^{13}C NMR spectra of compound **2-27**

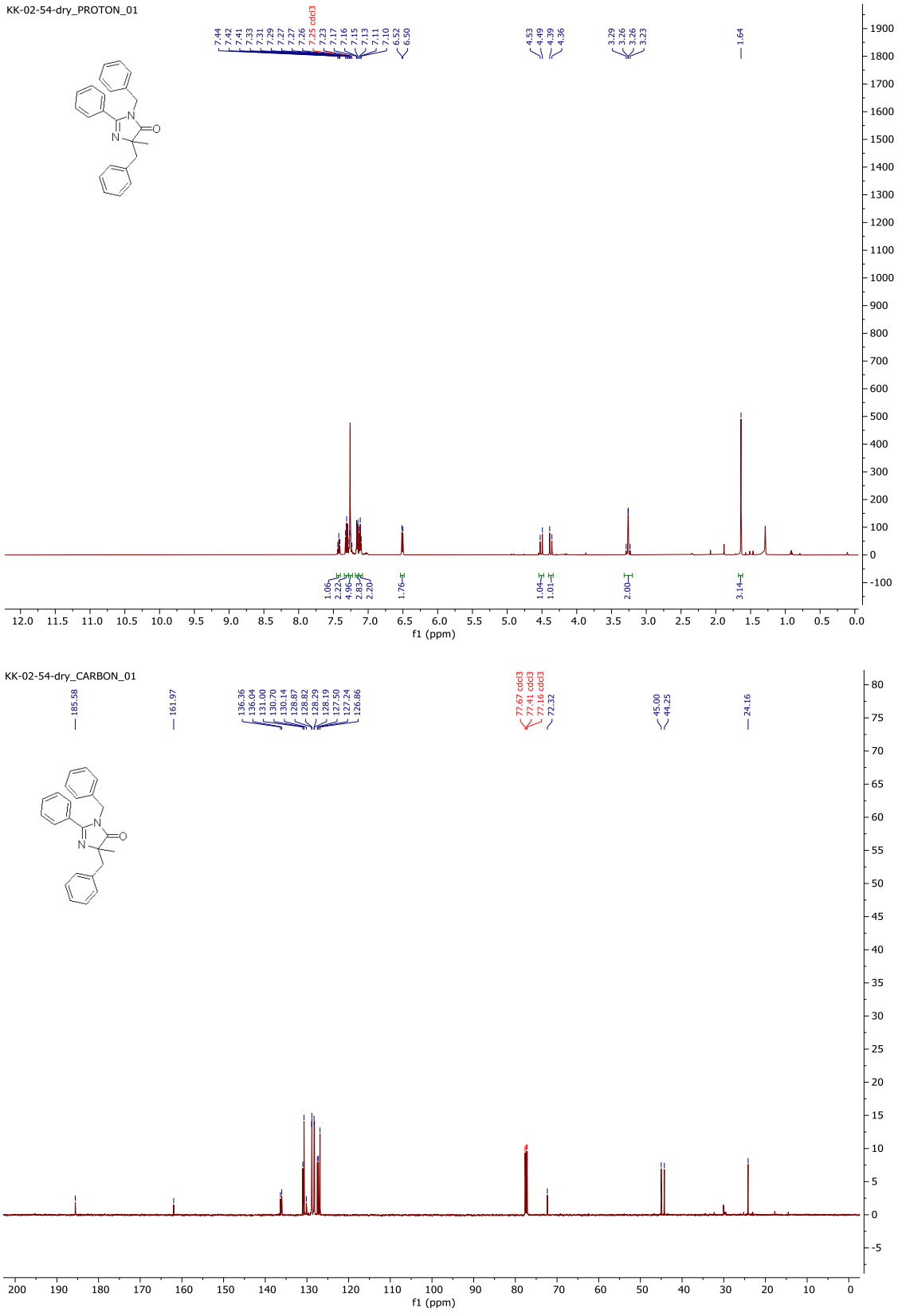
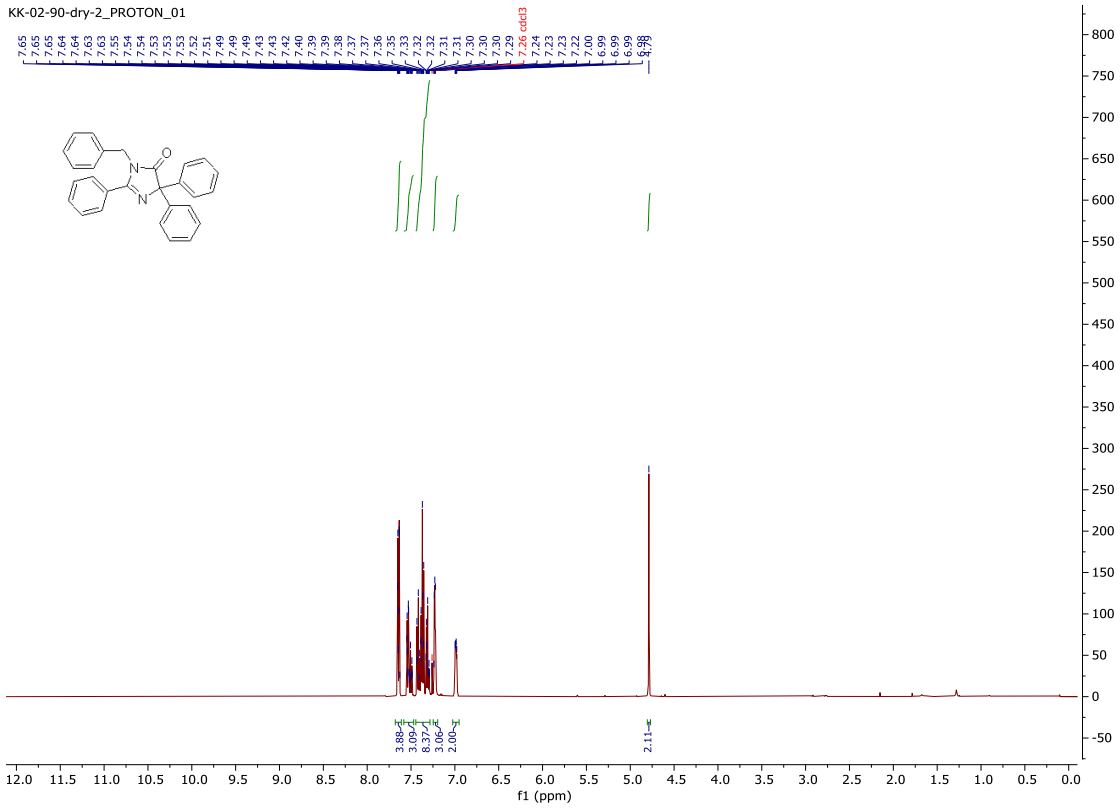


Figure 2.31 ^{1}H and ^{13}C NMR spectra of compound **2-28**



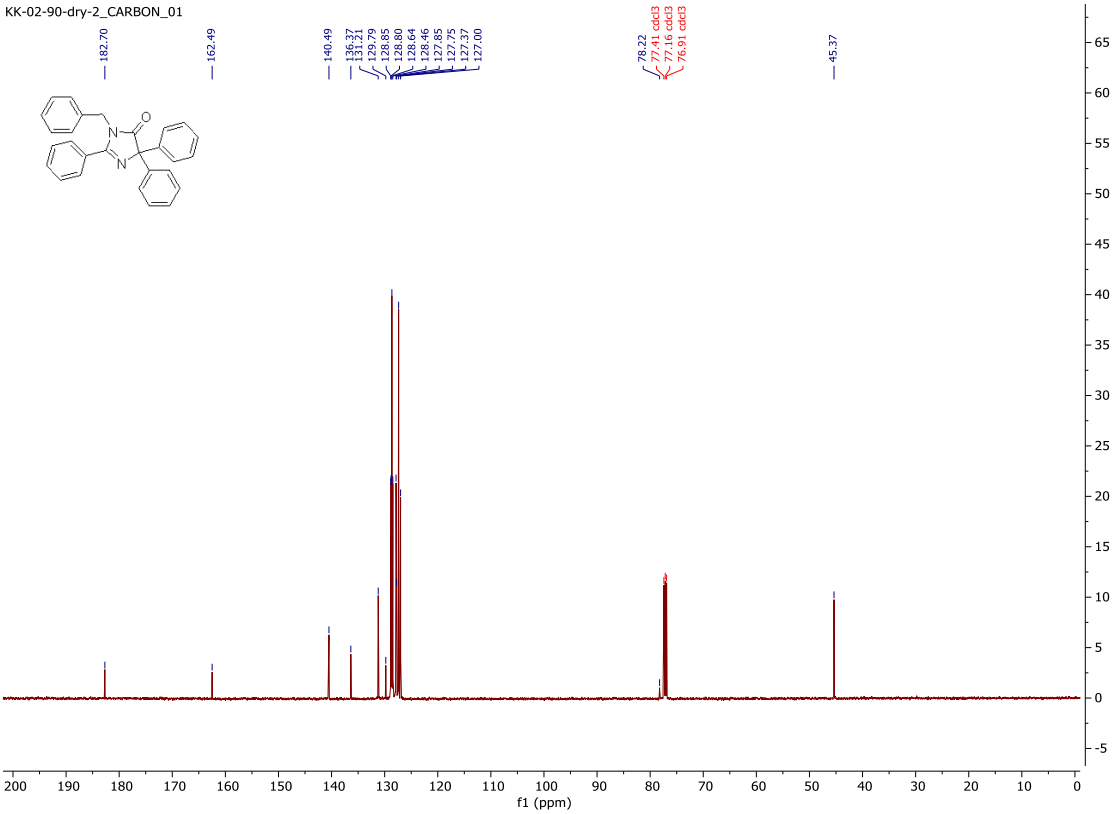


Figure 2.32 ^{1}H and ^{13}C NMR spectra of compound **2-29**

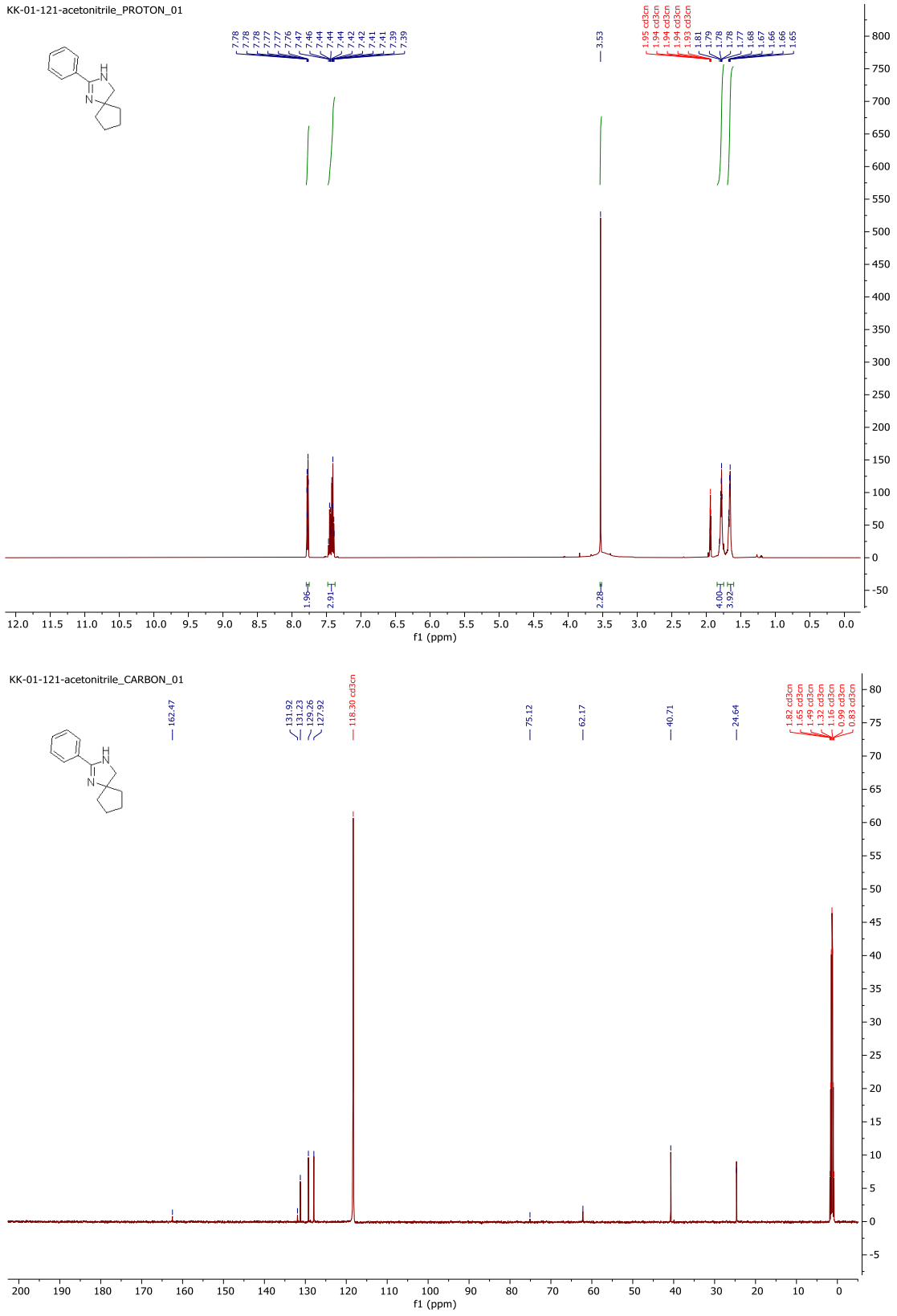
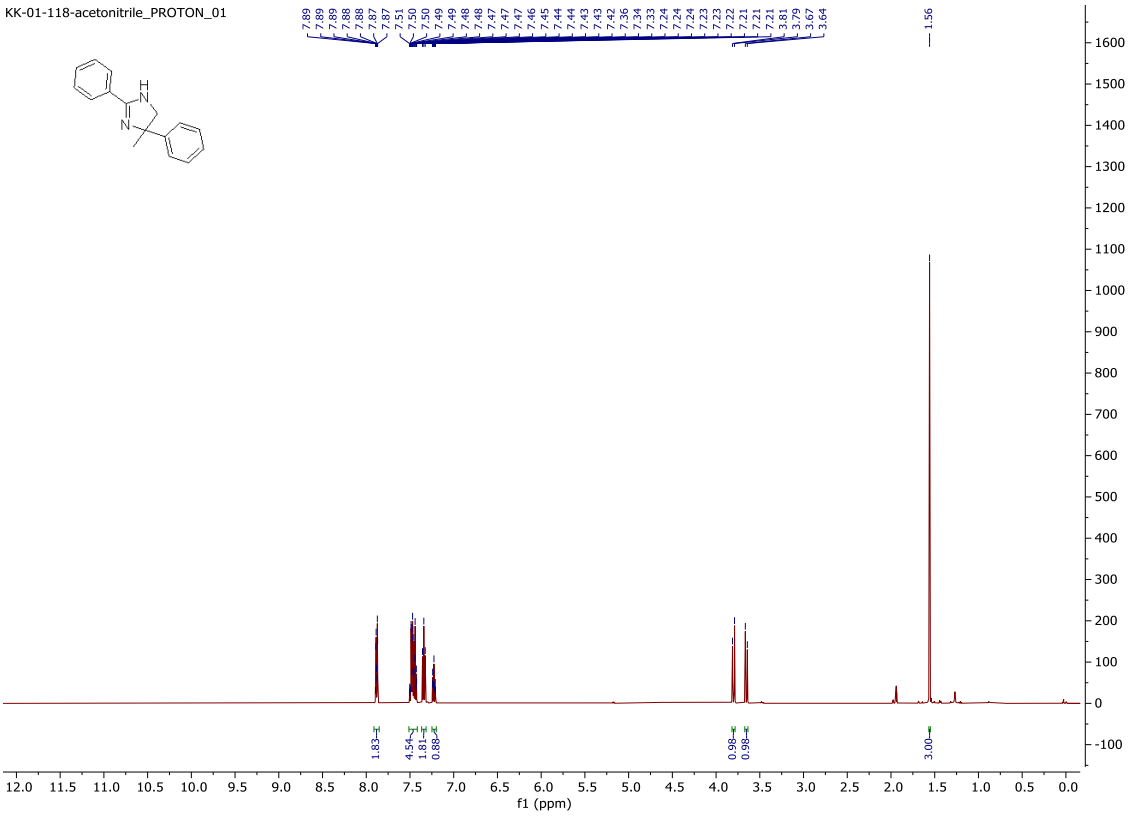


Figure 2.33 ^{1}H and ^{13}C NMR spectra of compound **2-30**



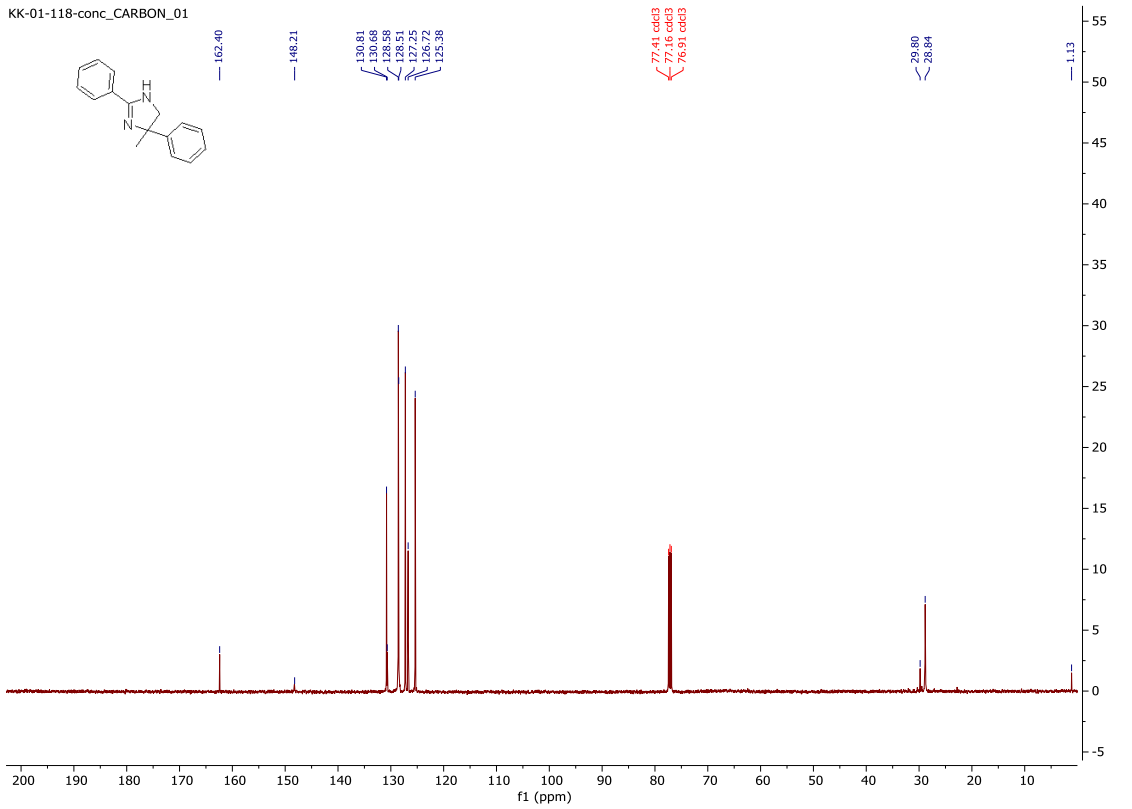


Figure 2.34 ^{1}H and ^{13}C NMR spectra of compound **2-31**

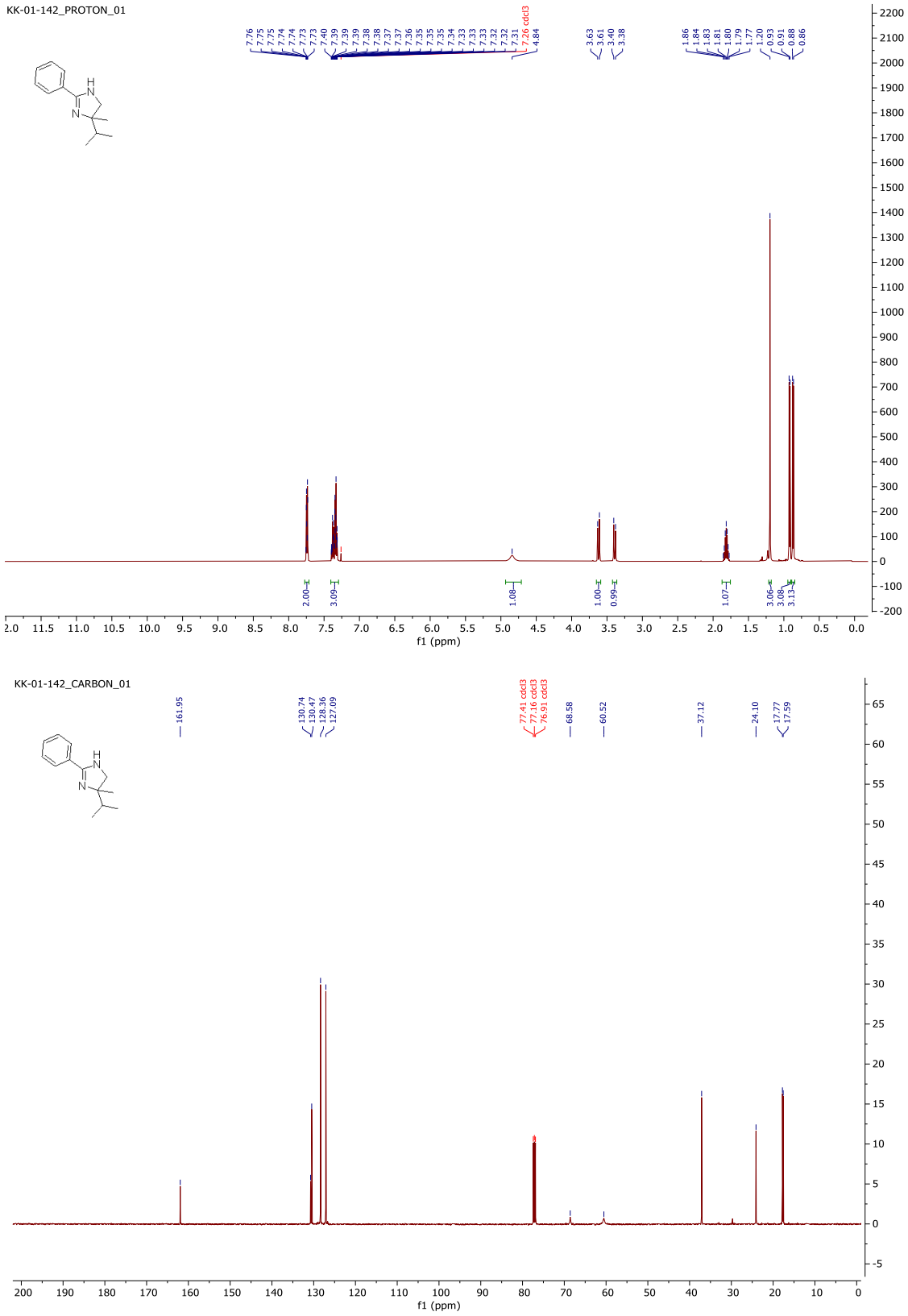
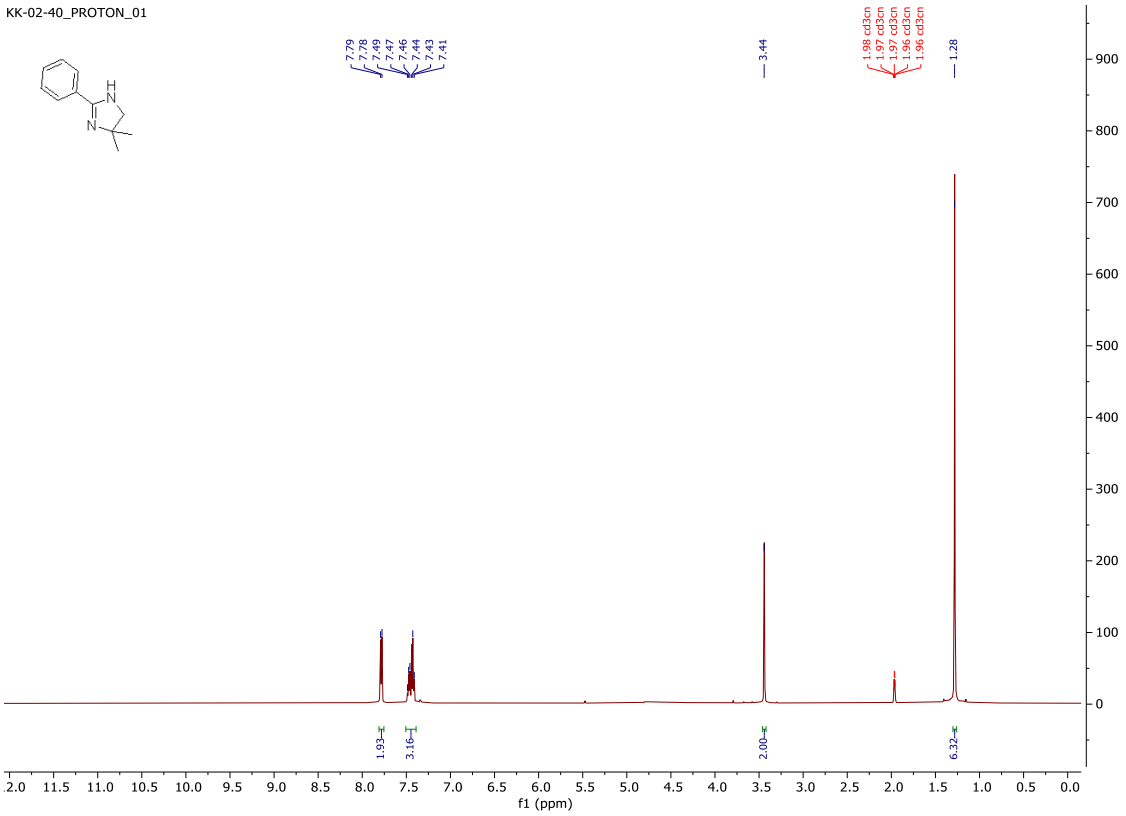


Figure 2.35 ^{1}H and ^{13}C NMR spectra of compound **2-32**



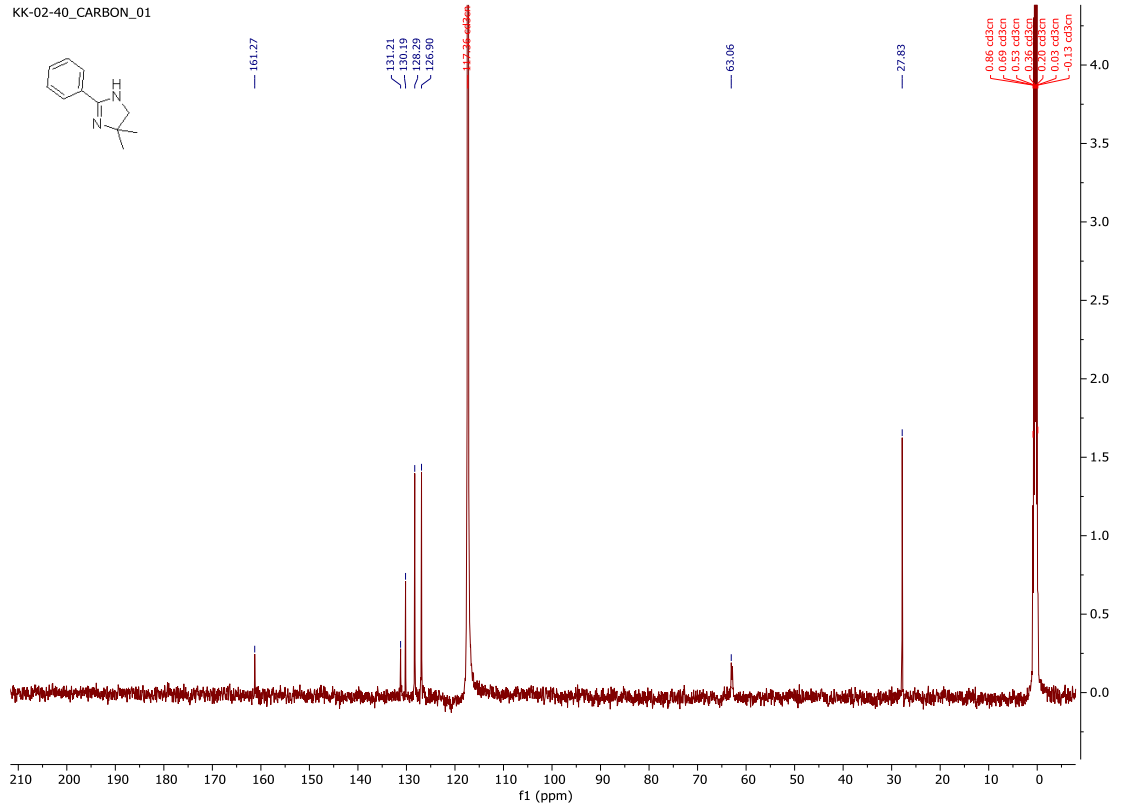


Figure 2.36 ^{1}H and ^{13}C NMR spectra of compound **2-33**

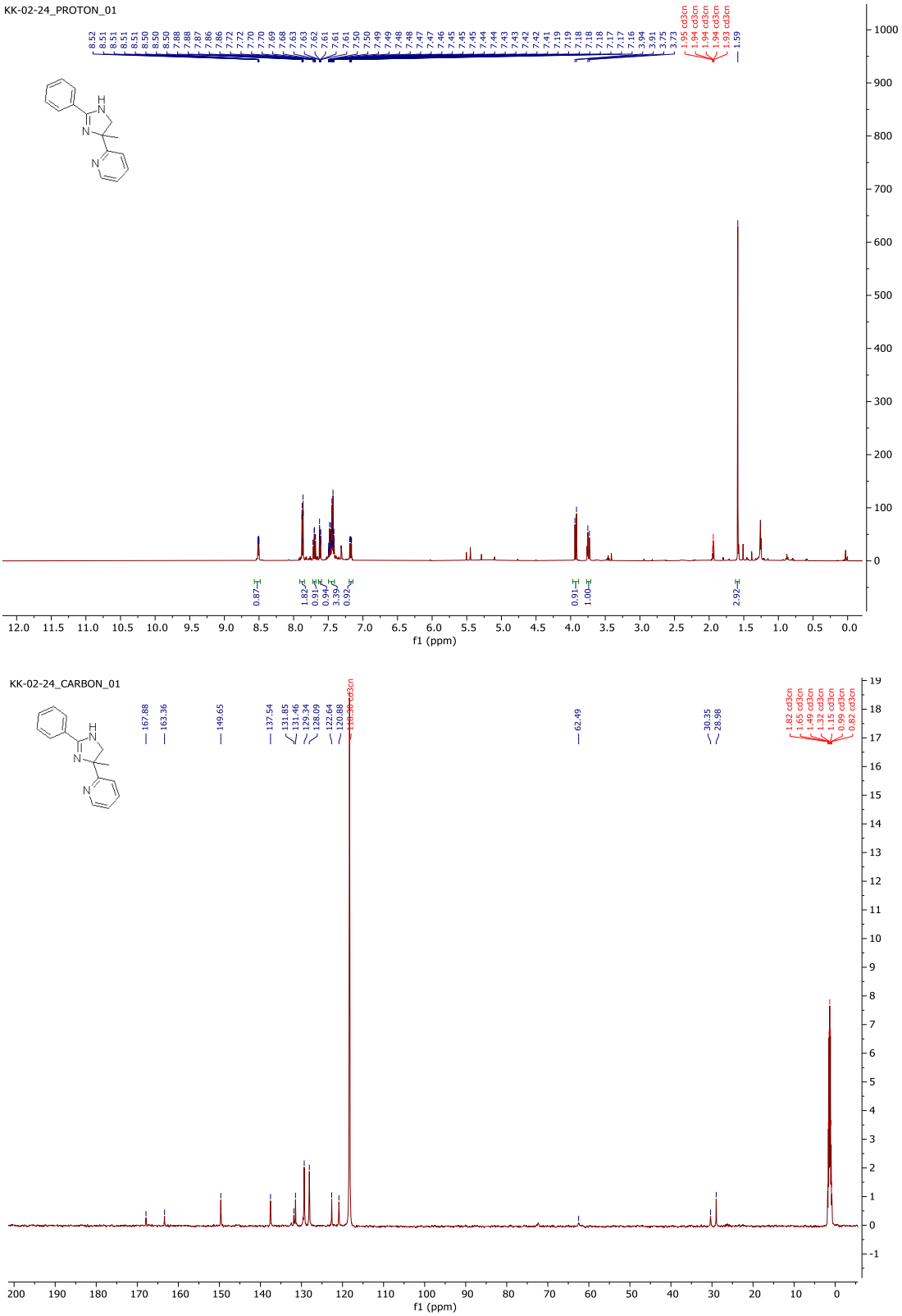
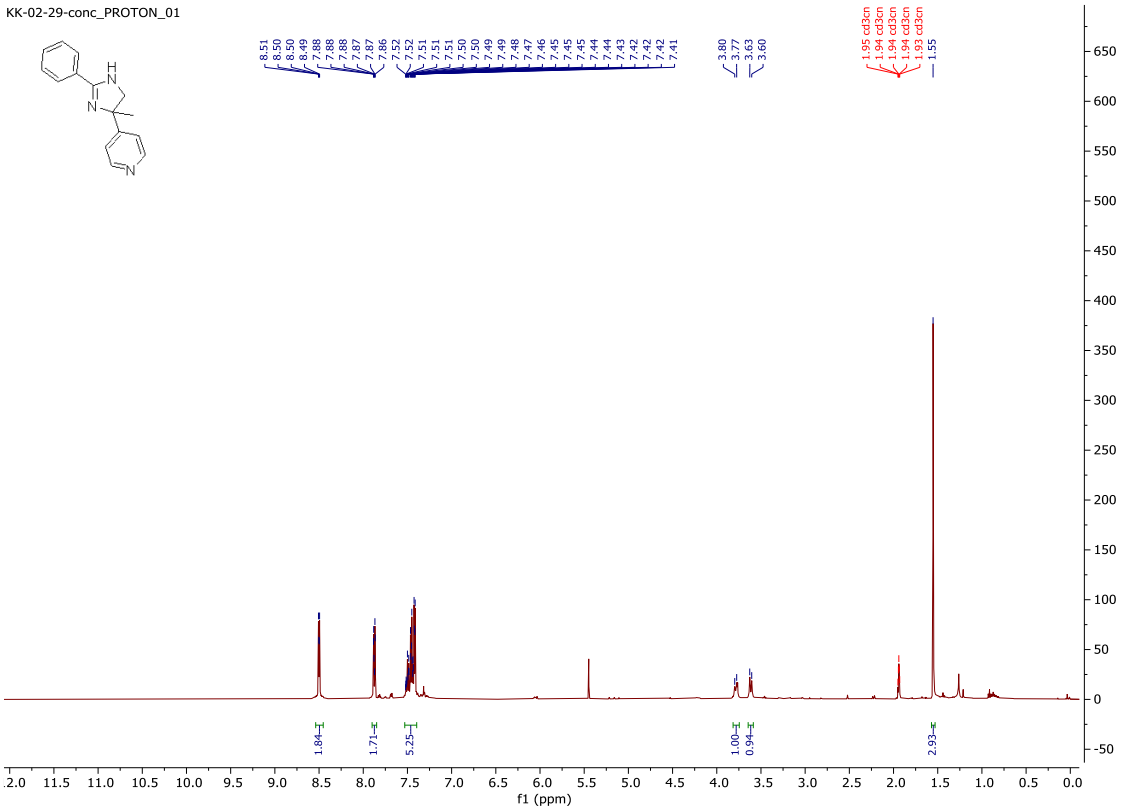


Figure 2.37 ^{1}H and ^{13}C NMR spectra of compound **2-34**



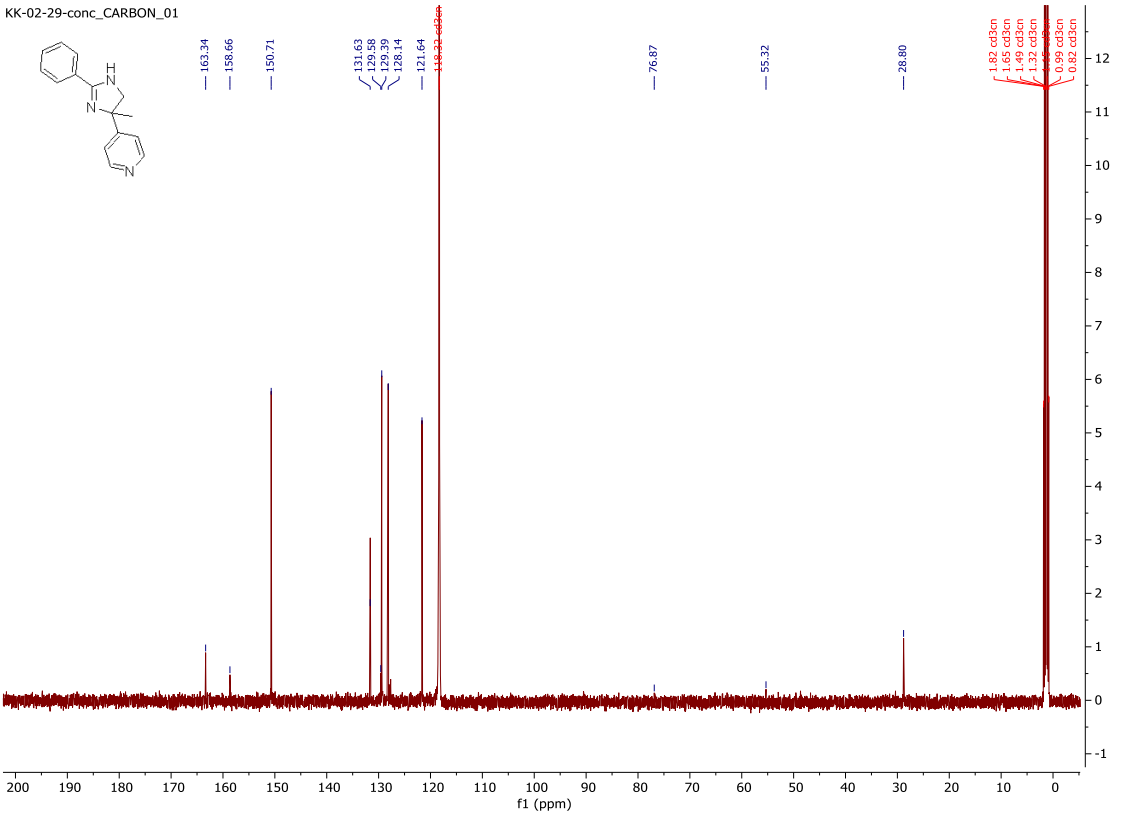
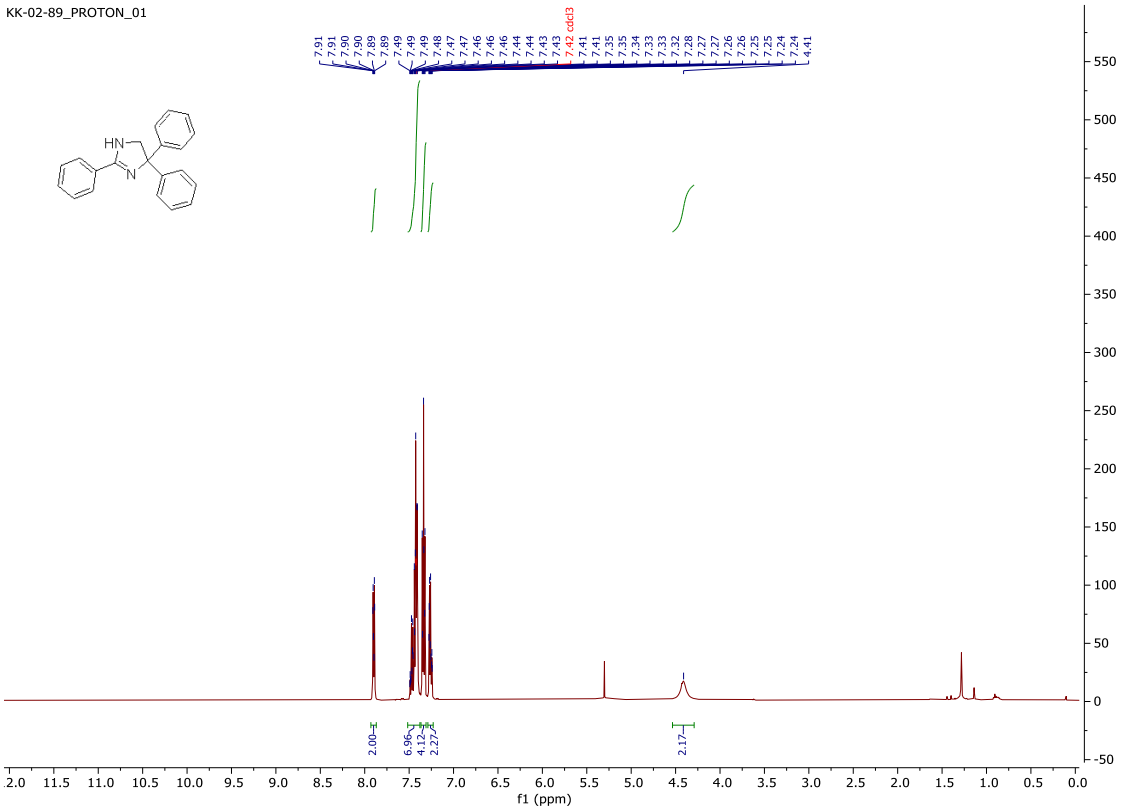


Figure 2.38 ^{1}H and ^{13}C NMR spectra of compound **2-35**



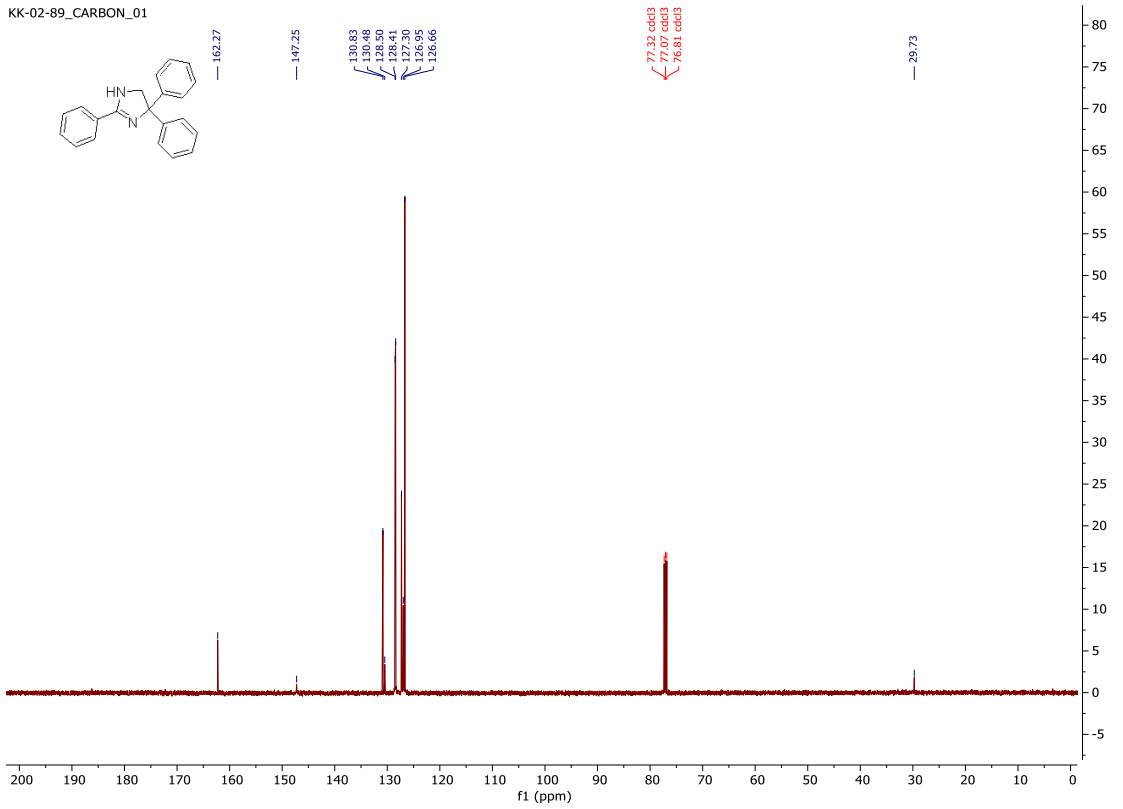
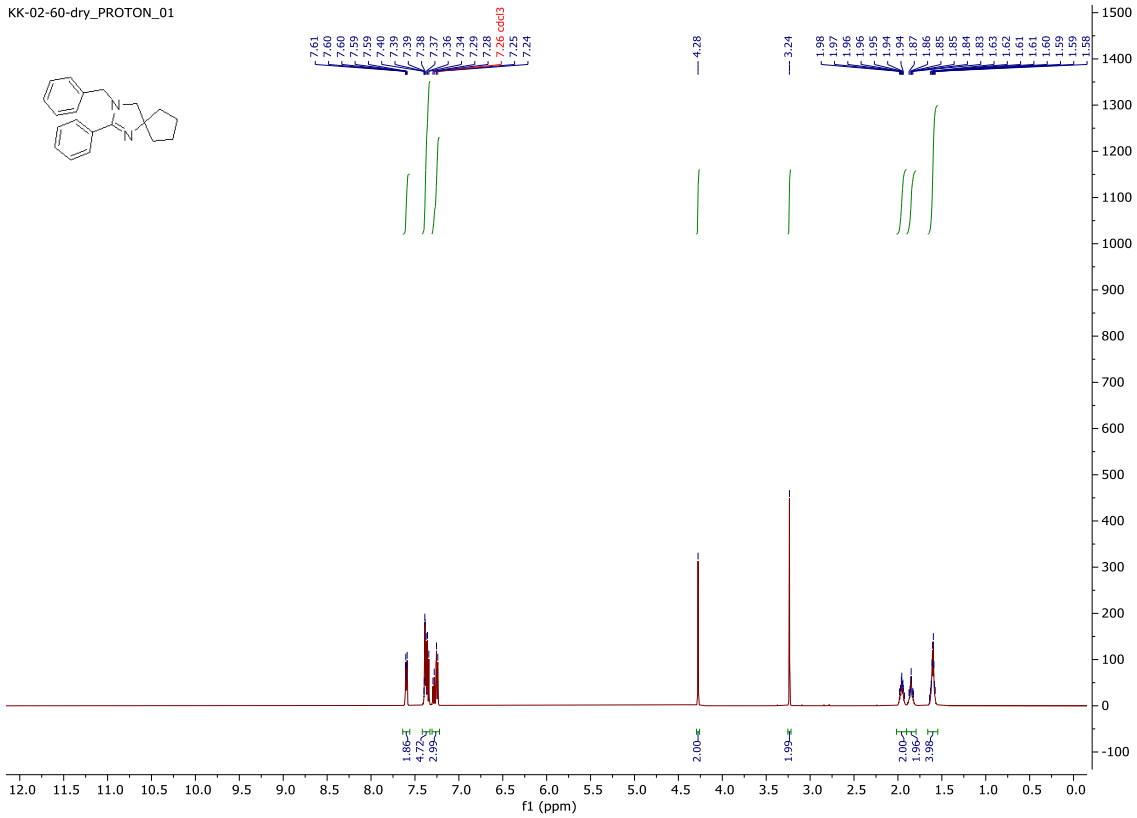


Figure 2.39 ^{1}H and ^{13}C NMR spectra of compound **2-36**



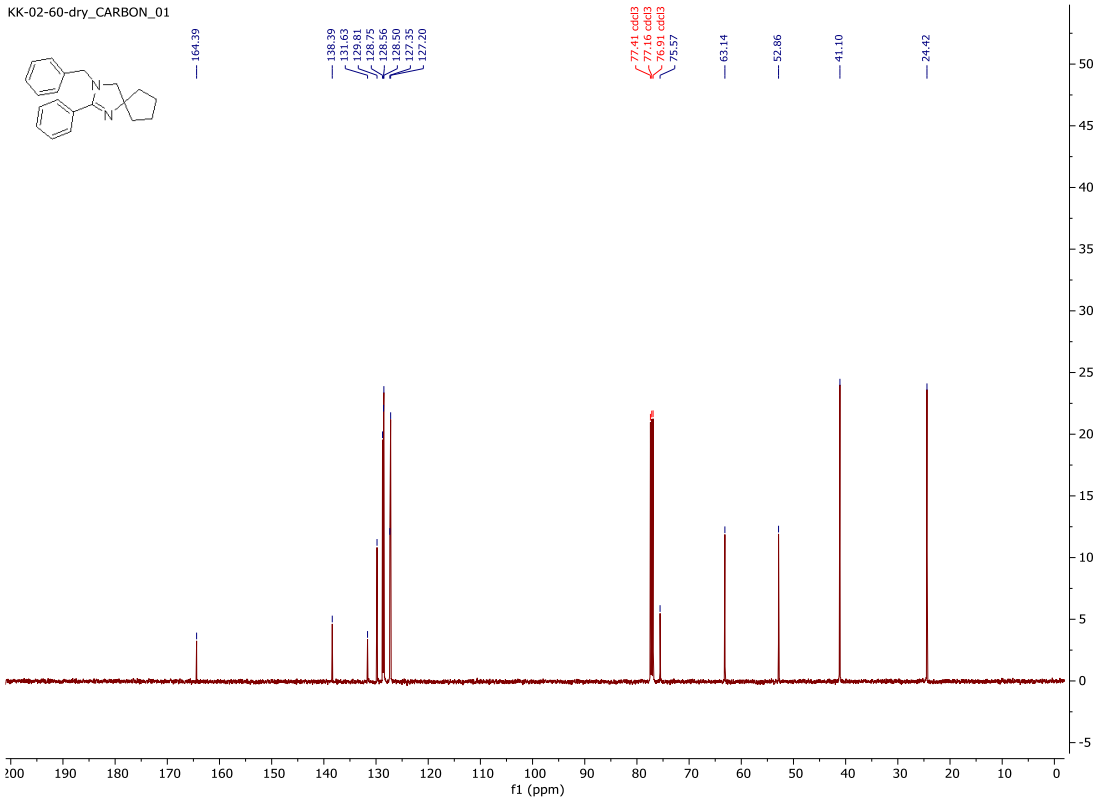


Figure 2.40 ^{1}H and ^{13}C NMR spectra of compound **2-37**

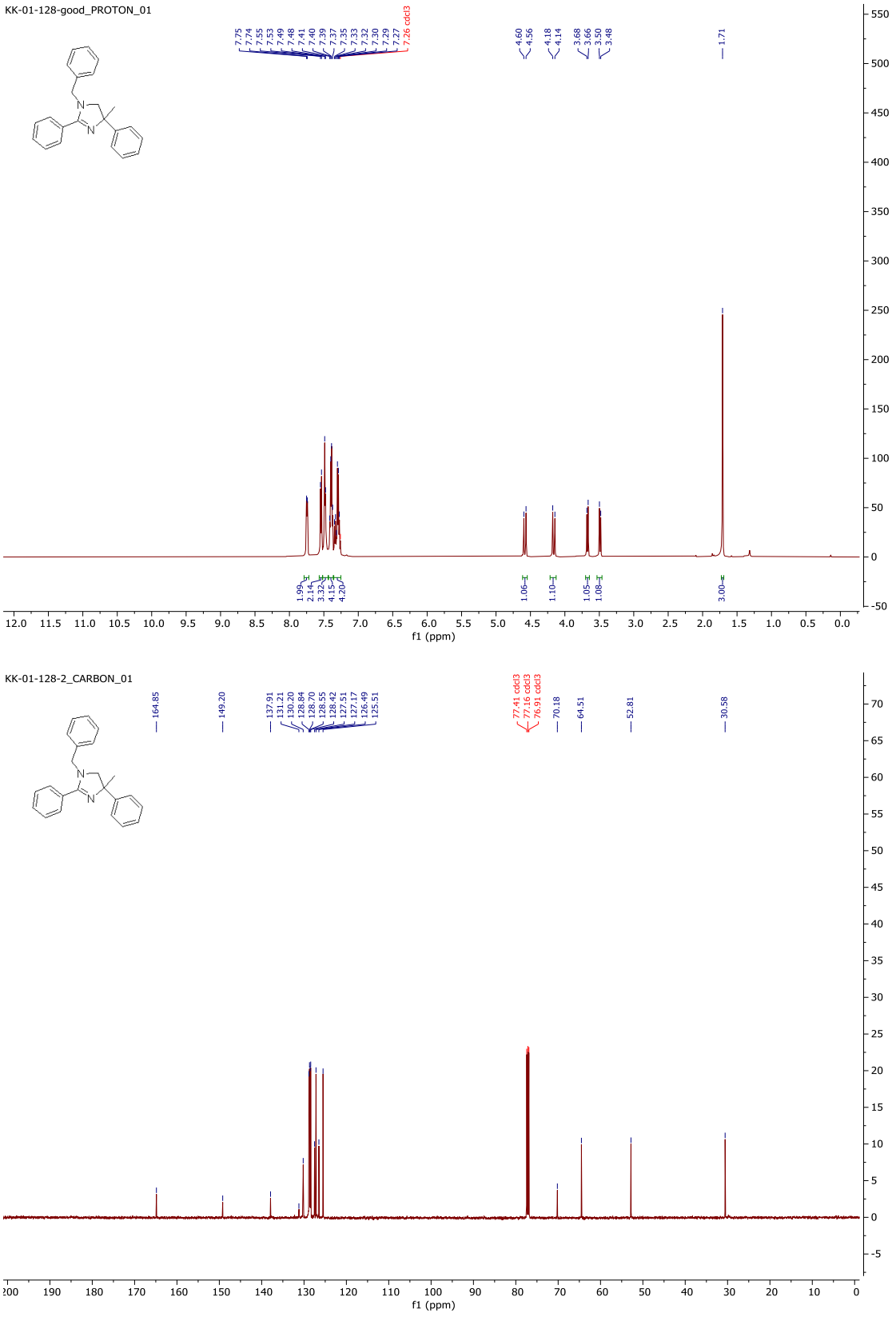


Figure 2.41 ^{1}H and ^{13}C NMR spectra of compound **2-38**

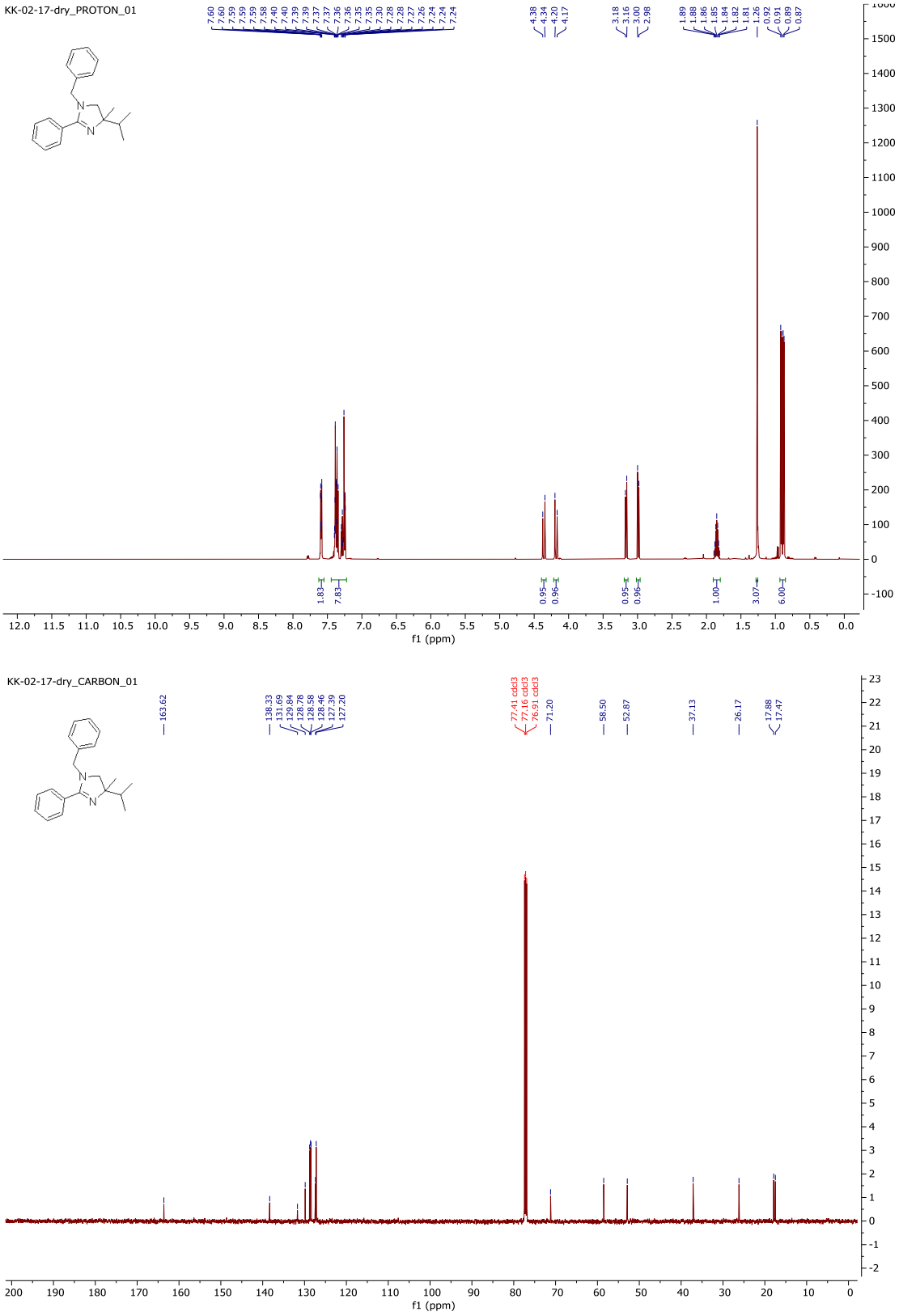
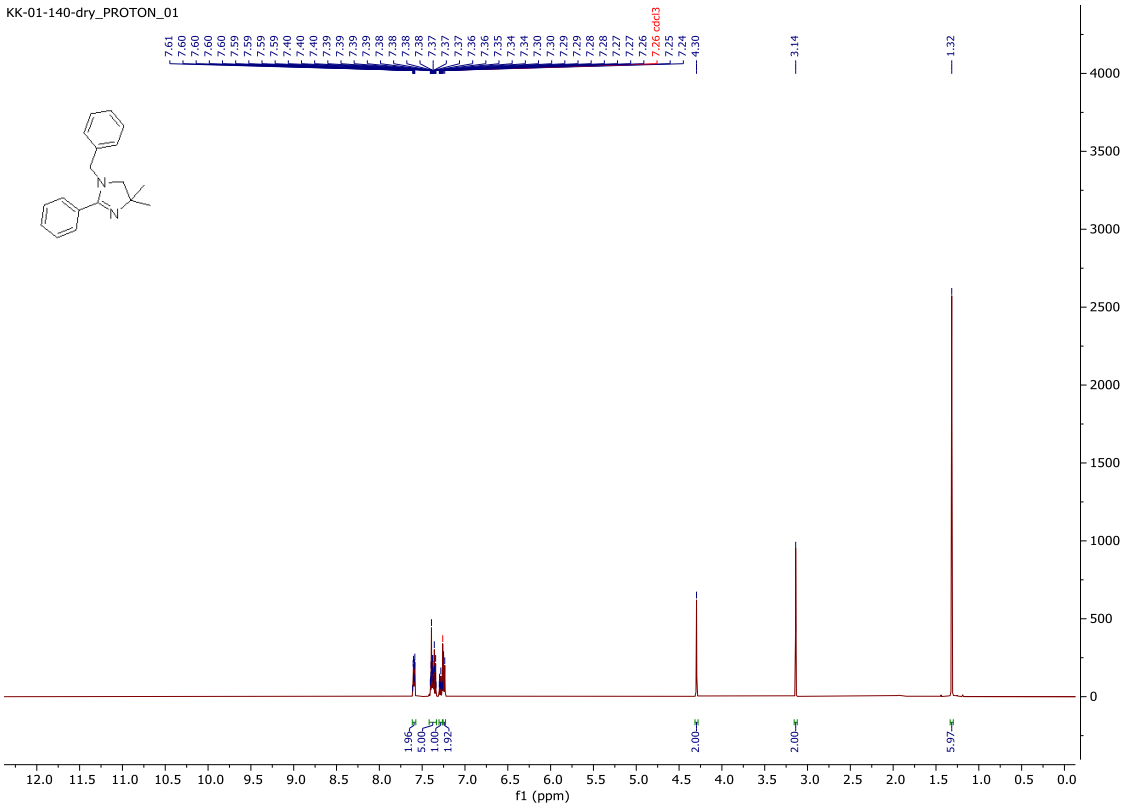


Figure 2.42 ^{1}H and ^{13}C NMR spectra of compound **2-39**



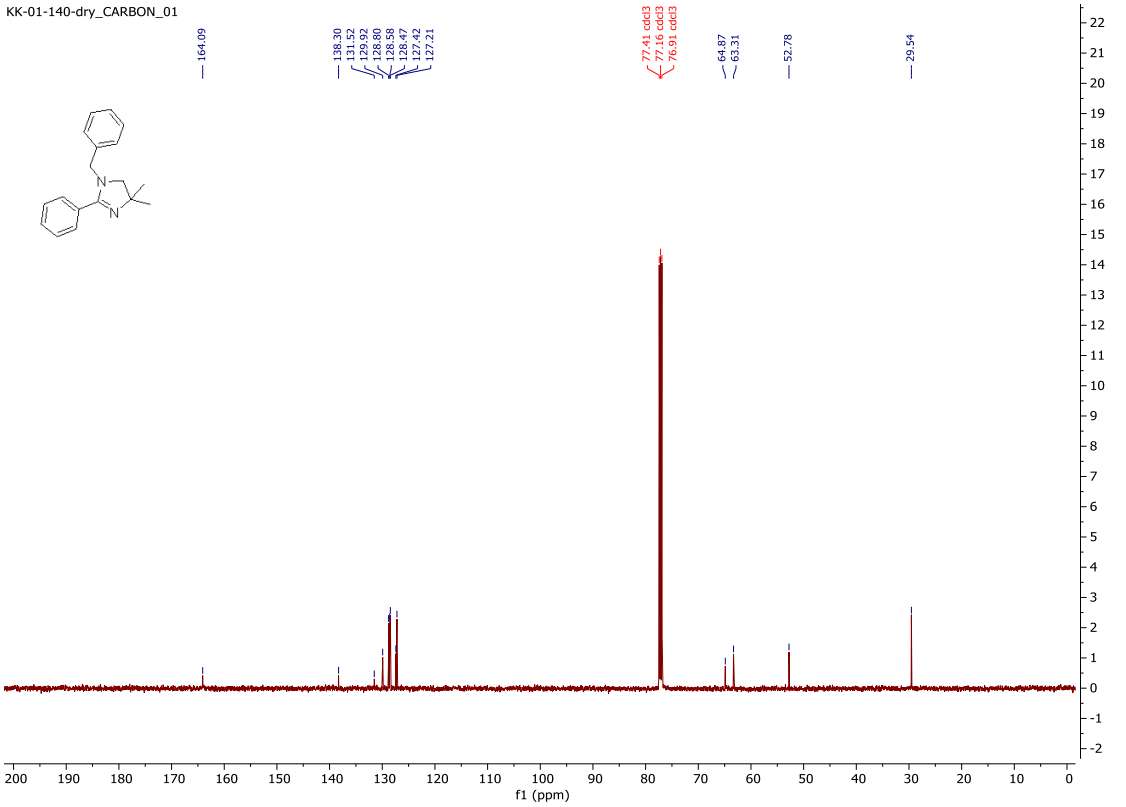


Figure 2.43 ^{1}H and ^{13}C NMR spectra of compound **2-40**

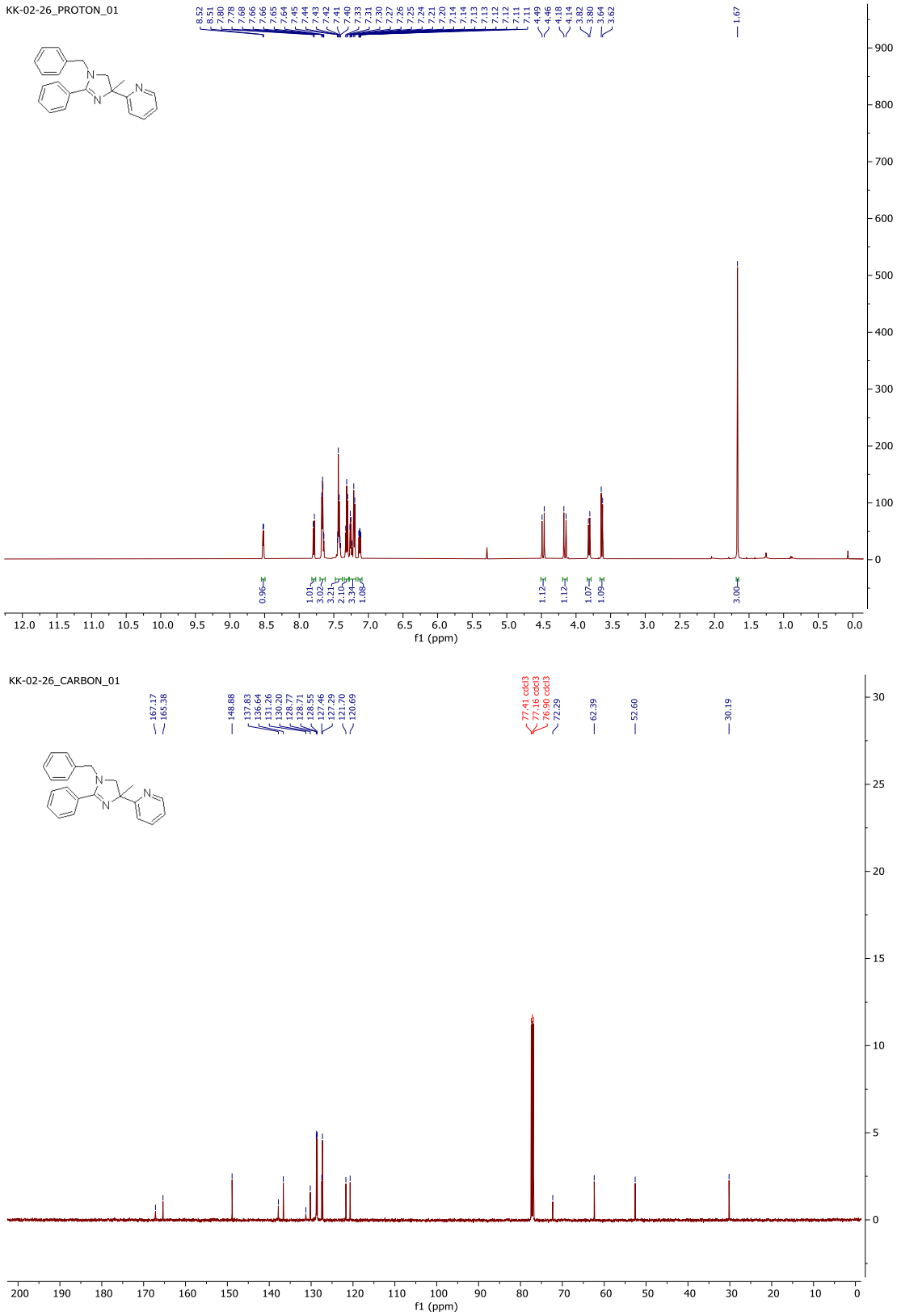
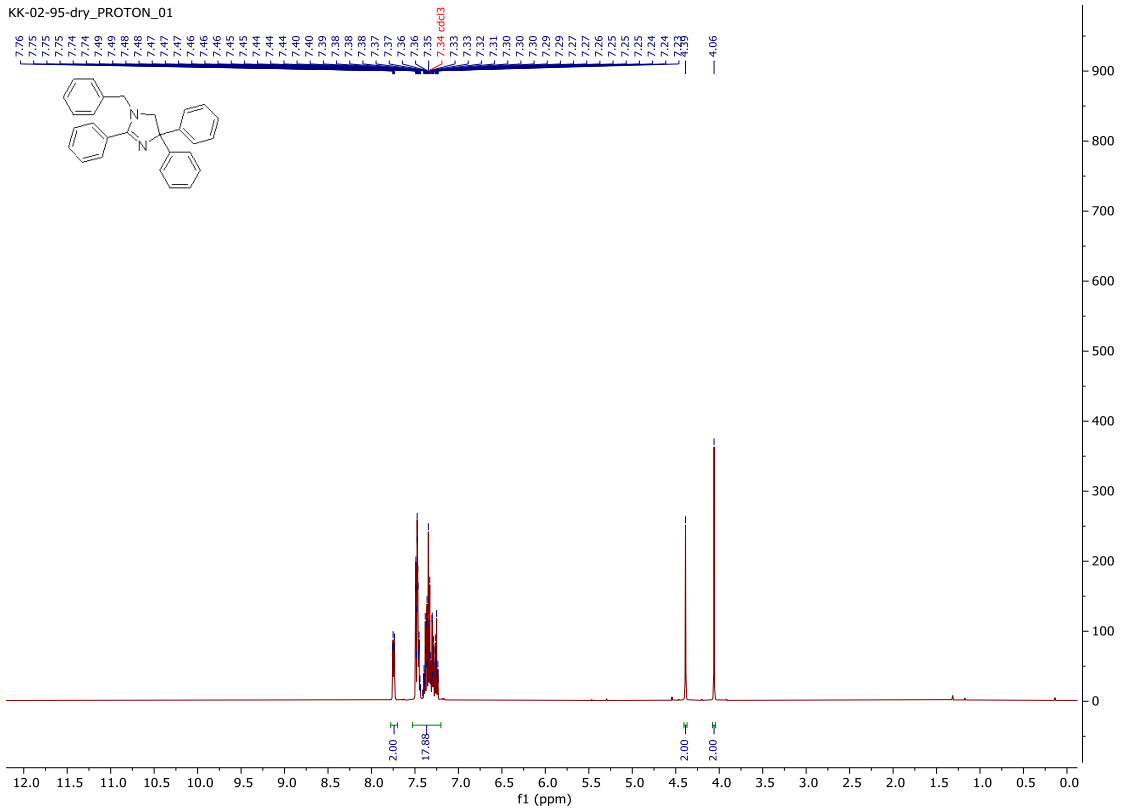
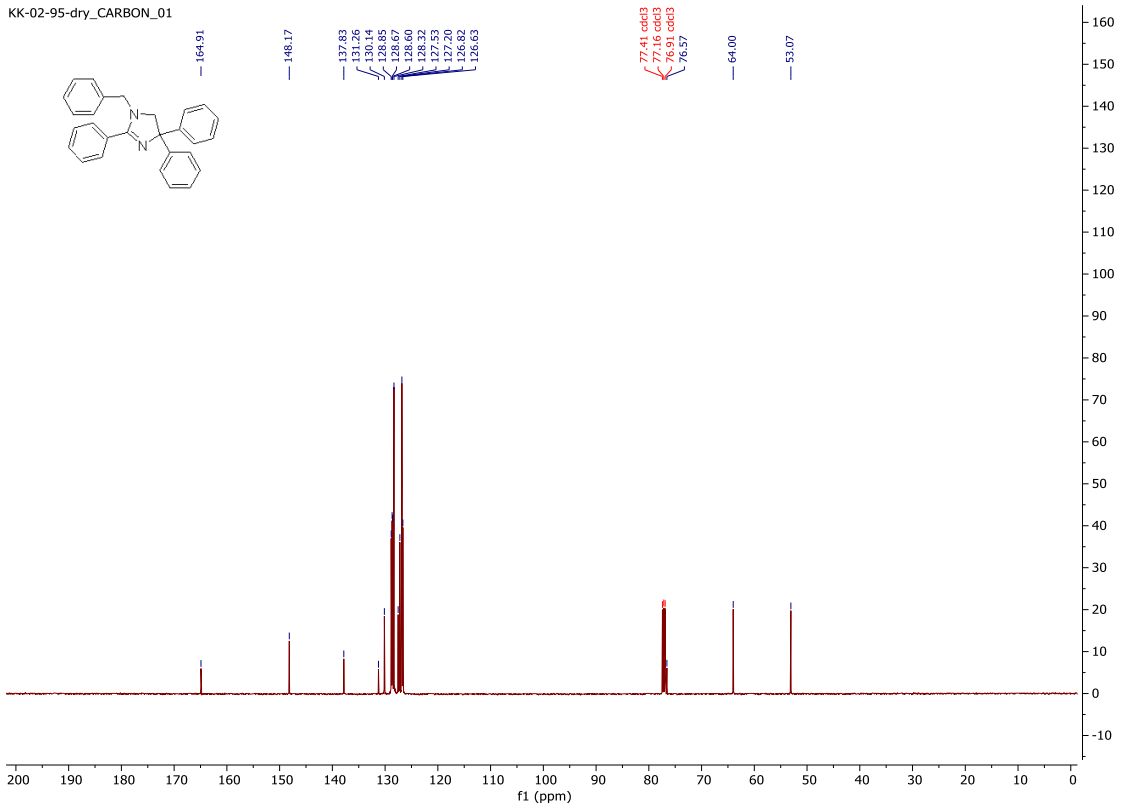


Figure 2.44 ^{1}H and ^{13}C NMR spectra of compound **2-41**





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3 Chapter 3: (4*H*) Imidazol-4-one containing natural products and their known total syntheses*

3.1 Introduction

There are many isolated (4*H*)-imidazol-4-one containing natural products known to date. To centralize this information, I wrote a review article, published in 2020, titled "The preparation of (4*H*)-imidazol-4-ones and their application in the total synthesis of natural products".[1] These natural products can be separated into three main categories: indole alkaloids, pyrrole alkaloids, and other 2-aminoimidazol-4-one alkaloids. Chapter 3 of this dissertation is an excerpt from the abovementioned review article and will discuss most known (4*H*)-imidazol-4-one containing natural products, their biological activities, and efforts towards their total syntheses.

3.2 Indole alkaloids

3.2.1 Aplysinopsin

The first aplysinopsin derivatives were isolated by Kazlauskas, Rymantas, and co-workers in 1977 from the dictoyocceratid sponge *Aplysinopsis*.[2] This family of natural products is derived from tryptophan and has been isolated from a number of different sources, including sponges and scleractinian corals.[3] There are over 30 known variations of aplysinopsin, of which the imidazolone containing derivatives are displayed in **Figure 3.1**.[4–8] There are a number of hydantoin-containing aplysinopsin analogues as well, but for the purpose of this review we will only be discussing the (4*H*)-imidazolon-4-one containing scaffolds. The natural derivatives of aplysinopsin differ by variations in the

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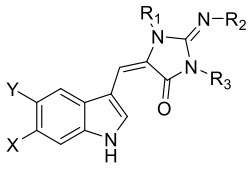
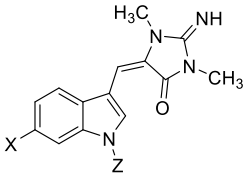
structure of the imidazolone ring (oxidation state, position and number of N-methylations), bromination pattern of the indole, presence and absence of a double bond on the 5 position of the imidazol-4-one ring, dimerization, and the stereochemistry.

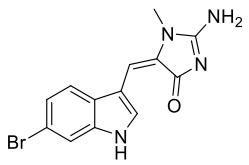
Figure 3.1 (4*H*)-imidazol-4-one containing derivatives of aplysinopsin



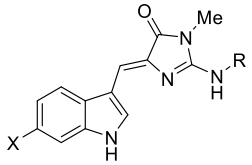
aplysinopsin derivatives
E-isomer dominant,
11 examples
X = H, Br
Y = H, Br
R_1 = CH_3, H
R_2 = H, CH_3, CH_2CH_3
R_3 = H, CH_3



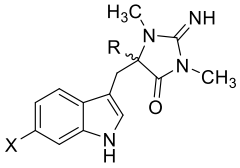
dimethylated aplysinopsins
E-isomer dominant,
3 examples
X = H, Br
Z = CH_3, $OCCH_2CH_3$



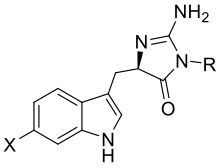
(*E*)-6-bromo-12-*nor*-aplysinopsin



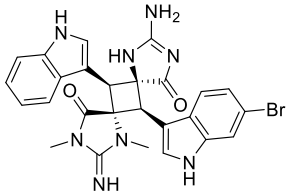
aplysinopsin derivatives
Z-isomer dominant
4 examples
X= H, Br
R= H, Me



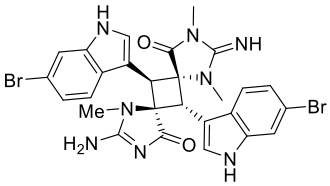
dihydroaplysinopsin derivatives
Racemic mixtures isolated
5 examples
X = H, Br
R = H, OH, OCH_3, OCH_2CH_3



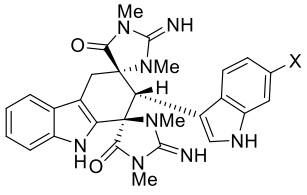
(*R*)-dihydroaplysinopsin
X = H, Br
R = H, CH_3



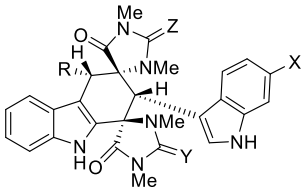
dictazole A



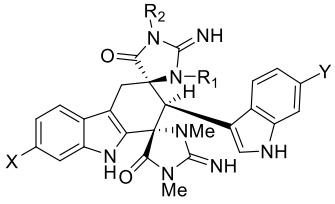
dictazole B



tubastrindole A-B
2 examples, X= H,Br



tubastrindole C-H
6 examples,
X= H,Br
Y= NH, O
Z= NH, O
R= H, OH



dictazoline A-E
5 examples
X = H, Br
Y = H, Br
R_1 = H, CH_3
R_2 = H, CH_3

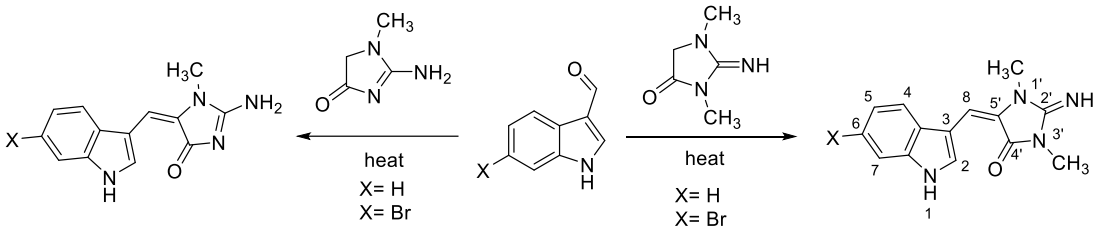
Aplysinopsins are known for their range of biological activities, including anticancer, antimicrobial, and antiplasmodial activities.[4] However, the most significant biological activity is modulation of neurotransmission through regulation of monoaminooxidase (MAO), serotonin receptors, and nitric oxide synthase (NOS)

activities.[9] Methylaplysinopsin is a potent reversible MAO inhibitor, found to decrease MAO concentrations below 1ng/mL over 4-8 hours in vivo.[10] 6-bromoaplysinopsin was found to have an affinity for human serotonin 5-HT2 receptors with a similar Ki as serotonin.[4] 5,6-dibromo-2'-demethylaplysinopsin (*Z*) showed 100% inhibition of nNOS at 125 μg/mL and 32% inhibition of iNOS.[9] Most recently, tubastrindole B was found to be a glycine-gated chloride channel receptor α1 selective GlyR antagonist (IC_{50}=25.9 μM with a IC_{50} > 300 μM for α3).[11] Aplysinopsin was not found to have any inhibitory activity in comparison. GlyRs play a pivotal role in running inhibitory neurotransmission in the spinal cord, brain stem and retina. This could be beneficial in the treatment of inflammatory pain, opioid-induced breathing disorders, epilepsy, and movement disorders.[11]

Two of the original total syntheses for (*E*)-aplysinopsin and its 6-bromo derivative were reported by Guella et. al (1988) and Fattorusso et. al (1985).[12,13] Their syntheses are summarized in **Scheme 3.1** and include a method to produce exo and endo products. The key step is a Knoevenagel condensation of methyl creatinine with indol-3-carboxaldehyde and its 6-bromo derivative. Additionally, Guella et. al. studied the photoisomerization of the (*E*) isomer and found they were able to increase the amount of (*Z*) isomer to establish H-C, C=O heteronuclear coupling constants for both.[13] Knowing that the (*E*) isomer has an appreciably larger coupling constant (10.5 Hz) than (*Z*) (5.1 Hz) has allowed researchers to quickly determine whether they synthesized the (*E*) or (*Z*) isomer in future studies. This synthesis has since been used to make a variety of analogues,[6,14] including 5,6-dibromo-2'-demethylaplysinopsin[15] and several series of *N*-alkylated aplysinopsin derivatives used for biological testing.[16–18] The conditions for this reaction vary but

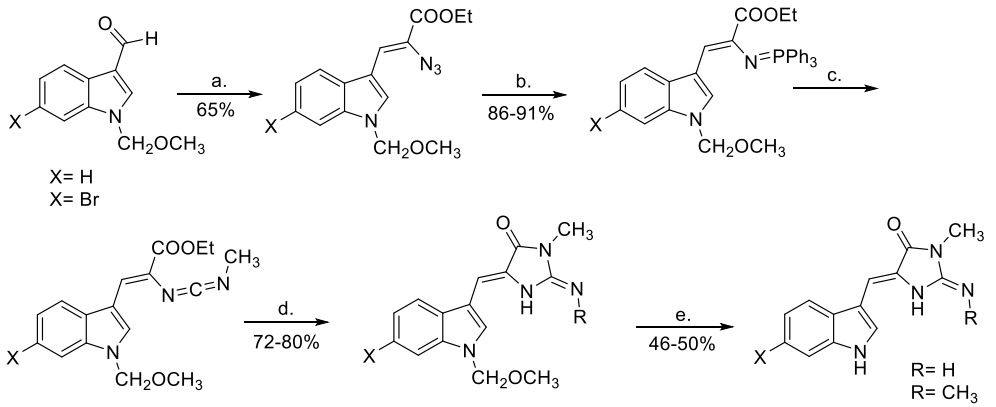
typically require heat and addition of a base, such as piperidine. A microwave-assisted method has been reported as well, where the reaction was microwaved for 30-60 seconds with creatinine and NaOAc.[18]

Scheme 3.1 First reported total synthesis of (*E*)-aplysinopsin derivatives



Since this first synthesis of aplysinopsin was published, many other groups have reported variations of the total synthesis of aplysinopsin and its derivatives.[11,14,19,20] A particularly interesting synthesis was reported by Molina et. al.[21,22] The authors reported synthesizing several aplysinopsin derivatives through a tandem aza-Wittig/heterocumulene-mediated annulation (**Scheme 3.2**). Using this method, four different analogues of aplysinopsin were synthesized in moderate yields, all containing *Z*-isomerization. This paper was one of the first reports of synthesizing (*Z*)-aplysinopsin derivatives.[21]

Scheme 3.2 Iminophosphorane-mediated ring formation to produce (*Z*)-aplysinopsin derivatives

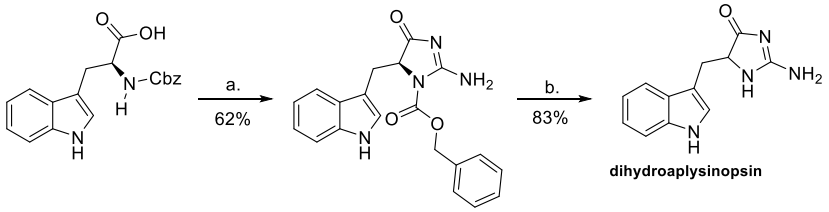


Reagents: a) $N_3CH_2COOC_2H_5$, NaOEt, EtOH, -15 °C, b) PPh_3, CH_2Cl_2, rt, c) CH_3-NCO, toluene, rt, d) NH_2R, toluene, 45 °C, e) HCOOH, reflux

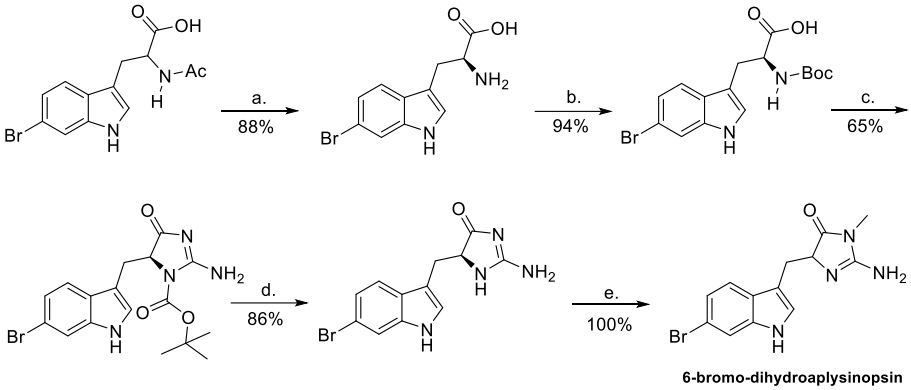
There are other, unique derivatives within the aplysinopsin family that cannot be synthesized via the same means as reported above. Examples include dihydroaplysinopsin and 6-bromo-dihydroaplysinopsin, which were isolated and synthesized in 2015 by Shaker et. al. (**Scheme 3.3**).[8] The formation of (4*H*)-imidazol-4-one involved *L*-tryptophan being treated with N-hydroxysuccinimide and *N*,*N*-dicyclohexylcarbodiimide to form an activated NHS-ester. This was then introduced to aqueous sodium cyanamide, which produced the protected imidazolone in good yields. Because they started with optically pure amino acids (L-tryptophan), Shaker used the configuration of the final synthesized products, which were determined as (*S*) even after partial racemization occurred, to determine the configuration of the natural products as (*R*) based on measured optical rotation.[8]

Another unique scaffold from the aplysinopsin family is dictazole. Dictazole A and B are distinctive aplysinopsins, formed through the dimerization of two aplysinopsin monomers. Several papers report on the synthesis of dictazole A and B through a [2+2] cycloaddition of aplysinopsin monomers. The first report of the formation of dictazole B was in 2014 by Skiredj et. al.[5] The authors were able to isolate 14% of dictazole B through a heterodimerization of an endo and exo monomer of 6-bromoaplysinopsin (**Scheme 3.4**). Additionally, they isolated 19% yield of the anti, head-to-tail homodimer of the exo starting material. The same group has published several other variations to this synthesis in similar yields.[23,24]

Scheme 3.3 Total synthesis of dihydroaplysinopsin and its 6-bromo derivative

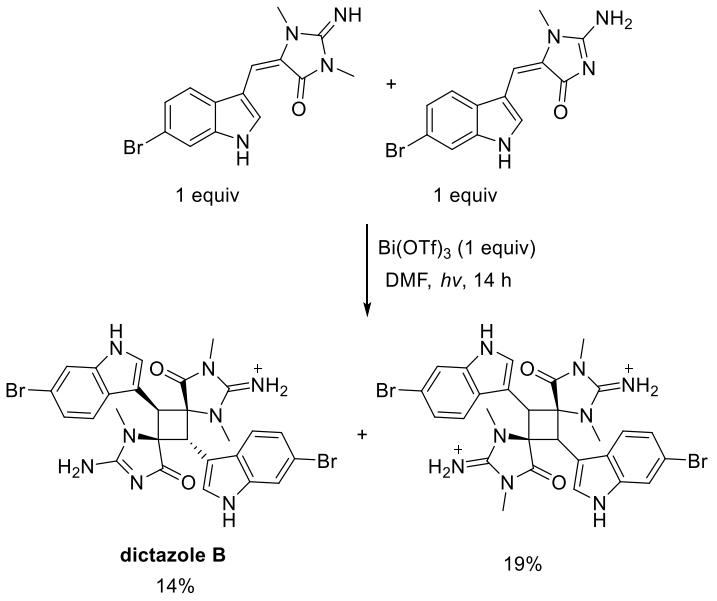


Reagents a) 1. THF, DCC, NHS, 0 °C, 3 h, 2. NaHNCN, H_2O, rt, 16 h, b) MeOH, Pd/C, H_2, rt, 1 h



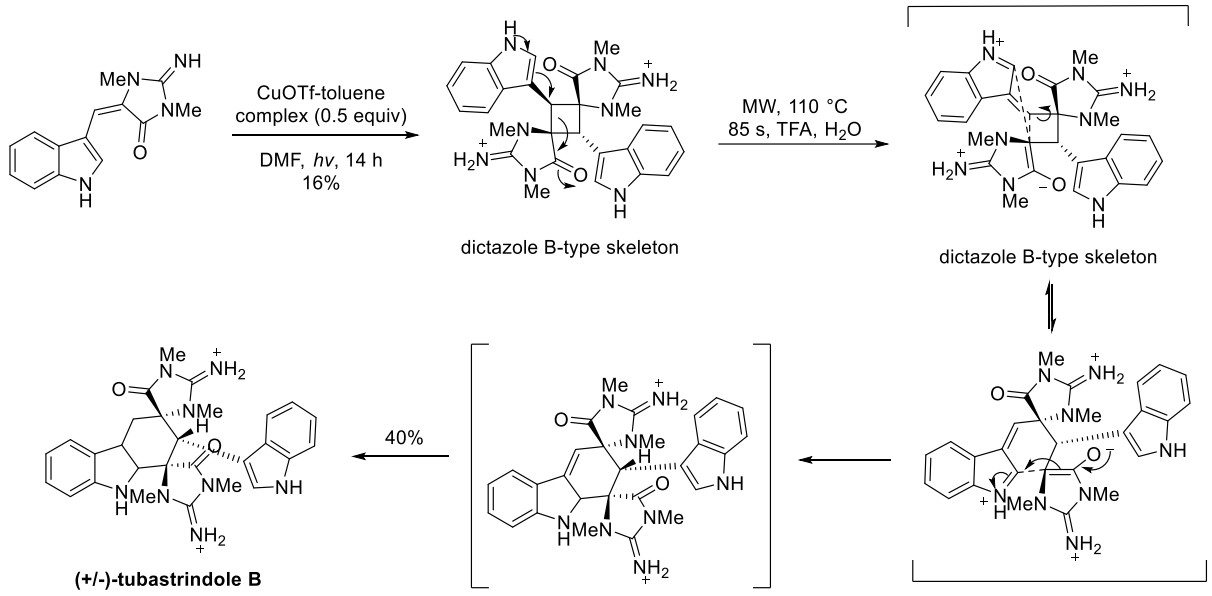
Reagents a) Borate buffer, $CoCl_2$ * 6 H_2O, pH = 8.5, L-aminoacyclase, 38 °C, 48 h, b) 1,4 dioxane, H_2O, KOH, Boc_2O, rt, 16 h, c) 1. THF, DCC, NHS, 2 h 2. NaHNCN, H_2O, rt, 16 h, d) MeCN, $SnCl_4$,0 °C, under Ar, 45 min, e) MeCN, EtOH, MeI, rt, 24 h

Scheme 3.4 The first total synthesis of dictazole B



Skiredj et. al. also worked on the total synthesis of tubastrindole B, the ring expanded product of dictazole B.[25] **Scheme 3.5** illustrates their reported aplysinopsin cascade, where ring expansion of dictazole B occurred upon addition of heat and trifluoroacetic acid. This mimics the proposed biomimetic formation of tubastrindole A-H and dictazoline A-E.[25]

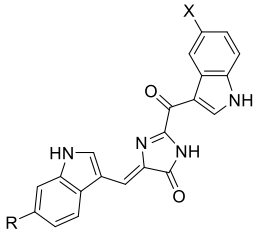
Scheme 3.5 The first total synthesis of tubastrindole B



3.2.2 Rhopaladins A–D

Rhopaladins A-D were first isolated by Sato et. al. in 1998 from the marine tunicate *Rhopalaea sp*.[26] Their structures are shown in **Figure 3.2**. Their geometry was determined as (*Z*) by running a NOESY experiment on rhopaladin C. Sato et. al. reported that rhopaladin C showed some antibacterial activity and rhopaladin B exhibited inhibitory activity against *c-erb*-2 kinase and cyclin dependent kinase 4 (IC_{50} = 7.4 and 12.5 μg/mL, respectively).[26]

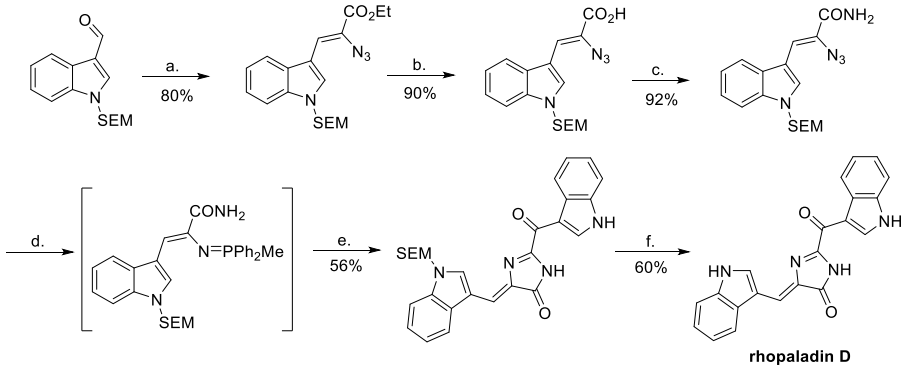
Figure 3.2 Rhopaladin A-D



rhopaladin A R=OH, X=Br
rhopaladin B R=OH, X=H
rhopaladin C R=H, X=Br
rhopaladin D R=H, X=H

The first total synthesis of rhopaladin D was reported by Fresneda et. al. in 2000 (**Scheme 3.6**).[27] The key step to imidazol-4-one formation was an intermolecular aza-Wittig reaction, where the intermediate reacted with indolyl-3-glyoxylyl chloride to produce an imidoyl chloride, which cyclized to form the central imidazolone ring. This total synthesis was completed in 6 steps, with an overall yield of 19%. However, after step (e.), the product was isolated as a 6:4 mixture of *E*/*Z* isomers and, even after chromatographic separation, the *Z* isomer underwent isomerisation to *E* isomer upon sunlight irradiation. Thus, the rhopaladin D they synthesized was a mixture of *E*/*Z* isomers as well. The other three rhopaladin analogues have yet to succumb to a total synthesis.

Scheme 3.6 First total synthesis of rhopaladin D

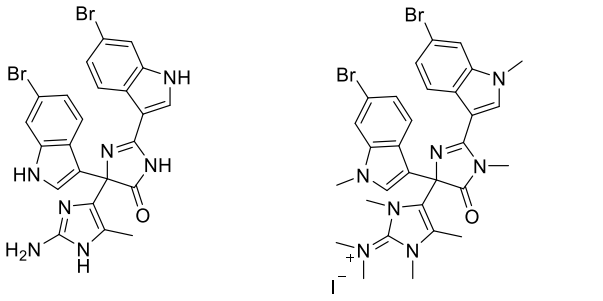


Reagents a) N_3CH_2COOEt, NaOEt, EtOH, -15 °C; b) LiOH, THF, H_2O; c) carbonyldiimidazole (CDI), NH_3, DMF; d) PPh_2Me, THF; e) indolyl-3-glyoxylyl chloride, polymer-bound BEMP, THF; f) TBAF, THF, reflux.

3.2.3 Nortopsentin D

Nortopsentin D was isolated in 1996 from the axinellid sponge *Dragmacidon sp*.[28] It is part of a family of imidazolediylbis(indole) alkaloids. While most of this family is comprised of imidazole linkers, nortopsentin D contains a (4*H*)-imidazol-4-one as its core scaffold (**Figure 3.3**). This natural product was tested for its cytotoxicity against KB tumor cells, antibacterial activity (*S. Aureus*), and antifungal activity (*Candida albicans*), but it proved inactive for all three. However, when methylated (Figure 4), nortopsentin D proved to be highly cytotoxic, with an EC_{50}=0.014 μg/mL, or 18 nM.[28] The total synthesis of nortopsentin D has yet to be reported.

Figure 3.3 Structures of nortopsentin D and its methylated derivative



nortopsentin D
Non-toxic

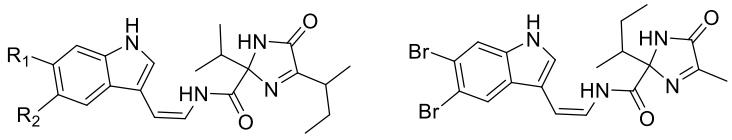
methylated derivative of nortopsentin D
High cytotoxic activity: EC_{50} = 18 nM

3.2.4 Kottamides A-D

Kottamides A-D are 2,2,5-trisubstituted (4*H*)-imidazol-4-one alkaloids isolated in 2002 from the New Zealand ascidian *Pycnoclavella kottae* (**Figure 3.4**).[29] The stereochemistry of C5 was never deduced for kottamides A-D, however they were able to confirm the *Z*-configuration of the enamide using J_{HH} measurements. Kottamides A-D were found to have a range of biological activities, including anti-inflammatory and anti-metabolic, as well as cytotoxicity towards several tumor cell lines. Kottamide D was

found to have potent anti-metabolic activity in cells using MTT assays (IC_{50}=6-10 µM).[29] Kottamides A-D were also assayed for cytotoxic and antimicrobial properties. All four were found to have moderate activity against P388 cells. Kottamide A was tested for cytotoxicity/antiviral activity against the African Green Monkey kidney cell line (BSC-1) infected with the RNA virus PV110. It was found to have some antiviral activity (zone size 1-2 mm) and moderate cytotoxicity (zone size >4.5 mm, 240 µg loading).[29] The total synthesis of kottamide A-D has yet to be elucidated.

Figure 3.4 Kottamides A-D



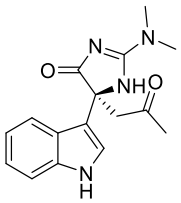
kottamide A R_1=Br, R_2=Br
kottamide B R_1=Br, R_2=H
kottamide C R_1=H, R_2=Br

kottamide D

3.2.5 Other indole alkaloids

An unnamed (4*H*)-imidazol-4-one containing indole alkaloid natural product was isolated from the marine tunicate *Dendrodoa grossularia* in 1998 by Riche and coworkers **(Figure 3.5)**.[30] They were able to identify the relative stereochemistry of compound **1** by measuring the optical rotation and isolating a single-crystal X-ray structure of the compound.

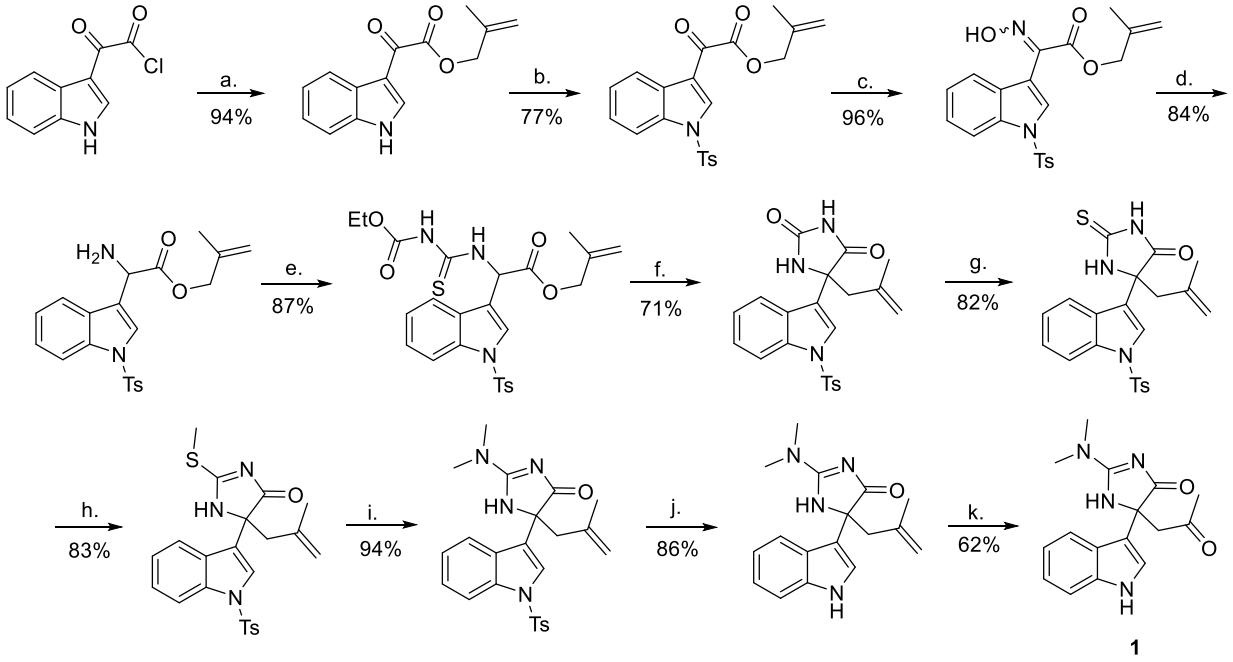
Figure 3.5 Indole alkaloid isolated from *D. grossularia*



1

The first total synthesis of compound **1** was completed by Hupp and Tepe in 2008 (**Scheme 3.7**).[31,32] In this synthesis, the quaternary carbon was formed via a oxazole rearrangement reaction, which produced an hydantoin product. The hydantoin was then converted into a thiohydantoin, which was reacted to form a 2-aminoimidazolone through standard conditions. In total, this total synthesis took 14 steps and had an overall yield of 12%. One thing to note with this total synthesis is the product was a racemate of compound **1**.

Scheme 3.7 The total synthesis of indole alkaloid **1**



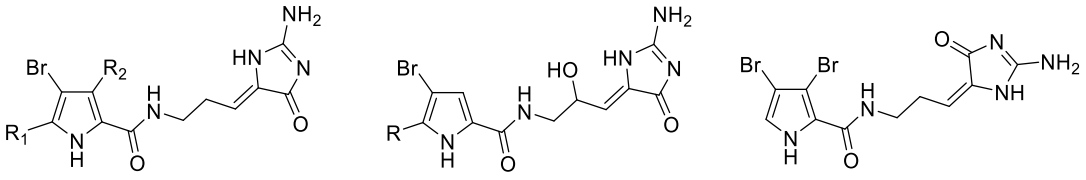
Reagents a) 2-methylprop-2-en-1-ol, ethyl acetate, rt, 16 h; b) TsCl, DMAP, DIPEA, CH_2Cl_2, rt, 16 h; c) NH_2OH*HCl, dioxane/H_2O, pyridine, reflux, 16 h; d) Zn, AcOH, 0 °C, 2 h; e) *O*-ethyl carbonisothiocyanatidate, CH_2Cl_2, rt, 16 h; f) 1. EDCI, Et_3N, CH_2Cl_2, 0 °C to reflux, 9 h, 2. NaOMe, MeOH, rt, 4 h, 3. HCl (aq.), 5 min; g) Lawesson's reagent, toluene, reflux, 24 h; h) MeI, DMAP, DIPEA, CH_2Cl_2, rt, 2 h; i) dimethylamine in THF, sealed tube, 75 °C, 4 h; j) KOEt, EtOH, reflux, 24 h; k) 1. OsO_4, NMO, rt, 4 h then 2. $NaIO_4$, 0 °C, 2 h

3.3 Pyrrole alkaloids

3.3.1 Dispacamide

Dispacamide and its mono-brominated derivative were first isolated by Caffieri et. al. in 1996 from four caribbean *Agelas* sponges.[33] Caffieri et. al. later isolated dispacamides C and D, which are racemic mixtures of the 9-hydroxyl derivatives of dispacamides A and B, in 1997 from the same sponges.[34] In 2014, dispacamide E was isolated by Ebada et. al. from two Indonesian *Stylissa* sponges.[35] These natural products are known precursors to the oroidin family and their cyclized derivatives, like hymenialdisine.[36] Their structures are displayed in **Figure 3.6**. Dispacamides have proven to have a range of useful biological activities, including antihistamine and antimalarial activity. Dispacamides C and D have been found to have impressive antihistamine activity when tested on isolated guinea pig ileum; with just 1 μM, the response to histamine was almost completely abolished.[34] Moreover, dispacamide B was found to have potent antiplasmodial activity (IC_{50}=1.34 μg/mL) against the multiple-drug resistant strain of *P. falciparum*, while being devoid of any cytotoxicity towards rat myoblast cells at 90 μg/mL.[37]

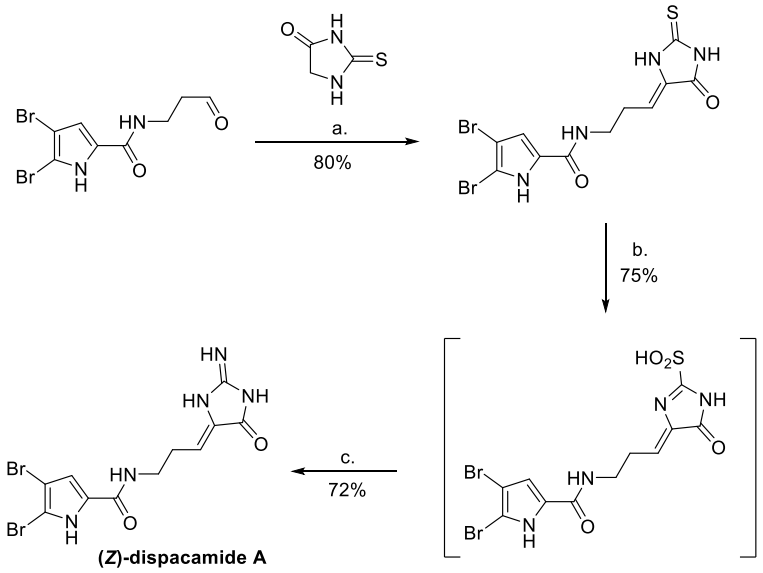
Figure 3.6 Dispacamide derivatives



The first total synthesis of dispacamide A was reported in 1997 by Lindel et al.[38] In this synthesis, the authors coupled an aliphatic aldehyde with thiohydantoin using piperidine to provide stereochemically pure (*Z*)-alkylidene thiohydantoin. This was then *S*-methylated and converted to a 2-amino-4-imidazolone upon treatment with heat and NH_3/NH_4Cl in methanol. They also reported an attempt to directly coupling creatinine with an aliphatic aldehyde, which was unsuccessful. The full synthesis is shown in **Scheme 3.8**.

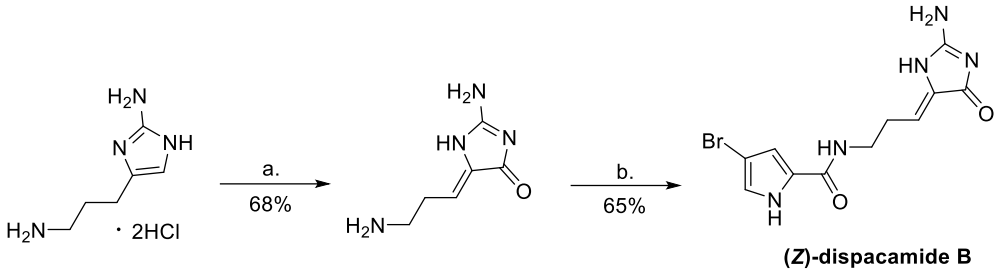
Dispacamide B was first synthesized a year later, in 1998, by Olofson et. al (**Scheme 3.9**).[39] In this synthesis, the key step is the oxidation of imidazole to imidazol-4-one using bromine and DMSO. The full synthesis, shown in **Scheme 3.9**, was performed in two steps and had an overall yield of 44%. Ando et. al. has reported similar syntheses, using tetra-n-butylammonium tribromide-DMSO to convert the 2-aminoimidazole to 2-amino-4-imidazolone.[40,41]

Scheme 3.8 The first total synthesis of dispacamide A



Reagents a) piperidine, EtOH/H_2O (8:2), rt, 4 h; b) aq. 70% TBHP, MeOH, rt, 12 h; c) NH_3,

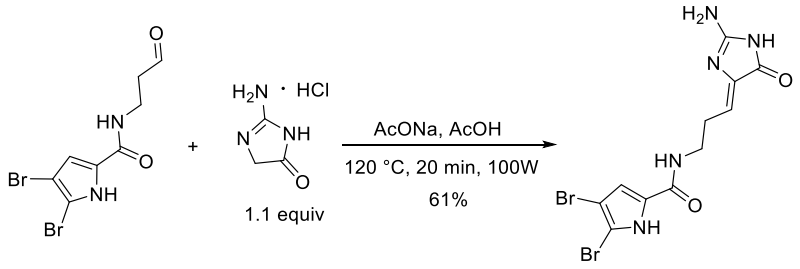
Scheme 3.9 The first total synthesis of monobromo-dispacamide



Reagents a) Br_2, DMSO, rt, b) 4-bromo-2-(trichloroacetyl)pyrrole, DMF, rt

The most recently reported total synthesis of dispacamide A was in 2012, where the authors directly condensed creatinine with an alkyl aldehyde under acidic conditions with heat to complete their total synthesis in 61% yield.[42] This reaction accomplished what Lindel et al. unsuccessfully attempted in 1997 by using a different set of conditions.[38] This one step, microwave-assisted procedure is shown in **Scheme 3.10**.

Scheme 3.10 Microwave-assisted total synthesis of dispacamide A

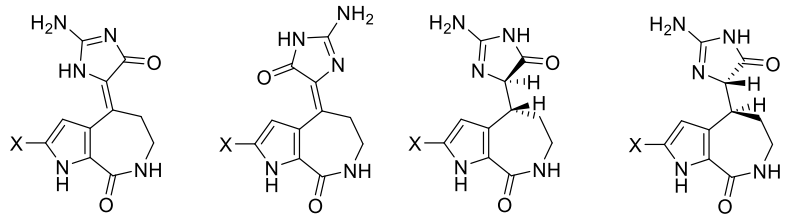


3.3.2 Hymenialdisine

Hymenialdisine was first isolated in 1981 by C. A. Mattia et. al. from the sponge *Acanthella aurantiaca*.[43] Since then, a few other derivatives of hymenialdisine have been isolated from various marine sponges (see **Figure 3.7**).[44–46] Hymenialdisine has been found to have a number of different biological activities, most notable as a nM kinase inhibitor (by competing with ATP for binding the kinases), resulting in inhibition of the

pro-inflammatory transcription factor, NF-ƙB.[47–50] Additionally, derivatives of hymenialdisine were found to be highly potent inhibitors of checkpoint kinase II (IC_{50} = 8 nM).[51–54] Moreover, it has been found to have some antitumor and anti-inflammatory activity.[55]

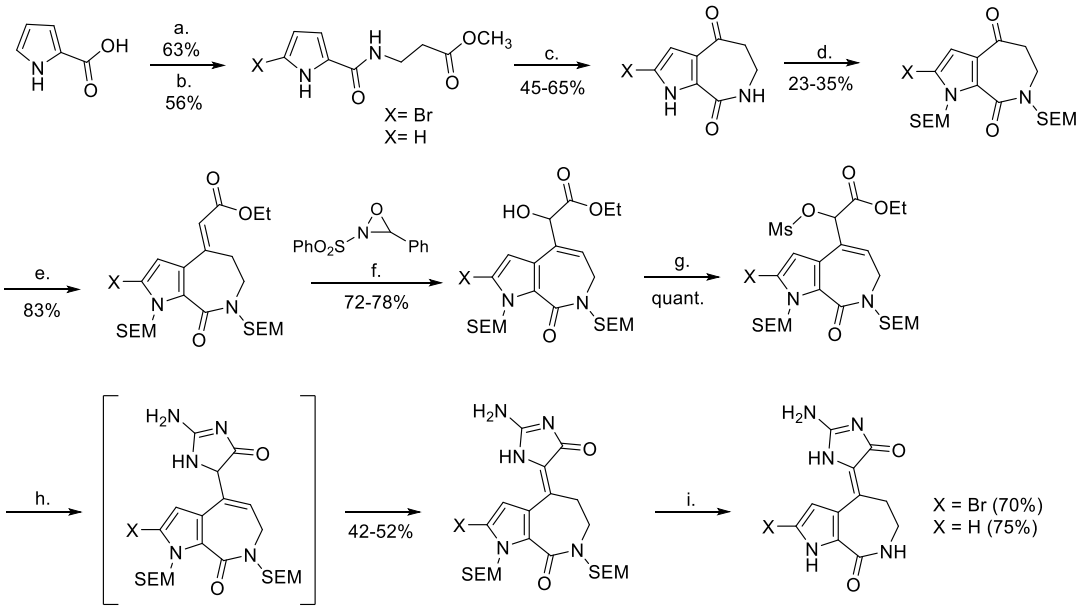
Figure 3.7 Isolated derivatives of hymenialdisine



The first total synthesis of (*Z*)-hymenialdisine and (*Z*)-debromo-hymenialdisine was reported in 1995 by Annoura and Tatsuoka.[56] This synthesis required 9 steps, starting from a 2-carboxyl-pyrolle, and had an overall yield of 1.5%. The imidazolone formation step took a α-ether-ester and cyclized it with guanidine to produce an imidazol-4-one (**Scheme 3.11**).

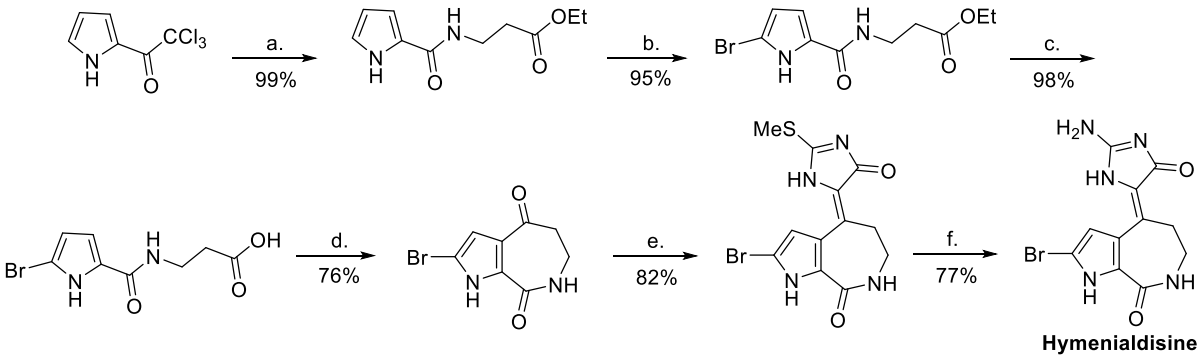
In 1997, Horne and co-workers reported a different synthesis of (*Z*)-hymenialdisine and (*Z*)-debromo-hymenialdisine, where the formation of the imidazolone ring came from the oxidation of 2-amino-4-bromoimidazole under acidic conditions with heat (**Scheme 3.12**).[57] This reaction took only three steps and had an overall yield of 23% and 18%, respectively.

Scheme 3.11 The first total synthesis of hymenialdisine and debromohymenialdisine by Annoura and Tatsuoka



Reagents a) $SOCl_2$, cat. DMF, toluene, 60 °C, 1 h, then $H_2NCH_2CH_2COOMe$, Et_3N, CH_2Cl_2, rt, 3 h; b) NBS, THF, rt, 2 h; c) 10% aq. NaOH-MeOH (2:1), rt, 5 h, then PPA-P_2O_5, 100°C, 1 h; d) NaH (2 eq.), SEMCl (2 eq.), DMF, rt, 2 h; e) $(EtO)_2POCH_2COOEt$, NaH, DME, 50 °C, 24 h; (f) KHMDS, THF, -78 °C, 2 h g) MsCl, Et_3N, CH_2Cl_2, 0 °C; h) guanidine, DMF, 50 °C, 5 h; i) 5% aq. HCl-MeOH (1:1), 80 °C, 2 h.

Scheme 3.12 Synthesis of hymenialdisine and debromohymenialdisine through the oxidation of 2-amino-4-bromoimidazole

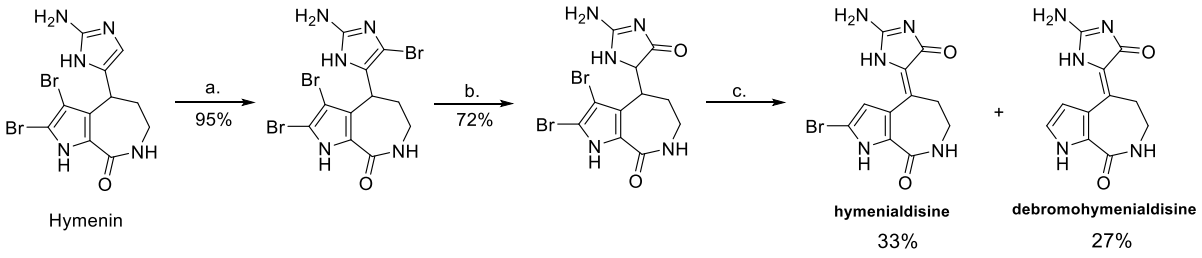


Reagents a) $H_2NCH_2CH_2COOEt$-HCl, Et_3N, DCM, rt; b) DBDMH, MeOH/THF, -78 °C- rt; c) LiOH or KOH, EtOH, H_2O, rt, 18 h; d) P_2O_5, $MeSO_3H$, 110 °C, 2 h; e) 2-(methylthio)-1,5-dihydro-4*H*-imidazol-4-one, $TiCl_4$, py., THF, -10 °C- rt; f) NH_4OH, THF, sealed tube, 110 °C.

In recent years, Saleem and Tepe reported a new total synthesis for hymenialdisine and its de-brominated derivative, illustrated in **Scheme 3.13**.[58] In this synthesis, 2-

(methylthio)-1,5-dihydro-4*H*-imidazol-4-one was condensed with 2-bromoaldisine. The methylthio group was converted to an amino substituent via reaction with ammonium hydroxide. This total synthesis took 6 steps and had an overall yield of 44%. Another paper used a very similar condensation reaction between 2-methylthiol-imidazol-4-one and 2-bromoaldisine, at somewhat lower yields.[59] The (*E*)-hymenialdisine and dihydrohymenialdisine derivatives have yet to be synthesized.

Scheme 3.13 An efficient total synthesis of hymenialdisine



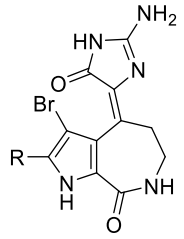
Reagents a) Br_2, TFA, rt; b) AcOH/H_2O, reflux c) CH_3SO_3H, HBr (cat.), 90 °C, sealed tube, 12 h.

3.3.3 Spongiacidins A and B

Spongiacidins A and B were isolated by Inaba et. al. in 1998 from the sponge *Hymeniacidon*.[60] Their structures are shown in **Figure 3.8**. This class of natural products is part of the hymenialdisine family, differing by their bromo substitution pattern and alkene stereochemistry. Spongiacidin A was identified as being cytotoxic to L5178Y and HCT116 cancer cell lines. It was also found to be a protein kinase inhibitor.[61] Unlike hymenialdisine, there have been no reports of the total synthesis of spongiacidins A or B. This may be in part due to the spontaneous conversion of spongiacidin's (*E*)-isomer to the (*Z*)-isomer, driven by the reduction of steric strain upon conversion.[62] The proposed isomerization is shown in **Scheme 3.14**. The steric strain exerted between the C-3

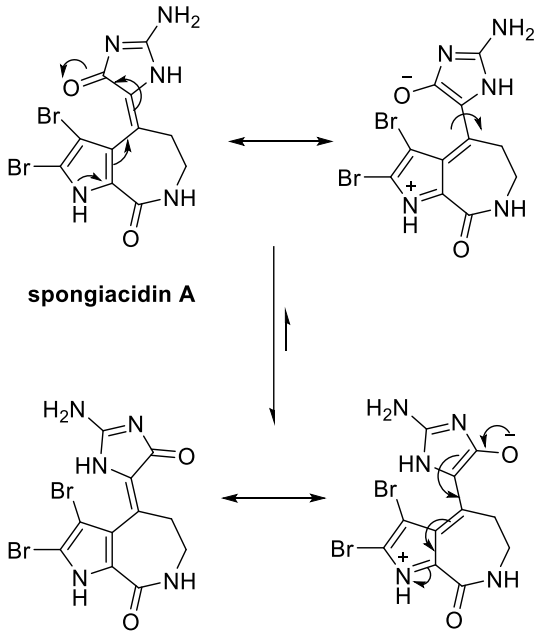
bromine and oxygen on C-15 prevents a straightforward synthesis of the (*E*)-isomer required to produce spongiacidin.

Figure 3.8 Spongiacidin A and B



(*E*)-spongiacidin A R=Br
(*E*)-spongiacidin B R=H

Scheme 3.14 Isomerization of (*E*)-spongiacidin A to the (*Z*)-isomer



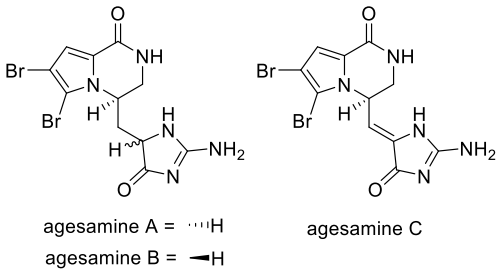
spongiacidin A

3.3.4 Agesamines A-C

Agesamine A and B were isolated by Katsuki et. al. in 2019 from the sponge *Agelas sp*.[63] Agesamine C was isolated by Kovalerchik et. al. in 2020 from the sponge *Agelas oriodes*.[64] Their structures are illustrated in **Figure 3.9**. Agesamine A and B were

found to have some cytotoxicity towards HeLa cells, and although the synthesis of the related hydantoin analogue has been reported,[65,66] the total syntheses of agesamines A-C have yet to be completed.

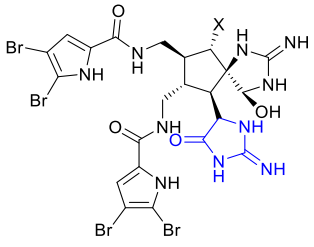
Figure 3.9 Agesamine derivatives



3.3.5 Donnazoles A and B

Donnazoles A and B were isolated by Al-Mourabit and coworkers in 2012 from the marine sponge *Axinella donnani* (**Figure 3.10**).[67] These natural products are dimeric members of the same class of pyrrole-aminoimidazole (PAI) alkaloids as oroidin, sceptrin, massadine, and palau'amine. The structure's absolute configuration was determined by comparison of their circular dichroism (CD) with sceptrin. To date, there has been no biological activity reported for donnazoles A and B. While some efforts have been made towards developing the methodology needed to produce donnazoles A and B,[68] they have yet to be synthesized.

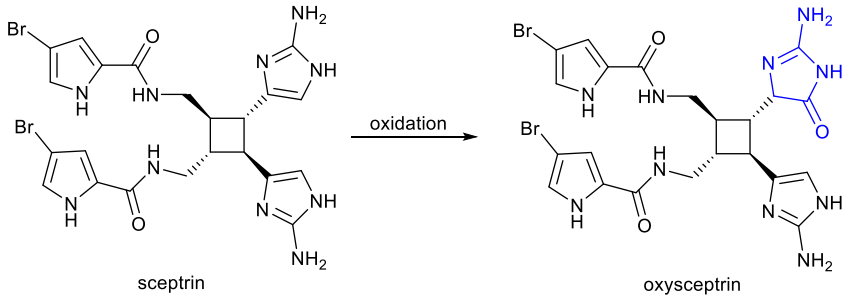
Figure 3.10 Donnazoles A and B



3.3.6 Oxysceptrin

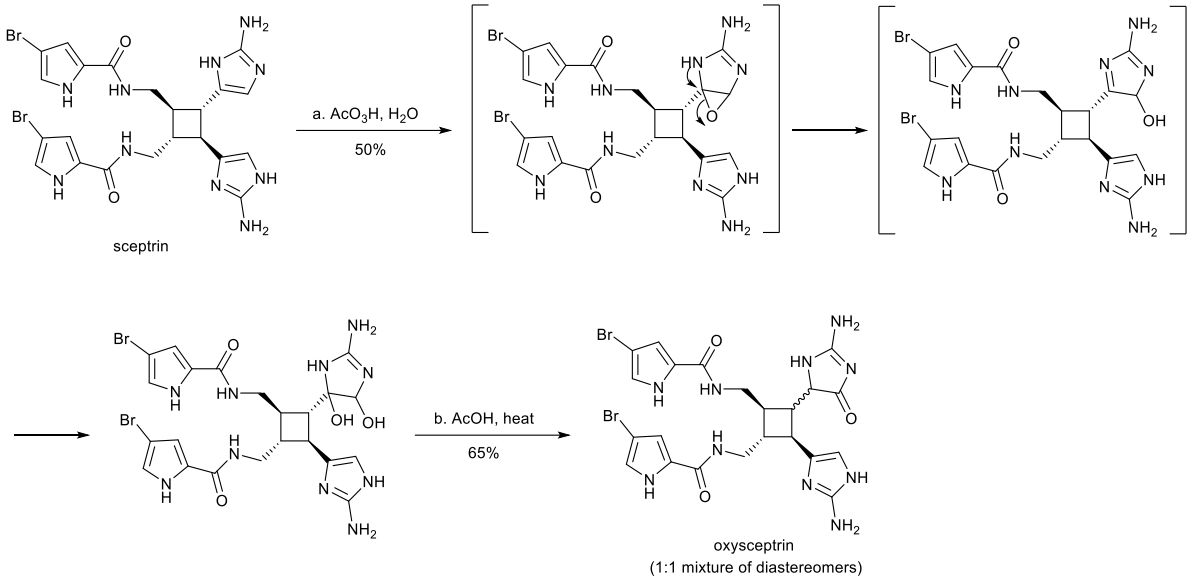
Oxysceptrin was first reported by Rinehart and coworkers, isolated from the Caribbean sponge *Agelas conifera* in 1991.[69] Ohizumi and coworkers later isolated this natural product from the marine sponge *Agelas nemoechinata*.[70] It is believed to be the product of the oxidation of sceptrin. The structures of oxysceptrin as well as the unoxidized sceptrin are displayed in **Figure 3.11**. Oxysceptrin exhibits some antiviral and antibacterial activity.[69,70]

Figure 3.11 Structures of oxysceptrin and sceptrin



The total synthesis of oxysceptrin was completed by Baran and coworkers in 2007, when they performed an oxidation of sceptrin to give oxysceptrin as a 1:1 mixture of diastereomers (**Scheme 3.15**).[71] In this synthesis, sceptrin was reacted with aqueous peracetic acid to give 50% yield of diol product, along with a recovered 35% of sceptrin. The diol was then converted to a ketone using acetic acid and heat. This synthesis was completed in two steps from sceptrin, with an overall yield of 32%.

Scheme 3.15 The first total synthesis of oxysceptrin

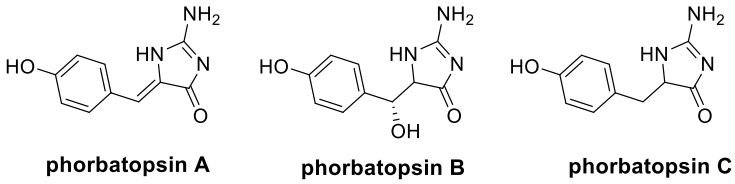


3.4 Other 2-amino-(4*H*)-imidazol-4-one alkaloids

3.4.1 Phorbatopsins A-C

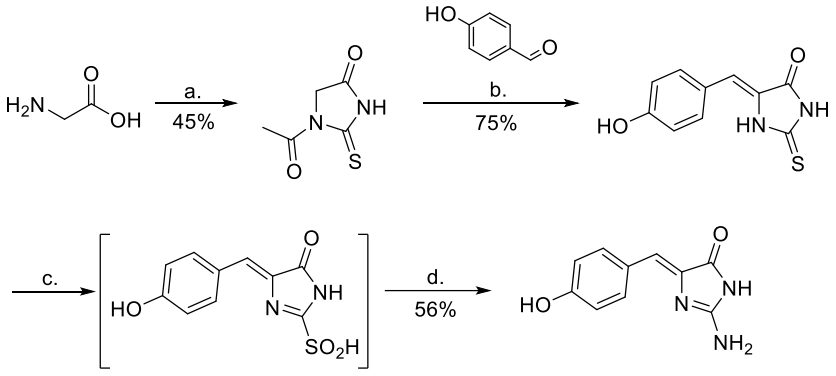
Phorbatopsins A-C were isolated by Nguyen et. al. in 2012 from the mediterranean sponge *Phorbas topsenti* (**Figure 3.12**).[72] One biological application for these compounds is their antioxidant activity, which was tested using the Oxygen Radical Absorbance Capacity (ORAC) assay. All were shown to have some activity, with phorbatopsin A being most active, having an ORAC value of 0.88 (which is comparable to the positive control Trolox's ORAC value of 1). Derivatives of phorbatopsin A were also tested for antitumor activity, with several derivatives having over 90% inhibition at a concentration of 50 μM.[73]

Figure 3.12 Isolated phorbatopsin analogues



The first total synthesis of phorbatopsin A was reported in 2013 (**Scheme 3.16**).[73] In this synthesis, glycine was treated with NH_4SCN under acidic conditions. The formed thiohydantoin was then condensed with 4-hydroxybenzaldehyde and converted to a 2-aminoimidazolone using TBHP and ammonia. Phorbatopsin A was produced in four steps with an overall yield of 19%. Phorbatopsins B and C have yet to be synthesized.

Scheme 3.16 The first total synthesis of phorbatopsin A

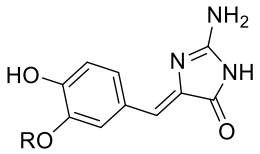


Reagents a) NH_4SCN, Ac_2O, AcOH, reflux, 2 h; b) NaOAc, AcOH, reflux, 5 h; c) TBHP, MeOH, rt, 2 h; d) NH_3, MeOH, rt, 10 h

3.4.2 Polyandrocarpamines A and B

Polyandrocarpamines A and B were isolated by Davis et. al. in 2002 from the Fijian ascidian *Polyandrocarpa* sp.[74] Their structures are shown in **Figure 3.13**. Polyandrocarpamine A was found to have selective cytotoxicity against the CNS cell line SF 268 with a GI value of 65 μM.[75] Then, in 2017, both derivatives were found to inhibit mammalian and protozoan DYRK and CLK kinases.[76]

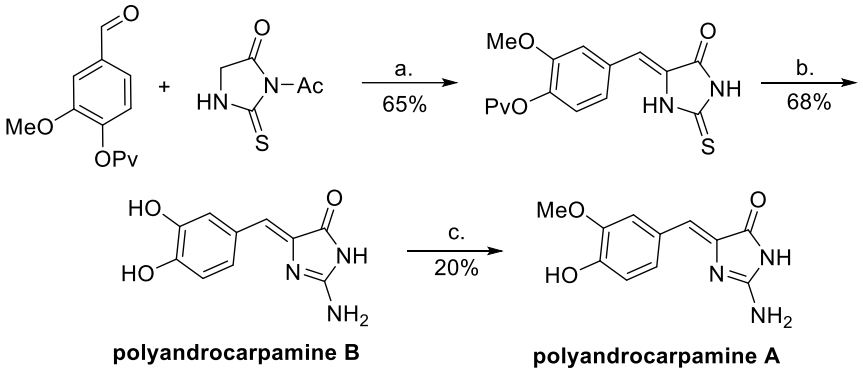
Figure 3.13 Polyandrocarpamine A and B



polyandrocarpamine A R=Me
polyandrocarpamine B R=H

The first total synthesis of polyandrocarpamine A and B was reported by Davis et. al. after their isolation in 2002.[74] This synthesis contains only three steps: condensation of an aryl aldehyde and thiohydantoin, then conversion to 2-aminoimidazolone using tetrabutyl hydrogen peroxide (TBHP) and ammonia. Polyandrocarpamine A was converted to polyandrocarpamine B through a demethylation using a boron tribromide dimethyl sulfide complex. The full synthesis of polyandrocarpamines A and B were completed in 44% and 9% overall yield, respectively (**Scheme 3.17**). In 2009, Davis et. al. reported a microwave assisted synthesis, producing polyandrocarpamine A and B in one step with yields of 56% and 80%, respectively.[77]

Scheme 3.17 The first total synthesis of polyandrocarpamine A and B



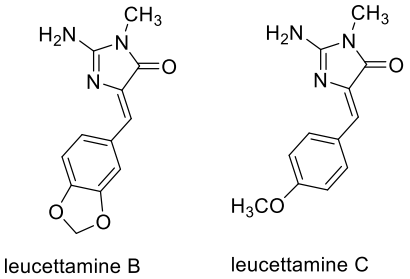
polyandrocarpamine B **polyandrocarpamine A**

Reagents a) NaOAc, AcOH, reflux, 2 h; b) TBHP (15 equiv), aq. NH_4OH, MeOH, rt, 72 h; c) BBr_3-SMe_2, DCE, reflux, 15 min.

3.4.3 Leucettamine B and C

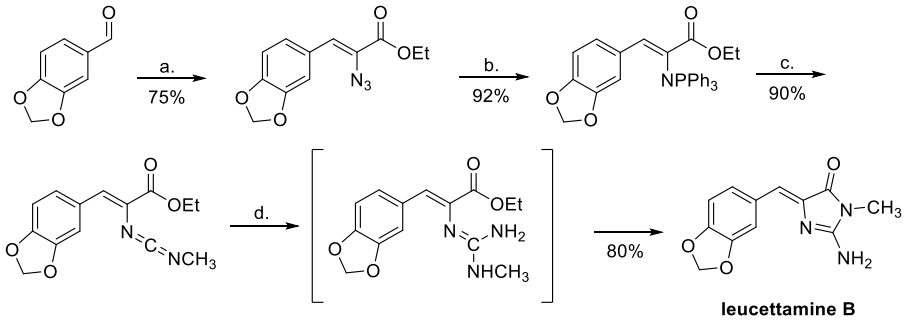
Leucettamine B was first isolated by Chan et. al. in 1993 from the marine sponge *Leucetta microraphis*,[78] and leucettamine C was isolated from *Leucetta* sponges in 2003.[79] These compounds have since been isolated from several other natural sources.[80,81] Their structures are shown in **Figure 3.14**. While leucettamine B and C have limited biological activity, derivatives of leucettamine B have been found to inhibit protein kinase activity.[76,82,83]

Figure 3.14 Imidazolone-containing leucettamine natural products



Leucettamine B was first synthesized in 1994 by Molina et. al.[84] The synthesis was completed in four steps, with an overall yield of 50% (**Scheme 3.18**). In this synthesis, the key transformation is an aza-Wittig/heterocumulene-mediated annulation to build the 2-aminoimidazolone ring.

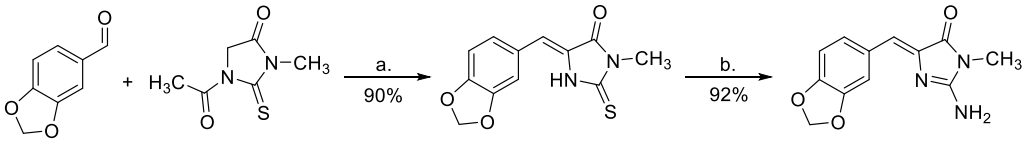
Scheme 3.18 The first total synthesis of leucettamine B



Reagents a) N_3CH_2COOEt, NaOEt, -15 °C; b) Ph_3P, CH_2Cl_2, rt; c) CH_3NCO, toluene, rt; d) NH_3, sealed tube, 45 °C

Another popular method of synthesizing leucettamine B is through the condensation of thiohydantoin and an aryl aldehyde or imine. This is then converted to a 2-amino-imidazole through varying conditions. The first report of this synthesis was in 1999 by Roue and Bergman, which produced leucettamine B in 83% yield.[85] Their synthesis is shown in **Scheme 3.19**. Since then, several other similar methods have been reported.[86–88]

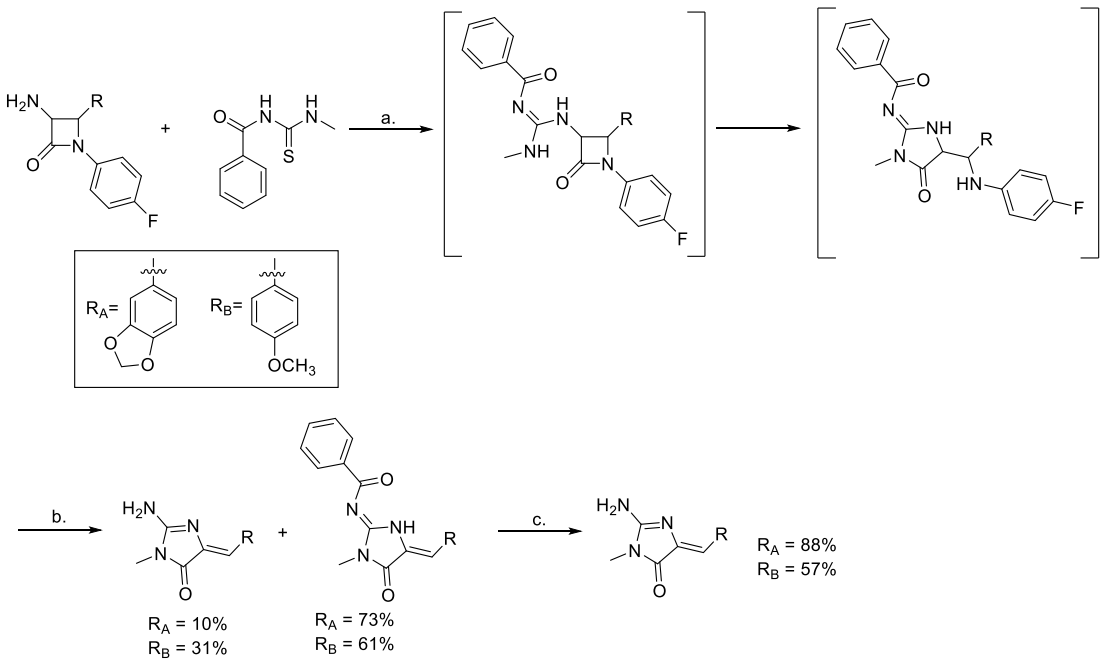
Scheme 3.19 Synthesis of leucettamine B via a thiohydantoin intermediate



Reagents a) CH_3CO_2H, CH_3COONa, heat; b) NH_3, TBHP

The most recently reported total synthesis of both leucettamines B and C was in 2017 by Drazic et. al.[89] Their synthesis is shown in **Scheme 3.20** and starts with a β-lactam, which first reacts with *N*-(methylcarbamothioyl)benzamide and then undergoes a ring expansion under basic conditions to produce the desired imidazol-4-one. This was the first report of the total synthesis of leucettamine C.

Scheme 3.20 Total synthesis of leucettamines B and C via β-lactam ring expansion

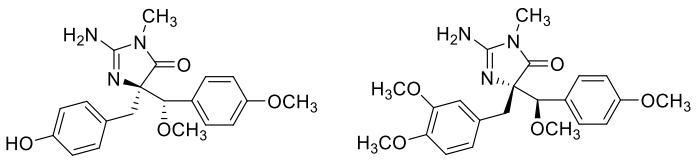


Reagents a) $HgCl_2$, Et_3N, DMF, rt, overnight; b) K_2CO_3, MeOH, rt, overnight; c) K_2CO_3, MeOH, 50 °C, overnight.

3.4.4 Calcaridines A and B

(+)-Calcaridine A was first isolated by Edrada et. al. in 2003 from the sponge *Leucetta*.[90] (-)-Calcaridine B was isolated by Tang et. al. in 2019 from the marine sponge *Leucetta chagosensis*.[91] Their structures are shown in **Figure 3.15**. (-)-Calcaridine B was found to exhibit mild cytotoxicity toward the MCF-7 cancer cell line with an IC_{50} value of 25.3 μM, whereas (+)-calcaridine A has no known biological activity to date.

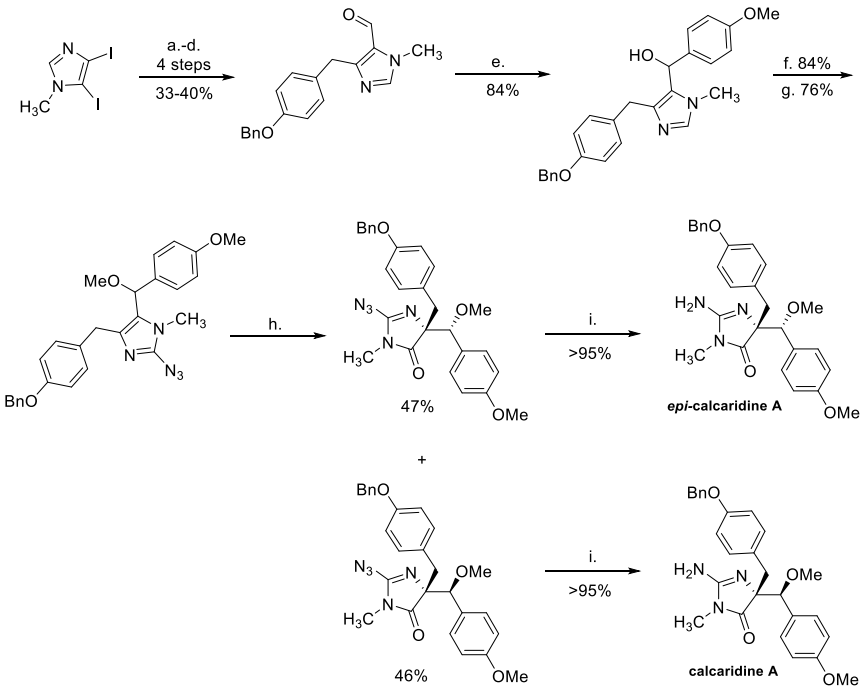
Figure 3.15 Calcaridine A and B



(+) calcaridine A **(-) calcaridine B**

The first total synthesis of calcaridine A was reported by Koswatta et. al.[92,93] In this synthesis (**Scheme 3.21**), a 2-azidoimidazole is converted to a 2-amino-4-imidazolone through sequential oxidation and reduction after a number of alkylation steps. This total synthesis was inspired by the proposed biomimetic synthesis. In this proposed pathway, calcaridine A is said to be derived from the rearrangement and/or oxidation of naamine A, an imidazole-containing natural product, also isolated from *Leucetta* sponges. The downside to this synthesis is the isolation of both (+)-calcaridine A and its epimer. They attempted to convert the individual diastereomers into each other upon reaction with catalytic HCl in methanol, but found no discernable epimerization, even at a range of different temperatures. (-)-Calcaridine B has yet to be synthesized.

Scheme 3.21 The first total synthesis of calcaridine A



Reagents a) EtMgBr, CH_2Cl_2, rt, then N-methyl-formanilide; b) ethylene glycol, *p*-TsOH, PhH, reflux; c) EtMgBr, THF, rt, then aryl aldehyde; d) HCl (aq), THF, reflux, then Et_3SiH, TFA, CH_2Cl_2, rt; e) 4-$MeOC_6H_4MgBr$, THF, reflux; f) NaH, THF, 0 °C-rt to 0°C, then MeI; g) BuLi, THF, -78 °C, then TsN_3; h) N-sulfonylaziridine. $CHCl_3$, rt; i) $Pd(OH)_2/C$, H_2, EtOH

3.5 Conclusions

In chapter 3 of this dissertation, the known imidazol-4-one containing natural products and their total syntheses were discussed in detail. **Table 3.1** summarizes the main imidazolone formation steps found in the mentioned total syntheses. Interestingly, most of the total syntheses followed one of a few common methods for imidazol-4-one formation. Seven of the natural products were formed via a conversion of thiohydantoin to imidazol-4-one, and the majority of these total syntheses also employed a Knoevenagel condensation to substitute the 5-position of the ring. All the natural products synthesized by this combination of reactions were 5-ethylidene-4-imidazolones. The aza-Wittig/heterocumulene-mediated annulation was also used to produce a couple 5-ethylidene-4-imidazolone containing natural products, namely aplysinopsin and leucettamine B. Two other approaches to synthesizing 5-ethylidene-4-imidazolones utilized in the total synthesis of natural products were an intermolecular aza-Wittig reaction, which was used to synthesize rhopaladin D, and a β-lactam ring expansion, used to synthesize leucettamines B and C.

Table 3.1 Summary of common transformations used in the synthesis of (4*H*)-imidazol-4-one containing natural products

Thiohydantoin conversion	Knoevenagel condensation	Aza-Wittig/Heterocumulene mediated annulation	Oxidative pinacol-like rearrangement of imidazole	Condensation of ester and guanidine/cyanamide
Dispacamide	**Dispacamide**	**Aplysinopsin**	**Hymenialdisine**	Hymenialdisine
Hymenialdisine	**Hymenialdisine**	**Leucettamine B**	**Monobromodispacamide**	Dihydroaplysinopsin
Polyandrocarpamine A	**Polyandrocarpamine A**		**Calcaridine A**	
Polyandrocarpamine B	**Polyandrocarpamine B**		**Oxysceptrin**	
Leucettamine B	**Leucettamine B**			
Indole alkaloid 1	**Aplysinopsin**			
Phorbatopsin A				

Moreover, a number of imidazol-4-one containing natural products have yet to be synthesized, namely rhopaladins A-C, nortopsentine D, kottamides A-D, dispacamides C-E, dihydro-hymenialdisine derivatives, spongiacidins A-B, agesamines A-C, donnazoles A-B, phorbatopsin B and C, and (-)-calcaridine B. Analyzing the natural products that have yet to be synthesized, most of them are 5-monosubstituted or 5,5-disubstituted imidazol-4-ones. While there are many preparative methods highlighted in chapter 1 to produce these imidazolones, the majority of them have not been utilized in any total syntheses. Two reactions used to produce 5-mono or 5,5-disubstituted-4-imidazolone containing natural products highlighted in section 3 are the oxidative pinacol-like rearrangement of imidazole to produce oxysceptrin and calcaridine A and the condensation of ester and guanidine or cyanamide, used to produce dihydroaplysinopsin. This chapter also highlighted several natural products that have yet to succumb to total synthesis. Here, the disparity between total syntheses of 5-ethylidene-4-imidazolones and

other imidazol-4-ones became apparent. Looking towards the future, it is evident more effort is needed in the realm of 5-monosubstituted and 5,5-disubstituted imidazol-4-ones, specifically highlighting enantioselective methods which can be used for the total synthesis of natural products. Overall, this review highlighted the importance of imidazol-4-ones in a variety of applications, and the preparative methods explored to date.

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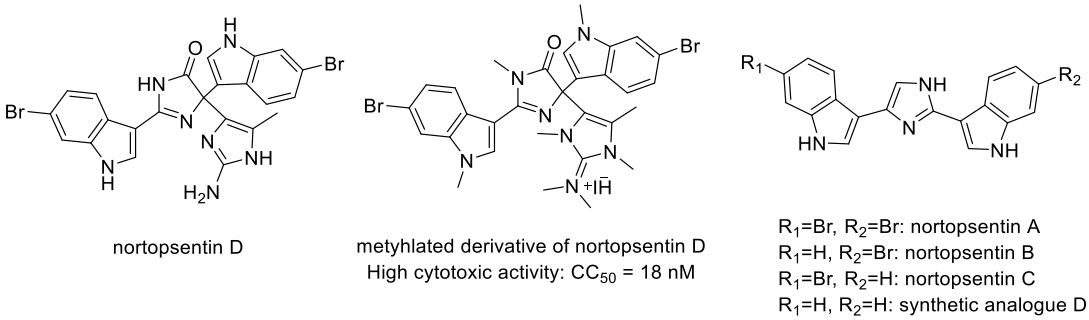
4 Chapter 4: First total synthesis of nortopsentin D*

4.1 Introduction

Nortopsentin D (**Figure 4.1**) was originally isolated in 1996 from the axinellid sponge *Dragmacidon sp*. in deep waters south of New Caledonia[1,2] and later from the sponge *A. dendromorpha*.[3] This is a fascinating structural variant of the nortopsentin family, whose methylated derivative has been shown to have high cytotoxicity towards tumoral cells (CC_{50} 18 nM) as well as antifungal activity against yeast.[1] Over the years, the nortopsentin family and its synthetic analogues have displayed a large range of biological activities in the areas of cytotoxicity, antiplasmodial, antibacterial, antifungal, and insecticidal activities.[1,4–10] Catalytic hydrogenation of nortopsentins A-C was previously reported to render the synthetic analogue D (**Figure 4.1**), which is unfortunately also sometimes referred to in the literature as nortopsentin D.[11–16]

Structurally, nortopsentin D is composed of a complex central trisubstituted (4*H*)-imidazol-4-one, with a 6-bromoindole at the C2 position and a 4-methyl-1*H*-imidazol-2-amine and 6-bromoindole at C5. The isolated natural product was determined to be a racemate, due to the nature of how nortopsentin D is assumed to be formed biogenetically. Nortopsentin D is one of several known 5,5-disubstituted (4*H*)-imidazol-4-one containing natural products that have yet to be synthesized, as highlighted in chapter 3 of this dissertation.[17] Chapter 4 of this dissertation will discuss the first reported total synthesis of nortopsentin D.

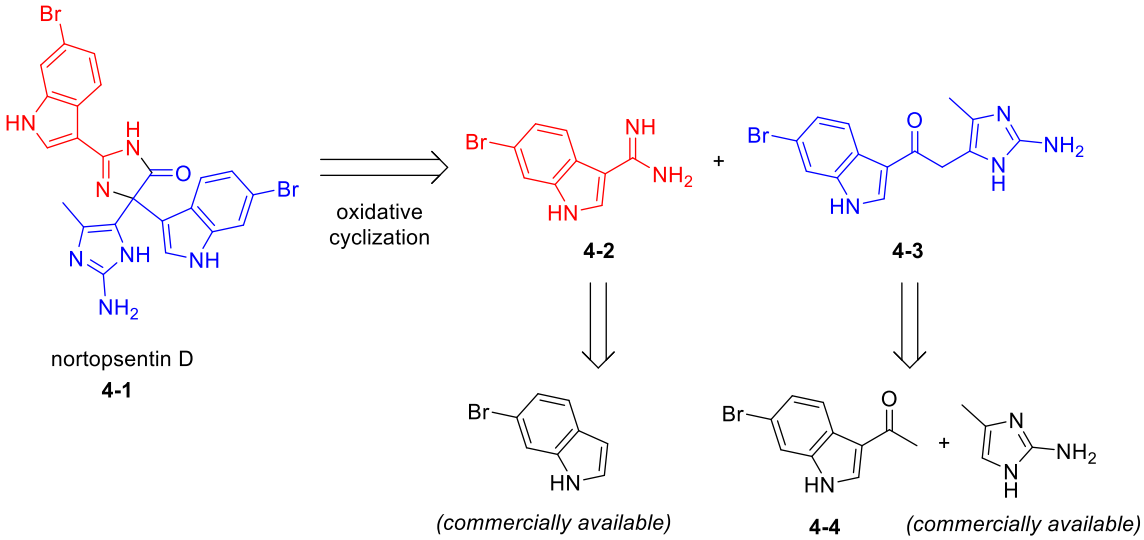
Figure 4.1 Natural products within the nortopsentin family



4.2 Results and discussion

My first-generation retrosynthesis for the total synthesis of nortopsentin D is shown in **Scheme 4.1.** In this retrosynthetic plan, nortopsentin D was broken down into two key intermediates- an amidine (**4-2**) and ketone (**4-3**). The amidine intermediate contains the 6-bromoindole seen at nortopsentin D's C2 position, and the ketone contains both the 6-bromoindole and 2-amino-4-methylimidazole seen at C5. It was proposed the cyclization of these two intermediates could be accomplished via an oxidative cyclization, the same as was used to synthesize imidazol-4-one small molecule activators in Chapter 2 of this thesis.[18]

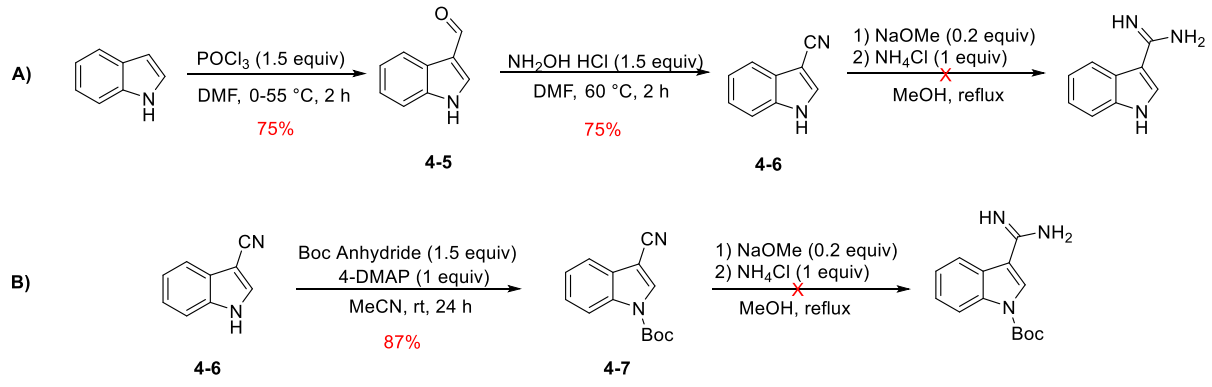
Scheme 4.1 First-generation retrosynthesis of nortopsentin D



4.2.1 Amidine synthesis

To synthesize the desired amidine intermediate, a previously reported method was adapted, as displayed in **Scheme 4.2**.[19] Starting with indole, which is commercially available, phosphoryl chloride was used to produce indole-3-formaldehyde (**4-5**) in 75% yield. The aldehyde was then converted to a nitrile by heating with hydroxylamine hydrochloride. Once the nitrile was formed, sodium methoxide was added to produce an iminoester which could undergo substitution for an amine upon the addition of ammonium chloride. Unfortunately, this reaction failed, and only starting material was visible when monitored by TLC. It is believed this reaction failed due to sodium methoxide's role in deprotonating the indole's free nitrogen, which prevents the formation of the desired imino ester. To avoid this issue, the free nitrogen was protected using di-tert-butyl dicarbonate, giving 87% yield of **4-7** (**Scheme 4.2B**). Unfortunately, when the amidine formation4 was attempted on **4-7**, the reaction failed once again. It was discovered refluxing with small amounts of base led to boc-deprotection instead of the expected product formation.

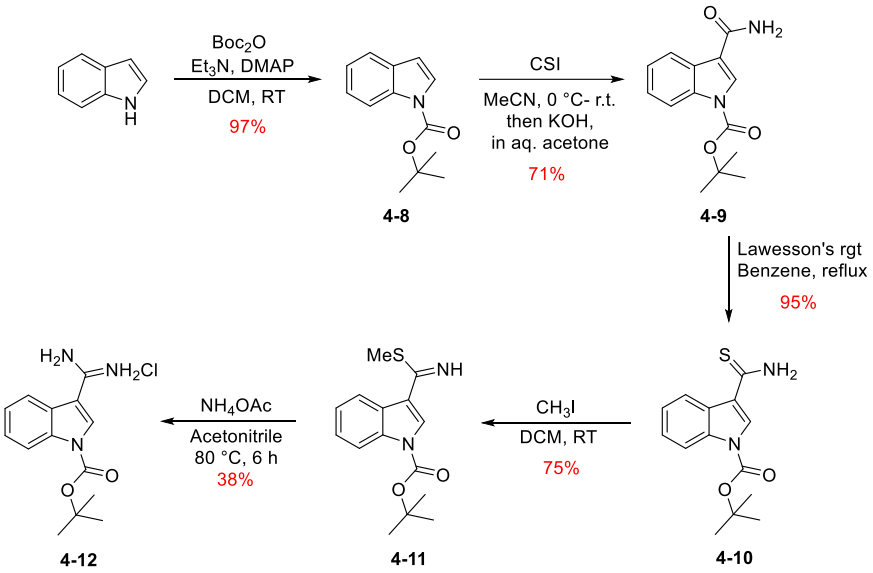
Scheme 4.2 Attempted synthesis of indole-3-amidine, via nucleophilic addition of amine to ntrile



At this point, a different approach was used for the formation of the desired indole-3-amidine, adapted from a previously reported synthesis of nortopsentin B and synthetic analogue D (**Scheme 4.3**).[20] The synthesis began with indole, which was *N*-protected using di-tert-butyl

dicarbonate, affording **4-8** in 97% yield. Functionalization of the C3 position of indole with an amide was performed using chlorosulfonyl isocyanate (CSI), followed by potassium hydroxide in aqueous acetone to produce **4-9** in 71% yield. Lawesson's reagent was used to convert the amide to a thioamide in 95% yield. The resultant compound was then converted to a methyl thiol imine using methyl iodide, giving **4-11** in 75% yield. The last step in the preparation of indole-3-amidine was the substitution of methyl thiol with an amine using ammonium acetate in acetonitrile. Overall, this pathway furnished the desired amidine **4-12** in 5 steps with an overall yield of 19%.

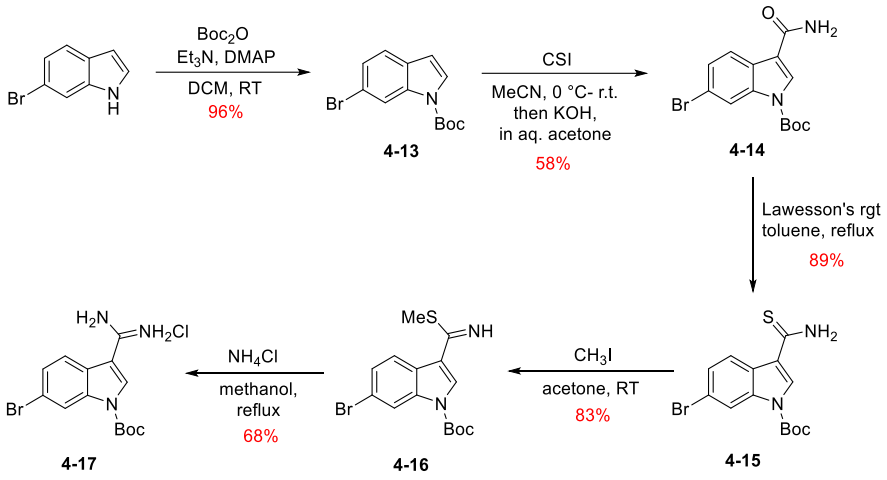
Scheme 4.3 Synthesis of tert-butyl 3-carbamimidoyl-1*H*-indole-1-carboxylate



Once the abovementioned synthetic pathway was optimized starting with indole, this pathway was repeated starting with the desired substrate, 6-bromoindole. The reactions behaved similarly to the model system (**Scheme 4.4**), following similar procedures to the previously mentioned. The synthesis began with 6-bromoindole, a common building block available in multigram quantities. Di-tert-butyl dicarbonate was used to protect the indole's nitrogen, affording **4-13** in 96% yield. Functionalization of the C3 position of indole with an amide was

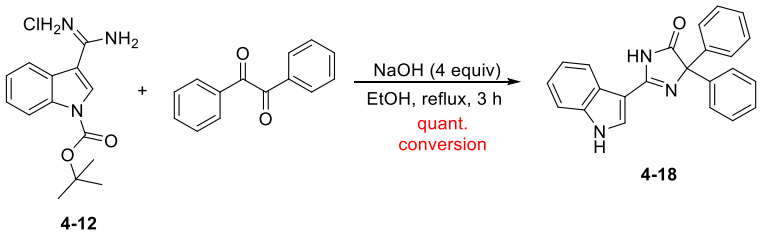
performed using chlorosulfonyl isocyanate (CSI), followed by potassium hydroxide in aqueous acetone to produce **4-14** in 58% yield. Lawesson's reagent was used to convert the amide to a thioamide in 89% yield. The resultant compound was then converted to a methyl thiol imine using methyl iodide, **4-16** in 83% yield. The last step in the preparation of the key amidine intermediate was the substitution of methyl thiol with an amine using ammonium chloride in methanol. Overall, this pathway furnished the desired amidine **4-17** in 5 steps with an overall yield of 28%.

Scheme 4.4 Synthesis of tert-butyl 6-bromo-3-carbamimidoyl-1*H*-indole-1-carboxylate



Once the desired amidine intermediate was produced, a model reaction of the imidazol-4-one formation step was performed. Here, **4-12** and benzil were condensed under basic conditions, using excess sodium hydroxide and heating in ethanol for a few hours. Complete conversion to the desired imidazol-4-one product was seen after 3 hours (**Scheme 4.5**). Additionally, it was observed the *N*-boc protecting group was removed during this reaction, leaving indole unprotected.

Scheme 4.5 Cyclization of indole-3-amidine (**4-12**) and benzil to produce an imidazol-4-one.



Asides from the above-mentioned methods, several other attempts were made at synthesizing the indole-3-amidine, specifically with a *N*-tosyl protecting group instead of *N*-boc. **Scheme 4.6** describes efforts towards synthesizing *N*-tosyl-indole-3-amidine. First, 6-bromoindole was C3 functionalized using chlorosulfonyl isocyante (CSI), to provide 6-bromo-1*H*-indole-3-carbonitrile (**4-19**) in 88% yield. From there, the nitrogen was protected using 4-toluenesulfonyl chloride under basic conditions (78%). The nitrile was then converted to a *N*-hydroxy-amidine (**4-21**) in 97% using hydroxyl amine and sodium bicarbonate. From there, a range of different conditions were attempted to reduce the *N*-hydroxy-amidine to amidine with varying success.

Scheme 4.6 Cyclization of indol-amidine and benzil to produce imidazolone

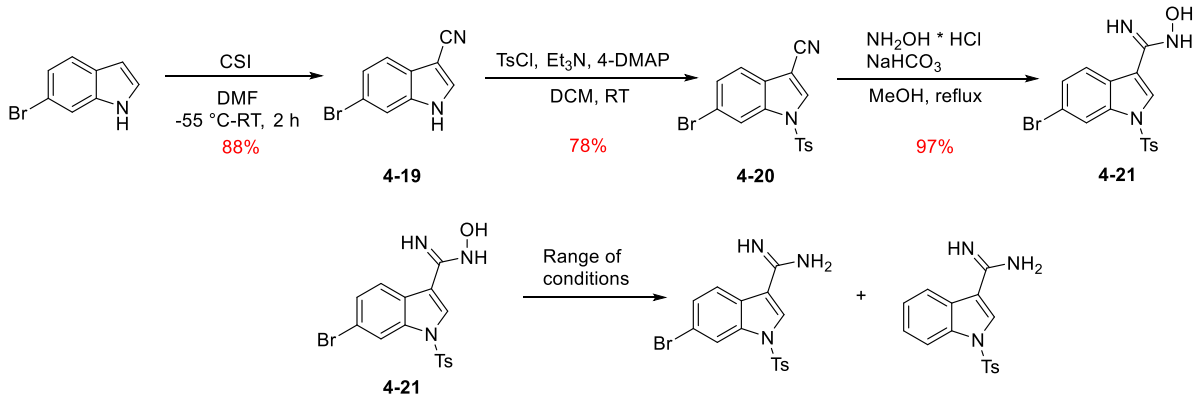


Table 4.1 summarizes efforts towards the reduction. Overall, problems with this method included poor conversion to the desired product or over-reduction, where the 6-bromo

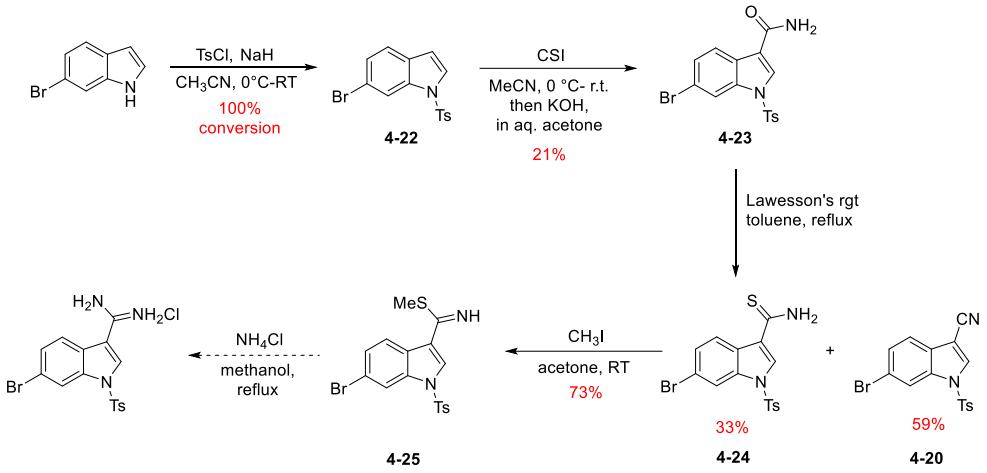
substituent was also reduced under metal-catalysed reductive conditions. Due to its unpredictive nature and poor regioselectivity, this method was not further pursued.

Following the same process used to synthesize **4-12** and **4-19**, the production of *N*-tosyl-indole-3-amidine was attempted, with lessened success (**Scheme 4.7**). Here, 6-bromoindole was *N*-tosylated using 4-toluenesulfonyl chloride and sodium hydride to produce **4-22** in over 100% conversion. Functionalizing the C3 position using CSI, followed by potassium hydroxide in aqueous acetone led to a mere 21% yield of desired product **4-23**, with a 55% recovery of starting material. Upon the addition of Lawesson's reagent, 33% yield of thioamide **4-24** was isolated after 1 hour of reacting and 59% yield of the 3-cyanoindole **4-20** was isolated. This was an interesting observation, as it speaks to the electron withdrawing nature of the *N*-tosylation, encouraging oxidation of the amide/thioamide to a nitrile. The isolated thioamide was converted to imido methyl thiol using methyl iodide, giving 73% of **4-25**. Unfortunately, the last step of this synthetic pathway has yet to be attempted.

Table 4.1 Efforts towards the reduction of 6-bromo-*N*-hydroxy-1-tosyl-1*H*-indole-3-carboximidamide to 6-bromo-*N*-tosyl-1*H*-indole-3-carboximidamide

Reaction conditions	**Yield of carboximidamide (mixture of brominated and debrominated products)**	**Notes**
Raney Ni, H_2, methanol, RT, overnight	NR	No reaction occurred
Acetic anhydride, acetic acid, RT, 30 min 10% $PdCl_2$, triethylsilane, 70 °C, 2 h	40%	Difficult workup- had to isolate as an HCl salt
Acetic anhydride, acetic acid, RT, 10 min Pd/C, H_2, RT, overnight	65%	Product not entirely clean. Evidence of debromination

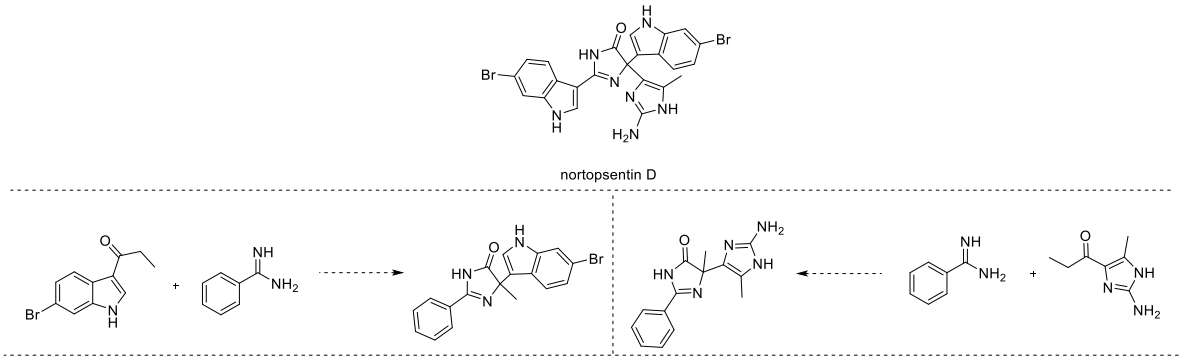
Scheme 4.7 Efforts towards the synthesis of *N*-tosyl-indole-3-amidine



4.2.2 Efforts towards synthesis of proposed ketone intermediate

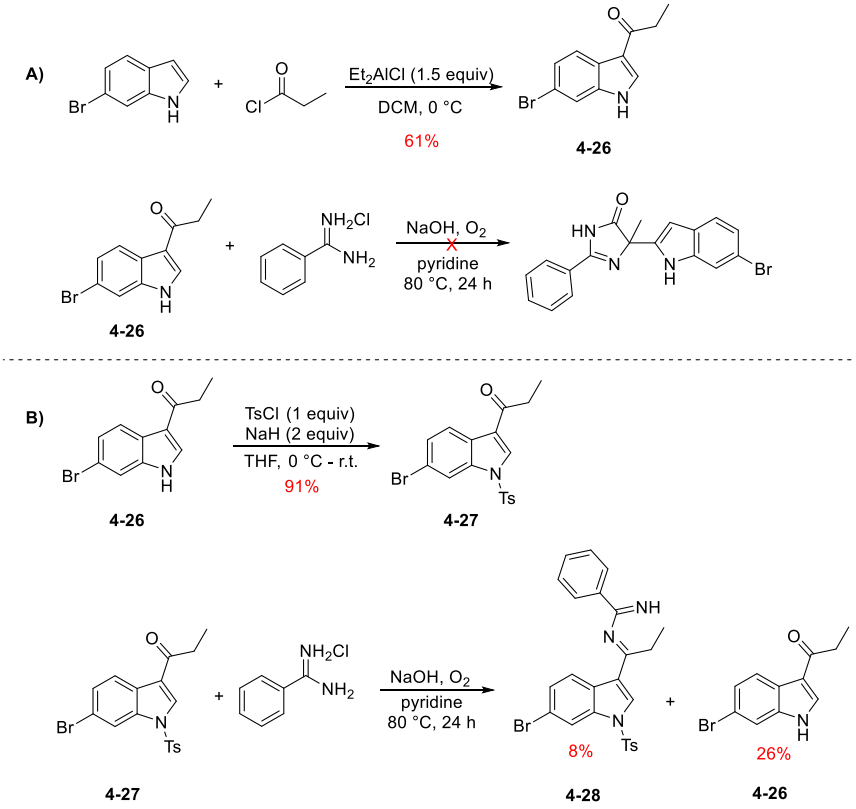
The other key intermediate necessary for this total synthesis was ketone (**4-3**), which combines the 6-bromoindole and 2-amino-4-methylimidazole present on C5 of nortopsentin D. Before exploring the synthesis of **4-3**, I proposed a model system to test the oxidative cyclization of the ketone and amidine. Here, I wanted to test the affect of each of the ketone's heterocycles individually to determine whether the heterocycle's electronics will affect the oxidative cyclization. Therefore, each heterocyclic ketone would be made as a propanone and reacted with benzamidine as a model for the actual cyclization, as described in **Scheme 4.8**.

Scheme 4.8 Proposed model systems to test the oxidative cyclization reaction conditions.



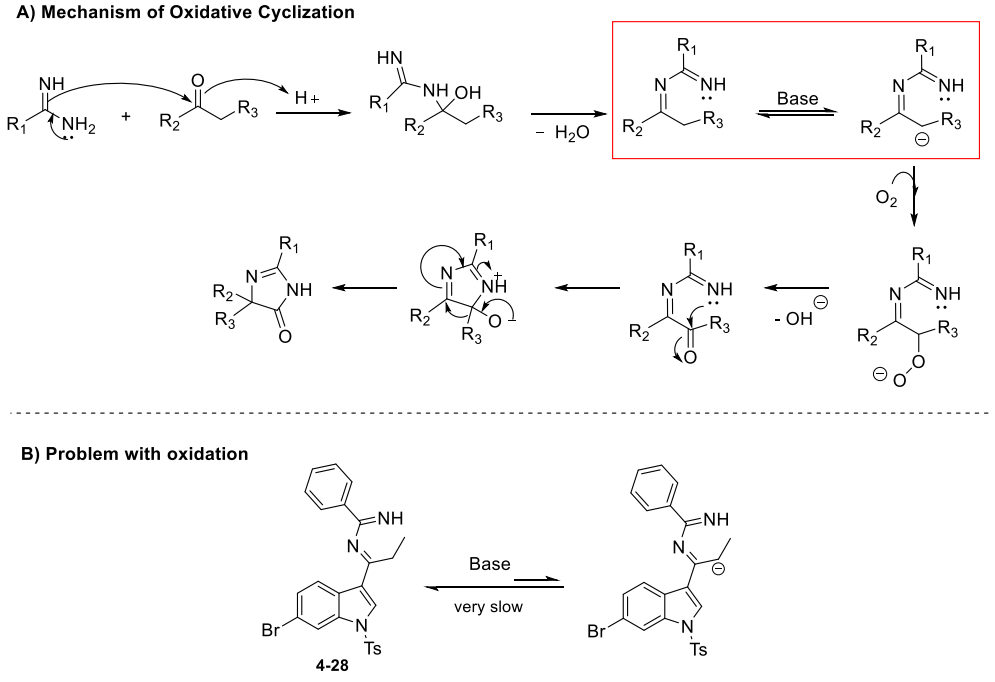
Scheme 4.9 describes the efforts made in testing 1-(6-bromo-1*H*-indol-3-yl)propan-1-one's ability to oxidatively cyclize with benzamidine. 6-bromoindole was coupled with propanal chloride to produce **4-26** in 61% yield. Once formed, cyclization of **4-26** and benzamidine was attempted, but no evidence of product formation was observed. One hypothesis as to why the cyclization failed is that the ketone was not a good enough electrophile for condensation due to the electron rich nature of the indole-conjugated carbonyl. To test this hypothesis, the indole's electron density was reduced by protecting the free nitrogen with a tosyl protecting group (**4-27**). Cyclization was attempted once again, and the isolated products included 26% yield of de-tosylated indole (**4-26**) (> 50% of starting material) and a minor amount of di-imine product **4-28** (8%). No imidazol-4-one product was observed or isolated.

Scheme 4.9 Attempts at condensation of 1-(6-bromo-1*H*-indol-3-yl)propan-1-one and benzamidine



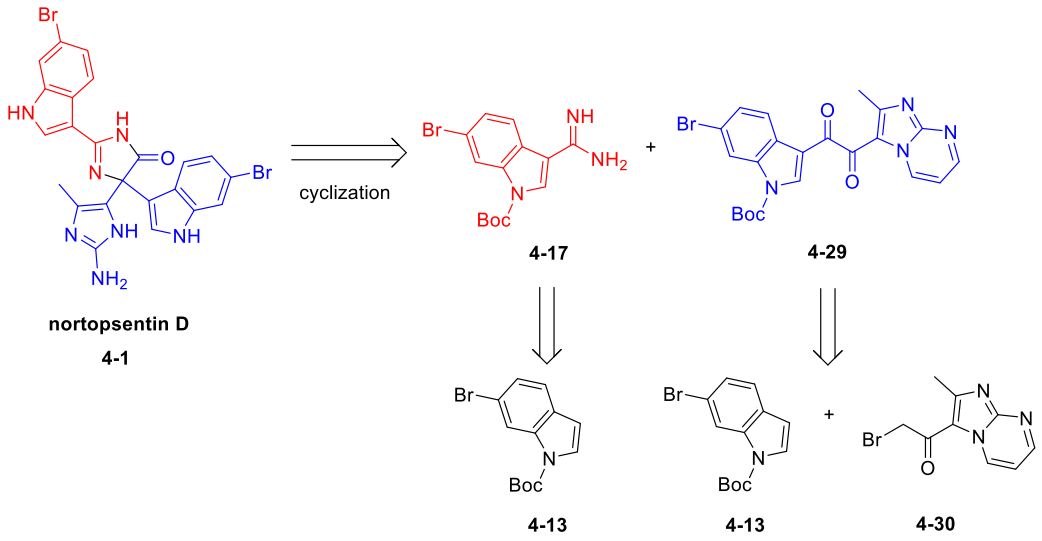
Unsuccessful formation of an imidazol-4-one product suggests there are complications with this method. First, the tosyl protecting group appears to be deprotected over time upon the addition of base and heat. Secondly, the isolated diimine sparks intrigue, as it suggests the condensation is taking place when the tosyl group is intact, however, the subsequent oxidation of the α-methylene did not take place. The mechanism for this reaction can be seen in **Scheme 4.10A**. Literature suggests the rate determining step for anionic autoxidations is the deprotonation of the methylene alpha to the diimine.[21–24] If oxidation is not occuring, it is likely due to a hinderance in the base's ability to deprotonate that alpha carbon (**Scheme 4.10B**). Here, it is believed the deprotonation of the methylene carbon is energetically unfavorable, even at elevated temperatures, due to the necessity to break a highly conjugated system in order to stablize the resultant anion via an enamine tautomer.

Scheme 4.10 Mechanism of the oxidative cyclization reaction to form an imidazol-4-one and the proposed problem with oxidation of **4-28.**



It became evident that the retrosynthetic plan for the synthesis of nortopsentin D would need to be altered to overcome this barrier towards oxidation. Thus, the second-generation retrosynthetic plan can be seen in **Scheme 4.11**. One change to this plan was an oxidation of ketone to dione before condensation of amidine and dione occurs. This should theoretically solve the oxidation problem observed in the first-generation retrosynthesis. Additionally, a range of different protective groups have been added to this retrosynthetic plan, including the protection of the free nitrogens on the indoles and the free nitrogens of 2-amino-4-methylimidazole. This modification was proposed to improve solubility of intermediates within a range of organic solvents, prevent undesirable side reactions, and ease the isolation/purification process throughout the synthetic pathway.

Scheme 4.11 Revised retrosynthesis for the total synthesis of nortopsentin D

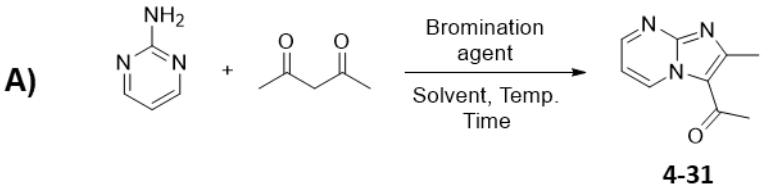


4.2.3 Efforts towards the synthesis of dione intermediate

Herein efforts towards the synthesis of dione **4-29** are described. First 2-bromo-1-(2-methylimidazo[1,2-a]pyrimidin-3-yl)ethan-1-one (**4-31**) was made. This protected form of the 2-amino-4-methylimidazole at C5 of nortopsentin D was proposed to improve the solubility of the intermediates as well as simplify isolation and purification. Additionally, the α-bromoketone is a

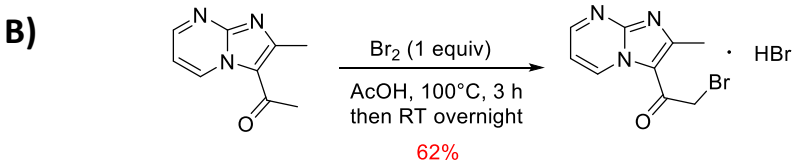
good handle for coupling with 6-bromoindole. Several different literature procedures were used when trying to optimize this reaction (**Scheme 4.12A**).[25,26] Here, 2,4-pentanedione was first brominated using a variety of bromination reagents and then cyclized with 2-aminopyrimidine to produce **4-31**. Most of the conditions screened led to roughly the same yield, varying only by about 10%. The final reaction conditions are CBr_4 in acetonitrile, heating at 75°C for 24 hours, which gave 32% yield of product, unfortunately much lower than literature reports.[25] The lower yields of this cyclization are attributed to the substrate's affinity for water and silica, making isolation of the product difficult. The methyl ketone was then brominated, following known literature examples for the bromination of methyl ketones (**Scheme 4.12B**).[27,28] Here, heating the methyl ketone with bromine in acetic acid produced **4-30** as a monohydrobromide in 62% yield.

Scheme 4.12 Production of 2-bromo-1-(2-methylimidazo[1,2-a]pyrimidin-3-yl)ethan-1-one



4-31

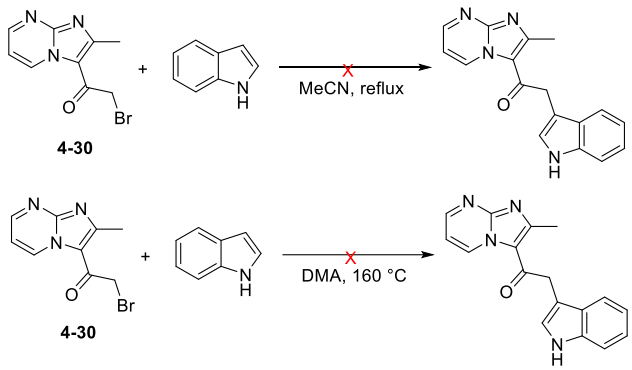
Bromination agent	Solvent	Temperature	Time	Yield
NBS (1.2 equiv.)	Water	80 °C	1 h	23%
NBS (1.2 equiv.)	Methanol	Reflux	3 h	24%
NBS (1.2 equiv.)	Water	Reflux	4 h	29%
CBr_4 (2 equiv.)	Acetonitrile	75 °C	24 h	32%



Once **4-30** was synthesized, several different methods were attempted in coupling the α-bromoketone to indole. First, **4-30** (desalted) and indole were stirred in either acetonitrile or dimethylacetamide while heating (**Scheme 4.13**), monitoring for any product formation via TLC.

Unfortunately, the only compounds recovered from these two reactions were starting materials. There was no evidence of coupled product.

Scheme 4.13 Attempts at coupling 2-bromo-1-(2-methylimidazo[1,2-a]pyrimidin-3-yl)ethan-1-one and indole upon the addition of heat.

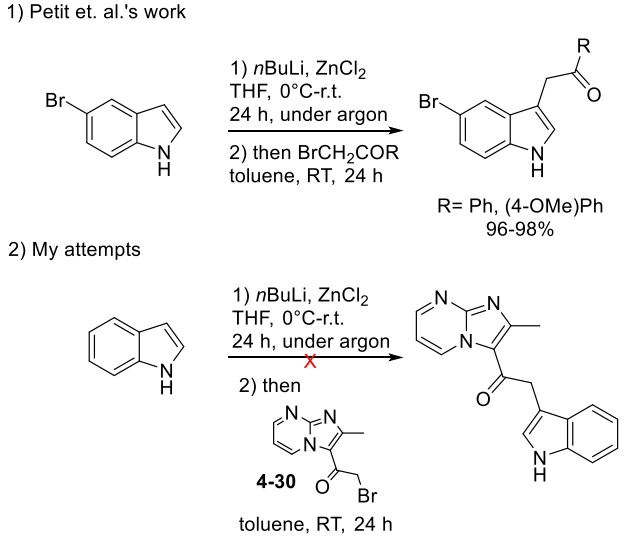


Another attempted method for coupling the α-bromoketone to indole was inspired by a paper by Petit et. al.[29] where they synthesized a ketone from indole and α-brominated acetophenone via a Friedel-Crafts type reaction using *n*BuLi and $ZnCl_2$. **Scheme 4.14** displays my attempt at this reaction, which unfortunately failed. It is believed this method did not work due to **4-30**'s lack of solubility in toluene, which hindered the inert atmosphere, as the vessel was opened to add **4-30**. The exposure to air disrupted the $ZnCl_2$-indole complex, preventing the coupling from occurring. No other solvents were used for this reaction; however, I do believe more exploration on this reaction may have led to the desired coupled product.

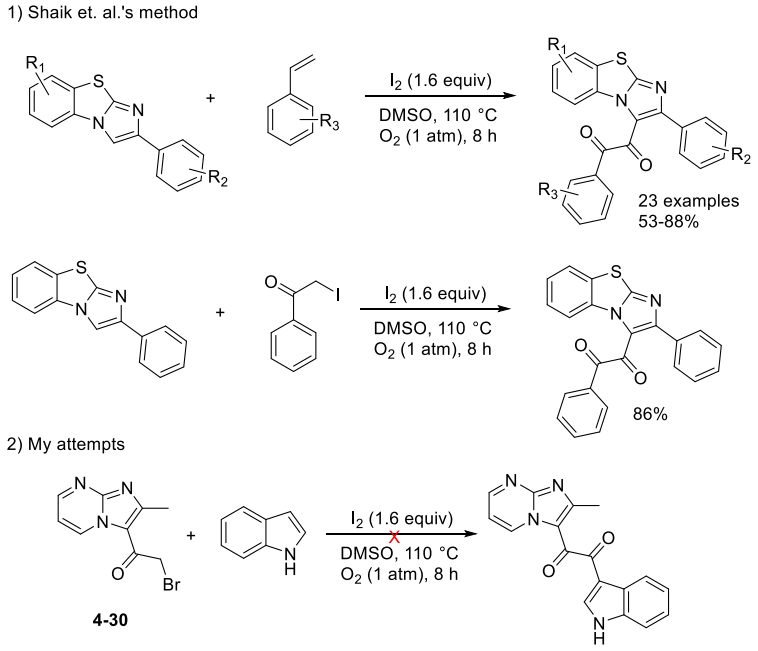
Several attempts were also made at first oxidizing the α-bromoketone to a dione and then coupling to indole. **Scheme 4.15** summarizes these efforts, using a method adapted from Shaik et. al.'s work.[30] Here, they reacted styrene and an α-iodoketone with benzo[*d*]imidazo[2,1-*b*]thiazoles through an oxidative cross coupling to produce a dione. The proposed mechanism of this reaction first involves a Kornblum oxidation[31] to produce an α-keto aldehyde, which then undergoes coupling activated by the aldehyde's coordination with iodine. When attempted on my scaffold, the

reaction failed, and no product formation was observed. This reaction was not explored further, as other methods were also underway.

Scheme 4.14 Attempted zinc-mediated coupling of indole to alpha-bromomethyl ketone.



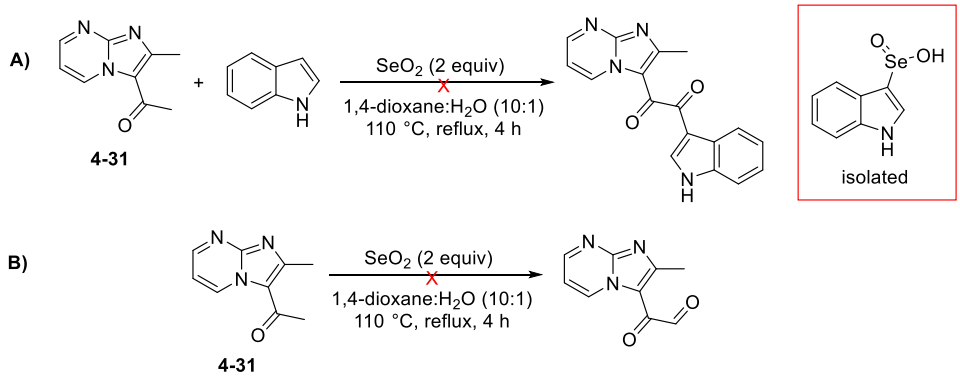
Scheme 4.15 Attempts at coupling via Kornblum oxidation



Based on the idea of oxidation followed by coupling, the use of selenium dioxide as an oxidation source was also attempted. **Scheme 4.16A** describes the first attempt at this reaction. Unfortunately, it was observed that selenium dioxide solely reacted with indole to produce a

selenic acid instead of oxidizing **4-31**. To test if the oxidation would occur without indole present, **4-31** was reacted with selenium dioxide, as shown in **Scheme 4.16B**. This reaction also failed, showing no evidence of the desired oxidation. Interestingly, literature suggests methyl ketones typically oxidize quite well with the help of selenium dioxide. At this time, it was also discovered that the methyl of the imidazole can also oxidize to an aldehyde upon the influence of selenium dioxide.[32]

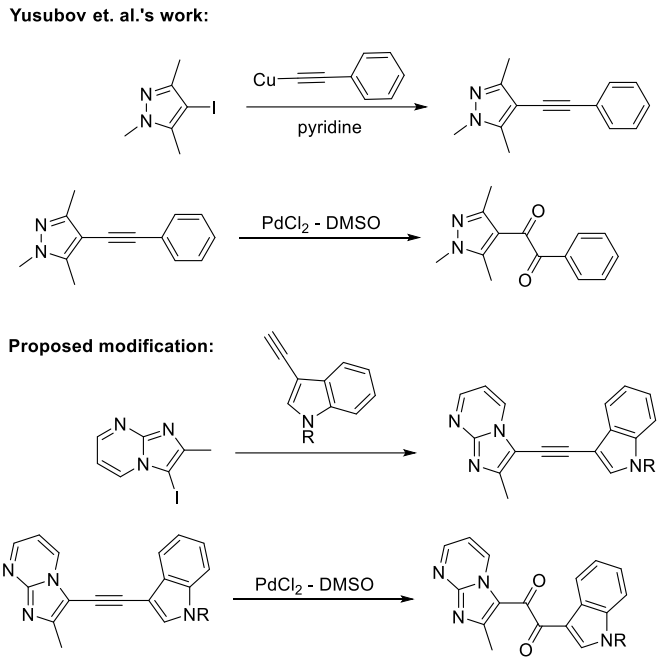
Scheme 4.16 Attempts at oxidation of methyl ketone using selenium dioxide



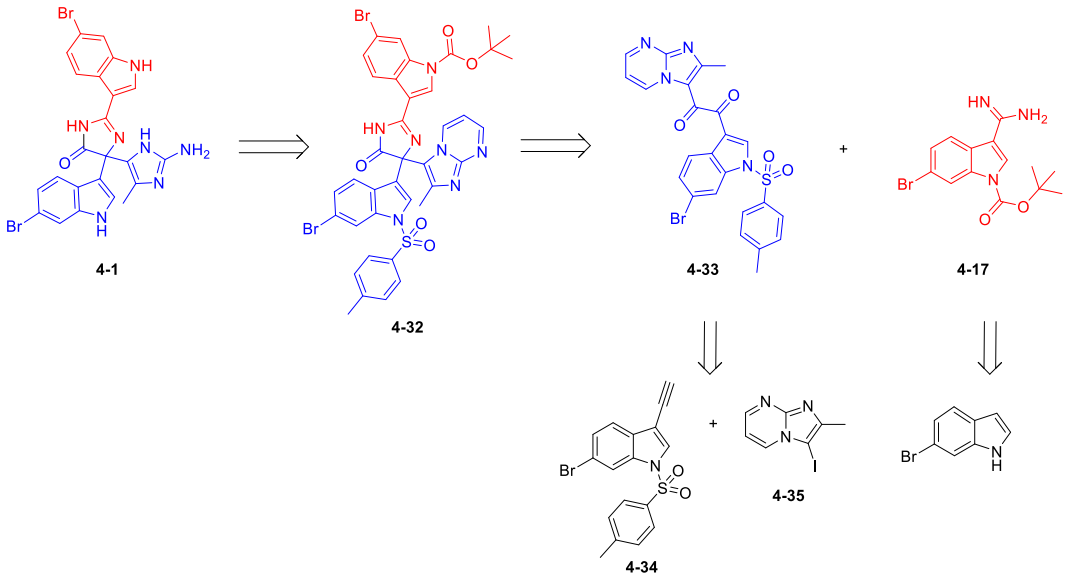
After the abovementioned attempts failed, it appeared a different approach was necessary to form the desired dione intermediate; specifically, the approach had to be a regioselective oxidation, to avoid oxidation of the methyl substituent on the 2-aminoimidazole. One proposed route was designed based on a paper by Yusubov, M. S. et. al.[33] where they synthesized unsymmetrical heteroaryl-1,2-diones through the oxidation of an internal alkyne. Here, aryl-alkynes and 4-iodopyrazoles were coupled via a copper-catalyzed reaction. The alkyne was then oxidized to a dione using $PdCl_2$ and DMSO. Interestingly, many of the reported pyrazoles and heterocycles contained methyl at oxidizable positions but were unoxidized via this regioselective process. **Scheme 4.17** summarizes their method as well as highlights a modified process for the total synthesis of nortopsentin D.

Based on the proposed modification shown in **Scheme 4.17**, a third-generation retrosynthetic plan was proposed (**Scheme 4.18**). The changes in retrosynthesis reflect the new intermediates required to perform an alkyne-aryl coupling reaction.

Scheme 4.17 Yusubov et. al.'s work with proposed modifications to produce the desired dione intermediate for the total synthesis of nortopsentin D.

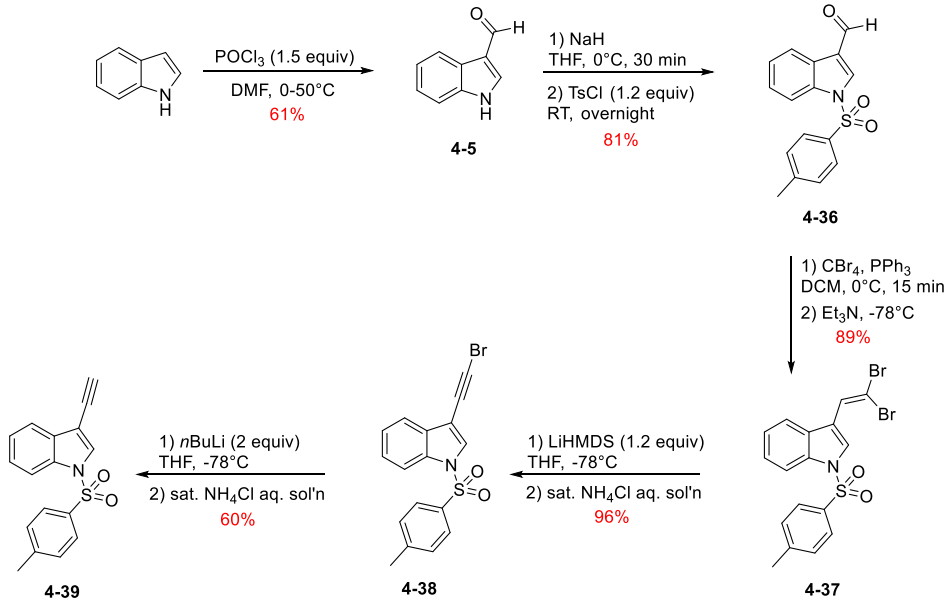


Scheme 4.18 Final retrosynthetic plan for the total synthesis of nortopsentin D



The first step in this new pathway was to produce the terminal alkyne and aryl iodide. **Scheme 4.19** describes the synthesis of 3-ethynyl-1-tosyl-1*H*-indole, adapted from Gagnon and Spino's synthesis of (+)-aspidofractinine.[34] As with previous methods, optimization of reactions was performed on indole instead of the more expensive 6-bromoindole substrate. First, phosphorus oxychloride was used to produce indole-3-carbaldehyde in 61% yield. Next, *N*-tosylation was performed using sodium hydride and 4-toluenesulfonyl chloride, providing 81% yield of the **4-36**. The aldehyde was then converted to a dibromo alkene via Wittig reaction with carbon tetrabromide and triphenylphosphine (89% yield).[34,35] The dibromo alkene was converted to a bromo-alkyne using lithium bis(trimethylsilyl)amide, which was subsequently converted to a terminal alkyne using n-butyl lithium.[36] This synthesis produced 3-ethynyl-1-tosyl-1*H*-indole (**4-39**) in 5 steps with a 25% overall yield.

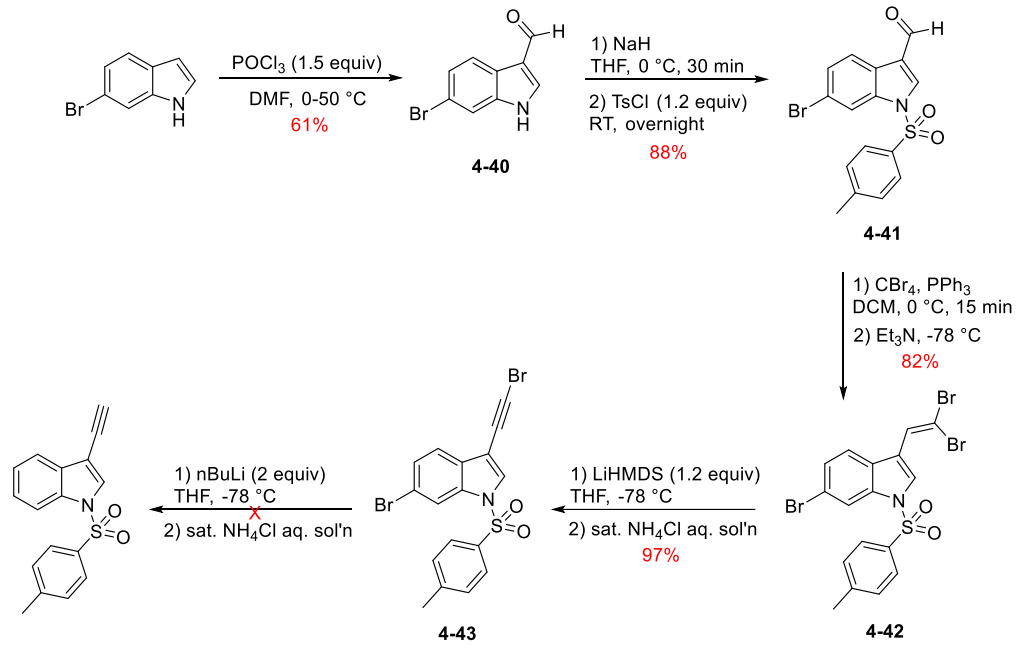
Scheme 4.19 Synthetic pathway to 3-ethynyl-1-tosyl-1*H*-indole (**4-39**)



Once optimized, the pathway displayed in **Scheme 4.19** was performed starting from 6-bromoindole (**Scheme 4.20**). Reaction yields were comparable to those reported in **Scheme 4.19**, however, it was discovered that *n*-butyl lithium reduced both the bromo alkyne and the 6-bromo

substituent on indole. This was determined via NMR and mass spectroscopy. Unfortunately, this method was not a valid pathway to synthesizing the desired alkyne.

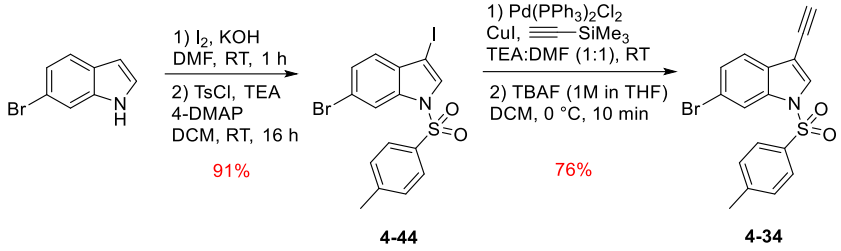
Scheme 4.20 Failed synthetic pathway to 6-bromo-3-ethynyl-1-tosyl-1*H*-indole



To avoid the debromination, **Scheme 4.21** displays the alternative method used to synthesize **4-34**. Starting with 6-bromoindole, 6-bromo-3-iodo-1-tosyl-1*H*-indole (**4-44**) was produced via iodination and subsequent *N*-tosylation, under the influence of triethylamine (TEA) and 4-dimethylaminopyridine (4-DMAP) and upon the addition of 4-toluenesulfonlyl chloride. This was followed by the Sonogashira coupling of trimethylsilylethyne under standard conditions, with a subsequent removal of the trimethylsilane using tetrabutylammonium fluoride (TBAF). This reaction produced the terminal alkyne **4-34** in 76% yield.[37,38] It should be noted that when the Sonogashira coupling was run at 60°C,[37,38] yields were considerably lower due to poor regioselective coupling at the C3-iodo versus C6-bromo positions. Reduction of the temperature to room temperature led to better control over the coupling regioselectivity,

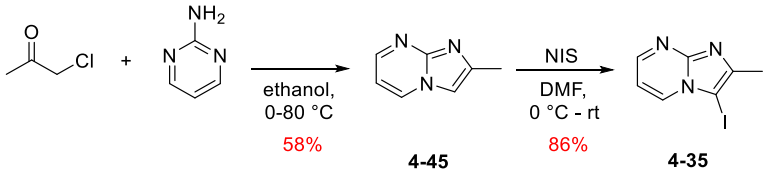
significantly favoring the desired C3 position. This two-step pathway led to the desired terminal alkyne **4-34** in an overall yield of 69%.

Scheme 4.21 Final synthetic pathway to 6-bromo-3-ethynyl-1-tosyl-1*H*-indole



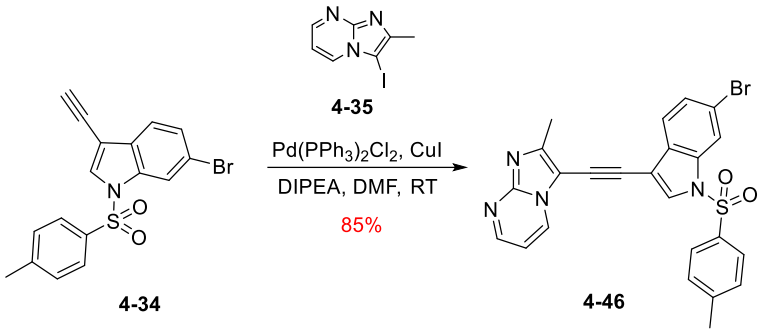
The other half of the dione began with the condensation of 1-chloroacetone and 2-aminopyrimidine, upon addition of heat, to produce 3-methylimidazo[1,2-a]pyrimidine (**4-45**) in 58% yield. This bicycle represents a protected form of the 4-methyl-1*H*-imidazol-2-amine observed on C5 of nortopsentin D. Iodination of 3-methylimidazo[1,2-a]pyrimidine with *N*-iodosuccinimide (NIS) led to the desired aryl iodide **4-35** in two steps with an overall yield of 50% (**Scheme 4.22**).

Scheme 4.22 Synthesis of 3-iodo-2-methylimidazo[1,2-a]pyrimidine



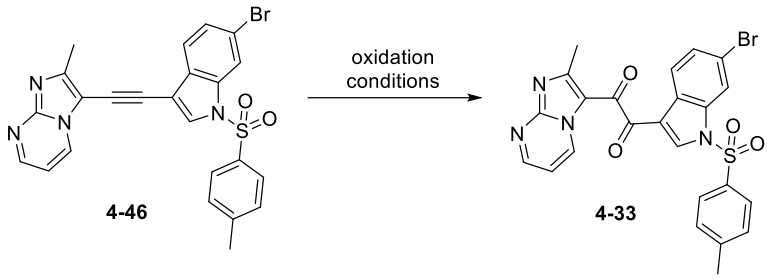
Once 3-iodo-2-methylimidazo[1,2-a]pyrimidine and 6-bromo-3-ethynyl-1-tosyl-1*H*-indole were produced, they were coupled together using a Sonogashira coupling reaction to produce **4-46** in 85% yield (**Scheme 4.23**).

Scheme 4.23 Sonogashira coupling of 3-iodo-2-methylimidazo[1,2-a]pyrimidine and 6-bromo-3-ethynyl-1-tosyl-1*H*-indole



Alkyne **4-46** underwent extensive experimentation to identify the best conditions for the oxidation of internal alkyne to dione. Unfortunately, it was found that the alkyne proved to be unstable under harsh oxidative conditions and high temperatures. Exposure of alkyne to a range of conditions (e.g. $KMnO_4$/TBAB,[39] $ICl/AgNO_3$,[40] $Pd(OAc)_2/AlCl_3$/DMSO/70-110 °C,[41] $PdCl_2$/DMSO/140 °C,[33] $RuCl_3/PhI(OAc)_2$,[42] 2-chloropyridine-*N*-oxide/$Ph_3PAuNTf_2$/75-85 °C[43,44]) led to low yields and a variety of complications, including decomposition of the starting material, loss of the tosyl protecting group and in some cases, over-oxidation. Additionally, the yields tended to be inconsistent, and some reactions were unrepeatable. These results are summarized in **Table 4.2**. The limitations of high temperature and harsh oxidation conditions were successfully overcome by use of mercuric nitrate monohydrate as an oxidation source. Within 8 hours at room temperature under air, 49% yield of the desired dione was produced. Additionally, the reaction could go for up to 16 hours without any change in yield or evidence of overoxidation. This method was originally reported by Jung and Deng in 2014 and later used as part of the total synthesis of lissodendrin B.[45,46] This oxidation proved to be a reliable, mild way of forming the desired dione intermediate.

Table 4.2 Optimization of the oxidation of alkyne to dione

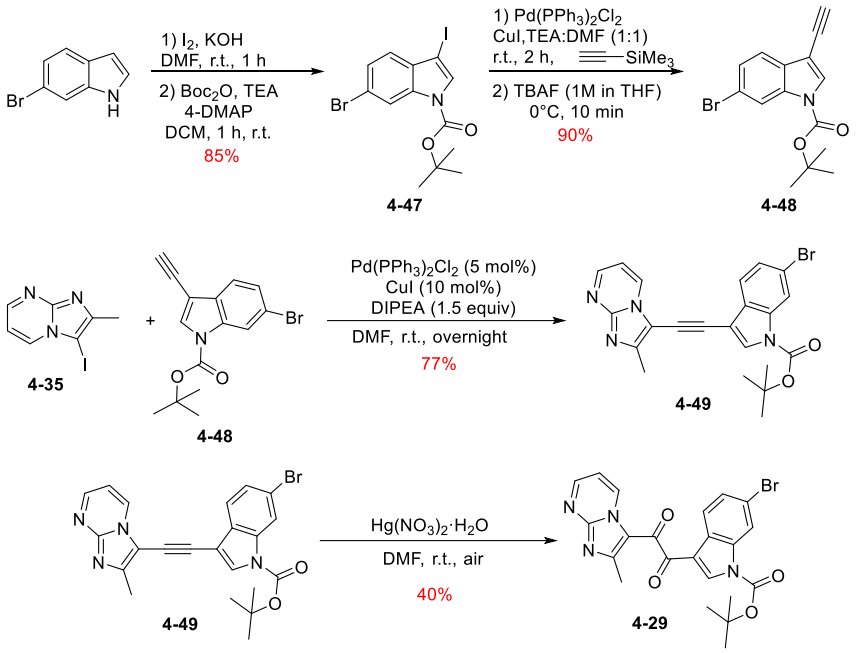


Entry	Oxidation reagents[a]	Temperature and time	Yield (%)
1	$KMnO_4$, TBAB, CH_2Cl_2	0 °C - RT, 5 h	NA
2	ICl, $AgNO_3$, CH_3CN	RT, 16 h	NA
3	$Pd(OAc)_2$, $AlCl_3$, DMSO	70 °C, 24 h	N.R.
4	$Pd(OAc)_2$, $AlCl_3$, DMSO	90 °C, 24 h	20
5	$Pd(OAc)_2$, $AlCl_3$, DMSO	110 °C, 24 h	12
6	$PdCl_2$, DMSO-*d*	140 °C, 5 h	30
7	$RuCl_3$, $PhI(OAc)_2$ CH_2Cl_2:H_2O (4:1)	RT, 30 min	19
8	2-chloropyridine-*N*-oxide [Au] cat. (10 mol%), CH_2Cl_2	reflux, 48 h in a pressure tube	19
9	2-chloropyridine-*N*-oxide [Au] cat. (10 mol%), $ClCH_2CH_2Cl$	75 °C, 48 h in a pressure tube	39[b]
10	2-chloropyridine-*N*-oxide [Au] cat. (10 mol%), $ClCH_2CH_2Cl$	85 °C, 48 h in a pressure tube	50[b]
11	$Hg(NO_3)_2 \cdot H_2O$ DMF	RT, 16 h	49

[a]Reaction conditions were based on previously reported literature examples, as referenced in the text. [b]Reaction yield was not consistent, and these results could not be repeated.

The dione formation pathway was also performed starting with boc-protected indole (**Scheme 4.24**), completed in four steps with an overall yield of 24%. The reasoning for using making the boc-protected dione for this was to allow for further exploration of cyclization conditions, as described in **section 4.2.4**.

Scheme 4.24 Synthesis of 6-bromo-3-ethynyl-1-boc-1*H*-indole

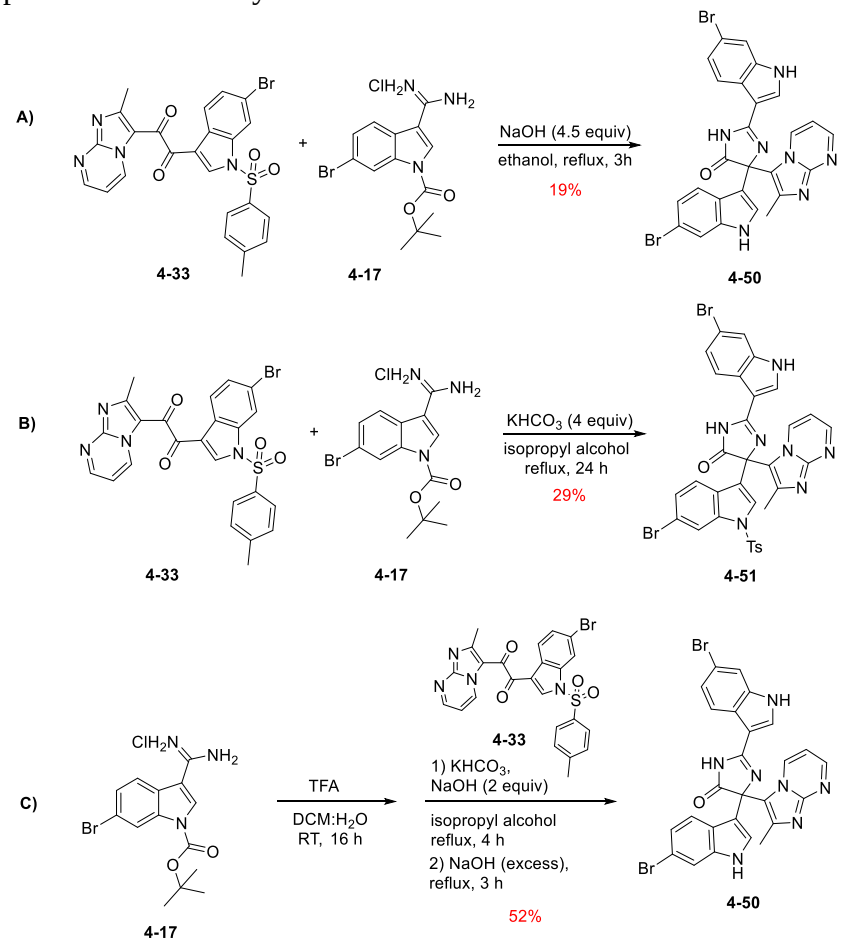


4.2.4 Final steps

Once amidine (**4-17**) and dione (**4-33**) were synthesized via reliable and multi-gram scalable routes, the formation of the core (4*H*)-imidazol-4-one was performed. Efforts in this cyclization are summarized in **Scheme 4.25**. First, following standard conditions reported for this condensation, the amidine and dione were reacted under basic conditions, using excess sodium hydroxide and refluxed in ethanol over a period of 3 hours.[47] The procedure provided 19% yield of the desired imidazol-4-one product (**Scheme 4.25A**), where both the *N*-boc and *N*-tosyl protecting groups were removed during the reaction. Interestingly, the main side product from this reaction was de-tosylated dione, which remained uncondensed even upon heating over an extended period. It was theorized that the presence of indole's *N*-tosyl, and correlatively the added electrophilicity, may be necessary for condensation with amidine. This hypothesis was tested by using a weaker, less nucleophilic base (potassium bicarbonate) and less nucleophilic solvent (isopropanol) in an effort to avoid *N*-tosyl deprotection (**Scheme 4.25B**). After refluxing

for 24 hours, 29% yield of cyclized product **4-51** was collected, where the indole's *N*-tosyl remained intact, supporting this hypothesis.

Scheme 4.25 Optimization of the cyclization of amidine and dione



Curiously, it was observed that the only cyclized product formed in **Scheme 4.25A** and **Scheme 4.25B** had lost the amidine's *N*-boc protecting group. This prompted a new hypothesis regarding the nucleophilic nature of the amidine: *N*-boc deprotection of the indole must occur before the amidine will condense with dione. **Scheme 4.25C** describes how this theory was tested. First, *N*-boc deprotection of amidine was performed, using trifluoroacetic acid (TFA). After reacting for 16 hours, volatiles were removed, and the reaction was basified (pH=8) in isopropanol using an excess of potassium bicarbonate and a minimal amount (2 equivalents) of sodium hydroxide. Once the solution was basic, dione was introduced into the reaction, and it

was heated until cyclization was complete. To simplify the isolation of this reaction, *N*-detosylation was also performed in the same pot, by adding excess sodium hydroxide and heating until the deprotection is complete. Overall, this modified procedure produced 52% yield of the desired imidazol-4-one product. Through this optimization process, it was determined that the condensation of amidine and dione is susceptible to changes in electron densities and can be manipulated to improve conversion to product. Even under the optimized conditions, the main observed side product formed was detosylated dione. More exploration into the stability of the tosyl protection group under basic conditions may lead to a further increase in conversion to the desired cyclized product.

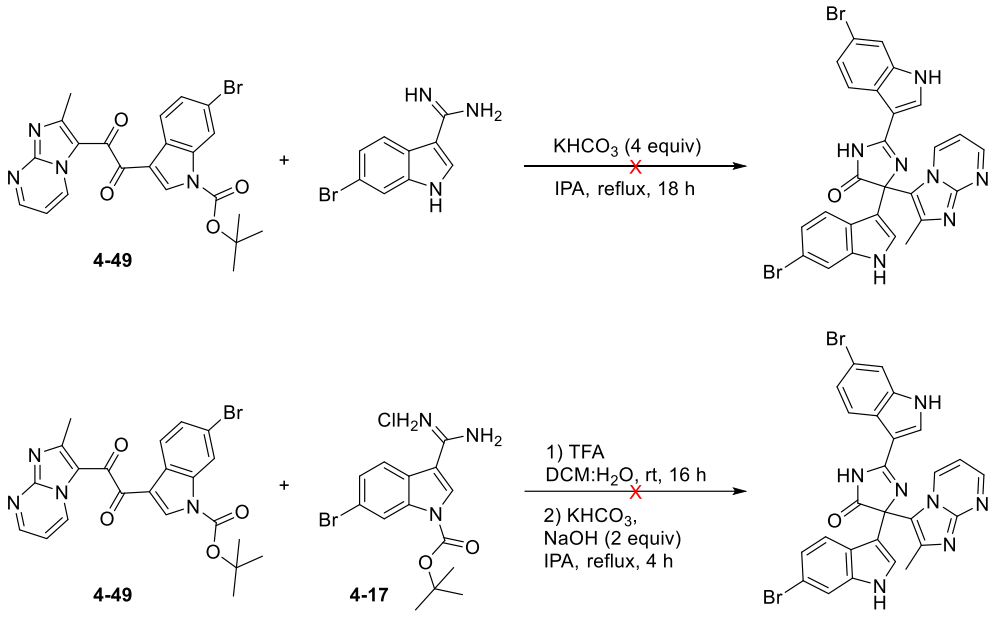
Table 4.3 summarizes all the conditions attempted during the optimization of the cyclization of amidine and dione. One of the most frustrating parts of this optimization was ensuring the amidine had been fully deprotected before the addition of dione; in fact, trifluoroacetic acid did not appear to completely deprotect the *N*-boc protecting group, even after 24 h. Another method used for the *N*-boc deprotection came from a paper specifically focused on the deprotection of *N*-boc from heteroarenes.[48] Unfortunately, it was found that in the presence of K_2CO_3 and water, the amidine hydrolyzes to an amide. In entry **9**, it was discovered that a majority of the amidine had been hydrolyzed to amide, and only 29% yield of cyclized product was isolated. This may also explain why the other reactions containing K_2CO_3 or Na_2CO_3 failed to provide cyclized product as well (entries **7** and **8**). Interestingly, refluxing the amidine with sodium hydroxide overnight before the addition of dione did lead to an increase in yield (entries **3** and **4**) as compared to adding amidine, sodium hydroxide and dione all at once, as in entry **1**. This suggests that under the influence of base and heat, some *N*-boc deprotection was occurring over time.

Table 4.3 Conditions attempted for the cyclization of amidine and dione

Entry	Reaction conditions	Yield of *N*-Ts-protected product (%)	Yield of deprotected product (%)	Notes
1	NaOH (4.5 equiv.) Ethanol, reflux, 4 h	NA	19	
2	$KHCO_3$ (4 equiv.) IPA, reflux, 18 h	29	NA	
3	1. Amidine, NaOH (2 equiv.), IPA, reflux, 18 h 2. Add dione, reflux, 2.5 h	38	NA	
4	1. Amidine, NaOH (2 equiv.), IPA, reflux, 18 h 2. Add dione, reflux, 7 h	35	NA	
5	1. Amidine, TFA, DCM:H_2O, rt, 24 h 2. Dione, NaOH (1.2 equiv.), $KHCO_3$ (15.4 equiv.), IPA, reflux, overnight 3. NaOH (3 equiv.), reflux, 2 h	NA	52	Three step process-repeated with similar results. This reaction is very dependent on the pH of the solution upon addition of NaOH. You must ensure the pH is around 8/9.
6	1. Amidine, TFA, DCM:H_2O, rt, 24 h 2. Dione, KOtBu (pH 8), IPA, reflux, 1 h	NA	NA	No evidence of cyclization TLC was messy after 1 hour of refluxing. Excess NaOH was added to the reaction and let the reaction reflux 30 more minutes. Still no cyclization observed.
7	1. Amidine, K_2CO_3 (3 equiv.) Methanol, reflux, 18 h 2. Dione, K_2CO_3 (1 equiv.), RT, 1.5 h	NA	NA	After addition of dione and K_2CO_3, the reaction was stirred at rt for 1.5 h. The TLC showed only deprotected dione present with no more starting material visible. No cyclization was observed.
8	Na_2CO_3 (1.6 equiv.) IPA, reflux, 18 h	NA	NA	Only deprotected dione isolated
9	1. Amidine, K_2CO_3 (3 equiv.), Methanol:H_2O, reflux, 18 h 2. Dione, IPA, reflux, 3 h	29	NA	Amidine hydrolyzed to amide under these conditions. 24% yield of deprotected dione was also isolated.

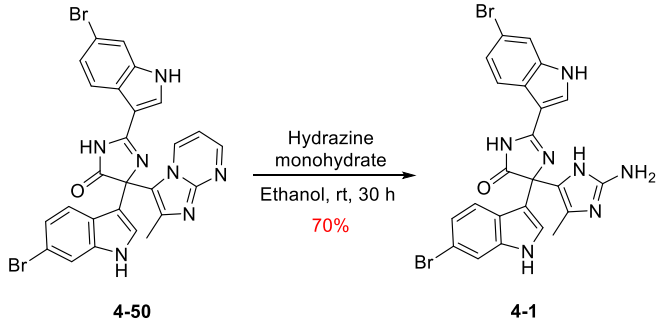
Lastly, an *N*-boc protected dione was also used for this cyclization reaction (**Scheme 4.26**). This reaction was attempted twice, and unfortunately no cyclization occurred. The only products isolated from this reaction were deprotected dione and amidine. It is believed that the *N*-boc group is not electron-withdrawing enough to encourage condensation of the amidine and dione, and thus the reaction just underwent boc-deprotection instead.

Scheme 4.26 Cyclization reactions using *N*-boc protected dione



Once the cyclization was optimized, I moved onto the last step of this total synthesis. Here, 3-methylimidazo[1,2-a]pyrimidine of was deprotected using hydrazine monohydrate, affording the title compound, nortopsentin D, in 70% yield (**Scheme 4.27**).

Scheme 4.27 Final step in the total synthesis of nortopsentin D

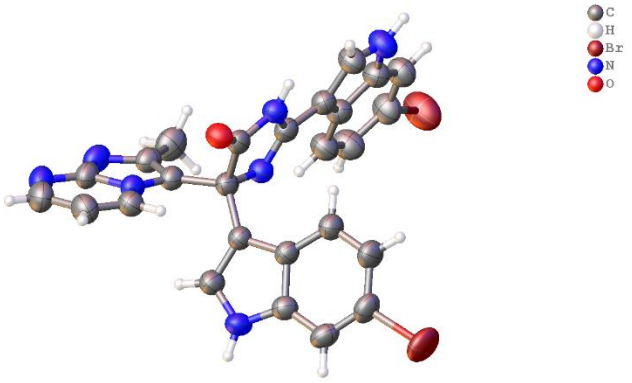


Spectroscopic data obtained from nortopsentin D is in full agreement with the original isolation paper (see **Table 4.4** for a tabulated spectral comparison of nortopsentin D to isolated natural product).[1] Slight changes in ppm shifts may be caused by a variation in the concentration of added TFA. Interestingly, and as Pietra and co-workers reported, several carbon peaks associated with the (4*H*)-imidazol-4-one ring were very broad and only made visible through enhanced apodization (exponential = 8 Hz). To further confirm the presence of the central heterocyclic ring, X-ray crystallography was used to determine the crystal structure of cyclized product (**Figure 4.2**). The X-ray crystallographic data confirms the presence of a central (4*H*)-imidazol-4-one ring. Additionally, the crystal confirmed the racemic nature of this natural product. As mentioned within the isolation paper, they were unable to determine any chiroptical data from the natural product, however it is believed that the biogenesis for this metabolite would lead to a racemate.[1]

Table 4.4 Tabulated spectral comparison of nortopsentin D to the isolated natural product

Spectrum	**nortopsentin D (1)**	**Isolated natural product**
^{1}H NMR (ppm in DMSO-d_6)	δ 12.10 (s, br., 1H), 11.99 (s, br., 1H), 11.26 (s, br., 2H), 8.28 (d, *J* = 8.5 Hz, 1H), 8.23 (s, 1H), 7.73 (d, *J* = 1.8 Hz, 1H), 7.61 (d, *J* = 1.8 Hz, 1H), 7.55 (d, *J* = 8.3 Hz, 1H), 7.34 (d, d, *J* = 8.5, 2.1 Hz, 2H), 7.14 (dd, *J* = 8.6, 1.8 Hz, 1H), 6.93 (s, br., 2H), 2.03 (s, 3H).	δ 12.93 (s, br, 1H), 12.09 (s, br, 1H), 11.43 (s, br, 1H), 8.35 (s, 1H), 8.26 (d, J = 8.7 Hz, 1H), 7.75 (d, J = 1.5 Hz, 1H), 7.62 (d, J = 1.8 Hz, 1H), 7.50 (d, J = 8.7 Hz, 1H), 7.41 (d, J = 2.7 Hz, 1H), 7.39 (dd, J = 8.7, 1H), 7.16 (dd, J = 8.7, 1.8 Hz, 1H), 7.05 (s, br, 2H), 1.95 (s, 3H).
^{13}C NMR (ppm in DMSO-d_6)	δ 182.58 (br.), 158.50 (br.), 145.90, 137.49, 137.41, 131.04, 124.58, 124.08, 123.99, 123.85, 122.86, 121.78, 121.08, 119.31, 118.65, 115.22, 114.64, 114.15, 114.09, 111.80, 104.56, 67.70 (br.), 9.17.	δ 181.62, 156.75, 145.98, 137.62, 137.58, 130.92, 124.49, 124.39, 124.04, 123.96, 123.15, 121.95, 121.43, 119.23, 118.74, 115.35, 114.82, 114.36, 114.24, 112.06, 104.78, 69.36, 9.53.
Mass Spectroscopy	HRMS (ESI-TOF) m/z [M+H]: 565.9953, 566.9979, 567.9937, 568.9966, 569.9926, 570.9950.	FAB-MS (glycerol, H^+, matrix): 566, 568, 570

Figure 4.2 X-ray crystal structure of cyclized imidazol-4-one containing product **4-50**.



4.3 Conclusions

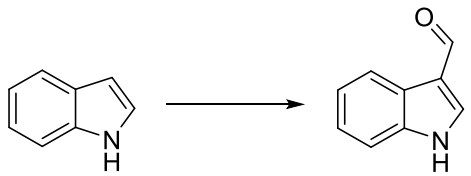
In conclusion, I have accomplished the first total synthesis of nortopsentin D.[49] This highly convergent synthesis only included 7 linear steps with an overall yield of 1.5%. The structure reported in Mancini et. al.'s original isolation report has been confirmed through both NMR and X-ray crystallography. This pathway features a unique method for the formation of the 5,5-disubstituted (4*H*)-imidazol-4-one ring, which can be envisioned for use in multiple other total syntheses. Currently, biological studies on nortopsentin D and some of its intermediates are underway within the Tepe lab.

4.4 Experimental section

General information

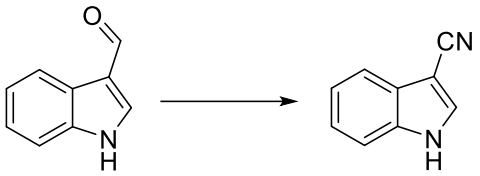
Reactions were carried out under a nitrogen atmosphere in flame-dried glassware. Solvents and reagents were purchased from commercial suppliers and used without further purification, unless otherwise mentioned. Anhydrous THF was distilled over sodium (dryness was monitored by the color of the solution, as indicated by benzophenone's ketyl radical), acetonitrile and triethylamine were distilled over calcium hydride, and toluene and dichloromethane were dried over molecular sieves directly before use. Magnetic stirring was used for all reactions. Yields refer to chromatographically and spectroscopically pure compounds

unless otherwise noted. Infrared spectra were recorded on a Jasco Series 6600 FTIR spectrometer. 1H and 13C NMR spectra were recorded on a Varian Unity Plus-500 or 600 spectrometer. Chemical shifts are reported relative to the residue peaks of the solvent (CDCl3: 7.26 ppm for 1H and 77.0 ppm for 13C) (DMSO-d6: 2.50 ppm for 1H and 39.5 ppm for 13C) (Acetone-d6: 2.05 ppm for 1H and 28.9 ppm for 13C) (CD3OD: 3.31 ppm for 1H and 47.6 ppm for 13C). The following abbreviations are used to denote the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, and m = multiplet. The following abbreviation is used to denote a broad signal: br = broad. HRMS were obtained at the Mass Spectrometry Facility of Michigan State University with a Micromass Q-ToF Ultima API LC-MS/MS mass spectrometer. Column chromatography was performed using a Teledyne ISCO CombiFlash® NextGen system with prepacked columns (RediSep® Normal-phase silica, 20-40 microns and RediSep® Rf Gold Reversed-Phase C18 silica, 20-40 microns). TLCs were performed on pre-coated 0.25 mm thick silica gel 60 F254 plates and pre-coated 150 um thick, C18 reverse phase F254 plates, visualized using UV light and iodine staining.



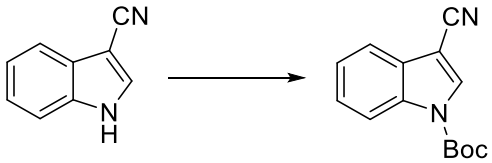
1*H*-indole-3-carbaldehyde (4-5): Indole (3 g, 25.6 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 30 mL of DMF. The reaction was cooled to 0 °C. Then, phosphoryl chloride (5.9 g, 38.5 mmol) was added to the reaction dropwise. The solution was stirred for 2 hours at 55 °C. When complete, the mixture was poured into 40 mL of ice water. A 20% NaOH solution was used to adjust the pH to 9. A precipitate fell out and the produce was filtered off. Crude product was recrystallized from ethanol to give a red solid (2.77 g, 75% yield).

^{1}H NMR (500 MHz, DMSO-*d6*) δ 12.13 (s, br., 1H), 9.93 (s, 1H), 8.27 (s, 1H), 8.09 (d, *J* = 7.3 Hz, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.22 (m, 2H). ^{13}C NMR (126 MHz, DMSO-*d6*) δ 185.03, 138.53, 137.10, 124.16, 123.51, 122.18, 120.88, 118.21, 112.47. IR (neat): 1628, 1611, 1575, 1520 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+H)$^{+}$] calc for ($C_9H_8NO^{+}$): 146.0606 Found: 146.0624. m.p.: 195 °C.



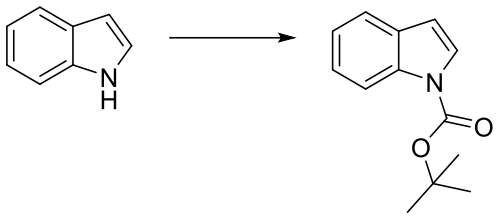
1*H*-indole-3-carbonitrile (4-6): Indole-3-carbaldehyde (0.5 g, 3.4 mmol) was added to a clean, dry round bottom under nitrogen and was dissolved in 5 mL DMF. Hydroxylamine hydrochloride (0.36 g, 5.2 mmol) was added to the reaction. The reaction stirred at 110 °C and monitored for completion via TLC. When complete, the reaction was cooled to room temperature and then poured into ice water (30 mL). The precipitate was then filtered off and washed with water. The crude product was recrystallized from ethanol to produce a red solid (0.36 g, 75% yield).

^{1}H NMR (500 MHz, Acetone-*d6*) δ 10.14 (s, br., 1H), 8.01 (d, *J* = 3.1 Hz, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 1H), 7.46 – 7.34 (m, 2H). ^{13}C NMR (126 MHz, Acetone-*d6*) δ 135.32, 133.63, 127.07, 123.84, 122.02, 118.86, 115.92, 112.80, 85.63. IR (neat): 3220, 2226, 1524 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+H)$^{+}$] calc for ($C_9H_7N_2^{+}$): 143.0609 Found: 143.0617. m.p.: 176 °C



tert-butyl 3-cyano-1*H*-indole-1-carboxylate (4-7): Indole-3-cyanide (1 g, 7 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 10-20 mL of anhydrous acetonitrile. Then, DMAP (0.86 g, 7 mmol) was added to the reaction. Boc anhydride (2.4 mL, 10.5 mmol) was added to the reaction, and the solution was stirred until complete, as monitored via TLC. When complete, 40 mL of water was added, and the solution becomes cloudy and white. This was extracted into ethyl acetate (3 x 40 mL). The organics were washed with an aqueous 1 M HCl solution (50 mL) and a saturated aqueous brine solution (50 mL). The organics were then dried with sodium sulfate and concentrated *in vacuo* to give pure product as a solid (1.48 g, 87% yield).

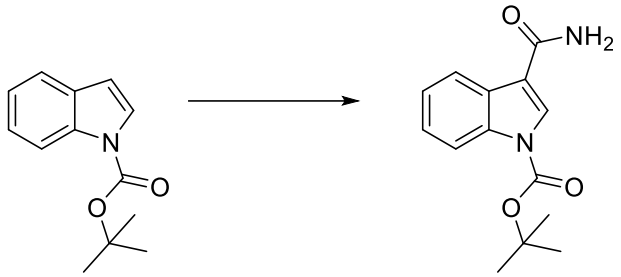
^{1}H NMR (500 MHz, Acetone-*d6*) δ 8.43 (s, 1H), 8.22 (d, *J* = 8.3 Hz, 1H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.49 (m, 1H), 7.43 (m, 1H), 1.72 (s, 9H). ^{13}C NMR (126 MHz, Acetone-*d6*) δ 148.16, 134.35, 134.28, 128.03, 126.11, 124.27, 119.23, 115.66, 113.59, 91.61, 85.72, 27.19. IR (neat): 2230, 1738, 1446 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+H)$^{+}$] calc for ($C_{14}H_{15}N_2O_2^{+}$): 243.1134 Found: 243.1175. m.p.: 127 °C



tert-butyl 1*H*-indole-1-carboxylate (4-8): Indole (5 g, 42.7 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 100 mL anhydrous dichloromethane. Trimethylamine (17.8 mL, 128 mmol) and 4-DMAP (1.0 g, 8.5 mmol) were then added to the reaction. Then, boc anhydride (10.8 mL, 46.9 mmol) was added dropwise to the solution. The reaction was stirred for 20 hours at room temperature. Once complete, as monitored via TLC, the reaction was quenched with 100 mL of water. Organics were extracted into dichloromethane (3 x

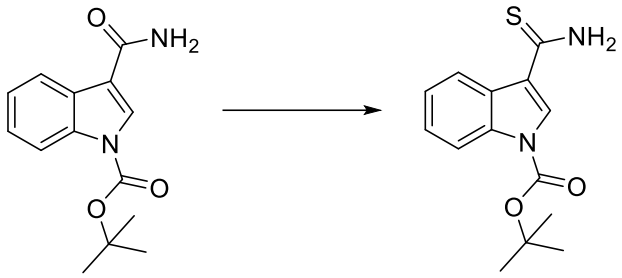
100 mL). The organics were dried over Na_2SO_4, concentrated *in vacuo* and purified via silica plug column (100% hexanes) to produce a clear oil (9.1 g, 97% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (d, *J* = 8.1 Hz, 1H), 7.65 (d, *J* = 3.7 Hz, 1H), 7.61 (d, *J* = 7.7 Hz, 1H), 7.37 (m, 1H), 7.28 (m, 1H), 6.61 (d, *J* = 3.7, 1H), 1.72 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.89, 135.26, 130.65, 125.96, 124.27, 122.71, 121.02, 115.24, 107.37, 83.69, 28.28. IR (neat): 1729 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for major fragment ($C_9H_8NO_2^+$): 162.0555 Found: 162.0544.



tert-butyl 3-carbamoyl-1*H*-indole-1-carboxylate (4-9): 4-8 (3.0 g, 1.8 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 10 mL anhydrous acetonitrile. The solution was cooled to 0 °C. Chlorosulfonyl isocyonate (1.2 mL, 13.8 mmol) was added to the reaction over a period of 10 minutes. The reaction was stirred and warmed up to room temperature over 2-3 hours. After completion, as monitored by TLC, a solution of acetone and water (9 mL to 1 mL) was added to the reaction. Then, the reaction was rendered alkaline using a 20% aqueous solution of NaOH. Organics were extracted into ethyl acetate (3 x 30 mL), and then washed with a brine solution. Organics were then dried with Na_2SO_4 and concentrated *in vacuo*. The product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). Pure product was a white solid (2.5 g, 71% yield).

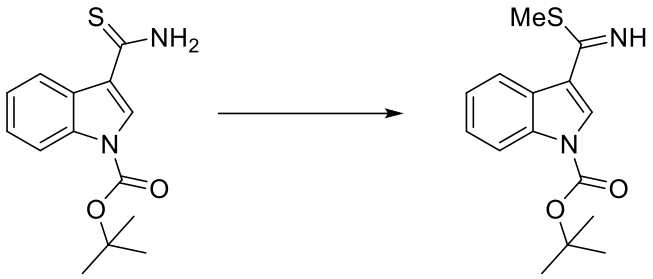
^{1}H NMR (500 MHz, Acetone-*d6*) δ 8.39 (s, 1H), 8.33 (d, *J* = 7.9 Hz, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 7.38 (m, 1H), 7.32 (m, 1H), 6.57 (s, br., 1H), 1.71 (s, 9H). ^{13}C NMR (126 MHz, Acetone-*d6*) δ 165.21, 149.15, 135.46, 128.65, 127.91, 124.72, 123.29, 122.14, 115.34, 114.83, 84.49, 27.28. IR (neat): 3513, 3470, 1740, 1651 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+Na)$^{+}$] calc for sodium salt ($C_{14}H_{16}N_2O_3Na^{+}$): 283.1100 Found: 283.1062. m.p.: 130 °C



tert-butyl 3-carbamothioyl-1*H*-indole-1-carboxylate (4-10): 4-9 (0.2 g, 0.77 mmol) and Lawesson's reagent (0.155 g, 0.38 mmol) were added to a clean, dry round bottom flask under nitrogen and dissolved in 10 mL of benzene. The reaction was stirred and refluxed for an hour (until the solution turned to a yellow/orange color. If your solution turned red, the reaction ran too long). Once complete, the organics were concentrated. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). Pure product was a yellow solid (0.20 g, 95% yield).

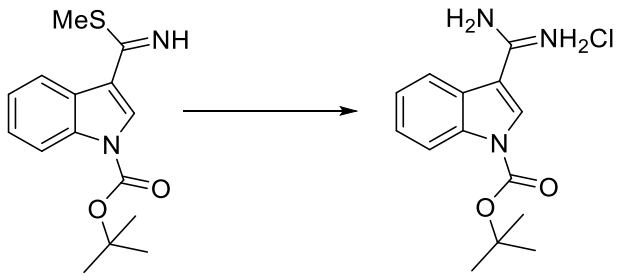
^{1}H NMR (500 MHz, Acetone-*d6*) δ 8.73 (s, br., 2H), 8.58 (d, *J* = 7.6 Hz, 1H), 8.33 (s, 1H), 8.19 (d, *J* = 8.3 Hz, 1H), 7.41 – 7.30 (m, 2H), 1.70 (s, 9H). ^{13}C NMR (126 MHz, Acetone-*d6*) δ 195.43, 149.84, 136.60, 128.66, 127.40, 125.65, 124.09, 123.12, 121.91, 115.73, 85.55, 28.10.

HRMS (ESI-TOF) m/z: [(M+H)$^{+}$] calc for major fragment ($C_{10}H_9N_2O_2S^{+}$): 221.0385 Found: 221.0389. m.p.: 71 °C



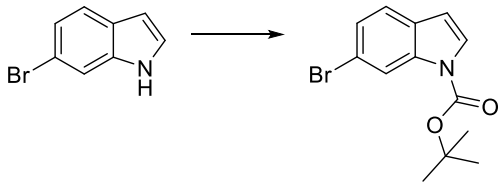
tert-butyl 3-(imino(methylthio)methyl)-1*H*-indole-1-carboxylate (4-11): 4-10 (1.25 g, 4.52 mmol) was dissolved in 25 mL of DCM in a clean, dry round bottom flask under nitrogen. Methyl iodide (0.84 mL, 13.57 mmol) was then added to the flask. The reaction was stirred at room temperature for 48 hours. After completion, as monitored by TLC, 15 mL of DCM was added. The reaction was washed with a saturated sodium bicarbonate solution (2 x 30 mL). Then the organics were washed with brine (2 x 30 mL). The organics were dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). Pure product was a yellow solid (0.99 g, 75%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.44 (s, br., 1H), 8.29 – 8.15 (m, 2H), 8.09 (s, 1H), 7.35 (m, 2H), 2.43 (s, 3H), 1.70 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.35 (br., visible only with enhanced apodization, exponential = 8 Hz), 149.24, 135.64, 127.38, 127.22, 125.21, 123.73, 121.75, 119.87, 115.18, 84.6ww8, 28.14, 12.17. IR (neat): 3300, 3142, 1726 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for major fragment ($C_{10}H_8N_2O_2S^+$): 235.05 Found: 235.0566. m.p.: 94 °C



tert-butyl 3-carbamimidoyl-1*H*-indole-1-carboxylate (4-12): 4-11 (0.21 g, 0.72 mmol) and ammonium chloride (0.042 g, 0.78 mmol) were added to a clean, dry round bottom under nitrogen and methanol (5 mL) was added to the reaction. The reaction was stirred and refluxed for 3 hours. After completion, the reaction was cooled and diluted in ethyl acetate (100 mL). The organic solution was washed with brine (2 x 50 mL). The ethyl acetate layer was concentrated down to give clean product. Product is a white solid (79 mg, 38% yield).

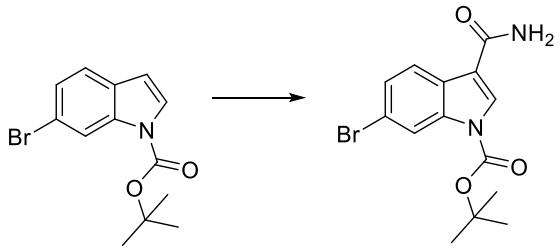
^{1}H NMR (500 MHz, CD_3OD) δ 8.46 (s, 1H), 8.28 (d, *J* = 8.4 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.50 (m, 1H), 7.44 (m, 1H), 1.73 (s, 9H). ^{13}C NMR (126 MHz, CD_3OD) δ 161.45, 148.41, 135.53, 130.70, 125.76, 125.52, 123.97, 119.50, 115.32, 109.06, 85.85, 26.78. IR (neat): 3472, 3324, 3154, 1745, 1660 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calc for ($C_{14}H_{18}N_3O_2^+$): 260.1399 Found: 260.1389. m.p.: 137 °C



tert-butyl 6-bromo-1*H*-indole-1-carboxylate (4-13): Commercially available 6-bromoindole (5 g, 25.5 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in anhydrous dichloromethane (50 mL). To this solution, trimethylamine (10.7 mL, 76.5 mmol) and 4-dimethylaminopyridine (0.62 g, 5.1 mmol) were added. Di-tert-butyldicarbonate (6.4 mL, 28 mmol) was then added dropwise to the solution. This reaction was stirred for 20 hours at room temperature. Once complete (as monitored via TLC), the reaction was quenched using water (40 mL), and the crude organics were extracted into dichloromethane (3 X 50 mL). The organics were dried over Na_2SO_4, concentrated *in vacuo* and purified using automated CombiFlash

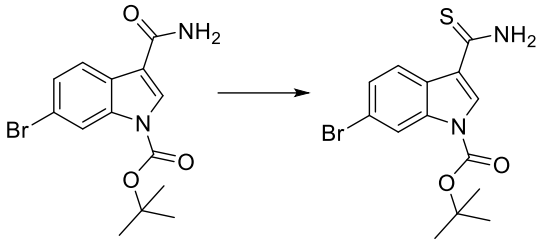
chromatography (silica gel, 20-40 microns, 100% hexanes) to produce a white solid (7.19 g, 96% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.57 (d, *J* = 3.7 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.35 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.54 (d, *J* = 3.7, 1H), 1.69 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.35, 135.86, 129.30, 126.33, 125.87, 121.95, 118.37, 117.91, 107.04, 84.22, 28.13. IR (neat): 1731 cm^{-1}. m.p.: 74 °C. Mass spectroscopy data was not possible to identify. Known compound, data matches literature values (P. Caramenti, R. K. Nandi, and J. Waser., *Chem. Eur. J* 2018, **24**, 10049 – 10053.)



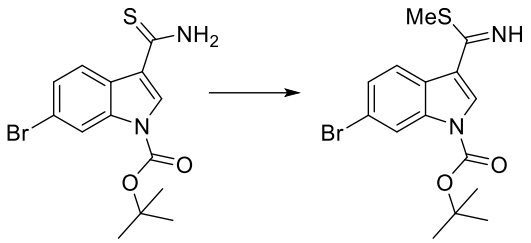
tert-butyl 6-bromo-3-carbamoyl-1*H*-indole-1-carboxylate (4-14): 4-13 (0.98 g, 3.3 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 10 mL of anhydrous acetonitrile. The solution was cooled to 0°C using an ice bath. Chlorosulfonyl isocyanate (0.29 mL, 3.3 mmol) was then added dropwise to the reaction over a period of 10 minutes. The reaction was stirred for 3 hours as it warmed to room temperature. Then, a solution of 9 mL of acetone and 1 mL of water was added to the reaction. The reaction was rendered alkaline upon addition of a 20% aqueous solution of NaOH. The organics were then extracted into ethyl acetate (3 x 30 mL). Organics were then combined and washed with brine, dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). The pure product was a white solid (0.67 g, 58% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.07 (s, 1H), 7.92 (d, *J* = 8.5 Hz, 1H), 7.37 (dd, *J* = 8.5, 1.8 Hz, 1H), 6.40 (s, br., 2H), 1.65 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.19, 148.77, 136.05, 128.59, 127.09, 126.53, 122.54, 119.03, 118.42, 115.01, 85.73, 28.11. IR (neat): 3421, 3349, 1739, 1648 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{14}H_{16}BrN_2O_3^+$) 282.9718 Found 282.9715. m.p.: 108 °C



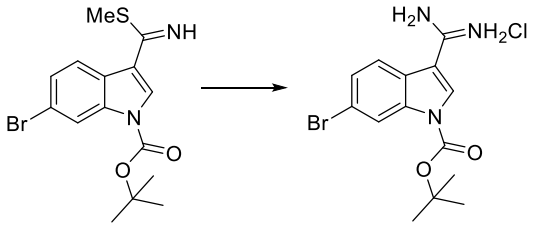
tert-butyl 6-bromo-3-carbamothioyl-1*H*-indole-1-carboxylate (4-15): 4-14 (0.8 g, 2.36 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 60 mL of anhydrous toluene. Lawesson's reagent (0.95 g, 2.36 mmol) was then added to the solution. The reaction was stirred and gently heated for 30 minutes, until the solution became homogenous and turned yellow in color. (Note: If this reaction is allowed to go too long, the solution will darken in color and undesired side products will form.) Once the reaction was complete, the solution was concentrated, and the crude reaction was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). Pure product is a yellow solid (0.75 g, 89% yield).

^{1}H NMR (500 MHz, Acetone-d_6) δ 8.78 (s, br., 2H), 8.59 (d, *J* = 8.6 Hz, 1H), 8.38 (d, *J* = 1.9 Hz, 1H), 8.33 (s, 1H), 7.50 (dd, *J* = 8.6, 1.9 Hz, 1H), 1.71 (s, 9H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 194.91, 149.59, 137.35, 128.14, 127.33, 127.18, 125.03, 121.49, 118.92, 118.69, 86.26, 28.07. IR (neat): 3247, 3119, 2218 cm^{-1}. mp: 179°C. Mass spectroscopy data was not possible to identify. Known compound, data matches literature values (C. Moody and J. Roffey, *ARKIVOC*, 2000, **3**, 393–401.)



tert-butyl 6-bromo-3-(imino(methylthio)methyl)-1*H*-indole-1-carboxylate (4-16): 4-15 (0.5 g, 1.4 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 30 mL of acetone. Methyl iodide (0.52 mL, 8.4 mmol) was added to the solution dropwise. The reaction was stirred at room temperature for 72 hours, or until the reaction was completed as monitored via TLC. Once complete, 30 mL of ethyl acetate was added to the reaction, and the reaction was washed with saturated sodium bicarbonate solution (2 x 30 mL) and brine (2 x 30 mL). Organics were then separated, dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). Pure product was a yellow solid (0.46 g, 89%).

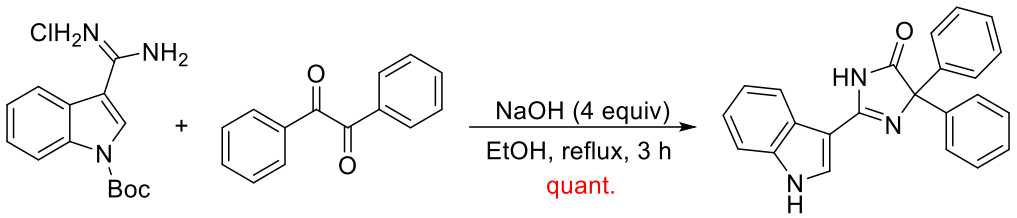
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.42 (s, br., 1H), 8.33 (s, 1H), 8.10 (s, 1H), 7.99 (s, 1H), 7.39 (dd, *J* = 8.5, 1.8 Hz, 1H), 2.39 (s, 3H), 1.67 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.22 (br., visible only with enhanced apodization, exponential = 8 Hz), 148.75, 136.20, 127.45, 126.89, 126.21, 123.22, 119.62, 118.95, 118.25, 85.22, 28.07, 12.02. IR (neat): 2972, 1741 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{15}H_{18}BrN_2O_2S^+$) 369.0272 Found 369.0263. m.p.: 141 °C



tert-butyl 3-carbamimidoyl-1*H*-indole-1-carboxylate (4-17): 4-16 (0.38 g, 1 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 10 mL of methanol.

Ammonium chloride (0.1 g, 1.2 mmol) was added to the solution. The reaction was stirred and refluxed for 24 hours, monitoring progress via TLC. Once the reaction was complete it was cooled, and the solution was concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, dichloromethane/methanol gradient 0-10%). Pure product was a white solid (0.26 g, 68% yield).

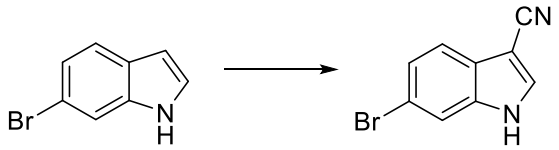
^{1}H NMR (500 MHz, CD_3OD) δ 8.45 (s, 1H), 8.45 (d, *J* = 1.8 Hz, 1H), 7.74 (d, *J* = 8.6 Hz, 1H), 7.56 (dd, *J* = 8.6, 1.8 Hz, 1H), 1.72 (s, 9H). ^{13}C NMR (126 MHz, CD_3OD) δ162.42, 149.42, 137.52, 132.65, 128.52, 125.94, 122.46, 120.60, 119.69, 110.46, 87.81, 28.14. IR (neat): 3302, 3119, 1649, 1569 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{14}H_{17}BrN_3O_2^+$) 338.0499 Found: 338.0511. m.p.: salt, >200°C.



2-(1*H*-indol-3-yl)-5,5-diphenyl-3,5-dihydro-4*H*-imidazol-4-one (4-18): 4-12 (25 mg, 0.084 mmol), benzil (17.8 mg, 0.084 mmol), and sodium hydroxide (13.5 mg, 0.336 mmol) were added to a clean, dry round bottom flask under nitrogen. This was dissolved in 5 mL ethanol. The reaction was stirred and refluxed for 2 to 3 hours. Once complete, pour reaction into 30 mL ethyl acetate and washed with water and brine (30 mL). Extract into ethyl acetate 3 times. Dry the organics with Na_2SO_4 and concentrate in vacuo. Crude product was characterized without purification (36 mg crude, over 100% yield).

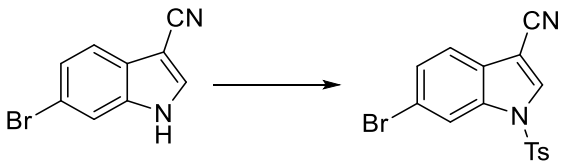
^{1}H NMR (500 MHz, Acetone-*d6*) δ 8.67 (dd, *J* = 6.4, 2.8 Hz, 1H), 8.19 (s, 1H), 7.74 (d, *J* = 6.8 Hz, 4H), 7.58 – 7.49 (m, 2H), 7.34 (m, 5H), 7.29 – 7.24 (m, 4H), 2.09 (s, 3H). ^{13}C NMR (126 MHz, Acetone-*d6*) δ 182.78, 155.32, 142.24, 137.03, 128.62, 128.46, 128.29, 128.06, 127.60,

127.21, 127.11, 126.94, 125.74, 123.05, 122.36, 121.19, 111.82, 105.82, 77.41, 29.72. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{23}H_{18}N_3O^+$) 352.14 Found: 352.14.



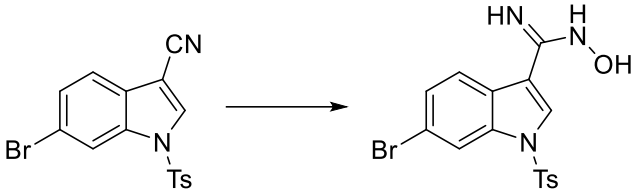
6-bromo-1*H*-indole-3-carbonitrile (4-19): 6-bromoindole (2 g, 10.2 mmol) was added to a clean, dry round bottom flask under argon and dissolved in 30 mL of anhydrous dimethylformamide. The solution was cooled to -50°C, and then chlorosulfonyl isocyanate (1.1 mL, 12.2 mmol) was added to the solution dropwise. The solution was stirred, and the temperature was raised to room temperature over the period of 2 hours. Once complete, as monitored by TLC, the reaction was poured into 50 mL of ice water, and the organics were extracted into ethyl acetate (3 x 50 mL). The organics were dried over Na2SO4 and concentrated in vacuo. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). Pure product was a yellow solid (1.98 g, 88%).

^{1}H NMR (500 MHz, Acetone-*d6*) δ 11.34 (s, br. 1H), 8.14 (s, 1H), 7.81 (d, *J* = 1.8 Hz, 1H), 7.64 (d, *J* = 8.5 Hz, 1H), 7.41 (dd, *J* = 8.5, 1.8 Hz, 1H). ^{13}C NMR (126 MHz, Acetone-*d6*) δ 136.36, 134.44, 126.13, 124.95, 120.31, 116.55, 115.67, 114.90, 86.30. IR (neat): 3244, 2220 cm^{-1}. mp: 189 °C. Known compound, data matches literature values (*J. Agric. Food Chem*. 2018, **66**, 4062−4072.)



6-bromo-1-tosyl-1*H*-indole-3-carbonitrile (4-20): 4-19 (2 g, 9.0 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 60 mL of anhydrous dichloromethane. Triethylamine (1.9 mL, 13.4 mmol) and 4-DMAP (0.22 g, 1.8 mmol) were then added to the solution. Then 4-toluenesulfonyl chloride (2.2 g, 11.6 mmol) was added to the reaction, and it was stirred at room temperature for 3 hours. Once complete, as monitored by TLC, the solution was concentrated *in vacuo*, and the crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). Pure product was a white solid (2.61 g, 78%).

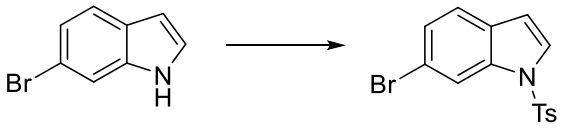
^{1}H NMR (500 MHz, Acetone-*d6*) δ 8.64 (s, 1H), 8.23 (d, *J* = 1.7 Hz, 1H), 8.07 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 8.4 Hz, 1H), 7.60 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.49 (d, *J* = 8.3 Hz, 2H), 2.39 (s, 3H). ^{13}C NMR (126 MHz, Acetone-*d6*) δ 147.17, 135.25, 134.21, 133.75, 130.69, 128.24, 127.53, 127.39, 121.53, 119.62, 116.60, 112.50, 93.30, 20.69. IR (neat): 3233, 2222 cm^{-1}. m.p.: 175 °C. Mass spectroscopy information could not be determined. Known compound, data matches literature values (*J. Agric. Food Chem*. 2018, **66**, 4062−4072.)



6-bromo-*N*-hydroxy-1-tosyl-1*H*-indole-3-carboximidamide (4-21): Hydroxylamine hydrochloride (0.97 g, 13.9 mmol) was added to a clean, dry round bottom flask under nitrogen, and it was dissolved in 50 mL of methanol. Sodium bicarbonate (1.2 g, 13.9 mmol) was added to the solution, and it stirred for 30 minutes. Then, **4-20** (2.6 g, 7.0 mmol) was added to the reaction, and it was stirred and refluxed for 5 hours. When complete, as monitored by TLC, the reaction was cooled to room temperature and concentrated *in vacuo*. Organics were dissolved in

50 mL of ethyl acetate and washed with 50 mL of water. The organics were then dried with Na_2SO_4 and concentrated *in vacuo* to give clean product as a white solid (2.75 g, 97% yield).

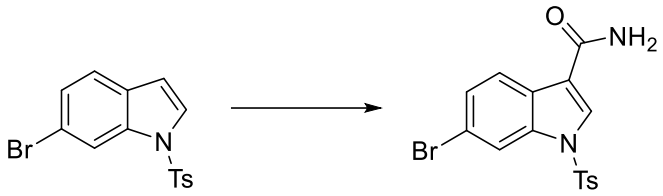
^{1}H NMR (500 MHz, Acetone-*d6*) δ 9.16 (s, br., 1H), 8.20 (s, 1H), 8.15 (m, 2H), 7.92 (d, *J* = 8.5 Hz, 2H), 7.48 – 7.36 (m, 3H), 5.64 (s, br., 2H), 2.35 (s, 3H). ^{13}C NMR (126 MHz, Acetone-*d6*) δ 148.29, 147.00, 136.66, 135.39, 131.20, 127.92, 127.84, 127.73, 126.24, 126.19, 119.06, 117.25, 116.76, 21.45. IR (neat): 3482, 3381, 3298, 1596 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{16}H_{15}N_3O_3SBr^+$) 408.0017, 409.9998 Found: 408.0015, 409.9997. m.p.: decomposed at 160 °C.



6-bromo-1-tosyl-1H-indole (4-22): 6-bromo-1H-indole (1 g, 5.1 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 20 mL of anhydrous acetonitrile. The solution was cooled to 0°C. Sodium hydride (0.41 g, 60 wt% in mineral oil, 10.2 mmol) was added to the solution, and it was stirred for 10 minutes. Then, 4-toluenesulfonyl chloride (1.1 g, 5.6 mmol) was added to the reaction. The reaction was allowed to stir and warm up to room temperature over a period of 2 hours. Once complete, as monitored via TLC, the reaction was quenched by slowly adding a saturated aqueous solution of ammonium chloride (10 mL). The organics were extracted into ethyl acetate (20 mL x 3). The organics were then washed with brine solution (20 mL) and dried with Na_2SO_4. The organics were then concentrated in vacuo to give the product as a clean, white solid (1.9 g, over 100% yield isolated).

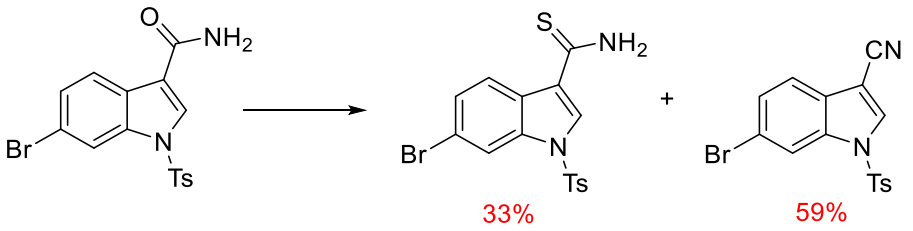
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.76 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 3.7 Hz, 1H), 7.37 (d, *J* = 8.3 Hz, 1H), 7.33 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.24 (d, *J* = 8.2 Hz, 2H), 6.61 (d, *J* = 3.7 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.32, 135.45, 135.00, 130.05, 129.54,

126.81, 126.66, 126.54, 122.49, 118.23, 116.56, 108.78, 21.60. IR (neat): 3137, 3109, 2925, 1595 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{15}H_{13}BrSNO_2^+$) 349.9850, 351.9830 Found: 349.9846, 351.9815. m.p.: 129 °C.



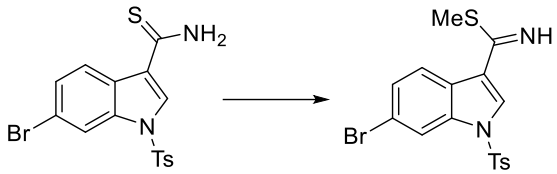
6-bromo-1-tosyl-1*H*-indole-3-carboxamide (4-23): 4-22 (1.79 g, 5.5 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 20 mL of anhydrous acetonitrile. The solution was cooled to 0°C using an ice bath. Chlorosulfonyl isocyanate (0.57 mL, 6.6 mmol) was then added dropwise to the reaction over a period of 10 minutes. The reaction was stirred for 3 hours as it warmed to room temperature. Then, a solution of 18 mL of acetone and 2 mL of water was added to the reaction. The reaction was rendered alkaline upon addition of a 20% aqueous solution of NaOH. The organics were then extracted into ethyl acetate (3 x 40 mL). Organics were then combined and washed with brine, dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). The pure product was a white solid (0.45 g, 21% yield). There was also a 55% recovery of the starting 6-bromo-1-tosyl-1H-indole.

^{1}H NMR (500 MHz, Acetone-*d6*) δ 8.45 (s, 1H), 8.21 (d, *J* = 8.5 Hz, 1H), 8.14 (d, *J* = 1.7 Hz, 1H), 7.95 (d, *J* = 8.5 Hz, 2H), 7.48 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.44 (d, *J* = 7.8 Hz, 2H), 6.70 (s, br., 1H), 2.37 (s, 3H). ^{13}C NMR (126 MHz, Acetone-*d6*) δ 164.19, 146.50, 135.36, 134.36, 130.48, 128.71, 128.11, 127.28, 127.12, 124.26, 118.31, 116.68, 115.86, 20.62. IR (neat): 3406, 3199, 3099, 1646, 1610 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{16}H_{14}N_2O_3SBr^+$) 392.9908, 394.9889 Found: 392.9914, 394.9893. m.p.: >200 °C.



6-bromo-1-tosyl-1*H*-indole-3-carbothioamide (4-24): 4-23 (0.35 g, 0.89 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 30 mL of anhydrous toluene. Lawesson's reagent (0.2 g, 0.49 mmol) was then added to the solution. The reaction was stirred and gently heated for 1.5 hours, Once the reaction was complete, the solution was concentrated *in vacuo*, and the crude reaction was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). Pure product is a yellow solid (0.12 g, 33% yield). Additionally, 0.2 g, 59% yield of 6-bromo-1-tosyl-1H-indole-3-carbonitrile (**4-20**) was isolated from the reaction.

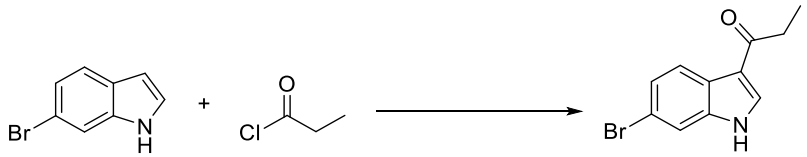
^{1}H NMR (500 MHz, Acetone-*d6*) δ 8.89 (s, br., 2H), 8.57 (d, *J* = 8.6 Hz, 1H), 8.38 (s, 1H), 8.16 (d, *J* = 1.8 Hz, 1H), 8.00 (d, *J* = 8.5 Hz, 2H), 7.51 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.45 (d, *J* = 8.3 Hz, 2H), 2.38 (s, 3H). ^{13}C NMR (126 MHz, Acetone-*d6*) δ 194.18, 147.51, 136.51, 135.10, 131.39, 128.72, 128.14, 127.99, 127.12, 125.67, 122.86, 119.26, 116.71, 21.51. IR (neat): 3015, 2929, 1582, 1568 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+H)$^{+}$] calcd for ($C_{16}H_{14}N_2O_2S_2Br^{+}$) 408.9680, 410.9659 Found: 408.9689, 410.9653. m.p.: 145 °C.



methyl 6-bromo-1-tosyl-1*H*-indole-3-carbimidothioate (4-25): 4-24 (0.1 g, 0.24 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 10 mL of acetone.

Methyl iodide (0.1 mL, 1.5 mmol) was added to the solution dropwise. The reaction was stirred at room temperature for 24 hours, or until the reaction was completed as monitored via TLC. Once complete, 20 mL of ethyl acetate was added to the reaction, and the reaction was washed with saturated sodium bicarbonate solution (2 x 20 mL) and brine (2 x 20 mL). Organics were then separated, dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). Pure product was a white solid (75 mg, 73%).

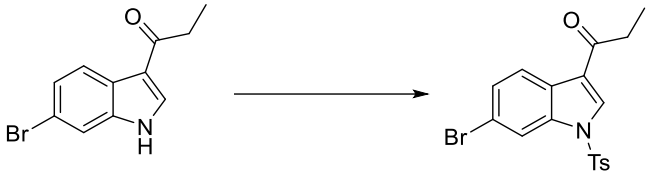
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (d, *J* = 1.7 Hz, 1H), 8.12 (d, *J* = 8.5 Hz, 1H), 8.05 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.42 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.28 (d, *J* = 8.5 Hz, 2H), 2.42 (s, 3H), 2.37 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.51, 145.99, 135.76, 134.57, 130.37, 127.95, 127.74, 127.13, 126.66, 123.91, 121.10, 119.33, 116.45, 21.75, 12.18. IR (neat): 3309, 3102, 1592, 1530 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{17}H_{16}N_2O_2S_2Br^+$) 422.9836, 424.9816 Found: 422.9837, 424.9817. m.p.: 141 °C.



1-(6-bromo-1*H*-indol-3-yl)propan-1-one (4-26): 6-bromo-1*H*-indole (0.98 g, 5 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in chloroform (20 mL). The solution was cooled to 0 °C. $AlEt_2Cl$ (4.2 mL, 1.8 M in toluene, 7.5 mmol) was added to the solution dropwise. The reaction was stirred for 30 minutes. Next, propionyl chloride (0.65 mL, 7.5 mmol) diluted in chloroform (20 mL) was added to the reaction dropwise. The reaction stirred for 2 hours at 0 °C. When complete, a pH 7 aqueous buffer was added to quench the reaction. A precipitate formed upon addition of the buffer, and the solids were filtered off. The precipitate was washed with dichloromethane and dried under reduced pressure. The precipitate

was then dissolved in acetone, and the residual solid was filtered off. Pure product was recrystallized out of the acetone by concentrating off half of the volume *in vacuo* and cooling the solution to 0 °C to give a white solid. (0.77 g, 61% yield)

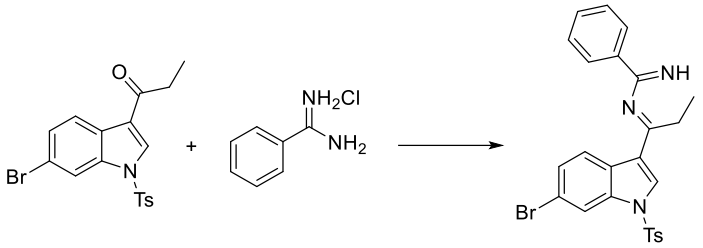
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (s, br., 1H), 8.34 (s, 1H), 8.12 (d, J = 8.5 Hz, 1H), 7.65 (d, J = 1.8 Hz, 1H), 7.31 (dd, J = 8.5, 1.8 Hz, 1H), 2.87 (q, J = 7.4 Hz, 2H), 1.10 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 196.36, 137.97, 134.86, 124.94, 124.90, 123.47, 116.39, 115.70, 115.17, 32.29, 9.39. IR (neat):3138, 3036, 2965, 2903, 1628, 1516, 1413, 1285 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+H)$^{+}$] calcd for ($C_{11}H_{11}BrNO^{+}$) 252.0024. Found: 252.0016. m.p. >200 °C.



1-(6-bromo-1-tosyl-1*H*-indol-3-yl)propan-1-one (4-27): 4-26 (0.76 g, 3.0 mmol) was added to a clean, dry flask under nitrogen and dissolved in 30 mL of anhydrous THF. The reaction was cooled to 0 °C, and then sodium hydride (0.24 g, 60% in mineral oil, 6 mmol) was added all at once. The reaction was stirred for 5 minutes. Then 4-toluenesulfonyl chloride (0.57 g, 3 mmol) was added all at once. The solution was stirred until the reaction was complete, as monitored by TLC. The reaction was cooled to 0 °C and quenched with a saturated aqueous $NaHCO_3$ solution. Organics were extracted into ethyl acetate x3 and dried with Na_2SO_4. Organics were concentrated *in vacou* to afford clean product as a white solid (1.1 g, 91% yield).

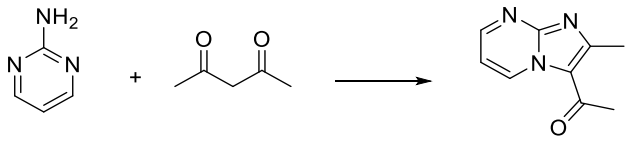
^{1}H NMR (500 MHz, Acetone-d_6) δ 8.63 (s, 1H), 8.23 (d, J = 8.5 Hz, 1H), 8.16 (d, J = 1.7 Hz, 1H), 8.04 (d, J = 8.5 Hz, 2H), 7.53 (dd, J = 8.5, 1.8 Hz, 1H), 7.46 (d, J = 8.7 Hz, 2H), 3.06 (q, J = 7.3 Hz, 2H), 2.39 (s, 3H), 1.16 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 196.90, 147.58, 136.32, 135.13, 133.89, 131.42, 128.77, 128.23, 127.82, 125.24, 121.40, 119.52, 116.81,

33.39, 21.52, 8.38. IR (neat): 1675, 1591, 1535, 1379 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{18}H_{17}NO_3SBr^+$) 406.0113, 408.0093 Found: 406.0121, 408.0103. m.p.: 135 °C.



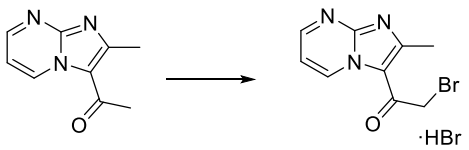
(*E*)-*N*-(1-(6-bromo-1-tosyl-1*H*-indol-3-yl)propylidene)benzimidamide (4-28): 4-27 (73 mg, 0.18 mmol), benzamidine hydrochloride (28 mg, 0.18 mmol), and sodium hydroxide (32 mg, 0.81 mmol) were added to a clean, dry round bottom flask under nitrogen and dissolved in 10 mL of pyridine. The reaction was stirred for 30 hours at 80 °C. Once complete, the solvent was removed *in vacuo*, and the crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, 100% hexanes). Pure product was a solid (7.2 mg, 8% yield). Other side products include **4-26** (over 50% of starting indole deprotected) and tosylated benzamidine (3.1 mg).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 – 8.46 (m, 2H), 7.96 (s, 1H), 7.91 (d, *J* = 1.9 Hz, 1H), 7.57 (d, *J* = 8.3 Hz, 2H), 7.53 (m, 3H), 7.33 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.27 (m, 2H), 6.93 (d, *J* = 8.1 Hz, 1H), 6.13 (s, br., 1H), 2.45 – 2.37 (m, 5H), 1.19 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.11, 164.79, 157.32, 144.90, 137.32, 136.04, 135.84, 132.02, 131.23, 130.14, 128.79, 128.51, 128.25, 127.27, 126.07, 124.63, 123.89, 123.64, 28.45, 21.79, 12.25. IR (neat): 3250, 2360, 1566, 1493, 1388 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{25}H_{23}BrN_3O_2S^+$) 510.0676 Found: 510.0733. m.p.: 188 °C.



1-(2-methylimidazo[1,2-a]pyrimidin-3-yl)ethan-1-one (4-31): 2,4-pentadione (2.17 mL, 21 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 5 mL of anhydrous acetonitrile. Then carbon tetrabromide (13.9 g, 42 mmol) was added to the solution. After letting the reaction stir at room temperature for 5 minutes, 2-aminopyrimidine (2 g, 21 mmol) was added. The reaction was stirred at 80 °C for 24 hours. When complete, the reaction was diluted in ethyl acetate (50 mL). The organics was washed with a saturated aqueous solution of sodium bicarbonate (30 mL) and brine (30 mL). The organics were then extracted into ethyl acetate 4-5 times. All organic layers were combined, dried with Na_2SO_4, and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient with 1% TEA). Pure product was a white solid (1.23 g, 33% yield).

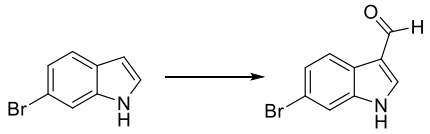
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.90 (dd, *J* = 6.9, 2.1 Hz, 1H), 8.66 (dd, *J* = 4.2, 2.1 Hz, 1H), 7.05 (dd, *J* = 6.9, 4.2 Hz, 1H), 2.80 (s, 3H), 2.59 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 188.37, 154.59, 153.13, 149.57, 136.59, 120.12, 110.76, 30.28, 18.34. IR (neat): 1623 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_9H_{10}N_3O^+$) 176.0824 Found: 176.0831. m.p.: 104 °C



2-bromo-1-(2-methylimidazo[1,2-a]pyrimidin-3-yl)ethan-1-one (4-30): 4-31 (0.41 g, 2.37 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 5 mL of glacial acetic acid. Then bromine (0.14 mL, 2.72 mmol) in 1 mL of acetic acid was added to the solution dropwise. The solution was stirred at 100 °C for 3 hours. Once complete, the reaction

was cooled to room temperature and stirred overnight. The precipitate that formed was filtered off and washed several times with acetone and a small amount of ethanol. The resultant light brown solid was taken up into acetone with a little ethanol and stirred at room temperature for 5 hours. The solid was again filtered off and washed with acetone. The solid was then dried *in vacuo* to afford pure product as a monohydrobromide salt (0.49 g, 62% yield)**.**

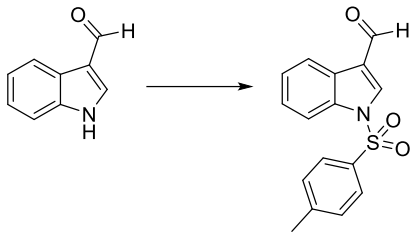
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.92 (dd, J = 6.9, 1.9 Hz, 1H), 8.99 (dd, J = 4.4, 1.9 Hz, 1H), 7.62 (dd, J = 6.9, 4.4 Hz, 1H), 4.94 (s, 2H), 2.88 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.01, 156.58, 149.12, 146.52, 137.82, 117.43, 113.88, 36.38, 15.63. IR (neat): 1676, 1631 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_9H_9N_3OBr^+$) 253.9929 Found: 253.9928. m.p.: salt, >200°C.



6-bromo-1H-indole-3-carbaldehyde (4-40): 6-bromoindole (1.95 g, 10.0 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 20 mL of DMF. The solution was stirred and cooled to 0°C. $POCl_3$ (2.29 g, 14.9 mmol) was then added to the solution dropwise. The reaction was then stirred for 2 hours at 55°C. Once complete, the mixture was poured into 40 mL of ice water. A 20% aqueous solution of NaOH was used to adjust the pH of the solution to 9. At the basic pH, a precipitate fell out and was then filtered off. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, hexanes/ethyl acetate gradient) to produce the pure product as a white solid (1.4 g, 61% yield).

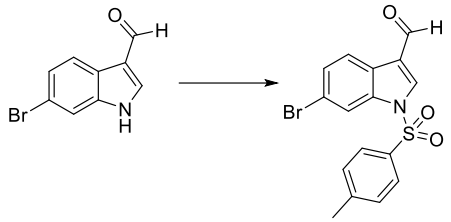
^{1}H NMR (500 MHz, Acetone-d_6) δ 10.04 (s, 1H), 8.26 (s, 1H), 8.16 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 1.7 Hz, 1H), 7.40 (dd, J = 8.4, 1.7 Hz, 1H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 185.41, 139.02, 138.73, 126.08, 124.44, 123.67, 119.85, 117.33, 115.99. IR (neat): 3133, 1634 cm^{-1}.

HRMS (ESI-TOF) m/z: [(M+H)$^+$] calcd for ($C_9H_7NOBr^+$) 223.9711, 225.9691 Found: 223.9718. 225.9698. m.p.: 201 °C.



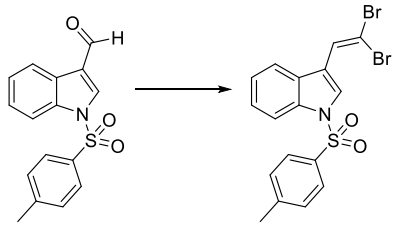
1-tosyl-1*H*-indole-3-carbaldehyde (4-36): 30 mL of anhydrous THF was cooled down to 0°C in a clean, dry round bottom flask under nitrogen, and **4-5** (1 g, 6.8 mmol) was added to the solution. Sodium hydride (0.55g, 13.8 mmol) was added, and the reaction stirred for 30 minutes at 0°C. Tosyl chloride (1.6 g, 8.3 mmol) in 10 mL anhydrous THF was added to the reaction dropwise over a period of 10 minutes. The reaction was then stirred overnight at room temperature. When complete, as monitored via TLC, the reaction was quenched with water (50 mL). The organics were extracted into DCM (3 x 40 mL). The organics were then washed with brine (50 mL) and dried with Na_2SO_4. The organics were concentrated *in vacuo*, and the crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, hexanes/ethyl acetate gradient) to provide pure product as a white solid (1.65 g, 81% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.08 (s, 1H), 8.26 (s, 1H), 8.24 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 8.3 Hz, 1H), 7.84 (d, *J* = 8.5 Hz, 2H), 7.38 (t, *J* = 8.5 Hz, 1H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.23 (d, *J* = 8.5 Hz, 2H), 2.29 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 185.47, 146.15, 136.39, 135.10, 134.14, 130.27, 127.17, 126.25, 126.21, 124.99, 122.50, 122.24, 113.20, 21.55. IR (neat): 3125, 1650, 1596 cm^{-1}. IR (neat): 3132, 1663, 1593 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+H)$^+$] calcd for ($C_{16}H_{14}NO_3S^+$) 300.0694 Found: 300.0699. m.p.: 146 °C.



6-bromo-1-tosyl-1*H*-indole-3-carbaldehyde (4-41): The procedure to produce **4-36** was used. The product was a white solid (1.9 g, 88% yield).

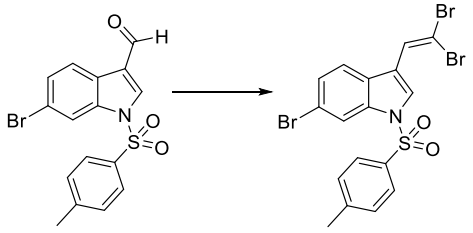
^{1}H NMR (500 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.19 (s, 1H), 8.12 (d, *J* = 1.7 Hz, 1H), 8.10 (d, *J* = 8.5 Hz, 1H), 7.84 (d, *J* = 8.5 Hz, 2H), 7.46 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.32 (d, *J* = 8.5 Hz, 2H), 2.39 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 185.07, 146.55, 136.32, 135.79, 134.04, 130.53, 128.50, 127.24, 125.11, 123.75, 122.00, 120.07, 116.38, 21.74. IR (neat): 3125, 1650, 1596 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{16}H_{13}NO_3SBr^+$) 377.9799, 379.9780 Found: 377.9804, 379.9783. m.p.: 160 °C.



3-(2,2-dibromovinyl)-1-tosyl-1*H*-indole (4-37): Carbon tetrabromide (1.11 g, 3.34 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 10 mL of dichloromethane. The solution was cooled to 0°C. Triphenylphosphine (1.66 g, 6.35 mmol) was added, and the solution stirred at 0°C for 15 minutes. Then, triethylamine (0.23 mL, 1.67 mmol) was added, and the mixture was cooled to -78°C. Lastly, **4-36** (0.5 g, 1.67 mmol) was added to the solution (dissolved in 10 mL of dichloromethane). The reaction was stirred at -78°C for 2 hours and then 1 hour at room temperature. Once complete, the reaction was filtered over a silica gel pad, eluting with dichloromethane. The organics were concentrated *in vacuo* to give a yellow

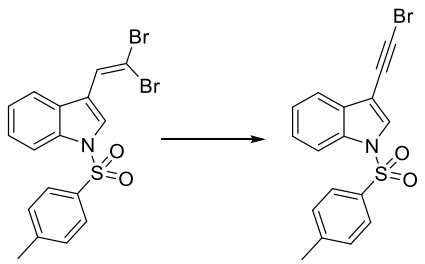
oil. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, hexanes/ethyl acetate gradient) to provide pure product as a white solid (0.68 g, 89% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.02 (d, *J* = 8.3 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 2H), 7.57 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 2H), 2.37 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.46, 134.93, 134.14, 130.14, 129.50, 127.00, 126.97, 125.54, 125.06, 123.73, 118.93, 117.43, 113.76, 90.42, 21.72. IR (neat): 3158, 2368, 2355, 1593 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{17}H_{13}Br_2NO_2S^+$) 453.9112, 455.9092, 457.9073 Found: 453.9111, 455.9090, 457.9068. m.p.: 131 °C.



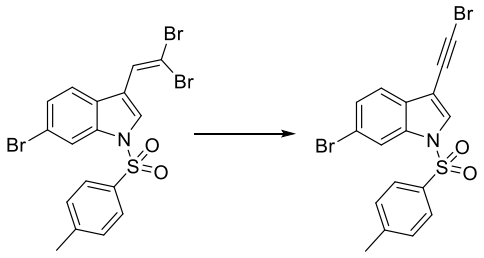
6-bromo-3-(2,2-dibromovinyl)-1-tosyl-1*H*-indole (4-42): The procedure to produce **4-37** was used. The product was a white solid (2.24 g, 81%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (s, 1H), 8.17 (s, 1H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.47 (s, 1H), 7.42 – 7.35 (m, 2H), 7.28 (d, *J* = 8.4 Hz, 2H), 2.37 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.84, 134.80, 134.73, 130.35, 128.34, 127.13, 127.04, 126.47, 125.38, 120.18, 119.26, 117.31, 116.85, 91.19, 21.80. IR (neat): 3148, 2362, 1592, cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{17}H_{12}Br_3NO_2S^+$) 533.8197, 535.8177 Found: 533.8185, 535.8174. m.p.: 189 °C.

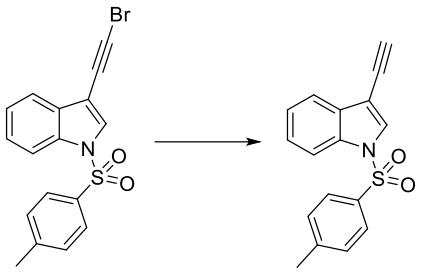


3-(bromoethynyl)-1-tosyl-1*H*-indole (4-38): 4-37 (0.3 g, 0.66 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 10 mL of anhydrous THF. The solution was cooled to -78 °C. LiHMDS (0.79 mL, 1M in THF, 0.79 mmol) was then added dropwise to the reaction. The reaction was stirred for 20 minutes. To quench, a saturated aqueous solution of NH_4Cl (10 mL) was added to the reaction. The organics were extracted into ethyl acetate (2 x 10 mL). The organics were then dried using Na_2SO_4 and concentrated *in vacuo* to give a crude product. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, hexanes/ethyl acetate gradient) to produce the pure product as a colorless oil (0.24 g, 96% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.3 Hz, 1H), 7.74 (m, 3H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.27 – 7.22 (m, 1H), 7.19 (d, *J* = 8.2 Hz, 2H), 2.30 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.57, 134.85, 134.09, 130.78, 130.17, 129.94, 127.05, 125.70, 123.96, 120.59, 113.71, 104.83, 71.62, 53.61, 21.72.

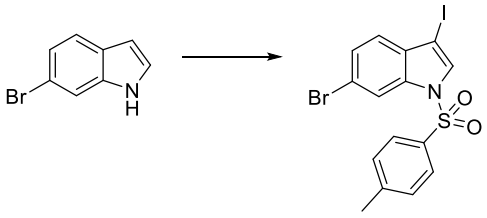


6-bromo-3-(bromoethynyl)-1-tosyl-1*H*-indole (4-43): The procedure to produce **4-38** was used. Product was a white solid (1.82 g, 97% yield). IR (neat): 3126, 1739, 1595 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{17}H_{11}Br_2NO_2S^+$) mass not found. m.p.: 104 °C.



3-ethynyl-1-tosyl-1*H*-indole (4-39): **4-38** (1.28 g, 3.42 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 50 mL of anhydrous THF. The solution was stirred and cooled to -78 °C. n-butyllithium (2.74 mL, 6.85 mmol, 2.5M in hexanes) was added to the solution dropwise. The reaction was stirred at -78 °C for 10 minutes. Then, a saturated aqueous solution of NH_4Cl (50 mL) was added to the reaction. The organics were extracted in ethyl acetate (2 x 50 mL). The organics were then washed with brine (50 mL), dried with Na_2SO_4, and concentrated *in vacuo*. The crude product was purified via recrystallization (ethyl acetate/hexanes) to provide a light pink solid (0.62 g, 60% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.3 Hz, 1H), 7.80 (s, 1H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.37 (t, *J* = 7.1 Hz, 1H), 7.30 (t, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 8.5 Hz, 2H), 3.27 (s, 1H), 2.33 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.55, 134.86, 134.13, 130.84, 130.15, 130.02, 127.05, 125.66, 123.93, 120.59, 113.66, 104.13, 81.71, 75.04, 21.69. IR (neat): 3262, 3129, 1594 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{17}H_{14}NO_2S^+$) 296.0745 Found: 296.0742. m.p.: 170 °C.

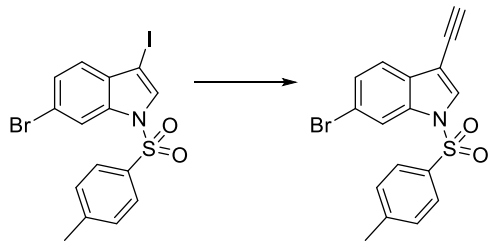


6-bromo-3-iodo-1-tosyl-1*H*-indole (4-44): Commercially available 6-bromoindole (2.0 g, 10.2 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 20 mL of

dimethylformamide. Potassium hydroxide (1.4 g, 25.5 mmol) was added to the reaction, and it was stirred for 5 minutes. Then, I_2 (2.6 g, 10.2 mmol) was added to the reaction. The reaction was stirred for 1 hour at room temperature. Once complete, the reaction was quenched with a saturated aqueous $Na_2S_2O_3$ solution (20 mL) and extracted into ethyl acetate (40 mL). The organics were dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was then dissolved in anhydrous THF, and the solution was cooled to 0°C. Triethylamine (2.8 mL, 20.4 mmol) and 4-dimethylaminopyridine (0.25 g, 2.4 mmol) were added to the solution, and it was stirred for 10 minutes. Then, a freshly recrystallized batch of 4-toluenesulfonyl chloride (2.3 g, 12.2 mmol) was added to the reaction slowly, and it was stirred at room temperature overnight. Once complete as monitored by TLC, dichloromethane (50 mL) was added to the reaction, and the organics were washed with brine solution (50 mL). The organics were then dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). The pure product was a white solid (4.4 g, 91% yield).

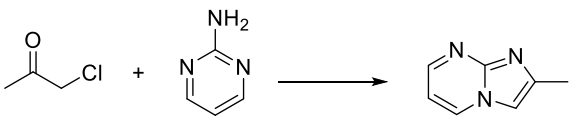
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, *J* = 1.7 Hz, 1H), 7.79 (d, *J* = 8.5 Hz, 2H), 7.66 (s, 1H), 7.42 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.27 (d, *J* = 8.1 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 1H), 2.37 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 145.85, 134.91, 134.69, 131.49, 130.33, 130.29, 127.41, 127.05, 123.31, 119.70, 116.47, 66.40, 21.76. IR (neat): 2980, 2950, 2605, 2499 cm^{-1}. m.p.: 133°C. Known compound, data matches literature values (J. Meizhong and P. Nicholas, WO2020132597, 2018).



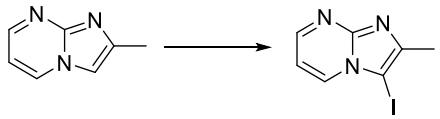
6-bromo-3-ethynyl-1-tosyl-1*H*-indole (4-34): **4-44** (0.5 g, 1.05 mmol) was added to a clean, dry three-neck flask under argon and dissolved in 6 mL of anhydrous dimethylformamide and 6 mL of anhydrous triethylamine. The solution was sparged with argon for 30 minutes. Once complete, $Pd(PPh_3)_2Cl_2$ (7.4 mg, 0.01 mmol) and CuI (4 mg, 0.02 mmol) were added to the reaction. The reaction was then sealed and put under argon via argon balloon. Trimethylsilylethyne (0.15 mL, 1.05 mmol) was added to the reaction dropwise as it stirred at room temperature for 3 hours. Once complete, as monitored by TLC, the reaction was quenched with water (15 mL). Organics were extracted into dichloromethane (3 x 30 mL), dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was then dissolved in 30-40 mL of dichloromethane and cooled to 0°C. Tetrabutylammonium fluoride (1.16 mL, 1M in THF) was added to the reaction dropwise as it stirred for 10 minutes. When complete, as monitored by TLC, the crude reaction was concentrated *in vacuo* and purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). The pure product was a white solid (0.3 g, 76%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, *J* = 1.7 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.75 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.40 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.26 (d, *J* = 8.1 Hz, 2H), 3.27 (s, 1H), 2.35 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.92, 134.72, 134.62, 130.34, 130.31, 129.70, 127.35, 127.07, 121.78, 119.42, 116.73, 104.06, 82.12, 74.43, 21.75. IR (neat): 3297, 2113 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{17}H_{13}BrNO_2S^+$) 373.9845, 375.9831 Found: 373.9849, 375.9831. m.p.: 99 °C.



3-methylimidazo[1,2-a]pyrimidine (4-45): Commercially available 2-aminopyrimidine (4 g, 42 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 50 mL of ethanol. The solution was stirred at room temperature, and commercially available chloroacetone (3.7 mL, 46 mmol) was added to the reaction dropwise. After an hour of stirring at room temperature, the reaction was stirred and refluxed for 20 hours. Once complete, the reaction was cooled to room temperature and concentrated *in vacuo*. To workup, the crude product was dissolved in 30 mL of water, and the pH was adjusted to 8-9 using a saturated aqueous solution of Na_2CO_3. The organics were extracted into dichloromethane (3 x 50 mL). The organics were then dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%, then dichloromethane with 5% methanol). The product was an off-white solid (3.27 g, 58% yield).

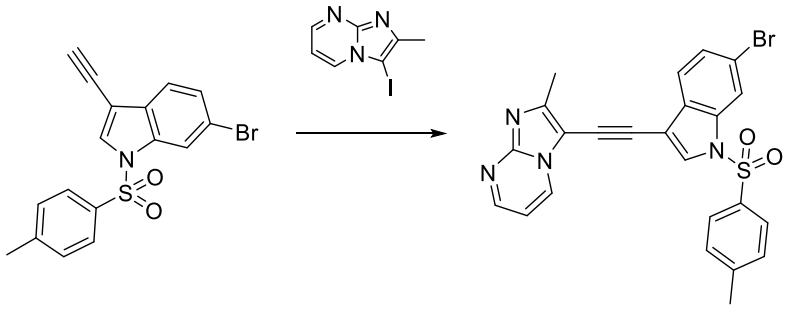
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (dd, *J* = 4.1, 2.0 Hz, 1H), 8.36 (dd, *J* = 6.7, 2.0 Hz, 1H), 7.29 (s, 1H), 6.77 (dd, *J* = 6.7, 4.1 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.28, 148.97, 145.68, 132.59, 108.17, 107.79, 14.86. IR (neat): 3104, 3059, 2939, 2601, 2531, 2488, 2364 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_7H_8N_3^+$) 134.0713 Found: 134.0722. m.p.: decomposed at 143°C.



2-iodo-3-methylimidazo[1,2-a]pyrimidine (4-35): **4-45** (1.0 g, 7.5 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 10 mL of anhydrous dimethylformamide.

The solution was cooled to 0°C in an ice bath. N-iodosuccinimide (2.0 g, 9.0 mmol) was added to the reaction portion wise. The reaction was stirred and allowed to warm to room temperature overnight. Once complete, a saturated aqueous solution of sodium bicarbonate (20 mL) was added to the reaction, and the solution was stirred for 1 hour. The reaction was then diluted in 40 mL of dichloromethane and washed with a saturated aqueous solution of sodium bicarbonate (20 mL), an aqueous solution of sodium thiosulfate (30 mL), and a brine solution (30 mL). The organics were dried over Na_2SO_4 and concentrated *in vacuo* to provide a pure white solid (1.67 g, 86% yield).

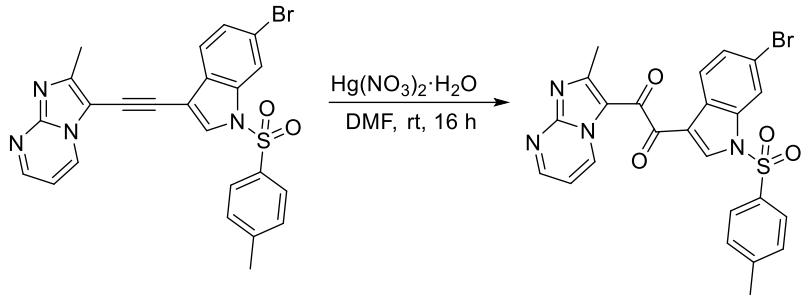
^{1}H NMR (500 MHz, Acetone-d_6) δ 8.59 (dd, J = 6.8, 1.9 Hz, 1H), 8.47 (dd, J = 4.1, 1.9 Hz, 1H), 7.13 (dd, J = 6.8, 4.1 Hz, 1H), 2.44 (s, 3H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 151.74, 150.50, 149.96, 134.80, 110.17, 61.61, 15.44. IR (neat): 3087, 3053, 2988 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_7H_7IN_3^+$) 259.9679 Found: 259.9700. m.p.: 118°C.



2-((6-bromo-1-tosyl-1*H*-indol-3-yl)ethynyl)-3-methylimidazo[1,2-a]pyrimidine (4-46): **4-34** (1 g, 2.7 mmol) and **4-35** (0.9 g, 3.5 mmol) were added to a clean, dry round bottom 3-neck flask under argon and dissolved in 15 mL of dimethylformamide. Diisopropylethylamine (0.7 mL, 4.0 mmol) was added to the reaction, and the solution was sparged with argon for 30 minutes. After sparging with argon, $Pd(PPh_3)_2Cl_2$ (94 mg, 0.13 mmol) and CuI (51 mg, 0.27 mmol) were added to the reaction, and it was sealed and put under argon using a balloon filled with argon. The reaction was stirred at room temperature for 16 hours. Once the reaction was complete, it was

quenched with 30 mL of water, and the organics were extracted into dichloromethane (3 x 30 mL). The organics were dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). The pure product was a yellow solid. (1.15 g, 85% yield).

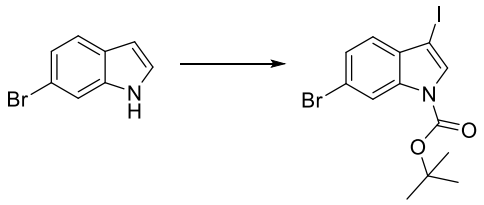
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (dd, *J* = 4.2, 2.0 Hz, 1H), 8.53 (dd, *J* = 6.7, 2.1 Hz, 1H), 8.18 (d, *J* = 1.7 Hz, 1H), 7.83 (s, 1H), 7.82 (d, *J* = 8.2 Hz, 2H), 7.52 (d, *J* = 8.3 Hz, 1H), 7.44 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 2H), 6.98 (dd, *J* = 6.7, 4.1 Hz, 1H), 2.64 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.27, 150.53, 148.02, 146.10, 134.87, 134.57, 132.44, 130.42, 129.50, 129.28, 127.54, 127.13, 121.63, 119.63, 116.88, 109.34, 104.78, 104.30, 91.28, 79.97, 21.80, 14.97. IR (neat): 2982, 2625, 2609, 2502, 2361 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{24}H_{18}BrN_4O_2S^+$) 505.0328, 507.0316 Found: 505.0337, 507.0320. m.p.: 204°C.



1-(6-bromo-1-tosyl-1*H*-indol-3-yl)-2-(3-methylimidazo[1,2-a]pyrimidin-2-yl)ethane-1,2-dione (4-33): 4-46 (0.1 g, 0.2 mmol) was added to a clean, dry round bottom flask under air and dissolved in 3 mL of anhydrous DMF. Mercuric nitrate monohydrate (0.14 g, 0.4 mmol) was added to the reaction, and it was stirred at room temperature for 18 hours. Once complete as determined by TLC, 10 mL of water was added to the reaction. Organics were extracted into ethyl acetate (3 x 40 mL). The organics were combined and washed with water (50 mL) and a saturated brine solution (50 mL). The organics were dried with Na_2SO_4 and concentrated *in*

vacuo. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%). The pure product was a yellow solid (52 mg, 49% yield).

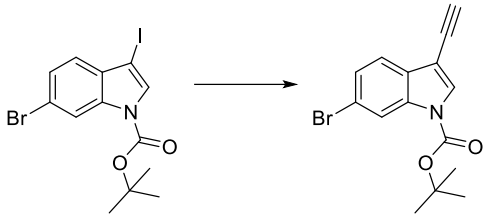
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.70 (dd, *J* = 6.7, 2.0 Hz, 1H), 8.58 (dd, *J* = 4.3, 2.1 Hz, 1H), 8.05 (s, 1H), 8.01 (d, *J* = 8.5 Hz, 1H), 7.89 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 8.5 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 2H), 7.00 (m, 1H), 2.24 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 187.36, 182.24, 159.14, 154.59, 151.22, 146.88, 136.91, 136.15, 135.64, 133.82, 130.71, 128.98, 127.45, 126.17, 124.07, 120.40, 117.49, 116.57, 116.51, 111.67, 21.86, 17.45. IR (neat): 2915, 2849, 1653, 1598 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{24}H_{18}BrN_4O_4S^+$) 537.0232, 539.0214 Found: 537.0243, 539.0214. m.p.: 141°C.



tert-butyl 6-bromo-3-iodo-1*H*-indole-1-carboxylate (4-47): 6-bromoindole (1 g, 5.1 mmol) was dissolved in 10 mL of DMF. KOH (0.72 g, 12.8 mmol) was added to the solution. Then, I_2 (1.3 g, 5.1 mmol) was added to the solution. The reaction stirred at room temperature for 1 hour. Then the reaction was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (40 mL) and then extracted into ethyl acetate (3 x 40 mL). The organics were dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product. The crude product was then dissolved in dichloromethane (20 mL) and trimethylamine (2.13 mL, 15.3 mmol) was added. 4-DMAP (0.12 g, 1.0 mmol) was then added to the solution. Lastly, boc anhydride (1.3 mL, 5.6 mmol) was added, and the reaction was stirred for 1 hour at room temperature. Once complete, the reaction was diluted in dichloromethane (50 mL) and washed with water (50 mL). The organics were separated, dried

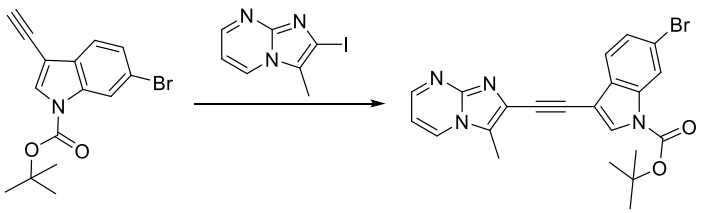
with Na_2SO_4 and concentrated *in vacuo*. The product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%)t o produce a colorless oil (1.8 g, 85% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.67 (s, 1H), 7.41 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.24 (d, *J* = 8.3 Hz, 1H), 1.67 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.40, 135.47, 131.13, 130.60, 126.70, 122.76, 119.43, 118.32, 85.03, 65.00, 28.22. IR (neat): 1732 cm^{-1}. m.p.: 148 °C. Mass spectroscopy data could not be determined. Known compound, data matches literature values (*Org. Biomol. Chem*., 2017, **15**, 6194–6204).



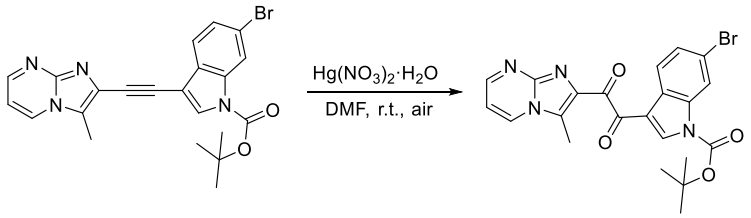
tert-butyl 6-bromo-3-ethynyl-1*H*-indole-1-carboxylate (4-48): The procedure to make **4-34** was followed. Pure product was a white solid (1.03 g, 90% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.77 (s, 1H), 7.54 (d, *J* = 8.3 Hz, 1H), 7.43 (dd, *J* = 8.3, 1.7 Hz, 1H), 3.25 (s, 1H), 1.68 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.69, 135.27, 130.31, 129.42, 126.72, 121.28, 119.19, 118.64, 102.37, 85.17, 81.23, 75.29, 28.20. IR (neat): 3291, 1736 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{15}H_{15}BrNO_2^+$) 320.0286 Found: 320.0279. m.p.: 109 °C.



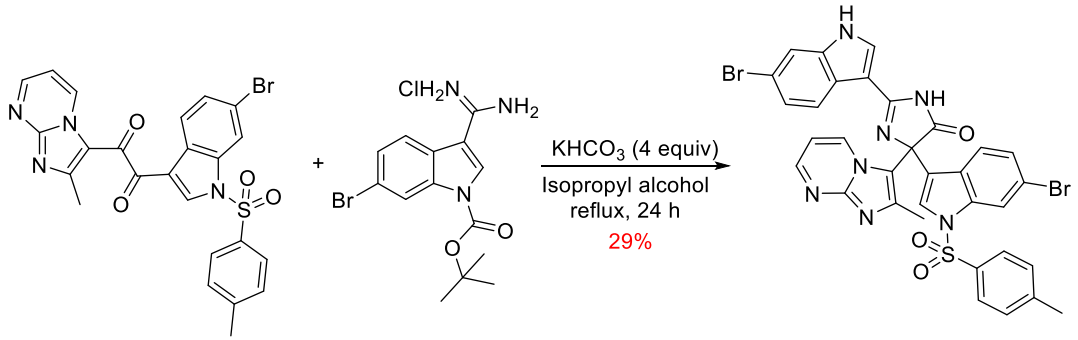
tert-butyl 6-bromo-3-((3-methylimidazo[1,2-a]pyrimidin-2-yl)ethynyl)-1H-indole-1-carboxylate (4-49): The procedure to make **4-46** was followed. Pure product was a light-yellow solid (0.87 g, 77% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (m, 2H), 8.41 (s, 1H), 7.86 (s, 1H), 7.57 (d, *J* = 8.3 Hz, 1H), 7.46 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.00 (dd, *J* = 6.6, 4.2 Hz, 1H), 2.67 (s, 3H), 1.69 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.76, 150.46, 148.67, 147.91, 135.46, 132.44, 129.67, 129.04, 126.94, 121.12, 119.44, 118.87, 109.32, 105.09, 102.64, 92.19, 85.47, 79.13, 28.22, 14.94. IR (neat): 1732 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{22}H_{20}BrN_4O_2^+$) 451.0770, 453.0752 Found: 451.0784, 453.0766. m.p.: >250 °C.



tert-butyl 6-bromo-3-(2-(3-methylimidazo[1,2-a]pyrimidin-2-yl)-2-oxoacetyl)-1H-indole-1-carboxylate (4-29): The procedure to make **4-33** was followed. Pure product was a light yellow solid (84 mg, 40% yield).

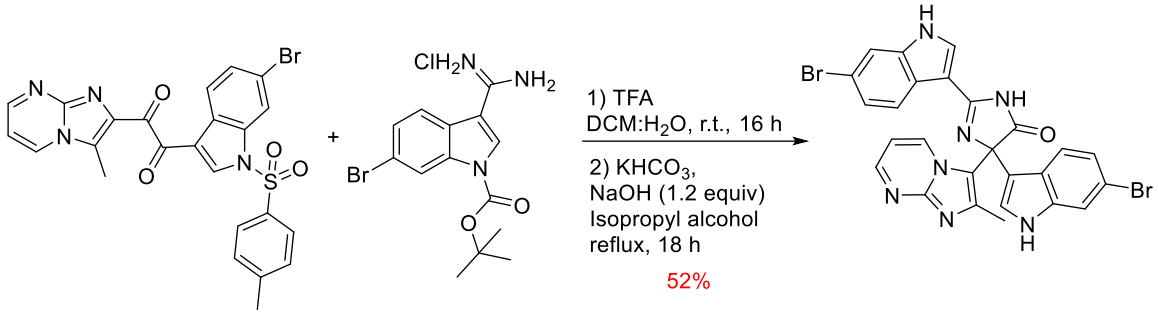
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.92 (dd, *J* = 6.8, 2.1 Hz, 1H), 8.80 (dd, *J* = 4.4, 2.1 Hz, 1H), 8.38 (s, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 8.22 (s, 1H), 7.53 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.22 (dd, *J* = 6.8, 4.3 Hz, 1H), 2.52 (s, 3H), 1.65 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 187.72, 182.66, 158.78, 154.47, 150.98, 148.20, 136.86, 136.50, 136.22, 128.43, 125.88, 123.54, 120.25, 118.73, 117.54, 115.69, 111.64, 86.88, 28.04, 17.28. IR (neat): 1748, 1650, 1597 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{22}H_{20}BrN_4O_4^+$) 483.0668, 485.0651 Found: 483.0667, 485.0649. m.p.: decomposed at >200 °C.



5-(6-bromo-1-tosyl-1*H*-indol-3-yl)-2-(6-bromo-1*H*-indol-3-yl)-5-(3-methylimidazo[1,2-a]pyrimidin-2-yl)-3,5-dihydro-4*H*-imidazol-4-one (4-51): 4-17 (25 mg, 0.067 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 5 mL of isopropyl alcohol. Potassium bicarbonate (22 mg, 0.22 mmol) was added to the reaction, and it stirred at room temperature for 10 minutes. **4-33** (30 mg, 0.056 mmol) was then added to the reaction, and the reaction stirred and refluxed overnight. Once the cyclization was complete as monitored by TLC, the reaction was concentrated in vacuo, and the crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, dichloromethane/methanol gradient 0-10%). The pure product was an off-white solid (12 mg, 29% yield).

^{1}H NMR (500 MHz, Acetone-d_6) δ 11.52 (s, br.,1H), 11.29 (s, br., 1H), 8.93 (dd, J = 7.0, 2.0 Hz, 1H), 8.44 – 8.38 (m, 3H), 8.14 (s, 1H), 7.94 (d, J = 8.1 Hz, 2H), 7.87 (s, 1H), 7.80 (s, 1H), 7.50 (d, J = 8.6 Hz, 1H), 7.42 (d, J = 8.0 Hz, 2H), 7.32 (m, 2H), 6.81 (dd, J = 7.0, 4.1 Hz, 1H), 2.61 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 181.37, 158.45, 150.22, 148.65, 147.12, 143.83, 138.95, 137.14, 135.32, 135.03, 131.65, 131.28, 128.60, 127.89, 127.72, 126.01, 125.50, 125.41, 124.36, 123.85, 120.47, 119.31, 117.21, 117.10, 115.98, 114.74, 108.75, 106.23, 71.35, 21.48, 16.63. IR (neat): 3208 (broad), 2359, 1707, 1598 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+H)$^{+}$] calcd for ($C_{26}H_{18}Br_2N_7O$+) 756.0028, 757.0057, 758.0010, 759.0037, 759.9996,

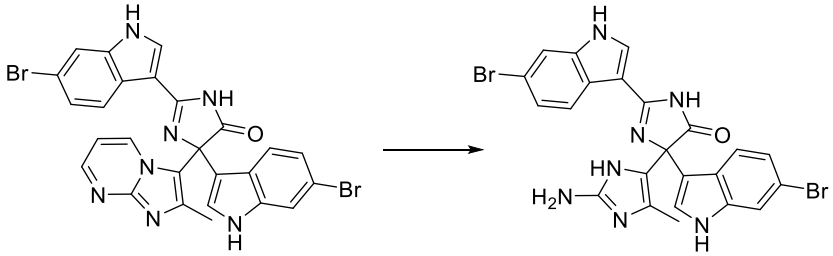
761.0018, 762.0012. Found: 756.0023, 757.0052, 758.0013, 759.0033, 759.9991, 761.0014, 762.0009. m.p.: decomposed at >200°C.



2,5-bis(6-bromo-1*H*-indol-3-yl)-5-(3-methylimidazo[1,2-a]pyrimidin-2-yl)-3,5-dihydro-4*H*-imidazol-4-one (4-50): A solution of **4-17** (22 mg, 0.06 mmol) in 5 mL of dichloromethane was added to a clean, dry round bottom flask under nitrogen. Trifluoroacetic acid (0.3 mL) and a drop of water were added to the reaction. The reaction was then stirred at room temperature for 24 hours. Once complete, the reaction was concentrated in vacuo. Once dry, the residue was dissolved in 5 mL isopropyl alcohol and potassium bicarbonate (76 mg, 0.75 mmol) was added to the reaction. Sodium hydroxide (2.3 mg, 0.06 mmol) was then added to the reaction, bringing the pH of the reaction from 1 to 8. Once the reaction turned alkaline, **4-33** (26 mg, 0.05 mmol) was added to the reaction. The reaction was refluxed overnight. Three more equivalents of sodium hydroxide were added, and the reaction was refluxed for another 2 hours. Once the reaction was complete based on TLC, the crude mixture was concentrated *in vacuo*, and the product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, dichloromethane/methanol gradient 0-10%) to give a brown solid (15 mg, 46-52% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.01 (s, br., 1H), 11.92 (s, br., 1H), 11.43 (s, br., 1H), 8.68 (d, J = 4.9 Hz, 1H), 8.39 (s, 1H), 8.24 (d, J = 8.6 Hz, 1H), 8.19 (s, 1H), 7.73 (s, 1H), 7.58 (s, 1H), 7.37 (d, J = 2.6 Hz, 1H), 7.32 (d, J = 8.6 Hz, 1H), 7.12 (d, J = 8.6 Hz, 1H), 7.05 – 6.99 (m, 1H), 6.84 (dd, J = 7.0, 4.2 Hz, 1H), 2.54 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.03, 156.37,

149.15, 146.99, 141.94, 137.75, 137.68, 134.08, 130.85, 124.79, 124.28, 124.23, 124.08, 123.12, 122.16, 120.83, 115.59, 114.99, 114.53, 114.46, 111.43, 107.68, 104.84, 104.54, 70.79, 16.11. IR (neat): 3174, 3101 (broad), 2923, 2844, 1611 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{26}H_{18}Br_2N_7O+$) 601.9940, 602.9968, 603.9921, 604.9948, 605.9905. Found: 601.9947, 602.9975, 603.9929, 604.9955, 605.9913. m.p.: 208°C.



2-amino-2',4'-bis(6-bromo-1*H*-indol-3-yl)-5-methyl-1',4'-dihydro-1*H*,5'*H*-[4,4'-biimidazol]-5'-one (4-1): **4-50** (47 mg, 0.08 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 2.5 mL of ethanol. Hydrazine monohydrate (5 mL) was then added to the reaction. The reaction was stirred at room temperature for 30 hours. Once the reaction was complete as determined by TLC, the solution was concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (C18 derivatized silica, 20-40 microns, water/methanol gradient 0-100%) to give a yellow to red solid (31 mg, 70% yield).

^{1}H NMR (500 MHz, DMSO-d_6) (NMR taken with stoichiometric amount of TFA) δ 12.10 (s, br., 1H), 11.99 (s, br., 1H), 11.26 (m, br., 2H), 8.28 (d, J = 8.5 Hz, 1H), 8.23 (s, 1H), 7.73 (s, 1H), 7.61 (s, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.34 (m, 2H), 7.14 (dd, J = 8.6, 1.8 Hz, 1H), 6.93 (s, br., 2H), 2.03 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) (Apodization set to exponential = 8 Hz for broad peaks) δ 182.58, 158.50, 145.90, 137.49, 137.41, 131.04, 124.58, 124.08, 123.99, 123.85, 122.86, 121.78, 121.08, 119.31, 118.65, 115.22, 114.64, 114.15, 114.09, 111.80, 104.56, 67.70, 9.17. IR (neat): 3722, 3633, 3225 (broad), 2924, 2359, 1685, 1559, 1449 cm^{-1}. HRMS (ESI-

TOF) m/z: $[(M+H)^+]$ calcd for ($C_{23}H_{18}Br_2N_7O^+$) 565.9940, 566.9968, 567.9921, 568.9948, 569.9904, 570.9929 Found: 565.9953, 566.9979, 567.9937, 568.9966, 569.9926, 570.9950. m.p.: decomposed at 160°C.

APPENDIX

APPENDIX

Figure 4.3 ^{1}H and ^{13}C NMR spectra of compound **4-5**

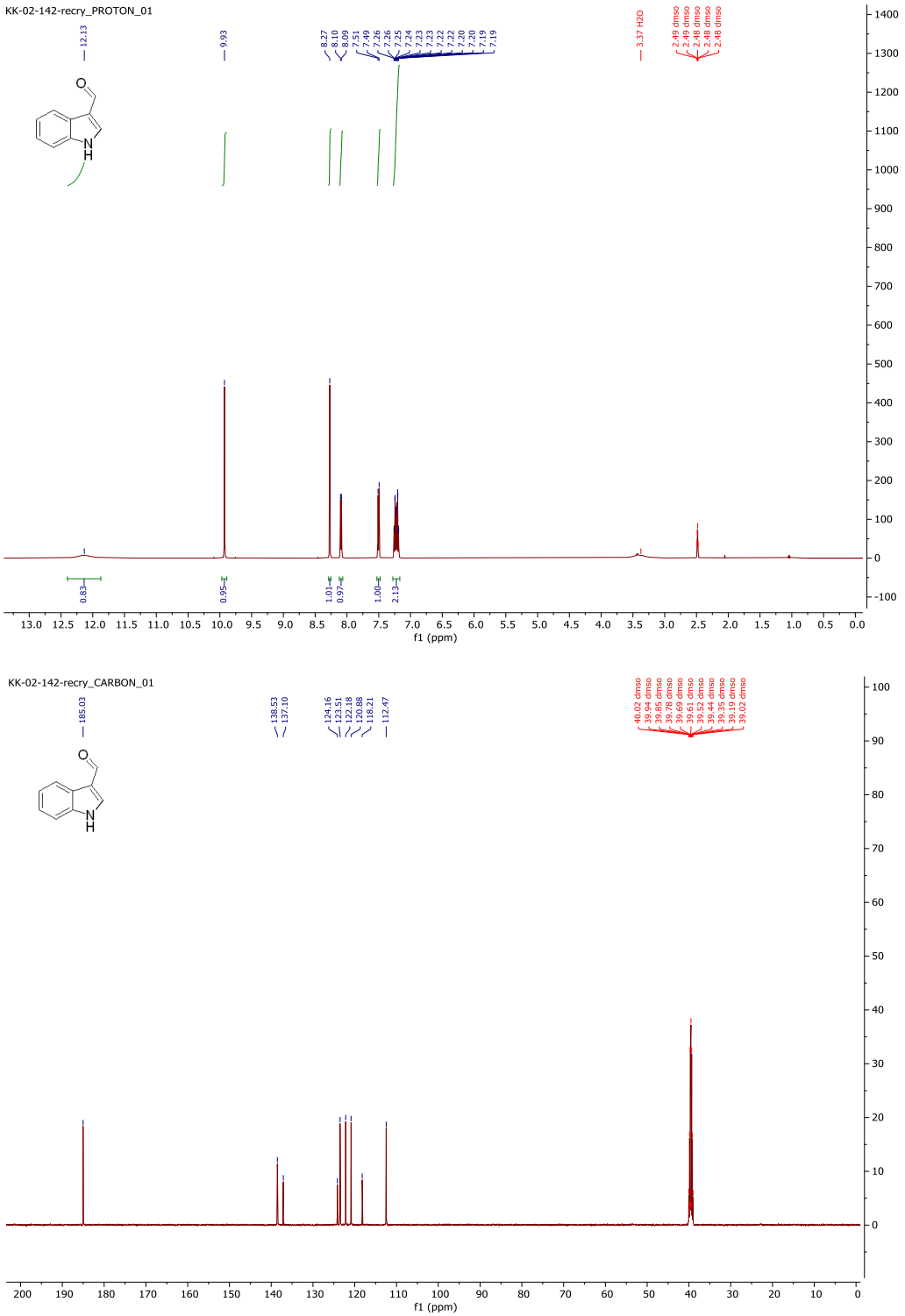
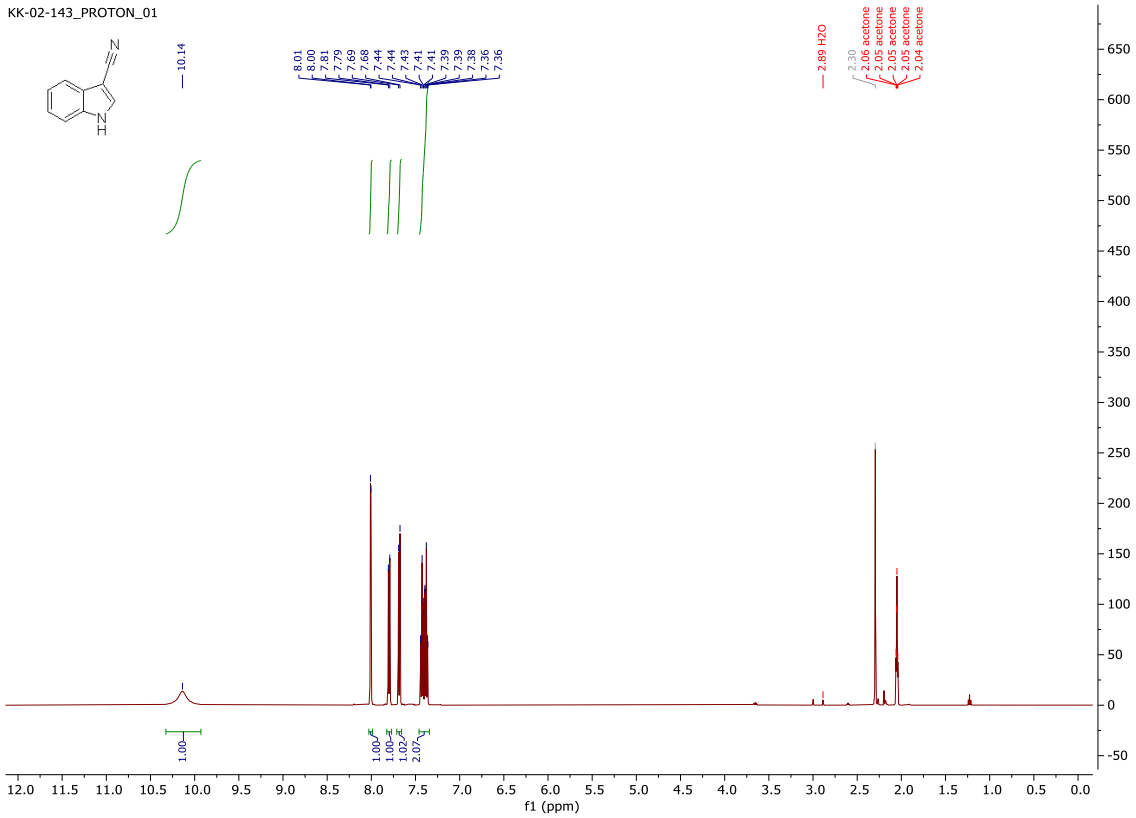


Figure 4.4 ^{1}H and ^{13}C NMR spectra of compound **4-6**



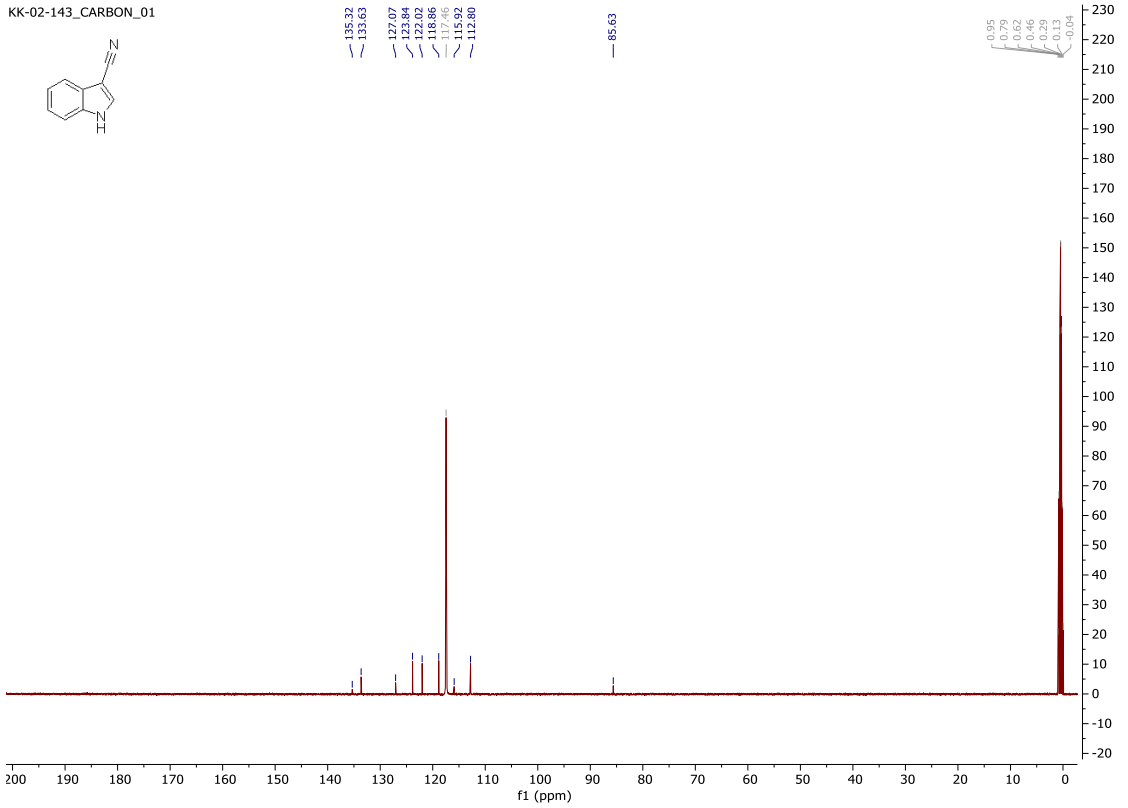


Figure 4.5 ^{1}H and ^{13}C NMR spectra of compound **4-7**

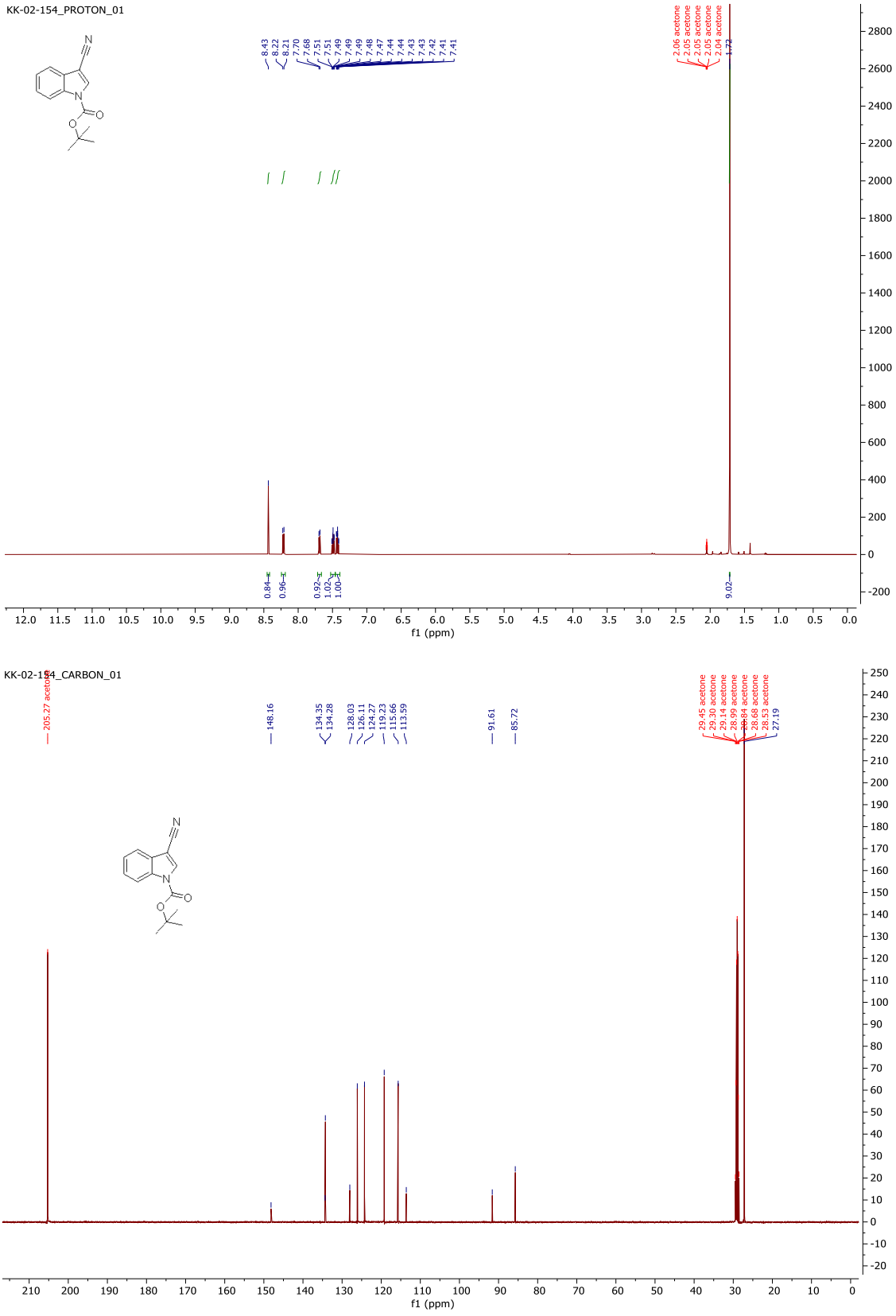


Figure 4.6 ^{1}H and ^{13}C NMR spectra of compound **4-8**

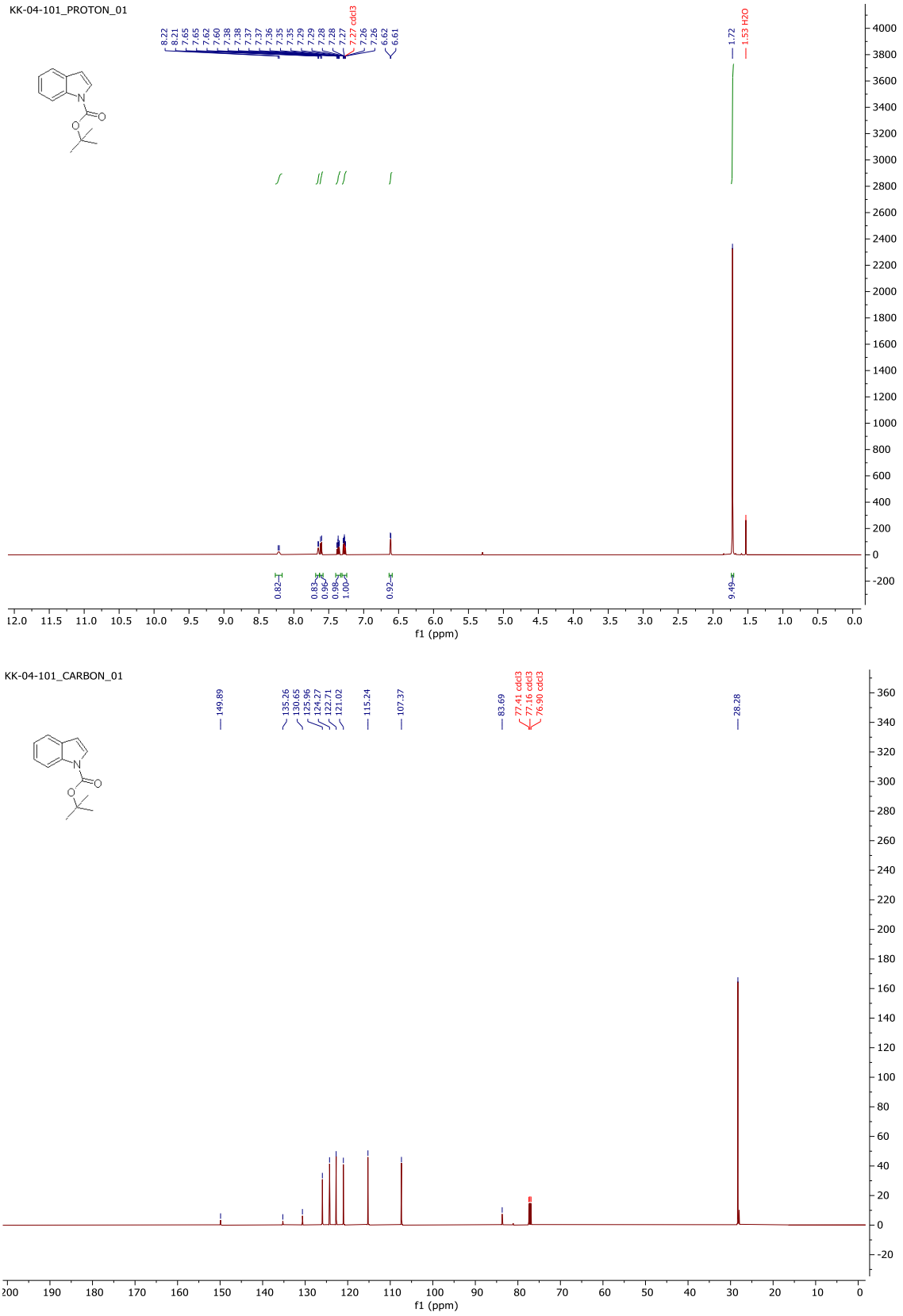


Figure 4.7 ^{1}H and ^{13}C NMR spectra of compound **4-9**

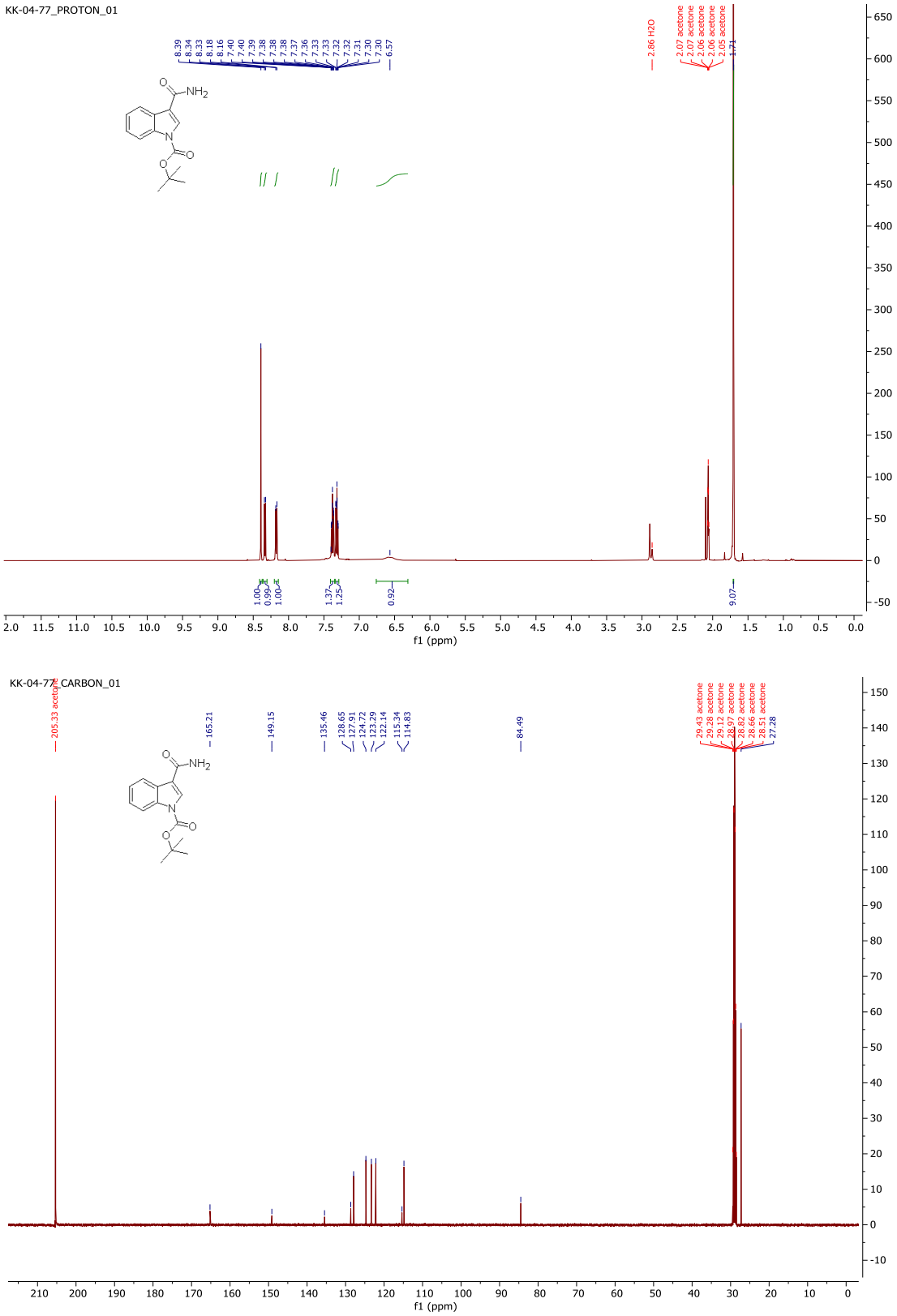


Figure 4.8 ^{1}H and ^{13}C NMR spectra of compound **4-10**

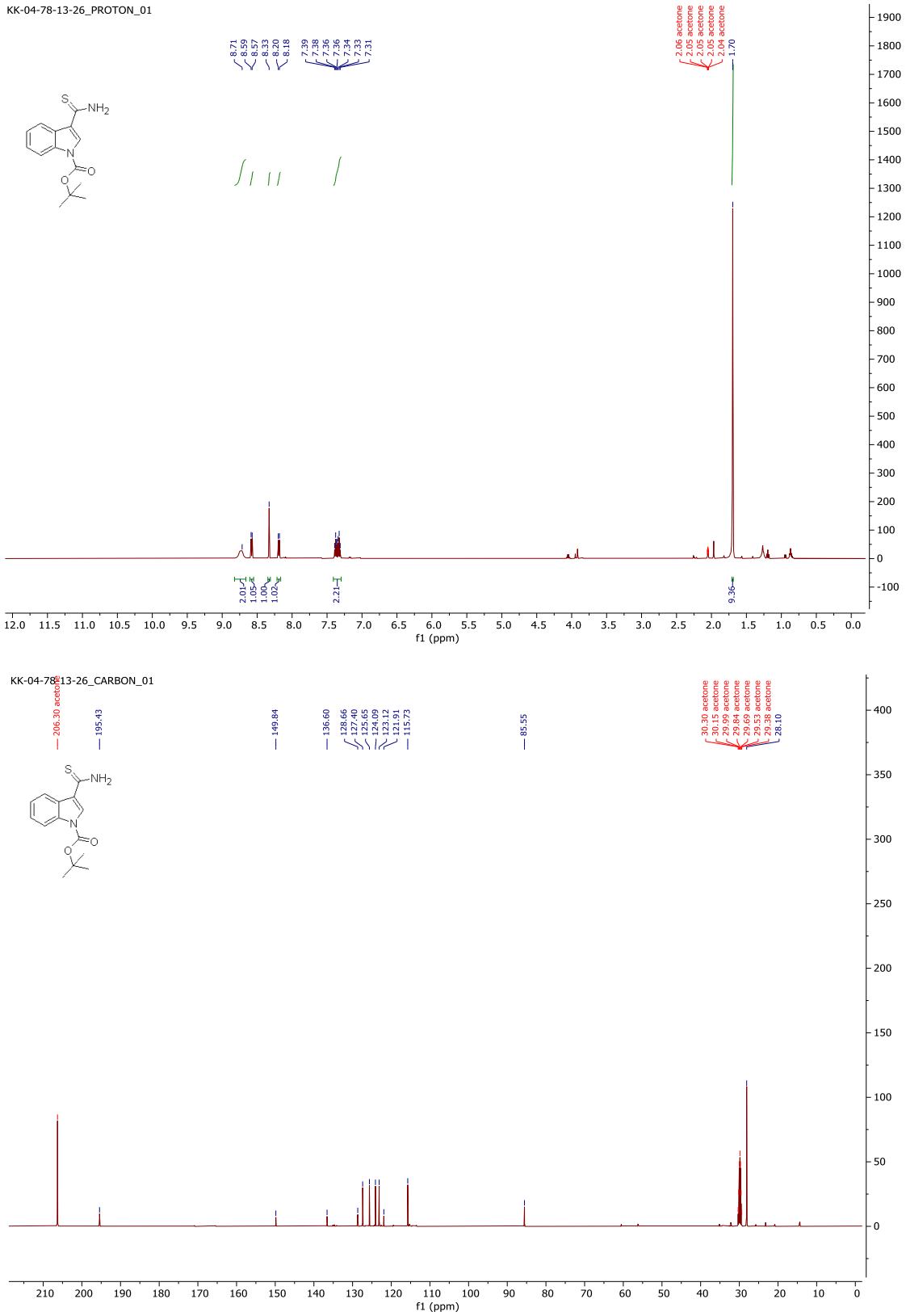


Figure 4.9 ^{1}H and ^{13}C NMR spectra of compound **4-11**

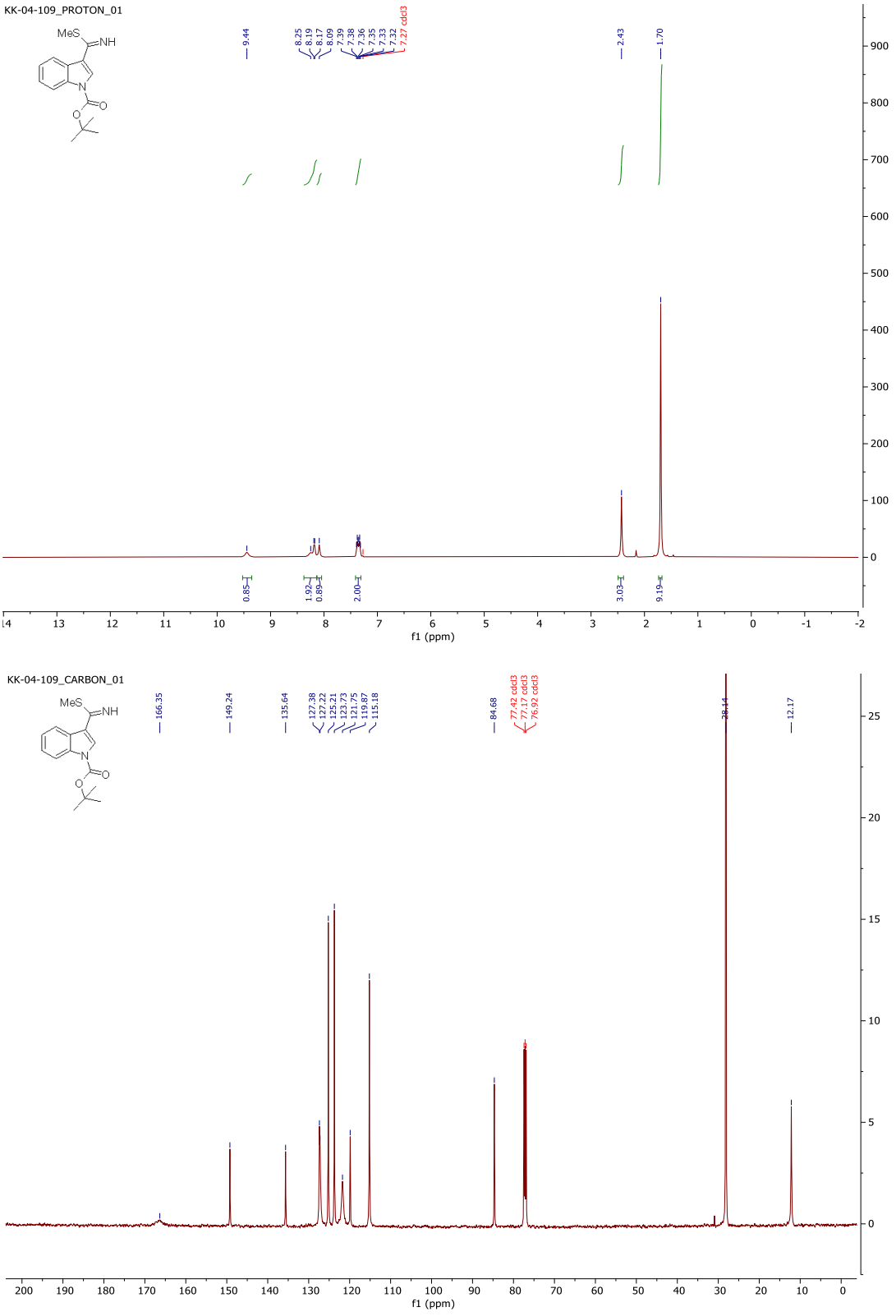


Figure 4.10 ^{1}H and ^{13}C NMR spectra of compound **4-12**

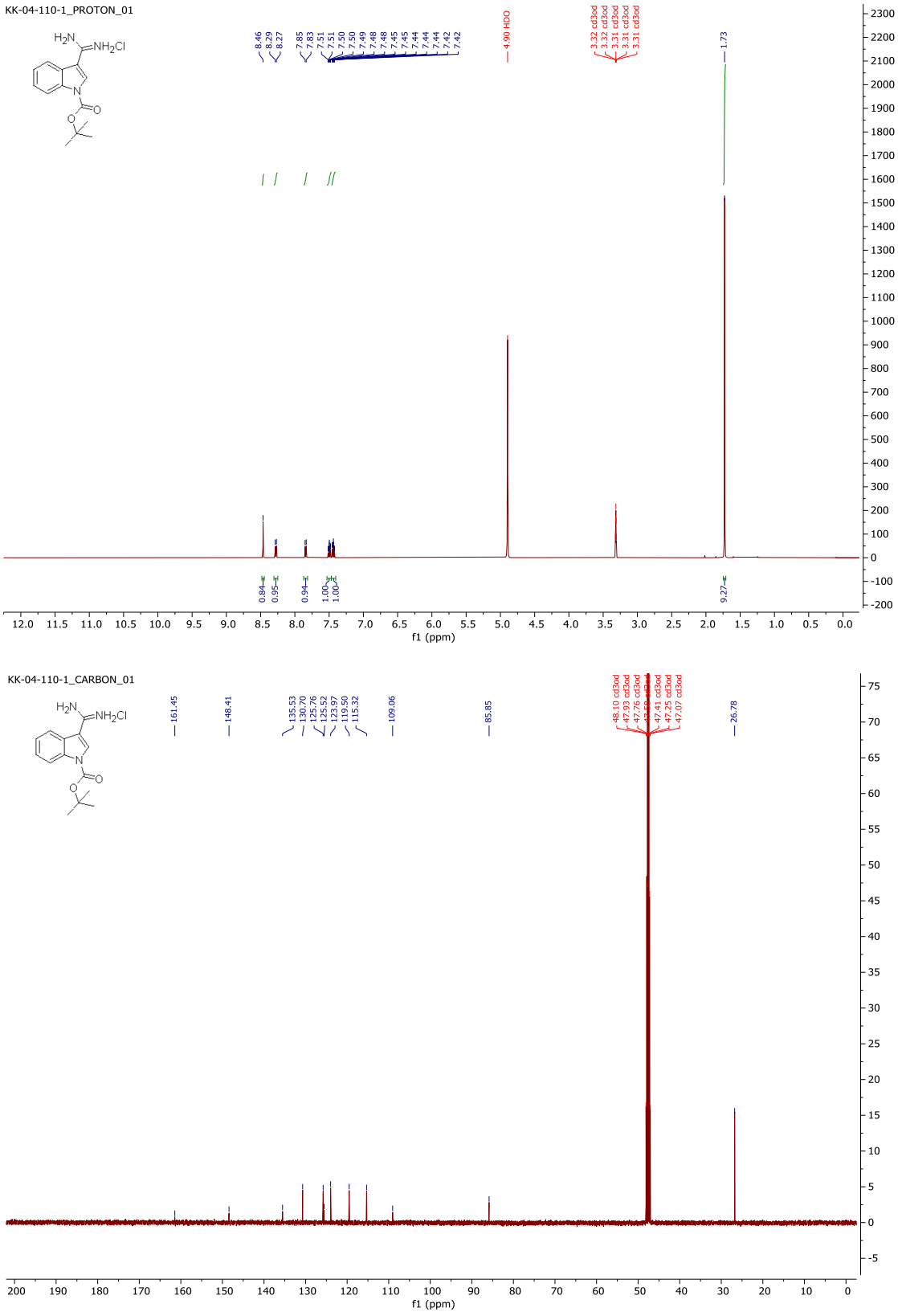


Figure 4.11 ^{1}H and ^{13}C NMR spectra of compound **4-13**

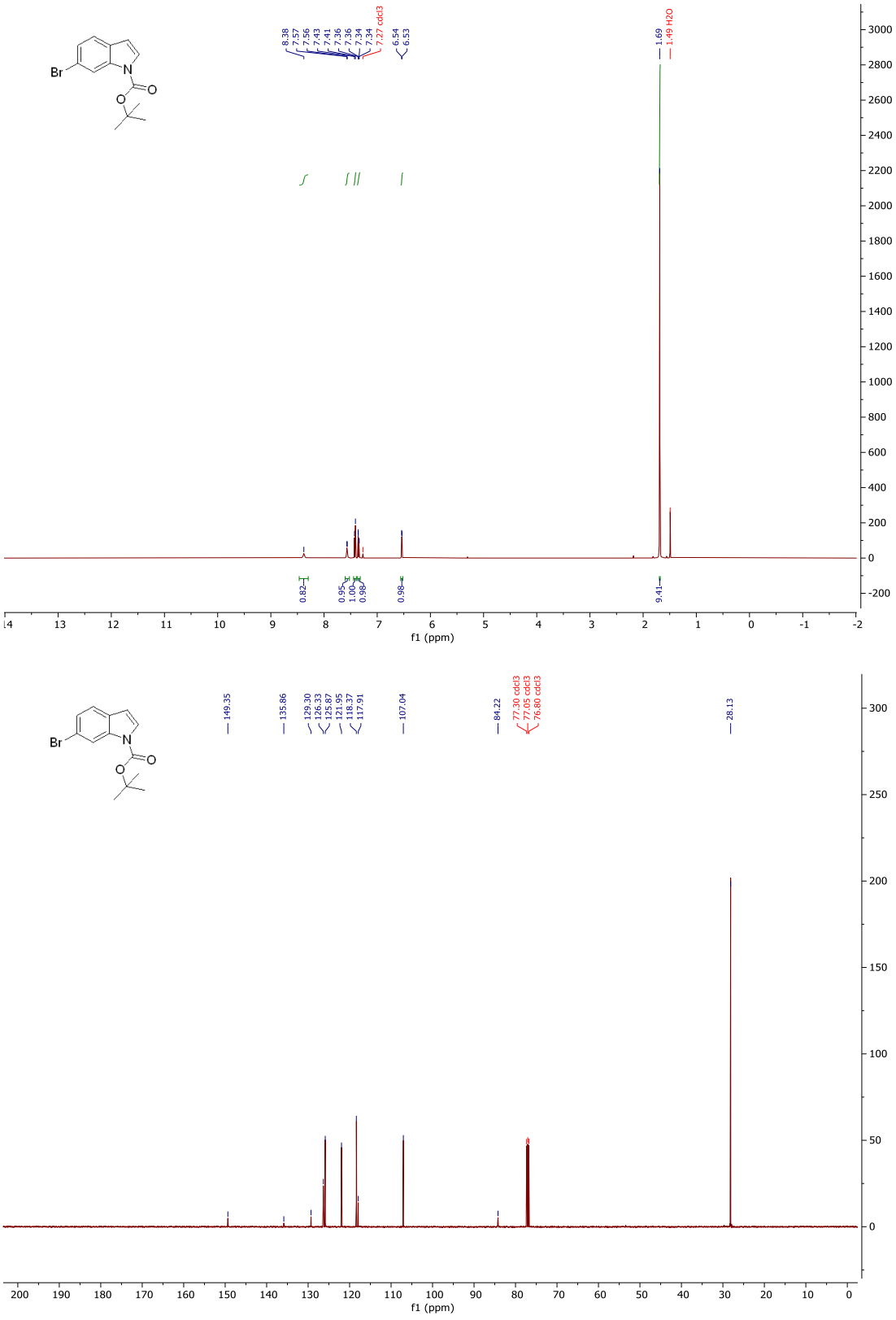


Figure 4.12 ^{1}H and ^{13}C NMR spectra of compound **4-14**

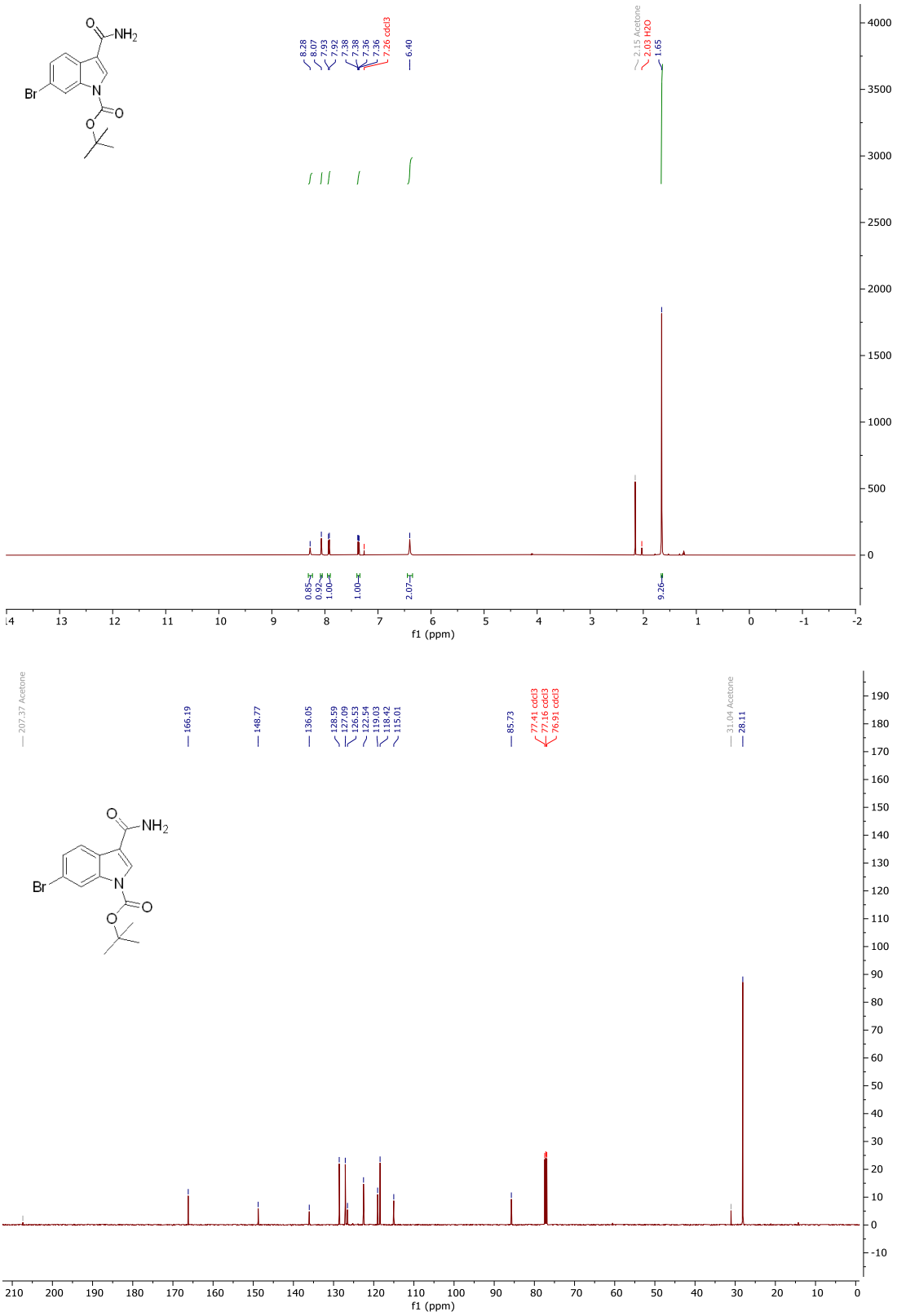


Figure 4.13 ^{1}H and ^{13}C NMR spectra of compound **4-15**

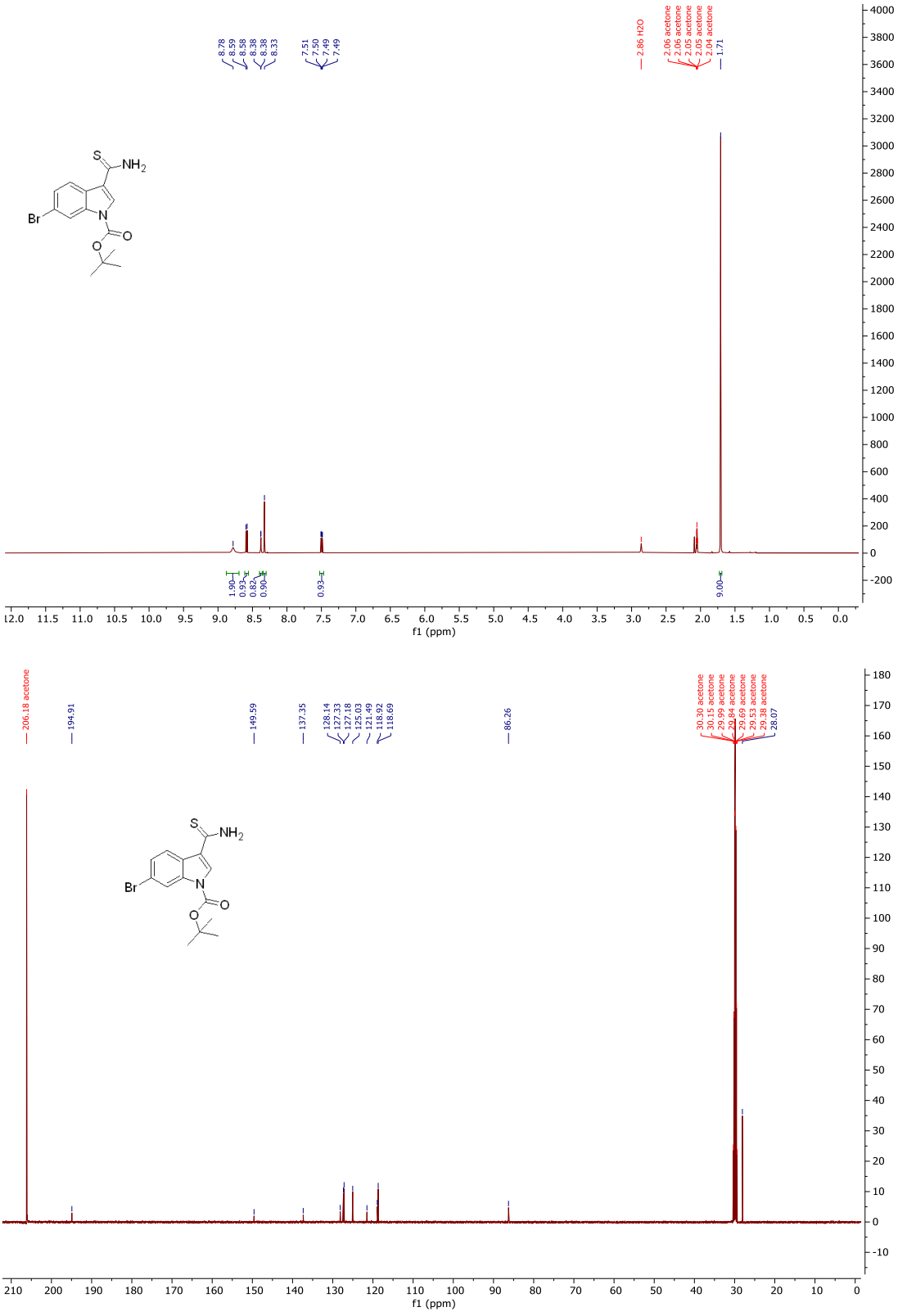


Figure 4.14 ^{1}H and ^{13}C NMR spectra of compound **4-16**

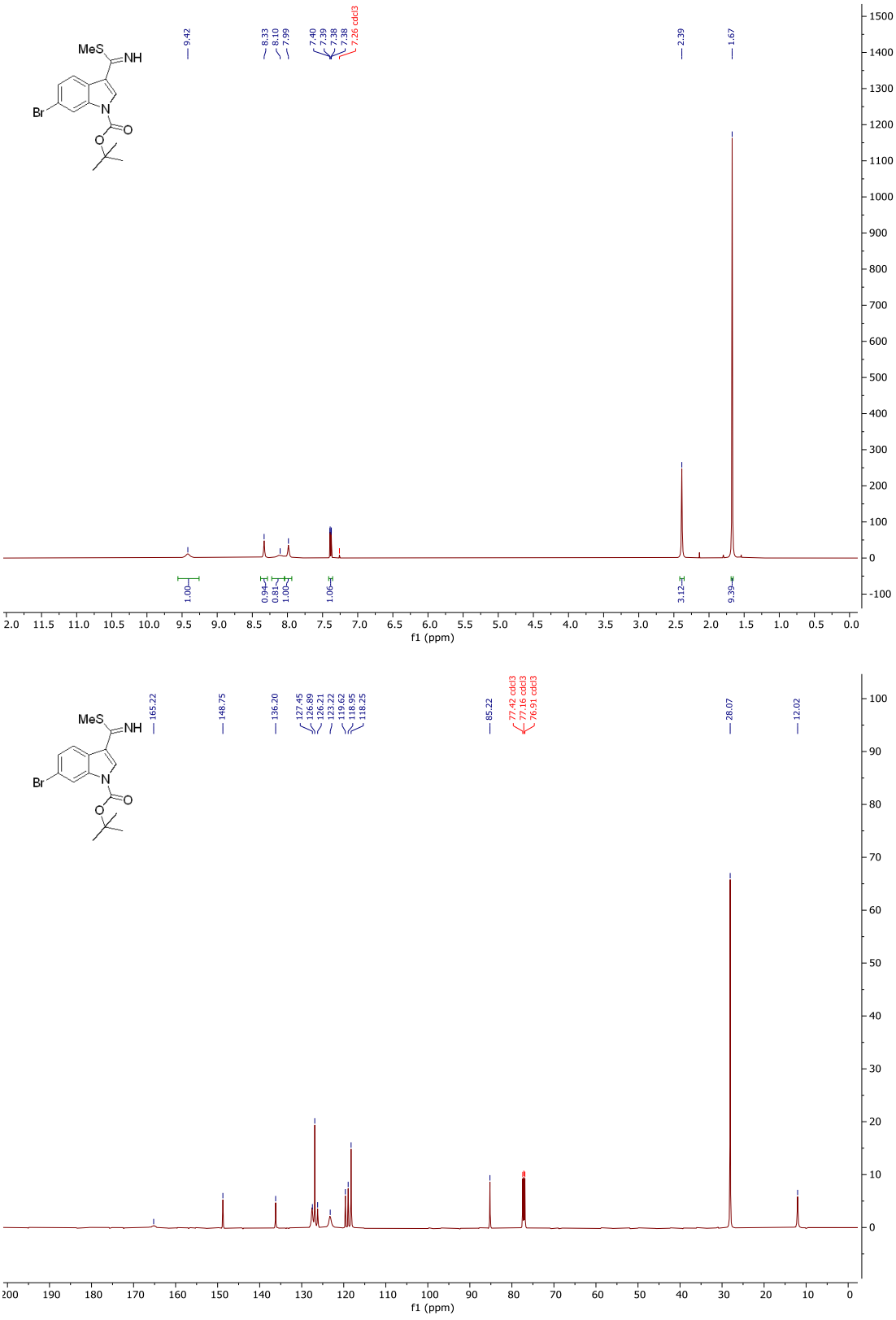


Figure 4.15 ^{1}H and ^{13}C NMR spectra of compound **4-17**

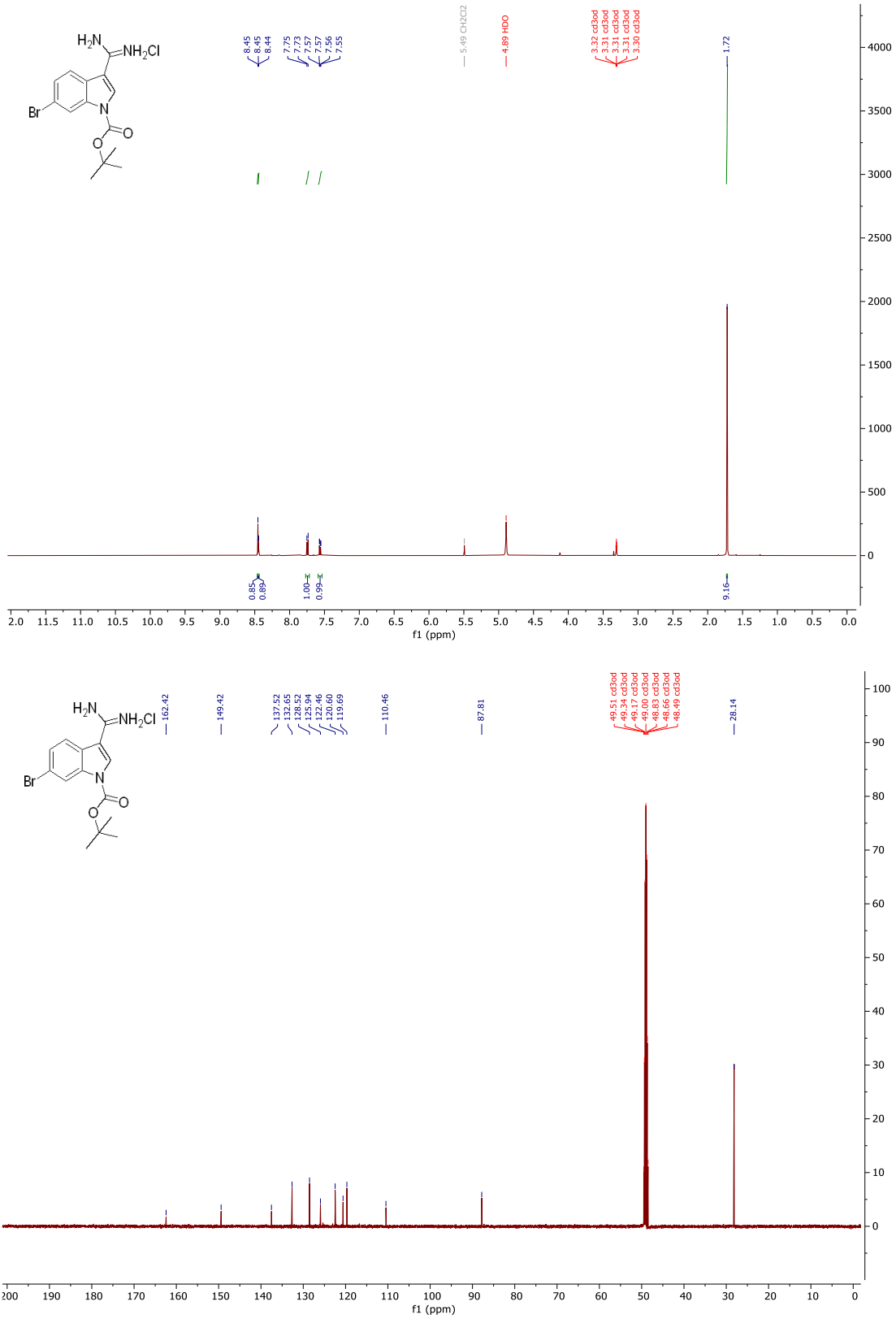


Figure 4.16 ^{1}H and ^{13}C NMR spectra of compound **4-18**

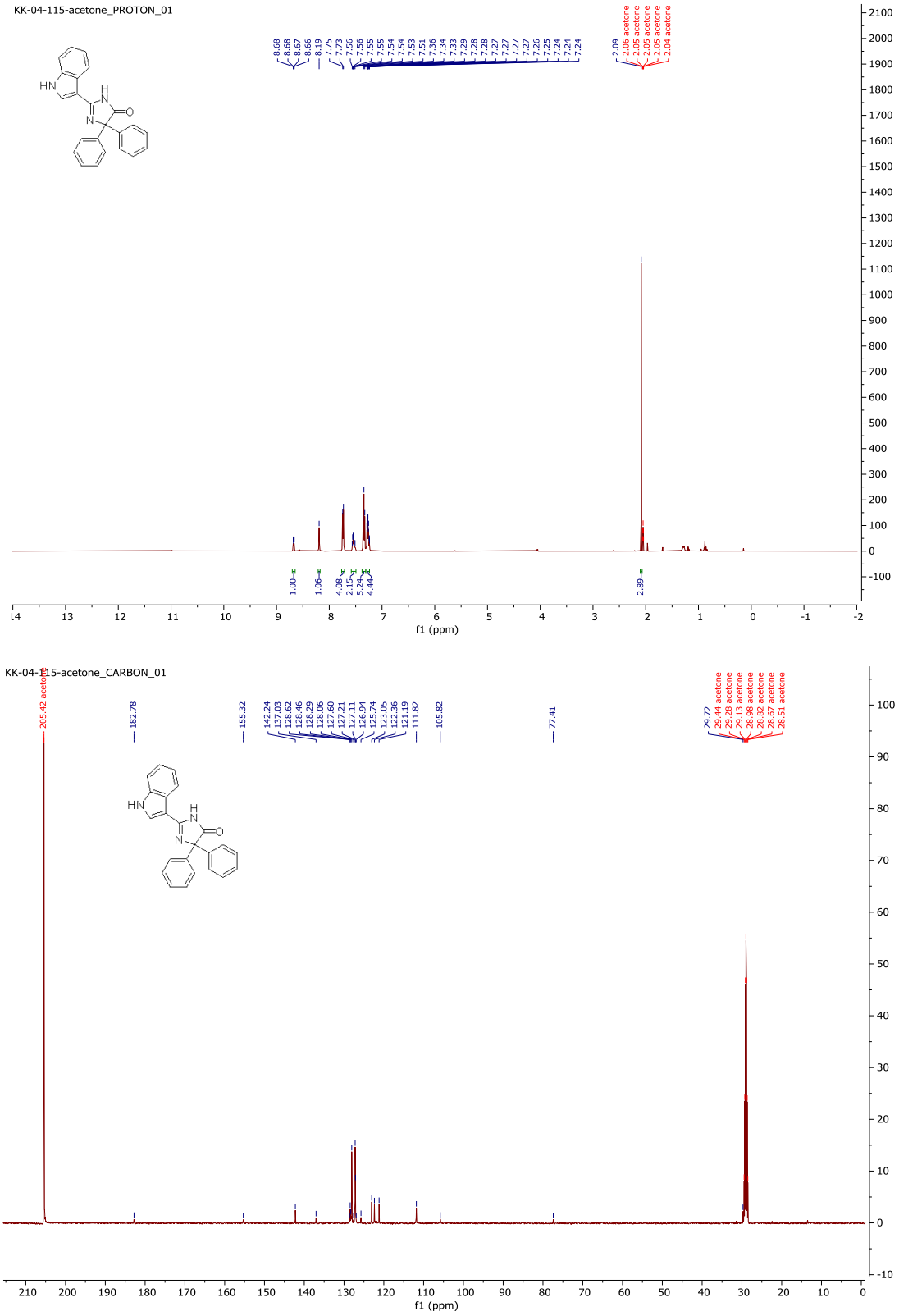
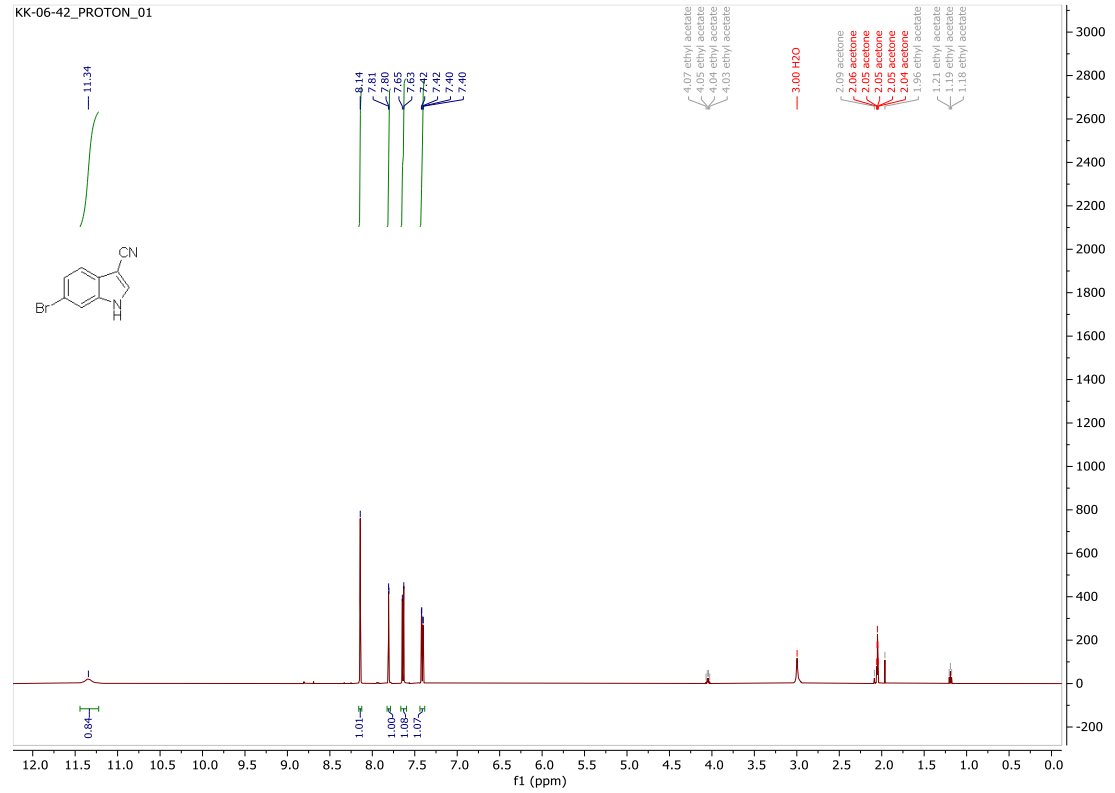


Figure 4.17 ^{1}H and ^{13}C NMR spectra of compound **4-19**



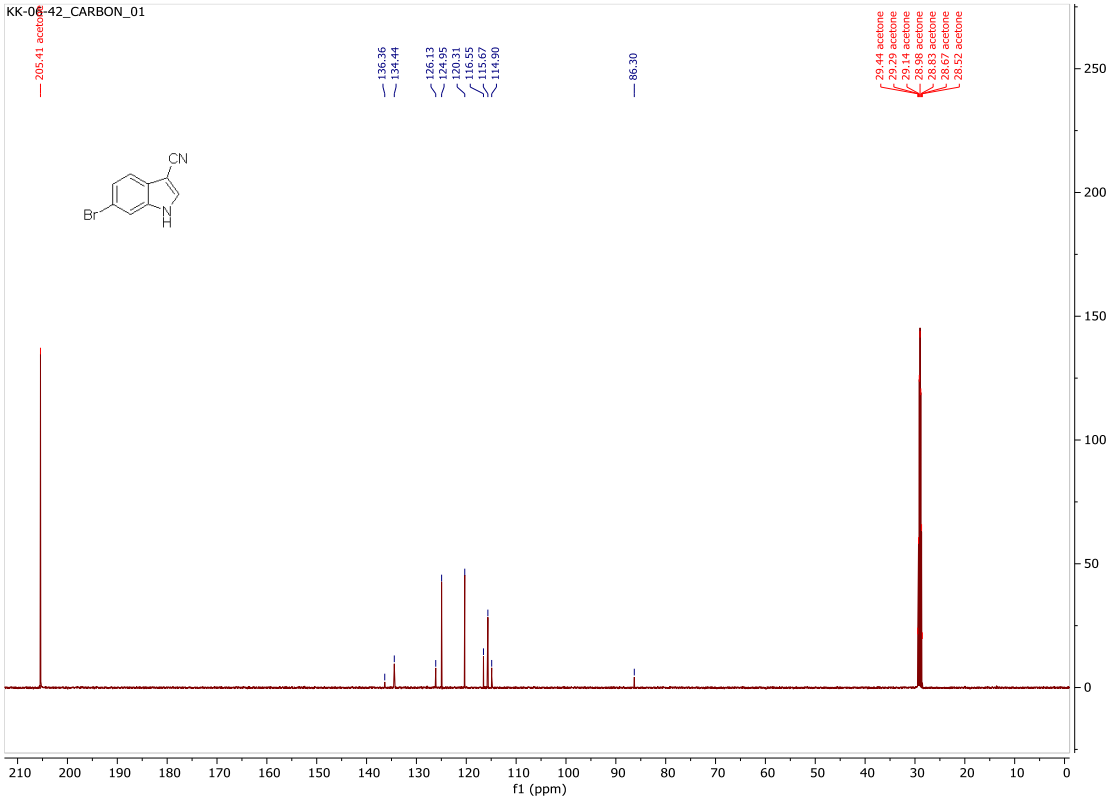
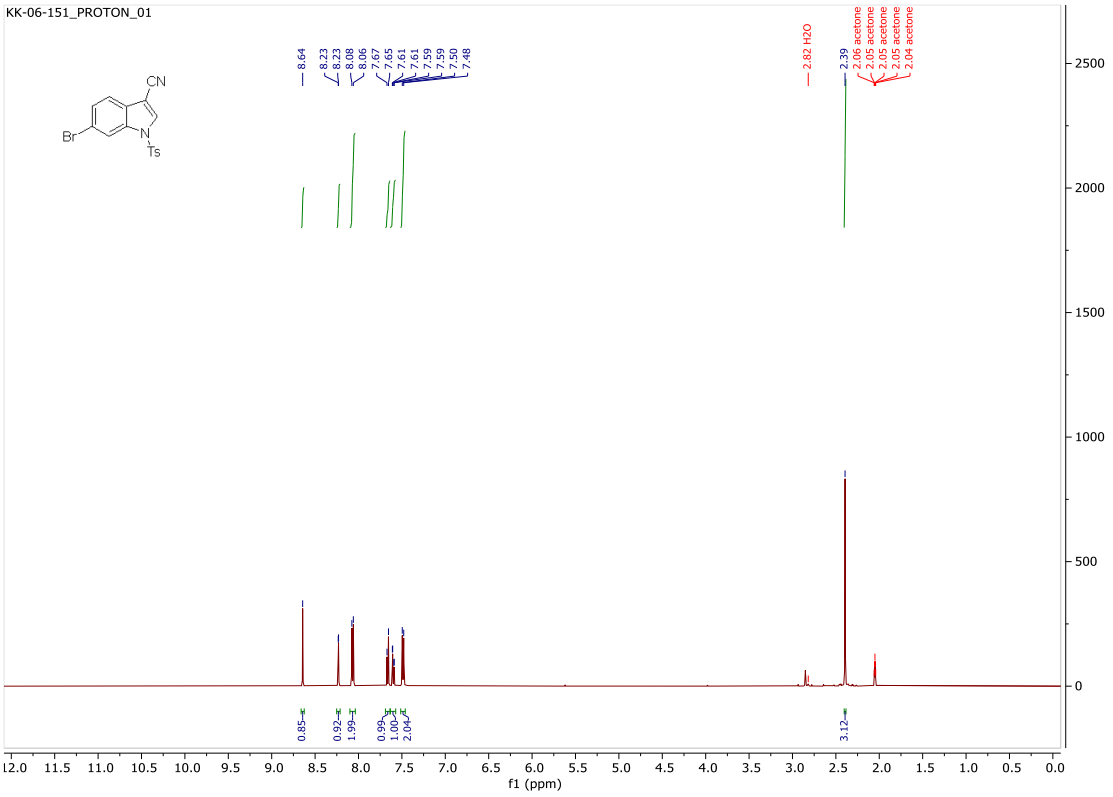


Figure 4.18 ^{1}H and ^{13}C NMR spectra of compound **4-20**



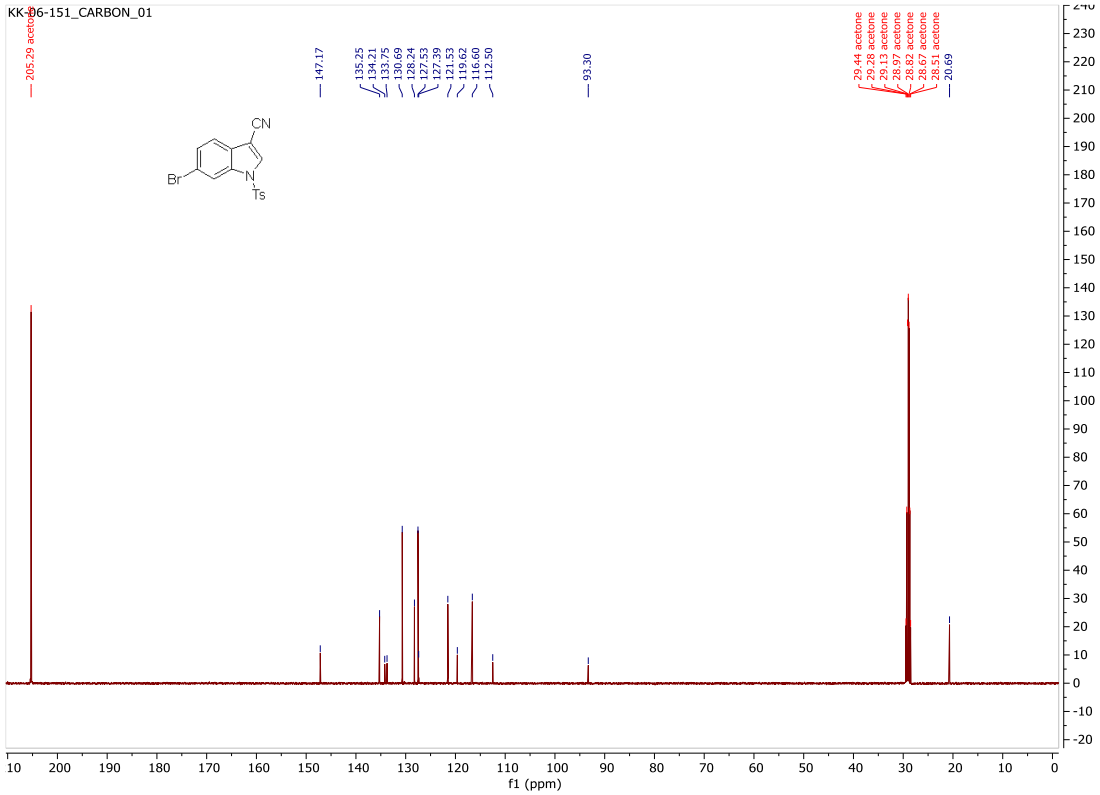
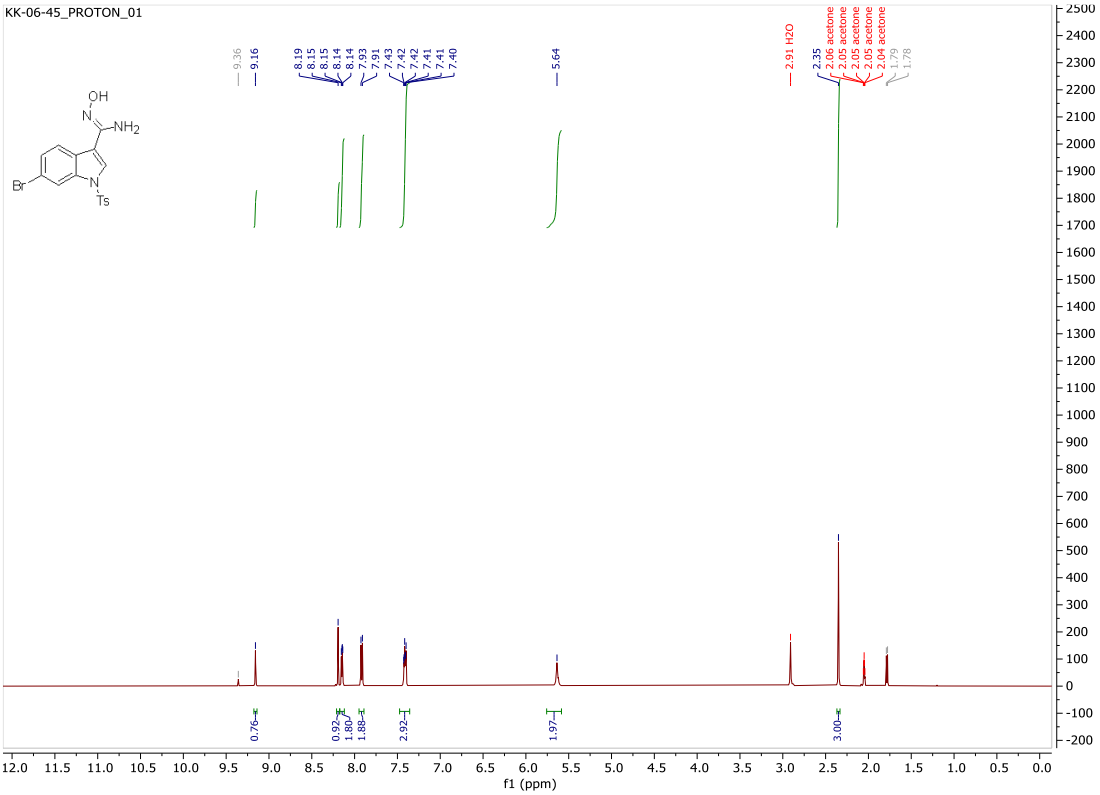


Figure 4.19 ^{1}H and ^{13}C NMR spectra of compound **4-21**



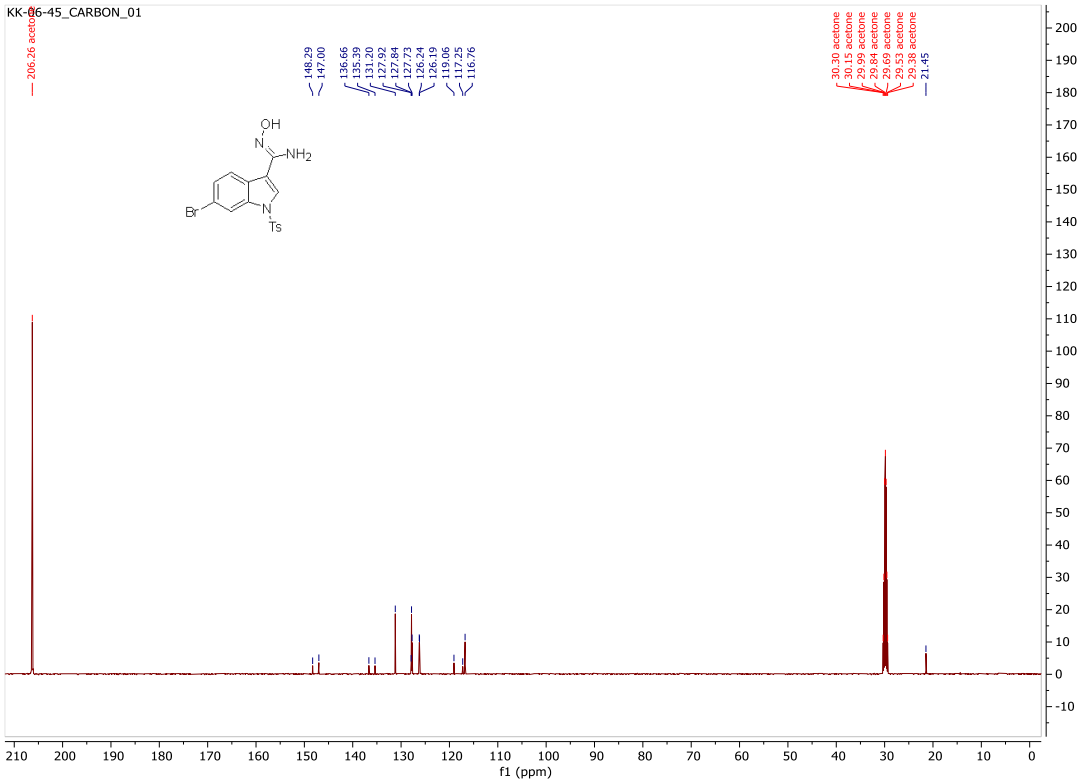


Figure 4.20 ^{1}H and ^{13}C NMR spectra of compound **4-22**

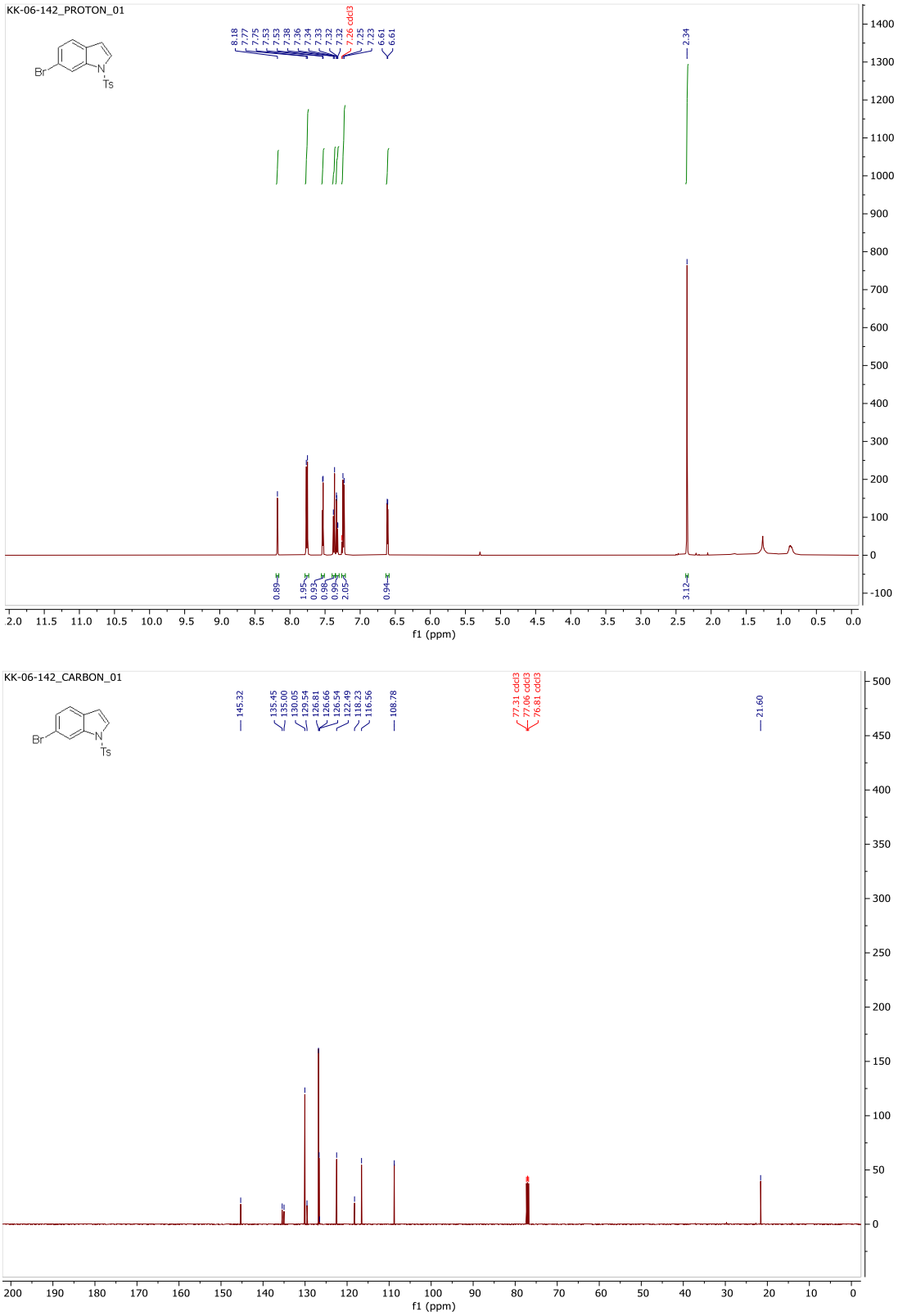
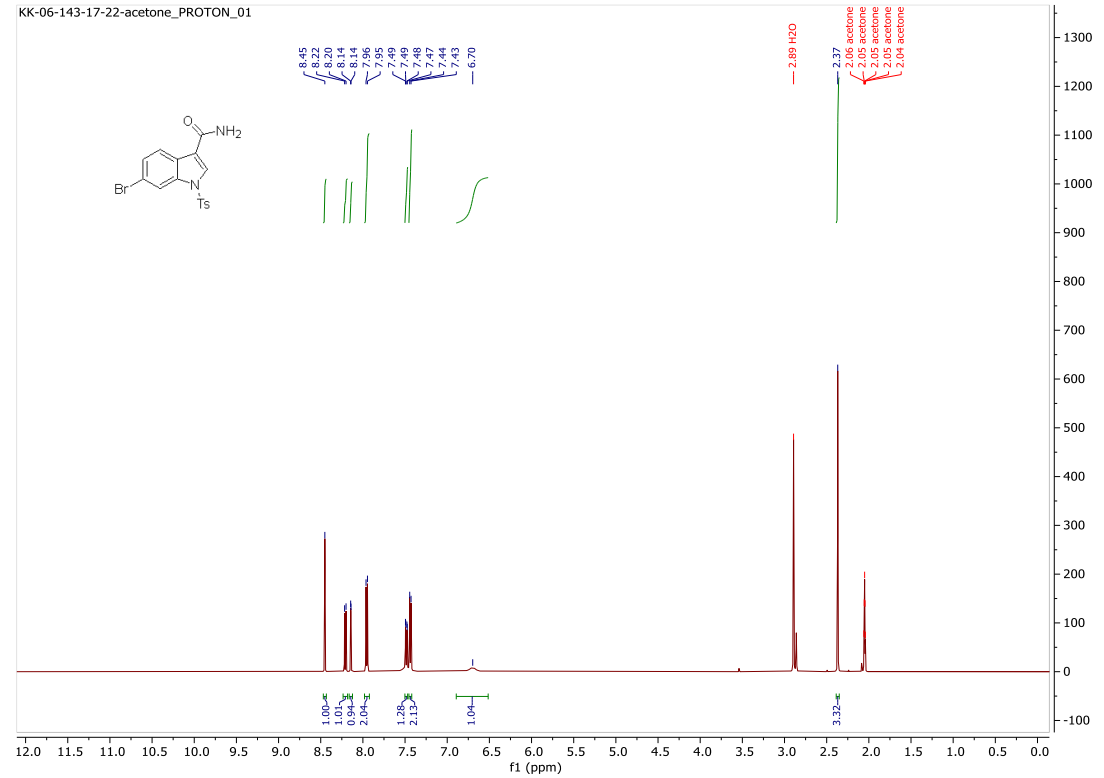


Figure 4.21 ^{1}H and ^{13}C NMR spectra of compound **4-23**



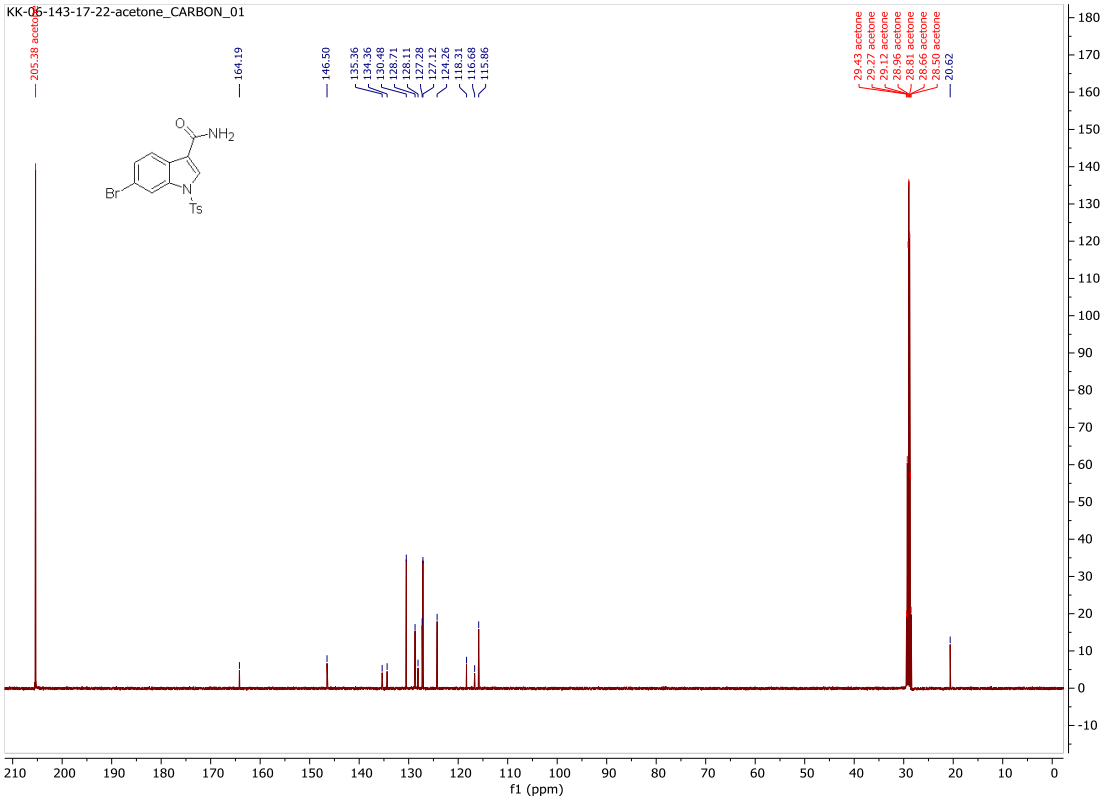
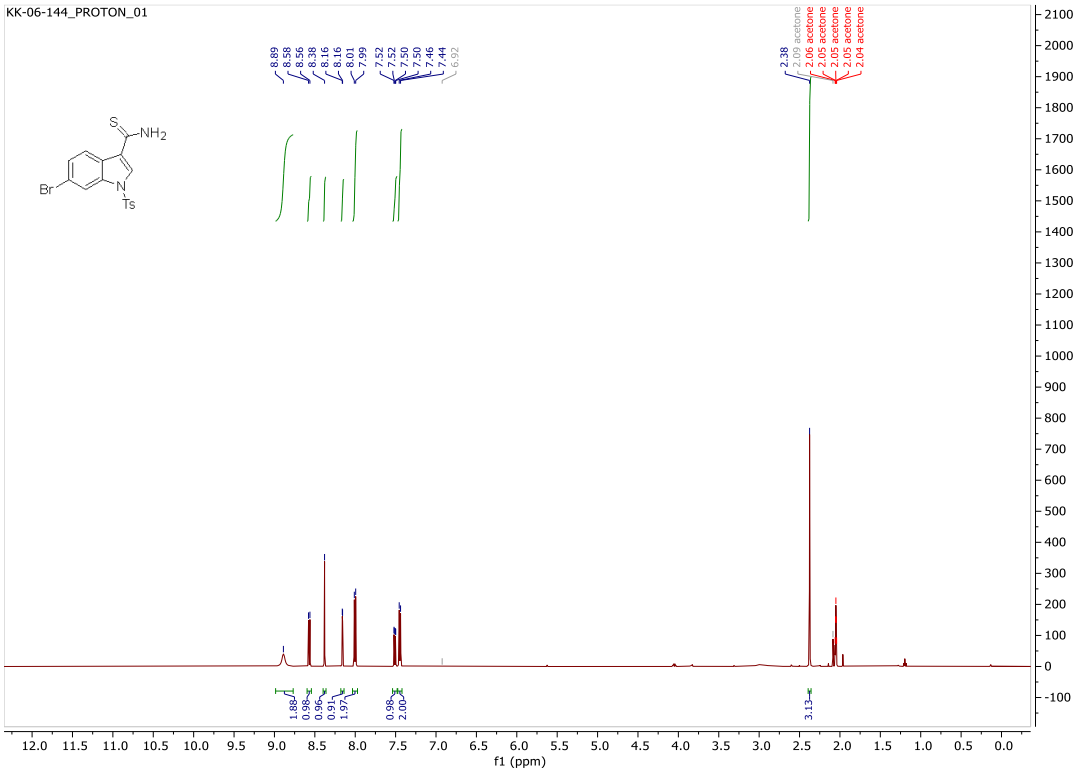


Figure 4.22 ^{1}H and ^{13}C NMR spectra of compound **4-24**



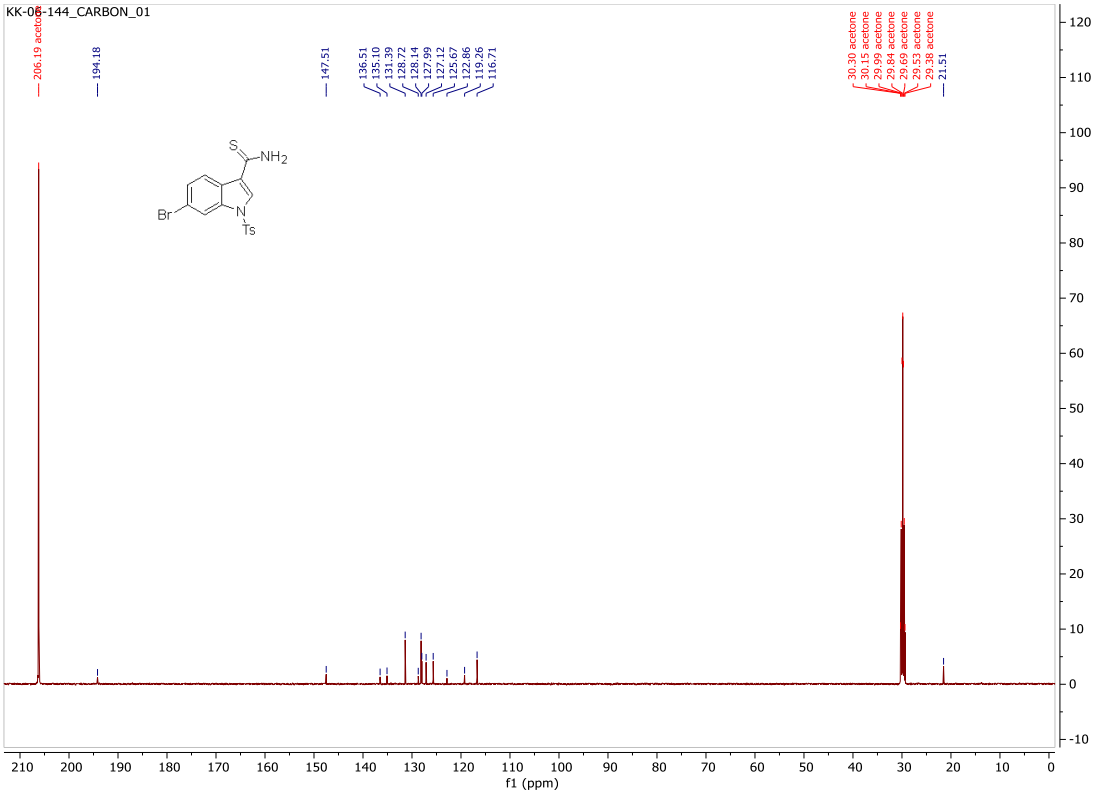
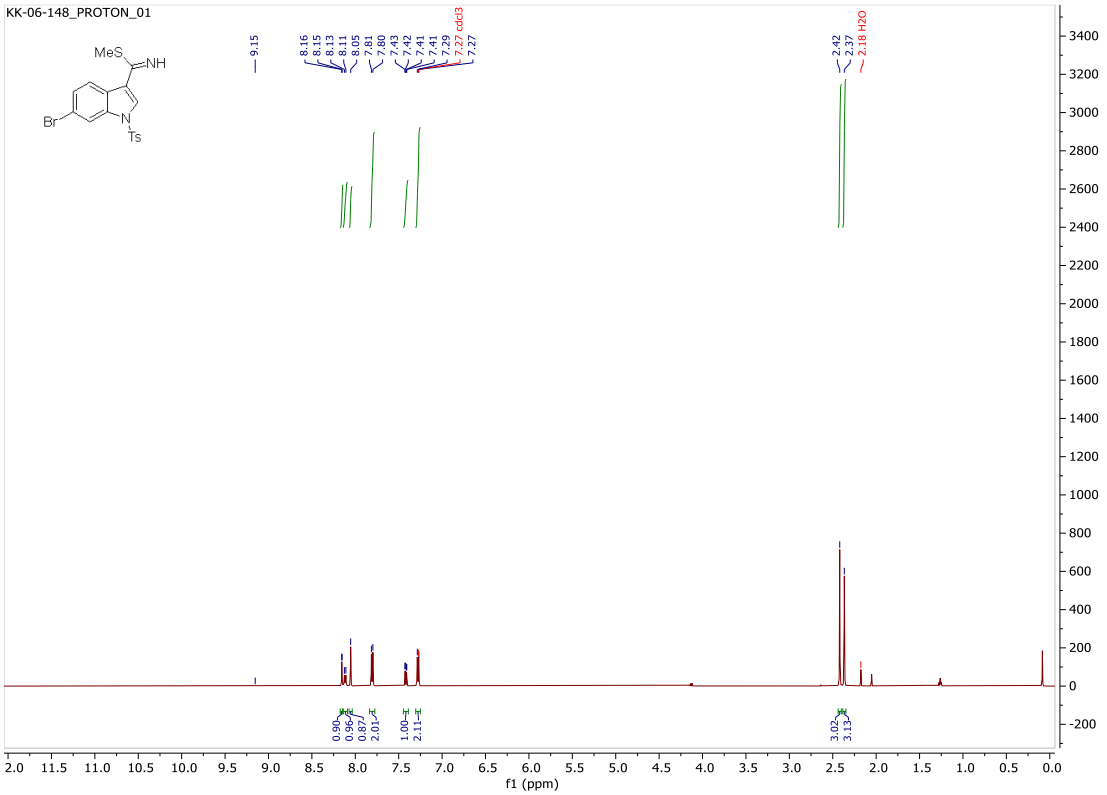


Figure 4.23 ^{1}H and ^{13}C NMR spectra of compound **4-25**



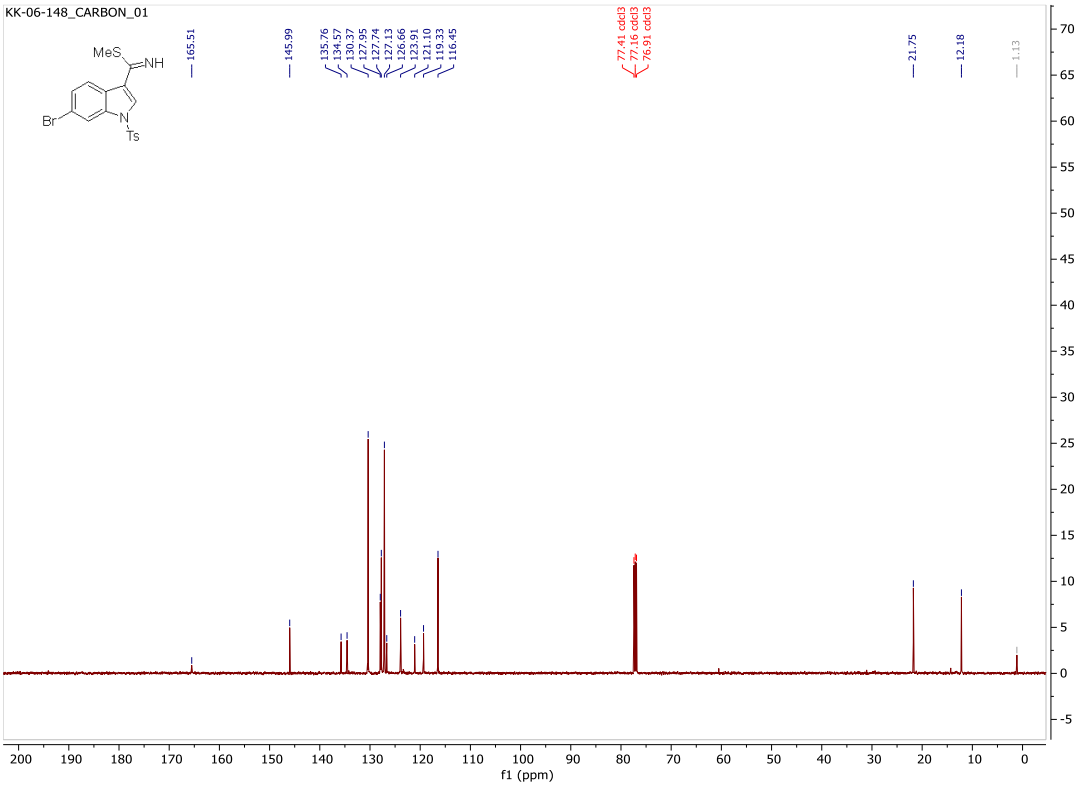


Figure 4.24 ^{1}H and ^{13}C NMR spectra of compound **4-26**

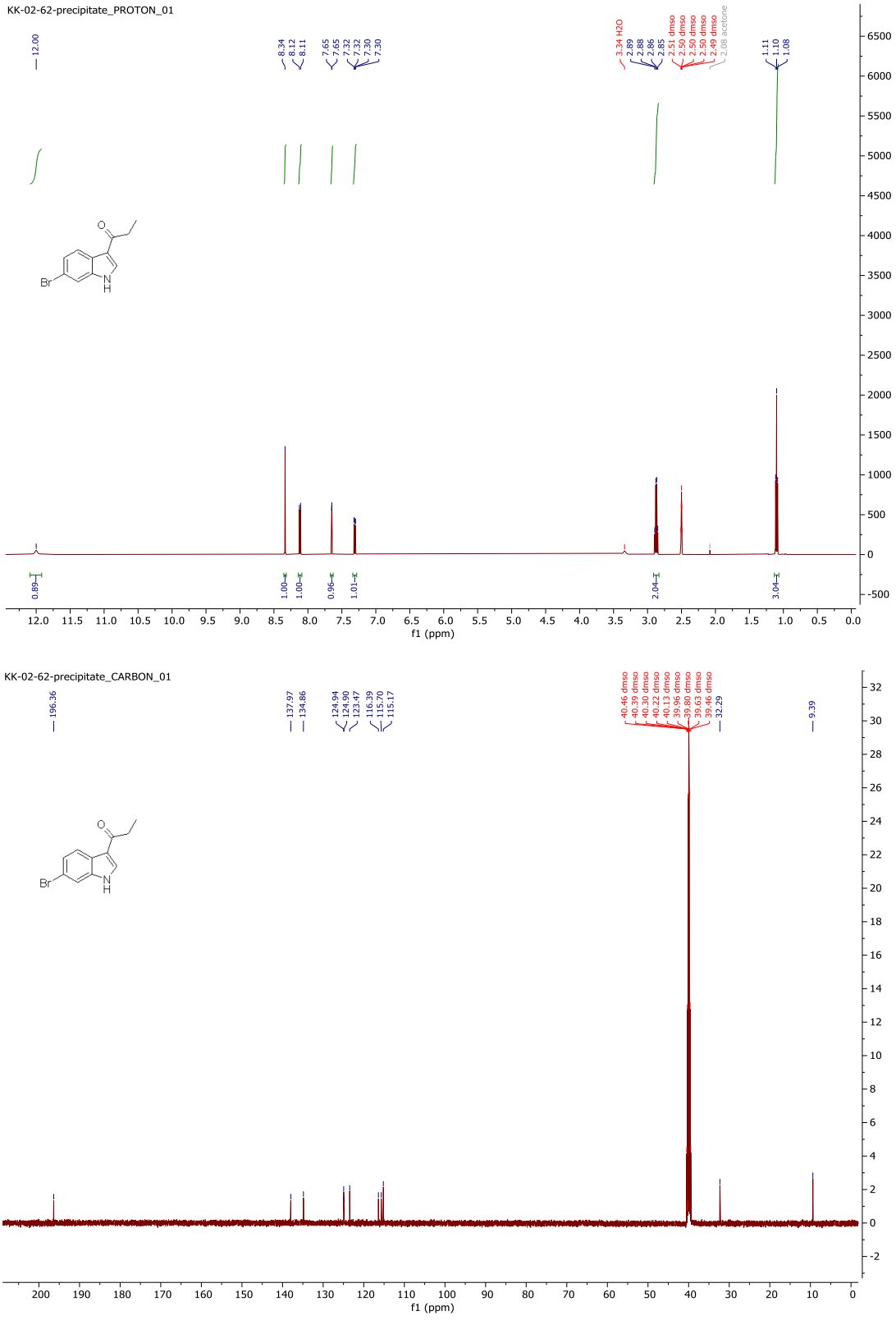


Figure 4.25 ^{1}H and ^{13}C NMR spectra of compound **4-27**

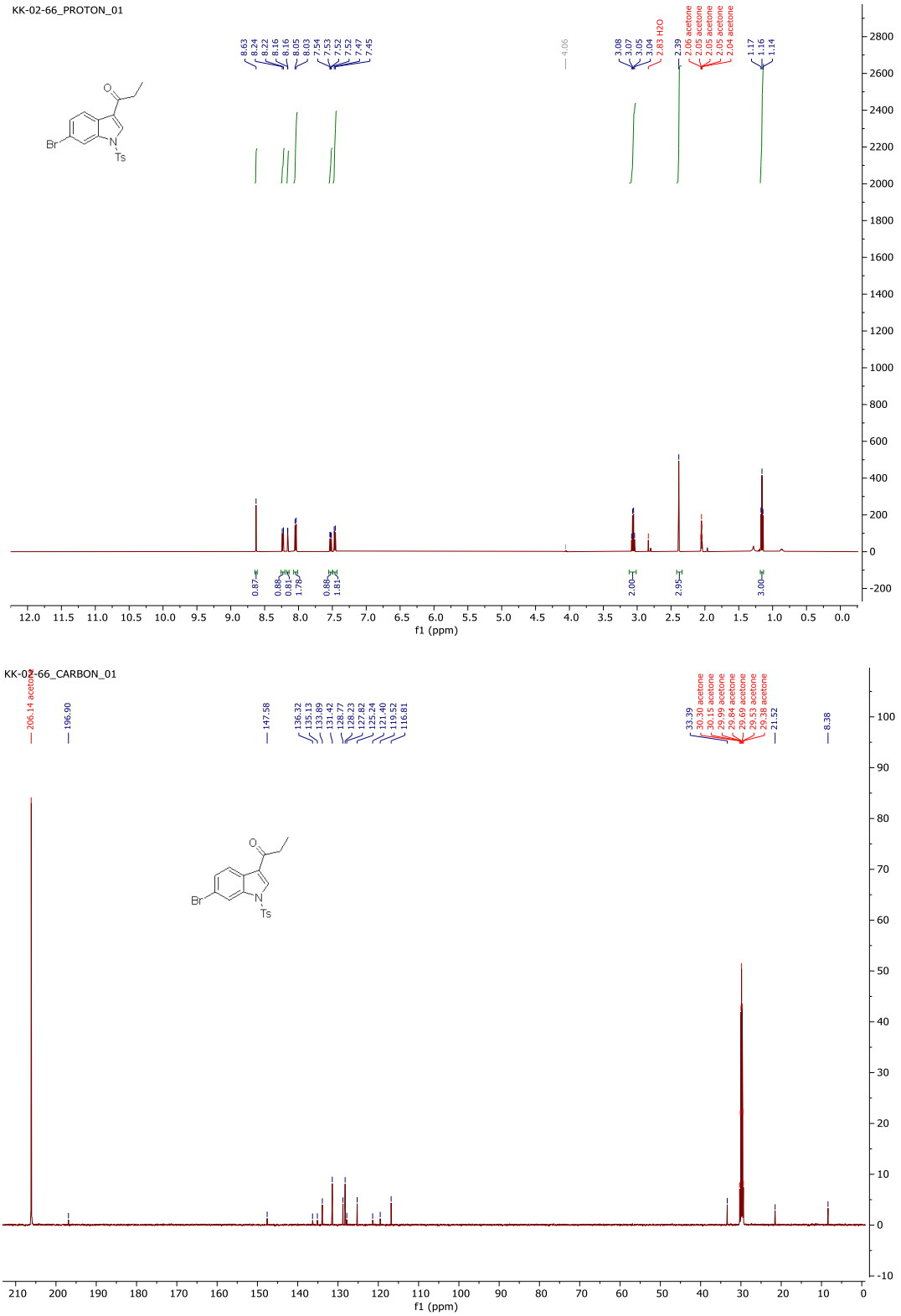


Figure 4.26 ^{1}H and ^{13}C NMR spectra of compound **4-28**

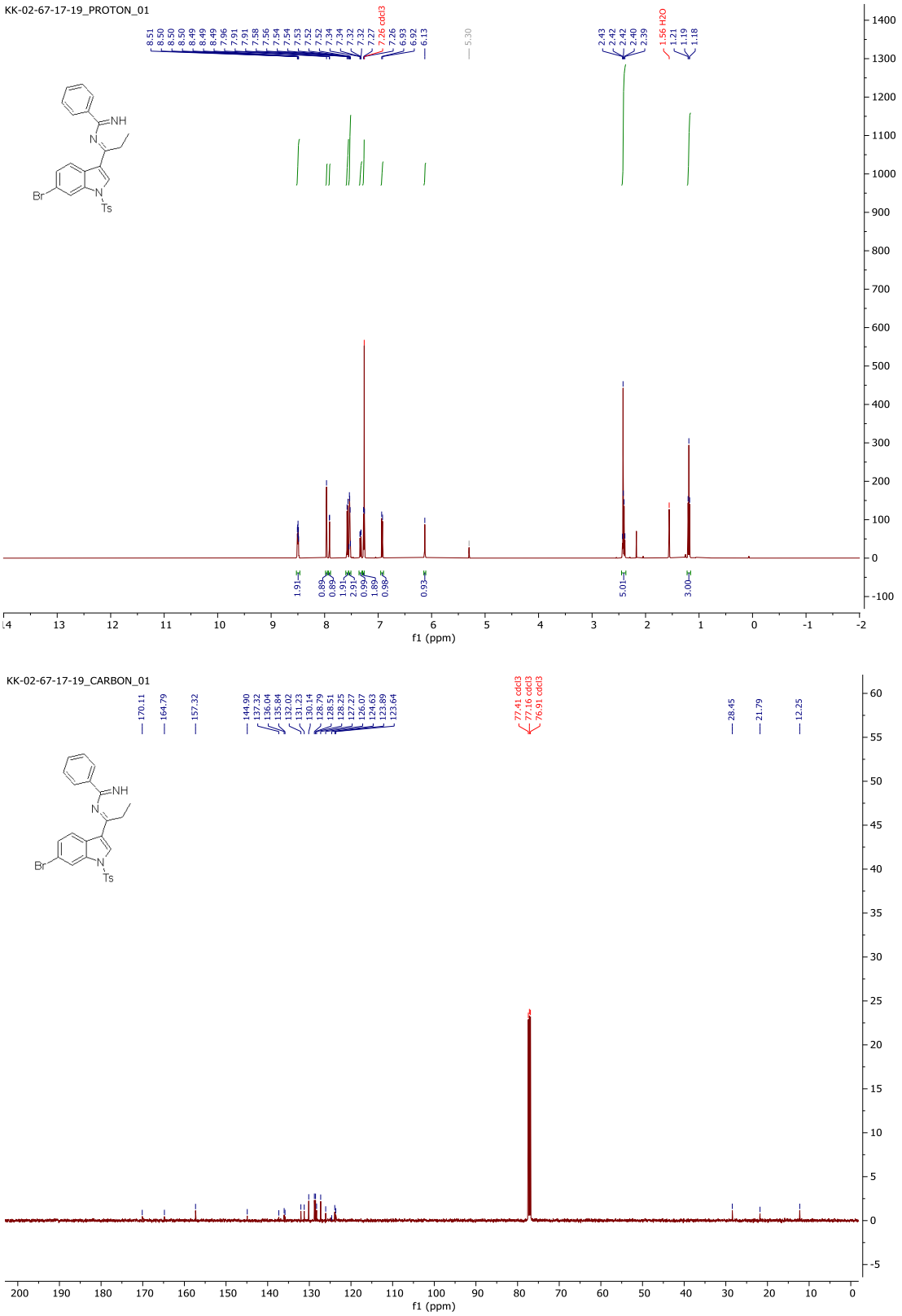


Figure 4.27 ^{1}H and ^{13}C NMR spectra of compound **4-31**

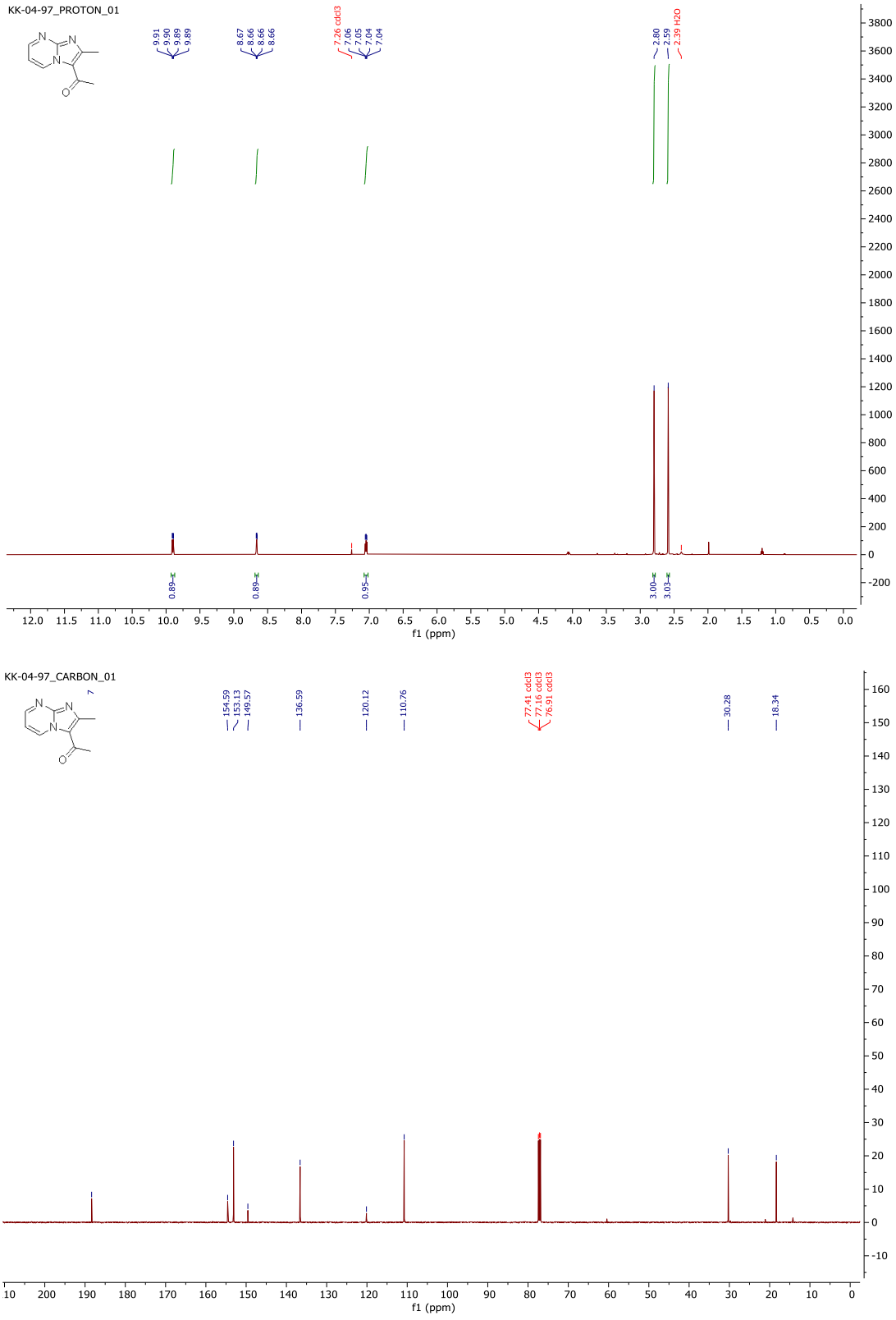
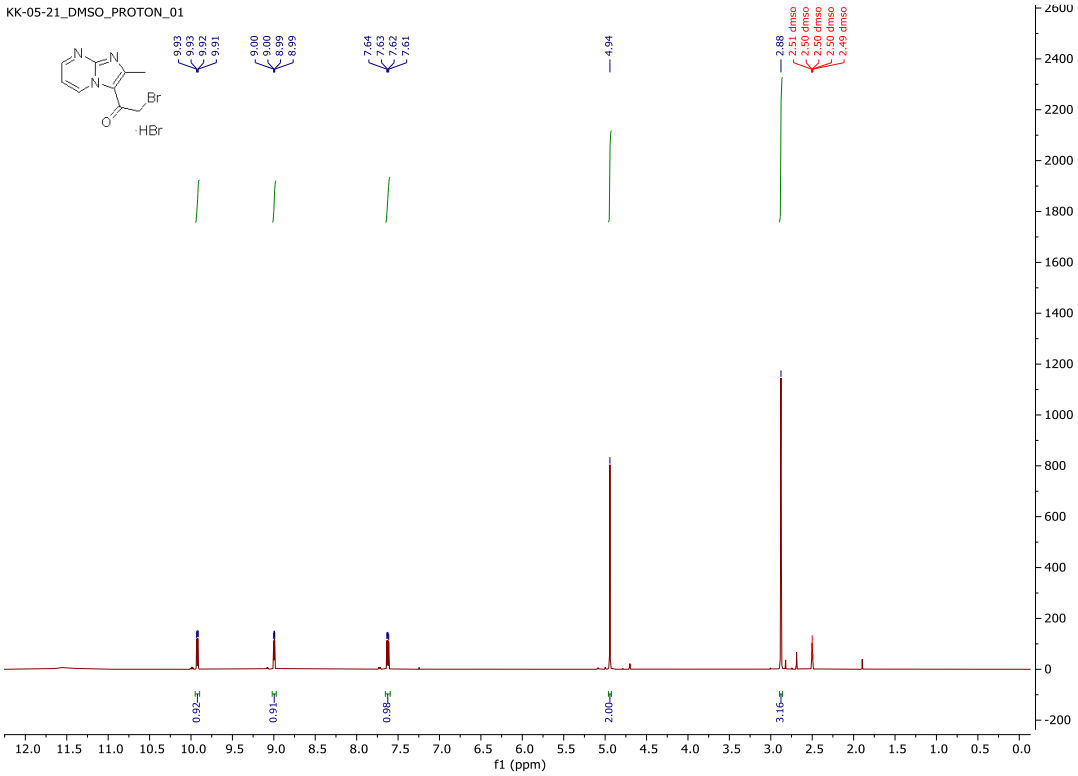


Figure 4.28 ^{1}H and ^{13}C NMR spectra of compound **4-30**



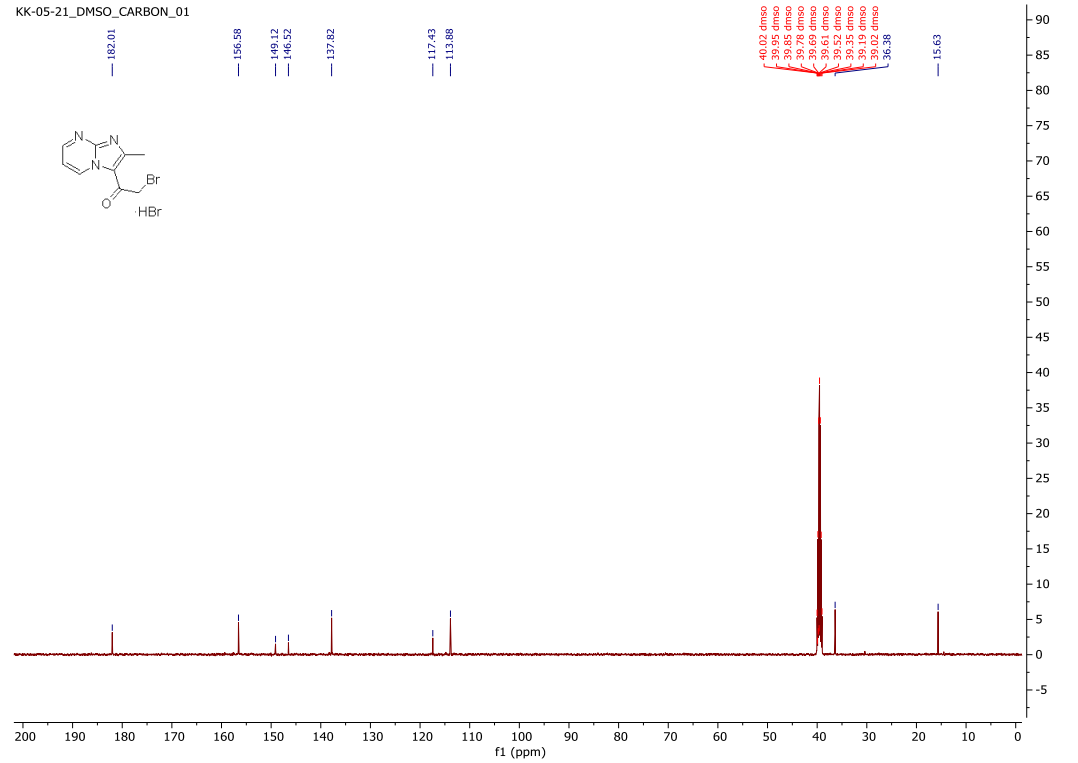


Figure 4.29 ^{1}H and ^{13}C NMR spectra of compound **4-36**

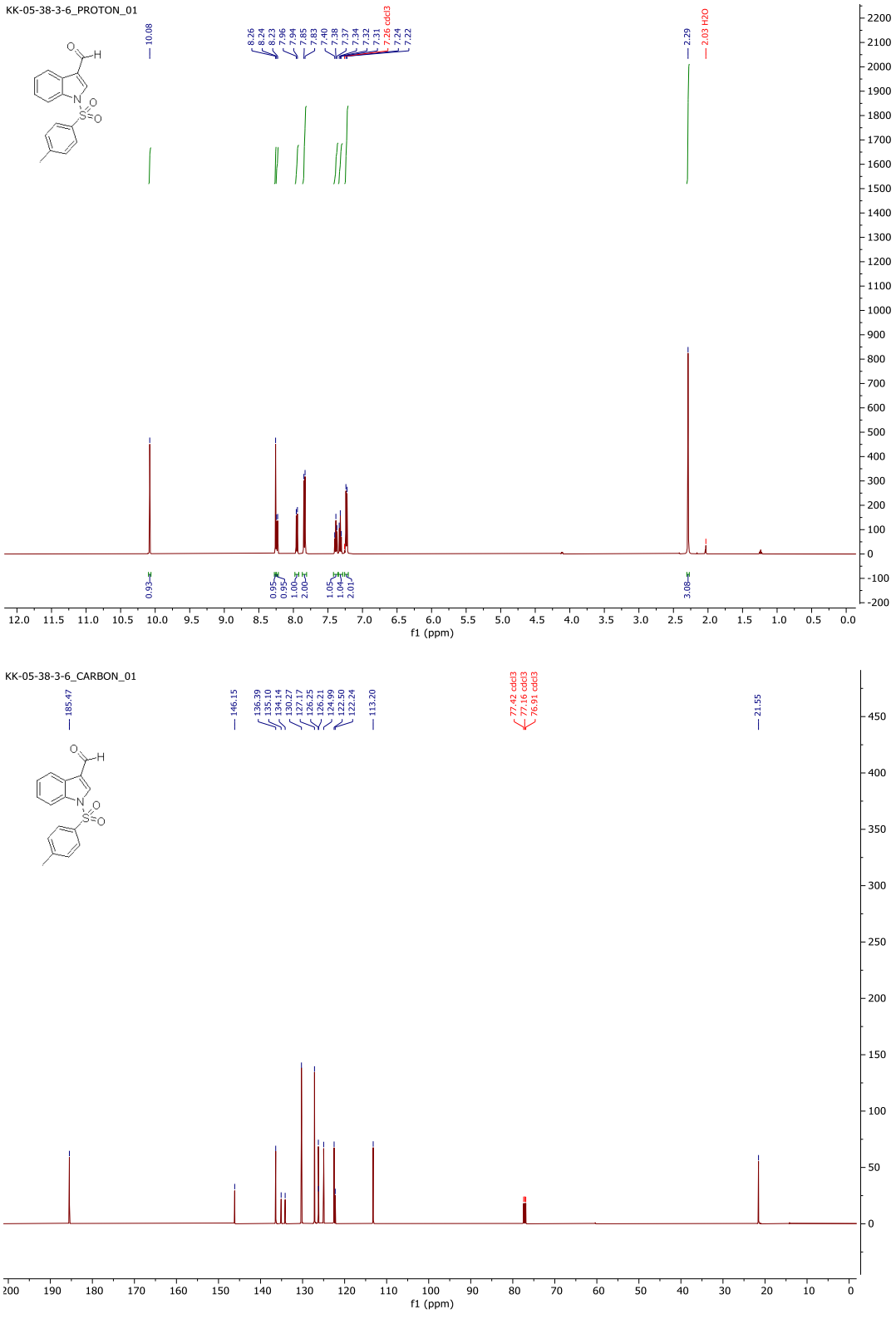


Figure 4.30 ^{1}H and ^{13}C NMR spectra of compound **4-37**

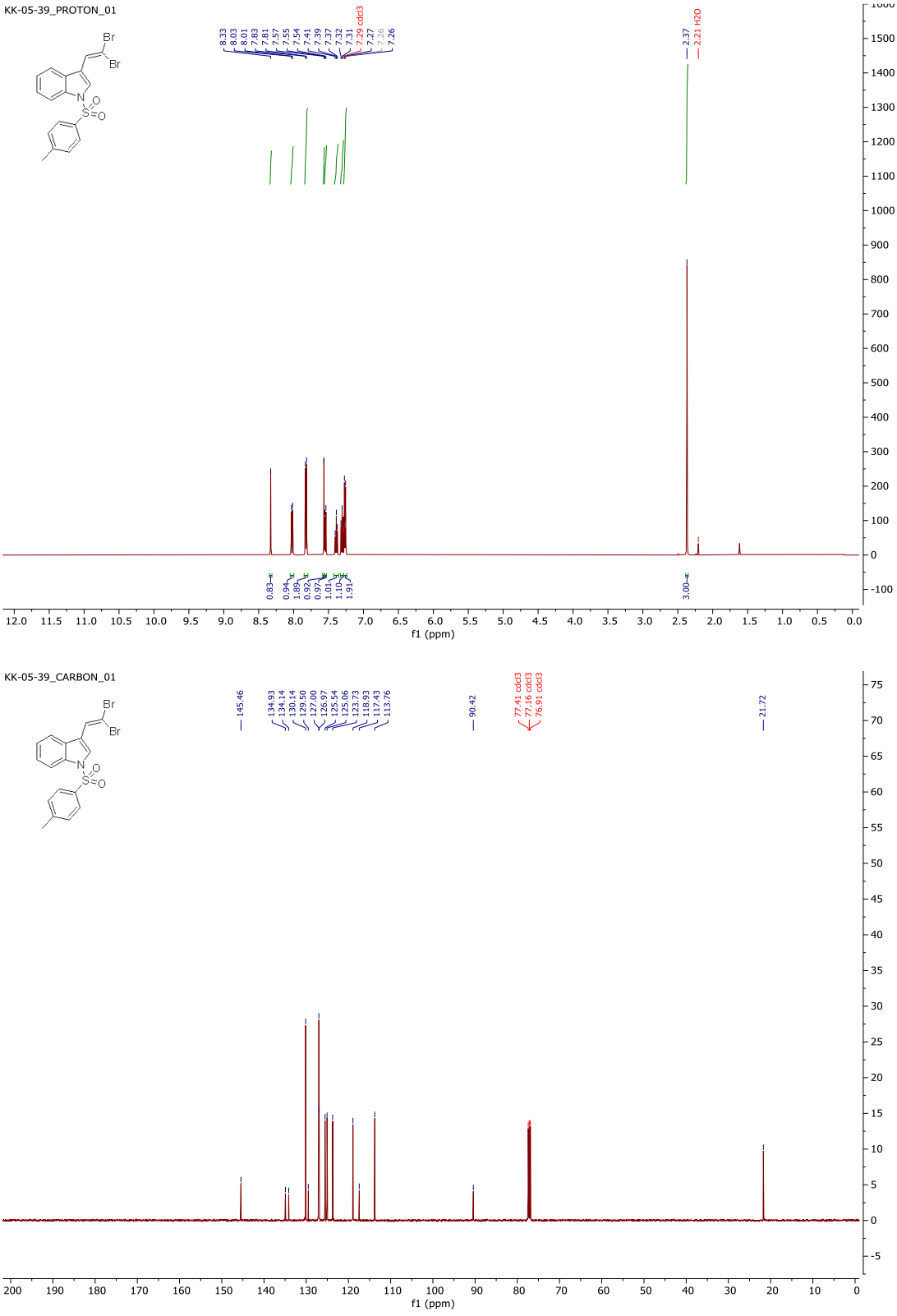
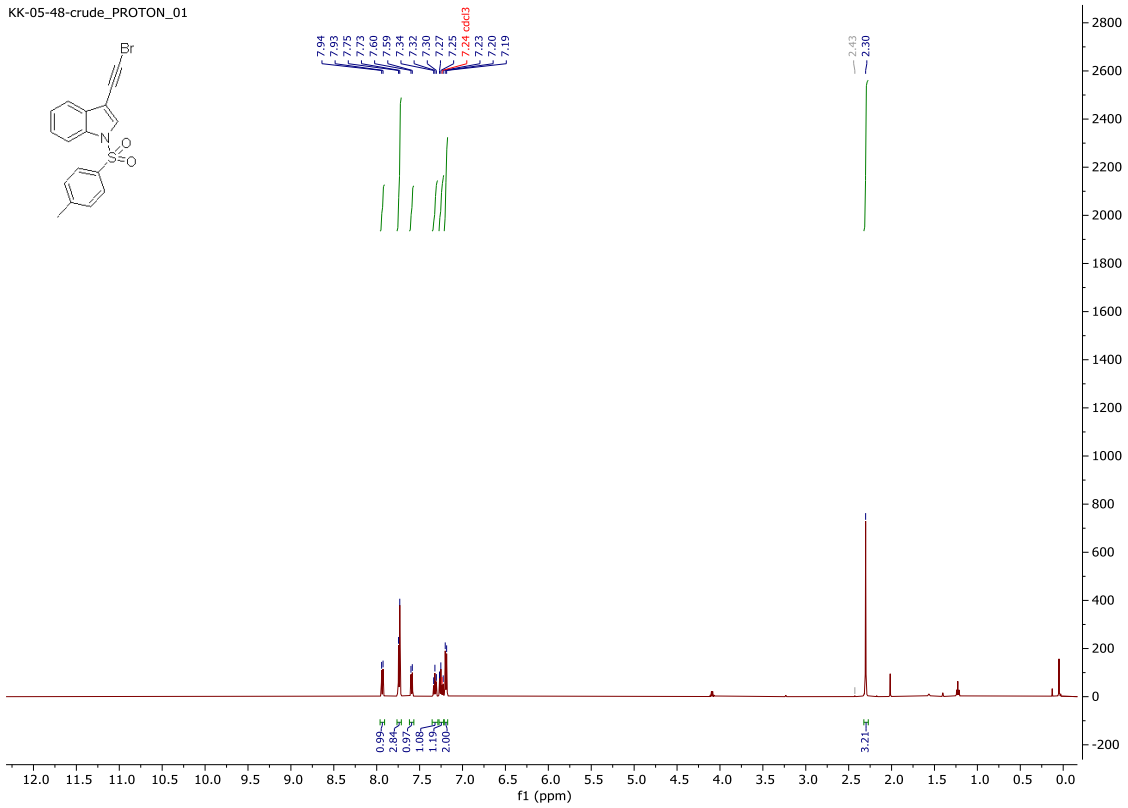


Figure 4.31 ^{1}H and ^{13}C NMR spectra of compound **4-38**



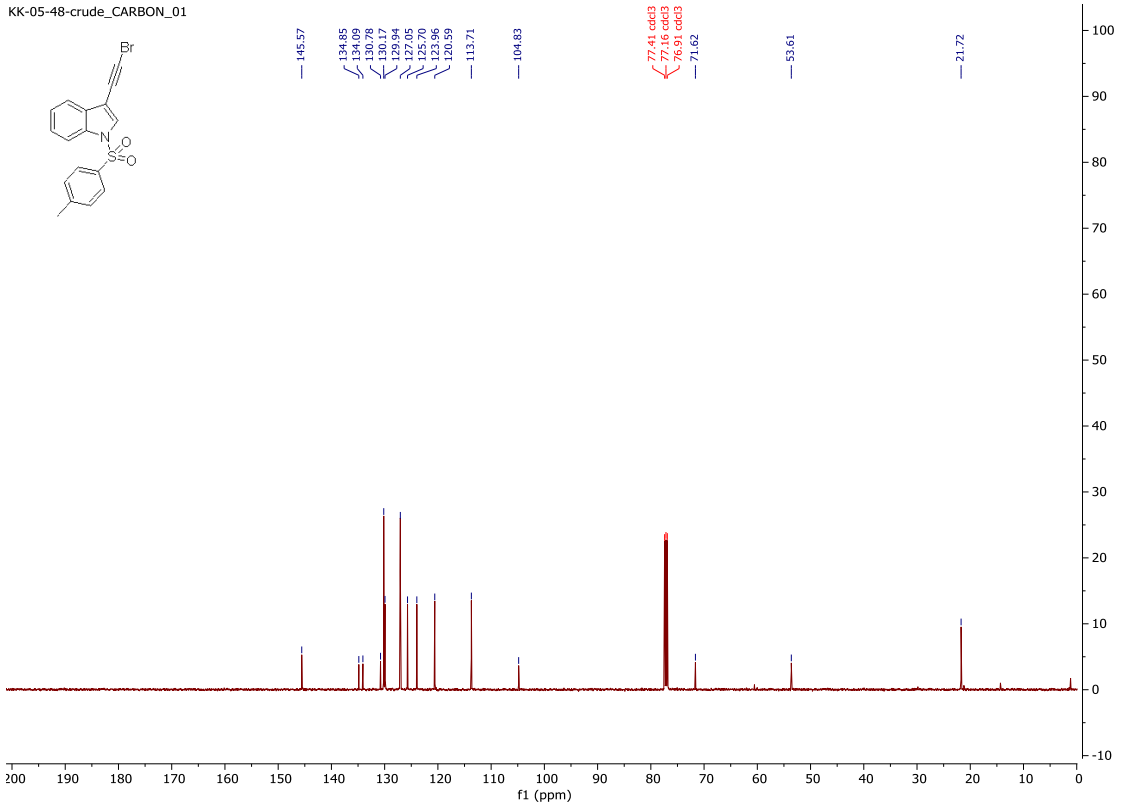
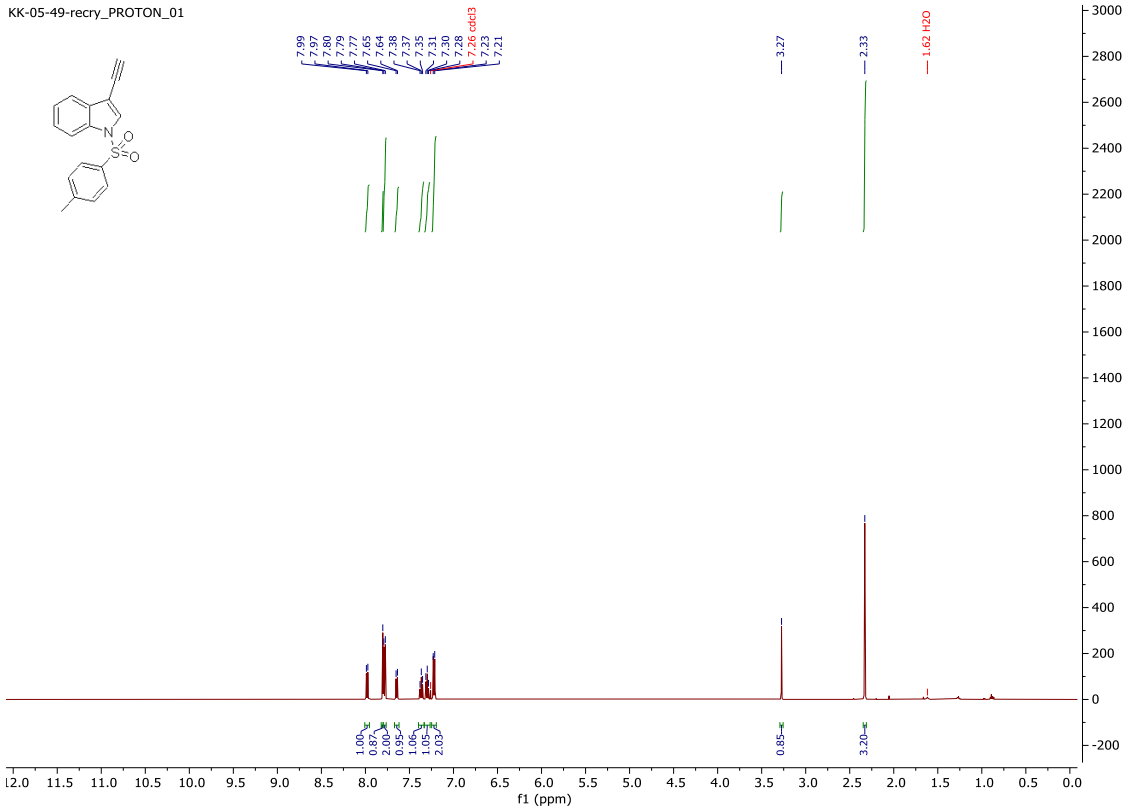


Figure 4.32 ^{1}H and ^{13}C NMR spectra of compound **4-39**



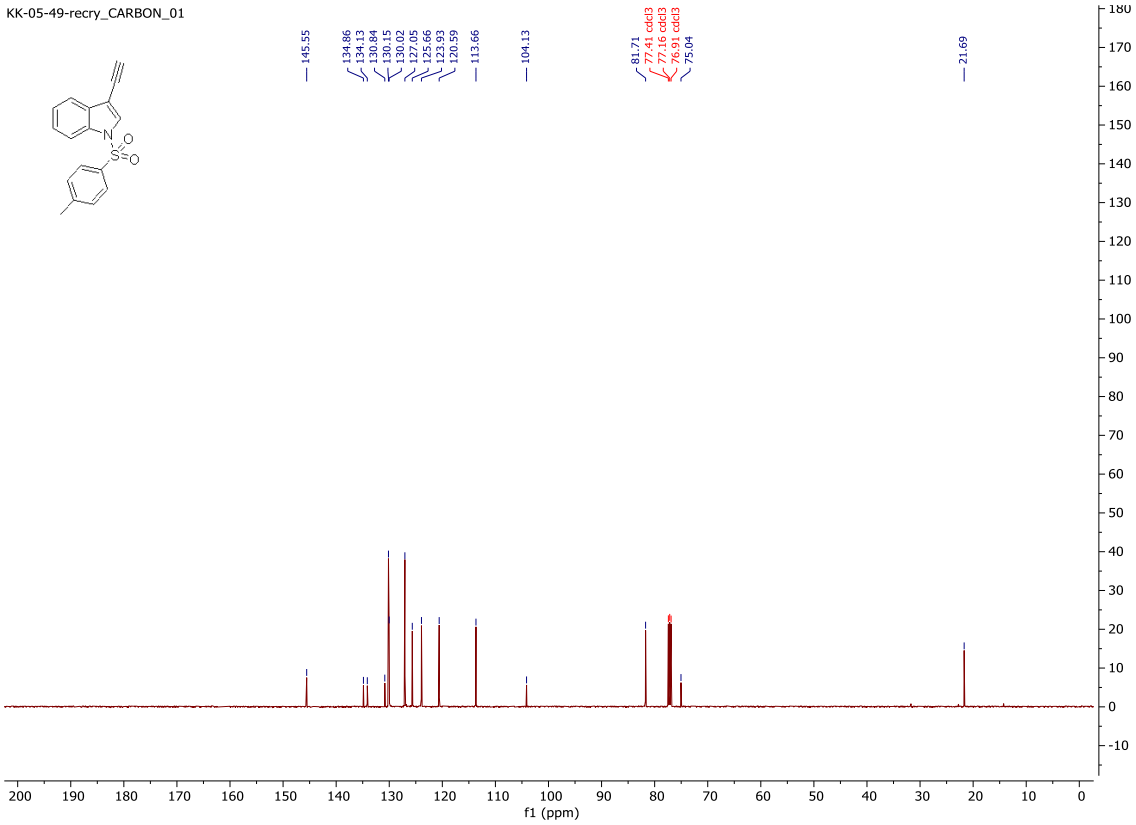
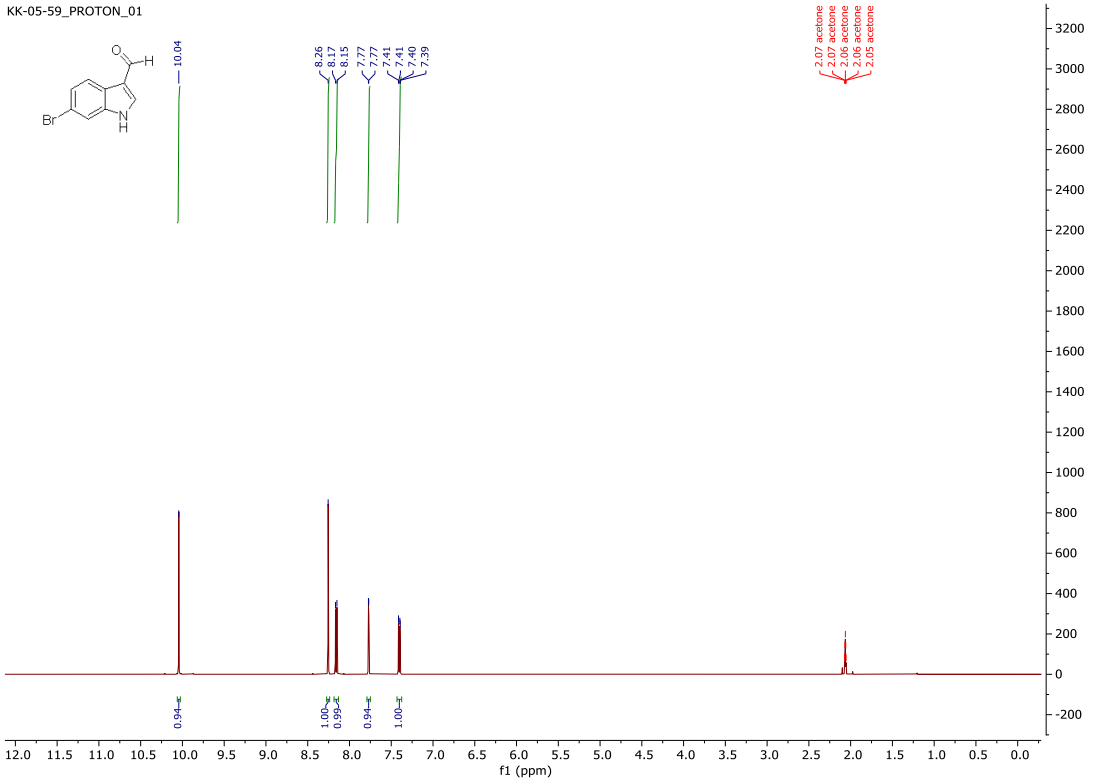


Figure 4.33 ^{1}H and ^{13}C NMR spectra of compound **4-40**



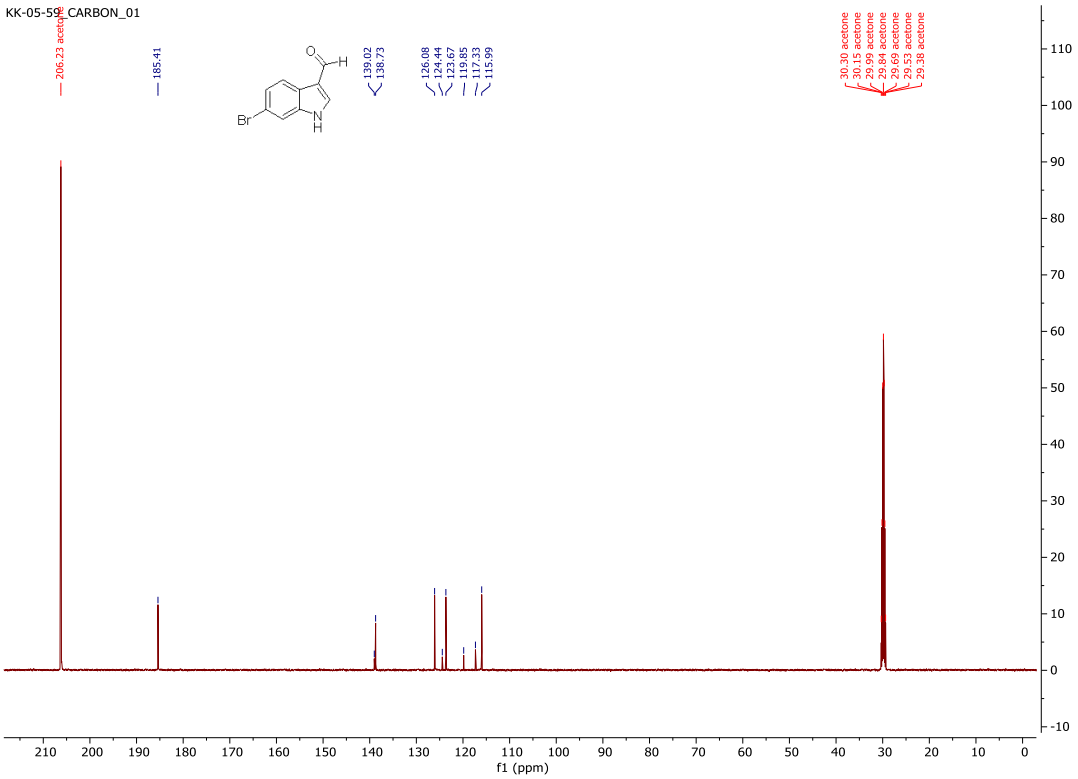
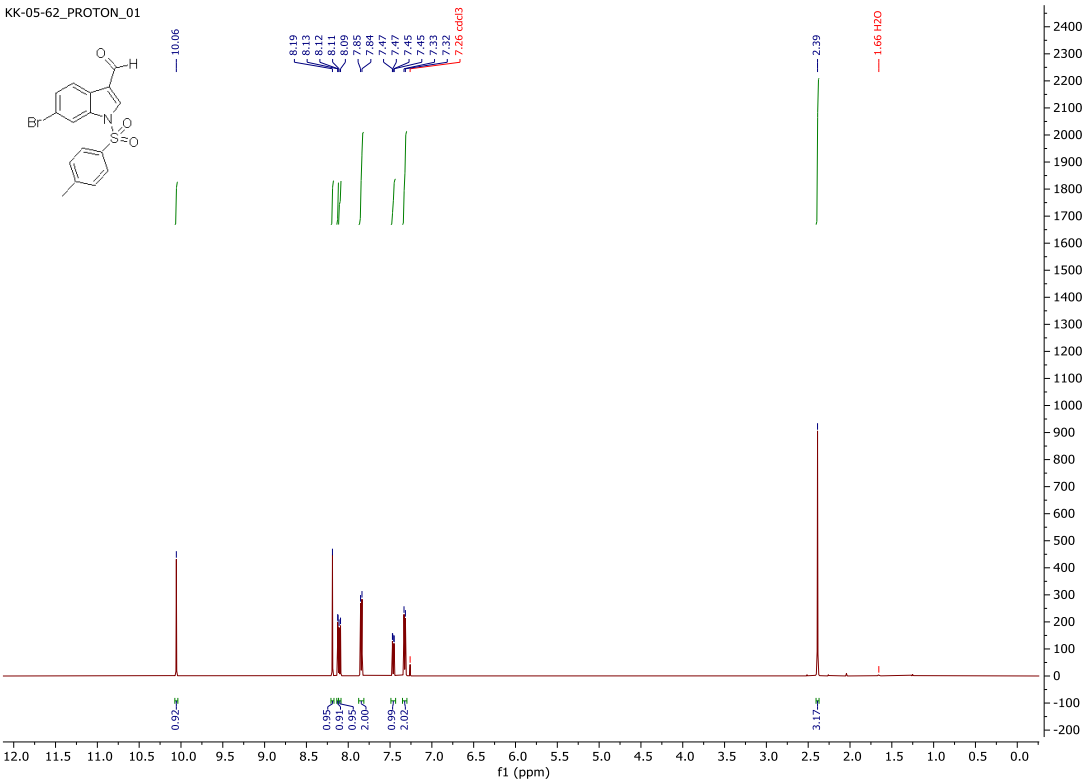


Figure 4.34 ^{1}H and ^{13}C NMR spectra of compound **4-41**



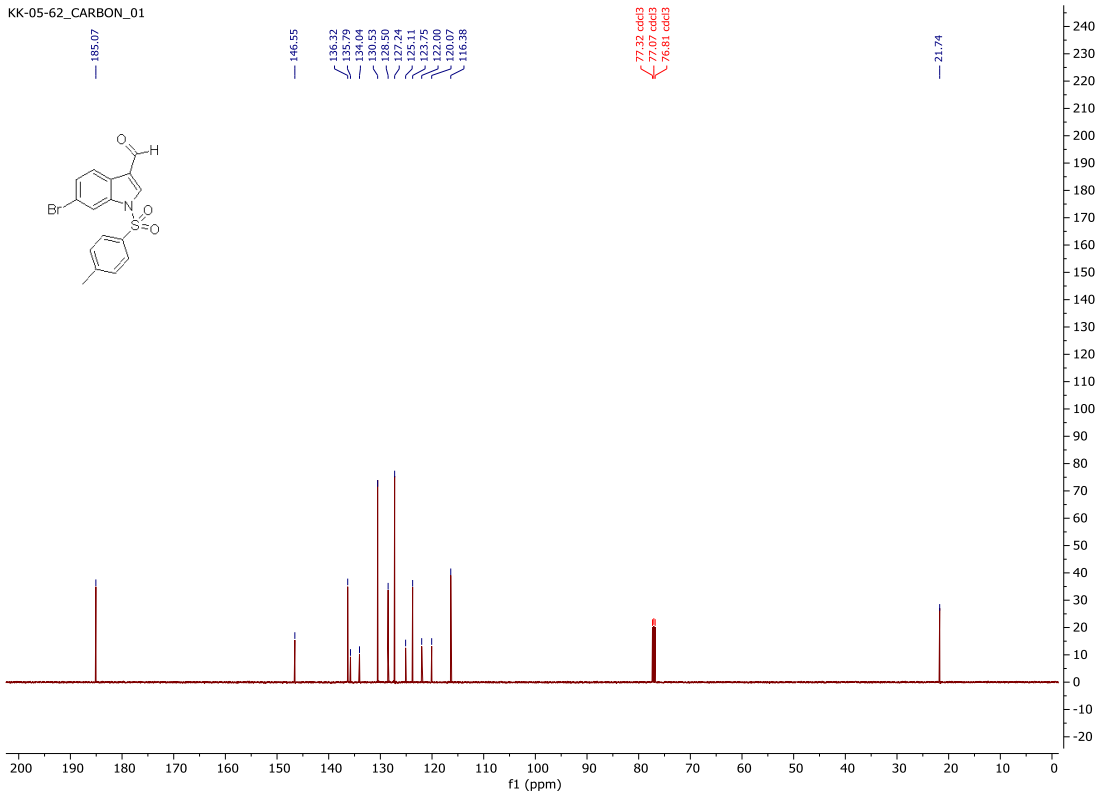
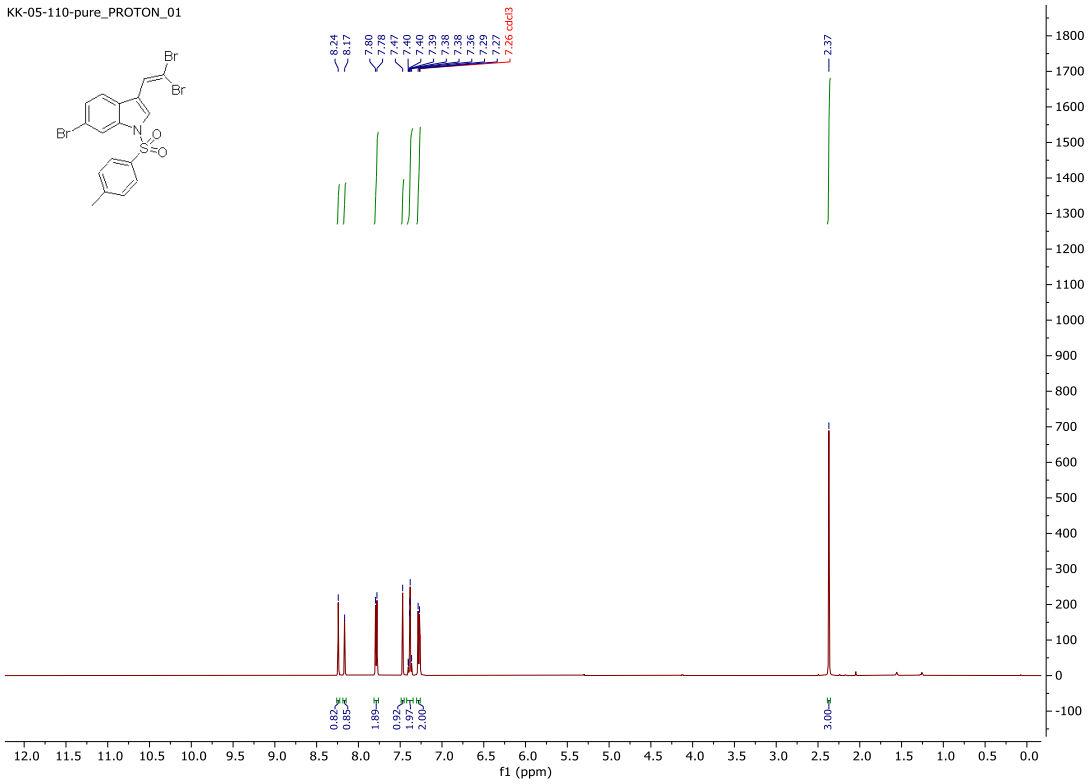


Figure 4.35 ^{1}H and ^{13}C NMR spectra of compound **4-42**



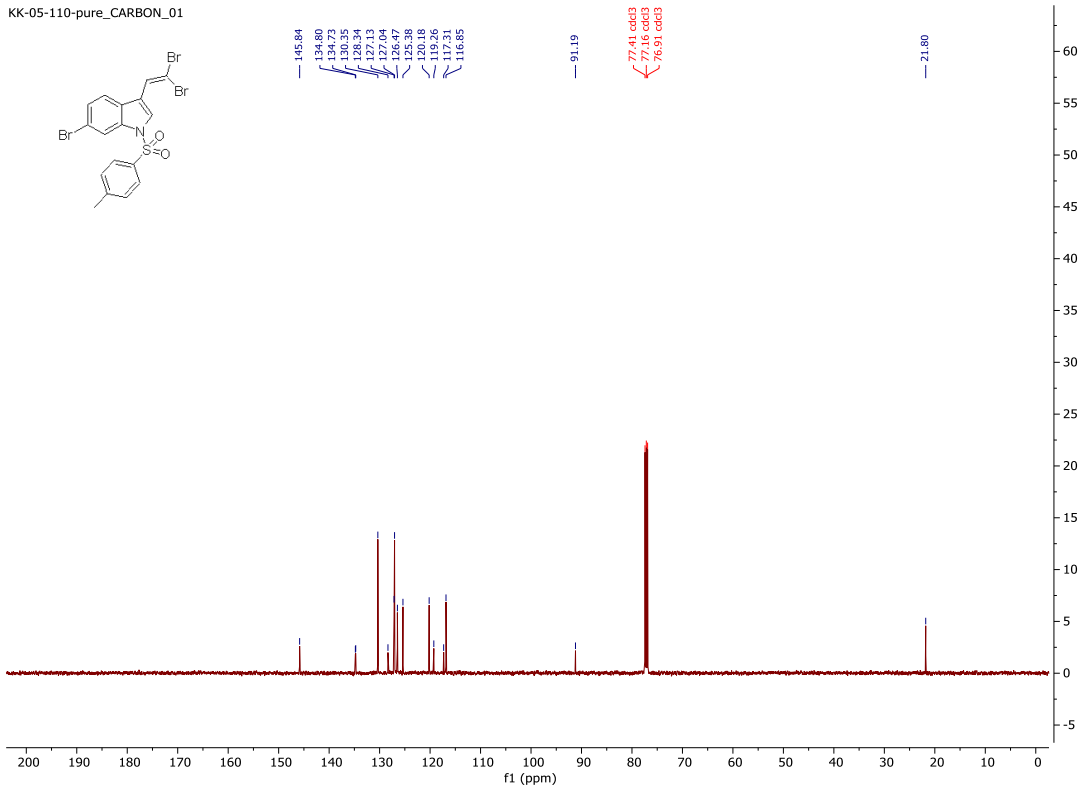
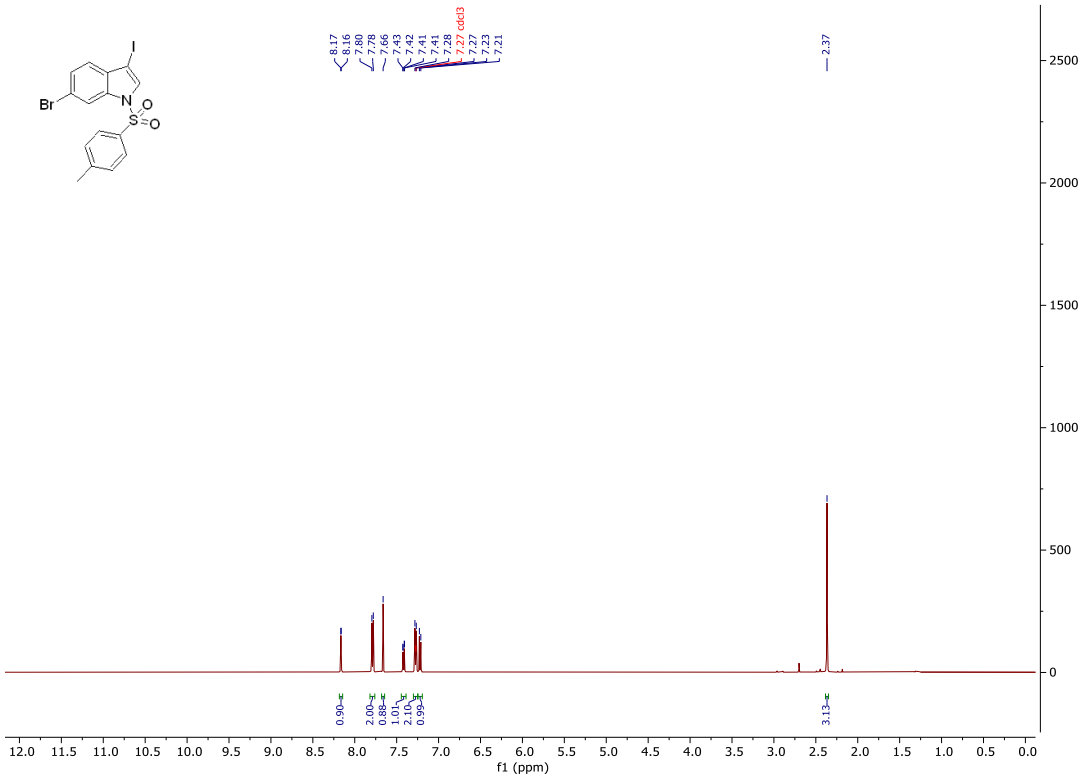


Figure 4.36 ^{1}H and ^{13}C NMR spectra of compound **4-44**



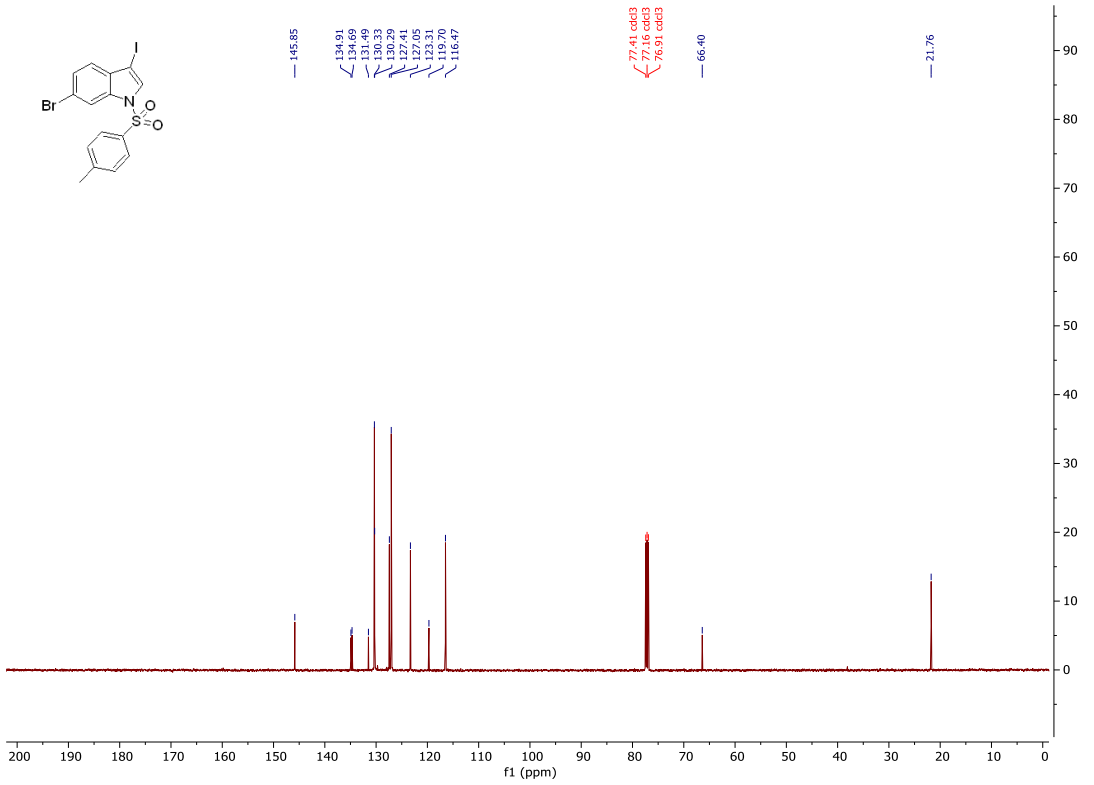
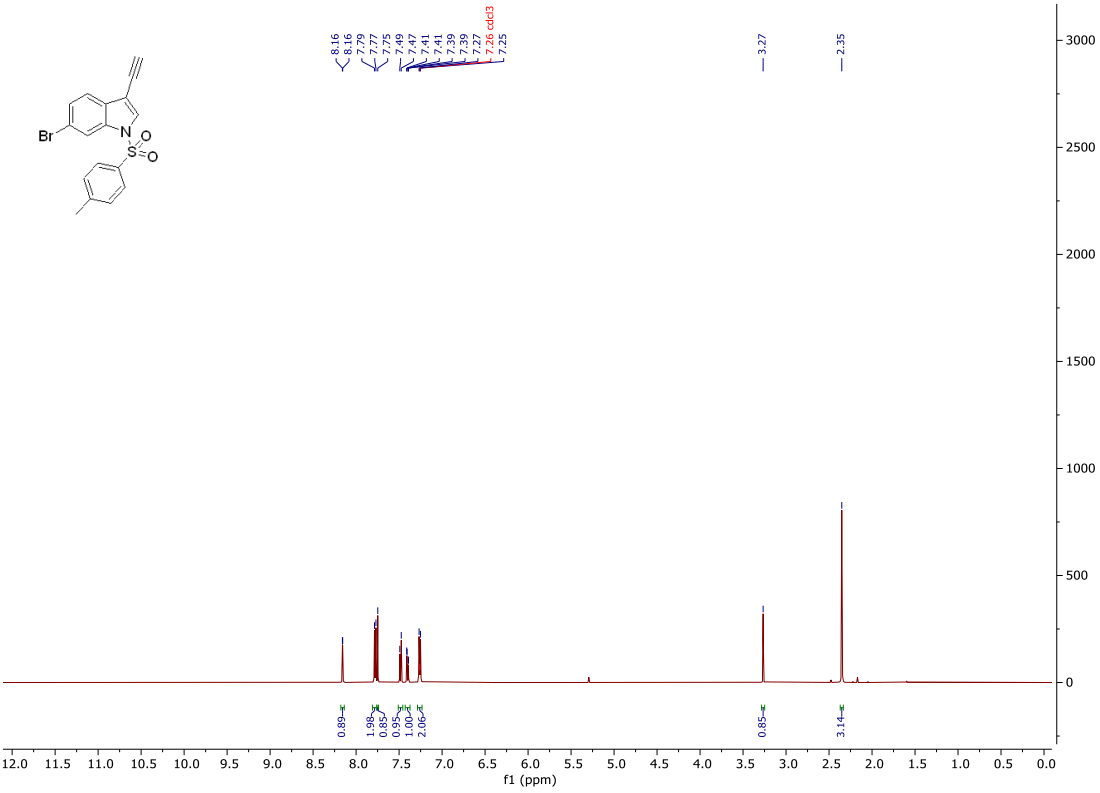
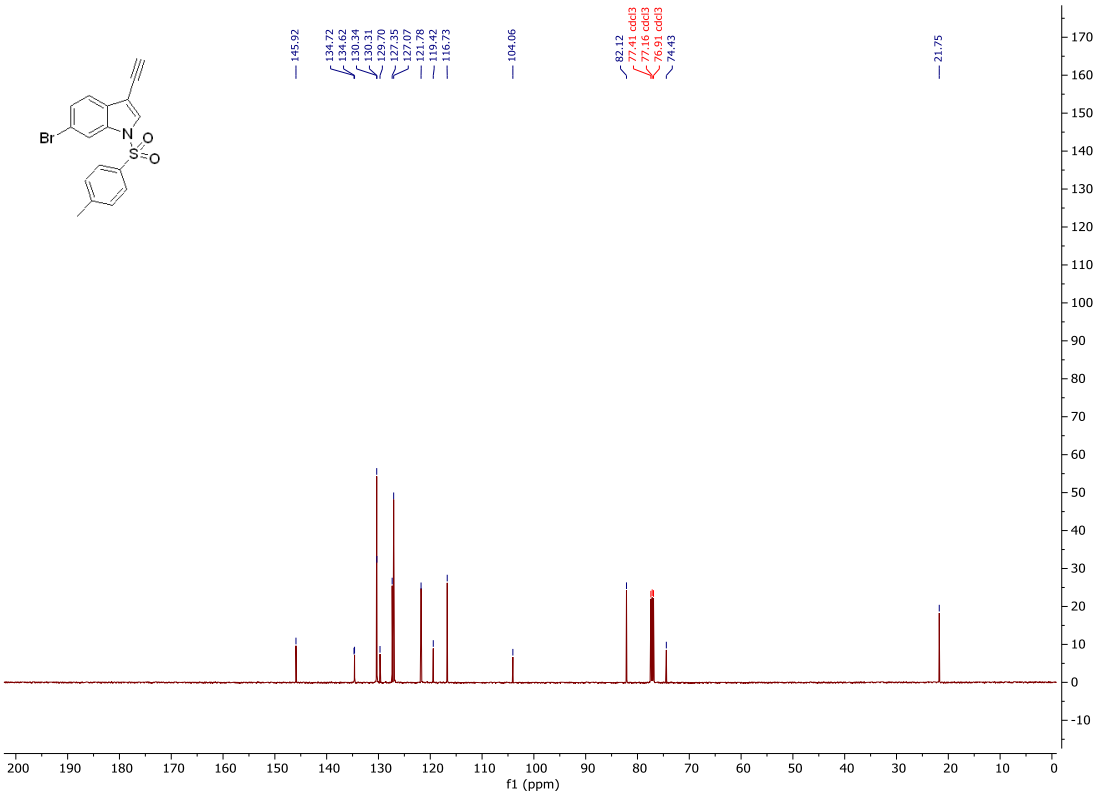


Figure 4.37 ^{1}H and ^{13}C NMR spectra of compound **4-34**





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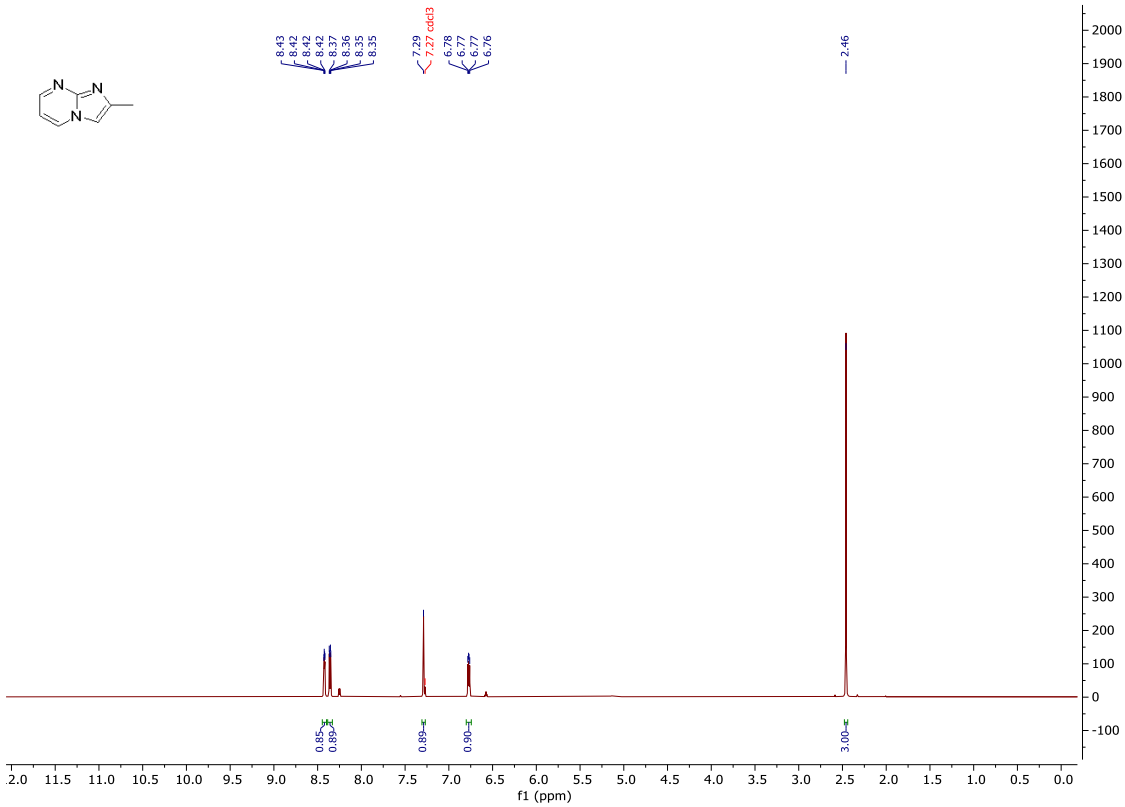

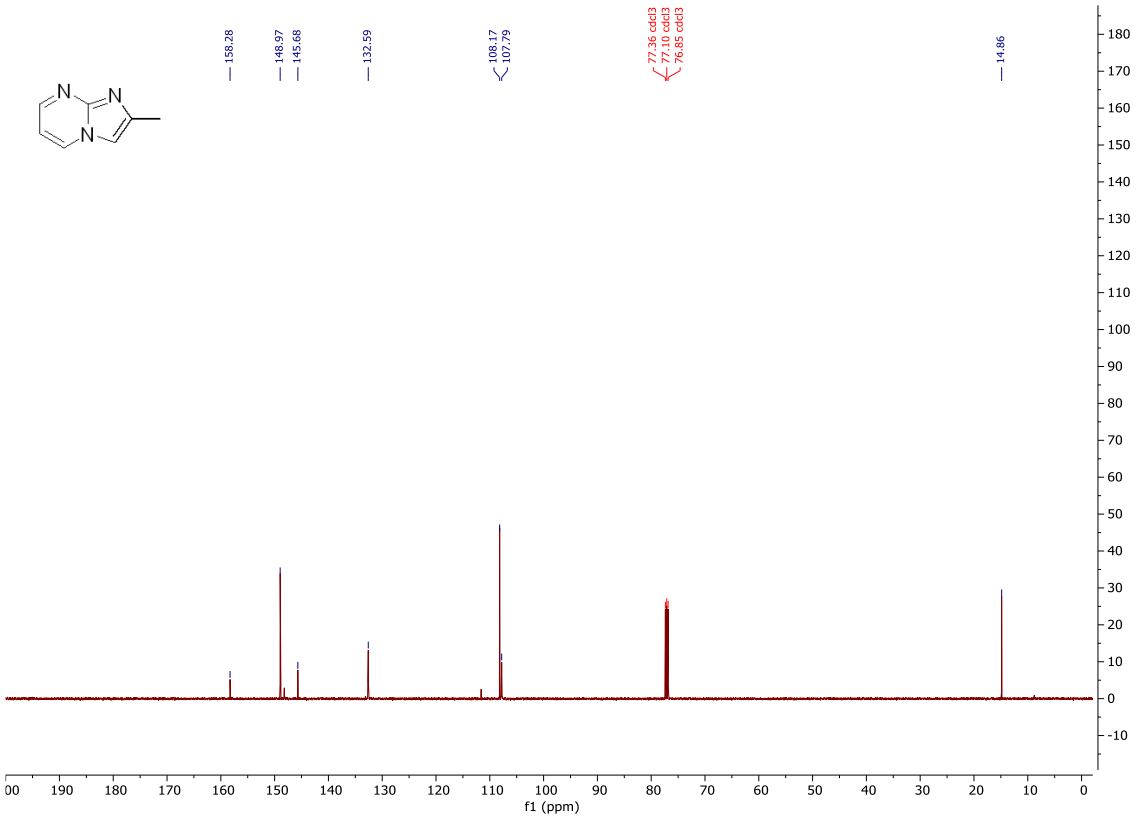

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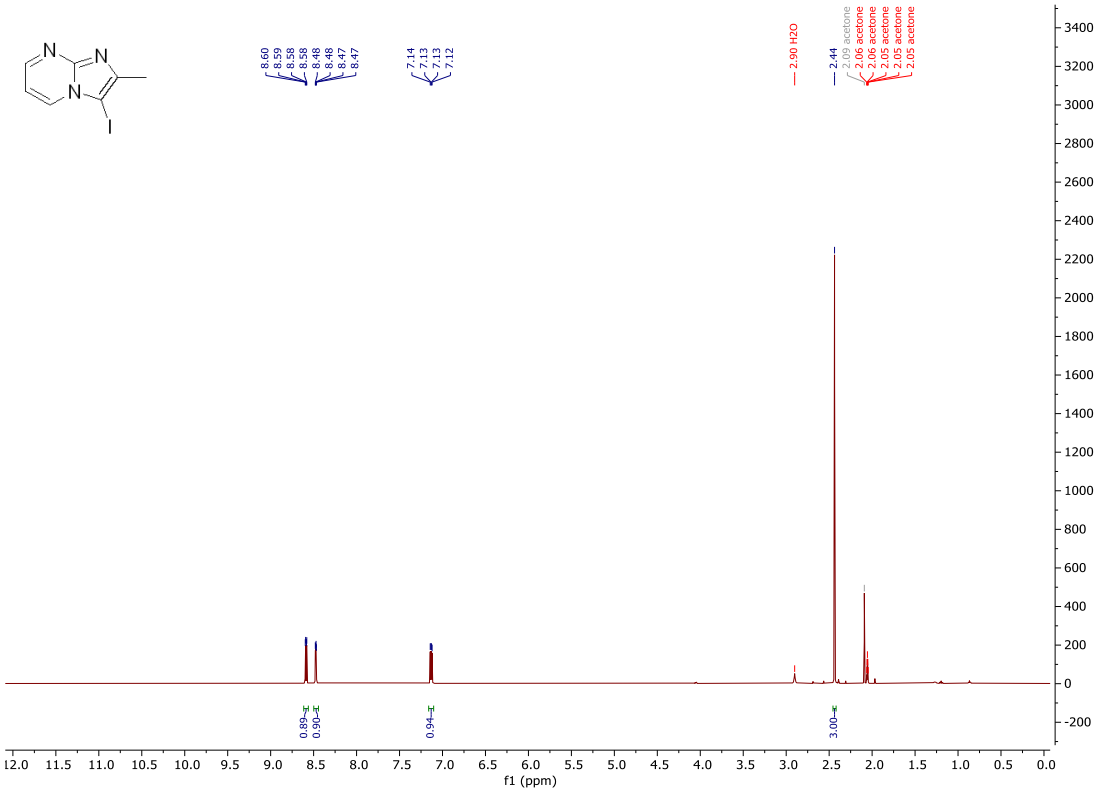


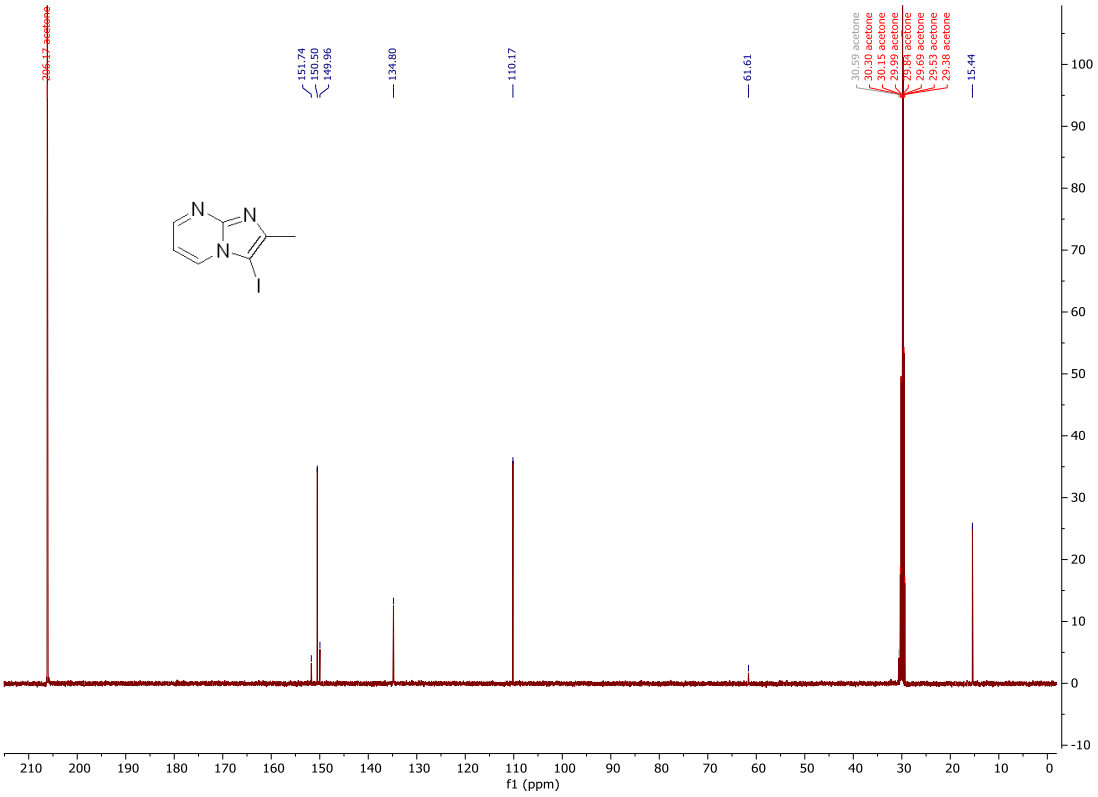
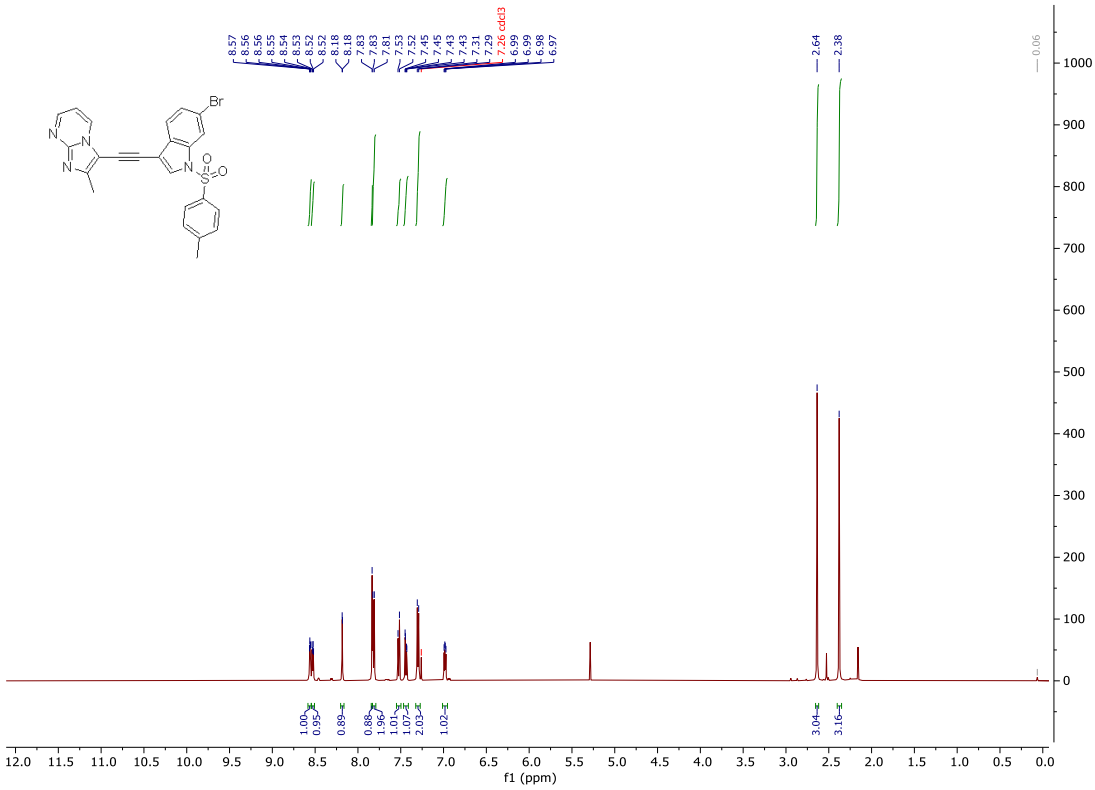


Figure 4.40 ^{1}H and ^{13}C NMR spectra of compound **4-46**



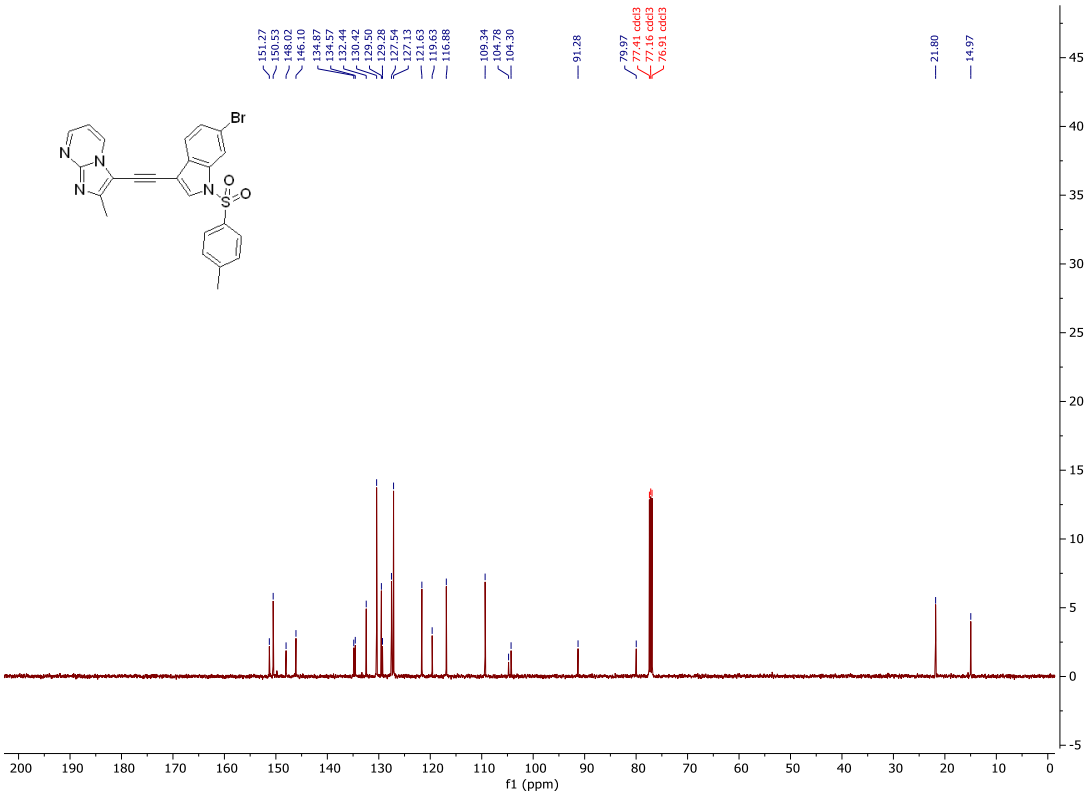
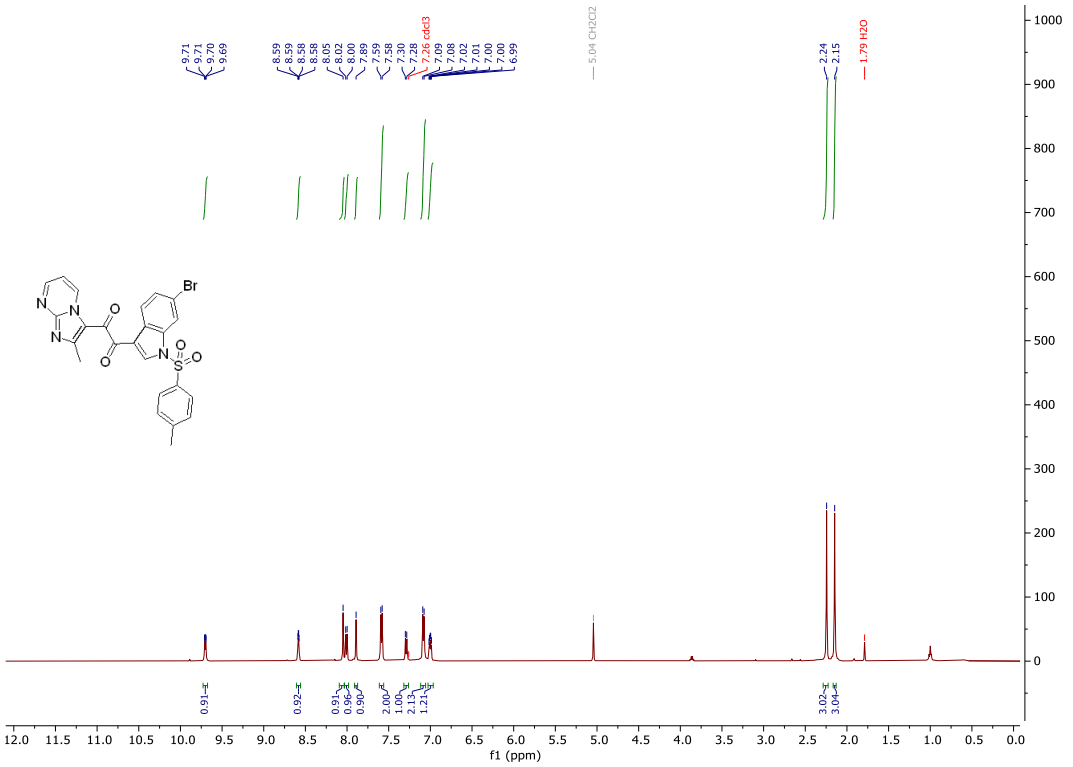


Figure 4.41 ^{1}H and ^{13}C NMR spectra of compound **4-33**



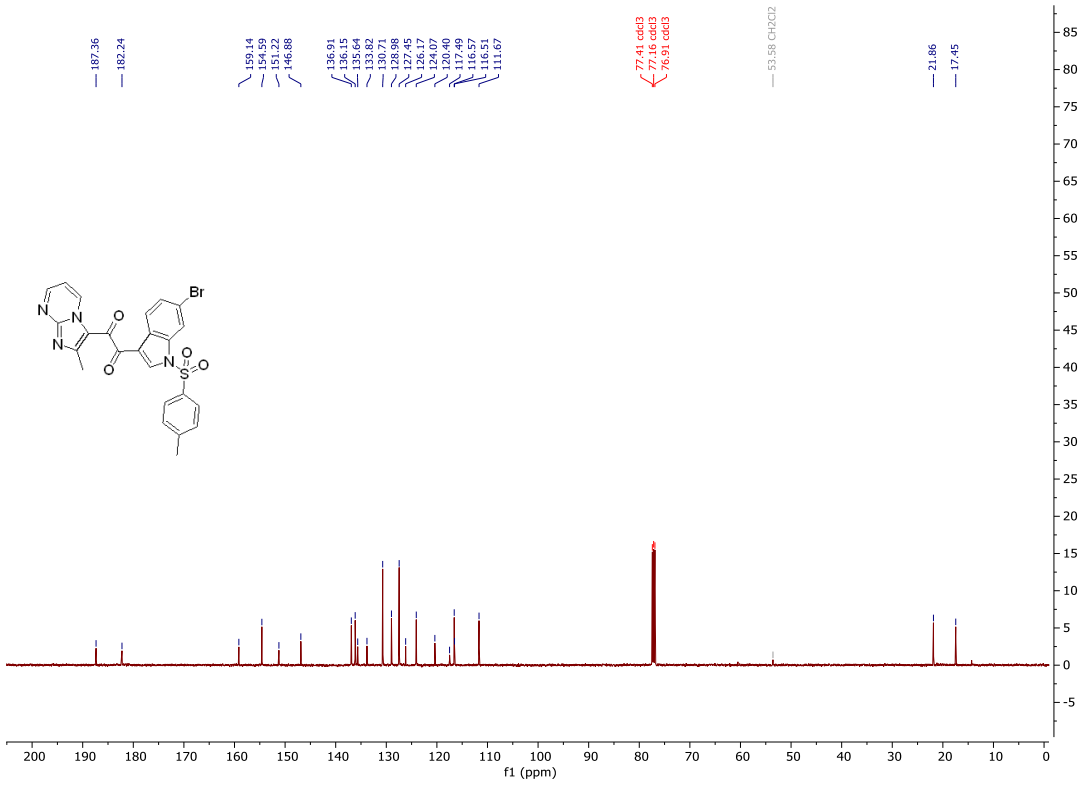


Figure 4.42 ^{1}H and ^{13}C NMR spectra of compound **4-47**

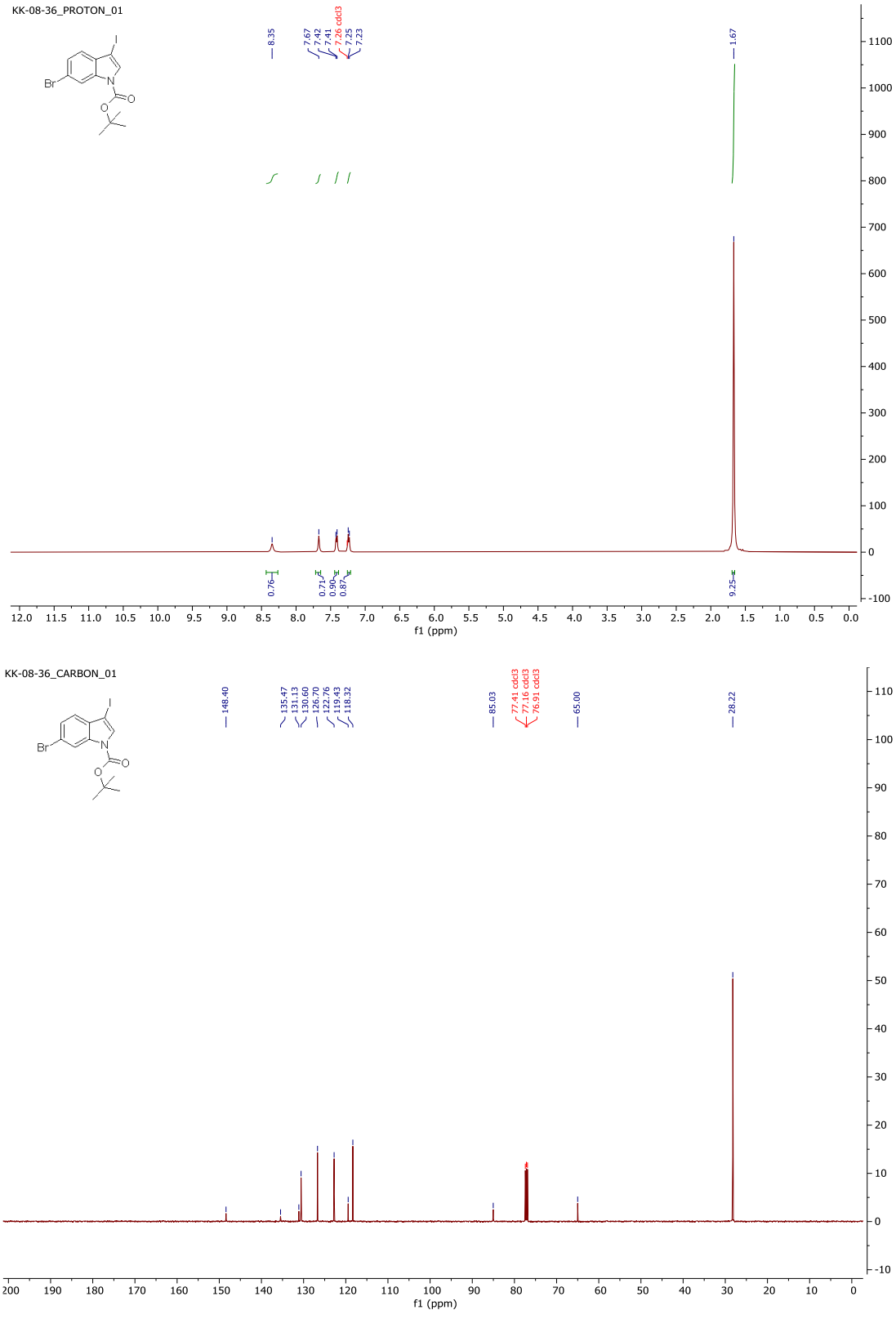
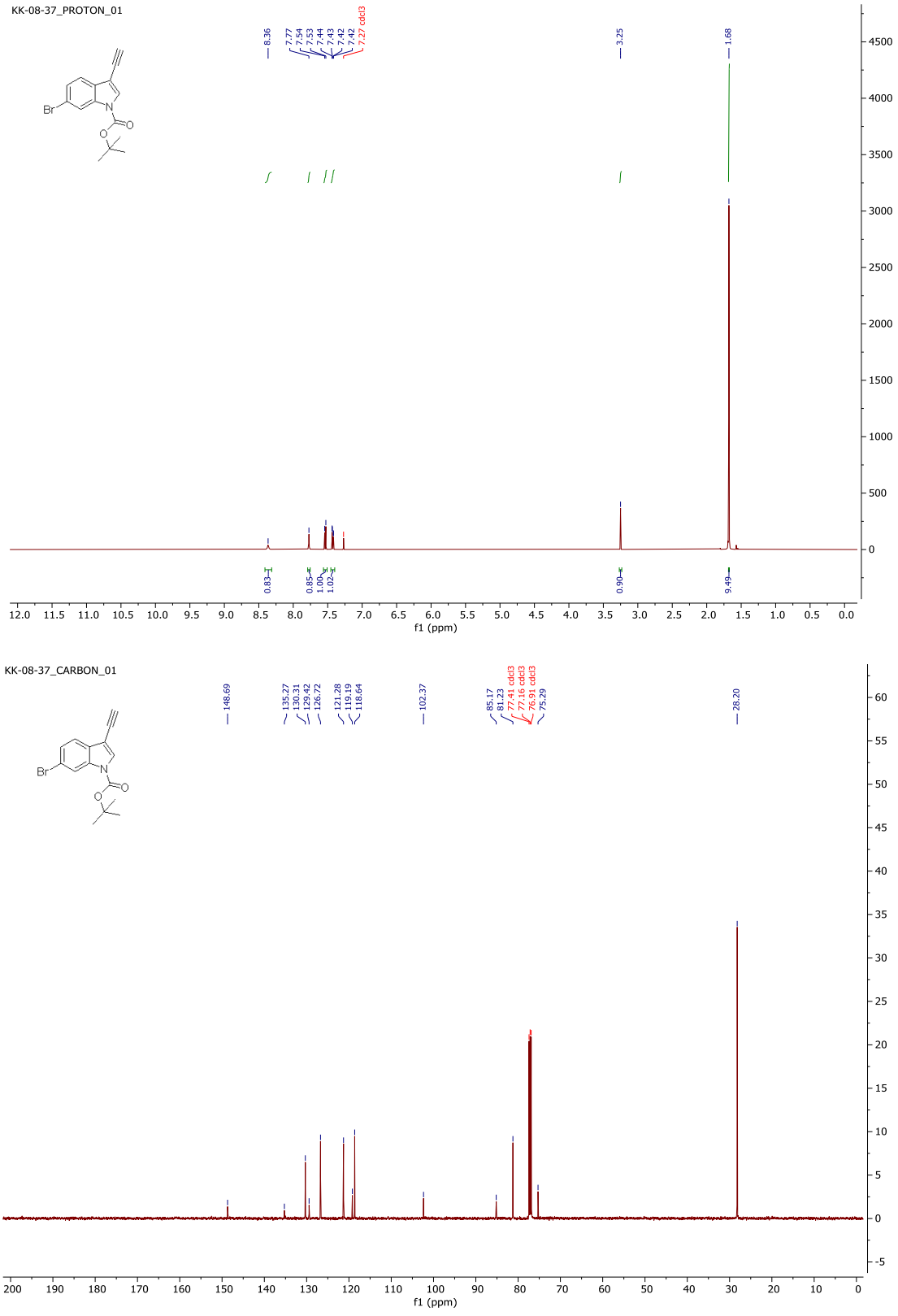


Figure 4.43 ^{1}H and ^{13}C NMR spectra of compound **4-48**



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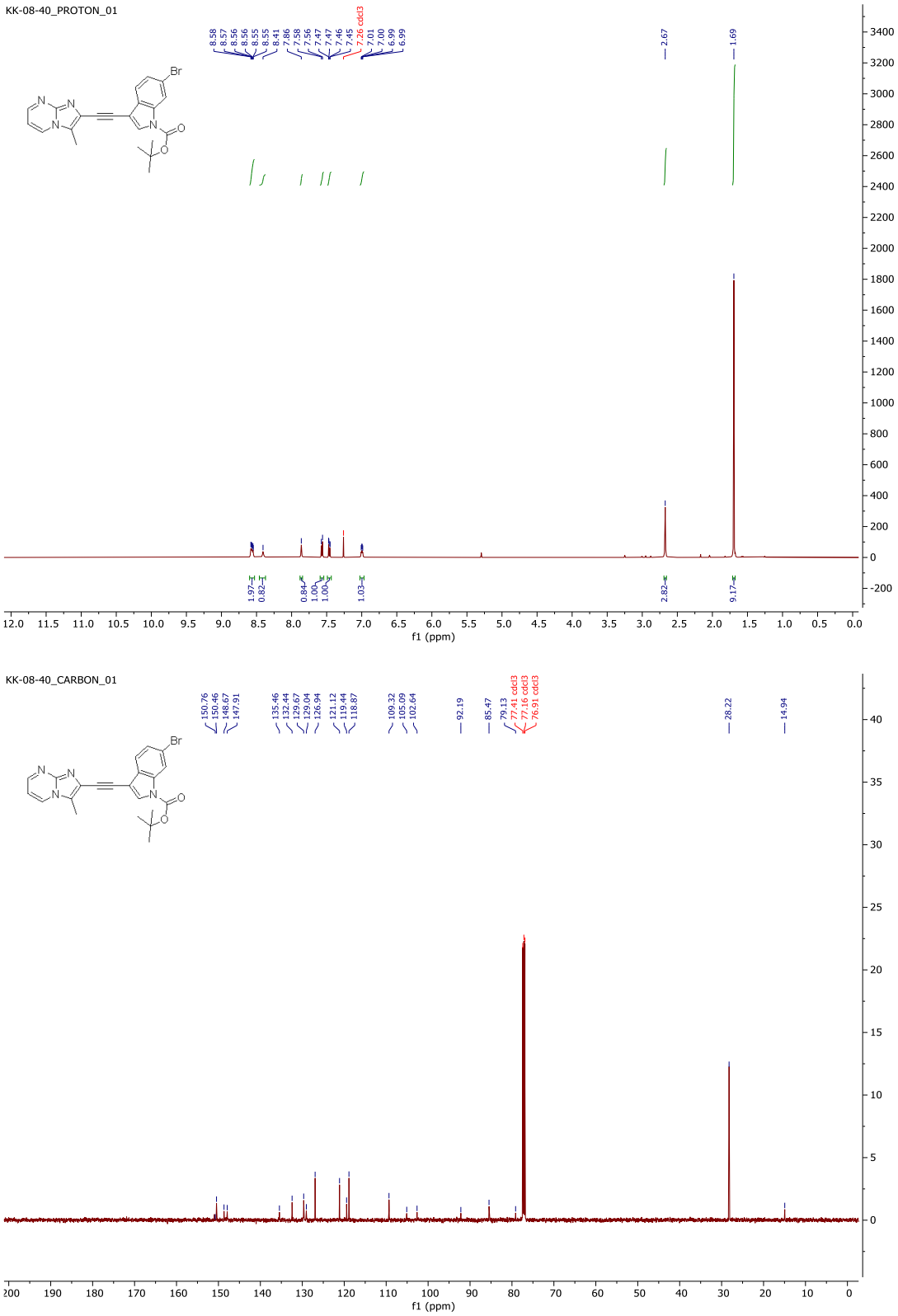
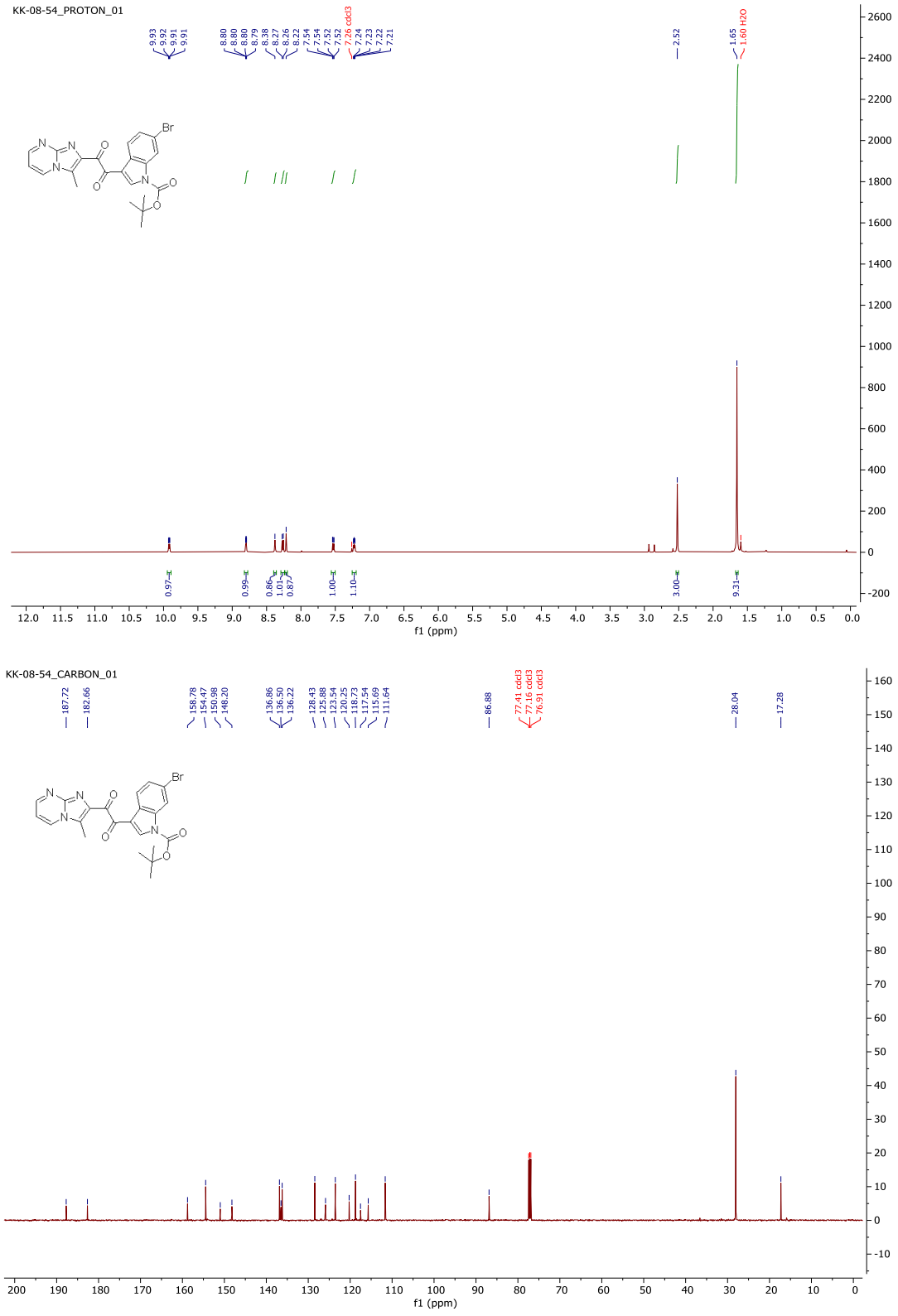


Figure 4.45 ^{1}H and ^{13}C NMR spectra of compound **4-29**



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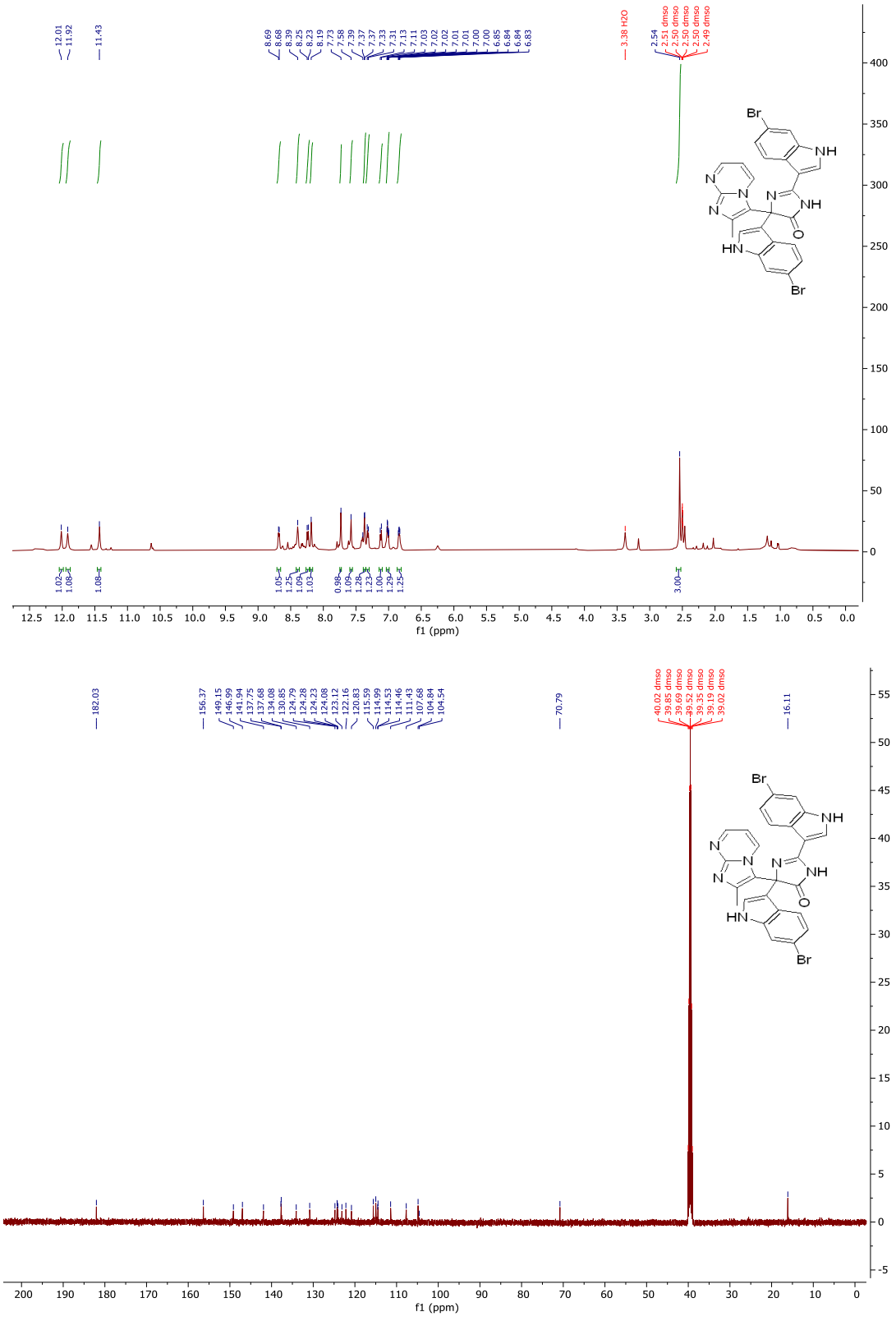
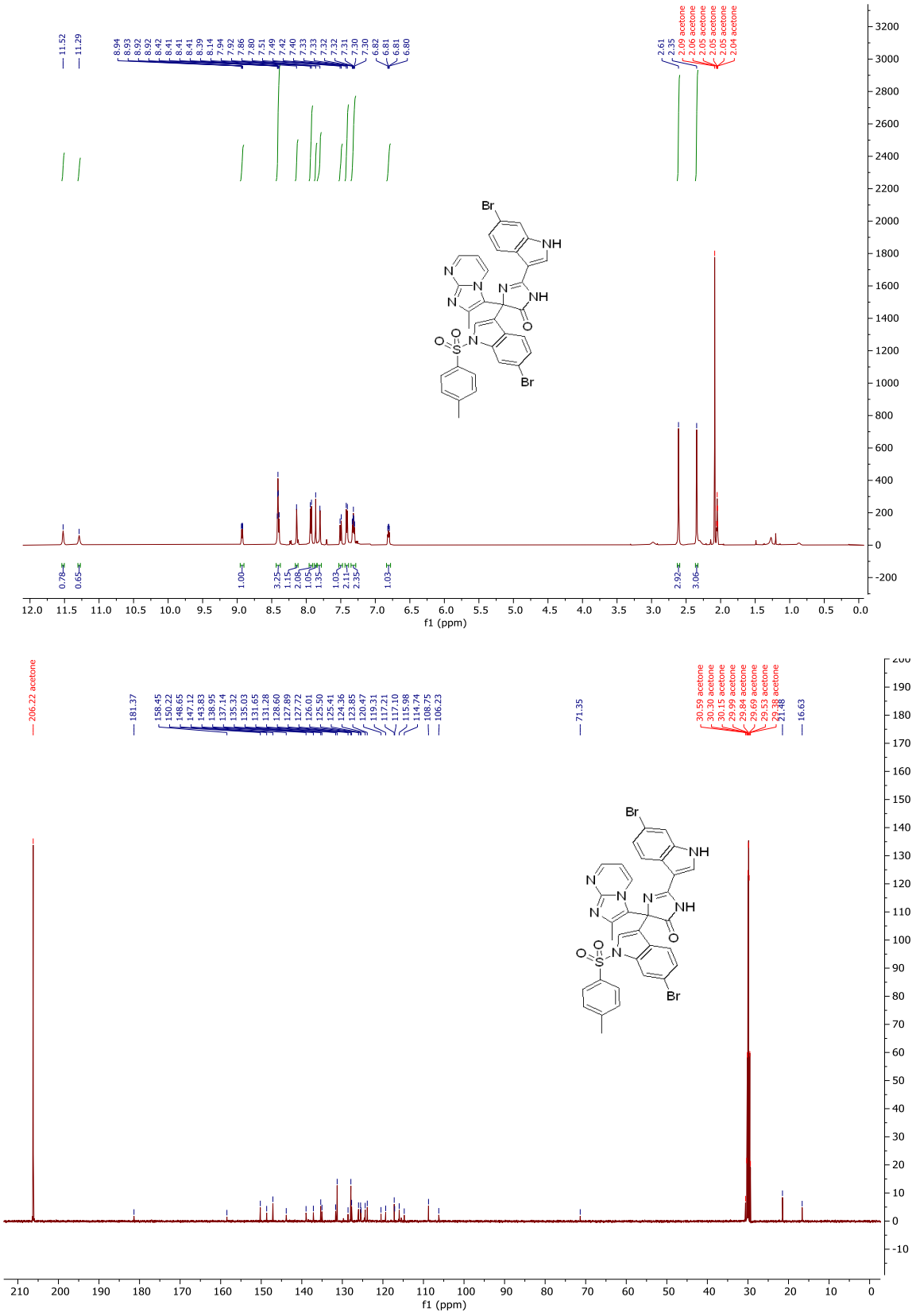


Figure 4.47 ^{1}H and ^{13}C NMR spectra of compound **4-51**



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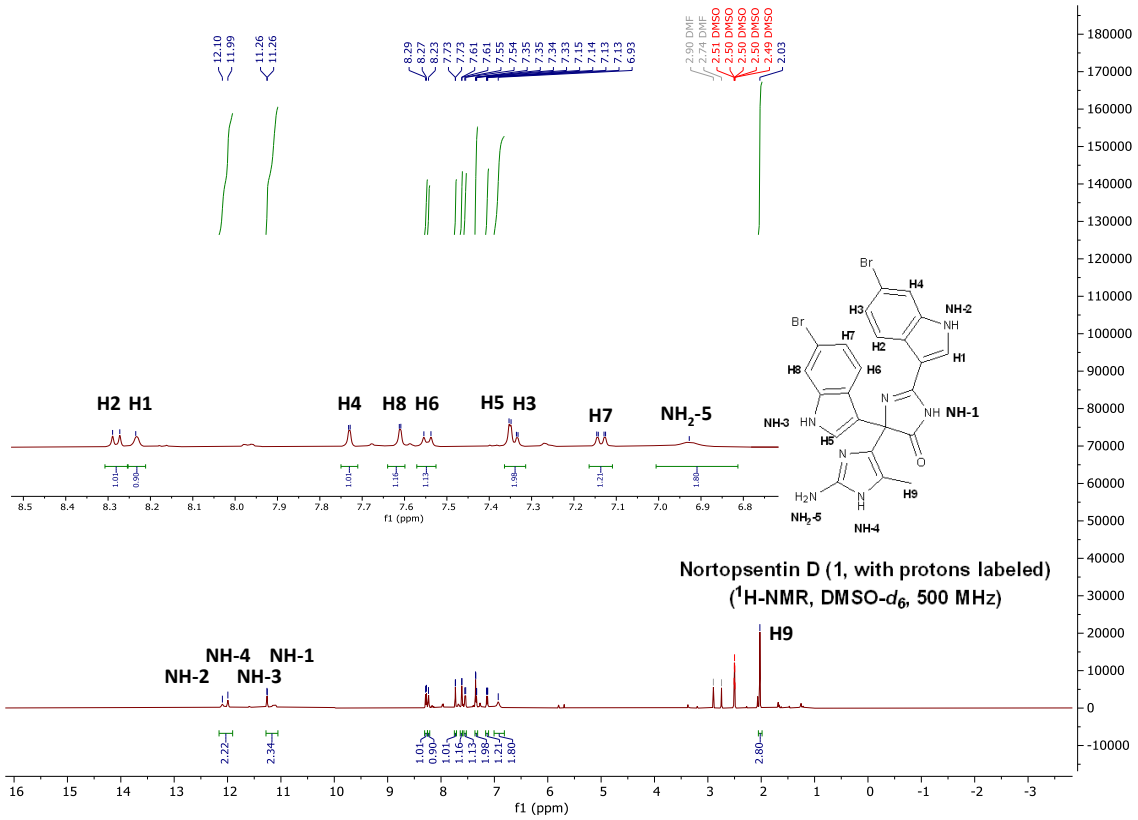


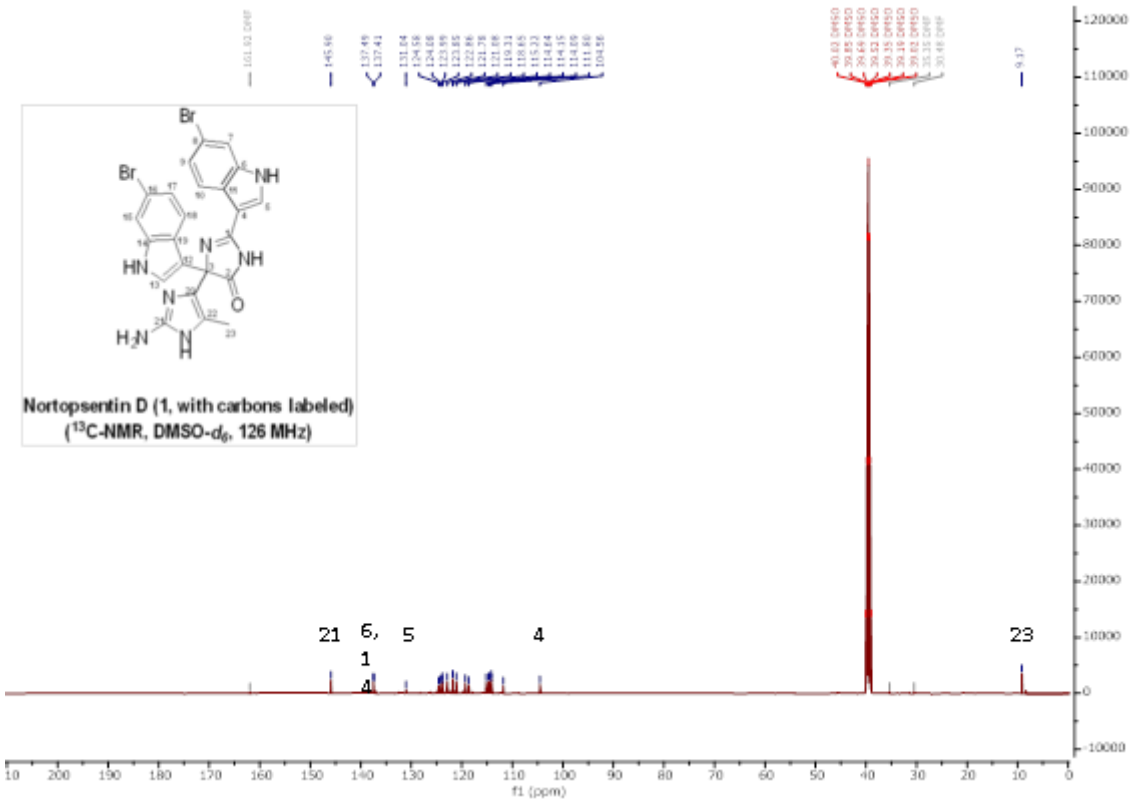
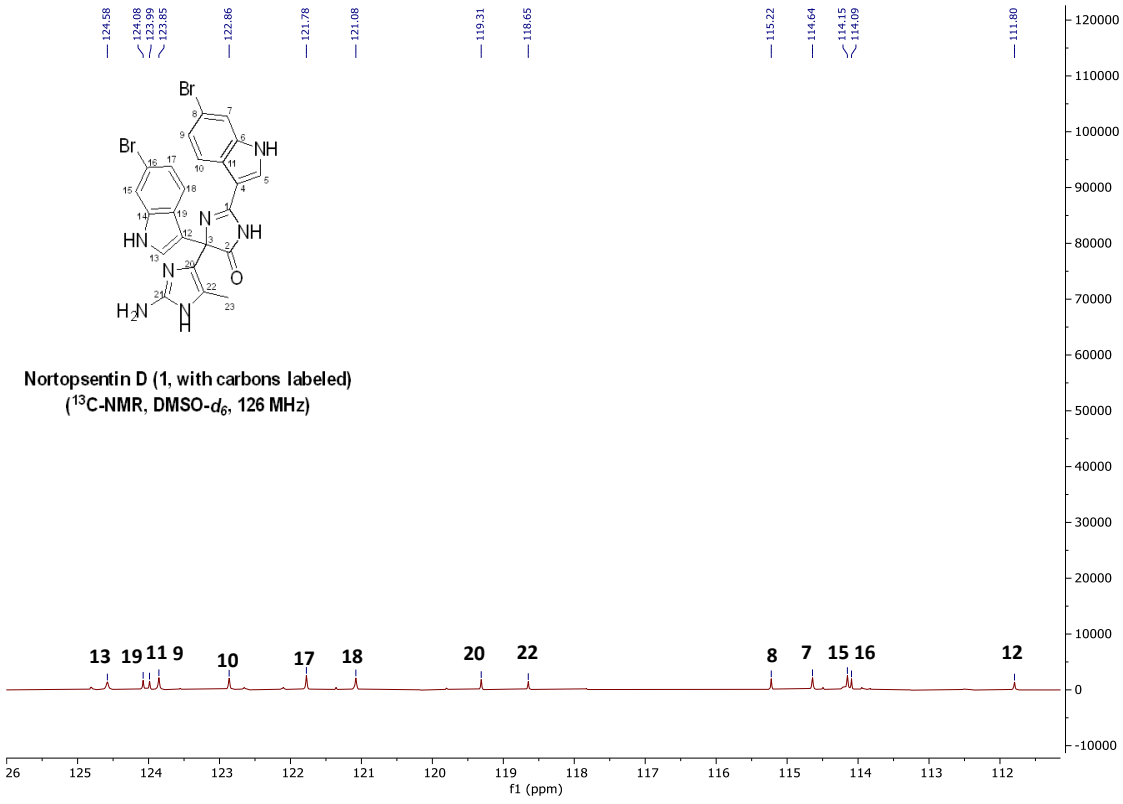
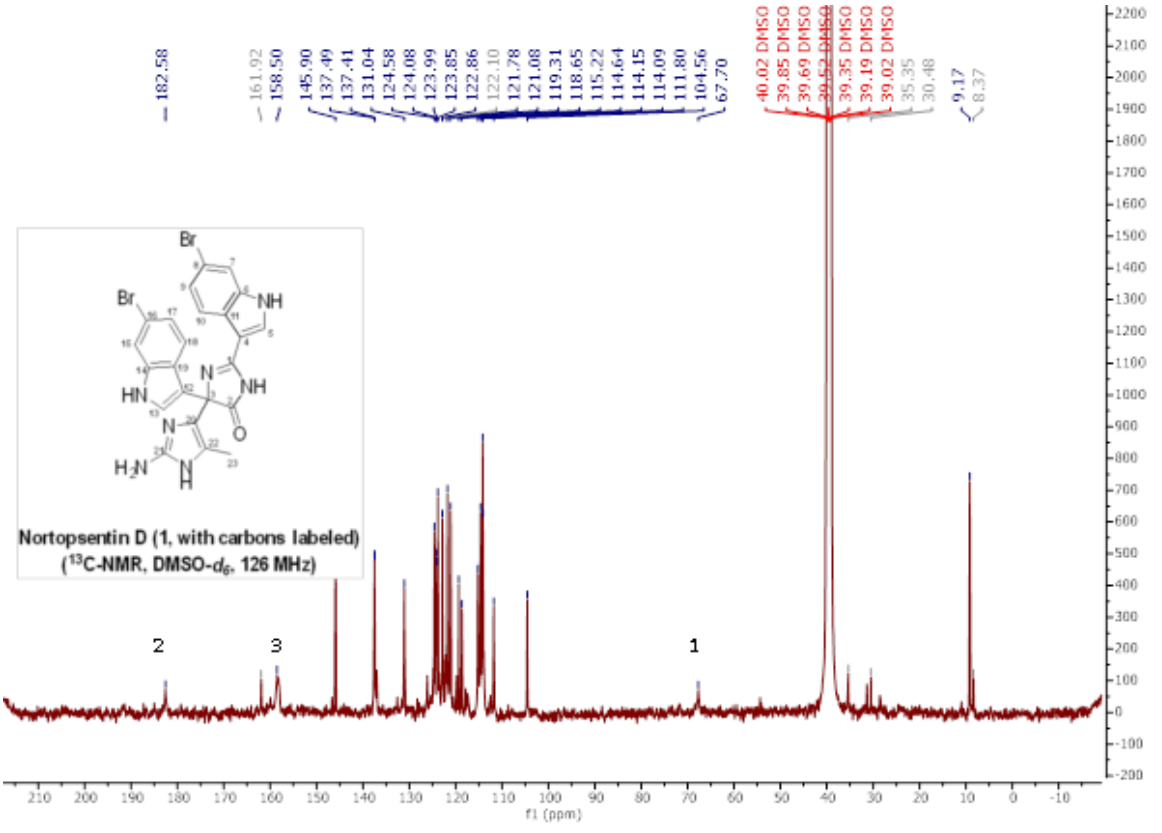


Figure 4.48 (cont'd).



Nortopsentin D (1, with carbons labeled)
(^{13}C-NMR, DMSO-d_6, 126 MHz)



Nortopsentin D (1, with carbons labeled)
(^{13}C-NMR, DMSO-d_6, 126 MHz)

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REFERENCES

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5 Chapter 5: Design and synthesis of fluspirilene analogues for the treatment of neurodegeneration*

5.1 Introduction

While the previously synthesized small molecule imidazol-4-ones showed no activity towards the proteasome, a promising new scaffold was determined from a high throughput screen on the NIH Clinical Collection and Prestwick libraries.[1] In this screen, purified human 20S proteasome was incubated with compounds (10 μM), followed by the introduction of a fluorogenic chymotrypsin-like (CT-L) peptide substrate (Suc-LLVY-7-amino-4-methylcoumarin (AMC)). An enhancd rate of proteolytic cleavage was measured by an increase in 7-amino 4-methylcoumarin fluorescence, which came from compounds able to induce an active conformation of the 20S proteasome, via gate-opening or other allosteric modulations. One of the active compounds from this screen was fluspirilene, an antipsychoitc drug known for its use in the treatment of schizophrenia. This promising new scaffold's 20S proteasone activity was further assessed by Taylor Fiolek via a series of assays on the three fluorogenic peptide substrates found within the catalytic core of the proteasome, aka a chymotryptic-like (CT-L), a trypsin-like (T-L) and a caspase-like (Casp-L) substrate, as well as the combination of all three substrates.[2] **Figure 5.1** illustrates that fluspirilene was able to activate all of the catalytic sites, achieving a doubling of activity (AC_{200}) at 2.2 μM (i.e. AC_{200} 2.2 μM), with a maximum fold enhancement of nearly 10-fold (i.e. 1000%).

While fluspirilene shows great promise as a potential proteasome activator and has good drug-like properties which allow it to penetrate the blood brain barrier (BBB), it's off-target affects must be considered.[3,4] As a potent dopamine D2 receptor antagonist, fluspirilene has been

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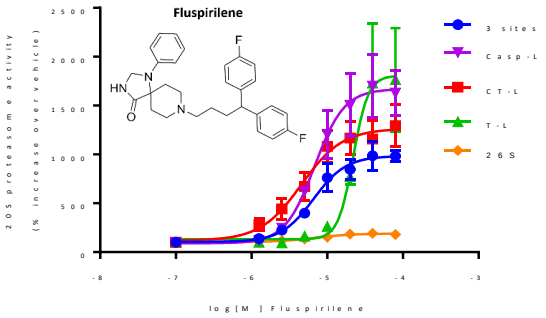
used to treat schizophrenia and thus cannot be used as a proteasome activator without modification. To reduce the dopamine D2 receptor activity, previous studies have shown that disrupting or eliminating the critical basic amine's interaction with the D2 receptor prevents D2R binding.[1] Thus, I aimed to design a modified fluspirilene scaffold that would maintain or increase its proteasome activity but diminish the effects on the D2 receptor binding.

Figure 5.1 Concentration response (0–80 μM) curve of fluspirilene, identifying the rate of proteolysis of fluorogenic peptide substrates by purified 20S proteasome as well as presenting the calculated AC_{200} and max fold increases in activity over the vehicle control. This work was performed by Taylor Fiolek.[2]



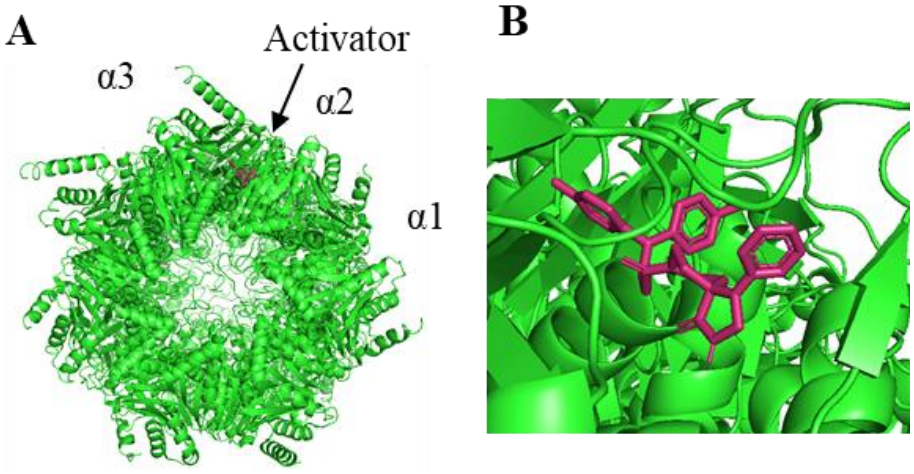
Catalytic site	3 sites	CT-L	T-L	Casp-L
Substrate	Combo	Suc-LLVY -AMC	Boc-LRR -AMC	Z-LLE -AMC
AC_{200} (μM)	2.2 ± 0.2	1.0 ± 0.3	10.9 ± 3.0	2.2 ± 0.3
Max Fold Increase	9.8 ± 0.5	12.9 ± 2.2	17.7 ± 5.3	16.3 ± 2.3

5.2 Results and discussion

With molecular docking as a guide, *N*-acylated fluspirilene (**5-12**) was developed to eliminate fluspirilene's D2 receptor activity. Here, conversion of the piperidine's amine to an amide has reduced the basicity of the critical basic amine predicted to bind to the D2 receptor. Docking studies show that fluspirilene and *N*-acylated fluspirilene dock similarly within the proteasome. These molecular docking studies were supported through computational resources and services provided by the Institute for Cyber-Enabled Research at Michigan State University and performed using Autodock Vina. Blind docking was performed on the entirety of the 20S proteasome, which allowed for true unbiased conformational preference. Both scaffolds were

found to bind preferentially to the α2-3 intersubunit pocket (**Figure 5.2A** and **Figure 5.2B**). Interestingly, all of the Tepe lab's previosuly reported 20S activators have docked preferentially in the α1-2 intersubunit pocket of the 20S proteasome.[1] Since fluspirilene and *N*-acylated fluspirilene were predicted to behave through a new, unique mechanism of action, two compounds were designed as negative controls in an effort to test the importance of the α2-3 intersubunit pocket for proteasomal activity. Compounds **5-15** and **5-7** were devised to show lessened activity towards the proteasome, built on assumptions regarding the importance of the difluoro substituents and the role of the diphenyl tail's optimal 3D conformation, respectively. These three new analogues of fluspirilene (compounds **5-7**, **5-12**, and **5-17**) were designed and synthesized to develop a better understanding of the in-pocket binding interactions of this newly discovered 20S proteasome activator.

Figure 5.2 A) Preferred docking site of fluspirilene (**5-16**) and *N*-acylated-fluspirilene (**5-12**) within the α2-3 intersubunit binding pocket of the 20S proteasome's α-ring B) Zoomed in image of compound **3-39** docked in the α2-3 intersubunit binding pocket

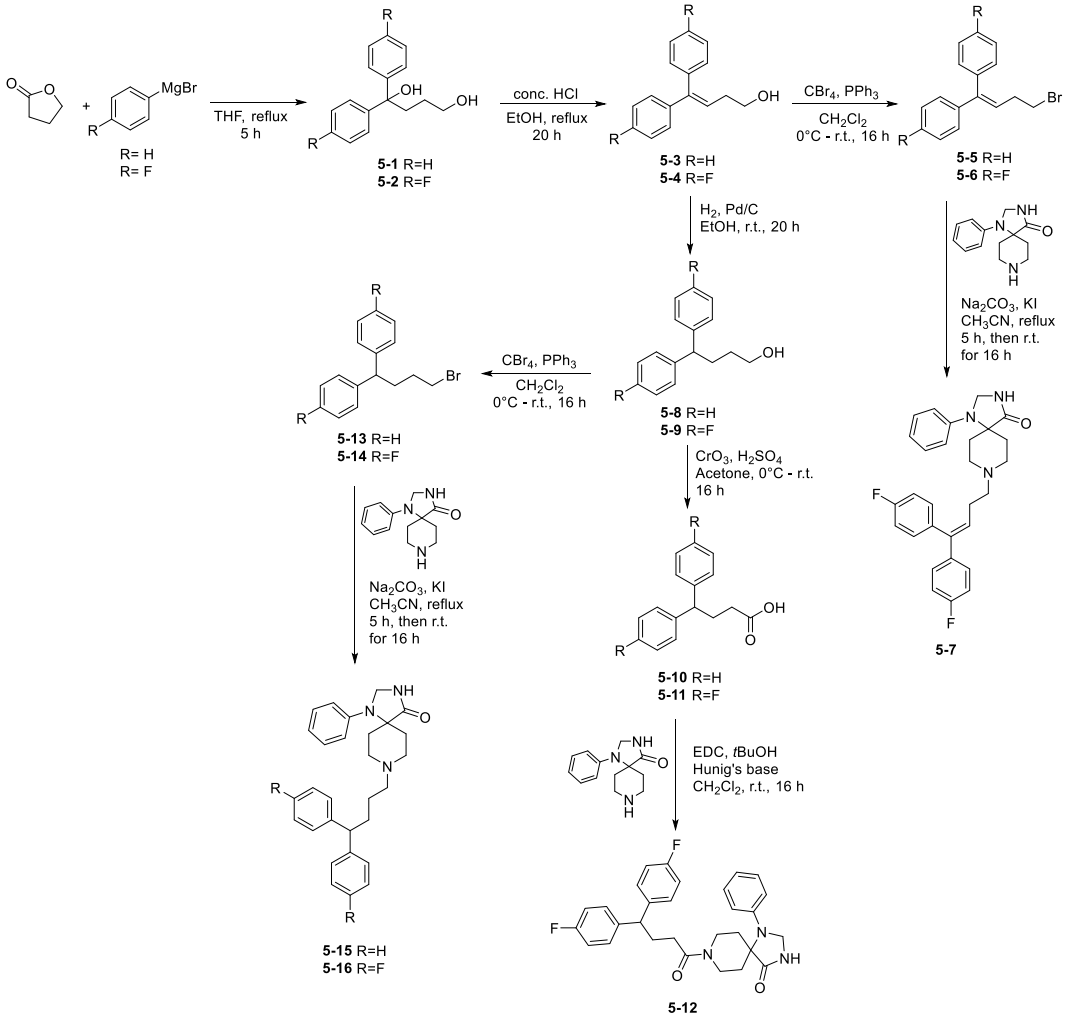


Fluspirilene and its derivatives were synthesized according to literature.[5] As shown in **Scheme 5.1**, the diphenyl tails were produced using a Grignard reaction between dihydrofuran-

2(3*H*)-one and two equivalents of aryl magnesium bromide. Subsequent dehydration of the tertiary alcohol was performed via reflux in ethanol with addition of hydrochloric acid. The formed alkenes (compounds **5-3** and **5-4**) were reduced using hydrogen gas with palladium on carbon in ethanol overnight to afford compounds **5-8** and **5-9**. Bromination was performed using carbon tetrabromide and triphenylphosphine to produce compounds **5-5**, **5-6**, **5-13**, and **5-14**. Lastly, nucleophilic substitution of the diphenyl butyl halides with 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one produced fluspirilene derivatives **5-7**, **5-15**, and **5-16**. To produce the acylated scaffold **5-12**, compound **5-9** was oxidized to a carboxylic acid using Jones reagent, which was then coupled to 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one using EDC.

All of the synthesized fluspirilene analgous (**5-7**, **5-12**, **5-15** and **5-16**) and their intermediates (**5-1** – **5-6, 5-8** – **5-11**, and **5-13** – **5-14**) were tested for 20S proteasome activity by Taylor Fiolek, as shown in **Figure 5.3**.[2] Here, to assess their activity, the standard fluorogenic peptide assay was employed using the combination of all three catalytic site substrates. As expected, none of the intermediates had any effects on the assay, however all of the fluspirilene analogues displayed some degree of activity. *N*-acylated fluspirilene (compound **5-12**) showed comparable potency (AC_{200} 1.9 µM) to that of fluspirilene (**Figure 5.3**), and a superior max fold enhancement (>2000%). These results support *N*-acylated fluspirilene (compound **5-12**) as a promising scaffold to develop more analogs from. Furthermore, *N*-acylated fluspirilene lacks the basic nitrogen known to be critical for D2R activity.[1]

Scheme 5.1 Synthetic pathway to fluspirilene compounds



BIOVIA Discovery Studio 2020 was employed to examine the α-pocket interactions of the synthesized fluspirilene analogues within the α2-3 intersubunit pocket (**Figure 5.4**) to further explore why fluspirilene and compound **5-12** show favorable 20S proteasomal activity while compounds **5-7** and **5-15** exhibit lessened activity. Regarding fluspirilene and *N*-acylated fluspirilene (compound **5-12**), strong hydrogen bond interactions are seen in multiple binding modes between the imidazol-4-one's amide N-H and a variety of amino acid residues including LYS77, ILE65, ASN84, TYR75, and GLN111 (**Figure 5.4A** and **Figure 5.4B**). While compounds **5-7** and **5-15** predict a hydrogen bond interaction between the amide's carbonyl and

proteasome amino acid residues, there are no connections between the amide's N-H and any of the above-mentioned residues (**Figure 5.4C** and **Figure 5.4D**). Furthermore, compounds **5-7** and **5-15** exhibit less preference for the α2-3 intersubunit pocket. This suggests a strong N-H hydrogen bond interaction may be necessary for preferential α2-3 pocket binding. Fluspirilene, compound **5-12**, and compound **5-15** display some pi-pi interactions with the diphenyl tail, specifically interacting with TYR154, PHE 60, and PHE 61, while compound **5-7** illustrates no pi-pi interactions between the aforementioned amino acid residues and diphenyl tail. This suggests locking the conformation of the diphenyl tail with a double bond may prevent binding via a limitation in the scaffold's conformational flexibility. Further exploration is needed to explain the significance of the difluoro substituents annd their impact, if any, on proteasomal activity.

Figure 5.3 Rate of proteolysis of a combination of the three catalytic site fluorogenic peptide substrates by purified 20S proteasome in the presence of a concentration response of fluspirilene intermediates and analogues (**5-1** – **5-16**) This work was performed by Taylor Fiolek.[2]

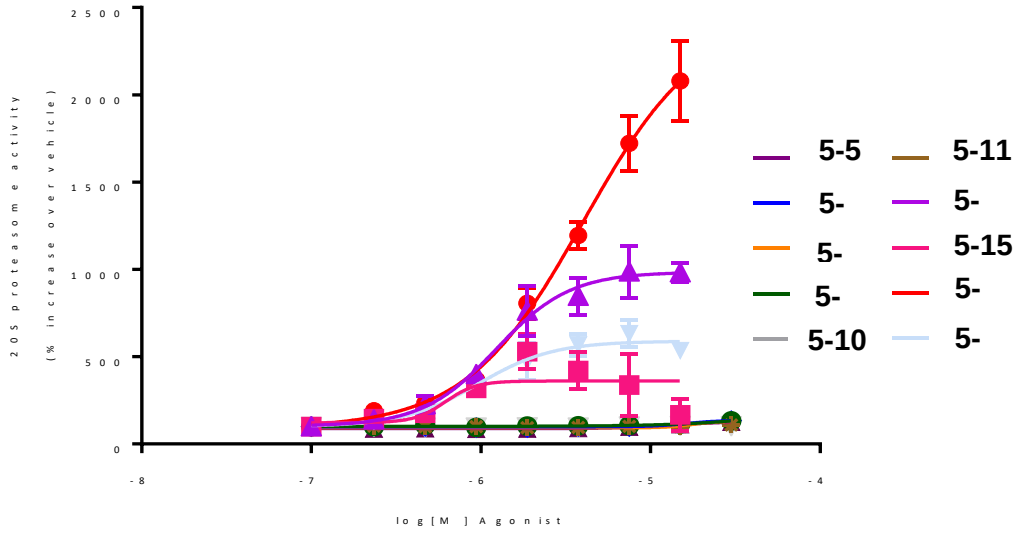
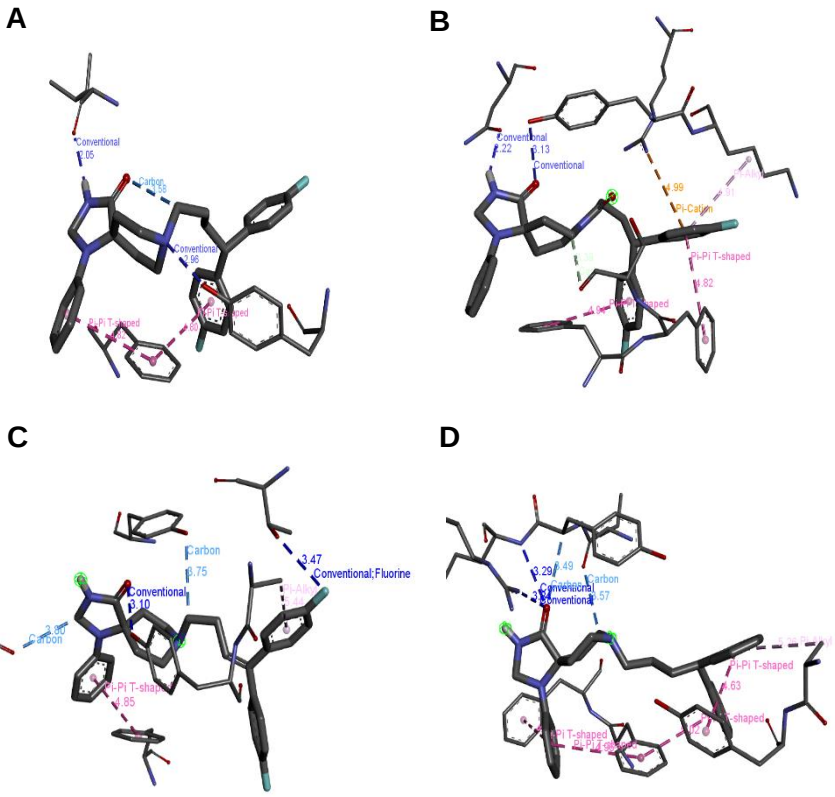


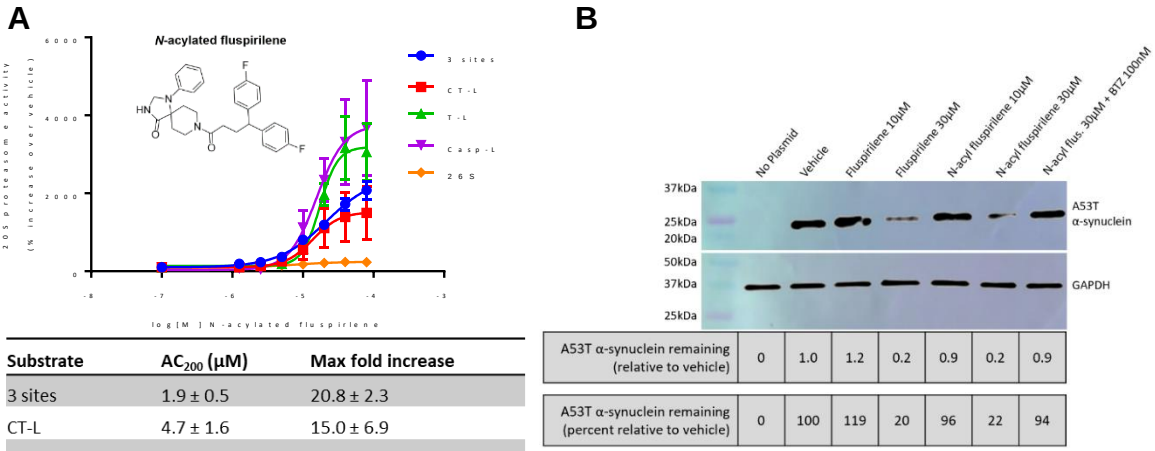
Figure 5.4 Binding models of fluspirilene and the three synthetic analogues, observed utilizing BIOVIA Discovery Studio 2020 A) fluspirilene (**5-16**) B) Compound **5-12** C) Compound **5-7** D) Compound **5-15**



Lastly, Taylor performed several additional assays to solidify the durability of *N*-acylated fluspirilene (compound **5-12**) as a 20S proteasome activator (**Figure 5.5**), testing the compound within a fluorogenic peptide assay, using the three individual substrates as well as a combination of the three and a cellular analysis on A53T mutant cells. As previously mentioned, the *N*-acylated fluspirilene analog performed similarly to fluspirilene within the fluorogenic peptide assay, with an AC_{200} of 1.9 μM using the combination of all three peptide substrate probes (**Figure 5.5A**). For the cellular assy, A53T α-synuclein plasmid was rapidly transfected into HEK-293T cells, which was probed for A53T alpha-synuclein protein in the presence and absence of compound, and cycloheximide was added to block protein synthesis ensuring

observed effects were due to chainges in protein level. A53T has been linked to early on-set familial Parkinson's disease and appears to oligomerize faster than wild-type protein.[6] The effects of fluspirilene and *N*-acylated fluspirilene (**5-12**) on the degradation of the pathogenic A53T a-synuclein mutant in cells was examined, showing that both fluspirilene and *N*-acylated fluspirilene successfully reduced the accumulation of A53T alpha-synuclein protein within 8 hours of treament, in a concentration dependent manner. Additionally, when a proteasome inhibitor such as bortezomib (BTZ) was added in with 20S proteasome enhancer *N*-acylated fluspirilene, the proteasome enhancement was nullified, which further supports the proteasome as the protease affected by *N*-acylated fluspirilene, as seen in **Figure 5.5B**.

Figure 5.5 A) Extended fluorogenic peptide analysis of *N*-acylated fluspirilene and calculated AC_{200} and max fold increases in activity over the vehicle control (n=3) These data were collected in triplicate and are shown with calculated standard deviations. B) Fluspirilene and *N*-acylated fluspirilene (**5-12**) enhanced proteasomal degradation of cellular A53T α-synuclein in transiently transfected HEK-293T cells. This work was performed by Taylor Fiolek.[2]



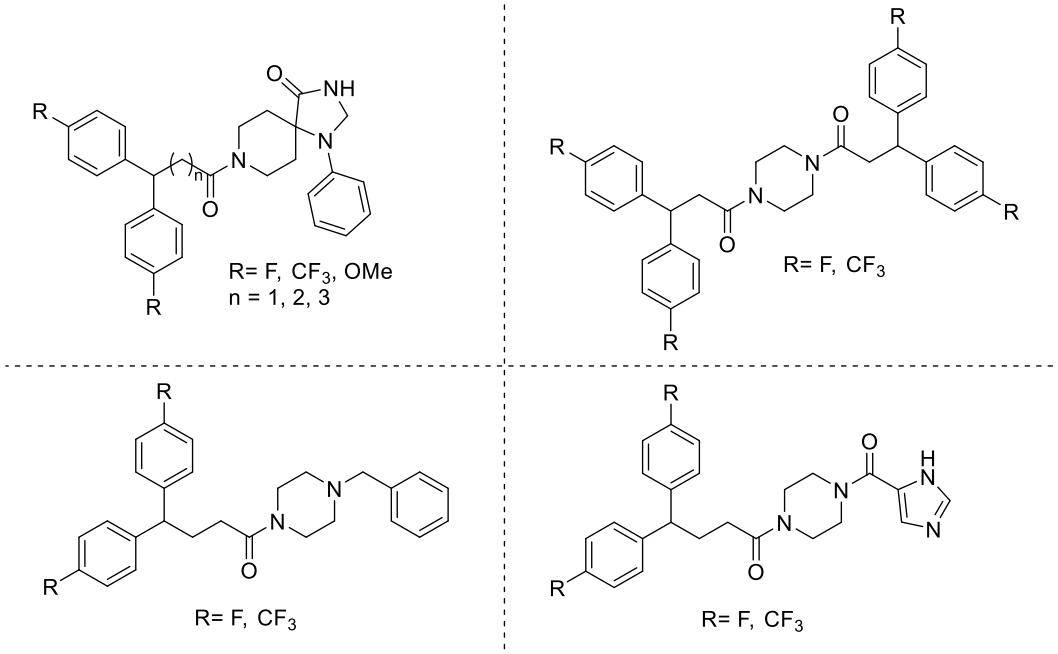
Substrate	AC_{200} (μM)	Max fold increase
3 sites	1.9 ± 0.5	20.8 ± 2.3
CT-L	4.7 ± 1.6	15.0 ± 6.9
T-L	5.6 ± 0.8	30.6 ± 7.4
Casp-L	4.1 ± 0.6	36.8 ± 12.2

A53T α-synuclein remaining (relative to vehicle)	0	1.0	1.2	0.2	0.9	0.2	0.9
A53T α-synuclein remaining (percent relative to vehicle)	0	100	119	20	96	22	94

5.3 Conclusions

Fluspirilene and its *N*-acylated analog **5-12** have proven to effectively enhance the activity of all three catalytic sites of the 20S proteasome as well as shown in-cell degradation of α-synuclein. Additionally, this class of compounsd is the first class of proteasome activators to bind selectively within the α2-3 intersubunit pocket. Based on the results of this study, there is a lot of potential for further development on both the *N*-acylated fluspirilene and related scaffolds. **Figure 5.6** displays some proposed scaffold modifications that may improve potency towards proteasome activation, as supported by docking studies. These proposed scaffolds are mainly α2-3 selective analogues that show equal to improved binding affinity as compared to *N*-acylated fluspirilene. I believe these newly proposed scaffolds could lead to potent and selective proteasome enhancer.

Figure 5.6 Proposed scaffolds for future development of the fluspirilene scaffold



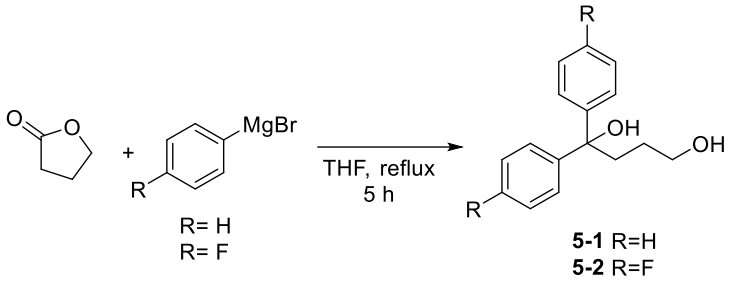
5.4 Experimental

General information

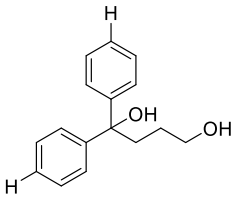
Human 20S proteasome and fluorogenic substrates N-succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin (Suc-LLVY-AMC), carboxyl benzyl-Leu-Leu-Glu-7- amido-4-methylcoumarin (Z-LLE-AMC), tert-butyloxycarbonyl-LeuArg-Arg-7-amido-4-methylcoumarin (Boc-LRR-AMC), and bortezomib were obtained from Boston Biochem, Inc. (Cambridge, MA). The recombinant wild type α-synuclein was obtained from Abcam (Cambridge, MA). Rabbit polyclonal anti-α-synuclein, mouse monoclonal anti-α-synuclein, and goat anti-rabbit HRP-linked antibody were purchased from Santa Cruz Biotechnologies (Dallas, TX). Unless otherwise noted, chemicals were purchased from commercial suppliers and used without further purification. Reactions were carried out under a nitrogen atmosphere in flame-dried glassware. Solvents and reagents were purchased from commercial suppliers and used without further purification. Anhydrous THF was distilled over sodium and benzophenone directly before use. Magnetic stirring was used for all reactions. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. Infrared spectra were recorded on a Jasco Series 6600 FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus-500 or 600 spectrometer. Chemical shifts are reported relative to the residue peaks of the solvent ($CDCl_3$: 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C) (DMSO-*d6*: 2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C). The following abbreviations are used to denote the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, and m = multiplet. HRMS were obtained at the Mass Spectrometry Facility of Michigan State University with a Micromass Q-ToF Ultima API LC-MS/MS mass spectrometer.

Molecular docking studies:

Docking was performed using PyRx's Vina Wizard program, supported through computational resources and services provided by the Institute for Cyber-Enabled Research at Michigan State University. The macromolecule was defined as the closed gate human proteasome (h20S), obtained from the PDB database (PDB ID: 4R3O). Small molecule ligands were generated in Perkin Elmer's Chem3D, where they were first minimized using MM2 force field then converted into PDB files. The designed ligands and 4R3O (macromolecule) were then uploaded into PyRx. Vina was run on a maximized grid of the h20S proteasome (grid box 153.2 x 138.0 x 189.4 Å) to allow for unbiased docking within the entirety of the proteasome. Each ligand was run through the Vina program three times at an exhaustiveness of 1000. Once complete, the top nine reported docking states were analyzed using Schrödinger's PyMOL Molecular Graphics System. Here it was discovered that the fluspirilene and *N*-acylated fluspirilene (16) docked preferentially in the α2-3 intersubunit binding pocket (Figure 2). To further explore the interactions between N-acylated fluspirilene (16) and the α2-3 subunit pocket, BIOVIA Discovery Studio 2020 was used. In this program, the receptor-ligand interactions function was used to explore how h20S (receptor) is interacting with N-acylated fluspirilene (ligand). All 9 states were analyzed for ligand-peptide interactions. The predicted key interactions are discussed within the paper (Figure 4). To support our docking driven hypothesis regarding the importance of the α2-3 binding pocket for proteasomal activation, ligands predicted to have varying preference for the α2-3 binding pocket were synthesized and checked for 20S activation in vitro.



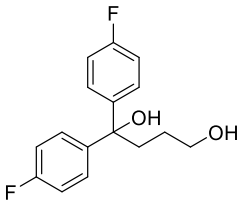
General method to synthesis diols via a Grignard reaction: THF (15 mL) was added to a clean, dry round-bottom flask under nitrogen. The appropriate aryl magnesium bromide (2M in diethyl ether, 2.5 equiv.) was added to the solution, and it was stirred with a magnetic stir bar. Then, a solution of gamma-butyrolactone（1 equiv,) in dry tetrahydrofuran (THF) (10 mL) was added dropwise to the mixture. The reaction was stirred and heated at reflux for 5h. When complete, the reaction was quenched with a few drops of 1M HCl aq. solution, and the THF was evaporated *in vacuo*. The crude mixture was extracted with diethyl ether (3 × 30 mL), and the organic layer was washed with a saturated brine solution (50 mL). The organics were dried over anhydrous sodium sulfate. The organic solvent was filtered, concentrated *in vacuo*, and purified using automated CombiFlash chromatography (silica gel, 20-40 microns, ethyl acetate/hexanes gradient 0-100%) to provide the pure product. Any changes to the procedure are mentioned under the specific compound.



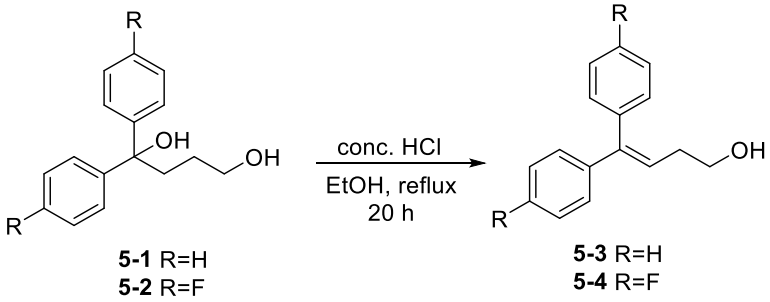
1,1-diphenylbutane-1,4-diol (5-1) The pure product was a white solid (quantitative yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.5 Hz, 4H), 7.29 (t, *J* = 7.0 Hz, 4H), 7.20 (t, *J* = 6.6 Hz, 2H), 3.60 (t, *J* = 6.0 Hz, 2H), 2.91 (s, 2H), 2.40 (t, *J* = 7.3 Hz, 2H), 1.56 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.22, 128.21, 126.82, 126.20, 77.90, 63.10, 39.05, 27.15.

IR: 3355, 2956, 2932 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M-H)^-$] calcd for ($C_{16}H_{17}O_2^-$) 241.1228 Found 241.1227. m.p.: 86 °C.

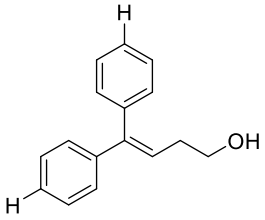


1,1-bis(4-fluorophenyl)butane-1,4-diol (5-2) The pure product was a white solid (2.23g, 69%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.33 (m, 4H), 6.97 (t, *J* = 8.7 Hz, 4H), 3.59 (t, *J* = 5.8 Hz, 2H), 3.23 (s, br., 2H), 2.35 (t, *J* = 7.2 Hz, 2H), 1.52 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.62 (d, *J* = 245.6 Hz), 142.80 (d, *J* = 3.2 Hz), 127.79 (d, *J* = 7.8 Hz), 114.90 (d, *J* = 21.1 Hz), 77.08, 62.82, 39.31, 26.79. IR: 3303, 3177 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M-H)^-$] calcd for ($C_{16}H_{15}F_2O_2^-$) 277.1040; Found 277.1038. m.p.: 99 °C.



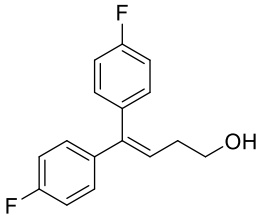
General method to eliminate the tertiary alcohol: The appropriate diol (1 equiv, 7.9 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 30 mL of ethanol. Then, concentrated HCl (11.5 mL) was added to the solution. The reaction was stirred with a magnetic stir bar and heated at reflux for 20 hours. When complete, the reaction was brought to a pH 7 with an aqueous solution of sodium bicarbonate, and the organics were collected and dried over anhydrous sodium sulfate. The organic solvent was filtered, concentrated *in vacuo*, and purified using automated CombiFlash chromatography (silica gel, 20-40 microns, gradient 0-

100% ethyl acetate in hexanes) to provide the pure product. Any changes to the procedure are mentioned under the specific compound.



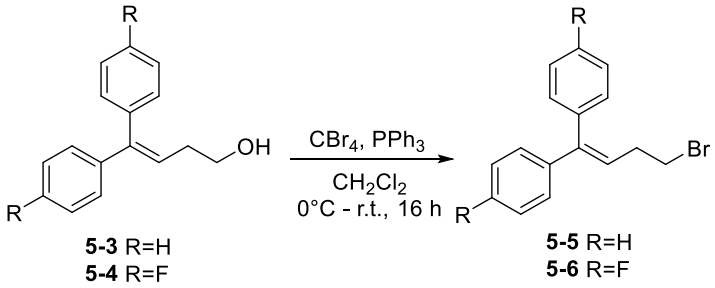
4,4-diphenylbut-3-en-1-ol (5-3) The product was a clear oil (0.48 g, 52%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 – 7.37 (m, 2H), 7.37 – 7.18 (m, 8H), 6.14 (t, *J* = 7.5 Hz, 1H), 3.73 (t, *J* = 6.6 Hz, 2H), 2.46 – 2.38 (m, 2H), 1.66 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.01, 142.45, 139.89, 129.90, 128.28, 128.14, 127.26, 127.12, 127.10, 125.42, 62.49, 33.34. IR: 3307, 3054, 3022, 2874 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{16}H_{17}O^+$) 225.1279 Found 225.1274.



4,4-bis(4-fluorophenyl)but-3-en-1-ol (5-4) The product was a clear oil (1.19 g, 58%).

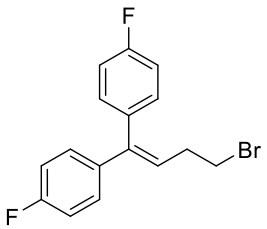
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (m, 4H), 7.06 (t, *J* = 8.7 Hz, 2H), 6.95 (t, *J* = 8.7 Hz, 2H), 6.05 (t, *J* = 7.5 Hz, 1H), 3.71 (t, *J* = 6.5 Hz, 2H), 2.37 (q, *J* = 6.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.16 (d, *J* = 21.6 Hz), 161.20 (d, *J* = 21.1 Hz), 142.21, 138.55 (d, *J* = 3.3 Hz), 135.60 (d, *J* = 3.7 Hz), 131.54 (d, *J* = 7.8 Hz), 128.88 (d, *J* = 7.8 Hz), 125.73, 115.40 (d, *J* = 21.2 Hz), 115.09 (d, *J* = 21.4 Hz), 62.52, 33.34. IR: 3306, 3043, 2879 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{16}H_{15}F_2O^+$) 261.1091 Found 261.1088.



5-3 R=H
5-4 R=F

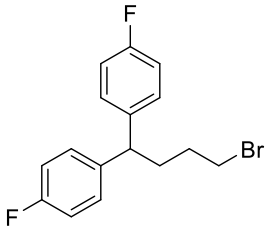
5-5 R=H
5-6 R=F

General method to brominate the alkyl tails: The appropriate alcohol (1 equiv, 0.96 mmol) and carbon tetrabromide (1.25 equiv, 1.15 mmol) were added to a clean, dry round-bottom flask under nitrogen and were dissolved in dry dichloromethane (10 mL). The solution was cooled to 0 °C. Triphenylphosphine (1.9 equiv, 1.82 mmol) was added to the reaction slowly at 0 °C, and the reaction mixture was stirred with a magnetic stir bar and allowed to warm to room temperature naturally. After stirring for 5 hours, the reaction was filtered and washed with petroleum ether (20 mL). The filtrate was concentrated *in vacuo* and purified using automated CombiFlash chromatography (silica gel, 20-40 microns, gradient 0-100% ethyl acetate in hexanes) to provide the pure product. Any changes to the procedure are mentioned under the specific compound.



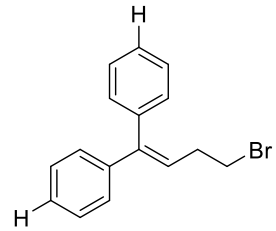
4,4'-(4-bromobut-1-ene-1,1-diyl)bis(fluorobenzene) (5-6) The product was a clear oil (0.27 g, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 – 7.06 (m, 6H), 7.01 – 6.95 (m, 2H), 6.03 (t, *J* = 7.2 Hz, 1H), 3.44 (t, *J* = 6.8 Hz, 2H), 2.68 (q, *J* = 6.9 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.20 (d, *J* = 26.2 Hz), 161.23 (d, *J* = 26.0 Hz), 142.32, 138.13 (d, *J* = 3.2 Hz), 135.27 (d, *J* = 3.7 Hz), 131.32 (d, *J* = 8.1 Hz), 128.88 (d, *J* = 8.1 Hz), 126.03, 115.46 (d, *J* = 21.4 Hz), 115.09 (d, *J* = 21.3 Hz), 32.80, 32.56. IR: 2922, 1658, 1599, 1504 cm^{-1}. Mass spectroscopy data could

not be determined. Known compound, data matches literature values (G. Chen, H. Xia, Y. Cai, D. Ma, J. Yuan and C. Yuan, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 234–239).



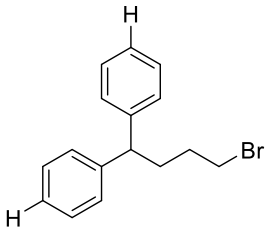
4,4'-(4-bromobutane-1,1-diyl)bis(fluorobenzene) (5-14) The product was a clear oil (0.13 g, 52%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (m, 4H), 6.99 (t, *J* = 8.7 Hz, 4H), 3.91 (t, *J* = 7.9 Hz, 1H), 3.42 (t, *J* = 6.6 Hz, 2H), 2.17 (m, 2H), 1.82 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.44 (d, *J* = 244.9 Hz), 140.00 (d, *J* = 3.2 Hz), 129.08 (d, *J* = 7.8 Hz), 115.42 (d, *J* = 21.1 Hz), 49.04, 34.33, 33.63, 31.00. IR: 2932 cm^{-1}. Known compound, data matches literature values (G. Chen, H. Xia, Y. Cai, D. Ma, J. Yuan and C. Yuan, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 234–239).

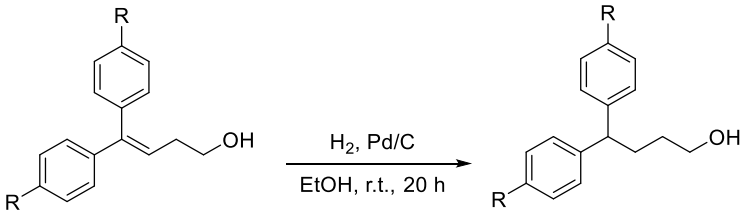


(4-bromobut-1-ene-1,1-diyl)dibenzene (5-5) The product was a clear oil (0.24 g, 76%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 – 7.38 (m, 2H), 7.38 – 7.23 (m, 6H), 7.22 – 7.18 (m, 2H), 6.11 (t, *J* = 7.3 Hz, 1H), 3.45 (t, *J* = 6.9 Hz, 2H), 2.71 (q, *J* = 7.1 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.39, 142.21, 139.71, 129.82, 128.47, 128.29, 127.45, 127.42, 127.41, 125.82, 33.04, 32.83. IR: 3437, 2924, 2853, 1717, 1657 cm^{-1}. Known compound, data matches literature values (G. Chen, H. Xia, Y. Cai, D. Ma, J. Yuan and C. Yuan, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 234–239).



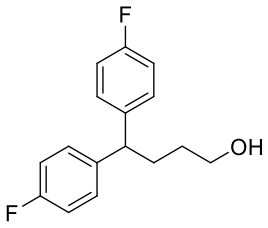
(4-bromobutane-1,1-diyl)dibenzene (5-13) The product was a clear oil (0.094 g, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.25 (m, 8H), 7.26 – 7.18 (m, 2H), 3.96 (t, *J* = 7.9 Hz, 1H), 3.44 (t, *J* = 6.6 Hz, 2H), 2.25 (q, *J* = 7.9 Hz, 2H), 1.87 (p, *J* = 6.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.56, 128.64, 127.88, 126.41, 50.68, 34.21, 33.93, 31.25. IR: 2936, 1602, 1504 cm^{-1}. Known compound, data matches literature values (G. Chen, H. Xia, Y. Cai, D. Ma, J. Yuan and C. Yuan, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 234–239).



5-3 R=H
5-4 R=F

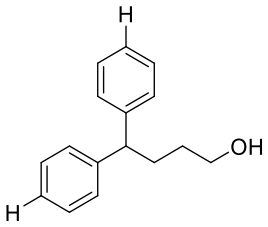
5-8 R=H
5-9 R=F

General method to hydrogenate alkenes: Palladium on carbon (25 mol%, 0.70 mmol) was added to a clean, dry round bottom flask and suspended in ethanol (10 mL). Then the appropriate alkene (1 equiv, 2.88 mmol) was added to the suspension, and it was stirred with a magnetic stir bar under a hydrogen atmosphere overnight at room temperature. When complete, the reaction was filtered through celite®, and the filtrate was concentrated *in vacuo*. The crude product was used without purification.



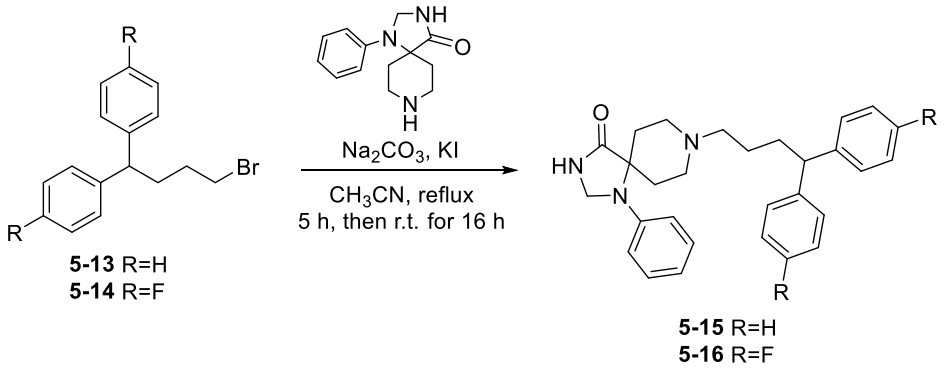
4,4-bis(4-fluorophenyl)butan-1-ol (5-9) The product was a clear oil (0.73 g, 97%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 – 7.14 (m, 4H), 7.02 – 6.94 (m, 4H), 3.90 (t, *J* = 7.9 Hz, 1H), 3.66 (t, *J* = 6.4 Hz, 2H), 2.12 – 2.04 (m, 2H), 1.56 – 1.44 (m, 3H, broadness suggests 1H is acidic). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.46 (d, *J* = 244.6 Hz), 140.55 (d, *J* = 3.1 Hz), 129.21 (d, *J* = 7.7 Hz), 115.40 (d, *J* = 21.1 Hz), 62.79, 49.66, 32.21, 31.20. IR: 3342, 2938, 2867 cm^{-1}. HRMS (ESI-TOF) m/z: [(M-H)$^-$] calcd for ($C_{16}H_{15}F_2O^-$) 261.1091 Found 261.1089.



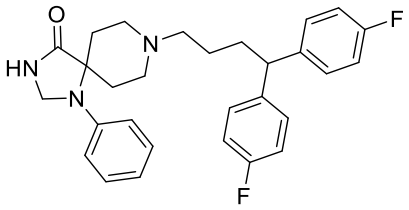
4,4-diphenylbutan-1-ol (5-8) The product was a clear oil (0.23 g, 93%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 – 7.24 (m, 8H), 7.24 – 7.16 (m, 2H), 3.93 (t, *J* = 7.9 Hz, 1H), 3.66 (t, *J* = 6.5 Hz, 2H), 2.19 – 2.11 (m, 2H), 1.60 – 1.51 (m, 2H), 1.43 (s, br., 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.93, 128.48, 127.85, 126.19, 62.88, 51.14, 31.85, 31.31. IR: 3320, 2934, 2864 cm^{-1}. HRMS (ESI-TOF) m/z: [(M-H)$^-$] calcd for ($C_{16}H_{17}O^-$) 225.1279 Found 225.1273.



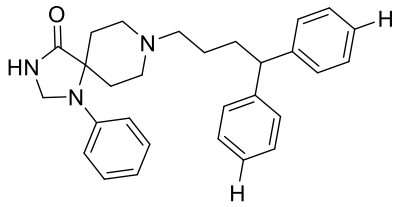
General method to condense brominated tails and compound: To a clean, dry round bottom flask containing acetonitrile (10 mL) was added 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (1 equiv, 0.25 mmol). Then sodium carbonate (3 equiv, 0.74 mmol) and a catalytic amount of KI

(10 mol%, 0.025 mmol) were added to the reaction. Lastly, the appropriate alkyl bromide (1 equiv, 0.25 mmol) was added to the solution. The solution was stirred with a magnetic stir bar at reflux for 5 hours and then stirred at room temperature overnight. When complete, the reaction was poured into 40 mL of ethyl acetate and washed with water (20 mL) and brine (20 mL). The organics were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was purified using automated CombiFlash chromatography (silica gel, 20-40 microns, gradient 0-100% ethyl acetate in hexanes) to provide the pure product. Any changes to the procedure are mentioned under the specific compound.



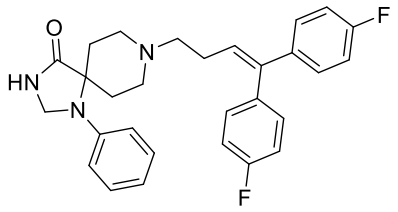
8-(4,4-bis(4-fluorophenyl)butyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (5-16) The product was a white solid (97 mg, 66%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (s, br., 1H), 7.27 (t, *J* = 7.9 Hz, 2H), 7.18 (dd, *J* = 8.5, 5.4 Hz, 4H), 6.97 (t, *J* = 8.7 Hz, 4H), 6.91 (d, *J* = 8.2 Hz, 2H), 6.86 (t, *J* = 7.3 Hz, 1H), 4.73 (s, 2H), 3.91 (t, *J* = 7.8 Hz, 1H), 2.73 (d, *J* = 50.4 Hz, 6H), 2.47 (s, 1H), 2.09 – 2.01 (m, 3H), 1.72 (d, *J* = 13.3 Hz, 2H), 1.49 (p, *J* = 7.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.39, 161.33 (d, *J* = 244.3 Hz), 143.08, 140.58 (d, *J* = 2.9 Hz), 129.28, 129.10 (d, *J* = 7.7 Hz), 119.01, 115.44, 115.25 (d, *J* = 21.0 Hz), 60.43, 59.43, 58.25, 49.77, 49.65, 33.89, 21.08, 14.22. IR: 2926, 2821, 1706, 1600, 1502 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M+H)^+$] calcd for ($C_{29}H_{32}F_2N_3O^+$) 476.2513 Found 476.2512. m.p.: 185 °C.



8-(4,4-diphenylbutyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (5-15) The product was a white solid (55 mg, 62%).

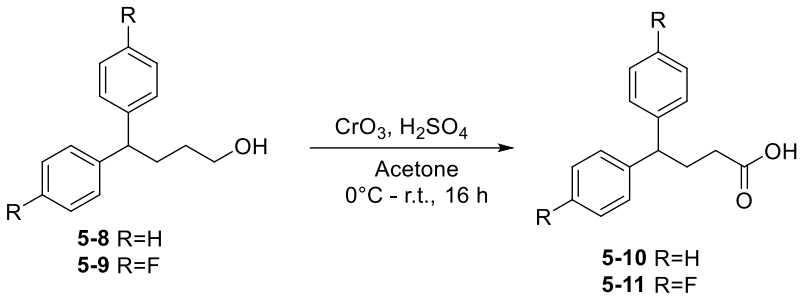
^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 7.36 – 7.23 (m, 8H), 7.17 (dt, *J* = 14.1, 7.2 Hz, 4H), 6.82 (d, *J* = 8.2 Hz, 2H), 6.72 (t, *J* = 7.3 Hz, 1H), 4.55 (s, 2H), 2.65 (m, 2H), 2.62 – 2.46 (m, 5H), 2.33 (t, *J* = 7.3 Hz, 2H), 2.10 – 2.03 (m, 2H), 1.52 (d, *J* = 13.0 Hz, 2H), 1.35 (p, *J* = 7.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 176.70, 145.76, 143.73, 129.42, 128.83, 128.01, 126.42, 117.86, 114.38, 59.07, 58.57, 58.00, 50.79, 49.86, 33.20, 28.80, 25.55. IR: 3186, 3060, 2932, 2817, 1704, 1599, 1502 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+H)$^{+}$] calcd for ($C_{29}H_{34}N_3O^{+}$) 440.2702 Found 440.2699. m.p.: 196 °C.



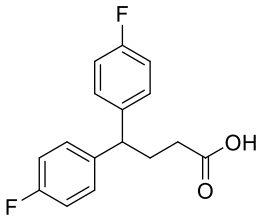
8-(4,4-bis(4-fluorophenyl)but-3-en-1-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (5-7) The product was a white solid (40 mg, 34%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (s, br., 1H), 7.39 – 7.32 (m, 2H), 7.32 – 7.21 (m, 4H), 7.21 – 7.03 (m, 4H), 7.03 – 6.93 (m, 3H), 6.15 (t, *J*= 7.4 Hz, 1H), 4.83 (s, 2H), 3.00 – 2.63 (m, 9H), 2.44 (m, 1H), 1.81 (d, *J* = 13.4 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.42, 163.19 – 162.89 (m), 161.28 – 160.87 (m), 143.09, 131.40 (d, J = 7.9 Hz), 130.53 (d, J = 8.1 Hz), 129.28, 128.78 (d, J = 7.7 Hz), 118.91, 115.72 (d, J = 21.5 Hz), 115.58, 115.36 – 115.19 (m), 115.00 (d, J = 21.3 Hz), 62.46, 59.43, 58.15, 49.65, 40.35, 30.97, 29.09, 27.80. IR: 3189, 3061, 2951, 2919, 2824,

1719, 1598, 1503 cm^{-1}. HRMS (ESI-TOF) m/z: $[(M+H)^+]$ calcd for ($C_{29}H_{29}F_2N_3O^+$) 474.2357 Found 474.2357. m.p.: 170 °C.

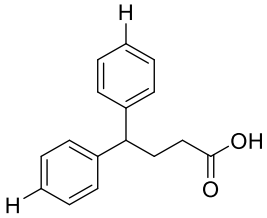


General method to oxidize primary alcohol to carboxylic acid: The appropriate alcohol (1 equiv, 0.76 mmol) was added to a clean, dry round bottom flask and dissolved in acetone (10 mL) and cooled to 0°C. Jones reagent (2.5 M in H_2O, 1 equiv.) was then added dropwise to the reaction. The solution was stirred with a magnetic stir bar at 0 °C for one hour. During this time, the solution turned from green to orange, and a precipitate formed. The reaction stirred at room temperature overnight. After the reaction was complete, isopropanol (10 mL) was added, and the solution returned to a green color. The mixture was filtered to remove any precipitate. The filtrate was evaporated and dissolved in diethyl ether (30 mL). The organics were washed with a saturated sodium bicarbonate aqueous solution and then a saturated brine solution. The organics were then dried over anhydrous sodium sulfate and concentrated *in vacuo*. The product was used without further purification.



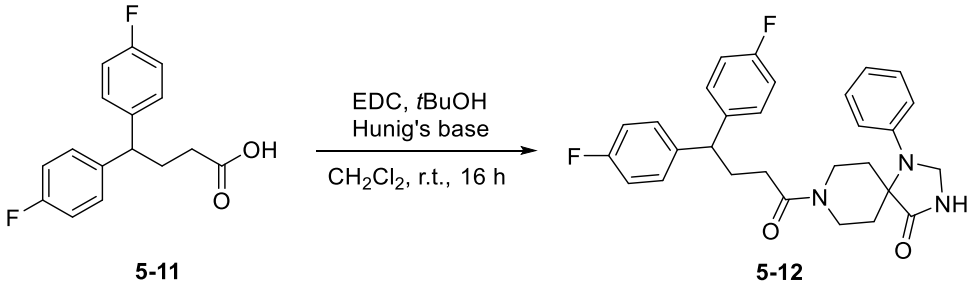
4,4-bis(4-fluorophenyl)butanoic acid (5-11) The product was an oil (0.19 g, 91%). The crude product was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 11.27 (br. s, 1H), 7.19 (m, 4H), 7.00 (t, *J* = 8.5 Hz, 4H), 3.95 (t, *J* = 7.1 Hz, 1H), 2.34 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.89, 161.53 (d, *J* = 244.9 Hz), 139.48 (d, *J* = 3.2 Hz), 129.17 (d, *J* = 7.8 Hz), 115.48 (d, *J* = 21.1 Hz), 48.86, 32.45, 30.60. IR: 3040, 2934, 1705, 1603, 1505 cm^{-1}. HRMS (ESI-TOF) m/z: [$(M-H)^-$] calcd for ($C_{16}H_{13}F_2O_2^-$) 275.0883 Found 275.0886.



4,4-diphenylbutanoic acid (5-10) The product was a white solid (69 mg, 72%). The crude product was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 11.44 (s, 1H), 7.35 – 7.18 (m, 10H), 3.98 (t, *J* = 7.8 Hz, 1H), 2.48 – 2.26 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.03, 144.05, 128.70, 127.94, 126.56, 50.43, 32.62, 30.38. HRMS (ESI-TOF) m/z: [$(M-H)^-$] calcd for ($C_{16}H_{15}O_2^-$) 239.1072 Found 239.1069. m.p.: 94 °C.



5-11 **5-12**

8-(4,4-bis(4-fluorophenyl)butanoyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (5-12)

Compound **5-11** (1 equiv., 0.25 mmol) was added to a clean, dry round bottom flask under nitrogen and dissolved in 5 mL of dichloromethane. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI·HCl) (1.2 equiv, 0.3 mmol) was added to the reaction. 1-

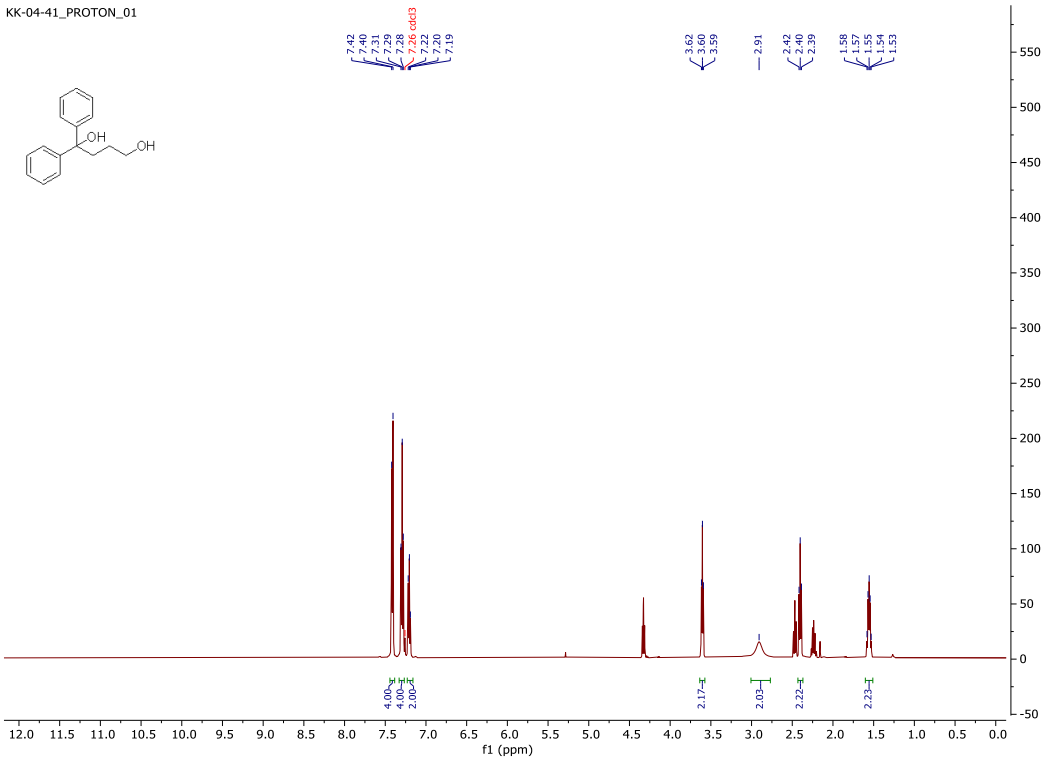
phenyl-1,3,8-triazaspiro[4.5]decan-4-one (1 equiv., 0.25 mmol) was added to the reaction in 5 mL of dichloromethane. Then, tert-butyl alcohol (1.2 equiv., 0.3 mmol) was added, and the reaction was stirred at room temperature for 5 minutes. Hunig's base (2.9 equiv., 0.72 mmol) was then added, and the reaction was stirred with a magnetic stir bar at room temperature overnight. After completion, as monitored by TLC, water was added to the solution to quench the reaction. The organics were extracted into dichloromethane (20 mL x 3) and then washed with a 1% aqueous HCl solution (20 mL) and brine (20 mL). The organics were dried over anhydrous sodium sulfate, concentrated *in vacuo* and purified using automated CombiFlash chromatography (silica gel, 20-40 microns, gradient 0-10% methanol in dichloromethane) to produce the pure product as a white solid (34 mg, 28%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (s, br., 1H), 7.24 (m, 2H), 7.17 (m, *J* = 8.2, 5.3 Hz, 4H), 6.95 (m, 4H), 6.89 (t, *J* = 7.4 Hz, 1H), 6.73 (d, *J* = 8.1 Hz, 2H), 4.73 (s, 2H), 4.54 (d, *J* = 10.4 Hz, 1H), 3.96 (t, *J* = 7.7 Hz, 1H), 3.77 (t, *J* = 13.1 Hz, 1H), 3.55 (d, *J* = 15.9 Hz, 1H), 3.39 (t, *J* = 12.9 Hz, 1H), 2.49 – 2.22 (m, 6H), 1.78 (d, *J* = 14.1 Hz, 1H), 1.72 (d, *J* = 13.9 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.33, 170.83, 161.42 (d, *J* = 244.8 Hz), 142.70, 139.95 (d, *J* = 2.9 Hz), 129.43, 129.14 (d, *J* = 7.9 Hz), 120.01, 116.04, 115.38 (d, *J* = 21.3 Hz), 59.52, 59.41, 48.93, 41.80, 38.19, 31.28, 31.05, 29.77, 29.15. IR: 3228, 2930, 1711, 1601, 1505 cm^{-1}. HRMS (ESI-TOF) m/z: [(M+H)+] calcd for ($C_{29}H_{29}F_2N_3O_2^+$) 490.2306 Found 490.2302. mp: 215 °C.

APPENDIX

APPENDIX

Figure 5.7 ^{1}H and ^{13}C NMR spectra of compound **5-1**



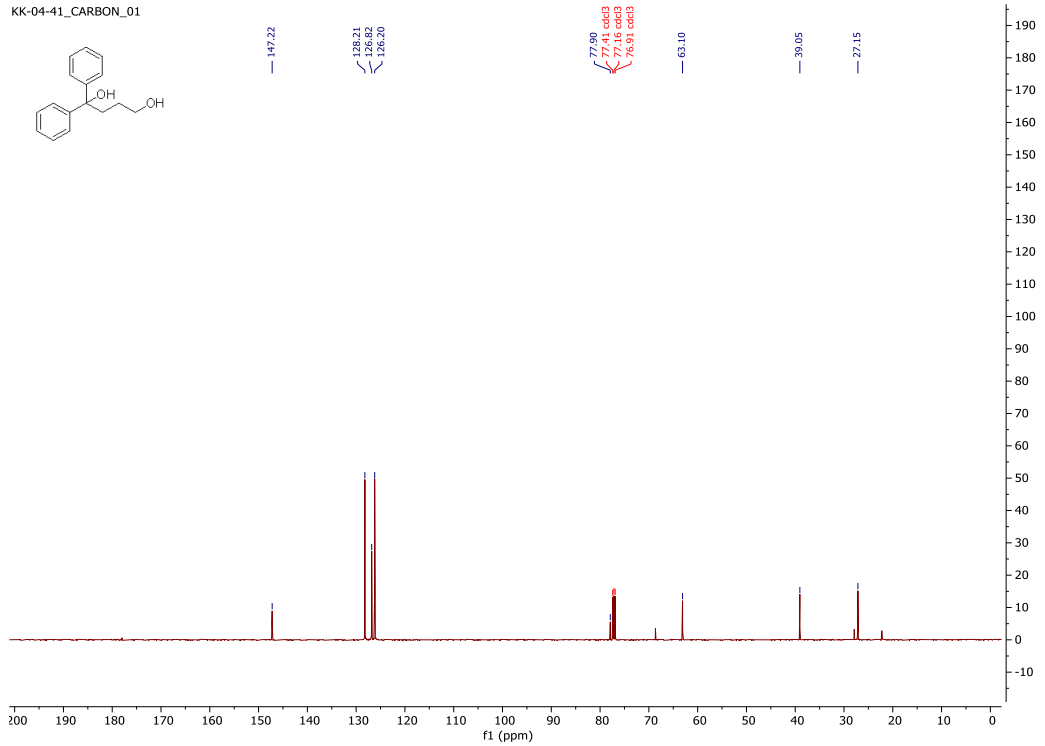


Figure 5.8 ^{1}H and ^{13}C NMR spectra of compound **5-2**

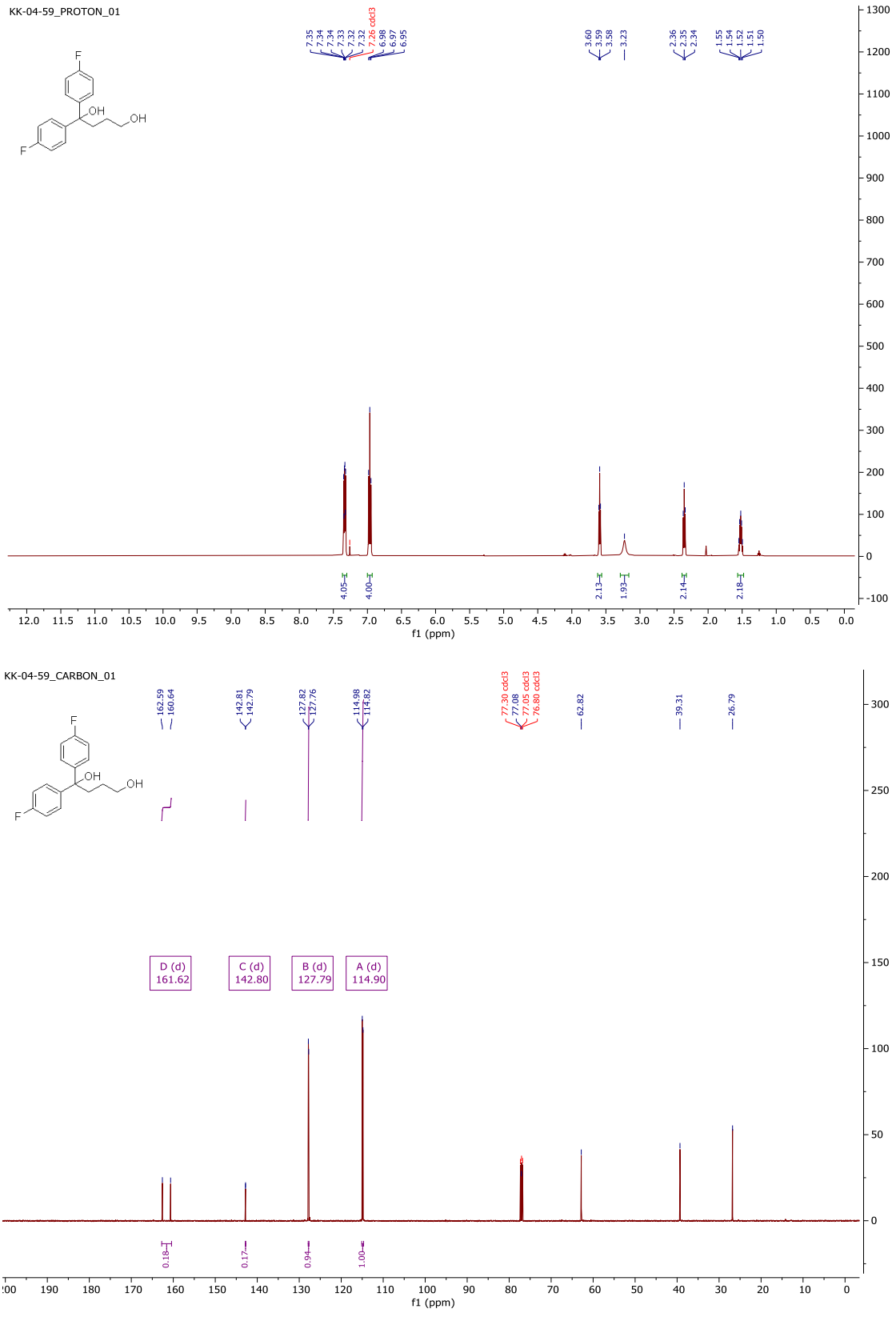


Figure 5.9 ^{1}H and ^{13}C NMR spectra of compound **5-3**

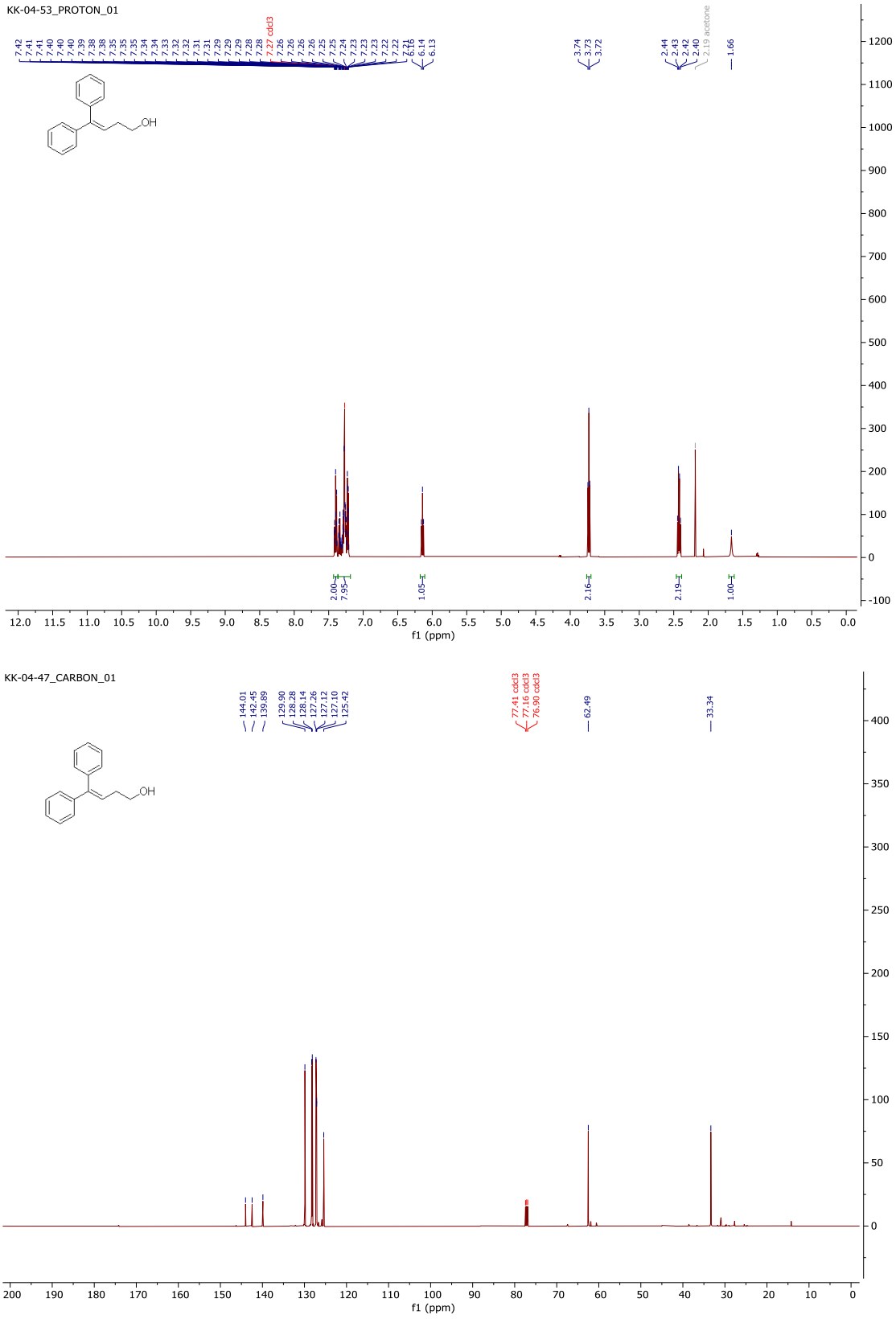


Figure 5.10 ^{1}H and ^{13}C NMR spectra of compound **5-4**

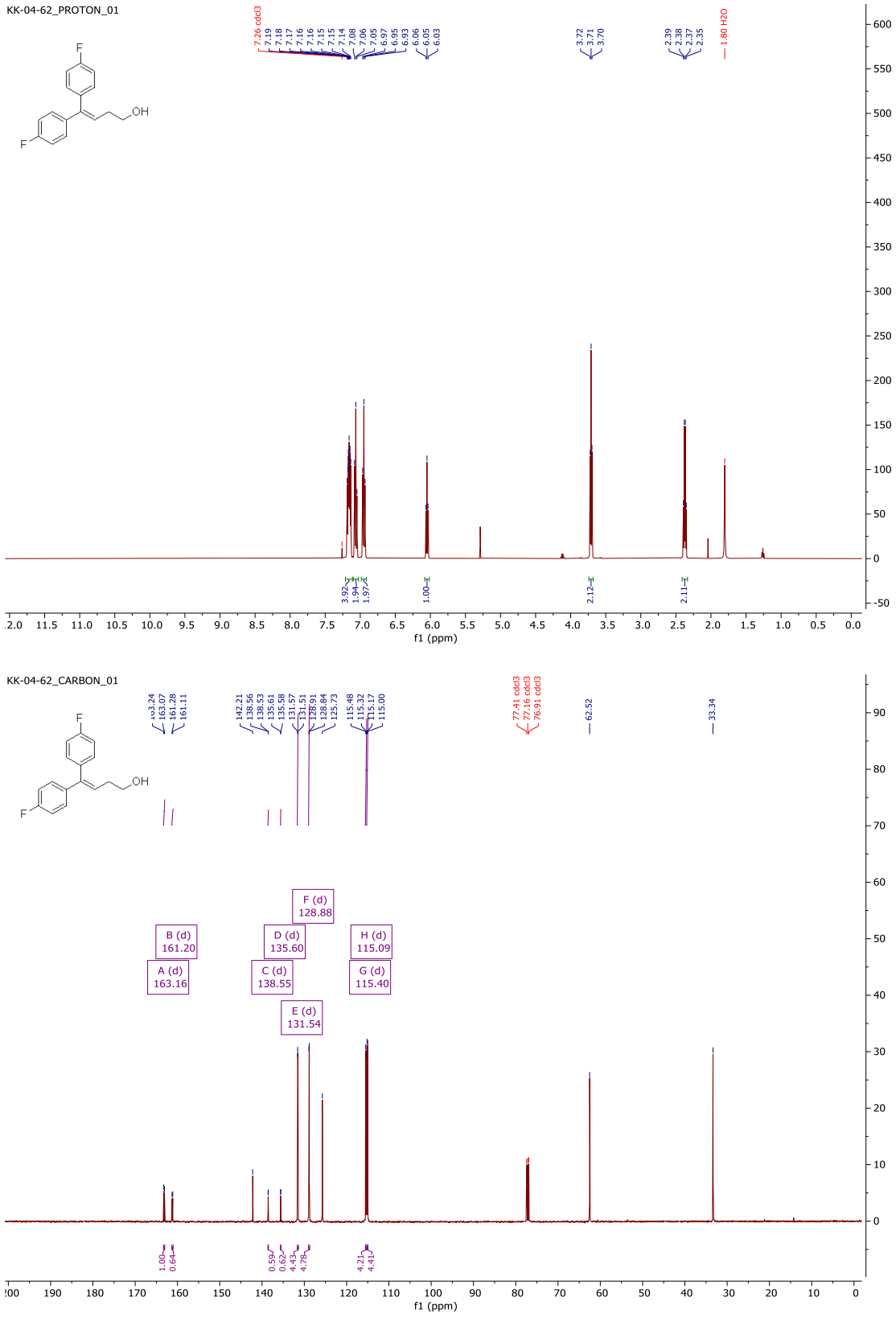


Figure 5.11 ^{1}H and ^{13}C NMR spectra of compound **5-5**

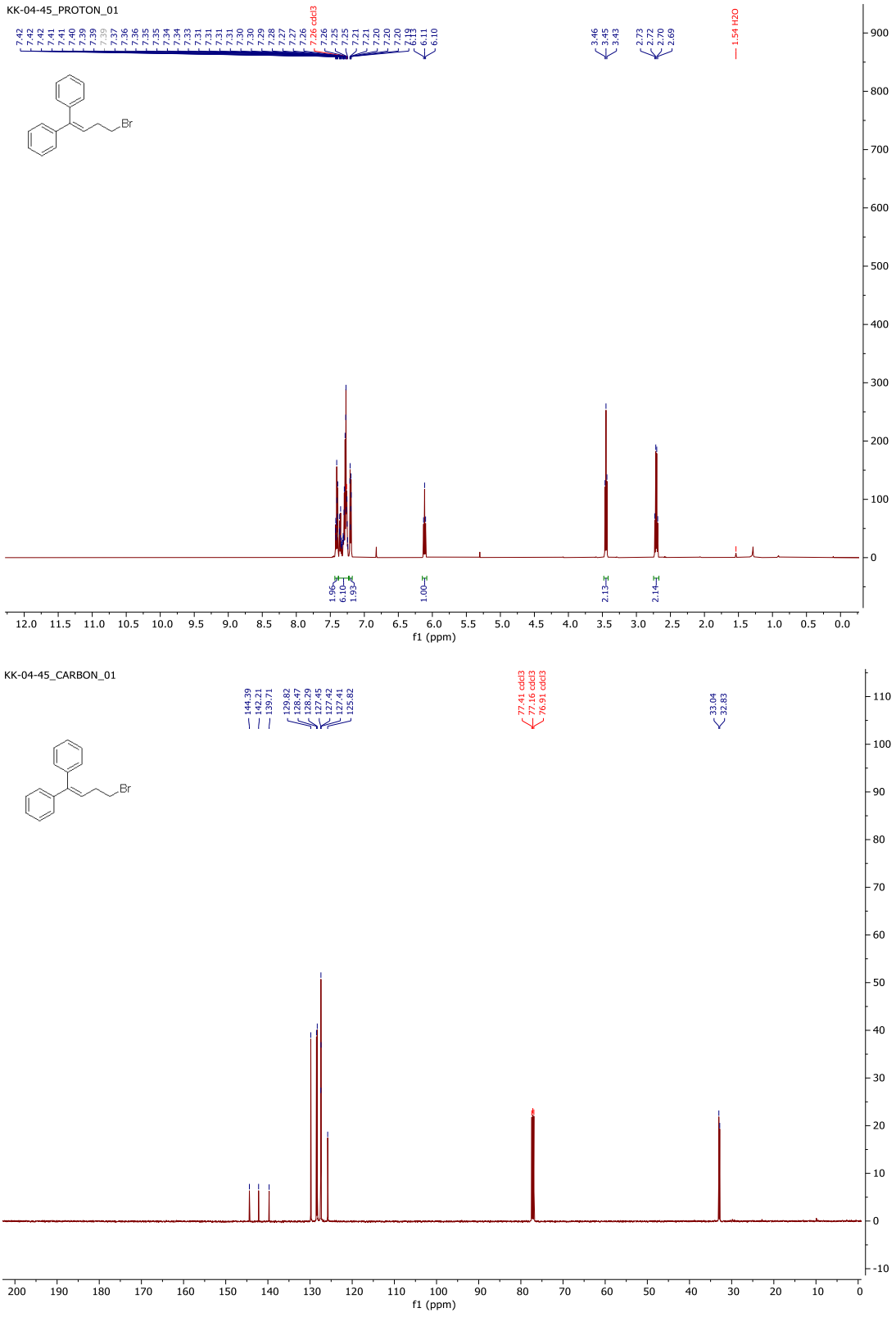
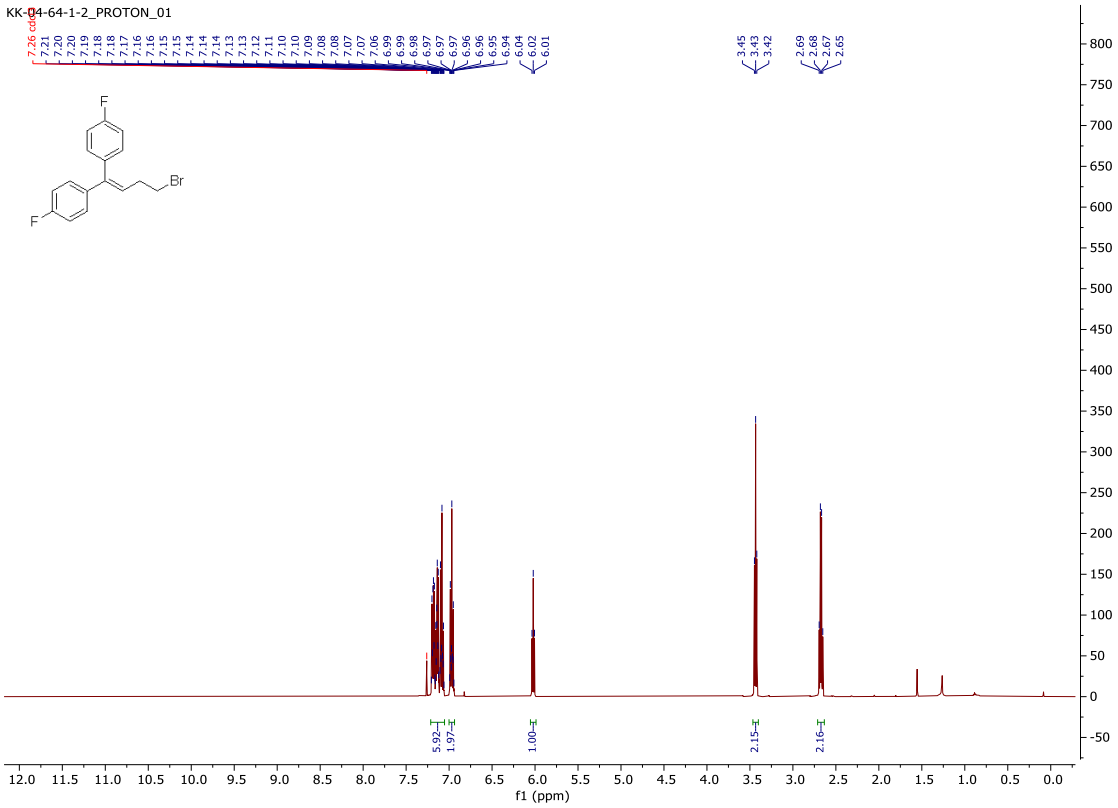


Figure 5.12 ^{1}H and ^{13}C NMR spectra of compound **5-6**



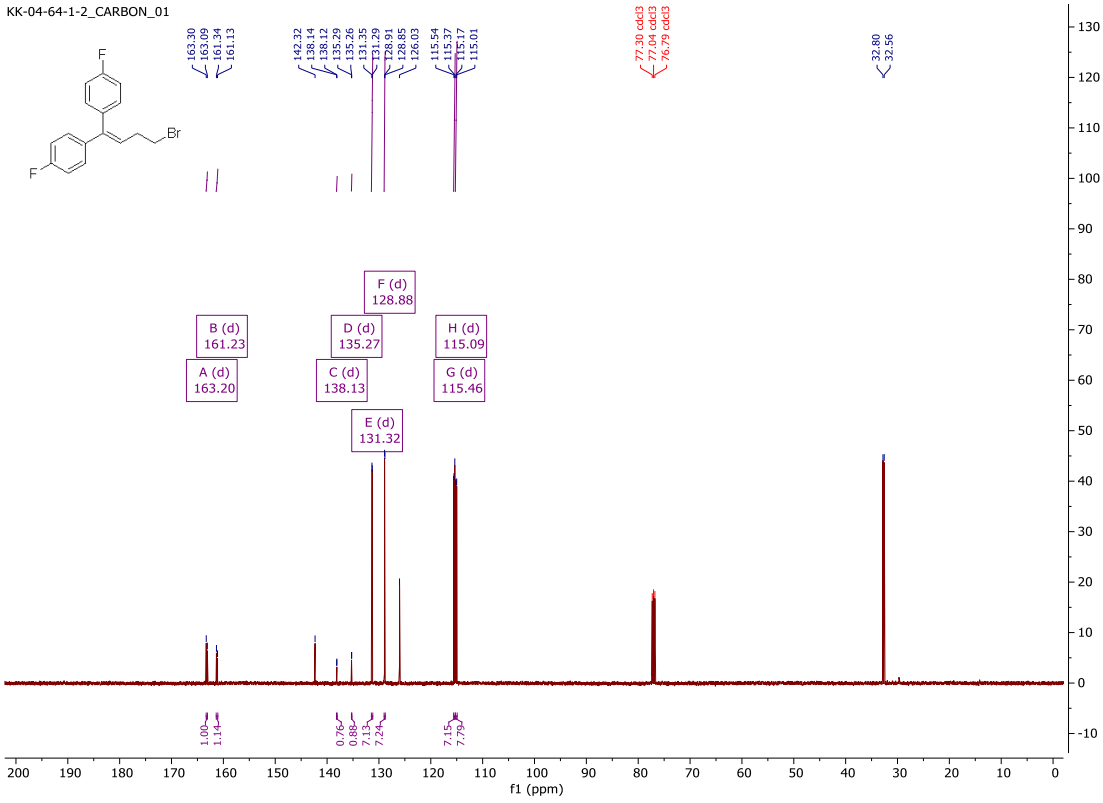


Figure 5.13 ^{1}H and ^{13}C NMR spectra of compound **5-7**

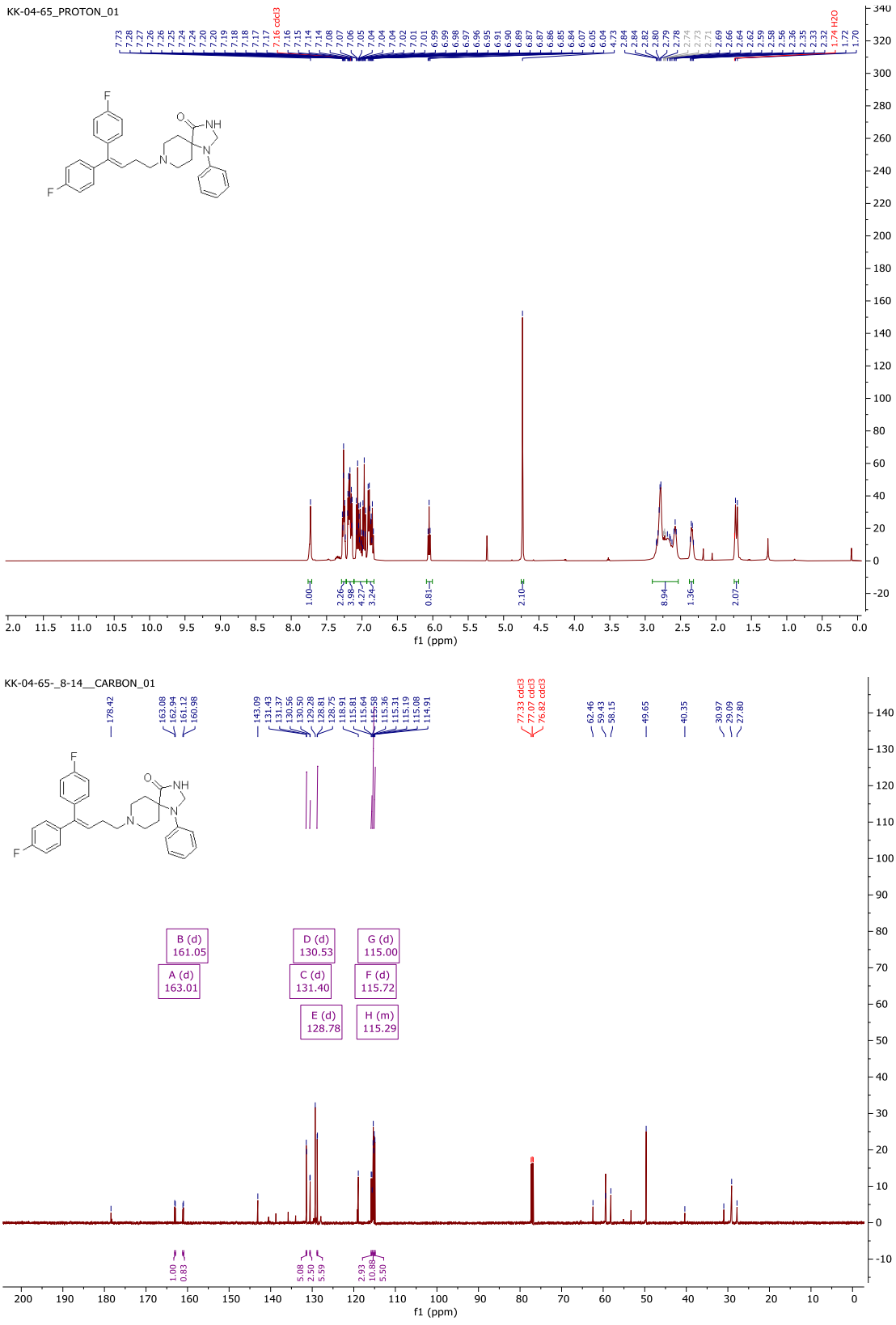
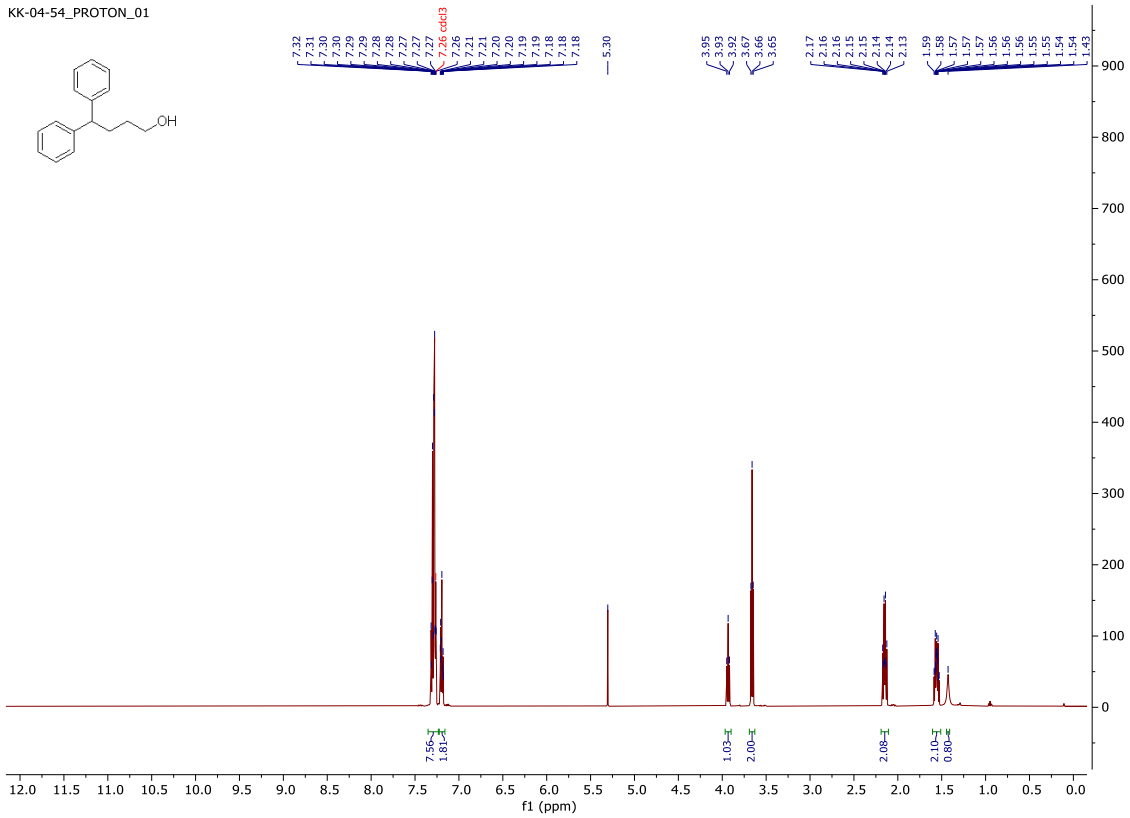


Figure 5.14 ^{1}H and ^{13}C NMR spectra of compound **5-8**



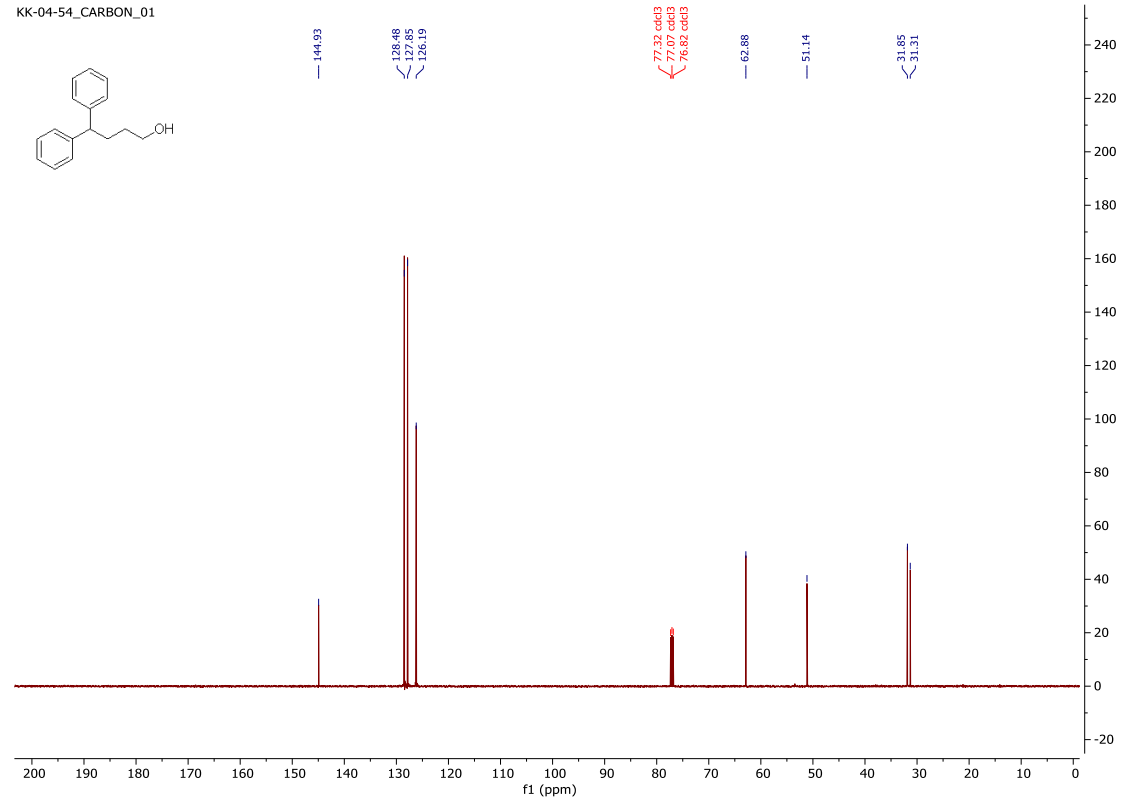


Figure 5.15 ^{1}H and ^{13}C NMR spectra of compound **5-9**

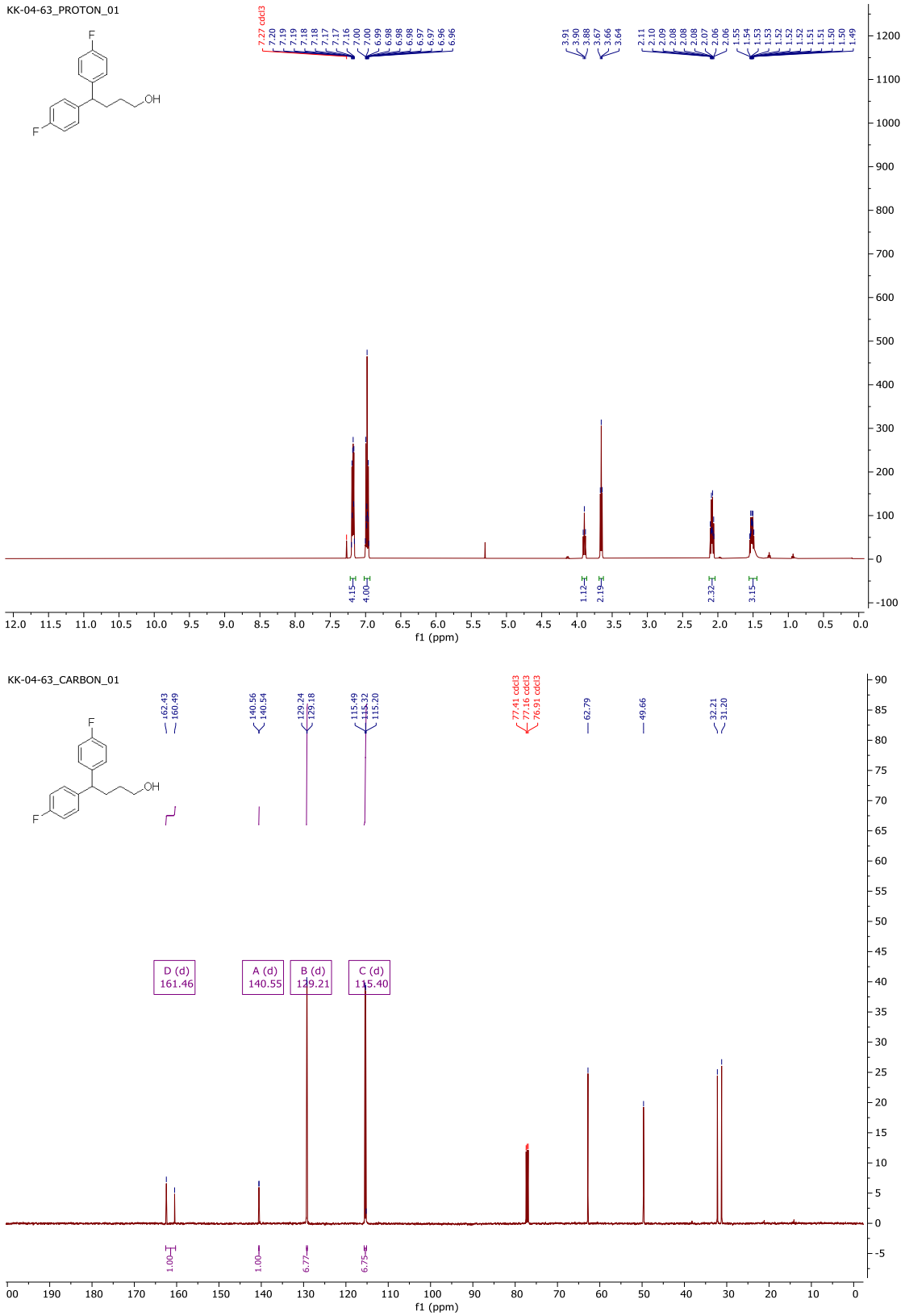


Figure 5.16 ^{1}H and ^{13}C NMR spectra of compound **5-10**

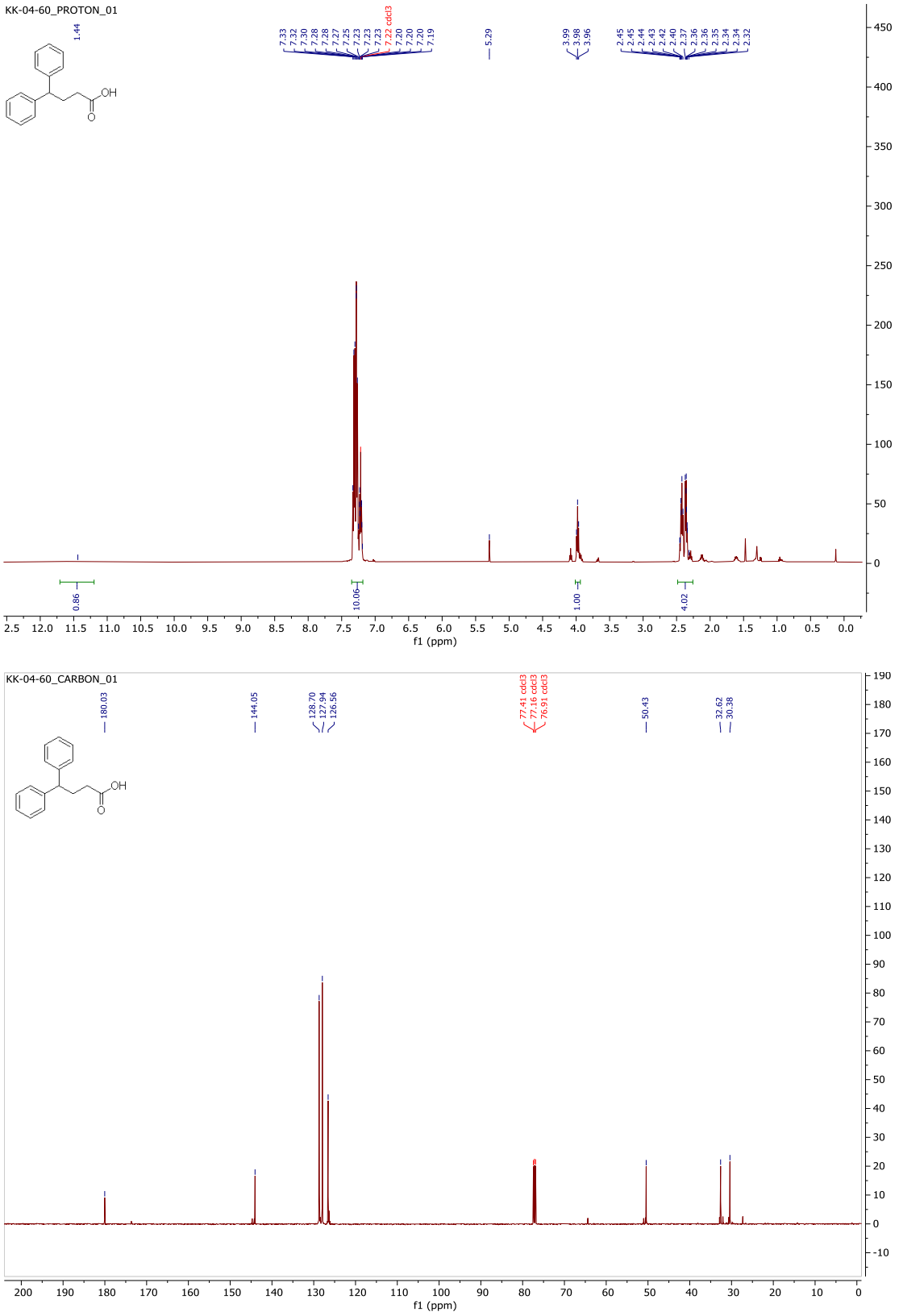


Figure 5.17 ^{1}H and ^{13}C NMR spectra of compound **5-11**

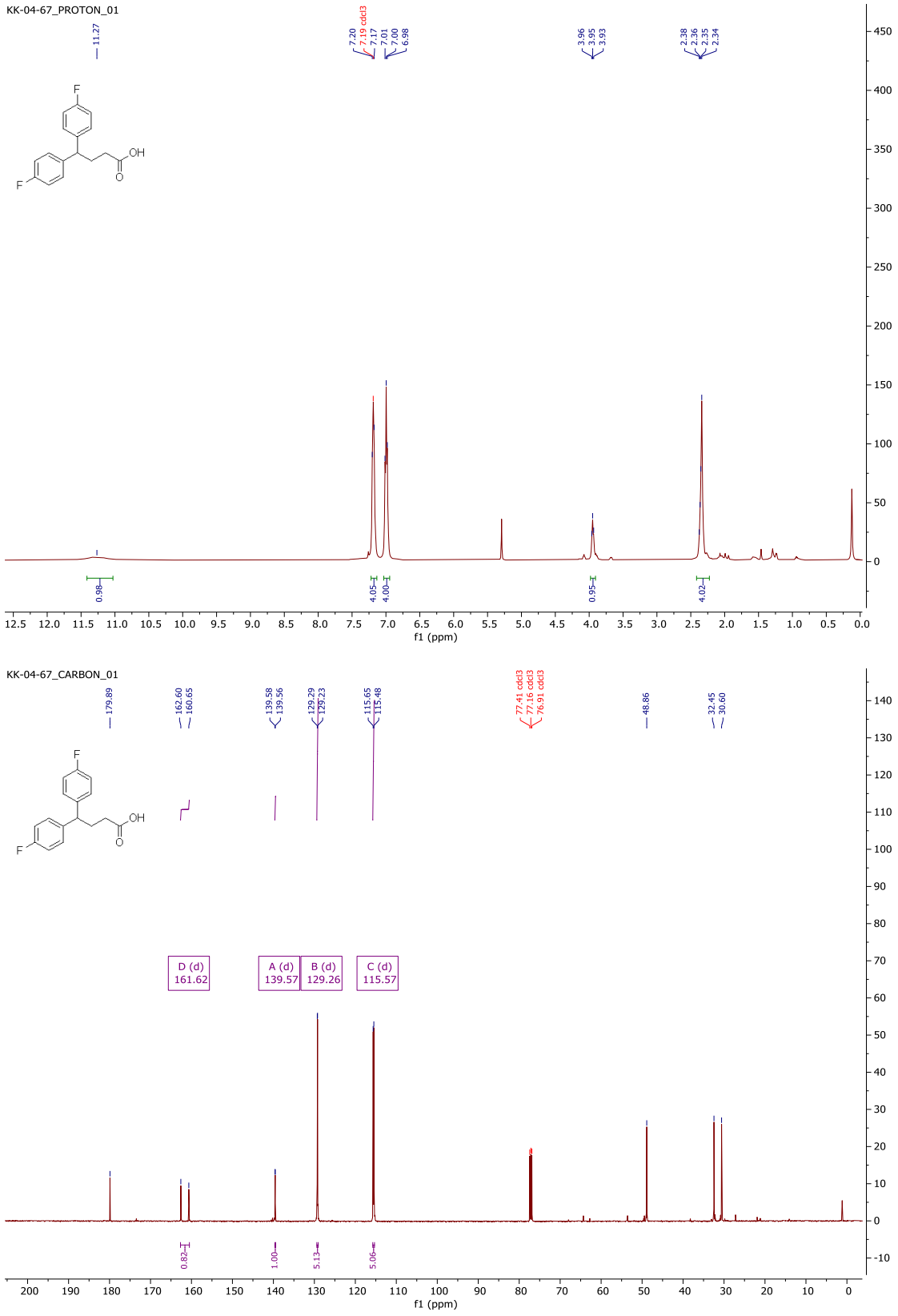
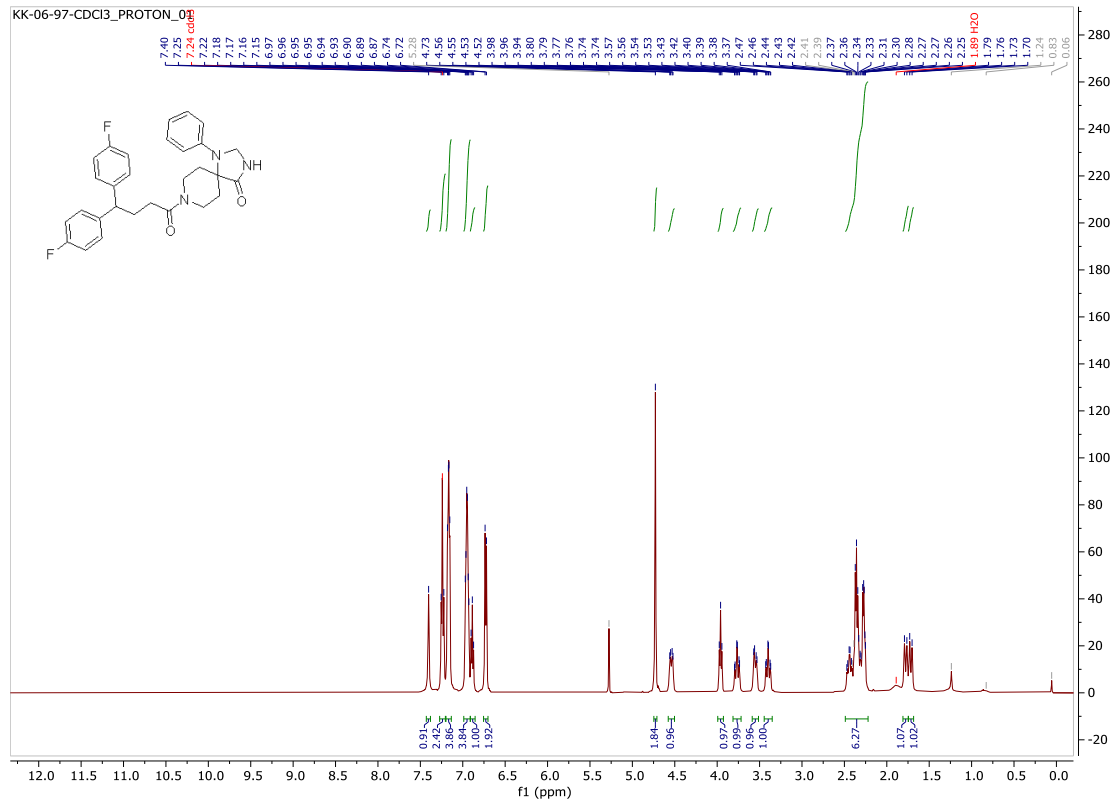


Figure 5.18 ^{1}H and ^{13}C NMR spectra of compound **5-12**



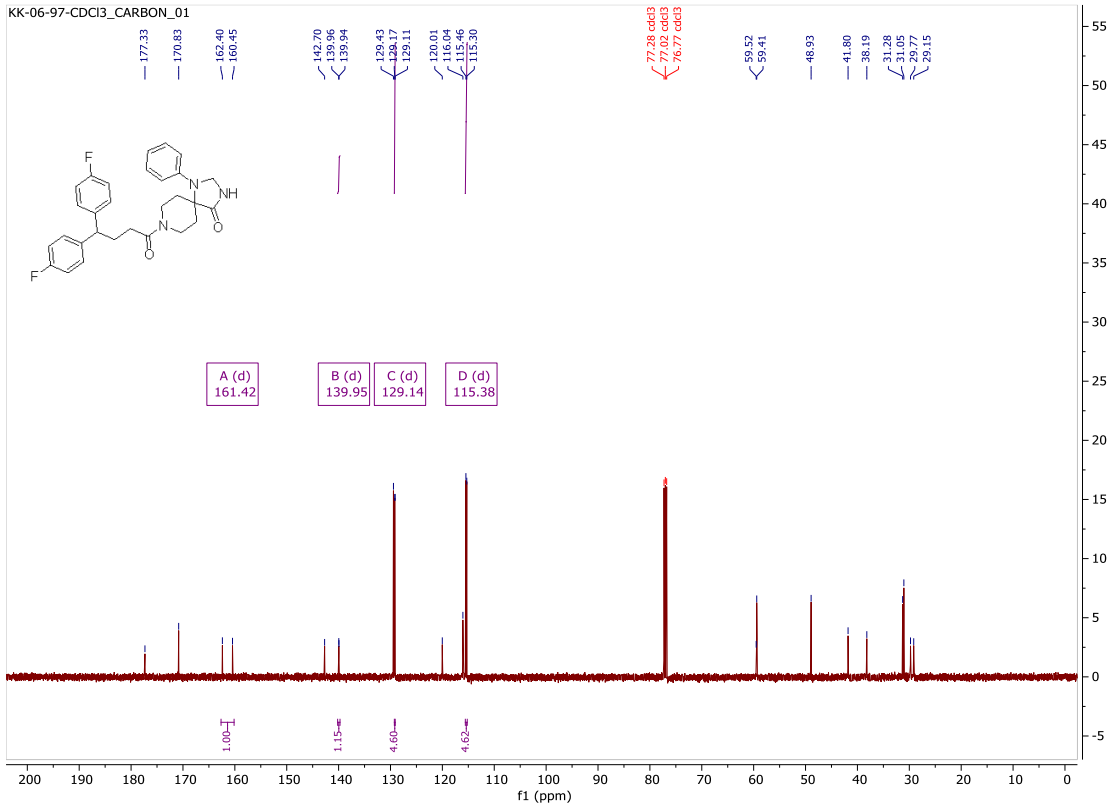
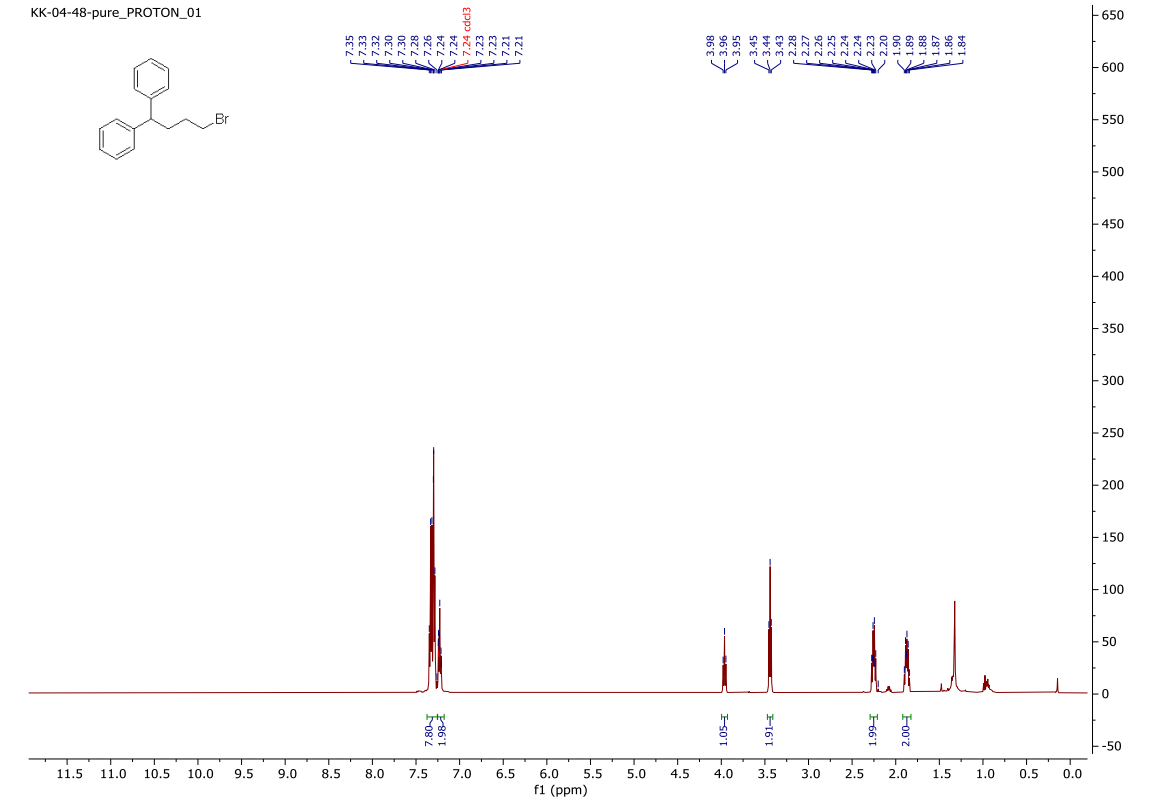


Figure 5.19 ^{1}H and ^{13}C NMR spectra of compound **5-13**



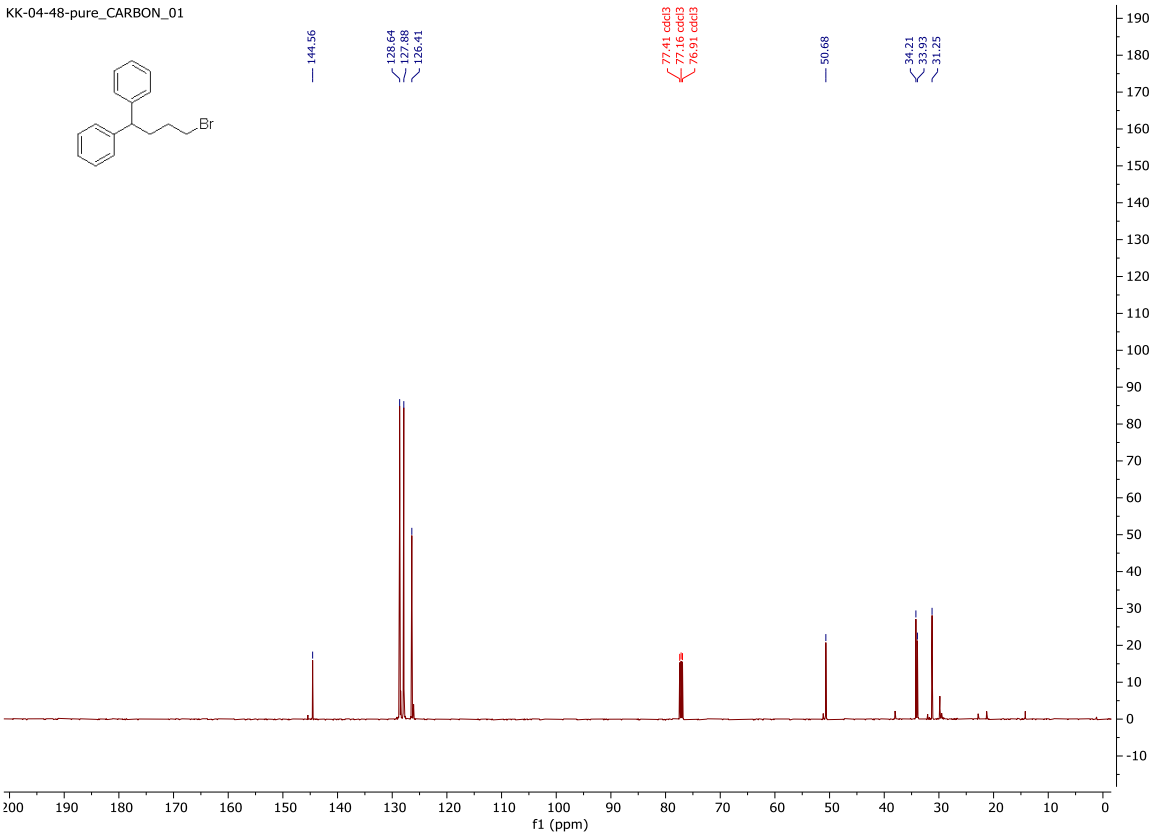
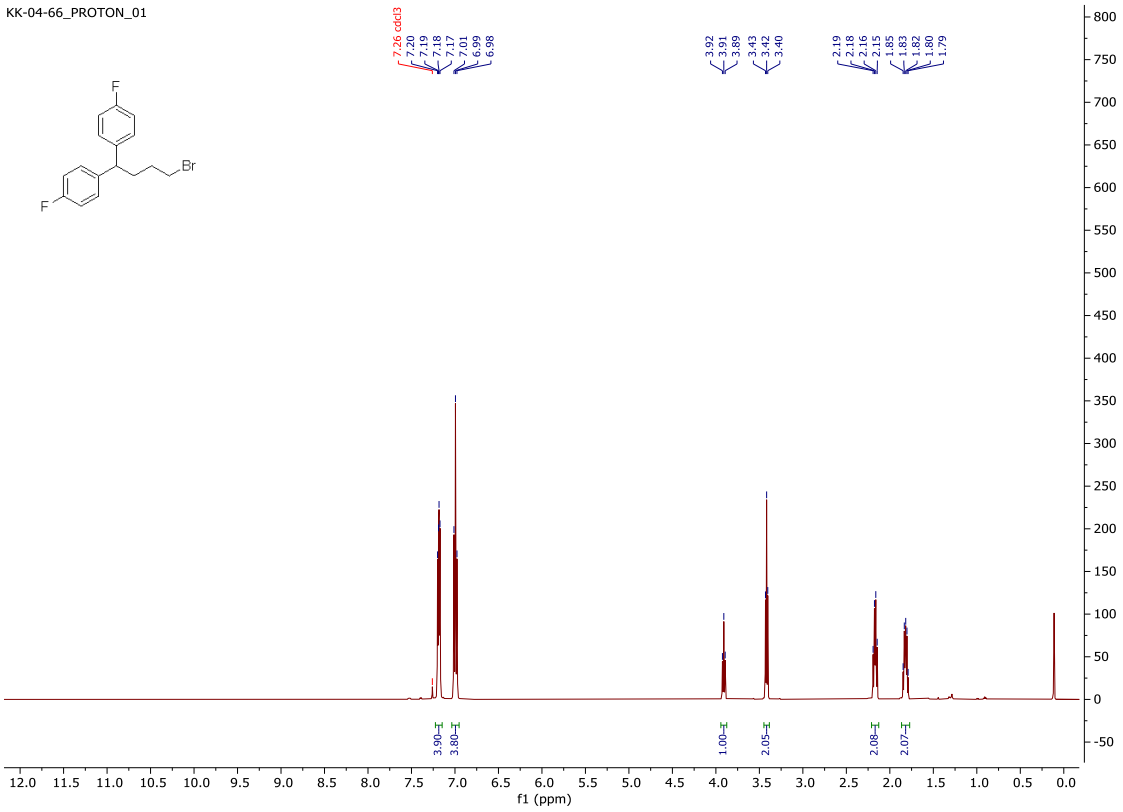


Figure 5.20 ^{1}H and ^{13}C NMR spectra of compound **5-14**



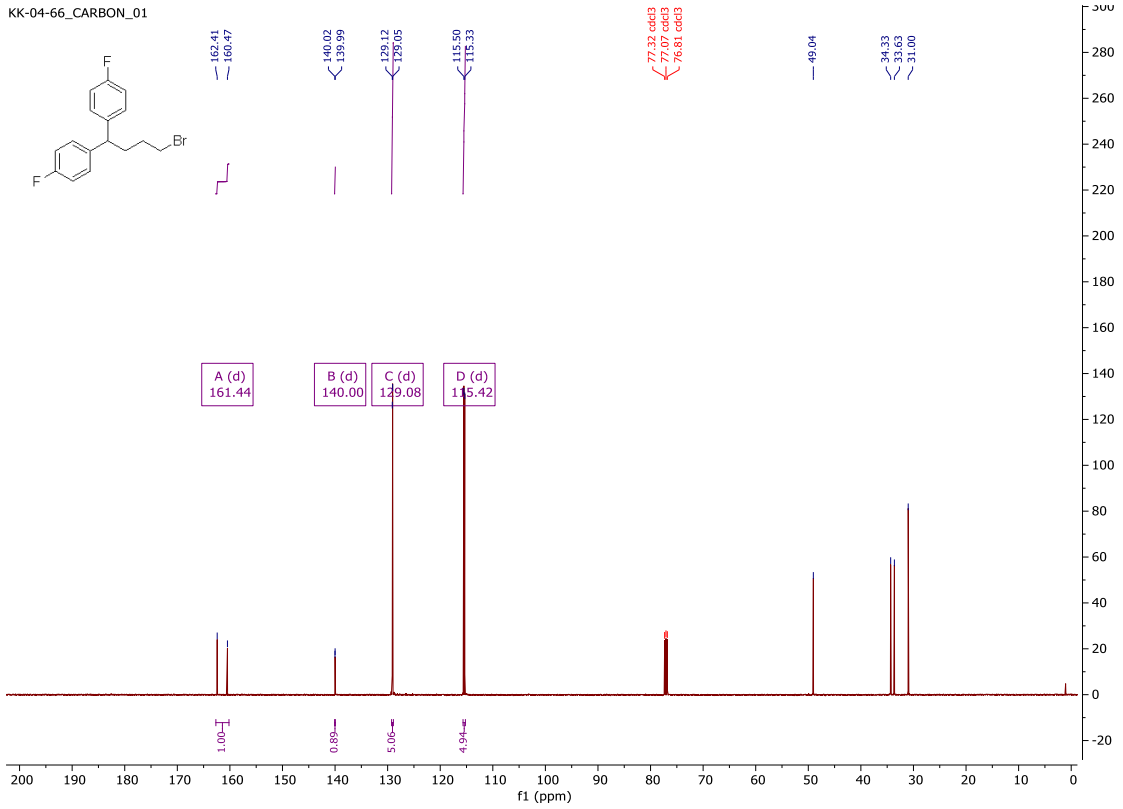


Figure 5.21 ^{1}H and ^{13}C NMR spectra of compound **5-15**

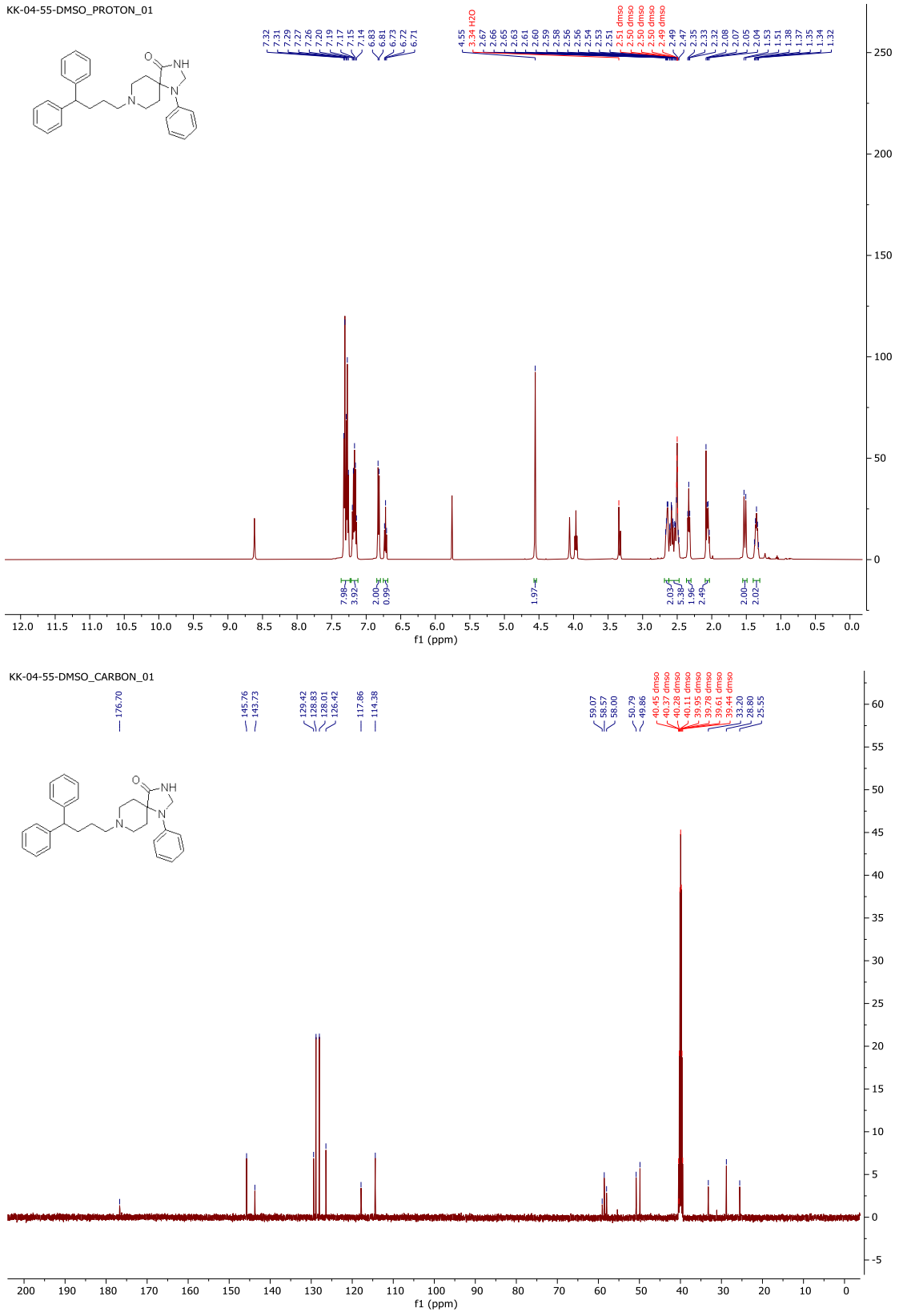
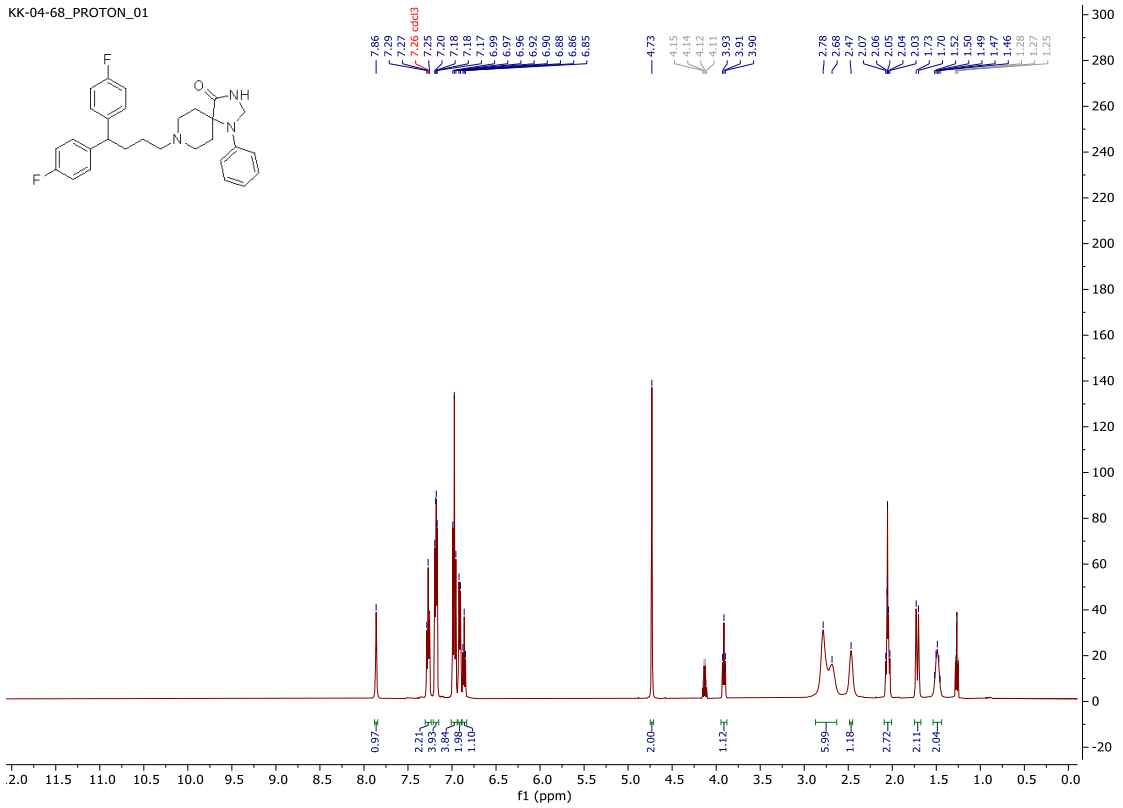
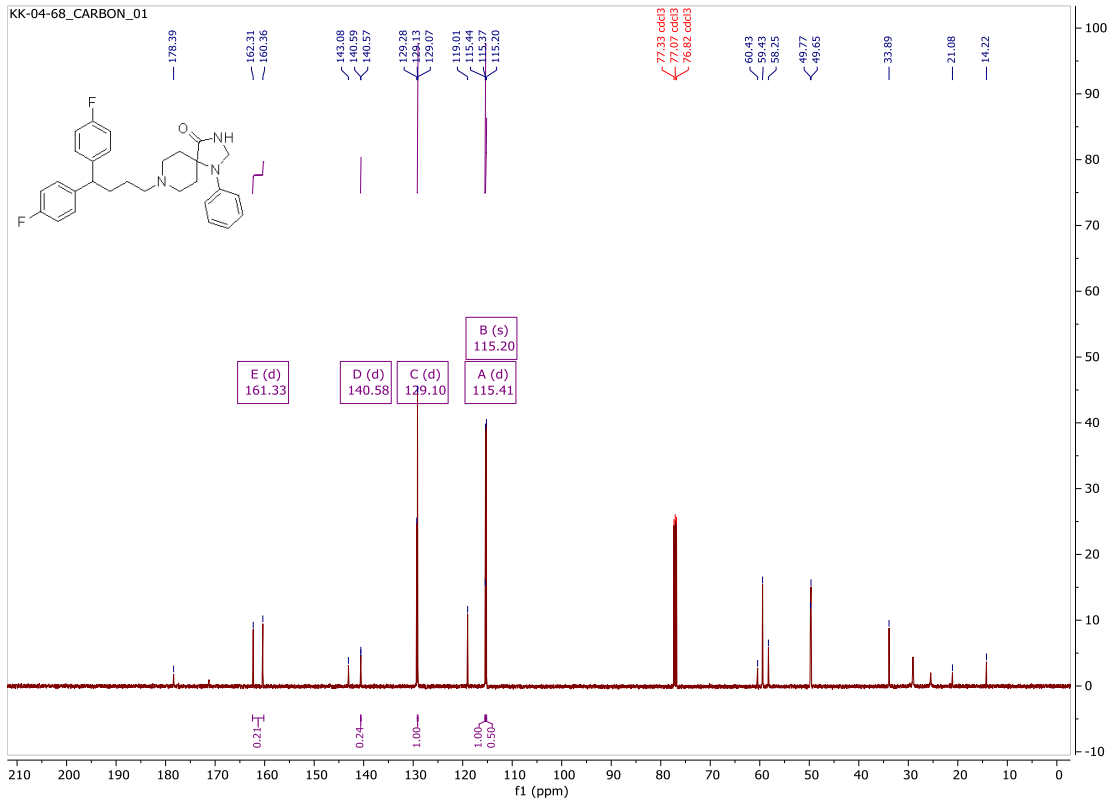


Figure 5.22 ^{1}H and ^{13}C NMR spectra of compound **5-16**





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6 Chapter 6: Conclusion and future works

This dissertation covers the work I completed while in the PhD program in Dr. Jetze Tepe's lab at Michigan State University. My work focused mainly on (4*H*)-imidazol-4-ones and their application in both the first total synthesis of nortopsentin D and small molecule design for proteasome activation. Herein, several new, but under-developed methods for the preparation of imidazol-4-ones were highlighted. While incomplete, these methods have the potential to lead to new preparative methods for the synthesis of (4*H*)-imidazol-4-ones and cyanamides, if further assessed. Additionally, this exploration inspired a retrosynthetic plan for the first total synthesis of nortopsentin D.

The first total synthesis of nortopsentin D was accomplished in 7 linear steps with an overall yield of 1.6%. Development of this total synthesis went through several iterations of retrosynthesis, with the final pathway involving a condensation of amidine and dione, followed by cyclization via pinacol-like rearrangement. While this convergent method had never been attempted before for the synthesis of a natural product, it can be envisioned for use in several other total syntheses of 5,5-disubstituted-imidazol-4-one containing natural products.

Lastly, a range of (4*H*)-imidazol-4-ones were highlighted as potential new leads for the treatment of neurodegeneration through proteasome activation. Specifically, one scaffold shows great promise as a proteasome activator: *N*-acylated fluspirilene. This small molecule represents the Tepe lab's first scaffold to dock within the α2-3 intersubunit pocket of the proteasome and cause a significant increase in IDP degradation *in vitro* (AC_{200} 1.9 μM, max fold enhancement >2000%) and in cells. Several proposed modifications to the scaffold have been designed via molecular docking studies. As future work for this project, these modifications should be

synthesized and tested within our lab to produce a more potent α2-3 intersubunit pocket proteasome activator.