PALACKÝ UNIVERSITY OLOMOUC

FACULTY OF SCIENCE

Department of Chemical Biology

(Un)Natural product synthesis: From phenolics and nitro fatty acids to unnatural α-amino acids

A Thesis Submitted for the Degree of Doctor of Sciences

Daniel Chrenko

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Bibliografická identifikace

Jméno a příjmení autora	Mgr. Daniel Chrenko
Název práce	Syntéza (ne)přírodních látek: Od fenolických látek a nitro mastných kyselin k nepřirozeným α-aminokyselinám.
Typ práce	Disertační práce
Pracoviště	Katedra Chemické biologie Přírodovědecká fakulta, Univerzita Palackého v Olomouci
Vedoucí práce	doc. RNDr. Jiří Pospíšil, Ph.D.

Abstrakt Předložená doktorská práce se primárně zabývá syntézou přírodních látek, zejména pak sekundárních metabolitů rostlin, látek přírodním látkám obdobných, studiem jejich biologické aktivity a v neposlední řadě také vývojem syntetických metod jež by přípravu těchto látek umožnily. Konkrétně se v práci zabýváme přípravou fenolických sekundárních metabolitů a jejich derivátů s neolignanovým benzofuranovým skeletem u kterých je očekávána anthelmintická a antiparkinsonová aktivita. Následně se zabýváme syntézou nitro mastných kyselin a vývojem syntetických metod umožňujících jejich stereoselektvní přípravu. Ve třetí části se zabýváme přípravou heteroarylsulfonamidů a z nich následně odvozených heteroaryl alkyl amino kyselin. Tato příprava je založena na konceptu paměti chirality.

Klíčová slova	sekundární metabolity; přírodní produkty; syntetické metody; paměť chirality; olefinační metody; neolignany; nitrované mastné kyseliny; aminokyseliny
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Supervisor	assoc. prof. Jiří Pospíšil, Ph.D.

Abstract The submitted thesis is primarily concerned with the synthesis of natural products, especially secondary metabolites of plants, substances similar to natural substances, the study of their biological activity and last but not least the development of synthetic methods that would enable the synthesis of these substances. Namely, we are concerned with the preparation of phenolic secondary metabolites and their derivatives with a neolignane benzofuran skeleton, which are expected to have anthelmintic and antiparkinsonian activity. Subsequently, we focus on the synthesis of nitro fatty acids and the development of synthetic methods that enable their stereoselective preparation. In the third part, we deal with the preparation of heteroaryl sulfonamides and the heteroaryl alkyl amino acids derived from them. This preparation of heteroaryl alkyl amino acids is based on the concept of memory of chirality.

Keywords	secondary metabolites; natural products; synthetic methods; memory of chirality; olefination methods; neolignans; nitro fatty acids; amino acids
Number of pages	286
Number of appendixes	4
Language	English

I hereby declare that I have written this Thesis independently and listed all literature sources.

In Olomouc, Daniel Chrenko

„I have no special talent. I am only passionately curious.“

Albert Einstein

Acknowledgement

It would never have been possible to complete this thesis without the help and support of numerous people. First and foremost, I would like to express my heartfelt gratitude to Jiří Pospíšil for offering me the opportunity to join his research group four years ago. He has been an exceptional supervisor, always available when needed the most. Over these four years, he dedicated significant time to help, advise, and teach me, not only in chemistry but also on a personal level. He never said no to my crazy ideas, and for that, I am immensely grateful.

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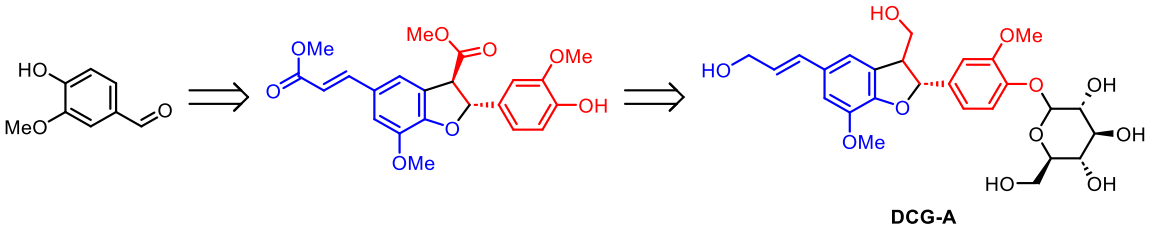
Table of contents

Aims of the Thesis

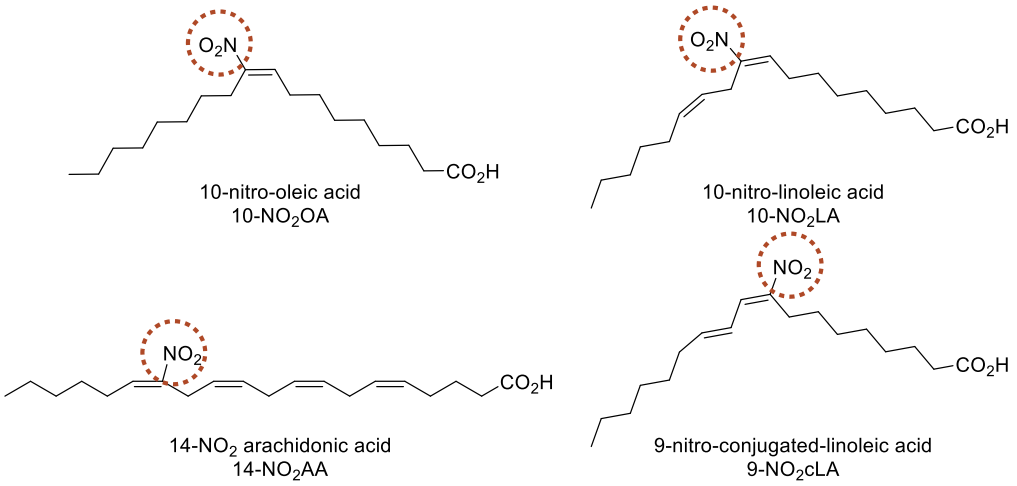
My thesis focuses on two main fields, natural product synthesis and synthetic method development. Throughout the work, the two themes are intertwined and constantly refer to each other and one inspires the other... And now it is up to me to attempt the almost impossible: to retell the story of my Ph.D.

My first interest was in the synthesis of a well-established class of plant secondary metabolites, phenolic compounds with a phenylpropanoid-dimer skeleton, more precisely, the compounds inspired by dehydrodiconiferyl alcohol glucoside (**DCG-A**). Compounds that contain a benzofuran core in its structure and that are well known for their biological activities that range from anthelmintic, anticancer, and antiviral.



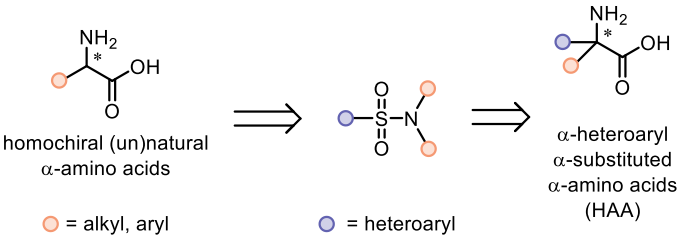
DCG-A

My second interest was in the synthesis of novel classes of natural products (nitro fatty acids) that presented a new synthetic challenge, stereodefined synthesis of multiple 1,2-di and trisubstituted alkenes. To achieve such a goal, a new synthetic pathway toward NO_2 fatty acids (NO_2FA) and 1,2-disubstituted alkenes had to be developed. We succeeded and as "the imaginary cherry on top of the cake," we capitalized on the gathered experience in the first total synthesis total synthesis of 14-NO_2 arachidonic acid.



Aims

Finally, our attention focused on the development of novel methodologies designed to prepared previously inaccessible heteroarylsulfenamides, structural motive that is commonly exploited in the medicinal chemistry field. For us the class of heteroaryl sulfones was dare for different reasons, we wished to apply it in the context of the concept of 'memory of chirality' to prepare a novel class of amino acids (α-heteroaryl α-substituted α-aminoacids).



In short, my thesis led me from phenylpropanoids based secondary metabolites synthesis, to the synthesis of nitro fatty acids, potential nuclear factor erythroid 2-related factor 2 (Nrf2) activators; and at the same time, the synthetic endeavors connected with stereoselective olefin synthesis, a topic so dear to my supervisor, led me *via* heteroarylsulfonamide synthesis to a new type of unnatural amino acids that could be used in the future as organocatalysts or be incorporated in DNA to form a novel Xeno nucleic acids.

This 'development' of my research then obviously predetermines the content of my thesis that, as a consequence, is divided into three separate parts. **1**) Neolignans, **2**) Nitro fatty acids, **3**) Sulfonamides and novel amino acids.

Abbreviations

°C = degree Celsius
2,6-lutidine = 2,6,-dimethylpyridine
AA – amino acids
Ac = acetyl
AcCN = acetonitrile
Ag_2O = silver oxide
$AgCO_3$ = silver nitrate
$AgNO_2$ = silver nitrite
AgOTf = silver trifluoromethanesulfonate
Ar = aryl
ARA = arachidonic acid
BBr_3 = boron tribromide
BCl_3= boron trichloride
BF_3= boron trifluoride
Bn = benzyl
BT = benzo[*d*]thiazol
Bz = benzoyl
CAL-B = *Candida antartica* lipase B
cat. = catalytic
CBr_4 = carbon tetrabromide
CuOTf = Copper(I) trifluoromethanesulfonate
d = doublet
d.r. = diastereomeric ratio
DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene
DCC = N,N'-dicyclohexylcarbodiimid
DCE = 1,2-dichloroethane
DCG-A = dehydrodiconiferyl alcohol glucoside
DCM = dichloromethane
DFT = density functional theory
DHB = 2,3-dihydrobenzofuran
DIAD = diisopropyl azodicarboxylate
DIBAL-H = diisobutyl aluminium hydride
DIC = N,N'-diisopropylcarbodiimide
DIPEA = N,N-diisopropylethylamine
DMAP = 4-dimethyl aminopyridine
DMB = 2,4-dimethoxybenzyl
DMF = dimethylformamide
DMP = Dess-Martin reagent
DMSO = dimethylsulfoxide
e.r. = enantiomeric ratio
equiv. = equivalent
Et_2O = diethylether
EtOAc = ethyl acetate
EtOH = ethanol
EWG = electron withdrawing group
FAs = fatty acids
$FeCl_3$ = Iron trichloride
GC = Gas chromatography
h = hour
H_2O_2 = hydrogen peroxide
HAA = α-heteroaryl α-substituted α-amino acids
HCl = hydrochloric acid
HMPA = hexamethylphosphoramide
HPLC = high-performance liquid chromatography
HRMS = high resolution mass spectroscopy
HRP = horseradish peroxidase
iPrOH = isopropanol
J = coupling constant
K_2CO_3 = potassium carbonate
KHMDS = potassium bis(trimethylsilyl)amide
LA = linoleic acid
LC-MS = Liquid chromatography–mass spectrometry
LDA = lithium diisopropylamide
LiHMDS = lithium bis(trimethylsilyl)amide
LiOH = Lithium hydroxide
m = multiplet
Me = methyl
MeCN = acetonitrile
MeOH = methanol
$MgBr_2$ = magnesium bromide
$MgSO_4$ = magnesium sulfate
min. = minute
MOM = methoxymethyl
MS = mass spectroscopy
MTBE = methyl *t*-butyl ether
n.r. = no reaction
Na_2SO_4 = sodium sulfate

NaHMDS = sodium bis(trimethylsilyl)amide
NaI = sodium iodide
NaOH = sodium hydroxide
NaOMe = sodium methoxide
NCS = N-chlorosuccinimide
NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells
NH_3 = ammonia
NMR = Nuclear magnetic resonance spectroscopy
NSAID = Nonsteroidal anti-inflammatory drug
Nrf2 = nuclear factor erythroid 2-related factor 2
OA = oleic acid
PE = petroleum ether
PG = protecting group
Ph = phenyl
PPh_3 = triphenylphosphine
ppm = parts per million
*p*TSA = *p*-toluenesulfonic acid
py = pyridine
q = quartet
QM = quinone methide
rpm = rotations per minute
RT = room temperature
s = singlet
S.M. = Starting material
SA = stearic acid
SAR = dtructure-activity relationship
SEM = 2-(Trimethylsilyl)ethoxymethyl
SiO_2 = Silica (silicon dioxide)
$SnCl_4$ = tin chloride
t = triplet
TBAF = tetrabutylammonium fluoride
TBS = *t*-butyldimethylsilyl
TEA = triethylamine
TES = triethylsilyl
TFA = trifluoroacetic acid
THF = tetrahydrofuran
TIPS = triisopropylsilyl
TLC = thin layer chromatography
TMS = trimethylsilyl
OTf = trifluoromethane sulfonate
UFAs = unsaturated fatty acids
$ZnBr_2$ = zinc bromide

CHAPTER I

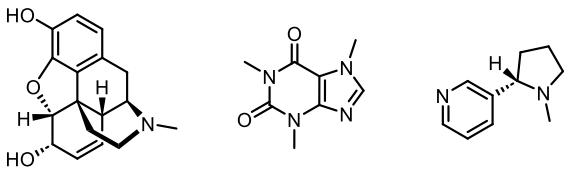
1. Secondary metabolites

1.1. Introduction

Plant secondary metabolites are a diverse group of organic compounds produced by plants and other living organisms, such as bacteria, fungi, and plants, which are not strictly necessary for their survival. In other words, they are not included in elemental processes such as growth, development, and reproduction. Such compounds are not essential for survival of the organism, but they play a key role in the organism's interactions with its environment (e.g. defense against herbivores, pathogens, and competitors, as well as communication with other organisms, etc.). And even though such function is not essential to the organism, it greatly increases quality of life, chances of reproduction, or chances of survival when facing to hostile environment pollinators and/or symbiotic partners.[1]

In general, secondary metabolites are divided, based on metabolic origin, into three large groups, alkaloids, terpenoids, and phenolic compounds.

1) Alkaloids: Nitrogen-containing compounds, often with a strong physiological effect on mammals. The most known alkaloids are caffeine, morphine, and nicotine (**Figure 1**). Alkaloids often function as defense compounds, deterring herbivores and pathogens due to their bitter taste and, in the extreme case, can even be lethal to the attacker due to their (neuro)toxic properties.[2]



morphine **1-1** caffeine **1-2** nicotine **1-3**

Figure 1: Structure of most known alkaloids.

2) Terpenoids (or isoprenoids): Compounds that originate from the five-carbon subunits of isoprene. Terpenoids participate in various functions, such as plant protection, signaling, or pollination. Examples of those include essential oils (limonene and menthol), carotenoids (β-carotene) and plant hormones (gibberellins and abscisic acid) (**Figure 2**).[3]

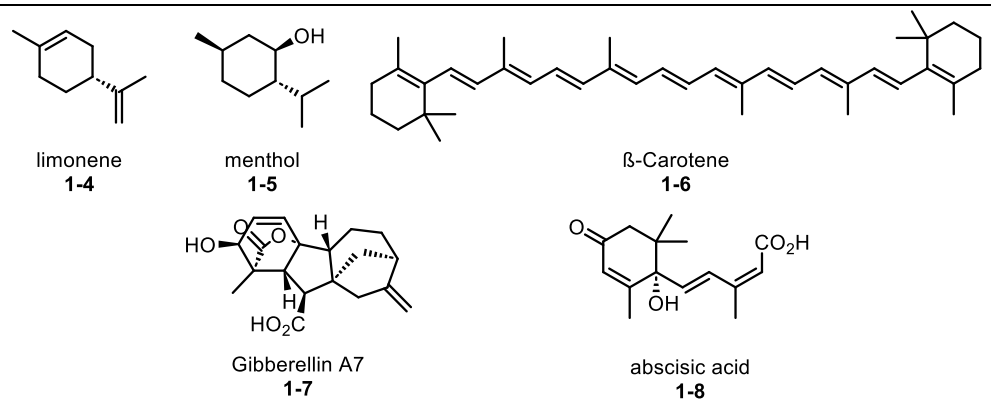


Figure 2: Structure of most known terpenoids.

3) Phenolic compounds: Group of compounds that include one- or more-phenyl subunit with one or more hydroxy groups. Phenolic compounds play a role in plant defense, signaling, and protection against ultraviolet light. Examples include flavonoids (anthocyanins and quercetin), lignans (sanguinolignan A) and neolignans (dehydrodiconiferyl alcohol 4-O-β-D-glucopyranoside), and most importantly lignin, a structural component of plant cell walls (**Figure 3**).[4,5]

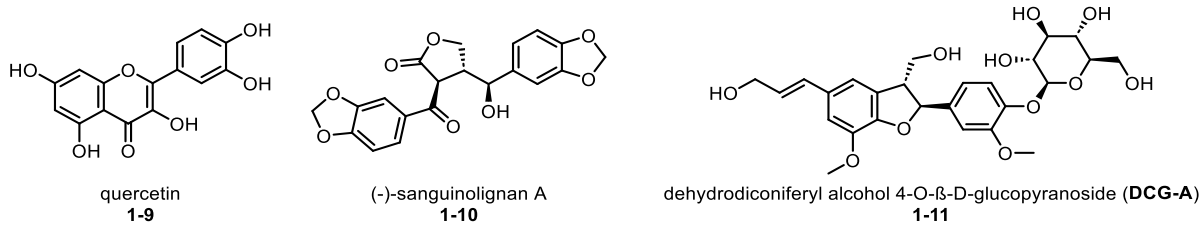


Figure 3: Structure of phenolic compounds.

At this point, I would like to emphasize that (mostly) plant secondary metabolites were extensively used by humans for various purposes mostly related to the healing processes over the past few thousand years, as can be demonstrated, e.g., on the use of salicylic acid (aspirin) to treat the feaver.[6] They are also often the basis for traditional medicines, and still now a days they serve as a key starting point during the development of new drugs.[5]

1.2. Phenylpropanoids

1.2.1. Introduction of phenylpropanoids

In plants, lignans and neolignans are formed by oxidative coupling of the hydroxycinnamic acid derivatives, these structures are formed by a β,β'-linkage between two units of phenylpropanoid. These units can have different side chains (C7-C9) in different oxidation states and vary in the substitution of the aromatic ring (C1-C6). For nomenclature purposes, phenylpropanoid monomers are typically numbered C1-C6 for the aromatic ring and C7-C9 for the propyl chain, with C1 and C7 atoms attached by covalent bond. In the second phenylpropanoid monomer, the numbering follows the same pattern, but with primed numbers (notated with a prime symbol, '). In the context of lignans and neolignans, the numbers mentioned (e.g., C8-C5' or C5-C5') represent the types of carbon-carbon (C-C) bonds formed during the oxidative coupling of two phenylpropanoid units. These numbers correspond to the positions on the aromatic rings where the coupling occurs. The dimerization proceeds *via* the oxidative radical pathway and the generated radical undergoes a further dimerization process. Based on the substitution pattern of the phenylpropanoid unit, the homocoupling occurs at different positions, and various types of dimers are formed. When the word **lignan** is used, a dimer generated by the C8-C8' dimerization process is described and the homodimerization that occurs *via* the C8 positions of both phenylpropanoid units is involved. Products of any other type of dimerization, for example, C8-C5', C5-C5' and C8-C4', are referred to as **neolignans**. The types of dimerization mentioned previously that produce a neolignan skeleton are only a few out of many possibilities, since additional substitution on the aromatic ring of the phenylpropanoid can lead to other possibilities such as, for example, the C8-C5'-dimers (**1-15b**) which are the target dimers of my thesis (**Figure 4**).[5,7]

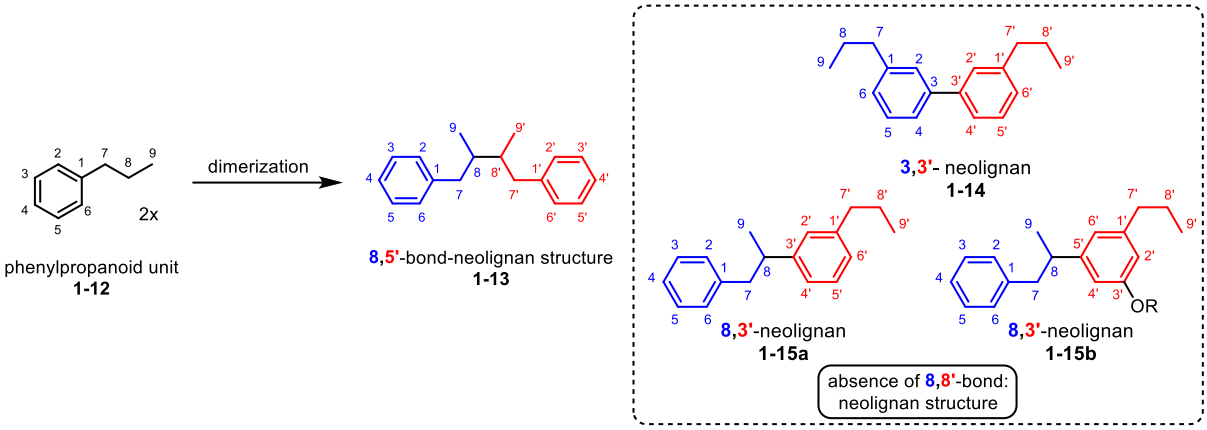
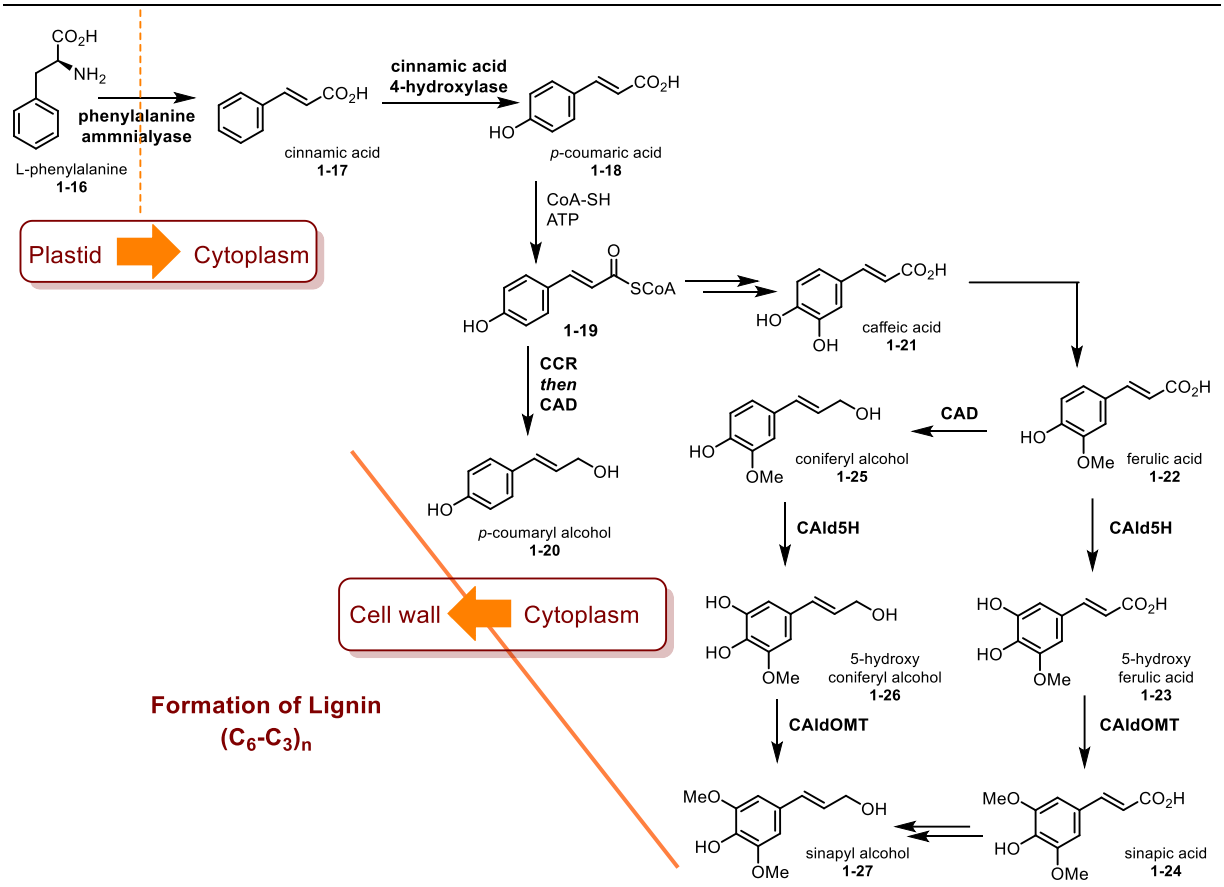


Figure 4: General scheme of neolignan dimerization. Only selected examples of neolignan dimers are shown.[5]

1.2.2. Biosynthesis

Phenolic compounds are plant secondary metabolites that originate in the shikimic acid biosynthesis pathway.[8,9] From a structural point of view, neolignans are homodimers of phenylpropanoids that originate from the metabolism of L-phenylalanine and with the help of enzymes such as phenylalanine ammonia-lyase L-phenylalanine is transformed to cinnamic acid which is then further hydroxylated with the help of cinnamic acid 4-hydroxylase and cytochrome P450 to *p*-coumaric acid (**Scheme 1**).[8] The biosynthesis of neolignans itself is an amazing process since one phenylpropanoid-based building block (*p*-coumaric or ferulic acid) undergoes *O*-methyltransferase-mediated methylation to yield cinnamic acid derivative which can further undergo lactase or peroxidase-triggered homodimerization that can yield only up to three products. However, countless (non)enzymatic transformations such as acid-catalysed cyclization, methylation, and/or oxidation literally open the doors to the world of structurally diverse phenolic secondary metabolites. Consequently, the structure and distribution of neolignans differ from plant to plant, biotope to biotope, and depend on the soil, climate, or exposure of the plants to stress.[5,10]



Scheme 1: Biosynthesis of phenylpropanoid monomers from L-phenylalanine (adapted from[5]).

1.2.3. Dimerization of phenylpropanoids

As demonstrated in the previous chapter, phenylpropanoid dimers (lignans and neolignans) are in plants generated during the oxidative radical dimerization step. This dimerization is mediated by laccases and peroxidases.[5,11,12] During the process configurationally unstable radical species are generated (as an example, the case of the C8-C5'-dimer is shown in **Figure 5**) and final product of the addition/post-addition transformations, the targeted neolignan **1-34** is formed as a racemic mixture of all possible stereoisomers (**Scheme 2**).

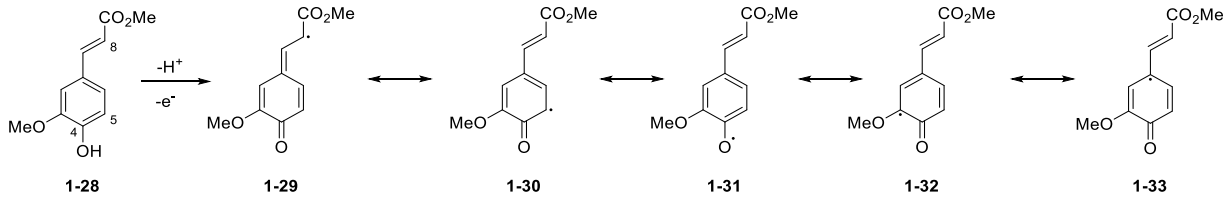
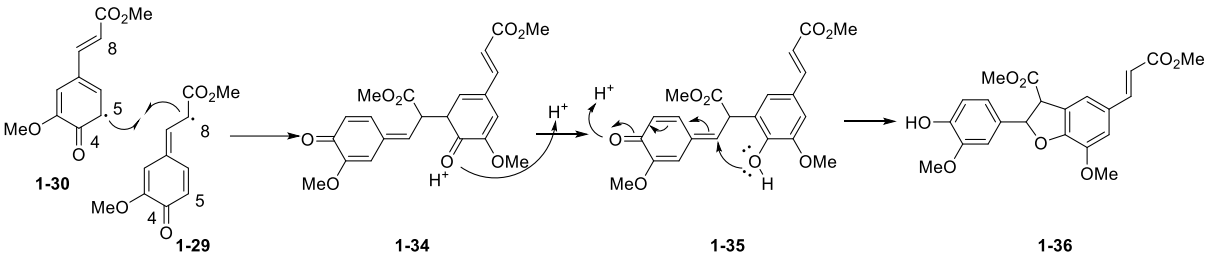


Figure 5: Dehydrogenation of methyl *p*-coumarate to show formation of phenoxy radical.[13]

The stereochemical outcome of plant-based enzymatic oxidative dimerization (many naturally occurring ligands and neolignans are isolated in enantiomerically enriched form) is then influenced by so-called dirigent proteins – proteins that are responsible for the streoisomeric outcome of the dimerization processes (**Scheme 2**).[14]



Scheme 2: 8-5' bimolecular phenoxy radical coupling of a methyl *p*-coumarate to neolignane structure.[15–17]

As mentioned previously, dimerization itself is *in planta* promoted by metalloenzymes containing peroxidases and lactases, iron and copper cantered metalloenzymes. Such information triggered the attention of various synthetic chemists and led to the development of various synthetic methodologies based on the use of Ag(I), Fe(III) or Cu(II) salts (Ag_2O[18], $FeCl_3$[19], $K_3[Fe(CN)_6]$[20]). The utilization of these salts has certain drawbacks, namely their high cost, limited reproducibility, and low reaction yields, accompanied by the formation of various side products. Moreover, the reactions themselves lack regio and stereocontrol that results in low yields of desired adducts and an obvious formation of dimers as racemic mixtures.[16] An alternative way, which is based on the use of oxidizing agents such as H_2O_2 in combination with isolated peroxidases such as horseradish peroxidase (HRP), also brings unsatisfactory results.[21] However, it was demonstrated that the choice of catalyst or oxidizing agent, as well as the reaction conditions (e.g. temperature, solvent, and concentration), can significantly influence the chemical selectivity (e.g., preference of C8-C8' over C8-C5' dimerization) and the yield of the dimerization process. Through careful optimization of these parameters, researchers could not only control the formation of lignan (C8-C8') or neolignane (C8-C5') structures, but also the stereochemistry of the consequent post-coupling reactions.[16,17,21]

1.2.4. Biological activity of neolignans

As mentioned in chapter 1.2., neolignans are a subclass of phenolic compounds originating from other than C8-C8' dimerization homocoupling of phenylpropanoid subunits. In total, plants produce virtually thousands of various structurally different neolignans (15 subtypes)

with a wide range of biological activity.[5,22] They exhibit antimicrobial activity against various bacteria, fungi, and viruses. Their mode of action can involve disrupting the microbial cell membrane, inhibiting the synthesis or function of essential enzymes, or interfere with the synthesis of cellular components, such as proteins or nucleic acids.[23] In some cases, antitumor activity[5] can be attributed to the ability of neolignan to induce apoptosis (programmed cell death), inhibit cell proliferation, and suppress tumor invasion and metastasis.[24] The whole class of such compounds displays a wide range of biological activities that goes far beyond the scope of this Thesis, therefore, I would like to pay attention only to those with the benzofurane-like core skeleton **1-39** (**Figure 6**).[5]

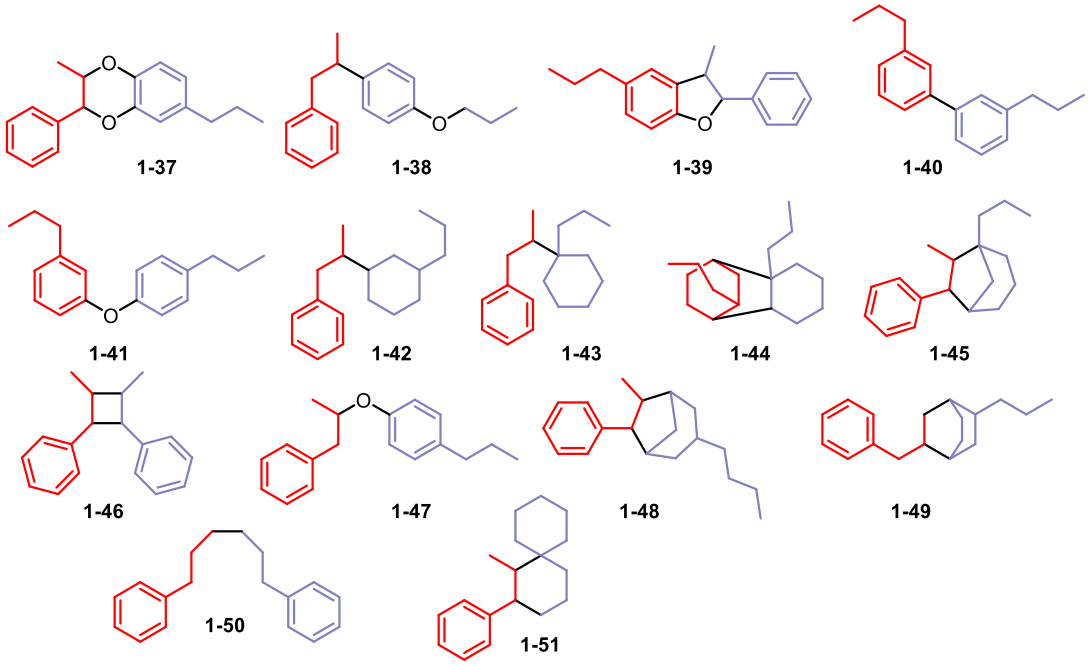


Figure 6: Most accepted structural subtypes of neolignans (adapted from [5]).

Most neolignans are non-toxic and in many cases presumably even have a positive effect on human bodies. The diverse biological activities of these phenolic compounds range from antiparasitic, anticancer, anti-inflammatory, neuroprotective, antibacterial, antifungal, and antiallergenic.[5]

Licarin A (**1-52**) isolated from *Nectandra glabrescens Benth* and **burchellin** (**1-53**) isolated from *Ocotea cymbarum Kunth* exhibit antitrypanosomal activity by interfering with essential biochemical processes in the parasite, leading to its inhibition and death (**Figure 7**). **Licarin A** caused inhibition of *in vitro* growth of the epimastigote form of *T. cruzi* (Dm28 strain), four

days (48.5 ± 2.6 µM) and seven days (77.2 ± 4.9 µM) after treatment with a dose of 310 µM, compared to the respective control groups (81 ± 6.1 µM and 141 ± 6.2 µM). The IC_{50}/96 h was 462.7 µM. **Burchellin** caused partial inhibition *in vitro*, four days (10.8 ± 0.6 µM) and seven days (12.7 ± 1.1) after treating the developing parasites at a dose of 100 µg/mL (294 µM), compared to the respective control groups (15.0 ± 1.7 µM and 16.0 ± 1.5 µM). IC_{50}/96 h for **burchelin** was of 756 µM. Each value represents the mean number of parasites ($x10^6$) from six individual samples. Both compounds show effectiveness against epimastigote and trypomastigote forms.[25]

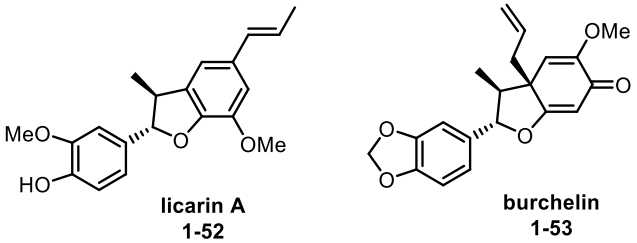


Figure 7: Structure of the licarin A (**1-52**) and burchelin (**1-53**).

Some neolignans found in Chinese olives, such as **cinncassin D** (**1-54**) or **picrasmalignan** (**1-55**), have been shown to modulate the inflammatory response by inhibiting the production of pro-inflammatory mediators, such as cytokines and prostaglandins. For **cinncassin D** IC_{50} values ranging from 18.1 to 30.2 µM based on its configuration. For **picrasmalignan**, the IC_{50} values ranged from 6.0 to 18.8 µM. Both compounds exhibited pronounced inhibitory effects on lipopolysaccharide-induced nitric oxide production in RAW 264.7 macrophages. This activity could be beneficial in the management of chronic inflammatory diseases, including arthritis, asthma, and inflammatory bowel diseases (**Figure 8**).[26]

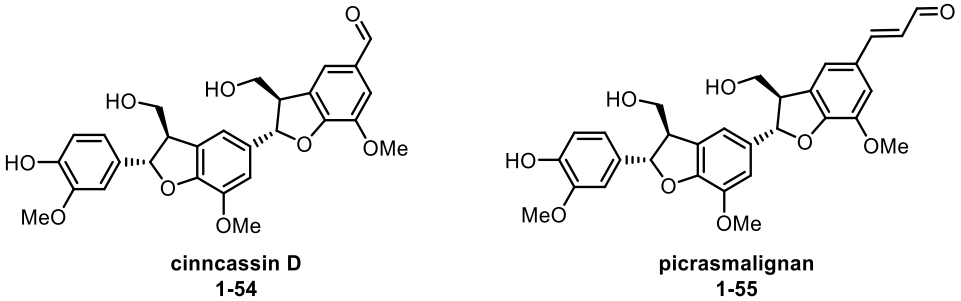
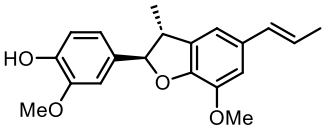


Figure 8: Structure of the cinncassin D (**1-54**) and picrasmalignan (**1-55**).

On the other hand **(–)-licarin A** (**1-52**) and **eupomatenoid-7** (**1-56**) have demonstrated neuroprotective activity and protect neuronal cells from damage caused by numerous factors, such as oxidative stress, excitotoxicity, and inflammation. At the same time, **licarin A**

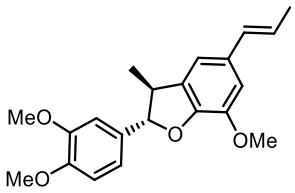
was not toxic at the concentration tested (10 µM). This property could be beneficial in the prevention or treatment of neurodegenerative diseases, such as Alzheimer's and Parkinson's disease (**Figure 9**).[27]



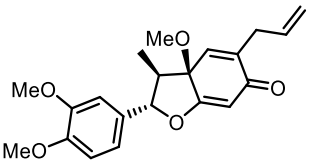
eupomatenoid-7
1-56

Figure 9: Structure of the eupomatenoid-7 (**1-56**).

Acuminatin (**1-57**) and **denudatin B** (**1-58**) (**Figure 10**) isolated from *Piper betle* showed antibacterial activity by inhibiting the multidrug antibiotic efflux pump NorA. In a study involving cotreatment with antibiotic norfloxacin in a strain of *S. aureus* that overexpresses the efflux pump, the compounds synergistically displayed MIC values of 2 mg/L each and fractional inhibition concentration indices of 0.13-0.25.[28]



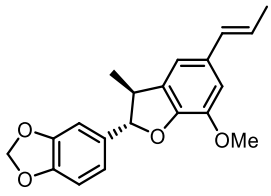
acuminatin
1-57



denudatin B
1-58

Figure 10: Structure of the **acumanatin** (**1-57**) and **denudatin B** (**1-58**).

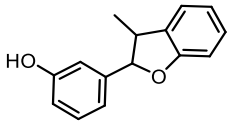
Interestingly, in the study where three sialidase isoforms (NanA–C) were evaluated, **licarin B** (**1-59**) (**Figure 11**) showed significant activity against NanA with IC_{50} = 1.5 ± 0.4 µM, NanB with IC_{50} = 14.0 ± 1.6 µM and NanC with IC_{50} = 30.7 ± 5.6 µM.[23]



licarin B
1-59

Figure 11: Structure of the licarin B (**1-59**).

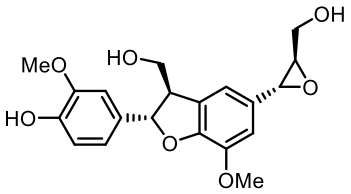
Some neolignans also exhibit antifungal activity. An example is **conocarpan** (**1-60**) (**Figure 12**) isolated from *piper rivinoides* that displayed antifungal activity against *candida albicans* strains (PRI, IC_{50} =9.20 ± 1.20 µg/mL; ATCC 10231 = 19.60 ± 2.30 µg/mL) however they were also found to be cytotoxic to mammalian cells.[29]



conocarpan
1-60

Figure 12: Structure of the conocarpan (**1-60**).

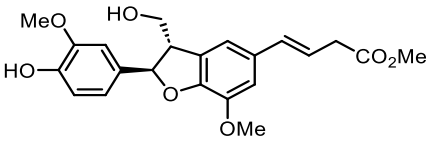
Lyciumnan (**1-61**) (**Figure 13**), an epoxide containing benzofuran neolignan found in *Lycium barbarum L* (used in traditional Chinese medicine) displayed moderate cytotoxic activities against cell lines A549 (IC_{50} = 37.1 ± 3.3 µM) HeLa (IC_{50} = 54.1 ± 3.9 µM) and PC-3 (IC_{50} = 51.7 ± 3.6 µM).[24]



lyciumnan
1-61

Figure 13: Structure of the lyciumnan (**1-61**).

Gardenofolin D (**1-62**) (**Figure 14**) also showed activity against HeLa cell lines with IC_{50} = 21.0 µM. The compound was further studied for its effects on cell morphology and apoptosis. Morphological experiments indicated that it induces apoptosis of HeLa cells at 25µM.[30]



gardenofolin D
1-62

Figure 14: Structure of the gardenofolin D (**1-62**).

Three new dehydrobenzofuran neolignans, **mappiodoinins A-C** (**1-63** to **1-65**) (**Figure 15**), together with nine known analogues, were isolated from the stems and leaves of *M. iodoies*. Cytotoxicity against several human cancer cell lines of were investigated. For HL-60, IC_{50} ranged from 0.78 to 2.16 µM, for the SMMC-7721 cell line, IC_{50} ranged from 1.06 to 5.32 µM, for A-549, IC_{50} ranged from 2.19 to 5.03 µM, for MCF-7, IC_{50} ranged from 1.28 to 3.69 µM and for the SW-480 cell line, IC_{50} ranged from 0.16 to 1.89 µM. This may be the reason *M. iodoies* had been used to treat tumors in China traditional medicine.[31]

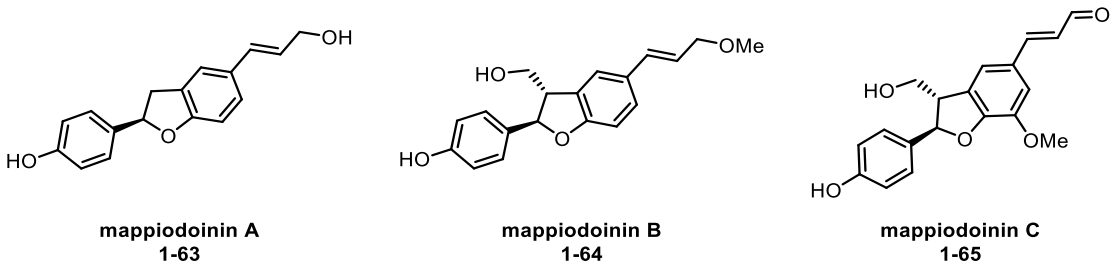


Figure 15: Structure of the mappiodoinin A-C (**1-63** to **1-65**).

Illiciumlignan D (**1-66**) (**Figure 16**) isolated from the branches and leaves of *Illicium wardii* was tested on the ovarian cancer cell line SKOV3, with the IC_{50} = 8.67 µM.[32]

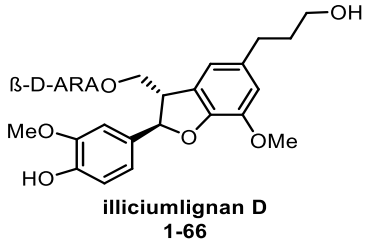


Figure 16: Structure of the illiciumlignan D (**1-66**).

Sambucasinol A-C (**1-67** to **1-69**) (**Figure 17**) were isolated from *S. williamsii twigs* and all showed a potent inhibitory effect on NO production in LPS-stimulated BV-2 cells and NGF secretion in C6 cells with IC_{50} values ranging from 6.82 to 14.70 µM. Compounds also showed consistent cytotoxic activity against cancer cell lines A549, SKOV3, SK-MEL-2 and XF498 with IC_{50} values ranging from 11.07 to 19.62 µM. Therefore, **sambucasinol A-C** are compounds that could be useful for the development of new anti-inflammatory, neuroprotective and anticancer agents.[31]

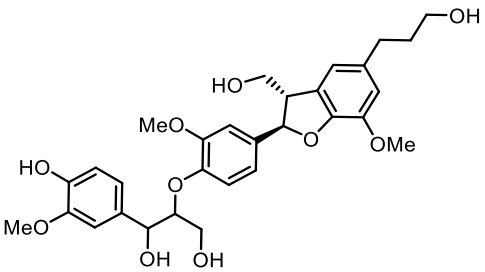
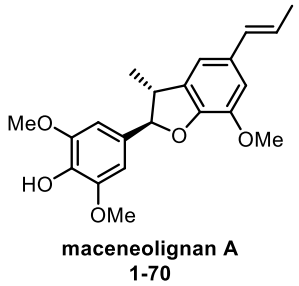


Figure 17: Structure of the sambucasinol A-C (**1-67** to **1-69**).

Neolignans also exhibit antiallergic activities – **Maceneolignan** (**1-70**) (**Figure 18**) isolated from the aril of *Myristica fragrans* (*Myristicaceae*) inhibited antigen-stimulated tumor

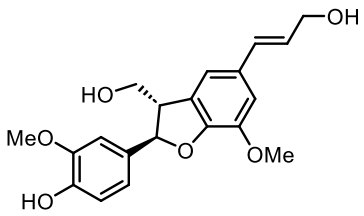
necrosis factor-α production, an important process in the late phase of type I allergic reactions with the TNF-a inhibitor, IC_{50} = 48.40 µM. [33]



maceneolignan A
1-70

Figure 18: Structure of the maceneolignan A (**1-70**).

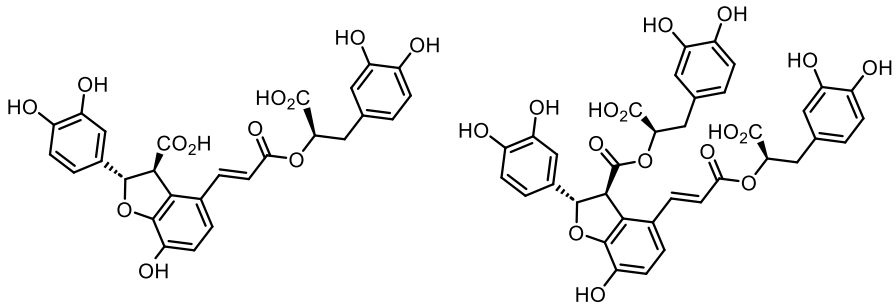
Dehydrodiconiferyl alcohol (**1-71**) (**Figure 19**), a compound originally isolated from *Cucurbita moschata*, inhibits osteoclast differentiation and promotes bone morphogenetic protein-2 (BMV-2)–induced osteoblastongenesis acting as an estrogen receptors agonist. This estrogenic effect may serve as a treatment for postmenopausal and ovariectomy-induced bone loss.[34,35]



dehydrodiconiferyl alcohol
1-71

Figure 19: Dehydrodiconiferyl alcohol (**1-71**).

Lithospermic acid (**1-72**) and **salviaonolic acid B** (**1-73**), compounds isolated *from Salvia miltiorrhiza*, are nontoxic and active against HIV by inhibiting HIV-1 integrase, with the IC_{50} of 0.83 µM and 0.48 µM, respectively. These two compounds hold promise as novel therapeutic agents against AIDS due to their high potency and absence of cytotoxicity.[36,37]



lithospermic acid
1-72

salvianolic acid B
1-73

Figure 20: Structure of the lithospermic acid (**1-72**) and salvianolic acid B (**1-73**).

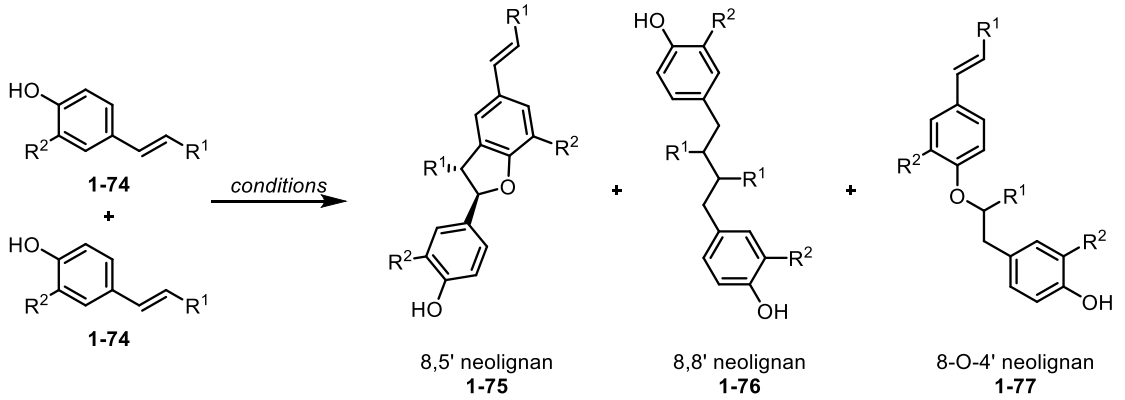
1.3. Synthesis of benzofurans

1.3.1. Synthesis based on enzymes and transitional metals

Several synthetic approaches to **1-39** neolignan (C8-C5') core are based on a biomimetic approach that proceeds *via* transition metal or enzyme (disguised transitions metal) mediated oxidative homocoupling of appropriate phenylpropanoid precursors. As was demonstrated in **Figure 5** (homocoupling intermediates), in such a case, the selected substrate must possess the phenolic OH group in *para* position to the attached phenylpropanoid C3 subunit. Therefore, only a few phenolic monomers can undergo dimerization (ferulic, coumaric, and caffeic acid). The key disadvantage of such biomimetic approach lies in the narrow scope of the substrates available for the coupling, reliability of the protocols that lacks general reproducibility, low reaction yields accompanied with various side reaction formation (most common side products are products of 8-O-4' and 8,8' dimerization), and lack in the stereochemistry of the post-coupling steps. The overview of products that are produced by various homodimerization methods is shown in **Table 1**.

These results play major roles in suggesting that the reaction solvent and water content in the reaction system affect the reaction products obtained from the oxidation either by enzymes or by transitional metals.

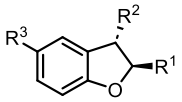
Table 1: Different dimerization conditions.



Entry	Conditions	R^1	R^2	Yield 8,5'	Yield 8,8'	Yield 8-O-4'	Ref.
1	$FeCl_3$, EtOH/water	CH_3	OCH_3	30 %	-	-	38
2	$FeCl_3$, acetone/water	CH_3	OCH_3	53 %	2 %	-	19
3	Ag_2O, DCM	CH_2OH	OCH_3	50 %	-		7
4	Ag_2O, dioxane	CH_2OH	OCH_3	14 %	-	70 %	
5	Ag_2O, benzene/acetone	$COOCH_3$	OCH_3	42-50 %	-	-	18,39
6	Ag_2O, benzene/acetone	$COOCH_3$	H	35 %	-	-	18
7	$K_3[Fe(CN)_6]$	$COOCH_3$	OCH_3	19 %	-	-	20
8	$K_3[Fe(CN)_6]$	$COOCH_3$	H	57 %	-	-	20
9	HRP/H_2O_2	CH_3	OCH_3	56 %	13 %	22 %	40
10	HRP/H_2O_2	$COOCH_3$	H		16 %	-	41
11	Laccase (*Rhus vernicifera*)	CH_3	OCH_3	43 %	-	22 %	42

1.3.2. Non-biomimetic approaches to dihydrofuran neolignans

2,3-Dehydrobenzofuran (**DHB**) belongs to the class of organic compounds with the coumaran skeleton, which entails a benzene ring fused to a 2,3-DHB ring (**Figure 21**). Not many methods have been described for the synthesis of *anti* 2,3-disubstituted DHBs, but there are a vast number of developed methods for formation of the benzofuran skeleton.

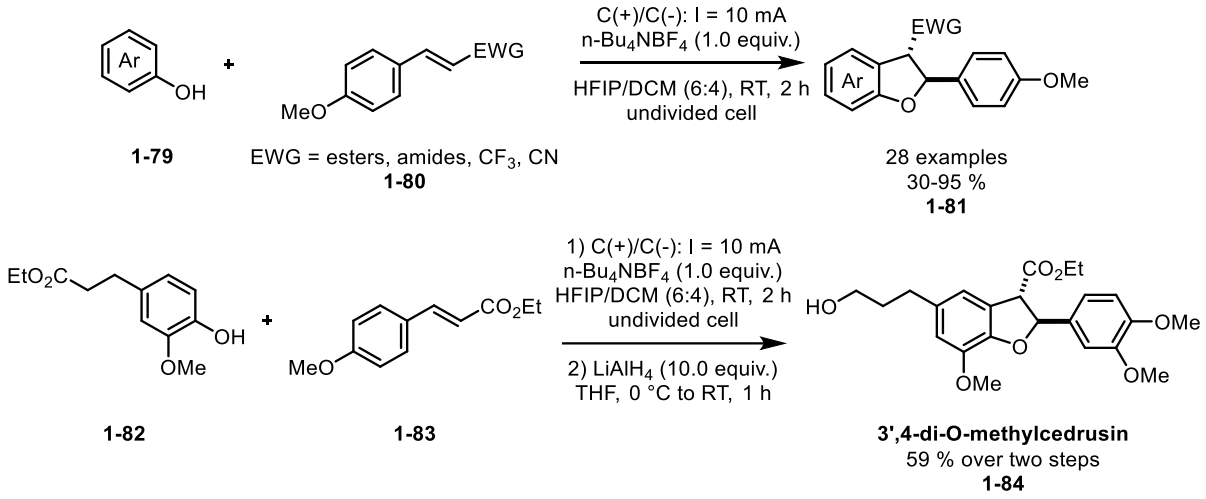


anti-2,3-DHB
1-78

Figure 21: Structure of the *anti*-2,3-DHB (**1-78**).

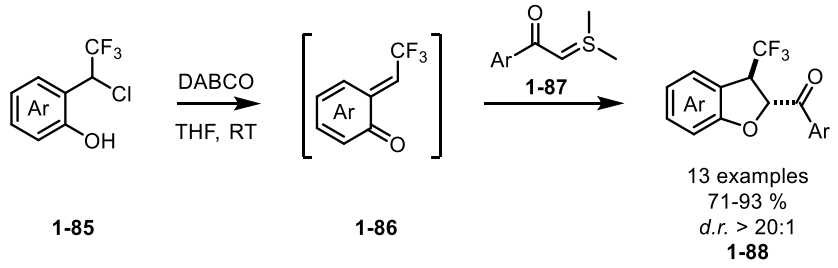
The aim of this chapter is to briefly summarize the most important and recent methods in the synthesis of analogues of *anti*-2,3-disubstituted C8-C5' neolignans.

In 2020, Wang and his coworkers reported an electro-oxidative [3+2] annulation reaction of phenols **1-79** and electron-deficient alkenes **1-80** for the synthesis of various chiral derivatives of 2,3-dehydrobenzofuran-3-ol **1-81**. The reaction might be used to synthesize alkyl amino, trifluoromethyl, and cyano substituted products. The natural product 3',4-di-O-methylcedrusin **1-84** was synthesized using this approach (**Scheme 3**).[43]



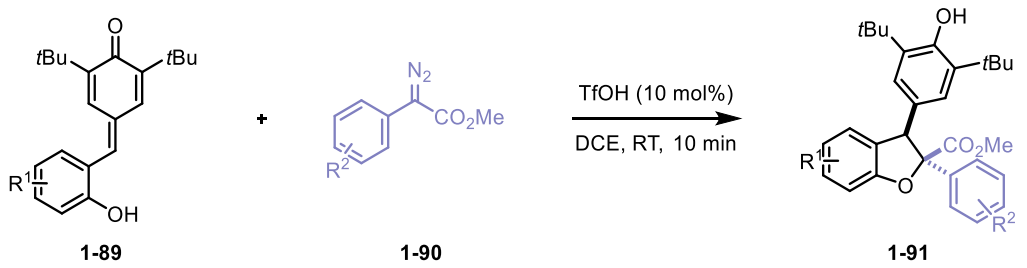
Scheme 3: Cu(I)-catalysed asymmetric synthesis of 2,3-dehydrobenzofuran-3-ol derivatives **1-81** and synthesis of 3',4-di-O-methylcedrusin **1-84**.[43]

In 2020 Srivari Chandrasekhar and co-workers described an effective synthesis of CF_3 functionalized DHBs **1-88** by annulation of *ortho*-hydroxy-CF_3-benzyl chlorides **1-85** with sulfur ylides **1-87** under basic conditions in a highly diastereoselective manner (**Scheme 4**).[44]



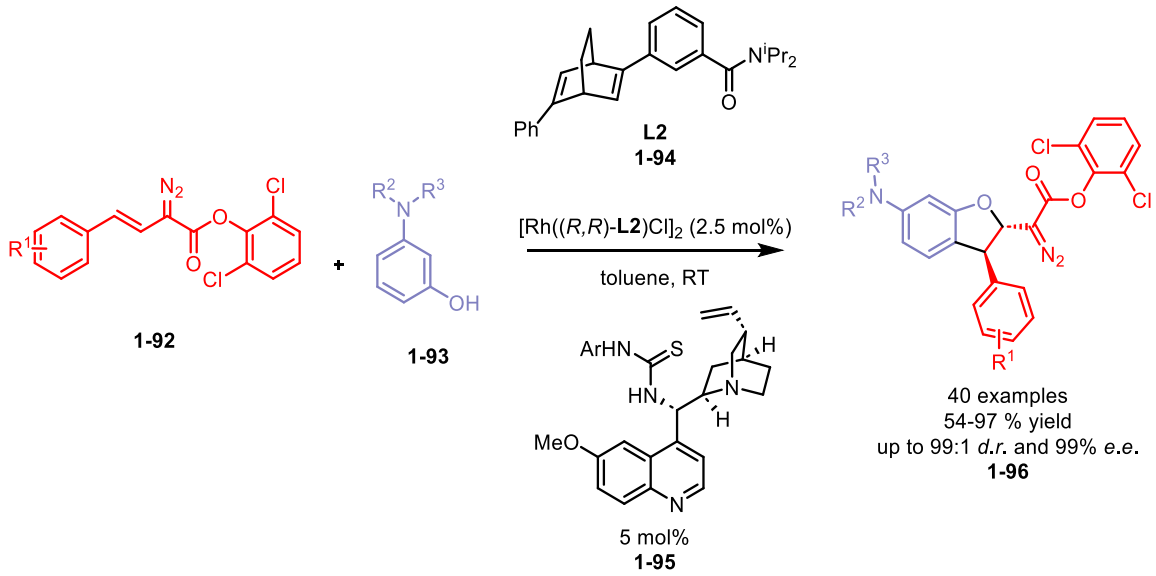
Scheme 4: Diastereoselective synthesis of CF_3-dehydrobenzofurans **1-88** by [4+1] annulation of *in situ*-generated CF_3-*o*-quinone methides **1-86** and sulfur ylides **1-87**.[44]

In 2021, Fener Chen and co-workers developed a methodology for the construction of 2,3-DHB scaffolds containing a quaternary carbon center at the position C2 in **1-91** by the [4+1] annulation reaction of *p*-quinone methides (QM) **1-89** and α-aryl diazoacetates **1-90** (C1 synthon) using a catalytic amount of TfOH (**Scheme 5**).[45]



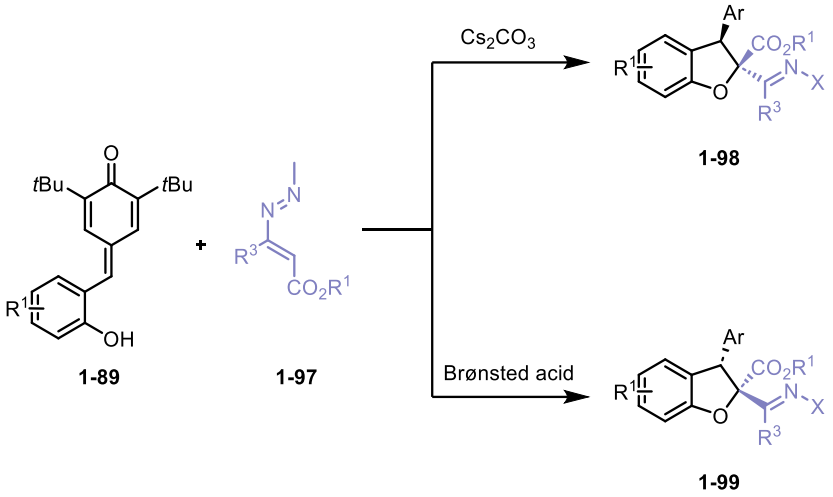
Scheme 5: TfOH-catalysed [4+1] annulation of *p*-QMs **1-89** with α-Aryl Diazoacetates **1-90.**[45]

Ming-Hua Xu and co-workers in 2021 achieved the synthesis of enantioenriched 2,3-disubstituted dihydrobenzofurans **1-94** utilizing arylvinyldiazoacetates **1-92** and aminophenons **1-93** using a chiral rhodium catalyst and organocatalyst **1-95** which independently control the formation of two stereogenic centers through one-pot C-H functionalization/oxa-Michael addition cascade reaction with excellent diastereomeric ration up to 99 % and excellent yields (**Scheme 6**).[46]



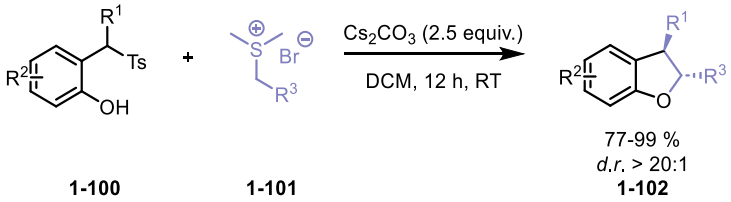
Scheme 6: Stereodivergent synthesis of enantioenriched 2,3-disubstituted dihydrobenzofurans **1-96** *via* a one-pot C–H functionalization/oxa-Michael addition cascade.[46]

In 2023, Mei group developed a diastereodivergent formal [4+1] cycloaddition of *p*-QMs **1-89** with azoalkenes **1-98** *via* the domino oxa-1,4-addition/1,6-addition process in good yields and with reversible diastereoselectivities (**Scheme 7**).[47]



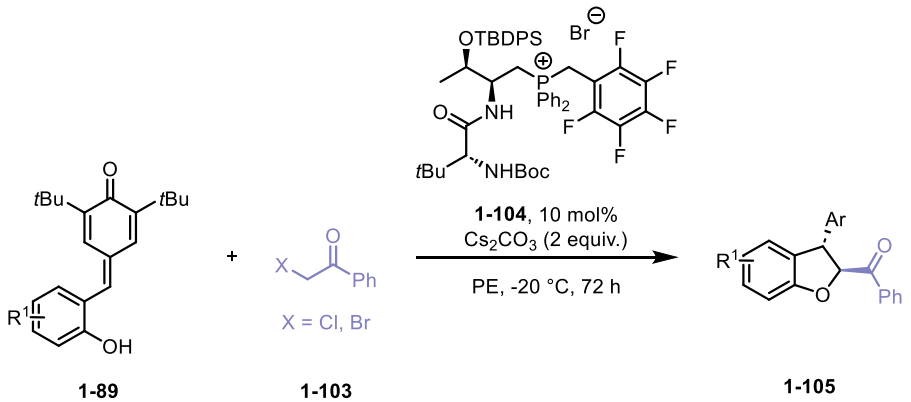
Scheme 7: Diastereodivergent formal [4+1] cycloaddition of azoalkenes **1-97** as one-carbon synthons.[47]

In 2013, a group of Zhou developed a mild method for the generation of *o*-QMs intermediates from 2-tosylalkylphenols **1-100** under basic conditions and their reaction with sulfur ylides **1-101** resulted in the selective synthesis of anti-2,3-DHBs (**Scheme 8**).[48]



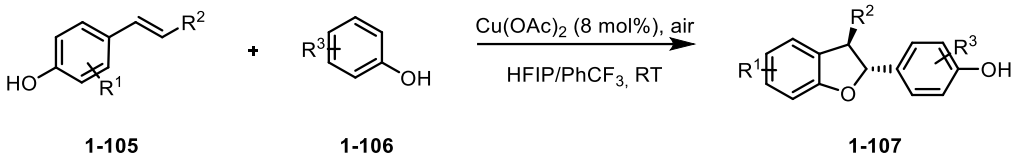
Scheme 8: A mild method for generation of *o*-quinone methides under basic conditions. The facile synthesis of *trans*-2,3-dihydrobenzofurans **1-102**.[48]

In 2019, a group of Wang developed enantioselective [4+1] annulation reaction between hydroxyl-substituted *p*-QMs with α-halogenated ketones by a dipeptide based bifunctional phosphonium salt catalyst (**Scheme 9**).[49]



Scheme 9: Bifunctional phosphonium salt directed enantioselective formal [4+1] annulation of hydroxyl-substituted *p*-QMs with α-halogenated ketones **1-103**.

In 2021, the Liu group developed a biomimetic selective methodology of using copper acetate as a catalyst and air as the terminal oxidant (oxidative cross coupling) (**Scheme 10**).[7]



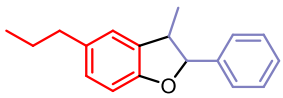
1-105 **1-106** **1-107**

Scheme 10: Biomimetic selective synthesis of 8,5' neolignan analogues **1-107**.[7]

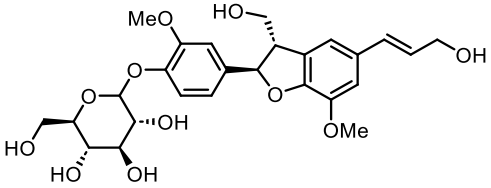
1.4. Results and discussion*

1.4.1. Introduction

Our research group has a long-standing interest in exploring the biological activities associated with plant secondary metabolites – more specifically, our focus lies in the synthesis of neolignan derivatives with the benzofuran structure (**1-39**) and biological evaluation of prepared library against nematodes in a model *Caenorhabditis elegans* and biological evaluation of antiparkinson activity. This part of the Thesis builds on previous gained knowledge in the synthesis and application of lignans and neolignans within our group. [5,50–52] Our long-standing goal is to develop a straightforward general synthetic route to dehydrodiconiferyl alcohol glucoside (**DCG-A**, **1-108**, **Figure 22**).



1-39



dehydrodiconiferyl alcohol glucoside, DCG-A
1-108

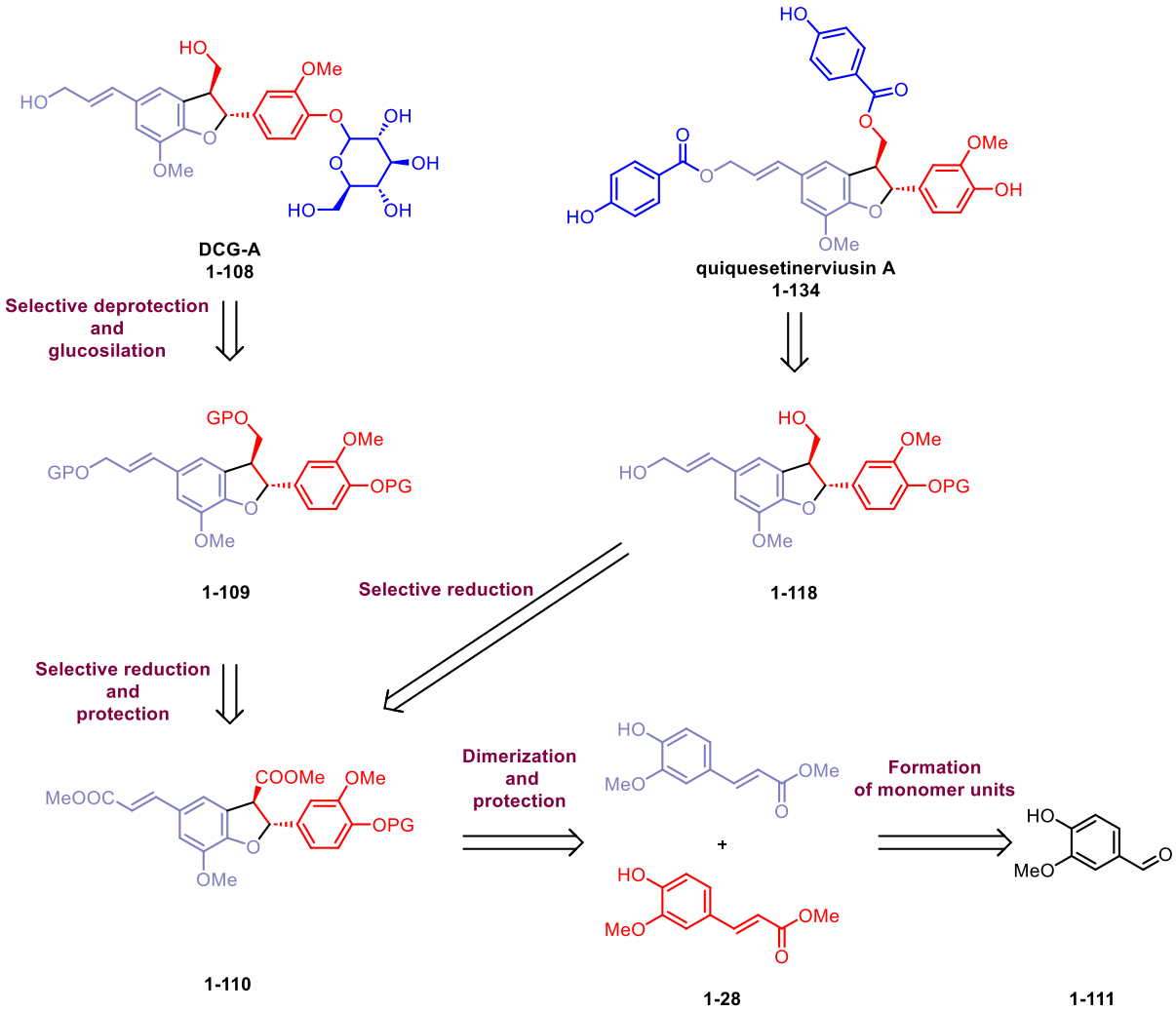
Figure 22: Structure of the general dihydrobenzofurane neolignan core **1-39** and **DCG-A**.

1.4.2. Retrosynthesis plan

Our strategy in achieving the goal of this project was to design a synthesis that would start from a simple and commercially available building block and allow us to prepare not only the **DCG-A** molecule but also analogues of other naturally occurring molecules that would allow us to constitute the whole neolignan-based chemical library suitable for further evaluation of their biological activity. An overview of our retrosynthetic analysis that was supposed to bring us to the **DCG-A** synthesis is outlined in **Scheme 11**.

* This work was supported by the European Regional Development Fund-Project 'Centre for Experimental Plant Biology' (no. CZ.02.1.01/0.0/0.0/16_019/0000738) and by the Palacký University Internal Grant Agency (IGA_PrF_2021_011, IGA_PrF_2021_024, IGA_PrF_2022_012, IGA_PrF_2022_016, IGA_PrF_2022_022). The research presented in this chapter were in part published in the Bachelor theses of Sylvie Lévayová (for details see further) and the manuscript is currently under preparation. The work was done in collaboration with Dr. Alena Kadlecová (anthelmintic activity evaluation), Dr. Hana Vylíčilová (cytokinin essays) and Dr. Gabriel Gonzalez (antiparkinson activity evaluation). As a primary researcher in the project, I was responsible for designing and conducting experimental procedures and measurements, analyzing the data, and interpreting the results. Additionally, I am writing a first draft of the manuscript.

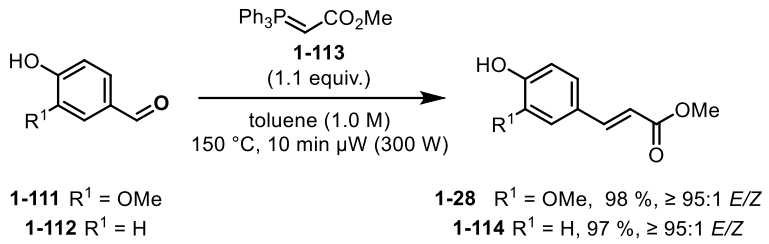
From a retrosynthetic point of view, the plan was divided into three parts and was based on the first (and only) total synthesis of **DCG-A** published in 1998 by Wong group[53] where DCG-A was prepared from the diol-protected neolignan dimer **1-109** by a synthetic sequence that comprised selective deprotection of the phenolic -OH group followed by glycosylation of **1-109**. Therefore, we decided to base our retrosynthetic proposal on the exploitation of the same diol **1-109**, which could be prepared by selective reduction of methyl esters to alcohols followed by introduction of a protecting group of **1-110.** This dimer **1-110** could be prepared by dimerization of the neolignan monomers **1-28** and introduction of the protective group in the phenolic hydroxy group. Compound **1-110** could further allow us the synthesis of few more natural products with the same molecular scaffold as in **DCG-A** as demonstrated on the quiquesetinerviusin A (**1-134**) (**Scheme 11**).



Scheme 11: Retrosynthetic plan towards **DCG-A** and **quiquesetinerviusin A**.

1.4.3. Towards DCG-A: Formation of monomer units of phenylpropanoids

Based on our proposal, the synthesis of the **DCG-A** should take 8 steps. We have started our investigation with the first step of the 8-step reaction sequence with the formation of monomer units of methyl ferulate **1-28** together with methyl coumarate **1-114**. We utilized method developed in our group[51] that explore microwave-initiated preparation of phenylpropanoids *via* Wittig reaction and allowed us to obtain both desired monomers in excellent yield and *E* selectivity. The values of selectivity and the reaction yields corresponded to those mentioned in the original protocol; therefore, no optimization of the reaction was required (**Scheme 12**).

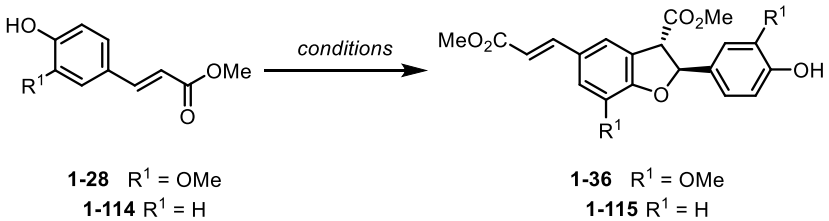


Scheme 12: Microwave-Assisted Synthesis of monomers **1-28** and **1-114**.[51]

1.4.4. Homocoupling dimerization

The next step was the homocoupling dimerization of the two monomers **1-28** and **1-114**. The dimerization reaction is an oxidative radical reaction that is well described in the literature[21,38–41],hence we screened the best conditions in the literature. Due to the low yields, we have performed optimization of the homocoupling reaction ourselves. Products **1-36** and **1-115** were isolated as white solids in 32 % and 48 % optimized yields, respectively. The conditions and results obtained are summarized in **Table 2**.

Table 2: Dimerization reaction optimization.



Entry	Conditions	R^1	Yields [%][a]
1	$K_3[Fe(CN)_6]$ (2.1 equiv.), DCM/$CHCl_3$ 1:9 (*V*/*V*), RT	CH_3	22
2	$K_3[Fe(CN)_6]$ (2.1 equiv.), DCM/$CHCl_3$ 1:9 (*V*/*V*), RT	H	17
3	Ag_2O (2.1 equiv.), acetone/benzene 2:1 (*V*/*V*), RT	CH_3	28

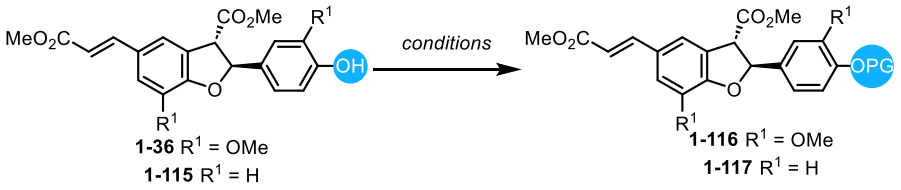
Entry	Conditions	R^1	Yields [%][a]
4	Ag_2O (1.0 equiv.), benzene/acetone 2:1 (*V*/*V*), RT	H	32
5	Ag_2O (1.0 equiv.), toluene/acetone 2:1 (*V*/*V*), RT	CH_3	48

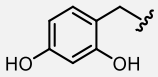
a) Refers to isolated yield after the column chromatography on silica gel.

1.4.5. Introduction of the phenolic protecting group

The third step was the introduction of a suitable protection group. We have decided to introduce various protecting groups, which would be orthogonal in reactivity to a reductive condition employed in the next step and could be removed using various reaction mechanisms and under various reaction conditions. Our starting materials **1-36** and **1-115** were selectively protected by dihydropyrane (**DHP**), 2-(trimethylsilyl)ethoxymethyl (**SEM**), 2,4-dimethoxybenzyl, triisopropylsilyl (**TIPS**), methoxymethyl (**MOM**) and *tert*-butyldimethylsilyl (**TBS**) in good to excellent yields. The results are shown in **Table 3** below.

Table 3: Conditions for introduction of different protecting groups.



Entry	Conditions	R^1		OPG	Yields [%][a]
1	DHP (4.0 equiv.), *p*TSA (cat.), RT, 18 h	H	**1-117a**	DHP	65
2	DIPEA (4.0 equiv.), SEMCl (2.0 equiv.) DCM (0.2 M), 0 °C to RT, 18 h	H	**1-117b**	SEM	70
3	2,4-dimethoxybenzyl 2,2,2-trichloroacetimidate (1.2 equiv.), *p*TSA (cat.), DCM (0.25 M), RT, 18 h	H	**1-117c**		95
4	TEA (3.0 equiv.), TIPSCl (1.3 equiv.) DCM (0.4 M), 0 °C to RT, 18 h	H	**1-117d**	TIPS	78
5	MOMCl (3.0 equiv., 3.5 M sol. in toluene), DIPEA (2.0 equiv.), DCM (0.5 M), 0 °C to RT, 18 h	H	**1-117e**	MOM	95
6	MOMCl (3.0 equiv., 3.5 M sol. in toluene), DIPEA (2.0 equiv.), DCM (0.5 M), 0 °C to RT, 18 h	CH_3	**1-116a**	MOM	94
7	TEA (3.0 equiv.), TBSCl (1.25 equiv.) DCM (0.5 M), 0 °C to RT, 18 h	H	**1-117f**	TBS	96
8	TEA (3.0 equiv.), TBSCl (1.25 equiv.) DCM (0.5 M), 0 °C to RT, 18 h	CH_3	**1-116b**	TBS	94

a) Refers to isolated yield after the column chromatography on silica gel.

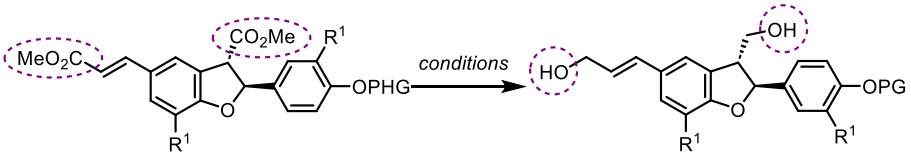
1.4.6. Reduction step

The fourth step of the sequence was the reduction of both ester groups in **1-116** and **1-117** to the corresponding alcohols **1-118** and **1-119**. At this point, our precious experience in the neolignan-core bearing compounds[50] was used, and DIBAL-H was selected as the most convenient reducing agent. The results are summarized in

Table 4.

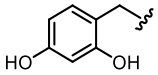
We have decided to continue in the synthesis only with the **TBS** protected dimers **1-118b** and **1-119f** and **MOM** protected dimers **1-118a** and **1-118e**, **2,4-dimethoxybenzyl** and **TIPS** protecting groups (unstable under reaction conditions or reaction work-up), and the **DHP** or **SEM** protecting groups (low yielding protocols), were discarded. It should be also noted, that when TBS protected dimers **1-116** and **1-117** were reduced, the reducing agent, DIBAL-H, had to be used in large excess (from 6 equiv. to 11 equiv.) to drive the reaction to completion, otherwise partially reduced products **1-120** and **1-121** were isolated (**Scheme 13**).

Table 4: Optimization of the reduction of esters to alcohols.



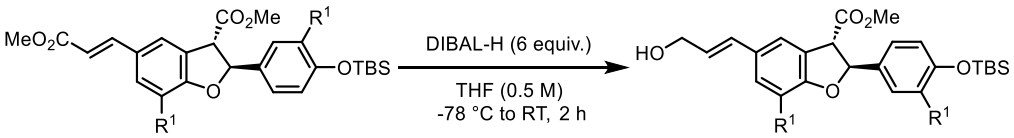
1-116 R^1 = OMe
1-117 R^1 = H

1-118 R^1 = OMe
1-119 R^1 = H

Entry	Conditions	R^1	product	OPG	Yields [%][a]
1	DIBAL-H (4.5 equiv., 1 M in THF) DCM (0.5 M), -78 °C to RT, 3 h	H	**1-119e**	MOM	34
2	DIBAL-H (4.5 equiv., 1 M in THF) THF (0.5 M), -78 °C to RT, 3 h	H	**1-119e**	MOM	89
3	DIBAL-H (4.5 equiv., 1 M in THF) THF (0.5 M), -78 °C to RT, 3 h	CH_3	**1-118a**	MOM	72
4	DIBAL-H (4.5 equiv., 1 M in THF) THF (0.5 M), -78 °C to RT, 3 h	H	**1-119a**	DHP	15
5	DIBAL-H (4.5 equiv., 1 M in THF) THF (0.5 M), -78 °C to RT, 3 h	H	**1-119b**	SEM	21
6	DIBAL-H (4.5 equiv., 1 M in THF) THF (0.5 M), -78 °C to RT, 3 h	H	**1-119c**		N.D.[b]
7	DIBAL-H (4.5 equiv., 1 M in THF)	H	**1-119d**	TIPS	N.D.[b]

Entry	Conditions	R^1	product	OPG	Yields [%][a]
8	THF (0.5 M), -78 °C to RT, 3 h DIBAL-H (4.5 equiv., 1 M in THF) DCM (0.5 M), -78 °C to RT, 3 h	CH_3	**1-118b**	TBS	39[c]
9	DIBAL-H (5 equiv., 1 M in THF) THF (0.5 M), -78 °C to RT, 3 h	H	**1-119f**	TBS	54[c]
10	DIBAL-H (5 equiv., 1 M in THF) THF (0.5 M), -78 °C to RT, 3 h	CH_3	**1-118b**	TBS	51[c]
11	DIBAL-H (6 equiv., 1 M in THF) THF (0.5 M), -78 °C to RT, 4 h	CH_3	**1-118b**	TBS	60[c]
12	1) DIBAL-H (6 equiv., 1 M in THF), THF (0.5 M), -78 °C to RT, 2 h 2) DIBAL-H (5 equiv., 1 M in THF), THF (0.5 M), RT, 2 h	H	**1-119f**	TBS	90
13	1) DIBAL-H (6 equiv., 1 M in THF), THF (0.5 M), -78 °C to RT, 2 h 2) DIBAL-H (5 equiv., 1 M in THF), THF (0.5 M), RT, 2 h	CH_3	**1-118b**	TBS	86

a) Refers to isolated yield after the column chromatography on silica gel.
b) Yield not determined, product decomposition.
c) Incomplete reduction, product(s) of partial reduction of at least of the ester group observed.



1-116 R^1 = OMe
1-117 R^1 = H

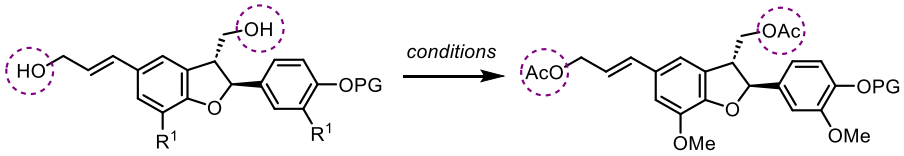
1-120 R^1 = OMe
1-121 R^1 = H

Scheme 13: Partial reduction of TBS-containing starting materials.

1.4.7. Hydroxy Group Protection - Step Five

The fifth step was the introduction of an acetyl protecting group on both primary alcohols presented on side chains of TBS and MOM protected benzofuran cores. This was achieved with acetyl chloride in excellent yields for MOM and TBS protected derivatives and the desired products **1-122** and **1-123** were isolated in excellent yields (**Table 5**).

Table 5: Introduction of acetyl protecting group.



1-118 R^1 = OMe
1-119 R^1 = H

1-122 R^1 = OMe
1-123 R^1 = H

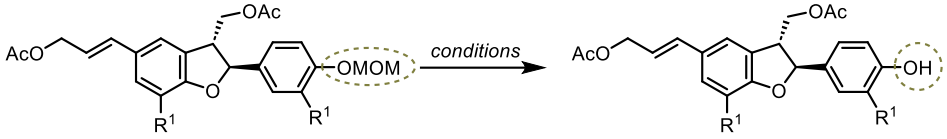
Entry	Conditions	R^1	product	OPG	Yields [%][a]
1	AcCl (2.2 equiv.), TEA (3.0 equiv.) DCM (0.25 M), 0 °C to RT, 18 h	H	**1-123a**	MOM	95
2	AcCl (2.2 equiv.), TEA (3.0 equiv.) DCM (0.25 M), 0 °C to RT, 18 h	CH_3	**1-122a**	MOM	99
3	AcCl (2.2 equiv.), TEA (3.0 equiv.) DCM (0.25 M), 0 °C to RT, 18 h	H	**1-123b**	TBS	94
4	AcCl (2.2 equiv.), TEA (3.0 equiv.) DCM (0.25 M), 0 °C to RT, 18 h	CH_3	**1-122b**	TBS	98

a) Refers to isolated yield after the column chromatography on silica gel.

1.4.8. Selective phenolic protecting group removal

The sixth step of the sequence was the selective removal of the phenolic protecting group, a simple step that proved to be more than difficult. The removal of the MOM protecting group will first be discussed (**Table 6**). Various reaction conditions were evaluated; however, only **entries 13** and **14** resulted in partial conversion of the starting material. Only when *in situ* generated strong Lewis acid TMSI was used (TMSCl/NaI system**, entry 17**), the conversion of the starting material was quantitative; however, the desired product was isolated only with a 20% yield. Unfortunately, when the reaction was carried out on a larger semipreparative scale (0.5 mmol), only decomposition of the starting material/product was observed. Therefore, we concluded that the MOM protecting group is not suitable to be further exploration in this synthetic scheme.

Table 6: Attempted deprotection of the MOM protected group from compounds **1-122** and **1-123**.



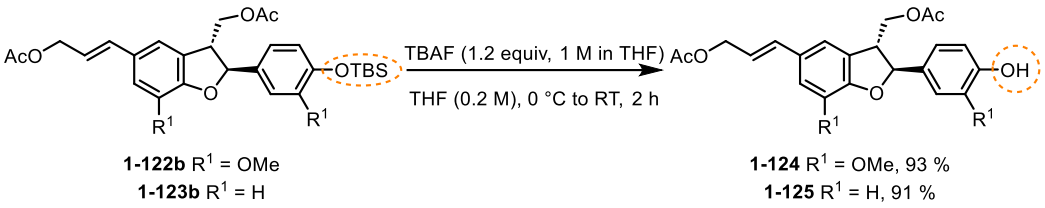
1-122-MOM R^1 = OMe
1-123-MOM R^1 = H

1-124 R^1 = OMe
1-125 R^1 = H

Entry	Conditions	R^1	Comments
1	$ZnBr_2$ (3.0 equiv.), Et_2O (0.05 M), 0 °C to RT, 18 h	H	decomposition[b]
2	*p*TSA (1.1 equiv.), MeOH (0.05 M), 30 °C, 2 h[54]	H	decomposition[b]
3	$ZnBr_2$ (1.0 equiv.), lithium thioethoxide (2.0 equiv.) DCM (0.05 M), 0 °C to RT, 18 h[55]	H	decomposition[b]
4	ZnI_2 (4.0 equiv.), Et_2O (0.05 M), 0 °C to RT, 18 h	CH_3	decomposition[b]
5	HCl (1.0 equiv., 1 M), MeOH (0.05 M), RT, 1 h[56]	H	decomposition[b]
6	HCl (20.0 equiv., 4 M in dioxane), THF (0.05 M), 0 °C, 20 min[53]	H	decomposition[b]
7	AcCl (2.0 equiv., 1 M), MeOH (0.1 M), RT, 1 h[57]	H	decomposition[b]
8	TFA (2.0 equiv., 0.0375 M), MeOH (0.1 M), RT, 1 h[58]	H	decomposition[b]
9	CBr_4 (0.1 equiv.), *i*PrOH (0.2 M), 85 °C, 2h [59]	H	decomposition[b]
10	CBr_4 (0.2 equiv.), PPh_3 (0.2 equiv.), DCM (0.2 M), 40 °C, 2 h[60]	H	decomposition[b]
11	$SnCl_4$ (0.1 equiv.), DCM (0.2 M), 0 °C, 1 h[61]	H	decomposition[b]
12	$MgBr_2{\cdot}Et_2O$ complex (5.0 equiv.), DCM (0.5 M), 0 °C, 1 h	H	decomposition[b]
13	TMSOTf (2.0 equiv.), 2,2'-dipyridyl (3.0 equiv.) AcCN (0.1 M), 0 °C, 8 h	H	**1-124** (traces) and decompostition[b]
14	TMSOTf (2.0 equiv.), 2,2'-dipyridyl (3.0 equiv.) AcCN (0.1 M), 0 °C, 8 h	CH_3	**1-124** (traces) and decompostition[b]
15	$BF_3{\cdot}Et_2O$ complex (5.0 equiv.), DCM (0.05 M), 0 °C, 2 h	CH_3	decomposition[b]
16	$BF_3{\cdot}Et_2O$ complex (0.5 equiv.), DCM (0.05 M), 0 °C, 1 h	CH_3	decomposition[b]
17	NaI (5.0 equiv.), TMSCl (1.5 equiv.), AcCN (0.02 M), RT, 4 h [62]	CH_3	20 %[a]
18	NaI (5.0 equiv.), TMSCl (5.0 equiv.), AcCN (0.04 M) DCM (0.04 M), RT, 4 h [63]	CH_3	decomposition[b]

a) Isolated yield after column chromatography on silica gel.
b) Based on the analysis of the ^{1}H NMR spectra of the crude reaction mixture.

The removal of the TBS group from compounds **1-122b** and **1-123b** was quite straightforward, since both compounds provided by treatment with TBAF in THF the desired desilylated products **1-124** and **1-125** in excellent yields of 93 % and 91 %, respectively. Accidentally, it was also observed that the reaction could also be carried out at -40°C without any observable decrease in the reaction yields. (**Scheme 14**).



1-122b R^1 = OMe
1-123b R^1 = H

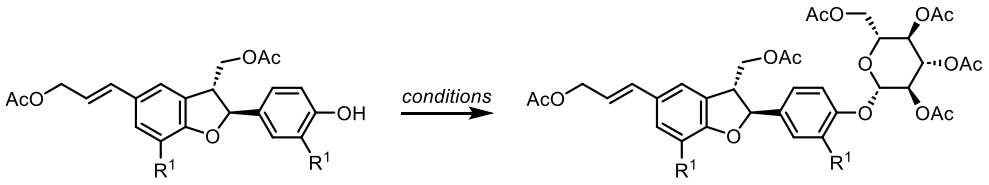
1-124 R^1 = OMe, 93 %
1-125 R^1 = H, 91 %

Scheme 14: Deprotection of TBS group.

1.4.9. Introduction of glucose

Having a coupling partner available for glucose introduction, a glycosylation reaction could be attempted (**Table 7**). Our first attempts at the reunion of the glucose derivative with the phenolic compounds started with well-known protocols in the literature that exploit the use of $AgCO_3$[64] and Ag_2O[65] salts as a coupling promoters. However, in all attempted cases, the desired products **1-126** and **1-127** were not formed since either no conversion of the starting phenols **1-124** or **1-125** was observed or the conversion was very low, and no traces of products were detected (**entry 1-8**). The use of stronger Lewis acids such as AgOTf[66] or $BF_3.Et_2O$ led to substrate decomposition (**entries 9-11**), and even the change in the glucose mode of activation (from bromide to imidate[67]) did not result in the product formation (**entry 12-14**). Finally, the Mitsunobu protocol saved the day and allowed us to isolate the desired **1-126** in 52 % yield. By slight modifications in the reaction conditions (reaction time and temperature), the desired product **1-126** was isolated in 91 % (**entry 17**). Those optimized reaction conditions were not attempted on phenol **1-125** for two reasons: (**1**) at that moment we have run out of the starting material and (**2**) before it could be rebuilt, the results of the biological evaluation of **DCG-A** for its cytokinin-like properties were obtained and the project was cancelled.

Table 7: Coupling of the phenolic intermediates **1-124** and **1-125** with glucose derivatives.



1-124 R^1 = OMe
1-125 R^1 = H

1-126 R^1 = OMe
1-127 R^1 = H

Entry	Conditions	R^1	Yields [%][a] /comments
1	2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (2.25 equiv.), py (0.05 M), $AgCO_3$ (4.0 equiv.), RT, 48 h	H	No conversion[b]
2	2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (1.1 equiv.), py (0.05 M), K_2CO_3 (10.0 equiv.), $AgCO_3$ (4.0 equiv.), RT, 24 h	CH_3	Low conversion[b]
3	2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (1.1 equiv.), acetone (0.03 M), K_2CO_3 (10.0 equiv.), $AgCO_3$ (4.0 equiv.), RT, 24 h	H	No conversion[b]
4	2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (1.1 equiv.), DCM (0.05 M), 2,6-lutidine (4.0 equiv.), $AgCO_3$ (4.0 equiv.), RT, 18 h	CH_3	Low conversion[b]
5	2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (1.2 equiv.), DCM (0.05 M), K_2CO_3 (10.0 equiv.), $AgCO_3$ (4.0 equiv.), RT, 24 h	CH_3	Low conversion[b]
6	2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (1.2 equiv.), 2,6-lutidine (4.0 equiv.), Ag_2O (4.0 equiv.), DCM (0.05 M), RT, 18 h	CH_3	decomposition[b]
7	2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (1.2 equiv.), Ag_2O (4.0 equiv.), py (0.05 M), RT, 18 h	CH_3	decomposition[b]
8	2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (1.2 equiv.), Ag_2O (4.0 equiv.), AcCN (0.05 M), RT, 18 h	CH_3	Low conversion[b]
9	2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (1.2 equiv.), AgOTf (4.0 equiv.), AcCN (0.09 M), RT, 18 h	CH_3	decomposition[b]
10	2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (1.2 equiv.), AgOTf (4.0 equiv.), py (0.05 M), RT, 18 h	CH_3	decomposition[b]
11	2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl bromide (1.0 equiv.), $BF_3{\cdot}Et_2O$ complex (1.1 equiv.), DCM (0.3 M), 0 °C to RT, 8 h	CH_3	decomposition[b]
12	(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (0.7 equiv.), TMSOTf (0.2 equiv.), DCM (0.3 M), 0 °C to RT, 18 h[67]	H	decomposition[b]

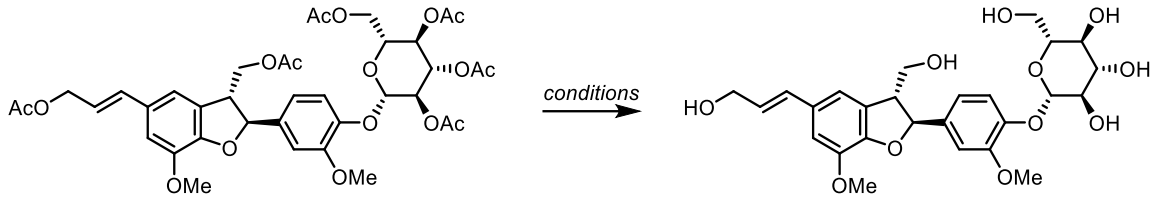
Entry	Conditions	R^1	Yields [%][a] /comments
13	(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-(2,2,2-trichloro-1-iminoethoxy) tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (0.7 equiv.), TMSOTf (0.2 equiv.), DCM (0.3 M), 0 °C to RT, 18 h[67]	H	decomposition[b]
14	(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-(2,2,2-trichloro-1-iminoethoxy) tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (1.0 equiv.), TMSOTf (0.1 equiv.), TEA (1.0 equiv.) DCM (0.1 M), -30 °C to RT, 18 h [68]	H	decomposition[b]
15	2,3,4,6-Tetra-O-acetyl-D-glucopyranose (1.5 equiv.), PPh_3 (1.5 equiv.), DIAD (1.5 equiv.), THF (0.02 M), 30 °C, 30 min µW (300 W)	CH_3	52[a]
16	2,3,4,6-Tetra-O-acetyl-D-glucopyranose (2. equiv.), PPh_3 (1.5 equiv.), DIAD (1.5 equiv.), THF (0.02 M), 40 °C, 30 min µW (300 W)	CH_3	74[a]
17	2,3,4,6-Tetra-O-acetyl-D-glucopyranose (2.0 equiv.), PPh_3 (1.5 equiv.), DIAD (1.5 equiv.), THF (0.02 M), 50 °C, 45 min µW (300 W)	CH_3	91[a]

a) Isolated yield after column chromatography on silica gel.
b) Based on the ^{1}H NMR spectra of the crude reaction mixture analysis.

1.4.10. Final step – selective acetyl groups

The last step of the sequence was the deprotection of the six acetyl groups of **1-126** (**Table 8**). First, a simplest reaction protocol based on the use of MeONa [69] was attempted; however, decomposition of the starting material or product was observed (**entry 1** and **2**). The change in reaction protocols (temperature, concentration, solvents, MeONa equivalents) furnished the desired conjugate **1-108** (**DCG-A**) only in low yields (**entry 3** to **5**). Thus, we moved our attention to the use of K_2CO_3[70], where the use of excess base (**entry 6**) led to complete decomposition while when 0.4 equivalents of K_2CO_3 were used, the desired product was isolated with a yield of 28 % (**entry 7**). The use of mild base TEA in a solvent mixture of MeOH/THF/H_2O resulted in the product **DCG-A** formation in 37 % yield. The best yield of compound **1-108** was achieved by employing 7N NH_3 in MeOH (**entry 8**). Under such reaction conditions, the desired product was finally obtained in 61 % isolated yield.

Table 8: Final step of the synthesis – acetyl group removal.



1-126 **DCG-A, 1-108**

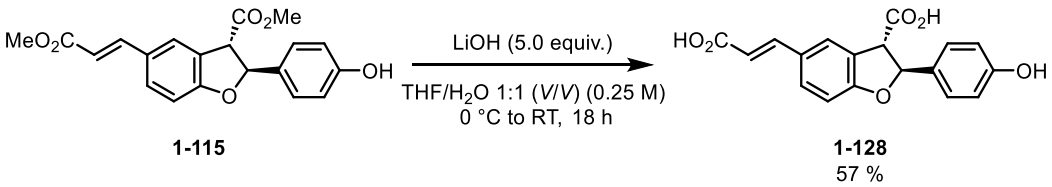
Entry	Conditions	Yields [%][a] /comments
1	NaOMe (1.0 equiv., 0.1 M), AcCN (1 M), 0 °C to RT, 8 h[c]	decomposition[b]
2	NaOMe (1.0 equiv., 0.1 M), MeOH (0.1 M), 0 °C to RT, 8 h[c]	decomposition[b]
3	NaOMe (0.3 equiv., 0.1 M), MeOH (0.02 M), 0 °C to RT, 1 h[c]	20
4	NaOMe (1.0 equiv., 0.1 M), AcCN (0.1 M), 0 °C to RT, 4 h[c]	33
5	NaOMe (1.0 equiv., 0.1 M), MeOH (0.1 M), 0 °C to RT, 3 h[d]	41
6	K_2CO_3 (7.0 equiv.), MeOH (0.1 M), 0 °C to RT, 1 h	decomposition[b]
7	K_2CO_3 (0.4 equiv.), MeOH (0.04 M), 0 °C to RT, 2 h	28
8	TEA (0.08 M), MeOH (0.08 M), THF (0.08 M), H_2O (0.08 M), RT, 18 h[71]	37
9	7N NH_3 in MeOH (0.05 M), RT, 24 h	61

a) Isolated yield after column chromatography on silica gel.
b) Based on the ^{1}H NMR spectra of the crude reaction mixture analysis.
c) Commercially available MeONa was used.
d) Freshly prepared MeONa generated by dissolving Na in MeOH was used.

1.4.11. Further reactivity of 8,5' dimers

As mentioned in the introduction, our goal of the project was to constitute a library of the desired neolignan core containing compounds that would be structurally related to **DCG-A**. Thus, various modifications of already prepared compounds were introduced with the aim of broadening the functional group and structural variety. Our attempts towards this goal are summarized within this chapter.

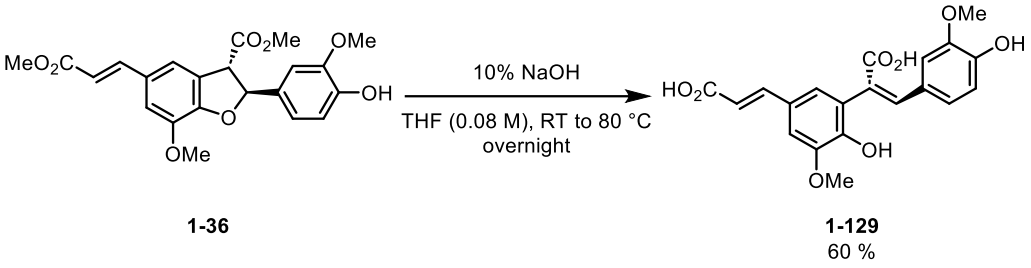
Carboxylic diacid 1-128. One of our goals was to prepare the dicarboxylic acid derivative **1-128**. To do so, we have tried NaOH in H_2O which resulted in decomposition. However, LiOH in the mixture of THF/H_2O resulted in a 57 % isolated yield (**Scheme 15**).



1-115 **1-128**
57 %

Scheme 15: Ester groups hydrolysis that led to diacid **1-128**.

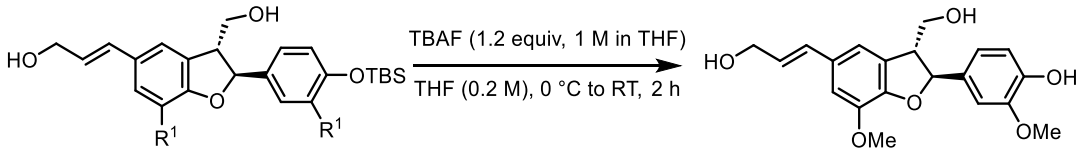
Opened diacid. Interestingly, if NaOH in THF was used to promote carboxylic acid hydrolysis, along with ester hydrolysis, the benzofuran motive also undergoes the base-initiated opening and generates compound **1-129** with a yield of 60 % (**Scheme 16**).



1-36 | **1-129** 60 %

Scheme 16: Formation of 8,5'-diferulic acid **1-129**.

Diol. Dehydrodi-*p*-coumaryl alcohol **1-131** and dehydrodiconiferyl alcohol **1-130** were prepared by TBAF-triggered TBS group removal in **1-118b** and **1-119f** (**Scheme 17**).

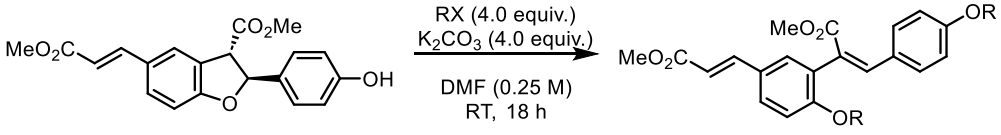


1-118b R^1 = OMe
1-119f R^1 = H

1-130 R^1 = OMe, 80 %
1-131 R^1 = H, 83 %

Scheme 17: Deprotection of the TBS group.

Opened diesters. It was observed that if phenol **1-115** is reacted with the mild base in the presence of alkylating reagents, instead of the phenolic group alkylation product, the reaction yields the product of the benzofuran opening where both *in situ* generated phenolic groups are alkylated (**Scheme 18**). Using such a protocol, three different products, all of which vary in the alkylating agent used, were prepared in good to very good yields.

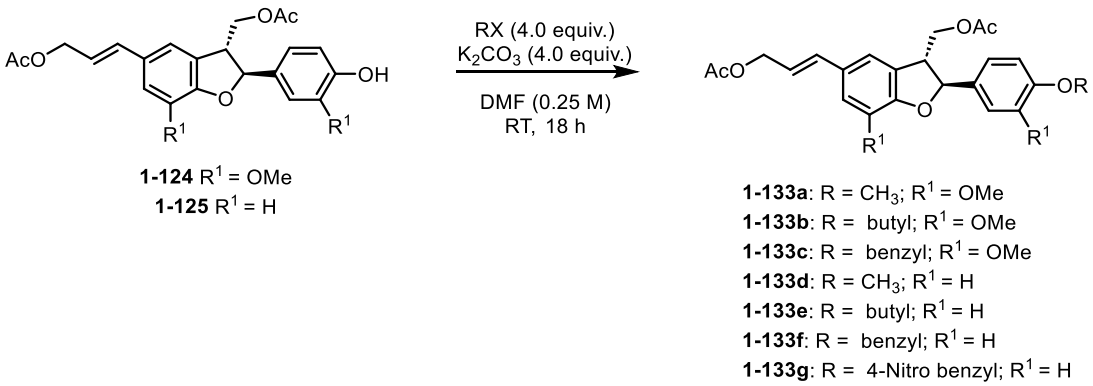


1-115

1-132a: R = CH_3; 79 %
1-132b: R = butyl; 58 %
1-132c: R = benzyl; 78 %

Scheme 18: Ring opening under basic alkylating conditions.

Alkylated phenol. Interestingly, when the same reaction conditions were applied to the reduced and acylated form of the same starting material, no product of the benzofurane skeleton was observed (**Scheme 19**).



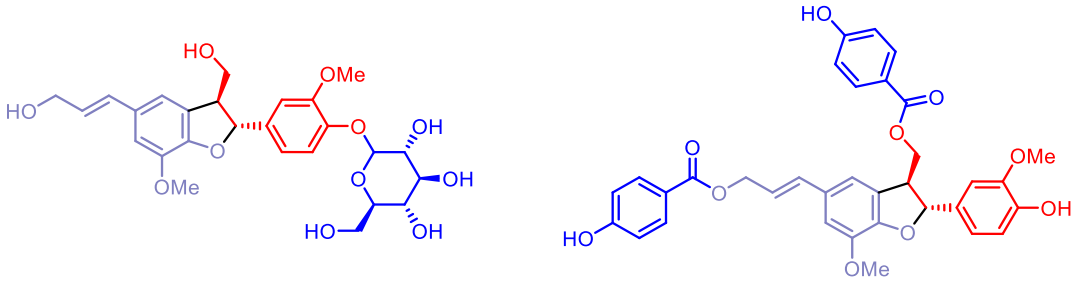
1-124 R^1 = OMe
1-125 R^1 = H

1-133a: R = CH_3; R^1 = OMe
1-133b: R = butyl; R^1 = OMe
1-133c: R = benzyl; R^1 = OMe
1-133d: R = CH_3; R^1 = H
1-133e: R = butyl; R^1 = H
1-133f: R = benzyl; R^1 = H
1-133g: R = 4-Nitro benzyl; R^1 = H

Scheme 19: Alkylation of **1-124** with various alkylating agents.

1.4.12. Toward quiquesetinerviusin A†

Quiquesetinerviusin A **1-134** is a natural product with a scaffold similar to **DCG-A** (**1-108**) (**Figure 23**). It was isolated from the *Calamus quiquesetinervius* stem in 2010 and its antioxidant activity was determined to be IC_{50} = 23.4 ± 3.7 µM.[72] Quiquesetinerviusin A (**1-134**) was first isolated from a terrestrial plant, however, later it was discovered that the **1-134** type compound can be found more commonly in marine plants, more specifically in the *Posidonia oceanica* family (first identification in 2012).[73]



DCG-A
1-108

quiquesetinerviusin A
1-134

Figure 23: Structure of DCG-A (**1-108**) and quiquesetinerviusin A (**1-134**).

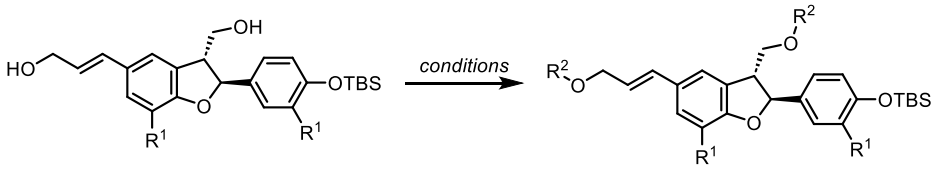
Although the first total synthesis of **quiquesetinerviusin A** was achieved already in 2017[74], no extensive biological evaluation of the targeted compounds was yet performed. To shed more light on the biological activity of the compound and with another objective that placed this compound as a possible 'marker' allowing the determination of the age of the marine

† Work in this subchapter was done in collaboration with Sylvie Lévayová, whom I co-supervised during her bachelor Thesis project. As a consequence, some results are based on the previous work of Sylvie Lévayová and were published in her Bachelor Thesis. After her Thesis was defended, the synthetic route was reconsidered, and several steps were re-optimized, changed or resynthesized.

grass layers in sediments, our intermediates previously prepared and used for the DCG-A (**1-108**) synthesis were reused in the quiquesetinerviusin A (**1-134**) synthesis.

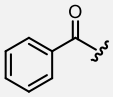
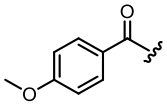
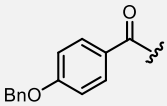
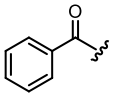
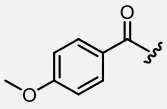
The synthesis began from the previously prepared diols **1-118b** and **1-119f**, which were acetylated with the corresponding benzoyl chlorides (**Table 9**). Using standard reaction conditions, the benzoyl derivative **1-135a** (42%), the 4-methoxybenzoyl derivatives **1-135b** (62%) and **1-135c** (61 %) were prepared along with their *des*-Methoxy analogues **1-136a** (54 %) and **1-136b** (62 %) in good to very good yields.

Table 9: Benzoylation of the free hydroxy groups. R^2 group introduction.



1-118b R^1 = OMe
1-119f R^1 = H

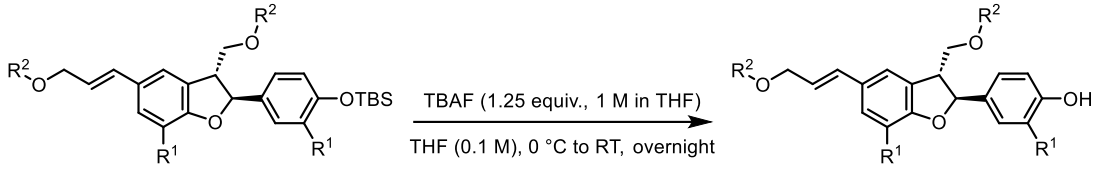
1-135 R^1 = OMe
1-136 R^1 = H

Entry	Conditions	R^1	R^2	Product	Yields [%][a]
1	BzCl (4.2 equiv.), TEA (6.0 equiv.) DCM (0.2 M), RT, 18 h	CH_3		**1-135a**	42
2	4-methoxy BzCl (4.2 equiv.), TEA (6.0 equiv.) DCM (0.2 M), RT, 18 h	CH_3		**1-135b**	62
3	4-(benzyloxy)benzoyl chloride (4.2 equiv.) TEA (6.0 equiv.), DCM (0.2 M), RT, 18 h	CH_3		**1-135c**	61
4	BzCl (4.4 equiv.), TEA (6.0 equiv.) DCM (0.2 M), RT, 18 h	H		**1-136a**	54
5	4-methoxy BzCl (4.2 equiv.), TEA (6.0 equiv.) DCM (0.2 M), RT, 18 h	H		**1-136b**	62

a) Isolated yield after column chromatography on silica gel.

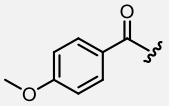
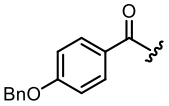
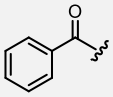
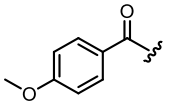
Similarly, the TBS group could be removed using TBAF in THF and the desired products **1-137a** (59 %), **1-137b** (61 %) and its *des*-Methoxy analogues **1-138a** (88%) and **1-138b** (71 %) were prepared in good yields. (**Table 10**).

Table 10: Removal of the TBS group in **1-135** and **1-136**.



1-135 R^1 = OMe
1-136 R^1 = H

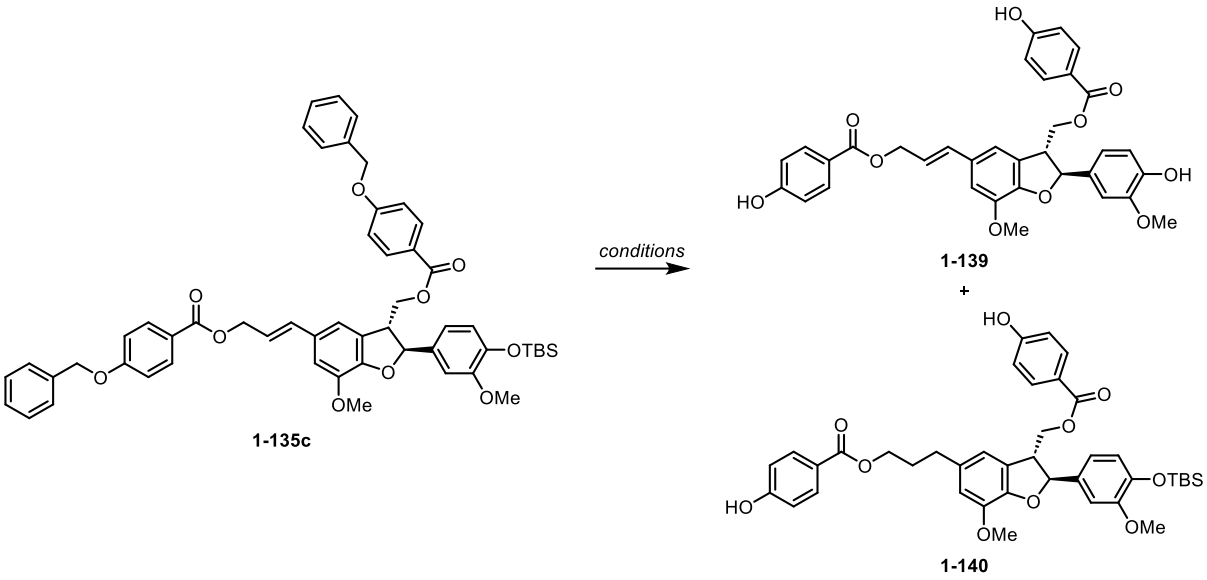
1-137 R^1 = OMe
1-138 R^1 = H

Entry	R^1	R^2	Product	Yields [%][a]
1	CH_3		**1-137a**	59
2	CH_3		**1-137b**	61
3	H		**1-138a**	54
4	H		**1-138b**	62

a) Isolated yield after column chromatography on silica gel.

Ultimately, our goal was to prepare quiquesetinerviusin A **1-134**. Various Lewis acids were used to selectively remove the benzyl protecting group from **1-137**, but none of the attempted conditions resulted in the formation of the desired product (**entry 1** to **3**). Disappointed with the result, the Pd/C promoted hydrogenation was used (**entry 4**). As expected, the benzyl group was removed; however, the olefinic bond was reduced at the same time. The product of deprotection/reduction, compound **1-140**, a TBS protected dihydroquiquesetinerviusin A was isolated in a yield of 67% (**Table 11**).

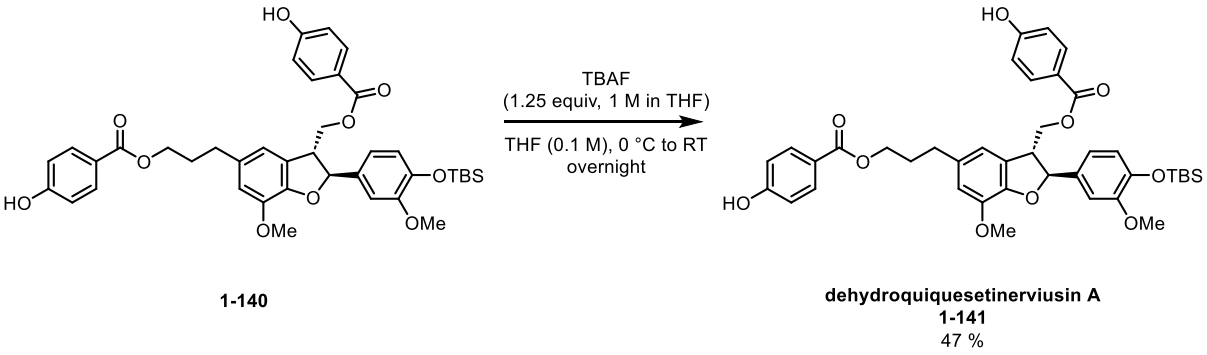
Table 11: Compound **1-139** and **1-140** synthesis.



Entry	Conditions	Product 1-139 [%][a]	Product 1-140 [%][a]	Comments
1	BCl_3 (3.5 equiv.), DCM (0.2 M) -78 °C to RT, 2 h	-	-	decomposition[b]
2	BCl_3 (3.5 equiv.), DCM (0.2 M) -78 °C to RT, 30 min	-	-	decomposition[b]
3	BBr_3 (2.5 equiv.), DCM (0.2 M) -78 °C to RT, 1 h	-	-	decomposition[b]
4	Pd/C (20 mol%), H_2, MeOH (0.05 M), RT, 2h	-	67	

a) Isolated yield after column chromatography on silica gel.
b) Based on the ^1H NMR spectra of the crude reaction mixture analysis.

The last step of the synthesis, protection of the TBS group, resulted in the formation of a dehydroquiquesetinerviusin A-**1-141** with a yield of 47 %.



Scheme 20: TBS group removal that yielded compound **1-141**.

1.5. Biological activity evaluation results

1.5.1. Anthelmintic activity

C. elegans is a free-living soil nematode commonly used as a model to study various aspects of human diseases[75], thus it is routinely used in drug screenings.[76] The toxicity of our compounds was evaluated by the chitinase assay followed by microscopic evaluation. The constituted library of compounds containing compounds that were prepared during my theses was tested for anthelmintic activity at concentrations of 50 and 5 µM concentrations. The standard anthelmintic drug ivermectin was used as a positive control in the experiment and DMSO was used as a negative control (**Figure 24**).

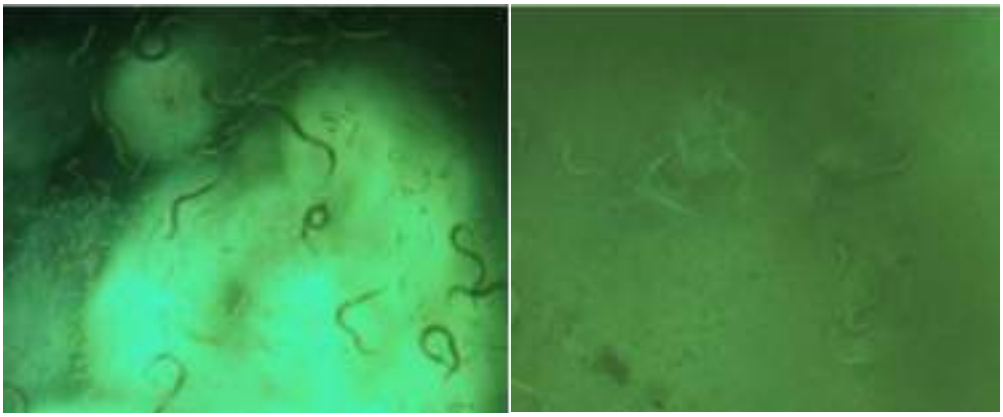


Figure 24: Typical microscope images obtained that are used to evaluate anthelmintic activity. A negative control (DMSO, left) and a positive control (ivermectin, right).

Based on the library evaluation, a compound **1-117c** was identified as a promising **HIT** compound, since it reduced the signal in the chitinase assay (quantifying egg hatching) at a concentration of 50 µM and 5 µM concentration. Activity values for representative examples of compounds from the evaluated chemical library are shown in **Figure 25**. All other compounds show none or unsignificant activity in the test.

The hit compound **1-117c** was further evaluated for its **structure-activity relationship** (SAR, **Figure 26**). Our study proved that part A of **1-117c** is essential to maintain the activity of the molecule. Based on the results obtained, we hypothesized that the Michael acceptor generated *in situ* in **1-117c** could be responsible for the observed biological activity (**Figure 27**). However, none of the prepared compounds that would possess such a structural motive (including the open form of neolignan) showed similar biological activity as compound **1-117c**, suggesting that our hypothesis is wrong.

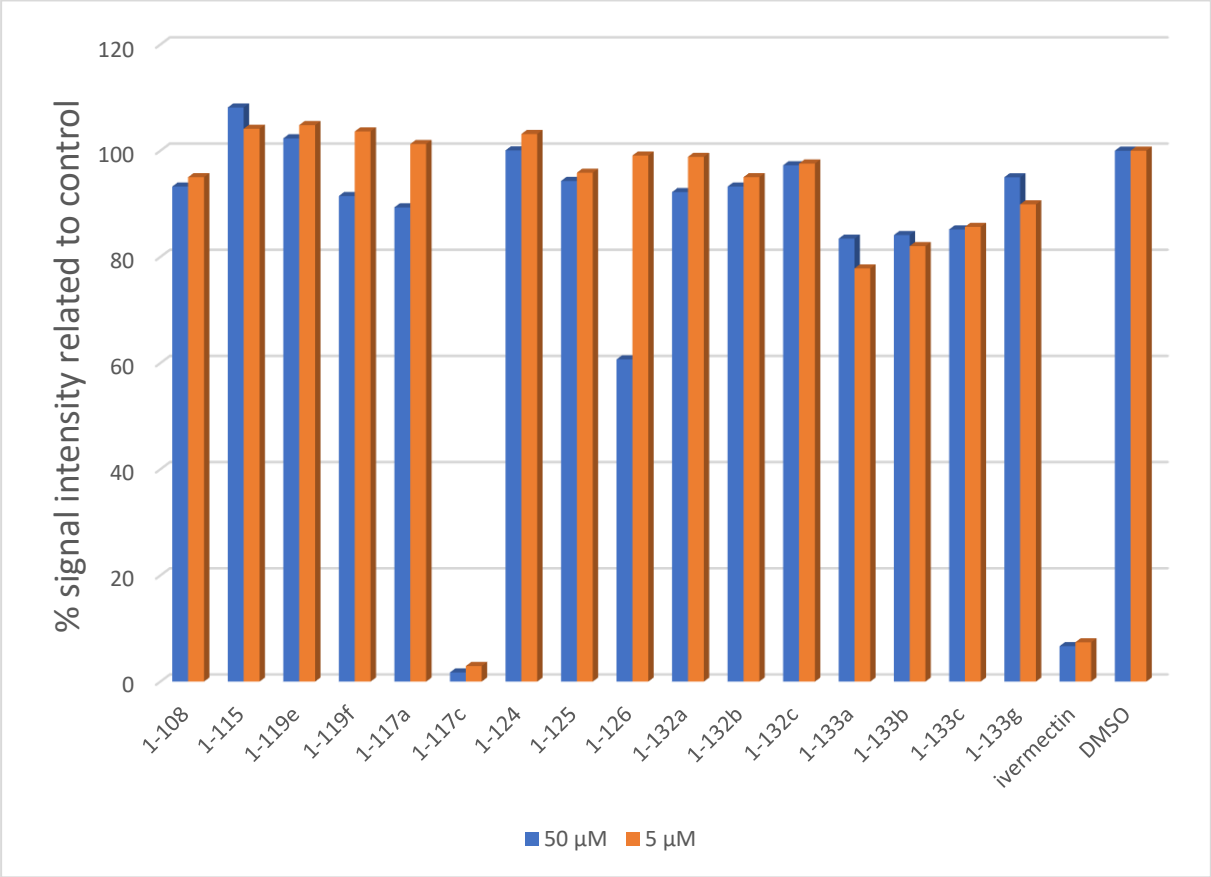


Figure 25: Anhelmintic activity of the evaluated chemical library (selected examples). Compounds were evaluated for their activity against the *Caenorhabditis elegans* model nematode by means of a chitinase assay. The depicted values are the average values from 3 independent experiments ± S.E.M. DMSO was used as negative control and ivermectine as positive control.

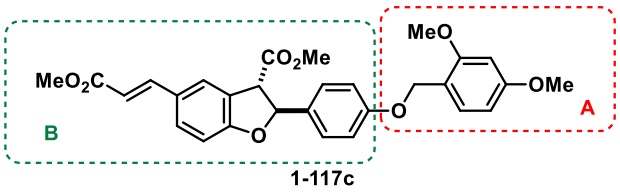


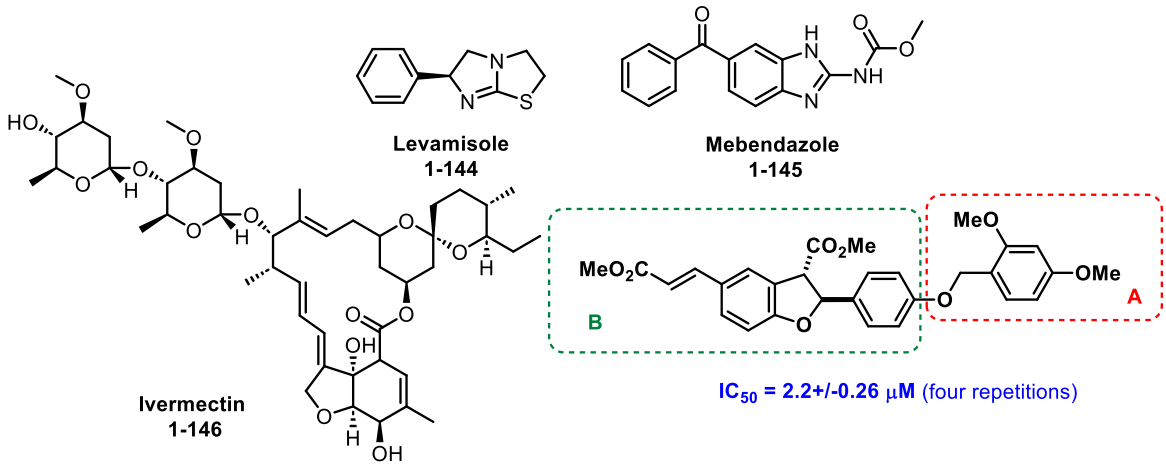
Figure 26: HIT compound **1-117c** and its evaluation within the SAR study (structural modifications). In turn, fragments A or B were modified.

Figure 27: The action of **1-117c** against *C. elegans* proceeds *via* an unknown mode of action that is currently under investigation.

Meanwhile, and to check if we should further develop our HIT compound, we have decided to evaluate compound **1-117c** against Invermectin, levamisol, and mebendazole (most commonly used drugs) resistant strains of *C. elegans* (**Table 12**). The compound **1-117c** has

been shown **to be highly active** against such strains, and therefore we are continuing in its development since (**a**) the mode of action of **1-117c differs** and (**b**) the mode of action is currently unknown.

Table 12: Commonly used antihelmintic drugs and IC_{50} values determined for **1-117c** in WT and *C. elegans* resistant lines.

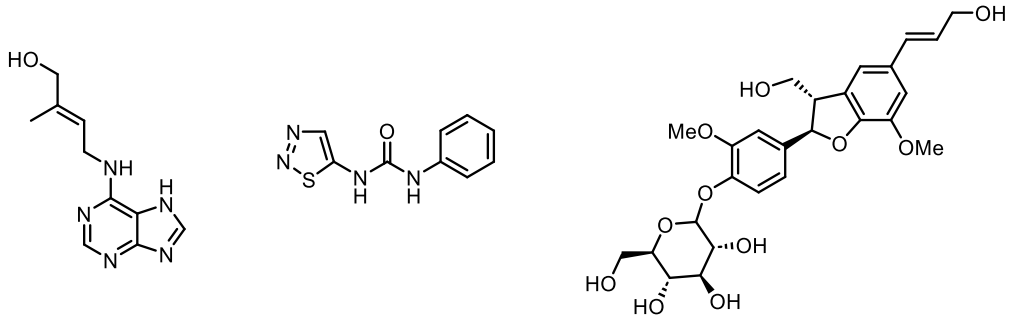


strains of *C. elegans*	IC_{50}	SD	S.E.M.
N2 (WT)	2,20	0,52	0,26
CB221 (resistant to levomine)	1,85	0,17	0,10
ZZ15 (levamisole resistant)	3,09	1,75	1,01
CB3474 (metadazole resistant)	3,00	0,53	0,27
DA1316 (resistant to ivermectin)	2,96	0,23	0,13

1.5.2. Cytokinin activity

The second type of biological activity that we have evaluated during my thesis was the cytokinin activity. Cytokinins represent an important group of plant hormones that regulate plant growth and development. Naturally occurring cytokinins are adenine derivatives, such as *trans*-zeatine (**1-147**) or phenylurea derivatives, such as thidiazuron (**1-148**) (**Figure 28**).[77] These compounds are plant hormones, which promote cell division or cytokinesis in plant shoots and roots. Interestingly, more than three decades ago, **DCG-A,** a phenylpropanoid-derived plant metabolite, was identified as a compound with cytokinin-substituting and cell division-promoting activity.[78] Despite the data published in 1991, there were no follow-up studies of **DCG-A** mode of action, and its original activity has never been explored to the best of our knowledge by other researchers. At the same time as us, in 2023, Witvriuw et al. prepared **DCG-A** using its original synthetic protocol and carried

out various experiments and assays with the aim of proving the published activities. Unfortunately, no activity was observed. Based on those results, the group concluded that the original publication was wrong, and that **DCG-A** has no cytokinin-substituting activity.[79]



***trans*-zeatine**
1-147

thidiazuron
1-148

DCG-A
1-108

Figure 28: Structure of *trans*-zeatine **1-147,** thidiazuron **1-148**, and **DCG-A**.

This observation and conclusions were independently confirmed by our own research group by using different cytokinin bioassays approximately at the same time (*Amaranthus, tobacco callus,* wheat leaf senescence, and root elongation inhibition assay). In all these tests, **DCG-A** cytokinin activity was not observed (*trans*-zeatine was used as a positive standard) (**Figure 29** and **Figure 30**).

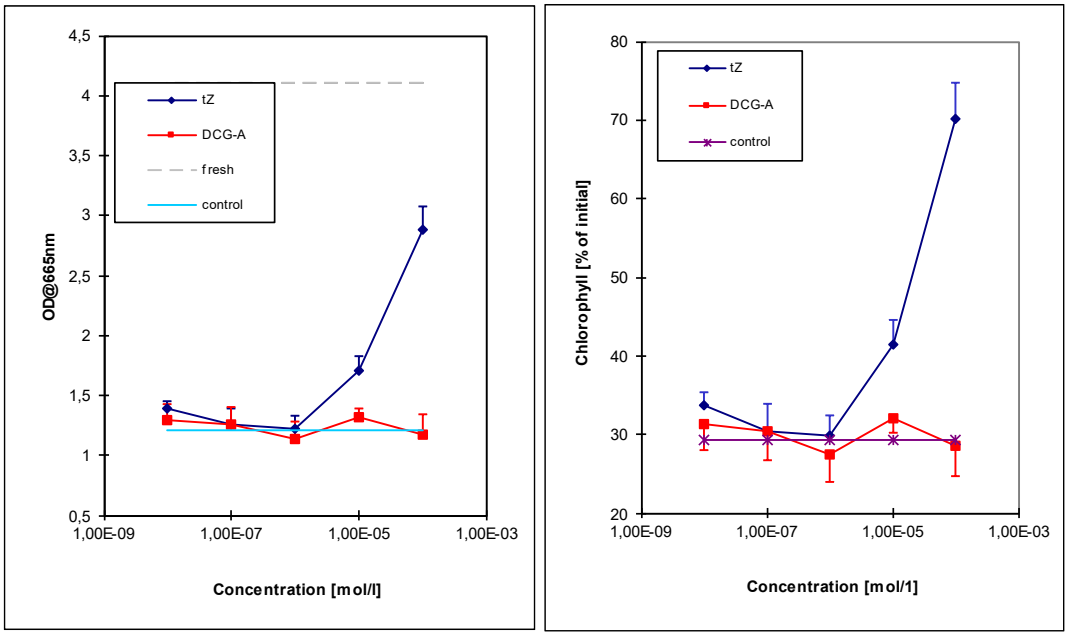


Figure 29: Dose response curves in *Amaranthus caudatus* var. *Artropurpurea* cotyledons treated with DCG-A and *trans*-zeatine. The dashed lines indicate values for the control treatment without any cytokinin. The error bars represent the standard deviation (n = 5).

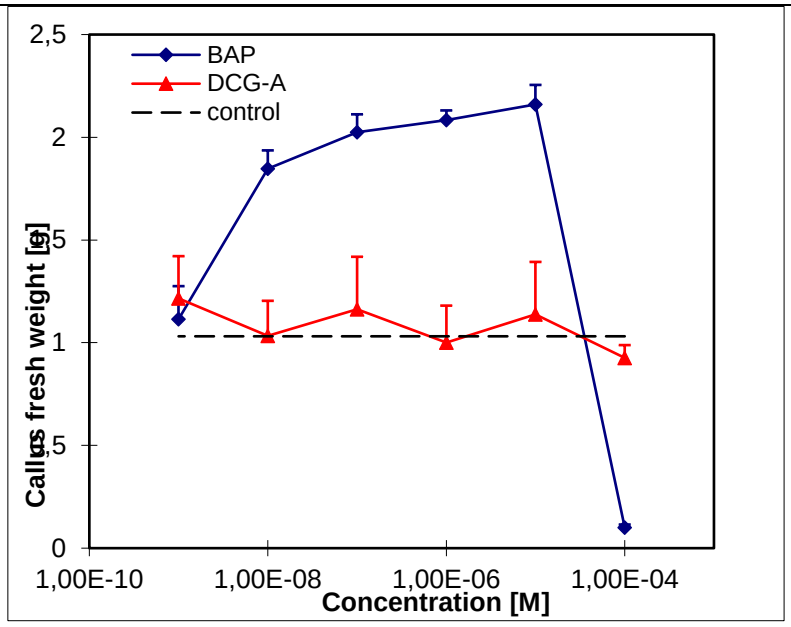


Figure 30: Tobacco callus bioassay, dose-response curves for cytokinin-dependent tobacco callus growth treated with a DCG-A and *trans*-zeatine. The dashed lines indicate values for the control treatment without any cytokinin. The error bars represent the standard deviation (n = 6).

1.6. Conclusions

We have successfully developed and optimized a reaction pathway for the synthesis of **DCG-A** (**1-108**) and its analogues in moderate to good overall yields. Additionally, we have explored the reactivity of 8,5' dimers and constituted a chemical library of 50 compounds of such a structural motive. Furthermore, with a bachelor student Silvie Levayová we have described a pathway towards **dehydroquiquesetinerviusin A** (**1-141**) and its analogues.

Our prepared compounds were tested for their anthelmintic activity (evaluated by Dr. A. Kadlecová) and cytokinin activity (evaluated by Dr. H. Vylíčilová).

In particular, compound **1-117c** was identified to be highly active in a chitinase assay (quantifying egg hatching) at a concentration of 50 µM and at a concentration of 5 µM. The IC_{50} values for this compound were stated to be 2.20 µM ± 0.26. In addition, this compound showed activity against *C. elegans* strains resistant to invermectin, levamisole, and mebendazole (commonly used anthelmintic drugs), indicating a different mechanism of action, which is currently under investigation.

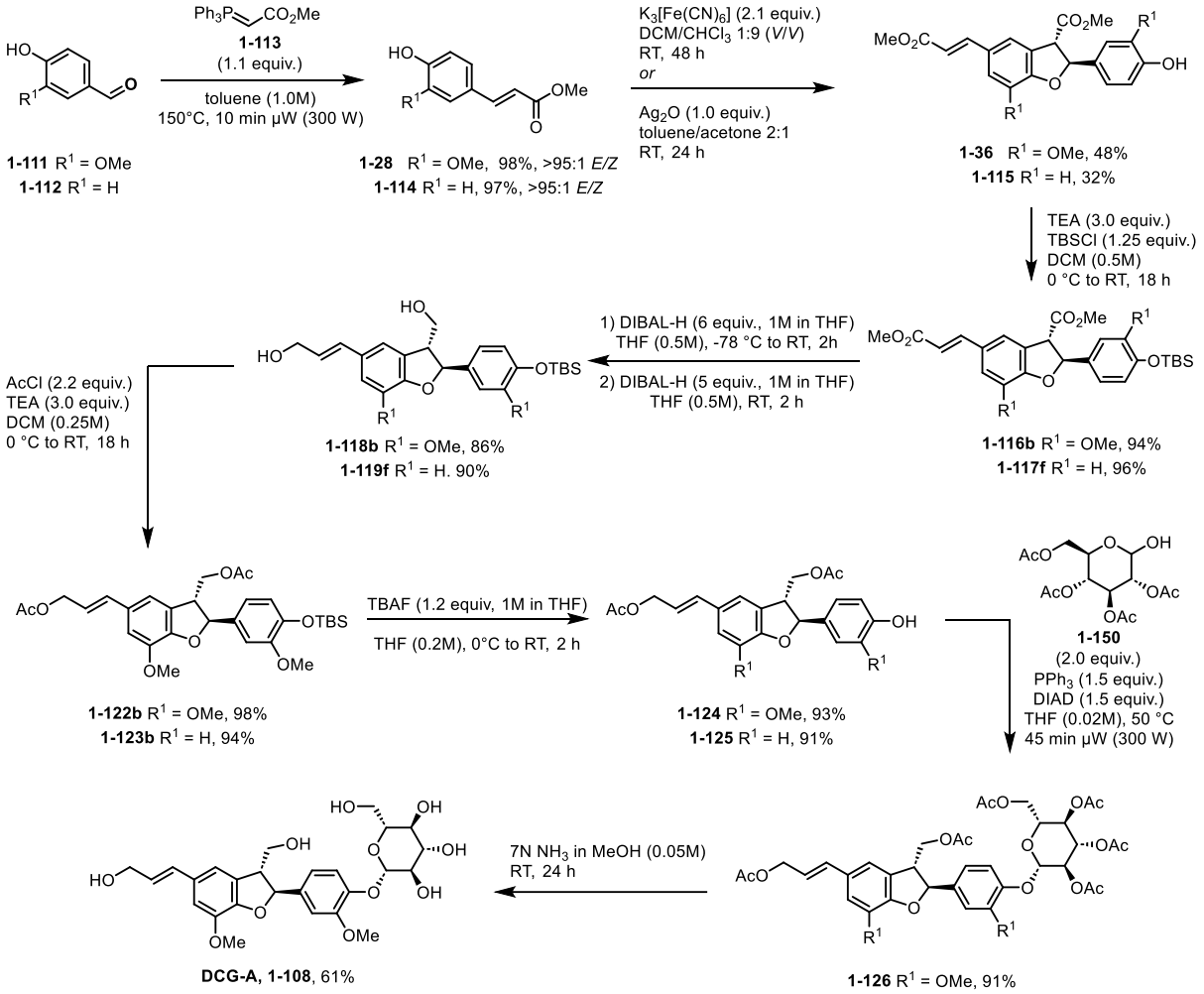
Next, cytokinin assays demonstrated that the **DCG-A** compound does not possess cytokinin activity, as it did not show significant cell division-promoting effects in standard callus and senescence assays.

In summary, our study not only established a robust synthetic pathway to **DCG-A** and its analogues but also provided valuable insights into their biological activities, highlighting the potential of these compounds in various biomedical applications. Future work will focus on further elucidating the mechanisms of action and expanding the biological evaluation of these compounds.

1.7. Experimental section

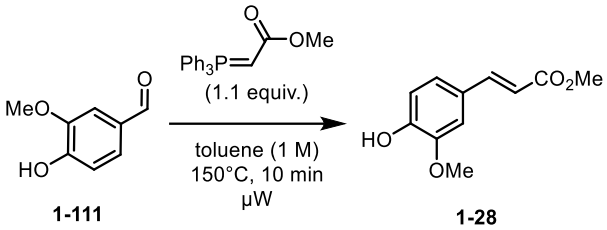
All starting materials were purchased from commercial suppliers and used without further purification, unless otherwise stated. All reactions were performed in round-bottom flasks fitted with rubber septa using standard laboratory techniques under positive pressure of argon (Air Liquide, >99.5% purity). In all reactions, unless stated otherwise, anhydrous solvents furnished by the Merck (Sigma-Aldrich) Company were used. The purification of reaction products was carried out by column chromatography using standard grade silica gel (60 Å, 230–400 mesh), or by preparative thin layer chromatography glass plates precoated with silica gel (silica gel G-200 F 254 , particle size 0.040−0.063 mm). Analytical thin-layer chromatography was performed on a thin-layer chromatography (TLC) aluminum plates pre-coated with silica gel (silica gel 60 F 254). Visualization was accomplished with UV light, phosphomolybdic acid and potassium permanganate stains, followed by heating. The ^{1}H NMR and ^{13}C{^{1}H} NMR spectra were measured on JEOL ECA400II (400 and 101 MHz) or JEOL 500 ECA (500 and 126 MHz) in chloroform-*d*, acetone-d_6, DMSO-d_6 or methanol-d_4. Chemical shifts are reported in ppm, and their calibration was carried out (a) in the case of ^{1}H NMR experiments on the residual peak of non-deuterated solvent δ ($CDCl_3$) = 7.26 ppm or δ (CD_3OD) = 3.31 ppm, δ (DMSO-d_6)= 2.50 ppm, δ (acetone-d_6)= 2.05 ppm and in the case of ^{13}C NMR experiments on the middle peak of the ^{13}C signal in deuterated solvent δ ($CDCl_3$) = 77.16 ppm, δ (CD_3OD) = 49.00 ppm, (DMSO-d_6)= 39.52 ppm, (acetone-d_6)= 29.84 ppm. The proton coupling patterns are represented as a singlet (s), a doublet (d), a doublet of a doublet (dd), a triplet (t), a triplet of a triplet (tt), and a multiplet (m). High-resolution mass spectrometry (HRMS) was performed on LC chromatograph (Dionex UltiMate 3000, Thermo Fischer Scientific, MA, USA) and mass spectrometer Exactive Plus Orbitrap high-resolution (Thermo Fischer Scientific, MA, USA) with electrospray ionization (ESI) and a time-of-flight analyzer operating in a positive or negative full scan mode in the range of 100 − 1700 m/z. High-performance liquid chromatography (HPLC) was performed using an Agilent 1290 Infinity II system with UV-VIS detector and an Agilent InfinityLab LC/MSD mass detector. Purification using semiprep HPLC was carried out on Agilent 1290 Infinity II with UV-VIS and mass detector Agilent InfinityLab LC/MSD using the C18 reverse-phase column (Agilent 5Prep-C18 10x21.2 mm). The gradient was formed from water and methanol with a flow rate of 20 mL/min.

1.7.1. Synthesis of DCG-A and related compounds



Scheme 21: Synthesis of DCG-A and related compounds.

Methyl ferulate (**1-28**)



Was prepared according to[51]

Vanillin **1-111** (6 g, 39.4 mmol, 1.0 equiv.) was dissolved in toluene (1 M, 39.4 mL), and phosphonium ylide (14.7 g, 43.4 mmol, 1.1 equiv.) was added. Mixture was placed to μW vial at RT. The vial was closed with Teflon tap and the whole mixture was heated to 150 °C for 10 min in the microwave reactor (300 W). After cooling to RT, organic solvents were removed under reduced pressure and the conversion of the ester was analyzed with help of the TLC. In case of incomplete conversion, the reaction was repeated. When the starting Vanillin was consumed, the crude product was purified by flash column chromatography (SiO_2; petroleum ether/EtOAc = 4:1 – 1:1) to afford **1-28** (8.1 g, 98 %).

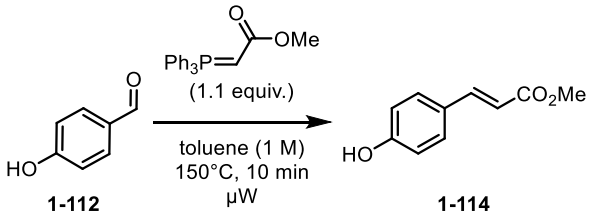
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): δ 7.35 (dd, *J* = 7. 6, 1.5 Hz, 1H), 7.21 (td, *J* = 7.8, 1.7 Hz, 1H), 6.96 (td, *J* = 7.6, 1.2 Hz, 1H), 6.79 (dd, *J* = 7.9, 1.1 Hz, 1H), 5.18 (s, 1H), 3.73 (s, 2H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 152.8, 129.5, 129.3, 121.2, 117.7, 116.8, 115.1, 18.3.

MS (ESI) *m/z* (%): 209 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{11}H_{13}O_4$: 209.0808; found: 209.0815.

Methyl *p*-coumarate (**1-114**)



Was prepared according to[51]

4-hydroxybenzaldehyde **1-112** (6 g, 39.4 mmol, 1.0 equiv.) was dissolved in toluene (1 M, 39.4 mL), and phosphonium ylide (14.7 g, 43.4 mmol, 1.1 equiv.) was added. Mixture was placed to μW vial at RT. The vial was closed with Teflon tap and the whole mixture was heated to

150 °C for 10 min in the microwave reactor (300 W). After cooling to RT, organic solvents were removed under reduced pressure and the conversion of the ester was analyzed with help of the TLC. In case of incomplete conversion, the reaction was repeated. When the starting Vanillin was consumed, the crude product was purified by flash column chromatography (SiO_2; petroleum ether/EtOAc = 4:1 – 1:1) to afford **1-114** (8 g, 97 %).

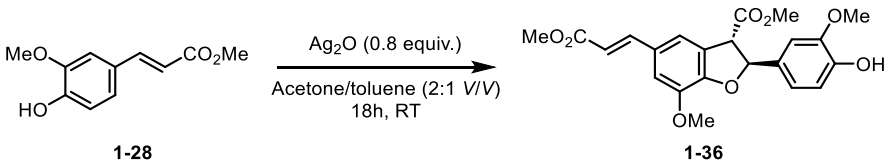
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): δ 7.64 (d, *J* = 15.9 Hz, 1H), 7.49 – 7.39 (m, 2H), 6.88 – 6.82 (m, 2H), 6.31 (d, *J* = 16.0 Hz, 1H), 5.18 (s, 1H), 3.80 (s, 3H).

^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$): 168.0, 157.6, 144.6, 130.1, 127.4, 115.9, 115.4, 51.8.

MS (ESI) *m/z* (%): 178 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{10}H_{11}O_3$: 179.0703; found: 179.0702.

(±)-dehydrodiferrulate dimethyl ester (**1-36**)



1-28 **1-36**

1-28 (16.4 g, 78.8 mmol, 1.0 equiv.) was dissolved in dry toluene/acetone (2:1 *V*/*V*, 600 mL), and flask was covered with aluminum foil. Next Ag_2O (14.6 g, 63 mmol, 0.8 equiv.) was added. After stirring for 18 h under aluminum foil, the mixture was filtered through a silica pad and evaporated under reduced pressure. The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 6:1 → 2:1) to afford **1-36** as a white solid (7.6 g, 48 %).

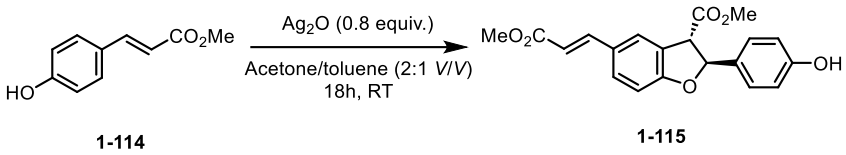
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): δ 7.65 (d, *J* = 15.9 Hz, 1H), 7.19 (t, *J* = 1.4 Hz, 1H), 7.02 (d, *J* = 2.0 Hz, 1H), 6.91 (dd, *J* = 3.6, 1.4 Hz, 3H), 6.32 (d, *J* = 15.9 Hz, 1H), 6.11 (d, *J* = 8.2 Hz, 1H), 5.64 (s, 1H), 4.35 (dt, *J* = 8.1, 0.8 Hz, 1H), 3.92 (s, 3H), 3.88 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 170.9, 167.8, 150.1, 146.8, 146.2, 144.9, 144.9, 131.5, 128.7, 125.8, 119.6, 118.1, 115.7, 114.7, 112.2, 108.8, 87.6, 56.3, 56.2, 55.6, 53.0, 51.8.

MS (ESI) *m/z* (%): 415 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{22}H_{23}O_8$: 415.1387 found: 415.1391.

(±) dehydrodicoumaric acid dimethyl ester (**1-115**)



1-114 **1-115**

1-114 (22 g, 123 mmol, 0.5 equiv.) was dissolved in solution of chloroform/dichloromethane (9:1 *V*/*V*, 610 mL), and the resulting mixture was stirred at RT for 5 min. Next potassium ferricyanide (97.6 g, 296 mmol, 1.2 equiv.) was dissolved in $NaHCO_3$ (0.45 M, 560 mL), and resulting brown solution was added dropwise into the mixture during 1h under argon atmosphere. Resulting mixture was stirred for 48 h at RT under argon atmosphere. Mixture was than evaporated and the water phase was extracted with EtOAc (4 x 400 mL), and combined organic layers were washed with brine (400 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 8:1 → 2:1) to afford **1-115** as a white solid (7 g, 32 %).

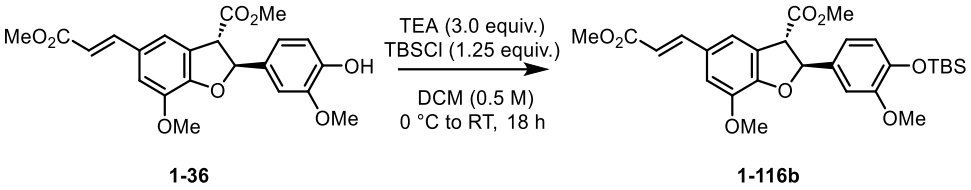
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): δ 7.66 (d, *J* = 15.9 Hz, 1H), 7.55 (t, *J* = 1.5 Hz, 1H), 7.42 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.27 (d, *J* = 2.1 Hz, 1H), 7.26 (d, *J* = 2.2 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.85 – 6.82 (m, 2H), 6.32 (d, *J* = 16.0 Hz, 1H), 6.09 (d, *J* = 7.5 Hz, 1H), 5.59 – 5.16 (m, 1H), 4.27 (d, *J* = 7.5 Hz, 1H), 3.84 (s, 3H), 3.80 (s, 4H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): δ 171.0, 168.1, 161.3, 156.2, 144.8, 132.2, 131.0, 127.9, 127.7, 125.2, 125.1, 115.8, 115.3, 110.5, 86.5, 55.2, 53.1, 51.9.

MS (ESI) *m/z* (%): 355 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{20}H_{19}O_6$: 355.1176; found: 355.1180.

Methyl 2-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)-7-methoxy-5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)-2,3-dihydrobenzofuran-3-carboxylate (**1-116b**)



1-36 **1-116b**

1-36 (1.1 g, 2.65 mmol, 1.0 equiv.) was dissolved in dry DCM (0.5 M, 6.21 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C TBSCl (0.5 g, 3.32 mmol, 1.25 equiv.) was added following addition of TEA (1.11 mL, 7.96 mmol, 3 equiv.). The resulting mixture was stirred at RT overnight. Next day 50 mL of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 40 mL). Combined organic layers were washed with brine (40 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO2; petroleum ether/EtOAc = 2:1) to afford **1-116b** as a white solid (1.33 g, 94 %).

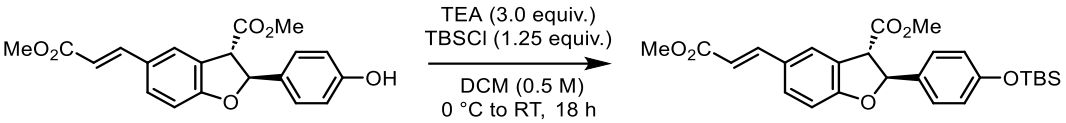
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.65 (d, *J* = 15.9 Hz, 1H), 7.22 – 7.17 (m, 1H), 7.03 – 7.00 (m, 1H), 6.88 – 6.79 (m, 3H), 6.32 (dd, *J* = 15.9, 0.7 Hz, 1H), 6.12 (d, *J* = 8.0 Hz, 1H), 4.36 (dd, *J* = 8.1, 1.0 Hz, 1H), 3.92 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 0.98 (s, 10H), 0.13 (s, 6H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 170.9, 167.8, 151.3, 150.1, 145.7, 144.9, 144.9, 133.0, 128.7, 125.9, 121.2, 118.9, 118.1, 115.7, 112.1, 110.3, 87.6, 56.3, 55.7, 55.6, 53.1, 51.8, 25.8, 18.6, -4.5.

MS (ESI) *m/z* (%): 529 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{28}H_{37}O_8Si$: 529.2252; found:529.2253.

Methyl 2-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)-2,3-dihydrobenzofuran-3-carboxylate (**1-117f**)



1-115 **1-117f**

1-115 (1.1 g, 3.1 mmol, 1.0 equiv.) was dissolved in dry DCM (0.5 M, 6.21 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C TBSCl (0.59 g, 3.88 mmol, 1.25 equiv.) was added following addition of TEA (1.29 ml, 9.31 mmol, 3 equiv.). The resulting mixture was stirred at RT overnight. Next day 50 ml of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 40 mL). Combined organic layers were washed with brine (40 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO2; petroleum ether/EtOAc = 2:1) to afford **1-117f** as a white solid (1.42 g, 96 %).

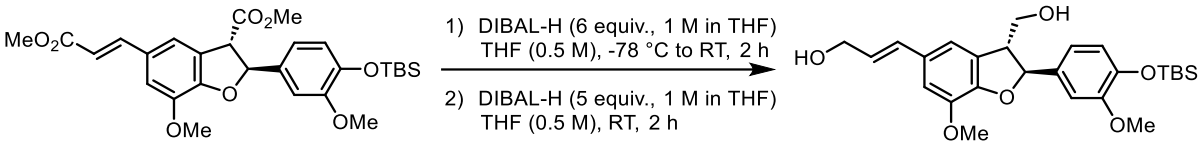
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.66 (d, *J* = 16.0 Hz, 1H), 7.57 – 7.55 (m, 1H), 7.43 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.26 – 7.22 (m, 2H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.87 – 6.78 (m, 2H), 6.32 (d, *J* = 16.0 Hz, 1H), 6.10 (d, *J* = 7.5 Hz, 1H), 4.28 (d, *J* = 7.5 Hz, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 0.97 (s, 9H), 0.21 – 0.15 (m, 6H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.0, 167.9, 161.3, 156.2, 144.7, 132.7, 130.9, 127.9, 127.3, 125.2, 125.1, 120.5, 115.4, 110.4, 86.6, 55.2, 53.0, 51.8, 29.8, 25.8, 18.3, -4.3.

MS (ESI) *m/z* (%): 469 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{26}H_{33}O_6Si$: 469.2041; found: 469.2044.

(*E*)-3-(2-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl)prop-2-en-1-ol (**1-118b**)



1-116b **1-118b**

A solution of ester **1-116b** (3 g, 5.67 mmol, 1.0 equiv.) in THF (0.25 M, 22.7 mL) was cooled to - 78 °C and DIBAL-H (1 M in THF, 34 mL, 34 mmol, 6 equiv.) was added dropwise over 10 minutes. The resulting mixture was stirred at - 78 °C for 30 min before the cooling bath removal. To achieve full reduction, after 1h at RT, 5 more equivalents of DIBAL-H were added, and the reaction mixture was stirred for another 1 hour at RT. Than reaction was cooled to -78 °C and Aqueous saturated solution of Rochel salt (100 mL) was added carefully dropwise to quench the reaction. After addition of Rochel salt, solution was diluted with EtOAc 100 mL. The resulting mixture was stirred at RT till a milky suspension turned into the clear biphasic solution (usually takes around 16 h). Resulting phases were separated and the aqueous layer was extracted with DCM (3x 75mL). Combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2; DCM/MeOH = 95:5) to afford **1-118b** as a colorless oil (2.3 g, 86 %).

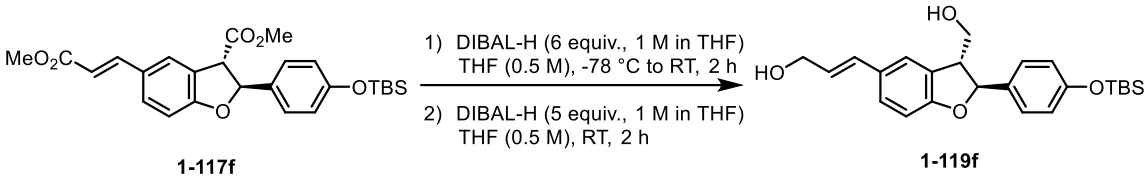
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 6.92 – 6.87 (m, 3H), 6.84 (dd, J = 8.1, 2.1 Hz, 1H), 6.80 (d, J = 8.1 Hz, 1H), 6.57 (dt, J = 15.8, 1.6 Hz, 1H), 6.25 (dt, J = 15.8, 5.9 Hz, 1H), 5.57 (d, J = 7.2 Hz, 1H), 4.31 (dd, J = 6.0, 1.5 Hz, 2H), 3.99 (dd, J = 11.0, 5.9 Hz, 1H), 3.93 – 3.91 (m, 1H), 3.91 (s, 3H), 3.77 (s, 3H), 3.64 (q, J = 5.8 Hz, 1H), 0.98 (s, 10H), 0.13 (s, 6H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 151.2, 148.6, 145.3, 144.6, 134.4, 131.5, 130.9, 128.3, 126.5, 121.0, 118.9, 114.9, 110.5, 110.3, 88.4, 64.1, 64.0, 56.1, 55.7, 53.6, 25.8, 18.6, -4.5.

MS (ESI) *m/z* (%): 473 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{26}H_{37}O_6Si$: 473.2354; found: 473.2355.

(*E*)-3-(2-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-3-(hydroxymethyl)-2,3-dihydrobenzofuran-5-yl)prop-2-en-1-ol (**1-119f**)



1-117f **1-119f**

A solution of ester **1-117f** (1.4 g, 2.99 mmol, 1.0 equiv.) in THF (0.25 M, 5.97 mL) was cooled to - 78 °C and DIBAL-H (1 M in THF, 17.9 mL, 17.9 mmol, 6 equiv.) was added dropwise over 10 minutes. The resulting mixture was stirred at - 78 °C for 30 min before the cooling bath removal. To achieve full reduction, after 1h at RT, 5 more equivalents of DIBAL-H were added, and the reaction mixture was stirred for another 1 hour at RT. Than reaction was cooled to -78 °C and Aqueous saturated solution of Rochel salt (100 mL) was added carefully dropwise to quench the reaction. After addition of Rochel salt, solution was diluted with EtOAc 100 mL. The resulting mixture was stirred at RT till a milky suspension turned into the clear biphasic solution (usually takes around 16 h). Resulting phases were separated and the aqueous layer was extracted with DCM (3x 75mL). Combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2; DCM/MeOH = 95:5) to afford **1-119f** as a colorless oil (1.11 g, 90 %).

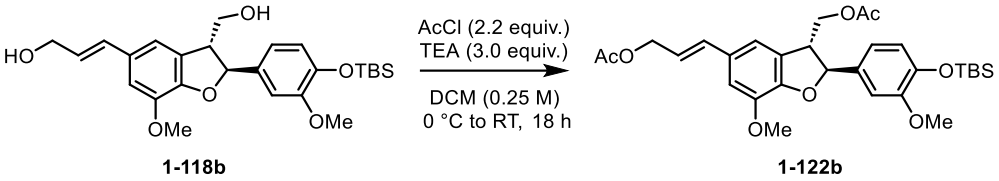
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.29 (t, *J* = 1.4 Hz, 1H), 7.25 – 7.21 (m, 3H), 6.85 – 6.79 (m, 3H), 6.57 (d, *J* = 15.9, 1.6 Hz, 1H), 6.23 (dt, *J* = 15.8, 6.0 Hz, 1H), 5.56 (d, *J* = 6.4 Hz, 1H), 4.30 (dd, *J* = 6.0, 1.5 Hz, 2H), 3.97 (dd, *J* = 10.9, 6.2 Hz, 1H), 3.92 (dd, *J* = 10.9, 5.1 Hz, 1H), 3.56 (q, *J* = 5.9 Hz, 1H), 0.97 (s, 9H), 0.18 (s, 6H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 160.0, 155.8, 134.2, 131.3, 129.9, 128.1, 127.6, 127.3, 126.0, 122.5, 120.4, 109.7, 87.3, 64.4, 64.0, 53.2, 25.8, 18.3, -4.3.

MS (ESI) *m/z* (%): 301 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{24}H_{33}O_4Si$: 413.2143; found: 413.2165.

(*E*)-3-(3-(acetoxymethyl)-2-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl)allyl acetate (**1-122b**)



1-118b **1-122b**

Diol **1-118b** (1.72 g, 3.64 mmol, 1.0 equiv.) was dissolved in DCM (0.25 M, 14.55 mL), and solution was cooled to 0 °C. Than TEA (1.51 mL, 10.9 mmol, 3 equiv.) was added following by acetyl chloride (0.57 mL, 8.01 mmol, 2.2 equiv.). The reaction mixture was stirred at room temperature overnight. Reaction was quenched by addition of water (50 mL). The aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **1-122b** as a colorless oil (2.0 g, 98 %).

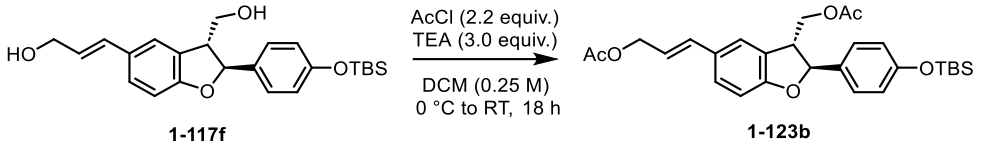
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 6.89 – 6.86 (m, 3H), 6.84 – 6.79 (m, 2H), 6.60 (dd, *J* = 15.7, 1.3 Hz, 1H), 6.16 (dt, *J* = 15.8, 6.7 Hz, 1H), 5.47 (d, *J* = 7.5 Hz, 1H), 4.71 (dd, *J* = 6.7, 1.3 Hz, 2H), 4.45 (dd, *J* = 11.2, 5.4 Hz, 1H), 4.30 (dd, *J* = 11.1, 7.6 Hz, 1H), 3.91 (s, 3H), 3.82 – 3.78 (m, 1H), 3.77 (s, 3H), 2.10 (s, 3H), 2.01 (s, 3H), 0.98 (s, 9H), 0.13 (d, *J* = 0.9 Hz, 6H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.1, 171.0, 151.3, 148.4, 145.4, 144.5, 134.6, 133.8, 130.5, 127.9, 121.2, 121.0, 119.1, 115.4, 110.5, 110.2, 89.1, 65.5, 65.4, 56.1, 55.7, 50.3, 25.8, 21.2, 21.0, 18.6, -4.5.

MS (ESI) *m/z* (%): 557 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{30}H_{41}O_8Si$: 595.2124; found: 595.2123.

(*E*)-3-(3-(acetoxymethyl)-2-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-2,3-dihydrobenzofuran-5-yl)allyl acetate (**1-123b**)



1-117f **1-123b**

Diol **1-117f** (0.45 g, 1.09 mmol, 1.0 equiv.) was dissolved in DCM (0.25 M, 4.36 mL), and solution was cooled to 0 °C. Than TEA (0.46 mL, 3.27 mmol, 3 equiv.) was added following by acetyl chloride (0.17 mL, 2.4 mmol, 2.2 equiv.). The reaction mixture was stirred at room temperature overnight. Reaction was quenched by addition of water (30 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **1-123b** as a colorless oil (0.51 g, 94 %).

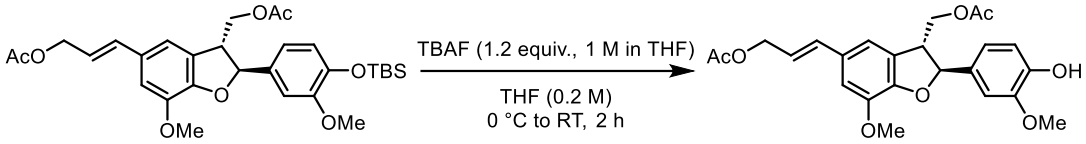
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.28 – 7.26 (m, 1H), 7.26 – 7.20 (m, 3H), 6.84 – 6.80 (m, 3H), 6.61 (d, *J* = 15.9 Hz, 1H), 6.14 (dt, *J* = 15.7, 6.7 Hz, 1H), 5.46 (d, *J* = 6.7 Hz, 1H), 4.70 (dd, *J* = 6.6, 1.3 Hz, 2H), 4.44 (dd, *J* = 11.1, 5.5 Hz, 1H), 4.29 (dd, *J* = 11.1, 7.8 Hz, 1H), 3.74 – 3.69 (m, 1H), 2.09 (s, 3H), 2.04 (s, 3H), 0.97 (s, 9H), 0.18 (s, 6H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.0, 170.9, 160.0, 155.9, 134.3, 133.5, 129.6, 128.5, 127.3, 127.0, 122.8, 120.8, 120.4, 109.8, 100.0, 88.0, 65.7, 65.4, 50.0, 25.8, 21.2, 20.9, 18.3, -4.3.

MS (ESI) *m/z* (%): 497 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{28}H_{36}KO_6Si$: 535.1913; found: 535.1913.

(±)-dehydrodiconiferyl diacetate (**1-124**)



1-122b **1-124**

1-122b (1.9 g, 3.41 mmol, 1.0 equiv.) was dissolved in THF (0.2 M, 17.1 mL), and solution was cooled to 0 °C. After 5 minutes TBAF (4.1 mL, 1 M, 4.1 mmol, 1.2 equiv.) was added dropwise and the solution was stirred at RT for 2h. Reaction was quenched by addition of NH_4Cl (50 mL). The aqueous layer was extracted with DCM (3 × 30 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **1-124** as a colorless oil (1.4 g, 93 %).

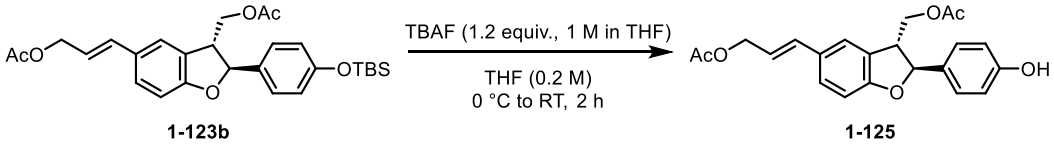
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 6.91 – 6.86 (m, 5H), 6.60 (d, *J* = 15.8 Hz, 1H), 6.16 (dt, *J* = 15.8, 6.7 Hz, 1H), 5.63 (s, 1H), 5.47 (d, *J* = 7.3 Hz, 1H), 4.71 (dd, *J* = 6.6, 1.3 Hz, 2H), 4.44 (dd, *J* = 11.2, 5.4 Hz, 1H), 4.30 (dd, *J* = 11.1, 7.4 Hz, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.80 – 3.75 (m, 1H), 2.10 (s, 3H), 2.03 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.1, 171.0, 148.4, 146.8, 146.0, 144.6, 134.5, 132.4, 130.6, 127.8, 121.3, 119.7, 115.4, 114.4, 110.6, 108.7, 89.0, 65.4, 65.4, 56.1, 50.4, 21.2, 21.0.

MS (ESI) *m/z* (%): 443 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{24}H_{26}O_8K$: 481.1259; found: 481.1262.

(±)-dehydrodi-*p*-coumaryl diacetate (**1-125**)



1-123b **1-125**

1-123b (1 g, 2.01 mmol, 1.0 equiv.) was dissolved in THF (0.2 M, 10.1 mL), and solution was cooled to 0 °C. After 5 minutes TBAF (2.42 mL, 1 M, 2.42 mmol, 1.2 equiv.) was added dropwise and the solution was stirred at RT for 2h. Reaction was quenched by addition of NH_4Cl (30 mL). The aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The

crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **1-125** as a colorless oil (0.7 g, 91 %).

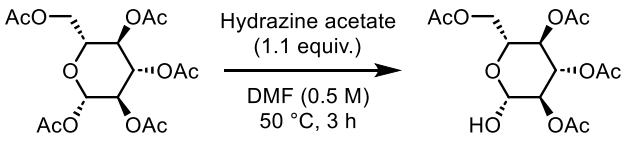
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.25 – 7.17 (m, 3H), 6.81 (dd, *J* = 8.3, 2.9 Hz, 3H), 6.60 (d, *J* = 15.8 Hz, 1H), 6.49 (s, 1H), 6.13 (dt, *J* = 15.9, 6.6 Hz, 1H), 5.44 (d, *J* = 6.6 Hz, 1H), 4.74 – 4.68 (m, 2H), 4.43 (dd, *J* = 11.1, 5.5 Hz, 1H), 4.29 (dd, *J* = 11.1, 7.7 Hz, 1H), 3.70 (q, *J* = 6.6 Hz, 1H), 2.10 (s, 3H), 2.04 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.6, 171.4, 159.8, 156.3, 134.4, 132.5, 129.6, 128.5, 127.5, 126.9, 122.8, 120.6, 115.7, 109.8, 87.8, 65.7, 65.6, 49.8, 21.2, 20.9.

MS (ESI) *m/z* (%): 383 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{22}H_{22}KO_6$: 421.1048; found: 421.1048.

2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (**1-150**)

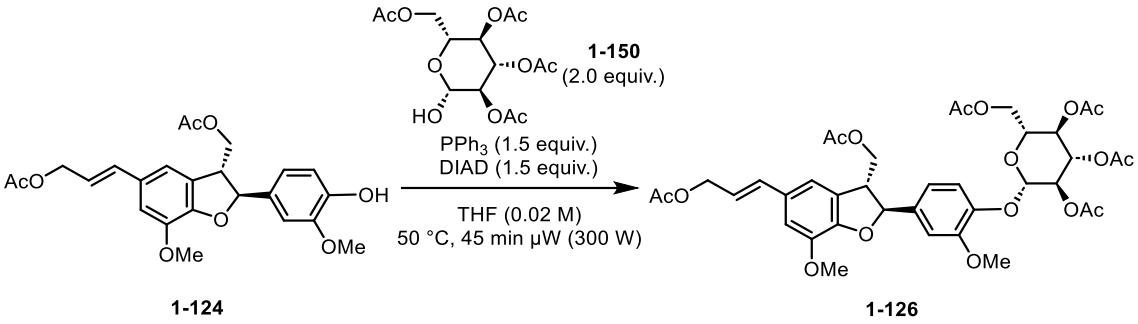


1-149 **1-150**

Prepared according to a literature. Spectroscopic data match those in the literature.[80]

Penta-O-acetyl-β-D-glucopyranose **1-149** (1 g, 2.51 mmol, 1.0 equiv.) was dissolved in DMF (0.5 M, 5.02 mL). Hydrazine acetate (0.26 g, 2.76 mmol, 1.1 equiv.) was added and the solution was warmed to 50 °C. Reaction was stirred for 3 h at 50 °C before it was cooled to RT and diluted by EtOAc (30 mL) and quenched by addition of water (30 mL). Mixture was transferred to a separatory funnel and was washed by NaCl (3x 30 mL). Organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-150** as a colorless oil (0.8 g, 91 %).

(±)-dehydrodiconiferyl alcohol-4-β-D-glucoside-hexaacetate (**1-126**)



1-124 (0.1 g, 0.23 mmol, 1.0 equiv.) was dissolved in THF (0.02 M, 11.3 mL) in a microwave vial and **1-150** (0.16 g, 0.45 mmol, 2 equiv.) and PPh_3 (0.09 g, 0.34 mmol, 1.5 equiv.) was added followed by addition of DIAD (0.7 µL, 0.34 mmol, 1.5 equiv.). The vial was closed with Teflon tap and the whole mixture was heated to 50 °C for 45 min in the microwave reactor (100 W). After cooling to RT, organic solvents were removed under reduced pressure. The crude product was purified semipreparative chromatography using MeOH/water as an eluent to afford **1-126** as a colorless oil (0.7 g, 91 %).

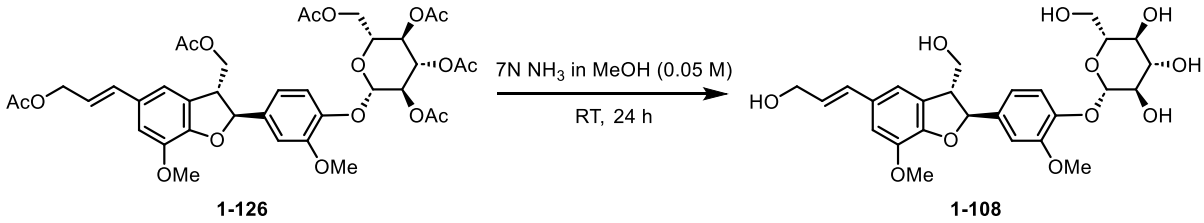
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.08 (dt, *J* = 8.3, 3.1 Hz, 1H), 6.92 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.90 – 6.85 (m, 3H), 6.60 (d, *J* = 15.8 Hz, 1H), 6.16 (dt, *J* = 15.9, 6.6 Hz, 1H), 5.50 (d, *J* = 6.9 Hz, 1H), 5.29 – 5.25 (m, 2H), 5.16 (dt, *J* = 10.0, 4.2 Hz, 1H), 4.95 – 4.89 (m, 1H), 4.71 (d, *J* = 6.6 Hz, 2H), 4.47 – 4.40 (m, 1H), 4.33 – 4.23 (m, 2H), 4.18 – 4.12 (m, 1H), 3.91 (s, 3H), 3.82 – 3.77 (m, 3H), 3.77 – 3.72 (m, 2H), 2.10 (s, 3H), 2.07 (s, 6H), 2.06 – 2.01 (m, 9H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.0, 170.9, 170.8, 170.4, 169.6, 169.5, 151.1, 150.9, 148.3, 146.2, 144.5, 137.2, 134.4, 130.8, 127.5, 121.4, 120.4, 120.3, 118.8, 118.5, 115.4, 110.7, 110.3, 100.9, 88.3, 88.2, 72.7, 72.1, 71.3, 68.4, 65.4, 65.3, 62.0, 56.2, 56.2, 56.1, 50.5, 21.2, 21.0, 20.9, 20.8, 20.7.

MS (ESI) *m/z* (%): 773 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{38}H_{44}KO_{17}$: 811.2216; found: 811.2201.

(±)-dehydrodiconiferyl alcohol 4-O-β-D-glucopyranoside (**1-108**)



1-126 **1-108**

Glucoside **1-126** (0.24 g, 0.31 mmol, 1.0 equiv.) was dissolved in 7N ammonia methanol solution (0.025 M, 12.6 mL). The reaction was stirred overnight at RT. After there was no starting material on TLC, the reaction was stopped by addition of 10 mL of water and was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified semipreparative chromatography using MeOH/water as an eluent to afford **1-108** as a yellow solid (0.1 g, 61 %).

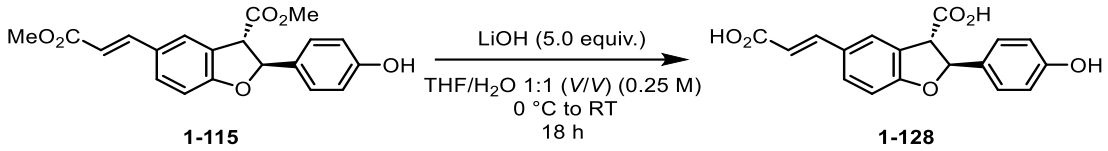
^{1}H NMR (500 MHz, Acetone-d^6) δ (ppm): 7.14 (d, *J* = 8.3 Hz, 1H), 7.08 (d, *J* = 2.0 Hz, 1H), 6.97 (d, *J* = 5.4 Hz, 2H), 6.93 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.53 (dd, *J* = 15.9, 1.7 Hz, 1H), 6.24 (dt, *J* = 15.8, 5.5 Hz, 1H), 5.62 (d, *J* = 6.1 Hz, 1H), 4.91 (d, *J* = 7.2 Hz, 1H), 4.39 – 4.33 (m, 1H), 4.27 (s, 1H), 4.19 (d, *J* = 5.4 Hz, 2H), 3.94 – 3.89 (m, 1H), 3.88 (s, 3H), 3.86 – 3.82 (m, 2H), 3.81 (s, 3H), 3.71 – 3.65 (m, 2H), 3.60 – 3.51 (m, 3H), 3.49 – 3.41 (m, 4H).

^{13}C{^{1}H} NMR (126 MHz, Acetone-d^6) δ (ppm): 150.8, 148.9, 147.6, 145.2, 137.7, 132.1, 130.4, 130.1, 128.5, 119.0, 117.9, 116.1, 111.7, 111.4, 111.4, 102.5, 88.2, 77.8, 74.6, 71.2, 64.6, 63.3, 62.5, 57.6, 56.5, 56.4, 54.9, 18.8.

MS (ESI) *m/z* (%): 521 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+NH_4]^+$ calculated for $C_{26}H_{36}NO_{11}$: 538.2283; found: 538.2290.

(±)-dehydrodi-*p*-coumaric acid (**1-128**)



1-115 (0.30 g, 0.64 mmol, 1.0 equiv.) was dissolved in THF (0.5 M, 0.7 mL), and the solution was cooled to 0 °C. After 5 minutes at a solution of LiOH (0.14 g, 3.2 mmol, 5.0 equiv.) in water (0.5 M, 0.7 mL). The solution was slowly allowed to warm to room temperature while stirring overnight. The following day, the mixture was quenched by addition of 1 M HCl (15 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; DCM:MeOH:AcOH = 20:1:0.1) to afford **1-128** as white solid (0.12 g, 57 %).

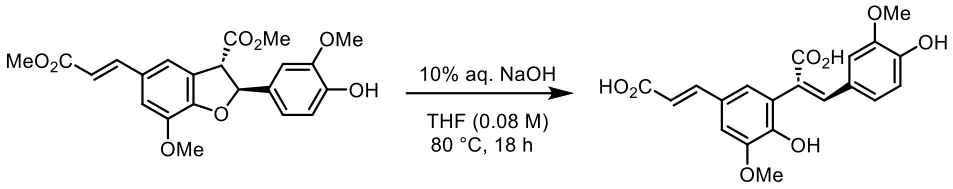
^{1}H NMR (500 MHz, Acetone-d$_6$) δ (ppm): 7.77 – 7.76 (m, 1H), 7.67 (d, *J* = 16.0 Hz, 1H), 7.62 – 7.59 (m, 1H), 7.32 – 7.28 (m, 2H), 6.92 (d, *J* = 8.3 Hz, 1H), 6.89 – 6.85 (m, 2H), 6.40 (d, *J* = 16.0 Hz, 1H), 6.04 (d, *J* = 7.3 Hz, 1H), 4.41 – 4.36 (m, 1H), 2.94 (bs, 2H).

^{13}C{^{1}H} NMR (126 MHz, Acetone-d$_6$) δ (ppm): 172.0, 168.0, 162.1, 158.6, 145.3, 132.1, 131.6, 128.7, 128.5, 127.1, 126.0, 116.5, 116.3, 110.7, 87.8, 55.5.

MS (ESI) *m/z* (%): 327

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{18}H_{15}O_6$: 327.0863; found 327.0869.

8,5'-diferulic acid (**1-129**)



1-36 **1-129**

1-36 (0.3 g, 0.72 mmol, 1.0 equiv.) was dissolved in THF (0.08 M, 9 mL), and 10% aq. NaOH (9 mL) was added. The resulting mixture was stirred at RT. for 30 min before it was warmed to 80 °C overnight. Next day the resulting mixture was cool to RT and acidified with 2 M aq. HCl to pH 2. The water phase was water phase was extracted with EtOAc (3 x 20 mL). Combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; $CHCl_3$:EtOAc:AcOH = 20:1:0.1-10:1:0.1) to afford **1-129** as a white solid (0.167 g, 60 %).

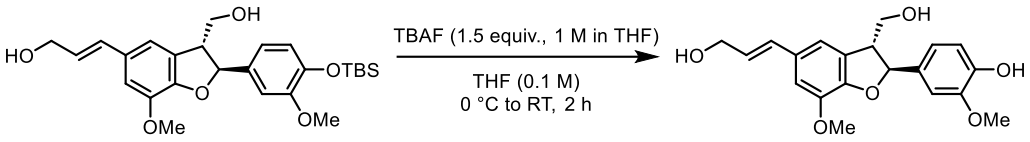
^{1}H NMR (500 MHz, Acetone-d^6) δ (ppm): 7.81 (s, 1H), 7.59 (d, *J* = 15.9 Hz, 1H), 7.39 (d, *J* = 2.0 Hz, 1H), 7.04 (d, *J* = 1.9 Hz, 1H), 6.86 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.74 (d, *J* = 2.0 Hz, 1H), 6.72 (d, *J* = 8.2 Hz, 1H), 6.39 (d, *J* = 15.9 Hz, 1H), 3.97 (s, 3H), 3.46 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Acetone-d^6) δ (ppm): 168.6, 168.0, 149.1, 148.0, 147.9, 145.7, 141.7, 127.7, 127.4, 126.4, 125.7, 125.2, 116.3, 115.6, 113.3, 110.2, 56.6, 55.5.

MS (ESI) *m/z* (%): 387 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{20}H_{19}O_8$: 387.1074; found: 387.1081.

(±)-dehydroconiferyl aldehyde (**1-130**)



1-118b **1-130**

1-118b (0.25 g, 0.52 mmol, 1.0 equiv.) was dissolved in THF (0.1 M, 5.2 mL), and solution was cooled to 0 °C. After 5 minutes TBAF (0.21 g, 0.78 mmol, 1.5 equiv.) was added and the solution was stirred overnight at RT. Reaction was quenched by addition of NH_4Cl (50 mL). The aqueous layer was extracted with DCM (3 × 30 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum DCM:MeOH = 10:1) to afford **1-130** as a white solid (0.15 g, 80 %).

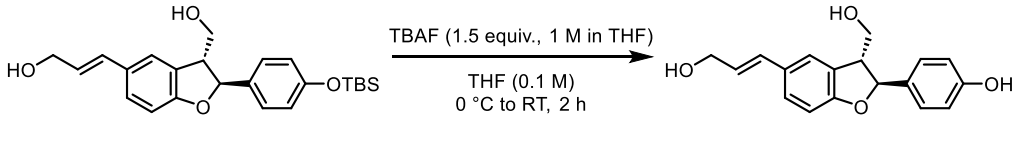
^{1}H NMR (500 MHz, Acetone-d^6) δ (ppm): 7.03 (d, *J* = 2.0 Hz, 1H), 6.97 (t, *J* = 1.2 Hz, 1H), 6.94 (d, *J* = 1.6 Hz, 1H), 6.87 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.80 (d, *J* = 8.1 Hz, 1H), 6.52 (dt, *J* = 15.9, 1.7 Hz, 1H), 6.24 (dt, *J* = 15.8, 5.6 Hz, 1H), 5.56 (d, *J* = 6.5 Hz, 1H), 4.19 (dd, *J* = 5.6, 1.6 Hz, 2H), 3.85 (s, 6H), 3.81 (s, 3H), 3.53 (q, *J* = 6.4 Hz, 1H).

^{13}C{^{1}H} NMR (126 MHz, Acetone-d^6) δ (ppm): 148.8, 148.3, 147.1, 145.1, 134.3, 131.8, 130.5, 130.3, 128.2, 119.5, 116.0, 115.6, 111.5, 110.4, 88.4, 69.3, 69.2, 64.4, 63.2, 56.3, 56.2, 54.6.

MS (ESI) *m/z* (%): 357 $[M-H]^-$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{20}H_{23}O_6$: 357.1333 found: 357.1338.

(±)-dehydrodi-*p*-coumaryl alcohol (**1-131**)



1-119f **1-131**

1-119f (0.2 g, 0.48 mmol, 1.0 equiv.) was dissolved in THF (0.1 M, 4.85 mL), and solution was cooled to 0 °C. After 5 minutes TBAF (0.19 g, 0.73 mmol, 1.5 equiv.) was added and the solution was stirred overnight at RT. Reaction was quenched by addition of NH_4Cl (50 mL). The aqueous layer was extracted with DCM (3 × 30 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product

was purified by column chromatography (SiO_2; petroleum DCM:MeOH = 10:1) to afford **1-131** as a white solid (0.12 g, 83 %).

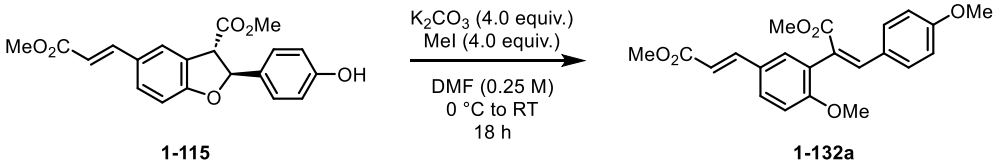
^{1}H NMR (500 MHz, Acetone-d^6) δ (ppm): δ 8.40 (bs, 1H), 7.43 – 7.36 (m, 1H), 7.28 – 7.17 (m, 3H), 6.84 – 6.81 (m, 2H), 6.74 (d, *J* = 8.2 Hz, 1H), 6.54 (dt, *J* = 15.7, 1.7 Hz, 1H), 6.23 (dt, *J* = 15.9, 5.5 Hz, 1H), 5.55 (d, *J* = 6.0 Hz, 1H), 4.19 (dd, *J* = 5.6, 1.5 Hz, 3H), 3.95 – 3.73 (m, 3H), 3.48 (q, *J* = 6.2 Hz, 1H).

^{13}C{^{1}H} NMR (126 MHz, Acetone-d^6) δ (ppm): 160.5, 158.1, 134.1, 131.0, 130.3, 129.5, 128.2, 128.1, 128.1, 123.5, 116.1, 109.7, 88.0, 68.1, 64.8, 64.7, 63.4, 63.3, 54.4, 26.1.

MS (ESI) *m/z* (%): 297 $[M-H]^-$ (100).

HRMS (ESI) *m/z:* $[M-H]^-$ calculated for $C_{18}H_{17}O_4$: 297.1121; found: 297.1126.

methyl (*Z*)-2-(2-methoxy-5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)phenyl)-3-(4-methoxyphenyl) acrylate (**1-132a**)



1-115 (0.1 g, 0.28 mmol, 1.0 equiv.) was dissolved in DMF (0.25 M, 1.1 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C K_2CO_3 (0.16 g, 0.1.13 mmol, 4.0 equiv.) was added following addition of methyl iodide (0.07 mL, 1.13 mmol, 4.0 equiv.). The resulting mixture was stirred at RT overnight. Next day 10 mL of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 10 mL). Combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-132a** as a white amorphous white solid (0,04 g, 79 %).

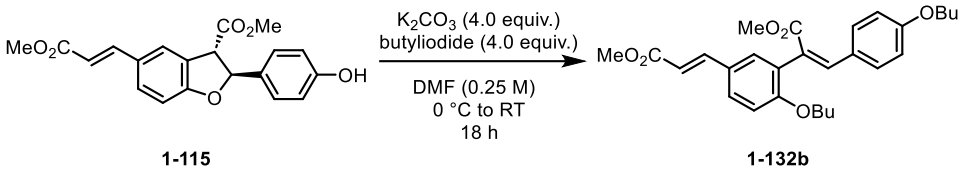
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.82 (s, 1H), 7.59 (d, *J* = 16.0 Hz, 1H), 7.54 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.28 (d, *J* = 2.3 Hz, 1H), 7.06 – 6.98 (m, 2H), 6.98 (d, *J* = 8.6 Hz, 1H), 6.72 – 6.65 (m, 2H), 6.24 (d, *J* = 16.0 Hz, 1H), 3.78 (s, 3H), 3.75 (s, 6H), 3.75 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 168.4, 167.8, 160.5, 159.5, 144.4, 141.0, 132.1, 131.0, 130.0, 127.5, 127.2, 126.3, 126.0, 115.7, 113.9, 111.5, 56.0, 55.3, 52.4, 51.7.

MS (ESI) *m/z* (%): 383 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{22}H_{25}O_6$: 383.1489; found: 383.1488.

methyl (*Z*)-2-(2-butoxy-5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)phenyl)-3-(4-butoxyphenyl) acrylate (**1-132b**)



1-115 **1-132b**

1-115 (0.1 g, 0.28 mmol, 1.0 equiv.) was dissolved in DMF (0.25 M, 1.1 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C K_2CO_3 (0.16 g, 0.1.13 mmol, 4.0 equiv.) was added following addition of 1-Iodobutane (0.13 mL, 1.13 mmol, 4.0 equiv.). The resulting mixture was stirred at RT overnight. Next day 10 mL of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 10 mL). Combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **1-132g** as a white solid (0.075 g, 58 %).

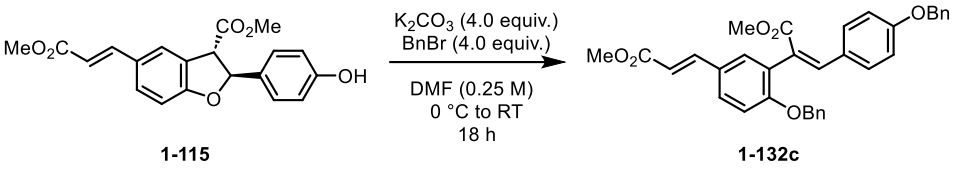
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.03 (d, *J* = 2.4 Hz, 1H), 7.71 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.65 (dd, *J* = 16.0, 4.6 Hz, 2H), 7.50 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.41 (d, *J* = 1.9 Hz, 1H), 7.35 – 7.27 (m, 2H), 7.00 (dd, *J* = 10.7, 8.5 Hz, 2H), 6.88 (d, *J* = 6.5 Hz, 2H), 6.39 (d, *J* = 16.0 Hz, 1H), 6.32 (d, *J* = 16.0 Hz, 1H), 4.08 (t, *J* = 6.6 Hz, 2H), 3.95 (t, *J* = 6.5 Hz, 5H), 3.92 (s, 4H), 3.80 (s, 5H), 1.76 (p, *J* = 6.7 Hz, 4H), 1.53 – 1.42 (m, 4H), 0.98 (td, *J* = 7.4, 3.0 Hz, 6H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 186.2, 172.4, 167.8, 167.4, 165.2, 162.6, 161.0, 159.6, 144.4, 143.0, 135.7, 131.9, 130.6, 128.9, 128.6, 127.7, 126.9, 126.8, 124.0, 123.2, 117.5, 116.0, 114.4, 113.1, 111.5, 94.1, 85.6, 69.3, 67.9, 53.2, 52.6, 51.9, 51.8, 31.4, 31.0, 29.8, 19.4, 19.1, 14.0, 13.9.

MS (ESI) *m/z* (%): 467 [M+H]+ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{28}H_{35}O_6$: 467.2428; found: 467.2427.

methyl (*Z*)-2-(2-(benzyloxy)-5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)phenyl)-3-(4-(benzyloxy)phenyl) acrylate (**1-132c**)



1-115 **1-132c**

1-115 (0.1 g, 0.28 mmol, 1.0 equiv.) was dissolved in DMF (0.25 M, 1.1 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C K_2CO_3 (0.16 g, 0.1.13 mmol, 4.0 equiv.) was added following addition of benzyl bromide (0.13 mL, 1.13 mmol, 4.0 equiv.). The resulting mixture was stirred at RT overnight. Next day 10 mL of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 10 mL). Combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **1-132c** as a white solid (0.12 g, 79 %).

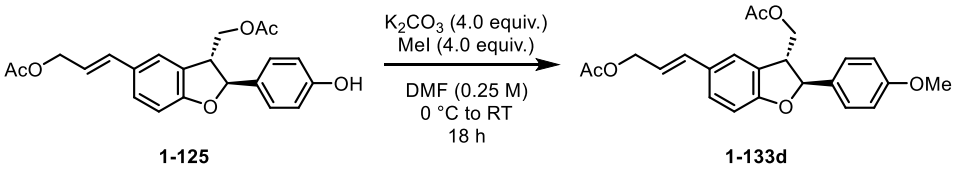
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.55 (d, *J* = 15.9 Hz, 1H), 7.42 – 7.37 (m, 4H), 7.37 – 7.35 (m, 1H), 7.35 – 7.30 (m, 1H), 7.25 – 7.19 (m, 3H), 7.14 – 7.10 (m, 2H), 6.89 – 6.84 (m, 5H), 6.70 (d, *J* = 1.8 Hz, 1H), 6.07 (d, *J* = 15.9 Hz, 1H), 5.63 (s, 1H), 5.03 (s, 2H), 3.81 (d, *J* = 13.1 Hz, 1H), 3.78 (s, 3H), 3.22 (s, 3H), 3.08 (d, *J* = 13.1 Hz, 1H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.3, 168.0, 162.2, 159.1, 145.0, 136.8, 135.5, 130.8, 130.7, 129.7, 128.7, 128.3, 128.2, 128.1, 127.9, 127.7, 127.6, 127.5, 127.2, 115.1, 114.7, 110.0, 92.7, 70.0, 64.4, 52.1, 51.7, 45.3, 29.8.

MS (ESI) *m/z* (%): 535 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{34}H_{31}O_6$: 535.2272; found:535.2118.

(*E*)-3-(3-(acetoxymethyl)-2-(4-methoxyphenyl)-2,3-dihydrobenzofuran-5-yl)allyl acetate (**1-133d**)



1-125 (0.05 g, 0.13 mmol, 1.0 equiv.) was dissolved in DMF (0.13 M, 1 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C K_2CO_3 (0.07 g, 0.52 mmol, 4.0 equiv.) was added following addition of methyl iodide (0.032 mL, 0.52 mmol, 4.0 equiv.). The resulting mixture was stirred at RT overnight. Next day 10 mL of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 10 mL). Combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-133d** as a white amorphous solid (0.04 g, 79 %).

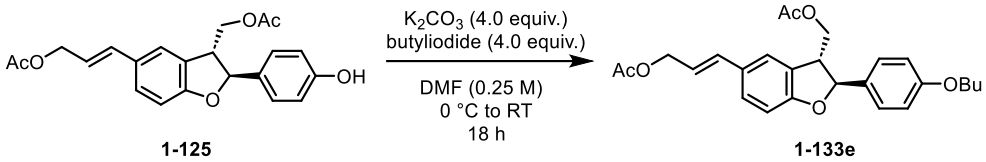
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.28 – 7.22 (m, 4H), 6.90 – 6.86 (m, 2H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.59 (d, *J* = 15.8 Hz, 1H), 6.13 (dt, *J* = 15.8, 6.6 Hz, 1H), 5.46 (d, *J* = 6.6 Hz, 1H), 4.69 (dd, *J* = 6.7, 1.3 Hz, 2H), 4.42 (dd, *J* = 11.1, 5.5 Hz, 1H), 4.28 (dd, *J* = 11.1, 7.8 Hz, 1H), 3.79 (s, 3H), 3.70 (q, *J* = 6.6 Hz, 1H), 2.08 (s, 3H), 2.03 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.1, 171.0, 160.0, 159.8, 134.4, 133.0, 129.7, 128.5, 127.4, 127.0, 122.8, 120.9, 114.2, 109.9, 87.9, 65.7, 65.5, 55.5, 50.0, 21.2, 21.0.

MS (ESI) *m/z* (%): 397

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{23}H_{25}O_6K$: 435.1204; found:435.1192.

(*E*)-3-(3-(acetoxymethyl)-2-(4-butoxyphenyl)-2,3-dihydrobenzofuran-5-yl)allyl acetate (**1-133e**)



1-125 (0.05 g, 0.13 mmol, 1.0 equiv.) was dissolved in DMF (0.13 M, 1 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C K_2CO_3 (0.07 g, 0.52 mmol, 4.0 equiv.) was added following addition of 1-Iodobutane (0.06 mL, 0.52 mmol, 4.0 equiv.). The resulting mixture was stirred at RT overnight. Next day 10 mL of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 10 mL). Combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-133e** as a white solid (0.052 g, 91 %).

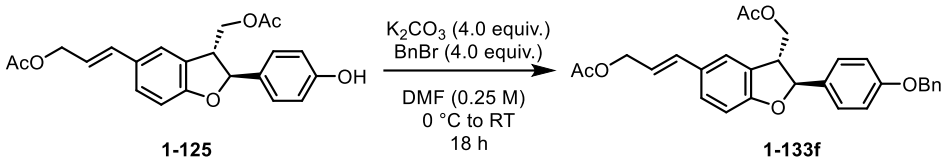
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.28 – 7.24 (m, 4H), 6.90 – 6.86 (m, 2H), 6.83 (d, *J* = 8.3 Hz, 1H), 6.61 (d, *J* = 15.8 Hz, 1H), 6.15 (dt, *J* = 15.8, 6.6 Hz, 1H), 5.47 (d, *J* = 6.6 Hz, 1H), 4.71 (dd, *J* = 6.7, 1.3 Hz, 2H), 4.44 (dd, *J* = 11.2, 5.5 Hz, 1H), 4.30 (dd, *J* = 11.1, 7.8 Hz, 1H), 3.95 (t, *J* = 6.5 Hz, 2H), 3.72 (q, *J* = 6.5 Hz, 1H), 2.10 (s, 3H), 2.05 (s, 3H), 1.79 – 1.72 (m, 2H), 1.53 – 1.44 (m, 2H), 0.97 (t, *J* = 7.4 Hz, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.0, 171.0, 160.0, 159.4, 134.4, 132.7, 129.6, 128.5, 127.3, 127.0, 122.8, 120.8, 114.8, 109.8, 87.9, 67.8, 65.6, 65.4, 50.0, 31.4, 21.2, 20.9, 19.3, 14.0.

MS (ESI) *m/z* (%):

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{26}H_{30}O_6K$: 477.1674; found: 477.1677.

(*E*)-3-(3-(acetoxymethyl)-2-(4-(benzyloxy)phenyl)-2,3-dihydrobenzofuran-5-yl)allyl acetate (**1-133f**)



1-125 (0.05 g, 0.13 mmol, 1.0 equiv.) was dissolved in DMF (0.13 M, 1 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C K_2CO_3 (0.07 g, 0.52 mmol, 4.0 equiv.) was added following addition of benzyl bromide (0.062 mL, 0.52 mmol, 4.0 equiv.). The resulting mixture was stirred at RT overnight. Next day 10 mL of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 10 mL). Combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-133f** as a white solid (0.05 g, 81 %).

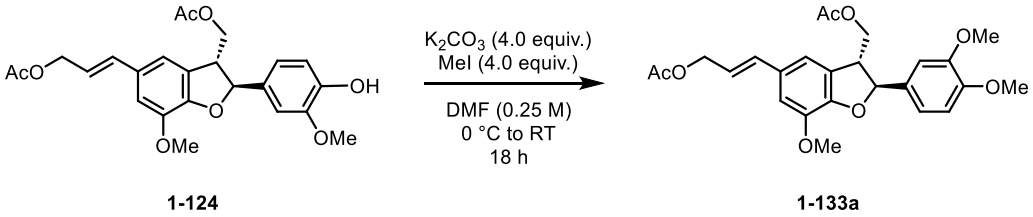
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.41 (dd, *J* = 15.6, 7.4 Hz, 4H), 7.35 – 7.30 (m, 2H), 7.29 – 7.23 (m, 3H), 6.99 – 6.94 (m, 2H), 6.83 (d, *J* = 8.2 Hz, 1H), 6.61 (d, *J* = 15.8 Hz, 1H), 6.14 (dt, *J* = 15.7, 6.7 Hz, 1H), 5.47 (d, *J* = 6.5 Hz, 1H), 5.07 (s, 2H), 4.74 – 4.66 (m, 2H), 4.44 (dd, *J* = 11.1, 5.5 Hz, 1H), 4.29 (dd, *J* = 11.1, 7.8 Hz, 1H), 3.71 (q, *J* = 6.6 Hz, 1H), 2.10 (s, 3H), 2.04 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.4, 171.2, 159.9, 156.3, 134.4, 132.6, 129.6, 128.5, 127.6, 127.0, 122.8, 120.7, 115.7, 109.8, 87.9, 65.7, 65.6, 49.9, 21.2, 21.0.

MS (ESI) *m/z* (%): 473

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{29}H_{28}O_6K$: 511.1517; found: 511.1519.

(*E*)-3-(3-(acetoxymethyl)-2-(3,4-dimethoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl) allyl acetate (**1-133a**)



1-124 **1-133a**

1-124 (0.05 g, 0.113 mmol, 1.0 equiv.) was dissolved in DMF (0.13 M, 1 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C K_2CO_3 (0.06 g, 0.45 mmol, 4 equiv.) was added following addition of methyl iodide (0.028 mL, 0.45 mmol, 4 equiv.). The resulting mixture was stirred at RT overnight. Next day 10 mL of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 10 mL). Combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-133a** as a white solid (0.039 g, 76 %).

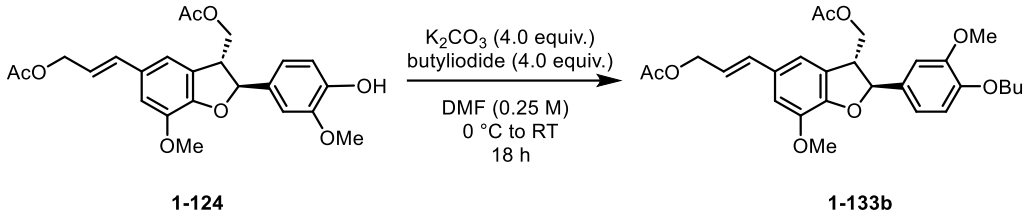
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 6.94 – 6.87 (m, 4H), 6.85 – 6.81 (m, 1H), 6.60 (d, *J* = 15.8 Hz, 1H), 6.16 (dt, *J* = 15.8, 6.6 Hz, 1H), 5.49 (d, *J* = 7.3 Hz, 1H), 4.71 (dd, *J* = 6.6, 1.2 Hz, 2H), 3.91 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.80 – 3.76 (m, 1H), 2.10 (s, 3H), 2.03 (s, 3H).

^{13}C{^{1}H} NMR (^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.1, 171.0, 149.3, 148.4, 144.5, 134.5, 132.9, 130.6, 127.8, 121.3, 118.9, 115.4, 111.0, 110.6, 109.3, 88.8, 65.4, 65.4, 56.1, 56.1, 56.0, 50.4, 33.2, 21.2, 21.0, 20.2.

MS (ESI) *m/z* (%): 457

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{25}H_{28}O_8K$: 495.1416; found: 495.1416.

(*E*)-3-(3-(acetoxymethyl)-2-(4-butoxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl) allyl acetate (**1-133b**)



1-124 **1-133b**

1-124 (0.05 g, 0.113 mmol, 1.0 equiv.) was dissolved in DMF (0.13 M, 1 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C K_2CO_3 (0.06 g, 0.45 mmol, 4 equiv.) was added following addition of 1-Iodobutane (0.051 mL, 0.45 mmol, 4 equiv.). The resulting mixture was stirred at RT overnight. Next day 10 mL of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 10 mL). Combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-133b** as a white amorphous solid (0.041 g, 73 %).

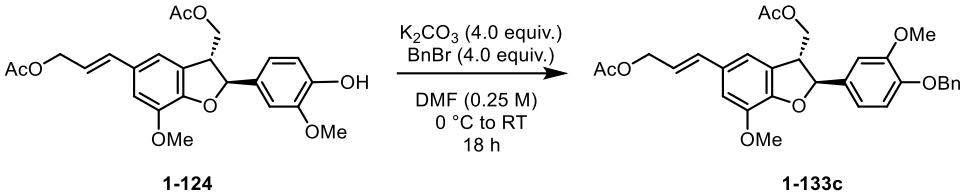
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 6.92 – 6.87 (m, 4H), 6.83 (d, *J* = 8.8 Hz, 1H), 6.60 (d, *J* = 15.8 Hz, 1H), 6.15 (dt, *J* = 15.8, 6.6 Hz, 1H), 5.48 (d, *J* = 7.3 Hz, 1H), 4.71 (d, *J* = 6.6 Hz, 2H), 4.43 (dd, *J* = 11.1, 5.4 Hz, 1H), 4.30 (dd, *J* = 11.2, 7.4 Hz, 1H), 4.00 (t, *J* = 6.8 Hz, 2H), 3.90 (s, 3H), 3.83 (s, 3H), 3.81 – 3.76 (m, 1H), 2.10 (s, 3H), 2.03 (s, 3H), 1.81 (p, *J* = 6.9 Hz, 2H), 1.48 (h, *J* = 7.4 Hz, 2H), 0.96 (t, *J* = 7.4 Hz, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.1, 170.9, 149.7, 148.9, 148.4, 144.5, 134.5, 132.7, 130.5, 127.8, 121.2, 118.9, 115.4, 112.7, 110.6, 109.8, 88.8, 68.8, 65.4, 65.4, 56.2, 56.1, 50.3, 31.3, 21.2, 21.0, 19.3, 14.0.

MS (ESI) *m/z* (%): 499

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{28}H_{34}O_8K$: 537.1885; found: 537.1885.

(*E*)-3-(3-(acetoxymethyl)-2-(4-(benzyloxy)-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl)allyl acetate (**1-133c**)



1-124 **1-133c**

1-124 (0.05 g, 0.113 mmol, 1.0 equiv.) was dissolved in DMF (0.13 M, 1 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C K_2CO_3 (0.06 g, 0.45 mmol, 4 equiv.) was added following addition of benzyl bromide (0.054 mL, 0.45 mmol, 4 equiv.). The resulting mixture was stirred at RT overnight. Next day 10 mL of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 10 mL). Combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-133c** as a white solid (0.055 g, 92 %).

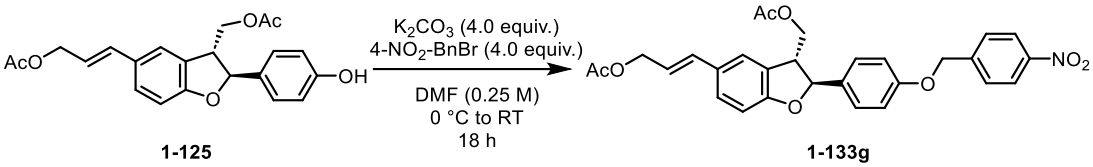
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.42 – 7.40 (m, 2H), 7.37 – 7.33 (m, 2H), 7.31 – 7.27 (m, 1H), 6.92 (d, *J* = 1.8 Hz, 1H), 6.88 (s, 2H), 6.85 – 6.82 (m, 2H), 6.60 (d, *J* = 15.9 Hz, 1H), 6.15 (dt, *J* = 15.7, 6.6 Hz, 1H), 5.47 (d, *J* = 7.3 Hz, 1H), 5.15 (s, 2H), 4.71 (dd, *J* = 6.7, 1.3 Hz, 2H), 4.43 (dd, *J* = 11.2, 5.4 Hz, 1H), 4.29 (dd, *J* = 11.2, 7.5 Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H), 3.80 – 3.75 (m, 1H), 2.10 (s, 3H), 2.01 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.1, 170.9, 149.9, 148.3, 144.5, 137.1, 134.5, 133.4, 130.6, 128.7, 128.0, 127.7, 127.3, 121.2, 118.8, 115.4, 113.9, 110.6, 109.8, 88.7, 71.1, 65.4, 65.4, 56.2, 56.1, 50.3, 21.2, 20.9.

MS (ESI) *m/z* (%): 533

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{31}H_{33}O_8$: 533.2170; found: 533.2193.

(*E*)-3-(3-(acetoxymethyl)-2-(4-((4-nitrobenzyl)oxy)phenyl)-2,3-dihydrobenzofuran-5-yl)allyl acetate (**1-133g**)



1-125 (0.05 g, 0.13 mmol, 1.0 equiv.) was dissolved in DMF (0.13 M, 1 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C K_2CO_3 (0.07 g, 0.52 mmol, 4.0 equiv.) was added following addition of 4-nitrobenzyl bromide (0.113 g, 0.52 mmol, 4.0 equiv.). The resulting mixture was stirred at RT overnight. Next day 10 mL of NH_4Cl was added and the water phase was water phase was extracted with DCM (3 x 10 mL). Combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-133g** as a white solid (0.046 g, 67 %).

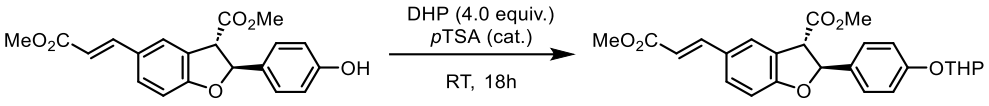
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.27 – 8.20 (m, 2H), 7.62 – 7.58 (m, 2H), 7.56 – 7.52 (m, 1H), 7.31 – 7.26 (m, 3H), 6.97 – 6.92 (m, 2H), 6.83 (d, *J* = 8.2 Hz, 1H), 6.61 (d, *J* = 15.8 Hz, 1H), 6.14 (dt, *J* = 15.7, 6.6 Hz, 1H), 5.48 (d, *J* = 6.5 Hz, 1H), 5.17 (s, 2H), 4.85 – 4.83 (m, 1H), 4.70 (d, *J* = 6.5 Hz, 2H), 4.44 (dd, *J* = 11.1, 5.5 Hz, 1H), 4.29 (dd, *J* = 11.1, 7.8 Hz, 1H), 3.70 (q, *J* = 6.6 Hz, 1H), 2.10 (s, 3H), 2.05 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.1, 171.0, 159.9, 158.3, 147.7, 144.4, 134.3, 134.0, 129.8, 128.6, 127.7, 127.5, 127.1, 126.8, 124.0, 123.9, 122.9, 121.0, 115.1, 109.9, 68.8, 65.7, 65.4, 64.1, 50.1, 21.2, 21.0.

MS (ESI) *m/z* (%): 518

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{29}H_{27}NO_8K$: 556.1368; found: 556.1368.

Methyl 5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)-2-(4-((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)-2,3-dihydrobenzofuran-3-carboxylate (**1-117a**)



1-115 **1-117a**

1-115 (0.60 g, 1.69 mmol, 1.0 equiv.) was dissolved in 3,4-dihydro-2*H*-pyran (0.62 mL, 6.77 mmol, 4.0 equiv.) and catalytic amount of *p*-toluene sulfonic acid was added. Mixture was stirred overnight at RT. The mixture was diluted with diethyl ether (20 mL), and 1 M NaOH (20 mL) was added. The aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **1-117a** as a colorless oil (0.48 g, 65 %).

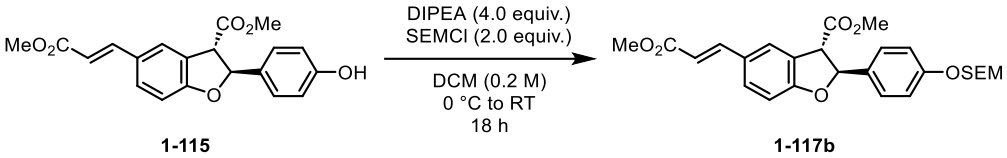
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.66 (d, *J* = 15.9 Hz, 1H), 7.57 – 7.55 (m, 1H), 7.43 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.32 – 7.28 (m, 2H), 7.07 – 7.03 (m, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.32 (d, *J* = 16.0 Hz, 1H), 6.14 – 6.08 (m, 1H), 5.44 – 5.41 (m, 1H), 4.30 – 4.26 (m, 1H), 3.90 – 3.86 (m, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 3.61 – 3.56 (m, 1H), 2.04 – 1.94 (m, 1H), 1.90 – 1.80 (m, 2H), 1.74 – 1.51 (m, 4H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.0, 167.9, 161.3, 157.4, 144.7, 132.9, 130.9, 127.9, 127.6, 127.3, 125.2, 125.0, 116.8, 115.8, 115.3, 110.4, 96.3, 86.5, 62.1, 55.1, 53.0, 51.8, 30.3, 25.2, 18.7.

MS (ESI) *m/z* (%): 439

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{25}H_{27}O_7$: 439.1751; found: 439.1759.

Methyl 5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)-2-(4-((2-(trimethylsilyl)ethoxy)methoxy)phenyl) -2,3-dihydrobenzofuran-3-carboxylate (**1-117b**)



1-115 **1-117b**

1-115 (0.20 g, 0.56 mmol, 1.0 equiv.) was dissolved in DCM (0.2 M, 2.8 mL), and the solution was cooled to 0 °C. After 5 minutes at 0 °C SEMCl (0.22 mL, 1.13 mmol, 2.0 equiv.) and DIPEA (0.40 mL, 2.26 mmol, 4.0 equiv.) was added. The solution was slowly allowed to warm to room temperature while stirring overnight. The following day, the mixture quenched by addition of NH_4Cl (15 mL), and the water phase was extracted with Et_2O (3 × 25 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **1-117b** as a colorless oil (0.19 g, 70 %).

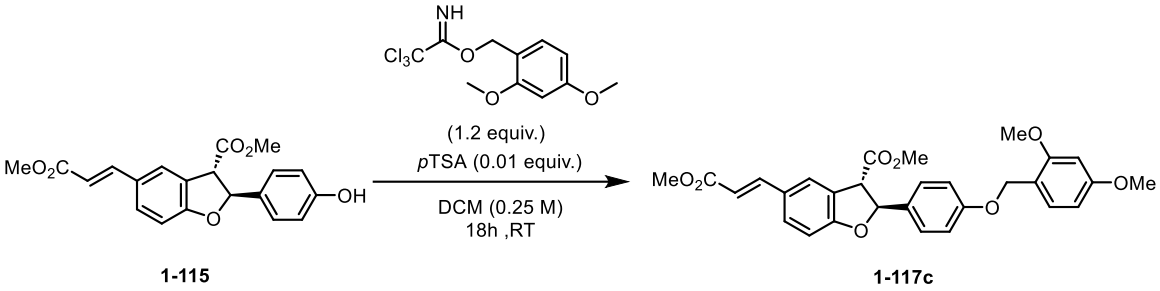
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.66 (d, *J* = 16.1 Hz, 1H), 7.56 (t, *J* = 1.5 Hz, 1H), 7.43 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.33 – 7.29 (m, 2H), 7.06 – 7.02 (m, 2H), 6.89 (d, *J* = 8.3 Hz, 1H), 6.32 (d, *J* = 15.9 Hz, 1H), 6.11 (d, *J* = 7.7 Hz, 1H), 5.21 (s, 2H), 4.28 (d, *J* = 7.4 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 3.76 – 3.71 (m, 2H), 0.97 – 0.92 (m, 2H), -0.01 (s, 9H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 170.9, 167.9, 161.3, 157.9, 144.7, 133.1, 130.9, 128.0, 127.4, 125.2, 125.1, 116.6, 115.4, 110.5, 93.0, 86.5, 66.4, 55.2, 53.0, 51.8, 18.2, -1.3.

MS (ESI) *m/z* (%): 485

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{26}H_{33}O_7Si$: 485.1990; found: 485.2002.

Methyl 2-(4-((2,4-dimethoxybenzyl)oxy)phenyl)-5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)-2,3-dihydrobenzofuran-3-carboxylate (**1-117c**)



Formation of imidate: DBU (1.77 mL, 11.9 mmol, 0.1 equiv.) was added to a solution of 2,4-dimethoxybenzylalkohol (20 g, 119 mmol, 1.0 equiv.) in DCM (200 mL, 0.3 M) under argon atmosphere. The resulting mixture was stirred at RT for 15 min before it was cooled to 0 °C. After 5 min at 0 °C, trichloracetonitril (14.3 mL, 143 mmol, 1.2 equiv.) was added and the resulting mixture was stirred at RT overnight. The resulting mixture was evaporated to dryness. The crude product was used in the next step without further purification. Spectroscopic data match those in the literature.[81] Imidate (1.33 g, 3.75 mmol, 1.0 equiv.) was dissolved in DCM (0.25 M, 15 mL) at RT and imidate (1.41 g, 4.5 mmol, 1.2 equiv.) was added at one portion. After 5 minutes, *p*TSA (6.5 mg, 0.038 mmol, 1 mol%) was added. After stirring for 18 h, the mixture was evaporated. The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 4:1 → 2:1) to afford **1-117c** as a white solid (1.8 g, 95 %).

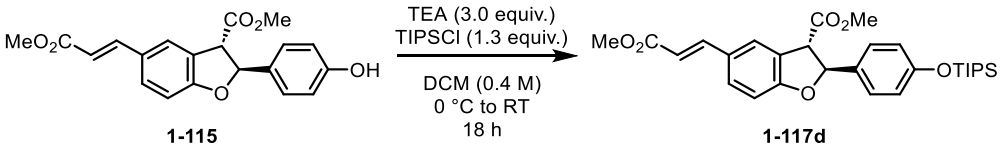
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): δ 7.66 (d, *J* = 16.0 Hz, 1H), 7.57 – 7.55 (m, 1H), 7.42 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.33 – 7.28 (m, 3H), 7.00 – 6.96 (m, 2H), 6.89 (d, *J* = 8.3 Hz, 1H), 6.50 – 6.47 (m, 2H), 6.32 (d, *J* = 15.9 Hz, 1H), 6.10 (d, *J* = 7.6 Hz, 1H), 5.02 (s, 2H), 4.29 (d, *J* = 7.5 Hz, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): δ 171.0, 167.9, 161.3, 161.1, 159.5, 158.4, 144.7, 132.0, 131.0, 130.3, 127.9, 127.4, 125.3, 125.1, 117.5, 115.3, 110.5, 104.3, 98.6, 86.6, 65.1, 55.6, 55.5, 55.2, 53.0, 51.8.

MS (ESI) *m/z* (%): 505 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{29}H_{28}O_8K$: 543.1416; found: 543.1417.

Methyl 5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)-2-(4-((triisopropylsilyl)oxy)phenyl)-2,3-dihydrobenzofuran-3-carboxylate (**1-117d**)



1-115 (0.10 g, 0.28 mmol, 1.0 equiv.) was dissolved in DCM (0.4 M, 0.7 mL), and the solution was cooled to 0 °C. After 5 minutes at 0 °C TEA (0.12 mL, 0.85 mmol, 3.0 equiv.) and TIPSCl (0.08 mL, 0.37 mmol, 1.3 equiv.) was added. The solution was slowly allowed to warm to room temperature while stirring overnight. The following day, the mixture was poured into cold water and extracted with Et_2O (3 × 25 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 5:1) to afford **1-117d** as a colorless oil (0.11 g, 78 %).

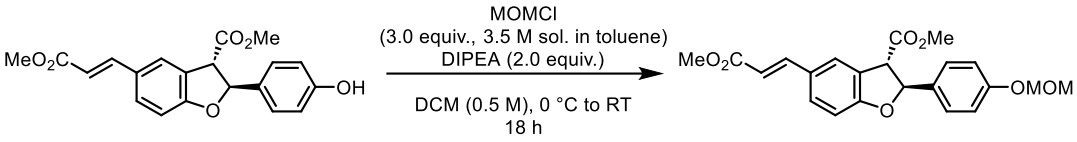
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.66 (d, *J* = 16.0 Hz, 1H), 7.56 (s, 1H), 7.42 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.24 – 7.21 (m, 2H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.88 – 6.85 (m, 2H), 6.32 (d, *J* = 16.0 Hz, 1H), 6.10 (d, *J* = 7.5 Hz, 1H), 4.28 (d, *J* = 7.3 Hz, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 1.25 (ddt, *J* = 8.1, 6.1, 3.2 Hz, 3H), 1.09 (d, *J* = 7.4 Hz, 18H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.0, 167.9, 156.6, 144.7, 132.4, 130.9, 127.9, 127.3, 125.3, 125.1, 120.3, 115.4, 110.5, 86.6, 55.2, 53.1, 51.8, 18.0, 12.8.

MS (ESI) *m/z* (%): 511

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{29}H_{39}O_6Si$: 511.2510; found: 511.2504.

Methyl 5-((E)-3-methoxy-3-oxoprop-1-en-1-yl)-2-(4-(methoxymethoxy)phenyl)-2,3-dihydrobenzofuran-3-carboxylate (**1-117e**)



1-115 **1-117e**

1-115 (1.33 g, 3.75 mmol, 1.0 equiv.) was dissolved in DCM (0.5 M, 7.5 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C DIPEA (1.3 mL, 7.51 mmol, 2.0 equiv.) was added following dropwise addition of methoxymethyl chloride solution in toluene (3.22 mL, 3.5 M, 11.3 mmol, 3.0 equiv.) during 1 h. The resulting mixture was stirred at RT overnight. Next day 50 mL of NH_4Cl was added and the water phase was water phase was extracted with EtOAc (3 x 100 mL). Combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-117e** as a white solid (1.42 g, 95 %).

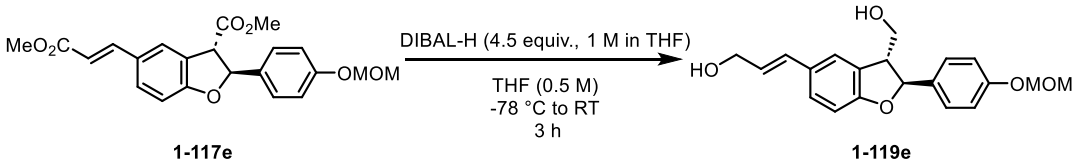
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm 7.69 – 7.60 (m, 2H), 7.56 (s, 1H), 7.49 – 7.37 (m, 2H), 7.33 – 7.29 (m, 2H), 7.05 – 7.02 (m, 2H), 7.01 – 6.97 (m, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.32 (d, *J* = 15.9 Hz, 1H), 6.11 (d, *J* = 7.5 Hz, 1H), 5.16 (s, 2H), 4.28 (d, *J* = 7.6 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.46 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 170.9, 167.8, 161.2, 157.6, 144.6, 133.3, 132.3, 130.9, 130.0, 128.0, 127.4, 125.2, 125.0, 116.6, 115.4, 110.4, 94.4, 86.4, 56.1, 55.2, 53.0, 51.7.

MS (ESI) *m/z* (%): 399 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{22}H_{23}O_7$: 399.1438; found: 399.1438.

(*E*)-3-(3-(hydroxymethyl)-2-(4-(methoxymethoxy)phenyl)-2,3-dihydrobenzofuran-5-yl)prop-2-en-1-ol (**1-119e**)



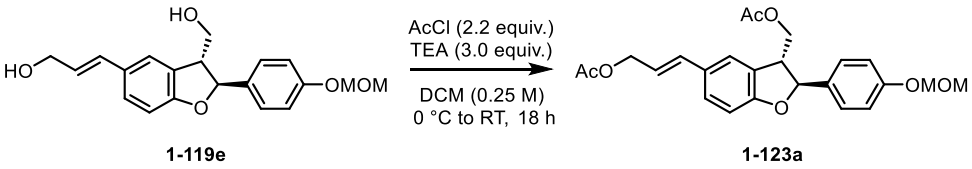
A solution of ester **1-117e** (1.3 g, 3.26 mmol, 1.0 equiv.) in THF (0.5 M, 6.53 mL) was cooled to - 78 °C and DIBAL-H (1 M in THF, 16.3 mL, 16.3 mmol, 4.5 equiv.) was added dropwise over 10 minutes. The resulting mixture was stirred at -78 °C for 30 min before the cooling bath removal. The reaction mixture was stirred for another 2 hours at RT. Than reaction was cooled to - 78 °C and aqueous saturated solution of Rochel salt (100 mL) was added carefully dropwise to quench the reaction. After addition of Rochel salt, solution was diluted with EtOAc 100 mL. The resulting mixture was stirred at RT till a milky suspension turned into the clear biphasic solution (usually takes around 16 h). Resulting phases were separated and the aqueous layer was extracted with DCM (3x 75mL). Combined organic layers were washed with brine (50 mL), dried over Na2SO4, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2; DCM/MeOH = 95:5) to afford **1-119e** as a colorless oil (0.7 g, 63 %).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.32 – 7.29 (m, 2H), 7.28 – 7.27 (m, 1H), 7.25 – 7.22 (m, 1H), 7.04 – 7.00 (m, 2H), 6.83 (d, *J* = 8.2 Hz, 1H), 6.61 – 6.53 (m, 1H), 6.26 – 6.18 (m, 1H), 5.58 (d, *J* = 6.3 Hz, 1H), 5.17 (s, 2H), 4.31 – 4.27 (m, 2H), 4.00 – 3.90 (m, 2H), 3.56 (q, *J* = 5.7 Hz, 1H), 3.46 (s, 3H).

MS (ESI) *m/z* (%): 343

HRMS (ESI) *m/z:* $[(M+H)^+[-H_2O]]^+$ calculated for $C_{20}H_{21}O_4$: 325.1434; found: 325.1435.

(*E*)-3-(3-(acetoxymethyl)-2-(4-(methoxymethoxy)phenyl)-2,3-dihydrobenzofuran-5-yl)allyl acetate (**1-123a**)



1-119e **1-123a**

Diol **1-119e** (0.6 g, 1.75 mmol, 1.0 equiv.) was dissolved in DCM (0.5 M, 3.5 mL), and solution was cooled to 0 °C. Than TEA (0.73 mL, 5.26 mmol, 3.0 equiv.) was added following by acetyl chloride (0.27 mL, 3.86 mmol, 2.2 equiv.). The reaction mixture was stirred at room temperature overnight. Reaction was quenched by addition of water (30 mL). The aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **1-123a** as a yellowish oil (0.7 g, 93 %).

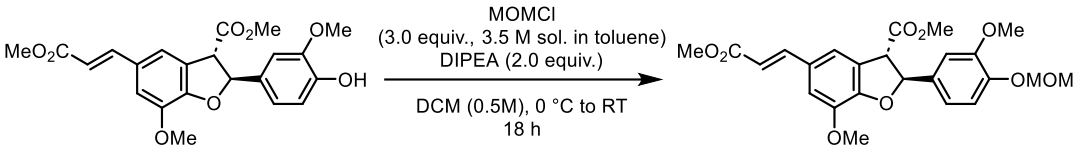
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.31 – 7.26 (m, 3H), 7.26 – 7.23 (m, 1H), 7.05 – 6.98 (m, 2H), 6.83 (d, *J* = 8.3 Hz, 1H), 6.61 (d, *J* = 15.9 Hz, 1H), 6.14 (dt, *J* = 15.8, 6.7 Hz, 1H), 5.48 (d, *J* = 6.5 Hz, 1H), 5.17 (s, 2H), 4.70 (dd, *J* = 6.7, 1.3 Hz, 2H), 4.44 (dd, *J* = 11.1, 5.5 Hz, 1H), 4.29 (dd, *J* = 11.1, 7.8 Hz, 1H), 3.70 (q, *J* = 6.6 Hz, 1H), 3.47 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.1, 171.0, 160.0, 157.4, 134.34, 134.26, 129.7, 128.5, 127.3, 126.9, 122.8, 120.9, 116.6, 109.9, 94.5, 87.7, 65.7, 65.5, 60.6, 56.1, 50.1, 21.2, 21.0, 14.3.

MS (ESI) *m/z* (%): 427

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{24}H_{26}O_7K$: 465.1310; found: 465.1310.

Methyl 7-methoxy-5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)-2-(3-methoxy-4-(methoxymethoxy) phenyl)-2,3-dihydrobenzofuran-3-carboxylate (**1-116a**)



1-36 **1-116a**

1-36 (2.0 g, 4.83 mmol, 1.0 equiv.) was dissolved in DCM (0.5 M, 9.65 mL), and the mixture was cooled to 0 °C. After 5 minutes at 0 °C DIPEA (1.67 mL, 9.65 mmol, 2.0 equiv.) was added following dropwise addition of methoxymethyl chloride solution in toluene (5.52 mL, 3.5 M, 19.3 mmol, 3.0 equiv.) during 1 h. The resulting mixture was stirred at RT overnight. Next day 50 mL of NH_4Cl was added and the water phase was water phase was extracted with EtOAc (3 x 100 mL). Combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-116a** as a white solid (2.05 g, 93 %).

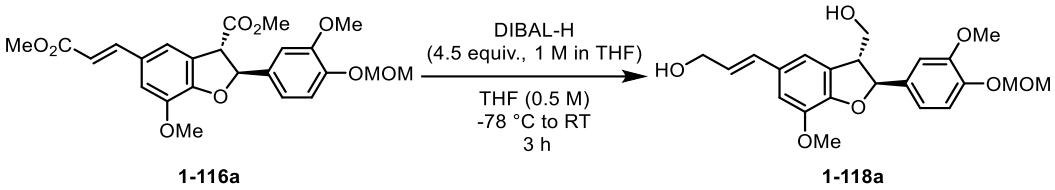
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.65 (d, *J* = 15.9 Hz, 1H), 7.19 – 7.18 (m, 1H), 7.15 – 7.11 (m, 1H), 7.03 – 7.02 (m, 1H), 6.95 – 6.89 (m, 2H), 6.32 (d, *J* = 15.9 Hz, 1H), 6.14 (d, *J* = 8.2 Hz, 1H), 5.22 (s, 2H), 4.35 (d, *J* = 8.1 Hz, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H), 3.49 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 170.8, 167.8, 150.1, 146.9, 144.9, 133.8, 128.8, 125.7, 118.9, 118.1, 116.4, 115.7, 112.2, 109.8, 95.5, 87.4, 56.4, 56.3, 56.1, 55.6, 53.1, 51.8.

MS (ESI) *m/z* (%): 459

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{24}H_{27}O_9$: 459.1650; found: 459.1651.

(*E*)-3-(3-(hydroxymethyl)-7-methoxy-2-(3-methoxy-4-(methoxymethoxy)phenyl)-2,3-dihydrobenzofuran-5-yl) prop-2-en-1-ol (**1-118a**)



1-116a **1-118a**

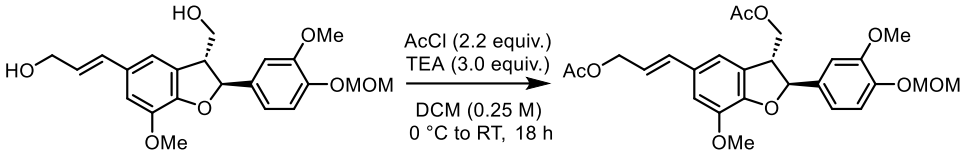
A solution of ester **1-116a** (2.0 g, 4.36 mmol, 1.0 equiv.) in THF (0.5 M, 6.53 mL) was cooled to - 78 °C and DIBAL-H (1 M in THF, 21.8 mL, 21.8 mmol, 4.5 equiv.) was added dropwise over 10 minutes. The resulting mixture was stirred at - 78 °C for 30 min before the cooling bath removal. The reaction mixture was stirred for another 2 hours at RT. Than reaction was cooled to - 78 °C and aqueous saturated solution of Rochel salt (100 mL) was added carefully dropwise to quench the reaction. After addition of Rochel salt, solution was diluted with EtOAc 100 mL. The resulting mixture was stirred at RT till a milky suspension turned into the clear biphasic solution (usually takes around 16 h). Resulting phases were separated and the aqueous layer was extracted with DCM (3x 75mL). Combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2; DCM/MeOH = 95:5) to afford **1-118a** as a colorless oil (1.16 g, 66 %).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.14 – 7.08 (m, 1H), 6.97 – 6.94 (m, 1H), 6.94 – 6.87 (m, 3H), 6.59 – 6.53 (m, 1H), 6.24 (dt, *J* = 15.8, 5.9 Hz, 1H), 5.61 (d, *J* = 7.0 Hz, 1H), 5.21 (s, 2H), 4.31 (dd, *J* = 6.0, 1.5 Hz, 2H), 3.98 (dd, *J* = 11.0, 6.0 Hz, 1H), 3.94 – 3.92 (m, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 3.63 (q, *J* = 5.8 Hz, 1H), 3.50 (s, 3H).

MS (ESI) *m/z* (%): 403

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{22}H_{26}O_7K$: 441.1310; found: 441.1312.

(*E*)-3-(3-(acetoxymethyl)-7-methoxy-2-(3-methoxy-4-(methoxymethoxy)phenyl)-2,3-dihydrobenzofuran-5-yl)allyl acetate (**1-122a**)



1-118a **1-122a**

Diol **1-118a** (0.41 g, 1.03 mmol, 1.0 equiv.) was dissolved in DCM (0.5 M, 4.1 mL), and solution was cooled to 0 °C. Than TEA (0.43 mL, 3.09 mmol, 3.0 equiv.) was added following by acetyl chloride (0.16 mL, 2.27 mmol, 2.2 equiv.). The reaction mixture was stirred at room temperature overnight. Reaction was quenched by addition of water (30 mL). The aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **1-122a** as a colorless oil (0.44 g, 88 %).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.10 (d, *J* = 8.3 Hz, 1H), 6.95 – 6.88 (m, 2H), 6.87 (s, 2H), 6.58 (d, *J* = 15.8 Hz, 1H), 6.14 (dt, *J* = 15.8, 6.6 Hz, 1H), 5.49 (d, *J* = 7.0 Hz, 1H), 4.69 (d, *J* = 6.6 Hz, 2H), 4.44 – 4.40 (m, 1H), 4.31 – 4.26 (m, 1H), 3.89 (s, 3H), 3.84 (s, 3H), 3.76 (q, *J* = 7.2 Hz, 1H), 3.48 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H).

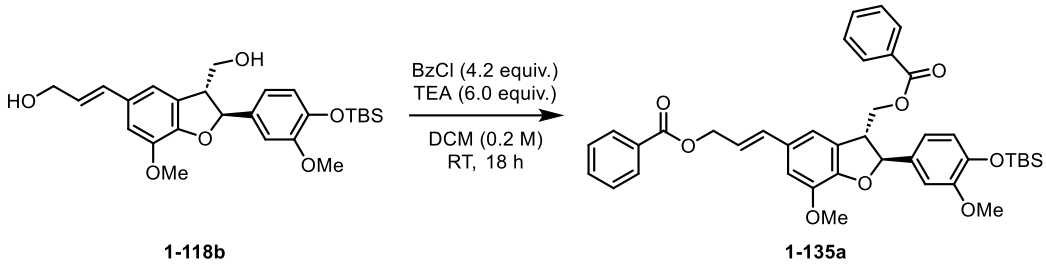
^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 170.9, 170.8, 150.0, 148.3, 146.6, 144.5, 134.6, 134.4, 130.6, 127.6, 121.2, 118.7, 116.2, 115.4, 115.4, 110.6, 109.6, 95.5, 88.5, 65.4, 65.3, 65.2, 56.2, 56.1, 56.0, 56.0, 55.9, 50.4, 21.1, 20.9.

MS (ESI) *m/z* (%): 487

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{26}H_{30}O_9K$: 525.1521; found: 525.1523.

1.7.2. Synthesis of dehydroquiquesetinerviusin A and related compounds

(*E*)-3-(3-((benzoyloxy)methyl)-2-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl)allyl benzoate (**1-135a**)



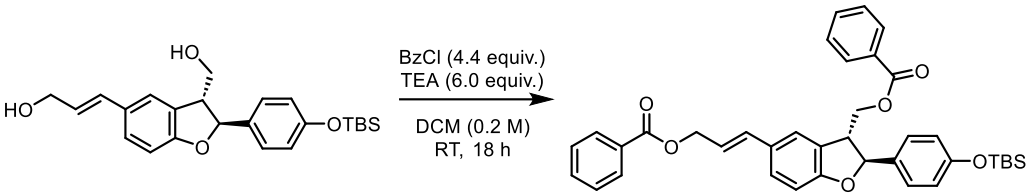
1-118b **1-135a**

1-118b (0.3 g, 0.63 mmol, 1.0 equiv.) was dissolved in DCM (0.2 M, 3.1 mL), and solution was cooled to 0 °C. After 5 minutes TEA (0.53 mL, 3.77 mmol, 6.0 equiv.) and benzoyl chloride (0.32 mL, 2.76 mmol, 4.4 equiv.) was added to the solution. The solution was stirred at RT overnight. Reaction was quenched by addition of NH_4Cl (15 mL). The aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-135a** as a white solid (0.18 g, 42 %).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.10 – 8.07 (m, 2H), 7.96 – 7.93 (m, 2H), 7.59 – 7.54 (m, 2H), 7.46 – 7.39 (m, 4H), 6.99 – 6.97 (m, 1H), 6.92 (d, *J* = 1.5 Hz, 1H), 6.87 – 6.84 (m, 2H), 6.80 – 6.77 (m, 1H), 6.71 – 6.66 (m, 1H), 6.27 (dt, *J* = 15.7, 6.5 Hz, 1H), 5.59 (d, *J* = 7.3 Hz, 1H), 4.97 (dd, *J* = 6.6, 1.2 Hz, 2H), 4.71 (dd, *J* = 11.2, 5.4 Hz, 1H), 4.54 (dd, *J* = 11.1, 7.9 Hz, 1H), 3.99 – 3.95 (m, 1H), 3.92 (s, 3H), 3.70 (s, 3H), 0.98 (s, 9H), 0.12 (s, 6H).

MS (ESI) *m/z* (%): 680 $[M+H]^+$ (100).

(*E*)-3-(3-((benzoyloxy)methyl)-2-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-2,3-dihydrobenzofuran-5-yl)allyl benzoate (**1-136a**)



1-119f **1-136a**

1-119f (0.25 g, 0.61 mmol, 1.0 equiv.) was dissolved in DCM (0.2 M, 3.1 mL), and solution was cooled to 0 °C. After 5 minutes TEA (0.51 mL, 3.64 mmol, 6.0 equiv.) and benzoyl chloride (0.31 mL, 2.76 mmol, 4.4 equiv.) was added to the solution. The solution was stirred at RT overnight. Reaction was quenched by addition of NH_4Cl (15 mL). The aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-136a** as a white solid (0.21 g, 54 %).

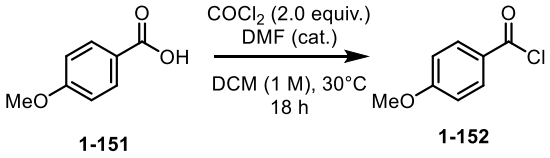
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.10 – 8.07 (m, 2H), 7.99 – 7.93 (m, 2H), 7.59 – 7.53 (m, 2H), 7.47 – 7.40 (m, 4H), 7.38 (t, *J* = 1.4 Hz, 1H), 7.29 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.26 – 7.23 (m, 2H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.83 – 6.77 (m, 2H), 6.70 (d, *J* = 15.8 Hz, 1H), 6.25 (dt, *J* = 15.8, 6.6 Hz, 1H), 5.56 (d, *J* = 6.6 Hz, 1H), 4.98 – 4.94 (m, 2H), 4.69 (dd, *J* = 11.1, 5.5 Hz, 1H), 4.55 (dd, *J* = 11.1, 7.8 Hz, 1H), 3.89 (q, *J* = 6.6 Hz, 1H), 0.97 (s, 9H), 0.18 (s, 6H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 166.6, 166.5, 160.1, 156.0, 134.4, 133.5, 133.4, 133.1, 130.4, 129.80, 129.77, 129.67, 128.7, 128.6, 128.5, 127.4, 127.1, 122.9, 121.0, 120.5, 109.9, 88.2, 66.4, 65.9, 50.1, 25.8, 18.3, -4.3.

MS (ESI) *m/z* (%): 620 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+NH_4]^+$ calculated for $C_{38}H_{44}NO_6Si$: 638.2932; found: 638.2947.

4-methoxybenzoyl chloride (**1-152**)



1-151 **1-152**

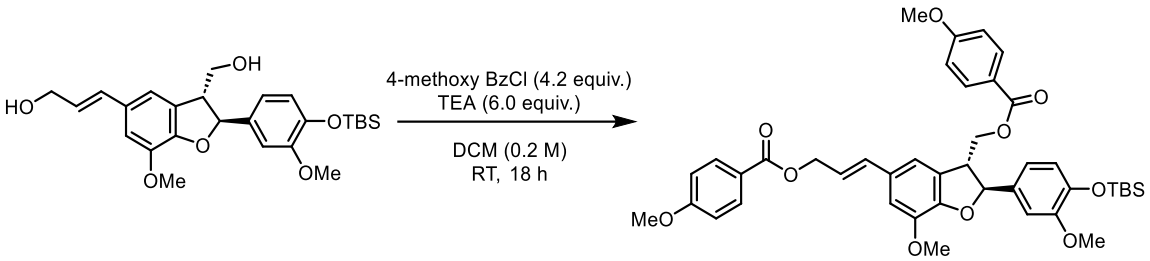
To the solution of 4-methoxybenzoic acid **1-151** (1 g, 7.24 mmol, 1.0 equiv.) in DCM (1 M, 7.24 mL) was added oxalyl chloride (1.26 mL, 14.5 mmol, 2.0 equiv.) and few drops of DMF. The solution was warmed to 30 °C and left stirring overnight. Next day reaction evaporated to dryness. The crude product **1-152** was used in the next step without further purification.

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.08 (dd, *J* = 8.9, 1.9 Hz, 2H), 6.99 – 6.93 (m, 2H), 3.90 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 167.3, 165.5, 134.2, 125.6, 114.4, 55.9.

MS (ESI) *m/z* (%): 170 $[M+H]^+$ (100).

(*E*)-3-(2-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)-7-methoxy-3-(((4-methoxybenzoyl)oxy)methyl)-2,3-dihydrobenzofuran-5-yl)allyl 4-methoxybenzoate (**1-135b**)



1-118b **1-135b**

1-118b (0.2 g, 0.42 mmol, 1.0 equiv.) was dissolved in DCM (0.2 M, 4.2 mL), and solution was cooled to 0 °C. After 5 minutes TEA (0.35 mL, 2.51 mmol, 6.0 equiv.) and **1-152** (0.3 g, 1.76 mmol, 4.2 equiv.) was added to the solution. The solution was stirred at RT overnight. Reaction was quenched by addition of NH_4Cl (15 mL). The aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-135b** as a white solid (0.19 g, 62 %).

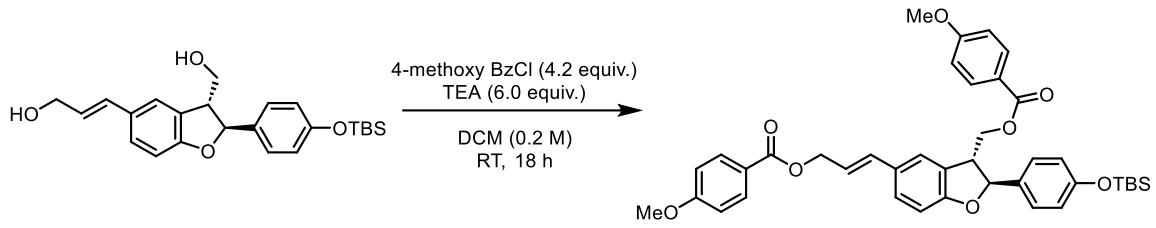
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.05 – 8.02 (m, 2H), 7.91 – 7.87 (m, 2H), 7.00 – 6.95 (m, 2H), 6.95 – 6.91 (m, 3H), 6.90 – 6.87 (m, 2H), 6.87 – 6.84 (m, 2H), 6.79 (d, *J* = 7.9 Hz,

1H), 6.69 – 6.64 (m, 1H), 6.26 (dt, *J* = 15.8, 6.5 Hz, 1H), 5.57 (d, *J* = 7.3 Hz, 1H), 4.93 (dd, *J* = 6.6, 1.3 Hz, 2H), 4.68 (dd, *J* = 11.1, 5.3 Hz, 1H), 4.50 (dd, *J* = 11.1, 8.0 Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.70 (s, 3H), 0.98 (s, 9H), 0.12 (s, 6H).

$^{13}C\{^{1}H\}$ NMR (126 MHz, Chloroform-d) δ (ppm): 166.4, 166.2, 163.7, 163.5, 151.2, 148.5, 145.4, 144.6, 134.3, 133.9, 133.0, 132.5, 131.8, 130.7, 127.9, 122.8, 122.2, 121.6, 121.0, 119.1, 115.5, 114.3, 113.84, 113.76, 110.6, 110.3, 89.5, 66.0, 65.6, 56.1, 55.6, 55.5, 50.6, 25.8, 18.6, -4.5.

MS (ESI) *m/z* (%): 740 $[M+H]^+$ (100).

(*E*)-3-(2-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-3-(((4-methoxybenzoyl)oxy)methyl)-2,3-dihydrobenzofuran-5-yl)allyl 4-methoxybenzoate (**1-136b**)



1-119f **1-136b**

1-119f (0.21 g, 0.52 mmol, 1.0 equiv.) was dissolved in DCM (0.2 M, 5.2 mL), and solution was cooled to 0 °C. After 5 minutes TEA (0.44 mL, 3.14 mmol, 6.0 equiv.) and **1-152** (0.38 g, 2.2 mmol, 4.2 equiv.) was added to the solution. The solution was stirred at RT overnight. Reaction was quenched by addition of NH_4Cl (15 mL). The aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-136b** as a white solid (0.19 g, 62 %).

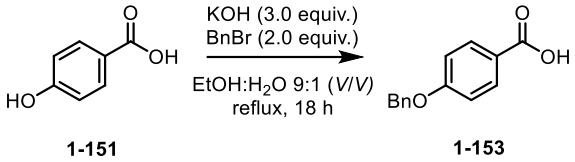
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.04 – 8.02 (m, 2H), 7.92 – 7.87 (m, 2H), 7.38 – 7.35 (m, 1H), 7.28 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.25 – 7.22 (m, 2H), 6.93 – 6.89 (m, 4H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.82 – 6.78 (m, 2H), 6.68 (dd, *J* = 15.9, 1.4 Hz, 1H), 6.25 (dt, *J* = 15.9, 6.6 Hz, 1H), 5.55 (d, *J* = 6.6 Hz, 1H), 4.92 (dd, *J* = 6.6, 1.3 Hz, 2H), 4.66 (dd, *J* = 11.0, 5.5 Hz, 1H), 4.51 (dd, *J* = 11.1, 7.8 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 0.97 (s, 9H), 0.18 (s, 6H).

$^{13}C\{^{1}H\}$ NMR (126 MHz, Chloroform-d) δ (ppm): 166.4, 166.2, 163.7, 163.5, 160.0, 156.0, 134.1, 133.0, 132.5, 131.8, 129.8, 128.6, 127.4, 127.2, 122.9, 122.8, 122.2, 121.2, 120.4, 117.2, 114.3, 113.9, 113.7, 109.9, 66.1, 65.7, 55.6, 25.8, 18.3, -4.3.

MS (ESI) *m/z* (%): 680 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{40}H_{45}O_8Si$: 681.2878; found: 681.2880.

4-(benzyloxy)benzoic acid (**1-153**)



1-151 **1-153**

KOH (2.38 g, 86.7 mmol, 3.0 equiv.) and benzyl bromide (6.91 mL, 57.8 mmol, 2.0 equiv.) were added to a solution of 4-hydroxybenzoic acid **1-151** (4 g, 28.9 mmol, 1.0 equiv.) in EtOH:H2O 9:1 (90:10 mL). The mixture was heated under reflux for 20 h. Next a 20% KOH solution (20 mL) was added, and the mixture was maintained under reflux for another 4 h. The mixture was then diluted with H_2O (60 mL) and acidified with 20% HCl solution. The precipitate formed was filtered and washed with water and *n*-hexane to give the pure **1-153** as a white solid (6.6g, 99 %).

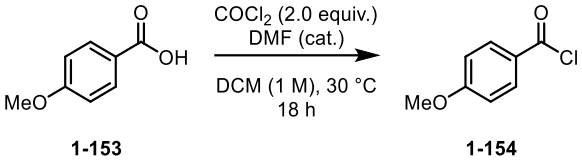
^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.47 – 7.40 (m, 2H), 7.04 – 6.99 (m, 2H), 6.99 – 6.92 (m, 2H), 6.91 – 6.87 (m, 1H), 6.68 – 6.62 (m, 2H), 4.73 (s, 2H).

$^{13}C\{^{1}H\}$ NMR (126 MHz, DMSO-d_6) δ (ppm): 167.0, 162.0, 136.6, 131.4, 128.5, 128.1, 127.9, 123.2, 114.6, 69.5.

MS (ESI) *m/z* (%): 228 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+NH_4]^+$ calculated for $C_{14}H_{13}O_3$: 229.0859; found: 229.0861.

4-(benzyloxy)benzoyl chloride (**1-154**)



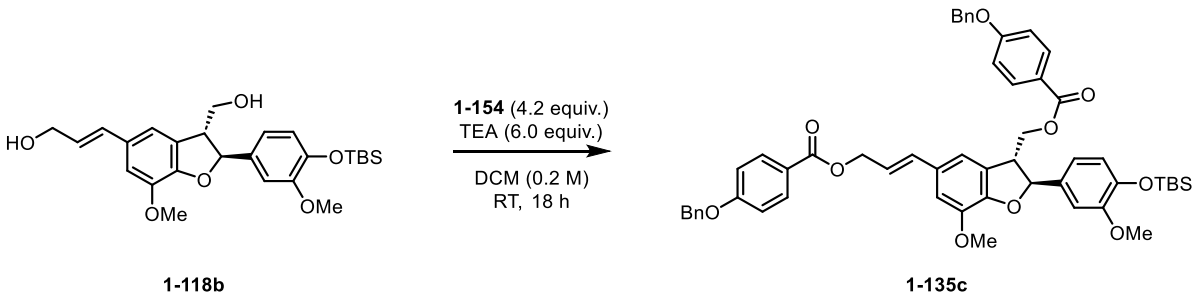
To the solution of **1-153** (0.5 g, 2.17 mmol, 1.0 equiv.) in DCM (1 M, 4.34 mL) was added oxalyl chloride (0.38 mL, 4.34 mmol, 2 equiv.) and few drops of DMF. The solution was warmed to 30 °C and left stirring overnight. Next day reaction evaporated to dryness. The crude **1-154** product was used in the next step without further purification.

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.12 – 8.06 (m, 2H), 7.47 – 7.40 (m, 4H), 7.06 – 7.01 (m, 2H), 5.16 (s, 2H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 167.3, 164.6, 135.7, 134.2, 128.9, 128.6, 127.7, 125.8, 115.2, 70.6.

MS (ESI) *m/z* (%): 246 $[M+H]^+$ (100).

(*E*)-3-(3-(((4-(benzyloxy)benzoyl)oxy)methyl)-2-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl)allyl 4-(benzyloxy)benzoate (**1-135c**)



1-118b (0.5 g, 1.05 mmol, 1.0 equiv.) was dissolved in DCM (0.2 M, 21 mL), and solution was cooled to 0 °C. After 5 minutes TEA (0.88 mL, 6.28 mmol, 6.0 equiv.) and **1-154** (1.15 g, 4.61 mmol, 4.2 equiv.) was added to the solution. The solution was stirred at RT overnight. Reaction was quenched by addition of NH_4Cl (15 mL). The aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 10:1 → 2:1) to afford **1-135c** as a white solid (0.55 g, 61 %).

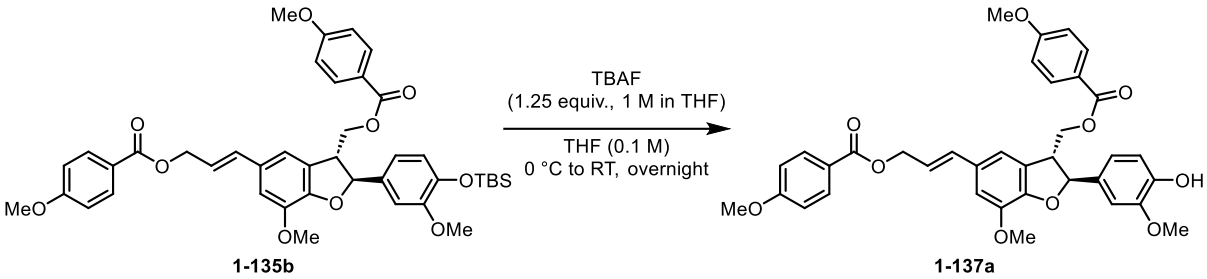
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.06 – 8.02 (m, 2H), 7.92 – 7.88 (m, 2H), 7.44 – 7.38 (m, 8H), 7.36 – 7.33 (m, 2H), 7.01 – 6.95 (m, 5H), 6.92 (s, 1H), 6.89 – 6.83 (m, 2H), 6.82 –

6.77 (m, 1H), 6.67 (d, *J* = 15.6 Hz, 1H), 6.26 (dt, *J* = 16.0, 6.5 Hz, 1H), 5.58 (d, *J* = 7.3 Hz, 1H), 5.11 (d, *J* = 2.8 Hz, 4H), 4.93 (d, *J* = 6.5 Hz, 2H), 4.68 (dd, *J* = 11.1, 5.3 Hz, 1H), 4.50 (dd, *J* = 11.1, 7.9 Hz, 1H), 3.98 – 3.93 (m, 1H), 3.92 (s, 3H), 3.69 (s, 3H), 0.98 (s, 9H), 0.13 (s, 6H).

$^{13}C\{^{1}H\}$ NMR (126 MHz, Chloroform-d) δ (ppm): 166.3, 166.1, 162.8, 162.7, 151.2, 148.5, 145.4, 144.6, 136.35, 136.28, 134.3, 133.9, 132.0, 131.9, 130.7, 128.8, 128.39, 128.36, 127.8, 127.64, 127.62, 123.0, 122.4, 121.6, 121.0, 119.0, 115.5, 114.7, 114.6, 114.5, 110.7, 110.2, 89.4, 70.2, 66.0, 65.6, 56.1, 55.5, 50.6, 29.8, 25.8, 25.8, 18.6, -4.5.

MS (ESI) *m/z* (%): 893 $[M+H]^+$ (100).

(*E*)-3-(2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-(((4-methoxybenzoyl)oxy)methyl)-2,3-dihydrobenzofuran-5-yl)allyl 4-methoxybenzoate (**1-137a**)



1-135b **1-137a**

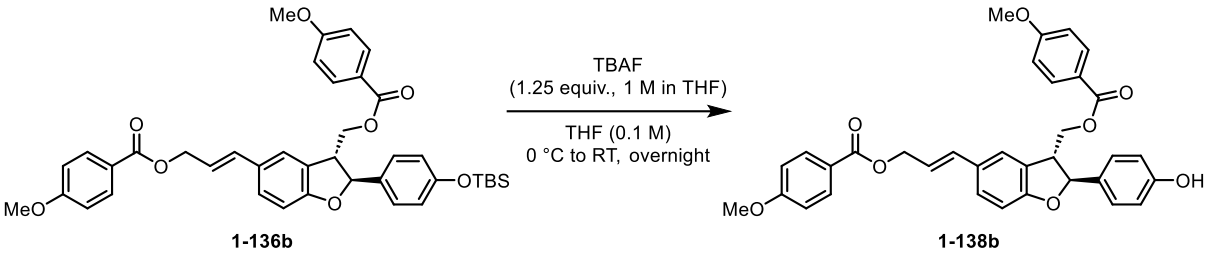
1-135b (0.185 g, 0.247 mmol, 1.0 equiv.) was dissolved in THF (0.1 M, 2.5 mL), and solution was cooled to 0 °C. After 5 minutes TBAF (0.309 mL, 0.309 mmol, 1 M, 1.25 equiv.) was added and the solution was stirred at RT overnight. Reaction was quenched by addition of NH_4Cl (15 mL). The aqueous layer was extracted with DCM (3 × 15 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 4:1 → 1:1) to afford **1-137a** (0.092 g, 59 %).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.05 – 8.02 (m, 2H), 7.90 – 7.86 (m, 2H), 6.97 (d, *J* = 1.4 Hz, 1H), 6.95 – 6.85 (m, 9H), 6.67 (d, *J* = 15.8 Hz, 1H), 6.30 – 6.24 (m, 1H), 5.61 (s, 1H), 5.57 (d, *J* = 7.4 Hz, 1H), 4.93 (dd, *J* = 6.6, 1.3 Hz, 2H), 4.68 (dd, *J* = 11.2, 5.4 Hz, 1H), 4.50 (dd, *J* = 11.1, 7.9 Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.79 (s, 3H).

$^{13}C\{^{1}H\}$ NMR (126 MHz, Chloroform-d) δ (ppm): 166.4, 166.2, 163.7, 163.5, 148.4, 146.8, 145.9, 144.6, 134.3, 132.43, 132.40, 131.8, 130.7, 127.8, 122.8, 122.1, 121.7, 119.8, 115.5, 114.4, 113.93, 113.91, 113.83, 113.75, 110.6, 108.8, 89.4, 65.9, 65.6, 56.1, 56.0, 55.61, 55.58, 50.7.

MS (ESI) *m/z* (%): 627 $[M+H]^+$ (100); **HRMS (ESI) *m/z:*** $[M+NH_4]^+$ calculated for $C_{36}H_{38}O_{10}N$: 644.2490; found: 644.2504.

(*E*)-3-(2-(4-hydroxyphenyl)-3-(((4-methoxybenzoyl)oxy)methyl)-2,3-dihydrobenzofuran-5-yl)allyl 4-methoxybenzoate (**1-138b**)



1-136b **1-138b**

1-136b (0.1 g, 0.15 mmol, 1.0 equiv.) was dissolved in THF (0.2 M, 0.7 mL), and solution was cooled to 0 °C. After 5 minutes TBAF-3H_2O (0.05g, 0.18 mmol, 1.25 equiv.) was added and the solution was stirred at RT overnight. Reaction was quenched by addition of NH_4Cl (15 mL). The aqueous layer was extracted with DCM (3 × 15 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 3:1 → 2:1) to afford **1-138b** (0.059 g, 71 %).

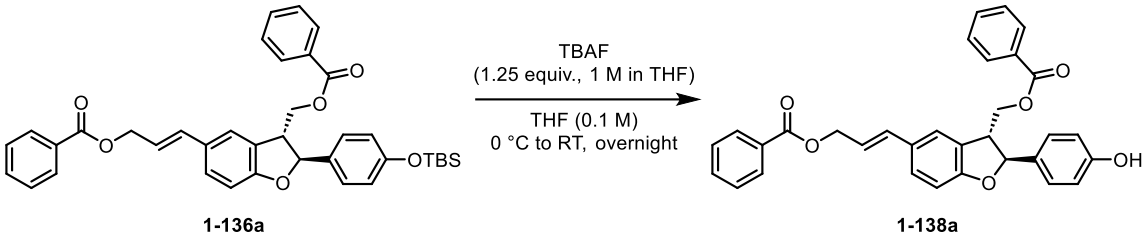
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.03 (d, *J* = 9.0 Hz, 2H), 7.90 (d, *J* = 8.8 Hz, 2H), 7.36 (d, *J* = 1.8 Hz, 1H), 7.27 (t, *J* = 8.8 Hz, 3H), 6.92 (d, *J* = 8.1 Hz, 2H), 6.90 (d, *J* = 8.1 Hz, 2H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 2H), 6.68 (d, *J* = 15.8 Hz, 1H), 6.25 (dt, *J* = 15.8, 6.5 Hz, 1H), 5.55 (d, *J* = 6.5 Hz, 1H), 4.92 (dd, *J* = 6.6, 1.2 Hz, 2H), 4.76 (s, 1H), 4.66 (dd, *J* = 11.0, 5.5 Hz, 1H), 4.51 (dd, *J* = 11.0, 7.8 Hz, 1H), 3.86 (s, 3H), 3.86 (s, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 166.4, 166.3, 163.7, 163.5, 160.0, 155.9, 134.1, 133.1, 131.8, 129.8, 128.6, 127.7, 127.1, 122.9, 122.8, 122.1, 121.2, 115.7, 113.9, 113.7, 109.9, 88.1, 66.1, 65.7, 55.60, 55.57, 50.2.

MS (ESI) *m/z* (%): 565 $[M-H]^-$ (100).

HRMS (ESI) *m/z:* $[M-H]^-$ calculated for $C_{34}H_{29}O_8$: 565.1868; found: 565.1868.

(*E*)-3-(3-((benzoyloxy)methyl)-2-(4-hydroxyphenyl)-2,3-dihydrobenzofuran-5-yl)allyl benzoate (**1-138a**)



1-136a (0.5 g, 0.727 mmol, 1.0 equiv.) was dissolved in THF (0.1 M, 7.27 mL), and solution was cooled to 0 °C. After 5 minutes TBAF (0.909 mL, 1 M, 0.18 mmol, 1.25 equiv.) was added and the solution was stirred at RT overnight. Reaction was quenched by the addition of NH_4Cl (25 mL). The aqueous layer was extracted with DCM (3 × 30 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-138a** (0.363 g, 88 %).

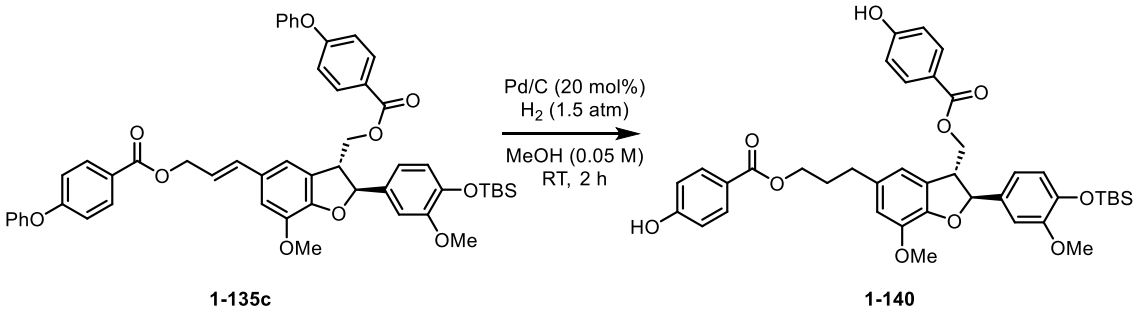
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.09 – 8.05 (m, 2H), 7.96 – 7.92 (m, 2H), 7.58 – 7.53 (m, 2H), 7.47 – 7.41 (m, 4H), 7.37 (s, 1H), 7.31 – 7.29 (m, 1H), 7.28 – 7.24 (m, 2fH), 6.86 (d, *J* = 8.3 Hz, 1H), 6.83 – 6.79 (m, 2H), 6.70 (d, *J* = 15.8 Hz, 1H), 6.25 (dt, *J* = 15.9, 6.6 Hz, 1H), 5.55 (d, *J* = 6.5 Hz, 1H), 5.12 (s, 1H), 4.96 (d, *J* = 6.6 Hz, 2H), 4.69 (dd, *J* = 11.1, 5.5 Hz, 1H), 4.54 (dd, *J* = 11.1, 7.8 Hz, 1H), 3.88 (q, *J* = 6.5 Hz, 1H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 166.7, 166.5, 160.0, 156.0, 134.4, 133.4, 133.2, 133.0, 130.3, 129.81, 129.77, 129.7, 128.7, 128.6, 128.5, 127.7, 127.0, 122.9, 121.0, 115.7, 109.9, 88.1, 66.4, 66.0, 50.1.

MS (ESI) *m/z* (%): 507 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+NH_4]^+$ calculated for $C_{32}H_{30}O_6N$: 524.2068; found: 524.2072.

3-(2-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)-3-(((4-hydroxybenzoyl)oxy)methyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl)propyl 4-hydroxybenzoate (**1-140**)



1-135c **1-140**

1-135c (0.113 g, 0.127 mmol, 1.0 equiv.) was dissolved in MeOH (0.05 M, 2.53 mL), and 20mol% Pd/C (0.027 g, 0.0025 mmol, 0.2 equiv.) was added under Argon atmosphere. Then the Argon atmosphere was changed for Hydrogen atmosphere (1 atm) and the solution was stirred at RT overnight. Reaction mixture was filtered through®, filter cake was washed by EtOAc (2x 10 mL), and solvent was concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **1-140** (0.067 g, 47 %).

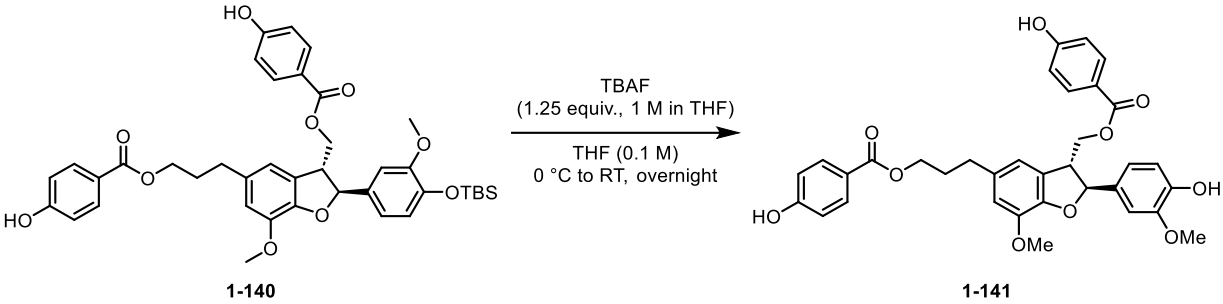
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.94 – 7.91 (m, 2H), 7.85 – 7.81 (m, 2H), 6.90 – 6.77 (m, 5H), 6.69 (d, *J* = 19.0 Hz, 2H), 5.97 (bs, 2H), 5.52 (d, *J* = 7.6 Hz, 1H), 4.66 – 4.61 (m, 1H), 4.53 – 4.46 (m, 1H), 4.34 – 4.29 (m, 2H), 3.92 (q, *J* = 7.2 Hz, 1H), 3.87 (s, 3H), 3.71 (d, *J* = 1.4 Hz, 3H), 2.09 – 2.03 (m, 2H), 0.98 – 0.97 (m, 9H), 0.13 – 0.12 (m, 6H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 166.8, 166.4, 160.4, 160.2, 151.2, 146.4, 145.2, 144.3, 135.1, 134.2, 132.1, 132.0, 127.5, 122.8, 122.2, 121.0, 119.2, 116.4, 115.5, 115.4, 112.6, 110.4, 89.0, 66.2, 64.3, 56.1, 55.6, 50.8, 32.4, 30.8, 25.8, 18.6, -4.5.

MS (ESI) *m/z* (%): 515 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M-H]^-$ calculated for $C_{40}H_{45}O_{10}Si$: 713.2788; found: 713.2794.

3-(2-(4-hydroxy-3-methoxyphenyl)-3-(((4-hydroxybenzoyl)oxy)methyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl)propyl 4-hydroxybenzoate (**1-141**)



1-140 **1-141**

1-140 (0.056 g, 0.078 mmol, 1.0 equiv.) was dissolved in THF (0.1 M, 0.78 mL), and solution was cooled to 0 °C. After 5 minutes TBAF-3H_2O (0.031 g, 0.098 mmol, 1.25 equiv.) was added and the solution was stirred at RT overnight. Reaction was quenched by addition of NH_4Cl (5 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 2:1 → 1:2, 1% AcOH) to afford **1-141** (0.022 g, 47 %).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.93 (d, *J* = 8.4 Hz, 2H), 7.80 (d, *J* = 8.4 Hz, 2H), 6.93 – 6.90 (m, 2H), 6.88 – 6.84 (m, 3H), 6.82 – 6.78 (m, 2H), 6.71 (s, 1H), 6.68 (s, 1H), 5.50 (d, *J* = 7.6 Hz, 1H), 4.64 (dd, *J* = 11.0, 5.5 Hz, 1H), 4.49 (dd, *J* = 11.0, 7.7 Hz, 1H), 4.31 (t, *J* = 6.4 Hz, 2H), 3.95 – 3.88 (m, 1H), 3.87 (s, 3H), 3.79 (s, 3H), 2.73 (t, *J* = 7.6 Hz, 2H), 2.09 – 2.02 (m, 2H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 166.6, 166.3, 160.3, 160.1, 146.8, 146.4, 145.8, 144.3, 135.1, 132.6, 132.1, 132.0, 127.6, 122.9, 122.2, 119.8, 116.4, 115.43, 115.40, 114.4, 112.6, 108.9, 89.1, 66.0, 64.2, 56.1, 56.0, 50.8, 32.4, 30.8.

MS (ESI) *m/z* (%): 599 $[M-H]^-$ (100).

HRMS (ESI) *m/z:* $[M-H]^-$ calculated for $C_{34}H_{31}O_{10}$: 599.1912; found: 599.1927.

CHAPTER II

2. Nitro fatty acids

2.1. Nitro Fatty Acids: An Introduction

Nitro fatty acids (**NO_2FAs**) and the corresponding nitrated lipids belong to a class of compounds formed endogenously by reaction of unsaturated fatty acids (UFAs) with reactive nitrogen species (for example, monooxide radical ($NO^{\cdot}$) or nitrite anions (NO_2^-)). These highly reactive nitrated organic species have been found in tissues and biological fluids of mammals; however, the most interesting is the fact that **NO_2FA** were found mainly in the brain. They have also been reported to form in healthy human plasma and urine.[82,83] Examples of nitrated FAs are shown in **Figure 31**.

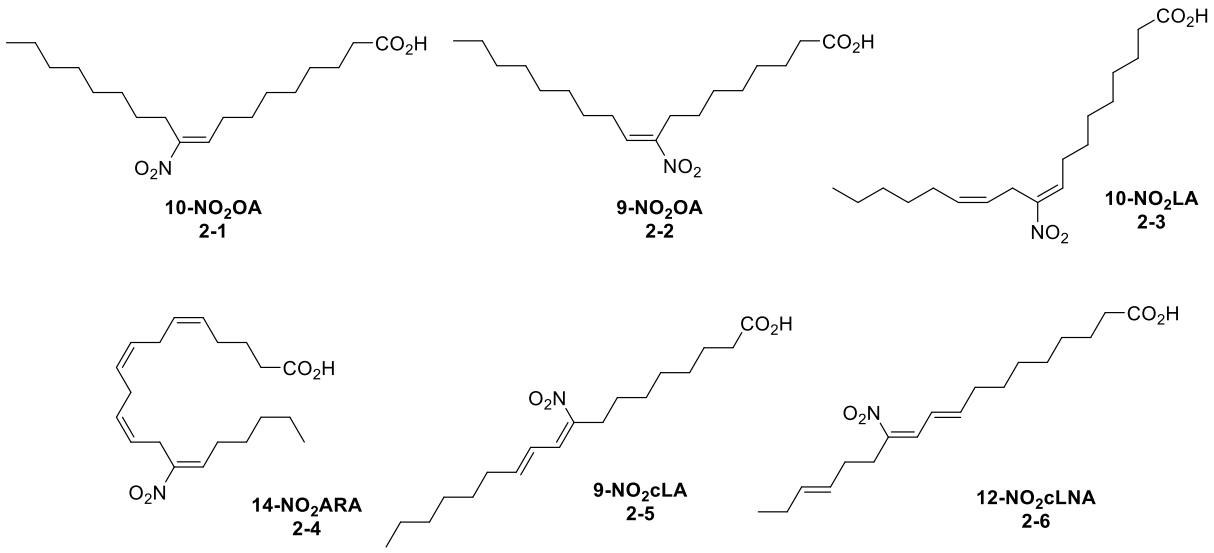
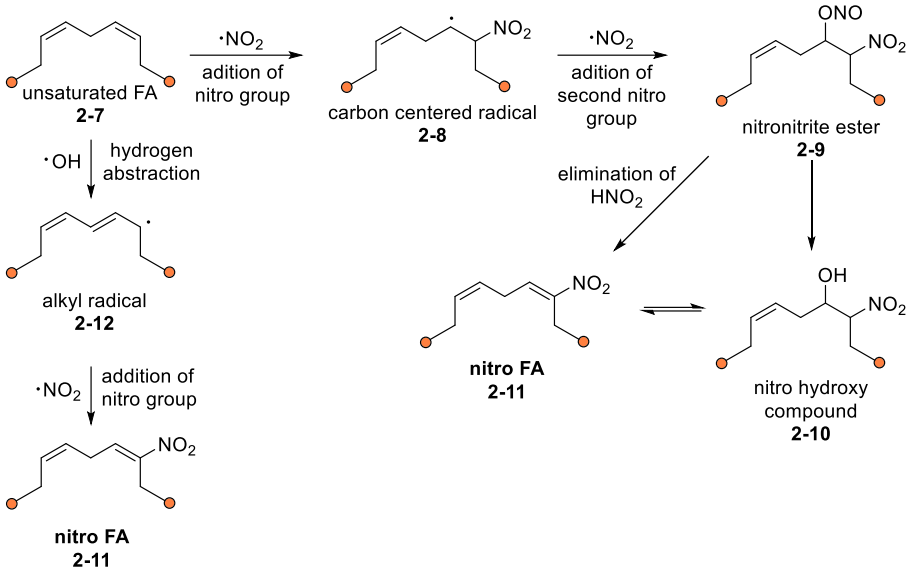


Figure 31: Examples of different NO_2FAs detected in mammals. Structure of 9- and 10-NO_2 oleic acid **2-1** and **2-2**, 10-NO_2 linoleic acid **2-3**, 14-NO_2 arachidonic acid **2-4**, 9-NO_2 conjugated linoleic acid **2-5** (nitro rumenic acid) and 12-NO_2 octadeca-9,11,15-trienoic acid **2-6** (nitro rumenelic acid).

From advanced studies, it is known that **NO_2FAs** are generated from fatty acids (**FAs**) by nitration in hydrophobic compartments such as the lipid bilayer of cellular membranes or the lipophilic core of lipoproteins,[84] and during digestion[85,86]. Reactive nitrogen species can modify unsaturated **FA** and produce various derivatives of **FA** that contain functional groups of hydroxyl, hydroperoxy, nitro, and nitrohydroxy functional groups.[84] Although, the exact formation of **NO_2FAs** *in vivo* remains unknown, two different mechanisms were proposed. One mechanism suggests that the first generation of carbon-centered radical **2-8** is formed which is further transformed to nitronitrite **2-9** and nitrohydroxy **2-10** compounds. The further elimination of nitrous acid (HNO_2) or water yields the final product, **NO_2FA**

derivative **2-11** (**Scheme 22**). The second mechanism involves the formation of the alkyl radical **2-12** that further undergoes to the addition of nitro group addition.[87,88]



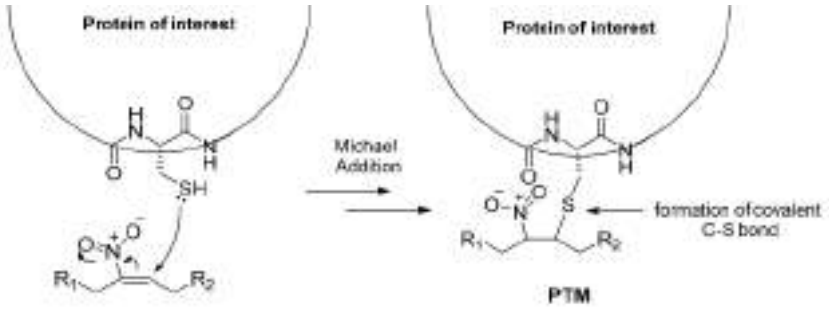
Scheme 22: Endogenous nitration of UFAs.[87]

In general, the structure of **NO_2FAs** contains at least one nitro olefinic bond, giving them a property of Michael acceptors. Therefore, these compounds are highly reactive to nucleophiles and can react endogenously with Michael donors forming a covalent Michael addition product. Such donors (nucleophiles) are, for example, the amino acids cysteine and lysine.[89–91]

2.1.1. Biological activity

The presence of Michael acceptor motive within the **NO_2FAs** structure predetermines that **NO_2FAs** should be expected to be a highly reactive species. And, as a consequence, various biological activities observed in the presence of **NO_2FAs** are attributed to these structures. For example, they form adducts with cysteine residues due to the high nucleophilic reactivity of the –SH group. This formation of adducts between cysteine and **NO_2FAs** plays a crucial role in many biochemical pathways that result in the activation of Nrf2. This activation induces the expression of genes responsible for antioxidant and cell protection function and induces anti-inflammatory cell singaling.[87,92–94] Addition of cysteine residues from a protein of interest causes structural change in protein and its function. This change

is called post-translation modification (**Scheme 23**).[87] Therefore, **NO_2FAs** are expected to be a Nrf2 activator.



Scheme 23: Post-translational modification of protein of interest (adapted from[87]).

NO_2FAs are also reported to interact with p65 and p50 cysteine residues of nuclear factor κB preventing the formation of various pro-inflammatory mediators responsible for diseases such as vascular inflammation and pro-fibrotic responses.[95] They also interact with regulatory proteins, such as keap-1 and peroxisome proliferator-activated receptor γ (PPARγ), heat shock proteins, 5-lipoxygenase, and others, in order to regulate many diseases.[95–98] To understand the way **NO_2FAs** interact with proteins is the foremost priority for the SAR to maximize the therapeutic effect of **NO_2FAs** and minimize harmful side effects.

One of the most studied **NO_2FAs** is 10-NO_2 oleic acid **2-1** (clinical name CXA-10, or generally 10-NO_2OA) was already in **PHASE II** clinical studies against pulmonary arterial hypertension, focal segmental glomerulosclerosis, and asthma. 10-NO_2OA **2-1** was also reported to significantly increase the antiproliferative effects of some known antineoplastic DNA damaging agents such as olaparib **2-13**, cisplatin **2-14,** and doxorubicin **2-15** against triple negative breast cancer (**Figure 32**).[99] 10-NO_2OA significantly reduces the growth of triple negative breast cancer epithelial cells by inhibiting the nuclear factor kappa-light chain enhancer of the activated B cells (NF-κB) signaling pathway. This inhibition occurs through the ability of 10-NO_2OA to modify specific cysteine residues in the NF-κB subunit kinase IKKβ and NF-κB RelA protein. This modification prevents DNA binding and subsequent degradation of proteins induced by the RelA protein. Additionally, 10-NO_2OA inhibits NF-κB activity induced by TNFα, leading to suppression of target genes responsible for the expression of metastasis-related proteins such as ICAM-1 and the urokinase-type plasminogen activator.[99,100]

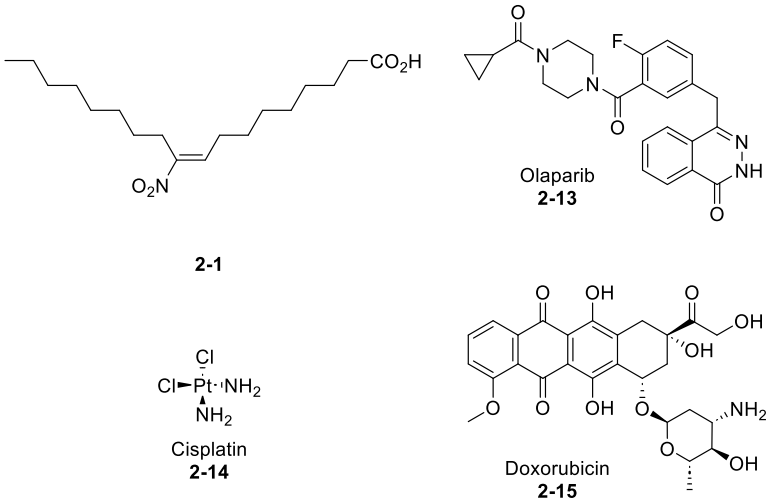


Figure 32: Structure of 10-NO_2OA **2-1** in comparison to other antineoplastic DNA-damaging agents.

In contrast, another variant of the well-known 10-NO_2OA, namely 9-nitro oleic acid **2-2** (**9-NO_2OA**), has demonstrated its potential as a cytotoxic agent against the colorectal cancer cell lines HCT-116 and HT-29. The presence of 9-NO_2OA significantly inhibited the cell viability of these cancer cells by inducing caspase-dependent apoptosis through the intrinsic apoptotic pathway. This anticancer effect can be attributed to the antioxidant properties of **NO_2FAs**, with 9-NO_2OA specifically targeting mitochondria and causing disruption of their membrane and respiratory functions. Ultimately, this disruption leads to apoptosis of cancer cells.[101]

Recent research has shown that NO_2OA (a mixture of regio isomers was used in this study) plays a key role in the regulation of pluripotency and differentiation of mouse embryonic stem cells through STAT3 signaling. 9/10-NO_2OA was shown to influence mESC pluripotency by regulating STAT3 phosphorylation. It also affected cardiac differentiation and directed mESCs to a neural fate.[102]

Nitro linoleic acid **2-3** (NO_2LA) and nitro arachidonic acid **2-4** (NO_2ARA) belonging to polyunsaturated **FAs** (**Figure 33**).

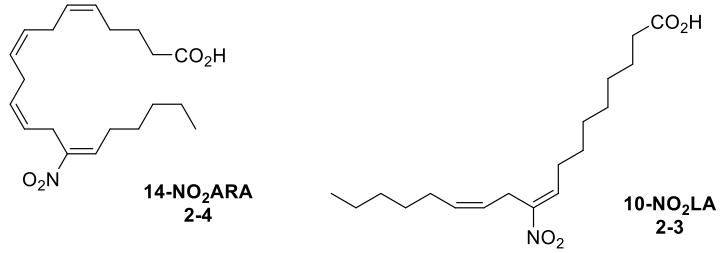


Figure 33: Examples of polyunsaturated FAs. Structure of 14-NO_2ARA **2-4** and 10-NO_2LA **2-3**.

These FAs are targets for peroxidation by nonenzymatic autocatalytic oxidation reactions due to their unsaturated double bonds.[103–105] Arguably, more important of these two,

arachidonic acid (**ARA**), a 20-carbon polyunsaturated FA with four double bonds is a precursor to prostaglandins and thromboxanes and a powerful signaling molecules. ARA nitration by inflammatory stimuli alters the 'normal metabolic pathway' of ARA leading to new responses.[106] Since NO_2ARA is the most abundant polyunsaturated FA in biological membranes, NO_2ARA could therefore function as a new signaling molecule and a specific biological marker of inflammation. Until today, NO_2ARA was prepared only by acidic nitration ($NaNO_2$) and was characterized as a mixture of 9-, 12-, 14-, and 15-NO_2 isomers. Biological evaluation of this NO_2ARA mixture showed that the compound exhibited anti-inflammatory activity by inhibiting POX activity, being a new poorly reversible POX inhibitor of PGHS-2103, the ability to release NO, induces vasorelaxation due to its capacity to release NO, and modulates macrophage activation and NOS2 expression.[107,108] Additionally, NO_2ARA exhibits antioxidant[109] and neuroprotective activity [110] due to the Nrf2 activation.

Conjugated FAs (**Figure 34**) are commonly found in a diet composed mainly of rumenic acid (conjugated linoleic acid, cLA) and rumelenic acid (conjugated α-linolenic acid, cLNA). To date, there is no clear consensus on the role of cLA in inflammatory diseases. Under normal conditions, these compounds are nitrated in the gastric compartment, mainly forming 9-NO_2cLA **2-5**, 12-NO_2cLA **2-6,** and 9-NO_2cLNA.[111]

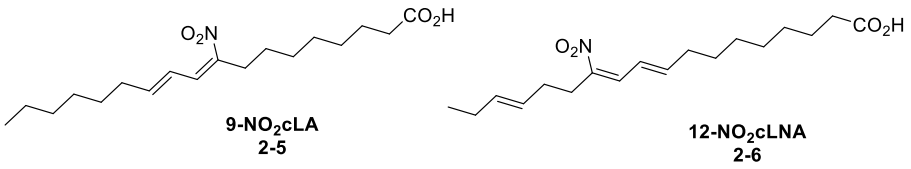
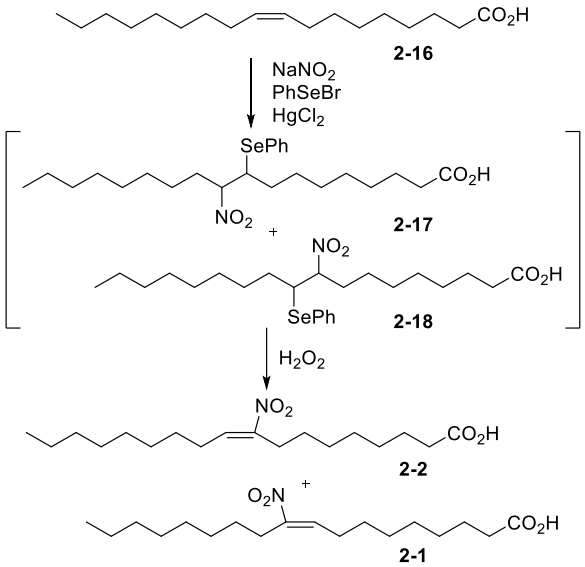


Figure 34: Examples of conjugated FAs. Structures of 9-NO_2cLA **2-5**, 12-NO_2cLA **2-6**.

In conclusion, the activity of **NO_2FAs** in various biological systems is evident. Furthermore, **NO_2FAs** are endogenous compounds produced by our body. Thus, their potential as a possible drug candidate is huge. However, the specific mode of action underlying their effects remains largely unknown. Previous studies have often focused on mixtures of stereo and regio isomers, which limits our understanding of the individual contributions of each regio isomer. Therefore, it is crucial to synthesize and isolate pure regio isomers of **NO_2FAs** to further investigate their biological activities. From our point of view, the biological testing of **NO_2FAs** should be carried out again but this time only pure regio and stereoisomers should be used to provide exact insights into the mechanisms of action of those molecules and improve our understanding of the various biological effects exhibited by **NO_2FAs**.

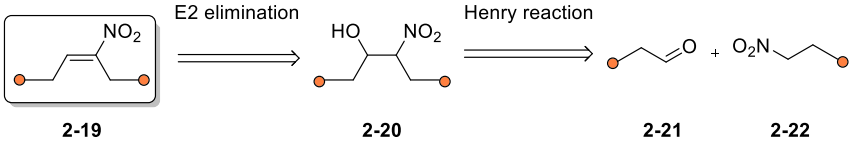
2.1.2. Synthesis of NO_2FAs

As mentioned previously, **NO_2FAs** are endogenously biosynthesized during inflammation by reactive nitrogen species.[87,88] Their importance and potential for additional applications however, led chemists to develop additional synthetic routes to their preparation. Currently, two different pathways are synthetically used. The first is biomimetic and explores the direct nitration of a double bond of unsaturated FAs which, for example, in the case of oleic acid **2-16**, delivers a mixture of 9- and 10-NO_2OAs **2-1** and **2-2** as final products (**Scheme 24**).[112,113] The situation is complicated when more double bonds are present. As mentioned in the previous chapter, the position of the nitro group is very important, therefore having a mixture of regio isomers is not optimal for biological evaluation and expensive separation of these isomers must (should) be performed.



Scheme 24: Direct nitration radical protocol (nitro-selenation/oxidation).[113]

The second approach is based on step-by-step synthesis which usually involves Henry reaction between nitro alkane/alkene **2-22** and aldehyde **2-21** followed by E2 elimination of the hydroxy group from nitro/hydroxy compound **2-20**. In such a case, the regio-defined isomer **2-19** with various degrees of *E*/*Z*-stereoselectivity is formed (**Scheme 25**).[114,115]

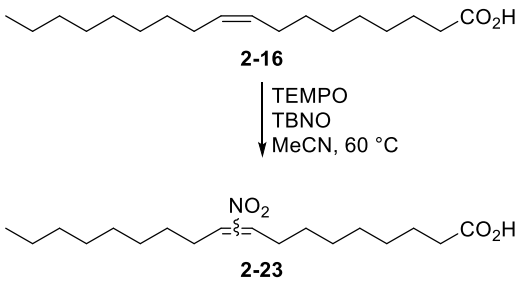


Scheme 25: Two possible retrosynthetic approaches towards NO_2FAs **2-19**.

2.2. Results and discussion°

2.2.1. Introduction

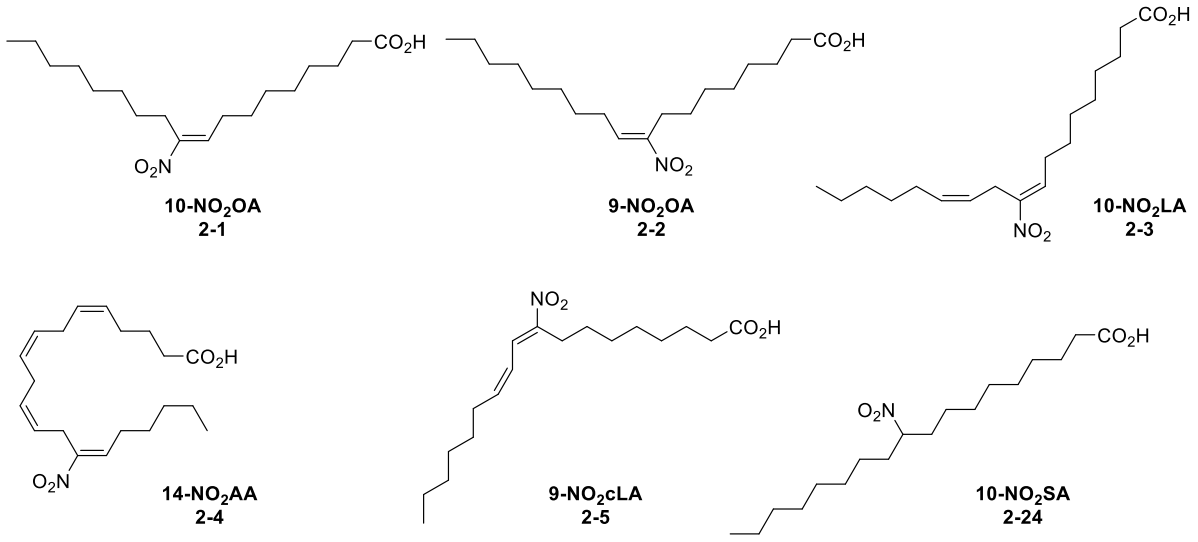
Over the last 3 years our group is interested in the **NO_2FAs** and their physical and biological properties. In 2022, our group has started a new collaboration with a group of Prof. Jan Vacek from Palacký University. The goal of this collaboration was to prepare **NO_2FAs** with stereo and regio control since most biological assays[116–118], redox properties evaluation[119], stability testing and measurement of electrophilic characteristics[120–122] were performed on mixtures of positional isomers (especially on mixture of 9-/10-NO_2 oleic acid **2-23**) prepared by relatively new optimized direct nitration radical protocol of oleic acid **2-16**[118], which eliminated nitro-selenation/oxidation[113] to avoid mercury contamination (**Scheme 26**).



Scheme 26: Novel protocol for direct nitration.[118]

Our initial phase included the synthesis of specific regio and stereo isomers including 9- and 10-NO_2 oleic acid **2-1** and **2-2**, 10-NO_2 linoleic acid **2-3**, 14-NO_2 arachidonic acid **2-4**, 9-NO_2 conjugated linoleic acid **2-5** (nitro rumenic acid) and as a control for mechanistic and biological studies a fully saturated 10-NO_2 stearic acid **2-24** (**Scheme 27**).

°This work was supported by Grant Agency of the Czech Republic (no.23-06051S) and by the Palacký University Internal Grant Agency (IGA_PrF_2022_016, IGA_PrF_2022_022, IGA_PrF_2023_031 and IGA_PrF_2023_020). Part of the results presented in this chapter were published in a full paper that covers the development of novel modification of Julia-Kocienski olefination (stereoselective synthesis of *E* and *Z* olefins; I am shared 1st author: *Adv. Synth. Catal.* **2024**, *366*, 480-487, doi: 10.1002/adsc.202301054) and the olefination reaction was further put in context of all olefination reactions of such type in recent review (I am 1st author, *Molecules* **2024**, *29*(12), 2719; doi: 10.3390/molecules29122719). As a primary researcher in the project, I was responsible for designing and conducting experimental procedures and measurements, analyzing the data, and interpreting the results. Additionally, I am now writing a synthesis related part of the manuscripts that are in preparation and will be published with our colleagues. Additional research that is based on compounds I prepared is summarized in chapter 2.3.

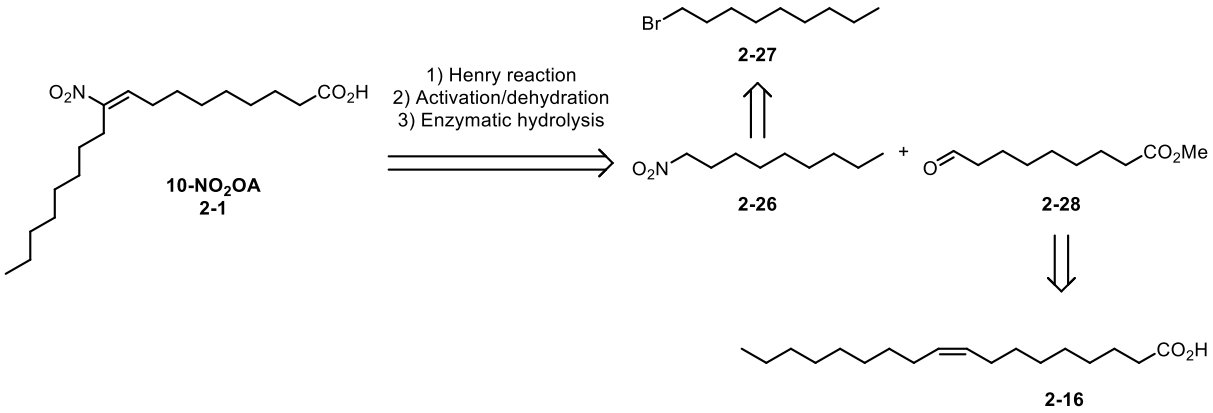


Scheme 27: Structures of targeted nitrated FAs.

In following chapters, the synthesis of each NO_2FA will be discussed separately.

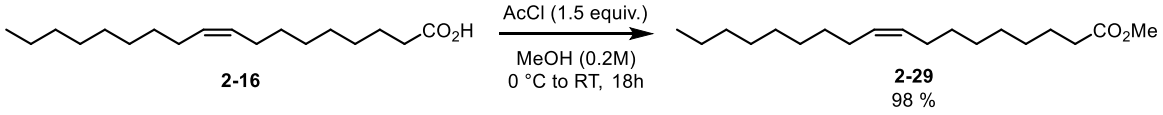
2.2.2. 10-NO_2 oleic acid

First, we tackled the synthesis of the 10-NO_2OA **2-1**. Our retrosynthetic plan was based on the Henry reaction[123] that required a reunion of the nitrononane **2-26** prepared from 1-bromononane **2-27** and of the methyl 9-oxononanoate **2-28** readily available from oleic acid **2-16** (**Scheme 28**).



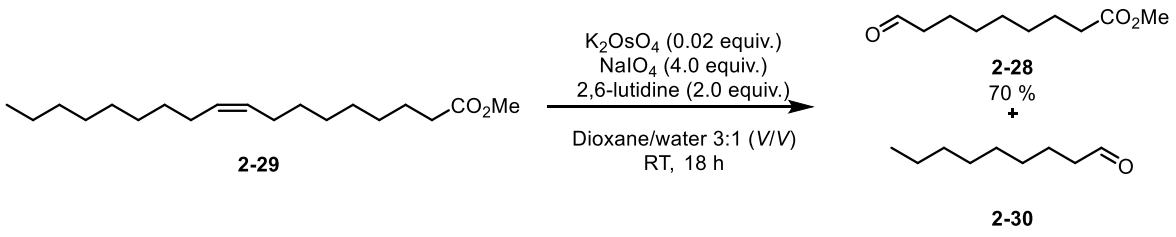
Scheme 28: Retrosynthetic plan towards 10-NO_2OA **2-1**.

The synthesis of 9-oxononanoate **2-28** began with the transformation of oleic acid to its methyl ester **2-29**. The methyl ester **2-29** was prepared from oleic acid **2-16** using AcCl/MeOH protocol in a yield of 98 % yield (**Scheme 29**).



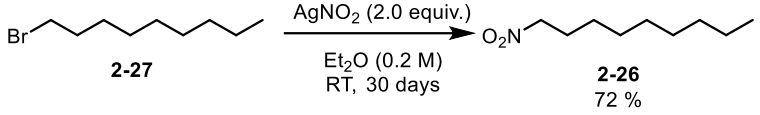
Scheme 29: Acylation of oleic acid **2-16**.

Oxidative cleavage of the olefinic bond in **2-29** carried out with the help of potassium osmate(VI) dihydrate in the presence of $NaIO_4$ (oxidant) and 2,6-lutidine yielded aldehyde **2-28** in 70 % yield (**Scheme 30**).



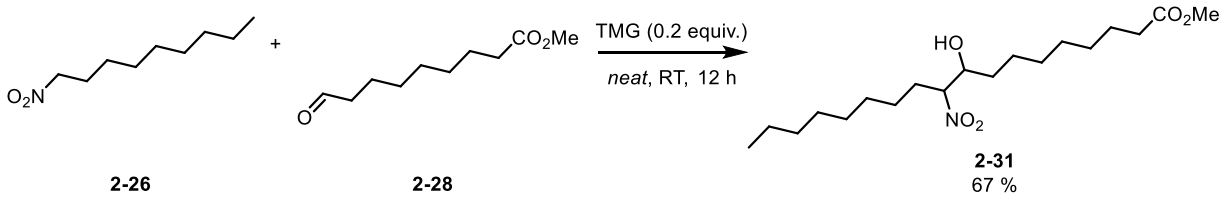
Scheme 30: Oxidative cleavage of methyl ester of oleic acid **2-29**.

Aldehyde **2-28** was then reacted with the nitro reagent **2-26** that was prepared by nitro/bromide exchange in the presence of silver nitrite. After 30 days of stirring in the dark, the reaction produced the desired product in a yield of 72 % yield (**Scheme 31**).[124]



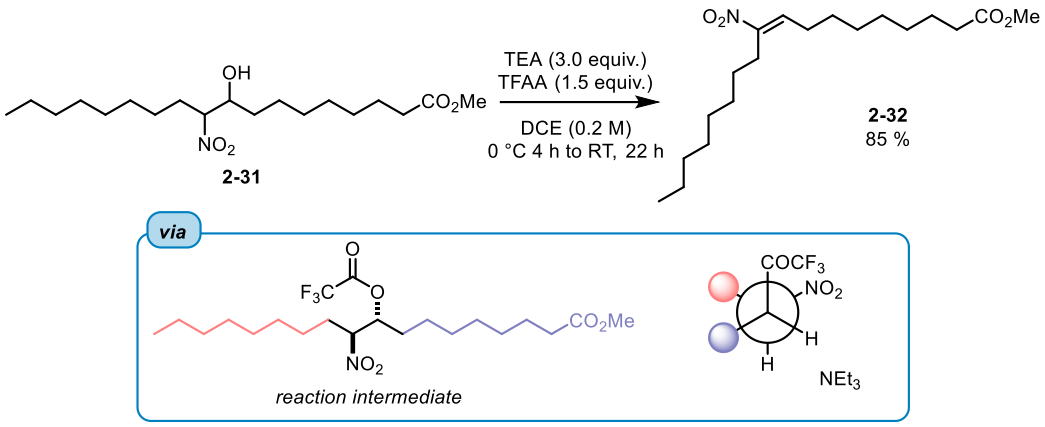
Scheme 31: Nitro/bromine exchange. Synthesis of the nitrogen compound **2-26**.

The reaction of nitro alkane **2-26** and with aldehyde **2-28** produced nitro aldol (Henry reaction) **2-31** with a yield of 67 %. The addition step of the sequence is crucial, since (**a**) it fixes the regiochemistry of the nitro group by combination of known precursors (nitro group is at the C10 position), and (**b**) it determines the *E*/*Z* stereochemical outcome of the subsequent *antiperiplanar* E2 elimination (**Scheme 32**). Under thermodynamic reaction conditions (use of TMG as a base) produces the predominantly *anti*-**2-31** adduct is produced. The desired adduct **2-31** must be immediately submitted to the elimination reaction to avoid any unwanted degradation. The elimination step that is mediated by the TFAA anhydride generates an activated intermediate *in situ* that in the presence of base undergoes an *anti*-elimination to yield *E*-olefin (*E*)-**2-32** in 85 % yield. The reaction proceeds under very mild conditions, yields the desired product as virtually a single stereoisomer (no traces of *Z* isomer as judged by the ^{1}H NMR spectra of the crude reaction mixture analysis), and only a small amount of unwanted side reaction products (in most cases) (**Scheme 33**).



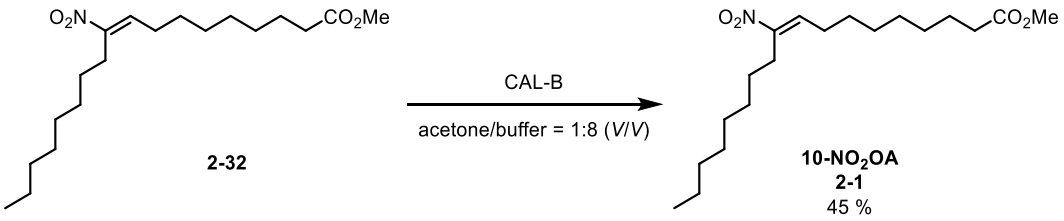
Scheme 32: Formation of hydroxy nitro intermediate **2-31**.

Next step, activation/dehydration generated the nitroalkene moiety **2-32** in an 85 % yield. Notably, this reaction proceeds under mild conditions, and yields clean stereoisomers, with very few side products observed (in most cases) (**Scheme 33**).



Scheme 33: Elimination of **2-31** that gives (*E*)-10-NO_2 methyl oleate **2-32**.

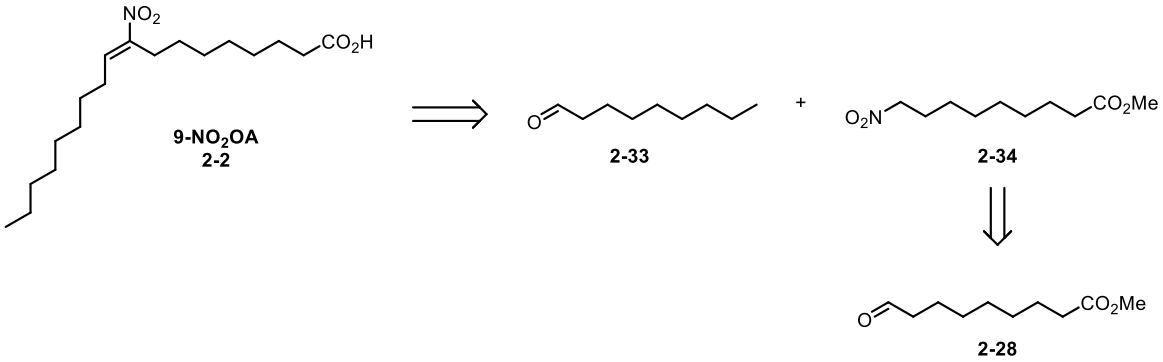
The final step of the synthesis involves the hydrolysis of the ester group in 10-NO_2 methyl oleate **2-32**. After several unsuccessful attempts and a very careful literature search of the reaction conditions, we opted for ester hydrolysis under mild enzyme-promoted conditions using lipase B from *Candida antarctica* (CAL-B). Conditions that should avoid side product formations and the final product, acid **2-1** degradation. This resulted in the formation of 10-NO_2OA **2-1** with a yield of 45 % and a 95:5 *E*/*Z* ratio (**Scheme 34**).



Scheme 34: Formation of 10-NO_2OA (**2-1**).

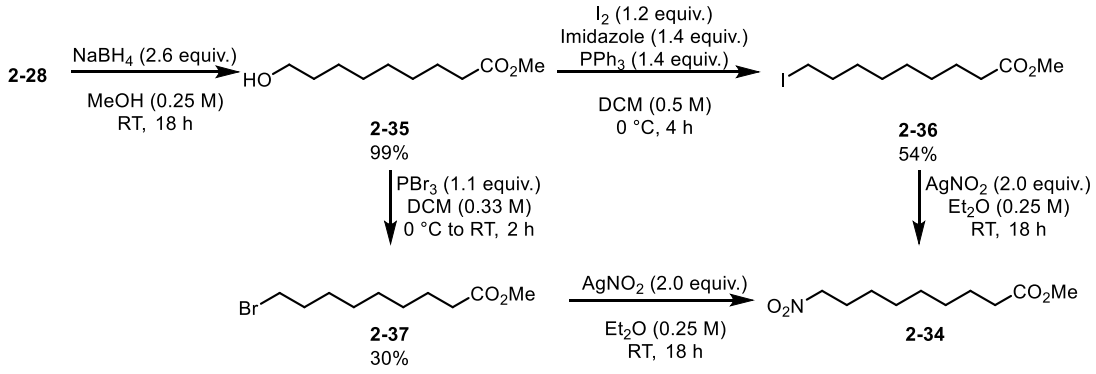
2.2.3. 9-NO_2 oleic acid

The retrosynthetic strategy for the synthesis of 9-NO_2OA **2-2** involves the same methodology, but the use of two different coupling partners for the Henry reaction. In this case, aldehyde **2-33** and methyl 9-nitrononanoate **2-34** must be used. While the first partner, aldehyde **2-33**, is commercial, the nitro derivative **2-28** must be prepared from aldehyde **2-28** (**Scheme 35**).



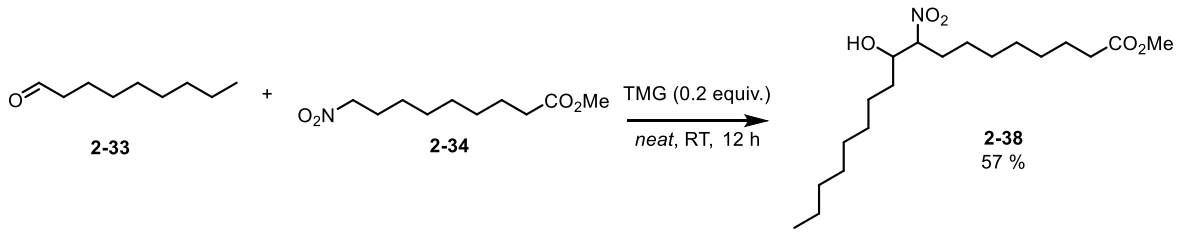
Scheme 35: Retrosynthetic plan towards 9-NO_2OA, **2-2**.

Thus, the nitro compound **2-34** is prepared from aldehyde **2-28** by reducing the aldehyde to alcohol **2-35** and the resulting alcohol is transformed into bromide **2-37** using PBr_3 in 30 % yield (**Scheme 36**). The bromide **2-37** was then transformed to methyl 9-nitrononanoate **2-34** in the presence of $AgNO_2$. However, the substitution reaction proceeds very slowly, and the desired product was isolated only in 23 % yield. The product of O-substitution (-ONO derivative) was isolated in 45 % isolated yield. To increase the isolated yield of a nitroalkane **2-34**, iodide **2-36** was prepared and reacted instead of the corresponding bromine derivative. This slight change resulted in an increased reaction yield and the final methyl 9-nitrononanoate **2-34** was isolated in 88 % yield. The whole protocol in addition eliminated all undesired side products.



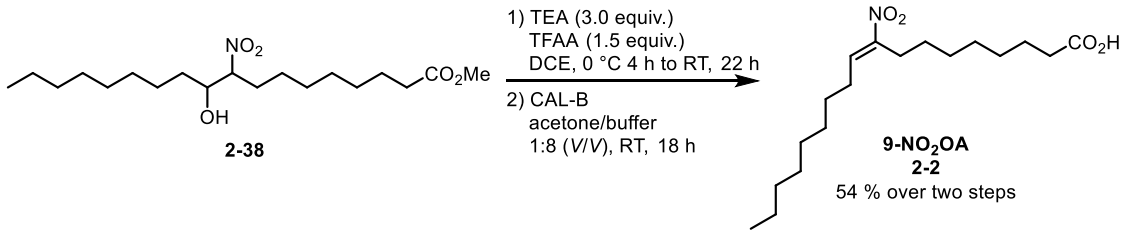
Scheme 36: Formation of methyl 9-nitrononanoate (**2-34**).

When the synthesis of methyl 9-nitrononanoate **2-34** was accomplished, a Henry reaction could be performed (**Scheme 37**). The desired Henry adduct **2-38** was isolated in a satisfactory 57 % yield.



Scheme 37: Formation of the Henry adduct **2-38**.

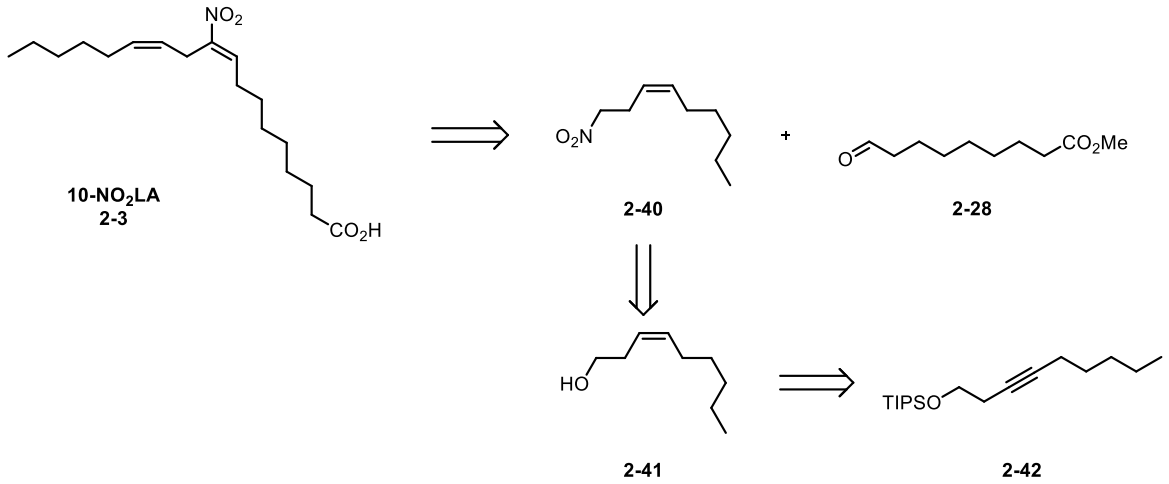
Intermediate **2-38** then reacted in the presence of TFAA and TEA and produced selectively 9-NO_2 methyl oleate **2-39** with an isolated yield of 81 % and >95:5 *E*/*Z* selectivity. The final step again involved enzymatic hydrolysis of ester promoted by CAL-B that furnished the desired 9-NO_2 oleic acid **2-2** in 66 % yield (54 % over two steps) and >95:5 *E*/*Z* selectivity (**Scheme 38**).



Scheme 38: Formation of **9-NO_2OA** (**2-2**).

2.2.4. 10-NO_2 linoleic acid

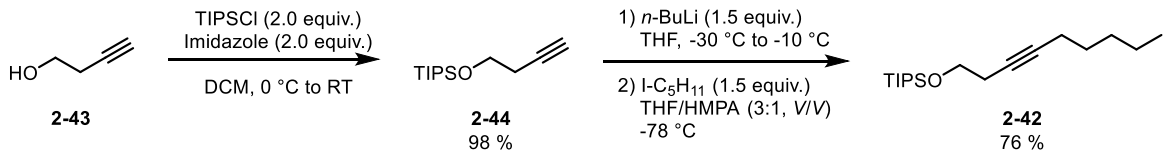
Synthesis of 10-NO_2LA proved to be more challenging compared to previous synthesis of nitro oleic acid, as it involved the construction of the first partner, nitro alkene **2-40**, before the subsequent Henry reaction with aldehyde **2-28** could be attempted. Nitro alkene **2-40** was designed to be synthesized from alcohol **2-41**, which could be prepared from TIPS-protected alkyne **2-42** (**Scheme 39**). Alternatively, our recently developed protocol, which employs the modified Julia-Kocienski reaction could be also used (for details, see chapter 2.5.8).[125]



Scheme 39: Retrosynthetic plan towards polyunsaturated **10-NO_2LA**, **2-3**.

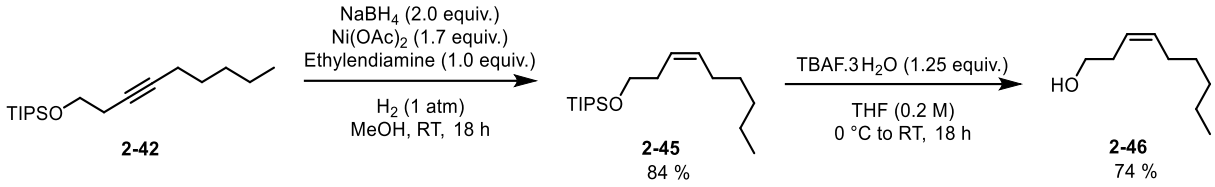
- **"Classical approach"**

First, we have employed the standard organic chemistry-based approach, where the synthesis began with TIPSCl protection of 3-butynol **2-43** (98 % yield) that was followed by alkylation of the terminal alkyne with 1-iodopentane (**Scheme 40**). The substitution reaction was performed with the help of a lithium acetylene-type reagent, which is known to have low reactivity in substitution reactions. To increase its reactivity, we added HMPA, a well-known lithium scavenger solvent, to the reaction mixture to generate a more reactive 'naked' alkyne anion. Under such reaction conditions, the substitution reaction proceeded smoothly and produced the desired **2-42** product with a yield of 76 %.



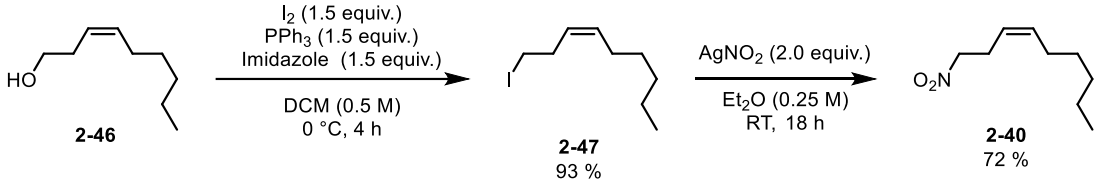
Scheme 40: TIPS protected internal alkyne **2-42** synthesis.

Subsequently, a (*Z*) selective reduction of the triple bond was carried out using $NaBH_4$/$Ni(OAc)_2$/ethylendiamine protocol, yielding TIPS-protected (*Z*) alkene **2-45** in 84 % yield and >95:5 *E*/*Z* selectivity. The deprotection of the TIPS group mediated by TBAF then resulted in the formation of alcohol **2-46** with a yield of 74 % (**Scheme 41**).



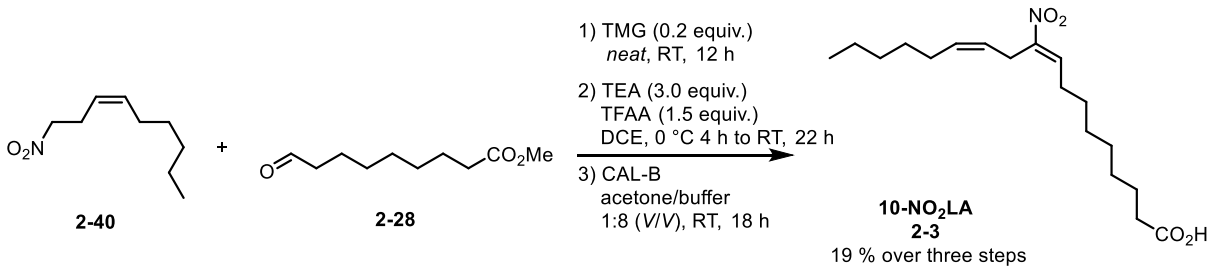
Scheme 41: Preparation of (*Z*)-alkene **2-46**.

Generated alcohol was transformed into the corresponding iodide **2-47** with help of I_2/PPh_3/imidazole protocol (93 % yield), and the reaction of **2-47** with $AgNO_2$ finally produced the nitro coupling partner **2-40** in 72 % (**Scheme 42**).



Scheme 42: Preparation of nitro alkene **2-40**.

The nitrogen coupling partner was then reacted with aldehyde **2-28** under the standard Henry coupling reaction protocol. The generated adduct was eliminated in the presence of TFAA/TEA, and the prepared ester was hydrolyzed to produce the desired **10-NO_2LA** (**2-3**). The overall yield of the transformation (3 steps) was 19 % and the acid was formed in >95:5; 5:>95 *E*/*Z*,*E*/*Z* ratio (**Scheme 43**).



Scheme 43: End game in the synthesis of **10-NO_2LA** (**2-3**).

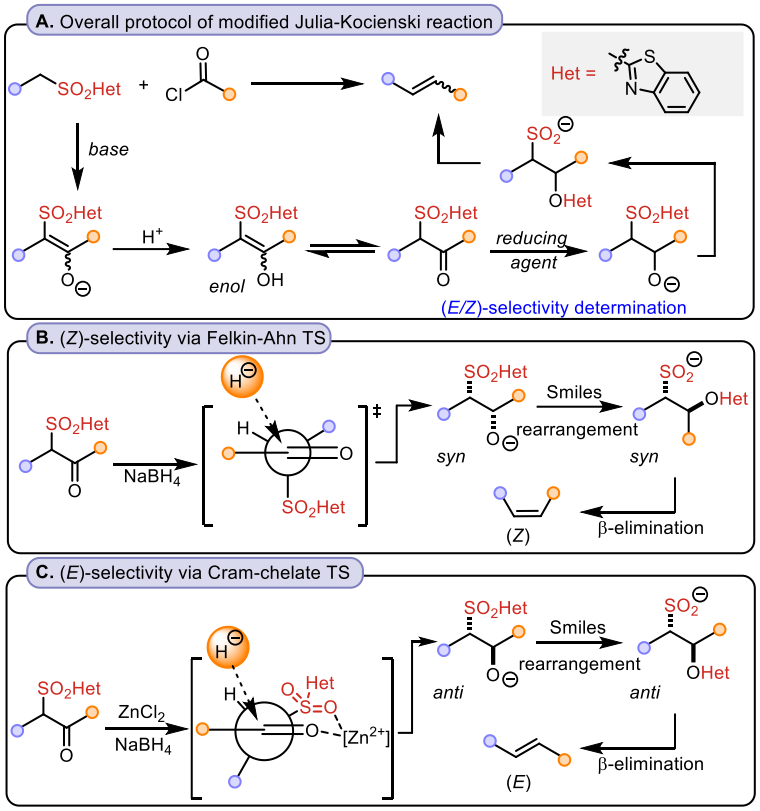
- **Modified Julia-Kocienski approach**

Alternatively, the *Z* olefin which is used in the preparation of **10-NO_2LA** (**2-3**) could also be prepared using our recently developed protocol, which transforms the well-known Julia-Kocienski reaction into a new type of the olefination reaction that allows for the stereoselective formation of *E* as well as *Z* olefins (

Scheme 44A).[125,126] The reaction sequence is based on the reaction of alkyl BT-sulfone with acyl chloride in the presence of an excess of a non-nucleophilic base. The reunion of the two reagents generates the desired b-keto sulfone that is present in the reaction mixture in the form of the corresponding enolate. The addition of MeOH results in *in situ* protonation of the enolate to the corresponding enol that, when present in its keto-form, can be reduced to the corresponding b-hydroxy sulfone by an external reducing agent. The reduction is the key for the stereochemical outcome of the reaction, since if the reduction proceeds under the Felkin-Ahn transition state conditions ($NaBH_4$), the *Z* olefin is formed (

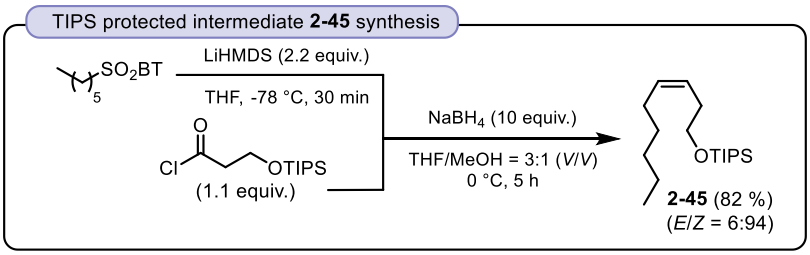
Scheme 44B). On the other hand, when the reduction proceeds under the Cram-chelate conditions ($NaBH_4/ZnCl_2$) then produce the desired olefin as the *E* isomer (

Scheme 44C). The reason is that stereoselective reduction produces *syn* or *anti*-hydroxy BT sulfone that is further transformed *via* the stereospecific *anti*-elimination process and yields *E* or *Z* olefin.



Scheme 44: Modified Julia-Kocienski Like olefination method.[125]

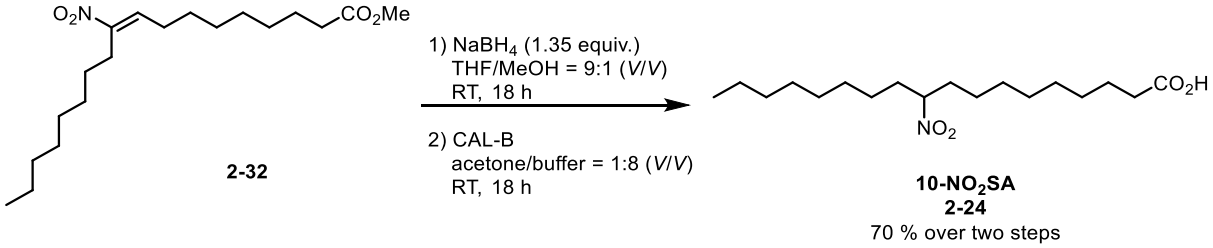
In the context of our project, the reaction intermediate, TIPS-ether **2-45**, was prepared using this protocol in an 82% yield and 6:94 *E*/*Z* ratio (**Scheme 45**).



Scheme 45: Application of modified Julia-Kocienski Like olefination method in the preparation of TIPS-ether **2-45**.

2.2.5. 10- NO_2 stearic acids

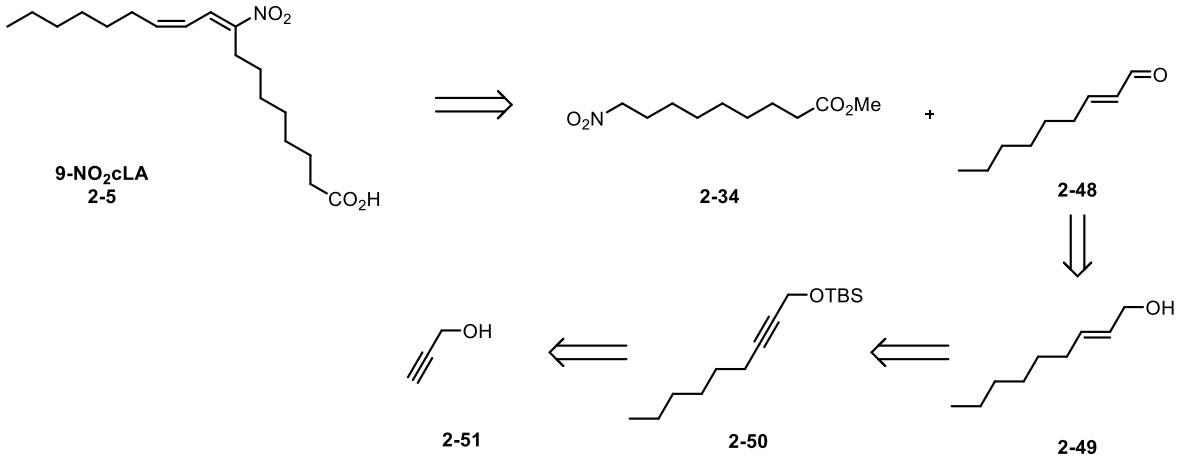
The synthesis of the 10-nitro derivative of stearic acid (**SA**) **2-24** involved a straightforward reduction of $NaBH_4$ **2-32** followed by enzyme-promoted ester hydrolysis. This two-step reaction sequence resulted in the formation of compound **2-24** and yielded the desired product with a yield of 70 % (**Scheme 46**).



Scheme 46: Preparation of **10-NO_2SA**, **2-24**.

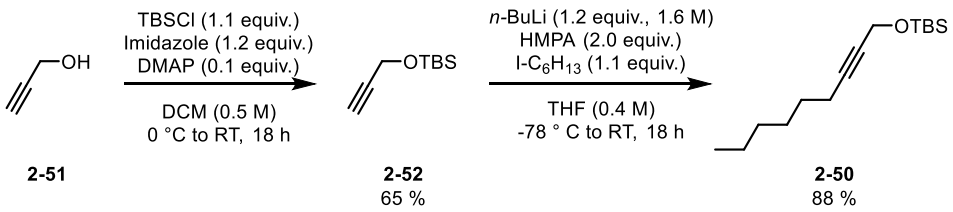
2.2.6. 9-NO_2 conjugated linoleic acid

The next challenge was the synthesis of 9-NO_2 conjugated linoleic acid (**9-NO_2cLA**, **2-5**) synthesis. The compound itself is known for its lability to both a basic and an acidic environment, and therefore its synthesis was quite a challenge. Our retrosynthesis was based on the Henry reaction between methyl 9-nitrononanoate **2-34** and aldehyde **2-48**. Aldehyde **2-48** was expected to be prepared from (*E*)-alcohol **2-49**, which, in turn, can be prepared from propargyl alcohol **2-51** (**Scheme 47**).



Scheme 47: Retrosynthetic plan of **9-NO_2cLA** (**2-5**).

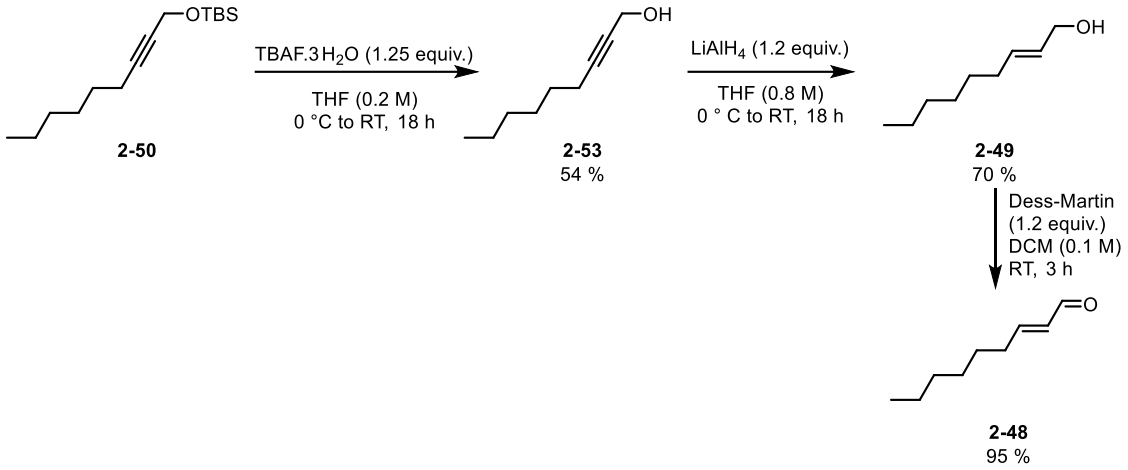
The synthesis of the first coupling partner, methyl 9-nitrononanoate **2-34**, was already described in the context of the synthesis of 9-NO_2OA and therefore will not be discussed. However, aldehyde **2-48** synthesis was not yet described (**Scheme 48**). As mentioned above, the synthesis started with propargyl alcohol **2-51** that was transformed to its TBS-ether **2-52** (65 % yield). Alkyn **2-52** was transformed into the corresponding organolithiated species that was further alkylated in the presence of HMPA with 1-iodohexane. Alkylated alkyne **2-50** was isolated in 88 % yield.



Scheme 48: Preparation of protected alkyne **2-50**.

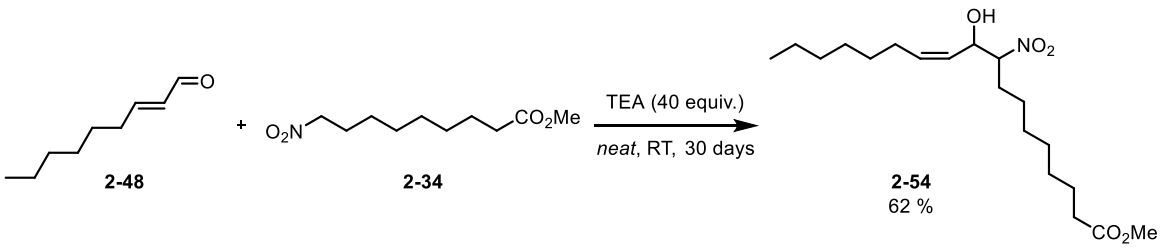
The removal of the TBAF-mediated TBS group produced alcohol **2-53** with a yield of 54 % and its (*E*) selective reduction of $LiAlH_4$ resulted in the formation of *E*-allylic alcohol **2-49**

with a yield of 70 % and >95:5 *E*/*Z* ratio. Dess-Martin periodinate promoted oxidation of allylic alcohol to aldehyde, yielding unsaturated aldehyde **2-48** in a yield of 95 % (**Scheme 49**).



Scheme 49: Preparation of aldehyde **2-48**.

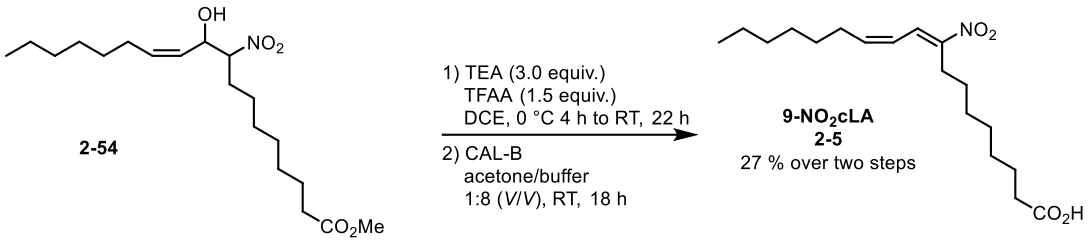
Having both reaction partners for the Henry reaction, the coupling step was attempted. First, a published protocol by Woodcock et al.[111] was evaluated. However, our substrates and those used in the work of Woodcock et al. differ, since in the referenced paper, *tert*-butyl 9-nitrononanoate is used as the starting material. We used a 9-nitrononanoate methyl ester **2-34**. The difference proved to be crucial since in our case, no product of the reaction was observed even after 3-5 days (reaction time of the original protocol). The desired product formation **2-54** was detected only after 10 days from the beginning of the reaction. Given the sluggish reaction progress, the reaction was monitored for an extended period of 30 days, after which the workup that consisted of TEA removal by stream of N_2 was used. In 30 days, the conversion of the methyl ester was only 70 % and the isolated yield of **2-54** was 62 %. Nitro-hydroxyl adduct **2-54** obtained was immediately used in the next step due to the reported low stability of the adduct (**Scheme 50**).



Scheme 50: Henry reaction. Adduct **2-54** synthesis.

The elimination step and the additional hydrolysis step of methyl ester required for the transformation of **2-54** to 9-NO_2cLA **2-5** proceeded without any further problems

and the desired product 9-NO_2cLA **2-5** was isolated in two mentioned steps in a modest isolated yield of 27 % and >95:5; >95:5 *E*/*Z*,*E*/Z selectivity (**Scheme 51**).



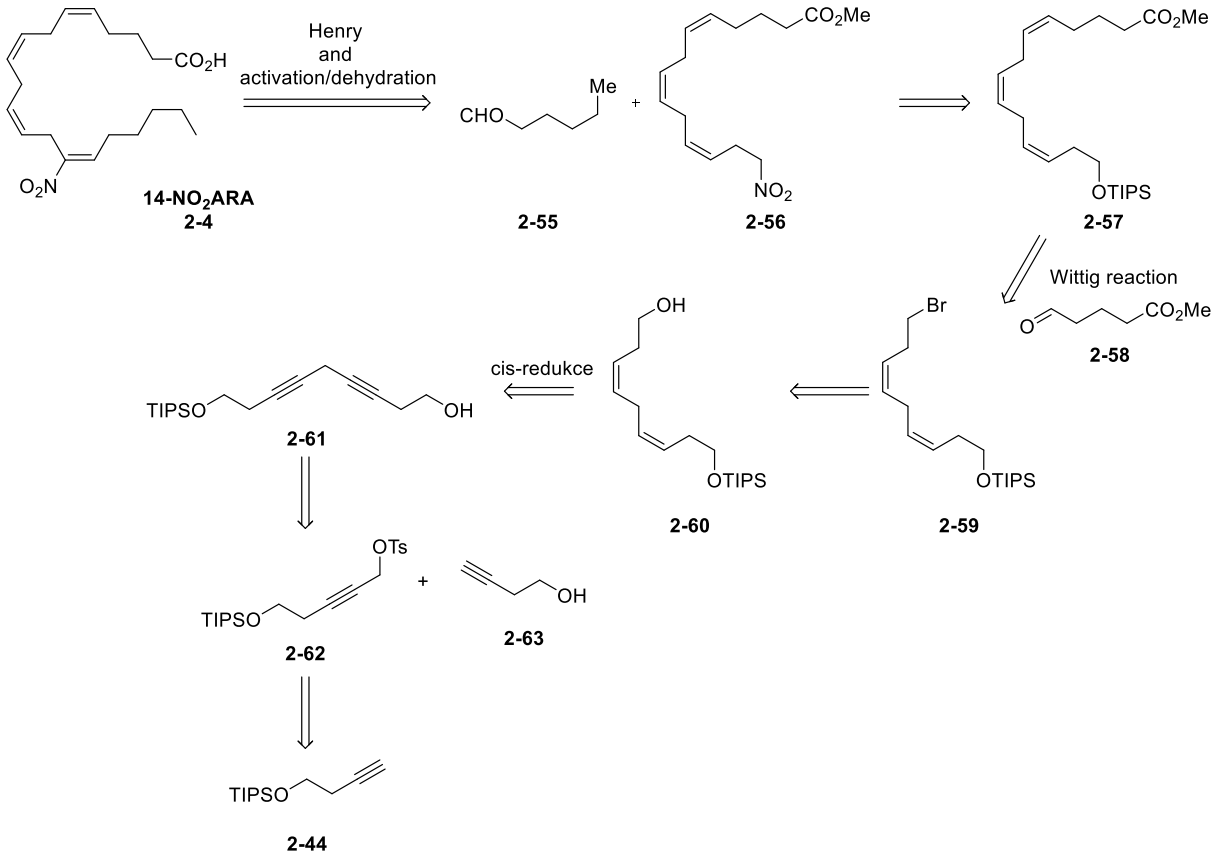
Scheme 51: Final steps of **9-NO_2cLA** (**2-5**) synthesis.

2.2.7. 14-NO_2 arachidonic acid

The next target of the synthesis was the synthesis of 14-NO_2 arachidonic acid (14-NO_2ARA, **2-4**). Our interest in this molecule is dated back to the year 2022 when our collaborators from the faculty of medicine and dentistry UPOL showed great interest in the evaluation of the molecule and we have found that there is no reported total synthesis of the molecule. Our journey to complete the synthesis of this molecule are described in the next few pages of this thesis.

2.2.7.1. First approach

First approach towards 14-NO_2ARA **2-4** was based on the similar retrosynthetic pathway described previously for the preparation of simpler NO_2FAs (**Scheme 52**). Compound **2-4** that contains three skipped (*Z*) 1,2-disubstituted olefins and one (*E*) (nitro containing) olefin was retrosynthetically divided along the nitro olefinic bond that could be established *via* Henry reaction/elimination protocol. This retrosynthetic step left us with aldehyde **2-55** and nitro-containing triene **2-56**. Triene **2-56** could be traced back to TIPS-ether **2-44**. However, to reach all *Z* triene **2-56** from the alcohol derivative **2-44** would require several steps where we identified two key ones: installation of the ester group *via* the *Z*-selective Wittig reaction (step inspired by the work of Suto et al.[127]) and dialkyne **2-61** synthesis and further all *Z* reduction.

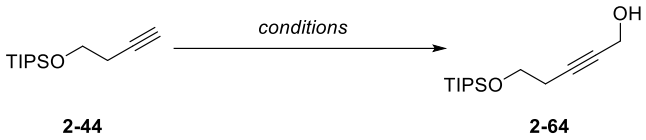


Scheme 52: Our first retrosynthetic approach to **14-NO$_2$ARA** (**2-4**).

The synthesis of the targeted molecule started with the TIPS protected 3-butynol **2-44** preparation. TIPS-ether **2-44** was then formylated using the published procedure[128], where the first step involved the formation of an aldehyde by the reaction of the generated alkyne anion with DMF, followed by the reduction of the aldehyde to alcohol (**Table 13**, **entry 1**). However, in our hands, this protocol proved to be low-yielding (product **2-64** was isolated only 40% in two steps) and we faced reproducibility issues due to the rapid decomposition of the aldehyde-containing intermediate. The issue becomes significant when the reaction was carried out in a larger scale. Therefore, an alternative one-step protocol based on the use of paraformaldehyde was used (**Table 13**, **entry 2**).[129] In this case the desired product was formed in 80 % isolated yield.

Tosylation of the alcohol **2-64** followed by copper(I)-promoted substitution reaction of the resulting activated propargylic alcohol in **2-62** with 3-butynol group produces the desired skipped dialkyne **2-61** in 95 % yield (**Scheme 53**).

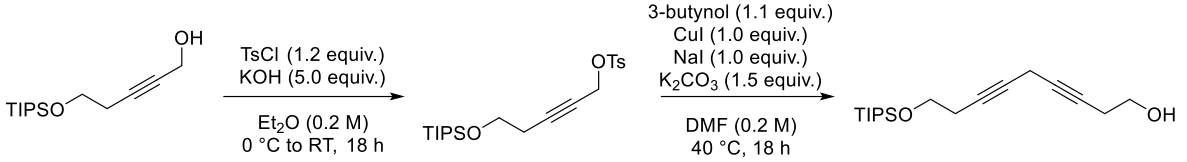
Table 13: Alcohol **2-64** synthesis.



2-44 **2-64**

Entry	Conditions	Yields [%][a]
1	1) *n*-BuLi (2.5 equiv., 1.6 M), DMF (2.0 equiv.), THF (0.4 M), -40 °C to RT, 2 h. 2) 2) $NaBH_4$ (2.0 equiv.), MeOH (0.2 M), 0 °C, 15 min.	40
2	*n*-BuLi (1.1 equiv., 1.6 M), paraformaldehyde (2.5 equiv.), THF (0.4 M), -78 °C to RT, 12 h	80

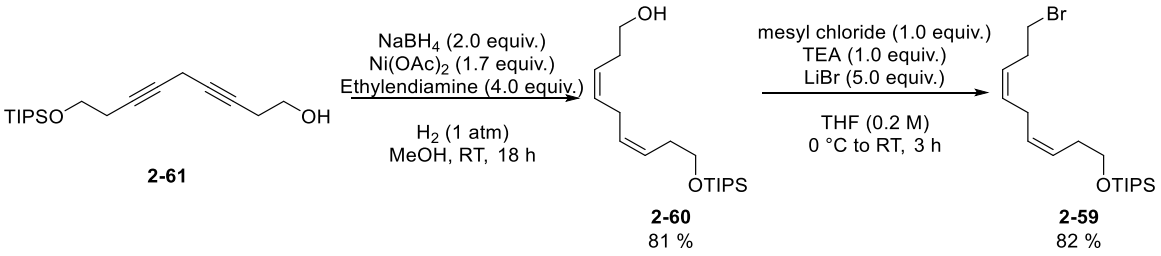
a) Isolated yield after column chromatography on silica gel.



2-64 **2-62** 95 % **2-61** 63 %

Scheme 53: Alkyne **2-61** preparation that features the copper(I) promoted substitution of activated propargylic alcohol.

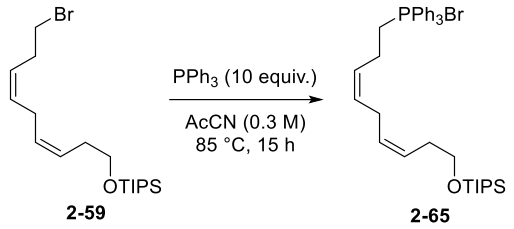
After diene **2-61 formation**, a *cis* reduction of the two triple bonds to the corresponding *Z* olefins was carried out under the hydrogen atmosphere with the help of the NiH_2 reducing agent (generated *in situ* from the $NaBH_4$/$Ni(OAc)_2$/ethylendiamine mixture) (**Scheme 54**). Skipped *Z,Z* dialkene **2-60** was isolated in 81 % and 5:>95; 5:>95 *E*/*Z*,*E*/*Z* ratio. Subsequently, bromide **2-59** was prepared from the alcohol **2-60** with a yield of 82 % over two steps. The reaction proceeded *via in situ* generated mesylate intermediate.



2-61 **2-60** 81 % **2-59** 82 %

Scheme 54: Formation of bromide **2-59**.

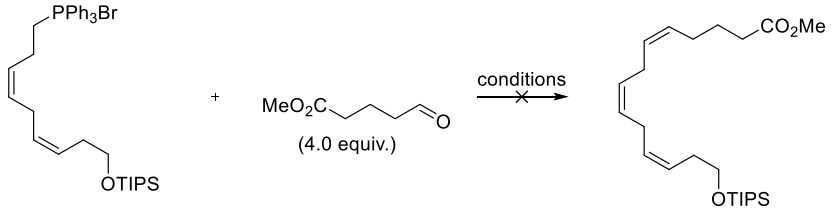
The reaction of the bromide **2-59** with PPh_3 then yielded the desired phosphonium salt **2-65** (**Scheme 55**). At this stage, we failed to purify the Wittig salt and even though it appeared not as important, our inability to purify the **2-65** salt to >95% purity proved to be crucial in the next Wittig olefination step.



Scheme 55: Formation of triphenylphosphine bromide **2-65**.

All our attempts to react crude phosphonium salt **2-65** with aldehyde **2-58** under various Wittig olefination conditions were unsuccessful and the desired product **2-57** was never observed (**Table 14**). Instead, various unidentified products were isolated; however, their structure was not clearly identified due to the spectra complexity and our inability to obtain them in pure form as an individual entity.

Table 14: Optimization of Wittig reaction between salt **2-65** and aldehyde **2-58**.



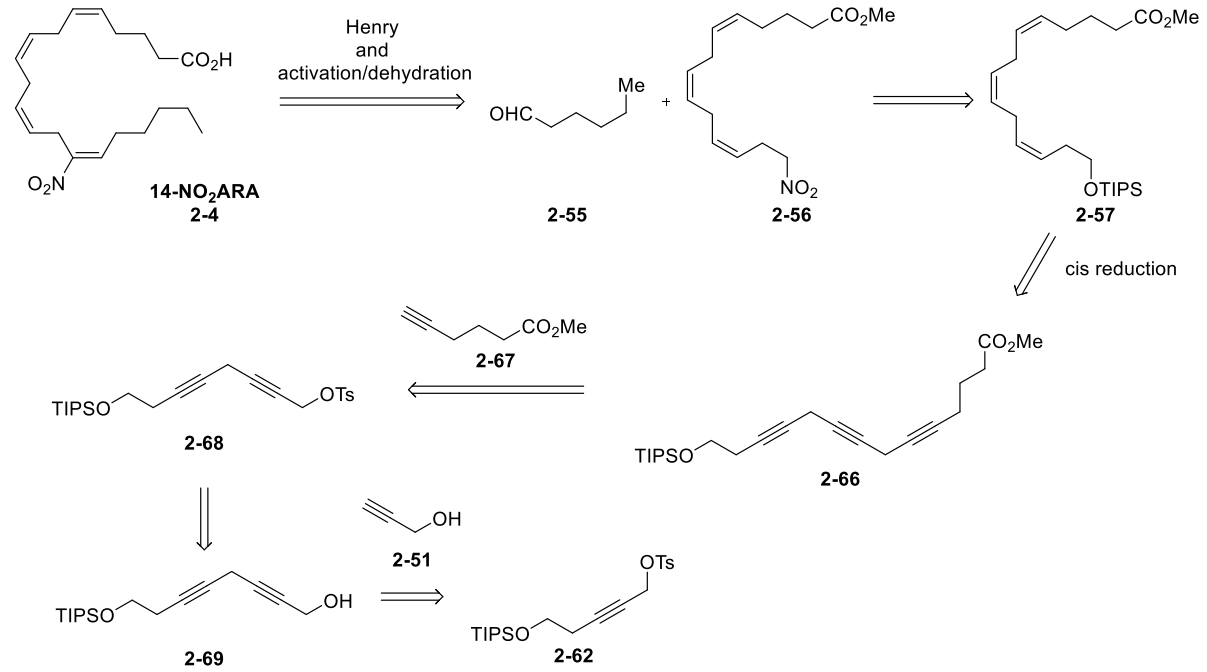
Entry	Conditions	Comments[a]
1	NaHMDS (2.1 equiv.), THF (0.1 M), 2 h	No desired product observed
2	NaHMDS (2.1 equiv.), THF (0.1 M), 4 h	No desired product observed
3	LiHMDS (2.1 equiv.), THF (0.1 M), 4 h	No desired product observed
4	LiHMDS (3.1 equiv.), THF (0.1 M), 18 h	decomposition

a) Based on the ^{1}H NMR spectra of the crude reaction mixture analysis.

Considering the problems related to the Wittig reaction (no traces of product) and our inability to purify the phosphonium salt **2-65**, our further efforts in this direction were abandoned and our synthetic strategy was revised.

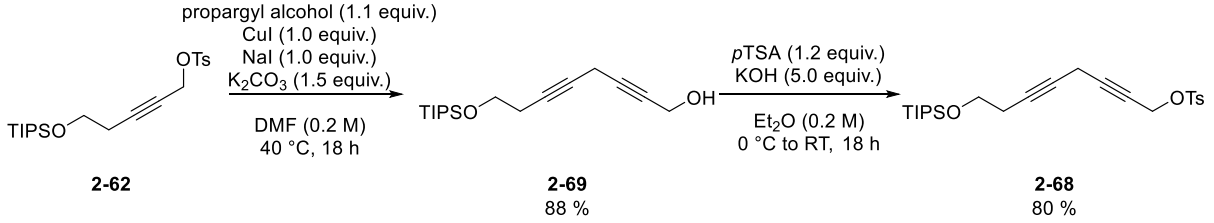
2.2.7.2. Second approach

In our second approach to Henry coupling partner **2-56,** we have replaced the trouble-making Wittig reaction with a step-by-step buildup strategy.[130] The final steps of the synthesis thus were unchanged, but TIPS-ether **2-57** should be prepared *via* all *cis* reduction of skipped trialkyne **2-66** intermediate. The skipped alkyne motive supposed to be built up *via* a sequence of copper(I)-promoted substitution reactions of activated propargylic hydroxyls with appropriate alkynes (**Scheme 56**).



Scheme 56: Second retrosynthetic analysis of **14-NO$_2$ARA** (**2-4**).

Building on the progress made in our initial approach, we utilized intermediate **2-62** and successfully synthesized product **2-69** in a yield of 88 % by utilization of copper(I)-promoted substitution reaction (**Scheme 57**). The tosylation of the resulting alcohol then furnished the desired activated propargylic alcohol **2-68** in 80 %.

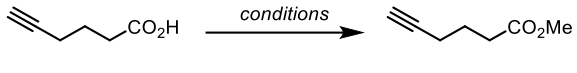


Scheme 57: Formation of tosylate **2-68**.

The second substitution reaction should then be carried out. However, first we had to prepare the required nucleophile, ester **2-67** (**Table 15**). Its synthesis started from the commercially

available 5-hexynoic acid **2-70** that was first esterified under acidic conditions (**entry 1** and **2**) using the MeOH/*p*TSA system or the $SOCl_2$/MeOH system. However, both methods yielded suboptimal results, since the desired ester **2-67** was isolated in reaction yields of 68 % (**entry 1**) and 49 % (**entry 2**), respectively. Consequently, we employed freshly generated diazomethane (**entry 3**) as a methylating agent. Under such conditions, the desired ester was formed in a quantitative manner**.**

Table 15: Methyl ester **2-67** synthesis. Optimalization of the protocol.

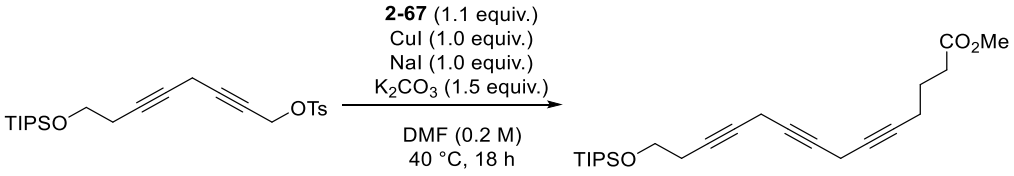


2-70 **2-67**

Entry	Conditions	Yields [%][a]
1	MeOH (5 M), *p*TSA (0.01 equiv.), DCM (2.5 M), reflux, 24 h	68
2	Thionyl chloride (3.0 equiv.), MeOH (0.2 M),0 °C to RT, 18 h	49
3	CH_2N_2 (2.2 equiv.), Et_2O (0.2 M), 0 °C, 30 min.	99

a) Isolated yield after column chromatography on silica gel.

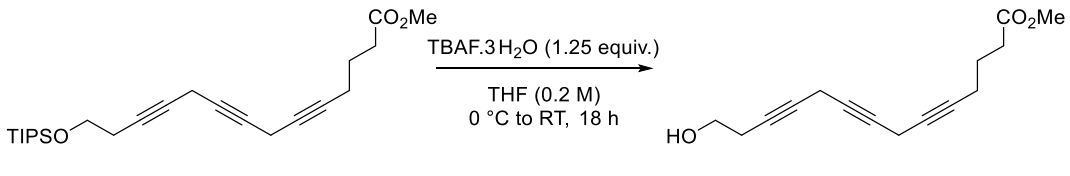
Subsequent reaction with methyl ester of 5-hexynoic acid **2-67** led to the formation of a product **2-66** which embody 3 triple bonds, unfortunately in an unimpressive yield of 23 % and low purity (**Scheme 58**).



2-68 **2-66**
23 %

Scheme 58: Formation of alkyne **2-66**.

Although, the deprotection of **2-66** resulted in the formation of an alcohol **2-71**, with another discouraging yield of only 24 % and again in low purity (**Scheme 59**).



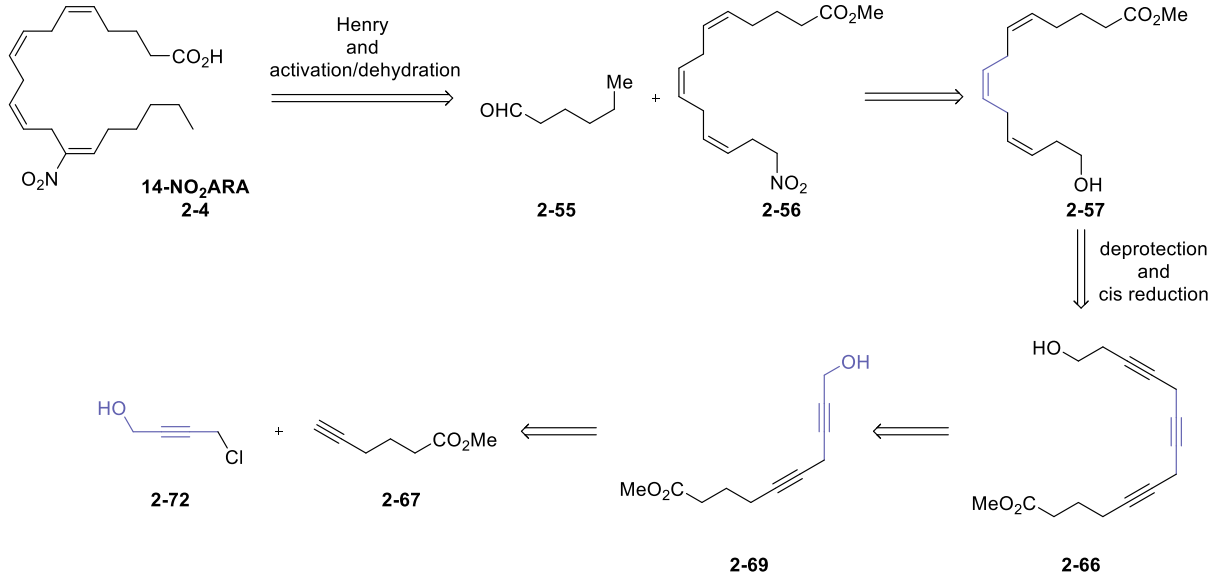
2-66 **2-71**
24 %

Scheme 59: Deprotection of **2-66**.

At this point, because of the low reaction yields, particularly in the last two steps, we have decided to abandon this pathway and explore a third alternative approach.

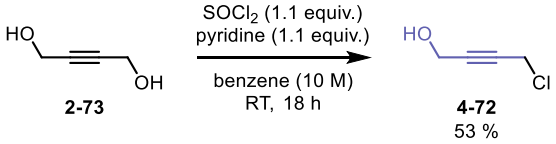
2.2.7.2.1. Third approach

The third and final approach is again based on the literature precedented transformations[130,131] and similarly to the second approach the key intermediate was trialkyn **2-66** (**Scheme 60**). However, while[125,126] the final steps remain the same, the opening transformations are way different. In this approach, we have decided to build up the whole carbon chain starting from the methyl ester of 5-hexynoic acid **2-67** and 4-chlorobut-2-yn-1-ol **2-72**



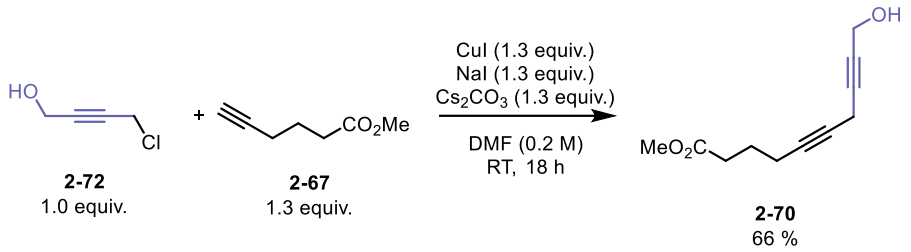
Scheme 60: Third approach to **14-NO$_2$ARA** (**2-4**). Retrosynthetic consideration.

The first step of the sequence is the monochlorination of the diol **2-73** that was carried out with the help of $SOCl_2$ (**Scheme 61**). The reaction proceeded in benzene and in the presence of pyridine and yielded the desired hydroxy chloride **4-72** in 53 % yield.



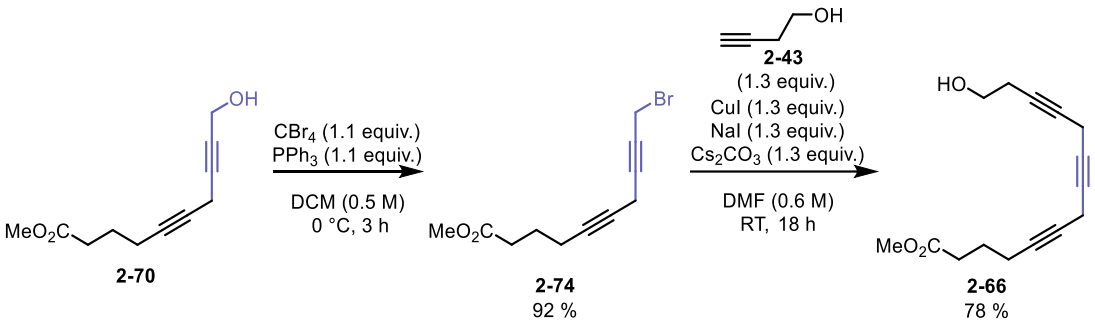
Scheme 61: Chlorination of **2-73**.

Next, the copper(I) promoted substitution reaction of alkyne **2-67** with the previously generated chloride **4-72** yielded alcohol **2-70** in a yield of 66 % (**Scheme 62**).[130]



Scheme 62: Coupling of chloride **2-72** and methyl 5-hexynoate **2-67**.

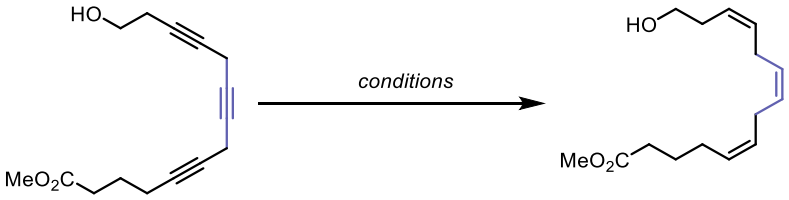
The alcohol **2-70** was then converted to the corresponding bromide **2-74** using the Appel reaction conditions (92 %), and the bromide **2-74** was reacted again in the presence of copper(I) salts with 3-butynol **2-43** and yielded alkyne **2-66** with three skipped triple bonds in a 78 % yield (**Scheme 63**).



Scheme 63: Formation of alkyne **2-66**.

At this stage a challenging all *cis* reduction of alkynes to olefins in **2-66** was evaluated (**Table 16**). First, hydrogenation promoted with the Lindlar catalyst was attempted, but only partial reduction of alkyne or partial/complete overreduction of alkynes to alkanes was observed (**entry 1**-**5**). Therefore, we switched the reducing system and used NiH_2 as a reducing agent (generated *in situ* from $NaBH_4$/$Ni(OAc)_2$/ethylendiamine in the presence of H_2). Under these conditions, the desired product was isolated in the yield of 42 % and 46 %, respectively (**entry 6** and **7**). Change in the solvent from methanol to ethanol resulted in complete decomposition (**entry 8**), but when the chelating diamine ligand equivalents were decreased from 4.0 to 2.0, the desired product **2-57** was formed in an increased 57 % yield (**entry 9**)

Table 16: Optimization of reduction of alkyne **2-66** to triple skipped alkene **2-57**.

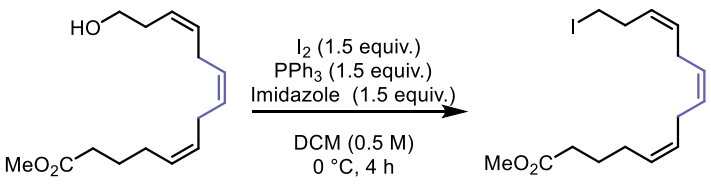


2-66 **2-57**

Entry	Conditions	Yields [%][a]	comments
1	Lindlar catalyst (20 mg/1 mmol), MeOH (0.5 M), 4 h	-	partial overreduction
2	Lindlar catalyst (20 mg/1 mmol), quinoline (0.02 equiv.), H_2 (1 atm), Et_2O (0.5 M), RT, 3.5 h	-	no conversion
3	Lindlar catalyst (20 mg/1 mmol), quinoline (0.3 equiv.) H_2 (1 atm), THF/Et_2O (0.115 M, 1:1 (*V*/*V*)), RT, overnight	-	complete overreduction
4	Lindlar catalyst (60 mg/1 mmol), quinoline (0.3 equiv.) H_2 (1 atm), Et_2O (0.5 M), RT, 3 h	-	Partial overreduction
5	Lindlar catalyst (60 mg/1 mmol), quinoline (0.3 equiv.) H_2 (1 atm), Et_2O (0.25 M), RT, overnight	-	complete overreduction
6	$NaBH_4$ (1.3 equiv.), $Ni(OAc)_2$ (1.2 equiv.), ethylendiamine, (4.0 equiv.), H_2 (1 atm), MeOH (0.5 M), RT, 3 h	42	-
7	$NaBH_4$ (1.3 equiv.), $Ni(OAc)_2$ (1.2 equiv.), ethylendiamine, (4.0 equiv.), H_2 (1 atm), MeOH (0.75 M), RT, 4 h	46	-
8	$NaBH_4$ (1.3 equiv.), $Ni(OAc)_2$ (1.2 equiv.), ethylendiamine, (2.0 equiv.), H_2 (1 atm), EtOH (0.75 M), RT, 18 h	-	decomposition
9	$NaBH_4$ (1.3 equiv.), $Ni(OAc)_2$ (1.2 equiv.), ethylendiamine, (2.0 equiv.), H_2 (1 atm), MeOH (0.75 M), RT, 4 h	57	-

a) Isolated yield after column chromatography on silica gel.
b) Based on the ^1H NMR spectra of the crude reaction mixture analysis.

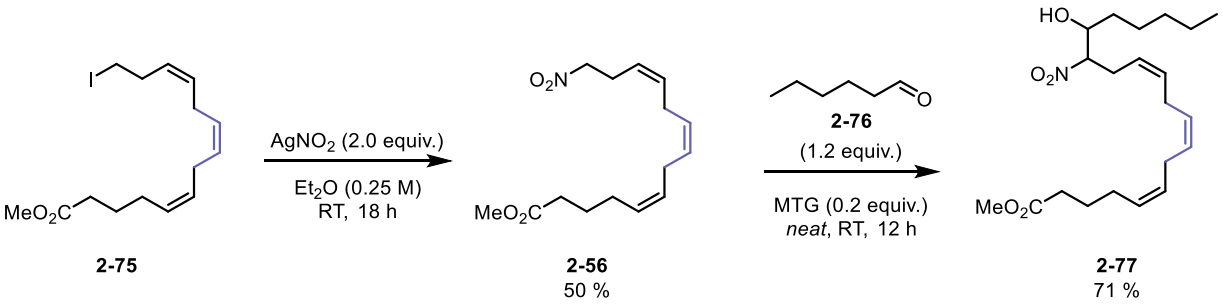
The prepared alcohol **2-57** was then transformed into iodide **2-75** using I_2/PPh_3/imidazole system (65 % yield) (**Scheme 64**) and the iodo derivative **2-75** was transformed with the help of $AgNO_2$ into the corresponding nitro precursor for the Henry reaction (**Scheme 65**). Nitro derivative **2-56** was prepared in 50 % yield.



2-57 **2-75**
65 %

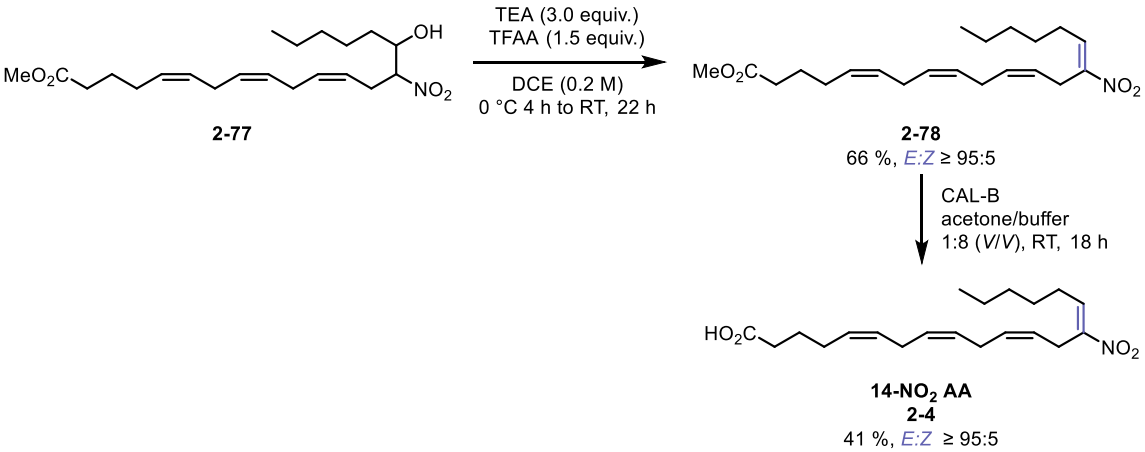
Scheme 64: Formation of Iodide **2-75**.

Finally, the Henry reaction of the nitro compound **2-56** with aldehyde **2-76** could be attempted (**Scheme 65**). Gratifyingly, the desired adduct **2-77** was formed under standard reaction conditions in just 12 h and a yield of 71 % (**Scheme 65**).



Scheme 65: Formation of nitro-alkene **2-56** and subsequent Henry reaction.

At this stage there were only two steps left in the sequence. First, adduct **2-77** underwent elimination reaction promoted with TFAA/TEA system and formed methyl ester of 14-nitro arachidonic acid **2-78** with a yield of 66 % and >95:5; 5:>95; 5:>95; 5:>95 *E/Z, E/Z, E/Z, E/Z* ratio. Subsequent enzymatic hydrolysis of **2-78** promoted with the CAL-B enzyme produced the final acid **2-4** in a yield of 41 % and an *E/Z, E/Z, E/Z, E/Z* ratio of >95:5; 5:>95; 5:>95; 5:>95 (**Scheme 66**).



Scheme 66: Elimination and ester hydrolysis. First synthesis of **14-NO_2ARA** (**2-4**).

2.2.8. 14-NO_2 anandamide

Anandamide (**2-79**) - the arachidonic acid ethanolamide, together with 2-arachidonoylglycerol (2-AG, **2-80**) was found in the brain and intestine, and both belong to lipids classified as endocannabinoids (**Figure 35**).

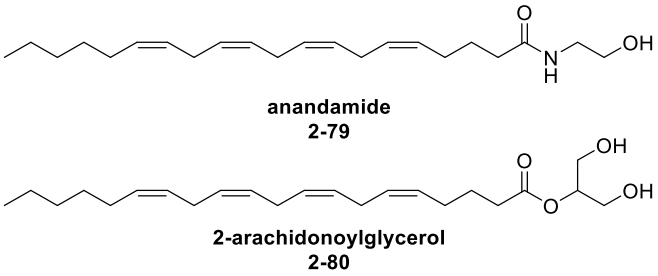


Figure 35: Structure of anandamide **2-79** and 2-arachidonoylglycerol **2-80**.

Cannabis sativa is a common plant that is known to contain various psychotropic compounds. Therefore, it is not surprising that the plant found its utility in many applications in medicine and is also known as a "readily available" intoxicant and/or as a drug. Recently, it has been reported that the plat has therapeutic effects, most probably due to cannabinoids.[132] Cannabinoids are products typically derived from cannabis plant flowers such as Δ^9-tetrahydrocannabinol (THC, **2-82**) and the cannabidiol (CBD, **2-81**) (**Figure 36**). The identification of THC structure in 1960s[133,134] and mechanistic bioactivity studies of THC led to the identification of cannabinoid receptors in the 1990s[135,136], as well as the identification of endogenous ligands of these receptors, endocannabinoids[137].

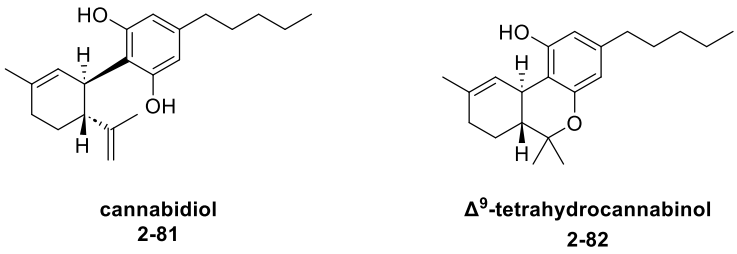
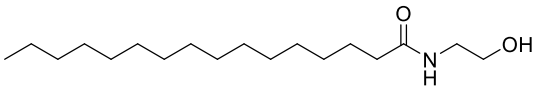


Figure 36: Structure of CBD **2-81** and THC **2-82**.

Cannabinoid receptors and endocannabinoids were found to be pleiotropic and involved in restoring homeostasis following pathological insults, suggesting their therapeutic opportunities.[138] The use of radiolabeled THC analogues led to the identification of binding sites in the brain, cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2) which led to the identification of CB1 and CB2 ligands.[136,137] **Anandamide** and

2-arachidonoylglycerol were found in the brain and intestine. They were also shown to activate CB1 and CB2 with high affinity and efficacy.[139–141]

Endocannabinoids play a crucial role in the regulation of various physiological processes, mainly including those related to the nervous system. Studies in animal models suggest that in neurological disorders, endocannabinoids can become dysregulated and contribute to disease in different ways.[142]For example, in Parkinson's disease[143,144], and Alzheimer's disease[145–147], Huntington disease[148,149] , Multiple sclerosis[150,151] and Epilepsy[152,153], altered levels or functions of endocannabinoids and their receptors have been observed, suggesting their potential as therapeutic targets. The action of endocannabinoids and their receptors in these neurological disorders is complex and diverse, often mitigating immune cell infiltration into the CNS and promoting anti-inflammatory responses through receptors such as CB2, TRPV1, or PPARγ.[154,155] Major efforts have been made to develop endocannabinoid-targeted drugs. The first promising drugs against Parkinson's disease, Alzheimer's disease, and multiple sclerosis are nabixomols (a mixture of THC and CBD), CBD, and palmitoylethanolamide (**Figure 36**, **Figure 37**). The efficacity of such drugs is attributed to their multitarget nature.



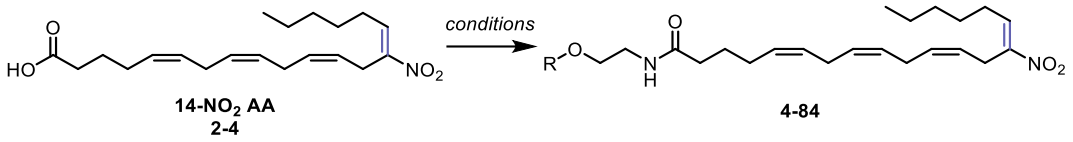
palmitoylethanolamide
2-83

Figure 37: Structure of palmitoylethanolamide **2-83**.

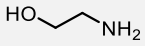
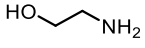
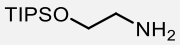
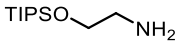
2.2.8.1. Preparation of 14-NO_2 anandamide

We have decided to prepare the nitrated version of anandamide at C14 position **2-84** and test its biological properties. Since we have successfully prepared 14-NO_2 arachidonic acid (**2-4**), we have expected that the synthesis of the nitro derivative of anandamide will not be a problem, especially since various total syntheses of anandamide have been reported in the litterature.[156–160] Initially, we have decided to allow 14-NO_2 arachidonic acid (**2-4**) to react with O-protected aminoethanol by means of amide coupling (**Table 17**). First conditions we tried focused on the use of CDI and DCC coupling reagents, but in all cases no product was observed. We believe that the main reason for this observation is the reactivity of the free amine group that presumably attacked the Michael acceptor (nitroolefin) within the **2-4** structure and caused the decomposition of the starting material.

Table 17: Transformation of 14-NO_2 arachidonic acid (**2-4**) into the 14-NO_2 anandamide derivative.



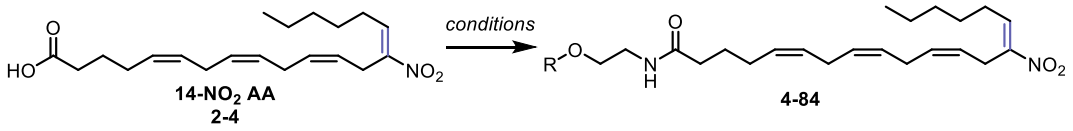
14-NO_2 AA 2-4 **4-84**

Entry	Conditions	Amine	Comments
1	**amine** (1.1 equiv.), CDI (1.1 equiv.), DCM (0.2 M), 0 °C to RT, 18 h		decomposition
2	**amine** (1.1 equiv.), CDI (1.1 equiv.), DCM (0.2 M), 0 °C to RT, 4 h		decomposition
3	**amine** (1.1 equiv.), CDI (1.1 equiv.), DCM (0.25 M), 0 °C to RT, 4 h		decomposition
4	**amine** (1.0 equiv.), DCC (1.0 equiv.), DMAP (0.2 equiv.) DCM (0.25 M), 0 °C to RT, 3 h		decomposition

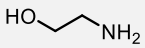
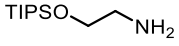
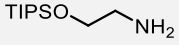
a) Based on the ^{1}H NMR spectra of the crude reaction mixture analysis.

For such a reason, we have decided to transform the free carboxylic acid to the corresponding acyl chloride and let it react with an excess of the base. (**Table 18**). We were hoping that the acyl chloride reactivity toward the nucleophilic amine will be faster than the corresponding addition of amine to the Michael acceptor, and thus that we will be able to terminate the reaction in time to isolate at least low yields of the desired product. Unfortunately, even in this case, only decomposition of the starting material occurred.

Table 18: Acyl halide-based transformation of **2-4** to 14-NO_2 anandamide derivative **4-84**.



14-NO_2 AA 2-4 **4-84**

Entry	Conditions	Amine	Comments[a]
1	**1)** Oxalyl chloride (2.0 equiv.), DMF (cat.) DCM (0.1 M), 0 °C to RT, 3 h **2)** amine (10.0 equiv.)		decomposition
2	Oxalyl chloride (4.0 equiv.), DMF (cat.) DCM (0.1 M), 0 °C to RT, 3 h		decomposition
2	**1)** Oxalyl chloride (4.0 equiv.), DMF (cat.) DCM (0.1 M), 0 °C to RT, 3 h **2)** amine (1.2 equiv.), K_2CO_3 (2.0 equiv.) DCM (0.1 M), 0 °C, 2 h		decomposition

a) Based on the ^{1}H NMR spectra of the crude reaction mixture analysis.

2.3. Biological evaluation

All newly prepared **NO_2FAs** were subjected to a biological activity evaluation, especially focusing on their activation of Nrf2 factor. Nrf2 is a well-known short-lived protein that works as a transcription factor, associated with the expression of numerous cytoprotective genes involved in xenobiotic metabolism and antioxidant responses.[161] Over the past decade, studies have highlighted its crucial role in combating oxidative stress and potential implications in neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Collaborative research has been conducted with Prof. Jan Vacek of Palacký University Olomouc, Faculty of Medicine, to explore the application of these compounds. Furthermore, the stability, redox properties, and electrophilic characteristics of methyl esters and acids were evaluated in collaboration with Prof. Ludvík Jiří and Dr. Liška Alan from the Jan Heyrovsky Institute of Physical Chemistry, Czech Academy of Sciences. Furthermore, the compounds were evaluated for their radioprotective properties in collaboration with Dr. Tomáš Perečko and Dr. Jana Perečková from the Institute of Biophysics, Czech Academy of Sciences. This activity, known to be related to Nrf2 activation in a wide variety of cells, is mediated by increasing DNA repair responses, neutralizing reactive oxygen species (ROS), reducing apoptosis, and regulating the cell cycle.[162]

2.4. Conclusion

In conclusion, this chapter presents our efforts to optimize the synthesis of NO_2FAs. We successfully developed efficient reaction conditions, leading to the synthesis of five NO_2FAs, including 9- and 10-NO_2 oleic acid (**2-1** and **2-2**), 10-NO_2 linoleic acid (**2-3**), 9-NO_2 conjugated linoleic acid (**2-5**, nitro rumenic acid) and we have disclosed the first total synthesis of 14-NO_2 arachidonic acid (**2-4**). Additionally, a saturated NO_2 acid, 10-NO_2 stearic acid (**2-24**) was prepared (**Figure 38**). The preparation of 14-NO_2 anandamide has so far not been successful and a different approach will have to be developed.

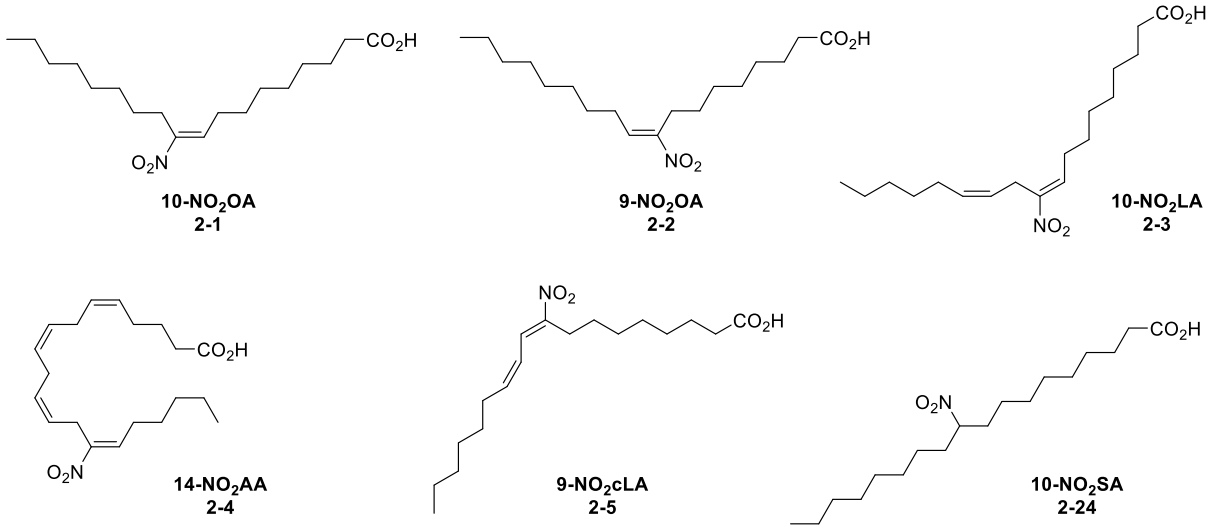


Figure 38: Structures of prepared FAs.

Biological evaluation, radioprotective properties, stability of NO_2FAs in water, their redox properties, and electrophilic characteristics of prepared compounds are currently being evaluated. The ongoing studies focus on detailed mechanistic investigations and further biological evaluations to fully elucidate the therapeutic potential of these NO_2FAs. We aim to explore their efficacy in various disease models and understand their mechanism of action at the molecular level. This work sets the foundation for the future development of NO_2FAs as potential therapeutic agents, because, for the first time, it allows the systematic study of **JUST ONE** stereoisomer of NO_2FAs that is in addition undoubtedly described and characterized.

The results from these collaborative efforts will be compiled and published in four independent articles, expected to be released in late 2024 and early 2025.

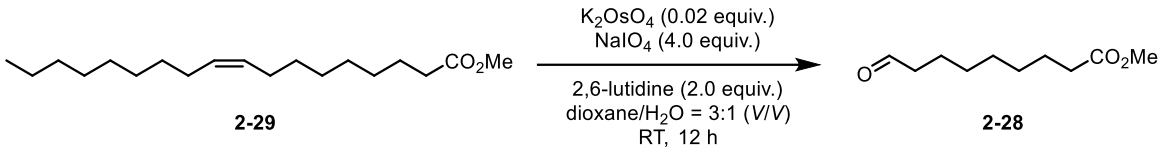
2.5. Experimental section

All starting materials were purchased from commercial suppliers and used without further purification, unless otherwise stated. All reactions were performed in round-bottom flasks fitted with rubber septa using standard laboratory techniques under positive pressure of argon (Air Liquide, >99.5% purity). In all reactions, unless stated otherwise, anhydrous solvents furnished by the Merck (Sigma-Aldrich) Company were used. Alternatively, tetrahydrofuran, acetonitrile and dichloromethane were dried using a solvent purification system equipped with alumina drying columns under argon. **Caution! HMPA and DCE are toxic. CARE SHOULD BE TAKEN!** Purification of reaction products was carried out by column chromatography using standard grade silica gel (60 Å, 230–400 mesh), or by preparative thin layer chromatography glass plates precoated with silica gel (silica gel G-200 F 254 , particle size 0.040−0.063 mm). Analytical thin-layer chromatography was performed on a thin-layer chromatography (TLC) aluminum plates pre-coated with silica gel (silica gel 60 F 254). Visualization was accomplished with UV light, phosphomolybdic acid and potassium permanganate stains, followed by heating. The ^{1}H NMR and ^{13}C{ ^{1}H} NMR spectra were measured on JEOL ECA400II (400 and 101 MHz) or JEOL 500 ECA (500 and 126 MHz) in Chloroform-d. Chemical shifts are reported in ppm, and their calibration was carried out (a) in the case of ^{1}H NMR experiments on the residual peak of non-deuterated solvent δ ($CDCl_3$) = 7.26 ppm or δ (CD_3OD) = 3.31 ppm, δ (DMSO-d_6)= 2.50 ppm, δ (acetone-d_6)= 2.05 ppm and in the case of ^{13}C NMR experiments on the middle peak of the ^{13}C signal in deuterated solvent δ ($CDCl_3$) = 77.16 ppm, δ (CD_3OD) = 49.00 ppm, (DMSO-d_6)= 39.52 ppm, (acetone-d_6)= 29.84 ppm. The proton coupling patterns are represented as a singlet (s), a doublet (d), a doublet of a doublet (dd), a triplet (t), a triplet of a triplet (tt), and a multiplet (m). High-resolution mass spectrometry (HRMS) was performed on LC chromatograph (Dionex UltiMate 3000, Thermo Fischer Scientific, MA, USA) and mass spectrometer Exactive Plus Orbitrap high-resolution (Thermo Fischer Scientific, MA, USA) with electrospray ionization (ESI) and a time-of-flight analyzer operating in a positive or negative full scan mode in the range of 100 − 1700 m/z. High-performance liquid chromatography (HPLC) was performed using an Agilent 1290 Infinity II system with UV-VIS detector and an Agilent InfinityLab LC/MSD mass detector. Purification using semiprep HPLC was carried out on Agilent 1290 Infinity II with UV-VIS and mass detector Agilent InfinityLab LC/MSD using

the C18 reverse-phase column (Agilent 5Prep-C18 10x21.2 mm). The gradient was formed from water and methanol with a flow rate of 20 mL/min. CAL-B - Lipase B Candida antarctica immobilized on Immobead 150 (≥2000 units/g IUBMB 3.1.1.3, EC number 232-619-9) was purchased from Merck.

2.5.1. 10-NO_2OA

methyl 9-oxononanoate (2-28)



2,6-lutidine (7.83 mL, 66.8 mmol, 2.0 equiv.), K_2OsO_4 (250 mg, 0.67 mmol, 0.02 equiv.) and $NaIO_4$ (28.8 g, 134 mmol, 4.0 equiv.) were sequentially added at room temperature to a stirred solution of **2-29** (10 g, 33.4 mmol, 1.0 equiv.) in dioxane/water = 3:1 (*V*/*V*, 300 mL). The reaction mixture was stirred at room temperature overnight before being filtered over a pad of Celite®. The filter cake was successfully washed with $CHCl_3$ (3x 200 mL). The combined filtrates were further washed with water (2x150 mL), brine (150 mL), dried over $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 1000:1) and gave a product **2-28** (4.36 g, 70 %) as a colorless oil.

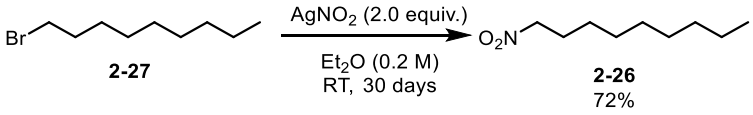
^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 9.76 (s, 1H), 3.66 (s, 3H), 2.42 (td, *J* = 7.3, 1.7 Hz, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.66 – 1.59 (m, 4H), 1.35 – 1.30 (m, 6H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 203.0, 174.4, 67.2, 60.6, 51.6, 44.0, 34.2, 29.1, 29.1, 29.0, 25.0, 22.1, 14.3.

R_f = 0.7 (PMA; petroleum ether:EtOAc = 10:1)

Data matched to those previously reported.[163]

1-nitrononane (2-26)



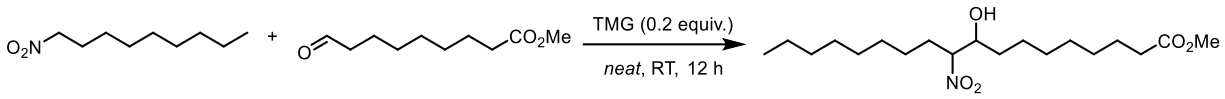
To a solution of bromide **2-27** (4 g, 19.3 mmol, 1.0 equiv.) in Et_2O (0.2 M, 96 mL) was added $AgNO_2$ (6 g, 38.6 mmol, 2.0 equiv.). The flask was stoppered and covered with aluminum foil to protect from light. The suspension was stirred for 30 days at RT. The mixture was diluted with EtOAc and filtered through a short plug of Celite® The filter pad was rinsed with additional amounts of EtOAc (2x 100 mL), and the combined filtrates were concentrated under reduced

pressure. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 1000:1) and gave a product **2-26** (2.43 g, 72 % yield,) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 4.37 (t, J = 7.1 Hz, 2H), 2.03 – 1.96 (m, 2H), 1.41 – 1.19 (m, 12 h), 0.87 (t, J = 6.9 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 75.9, 31.9, 29.4, 29.3, 29.0, 27.6, 26.4, 22.8, 14.2.

methyl 9-hydroxy-10-nitrooctadecanoate (2-31)



2-26 **2-28** **2-31**

Neat mixture of 1-nitrononane **2-26** (0.5 g, 2.28 mmol, 1.0 equiv.) and aldehyde **2-28** (0.4 g, 2.73 mmol, 1.2 equiv.) was cooled to 0 °C and 1,1,3,3-tetramethylguanidine (0.053 g, 0.2 mmol, 0.2 equiv.) was added. The resulting mixture was stirred for 18 h at room temperature before being cooled to 0 °C and the reaction was terminated with the addition of H_2O (10 mL). The resulting mixture was extracted with EtOAc (3×25 mL), and the combined organic phases were dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 50:1) to yield the adduct **2- 31** (0.55 g, 67 %; *d.r.* = 1.48:1) as a pale-yellow oil. Product **2-31** was obtained as a mixture of two diastereoisomers in a 1.48:1 *d.r.* ratio (based on the ^{1}H NMR spectra analysis).

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 4.43 (ttd, *J* = 9.3, 4.0, 1.7 Hz, 1H), 4.00 (ddq, *J* = 8.7, 4.7, 2.0 Hz, 0.5H), 3.85 (pd, *J* = 7.3, 6.4, 2.8 Hz, 0.5H), 3.66 (s, 3H), 2.34 (dd, *J* = 4.9, 3.0 Hz, 0.5H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.18 (dd, *J* = 8.0, 3.4 Hz, 0.5H), 2.14 – 1.95 (m, 1H), 1.83 – 1.72 (m, 1H), 1.63 – 1.59 (m, 2H), 1.53 – 1.40 (m, 3H), 1.33 – 1.23 (m, 18 h), 0.87 (t, *J* = 7.0 Hz, 3H).

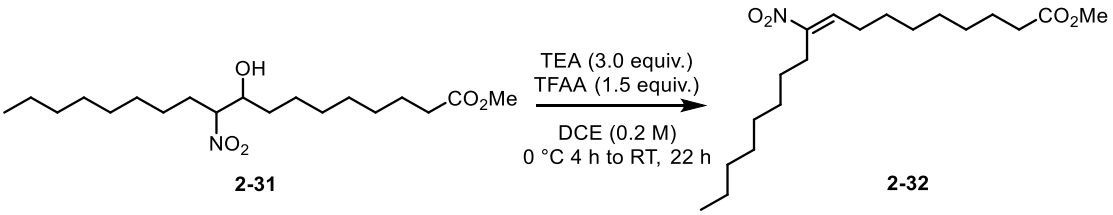
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 174.5, 93.1, 92.5, 72.4, 72.1, 51.6, 34.1, 33.6, 33.2, 31.9, 30.5, 29.3, 29.2, 29.2, 29.1, 29.0, 28.1, 26.1, 25.8, 25.6, 25.2, 24.9, 22.7, 14.2.

MS (ESI) *m/z* (%): 361 $[M+H]^+$.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{19}H_{38}NO_5$: 360.2744; found: 360.2748.

R_f = 0.19 (PMA, *n*-hexane:EtOAc = 10:1)

methyl (*E*)-10-nitrooctadec-9-enoate (2-32)



Adduct **2-31** (0.24 g, 0.66 mmol, 1.0 equiv.) was dissolved in DCE (3.3 mL, 0.2 M) and cooled to 0 °C. TEA (0.28 mL, 1.98 mmol, 3.0 equiv.) and TFAA (0.14 mL, 0.99 mmol, 1.5 equiv.) were sequentially added and the resulting mixture was stirred at 0 °C for 4 h and then at room temperature for next 22 h The whole mixture was cooled to 0 °C and water (10 mL) was added in order to terminate the reaction. The resulting mixture was extracted with EtOAc (3×15 mL), and organic phases were combined, dried over $MgSO_4$, and the volatiles were removed *in vacuo*. The crude product was purified using semipreparative chromatography (C18 reverse-phase column; MeOH:H_2O) to yield the desired nitro olefin **2-32** (0.19g, 85 %; *E/Z* ≥ 95:5 (based on the ^{1}H NMR spectra analysis) in a form of a colorless oil.

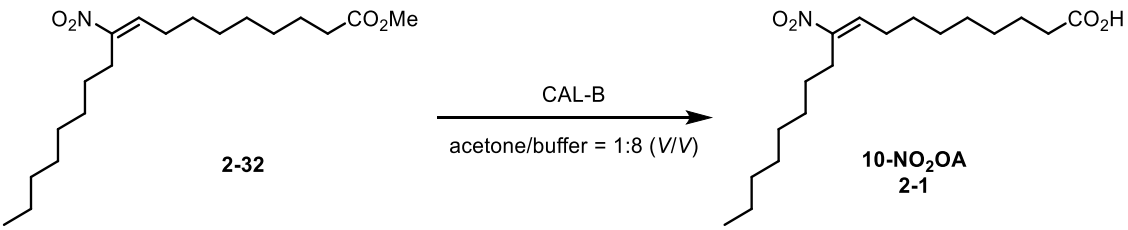
^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 7.07 (t, *J* = 7.9 Hz, 1H), 3.67 (d, *J* = 1.0 Hz, 3H), 2.60 – 2.53 (m, 2H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.21 (q, *J* = 7.6 Hz, 2H), 1.62 (p, *J* = 7.2 Hz, 2H), 1.48 (h, *J* = 8.8, 8.2 Hz, 4H), 1.38 – 1.23 (m, 16H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 174.4, 152.1, 136.4, 51.6, 34.1, 32.0, 29.4, 29.32, 29.29, 29.14, 29.11, 28.6, 28.12, 28.05, 26.5, 25.0, 22.8, 14.2.

MS (ESI) *m/z* (%): 343 $[M+H]^+$.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{19}H_{36}NO_4$: 342.2639; found: 342.2642.

(*E*)-10-nitrooctadec-9-enoic acid (10-NO_2OA, 2-1)



CAL-B (400 mg) was added to a solution of methyl ester **2-32** (0.3 g, 0.88 mmol, 1.0 equiv.) in acetone (11 mL, 0.08 M) and aqueous phosphate buffer (88 mL, 0.01 M, pH 7.4). The solution was vigorously stirred (magnetic stirrer, 1000 rpm) at room temperature for 18 h. 1 M HCl solution was added to adjust the pH of the solution to pH = 3. The whole mixture was

then extracted with EtOAc (5x20 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and the volatiles were removed *in vacuo*. The crude product was purified using semipreparative chromatography (C18 reverse-phase column; MeOH:H_2O) to yield the desired acid **10-NO_2OA, 2-1** (0.13 g, 45 %, *E/Z* ≥ 95:5; based on the ^{1}H NMR spectra analysis).

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 10.50 (bs, 1H), 7.07 (t, *J* = 7.9 Hz, 1H), 2.59 – 2.54 (m, 2H), 2.36 (t, *J* = 7.5 Hz, 2H), 2.21 (q, *J* = 7.6 Hz, 2H), 1.67 – 1.60 (m, 2H), 1.53 – 1.44 (m, 4H), 1.39 – 1.32 (m, 6H), 1.32 – 1.24 (m, 10H), 0.88 (t, *J* = 6.8 Hz, 3H).

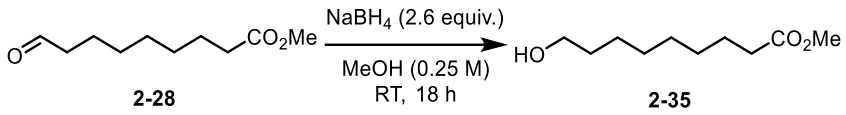
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 152.1, 136.4, 33.8, 32.0, 29.4, 29.38, 29.32, 29.1, 29.0, 28.6, 28.12, 28.05, 26.5, 24.7, 22.8, 14.2.

MS (ESI) *m/z* (%): 327 $[M-H]^-$.

HRMS (ESI) *m/z:* $[M-H]^-$ calculated for $C_{18}H_{32}NO_4$: 326.2337; found: 326.2340.

2.5.2. 9-NO_2OA

methyl 9-hydroxynonanoate (2-35)



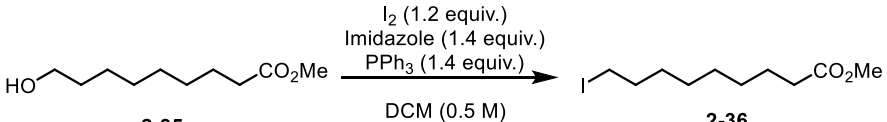
2-28 2-35

$NaBH_4$ (1.7 g, 44.2 mmol, 2.6 equiv.) was added to a cold (0 °C) solution of methyl oxoester **2-28** (3.2 g, 17 mmol, 1.0 equiv.) in MeOH (68 mL, 0.25 mL) and the resulting mixture was stirred at RT overnight. Water (50 mL), and EtOAc (100 mL) were added at room temperature and the resulting phases were separated. Aqueous layer was extracted with EtOAc (3x50 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and the volatiles were removed *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 10:1) to yield the desired nitro alkane **2-35** (3.17 g, 99 %) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 3.66 (d, J = 2.0 Hz, 3H), 3.63 (t, J = 6.6 Hz, 2H), 2.30 (td, J = 7.4, 2.0 Hz, 2H), 1.64 – 1.59 (m, 2H), 1.59 – 1.52 (m, 2H), 1.37 – 1.26 (m, 8H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 174.5, 63.2, 51.6, 34.2, 32.9, 29.3, 29.3, 29.2, 25.8, 25.0.

methyl 9-iodononanoate (2-36)



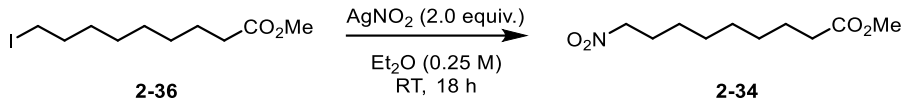
2-35 2-36

To an ice-cold solution of alcohol **2-35** (3.1 g, 2.14 mmol) in DCM (0.5 M, 33 mL) were added imidazole (1.57 g, 22.8 mmol, 1.4 equiv.), PPh_3 (6.05 g, 22.8 mmol, 1.4 equiv.), and I_2 (5 g, 19.6 mmol, 1.2 equiv.). After 3.5 h at 0 °C, the solution was diluted with EtOAc (100 mL), and a solution was filtered through a pad of silica gel to afford crude product. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 10:1) to yield the desired iodide **2-36** (4 g, 83 %) as a pale orange

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 3.67 (s, 3H), 3.18 (t, J = 7.0 Hz, 2H), 2.30 (t, J = 7.5 Hz, 2H), 1.81 (p, J = 7.1 Hz, 2H), 1.64 – 1.59 (m, 2H), 1.41 – 1.35 (m, 2H), 1.34 – 1.23 (m, 6H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 174.4, 51.6, 34.2, 33.6, 30.5, 29.2, 29.2, 28.5, 25.0, 7.4.

methyl 9-nitrononanoate (2-34)



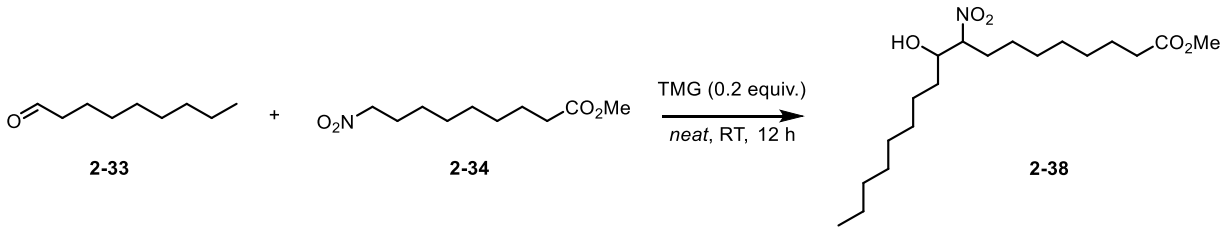
2-36 **2-34**

To a solution of iodide **2-36** (4 g, 13.3 mmol, 1.0 equiv.) in Et_2O (0.2 M, 66 mL) was added $AgNO_2$ (4.1 g, 26.6 mmol, 2.0 equiv.). The flask was stoppered and covered with aluminum foil to protect from light. The suspension was stirred overnight at RT. The mixture was diluted with EtOAc (100 mL) and filtered through a short plug of Celite®. The filter pad was rinsed with additional amounts of EtOAc (2x 100 mL), and the combined filtrates were concentrated under reduced pressure. Purification of the crude residue by flash column chromatography (SiO_2; petroleum ether:EtOAc = 4:1) afforded the desired nitro alkane **2-34** (4 g, 88 %) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 4.37 (t, *J* = 7.0 Hz, 2H), 3.66 (s, 3H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.00 (p, *J* = 7.1 Hz, 2H), 1.62 (q, *J* = 7.4 Hz, 2H), 1.41 – 1.26 (m, 8H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 174.4, 75.8, 51.6, 51.6, 34.1, 29.0, 29.0, 28.8, 27.5, 26.3, 24.9.

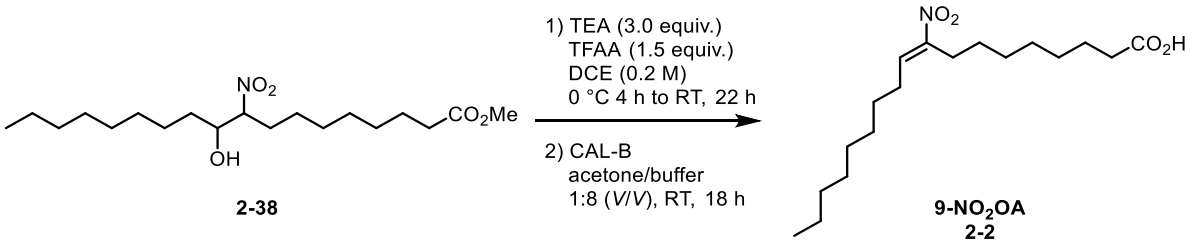
methyl 10-hydroxy-9-nitrooctadecanoate (2-38)



2-33 **2-34** **2-38**

Neat mixture of nitroalkane **2-34** (1.2 g, 5.47 mmol, 1.0 equiv.) and nonanal **2-33** (0.96 g, 6.56 mmol, 1.2 equiv.) was cooled to 0 °C and 1,1,3,3-tetramethylguanidine (0.14 g, 1.1 mmol, 0.2 equiv.) was added. The resulting mixture was stirred for 12 h at room temperature before being cooled to 0 °C and the reaction was terminated with the addition of H_2O (25 mL). The resulting mixture was extracted with EtOAc (3×30 mL), and the combined organic phases were dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product **2-38** was used directly without further purification in the next step.

(*E*)-9-nitrooctadec-9-enoic acid (9-NO_2OA, 2-2)



Adduct **2-38** (0.24 g, 0.66 mmol, 1.0 equiv.) was dissolved in DCE (3.3 mL, 0.2 M) and cooled to 0 °C. TEA (0.28 mL, 1.98 mmol, 3.0 equiv.) and TFAA (0.14 mL, 0.99 mmol, 1.5 equiv.) were sequentially added and the resulting mixture was stirred at 0 °C for 4 h and then at room temperature for next 22 h The whole mixture was cooled to 0 °C and water (10 mL) was added in order to terminate the reaction. The resulting mixture was extracted with EtOAc (3×15 mL), and organic phases were combined, dried over $MgSO_4$, and the volatiles were removed *in vacuo*. The crude product was purified using semipreparative chromatography (C18 reverse-phase column; MeOH:H_2O) to yield the desired methyl 9-nitro oleate **2-39** (0.13 g, 57 %).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 7.08 (t, *J* = 7.9 Hz, 1H), 3.66 (s, 3H), 2.60 – 2.53 (m, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.21 (q, *J* = 7.6 Hz, 2H), 1.61 (p, *J* = 7.2 Hz, 2H), 1.48 (h, *J* = 7.5 Hz, 4H), 1.37 – 1.22 (m, 16H), 0.90 – 0.83 (m, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 174.4, 151.9, 136.7, 51.6, 34.2, 31.9, 29.5, 29.4, 29.3, 29.2, 29.1, 29.1, 28.7, 28.2, 28.0, 26.5, 25.0, 22.8, 14.2.

CAL-B (400 mg) was added to a solution of methyl ester **2-39** (0.13 g, 0.38 mmol, 1.0 equiv.) in acetone (4.7 mL, 0.08 M) and aqueous phosphate buffer (38 mL, 0.01 M, pH 7.4). The solution was vigorously stirred (magnetic stirrer, 1000 rpm) at room temperature for 18 h. 1 M HCl solution was added to adjust the pH of the solution to pH = 3. The whole mixture was then extracted with EtOAc (5x20 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and the volatiles were removed *in vacuo*. The crude product was purified using semipreparative chromatography (C18 reverse-phase column; MeOH:H_2O) to yield the desired acid **9-NO_2OA, 2-2** (0.1 g, 81 %, *E/Z* ≥ 95:5; based on the ^{1}H NMR spectra analysis).

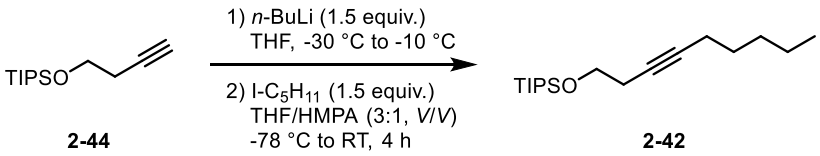
^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 11.53 (s, 1H), 7.07 (t, *J* = 7.9 Hz, 1H), 2.60 – 2.51 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.20 (q, *J* = 7.6 Hz, 2H), 1.62 (p, *J* = 7.4 Hz, 2H), 1.48 (h, *J* = 7.2 Hz, 4H), 1.37 – 1.29 (m, 6H), 1.32 – 1.21 (m, 10H), 0.92 – 0.83 (m, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm):152.1, 136.4, 33.9, 32.0, 29.4, 29.3, 29.3, 29.1, 29.0, 28.6, 28.1, 28.1, 26.5, 24.7, 22.8, 14.2.

HRMS (ESI) *m/z:* [M-H]$^-$ calculated for $C_{18}H_{32}NO_4$: 326.2337; found: 326.2326.

2.5.3. 10-NO_2LA

Triisopropyl(oct-3-yn-1-yloxy)silane (**2-42**)



A solution of TIPS protected acetylene **2-44** (10 g, 43.7 mmol, 1.0 equiv.) in THF (109 mL, 0.4 M) was cooled to -30 °C (dry ice/acetone) and *n*-BuLi (41 mL, 64 mmol, 1.5 equiv.; 1.6 M sol. in hexane) was added dropwise. The resulting mixture was brought to -10 °C and stirred for 1 hour. The whole mixture was cooled to -78 °C (dry ice/acetone) and a mixture of 1-iodopentane (8.7 mL, 43.7 mmol, 1.5 equiv.) in THF:HMPA = 3:1 (*V*/*V*; 88 mL) was added dropwise. The whole mixture was stirred at -78 °C for 1 h and then was allowed to worm up to room temperature over a period of 4 h. Water (50 mL) was added and the whole mixture was extracted with Et_2O (3x100 mL). The combined organic layers were washed with brine (50 mL), washed over Na_2SO_4, filtered and the volatiles were removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 100:1) and yielded acetylene **2-42** (9.86 g, 76 %) in the form of a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 3.77 (t, *J* = 7.4 Hz, 2H), 2.42 – 2.36 (m, 2H), 2.13 (tt, *J* = 7.2, 2.4 Hz, 2H), 1.48 (p, *J* = 7.0 Hz, 2H), 1.39 – 1.27 (m, 3H), 1.14 – 1.01 (m, 20H), 0.90 (t, *J* = 7.1 Hz, 3H).

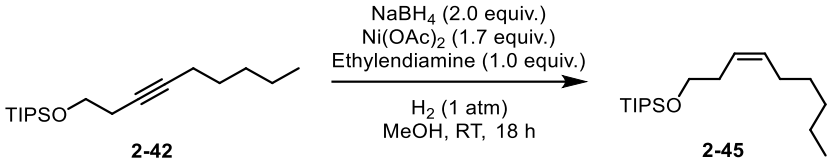
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 62.8, 31.2, 28.9, 23.4, 22.4, 18.9, 18.7, 18.1, 14.1, 12.12, 12.09.

MS (ESI) *m/z* (%): 298 $[M+H]^+$.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{18}H_{39}OSi$: 297.2608; found: 297.2605.

R_f = 0.45 (vanillin, orange spot; petroleum ether:EtOAc = 10:1)

(*Z*)-triisopropyl(non-3-en-1-yloxy)silane (**2-45**)



$NaBH_4$ (9.86 g, 65.8 mmol, 2.0 equiv.) was added to a stirred solution of $Ni(OAc)_2$ (14.2 g, 56 mmol, 1.7 equiv.) in dry MeOH (330 mL, 0.1 M) at room temperature under positive pressure of argon. Argon atmosphere (balloon) was replaced with hydrogen (double layer balloon) and ethylenediamine (3.29 mL, 32.9 mmol, 10 M, 1.0 equiv.) was added. After 5 min, alkyne **2-42** (9.86 g, 32.9 mmol, 1.0 equiv.) in MeOH (20 mL) was added and the resulting mixture was stirred for 4 h. The whole mixture was filtered through a pad of Celite® and the filter cake was washed with Et_2O (200 mL). Filtrate was diluted with brine (150 mL), and EtOAc (150 mL), and the resulting layers were separated. The aqueous phase was extracted with EtOAc (3x150 mL), and the combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered, and the volatiles were removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 100:1) and yielded a product **2-45** (8.25 g, 84 %) in the form of a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 5.48 – 5.35 (m, 2H), 3.68 – 3.65 (m, 2H), 2.30 (q, *J* = 7.2 Hz, 2H), 2.04 (q, *J* = 7.3 Hz, 2H), 1.35 – 1.27 (m, 6H), 1.06 (dd, *J* = 5.5, 2.3 Hz, 21H), 0.90 – 0.86 (m, 3H).

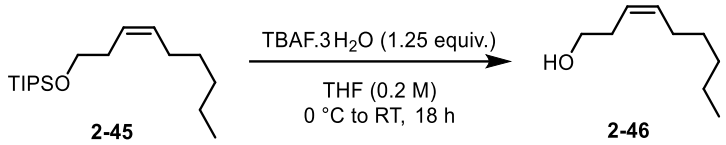
^{13}C{^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 132.0, 125.7, 63.4, 31.7, 31.4, 29.6, 27.5, 22.7, 18.2, 14.2, 12.2.

MS (ESI) *m/z* (%): 300 $[M+H]^+$.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{18}H_{39}OSi$: 299.2765; found: 299.2768.

R_f = 0.55 (PMA, petroleum ether:EtOAc = 10:1)

(*Z*)-non-3-en-1-ol (**2-46**)



Alkene **2-45** (6 g, 19.9 mmol, 1.0 equiv.) was dissolved in THF (99.5 mL, 0.2 M) and the resulting mixture was cooled to 0 °C (ice/water). TBAF.3H_2O (8 g, 24.9 mmol, 1.25 equiv.) was added dropwise and the resulting mixture was stirred at 0 °C for 3 h. Sat. aq. NH_4Cl (50 mL) was added, and the resulting mixture was extracted with EtOAc (3x100 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and the solvents were removed under reduced pressure. The crude product was purified by column flash column chromatography (SiO_2; petroleum ether:EtOAc = 10:1) and gave a product **2-46** (2.11 g, 74 %) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 5.62 – 5.51 (m, 1H), 5.42 – 5.29 (m, 1H), 3.67 – 3.57 (m, 2H), 2.33 (qd, *J* = 6.6, 1.5 Hz, 2H), 2.06 (qd, *J* = 7.3, 1.6 Hz, 2H), 1.39 – 1.23 (m, 7H), 0.88 (t, *J* = 6.9 Hz, 3H).

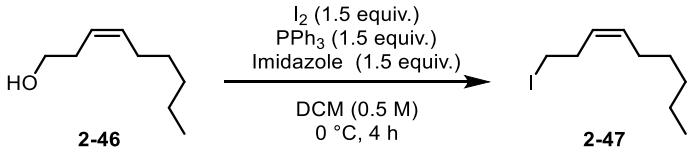
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 133.8, 125.1, 62.5, 31.6, 30.9, 29.5, 27.5, 22.7, 18.7, 14.2.

MS (ESI) *m/z* (%): 143 $[M+H]^+$.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_9H_{19}O$: 143.1430; found: 1431429.

R_f = 0.3 (vanillin, blue-green spot; petroleum ether:EtOAc = 4:1)

(*Z*)-1-iodonon-3-ene (**2-47**)



A solution of **2-46** (2.11 g, 14.7 mmol, 1.0 equiv.) in DCM (147 mL, 0.1 M) was cooled to 0 °C and PPh_3 (5.84 g, 22 mmol, 1.5 equiv.), imidazole (1.51 g, 22 mmol, 1.5 equiv.), and iodine (5.65 g, 22 mmol, 1.5 equiv.) was sequentially added. The resulting mixture was allowed to warm up to room temperature, and the reaction progress was monitored by TLC. Water (50 mL) was added to terminate the reaction and the resulting phases were separated. The aqueous phase was extracted with EtOAc (3x50 mL), and the combined organic phases were washed with brine (50 mL), dried over $MgSO_4$ and the volatiles were removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 10:1) and yielded **2-47** (3.45, 93 %) as colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 5.57 – 5.50 (m, 1H), 5.34 – 5.28 (m, 1H), 3.13 (t, *J* = 7.3 Hz, 2H), 2.63 (q, *J* = 7.3 Hz, 2H), 2.02 (q, *J* = 7.4 Hz, 2H), 1.38 – 1.25 (m, 6H), 0.89 (t, *J* = 6.8 Hz, 3H).

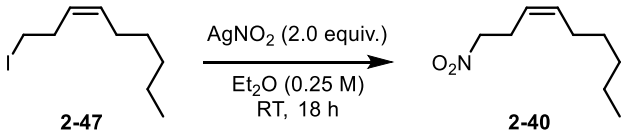
^{13}C{^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 132.9, 127.8, 31.7, 31.6, 29.3, 27.6, 22.7, 14.2, 5.7.

MS (ESI) *m/z* (%): 253 $[M+H]^+$.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_9H_{18}I$: 253.0448; found: 253.0450.

R_f = 0.8 (vanillin; petroleum ether:EtOAc = 10:1)

(*Z*)-1-nitronon-3-ene (**2-40**)



At room temperature, a silver nitrite (4.2 g, 27.1 mmol, 2.0 equiv.) was added in one portion to an aluminum foil-covered flask containing a stirred solution of **2-47** (3.45 g, 13.5 mmol, 1.0 equiv.) in Et_2O (54 mL, 0.25 M). The resulting mixture was stirred at room temperature for 18 h before being filtered through a short pad of Celite®. The filter cake was washed with EtOAc (3x50 mL), and the resulting layers were separated. The aqueous layer was extracted with EtOAc (3x50 mL), and the organic layers were combined, washed with brine, dried over

$MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc =10:1) and yielded a product **2-40** (1.67 g, 72 %) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 5.58 (dtt, *J* = 16.4, 7.5, 1.5 Hz, 1H), 5.29 (dtt, *J* = 16.5, 7.5, 1.7 Hz, 1H), 4.37 (t, *J* = 7.2 Hz, 2H), 2.83 – 2.68 (m, 2H), 2.08 – 2.01 (m, 2H), 1.36 – 1.25 (m, 6H), 0.89 (t, *J* = 6.9 Hz, 3H).

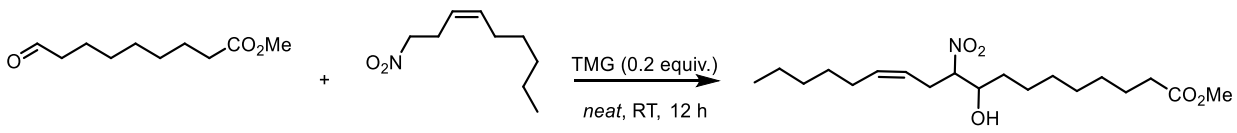
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 135.1, 122.2, 75.3, 31.6, 29.2, 27.4, 25.6, 22.7, 14.2.

MS (ESI) *m/z* (%): 172 $[M+H]^+$.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_9H_{18}NO_2$: 172.1332; found: 172.1335.

R_f = 0.4 (petroleum ether:EtOAc = 10:1)

methyl (*Z*)-9-hydroxy-10-nitrooctadec-12-enoate (2-84)



2-28 **2-40** **2-84**

Neat mixture of nitroalkane **2-40** (0.3 g, 1.73 mmol, 1.0 equiv.) and aldehyde **2-28** (0.49 g, 2.6 mmol, 1.2 equiv.) was cooled to 0 °C and 1,1,3,3-tetramethylguanidine (0.04 g, 0.35 mmol, 0.2 equiv.) was added. The resulting mixture was stirred for 12 h at room temperature before being cooled to 0 °C and the reaction was terminated with the addition of H_2O (5 mL). The resulting mixture was extracted with EtOAc (3×15 mL), and the combined organic phases were dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 50:1) to yield the adduct **2-84** (0.5 g, 81 %; *d.r.* = 1.17:1) in the form of a colorless oil. Product **2-84** was obtained as a mixture of two diastereoisomers in a 1.17:1 *d.r.* ratio (based on the ^{1}H NMR spectra analysis).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 5.62 – 5.53 (m, 1H), 5.33 – 5.24 (m, 1H), 4.48 – 4.39 (m, 1H), 4.05 (dq, *J* = 8.5, 4.2 Hz, 0.5H, *major diastereoisomer*), 3.91 – 3.85 (m, 0.5H, *minor diastereoisomer*), 3.66 (s, 3H), 2.94 – 2.76 (m, 1H), 2.62 – 2.52 (m, 1H), 2.36 (d, *J* = 4.8 Hz, 0.5H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.21 (d, *J* = 8.2 Hz, 0.5H), 2.02 (q, *J* = 7.4 Hz, 2H), 1.62 (p, *J* = 7.3 Hz, 2H), 1.58 – 1.39 (m, 4H), 1.34 – 1.26 (m, 12 h), 0.88 (t, *J* = 6.9 Hz, 3H).

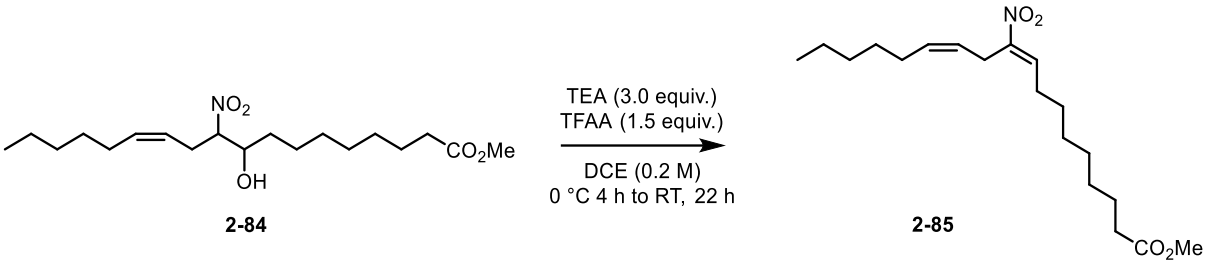
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 174.4, 135.5, 135.2, 122.3, 121.6, 92.3, 92.0, 72.2, 71.7, 51.6, 34.2, 33.7, 33.3, 31.6, 29.25, 29.22, 29.17, 29.07, 28.7, 27.4, 26.3, 25.6, 25.4, 25.0, 22.7, 14.2.

MS (ESI) *m/z* (%): 359 $[M+H]^+$.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{19}H_{36}NO_5$: 358.2588; found: 358.2590.

R_f = 0.2 (PMA; petroleum ether:EtOAc = 10:1)

methyl (9*E*,12*Z*)-10-nitrooctadeca-9,12-*d*ienoate (2-85)



Adduct **2-84** (0.5 g, 1.4 mmol, 1.0 equiv.) was dissolved in DCE (7 mL, 0.2 M) and cooled to 0 °C. TEA (0.59 mL, 4.2 mmol, 3.0 equiv.) and TFAA (0.3 mL, 2.1 mmol, 1.5 equiv.) were sequentially added and the resulting mixture was stirred at 0 °C for 4 h and then at room temperature for next 22 h. The whole mixture was cooled to 0 °C and water (10 mL) was added to terminate the reaction. The resulting mixture was extracted with EtOAc (3×25 mL), and organic phases were combined, dried over $MgSO_4$, and the volatiles were removed *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 20:1) to yield the desired nitro olefin **2-85** (0.26 g, 55 %; *E/Z* ≥ 95:5 (based on the ^{1}H NMR spectra analysis) in a form of a colorless oil.

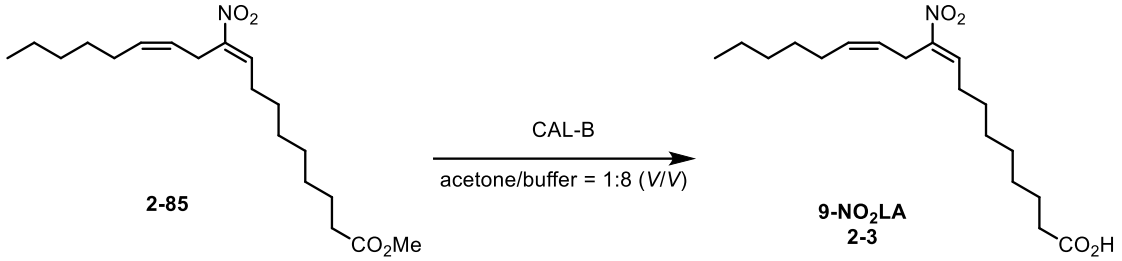
^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 7.08 (t, J = 7.9 Hz, 1H), 5.49 (dtt, J = 10.8, 7.3, 1.8 Hz, 1H), 5.25 (dtt, J = 10.5, 7.0, 1.7 Hz, 1H), 3.66 (s, 3H), 3.33 (dd, J = 7.0, 1.8 Hz, 2H), 2.30 (t, J = 7.5 Hz, 2H), 2.24 (q, J = 7.7 Hz, 2H), 2.12 (qd, J = 7.3, 1.6 Hz, 2H), 1.62 (dd, J = 9.9, 4.6 Hz, 2H), 1.49 (dt, J = 11.5, 7.2 Hz, 2H), 1.41 – 1.23 (m, 12H), 0.91 – 0.87 (m, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 174.3, 150.8, 136.6, 133.2, 123.4, 51.6, 34.1, 31.7, 29.29, 29.23, 29.12, 29.10, 28.5, 28.1, 27.5, 25.0, 22.7, 14.2.

MS (ESI) *m/z* (%): 340 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{19}H_{34}NO_4$: 340.2482; found: 340.2486.

(9*E*,12*Z*)-10-nitrooctadeca-9,12-dienoic acid (9-NO_2LA, 2-3)



CAL-B (400 mg) was added to a solution of methyl ester **2-85** (0.3 g, 0.24 mmol, 1.0 equiv.) in acetone (11 mL, 0.08 M) and aqueous phosphate buffer (88 mL, 0.01 M, pH 7.4). The solution was vigorously stirred (magnetic stirrer, 1000 rpm) at room temperature for 18 h. 1 M aq. HCl solution was added to adjust the pH of the solution to pH = 3. The whole mixture was then extracted with EtOAc (5x20 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and the volatiles were removed *in vacuo*. The crude product was purified using semipreparative chromatography (C18 reverse-phase column; MeOH:H_2O) to yield the desired acid **10-NO_2LA**, **2-3** (0.12 g, 42 %, *E/Z* ≥ 95:5; based on the ^{1}H NMR spectra analysis).

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): δ 11.14 (bs, 1H), 7.08 (t, *J* = 7.9 Hz, 1H), 5.49 (dtt, *J* = 10.9, 7.3, 1.8 Hz, 1H), 5.25 (dtt, *J* = 10.7, 6.9, 1.7 Hz, 1H), 3.34 (dd, *J* = 7.0, 1.8 Hz, 2H), 2.35 (t, *J* = 7.4 Hz, 2H), 2.24 (q, *J* = 7.6 Hz, 2H), 2.12 (q, *J* = 7.3 Hz, 2H), 1.65 – 1.61 (m, 2H), 1.53 – 1.47 (m, 2H), 1.38 – 1.29 (m, 12 h), 0.90 (t, *J* = 6.8 Hz, 3H).

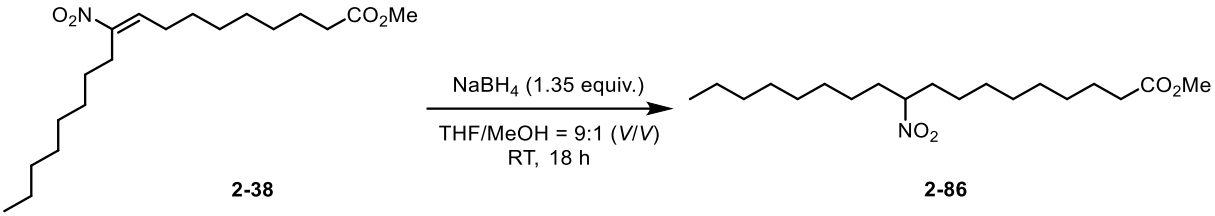
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 179.6, 150.8, 136.6, 133.2, 123.4, 34.0, 31.7, 29.3, 29.2, 29.1, 29.0, 28.5, 28.1, 27.6, 25.0, 24.7, 22.7, 14.2.

MS (ESI) *m/z* (%): 324 $[M-H]^-$ (100).

HRMS (ESI) *m/z:* $[M-H]^-$ calculated for $C_{18}H_{30}NO_4$: 324.2169; found: 324.2179.

2.5.4. 10-NO_2SA

methyl 10-nitrooctadecanoate (2-86)



$NaBH_4$ (0.058 g, 1.5 mmol, 1.35 equiv.) was added to a cold (0 °C) solution of methyl (*E*)-9-nitrooctadec-9-enoate **2-38** (0.400 g, 1.1 mmol, 1.0 equiv.) in THF/MeOH (5.5 mL, 9:1 (*V*/*V*)) and the resulting mixture was stirred at RT for 14 h. Water (5 mL), and EtOAc (5 mL) were added at room temperature and the resulting phases were separated. Aqueous layer was extracted with EtOAc (5x20 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and the volatiles were removed *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 10:1) to yield the desired nitro alkane **2-86** (0.27 g, 73 %) as a colorless oil.

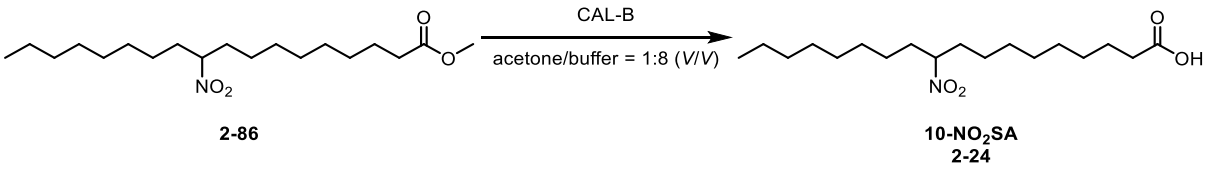
^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 4.45 (tt, *J* = 9.3, 4.5 Hz, 1H), 3.66 (s, 3H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.94 (dtd, *J* = 14.0, 8.9, 4.3 Hz, 2H), 1.67 (dp, *J* = 15.2, 5.4, 5.0 Hz, 2H), 1.59 (dt, *J* = 12.4, 6.1 Hz, 2H), 1.34 – 1.20 (m, 22H), 0.87 (t, *J* = 6.9 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 174.5, 89.2, 51.6, 34.2, 34.1, 34.0, 31.9, 29.4, 29.3, 29.20, 29.17, 29.1, 29.0, 25.93, 25.91, 25.0, 22.8, 14.2.

MS (ESI) *m/z* (%): 345 $[M+H]^+$.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{19}H_{38}NO_4$: 344.2795; found: 344.2796.

10-nitrooctadecanoic acid (10-NO_2SA, 2-24)



CAL-B (0.4 g) was added to a solution of methyl ester **2-86** (0.18 g, 0.52 mmol, 1.0 equiv.) in acetone (6.50 mL, 0.08 M) and aqueous phosphate buffer (52.4 mL, 0.01 M, pH 7.4). The solution was vigorously stirred (magnetic stirrer, 1000 rpm) at room temperature for 18 h. 1 M aq. HCl solution was added to adjust the pH of the mixture to pH = 3. The whole mixture was then extracted with EtOAc (5x20 mL), and the combined organic layers were dried over

$MgSO_4$, filtered, and the volatiles were removed *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 10:1) to yield the adduct **10-NO_2SA**, **2-24** (0.17 g, 96 %) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 11.15 (bs, 1H), 4.45 (tt, *J* = 9.3, 4.5 Hz, 1H), 2.34 (t, *J* = 7.5 Hz, 2H), 1.99 – 1.89 (m, 2H), 1.72 – 1.58 (m, 4H), 1.36 – 1.22 (m, 22H), 0.87 (t, *J* = 7.0 Hz, 3H).

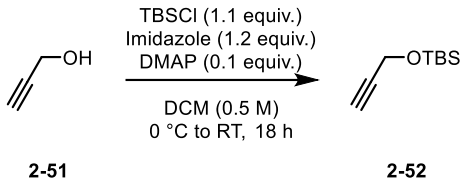
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 179.7, 89.2, 34.1, 34.0, 31.9, 29.4, 29.3, 29.19, 29.17, 29.11, 29.06, 29.02, 25.93, 25.90, 24.7, 22.8, 14.2.

MS (ESI) *m/z* (%): 329 $[M-H]^-$.

HRMS (ESI) *m/z:* $[M-H]^-$ calculated for $C_{18}H_{34}NO_4$: 328.2493; found: 328.2496.

2.5.5. 9-NO_2cLA

tert-butyldimethyl(prop-2-yn-1-yloxy)silane (2-52)



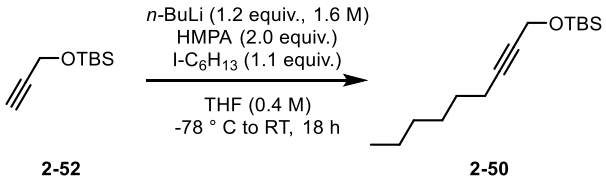
A solution of imidazole (22.0 g, 323 mmol, 1.2 equiv.) in DCM (539 mL, 0.5 M) was cooled to 0 °C and propargyl alcohol **2-51** (15.1 g, 269 mmol, 1.0 equiv.) was added. DMAP (3.36 g, 26.9 mmol, 0.1 equiv.) and TBSCl (51.3 mL, 296 mmol, 1.1 equiv.) were added and the whole mixture was stirred at room temperature overnight. Sat. aq. NH_4Cl (150 mL) was added, and the resulting layers were separated. The aqueous layer was extracted with DCM (3x150 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 100:1) and yielded product **2-52** (30.0 g, 65 %) in the form of a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 4.31 (m, 2H), 2.39 (td, *J* = 2.4, 0.8 Hz, 1H), 0.91 (m, 9H), 0.13 – 0.12 (m, 6H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 82.5, 73.0, 51.7, 26.1, 25.9, 18.4, -5.1.

R_f = 0.55 (hexane:EtOAc, 10:1)

tert-butyldimethyl(oct-2-yn-1-yloxy)silane (2-50)



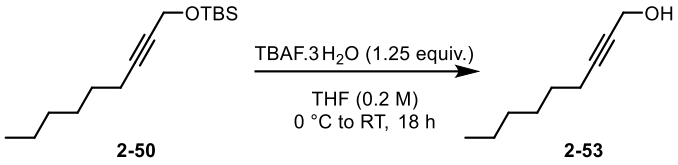
To a solution of TBS protected alcohol **2-52** (6.85 g, 40 mmol, 1.0 equiv.) in THF (100 mL, 0.4 M) at -78 °C was added *n*-BuLi (29.9 mL, 47.8 mmol, 1.6 M in hexane) dropwise and stirring was continued at -78 °C for 1 h. Then, 1-iodohexane (6.46 mL, 43.8 mmol, 1.1 equiv.) was added to the reaction mixture followed by HMPA (13.9 mL, 79.6 mmol, 2.0 equiv.). The reaction mixture was allowed to warm to RT and left stirring overnight. Sat. aq. NH_4Cl (100 mL) was added. The organic layer was separated, and the aqueous phase was extracted by EtOAc (3x 100mL). Combined organic layers were dried with $MgSO_4$, filtered and concentrated

under vacuum. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 100:1) and yielded product **2-50** (8.45 g, 88 %) in the form of a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 4.30 (t, *J* = 2.2 Hz, 2H), 2.19 (tt, *J* = 7.1, 2.2 Hz, 2H), 1.53 – 1.45 (m, 2H), 1.42 – 1.34 (m, 2H), 1.33 – 1.24 (m, 4H), 0.91 (d, *J* = 0.5 Hz, 9H), 0.88 (t, *J* = 7.0 Hz, 3H), 0.12 (d, *J* = 0.5 Hz, 6H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 85.7, 78.7, 52.2, 31.5, 28.7, 26.0, 25.9, 22.7, 18.9, 18.5, 14.2, -5.0.

non-2-yn-1-ol (2-53)

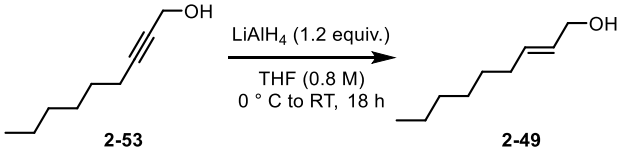


Alkene **2-50** (8.45 g, 32.9 mmol, 1.0 equiv.) was dissolved in THF (164 mL, 0.2 M) and the resulting mixture was cooled to 0 °C (ice/water). TBAF.3H_2O (13.2 g, 41.1 mmol, 1.25 equiv.) was added and the resulting mixture was stirred at 0 °C for 3 h. Sat. aq. NH_4Cl (100 mL) was added, and the resulting mixture was extracted with EtOAc (5x50mL). The combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and the solvents were removed under reduced pressure. The crude product was purified by column flash column chromatography (SiO2; petroleum ether:EtOAc = 40:1) and gave a product **2-53** (0.1 g, 24 %) as a yellowish oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 4.25 (dtd, *J* = 5.9, 2.2, 1.3 Hz, 2H), 2.21 (dddd, *J* = 7.2, 5.1, 2.2, 1.1 Hz, 2H), 1.59 – 1.55 (m, 2H), 1.54 – 1.45 (m, 3H), 1.40 – 1.34 (m, 2H), 1.33 – 1.24 (m, 4H), 0.91 (d, *J* = 1.2 Hz, 3H), 0.90 – 0.86 (m, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 86.9, 78.4, 51.6, 31.5, 28.7, 25.8, 22.7, 18.9, 14.2.

(*E*)-non-2-en-1-ol (2-49)



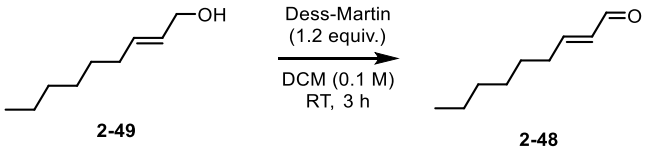
To a suspension of $LiAlH_4$ (0.89 g, 23.3 mmol, 1.2 equiv.) at 0 °C in dry THF (24.3 mL, 0.8 M) was added compound alcohol **2-53** (2.75 g, 19.4 mmol) dissolved in dry THF (19.4 mL, 1 M). The reaction was stirred at 0 °C for 10 min before it was allowed to warm to room temperature and allowed to stir overnight. Upon completion, the reaction was carefully quenched at 0 °C by the addition of water (2 mL) and 3 M NaOH followed by water (3x 15 mL). The mixture was filtered, and the filter cake was washed repeatedly with diethyl ether (5x 20 mL) and DCM (3x 5 mL). Concentration in vacuo followed by purification by column flash column chromatography (SiO2; petroleum ether:EtOAc = 4:1) and gave a product **2-49** (1.75 g, 70 %) as a yellowish oil.

R_f = =0.69 (hexane:EtOAc, 4:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 5.66 (qt, *J* = 15.4, 6.0 Hz, 2H), 4.08 (d, *J* = 5.5 Hz, 2H), 2.04 (q, *J* = 7.0 Hz, 2H), 1.40 – 1.33 (m, 2H), 1.32 – 1.22 (m, 6H), 0.88 (t, *J* = 6.7 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 133.8, 128.9, 64.0, 32.4, 31.9, 29.2, 29.0, 22.8, 14.2.

(*E*)-non-2-enal (2-48)



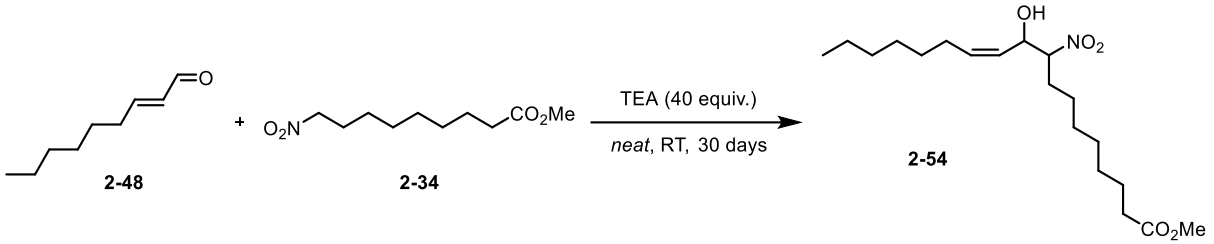
To a stirred solution of the alcohol **2-48** (1.5 g, 11.7 mmol, 1 equiv.) in DCM (117 mL, 0.1 M) was added Dess-Martin periodinane (6.37 g, 14.3 mmol, 1.22 equiv.). The resulting milky suspension was stirred for 3 h followed by the addition of sat. aq. $NaHCO_3$ solution (50 mL). Within 20 min the solution became clear and biphasic. The mixture was extracted with DCM (3x 50 mL), dried over $MgSO_4$, filtered and the solvents were removed under reduced pressure. The crude product was purified by column flash column chromatography (SiO_2; petroleum ether:EtOAc = 4:1) and gave a product **2-53** (1.5 g, 95 %) as a yellowish oil.

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 9.51 (d, *J* = 7.9 Hz, 1H), 6.85 (dt, *J* = 15.6, 6.8 Hz, 1H), 6.12 (ddt, *J* = 15.6, 7.9, 1.6 Hz, 1H), 2.38 – 2.29 (m, 2H), 1.51 (p, *J* = 7.3 Hz, 2H), 1.37 – 1.24 (m, 6H), 0.90 – 0.87 (m, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 194.4, 159.3, 133.1, 32.9, 31.7, 28.9, 27.9, 22.7, 14.2.

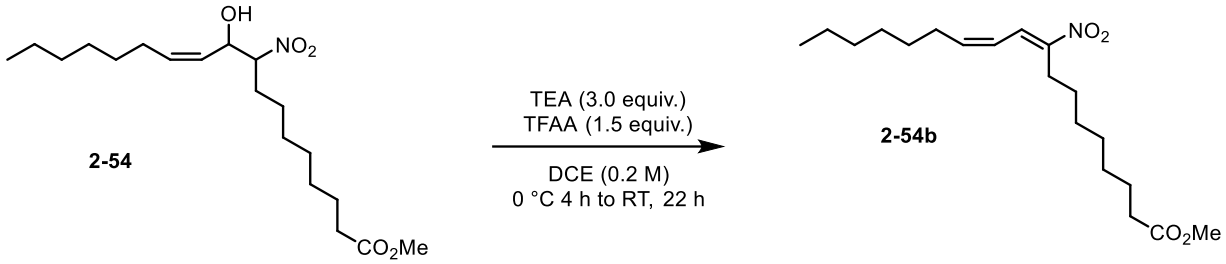
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for C9H16O: 141.1274; found: 141.1277.

methyl (*Z*)-10-hydroxy-9-nitrooctadec-11-enoate (**2-54**)



Aldehyde **2-48** (0.95 g, 6.7 mmol, 1.7 equiv.) and nitro ester **2-34** (0.86 g, 3.94 mmol, 1 equiv.) were mixed and TEA (22 mL, 158 mmol, 40 equiv.) was added. The reaction was monitored until starting materials were consumed. After 30 days, the reaction was evaporated with a stream of nitrogen to remove triethylamine solvent then the crude oil redissolved in 20 mL Et_2O. The ether solution was transferred to a separatory funnel and washed with 30 mL × 2 aqueous 0.1 M HCl, 30 mL × 2 water, and 30 mL brine. The resulting solution was dried over anhydrous sodium sulfate, filtered through a plug of silica gel and Celite®, then concentrated by rotary evaporation. The crude product was purified by gradient column flash column chromatography (SiO_2; petroleum ether:EtOAc = 30:1 → 1:1) to separate starting materials from the product. The product was isolated and gave a product **2-54** (1.15 g, 60 %, *d.r.* 31:69) as a yellowish oil together with isolated aldehyde (0.2 g) and nitro ester (0.18 g). The product **2-54** was then immediately used in the next step

methyl (9*E*,11*Z*)-9-nitrooctadeca-9,11-dienoate (**2-54b**)



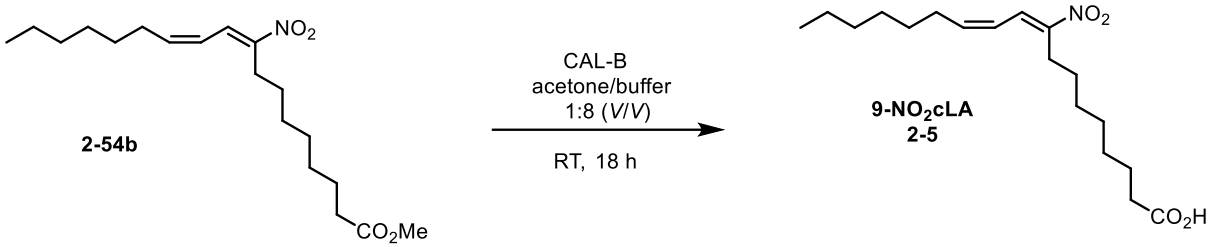
Adduct **2-54** (1.15 g, 3.2 mmol, 1.0 equiv.) was dissolved in DCE (16 mL, 0.2 M) and cooled to 0 °C. TEA (0.98 mL, 9.6 mmol, 3.0 equiv.) and TFAA (1.01 mL, 4.8 mmol, 1.5 equiv.) were sequentially added and the resulting mixture was stirred at 0 °C for 4 h and then at room temperature for next 22 h. The whole mixture was cooled to 0 °C and water (20 mL) was added to terminate the reaction. The resulting mixture was extracted with EtOAc (3×25 mL), and organic phases were combined, dried over $MgSO_4$, and the volatiles were removed *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 20:1) to yield the desired nitro olefin **2-54b** (0.5 g, 47 %; *E/Z* ≥ 95:5 (based on the ^{1}H NMR spectra analysis) in a form of a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 7.54 (d, *J* = 11.3 Hz, 1H), 6.34 (dt, *J* = 14.5, 7.0 Hz, 1H), 6.19 (ddt, *J* = 14.8, 11.4, 1.4 Hz, 1H), 3.66 (s, 3H), 2.71 – 2.60 (m, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.25 (q, *J* = 7.8, 7.2 Hz, 2H), 1.62 (dd, *J* = 10.1, 4.5 Hz, 2H), 1.50 (q, *J* = 6.3, 5.4 Hz, 2H), 1.50 – 1.37 (m, 2H), 1.39 – 1.22 (m, 12H), 0.89 (t, *J* = 6.8 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 174.4, 149.5, 149.2, 134.1, 123.7, 51.6, 34.2, 33.8, 31.7, 29.1, 29.1, 29.1, 29.0, 28.7, 28.2, 26.7, 25.0, 22.7, 14.2.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{19}H_{33}NO_4$: 340.2482; found: 340.2480.

(9*E*,11*Z*)-9-nitrooctadeca-9,11-dienoic acid, 9-NO_2cLA (**2-5**)



CAL-B (600 mg) was added to a solution of methyl ester **2-54b** (1.115 g, 3.2 mmol, 1.0 equiv.) in acetone (11 mL, 0.08 M) and aqueous phosphate buffer (88 mL, 0.01 M, pH 7.4). The solution was vigorously stirred (magnetic stirrer, 1000 rpm) at room temperature for 18 h. 1 M aq. HCl solution was added to adjust the pH of the solution to pH = 3. The whole mixture was then extracted with EtOAc (5x20 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and the volatiles were removed *in vacuo*. The crude product was purified using semipreparative chromatography (C18 reverse-phase column; MeOH:H_2O) to yield the desired acid **9-NO2cLA**, **2-3** (0.12 g, 42 %, *E/Z* ≥ 95:5; based on the ^{1}H NMR spectra analysis).

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 7.56 – 7.52 (m, 1H), 6.35 (dt, *J* = 14.5, 7.0 Hz, 1H), 6.19 (ddt, *J* = 15.0, 11.4, 1.4 Hz, 1H), 2.69 – 2.62 (m, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 2.27 – 2.22 (m, 2H), 1.63 (p, *J* = 7.9, 7.1 Hz, 2H), 1.52 (d, *J* = 7.3 Hz, 2H), 1.48 – 1.42 (m, 2H), 1.37 – 1.25 (m, 12H), 0.94 – 0.85 (m, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 178.3, 149.5, 149.2, 134.1, 123.7, 33.8, 33.8, 31.7, 29.1, 29.1, 29.0, 29.0, 28.7, 28.2, 26.7, 24.7, 22.7, 14.2.

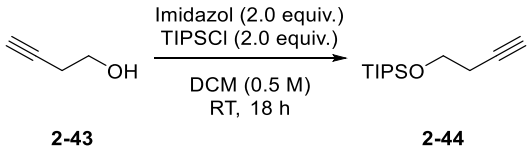
HRMS (ESI) *m/z:* $[M-H]^-$ calculated for $C_{18}H_{30}NO_4$: 324.2180; found: 324.2178.

R_f = 0.33 (hexane/EtOAc; 3:1)

2.5.6. 14-NO_2ARA

2.5.6.1. Fist approach

(but-3-yn-1-yloxy)triisopropylsilane (**2-44**)

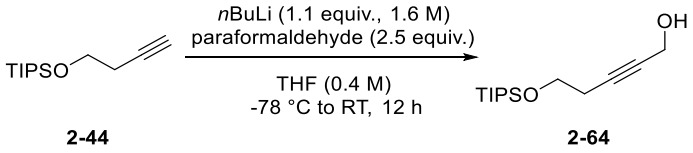


To a solution of imidazole (18.1 g, 263 mmol, 2.0 equiv.) and TIPSCl (58 mL, 263 mmol, 2.0 equiv.) in DCM, under nitrogen atmosphere, 3-butyn-1-ol **2-43** (10.0 mL, 131 mmol, 1.0 equiv.) was added and the reaction mixture was stirred at RT overnight. Then, water (200 mL), and the aqueous layer was extracted with EtOAc (3x 250 mL). Combined organic layers were then washed with saturated aqueous $NaHCO_3$ (200 mL), and brine (200 mL), dried over $MgSO_4$, filtered, and solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 1000:1) to afford **2-44** (26.5 g, 89 %) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 3.82 (t, *J* = 7.3 Hz, 2H), 2.44 (td, *J* = 7.3, 2.7 Hz, 2H), 1.96 (td, *J* = 2.7, 0.8 Hz, 1H), 1.12 – 1.04 (m, 21H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 81.7, 69.4, 62.1, 23.0, 18.1, 12.1.

5-((triisopropylsilyl)oxy)pent-2-yn-1-ol (**2-64**)



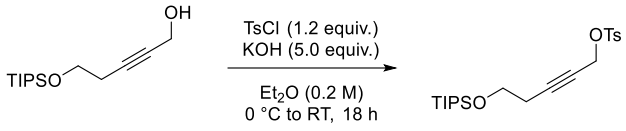
To a solution of (but-3-yn-1-yloxy)triisopropylsilane **2-44** (6 g, 26.2 mmol, 1.0 equiv.) in THF (65.6 mL, 0.4 M) at -78 °C was added dropwise *n*-BuLi (20.2 mL, 32.3 mmol, 1.2 equiv., 1.6 M in hexane) and stirring was continued at -78 °C for 1 h. Then, to the reaction mixture was added to a paraformaldehyde (2.24 g, 65.6 mmol, 2.5 equiv.) as a suspension in THF (65.6 mL, 0.4 M) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. NH_4Cl (50 mL) saturated aqueous solution was added, the organic layer was separated, and the aqueous layer was extracted with EtOAc (3x 100 mL). Combined organic layers were washed with brine (200 mL), dried over $MgSO_4$, filtered, and solvents were removed under reduced pressure. The crude product was purified by gradient column

chromatography (SiO_2; petroleum ether/EtOAc = 20:1 → 10:1) to afford **2-64** (5.4 g, 80 %) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 4.25 – 4.22 (m, 1H), 3.80 (td, *J* = 7.3, 1.2 Hz, 1H), 2.49 – 2.44 (m, 1H), 1.08 – 1.03 (m, 21H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 83.6, 79.6, 62.2, 51.5, 23.3, 18.1, 12.1.

5-((triisopropylsilyl)oxy)pent-2-yn-1-yl 4-methylbenzenesulfonate (2-62)



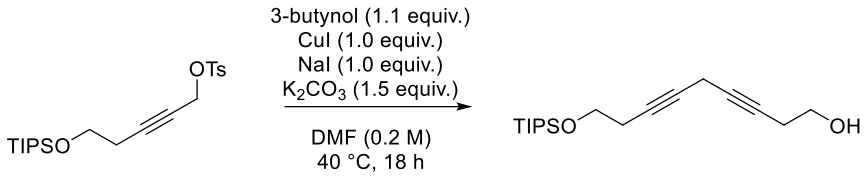
2-64 **2-62**

KOH (7.64 g, 116 mmol, 5.0 equiv.) was added to a solution of alcohol **2-62** (6 g, 18.5 mmol, 1.0 equiv.), *p*-toluenesulfonyl chloride (5.41 g, 27.8 mmol, 1.2 equiv.) and Et_2O (116 mL, 0.2 M) at 0 °C. The mixture was stirred for 2 h at 0 °C, after which the reaction was warmed to RT. Next day, the reaction was quenched with saturated aqueous NH_4Cl (50 mL) and extracted with EtOAc (3x 50 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (SiO_2; petroleum ether/EtOAc = 20:1) to afford tosylate **2-62** (9 g, 95 %) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 7.84 – 7.78 (m, 2H), 7.36 – 7.32 (m, 2H), 4.68 (t, *J* = 2.2 Hz, 2H), 3.68 (t, *J* = 7.2 Hz, 2H), 2.45 (s, 3H), 2.33 (tt, *J* = 7.2, 2.2 Hz, 2H), 1.07 – 1.00 (m, 21H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 145.0, 133.4, 129.8, 128.2, 87.6, 73.0, 61.6, 58.7, 23.2, 21.8, 18.0, 17.8, 12.4, 12.0.

9-((triisopropylsilyl)oxy)nona-3,6-diyn-1-ol (2-61)



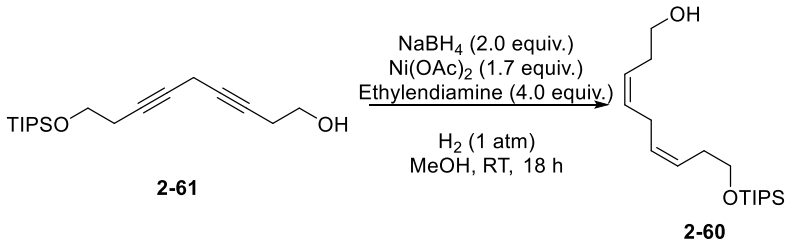
2-62 **2-61**

K_2CO_3 (7.68 g, 55.6 mmol, 3.0 equiv.) was added to a mixture of tosylate **2-62** (7.5 g, 18.1 mmol, 1.0 equiv.), 3-butyn-1-ol (1.45 mL, 19 mmol, 1.0 equiv.), CuI (3.51 g, 18.1 mmol, 1.0 equiv.), NaI (2.74 g, 18.1 mmol, 1.0 equiv.), and DMF (90 mL, 0.2 M) at RT. The mixture was heated to 40 °C, stirred for 18 h at this temperature, and quenched with saturated aqueous NH_4Cl (50 mL). The resulting mixture was filtrated through a pad of Celite®. After the organic layer was separated, the resulting aqueous layer was extracted with Et_2O (3x 50 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 20:1 → 10:1) to afford 1,4-diyne **2-61** (3.5 g, 63 %) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 3.78 (t, J = 7.3 Hz, 2H), 3.70 (q, J = 6.0 Hz, 2H), 3.13 (m, 2H), 2.44 (ddt, J = 8.2, 6.7, 2.2 Hz, 2H), 2.41 (dq, J = 7.3, 2.5 Hz, 2H), 1.11 – 1.01 (m, 21H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 77.8, 77.0, 76.8, 75.3, 62.3, 61.2, 23.3, 23.3, 18.1, 12.1, 12.1, 9.9.

(3*Z*,6*Z*)-9-((triisopropylsilyl)oxy)nona-3,6-dien-1-ol (2-60)



2-61 **2-60**

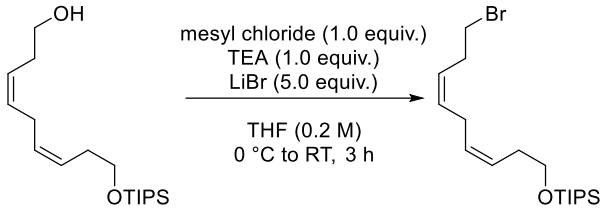
$NaBH_4$ (0.89 g, 23.1 mmol, 2.0 equiv.) was added to a stirred solution of $Ni(OAc)_2$ (5 g, 19.6 mmol, 1.7 equiv.) in dry MeOH (116 mL, 0.1 M) at RT under positive pressure of argon. Argon atmosphere (balloon) was replaced with hydrogen (double layer balloon) and ethylenediamine (4.6 mL, 46.2 mmol, 4.0 equiv., 10 M) was added. After 5 min, alkyne **2-61** (3.6 g, 11.6 mmol, 1.0 equiv.) in MeOH (20 mL) was added and the resulting mixture was stirred overnight. The whole mixture was filtered through a pad of Celite® and the filter cake

was washed with Et_2O (2x100 mL). Filtrate was diluted with brine (150 mL), and EtOAc (150 mL), and the resulting layers were separated. The aqueous phase was extracted with EtOAc (3x150 mL), and the combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered, and the volatiles were removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 20:1) and yielded a product **2-60** (2.93 g, 81 %) in the form of a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 5.54 (q, J = 8.1 Hz, 1H), 5.50 – 5.36 (m, 3H), 3.67 (dt, J = 18.4, 6.7 Hz, 4H), 2.85 (t, J = 6.7 Hz, 2H), 2.35 (dq, J = 13.4, 6.7 Hz, 4H), 1.06 (m, 21H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 131.5, 129.4, 126.7, 125.6, 63.2, 62.4, 31.4, 31.0, 26.0, 18.2, 18.1, 12.13, 12.08.

(((3*Z*,6*Z*)-9-bromonona-3,6-dien-1-yl)oxy)triisopropylsilane (**2-59**)



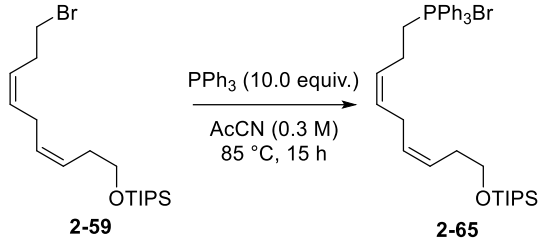
2-60 **2-59**

Mesyl chloride (1.37 mL, 17.4 mmol, 2.0 equiv.) was added to a solution of 1,4-diene **2-60** (2.74 g, 8.68 mmol, 1.0 equiv.), TEA (2.4 mL, 2.7 mmol, 2.0 equiv.), and THF (87 mL, 0.1 M) at 0 °C. After the solution was maintained for 1 h at 0 °C, lithium bromide (7.6 g, 86.8 mmol, 10.0 equiv.) was added to the solution. The resulting mixture was then heated to 50 °C, maintained for 3 h at this temperature after which it was cooled to RT and left overnight. Next day, the reaction was quenched with saturated aqueous $NaHCO_3$ (50 mL) and extracted with EtOAc (3x 50 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 20:1) and yielded a product **2-59** (2.65 g, 82 %) in the form of a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 5.58 – 5.47 (m, 1H), 5.49 – 5.35 (m, 3H), 3.69 (t, J = 7.0 Hz, 2H), 3.38 (t, J = 7.1 Hz, 2H), 2.82 (t, J = 7.0 Hz, 2H), 2.65 (q, J = 7.2 Hz, 2H), 2.33 (q, J = 7.0 Hz, 2H), 1.16 – 1.01 (m, 21H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 131.2, 129.1, 126.8, 126.4, 63.2, 32.5, 31.4, 30.9, 26.1, 18.2, 12.1.

bromotriphenyl((3*Z*,6*Z*)-9-((triisopropylsilyl)oxy)nona-3,6-dien-1-yl)-l5-phosphane (**2-65**)



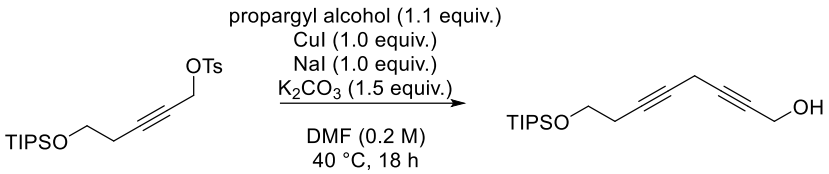
PPh_3 (18.5 g, 69.9 mmol, 10.0 equiv.) was dissolved in AcCN (23.3 mL, 0.3 M) and bromide **2- 59** (2.65 g, 6.99 mmol, 1.0 equiv.) was added at RT. The solution was heated to 85 °C, maintained for 15 h at this temperature, after which it was cooled and evaporated. The crude product was purified by flash column chromatography (SiO_2; petroleum ether:EtOAc = 10:1) and yielded a product **2-65** in the form of a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 7.91 – 7.85 (m, 6H), 7.79 (m, 4H), 7.70 (m, 5H), 5.64 (m, 1H), 5.42 – 5.32 (m, 2H), 5.28 – 5.20 (m, 1H), 4.00 (m, 2H), 3.61 (t, *J* = 6.9 Hz, 2H), 2.54 (t, *J* = 7.3 Hz, 2H), 2.46 (q, *J* = 8.6, 7.3 Hz, 2H), 2.17 (m, 2H, overlapping signal with impurity), 1.14 – 0.97 (m, 21H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 135.1, 134.0, 133.9, 130.7, 130.6, 130.4, 128.8, 126.8, 118.9, 118.2, 63.1, 31.4, 25.8, 23.3, 20.6, 18.2, 12.1.

2.5.6.2. Second approach

8-((triisopropylsilyl)oxy)octa-2,5-diyn-1-ol (2-69)



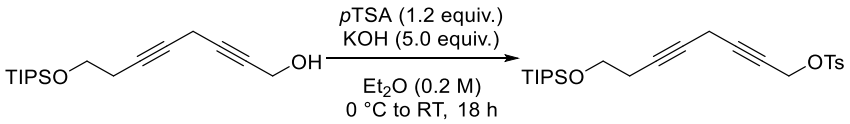
2-62 **2-69**

K_2CO_3 (4 g, 28.9 mmol, 1.5 equiv.) was added to a mixture of tosylate **2-62** (8 g, 19.3 mmol, 1.0 equiv.), propargyl alcohol (1.2 mL, 20.3 mmol, 1.0 equiv.), CuI (3.75 g, 19.3 mmol, 1.0 equiv.), NaI (2.92 g, 19.3 mmol, 1.0 equiv.), and DMF (96 mL, 0.2 M) at RT. The mixture was heated to 40 °C, stirred for 18 h at this temperature, and quenched with saturated aqueous NH_4Cl (50 mL). The resulting mixture was filtrated through a pad of Celite®. After the organic layer was separated, the resulting aqueous layer was extracted with Et_2O (3x 50 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 20:1 → 10:1) to afford 1,4-diyne **2-69** (4.97 g, 88 %) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 4.26 (dt, *J* = 6.2, 2.2 Hz, 2H), 3.78 (t, *J* = 7.3 Hz, 2H), 3.18 (p, *J* = 2.3 Hz, 2H), 2.41 (tt, *J* = 7.3, 2.4 Hz, 2H), 1.52 (t, *J* = 6.2 Hz, 1H), 1.09 – 1.03 (m, 21H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 80.7, 78.6, 78.2, 74.6, 62.3, 62.3, 62.2, 51.5, 51.4, 23.3, 23.2, 18.1, 12.1, 10.0.

8-((triisopropylsilyl)oxy)octa-2,5-diyn-1-yl 4-methylbenzenesulfonate (2-68)



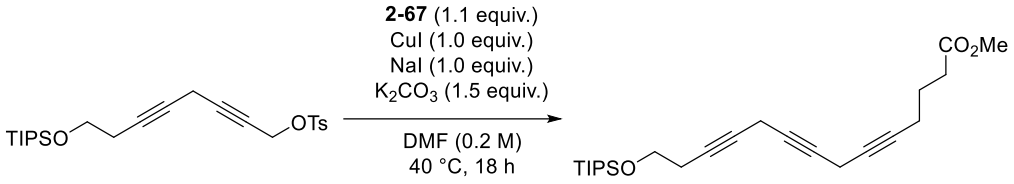
2-69 **2-68**

KOH (5.51 g, 83.5 mmol, 5.0 equiv.) was added to a solution of alcohol **2-69** (4.97 g, 16.7 mmol, 1.0 equiv.), *p*-toluenesulfonyl chloride (3.9 g, 20 mmol, 1.2 equiv.) and Et_2O (83 mL, 0.2 M) at 0 °C. The mixture was stirred for 2 h at 0 °C, after it was warmed to RT and left overnight. Next day, the reaction was quenched with saturated aqueous NH_4Cl (50 mL) and extracted with EtOAc (3x 50 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (SiO_2; petroleum ether/EtOAc = 20:1) to afford tosylate **2-68** (6 g, 80 %) as a colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 7.84 – 7.80 (m, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 4.69 (t, *J* = 2.2 Hz, 2H), 3.76 (t, *J* = 7.3 Hz, 2H), 3.04 (p, *J* = 2.3 Hz, 2H), 2.45 (s, 3H), 2.39 (tt, *J* = 7.3, 2.4 Hz, 2H), 1.08 – 1.02 (m, 21H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 145.2, 133.3, 129.9, 128.3, 84.5, 78.6, 73.6, 72.2, 62.2, 58.3, 23.2, 21.8, 18.1, 17.8, 12.4, 12.1, 10.0.

methyl 14-((triisopropylsilyl)oxy)tetradeca-5,8,11-triynoate (2-66)

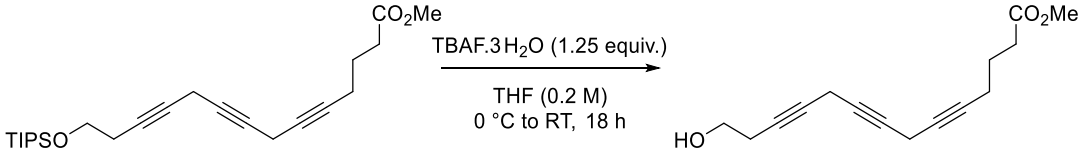


2-68 **2-66**

To a mixture of tosylate **2-68** (3.91 g, 8.63 mmol, 1.0 equiv.) in DMF (43 mL, 0.2 M), NaI (1.31 g, 8.63 mmol, 1.0 equiv.), CuI (1.68 g, 8.63 mmol, 1.0 equiv.), and K_2CO_3 (1.81 g, 12.9 mmol, 1.5 equiv.) were sequentially added at RT. After 2 min, methyl 5-hexynote **2-67** (1.21 g, 9.49 mmol, 1.1equiv.) was added and the resulting mixture was stirred at RT for 12 h. The whole mixture was then diluted with EtOAc (50 mL), and the resulting mixture was filtered through a pad of Celite®. Resulting filtrate was washed with saturated aq. NH_4Cl (20 mL), and the aqueous layer was back extracted with EtOAc (3x 50 mL). The combined organic phases were washed with brine (50 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **2-66** (0.87 g, 23 %).

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 3.77 (t, *J* = 7.3 Hz, 1H), 3.68 (d, *J* = 2.9 Hz, 2H), 3.13 (q, *J* = 2.0, 1.5 Hz, 2H), 2.50 – 2.37 (m, 2H), 2.25 (dddd, *J* = 16.2, 9.3, 7.0, 2.5 Hz, 1H), 1.82 (tt, *J* = 14.4, 7.2 Hz, 1H), 1.05 (d, *J* = 5.5 Hz, 21H).

methyl 14-hydroxytetradeca-5,8,11-triynoate (2-71)



2-66 **2-71**

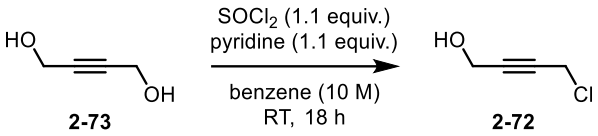
Alkene **2-66** (0.74 g, 1.81 mmol, 1.0 equiv.) was dissolved in THF (9.1 mL, 0.2 M) and the resulting mixture was cooled to 0 °C (ice/water). TBAF.$3H_2O$ (0.73 g, 2.27 mmol, 1.25 equiv.) was added and the resulting mixture was stirred at 0 °C for 3 h. Sat. aq. NH_4Cl (50 mL) was added, and the resulting mixture was extracted with EtOAc (5x25 mL). The combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and the solvents were removed under reduced pressure. The crude product was purified by column flash column chromatography (SiO2; petroleum ether:EtOAc = 10:1) and gave a product **2-71** (0.1 g, 24 %) as a yellowish oil.

^{1}H NMR (500 MHz, Chloroform-*d*) *δ* (ppm): 3.71 (t, *J* = 6.3 Hz, 2H), 3.68 (s, 3H), 3.15 (dp, *J* = 14.2, 2.4 Hz, 3H), 2.48 – 2.41 (m, 4H), 2.24 (tt, *J* = 6.9, 2.3 Hz, 2H), 1.82 (p, *J* = 7.2 Hz, 2H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 173.9, 79.6, 76.1, 75.0, 74.8, 74.6, 61.1, 51.7, 32.9, 23.9, 23.1, 18.2, 18.0, 11.9, 9.9, 9.8.

2.5.6.3. Third approach

4-chlorobut-2-yn-1-ol (2-72)



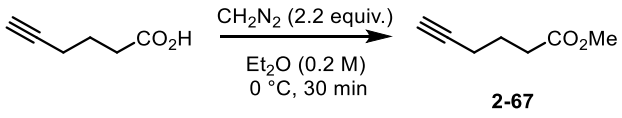
To a solution of but-2-yne-1,4-diol **2-73** (10.0 g, 116 mmol, 1 equiv.) in benzene (11.6 mL, 10 M), dry pyridine (10.3 mL, 128 mmol, 1.1 equiv.) was added. The mixture was cooled to 0 °C and stirred for 5 min before $SOCl_2$ (9.3 mL, 128 mmol, 1.1 equiv.) was added dropwise. The mixture was then allowed to stir overnight at RT. After consumption of the starting material (TLC), the mixture was poured into ice-water mixture (10 g/10 mL). The resulting layers were separated, and the aqueous layer was extracted with EtOAc (3x 50 mL). Combined organic layers were then washed with saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL), dried over $MgSO_4$, filtered, and solvents were removed under reduced pressure. The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 10:1 → 2:1) to afford **2-72** (6.39 g, 53 %).

R_f = 0.23 (petroleum ether/EtOAc 4:1)

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 4.33 (t, J = 3.0 Hz, 1H), 4.18 (dt, *J* = 2.5, 2.0 Hz, 2H), 1.74 (d, *J* = 2.6 Hz, 2H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 84.8, 80.7, 51.2, 30.4.

methyl hex-5-ynoate (2-67)

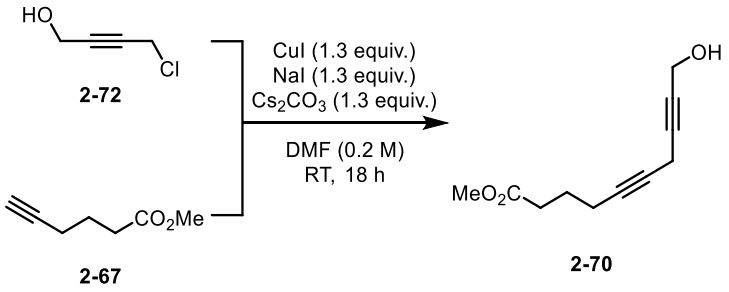


Freshly prepared ether solution of diazomethane [KOH (12 mL, 12 M) was slowly added to *N*-nitroso-*N*-methyl urea (8.4 g, 81.4 mmol, 2.2 equiv.) mixture in Et_2O (164 mL, 0.225 M), and stirred at 0 °C until all *N*-nitroso-*N*-methyl urea dissolves] was added dropwise to 5-hexynoic acid (4.15 g, 37 mmol, 1 equiv.) solution in Et_2O (16.4 mL, 2.25 M) at 0 °C. The resulting mixture was stirred at 0 °C for 1h and the N_2 was bubbled through the solution to remove an excess of the diazomethane (change of color from yellowish to clear). The whole mixture was then concentrated under reduced pressure, and the crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **2-67** (4.7g, 99 %).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 3.68 (s, 3H), 2.46 (t, *J* = 7.4 Hz, 2H), 2.27 (td, *J* = 7.0, 2.7 Hz, 2H), 1.97 (t, *J* = 2.7 Hz, 1H), 1.85 (p, *J* = 7.2 Hz, 2H).

^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$): 173.7, 83.4, 69.3, 51.7, 51.7, 32.8, 23.7, 18.0.

methyl 10-hydroxydeca-5,8-diynoate (2-70)



To a mixture of propargyl chloride **2-72** (2.7 g, 25.8 mmol, 1.0 equiv.) in DMF (52 mL, 0.5 M), NaI (5.08 g, 33.6 mmol, 1.3 equiv.), CuI (6.53 g, 33.6 mmol, 1.3 equiv.), and Cs_2CO_3 (4.69 g, 33.6 mmol, 1.3 equiv.) were sequentially added at RT. After 2 min, methyl hex-5-ynote **2-67** (4.32 g, 33.6 mmol, 1.3 equiv.) was added and the resulting mixture was stirred at RT for 12 h. The whole mixture was then diluted with EtOAc (50 mL), and the resulting mixture was filtered through a pad of Celite®. Resulting filtrate was washed with saturated aq. NH_4Cl (20 mL), and the aqueous layer was back extracted with EtOAc (3x 50 mL). The combined organic phases were washed with brine (50 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **2-70** (3.38 g, 66 %).

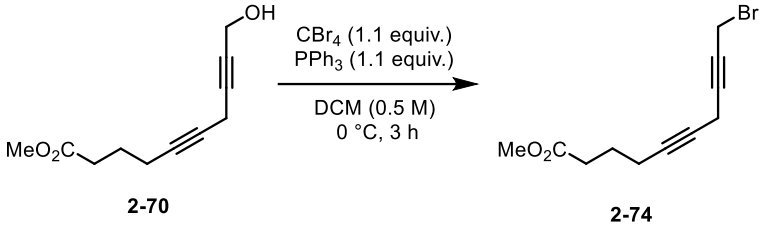
R_f = 0.3 (petroleum ether/EtOAc 7:3)

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 4.26 (t, *J* = 2.4 Hz, 2H), 3.68 (s, 2H), 3.18 (td, *J* = 2.3, 1.2 Hz, 2H), 2.44 (t, *J* = 7.5 Hz, 2H), 2.24 (dddd, *J* = 6.9, 5.7, 3.0, 1.8 Hz, 2H), 1.88 – 1.76 (m, 2H).

^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$): 173.8, 80.7, 79.9, 78.7, 74.6, 51.8, 51.4, 33.0, 23.9, 18.3, 10.0.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{11}H_{14}O_3$ $[M+Na]^+$: 217.0835; found: 217.0834.

methyl 10-bromodeca-5,8-diynoate (2-74)



2-70 **2-74**

Diyne **2-70** (5.07 g, 25.8 mmol, 1.0 equiv.) was dissolved in DCM (25.8 mL, 1 M) and PPh_3 (7.53 g, 28.4 mmol, 1.1 equiv.) and CBr_4 (9.52 g, 28.4 mmol, 1.1 equiv.) were added. After being stirred at 0 °C for 3 h, the mixture was diluted with hexane/EtOAc (1:1, *V*/*V*; 100 mL), and the whole mixture was filtered through a plug of silica. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (petroleum ether/EtOAc = 10:1) to afford **2-74** (6.1 g, 92 %).

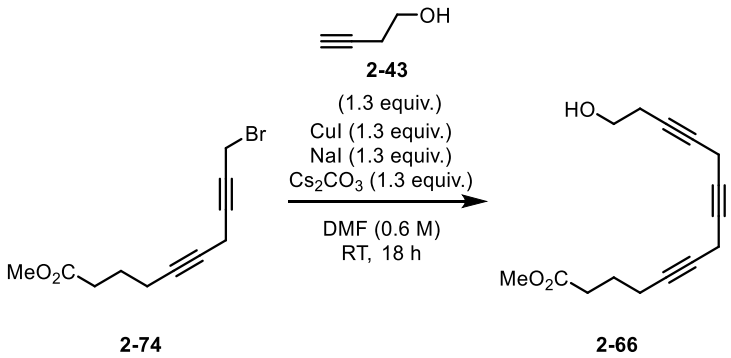
R_f = 0.6 (petroleum ether/EtOAc 7:1)

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 3.92 – 3.90 (m, 2H), 3.68 (s, 3H), 3.22 – 3.18 (m, 2H), 2.43 (t, *J* = 7.4 Hz, 2H), 2.26 – 2.22 (m, 2H), 1.82 (p, *J* = 7.0 Hz, 2H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 173.8, 82.0, 80.2, 75.5, 74.0, 51.7, 33.0, 23.9, 18.3, 14.9, 10.2.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{11}H_{14}O_2Br$: 257.0172; found: 257.0171.

Methyl 14-hydroxytetradeca-5,8,11-triynoate (2-66)



2-74 **2-66**

To a mixture of 3-butyn-1-ol (2.6 mL, 3.33 mmol, 1.3 equiv.), NaI (5.09 g, 3.33 mmol, 1.3 equiv.), CuI (6.47 g, 3.33 mmol, 1.3 equiv.), and Cs_2CO_3 (10.95 g, 33.3 mmol, 1.3 equiv.) in DMF (43 mL, 0.6 M) at RT was added bromide **2-66** (6.7 g, 25.8 mmol, 1.0 equiv.) in DMF (5 mL) at RT. After being stirred overnight at RT, the mixture was diluted with EtOAc (100 mL), and the whole mixture was filtered through a pad of Celite®. Filter cake was rinsed with EtOAc (2x100 mL),

and combined filtrates were washed with saturated aq. NH_4Cl (50 mL). The aqueous layer was back extracted with EtOAc (3x 50 mL), and combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 2:1) to afford **2-66** (5 g, 78 %).

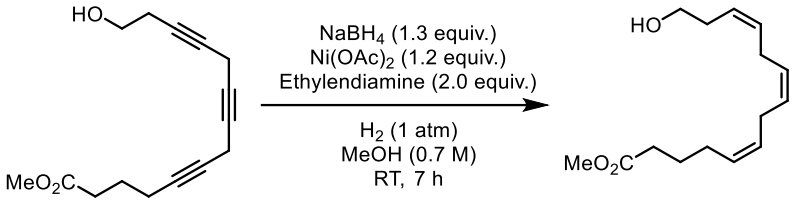
R_f = 0.5 (petroleum ether/EtOAc 1:1)

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 3.74 – 3.69 (m, 2H), 3.68 (s, 3H), 3.21 – 3.07 (m, 4H), 2.44 (*J* = 8.4, 4.8 Hz, 4H), 2.27 – 2.21 (m, 2H), 1.82 (p, *J* = 7.2 Hz, 2H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 173.9, 79.7, 76.3, 75.1, 74.9, 74.6, 61.2, 51.7, 33.0, 24.0, 23.2, 18.3, 10.0, 9.9.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{15}H_{19}O_3$: 247.1329; found: 247.1329.

Methyl (5*Z*,8*Z*,11*Z*)-14-hydroxytetradeca-5,8,11-trienoate (2-57)



2-66 **2-57**

To a solution of $Ni(OAc)_2{\cdot}4H_2O$ (2.47 g, 9.73 mmol, 1.21 equiv.) in MeOH (11.5 mL, 0.75 M) at 0 °C, $NaBH_4$ (403 mg, 10.5 mmol, 1.3 equiv.) was added. The flask was purged with hydrogen, and ethylenediamine (1.61 mL, 10 M, 2 equiv.) was added to the mixture. After 10 min, triyne **2-71** (2.0 g, 8.05 mmol, 1.0 equiv.) in MeOH (2.0 mL) was added. The mixture was stirred at RT for 5 h. Then the mixture was diluted with hexane/EtOAc (1:1, 50 mL), and the whole mixture was filtered through a pad of silica. Filter cake was washed with EtOAc (2x20 mL), and combined filtrates were washed with saturated aq. NH_4Cl (50 mL). The aqueous layer was back extracted with EtOAc (3x 50 mL), and combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 6:1 →2:1) to afford triene **2-57** (1.17 g, 58 %).

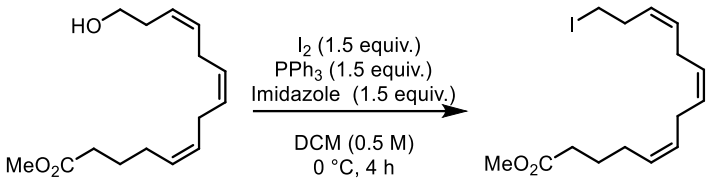
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 5.57 – 5.50 (m, 1H), 5.44 – 5.33 (m, 5H), 3.66 (s, 3H), 2.87 – 2.75 (m, 4H), 2.39 – 2.29 (m, 4H), 2.15 – 2.07 (m, 2H), 1.74 – 1.66 (m, 2H).

$^{13}C\{^{1}H\}$ NMR (126 MHz, Chloroform-d) δ (ppm): 174.3, 131.2, 129.1, 128.9, 128.5, 128.1, 125.8, 62.3, 51.7, 33.6, 31.0, 26.7, 25.9, 25.7, 24.9.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{15}H_{25}O_3$: 253.1798; found: 253.1799.

R_f = 0.5 (petroleum ether/EtOAc 2:1)

methyl (5*Z*,8*Z*,11*Z*)-14-iodotetradeca-5,8,11-trienoate (**2-75**)



2-57 **2-75**

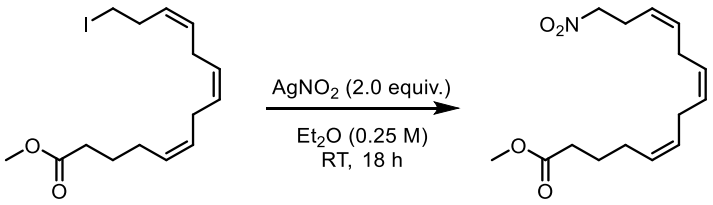
To an ice-cold solution of triene **2-57** (2.42 g, 9.5 mmol, 1 equiv.) in DCM (19 mL, 0.5 M), imidazole (0.91 g, 13.3 mmol, 1.4 equiv.), PPh_3 (3.53 g, 13.3 mmol, 1.4 equiv.), and I_2 (2.93 g, 11.4 mmol, 1.2 equiv.) were sequentially added. After 3 h at 0 °C, the solution was diluted with petroleum ether/ether mixture (4:1, 50 mL), and the whole mixture was filtered through a pad of silica to afford unstable iodide **2-75** (2.25 g, 65 %) that was used immediately in the next step of the synthesis.

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 5.56 – 5.46 (m, 1H), 5.40 – 5.30 (m, 5H), 3.65 (s, 3H), 3.13 (t, *J* = 7.2 Hz, 2H), 2.83 – 2.73 (m, 4H), 2.65 (q, *J* = 7.2 Hz, 2H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.13 – 2.04 (m, 2H), 1.73 – 1.64 (m, 2H).

$^{13}C\{^{1}H\}$ NMR (126 MHz, Chloroform-d) δ (ppm): 174.1, 130.5, 129.1, 128.8, 128.6, 128.4, 127.7, 51.6, 33.5, 31.6, 26.7, 26.6, 25.9, 25.7, 24.8, 5.3.

R_f = 0.65 (hexane/EtOAc 5:1)

methyl (5*Z*,8*Z*,11*Z*)-14-nitrotetradeca-5,8,11-trienoate (**2-56**)



2-75 **2-56**

To a solution of **2-75** (3.3 g, 9.02 mmol, 1.0 equiv.) in Et_2O (36.1 mL, 0.25 M) placed in aluminum foil wrapped flack was at RT added in one portion silver nitrite (2.8 g, 18 mmol, 2.0 equiv.). The resulting mixture was stirred for 12 h before it was filtered through a short pad of Celite®. Filter cake was washed with EtOAc (3x100 mL), and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 10:1) to afford triene **2-56** (1.26 g, 50 %).

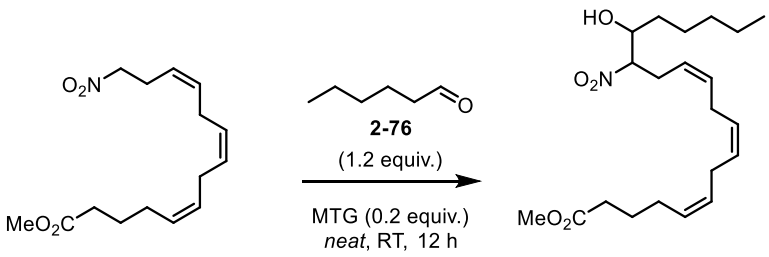
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 5.59 – 5.52 (m, 1H), 5.41 – 5.28 (m, 5H), 4.38 (t, *J* = 7.1 Hz, 2H), 3.65 (s, 3H), 2.85 – 2.80 (m, 2H), 2.80 – 2.74 (m, 4H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.12 – 2.05 (m, 2H), 1.74 – 1.64 (m, 2H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 174.1, 132.7, 129.2, 128.9, 128.7, 127.3, 122.9, 75.0, 51.6, 33.5, 26.6, 25.7, 25.5, 24.8.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{15}H_{24}NO_4$: 282.1700; found:282.1695.

R_f = 0.3 (hexane/EtOAc 10:1)

methyl (5*Z*,8*Z*,11*Z*)-15-hydroxy-14-nitroicosa-5,8,11-trienoate (**2-77**)



2-56 **2-77**

A mixture of nitroalkane **2-56** (1.25 g, 4.4 mmol, 1.0 equiv.) and hexanal **2-76** (0.66 mL, 5.3 mmol, 1.2 equiv.) (neat) was cooled to 0 °C and 1,1,3,3-tetramethylguanidine (113 µl, 0.88 mmol, 0.2 equiv.) was added. Resulting mixture was stirred at RT for 12 h before it was again cooled to 0 °C. H_2O (30 mL) was added and the whole mixture was extracted with EtOAc (3× 50 mL). The combined organic phases were dried over Na_2SO_4, filtered and the solvents were

removed under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 40:1 → 20:1) to afford triene **2-77** (1.2 g, 71 %).

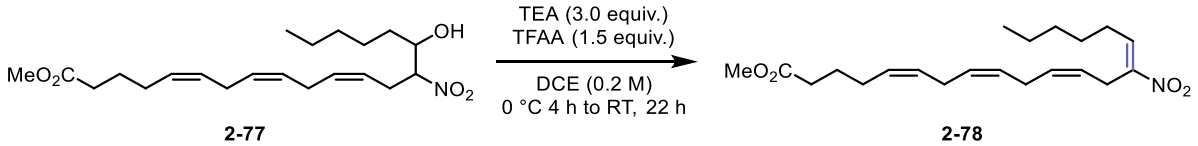
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 5.61 – 5.51 (m, 1H), 5.43 – 5.27 (m, 5H), 4.49 – 4.42 (m, 1H), 4.06 (dq, *J* = 8.7, 4.4 Hz, 1H), 3.92 – 3.84 (m, 1H), 3.67 (s, 3H), 2.91 – 2.77 (m, 4H), 2.64 – 2.59 (m, 1H), 2.35 – 2.29 (m, 3H), 2.14 – 2.09 (m, 2H), 1.71 (p, *J* = 7.5 Hz, 2H), 1.56 – 1.43 (m, 3H), 1.35 – 1.28 (m, 5H), 0.91 – 0.87 (m, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 174.3, 133.2, 129.2, 129.0, 128.8, 127.4, 122.4, 92.0, 71.7, 51.7, 33.8, 33.6, 31.6, 28.7, 26.7, 25.8, 25.7, 25.2, 24.9, 22.6, 14.1.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{21}H_{36}NO_5$: 382.2588; found:382.2587.

R_f = 0.25 (hexane/EtOAc 7:1)

methyl (5*Z*,8*Z*,11*Z*,14*E*)-14-nitroicosa-5,8,11,14-tetraenoate (2-78)



2-77 **2-78**

Adduct **2-77** (1.5 g, 3.9 mmol, 1.0 equiv.) was dissolved in DCE (19.5 mL, 0.2 M) and the resulting mixture was cooled to 0 °C. TEA (0.14 mL, 11.7 mmol, 3.0 equiv.) and TFAA (0.82 mL, 5.84 mmol, 1.5 equiv.) were sequentially added and the resulting reaction mixture was stirred at 0 °C for 4 h and then at RT for 22 h. The whole mixture was cooled to 0 °C, and water (100 mL) was added to terminate the reaction. The resulting mixture was extracted with EtOAc (3× 100 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude product was purified by column flash column chromatography (SiO_2; petroleum ether/EtOAc = 30:1) to afford tetraene **2-78** (0.93 g, 66 %; *E/Z* (newly generated olefinic bond) ≥ 95:5) as a colorless oil.

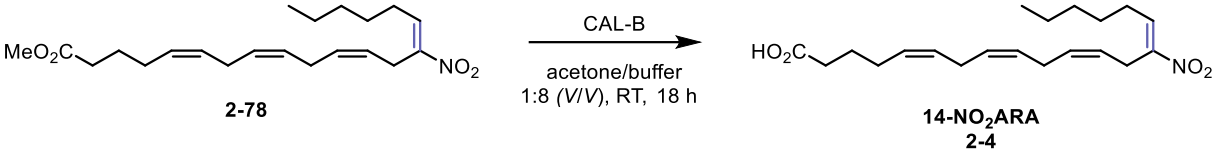
R_f = 0.4 (hexane/EtOAc 5:1)

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.12 (t, *J* = 7.9 Hz, 1H), 5.51 – 5.24 (m, 6H), 3.67 (s, 3H), 3.38 (d, *J* = 6.6 Hz, 2H), 2.93 (t, *J* = 6.5 Hz, 2H), 2.82 (t, *J* = 6.0 Hz, 2H), 2.15 – 2.08 (m, 2H), 1.71 (p, *J* = 7.5 Hz, 2H), 1.54 – 1.48 (m, 2H), 1.36 – 1.30 (m, 4H), 0.92 – 0.89 (m, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 174.2, 137.0, 130.9, 129.2, 128.9, 128.8, 127.6, 124.1, 51.6, 33.6, 31.6, 29.8, 28.3, 28.1, 26.7, 25.9, 25.8, 25.0, 24.9, 22.5, 14.1.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{21}H_{34}NO_4$: 364.2482; found: 364.2479.

(5*Z*,8*Z*,11*Z*,14*E*)-14-nitroicosa-5,8,11,14-tetraenoic acid; 14-nitro arachidonic acid (**14-NO_2ARA**, **2-4**)



CAL-B (1400 mg) was added to a solution of methyl ester **2-78** (880 mg, 0.24 mmol, 1.0 equiv.) in acetone (30 mL, 0.08 M) and aqueous phosphate buffer (240 mL, 0.01 M, pH 7.4). The solution was vigorously stirred (magnetic stirrer, 1000 rpm) at room temperature for 18 h. 1 M HCl was added to terminate the reaction and to adjust the pH of the solution to pH = 3. The whole mixture was then transferred to separatory funnel and layers were separated. Aqueous layer was extracted with EtOAc (5x80 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and the volatiles were removed *in vacuo*. The crude product was purified using semipreparative chromatography (C18 reverse- phase column; MeOH:H_2O) to yield the desired acid **14-NO_2ARA 2-4** (350 mg, 41 %, *E/Z* ≥ 95:5; based on the ^{1}H NMR spectra analysis).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 7.12 (t, *J* = 7.9 Hz, 1H), 5.50 – 5.24 (m, 6H), 3.37 (d, *J* = 6.7 Hz, 2H), 2.93 (t, *J* = 6.6 Hz, 2H), 2.83 (t, *J* = 6.1 Hz, 2H), 2.37 (t, *J* = 7.4 Hz, 2H), 2.25 (q, *J* = 7.6 Hz, 2H), 2.17 – 2.10 (m, 2H), 1.72 (p, *J* = 7.5 Hz, 2H), 1.54 – 1.46 (m, 2H), 1.35 – 1.30 (m, 4H), 0.91 – 0.87 (m, 3H).

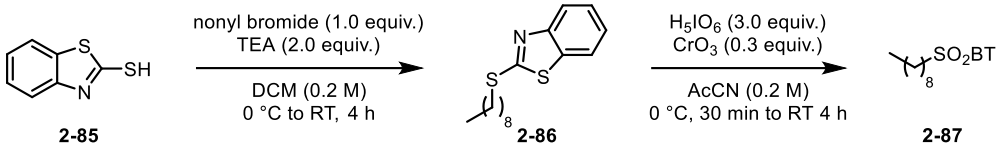
^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 177.9, 150.4, 137.1, 130.9, 129.1, 129.0, 128.9, 127.6, 124.1, 33.1, 31.6, 28.3, 28.1, 26.6, 25.9, 25.8, 25.0, 24.6, 22.5, 14.1.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{20}H_{32}NO_4$: 350.2326; found: 350.2331.

R_f = 0.45 (hexane/EtOAc 2:1)

2.5.7. oleic acid synthesis using modified Julia-Kocienski method

2-(nonylsulfonyl)benzo[*d*]thiazole (**2-87**)



A benzothiazole-2-thiol **2-85** (10.0 g, 60 mmol, 1.0 equiv.) and nonyl bromide (12.4 g, 60 mmol, 1.0 equiv.) were dissolved in DCM (0.2 M, 300 mL) and the mixture was cooled to 0 °C (ice/water, external). TEA (12.1 mL, 120 mmol, 2.0 equiv.) was added dropwise, and the resulting mixture was allowed to warm to RT and stirred for additional 4 hours. 2 M aq. HCl (150 mL) was added to the reaction mixture at RT, and the resulting layers were separated. The aqueous layer was extracted with DCM (3x100mL) and organic layers were combined, washed with water (50 mL), brine (50 mL), dried over $MgSO_4$, and the solvents were evaporated under reduced pressure. The crude product **2-86** was used in the next step without further purification.

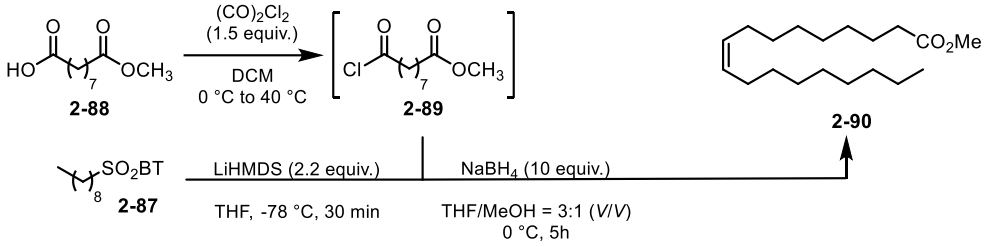
The crude sulfide **2-86** (10 g, 20 mmol, 1.0 equiv.) and periodic acid (13.7 g, 60 mmol, 3.0 equiv.) were dissolved in acetonitrile (0.2 M, 100 mL) and the resulting mixture was cooled to 0 °C. CrO_3 (0.6 g, 6 mmol, 0.3 equiv.) was added portion-wise, and the resulting mixture was stirred for an additional 30 minutes at 0 °C, before being allowed to warm to RT. The resulting mixture was stirred for an additional 4 hours before it was cooled to 0 °C and the reaction was terminated by adding sat. aq. Na_2SO_3 (150 mL). The whole mixture was filtered through a short pad of Celite®. Filter cake was washed with EtOAc (3x50 mL). Resulting layers were separated, and the organic phase was washed with sat. aq. Na_2SO_3 (2x50 mL), water (2x50mL), brine (2x20mL) and dried over $MgSO_4$. Solvents were removed under the reduced pressure and purified with help of column chromatography (SiO_2; petroleum ether:EtOAc = 10:1->4:1->2:1) to yield sulfone **2-87** (11.5 g, 59 % over two steps).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.24 (dd, J = 7.8, 1.5 Hz, 1H), 8.03 (dd, J = 7.7, 1.5 Hz, 1H), 7.66–7.57 (m, 2H), 3.54– 3.49 (m, 2H), 1.90–1.82 (m, 2H), 1.52–1.43 (m, 2H), 1.39-1.29 (m, 10H), 0.93 (t, J = 7.3 Hz, 3H).

^{13}C{^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 166.0, 152.9, 136.9, 128.1, 127.8, 125.6, 122.5, 54.6, 30.9, 27.7, 22.2, 22.1, 22.0, 21.9, 13.6.

MS (ESI) *m/z* (%): 326 $[M+H]^+$.

methyl oleate (2-90)



Oxalyl chloride (804 µL, 10.3 mmol, 1.65 equiv.) was added at RT to an azelaic acid monomethyl ester **2-88** (1.37 g, 6.75 mmol, 1.1 equiv.) and the resulting mixture was gently stirred till the evolution of the gas ceased (25 min). The resulting mixture was then stirred at reflux for additional 2h before it was cooled to RT. The whole mixture was then concentrated under vacuum (excess of $(CO)_2Cl_2$ removal) and the resulting acyl chloride **2-89** was dissolved in dry THF (5 mL) and cooled to -78 °C.

In a separated flask, sulfone **2-87** (2 g, 6.14 mmol; 1.0 equiv.) was dissolved in dry THF (31 mL, 0.2 M) and cooled to −78 °C (acetone/dry ice; external). After 5 minutes, LiHMDS (13.5 mL, 13.5 mmol; 2.2 equiv; 1.0 M solution in THF) was added dropwise over a period of 5 minutes. The reaction mixture turned light orange upon its addition. Previously prepared acyl chloride **2-89** was then added in one shot *via* HPLC Teflon cannula (ø 1.5 mm) at -78 °C.

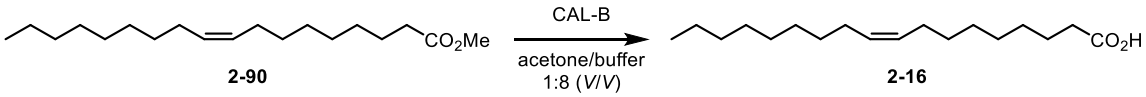
The resulting reaction mixture was allowed to stir at −78 °C for 30 minutes before the cooling bath was removed. The reaction mixture was then allowed to stir at RT for additional 30 min before MeOH (11 mL; THF/MeOH = 3:1 (*V*/*V*)) was added. After additional 5 min at RT, the reaction mixture was cooled to 0 °C and stirred for an additional 5 minutes. $NaBH_4$ (2.32 g, 61.4 mmol, 10 equiv.) was added portion wise at 0 °C and the resulting reaction mixture was stirred at 0 °C for an additional 6 h, before being quenched with 0.5M aq. HCl (40 mL). The resulting mixture was stirred at RT for 10 h. The whole reaction mixture was extracted with EtOAc (4x50 mL), and the organic layers were combined, washed with water (25 mL), brine (50 mL), dried over Na_2SO_4, filtered and solvents were removed under reduce pressure. The crude reaction mixture was purified by flesh column chromatography (SiO_2; petroleum ether:Et_2O = 20:1) and gave the desired product **2-90** (1.02 g, 63%) as a pure (*Z*)-isomer (^{1}H NMR, *E*/*Z* = 1:>99) in form of colorless oil.

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 5.44 – 5.26 (m, 2H), 3.66 (s, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.06 – 1.94 (m, 4H), 1.62 (p, *J* = 7.1 Hz, 2H), 1.28 (q, *J* = 9.9, 7.5 Hz, 21H), 0.88 (t, *J* = 6.7 Hz, 3H).

$^{13}C\{^{1}H\}$ NMR (126 MHz, Chloroform-d) δ (ppm):174.5, 130.1, 129.9, 51.6, 34.2, 32.0, 29.9, 29.8, 29.7, 29.5, 29.3, 29.3, 29.2, 27.4, 27.3, 25.1, 22.8, 14.3.

MS (ESI) *m/z* (%): 297 $[M+H]^+$, 319 $[M+Na]^+$.

oleic acid (2-16)



2-90 **2-16**

CAL-B (70 mg/mmol) was added to a solution of methyl ester **2-90** (0.3 g, 0.88 mmol, 1.0 equiv.) in acetone (11 mL, 0.08 M) and aqueous phosphate buffer (88 mL, 0.01 M, pH 7.4). The solution was vigorously stirred (magnetic stirrer, 1000 rpm) at room temperature for 18 h. 1 M HCl solution was added to adjust the pH of the solution to pH = 3. The whole mixture was then extracted with EtOAc (5x20 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and the volatiles were removed *in vacuo*. The crude product was purified using semipreparative chromatography (C18 reverse-phase column; MeOH:H_2O) to yield the desired acid **oleic acid, 2-16** (0.255g 82%, E/Z = 1:>99; based on the ^{1}H NMR spectra analysis).

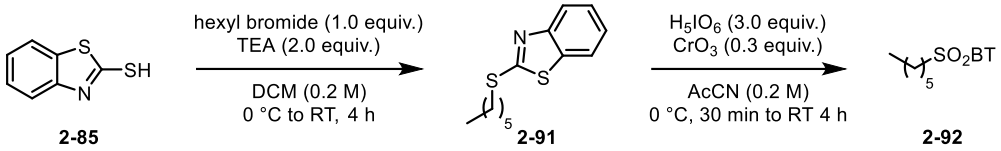
^{1}H NMR (500 MHz, methanol-d_4) δ (ppm):5.40 – 5.30 (m, 2H), 4.90 (bs, 1H), 2.28 (t, *J* = 7.5 Hz, 2H), 2.10 – 2.00 (m, 4H), 1.60 (p, *J* = 7.3 Hz, 2H), 1.43 – 1.25 (m, 20H), 0.90 (t, *J* = 6.8 Hz, 3H).

$^{13}C\{^{1}H\}$ NMR (101 MHz, methanol-d_4) δ (ppm): 177.7, 130.9, 130.8, 35.0, 33.1, 30.9, 30.8, 30.6, 30.5, 30.4, 30.3, 30.3, 30.2, 28.1, 26.1, 23.8, 14.5.

MS (ESI) *m/z* (%): 283 $[M+H]^+$, 305 $[M+Na]^+$.

2.5.8. linoleic acid synthesis using modified Julia-Kocienski method

2-(hexylsulfonyl)benzo[*d*]thiazole (**2-92**)



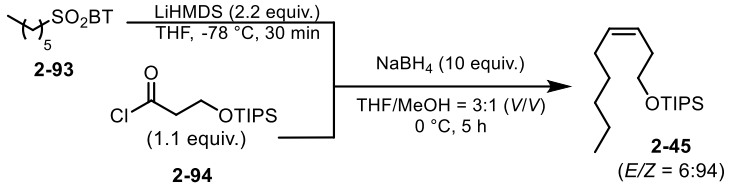
A benzothiazole-2-thiol **2-85** (10.0 g, 60 mmol, 1.0 equiv.) and hexyl bromide (9.87 g, 60 mmol, 1.0 equiv.) were dissolved in DCM (0.2 M, 300 mL) and the mixture was cooled to 0 °C (ice/water, external). TEA (12.1 mL, 120 mmol, 2.0 equiv.) was added dropwise, and the resulting mixture was allowed to warm to RT and stirred for additional 4 hours. 2 M aq. HCl (150 mL) was added to the reaction mixture at RT, and the resulting layers were separated. The aqueous layer was extracted with DCM (3x100mL) and organic layers were combined, washed with water (50 mL), brine (50 mL), dried over $MgSO_4$, and the solvents were evaporated under reduced pressure. The crude product **2-91** was used in the next step without further purification.

The crude sulfide **2-91** (10 g, 20 mmol, 1.0 equiv.) and periodic acid (13.7 g, 60 mmol, 3.0 equiv.) were dissolved in acetonitrile (0.2 M, 100 mL) and the resulting mixture was cooled to 0 °C. CrO_3 (0.6 g, 6 mmol, 0.3 equiv.) was added portion-wise, and the resulting mixture was stirred for an additional 30 minutes at 0 °C, before being allowed to warm to RT. The resulting mixture was stirred for an additional 4 hours before it was cooled to 0 °C and the reaction was terminated by adding sat. aq. Na_2SO_3 (150 mL). The whole mixture was filtered through a short pad of Celite®. Filter cake was washed with EtOAc (3x50 mL). Resulting layers were separated, and the organic phase was washed with sat. aq. Na_2SO_3 (2x50 mL), water (2x50mL), brine (2x20mL) and dried over $MgSO_4$. Solvents were removed under the reduced pressure and purified with help of column chromatography (SiO_2; petroleum ether:EtOAc = 10:1->4:1->2:1) to yield sulfone **2-92** (10 g, 58 % over two steps).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm):8.21 (ddd, J = 7.9, 1.4, 0.8 Hz, 1H), 8.01 (ddd, J = 7.7, 1.4, 0.8 Hz, 1H), 7.63 (ddd, J = 8.5, 7.2, 1.4 Hz, 1H), 7.58 (ddd, J = 8.5, 7.3, 1.6 Hz, 1H), 3.50 (t, J = 8.0 Hz, 2H), 1.87 (q, J = 7.7 Hz, 2H), 1.43 (q, J = 7.5 Hz, 2H), 1.27 (dq, J = 7.1, 3.6 Hz, 4H), 0.85 (t, J = 7.1 Hz, 3H). **^{13}C{^{1}H} NMR (101 MHz, Chloroform-d) δ (ppm):** 166.0, 152.8, 136.9, 128.1, 127.8, 125.6, 122.5, 54.8, 31.2, 28.0, 22.31, 22.28, 14.0.

MS (ESI) *m/z* (%): 284 $[M+H]^+$.

(*Z*)-triisopropyl(non-3-en-1-yloxy)silane (**2-45**)



Sulfone **2-93** (1.5 g, 5.29 mmol; 1.0 equiv.) was dissolved in dry THF (50 mL, 0.1M) and cooled to −78 °C (acetone/dry ice; external). After 5 minutes, LiHMDS (11.65 mL, 11.64 mmol; 2.2 equiv; 1.0 M solution in THF) was added over 2 min period. The reaction mixture turned light orange upon its addition. TiPSO-propionyl chloride **2-94** (1.54 g, 5.82 mmol, 1.1 equiv.) in dry THF (5 mL) was cooled to -78 °C (dry ice/acetone ; extern.) and then added in one shot *via* HPLC Teflon cannula (ø 1.5 mm) at -78 °C to the solution of sulfone 1e. The resulting reaction mixture was allowed to stir at −78 °C for 30 minutes before the cooling bath was removed. The reaction mixture was allowed to stir at RT for 20 min before MeOH (14 mL; THF/MeOH = 3:1 (V/V)) was added. The mixture was cooled to 0 °C and stirred for an additional 5 minutes, before NaBH4 (2.0 g, 53 mmol; 10 equiv.) was added at 0 °C. The resulting reaction mixture was stirred at 0 °C for an additional 4h, before being quenched with 0.5M aq. HCl (50 mL). The resulting mixture was stirred at RT for 4 h. Resulting phases were separated, and the aqueous phase was extracted with EtOAc (3 x 75 mL). Organic layers were combined and washed with water (50 mL), brine (50 mL), dried over Na2SO4, filtered and solvents were removed under reduced pressure. The residue was purified by column chromatography (SiO2; petroleum ether:EtOAc = 200:1->100:1) and yielded the desired product **2-45** (1.3 g, 82%, E/Z = 6:94) in form of colorless oil.

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 5.48 – 5.35 (m, 2H), 3.68 – 3.65 (m, 2H), 2.30 (q, *J* = 7.2 Hz, 2H), 2.04 (q, *J* = 7.3 Hz, 2H), 1.35 – 1.27 (m, 6H), 1.06 (dd, *J* = 5.5, 2.3 Hz, 21H), 0.90 – 0.86 (m, 3H).

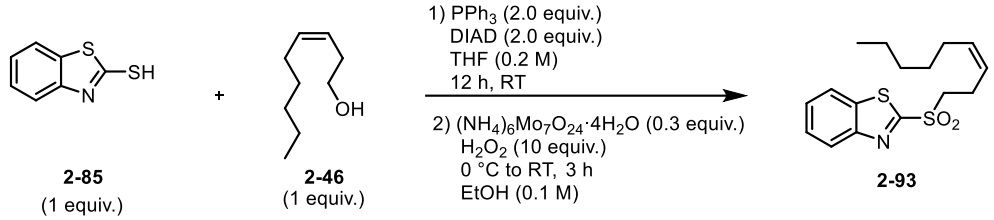
^{13}C{^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 132.0, 125.7, 63.4, 31.7, 31.4, 29.6, 27.5, 22.7, 18.2, 14.2, 12.2.

MS (ESI) *m/z* (%): 300 $[M+H]^+$.

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{18}H_{39}OSi$: 299.2765; found: 299.2768.

R_f = 0.55 (PMA, petroleum ether:EtOAc = 10:1)

(*Z*)-2-(non-3-en-1-ylsulfonyl)benzo[*d*]thiazole (**2-93**)



Alcohol **2-46** (0.128 g, 0.891 mmol, 1.0 equiv.), benzothiazole **2-85** (0.149 g, 0.891 mmol, 1.0 equiv.), and triphenylphosphine (0.467 g, 1.78 mmol, 2.0 equiv.) were dissolved in THF (9.0 mL, 0.1 M) at RT and DIAD (0.354 mL, 1.78 mmol, 2.0 equiv.) was added dropwise. The resulting mixture was allowed to stir at RT for 12 h and solvents were removed under reduced pressure. The residue was dissolved in EtOH (8.9 mL, 0.1 M) and the whole mixture was cooled to 0 °C (ice/water; extern). In separate flask was placed ammonium molybdate (0.334 g, 0.267 mmol, 0.3 equiv.) and H_2O_2 (0.505 mL, 8.91 mmol, 10 equiv.; 50% in H2O) was added to it. The resulting yellow clear solution was cooled to 0 °C and added with help of Pipette Pasteur to the solution of sulfide in EtOH. The resulting mixture was stirred at 0 °C for 3 h (TLC monitoring) before it was diluted with H_2O (20 mL). The resulting mixture was extracted with DCM (3x75 mL) and combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered and solvents were removed under reduced pressure. The residue was purified by column chromatography (SiO_2; petroleum ether:EtOAc = 5:1) and gave the desired sulfone **2-93** (0.265 g, 92%, E/Z = 1:>99) as a slightly yellow oil.

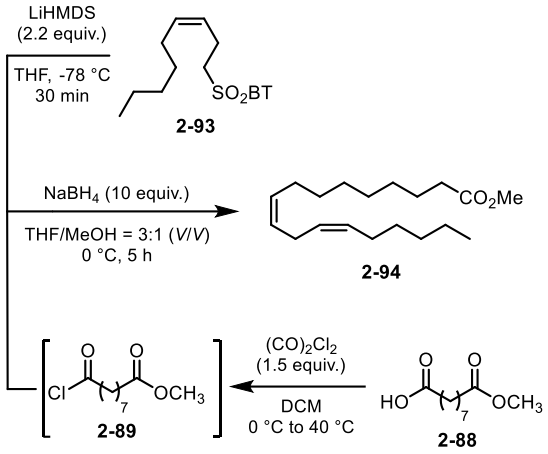
^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.23 (d, J = 8.3 Hz, 1H), 8.04 (d, J = 7.7 Hz, 1H), 7.69 – 7.58 (m, 2H), 5.48 (ddt, J = 10.6, 7.3, 1.0 Hz, 1H), 5.30 (qt, J = 10.5, 7.5, 7.1, 1.8, 0.8 Hz, 1H), 3.61 – 3.52 (m, 2H), 2.64 (q, J = 7.7 Hz, 2H), 1.99 (q, J = 7.2 Hz, 2H), 1.37 – 1.15 (m, 4H), 0.86 (t, J = 7.0 Hz, 3H).

^{13}C{^{1}H} NMR (101 MHz, Chloroform-d) δ (ppm): 166.25, 153.19, 137.24, 134.36, 128.51, 128.15, 125.93, 124.03, 122.82, 54.86, 31.86, 29.48, 27.64, 22.94, 21.02, 14.49.

MS (ESI) *m/z* (%): 324 $[M+H]^+$.

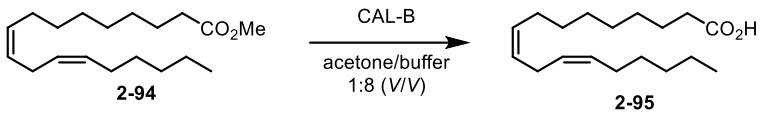
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{16}H_{21}NO_2S_2Na$ $[M+Na]^+$: 346.0906; found: 346.0912.

methyl linoleate (2-94)



Oxalyl chloride (0.583 mL, 6.80 mmol, 1.65 equiv.) was added at RT to an azelaic acid monomethyl ester **2-88** (1.0 g, 4.94 mmol, 1.2 equiv.) and the resulting mixture was gently stirred till the evolution of the gas ceased (25 min). The resulting mixture was then stirred at reflux for additional 2h before it was cooled to RT. The whole mixture was then concentrated under vacuum (excess of $(CO)_2Cl_2$ removal) and the resulting acyl chloride **2-89** was dissolved in dry THF (5 mL) and cooled to -78 °C. In a separated flask, sulfone **2-93** (1.33 g, 4.12 mmol, 1.0 equiv.) was dissolved in dry THF (20 mL, 0.2 M) and cooled to −78 °C (acetone/dry ice; external). After 5 minutes, LiHMDS (9.1 mL, 9.06 mmol; 2.2 equiv.; 1.0 M solution in THF) was added dropwise over a period of 5 minutes. The reaction mixture turned light orange upon its addition. Previously prepared acyl chloride **2-89** was then added in one shot to the resulting yellow-orange solution *via* HPLC Teflon cannula (ø 1.5 mm) at -78 °C. The resulting reaction mixture was allowed to stir at −78 °C for 30 minutes before the cooling bath was removed. The reaction mixture was then allowed to stir at RT for additional 30 min before MeOH (10 mL; THF/MeOH = 3:1 (*V*/*V*)) was added. After additional 5 min at RT, the reaction mixture was cooled to 0 °C and stirred for an additional 5 minutes. $NaBH_4$ (1.56 g, 41.2 mmol, 10 equiv.) was added portion wise at 0 °C and the resulting reaction mixture was stirred at 0 °C for an additional 6h, before being quenched with 0.5M aq. HCl (40 mL). The resulting mixture was stirred at RT for 10 h. The whole reaction mixture was extracted with EtOAc (4x75 mL), and the organic layers were combined, washed with water (50 mL), brine (50 mL), dried over Na2SO4, filtered and solvents were removed under reduced pressure. The crude reaction mixture was purified by column chromatography (SiO2; petroleum ether:EtOAc = 10:1) and gave the desired product **2-94** (0.630 g, 52%, *Z*/*E*:*Z*/*Z* = 1:>99) in form of yellowish oil. **^{1}H NMR (500 MHz, Chloroform-d) δ (ppm):** 5.41 – 5.28 (m, 4H), 3.66 (s, 3H), 2.81 – 2.68 (m, 1H), 2.38 – 2.25 (m, 2H), 2.05 (qd, J = 6.8, 1.3 Hz, 4H), 1.66 – 1.58 (m, 2H), 1.41 – 1.22 (m, 19H), 0.89 (t, J = 7.0 Hz, 3H). **^{13}C{^{1}H} NMR (101 MHz, Chloroform-d) δ (ppm):** 174.49, 130.37, 130.20, 128.18, 128.04, 51.61, 34.25, 31.67, 29.73, 29.49, 29.30, 29.26, 29.24, 27.34, 27.33, 25.76, 25.08, 22.72, 14.23. **MS (ESI) *m/z* (%):** 317 $[M+Na]^+$.

linoleic acid (2-95)



2-94 2-95

CAL-B (70 mg/mmol) was added to a solution of methyl ester **2-94** (0.3 g, 1 mmol, 1.0 equiv.) in acetone (12.7 mL, 0.08 M) and aqueous phosphate buffer (102 mL, 0.01 M, pH 7.4). The solution was vigorously stirred (magnetic stirrer, 1000 rpm) at room temperature for 18 h. 1 M HCl solution was added to adjust the pH of the solution to pH = 3. The whole mixture was then extracted with EtOAc (5x25 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and the volatiles were removed *in vacuo*. The crude product was purified using semipreparative chromatography (C18 reverse-phase column; MeOH:H_2O) to yield the desired acid **linoleic acid, 2-95** (0.22 g 75%, *E*/*Z* = 1:>99; based on the ^1H NMR spectra analysis).

$^{13}C\{^1H\}$ NMR (101 MHz, methanol-d_4) δ (ppm): 5.52 – 5.26 (m, 4H), 4.90 (bs, 1H) 2.78 (dd, J = 6.7, 5.5 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 2.13 – 2.03 (m, 4H), 1.68 – 1.54 (m, 2H), 1.43 – 1.25 (m, 14H), 0.94 – 0.85 (m, 3H).

$^{13}C\{^1H\}$ NMR (101 MHz, methanol-d_4) δ (ppm): 177.72, 130.93, 130.87, 129.11, 129.06, 34.96, 32.69, 30.71, 30.50, 30.33, 30.24, 28.18, 28.15, 26.54, 26.10, 23.65, 14.44.

MS (ESI) *m/z* (%):303 $[M+Na]^+$.

CHAPTER III

3. Sulfonamides

3.1. Introduction to Sulfonamides

Sulfonamides, also known under the name 'sulfa drugs', are a class of compounds that contain the R^1SO_2-NH_2 moiety that can vary in nitrogen atom substitution. For this reason, sulfonamides are further divided into three subcategories – non-substituted, primary and secondary sulfonamides (**Figure 39**).

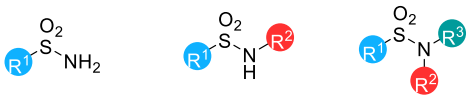


Figure 39: General structure of primary (left), secondary (middle) and tertiary (right) sulfonamides.

3.1.1. Biological activity of sulfonamides

Compounds that include a sulfonamide motive within their structure possess huge biological activities, such as antibacterial[164], oral hypoglycemic[165], antitumor[166], antiviral[164,167], antiepileptic[168], antihypertensive[169], antiprotozoal[170], antifungal[171], anticancer[164,167], anti-inflammatory[172], diuretic[173], activity against Alzheimer's [174] and Parkinson's disease [175], anti-inflammatory Activity[176] etc.

'Sulfa drugs' as antibacterial agents were first mentioned in the 1930s and in 1939 the Nobel prize was awarded to Gerhard Domagk for the discovery of the antibacterial effect of Prontosil, which bears a structure without substituted sulfonamide. Since then, these substances have gained popularity in the field of medicinal chemistry. However, eventually bacteria started to develop resistance to sulfonamides and penicillin replaced the use Prontosil as a first-line treatment.[177] While antibiotic resistance is an issue, sulfonamides are still commonly used to treat a variety of bacterial infections. The most widely used sulfonamides are, for example, celecoxib, zonisamide, and sulfasarazine which belong to primary sulfonamides. Celecoxib as a member of primary sulfonamides acts as a COX-2 inhibitor, which is a group of compounds that acts as a non-steroidal anti-inflammatory drug (NSAID).[178] NSAIDs are usually used to treat pain and inflamation.[178] Celecoxib is the 98th most prescribed medication in the United States. In 2020, zonisamide was the 278th most prescribed medication for the treatment of epilepsy and Parkinson's disease.[179] Sulfasarazine belongs to the family of secondary sulfonamides and is used against Crohn's disease and rheumatoid

arthritis.[180] Glyburide antidiabetic properties and therefore is used to treat type 2 diabetes[181] One of the few examples of tertiary sulfonamides is darunavir, which has been approved for the treatment of HIV infection as part of highly active antiretroviral therapy, in combination with other anti-HIV agents (**Figure 40**).[182]

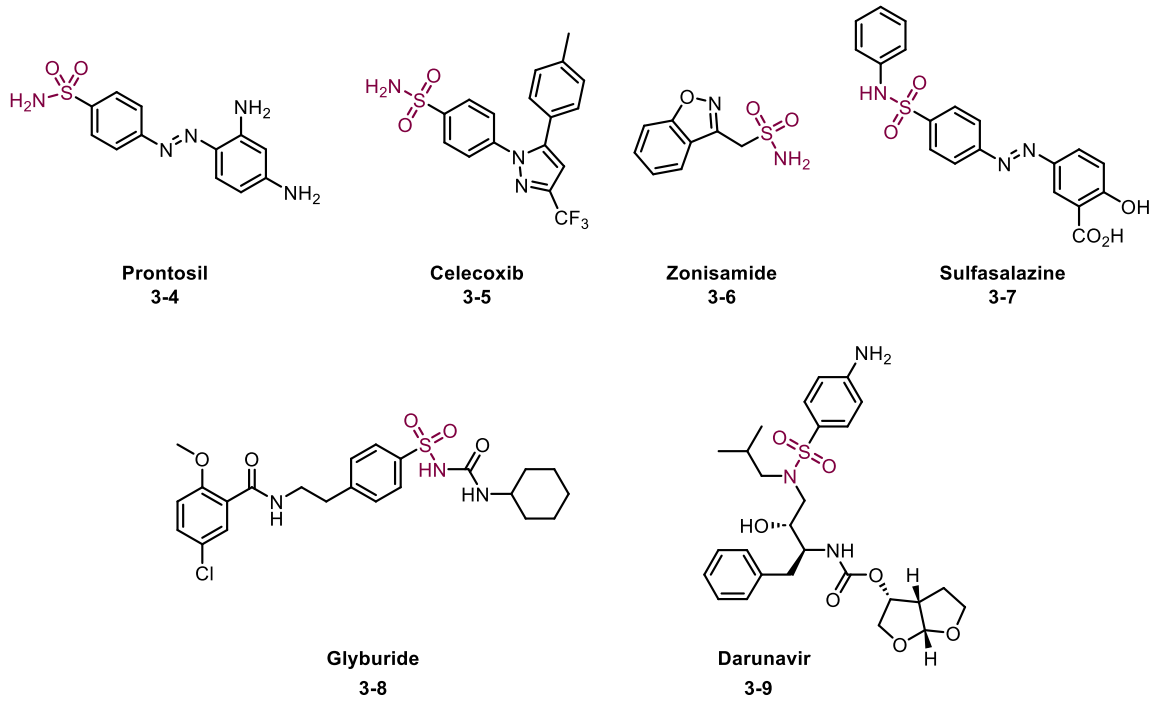


Figure 40: Structure of sulfonamides used as drugs.

Thanks to their extensive biological effects, sulfonamides are being revived and have recently become a point of interest for medicinal chemists, as new ways of using them in the fight against various types of infection have been discovered. Overall, sulfonamides remain an important group of drugs in the fight against infectious diseases and their research and development will continue in the future.

3.2. General approaches towards sulfonamides

The most used synthetic routes to sulfonamides have traditionally been divided into three approaches. The first approach involved the use of sulfonyl chlorides, which can be obtained by chlorosulfonylation of arenes,[183] sulfochlorination of alkyl chains,[184] or oxidation[185]/chlorination[186] of thiols (**Figure 41A**). However, these methods have certain limitations, including the low thermal stability of (heteroaryl)sulfonyl chlorides and relatively limited functional group tolerance.[187] These limitations led to the second approach, which used sulfinic acid salts (ester hydrolysis),[188] or by sulfonylation using DABSO (**Figure 41B**).[189] The third most common alternative is a well-known SuFEx approach. (**Figure 41C**). The last approach, which is newly added and rarely used, relies on the conversion of thiols into sulfenamides, which are subsequently oxidized to form sulfonamides. The drawback of this oxidation of sulfenamides to sulfonamides are low yields (**Figure 41D**).[190,191]

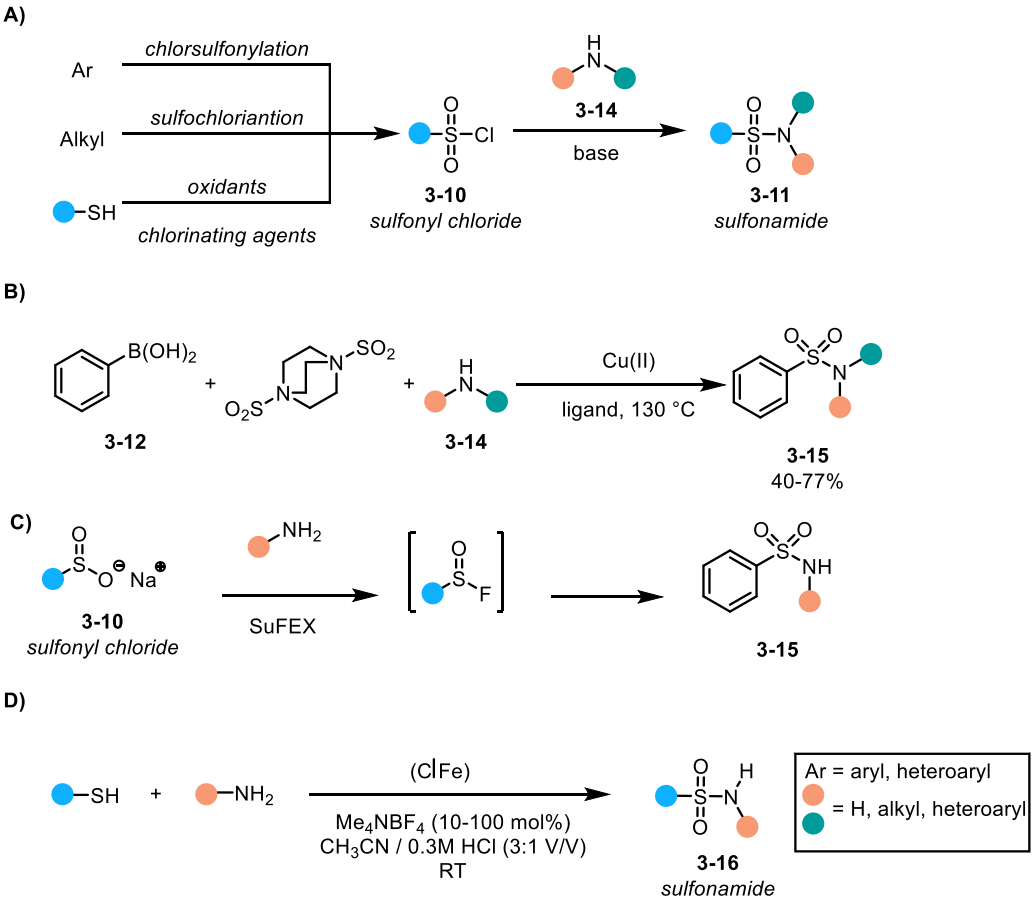
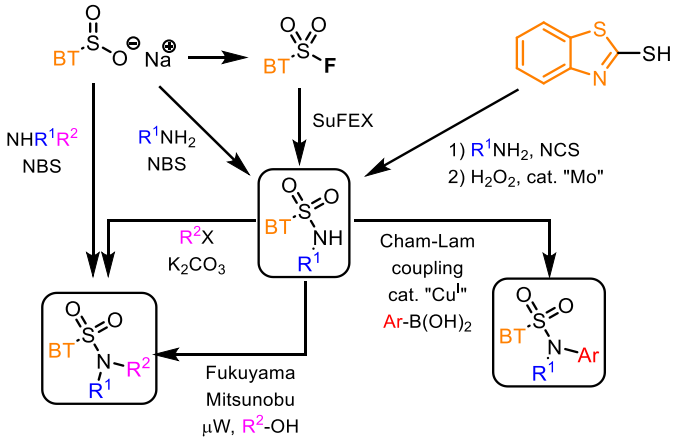


Figure 41: Approaches towards sulfonamides. **A**) Classical approach to sulfonamides. **B**) DABSO-based approach. **C**) SuFEX approach. **D**) Electrochemical oxidative coupling.

During recent years, our research group focused on the chemistry of **heteroaryl sulfonamides**.[187,192] While the above-mentioned methods are generally effective for the synthesis of alkyl and aryl sulfonamides, they present challenges when applied to heteroaryl sulfonamides.[187] These challenges include the instability of reaction intermediates, the need for harsh reaction conditions, and low reaction yields.[193–195] Therefore, our group focused on developing a method that would eliminate these challenges related to the synthesis of *N*-monoalkyl, *N,N*-dialkyl, and *N,N*-alkyl aryl benzothiazole sulfonamides. After a huge amount of time and efforts, a unified approach to *N*-substituted and *N,N*-disubstituted benzothiazole (BT) sulfonamides was developed by Zálešák et al.[187] It was demonstrated that various substrates and sulfonamides or their precursors need to be prepared using various synthetic strategies. The only criterion was that the developed methods are based on readily available building blocks that should be further interconnected through various strategies, including **1**) S-oxidation/S-N coupling with primary and secondary amines, **2**) S-N coupling /S-oxidation sequence, and **3**) S-oxidation/S-F bond formation/SuFEx approach. These three mentioned methods allowed the synthesis of a basic common intermediate that could be further functionalized to *N,N*-disubstituted BT-sulfonamides by base-promoted alkylation with alkyl halides and/or stereospecific Fukuyama Mitsunobu alkylation by reaction with alkyl alcohol. The aryl substituents were installed using the Cham-Lam coupling reaction. (**Scheme 67**).



Scheme 67: Overview of the general routes to BT sulfonamides (adapted from[187]).

3.3. Amino acids

The second class of compounds that I will discuss in my theses are amino acids **3-19** (AA) (**Figure 42**). Along with its non-disputable function in living organisms, where they serve as building blocks of proteins and play crucial roles in various biological processes, AAs are also common, structurally very useful, and chiral chemical pools for organic synthesis.

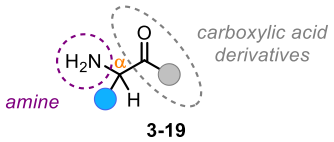


Figure 42: General structure of α-amino acids.

As mentioned above, AAs are essential for the structure and function of enzymes, receptors, and signaling molecules, as well as fundamental building blocks in biology (more than 500 AAs have been identified in nature).[196,197] The name comes from the fact that all AAs contain at least one amine and one carboxylic acid moiety, which makes them attractive building blocks. The presence of these functional groups results in relatively high hydrophilicity, a property that is currently exploited for the enhancement of drug delivery. The incorporation of AAs in drugs proved to improve the biopharmaceutical properties of targeted molecules such as permeability, stability, and solubility.[198]

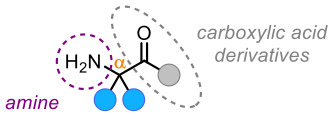
From a biosynthetic point of view, the biosynthesis of AAs is a complex process that involves numerous enzymatic steps. All 20 naturally occurring AAs that can be easily found in proteins are α-amino acids that differ with the side chains. And this difference strongly influences the inherent properties of AAs as well as of proteins and/or other compounds that are generated starting from them. [196,199]

The unique properties of AAs are explored in a wide range of applications across several scientific fields, from organic synthesis and biochemistry to material science. One of the reasons why they are so commonly used is that there is a desire to “escape from flatland”.[200] A concept that answers the search of the pharmaceutical industry for better drug and candidate selectivity, the concept of searching for compounds that selectively interact only with the chosen receptor, the target place of biological action. The situation is favored using drug candidates with several chiral centers, which allows for better selectivity of the drug candidate.

For this and other reasons, numerous approaches for amino acid synthesis were developed and are now available as a chemical tool for organist chemists. Such tools vary in many ways, and they range from enzymatic applications,[201] *via* transition-metal catalysis,[202] organocatalysis,[203] to radical chemistry-based[204] methods. And still there are many novel and modern synthetic ways that are waiting to be disclosed and added to the tool of modern organic synthetic chemist. Such methods aim to synthesize specific structural motives that include AAs or to develop a synthesis of previously unknown AAs with the goal of adding new synthetic possibilities and previously unknown building blocks to the hands of medicinal chemists.

3.4. α,α-disubstituted-α-amino-acids

The most interesting and for the moment developed motive in AA chemistry is the synthesis of enantioenriched α,α-disubstituted α-AAs **3-20**. All these compounds are non-proteinogenic AAs, since the α-carbon atom next to the carboxylic function does not have a hydrogen atom and instead another alkyl substituent is placed (**Figure 43**). The 'double' substitution of the α position gives special properties to the AAs. For example, double substitution blocks/changes the way proteins fold, blocks active centers of proteins, and is resistant to oxidation, etc. In the case of plants, the cyclic form of the disubstituted AAs (which contain a cyclopropane scaffold in the building block) serves as a source of ethylene, a plant signaling molecule.



3-20

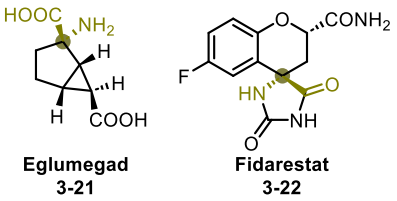
Figure 43: General structure of α,α-disubstituted α-AAs.

Interestingly, such a type of skeleton (α,α-disubstituted AA) is present in many natural products, as well as in synthetic drugs since recently. It is not surprising that the pharmaceutical industry is paying much more attention to the development of new synthetic routes for novel disubstituted AAs. The new type of structure, when incorporated into advanced molecular scaffolds, could further increase, for example, biostability, rigidity, increase biopharmaceutical properties, or allow for better selectivity of new or already existing drugs.[205–207] However, preparation of a carbon atom with four distinct non-hydrogen

substituents remains a synthetic challenge, where the steric hindrance generated between the substituents is one of them. In general, the synthesis of the quaternary homochiral stereogenic center requires a multistep approach with several functional group interconversions.

3.4.1. Biological activity of α,α-disubstituted α-AAs.

A few examples of biologically active synthetic molecules are mentioned in **Figure 44**. Eglumegad **3-21**, also known as LY-354740, is a compound developed by Eli Lilly and Company, which acts as a group-selective agonist for group II metabotropic glutamate receptors and is being investigated in the treatment of anxiety and drug addiction.[208] Another example can be fidarestat **3-22**, also known as SNK-860. Compound **3-22** belongs to the group of hydantoin heterocycles that are also known to be an aldose reductase inhibitor. This compound was in advanced Phase III clinical studies for the treatment of diabetic peripheral neuropathy; however, the study was discontinued in 2006.[209]



Eglumegad 3-21 **Fidarestat 3-22**

Figure 44: Examples of synthetic biologically active α,α-disubstituted αAA.[208,209]

Naturally occurring biologically active α,α-disubstituted α-AAs are more common that we think (**Figure 45**). One of the examples is **sphingofungin E 3-26** that was isolated from a fermentation of *Paecilomyces variotii*. This ingosine-like compound inhibits serine palmitoyl transferase, an enzyme essential in the biosynthesis of sphingolipids, with an IC_{50} of 7.2 nM.[210] **Lactacystin 3-23** and its cell-permeable β-lactone form, **omuralide 3-24**, are both compounds naturally synthesized by bacteria of the genus *Streptomyces lactacystenaeus*, first identified in 1991. Both have selective and potent irreversible inhibitory effects on the 20S proteasome, a large polymolecular protein that is responsible for the degradation of ubiquitin-labeled proteins.[206,211] **Coronatine 3-25** is a phytotoxin produced by *Pseudomanas syringae*, which is involved in chlorosis in leaves and that forces the stomata to reopen after closing in response to pathogen-associated molecular patterns.[212]

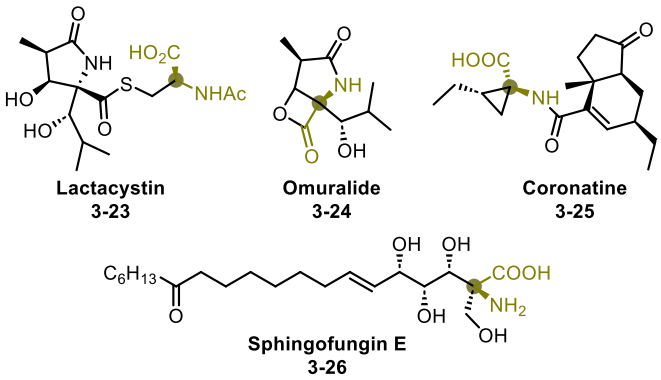
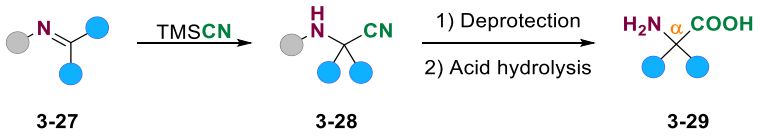


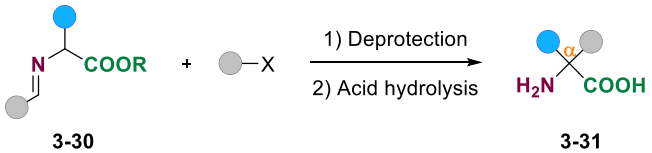
Figure 45: Examples of naturally occurring biologically active α,α-disubstituted αAA. [206,210–212]

3.4.2. Known methodologies of α,α-disubstituted αAA and their derivatives synthesis

Over the past few decades, numerous methods for the selective and enantioselective synthesis of α,α-disubstituted αAAs and their derivatives have been developed. The methods were developed with the aim to prepare natural products and other biologically active compounds. In general, developed methods require several steps (5-7 steps), to achieve the α,α-disubstituted AAs, and therefore the overall yields of the synthetic sequence are usually low.[213] Oldest, but still employed synthetic strategy that allows for α,α-disubstituted AA synthesis was described already in 1850. The method is called the Strecker synthesis and its asymmetric versions are still commonly used (**Scheme 68**).[213] Similarly, a bit younger coupling of Schiff-base enolates with various electrophiles derives α-amino esters (**Scheme 69**).[214,215]



Scheme 68. Strecker synthesis of α,α-disubstituted αAA.[213]

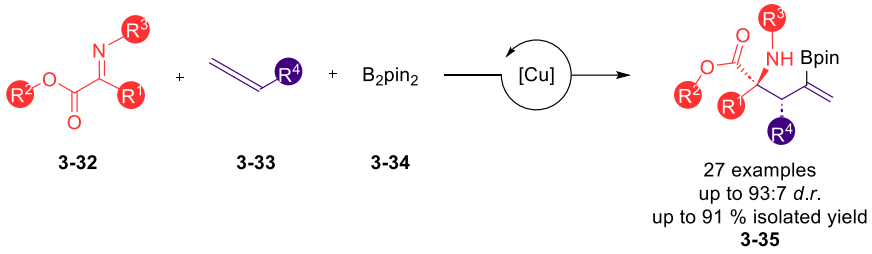


Scheme 69: Schiff-base synthesis of α,α-disubstituted αAA. [214,215]

The aim of this chapter is to briefly summarize the most important and recent methods employed in the synthesis of α,α-disubstituted α-AAs and its derivatives with a primary focus on methods developed in the past decade. These strategies include the formation of C-C

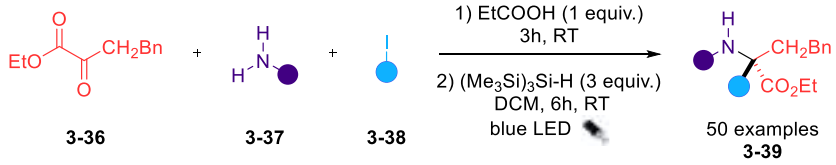
bonds using synergistic catalysis through common Cu/Pd, Ni/Cu and other cooperative systems to achieve the enantioselectivity[216–219], α-functionalization of general α-AAs (especially arylation and alkenylation)[220–222], direct α-C-H bond functionalization of α-amino acid[223] or hydrocarboxylation of amines or imines.[224]

In 2019, the Proctet group developed a copper-catalyzed borylative allylation conditions that allowed the modification of ketiminoesters **3-32** with allenes **3-33** and bis(pinacolato)diborane **3-34** and produced quaternary α-amino esters **3-35** (**Scheme 70**).[225]



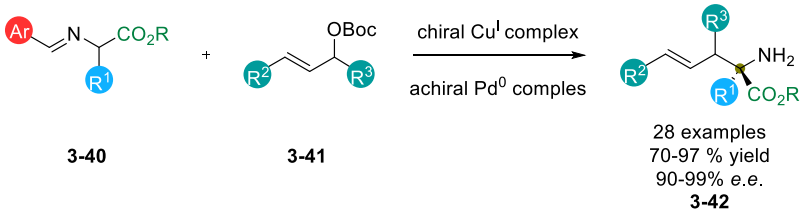
Scheme 70: Copper-catalyzed borylative allylation of ketiminoesters.[225]

In 2022, Gaunt group developed a protocol for the synthesis of α-tertiary amino esters **3-39** starting from primary alkylamine **3-37**, alkyl α-ketoesters **3-36**, and 1°, 2° or 3° alkyl iodides **3-38**. In this one-pot protocol, a broad scope of adducts could be prepared (**Scheme 71**).[226]



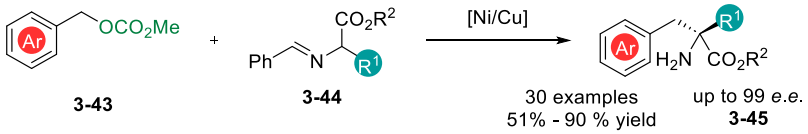
Scheme 71: Synthesis of α-tertiary amino ester *via* photoredox process.[226]

In 2017, Wang et al. described the enantioselective α-allylic alkylation of readily available aldimine esters **3-40** catalyzed by a synergistic Cu^{I}/Pd^{0} catalyst system to produce nonproteinogenic α,α-disubstituted αAAs **3-42** in high yields and with excellent enantioselectivity (**Scheme 72**).[216]



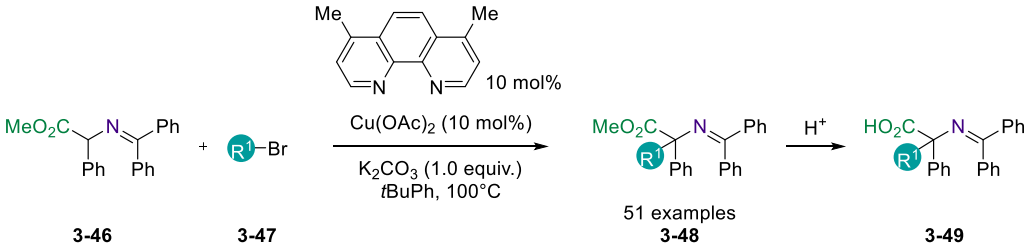
Scheme 72: Cu^{I}/Pd^{0} cooperative catalysis for enantioselective alkylation of aldimine esters.[216]

More recently, in 2022, Zhang's group reported the first synergistic asymmetric Ni/Cu-catalyzed benzylic alkylation of aldimine esters **3-44** in high yields and enantioselectivities (**Scheme 73**).[217]



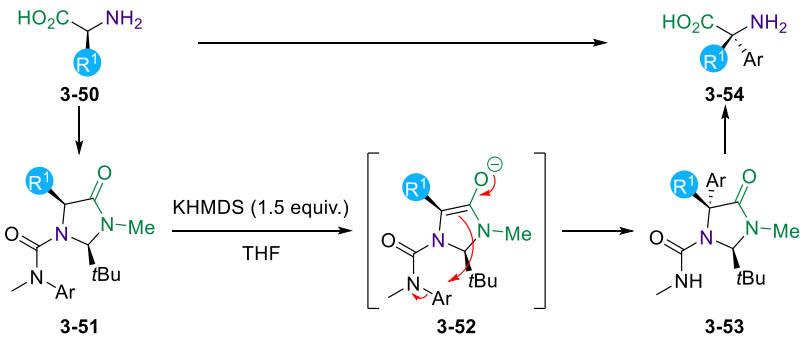
Scheme 73: Ni/Cu-catalysed benzylic alkylation of prochiral aldimine esters.[217]

In 2020, the Ohshima group developed a method that was different from traditional systems that used Schiff bases. In previous examples, Schiff bases generally reacted with primary and very limited secondary alkyl halides **3-47**. In this work, α,α-disubstituted α-amino acid derivatives are prepared in moderate to high yields by radical-radical coupling of two radical species that are obtained through a one-electron process. α,α-disubstituted α-AAs **3-49** are then obtained by acid hydrolysis (**Scheme 74**).[227]



Scheme 74: Amino acid Schiff bases merging with copper catalysis. [227]

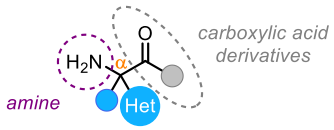
The Clayden Group reported in 2018 a construction of α,α-disubstituted α-AAs **3-54** *via* the α-arylation process where generated *N*-aryl ureas from AAs are converted to imidazolidinones **3-51**. Upon treatment with a base, the enolate **3-52** is formed in which the aromatic substituent of urea migrates stereoselectively, and the migration is directed by the bulky *tert*-butyl group. Hydrolysis of the product provides the quaternary α-aryl amino acids **3-54** (**Scheme 75**).[228]



Scheme 75: Metal-free stereoselective arylation of amino acids.[228]

3.5. α-heteroaryl α-substituted α-AAs

In our group, during our quest for heteroarylsulfonamides, we became interested in α-heteroaryl α-substituted α-amino acids (HAA), compounds that have proved to be virtually non-existing in chemical space of our universe. Indeed, only a very detailed literature search can reveal a few existing examples of molecules that would embody such a molecular scaffold. We are talking about HAAs with a heterocycle with at least two heteroatoms. From our point of view, such a molecular scaffold was very promising, since it combined the structure and functional potential of α,α-disubstituted α-AAs (used in the construction of non-natural peptides and proteins) and the power of the polyheteroatom containing heteroaryl structural motives (crucial for many drugs and drug-like molecules). As mentioned in previous chapters, a prominent subgroup of α-AAs are α,α-disubstituted α-AAs, AAs with unique pharmacological and biological capabilities. The heteroaryl group present in the α position, should even improve the unique chemical and biological properties already known for "simple" α,α-disubstituted α-AAs (**Figure 46**).



3-55

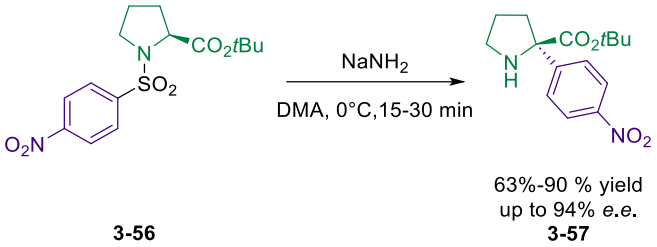
Figure 46: General structure of α-heteroaryl α-substituted α-AAs (HAA).

As mentioned above and to our knowledge, there are only few examples of ααHAAs in the literature[229–232], and in most cases, the heteroaryl moiety of HAA is restricted to the pyridine ring and/or HAA is formed as an unwanted side product or is present in racemic form. Since such compounds are mostly unknown, we reasoned that the development of a robust and general route to homochiral HAAs is of interest to synthetic chemists (development of a novel synthetic method potentially broadly applicable), medicinal, and material (new building blocks with potentially interesting properties).

3.5.1. Synthesis of α-heteroaryl α-substituted α-amino acid derivatives

As we could see in Chapter 3.4.2, various synthetic approaches to α,α-disubstituted α-AAs were developed, but only few were reported for ααHAAs. One of them is the reaction of proline-modified 4-NO_2phenyl sulfonamide **3-56**, which upon treatment with $NaNH_2$

undergoes Smiles rearrangement and yields α-arylated proline derivative **3-57** in good yield and enantioselectivity (**Scheme 76**). Unfortunately, it was shown that the reaction conditions are limited to proline AA and the phenyl ring must contain an electron withdrawing group (EWG) in the *para* position of the phenyl ring and thus are limited to mono and dinitrophenyl sulfonamides).[233]



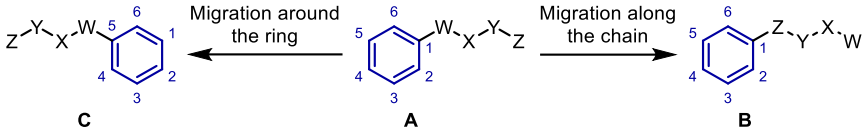
3-56 **3-57**

Scheme 76: Enantioselective rearrangement of proline sulfonamides.[233]

The reaction was first reported and further developed by Foschi et al.[233] and it is one of the examples where the stereoselectivity of the reaction is dictated by the concept of a **Memory of Chirality**. In detail, the chirality of the modified stereogenic center is maintained only due to the fast intramolecular **Smiles rearrangement** step. The presence of the EWG group(s) on the phenyl ring is important for the stabilization of the polar Meisenheimer complex intermediate. In the next two subchapters a concept of **Smiles rearrangement** and concept of **Memory of chirality**, which are relevant for the results and discussion of chapter 3.8, and therefore will be described in detail on the following pages.

3.6. Truce Smiles rearrangement

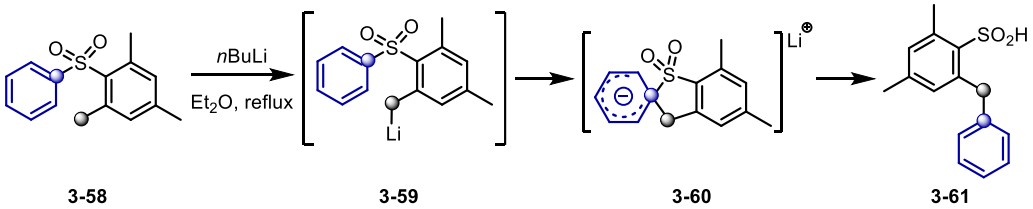
Arguably, one of the most efficient and elegant ways to functionalize aromatic rings involves selective migration of the aryl moiety (rearrangement reactions). A rearrangement changes the attachment point between the arene ring and the atom it was originally connected to, and it is thus an inherently high atom economy. Starting with a relatively simple substrate, rearrangement reactions facilitate the creation of more complex molecules. Additionally, these reactions often achieve selectivity that their intermolecular counterparts cannot, thanks to increased reactivity resulting from a lower entropy of activation. [234] These reactions involving arenes can be classified into two families: migration along the ring (**A** to **C**, e.g., Claisen rearrangement[235]) and migration along the chain (**A** to **B**, e.g., Smiles rearrangement[236], **Scheme 77**)



Scheme 77: Aryl migration strategies.

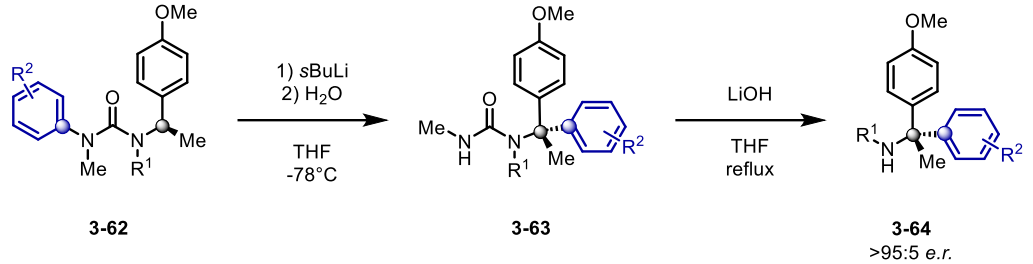
The Smiles rearrangement belongs to the migration along the chain family of rearrangements. This process has been widely studied and applied in a variety of fields, including drug discovery, materials science, and natural product synthesis.[234,237–240] One important aspect of Smiles rearrangement is the role of reaction conditions in controlling the outcome of the reaction. The reaction can proceed under acidic or basic conditions, and the choice of conditions can have a significant impact on the selectivity and efficiency of the reaction. For example, in some cases, the use of Lewis acids can improve the yield of the reaction and enhance the stereoselectivity, while in other cases Lewis acids can lead to undesired side reactions and reduced selectivity.[240] The choice of solvent can also affect the outcome of the reaction, with some solvents promoting faster reaction rates and others promoting greater selectivity. The mechanism of Smiles rearrangement has been the subject of much study and debate. Although the basic principles of the reaction are well established, the details of the reaction mechanism can vary depending on the specific reaction conditions and the structure of the starting material. Seminal base-catalyzed example of the Polar Truce-Smiles rearrangement mechanism is described in **Scheme 78**. In this example, a carbon-based nucleophile **3-59** is formed by lithiation of mesitylphenylsulfone **3-58**. Intermediate **3-59**

rearranges through the formation of a polar Meisenheimer spirocyclic intermediate **3-60**, delivering the corresponding lithium sulfonate. After protonation, the corresponding sulfinic acid **3-61** is obtained.[234]



Scheme 78: Typical tandem Polar Truce–Smiles rearrangement.[240]

One of the pioneering works in asymmetric Truce-smiles rearrangement was reported by Clayden et al.[233] where they prepared substituted diarylmethylamines **3-64** through the stereospecific intramolecular electrophilic aryl migration of chiral ureas **3-62** (**Scheme 79**).



Scheme 79: Clayden asymmetric Smiles rearrangement.[241]

Overall, Smiles rearrangement is a powerful tool in organic synthesis with a wide range of applications in drug discovery, materials science, and natural product synthesis. The reaction can be used to create complex molecules from simpler building blocks, and the ability to control the stereochemistry of the reaction makes it a valuable tool in the synthesis of chiral compounds and natural products.

3.7. Memory of chirality

Memory of chirality[242] is a concept introduced by Kawabata nearly 40 years ago that describes the 'conservation' of chirality in the case of reactions that proceed directly on the stereogenic center of the substrate (**Figure 47**). In short, the stereogenic center, for example on the sp^3 carbon atom, is destroyed (e.g., transformed to enolate), however, the original chirality of the starting material is 'conserved' ('memorized' / retained in the form of dynamic sp^2 chirality) and 'recuperated' by the reaction with the external reagent, most commonly electrophile. Memorization in this context means converting *the point chirality* (typically around the carbon stereocenter) to *the dynamic chirality* (typically through the "frozen" conformation to the 'special' chirality, chirality of the environment) and then recuperating back from the *dynamic chirality* to the *point chirality* (typically back to the carbon-based stereogenic center) chirality. During the process, either retention or inversion of chirality might occur.[243]

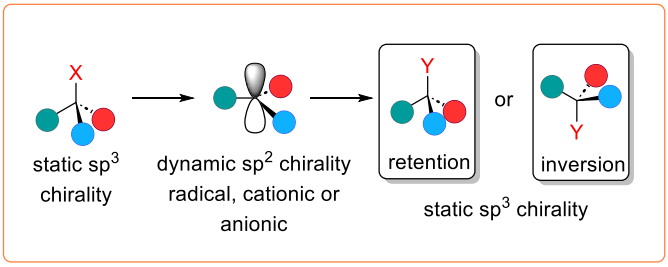
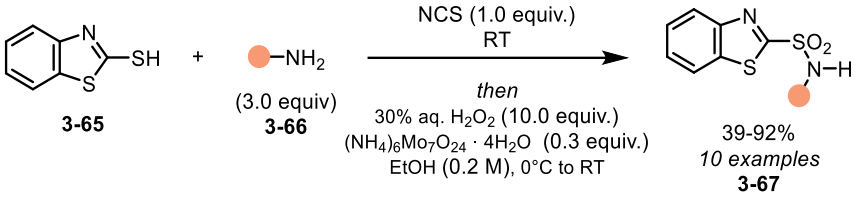


Figure 47: Concept of "Memory of chirality". Chirality is transferred from enantioenriched starting material to enantioenriched product.[243]

3.8. Results and discussion[#]

3.8.1. Introduction to sulfonamides synthesis

Over the past decade, we have been interested in exploring new ways to sulfonamides. As mentioned in Chapter 3.2 our group has developed a unified approach towards *N*-substituted and *N,N*-disubstituted BT sulfonamides (**Scheme 67**). From the newly developed routes to BT-sulfonamides, especially those based on the sulfenamide precursor gained much interest within our group due to its operational simplicity and high yield (**Scheme 80**). The problem with the method was that its scope and limitations were not fully disclosed.



Scheme 80: Approaches towards *N*-substituted benzo[*d*]thiazole-2-yl sulfonamides from benzo[*d*]thiazole-2-thiol and alkyl amines.

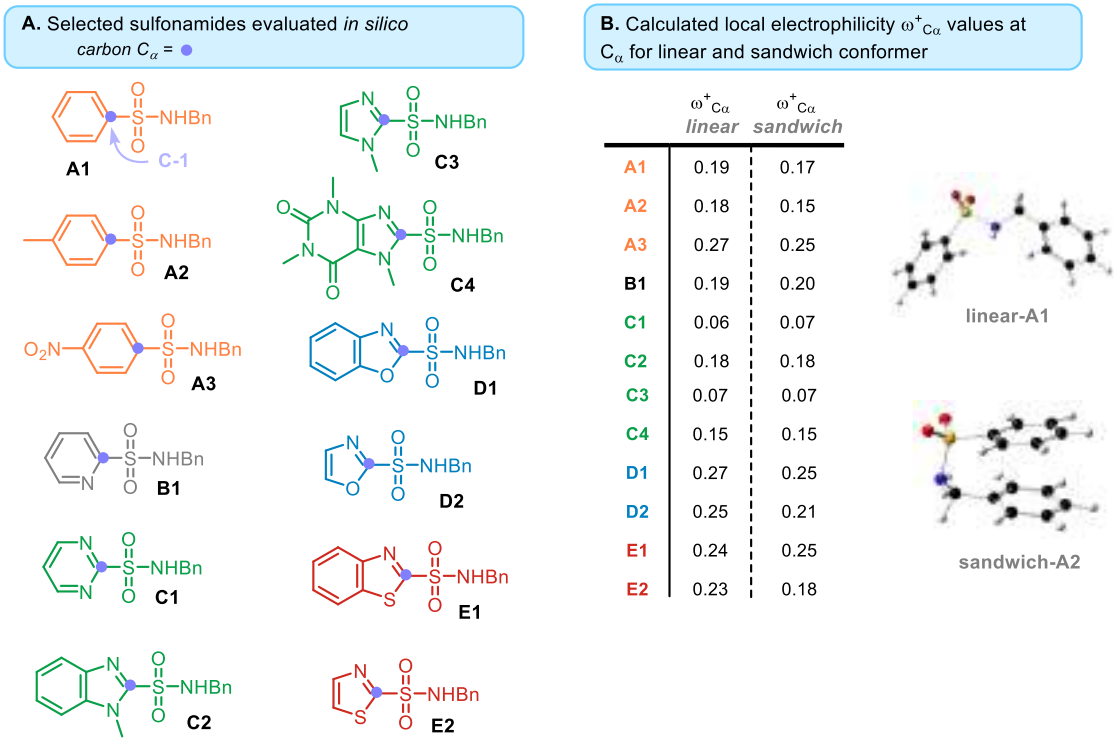
Thus, my first objective was to expand the application of this method to additional heterocyclic thiols, mainly nitrogen-containing heterocycles pyridine, pyrimidine, imidazole, benzo[*d*]imidazole and purine-2,6-dione, oxygen-containing heterocycles such as benzo[*d*]oxazole and oxazoles, and to sulfur-containing benenzo[*d*]thiazole and thiazole.

[#]This work was supported by the European Regional Development Fund-Project 'Centre for Experimental Plant Biology' (no. CZ.02.1.01/0.0/0.0/16_019/0000738) and by the Palacký University Internal Grant Agency (IGA_PrF_2022_016, IGA_PrF_2022_022, IGA_PrF_2023_031 and IGA_PrF_2023_020). Part of the research was published in *Org. Biomol. Chem.* **2022**, *20*, 3154-3159; DOI: 10.1039/D2OB00345G. The research presented in chapter 3.8.2. were in part carried out by MSc. Jozef Kristek, with whom I did collaborate on the method development. The results obtained by him are presented only in the Results and discussion part to enrich the discussion. No experimental data are present in such case in the experimental part, since those will be integral part of his Thesis. The manuscript, where we both will be shared 1st authors is currently under preparation. The work was done in collaboration with prof. Freija De Vleeschouwer (DFT calculations) and Dr. Tomáš Pospíšil (advanced NMR experiments). As a co-primary researcher (shared with MSc J. Kristek) in the project, I was responsible for designing and conducting experimental procedures and measurements, analyzing the data, and interpreting the results. Additionally, I am co-writing a first draft of the manuscript.

3.8.1.1. Evaluation of sulfonamides *in silico*

Our work on the extension of the methodology started with the *in silico* evaluation of the selected heterocycle stability and reactivity. The reason for such 'project opening' was our previous experience with the preparation of derivatives from BT where the stability of intermediates and compounds was sometimes disputable and slight changes in reaction or further storage conditions had a huge impact on the reaction yields or stability. And we expected that the instability is caused by the C_α on a sulfone/sulfonamide of the heterocycle that is undergoing nucleophilic attack. Therefore, selected structures were evaluated *via* DFT calculation methods by Prof. Freija De Vleeschouwer and her Ph.D. student Eline Desmedt from Vrije Universiteit Brussel. In particular, we were interested in the local electrophilicity $\omega_{C\alpha}^{+}$ at C_α carbon of selected heteroarylsulfenamides (

Figure 48).[192] The calculations found that the evaluated structures can be present in solution in two very different conformers, linear (expected) and sandwich (unexpected), and that local electrophilicity of such conformers might be significantly different (for example difference in case of **D1** and **D2**).



	$\omega^+_{C\alpha}$ *linear*	$\omega^+_{C\alpha}$ *sandwich*
A1	0.19	0.17
A2	0.18	0.15
A3	0.27	0.25
B1	0.19	0.20
C1	0.06	0.07
C2	0.18	0.18
C3	0.07	0.07
C4	0.15	0.15
D1	0.27	0.25
D2	0.25	0.21
E1	0.24	0.25
E2	0.23	0.18

Figure 48: Local electrophilicity $\omega_{C\alpha}^+$ at C_α carbon of selected heteroarylsulfenamides.

As a consequence, two different conclusions emerged from the calculations: (**1**) oxygen-containing heterocycles should be due to the highest electrophilicity index our ideal substrate for reaction optimization (should be the most prone to react with external electrophiles), and (**2**) the sandwich conformation brought us to the expectation that heteroarylsulfenamides might be a suitable substrate for the development of the 'Memory of Chirality' concept.

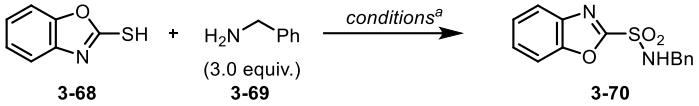
3.8.1.2. Optimization

As mentioned above, our optimization of the heteroaryl sulfonamide reaction conditions began with benzo[*d*]oxazole **3-68** since it was the most sensitive substrate to nucleophilic attack (based on *in silico* results). Initial conditions for the synthesis of benzo[*d*]oxazole sulfonamide **3-70** were taken from the previously developed reaction protocol, although only traces of the desired product were observed (

Table 19). Detailed investigation of the formation and degradation of sulfenamide (equivalents of H_2O_2 and other reagents, including reaction workup and product purification) followed by oxidation to sulfonamide together with purification (crystallization turned out to be the only method for purification method) then allowed us to isolate the desired product in

35 % yield (**entry 4**). This experience led us to conclude two things: (1) if one-pot protocol fails, the intermediate sulfenamide needs to be purified, and (2) oxidation step needs to be checked for the stability of generated sulfonamide (if product unstable, crystallization conditions need to be searched).

Table 19: Optimization of benzo[*d*]oxazole sulfonamide synthesis



3-68 **3-69** **3-70**

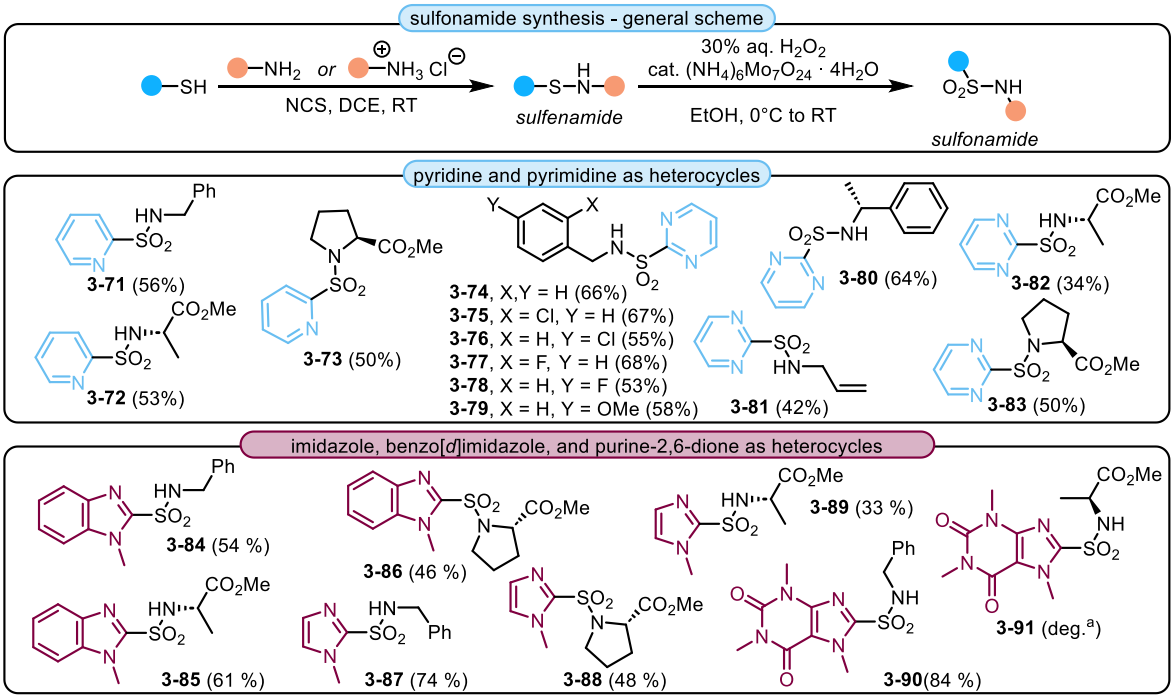
Entry	Conditions	NMR Yields [%][b]	Isolated Yield [%][c]
1	NCS (1.0 equiv.), DCM, RT, 30 min, then $(NH_4)_6MoO_4.4H_2O$ (0.3 equiv.), H_2O_2 (20 equiv.), EtOH, 0 °C to RT, 5h	34	0
2	NCS (1.0 equiv.), DCE, RT, 1h, then $(NH_4)_6MoO_4.4H_2O$ (0.3 equiv.), H_2O_2 (20 equiv.), EtOH, 0 °C to RT, 5h	59	8
3	NCS (1.0 equiv.), DCE, RT, 1h, then $(NH_4)_6MoO_4.4H_2O$ (0.3 equiv.), H_2O_2 (10 equiv.), EtOH, 0 °C (30 min) to RT, 12 h	69	16
4[d]	NCS (1.0 equiv.), DCE, RT, 1h, then $(NH_4)_6MoO_4.4H_2O$ (0.3 equiv.), H_2O_2 (10 equiv.), EtOH,0 °C (30 min) to RT, 12 h	68	35

[a] The reactions were performed on benzo[*d*]oxazole-2-thiol (1 mmol) and benzylamine (3 mmol) scale.
[b] NMR yield determined using dimethylsulfone as an internal standard.
[c] Obtained *via* crystallization.
[d] Reaction performed on benzo[*d*]oxazole-2-thiol (5 mmol) and benzyl amine (15 mmol) scale.

3.8.1.3. Scope and limitations of heteroaryl sulfonamides synthesis

Although a comprehensive description of the developed synthetic approach towards the final conjugates can be found in a published paper [192], it is important to provide to the reader a summary of scope and limitations of the method. All compounds that were prepared by me during this project are then summarized in the experimental part of this chapter and their full characterization is provided.

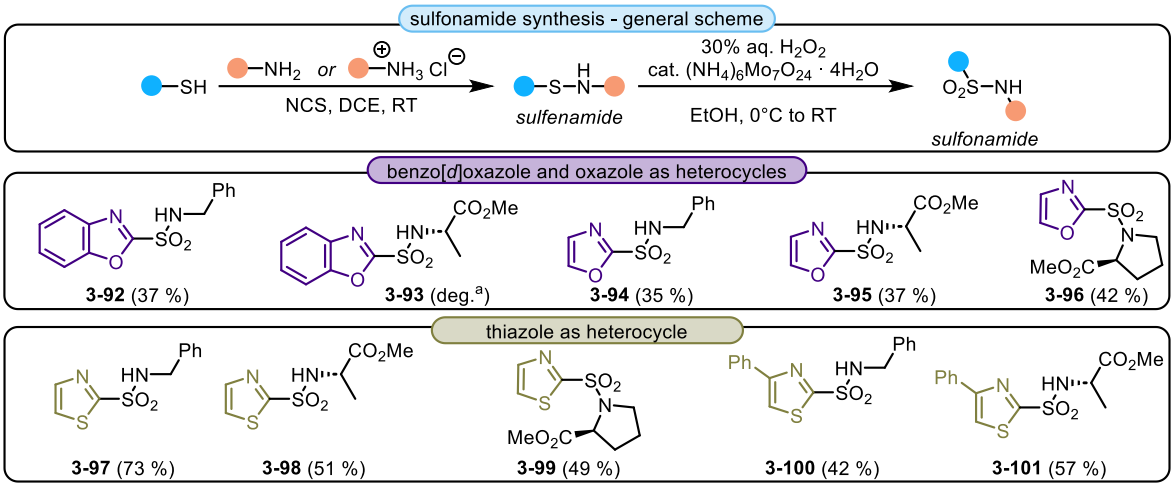
The optimal conditions (entry 4,**Table 19**) for a preparation of *N*-substituted sulfonamides were used as the standard reaction conditions, however, in some cases several deviations from the standard protocol were applied especially with the regard of the product isolation and purification. The scope of the reaction started with the evaluation of pyridines, pyrimidines, imidazoles, benzo[*d*]imidazoles and purine-2,6-diones (**Scheme 81**). All these substrates roved to be suitable for the reaction and the desired compounds were isolated in good to very good yields.



Scheme 81: Scope of the oxidative coupling between heteroaryl thiols (for pyridines, pyrimidines, imidazoles, benzo[*d*]imidazoles, purine-2,6-diones, benzo[*d*]oxazoles and oxazoles and thiazoles) and different amines. *Yields refer to pure isolated compounds after two steps. All reactions were typically performed on a 5 mmol scale of the corresponding heteroaryl thiol. [a]Sulfonamides detected by ^{1}H NMR; however, all attempts to isolate it failed.*

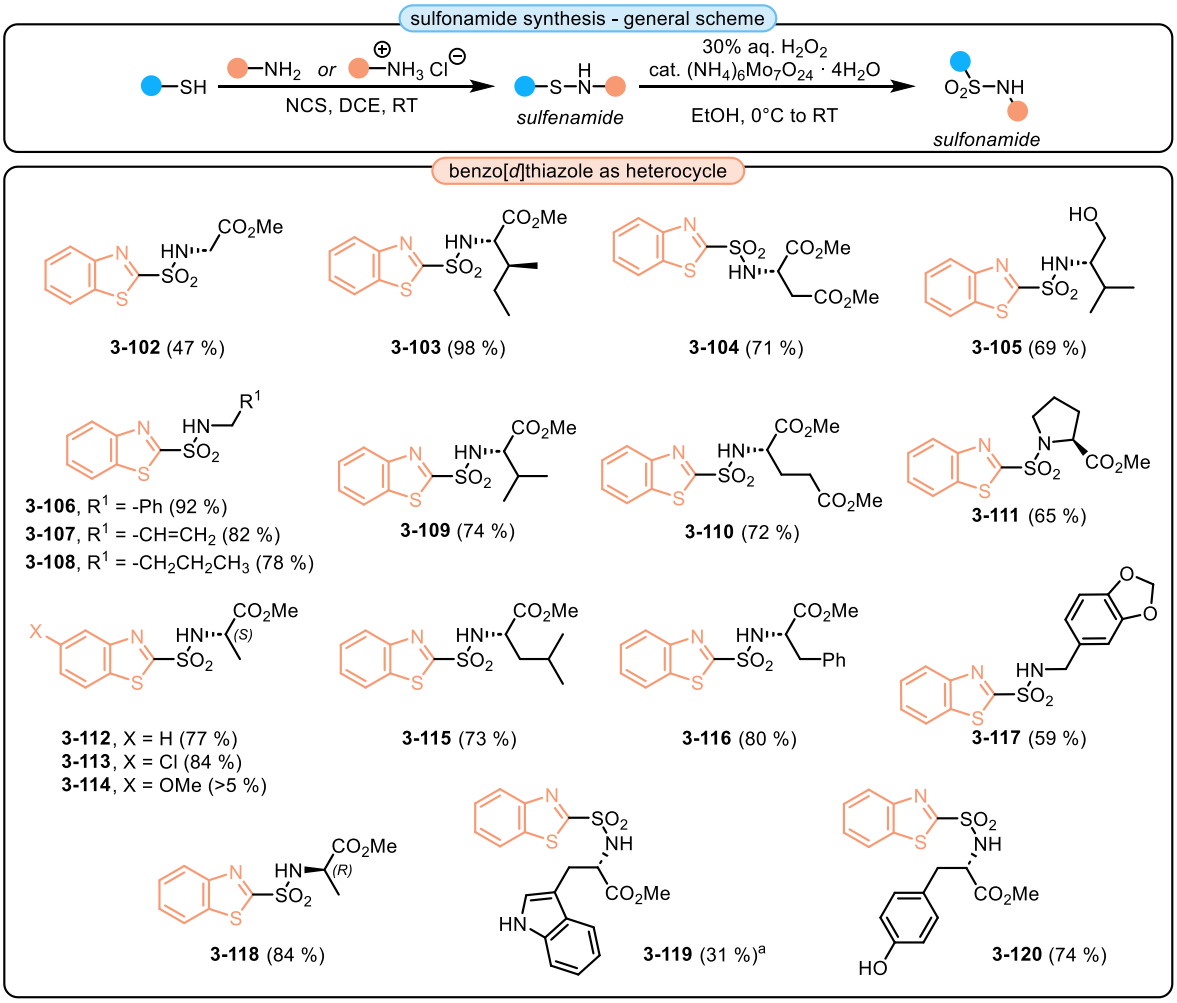
Next, benzo[*d*]oxazoles and oxazoles and thiazoles were evaluated (**Scheme 82**). In this case it was observed that the stability of generated products is in many cases low and desired

products degraded either during the reaction workup or during the purification. Interestingly, once the final compounds were pure, they proved to be bench stable for several months.



Scheme 82:Scope of the oxidative coupling between heteroaryl thiols (benzo[*d*]oxazoles and oxazoles and thiazoles) and different amines. *Yields refer to pure isolated compounds after two steps. All reactions were typically performed on a 5 mmol scale of the corresponding heteroaryl thiol.*

We have also quite substantially broadened the scope for benzo[*d*]thiazoles sulfonamides (**Scheme 83**) and included other amino acids (many of obtained sulfonamides derived from AA are not part of the original publication[192]), such as glycine **3-102**, both enantiomers of alanine **3-112** and **3-118**, isoleucine **3-103**, leucine **3-115**, proline **3-111**, valine **3-109**, phenylalanine **3-116**, tryptophan **3-119**, tyrosine **3-120**, and aspartic **3-104** and glutamic acid **3-110** besides other amines. Benzo[*d*]thiazoles sulfonamides proved to be the most stable group of sulfonamides generated *via* our protocol and their yields vary from good to excellent.



Scheme 83: Scope of the oxidative coupling between benzo[*d*]thiazole-2-thiols and different amines. *Yields refer to pure isolated compounds after two steps. All reactions were typically performed on a 5 mmol scale of the corresponding heteroaryl thiol.* [a]*Product* ***3-119*** *is sensitive to oxygen and upon concentration decomposes.*

On the other hand, several substrates prove to be inappropriate substrates for the reaction (**Figure 49**). The partially saturated heteroaryl sulfonamides **3-122** and **3-123**, purine **3-124,** and triazole **3-125** react in the first step and produce sulfenamides smoothly. However, the subsequent oxidation step to sulfonamide was unsuccessful, most probably due to the oxidative decomposition of the sulfenamide. In the case of **3-121** and aromatic amines, the first step of the sequence, formation of sulfenamide, failed. We believe that the presence of a free N-H bond on the heterocycle is responsible for this behaving. In the case of tetrazole **3-126** both steps, sulfenamide formation and further oxidation, occurred and the desired product was clearly identifiable from the ^{1}H NMR spectra of the crude reaction mixture analysis, however, the purification of the product failed regardless of the isolation technique used.

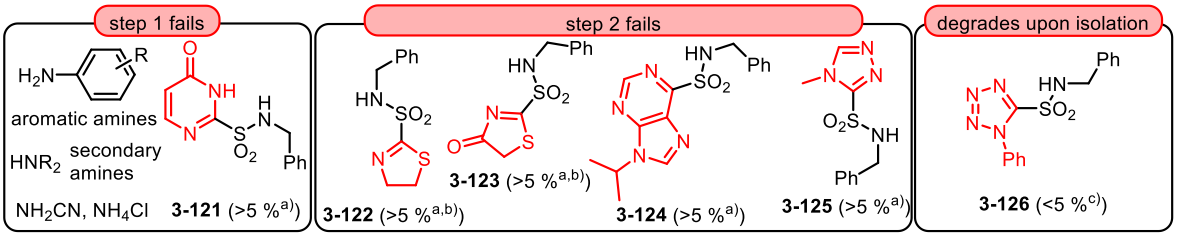
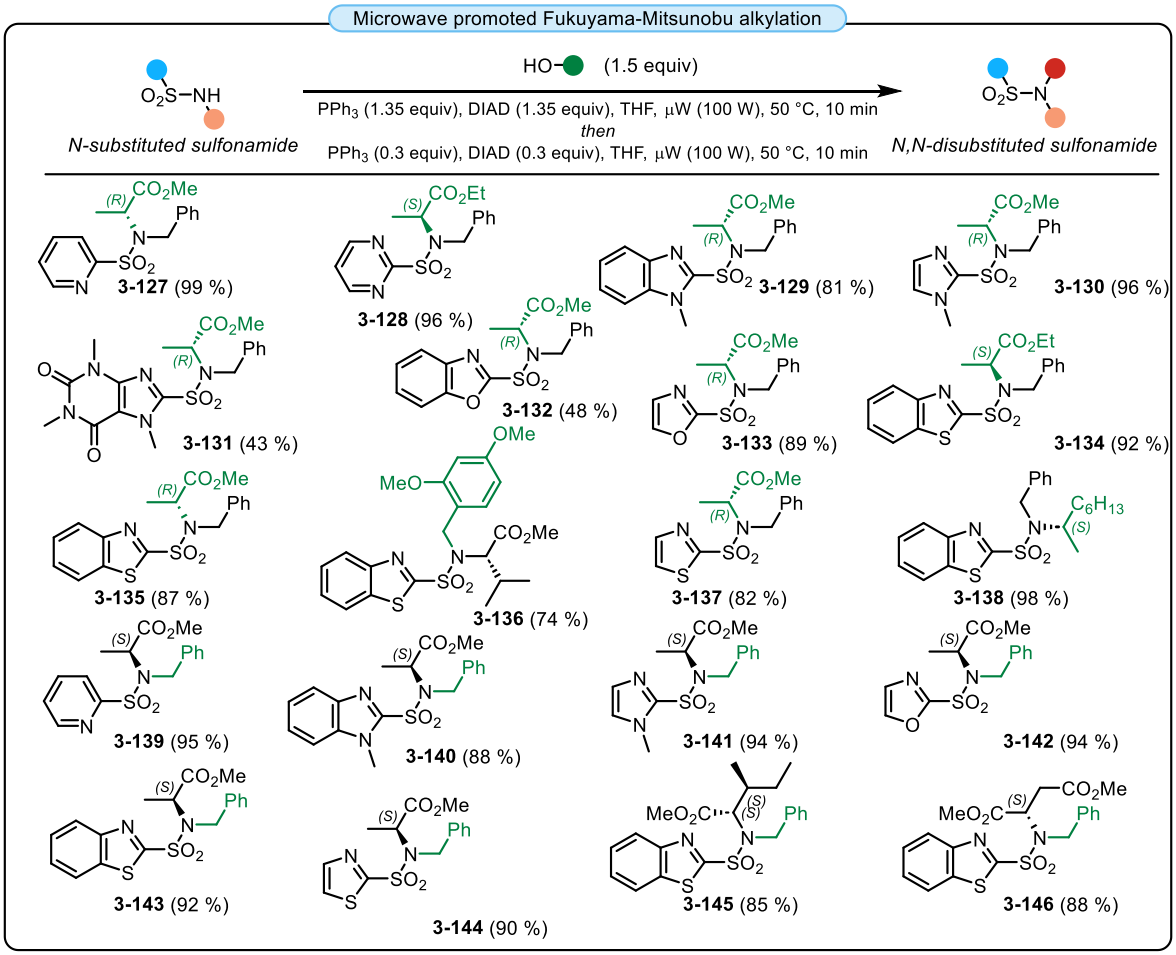


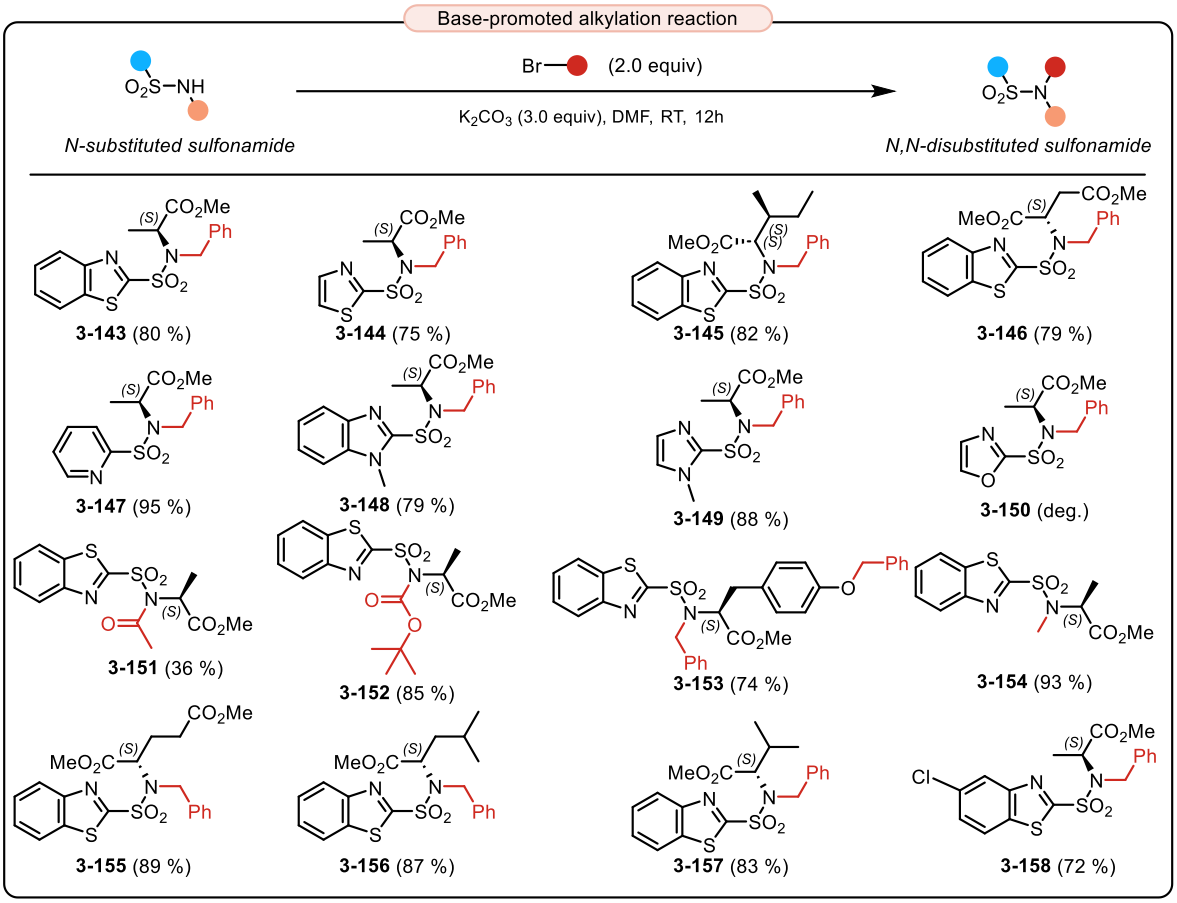
Figure 49: Limitations of the oxidative coupling between heteroaryl thiols and different amines. *[a]No product formation was observed. Only intermediate sulfenamide was detected. [b]The oxidative opening of the heterocycle occurred during the oxidation step. Only side products were formed [c]Desired product **3-126** isolated only as a mixture of **3-126** with phenyl tetrazole (1 : 5). All attempts to purify compound **3-126** failed due to compound **3-126** decomposition.*

Next, prepared *N*-monosusbtituted heteroaryl sulfonamides were transformed to a *N,N*-disubstituted one. To achieve selective alkylation, two different methods were used. The first method explores the use of alcohols as alkylating agents and is based on the microwave-promoted Fukuyama–Mitsunobu alkylation reaction (**Scheme 84**).



Scheme 84: *N,N*-disubstituted sulfonamide synthesis. Scope of the microwave-promoted Fukuyama-Mitsunobu alkylation reaction. *Yields refer to pure isolated compounds.*

The second method uses alkyl bromides as alkylating agents and proceeds under basic conditions (**Scheme 85**).



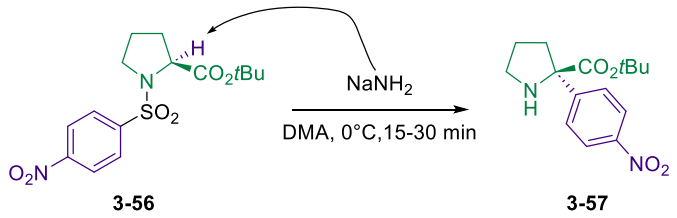
Scheme 85: *N*,*N*-disubstituted sulfonamide synthesis. Scope of the base-mediated alkylation reaction. *Yields refer to pure isolated compounds.*

In both cases, the expected products formed in good to excellent yields, and in the case of Fukuyama-Mitsunobu reaction, the reaction proceeded with complete inversion of the stereochemistry with regards to the original stereogenic center present in the starting alcohol. Again, *N*-substituted and *N*,*N*-disubstituted heteroaryl sulfonamides prepared by me during the scope and limitations determination are listed in the Experimental part with their full characterization data.

3.8.2. Introduction to α-heteroaryl α-substituted α-AAs synthesis

In Chapters **3.4.2** and **3.5**, we have discussed extensive methodologies for the synthesis of α,α-disubstituted αAA and their derivatives. The methods depicted are quite broad and applicable on many substrates; however, when it comes to α-heteroaryl α-substituted α-AAs the power of such methods is vanishing.

As mentioned earlier, *in silico* evaluation of simple heteroaryl sulfonamides demonstrated that such compounds can be readily found in sandwich-like conformation (**Figure 48**), and such information in combination with the knowledge of Kawabata's principle of Memory of chirality, led us to the conclusion that such AA-derived heteroaryl sulfonamides could be explorable substrates to a new previously unknown class of α-heteroaryl α-substituted α-AAs and their derivatives. In this context, the literature precedent [233] that describes the transformation of the proline-type sulfonamide **3-56** *via* Smiles rearrangement to α,α-disubstituted αAA ester **3-57**, was a great encouragement.



Scheme 86: Enantioselective rearrangement of proline sulfonamides.[233]

The primary motivation for the α-heteroaryl α-substituted α-AAs (**HAA**s) synthesis was, however, its application potential. We believe that this unique class of compounds can have a huge impact in at least two areas of research (**Figure 50**): (**1**) a design of a novel class of tunable organocatalysts, such as Hayashi-Jorgensen-like organocatalyst **3-161**, and (**2**) in a preparation of previously unexplored variety of Xeno nucleic acids, such as C8-Ala-Ado **3-163**. The with stability of such Xeno nucleic acids is obviously unknown, but we believe that it might be sufficient to be explorable in context of DNA or RNA.

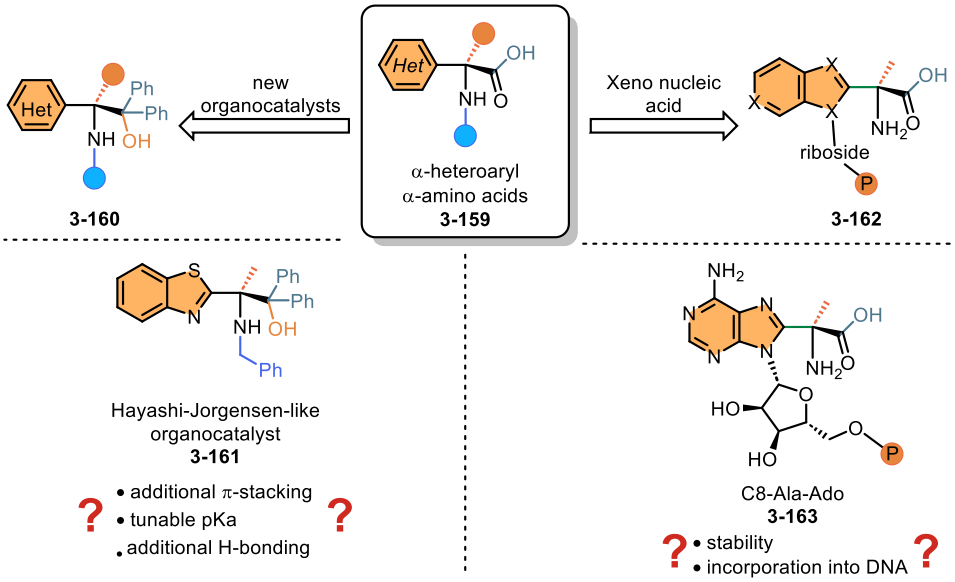


Figure 50: Two research domains where α-heteroaryl α-substituted α-AAs (**HAA**s) might play a key role in the future.

3.8.2.1. Evaluation of sulfonamides *in silico*

As previously, our first steps led to our colleagues from the theoretical department, and we led them to evaluate our idea of 'fixed' required for the Memory of Chirality concept the *in silico*. This approach that was used even later during our study allowed us to rationalize our ideas and definitely pushed us to test our Smiles rearrangement-based hypothesis towards the experimental evaluation. In particular we were interested in two outcomes: (**1**) the electrophilicity of the C_α electrophilic center in the heterocycle, and (**2**) the position of the acidic hydrogen atom placed in the a position to ester group (**Figure 51**). Theoretical approach suggested that both preliminary conditions are achieved, ((**1**) the C_α electrophilic center in the heterocycle is highly electrophilic, and (**2**) the acidic proton a to the ester is well suited to be deprotonated with a strong base) in the evaluated molecules at the same time. We thus conclude that the Smiles rearrangement is possible and can successfully occur.

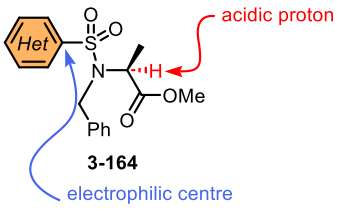
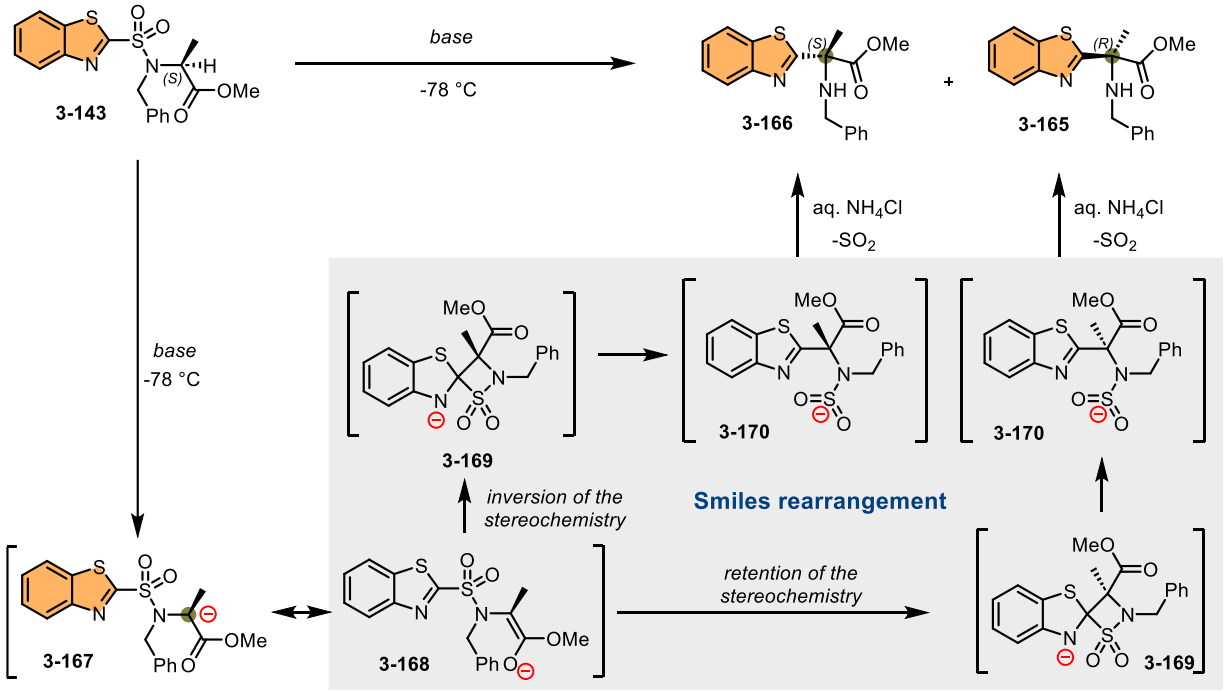


Figure 51: Necessity of the two reactive centers, (**1**) electrophilic center in the heterocycle, and (**2**) well-placed hydrogen atom in a position to ester group.

With this information in hand, we have taken advantage of our developed synthetic pathway to optically pure electron-deficient *N,N*-disubstituted heteroaryl sulfonamides

and used compound **3-143** as the model substrate for the base-promoted Smiles rearrangement (**Scheme 87**). We expected that the reaction will proceed according to the following scenario: sulfonamide **3-143** will be deprotonated with a nonnucleophilic base and the generated anion will be either (**A**) conformationally stable or, more likely, (**B**) the generated enolate will preserve the conformation the molecule kept before the deprotection. In addition, we expected that the Smiles rearrangement will be a rapid process that will proceed faster than the inevitable conformational change would occur. The corresponding α-amino ester with retention **3-165** or inversion **3-166** of configuration. First, the deprotonation to form **3-167** occurs, followed by tautomerization to its enol form **3-168**. Next, the formation of Meisenheimer complex **3-169** followed by Smiles rearrangement proceeds to form **3-170**, which upon aquatic workup protonates and releases SO_2.



Scheme 87: Expected reaction mechanism of heteroaryl sulfonamide **3-143** transformation to **HAA 3-165** or **3-166** that should proceed *via* Smiles rearrangement.

3.8.2.2. Optimization

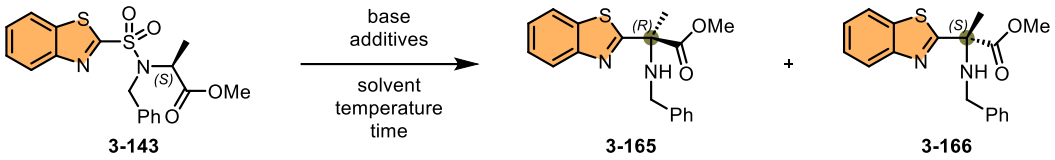
To validate our hypothesis, the reaction conditions that would yield the desired rearrangement product were searched (**Table 20**). In the first attempt, 6.0 equiv. of LDA was added to sulfonamide **3-143** at -78 °C and the reaction was allowed to stir at that temperature for 30 min (**Entry 1**). The degradation of the starting material

was observed, however, a comprehensive analysis of the ^{1}H NMR spectra of the crude reaction mixture revealed some traces of a new compound that was later identified as our product **3-166**. Next, KHMDS (1.5 equiv.) in Et_2O was used (**entry 2**). The reaction proceeded smoothly, and the spectra of the crude reaction mixture were quite clean. Additionally, based on the TLC analysis, the overall transformation proceeded within 10 min (conversion of the starting material). The desired product **3-166** was formed and after the HPLC separation method was established that the enantiomeric ratio of **3-166** to its enantiomer **3-165** is 81:19. The reaction yield was 92 %. Next, a screening of the optimal reaction conditions started. First, the influence of the solvents was established (**entries 2-5**) identifying THF as the best reaction solvent. Next, based on our longstanding tradition reaction of KHMDS at -78 °C in the presence of 18-crown-6 was carried out (**entry 6**).[244] The addition of 18-crown-6 should allow the Smiles rearrangement to proceed faster since the potassium cation should be scavenged and to further increase already nice enantiomeric ratio of the reaction. Much to our surprise, after the reaction workup and determination of the enantiomeric purity, the opposite enantiomer, compound **3-165**, was formed as a major product of the reaction (**3-165:3-166 =** 93:7). When 1.5 equiv. of KHMDS with 3.0 equiv. of 18-crown-6 was used at -95 °C, the enantiomeric ration increased slightly (**entry 7**, 95:5). The results were encouraging, but confusing. Thus, other bases such as LiHMDS and NaHMDS and additives were screened, and very interesting results were obtained. LiHMDS without any additional additives gave at -78 °C (**entry 8**, *e.r.* = 87:13) the same result as KHMDS/18-crown-6 mixture. The addition of HMPA led to a decrease in reaction selectivity (**entry 9**, 75:25). Decreasing the temperature from -78 °C to -90 °C also helped the selectivity, and *e.r.* increased to 96:4 (**entry 10**). NaHMDS without any additives provided only poor selectivity of 67:33 (**entry 11**), but when 3.0 equiv. of 18-crown-6 was added, the selectivity increased to excellent **97:3** (**entry 12**).

The conditions obtained were excellent; however, they did not answer the question how reversed selectivity could be repetitively achieved (**entry 5**, *e.r.* = 17:83). Our hypothesis was that the size of the cation and/or additive might play a role in the selectivity switch. The task of finding out if the hypothesis is correct was taken over by my colleague, MSc. Jozef Kristek, who devoted his time to searching for the right reaction conditions that would allow us to prepare enantiomer **3-166** as the main product of the reaction starting from the same

starting material as me, who is able to prepare enantiomer **3-165**. The results obtained during his optimization are for clarity of the discussion summarized in **Table 21** and **Table 22**.

Table 20: Optimization of the reaction conditions of the Smiles rearrangement.



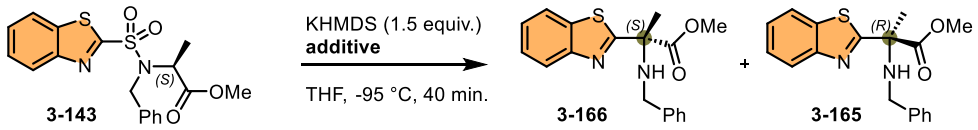
3-143 **3-165** **3-166**

Entry	Base (equiv.)	Additive (equiv.)	Solvent[a]	Temp. [°C]	Time [min]	Conv. [%][b]	Yield [%][c]	*e.r.*[d]
1	LDA (6.0)	-	THF	-78	30	≥98	n.d.	-
2	KHMDS (1.5)	-	Et_2O	-78	30	≥98	n.d.	19:81
3	KHMDS (1.5)	-	Et_2O	-95	30	≥98	n.d.	15:85
4	KHMDS (1.5)	-	DMF	-50	30	≥98	n.d.	17:83
5	KHMDS (1.5)	-	THF	-95	30	≥95	63	17:83
6	KHMDS (1.5)	18-crown-6 (3.0)	THF	-78	30	≥98	69	93:7
7	KHMDS (1.5)	18-crown-6 (3.0)	THF	-95	30	≥99	73	**90:10**
8	LiHMDS (1.5)	-	THF	-78	30	≥98	n.d.	87:13
9	LiHMDS (1.5)	HMPA (3.0)	THF	-78	30	≥98	n.d.	75:25
10	LiHMDS (1.5)	-	THF	-95	30	≥98	75	**97:3**
11	NaHMDS (1.5)	-	THF	-78	30	≥99	n.d.	67:33
12	NaHMDS (1.5)	18-crown-6 (3.0)	THF	-78	30	≥99	90	**97:3**

a) All reactions were performed in 0.1 M concentration; b) Based on ^{1}H NMR spectra of the crude reaction mixture or by HPLC; c) Refers to pure isolated product; d) Determined by chiral HPLC analysis.

First, screening of different additives that were added to the reaction mixture prior the base, KHMDS, was performed (**Table 21**). It was observed that product **3-166** in high enantiomeric ratios might be obtained. The best *e.r.* was obtained in the case CuOTf (**entry 14**).

Table 21: Evaluation of the role of the additional metal salts given to the reaction mixture *prior* cooling down to -95 °C.

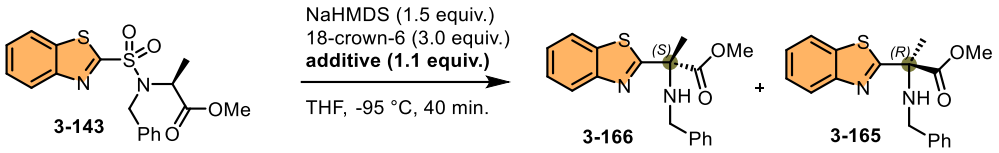


Entry[a]	Additive	Additive equiv.	Conv. [%][b]	*e.r.*[c]
1	LiCl	1.1	>98	46:53
2	NaCl	1.1	>98	13:87
3	KCl	1.1	>98	13:87
4	KCl	3.0	>98	13:87
5	KCl	5.0	>98	20:80
6	CsF	1.1	>98	13:87
7	$MgCl_2$	1.1	70	32:68
8	$CaCl_2$	1.1	>98	13:87
9	$Yb(OTf)_3$	1.1	>98	13:87
10	$Sc(OTf)_3$	1.1	70	25:75
11	$Zn(OTf)_2$	1.1	>98	12:88
12	$Zn(OTf)_2$	3.0	70	20:80
13	$Zn(OTf)_2$	5.0	30	35:65
14	CuOTf	1.1	>98	10:90
15	CuOTf	3.0	30	35:65
16	CuOTf	5.0	15	30:70
17	CuCl	1.1	>98	30:70
18	$ZnCl_2$	1.1	>98	82:18
19	AgOTf	1.1	>98	85:15
20	$AgNO_3$	1.1	>98	85:15

[a]Reactions were performed with sulfonamide **3-143** (20.0 mg, 0.05 mmol, 1.0 equiv) in THF (0.1 M) at -95 °C with KHMDS (0.08 mmol, 1.5 equiv) and different inorganic salts. [b]All conversions were determined by crude HPLC analysis or by NMR. [c]The enantiomeric ratio (e.r.) was determined by chiral HPLC analysis.

The same optimization was performed for the NaHMDS/18-crown-6 system (**Table 22**). Interestingly, in this case the reaction yielded the enantiomer **3-165** as the main product of the reaction suggesting that the addition of the 18-crown-6 additive hammers any influence that additional salt addition has on the reaction selectivity.

Table 22: Influence of added cations to the NaHMDS/18-crown-6 system on the reaction selectivity.



Entry[a]	Additive	Additive equiv.	Conv. [%][b]	*e.r.*[c]
1	LiCl	1.1	>98	99:1
2	NaCl	1.1	>98	82:18
3	KCl	1.1	>98	82:18
4	CsF	1.1	>98	81:19
5	$MgCl_2$	1.1	>98	86:14
6	$CaCl_2$	1.1	>98	94:6
7	$Yb(OTf)_3$	1.1	50	85:15
8	$Sc(OTf)_3$	1.1	40	81:19
9	$Zn(OTf)_2$	1.1	43	93:7
10	CuOTf	1.1	61	95:5

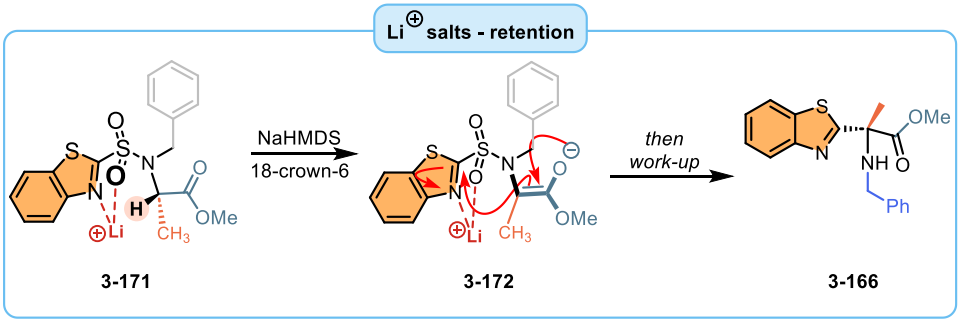
[a]Reactions were performed with sulfonamide **3-143** (20.0 mg, 0.05 mmol, 1.0 equiv) and 18-crown-6 (0.15 mmol, 3.0 equiv) in THF (0.1 M) at -95 °C with NaHMDS (0.08 mmol, 1.5 equiv) and different inorganic salts (0.06 mmol, 1.1 equiv). [b]All conversions were determined by crude HPLC analysis or by NMR. [c]The enantiomeric ratio (e.r.) was determined by chiral HPLC analysis.

To conclude, extensive optimization of the reaction conditions identified two sets of the reaction conditions that can selectively furnish compound **3-165** or **3-166** starting from the same homochiral starting material. When 1.5 equiv. of NaHMDS with 3.0 equiv. of 18-crown-6 and 1.1 equiv. of LiCl were used, stereoselectivite formation of the **3-165** enantiomer increased to 99:1 (**entry 1, Table 22**), and when 1.5 equiv. of KHMDS with 1.1 equiv. of CuOTf was used, the opposite enantiomer **3-166** was isolated in 95:5 *e.r.* (**entry 13, Table 20**).

3.8.2.3. Mechanistic studies of Li^+ and Cu^{2+} salts

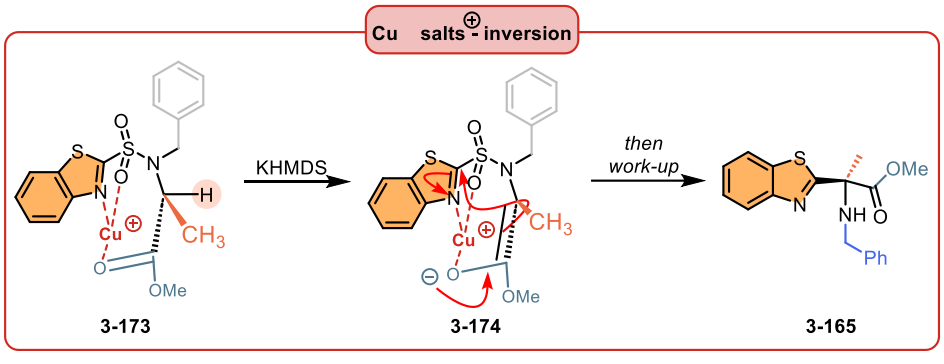
Given the intriguing results from the initial screening and our success in synthesizing both enantiomers from a same starting material, we reached out to our collaborators Dr. Freija De Vleeschouwer from Vrije Universiteit Brussel for assistance with density functional theory (DFT) calculations and Dr. Tomáš Pospíšil for NMR experiments. With their expertise, we computed the transitional states and proposed a coordination mechanism involving two distinct cations, shedding light on the reaction mechanism.

First, we propose that lithium(I) cation from LiCl coordinates to oxygen of the sulfonamide group and nitrogen of the BT-heteroaryl and forms intermediate **3-171**. Since the Li(I) is a small cation, it does not sterically interfere with the ester part of the molecule. Consequently, after deprotonation by NaHMDS, the nucleophilic attack of the enolate **3-172** happens from the front side of the molecule and the rearrangement proceeds with the retention of the stereochemistry. Aqueous work up then yields to product **3-166** and the reaction proceeds overall with retention of configuration (**Scheme 88**).



Scheme 88: Proposed formation of **3-166** using LiCl additive.

When copper triflate is added, the overall picture changes. Copper(I) coordinates presumably with the oxygen of the sulfonamide, the nitrogen of the BT group, and the oxygen of the ester group. The change in the cation size is a reason for the observed change in the mode of coordination. The generated conformer **3-173** than placing the acidic hydrogen atom outside of the complex and its subsequent deprotonation then forces the Smile rearrangement to proceed *via* the inversion of the configuration. The aqueous work then yields the final product with the opposite configuration, when compared to the original configuration of the starting material (**Scheme 89**).



Scheme 89: Proposed formation of **3-165** using CuOTf additive.

3.8.2.4. Determination of absolute configuration

Of course, the reaction mechanism could not be suggested before the determination of the absolute configuration of compounds **3-165** and **3-166** was achieved. We have tried many ways of doing it. First, the transformation of generated products to Mosher amides was carried out, but no product formation was observed. Co-crystallization of generated compounds with various chiral acids failed again presumably due to a low basicity of sulfonamide. Therefore, the only possibility left was to prepare a single crystal for X-ray analysis. Thus, the crystallization of **3-166** by dissolving it in DCM and allowing it to crystalize in the hexane atmosphere allowed us to obtain a monocrystal, which was suitable for X-Ray analysis. Single X-Ray analysis of the product was performed by Dr. Ivan Němec (Palacký University Olomouc) (**Figure 52**).

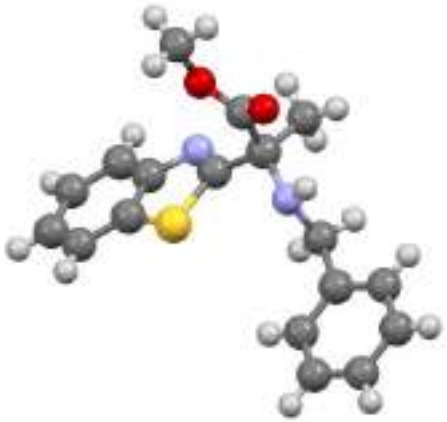
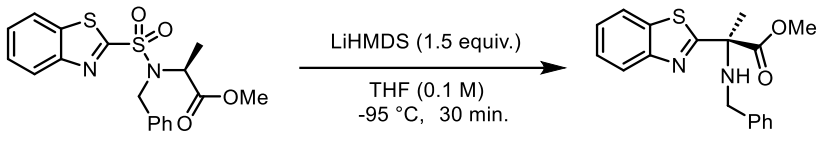


Figure 52: Crystallin structure of **3-166**.

3.8.2.5. Competitive reactivity in the presence of other functional groups

We also wanted to find out which functional groups are tolerated during the Smiles rearrangement. Therefore, we have performed a competitive reaction of our model substrate **3-143** with LiHMDS in the presence of 4-methoxyacetophenone (*p*Ka ≈ 21, keton group), *p*-anisaldehyd (*p*Ka ≈ 16, aldehyde group), ethylacetate (*p*Ka ≈ 25, ester group), silylated BT sulfonamide (*p*Ka ≈ 6) and monitored conversion (**Table 23**). Competitive experiments suggested that the reaction cannot be carried out in the presence of substrates with readily available (accessible) acidic hydrogen atoms that have lower pKa compared to the acidic proton in heteroaryl sulfone **3-143** (pKa ~ 25-28). However, if a sterically hindered acidic proton is present, the Smiles rearrangement might proceed smoothly.

Table 23: Functional group tolerance experiments.



3-143 **3-165**

Entry	Additive (1.0 equiv.)	p*Ka*	Product formation [%][a]
1	4-methoxyacetophenone	≈ 25	29
2	*p*-anisaldehyd	≈ 16	≤1
3	ethylacetate	≈ 25	≤1
4	silylated BT sulfonamide	≈ 6	≥99

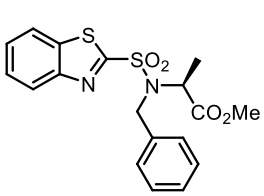
a) Based on ^{1}H NMR spectra of the crude reaction mixture

3.8.2.6. Effects of nitro group

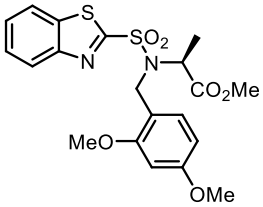
We have also prepared three other BT sulfonamides (**3-175** – **3-177**), together with the **3-143** model substrate according to our developed protocol[192] with different substitution on the benzyl group. These substrates should allow us to evaluate the influence of the substitution on the aromatic ring on the Smiles rearrangement (**Table 24**). In addition, the role of substitution might prove to be crucial when the pre-rearrangement conformation of the substrates is adapted.

From **Table 24** it is clear that the electron-donating dimethoxybenzyl **3-175** gives the best selectivity during the reaction for both inversion and retention products (**entry 5** and **6**) whereas electron-withdrawing 2-nitrobenzyl **3-176** gives the worst selectivity (**entries 10** and **11**). The change in the nitro group position to *para* makes the reaction proceed and if LiHMDS is used as a base, the desired product **3-177** (**entry 8**) can be isolated albeit with modes stereoselectivity. Those results had an important impact on our scope and limitations determination campaign. Since dimethoxybenzyl group increases the reaction stereoselectivity of the rearrangement process (presumably due to a better conformation stability) it was used in the cases where worse reaction yields, or selectivity were observed during the scope and the limitation evaluation.

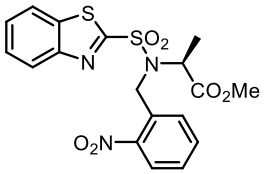
Table 24: Impact of the benzylic group substitution on the reaction yields and selectivity.



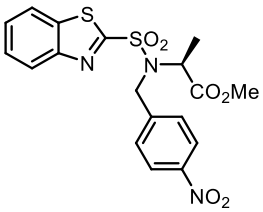
3-143



3-175



3-176



3-177

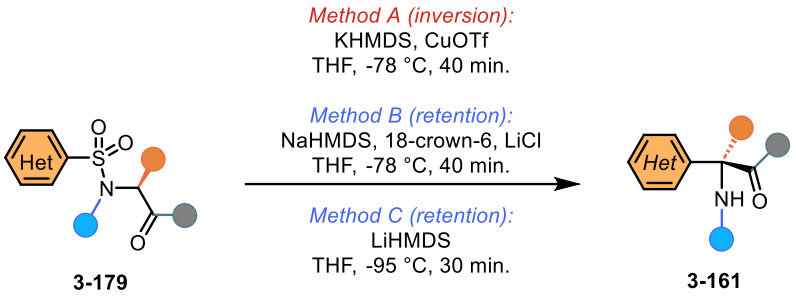
Entry	compound	base	salt/additive	Time [min]	Temp [°C]	Conv. [%][a]	*e.r.*
1	**3-143**	LiHMDS	-	30	-95	~99%	96 : 4
2	**3-143**	KHMDS	18-crown-6	30	-95	~99%	72 : 28
3	**3-143**	NaHMDS	LiCl, 18-crown-6	60	-95	~90%	98 : 2
4	**3-143**	KHMDS	CuOTf	30	-78	99%	10:90
5	**3-175**	KHMDS	CuOTf	60	-78	~75%	7 : 93
6	**3-175**	NaHMDS	LiCl, 18-crown-6	60	-78	~99%	99:1
7	**3-177**	KHMDS	CuOTf	60	-78	~30%	35:65
8	**3-177**	NaHMDS	LiCl, 18-crown-6	60	-78	~30%	95:5
9	**3-177**	LiHMDS	-	60	-78	~54%	-
10	**3-176**	KHMDS	CuOTf	60	-78	~<5%	-
11	**3-176**	NaHMDS	LiCl, 18-crown-6	60	-95	~<5%	-
12	**3-176**	KHMDS	18-crown-6	60	-78	~60%	-
13	**3-176**	LiHMDS	-	60	-78	~95%	76:24

a) Based on ^{1}H NMR spectra of the crude reaction mixture
b) Determined by chiral HPLC analysis.

3.8.2.7. Scope and limitations of α-heteroaryl α-substituted α-AA derivatives

With the proof-of-concept established and optimized reaction conditions in hand, the scope and limitations of the method could be established. Three protocols were evaluated: one for inversion of the stereochemistry - **Method A** (KHMDS (1.5 equiv.), CuOTf (1.1 equiv.), THF (0.1 M)) and two for retention of the stereochemistry - **Method B** and **Method C** (LiHMDS (1.5 equiv.), THF (0.1 M) or NaHMDS (1.5 equiv.), 18-crown-6 (3.0 equiv.), LiCl (1.1 equiv.), THF (0.1 M)). Two different methods for the retention of stereochemistry were selected since they proved to be complementary in their reaction outcomes (**Scheme 90**). At this point, it should be noted that all starting substrates for the rearrangement reaction, heteroaryl sulfonamides, were prepared according to a protocol developed in our group[192] and their synthesis, if not presented earlier, is summarized in the Experimental part. My part in this project focused on the evaluation of the role of *N*-alkyl substitution, amino acid

substitution (substitution excluding the ester part) part, and partially in the heterocycles scope. The influence and scope of the ester and heterocycle parts (majority) were the responsibility of my colleague Jozef Kristek.



Scheme 90: Optimal conditions for Smiles rearrangement.

First, the influence of the alkyl group on the nitrogen atom of sulfonamide was evaluated (**Figure 53**). Various groups were evaluated with respect to their structural diversity and steric hindrance. The screening of the influence of the alkyl chains (such as: -benzyl **3-167**, methyl **3-181**, butyl **3-182**, isopropyl **3-183**, allyl **3-185**, and **3-186**) revealed that in all cases the rearrangement proceeded in good yields and selectivity if retention of the configuration is considered. In case of inversion of the configuration, moderate to good selectivity was reached. Carbonyl groups (-$COCH_3$ **3-180** and *t*-butoxycarbonyl **3-187**) were also successfully incorporated, as well as various substituted benzylic groups.

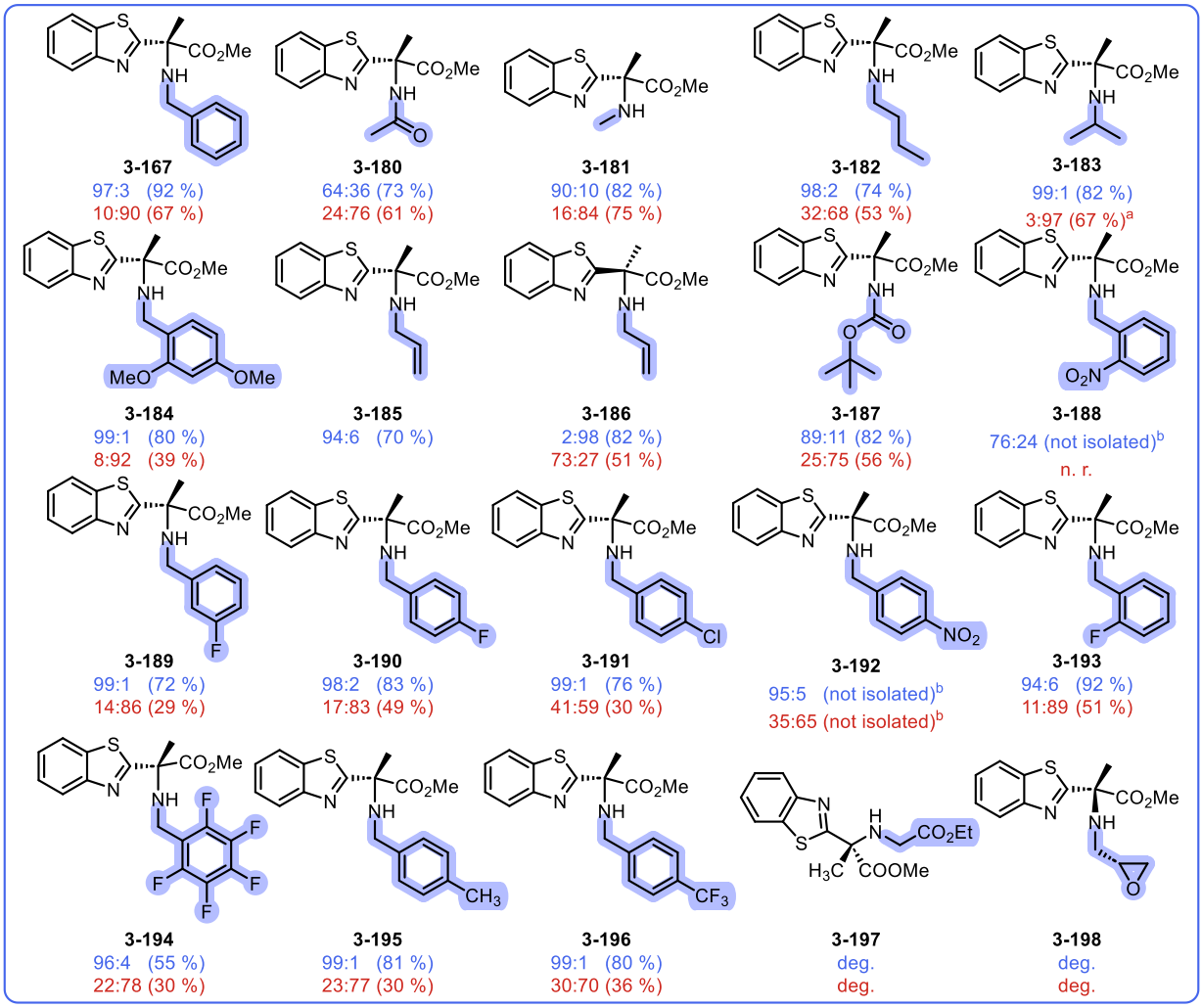


Figure 53: The scope and limitations of nitrogen substitution. *Yields refer to pure isolated compounds. [a]Partial conversion, yield calculated on the recuperated starting material; n.r. = no reaction; deg. = degradation of starting material [b]not purified, NMR conversion and HPLC were measured on crude reaction mixture.*

Next, the diversity of the heterocycles was investigated. We have successfully incorporated pyrimidine **3-199** and **3-207**, oxazole **3-200**, thiazol **3-201**, pyridine **3-203**, benzoimidazol **3-204**, imidazole **3-205,** as well as chlorinated BT sulfonamide **3-208** into the rearranged HAAs (**Figure 54**). Substituted BT heterocycles in different positions were not prepared due to the intermediate instability and therefore could not be tested under the reaction conditions. One of our goals was also the incorporation of the purine heterocycle, which we have successfully formed as a C6 rearranged product with alanine **3-206**, but due to the problematic separation of the peaks on the chiral HPLC, we were unable to determine the optical purity of the product.

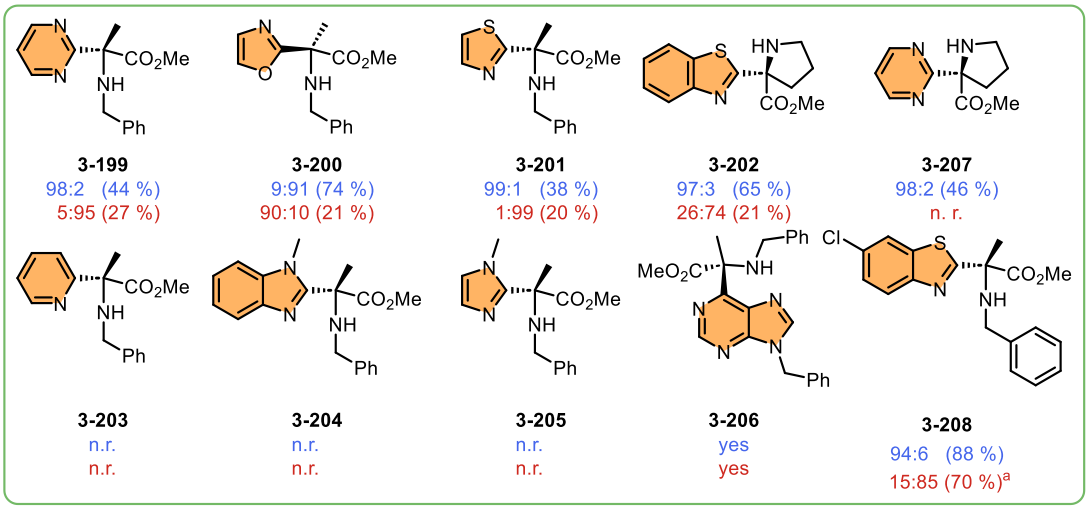


Figure 54: Heterocyclic scope and limitations. *[a]Partial conversion, yield calculated on the recuperated starting material; n.r. = no reaction; deg. = degradation of starting material; [b]not purified, NMR conversion and HPLC were measured on crude reaction mixture.*

We have also screened various ester groups as well as amides. In all cases, the products were obtained with good to excellent yields and enantioselectivity (**Figure 55**).

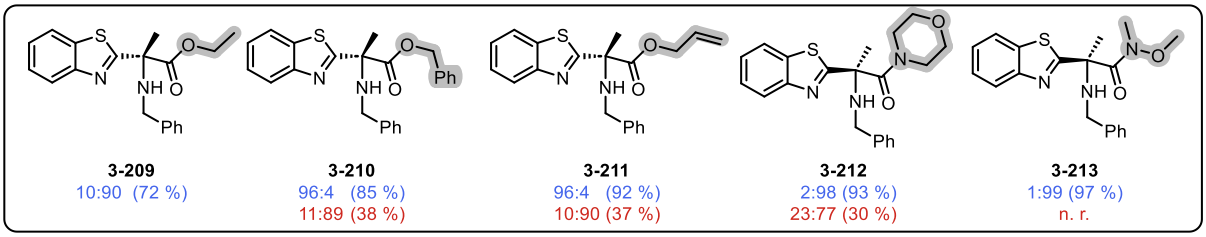


Figure 55: Scope and limitations of the ester and amide parts. *Yields refer to pure isolated compounds.*

A high degree of flexibility was found in the incorporation of different amino acids (**Figure 56**). This included methyl esters of alanine as our model substrate **3-167** and **3-184**, as well as valine **3-218** and **3-215**, leucine **3-214** and **3-217**, proline **3-224**, **3-202** and **3-207**, glutamic acid **3-178** and **3-219**, tryptophan **3-221**, phenylalanine **3-216** and benzyl-protected tyrosine **3-222**. Glycine **3-220** produced the product as racemate in both cases. The isoleucine substrate **3-209** was obtained only under modified conditions using LiHMDS with the addition of 6.0 equiv. of HMPA, resulting in the formation of the racemic product. Incorporation of methoxy groups **3-208** into the substrate did not help in this case.

It was observed that in case of more complex AA-based sulfonamide substrates, only the conditions for **retention** of the configuration (**Method B/C**) allowed the preparation of the desired HAA in good yield and stereoselectivity. In such cases, **Method A**, which is supposed to yield the product with inversion of the configuration, produced only an unchanged starting material (no racemization was observed) suggesting that the reaction conditions do not allow for the acidic proton removal.

However, in most cases, the products were obtained with good yields and excellent enantioselectivities. In some cases, partial and/or low conversion was observed. Other amino acid-containing starting materials, such as methionine, cysteine, lysine, and arginine, were problematic to prepare and therefore were not tested.

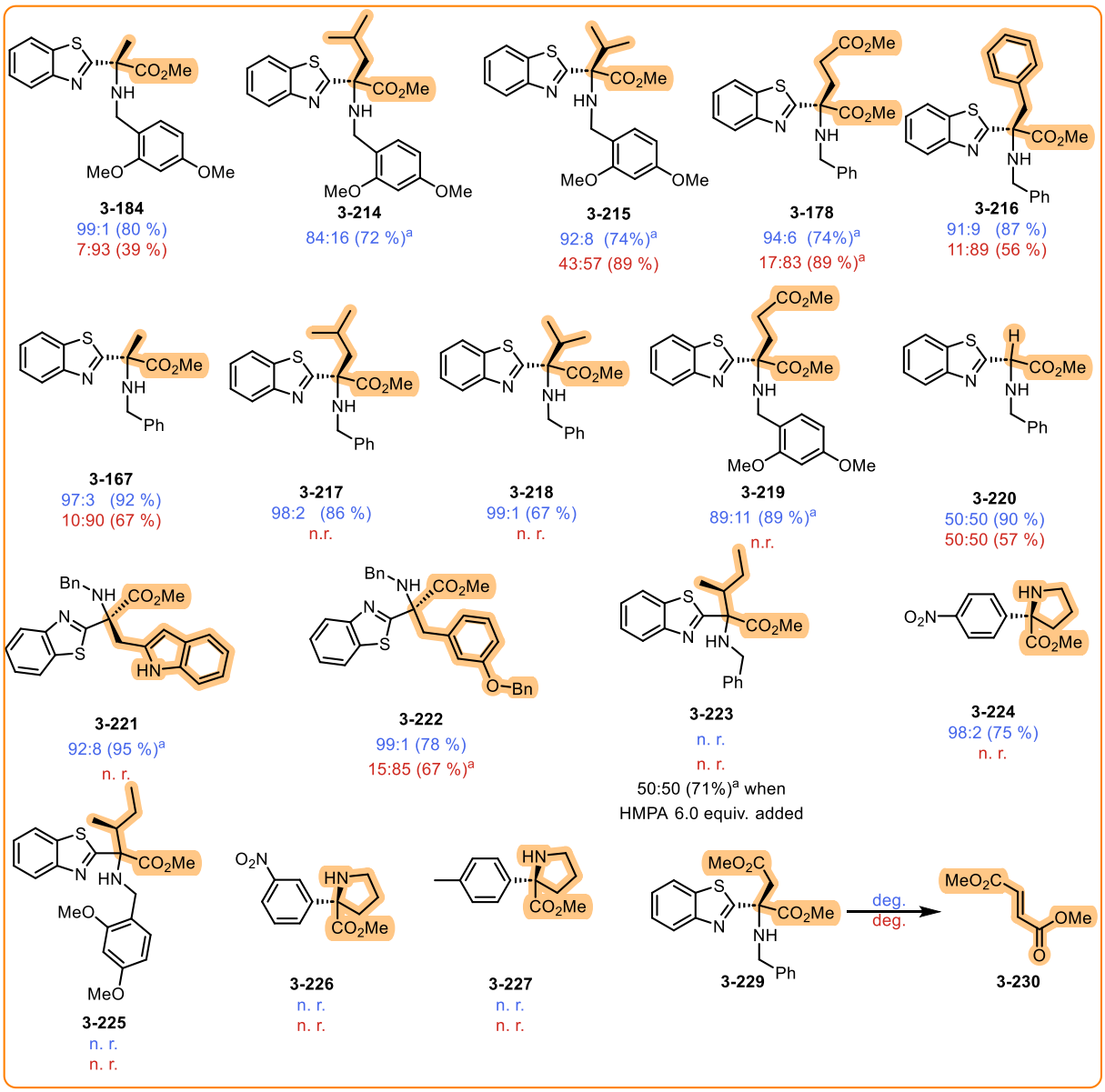


Figure 56: Amino ester part – scope and limitations. *[a]Partial conversion, yield calculated on the recuperated starting material; n.r. = no reaction; deg. = degradation of starting material; Yields refer to pure isolated compounds.*

Asparagic acid containing substrate **3-212** was degraded to dimethylformate **3-213** most probably due to competitive deprotonation of protons in β position

3.9. Conclusion

In this chapter, we successfully found conditions for our previously developed method for the heteroaryl sulfonamide synthesis, that broadened the scope of the method to additional heterocycles. This expansion included nitrogen-containing heterocycles such as pyridines, pyrimidines, imidazoles, benzo[*d*]imidazoles, and purine-26-diones, as well as oxygen-containing benzo[*d*]oxazoles and oxazoles, and sulfur-containing benzo[*d*]thiazoles and thiazoles. Our results showed that such heteroaryl sulfonamides are prone to nucleophilic attack at the α-carbon, making them suitable candidates for Smiles rearrangement.

The advantage of observed reactivity was used to develop a novel methodology for the asymmetric synthesis of quaternary α-heteroaryl α-substituted α-amino acids. Our mechanistic investigations, supported by detailed computational studies (DFT calculations), indicated that the selectivity of the rearrangement products is influenced by the concept of Memory of chirality. This allowed us to achieve selective synthesis of products with either inversion or retention of stereochemistry from a single starting material.

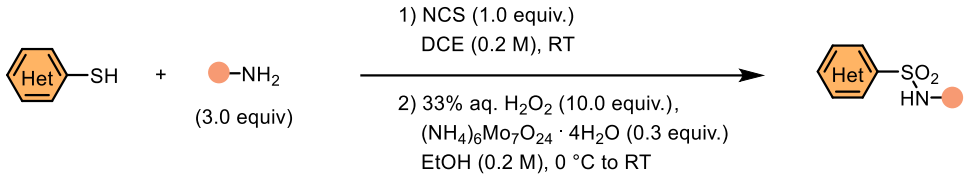
In conclusion, we have developed a robust and selective protocol for the synthesis of α-heteroaryl α-substituted α-AAs, which can be used further in the design of novel organocatalysts and/or the preparation of Xeno nucleic acids. This methodology provides significant advances in the field of asymmetric synthesis, offering new opportunities for the development of complex structures.

3.10. Experimental section

All starting materials were purchased from commercial suppliers and used without further purification, unless otherwise stated. All reactions were performed in round-bottom flasks fitted with rubber septa using standard laboratory techniques under positive pressure of argon (Air Liquide, >99.5% purity). Anhydrous Tetrahydrofuran (THF), dichloromethane (DCM) and 1,2-dichloroethane (DCE) were purchased from Sigma-Aldrich. **Caution! HMPA and DCE are toxic. CARE SHOULD BE TAKEN!** Purification of reaction products was carried out by column chromatography using standard grade silica gel (60 Å, 230–400 mesh), or by preparative thin layer chromatography glass plates precoated with silica gel (silica gel G-200 F_{254}, particle size 0.040−0.063 mm). Analytical thin-layer chromatography was performed on a thin-layer chromatography (TLC) aluminum plates pre-coated with silica gel (silica gel 60 F_{254}). Visualization was accomplished with UV light, phosphomolybdic acid, and potassium permanganate stains, followed by heating. Reactions run at temperatures of -78 °C (N_2/acetone) or -95 °C (N_2/methanol) were carried out using a cooling bath, and indicated temperatures refers to the cooling bath temperature. The determination of melting points was done on a Büchi melting point apparatus. The ^{1}H NMR and $^{13}C\{^{1}H\}$ NMR spectra were measured on JEOL ECA400II (400 and 101 MHz) or JEOL 500 ECA (500 and 126 MHz) in Chloroform-d or CD_3OD. Chemical shifts are reported in ppm, and their calibration was carried out (a) in the case of ^{1}H NMR experiments on the residual peak of non-deuterated solvent δ ($CDCl_3$) = 7.26 ppm or δ (CD_3OD) = 3.31 ppm, δ (DMSO-d_6)= 2.50 ppm, δ (acetone-d_6)= 2.05 ppm and in the case of ^{13}C NMR experiments on the middle peak of the ^{13}C signal in deuterated solvent δ ($CDCl_3$) = 77.16 ppm, δ (CD_3OD) = 49.00 ppm, (DMSO-d_6)= 39.52 ppm, (acetone-d_6)= 29.84 ppm. The proton coupling patterns are represented as a singlet (s), a doublet (d), a doublet of a doublet (dd), a triplet (t), a triplet of a triplet (tt), and a multiplet (m). High-resolution mass spectrometry (HRMS) on Agilent 6230 high-resolution mass spectrometer with electrospray ionization (ESI) and a time-of-flight analyzer operating in a positive or negative full scan mode in the range of 100 − 1700 m/z. High-performance liquid chromatography (HPLC) was performed using an Agilent 1290 Infinity II system with UV-VIS detector and an Agilent InfinityLab LC/MSD mass detector. Purification using semiprep HPLC was carried out on Agilent 1290 Infinity II with UV-VIS and mass detector Agilent InfinityLab LC/MSD using the C18 reverse-phase column (Agilent 5Prep-C18 10x21.2 mm). The gradient

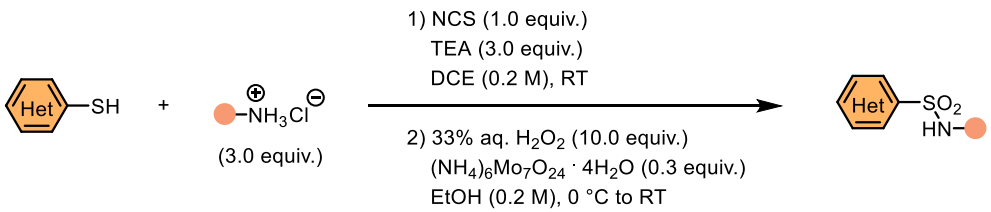
was formed from 15 mM aqueous ammonium acetate (buffer) and methanol with a flow rate of 20 mL/min. Chiral analysis was performed on Waters Alliance 2695 with autosampler and UV-VIS detector Waters 2996 PDA using chiral columns (CHIRAL ART Amylose-SA 250x4,6 mm, 5 µm; CHIRALCEL Cellulose OD-H, 250x4,6 mm, 5 µm; CHIRALCEL Cellulose OZ-H, 250x4,6 mm, 5 µm). All solvents used were HPLC-grade solvents purchased from Merk. The column employed and the respective solvent mixture are indicated for each experiment. Specific rotations ($[\alpha]_D^T$) were measured with Perkin Elmer Polarimeter 241 Automatic (Massachusetts, USA) at the indicated temperature. Measurements were performed in a 1 mL cell (50 mm length) with concentrations (g/(100 mL)) reported in corresponding solvent. All microwave irradiation experiments were carried out in a dedicated CEM-*d*iscover mono-mode microwave apparatus. The reactor was used in the standard configuration as delivered, including proprietary software. The reactions were carried out in 10- or 35-mL glass vials that were sealed with silicone/PTFE caps, which can be exposed to a maximum of 250 °C and 20 bar internal pressure. The temperature was measured with an IR sensor on the outer surface of the process vial. After the irradiation period, the reaction vessels were cooled to ambient temperature by gas jet cooling.

Method A



Heterocyclic thiol (1 mmol, 1.0 equiv.) and amine (3 mmol, 3.0 equiv.) were suspended in DCE (0.2 M) at RT and the resulting mixture was stirred at RT for 10 min. NCS (1 mmol, 1.0 equiv.) was added portion wise over a period of 5 min, and the whole mixture was stirred for additional 2 h at RT. The whole slurry was filtered, filter cake was washed with DCM, and the combined filtrates were evaporated *in vacuo*. Residue was dissolved in EtOH (0.2 M), cooled to 0 °C and a premixed cold (0 °C) bright yellow solution of 33% aq. H_2O_2 (10 mmol, 10.0 equiv.) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.3 mmol, 0.3 equiv.) was added with help of pipette Pasteur (*CAUTION: the use of metallic needle must be avoided!*). The resulting mixture was stirred at 0 °C for 0.5 h before it was allowed to warm to RT (cooling bath removed) and stirred for additional 8 h at RT. The whole mixture was cooled to 0 °C (ice/water) and sat. aq. Na_2SO_3 was added. The whole mixture was stirred at 0 °C for 10 min (*presence of peroxide was checked by iodide paper and if necessary additional of Na_2SO_3 was added*) before it was filtered. Filter cake was washed with EtOH, and the combined filtrates were concentrated under reduced pressure. The residue was diluted by water and the whole mixture was extracted with DCM. Combined organic layers were washed with H_2O, brine, dried over Na_2SO_4, and the volatiles were removed under reduced pressure. The crude product was washed with hexane and purified by recrystallization or by column chromatography.

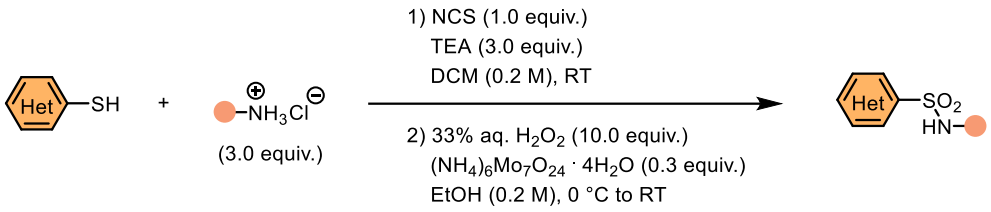
Method B



Heterocyclic thiol (1 mmol, 1.0 equiv.), TEA (3.0 mmol, 3.0 equiv.) and amine hydrochloride (3 mmol, 3.0 equiv.) were suspended in DCE (0.2 M) at RT and the resulting mixture was stirred at RT for 10 min. NCS (1 mmol, 1.0 equiv.) was added portion wise over a period of 5 min, and the whole mixture was stirred for additional 2 h at RT. The whole slurry was filtered, filter cake was washed with DCM, and the combined filtrates were evaporated *in vacuo*. Residue was dissolved in EtOH (0.2 M), cooled to 0 °C and a premixed cold (0 °C) bright yellow solution of

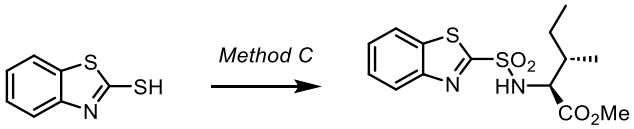
33% aq. H_2O_2 (10 mmol, 10 equiv.) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.3 mmol, 0.3 equiv.) was added with help of pipette Pasteur (*CAUTION: the use of metallic needle must be avoided!*). The resulting mixture was stirred at 0 °C for 0.5 h before it was allowed to warm to RT (cooling bath removed) and stirred for additional 8 h at RT. The whole mixture was cooled to 0 °C (ice/water) and sat. aq. Na_2SO_3 was added. The whole mixture was stirred at 0 °C for 10 min (*presence of peroxide was checked by iodide paper and if necessary additional of Na_2SO_3 was added*) before it was filtered. Filter cake was washed with EtOH, and the combined filtrates were concentrated under reduced pressure. The residue was diluted by water and the whole mixture was extracted with DCM. Combined organic layers were washed with H_2O, brine, dried over Na_2SO_4, and the volatiles were removed under reduced pressure. The crude product was washed with hexane and purified by recrystallization or by column chromatography.

Method C



Heterocyclic thiol (1 mmol, 1.0 equiv.), TEA (3.0 mmol, 3.0 equiv.) and amine hydrochloride (3 mmol, 3.0 equiv.) were suspended in DCM (0.2 M) at RT and the resulting mixture was stirred at RT for 10 min. NCS (1 mmol, 1.0 equiv.) was added portion wise over a period of 5 min, and the whole mixture was stirred until TLC indicated consumption of starting material. The whole slurry was filtered, filter cake was washed with DCM, and the combined filtrates were evaporated *in vacuo*. Residue was dissolved in EtOH (0.2 M), cooled to 0 °C and a premixed cold (0 °C) bright yellow solution of 33% aq. H_2O_2 (10 mmol, 10 equiv.) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.3 mmol, 0.3 equiv.) was added with help of pipette Pasteur (*CAUTION: the use of metallic needle must be avoided!*). The resulting mixture was stirred at 0 °C for 0.5 h before it was allowed to warm to RT (cooling bath removed) and stirred until TLC indicated consumption of starting material. Mixture was diluted with H_2O and DCM following extraction. Combined organic layers were washed brine, dried over Na_2SO_4, and the volatiles were removed under reduced pressure. The crude product was washed with hexane and purified by recrystallization or by column chromatography.

methyl (benzo[*d*]thiazol-2-ylsulfonyl)-L-isoleucinate (**3-103**)



3-65 **3-103**

Method C: Starting from 2-mercaptobenzothiazol **3-65** (1 g, 5.9 mmol, 1.0 equiv.) and L-isoleucine methylester hydrochloride (3.23 g, 17.8 mmol, 3.0 equiv.). The crude sulfenamide had to be purified prior the oxidation step, otherwise the decomposition occurred (column chromatography SiO_2; petroleum ether/EtOAc = 10:1 – 5:1). The crude sulfonamide **3-103** was purified by column chromatography (SiO2; petroleum ether/EtOAc = 4:1) to afford pure **3-103** (1.88 g, 98 %, *d.r.* ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.12 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.97 (dd, *J* = 7.7, 1.9 Hz, 1H), 7.60 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 7.55 (ddd, *J* = 8.4, 7.2, 1.3 Hz, 1H), 5.68 (d, *J* = 9.7 Hz, 1H), 4.32 (dd, *J* = 9.7, 4.9 Hz, 1H), 3.49 (s, 3H), 1.92 (dqt, *J* = 9.2, 6.8, 4.7 Hz, 1H), 1.42 (dtd, *J* = 14.8, 7.4, 4.4 Hz, 1H), 1.19 (ddq, *J* = 14.4, 9.2, 7.4 Hz, 1H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.6, 165.7, 152.4, 136.4, 127.8, 127.6, 125.1, 122.4, 61.5, 52.6, 38.6, 24.7, 15.6, 11.5.

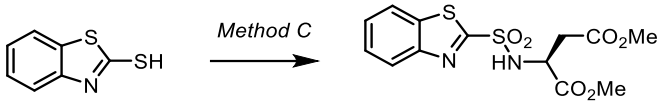
MS (ESI) *m/z* (%): 343 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{14}H_{19}N_2O_4S_2$: 343.0786; found: 343.0782.

R_f = 0.60 (hexane/ EtOAc = 3:1)

$[\alpha]_D^{25}$ = +36.5° (*c* 1.0, $CHCl_3$)

dimethyl (benzo[*d*]thiazol-2-ylsulfonyl)-L-aspartate (**3-104**)



3-65 **3-104**

Method C: Starting from 2-mercaptobenzothiazol **3-65** (1.0 g, 6.0 mmol, 1.0 equiv.) and L-aspartic acid dimethylester hydrochloride (3.58 g, 17.9 mmol, 3.0 equiv.). The crude product was purified by crystallization from 40% ethanol to afford **3-104** as white crystal (1.52 g, 71 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.15 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.98 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.59 (dtd, *J* = 21.7, 7.3, 1.4 Hz, 2H), 6.19 (d, *J* = 8.4 Hz, 1H), 4.67 (dt, *J* = 8.5, 4.3 Hz, 1H), 3.67 (s, 3H), 3.55 (s, 3H), 3.15 – 3.01 (m, 2H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.0, 170.0, 165.7, 152.4, 136.5, 127.9, 127.6, 125.2, 122.4, 53.3, 53.0, 52.4, 37.6.

MS (ESI) *m/z* (%): 358 $[M+H]^+$ (100).

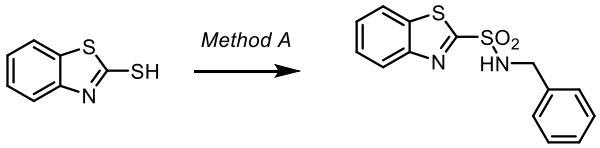
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{13}H_{15}N_2O_6S_2$: 359.0366; found: 359.0359.

R_f = 0.30 (hexane/ EtOAc = 2:1)

$[\alpha]_D^{23}$ = +38.0° (*c* 1.0, $CHCl_3$)

m. p. = 100-102 °C

N-benzylbenzo[*d*]thiazole-2-sulfonamide (**3-106**)



3-65 **3-106**

Method A: Starting from 2-mercaptobenzothiazol **3-65** (1.45 g, 8.7 mmol, 1.0 equiv.) and benzylamine (2.85 g, 26.1 mmol, 3.0 equiv.). The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 8:1) to afford **3-106** as yellow solid (2.48 g, 92 % yield).

^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm): 8.17 – 8.14 (m, 1H), 7.99 – 7.95 (m, 1H), 7.66 – 7.57 (m, 1H), 7.59 – 7.54 (m, 1H), 7.31 – 7.26 (m, 4H), 7.26 – 7.22 (m, 1H), 5.46 (t, *J* = 5.4 Hz, 1H), 4.44 (d, *J* = 6.1 Hz, 2H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 166.0, 152.4, 136.5, 135.7, 128.9, 128.3, 128.2, 127.8, 127.6, 125.2, 122.3, 48.2.

MS (ESI) *m/z* (%): 305 $[M+H]^+$ (100).

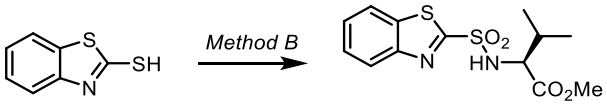
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{14}H_{13}N_2O_2S_2$: 305.0413; found: 305.0412.

R_f = 0.50 (hexane/ EtOAc = 3:1)

$[\alpha]_D^{23}$ = +38.1° (*c* 1.0, $CHCl_3$)

m. p. = 108-112 °C

methyl (benzo[*d*]thiazol-2-ylsulfonyl)-L-valinate (3-109)



3-65 **3-109**

Method B: Starting from 2-mercaptobenzothiazol **3-65** (1.0 g, 6.0 mmol, 1.0 equiv.) and L-valine methylester hydrochloride (3.0 g, 17.9 mmol, 3.0 equiv.). The crude product was purified by crystallization from 40% ethanol to afford **3-109** as white crystal (1.45 g, 74 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.11 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.97 (dd, *J* = 7.4, 1.2 Hz, 1H), 7.63 – 7.52 (m, 2H), 5.89 – 5.60 (m, 1H), 4.28 (dd, *J* = 9.9, 4.6 Hz, 1H), 3.50 (s, 3H), 2.17 (pd, *J* = 6.8, 4.7 Hz, 1H), 1.04 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.6, 165.7, 152.4, 136.4, 127.8, 127.6, 125.1, 122.4, 62.1, 52.7, 31.7, 19.1, 17.3.

MS (ESI) *m/z* (%): 329 $[M+H]^+$ (100).

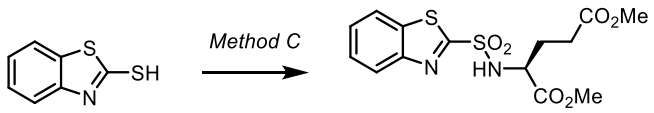
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{13}H_{17}N_2O_4S_2$: 329.0624; found: 329.0629.

R_f = 0.50 (hexane/ EtOAc = 3:1)

$[\alpha]_D^{24}$ = +52.8° (*c* 1.0, $CHCl_3$)

m. p. = 106-107 °C

dimethyl (benzo[*d*]thiazol-2-ylsulfonyl)-L-glutamate (3-110)



3-65 **3-110**

Method C: Starting from 2-mercaptobenzothiazol **3-65** (1.0 g, 6.0 mmol, 1.0 equiv.) and L-glutamic acid dimethylester hydrochloride (3.84 g, 17.9 mmol, 3.0 equiv.). The crude product was purified by crystallization from 40% ethanol to afford **3-110** as white crystal (1.61 g, 72 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.11 (ddd, *J* = 8.2, 1.4, 0.7 Hz, 1H), 7.97 (ddd, *J* = 7.9, 1.4, 0.7 Hz, 1H), 7.62 – 7.54 (m, 2H), 5.83 (d, *J* = 8.8 Hz, 1H), 4.50 (td, *J* = 8.7, 4.6 Hz, 1H), 3.66 (s, 3H), 3.59 (s, 3H), 2.61 – 2.48 (m, 2H), 2.31 – 2.22 (m, 1H), 2.01 (dddd, *J* = 14.2, 8.8, 7.7, 6.4 Hz, 1H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 173.1, 171.5, 165.6, 152.3, 136.4, 127.9, 127.6, 125.1, 122.4, 56.2, 53.1, 52.0, 29.7, 28.3.

MS (ESI) *m/z* (%): 373 $[M+H]^+$ (100).

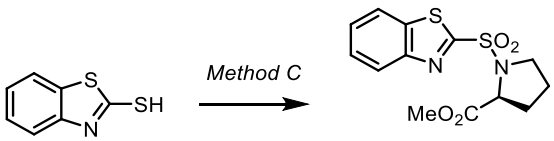
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{14}H_{17}N_2O_6S_2$: 373.0523; found: 373.0531.

R_f = 0.50 (hexane/ EtOAc = 1:1)

$[\alpha]_D^{23}$ = +38.1° (*c* 1.0, $CHCl_3$)

m. p. = 106-108 °C

methyl (benzo[d]thiazol-2-ylsulfonyl)-L-prolinate (3-111)



3-65 **3-111**

Method C: Starting from 2-mercaptobenzothiazol **3-65** (1.0 g, 5.8 mmol, 1.0 equiv.), 3.5 equiv. of TEA and L-proline methylester hydrochloride (2.2 g, 17.4 mmol, 3.0 equiv.). The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 3:2) to afford **3-111** as a slightly yellowish crystals (1.27 g, 65 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.18 (d, *J* = 8.2 Hz, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 7.65 – 7.59 (m, 1H), 7.57 (td, *J* = 7.7, 7.3, 1.2 Hz, 1H), 4.71 (dd, *J* = 8.6, 3.5 Hz, 1H), 3.85 – 3.77

(m, 1H), 3.74 (s, 3H), 3.64 (dt, *J* = 9.8, 7.4 Hz, 1H), 2.26 – 2.14 (m, 1H), 2.14 – 1.99 (m, 2H), 1.97 – 1.85 (m, 1H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 172.0, 164.5, 152.6, 136.3, 127.7, 127.5, 125.3, 122.2, 61.4, 52.7, 49.5, 31.1, 24.8.

MS (ESI) *m/z* (%): 327 $[M+H]^+$ (100).

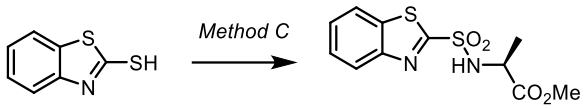
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{13}H_{15}N_2O_4S_2$: 327.0468; found 327.0467.

R_f = 0.30 (hexane/ EtOAc = 2:1)

$[\alpha]_D^{24}$ = -214.8° (*c* 1.01, $CHCl_3$)

m. p. = 74-75 °C

methyl (benzo[*d*]thiazol-2-ylsulfonyl)-L-alaninate (3-112)



3-65 **3-112**

Method C: Starting from 2-mercaptobenzothiazol **3-65** (1.45 g, 8.7 mmol, 1.0 equiv.) and L-alanine methylester hydrochloride (3.65 g, 26.1 mmol, 3.0 equiv.). The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 3:1) to afford **3-112** as orange solid (2.01g, 77 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.14 (dt, *J* = 7.5, 1.6 Hz, 1H), 7.97 (dt, *J* = 8.7, 1.3 Hz, 1H), 7.63 – 7.58 (m, 1H), 7.58 – 7.54 (m, 1H), 5.87 (d, *J* = 7.8 Hz, 1H), 4.52 – 4.43 (m, 1H), 3.60 (s, 3H), 1.51 (d, *J* = 7.1 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 172.4, 165.9, 152.4, 136.4, 127.9, 127.7, 127.6, 125.2, 122.4, 53.0, 52.6, 20.1.

MS (ESI) *m/z* (%): 301 $[M+H]^+$ (100).

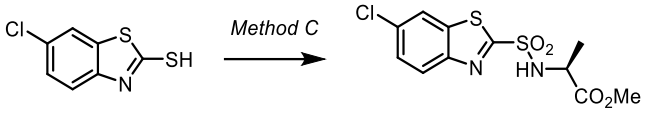
HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{11}H_{12}KN_2O_4S_2$: 338.9876; found: 338.9873.

R_f = 0.40 (hexane/EtOAc = 3:1)

$[\alpha]_D^{21}$ = +15.9° (*c* 0.45, $CHCl_3$)

m. p. = 116-118 °C

methyl ((6-chlorobenzo[*d*]thiazol-2-yl)sulfonyl)-L-alaninate (**3-113**)



3-231 **3-113**

Method C: Starting from 5-chloro-2-mercaptobenzothiazol **3-231** (0.33 g, 2.4 mmol, 1.0 equiv.) and L-alanine methylester hydrochloride (1,01 g, 7.3 mmol, 3.0 equiv.). The crude sulfenamide had to be purified prior the oxidation step, otherwise the decomposition occurred (column chromatography SiO_2; petroleum ether/EtOAc = 10:1 – 5:1). The crude sulfonamide **3-113** was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford pure **3-113** (0.44 g, 84 %, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.12 (d, *J* = 2.0 Hz, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 7.54 (dd, *J* = 8.6, 2.0 Hz, 1H), 5.86 (d, *J* = 8.1 Hz, 1H), 4.51 – 4.45 (m, 1H), 3.64 (s, 3H), 1.52 (d, *J* = 7.4 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 172.4, 167.8, 153.1, 134.6, 133.9, 128.6, 124.8, 123.2, 53.1, 52.6, 20.1.

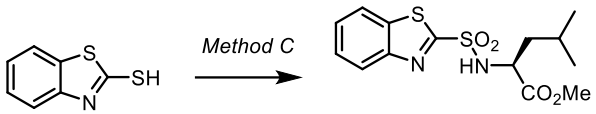
MS (ESI) *m/z* (%): 334 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{11}H_{12}ClN_2O_4S_2$: 334.9922; found: 334.9929.

R_f = 0.50 (hexane/ EtOAc = 3:1)

$[\alpha]_D^{25}$ = -36.8° (*c* 1.0, $CHCl_3$)

methyl (benzo[*d*]thiazol-2-ylsulfonyl)-L-leucinate (**3-115**)



3-65 **3-115**

Method C: Starting from 2-mercaptobenzothiazol **3-65** (1.0 g, 6.0 mmol, 1.0 equiv.) and L-Leucine methylester hydrochloride (3.2 g, 17.9 mmol, 3.0 equiv.). The crude product was purified by crystallization from 40% ethanol to afford **3-105** as white crystal (1.49 g, 73 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.17 – 8.09 (m, 1H), 8.01 – 7.93 (m, 1H), 7.62 – 7.54 (m, 2H), 5.50 (d, *J* = 10.6 Hz, 1H), 4.44 (td, *J* = 9.4, 5.5 Hz, 1H), 3.50 (s, 3H), 1.88 (dh, *J* = 8.3, 6.5 Hz, 1H), 1.63 – 1.55 (m, 2H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.94 (d, *J* = 6.7 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 172.6, 165.7, 152.4, 136.5, 127.8, 127.6, 125.1, 122.4, 55.6, 52.8, 42.5, 24.5, 22.9, 21.5.

MS (ESI) *m/z* (%): 343 $[M+H]^+$ (100).

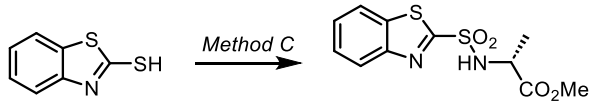
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{14}H_{19}N_2O_4S_2$: 343.0781; found: 343.0773.

R_f = 0.40 (hexane/ EtOAc = 3:1)

$[\alpha]_D^{22}$ = +27.8° (*c* 1.0, $CHCl_3$)

m. p. = 110-112 °C

methyl (benzo[*d*]thiazol-2-ylsulfonyl)-D-alaninate (3-118)



3-65 **3-118**

Method C: Starting from 2-mercaptobenzothiazol **3-65** (1.36 g, 7.9 mmol, 1.0 equiv.) and D-alanine methylester hydrochloride (3.33 g, 23.6 mmol, 3.0 equiv.). The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 6:1 → 4:1) to afford **3-118** as orange solid (2.01 g, 84 % yield, *e.r.* = ≥ 1:99).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.16 – 8.12 (m, 1H), 8.02 – 7.93 (m, 1H), 7.63 – 7.58 (m, 1H), 7.58 – 7.54 (m, 1H), 5.78 (d, *J* = 7.7 Hz, 1H), 4.48 (p, *J* = 7.3 Hz, 1H), 3.61 (s, 3H), 1.52 (d, *J* = 7.1 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 172.4, 165.9, 152.4, 136.5, 127.9, 127.6, 125.2, 122.4, 53.0, 52.6, 20.1.

MS (ESI) *m/z* (%): 301 $[M+H]^+$ (100).

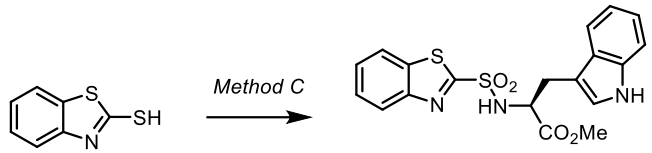
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{11}H_{13}N_2O_4S_2$: 301.0317; found: 301.0319.

R_f = 0.40 (hexane/EtOAc = 3:1)

$[\alpha]_D^{20}$ = -17.0° (*c* 1.0, $CHCl_3$)

m. p. = 115-117 °C

methyl (benzo[*d*]thiazol-2-ylsulfonyl)-L-tryptophanate (**3-119**)

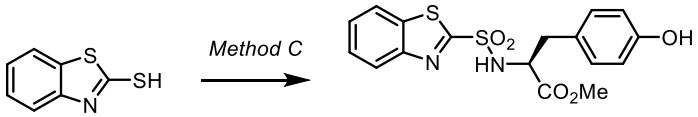


3-65 **3-119**

Method C: Starting from 2-mercaptobenzothiazol **3-65** (1.36 g, 8.1 mmol, 1.0 equiv.) and L-tryptophan methylester hydrochloride (6.3 g, 24.2 mmol, 3.0 equiv.). The crude sulfenamide had to be purified prior the oxidation step, otherwise the decomposition occurred (column chromatography SiO_2; petroleum ether/EtOAc = 10:1 – 5:1). **The solution was concentrated to ~ 0.1 M solution of sulfonamide 3-119 in DCM due to decomposition.** The product was used in the next step without further purification.

CAUTION! If concentrated to dryness the product decomposes!

methyl (benzo[*d*]thiazol-2-ylsulfonyl)-L-tyrosinate (**3-120**)



3-65 **3-120**

Method C: Starting from 2-mercaptobenzothiazol **3-65** (1.0 g, 5.8 mmol, 1.0 equiv.) and L-tyrosine methylester hydrochloride (4.03 g, 17.4 mmol, 3.0 equiv.). The crude sulfenamide had to be purified prior the oxidation step, otherwise the decomposition occurred (SiO_2; petroleum ether/EtOAc = 4:1). The crude sulfonamide **3-120** was purified by column chromatography (SiO2; petroleum ether/EtOAc = 4:1) to afford pure **3-120** (0.95 g, 74 %).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.15 – 8.10 (m, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.60 (t, *J* = 7.6 Hz, 1H), 7.56 (t, *J* = 7.6 Hz, 1H), 6.99 – 6.93 (m, 2H), 6.68 – 6.61 (m, 2H), 5.53 (d, *J* = 8.5 Hz, 1H), 4.79 (s, 1H), 4.68 (dt, *J* = 8.4, 5.7 Hz, 1H), 3.55 (s, 3H), 3.09 (qd, *J* = 14.0, 5.6 Hz, 2H). **^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm):** 171.0, 165.6, 155.0, 152.4, 136.5, 130.8, 127.8, 127.6, 126.7, 125.2, 122.4, 115.7, 57.7, 52.8, 38.7.

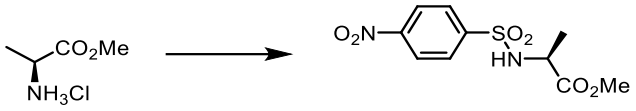
MS (ESI) *m/z* (%): 373 $[M+H]^+$ (100).

HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{17}H_{16}N_2O_5S_2$: 393.0573; found: 393.0575.

R_f = 0.50 (hexane/ EtOAc = 4:1)

$[\alpha]_D^{23}$ = +38.1° (*c* 1.0, $CHCl_3$) **m. p.** = 106-108 °C

methyl ((4-nitrophenyl)sulfonyl)-L-alaninate (3-232)



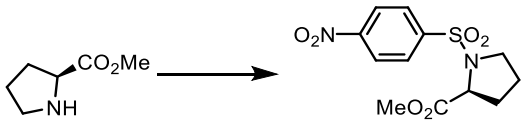
3-231 **3-232**

Prepared according to a literature. Spectroscopic data match those in the literature.[245]

To a solution L-alanine methyl ester hydrochloride **3-231** (1 g, 7.16 mmol, 1.0 equiv.) in DCM (10 mL, 0.5 M) was added TEA (3.0 mL, 21.5 mmol, 3.0 equiv.) followed by 4-nitrobenzenesulfonyl chloride (1.76 g, 7.9 mmol, 1.1 equiv.) at 0 °C and the mixture was stirred at room temperature for 16 hours. The mixture was diluted with DCM, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness. The crude product **3-232** was used in the next step without further purification (1.95g, 99 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.37 – 8.33 (m, 2H), 8.07 – 8.03 (m, 2H), 4.07 (q, *J* = 7.2 Hz, 1H), 3.59 (s, 3H), 2.96 (q, *J* = 7.2 Hz, 1H), 1.43 (d, *J* = 7.2 Hz, 3H).

methyl ((4-nitrophenyl)sulfonyl)-L-prolinate (3-234)



3-233 **3-234**

Prepared according to a literature. Spectroscopic data match those in the literature.[246]

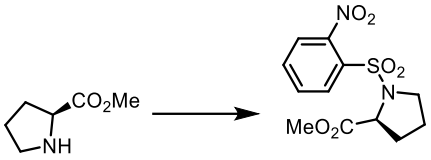
To a solution of *L*-Proline methyl ester **3-233** (0.34 g, 2.0 mmol, 1.0 equiv.) in DCM (8 mL, 0.25 M) was added TEA (0.84 mL, 6.0 mmol, 3.0 equiv.) followed by 4-nitrobenzenesulfonyl chloride (0.49 g, 2.2 mmol, 1.1 equiv.) at 0 °C and the mixture was stirred at room temperature for 16 hours. Then, the mixture was quenched with water and the layers were separated. The aqueous layer was extracted using DCM (3 x 50 mL). Combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **3-234** s a colorless oil (0.45 g, 72 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.39 – 8.36 (m, 2H), 8.10 – 8.07 (m, 2H), 4.47 (dd, *J* = 8.6, 3.8 Hz, 1H), 3.71 (s, 3H), 3.48 – 3.43 (m, 2H), 2.22 – 2.15 (m, 1H), 2.08 – 1.99 (m, 2H), 1.95 – 1.87 (m, 1H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 172.3, 150.2, 144.8, 128.8, 124.3, 60.7, 52.7, 48.4, 31.1, 24.9.

$[\alpha]_D^{22}$ = -69.5° (*c* 1.0, $CHCl_3$)

methyl ((2-nitrophenyl)sulfonyl)-L-prolinate (3-235)



3-233 **3-235**

Prepared according to a literature. Spectroscopic data match those in the literature.[246]

To a solution of *L*-Proline methyl ester **3-233** (0.40 g, 2.42 mmol, 1.0 equiv.) in DCM (9.7 mL, 0.25 M) was added TEA (1.02 mL, 7.26 mmol, 3.0 equiv.) followed by 2-nitrobenzenesulfonyl chloride (0.66 g, 2.91 mmol, 1.2 equiv.) at 0 °C and the mixture was stirred at room temperature for 16 hours. Then, the mixture was quenched with water and the layers were separated. The aqueous layer was extracted using DCM (3 x 50 mL). Combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **3-235** as a colorless oil (0.58 g, 77 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.74 (q, *J* = 1.9 Hz, 1H), 8.44 (ddt, *J* = 8.3, 2.3, 1.1 Hz, 1H), 8.23 (ddt, *J* = 4.1, 2.8, 1.9, 1.1 Hz, 1H), 7.75 (t, *J* = 8.0 Hz, 1H), 4.51 (ddd, *J* = 8.9, 3.8, 2.0 Hz, 1H), 3.72 (s, 3H), 3.51 – 3.41 (m, 2H), 2.25 – 2.15 (m, 1H), 2.10 – 2.03 (m, 1H), 2.03 – 1.89 (m, 2H).

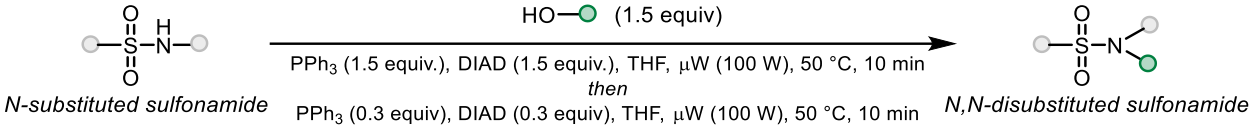
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 172.3, 148.4, 141.4, 133.1, 130.4, 127.3, 122.8, 60.7, 52.7, 48.4, 31.1, 24.9.

MS (ESI) *m/z* (%): 353 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{12\ h14}N_2O_6SK$: 353.0204; found: 353.0217.

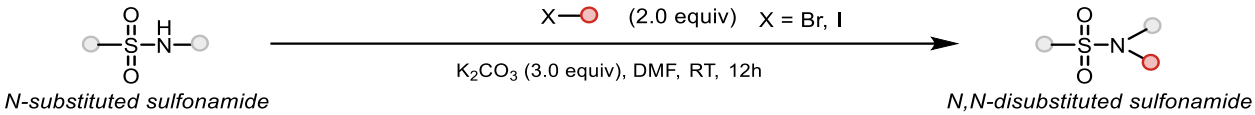
R_f = 0.40 (PE/ EtOAc = 2:1)

Method D: Fukuyama-Mitsunobu reaction protocol (FMR protocol)



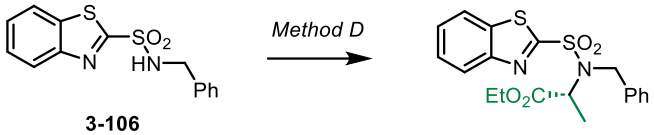
Sulfonamide (1.0 equiv.) and PPh_3 (1.5 equiv.) in dry THF (0.05 M) were placed to µW vial at RT, and alcohol (1.5 equiv.) followed by DIAD (1.5 equiv.) were added. The vial was closed with Teflon tap and the whole mixture was heated to 50 °C for 10 min in the microwave reactor (100 W). After cooling to RT, organic solvents were removed under reduced pressure and the conversion of the sulfonamide was analyzed with help of the ^1H NMR experiments. In case of incomplete conversion, the whole mixture was redissolved in dry THF (0.05 M), additional PPh_3 (0.3 equiv.) and DIAD (0.3 equiv.) were added and the whole procedure was repeated. In case of missing signals of the starting alcohol, an additional 0.3 equiv. of the corresponding alcohol was also added. When the starting sulfonamide was consumed, the crude product was purified by flash column chromatography on silica gel.

Method E: Alkylation protocol



Sulfonamide (1.0 equiv.) was dissolved in DMF (0.1 M) and K_2CO_3 (3.0 equiv.) was added. The resulting mixture was stirred at RT for 5 min, and alkyl bromide or alkyl iodide (2.0 equiv.) was added dropwise over a period of 5 min. The whole mixture was stirred at RT for 16 h, before it was diluted with H_2O. The whole mixture was extracted with EtOAc, and combined organic layers were washed with, dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure.

ethyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-benzyl-L-alaninate **3-134**



3-106 **3-134**

Prepared according to a literature. Spectroscopic data match those in the literature.[187]

Method D: Starting from sulfonamide **3-106** (0.1 g, 0.33 mmol, 1.0 equiv.) and alcohol L(-)lactate (0.04 mL, 0.33 mmol, 1.0 equiv.). The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **3-134** as a slightly yellow oil (0.13 g, 98 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm): 8.21 – 8.15 (m, 1H), 8.00 – 7.95 (m, 1H), 7.61 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 7.56 (ddd, *J* = 8.5, 7.2, 1.4 Hz, 1H), 7.44 – 7.41 (m, 2H), 7.34 – 7.25 (m, 3H), 4.93 (d, *J* = 16.4 Hz, 1H), 4.86 (q, *J* = 7.3 Hz, 1H), 4.54 (d, *J* = 16.4 Hz, 1H), 3.86 – 3.72 (m, 2H), 1.34 (d, *J* = 7.4 Hz, 3H), 0.96 (t, *J* = 7.1 Hz, 3H).

^{13}C {^{1}H} NMR (101 MHz, Chloroform-*d*) δ (ppm): 170.7, 165.6, 152.7, 137.0, 136.5, 128.6, 128.2, 127.9, 127.7, 127.5, 125.2, 122.2, 61.6, 56.4, 50.3, 16.9, 13.8.

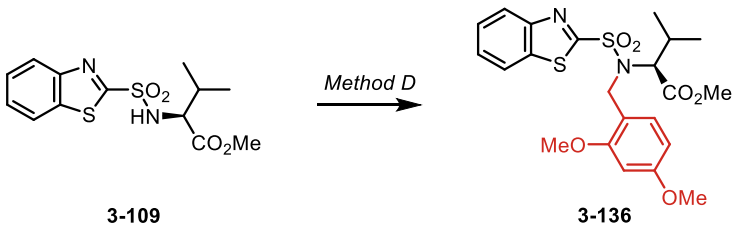
MS (ESI) *m/z* (%): 405 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{19}H_{21}N_2O_4S_2$: 405.0937; found: 405.0938.

R_f = 0.53 (hexane/ EtOAc = 3:1)

$[\alpha]_D^{22}$ = +26.3° (*c* 1.0, $CHCl_3$)

methyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-(2,4-dimethoxybenzyl)-L-valinate (**3-136**)



3-109 **3-136**

Method D: Starting from sulfonamide **3-109** (0.2 g, 0.61 mmol, 1.0 equiv.) and 2,4-dimethoxybenzylalkohol (0.21 g, 1.22 mmol, 2.0 equiv.). The crude product was purified by column chromatography (petroleum ether/EtOAc = 10:1) to afford **3-136** as a yellow oil (0.18 g, 61 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.17 (d, *J* = 8.2 Hz, 1H), 7.96 (d, *J* = 7.7 Hz, 1H), 7.62 – 7.57 (m, 1H), 7.57 – 7.51 (m, 2H), 6.45 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.37 – 6.34 (m, 1H), 4.85

(d, *J* = 16.2 Hz, 1H), 4.76 (d, *J* = 16.1 Hz, 1H), 4.28 (d, *J* = 10.3 Hz, 1H), 3.78 (s, 3H), 3.75 (s, 3H), 3.26 (s, 3H), 2.16 – 2.06 (m, 1H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.81 (d, *J* = 6.6 Hz, 3H).

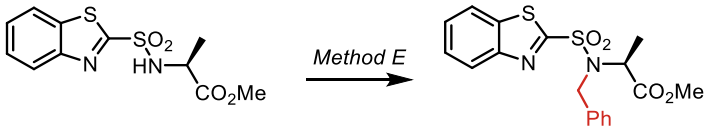
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 170.1, 165.9, 160.6, 158.2, 152.6, 136.6, 131.8, 130.4, 127.5, 127.4, 125.2, 122.2, 117.1, 104.2, 98.1, 66.9, 55.5, 55.4, 51.7, 44.5, 28.4, 20.0, 19.8.

MS (ESI) *m/z* (%): 479 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{14}H_{17}N_2O_5S_2$: 479.1311; found: 479.1305.

R_f = 0.38 (hexane/ EtOAc = 5:1)

methyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-benzyl-L-alaninate (**3-143**)



3-112 **3-143**

Method E: Starting from sulfonamide **3-112** (0.3 g, 1.0 mmol, 1.0 equiv.) and benzyl bromide (0.24 mL, 2.0 mmol, 2.0 equiv.). The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 10:1 → 4:1) to afford **3-143** as slightly yellow oil (0.33 g, 84 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm): 8.19 (ddd, *J* = 8.1, 1.4, 0.6 Hz, 1H), 8.03 – 7.93 (m, 1H), 7.62 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 7.56 (ddd, *J* = 8.0, 7.2, 1.4 Hz, 1H), 7.45 – 7.40 (m, 2H), 7.36 – 7.26 (m, 3H), 4.92 – 4.83 (m, 2H), 4.60 (d, *J* = 16.3 Hz, 1H), 3.34 (s, 3H), 1.36 (d, *J* = 7.3 Hz, 3H).

^{13}C {^{1}H} NMR (101 MHz, Chloroform-*d*) δ (ppm): 171.1, 165.6, 152.7, 136.8, 136.5, 128.6, 128.3, 127.9, 127.7, 127.5, 125.3, 122.3, 56.2, 52.3, 50.3, 16.6.

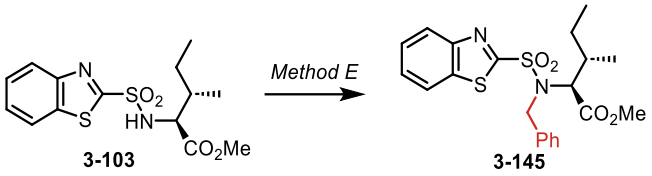
MS (ESI) *m/z* (%): 391 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{18}H_{19}N_2O_4S_2$: 391.0786; found: 391.0784.

R_f = 0.40 (hexane/ EtOAc = 3:1)

$[\alpha]_D^{23}$ = -29.1° (*c* 1.0, $CHCl_3$)

methyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-benzyl-L-isoleucinate (**3-145**)



Method E: Starting from sulfonamide **3-103** (0.365 g, 1.06 mmol, 1.0 equiv.) and benzyl bromide (0.25 mL, 2.11 mmol, 2.0 equiv.). The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **3-145** as colorless oil (0.24 g, 52 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.17 (ddd, *J* = 8.2, 1.3, 0.7 Hz, 1H), 7.98 (ddd, *J* = 8.0, 1.4, 0.7 Hz, 1H), 7.63 – 7.52 (m, 4H), 7.35 – 7.31 (m, 2H), 7.30 – 7.26 (m, 1H), 5.09 (d, *J* = 16.1 Hz, 1H), 4.73 (d, *J* = 16.1 Hz, 1H), 4.35 (d, *J* = 10.7 Hz, 1H), 3.26 (s, 3H), 1.70 – 1.61 (m, 1H), 1.47 (dtd, *J* = 15.1, 7.5, 2.4 Hz, 1H), 0.90 – 0.82 (m, 1H), 0.74 (d, *J* = 6.6 Hz, 3H), 0.38 (t, *J* = 7.4 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 170.4, 165.6, 152.5, 137.1, 136.5, 129.1, 128.5, 127.9, 127.7, 127.5, 125.1, 122.2, 65.8, 51.7, 50.2, 34.7, 25.5, 15.7, 10.4.

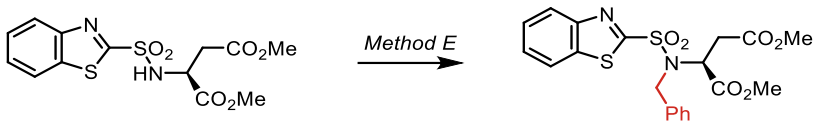
MS (ESI) *m/z* (%): 433 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{21}H_{24}KN_2O_4S_2$: 471.0809; found: 471.0811.

R_f = 0.50 (PE/ EtOAc = 2:1)

$[\alpha]_D^{23}$ = -32.7° (*c* 1.2, $CHCl_3$)

dimethyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-benzyl-L-aspartate (**3-146**)



3-104 **3-146**

Method E: Starting from sulfonamide **3-104** (1.0 g, 2.79 mmol, 1.0 equiv.) and benzyl bromide (0.67 mL, 5.58 mmol, 2.0 equiv.). The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 20:1 → 5:1) to afford **3-146** as a colorless solid (1.05 g, 84 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.20 – 8.17 (m, 1H), 8.01 – 7.98 (m, 1H), 7.63 (ddt, *J* = 8.1, 7.0, 1.1 Hz, 1H), 7.58 (ddt, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.43 (dd, *J* = 7.9, 1.6 Hz, 2H), 7.35 – 7.29 (m, 3H), 5.02 (dd, *J* = 8.4, 5.8 Hz, 1H), 4.89 (d, *J* = 15.7 Hz, 1H), 4.51 (d, *J* = 15.7 Hz, 1H), 3.53 (s, 3H), 3.34 (s, 3H), 2.99 (dd, *J* = 17.0, 8.4 Hz, 1H), 2.76 (dd, *J* = 17.0, 5.7 Hz, 1H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 170.6, 169.5, 165.7, 152.5, 136.5, 135.5, 128.9, 128.7, 128.4, 127.8, 127.6, 125.3, 122.3, 56.9, 52.7, 52.1, 52.0, 35.4.

MS (ESI) *m/z* (%): 449 $[M+H]^+$ (100).

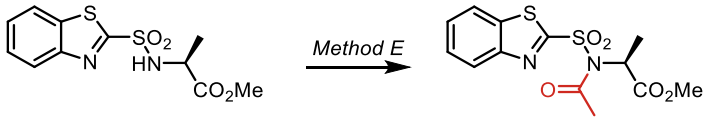
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{20}H_{21}N_2O_6S_2$: 449.0836; found: 449.0829.

R_f = 0.44 (PE/ EtOAc = 5:1)

$[\alpha]_D^{22}$ = -50.6° (*c* 1.0, $CHCl_3$)

m. p. = 110-112 °C

methyl *N*-acetyl-*N*-(benzo[*d*]thiazol-2-ylsulfonyl)-L-alaninate (**3-151**)



3-112 **3-151**

Sulfonamide **3-112** (0.5 g, 1.65 mmol, 1.0 equiv.) was dissolved in THF (8.2 mL, 0.2 M) followed by addition of NaH (60% suspension in mineral oil; 0.1 g, 5.5 mmol, 1.5 equiv.). The mixture was cooled to 0 °C and acetyl chloride (0.24 mL, 3.3 mmol, 2.0 equiv.) was added and the reaction mixture was stirred at RT for 16 h. After that H_2O mixture evaporated and the crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 10:1 → 4:1) to afford **3-151** as a slightly yellow oil (0.2g, 36 % yield, *e.r.* = ≥ 99:1).

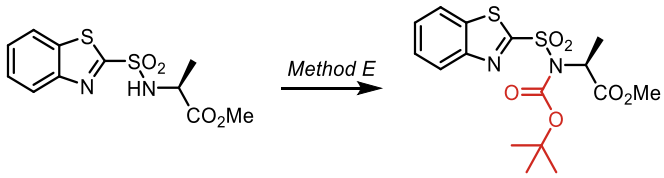
^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.21 (dd, *J* = 8.1, 1.8 Hz, 1H), 8.01 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.64 (ddd, J = 15.4, 7.2, 1.4 Hz, 2H), 5.06 (q, *J* = 6.9 Hz, 1H), 3.50 (s, 3H), 2.70 (s, 3H), 1.63 (d, *J* = 6.9 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 170.2, 169.9, 163.8, 152.2, 136.7, 128.6, 128.1, 125.8, 122.4, 56.6, 52.7, 25.9, 16.0.

MS (ESI) *m/z* (%): 343 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{13}H_{15}N_2O_5S_2$: 343.0417; found: 343.0426.

methyl N-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-(*tert*-butoxycarbonyl)-L-alaninate (**3-152**)



3-112 **3-152**

Method E: Sulfonamide **3-112** (0.2 g, 0.66 mmol, 1.0 equiv.) was dissolved in DCE (1.3 mL, 0.5 M) followed by addition of TEA (0.112 mL, 0.8 mmol, 1.2 equiv.). Subsequently, $(Boc)_2O$ (0.43 g, 2.0 mmol, 3.0 equiv.) was added and the reaction mixture was stirred at 60 °C for 16 h. Then, the mixture evaporated, and the crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 10:1 → 4:1) to afford **3-152** as a slightly yellow oil (0.225 g, 85 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.20 (dd, *J* = 8.5, 1.2 (Hz, 1H), 8.00 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.67 – 7.53 (m, 2H), 5.24 (q, *J* = 7.0 Hz, 1H), 3.75 (s, 3H), 1.77 (d, *J* = 7.0 Hz, 3H), 1.36 (s, 10H).

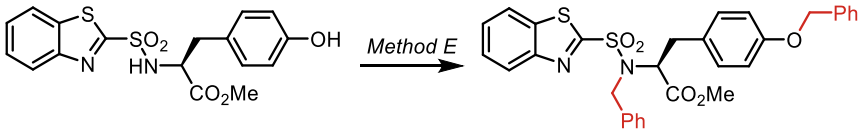
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 173.0, 170.4, 164.6, 151.8, 149.6, 137.1, 128.1, 127.7, 125.6, 122.3, 86.5, 86.4, 56.1, 52.8, 28.7, 27.9, 27.9, 16.6.

MS (ESI) *m/z* (%): 401 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{16}H_{21}N_2O_6S_2$: 401.0836; found: 401.0831.

R_f = 0.52 (hexane/ EtOAc = 4:1)

methyl (*S*)-2-(*N*-benzylbenzo[*d*]thiazole-2-sulfonamido)-3-(4-(benzyloxy)phenyl)propanoate (3-153)



3-120 **3-153**

Method E: Starting from sulfonamide **3-120** (0.3 g, 0.76 mmol, 1.0 equiv.) and benzyl bromide (0.27 mL, 2.3 mmol, 3.0 equiv.). The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 10:1) to afford **3-153** as a slightly yellow oil (0.95 g, 74 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.17 (d, *J* = 8.2 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.61 (td, *J* = 7.4, 1.2 Hz, 1H), 7.56 (td, *J* = 7.5, 1.3 Hz, 1H), 7.43 – 7.36 (m, 7H), 7.34 – 7.27 (m, 4H), 6.92 (d, *J* = 8.3 Hz, 2H), 6.73 (d, *J* = 8.4 Hz, 2H), 4.95 (s, 2H), 4.87 – 4.82 (m, 2H), 4.72 (d, *J* = 15.7 Hz, 1H), 3.22 (s, 3H), 3.13 (dd, *J* = 14.1, 8.5 Hz, 1H), 2.85 (dd, *J* = 14.1, 6.6 Hz, 1H).

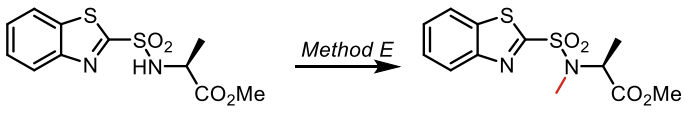
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 170.0, 165.8, 157.7, 152.6, 137.1, 136.5, 136.1, 130.3, 128.8, 128.7, 128.6, 128.6, 128.1, 128.1, 127.7, 127.6, 127.6, 125.2, 122.2, 114.8, 70.0, 61.9, 52.2, 50.8, 35.8, 29.8.

MS (ESI) *m/z* (%): 573 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{18}H_{18}N_3O_6S_2$: 573.1512; found: 573.1511.

$[\alpha]_D^{23}$ = -7.2° (*c* 1, $CHCl_3$)

methyl *N*-(benzo*[d]*thiazol-2-ylsulfonyl)-*N*-methyl-L-alaninate (3-154)



3-112 **3-154**

Method E: Starting from sulfonamide **3-112** (0.2 g, 0.67 mmol, 1.0 equiv.) and iodomethane (0.08 mL, 1.33 mmol, 2.0 equiv.). The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 8:1) to afford **3-154** as a colorless oil (0.193 g, 93 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.17 (ddd, *J* = 8.3, 1.2, 0.7 Hz, 1H), 7.97 (ddd, *J* = 8.1, 1.6, 0.7 Hz, 1H), 7.60 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 7.55 (ddd, *J* = 8.4, 7.3, 1.3 Hz, 1H), 4.93 (q, *J* = 7.3 Hz, 1H), 3.48 (s, 3H), 3.07 (s, 3H), 1.46 (d, *J* = 7.3 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.1, 164.9, 152.7, 136.4, 127.7, 127.5, 125.2, 122.2, 55.6, 52.5, 31.0, 15.6.

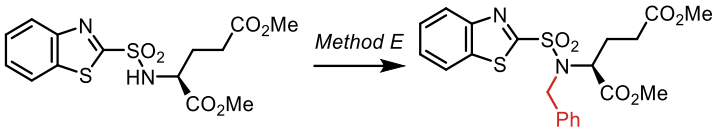
MS (ESI) *m/z* (%): 315 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{12\,h15}N_2O_4S_2$: 315.0468; found: 315.0478.

R_f = 0.35 (PE/ EtOAc = 2:1)

$[\alpha]_D^{23}$ = -30.3° (*c* 1.0, $CHCl_3$)

dimethyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-benzyl-L-glutamate (3-155)



3-110 **3-155**

Method E: Starting from sulfonamide **3-110** (1.0 g, 2.69 mmol, 1.0 equiv.) and benzyl bromide (0.65 mL, 5.48 mmol, 2.0 equiv.). The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 20:1 → 5:1) to afford **3-155** as a colorless solid (1.1 g, 89 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.18 (ddd, *J* = 8.2, 1.4, 0.7 Hz, 1H), 7.99 (ddd, *J* = 8.1, 1.4, 0.7 Hz, 1H), 7.62 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H), 7.59 – 7.55 (m, 1H), 7.48 – 7.45 (m, 2H), 7.34 – 7.27 (m, 3H), 5.00 (d, *J* = 15.9 Hz, 1H), 4.74 (dd, *J* = 9.9, 4.9 Hz, 1H), 4.45 (d, *J* = 15.9 Hz, 1H), 3.57 (s, 3H), 3.30 (s, 3H), 2.36 – 2.23 (m, 1H), 2.25 – 2.09 (m, 2H), 1.88 – 1.77 (m, 1H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 172.8, 170.1, 165.3, 152.6, 136.5, 136.2, 128.9, 128.7, 128.2, 127.7, 127.6, 125.2, 122.3, 60.1, 52.4, 51.8, 51.0, 29.9, 25.1.

MS (ESI) *m/z* (%): 463 $[M+H]^+$ (100).

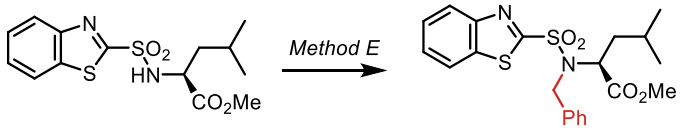
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{21}H_{23}N_2O_6S_2$: 463.0992; found: 463.1001.

R_f = 0.42 (PE/ EtOAc = 5:1)

$[\alpha]_D^{23}$ = -26.3° (*c* 0.4, $CHCl_3$)

m. p. = 58-60 °C

methyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-benzyl-L-leucinate (**3-156**)



3-115 **3-156**

Method E: Starting from sulfonamide **3-115** (1.0 g, 2.92 mmol, 1.0 equiv.) and benzyl bromide (0.71 mL, 5.84 mmol, 2.0 equiv.). The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 15:1 → 5:1) to afford **3-156** as a colorless solid (1.94 g, 87 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.19 – 8.16 (m, 1H), 7.99 (dt, *J* = 8.0, 1.0 Hz, 1H), 7.62 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 7.57 (ddt, *J* = 8.0, 7.1, 1.6 Hz, 1H), 7.50 – 7.46 (m, 2H), 7.33 (td, *J* = 6.9, 1.2 Hz, 2H), 7.30 – 7.27 (m, 1H), 5.02 (d, *J* = 16.3 Hz, 1H), 4.80 (dd, *J* = 8.3, 6.4 Hz, 1H), 4.51 (d, *J* = 16.4 Hz, 1H), 3.29 (s, 3H), 1.54 – 1.49 (m, 2H), 1.44 (dt, *J* = 13.1, 6.5 Hz, 1H), 0.86 (d, *J* = 6.3 Hz, 3H), 0.49 (d, *J* = 6.5 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.2, 165.4, 152.7, 137.1, 136.5, 128.6, 128.6, 127.9, 127.7, 127.5, 125.2, 122.3, 59.3, 52.3, 50.6, 39.1, 24.4, 22.4, 21.4.

MS (ESI) *m/z* (%): 433 $[M+H]^+$ (100).

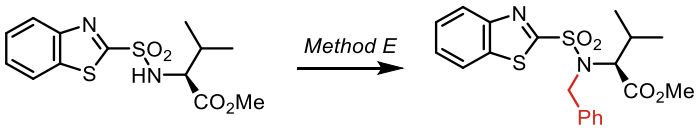
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{21}H_{25}N_2O_4S_2$: 433.1250; found: 433.1263.

R_f = 0.35 (PE/ EtOAc = 5:1)

$[\alpha]_D^{23}$ = -66.8° (*c* 1.2, $CHCl_3$)

m. p. = 100-102 °C

methyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-benzyl-L-valinate (**3-157**)



3-109 **3-157**

Method E: Starting from sulfonamide **3-109** (1.0 g, 3.04 mmol, 1.0 equiv.) and benzyl bromide (0.74 mL, 6.08 mmol, 2.0 equiv.). The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 15:1 → 5:1) to afford **3-157**as a colorless solid (1.19 g, 93 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.17 (ddd, *J* = 8.4, 1.2, 0.7 Hz, 1H), 7.98 (ddd, *J* = 8.1, 1.3, 0.7 Hz, 1H), 7.61 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H), 7.56 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.54

– 7.50 (m, 2H), 7.34 – 7.26 (m, 3H), 5.02 (d, *J* = 15.9 Hz, 1H), 4.69 (d, *J* = 15.9 Hz, 1H), 4.27 (d, *J* = 10.7 Hz, 1H), 3.24 (s, 3H), 2.04 – 1.94 (m, 1H), 0.81 (d, *J* = 6.6 Hz, 3H), 0.73 (d, *J* = 6.6 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 170.2, 165.8, 152.5, 136.8, 136.5, 129.3, 128.5, 128.0, 127.7, 127.5, 125.1, 122.2, 67.1, 51.8, 50.4, 28.7, 19.7, 19.4.

MS (ESI) *m/z* (%): 419 $[M+H]^+$ (100).

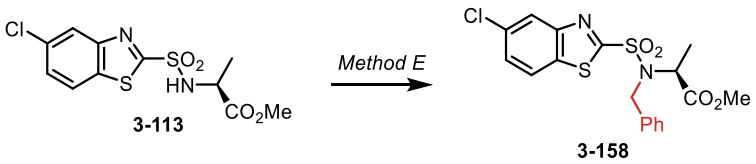
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{20}H_{23}N_2O_4S_2$: 419.1094; found: 419.1110.

R_f = 0.49 (PE/ EtOAc = 5:1)

$[\alpha]_D^{23}$ = -40.7° (*c* 1.0, $CHCl_3$)

m. p. = 110-112 °C

methyl *N*-benzyl-*N*-((6-chlorobenzo[*d*]thiazol-2-yl)sulfonyl)-L-alaninate (**3-158**)



Method E: Starting from sulfonamide **3-113** (0.39 g, 1.16 mmol, 1.0 equiv.) and benzyl bromide (0.28 mL, 2.32 mmol, 2.0 equiv.). The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 10:1 → 5:1) to afford **3-158** as a slightly yellowish oil (0.36 g, 72 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.17 (dd, *J* = 2.1, 0.5 Hz, 1H), 7.90 (dd, *J* = 8.6, 0.5 Hz, 1H), 7.54 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.42 – 7.39 (m, 2H), 7.34 – 7.30 (m, 2H), 7.29 – 7.25 (m, 1H), 4.91 – 4.82 (m, 2H), 4.56 (d, *J* = 16.3 Hz, 1H), 3.38 (s, 3H), 1.36 (d, *J* = 7.3 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.1, 167.5, 153.4, 136.6, 134.7, 133.7, 128.6, 128.4, 128.4, 128.2, 128.0, 124.8, 123.1, 56.3, 52.5, 50.3, 16.7.

MS (ESI) *m/z* (%): 425 $[M+H]^+$ (100).

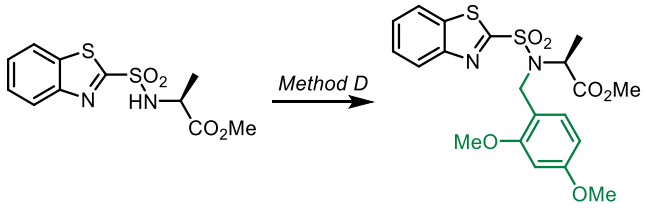
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{18}H_{18}ClN_2O_4S_2$: 425.0391; found: 425.0394.

R_f = 0.45 (hexane/ EtOAc = 3:1)

$[\alpha]_D^{23}$ = -24.9° (*c* 1.0, $CHCl_3$)

m. p. = 110-112 °C

methyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-(2,4-dimethoxybenzyl)-L-alaninate (**3-175**)



3-112 **3-175**

Method D: Starting from sulfonamide **3-112** (0.7 g, 2.33 mmol, 1.0 equiv.), 2,4-dimethoxybenzylalcohol (0.6 g, 3.50 mmol, 1.5 equiv.), PPh_3 (1.2 g, 4.66 mmol, 2.0 equiv.) and DIAD (0.9 mL, 4.66 mmol, 2.0 equiv.). The crude product was purified by gradient column chromatography (SiO_2; hexane/EtOAc = 10:1 → 3:1) to afford **3-175** as a slightly blue solid (0.78 g, 74 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): δ 8.21 – 8.15 (m, 1H), 7.99 – 7.94 (m, 1H), 7.63 – 7.57 (m, 1H), 7.57 – 7.52 (m, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 6.47 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.34 (d, *J* = 2.3 Hz, 1H), 4.85 (q, *J* = 7.3 Hz, 1H), 4.72 (d, *J* = 16.2 Hz, 1H), 4.57 (d, *J* = 16.3 Hz, 1H), 3.78 (s, 3H), 3.71 (s, 3H), 3.36 (s, 3H), 1.39 (d, *J* = 7.3 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.3, 165.7, 160.6, 157.9, 152.6, 136.5, 131.0, 127.6, 127.4, 125.2, 122.2, 117.0, 104.3, 98.0, 56.2, 55.5, 55.3, 52.3, 44.3, 16.0.

MS (ESI) *m/z* (%): 451 $[M+H]^+$ (100).

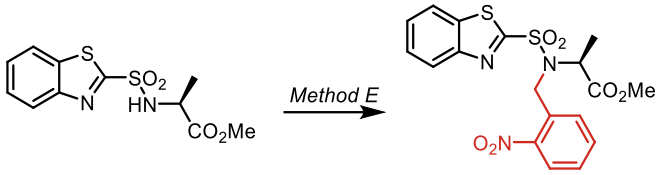
HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{20}H_{22}N_2O_6S_2$: 489.0551; found: 489.0555.

R_f = 0.28 (hexane/EtOAc = 3:1)

$[\alpha]_D^{31}$ = -12.7° (*c* 1.0, $CHCl_3$)

m. p. = 88-90 °C

methyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-(2-nitrobenzyl)-L-alaninate (**3-176**)



3-112 **3-176**

Method E: Starting from sulfonamide **3-112** (0.2 g, 0.66 mmol, 1.0 equiv.) and 2-nitrobenzyl bromide (0.29 mL, 1.33 mmol, 2.0 equiv.). The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 10:1 → 4:1) to afford **3-176** as a slightly yellow oil (0.26 g, 90 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.20 (dt, *J* = 8.4, 0.9 Hz, 1H), 8.06 (dt, *J* = 8.3, 1.1 Hz, 1H), 8.04 – 7.97 (m, 2H), 7.74 – 7.67 (m, 1H), 7.63 (ddt, *J* = 15.5, 7.2, 1.1 Hz, 1H), 7.58 (ddt, *J* = 15.4, 7.2, 1.1 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 1H), 5.31 (d, *J* = 18.8 Hz, 1H), 5.00 (q, *J* = 7.4 Hz, 1H), 4.95 (d, *J* = 18.8 Hz, 1H), 3.34 (s, 3H), 1.35 (d, *J* = 7.3 Hz, 3H).

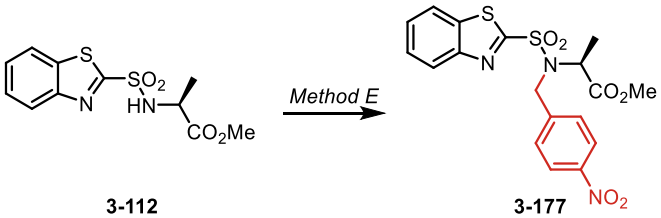
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 170.7, 164.2, 152.6, 147.6, 136.5, 133.9, 133.8, 130.0, 128.4, 128.0, 127.7, 125.3, 125.1, 122.3, 56.8, 52.6, 47.4, 16.6.

MS (ESI) *m/z* (%): 436 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{18}H_{18}N_3O_6S_2$: 436.0632; found: 436.0627.

R_f = 0.35 (hexane/ EtOAc = 4:1)

methyl *N*-(benzo*[d]*thiazol-2-ylsulfonyl)-*N*-(4-nitrobenzyl)-L-alaninate (**3-177**)



3-112 **3-177**

Method E: Starting from sulfonamide **3-112** (0.2 g, 0.66 mmol, 1.0 equiv.) and 4-nitrobenzyl bromide (0.29 mL, 1.33 mmol, 2.0 equiv.). The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 10:1 → 4:1) to afford **3-177** as a slightly yellow oil (0.27 g, 93 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.20 (ddt, *J* = 9.3, 8.2, 1.2 Hz, 3H), 8.00 (dq, *J* = 8.0, 1.0 Hz, 1H), 7.66 – 7.62 (m, 3H), 7.59 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H), 5.06 (d, *J* = 17.4 Hz, 1H), 4.96 (q, *J* = 7.4 Hz, 1H), 4.65 (d, *J* = 17.4 Hz, 1H), 3.38 (s, 3H), 1.35 (d, *J* = 7.4 Hz, 3H).

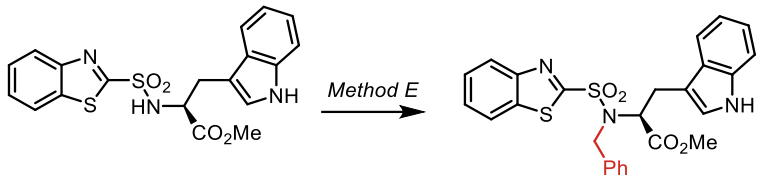
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.0, 164.7, 152.6, 147.6, 145.1, 136.4, 128.5, 128.0, 127.8, 125.3, 123.9, 122.3, 56.4, 52.6, 49.4, 17.0.

MS (ESI) *m/z* (%): 436 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{18}H_{18}N_3O_6S_2$: 436.0632; found: 436.0628.

R_f = 0.40 (hexane/ EtOAc = 4:1)

methyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-benzyl-L-tryptophanate (**3-236**)



3-119 **3-236**

Modified Method E: To the solution of sulfonamide **3-119** in DMF (0.1 M, 20.5 mL) was added followed by addition of K_2CO_3 (0.85 g, 6.14 mmol, 3 equiv.). Subsequently, benzyl bromide (0.49 mL, 4.09 mmol, 2 equiv.) was added dropwise and the reaction mixture was stirred at room temperature for 16 h. Then, the mixture was quenched with water and the layers were separated. The aqueous layer was extracted using EtOAc (3 x 20 mL). Combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude product was purified by column chromatography (SiO2; petroleum ether/EtOAc = 6:1 to 2:1) to afford **3-236** in 30 % yield over 2 steps (0.3g, *e.r.* = ≥ 98:2) as a slightly orange oil.

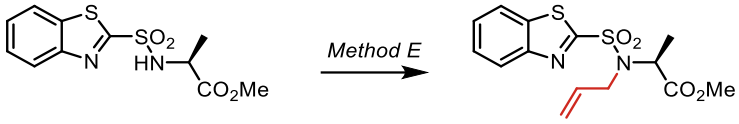
^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.16 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.94 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.79 (s, 1H), 7.61 (ddd, *J* = 8.2, 7.1, 1.3 Hz, 1H), 7.56 (ddd, *J* = 8.2, 7.2, 1.3 Hz, 1H), 7.45 – 7.41 (m, 3H), 7.30 – 7.25 (m, 2H), 7.23 (dd, *J* = 9.6, 2.5 Hz, 1H), 7.14 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 7.06 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 6.89 – 6.86 (m, 1H), 4.98 (ddd, *J* = 8.3, 6.6, 1.6 Hz, 1H), 4.88 (d, *J* = 15.9 Hz, 1H), 4.76 (d, *J* = 16.0 Hz, 1H), 3.38 (dd, *J* = 14.7, 8.5 Hz, 1H), 3.20 (s, 3H), 3.12 (dd, *J* = 14.7, 6.5 Hz, 1H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 170.2, 165.9, 152.6, 136.5, 136.2, 136.0, 128.8, 128.5, 128.0, 127.6, 127.5, 127.1, 125.1, 123.5, 122.3, 122.2, 119.7, 118.7, 111.2, 110.4, 60.6, 52.2, 50.8, 26.6.

MS (ESI) *m/z* (%): 506 $[M+H]^+$ (100). **HRMS (ESI) *m/z:*** $[M+K]^+$ calculated for $C_{26}H_{23}KN_3O_4S_2$: 544.0762; found: 544.0772.

R_f = 0.30 (hexane/ EtOAc = 3:1); $[\alpha]_D^{22}$ = -16.3° (*c* 0.9, $CHCl_3$)

methyl *N*-allyl-*N*-(benzo[*d*]thiazol-2-ylsulfonyl)-L-alaninate (**3-237**)



3-112 **3-237**

Method E: Starting from sulfonamide **3-112** (0.21 g, 0.70 mmol, 1.0 equiv.) and allyl bromide (0.12 mL, 1.40 mmol, 2.0 equiv.). The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 8:1) to afford **3-237** as a colorless oil (0.17 g, 72 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.17 (d, *J* = 7.5 Hz, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.60 (ddd, *J* = 8.3, 7.3, 1.2 Hz, 1H), 7.55 (ddd, *J* = 8.3, 7.3, 1.2 Hz, 1H), 5.93 (ddt, *J* = 16.4, 10.3, 6.2 Hz, 1H), 5.26 (dd, *J* = 17.2, 1.3 Hz, 1H), 5.16 (dd, *J* = 10.2, 1.2 Hz, 1H), 4.87 (q, *J* = 7.3 Hz, 1H), 4.24 (dd, *J* = 16.6, 6.1 Hz, 1H), 4.00 (dd, *J* = 16.6, 6.1 Hz, 1H), 3.47 (s, 3H), 1.50 (d, *J* = 7.3 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.4, 165.7, 152.6, 136.4, 134.6, 127.7, 127.5, 125.2, 122.3, 118.4, 56.1, 52.5, 49.2, 16.6.

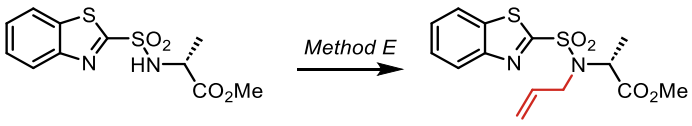
MS (ESI) *m/z* (%): 340 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{14}H_{17}N_2O_4S_2$: 341.0624; found: 341.0626.

R_f = 0.45 (PE/ EtOAc = 4:1)

$[\alpha]_D^{24}$ = +29.8° (*c* 1.0, $CHCl_3$)

methyl *N*-allyl-*N*-(benzo[*d*]thiazol-2-ylsulfonyl)-D-alaninate (**3-238**)



3-118 **3-238**

Method E: Starting from sulfonamide **3-118** (0.6 g, 2.0 mmol, 1.0 equiv.) and allyl bromide (0.35 mL, 4.0 mmol, 2.0 equiv.). The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 8:1) to afford **3-238** as a colorless oil (0.5 g, 74 % yield, *e.r.* = ≥ 1:99).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.17 (dt, *J* = 8.4, 0.9 Hz, 1H), 7.97 (dt, *J* = 8.0, 1.0 Hz, 1H), 7.60 (ddd, *J* = 8.2, 7.2, 1.3 Hz, 1H), 7.55 (ddd, *J* = 8.4, 7.1, 1.3 Hz, 1H), 5.93 (ddt, *J* = 17.2, 10.2, 6.1 Hz, 1H), 5.26 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.16 (dq, *J* = 10.2, 1.3 Hz, 1H), 4.87 (q, *J*

= 7.3 Hz, 1H), 4.24 (ddt, *J* = 16.7, 6.2, 1.6 Hz, 1H), 4.00 (ddt, *J* = 16.6, 6.2, 1.4 Hz, 1H), 3.46 (s, 3H), 1.50 (d, *J* = 7.4 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.4, 165.7, 152.6, 136.4, 134.6, 127.7, 127.5, 125.2, 122.3, 118.4, 56.1, 52.5, 49.2, 16.6.

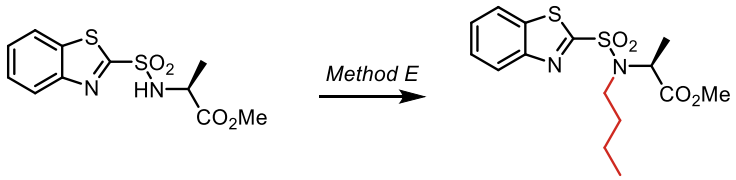
MS (ESI) *m/z* (%): 340 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{14}H_{17}N_2O_4S_2$: 341.0624; found: 341.0628.

R_f = 0.45 (PE/ EtOAc = 4:1)

$[\alpha]_D^{24}$ = -25.0° (*c* 0.8, $CHCl_3$)

methyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-butyl-L-alaninate (3-239)



3-112 **3-239**

Method E: Starting from sulfonamide **3-112** (0.6 g, 2.0 mmol, 1.0 equiv.) and 1-iodobutane (0.46 mL, 4.0 mmol, 2.0 equiv.). The crude product was purified by gradient column chromatography (SiO_2; petroleum ether/EtOAc = 10:1 → 5:1) to afford **3-239** as a colorless solid (0.55 g, 77 % yield, *e.r.* = ≥ 1:99).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.16 (dt, *J* = 8.7, 0.8 Hz, 1H), 7.97 (dt, *J* = 8.0, 0.9 Hz, 1H), 7.60 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 7.55 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H), 4.85 (q, *J* = 7.3 Hz, 1H), 3.57 (ddd, *J* = 15.6, 10.9, 5.1 Hz, 1H), 3.45 (s, 3H), 3.21 (ddd, *J* = 15.0, 11.0, 5.5 Hz, 1H), 1.83 – 1.74 (m, 1H), 1.67 – 1.60 (m, 1H), 1.51 (d, *J* = 7.3 Hz, 3H), 1.37 – 1.30 (m, 2H), 0.93 (t, *J* = 7.3 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.6, 165.6, 152.6, 136.4, 127.6, 127.5, 125.2, 122.2, 56.2, 52.5, 46.9, 33.3, 20.2, 16.7, 13.9.

MS (ESI) *m/z* (%): 357 $[M+H]^+$ (100).

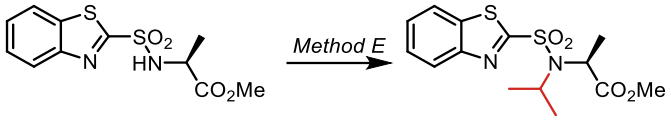
HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{15}H_{21}N_2O_4S_2$: 357.0937; found: 357.0945.

R_f = 0.55 (PE/ EtOAc = 2:1)

$[\alpha]_D^{23}$ = -50.1° (*c* 1.2, $CHCl_3$)

m. p. = 53-55 °C

methyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-isopropyl-L-alaninate (3-240)



3-112 **3-240**

Method D: Starting from sulfonamide **3-112** (0.11 g, 0.37 mmol, 1.0 equiv.) and isopropanol (0.06 mL, 0.73 mmol, 2.0 equiv.). The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) to afford **3-240** as a colorless oil (0.1 g, 80 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.13 (dt, *J* = 8.5, 0.9 Hz, 1H), 7.95 (dt, *J* = 7.6, 0.8 Hz, 1H), 7.58 (ddd, *J* = 8.3, 7.1, 1.4 Hz, 1H), 7.53 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 4.33 (q, *J* = 7.2 Hz, 1H), 4.27 (p, *J* = 6.8 Hz, 1H), 3.69 (s, 3H), 1.63 (d, *J* = 7.2 Hz, 3H), 1.38 (d, *J* = 6.8 Hz, 3H), 1.27 (d, *J* = 6.8 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.9, 167.7, 152.4, 136.4, 127.5, 127.3, 125.1, 122.2, 53.8, 52.7, 52.1, 21.9, 21.6, 17.6.

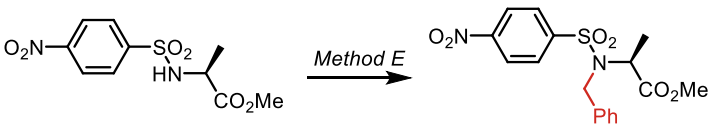
MS (ESI) *m/z* (%): 343 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{14}H_{19}N_2O_4S_2$: 343.0781; found: 343.0783.

R_f = 0.40 (PE/ EtOAc = 2:1)

$[\alpha]_D^{23}$ = +17.8° (*c* 1.1, $CHCl_3$)

methyl *N*-benzyl-*N*-((4-nitrophenyl)sulfonyl)-L-alaninate (3-241)



3-232 **3-241**

Prepared according to a literature. Spectroscopic data match those in the literature.[245]

To the mixture of nosyl-protected alanine methyl ester **3-232** (1.95 g, 6.76 mmol, 1.0 equiv.) and K_2CO_3 (0.6g, 7.4 mmol, 1.5 equiv.) in DMF (14 mL, 0.5 M) was added benzyl bromide (0.89 mL, 7.4 mmol, 1.05 equiv.) at 0 °C. The mixture was stirred overnight at room temperature for 16 h. Upon completion, the solid was removed by filtration through cotton wool. The filtrate was washed with HCl (1 M), saturated $NaHCO_3$ (15 mL) solution and brine (15 mL), dried with

anhydrous Na_2SO_4. The crude product **3-241** was used in the next step without further purification (2.3 g, 86 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.32 – 8.27 (m, 2H), 7.97 – 7.91 (m, 2H), 7.30 – 7.27 (m, 4H), 4.73 (q, *J* = 7.3 Hz, 1H), 4.64 (d, *J* = 15.9 Hz, 1H), 4.38 (d, *J* = 15.9 Hz, 1H), 3.53 (s, 3H), 1.36 (d, *J* = 7.3 Hz, 3H).

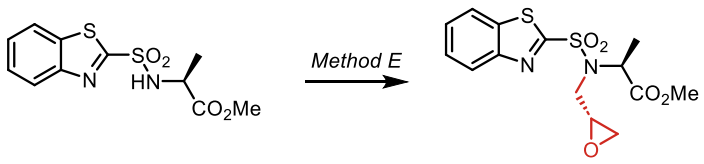
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.3, 150.0, 146.0, 136.4, 128.7, 128.7, 128.3, 128.0, 124.1, 56.01, 52.5, 49.8, 17.1.

MS (ESI) *m/z* (%): 379 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{17}H_{19}N_2O_6S_2$: 379.0964; found: 379.1031.

$[\alpha]_D^{23}$ = -34.9° (*c* 1.0, $CHCl_3$)

methyl *N*-(benzo[d]thiazol-2-ylsulfonyl)-*N*-(((*R*)-oxiran-2-yl)methyl)-L-alaninate (3-242)



3-112 **3-242**

Method D: Starting from sulfonamide **3-112** (0.09 g, 0.3 mmol, 1.0 equiv.) and (*R*)-Glycidol (0.04 mL, 0.6 mmol, 2.0 equiv.). The crude product was purified by semipreparative HPLC using gradient (MeOH:AcCN = 90:10 → 30:70 → 90:10) to afford **2-242** as a slightly yellow oil (0.066 g, 92 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-d) δ (ppm): 8.16 (dt, *J* = 8.3, 1.0 Hz, 1H), 8.00 – 7.96 (m, 1H), 7.61 (ddd, *J* = 8.4, 7.2, 1.3 Hz, 1H), 7.56 (ddd, *J* = 8.4, 7.2, 1.3 Hz, 1H), 4.89 (q, *J* = 7.3 Hz, 1H), 3.87 – 3.82 (m, 1H), 3.54 (s, 3H), 3.45 (dd, *J* = 16.0, 5.9 Hz, 1H), 3.36 – 3.31 (m, 1H), 2.84 (t, *J* = 4.3 Hz, 1H), 2.63 (dd, *J* = 4.7, 2.6 Hz, 1H), 1.49 (d, *J* = 7.3 Hz, 3H).

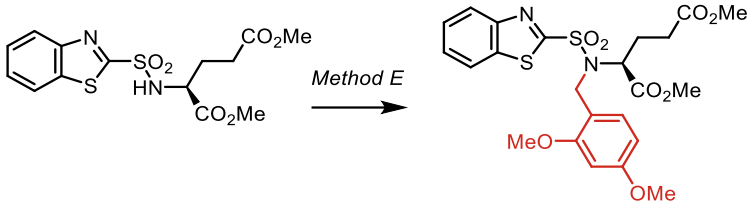
^{13}C {^{1}H} NMR (126 MHz, Chloroform-d) δ (ppm): 171.3, 165.4, 152.6, 136.4, 127.8, 127.6, 125.2, 122.3, 56.2, 52.7, 51.1, 48.3, 46.2, 15.9.

MS (ESI) *m/z* (%): 357 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{14}H_{17}N_2O_5S_2$: 357.0573; found: 357.0582.

$[\alpha]_D^{23}$ = -440° (*c* 0.25, DCM)

dimethyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-(2,4-dimethoxybenzyl)-L-glutamate (**3-243**)



3-107 **3-243**

Method D: Starting from sulfonamide **3-107** (0.2 g, 0.54 mmol, 1.0 equiv.) and 2,4-dimethoxybenzylalkohol (0.184 g, 1.07 mmol, 2.0 equiv.). The crude product was purified by column chromatography (petroleum ether/EtOAc = 10:1) to afford **3-243** as a yellow oil (0.2 g, 72 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.18 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.97 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.60 (ddd, *J* = 8.4, 7.2, 1.3 Hz, 1H), 7.55 (ddd, *J* = 8.3, 7.2, 1.2 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 1H), 6.47 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.32 (d, *J* = 2.4 Hz, 1H), 4.73 (d, *J* = 15.5 Hz, 1H), 4.73 – 4.64 (m, 2H), 4.62 (d, *J* = 15.4 Hz, 1H), 3.78 (s, 3H), 3.67 (s, 3H), 3.58 (s, 3H), 3.35 (s, 3H), 2.36 – 2.19 (m, 3H), 1.95 (qd, *J* = 12.0, 10.1, 3.4 Hz, 1H).

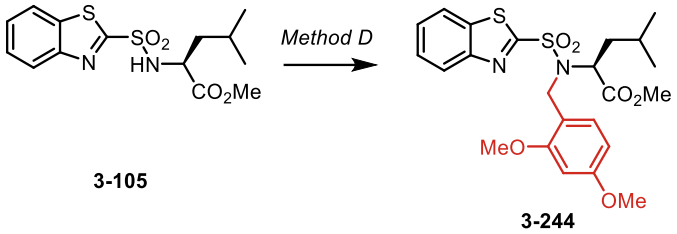
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 173.1, 170.4, 165.7, 161.1, 158.3, 152.7, 136.6, 132.3, 127.6, 127.5, 125.2, 122.2, 116.3, 104.6, 98.2, 60.0, 55.5, 55.3, 52.4, 51.7, 44.8, 30.2, 24.6.

MS (ESI) *m/z* (%): 523 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+K]^+$ calculated for $C_{23}H_{26}N_2O_8S_2$: 561.0768; found: 531.0755.

R_f = 0.35 (hexane/ EtOAc = 5:1)

methyl *N*-(benzo[*d*]thiazol-2-ylsulfonyl)-*N*-(2,4-dimethoxybenzyl)-L-leucinate (**3-244**)



Method D: Starting from sulfonamide **3-105** (0.2 g, 0.61 mmol, 1.0 equiv.) and 2,4-dimethoxybenzylalkohol (0.21 g, 1.22 mmol, 2.0 equiv.). The crude product was purified by column chromatography (petroleum ether/EtOAc = 10:1) to afford **3-244** as a colorless oil (0.2 g, 70 % yield, *e.r.* = ≥ 99:1).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.18 (dt, J = 8.3, 0.9 Hz, 1H), 8.01 – 7.95 (m, 1H), 7.60 (ddd, J = 8.4, 7.3, 1.4 Hz, 1H), 7.58 – 7.53 (m, 2H), 6.50 (dd, J = 8.5, 2.4 Hz, 1H), 6.37 (d, J = 2.4 Hz, 1H), 4.77 – 4.71 (m, 2H), 4.67 (d, J = 16.4 Hz, 1H), 3.80 (s, 3H), 3.72 (s, 3H), 3.31 (s, 3H), 1.58 (t, J = 7.0 Hz, 2H), 1.50 (dt, J = 13.2, 6.6 Hz, 1H), 0.86 (d, J = 6.3 Hz, 3H), 0.61 (d, J = 6.6 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.3, 165.6, 160.7, 157.8, 152.7, 136.6, 131.5, 130.4, 127.6, 127.4, 125.2, 122.2, 117.3, 104.5, 98.1, 59.2, 55.5, 55.3, 52.2, 44.1, 38.6, 24.6, 22.7, 21.6.

MS (ESI) *m/z* (%): 493 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{23}H_{29}N_2O_6S_2$: 493.1462; found: 493.1459.

R_f = 0.40 (hexane/ EtOAc = 5:1)

Method A: Smiles rearrangement

Method A: A tertiary sulfonamide (1 equiv.) was dissolved in THF (0.1 M), and the solution was cooled to -95 °C (N_2/methanol). After 10 minutes 18-crown-6 (3 equiv.) and the solution of KHMDS (1 M in THF, 1.5 equiv.) were added. The reaction was kept at -95 °C and the progress was monitored by TLC. Resulting mixture was quenched after 30 minutes (unless stated otherwise) by NH_4Cl (15 mL) and EtOAc (15 mL) was added. Resulting layers were separated and the aqueous layer was extracted with EtOAc (3 x 15 mL). Combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure to yield the crude product.

Method B: Smiles rearrangement

Method B: A tertiary sulfonamide (1 equiv.) was dissolved in THF (0.1 M), and the solution was cooled to -95 °C (N_2/methanol). After 10 minutes the solution of LiHMDS (1 M in THF, 1.5 equiv.) were added. The reaction was kept at -95 °C and the progress was monitored by TLC. Resulting mixture was quenched after 30 minutes (unless stated otherwise) by NH_4Cl (15 mL) and EtOAc (15 mL) was added. Resulting layers were separated and the aqueous layer was extracted with EtOAc (3 x 15 mL). Combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure to yield the crude product.

Method C: Smiles rearrangement

Method C: To a solution of HMDS (1.5 equiv.) in tetrahydrofuran (0.1 M) cooled in an ice-water bath was added *n*-BuLi (1.5 equiv.) *via* syringe. The mixture was stirred at room temperature for 15-30 min prior to use. After that, solution was cooled to -95 °C (N_2/methanol) and sulfonamide (1 equiv.) was added. After 30 minutes (unless stated otherwise) the reaction was quenched with sat. NH_4Cl and EtOAc (15 mL) was added. Resulting layers were separated and the aqueous layer was extracted with EtOAc (3 x 15 mL). Combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure to yield the crude product.

Method D: Smiles rearrangement

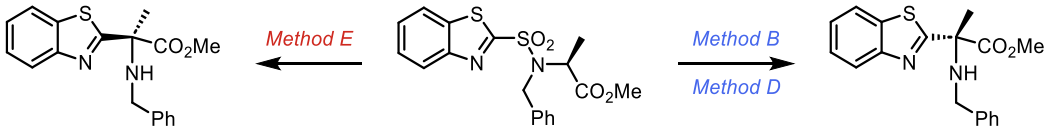
Method D: A tertiary sulfonamide (1 equiv.) was dissolved in THF (0.1 M), and the solution was cooled to -78 °C (N_2/acetone or dry ice/acetone). Then 18-crown-6 (3 equiv.) and LiCl (1.1 equiv.) were added following addition of NaHMDS (1 M in THF, 1.5 equiv.). The reaction was

kept at -78 °C and the progress was monitored by TLC. Resulting mixture was quenched after 30 minutes (unless stated otherwise) by NH_4Cl (15 mL) and EtOAc (15mL) was added. Resulting layers were separated and the aqueous layer was extracted with EtOAc (3 x 15 mL). Combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure to yield the crude product.

Method E: Smiles rearrangement inversion

Method E: A tertiary sulfonamide (1 equiv.) was dissolved in THF (0.1 M), and the solution was cooled to -78 °C (N_2/acetone or dry ice/acetone). Then CuOTf (1.1 equiv.) was added following addition of KHMDS (1 M in THF, 1.5 equiv.). The reaction was kept at -78 °C and the progress was monitored by TLC. Resulting mixture was quenched after 30 minutes (unless stated otherwise) by NH_4Cl (15 mL) and EtOAc (15 mL) was added. Resulting layers were separated and the aqueous layer was extracted with EtOAc (3 x 15 mL). Combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure to yield the crude product.

methyl 2-(benzo[*d*]thiazol-2-yl)-2-(benzylamino)propanoate



(-)-3-167 **3-143** **(+)-3-168**

The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) and obtained as colorless oil. **Method B**: Starting from 0.100 g (0.26 mmol, 1 equiv.) of **3-143**, yielded 0.061 g (73 %) of **(+)-3-167**, *e.r.* = ≥ 97:3 $[\alpha]_D^{20}$ = + 17.3° (*c* 1.2, $CHCl_3$); **Method D**: 0.2 g (0.51 mmol, 1 equiv.) of **(+)-3-143**, yielded 0.15 g (92 %) of **3-167**, *e.r.* = ≥ 96:4, $[\alpha]_D^{24}$ = - 11.7° (*c* 1, $CHCl_3$). **Method E**: Starting from 0.1 g (0.26 mmol, 1 equiv.) of **3-143**, yielded 0.055 g (67 %) of **(-)-3-167**, *e.r.* = ≥ 10:90, $[\alpha]_D^{24}$ = - 15.8° (*c* 1, $CHCl_3$).

^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm): 8.04 (dt, *J* = 8.1, 1.0 Hz, 1H), 7.89 (dt, *J* = 8.0, 0.9 Hz, 1H), 7.50 – 7.33 (m, 6H), 7.31 – 7.26 (m, 1H), 3.84 – 3.79 (m, 1H), 3.79 (s, 3H), 2.83 (bs, 1H), 1.96 (s, 3H).

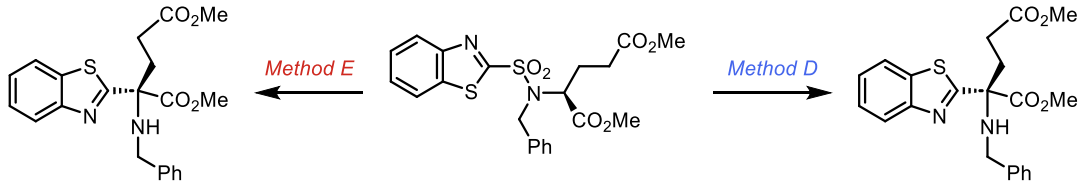
^{13}C NMR (101 MHz, Chloroform-*d*) δ (ppm): 175.2, 172.9, 153.5, 139.7, 135.6, 128.6, 128.4, 127.4, 126.1, 125.3, 123.5, 121.8, 66.5, 53.2, 48.2, 23.7.

MS (ESI) *m/z* (%) 327: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{18}H_{19}N_2O_2S$, 327.1167; found: 327.1156.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 11.95 min, t_{R2} = 14.95 min.

dimethyl 2-(benzo[*d*]thiazol-2-yl)-2-(benzylamino)pentanedioate



(+)-3-178 **3-155** **(-)-3-178**

Reaction time was increased to 1h and 3 equiv. of $LiNTMS_2$were used. The crude product was purified using preparative TLC (SiO_2; petroleum ether/EtOAc = 2:1) and obtained as white solid. **Method D**: 0.185 g (0.4 mmol, 1 equiv.) of **3-155**, yielded 0.089 g (55 %, 74 % after recuperating starting material) of **(-)-3-178**, *e.r.* = ≥ 94:6; $[\alpha]_D^{20}$ = - 12.1° (*c* 1.2, $CHCl_3$).

Method E: 0.05 g (0.1 mmol, 1 equiv.) of **3-155**, yielded 0.022 g (89 %) of **(+)-3-178** after recuperating starting material (conversion = 50 %), *e.r.* = ≥ 17:83, $[\alpha]_D^{22}$= +15.2 (*c* 0.5, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.05 (dt, *J* = 8.2, 0.8 Hz, 1H), 7.89 (dt, *J* = 7.9, 0.9 Hz, 1H), 7.49 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H), 7.45 – 7.37 (m, 3H), 7.40 – 7.30 (m, 3H), 7.33 – 7.24 (m, 2H), 3.78 (s, 3H), 3.78 – 3.66 (m, 5H), 3.63 (s, 3H), 2.90 – 2.80 (m, 1H), 2.80 – 2.68 (m, 1H), 2.46 (t, *J* = 8.1 Hz, 2).;

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 173.3, 173.0, 171.8, 153.3, 139.3, 135.4, 128.6, 128.6, 128.3, 128.2, 127.5, 126.2, 125.4, 123.6, 121.8, 68.7, 53.2, 51.9, 47.5, 30.1, 28.8.

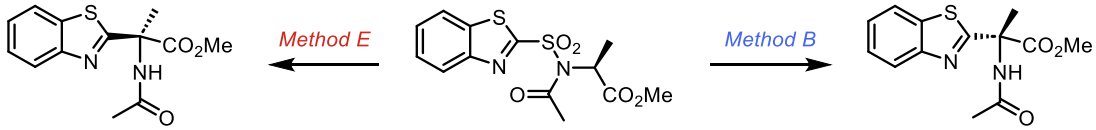
MS (ESI) *m/z* (%) 361: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{21}H_{23}N_2O_4S$, 399.1373; found: 399.1380.

HPLC (AD-H, Hexane : iPrOH = 80:20, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 15.20 min, t_{R2} = 18.38 min.

m. p. = 80 – 82 °C

methyl 2-acetamido-2-(benzo[d]thiazol-2-yl)propanoate (**3-180**)



(+)-3-180 **3-151** **(-)-3-180**

The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) and obtained as colorless oil. **Method D**: Starting from 0.1 g (0.29 mmol, 1 equiv.) of **3-151**, yielded 0.061 g (73 %) of **(-)-3-180**, *e.r.* = ≥ 64:36 $[\alpha]_D^{22}$ = -3.2° (*c* 0.6, $CHCl_3$).; **Method E**: 0.1 g (0.29 mmol, 1 equiv.) of **3-151** yielded 0.05 g (61 %) of **(+)-3-180**, *e.r.* = ≥ 24:76, $[\alpha]_D^{21}$ = +5.3° (*c* 1.1, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.03 (dt, *J* = 8.3, 0.9 Hz, 1H), 7.87 (dt, *J* = 8.0, 0.9 Hz, 1H), 7.58 (s, 1H), 7.50 (ddd, *J* = 8.3, 7.2, 1.2 Hz, 1H), 7.41 (ddd, *J* = 8.3, 7.2, 1.1 Hz, 1H), 3.75 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 171.0, 170.1, 169.6, 152.0, 135.7, 126.5, 125.9, 123.5, 121.9, 62.9, 53.8, 24.8, 23.6.

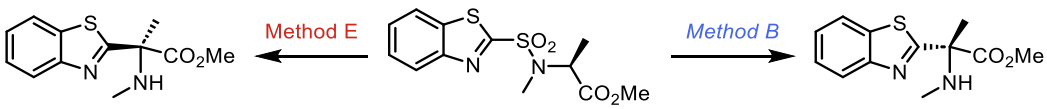
MS (ESI) *m/z* (%) 279: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{13}H_{14}N_2O_3S$: 279.0798 found: 279.0800.

R_f = (hexane/ EtOAc = 5:1)

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 12.00 min, t_{R2} = 12.53 min.

methyl 2-(benzo[*d*]thiazol-2-yl)-2-(methylamino)propanoate (**3-181**)



(-)-3-181 **3-154** **(+)-3-181**

The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 10:1) and obtained as colorless oil. **Method B**: 0.150 g (0.48 mmol, 1 equiv.) of **3-154**, yielded 0.098 g (82 %) of **(+)-3-181**, *e.r.* = ≥ 90:10 $[\alpha]_D^{20}$ = + 7 (*c* 1.2, $CHCl_3$). **Method E**: 0.042 g (0.134 mmol, 1 equiv.) of **3-154**, yielded 0.025 g (75 %) of **(-)-3-181**, *e.r.* = ≥ 16:84, $[\alpha]_D^{25}$ = - 6.5 ° (*c* 1, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.02 (dt, *J* = 8.1, 1.0, 0.6 Hz, 2H), 7.88 (dt, *J* = 8.0, 1.3, 0.6 Hz, 1H), 7.47 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H), 7.38 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 3.79 (s, 3H), 2.43 (s, 3H), 1.85 (s, 3H).

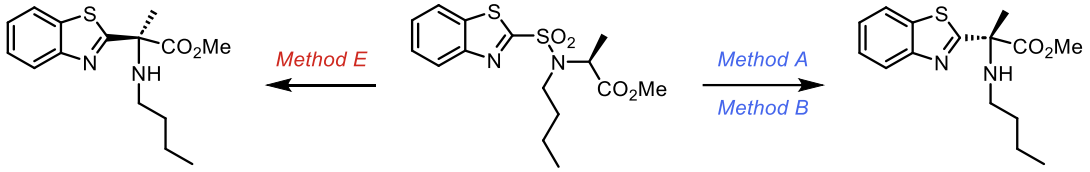
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 175.0, 173.0, 153.4, 135.5, 126.1, 125.3, 123.5, 121.8, 66.8, 53.2, 30.5, 22.8.

MS (ESI) *m/z* (%) 251: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{12\,h15}N_2O_2S$, 251.0849; found: 251.0849.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 10.55 min, t_{R2} = 12.98 min.

methyl 2-(benzo[*d*]thiazol-2-yl)-2-(butylamino)propanoate (**3-182**)



The crude product was purified using preparative TLC (SiO_2; petroleum ether/EtOAc = 4:1) and obtained as colorless oil. **Method A**: Starting from 0.150 g (0.42 mmol, 1 equiv.) of **3-239**, yielded 0.090 g (73 %) of **(+)-3-182**, *e.r.* = ≥ 77:23, $[\alpha]_D^{20}$ = + 2.2° (*c* 1, $CHCl_3$); **Method B**: 0.07 g (0.21 mmol, 1 equiv.) of **3-239**, yielded 0.091 g (74 %) of **3-182**, *e.r.* = ≥ 98:2, $[\alpha]_D^{21}$ = + 16.9° (*c* 1, $CHCl_3$). **Method E**: 0.05 g (0.14 mmol, 1 equiv.) of **3-239**, yielded 0.022 g (53 %) of **(-)-3-182** after recuperated starting material (conversion = 50 %), *e.r.* = ≥ 32:68, $[\alpha]_D^{20}$ = - 2.1° (*c* 1, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.01 (dt, *J* = 8.2, 0.9 Hz, 1H), 7.87 (dt, *J* = 8.0, 1.0 Hz, 1H), 7.45 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H), 7.36 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 3.77 (s, 3H), 2.66 – 2.51 (m, 2H), 2.49 (bs, 1H), 1.86 (s, 3H), 1.58 – 1.48 (m, 2H), 1.39 (h, *J* = 7.3 Hz, 2H), 0.91 (t, *J* = 7.3 Hz, 3H).

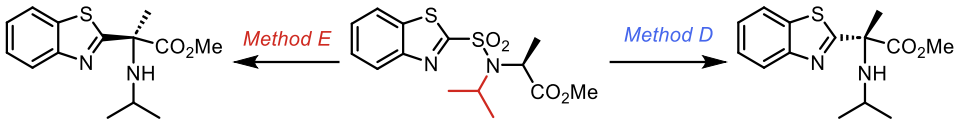
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 175.6, 173.1, 153.4, 135.5, 126.0, 125.9, 125.2, 125.1, 123.4, 121.8, 66.3, 53.1, 43.5, 32.7, 23.4, 20.5, 14.1.

MS (ESI) *m/z* (%): 293 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{15}H_{21}N_2O_2S$, 293.1324; found: 293.1326.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 9.07 min, t_{R2} = 10.67 min.

methyl 2-(benzo[*d*]thiazol-2-yl)-2-(isopropylamino)propanoate (**3-183**)



(-)-3-183 **3-240** **(+)-3-183**

The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 10:1) and obtained as colorless oil. **Method D**: Starting from 0.050 g (0.146 mmol, 1 equiv.) of **3-240**, yielded 0.033 g (82 %) of **(+)-3-183**, *e.r.* = ≥ 99:1, $[\alpha]_D^{20}$ = +14.2 (*c* 1, $CHCl_3$) ; **Method E**: 0.04 g (0.117 mmol, 1 equiv.) of **3-240**, yielded 0.022 g (67 %) of **(-)-3-183** after recuperated starting material (conversion = 92 %), *e.r.* = ≥ 3:97, $[\alpha]_D^{25}$ = = -11.2 (*c* 1, $CHCl_3$)

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.00 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.86 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.45 (tt, *J* = 8.7, 1.5 Hz, 1H), 7.36 (td, *J* = 7.6, 6.9, 1.4 Hz, 1H), 3.77 (s, 3H), 3.08 – 3.00 (m, 1H), 2.55 (bs, 1H), 1.86 (s, 3H), 1.10 (ddd, *J* = 5.9, 4.1, 1.7 Hz, 6H).

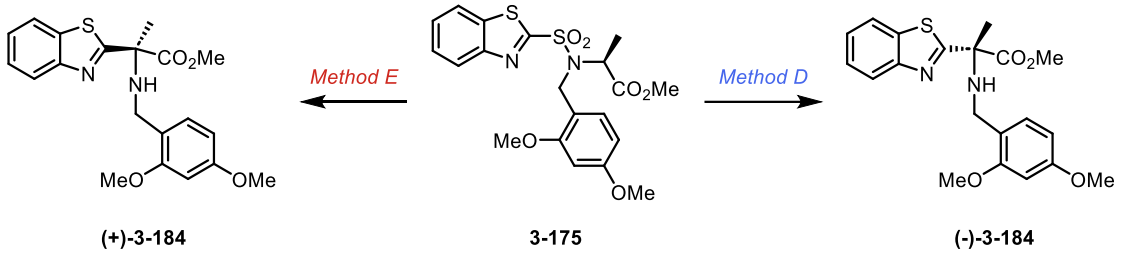
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 177.4, 174.2, 153.5, 135.5, 126.0, 125.1, 123.4, 121.8, 65.8, 53.1, 45.0, 25.3, 24.6, 23.8.

MS (ESI) *m/z* (%) 279: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{14}H_{19}N_2O_2S$, 279.1167; found: 279.1169.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 7.60 min, t_{R2} = 8.70 min.

methyl 2-(benzo[d]thiazol-2-yl)-2-((2,4-dimethoxybenzyl)amino)propanoate (**3-184**)



(+)-3-184 **3-175** **(-)-3-184**

The crude product was purified by gradient column chromatography (SiO_2; hexane/EtOAc = 15:1 → 5:1) and obtained as colorless oil. **Method D**: Starting from sulfonamide **3-175** (0.075 g, 0.17 mmol, 1.0 equiv.), yielded 0.031 g (80 %) of **(-)-3-184**, *e.r.* = 99:1, $[\alpha]_D^{31}$ = -6.8° (c 1, $CHCl_3$). **Method E**: Starting from sulfonamide **3-175** (0.075 g, 0.17 mmol, 1.0 equiv.), yielded 0.023 g (39 %) of **(+)-3-184**, *e.r.* = 8:92, $[\alpha]_D^{31}$ = +5.9° (c 1, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.02 (dt, J = 8.3, 0.8 Hz, 1H), 7.91 – 7.83 (m, 1H), 7.46 (ddd, J = 8.4, 7.3, 1.2 Hz, 1H), 7.37 (ddd, J = 8.3, 7.1, 1.1 Hz, 1H), 7.26 (d, J = 0.8 Hz, 1H), 6.51 – 6.40 (m, 2H), 3.80 (d, J = 1.4 Hz, 7H), 3.69 (s, 3H), 1.94 (s, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): δ 176.1, 172.9, 160.3, 158.6, 153.5, 135.6, 130.4, 126.0, 125.1, 123.4, 121.8, 120.2, 104.0, 98.6, 66.2, 55.5, 55.4, 53.1, 42.9, 23.4.

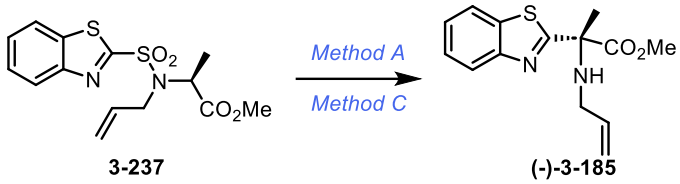
MS (ESI) *m/z* (%): 387 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{20}H_{22}N_2O_4S$: 387.1373; found: 387.1382.

R_f = 0.58 (hexane/EtOAc = 3:1)

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 15.35 min, t_{R2} = 18.32 min.

methyl 2-(allylamino)-2-(benzo[*d*]thiazol-2-yl)propanoate (3-185)



The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 10:1) and obtained as colorless oil. **Method A**: Starting from 0.075 g (0.22 mmol, 1 equiv.) of **3-237**, yielded 0.043 g (71 %) **(-)-3-185**, *e.r.* = ≥ 81:19, $[\alpha]_D^{24}$ = - 3.6° (*c* 1, $CHCl_3$); **Method C**: 0.045 g (0.13 mmol, 1 equiv.) of **3-237**, yielded 0.027 g (70 %) **(-)-3-185**, *e.r.* = ≥ 94:6, $[\alpha]_D^{24}$ = - 6.8° (*c* 1, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.02 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.87 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.46 (ddt, *J* = 8.1, 7.1, 1.1 Hz, 1H), 7.37 (ddt, *J* = 7.9, 7.0, 0.9 Hz, 1H), 6.00 – 5.91 (m, 1H), 5.33 – 5.22 (m, 1H), 5.18 – 5.09 (m, 1H), 3.78 (s, 3H), 3.34 – 3.20 (m, 2H), 1.90 (s, 3H).

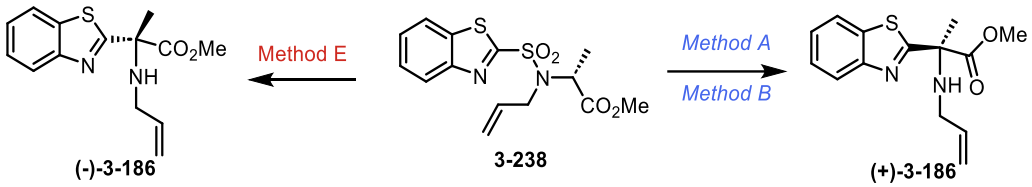
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 175.0, 172.8, 153.4, 136.0, 135.6, 126.1, 125.3, 123.5, 121.8, 116.7, 66.2, 53.2, 46.6, 23.4.

MS (ESI) *m/z* (%) 277: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{14}H_{17}N_2O_2S$, 277.1005; found: 277.1008.

HPLC (AD-H, Hexane: iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 9.70 min, t_{R2} = 11.12 min.

methyl 2-(allylamino)-2-(benzo[*d*]thiazol-2-yl)propanoate (3-186)



The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 10:1) and obtained as colorless oil. **Method A**: Starting from 0.15 g (0.44 mmol, 1 equiv.) of **3-238**, yielded 0.098 g (80 %) of **(+)-3-186**, *e.r.* = ≥ 24:76, $[\alpha]_D^{24}$ = + 1.3° (*c* 0.7, $CHCl_3$); **Method B**: 0.150 g (0.44 mmol, 1 equiv.) of **3-238**, yielded 0.1 g (82 %) of **(+)-3-186**, *e.r.* = ≥ 2:98, $[\alpha]_D^{24}$ = + 5.5° (*c* 0.7, $CHCl_3$). **Method E**: 0.05 g (0.15 mmol, 1 equiv.) of **3-238**, yielded 0.025 g (51 %) of **(-)-3-186** after recuperated starting material (conversion = 30 %), *e.r.* = ≥ 73:27, $[\alpha]_D^{25}$ = - 1.5 ° (*c* 1, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.02 (dt, *J* = 8.1, 1.0 Hz, 1H), 7.88 (dt, *J* = 7.8, 0.9 Hz, 1H), 7.47 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H), 7.38 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 5.96 (ddt, *J* = 17.0, 10.1, 5.8 Hz, 1H), 5.28 (dq, *J* = 17.1, 1.7 Hz, 1H), 5.14 (dq, *J* = 10.4, 1.4 Hz, 1H), 3.78 (s, 3H), 3.31 – 3.21 (m, 2H), 1.89 (s, 3H).

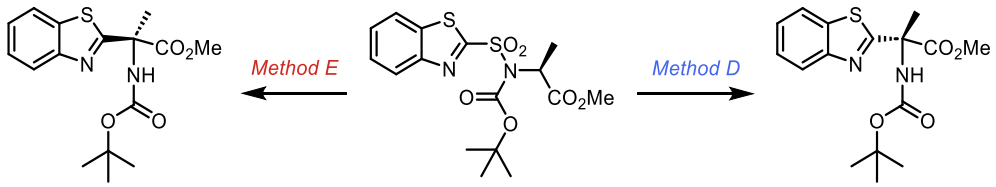
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 175.2, 173.0, 153.5, 136.1, 135.6, 126.1, 125.3, 123.5, 121.8, 116.5, 66.2, 53.2, 46.6, 23.5.

MS (ESI) *m/z* (%) 277: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{14}H_{17}N_2O_2S$, 277.1005; found: 277.1009.

HPLC (AD-H, Hexane: iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 9.68 min, t_{R2} = 11.13 min.

methyl 2-(benzo[*d*]thiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoate (3-187)



The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) and obtained as colorless oil. **Method D**: starting from 0.1 g (0.25 mmol, 1 equiv.) of **3-230**, yielded 0.069 g (82 %) of **(-)-3-187**, *e.r.* = ≥ 89:11 $[\alpha]_D^{22}$ = -14.8° (*c* 1.2, $CHCl_3$).; **Method E**: 0.02 g (0.05 mmol, 1 equiv.) of **3-230** yielded 0.01 g (56 %) of **(+)-3-187** (conversion = 60%), *e.r.* = ≥ 25:75, $[\alpha]_D^{21}$ = +5.2° (*c* 1.2, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.03 (dd, J = 8.0, 0.8 Hz, 1H), 7.86 (dd, J = 8.0, 1.4 Hz, 1H), 7.48 (ddd, J = 15.4, 8.6, 1.3 Hz, 1H), 7.40 (ddd, J = 15.1, 8.7, 1.3 Hz, 1H), 6.56 (bs, 1H), 3.75 (s, 3H), 2.13 (s, 3H), 1.48 – 1.38 (m, 9H).

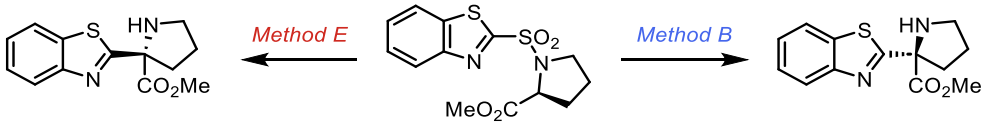
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 171.3, 170.7, 152.4, 135.9, 126.4, 125.7, 123.6, 121.8, 62.9, 53.7, 28.4.

MS (ESI) *m/z* (%) 337: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{16}H_{20}N_2O_4S$, 337.1217; found: 337.1215.

HPLC (OZ-H, Hexane : iPrOH = 95:5, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 12.85 min, t_{R2} = 14.00 min.

methyl 2-(benzo[*d*]thiazol-2-yl)pyrrolidine-2-carboxylate (**3-202**)



(-)-3-202 **3-111** **(+)-3-202**

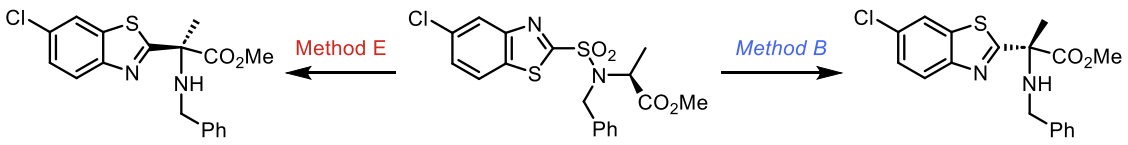
The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 10:1) and obtained as colorless oil. **Method B**: 0.1 g (0.3 mmol, 1 equiv.) of **3-110**, yielded 0.061 g (76 %) of **3-216**, *e.r.* = ≥ 98:2; $[\alpha]_D^{20}$ = + 22° (1.2 c; $CHCl_3$). **Method E**: Starting from sulfonamide **3-110** (0.050 g, 0.15 mmol, 1.0 equiv.), yielded 0.008 g (21 %) of **3-216** (conversion = 50 %), *e.r.* = 6:94; $[\alpha]_D^{22}$ = - 18° (1.0 c; $CHCl_3$).

^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm): 8.01 (ddd, *J* = 8.1, 1.2, 0.7 Hz, 1H), 7.86 (ddd, *J* = 8.0, 1.3, 0.6 Hz, 1H), 7.44 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H), 7.35 (ddd, *J* = 8.3, 7.3, 1.2 Hz, 1H), 3.80 (s, 3H), 3.36 (bs. 1H), 3.22 – 3.10 (m, 2H), 2.69 – 2.54 (m, 2H), 1.95 – 1.85 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ (ppm): 177.3, 173.1, 154.3, 135.9, 125.9, 125.0, 123.3, 121.8, 72.4, 53.6, 47.1, 38.0, 26.1.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 14.25 min, t_{R2} = 15.30 min.

methyl 2-(benzylamino)-2-(6-chlorobenzo[*d*]thiazol-2-yl)propanoate (**3-208**)



(+)-3-208 **3-158** **(-)-3-208**

The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 10:1) and obtained as colorless oil. **Method B**: 0.040 g (0.094 mmol, 1 equiv.) of **3-158**, yielded 0.030 g (88 %) of **(-)-3-208**, *e.r.* = ≥ 94:6; $[\alpha]_D^{20}$ = - 12.1° *(c* 1.2, $CHCl_3$). **Method E**: 0.032 g (0.075 mmol, 1 equiv.) of **3-158**, yielded 0.012 g (70 % after recuperating starting material) of **(+)-3-208** (conversion = 50 %), *e.r.* = ≥ 15:85, $[\alpha]_D^{23}$ = + 11 ° (*c* 1, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.02 (dd, *J* = 2.0, 0.5 Hz, 1H), 7.79 (dd, *J* = 8.5, 0.5 Hz, 1H), 7.44 – 7.41 (m, 2H), 7.37 – 7.34 (m, 3H), 7.30 – 7.27 (m, 1H), 3.82 – 3.75 (m, 5H; two signals overlap s 3H and q 2H), 2.86 (bs, 1H), 1.94 (s, 3H).

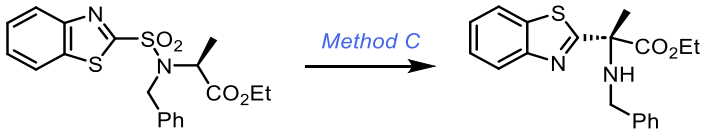
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 177.5, 172.7, 154.4, 139.5, 133.9, 132.1, 128.7, 128.4, 127.5, 125.8, 123.3, 122.6, 66.5, 53.3, 48.1, 23.7.

MS (ESI) *m/z* (%) 361: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{18}H_{18}ClN_2O_2S$, 361.0772; found: 361.0775.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 12.07 min, t_{R2} = 14.90 min.

ethyl 2-(benzo[*d*]thiazol-2-yl)-2-(benzylamino)propanoate (3-209)



3-134 **(-)-3-209**

The crude product was purified using preparative TLC (SiO_2; petroleum ether/EtOAc = 3:1) and obtained as colorless oil. **Method C**: 0.1 g (0.25 mmol, 1 equiv., *e.r.* = ≥ 90:10) of **3-130**, yielded 0.061 g (72 %) of **(-)-3-209**, *e.r.* = ≥ 90:10, $[\alpha]_D^{21}$ = - 3.4° (*c* 0.5, $CHCl_3$). **Method A**: 0.1 g (0.25 mmol, 1 equiv., *e.r.* = ≥ 90:10) of **3-134**, yielded 0.057 g (65 %) of **3-209**, *e.r.* = ≥ 50:50.

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.05 (dt, *J* = 8.2, 0.8 Hz, 1H), 7.89 (dt, *J* = 7.9, 0.8 Hz, 1H), 7.48 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.46 – 7.43 (m, 2H), 7.41 – 7.34 (m, 3H), 7.31 – 7.26 (m, 1H), 4.27 (q, *J* = 7.1 Hz, 2H), 3.84 – 3.77 (m, 2H), 2.85 (bs, 1H), 1.96 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 3H).

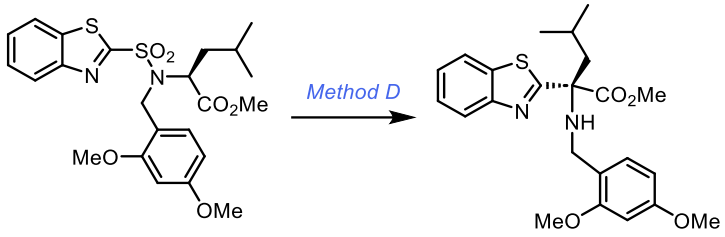
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 175.2, 172.3, 153.4, 139.7, 135.6, 128.6, 128.4, 127.4, 126.0, 125.2, 123.5, 121.8, 66.4, 62.2, 48.1, 23.6, 14.2.

MS (ESI) *m/z* (%): 341 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{19}H_{21}N_2O_2S$: 341.1318; found: 341.1319.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 11.05 min, t_{R2} = 13.65 min.

methyl 2-(benzo[*d*]thiazol-2-yl)-2-((2,4-dimethoxybenzyl)amino)-4-methylpentanoate (**3-214**)



3-244 **(-)-3-214**

The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) and obtained as colorless oil. **Method D**: 0.045 g (0.09 mmol, 1 equiv.) of **3-244** yielded 0.054 g (72 %) **(-)-3-214** after recuperating starting material (conversion = 60 %), *e.r.* = ≥ 84:16, $[\alpha]_D^{21}$ = -18.6° (*c* 0.7, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.05 (d, *J* = 8.2 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.46 (td, *J* = 8.3, 7.8, 1.5 Hz, 1H), 7.44 – 7.32 (m, 1H), 7.24 (d, *J* = 8.2 Hz, 1H), 6.49 – 6.40 (m, 2H), 3.79 (d, *J* = 6.8 Hz, 6H), 3.72 (d, *J* = 12.5 Hz, 1H), 3.68 (d, *J* = 1.1 Hz, 3H), 3.48 (d, *J* = 12.4 Hz, 1H), 3.09 (bs, 1H), 2.52 (dd, *J* = 14.7, 7.1 Hz, 1H), 2.37 (dd, *J* = 14.6, 4.7 Hz, 1H), 1.87 (ddt, *J* = 13.3, 11.2, 6.6 Hz, 1H), 0.93 (dd, *J* = 11.8, 6.7 Hz, 6H).

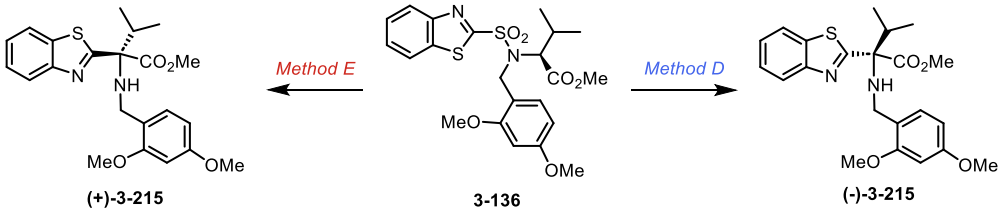
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 175.6, 172.7, 160.3, 158.6, 153.6, 135.9, 130.3, 125.9, 125.1, 123.5, 121.8, 120.4, 104.0, 98.6, 68.9, 55.5, 55.4, 52.9, 43.2, 42.2, 29.9, 24.4, 23.8, 23.4.

MS (ESI) *m/z* (%) 429: $[M+H]^+$ (100).

HRMS (ESI) *m/z*: $[M+H]^+$ calc. for $C_{23}H_{28}N_2O_4S$, 429.1843; found: 429.1845.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 11.47 min, t_{R2} = 12.27 min.

methyl 2-(benzo[*d*]thiazol-2-yl)-2-((2,4-dimethoxybenzyl)amino)-3-methylbutanoate (**3-215**)



The crude product was purified using preparative TLC (SiO_2; petroleum ether/EtOAc = 3:1) and obtained as colorless oil. **Method D**: 0.050 g (0.1 mmol, 1 equiv.) of **3-136** yielded 0.018 mg (42 %) of **(-)-3-215** (conversion = 50%) *e.r.* = ≥ 84:16, $[\alpha]_D^{21}$ = -16.3° (*c* 1, $CHCl_3$).

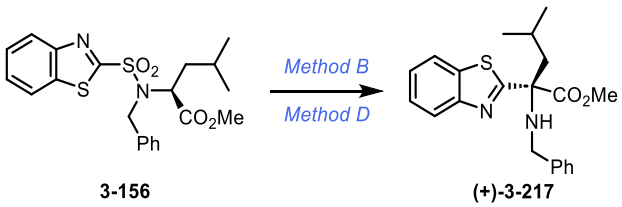
Method E: 0.050 g (0.1 mmol, 1 equiv.) of **3-136** yielded 0.016 mg (37, 75 %) of **(+)-3-215** after recuperating starting material (conversion = 52%) *e.r.* = ≥ 43:57$[\alpha]_D^{22}$ = +8.2° (*c* 1, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.07 (dt, J = 8.3, 1.4 Hz, 1H), 7.88 (dd, J = 8.0, 1.4 Hz, 1H), 7.49 – 7.43 (m, 1H), 7.39 – 7.34 (m, 1H), 7.30 – 7.22 (m, 2H), 6.46 (dt, J = 8.3, 2.0 Hz, 1H), 6.45 – 6.43 (m, 1H), 3.80 (s, 3H), 3.79 (s, 2H), 3.78 (s, 3H), 3.74 – 3.61 (m, 2H), 2.70 (hept, J = 13.8, 7.6, 3.4 Hz, 1H), 1.12 (dd, J = 6.7, 1.7 Hz, 3H), 0.99 (dd, J = 6.9, 1.7 Hz, 3H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 172.4, 172.2, 160.3, 158.6, 153.5, 135.4, 130.2, 125.7, 124.9, 123.5, 121.5, 120.5, 104.0, 98.6, 73.0, 55.5, 55.4, 52.4, 43.8, 35.9, 18.4, 17.6.

HPLC (OZ-H, Hexane : iPrOH = 95:5, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 16.25 min, t_{R2} = 29.68 min.

methyl 2-(benzo[*d*]thiazol-2-yl)-2-(benzylamino)-4-methylpentanoate (**3-217**)



The crude product was purified using preparative TLC (SiO_2; petroleum ether/EtOAc = 3:1) and obtained as colorless oil. **Method B**: Starting from 0.1 g (0.23 mmol, 1 equiv.) of **3-156**, yielded 0.134 g (73 %) of **(+)-3-217**, *e.r.* = ≥ 98:2; $[\alpha]_D^{23}$ = +9.2 (1 c, $CHCl_3$). **Method D**: Starting from 0.22 g (0.5 mmol, 1 equiv.) of **3-156**, yielded 0.134 g (86 %) of **(+)-3-217**, *e.r.* = ≥ 95:5; $[\alpha]_D^{24}$ = + 9.0 (1 c, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.06 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.89 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.48 (ddd, *J* = 8.2, 7.3, 1.3 Hz, 1H), 7.45 – 7.41 (m, 2H), 7.41 – 7.38 (m, 1H), 7.37 – 7.33

(m, 2H), 7.30 – 7.27 (m, 1H), 3.74 (s, 3H), 3.72 – 3.63 (m, 2H), 2.96 (bs, 1H), 2.53 (dd, *J* = 14.6, 7.3 Hz, 1H), 2.43 – 2.34 (m, 1H), 1.87 (hd, *J* = 6.8, 4.8 Hz, 1H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 3H).

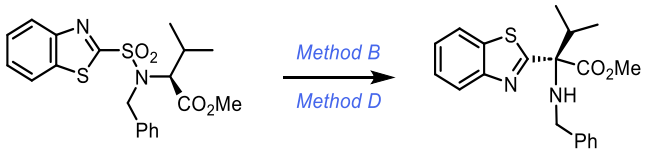
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 172.5, 153.4, 135.8, 128.6, 128.4, 127.4, 126.1, 125.3, 123.5, 121.9, 69.1, 53.1, 47.5, 43.3, 24.4, 24.1, 23.2.

MS (ESI) *m/z* (%) 369: $[M+H]^+$ (100).

HRMS (ESI) ***m/z:*** $[M+H]^+$ calc. for $C_{21}H_{25}N_2O_2S$, 369.1631; found: 369.1625.

HPLC (AD-H, Hexane : iPrOH = 80:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 10.72 min, t_{R2} = 11.58 min.

methyl 2-(benzo[*d*]thiazol-2-yl)-2-(benzylamino)-3-methylbutanoate (3-218)



3-157 **3-218**

The crude product was purified by semipreparative HPLC and obtained as colorless oil. **Method B**: Starting from 0.2 g (0.473 mmol, 1 equiv.) of **3-157** yielded 0.102 mg (75 %) of **(+)-3-218**, (conversion = 60 %); *e.r.* = ≥ 85:15; $[\alpha]_D^{22}$= +31,7 (*c* 0.6, $CHCl_3$).

Method D: Starting from 0.1 g (0.23 mmol, 1 equiv.) of **3-157** yielded 0.55 g (67 %) of **(+)-3-218**, *e.r.* = ≥ 99:1; $[\alpha]_D^{23}$ = +50.5 (1 c, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.09 (dt, *J* = 8.1, 0.8 Hz, 1H), 7.90 (ddd, *J* = 7.9, 1.3, 0.6 Hz, 1H), 7.50 – 7.42 (m, 3H), 7.40 – 7.33 (m, 3H), 7.31 – 7.26 (m, 1H), 3.85 (s, 3H), 3.78 (d, *J* = 12.5 Hz, 1H), 3.69 (d, *J* = 12.5 Hz, 1H), 2.69 (h, *J* = 6.8 Hz, 1H), 2.47 (bs, 1H), 1.12 (d, *J* = 6.7 Hz, 3H), 1.00 (d, *J* = 6.9 Hz, 3H).

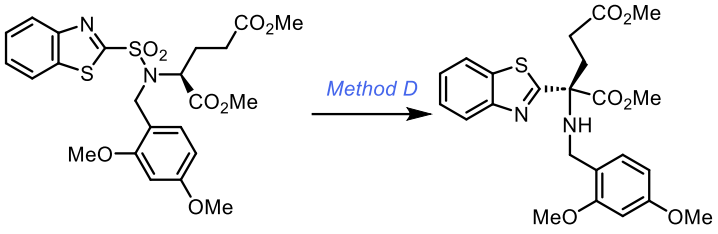
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 172.1, 171.7, 153.3, 139.8, 135.4, 128.6, 128.3, 127.4, 125.8, 125.1, 123.5, 121.5, 73.4, 52.6, 49.1, 36.9, 18.4, 17.7.

MS (ESI) *m/z* (%) 355: $[M+H]^+$ (100).

HRMS (ESI) ***m/z:*** $[M+H]^+$ calc. for $C_{20}H_{23}N_2O_2S$, 355.1475; found: 355.1471.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 10.12 min, t_{R2} = 14.18 min.

dimethyl 2-(benzo[*d*]thiazol-2-yl)-2-((2,4-dimethoxybenzyl)amino)pentanedioate (**3-219**)



3-243 **(-)-3-219**

The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) and obtained as colorless oil. **Method D**: Starting from 0.05 g (0.096 mmol, 1 equiv.) of **3-243**, yielded 0.022 g (58 %; 89 % after recuperating starting material) of **(-)-3-219** (conversion = 70 %), *e.r.* = ≥ 89:11 $[\alpha]_D^{22}$ = -18.6° (*c* 1.1, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.18 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.97 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.60 (ddd, *J* = 8.4, 7.2, 1.3 Hz, 1H), 7.55 (ddd, *J* = 8.3, 7.2, 1.2 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 1H), 6.47 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.32 (d, *J* = 2.4 Hz, 1H), 4.73 (d, *J* = 15.5 Hz, 1H), 4.73 – 4.64 (m, 2H), 4.62 (d, *J* = 15.4 Hz, 1H), 3.78 (s, 3H), 3.67 (s, 3H), 3.58 (s, 3H), 3.35 (s, 3H), 2.36 – 2.19 (m, 3H), 1.95 (qd, *J* = 12.0, 10.1, 3.4 Hz, 1H).

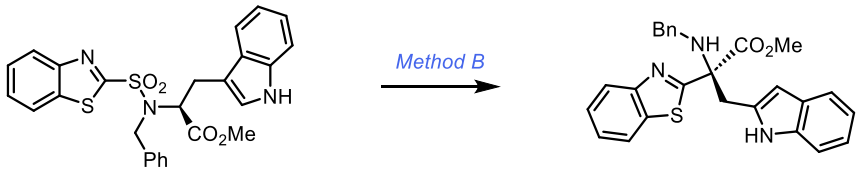
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 173.9, 173.6, 172.0, 160.4, 158.7, 153.5, 135.5, 130.5, 126.1, 125.3, 123.6, 121.8, 119.9, 104.0, 98.6, 68.4, 55.5, 55.4, 53.1, 51.8, 42.4, 29.8, 28.7.

MS (ESI) *m/z* (%) 459: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{17}H_{19}N_2O_4$, 459.1584; found: 459.1580.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 16.00 min, t_{R2} = 21.37 min.

methyl 2-(benzo[*d*]thiazol-2-yl)-2-(benzylamino)-3-(1*H*-indol-2-yl)propanoate (**3-221**)



3-236 **(+)-3-221**

The crude product was purified by preparative TLC (SiO_2; petroleum ether/EtOAc = 7:2) and obtained as colorless oil. **Method B**: Starting from 0.04 g (0.078 mmol, 1 equiv.) of **3-236**, yielded 0.025 g (73 %, 95 % rsm) **(+)-3-221**, *e.r.* = ≥ 92:8; $[\alpha]_D^{22}$= +18.2 (*c* 1.2, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.13 – 8.06 (m, 2H), 7.87 (dt, *J* = 8.0, 1.0 Hz, 1H), 7.60 (dt, *J* = 8.1, 0.9 Hz, 1H), 7.50 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 7.41 – 7.38 (m, 3H), 7.33 – 7.30 (m, 3H), 7.26 – 7.23 (m, 1H), 7.15 (ddd, *J* = 8.0, 6.9, 1.1 Hz, 1H), 7.09 – 7.07 (m, 1H), 7.05 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 4.18 (dd, *J* = 15.1, 0.8 Hz, 1H), 3.92 – 3.81 (m, 3H), 3.61 (s, 3H).

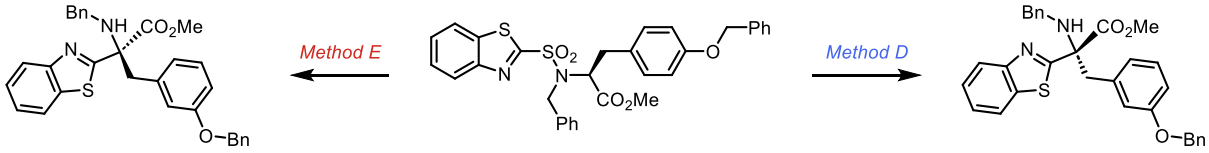
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 173.9, 171.9, 153.3, 139.7, 136.1, 135.8, 128.5, 128.3, 128.1, 127.3, 126.0, 125.3, 123.6, 123.4, 122.2, 121.8, 119.6, 118.9, 111.2, 109.2, 70.2, 53.1, 47.7, 31.5.

MS (ESI) *m/z* (%) 442: $[M+H]^+$ (100).

HRMS (ESI) *m/z*: $[M+H]^+$ calc. for $C_{26}H_{24}N_3O_2S$, 442.1584; found: 442.1578.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 16.63 min, t_{R2} = 19.25 min.

dimethyl 2-(benzo[*d*]thiazol-2-yl)-2-((2,4-dimethoxybenzyl)amino)pentanedioate (**3-222**)



(+)-3-222 **3-153** **(-)-3-222**

The crude product was purified by column chromatography (SiO_2; petroleum ether/EtOAc = 4:1) and obtained as colorless oil. **Method D**: Starting from 0.223 g (0.4 mmol, 1 equiv.) of **3-153**, yielded 0.158 g (78 %) of **(-)-3-222**, *e.r.* = ≥ 99:1 $[\alpha]_D^{21}$ = -25.6° (*c* 1.2, $CHCl_3$).; **Method E**: 0.03 g (0.05 mmol, 1 equiv.) of **3-153** yielded 0.018 g (67 %) of **(+)-3-222** (conversion was 50 %), *e.r.* = ≥ 15:85, $[\alpha]_D^{21}$ = +18.6° (*c* 1.2, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.09 (d, J = 8.2 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.50 (t, J = 7.7 Hz, 1H), 7.44 – 7.36 (m, 7H), 7.34 (t, J = 7.9 Hz, 3H), 7.32 – 7.22 (m, 2H), 7.06 (d,

J = 8.1 Hz, 2H), 6.88 – 6.81 (m, 2H), 5.01 (s, 2H), 3.91 (dd, J = 17.3, 13.4 Hz, 2H), 3.80 (d, J = 12.7 Hz, 1H), 3.71 (d, J = 1.4 Hz, 3H), 3.65 (d, J = 14.3 Hz, 1H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 173.6, 171.5, 158.1, 153.2, 139.5, 137.1, 136.0, 131.2, 128.7, 128.6, 128.3, 128.1, 127.7, 127.6, 127.4, 126.0, 125.4, 123.6, 121.9, 114.9, 70.8, 70.1, 53.0, 47.7, 40.4.

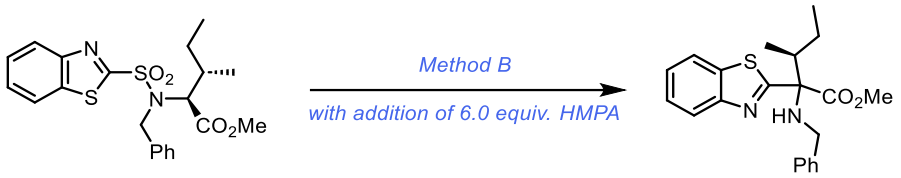
MS (ESI) *m/z* (%) 459: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{17}H_{19}N_2O_4$, 459.1584; found: 459.1580.

R_f = 0.65 (hexane/ EtOAc = 4:1)

HPLC (AD-H, Hexane : iPrOH = 80:20, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 19.32 min, t_{R2} = 27.33 min.

mixture of (3*S*) methyl 2-(benzo[*d*]thiazol-2-yl)-2-(benzylamino)-3-methylpentanoate (**3-223**)



3-145 **3-223**

The crude product was purified using preparative TLC (SiO_2; petroleum ether/EtOAc = 3:1) and obtained as colorless oil. **Modified method B**: Reaction time was increased to 1h, 3 equiv. of LiHMDS were used and HMPA (6.0 equiv.) was added. Starting from 0.050 g (0.114 mmol, 1 equiv., *e.r. =* = 99:1) of **3-145,** yielded (54 %; 78 % on recuperating starting material) of **3-223** 0.023 g, *e.r. =* ≥ 52:48. , (conversion = 55 %).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.08 (dd, *J* = 8.2, 3.7 Hz, 2H), 7.89 (d, *J* = 7.9 Hz, 2H), 7.49 – 7.43 (m, 6H), 7.40 – 7.33 (m, 6H), 7.30 – 7.26 (m, 2H), 3.85 (d, *J* = 3.9 Hz, 6H), 3.80 – 3.74 (m, 2H), 3.69 (dd, *J* = 12.4, 6.6 Hz, 2H), 2.40 – 2.33 (m, 2H), 1.95 – 1.83 (m, 2H), 1.13 (d, *J* = 6.7 Hz, 3H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.94 (t, *J* = 7.4 Hz, 3H), 0.91 – 0.87 (m, 3H).

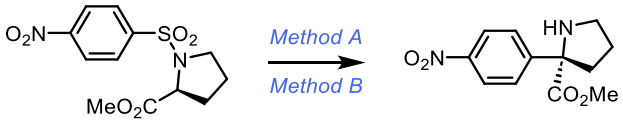
^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) *δ* (ppm): 172.3, 172.1, 172.1, 172.1, 153.4, 153.3, 139.9, 139.8, 135.4, 135.4, 128.6, 128.3, 128.3, 127.4, 127.4, 125.8, 125.1, 123.5, 123.5, 121.5, 73.7, 73.7, 52.6, 52.6, 49.1, 49.1, 44.0, 43.8, 25.4, 24.3, 14.6, 13.9, 12.7, 12.6.

MS (ESI) *m/z* (%) 369: $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calc. for $C_{18}H_{18}ClN_2O_2S$, 369.1631; found: 369.1633.

HPLC (AD-H, Hexane: iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 9.92 min, t_{R2} = 12.63 min.

methyl 2-(4-nitrophenyl)pyrrolidine-2-carboxylate (**3-224**)



3-234 **(-)-3-224**

The crude product was purified using preparative TLC (SiO_2; petroleum ether/EtOAc = 4:1) and obtained as colorless oil. **Method A**: Starting from 0.07 g (0.21 mmol, 1 equiv.) of **3-224**, yielded 0.041 g (74 %) of **(-)-3-224**, *e.r.* = ≥ 91:9, $[\alpha]_D^{20}$ = - 8° (*c* 1, $CHCl_3$); **Method B**: Starting from 0.07 g (0.21 mmol, 1 equiv.) of **3-234**, yielded 0.042 g (75 %) **(-)-3-224**, *e.r.* = ≥ 98:2, $[\alpha]_D^{21}$ = - 9.1° (*c* 1, $CHCl_3$).

^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 8.20 – 8.14 (m, 2H), 7.77 – 7.73 (m, 2H), 3.71 (s, 3H), 3.10 (dddd, *J* = 32.3, 10.0, 7.6, 6.3 Hz, 2H), 2.76 (ddd, *J* = 12.9, 7.7, 5.5 Hz, 1H), 2.05 (dt, *J* = 12.8, 8.2 Hz, 1H), 1.88 – 1.80 (m, 2H).

^{13}C {^{1}H} NMR (126 MHz, Chloroform-*d*) δ (ppm): 175.1, 150.5, 147.3, 127.5, 123.5, 72.3, 53.3, 46.2, 37.8, 25.2.

MS (ESI) *m/z* (%): 251 $[M+H]^+$ (100).

HRMS (ESI) *m/z:* $[M+H]^+$ calculated for $C_{12}H_{15}N_2O_4$: 251.1026; found: 251.1029.

HPLC (AD-H, Hexane : iPrOH = 90:10, 0.5 mL/min, 298 K, 220 nm): t_{R1} = 17.10 min, t_{R2} = 19.85 min.

4. Global conclusion

This thesis summarizes the successful development and optimization of several synthetic pathways and the exploration of their respective biological activities. We have established a robust method for the synthesis of DCG-A molecule **1-108** and its analogues, producing a library of 50 compounds with notable activities against various biological targets. Specifically, compound **1-117c** demonstrated significant anthelmintic activity, including efficacy against resistant strains of *C. elegans*, indicating a novel mechanism of action. Furthermore, the candidate worked on the development of a versatile 'non-symmetrical' route toward dihydrobenzofurans using squareamide or thiourea organocatalysts (since this project is not finished, it was not included in this Thesis).

In the second project, we have successfully optimized the synthetic pathway towards various NO_2FAs including (10- and 9-NO_2OA, 10-NO_2LA, 9-NO_2cLA, 14-NO_2ARA, and 10-NO_2SA). These compounds are currently being evaluated extensively in biological tests to determine their therapeutic potential, with promising preliminary results suggesting radioprotective properties. This work lay the foundation for the future development of NO_2FAs as potential therapeutic agents. Furthermore, the candidate partially worked on the development of a Julia-Kocienski olefination method and its application in the synthesis of various FA and NO_2FA.

1. Bon, D.J.-Y.D.; **Chrenko, D.**; Kováč, O.; Ferugová, V.; Lasák, P.; Fuksová, M.; Zálešák, F. & Pospíšil, J. *Adv. Synth.Catal.* **366**, 480–4 (**2024**).
2. **Chrenko, D.**; Pospíšil, J. *Molecules* , **29** (12), 2719 (**2024**).

In the third part, we have successfully expanded the scope of heteroaryl sulfonamide synthesis to include various heterocycles and leveraged these compounds in a novel developed methodology for the asymmetric synthesis of α-heteroaryl α-substituted α-amino acid derivatives which proceeds under the polar Truce-Smiles rearrangement. We have also successfully proposed a mechanism of rearrangement based on detailed experimental and computational studies.

3. Iakovenko, R. O.; **Chrenko, D.**; Kristek, J.; Desmedt, E.; Zálešák, F.; De Vleeschouwer, F. & Pospíšil, J. *Org. Biomol. Chem.* **20**, 3154–3159 (**2022**).

Overall conclusion

During a research stay at JKU Linz in 2022, in a group of prof. Mario Waser, the candidate worked on developing and applying new phase-transfer catalysts. The results obtained during the research stay were published in 2023.

4. Zebrowski, P., Röser, K., **Chrenko, D.**, Pospíšil, J. & Waser, M. *Synthesis* **55**, 1706–1713 (**2023**). (Included in Special Issue dedicated to Prof. Dr. Cristina Nevado, Recipient of the 2021 Dr. Margaret Faul Women in Chemistry Award)

Additionally, during the research stay at UZH Zurich in 2023, in the group of prof. Cristina Nevado, the candidate worked on the synthesis of new ligands for asymmetric dual Ni/photoredox catalysis and photoredox-dual catalyzed silylarylation of unactivated alkenes.

In summary, this thesis presents not only significant synthetic advancements, but also biological insight. Future research will continue to build on these foundations.

5. Literature

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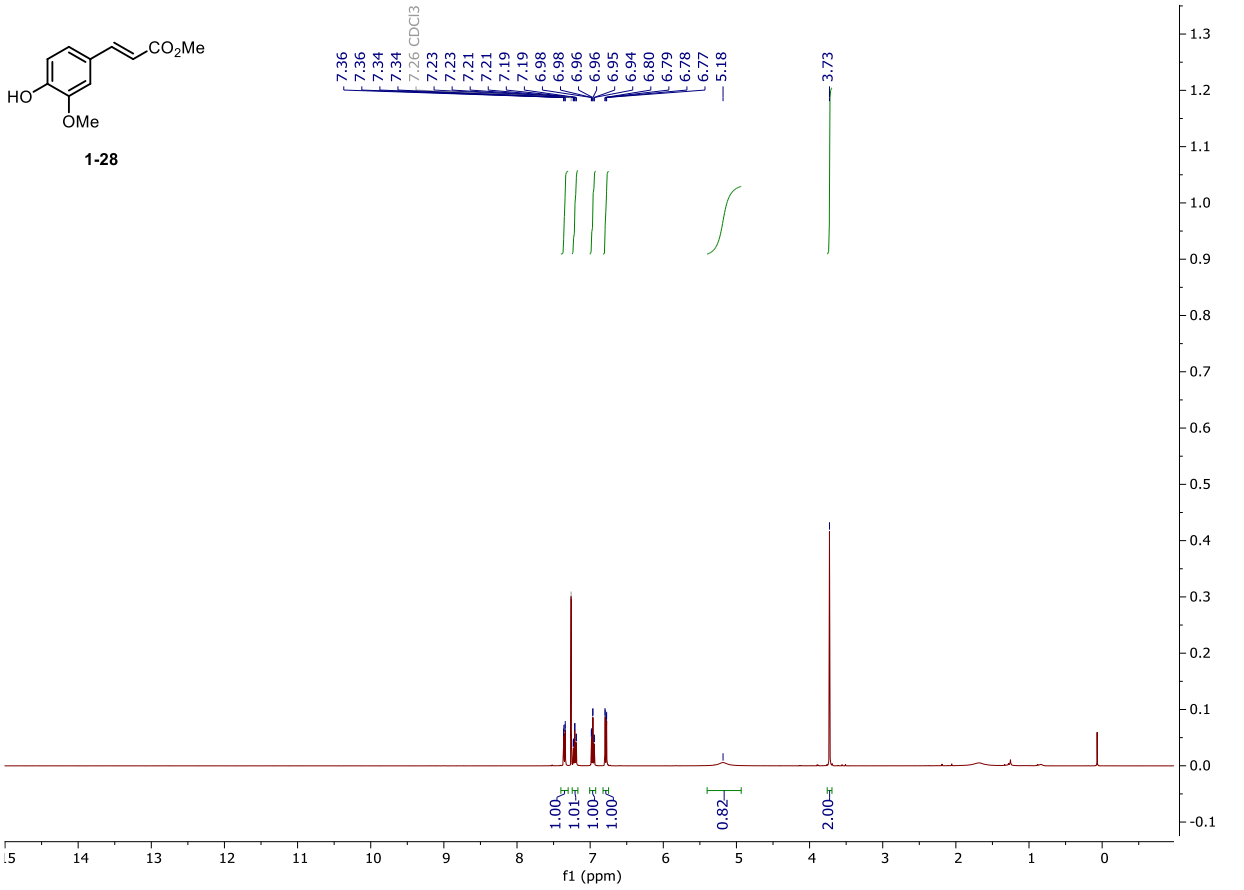
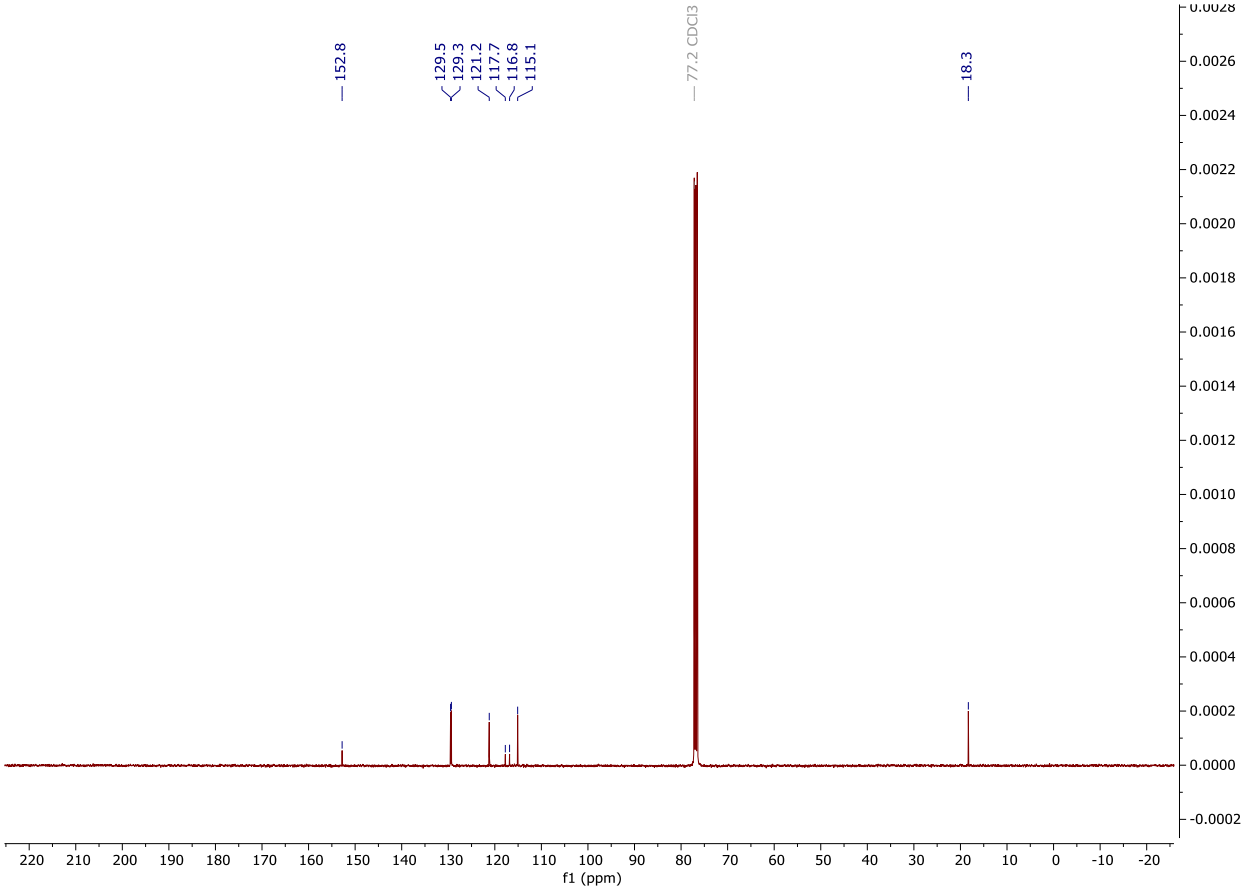
6. NMR and HPLC

The ^{1}H NMR and $^{13}C\{^{1}H\}$ NMR spectra were measured on Jeol ECA400II (400 and 101 MHz) or Jeol 500 ECA (500 and 126 MHz) in Chloroform-d or CD_3OD. Chemical shifts are reported in ppm, and their calibration was carried out (a) in the case of ^{1}H NMR experiments on the residual peak of non-deuterated solvent δ ($CDCl_3$) = 7.26 ppm or δ (CD_3OD) = 3.31 ppm, δ (DMSO-d_6)= 2.50 ppm, δ (acetone-d_6)= 2.05 ppm and in the case of ^{13}C NMR experiments on the middle peak of the ^{13}C signal in deuterated solvent δ ($CDCl_3$) = 77.16 ppm, δ (CD_3OD) = 49.00 ppm, (DMSO-d_6)= 39.52 ppm, (acetone-d_6)= 29.84 ppm. The proton coupling patterns are represented as a singlet (s), a doublet (d), a doublet of a doublet (dd), a triplet (t), a triplet of a triplet (tt), and a multiplet (m). ***NMR spectra were recorded from a pure compounds, purified by column chromatography, preparative TLC or in some cases semipreparative HPLC.***

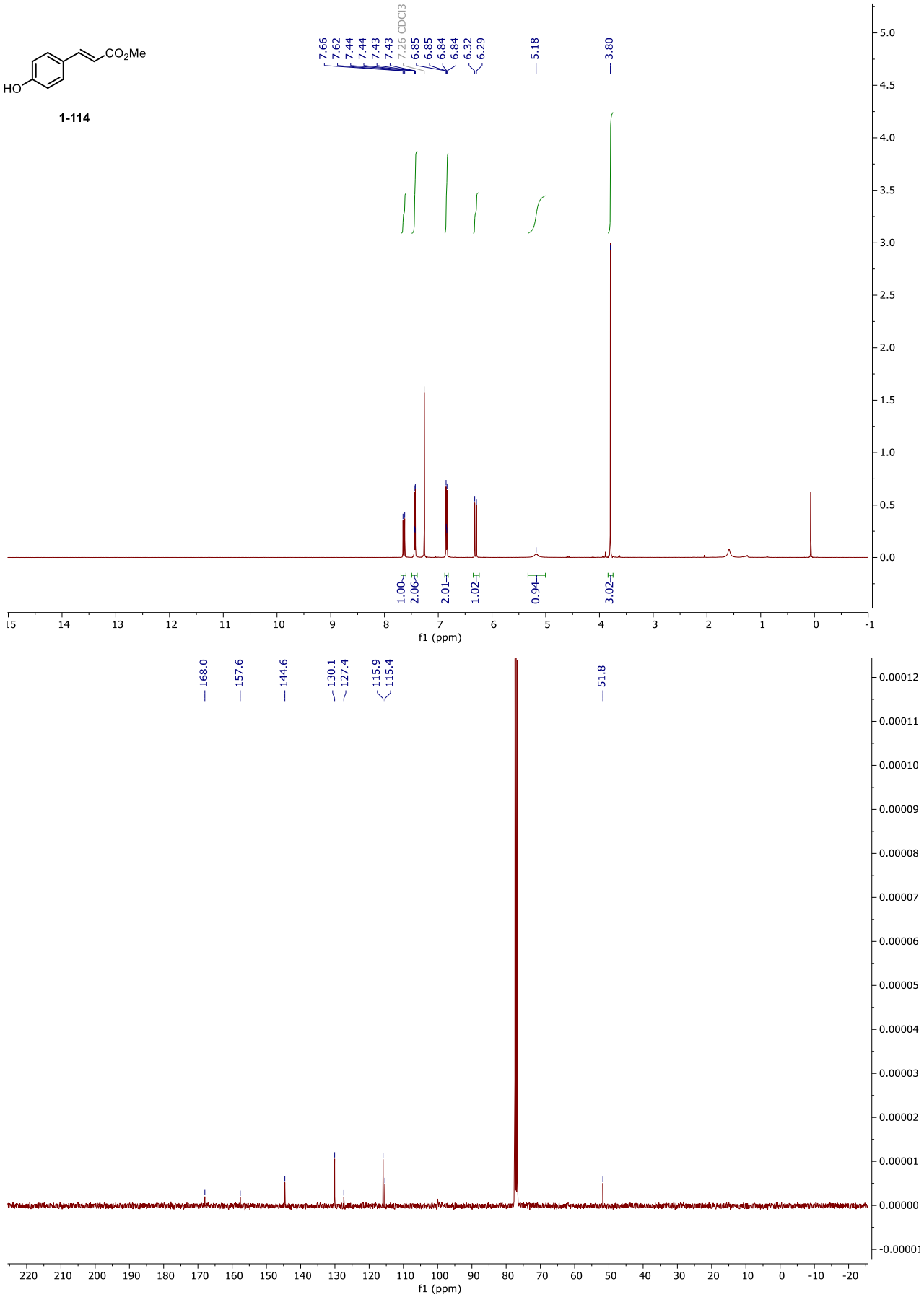
Chiral analysis was performed on Waters Alliance 2695 with autosampler and UV-VIS detector Waters 2996 PDA using chiral columns (CHIRAL ART Amylose-SA 250x4,6 mm, 5 µm; CHIRALCEL Cellulose OD-H, 250x4,6 mm, 5 µm; CHIRALCEL Cellulose OZ-H, 250x4,6 mm, 5 µm). All solvents used were HPLC-grade solvents purchased from Merk. The column employed and the respective solvent mixture are indicated for each experiment. ***HPLC chromatograms were recorded mostly from pure compounds but in some cases crude reaction mixture was measured instead.***

Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-28**

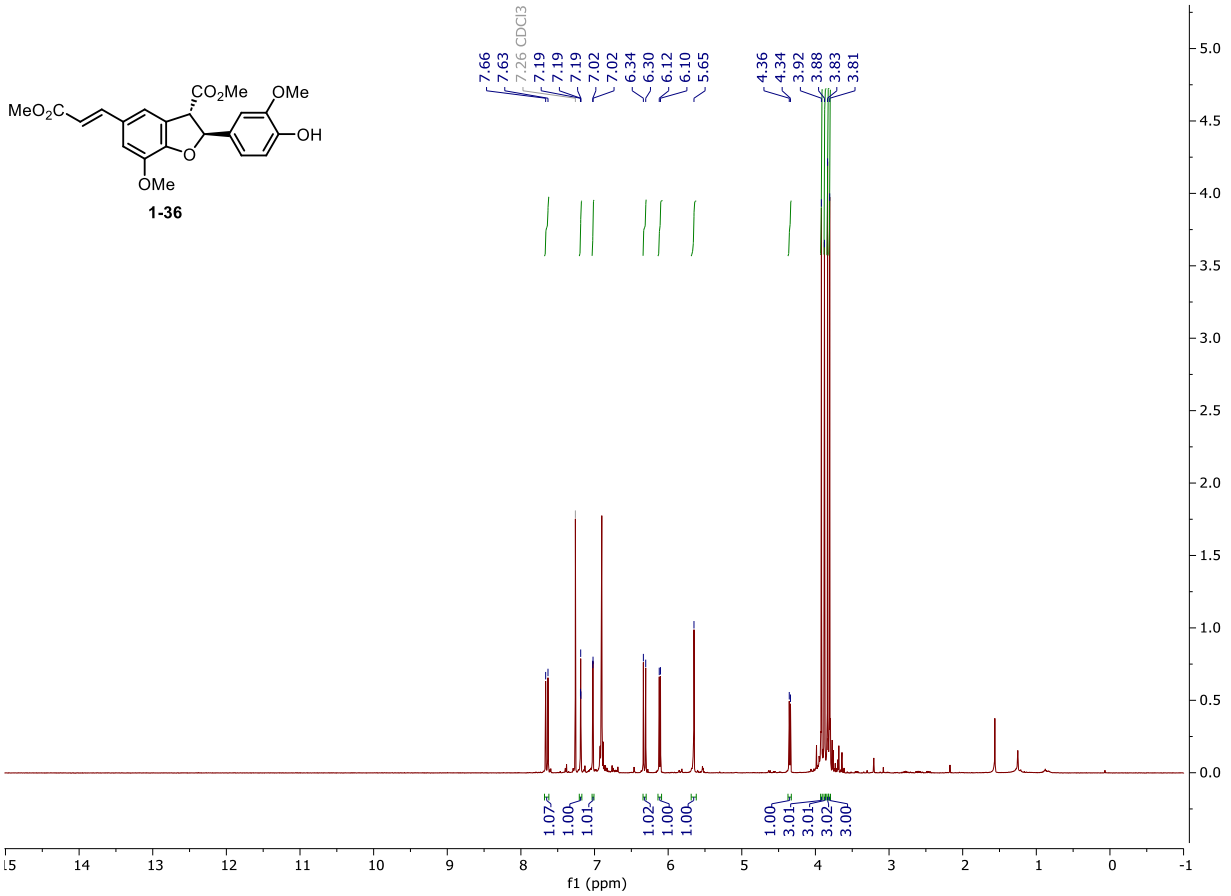


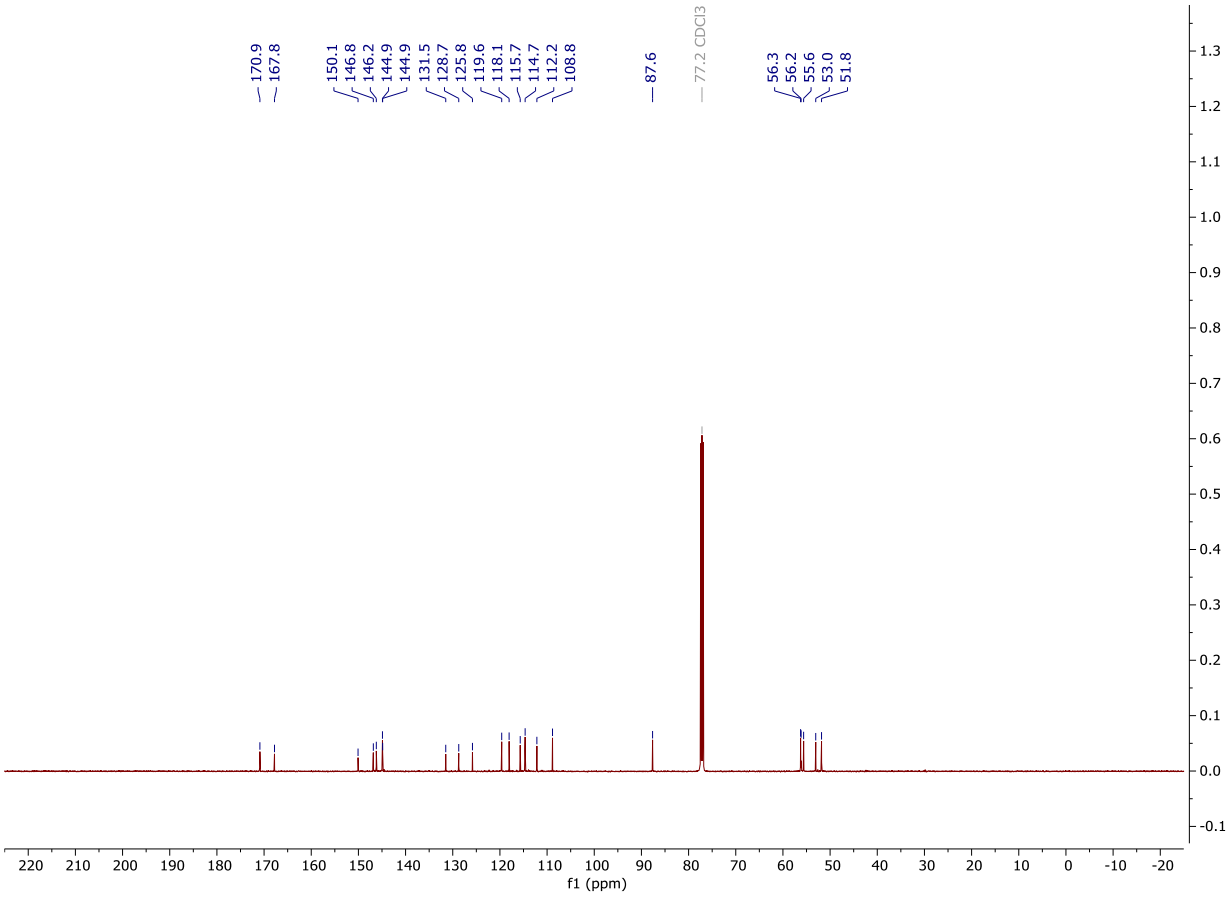


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-114**

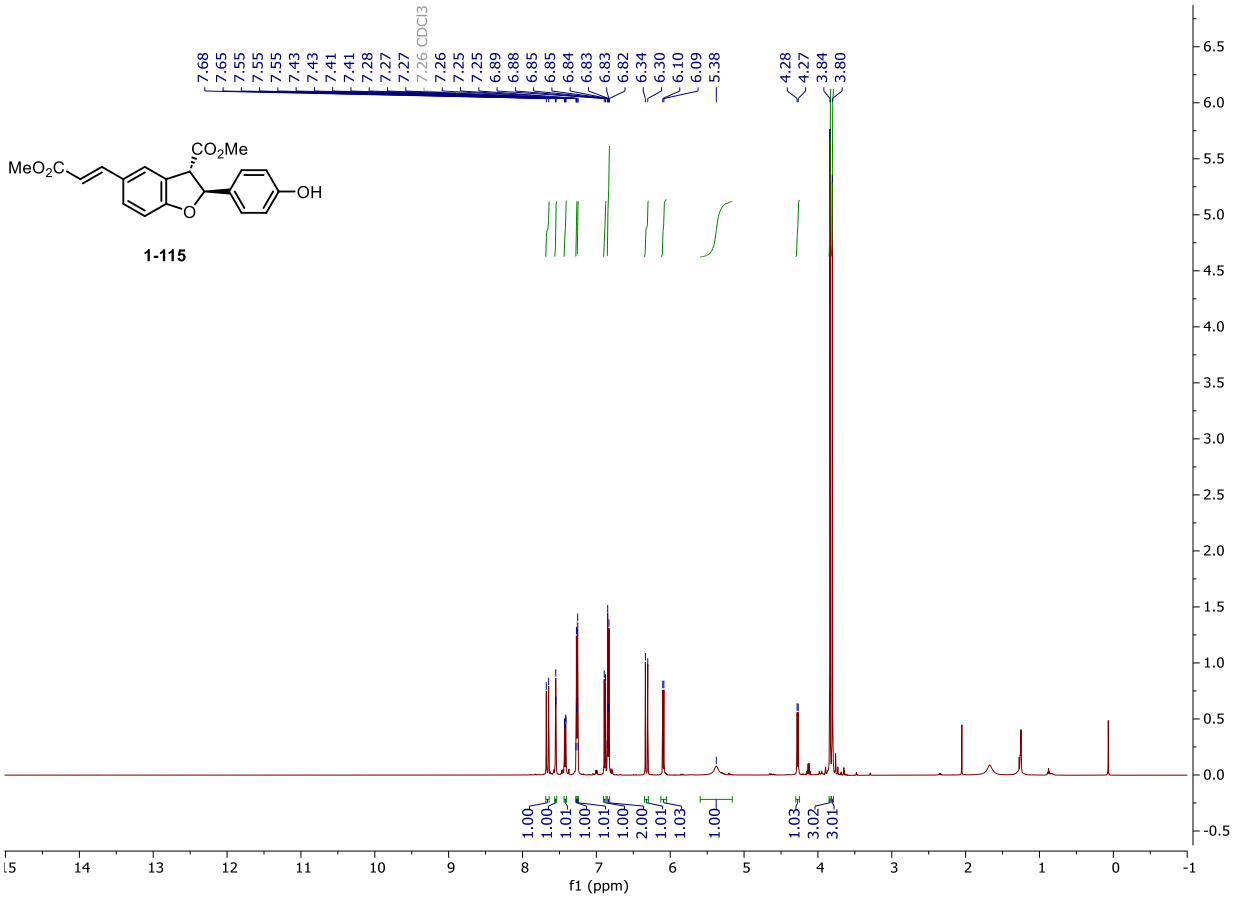


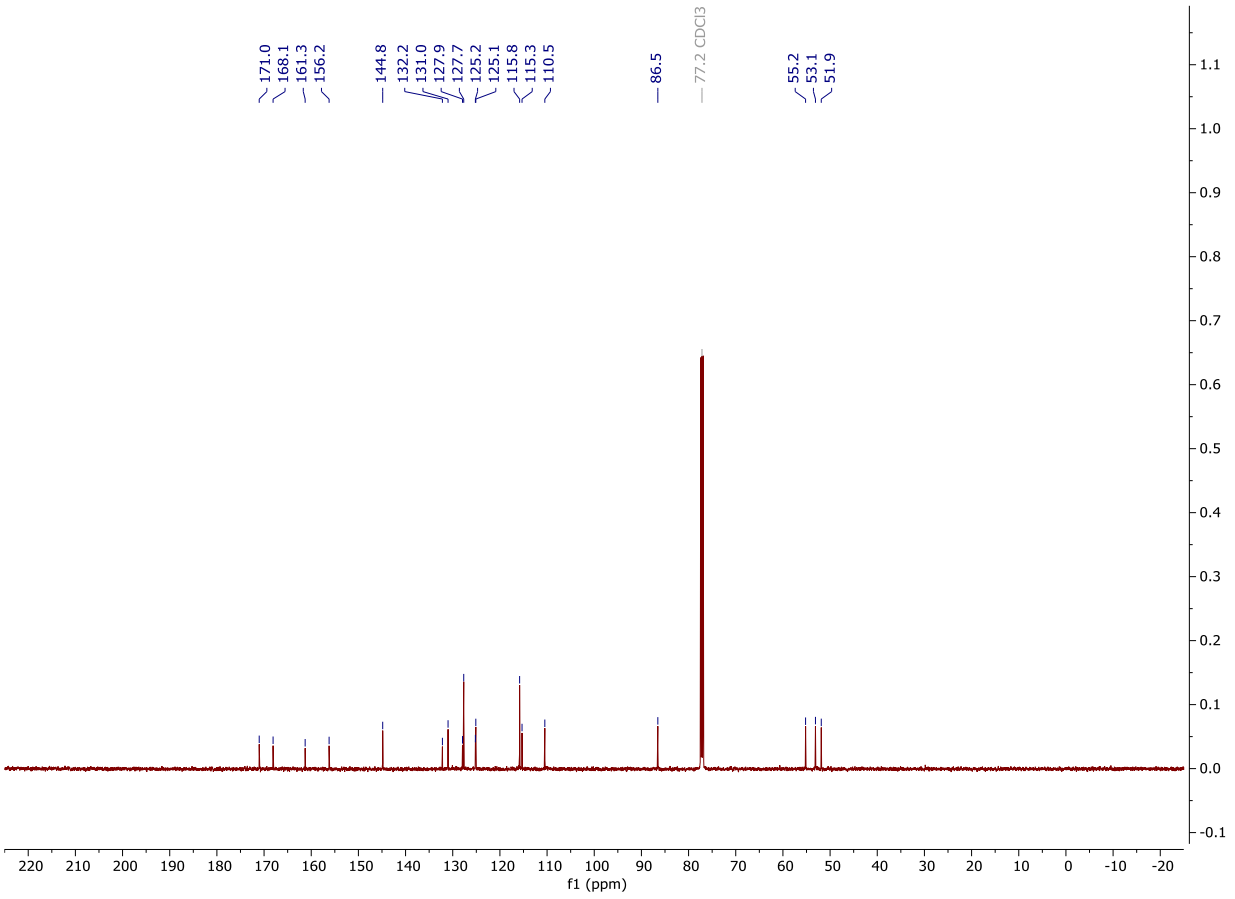
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-36**





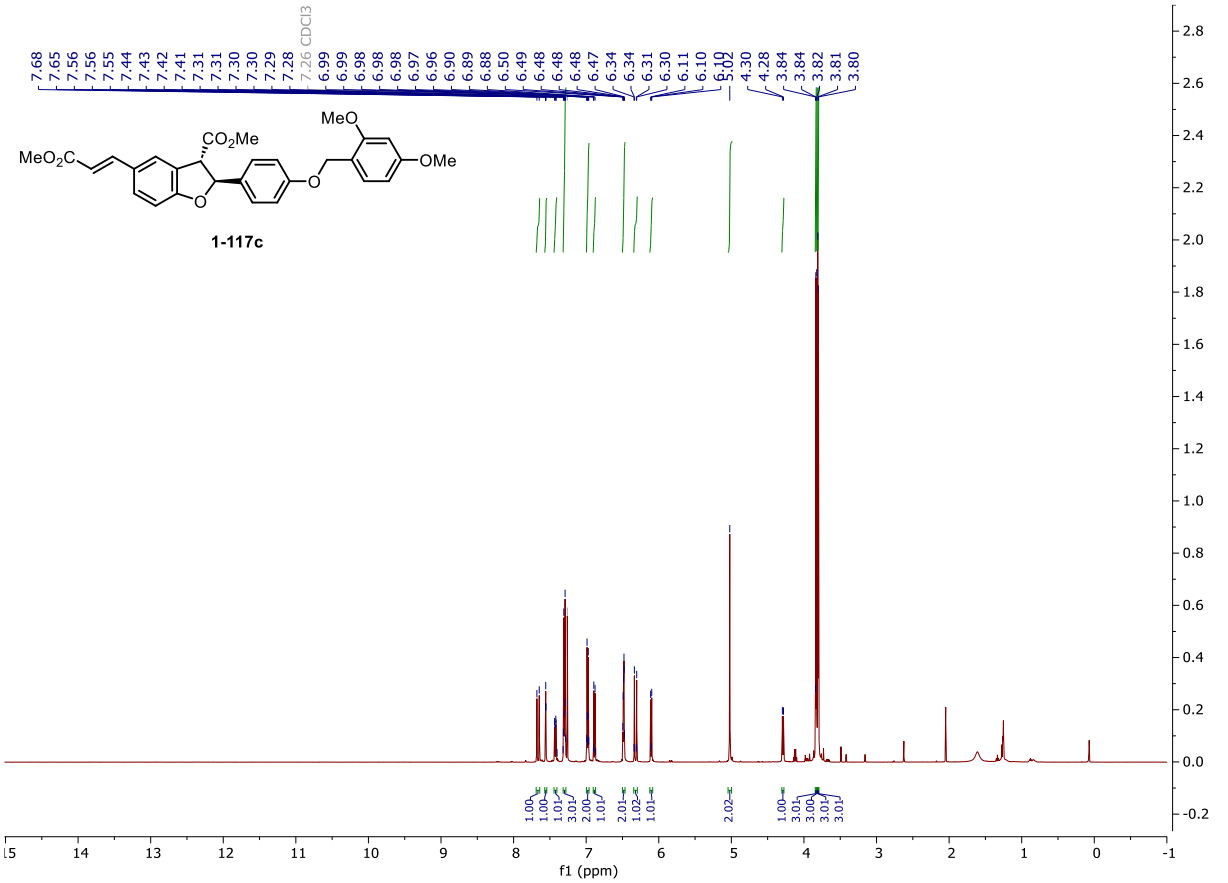
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-115**

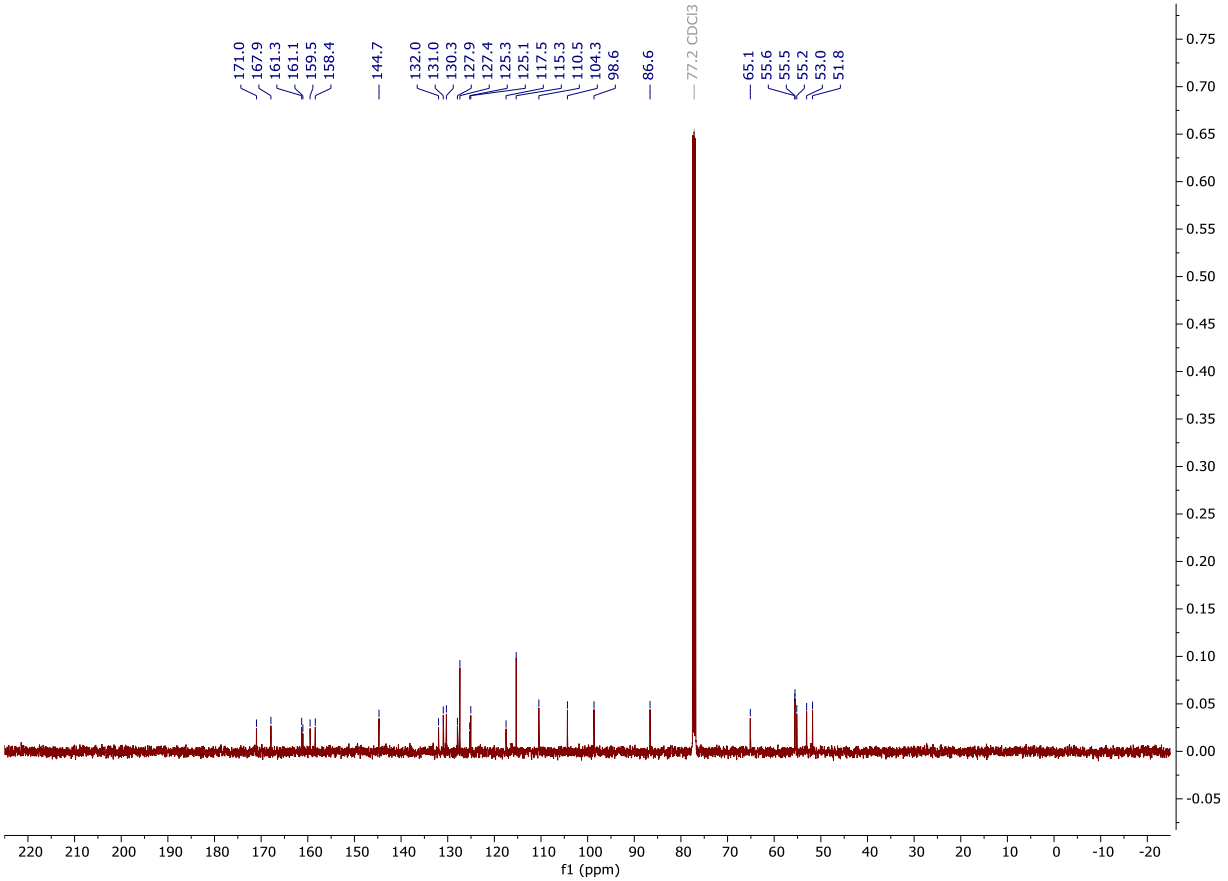




NMR and HPLC

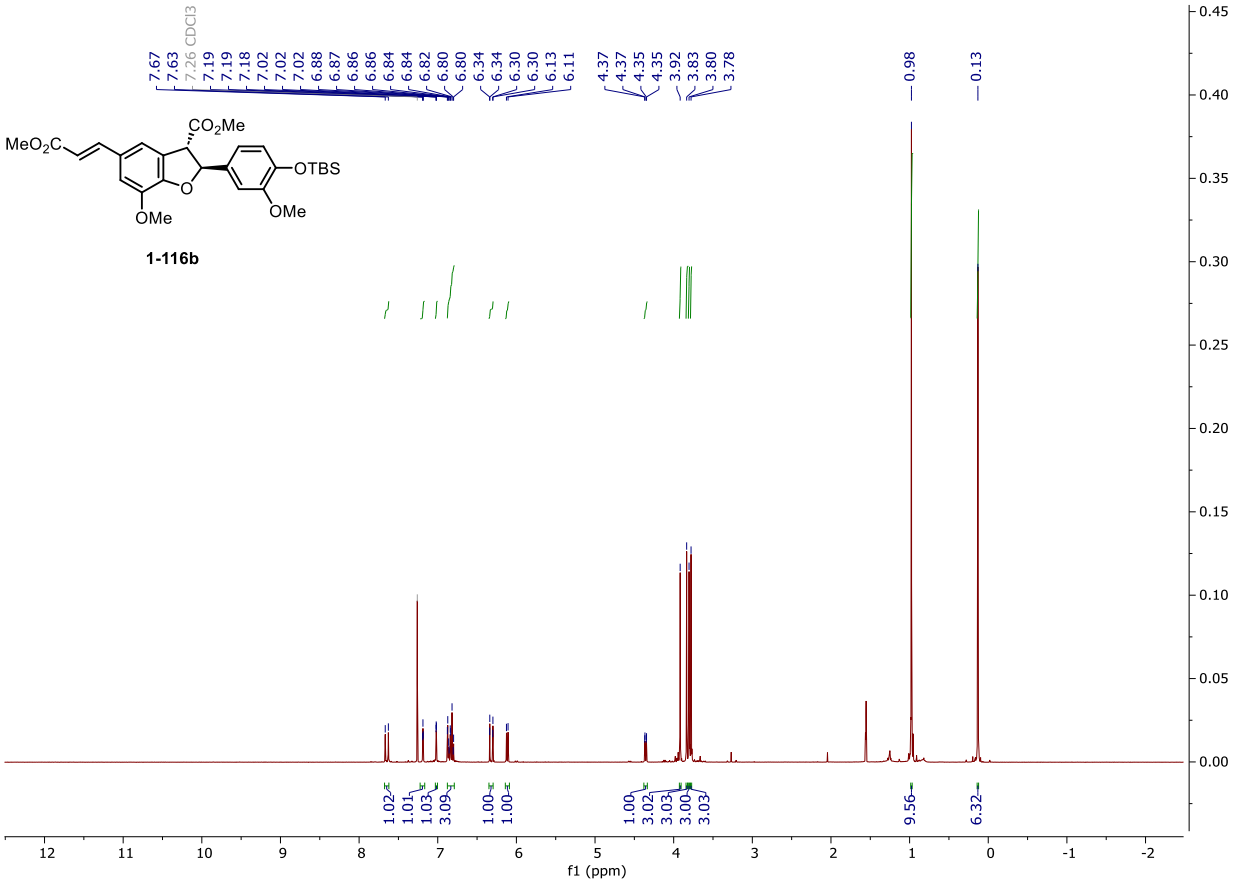
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-117c**

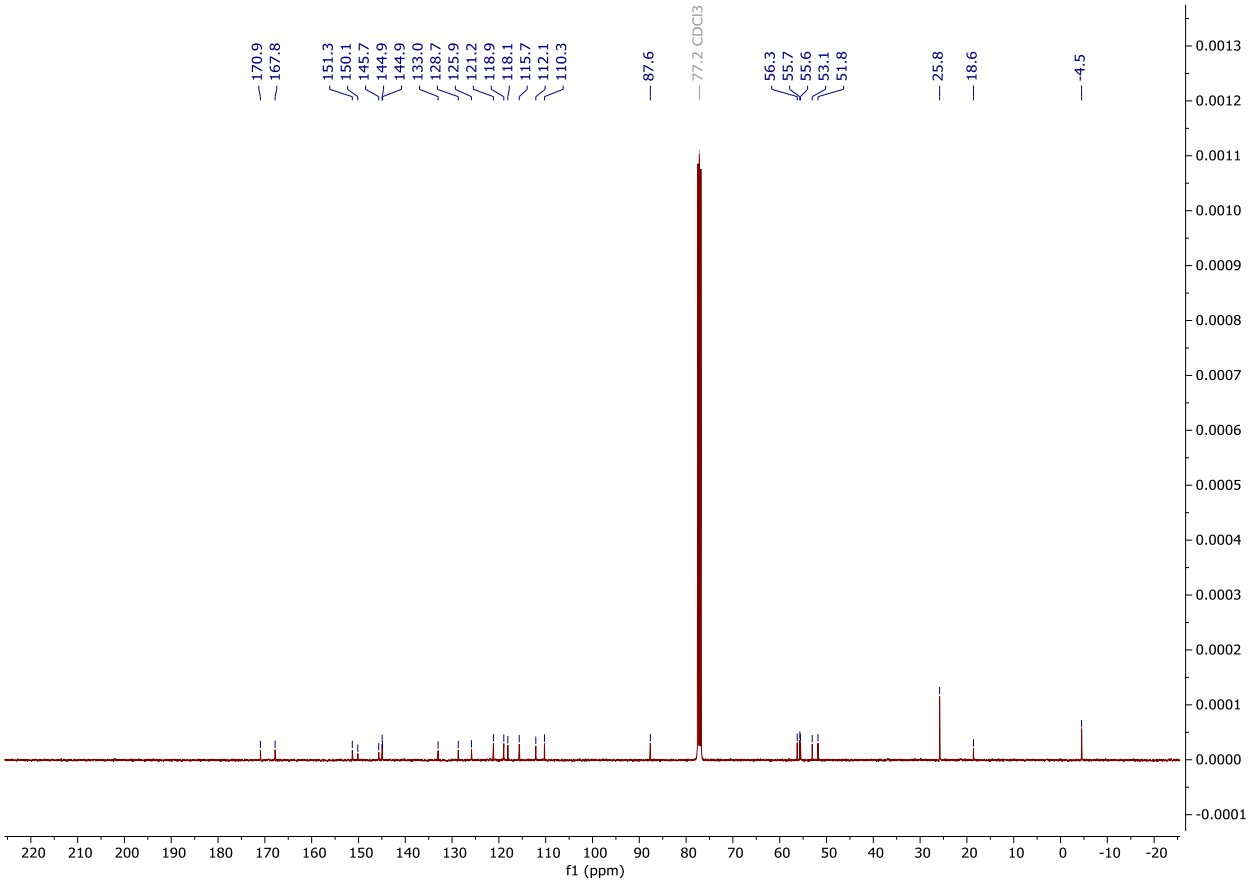




NMR and HPLC

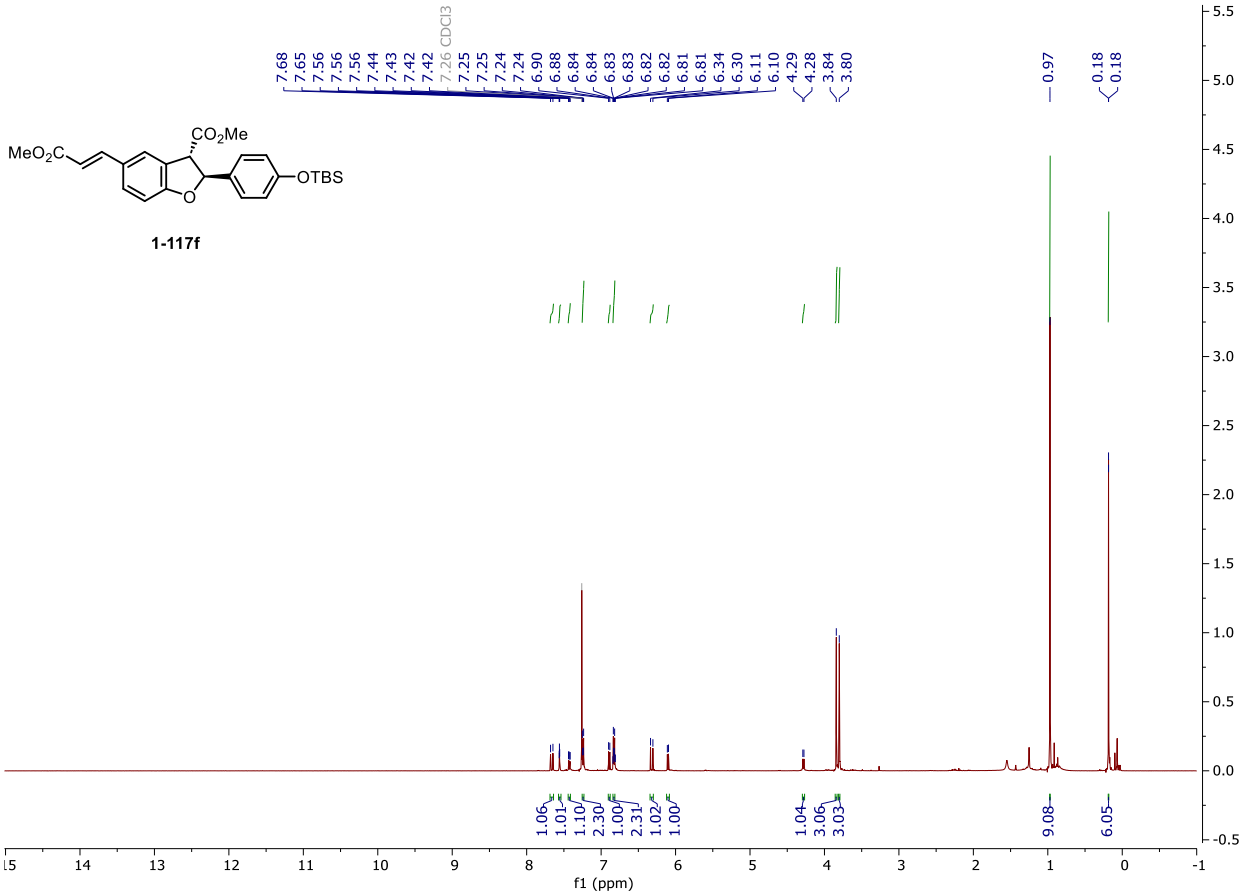
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-116b**

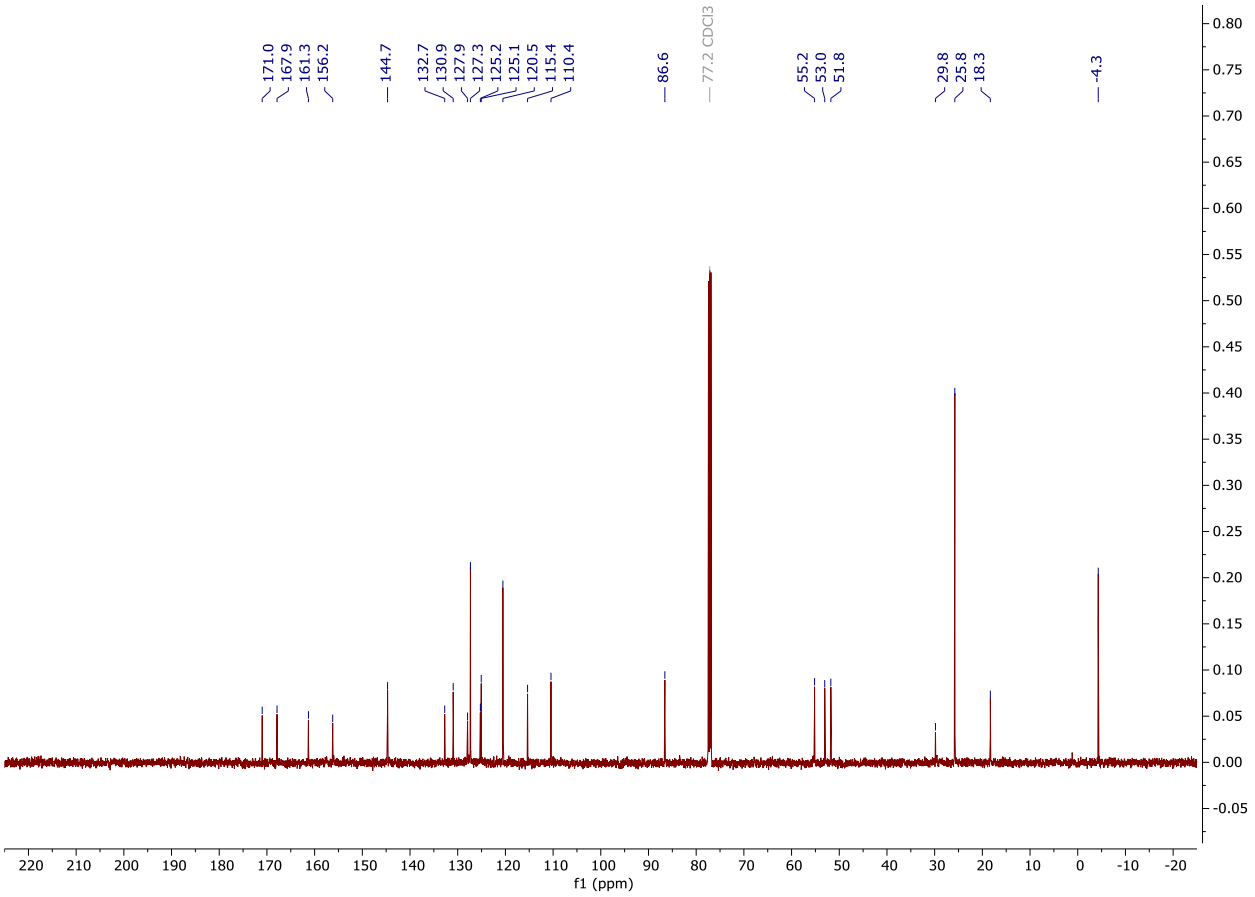




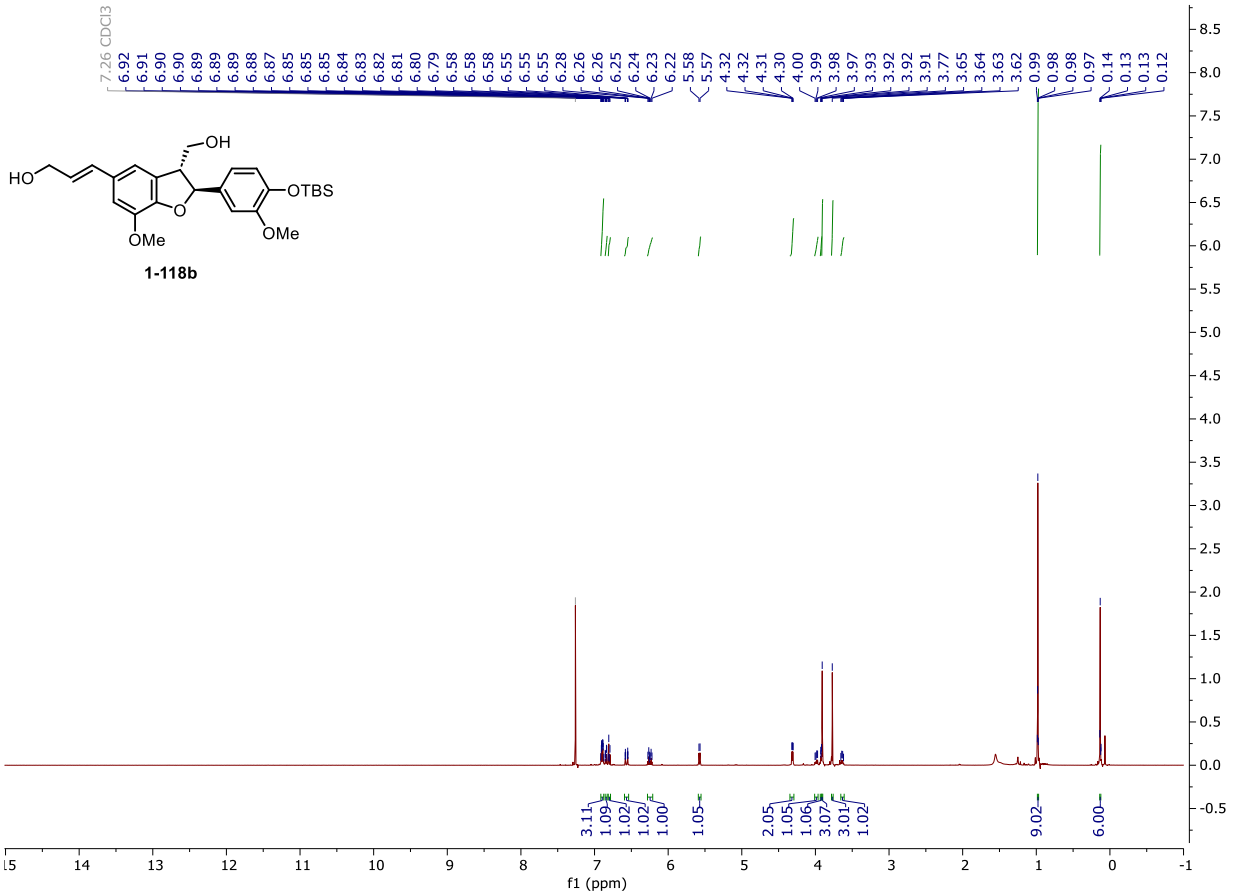
NMR and HPLC

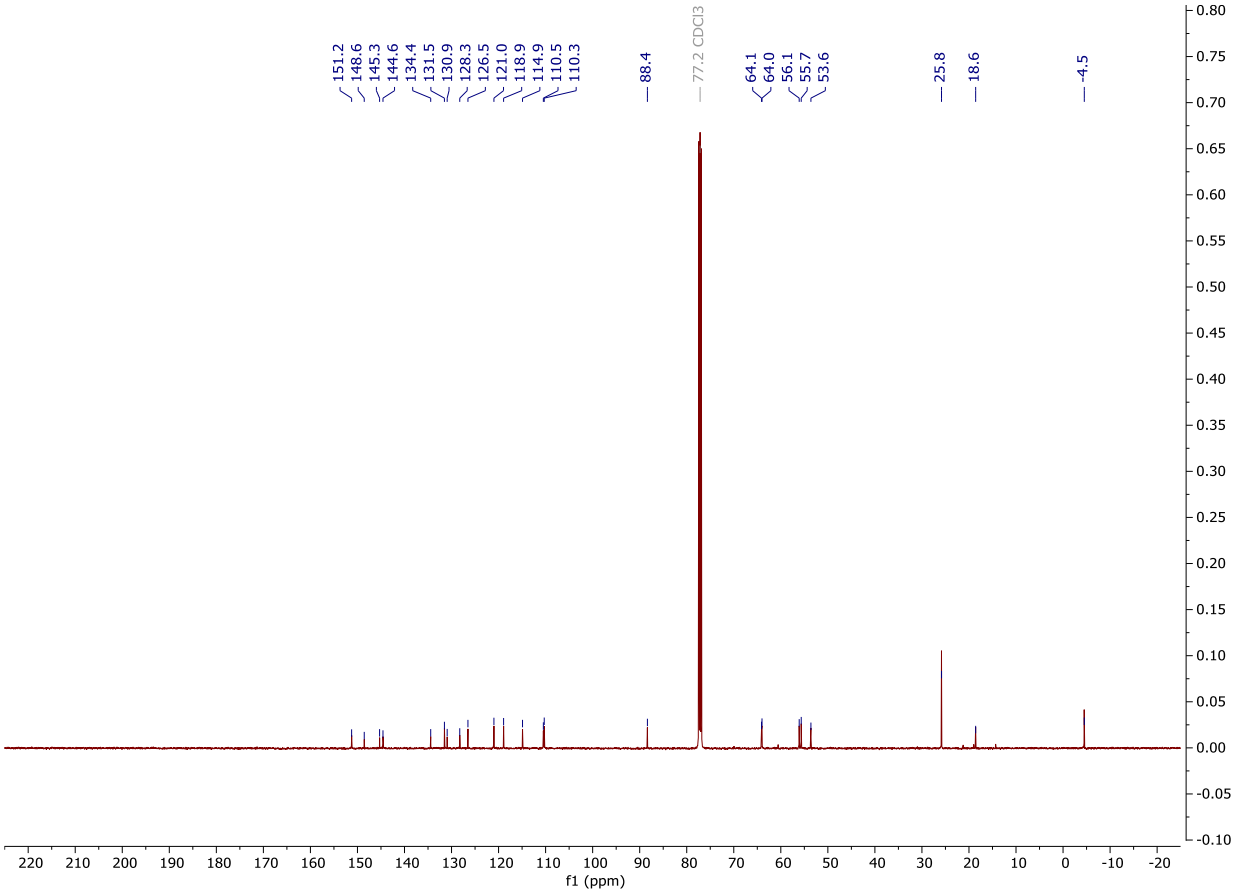
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-117f**



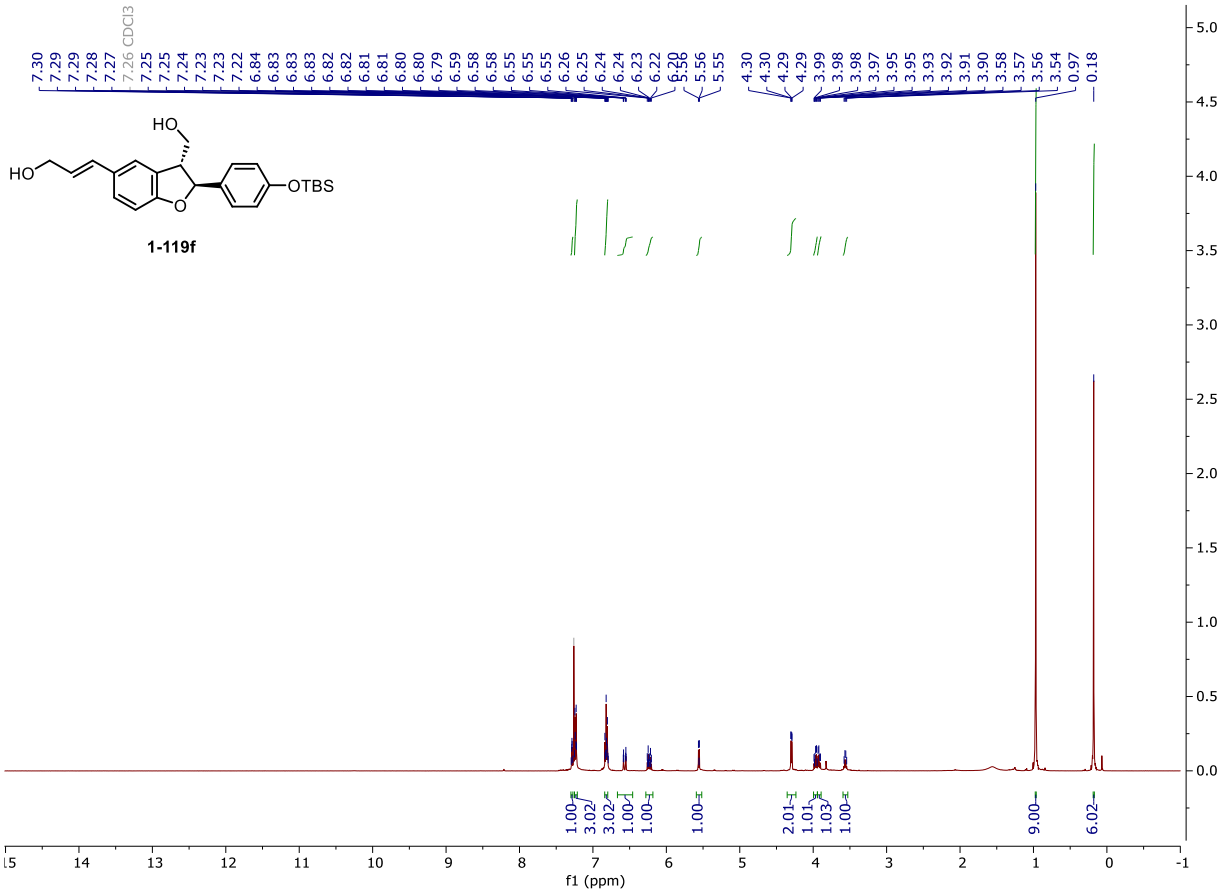


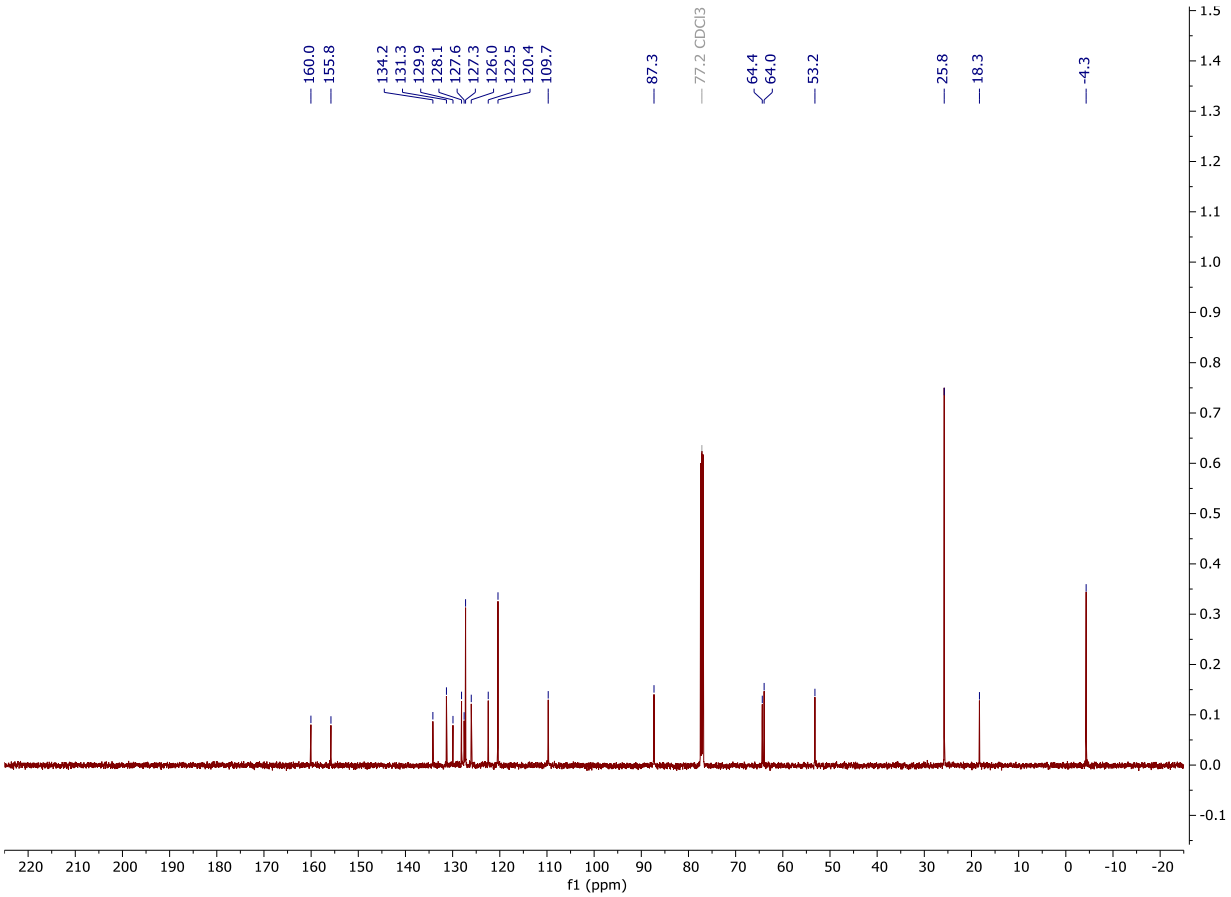
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-118b**





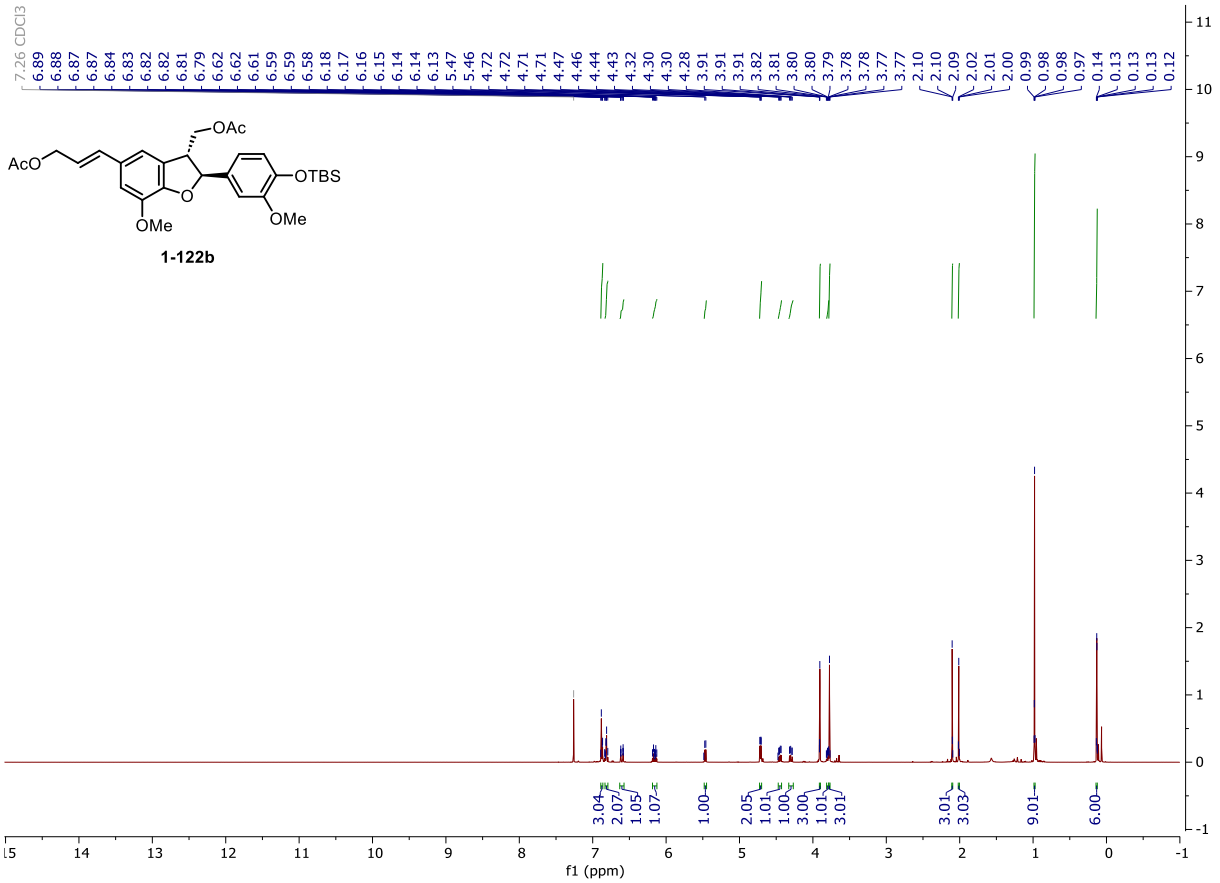
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-119f**

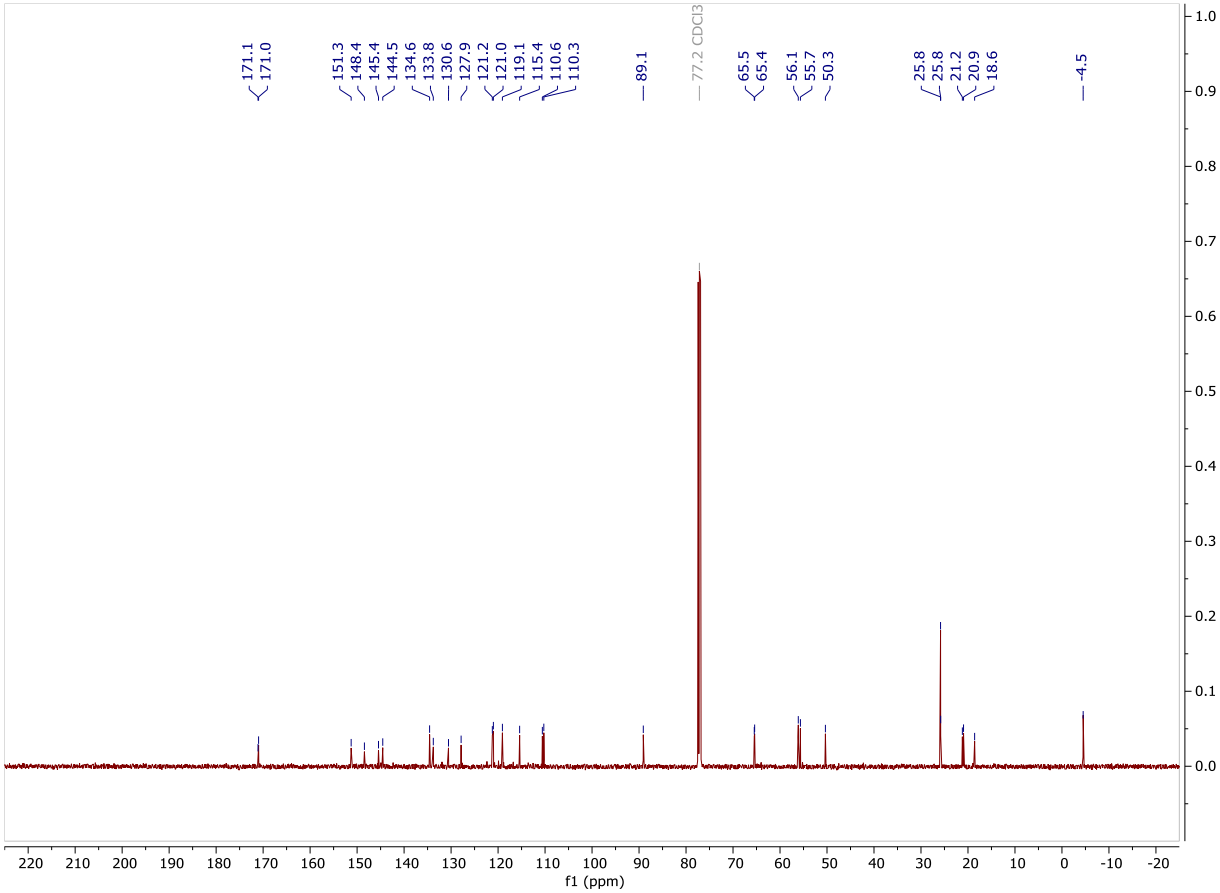




NMR and HPLC

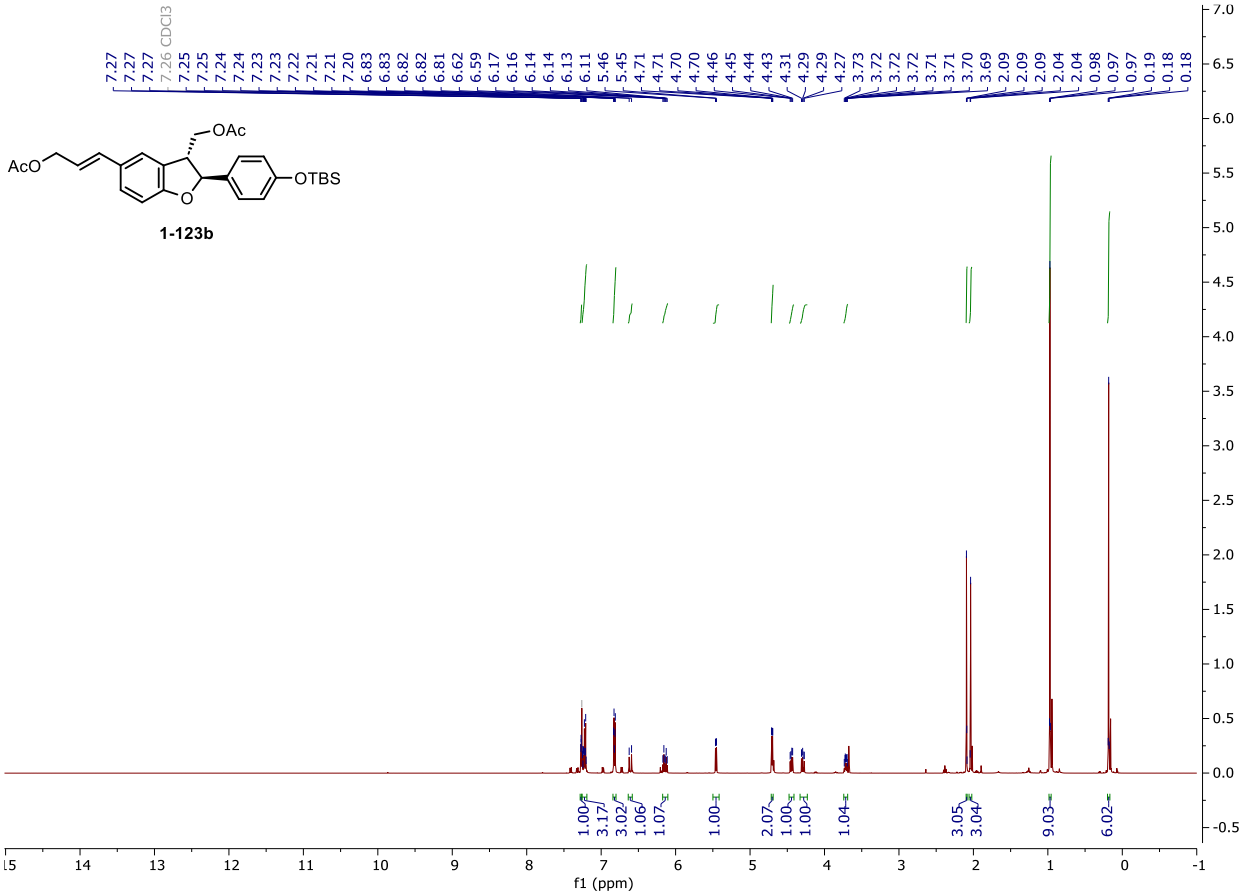
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-122b**

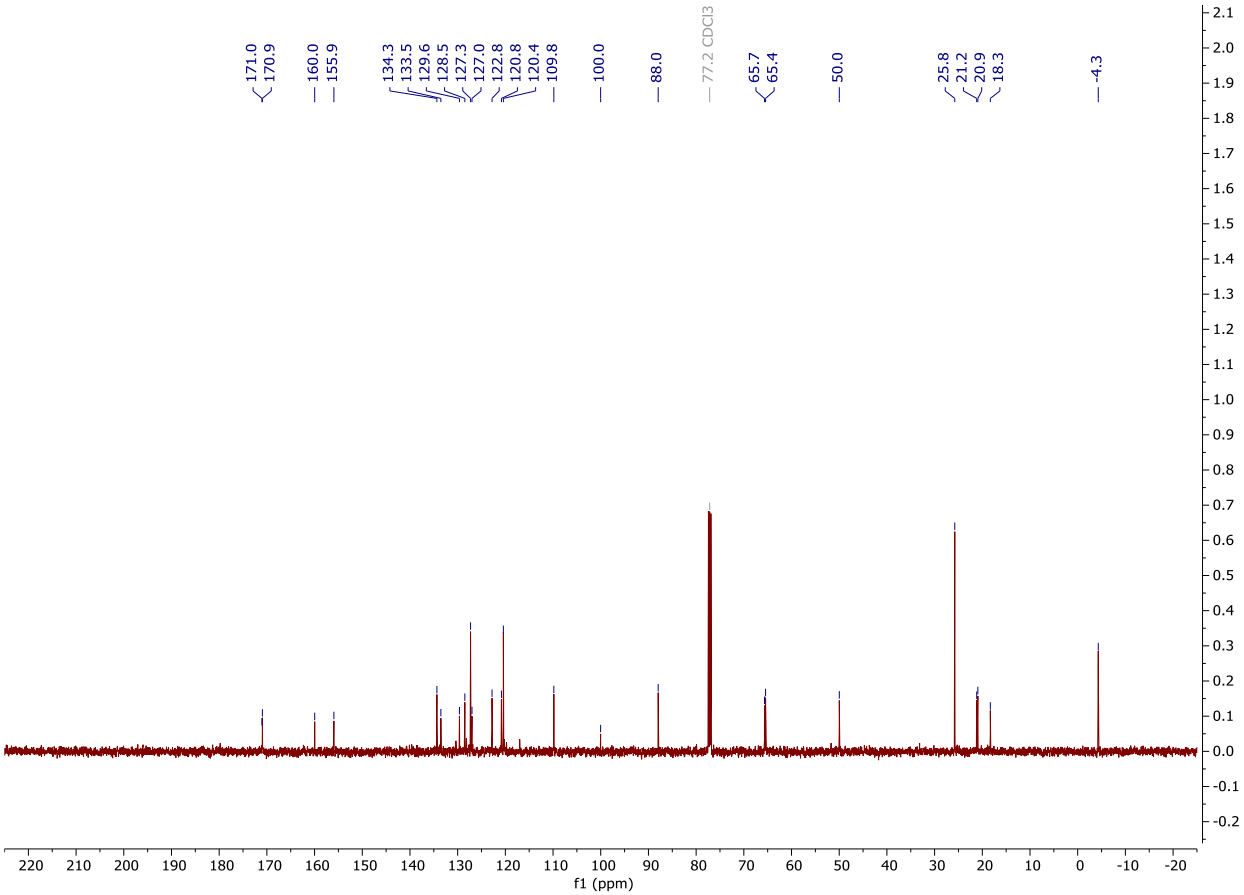




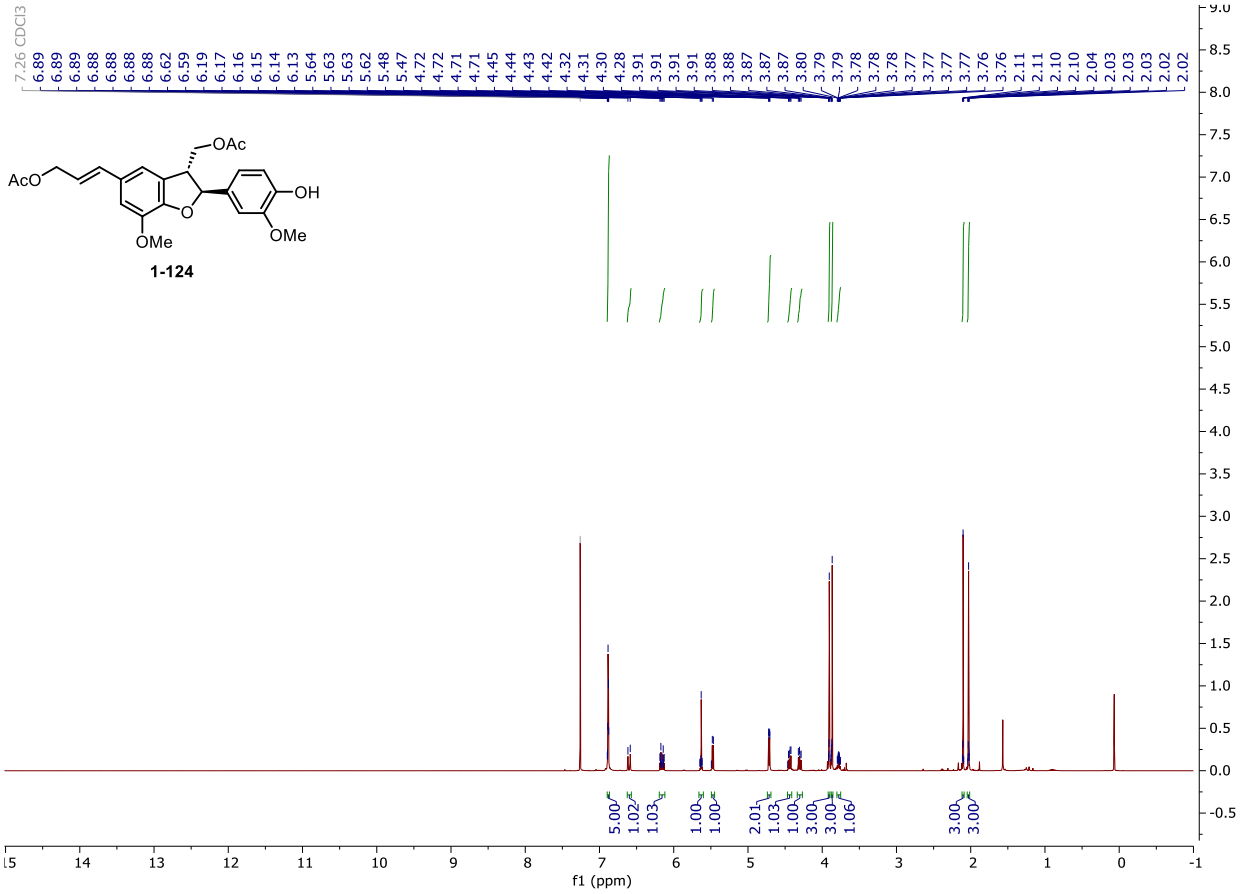
NMR and HPLC

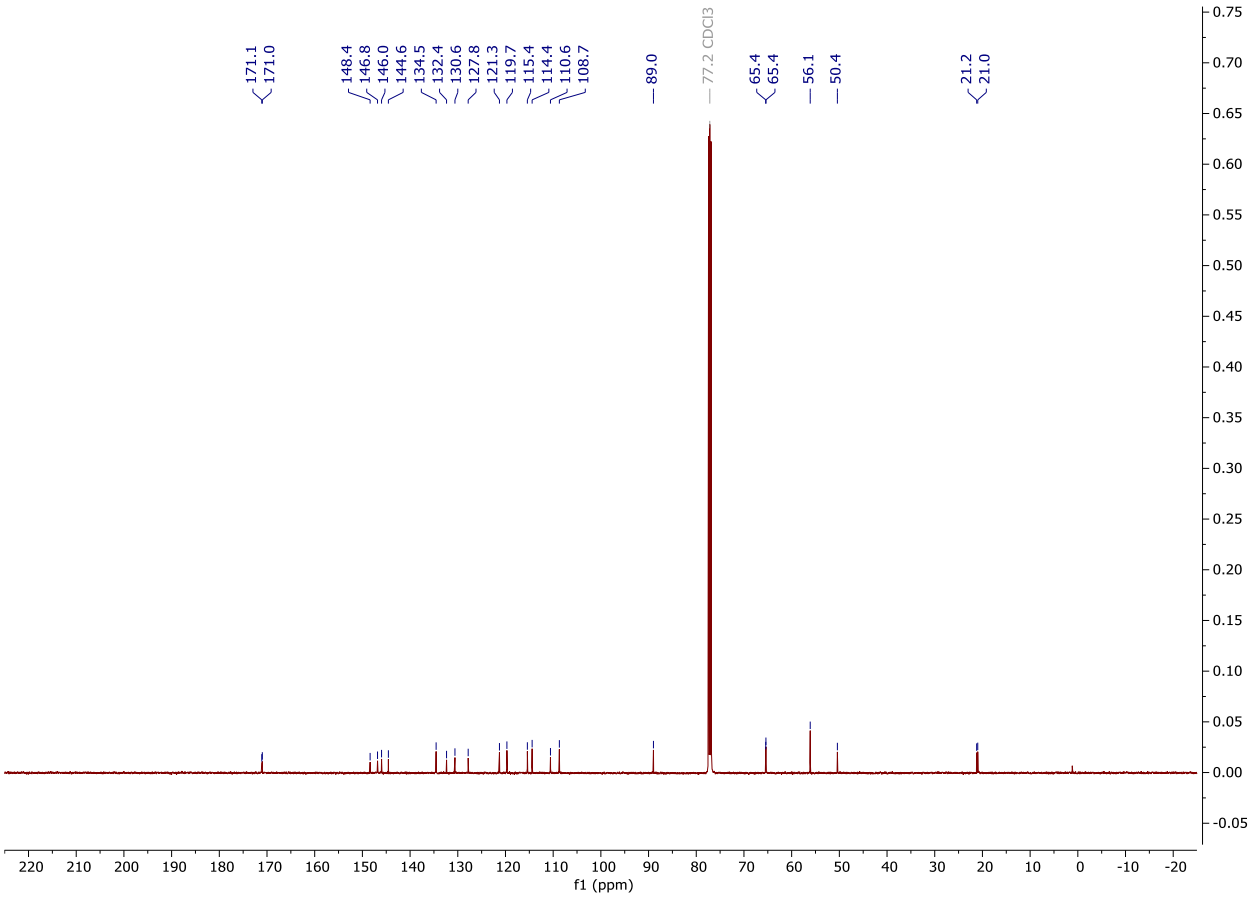
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-123b**



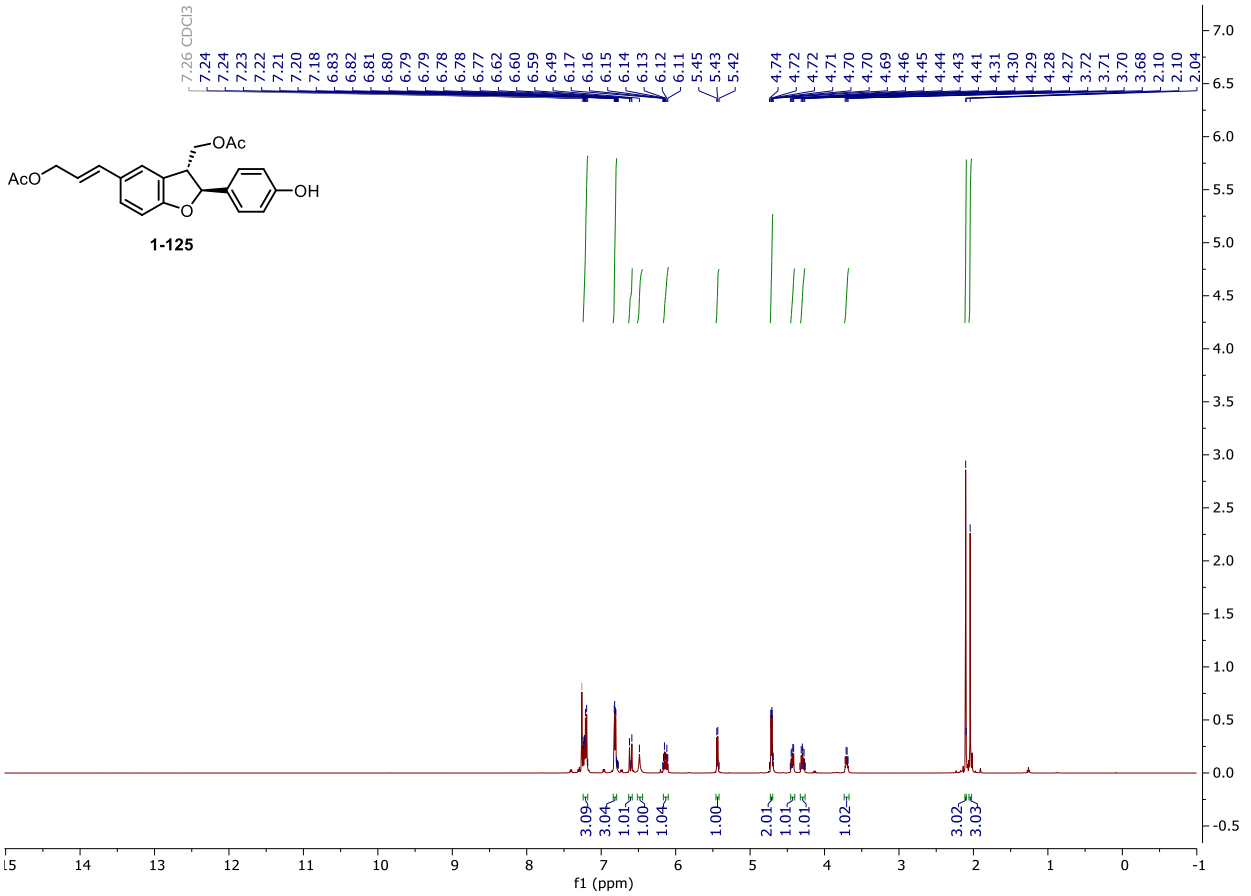


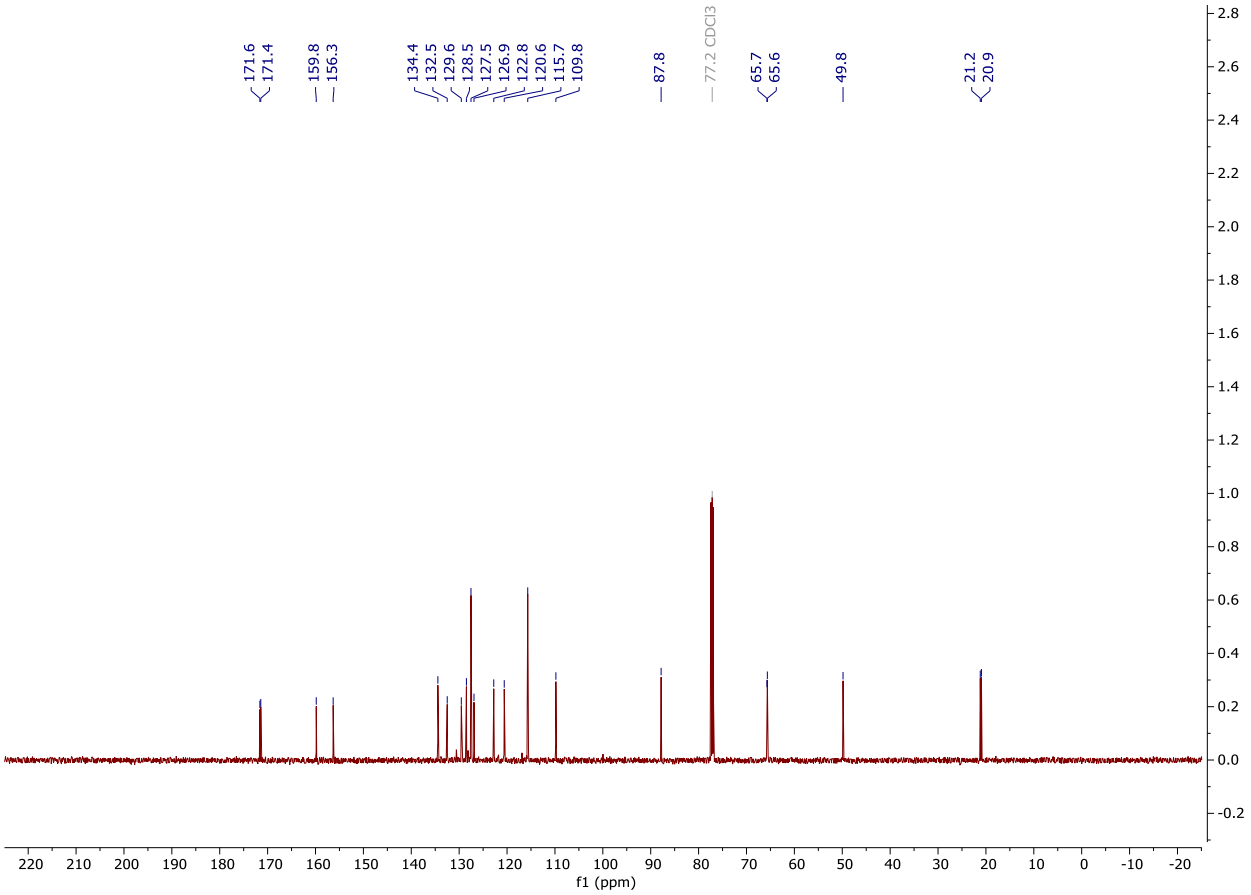
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-124**



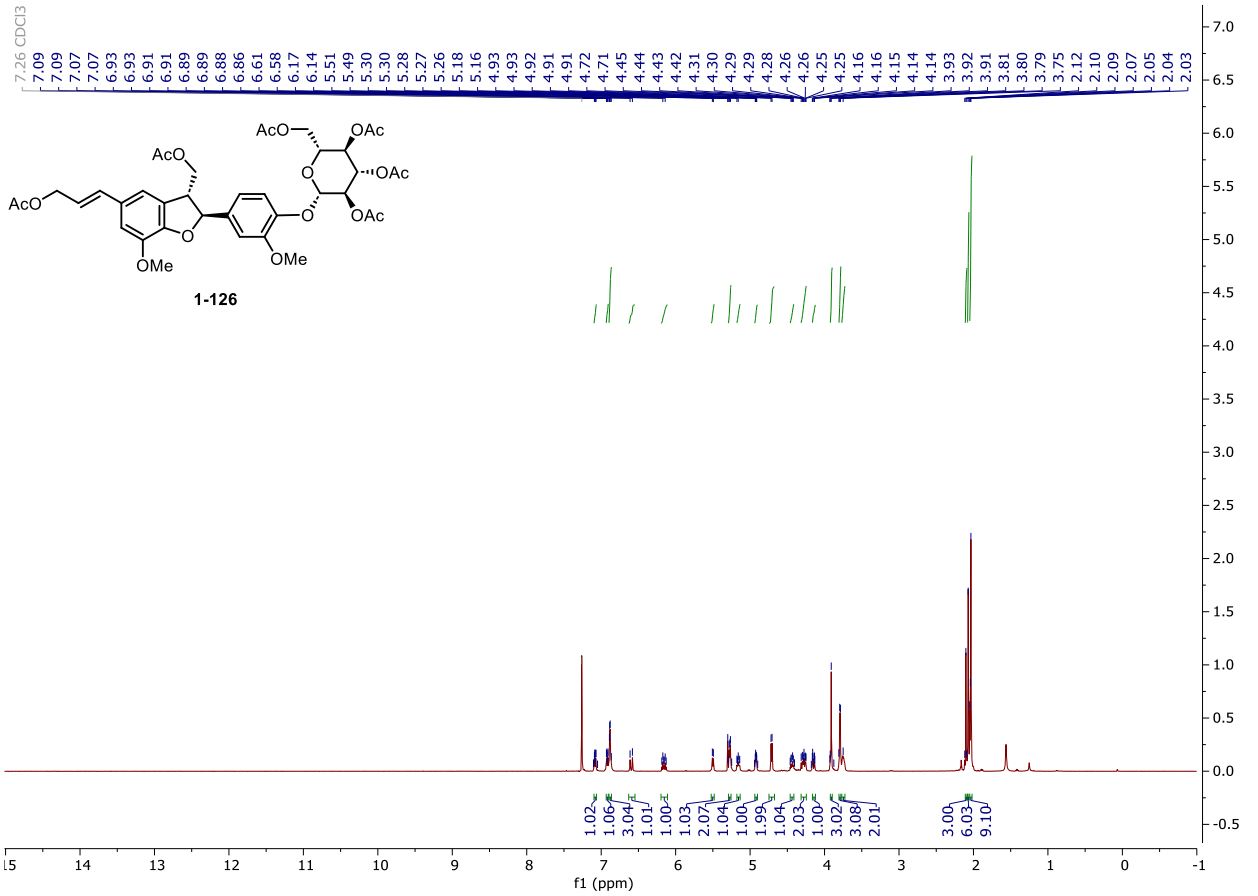


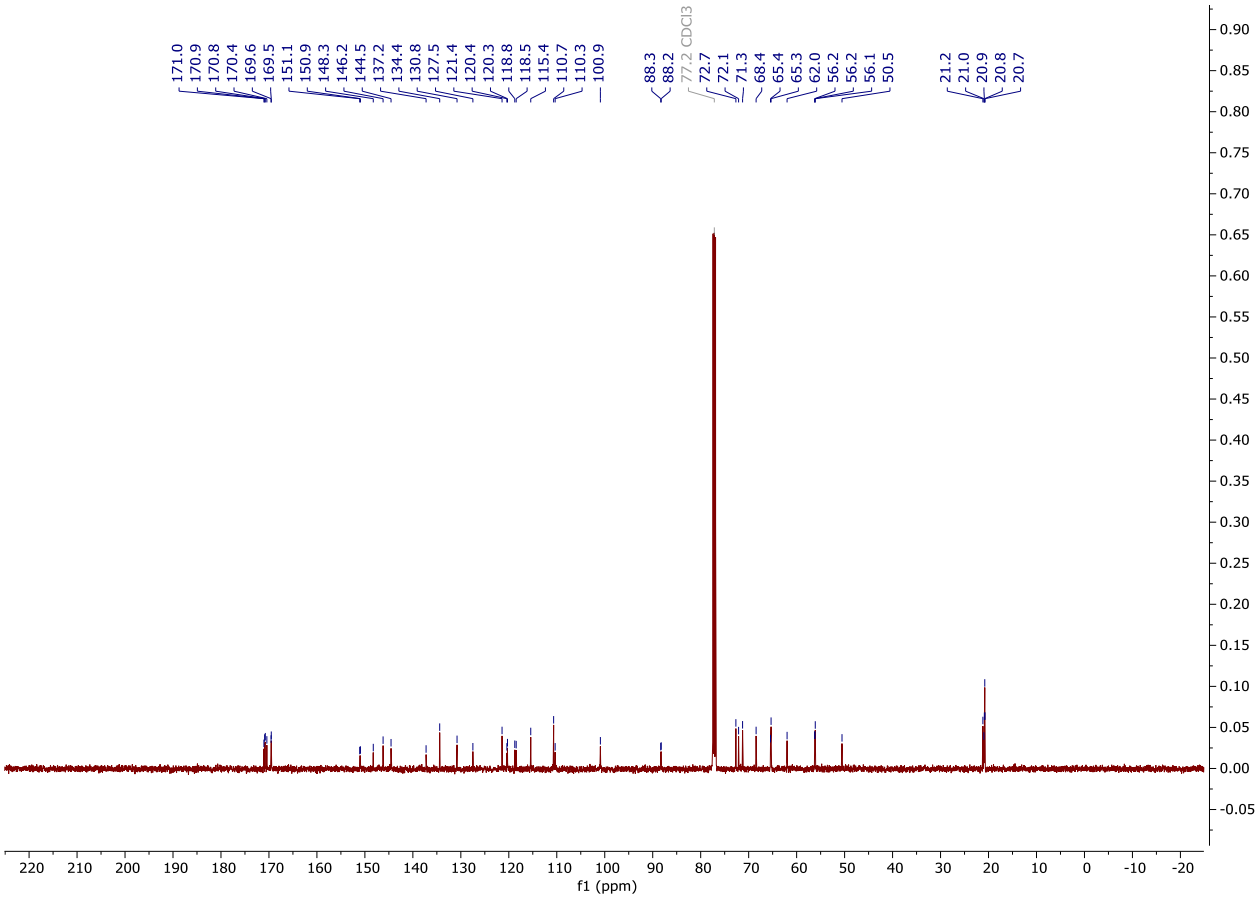
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-125**



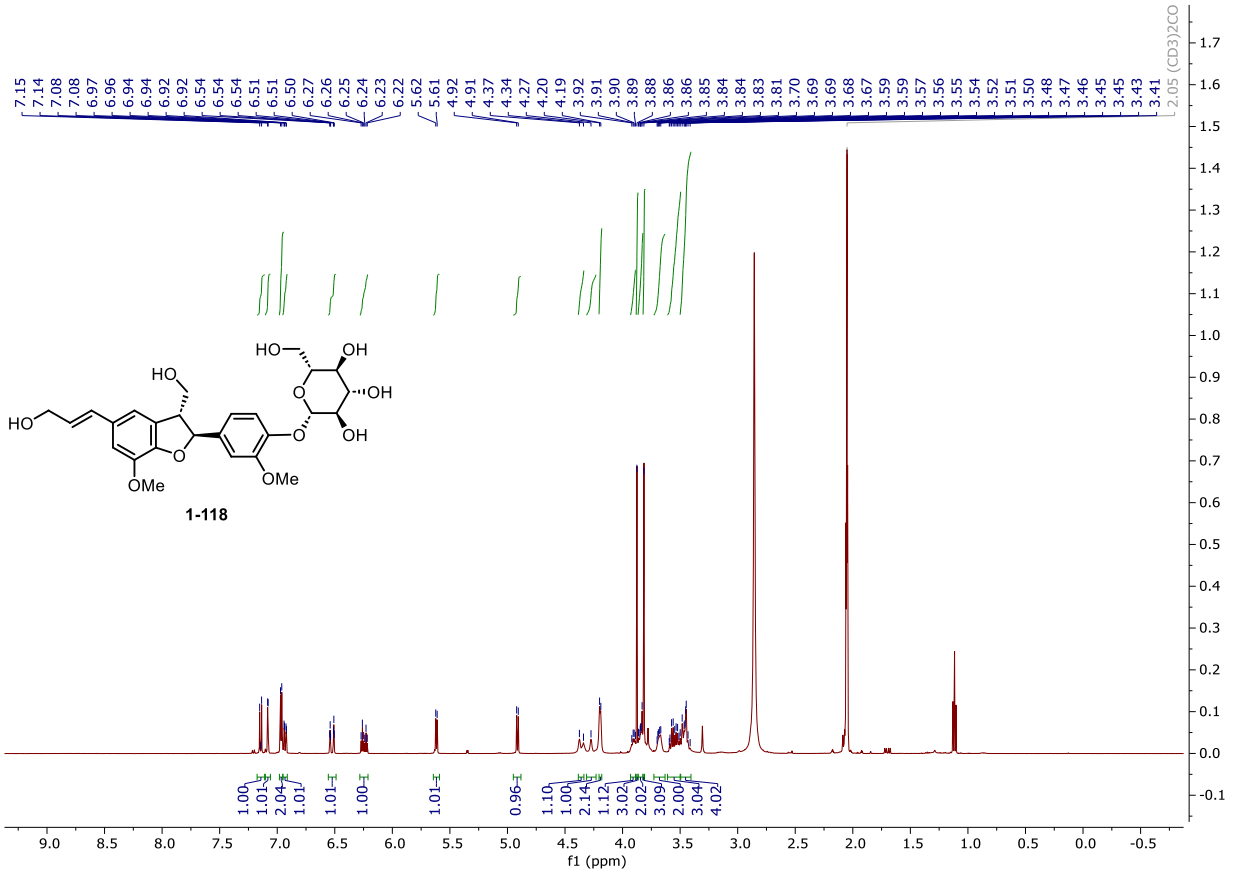


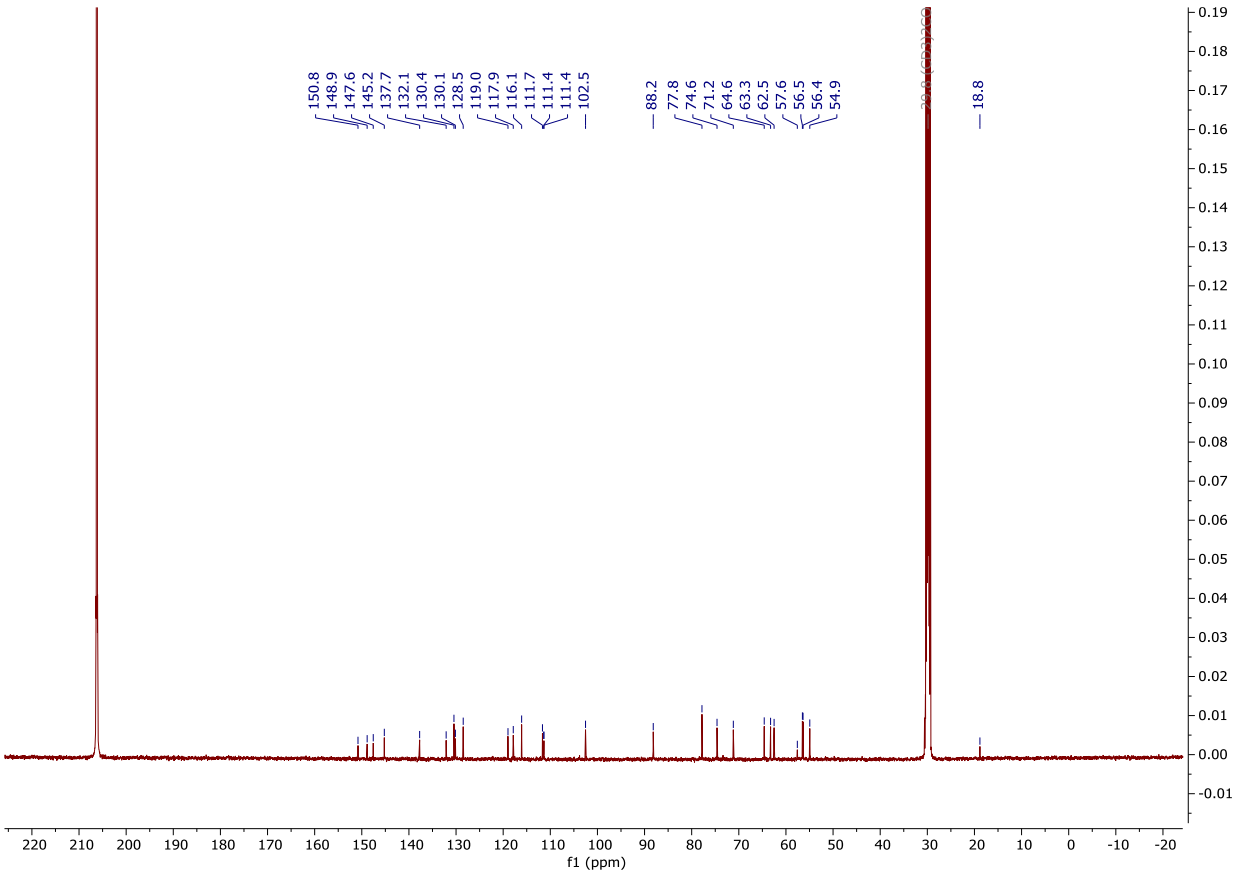
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-126**



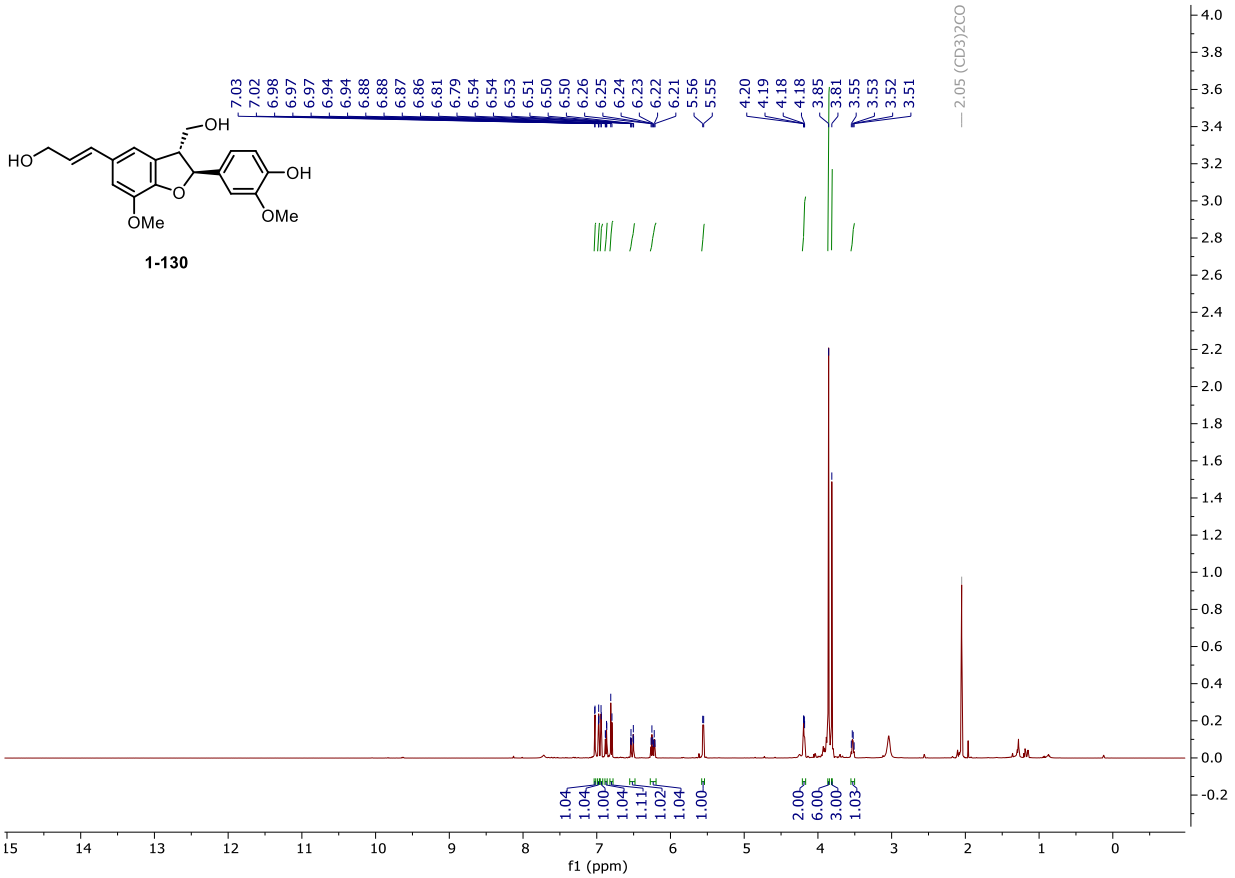


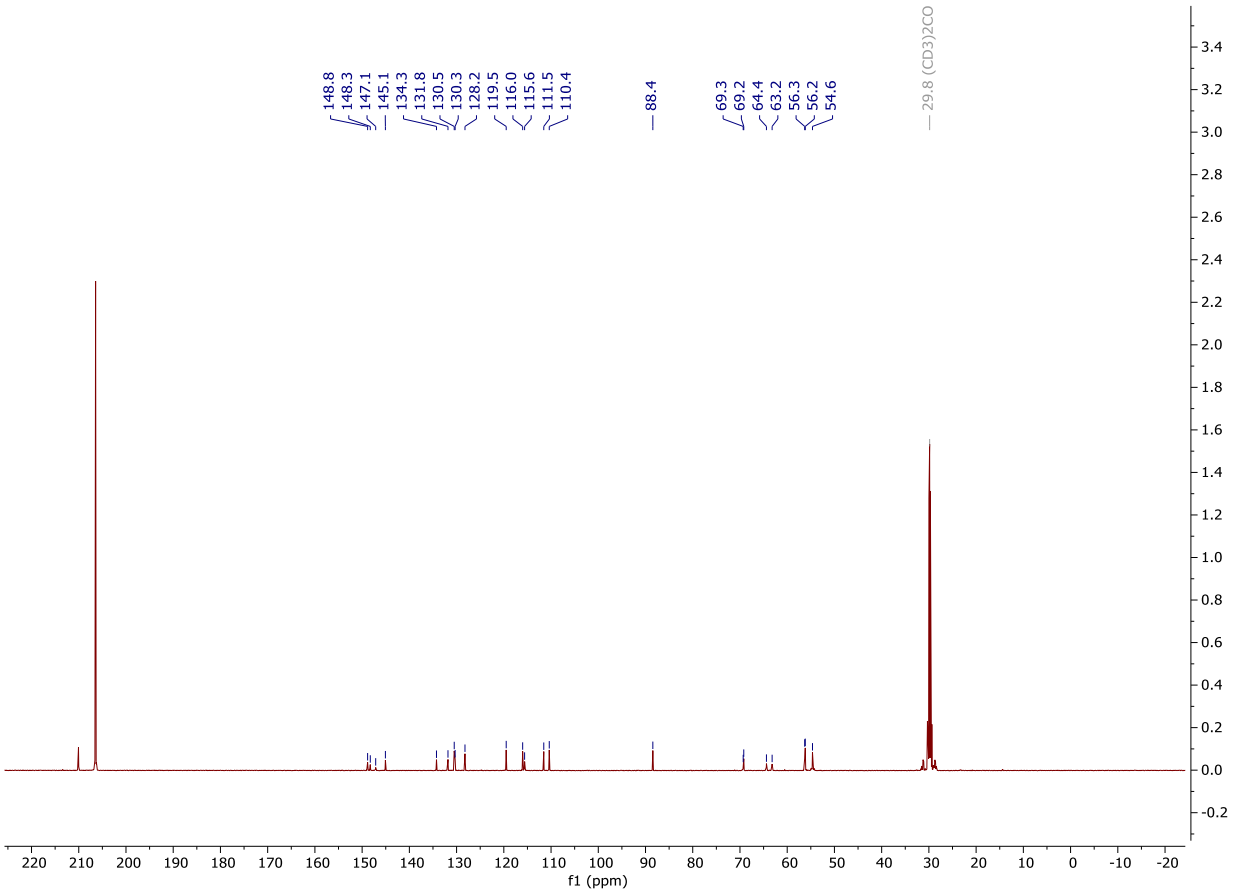
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of DCG-A, 1-108



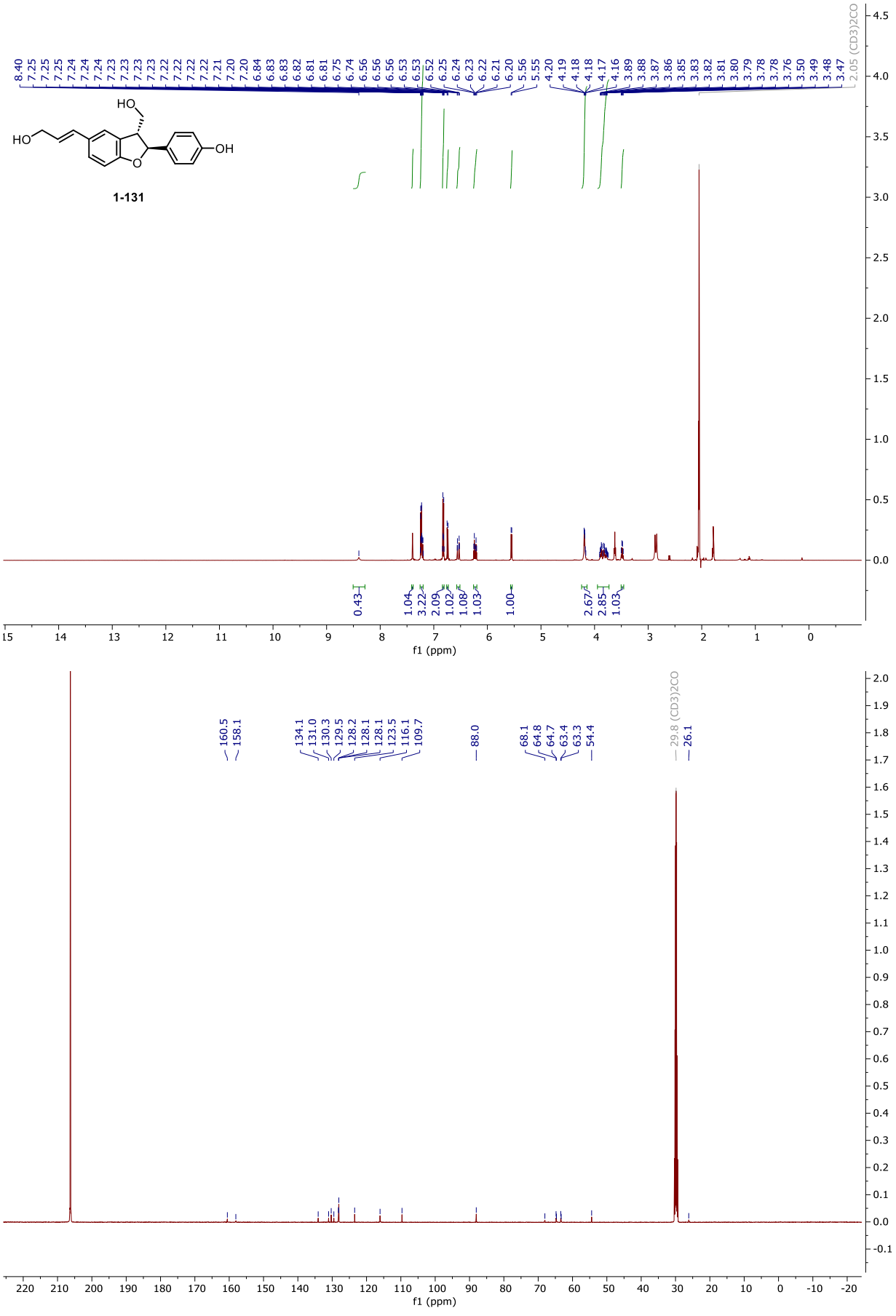


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-130**



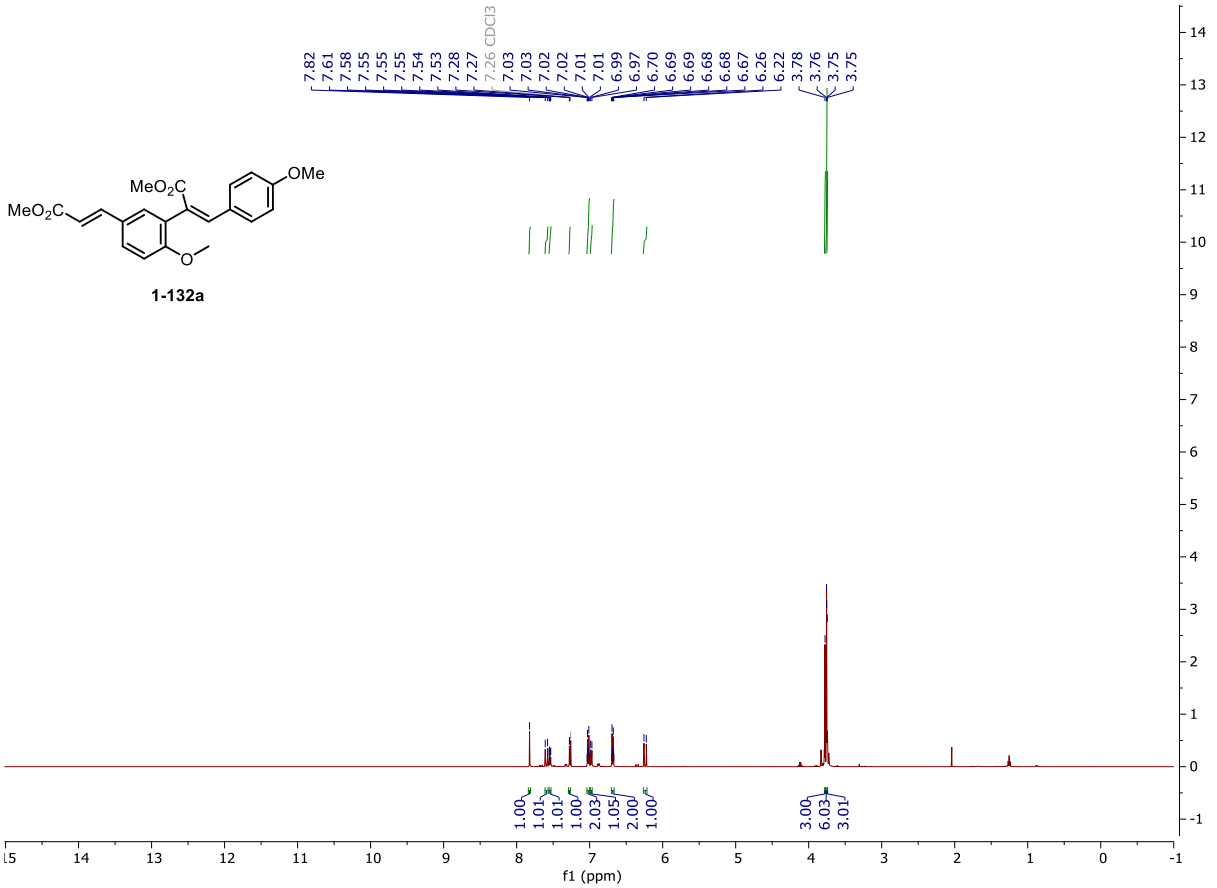


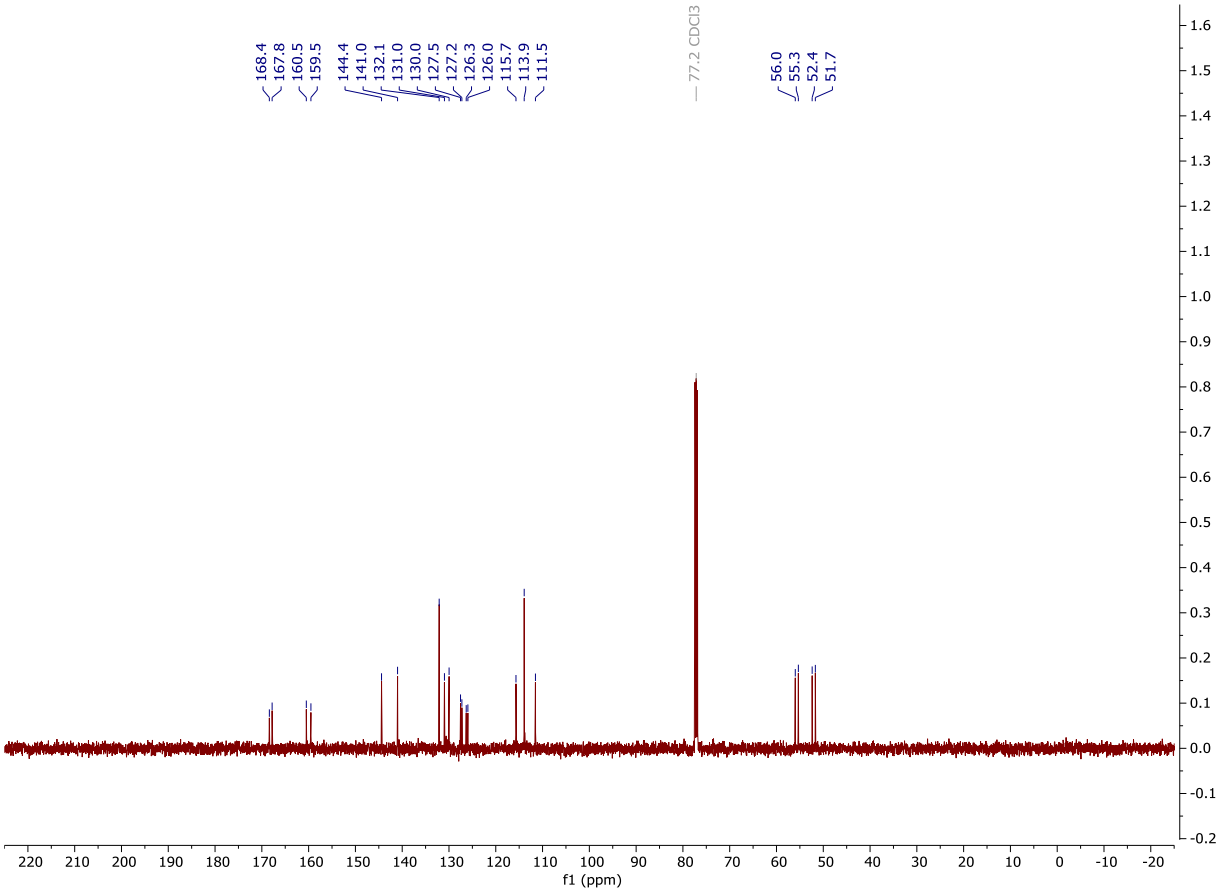
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-131**



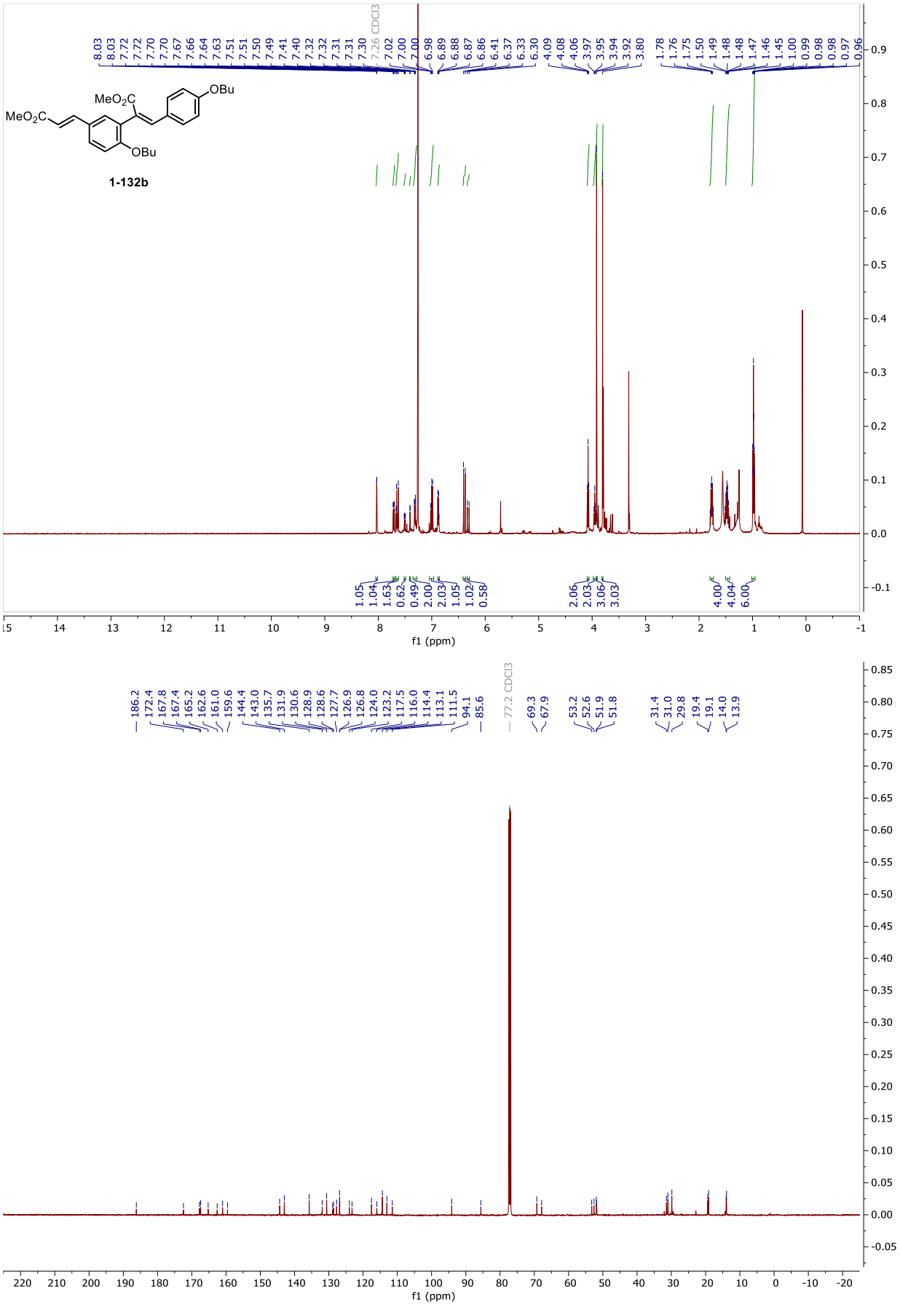
NMR and HPLC

Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-132a**



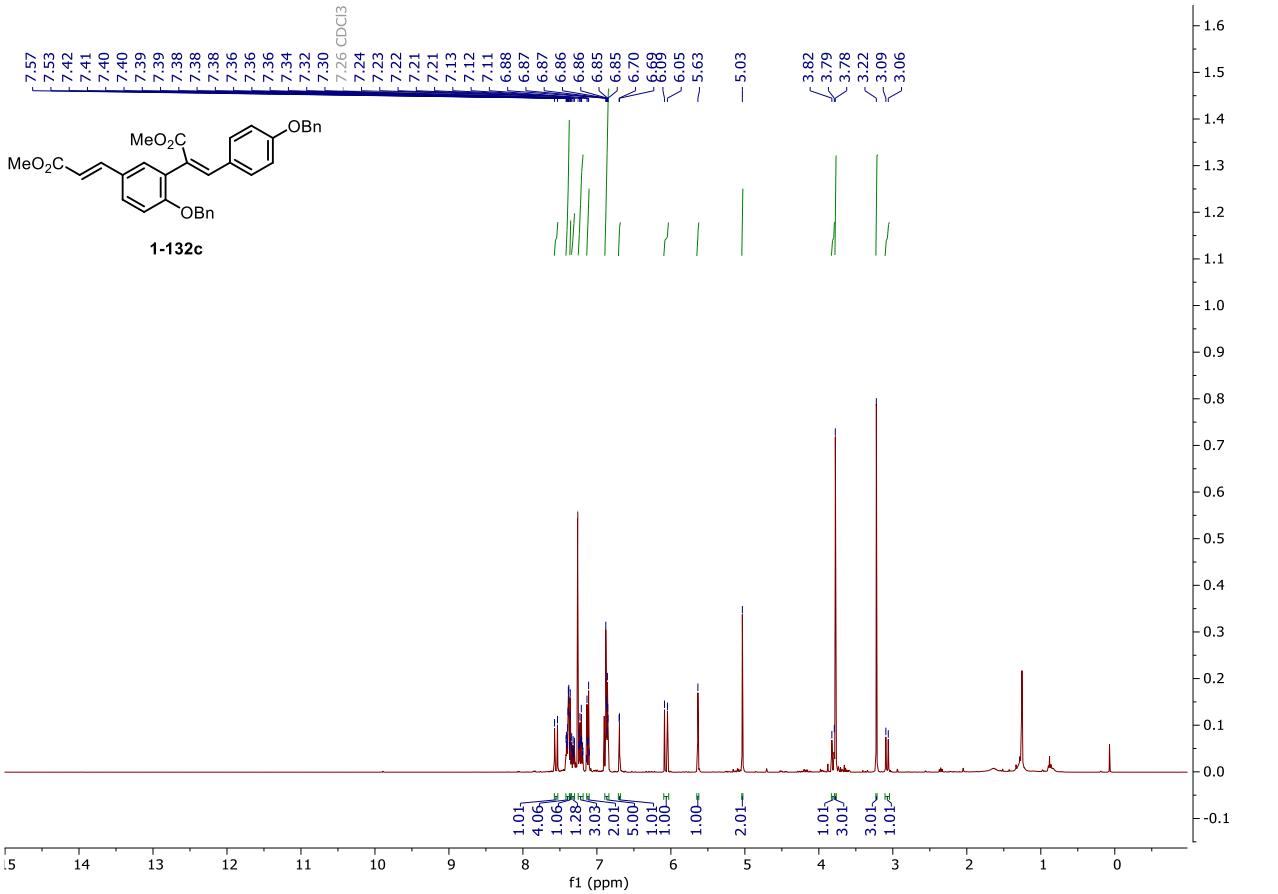


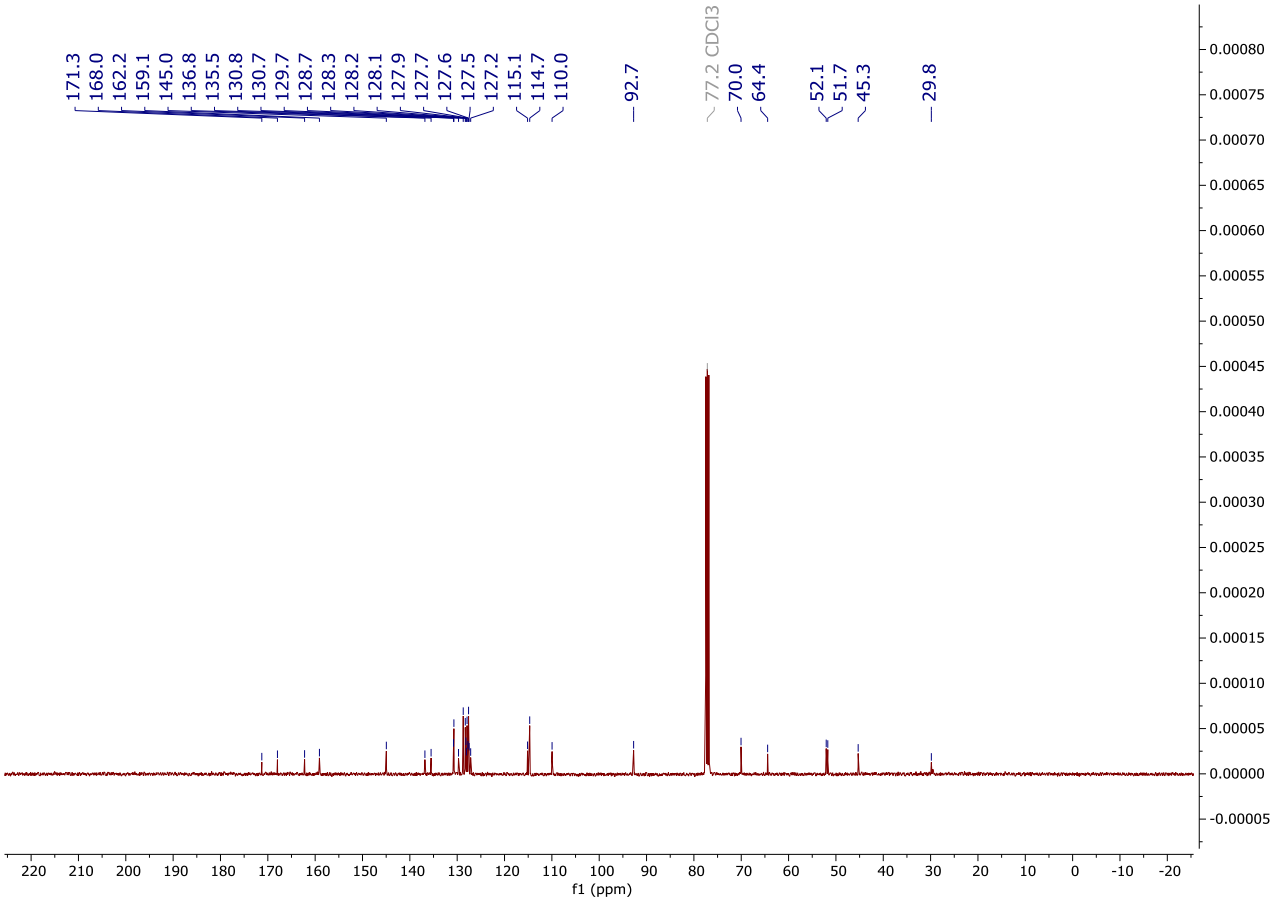
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-132b**



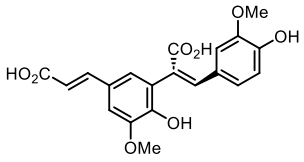
NMR and HPLC

Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-132c**

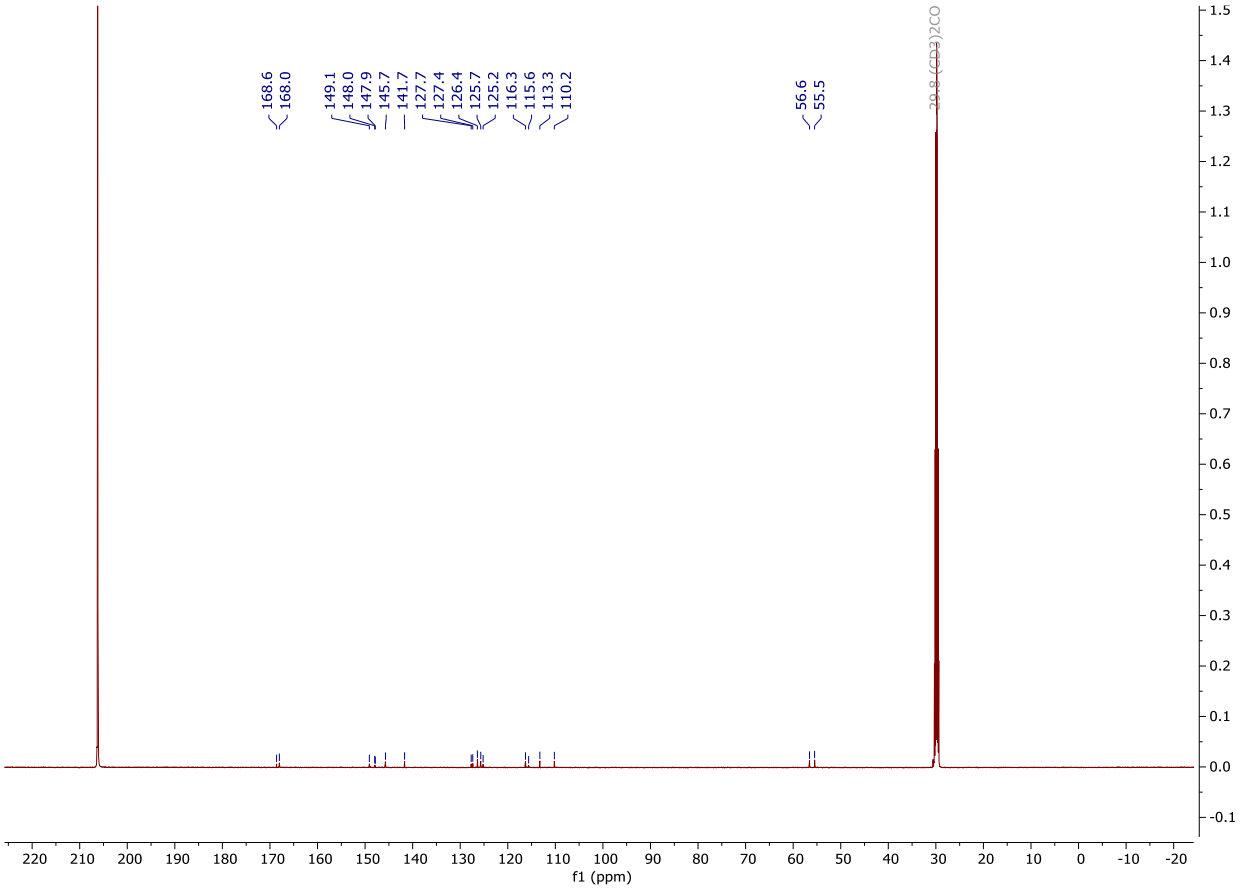




Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-129**

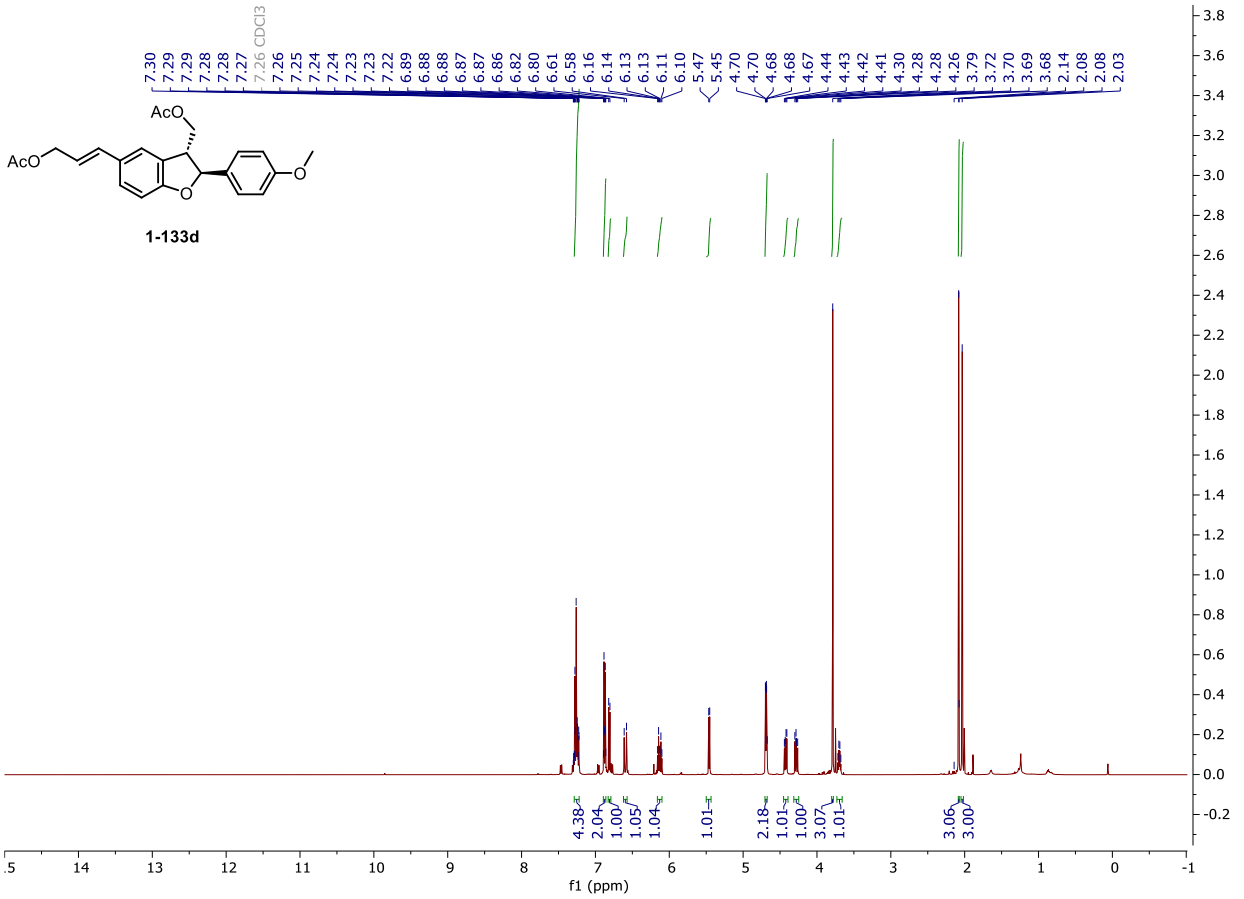


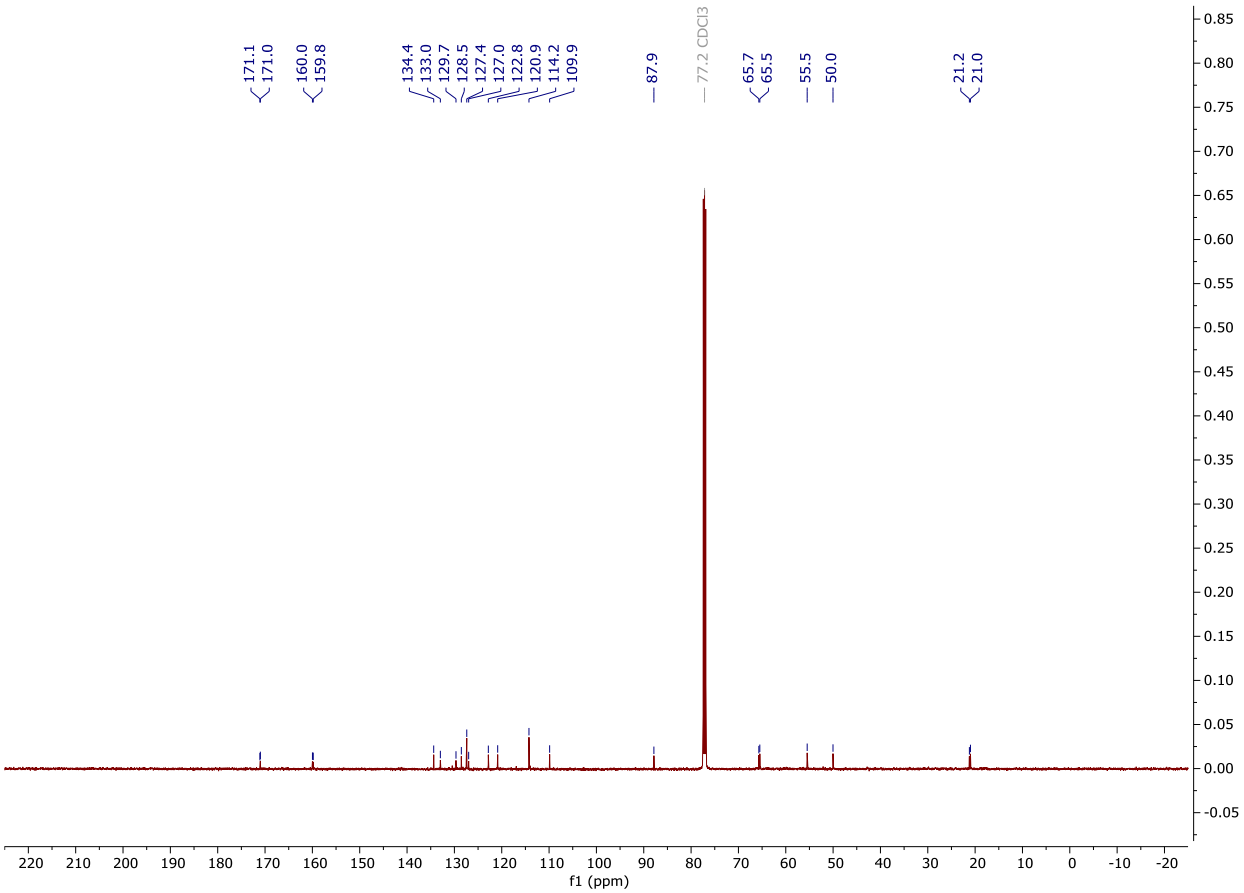
1-129



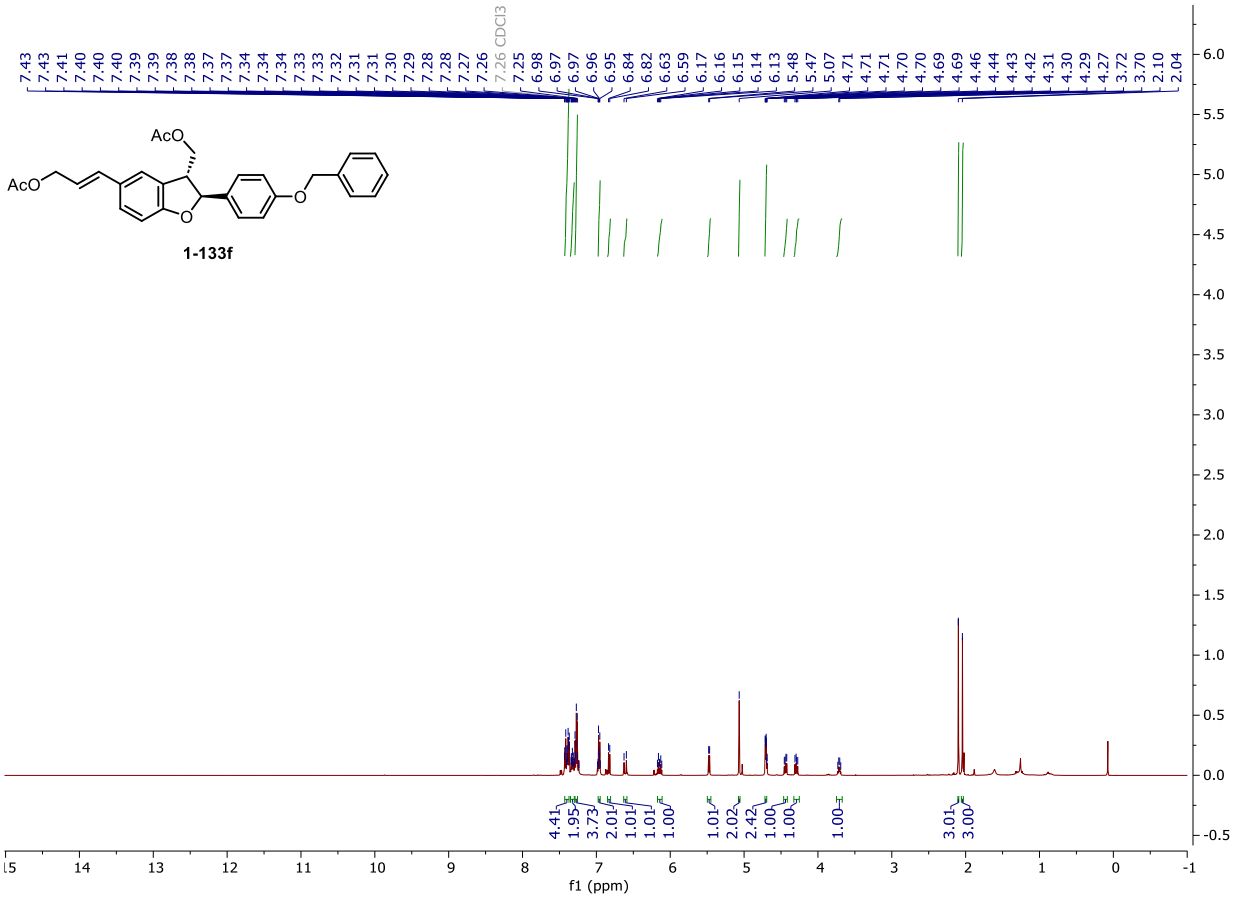
NMR and HPLC

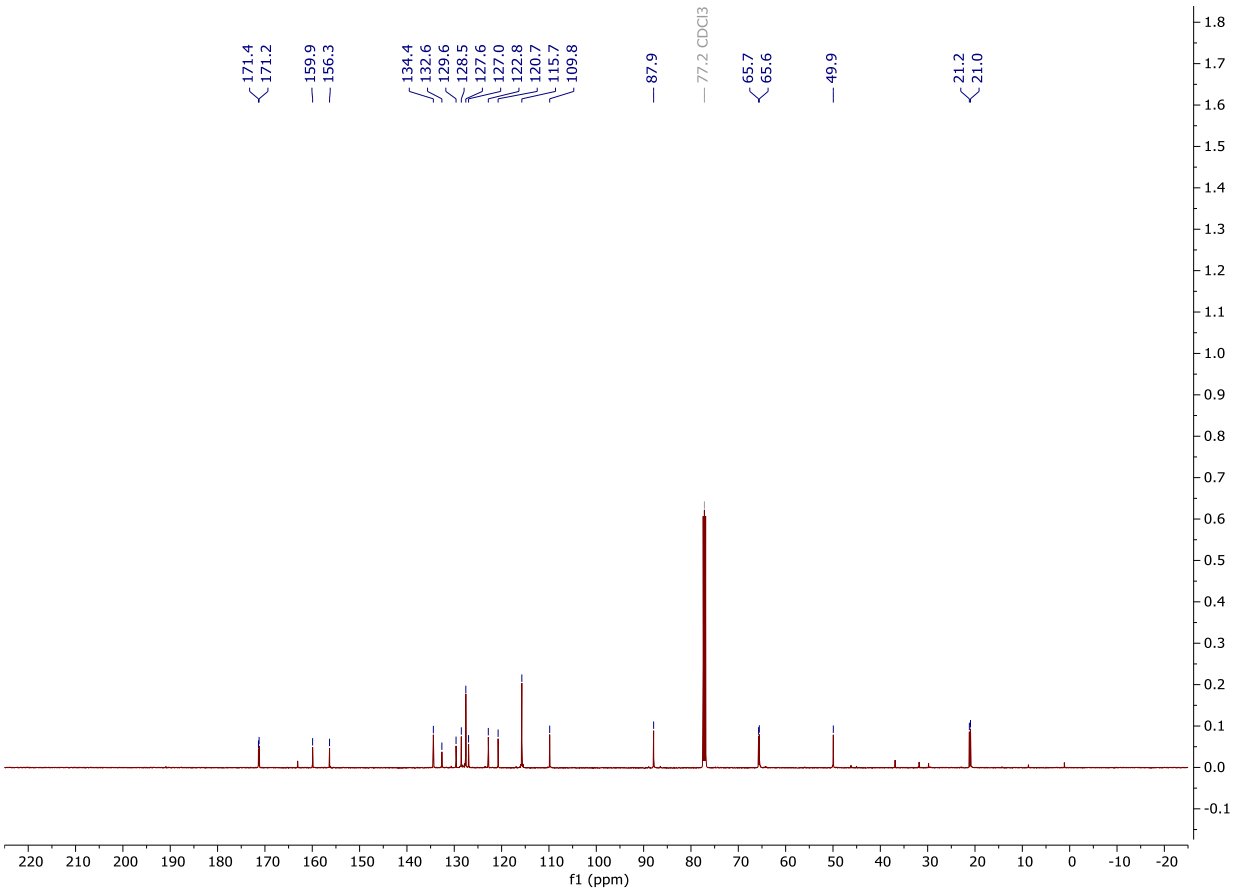
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-133d**



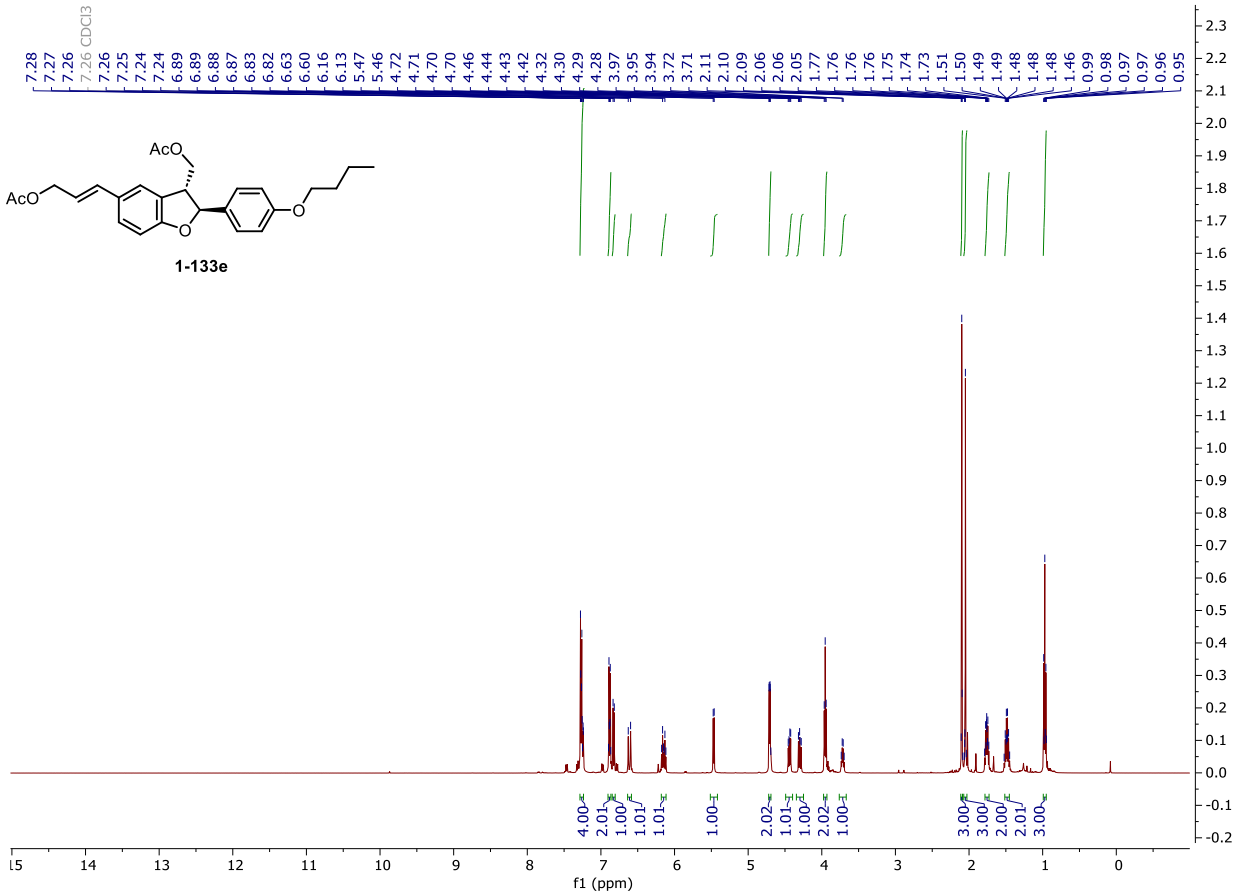


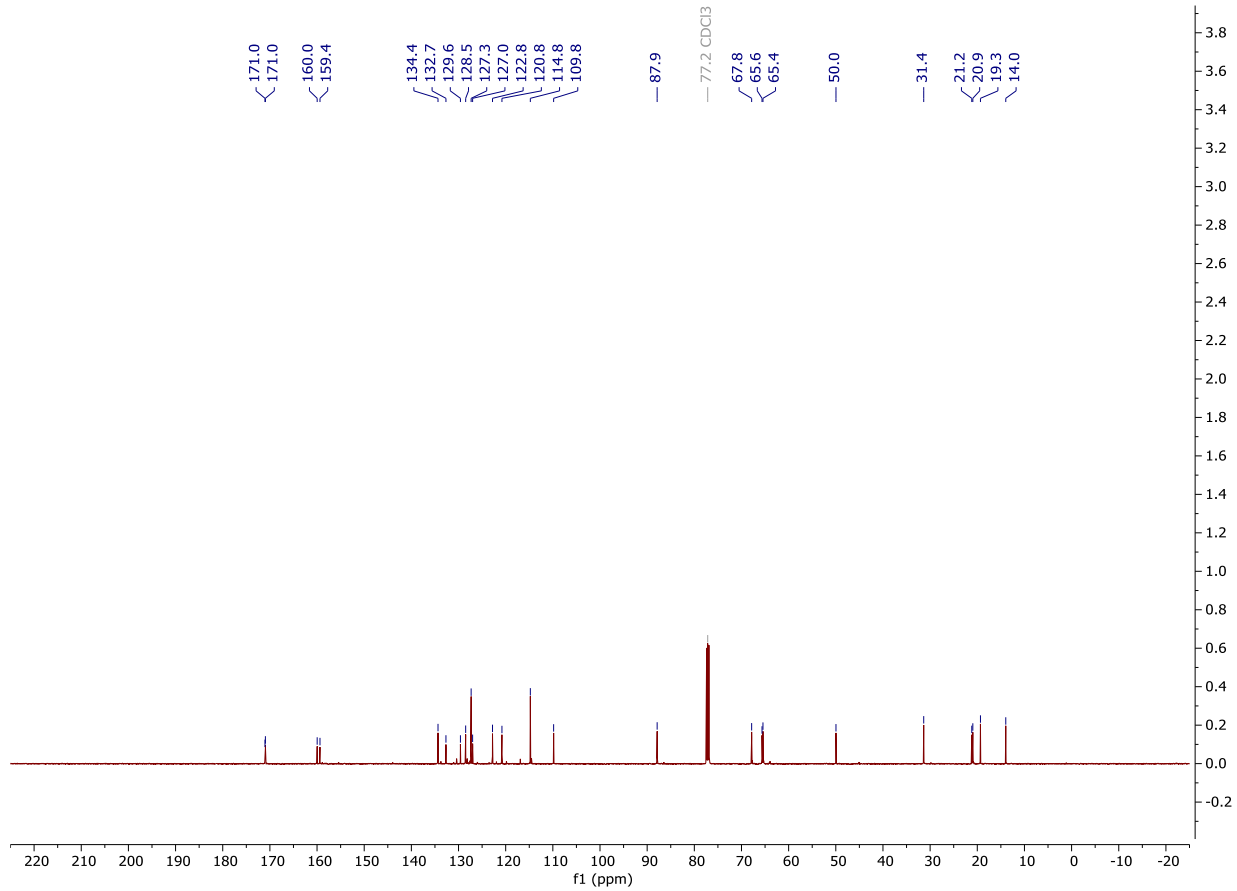
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-133f**



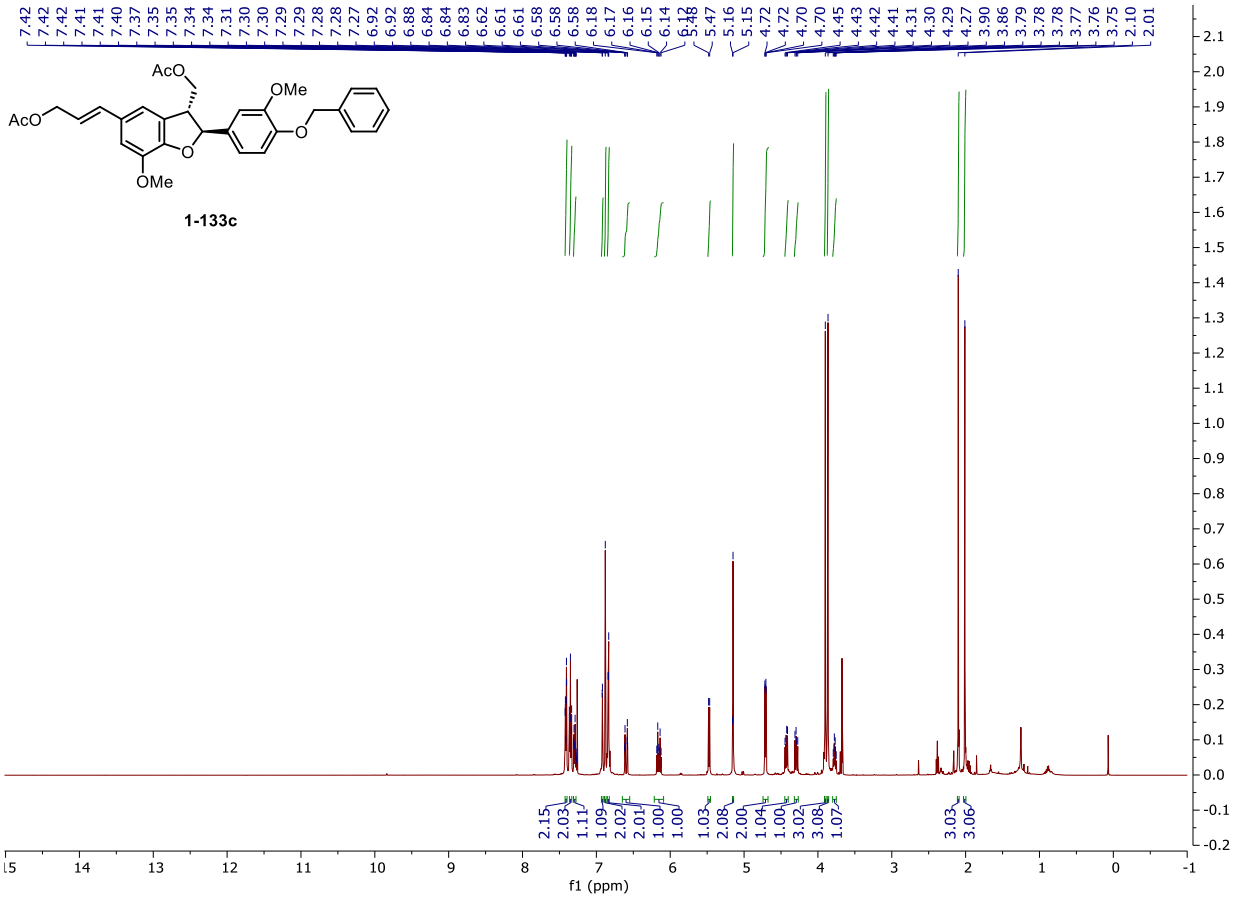


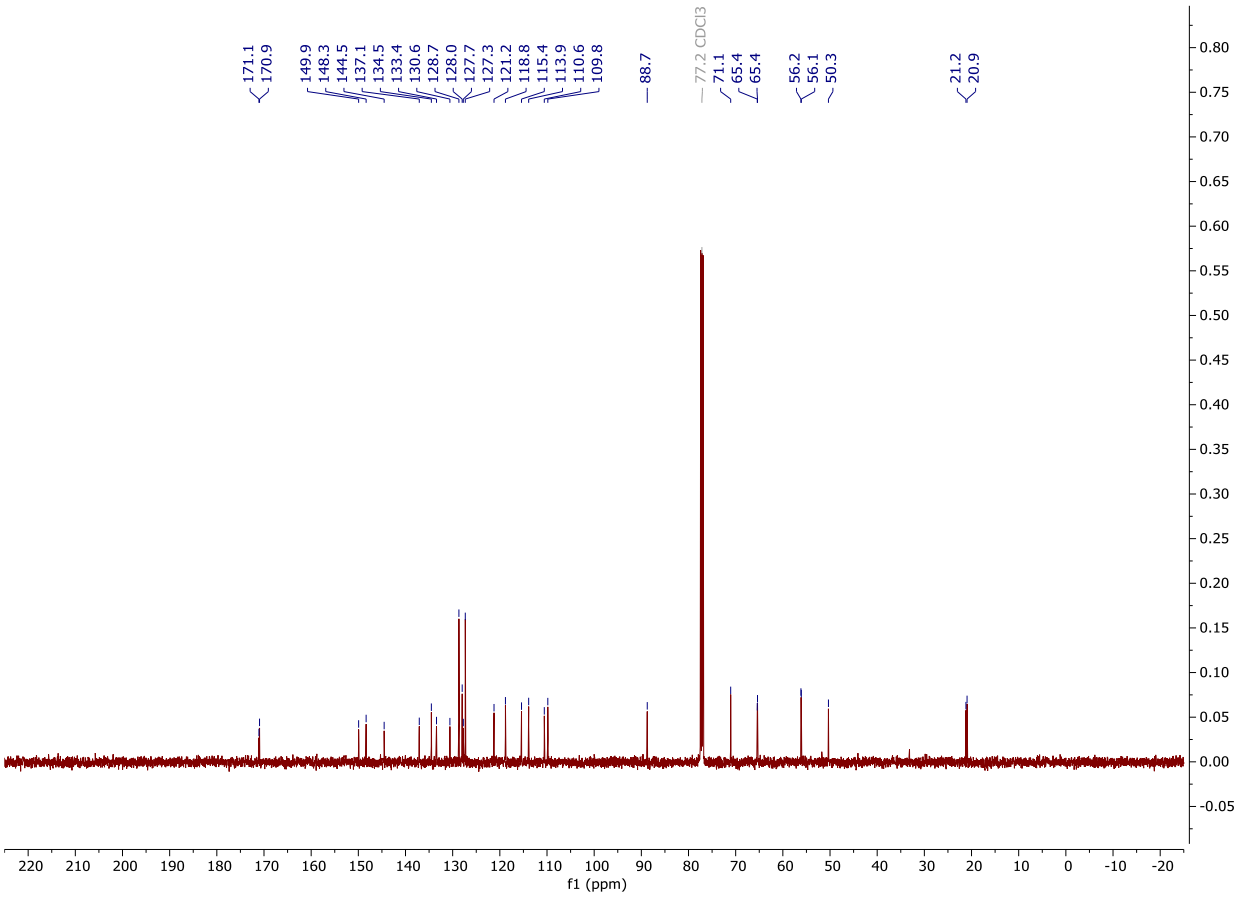
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-133e**



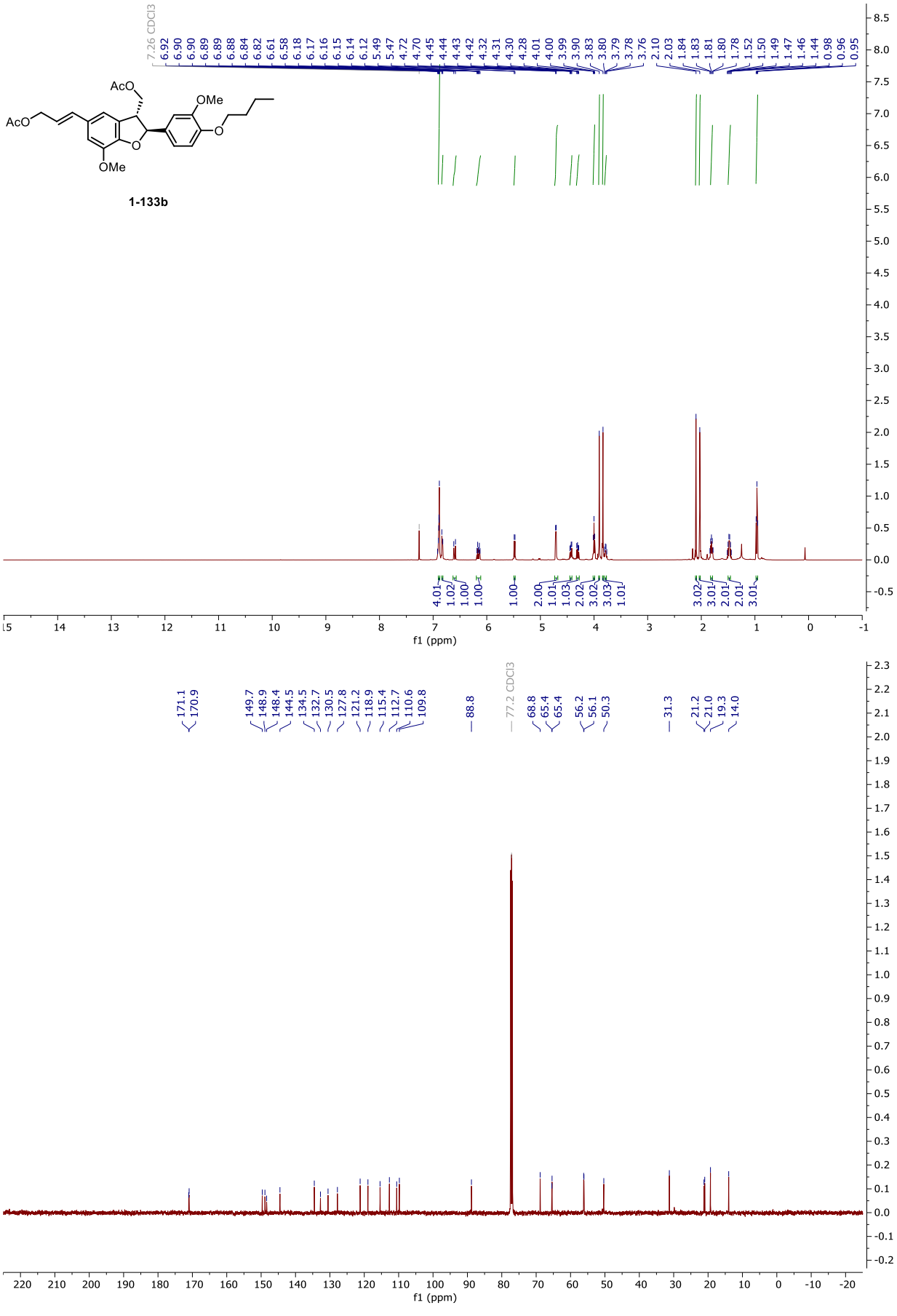


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-133c**

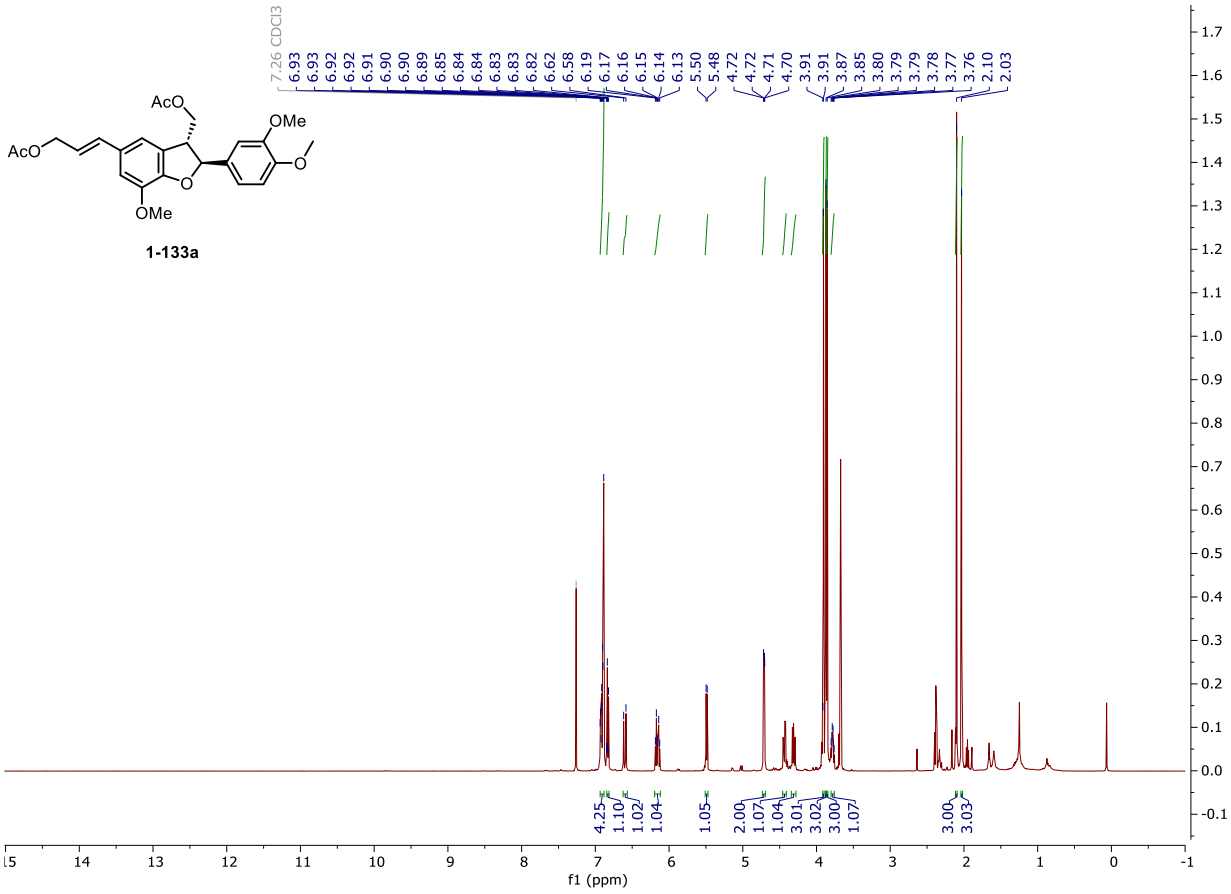


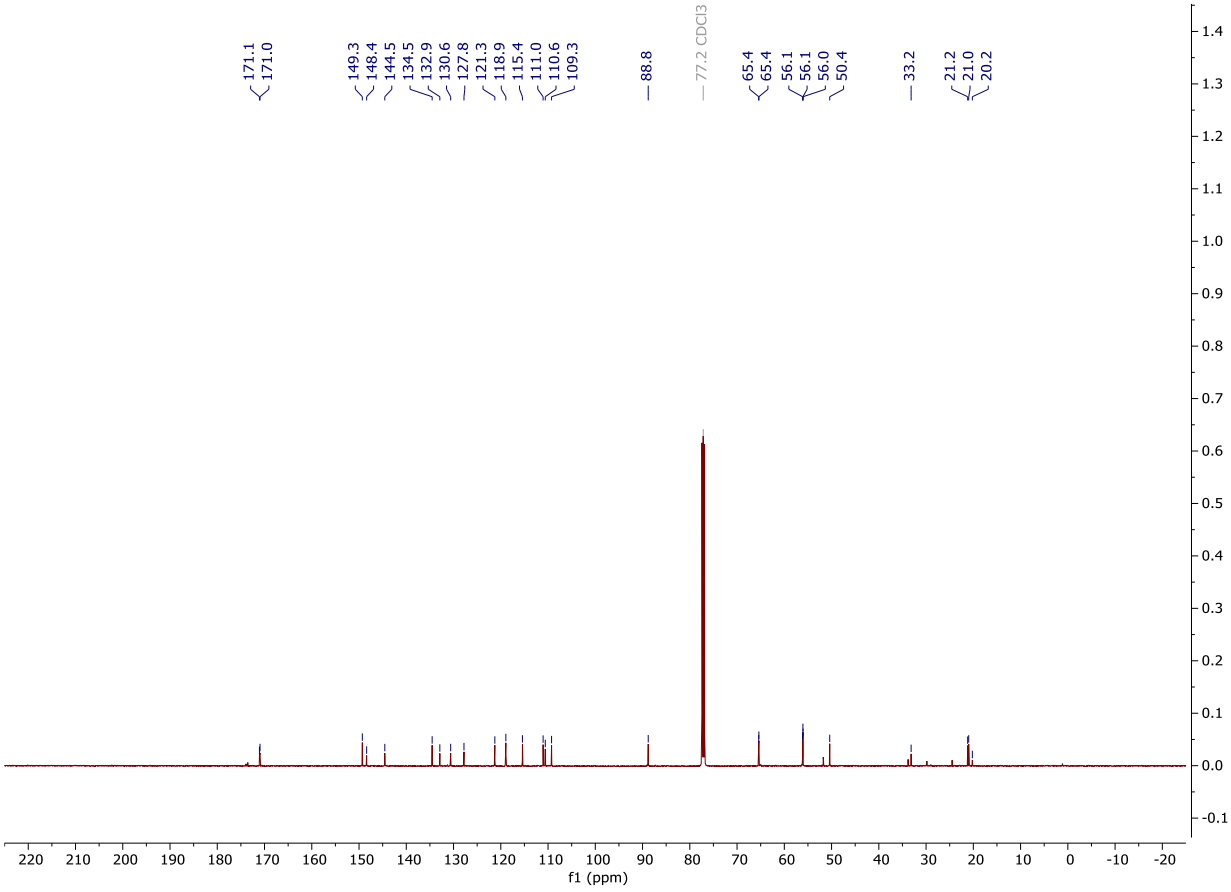


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-133b**

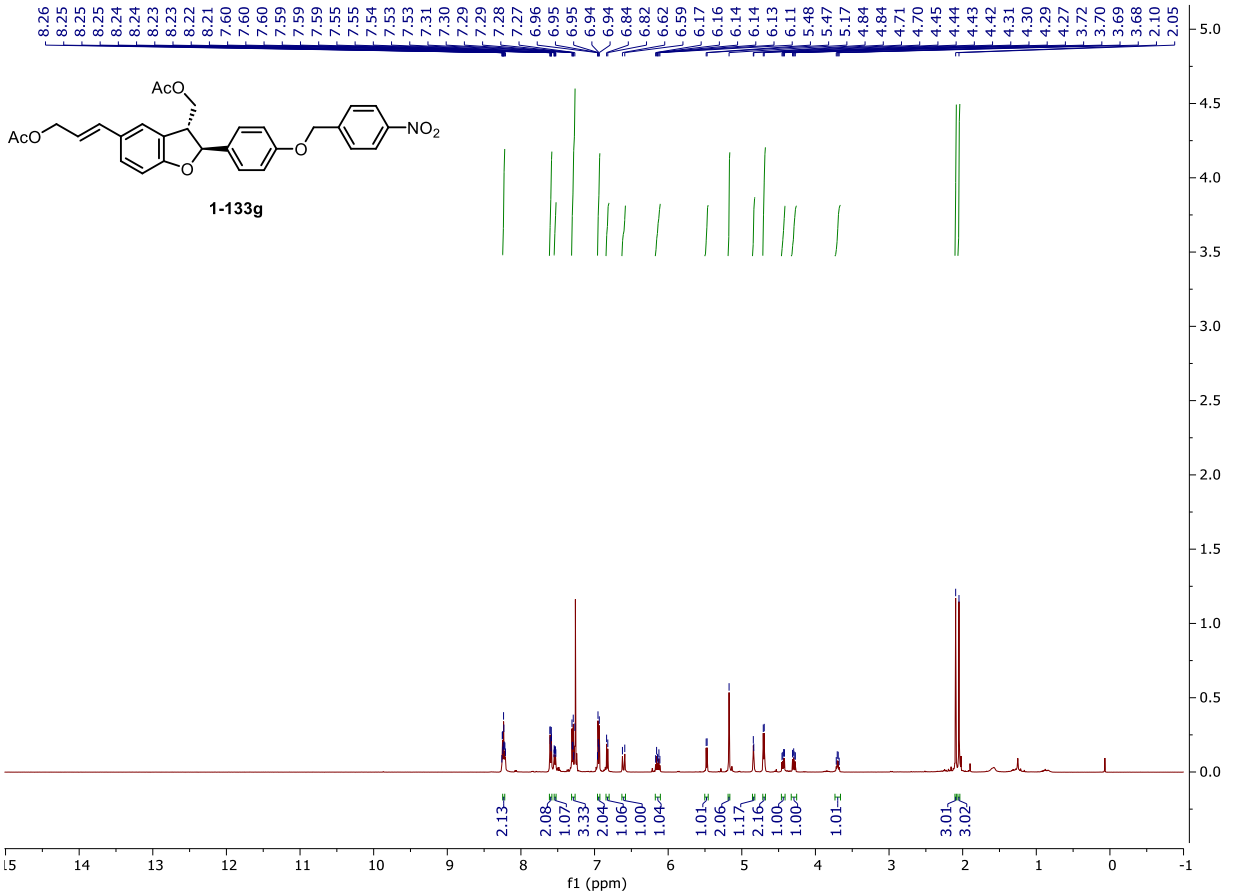


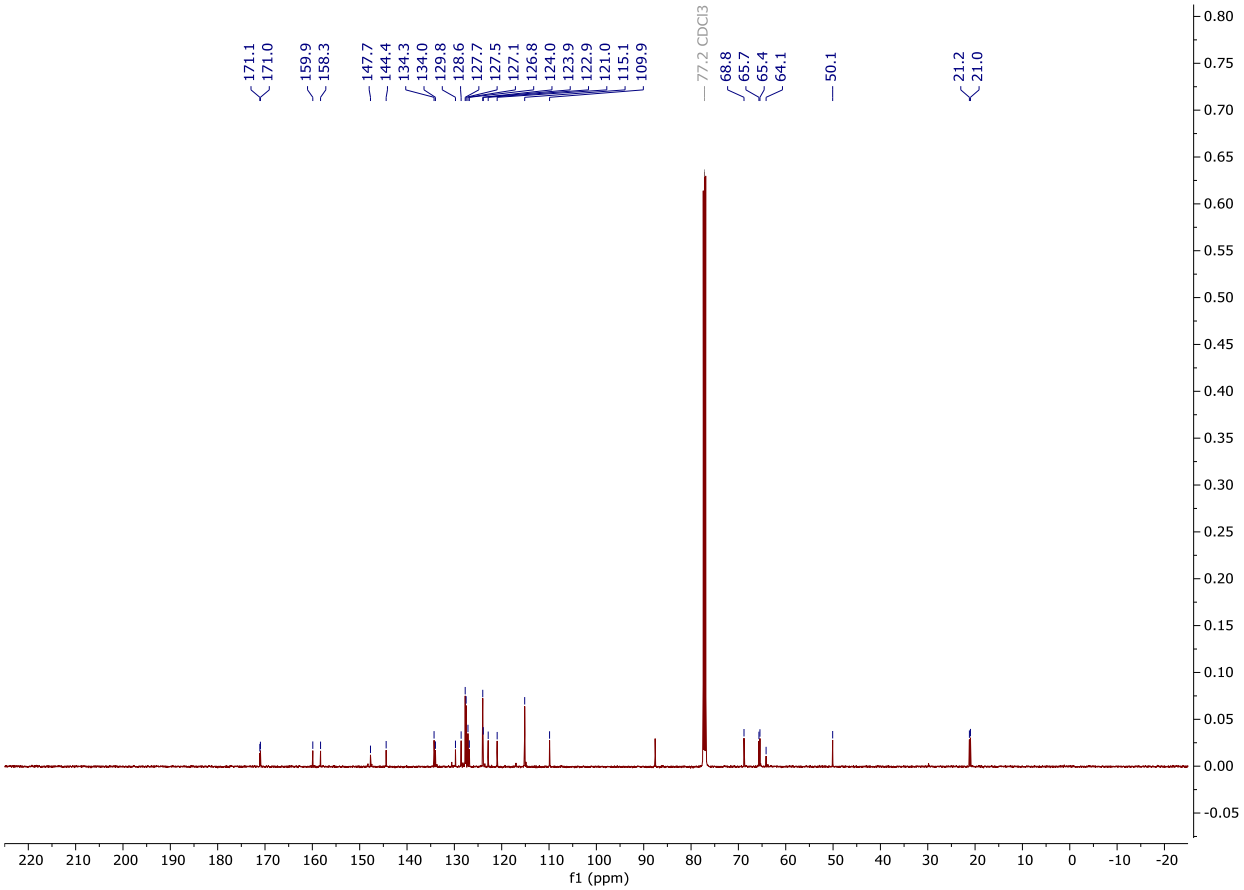
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-133a**



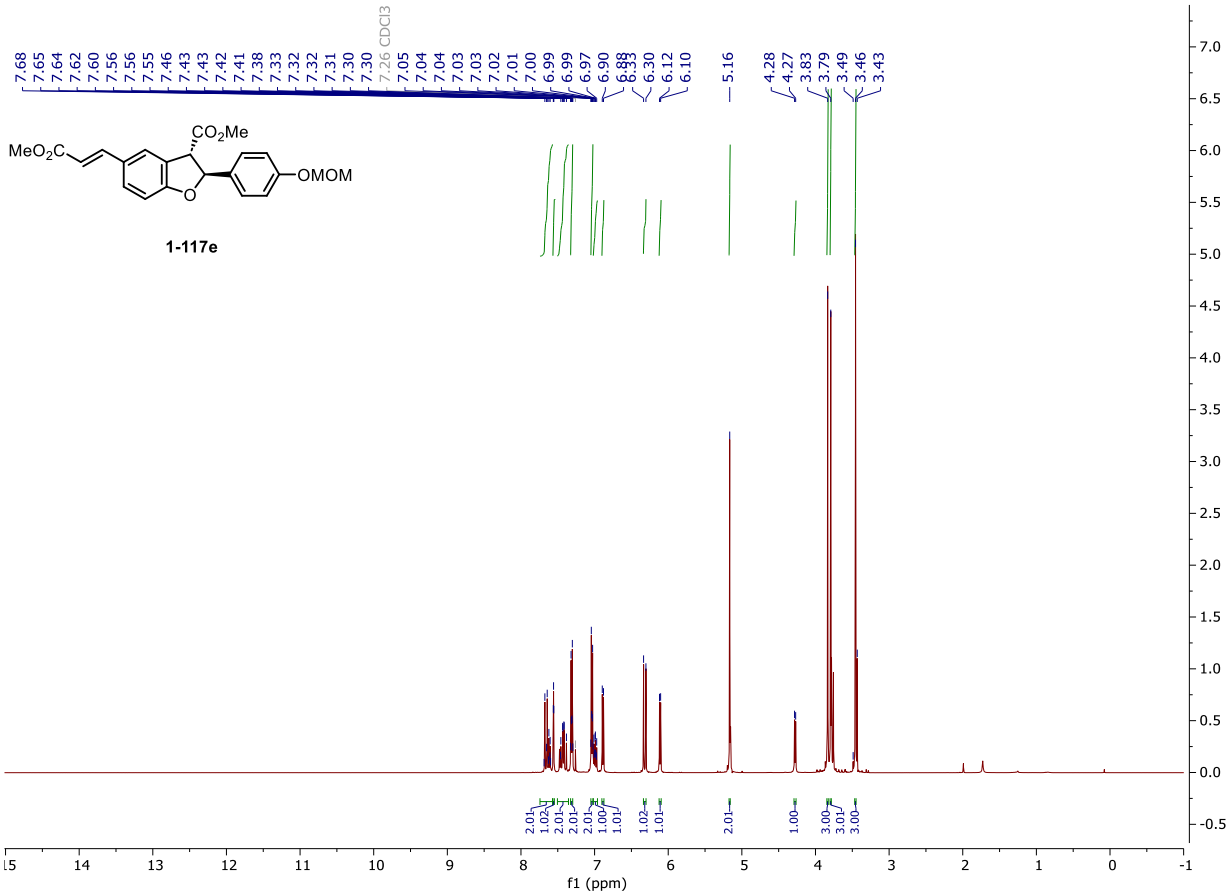


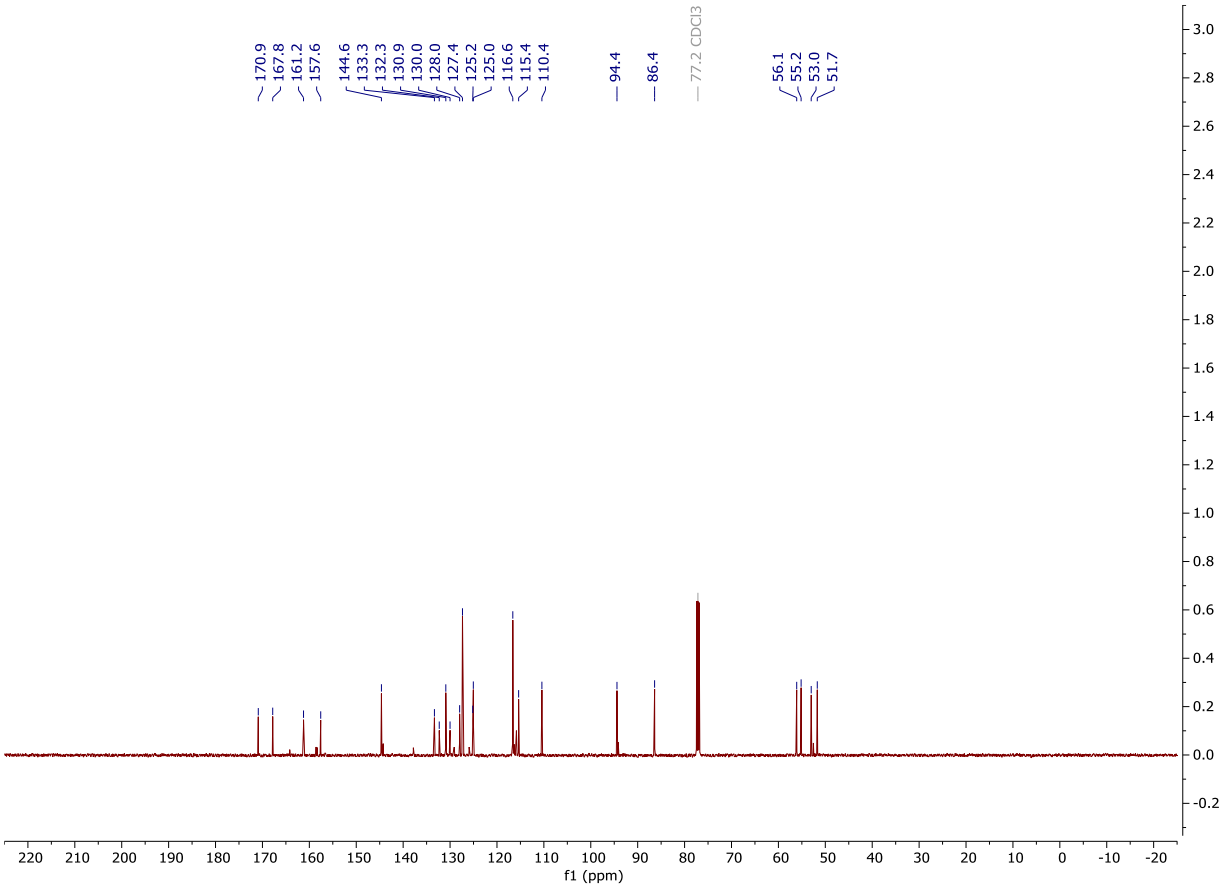
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-133g**





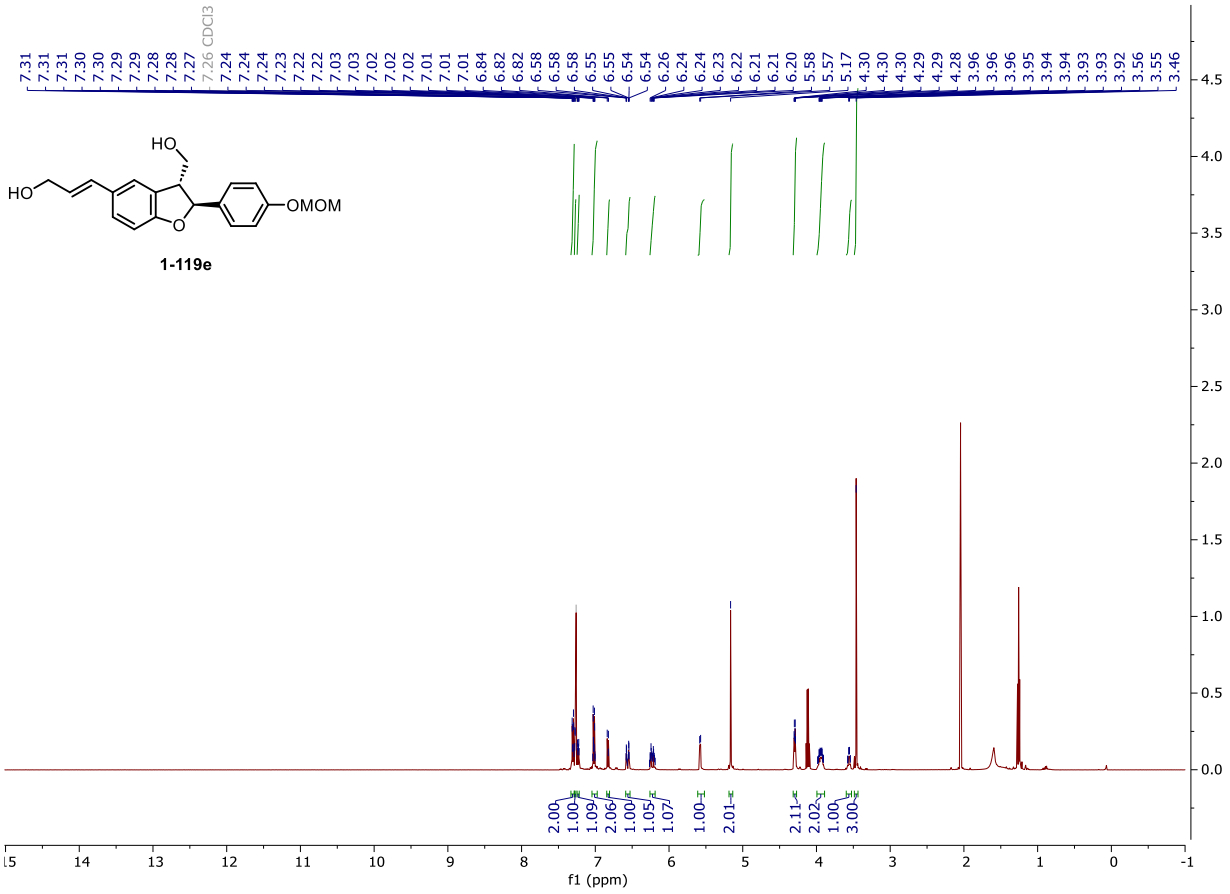
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-117e**





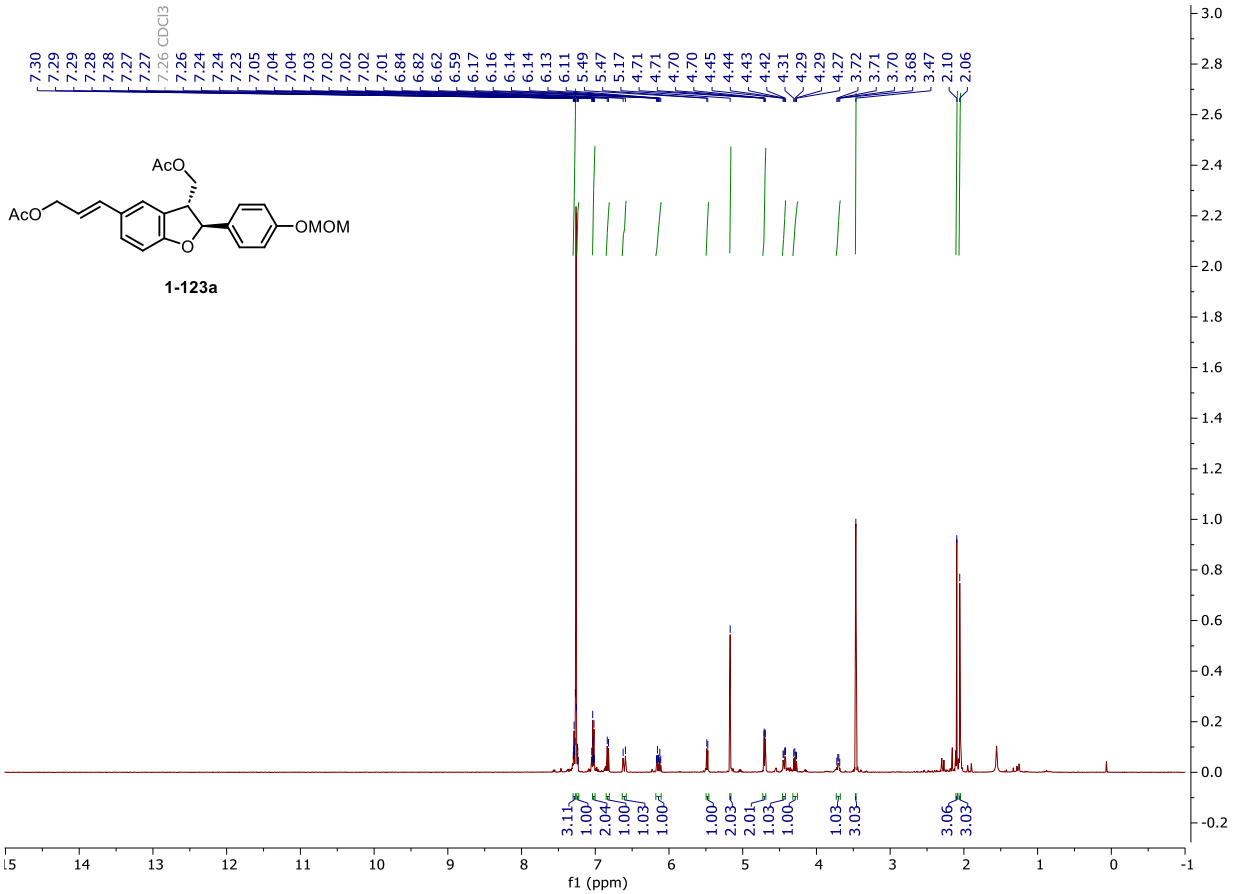
NMR and HPLC

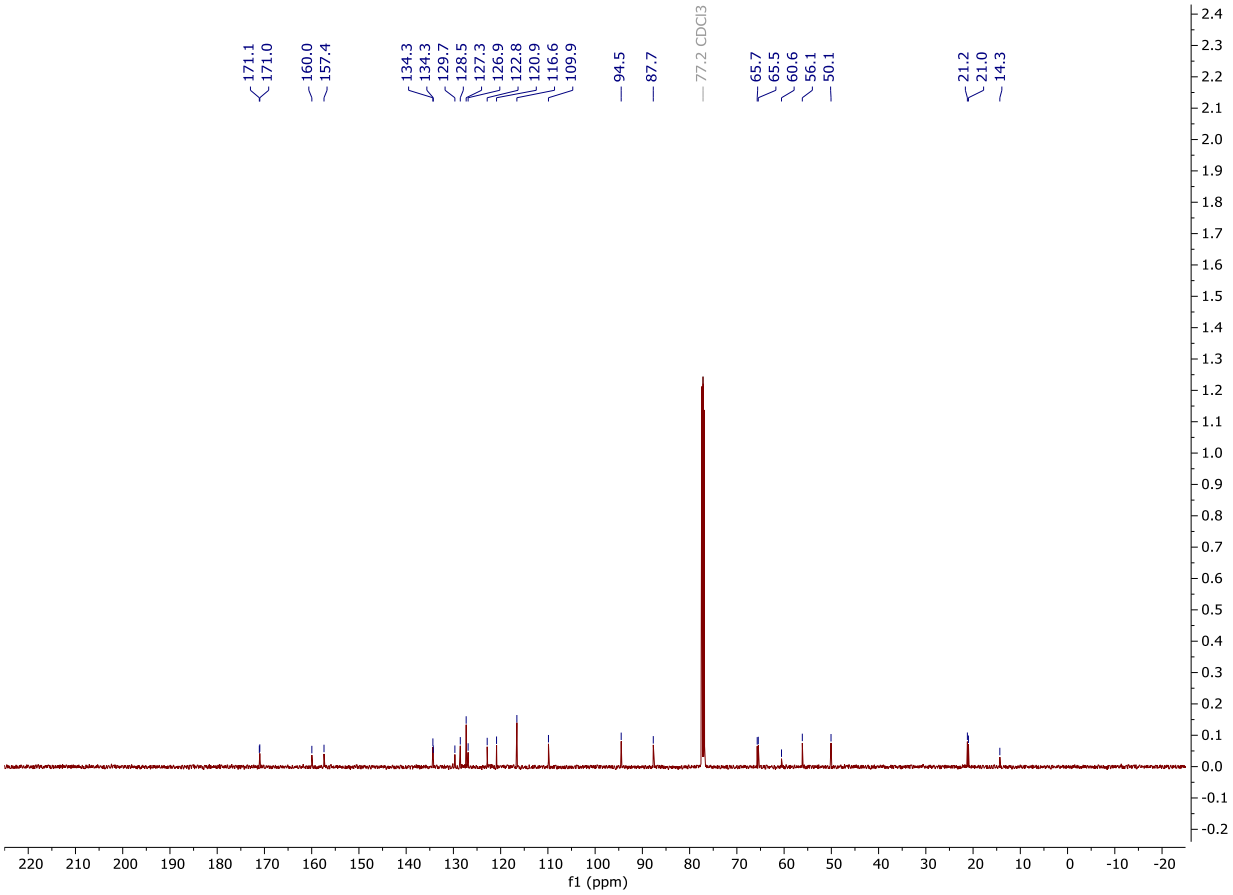
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-119e**



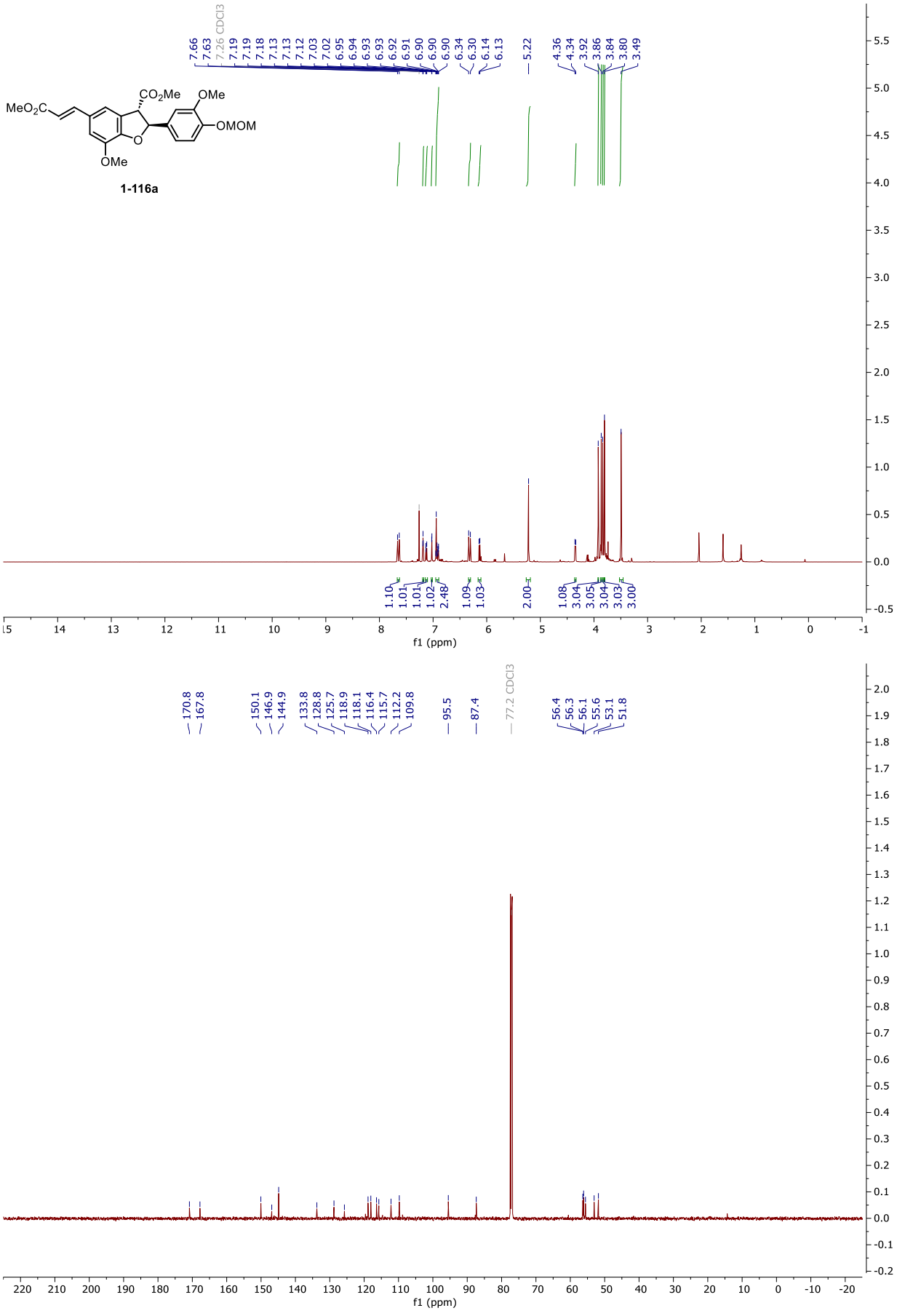
NMR and HPLC

Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-123a**

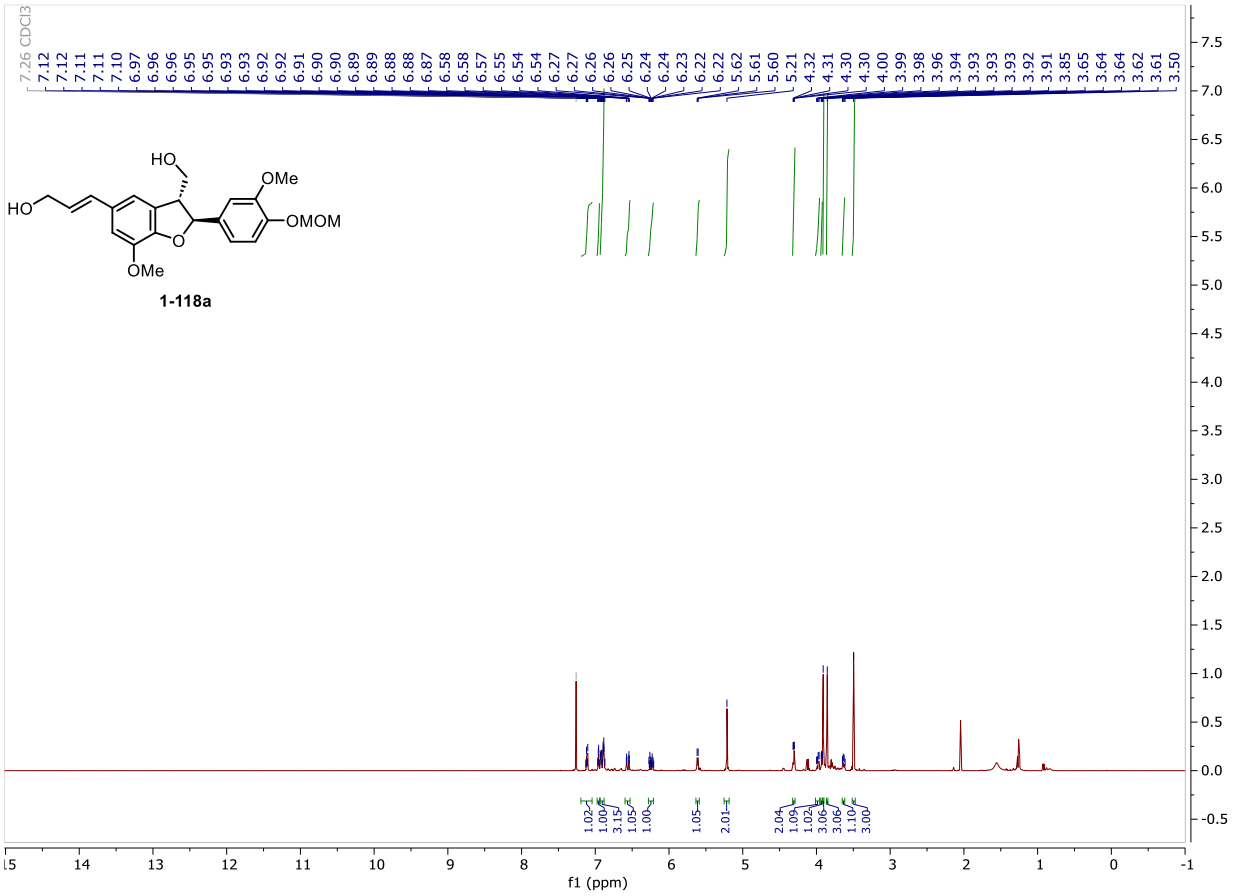




Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-116a**

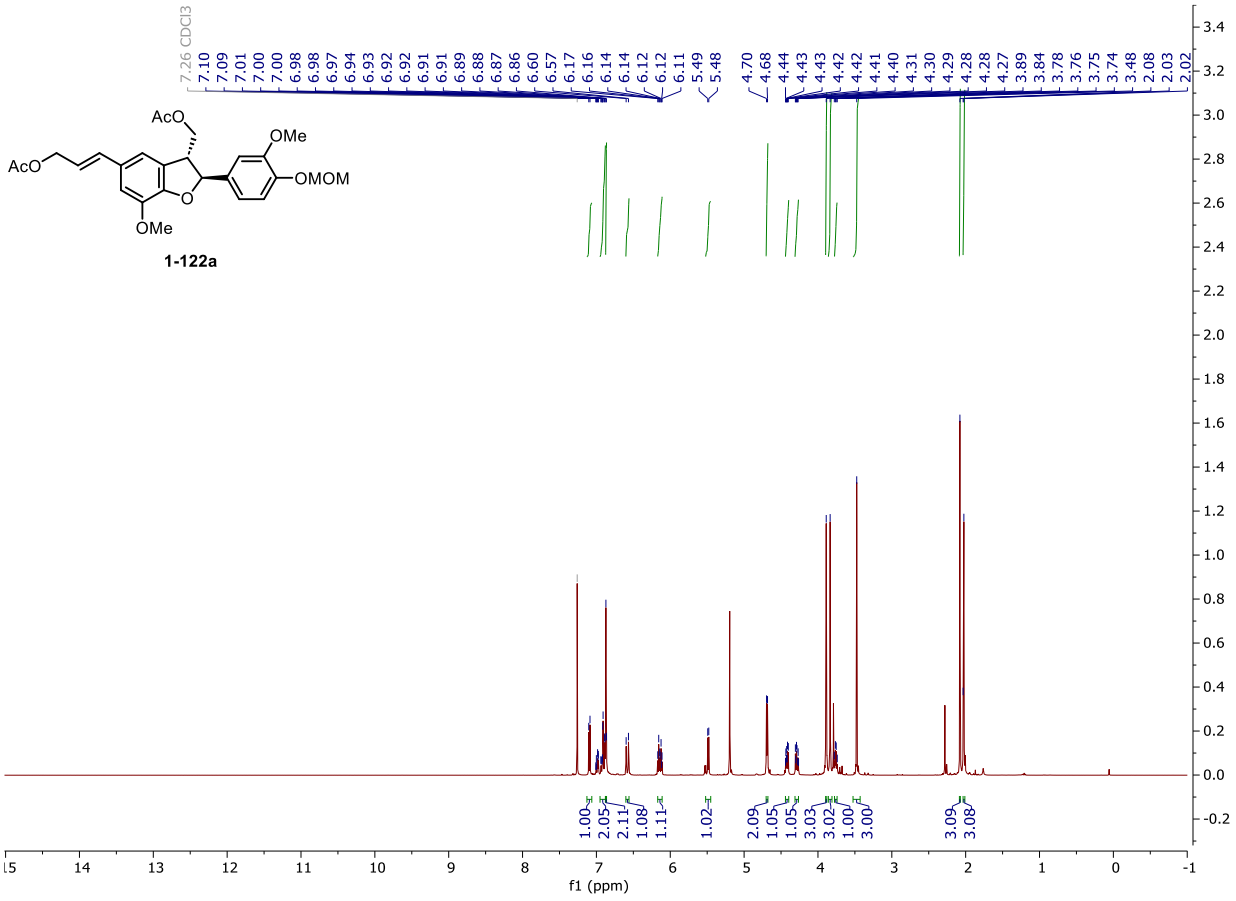


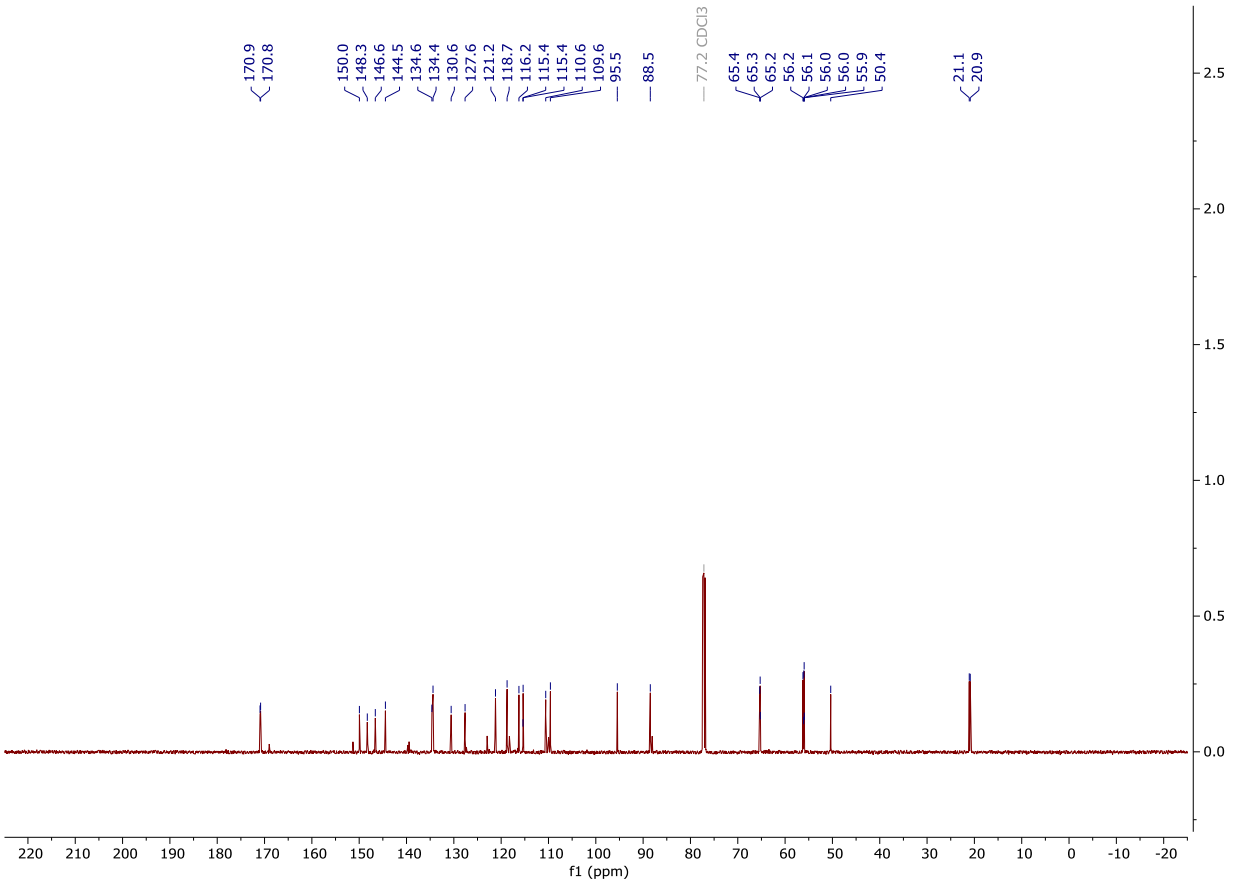
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-118a**



NMR and HPLC

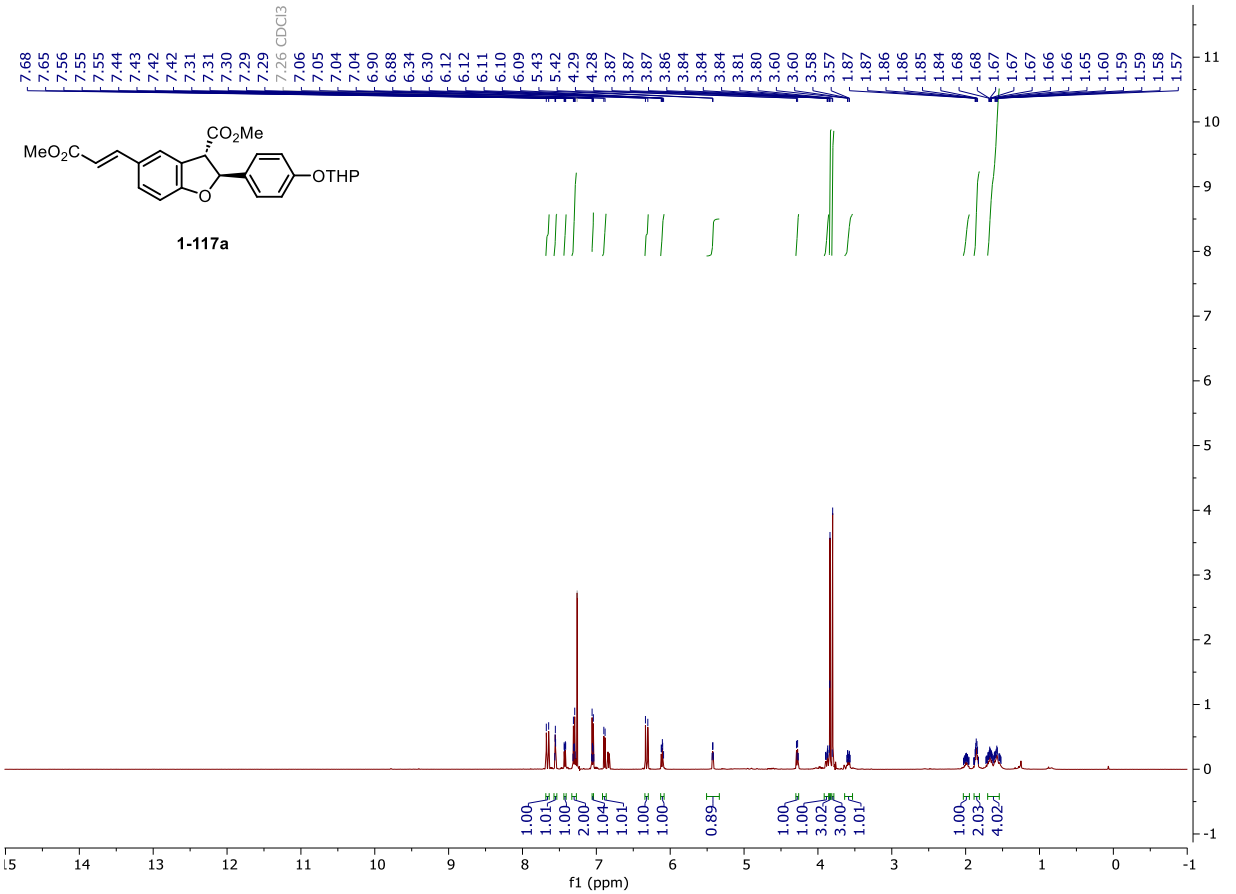
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-122a**

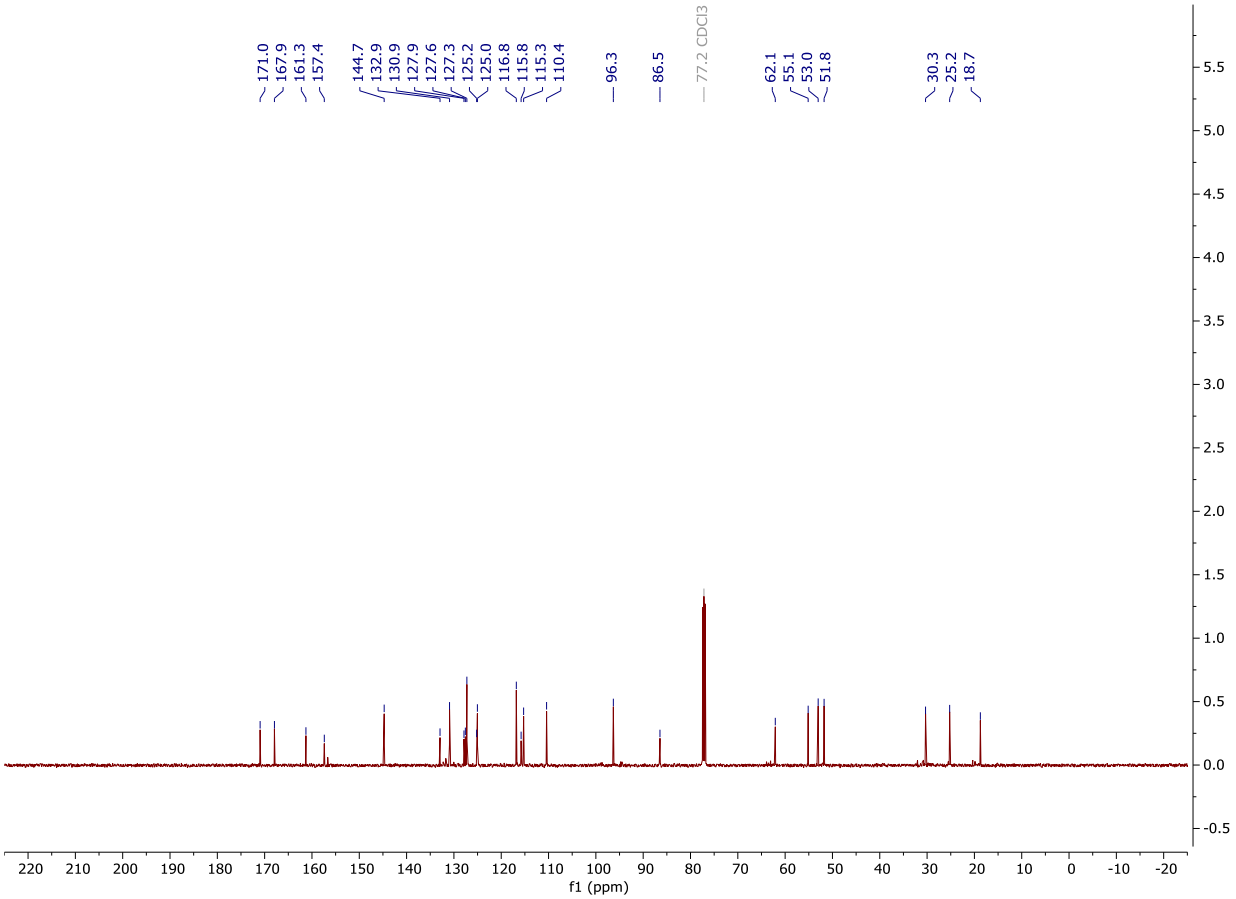




NMR and HPLC

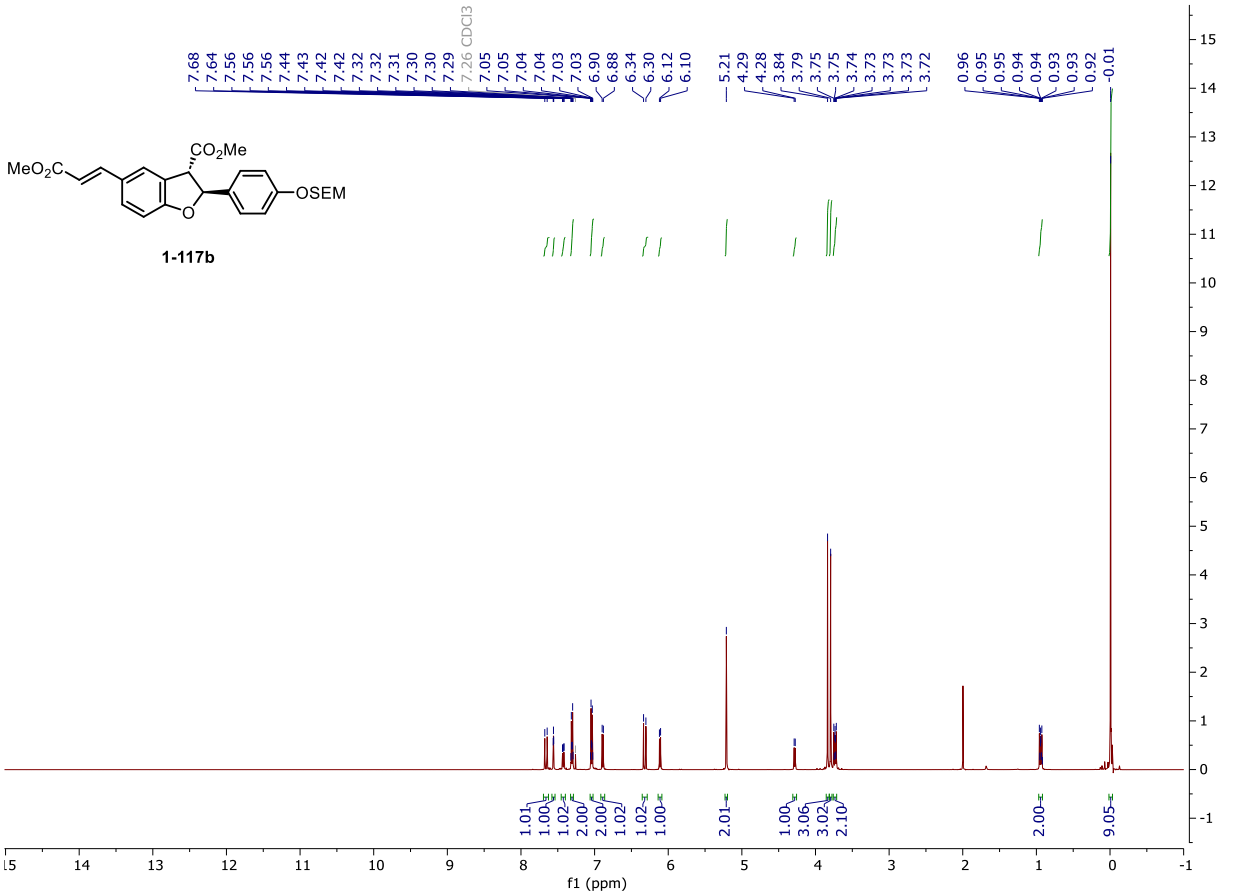
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-117a**

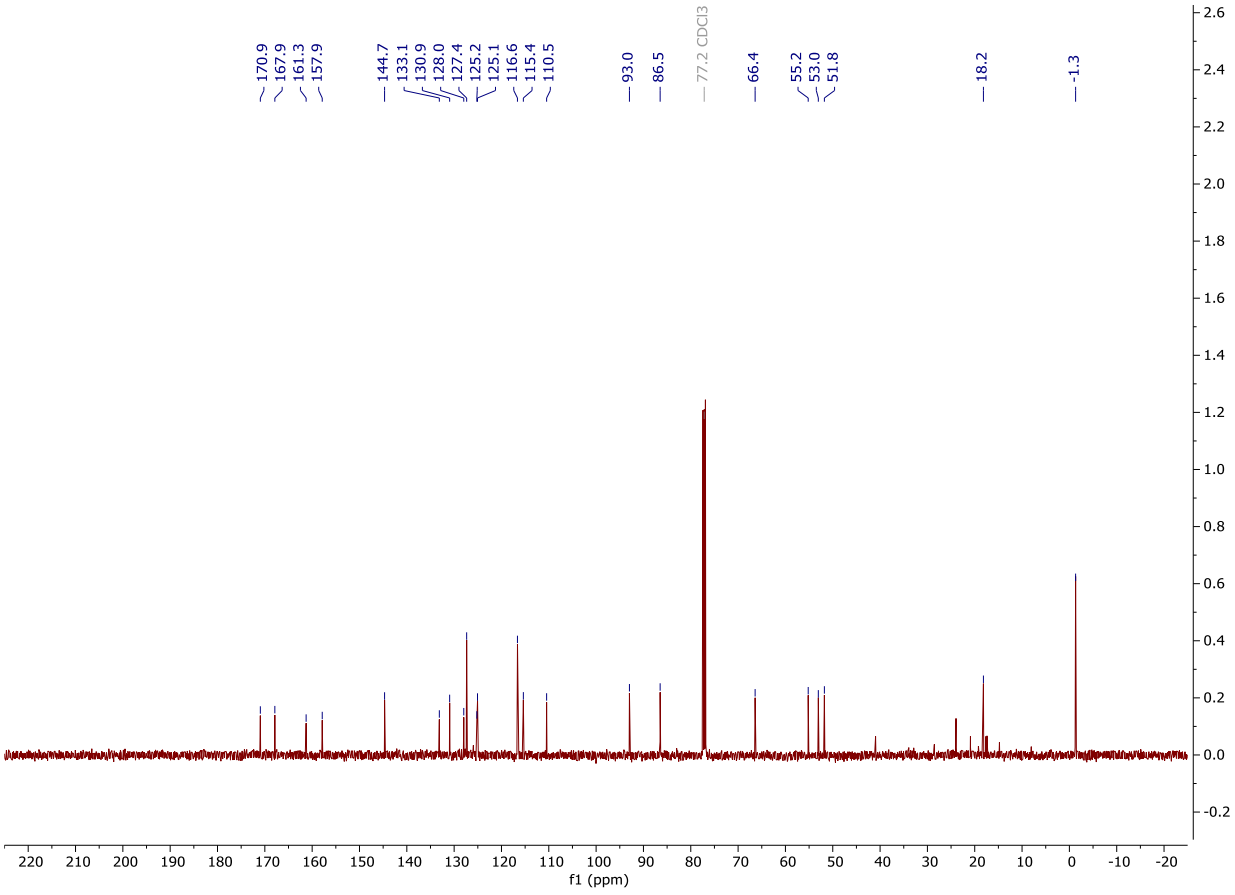




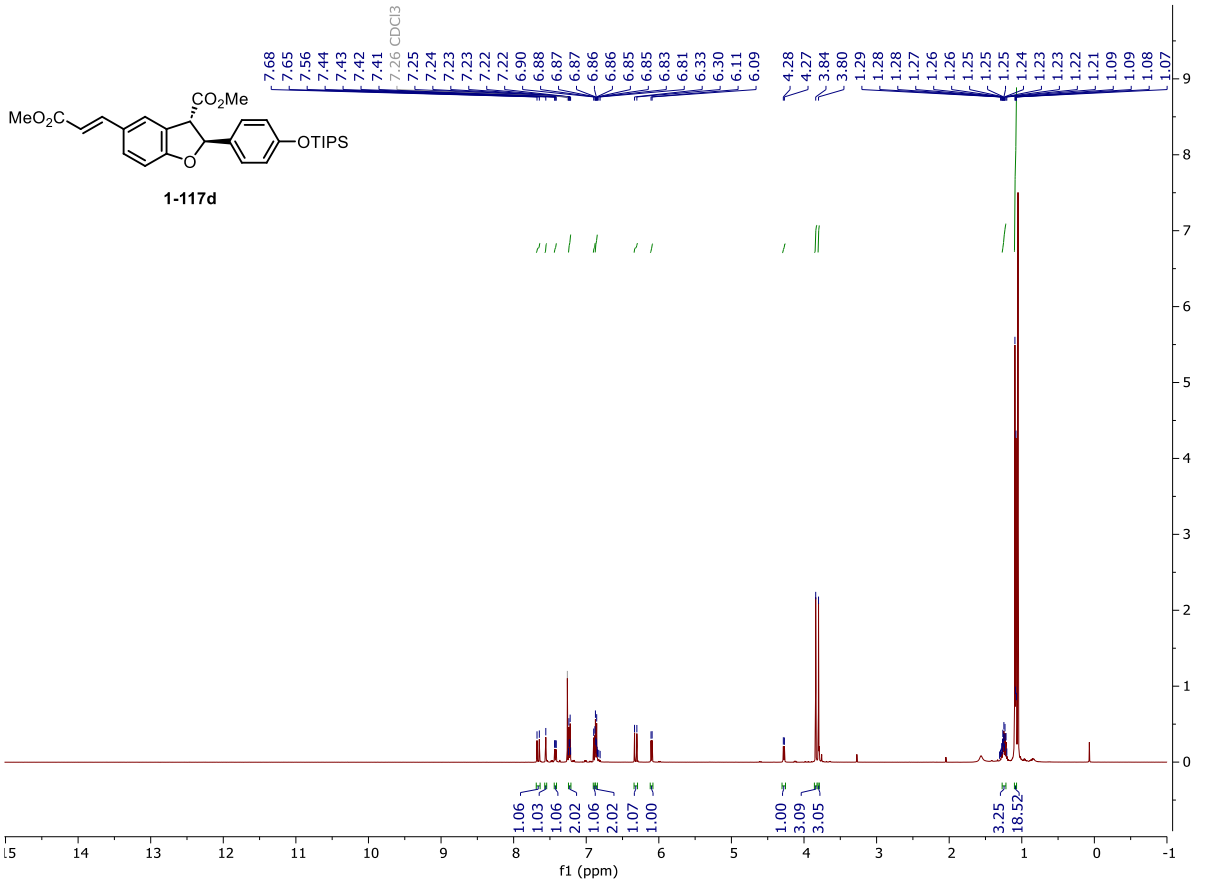
NMR and HPLC

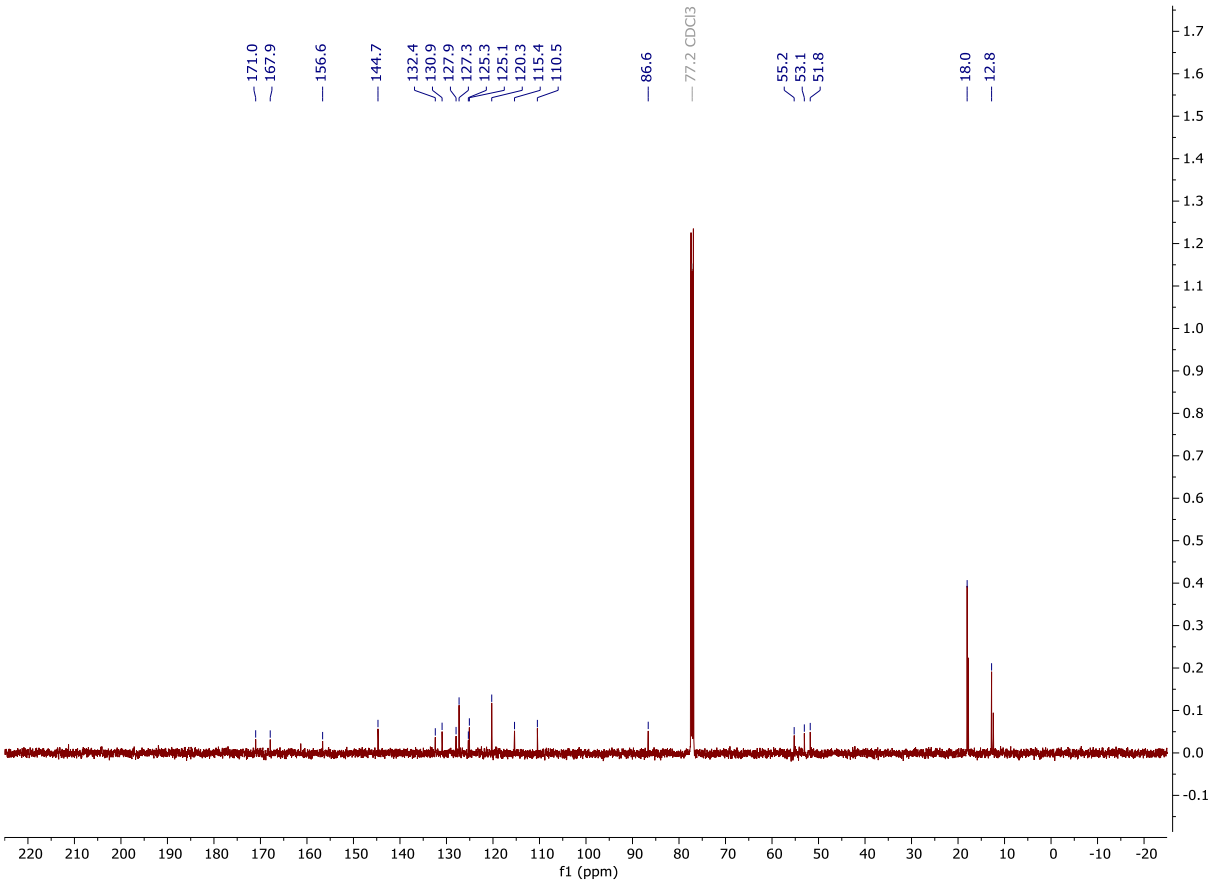
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-117b**



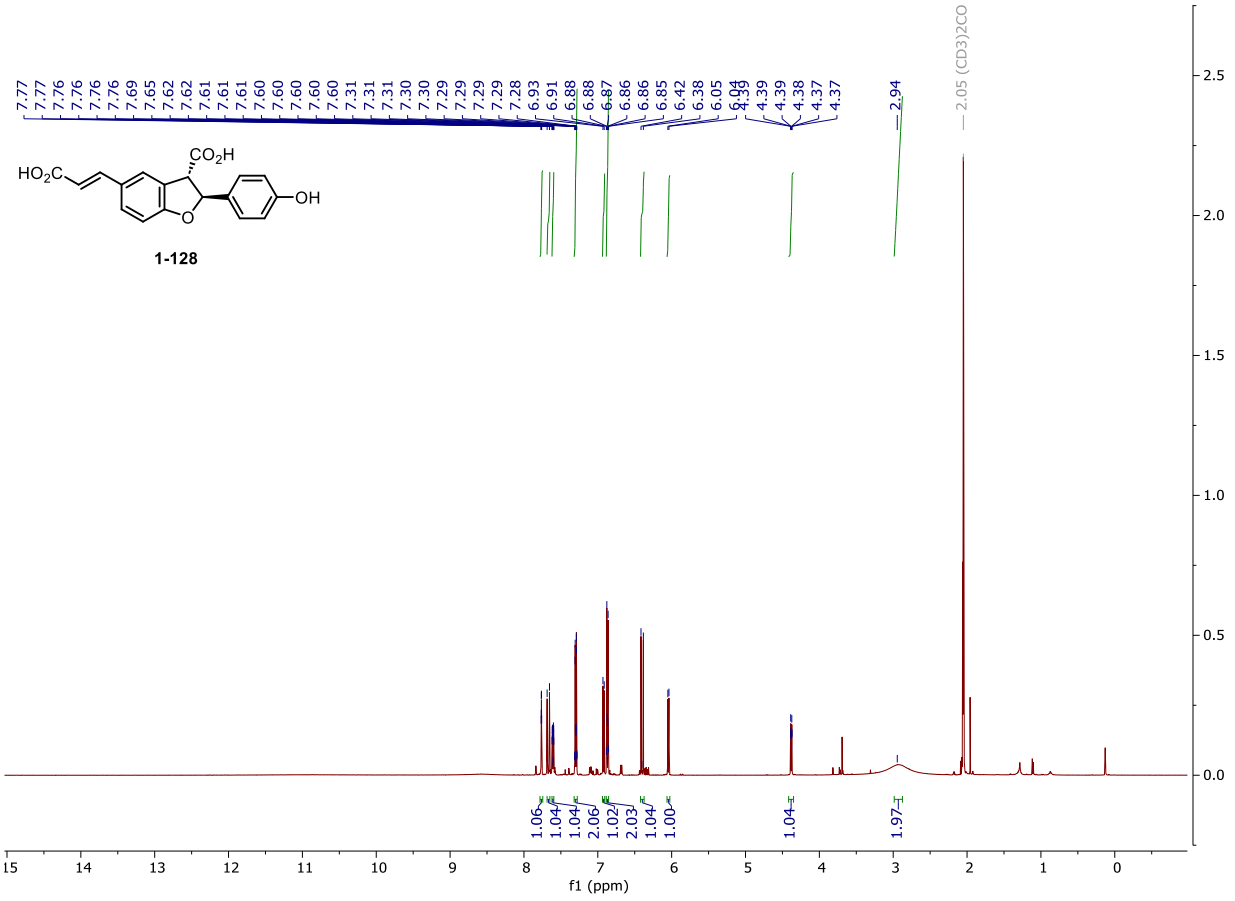


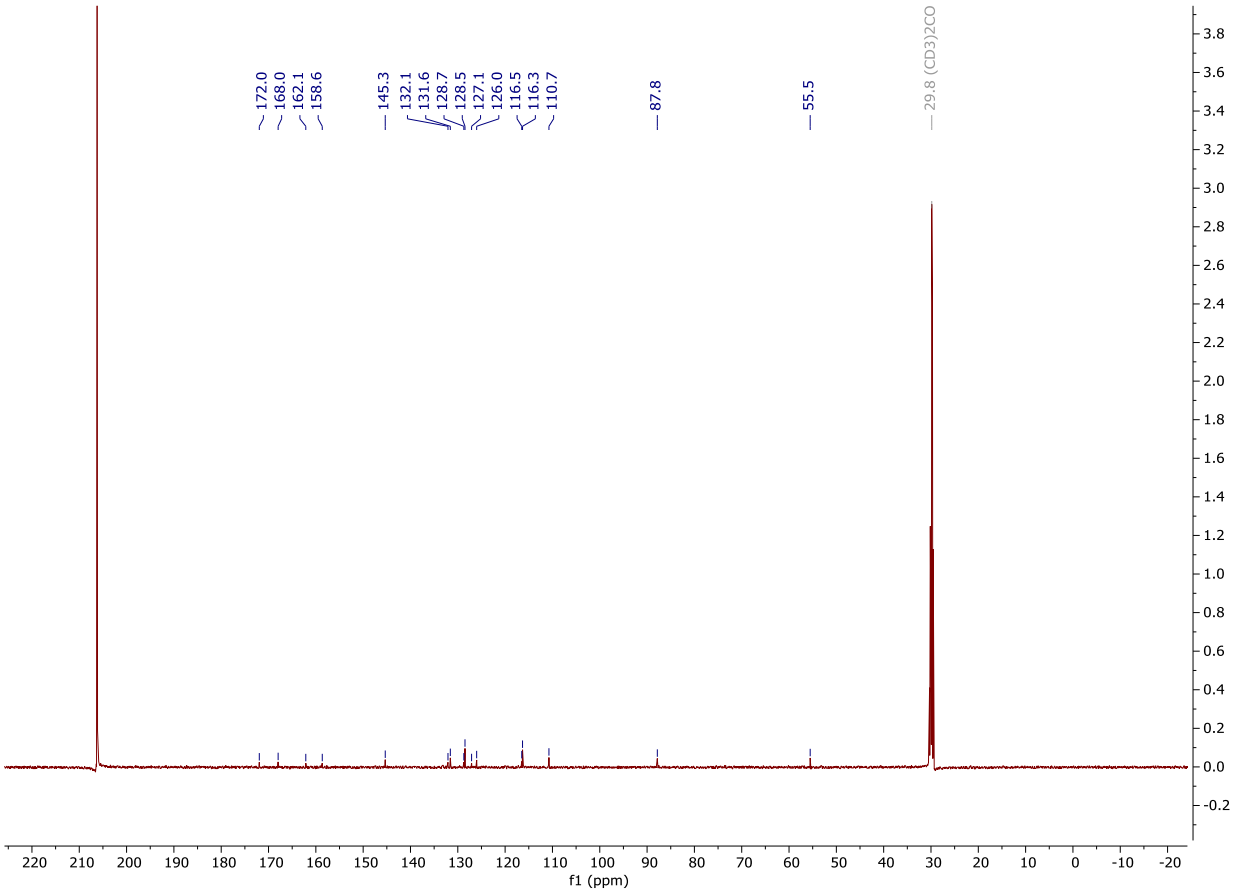
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-117d**





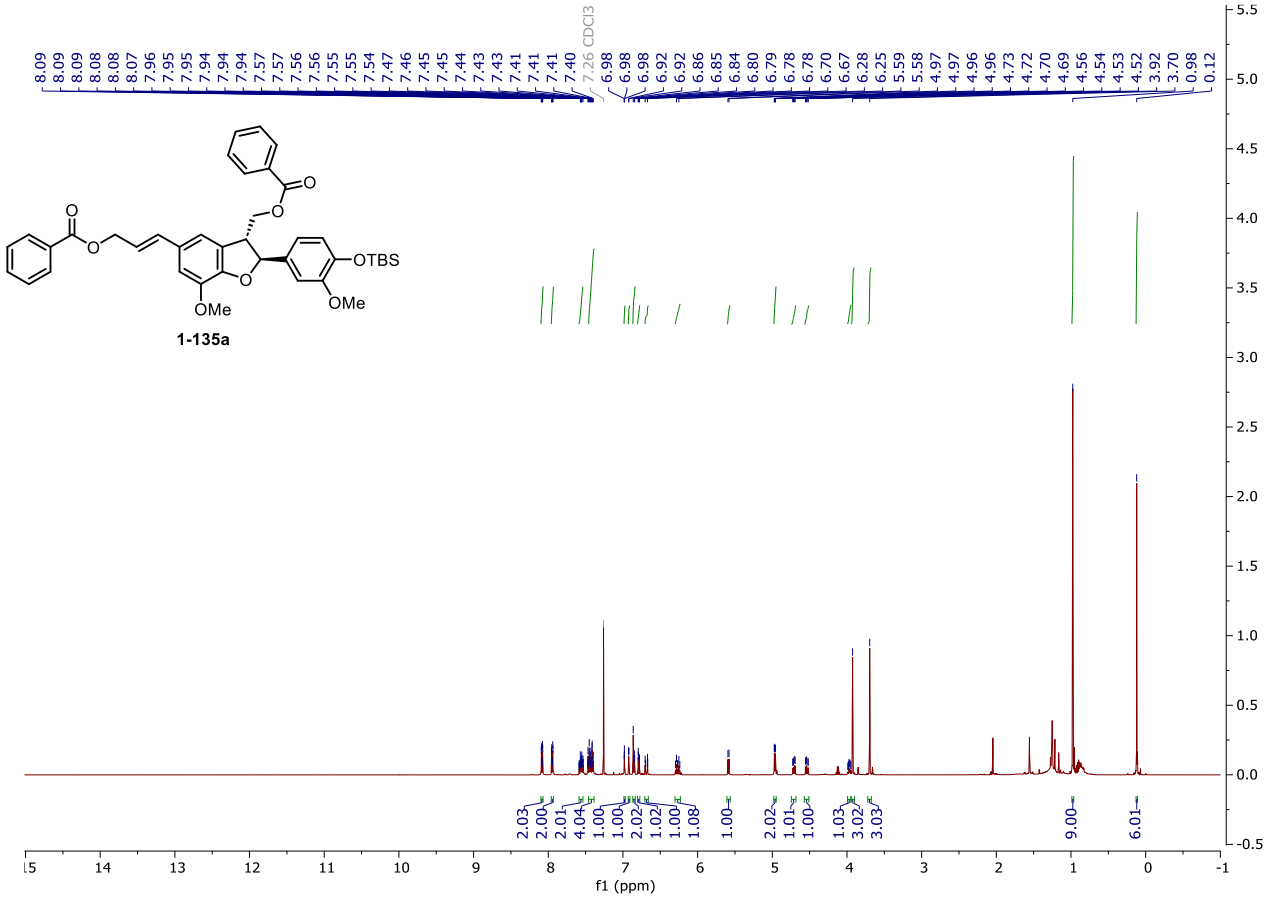
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-128**





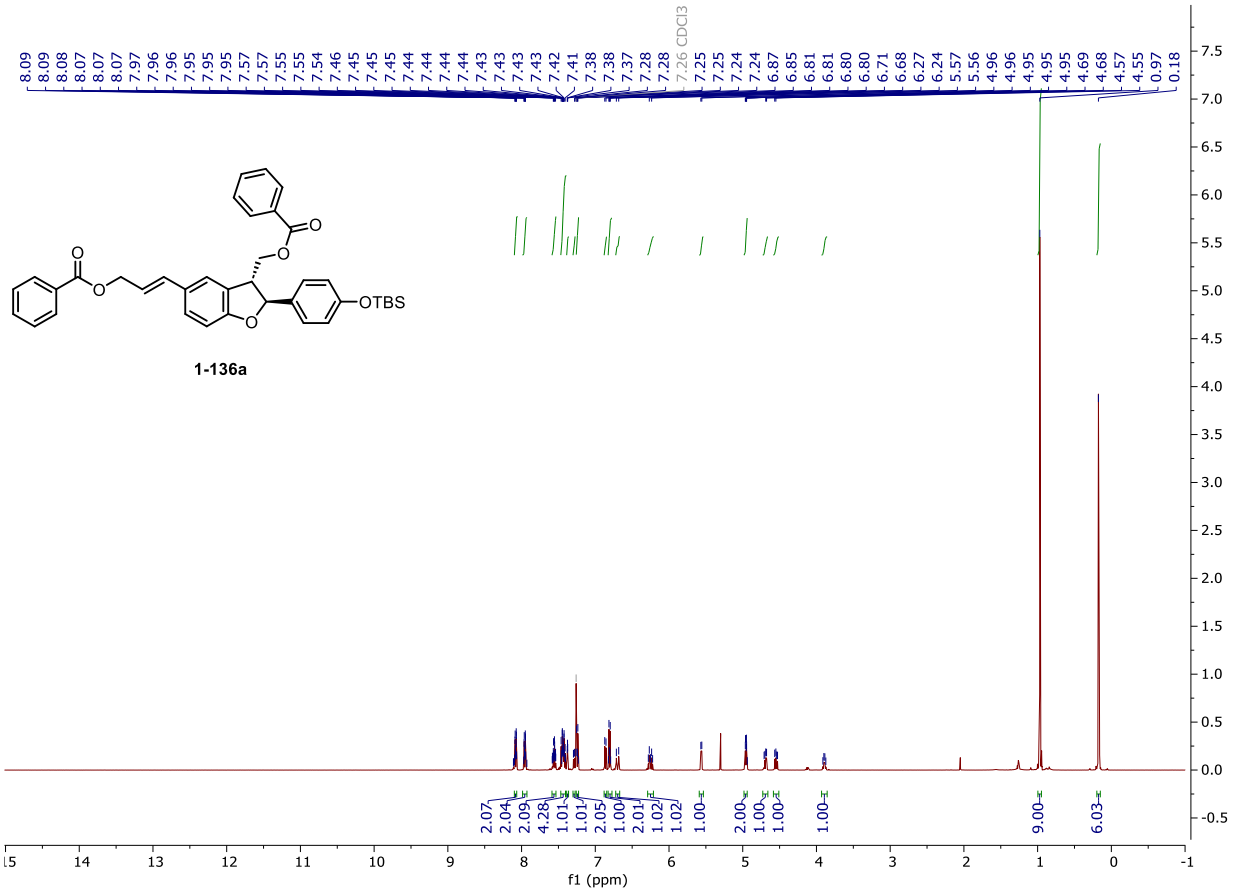
NMR and HPLC

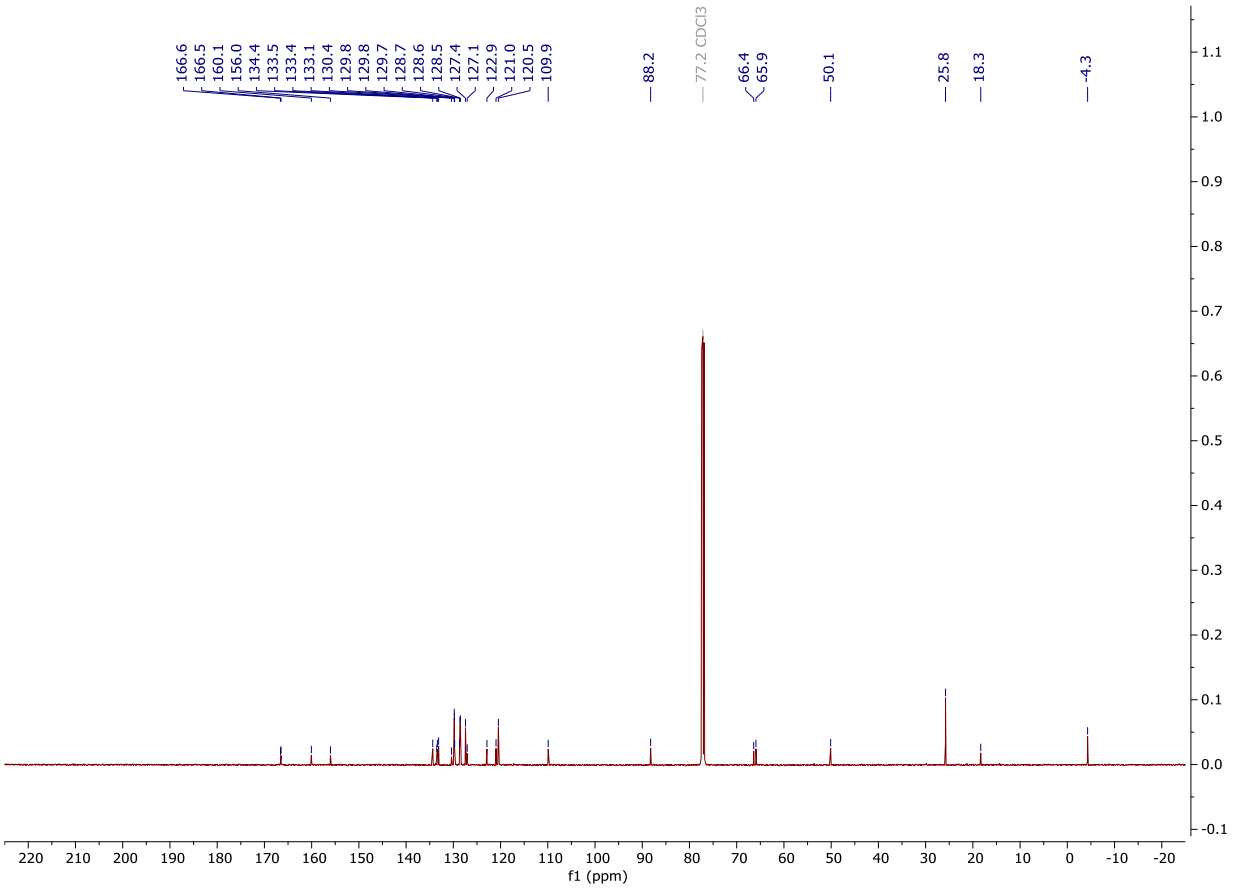
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-135a**



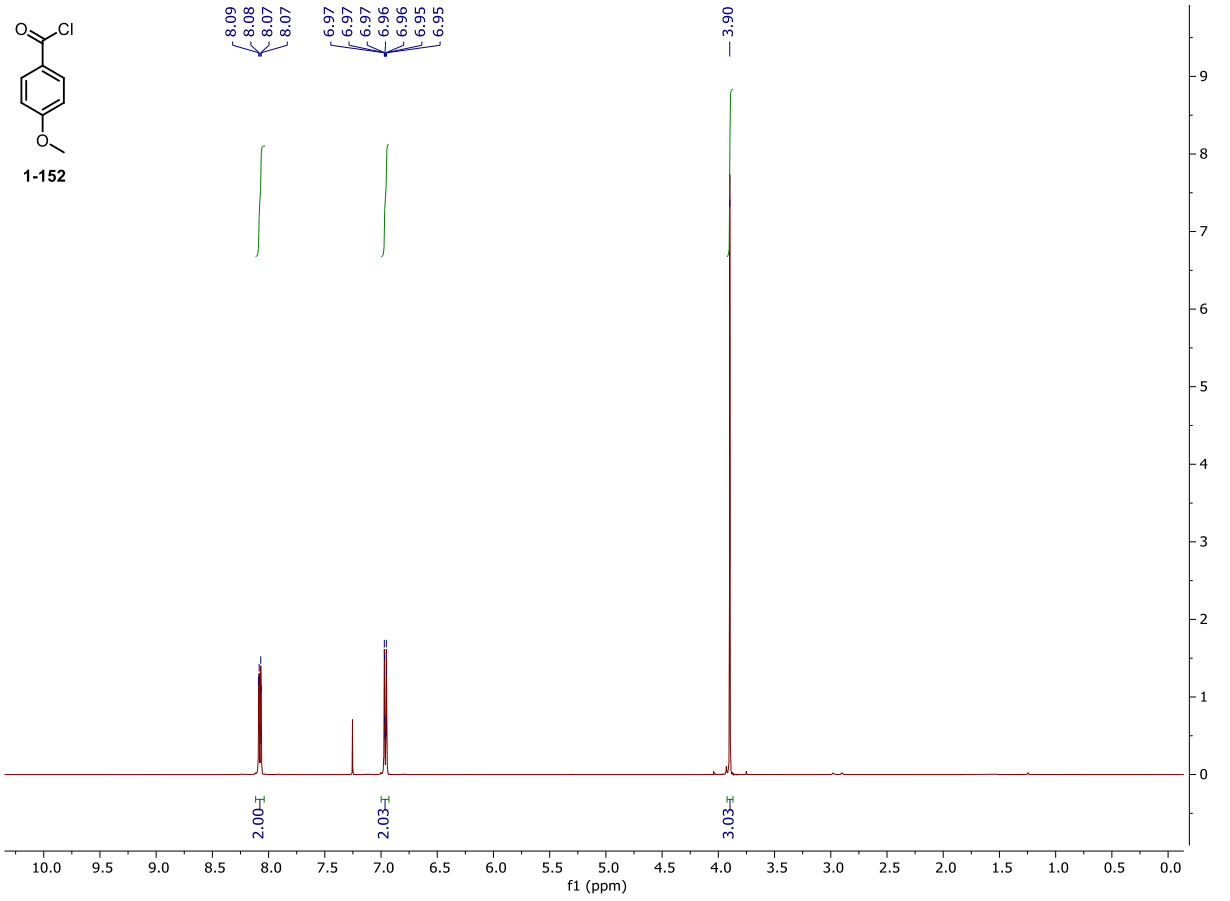
NMR and HPLC

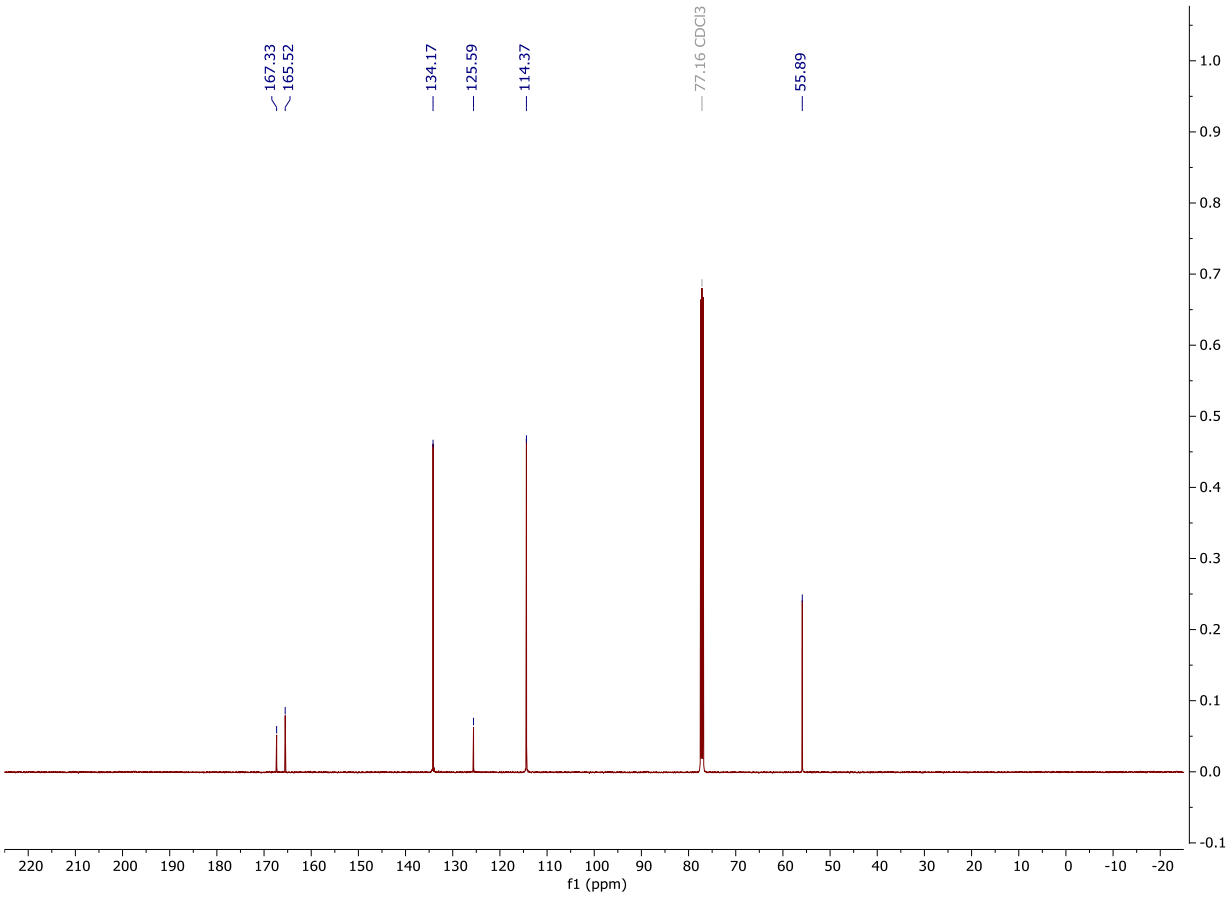
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-136a**



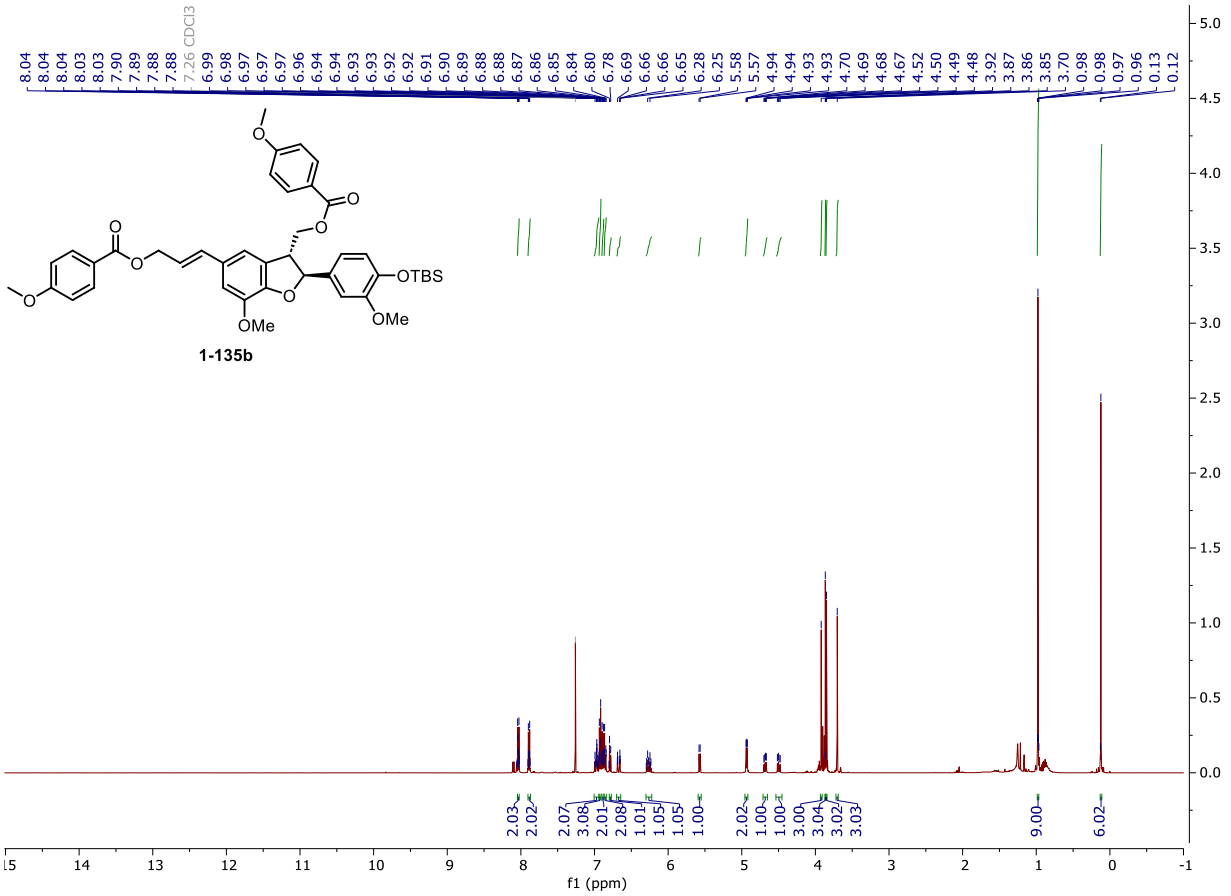


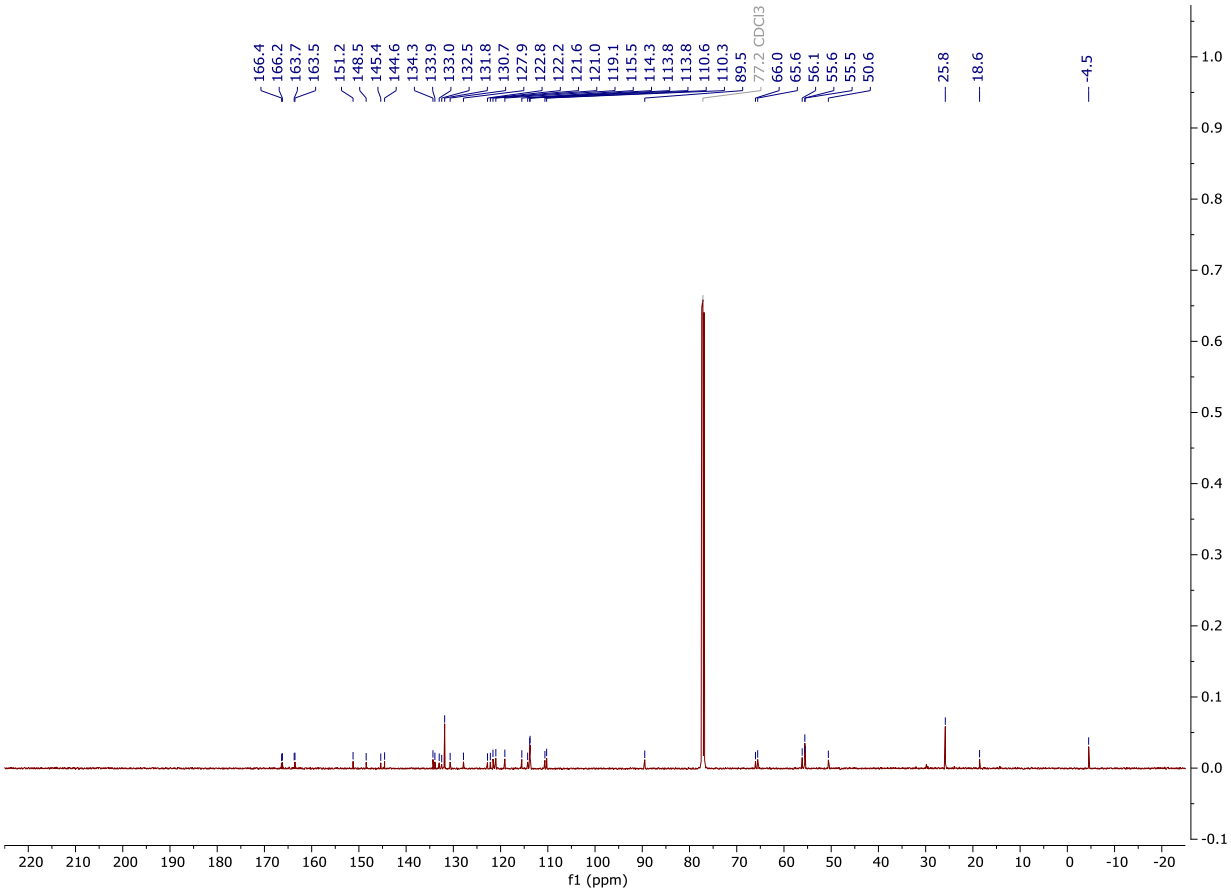
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-152**





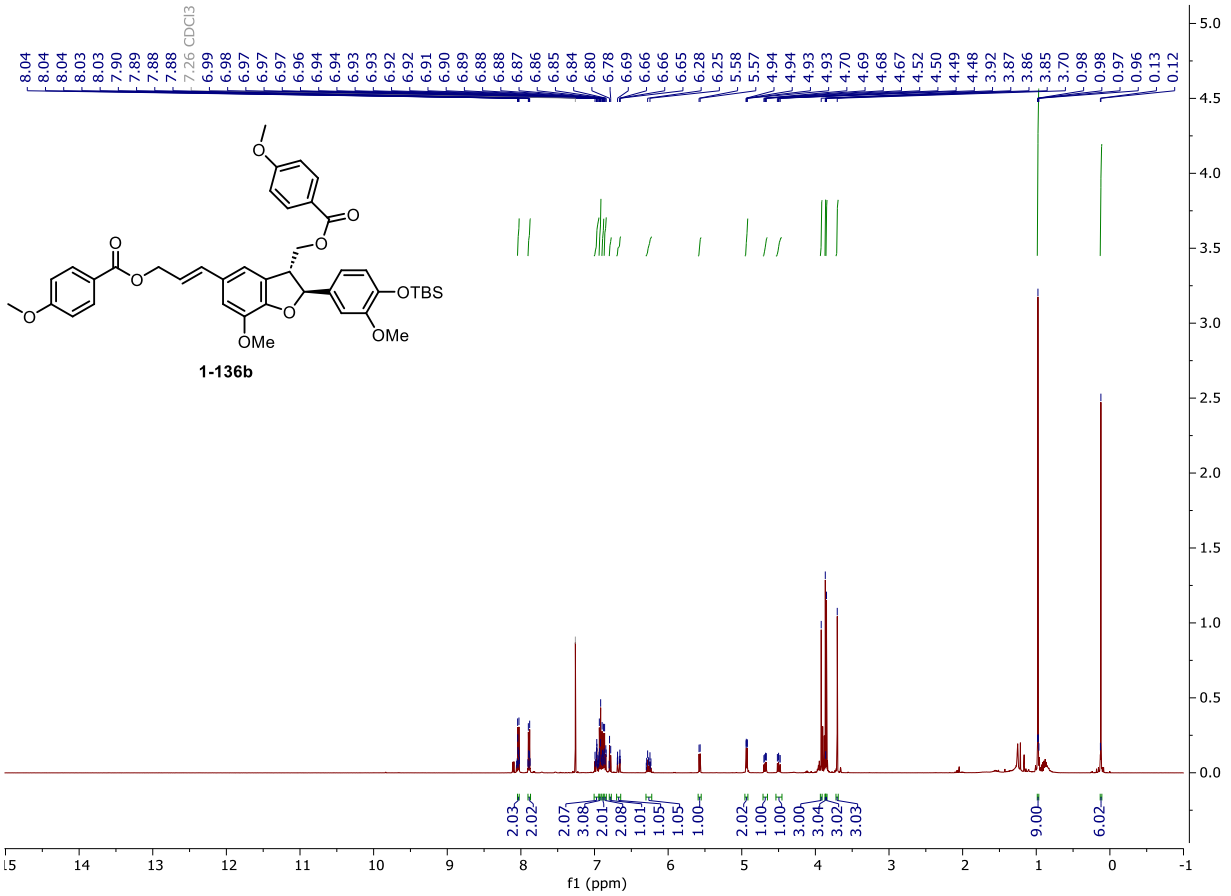
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-135b**

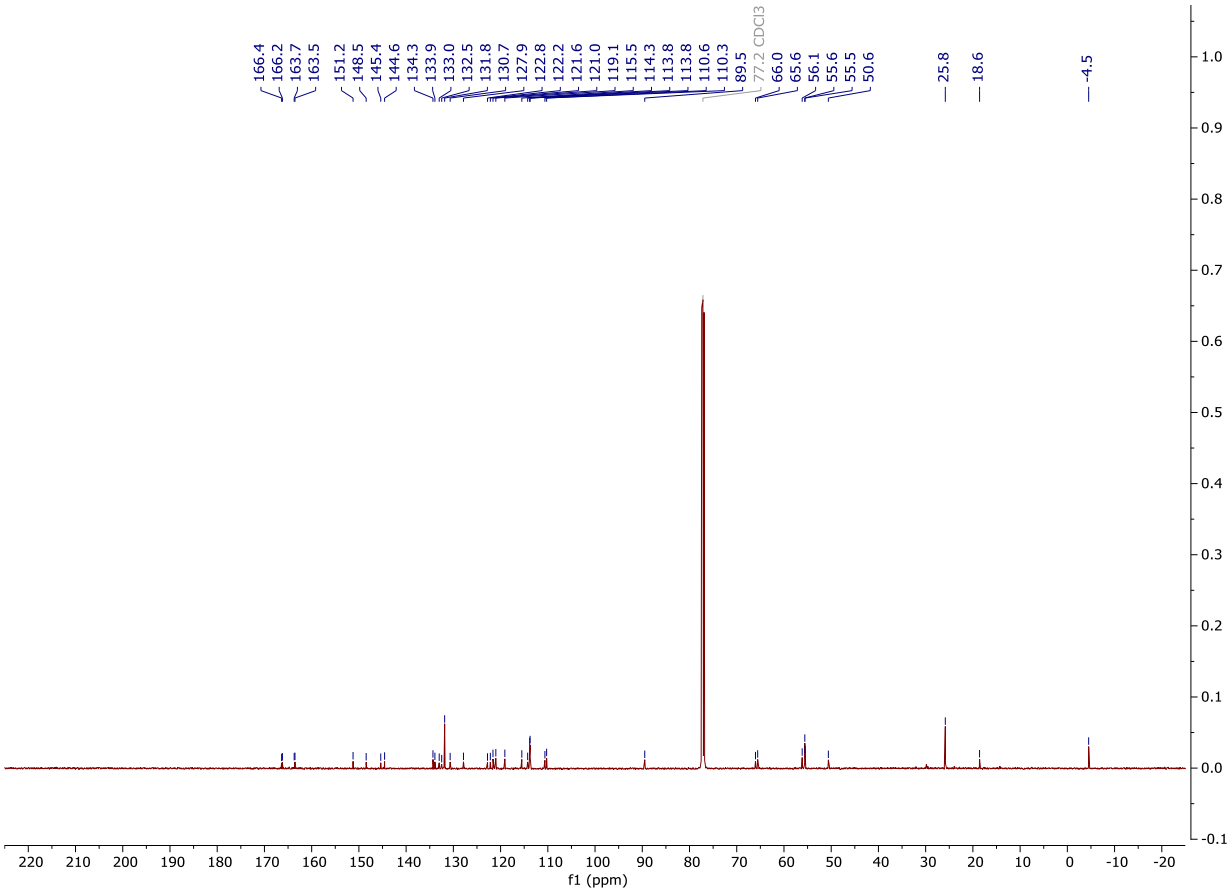




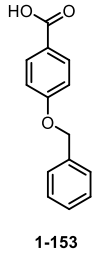
NMR and HPLC

Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-136b**

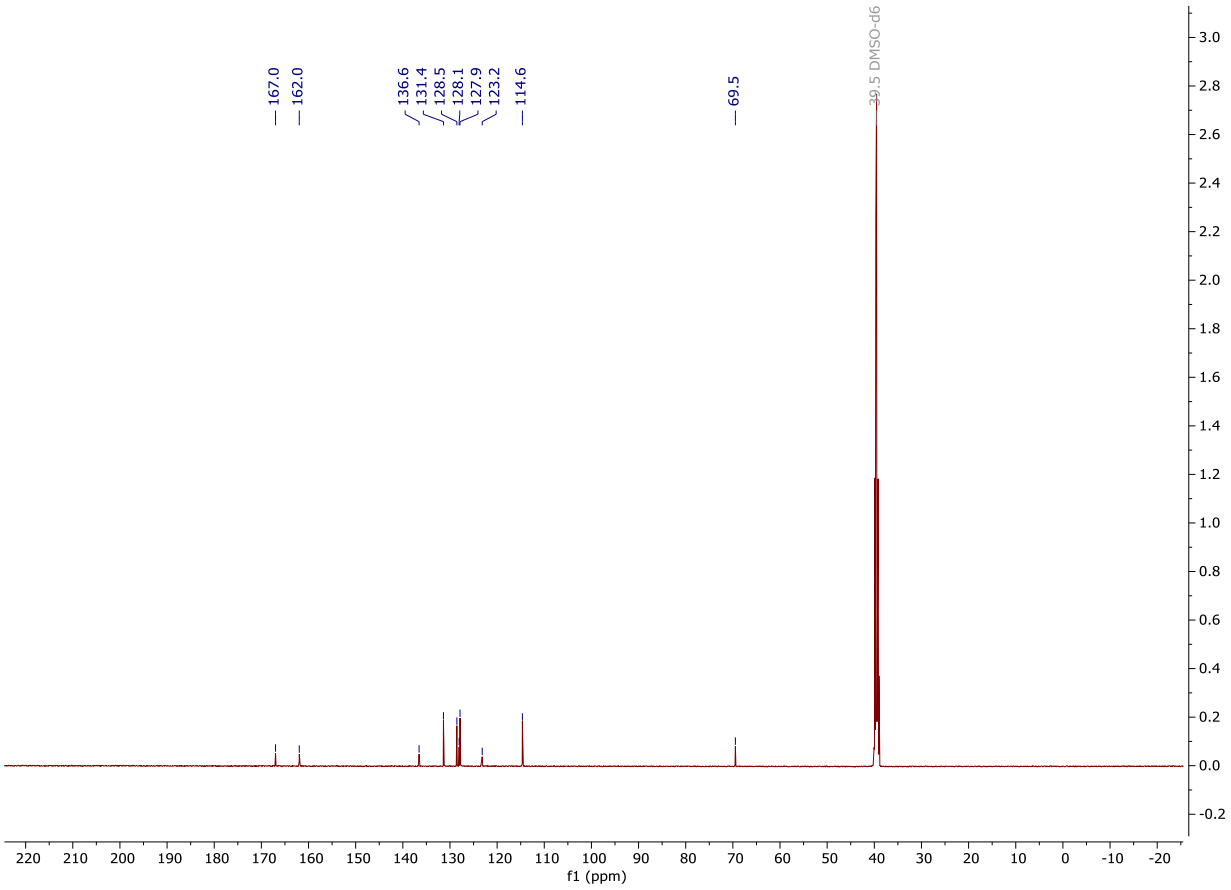




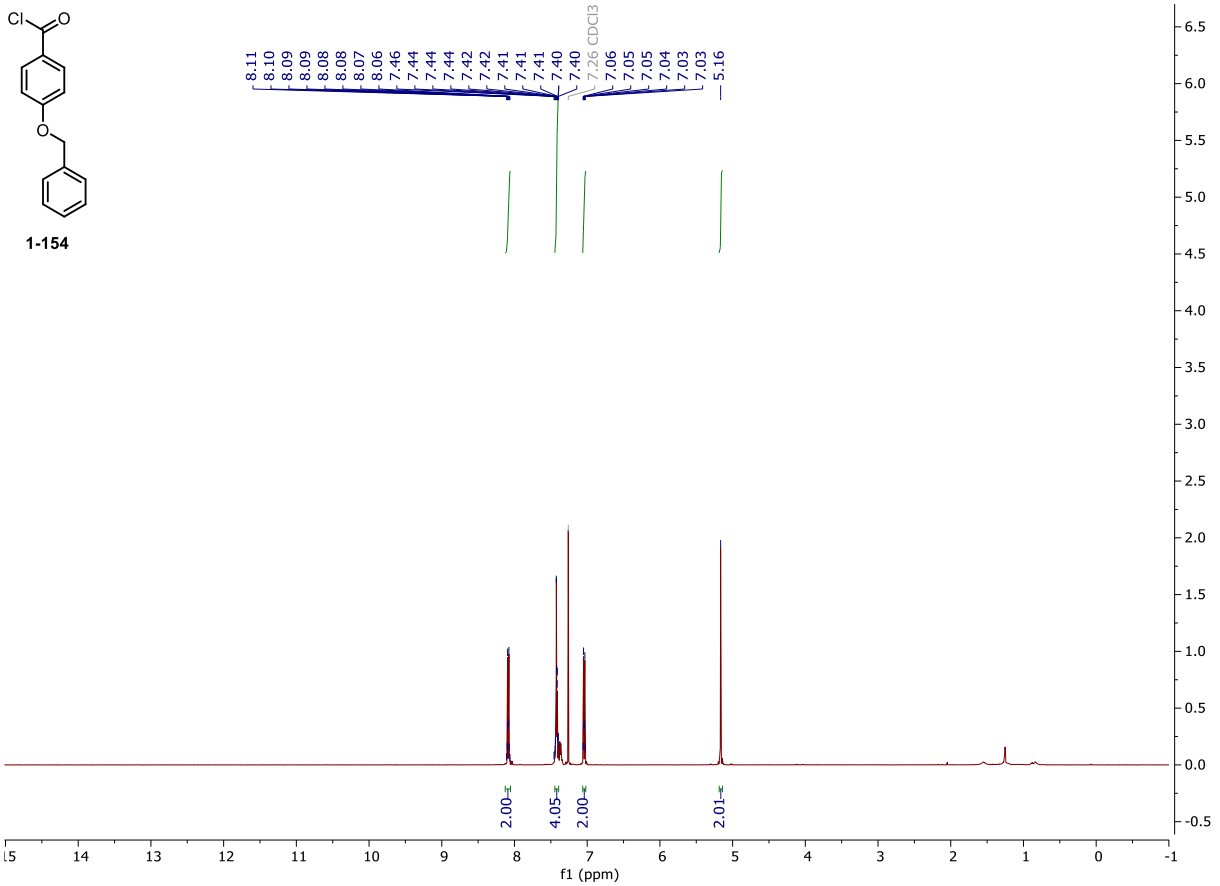
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-153**

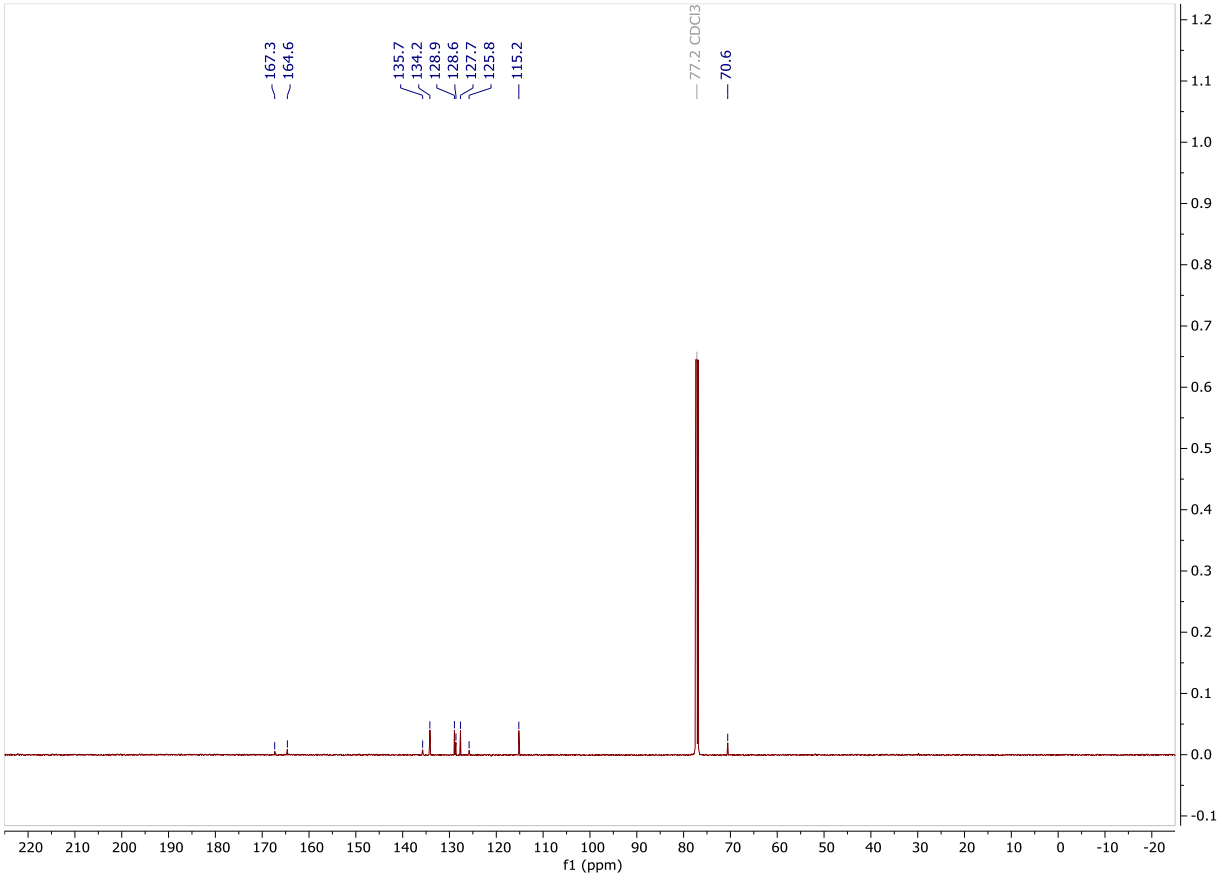


1-153



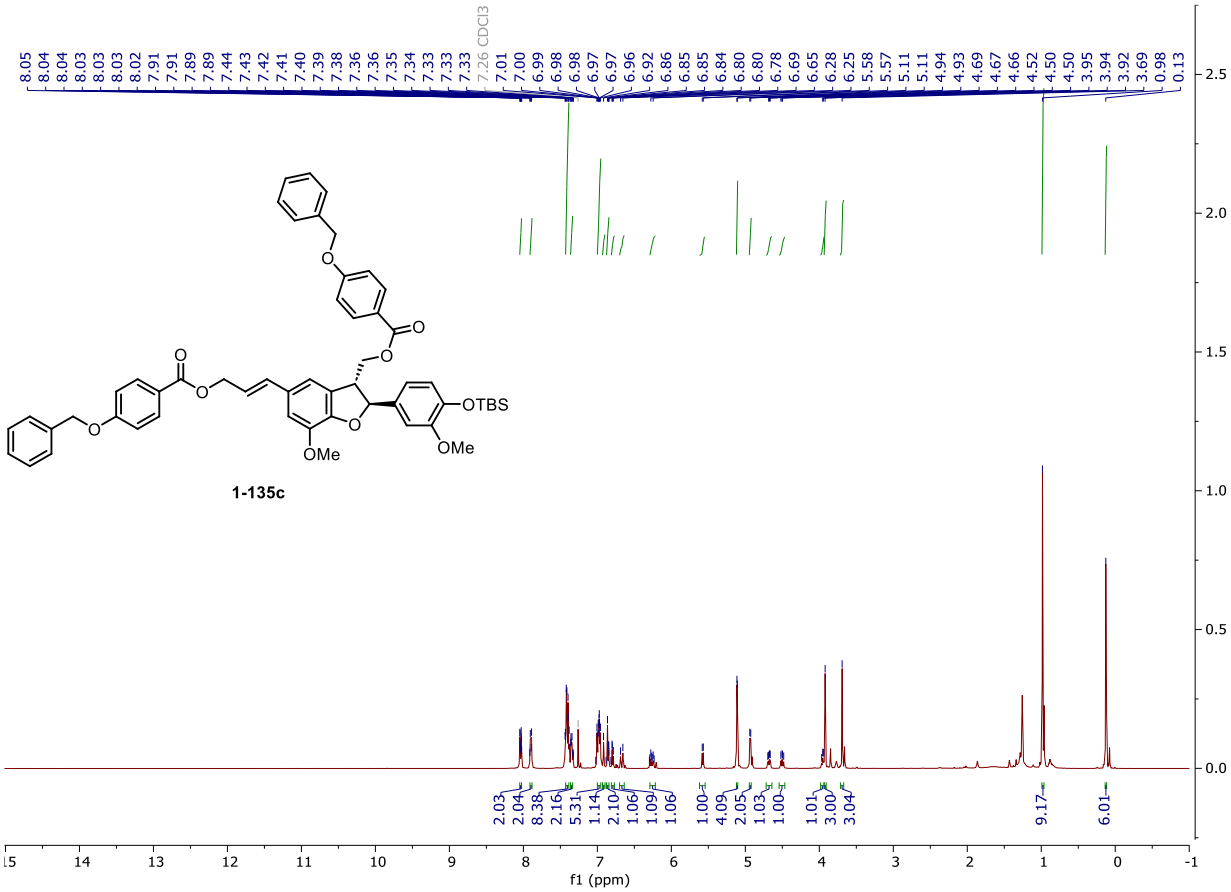
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-154**

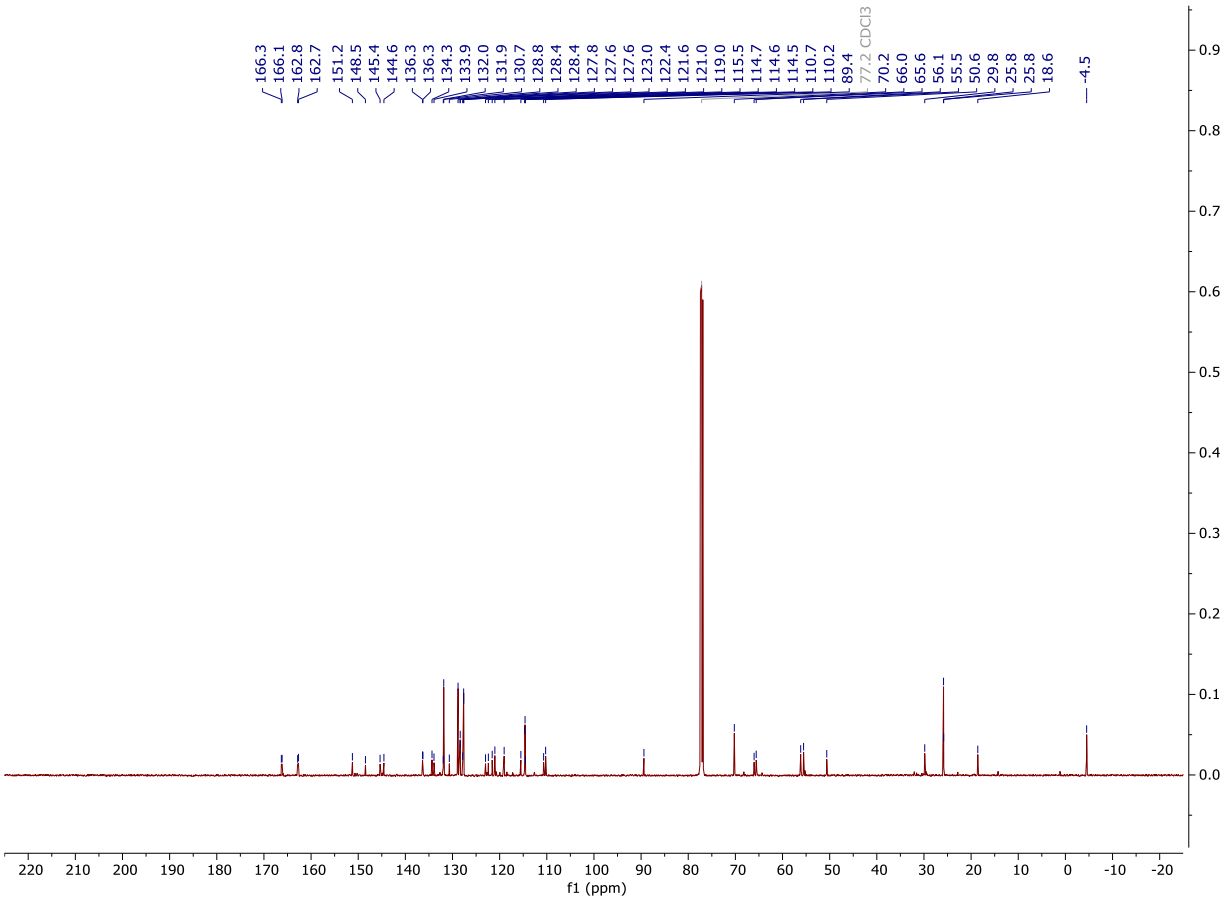




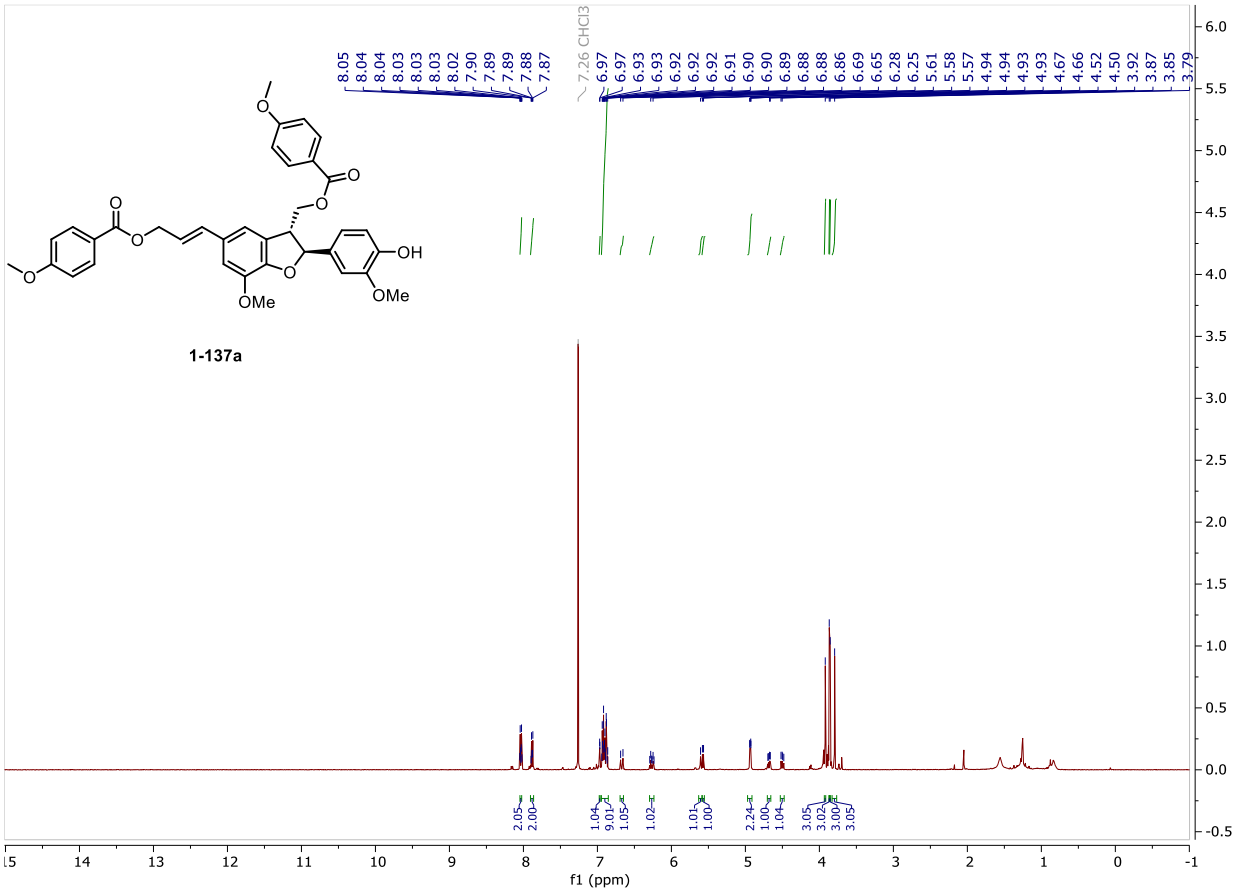
NMR and HPLC

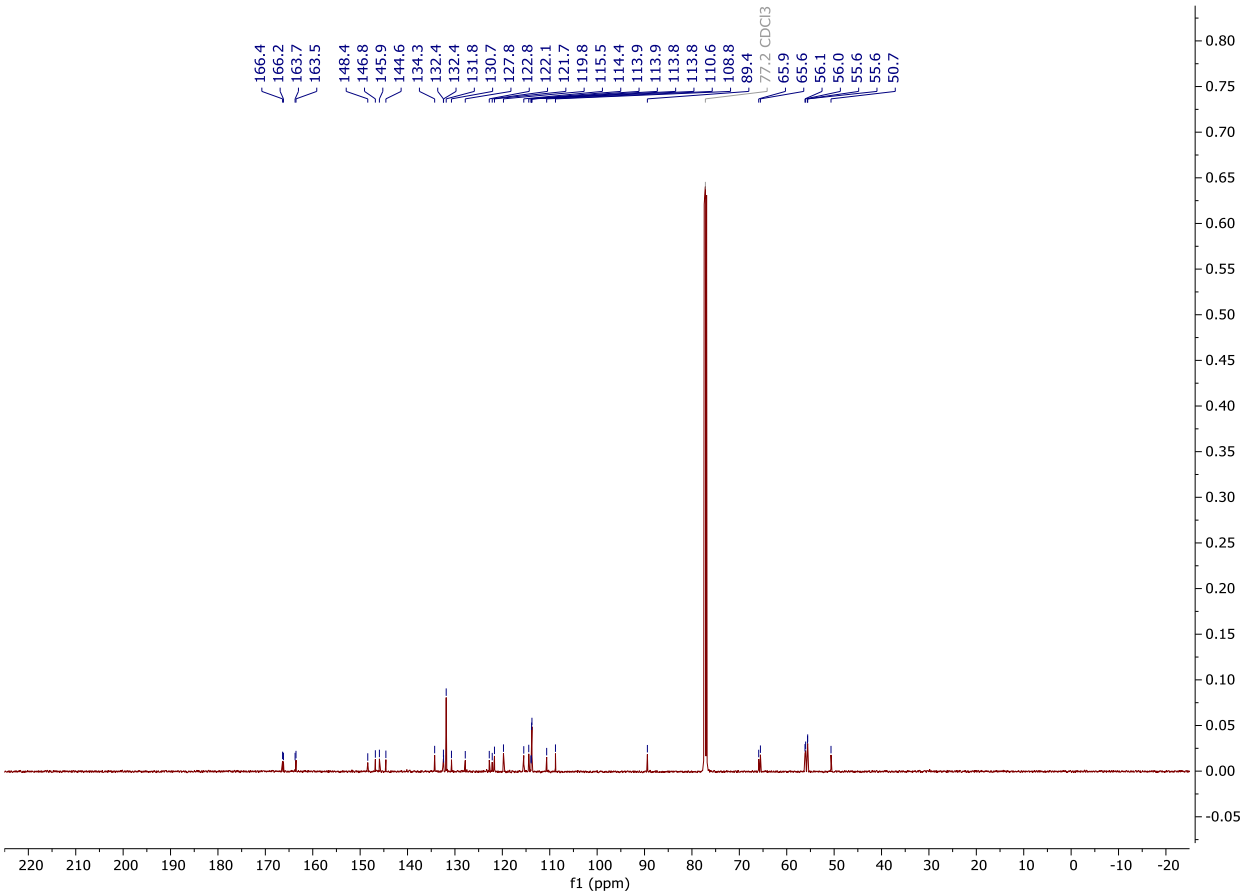
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-135c**



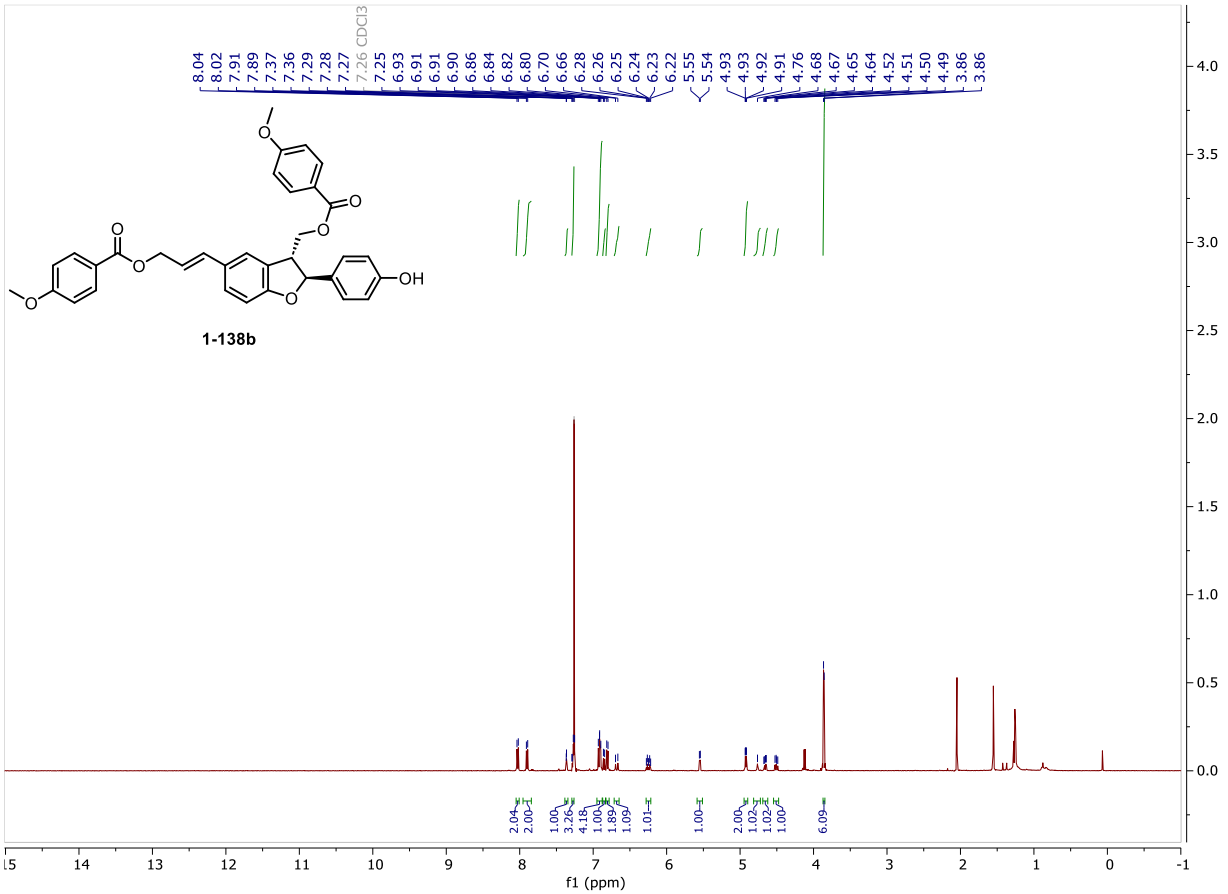


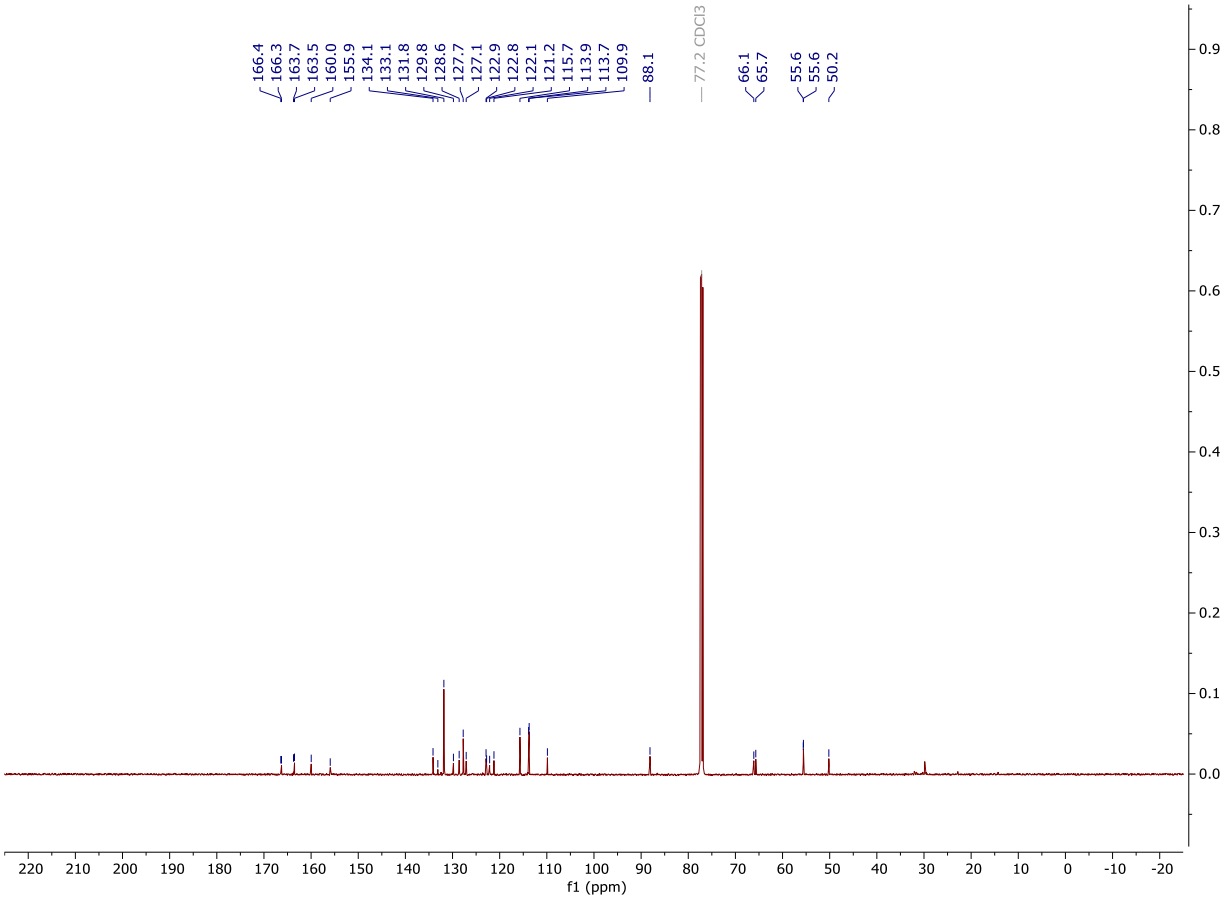
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-137a**



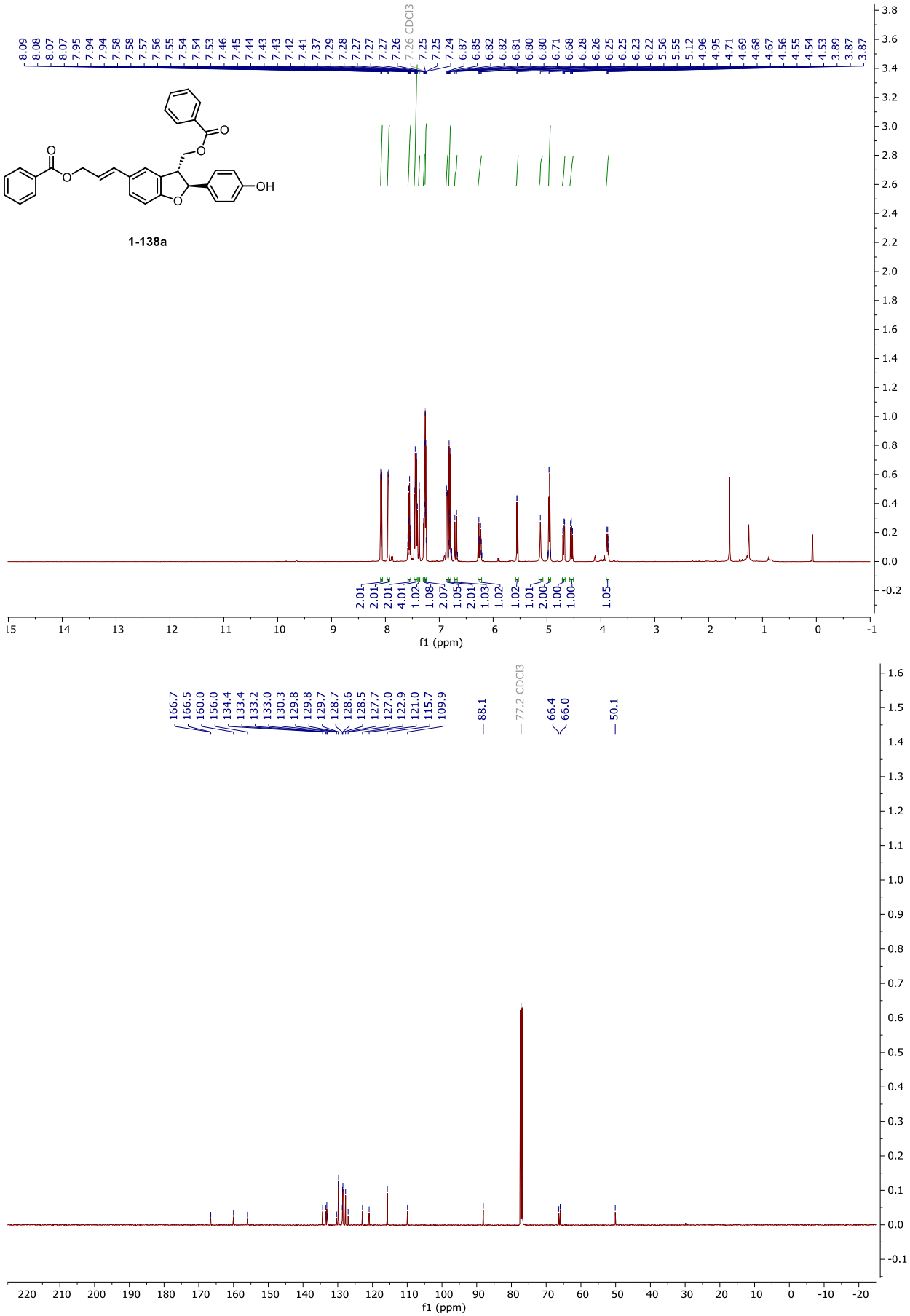


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-138b**

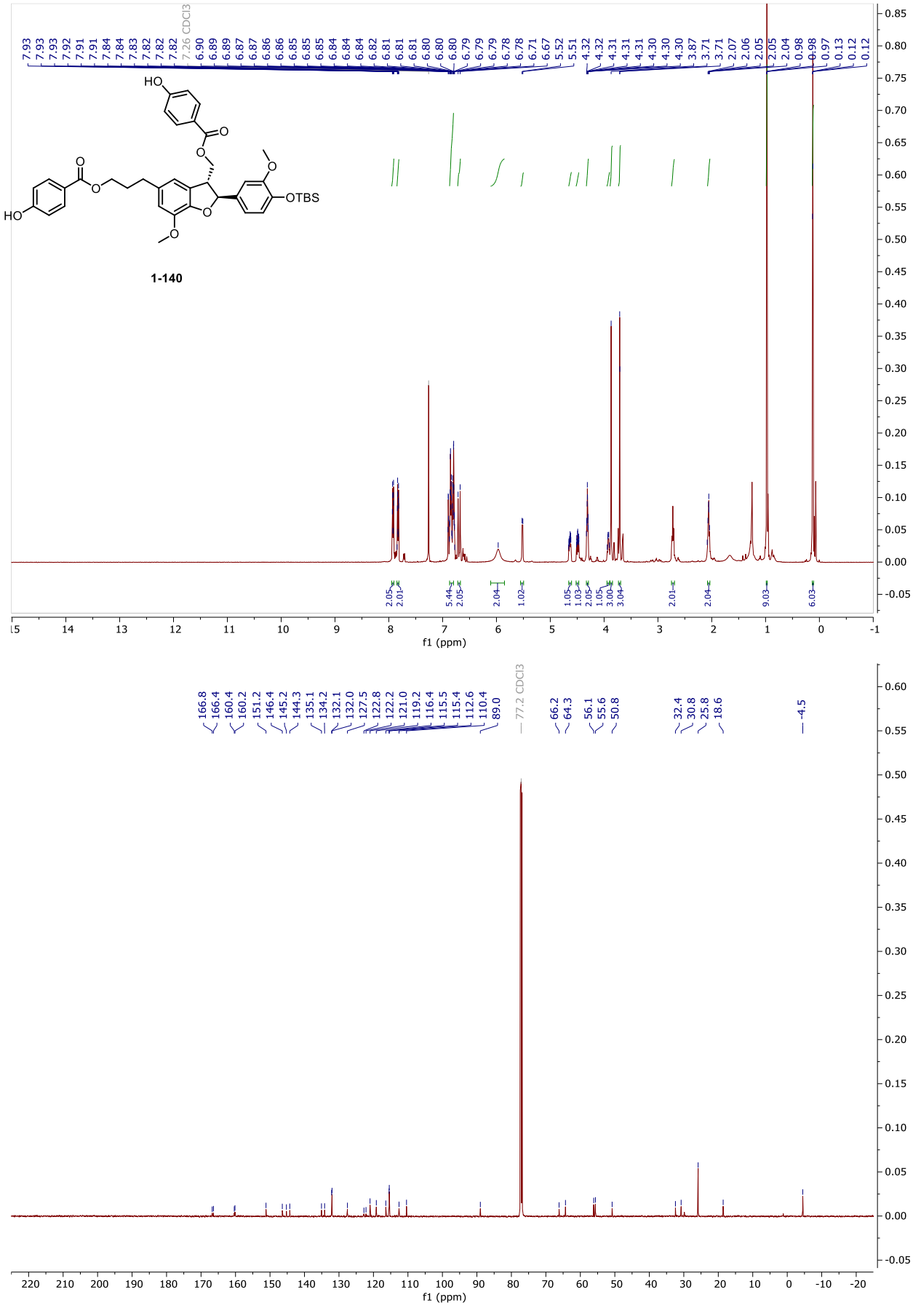




Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-138a**

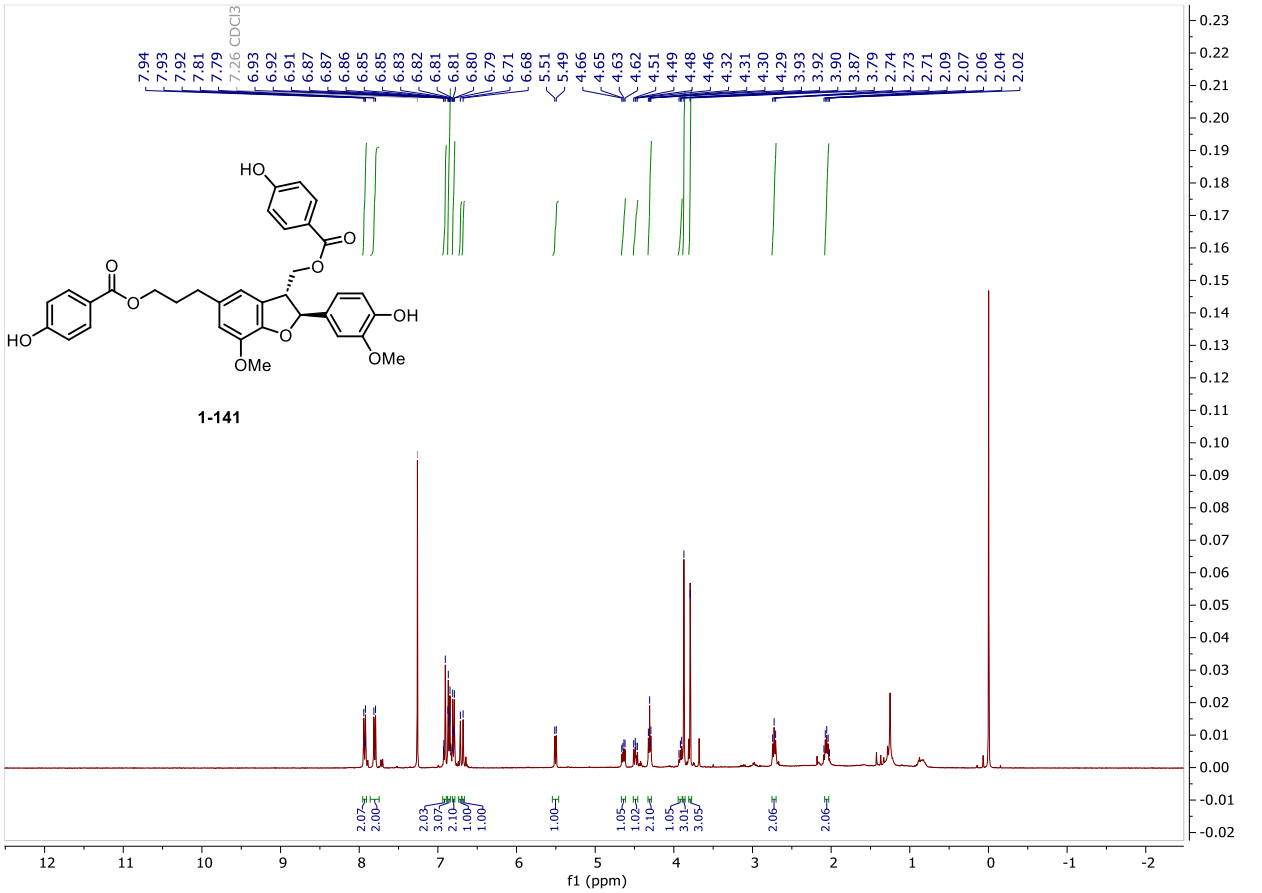


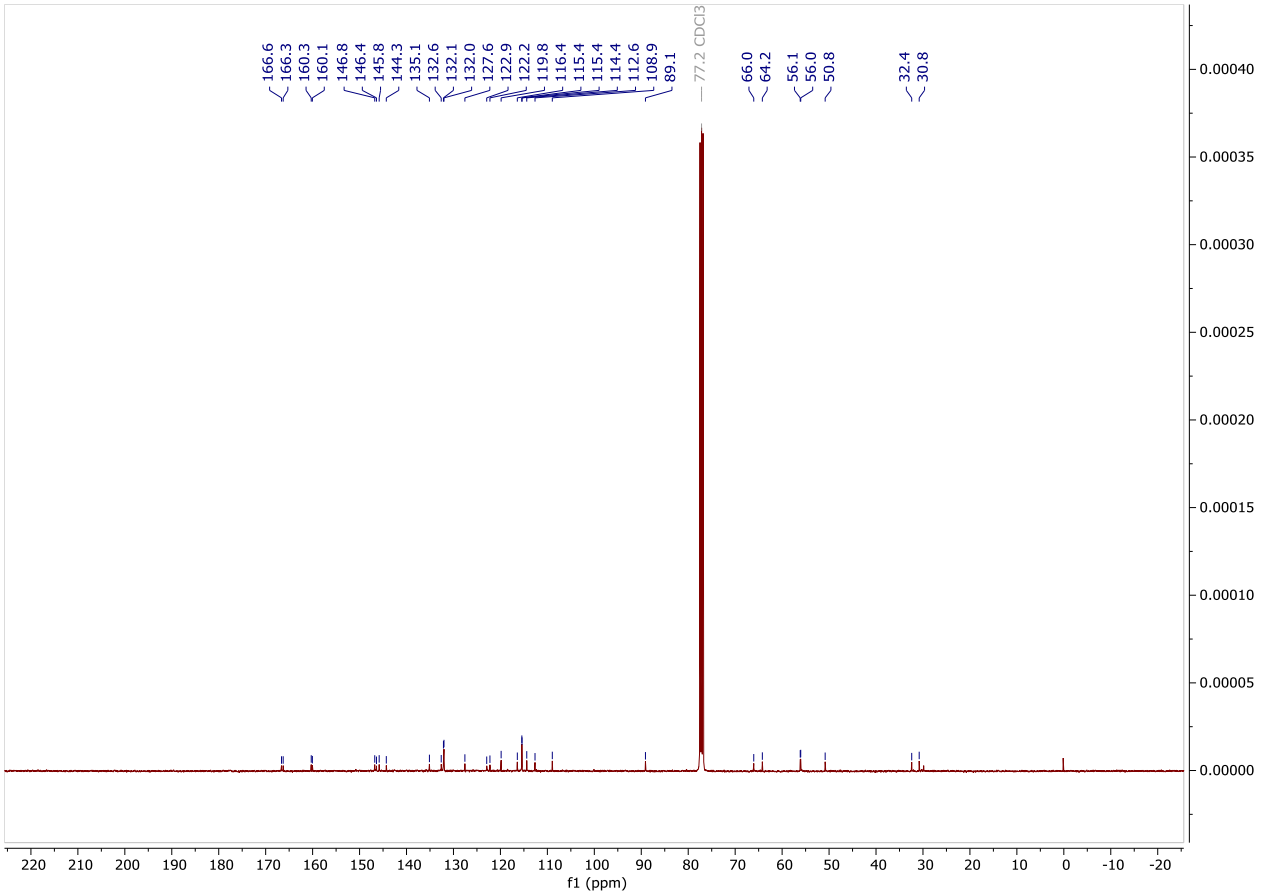
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-140**



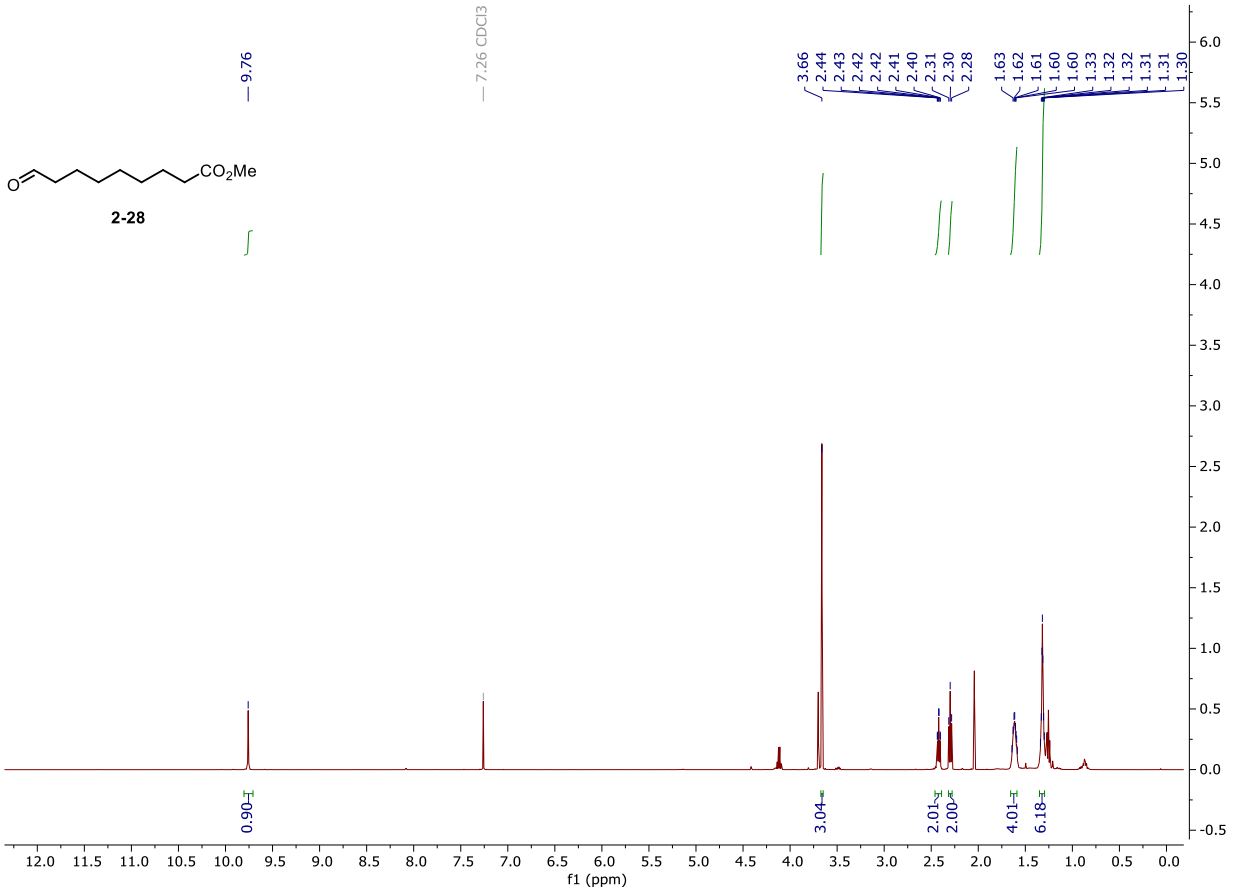
NMR and HPLC

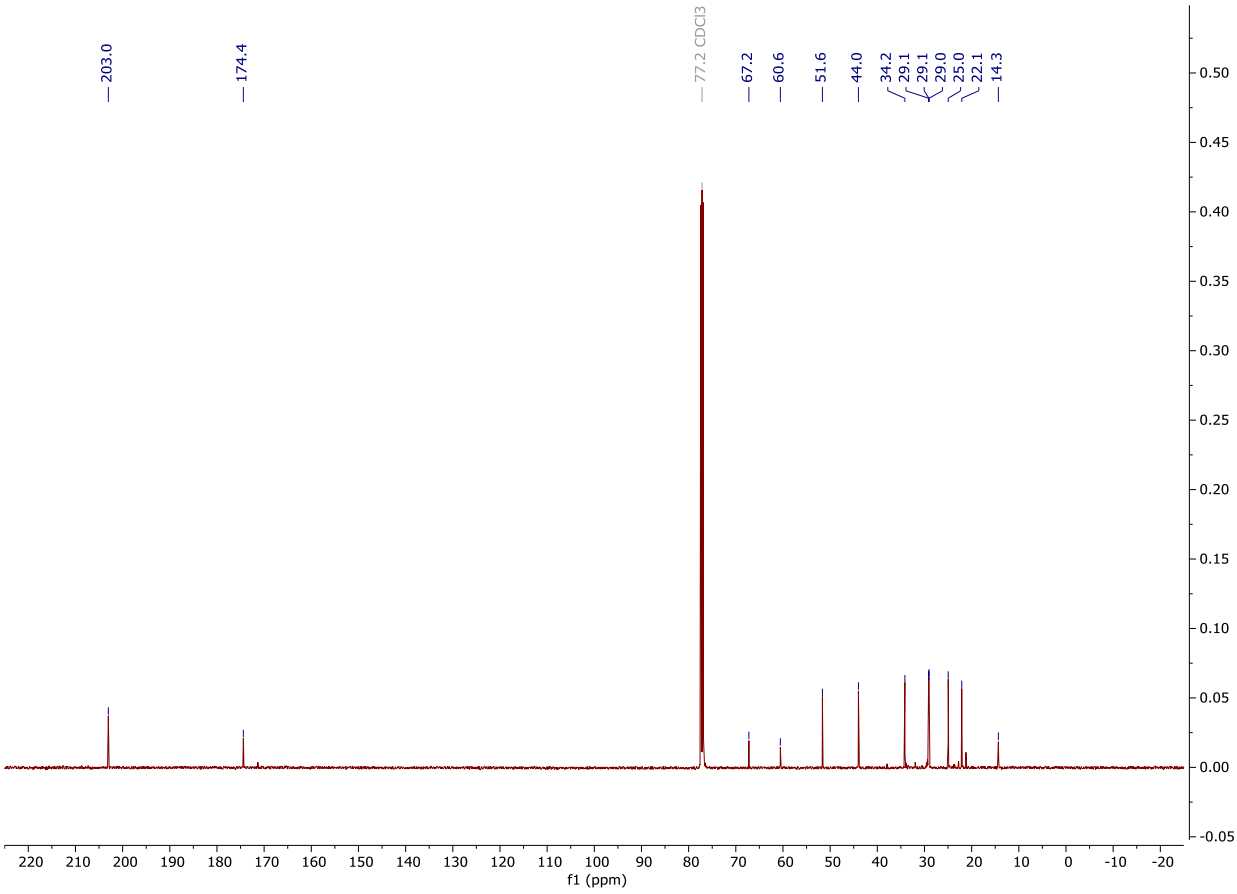
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **1-141**



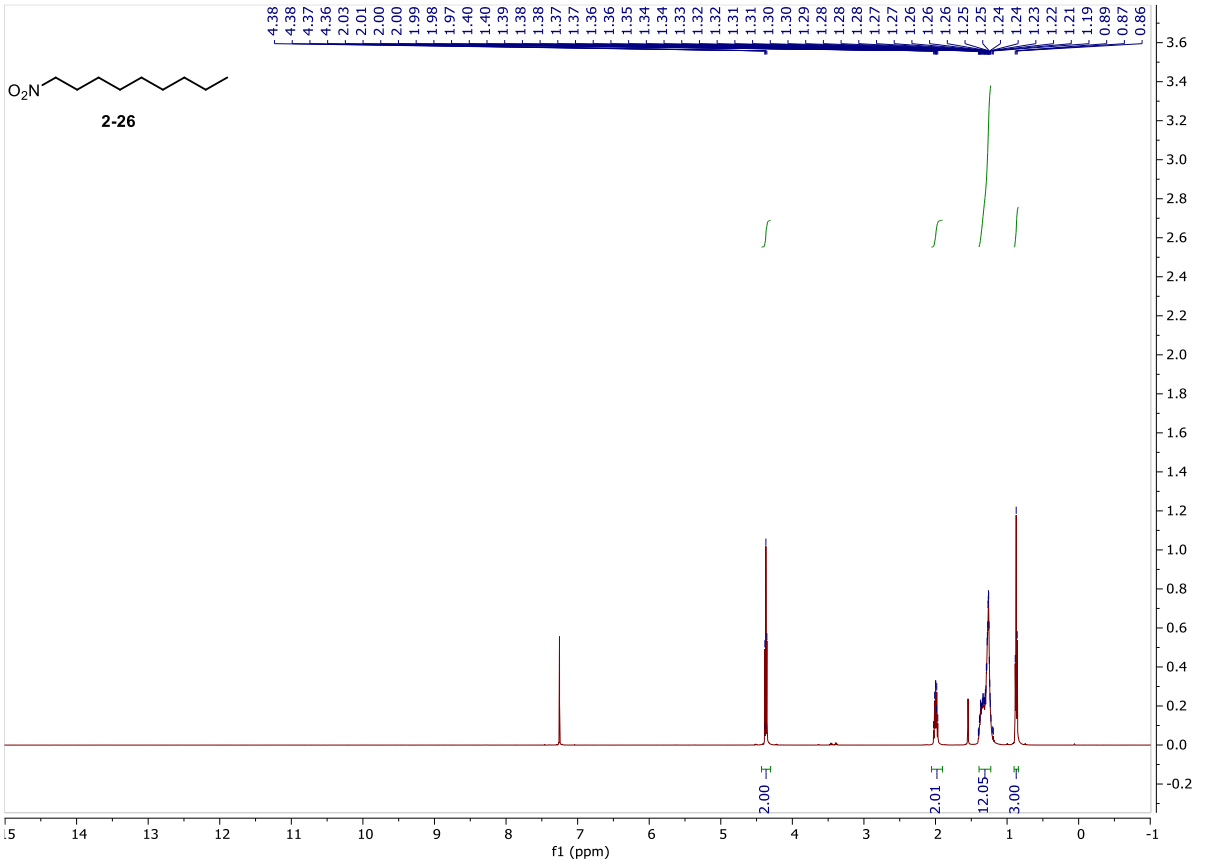


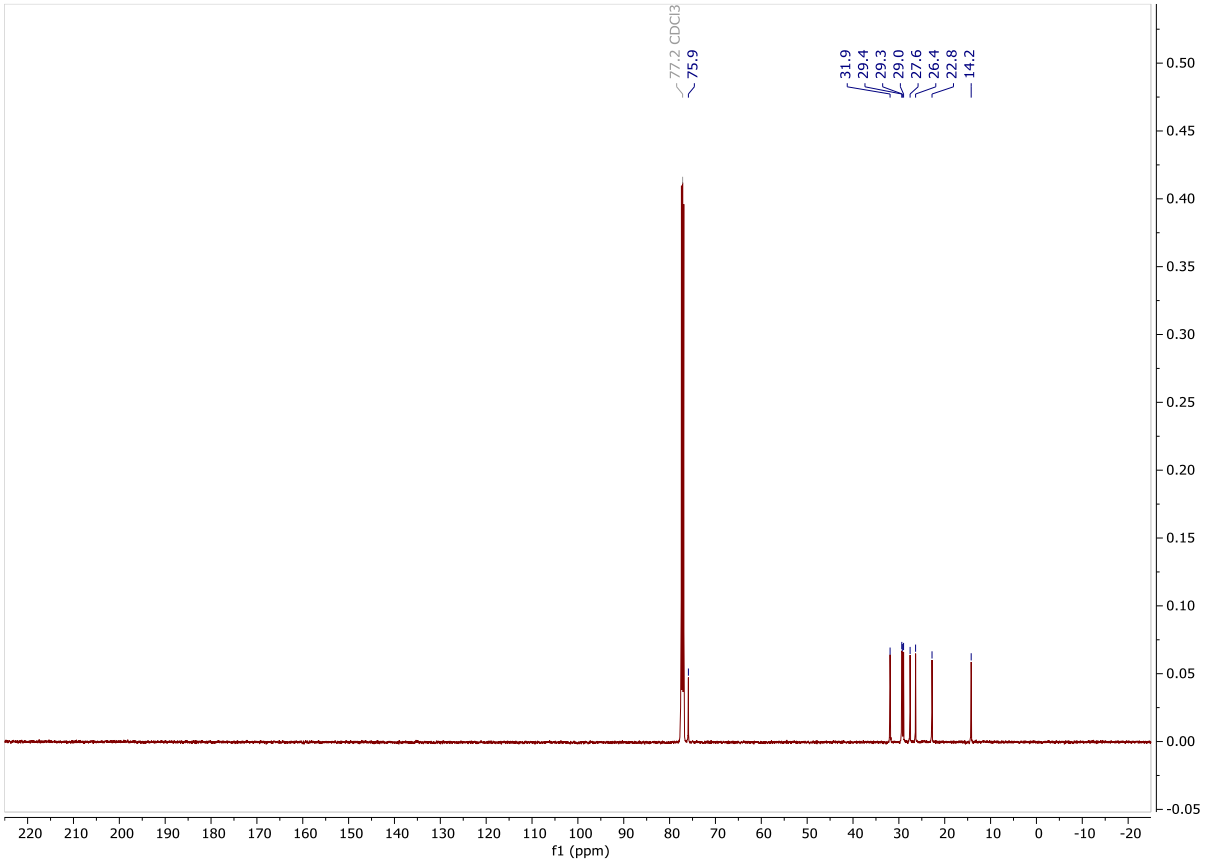
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-28**



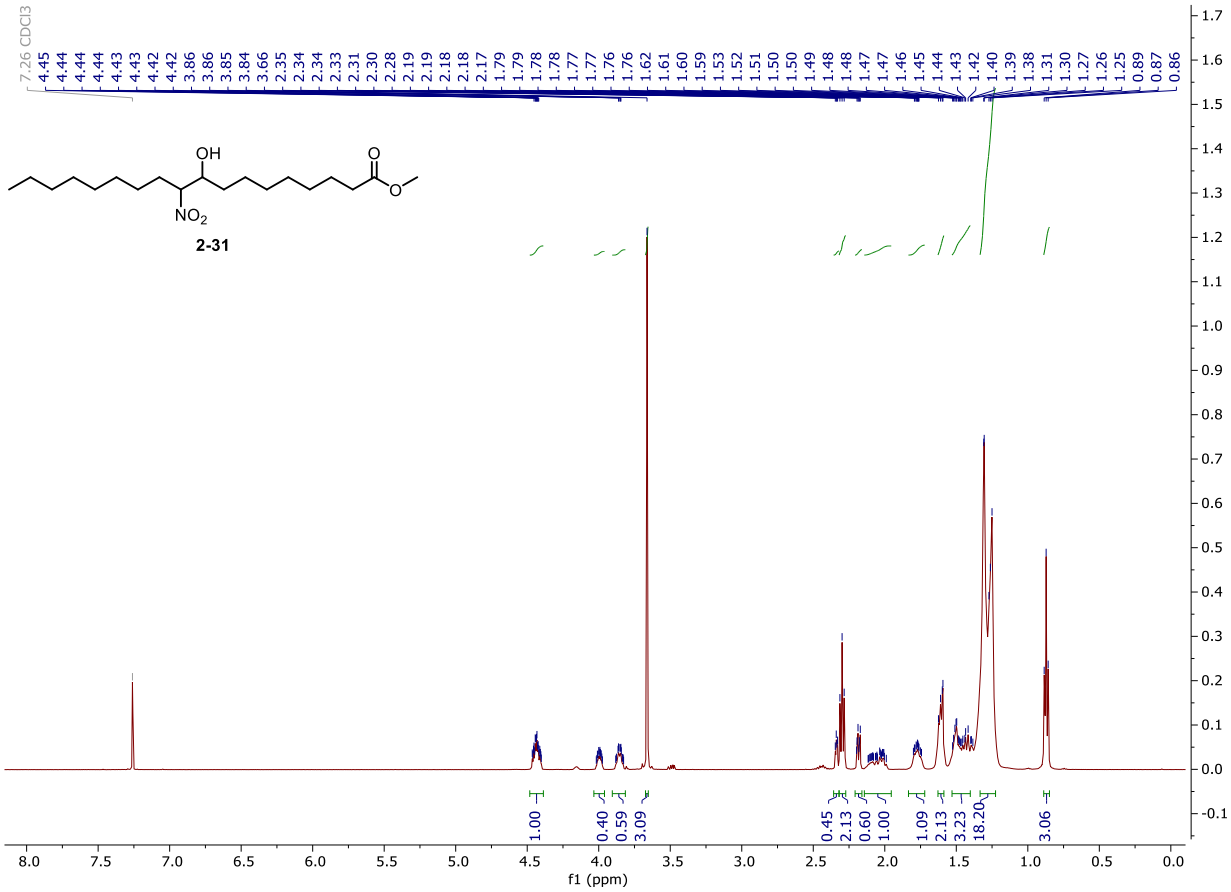


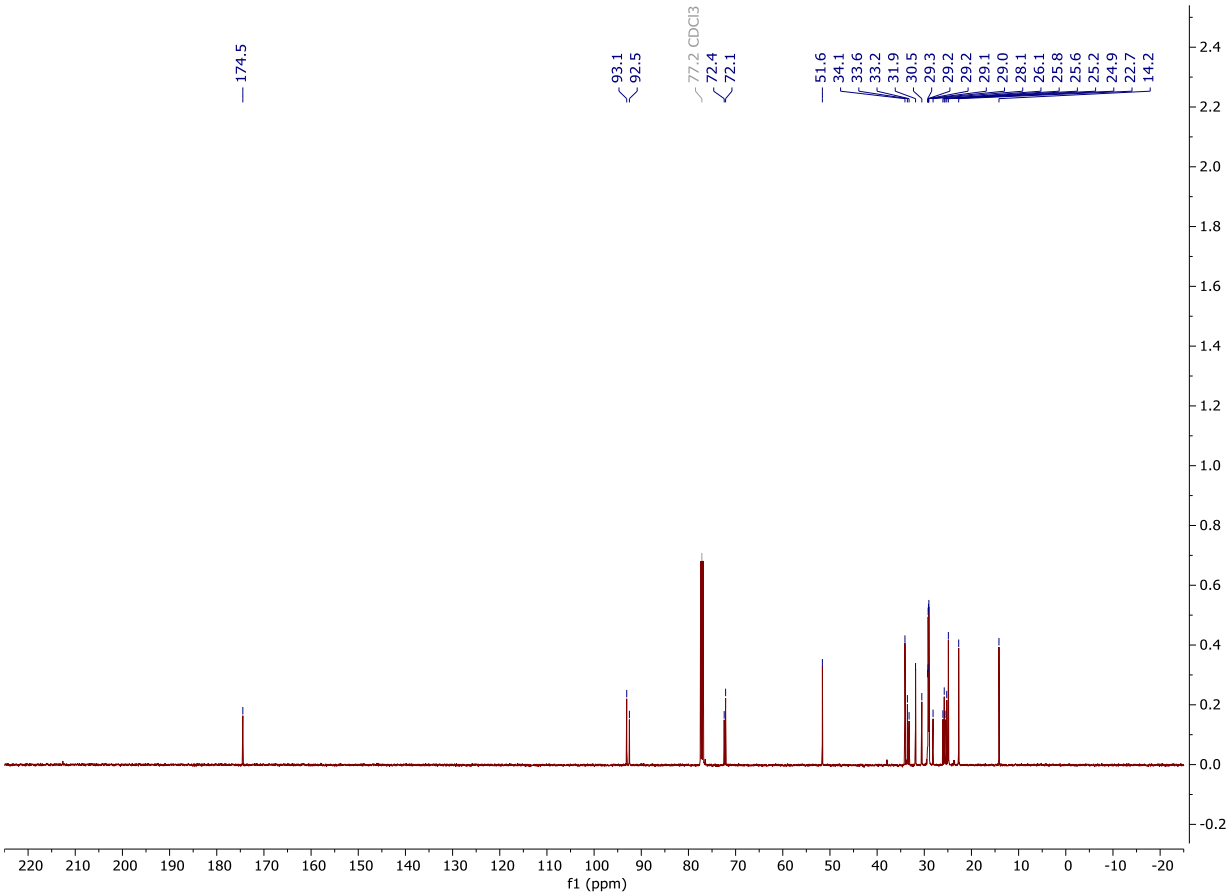
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-26**



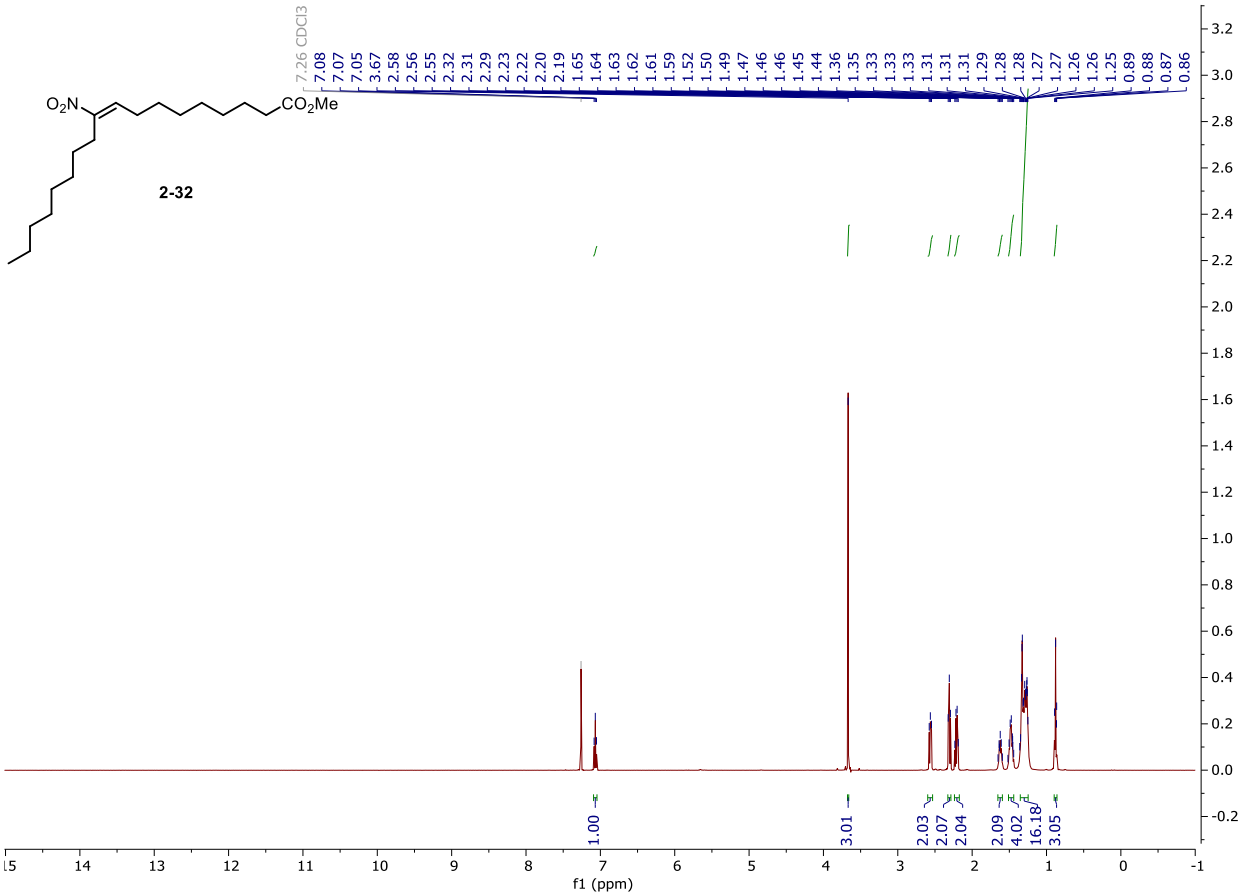


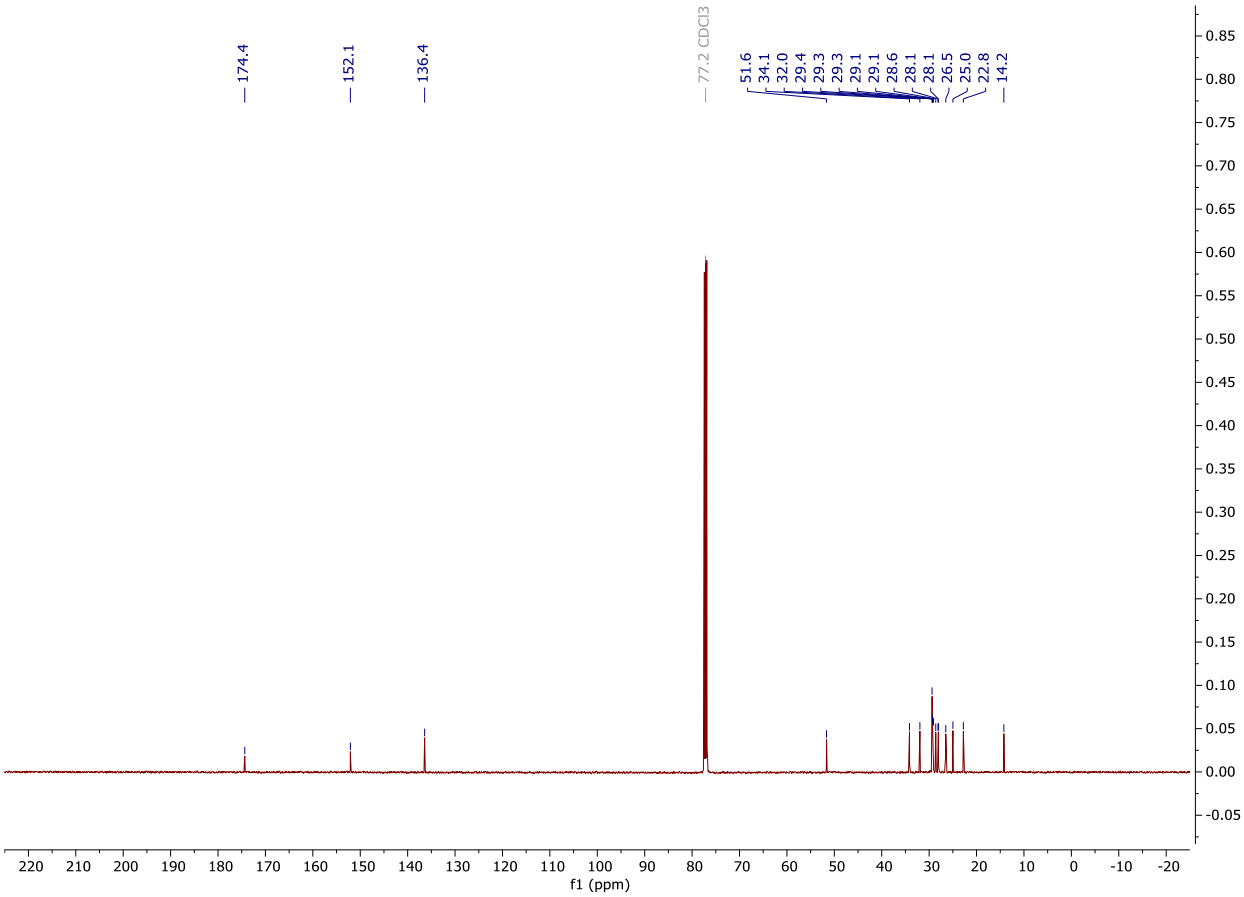
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-31**



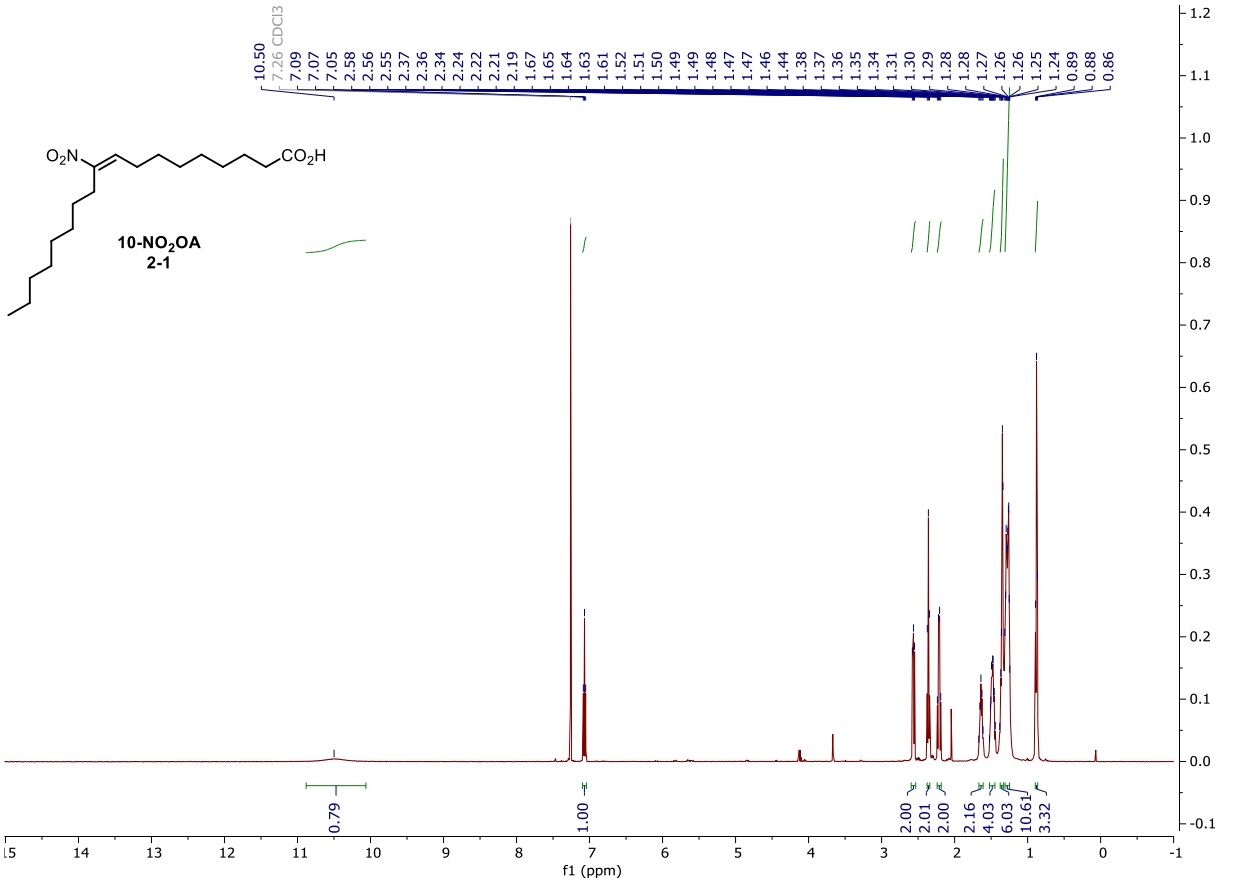


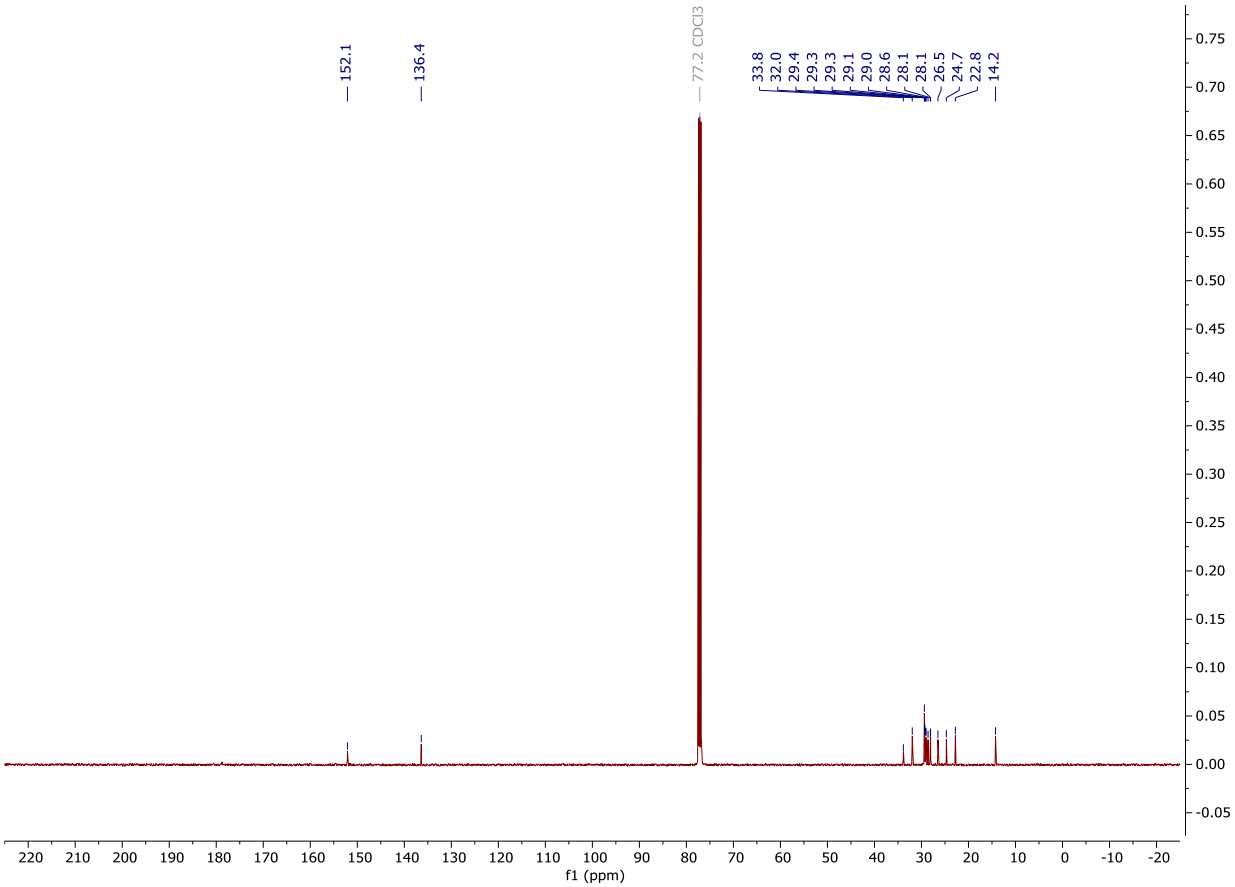
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-32**



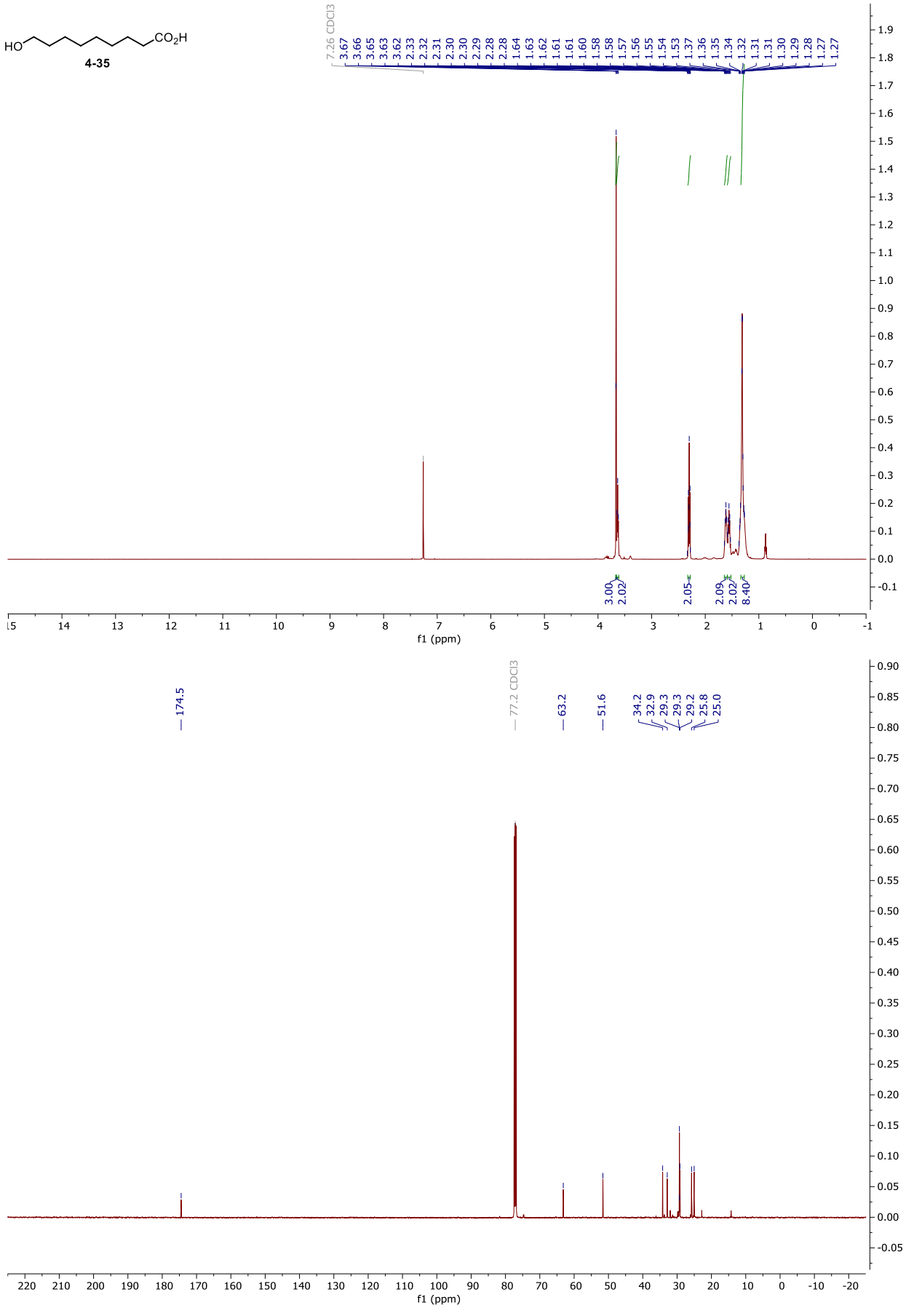


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **10-NO_2OA, 2-1**

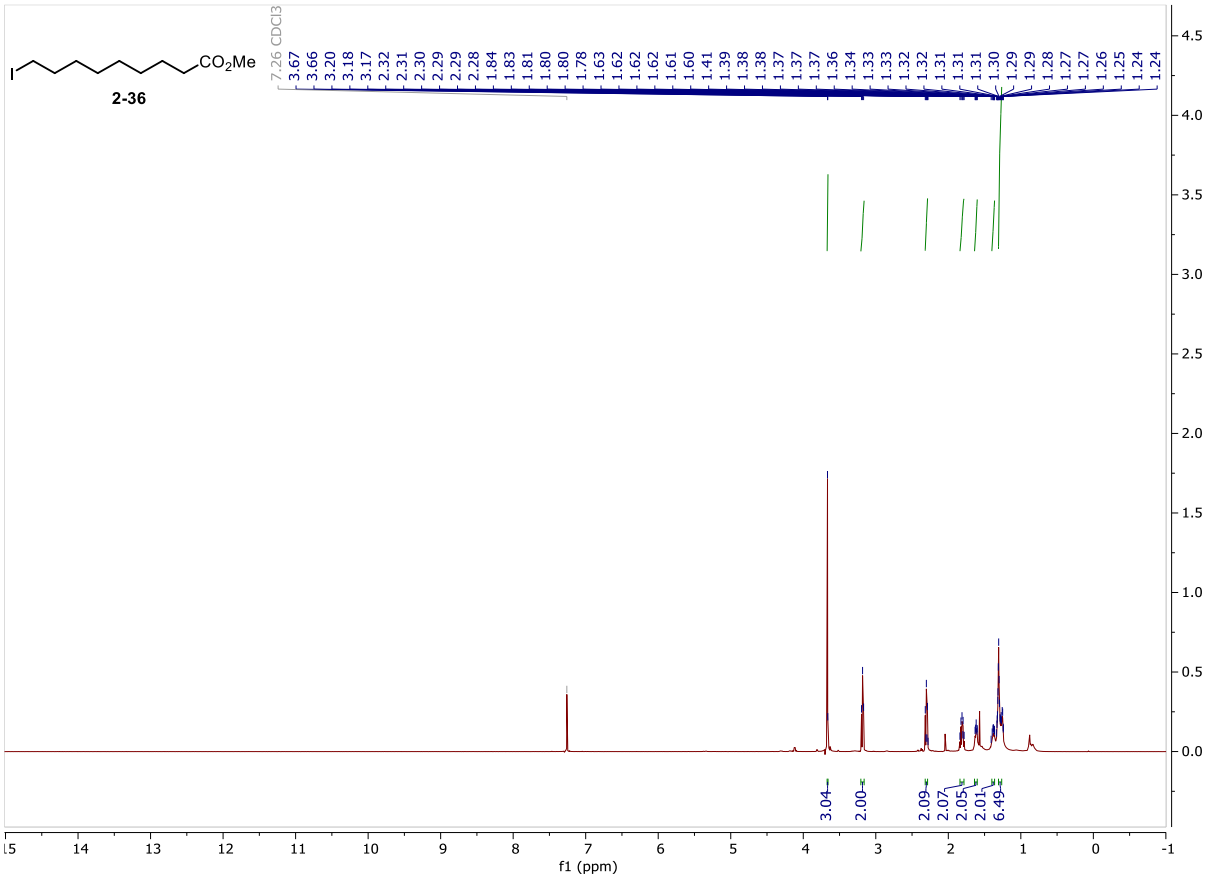


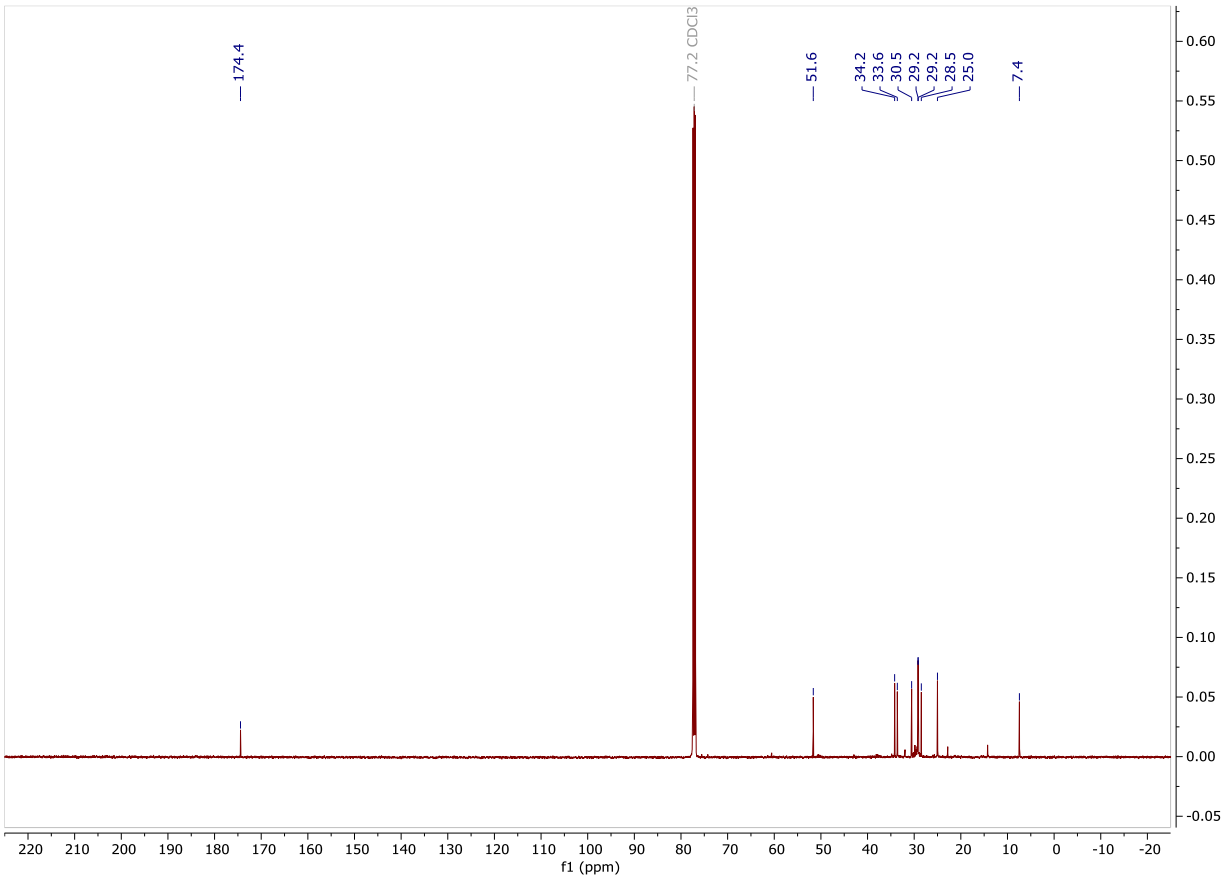


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-35**

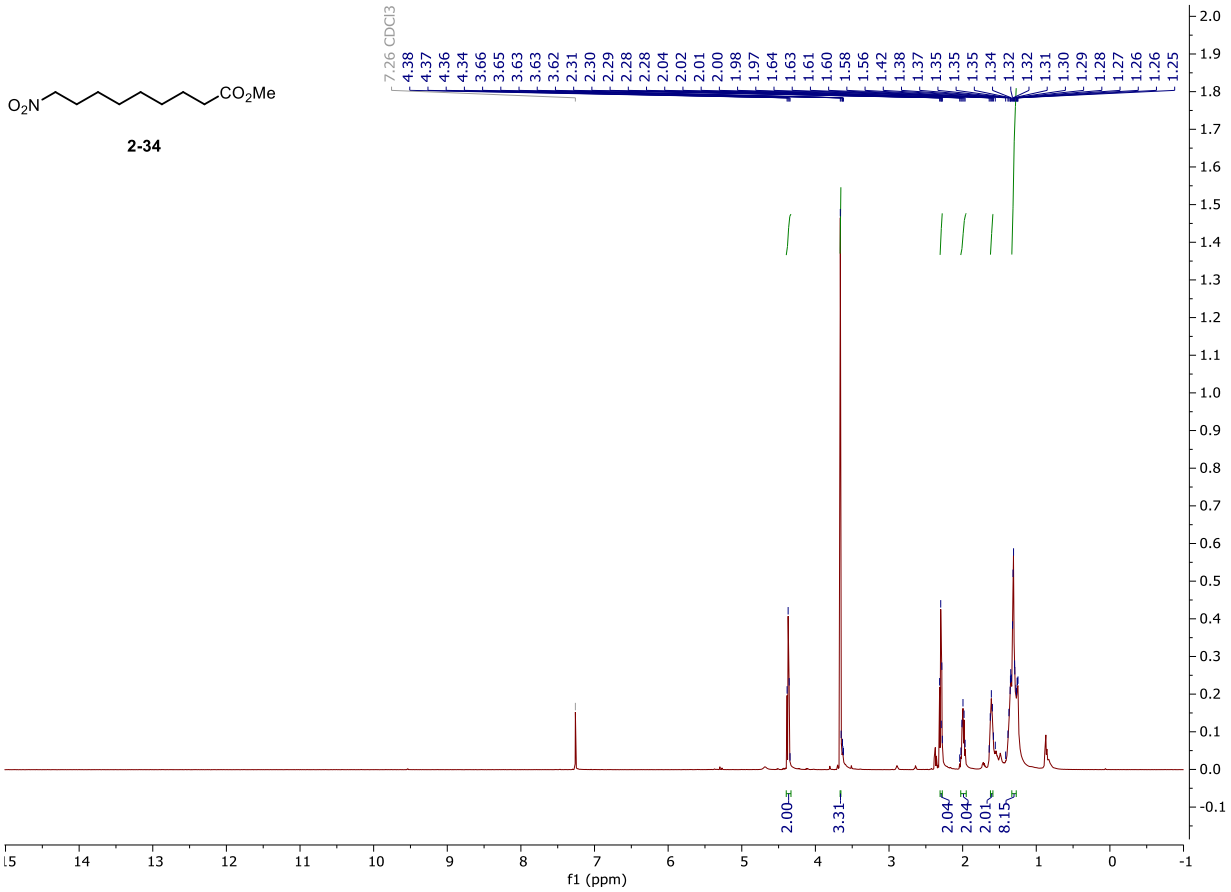


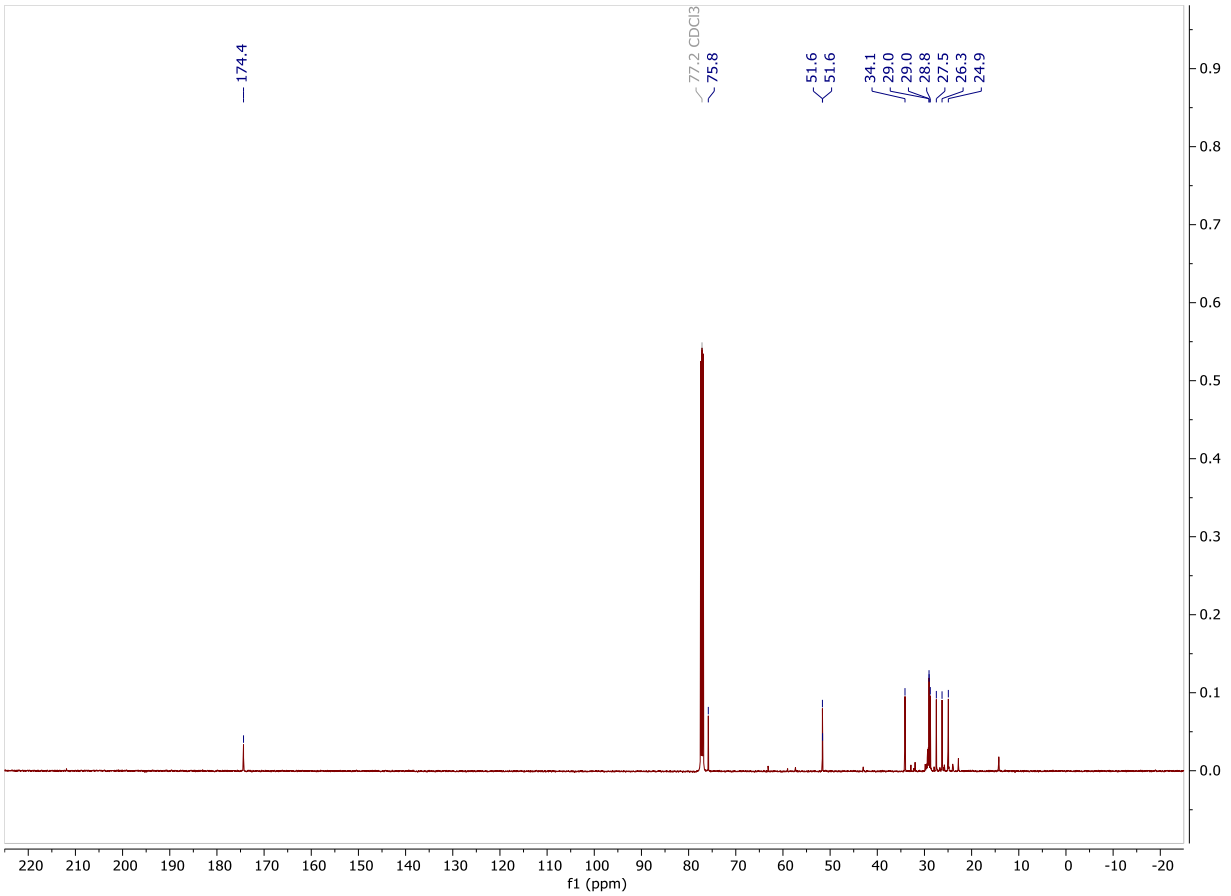
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-36**



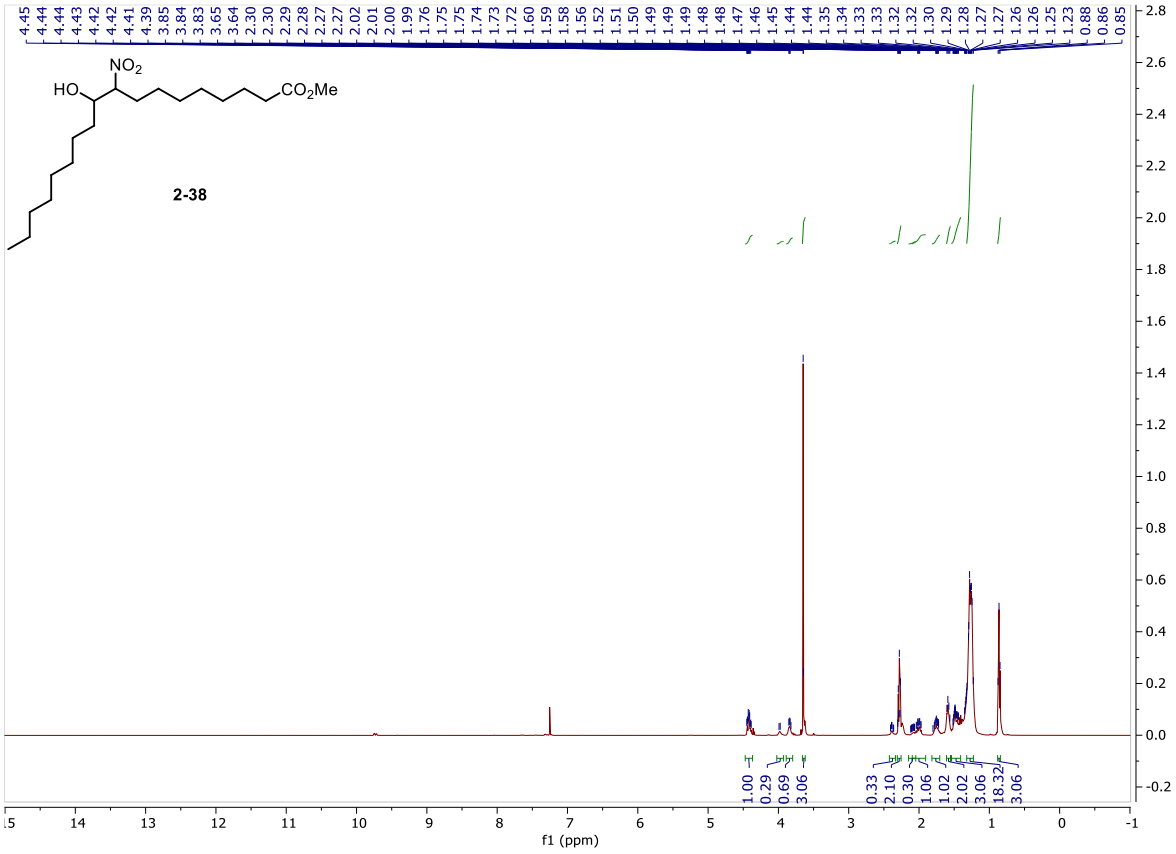


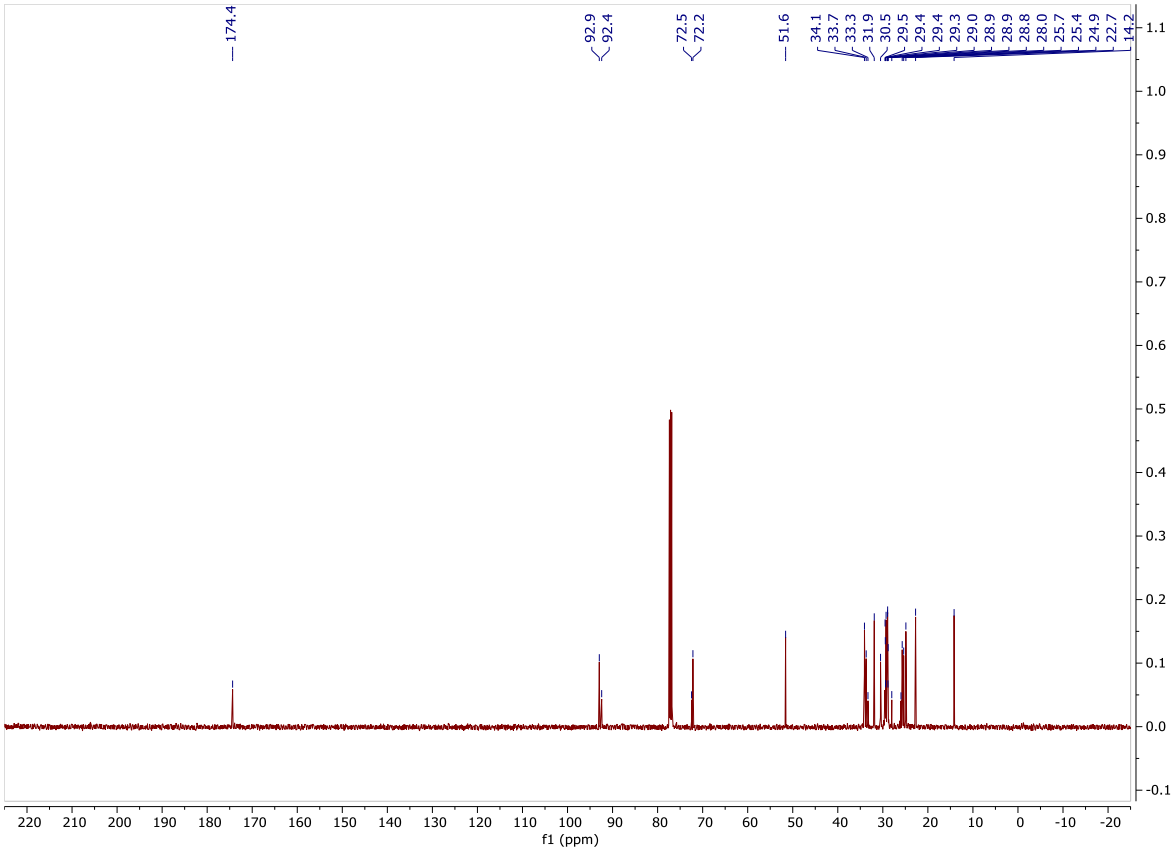
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-34**



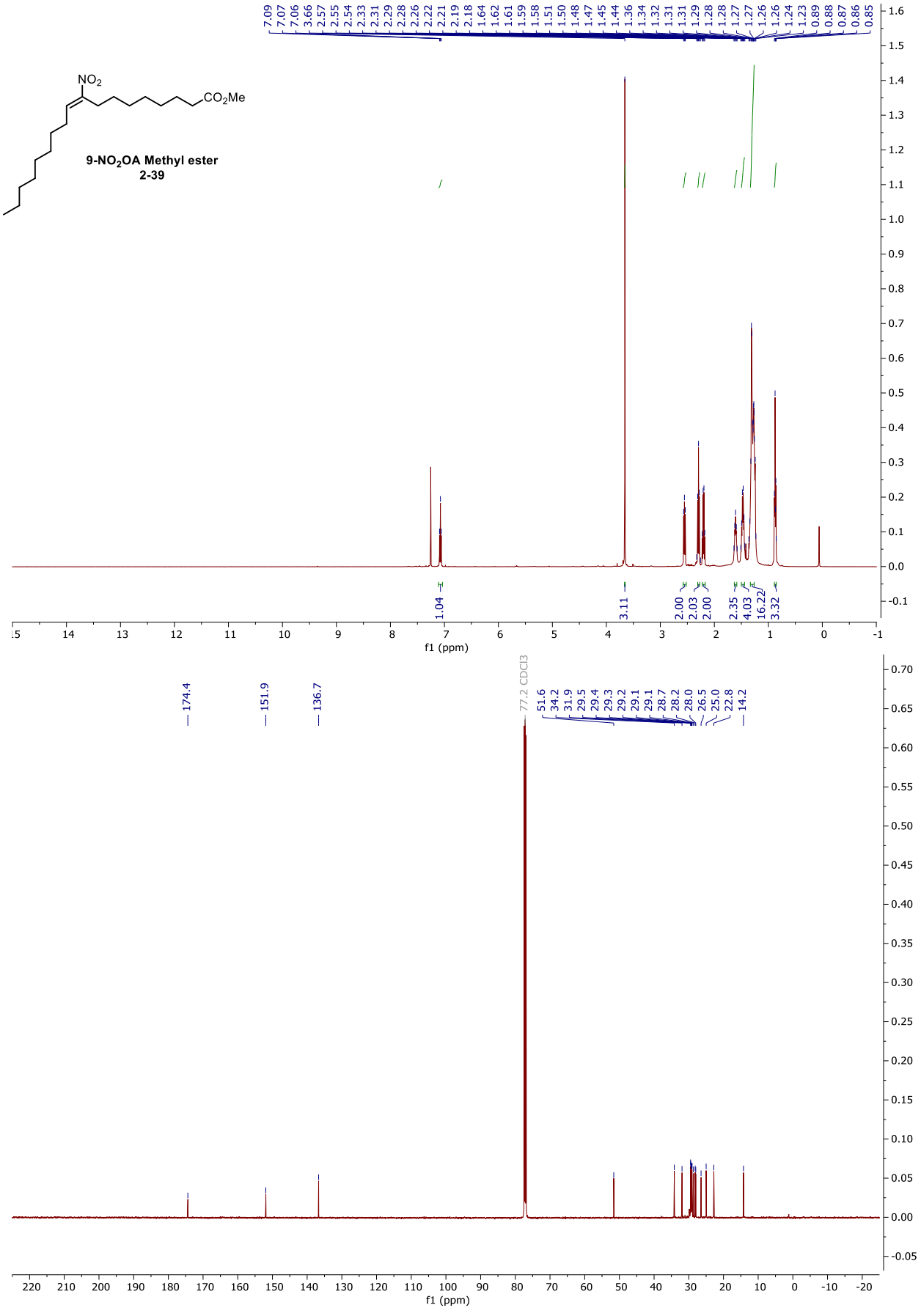


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-38**

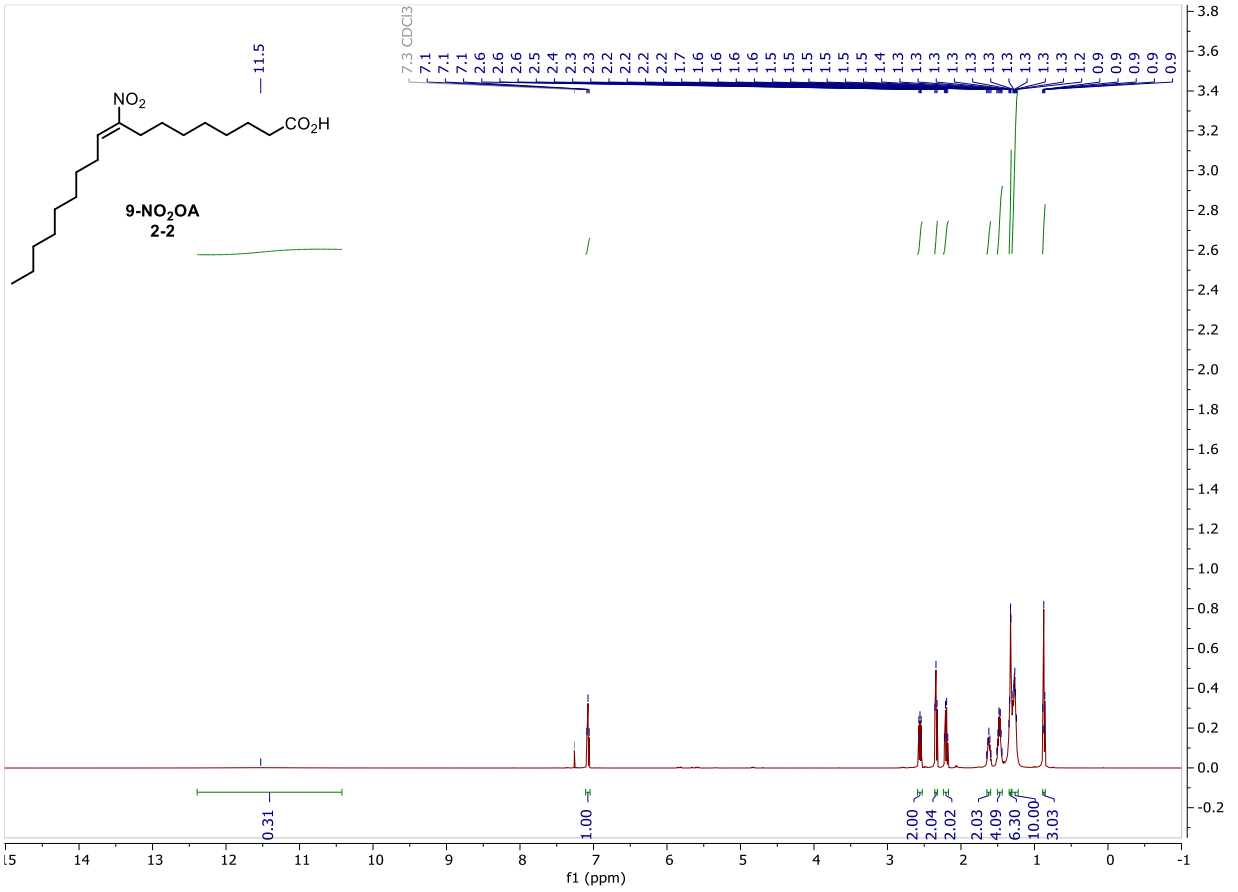


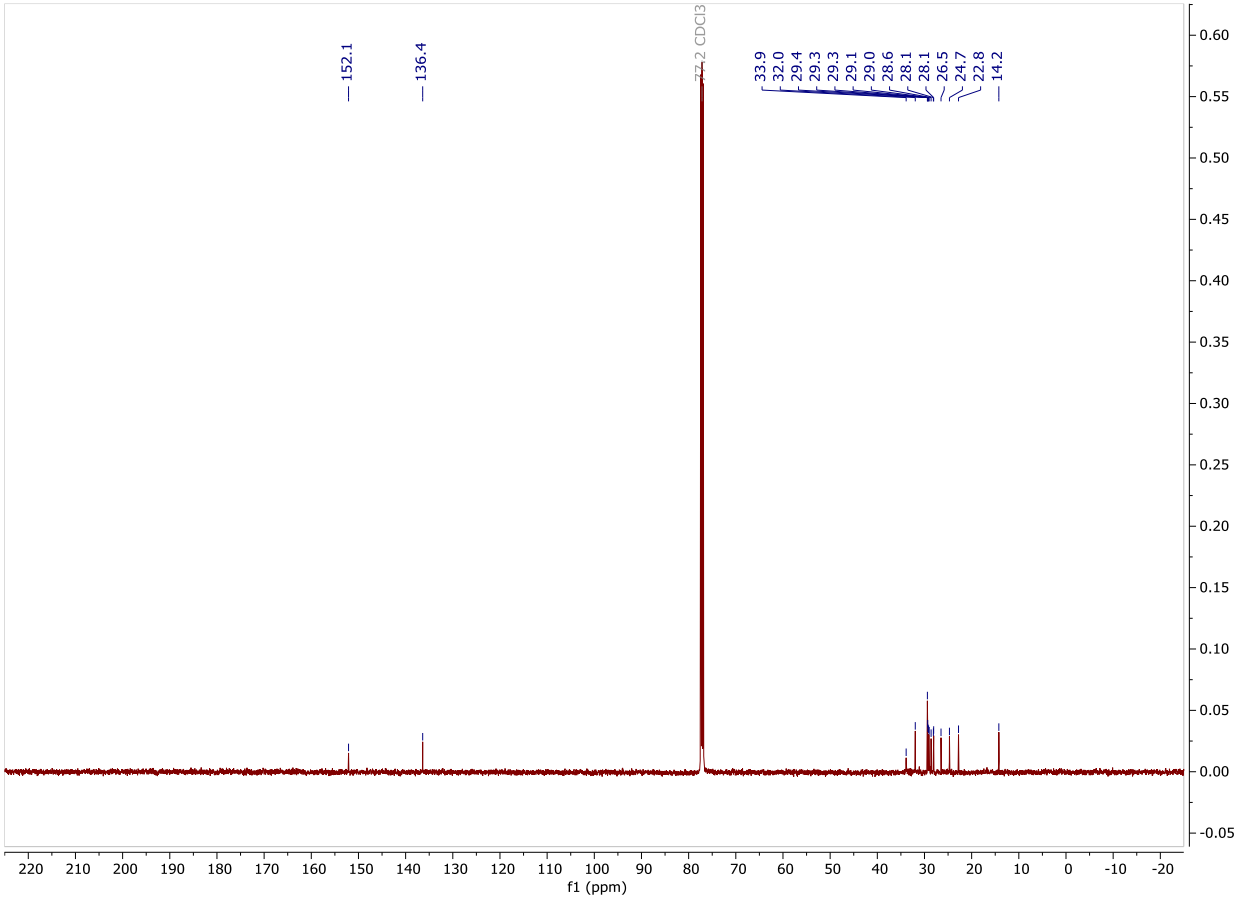


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-39**

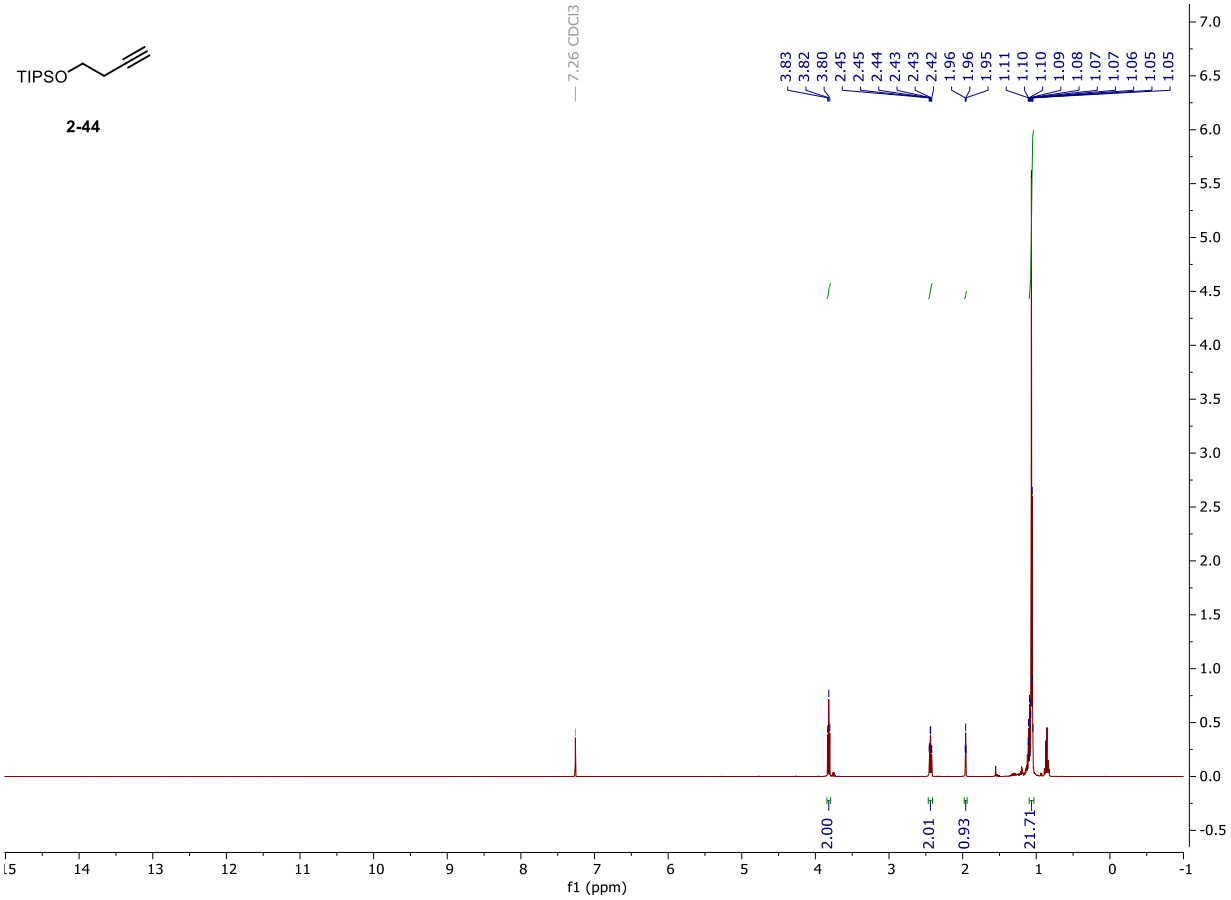


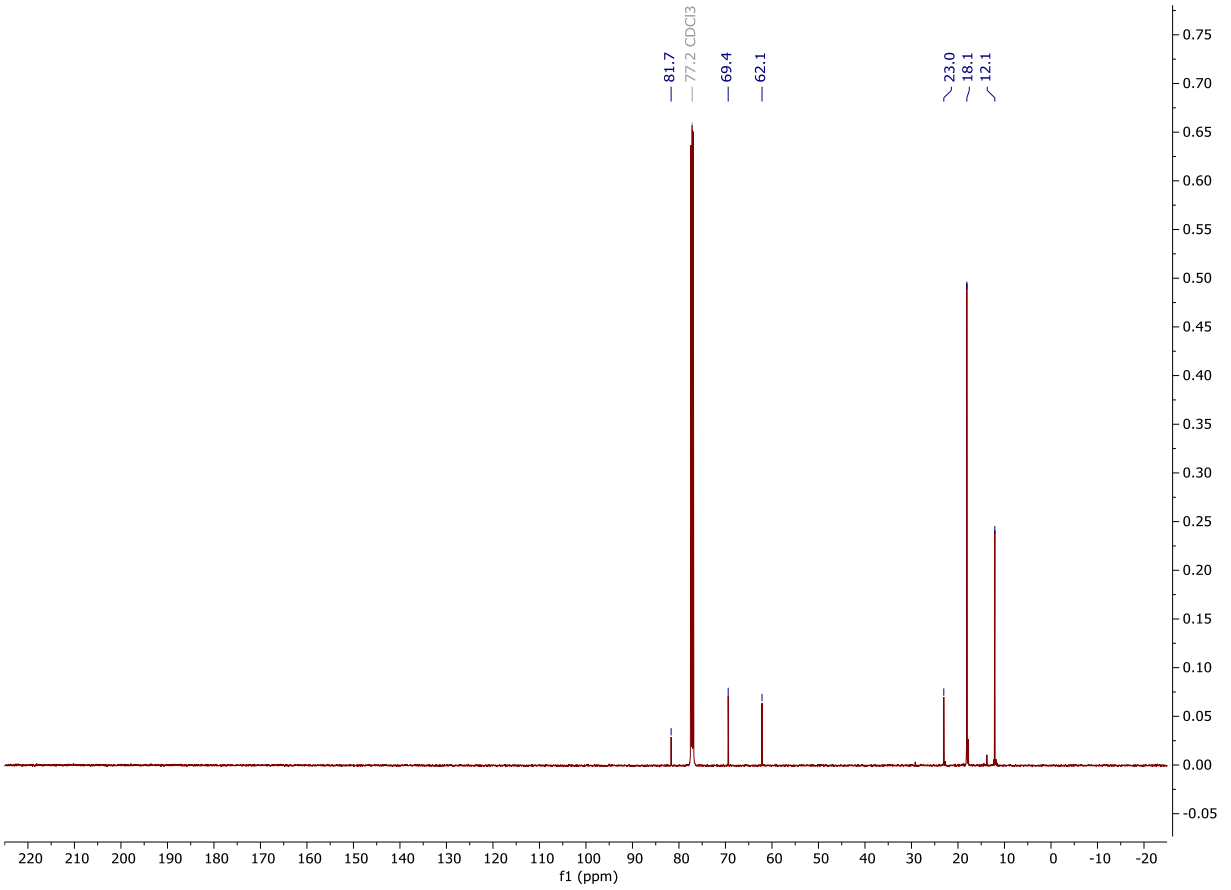
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **9-NO_2OA, 2-2**



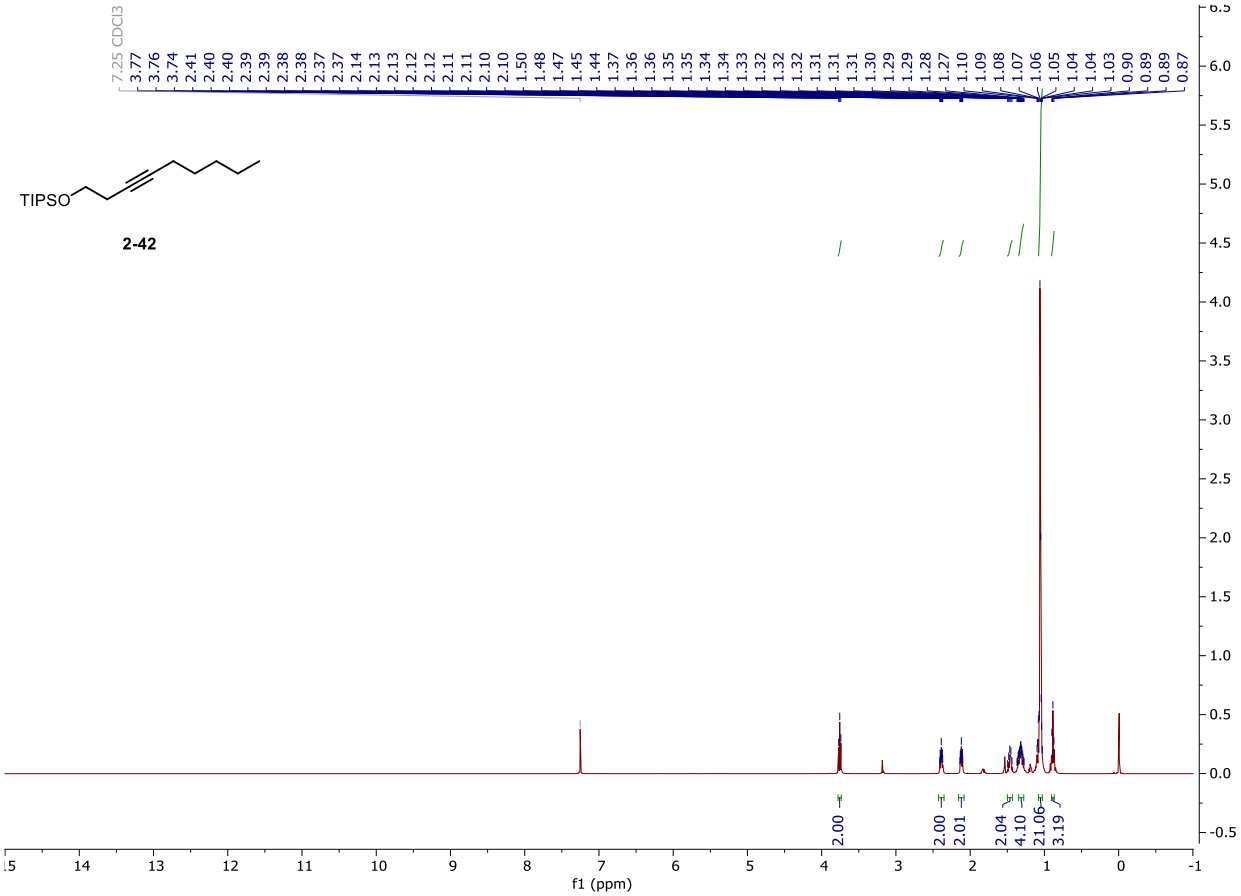


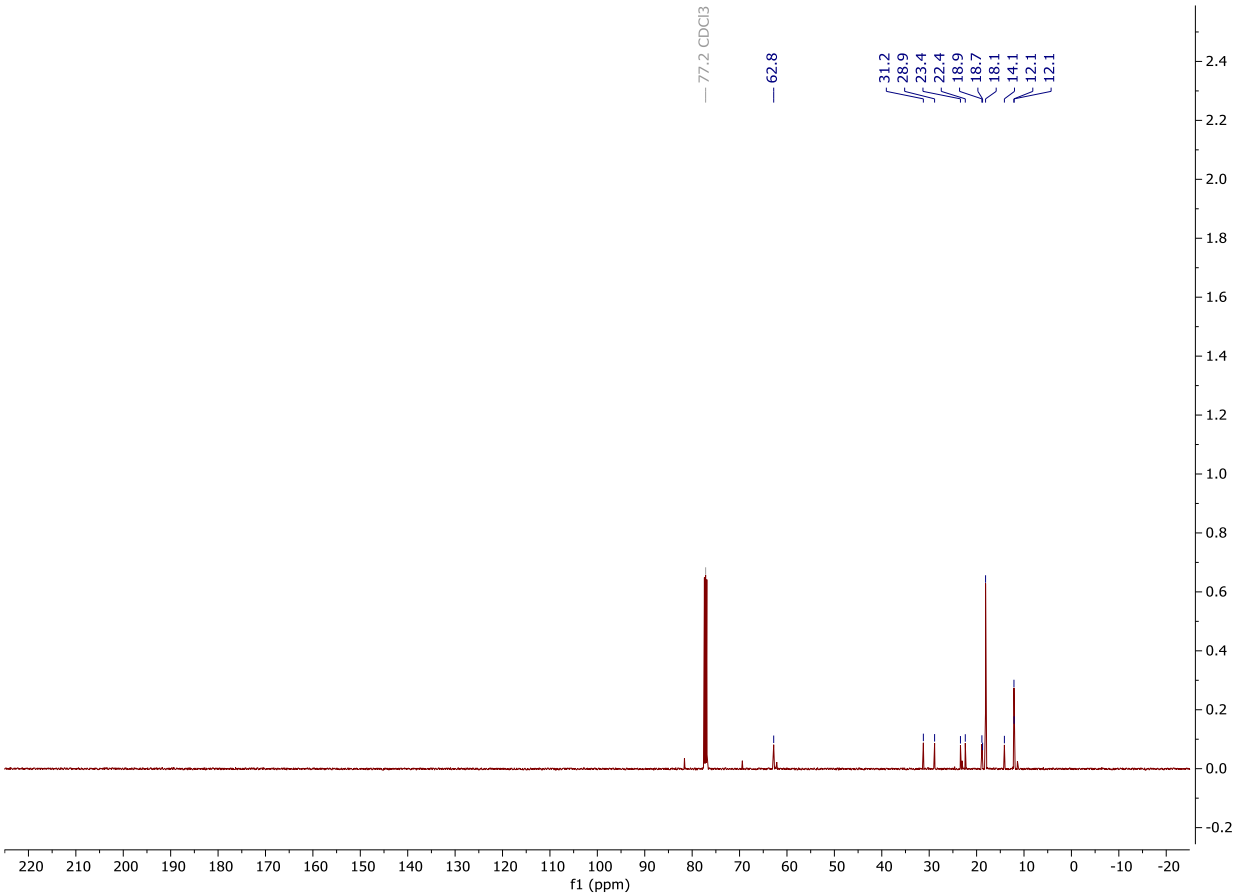
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-44**



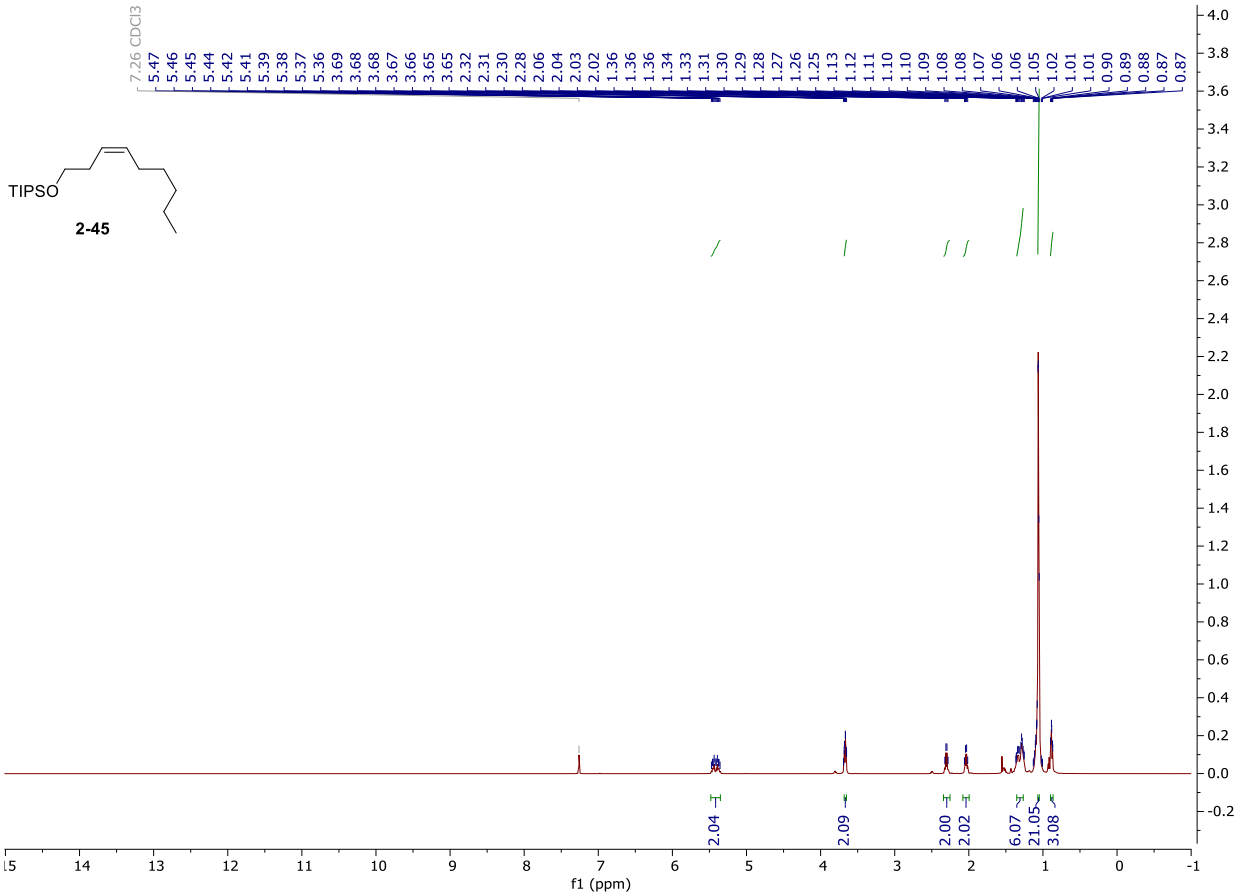


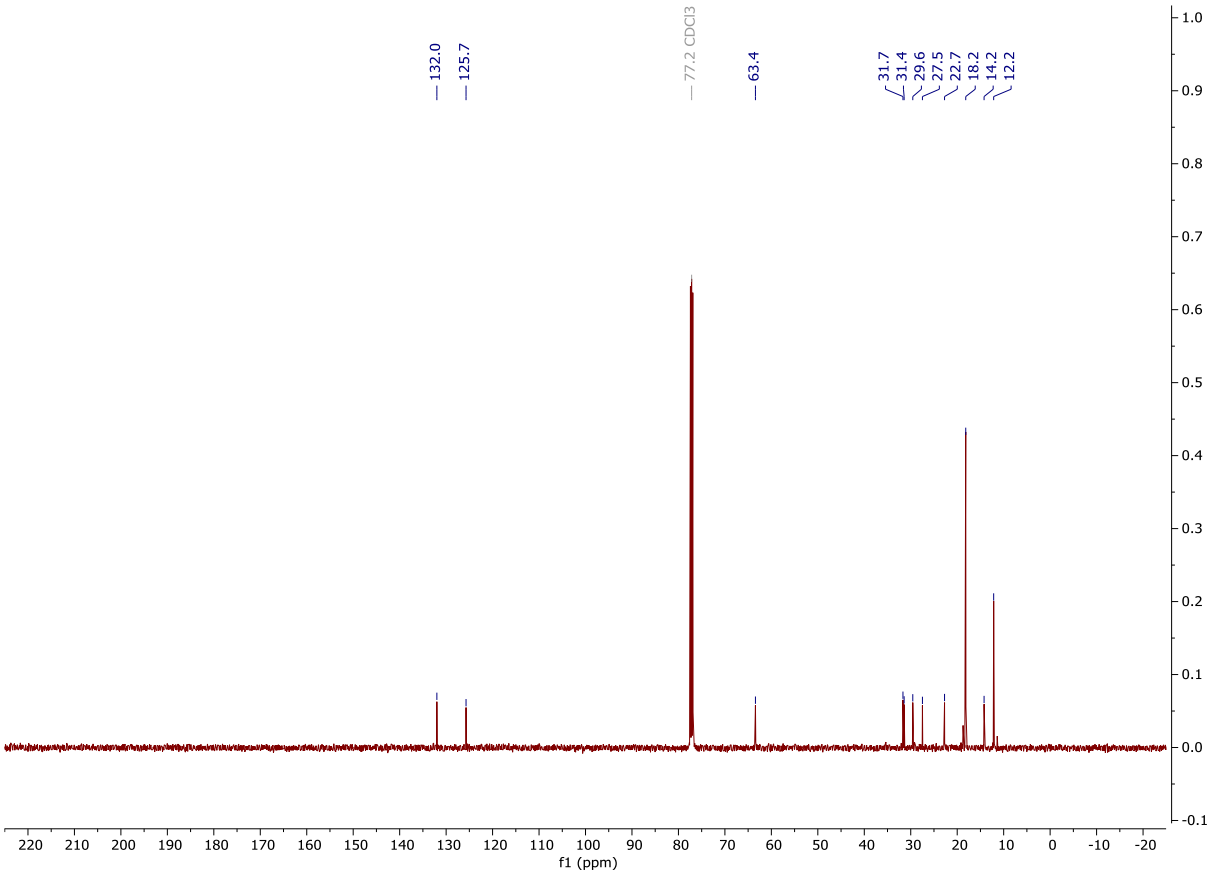
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-42**



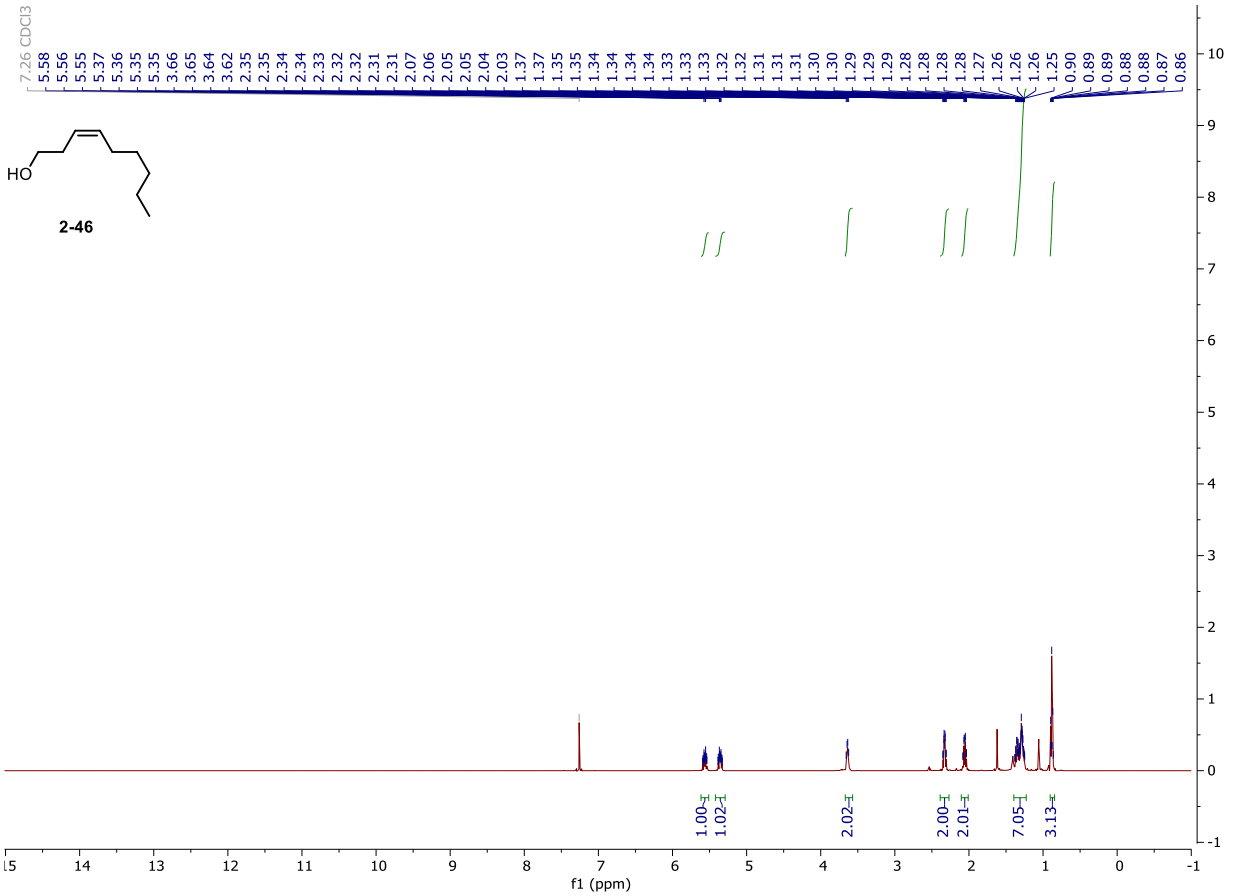


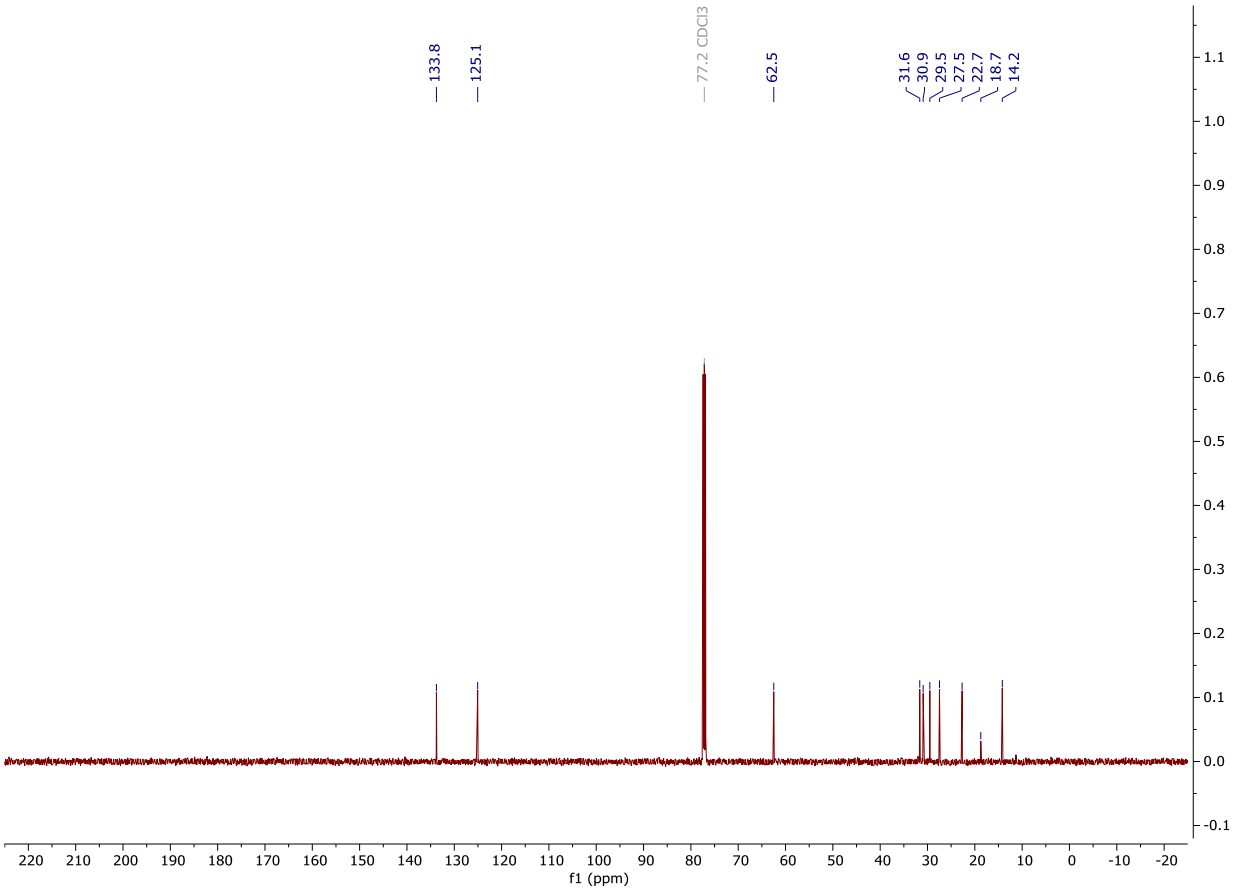
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-45**



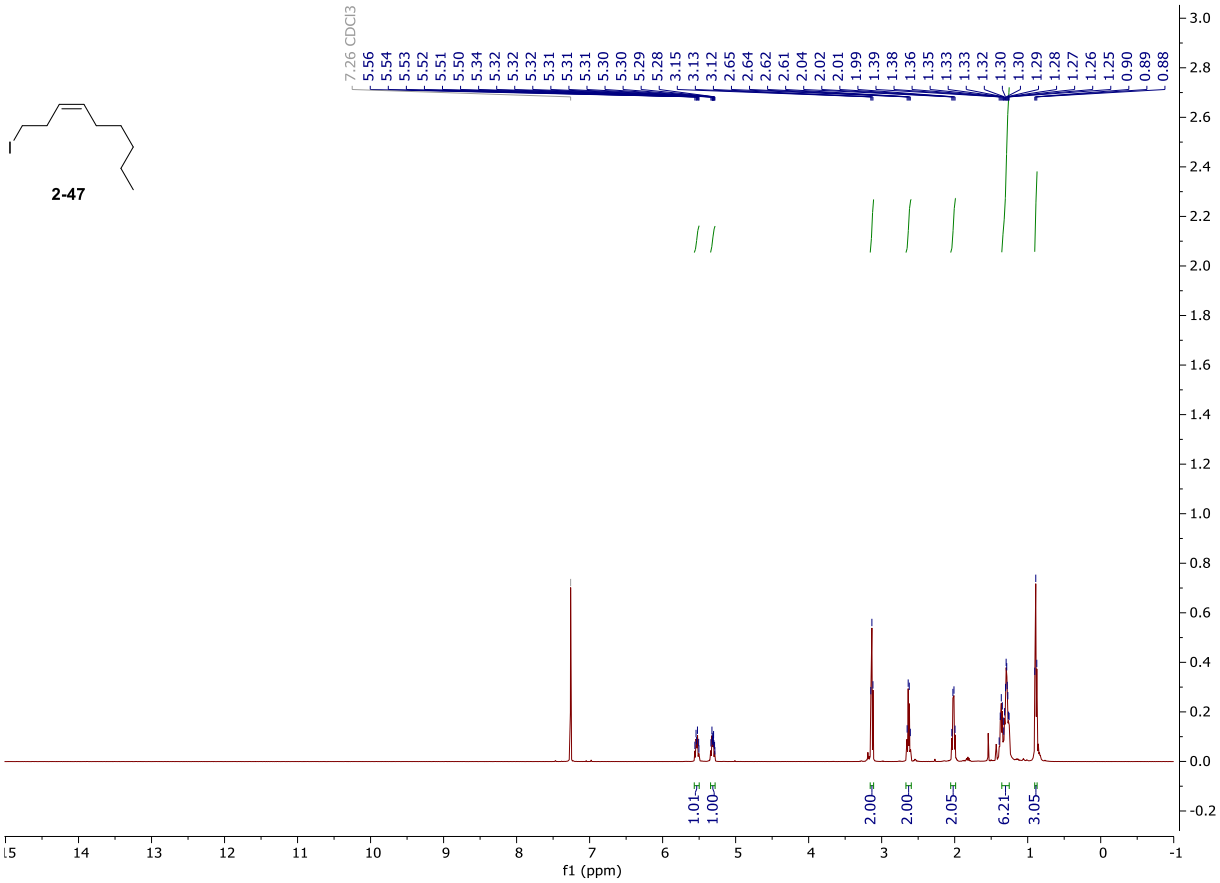


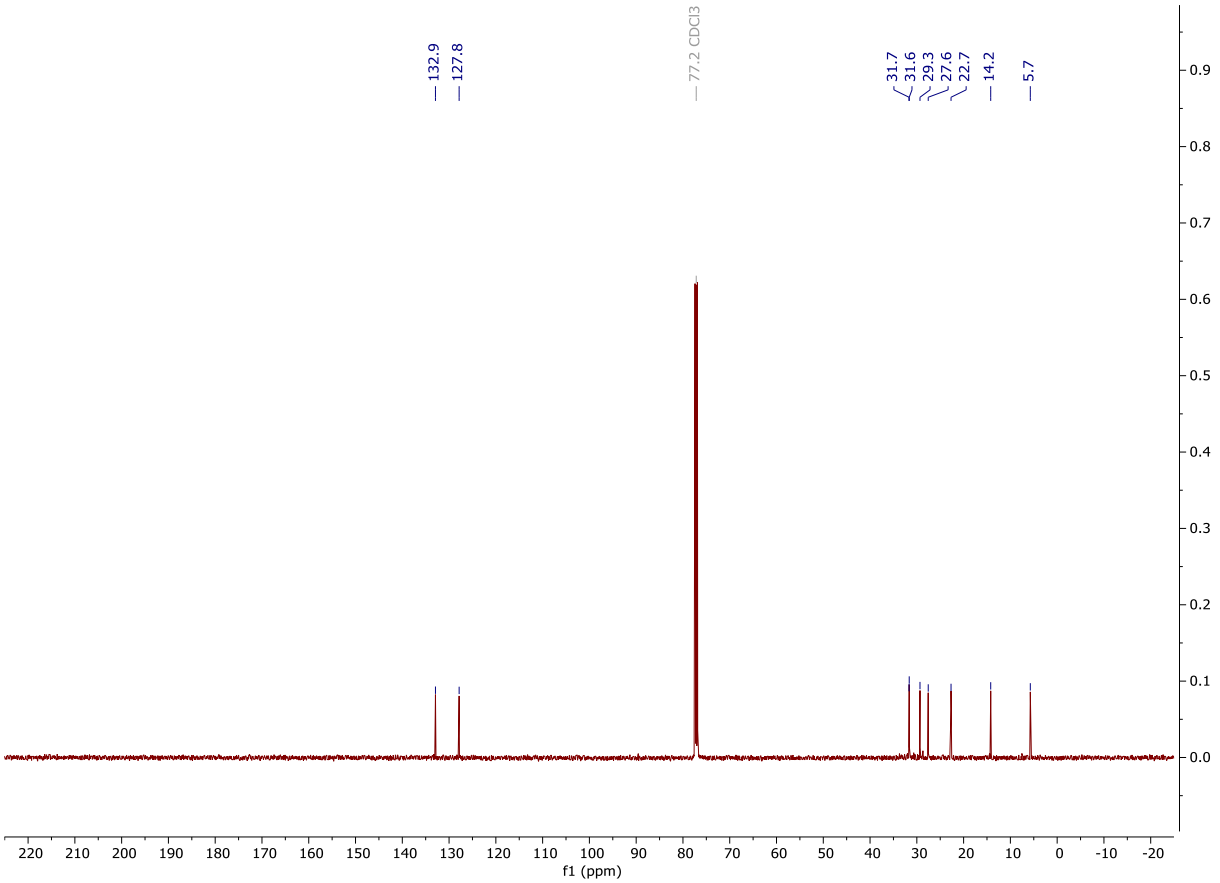
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-46**



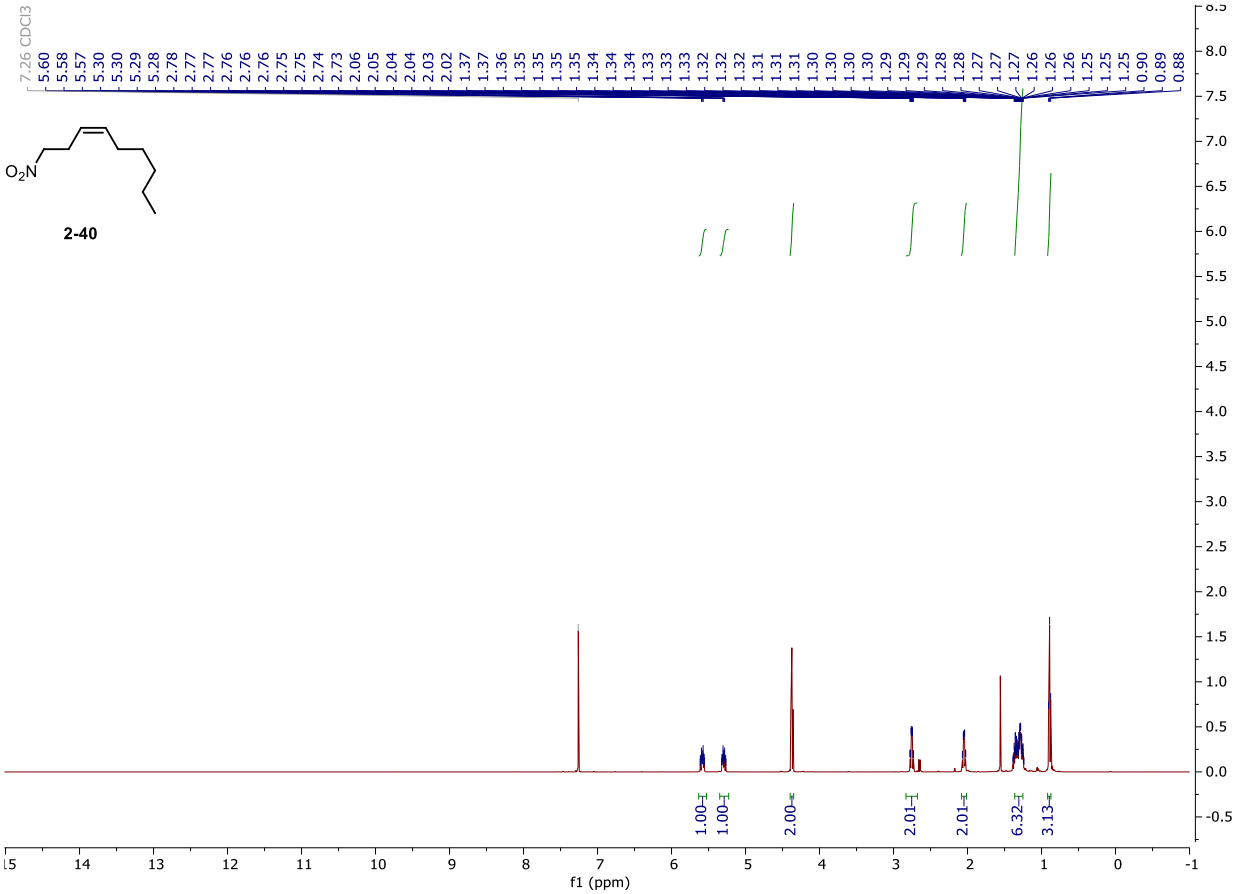


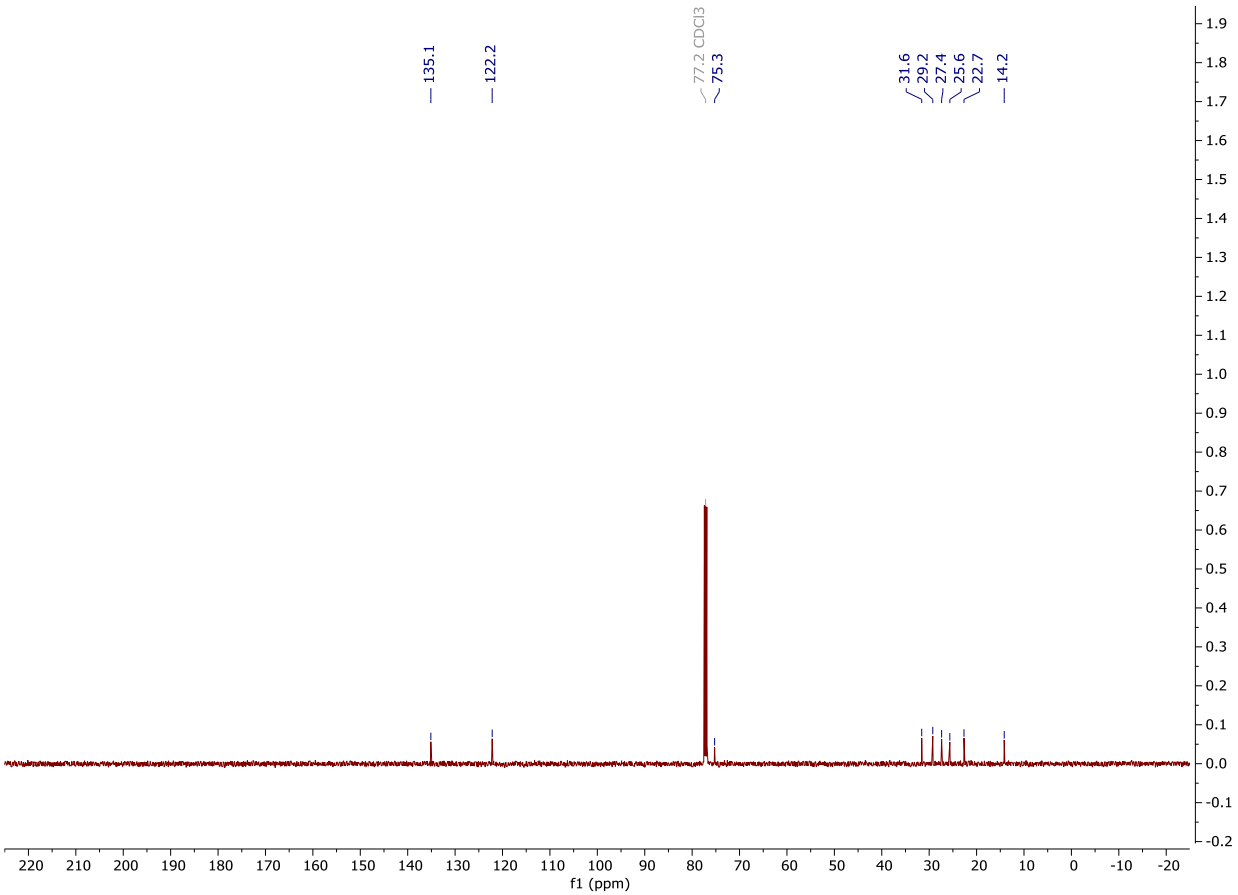
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-47**



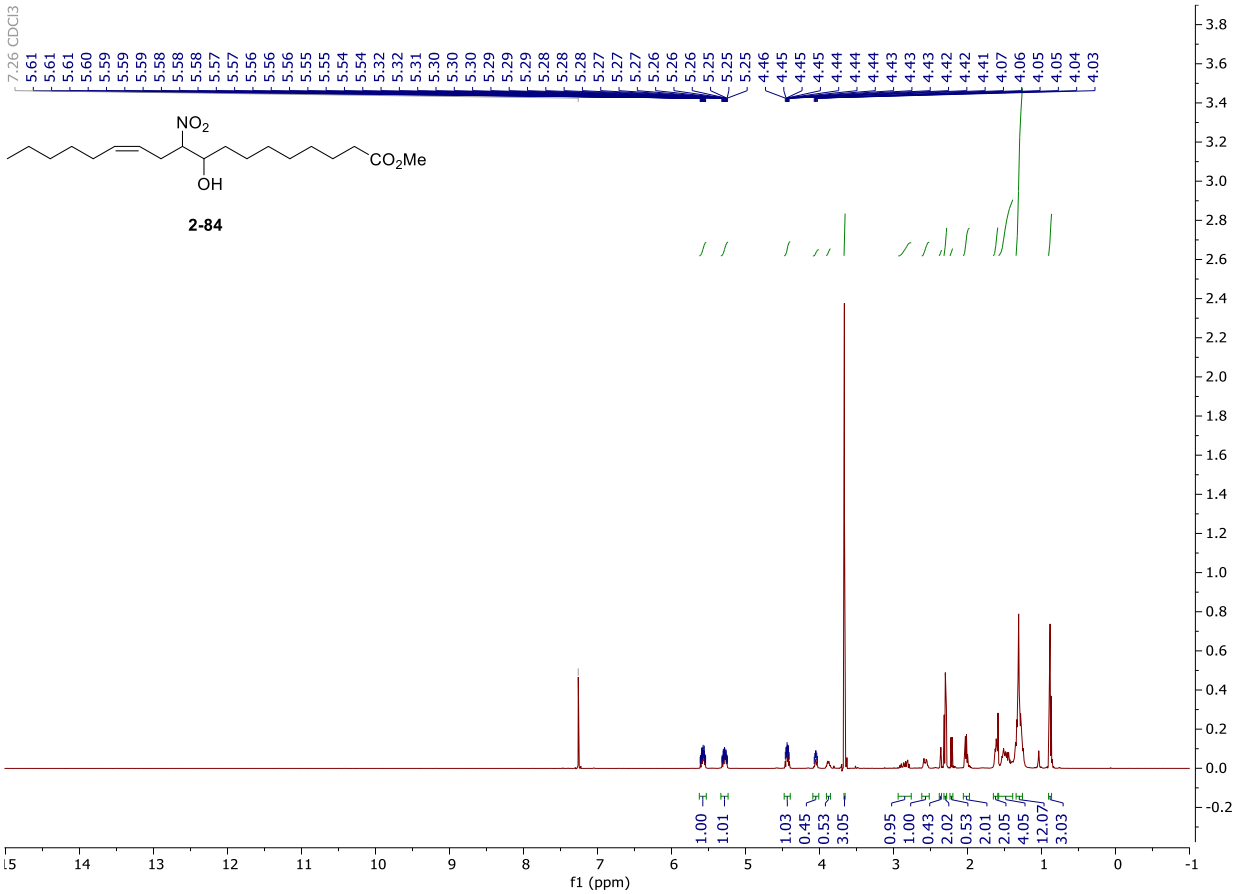


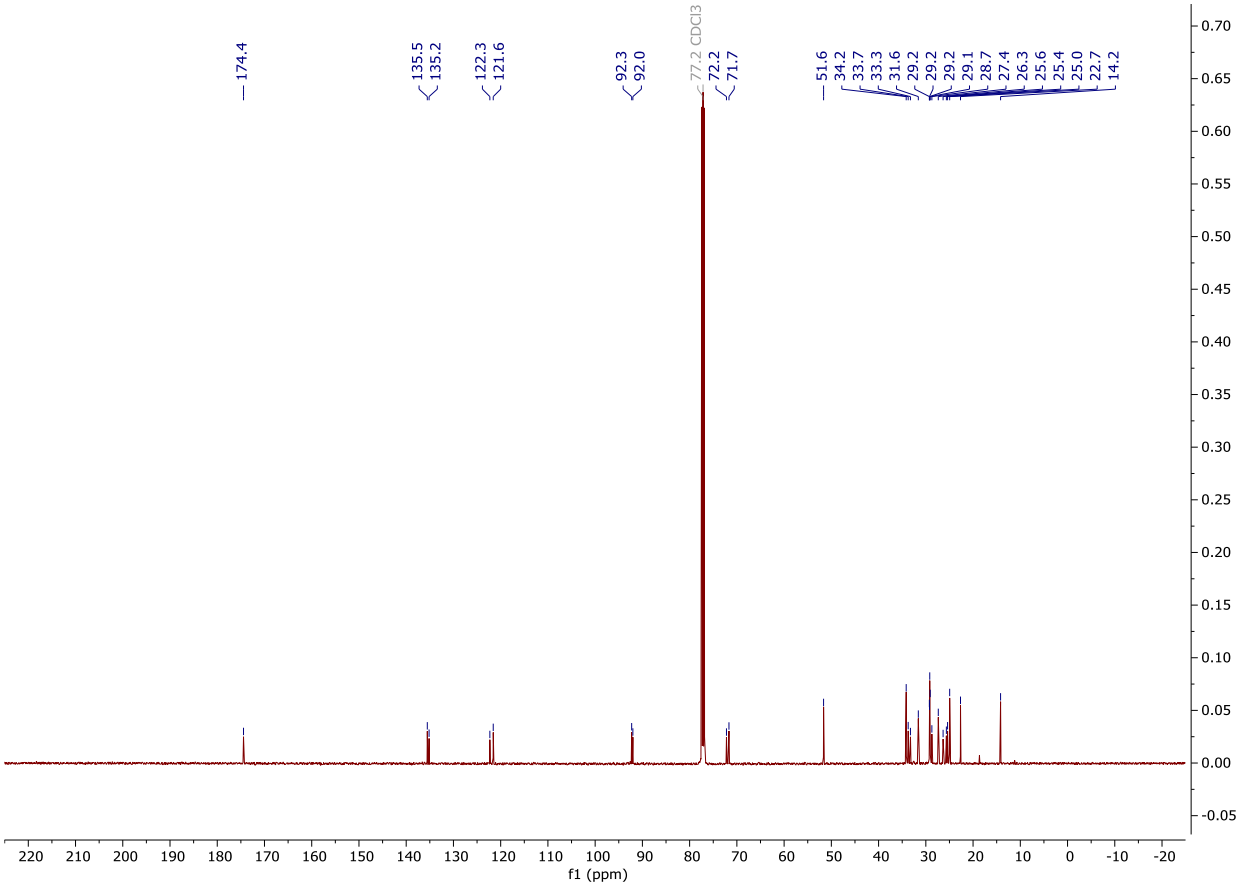
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-40**



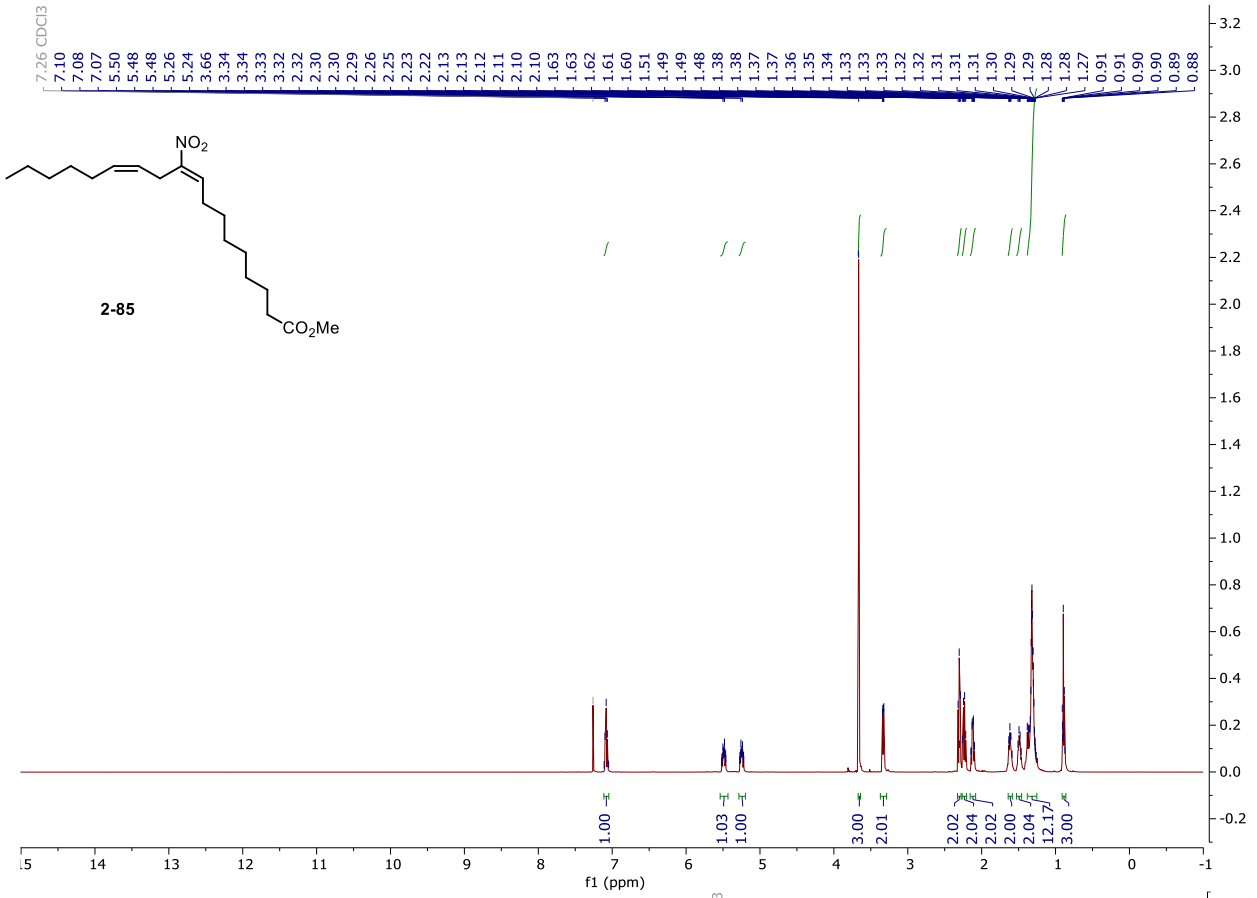


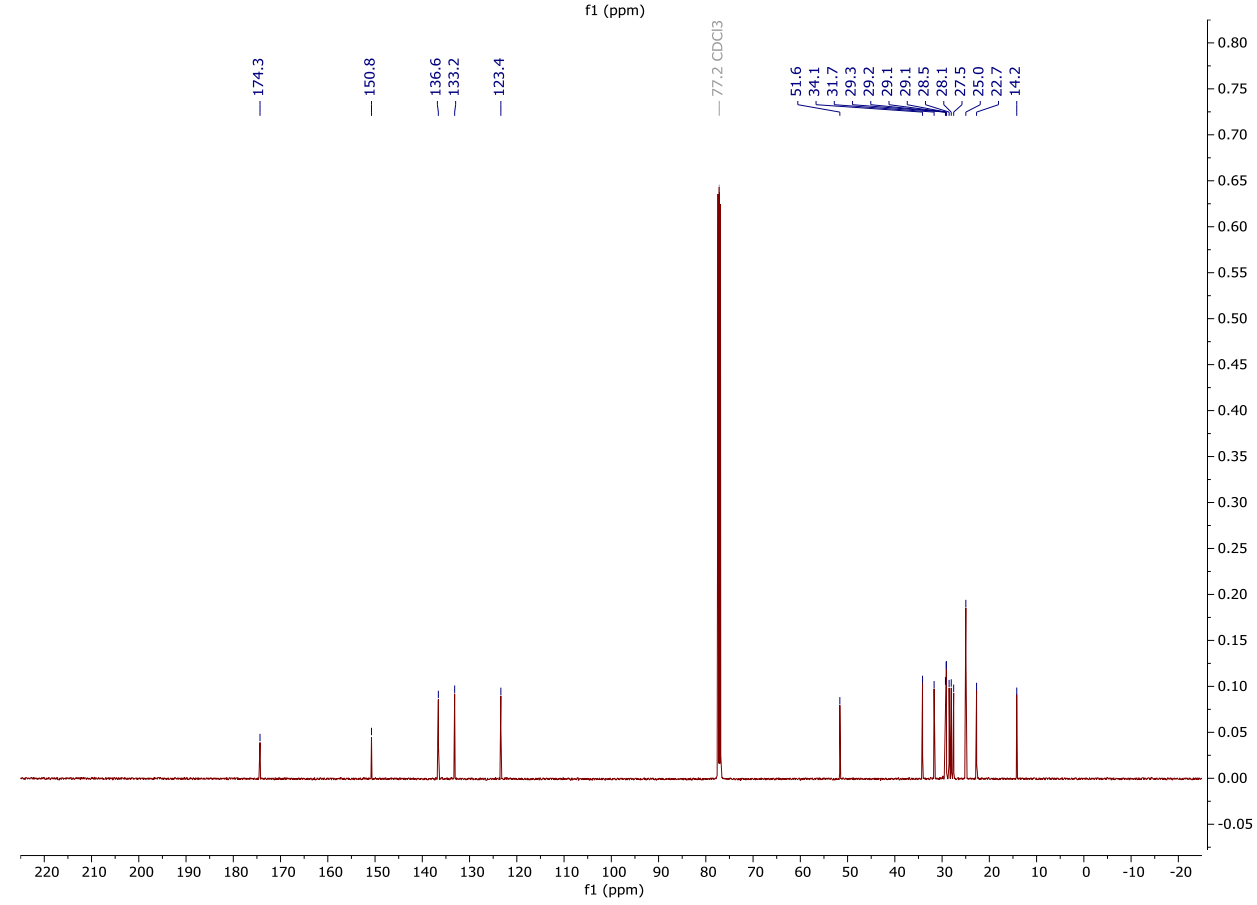
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-84**



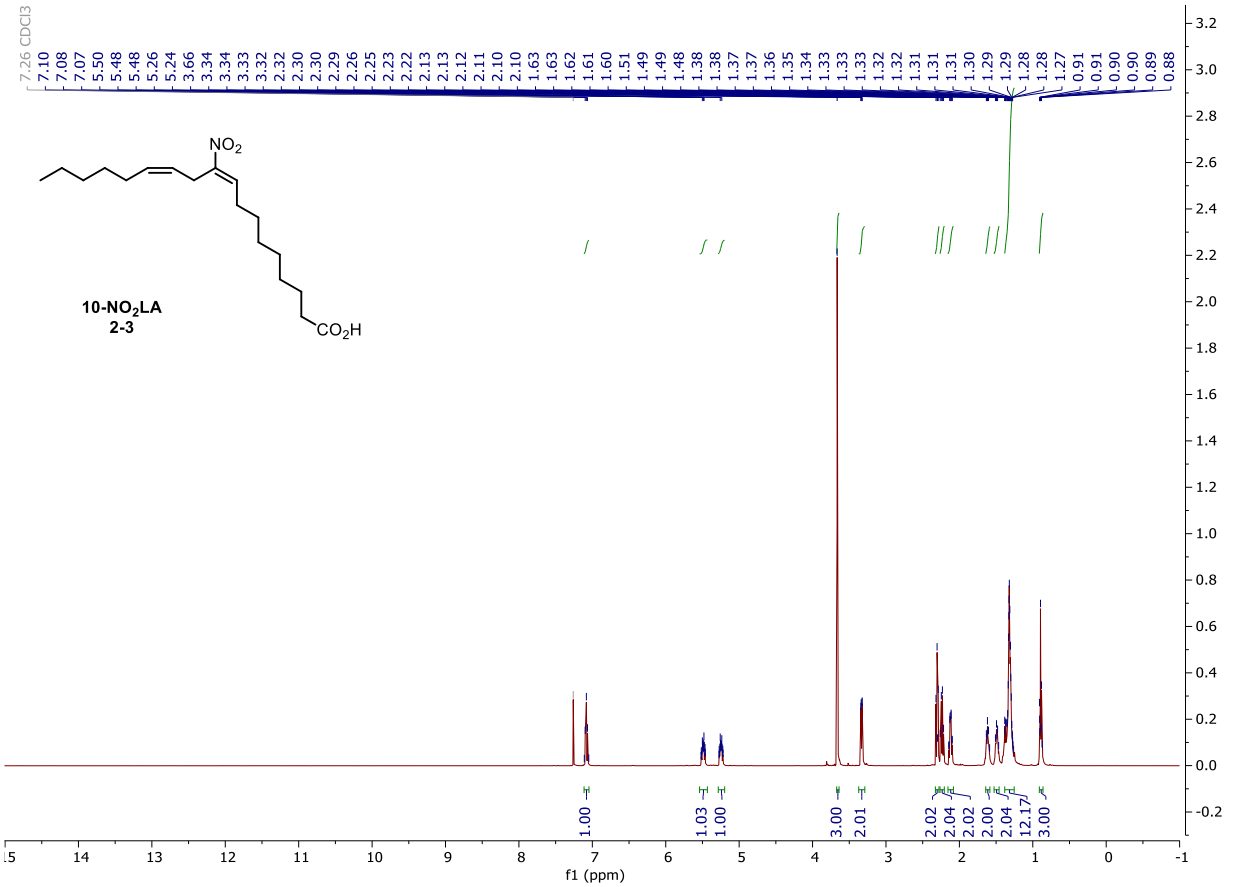


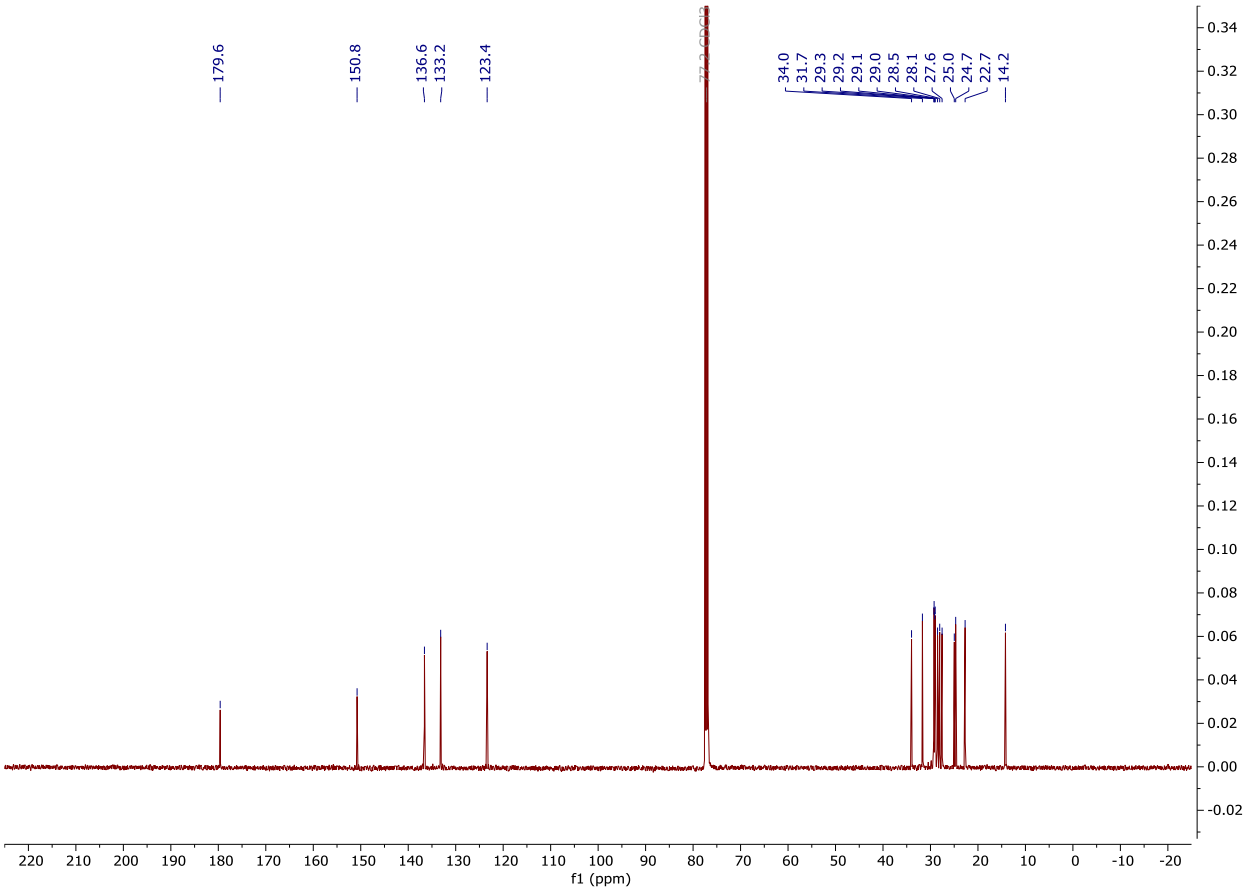
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-85**



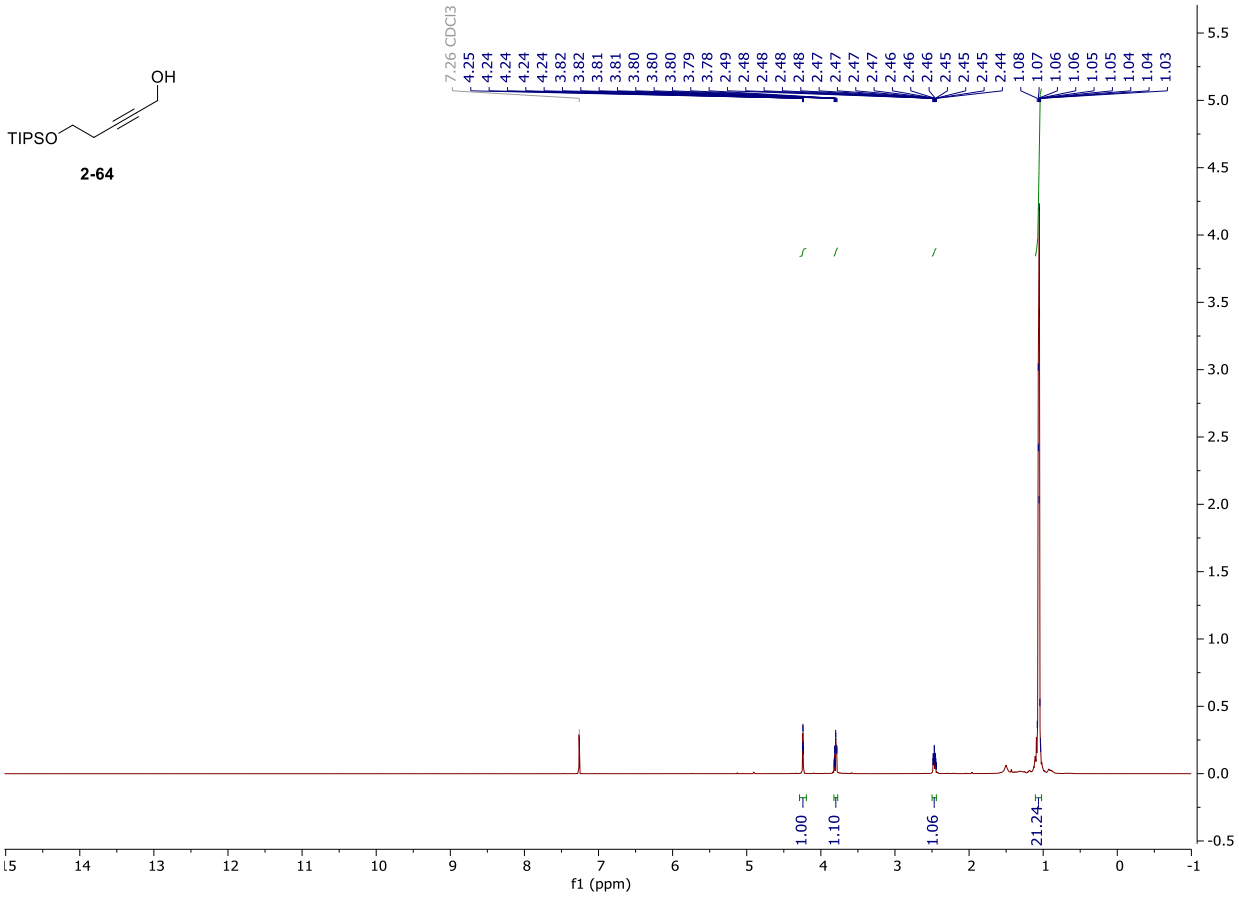


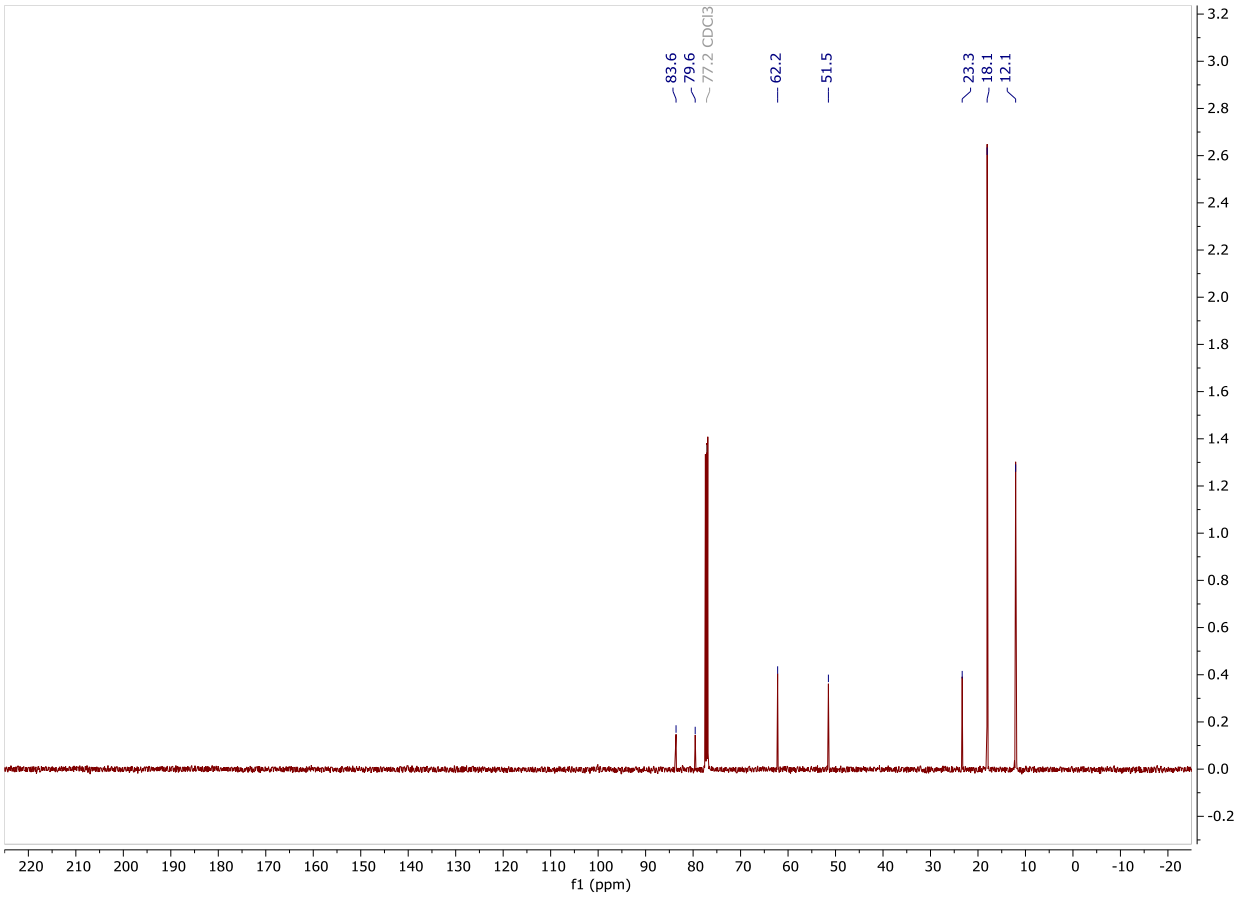
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of 10-NO_2LA, **2-3**



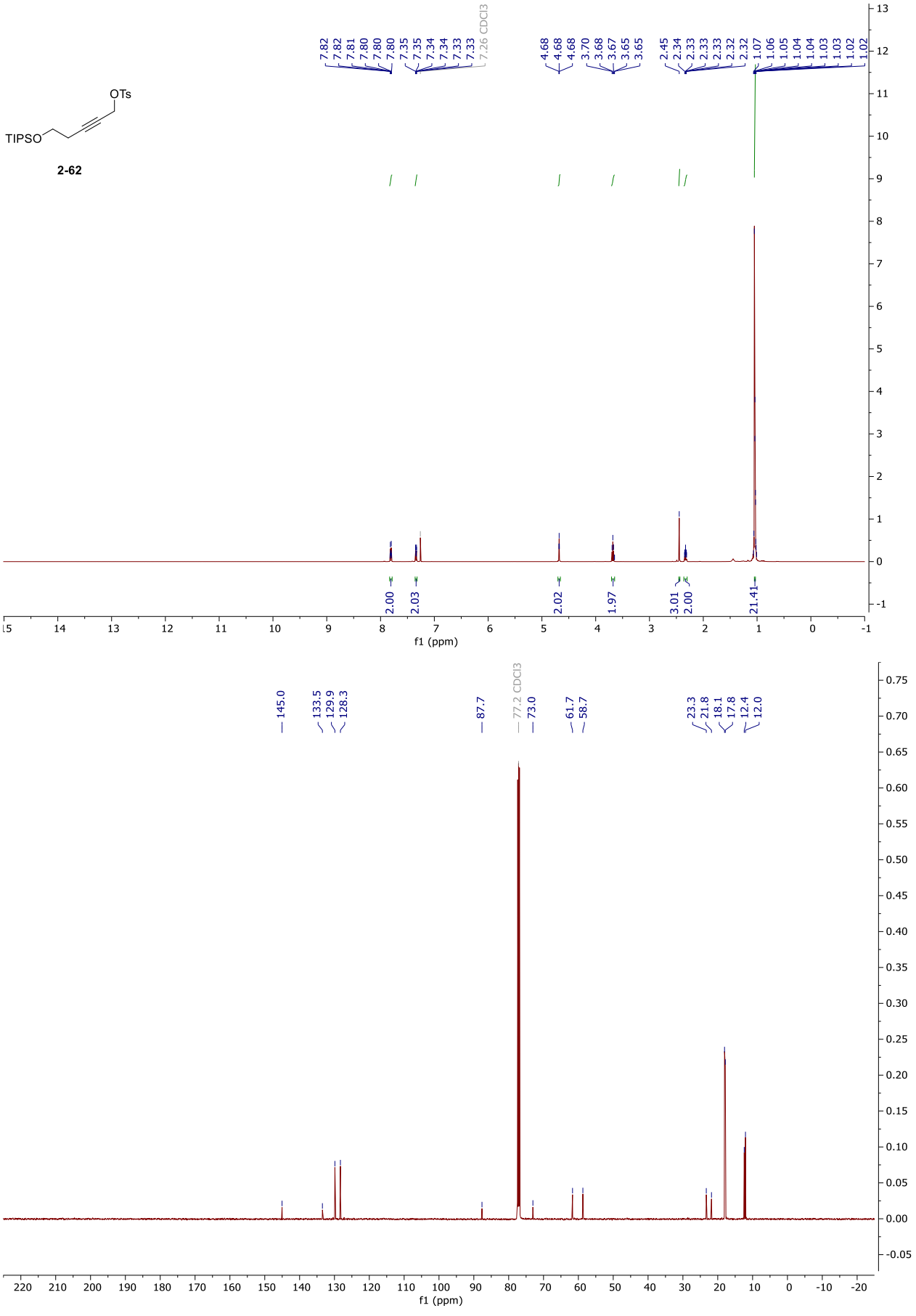


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-64**

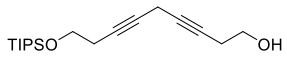




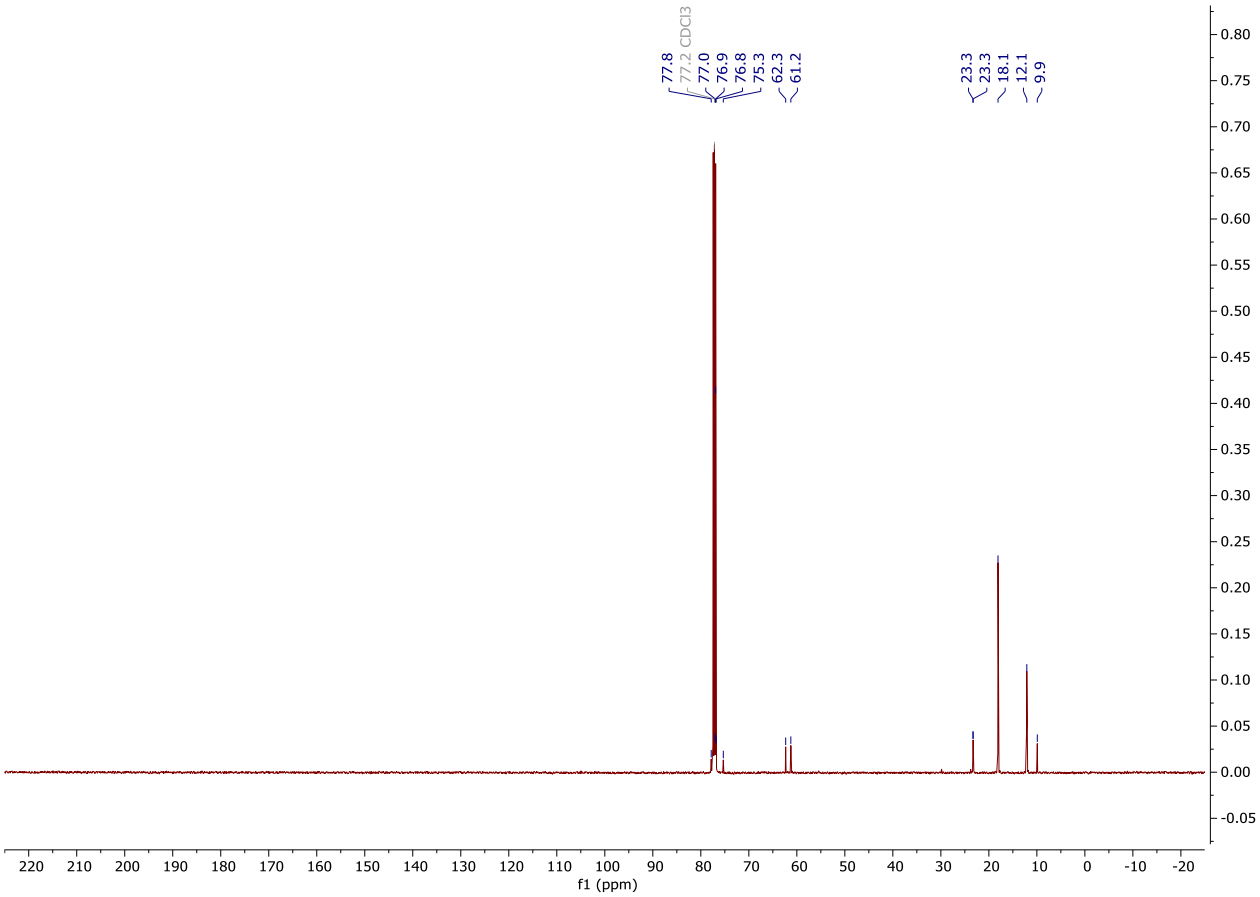
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-62**



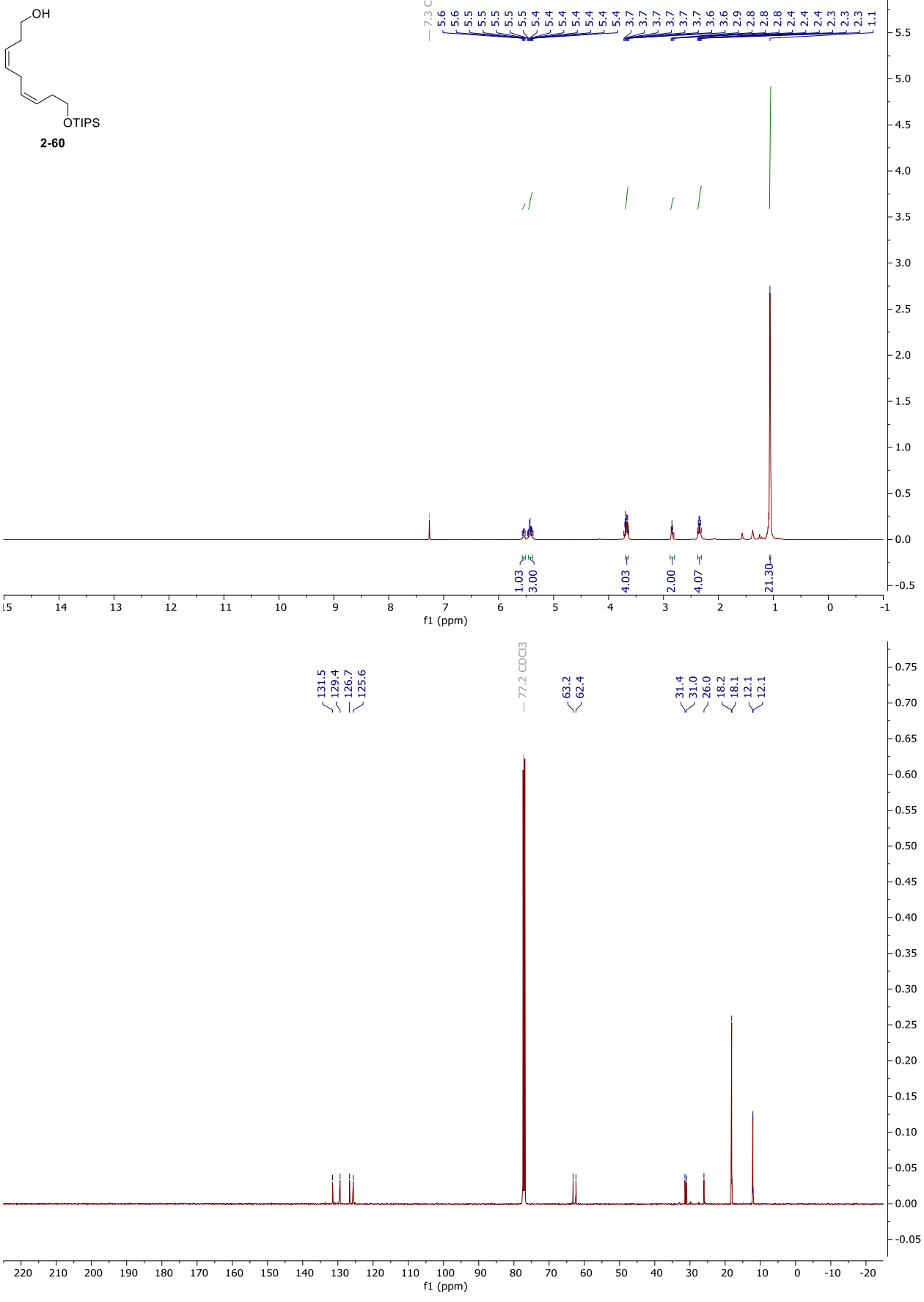
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-61**



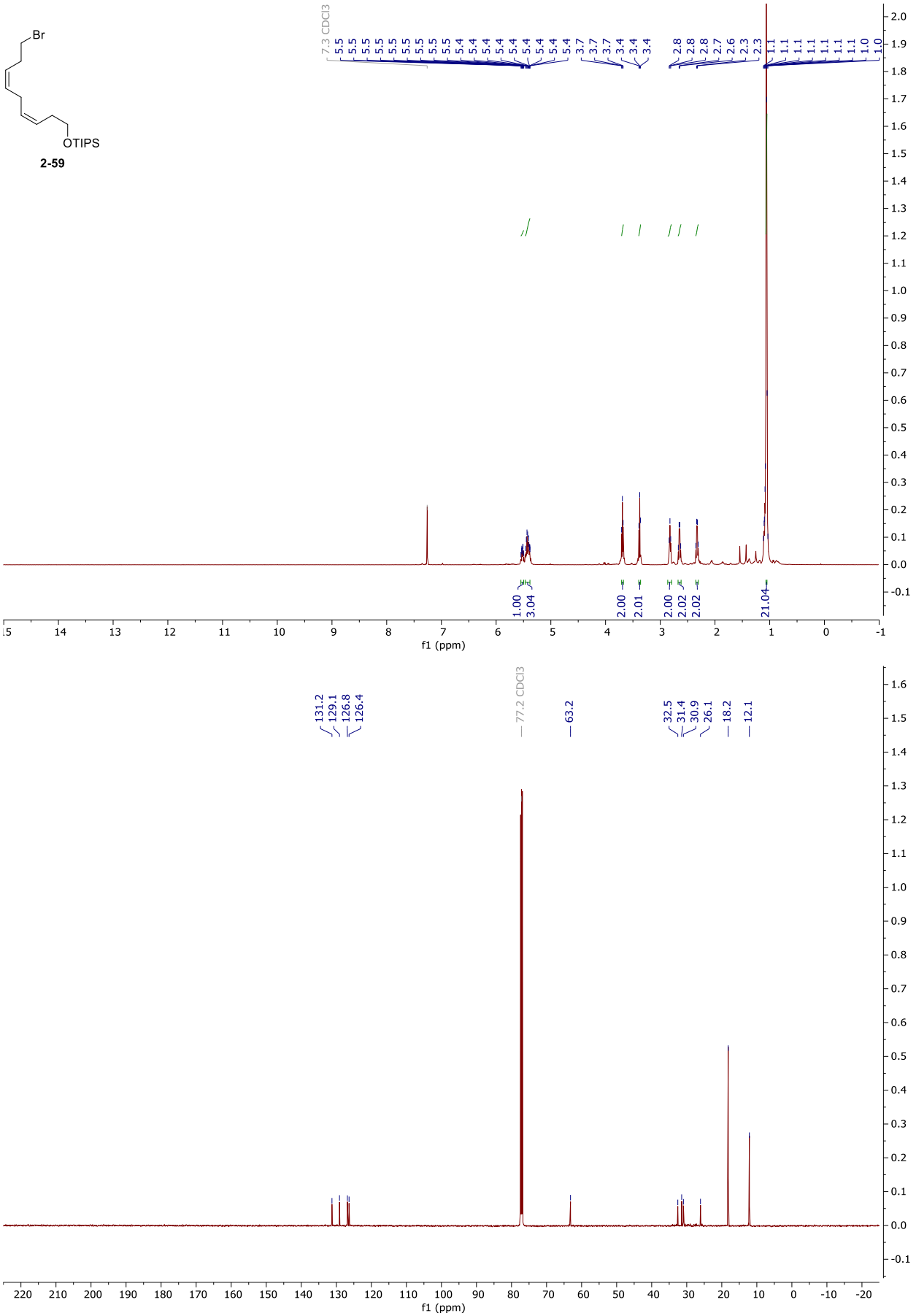
2-61



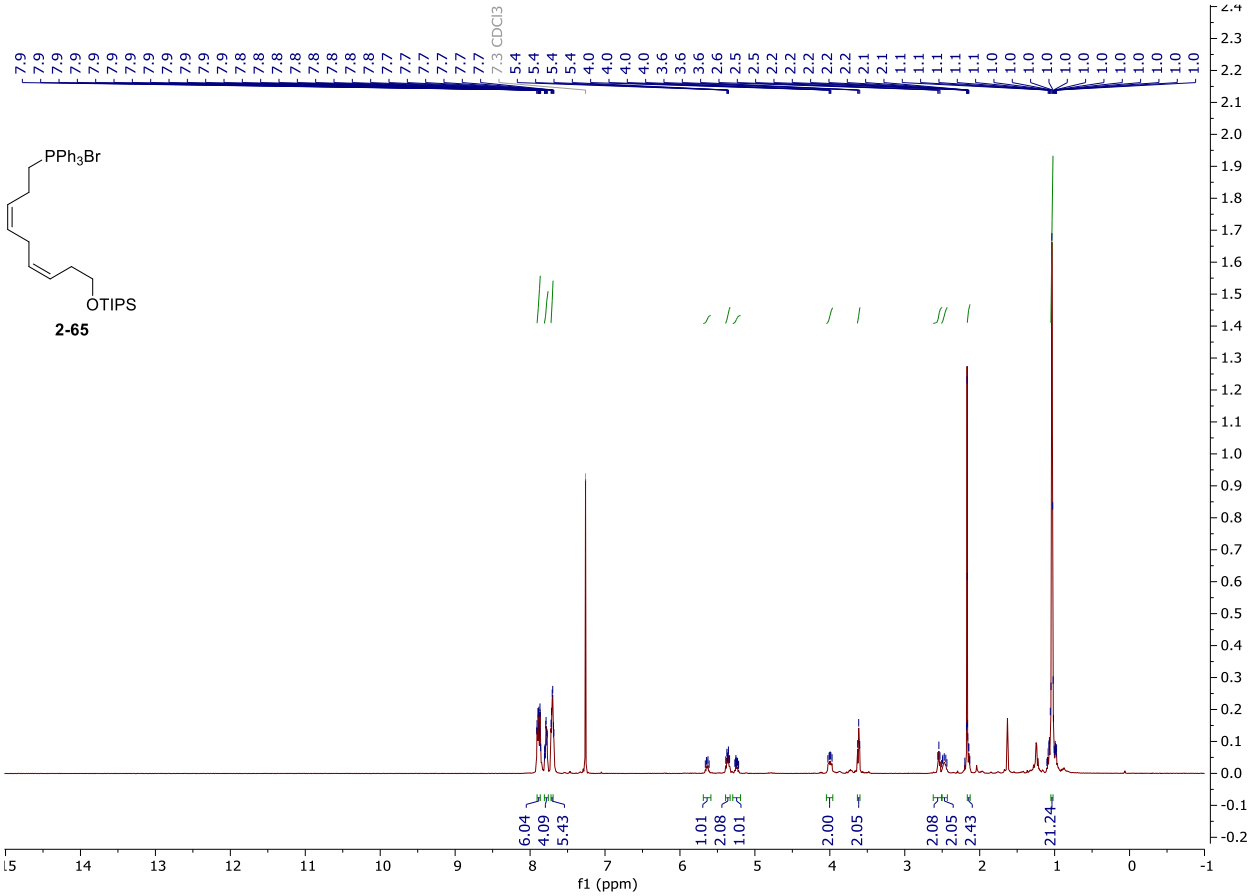
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-60**

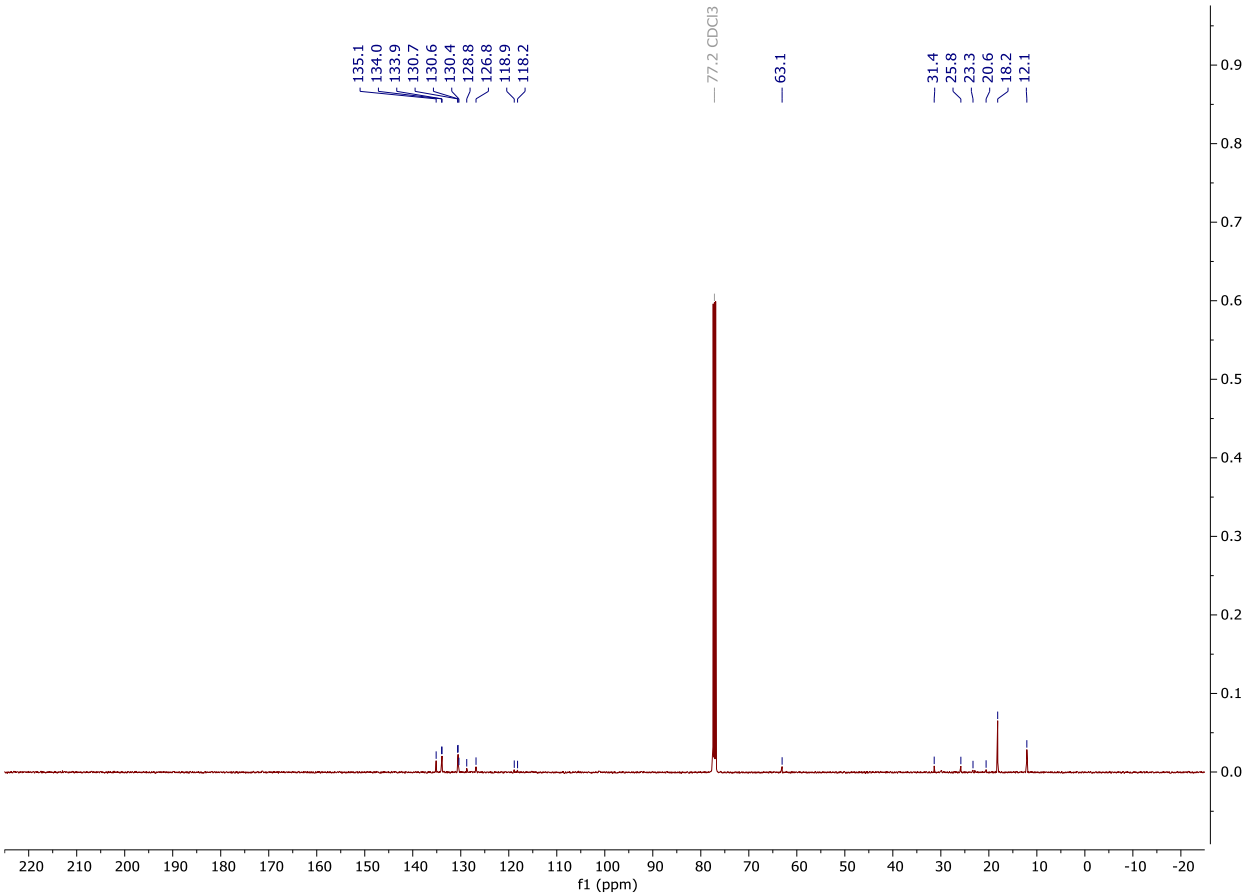


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-59**

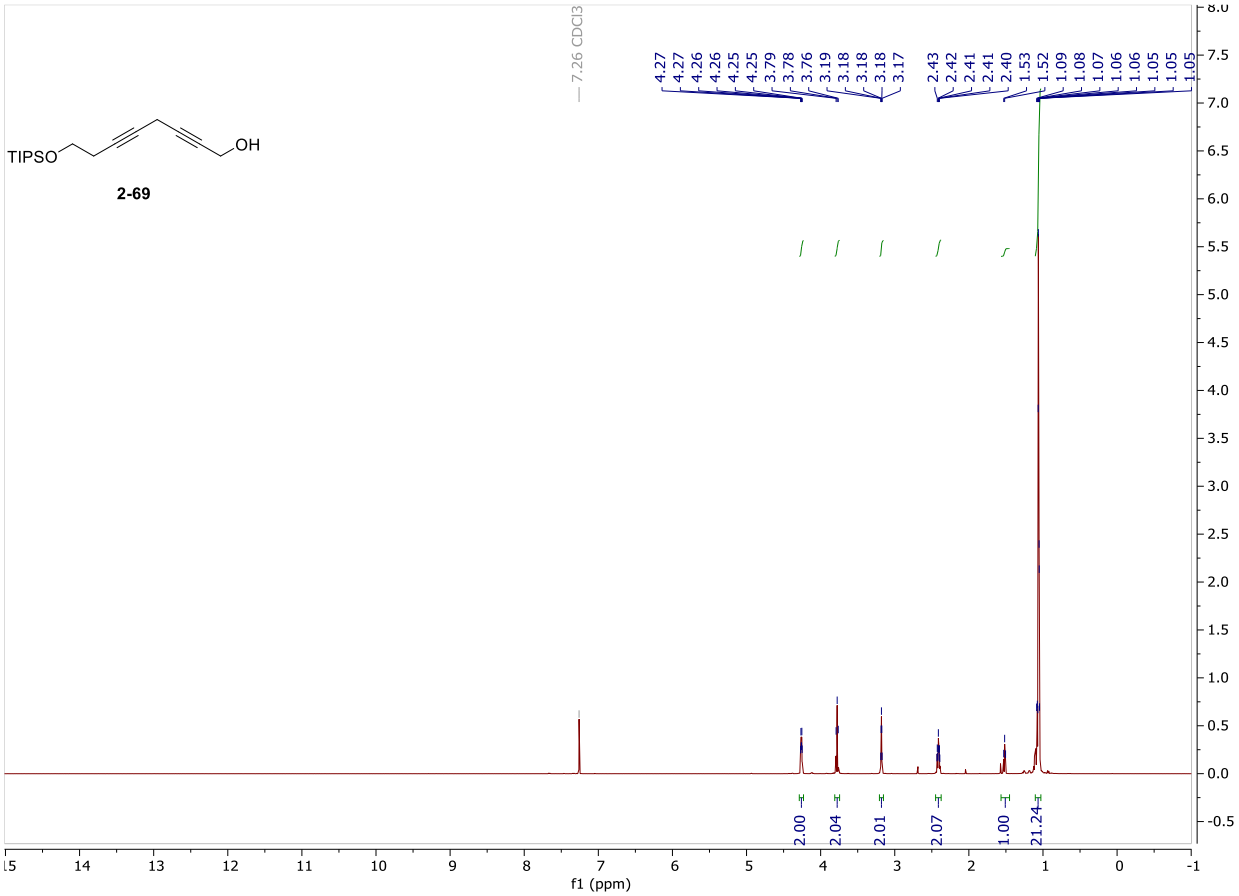


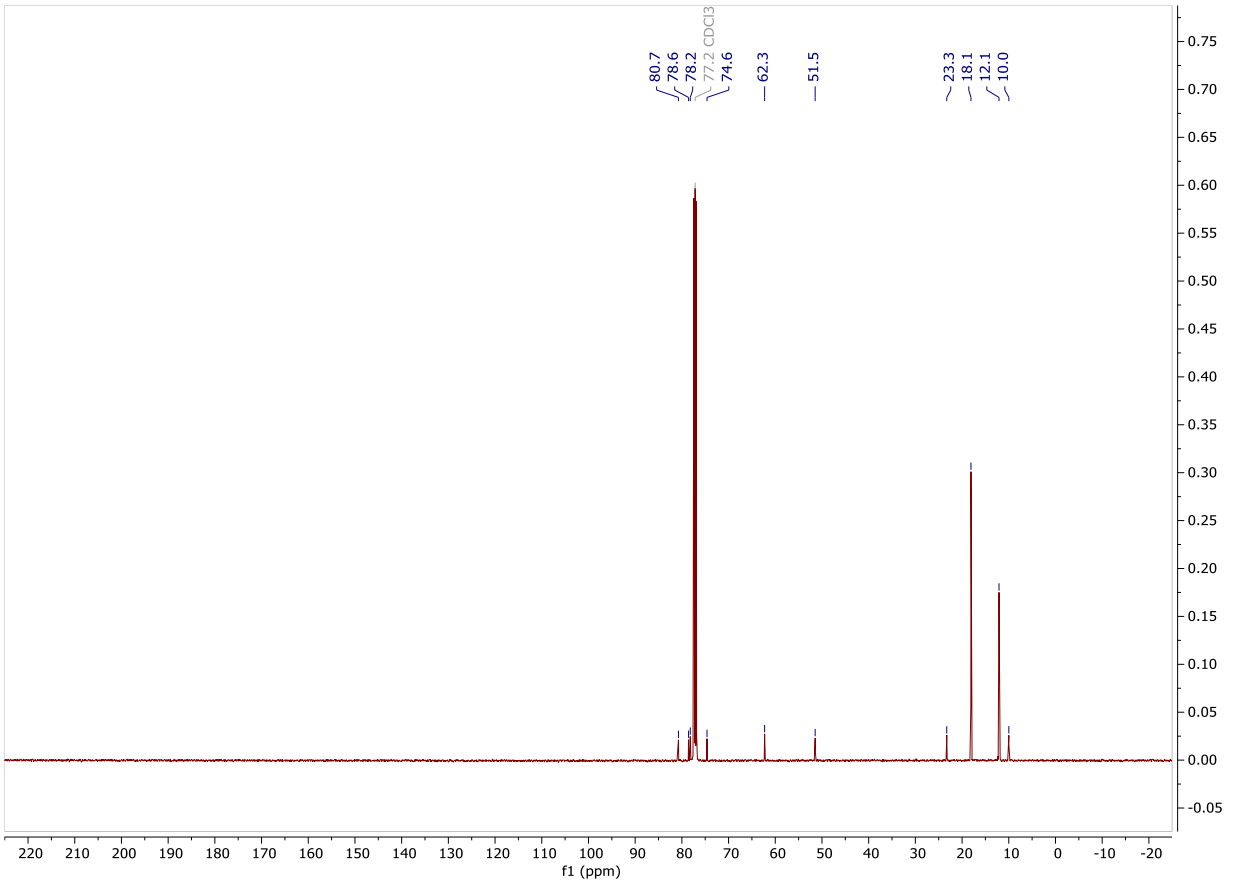
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-65**





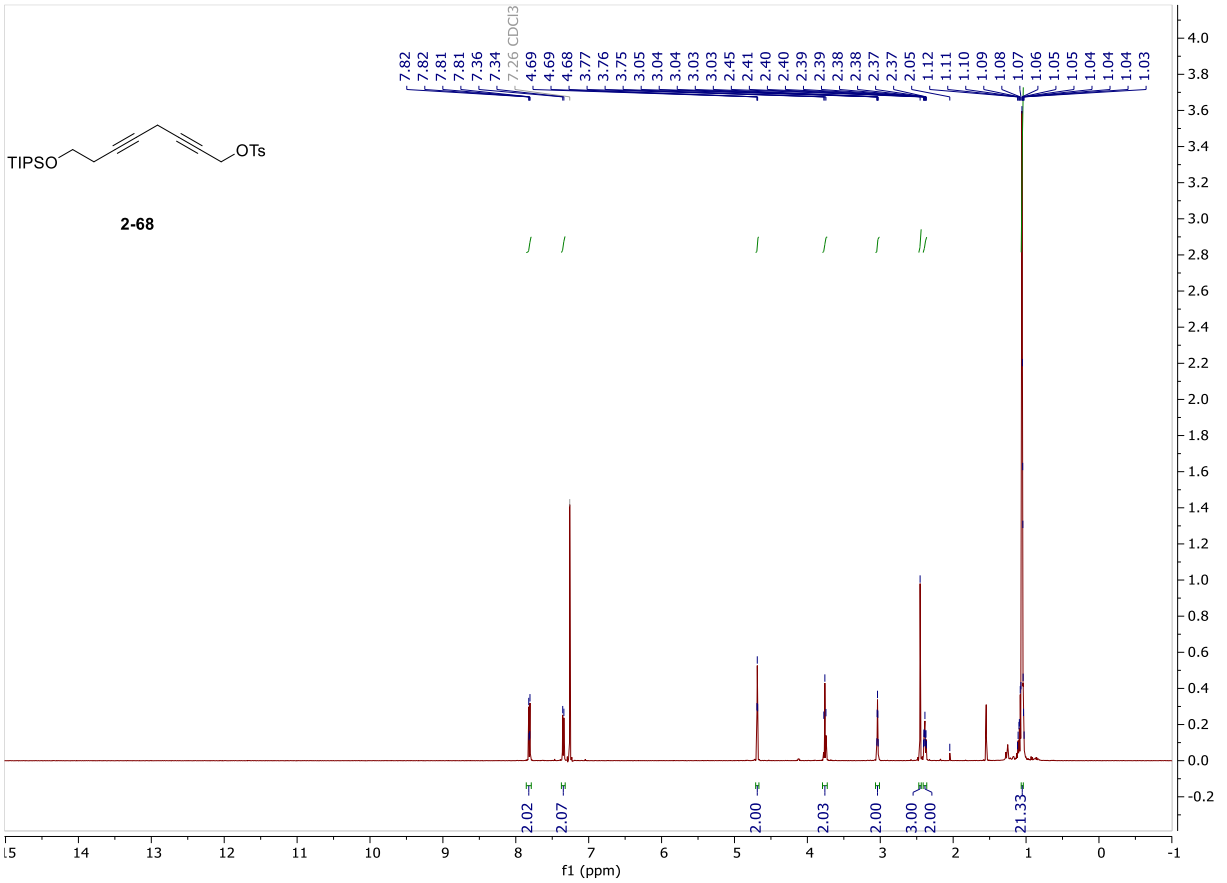
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-69**

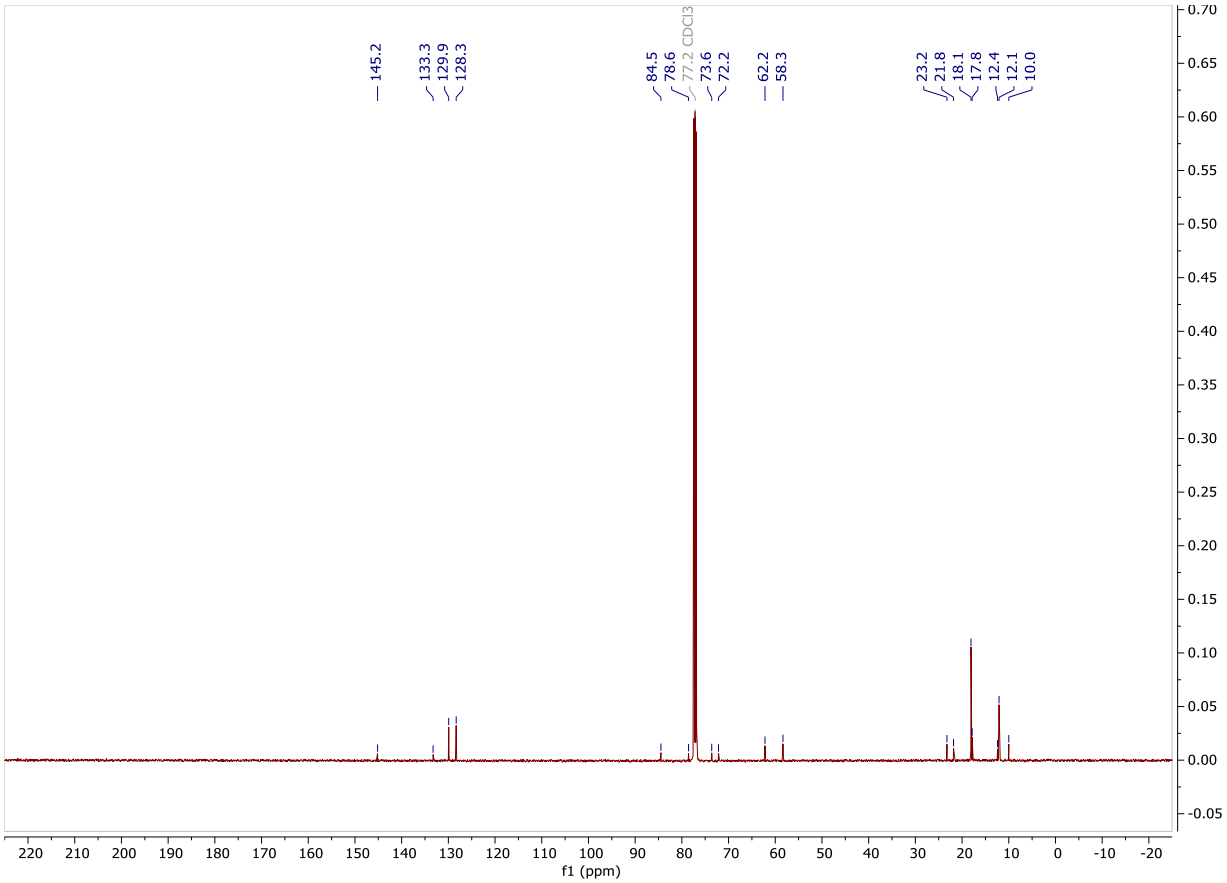




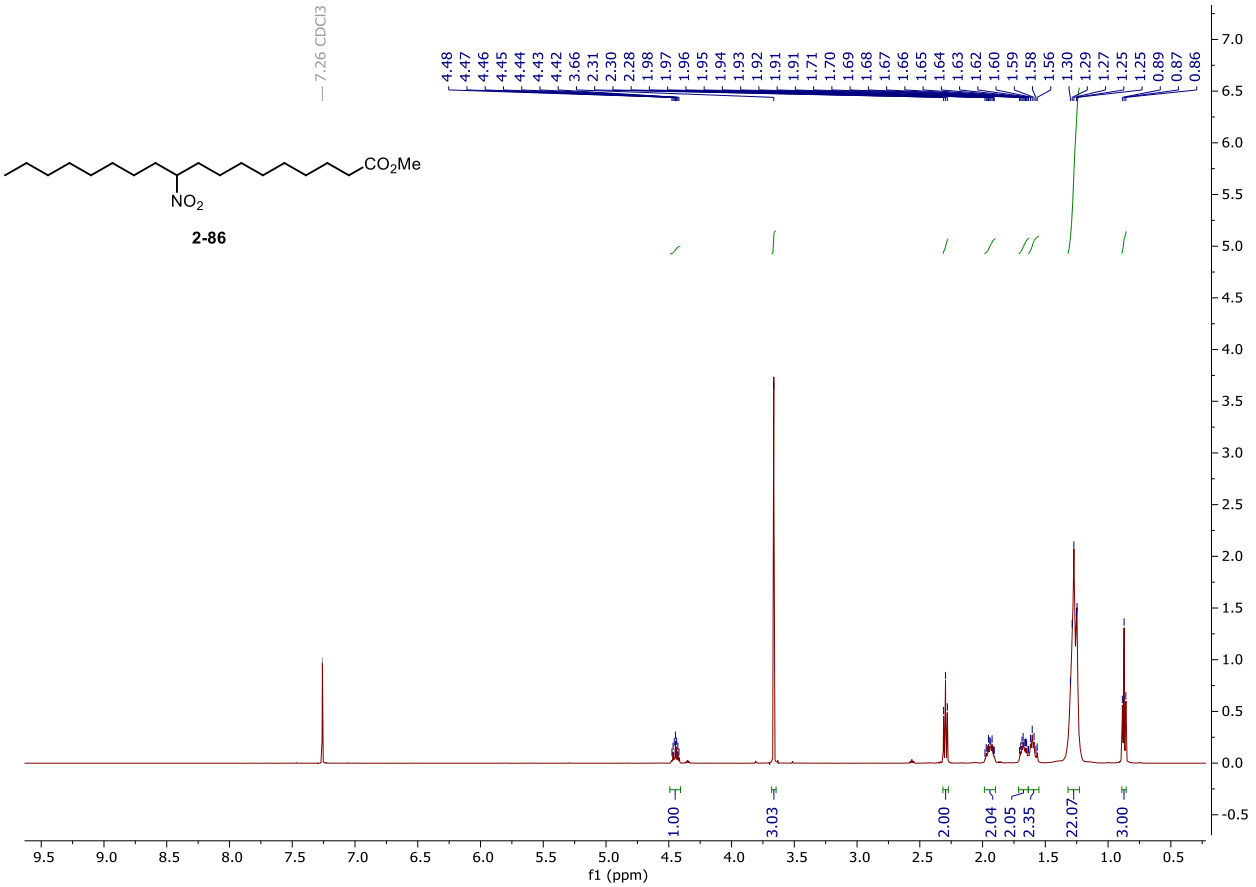
NMR and HPLC

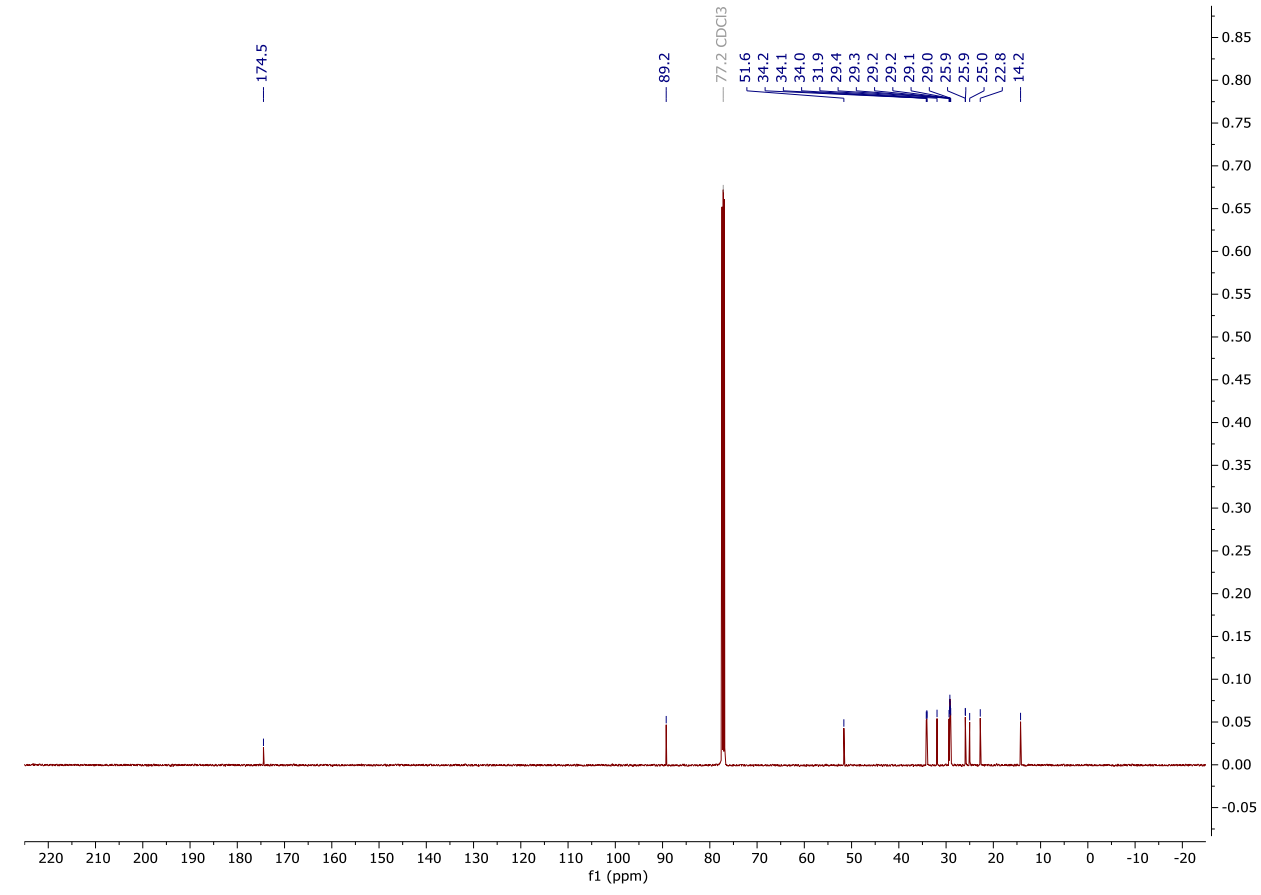
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-68**



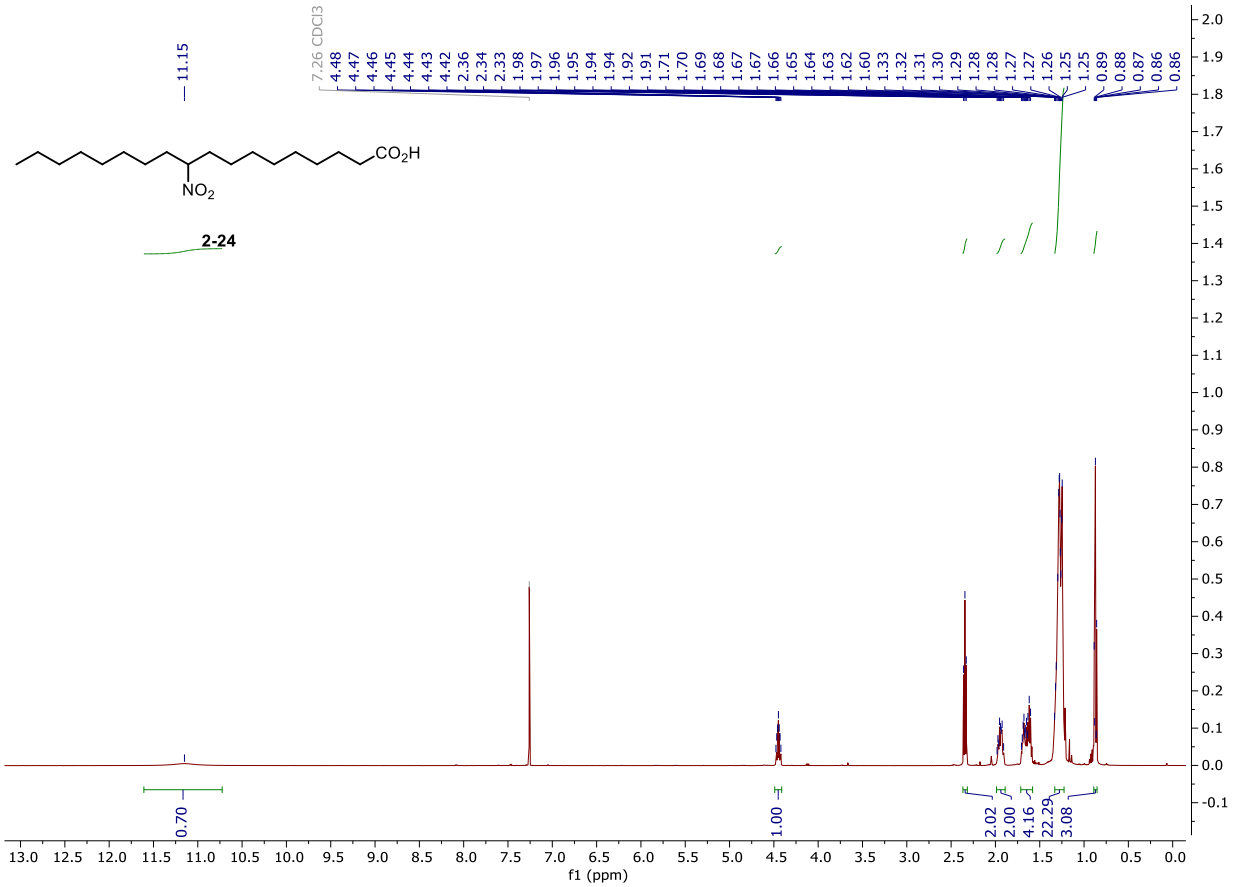


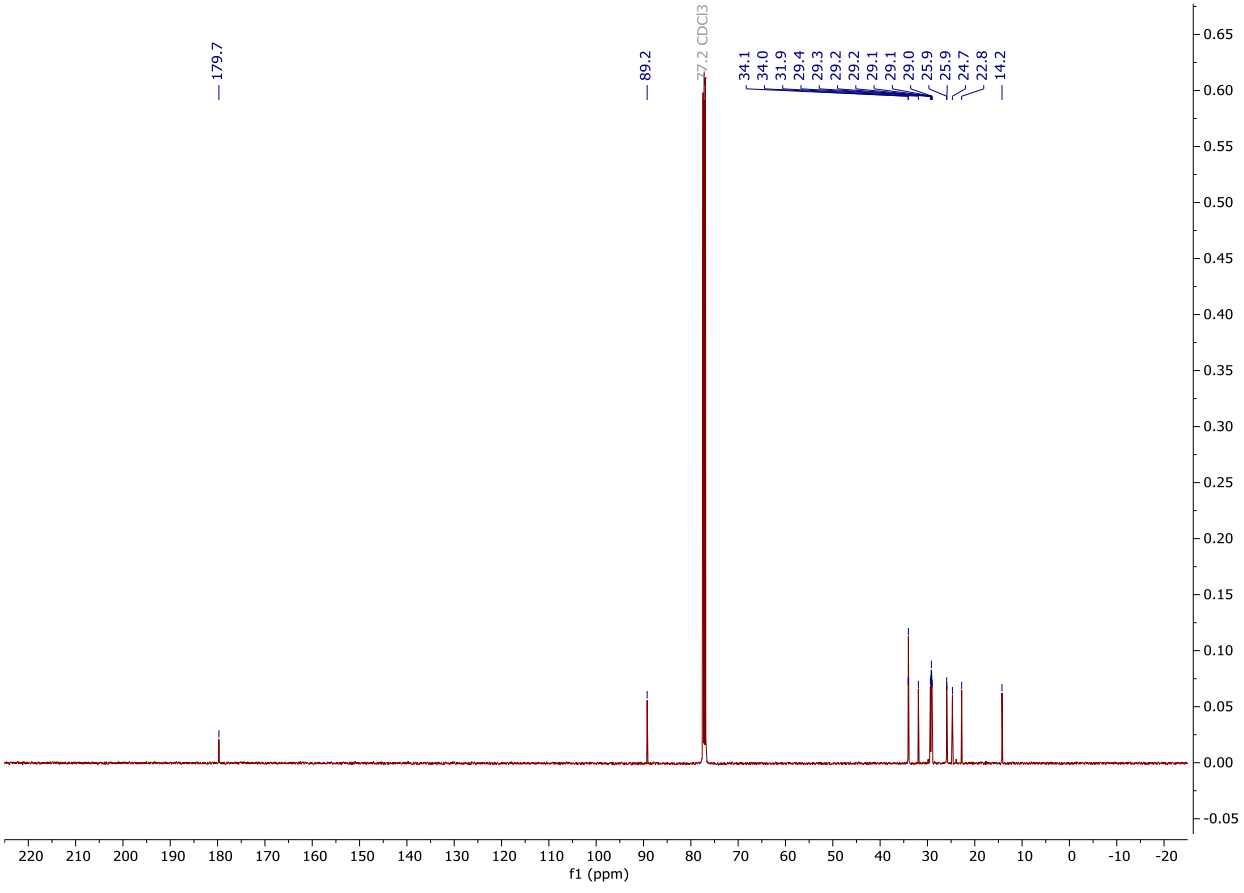
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-86**



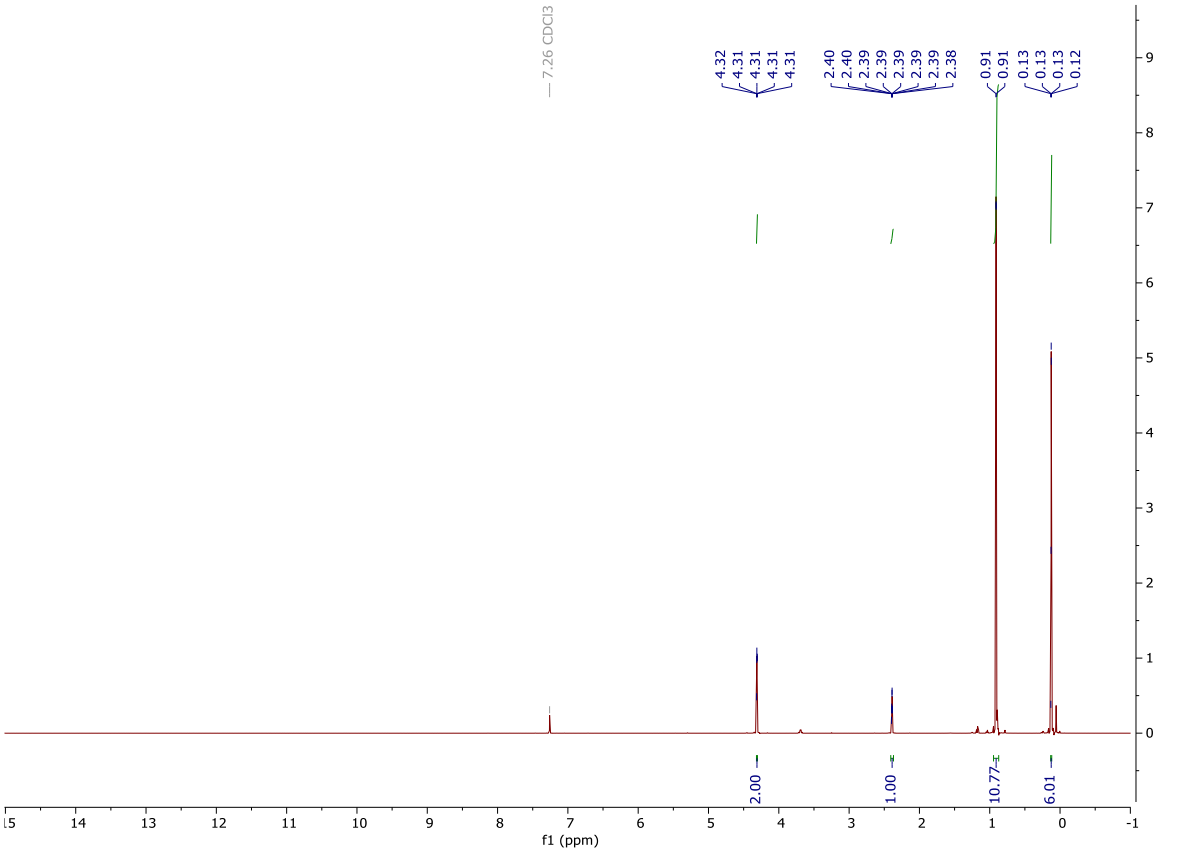


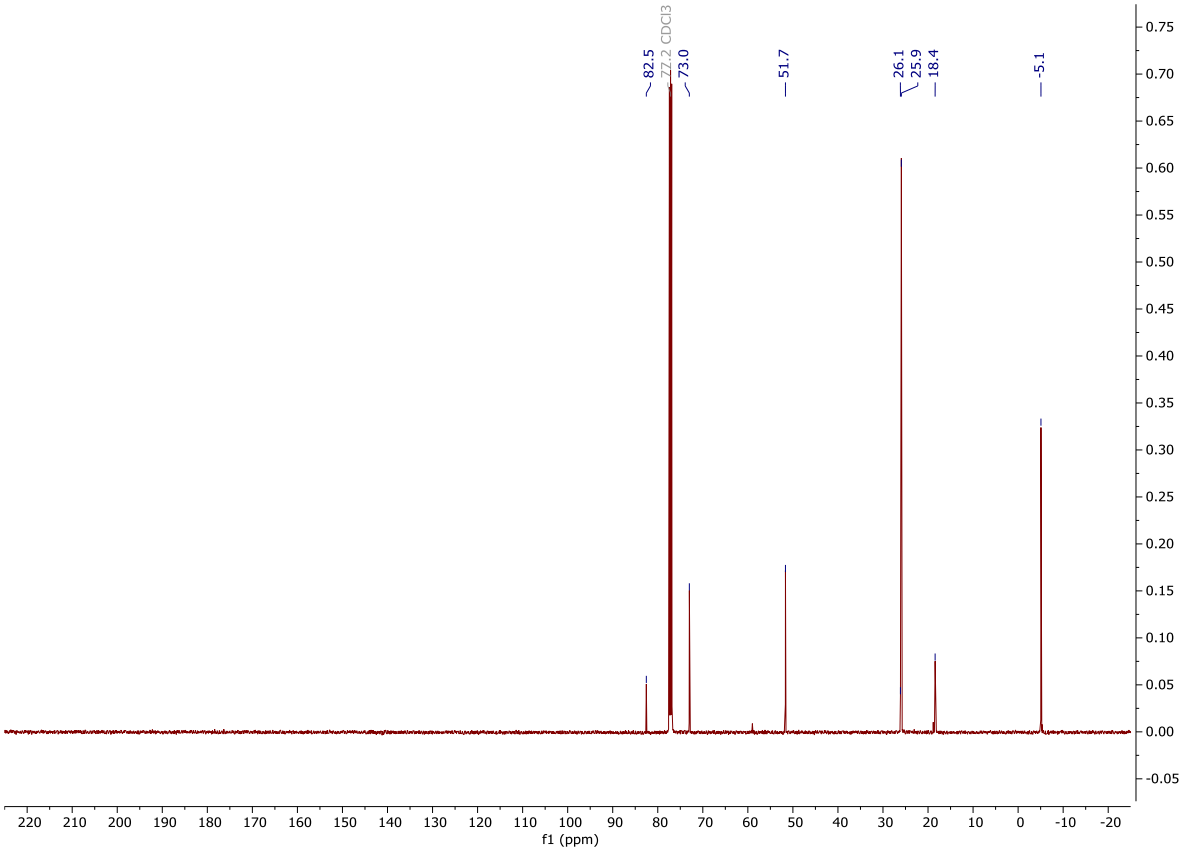
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **10-NO_2SA, 2-24**



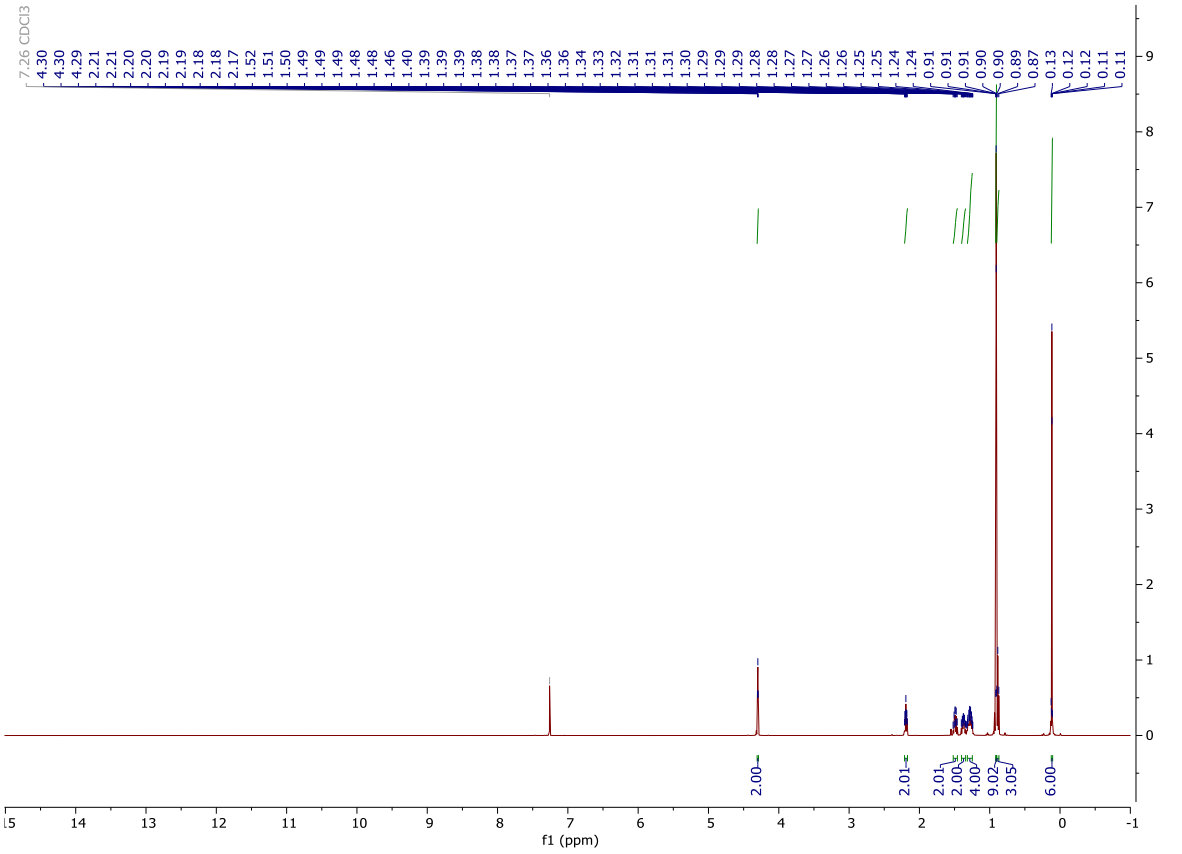


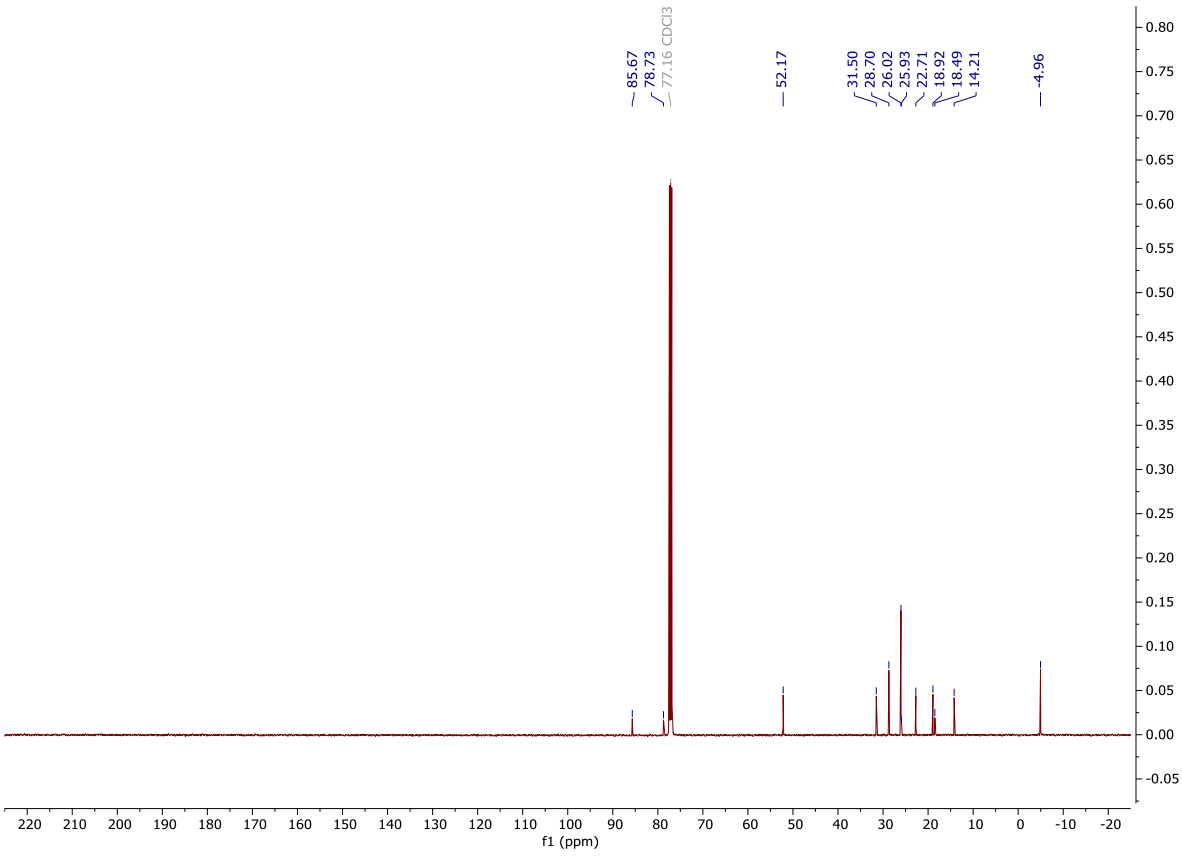
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-52**



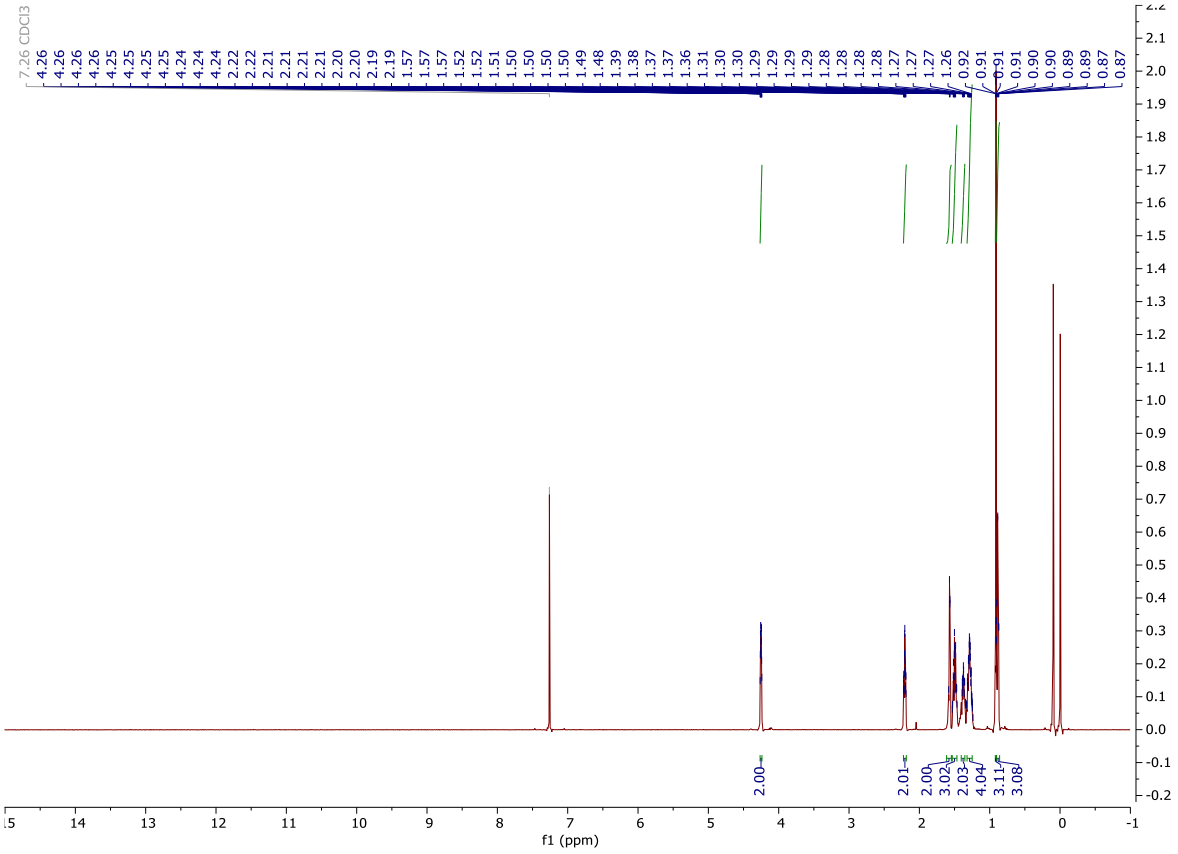


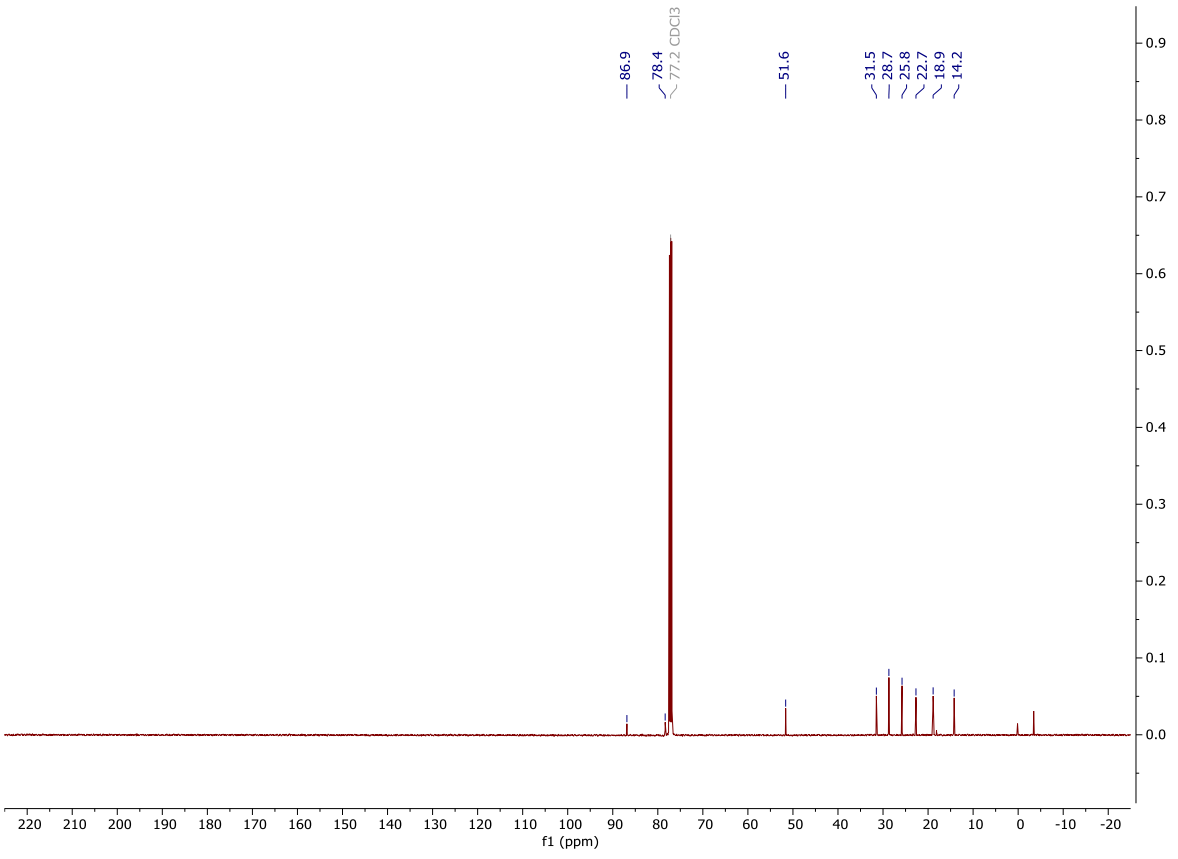
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-50**



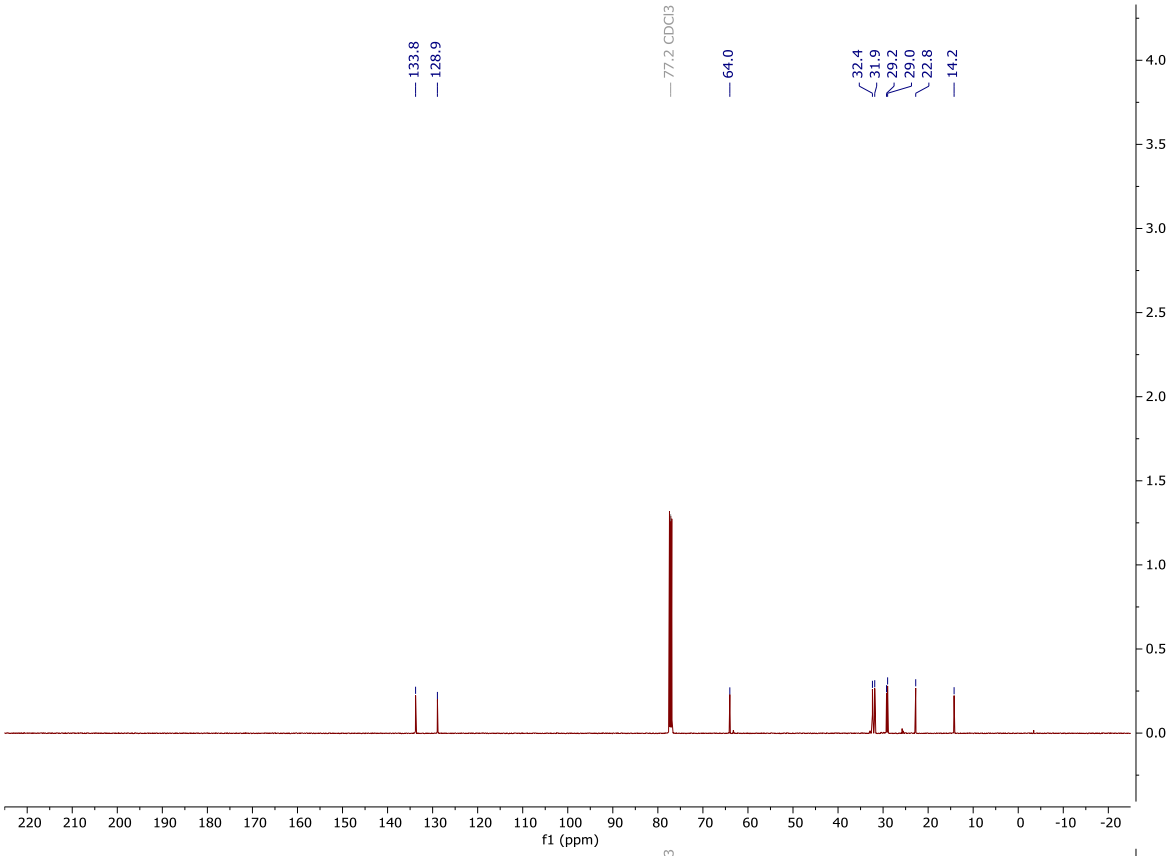


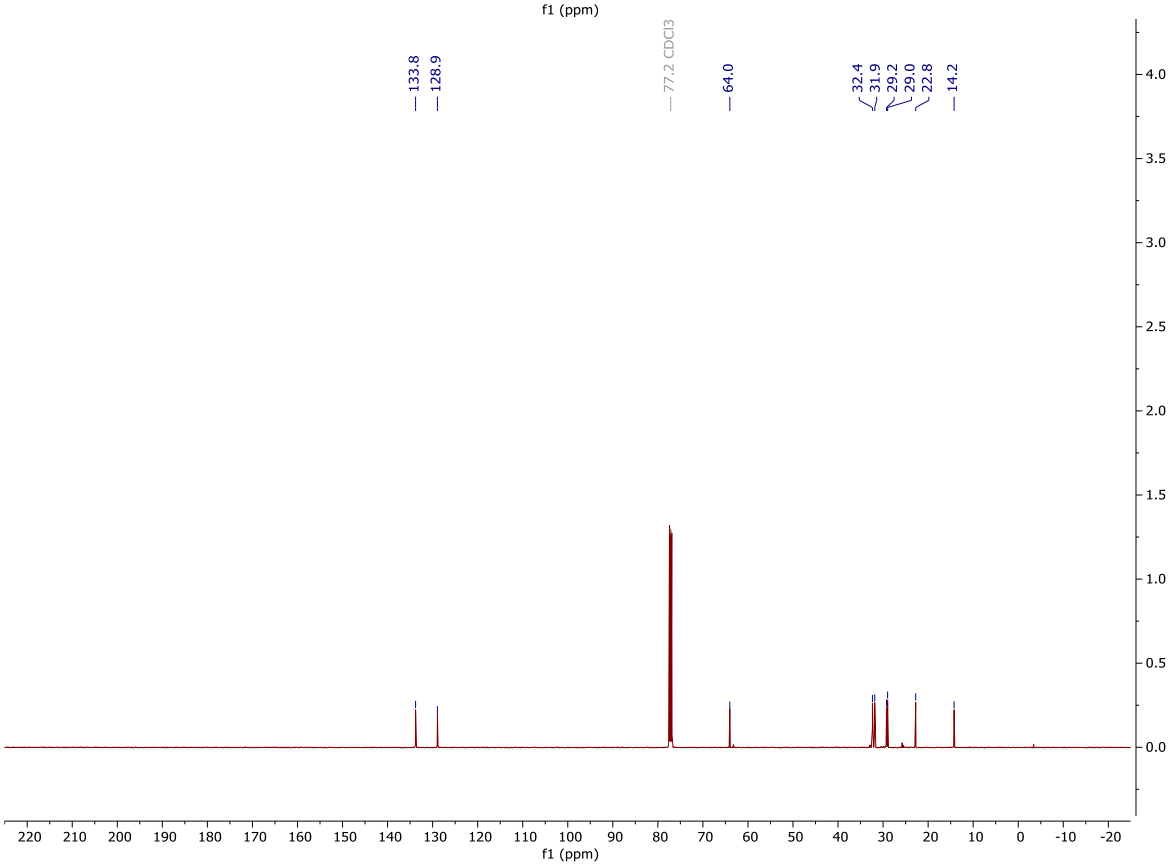
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-53**



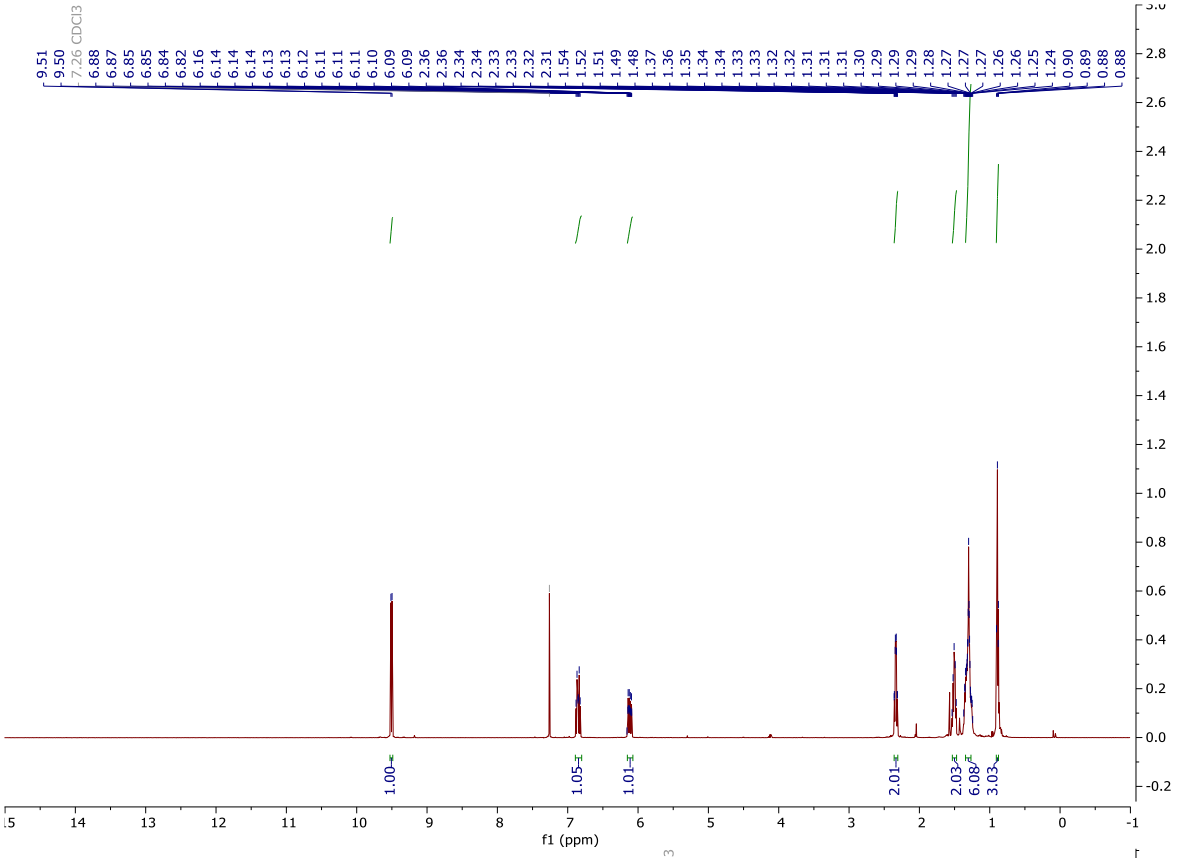


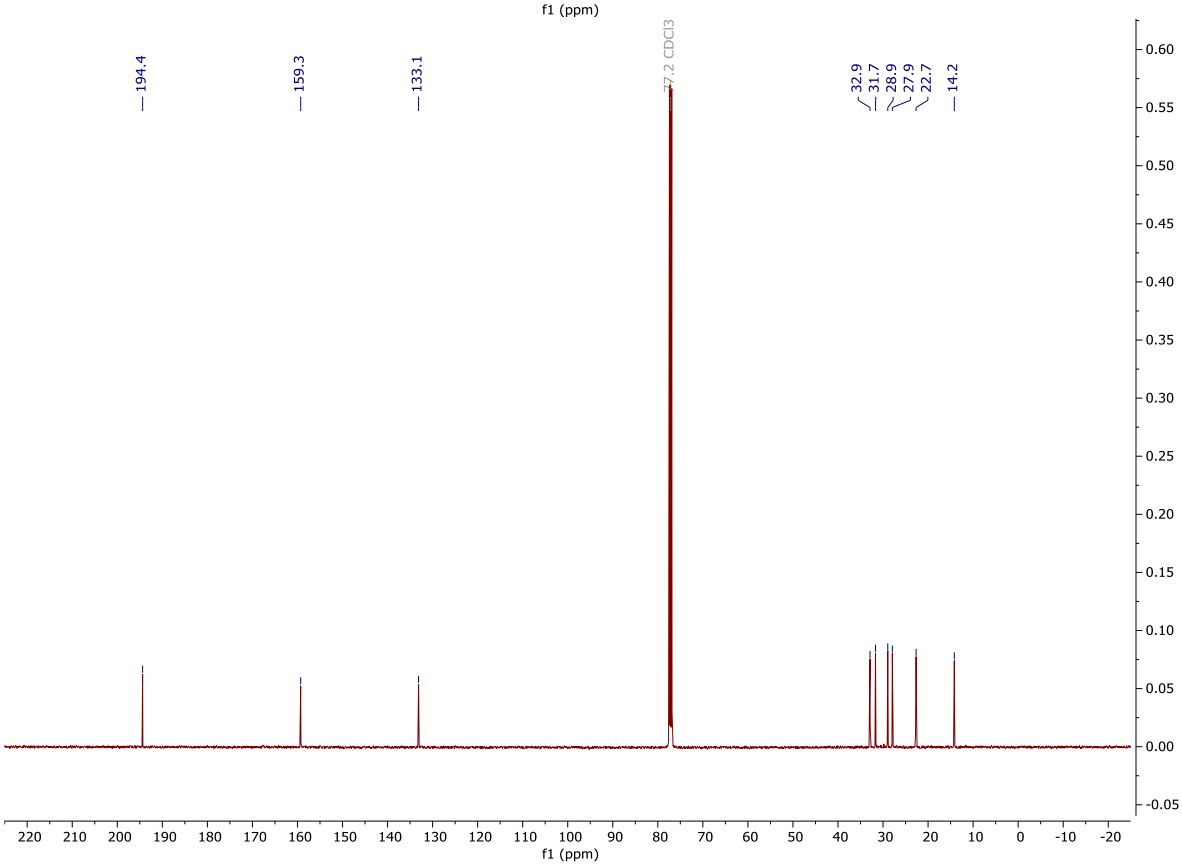
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-49**



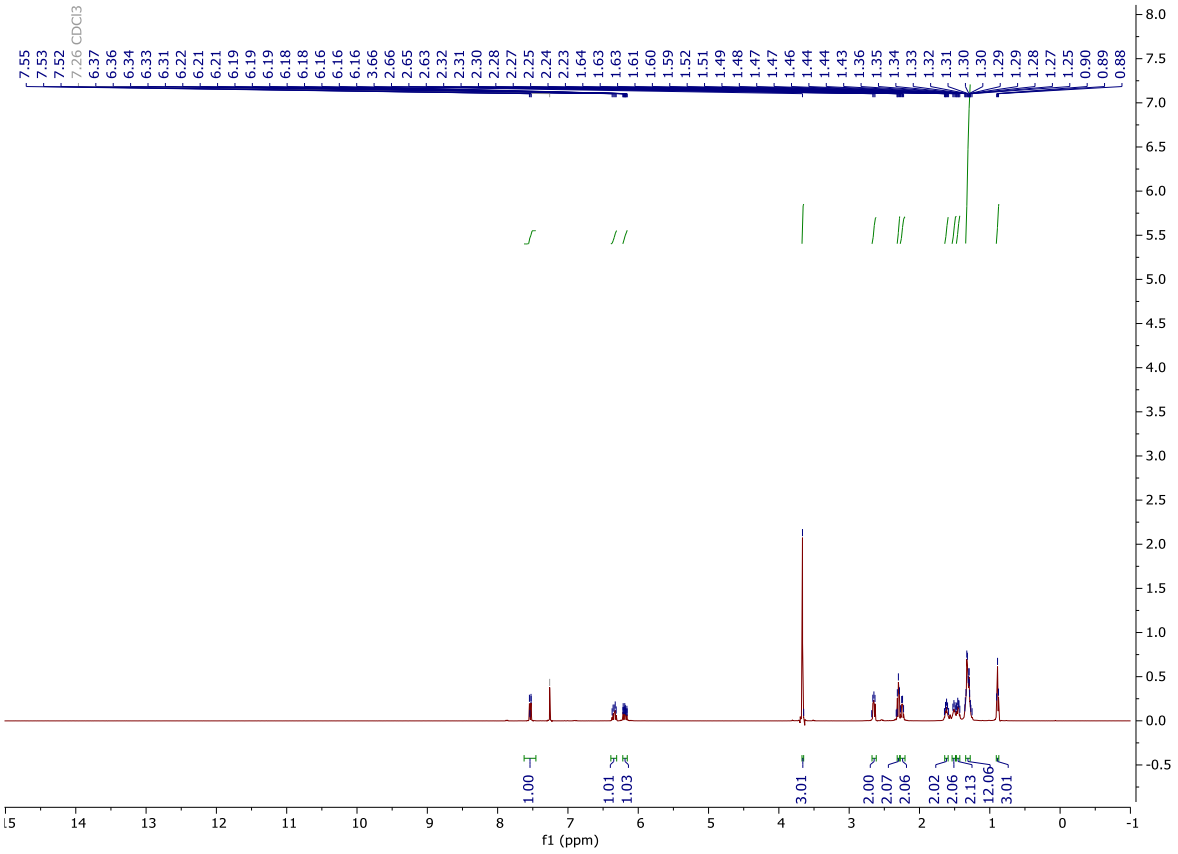


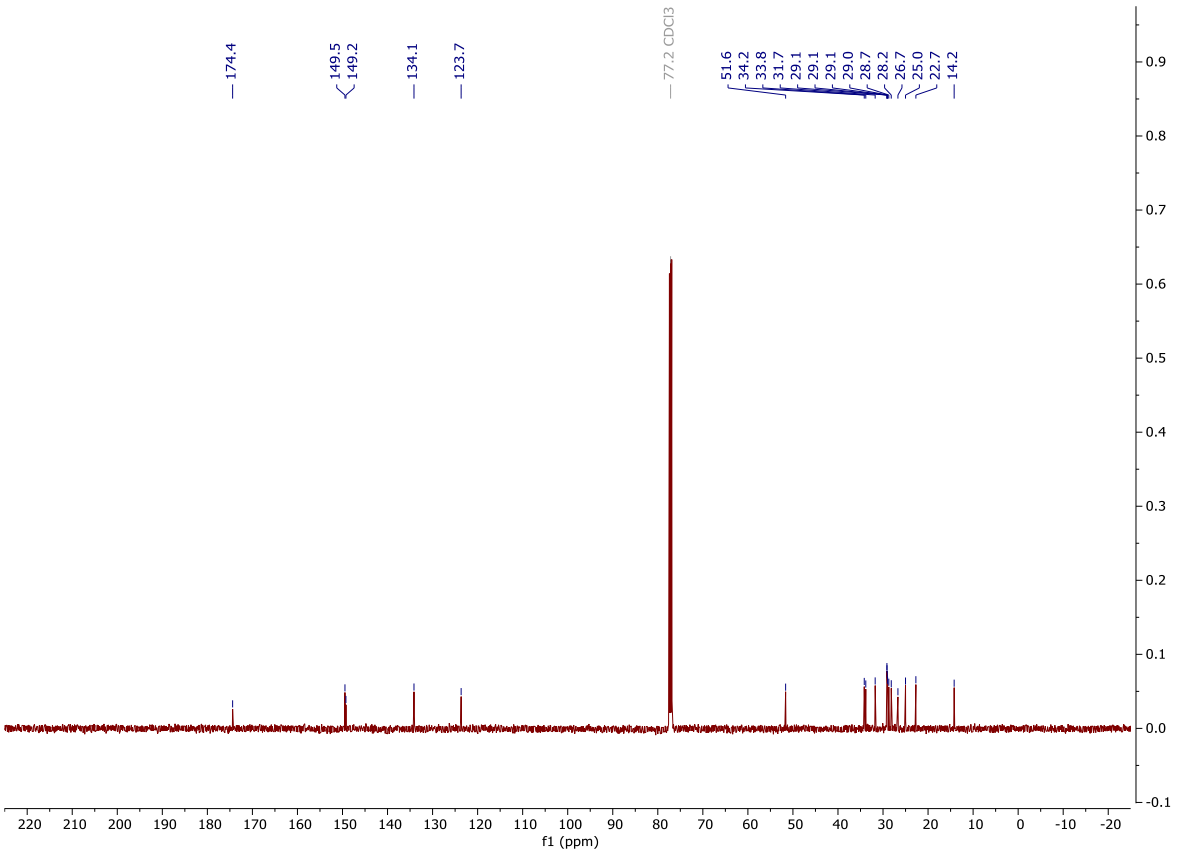
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-48**



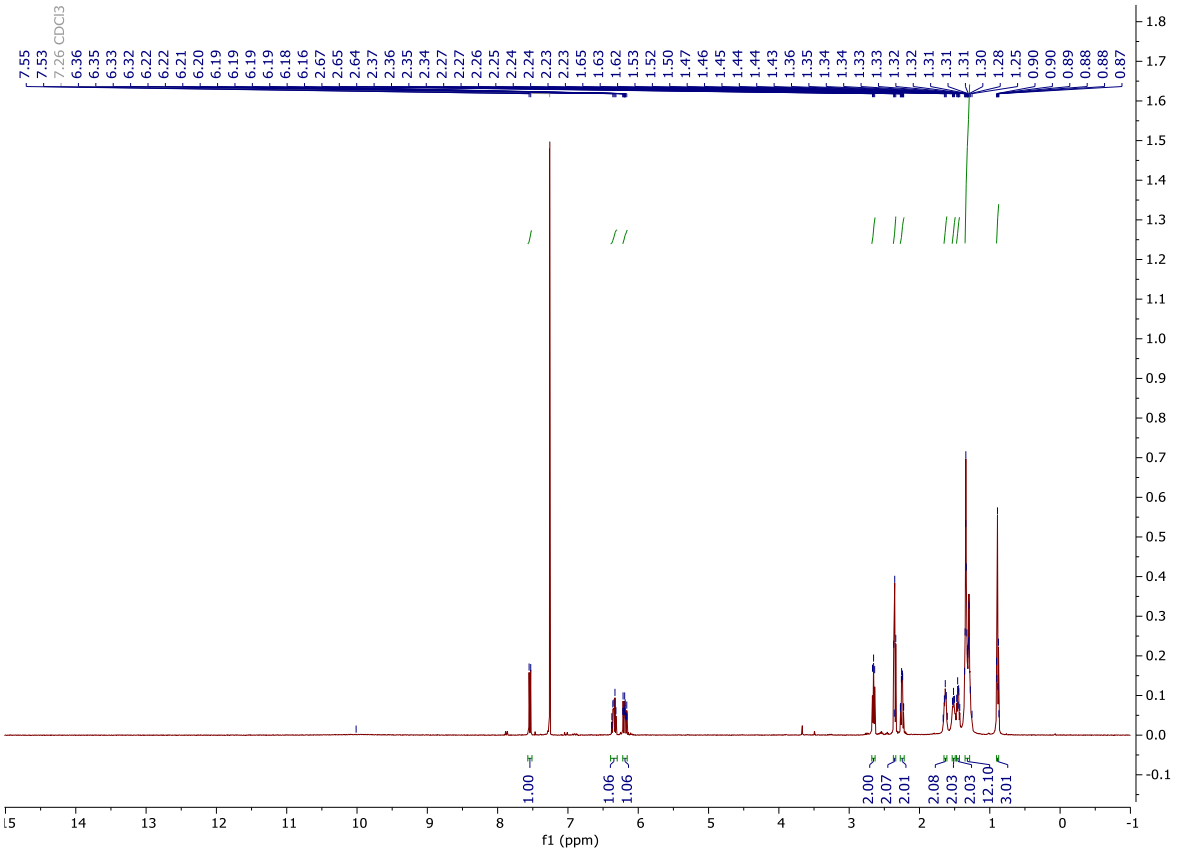


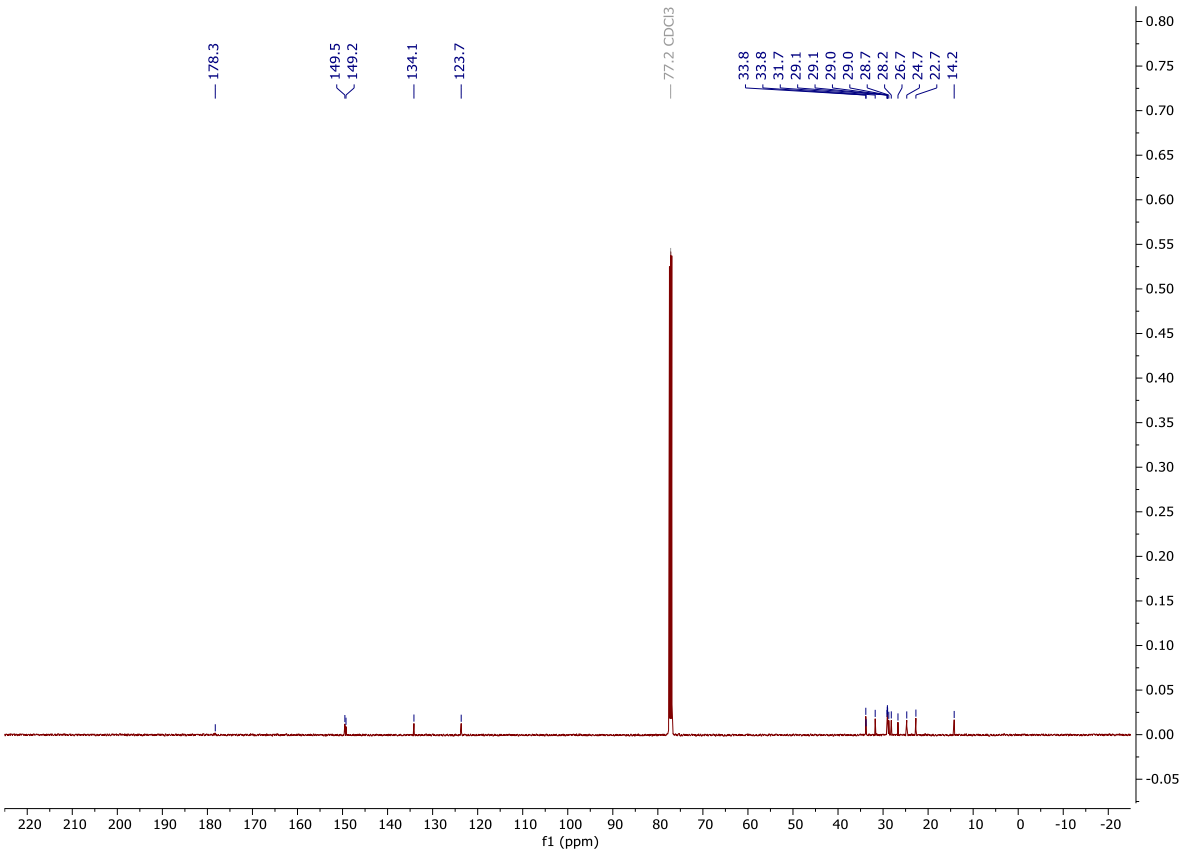
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-54b**



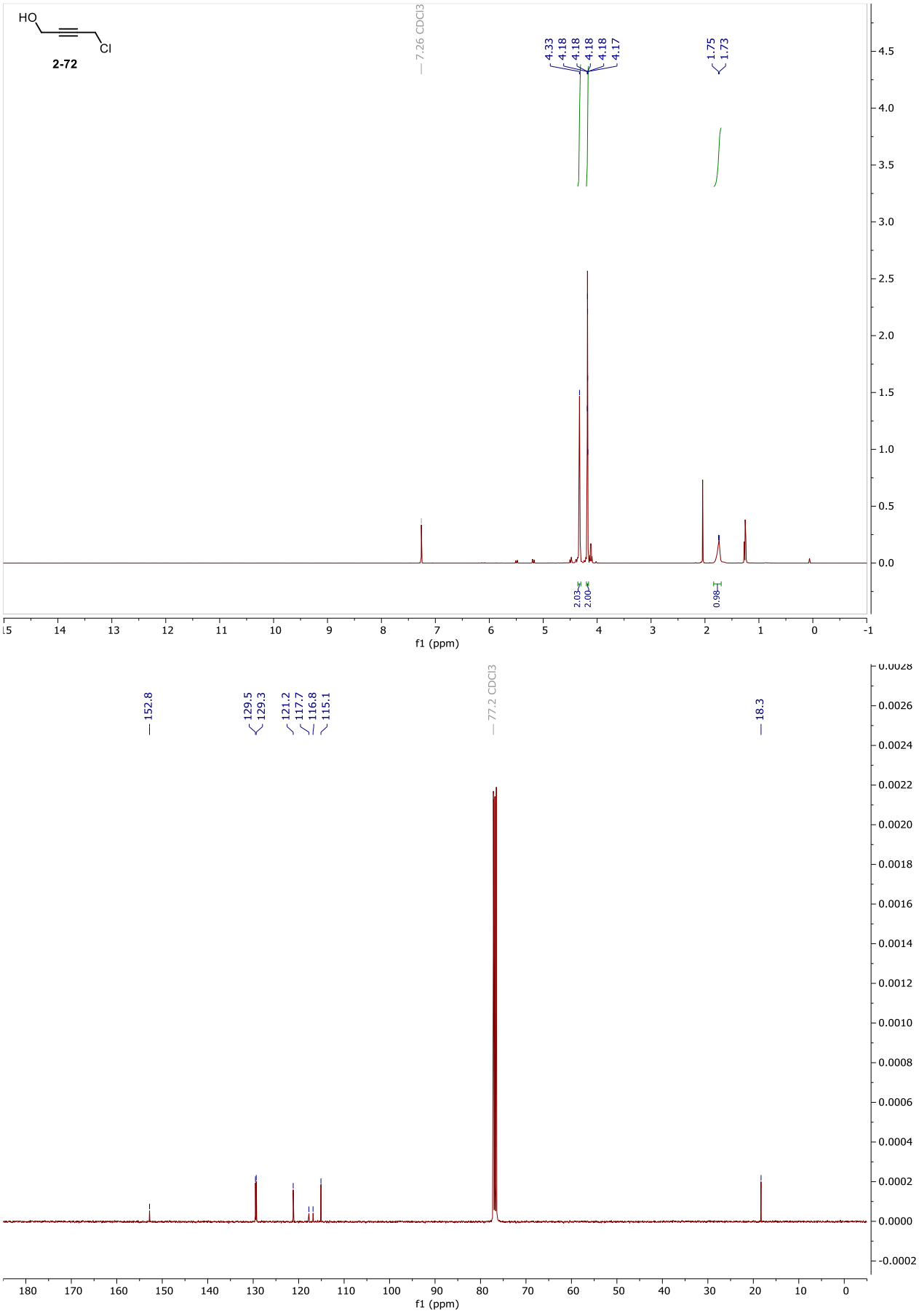


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **9-NO_2cLA, 2-5**

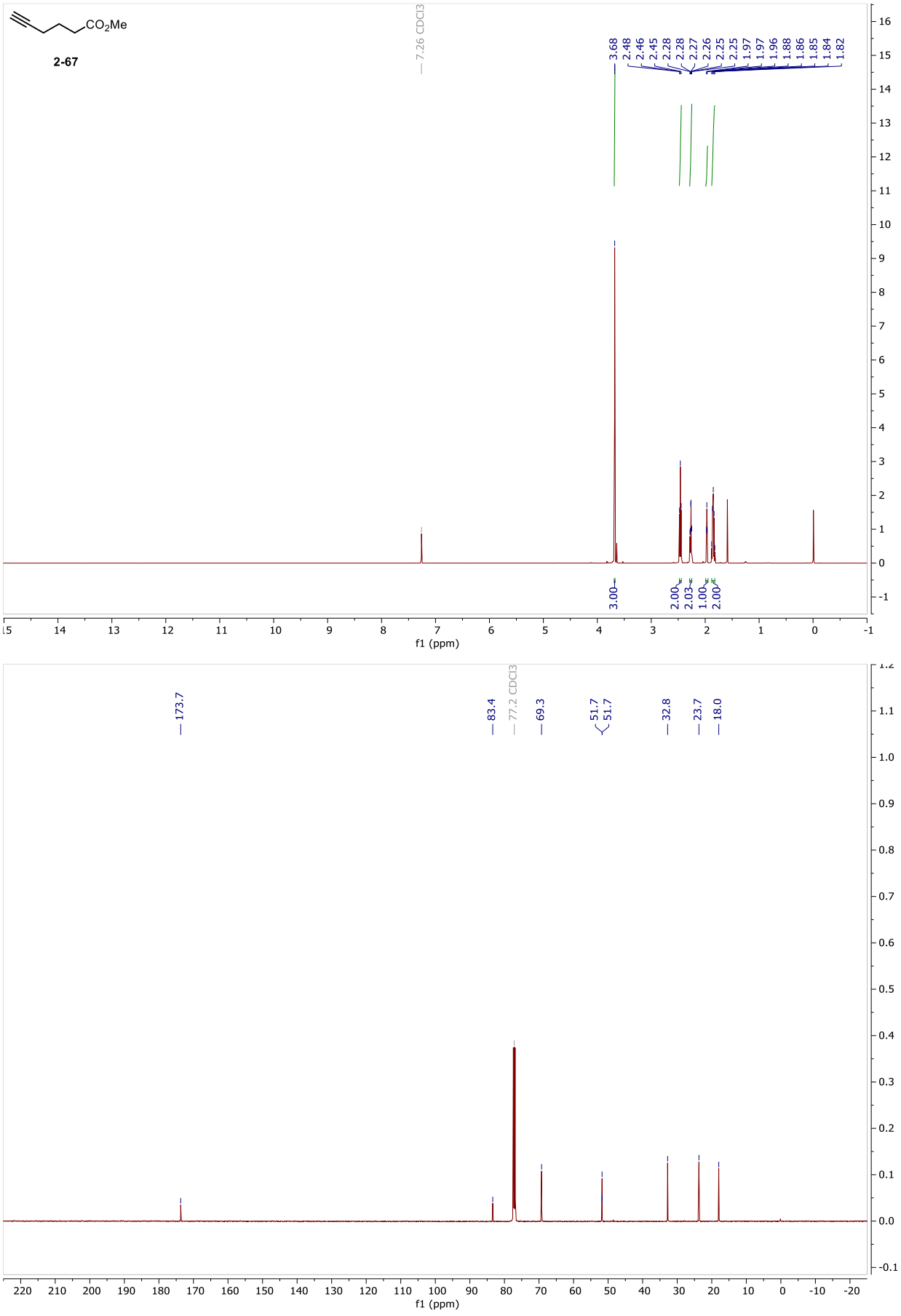




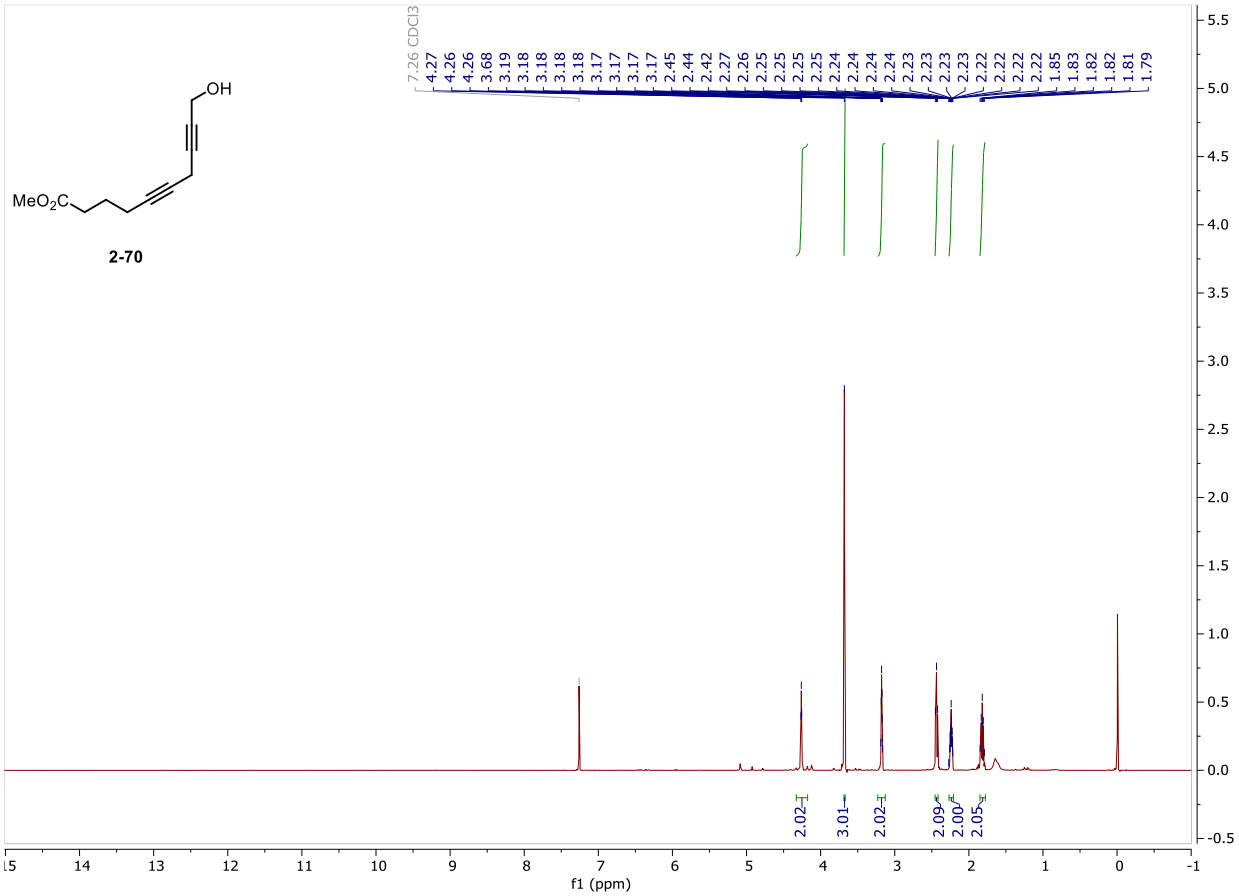
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-72**

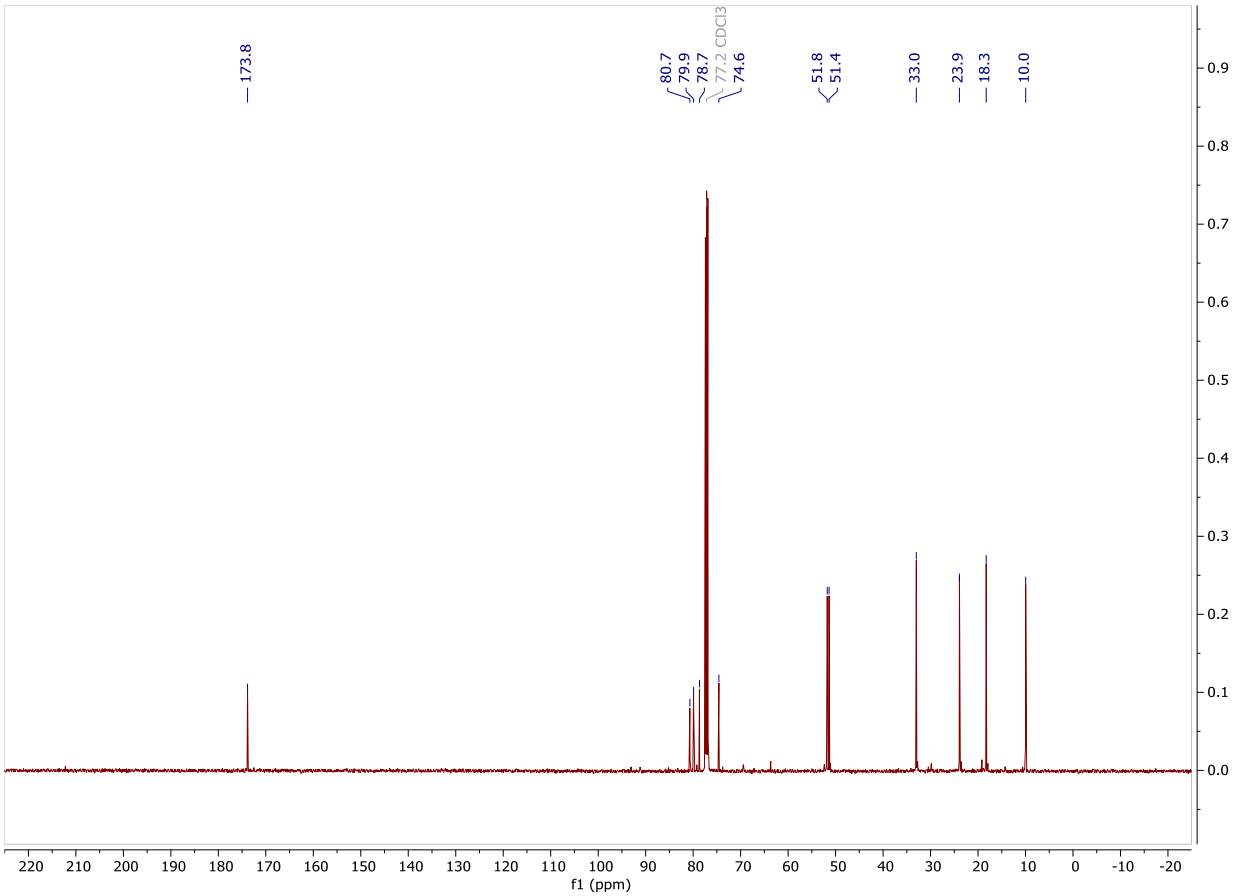


Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-67**

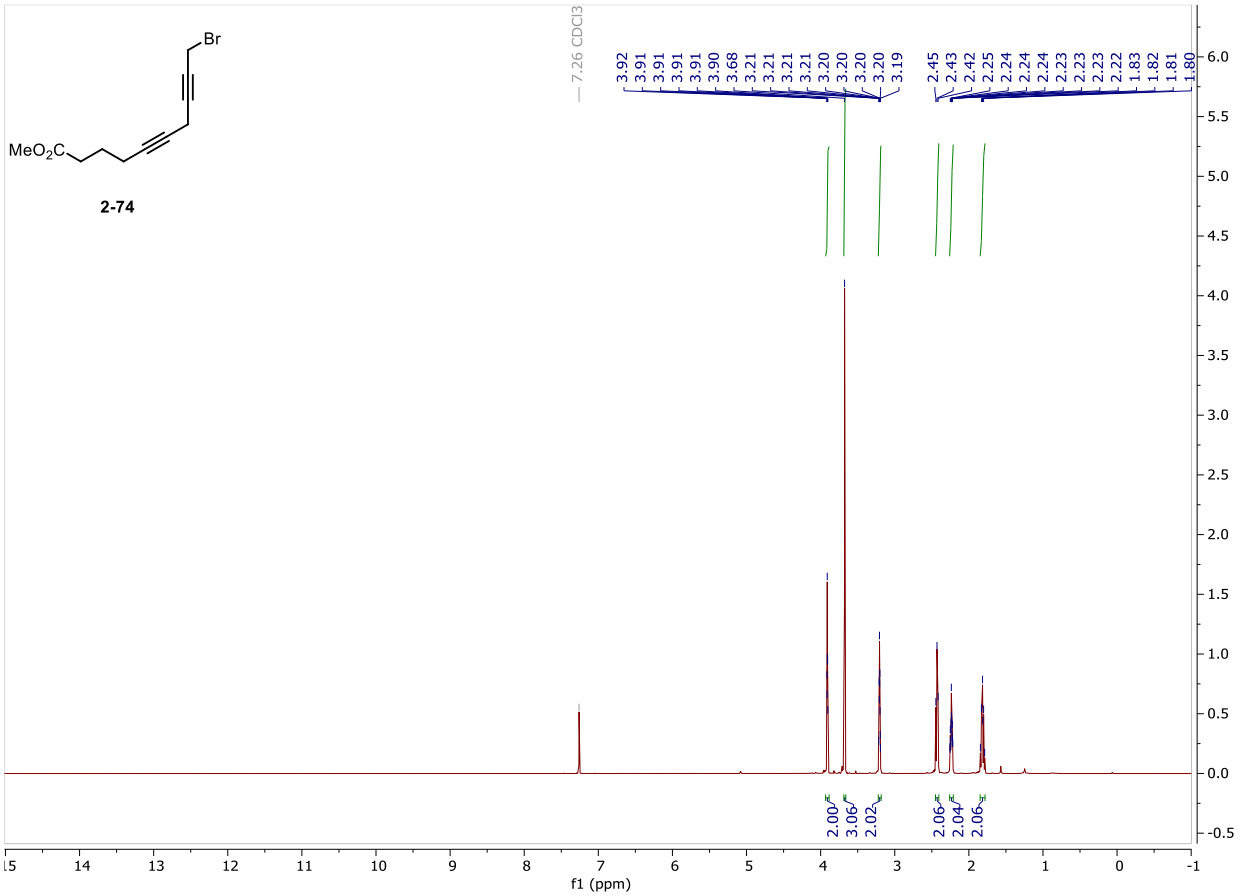


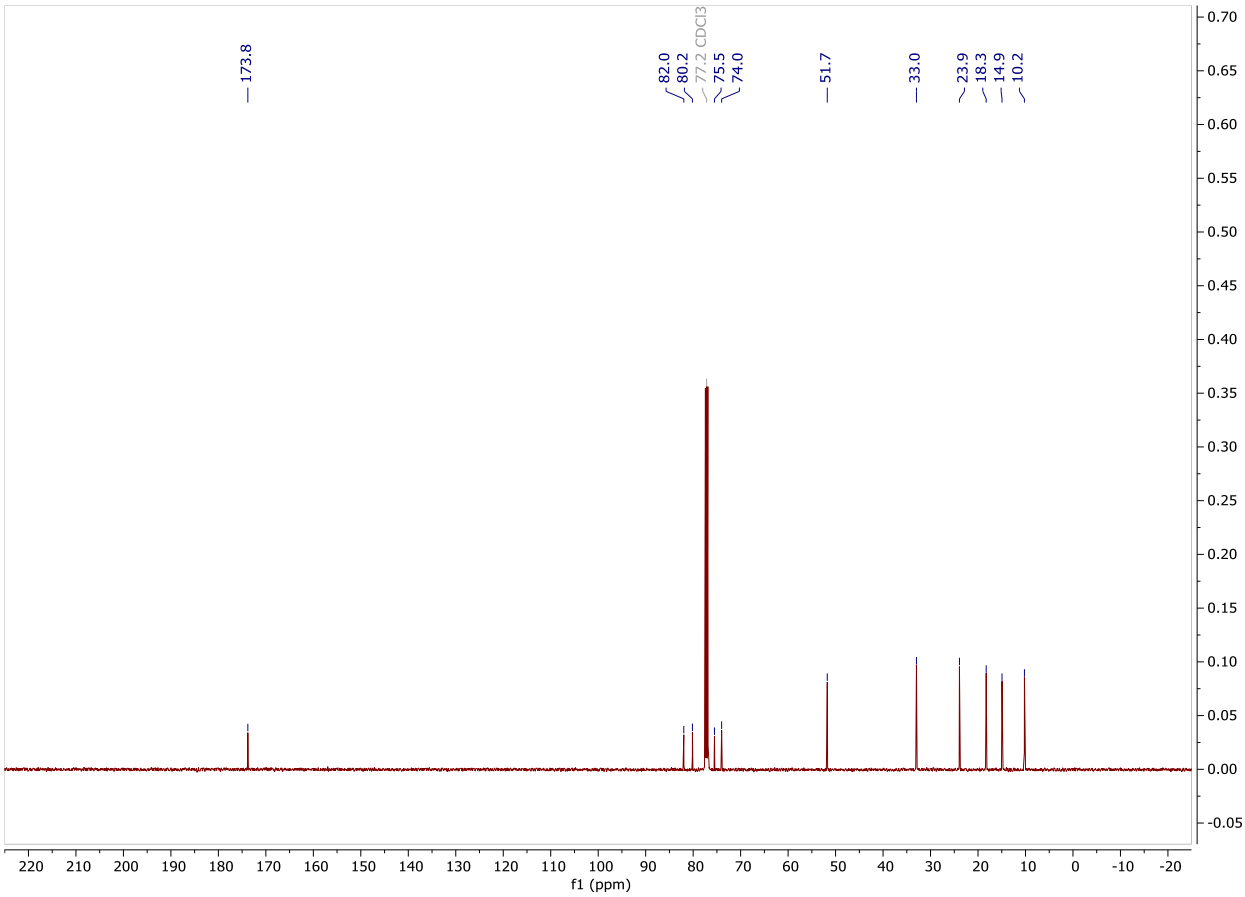
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-70**





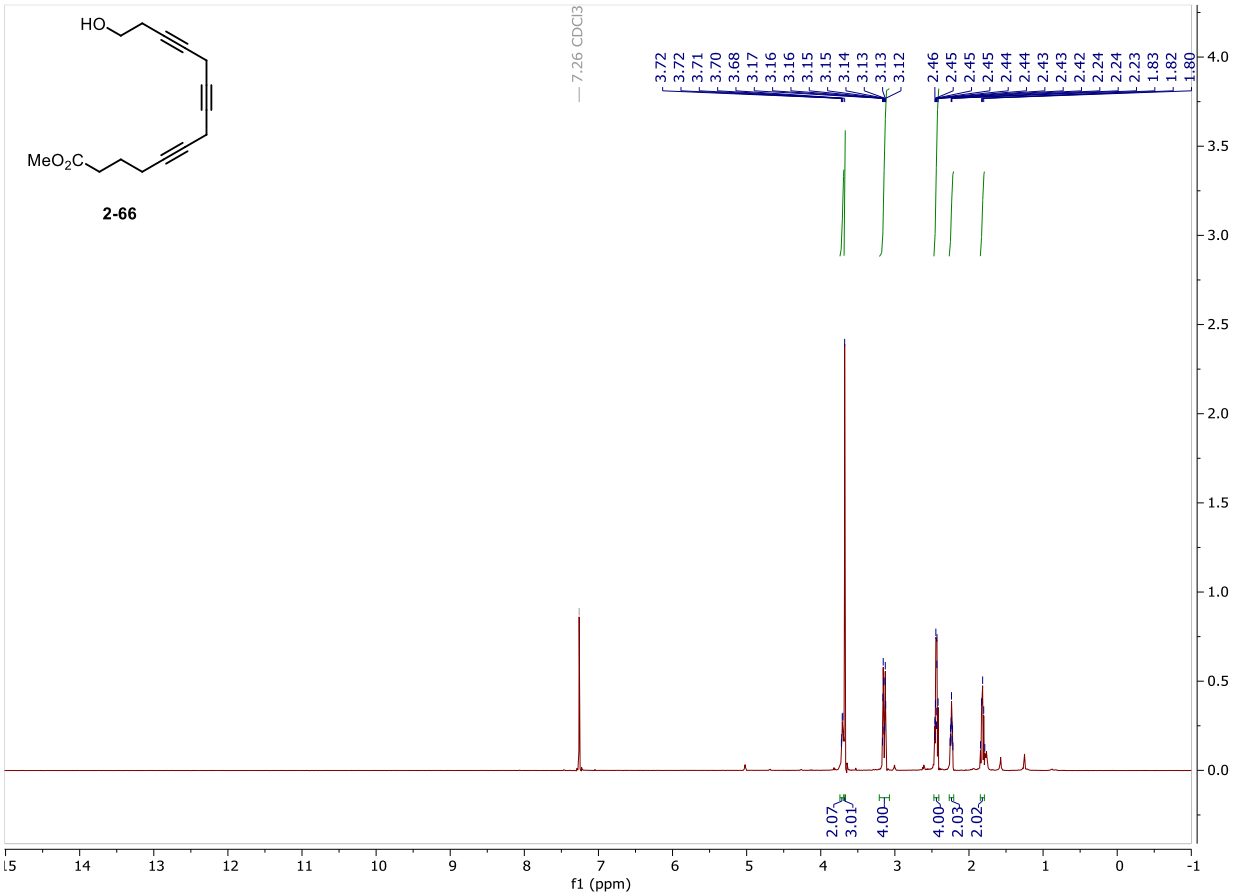
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-74**

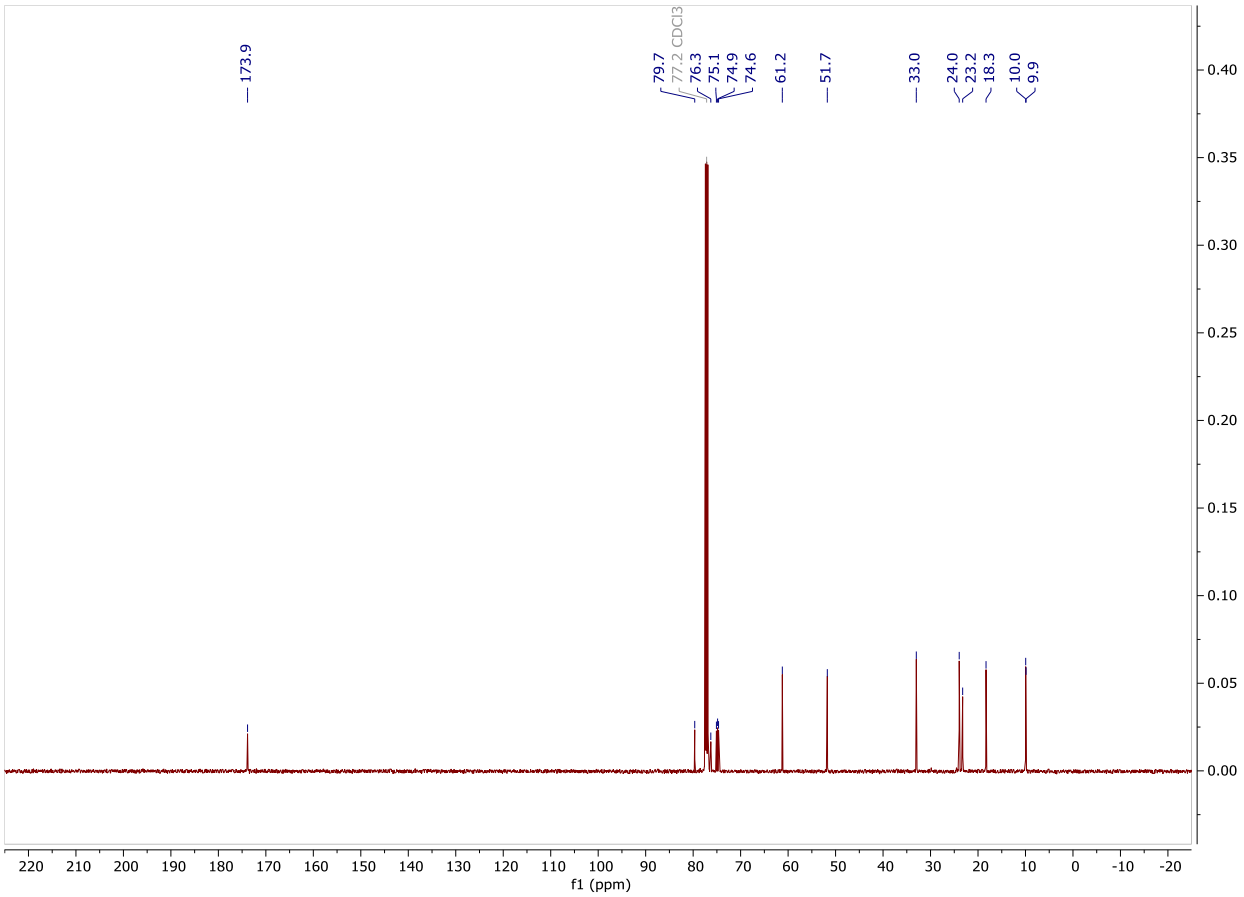




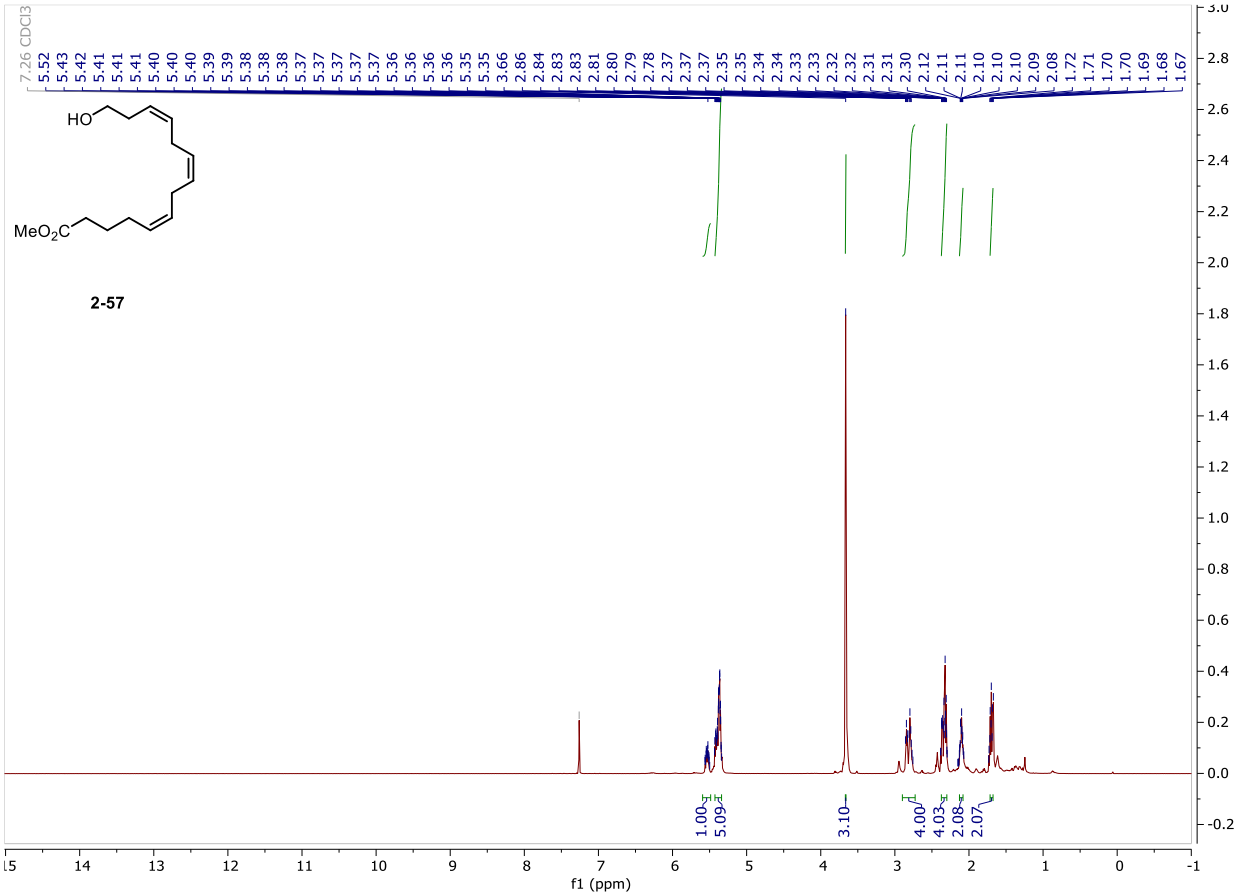
NMR and HPLC

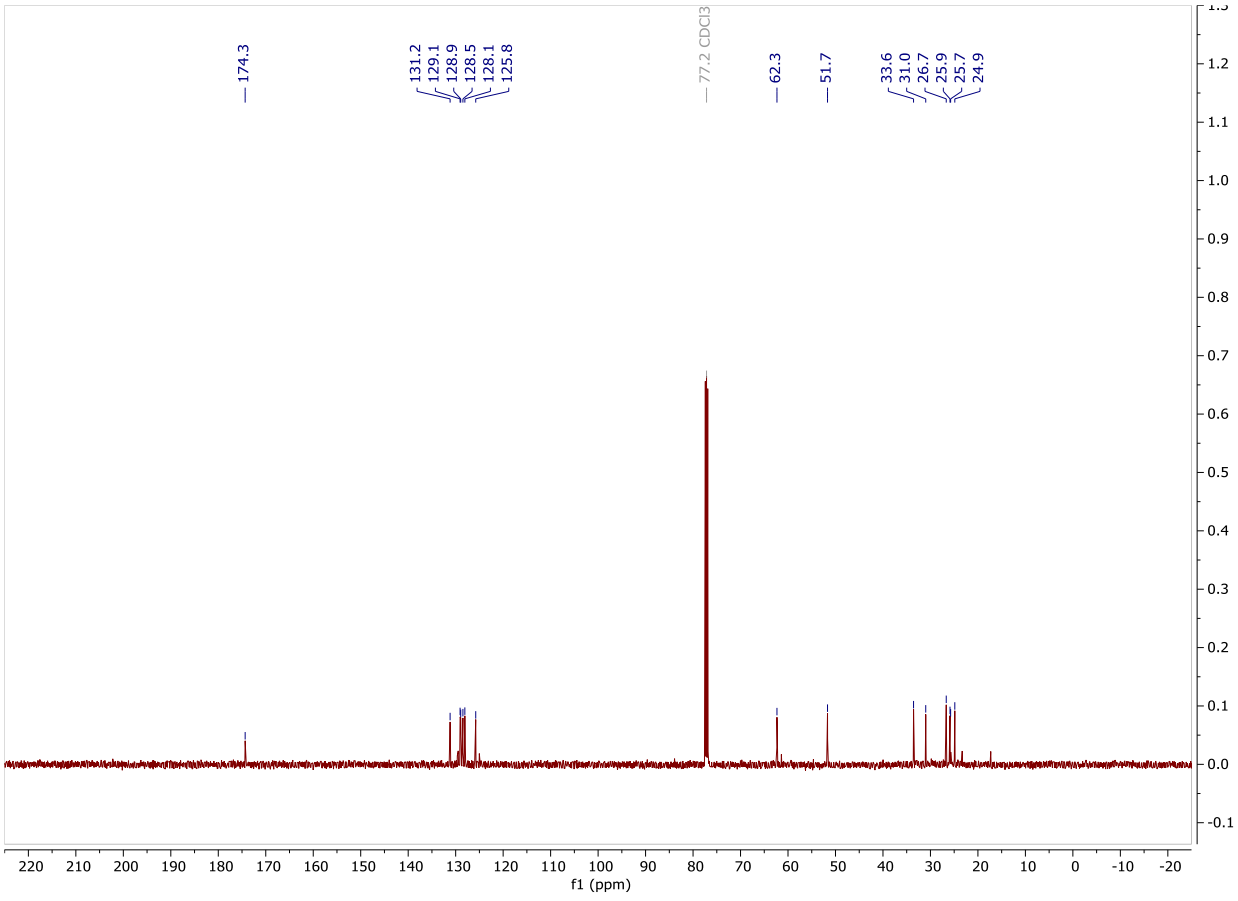
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-66**



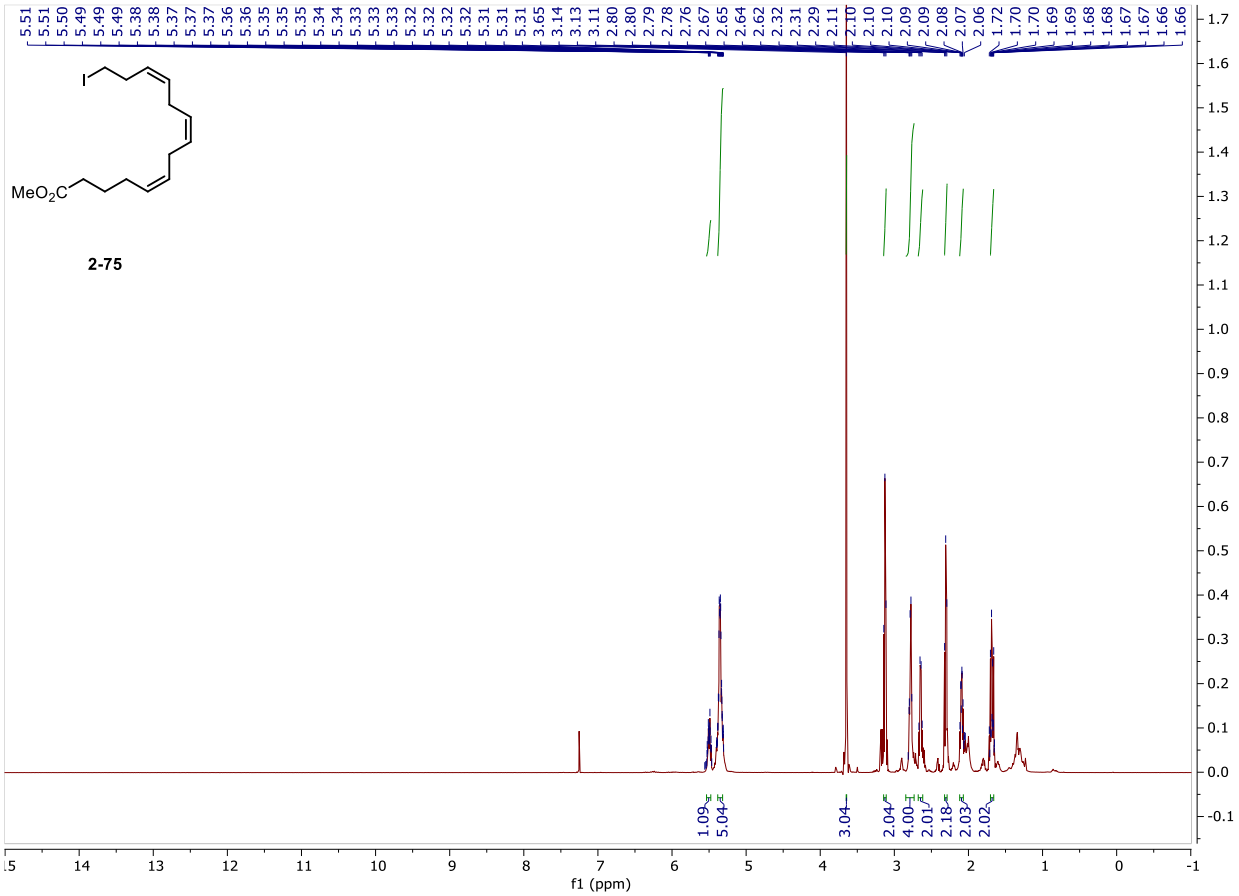


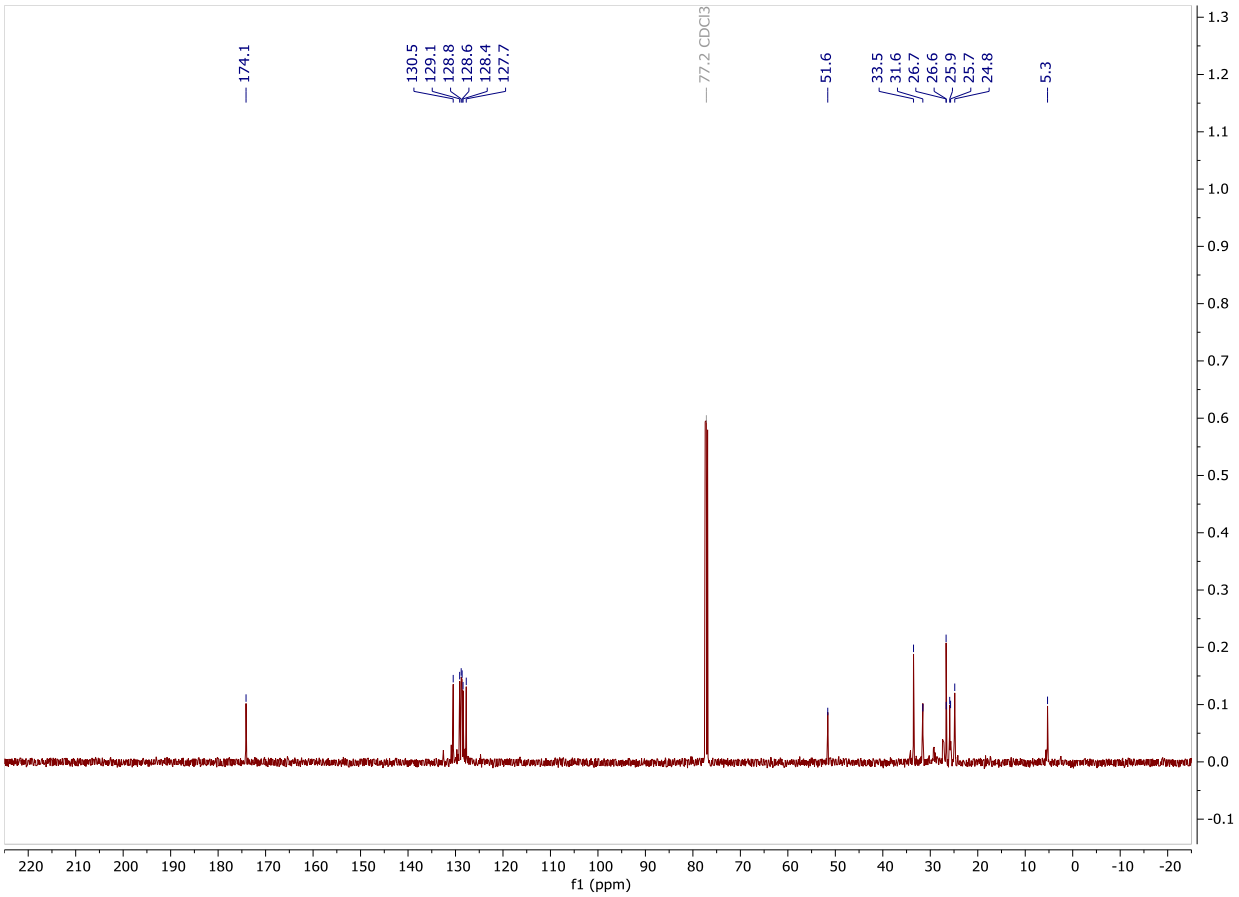
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-57**





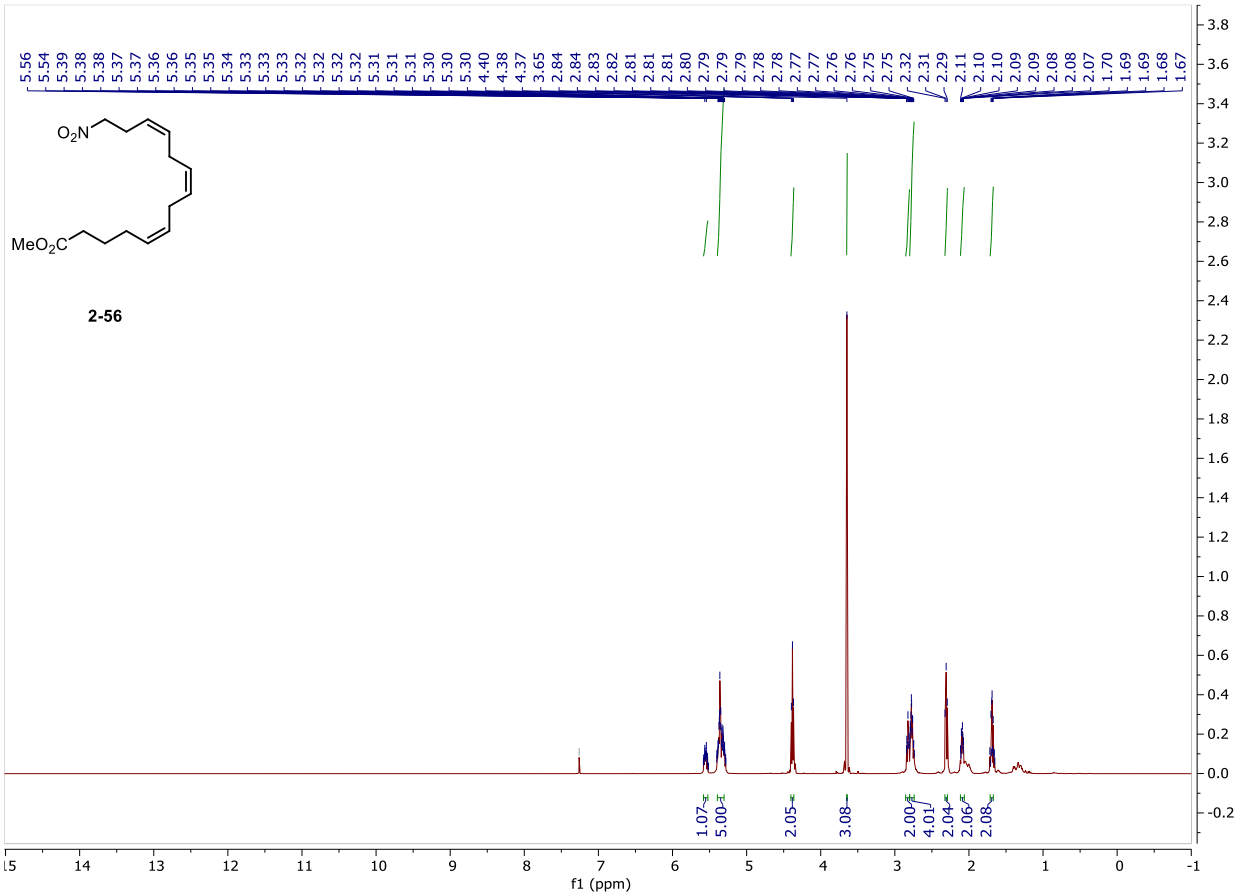
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-75**

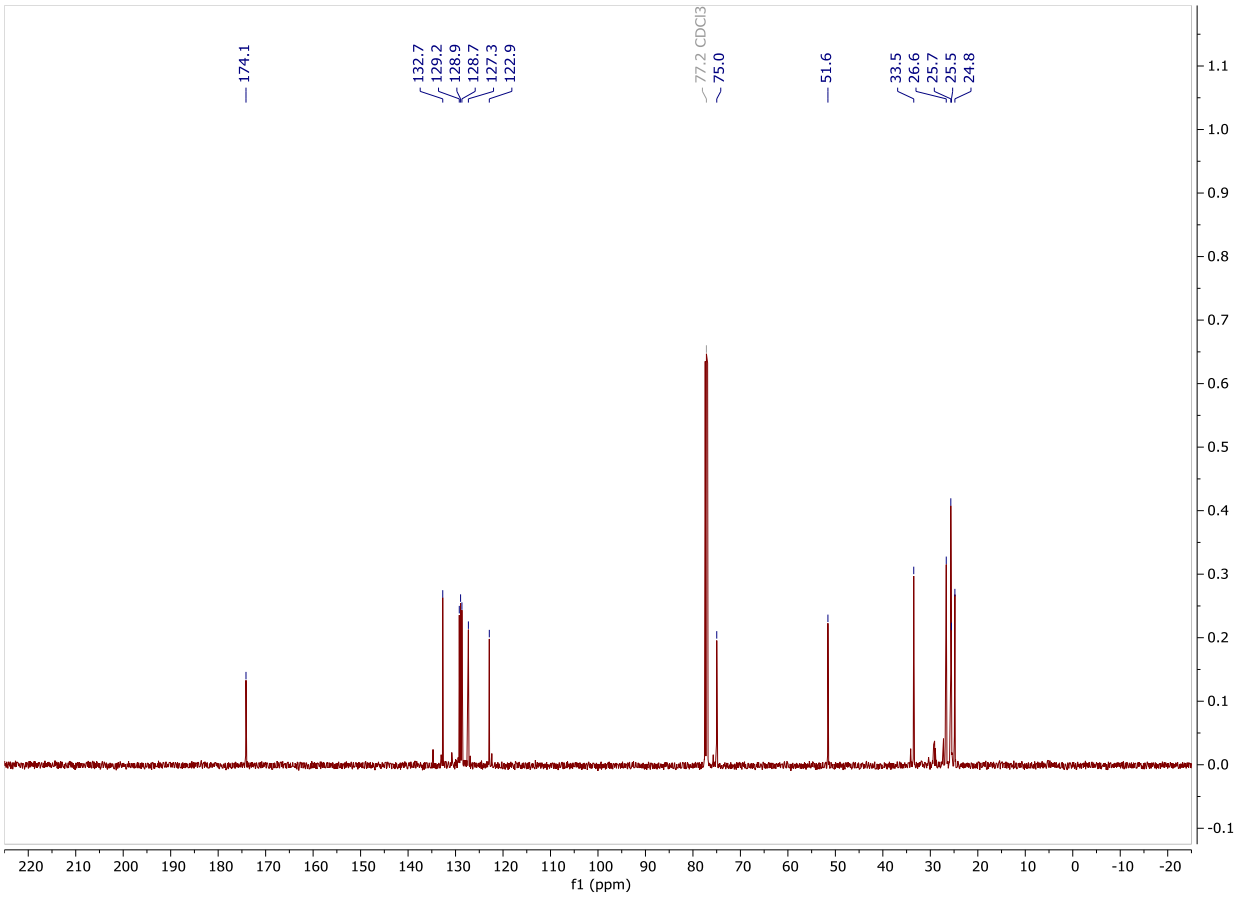




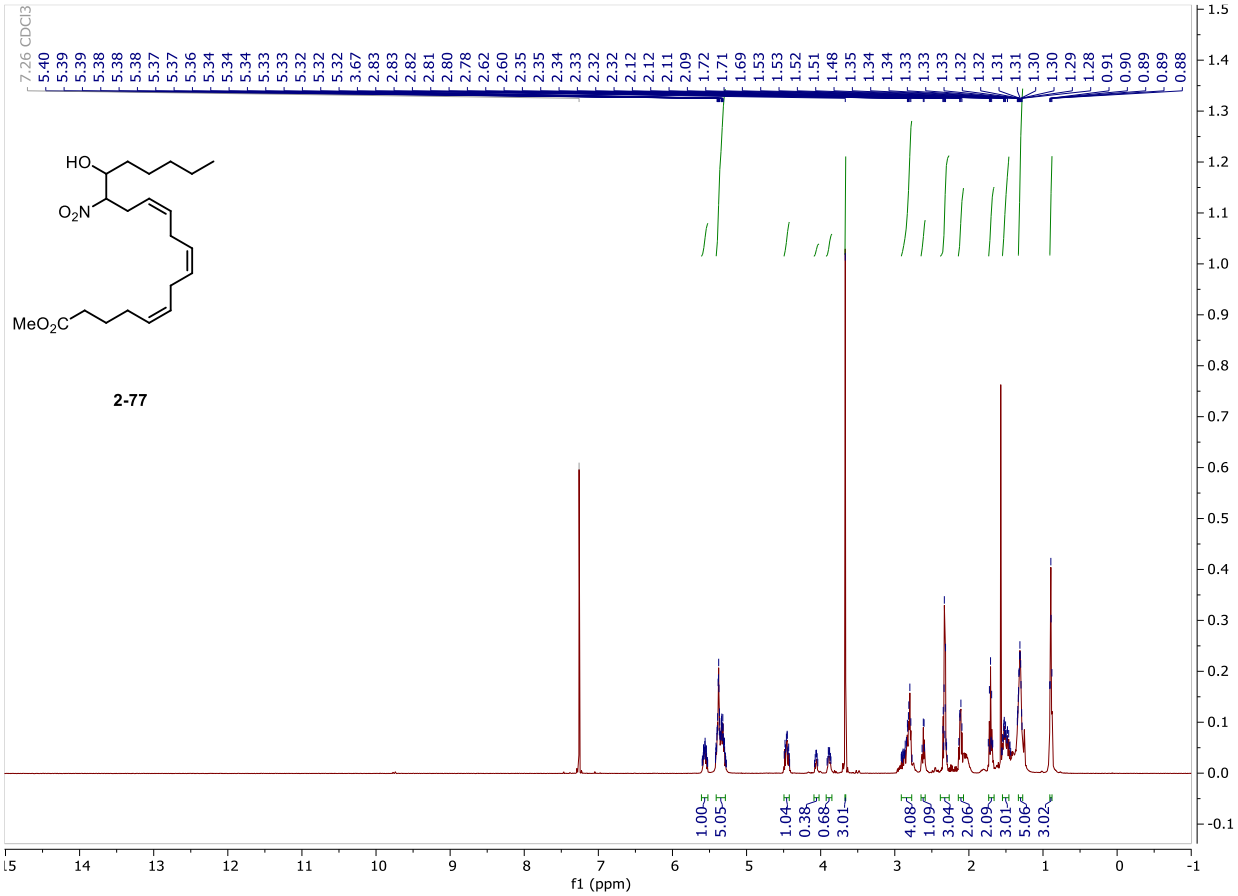
NMR and HPLC

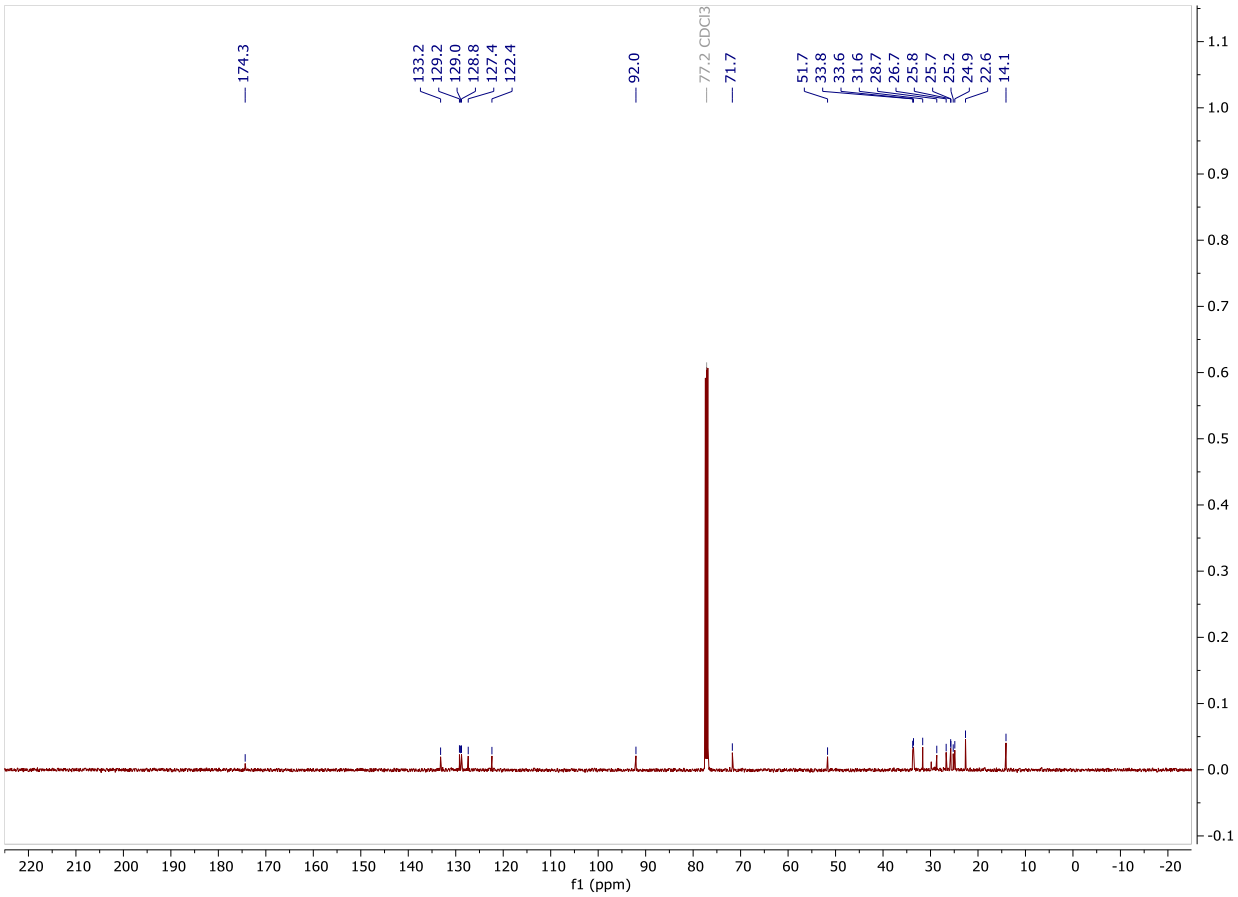
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-56**



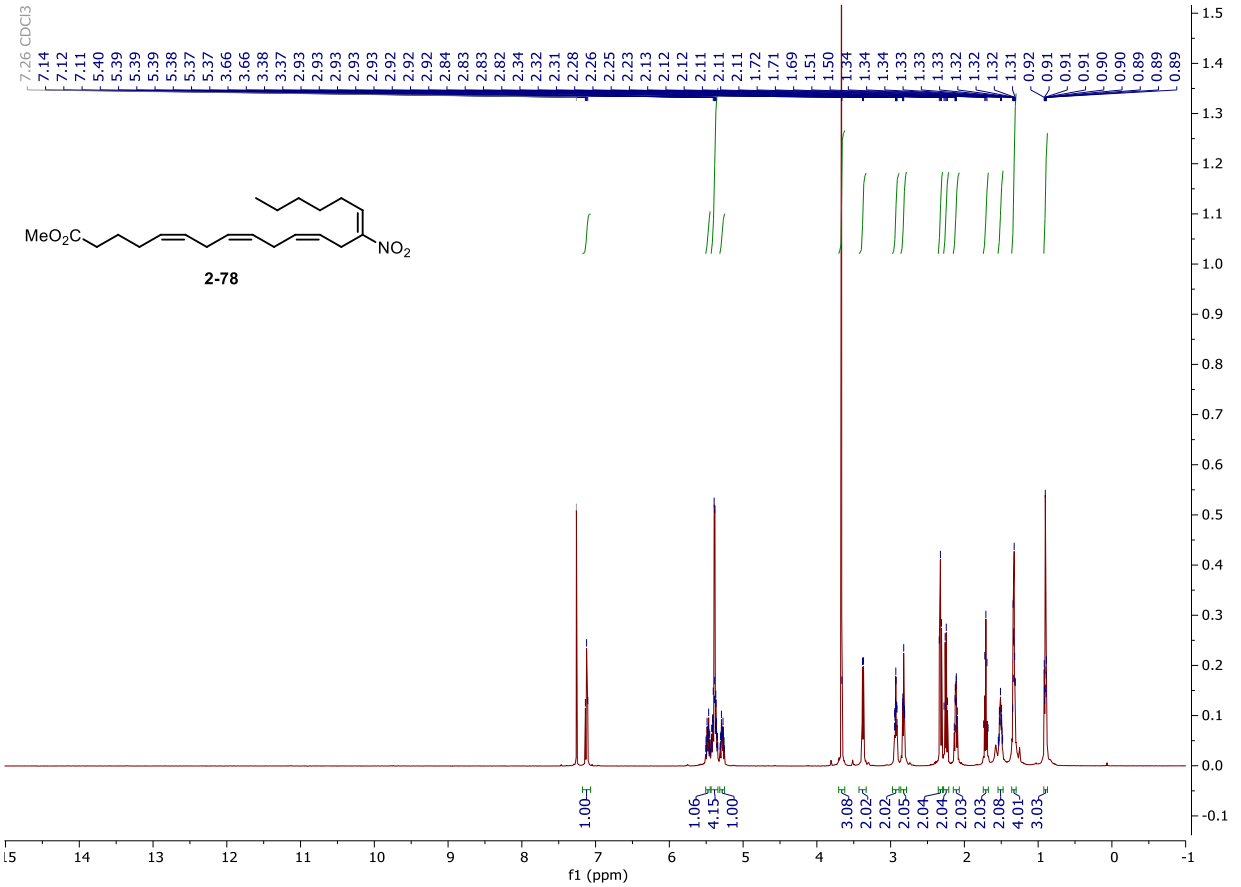


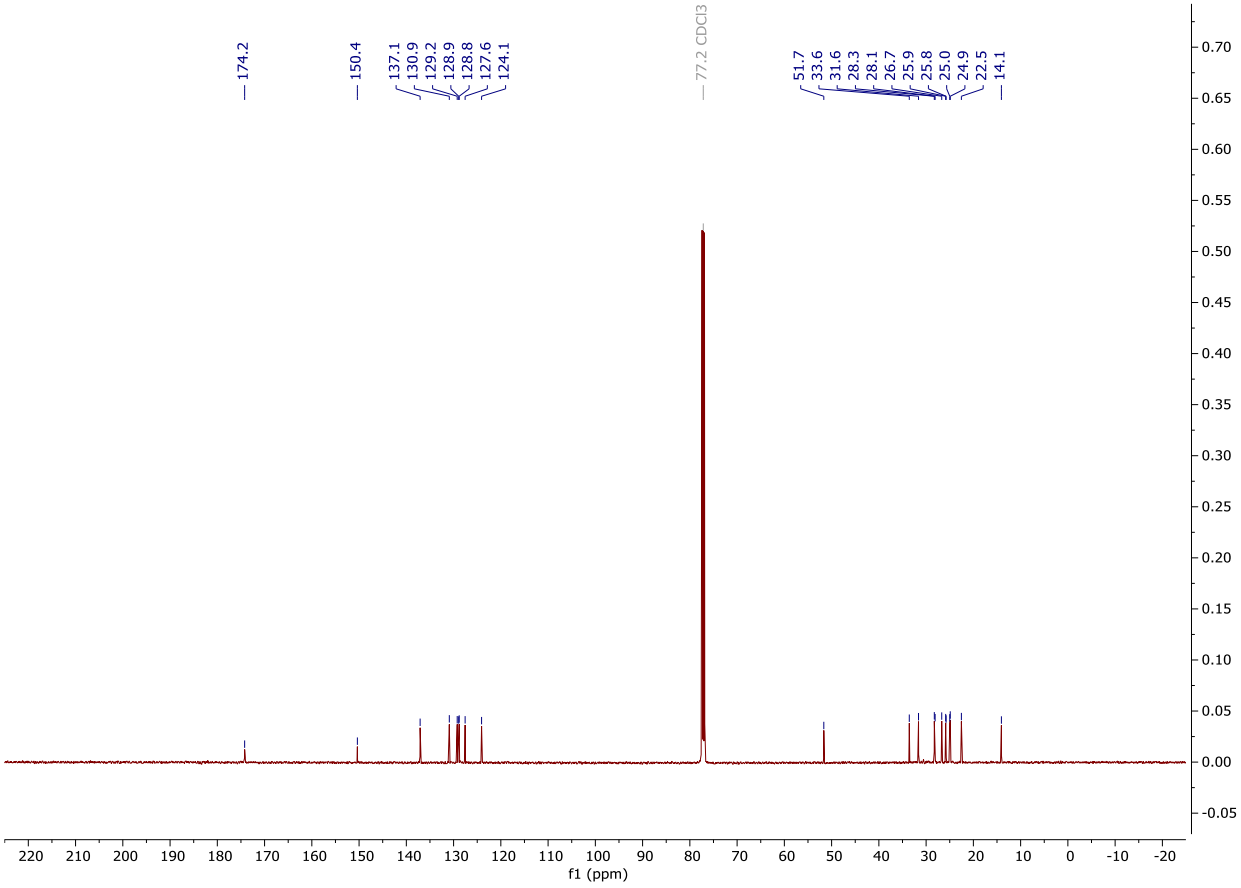
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-77**



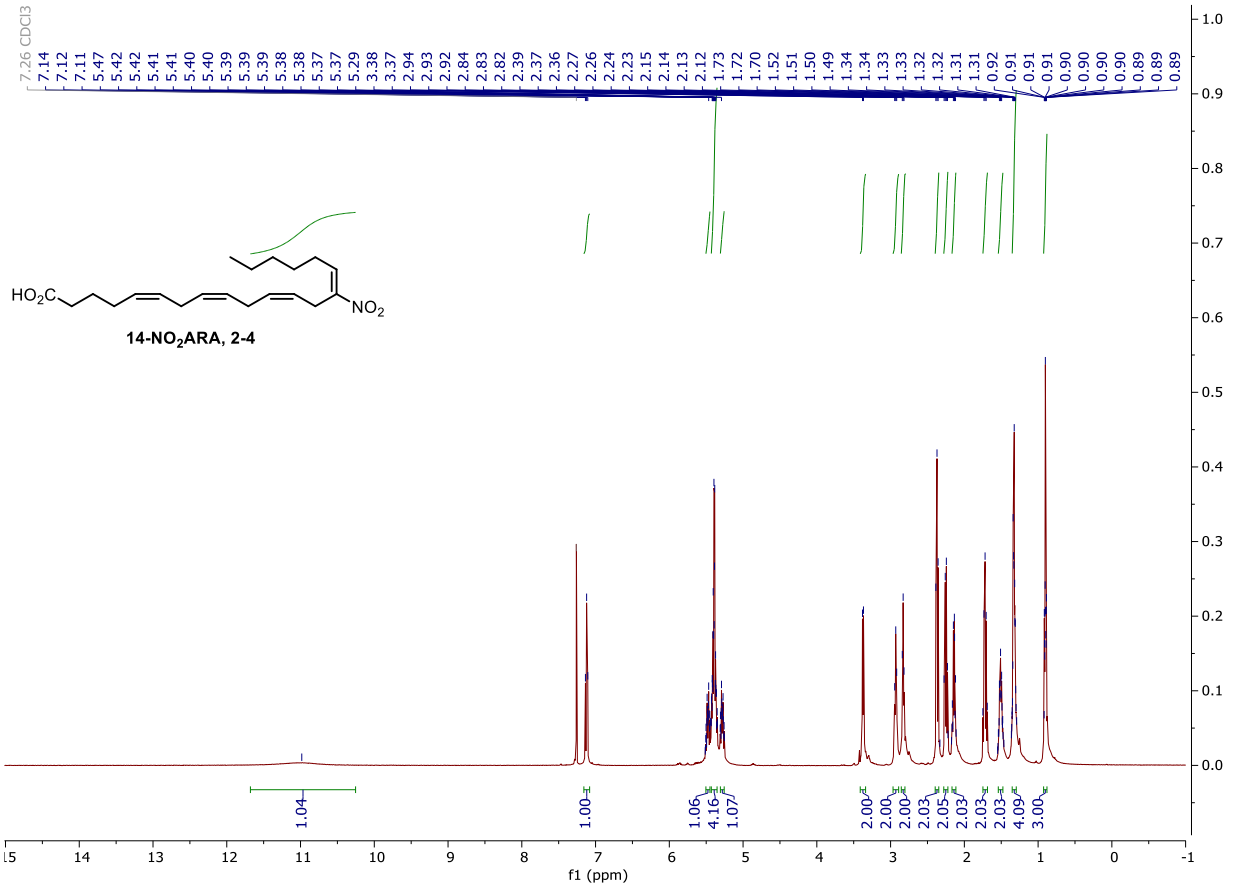


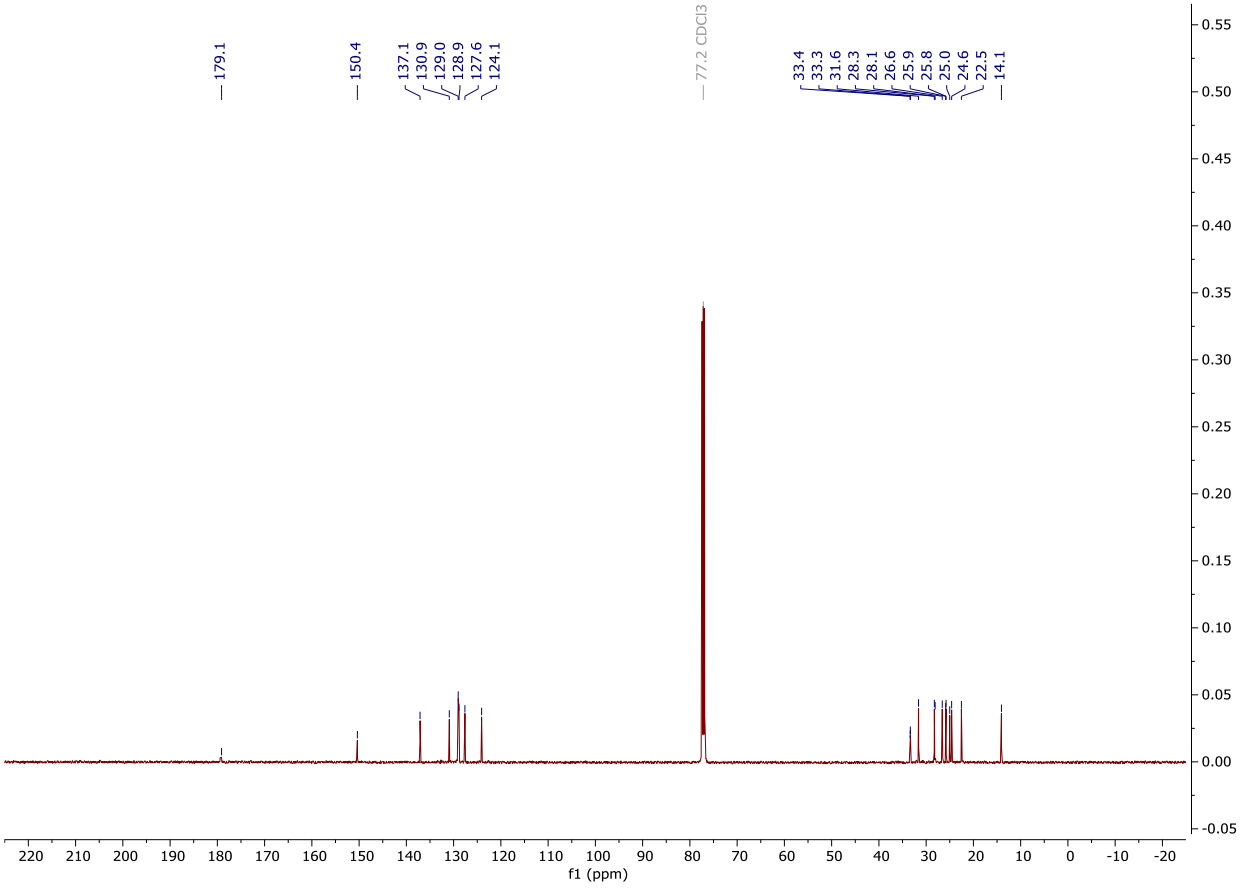
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-78**



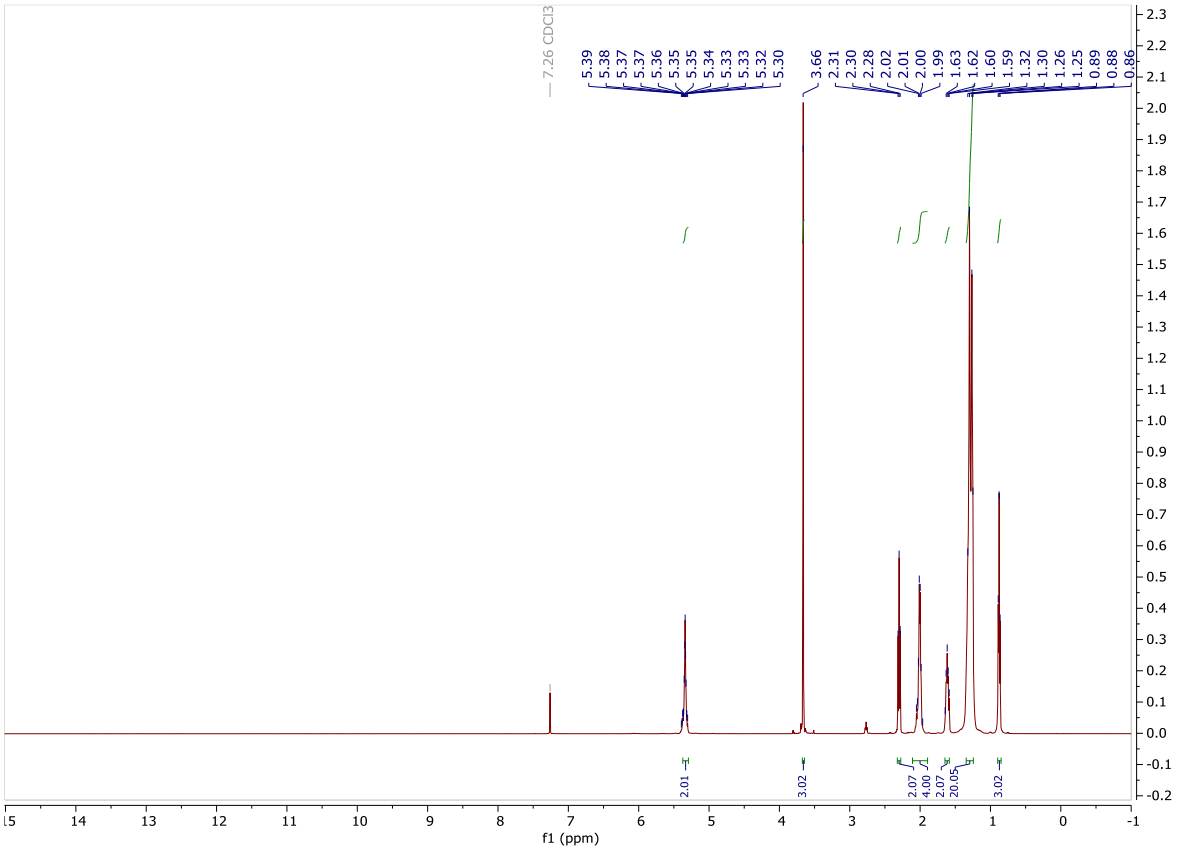


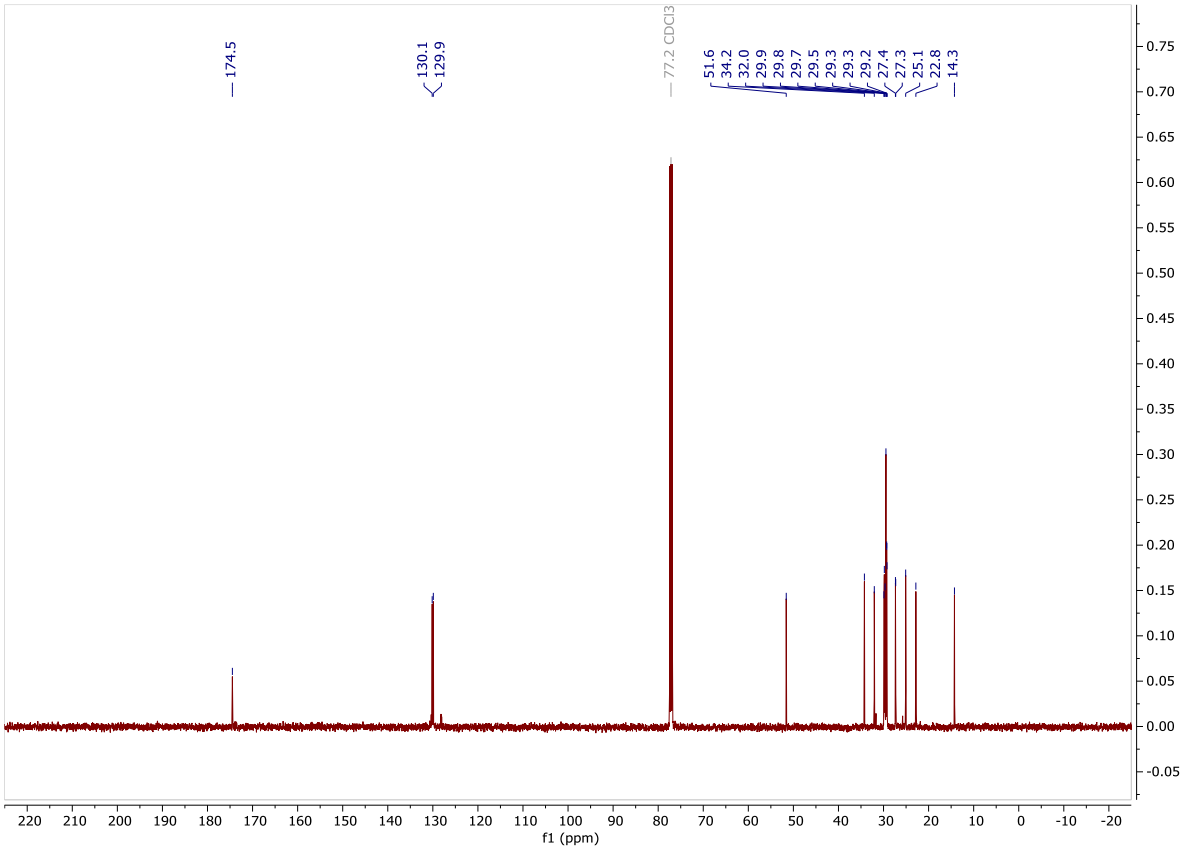
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **14-NO_2ARA, 2-4**



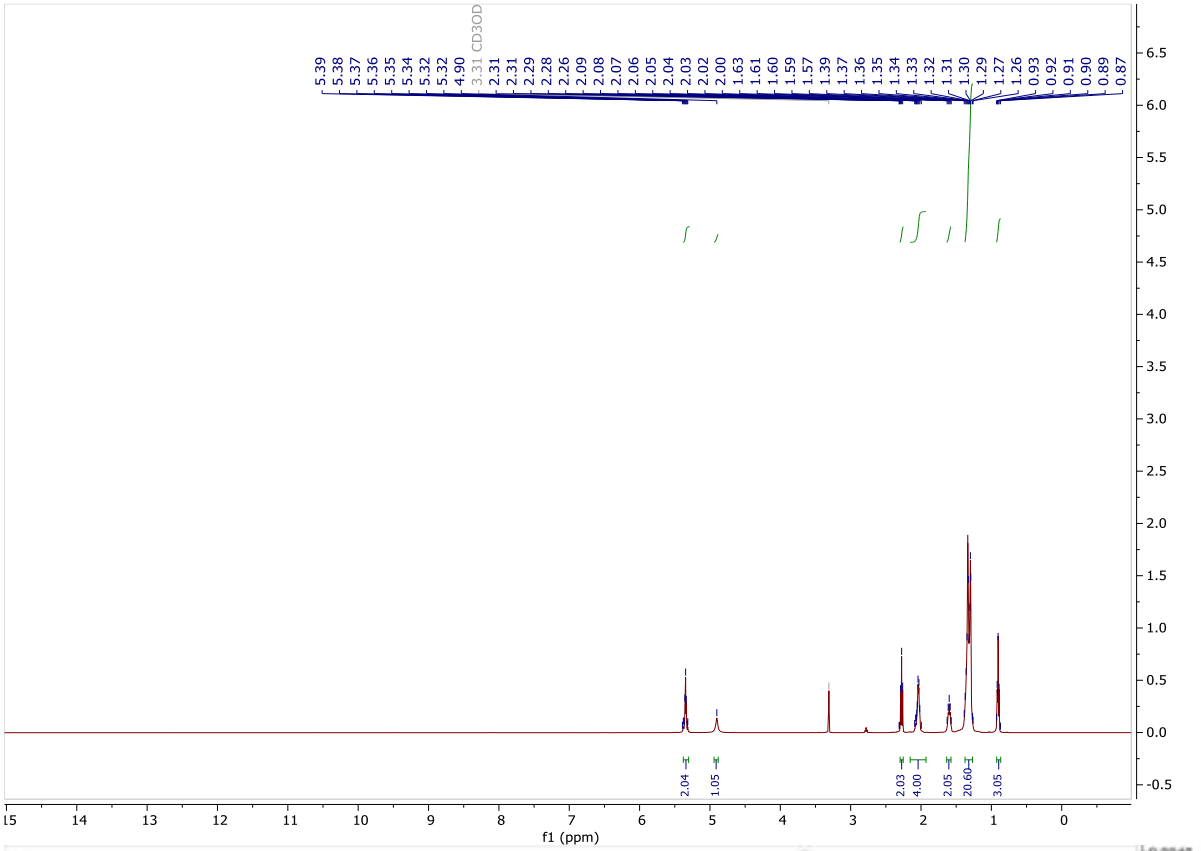


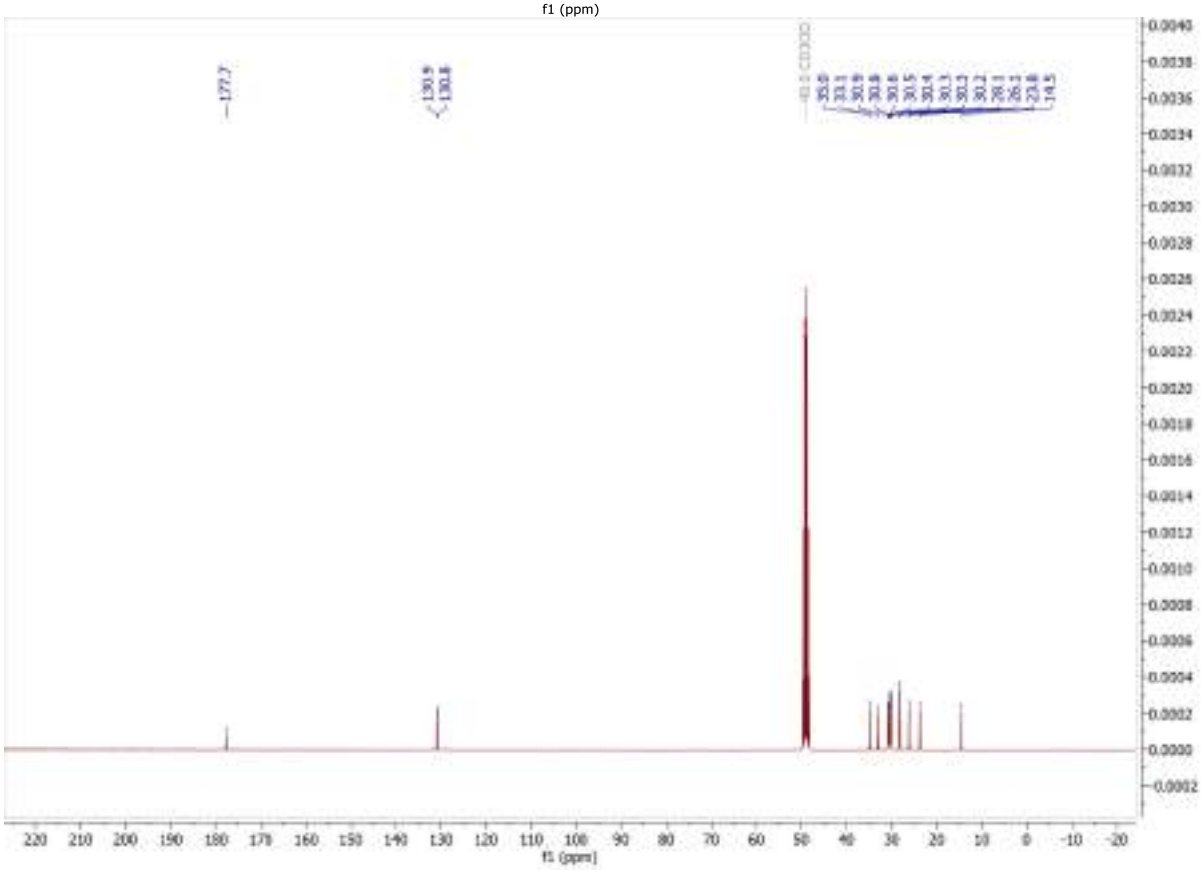
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-90**



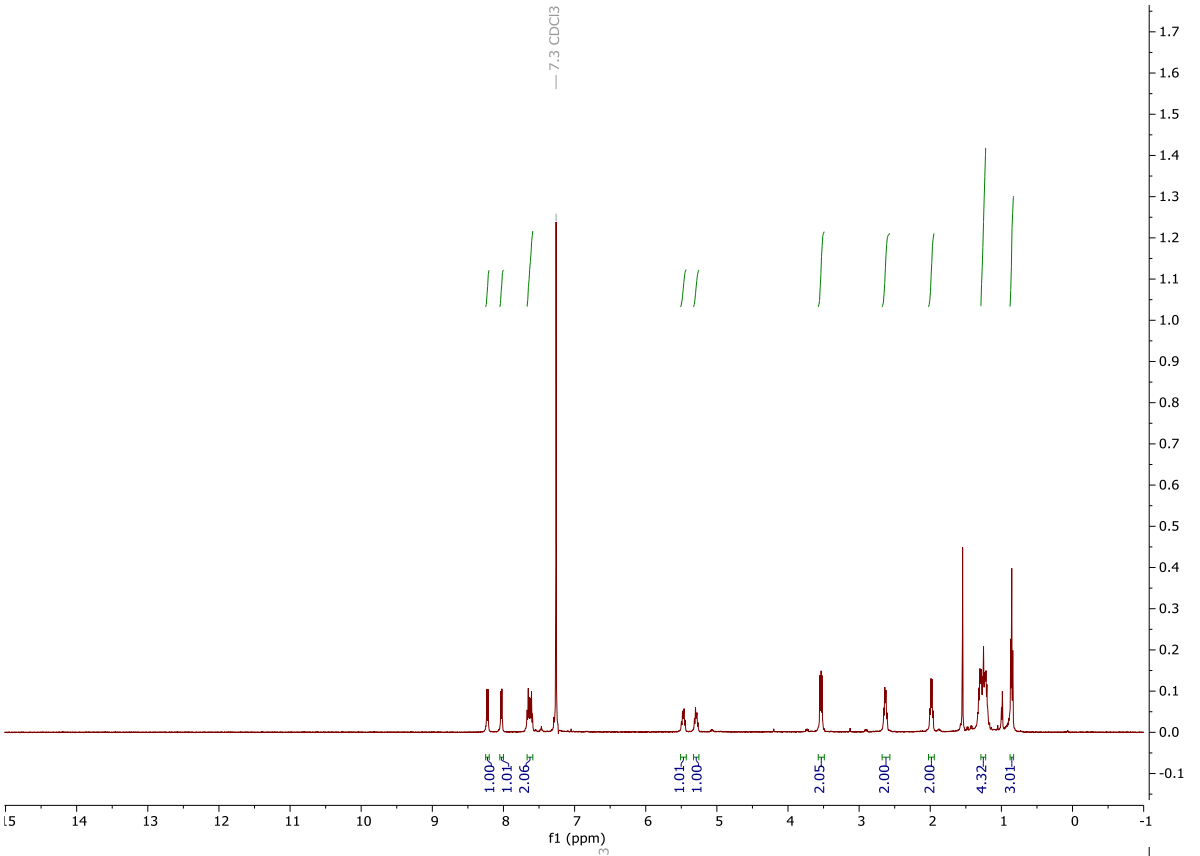


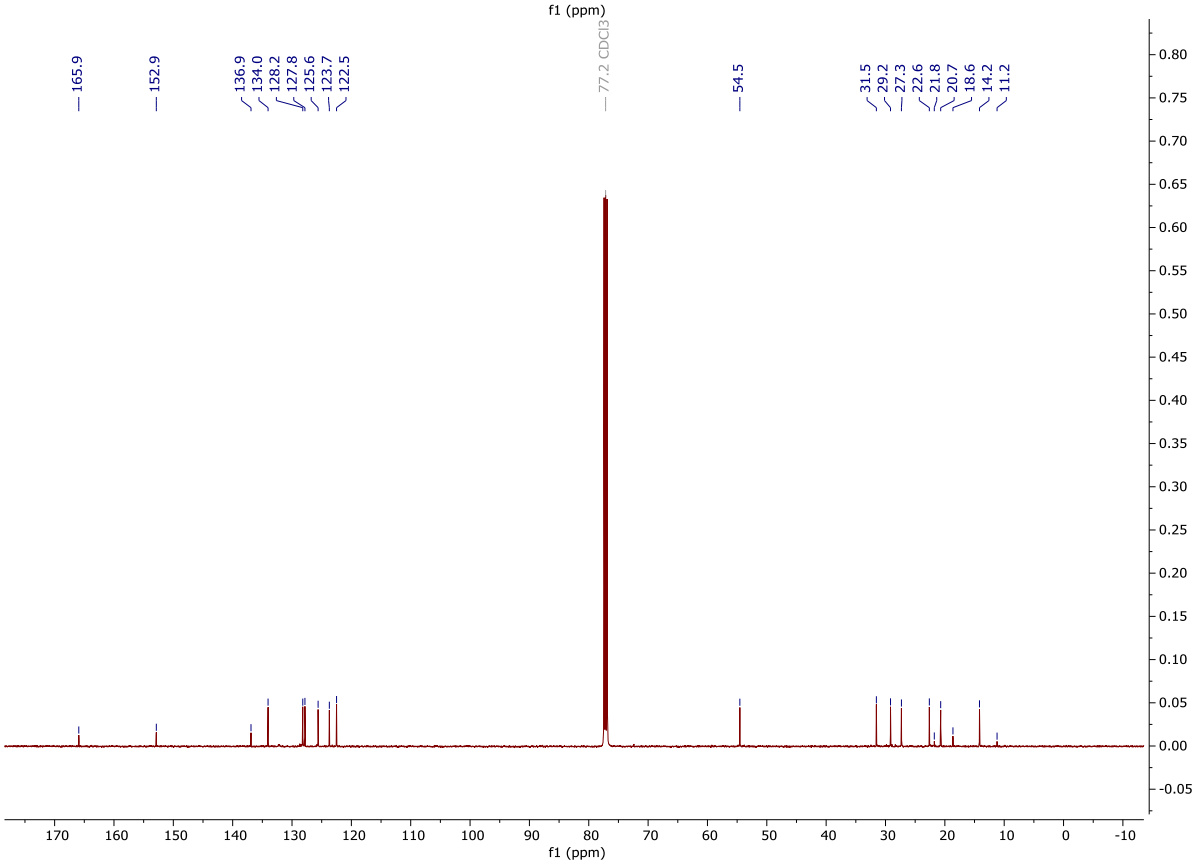
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-16**



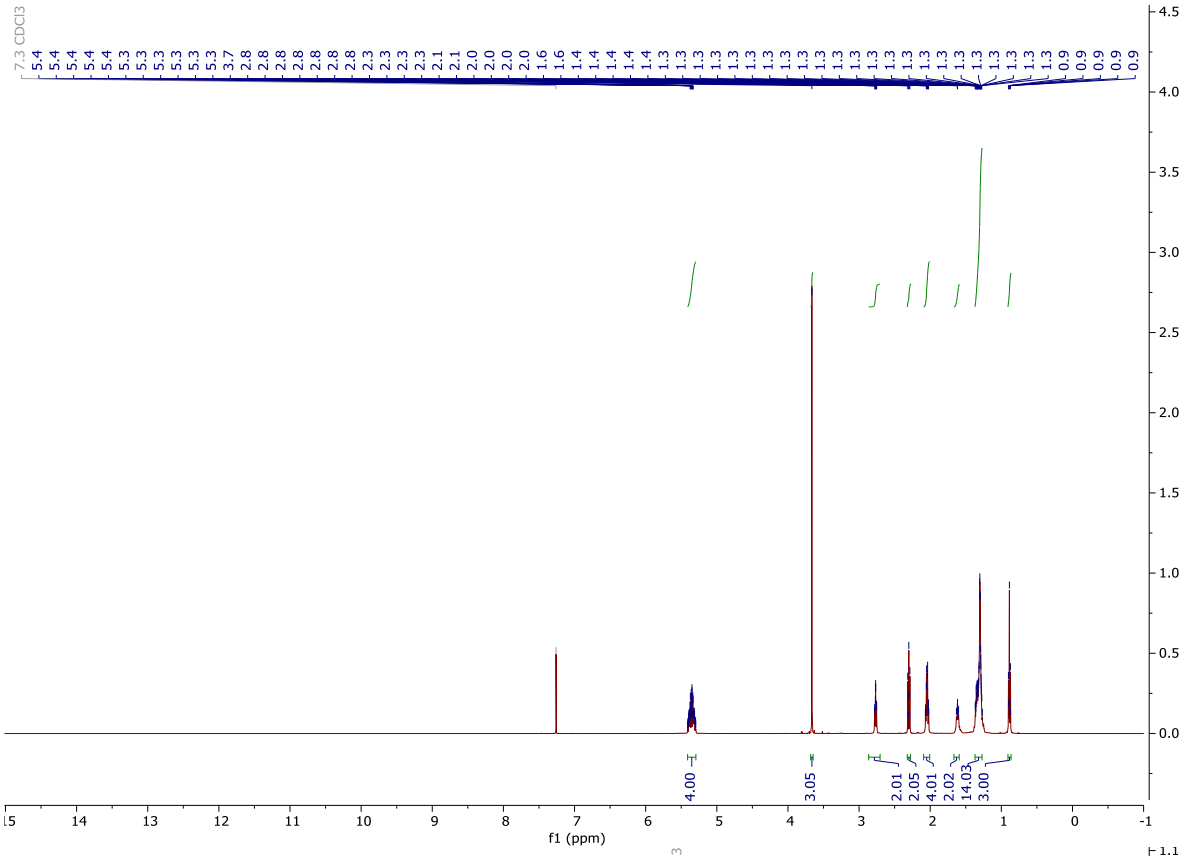


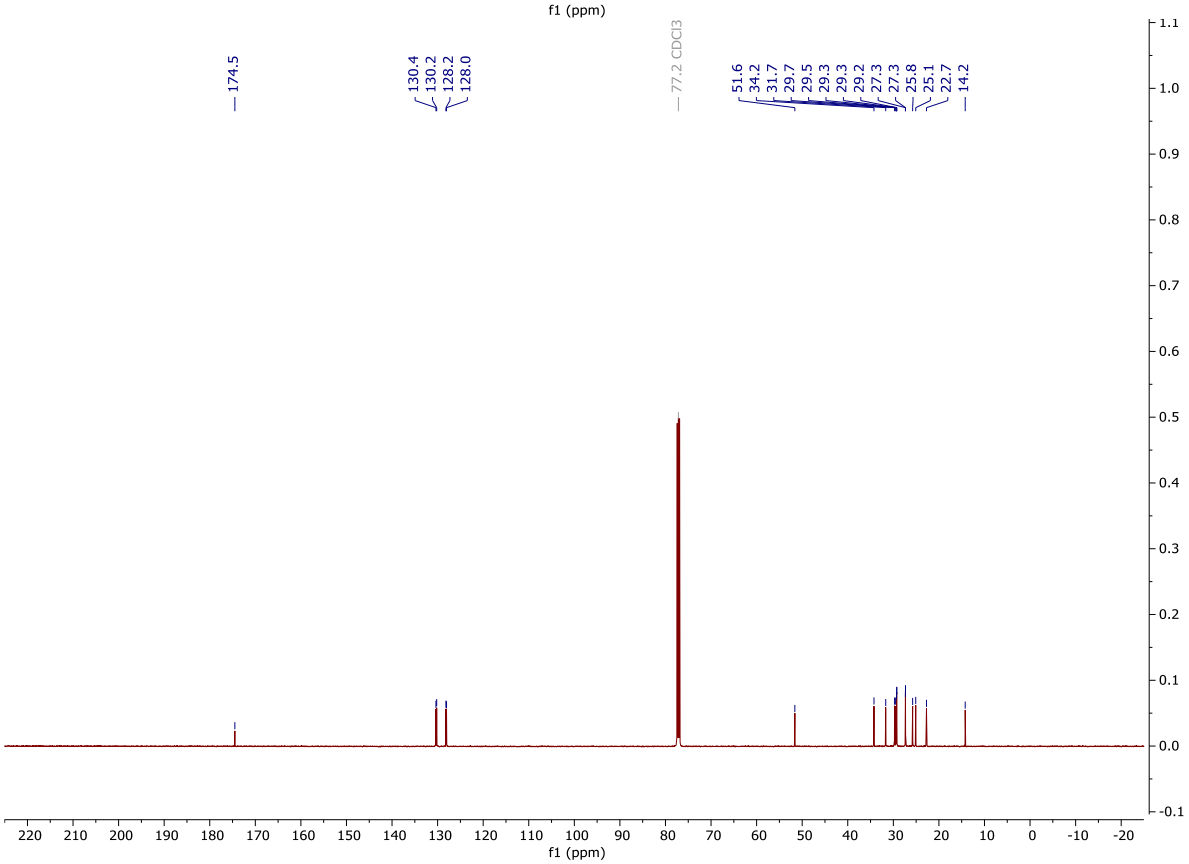
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-93**



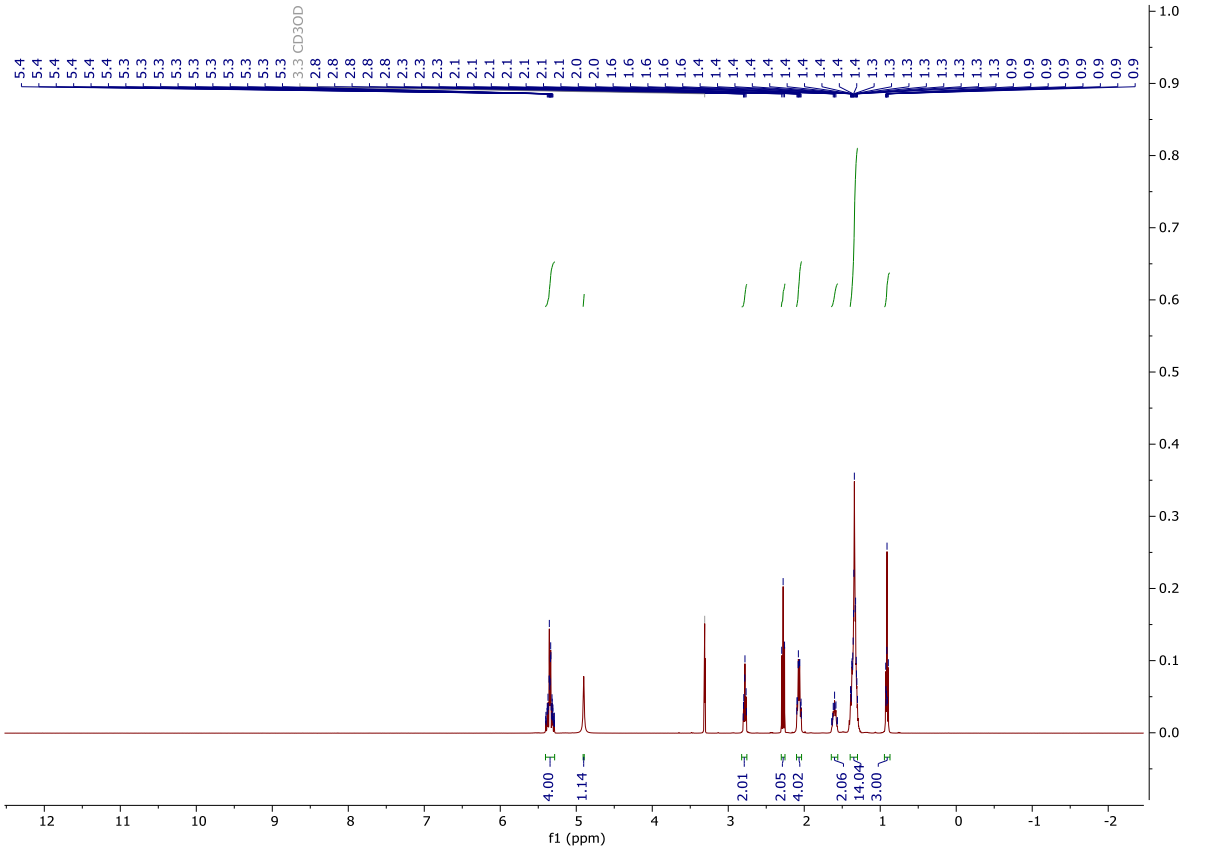


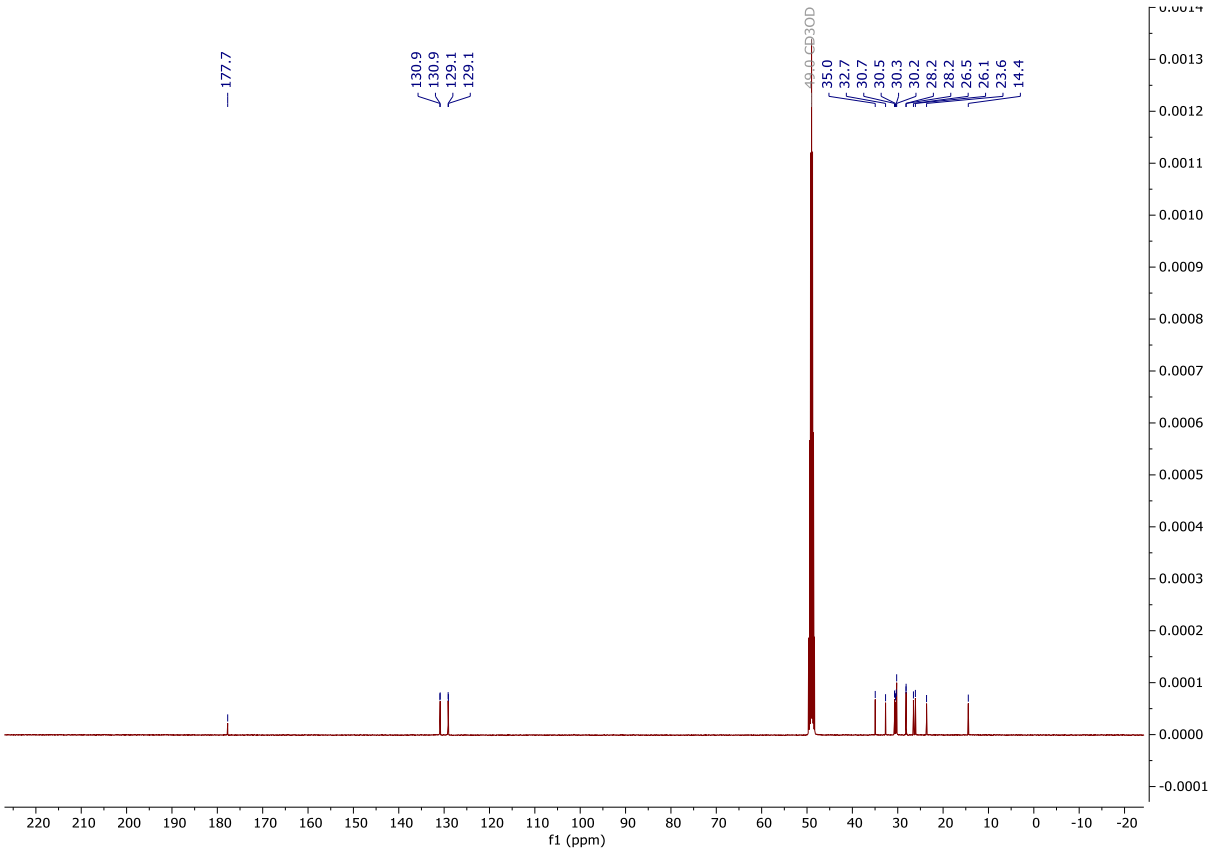
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-94**



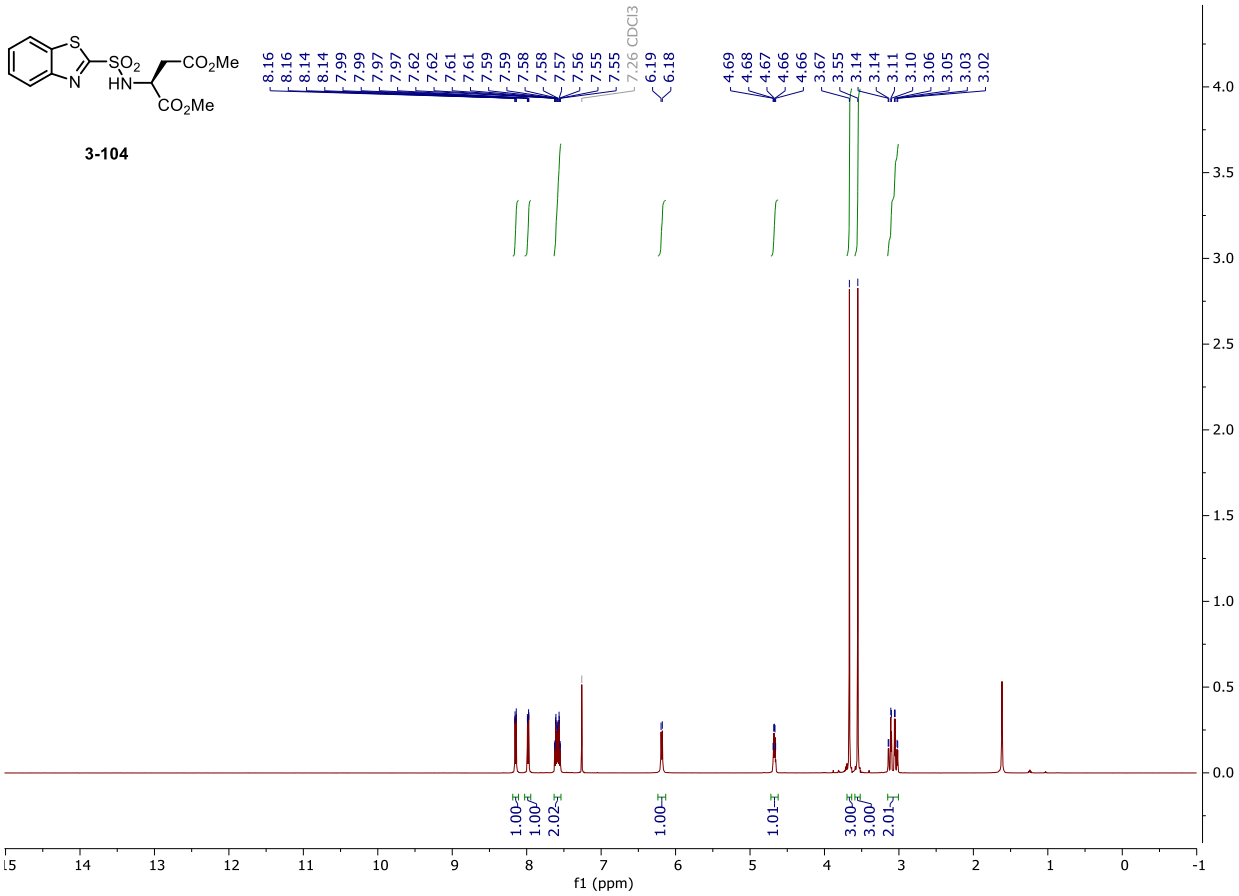


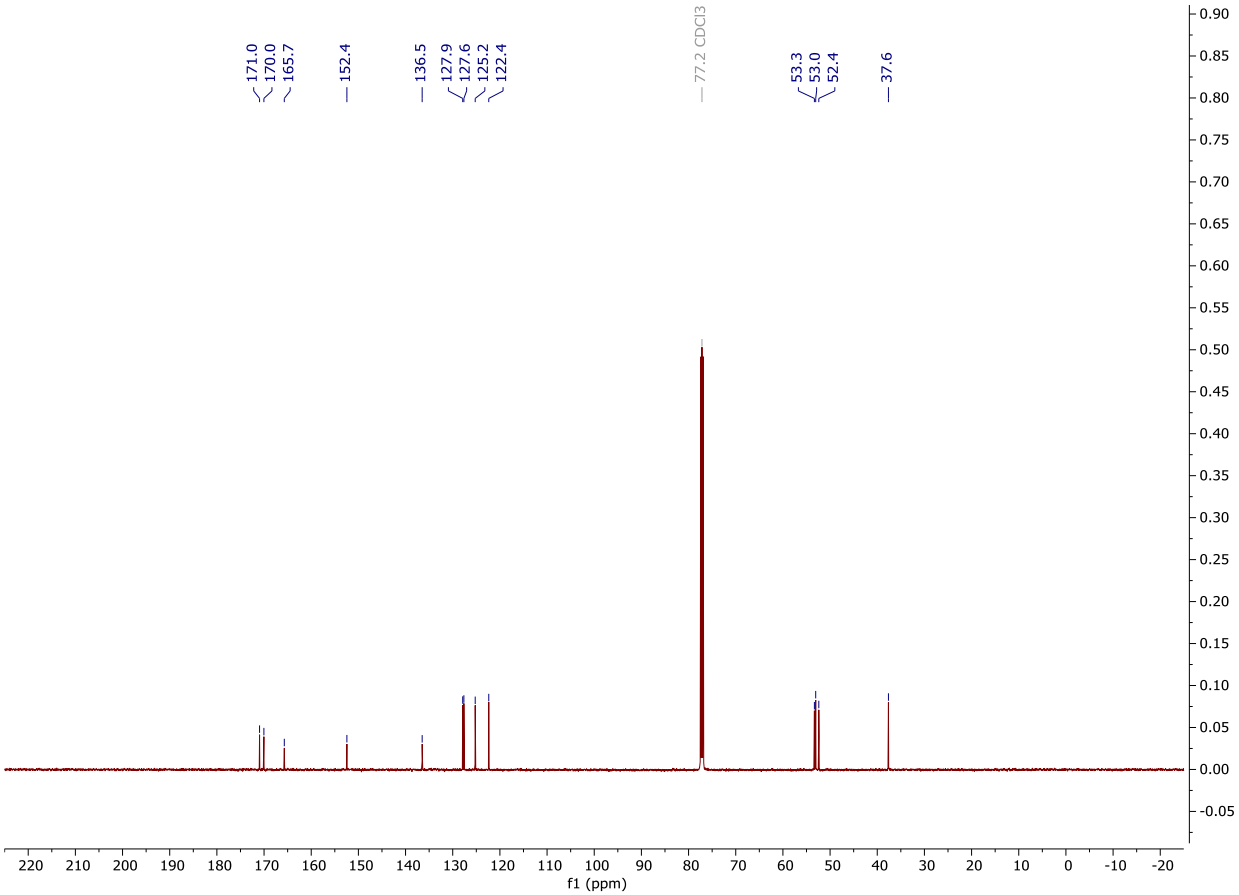
Copy of ^{1}H and $^{13}C\{^{1}H\}$ spectra of **2-95**



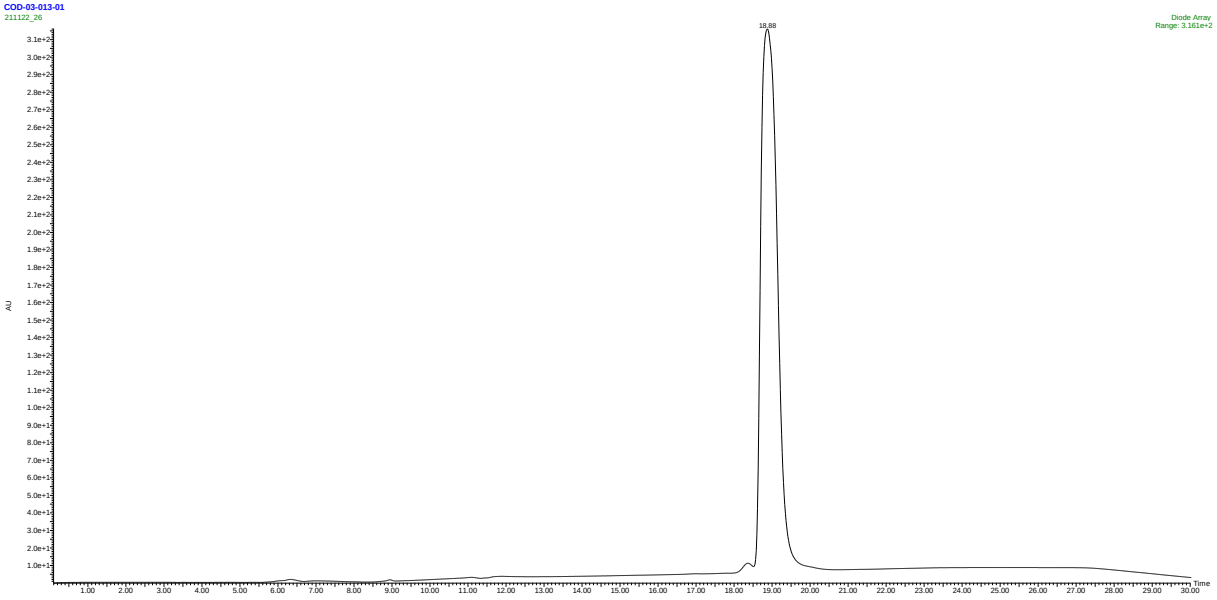


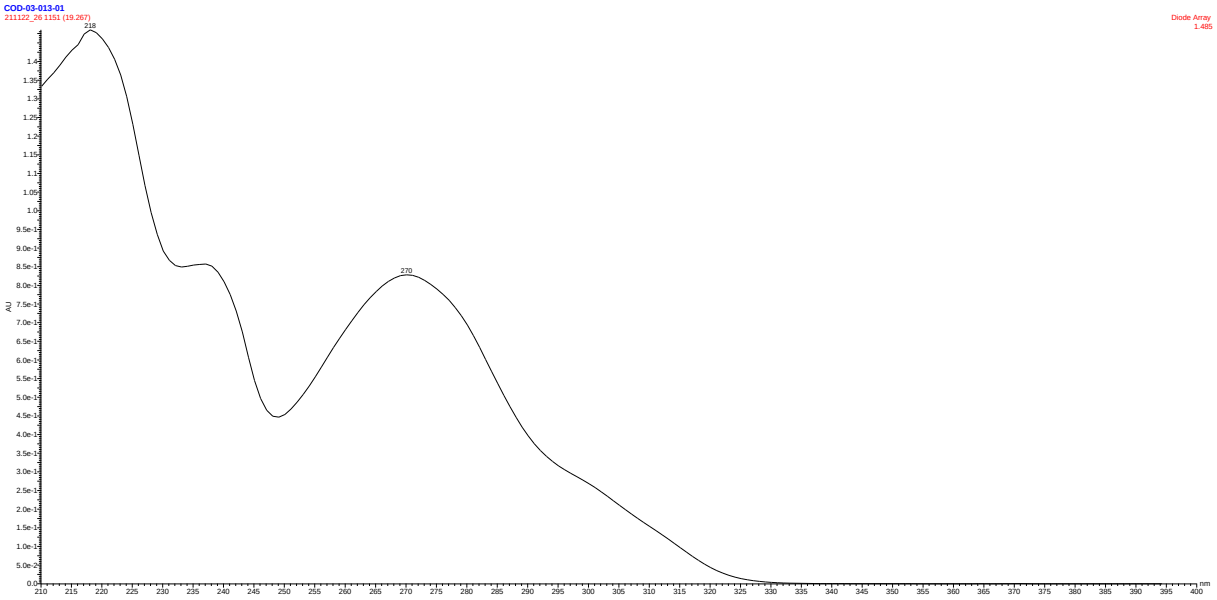
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-104**



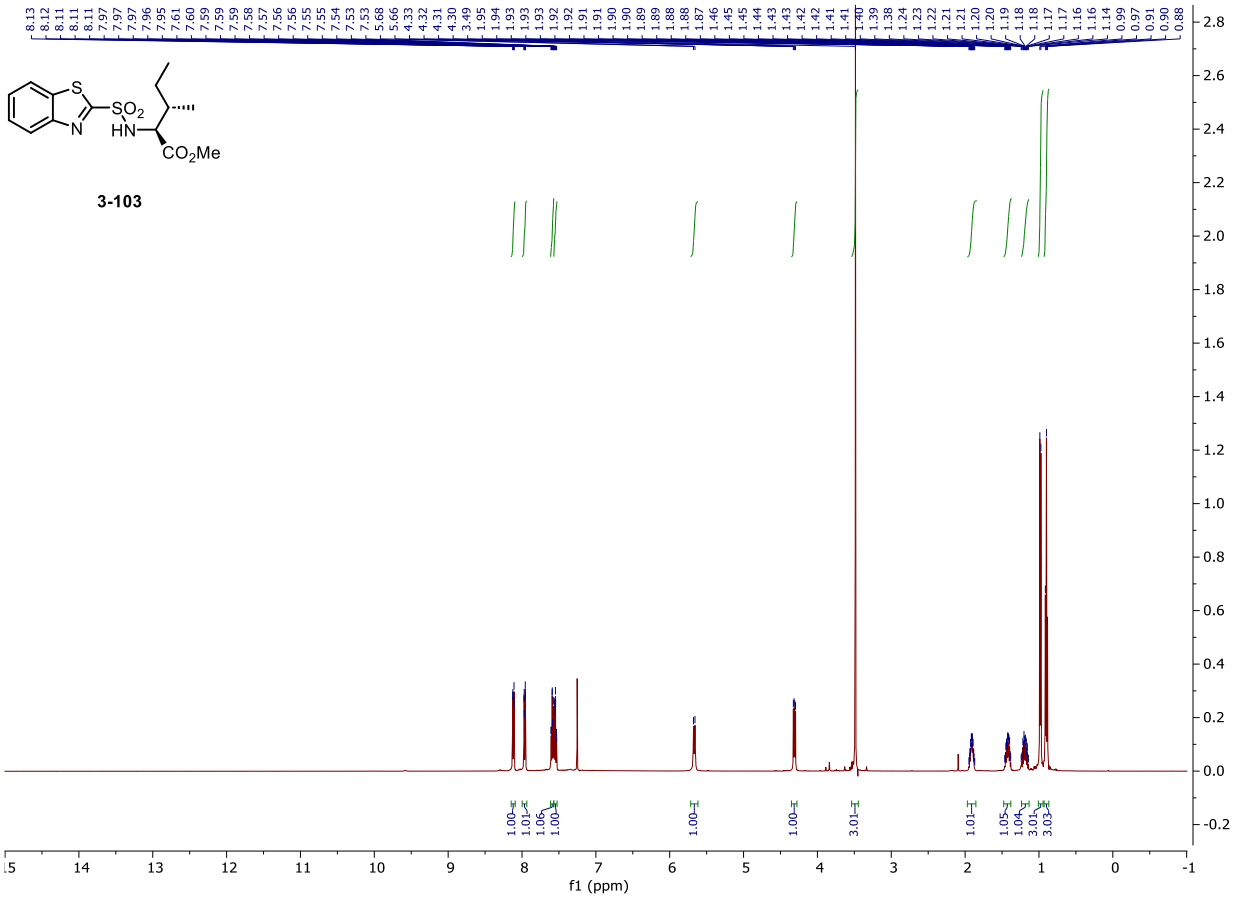


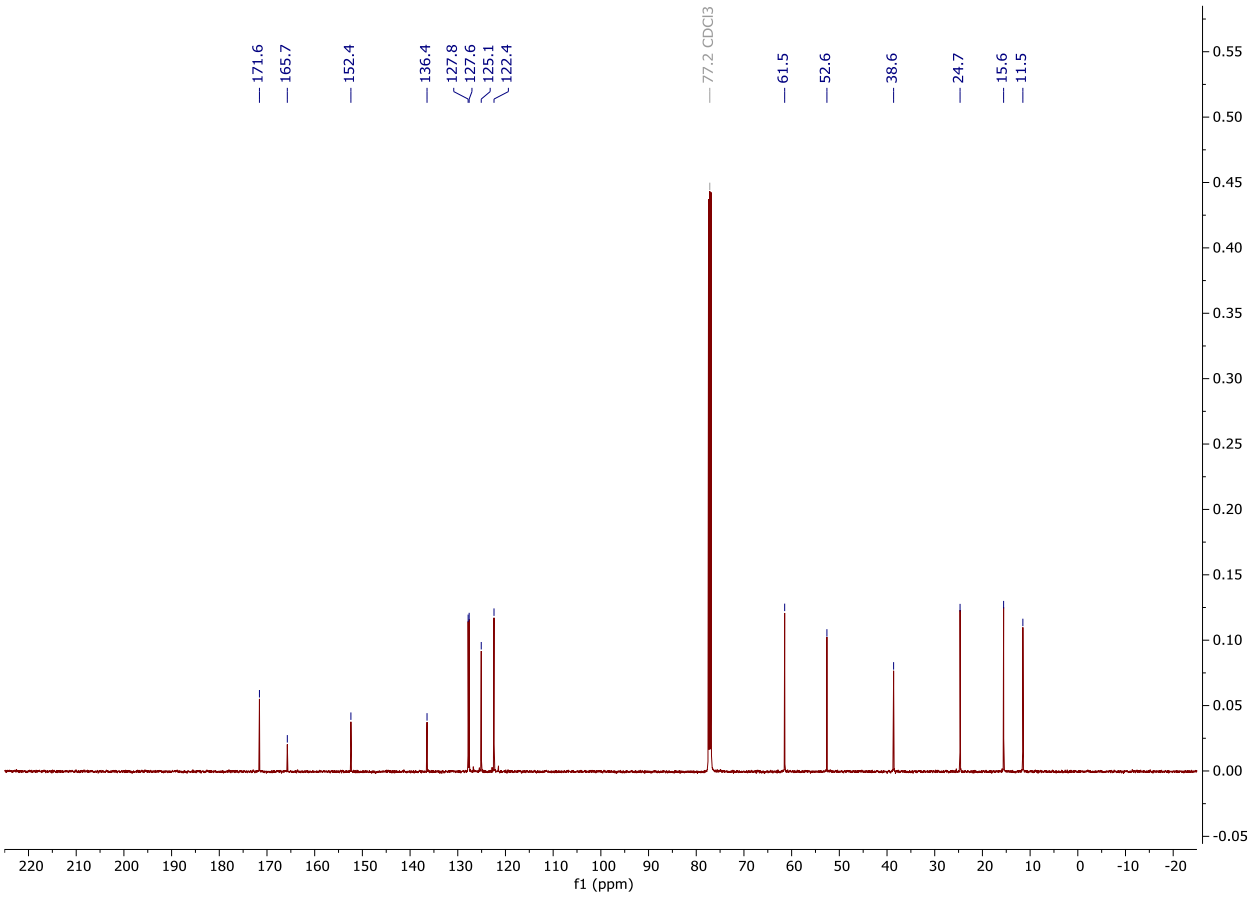
HPLC chromatograms of **3-104**



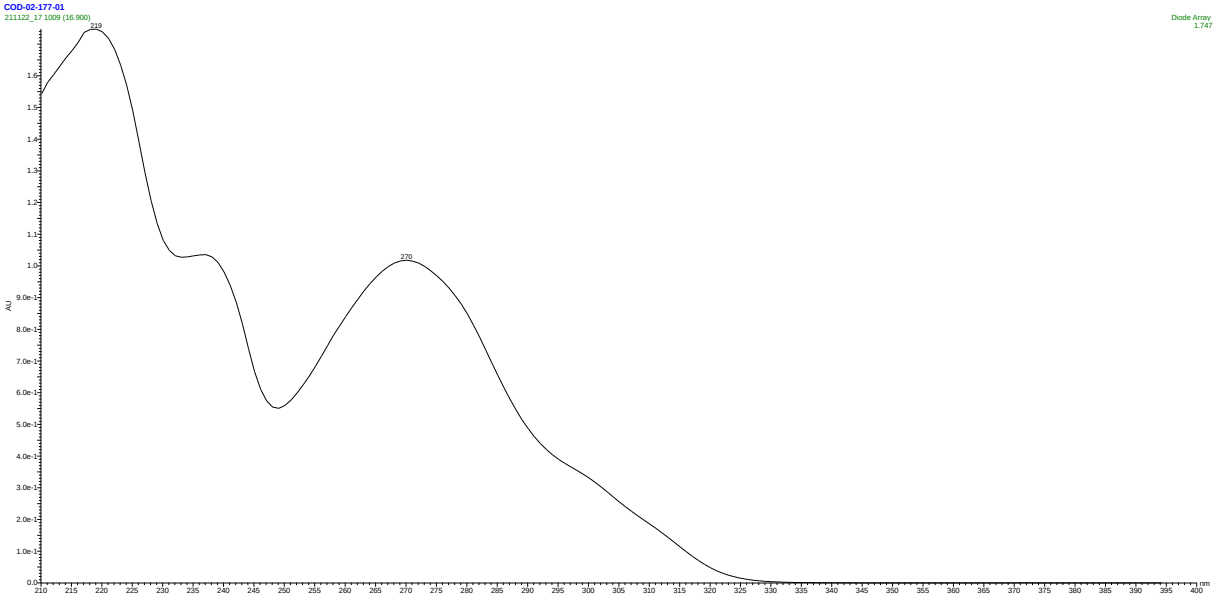


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-103**

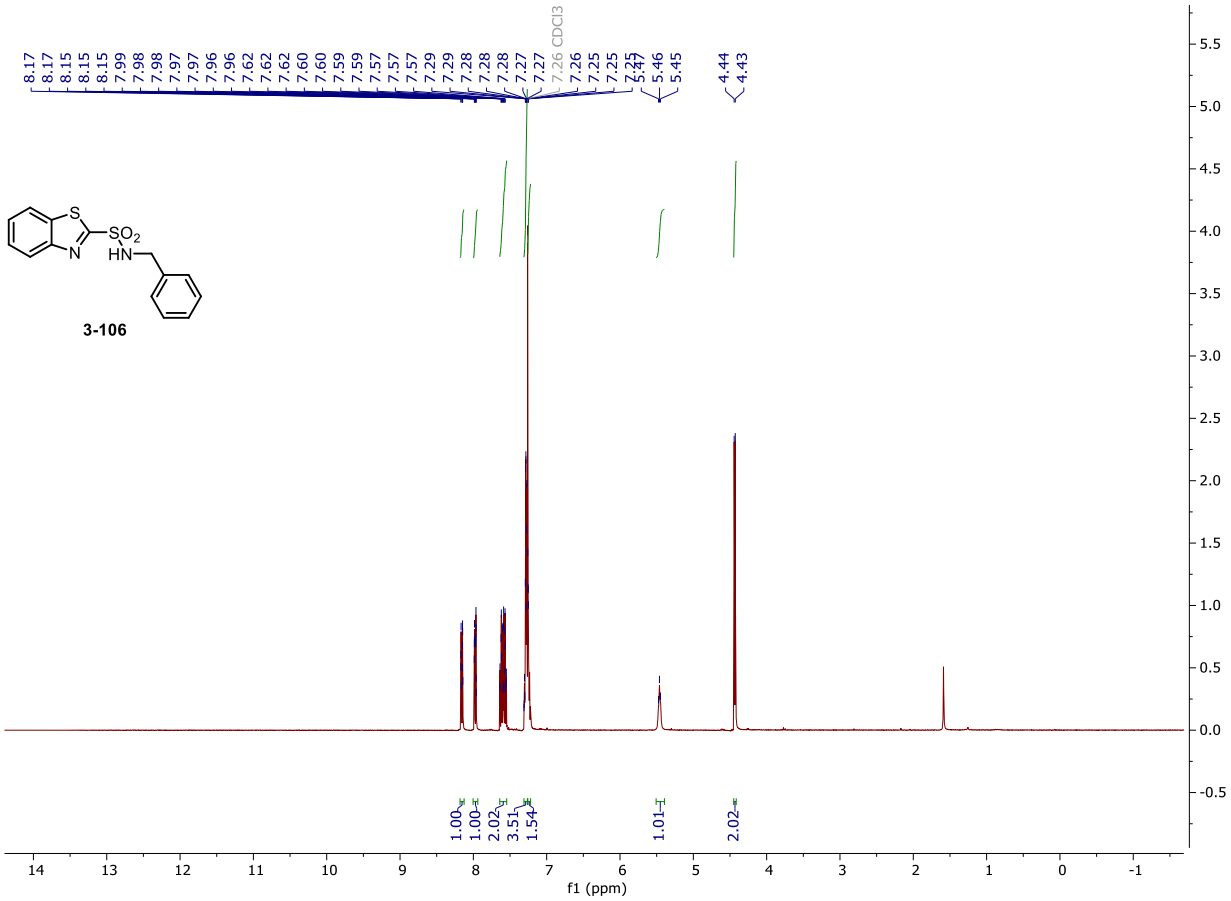


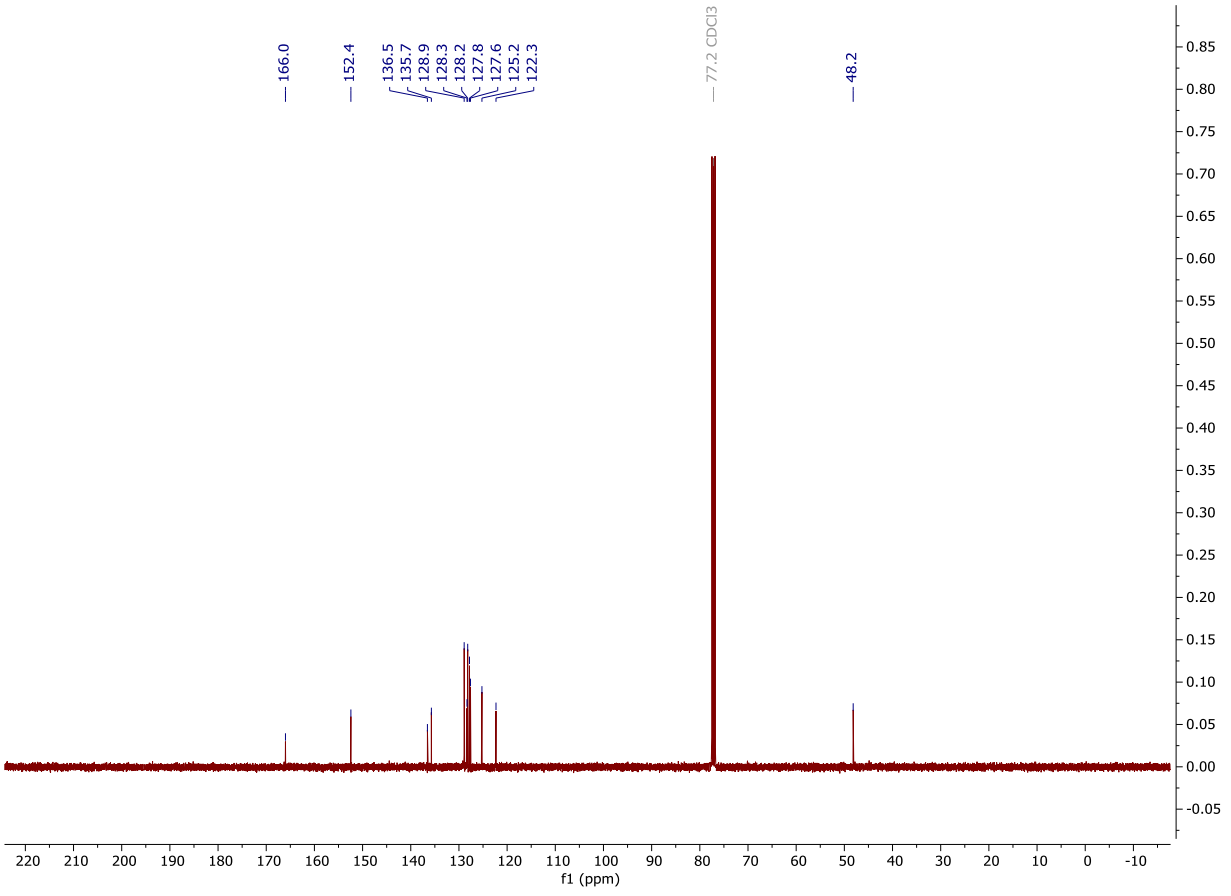


HPLC chromatograms of **3-103**

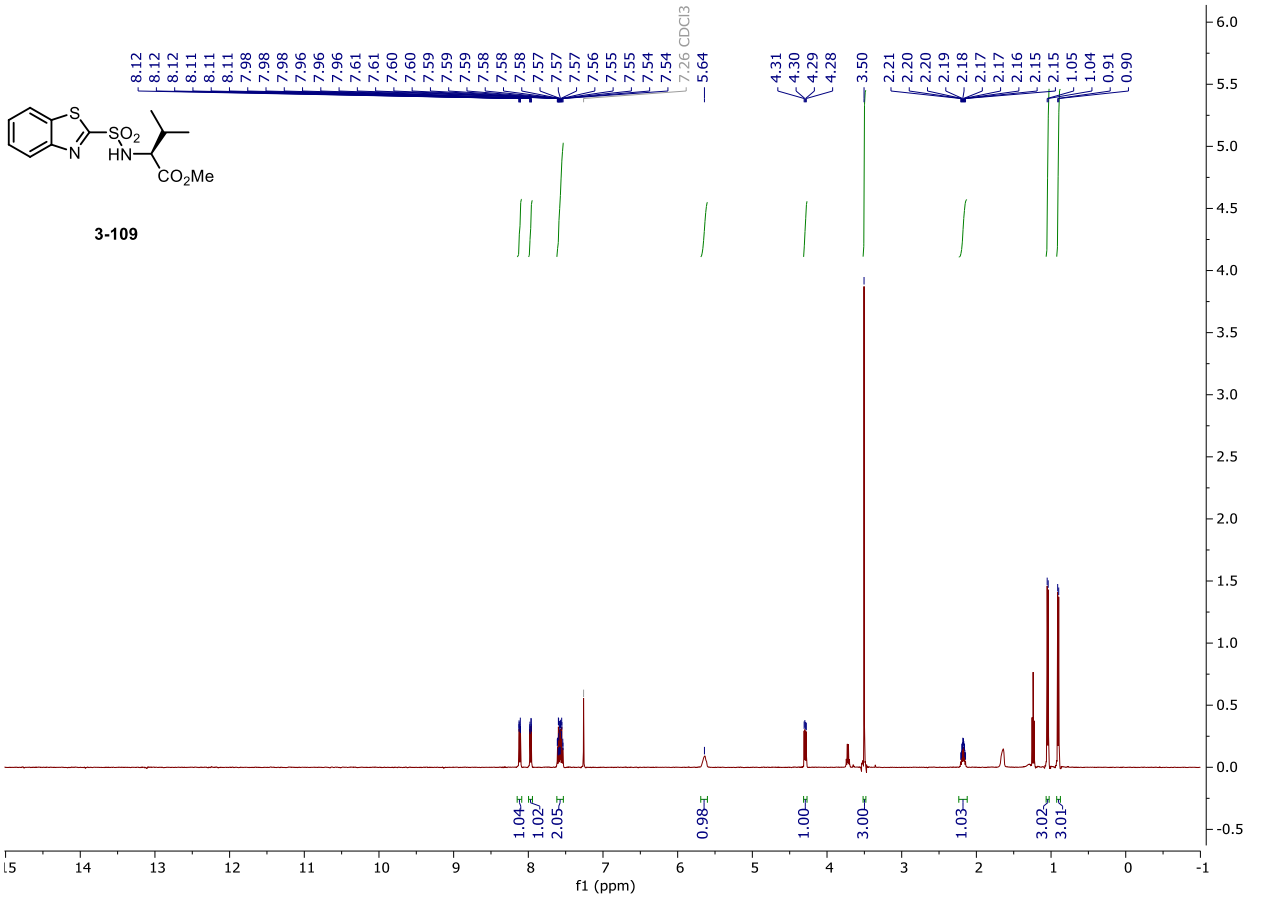


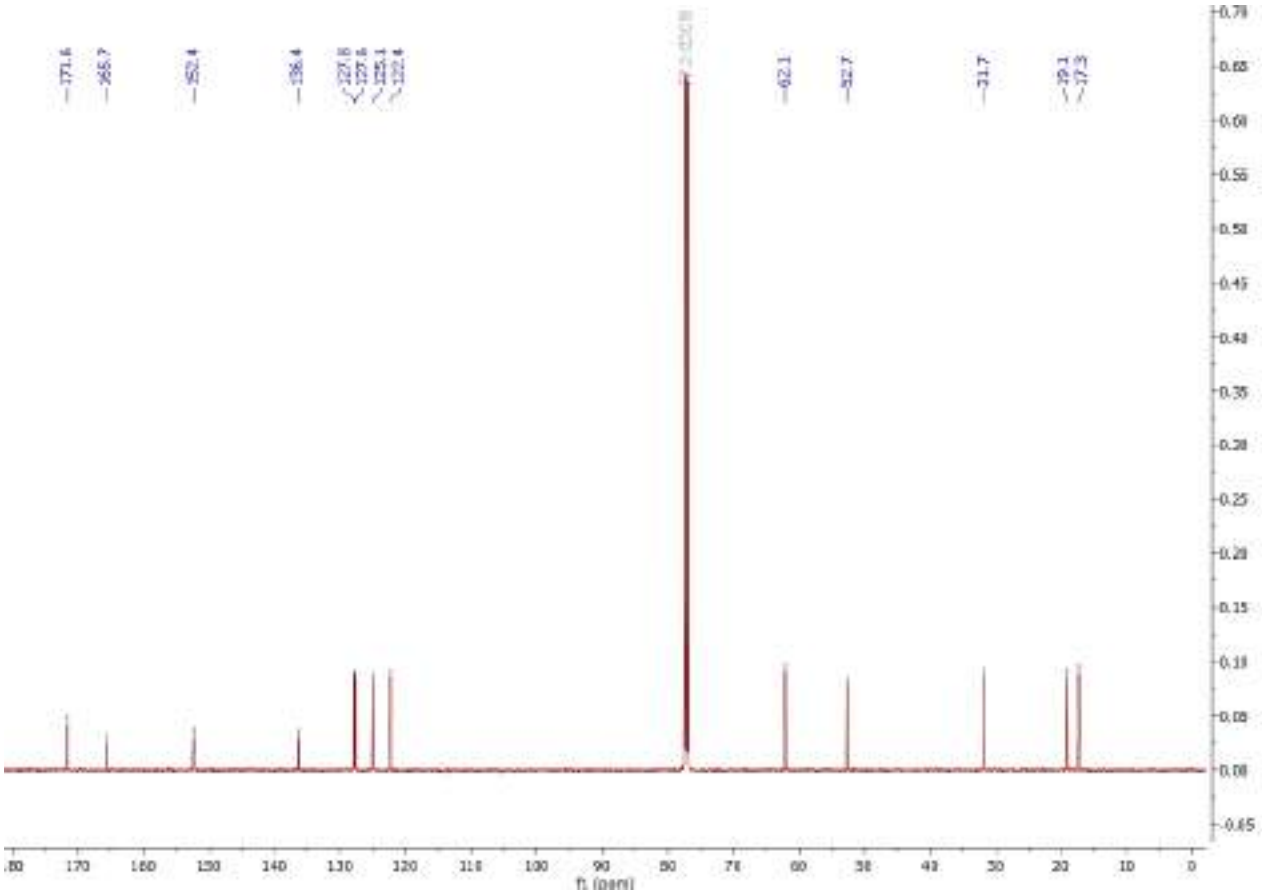
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-106**



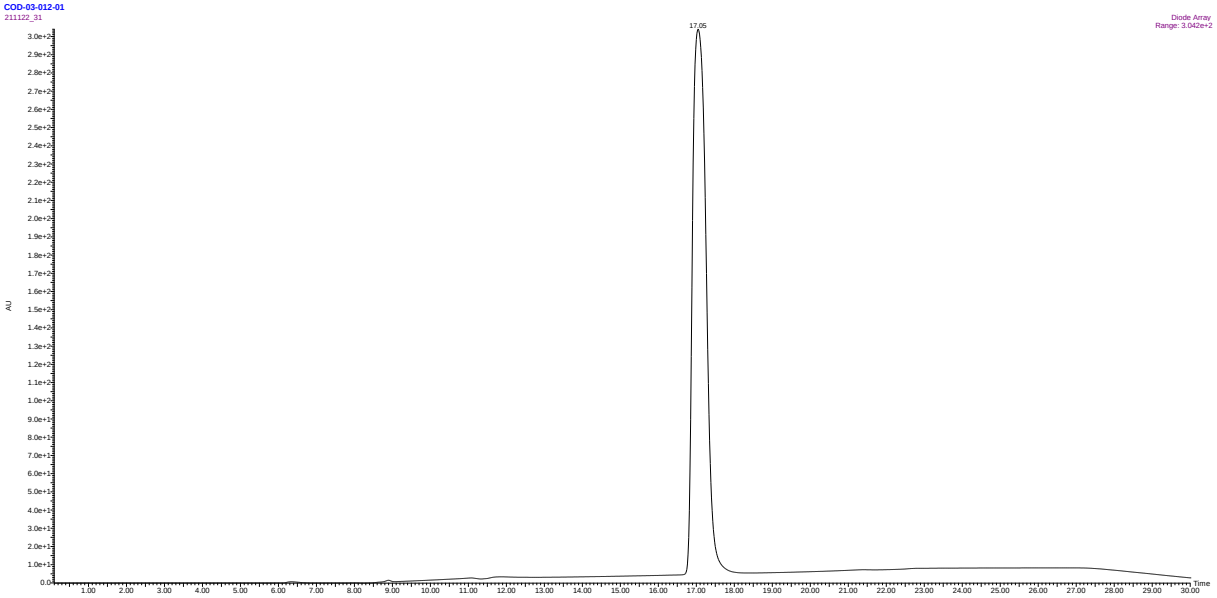


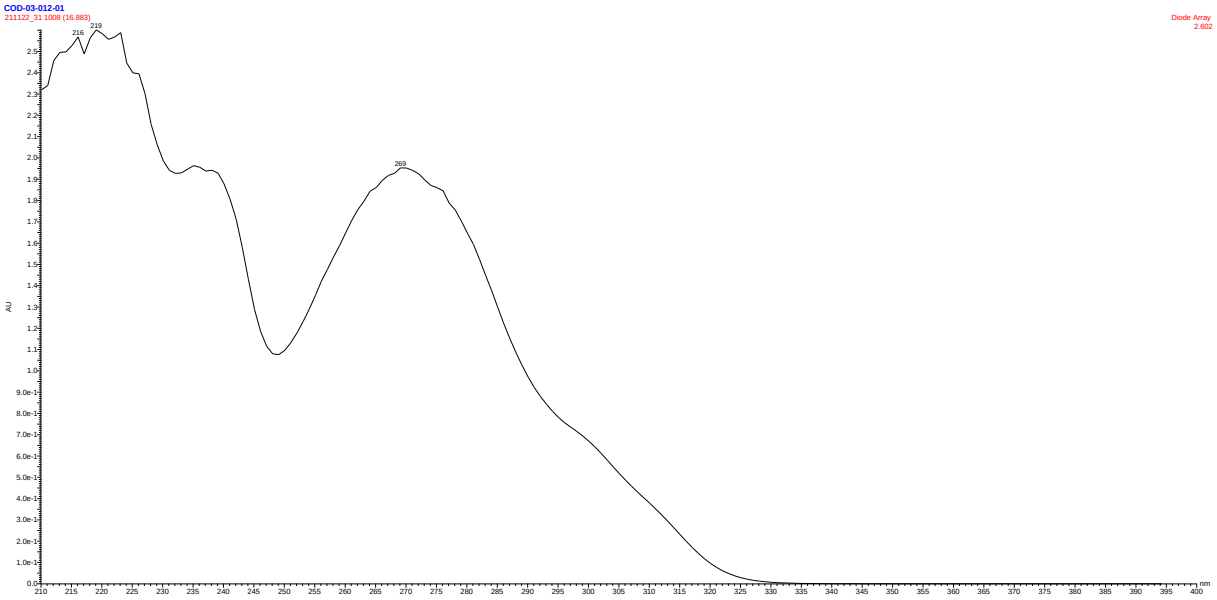
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-109**





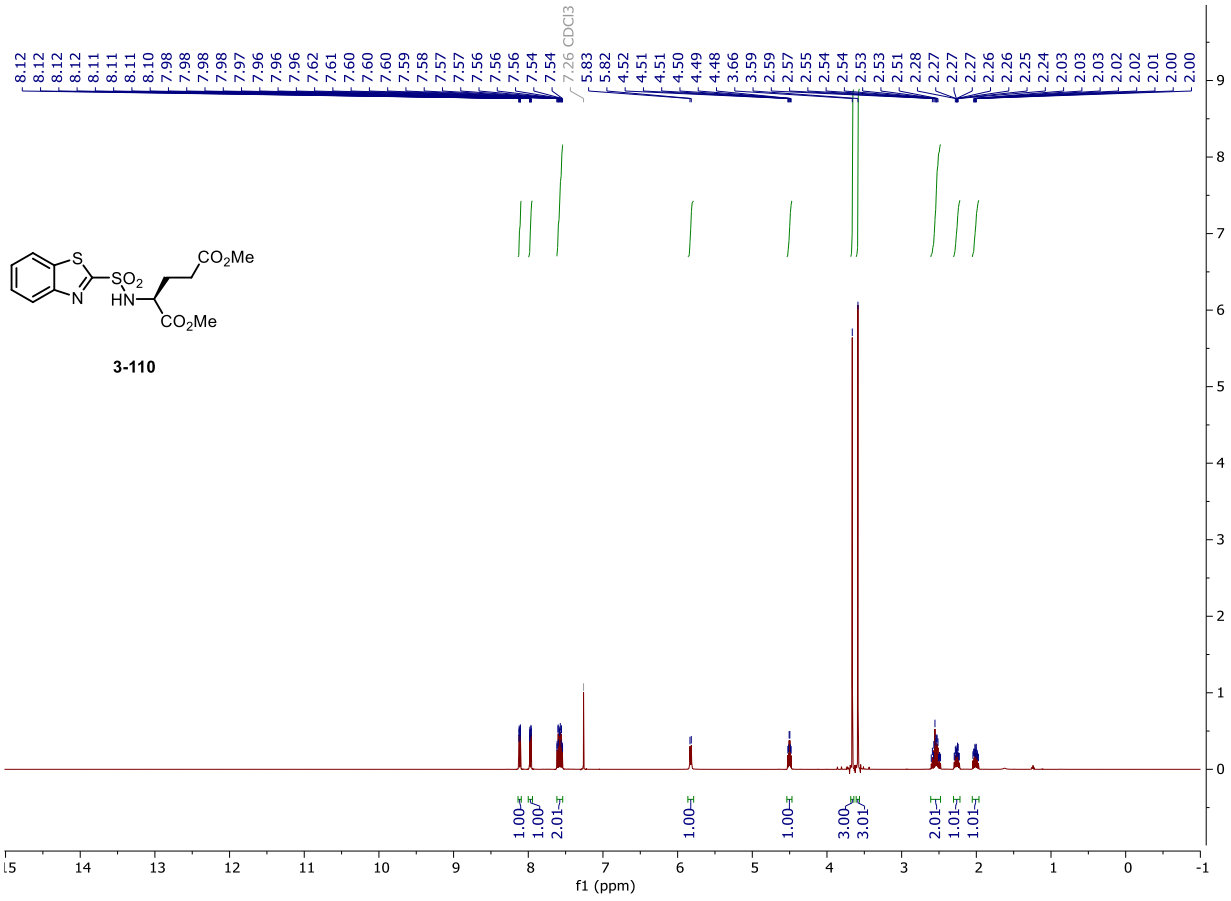
HPLC chromatograms of **3-109**

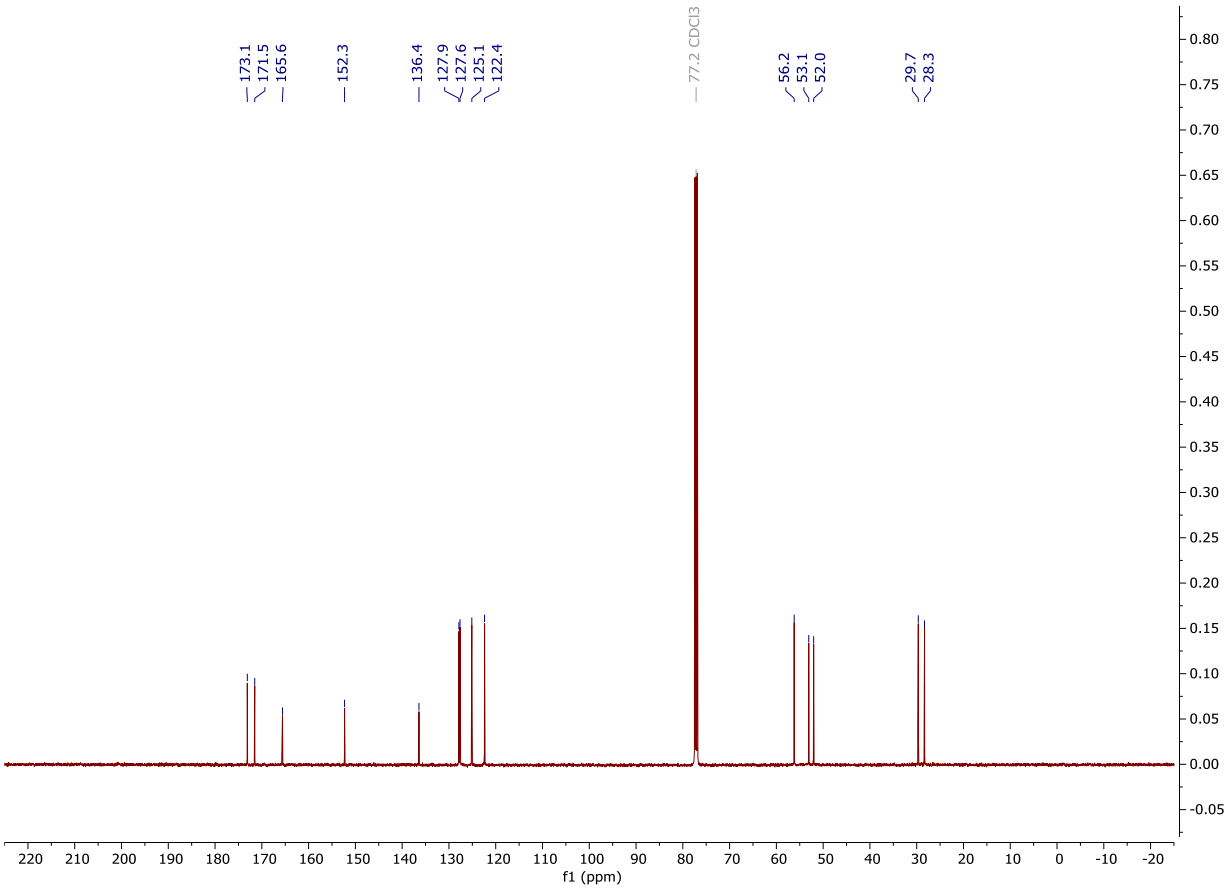




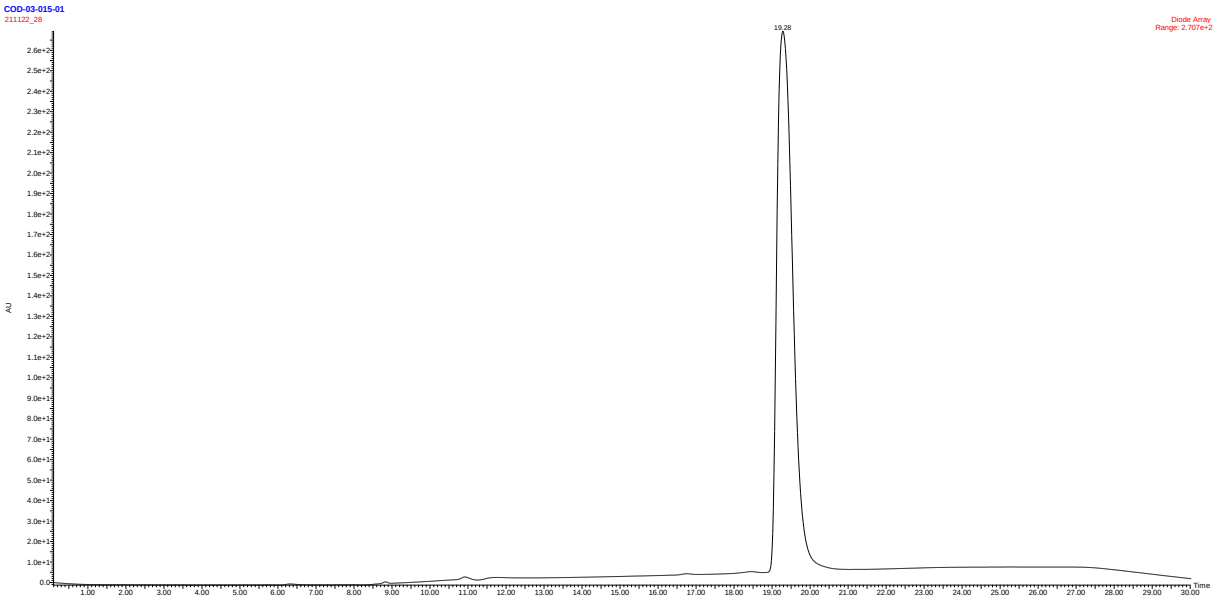
NMR and HPLC

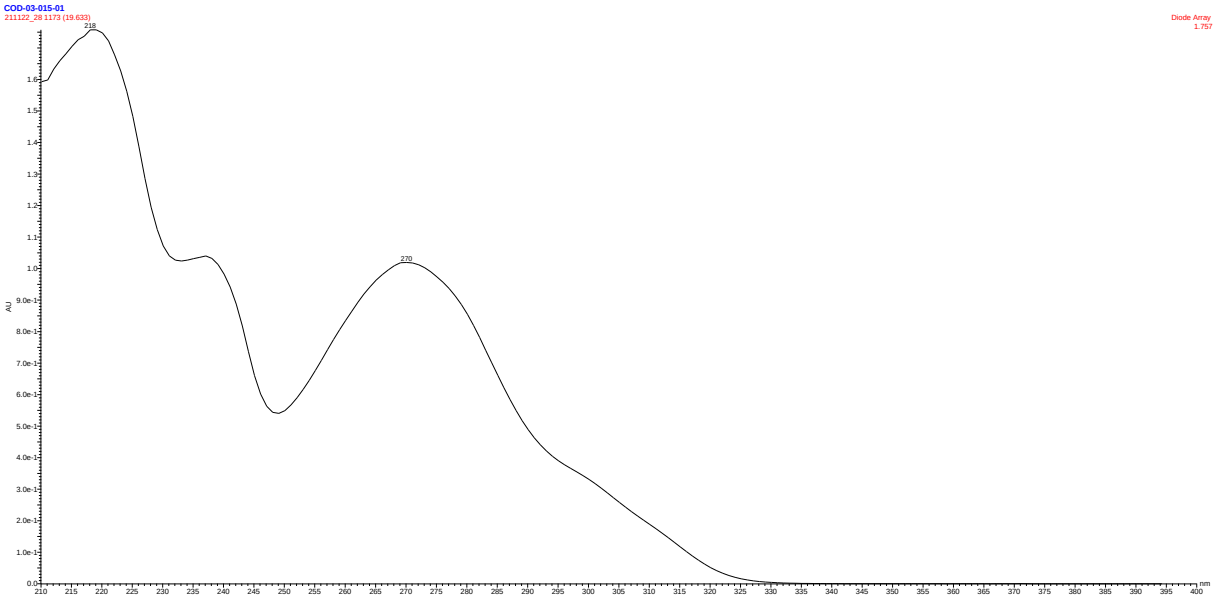
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-110**



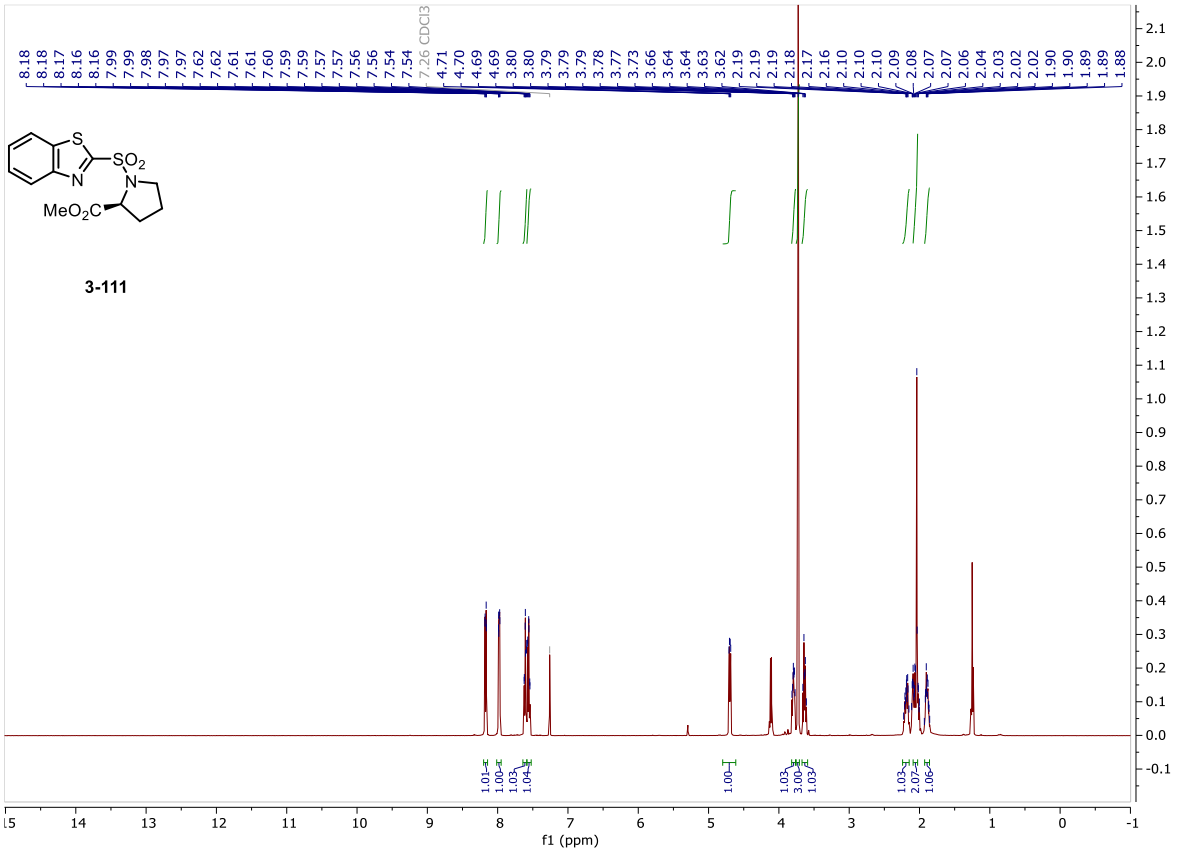


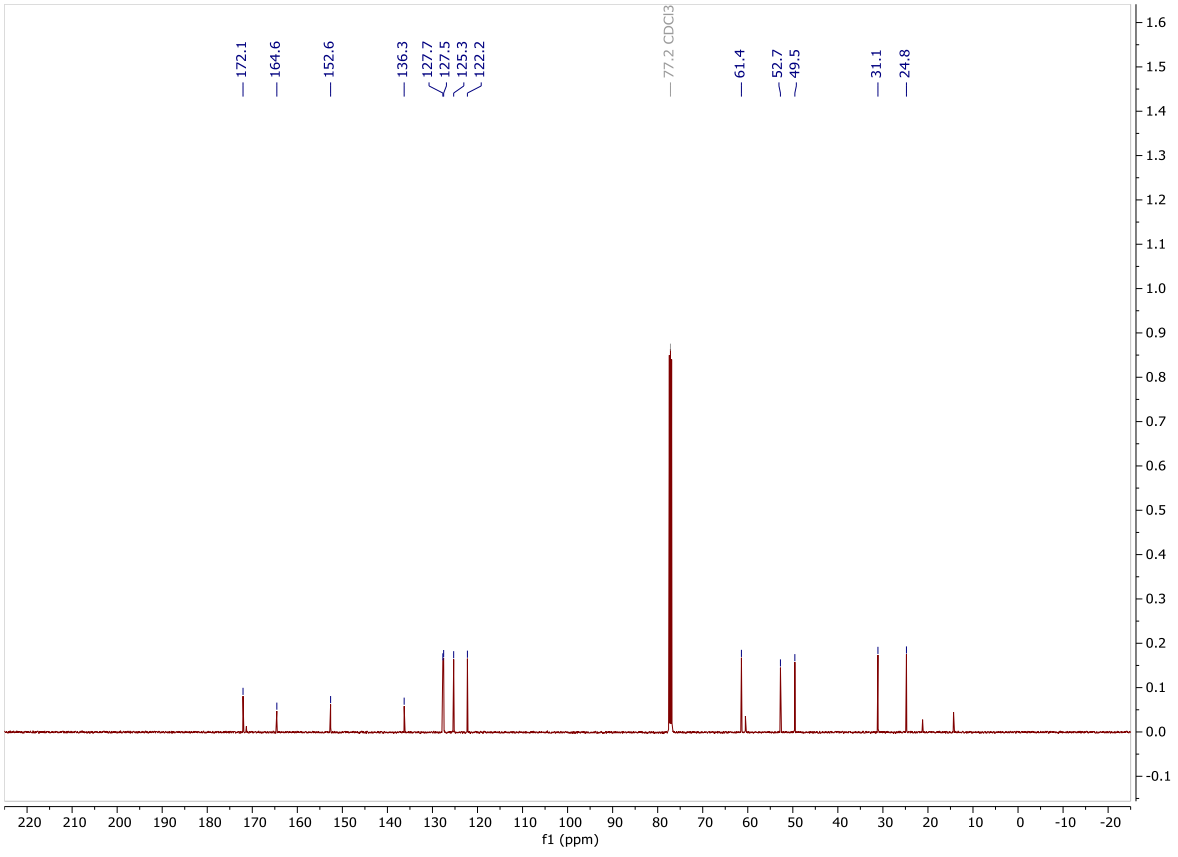
HPLC chromatograms of **3-110**



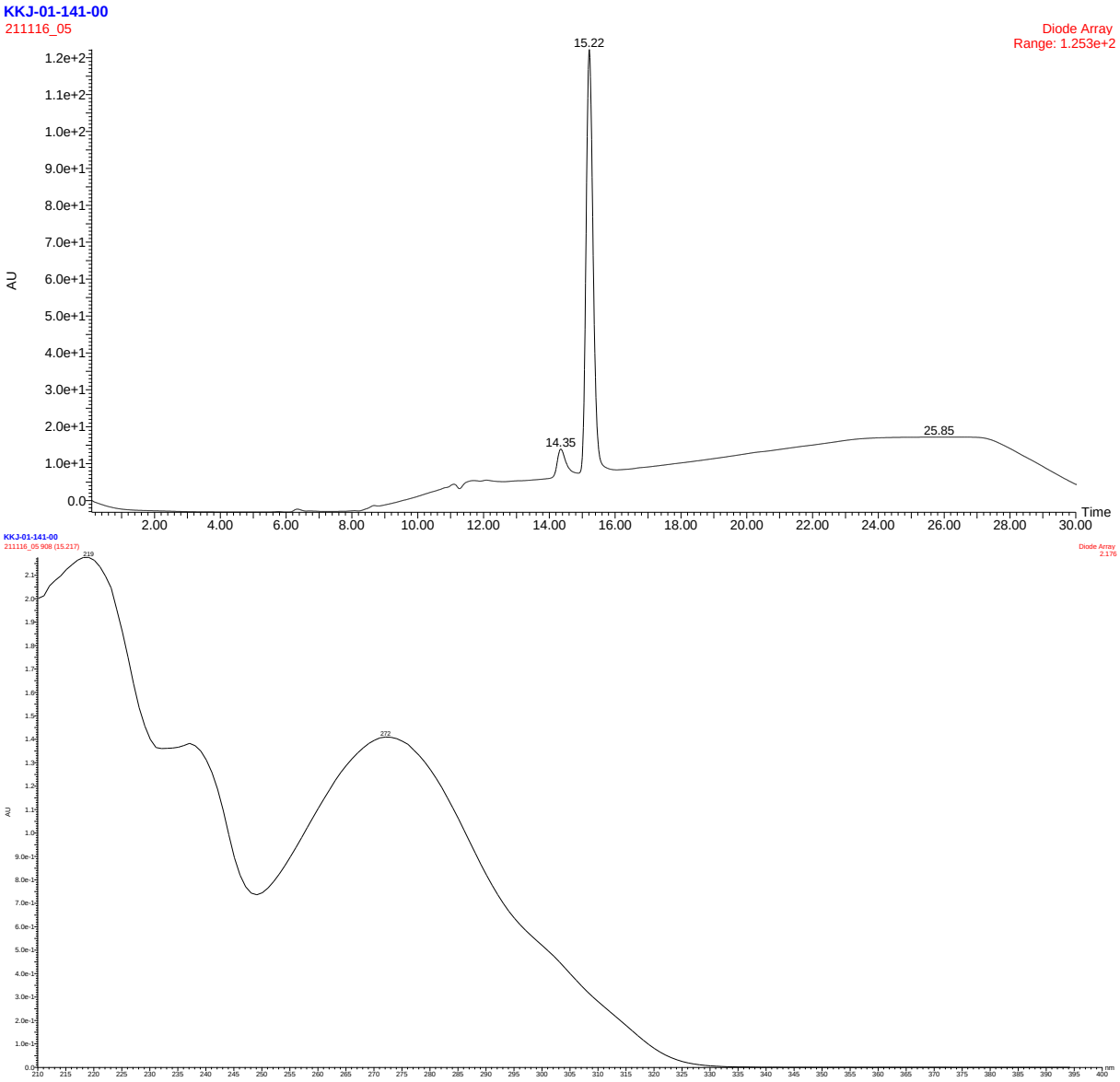


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-111**

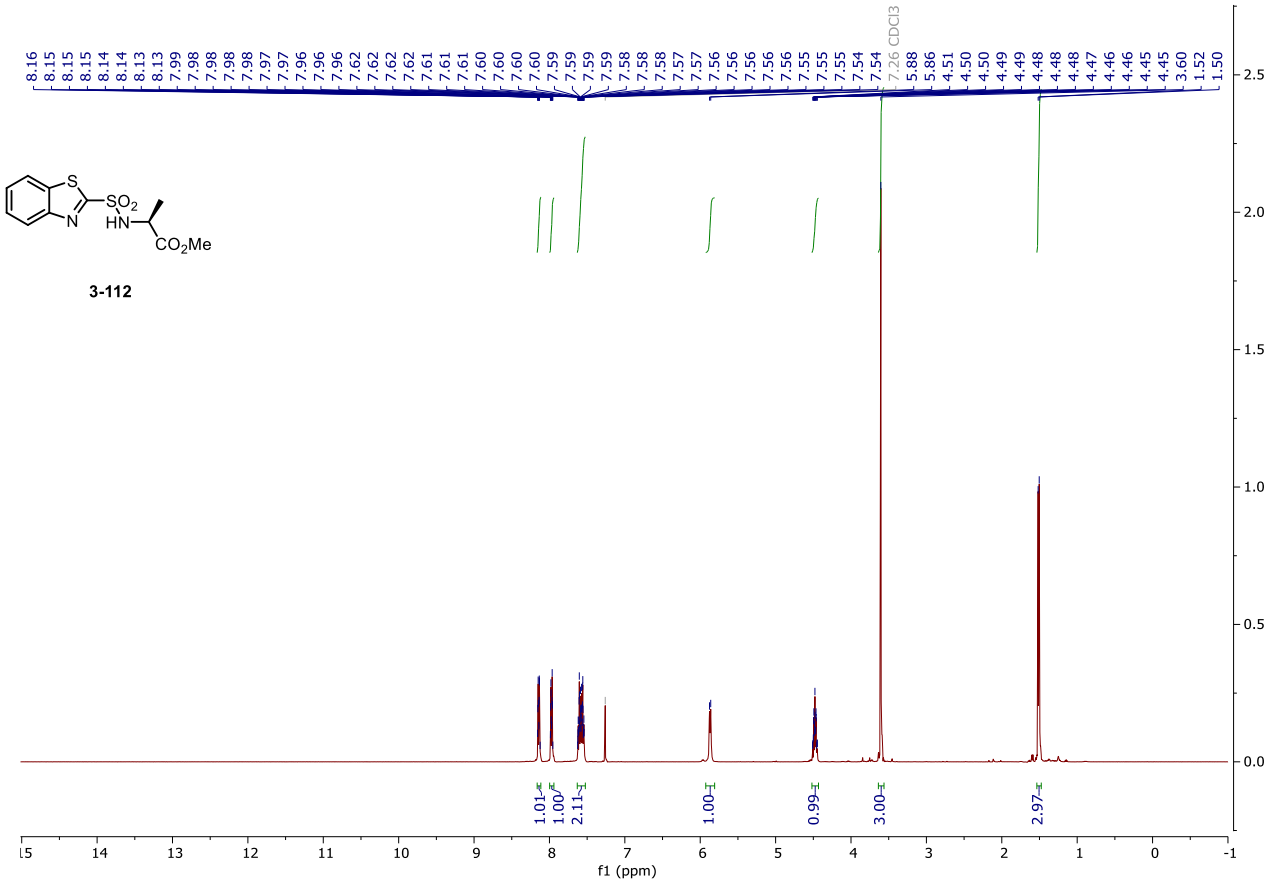


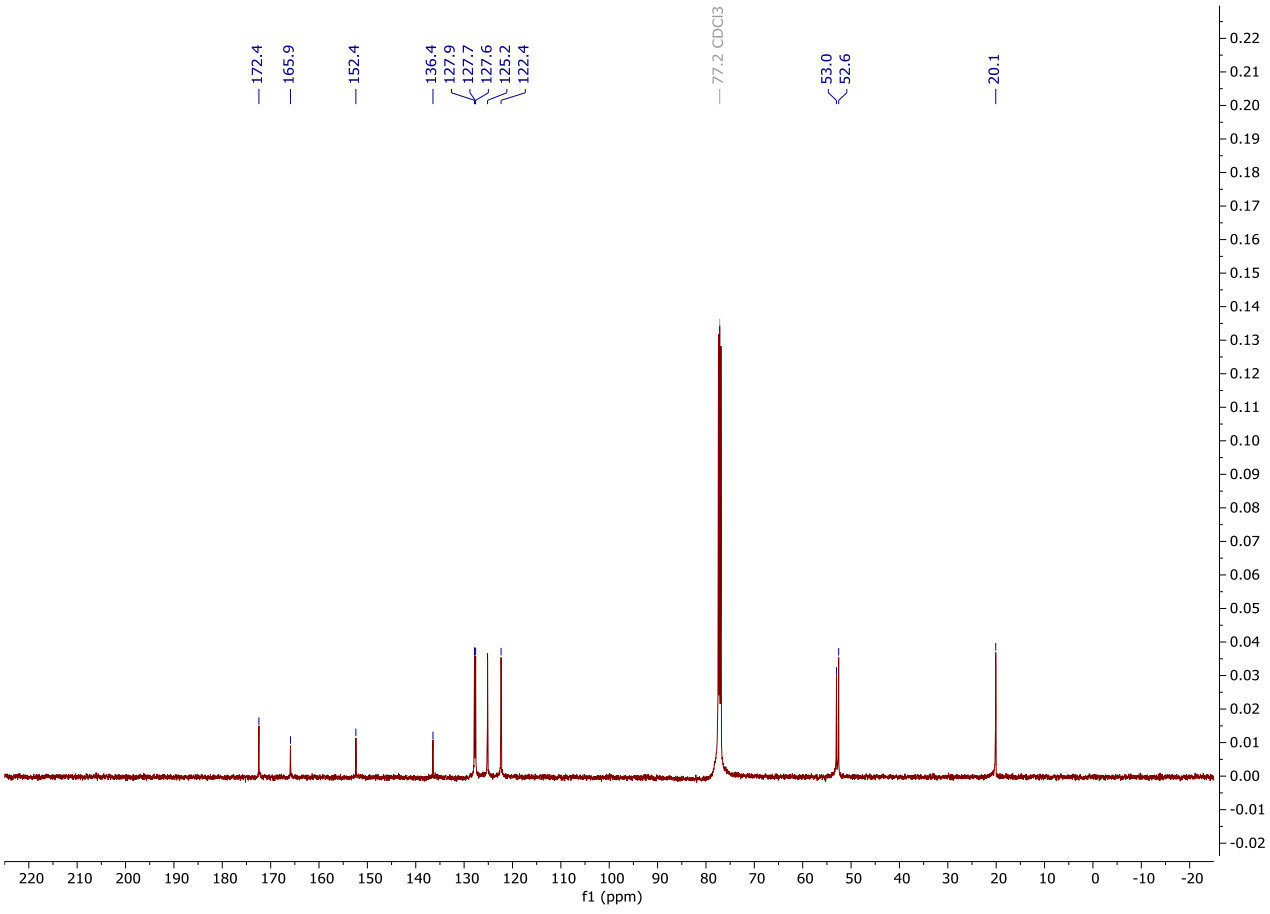


HPLC chromatograms of **3-111**

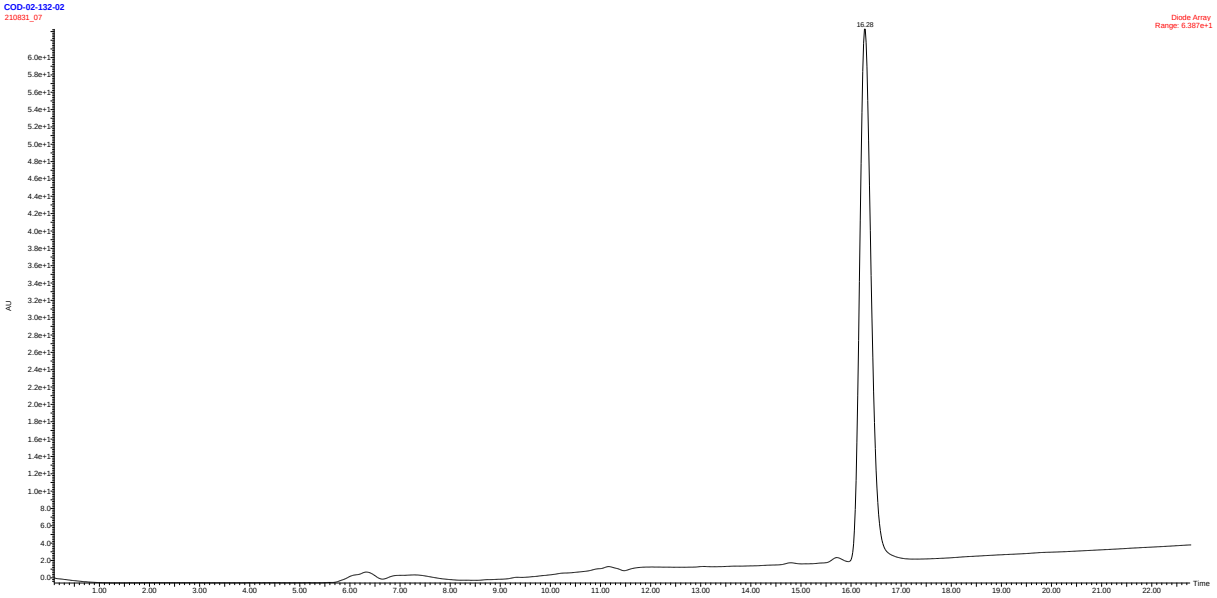


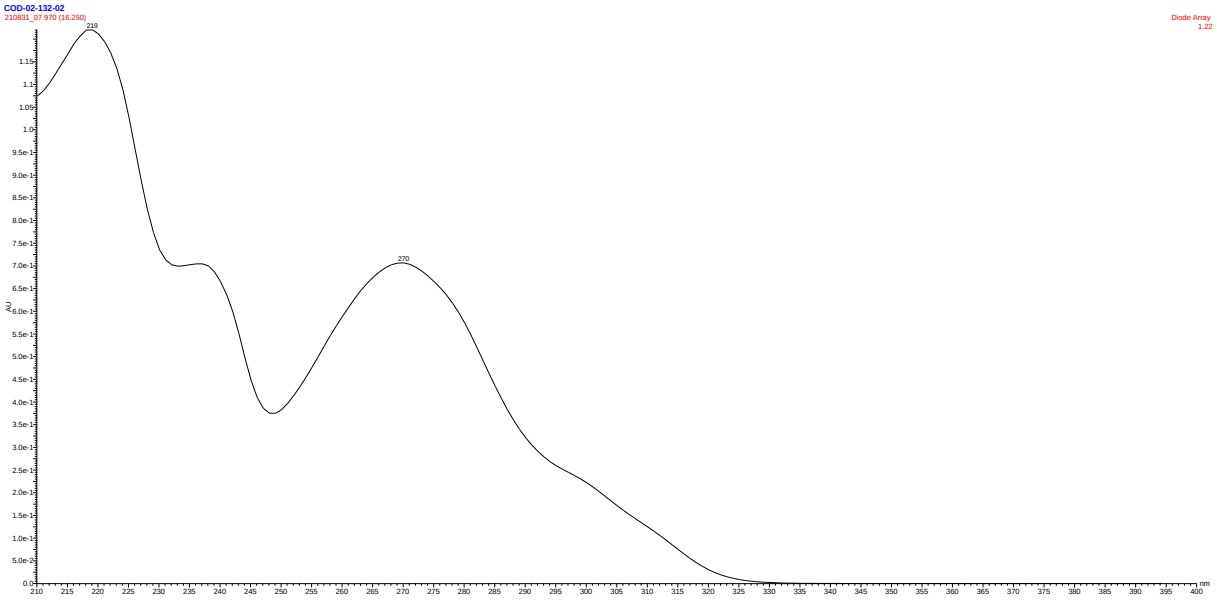
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-112**



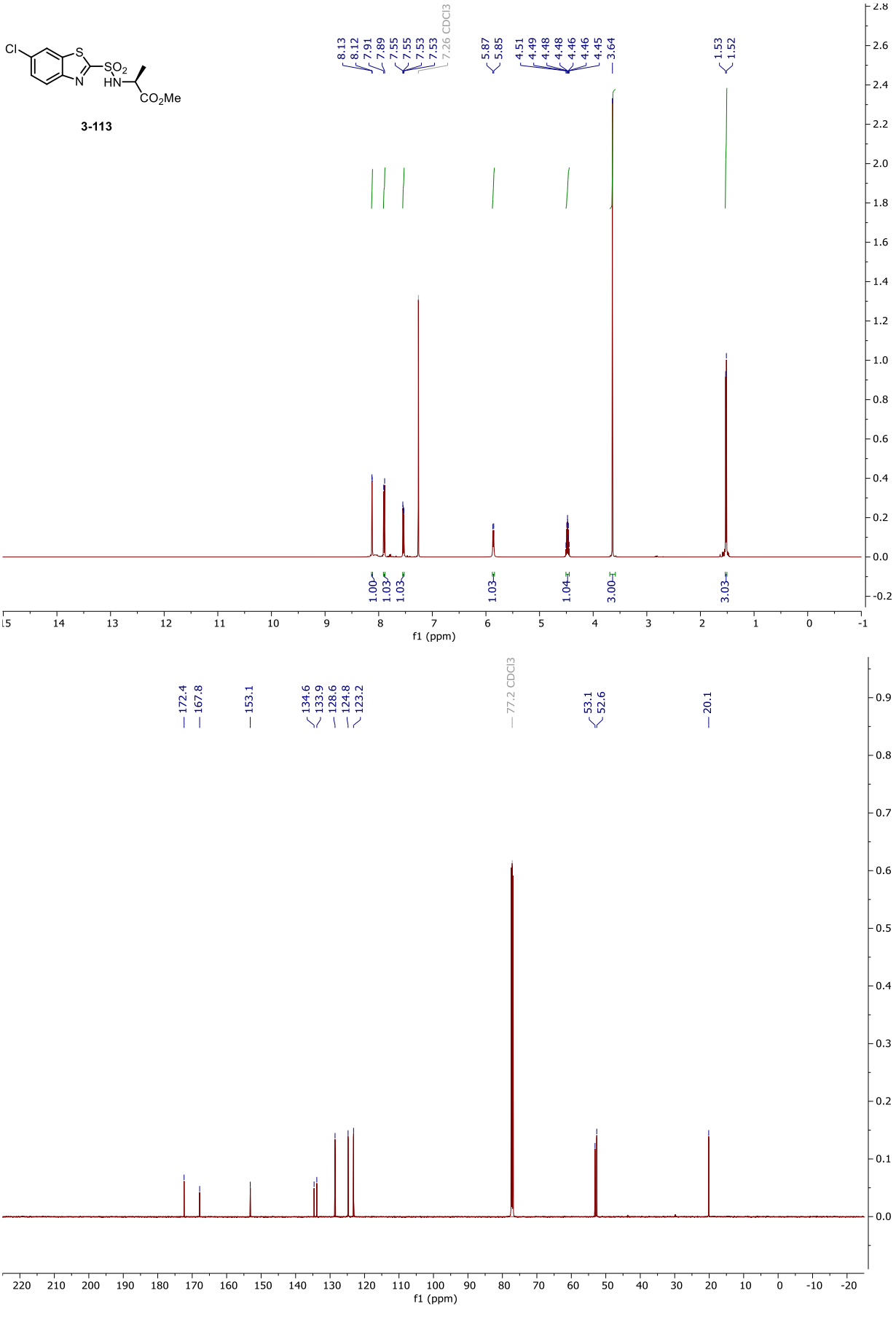


HPLC chromatograms of **3-112**

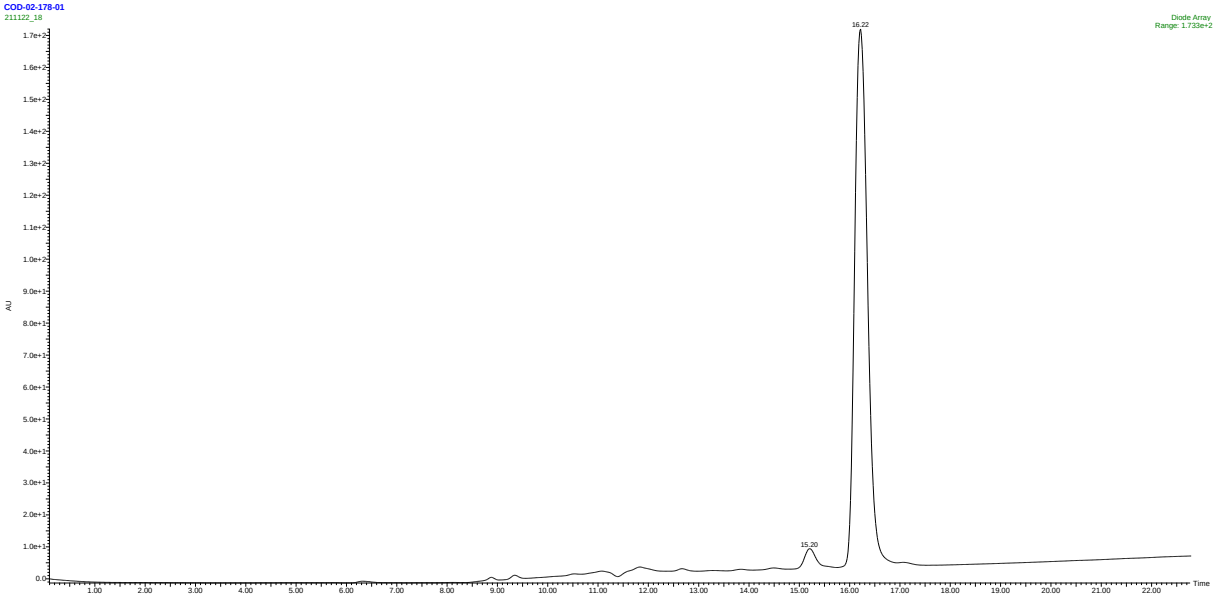


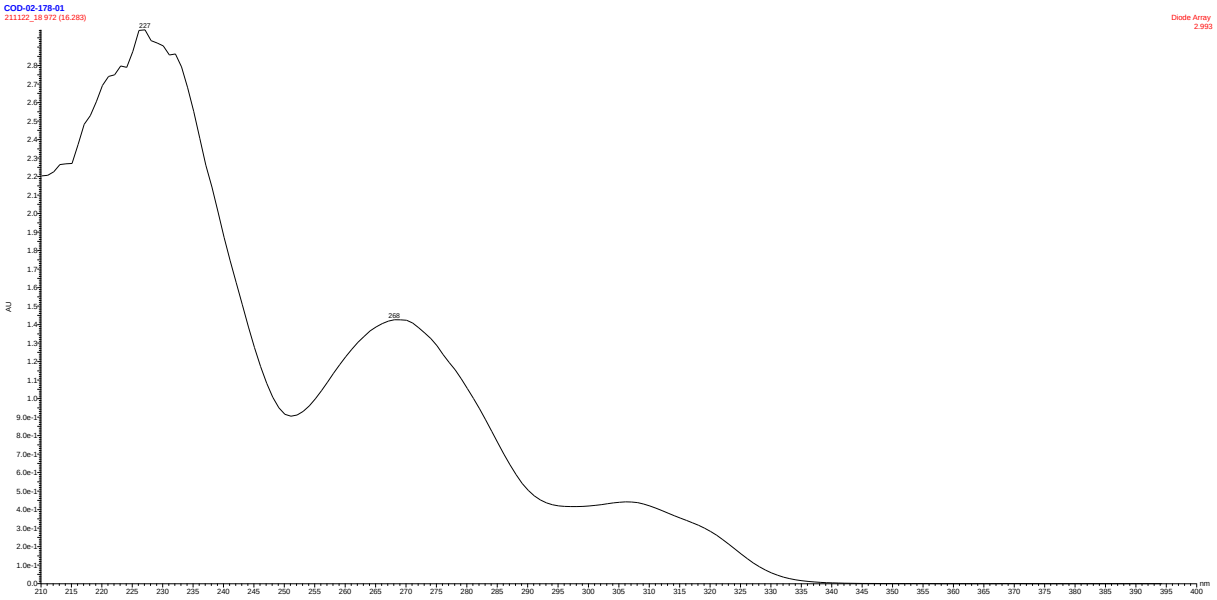


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-113**

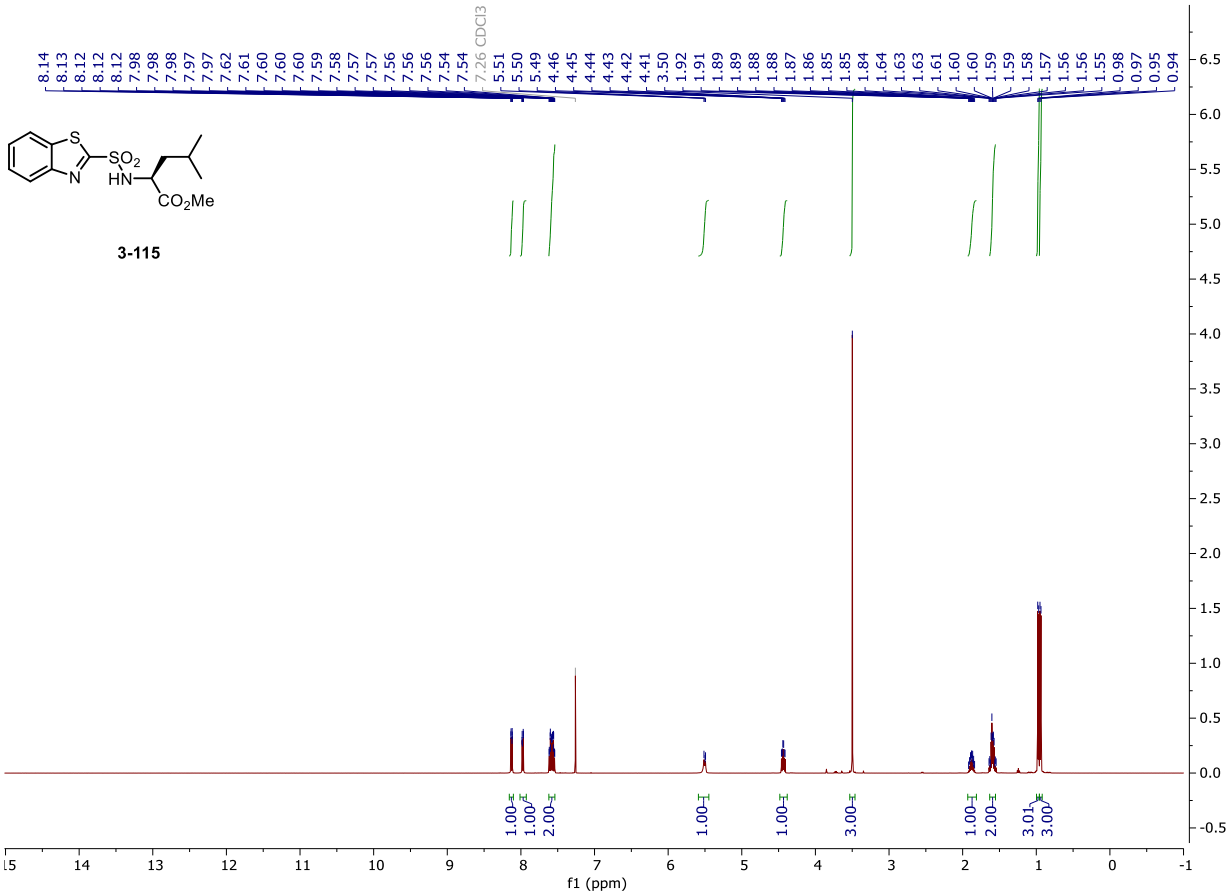


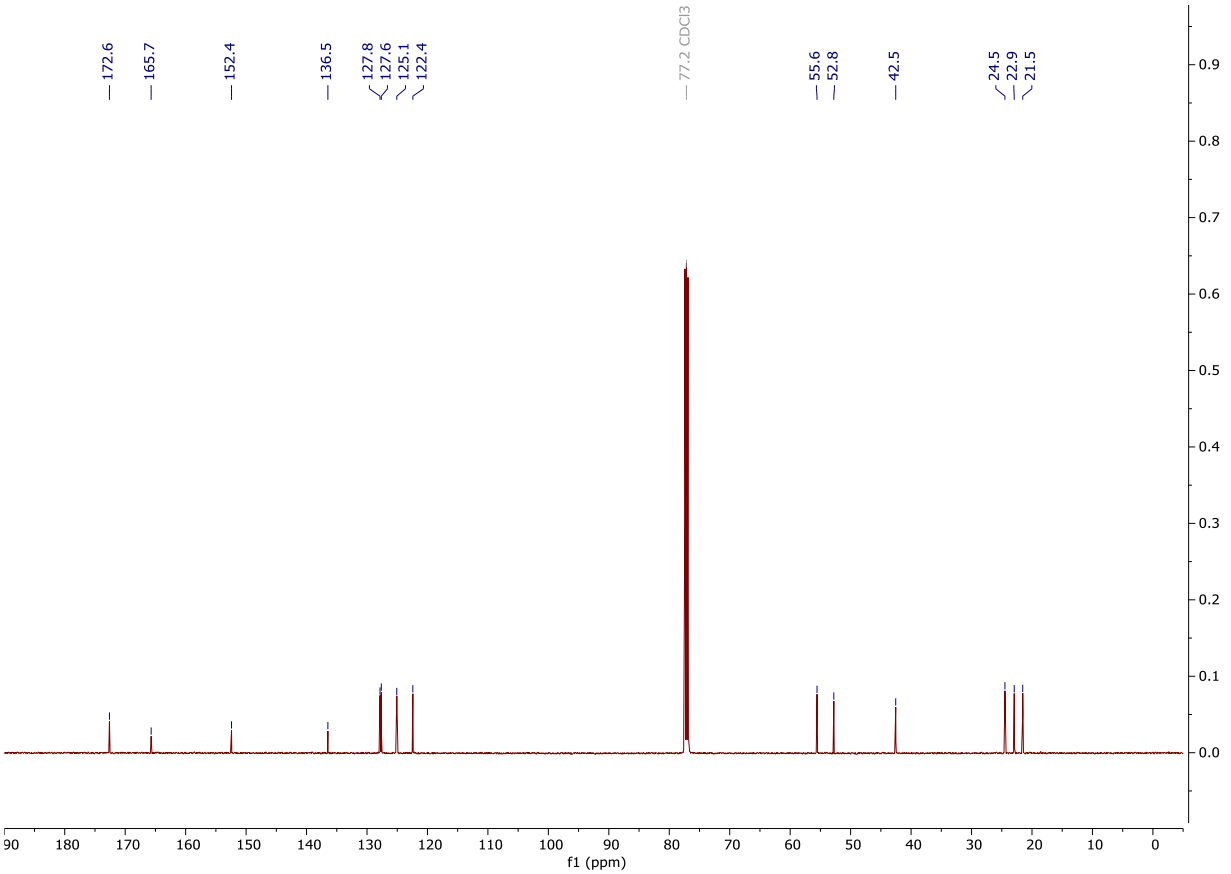
HPLC chromatograms of **3-113**



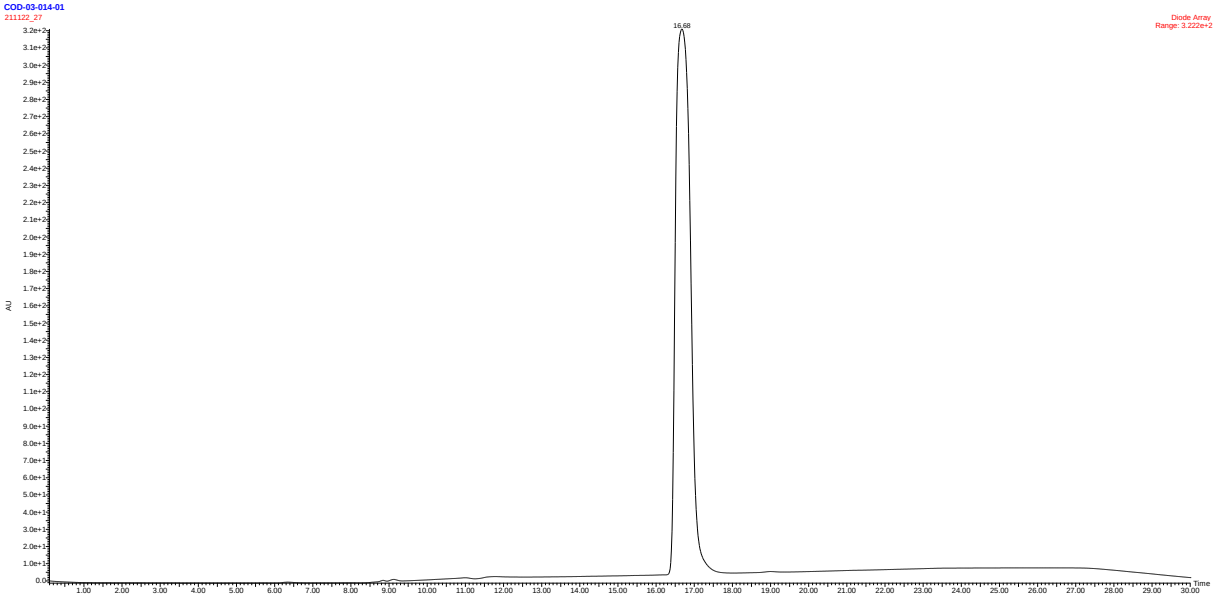


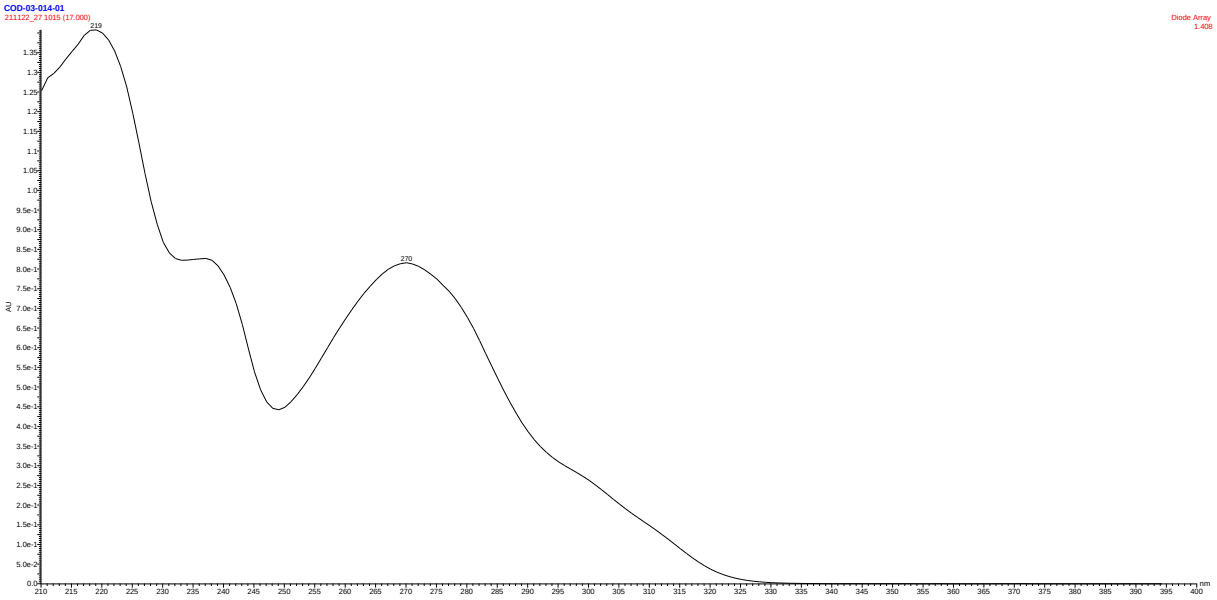
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-115**



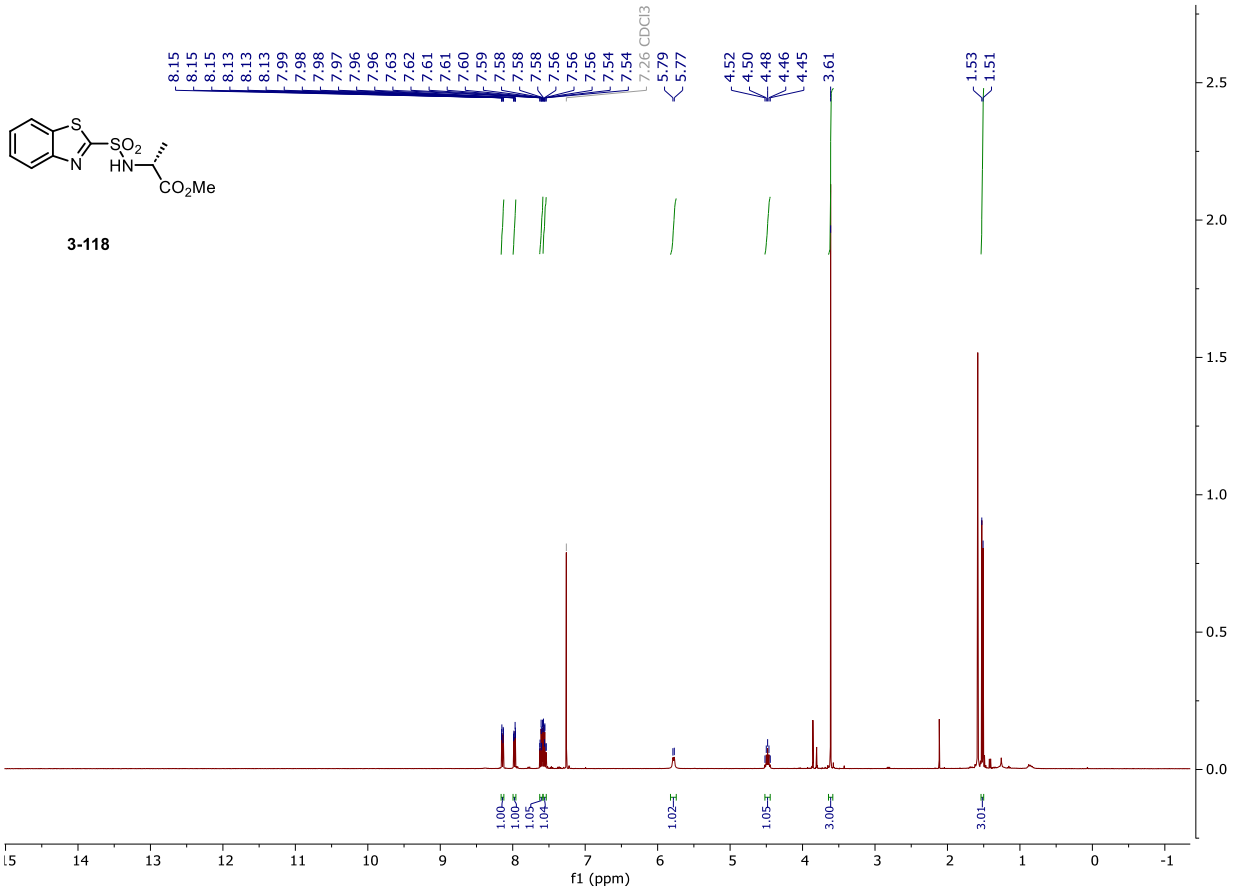


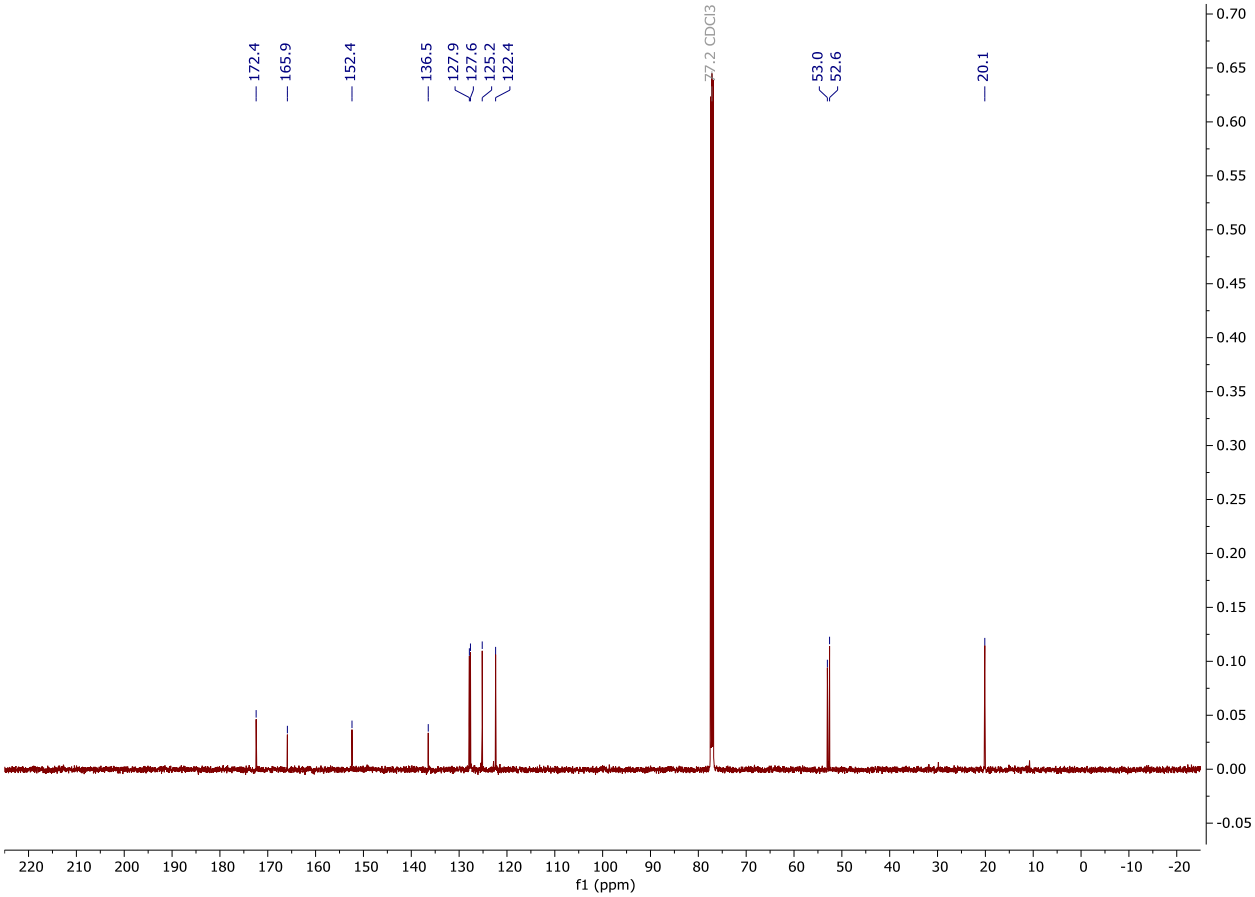
HPLC chromatograms of **3-115**





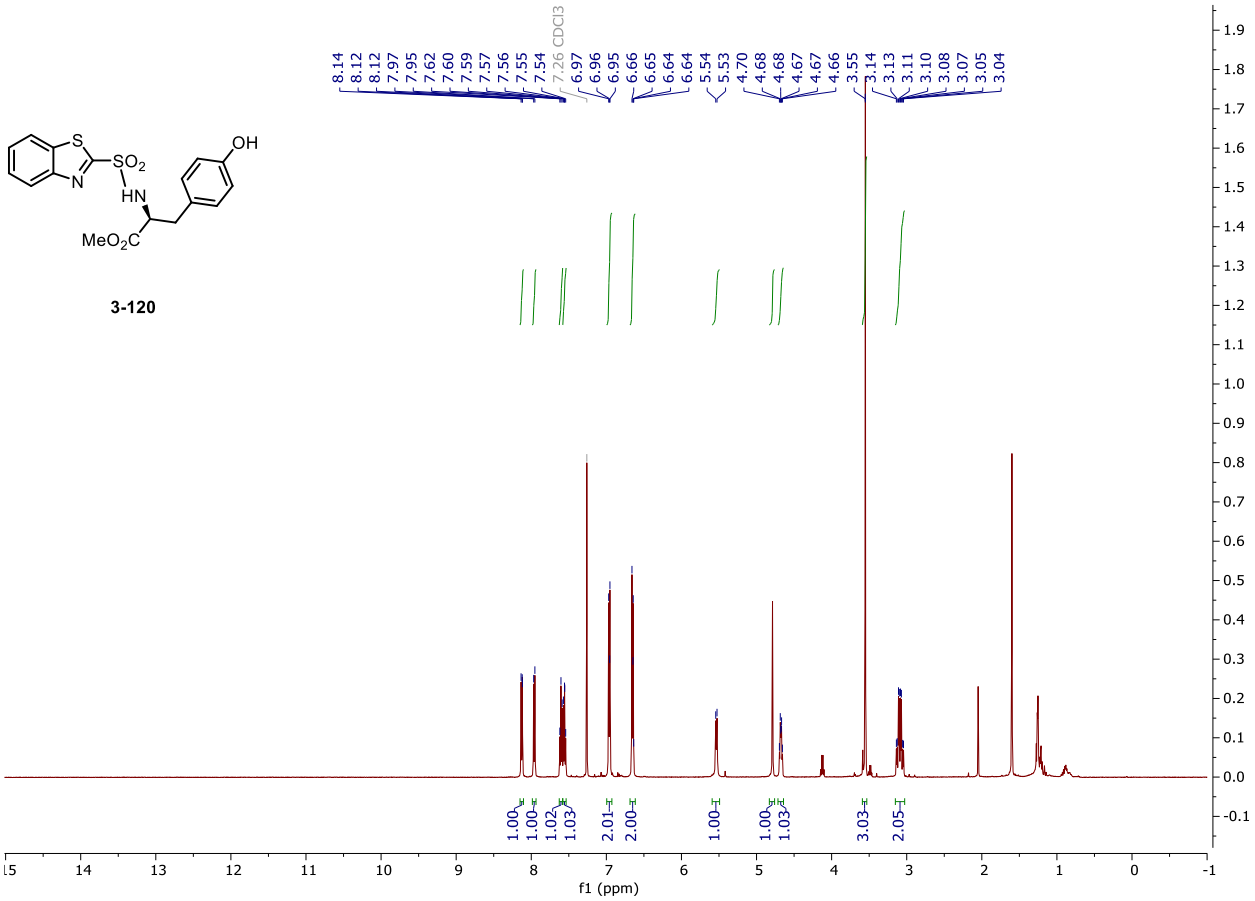
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-118**

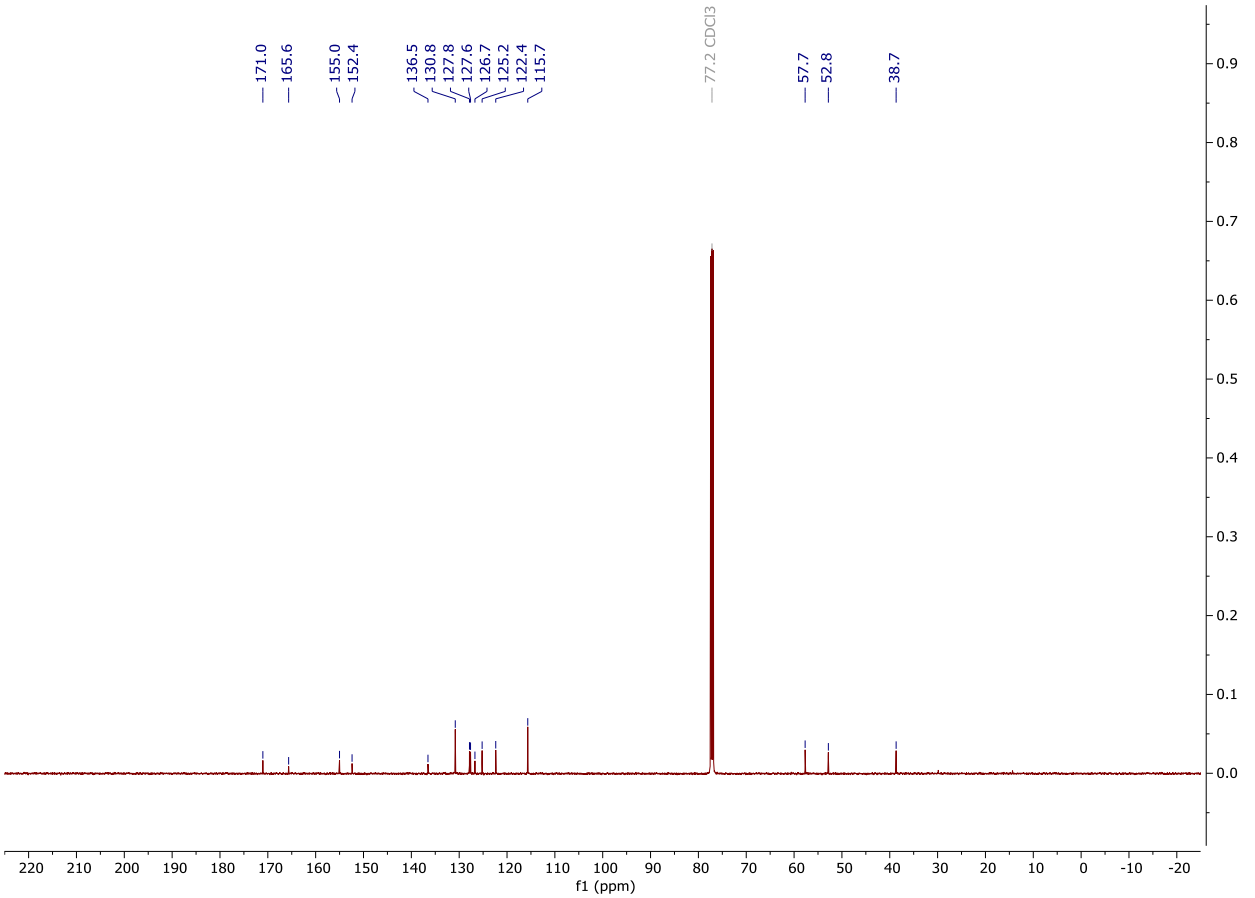




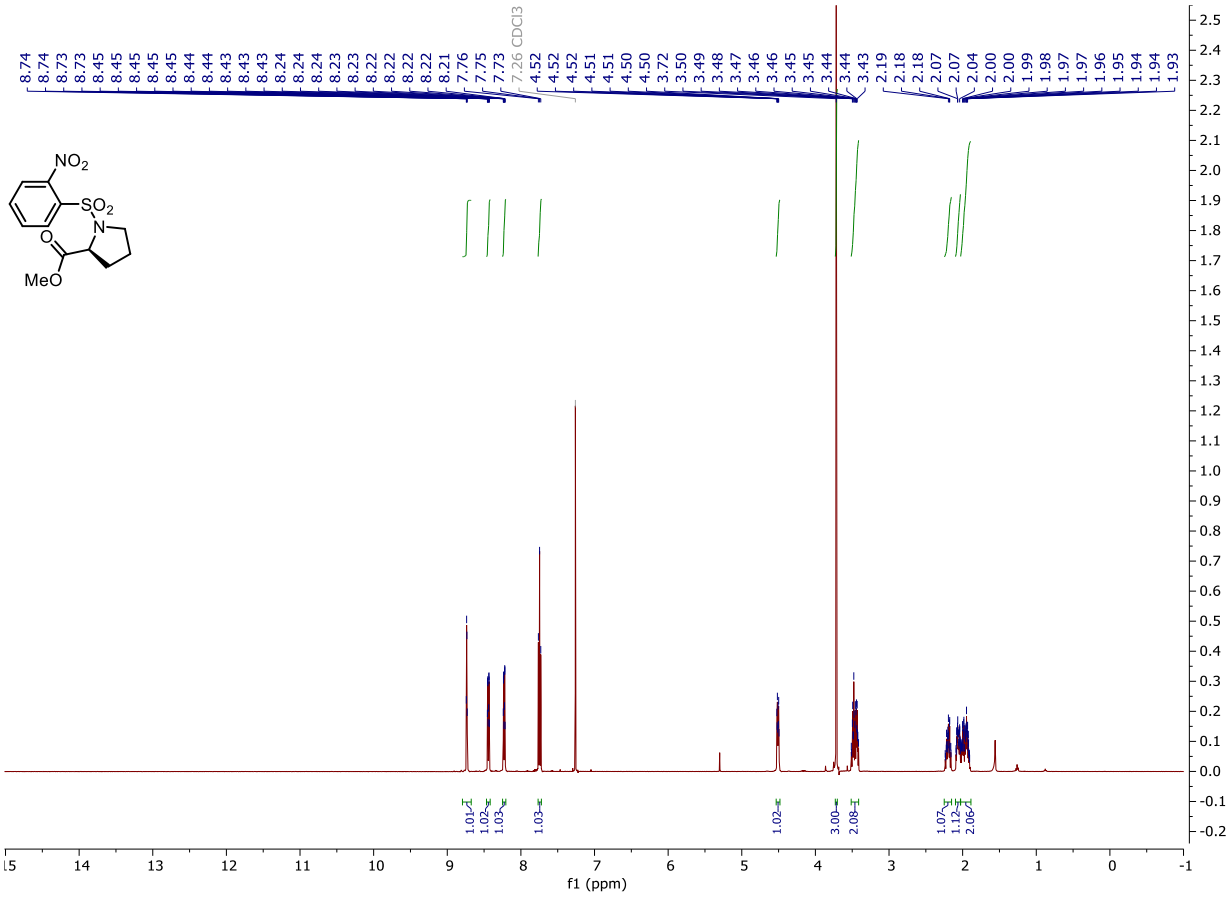
NMR and HPLC

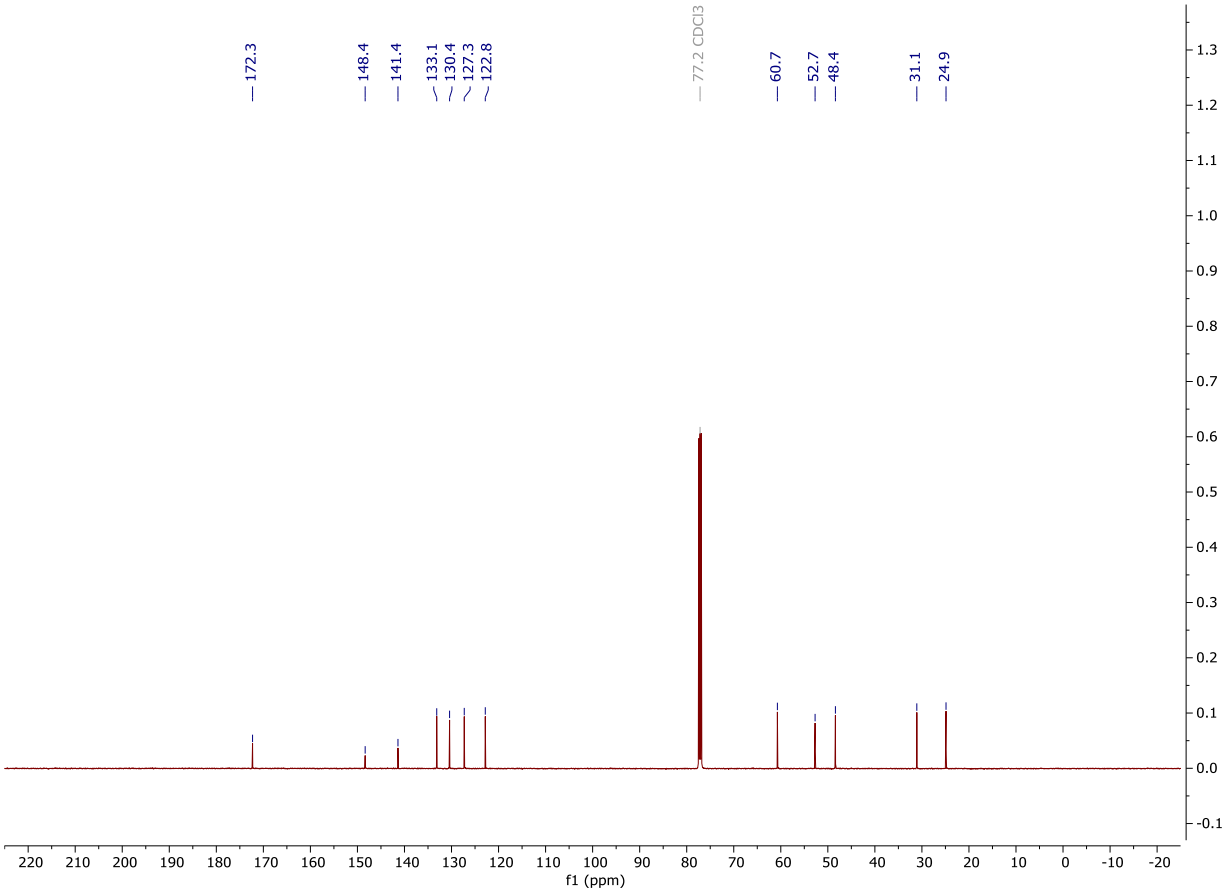
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-120**



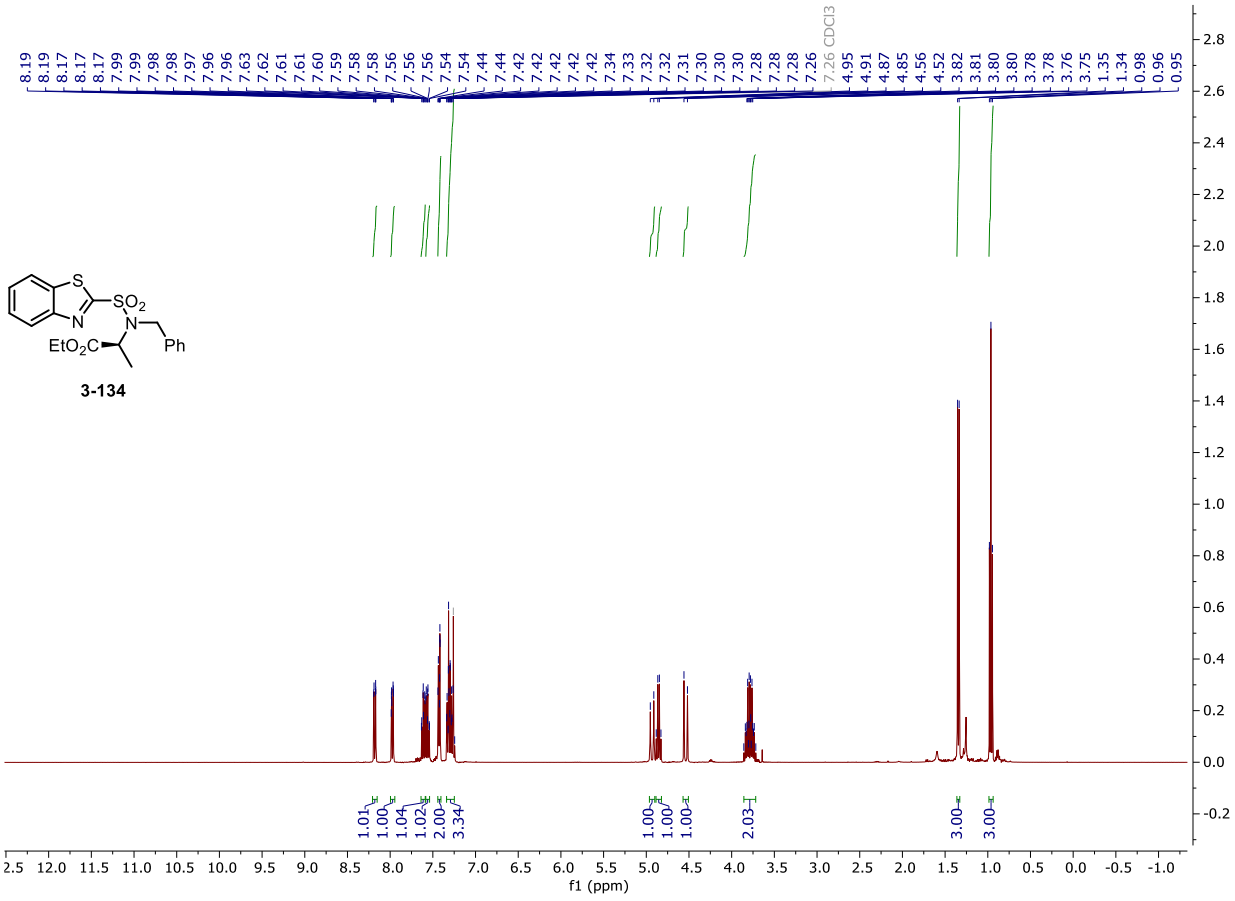


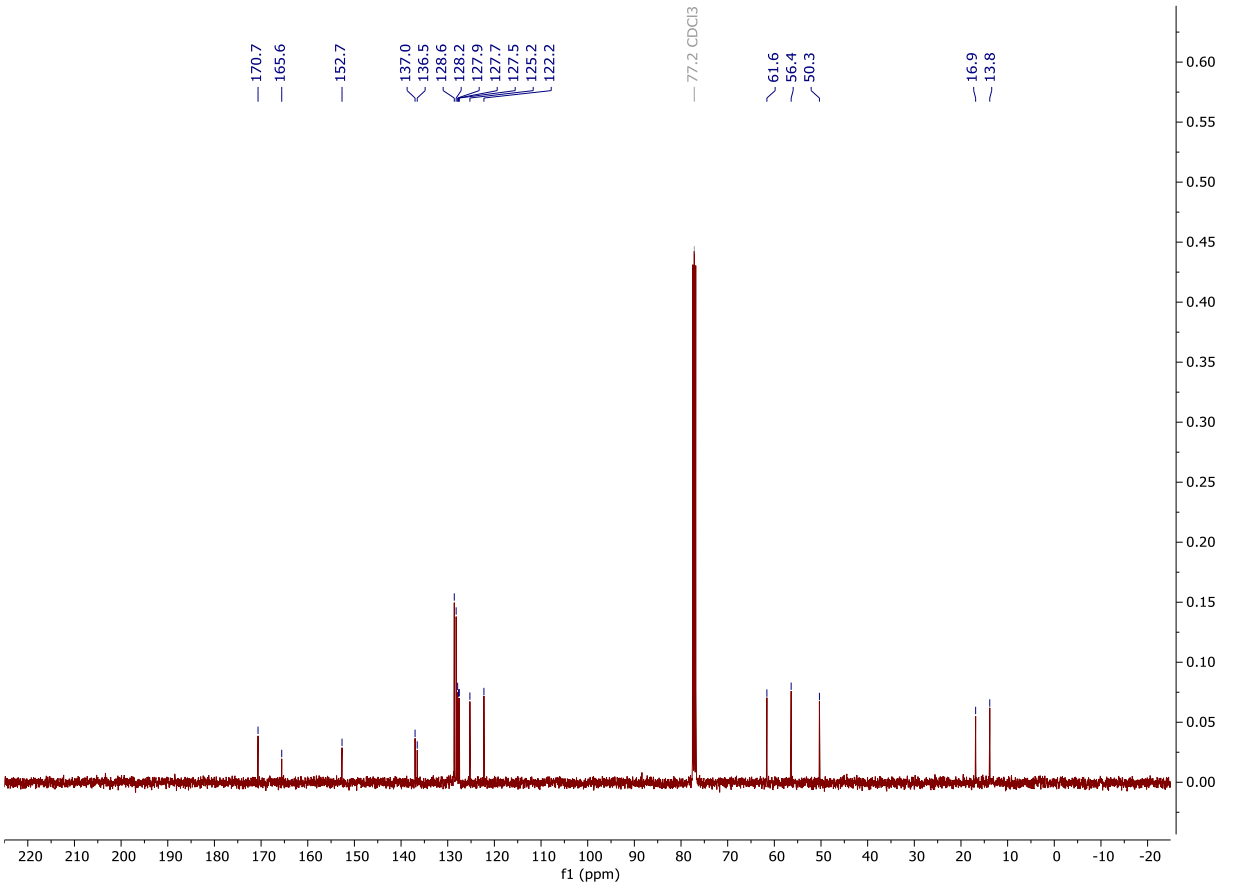
NMR and HPLC


8.74
8.74
8.73
8.73
8.45
8.45
8.45
8.45
8.44
8.44
8.43
8.43
8.43
8.24
8.24
8.24
8.23
8.23
8.22
8.22
8.22
8.22
8.21
7.76
7.75
7.73
7.26 CDCl3
4.52
4.52
4.52
4.51
4.51
4.50
4.50
3.72
3.50
3.49
3.48
3.47
3.46
3.46
3.45
3.45
3.44
3.44
3.43
2.19
2.18
2.18
2.07
2.07
2.04
2.00
2.00
1.99
1.98
1.97
1.97
1.96
1.95
1.94
1.94
1.93
NO2
SO2
N
O
MeO
1.01
1.02
1.03
1.03
1.02
3.00
2.08
1.07
1.12
2.06
f1 (ppm)

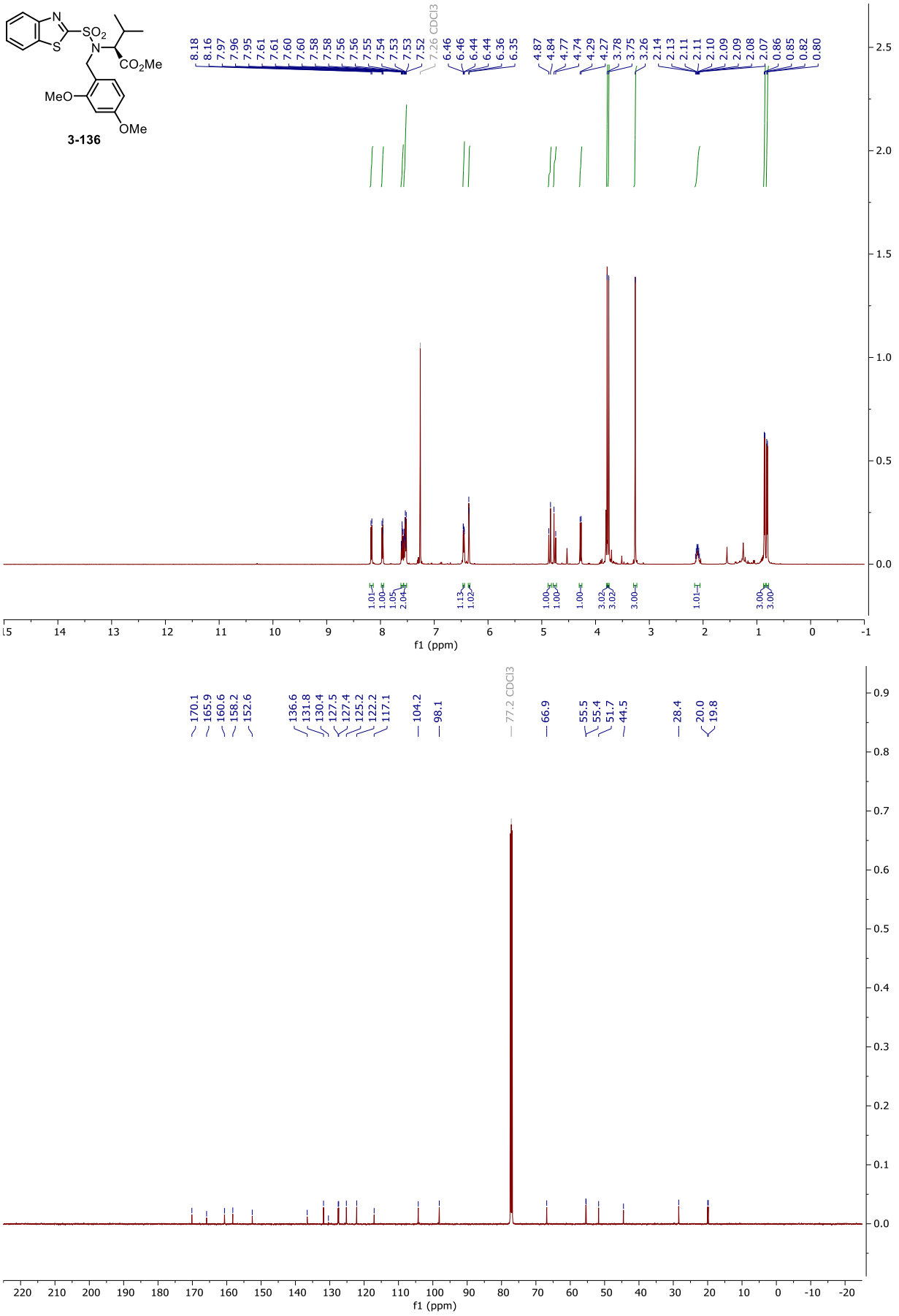

172.3
148.4
141.4
133.1
130.4
127.3
122.8
77.2 CDCl3
60.7
52.7
48.4
31.1
24.9
f1 (ppm)

Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-134**

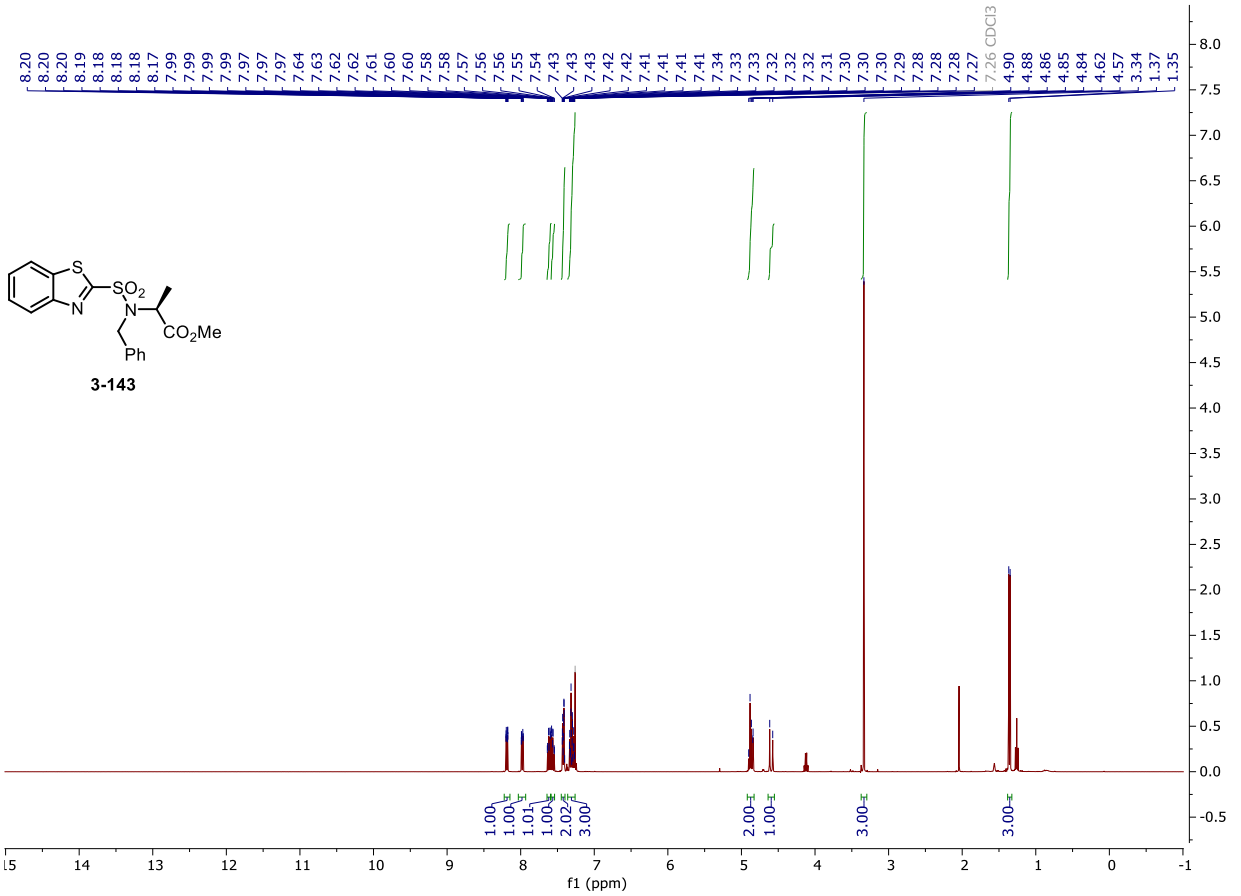


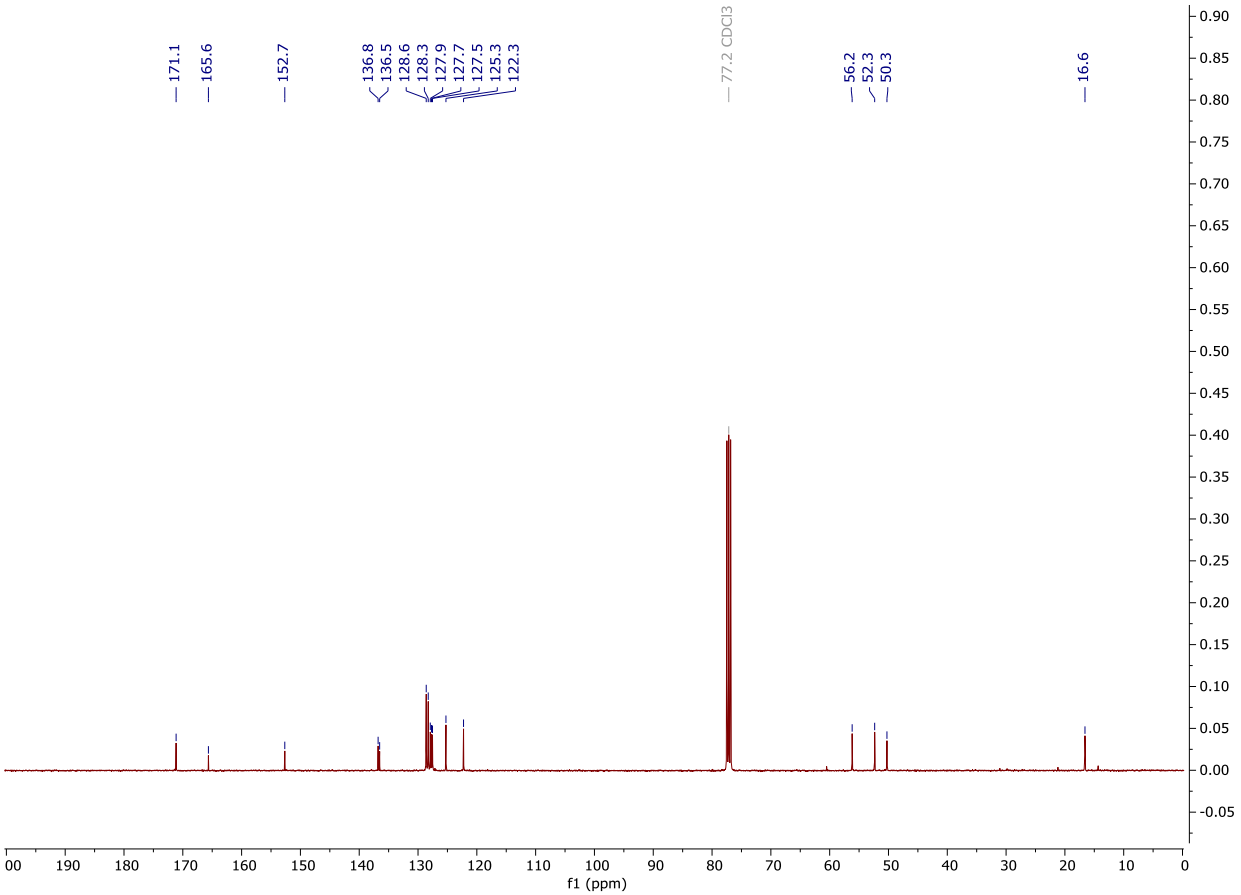


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-136**

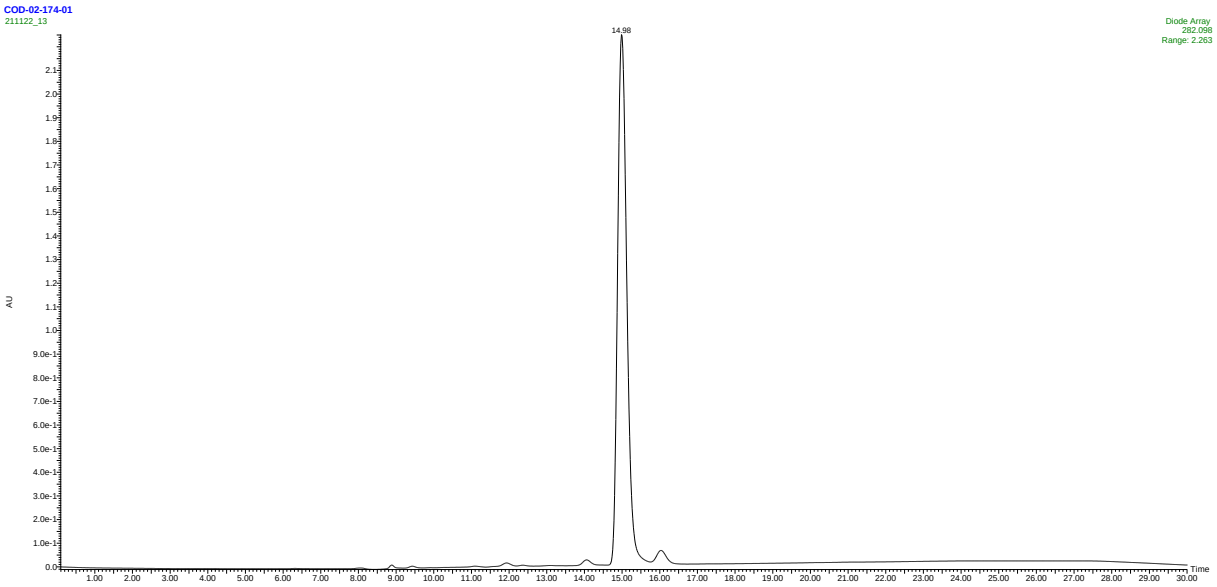


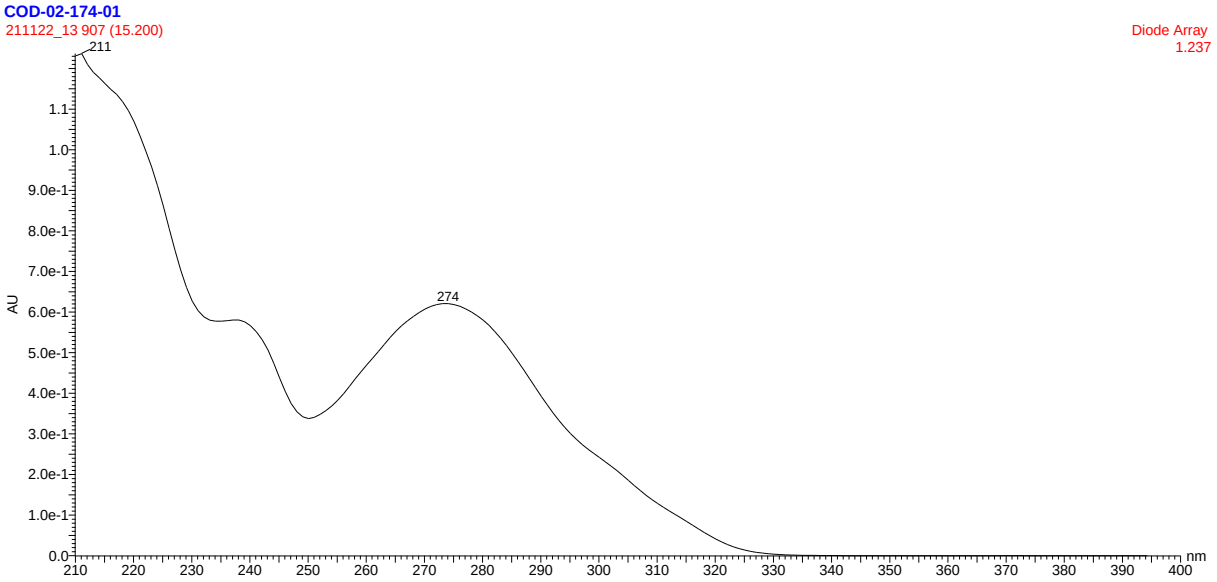
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-143**



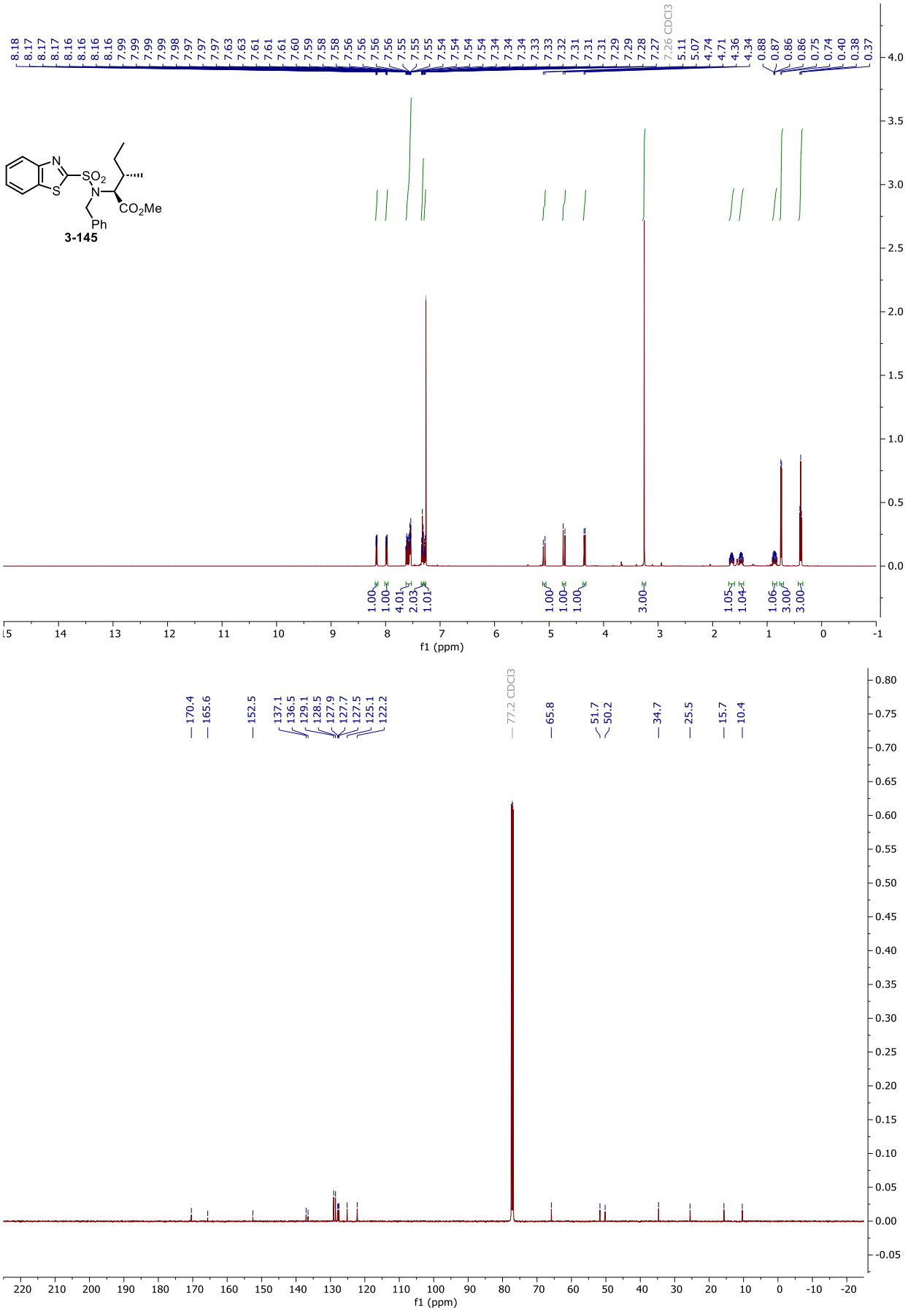


HPLC chromatograms of **3-143**

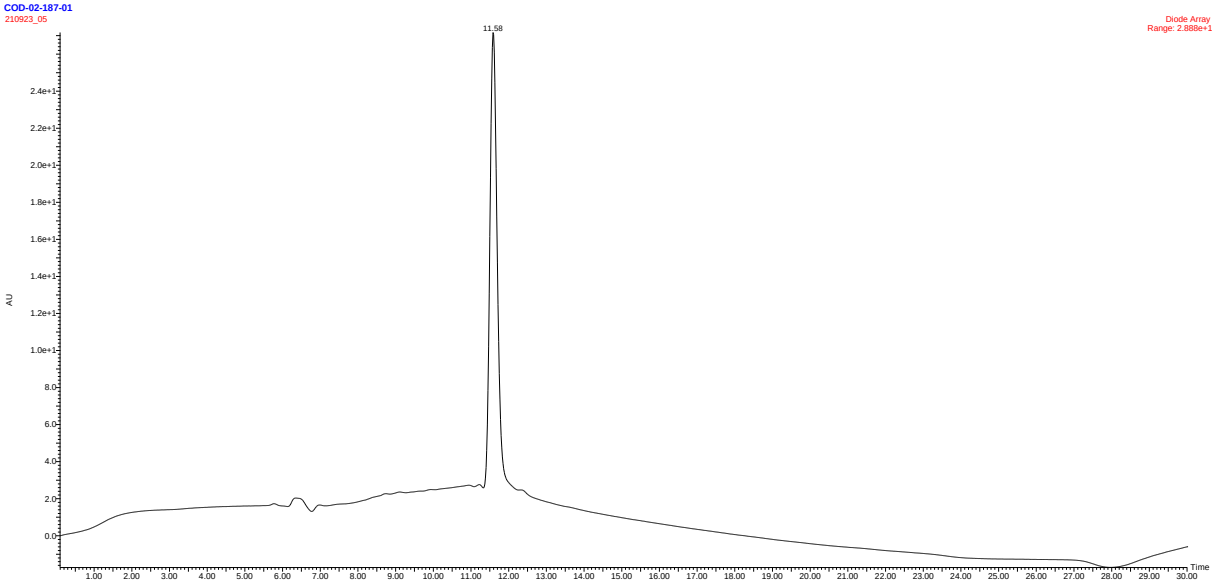


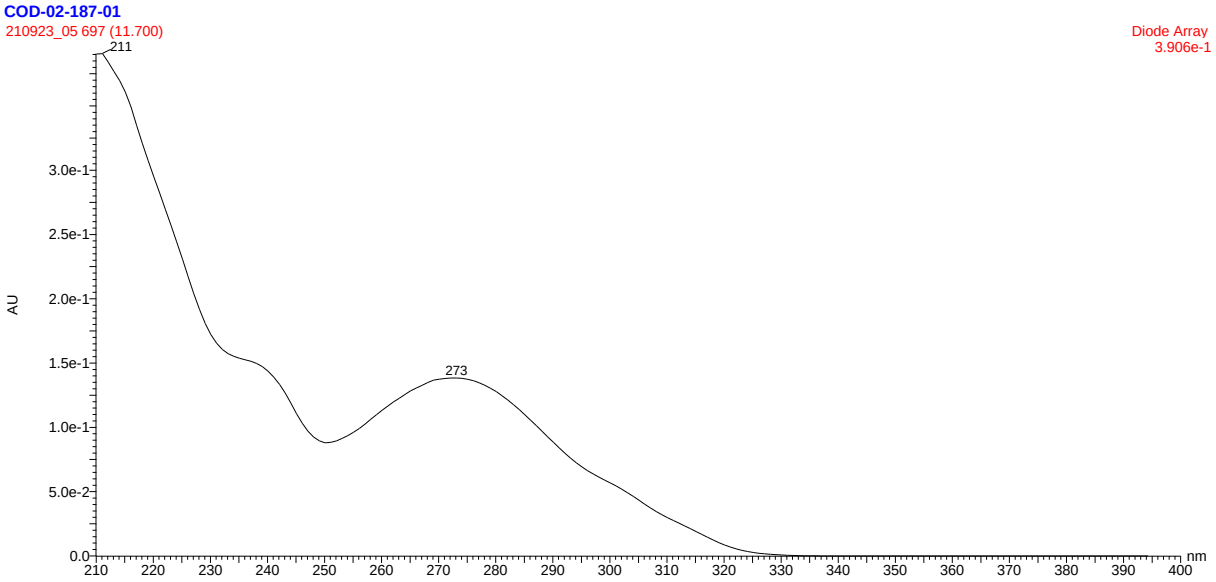


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-145**

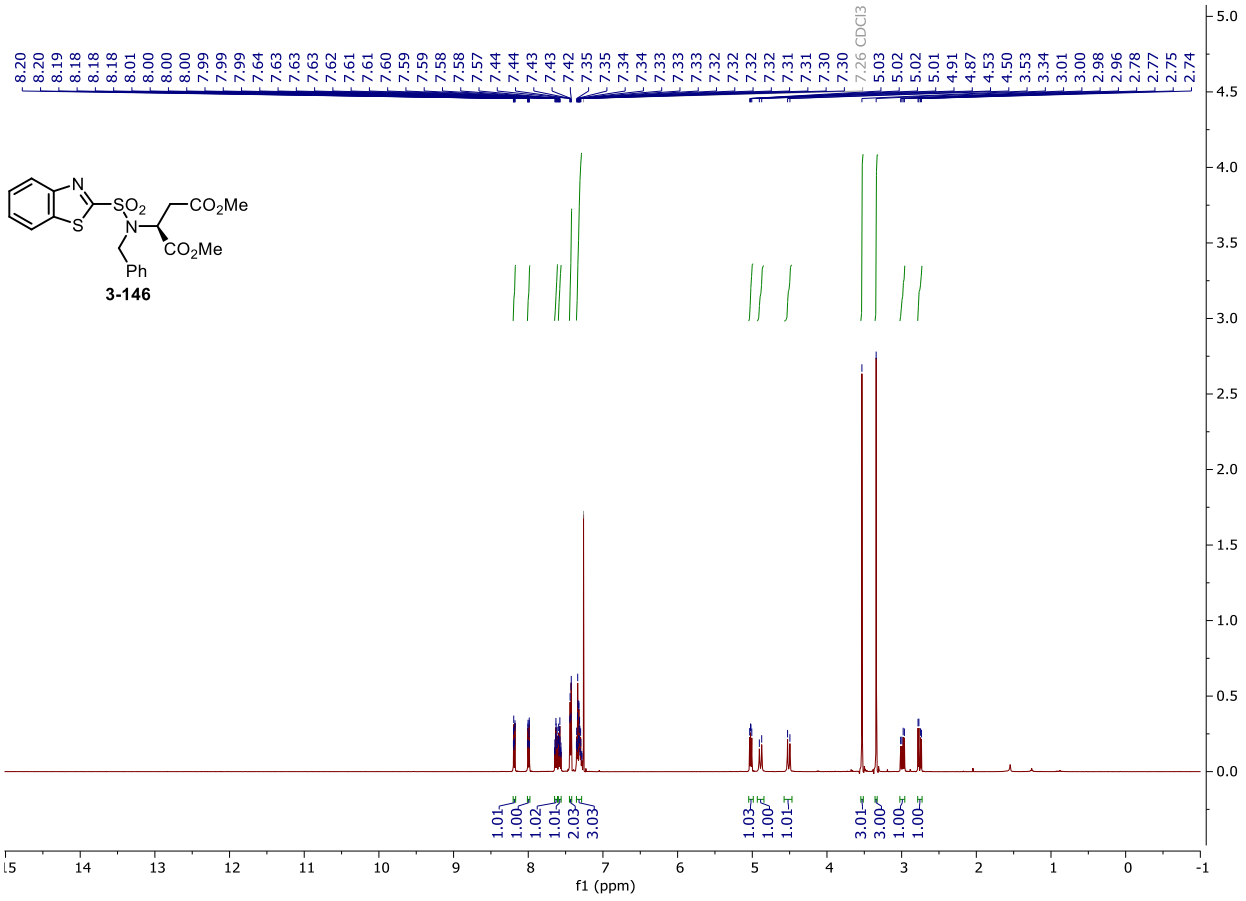


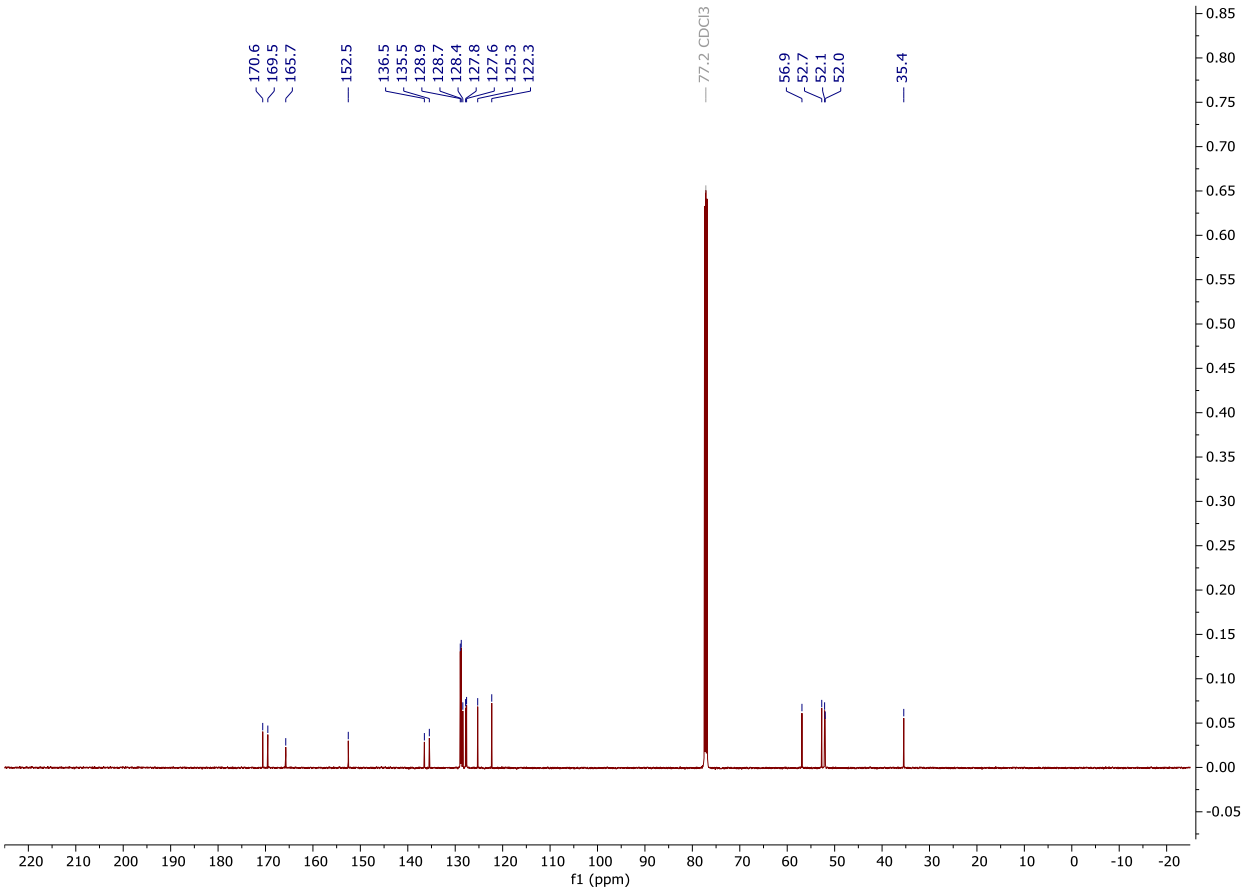
HPLC chromatograms of **3-145**



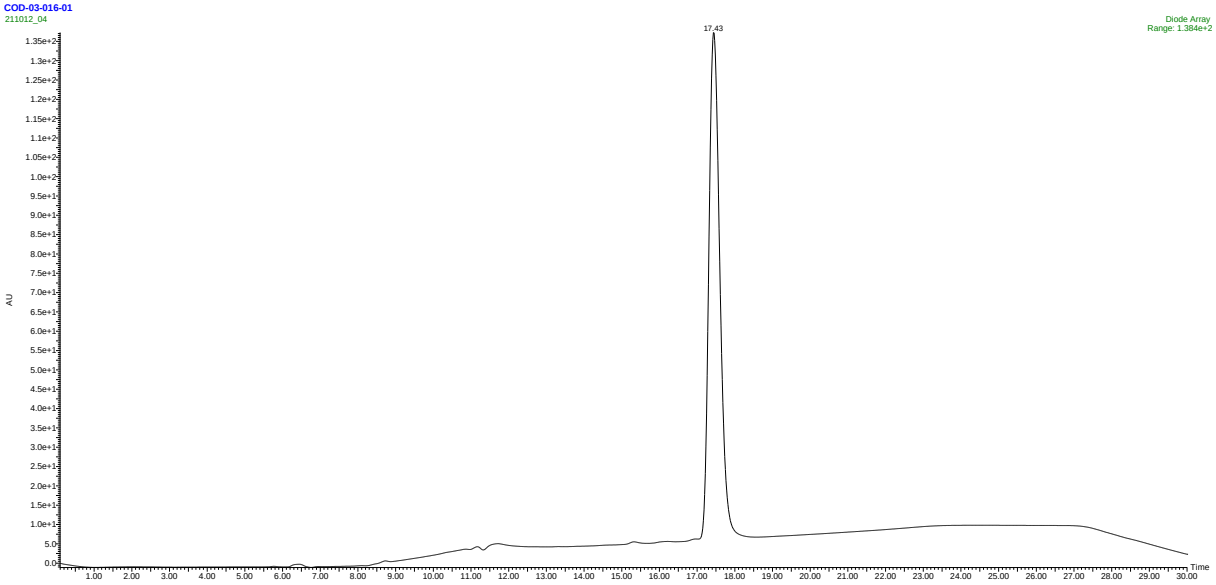


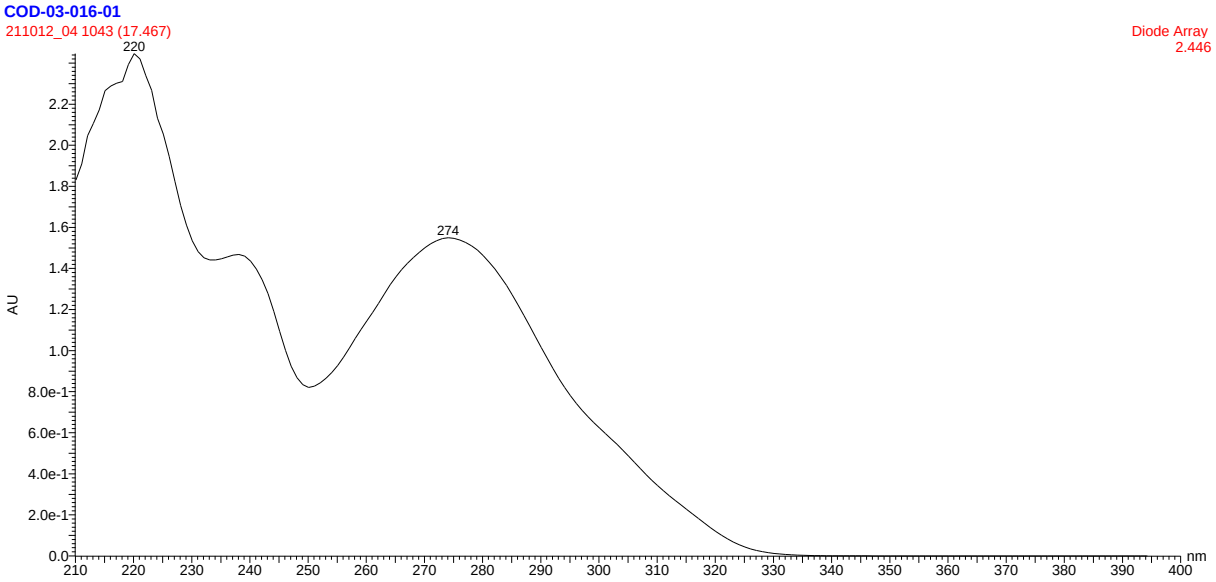
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-146**



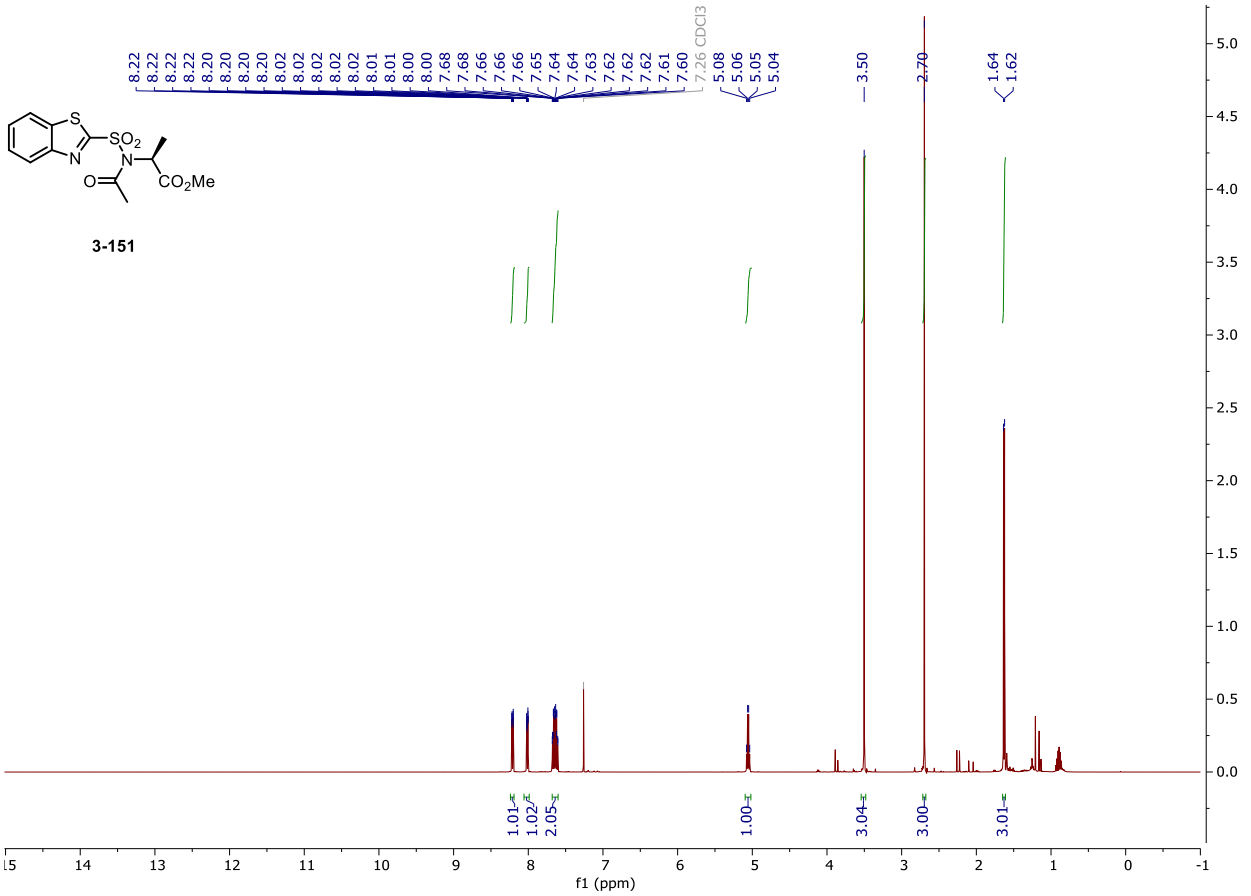


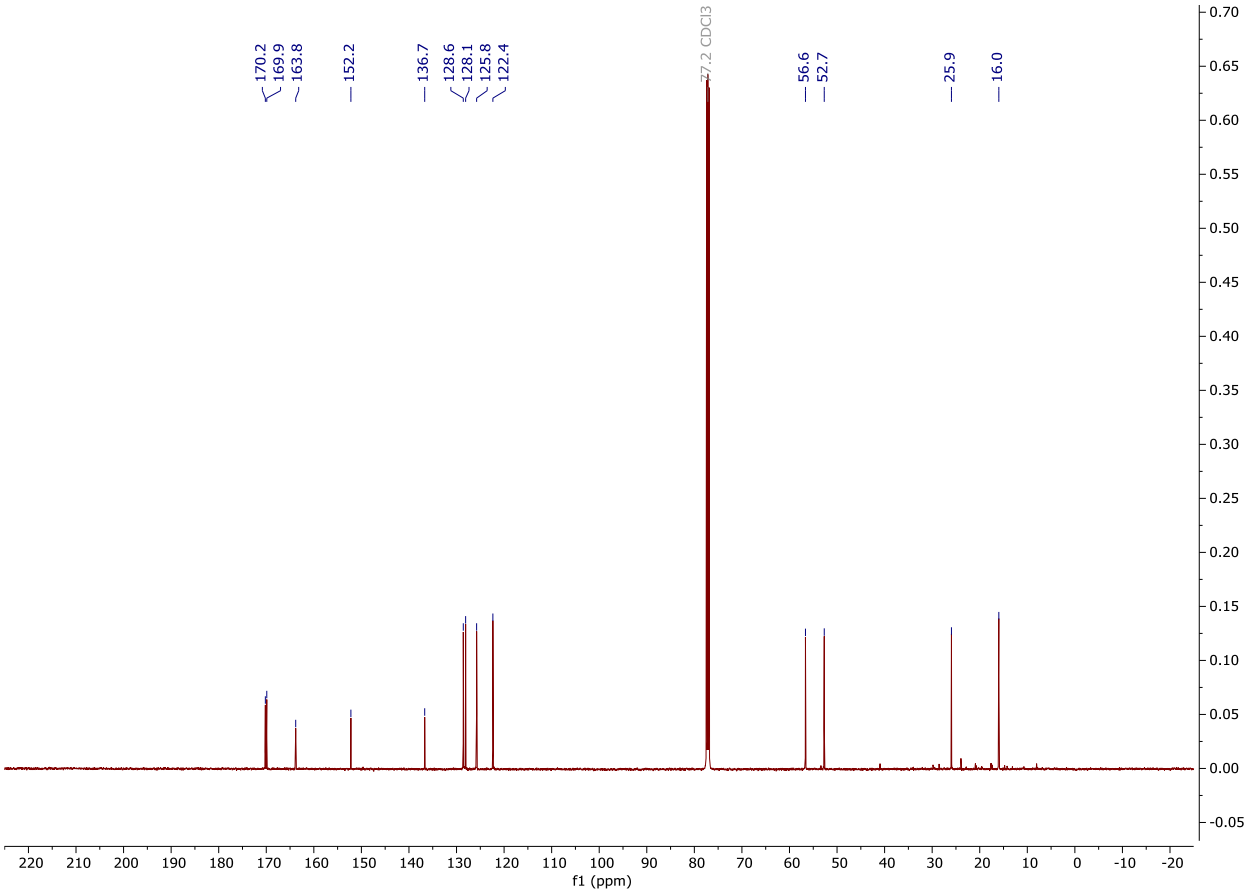
HPLC chromatograms of **3-146**



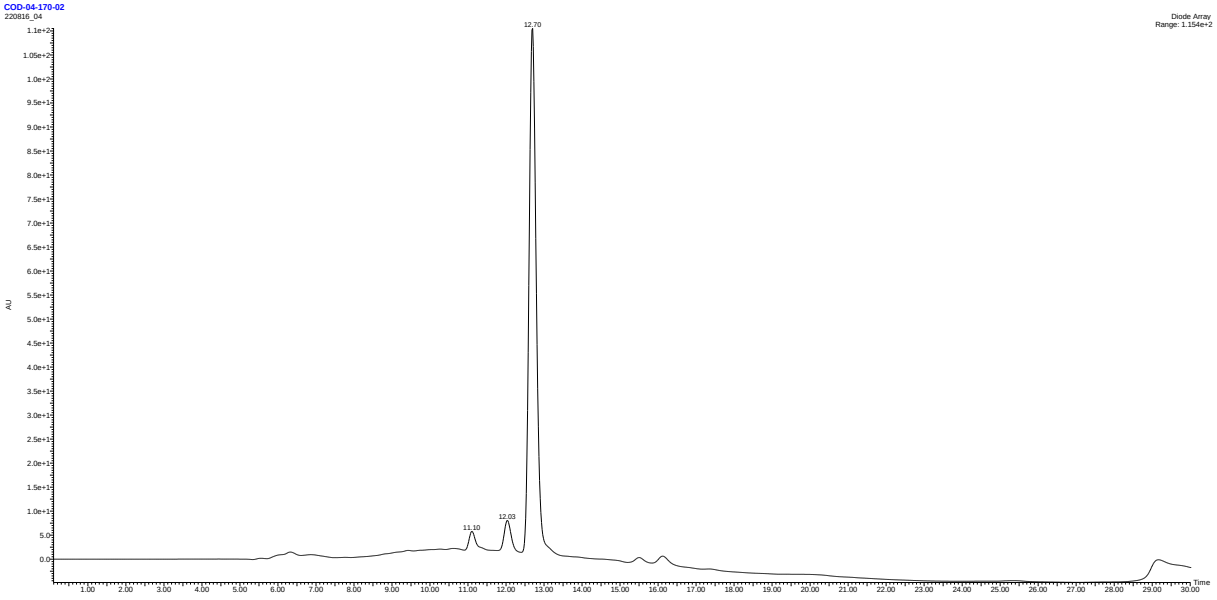


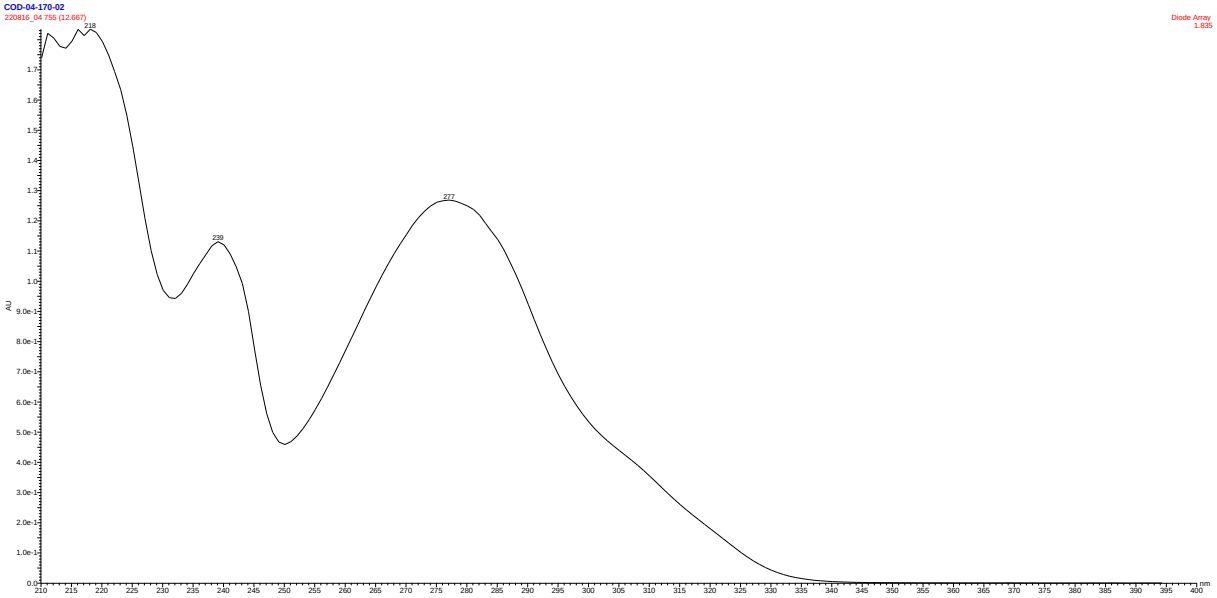
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-151**



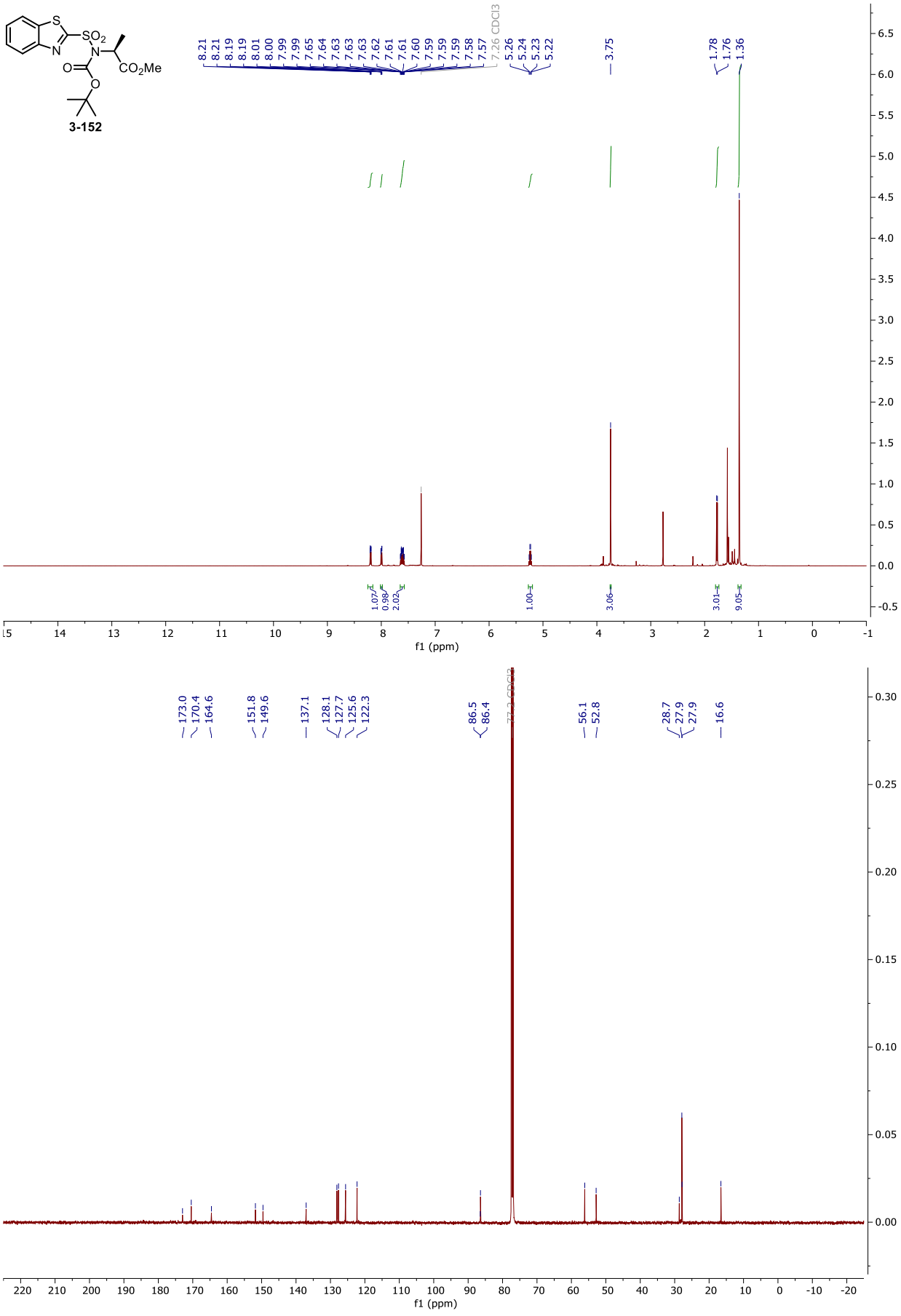


NMR and HPLC


COD-04-170-02
220816_04
Diode Array
Range: 1.154e+2
12.70
11.10
12.03
AU
Time

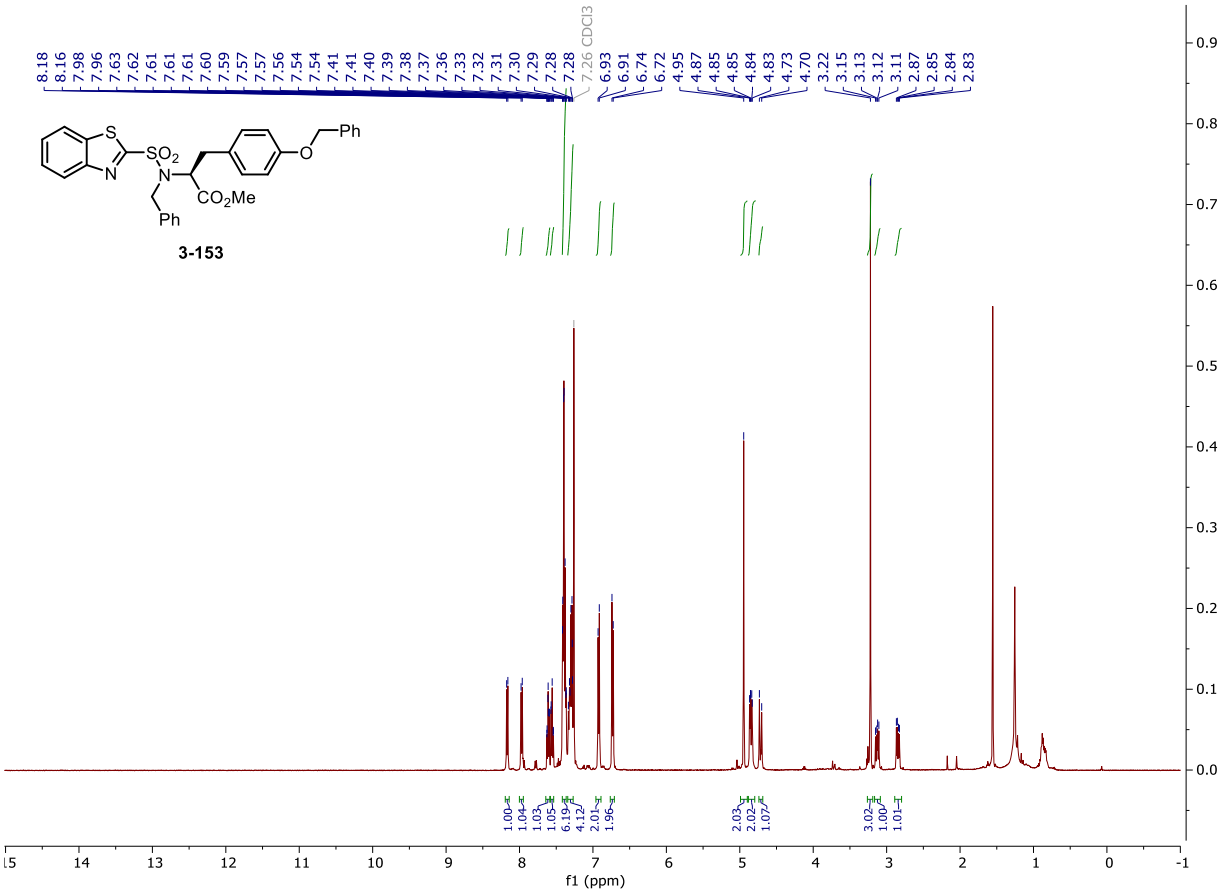

COD-04-170-02
220816_04 755 (12.667)
Diode Array
1.835
218
239
277
AU
nm

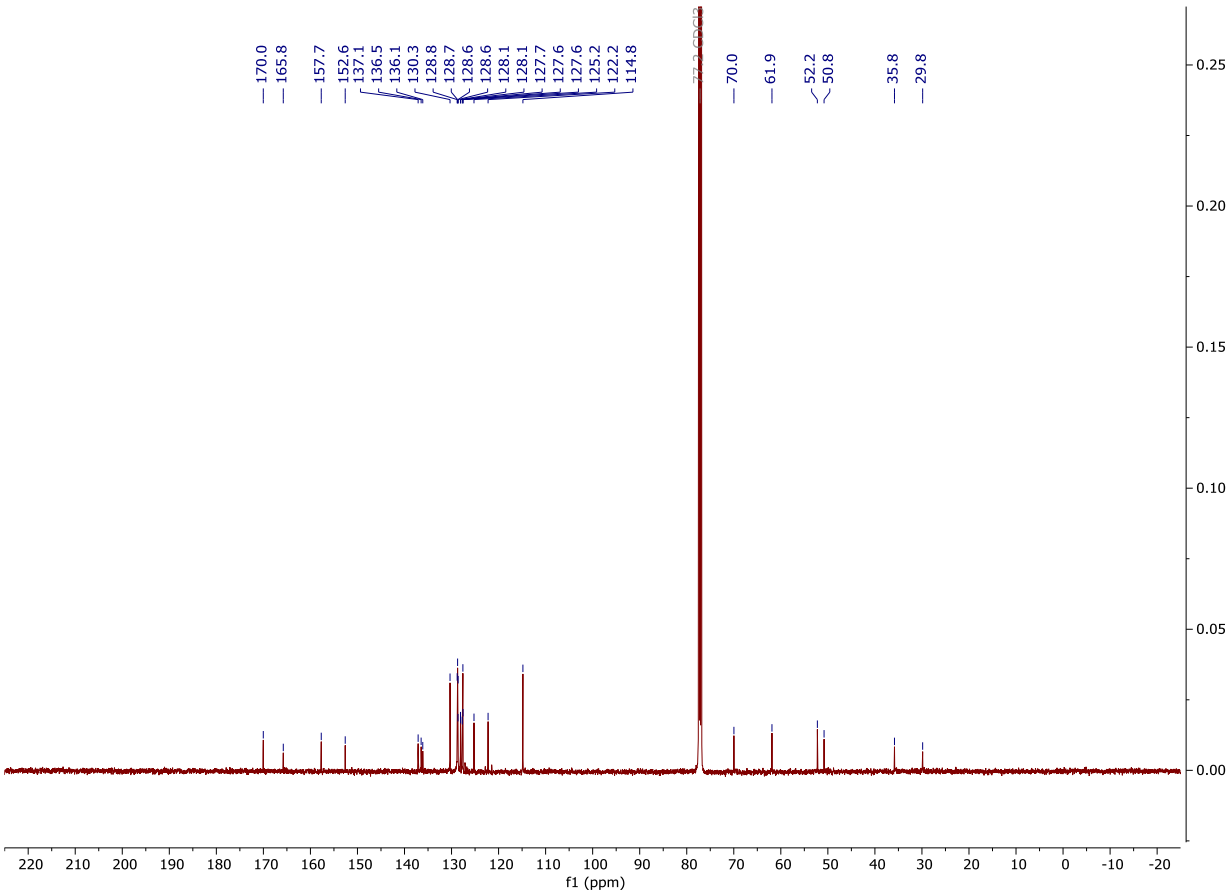
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-152**



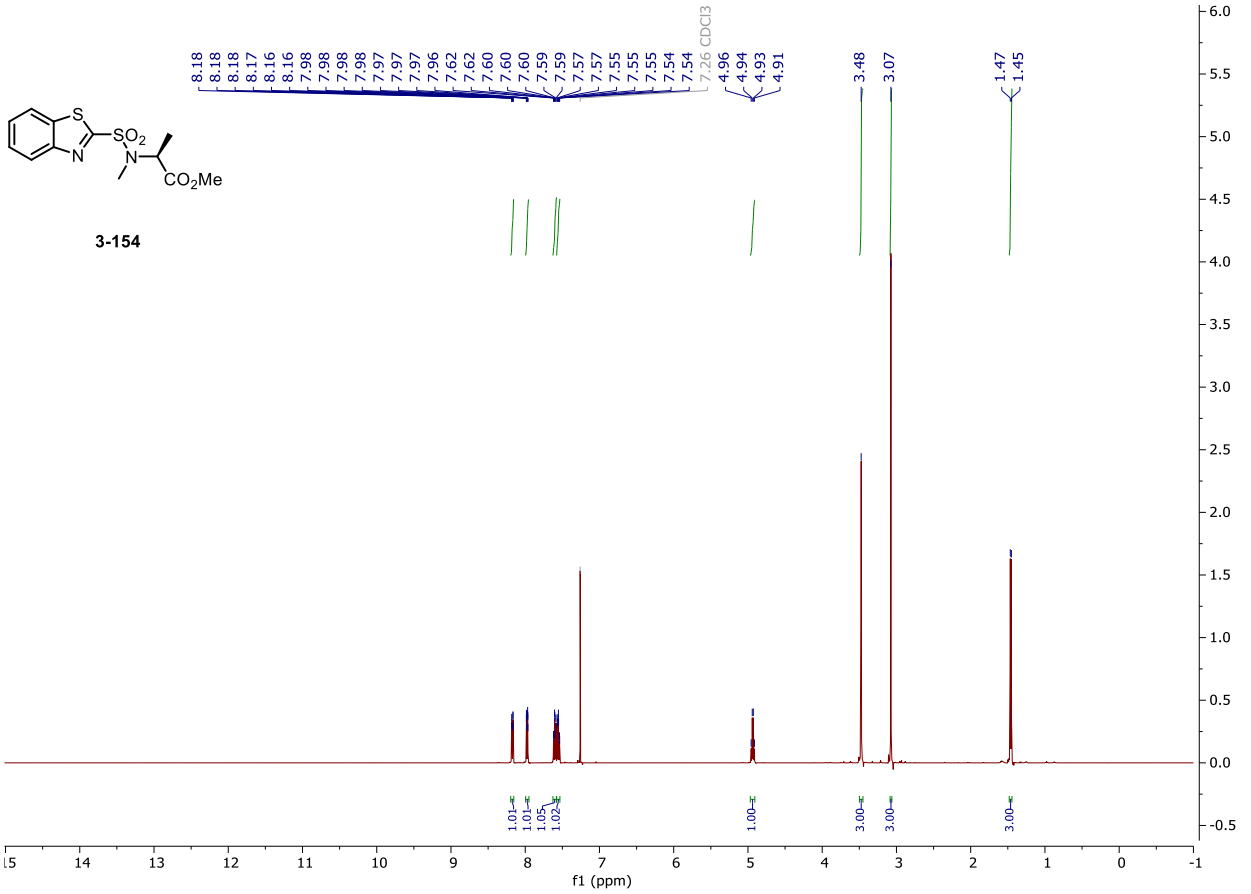
NMR and HPLC

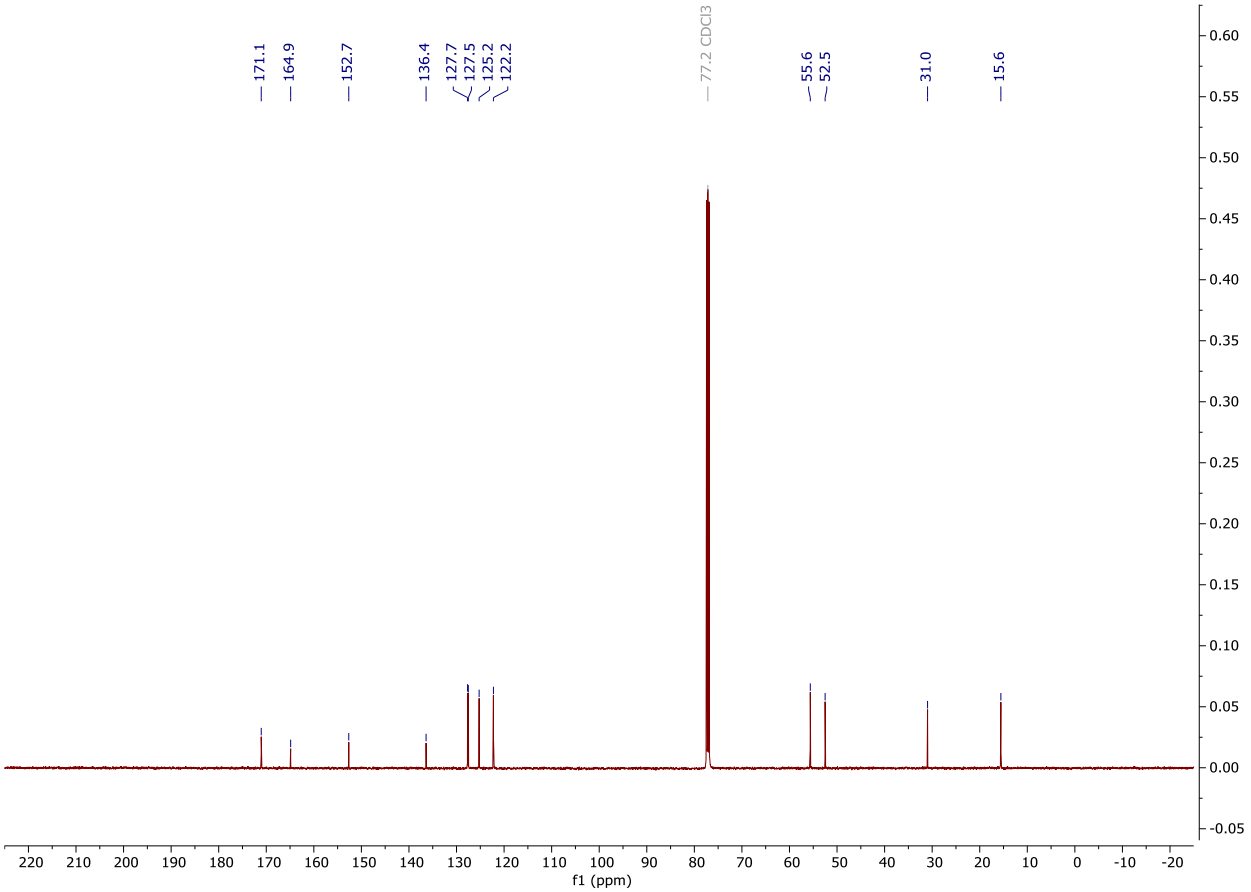
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-153**



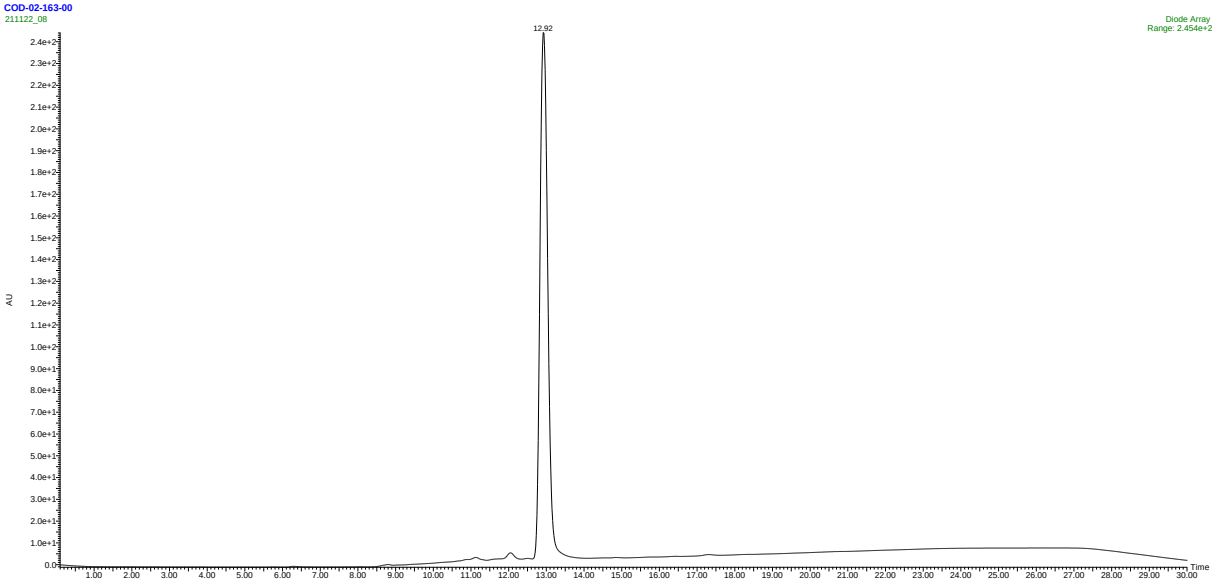


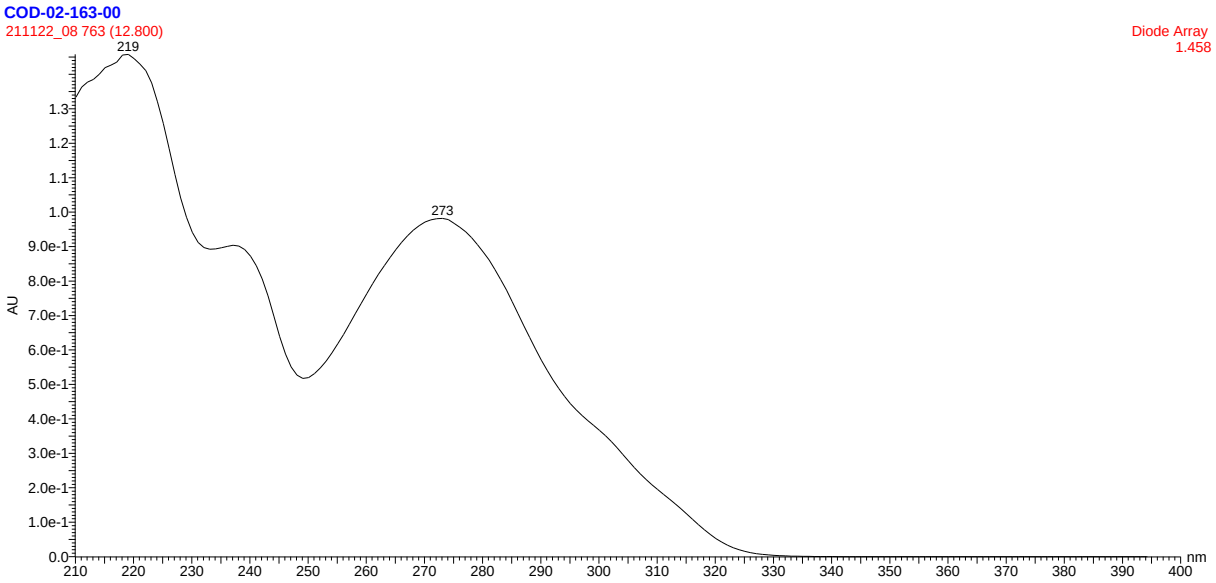
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-154**





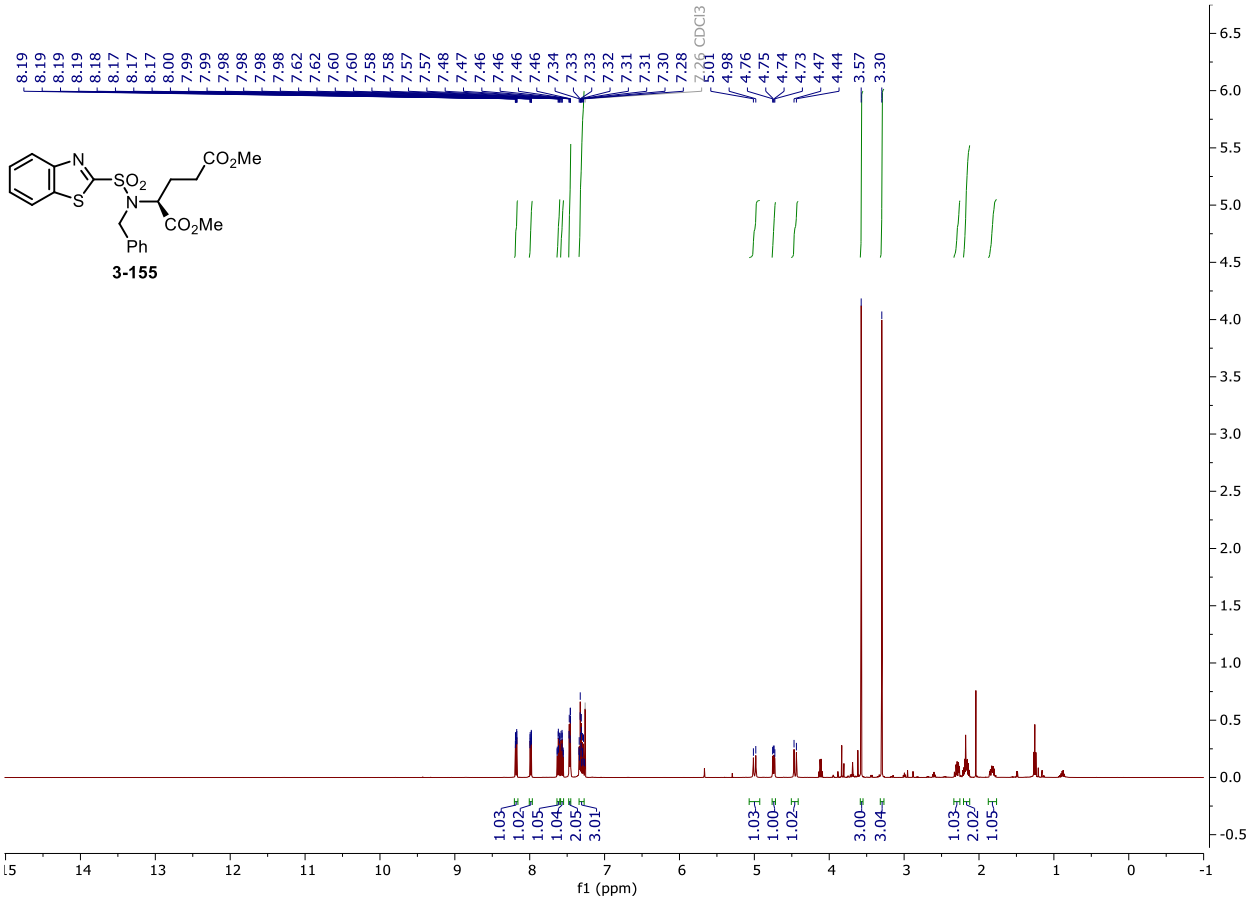
HPLC chromatograms of **3-154**

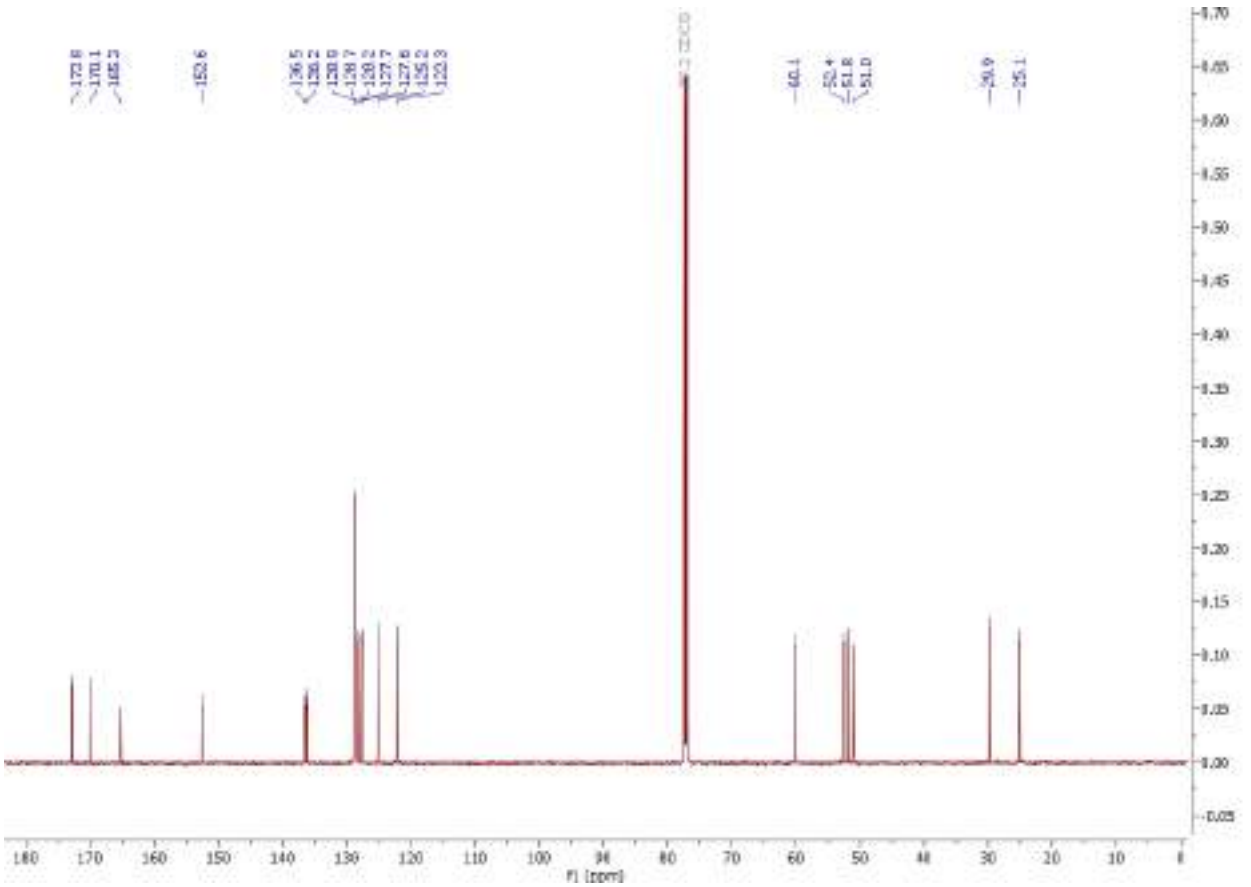




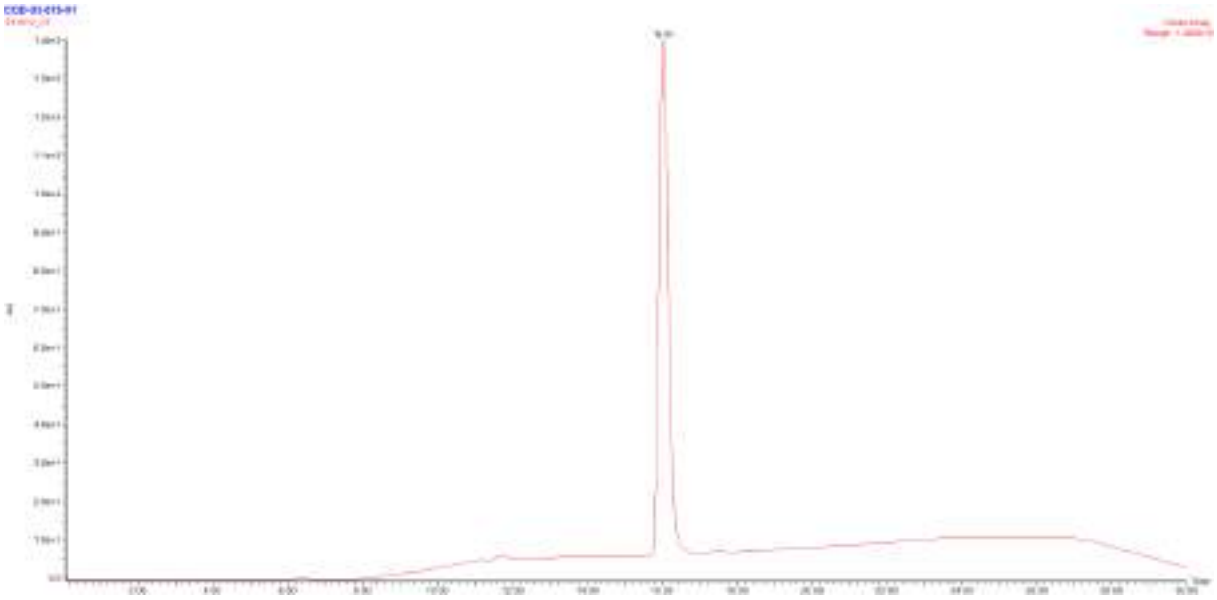
NMR and HPLC

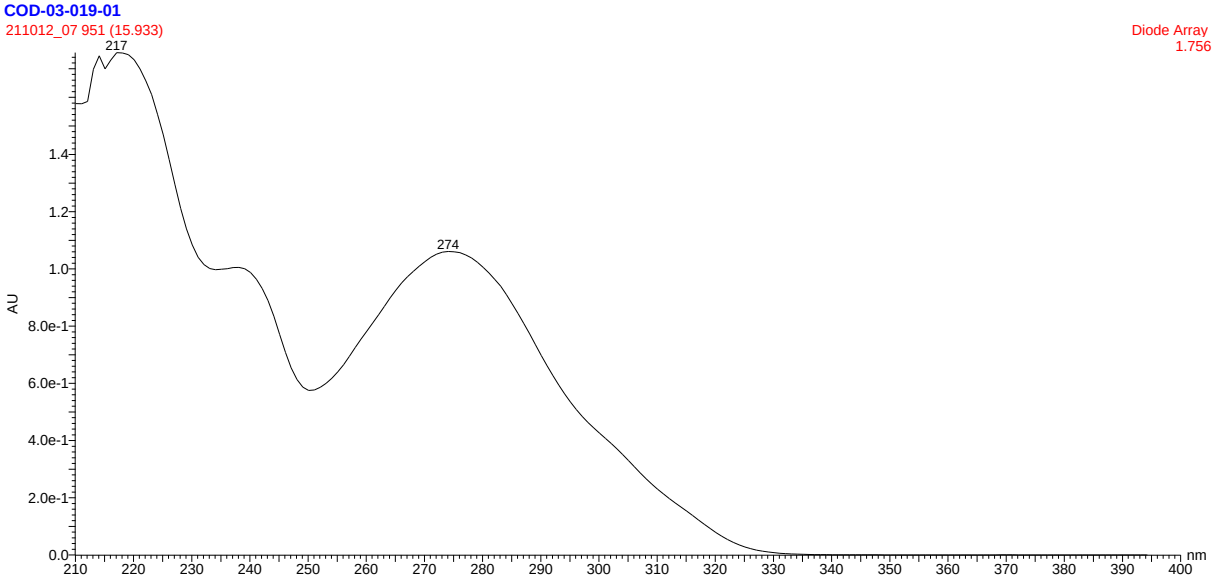
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-155**



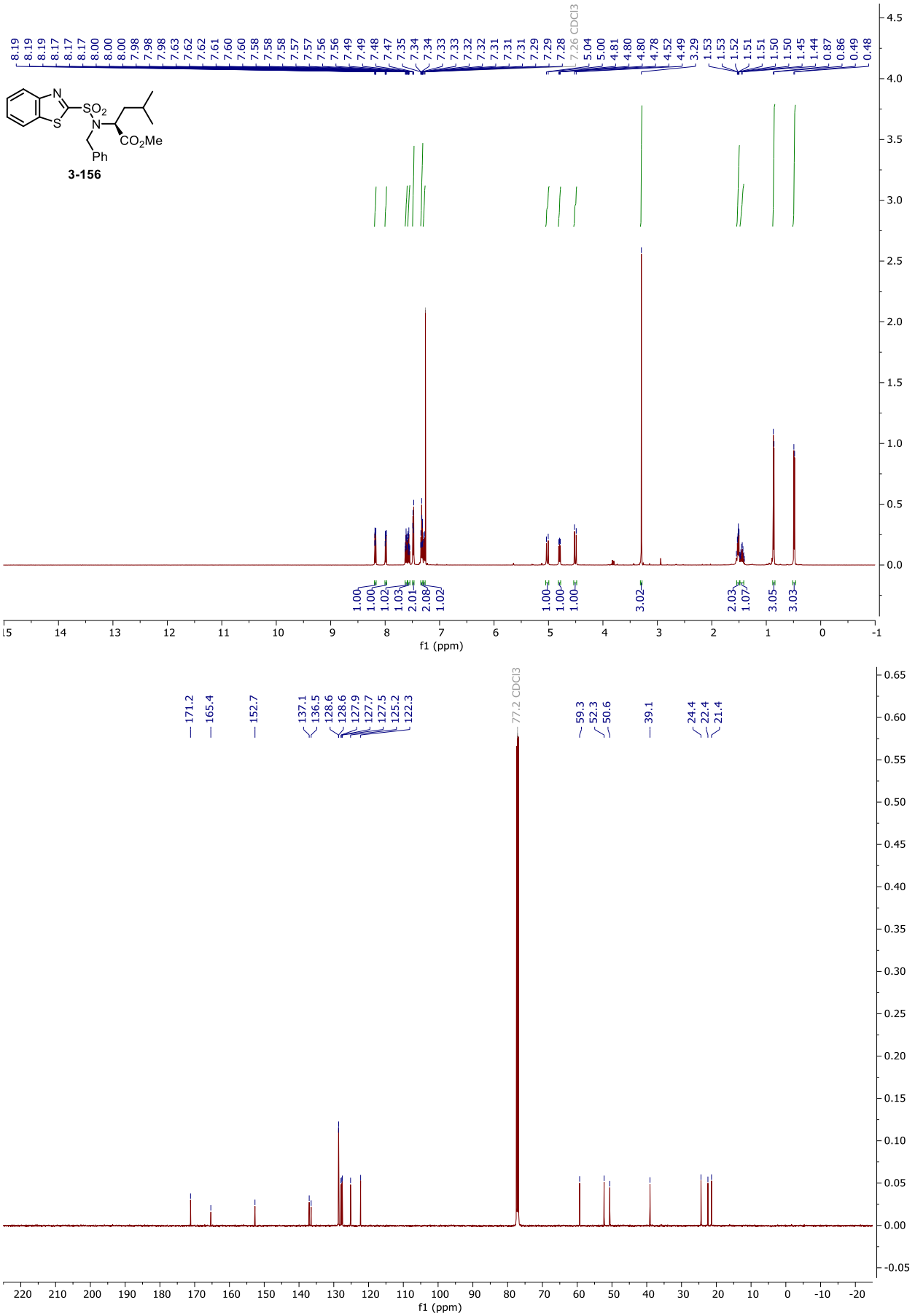


HPLC chromatograms of **3-155**

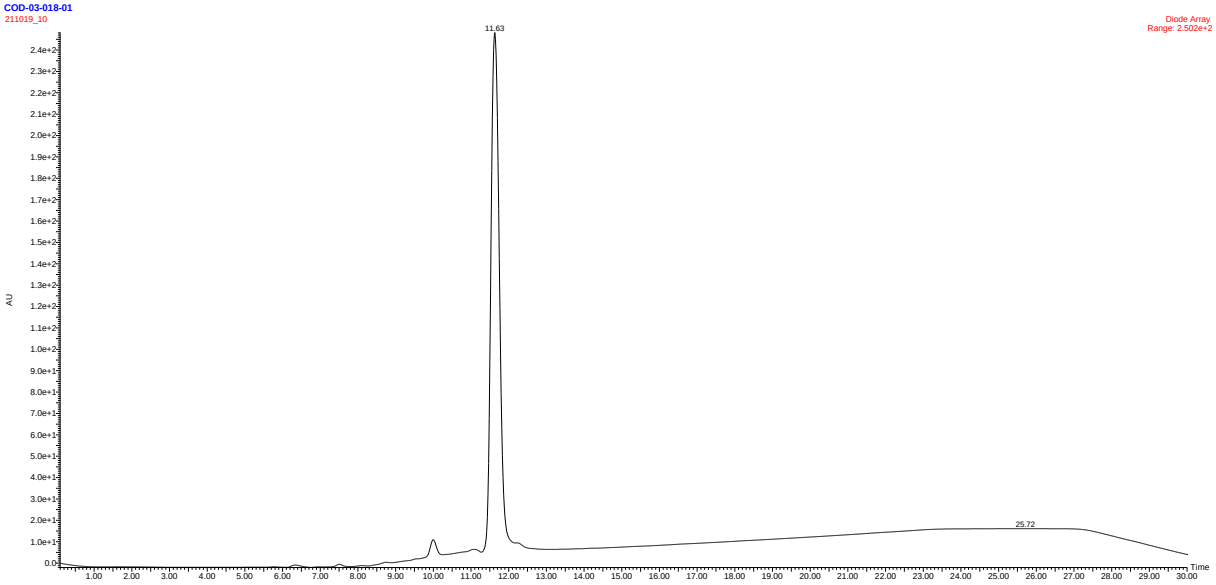


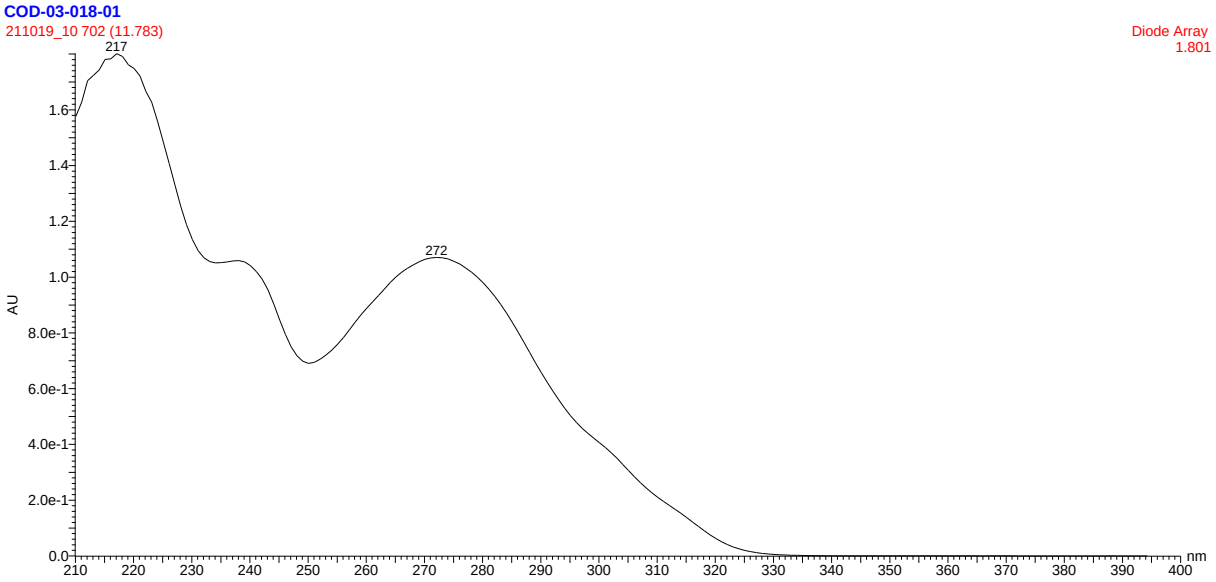


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-156**

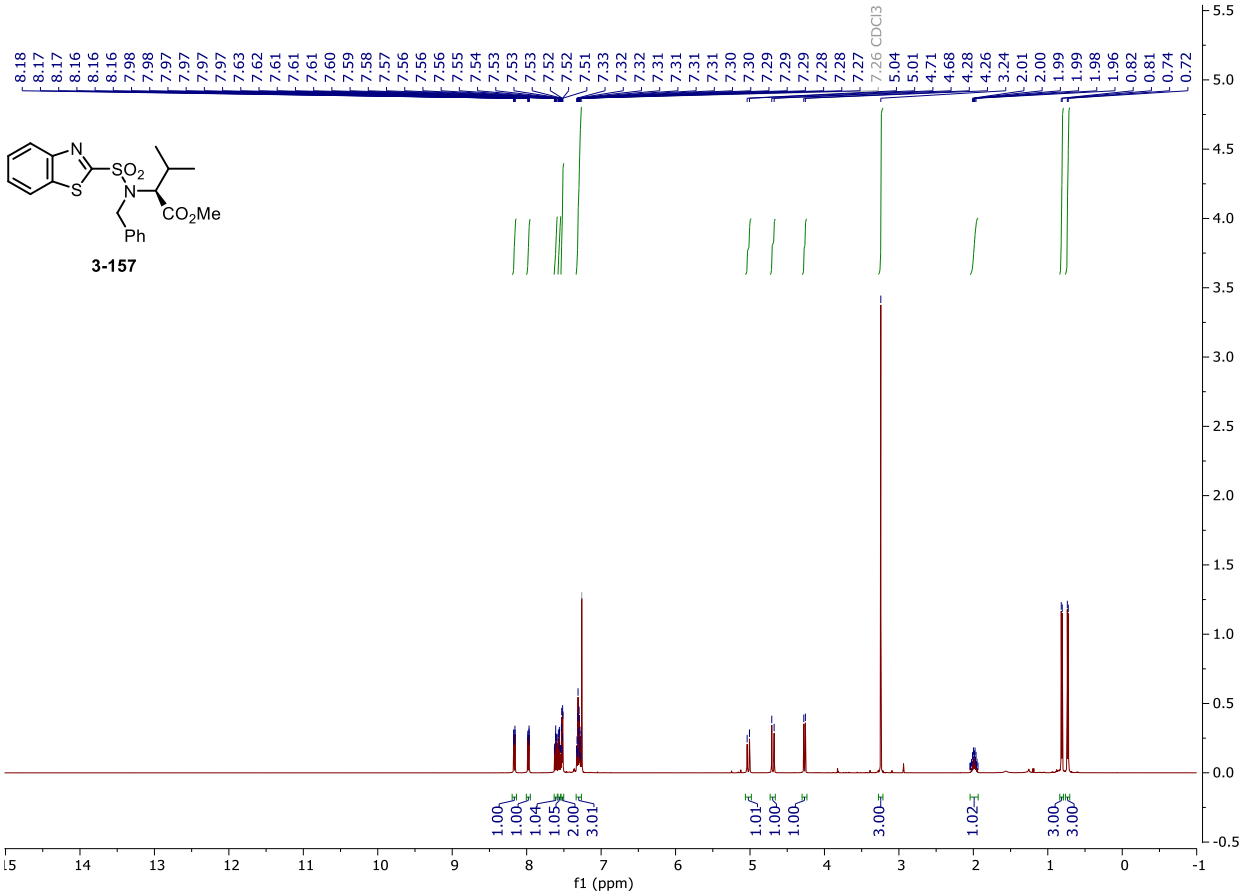


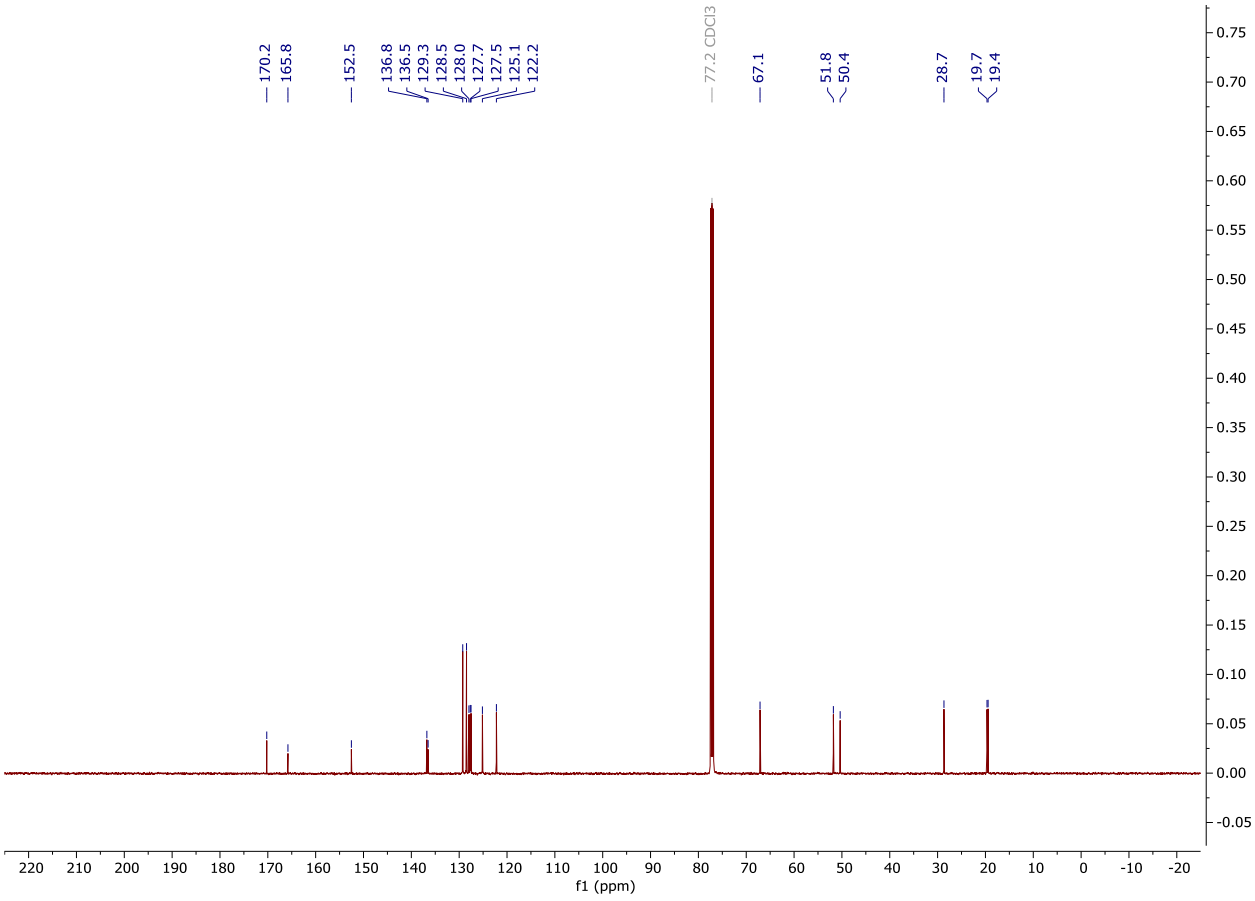
HPLC chromatograms of **3-156**



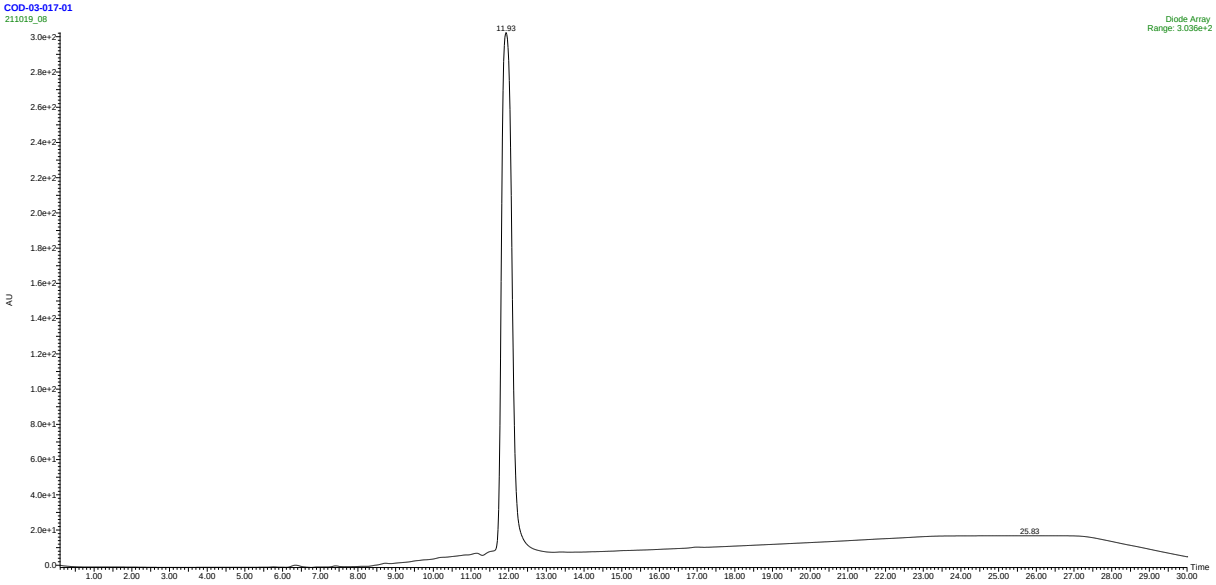


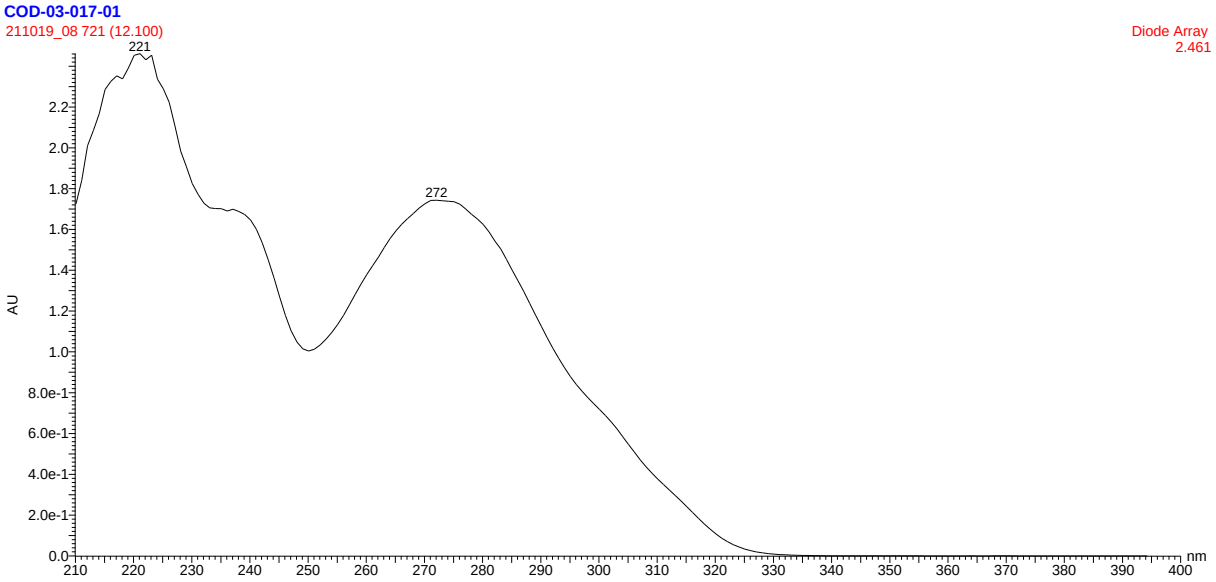
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-157**





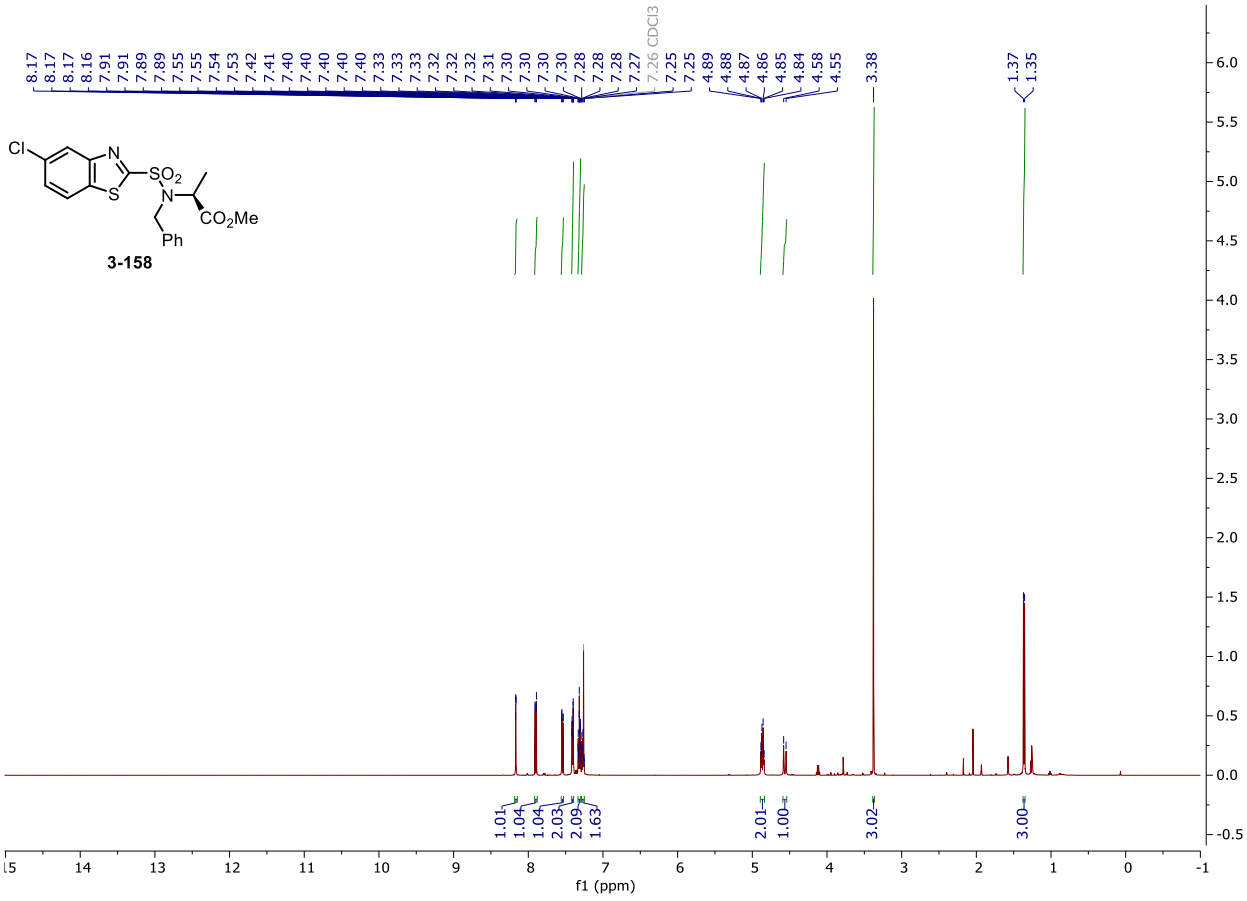
HPLC chromatograms of **3-157**

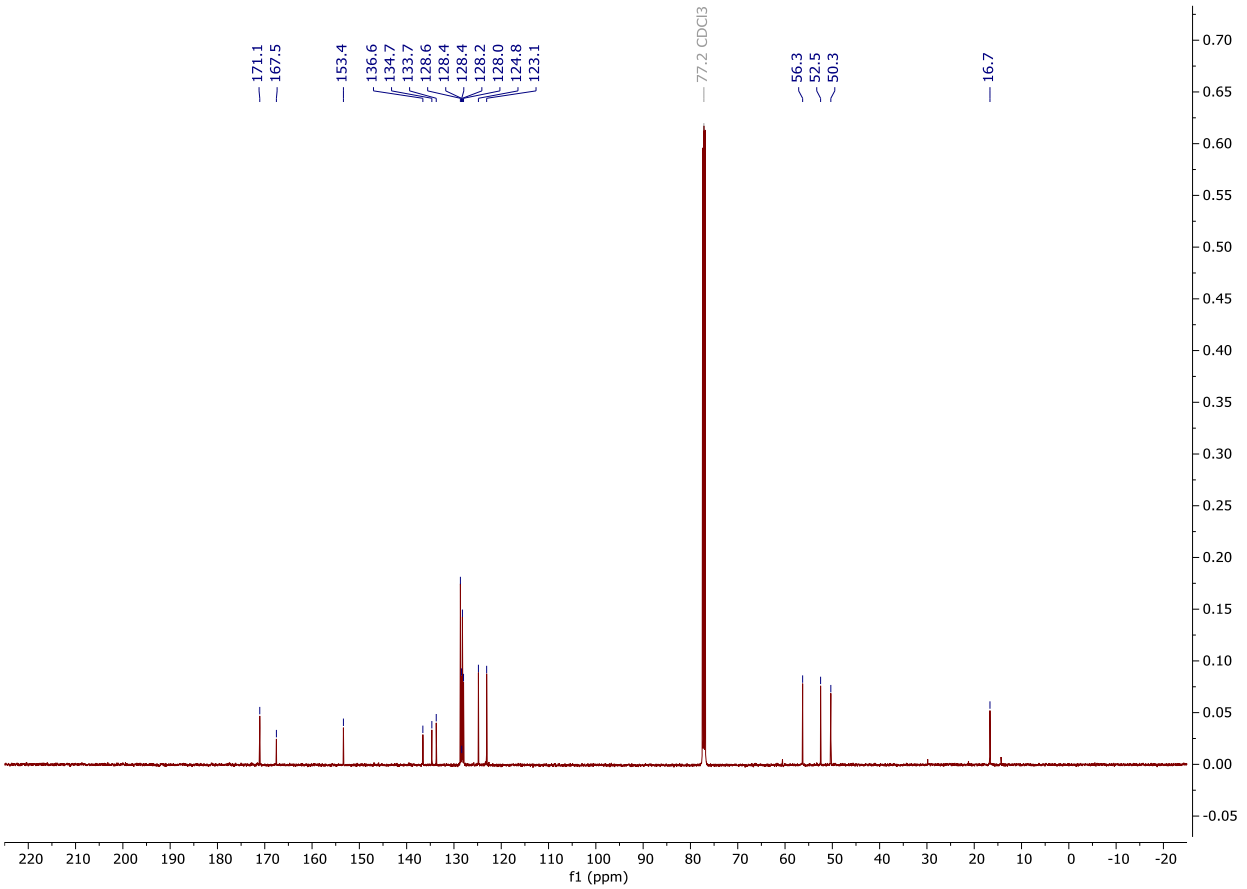




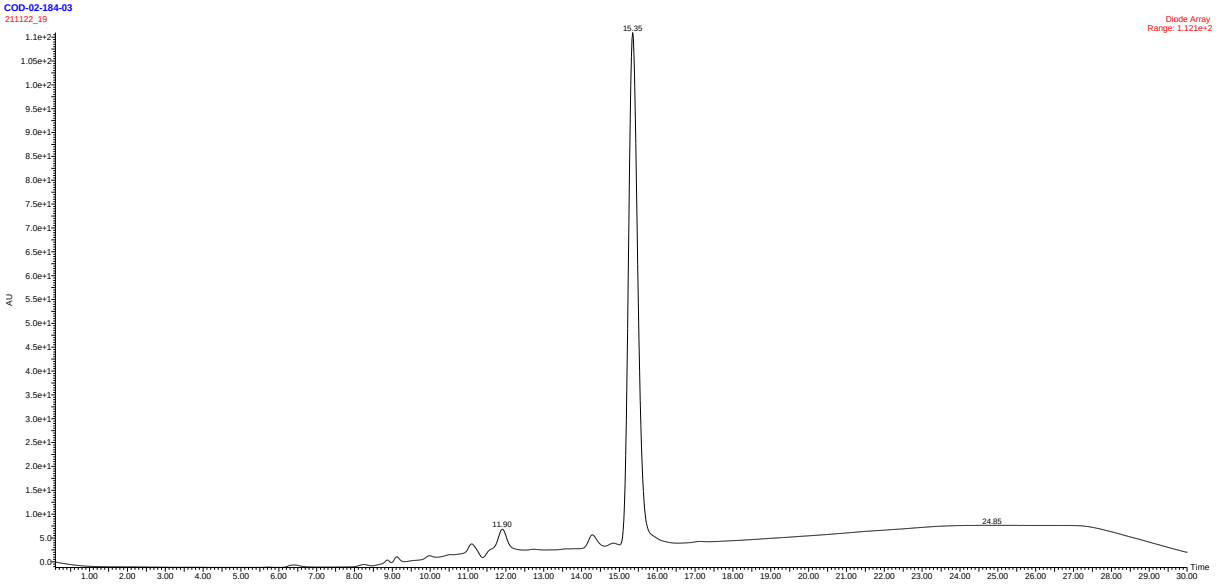
NMR and HPLC

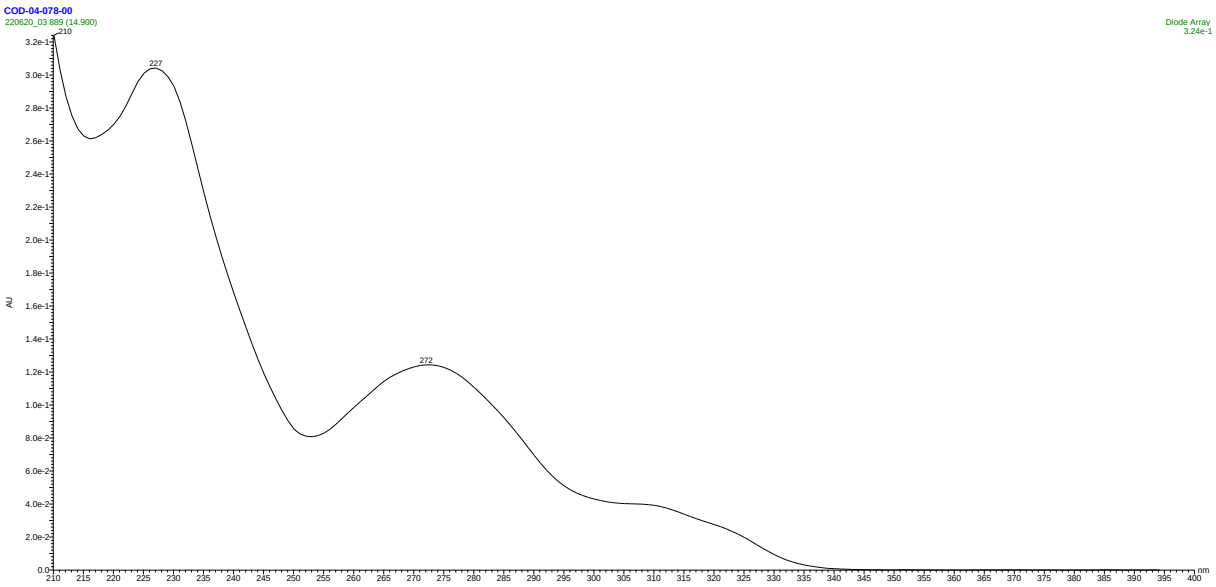
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-158**



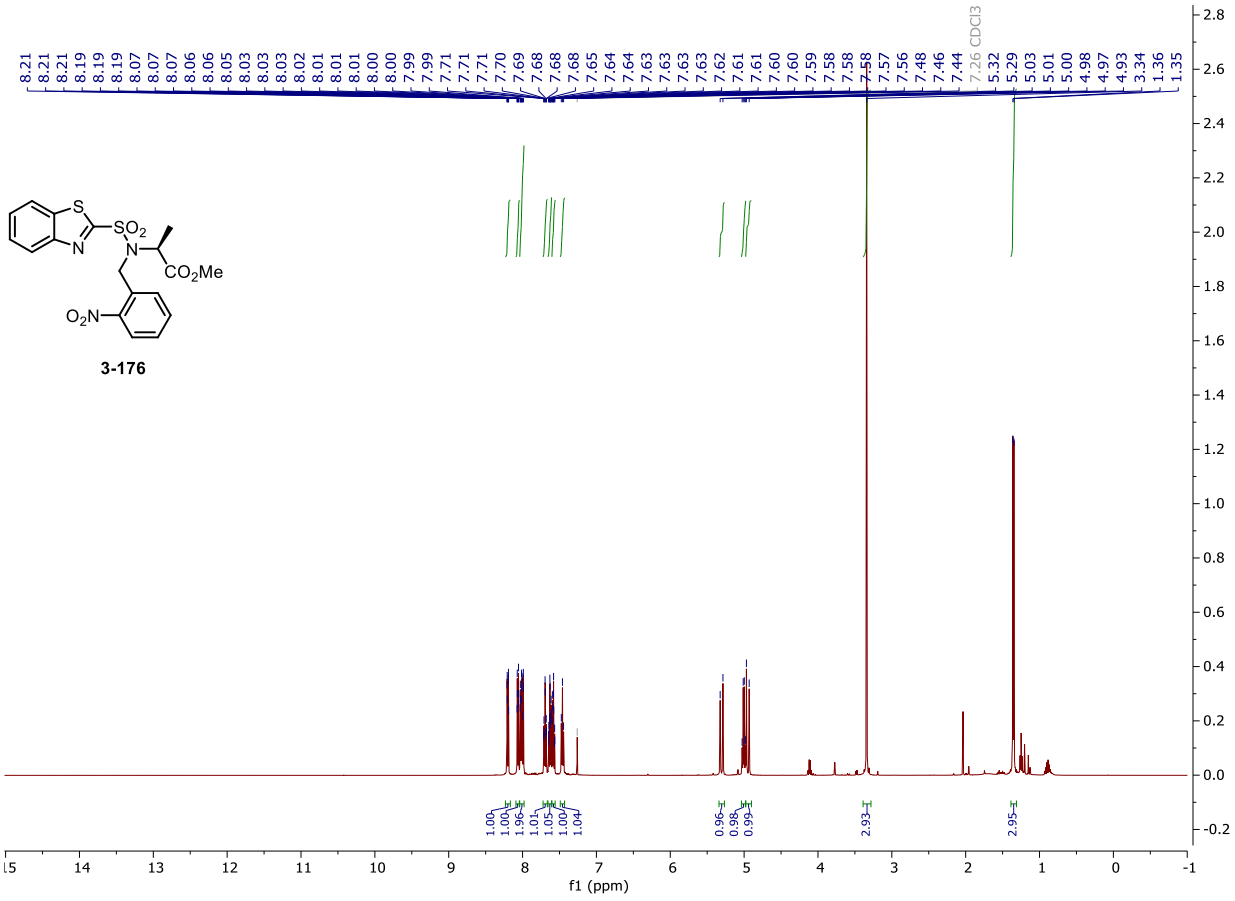


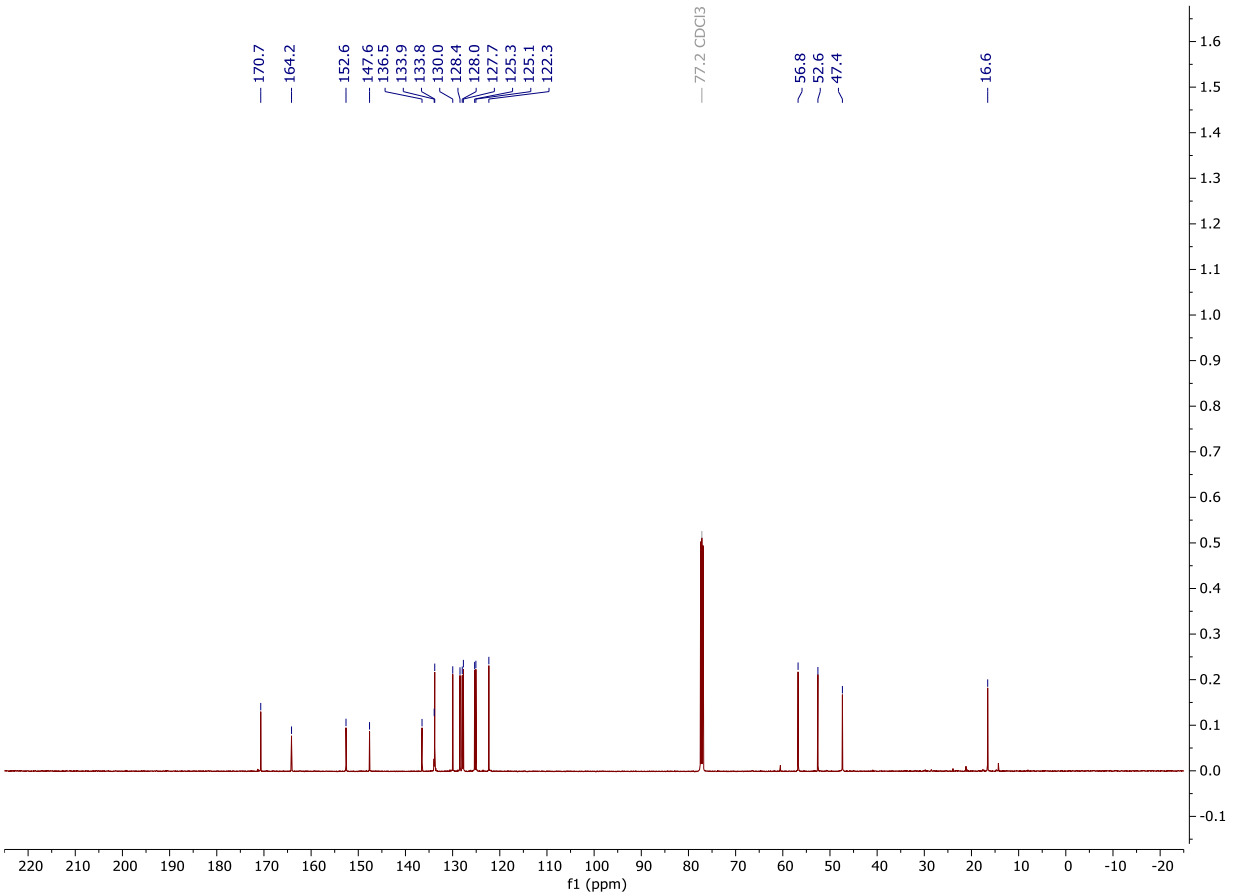
HPLC chromatograms of **3-158**



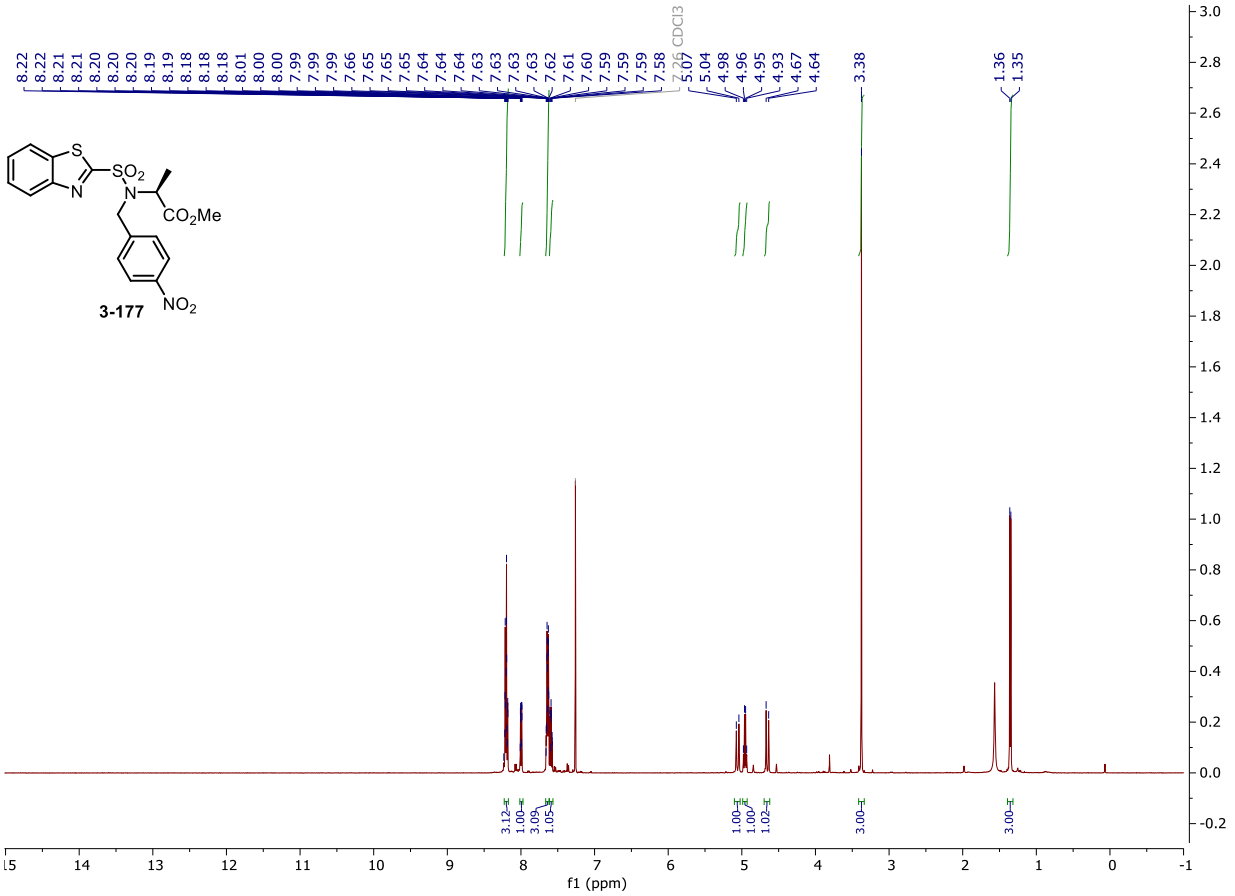


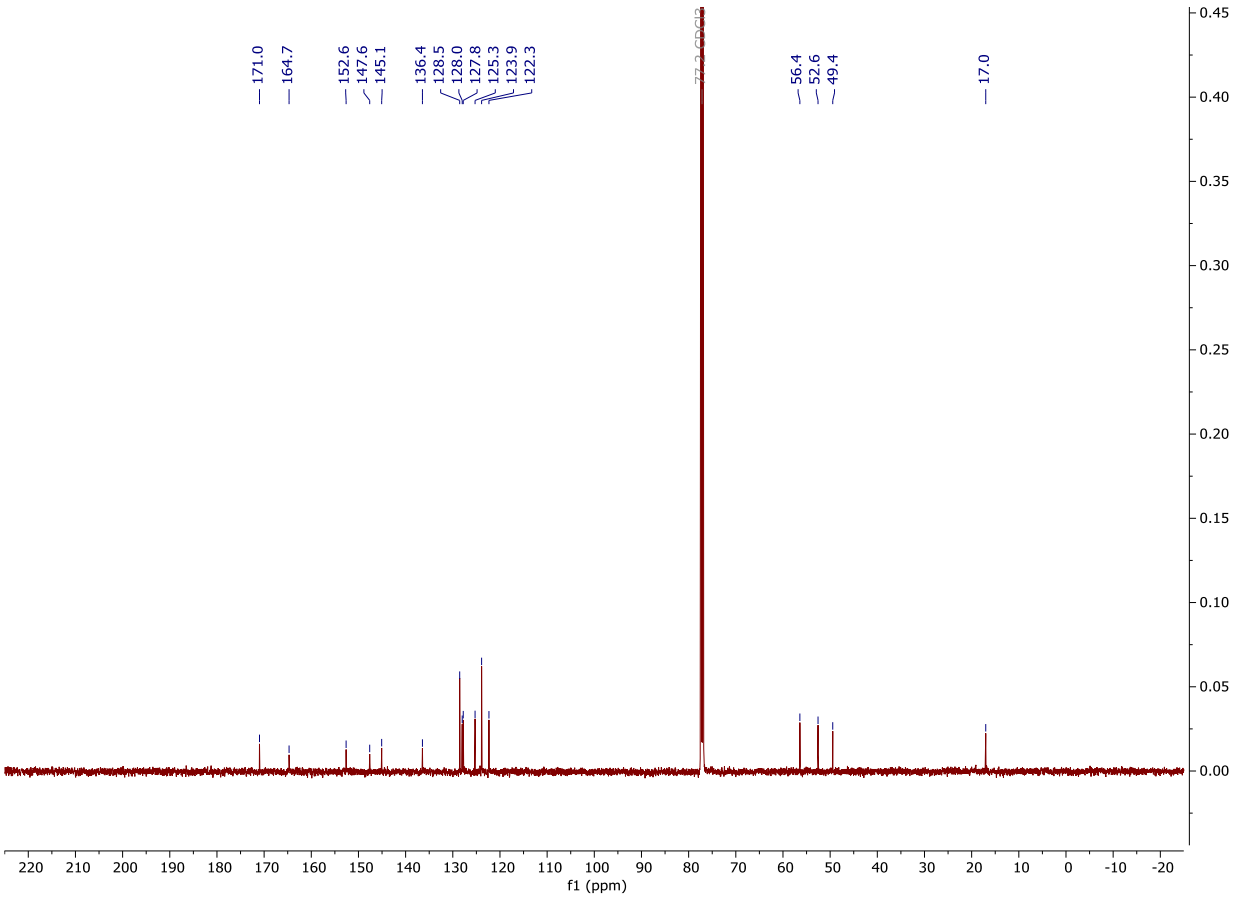
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-176**



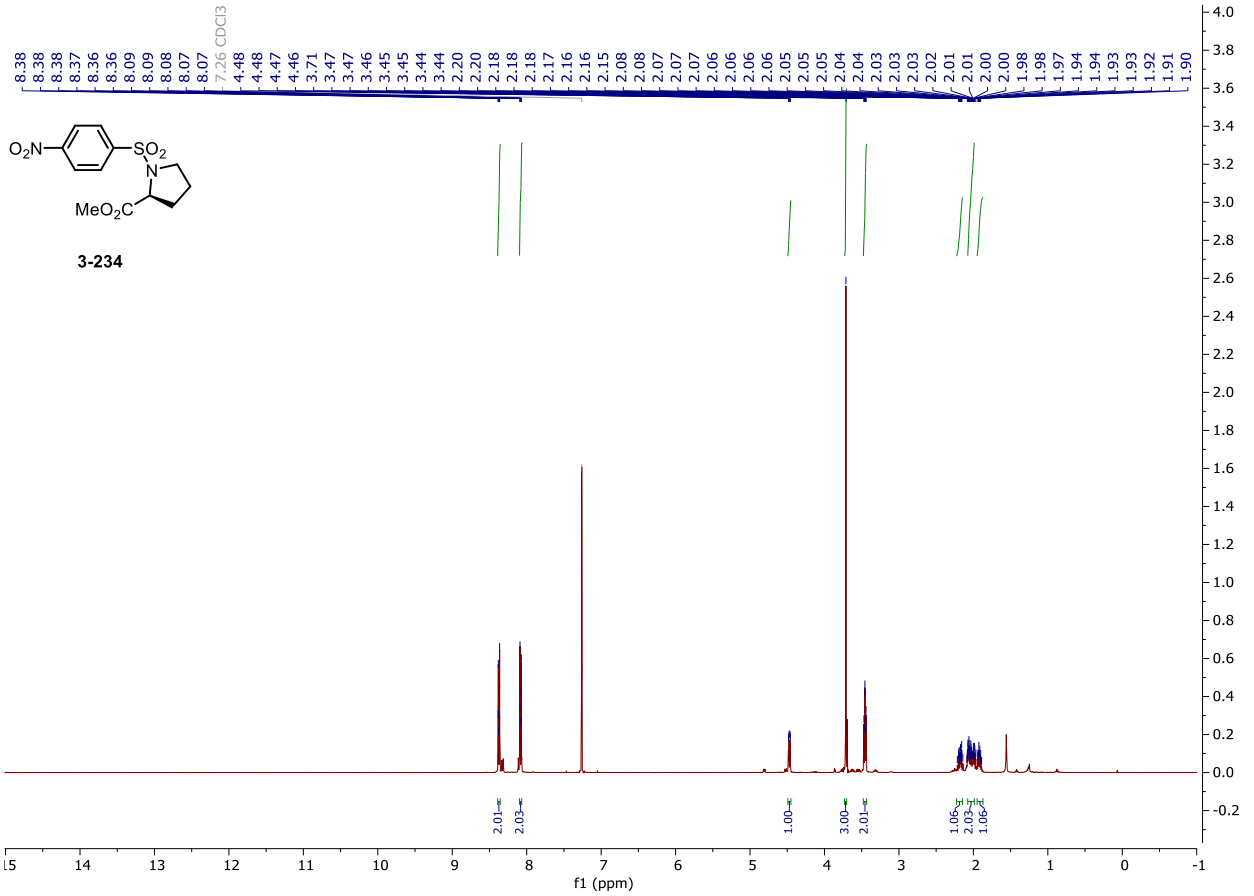


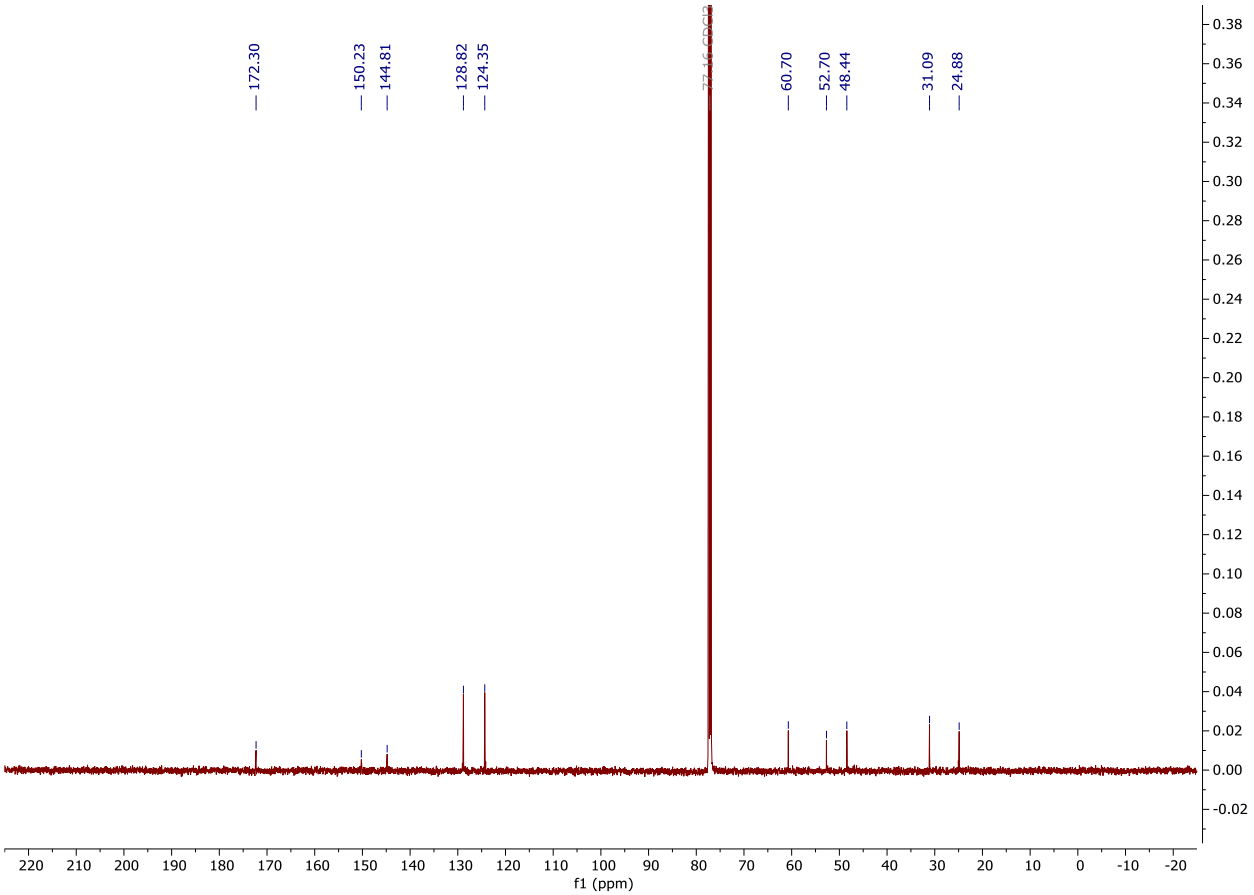
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-177**



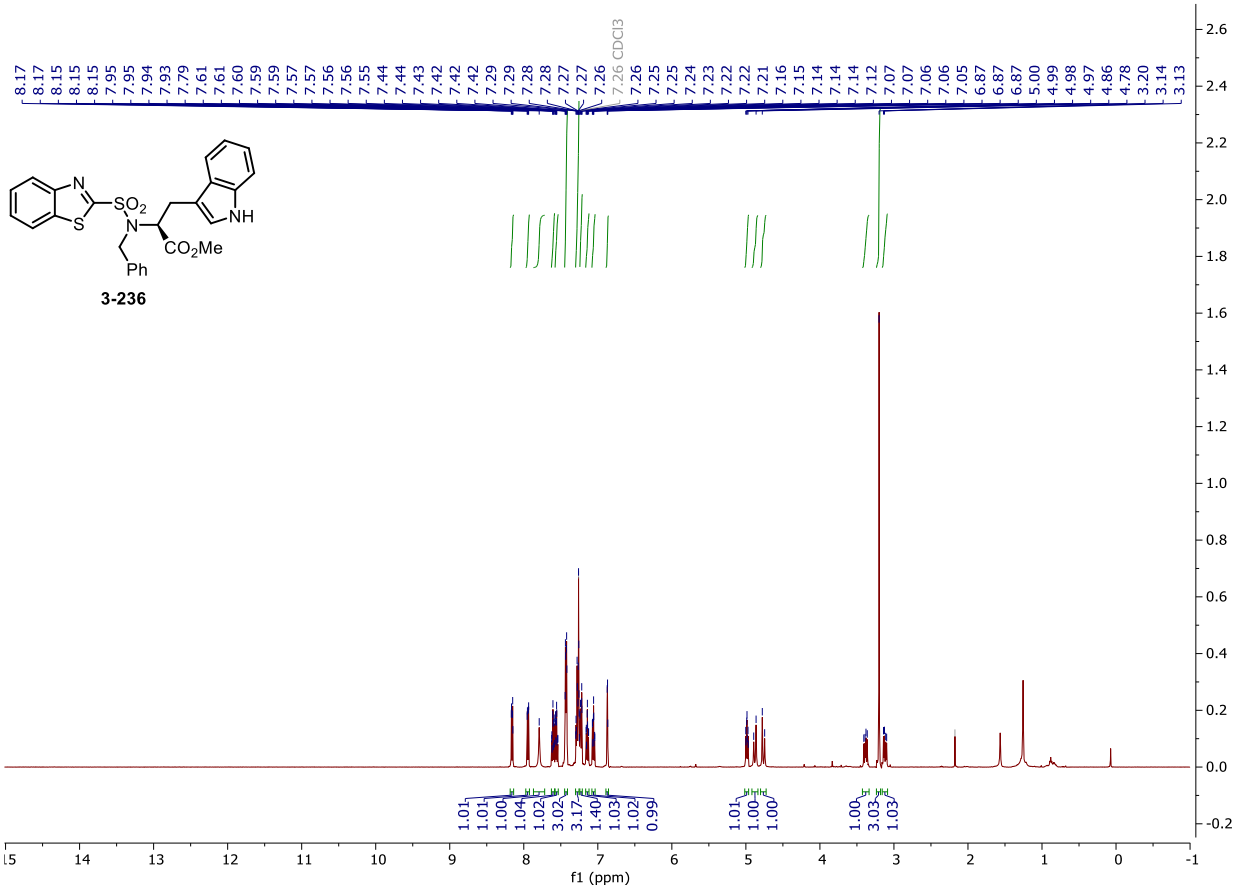


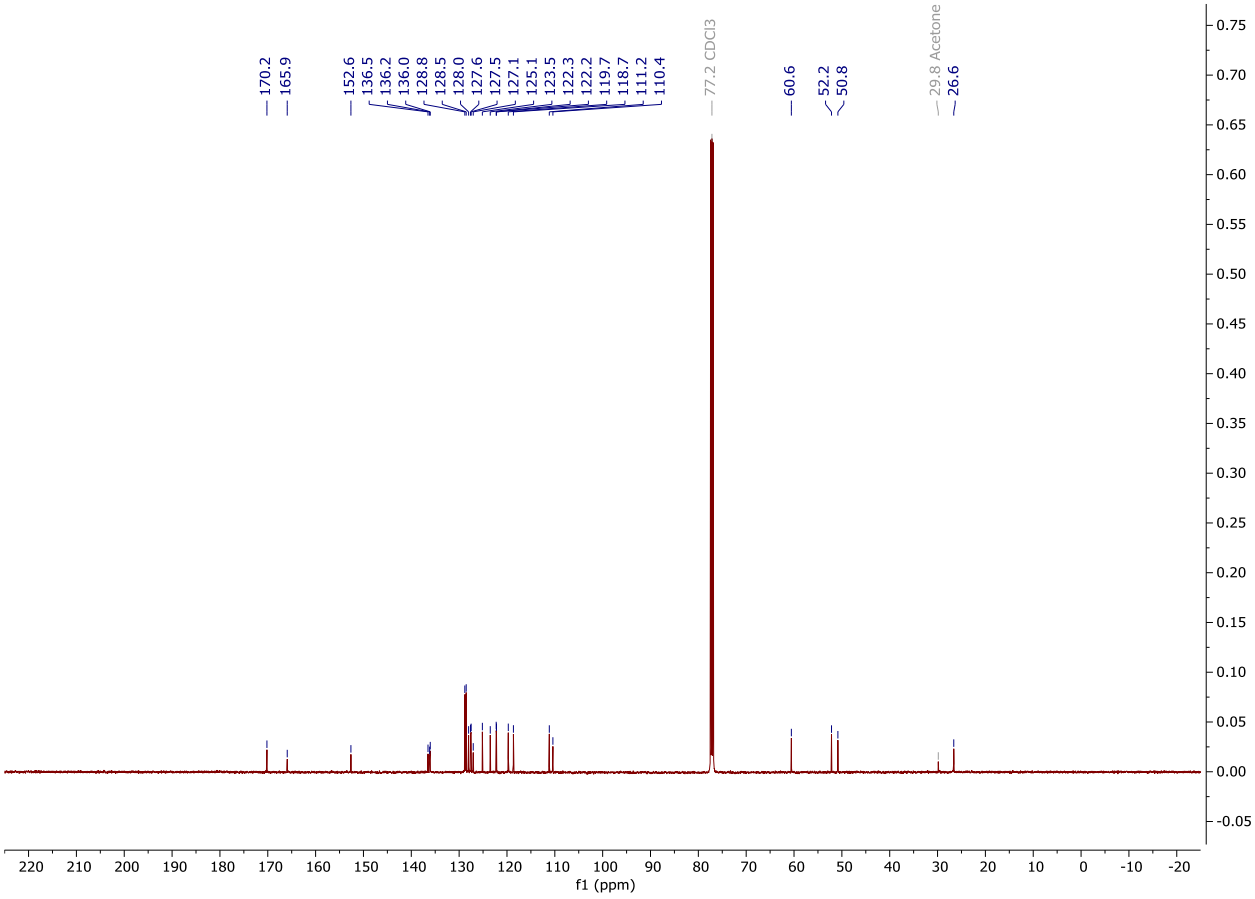
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-234**



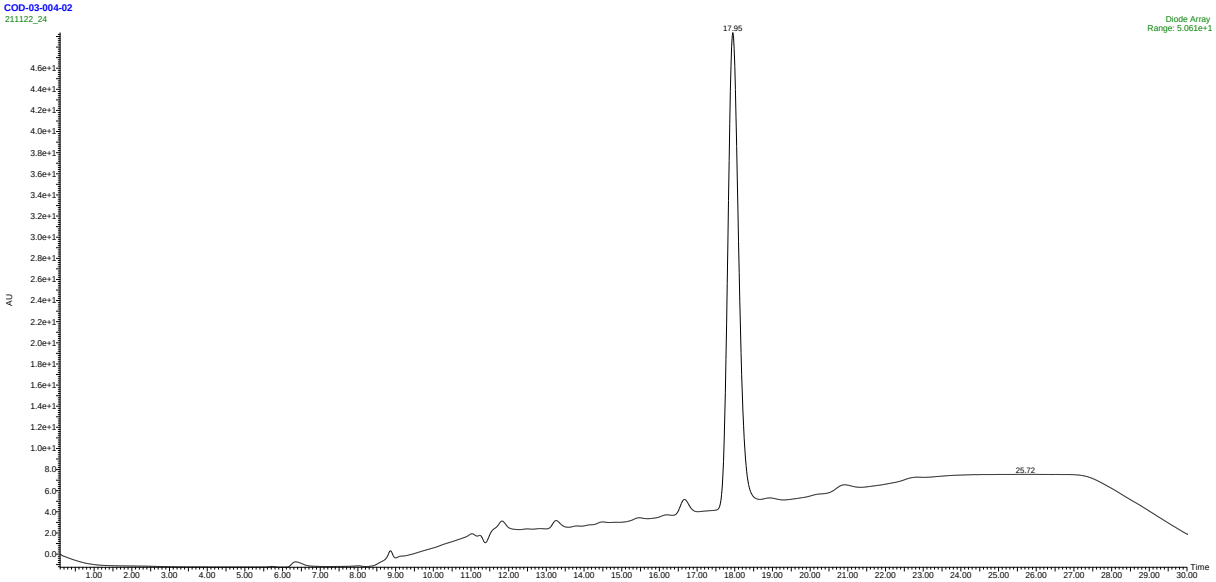


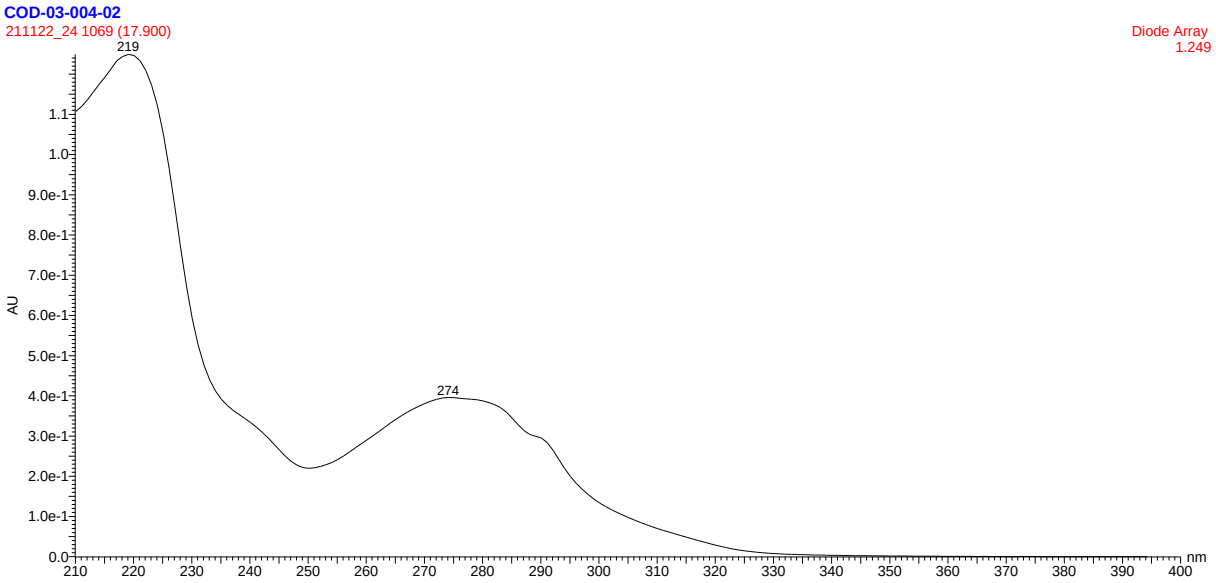
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-236**





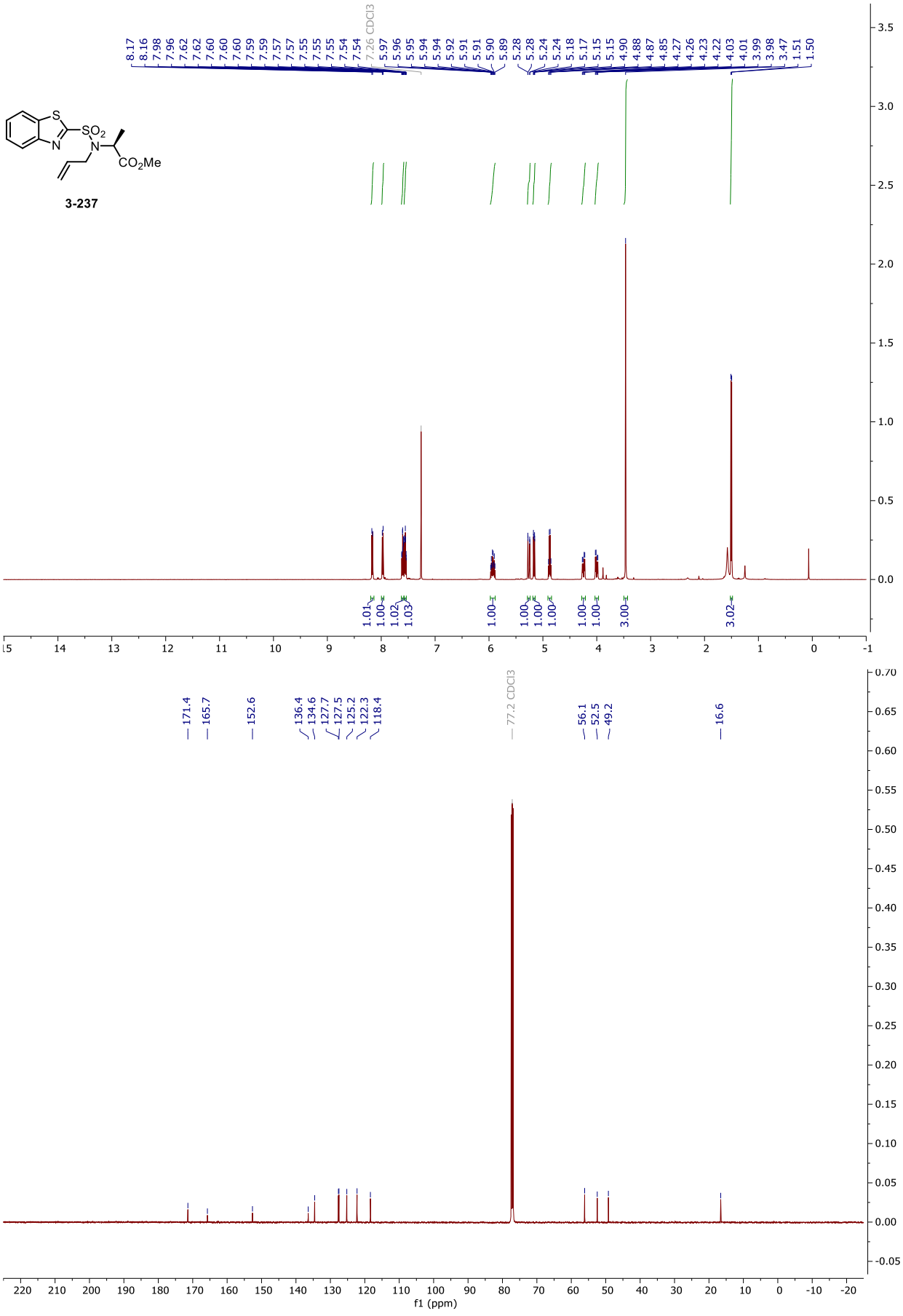
HPLC chromatograms of **3-236**



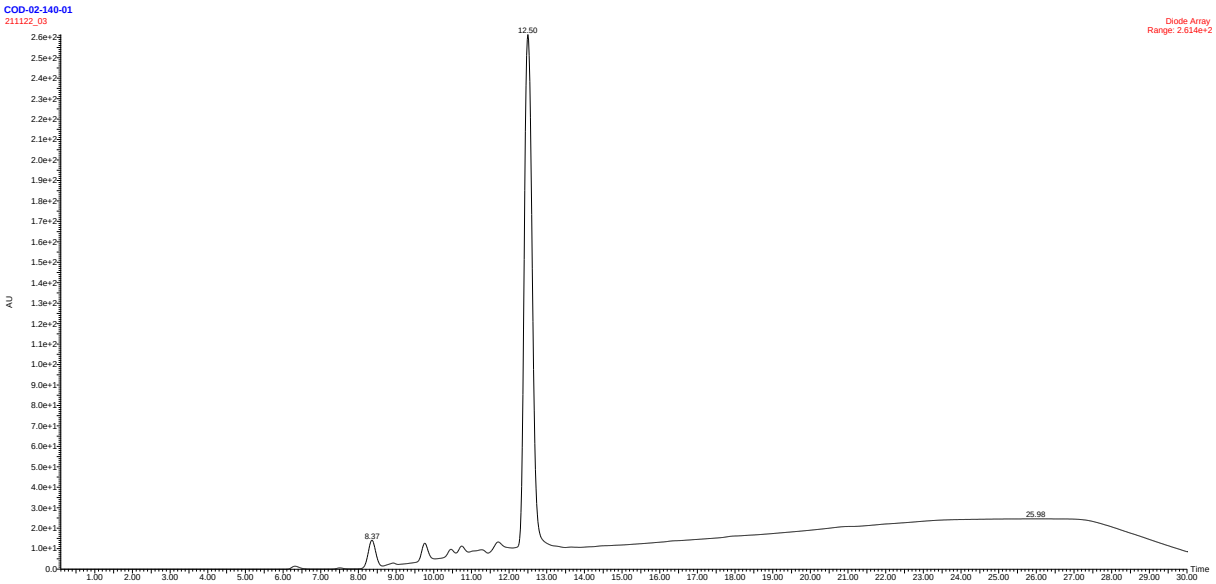


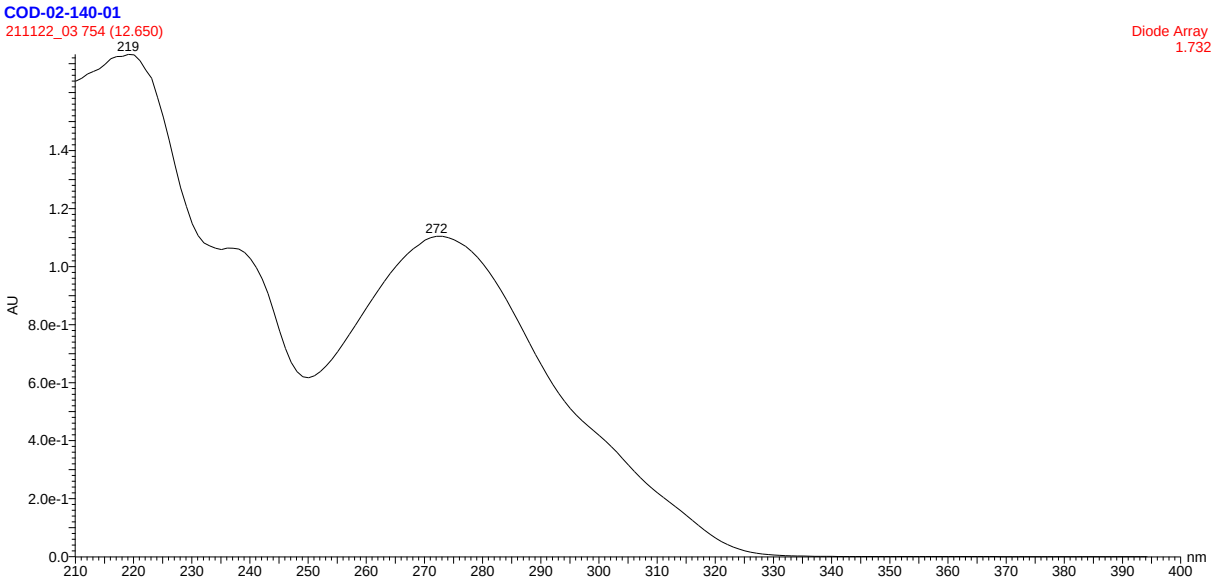
NMR and HPLC

Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-237**

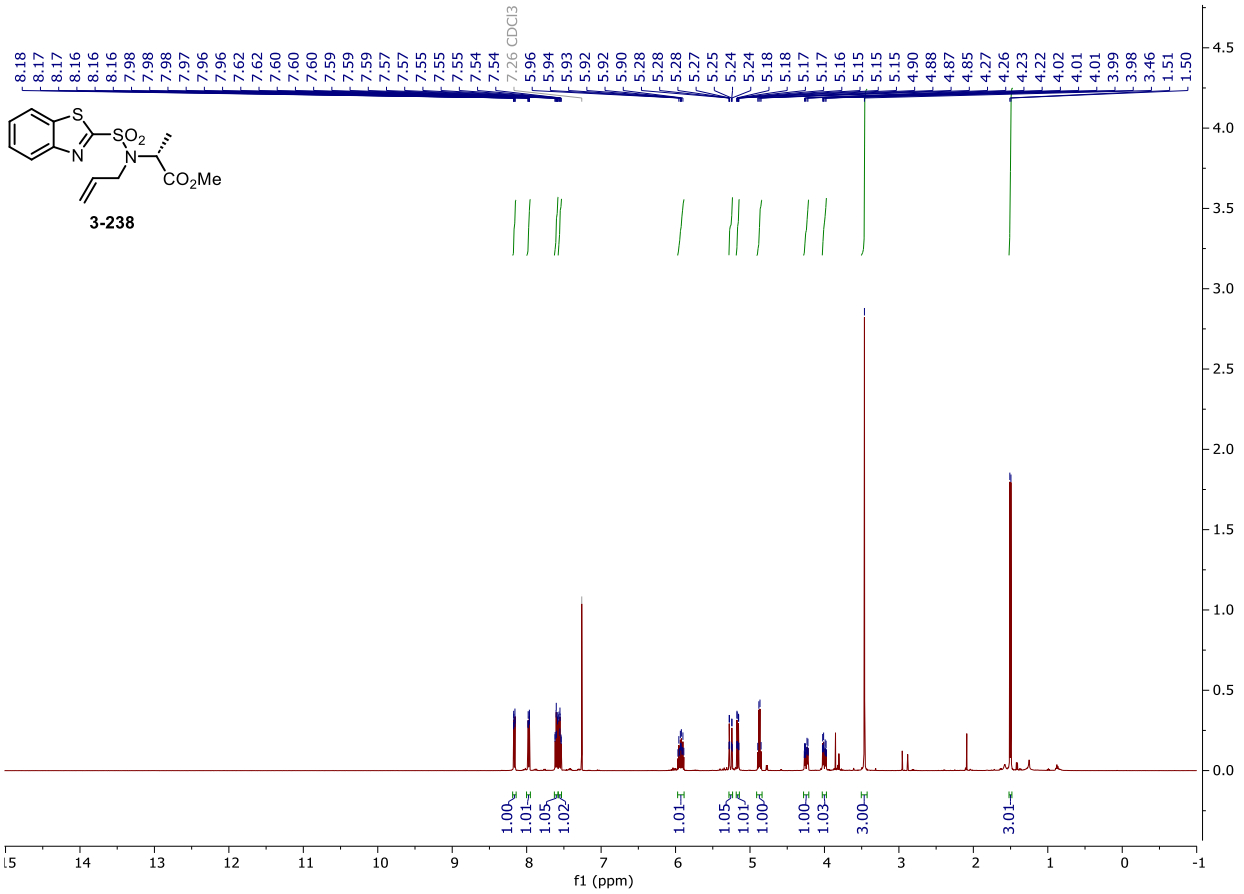


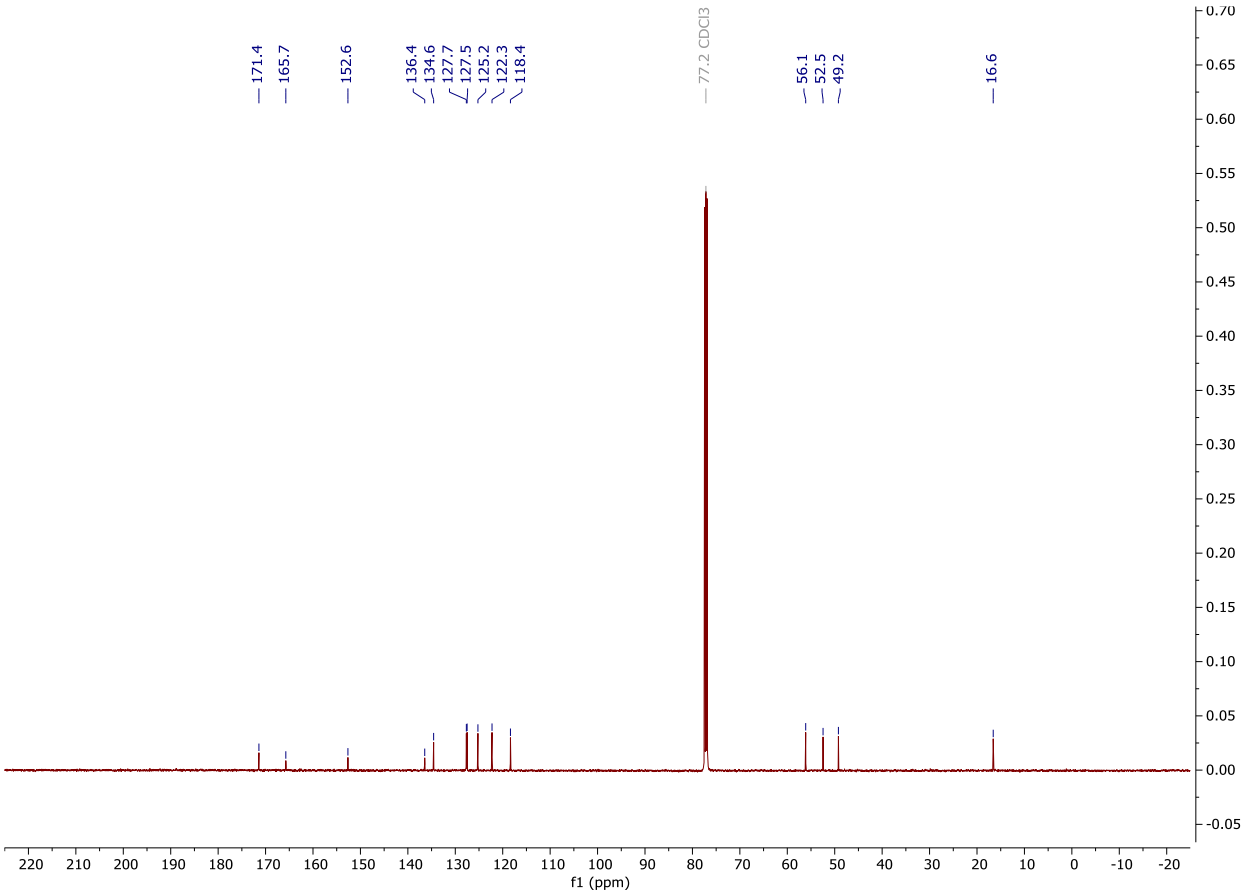
HPLC chromatograms of **3-237**



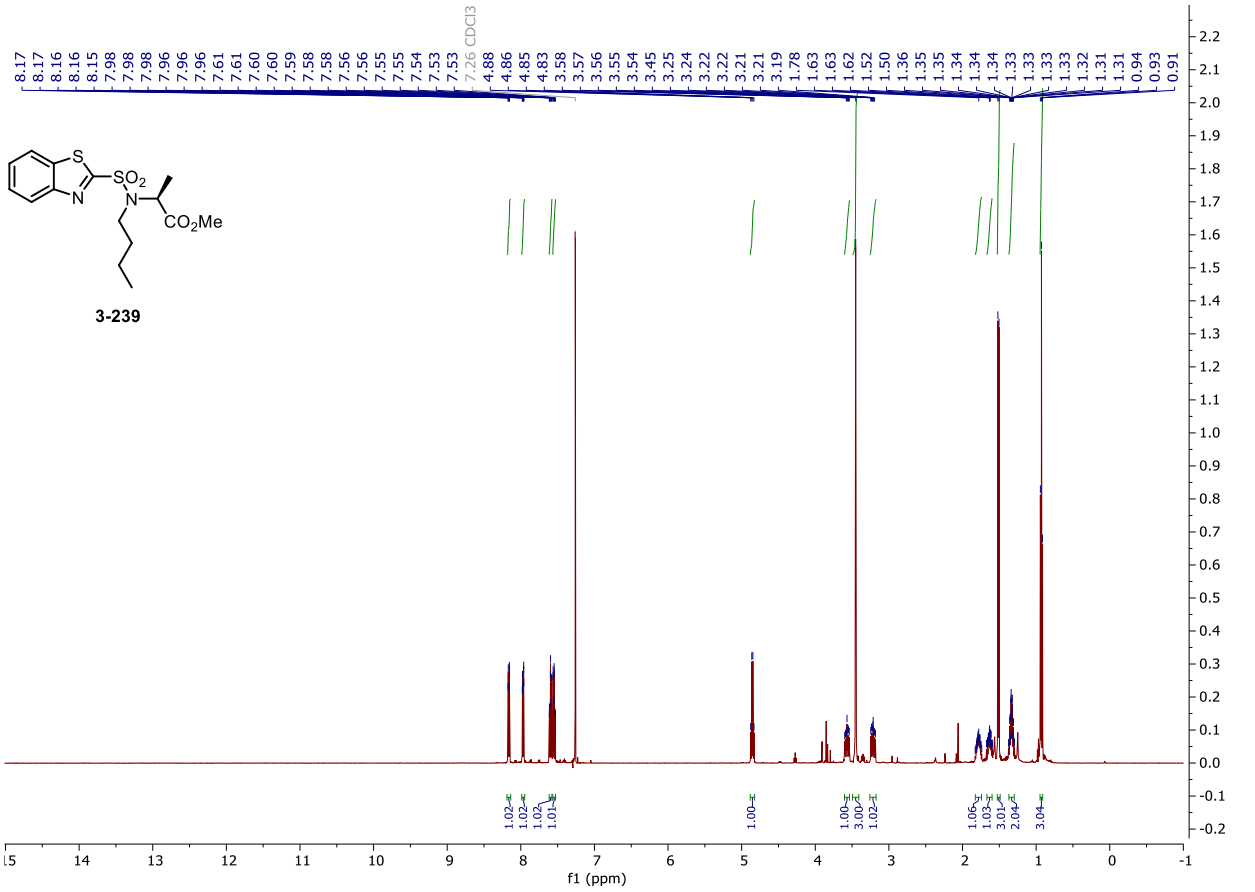


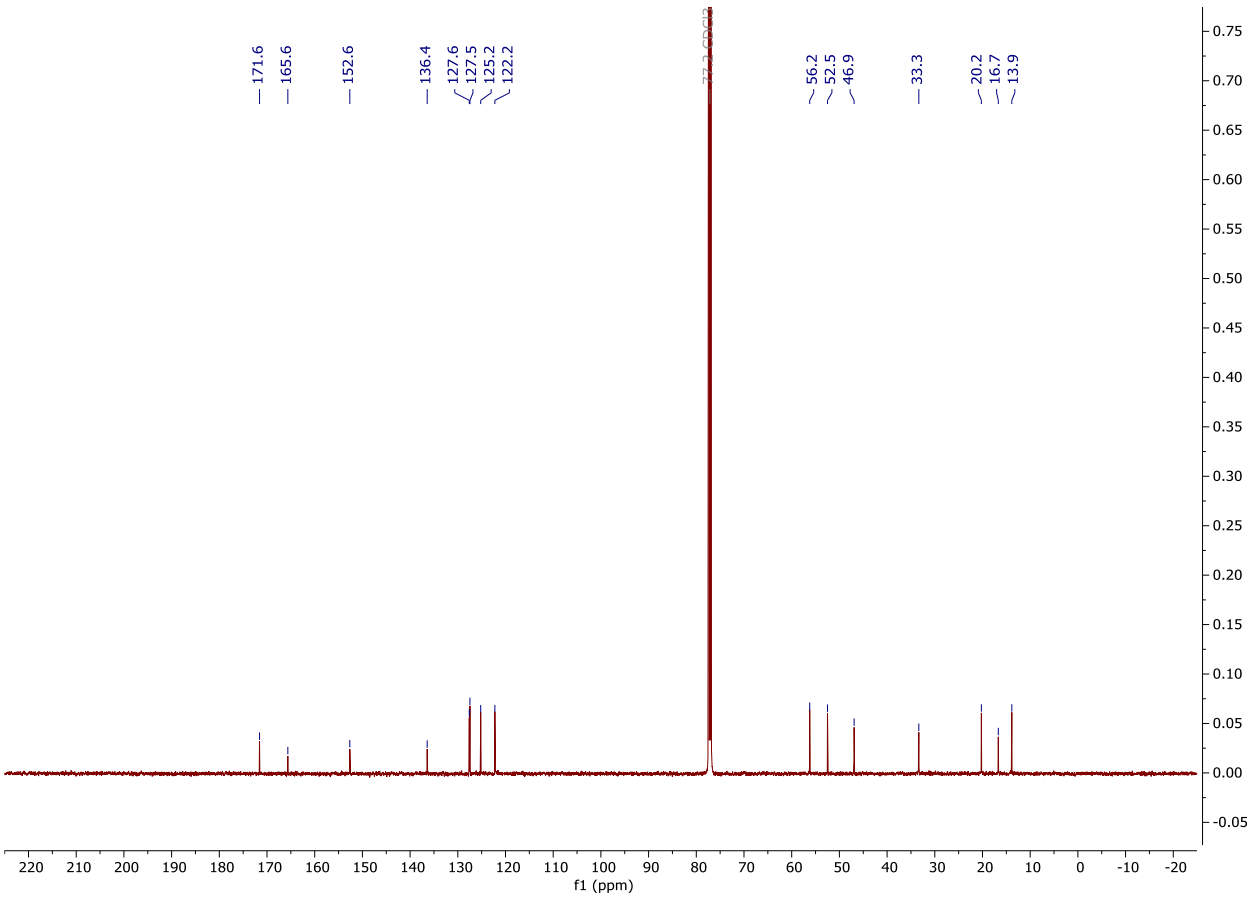
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-238**



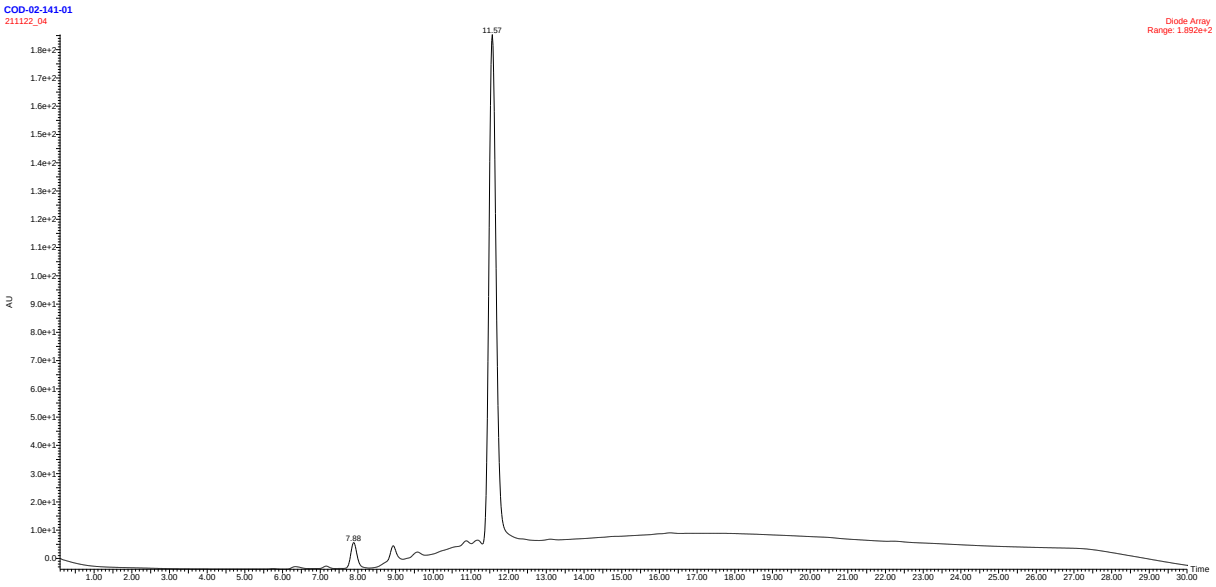


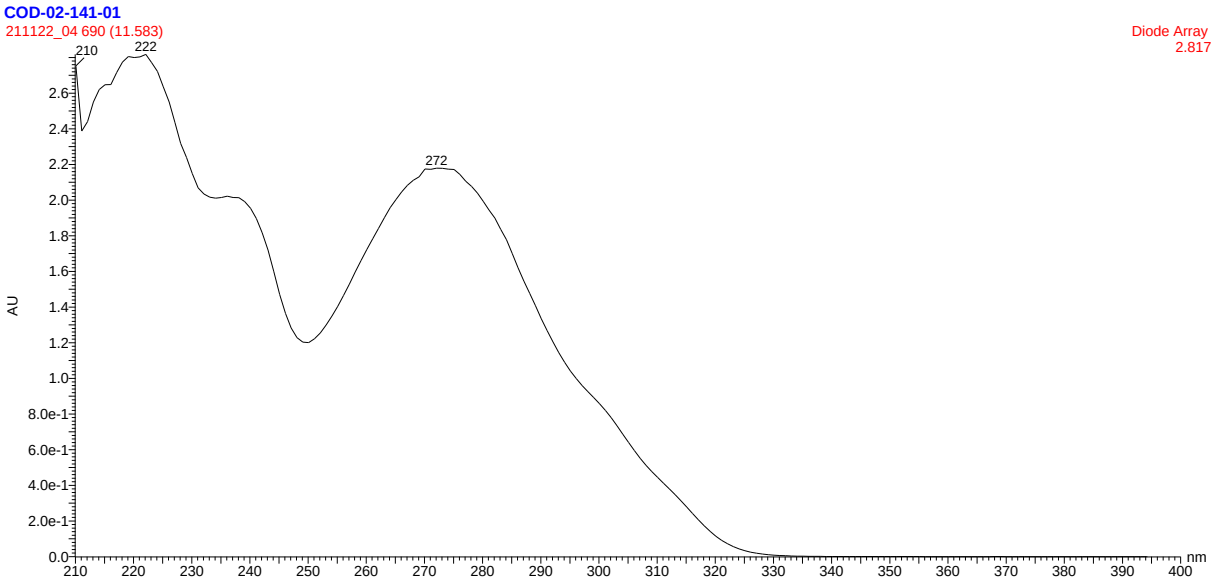
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-239**



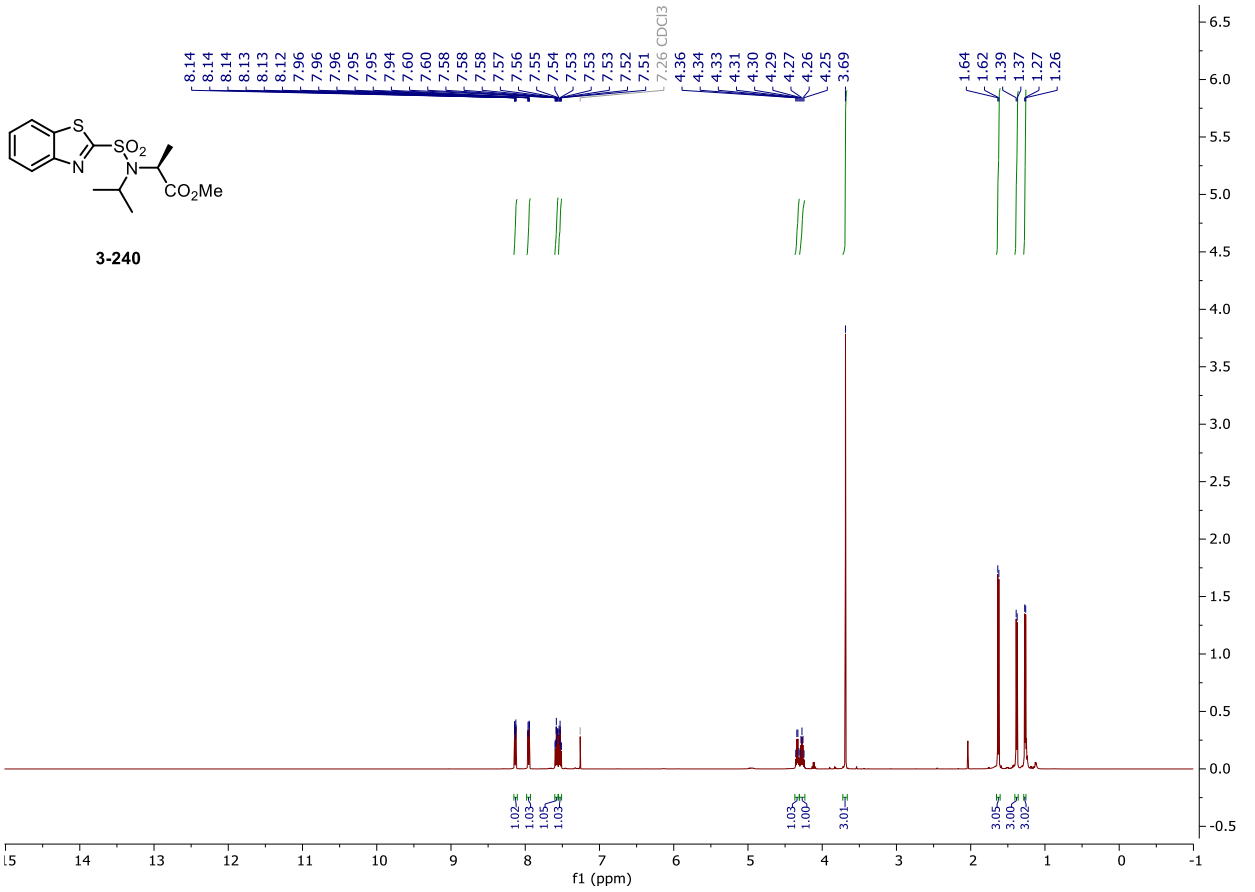


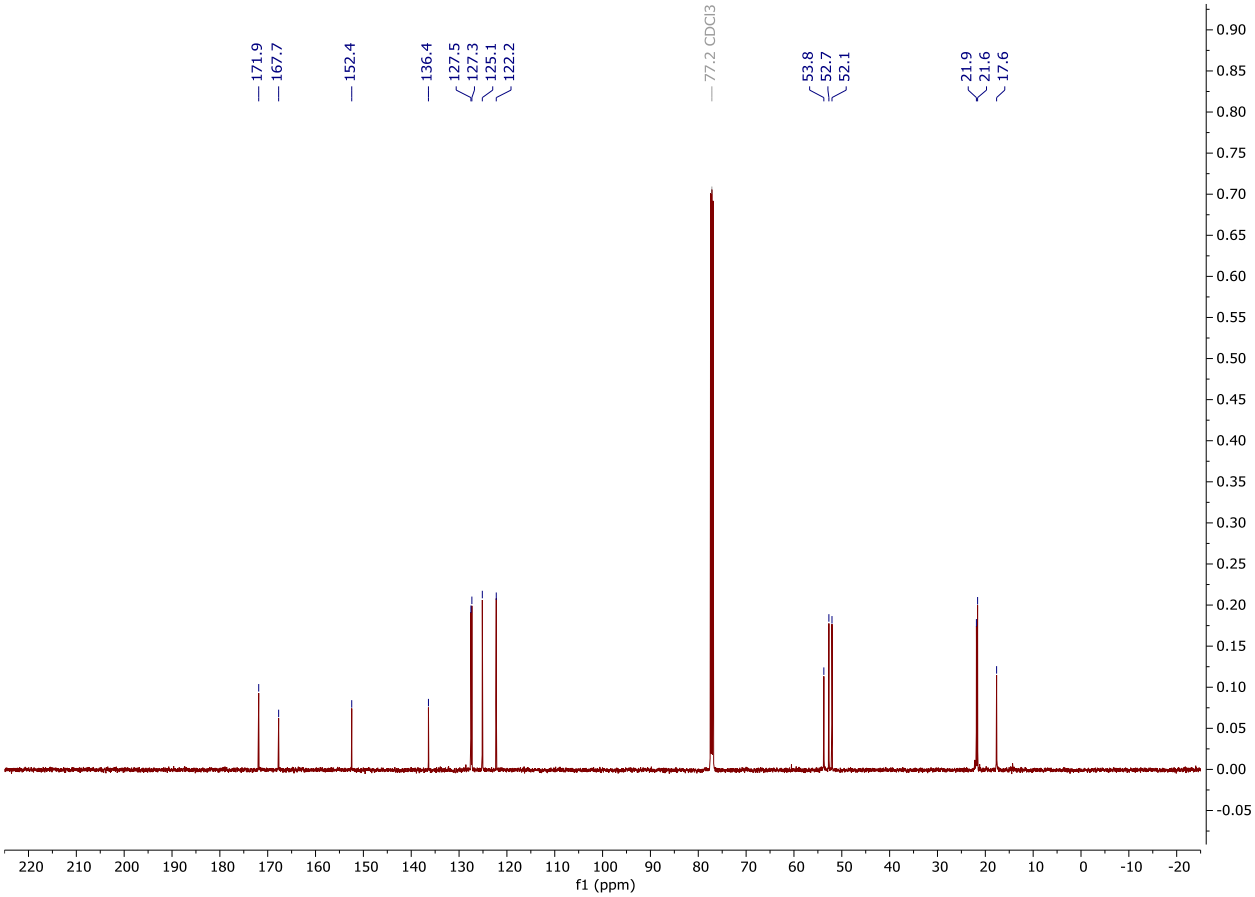
HPLC chromatograms of **3-239**



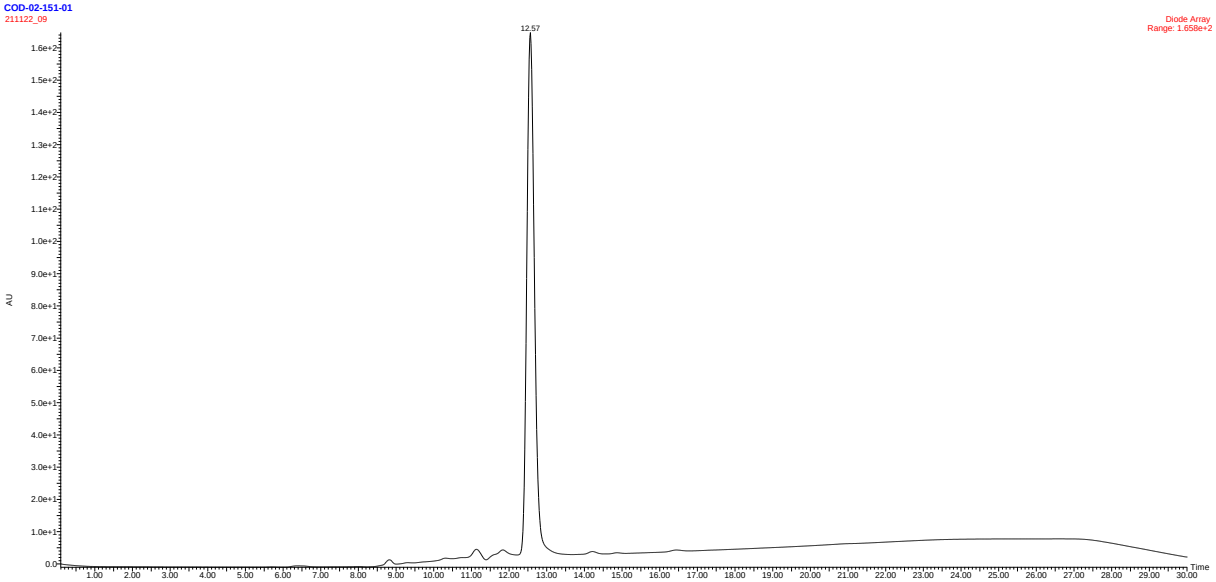


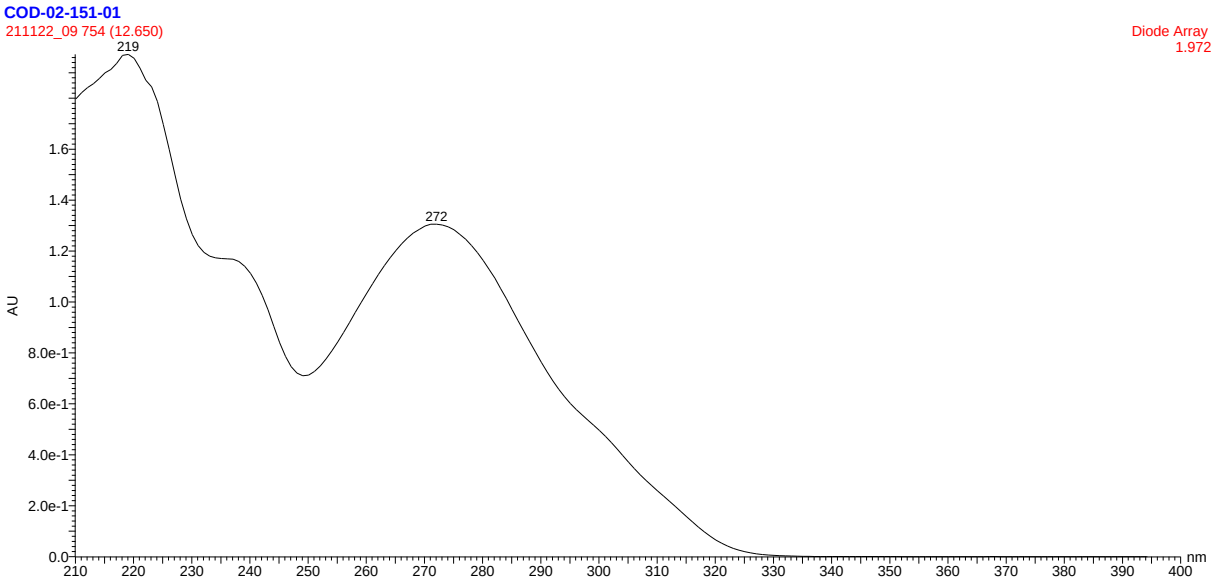
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-240**



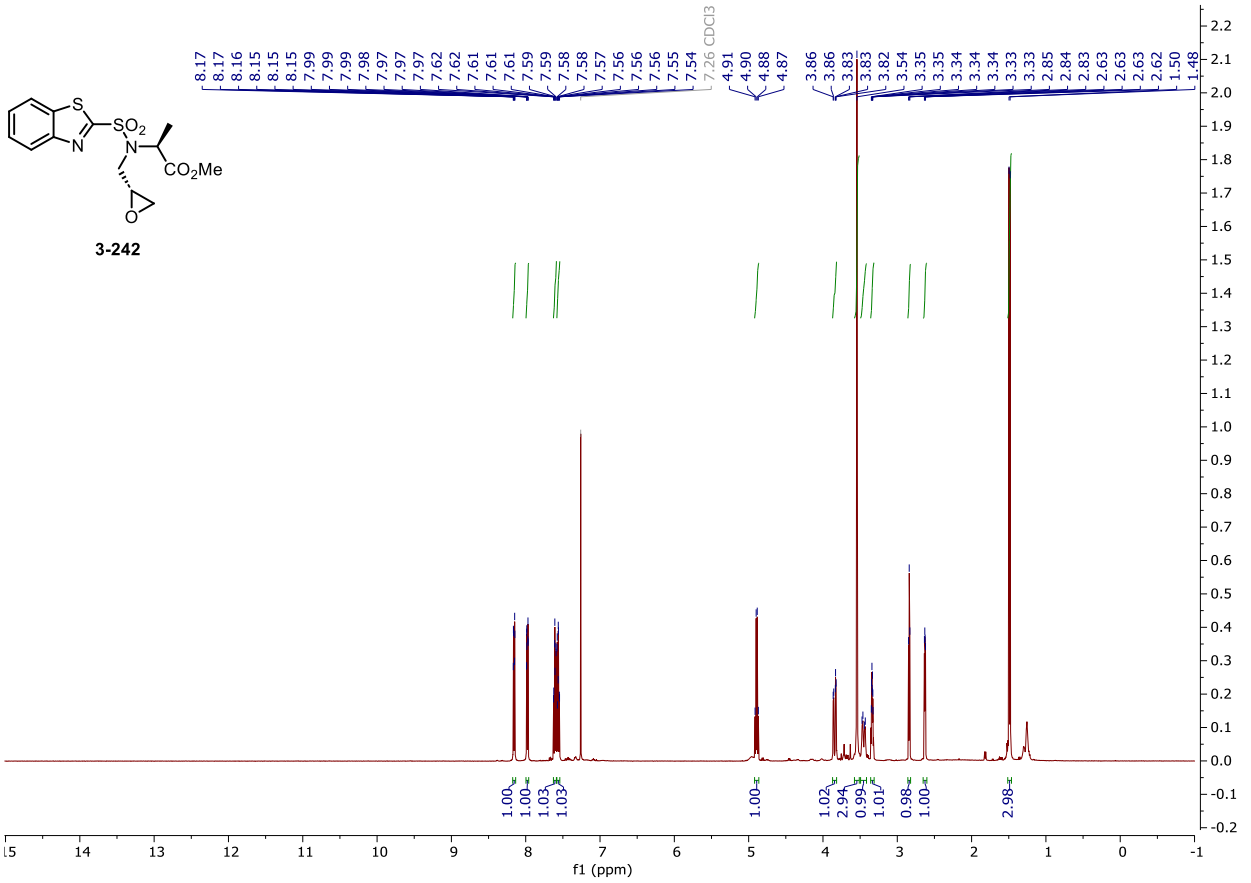


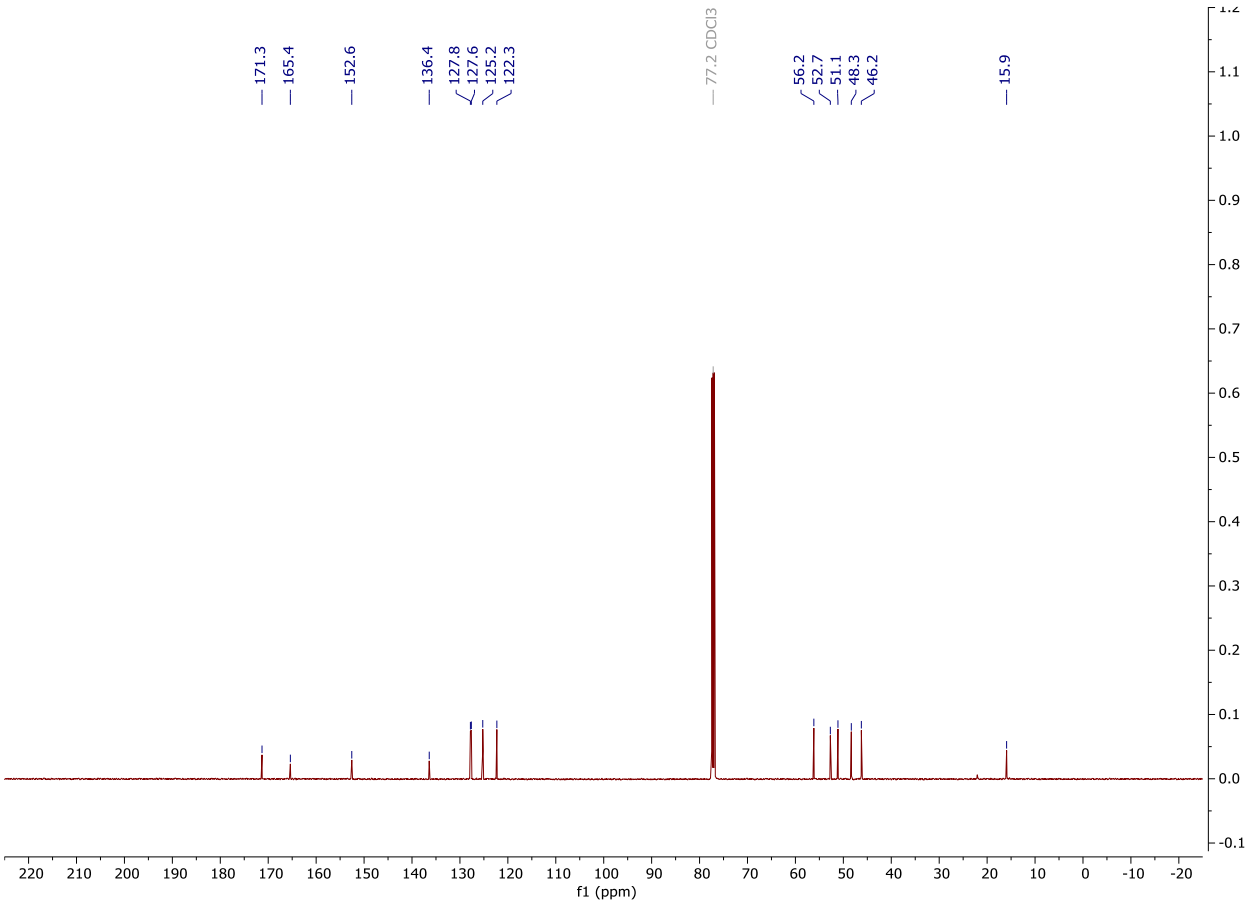
HPLC chromatograms of **3-240**



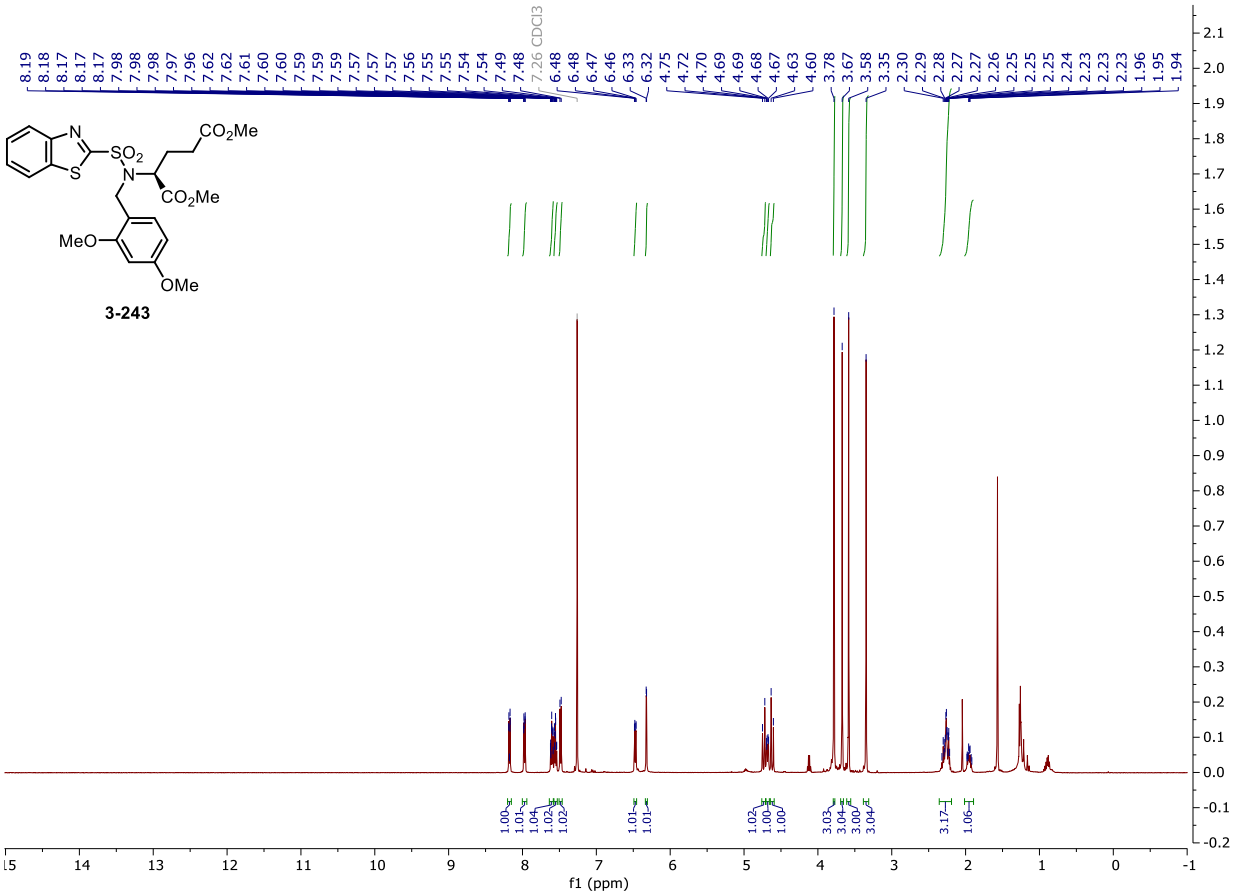


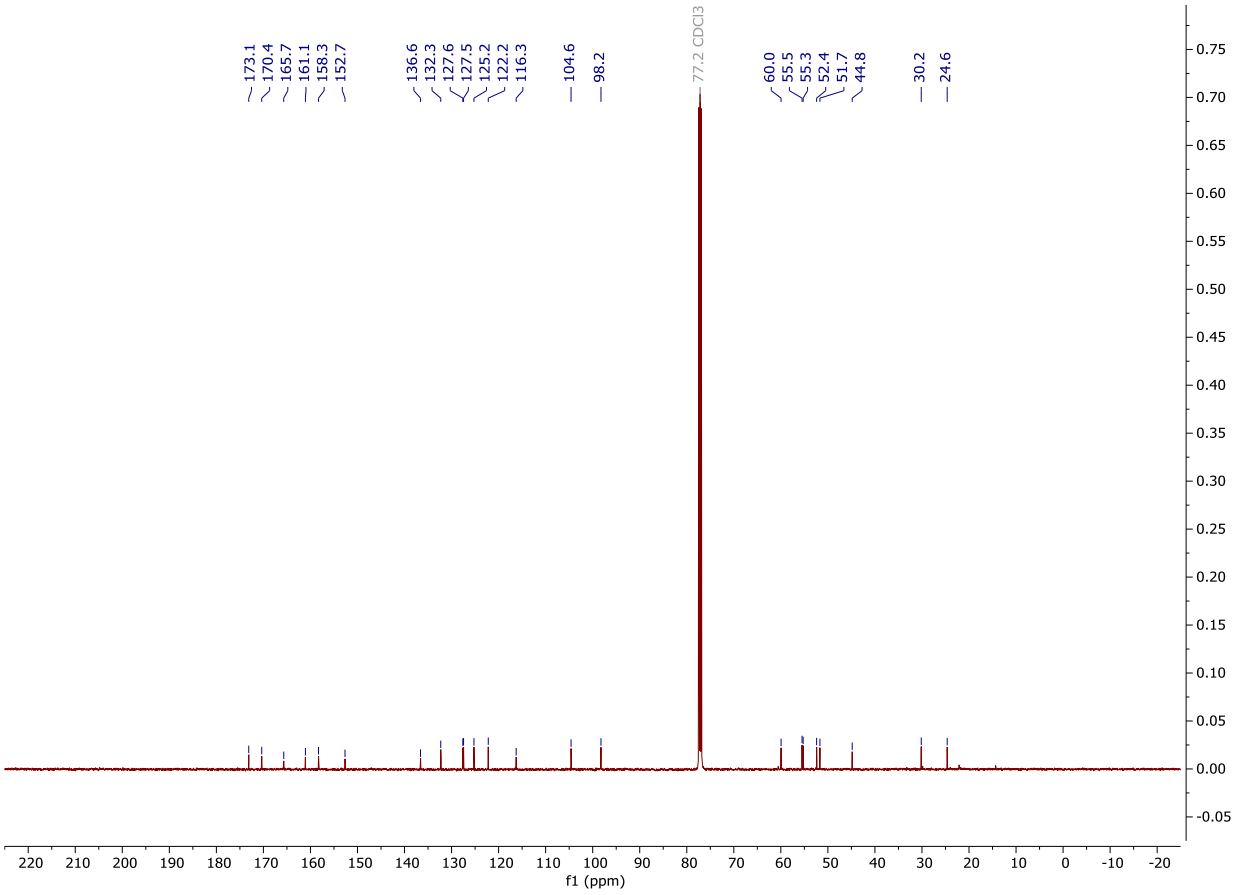
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-242**



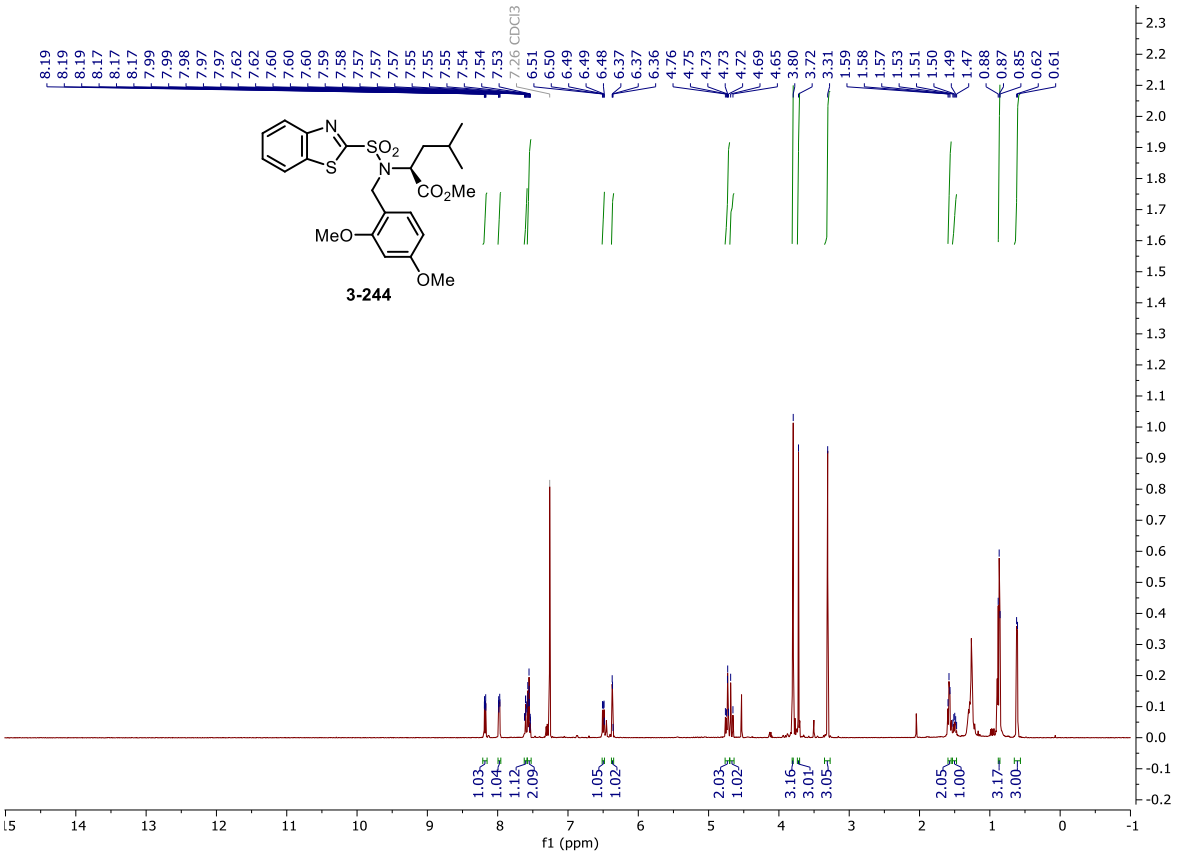


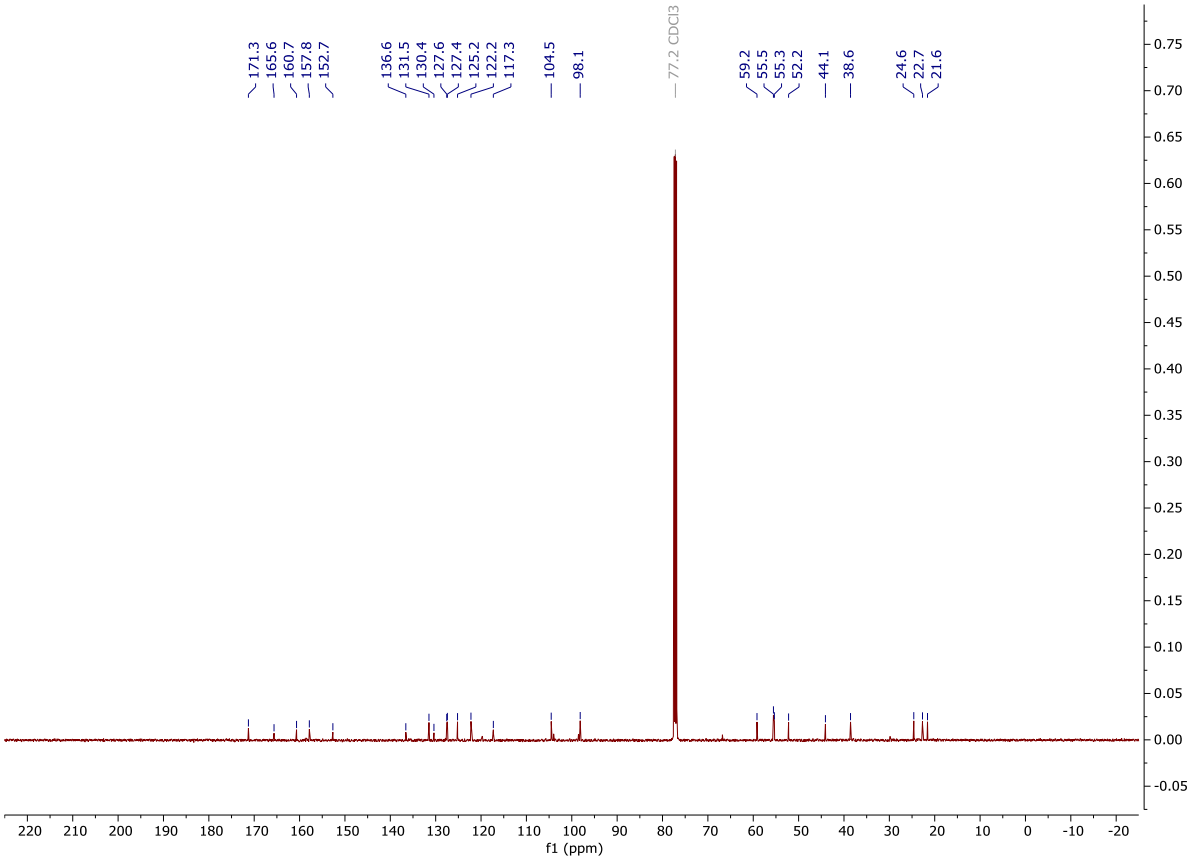
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-243**



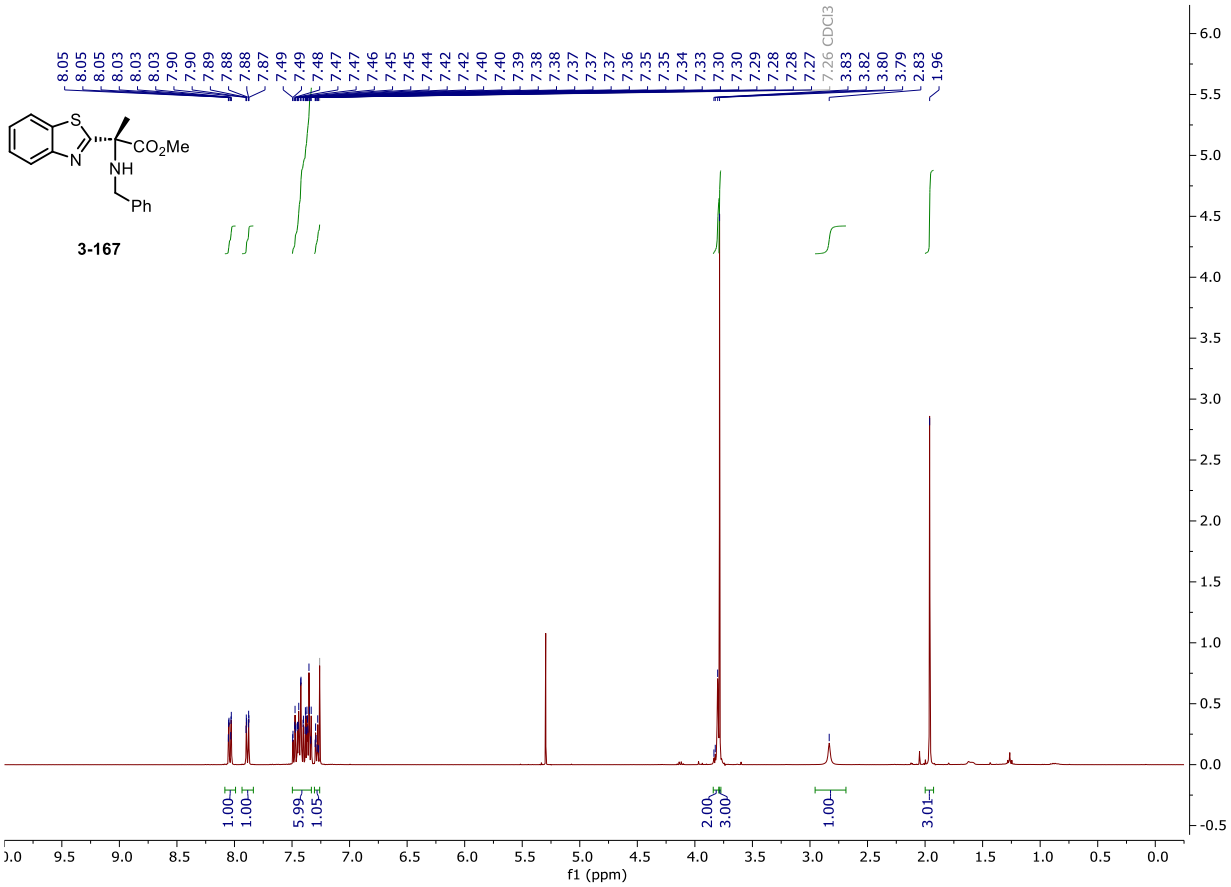


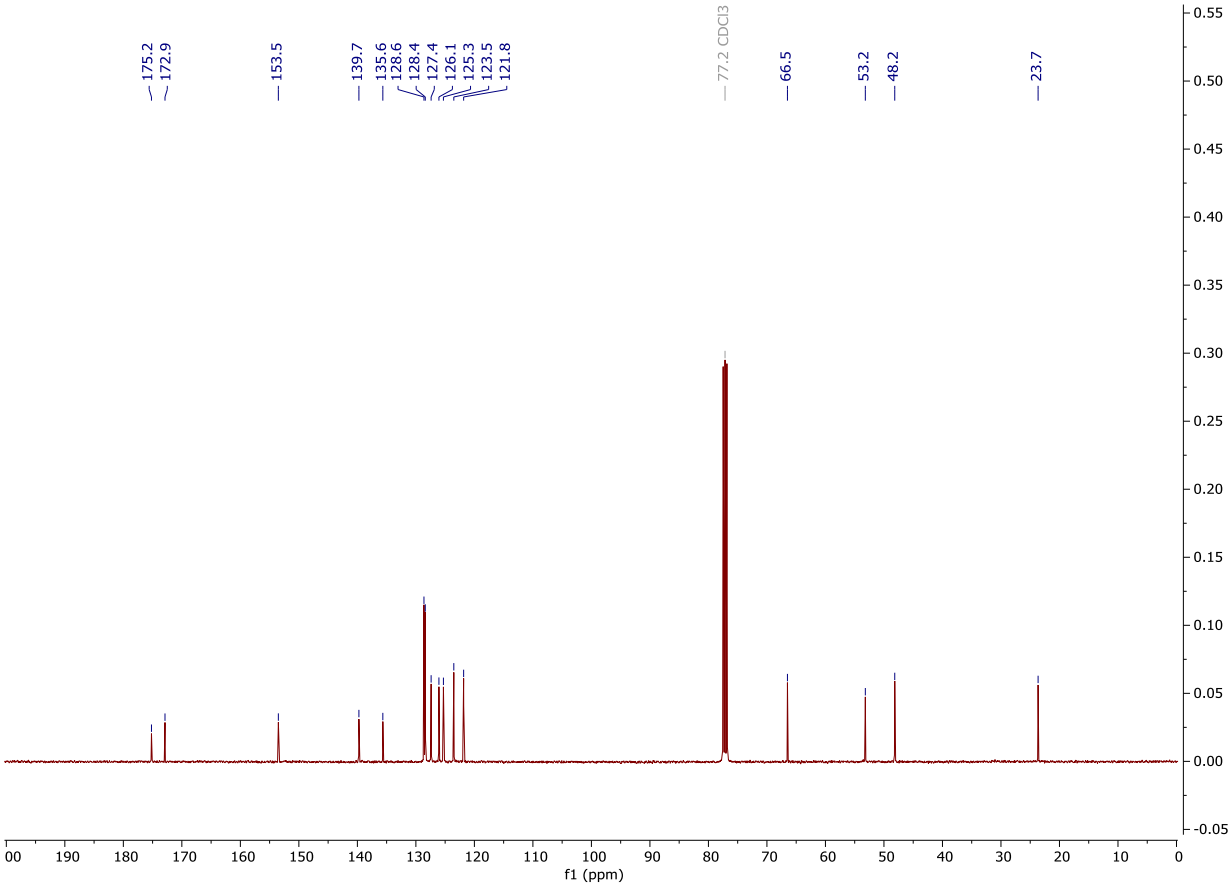
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-244**



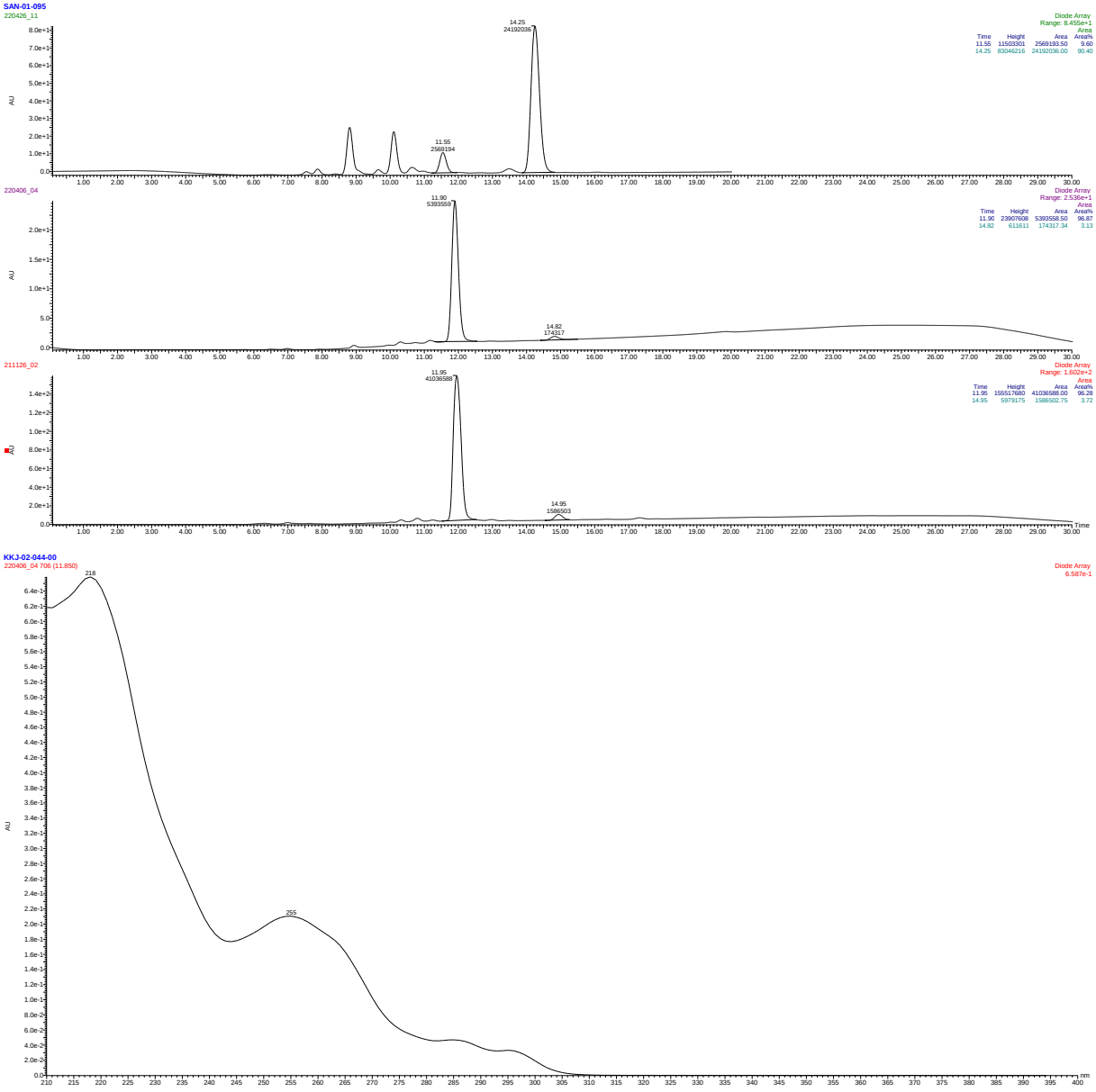


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-167**

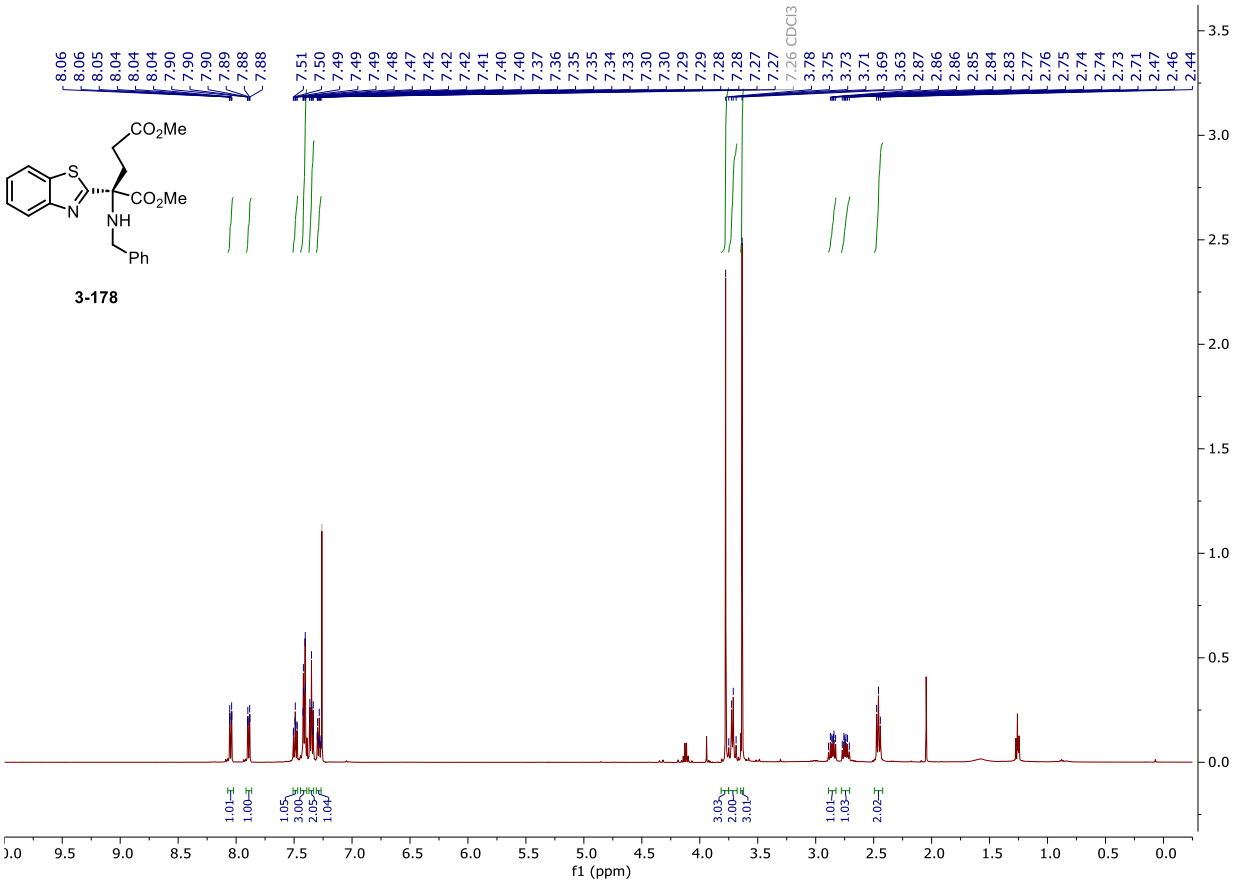


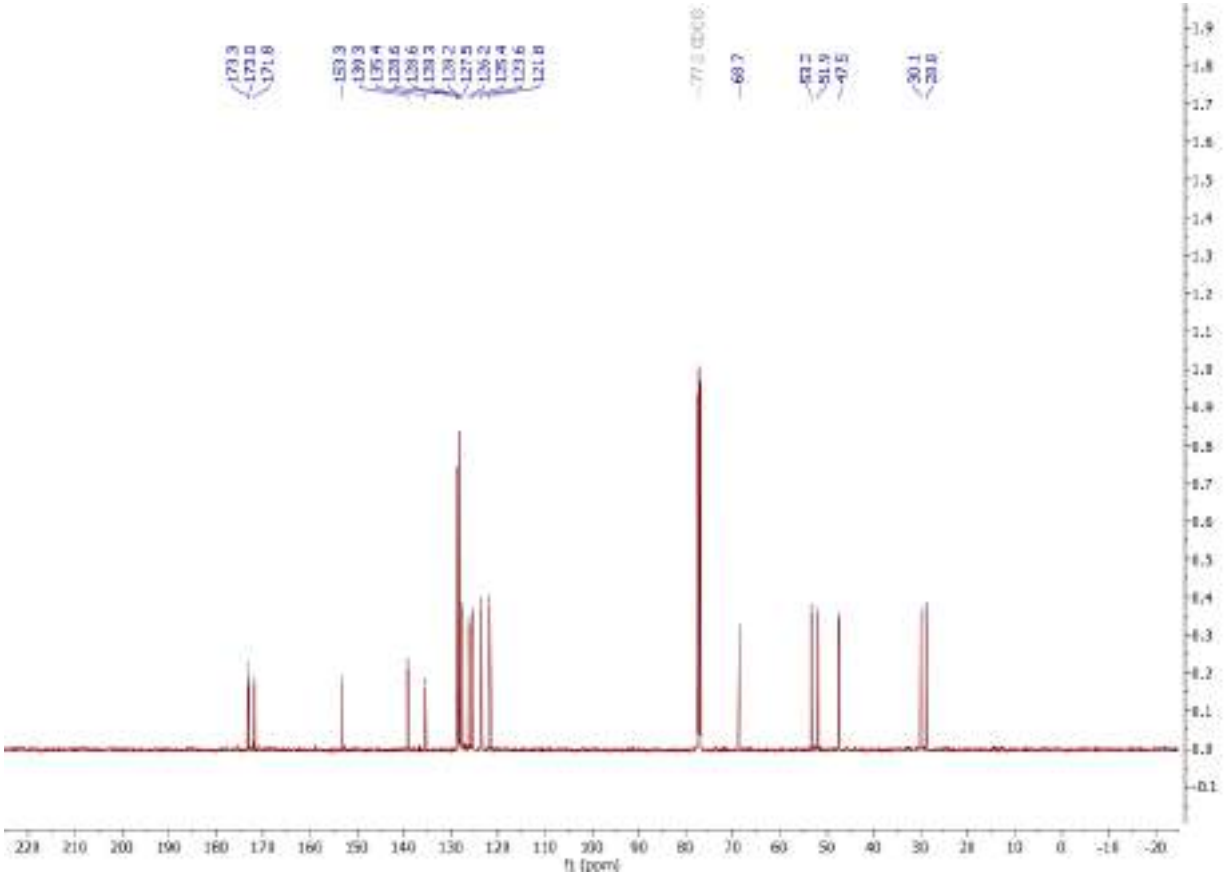


HPLC chromatograms of **3-167**

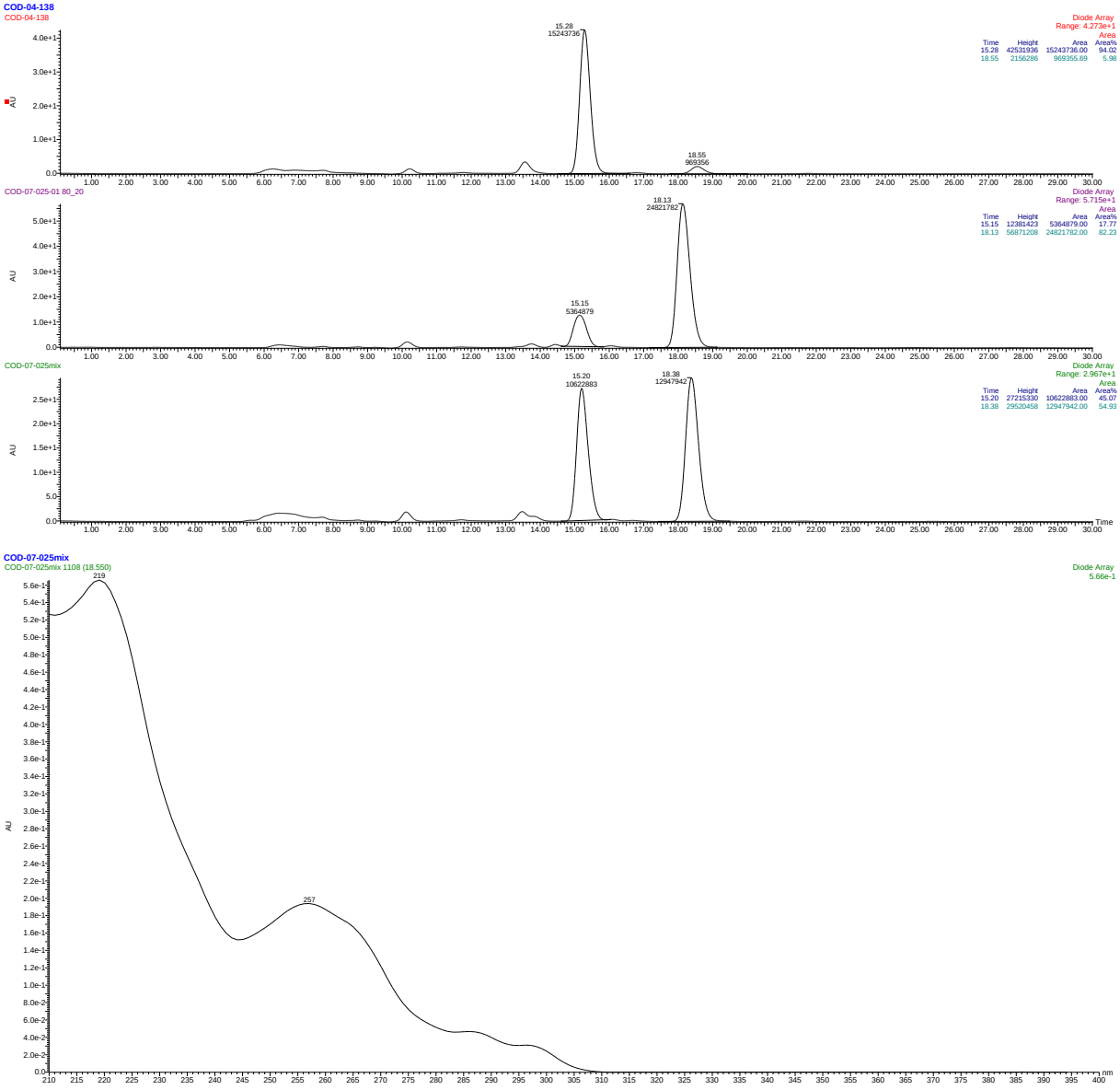


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-178**

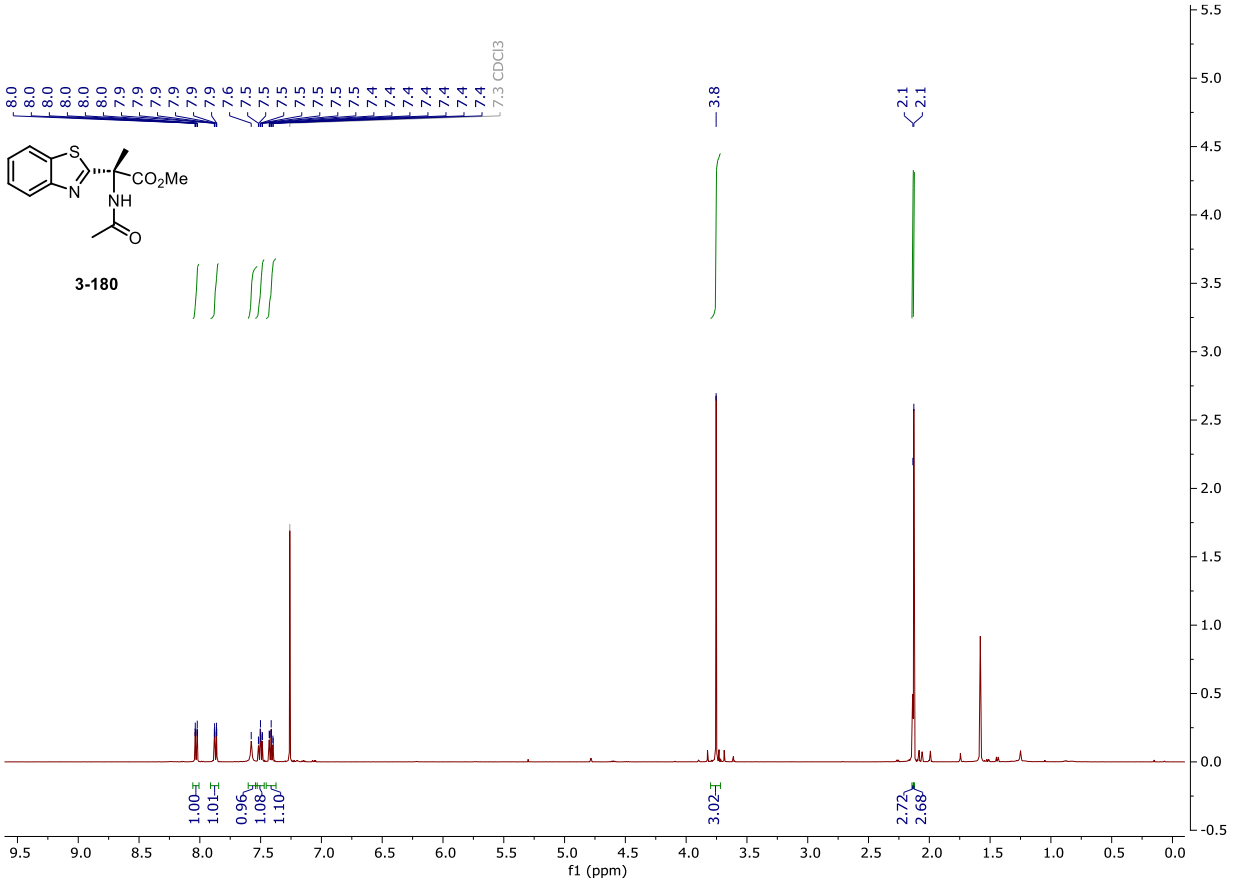


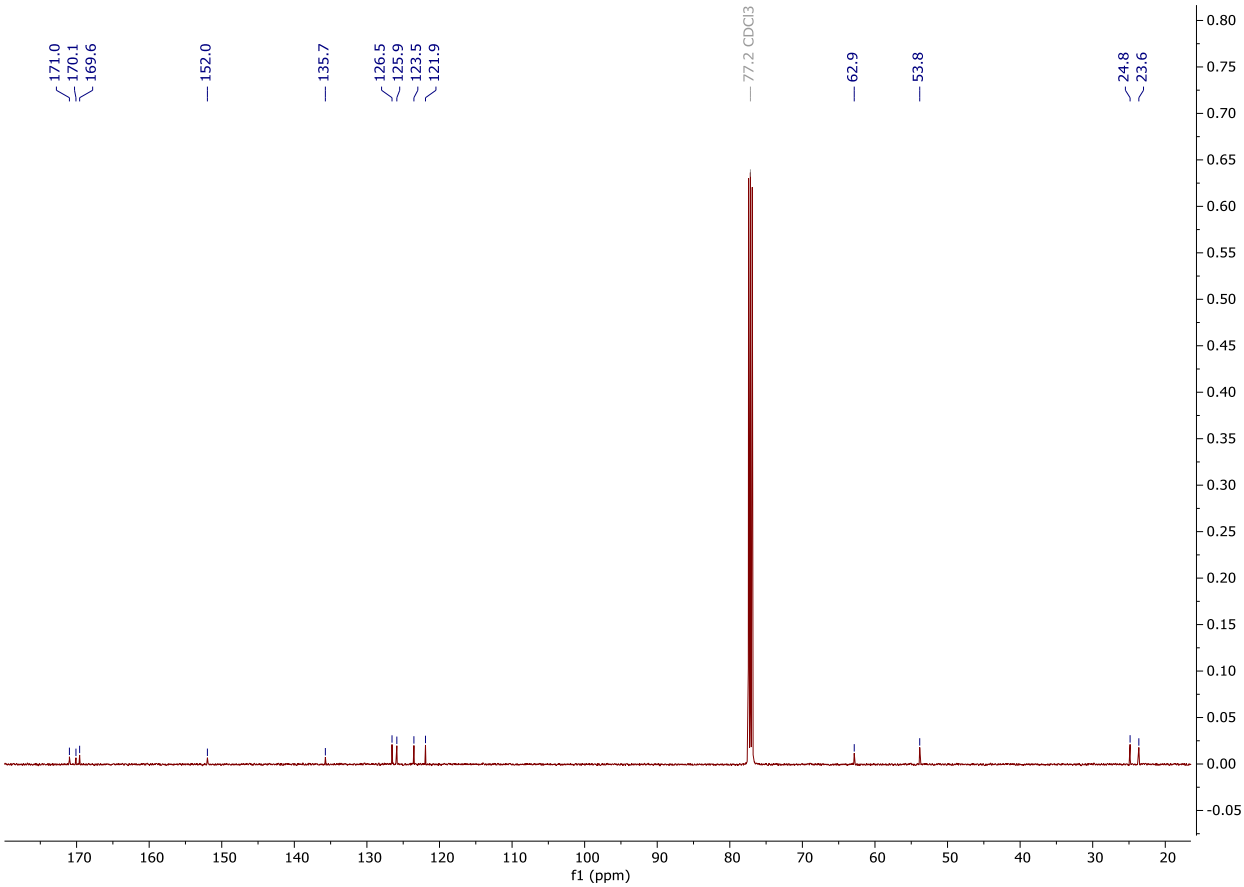


HPLC chromatograms of **3-178**

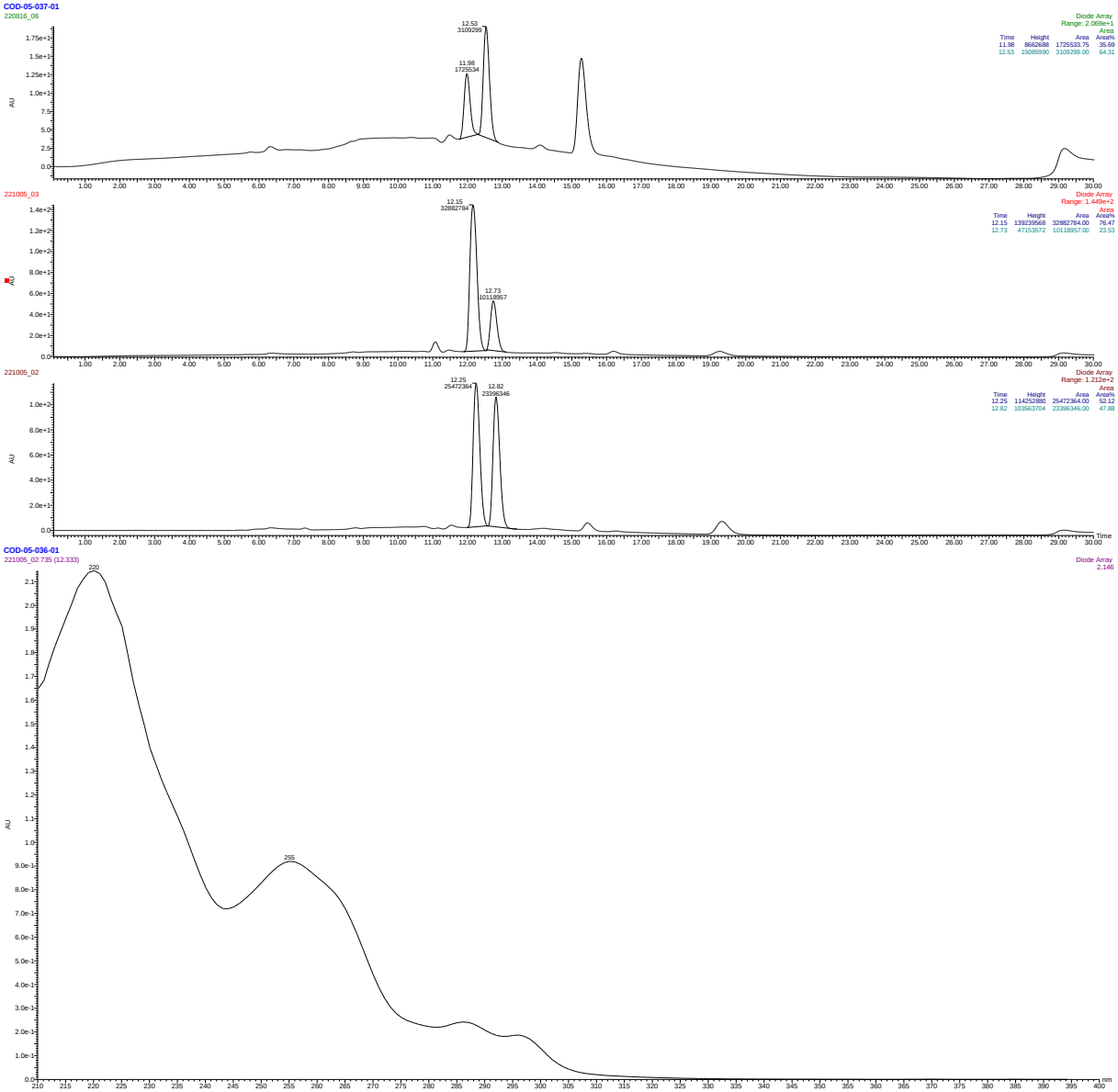


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-180**

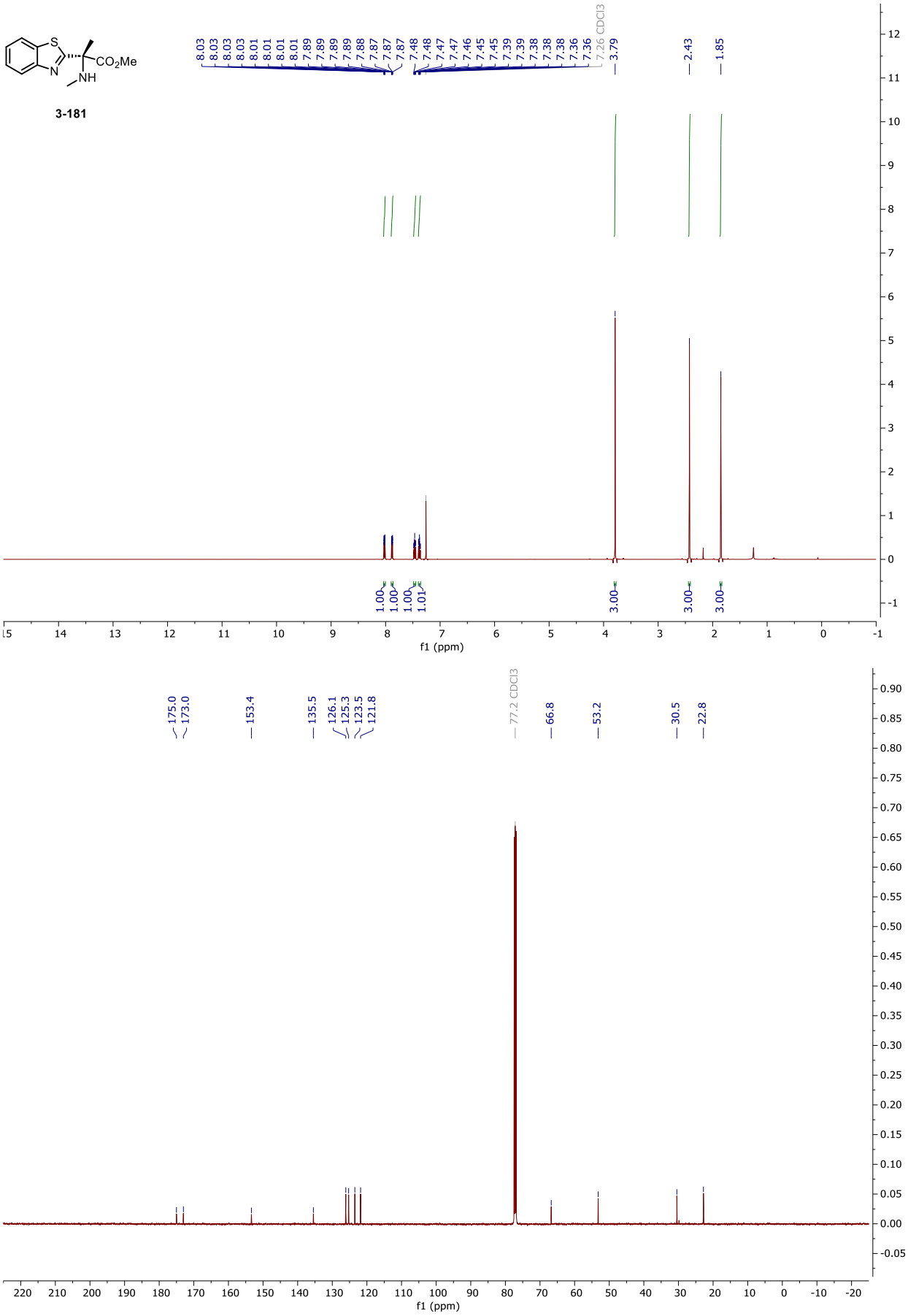




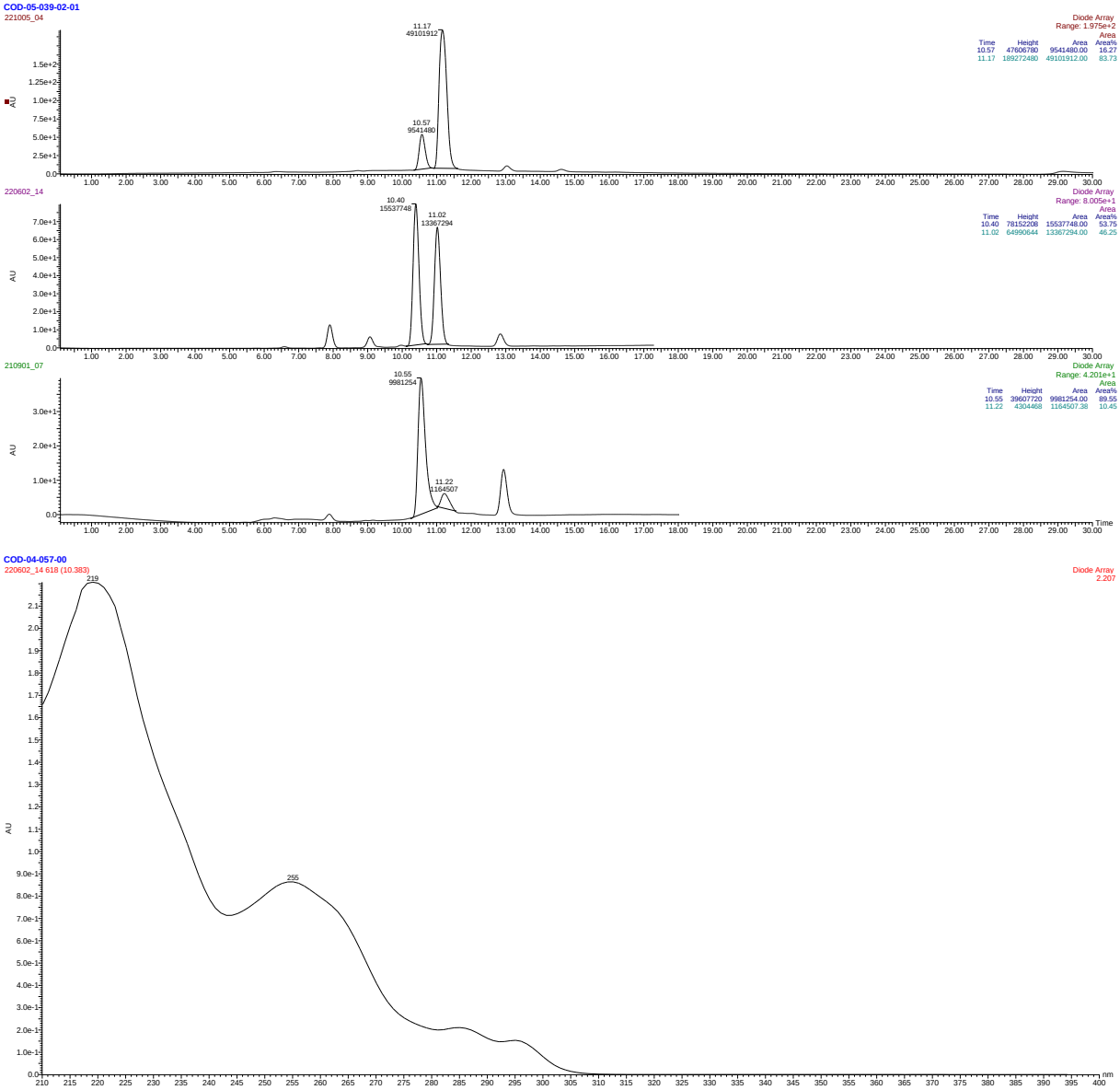
HPLC chromatograms of **3-180**



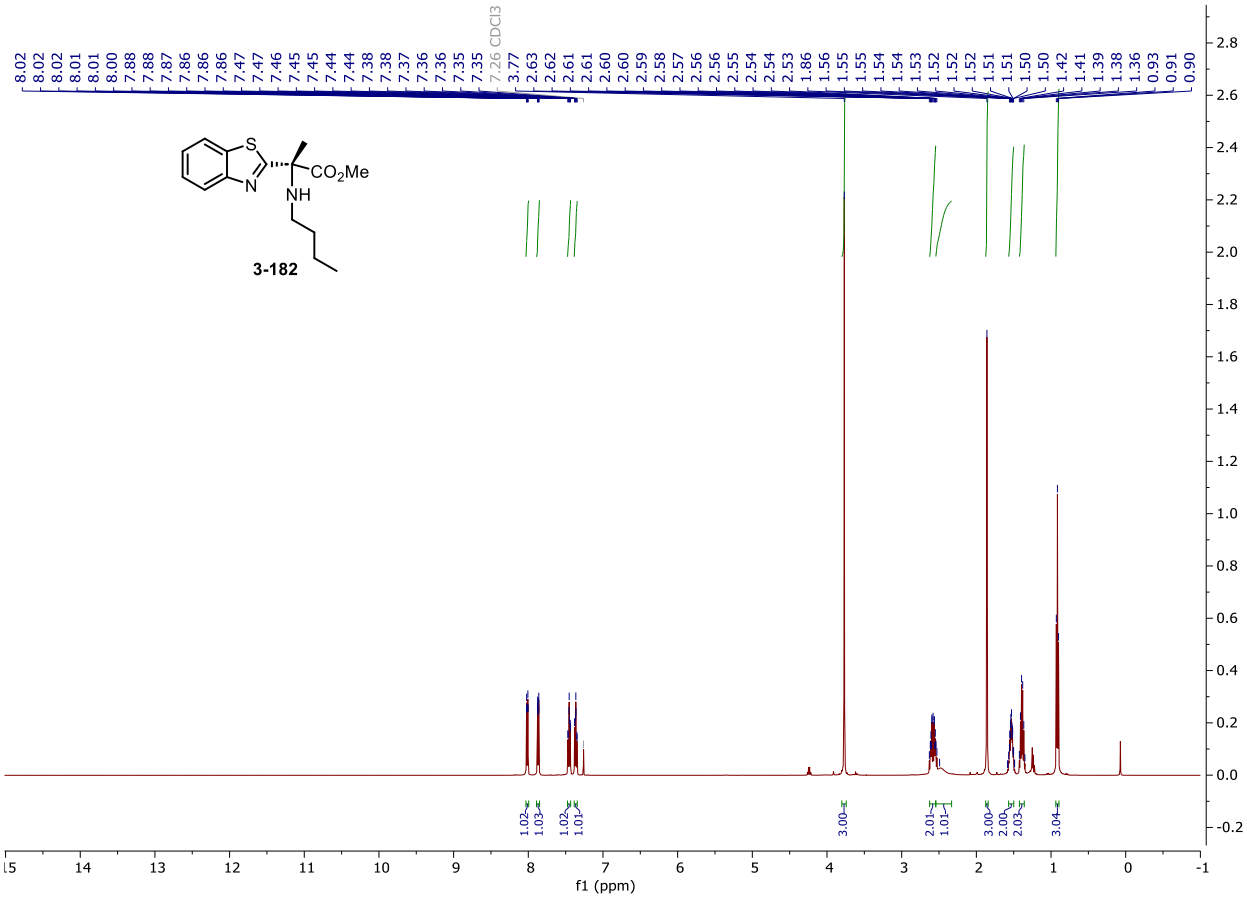
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-181**

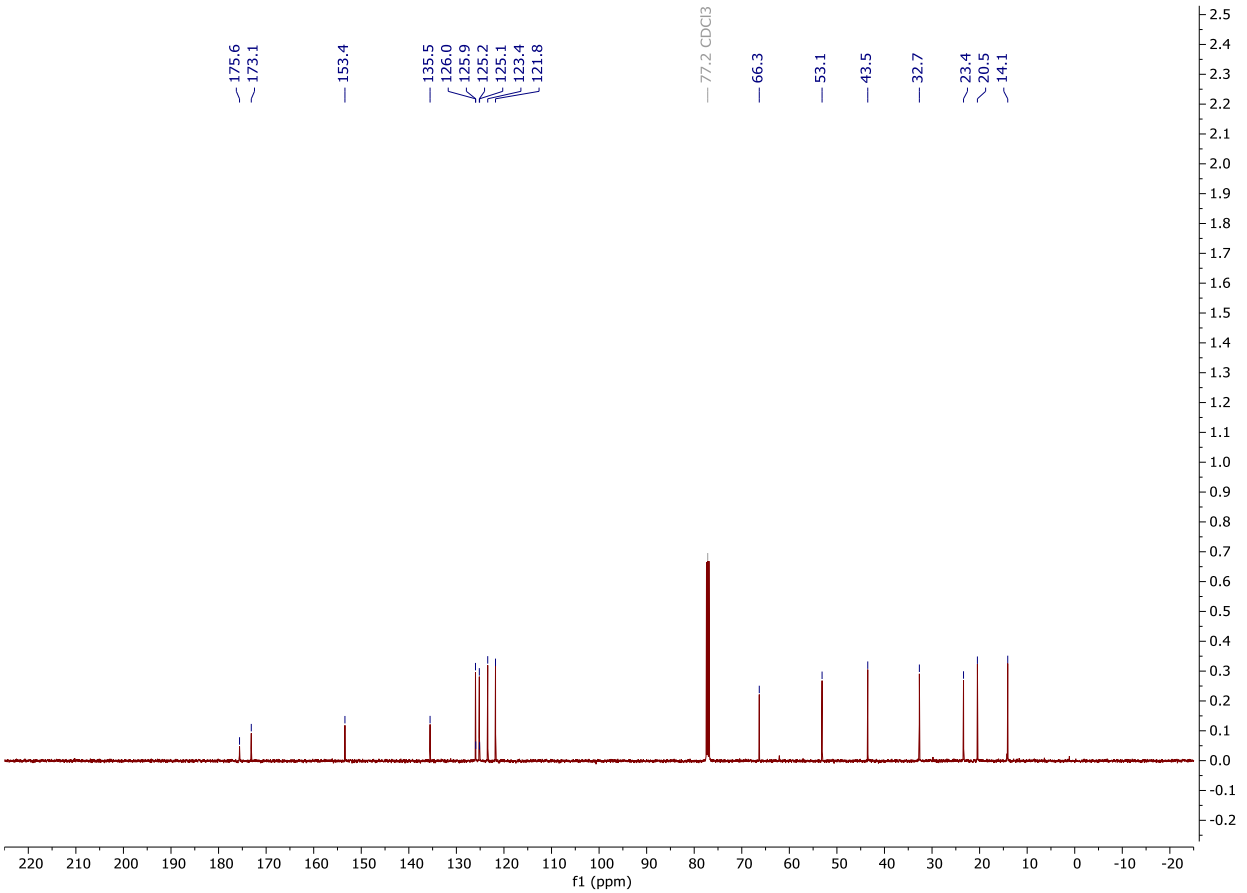


HPLC chromatograms of **3-181**

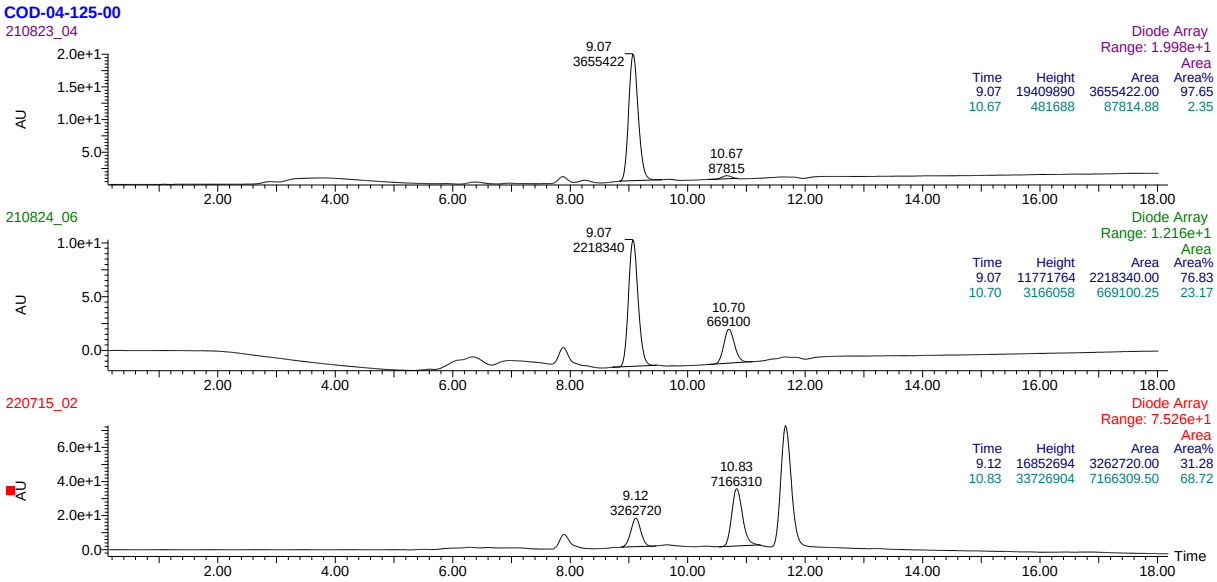


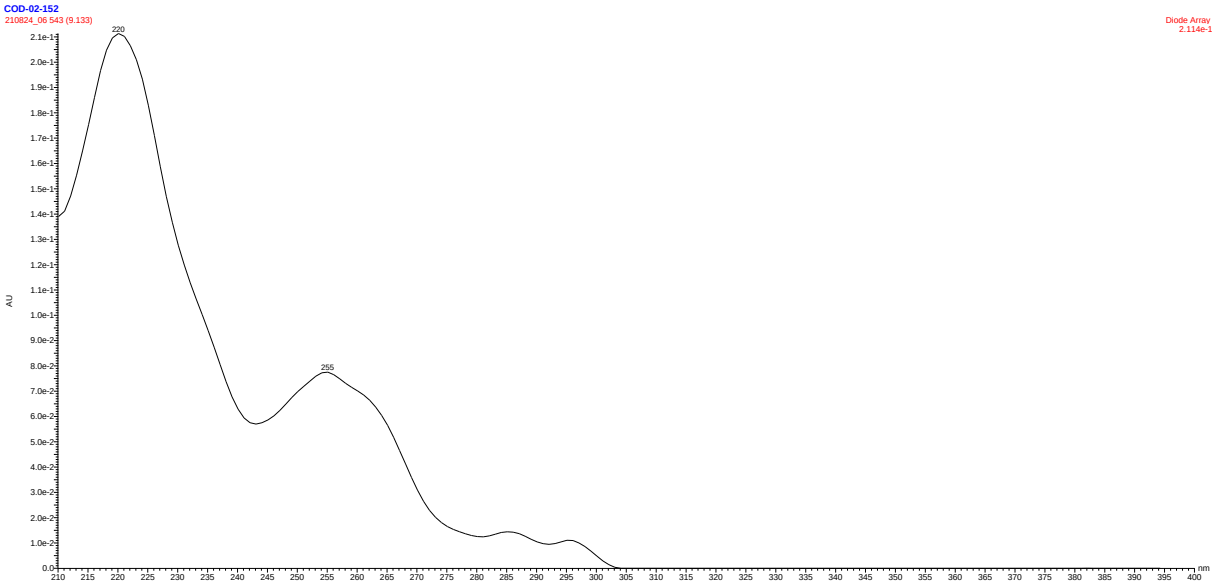
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-182**



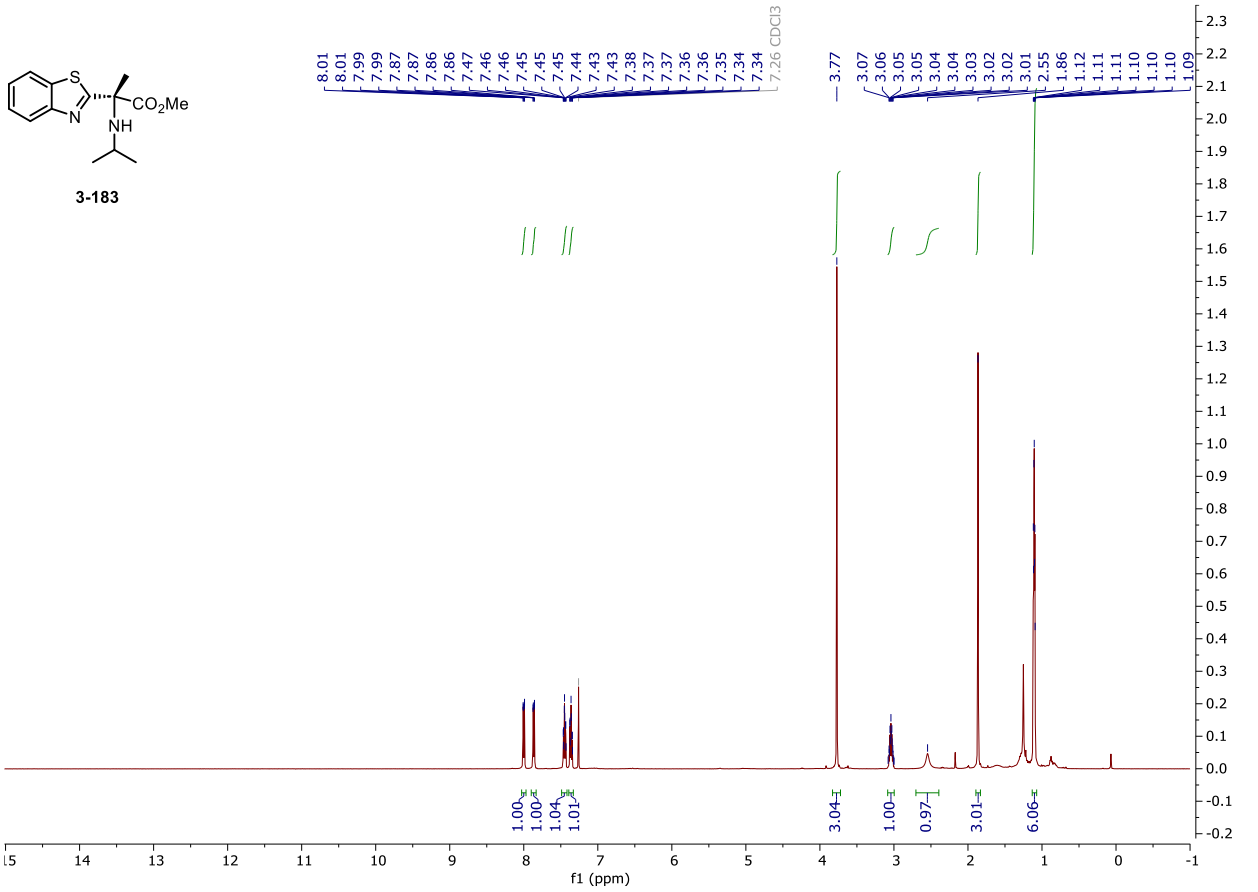


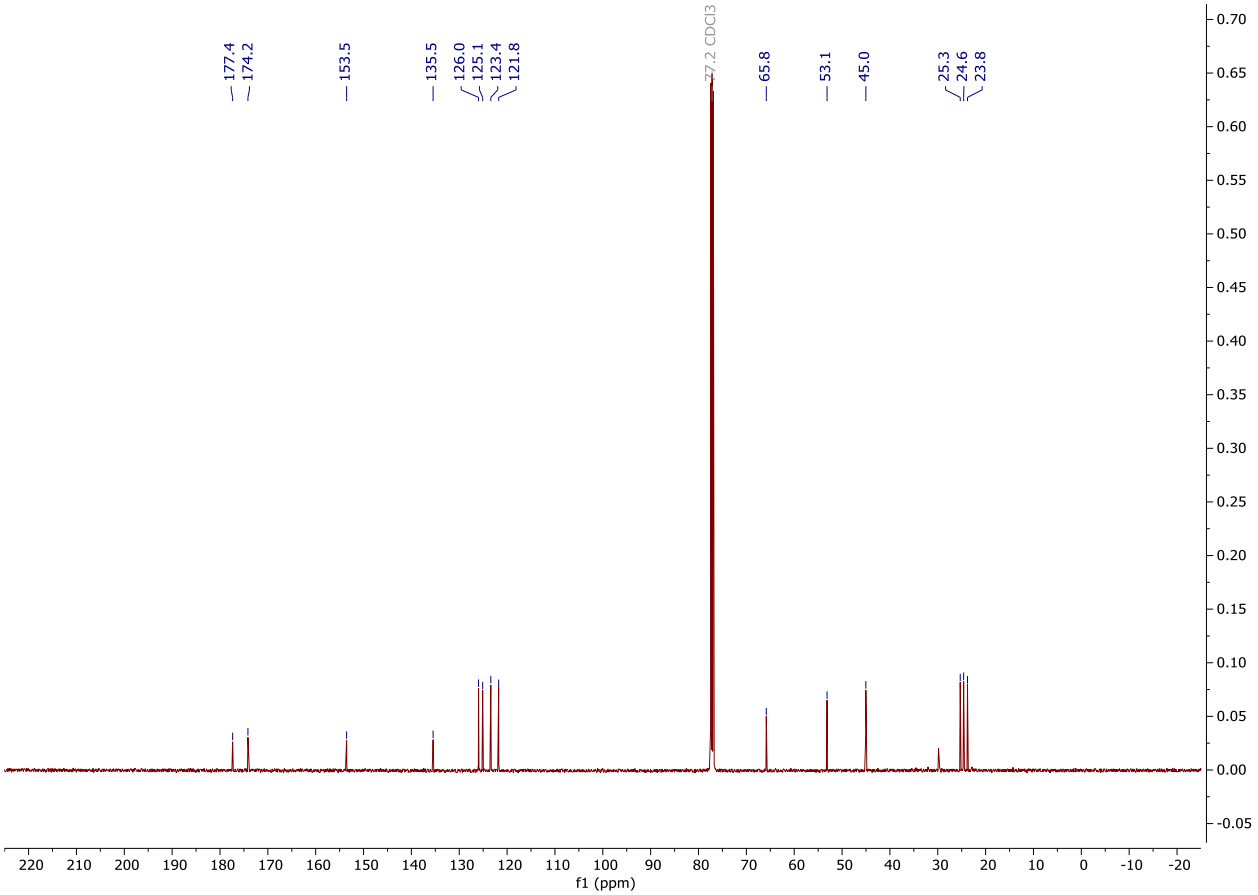
HPLC chromatograms of **3-182**



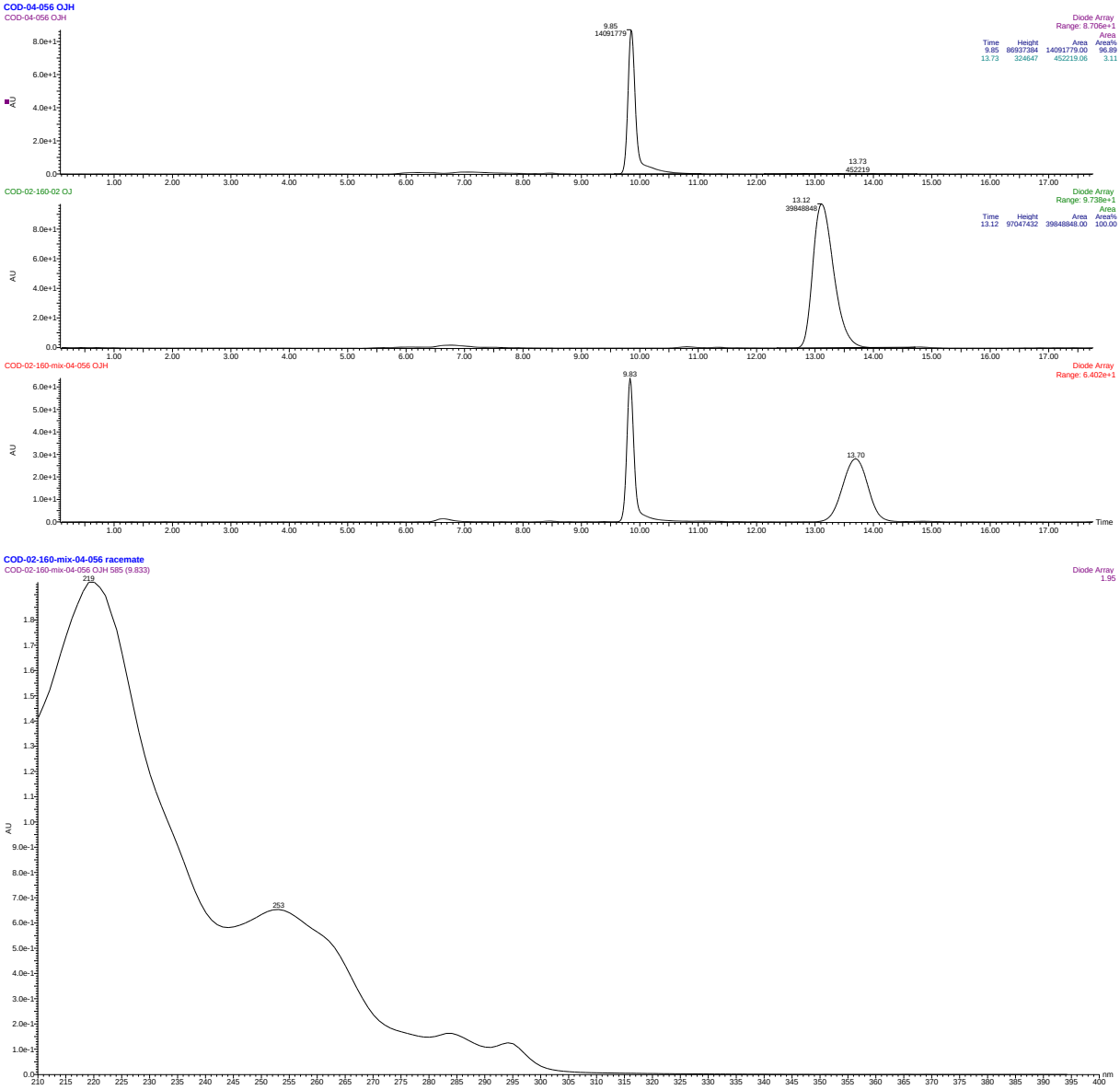


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-183**

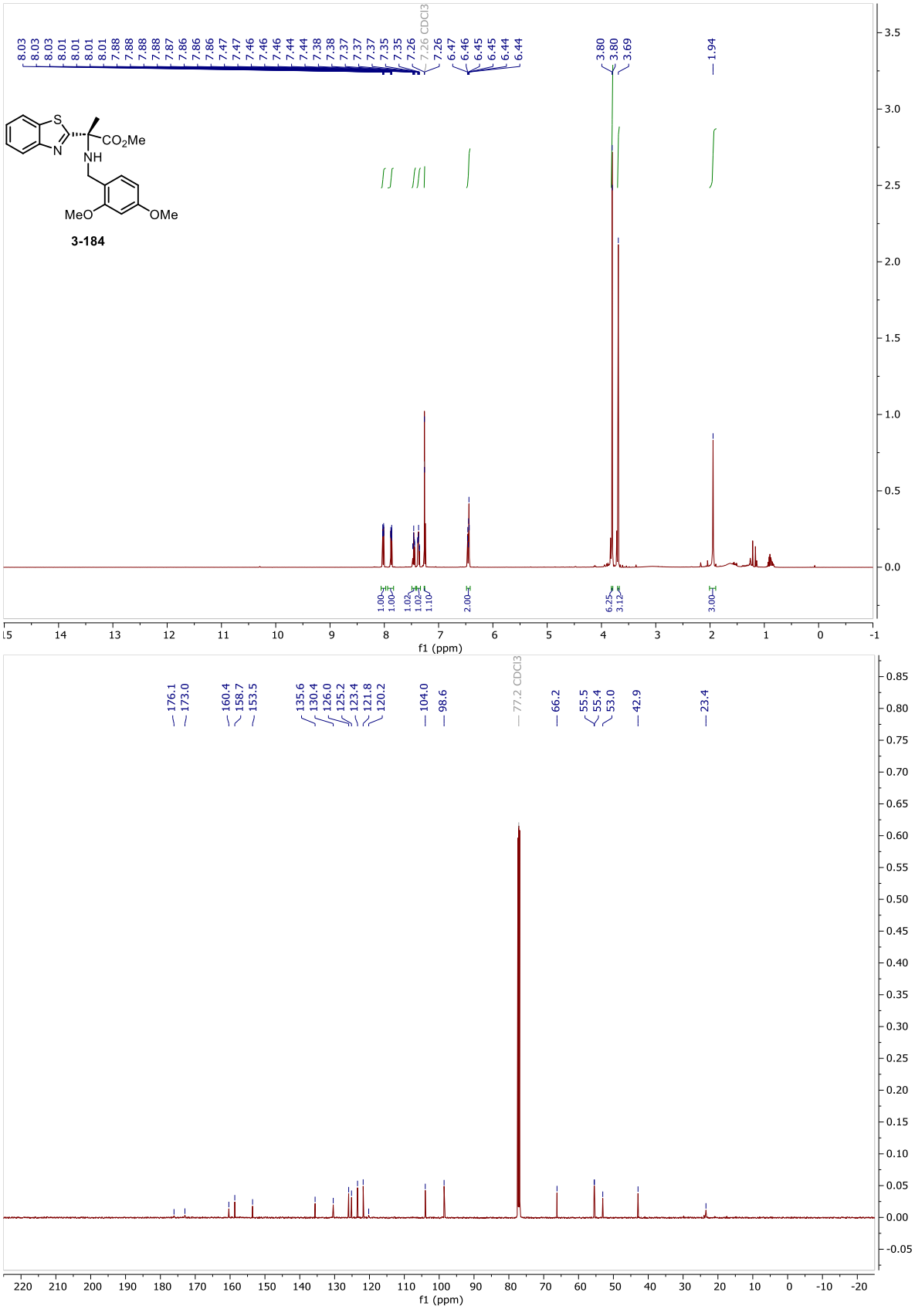




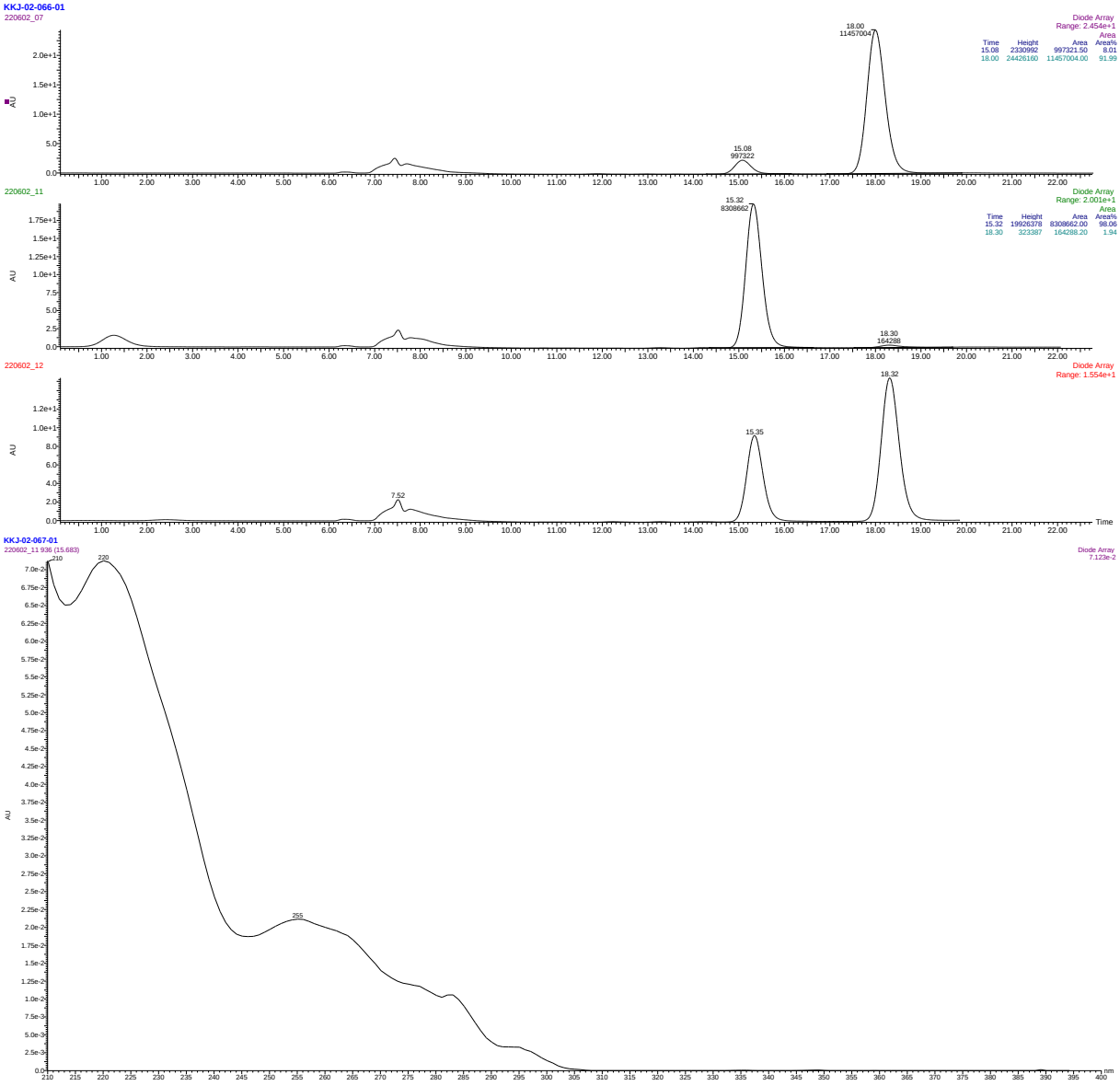
HPLC chromatograms of **3-183**



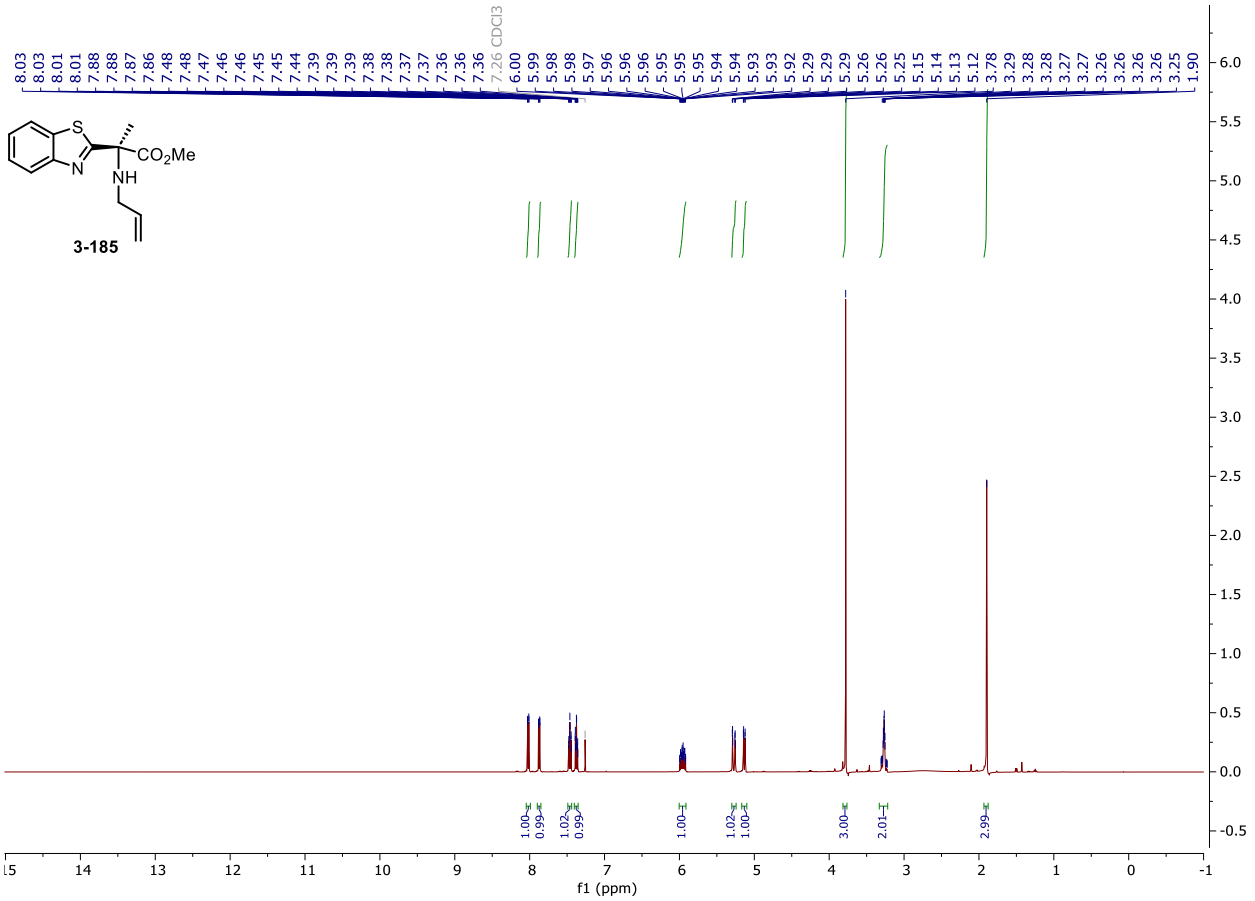
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-184**

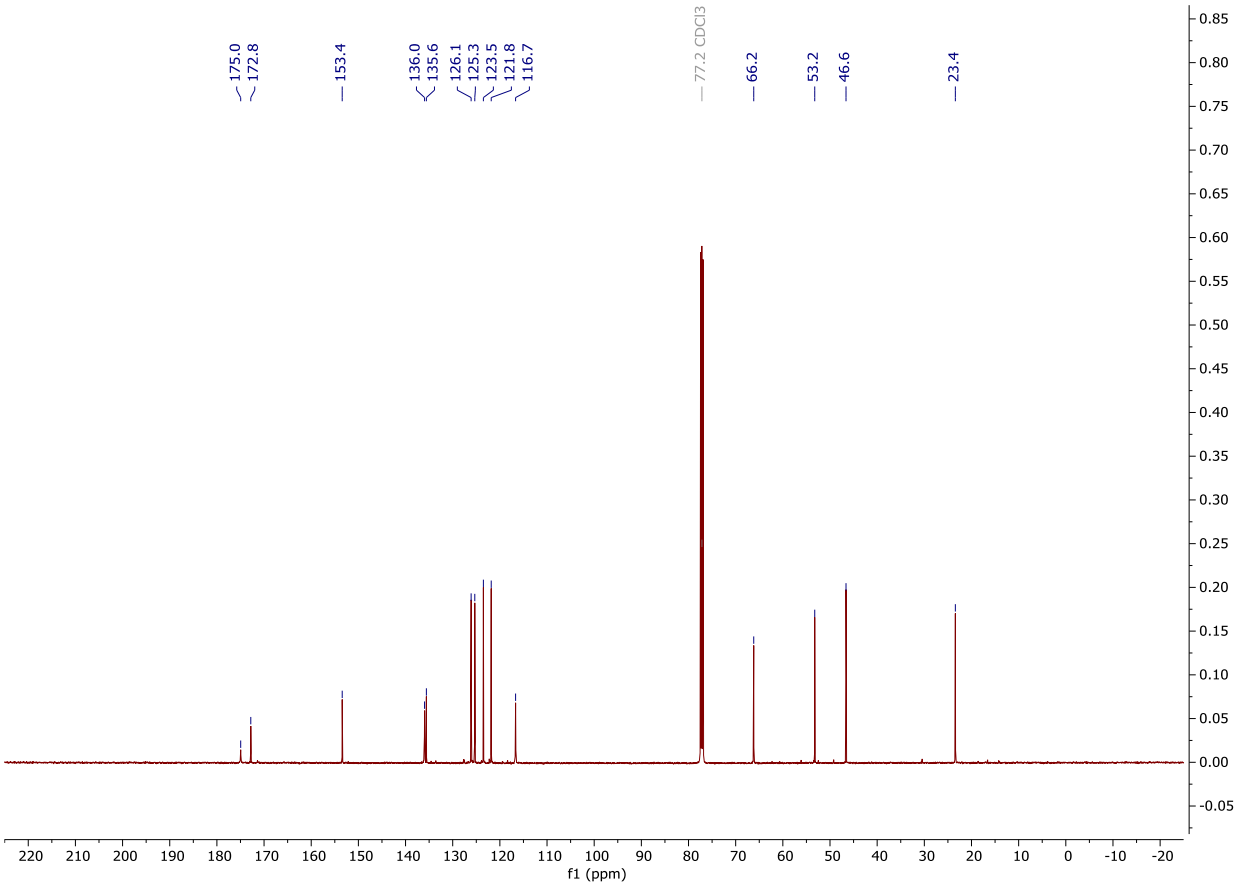


HPLC chromatograms of **3-184**

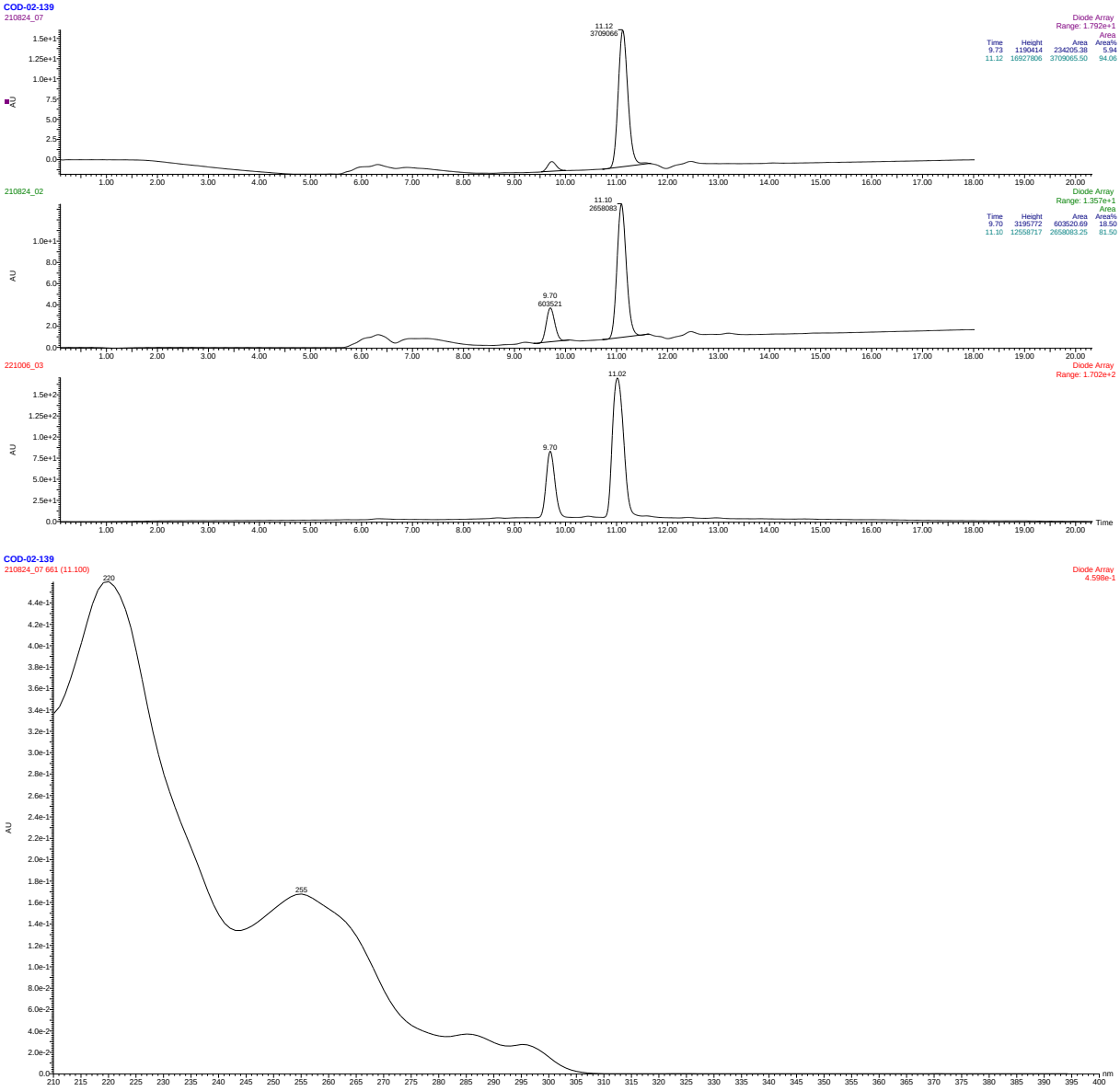


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-185**

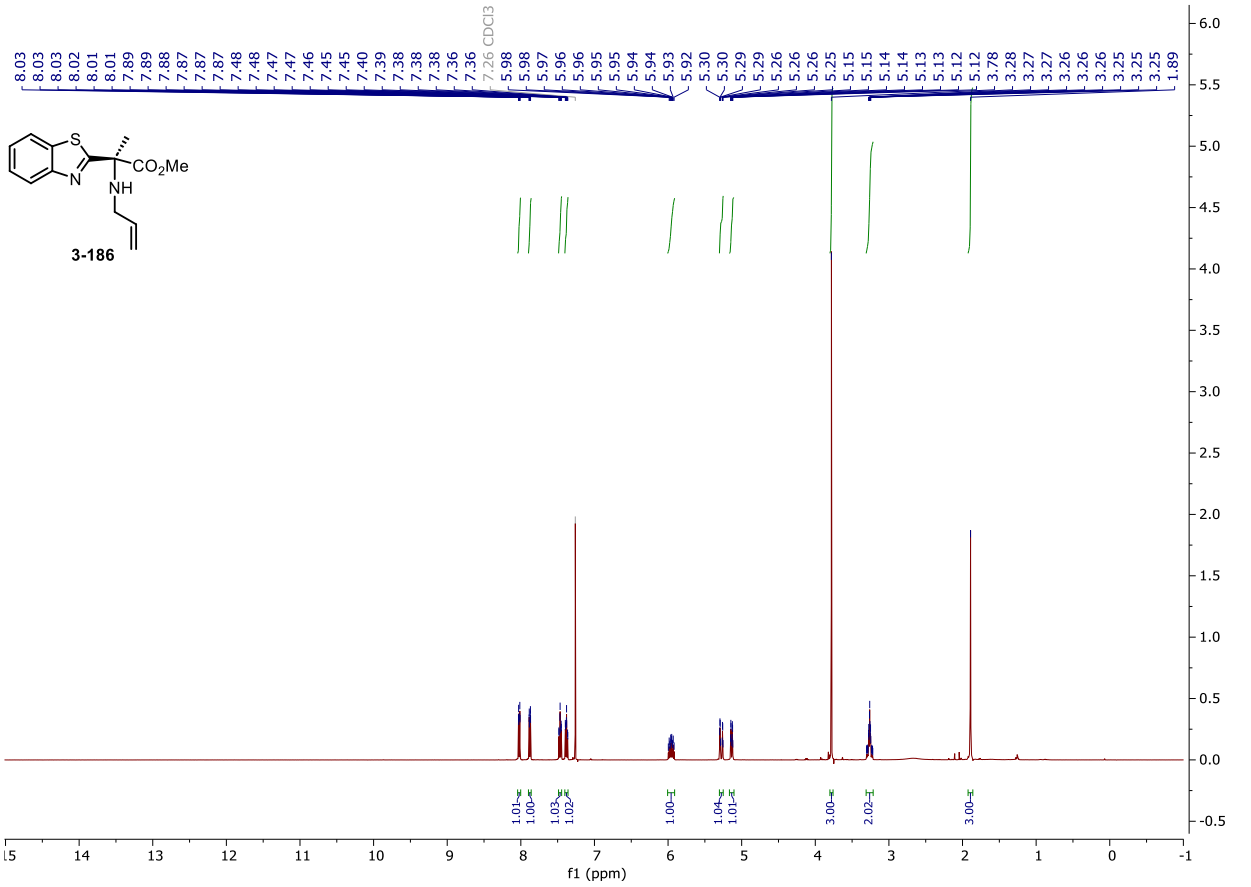


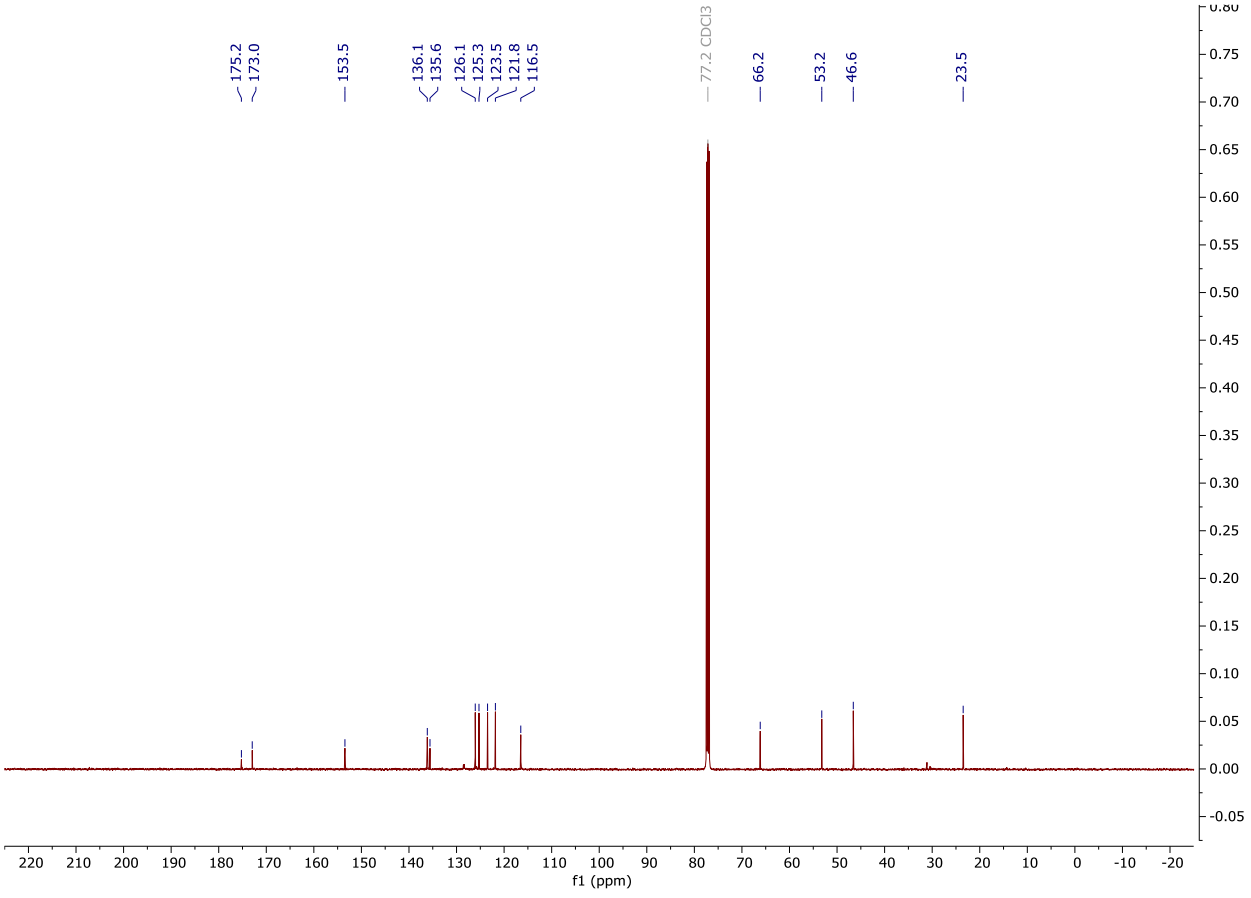


HPLC chromatograms of **3-185**

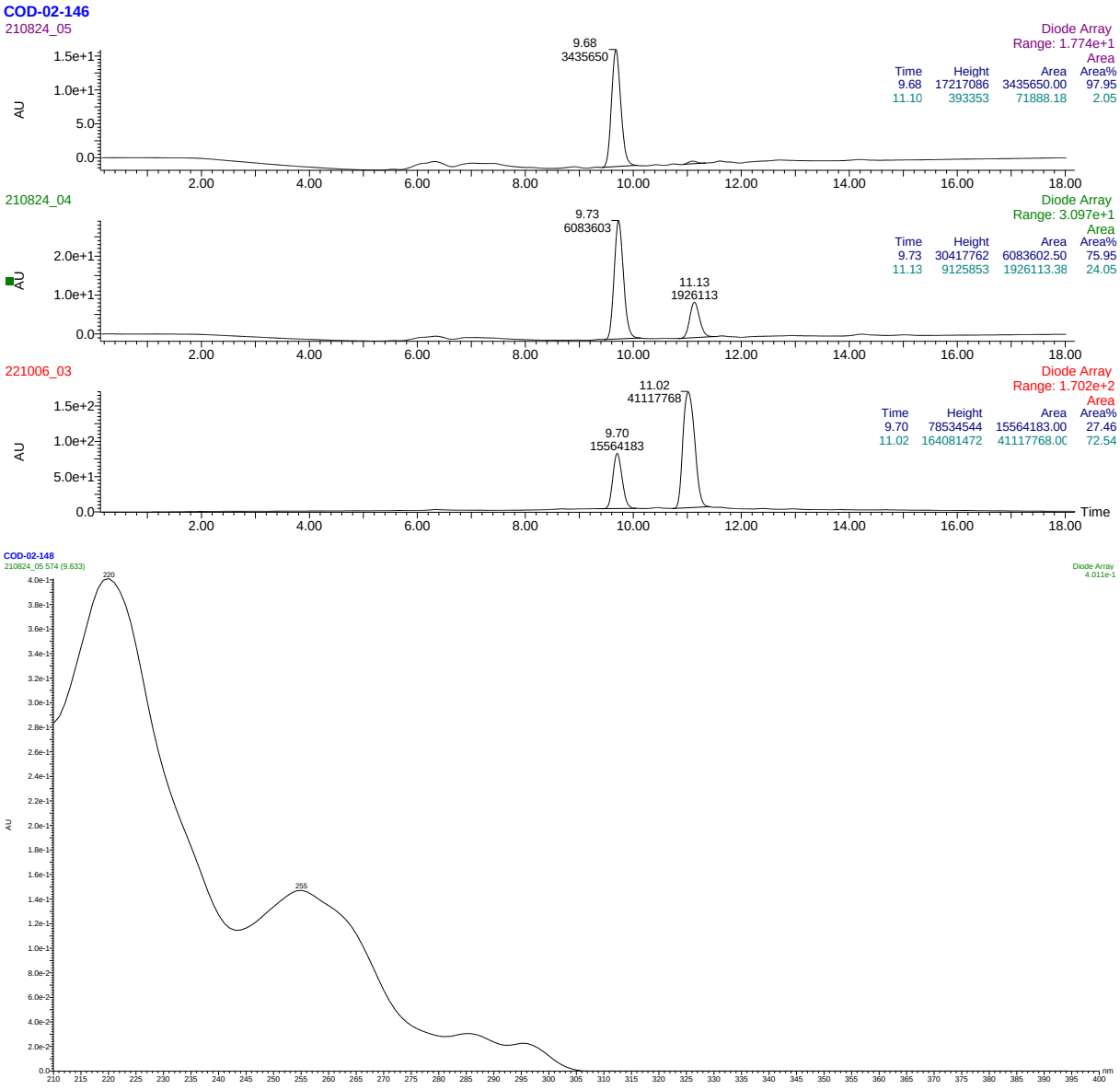


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-186**

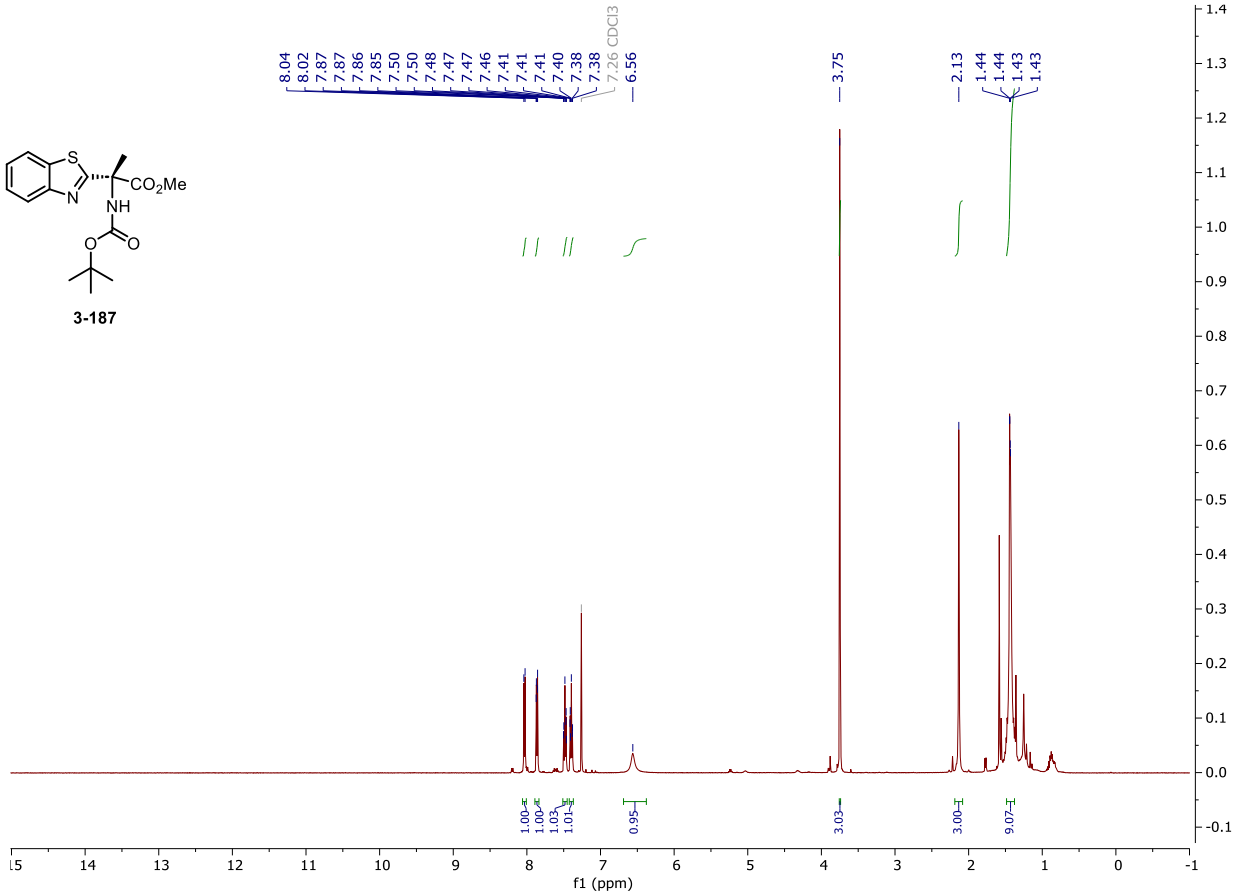


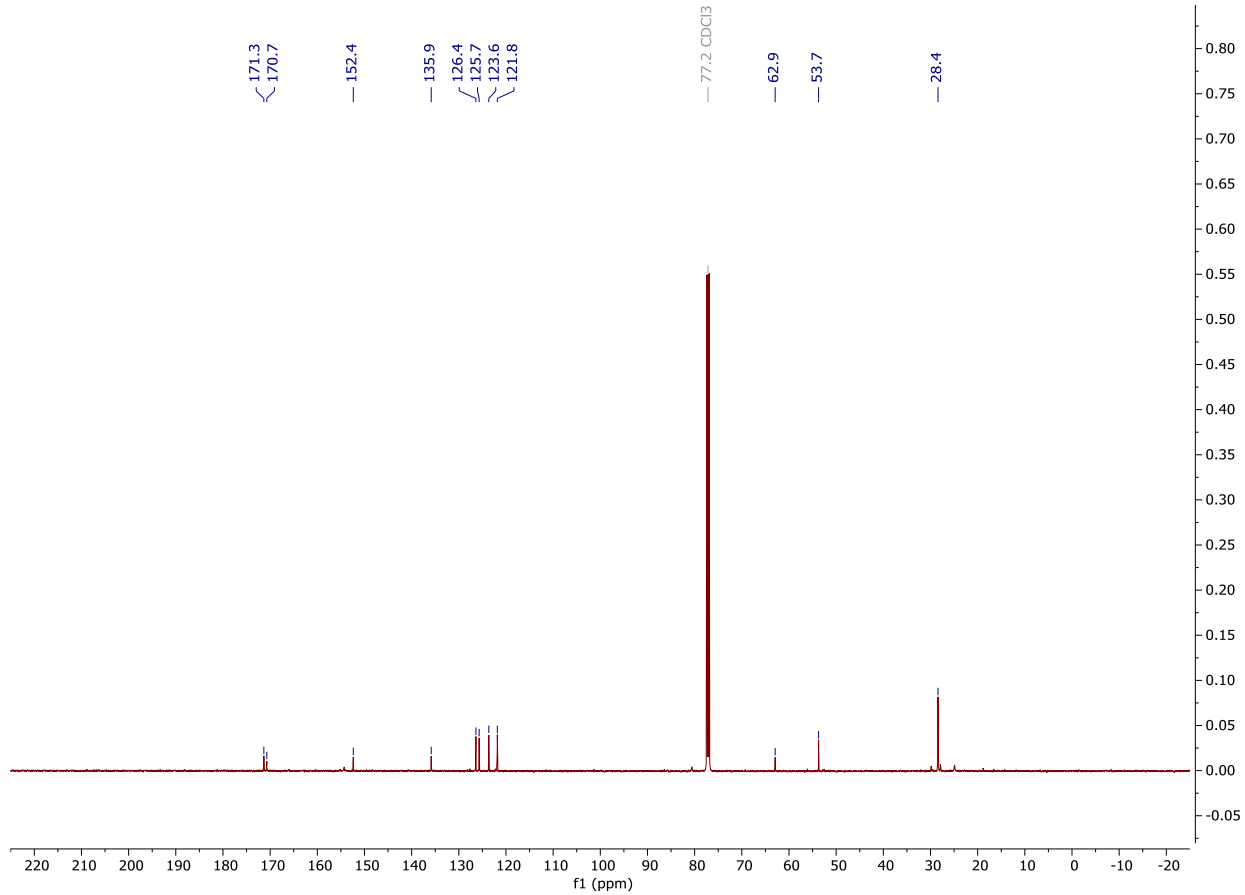


HPLC chromatograms of **3-186**



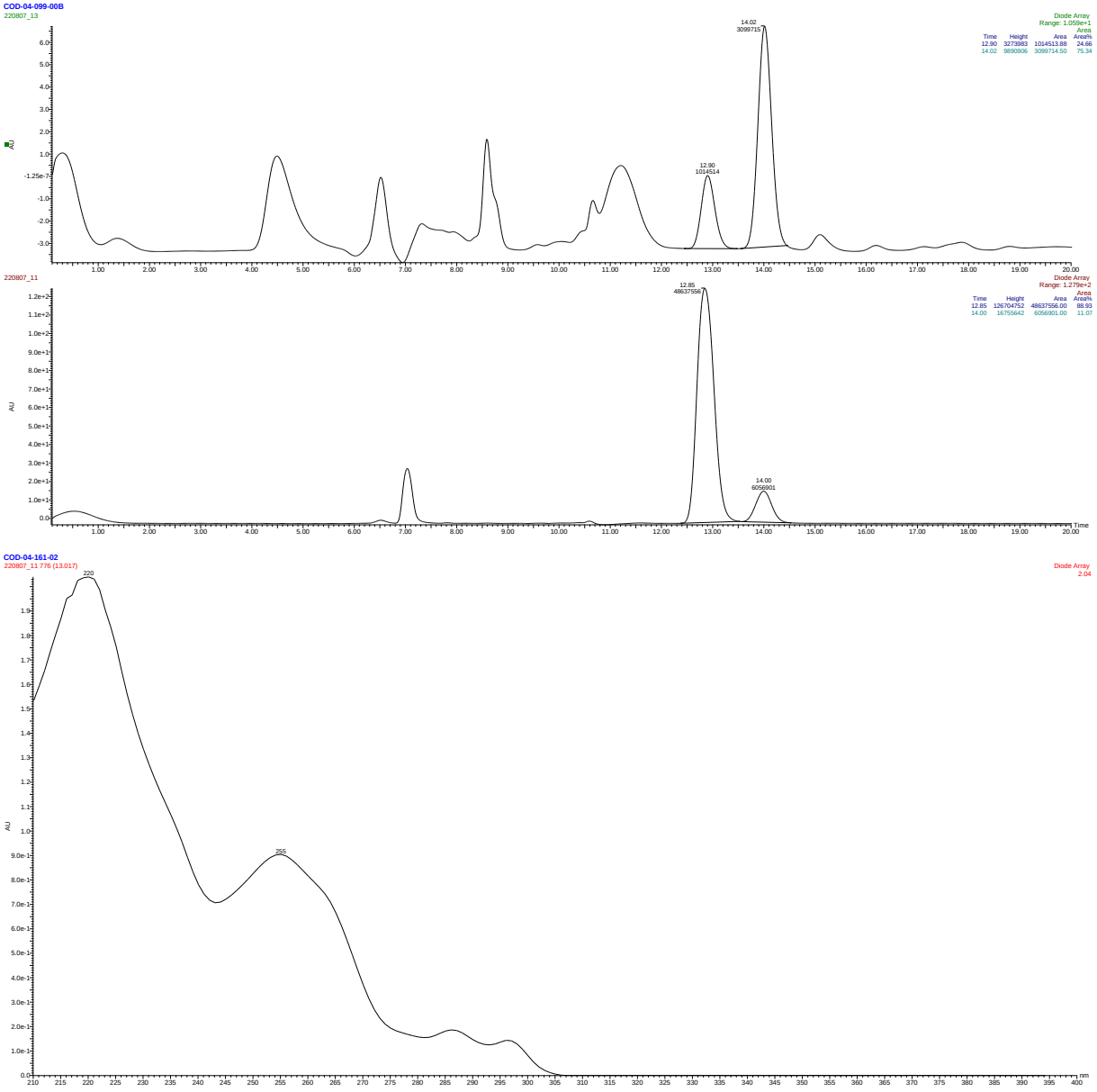
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of 3-187





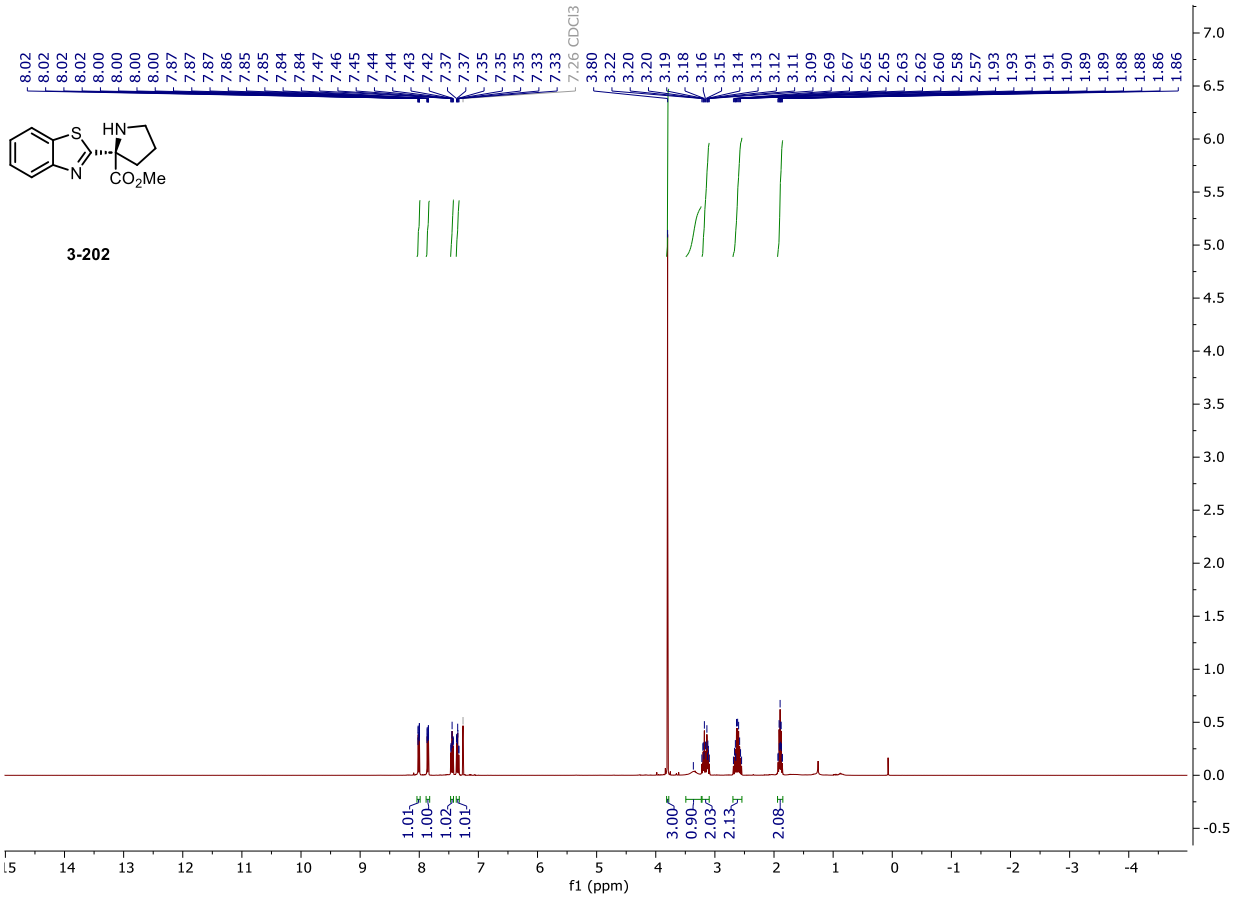
NMR and HPLC

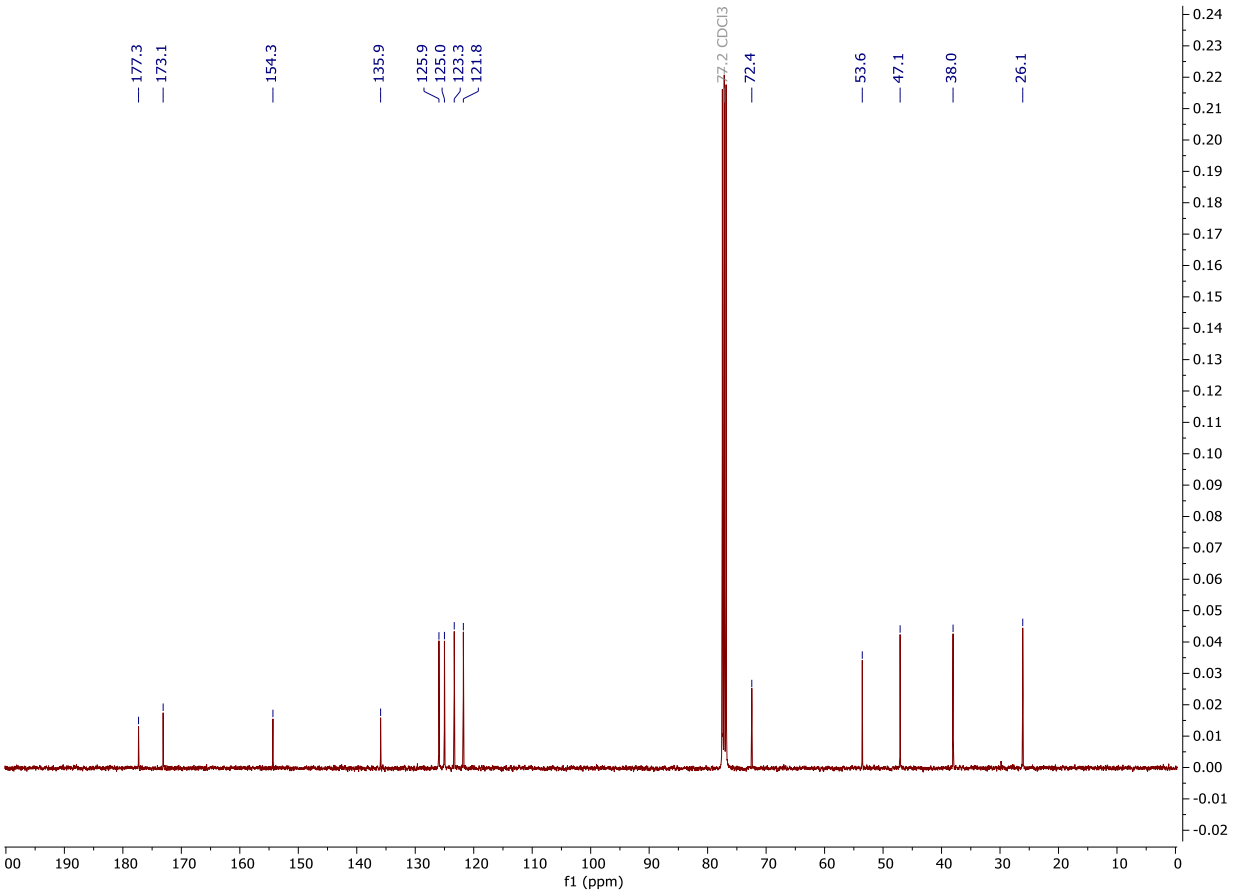
HPLC chromatograms of **3-187**



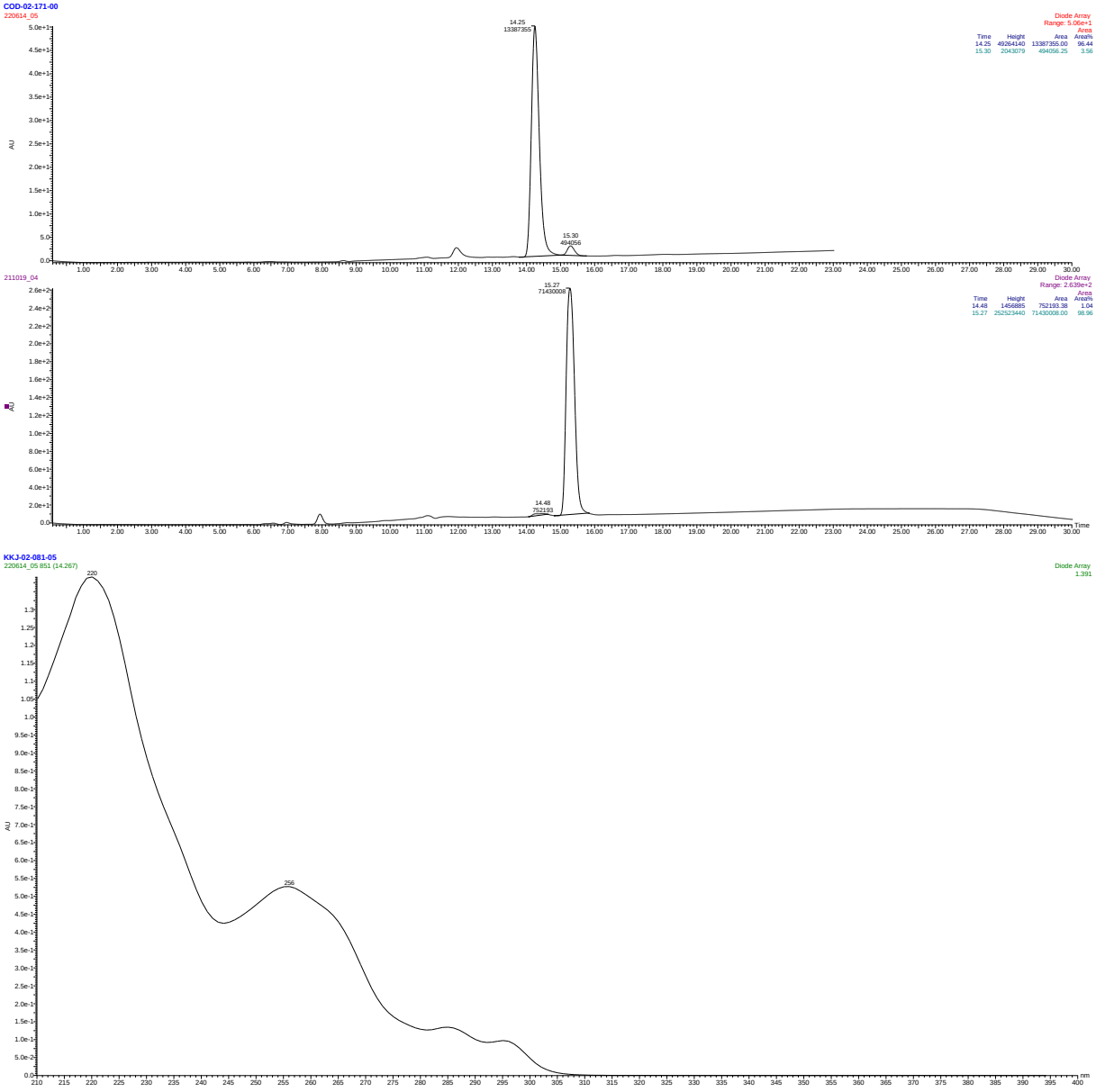
NMR and HPLC

Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of 3-202



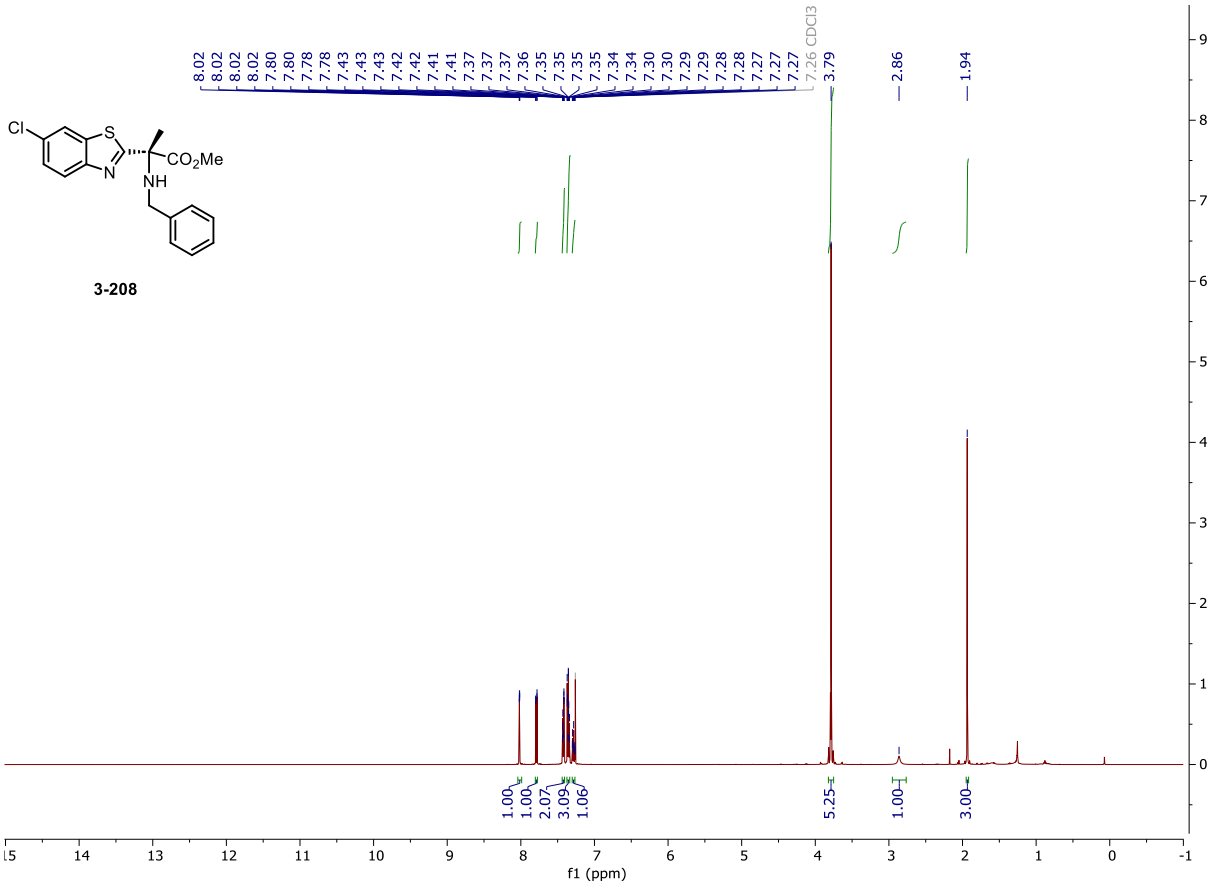


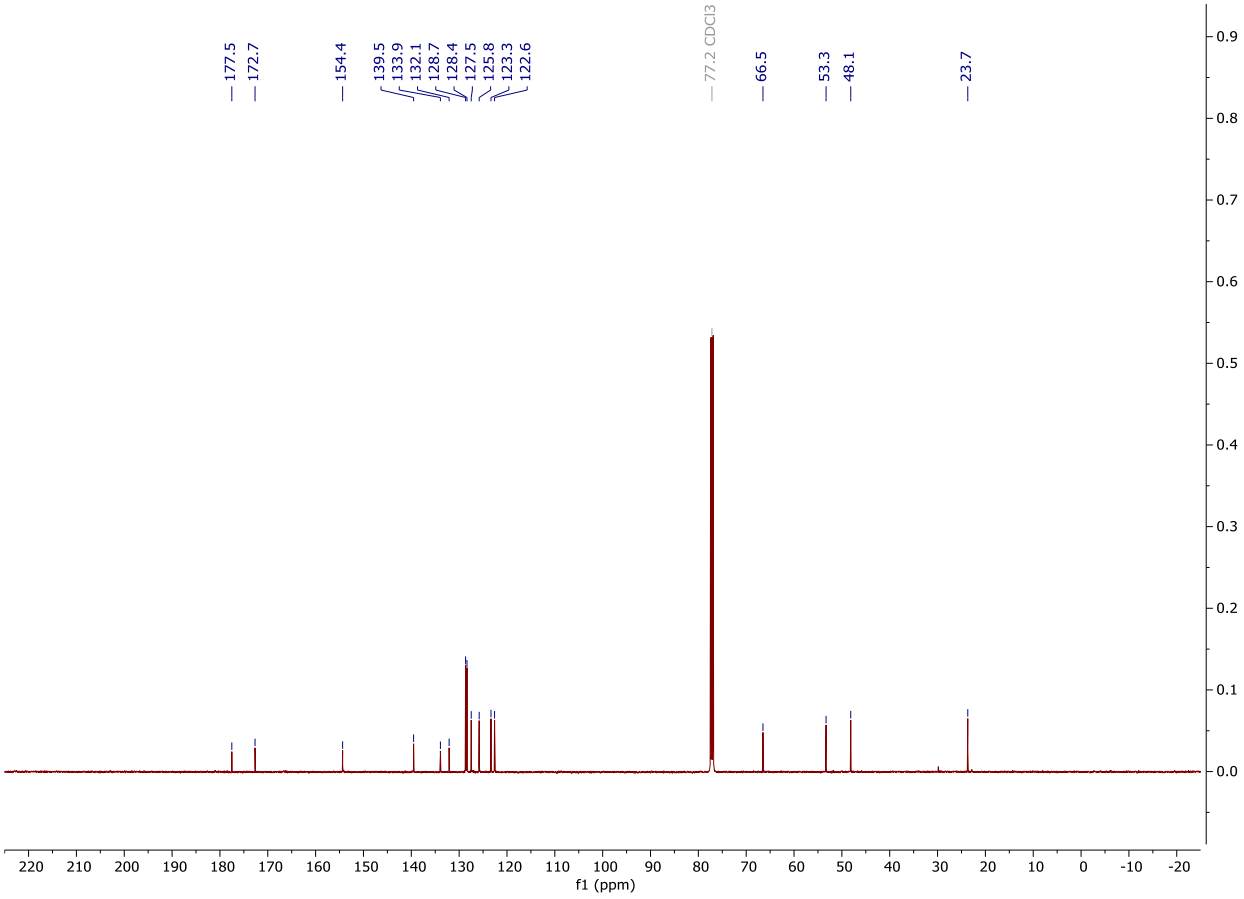
HPLC chromatograms of **3-202**



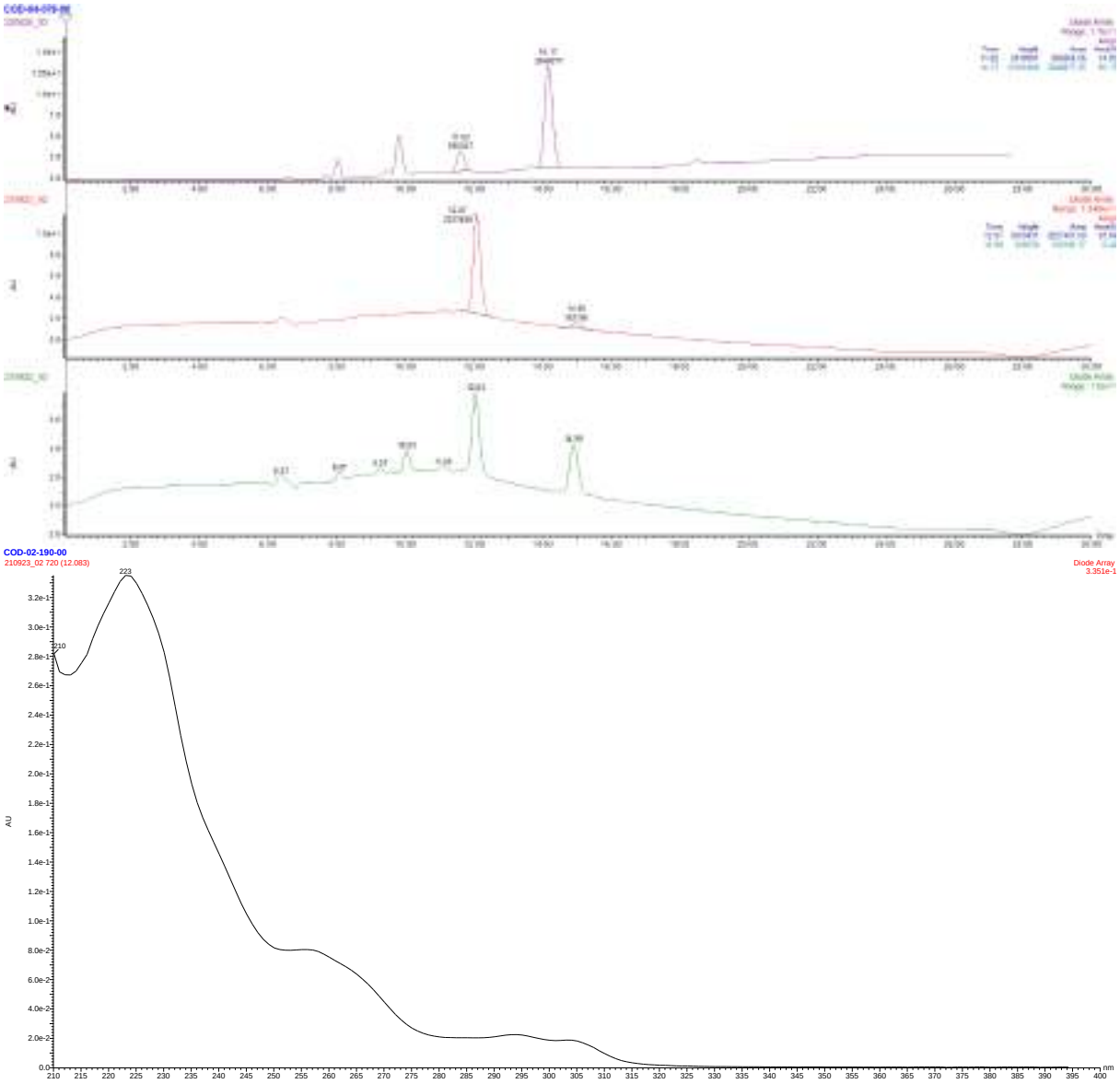
NMR and HPLC

Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of 3-208

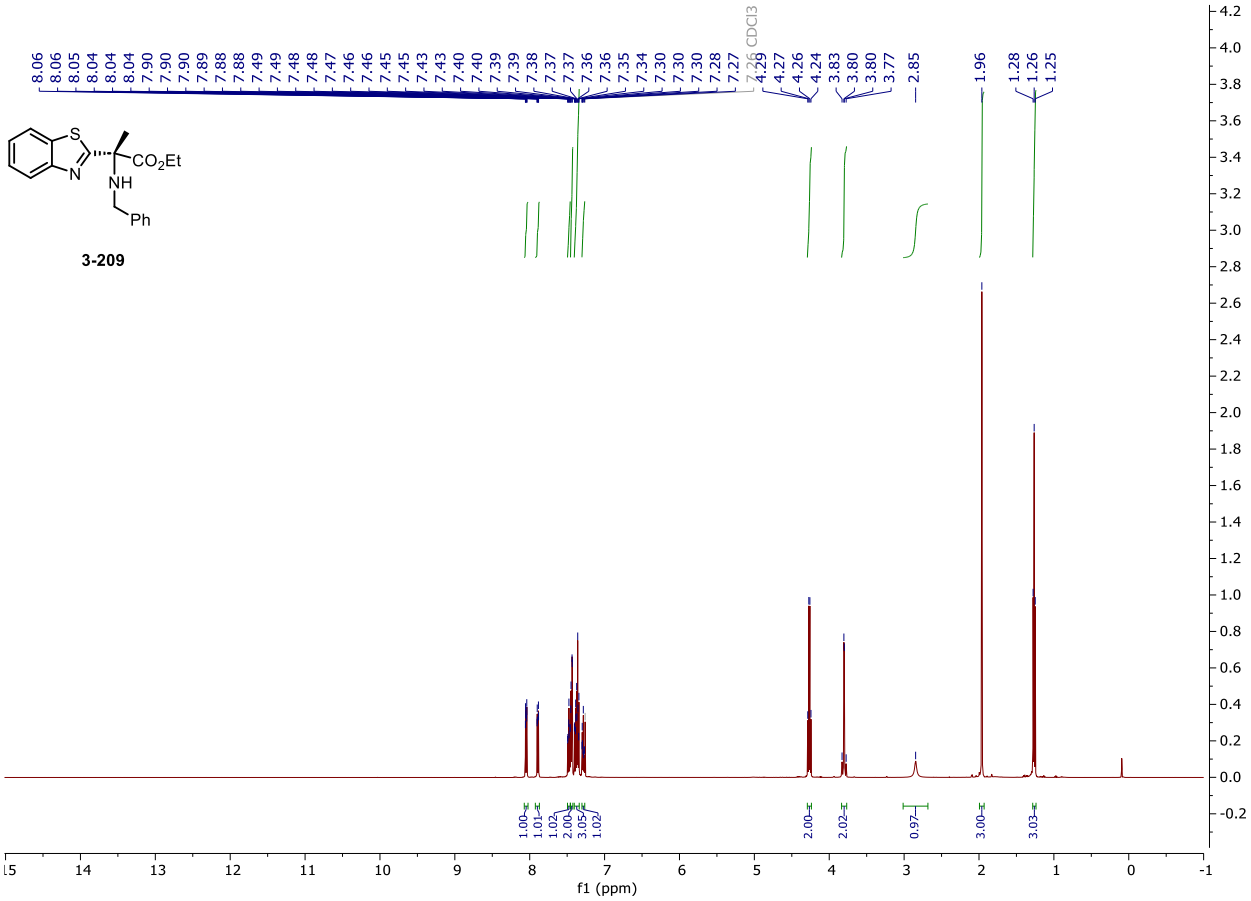


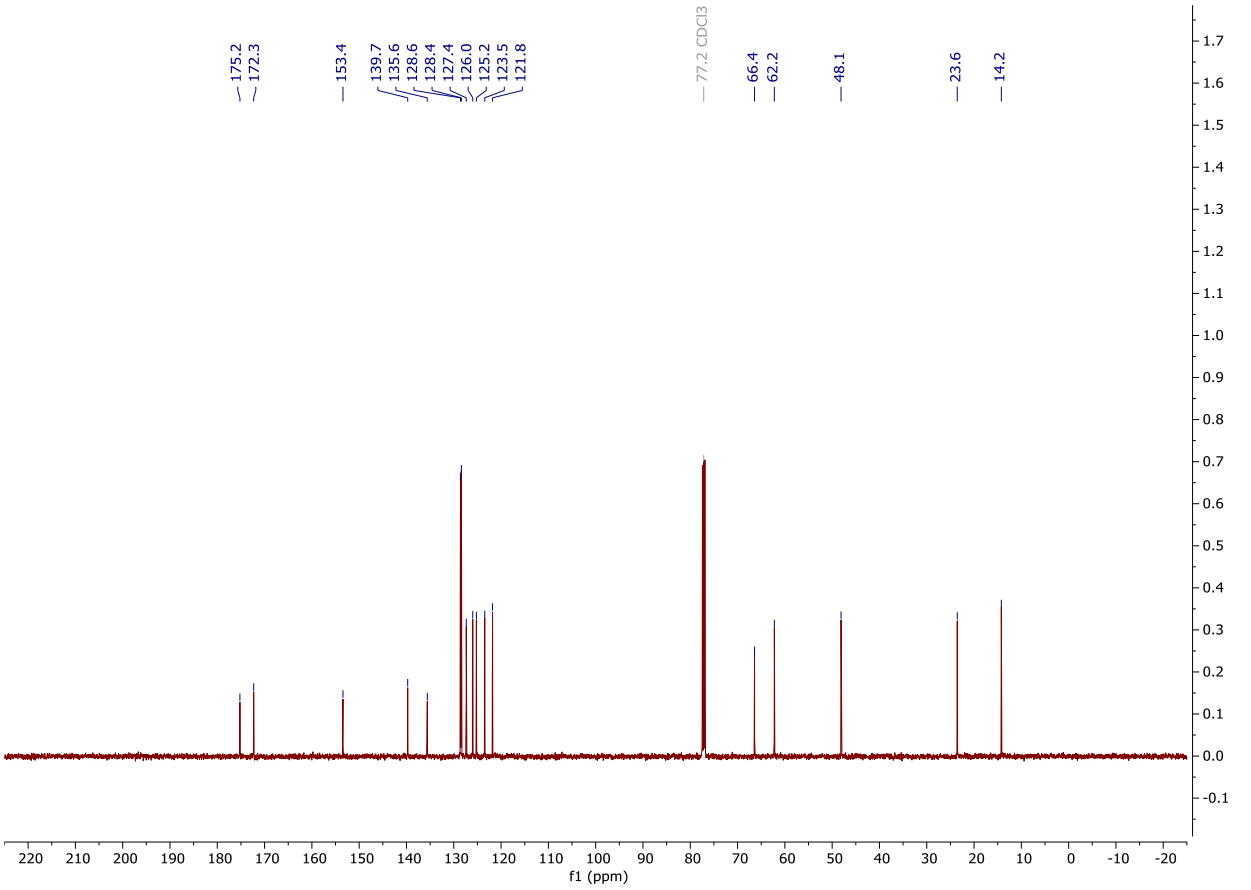


HPLC chromatograms of 3-208

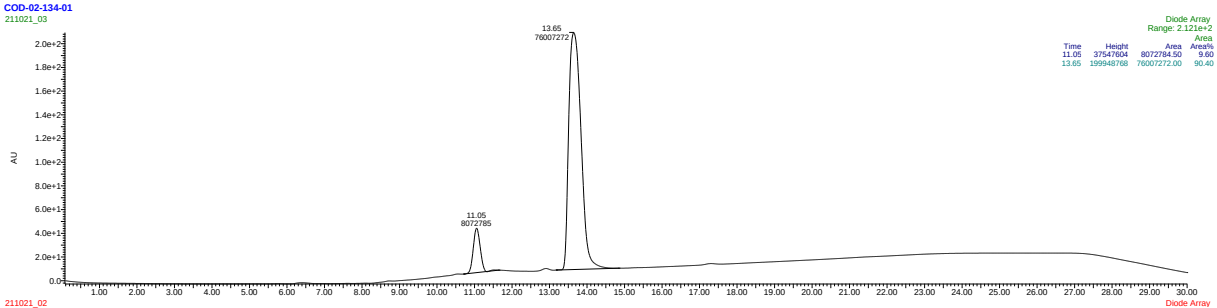


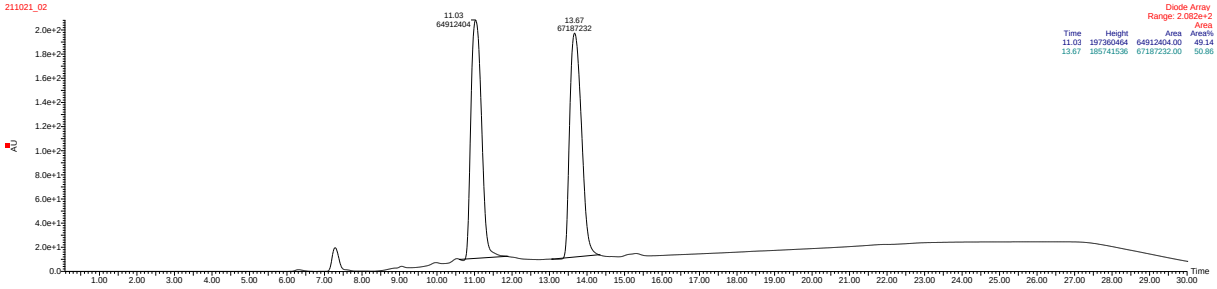
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-209**

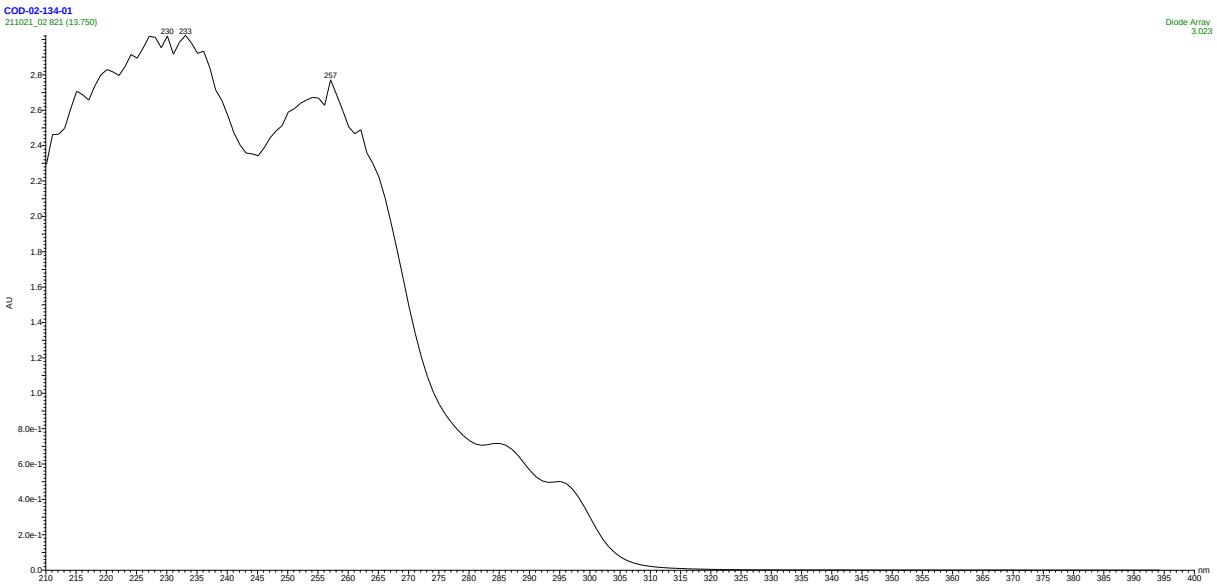




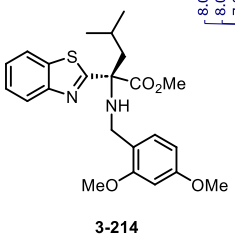
HPLC chromatograms of **3-209**



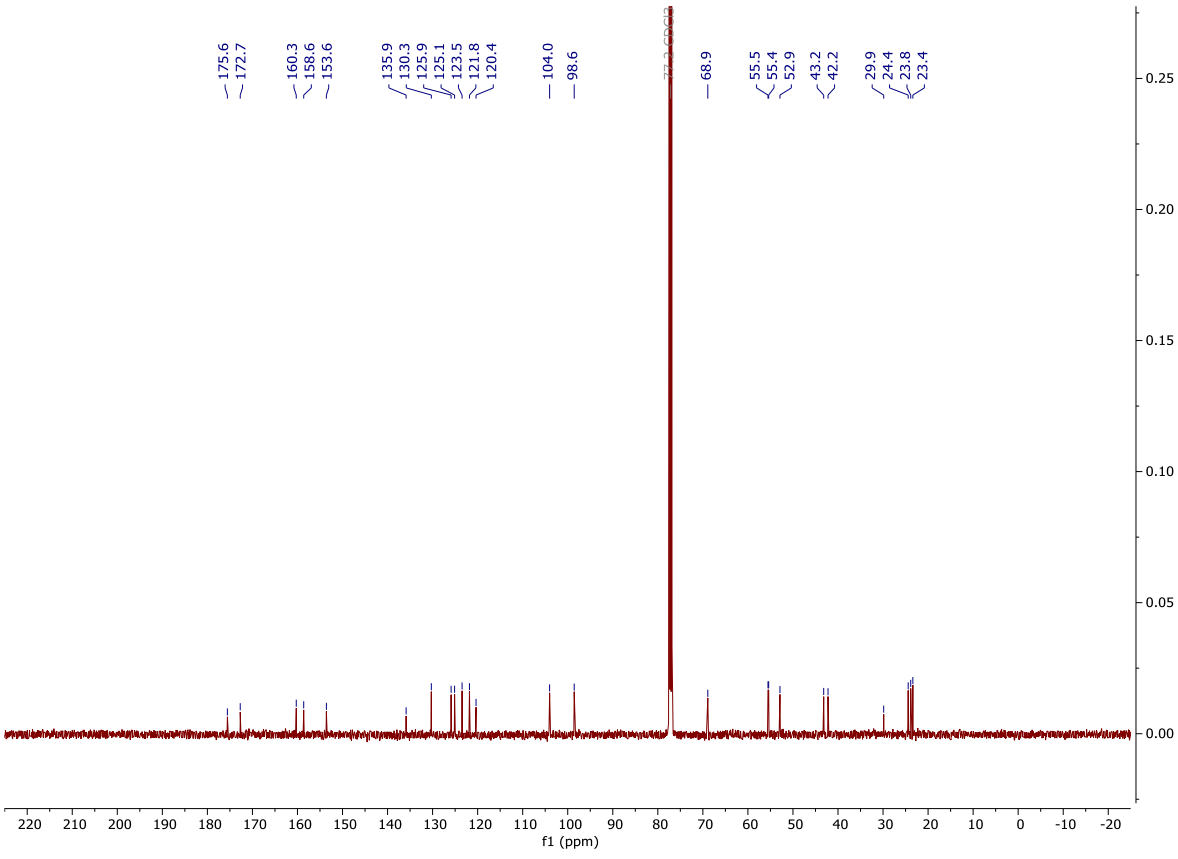




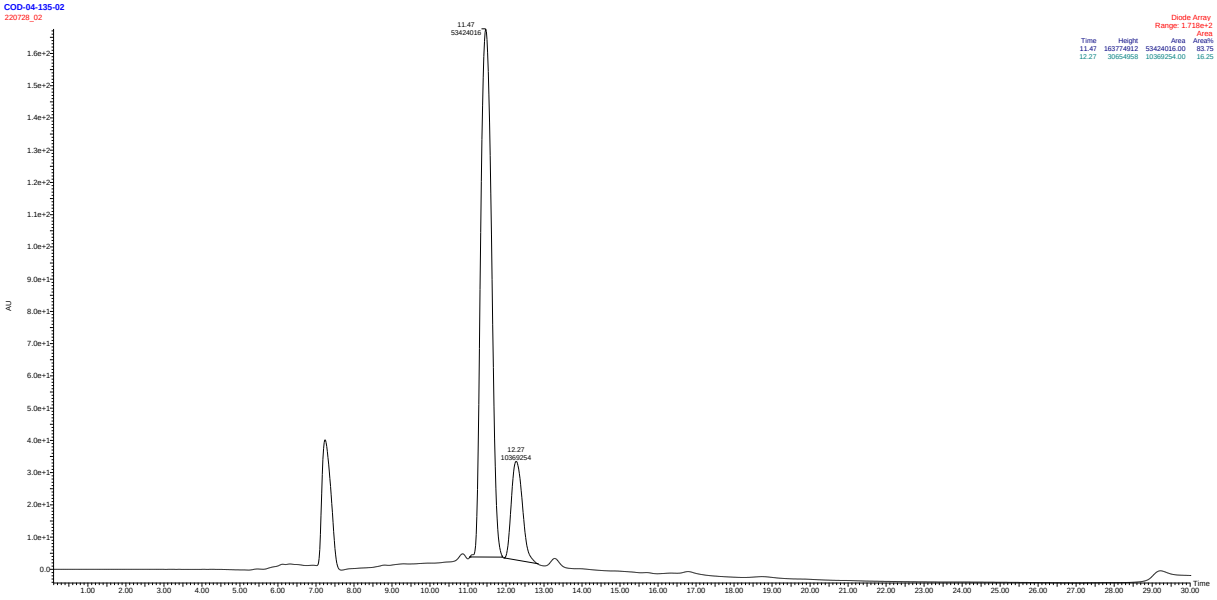
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-214**

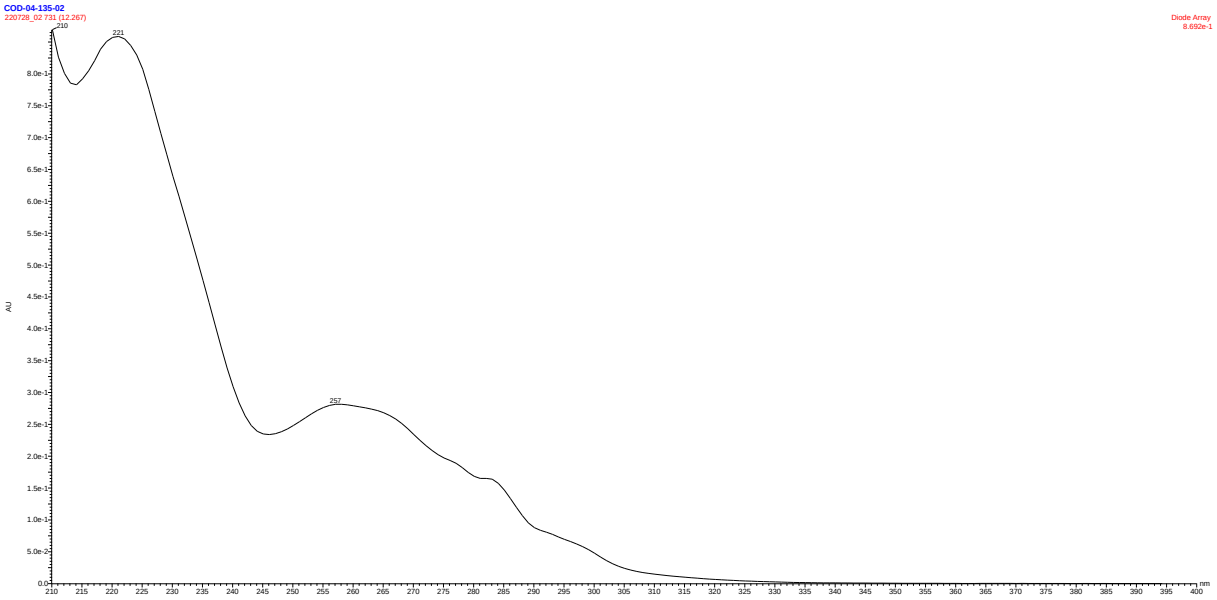


3-214

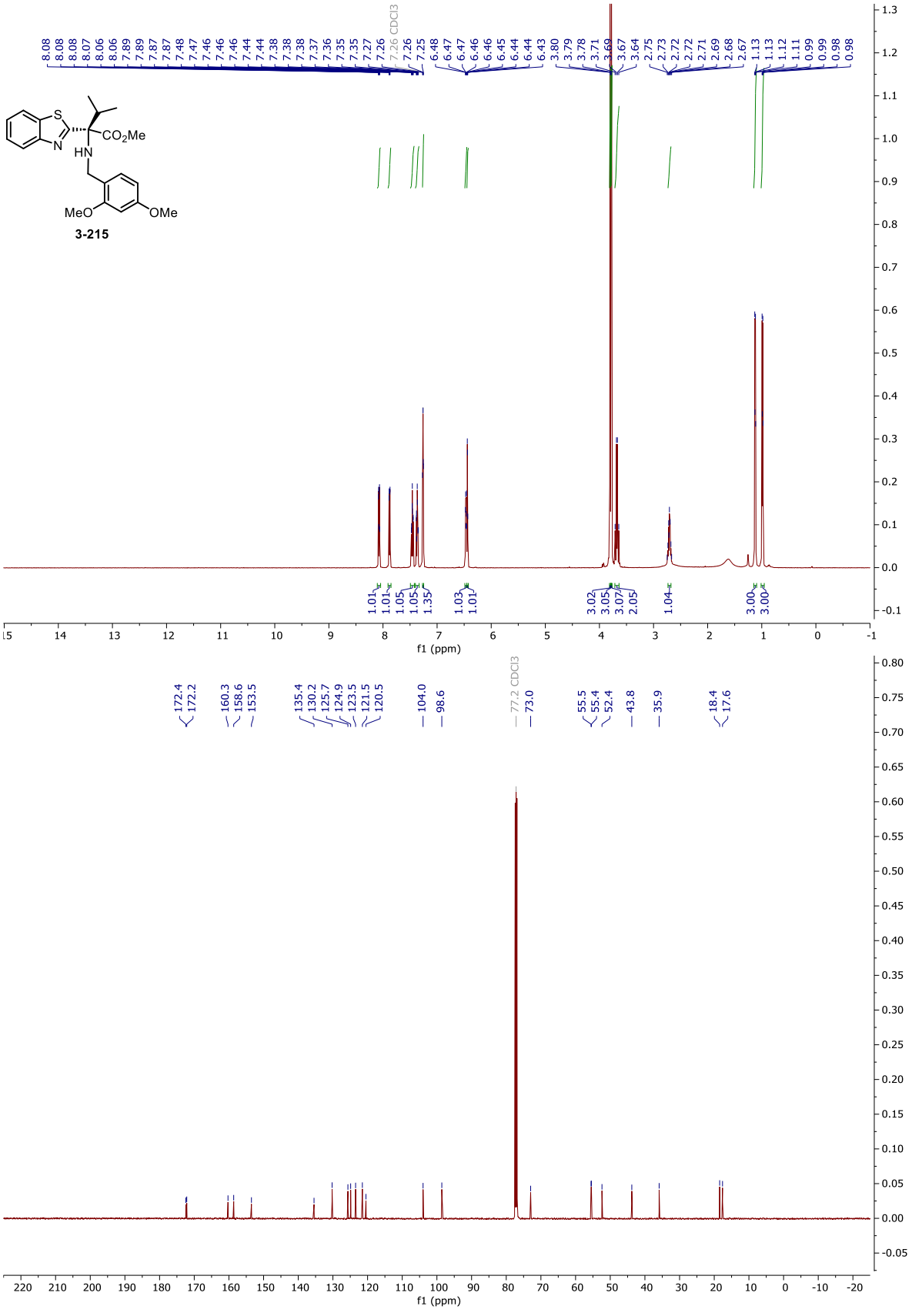


HPLC chromatograms of **3-214**



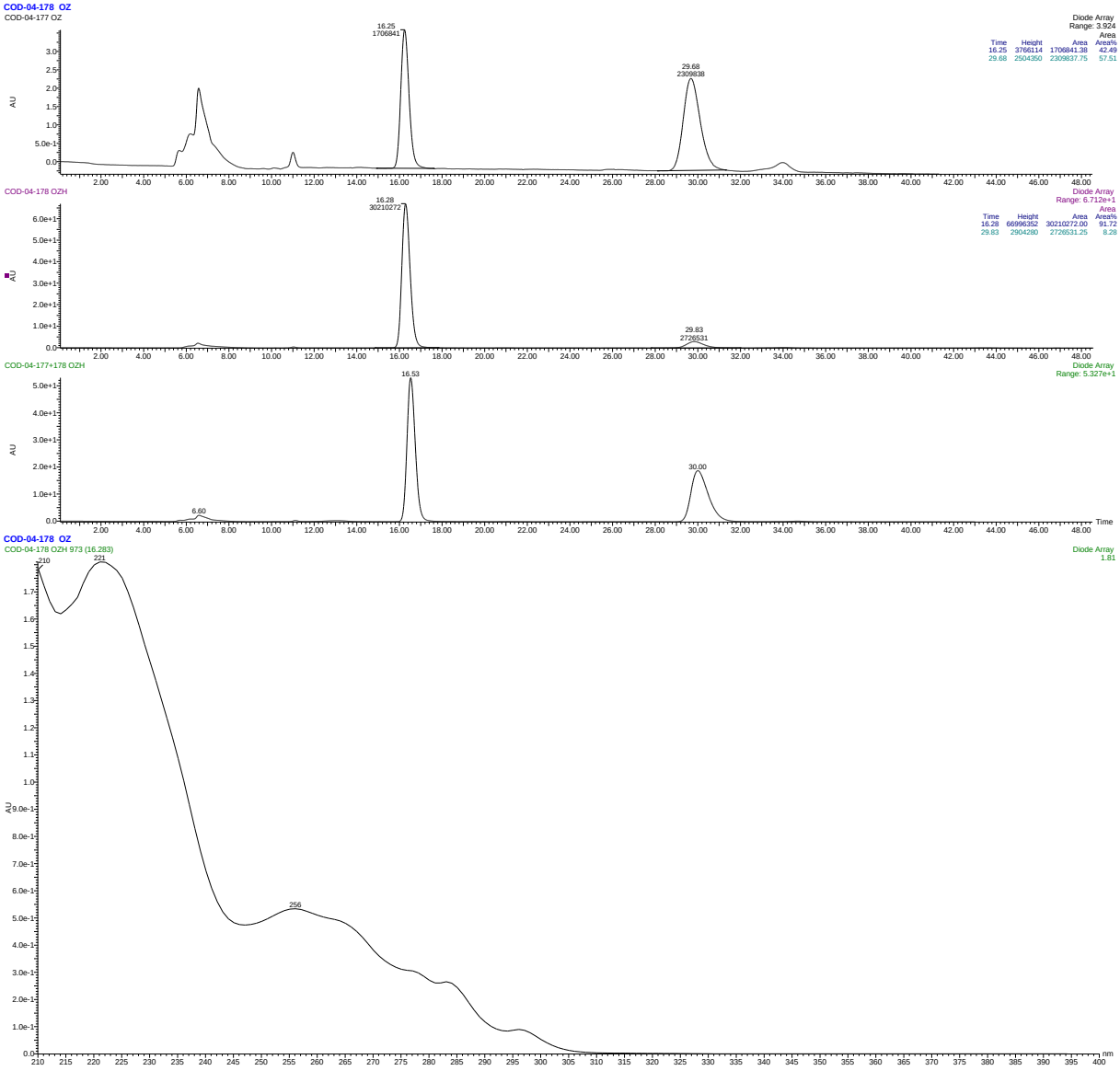


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-215**

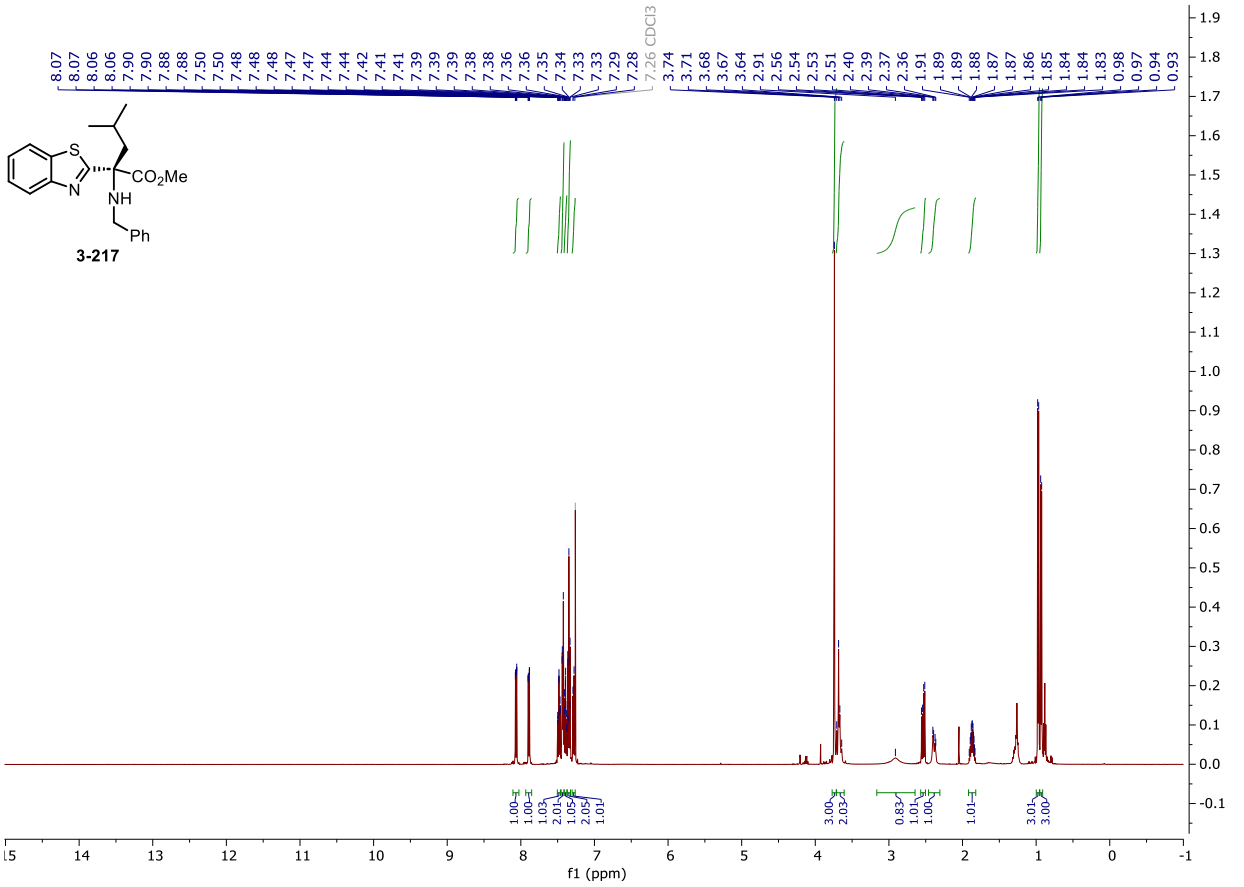


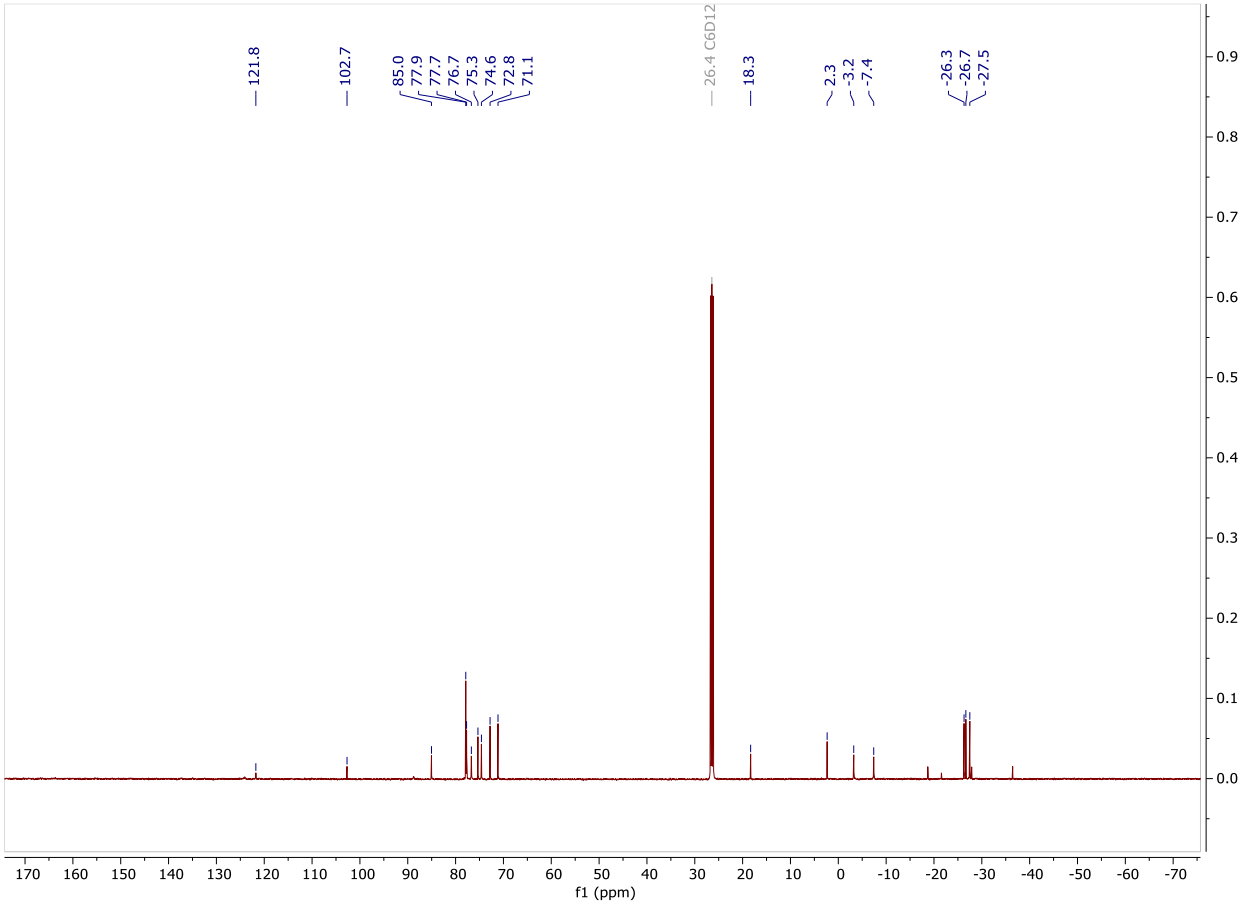
NMR and HPLC

HPLC chromatograms of **3-215**

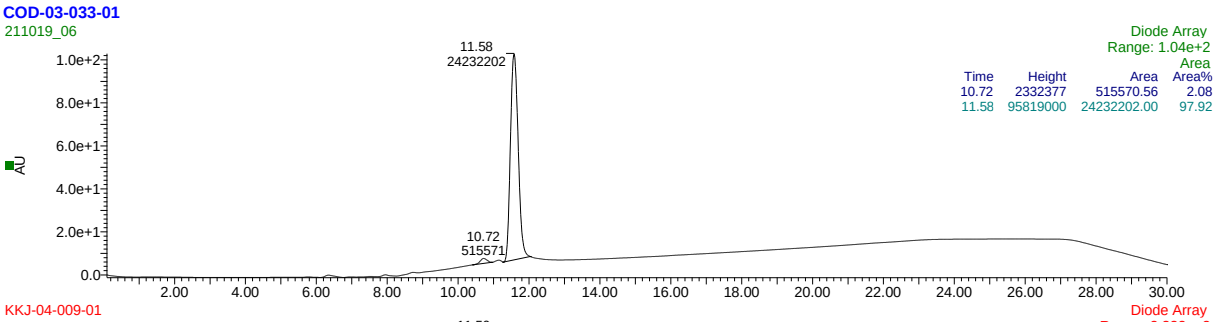


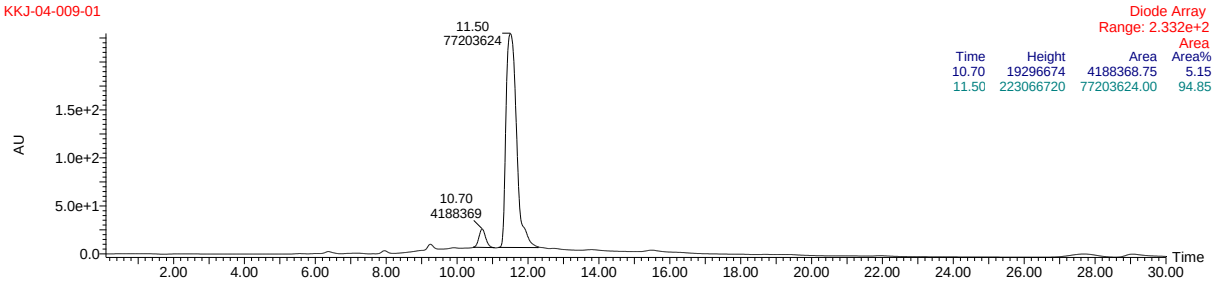
Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-217**

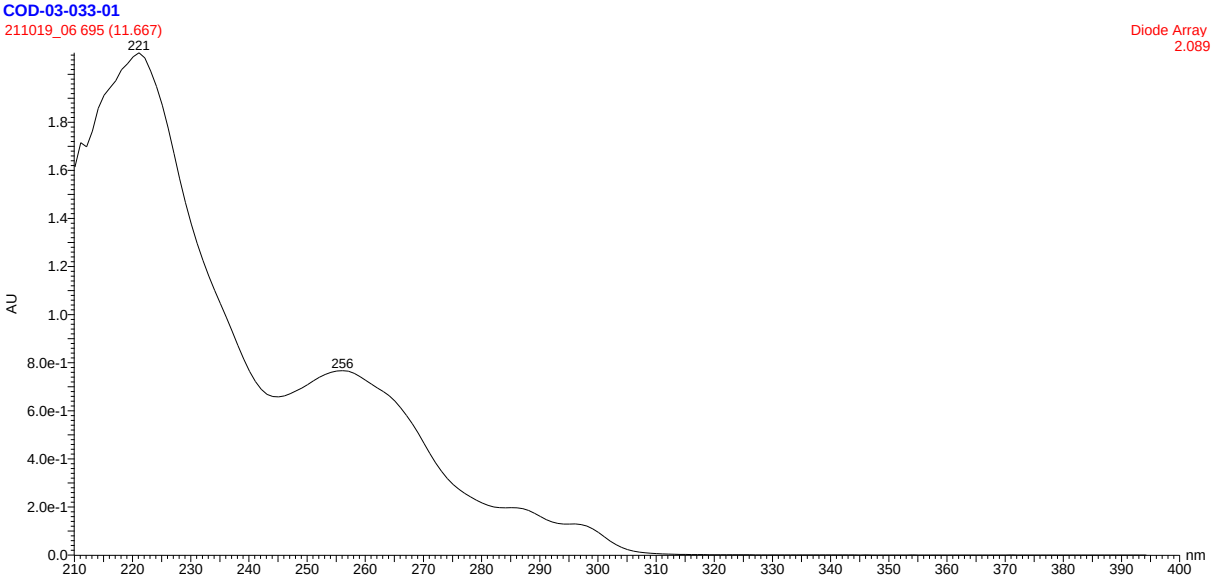




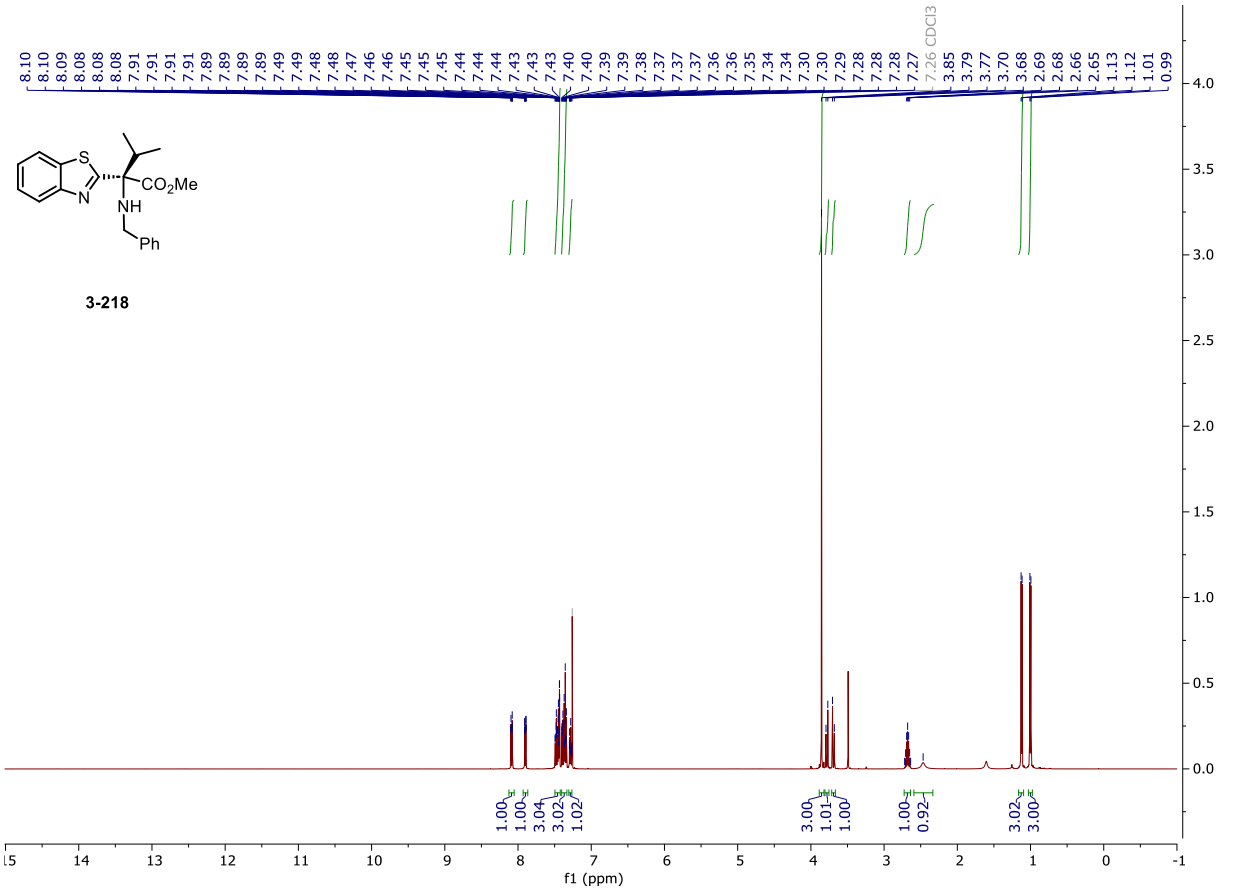
HPLC chromatograms of **3-217**

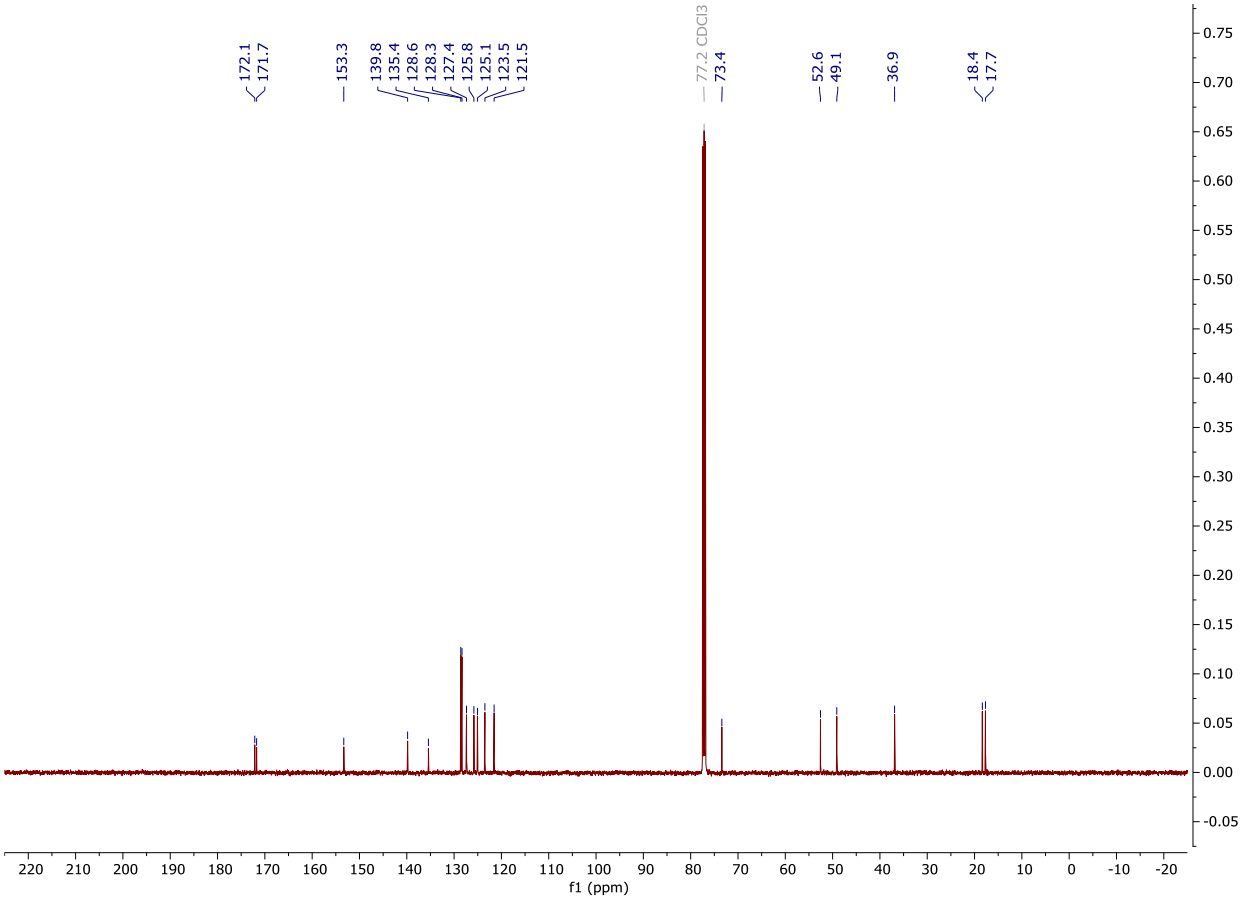




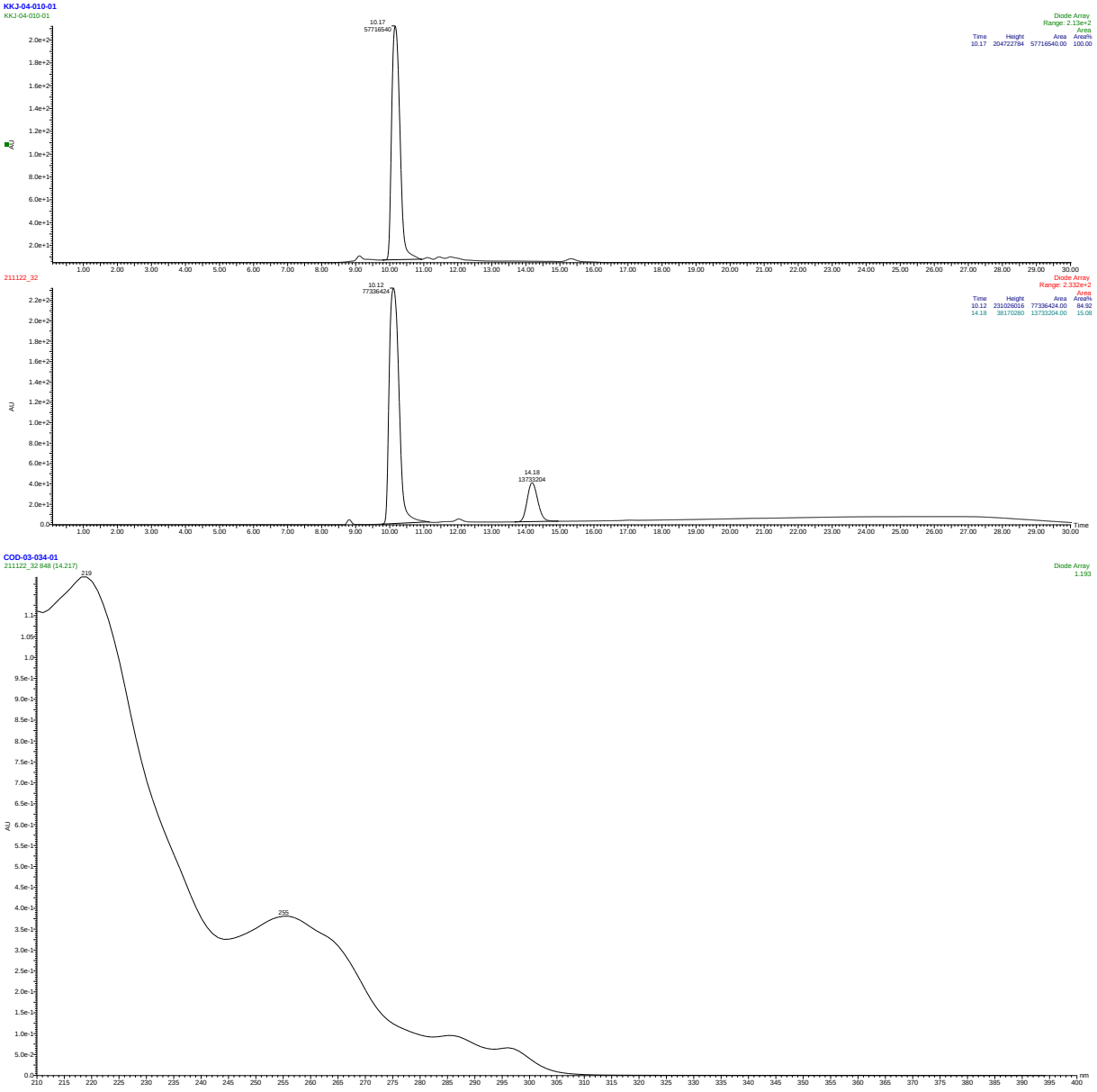


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-218**





HPLC chromatograms of **3-218**



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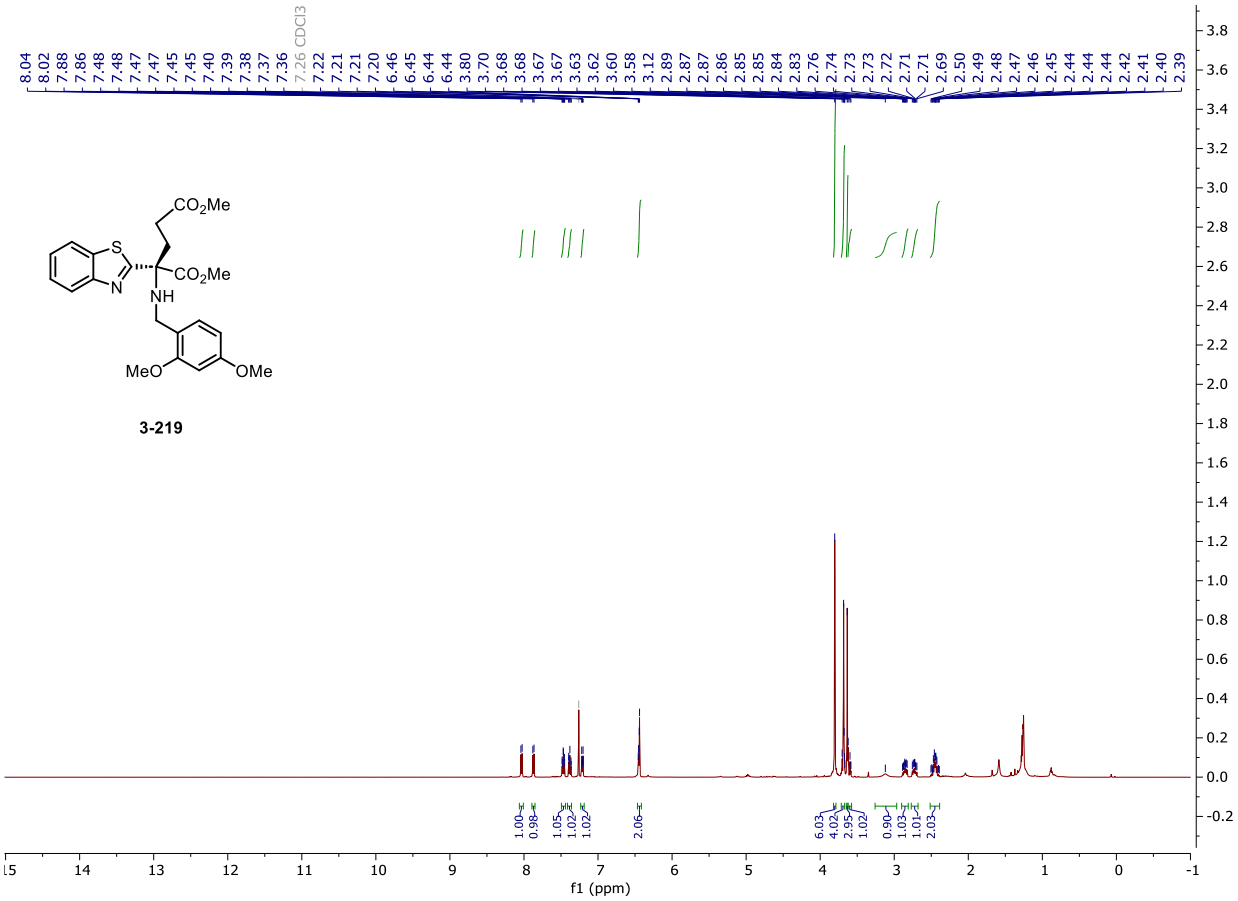

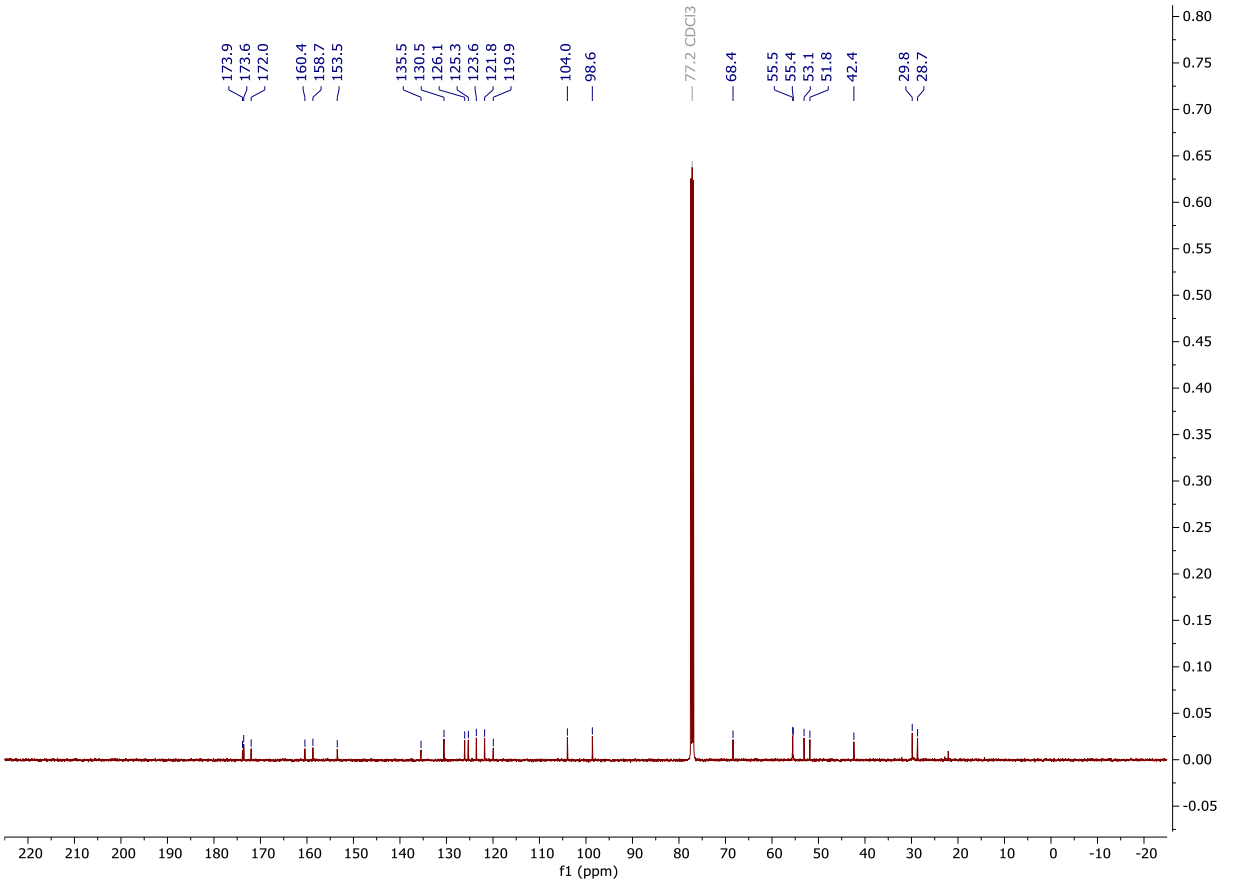

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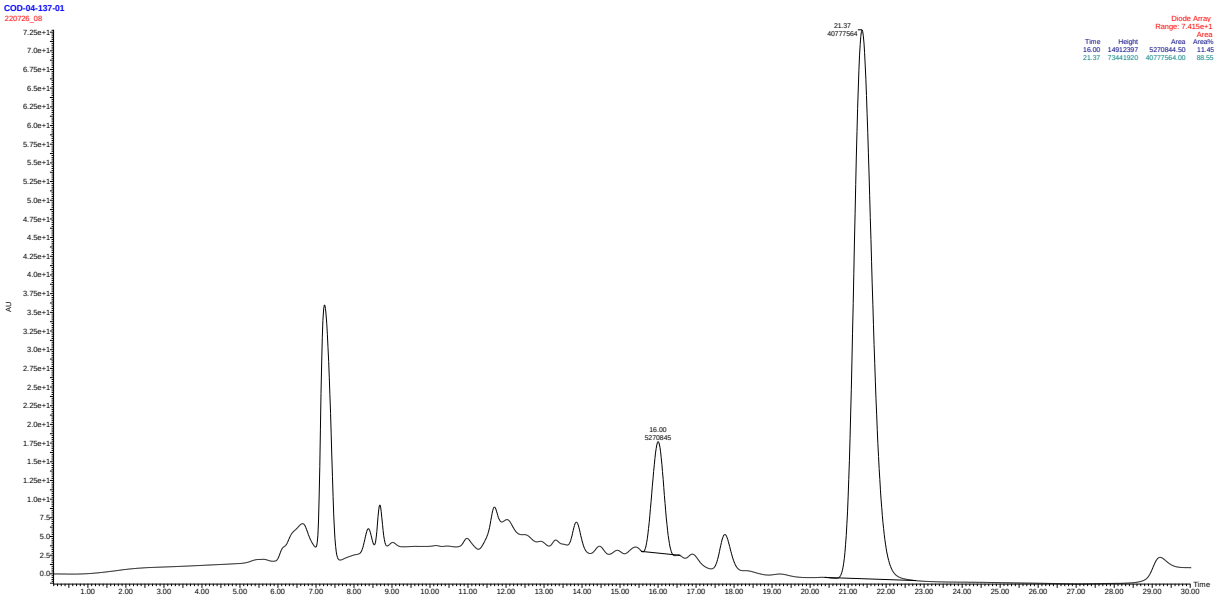

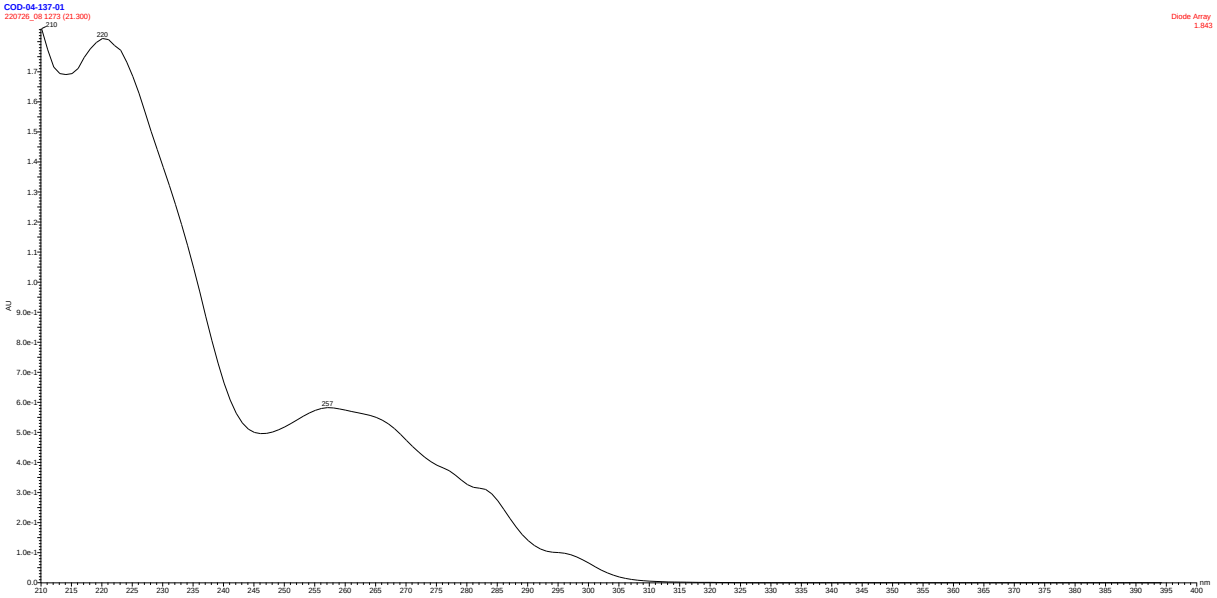

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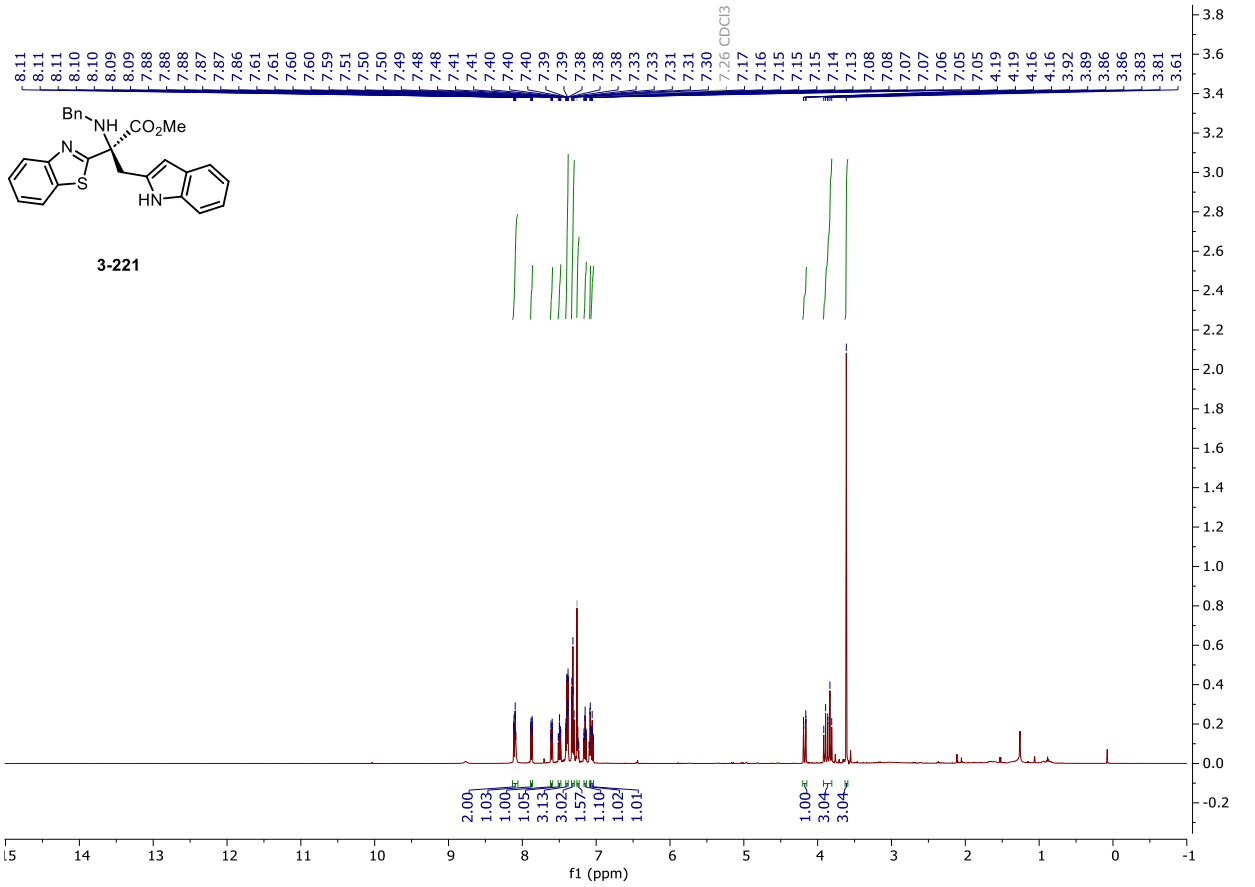

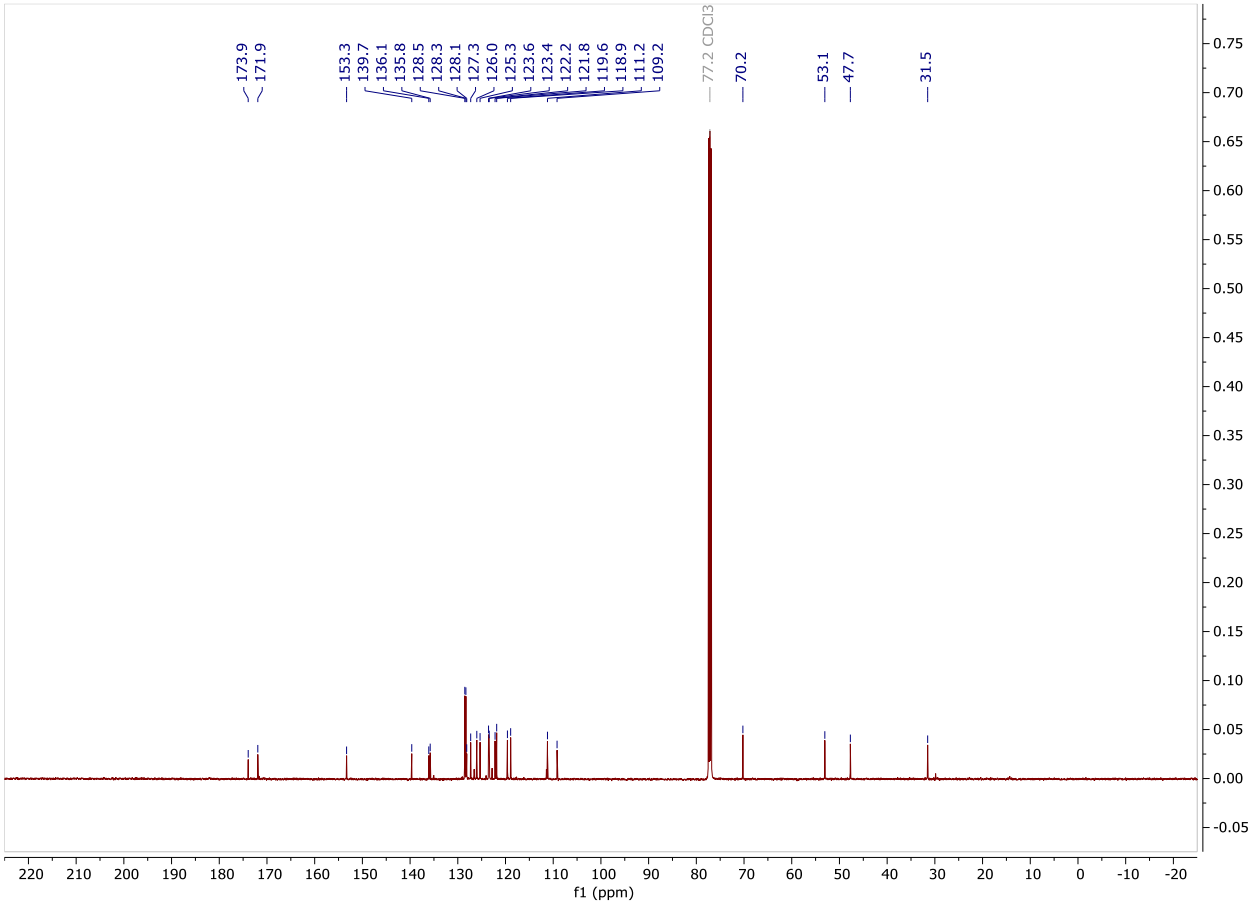

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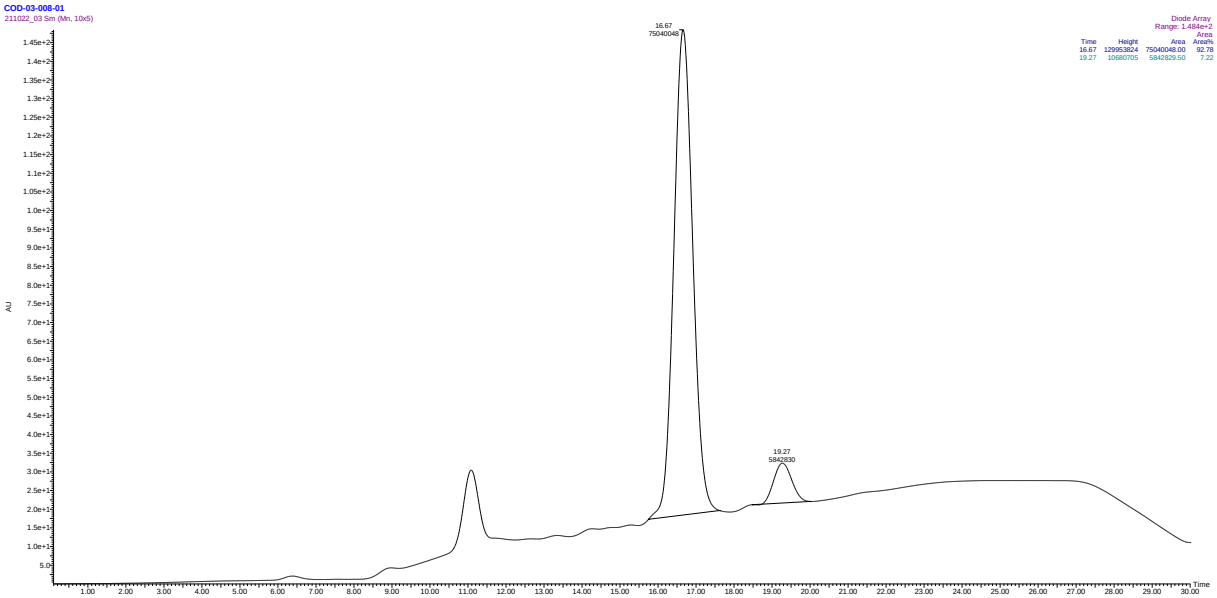
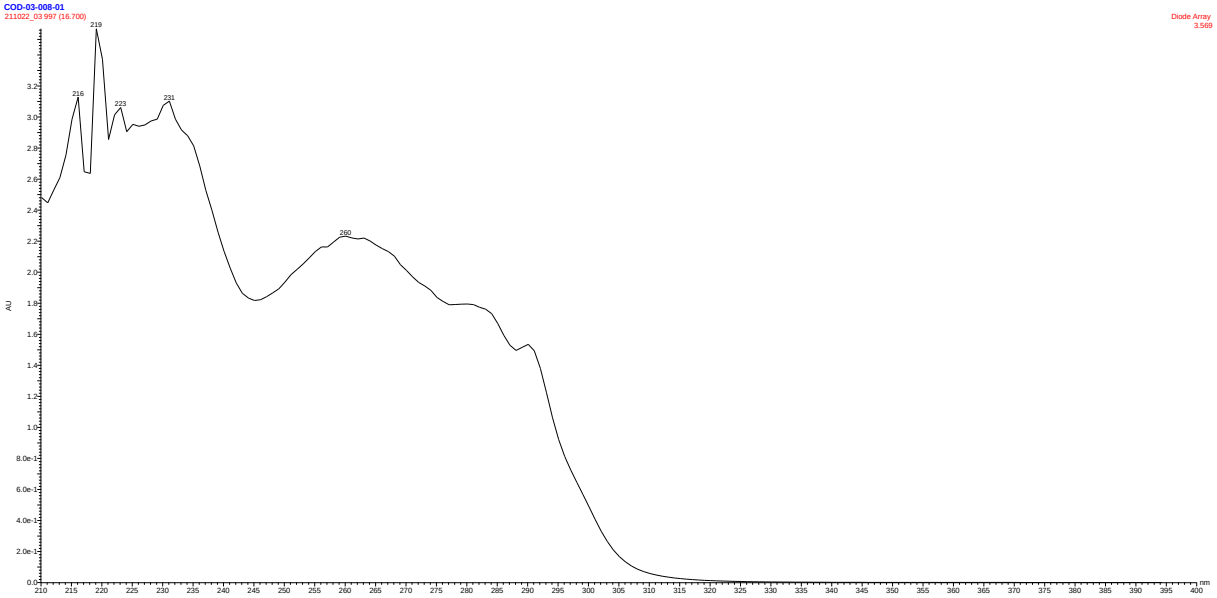
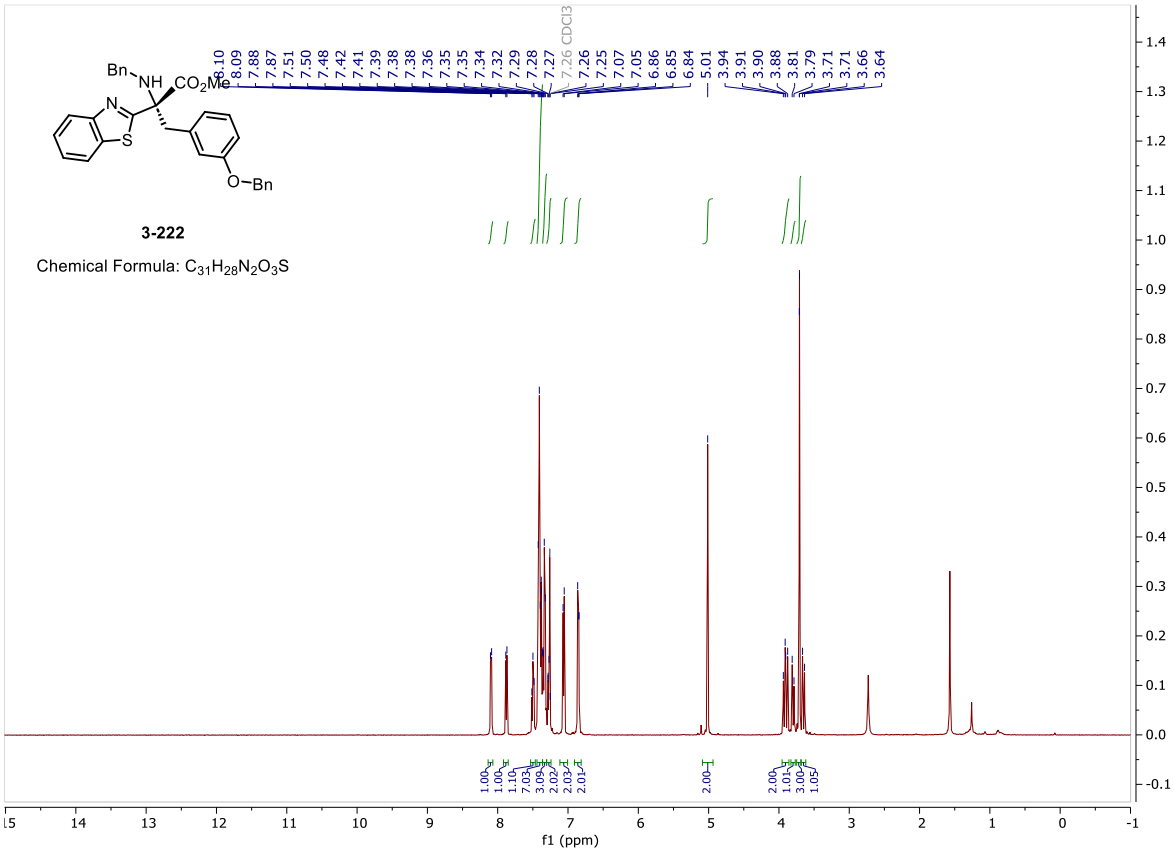
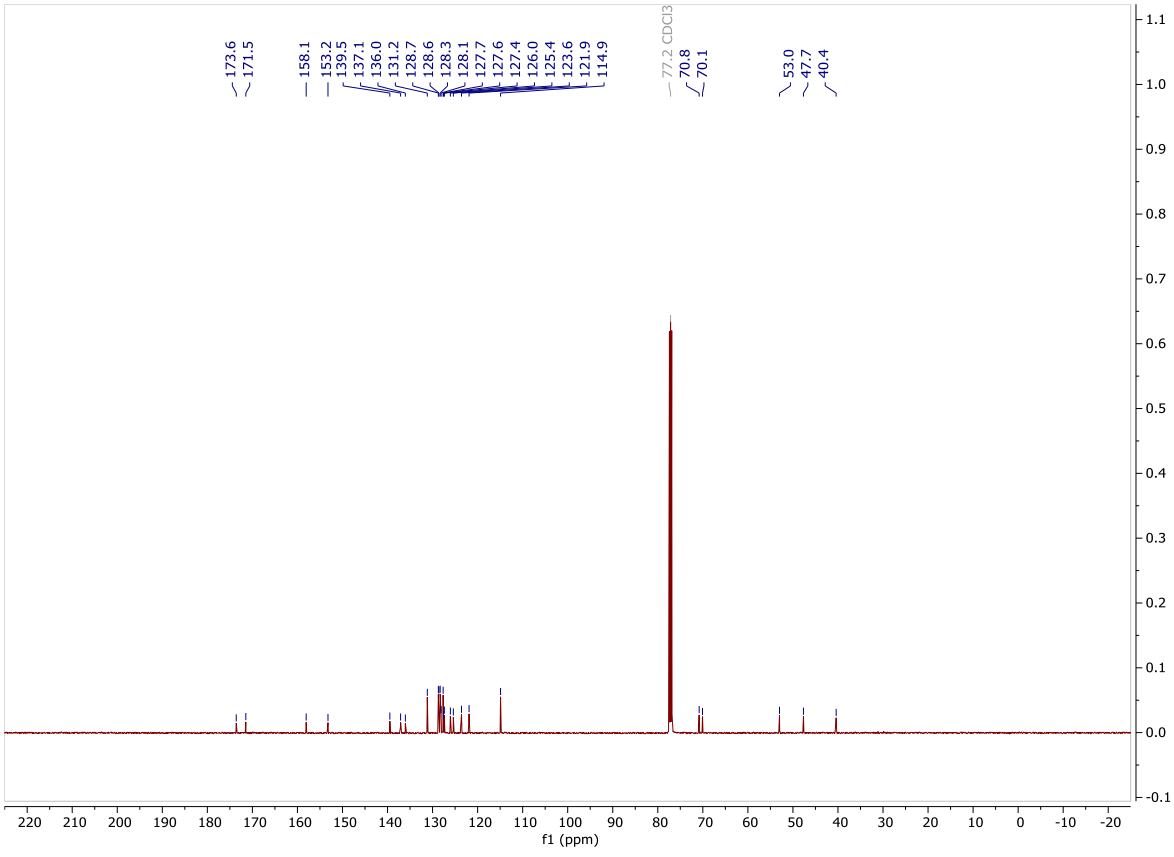




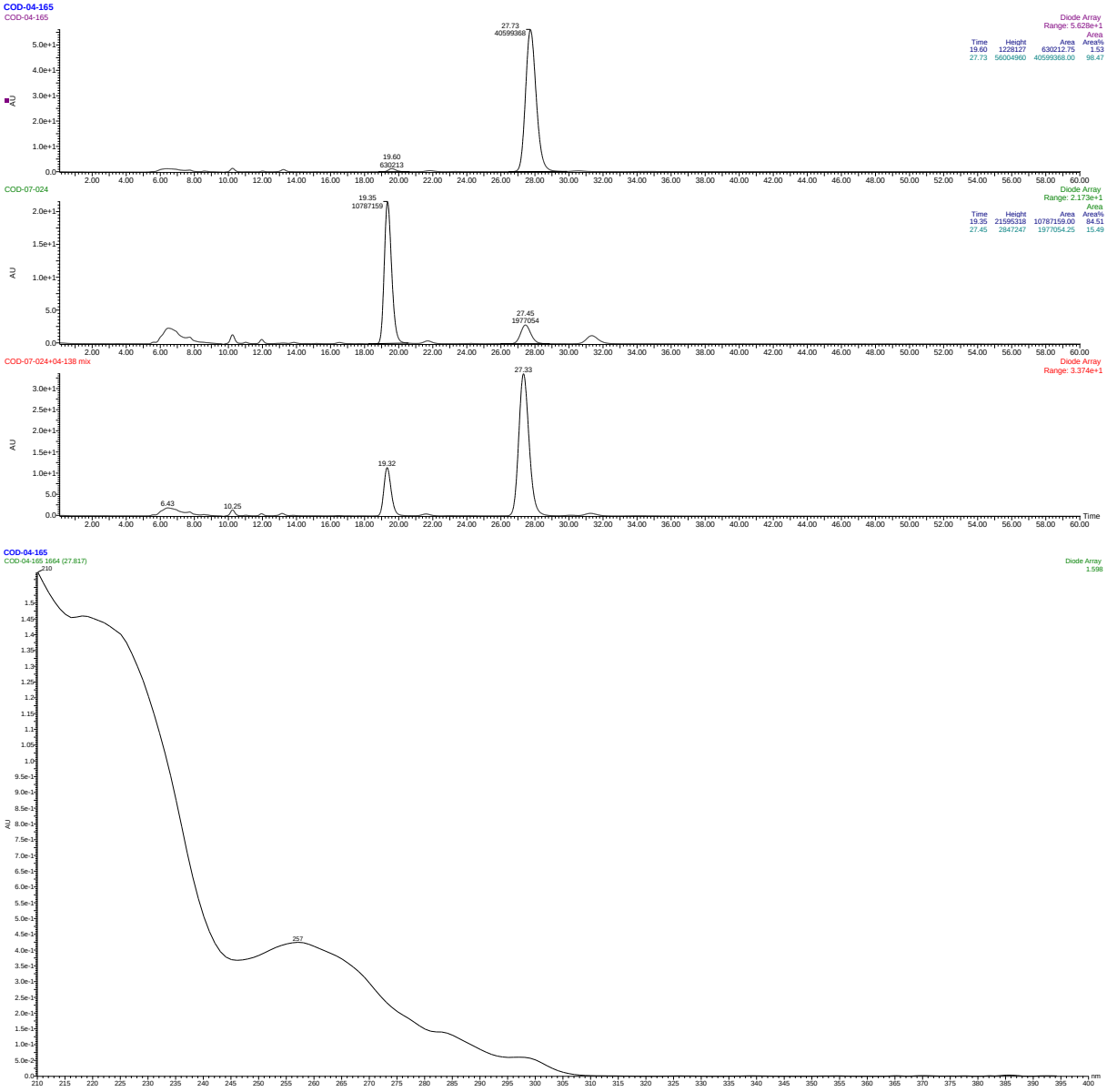
NMR and HPLC

Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-222**





HPLC chromatograms of **3-222**



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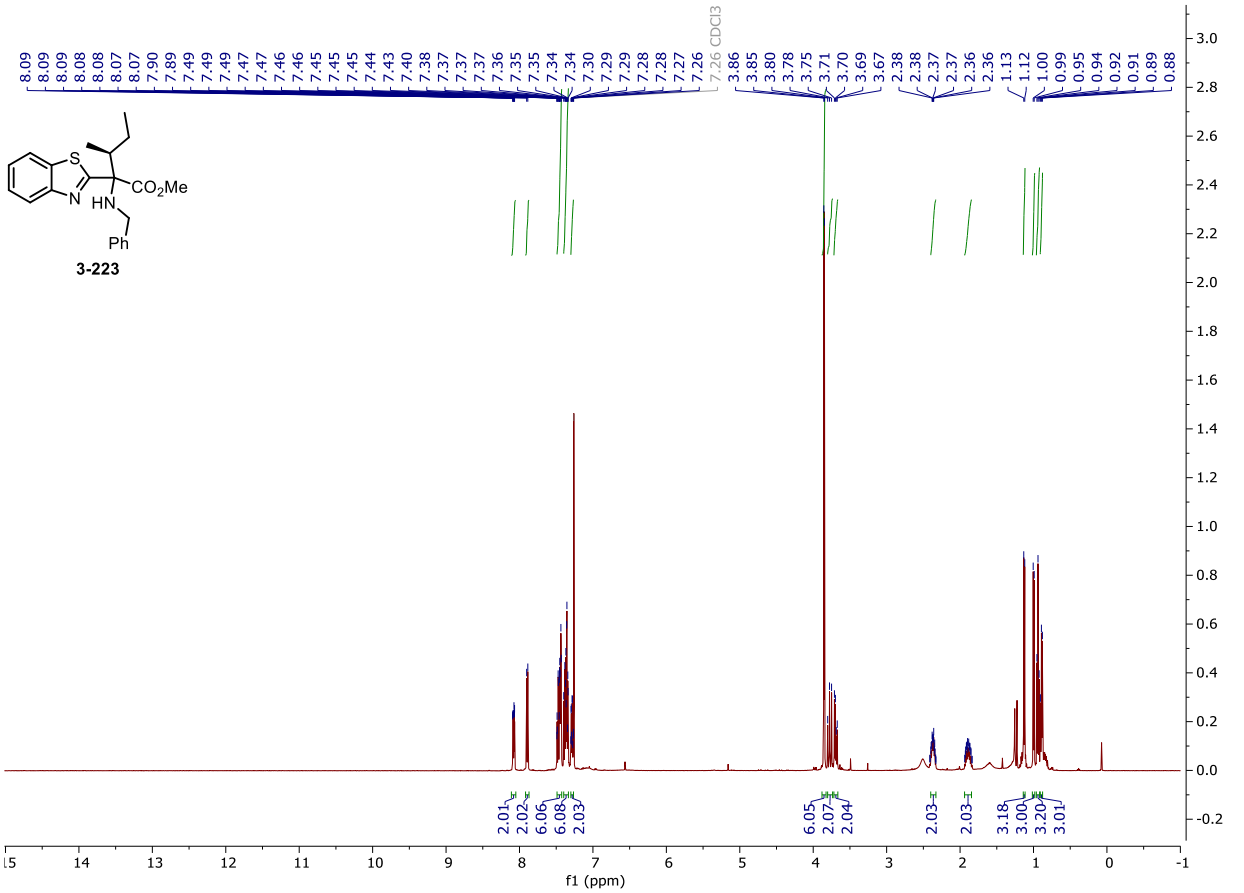
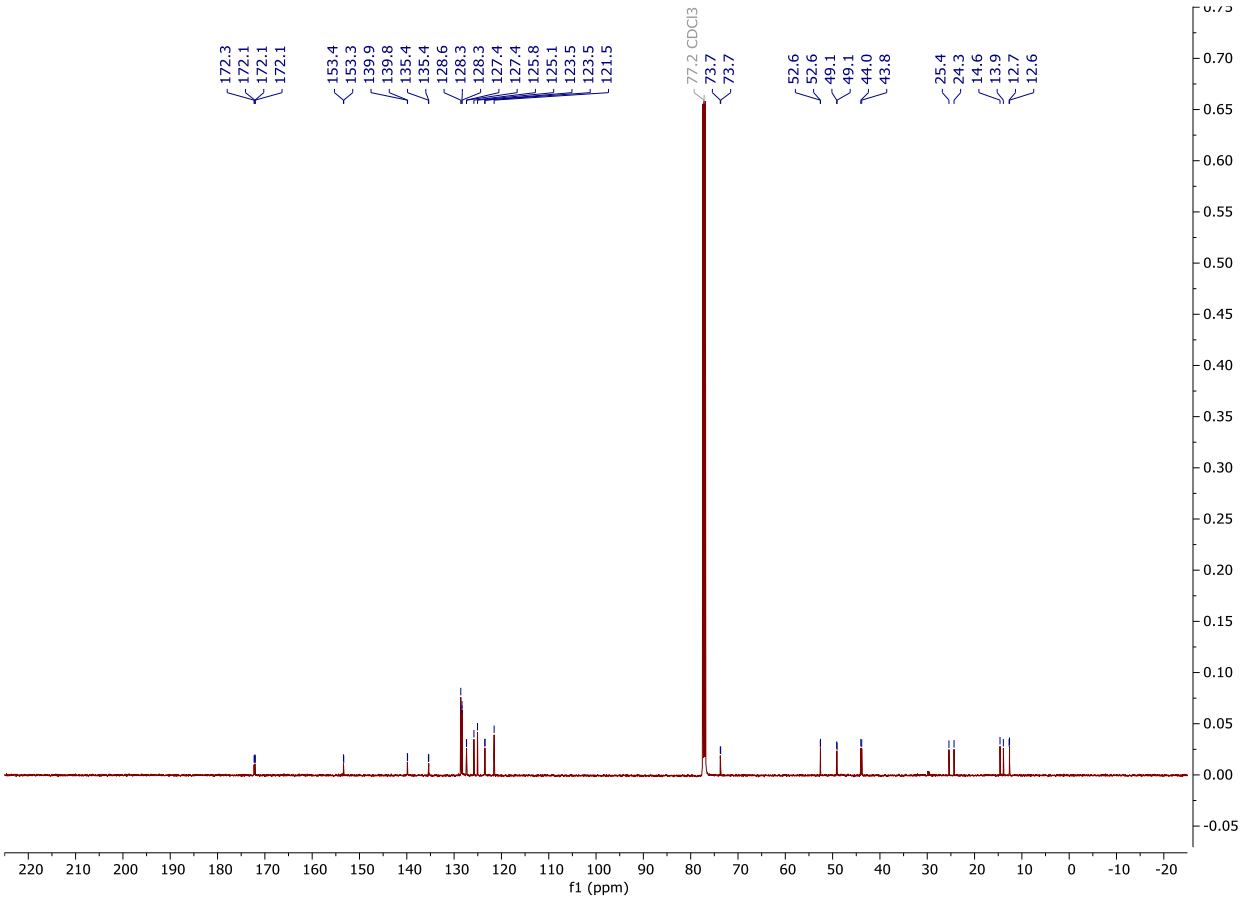
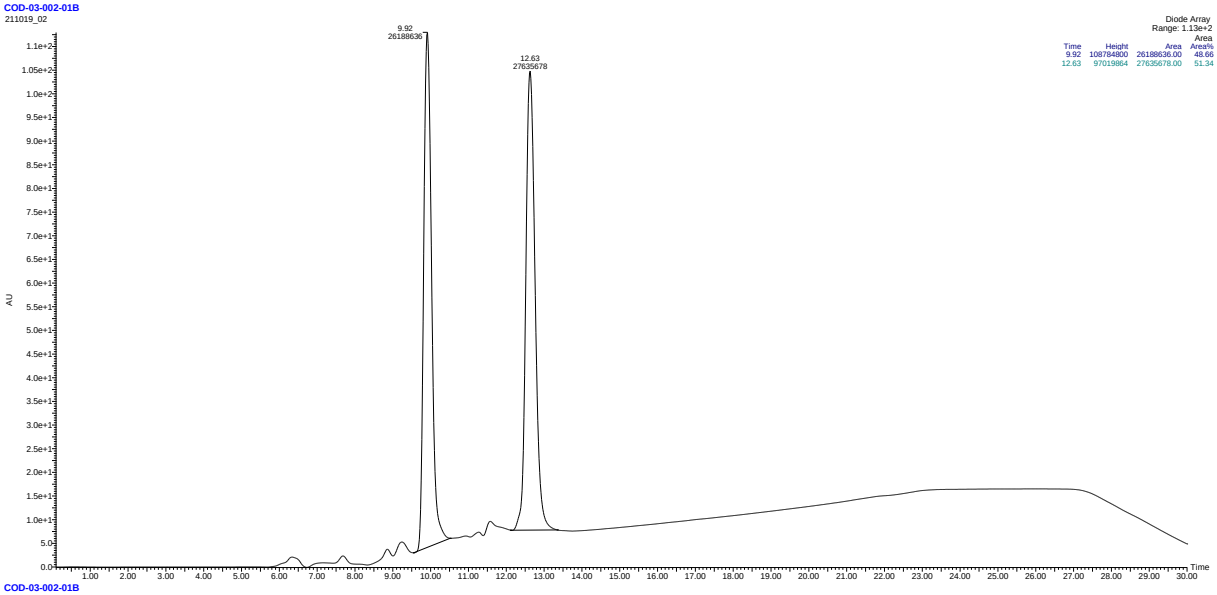
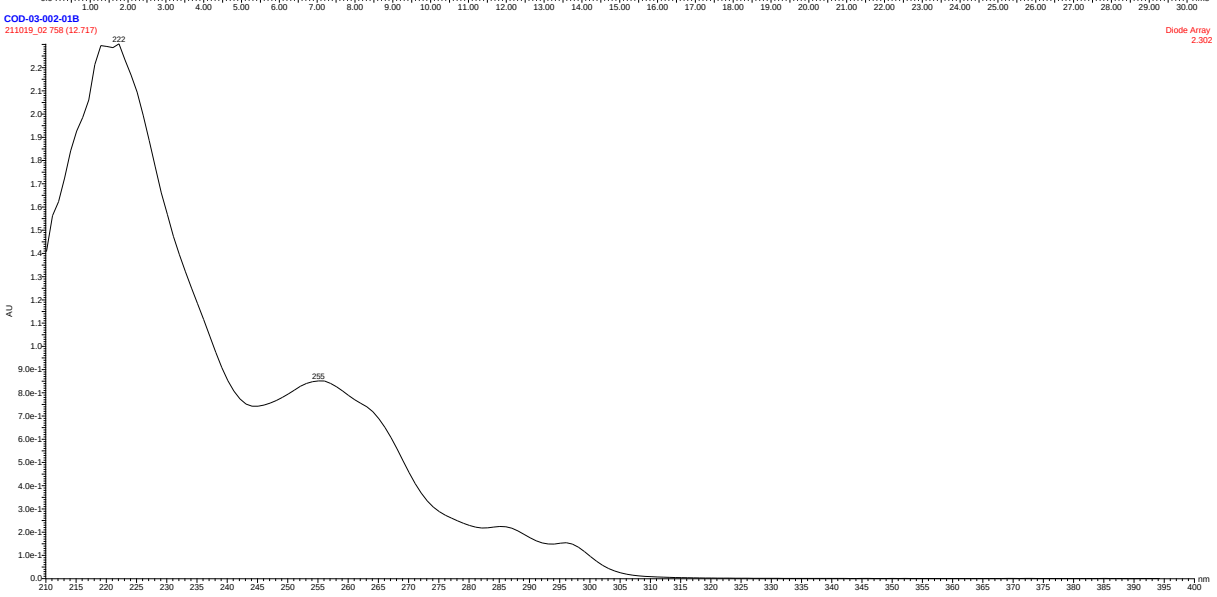


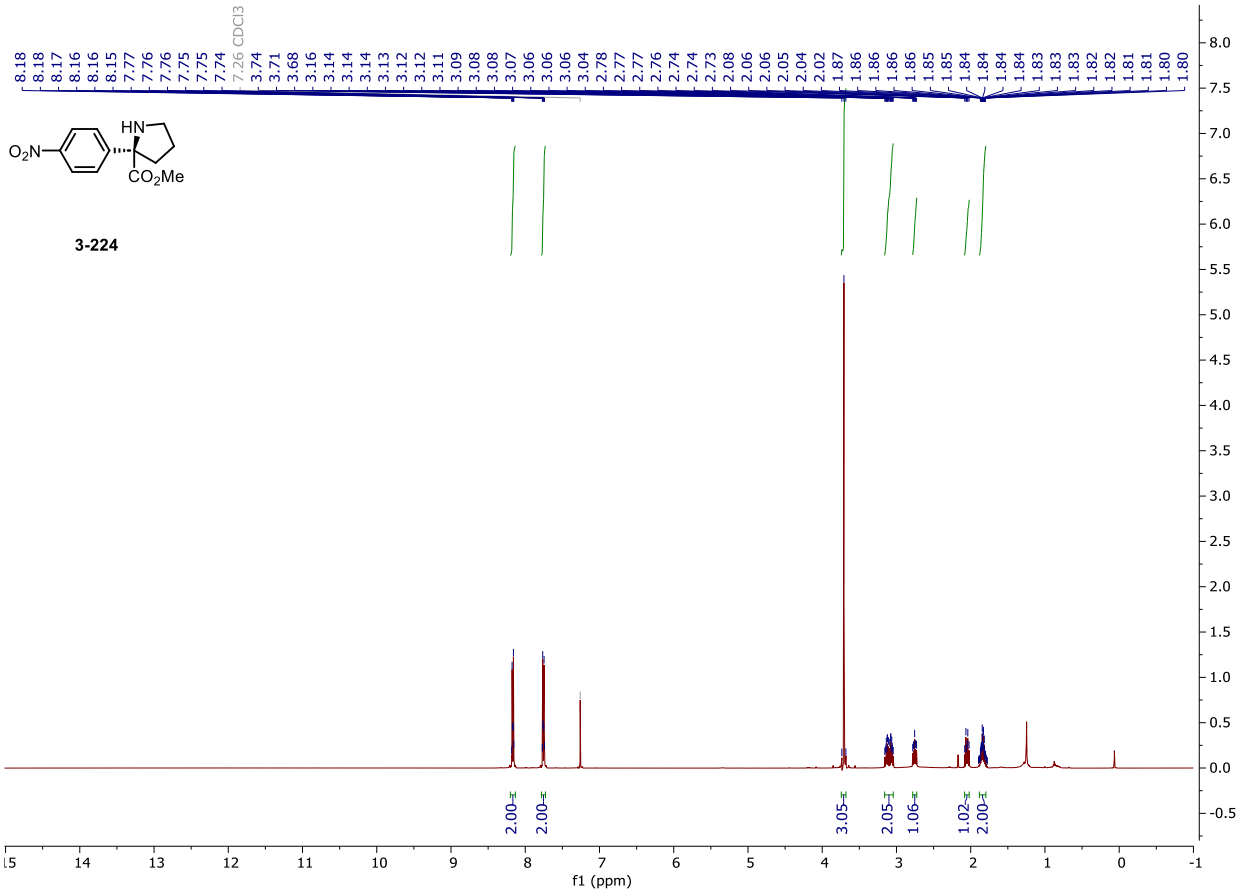


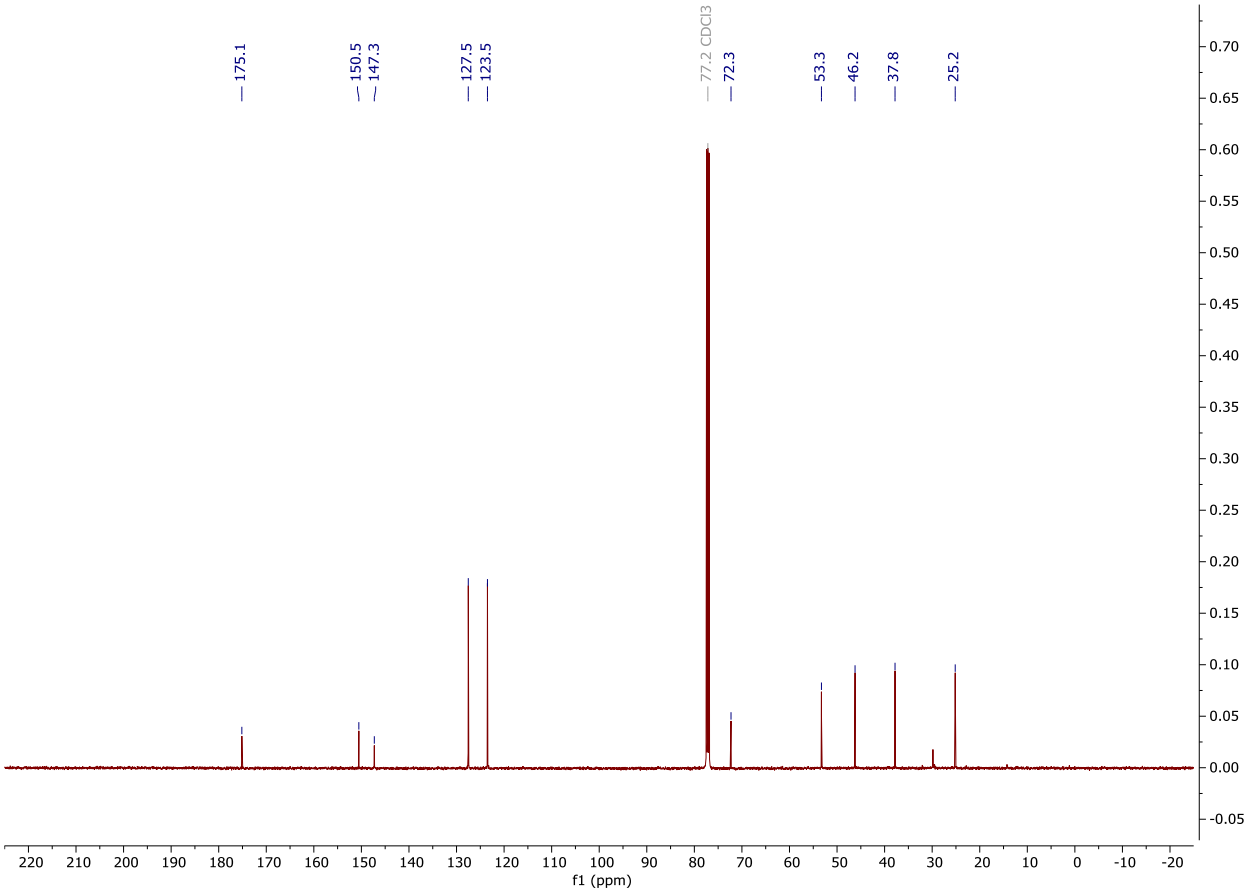
HPLC chromatograms of **3-223**



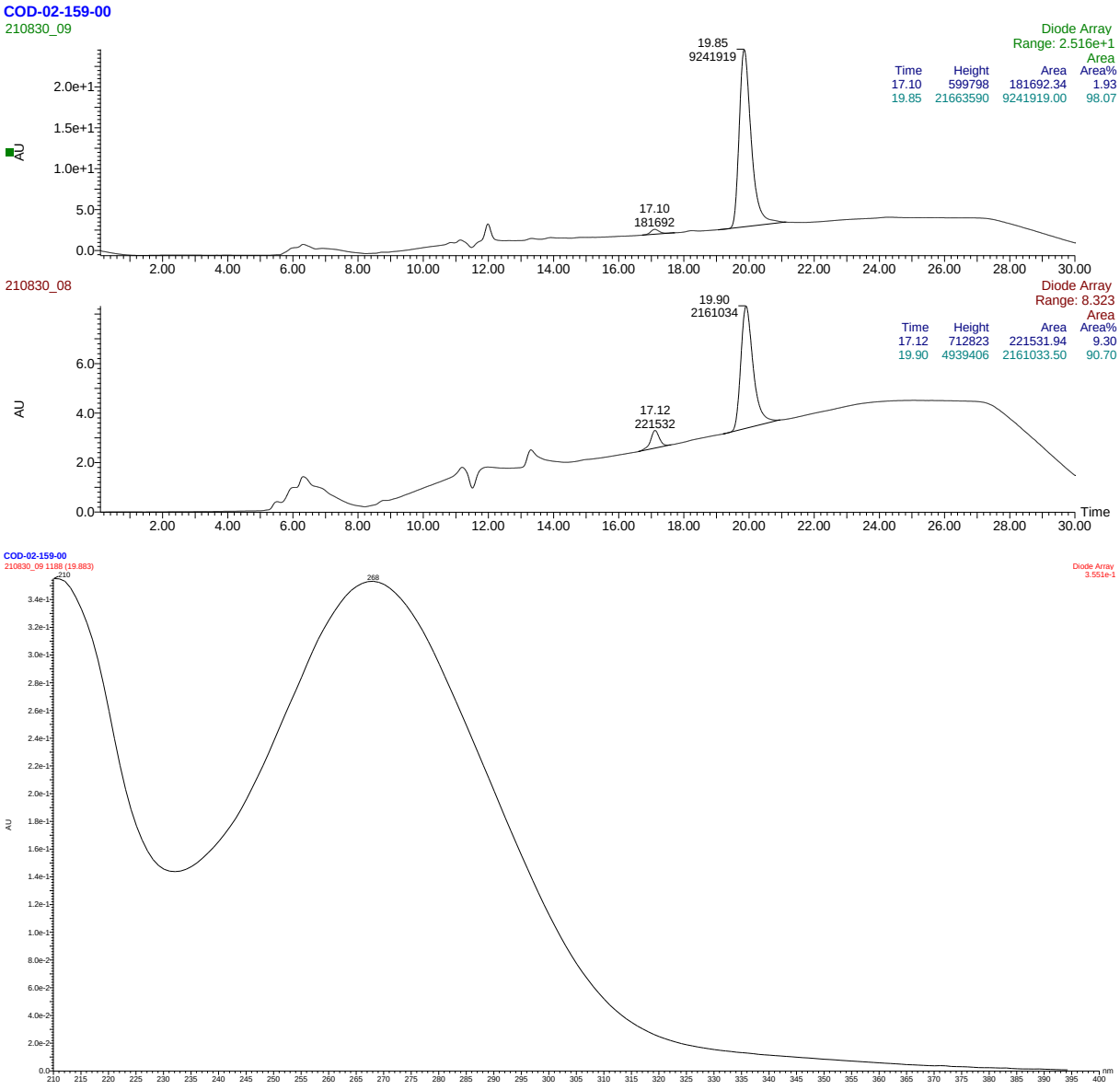


Copy of ^{1}H, $^{13}C\{^{1}H\}$ spectra of **3-224**





HPLC chromatograms of **3-224**



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Heteroaryl sulfonamide synthesis: scope and limitations†

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Heteroaryl sulfonamides are important structural motifs in the medicinal and agrochemical industries. However, their synthesis often relies on the use of heteroaryl sulfonyl chlorides, which are unstable and toxic reagents. Herein, we report a protocol that allows direct oxidative coupling of heteroaryl thiols and primary amines, readily available and inexpensive commodity chemicals. The transformation proceeds under mild reaction conditions and yields the desired *N*-alkylated sulfonamides in good yields. *N*-alkyl heteroaryl sulfonamides can be further transformed using a microwave-promoted Fukuyama–Mitsunobu reaction to *N*,*N*-dialkyl heteroaryl sulfonamides. The developed protocols thus enable the preparation of previously difficult to prepare sulfonamides (toxic reagents, harsh conditions, and low yields) under mild conditions.

Introduction

Although the structural motif of sulfonamide is rather rare in nature,[1] it occupies a privileged position in the medicinal and agrochemical areas of research and applications.[2–4] In fact, its metabolic stability[5] and carboxyl group isosterism[6] are presumably responsible for the high levels of biological activity that compounds with the sulfonamide group possess. The classical approach to their synthesis is based on the reunion of sulfonyl chlorides with amines (Fig. 1A).[7,8] This widely used method suffers from the toxicity and instability of sulfonyl chloride reagents that must, in addition, be prepared using strong oxidizing and chlorinating agents.[9–15] To overcome such a drawback, one-pot approaches[16,17] and, more recently, the copper-catalysed arylboronic acid and DABSO-based approach[18] were developed (Fig. 1B). Direct electrochemical oxidative coupling of thiols and amines was recently developed as a non-toxic alternative to the commonly used sulfonyl chloride route (Fig. 1C).[19,20] Despite all the tremendous progress, polyheteroatomic heteroaryl sulfonamides still remain a challenging target when their synthesis is attempted. In the case of heteroaryl sulfonamide synthesis, commonly used methods suffer from low stability of the required sulfonyl chlorides[21] or low conversion of sulfenamide to sulfonamide during the oxidation step.[19,22]

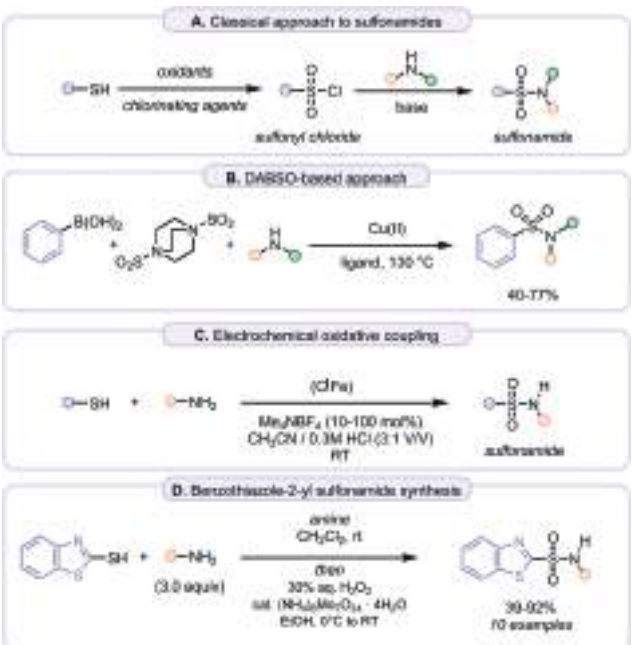

Fig. 1 Previous approaches to sulfonamides. (A) Commonly used route to sulfonamides *via* the corresponding sulfonyl chloride. (B) DABSO-based approach to sulfonamides starting from aryl boronic acids. (C) Electrochemical synthesis of *N*-alkyl sulfonamide. (D) Our previously developed approach to benzothiazole sulfonamides.

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Our interest in heteroaryl sulfonamide synthesis led us recently to develop a unified approach to *N*-substituted and *N*,*N*-disubstituted benzothiazole-2-yl sulfonamides from benzo[*d*]thiazole-2-thiol and alkyl amines, readily available bulk chemicals (Fig. 1D).[23] Simplicity of the developed protocol hand-in-hand with a quasi-nonexistent general method that would allow the synthesis of, for example, (benzo[*d*])oxazole-2-yl sulfonamides[19] led us to the idea of extending this method to other heterocyclic thiols.

Results and discussion

Preliminary results

Before extension of our oxidative coupling method to other heteroaromatic thiols, the electrophilicity of the C_α sulfonamide carbon in representative (hetero)aromatic thiols (**1a–10a**, Fig. 2A) was evaluated *in silico*. Previous observations and data obtained during our research in the field of benzothiazole-2-yl sulfones and sulfonamides showed that sulfone/sylfonamide-bearing C_α carbon in the heterocycle is prone to nucleophilic attack.[24–26] We exploited this behaviour, for example, in amine synthesis;[23] however, it also caused the instability of sulfones and sulfonamides on silica gel.[24,27,28] For those reasons we wondered if the quasi-nonexistence of several classes of heteroaryl sulfonamides in the literature[19] might be caused by their instability. To shed some light on the C_α atom electrophilicity in *N*-benzylated sulfonamides (Fig. 2A), the local electrophilicity index values ($\omega_{C_\alpha}^{+}$)[29,30] were calculated for two optimized conformations, linear and sandwich-like (Fig. 2B, the latter being more stable).[31] Computational data suggested that newly prepared heteroaryl sulfonamides **2a–10a** should not suffer from the C_α nucleophilic attack (similarly to known aryl sulfonamides **1**), since their $\omega_{C_\alpha}^{+}$ values are not exceeding those of sulfonamides **1a–c**. On the other hand, the stability of **2a–10a** in the presence of Lewis or Brønsted acid was still questionable.[32] Based on the calculations, benzoxazole sulfonamide **7a**, in its protonated form, is very prone for a nucleophilic attack at C_α and, therefore, it was selected as the target substrate for reaction development.[32]

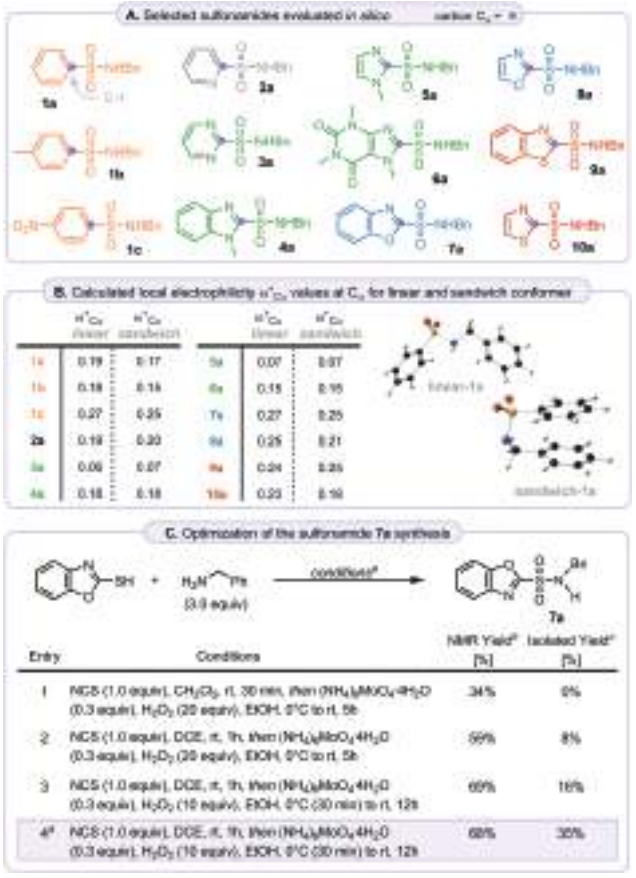

Fig. 2 *In silico* evaluation of the optimization of the reaction of sulfonamides **1a–10a** and sulfonamide **7a**. (A) Evaluated structures. (B) Calculated values of local electrophilicity $\omega_{C_\alpha}^{+}$ at carbon C_α for the two most stable conformers found during geometry optimization. (C) Representative examples of optimalization of the synthesis reaction of sulfonamide **7a**. [a] Reactions were performed on the benzo[*d*]oxazole-2-thiol (1 mmol) and benzylamine (3 mmol) scale. [b] NMR yield determined using dimethylsulfone as the internal standard. [c] Obtained *via* crystallization. [d] Reaction carried out on benzo[*d*]oxazole-2-thiol (5 mmol) and benzylamine (15 mmol) scale.

The optimization of the reaction started by reacting benzoxazole-2-thiol with benzylamine under previously developed reaction conditions for 2-mercaptobenzothiazol (Fig. 2C, entry 1).[23] Only traces of product **7a** were observed. Detailed investigation and optimization of the reaction steps (sulfenamide formation (entry 2) and its oxidation to sulfonamide (entry 3)) and the isolation and purification of product **7a** allowed us to prepare the desired sulfonamide in a reasonable 35% isolated yield (entry 4).[32] The low isolated yield is caused by the isolation process of **7a** (NMR-based yield is higher (68–69%), entries 3 and 4). In our hands, crystallization proved to be the only way of purification that allowed us to obtain pure sulfonamide **7a**.

Scope and limitations

Having identified optimal reaction conditions of the oxidative coupling, the scope and limitations were established (Fig. 3). It was observed that the applicability of the method is broad and that most of the heteroaryl sulfonamides can be generated in good yields using our two-step process. However, in many cases, generated *N*-substituted sulfonamides were unstable in solution and degraded during the purification process. Once obtained in pure form, if solid, they proved to be bench-stable over the period of 3 months. The observed instability was especially significant in the case of (benzo)oxazole sulfonamides **7** and **8**, heteroaryl sulfonamides substituted with electron-donating group ((–)-**9f**) or heteroaryl sulfonamides containing four nitrogens within the heterocycle (**6b** and **16**).[32]

The reaction sequence proved to be futile when secondary amines, aromatic amines, cyanamide, or ammonium chloride were used as the reaction partner. In such cases, the first step, the formation of sulfenamide, failed. The only exception to

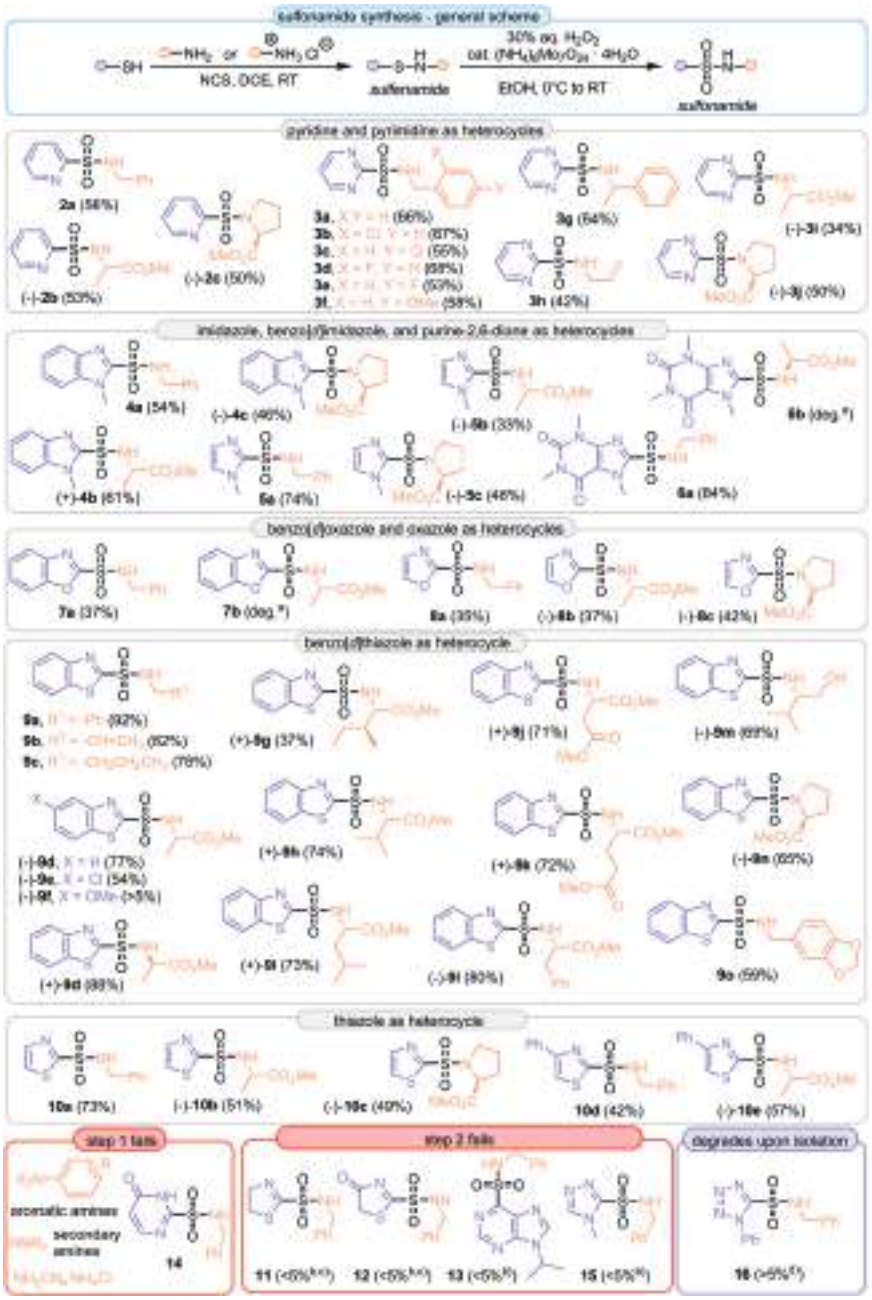

Fig. 3 Scope and limitations of the oxidative coupling between heteroaryl thiols and amines. Yields refer to pure isolated compounds after two steps. All reactions were typically performed on a 5 mmol scale of the corresponding heteroaryl thiol. [a] Sulfonamide detected by ^{1}H NMR; however, all attempts to isolate it failed. [b] No product formation was observed. Only intermediate sulfenamide was detected. [c] The oxidative opening of the heterocycle occurred during the oxidation step. Only side products were formed (see Scheme S3†). [d] Desired product **16** isolated only as a mixture of **16** with phenyl tetrazole **S9** (**16** : **S9** = 1 : 5). All attempts to purify compound **16** failed due to compound **16** decomposition.

this tendency was observed for proline esters (secondary amines observation exception). In such a case, the desired sulfonamides (−)-**2c**, (−)-**3j**, (−)-**4c**, (−)-**5c**, (−)-**8c**, (−)-**9n** and (−)-**10c** were isolated in good yields (42 to 62%). The reason behind this observation remains unclear. In the case of partially saturated heteroaryl sulfonamides (**11** and **12**), the formation of sulfenamide proceeded well; however, the oxidation step (from sulfenamide to sulfonamide) failed, and no traces of the desired sulfonamides were detected. The oxidative decomposition of the intermediate sulfenamide is presumably occurring.[33] The same situation was observed when the synthesis of sulfonamides **13** and **15** was attempted. However, in those cases, no identifiable products of oxidative decomposition were observed. In the case of heterocycle **14**, however, even the first step of the sequence failed presumably due to the presence of N–H bond on the heterocycle.

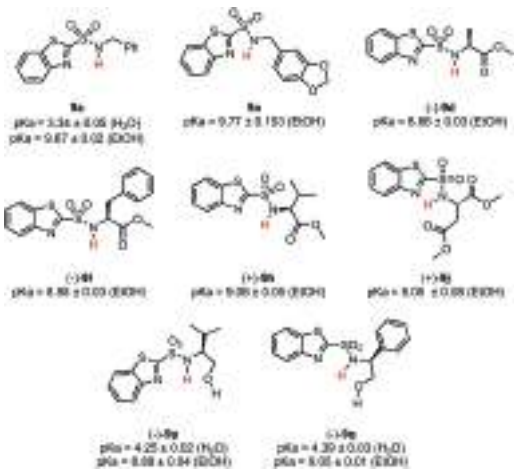

Fig. 4 Determined pK_a values of selected sulfonamides in H_2O and EtOH. Due to the low solubility of some of the sulfonamides in water, the pK_a values for all selected compounds were determined in EtOH.[32,34]

N,*N*-Disubstituted heteroaryl sulfonamide synthesis

At this stage, we reasoned that the observed instability of several *N*-substituted sulfonamides **2–10** might be caused by the acidity of the sulfonamide *N*-hydrogen. To shed some light on the acidity and to evaluate the possible H-bond donor ability of the targeted sulfonamides, the pK_a value of the selected sulfonamides was determined (Fig. 4). The pK_a values of the simple benzylated sulfonamides **9a** and **9o** were observed to be slightly greater than 9.5 (for comparative reasons,[34] all values were determined in EtOH due to a low solubility of several sulfonamides in H_2O). However, the pK_a values of amino acid-derived sulfonamides (sulfonamides (−)-**9d,l** and (+)-**9h**) were substantially lower ($pK_{a(EtOH)} \sim 8.6$ to 9 *vs.* 9.6–9.7).

Such an observation suggested that if an additional substitution of the sulfonamide nitrogen atom were introduced, the overall stability of the heteroaryl sulfonamides might increase.

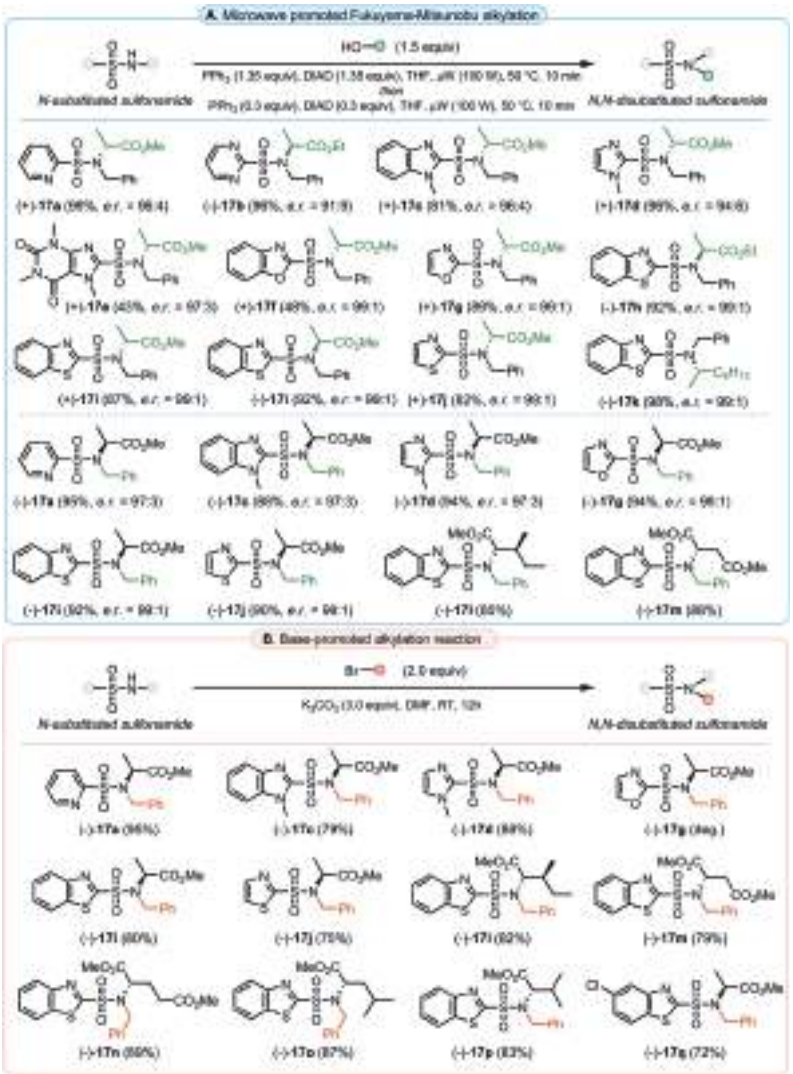

Fig. 5 Synthesis of *N*,*N*-disubstituted sulfonamide **17**. (A) Scope of the microwave-promoted Fukuyama–Mitsunobu alkylation reaction. (B) Scope of the base-mediated alkylation reaction.

Furthermore, proline-derived sulfonamides (−)-**2c**, (−)-**3j**, (−)-**4c**, (−)-**5c**, (−)-**8c**, (−)-**9n**, and (−)-**10c** proved to be reasonably stable and their lower isolated yields were mostly related to incomplete intermediate sulfenamide formation due to competitive disulfide formation during the first step of the sequence. Taking into account the low pK_a values of *N*-hydrogens in evaluated sulfonamides, two alkylation methods for preparation of *N*,*N*-disubstituted sulfonamides were designed (Fig. 5). In the first, *N*-alkyl sulfonamides reacted under our recently developed microwave-promoted Fukuyama–Mitsunobu alkylation conditions (Fig. 5A). The simple alkylation of amino acid-containing sulfonamides with benzyl bromide in the presence of base was also examined (Fig. 5B).

Due to the previously mentioned instability of (benzo) oxazole sulfonamides **7a**, compound **7a** was used as a model substrate for the development of alkylating conditions. After some evaluation, a slight modification of our recently developed microwave-promoted Fukuyama–Mitsunobu alkylation[32] conditions was found to be suitable (Fig. 5A).[23,35,36] Using such conditions, sulfonamide **7a** was transformed into *N*,*N*-dialkylated benzoxazole sulfonamide (+)-**17f** with a 48% yield. Pure (+)-**17f** was found to be shelf stable for at least 3 months. Using the same protocol, various *N*,*N*-dialkylated sulfonamides **17** (Fig. 5A) were prepared in good to excellent yields. Having easy access to *N*-benzylated sulfonamides (compounds **2a–10a**) and to the sulfonamides generated from L-alanine ((−)-**2b**, (−)-**3i**, (+)-**4b**, (−)-**5b**, (−)-**8b**, (−)-**9b**, and (−)-**10b**), synthesis of both enantiomeric forms of the corresponding sulfonamides **17a,c,d, g** and **i** could be accomplished. It was observed that *N*-alkylated sulfonamides can readily be benzylated with benzyl bromide in the presence of a base (Fig. 5B). The only exception were derivatives of (benz)oxazole sulfonamides **7a**, **8a**, and (−)-**8b** that degraded under the applied reaction conditions.

Conclusions

A short and efficient synthetic route to previously unreported *N*-alkylated heteroaryl sulfonamides based on the bulk chemicals (heteroaryl thiols and primary amines including α-amino esters) was developed. Prepared *N*-alkylated sulfonamides then served as a nucleophile in the microwave-promoted Fukuyama–Mitsunobu alkylation reaction and generated stereoselectively the desired *N*,*N*-disubstituted heteroaryl sulfonamides in good (benzoxazole sulfonamides) to excellent yields (other tested heterocycles).

Having demonstrated that various types of heteroaryl sulfonamide building blocks might be readily available starting from simple, inexpensive, and bulk chemicals, we expect that it will boost their use in the field of medicinal chemistry.

Author contributions

R. O. I. performed most of the experiments and analysed the experimental data. D. C., J. K., and F. Z. carried out the experiments and analyzed the experimental data. R. O. I. optimized the Fukuyama–Mitsunobu reaction. D. C. optimized the alkylation reaction. J. K. performed the pK_a determination. R. O. I. and D. C. partially designed the experimental plans. E. D. and F. D. V. carried out the DFT calculations and analysed the obtained results. J. P. initiated the project, led the project team, designed the experiments, and analysed the results. R. O. I. and J. P. co-wrote the paper with input from all authors. All authors have approved the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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Julia-Kocienski-Like Connective C–C and C=C Bond-Forming Reaction

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Dedicated in memory of professor István E. Markó.

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Abstract: In this paper, we present a one-pot protocol that enables a straightforward and selective transformation of alkyl benzothiazol-2-yl and phenyltetrazol-2-yl sulfones and acyl chlorides into ketones, E-olefins, Z-olefins, and even pyrroles. The final product of the reaction depends on the proper choice of the reaction workup. Notably, the protocol designed for olefin formation allows a switch between E- and Z-olefin formation by the correct choice of the reaction workup. These developed protocols facilitate the formation of all compounds under mild reaction conditions, as evidenced by the synthesis of (nitro)-fatty acids, and the concept can be extended to other product formations, as demonstrated by the synthesis of pyrroles.

Keywords: coupling reaction; olefination method; fatty acid synthesis; divergence in synthesis; stereoselectivity

Introduction

Carbon-carbon bond-forming reactions are the key connective reactions in organic synthetic chemistry. Among these, the stereoselective formation of unsaturated bonds, specifically olefins, holds fundamental significance due to their involvement in the synthesis of natural products, bioactive compounds, and materials. Presently, two commonly used methods for achieving stereoselective connective olefination are olefin cross-metathesis[1] and coupling of anion-stabilized reagents with aldehydes or ketones[2] (Figure 1). These methods generally operate under mild reaction conditions, exhibit good tolerance towards functional groups, and yield predominantly (*E*)-configured olefins. However, there are limited methods that address the selective formation of (*Z*)-olefins. In such cases, specific catalysts (e. g., cross-metathesis)[3] or modified

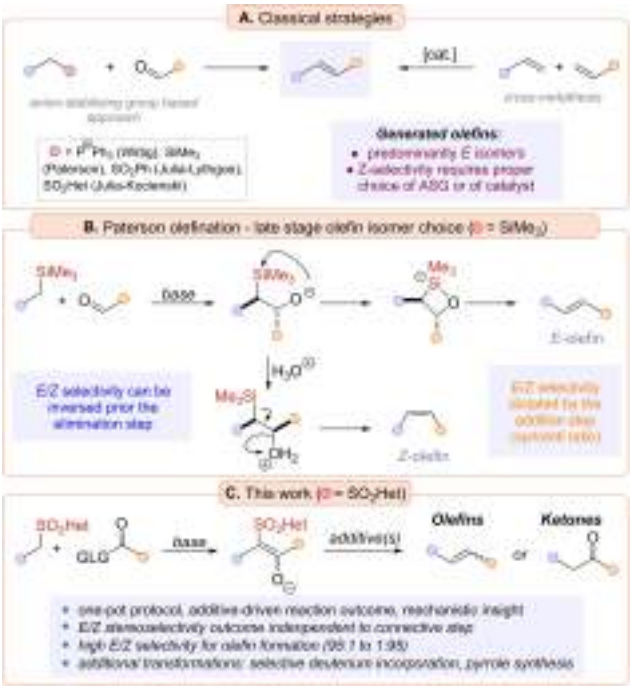

Figure 1. Connective olefination methods.

anion stabilizing groups (e. g., Still-Gennari modification of Horner-Wadsworth-Emmons olefination)[4] are utilized. The Peterson olefination,[2g,h] on the other hand, allows for the stereoselective formation of either (*E*) or (*Z*)-olefins from the same starting material by a simple alteration in the reaction work-up (acidic vs. basic).[5] However, even in this case, the stereoselectivity of the *in situ* generated adduct (*syn/anti*) is reflected in the final stereochemical outcome of the olefination.

The objective of our study was to devise an olefination method capable of selectively producing either (*E*)- or (*Z*)-olefins from identical starting materials. We considered whether in our group the extensively developed Julia-Kocienski olefination reaction[6] could be modified to meet these specific requirements. In previous endeavors, both our group[6b,c] and others[7] attempted to influence the stereochemical outcome of the Julia-Kocienski reaction using different approaches. However, the outcomes of such attempts proved to be highly substrate dependent.[8]

To overcome these limitations, we speculated that introducing the *syn/anti* configuration independently after the connective C–C bond-forming reaction could potentially achieve two objectives: (1) increase the *E/Z* selectivity, and (2) selectively generate (*E*) or (*Z*) olefins. Moreover, we considered that the utilization of in situ generated C–C bond intermediates might be extended to additional transformations, making this connective approach advantageous for a wide range of synthetic endeavors. Below, we summarize our findings from the aforementioned research efforts.

Results and Discussion

Preliminary Results

Our development of the novel connective Julia-Kocienski-type protocol began with identifying two key steps (C–C bond formation/stereochemistry introduction) that were later conducted in a one-pot manner. In our plan, the reaction between heteroaryl sulfone **1** and acyl halide **2** seemed the most suitable approach, as the in situ generated enolate **4** could likely be readily transformed to the β-keto sulfone **5** by an external proton source (Figure 2A). Subsequently, mild reducing agents like $NaBH_4$ were expected to facilitate the reduction of **5** to β-alkoxy sulfone **6**, which, through intramolecular Smiles rearrangement and further *anti*-elimination, would yield the desired olefin **3**.

The optimization of the reaction sequence began with the reaction of BT-sulfone **1a** with acyl chloride **2a** under previously described conditions (Figure 2B, entry 1).[9] The *in situ* generated adduct **4a** was then protonated with an excess of AcOH, and to test the hypothesis that adduct **4a** can be transformed into ketone **5** and the BT-sulfone group can be removed *in situ*, a reducing metal, Zn dust (10 equiv.), was added.[10] Encouragingly, the desired ketone **8a** was obtained in 73% yield.[11]

After validating the feasibility of *in situ* protonation/sulfone reduction of adduct **4a**, the second step of the sequence, the stereoselective reduction of β-hydroxy sulfone **5a**, was attempted (Figure 2B). After some reaction condition optimization,[11,12] it was observed that the addition of MeOH to the reaction mixture after the formation of adduct **4a**, followed by an excess of $NaBH_4$ (20 equiv.), generated the desired (*Z*)-**3a** olefin in 72% yield and with a 2:98 *E/Z*-selectivity (Figure 2B, entry 2). On the other hand, when *i*PrOH, followed by $ZnCl_2$ and $NaBH_4$, was added, olefin (*E*)-**3a** was obtained with a yield of 69% and a 98:2 *E/Z*-selectivity (Figure 2B, entry 3). Similar results were obtained when sulfone **1b** (Het = PT) was used as a starting material, although the desired products were isolated in somewhat lower yields (entries 4 to 6). It was also demonstrated that $Zn(BH_4)_2$[13] could be used as the reducing agent in the case of *E*-selective olefin formation protocol (entry 7).

Based on the obtained data[11] and in agreement with the work of Jørgensen *et al.*,[12] we believe that the stereochemistry of the olefin formation is governed by the stereoselective reduction of intermediate **5**. In the case of the (*Z*)-olefin, the reduction of intermediate **5** occurs via the Felkin-Ahn-like transition state, driving the formation of the (*Z*)-olefin (Figure 2C). The subsequent transformation of *syn* β-hydroxy

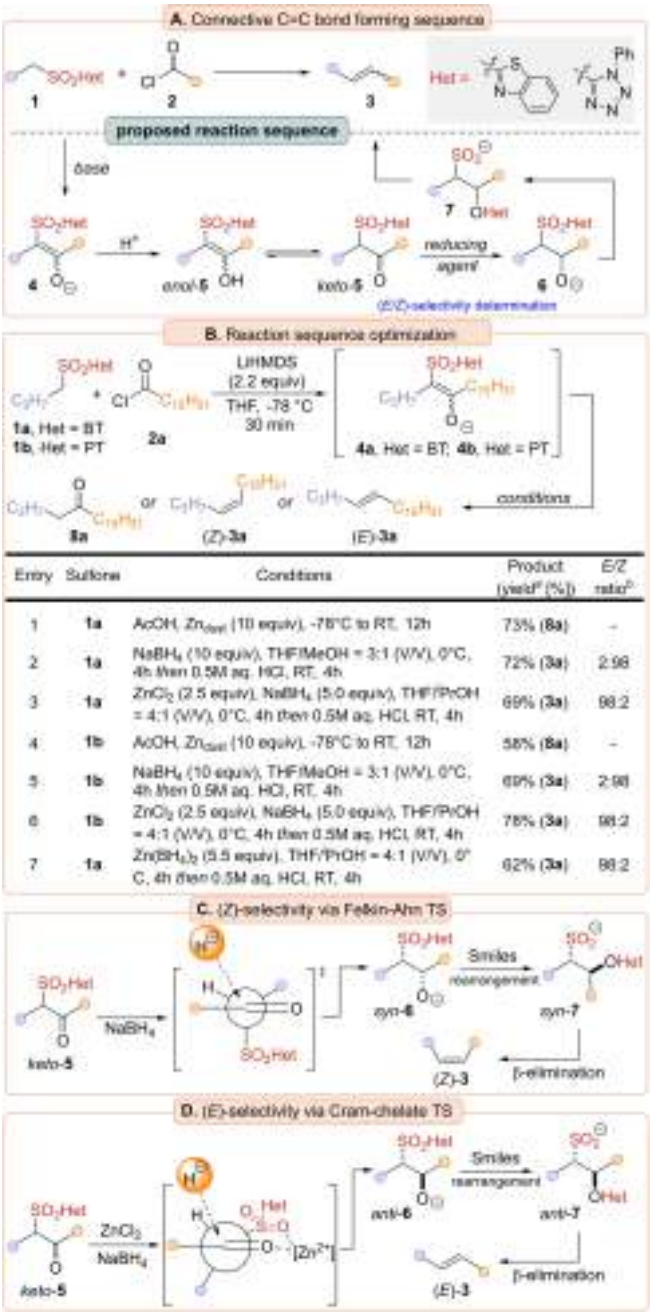

Figure 2. (A) Connective reaction sequence featuring olefin formation. (B) Reaction protocol optimization – selected representative examples. (C) Felkin-Ahn-based reaction mechanism presumably operating during the (*Z*)-olefin formation. (D) Cram-chelate-based reaction mechanism presumably operating during the (*E*)-olefin formation.

sulfone *syn*-**6**, involving Smiles rearrangement and β-anti-elimination processes, leads to the observed (*Z*)-olefin **3**.

In the case of (*E*)-olefin formation, the reduction of intermediate **5** proceeds via the Cram-chelate transition state, leading to the *anti* β-hydroxy sulfone *anti*-**6**. Subsequently, the Smiles rearrangement and β-anti-elimination reactions complete the formation of the (*E*)-olefin **3**.

Scope and Limitations

With suitable reaction conditions in hands, the scope and limitations of the transformations could be established (Figure 3). It was observed that various substituted acyl and aryl chlorides were readily transformed to the corresponding ketones **8a–i** in good to very good yields (Figure 3A). When succinyl dichloride was reacted with an excess of the corresponding sulfone **1** (2 equiv. of sulfone and 4.4 equiv. of LiHMDS were used), generated diketones **8j** and **k** were isolated as the only products of the reaction. In all evaluated cases, transformations using PT-containing sulfones **1** yielded the desired ketones **8** in lower isolated yield when compared to BT-substituted sulfones **1**. The condensation reaction and the formation of ketone **8** failed when oxalyl mono and dichlorides, α-chloro acetyl chloride, monochloro fumarate ester, or TFAA were used as acylating reagents (Figure 3D).

In the case of olefination reactions, it was observed that the (*Z*)-selective transformations (Figure 3B) proceed in high *Z*-selectivity (*E/Z* from 2:98 to 20:80) when alkyl sulfones **1** were reacted with acyl or benzoyl chlorides **2**. The *Z*-selectivity decreased when benzyl sulfones **1** were used as substrates (*E/Z* from 27:73 to 46:54). The special case was the use of 4-methoxybenzoyl chloride as a substrate for the reaction. In this case, only *E*-olefins **3f** and **3g** were formed as the main products (*E/Z* = 90:10 to 93:7). It is expected that this observation is caused by the competitive *syn* elimination of the generated *syn*-**6** adduct (*synperiplanar* elimination from *cisoid* conformation of intermediate *syn*-**7**) as previously described for such type of adducts containing the electron-donating group on the aromatic ring.[6a,11] When the *E*-selective protocol was evaluated (Figure 3C), the desired *E*-olefins **3** were generated as the main products of the reaction in *E/Z* ratios ranging from 98:2 to 81:19. In all of the evaluated cases, reactions carried out with PT-sulfones generated the desired product in somewhat lower reaction yields than those that occurred with the corresponding BT-sulfone.

Subsequently, we explored further extensions of the developed methods (Figure 4). First, the selective incorporation of the deuterium atom into the newly generated olefin **3** was evaluated (Figure 4A). Based on the proposed reaction mechanism, the use of $NaBD_4$ instead of $NaBH_4$ should lead to selective **3**-*d* olefin formation (Figures 2C and 2D). In all evaluated cases, the assumption proved to be correct, however, the deuterium incorporation into generated olefins **3a**-*d*, **h**-*d* and **i**-*d* was ranging between 88 to 92%.[11] From the *E/Z* selectivity point of view the results were in

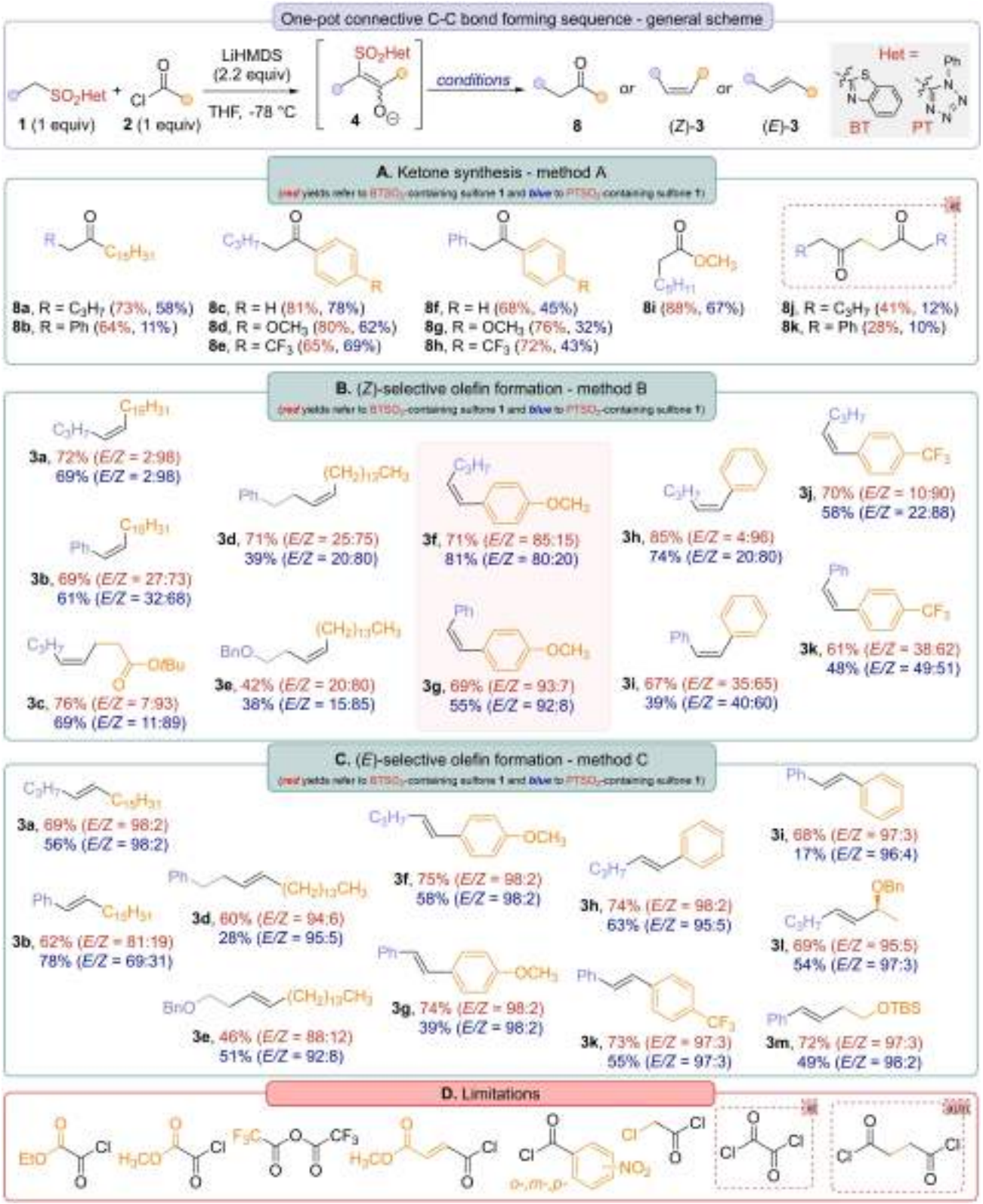

Figure 3. Scope and limitations of the connective method developed. (A) Scope of ketone formation. (B) Scope of the selective (*Z*)-olefin formation. The *E/Z* ration is based on the ^{1}H NMR spectra of the crude reaction mixture analysis. (C) Scope of selective (*E*)-olefin formation. The *E/Z* ratio is based on the ^{1}H NMR spectra of the crude reaction mixture analysis. (D) Substrates that failed to yield any olefine or ketone under the investigated reaction conditions. ***Conditions***. Method A: AcOH, Zn_{dust} (10 equiv.), −78 °C to RT, 12 h; Method B: $NaBH_4$ (10 equiv.), THF/MeOH = 3:1 (V/V), 0 °C, 4 h *then* 0.5 M aq. HCl, RT, 4 h; Method C: $ZnCl_2$ (2.5 equiv.), $NaBH_4$ (5.0 equiv.), THF/*i*PrOH = 4:1 (V/V), 0 °C, 4 h *then* 0.5 M aq. HCl, RT.

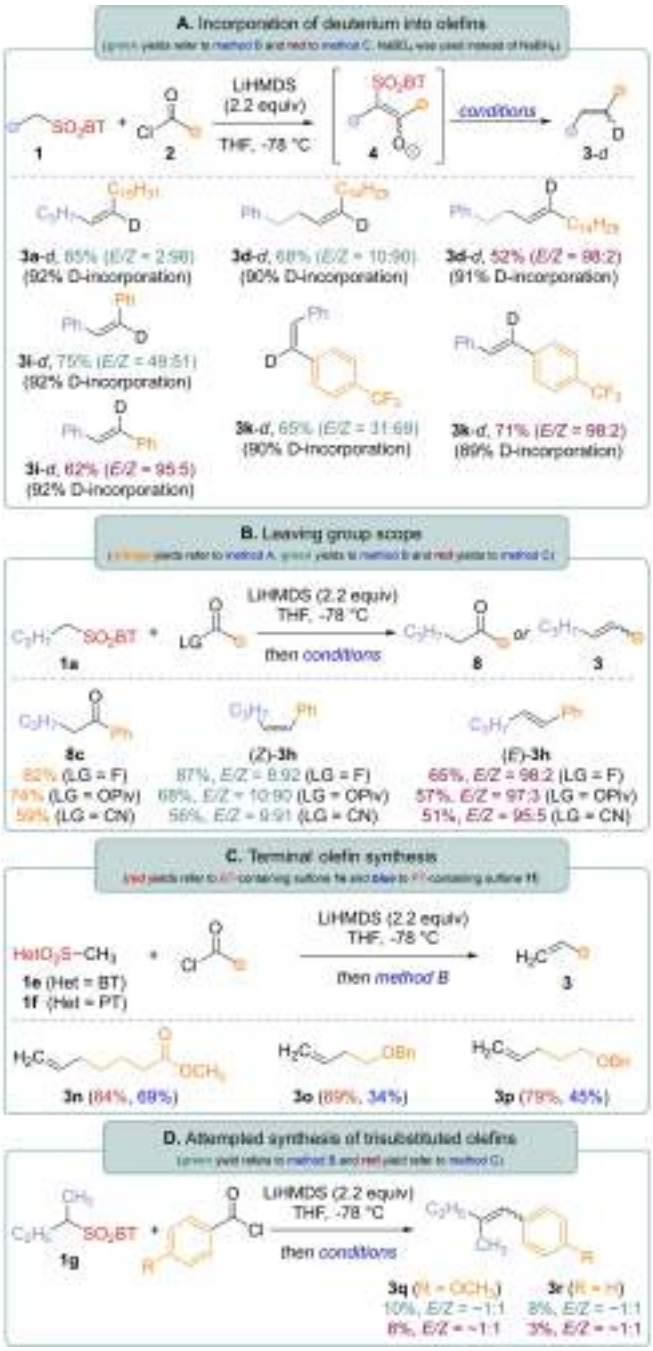

Figure 4. (A) Generation of deuterium-containing olefins **3**. *Deuterium* incorporation based on the ^{1}H-NMR spectra analysis. (B) Evaluation of the influence of the ability of the leaving group on the reaction sequence outcome. (C) One-pot protocol for the terminal olefin formation. (D) Attempt to apply our olefination method to the stereoselective trisubstituted olefin formation. ***Conditions***. Method A: AcOH, Zn_{dust} (10 equiv.), −78 °C to RT, 12 h; Method B: $NaBH_4$ (10 equiv.), THF/MeOH = 3:1 (V/V), 0 °C, 4 h *then* 0.5 M aq. HCl, RT, 4 h; Method C: $ZnCl_2$ (2.5 equiv.), $NaBH_4$ (5.0 equiv.), THF/*i*PrOH = 4:1 (V/V), 0 °C, 4 h *then* 0.5 M aq. HCl, RT, 4 h.

agreement with the previously observed trends. The only exception was the formation of stilbene **3i**-*d* via the *Z*-selective method B. In such a case, the generated olefin **3i**-*d* was formed in a 49:51 *E/Z* ratio. We speculate that the observed selectivity is caused by the competitive *syn*-elimination of the intermediate **7**.[6a]

To broaden the applicability of our methods, the influence of the three additional leaving groups on the acyl reagents, fluoride, pivaoylate and cyanide, were evaluated (Figure 4B). Gratifyingly, it was observed that all three leaving groups can be successfully used as chloride anion equivalents without any significant impact on the reaction yield and the *E/Z* selectivity.

Finally, olefin protocols were also evaluated in terms of terminal (Figure 4C) and trisubstituted (Figure 4D) olefin formation. In the first case, both the methyl heteroaryl sulfones **1e** (Het = BT) and **1f** (Het = PT) reacted smoothly and generated olefins **3n–p** with good to very good yields. Again, sulfone **1e** yielded the desired olefins in slightly better yields than the corresponding sulfone **1f**. In the case of the attempted stereoselective trisubstituted olefin **3q** formation (Figure 4D), the desired olefin was formed only in low reaction yields (11–17%) and selectivity (45:55 for the *Z*-selective protocol, and 63:37 for the *E* selective protocol).

Pyrrole Synthesis

Having easy access to 1,4-diketones (Figure 3A, compounds **8j** and **k**), a one-pot synthesis of the corresponding pyrroles **9** was attempted (Figure 5). After intensive reaction conditions optimization,[10] the optimal reaction conditions under which aniline and benzylamine could be used to generate the desired pyrrole **9a** (from aniline), **9b** (from benzyl amine), and **9c** (from *n*-butyl amine) in one-pot manner and isolated yields of 28%, 37%, and 21%, respectively.

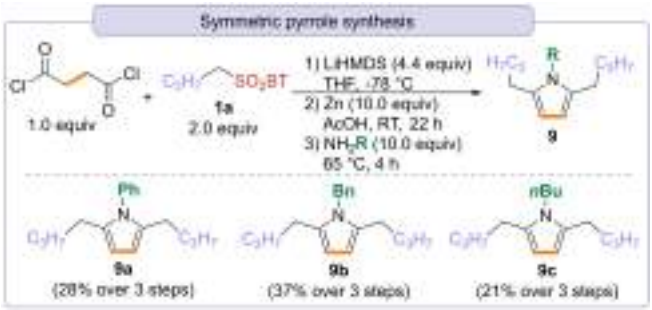

Figure 5. Three-step, one-pot protocol for symmetrically substituted pyrrole synthesis. *BT = benzo[d]thiazole-2-yl.*

Fatty Acid Synthesis

Finally, (*Z*)-olefination method (Method B) was also used to prepare selected (nitro)-fatty acids (Figure 6) in a short and efficient way. Indeed, starting from the readily available building blocks the synthesis of Oleic acid (**14**, 3 steps, 52% overall yield), Linoleic acid (**19**, 6 steps, 26% overall yield) and 10-NO_2-Linoleic acid (**24**, 7 steps, 8% overall yield) could be readily achieved.

Conclusion

We have developed a one-pot protocol that enables the generation of ketones, as well as *E* and *Z*-olefins, using commercially available starting materials. The remarkable aspect of this protocol is that the final product obtained is not influenced by the initial reaction conditions. Instead, the choice between the product being a ketone, *E*-olefin, or *Z*-olefin is determined during the reaction work-up process. It is important to emphasize that this olefination protocol allows for the independent synthesis of *E* and *Z* olefins from the same starting material. This selectivity adds to the versatility of the method. Thus, the described olefination sequence is equivalent to the well-known Petersen olefination, but allows the further extension of it to e. g. ketone synthesis.

Furthermore, we have demonstrated that the identified reaction conditions can be extended to other transformations. For example, we successfully applied the one-pot protocol to pyrrole synthesis, showcasing its potential for diverse applications in organic synthesis. Developed protocols can be applied in natural product synthesis, as demonstrated in the case of (nitro)-fatty acids. These findings underscore the significance of our protocol, which not only offers a versatile and efficient approach to ketone and olefin synthesis but also holds promise for various other synthetic endeavors and applications in the field of organic chemistry.

Experimental Section

General Procedure for Carbonyl Compound 8 Synthesis (Method A)

Sulfone **1** (1 mmol, 1.0 equiv.) was dissolved in dry THF (0.1 M) and cooled to −78 °C (acetone/dry ice). After 5 minutes, LiHMDS (2.2 mmol, 2.2 equiv., 1.0 M solution in THF) was added dropwise, and the reaction mixture immediately turned light orange. Subsequently, acyl chloride **2** (1.1 mmol, 1.1 equiv.) was added over 5 minutes. The resulting reaction mixture was allowed to stir at −78 °C for 30 minutes. Glacial AcOH (5 mL, 0.2 M) was added and the reaction mixture stirred at −78 °C for additional 5 minutes. To this mixture Zn powder (5 mmol, 5.0 equiv.) was added and the cooling bath was removed. The resulting reaction mixture was allowed to warm

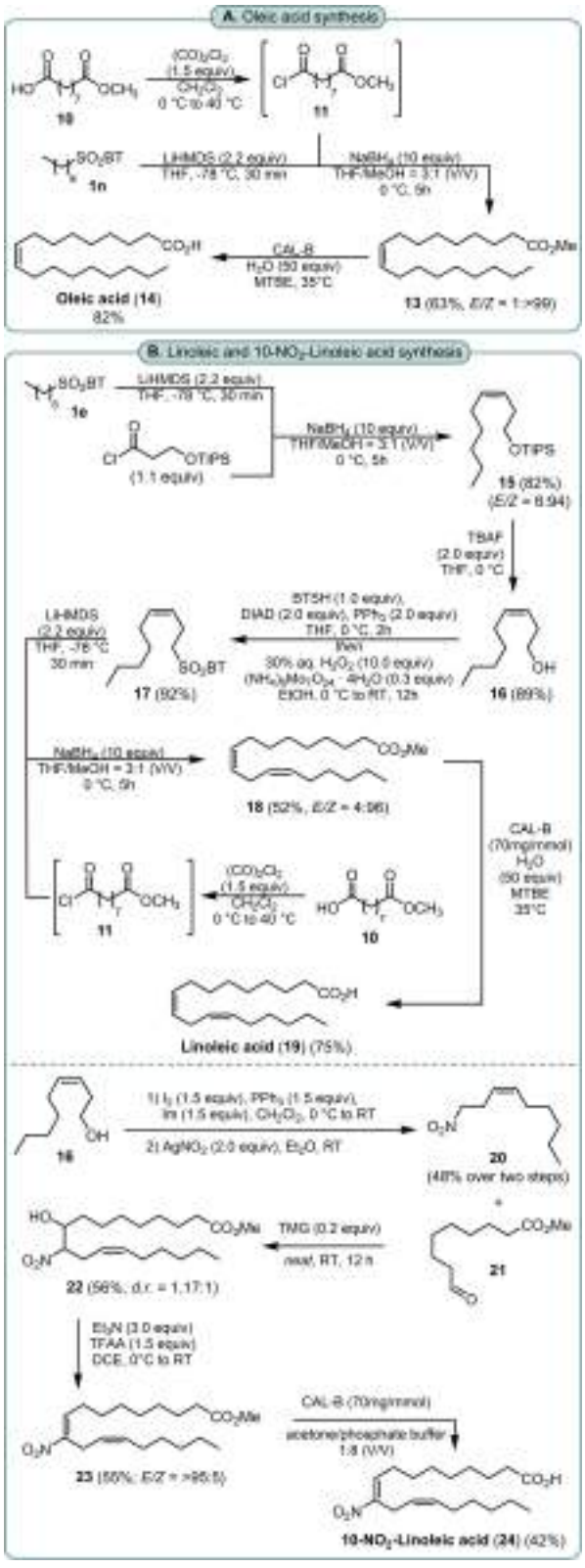

Figure 6. (A) Oleic acid synthesis based on the (*Z*)-selective Method B. (B) linoleic and 10-NO_2-Linoleic acid synthesis. *TMG = 1,1,3,3-Tetramethylguanidine, CAL-B = Candida antarctica Lipase B, Im = imidazole.*

spontaneously to RT, then stirred for 9 hours. Reaction was quenched with the addition of EtOAc (20 mL) and the whole solution was filtered through a pad of Celite®. The filter cake was washed with EtOAc (3×15 mL) and the combined filtrates were washed with saturated aqueous Na_2CO_3 (20 mL), brine (25 mL), dried over anhydrous Na_2SO_4 and solvents removed under reduce pressure to yield crude product. The crude product was purified by flash column chromatography on silica gel eluting with *n*-hexane/EtOAc to afford pure product **8**.

General Procedure for (*Z*)-Olefin 3 Synthesis (Method B)

Sulfone **1** (1 mmol, 1.0 equiv.) was dissolved in dry THF (10 mL, 0.1 M) and cooled to −78 °C (acetone/dry ice, external). After 5 minutes, LiHMDS (2.2 mmol; 2.2 equiv.; 1.0 M solution in THF) was added dropwise. The reaction mixture turned light orange upon its addition. Acyl chloride **2** (1.1 mmol, 1.1 equiv.) was added slowly over 5 minutes. The resulting reaction mixture was allowed to stir at −78 °C for 30 minutes before the cooling bath was removed. The reaction mixture was allowed to stir at RT for 20 min before MeOH (3.3 mL, THF/MeOH = 3:1 (V/V)) was added. The mixture was cooled to 0 °C and stirred for an additional 5 minutes, before $NaBH_4$ (10 mmol, 10 equiv.) was added at 0 °C. The resulting reaction mixture was stirred at 0 °C for an additional 4 h, before being quenched with 2 M aq. HCl (20 mL). The resulting mixture was stirred at RT for 4 h. Resulting phases were separated, and the aqueous phase was extracted with EtOAc (3×20 mL). Organic layers were combined and washed with water (10 mL), brine (15 mL), dried over Na_2SO_4, filtered and solvents were removed under reduce pressure to provide the crude product. The crude product was purified by flash column chromatography on silica gel eluting with *n*-hexane/EtOAc to afford pure product **3**.

General Procedure for (*E*)-Olefin 3 Synthesis (Method C)

Sulfone **1** (1 mmol, 1.0 equiv.) was dissolved in dry THF (0.1 M) and cooled to −78 °C (acetone/dry ice, external). After 5 minutes, LiHMDS (2.2 mmol, 2.2 equiv., 1.0 M solution in THF) was added dropwise. The reaction mixture turned light orange upon its addition. Acyl chloride **2** (1.1 mmol, 1.1 equiv.) was added slowly over 5 minutes. The resulting reaction mixture was allowed to stir at −78 °C for 30 minutes before the cooling bath was removed. The reaction mixture was allowed to stir at RT for 20 min before 2-propanol (2.5 mL, THF/2-propanol = 4:1 (V/V)) was added. The mixture was stirred at RT for an additional 5 minutes, before adding anhydrous $ZnCl_2$ (2.5 mmol, 2.5 equiv.) was added. The resulting suspension was cooled to 0 °C and stirred for an additional 5 minutes before $NaBH_4$ (5 mmol, 5 equiv.) was added. The resulting reaction mixture was then stirred at 0 °C for an additional 4 h, before being quenched with 2 M aq. HCl (20 mL). The resulting mixture was stirred at RT for 4 h. Resulting phases were separated, and the aqueous phase was extracted with EtOAc (3×20 mL). Organic layers were combined and washed with water (10 mL), brine (15 mL), dried over Na_2SO_4, filtered, and solvents were removed under reduce pressure to provide the crude product. The crude product was purified by flash column chromatography on silica gel eluting with *n*-hexane/EtOAc to afford pure product **3**.

Experimental procedures and characterization data for sulfones **1a**–**n**, olefins **3a**–**r**, ketones **8a**–**k**, pyrroles **9a**–**c**, oleic acid **13**, linoleic acid **19**, and 10-nitro-linoleic acid **24** preparation, as well as copy of ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of prepared compounds are included in the supporting information.

Author Contributions

D. J.-Y. B., D. C., O. K., and J. P. carried out most of the experiments. V. F. carried out all experiments connected with the PT-sulfones. F. Z., P. L. and M. F. contributed to the olefination protocol and validated developed protocols. O. K. carried out all pyrrole-linked experiments. D. C. carried out all fatty acid synthesis related experiments. J. P. wrote the original draft based on the data obtained from all coauthors. J. P. designed the project, was responsible for the acquisition of funding and led the project. All co-authors read the original draft and collaborated on its corrections/modifications/changes.

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Review

Latest Developments of the Julia–Kocienski Olefination Reaction: Mechanistic Considerations

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Abstract: Since its discovery, the Julia–Kocienski olefination reaction has over past 30 years become one of the key C-C connective methods that is used in late-stage natural product synthesis. The reaction proceeds under mild reaction conditions, with a wide substrate scope and functional group tolerance range and with high (*E*) selectivity. In this focused review, we discuss the reaction from a mechanistic point of view and disclose key features that play an important role in reaction selectivity. Finally, the mechanistic aspects of the newly developed modification of the Julia–Kocienski reaction, which allows the formation of both (*E*) and (*Z*) olefins from the same reaction partners, are discussed.

Keywords: Julia–Kocienski reaction; olefination; reaction selectivity; reaction mechanism

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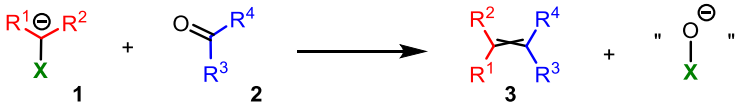
1. Introduction

Alkenes belong to a chemical functional group that is omnipresent in literally all natural products. Interestingly, since the early times when organic synthesis slowly became a 'useful' scientific discipline, many synthetic strategies have focused on the stereoselective synthesis of these structural motives. In particular, methods that allow for the connective stereoselective introduction of the olefin moiety have become very valuable tools for this goal. Over the past 100 years, many different connective olefination methods have been developed, although many of them follow the same retrosynthetic pathway [1]; they are based on the reunion of α-negative charge-stabilizing reagents **1** with aldehydes or ketones **2** (Table 1).

Table 1. Common carbonyl-based olefination methods used in organic synthesis.



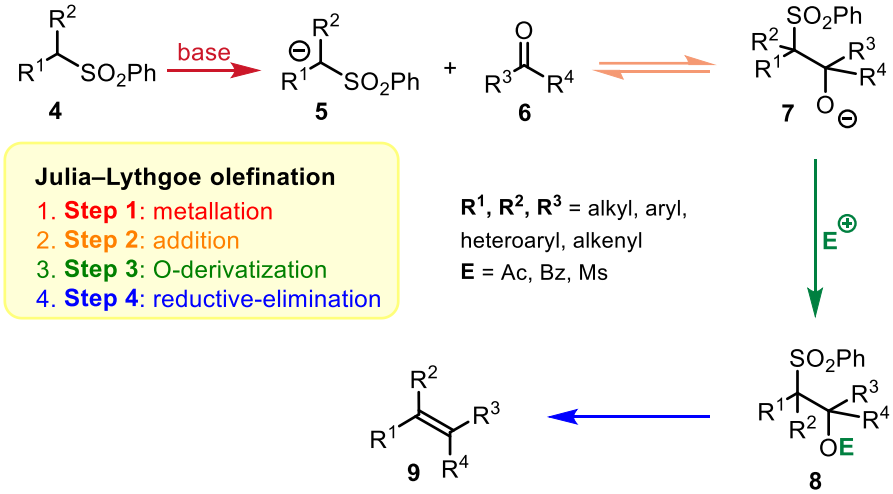
Activating Unit X	Olefination Method	Litt. Reference
$PhSO_2$	Julia–Lythgoe	Ref. [1]
$ActSO_2$	Julia–Kocienski	Ref. [1]
PhSO(NMe)	Johnson	Ref. [2]
R_3P^+	Wittig	Ref. [3]
$R_2P(=O)$	Wittig–Horner	Ref. [3]
$(RO)_2P(=O)$	Horner–Wadsworth–Emmons (HWE)	Ref. [4]
R_3Si	Peterson	Ref. [5]
R_2B	Boron–Wittig	Ref. [6]

Since the introduction of the Wittig reaction [7,8] in the late 1950s of the twentieth century, the Wittig [3], Horner–Wadsworth–Emmons [4], Johnson [2], Peterson [5], and Julia olefination [1] methods have established themselves as the most widely used olefination protocols. Each of these methods has its advantages and drawbacks, which have changed over time because each of the methods has gone through a long and interesting development process since its original disclosure. In this personalized, focused review, we wish to discuss the mechanism of the so-called modified Julia reaction [1,9–16], also known as the Julia one-pot, Silvestre–Julia, or Julia–Kocienski olefination reaction, as well as its development in terms of the reaction mechanism and selectivity. The last part of the review will focus on the recently developed modification of the Julia–Kocienski olefination transformation that allows selective formation of (*E*) or (*Z*) olefins by a simple change in the reaction workup, and its scope and limitations will be compared with the Petersen and Zweifel olefination methods; protocols that also allow selective (*E*) or (*Z*) olefin formation by simple change in the reaction workup.

2. Origins and Mechanism of the Julia–Kocienski Olefination Reaction

2.1. Julia–Lythgoe Olefination vs. Julia–Kocienski Olefination: A Comparison

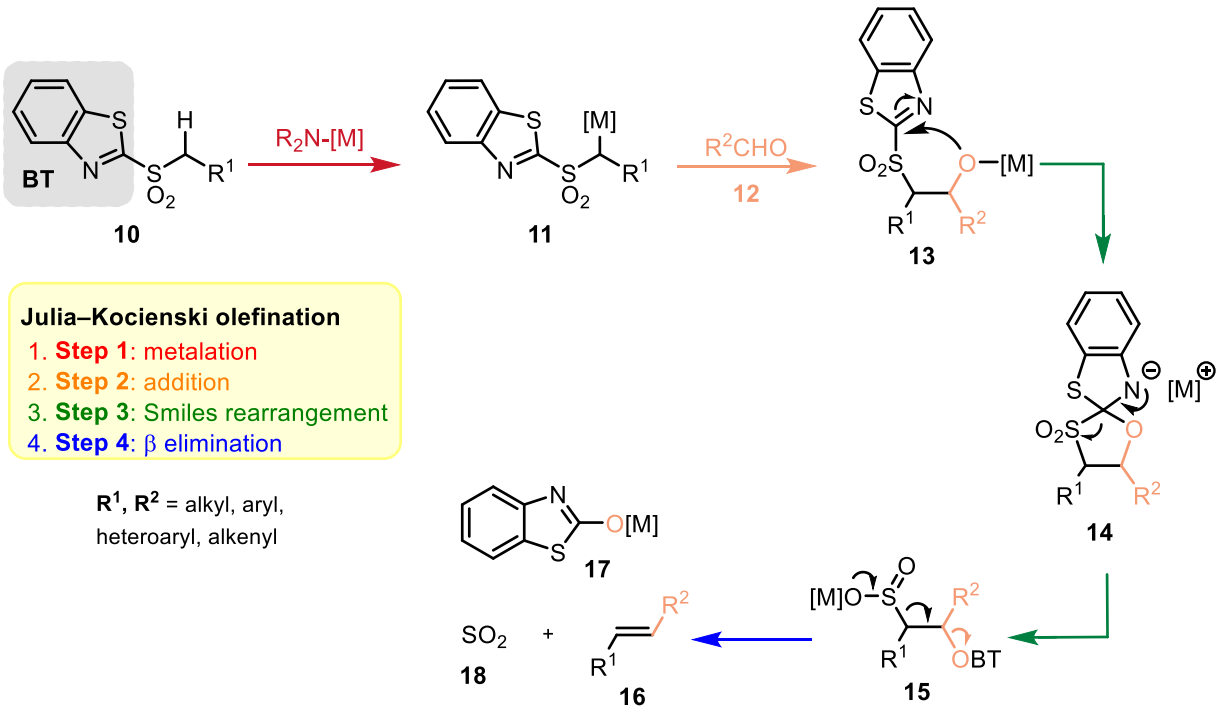
Classical Julia olefination, also known as Julia–Lythgoe olefination, was described for the first time in 1973 by (Mark) Julia and Paris [17] and was later developed by Kocienski and Lythgoe [18]. The original protocol was soon expanded for the beneficial *O*-derivatization step, consisting of four distinct stages carried out commonly in the two-pot protocol (Scheme 1): (1) the metalation of an alkylarylsulfone **4**; (2) the addition of the resulting carbanion species **5** to an aldehyde or ketone **6**; (3) the *O*-acylation (sulfonylation) of the adduct **7**; (4) the elimination of the β-acyl (sulfonyl) oxysulfone **8** intermediate. The addition of **5** to **6** typically yields product **7** as a mixture of all possible diastereoisomers; however, this is not of consequence because the stereochemical information encoded in **7** (or **8**) is lost during the elimination step. A common feature of Julia–Lythgoe olefination is its high (*E*)-stereoselectivity [1]—a consequence of the various radical mechanisms that operate in the final stage of reductive elimination [19].



Scheme 1. The Julia–Lythgoe olefination protocol.

The main drawbacks of Julia–Lythgoe olefination, namely the steric requirement-driven (*E*/*Z*) selectivity and the two-pot protocol, were in 1993 overcome by Silvestre Julia [20,21] (brother of Mark Julia). Their modification of the standard Julia–Lythgoe olefination protocol was based on the replacement of the phenylsulfonyl group with the benzo[*d*]thiazol-2-ylsulfonyl (BT) group (Scheme 2) [22–24]. The common features of the new transformation of the Julia–Lythgoe olefination reaction are the first two steps: (1) metalation; (2) the addition of metalated sulfone **11** to aldehyde **12**. Since in this case the aryl group in the alkyl aryl sulfone is an electron acceptor, the initially generated *β*-alkoxy

sulfone adduct **13** can undergo a spontaneous Smiles rearrangement (S to O migration of the heteroaryl group) to yield adduct **15**. The subsequent β-elimination of SO_2 (**18**) and of an aryloxide anion (**17**) in **15** directly forms olefin **16**.



Scheme 2. The Julia–Kocienski olefination reaction—a mechanistic overview.

As mentioned above, Silvestre Julia introduced the BT group as the only electron acceptor aryl group suitable for the Julia–Kocienski olefination reaction. However, this situation did not last long, and many other research groups introduced several different heteroaryl groups such as pyridin-2-yl (**PYR**) [20,25], 1-phenyl-1*H*-tetrazol-5-yl (**PT**) [13], 1-*tert*-butyl-1*H*-tetrazol-5-yl (**TBT**) [26], and 3,5-bis(trifluoromethyl)phenyl (**BTFP**) [20,27,28]. Interestingly, only the **PT** group introduced by Kocienski et al. [13,26] possessed sufficiently interesting properties (diminished side reactions such as homocoupling [13], high (*E*) selectivity) that remained along with the original BT group as the most widely used heteroaryl acceptor groups explored in olefination reactions (Figure 1).

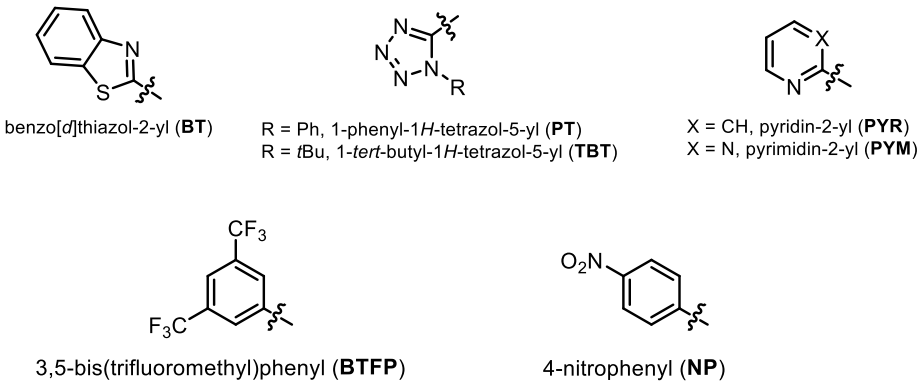


Figure 1. The most commonly used activators in Julia–Kocienski olefination.

The generalized scopes and limitations and the achieved (*E*/*Z*) selectivity rates observed for Julia–Lythgoe and Julia–Kocienski olefination are summarized in Table 2.

Table 2. Comparison of the Julia–Lythgoe and Julia–Kocienski olefination reactions—general features.

Key Features	Julia–Lythgoe	Julia–Kocienski
Practical Difference Origin of Stereoselectivity	Two-pot protocol Reductive elimination Step	One-pot protocol Addition step
Scope of olefin formation		
Terminal	✔	✔
1,2-disubstituted	✔	✔
Trisubstituted	✔	≈
Tetrasubstituted	≈	X
Scope of (*E*)-Stereoselectivity		
1,2-disubstituted	✔	✔
Trisubstituted	≈	X
Tetrasubstituted	≈	X
Scope of (*Z*)-Stereoselectivity		
1,2-disubstituted	X	✔ if the **TBT**-activating group is used;
Trisubstituted	X	X
Tetrasubstituted	X	X

Note: ✔—good to excellent; ≈—acceptable; X—unsatisfactory result(s).

2.2. Reaction Mechanism and Its Impact on the Selectivity of Julia–Kocienski Olefination

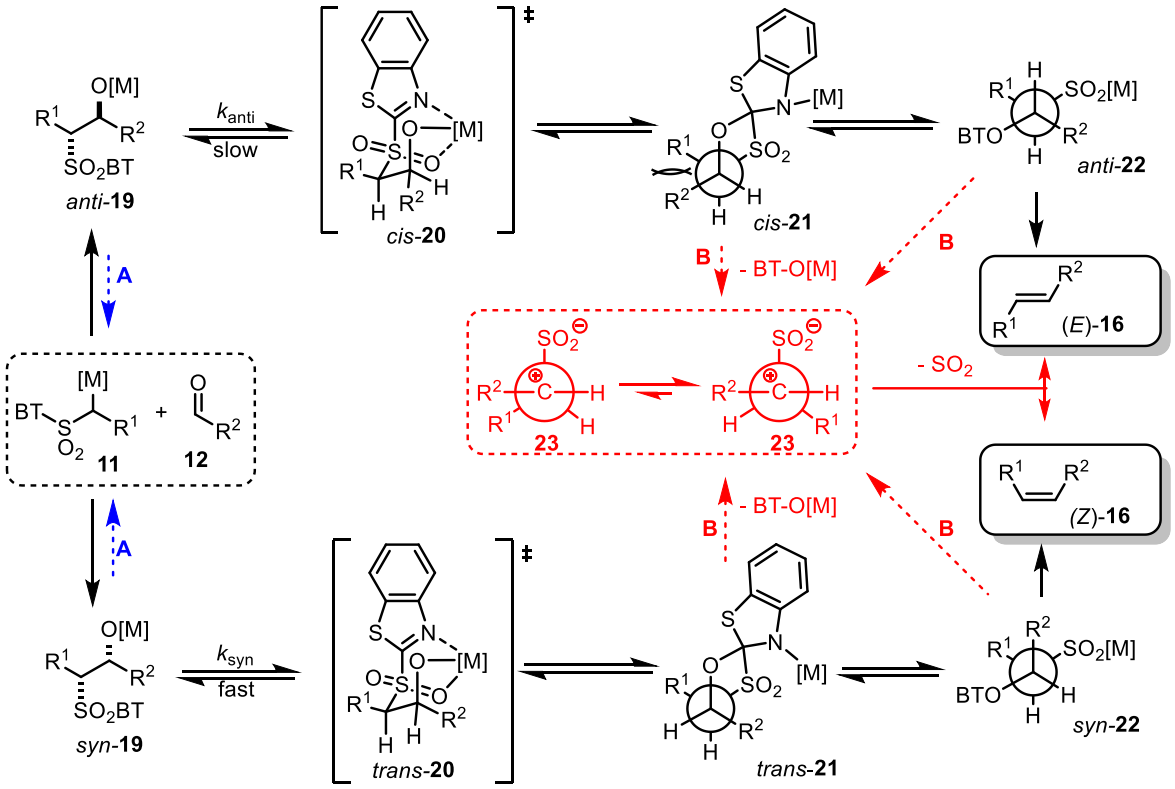
The Julia–Kocienski reaction mechanism was intensively studied by Silvestre Julia [20,21], and their studies were further extended by Kocienski and Blackmore [11–13,26]. Based on these excellent mechanistic studies, the reaction mechanism can be established with respect to the stereochemical outcomes of the reaction (Scheme 3). There are three key features of this mechanism that deserve a brief comment.

(1) The addition of metalated sulfone **11** to aldehyde **12** can provide *anti*-adduct *anti*-**19** via **TS1** or the *syn*-adduct *syn*-**19** via **TS2** (Figure 2). The selectivity in this step is extremely important, since all subsequent transformations of intermediate **19**, the Smiles rearrangement, and the β-elimination process are stereospecific. Thus, the *syn/anti*-selectivity of the addition step determines the final (*E*/*Z*) olefin ratio. Therefore, in theory, the (*E*/*Z*) selectivity of the reaction can be swapped from (*E*) to (*Z*) if proper reaction conditions are applied.

(2) When stabilized metalated sulfonyl anions **11** (R^1 = Ph, alkenyl, etc.) are used, the addition of **11** to **12** becomes reversible (Scheme 3, path A). In this case, the original kinetically driven *syn*/*anti*-ratio of adduct **19** becomes less important in comparison with the Smiles rearrangement reaction rates (transformation of **19** to **22**). In such cases, the rearrangement of *anti*-**19** adduct leading to (*E*) olefin **16** is slower compared to the rearrangement of *syn*-**19** to olefin (*Z*)-**16** due to repulsive 1,2-interactions in the transition state (see *cis*-**20**).

(3) For the elimination step, two borderline mechanisms are generally accepted. In the first, which is the most common, the rearranged intermediate **22** undergoes β-elimination. The elimination is stereospecific, and the *syn*-**19** adduct-rearranged intermediate *syn*-**22** furnishes the (*Z*) olefin and the *anti*-**19** adduct-rearranged intermediate, the compound *trans*-**22** (*trans* refers to the arrangement of R^1 and R^2 within the intermediate cycle), yields the (*E*) olefin. Alternatively, when (hetero)aryl aldehydes **12** (R^2 = (hetero)aryl) are used, an alternative elimination pathway (path B) is postulated to occur. In this case, the elimination pathway should proceed through the formation of an intermediate carbocation **23.** The steric requirements of R^1 and R^2 then play a crucial role in the final (*E*/*Z*) selectivity of the reaction. Path B was used

to explain the unexpected (*E*) selectivity of the coupling reactions carried out using (hetero)aryl aldehydes **12** as substrates.



Scheme 3. Detailed reaction mechanism of the Julia–Kocienski reaction. **A** refers to the retroaddition process that occurs for sulfones **11** with R^1 = (hetero)aryl, alken, alkyn (see point 2 below). **B** referes to previously proposed elimination process that was based on the cation **23** formation (see point 3 below). ‡ indicates the transition state.

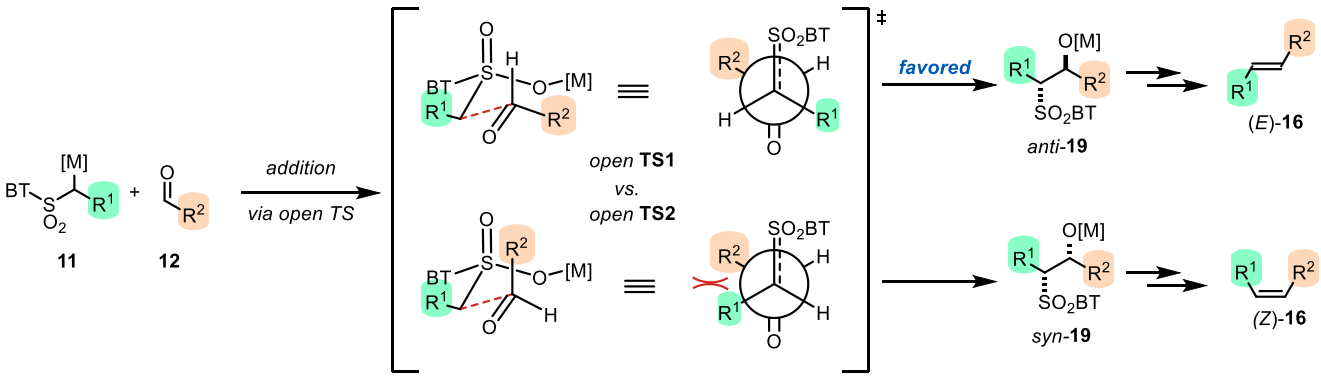
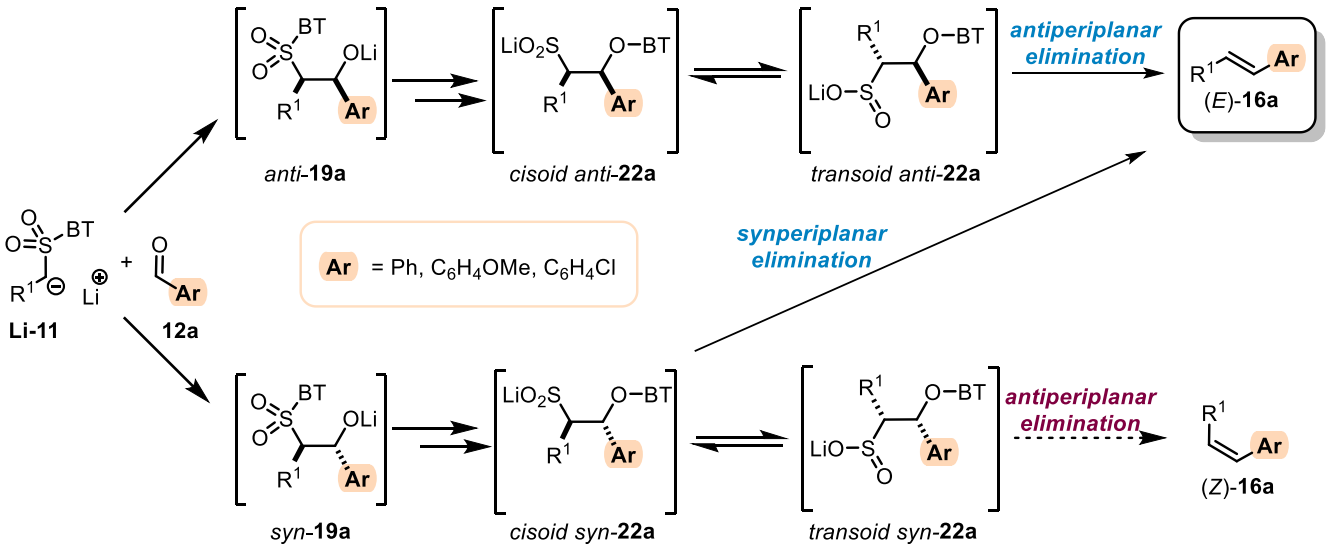


Figure 2. Addition of the metalated sulfone **11** to aldehyde **12**. Mechanistic rationale. ‡ indicates the transition state.

Recently, our group, in collaboration with Robiette's group, proposed an alternative explanation for the observed (*E*) selectivity of these reactions. Our explanation is based on a combined experimental and theoretical study that revealed that the key role in the elimination step is played by the rearrangement product **22a** (Scheme 4) [14]. In general, both the *anti*- and *syn*-**22a** intermediates can adopt the *cisoid* and *transoid* conformations. The conformational equilibrium is strongly influenced by the steric requirements of the R^1 and Ar groups, and in the case of the *anti*-**22a** intermediate, the *transoid* is preferred, while

in the case of *syn*-22*a*, the *cisoid* is preferred. Advanced experimental and theoretical studies have suggested that in the case of a *cisoid* conformation, competitive *syn* elimination can occur [14], explaining the almost exclusive formation of (*E*) olefins observed in the general structure **16a**.

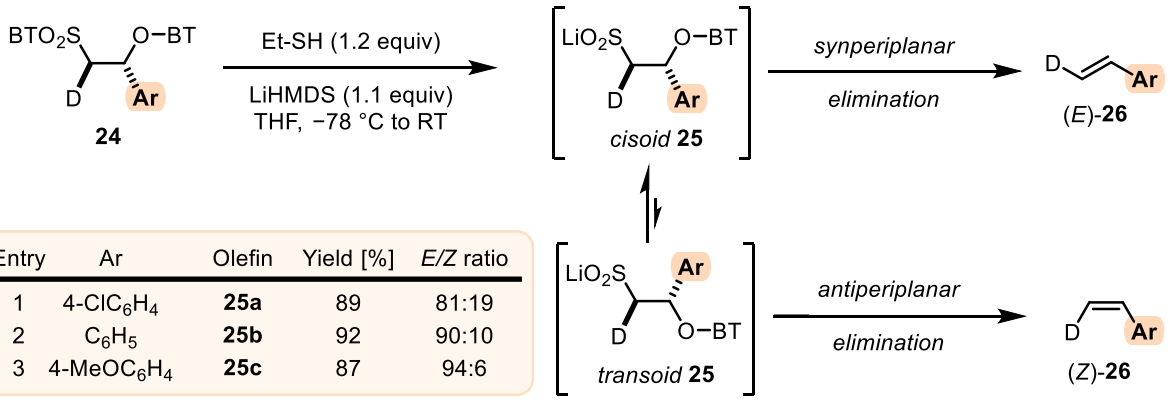


Scheme 4. The rationale for the observed high (*E*) selectivity in the Julia–Kocienski olefination of aromatic aldehydes.

Theoretical studies have also suggested that the *syn* elimination process should be more favored when the aryl substituent R^2 has electron-donating substituents and disfavored when an electron-deficient substituent is present. The postulated prediction was then evaluated using a stereodefined intermediate **24**, which was selectively transformed in situ to the corresponding lithiated anion **25**, which itself was allowed to undergo an elimination process (Scheme 5). With this approach, the generated anion cannot undergo the retroaddition process (it is an intermediate after the rearrangement step), and the nucleophile generated in situ (thiolate anion) is not basic enough to trigger the epimerization process of any of the two epimerizable stereogenic centers. Therefore, only (*Z*) olefin (*Z*)-**26** should be produced as the main product of the transformation. If the reaction proceeds through the carbocation-type intermediate of **23** (see Scheme 3), an approximately 50:50 ratio of the (*E*/*Z*) isomeric mixture can be expected. In all tested cases, the (*E*)-isomer (*E*)-**26**, the product of the *synperiplanar* elimination process, was produced as the main product of the reaction, strongly suggesting that the *syn* elimination process is the main process that operates during the Julia–Kocienski olefination reaction of alkyl sulfones with aryl aldehydes. The observed stronger preference for electron-donating group-containing intermediates to undergo preferentially *synperiplanar* elimination was also in agreement with the DFT-calculation-based prediction.

2.3. Recent Reaction Selectivity Improvements

The reaction mechanism proposed by Julia and Kocienski, which was later confirmed by our own studies, implies that the reaction selectivity is directly linked with the initial *syn*/*anti*-selectivity of the addition step. The adduct ration further directly influences the selectivity (*E*/*Z*) of the overall reaction, regardless of whether the reaction proceeds through the *antiperiplanar* elimination (for R^1 and R^2 = alkyl) or mixed *antiperiplanar* and *synperiplanar* (for R^1 or R^2 = (hetero)aryl) elimination in the final step. Unsurprisingly, most of the methods developed to influence the reaction selectivity in favor of one of the two isomers focus on the key addition step.

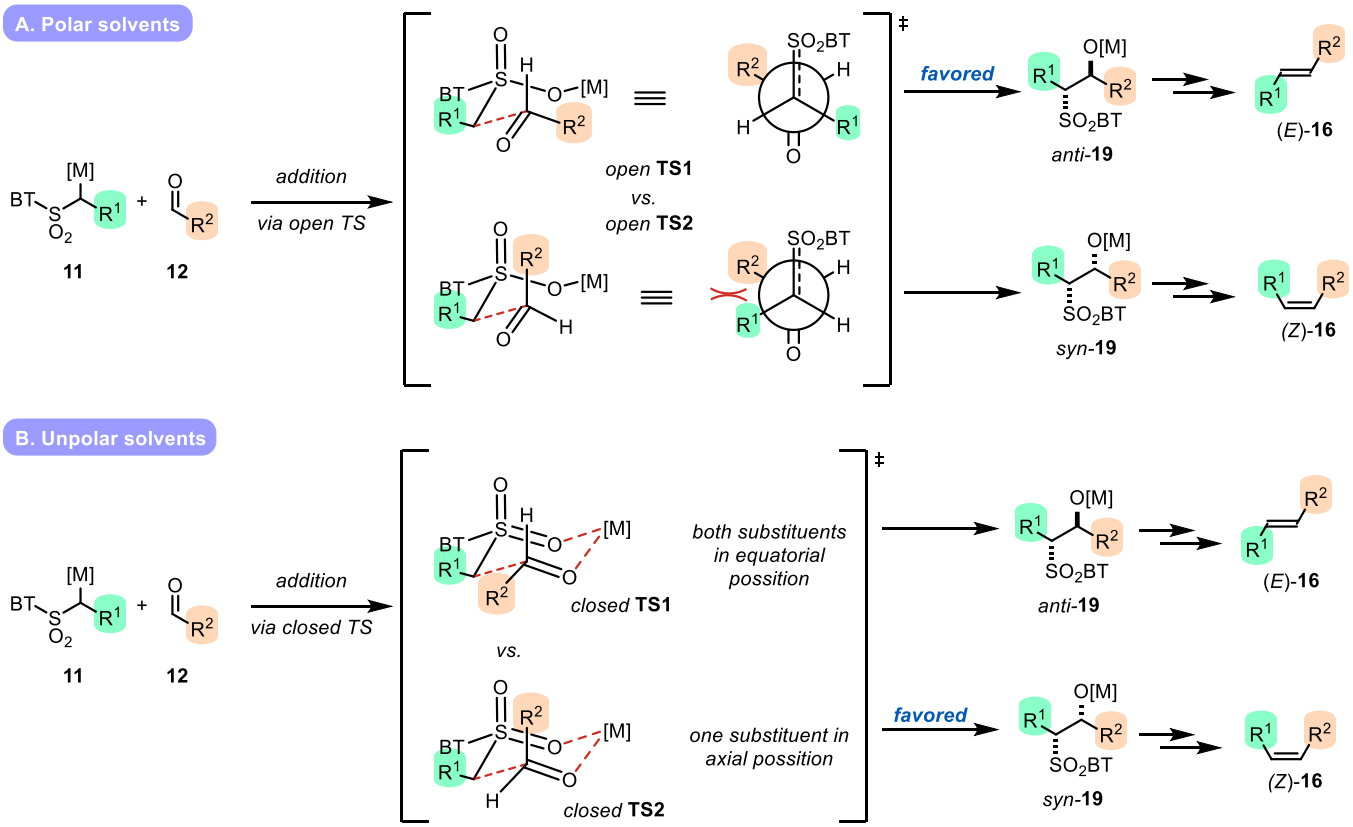


Entry	Ar	Olefin	Yield [%]	*E*/*Z* ratio
1	4-ClC_6H_4	**25a**	89	81:19
2	C_6H_5	**25b**	92	90:10
3	4-$MeOC_6H_4$	**25c**	87	94:6

Scheme 5. Stereoselectivity in the elimination step—a competition between the *synperiplanar* and *antiperiplanar* elimination processes.

2.3.1. Solvent Effect

The most important and straightforward way to influence the *syn*/*anti*-selectivity of the addition step is to choose the right solvent for the transformation. When polar solvents such as THF, DME, or DMF are used, *anti*-adduct *anti*-**19** is the preferred addition product due to its solvent stabilization potential (Scheme 6A). On the contrary, when nonpolar solvents such as toluene are used, the reaction proceeds via a closed transition state (Scheme 6B) and *syn*-adduct *syn*-**19** is preferred.

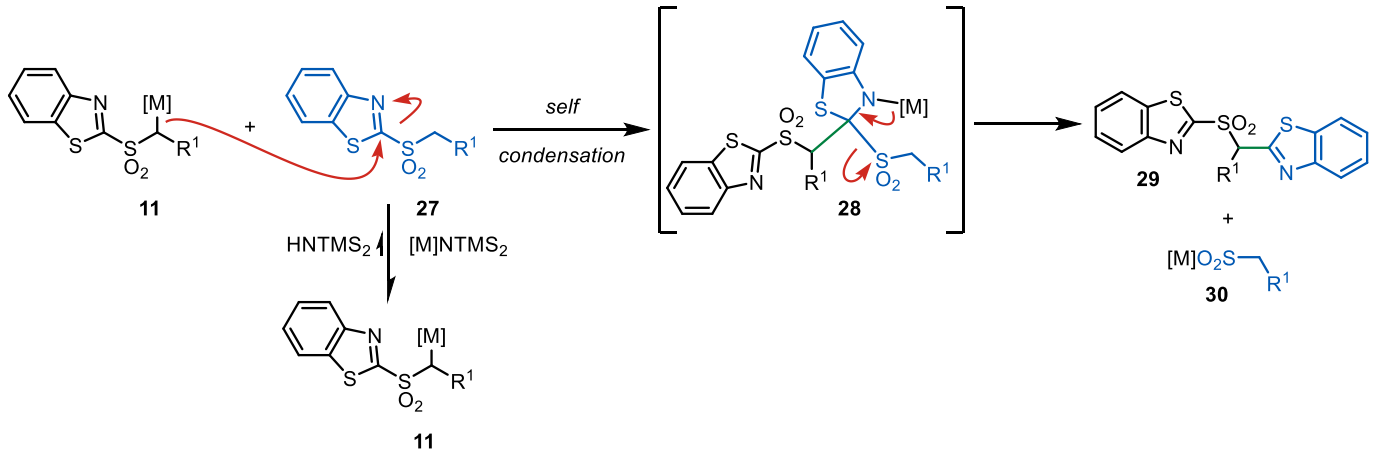


Scheme 6. The impact of the solvent's polarity on the stereochemical outcome of the Julia–Kocienski reaction. ‡ indicates the transition state.

It should be noted that although such an approach is generally applicable and correct, the role of the solvent might be further influenced by metal salts and additional cosolvents.

- Metal cation

The metal cation, which is always present in the reaction mixture as a 'residue' after the deprotonation step, has a key influence on the selectivity of the reaction. In general, cations with the character of a hard Lewis acid, such as Li^+, favor the formation of the (*E*) olefins. It is assumed that the observed (*E*) selectivity is caused by better stabilization of the generated anion **11**, which can be further added due to its lower reactivity to aldehyde **12**, which has better selectivity and favors the *anti*-adduct *anti*-**19**. On the contrary, when a large cation is used, such as K^+, the reaction can proceed preferentially either via closed TS or the solvent can increase the dissociation of the cation from **11**, thereby increasing the reactivity. The first case is typical for nonpolar solvents (e.g., toluene) because the solvent does not provide additional stabilization to the reagents or reaction intermediates. In the latter case, the dissociation of the cation from reagent **11** increases the reactivity of the anion and leads to faster production of the kinetic product of the addition step, *anti*-isomer *anti*-**19**. However, it should also be noted that an increase in anion **11**'s reactivity can also inevitably lead to the undesired self-condensation of reagent **11** (Scheme 7); thus, a compromise between selectivity and reactivity has to be reached.



Scheme 7. The self-condensation reaction that accompanies the reaction of anion **11**.

- Cosolvents

The addition of the cosolvents to the reaction mixture can also be beneficial when (*E*) selectivity is desired. It was observed that the addition of cosolvents such as DMPU or HMPA to reaction mixtures carried out in THF or DMF led to an increase in the (*E*) olefin selectivity of the desired product. It is believed that the cosolvent's role is in metal cation scavenging, with an impact similar to that described in the previous section (increased reactivity that favors *anti*-adduct formation).

2.3.2. Additives

Another way to increase the selectivity (*E*/*Z*) of the Julia–Kocienski reaction is by adding additives to the reaction mixture. Over the years, many different additives have been used for such purposes; however, only a few of them have had a significant effect. The relevant ones are listed below.

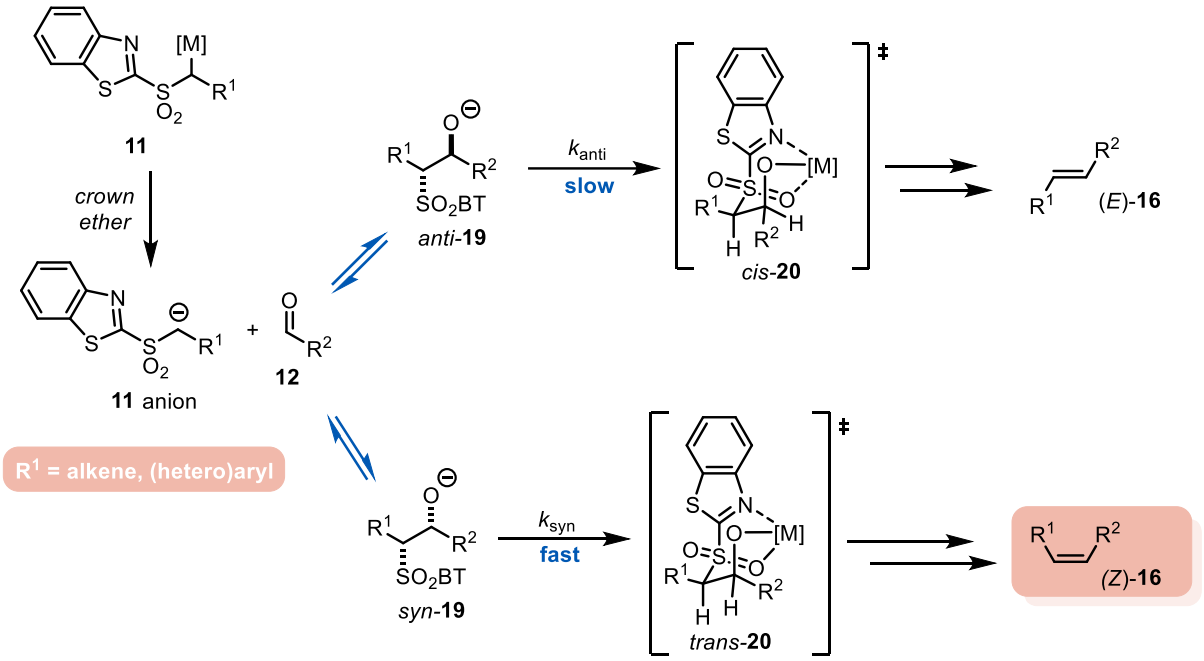
- Crown ethers

As mentioned in the previous section, the role of the (co-)solvent was shown to have a tremendous effect on the reaction yield and selectivity. As a modus operandi, it was postulated that polar solvents increase the reactivity of anion **11** due to a cation–anion separation (reaction kinetic) that leads to the preferential formation of *anti*-adducts (polar solvents) or *syn*-adducts (nonpolar solvents). As a disadvantage, the self-condensation of metalated sulfone **11** (Scheme 7) was observed. The use of specific cation-chelating

cosolvents such as HMPA or DMPU showed only limited success, even though in several cases it led to the diminished formation of self-condensation products and an increase in (*E*) selectivity.

Based on the same logic, to increase the reactivity of metalated sulfone **11** and increase the formation of the *anti*-adduct (kinetic product), an excess of crown ethers (18-crown-6 for K^+, 12-crown-6 for Li^+) [29] can be used during the reaction, as demonstrated in several recent total syntheses of natural products (e.g., zeaenol [30], paecilomycins E and F [31], amphidinolide E [32], and salarins A and C [33]).

However, it should be noted that if metalated sulfone **11** is used with a group in the lateral chain (R^1) that is capable of stabilizing the generated anion, the addition of generated anion **11** to aldehyde **12** is reversible (Scheme 8). Consequently, the *syn*/*anti* ratio of adducts **19** is in equilibrium and (*Z*) olefin (*Z*)-**16** is formed preferentially due to a faster ($k_{anti} < k_{syn}$) Smiles rearrangement step [34].



Scheme 8. Role of crown ethers in the Julia–Kocienski reaction. High (*Z*) selectivity in the case of stabilized metalated sulfones. ‡ indicates the transition state.

- Ammonium salts

The use of ammonium salts proved to also be beneficial, and in several cases of highly complex molecular scaffolds led to increases in the observed reaction yield and (*E*) selectivity [35,36]. It is believed that the role of ammonium salts is in the activation of aldehyde **12**, where due to its steric requirements it increases the *anti*-selectivity of the addition step. Note also that the role of the counter-anion of the ammonium salt is not innocent. The best (*E*) selectivity was observed when potassium-containing metalated sulfone **11** was reacted in the presence of TBAB (tetrabutylammonium bromide) and lithium-containing metalated sulfone **11** was reacted in the presence of TBAC (tetrabutylammonium chloride). Such observations suggest the beneficial formation of KBr and LiCl salts during the reaction.

- Chelating salts

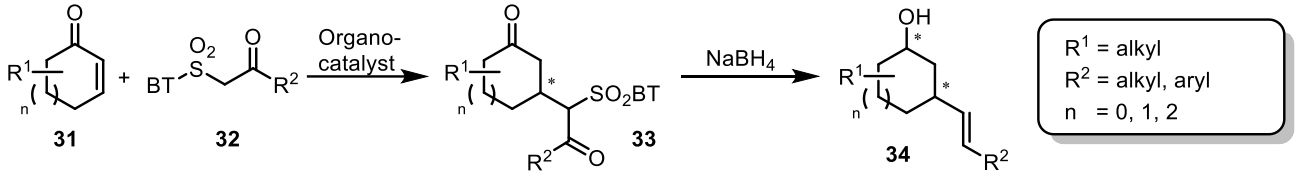
Similarly, metal cations (e.g., $CeCl_3$ [37,38], $MgCl_2$ [39], $ZnCl_2$, and LiBr) can be used to activate aldehyde **12** during the reaction. The addition of such a salt generally results in an increase in the reaction yield of the transformation. The (*E*/*Z*) selectivity of the transformation is influenced only if aldehydes bearing *α*-alkoxy substituents [39] are used

in the presence of an excess of $MgCl_2$ or $ZnCl_2$ (addition via the Cram chelate transition state) [40].

3. Julia–Kocienski Olefination—Extension to Carboxylic Acid Derivatives

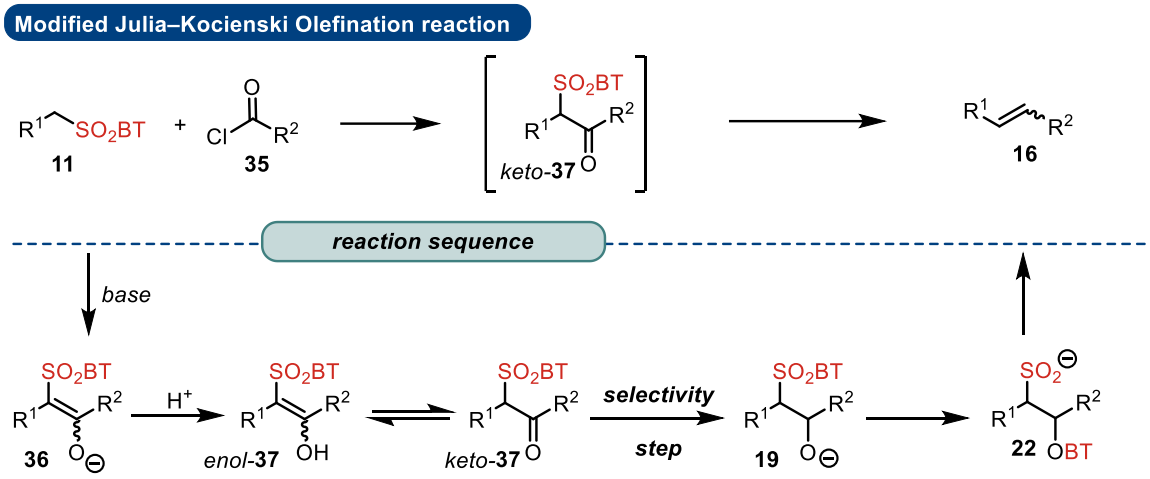
All of the olefination methods mentioned above are based on the reunion of the metalated sulfone **11**-type intermediate and a carbonyl-containing intermediate **12** (Scheme 2). The overall transformation can, thus, be regarded as an addition–rearrangement–elimination sequence, where the final (*E*/*Z*) selectivity of the newly olefinic bond is determined by the addition step. Therefore, the stereoselectivity is dictated by the reaction kinetic of the addition step (kinetic conditions) or by the kinetic of the rearrangement step (as the addition step is in equilibrium) (Scheme 3). Gueyrard's group also demonstrated that in some cases lactones can also be used as reaction partners in the Julia–Kocienski reaction and that the subsequent addition–rearrangement–elimination step then yields the corresponding enol ethers [16].

However, recently this paradigm changed, since we introduced the 'reaction work-up-driven selectivity' approach for the Julia–Kocienski reaction [41]. Analogous to the famous Peterson olefination reaction [5], we designed and optimized the new Julia–Kocienski protocol, which allows selective (*E*) or (*Z*) olefin formation via a simple change in the reaction work-up procedure. Our protocol is based on the seminal work by Jørgensen et al. [42,43], which demonstrated that *β*-keto BT sulfones **33** can be successfully transformed into the corresponding olefins **34** in high yields and with (*E*) stereoselectivity (Scheme 9).

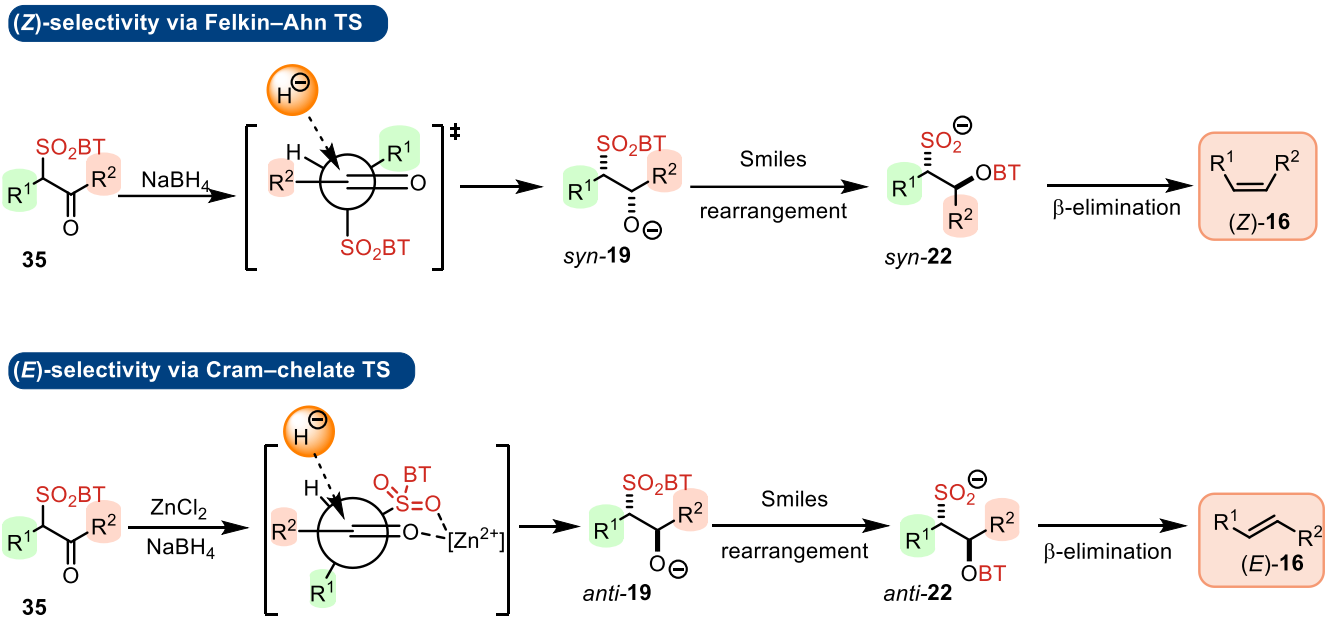


Scheme 9. The seminal work by Jørgensen et al. [42,43] demonstrated the possibility of the stereoselective transformation of *β*-keto sulfones into the corresponding (*E*) olefins **34**. * refers to the stereogenic center.

Based on these results, we designed a novel type of Julia–Kocienski reaction that allows the synthesis of the desired olefins **16**, starting from the metalated sulfone **11** and the acyl halides **35** (Scheme 10). In this sequence, the reunion of the two reagents (compounds **11** and **35**) is carried out using a previously described protocol [44,45]. The generated adduct **36** is then quenched in situ with the external source of the proton (the protic solvent, e.g., MeOH) and the *β*-keto sulfone **37** is formed. Compound **37** is present in the reaction mixture as a dynamic mixture of its keto and enol derivatives. When an external mild reducing agent (e.g., $NaBH_4$) is added, the keto form of *keto*-**37** is selectively reduced, and the nucleophilic hydride approach is directed according to the Felkin–Ahn model [46] (Scheme 11). Carbonyl reduction preferentially generates a *syn* derivative of *β*-hydroxy sulfone *syn*-**19**, and compound *syn*-**19** is further converted via the Smiles rearrangement–*β*-elimination sequence of the Julia–Kocienski olefination reaction to olefin (*Z*)-**16**. However, if chelating salts such as $ZnCl_2$ are added to the reaction mixture prior to $NaBH_4$, the reduction proceeds through the Cram chelate model and the *anti*-*β*-hydroxy sulfone *anti*-**19** is formed. Consequently, compound *anti*-**19** then generates, after the Smiles rearrangement–*β*-elimination sequence, desired (*E*) olefin (*E*)-**16**.



Scheme 10. Proposed reaction sequence for the modified Julia–Kocienski olefination reaction, where the stereoselectivity of the generated olefin is not determined in the addition step.

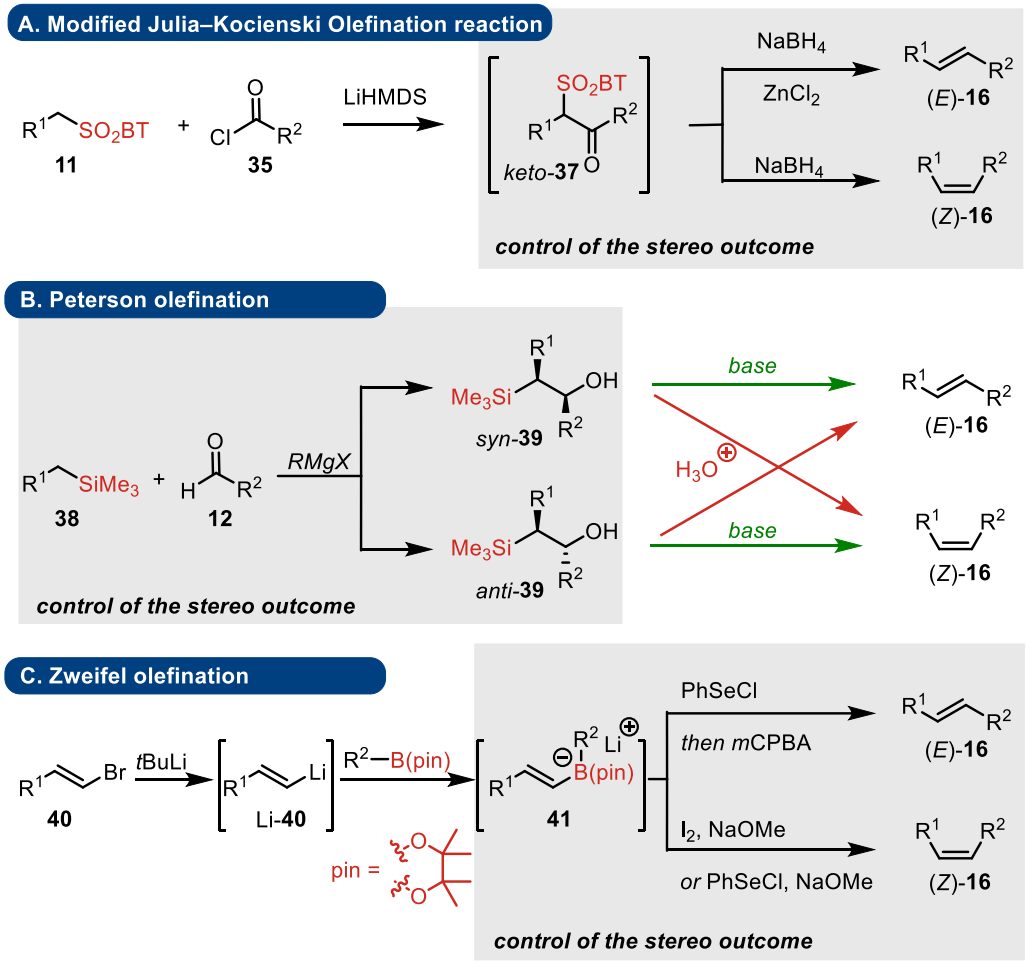


Scheme 11. The rational design behind the stereoselective modified Julia–Kocienski olefination reaction. ‡ indicates the transition state.

Although only the preliminary scope and limitations of the transformation were established (28 examples), the method was successfully applied in the context of (nitro)fatty acid synthesis [41].

4. Julia–Kocienski, Peterson, and Zweifel Olefination Reactions: A Brief Comparison

In the previous chapter, we reviewed the modified Julia–Kocienski olefination reaction and disclosed its preliminary scope and limitations. In this chapter, we discuss this type of reaction in the context of the two presumably most used coupling methods that allow the generation of (*E*) or (*Z*) olefins stereoselectively during the reaction work-up—Peterson olefination [5,47] and Zweifel [48,49] olefination. Scheme 12 highlights three general schemes of the three mentioned methods. Each of the methods will now be discussed from the substrate and stereo outcome control viewpoints.



Scheme 12. Modified Julia–Kocienski, Peterson, and Zweifel olefination reactions. Three different transition-metal-free types of connective coupling reactions that allow selective (*E*) and (*Z*) olefin formation.

4.1. Modified Julia–Kocienski Reaction

- Substrates

Modified Julia–Kocienski olefination in general reunites two type substrates, sulfone **11** and acyl halide **35**. Sulfone **11** is generally obtained from the corresponding alcohol in the two-step Mitsunobu reaction [50]–oxidation protocol. Both steps generally proceed under very mild reaction conditions, since the second oxidation step is generally performed using H_2O_2 in the presence of molybdenum or tungsten-based catalysts [51].

- Elimination step

The mechanism of the elimination step that occurs after the decisive step controlling the stereo outcome of the reaction—the reduction of the carbonyl—was discussed in detail in the previous chapter. For the carbonyl reduction step, it should be noted that its result is strongly influenced by the steric encumbrance of the substituents on the acyl chloride **35**. Furthermore, if the R^1 and R^2 groups are aryl, a competitive *syn* elimination process will occur to further hammer the stereoselectivity of the reaction (see Section 2.2 for more details).

- Presence of stereogenic centers

At the present time, there are no sufficient experimental data that would experimentally address the question of the tolerance of the method toward the stereogenic center's

stability. However, one could conclude that stereogenic centers that are base- and acid-sensitive in the α position of acyl halide **35** should not be tolerated. Similarly, base-sensitive centers in and further on positions in sulfone **11** or acyl halide **35** might also undergo epimerization under the applied reaction conditions.

4.2. Peterson Olefination

Peterson olefination is seemingly 'the most classical' olefination transformation of the three methods discussed. It explores one of the 'classical' precursors of the olefination coupling, aldehyde, and the ratio (*E*/*Z*) of the formed olefin is determined in the first addition step [5,47]. However, there are also two characteristics that separate this type of connective method from the others: (1) the stability of the organosilicon compounds allows for further pre- or post-addition step transformations of β-hydroxy silanes that allow the stereoselective formation of enamines [52,53] or vinyl sulfones [54]; (2) due to the commercial availability of the TMS-CH_2-MgCl reagent, Peterson olefination is commonly used to generate vinyl olefins from sterically hindered or perfluorinated ketones [55]. However, both trends are beyond the scope of this focused review and will not be discussed.

- Substrates

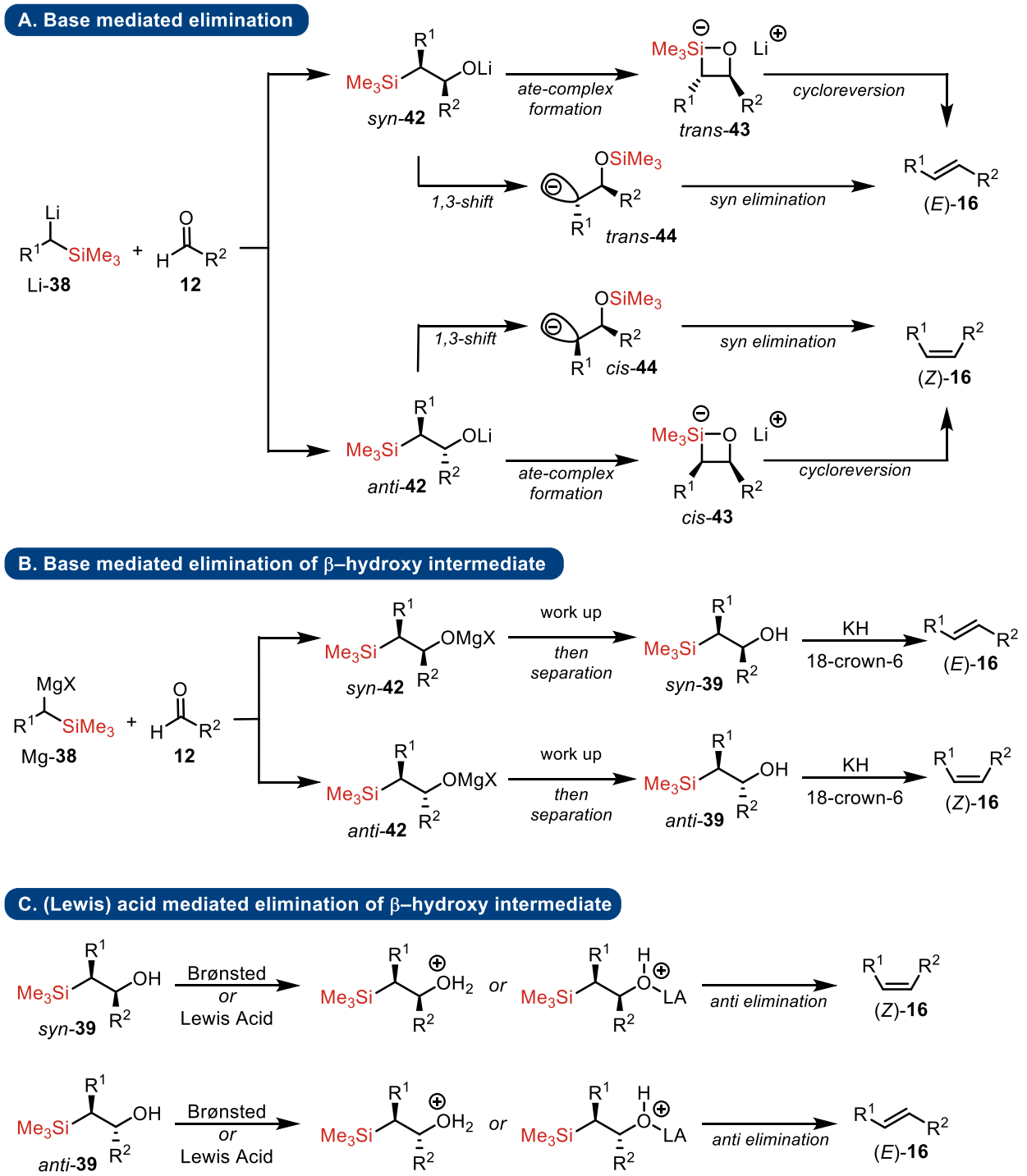
The transformation is based on the reunion of the two substrates, aldehyde **12** and silane **38** (Scheme 12). While the synthesis of the aldehyde **12** coupling partner is well documented and can be achieved via various means, under very mild reaction conditions, and on rather complex substrates, the formation of the silicon-containing partner **38** was for decades rather tricky. However, recent (past two decades) developments, especially in the field of transition-metal-mediated hydrosilylation reactions, have made available even complex silanes **38** [56,57].

As mentioned previously, the stereoselectivity outcome of the reaction is determined in the first addition step of the reaction. The addition of an anion generated from **38** to aldehyde **12** generally proceeds with reasonably good diastereoselectivity, and the influence of (co)solvents and ions is similar to those observed for the Julia–Kocienski olefination reaction (see Scheme 6). The generated adducts, anion *syn*-**42** and *anti*-**42**, then spontaneously undergo elimination via the pentacoordinate 1,2-oxasiletanide intermediate, which subsequently undergoes cycloreversion (see the elimination step below). However, when using the α-silyl organomagnesium reagent Mg-**38**, due to a strong magnesium–oxygen bond, the corresponding adduct **42** is generally sufficiently stable and can be trapped in the form of β-hydroxy silane **39** (Scheme 12B). Both generated diastereoisomers, *syn*- and *anti*-**39**, can be further separated and submitted to the stereoselective elimination step (vide infra).

- Elimination step

As mentioned above, the elimination step in Peterson olefination generally proceeds spontaneously immediately after the addition step (Scheme 13A). In such cases, it is generally accepted that the reaction proceeds through the formation of the 1,2-oxasiletanide intermediate through the addition–cycloreversion mechanism or through the 1,3 migration–*syn*periplanar β-elimination mechanism. The reaction is stereoselective and the configuration of adduct **42** is reflected in the final *E*/*Z* ratio of the olefinic product **16**, since the *syn*-**42** adduct yields (*E*) olefin (*E*)-**16** and the *anti*-**42** adduct yields (*Z*) olefin (*Z*)-**16**.

Pure (*E*) or (*Z*) olefins can be obtained if interrupted Peterson olefination is performed. In such a case, the intermediate β-hydroxy silane **39** is isolated and the two diastereoisomers are separated. In this case, if submitted to basic conditions, the same olefin type ((*E*/*Z*) configuration) as in the one-pot protocol is obtained (Scheme 13B). However, if the intermediates *syn*- and *anti*-**39** are submitted to Brønsted or Lewis acid reaction conditions, the reaction proceeds through the *anti*periplanar β-elimination process and the stereochemical result of the reaction is the opposite of that from base-mediated elimination. The *syn*-**39** isomer then produces (*Z*) olefin (*Z*)-**16** and the *anti*-**39** yields (*E*) olefin (*E*)-**16** (Scheme 13C).



Scheme 13. (**A**) The mechanism of Peterson olefination carried out in a one-pot manner. The mechanism of base-mediated elimination is depicted. (**B**) The Peterson olefination sequence carried out as a two-step protocol. The elimination of the *β*-hydroxy intermediate proceeds under basic conditions. (**C**) The second step of the Peterson olefination reaction, in which the elimination of the *β*-hydroxy intermediate proceeds under Brønsted or Lewis acid conditions.

- Presence of stereogenic centers

Similarly to Julia–Kocienski olefination, the substrates used as starting materials in the Peterson olefination reaction have stereogenic centers in the α position that are base- and acid-sensitive to the carbonyl group in **12**. Similarly, the base-sensitive centers

in β and in positions close to the silicon group in silane **38** or aldehyde **12** could also undergo epimerization.

4.3. Zweifel Olefination

The Zweifel olefination protocol differs from the Julia–Kocienski and Peterson olefination reactions in many ways. Firstly, the olefinic bond found in the final product is already present in one of the two starting substrates, normally in the vinyl halide **40** (Scheme 12C). In its original form, Zweifel olefination is 'nothing more' than the Suzuki–Miyaura coupling-like reaction while being free of transition metals, which proceeds with the inversion of the stereochemistry when it comes to the double-bond geometry [48]. This statement is oversimplified, especially when the stereo outcome of the reaction is considered, although still states the point that the reaction is not stereodivergent, as is the case of the two previously discussed reactions. However, the situation has changed less than a decade ago, when Aggarwal and co-workers introduced a new PhSeCl-based reaction work-up protocol [58], which in combination with a base (NaOMe) or oxidant (*m*CPBA) was able to selectively produce (*E*) or (*Z*) olefins starting from the same vinyl boronic ester starting material. The difference between the newly developed reaction and the previous Zweifel olefination protocol is rather important; therefore, one could consider renaming the Zweifel stereodivergent olefination protocol as Zweifel–Aggarwal olefination.

- Substrates

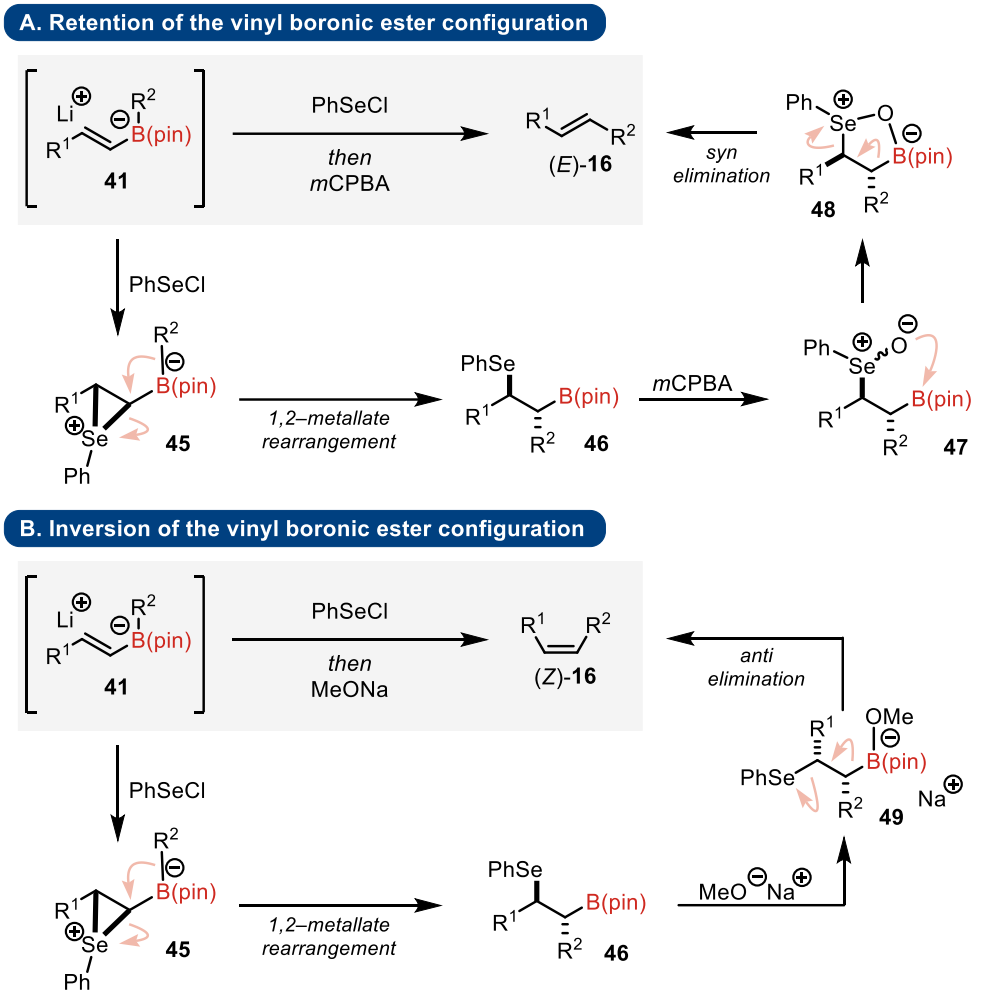
The typical substrates in the Zweifel stereodivergent reaction are vinyl halide **40**, which is further transformed in situ to the corresponding lithiated species Li-**40**, and a boronic ester (normally pinacol alkyl borane). The synthesis of any of the two substrates need not to be discussed in detail, since many methods can be employed. However, what should be highlighted is that the boronic ester substrates can be readily prepared in an enantioenriched form (a stereogenic center to a boronic ester), and that the stereogenic center is due to boron migration properties conserved during the reaction (vide infra).

- Elimination step

Similarly to the previous two olefination methods, the stereodivergence of the Zweifel–Aggarwal olefination reaction is introduced in the elimination step. The common intermediate of the reaction is the borate complex **41** (shown for the (*E*) isomer), which is then reacted with the PhSeCl reagent to form intermediate **45** (Scheme 14). Intermediate **45** is highly reactive and initiates the spontaneous stereospecific 1,2-metallated migration of the alkyl group R^2 from the boron atom. Intermediate **46** is then treated with *m*CPBA (Scheme 14A) or MeONa (Scheme 14B). In the first case, *m*CPBA oxidizes phenyl selenium to selenium oxide **47** and the generated selenium oxide **47** undergoes an intramolecular *syn* elimination process that proceeds through the cyclic intermediate **48**. In this case, the transformation proceeds with the preservation of the configuration if the original configuration of the vinyl boronate intermediate **41** is considered.

In the second case (Scheme 14B), the addition of the methanolate anion generates complex **49**. Complex **49** then spontaneously releases the phenyl selenium anion as a good leaving group via the *anti*-elimination process. In this case, a complete transformation takes place, with a formal inversion of the configuration compared to the original configuration on the vinyl boronate intermediate **41**.

Overall, starting from the same readily available 1-halide-2-alkyl/aryl olefin, both (*E*) and (*Z*) olefins **16** can be readily and stereoselectively generated.



Scheme 14. Stereodivergent Zweifel olefination reaction mechanisms (**A**) for the transformation that preserves the original vinyl boronic ester configuration and (**B**) the transformation that inverses the original vinyl boronic ester configuration.

- Presence of stereogenic centers

The Zweifel–Aggarwal olefination method has an advantage over the Julia–Kocienski and Peterson olefination methods, which results from the structure of the starting reagents; namely, it does not contain labile stereogenic centers in the α-position to the aldehyde or acyl halide. Stereogenic centers in the α-position to the boron atom or in the allylic position in the case of the second reacting partner are generally very stable under the standard reaction conditions, and in the case of boron-containing reagents, they are also readily available using various synthetic methods. From a mechanistic point of view, the 1,2-metallated rearrangement proceeds while the configuration is preserved [59], meaning this method is highly suitable for 1,2-disubstituted olefins with a stereogenic center in the allylic position. This strategy has already been exploited several times in the context of natural product synthesis [60].

5. Conclusions

Since its first dissemination in 1993, the reaction sequence that is now referred to as the Julia–Kocienski reaction has become a very popular late-stage connective method in natural product synthesis because it combines highly efficient (reaction yield) and selective (predominantly (*E*)-selective) connective methods that proceed in a one-pot protocol under mild reaction conditions and with broad substrate and functional group tolerances. The past

30 years of reaction development have also identified key mechanistic properties that allow for better control of the reaction selectivity. Moreover, we have recently introduced a novel modification of the Julia–Kocienski reaction that not only increases the starting material scope (since it allows for the use of previously inaccessible carboxylic acid derivatives as substrates) but also allows for selective (*E*) or (*Z*) olefin formation. In addition, this method allows for the first time the development of the Julia–Kocienski olefination reaction for the independent formation of (*E*) or (*Z*) olefins, starting from the same starting materials and using simple reaction work-up protocol alternation.

Within this focused review, we wished to shed some light on the Julia–Kocienski reaction's development and highlight the latest evolution, which resulted in the transition of the Julia–Kocienski olefination reaction into a stereodivergent method. In this context, the modified Julia–Kocienski olefination reaction was compared with the two other methods that allow the 'workup-based' stereodivergent formation of (*E*) and (*Z*) olefins, the Peterson olefination and modified Zweifel olefination methods (we propose naming the latter Zweifel–Aggarwal olefination to distinguish it from the original Zweifel olefination method).

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Palacký University Olomouc

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Summary of the Doctoral Thesis

(Un)Natural product synthesis: From phenolics and nitro fatty acids to unnatural α-amino acids

P1527 Biology

1501V019 Experimental biology

Supervisor

doc. RNDr. Jiří Pospíšil, Ph.D.

Olomouc

2024

The presented Ph.D. Thesis was carried out at the Department of Chemical Biology, Faculty of Science, Palacký University, Šlechtitelů 241/27, 783 71 Olomouc, the Czech Republic within the Ph.D. program of Experimental Biology, Faculty of Science, Palacký University in Olomouc during 2020-2024.

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The Ph.D. Thesis and expert reviews will be available 14 days before the defence in the Study Department of Faculty of Science (Mgr. Martina Karásková), Palacký University, 17. listopadu 12, Olomouc.

After the defence, the Ph.D. Thesis will be stored in the Library of the Biological Departments, Faculty of Science, Palacký University, Šlechtitelů 27, Olomouc – Holice.

prof. Ing. Miroslav Strnad, CSc. DSc.
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Study Program Experimental Biology
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Content

1. Introduction

Presented Thesis deals with natural substances, especially their targeted synthesis, and then the development of new synthetic methods that would allow us to prepare both natural substances, whether of plant or other origin, and substances of non-natural origin, only inspired by nature, origin. Thus, the thesis is divided into three indirectly related parts.

In the first part, plant secondary metabolites are a diverse group of organic compounds produced by plants and other living organisms, such as bacteria, fungi, and plants, which are not strictly necessary for their survival. In other words, they are not included in elemental processes such as growth, development, and reproduction. These compounds are not essential for the survival of the organism, but they play an important role in the interactions of the organism with its environment.[1] In plants, phenolics, and especially its subgroup neolignans, belong to the most abundant groups of phytocompounds. These compounds are formed by oxidative coupling of hydroxycinnamic acid derivatives by a β,β'-linkage between two phenylpropanoid units. These compounds have broad biological activities ranging from antiparasitic, anticancer, anti-inflammatory, neuroprotective, antibacterial, antifungal to antiallergenic.[5] Every year new neolignans are identified and therefore new synthetic routes and their biological activity is searched.[2,3]

In the second part, the domain of endogenic natural products, nitrated fatty acids, is tackled. Nitro fatty acids (NO_2FAs) and corresponding nitrated lipids are formed endogenously by reaction of unsaturated fatty acids (UFAs) with reactive nitrogen species (for example monoxide ($NO^{\cdot}$) or nitrite anions (NO_2^-)). These highly reactive nitrated organic species have been found in tissues and biological fluids but mainly in the brain. They are also reported to form in healthy human plasma and urine.[4,5] They have a vast biological activity, but their mechanism of action has yet to be found. [6–9]

Finally, the knowledge and experience gathered within the Julia-Kocienski olefination project was applied to the development of the heteroaryl sulfonamide synthesis protocol. Protocol that proved to be crucial for the development of chiral memory-based synthesis of a new class of homochiral α-heteroaryl α-substituted α-amino acid synthesis. Overall, two new synthetic methodologies were disclosed allowing the preparation of heteroaryl sulfonamides, compounds with a rich biological activity ranging from antitumor[10], antiviral[11,12], to antifungal[13], anticancer,[11,12] or previously undescribed α-amino acids with unknown potential biological activity.

2. Aims and scope

As mentioned in chapter 1, our research group is interested among others in **(1)** the chemistry of phenolic compounds (plan secondary metabolites); **(2)** the chemistry of benzothiazole (BT) sulfones and their various derivatives, especially in the context of various olefination methods; and more recently also in **(3)** the chemistry of NO_2FAs. Correspondingly, presented thesis is separated into three main chapters gathered around the previously mentioned centre of interest.

The aim of the first part of this Thesis is to increase our knowledge about the synthesis and biological evaluation of the anthelmintic, antiparkinsonian and cytokinin activity of neolignan compounds. My goal in this part was to develop a versatile biomimetic synthetic approach that would allow the synthesis of various unsymmetrical neolignans with a dihydrobenzofuran core.

The second part of the thesis focuses on the synthesis, biological evaluation, and characterization of NO_2FAs. Although the NO_2FA class of compounds is known for almost three decades, its mechanism of action is still hidden under the shadow. We presume that such a situation is mainly caused by a nonspecific manner of their synthesis, which yields mixtures of positional and stereo isomers and therefore complicates a biological evaluation of compounds that are evaluated for their biological activity. My goal was to develop a synthetic protocol that would allow stereo- and regio-controlled synthesis of NO_2FAs.

In the third part of the Thesis the stereocontrolled synthesis of novel previously unknown homochiral α-heteroaryl α-substituted α-amino acid is pursued. Such class of α-amino acids that contains quaternary stereogenic centre might be of interest to chemical biology field in many ways, and therefore it broad such class of molecules into the centre of our interest. My goal within this project was to help explore the scope and limitations of this method.

The overall aims of this doctoral Thesis are:

1) Provide theoretical summary for each targeted topic in clear and organized way.
2) Targeted synthesis and characterization of selected dihydrobenzofuran-core containing (un)natural compounds.
3) Methodology development that would lead to, and synthesis and characterization of NO_2FAs.

   Determination of scope and limitations of methods that provide heteroaryl sulfonamides and unnatural homochiral quaternary α heteroaryl α amino acids.

3. General methods

All starting materials were purchased from commercial suppliers and used without further purification, unless otherwise stated. All reactions were carried out in round bottom flasks fitted with rubber septa using standard laboratory techniques under positive pressure of argon (Air Liquide, >99.5% purity). In all reactions, unless stated otherwise, anhydrous solvents furnished by the Merck (Sigma-Aldrich) Company were used. The purification of the reaction products was carried out by column chromatography using standard grade silica gel (60 Å, 230–400 mesh), or by preparative thin layer chromatography glass plates precoated with silica gel (silica gel G-200 F^{254}, particle size 0.040−0.063 mm). Analytical thin-layer chromatography was performed on a thin-layer chromatography (TLC) aluminium plates precoated with silica gel (silica gel 60 F^{254}). Visualization was accomplished with UV light, phosphomolybdic acid, and potassium permanganate stains, followed by heating. Reactions carried out at temperatures of -78 °C (N_2/acetone) or -95 °C (N_2/methanol) were carried out using a cooling bath, and the indicated temperatures refer to the external temperature of the cooling bath. The determination of melting points was made on a Büchi melting point apparatus. The ^{1}H NMR and $^{13}C\{^1H\}$ NMR spectra were measured on JEOL ECA400II (400 and 101 MHz) or JEOL 500 ECA (500 and 126 MHz) in chloroform-*d*, acetone-d_6, DMSO-d_6 or methanol-d_4. Chemical shifts are reported in ppm, and their calibration was carried out (a) in the case of ^{1}H NMR experiments on the residual peak of non-deuterated solvent δ ($CDCl_3$) = 7.26 ppm or δ (CD_3OD) = 3.31 ppm, δ (DMSO-d_6)= 2.50 ppm, δ (acetone-d_6)= 2.05 ppm and in the case of ^{13}C NMR experiments on the middle peak of the ^{13}C signal in deuterated solvent δ ($CDCl_3$) = 77.16 ppm, δ (CD_3OD) = 49.00 ppm, (DMSO-d_6)= 39.52 ppm, (acetone-d_6)= 29.84 ppm. The proton coupling patterns are represented as a singlet (s), a doublet (d), a doublet of a doublet (dd), a triplet (t), a triplet of a triplet (tt), and a multiplet (m). High-resolution mass spectrometry (HRMS) on Agilent 6230 high-resolution mass spectrometer with electrospray ionization (ESI) and a time-of-flight analyser operating in a positive or negative full scan mode in the range of 100 − 1700 m/z. High-performance liquid chromatography (HPLC) was performed using an Agilent 1290 Infinity II system with UV-VIS detector and an Agilent Infinity Lab LC/MSD mass detector. Purification using semi-prep HPLC was carried out on Agilent 1290 Infinity II with UV-VIS and Agilent Infinity Lab LC / MSD mass detector using the C18 reverse phase column (Agilent 5Prep-C18 10x21.2 mm). The gradient

was formed from 15 mM aqueous ammonium acetate (buffer) and methanol with a flow rate of 20 mL/min.

Chiral analysis was performed on Waters Alliance 2695 with autosampler and UV-VIS detector Waters 2996 PDA using chiral columns (CHIRAL ART Amylose-SA 250x4,6 mm, 5 µm; CHIRALCEL Cellulose OD-H, 250x4,6 mm, 5 µm; CHIRALCEL Cellulose OZ-H, 250x4,6 mm, 5 µm). All solvents used were HPLC-grade solvents purchased from Merk. The column employed and the respective solvent mixture are indicated for each experiment. Specific rotations ($[\alpha]_D^T$) were measured with Perkin Elmer Polarimeter 241 Automatic (Massachusetts, USA) at the indicated temperature. Measurements were performed in a 1 ml cell (50 mm length) with concentrations (g/(100 ml)) reported in the corresponding solvent. All microwave irradiation experiments were carried out in a dedicated CEM-discover monomode microwave apparatus. The reactor was used in the standard configuration as delivered, including proprietary software. The reactions were carried out in 10- or 35-mL glass vials that were sealed with silicone/PTFE caps, which can be exposed to a maximum of 250 °C and 20 bar internal pressure. The temperature was measured with an IR sensor on the outer surface of the process vial. After the irradiation period, the reaction vessels were cooled to ambient temperature by gas jet cooling.

All procedures of the preparation of the compounds are well described in the experimental part of the Thesis for each project separately.

The numbering of molecules in this Thesis summary is for clarity reasons the same as in the Thesis manuscript.

4. Overview of achieved results

4.1. Benzofuran-based Neolignans

As mentioned in the introductory part, neolignans belong to the secondary metabolites of the plant. Within a plethora of known phenolic neolignans that can be found in plants, our main attention focused on the neolignans with the dihydrobenzofuran skeleton (**Figure 1**). Our primary target was the synthesis of natural compounds **DCG-A** (**1-108**) and **quiquesetinerviusin A** (**1-134**) since the first was supposed to have interesting cytokinin-like biological properties and the second could be used as a standard in the determination of the age of marine sedimentation.

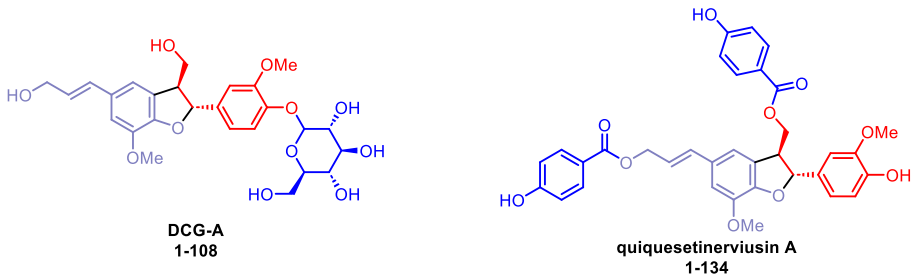
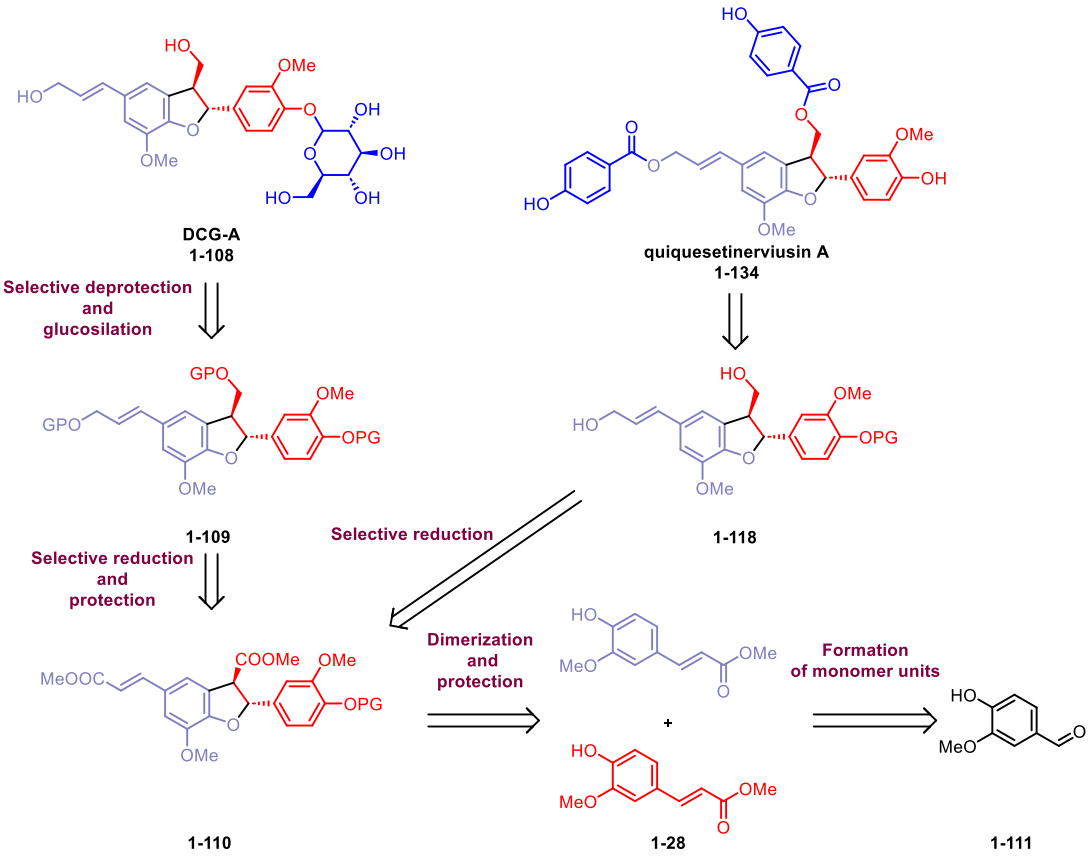


Figure 1

Our strategy was to design a retrosynthetic plan that would start from simple and commercially available building blocks and allow us to prepare not only the **DCG-A** molecule but also analogues of naturally occurring molecules. An overview of our retrosynthetic analysis of **DCG-A** (**1-108**) and **quiquesetinerviusin A** (**1-134**) is provided in **Scheme 1**.

Previous (and only) total synthesis of **DCG-A** published in 1998 by Wong *et al.*[14] based the synthesis of **DCG-A** on the globally protected diol **1-109** where selective deprotection of the phenolic hydroxy group allowed for the selective glycosylation step.

Thus, our retrosynthetic proposal focused on the use of diol **1-109**, which could be easily prepared by selective reduction of ester groups in **1-110**. Dimer **1-110** can be prepared by biomimetic dimerization of neolignan monomers **1-28** and introduction of the protecting group to the phenolic hydroxy group. The dimer **1-110** can also be used in the synthesis of **quiquesetinerviusin A**, where selective reduction of esters in **1-110** produces alcohol **1-118**, which can in turn be transformed into its diacylated derivative **1-134**.



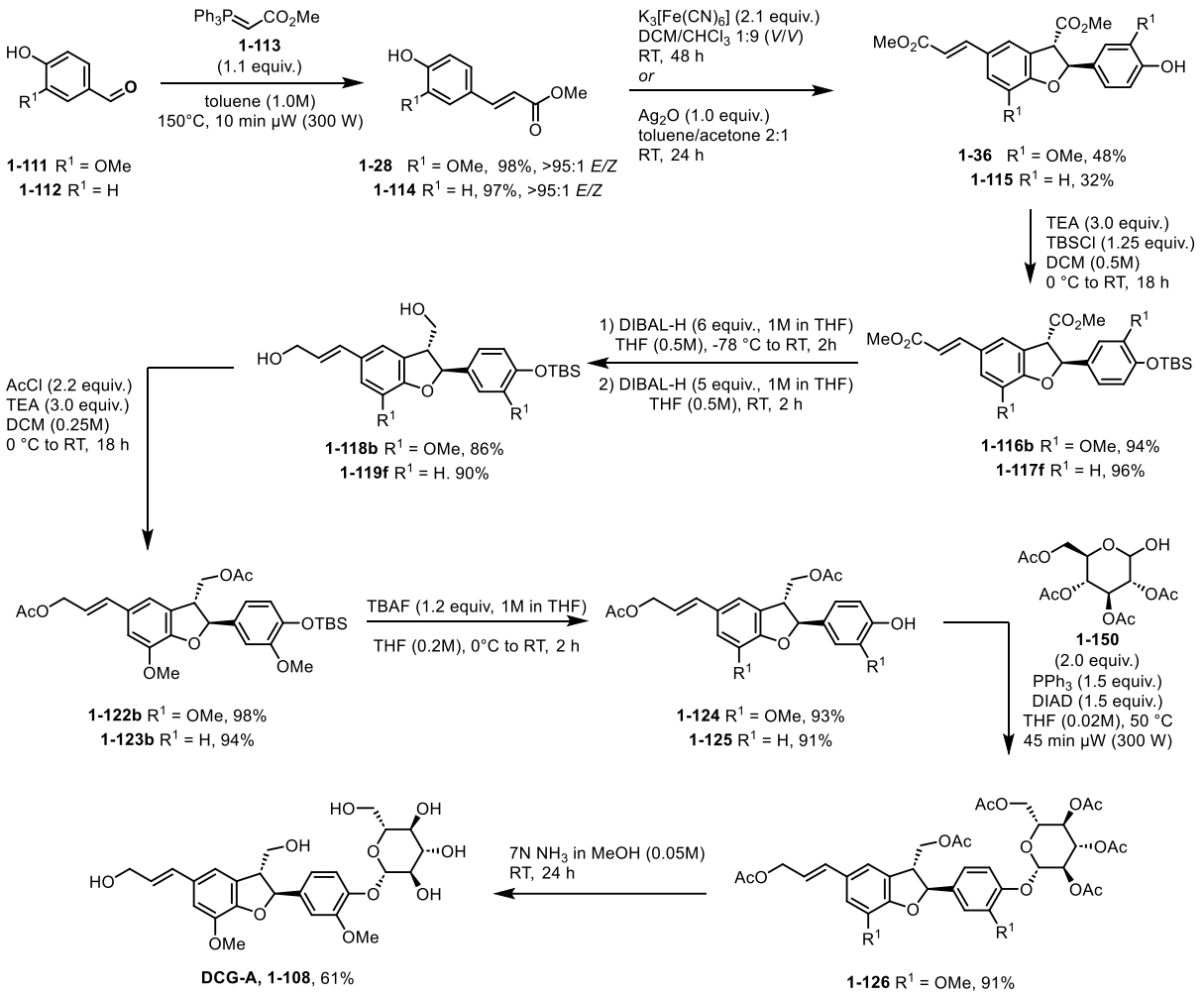
Scheme 1

We have optimized each step of the synthesis and successfully prepared **DCG-A** (over 8 steps and in an overall yield of 19%) using the following synthetic sequence (**Scheme 2**). (1) formation of monomers, 2) dimerization, 3) protection of phenolic -OH, 4) reduction of esters to alcohol, 5) protection of formed alcohols, 6) deprotection of phenolic -OH, 7) Mitsunobu reaction with peracetylated glucose and 8) deprotection of acetyl groups. Structural analogues without aromatic methoxy groups were prepared from 4-hydroxybenzaldehyde **1-112** along with the same synthetic sequence.

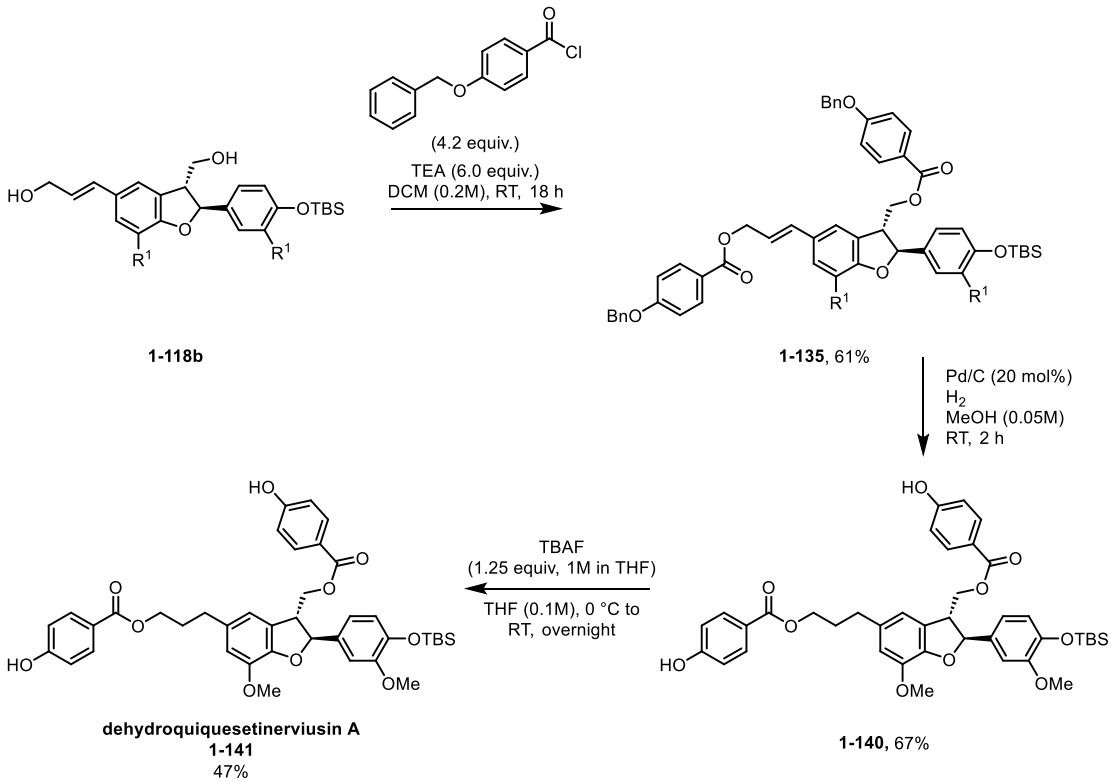
During the synthesis of quiquesetinerviusin A, we have faced many challenges, especially in the protection/deprotection of the phenolic and benzylic protective group steps, during which the double bond was reduced and **dehydroquiquesetinerviusin A** was isolated **Scheme 3**.

At the same time, we have created a library of 50 dihydrobenzofuran-skeleton-containing molecules, which were tested for their anthelmintic (1 HIT identified), antiparkinson (3 HITs identified), and anticancerous activity. **DCG-A** was additionally tested for cytokinin activity, since such activity was described in the pilot publication featuring this compound back in 1991.[15]

The relevant procedures as well as characterizations of prepared compounds are described in detail in the experimental section of **chapter 1** of the dissertation Thesis.



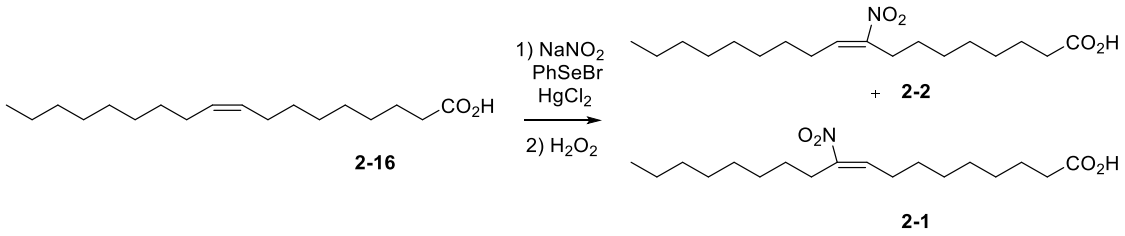
Scheme 2



Scheme 3

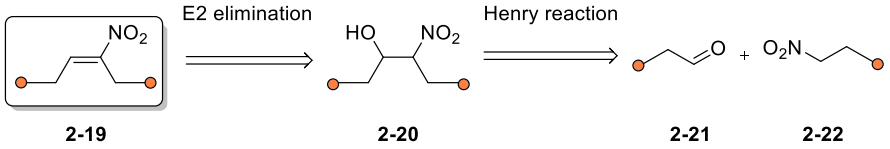
4.2. Nitro fatty acids

In this part of the thesis, the focus was on the preparation of endogenous NO_2FAs with absolute stereo and regio control. The formation of these natural compounds *in vivo* remains unknown, but in general these compounds are produced during digestion and oxidative stress by reaction with reactive oxygen and nitrogen species.[6,16] Over the past 20 years, these compounds were prepared mainly in the laboratory by direct nitration from the original fatty acids, as shown in the example of oleic acid **2-16** in **Scheme 4**. This method was well established due to its simplicity, but it has several drawbacks, such as low yields and the formation of inseparable and unpredictable mixtures of stereo- and regio-isomers.



Scheme 4

Since preparation of these compounds in this way produces complex product mixtures, a considerable amount of time and resources were spent in our as well as other scientific groups on connective approaches that would allow for the stereoselective Henry reaction of aldehyde and nitro alkane/alkene followed by elimination of E2 of the generated adduct (**Scheme 5**).



Scheme 5

By utilizing above mentioned connective approach we have successfully prepared 5 different members of NO_2FA family including **10-nitro oleic acid** (**2-1**), **9-nitro oleic acid** (**2-2**), **10 nitro linoleic acid** (**2-3**), **9-nitro conjugated linoleic acid** (**2-4**) and related **10-nitro stearic acid** (**2-24**) that was required to be used as a control in biological assays (**Figure 2**). Within this campaign I was also involved in the development of novel modification of Julia-Kocienski olefination reaction that allows for stereoselective preparation of (*E*) as well as (*Z*) olefins starting from the same starting material.

Exact procedures and preparation of starting materials for subsequent Henry reaction can be found in the experimental section of **Chapter 2**.

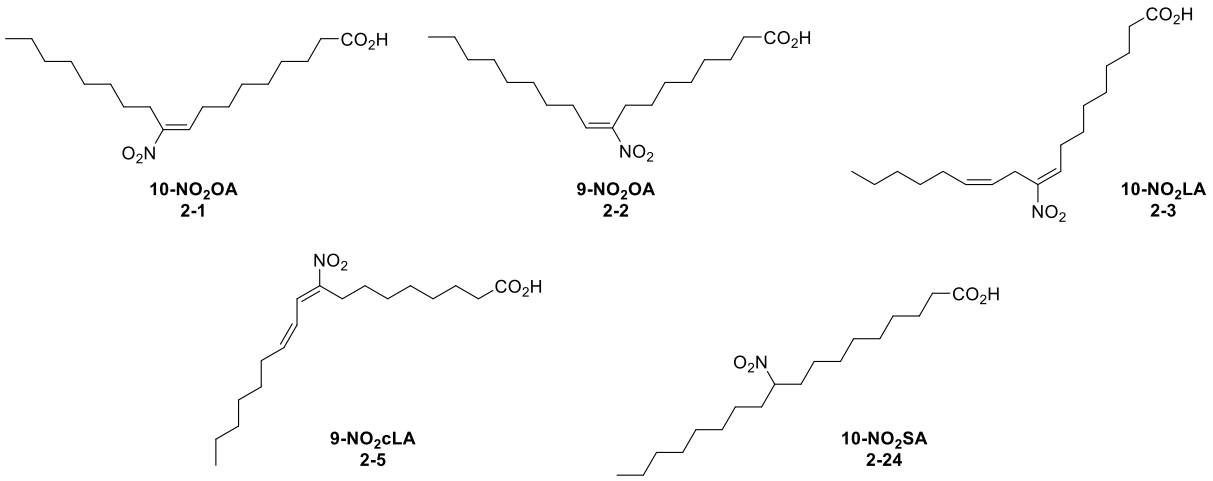
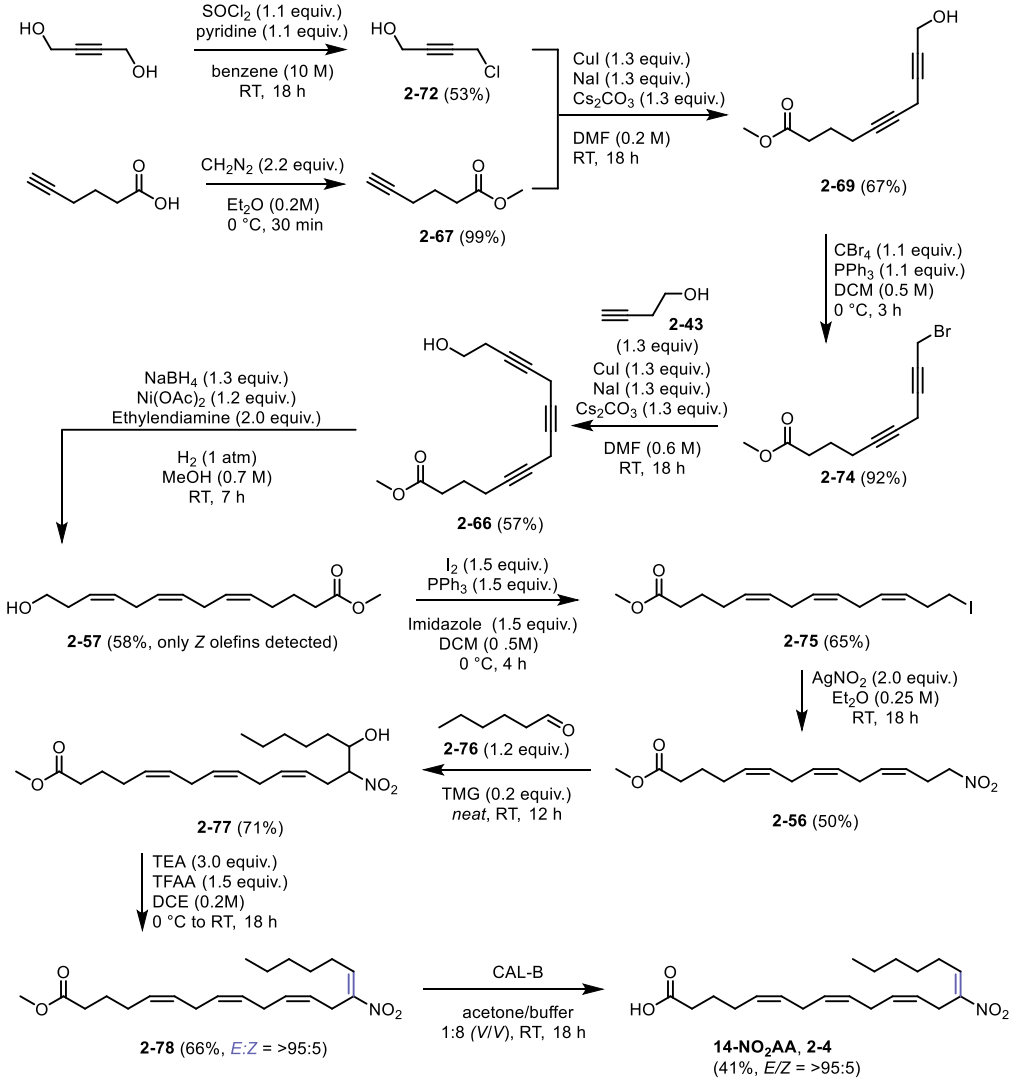


Figure 2

In this campaign we have also successfully accomplish a **first total synthesis** of 14-nitro arachidonic acid, that is based on the use of a nitro compound **2-56 build-up strategy** followed by the Henry reaction with hexanal **2-76** and subsequent elimination of E2 (**Scheme 6**, 9 steps, 1.3% overall yield).



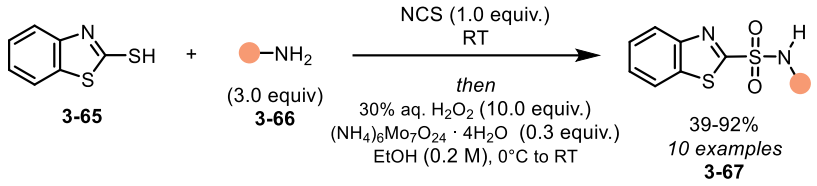
Scheme 6

Notable is the fact that in the last step of all NO_2FA syntheses, the enzyme-based demethylation of methyl ester derivatives was used. The reaction proceeds at pH = 7.4 and eliminates most of the generation of side products generation that under normal reaction conditions accompany ester group hydrolysis. Thus, the final NO_2FAs are produced with much better overall yields and without compromising the final stereochemistry. Additionally, the method eliminates the formation of various salts that are generated when acidic or basic deprotection conditions are used, and this causes difficulty in the purification of the final product purification.

All prepared nitro fatty acids are being evaluated for their stability in water, redox properties, biological activity (tested as Nrf2 activators) and radioprotective properties in mice, as well as analytical standards to evaluate their possible presence in plants and freshwater algae.

4.3. Benzothiazolesulfonamide and unnatural quaternary heteroaryl amino acids synthesis

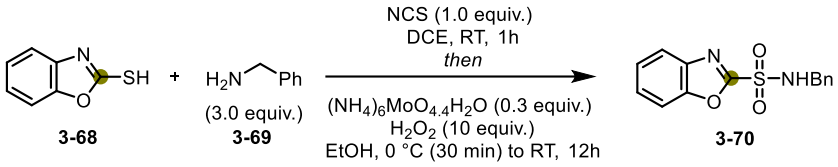
The third part of the thesis focuses on the synthesis of heteroaryl sulfones, a method recently developed in our group that allows the synthesis of *N*-substituted and *N,N*-disubstituted benzothiazole-2-yl sulfonamides from benzo[*d*]thiazole-2-thiols and alkyl amines (**Scheme 7**), and its extension to the synthesis of amino acids (**Figure 3**).



3-65 **3-66** **3-67**

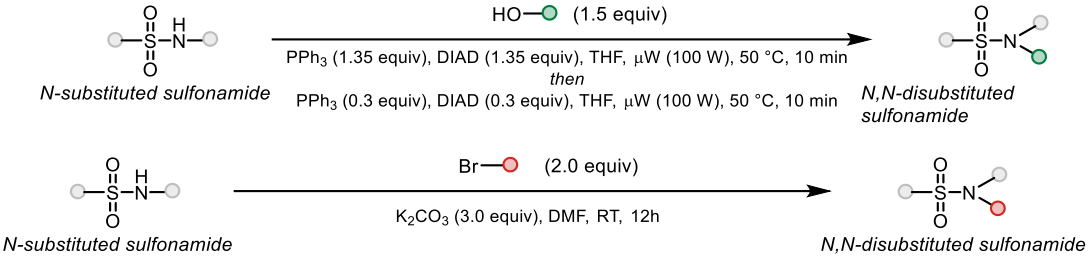
Scheme 7

First, an extension of the reaction methodology toward other heterocycles was attempted. Thus, other heteroaryl scaffolds such as **nitrogen-containing** pyridine, pyrimidine, imidazole, benzo[*d*]imidazole, and purine-2,6-dione, as well as **oxygen-containing** benzo[*d*]oxazole and oxazoles and **sulfur-containing** benzo[*d*]thiazole and thiazole were investigated. The optimization of this method was performed on benzo[*d*]oxazole. After thoughtful optimization, optimal conditions were found (**Scheme 8**) and the scope and limitation of the method could be established.



Scheme 8

It was observed that the targeted products are in many cases unstable in solution and/or degraded during purification, but once purified and isolated as a pure solid compound, they proved to be bench-stable for several months. Finally, two synthetic protocols were established. The first is based on base-promoted alkylation, and the second on microwave-promoted Fukuyama-Mitsunobu reaction were developed (**Scheme 9**).



Scheme 9

A comprehensive description of the synthetic approaches developed towards the final compounds are summarized in the full paper[17] where we describe the synthesis of more than 50 final and newly prepared and characterized heteroaryl sulfones. 24 of those were prepared by me.

Having such a broad library of previously unknown *N,N*-disubstituted heteroaryl sulfonamides in hand, we wanted to tackle the main objective for which the synthetic method was developed. Application of such a product in memory of chirality-based synthesis of a previously unknown class of α-heteroaryl α-substituted α-AAs and their derivatives. From preliminary DFT calculation-based evaluation and our experience with the Julia-Kocienski olefination reaction, it was expected that a hydrogen atom α to the nitrogen atom of the sulfonamide **3-159** is acidic and it was expected that if deprotonated a Smiles rearrangement might occur (**Figure 3**). And if the generation/rearrangement process is fast enough, the chiral information included in the amino acid should be cleanly transferred to the newly established amino acid **3-159**.

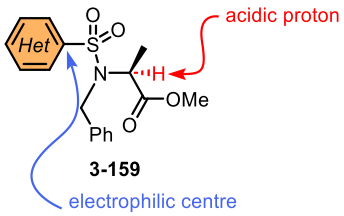
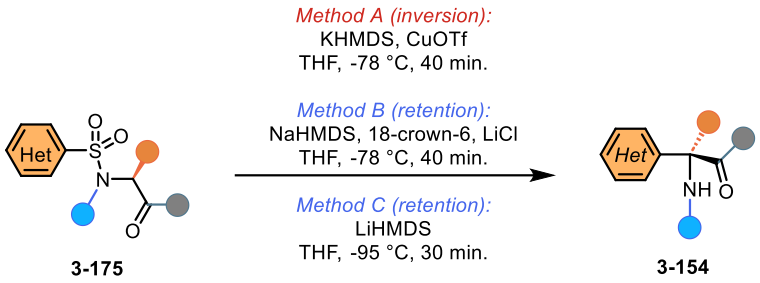


Figure 3

With this information in hand, we have successfully designed, optimized, and developed a synthetic protocol based on the concept of 'memory of chirality' that allows us to prepare both possible enantiomers of previously unknown α-heteroaryl α-substituted α-AAs starting from natural and unnatural (L)-α-AAs.

The general transformation is depicted in **Scheme 10** and the concept behind it is described in detail in **Scheme 11** using L-alanine amino acid-based sulfonamide. **3-99** as a model substrate. Using developed protocols, derivative **3-99** can be transformed into the desired product **3-161** (reaction proceeds with the retention of the chirality on the stereogenic center; **Method A** (KHMDS (1.5 equiv.), CuOTf (1.1 equiv.), THF (0.1M)), or in its antipod **3-160** (reaction proceeds with the inversion of the chirality on the stereogenic center; **Method B**: NaHMDS (1.5 equiv.), 18-crown-6 (3.0 equiv.), LiCl (1.1 equiv.), THF (0.1M); **Method C:** LiHMDS (1.5 equiv.), THF (0.1M))

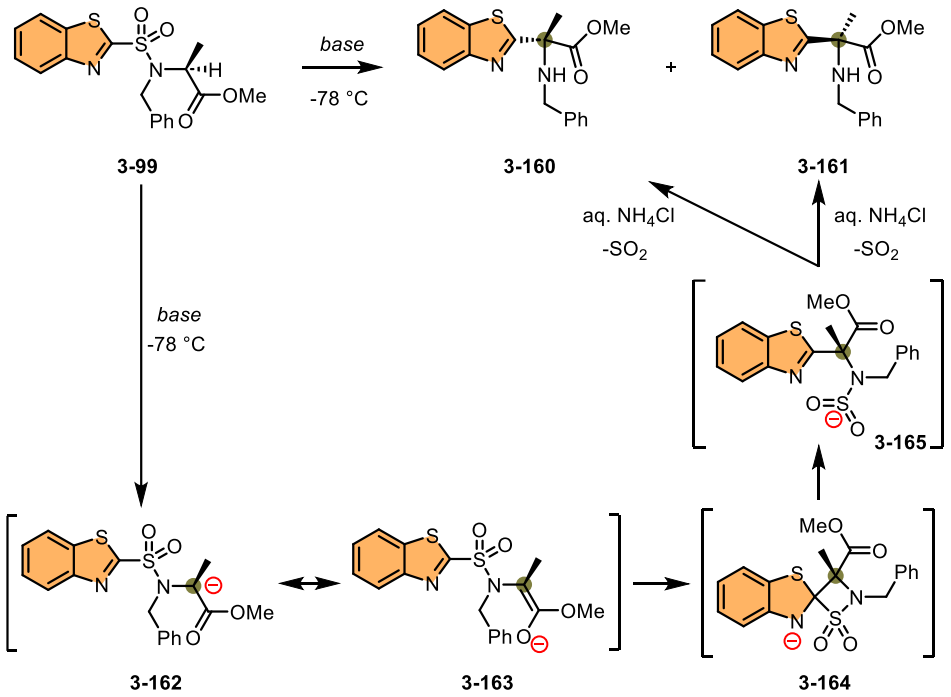


Scheme 10

From the mechanistic point of view, the reaction presumably proceeds via the following manner: the deprotonation of **3-99** forms anion **3-162** which immediately adopts its more stable enol form **3-163**. Next, the formation of Meisenheimer complex **3-164** followed by Smiles rearrangement proceeds to form adduct **3-165**, which upon aqueous acidic workup releases SO_2.

I was personally involved within this project in the optimization of the reaction conditions and further on the evaluation of scopes and limitations of the method by varying the alkyl group, amino acid part and (partially) also the heterocyclic part. Although the results and

discussion part of Chapter 3 include all the spectrum of prepared α-heteroaryl α-substituted α-AAs, the experimental part is limited only to those I prepared. These which were prepared by my colleague, Mgr. Jozef Kristek are not included in the experimental part.



Scheme 11

5. Conclusion and perspectives

In the first part of the thesis that focuses on phenylpropanoids, we have successfully developed and employed a synthetic pathway to the **DCG-A** molecule (**1-108**) and constituted a library of 50 **DCG-A**-like compounds. In collaboration with Dr. Kadlecová, compound **1-117c** was identified as a potent anthelmintic compound by being active against *C. elegans* in a chitinase assay (quantifying egg hatching) at a concentration of 50 µM and at 5 µM. The IC_{50} values of this compound were stated to be 2.20 µM ± 0.26. Furthermore, it was also observed that this compound is also active against *C. elegans* strains resistant to ivermectin, levamisol, and mebendazole, a commonly used anthelmintic drug.

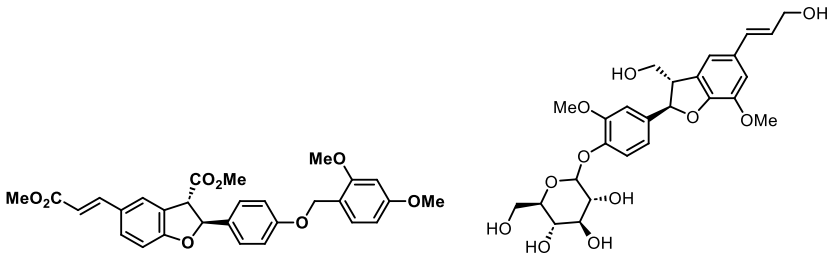


Figure 4

In addition, the constituted library was also evaluated for its antiparkinson activity (work done in collaboration with Dr. Gonzalez) and three hit compounds were identified. Dr. Gonzales team is now working towards the determination of their mode of action. Finally, cytokinin assays evaluation of prepared compounds were performed (done by Dr. H. Vylíčilová) to determine whether the cytokinin activity of the **DCG-A** compound corresponds to the previous literature report. Unfortunately, it was found that in standard callus and senescence cytokinin assays, the **DCG-A** compound does not exhibit any cytokinin activity.

In the second part of the thesis, we have successfully prepared 5 NO_2-FAs, including 9- and 10-NO2 oleic acid **2-1** and **2-2**, 10-NO_2 linoleic acid **2-3**, 9-NO_2 conjugated linoleic acid **2-5** (nitro rumenic acid) and we successfully accomplished a first total synthesis of 14-NO_2 arachidonic acid **2-4**. Furthermore, a saturated NO_2 acid, 10-NO_2 stearic acid **2-24, which** served as a control compound during the biological evaluation of its unsaturated equivalent, was prepared (**Figure 5**).

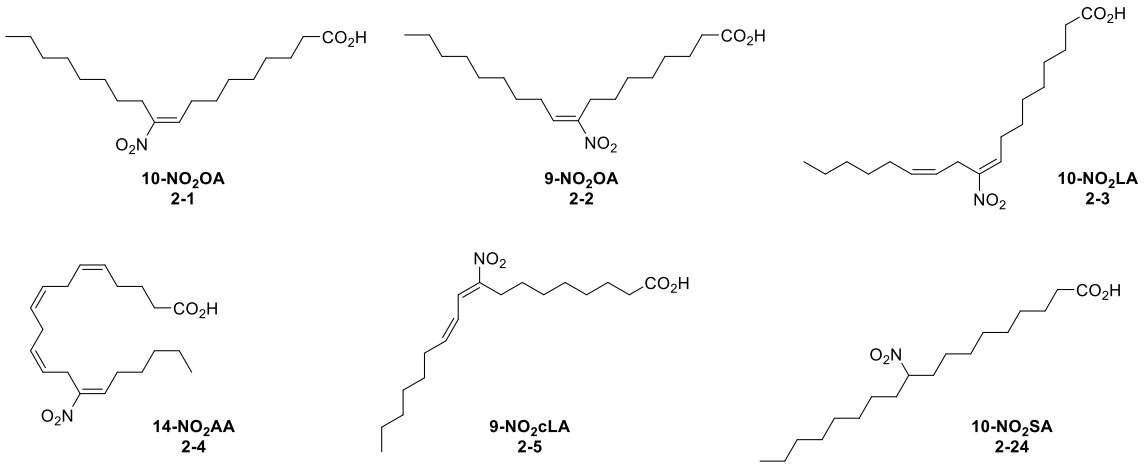
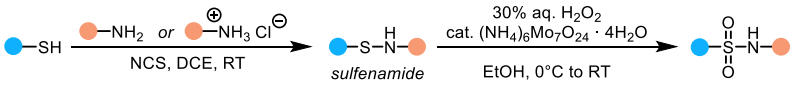


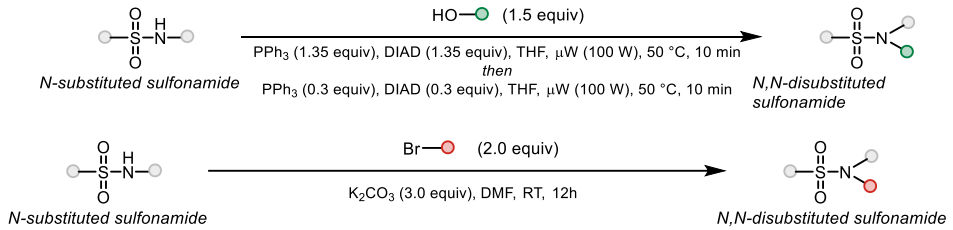
Figure 5

The third part of the Thesis focused on a scope and limitation evaluation of recently developed method of *N*-substituted and *N,N*-disubstituted benzo[*d*]thiazole sulfone amide synthesis. The extension of the method to various heteroaryls and *N*-substitution was found and a novel and more general method for the synthesis of heteroaryl sulfonamides was developed (**Scheme 12**).



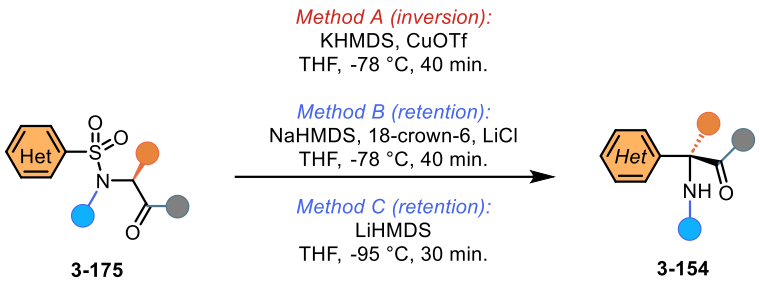
Scheme 12

Additional methods of further *N*-alkyl-substituted heteroaryl sulfonamide transformation to *N,N*-disubstituted heteroaryl sulfonamide based on (A) a microwave-promoted Fukuyama-Mitsunobu alkylation or (B) a base-promoted alkylation reaction were also found (**Scheme 13**).



Scheme 13

The newly formed synthetic route to *N,N*-disubstituted heteroaryl sulfonamides was then explored in a new memory of chirality-based methodology that allowed us for the first time to prepare enantioselectivity enriched α-**h**eteroaryl α-substituted α-**a**mino **a**cids (HAA). The most interesting part about this transformation is the fact that starting from the same homochiral *N*-substituted heteroaryl sulfonamide, both enantiomers of the newly formed HAA product could be formed independently and with high stereopurity purity (**Scheme 14**).



Scheme 14

Mechanistic investigations, including a detailed computational study, suggest that the selectivity can be directly correlated with the conformation adopted by the starting material, heteroaryl sulfone, prior to the reaction that is triggered by the addition of the base. The conformation seems unchanged (the reaction is fast) during the deprotonation/Smiles rearrangement key step of the transformation. Such a suggestion is in agreement with the memory of the chirality concept.

6. List of author´s publications and conference contributions

(underlined authors contributed equally and are in alphabetical order)

1. Iakovenko, R. O.; **Chrenko, D.**; Kristek, J.; Desmedt, E.; Zálešák, F.; De Vleeschouwer, F. & Pospíšil, J. *Org. Biomol. Chem.* **20**, 3154–3159 (2022). DOI: 10.1039/D2OB00345G.
2. Zebrowski, P., Röser, K., **Chrenko, D.**, Pospíšil, J. & Waser, M. *Synthesis* **55**, 1706–1713 (2023). DOI: 10.1055/a-1948-5493 (*Included in Special Issue dedicated to Prof. Dr. Cristina Nevado, Recipient of the 2021 Dr. Margaret Faul Women in Chemistry Award).*
3. Bon, D.J.-Y.D.; **Chrenko, D.**; Kováč, O.; Ferugová, V.; Lasák, P.; Fuksová, M.; Zálešák, F. & Pospíšil, J. *Adv. Synth. Catal.* ***366***, 480–4 (2024). DOI: 10.1002/adsc.202301054.
4. **Chrenko, D.** & Pospíšil*, J. *Molecules* ***29***(12), 2719 (2024). DOI : 10.3390/molecules29122719.

Conference contribution – oral presentations:

1. Chemistry and Biology of phytohormones and related substances 2023, Radějov, CZE (5/2024), *Bioactive lipids: Synthesis and application of nitro fatty acids*

2. 23rd Interdisciplinary meeting of young life scientist, Milovy, CZE (05/2024), *Julia-Kocienski-like connective C-C and C=C bond-forming reaction and its application*

3. 4th Annual CNPD (Centre for Natural Products Discovery) Conference 2023, Liverpool, UK (06/2023), *Neolignans: Natural products with a potential to kill*

4. Chemistry and Biology of phytohormones and related substances 2023, Velké Losiny, CZE (5/2023), *The Nitro Revolution: Unlocking the Potential of NO2-Fatty Acids for Radioprotection and More* – **Best talk award**

5. 21st Interdisciplinary meeting of young life scientist, Milovy, CZE (05/2022), *Dual organocatalysis-based approach to 2,3-dihydrobenzofuran skeleton*

6. Chemistry and Biology of phytohormones and related substances 2022, Skalský dvůr, CZE (05/2022), *Having fun with benzothiazolsulfonamides*

7. EXBIO-PCR 2021, Olomouc, CZE (9/2021), *Neolignans: powerful plant secondary metabolites*

8. Chemistry and Biology of phytohormones and related substances 2021, Malenovice, CZE (09/2021), *Neolignans: powerful plant secondary metabolites* – **Best talk award**

Conference contribution – poster presentations:

1. 56th Conference: Advances in Organic, Bioorganic and Pharmaceutical Chemistry, Liblice, CZE (11/2022), *Organocatalyzed tandem cyanohydrin / acyltransfer reaction*
2. The 19th Blue Danube Symposium on Heterocyclic Chemistry, Bratislava, SK (08/2022), *Neolignans: Powerful plant secondary metabolites*
3. 3rd Alpine Winter conference on Medicinal and Synthetic Chemistry, online (01/2022)

7. Research stay abroad:

09/2023 – 12/2023 University of Zürich, Switzerland

Group of Prof. Dr. Cristina Nevado

Topic: Synthesis of novel ligand families for Ni dual/photoredox catalysis and asymmetric arylsilylation of electron-deficient alkenes.

01/2022 – 03/2022 Johannes Kepler Universität Linz, Austria

Group of Prof. Dr. Mario Waser

Topic: Synthesis and application of novel quaternary ammonium salt catalysts and their application in asymmetric β-addition of isoxazolidin-5-ones to allenoates.

8. Prizes, awards, scholarships, membership, and teaching

Deans Award UPOL 2024 – 3rd place for best dissertation Thesis in the category of chemistry.

Palacký Endowment Foundation 2023 (funding of the research stay abroad at UZH Zurich) – 116 600 CZK.

Mobility UP scholarship 2022 (funding of the research stay abroad at JKU Linz).

Chemistry and Biology of phytohormones and related substances 2023 – **Best Talk Award**.

Chemistry and Biology of phytohormones and related substances 2021 – **Best Talk Award**.

Member of Czech chemical society (since 2020).

Teaching

Chemistry for Biologists 2 – laboratory course (2020-2024 – 8 terms, 4h/week)

Chemistry for Petroleum engineering – (2021, 50 hours) – Erbil, Iraq, Kurdistan

Cosupervisor of 1 master, 2 bachelor and 2 high school students

Janůjová Eva – Synthesis of new substances derived from (-)-sanguinolignan A and their antihelmintic activity (master Thesis defended 06/2021)

Hendrychová Romana – Biological properties of cyclophenyl lactones: Influence of the phenylsulfone (bachelor Thesis defended 06/2021)

Levayová Sylvie – Structure of neolignans isolated from Posidonia oceanica (bachelor Thesis defended 06/2023)

Dopitová Barbora – Secondary metabolites of plant origin – development of novel methods for Synthesis and biological evaluation (2022-2023, high school student work, Badatel – 1st place in conference of young scientists - biotechnology)

Adam Horák – Synthesis of plant secondary metabolites with dihydropyran skeleton – syntehsis of obolacton (2023-2024, high school student work, Badatel – 1st place in conference of young scientists)

9. Souhrn (in Czech)

Tato disertační práce se zabývá syntézou přírodních a nepřírodních látek a vývojem nových syntetických metod umožňujících přípravu těchto látek. Práce se zaměřuje na **1)** syntézu a biologickou evaluaci fenolických sloučenin, **2)** vývoj metodologie pro syntézu a charakterizaci nitro mastných kyselin a jejich biologickou evaluaci a **3)** vývoj chemie benzothiazol sulfon amidů a nových homochirálních α-heteroaryl α-substituovaných α-aminokyselin.

Cíl první části disertační práce, je rozšířit rozsah našich znalostí o syntéze a biologických vlastnostech. Hlavním cílem je syntéza přírodních látek strukturně podobných látce DCG-A **1-108** a quiquesetinerviusin A **1-134**. Úspěšně byla připravena knihovna 50 sloučenin, které byly testovány na aktivitu proti parazitům (*C. elegans*) a Parkinsonově chorobě. Látka DCG-A byla také testována na její cytokininovou aktivitu.

V druhé části disertační práce je pozornost věnována přípravě nitro mastných kyselin s absolutní stereo- a regio- kontrolou. Především z důvodu toho, že v posledních 20 letech byly tyto sloučeniny v laboratoři připravovány převážně přímou nitrací původních mastných kyselin, což mělo několik nevýhod, jako nízké výtěžky a vznik neoddělitelných směsí stereo- a regioizomerů. Úspěšně jsme vyvinuli novou metodika využívající stereoselektivní Henryho reakci aldehydu a nitroalkanu/alkenu následovanou E2 eliminací. Celkově bylo připraveno 5 různých nitro mastných kyselin (10-nitrooleová kyselina (**2-1**), 9-nitrooleová kyselina (**2-2**), 10-nitrolinolová kyselina (**2-3**), 9-nitrokonjugovaná linolová kyseliny (**2-4**) a 10-nitrostearové kyseliny (**2-24**), která byla použita jako kontrola v biologických testech (z důvodu absence dvojné vazby). Dále byla úspěšně provedena první totální syntéza 14-nitroarachidonové kyseliny (**2-5**). Všechny připravené nitro mastné kyseliny jsou v tuto chvíli testovány na jejich stabilitu ve vodě, redoxní vlastnosti, biologickou aktivitu (testovány jako Nrf2 aktivátory) a radioprotektivní vlastnosti u myší. Látky jsou také používány jako analytické standardy pro jejich možnou přítomnost v rostlinách a sladkovodních řasách.

Třetí kapitola se soustředí na syntézu heteroaryl sulfon amidů. Především na rozšíření metody nedávno vyvinuté v naší výzkumné skupině, která umožňuje připravit *N*-substituované a *N,N*-disubstituované benzothiazol-2-yl sulfonamidy z benzo[*d*]thiazol-2-thiolu and alkyl aminů a rozšíření této metody pro přípravu nepřírodních α-amino kyselin. Úspěšně se nám povedlo rozšířit metodu o další heterocykly jako jsou pyridin a pyrimidin, imidazol, benzo[*d*]imidazol, benzo[*d*]oxazol, oxazol, benzo[*d*]thiazol, a thiazol. Po optimalizaci, jsme připravili knihovnu přibližně 50 látek *N*-substituovaných a *N,N*-disubstituovaných heteroaryl

sulfonamidů. Tato knihovna byla poté za využití konceptu “paměti chirality” použita pro přípravu obou enantiomerů doposud neznámých α-heteroaryl α-substitutuovaných α-amino kyselin pomocí bazického polárního Truce-Smiles přesmyku. Pomocí DFT výpočtů a analýzy experimentálních dat, jsme úspěšně navrhli mechanismus reakce.

10. References

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