Synthesis of biologically active enones: 2,3-dihydro-1,3,4-oxadiazoles, α-X-cyclopentenones and attempts towards limnophilaspiroketone and zerumbone

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften

(Dr. rer. nat.)

an der Fakultät der Chemie und Pharmazie

der Universität Regensburg

vorgelegt von

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aus

Jordanien

2015

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Promotionsgesuch eingereicht am: 23.02.2015

Promotionskolloquium am: 12.03.2015

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Abreviations

Ac_2O	Acetic anhydride
aq.	Aqueous
ARE	Antioxidant response element
ATPH	Aluminum tris(2,6-diphenylphenoxide)
CAN	Ceric ammonium nitrate
COX-2	Cyclooxygenase-2
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EI	Electron ionization
eq.	Equivalent
ES-MS	Electron spray-mass spectrometry
HO-1	Heme oxygenase-1
HRMS	High resolution mass spectrometry
HGC	Hovedya-Grubbs catalyst
IKK	IκB kinase
iNOS	Inducible NO synthase
IR	Infrared
IκBα	inhibitor of kappa B
IBX	2-Iodoxybenzoic acid
Keap1	Kelch-like ECH-associated protein1
LDA	Lithium diisopropylamide
LiHMDS	Lithium hexamethyldisilazide
MS	Mass spectrometry
NCS	*N*-Chlorosuccinimide

Abreviations

NF-κB	Nuclear factor-kappa B
NIS	*N*-Iodosuccinimide
NMP	*N*-Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
Nrf2	Nuclear factor-erythroid-2-related factor 2
PCC	Pyridinium chlorochromate
r.t.	Room temperature
THF	Tetrahydrofuran
TLC	Thin Layer chromatography
TRIS-HCl	Tris(hydroxymethyl)aminomethane hydrochloride

1. Introduction

1.1. α,β-Unsaturated carbonyl compounds

The enone unit is one of the most important moiety that exist in natural and unnatural compounds. This moiety plays an important role in medicinal chemistry and industry as a starting material (e.g. ethyl acrylate, a monomer for the production of acrylic polymer).[1] These compounds could be listed as: α,β-unsaturated ketones, aldehydes, esters and amides (Figure 1).[2-4]

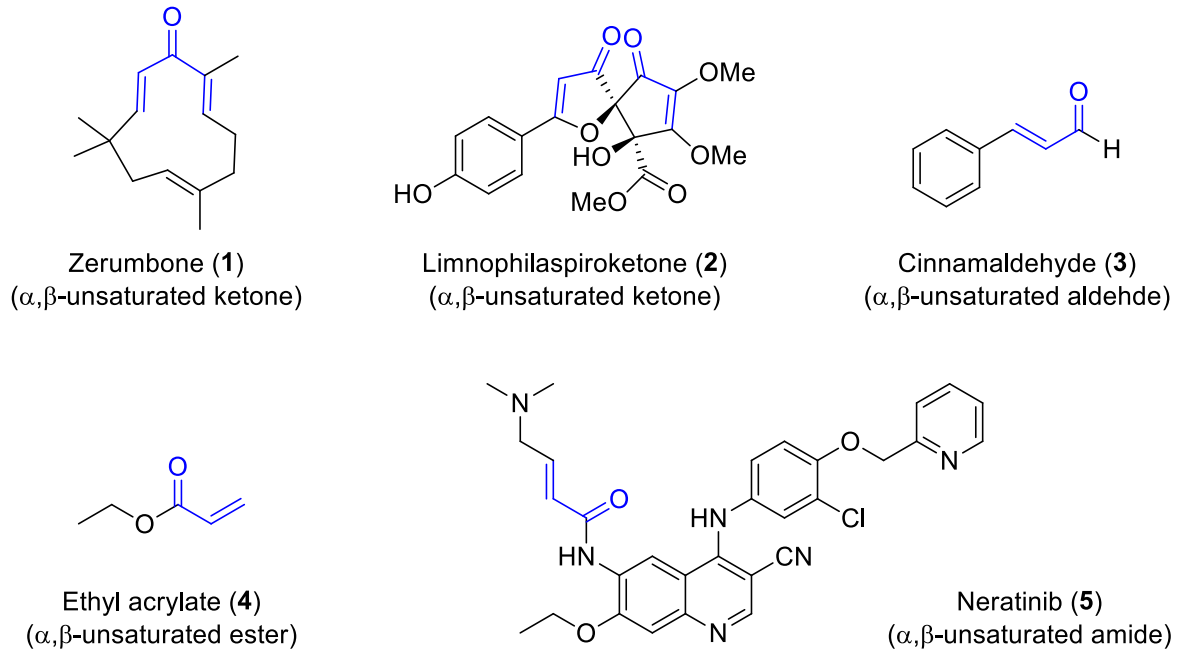


Figure 1: Examples of α,β-unsaturated carbonyl compounds.

1.1.1. The role of α,β-unsaturated carbonyl compounds in inflammation

Inflammation is a normal response of living tissues against injury, in order to repair the infected cells or defense against pathogens. Transcription factors play an important role in the inflammation process, in the normal cells the transcription factor Nrf2 (nuclear factor-erythroid-2-related factor 2) is deactivated by a protein called Keap1 (Kelch-like ECH-associated protein1). During the inflammation process the thiol residues in Keap1 can be oxidized to form disulfide bonds or react as a Michael donor with the α,β-unsaturated carbonyl compounds. In both cases the result is a free Nrf2 which can pass through the nucleus and binds to the antioxidant response elements, which cause anti-inflammatory protein production such as heme oxygenase 1 (HO-1) (Figure 2).[5-6]

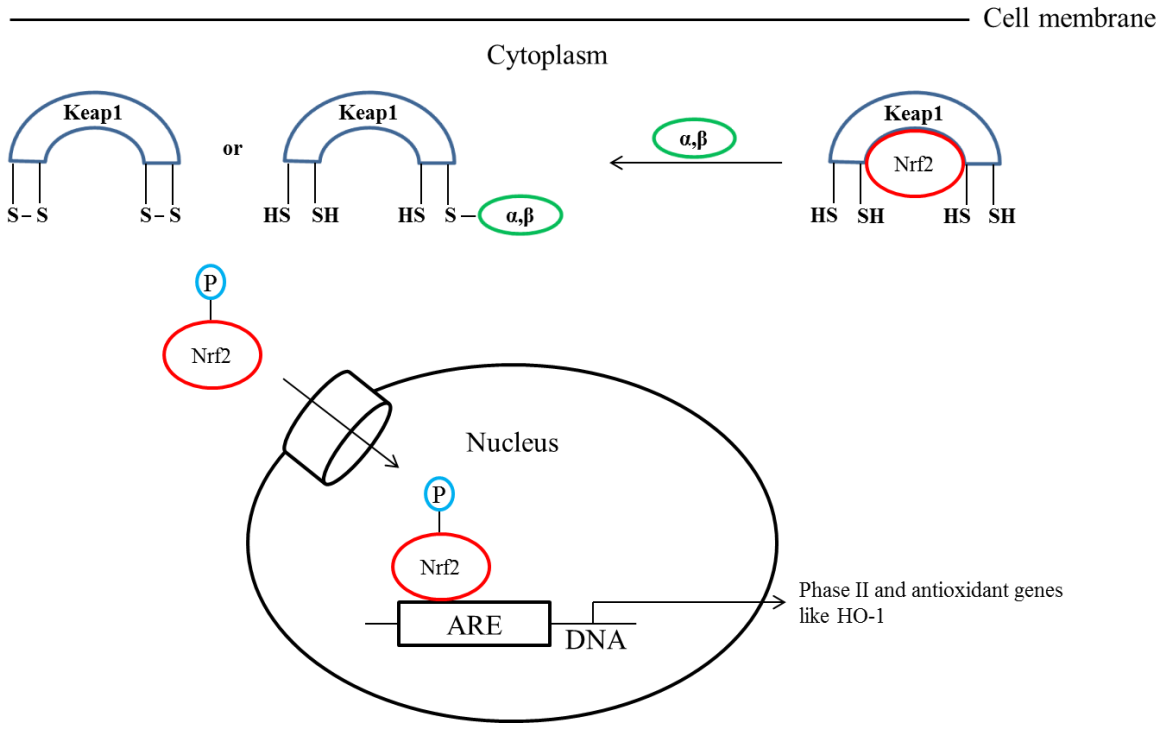


Figure 2: Mechanism of Nrf2 activation.

One other important factor for control of inflammation is the transcriptional factor NF-κB (nuclear factor-kappa B). In the unstimulated cells the NF-κB exists in the cytoplasm as a dimer of NF-κB1 (p50) and Rel A (p65), which binds with the inhibitor IκBα. The process to activate the NF-κB starts from the signals come to the cytoplasm in which the IκBα is phosphorylated by the IKK complex (IκB kinase) followed by ubiquitination of the IκBα, as a result, the NF-κB becomes free and active to penetrate inside the nucleus and bind with the DNA to produce proinflammatory genes like iNOS (inducible NO synthase) and COX-2 (cyclooxygenase-2). Different stimuli can activate the NF-κB such as T and B cell mitogens, bacterial lipopolysaccharide, viruses, UV-light and tumor necrosis factor (TNF). Thus the NF-κB pathway is very important in the cancer therapeutic chemopreventive approach since inhibition of this way using synthetic drugs (such as α,β-unsaturated carbonyl compounds) can either prevent cancer development or inhibit cancer cell growth (Figure 3).[5-6]

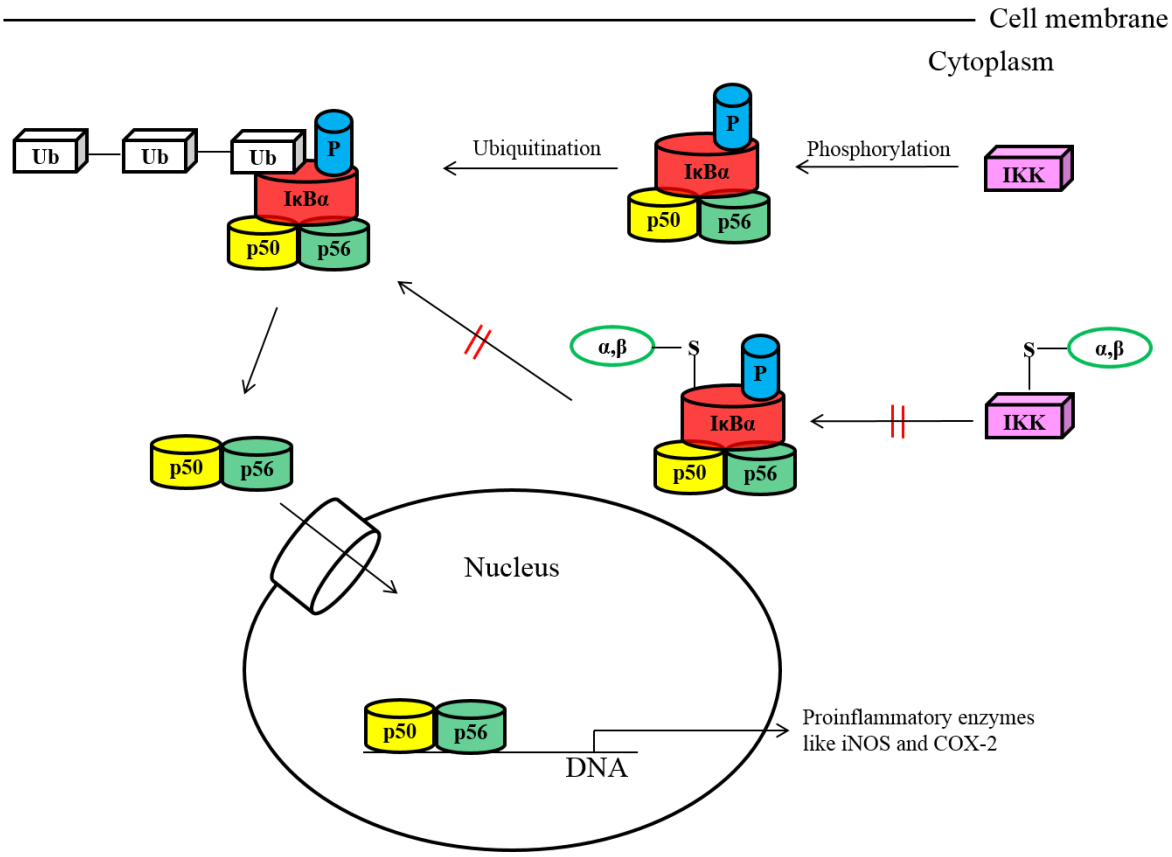


Figure 3: Regulation of NF-κB pathway by α,β-unsaturated carbonyl compounds, (NF-κB: nuclear factor-kappa B; a heterodimer of p50 and p65 proteins; IKK: IκB kinase; IκBα: inhibitor of kappa B, α; Ub-Ub-Ub: ubiquitination; α,β: α,β-unsaturated carbonyl compound; iNOS: inducible NO synthase; COX-2: cyclooxygenase-2).

1.1.2. Reactivity of α,β-unsaturated carbonyl compounds

The reactivity of many α,β-unsaturated carbonyl compounds depends on their behavior as Michael acceptors. Nevertheless, enone moieties are able to have radical scavenging properties, reductive potential and double bond isomerization (Figure 4).[2]

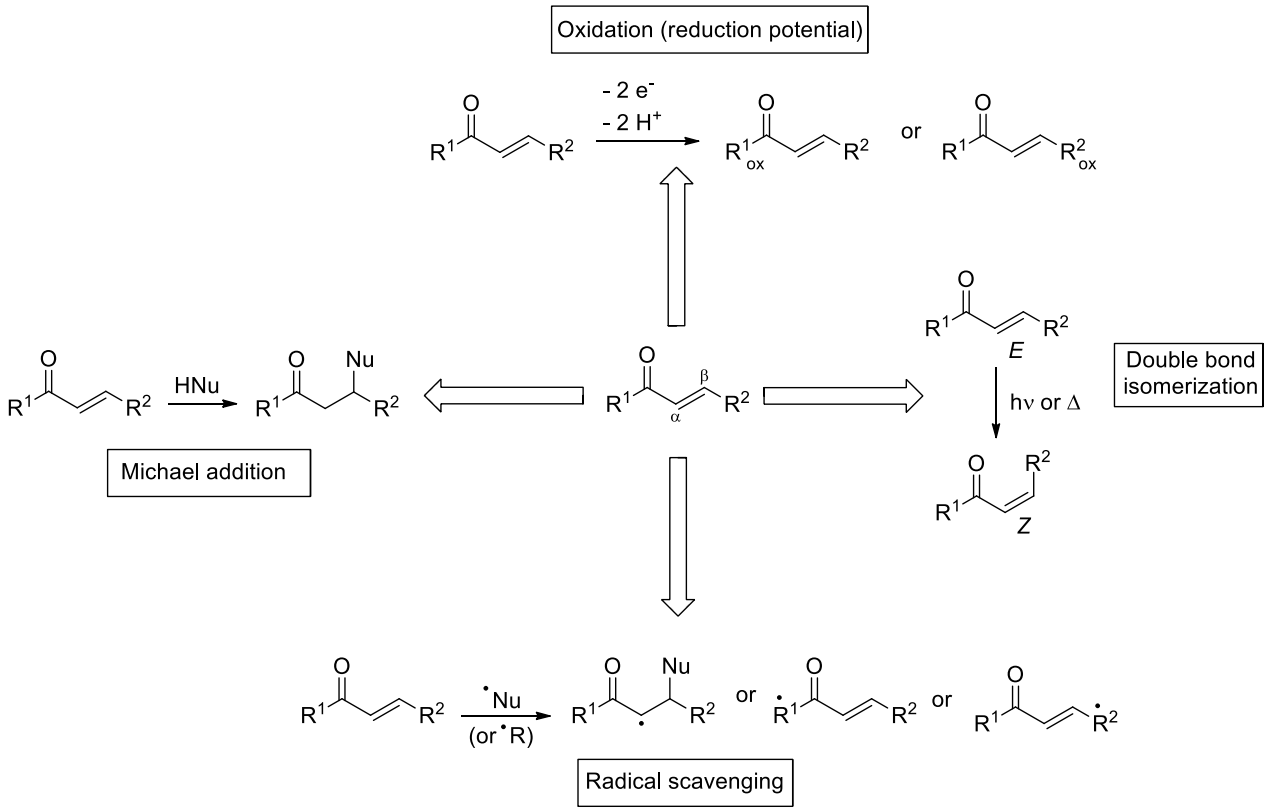


Figure 4: Possible reactivities of α,β-unsaturated compounds.[2]

The activity of the Michael system correlates strongly to the substituents on both α- and β- positions which lead to variable biological activities. Honda *et al*.[7] showed that introducing a cyano group on the α-position of the oleanolic acid derivatives (Figure 5) can enhance the inhibition of production of NO induced in mouse macrophages.

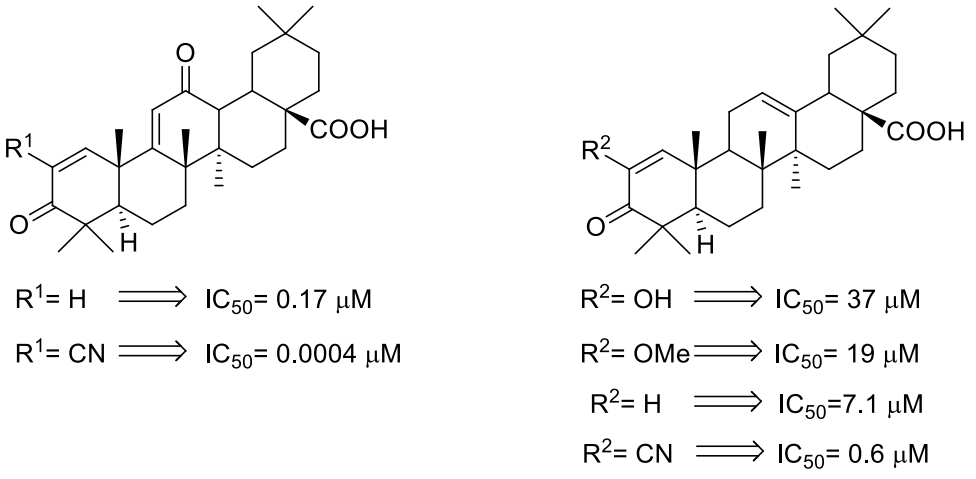
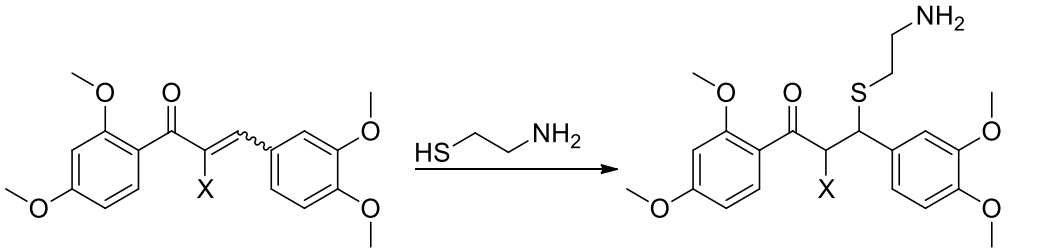


Figure 5: Effect of α-substituted on the inhibition of NO production in mouse macrophages.[7]

In order to measure the reactivity of α,β-unsaturated carbonyl compounds as Michael accepters, Amslinger and Al-Rifai developed kinetic thiol assay, to assess the reactivity of chalcones towards thiols. Second order-rate constants (k_2) were used to compare the reactivity of different chalcones. The optimized conditions were using solvent mixture (100 mM TRIS-HCl, pH=7.4, 2 mM EDTA/ethylene glycol (20:80)), the thiol was cysteamine, the wavelength determined after UV-Vis scans and LC-MS measurements and the thiol-adduct product was characterized by LC-MS measurements. Under these conditions different α-X-chalcones were screened with k_2 values in the range of 0.004 $M^{-1}s^{-1}$ for α-COOH-chalcones to 5750 $M^{-1}s^{-1}$ for α-CN-chalcones (Scheme 1).[8] These results showed again the effect of the α-substitutions on the behavior of the α,β-unsaturated carbonyl compounds to be Michael acceptors.



X: CN, NO_2, CF_3, Br, Cl, I, F, H, COOH, COOEt, Me, Ph, *p*-NO_2-C_6H_4 and *p*-MeO-C_6H_4.

Scheme 1: Reaction of α-X-chalcones with cysteamine.

1.1.3. Biological activities of α,β-unsaturated carbonyl compounds

α,β-Unsaturated carbonyl compounds display a wide spectrum of biological activities such as antibacterial, anti-inflammatory and anticancer. Kaempferol (**6**) (Figure 6) is a flavonol that presents at high level in tea, broccoli, grapes and strawberries. It can prevent the formation of reactive oxygen species (ROS) in RAW264.7 cells, a mouse macrophage cell line.[9] New study on curcumin (**7**) showed that curcumin can inhibit the proliferation and invasion of MHCC97-H cells. The result provides supporting to curcumin clinical use.[10] Caffeic acid phenethyl ester (CAPE) (**8**) was tested against six different cancer cell lines and showed high selectivity as antiproliferative of liver-metastatic murine colon 26-L5 carcinoma cell line up to (EC_{50} = 0.02 µM).[11] A natural product dicerandrol C (**9**) which is a metabolite of *Phomopsis longicolla* the fungi isolated from the red seaweed *Bosterychia radicans* had significant antibacterial activity against *Staphylococcus aureus* (ATCC 6538) and *Staphylococcus*

saprophyticus (ATCC 15305) with minimum inhibitory concentrations of 1.33 µM and 2.66 µM, respectively.[12]

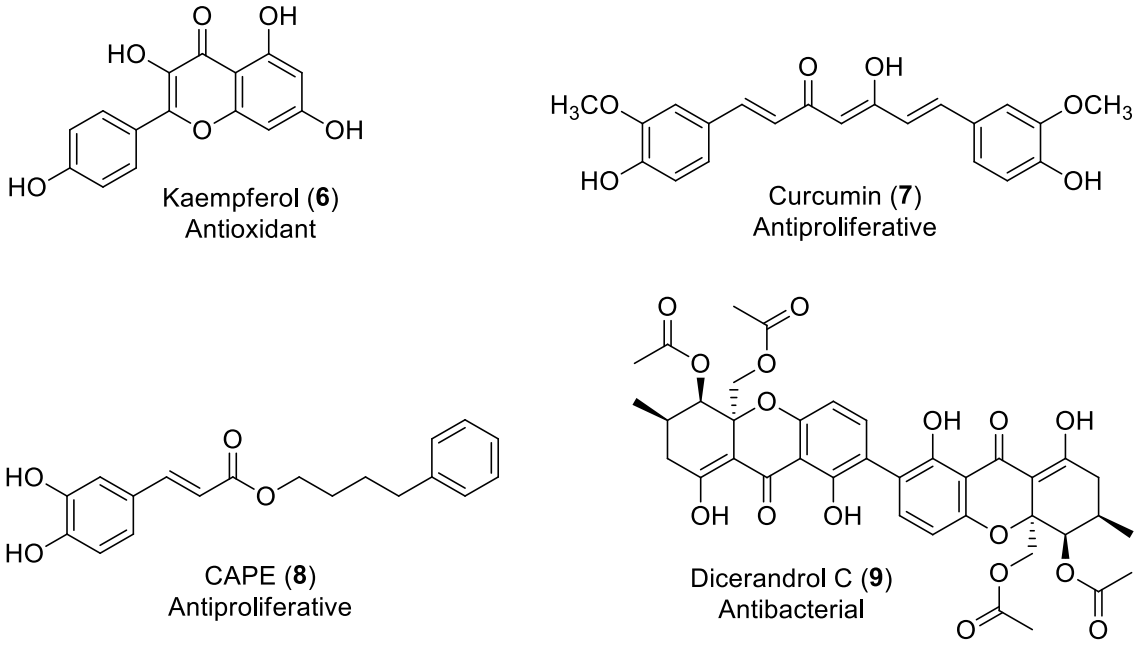
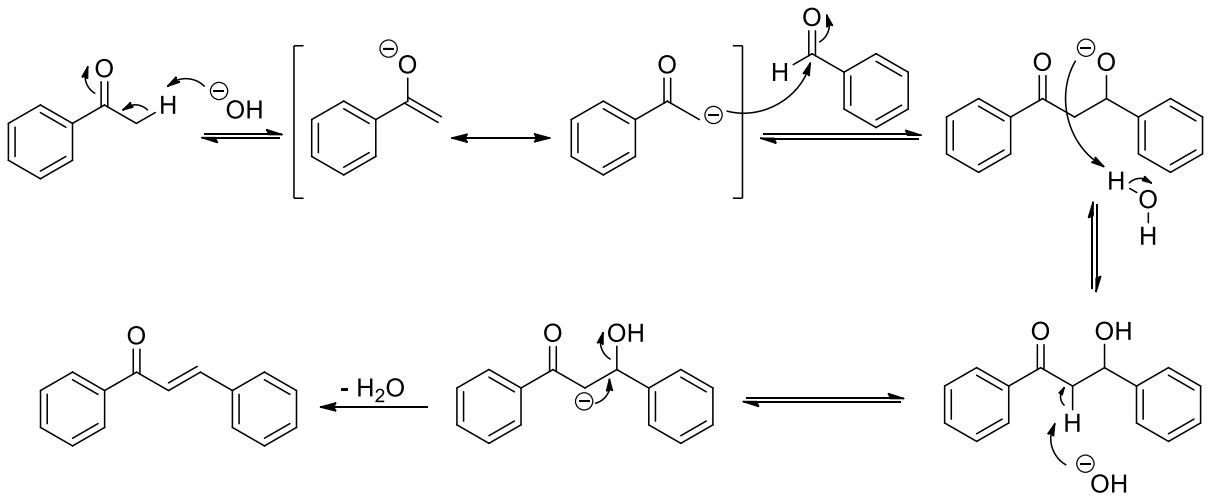


Figure 6: Some biological active α,β-unsaturated carbonyl compounds.

1.1.4. Synthesis of α,β-unsaturated carbonyl compounds

The most common method to form the enone unit is the condensation reaction between an aldehydes and a ketones in acidic, basic medium and sometimes catalysts. The reaction conditions depend on the aldehydes and the ketones. The Claisen-Schmidt condensation is one of the best strategies to form the enone in basic medium. Scheme 2 shows the mechanism of this reaction to produce the chalcone in which the base deprotonates the ketone to form the enolate that attacks the aldehyde to produce a β-hydroxyketone, followed by dehydration to afford the desired chalcone.[13]

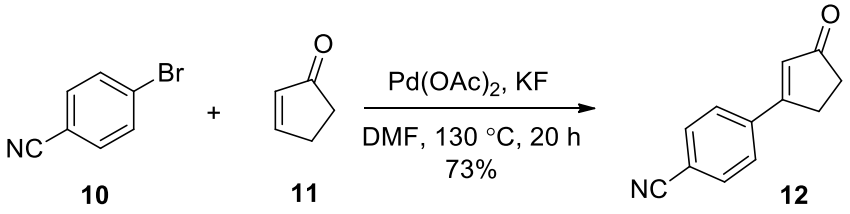


Scheme 2: Mechanism of the Claisen-Schmidt condensation.

Other methods to form such an enone are usage of salts,[14] or using Lewis acids like $AlCl_3$ and $TiCl_4$ to couple α,β-unsaturated acyl chlorides with a vinyl moiety.[15]

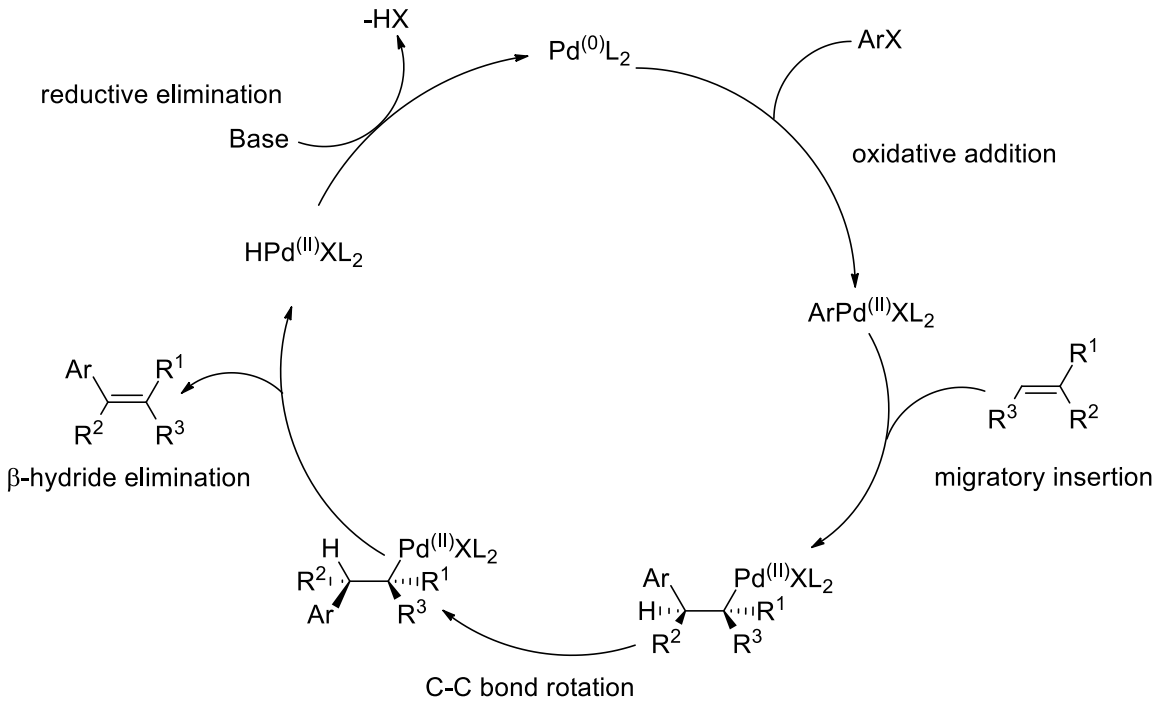
The Wittig reaction was also used to produce the α,β-unsaturated carbonyl compound, as an example Kodama *et al*.[16] coupled an aldehyde with phosphonium yild in the total synthesis of zerumbone (**1**).

Additionally, the Heck reaction using palladium catalyst is very useful in some cases to form α,β-unsaturated carbonyl derivatives by coupling aryl halides (specially aryl iodide) with vinyl ketones or aldehydes (Scheme 3).[17]



Scheme 3: Derivatization of an α,β-unsaturated carbonyl compound via Heck reaction.

The proposed mechanism of Heck reaction starts with generation of $Pd^{(0)}$ catalyst followed by oxidative addition into C-X bond, then syn addition of Ar-$Pd^{(II)}XL_2$ to vinyl moiety, in some cases C-C bond rotation occurs to form less steric hindrance skeleton. The final product generates by syn β-hydride elimination to regenerate $Pd^{(0)}L_2$ (Scheme 4).[18]

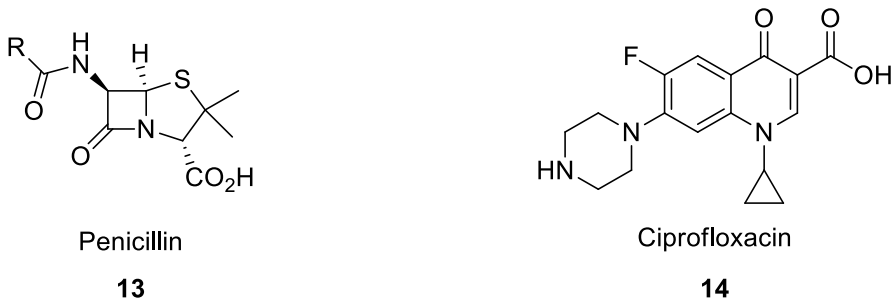


Scheme 4: General mechanism of the Heck reaction.

Due to a wide use of Heck reaction especially in natural product synthesis, different Pd-catalysts have been used such as $Pd(OAc)_2$, $Pd_2(dba)_3$ and $PdCl_2(PPh_3)_2$.[19]

1.2. Antimicrobial activity and multidrug resistance

Infections microorganisms by bacteria, fungi and viruses cause a continuous and serious threat to human health and life since decades, which lead to continuous drug development. The antimicrobial agents could be natural products such as penicillin (**13**) or synthesized such as ciprofloxacin (**14**) (Figure 7).



Penicillin **13**

Ciprofloxacin **14**

Figure 7: Structures of penicillin (**13**) and ciprofloxacin (**14**).

Antimicrobial agents act selectively on the microbial functions with minimal effects or without affecting steward functions. In general, antimicrobial agents can be described

as either bacteriostatic or bactericidal. Bacteriostatic antimicrobial agents only inhibit the growth or the multiplication of the bacteria giving the immune system of the host time to clear them from the body, in this case the bacteria elimination process depends on the competence of the immune system. Examples for the bacteriostatic compounds are chloramphenicol which inhibits the protein synthesis in the bacteria, while sulfonamides inhibit folate synthesis. The second type of antimicrobial agents is bactericidal agents which kill the bacteria and therefore with or without a competent immune system of the steward the bacteria will be killed, examples are penicillins (**13**) and cephalosporins which inhibit the cell wall synthesis, while quinolones inhibit DNA synthesis.[20-21]

In order to survive, the microorganism developed different methods to resist the effect of antimicrobial agents which is called multidrug resistance (MDR). The microorganism can resist the drugs in different ways (Figure 8), such as drug efflux, in which the microorganism pumps the drug out of the cell. A second method is altering the cell wall permeability which leads to decrease of the drug concentration in the cell, a third is mutation in the cell that causes changes in the drug target in which the drug can't bind to the target any more, a forth is the up regulation of the target protein in the cell. In this case the drug deactivates few targets but there still alternative targets which still make the desired functions for the cell. Finally MDR can occur by applying the cell specific enzymes to deactivate the drug or destroy it.[22]

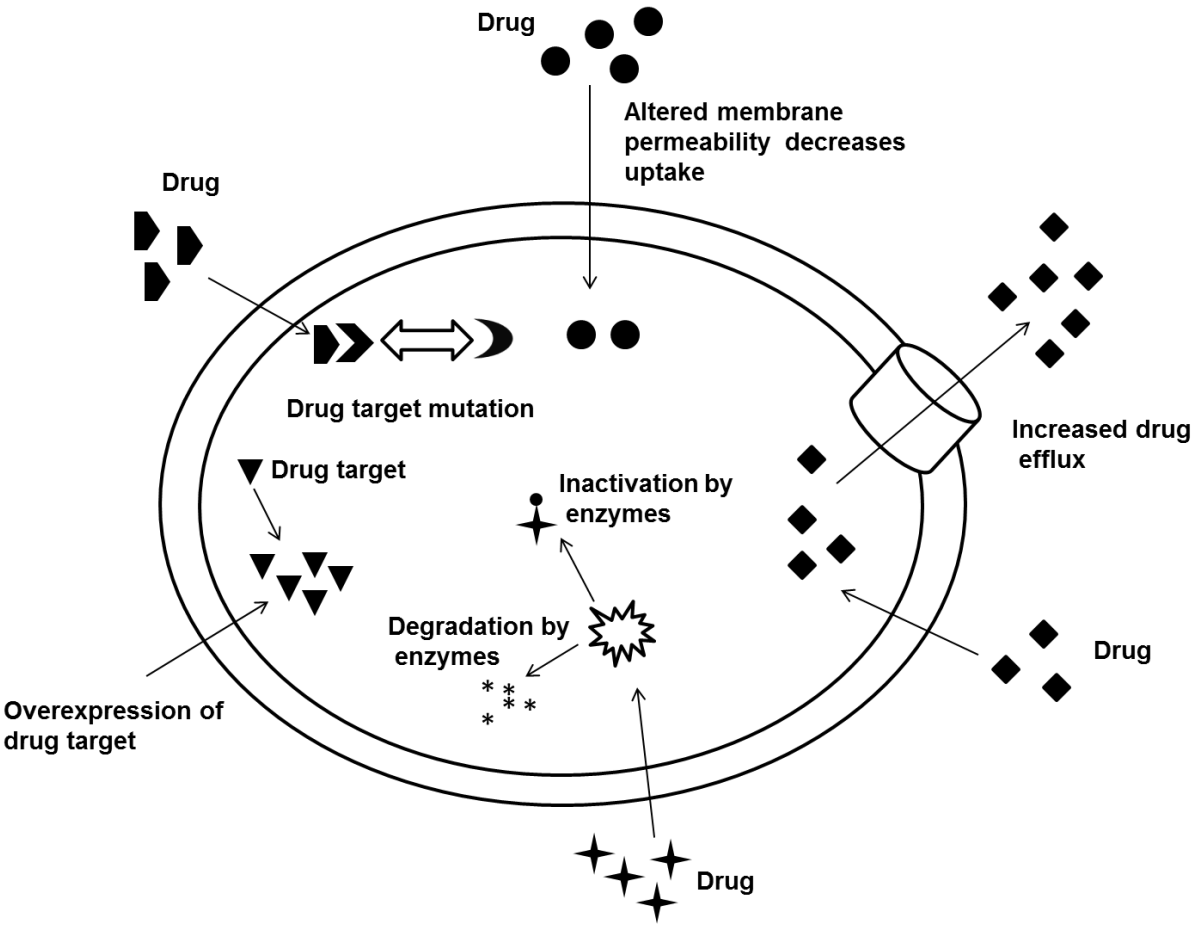


Figure 8: Mechanisms of multidrug resistance (with permission).[22]

Due to MDR, the drug will be inefficacious and not available useful to treat the infection, which means a big challenge for scientists to search for new antimicrobial agents.

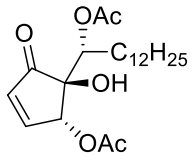
1.3. The cyclopentenone unit in natural products

The cyclopentenone skeleton (which contains α,β-unsaturated carbonyl) is a common moiety exists in natural products. The hygrophorone (**15**) (Figure 9), a cyclopentenone natural product derived from the fruiting bodies of the mushroom *Hygrophorus* shows promising antifungal activity.[23] Haneishi *et al*. succeeded to isolate novel antibacterial cyclopentenone derivatives from the culture filtrate of a *streptomycete* called methylenomycins A (**16**) and methylenomycins B (**17**), which showed inhibition against different gram-positive and gram-negative bacteria.[24]

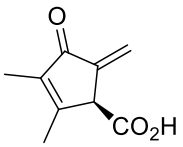
Liao *et al*.[25] in 2005 were able to isolate and characterize lathyranoic acid A (**18**), a natural product from the seeds of *Euphorbia lathyris* and a common Traditional

Chinese Medicine used for ascites, coprostasis, anuresis, amenorrhea, venous stasis, terminal schistosomiasis, scabies, and snakebite.

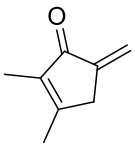
The methanol extracted fraction from the fruiting bodies of *Tylopilus eximius* shows antibacterial properties, this extract has a compound with a cyclopentenone moiety with two phenolic groups and with a hydroxyl group in the α-position called tylopilusin A (**19**), which inhibits the yellow pigment (staphyloxanthin) produced by methicillin-resistant *Staphylococcus aureus* (MRSA). The yellow pigment in the *Staphylococcus aureus* works as an antioxidant to protect it from reactive oxygen species (ROS) produced by the host immune system (Figure 9).[26]



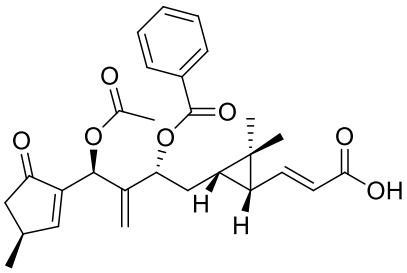
4,6-Diacetylhygrophorone A (**15**)



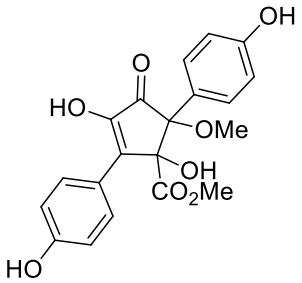
Methylenomycins A (**16**)



Methylenomycins B (**17**)



Lathyranoic acid A (**18**)

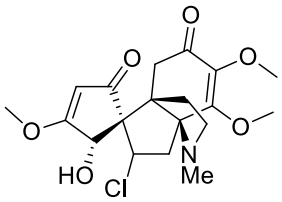


Tylopilusin A (**19**)

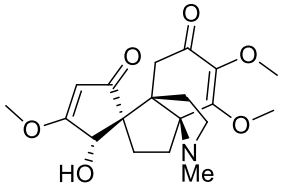
Figure 9: Some biologically active cyclopentenone natural products.

1.3.1. Spirocyclopentenones in natural products

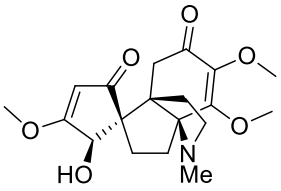
In addition to the cyclopentenone unit in some natural products, there are many natural products which contain a spirocyclopentenone unit. Members of the acutumine family (**20**, **21** and **22**) (Figure 10) are natural products from *Menispermum dauricum*, a plant widely used in the Traditional Medicine of China with analgesic and antipyretic properties, from which Yu *et al*.[27] in 2002 isolated acutumine derivatives, the spirocyclopentenone isolated compounds show selective inhibition of T-cell growth. Another example is hypserpanine A (**23**) which was isolated from *Hypserpa nitida* and show an interesting activity as antihepatitis B.[28]



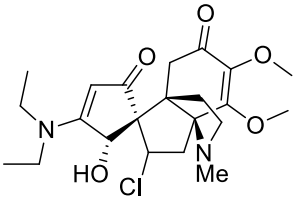
Acutumine (**20**)



Dechlorioacutumine (**21**)



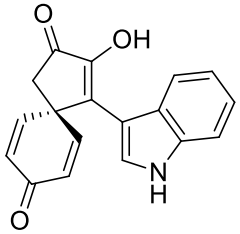
1-Epidechlorioacutumine (**22**)



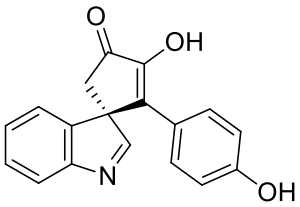
Hypserpanine A (**23**)

Figure 10: Acutumine natural products.

Park *et al*.[29] recently described new secondary metabolites from the 24 h broth culture of *Lysinibacillus fusiformis* KMC003 derived from acidic coal-mine drainage which have a spirocyclopentenone moiety with a hydroxyl group in the α-position called spirobacillene A (**24**) and spirobacillene B (**25**). The newly isolated compounds show weak antibacterial properties (Figure 11).



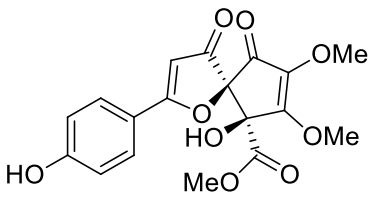
Spirobacillene A (**24**)



Spirobacillene B (**25**)

Figure 11: Structures of spirobacillene A (**24**) and spirobacillene B (**25**).

Another example for the spirocyclopentenone in natural products was isolated by Jang *et al*.[4] in 2005 from *Limnophila geoffrayi*, a plant used in the Traditional Medicine of Thailand due to its antipyretic, expectorant and galactagogue prosperities. The new isolated compound which has two enone units, a spirocyclopentenone system and a phenolic moiety is called limnophilaspiroketone (**2**) (Figure 12). Moreover, Suksaamrarm *et al*.[30] isolated some flavones from the same plant as antimycobacterial and antioxidant agents. The essential oil received from *Limnophila geoffrayi* by Thongdon-A and Inprakhon in 2009 show highly antimicrobial and strong insecticidal activities.[31]



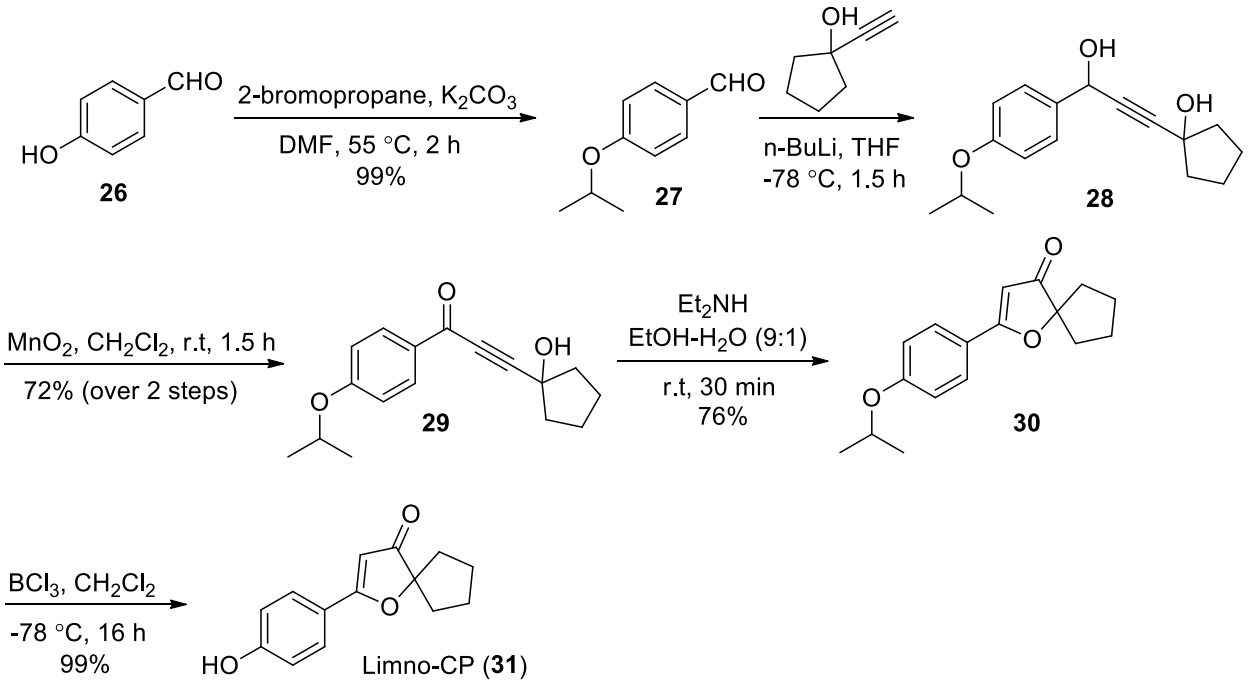
Limnophilaspiroketone (**2**)

Limnophila geoffrayi

Figure 12: Structure of limnophilaspiroketone (**2**) and image of *Limnophial geoffryi*.

1.3.2. Derivatives of limnophilaspiroketone (Limno-CPs)

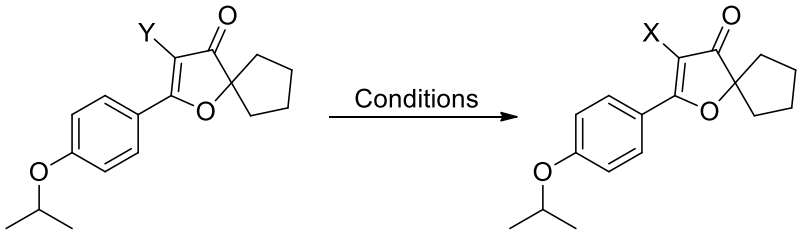
Since limnophilaspiroketone has a unique skeleton, Sabine Amslinger and Simon Lindner were able to synthesize derivatives which have a similar skeleton to the natural product limnophilaspiroketone called Limno-CPs.[32] The sequence started with protection of 4-hydroxybenzaldehyde (**26**) with *i*Pr group to form compound **27** in 99% yield after 2 h (Scheme 5). After that aldol condensation was applied between compound **27** with 1-ethynylcyclopentanol, the produced benzyl alcohol (**28**) was oxidized by MnO_2 to the corresponding carbonyl group (**29**). Then the key step comes by cyclization using Et_2NH in EtOH-H_2O mixture afford *i*Pr-Limno-CP (**30**) in 76% yield. Finally isopropyl deprotection by BCl_3 produced the desired product (**31**) in overall yield 54%.



Scheme 5: Synthesis of Limno-CP (**31**).[32]

For more investigations different substituents were introduced in the α-position of the enone unit to the prepared Limno-CP in order to study the behavior of the derivatives as Michael acceptors. Table 1 shows the conditions and the yields of the synthesized compounds.

Table 1: Synthesis of α-X-Limno-CPs.[32]



Y Starting material	Conditions	X Product	Yield (%)
H	NFSI, THF, reflux, 16 h	F	5
H	NCS, MeOH, reflux, 16 h	Cl	91
H	NBS, MeCN, 0 °C, 30 min	Br	88
H	Br_2, Et_3N, $CHCl_3$, 0 °C, 3 h	Br	92
H	NIS, MeCN, reflux, 16 h	I	88
H	I_2, PDC, CH_2Cl_2, r.t, 3 h	I	72
Br	NaCN, $NiBr_2$, NMP, µw, 200 °C, 10 min	CN	89
CN	aq 12 M NaOH-1,4-dioxane (1:1), r.t, 1.5 h	$CONH_2$	73
Br	$PhB(OH)_2$, $Pd_2(dba)_3$, PPh_3, Et_2NH, PhMe-EtOH-H_2O, reflux, 20 h	Ph*	79

*Similar structure to the COX-2 inhibitor Rofecoxib.

The synthesized compounds were deprotected using BCl_3 to form a set of phenolic derivatives in 82-99% yield after 3-40 h. All the compounds were tested as Michael acceptors by the kinetic thiol assay and none of them showed Michael acceptor activity as no reaction was observed, which reflect the low electrophilicity of the system.[32]

1.4. Zerumbone

Zerumbone (**1**) (Figure 13), a cyclic 11-membered ring natural product with α,β-unsaturated carbonyl group. It is the main component of the essential oil of *Zingiber zerumbet* Smith. The first isolation of this compound was in 1960, and since that the attention was increased.[33]

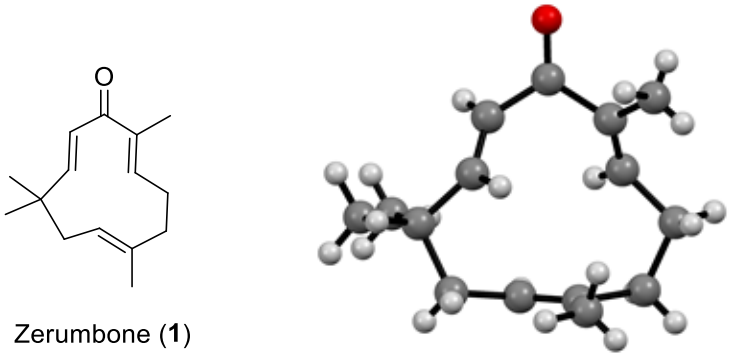


Figure 13: Structure of zerumbone (**1**) and the X-ray structure.[34]

1.4.1. Biological activity of zerumbone and its derivatives

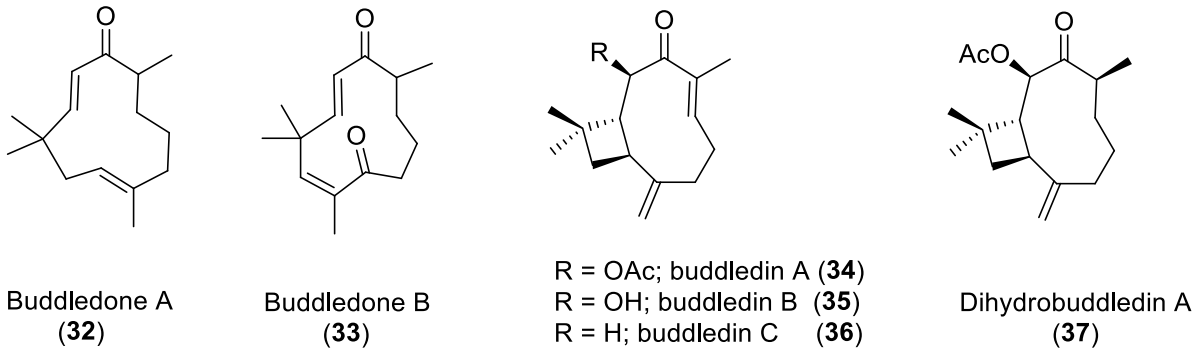
Xian *et al*. showed in 2006 that zerumbone (**1**) is able to significantly suppress the proliferation of leukemia NB4 cells by inducing G2/M cell cycle arrest, followed by apoptosis with an IC_{50} value 10 μM.[35] Furthermore, Kim *et al*. studied the effect of zerumbone (**1**) in vivo in colon and lung cancer in mice. The study shows the ability of zerumbone (**1**) as chemopreventive of colon and lung cancer in mice. These properties came from the antiproliferative, apoptosis inducing, anti-inflammatory and suppression of NF-κB and HO-1.[36] Similarly, Abdelwahab *et al*.[37] found in 2011 that zerumbone (**1**) is able to inhibit proliferative of T-acute lymphoblastic leukemia cells. In addition, zerumbone shows promising activity as anticholinesterase.[38]

Recently, a study showed the attractive activity of zerumbone (**1**) in vitro (MCF-7 and MDA-MB-231 cells) and (MDA-MB-213 cells) to inhibit growth of human breast cancer cells in association with apoptosis induction.[39] Moreover, zerumbone (**1**) inhibited the NF-κB-dependent proangiogenic, which is related to block the pancreatic cancer-associated angiogenesis.[40]

Shin *et al*.[41] reported the ability of zerumbone (**1**) to activate Nrf2 which induces HO-1 in mouse skin and cultured murine epidermal cells. Importantly, the derivatives produced by reducing the carbonyl group to the alcohol or remove it completely did

not show any activity. This proves the important role of the α,β-unsaturated carbonyl group in the biological properties of the zerumbone. Zerumbone (**1**) also inhibits RANKL, a receptor that activates NF-κB and is responsible for many diseases such as cancer, chronic inflammatory and bone resorption by inhibition of IκBα kinase, IκBα phosphorylation and IκBα degradation.[42]

Natural zerumbone derivatives have been isolated from *Buddleja* species (Figure 14). The plant is used in Chinese Traditional Medicine. The flowers of *Buddleja officinalis*, are Traditional Chinese Medicine used for the treatment of conjunctival congestion and clustered nebulae, whereas the roots of *Buddleja asiatica Lour* are used as an anti-inflammatory agent. Similar usage of *Buddleja globosa* is reported from Chile, where the leaves and flowers are used for washing injury and treating ulcers.[43]



Buddledone A (**32**)

Buddledone B (**33**)

R = OAc; buddledin A (**34**)
R = OH; buddledin B (**35**)
R = H; buddledin C (**36**)

Dihydrobuddledin A (**37**)

Figure 14: Some naturally occuring zerumbone analogues.

Further investigations of zerumbone led Kitayama *et al*.[44] to synthesize new antibacterial derivatives. The new derivatives **38**, **39**, **40** and **41** (Figure 15) showed selective inhibition of gram-positive bacteria (*Bacillus subtilis* 168), especially the open chain analogues **40** and **41**. While all the derivatives did not show any activity against gram-negative bacteria (*Escherichia coli* MC4100).

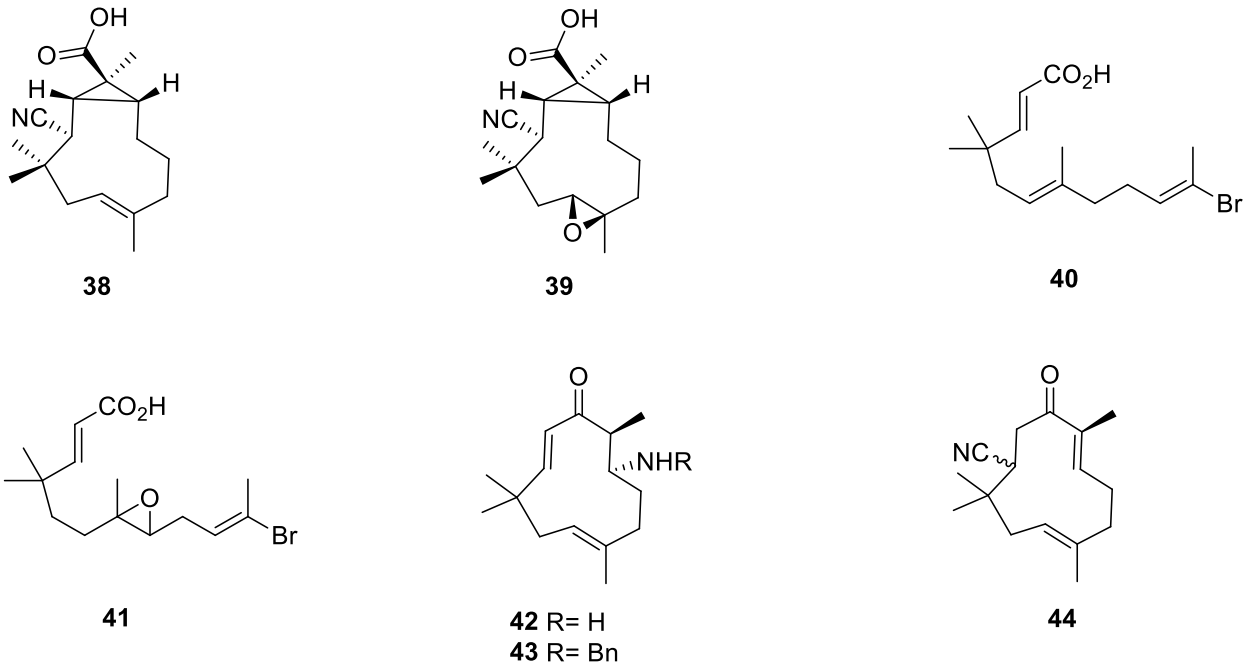


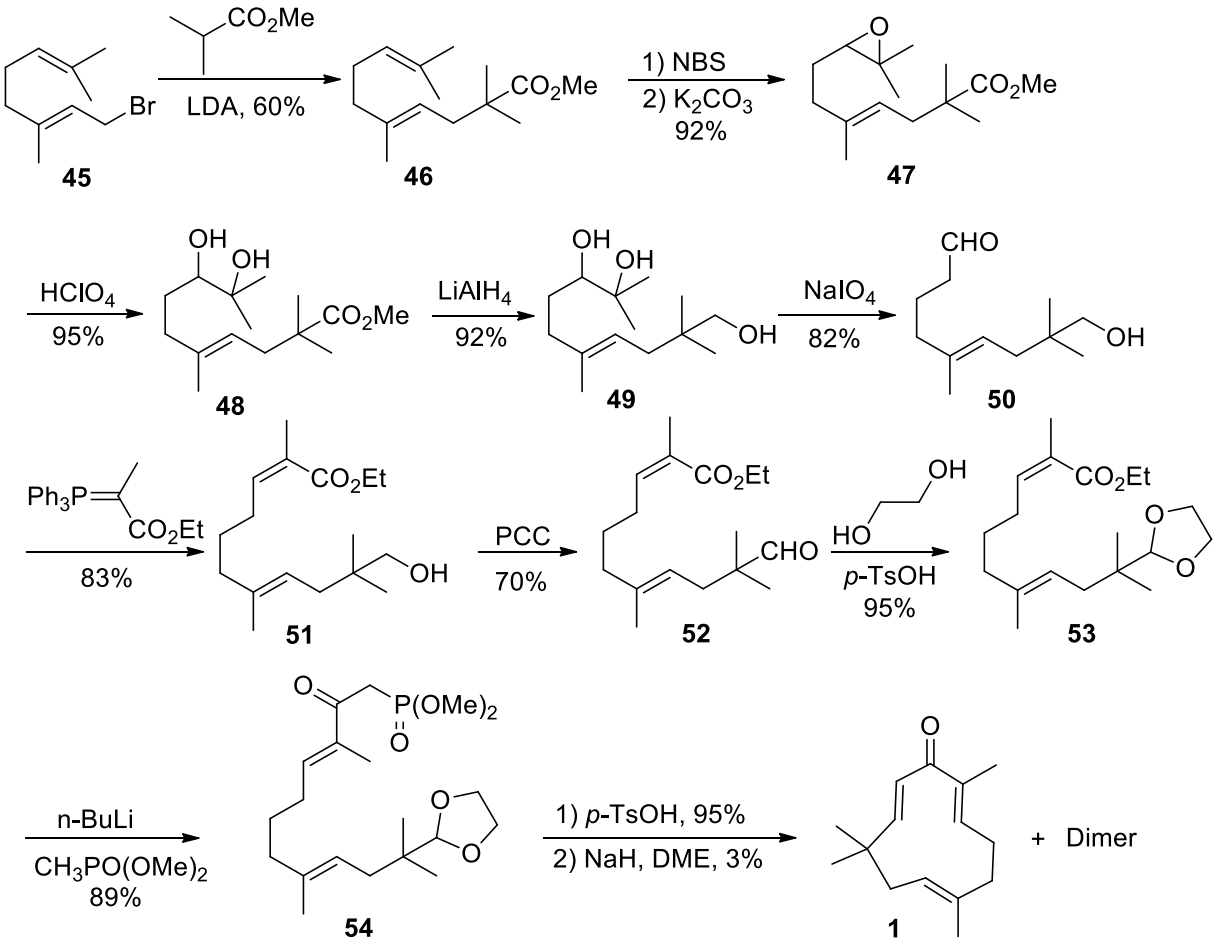
Figure 15: Some antibacterial (**38-41**) and antimalarial (**42-44**) synthetic zerumbone derivatives.

Amine and cyano zerumbone derivatives **42**, **43** and **44** (Figure 15) displyed antimalarial activity with IC_{50} values 5.6, 7.0 and 7.5 µM, respectively. The IC_{50} values against normal cell line were 26-47 µM which make them not suitable as antimalarial drugs.[45] Furthermore, some zerumbone analogues were able to suppress NO generation and the study showed the importance of the conjugated system for this behavior.[46]

1.4.2. Total synthesis of zerumbone

Kodoma *et al*.[16] published in 1987 the first total synthesis of zerumbone (Scheme 6). The sequence started with a substitution reaction between geranyl bromide **45** and methyl isobutyrate to produce ester **46**, followed by a selective epoxidation using NBS and K_2CO_3. The epoxide was converted to the vicinal diol **47** by perchloric acid. The next step was to reduce the ester **48** to the corresponding alcohol **49** by $LiAlH_4$, then sodium periodate was used to cleave the bond in the vicinal diol to give the corresponding aldehyde **50**. An ester group was introduced to the aldehyde by Wittig reaction giving compound **51**, followed by oxidation of the alcohol to aldehyde **52** which is protected by ethylene glycol (**53**). Then, the ketophosphonate **54** is prepared by coupling the ester group in **53** with dimethyl methyl phosphonate using n-BuLi as a base. Deprotection of the aldehyde and treating it with sodium hydride gives the

desired zerumbone (**1)** in 3% overall yield. At the same time 55% from the dimer was obtained, many conditions were screened to suppress the dimer including increased the dilution factor but still high yield was observed.



Scheme 6: Total synthesis of zerumbone.[16]

1.5. 1,3,4-Oxadiazole derivatives

The 1,3,4-oxadiazole is a five membered heterocyclic ring compound containing one oxygen atom in position 1 and two nitrogen atoms in positions 3 and 4. This skeleton gives the compound special properties which make it one of the most regular studied oxadiazole isomer **58** (Figure 16).

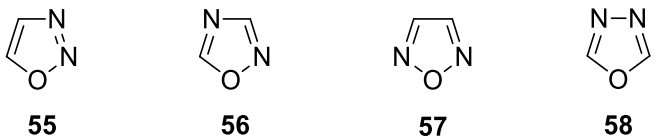


Figure 16: Oxadiazole isomers.

1.5.1. Biological activity of 1,3,4-oxadiazole derivatives

As a special feature, compounds containing the 1,3,4-oxadiazole moiety have broad biological activities including antibacterial, antifungal, anti-inflammatory, antiviral, anticancer and antihypertensive. Figure 17 shows two examples of compounds containing the 1,3,4-oxadiazole unit in late stage clinical development.[47]

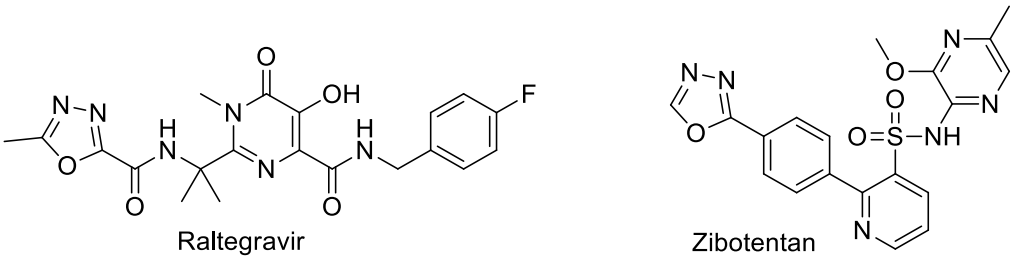


Figure 17: Structures of raltegravir and zibotentan, drugs that are in late stage clinical development.

In order to enhance the biological activity of the 1,3,4-oxadiazole derivatives, some examples in the literature combined this unit with another biologically active moiety like an enone unit. Figure 18 shows the 1,3,4-oxadiazole ring in combination with an α,β-unsaturated carbonyl moiety to give antibacterial compounds.[48-49]

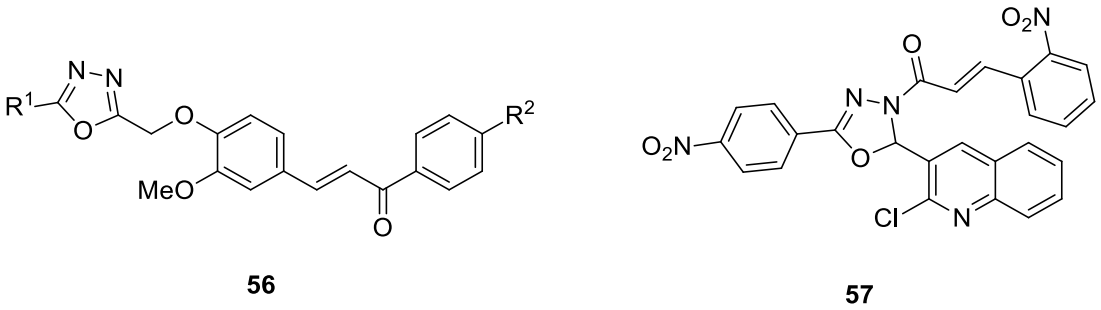


Figure 18: Antibacterial compounds with 1,3,4-oxadiazole unit combined with the enone moiety.

Some substitutions on the 1,3,4-oxadiazole ring like the pyridinyl moiety showed interesting biological activities, such as 2,3-dihydro-1,3,4-oxadiazole **58** is analgesic active agent while compound **59** is an anticancer agent (Figure 19).[50-51] On the other hand, a thiol group in position 2 of the ring demonstrated a wide spectrum of biological activities such as antibacterial activity (e.g. compound **60)**,[52] antidiabetic[53], anti-inflammatory and analgesic activity like compound **61**.[54]

Recently, Zhang *et al*.[55] prepared a new 1,3,4-oxadiazole-2-thiol derivative **62** with pyridinyl moiety in position 5. The compound showed high activity against four

different cancer cell lines HEPG2, MCF7, SW1116 and BGC823 with IC_{50} values 0.76-1.54 µM (Figure 19).

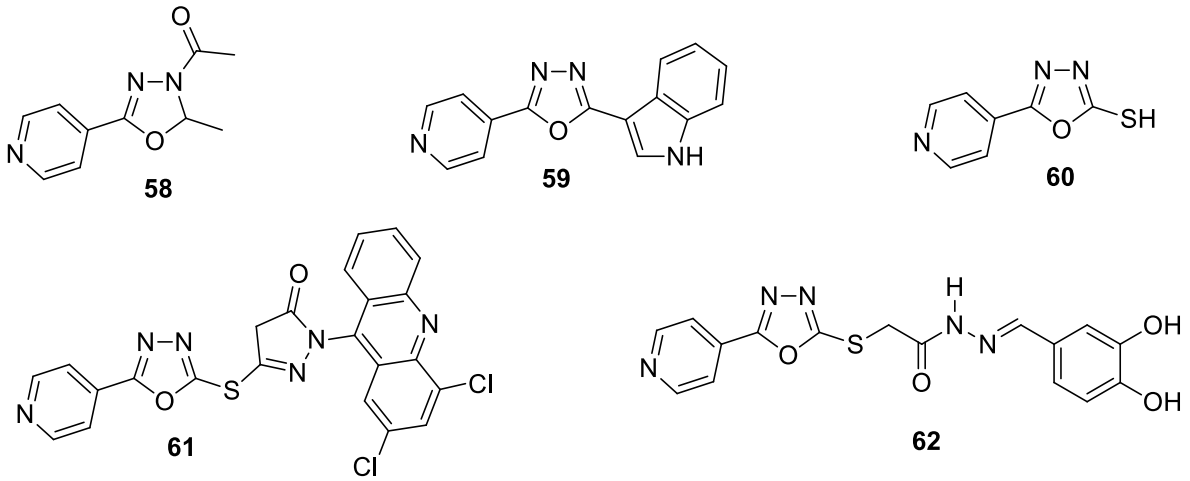


Figure 19: Biologically active 1,3,4-oxadiazole derivatives with a pyridinyl unit and some with a sulfur bridge.

1.5.2. Concepts to synthesize 1,3,4-oxadiazole derivatives

Several synthetic methods have been reported for the preparation of dialkyl and diaryl 1,3,4-oxadiazoles, Figure 20 summarize some of these methods.[47]

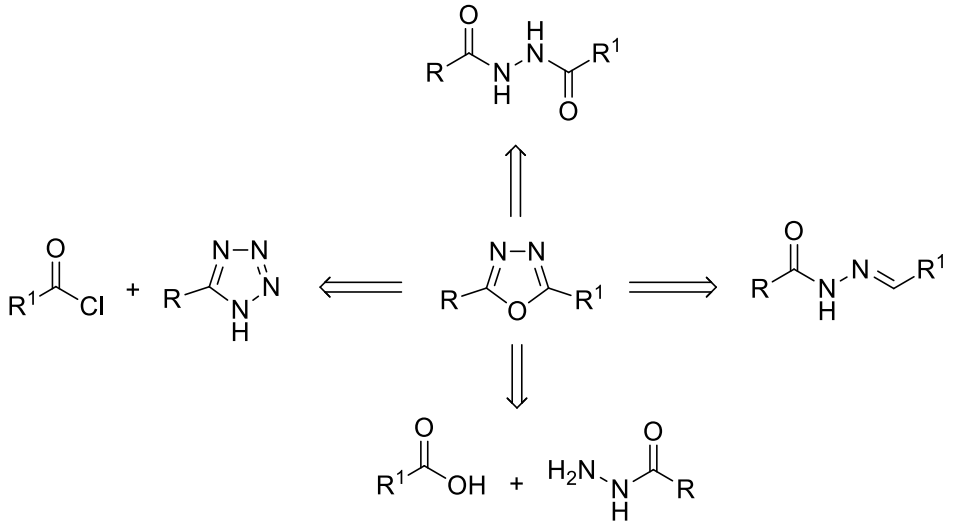
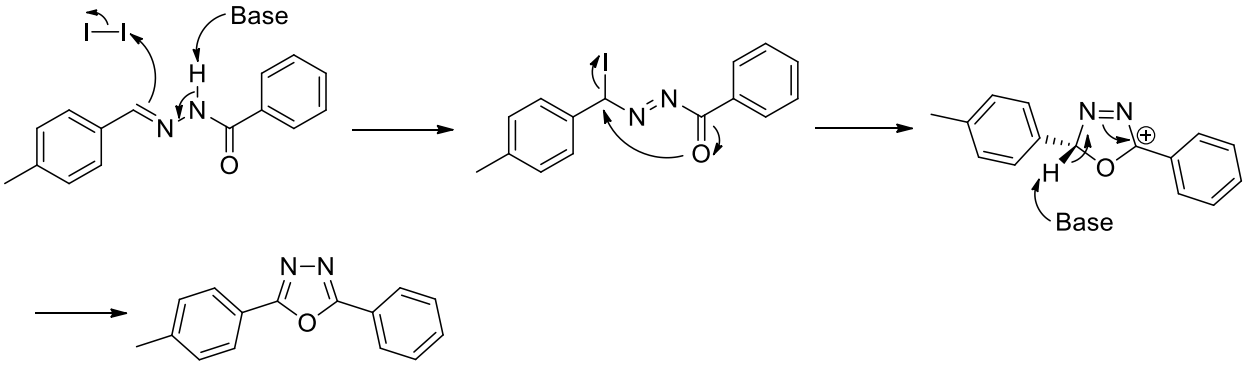


Figure 20: Concepts to synthesize disubstituted 1,3,4-oxadiazole derivatives.[47]

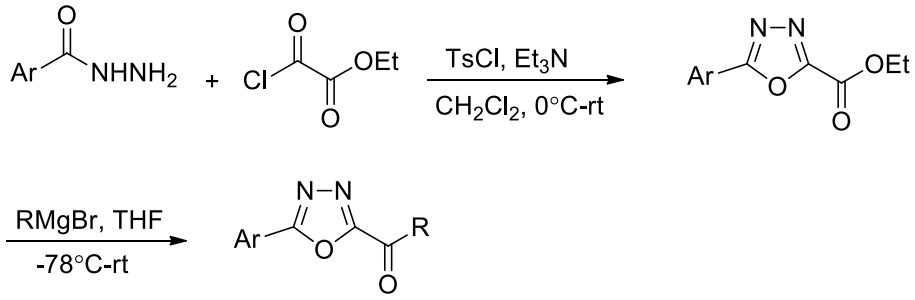
Ya *et al*.[56] developed an oxidation cyclization of hydrazones to 1,3,4-oxadiazoles using stoichiometric amount of iodine in presence of potassium carbonate. The proposed reaction mechanism is shown in Scheme 7.



Scheme 7: Proposed mechanism of 1,3,4-oxadiazole ring formation using iodine.[56]

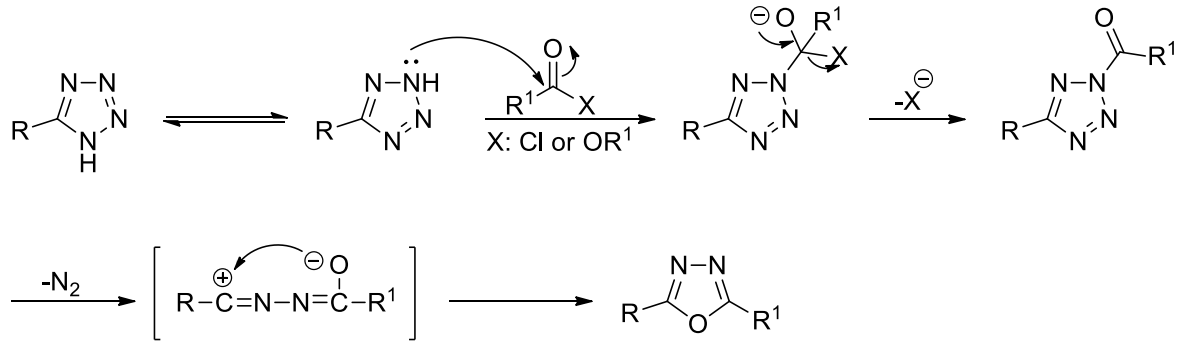
In some cases the cyclization process can be done using an oxidizing agent like CAN,[57] or using a catalyst such as $Cu(OTf)_2$.[58]

1,3,4-oxadiazole derivatives can also be prepared starting from an acid chloride and hydrazide to produce a 2,5-disubstituted-1,3,4-oxadiazole ring in a good yield (Scheme 8).[59] This sequence uses mild conditions and a nontoxic agent like TsCl and Et_3N, CDI, EDC and $SOCl_2$ to form the ring, while other methods use some aggressive conditions such as $POCl_3$ under reflux.[60]



Scheme 8: Synthesis of the 2,5-disubstituted-1,3,4-oxadiazole compounds.

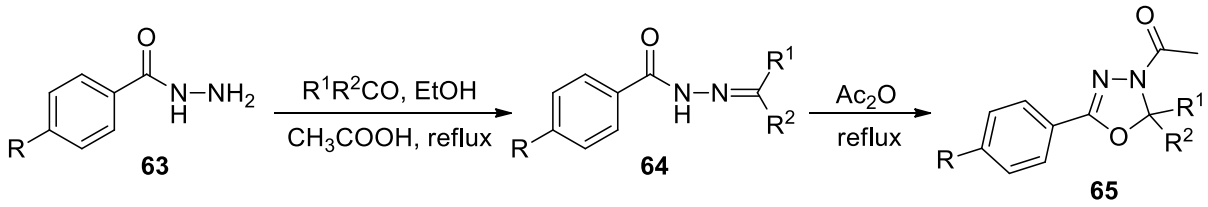
Although the Huisgen reaction (Scheme 9) is less common than the other methods to synthesize 1,3,4-oxadiazole compounds, it is useful in some cases. In this reaction a tetrazole compound is combined with an acid chloride or an acid anhydride.[61] Firstly, the tetrazole attacks the carbonyl in the acid chloride or anhydride to form the amide, the formed tetrazole amide is unstable so, extrusion of N_2 gas occurs to generate 1,5-dipole which is subsequently cyclized to the desired product.[62]



Scheme 9: Mechanism of the Huisgen reaction.[62]

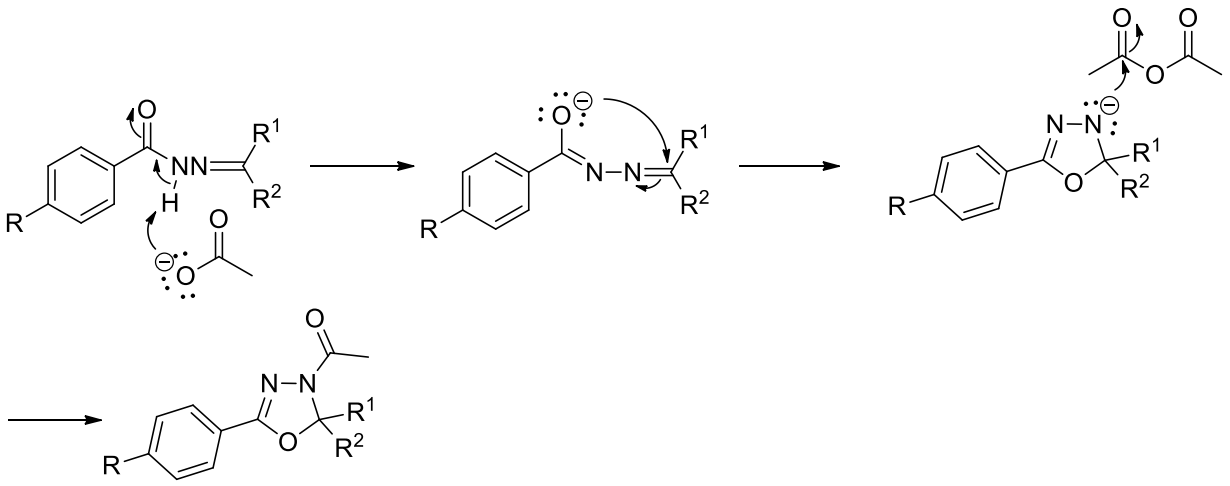
It is possible also to generate 1,3,4-oxadiazole ring from the reaction between carboxylic acids and hydrazide using $POCl_3$. This method is unfavorable due to the aggressive reagent ($POCl_3$) nevertheless, it is used in some cases.[63]

The common method to synthesize 2,3-dihydro-1,3,4-oxadiazole ring is by generating the hydrazide **63** (Scheme 10) followed by Schiff base formation with an aldehyde or a ketone to form the hydrazone **64**. The last step in this method is refluxing the hydrazone in acetic anhydride to form the desired product **65**.[64]



Scheme 10: Synthesis of 2,3-dihydro-1,3,4-oxadiazole derivatives using the Schiff base strategy.[64]

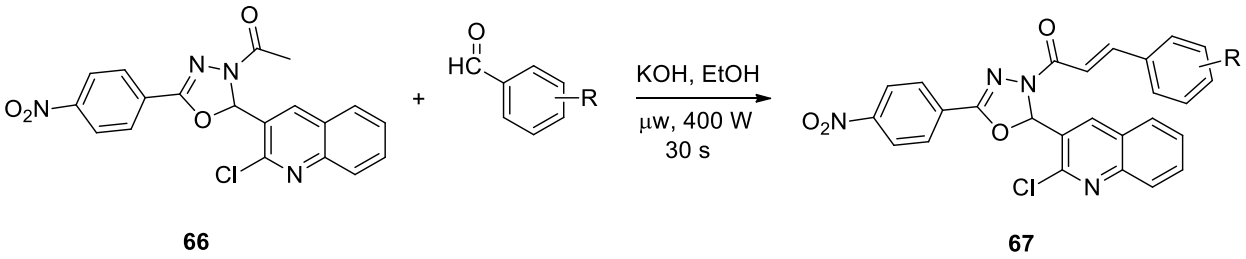
The mechanism of the cyclization (Scheme 11) starts with deportonation of the amide (N-H). The produced oxygen anion attacks the carbon atom of the C=N bond, the results is forming the 2,3-dihydro-1,3,4-oxadiazole with nitrogen anion in position 3. Finally, the anion attacks the acetic anhydride to add the acetyl group to position 3 of the ring and regenerate the acetate again.[49]



Scheme 11: Mechanism of 2,3-dihydro-1,3,4-oxadiazole ring formation.

1.5.3. Synthesis of 2,3-dihydro-1,3,4-oxadiazoles with an additional enone unit

In order to combine two biological active moieties, namely the 1,3,4-oxadiazole ring and an α,β-unsaturated system, Desai and Dodiya[49] prepared 2,3-dihydro-1,3,4-oxadiazole with an acetyl group at position 3, then the Claisen-Schmidt condensation is applied using KOH in ethanol using microwave to insert the enone unit at position 3 of the ring in a 63-78% yield (Scheme 12).[49] Kaur *et al*.[65] used the same method except that they used NaOH in refluxing ethanol.



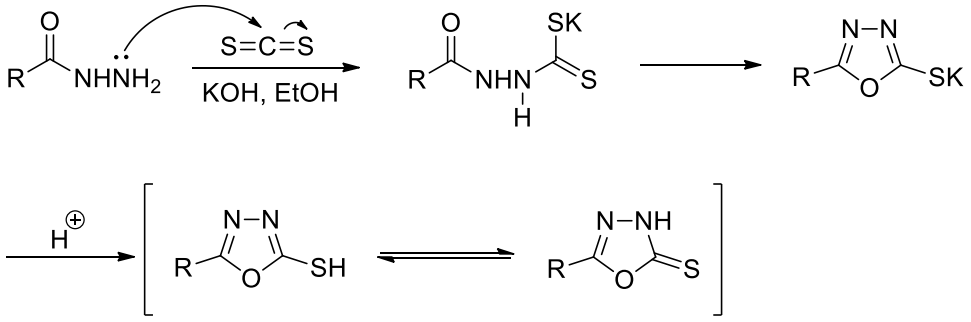
66 **67**

Scheme 12: Synthesis of 1,3,4-oxadiazole ring with an α,β--unsaturated carbonyl system by the Claisen-Schmidt condensation.

1.5.4. Synthesis of 1,3,4-oxadiazole-2-thiol derivatives

The most common method to introduce the thiol group to the oxadiazole ring is the reaction between the hydrazides with carbon disulfide in ethanol and KOH followed by acidification to produce 1,3,4-oxadiazole-2-thiol, which exists as thiol-thione tautomerism. In this mechanism the hydrazone nitrogen atom attacks the carbon in the carbon disulfide in alcoholic KOH to form potassium salt followed by cyclization to

produce 1,3,4-oxadiazole-2-thiopotassium salt which is finally protonated by HCl forming the desired product (Scheme 13).[66]



Scheme 13: Mechanism of 1,3,4-oxadiazole-2-thiol formation.[66]

2. Aim of the work

The present thesis focuses on the synthesis of a library of compounds containing an α,β-unsaturated carbonyl group. First of all, new derivatives from the natural product limnophilaspiroketone are intended to be synthesized including N-Limno-CP, S-Limno-CP and methylene-Limno-CP. Consequently, new method has to apply in order to attempt synthesize the natural product limnophilaspiroketone, since there is no total synthesis available until now for the synthesis of limnophilaspiroketone.

Starting from the biological activity of the natural product zerumbone, which has gained attention recently, a new strategy is applied in order to synthesize zerumbone and some analogues. The method described by Kodama *et al*.[16] to synthesize zerumbone has 13 steps with an overall yield 0.6%. All research on zerumbone still uses the isolated zerumbone from the plant *Zingiber zerumbet* Smith, so a new method is necessary to produce the zerumbone with an efficient synthetic method.

Also, a series of heterocyclic compounds are prepared which combine three important biological moieties including: 1,3,4-oxadiazole ring, α,β-unsaturated carbonyl group and pyridinyl unit. Since all these building blocks have interesting biological activity and no examples in the literature in which all these parts are combined in one skeleton. In this work 20 derivatives are synthesized with different substituents and different positions in order to study the relationship between the substituents and the biological activities of the synthesized compounds. The new derivatives are evaluated as antiproliferative, antibacterial and antifungal agents.

Finally, the compounds are evaluated for their Michael acceptor activity with thiols, and it is attempted to find a relationship between the Michael acceptor activity and the biological properties of the compounds.

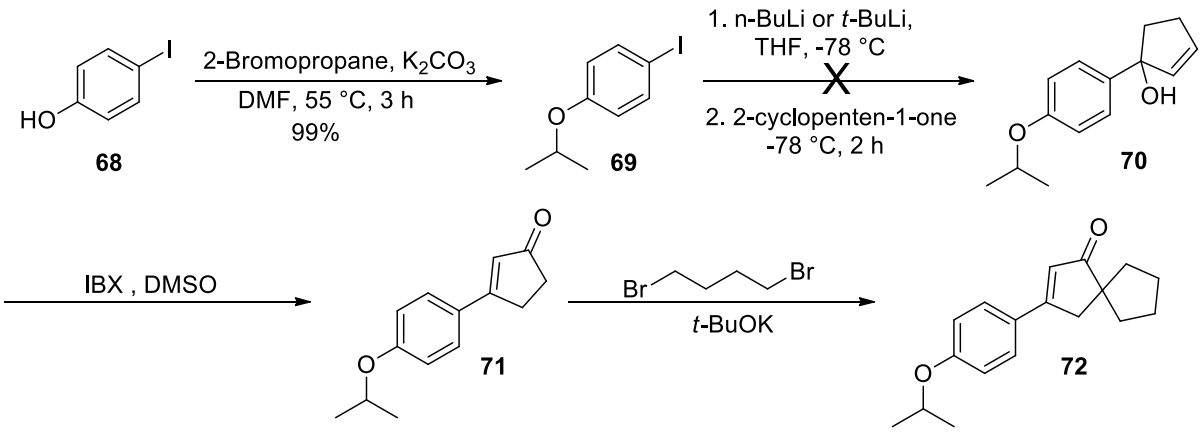
3. Results and discussion

3.1. Methods to synthesize Limno-CPs derivatives

3.1.1. Synthesis of methylene-Limno-CP and α-X-methylene-Limno-CP

The attractive biological activity of the *Limnophila geoffrayi* a plant used in the Traditional Medicine of Thailand, encouraged us to synthesize derivatives of the natural product limnophilaspiroketone. The derivative methylene-Limno-CP synthesized first followed by introducing substituents in the α-position.

The first step in the sequence (Scheme 14) was the protection of the phenolic group of 4-hydroxy iodobenzene (**68**) with isopropyl group to afford **69** in quantitative yield. Then lithium-iodide exchange was applied followed by adding 2-cyclopenten-1-one in order to generate compound **70**. The exchange was failed, and no product was observed with both n-BuLi and *t*-BuLi.

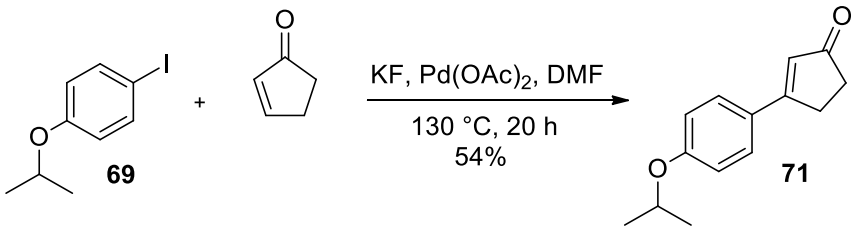


Scheme 14: Attempts to synthesize *i*Pr-methylene-Limno-CP (**72**).

The reason could be related to the strong electron donating isopropoxy group in the para position, which lead to increase the electron density in the C-I bond and decrease the affinity of the exchange.

Since the first pathway failed, a new strategy was applied to synthesize compound **71** directly from compound **69**. This time a Heck reaction (Scheme 15) was used to couple 4-isopropoxyiodobenzene (**69**) with 2-cyclopenten-1-one using palladium acetate as a catalyst and potassium fluoride as base.[17] The reaction took place at 130 °C to afford the aryl cyclopentenone **71** in 54% yield after 20 h. Since the bromo

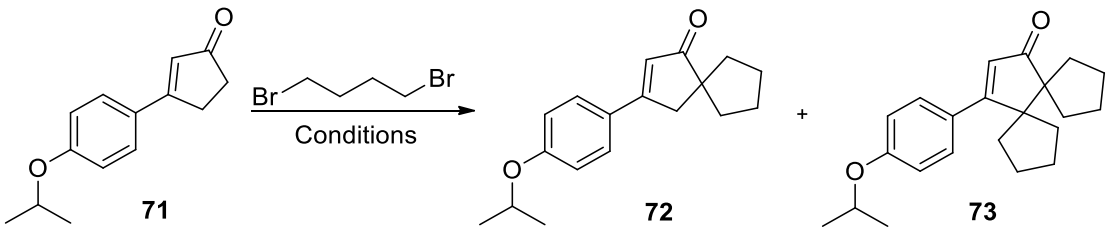
derivative is cheaper than the iodo the same condition was used to prepare compound **71** from the 4-isopropoxybromobenzene but only traces from the desired product was produced with very weak conversion. This happens because the cleavage of the C-I bond is easier than the C-Br in the oxidative addition step during the Heck reaction (Scheme 15).



Scheme 15: Synthesis 3-(4-isopropoxyphenyl)cyclopent-2-enone (**71**) by the Heck reaction.

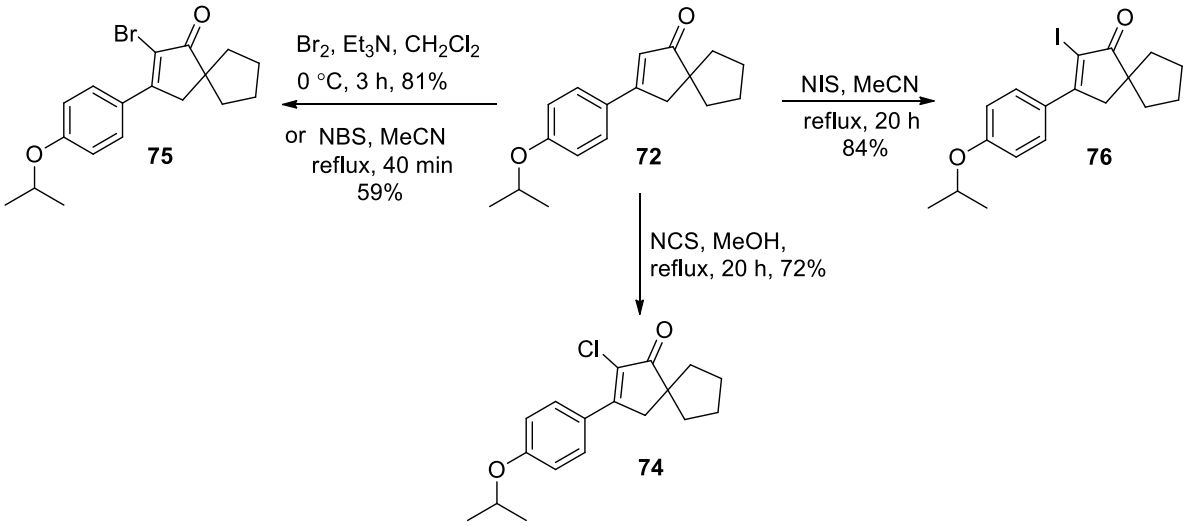
With compound **71** in hand, the next step was forming a spirosystem with cyclopentenone ring to produce *i*Pr-methylene-Limno-CP (**72**). Table 2 shows the conditions under which the desired product was prepared. Generally, the reactions gave multiple products since the S_N2 reaction for 1,4-dibromobutane is competing with an elimination reaction to form alkenes mixture. Also the allylic position on the cyclopentene-1-one is able to undergo a substitution reaction and 5% of the dispirosystem **73** was isolated. The best result was acquired by refluxing in benzene with *t*-BuOK[67] giving the compound **72** in 37% yield.

Table 2: Synthesis of α-Limno-CPs



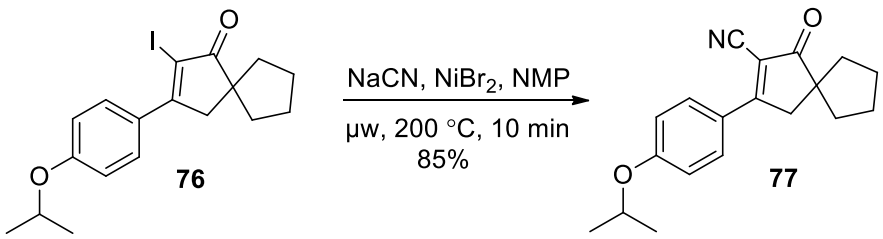
Entry	Conditions	Yield (%) of **72**	Yield (%) of **73**
1	n-BuLi, diisopropylamine, THF, -78 °C, 2 h	Complex mixture	-
2	$NaNH_2$, Et_2O, reflux, 24 h	Traces	-
3	*t*-BuOK, benzene, r.t., 3 h	22	-
4	*t*-BuOK, benzene, reflux, 3 h	37	5

By preparing compound **72** we were able to introduce substituents in the α-position. Firstly, α-bromo-methylene-Limno-CP **75** was produced using Br_2 followed by eliminating HBr with Et_3N in 81% yield after 3 h (Scheme 16). The second way to synthesize the α-bromo-methylene-Limno-CP was using NBS in MeCN at 0 °C for 40 min which gave the product **75** in 88% yield. α-Chloro-methylene Limno-CP **74** and α-iodo-methylene-Limno-CP **76** were synthesized using NCS and NIS, respectively, by refluxing for 20 h to afford 72% and 84% yield, respectively.



Scheme 16: Synthesis of α-X-methylene-Limno-CPs (X: Cl, Br and I).

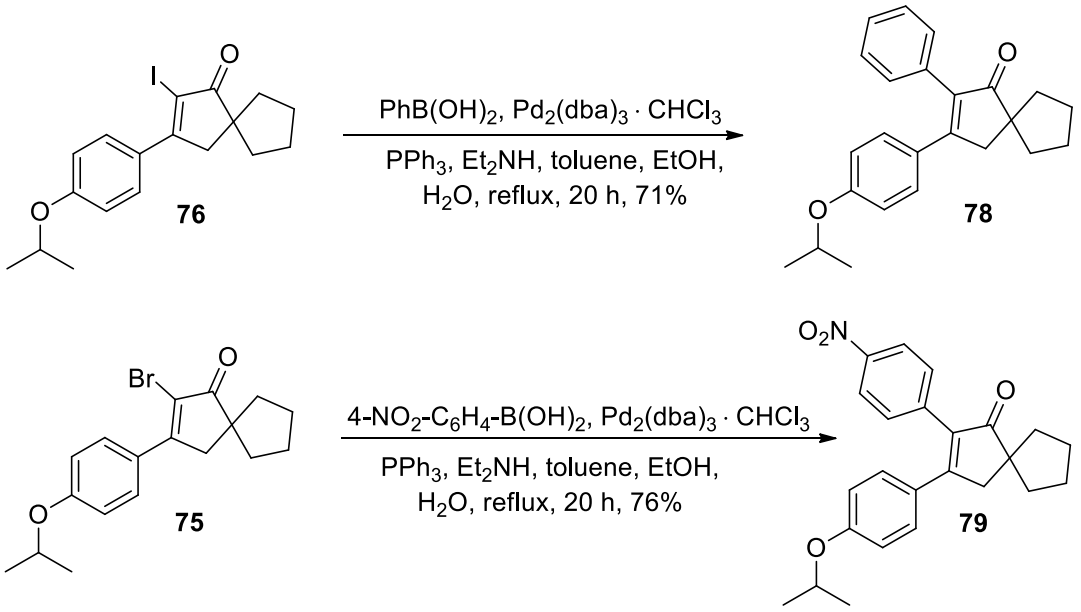
The α-CN-methylene-Limno-CP **77** (Scheme 17) was obtained from the iodo derivative **76** using microwave irradiation, NaCN as a nitrile source and $NiBr_2$ as a catalyst at 200 °C. The yield was 85% after just 10 min using high boiling point solvent *N*-methyl-2-pyrrolidone (NMP).[32]



Scheme 17: Synthesis of α-CN-methylene Limno-CP **77**.

The α-I and α-Br-methylene-Limno-CP were the key intermediates in the synthesis of other derivatives.

Suzuki coupling[32] (Scheme 18) was applied to produce α-aryl-methylene-Limno-CP. Herein, α-C_6H_5-methylene-Limno-CP **78** was prepared from α-I-methylene-Limno-CP **76** with 71% yield after 20 h and α-4-NO_2-C_6H_4-methylene-Limno-CP **79** was prepared from the α-Br-methylene-Limno-CP **75** with 76% yield after 20 h

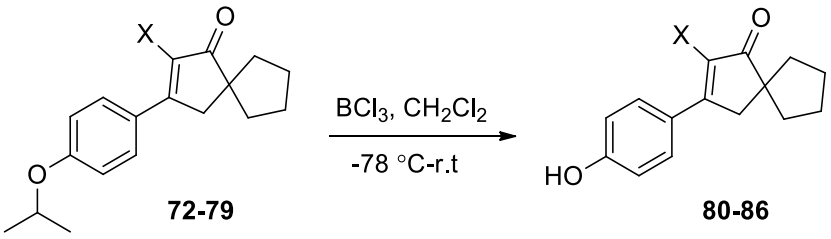


Scheme 18: Synthesis of α-aryl-methylene-Limno-CPs by the Suzuki coupling.

Trials to form α-F-methyleneLimno-CP failed despite the fact that different conditions such as selectfluor/MeCN,[68] NFSI/THF[32] and Bu_4NBr/KF/DMSO[69] were screened and no conversion of the starting material was observed.

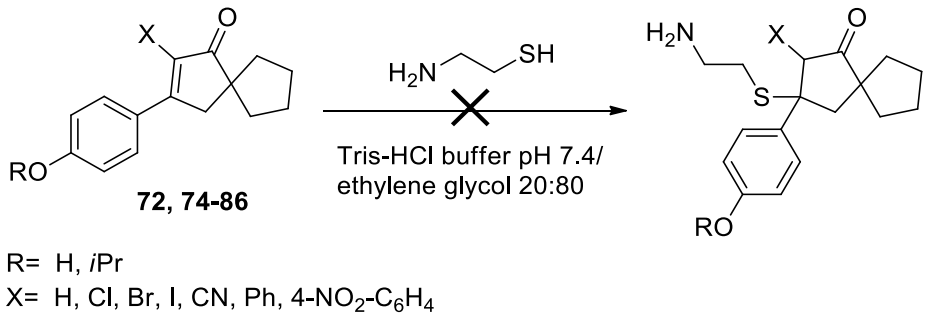
To generate the free hydroxyl group in the methylene-Limno-CPs six equivalents BCl_3 were used at -78 °C to obtain a complete deprotection with excellent yields. In general, all reactions gave excellent yields (91-96%) (Table 3), as well as showing fast reactions in most cases except **72** and **79** which needed 16 h for a complete conversion.

Table 3: Deprotection of methylene-Limno-CP derivatives.



Starting material	X	Time (h)	Product	Yield (%)
72	H	16	**80**	93
74	Cl	1	**81**	90
75	Br	1	**82**	91
76	I	1	**83**	96
77	CN	3	**84**	96
78	Ph	1	**85**	95
79	*p*-NO_2-C_6H_4	16	**86**	91

With all compounds in hand, we tried to compare the Michael acceptor activities of the synthesized derivatives, therefore a 96-well plate based thiol assay was used to determine the second order-rate constants this work was done by my colleague Hermina Petkes in Amslinger group. The reactions (Scheme 19) were carried out in a buffer containing 100 mM TRIS-HCl at pH 7.4, 2 mM EDTA–ethylene glycol 20:80 under pseudo-first-order conditions at a concentration of 40 µM for methylene-Limno-CPs and 500 fold of cysteamine as a thio Michael donor. None of the synthesized compounds was active towards a Micheal addition of cysteamine. This proved, that these molecules have an electron rich α,β-unsaturated carbonyl system and also steric hindrance with two substitutes in the β-position which lead to poor Michael acceptors.



Scheme 19: Reaction of methylene-Limno-CPs as thiol-Michael addition with cysteamine.

3.1.2. Attempts for the synthesis of N-Limno-CP and S-Limno-CP

The next derivatives related to the natural product limnophilaspiroketone are S-Limno-CP (**87**) and N-Limno-CP (**88**) where the oxygen atom in the furan ring in the Limno-CP (**31**) is replaced by sulfur and nitrogen, respectively (Figure 21).

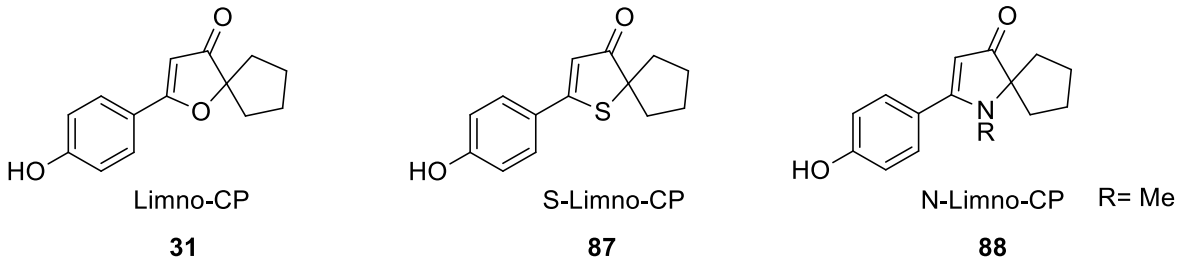
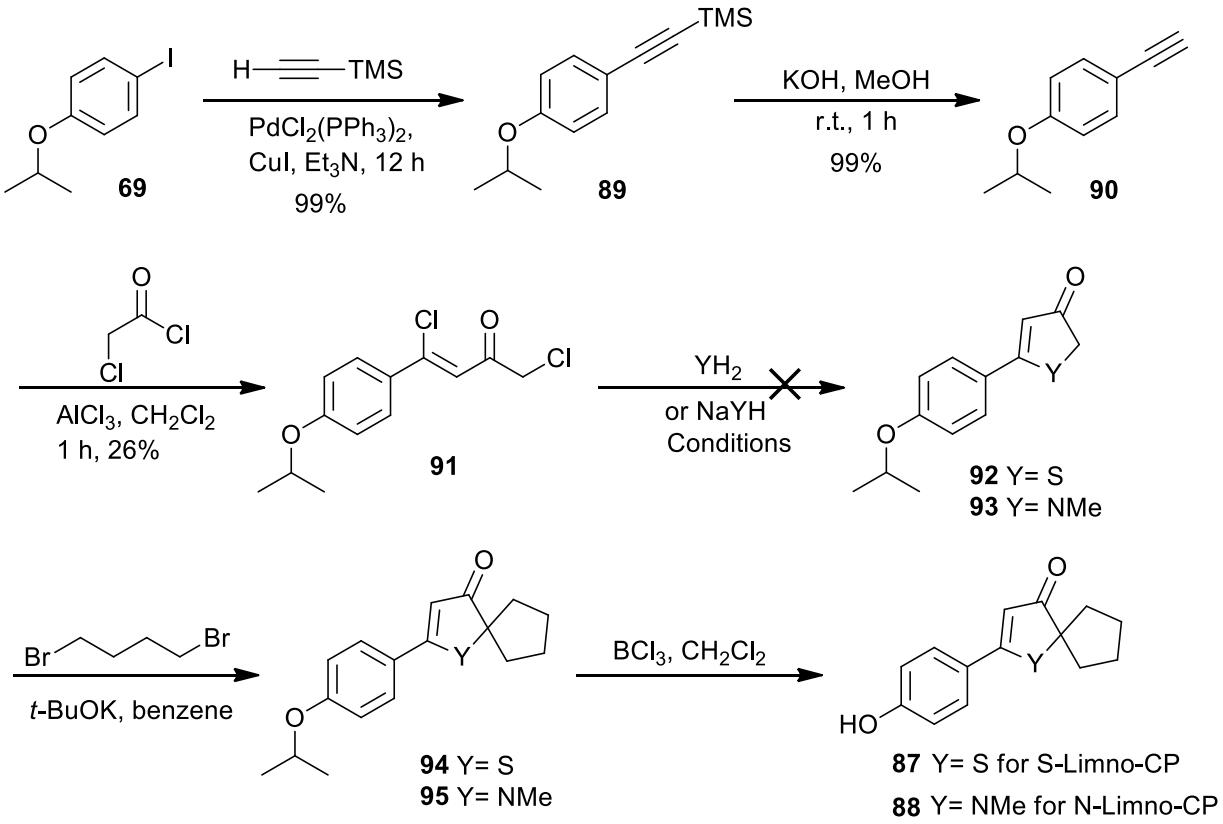


Figure 21: Structures of Limno-CP analogues.

To prepare S-Limno-CP (**87**) and N-Limno-CP (**88**), intermediate **91** (Scheme 20) was needed in which the ring closing could take place by NaSH for the S-Limno-CP and RNH_2 for the N-Limno-CP. The desired intermediate **91** can be synthesized starting from *p*-isopropoxyiodobenzene (**69**) using a Sonogashira coupling with ethynyltrimethylsilane and a palladium catalyst. The reaction worked smoothly to give compound **89** with quantitative yield after 12 h. The next step was the removal of the TMS group in order to generate the terminal alkyne. In this case a solution of KOH/MeOH at room temperature was enough to produce compound **90** in quantitative yield after just 1 h. Then the desired intermediate **91** could be prepared by the Friedel-Crafts acylation of terminal alkyne **90** with chloroacetyl chloride using $AlCl_3$.[70-71] The reaction took place at -78 °C to room temperature for around 1 h affording 26% yield.

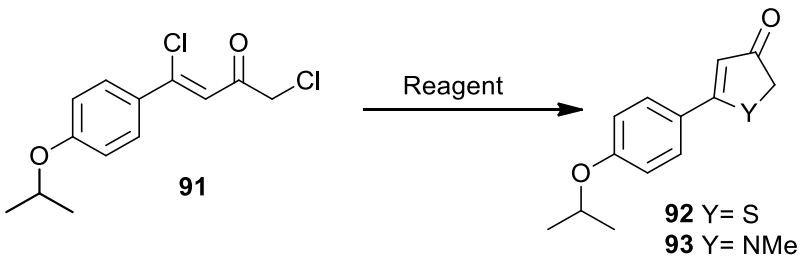
The intermediate **91** was ready then to generate the five membered heterocyclic rings, using NaSH for *i*Pr-S-Limno-CP (**94**) and primary amines for *i*Pr-N-Limno-CP (**95**)



Scheme 20: Attempts to synthesize S-Limno-CP (**87**) and N-Limno-CP (**88**).

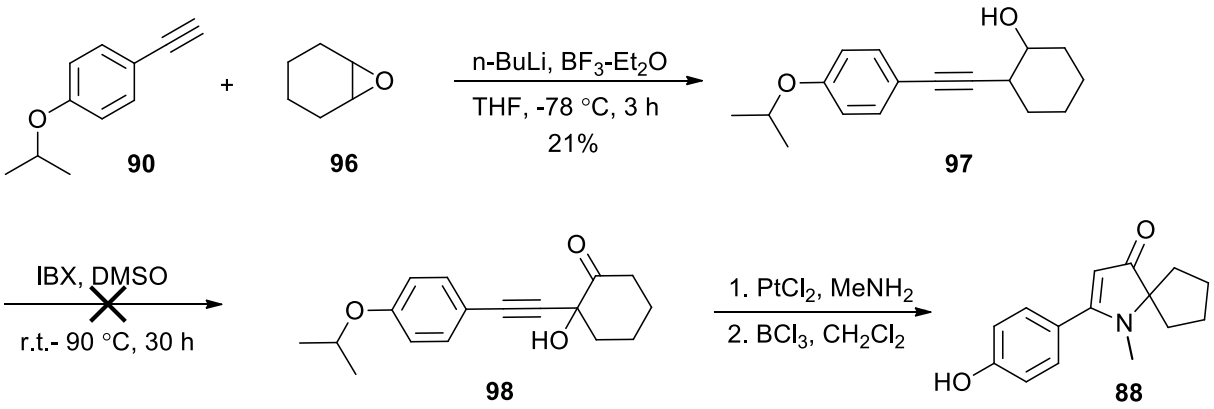
To synthesize compound **92**, NaSH·x H_2O was used. Despite of screening different solvents and solvent free conditions (Table 4, Entry 1, 2, 3 and 4), complex mixtures were always observed even with cooling to -78 °C. The reaction was very fast and the starting material was completely converted within 10 min and complex mixture was opserved in the TLC. The same was observed when methyl amine (Table 4, Entry 5) was used in order to synthesize compound **93**. This suggests that intermediate **91** is not stable under the screened conditions to produce the desired derivatives S-Limno-CP (**87**) and N-Limno-CP (**88**), which mean no further possibilities for this pathway.

Table 4: Approaches to synthesize compounds **92** and **93**.



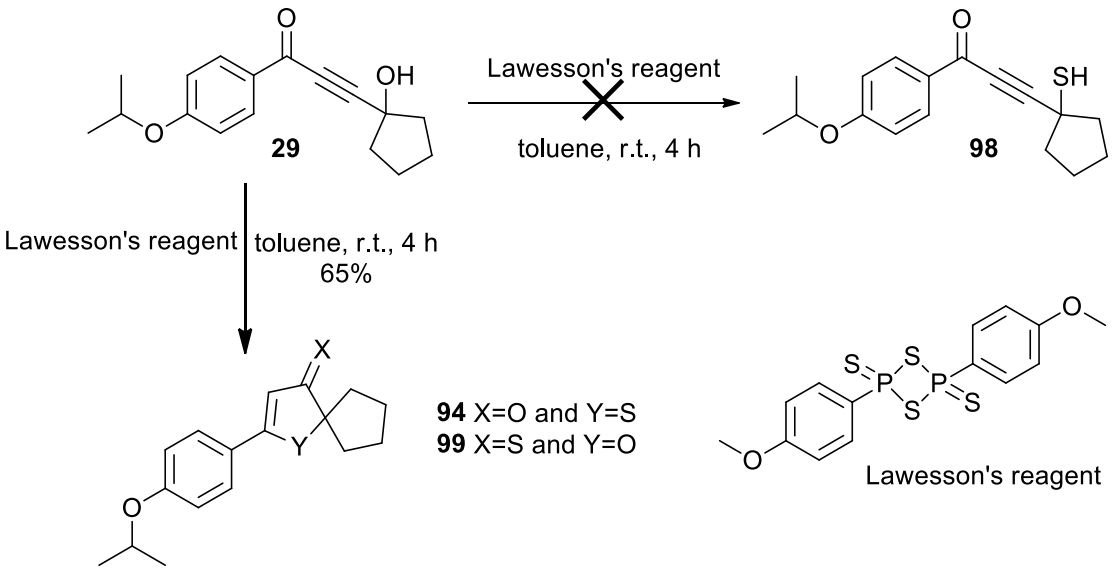
Entry	Y	Reagent	Solvent	T (°C)	t (min)	Result
1	S	NaHS·x H_2O	acetone	r.t.	10	decomposition
2	S	NaHS·x H_2O	CH_2Cl_2	-10	10	decomposition
3	S	NaHS·x H_2O	CH_2Cl_2	-78	10	decomposition
4	S	NaHS·x H_2O	-	-10	10	decomposition
5	MeN	$MeNH_2$, THF	THF	-10	30	decomposition

An alternative method to synthesize N-Limno-CP utilizes the reaction of terminal alkyne **90** with 1,2-epoxycyclohexane (**96**) to form the secondary alcohol **97** (Scheme 21). The reaction took place with boron trifluoride etherate[72] at -78 °C for 3 h to give only 21% yield of the desired product **97** and multiple by products. The next step was an oxidation of the alcohol **97** using IBX in DMSO[73] in order to synthesize compound **98**, no reaction at all was observed even at 90 °C.



Scheme 21: Attempts to synthesize N-Limno-CP (**88**).

Referring to the cyclization step in the mechanism of Limno-CP synthesis, the OH group in compound **29** (Scheme 22) could be replaced with a thiol group to form compound **98**, then by the same cyclization process as in the Limno-CP could be applied. For the thiol generation, the Lawesson's reagents was used under the same conditions in Nishio, T procedure[74] Surprisingly, new compound was observed in 66% yield after 4 h at room temperature. The mass spectrum showed mass peak at 288.12, while the ^{1}H-NMR and ^{13}C-NMR show a cyclic product. In this case there are two possible isomers: the first possible skeleton is *i*Pr-S-Limno-CP (**94**) while the second possible isomer is the *i*Pr-thiocarbonyl-Limno-CP (**99**).



Scheme 22: Treatment of compound **29** with the Lawesson's reagent.

In order to distinguish between the two isomers and determine the correct skeleton, two experiments were done. The first experiment was measuring the IR, in this case it was significant that the band at around 1675 cm^{-1} which corresponds to the conjugated α,β-unsaturated carbonyl group was missing (Figure 22), while a strong bands can be observed at 1117 cm^{-1} related to the thiocarbonyl group.

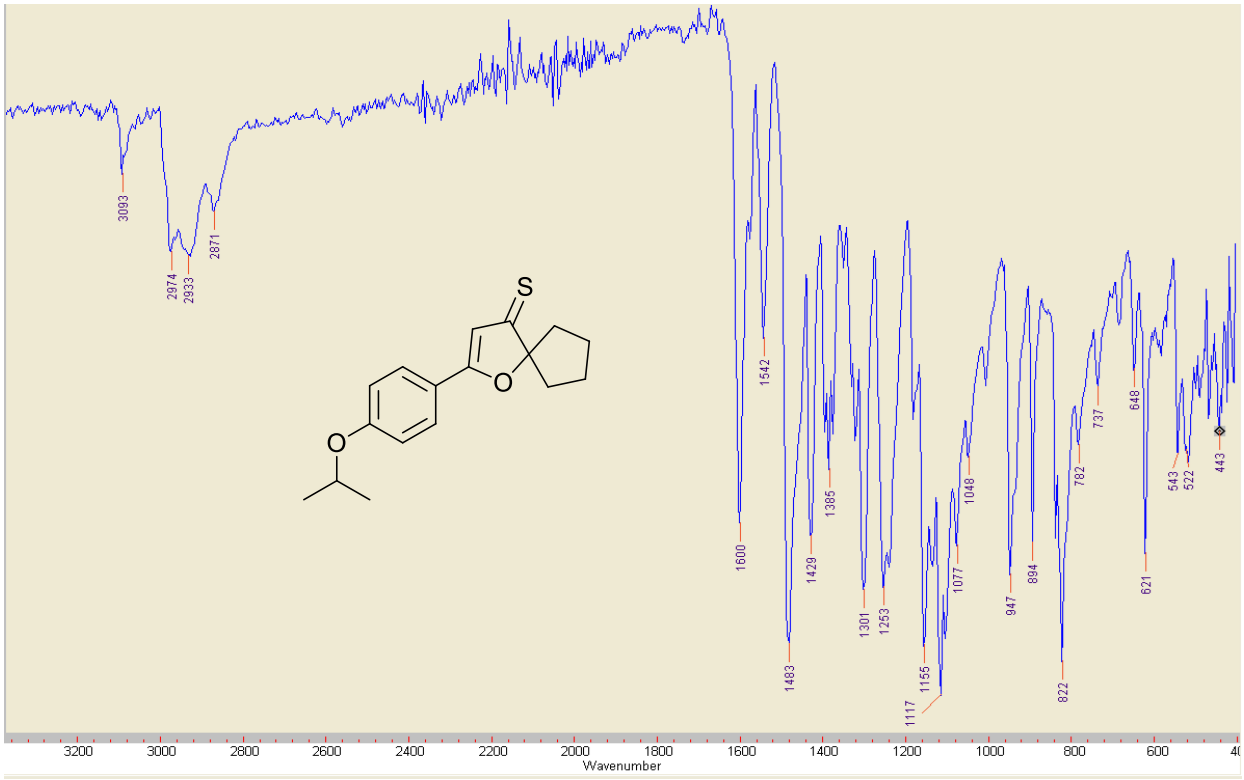


Figure 22: IR spectrum showing thiocarbonyl-Limno-CP (**99**).

The band at 1117 cm^{-1} matchs the range of conjugated thiocarbonyl group (Figure 23),[75-76] therefore, the IR data most likely shows the thiocarbonyl compound **99**.

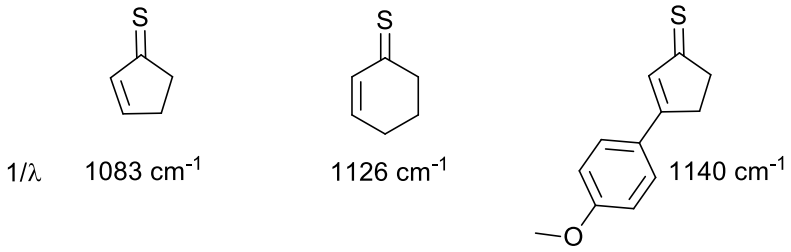


Figure 23: Wave numbers 1/λ (cm^{-1}) for conjugated thiocarbonyl group (C=S).[75-76]

The second experiment aimed at proving the product was a treatment of the *i*Pr-Limno-CP (**30**) with the Lawesson's reagent in order to convert the carbonyl group to the thiocarbonyl group by refluxing in toluene for 48 h. This produced the thiocarbonyl-Limno-CP (**99**) in 45% yield. At this point by comparing the ^{1}H-NMR spectrum of the product with the ^{1}H-NMR spectrum of the unknown compound (Figure 24), it was clear that the two spectra are identical which proves that the unknown product is thiocarbonyl-Limno-CP (**99**).

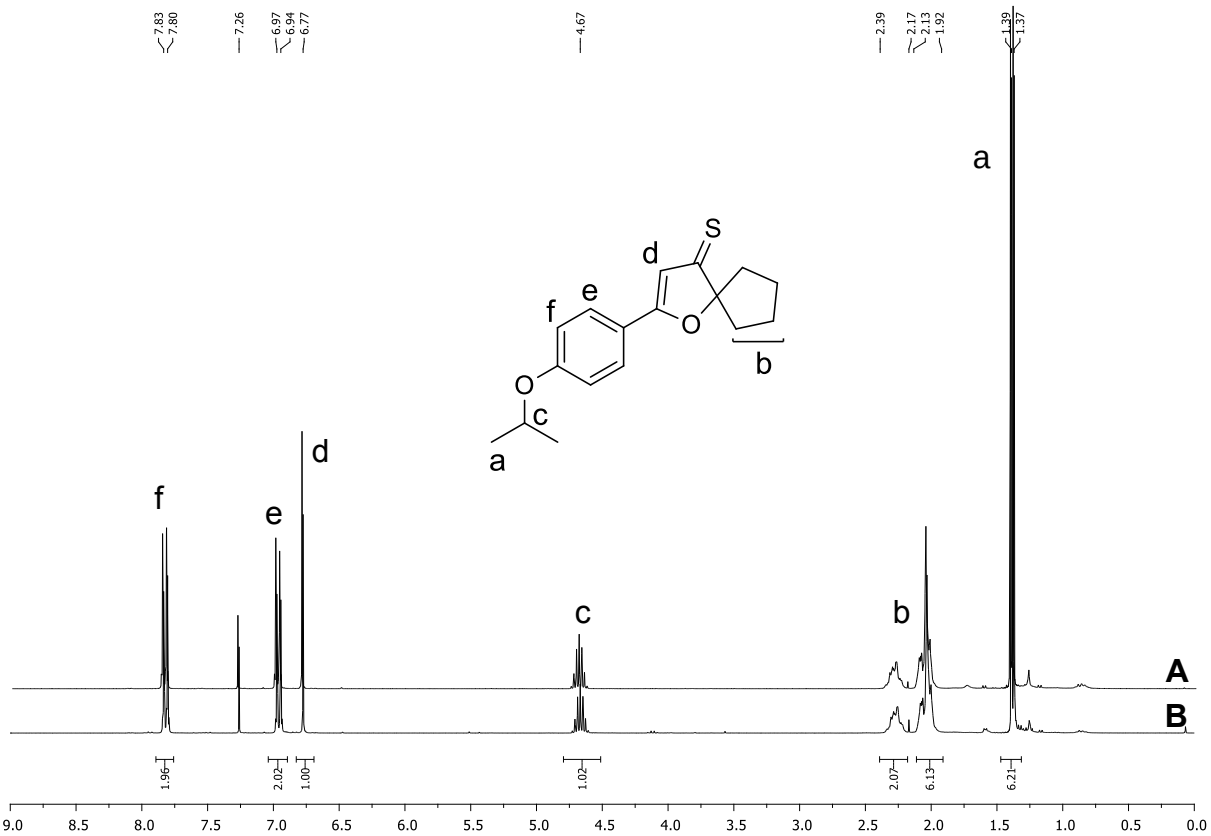
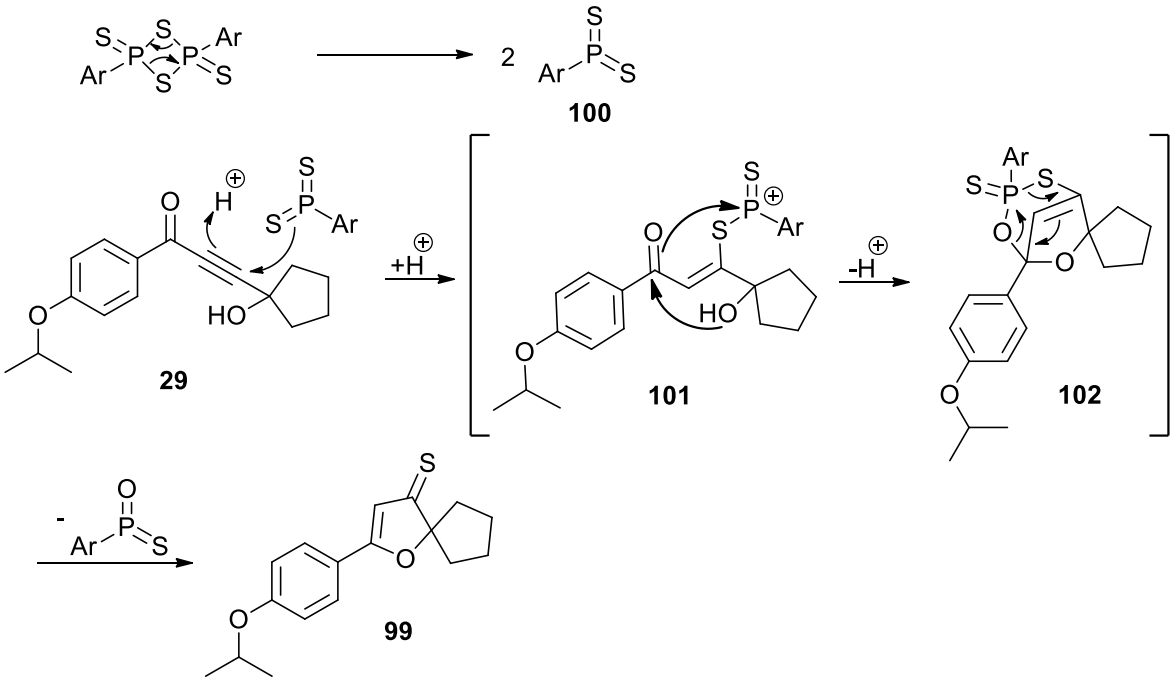


Figure 24: ^{1}H-NMR spectrum showing thiocarbonyl-Limno-CP (**99**), **A**: 1H NMR for **99** from the starting material **29**, **B**: 1H NMR for **99** from the starting material **30**.

The proposed mechanism for formation of thiocarbonyl compound **99**, start with decomposition of Lawesson's reagent to dithiophosphine ylide **100** (Scheme 23): The sulfur atom in the ylide attacks the β-position of the unsaturated carbonyl moiety while the α-position is protonated to form compound **101**. The hydroxyl group in **101** attacks the carbonyl group and simultaneously the oxygen atom in the carbonyl group attacks the phosphorus cation in the dithiophosphine ylide to form six membered ring fused with furan ring **102**. Finally, six membered ring in compound **102** opens to form the thiocarbonyl compound **99**. The driving force is forming the P=O bond in the by-product.



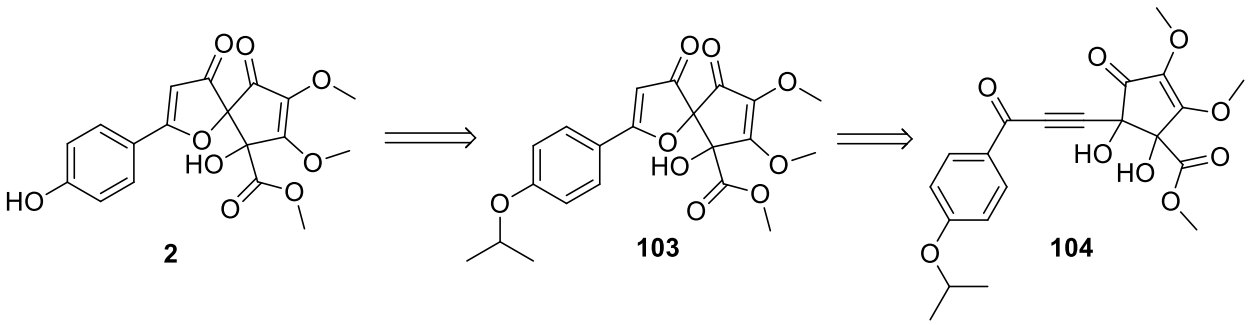
Scheme 23: Proposed mechanism to synthesize compound **99**.

3.2. Limnophilaspiroketone

The natural product limnophilaspiroketone (**2**) was isolated as a racemic mixture, with interesting skeleton combining two α,β-unsaturated systems with a phenolic moiety and also with a spirosystem. These specifications are encouraging to try synthesize such a compound.

3.2.1 Retrosynthesis of limnophilaspiroketone

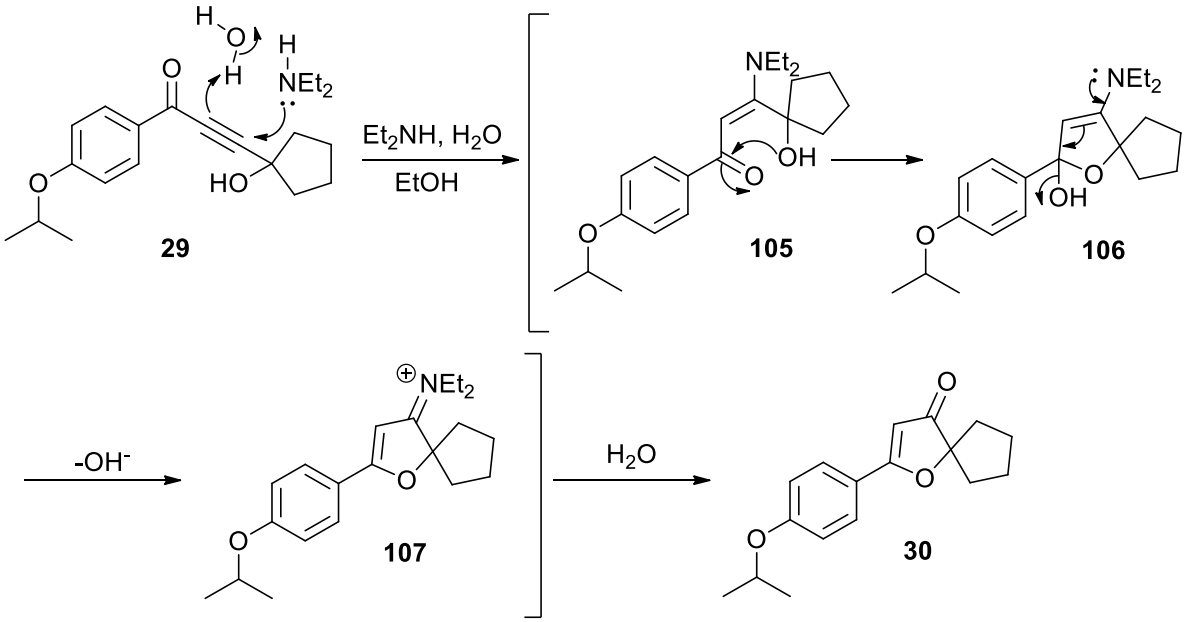
To synthesize limnphilaspiroketone (**2**), firstly the 3(2*H*)-furanone ring could be formed by the method used by Sabine Amslinger and Simon Lindner on the synthesis of Limno-CP from the corresponding alcohol (Scheme 24).[32]



Scheme 24: Retrosynthesis leading to cyclization of compound **104** to form limnphilaspiroketone (**2**).

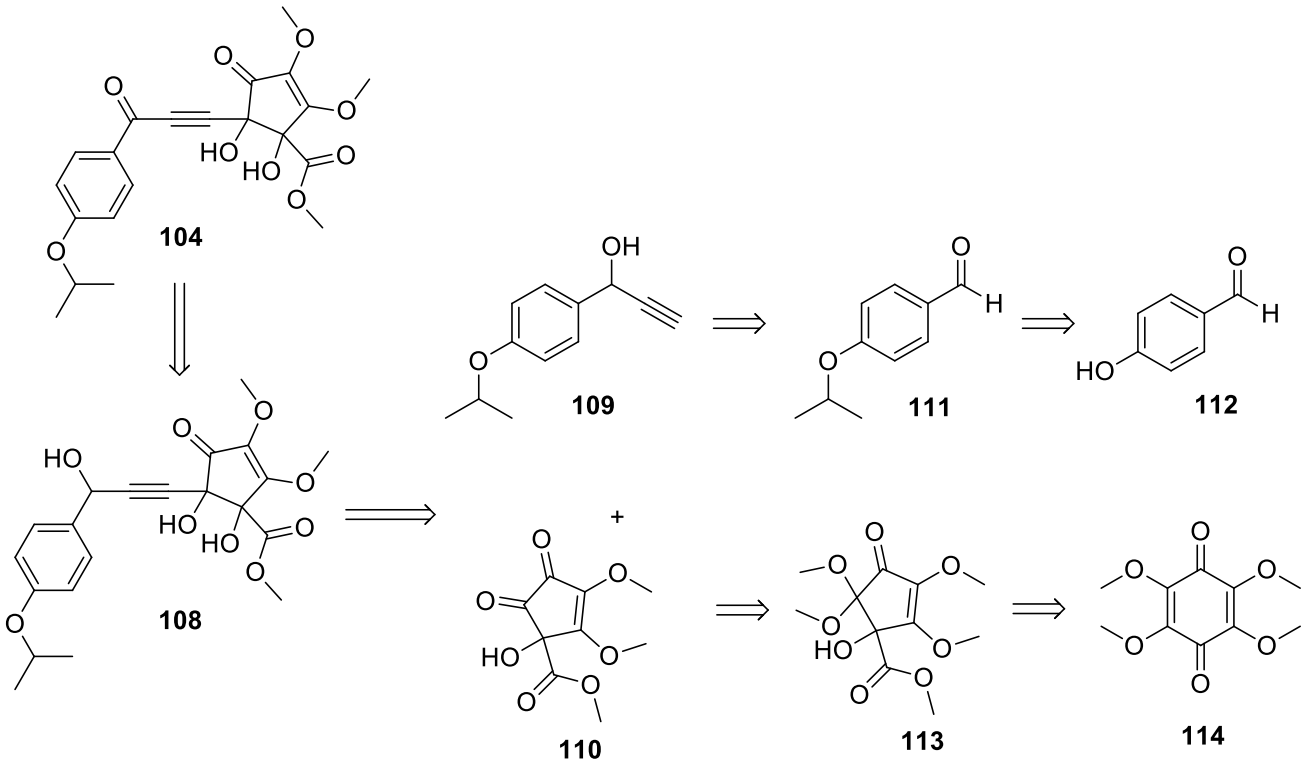
The proposed mechanism (Scheme 25) shows that diethylamine attacks the β-position of the α,β-unsaturated carbonyl system in compound **29**, while the α-position

is protonated by water to form **105**. Subsequently, the *tert*-alcohol attacks the carbonyl group to form the furan ring in **106**, after that the nitrogen atom forms iminium cation **107** under elimination of hydroxide ion. Finally, the iminium cation is converted to carbonyl group in the presence of water to give the desired product **30**.[32]



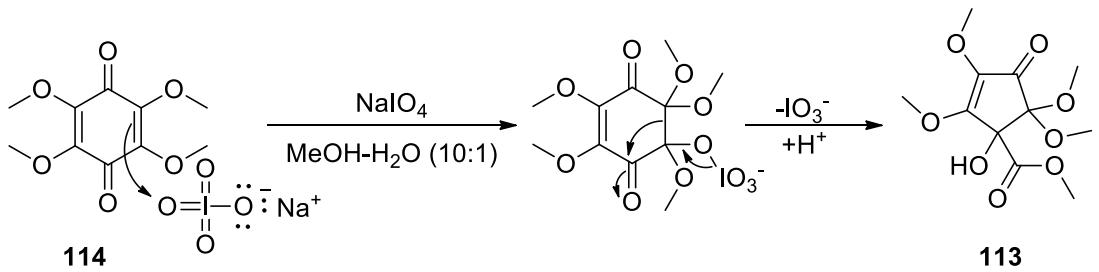
Scheme 25: Proposed mechanism of the cyclization reaction to form 3(2*H*)-furanone ring (**30**).[32]

Compound **104** (Scheme 26) can be derived from the corresponding benzyl alcohol **108** by oxidation reaction using PCC or MnO_2 in CH_2Cl_2. The other two *tert*-alcohols will not be oxidized. The alkyne **108** can be generated from two moieties: the terminal alkyne **109** and the cyclopentenone compound **110**. This reaction could be done by treating the alkyne with n-BuLi followed by nucleophilic addition selectively to the non-conjugated carbonyl group in compound **110**. Since compound **110** has three carbonyl groups where one is an ester, which is the poorest electrophile comparing to the other carbonyl groups. The second carbonyl group forms an enone system which causes decrease in the electrophilicity. In this case the priority is to the third non-conjugated carbonyl group which can react with the terminal alkyne **109**.



Scheme 26: Retrosynthesis of compound **104**.

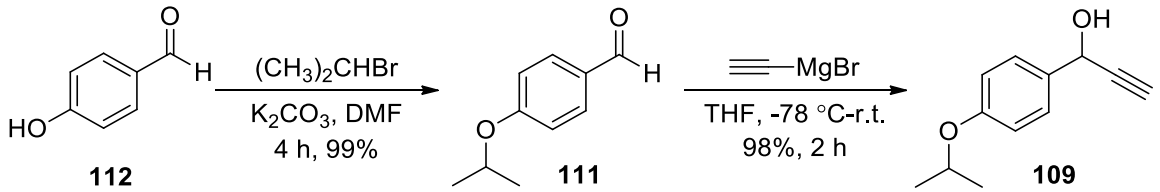
The analogue **109** (Scheme 26) could be synthesized by the Grignard reaction between the protected benzaldehyde **111** and ethynylmagnesium bromide. Compound **110** could be generated from the deprotection of the carbonyl group in the known compound **113**. Gottfried *et al*.[77] synthesized in 1985 compound **113** (Scheme 27) from tetramethoxy-1,4-benzoquinone (**114**) with sodium metaperiodate in methanol. The mechanism of the reaction starts by attacking the oxygen atom in the sodium metaperiodate by the double bond from the tetramethoxy-1,4-benzoquinone, followed by ester rearrangement to constrict the ring from a six to a five membered ring.



Scheme 27: Gottfried *et al*.[77] mechanism to form cyclopentenone **113** from tetramethoxy-1,4-benzoquinone.

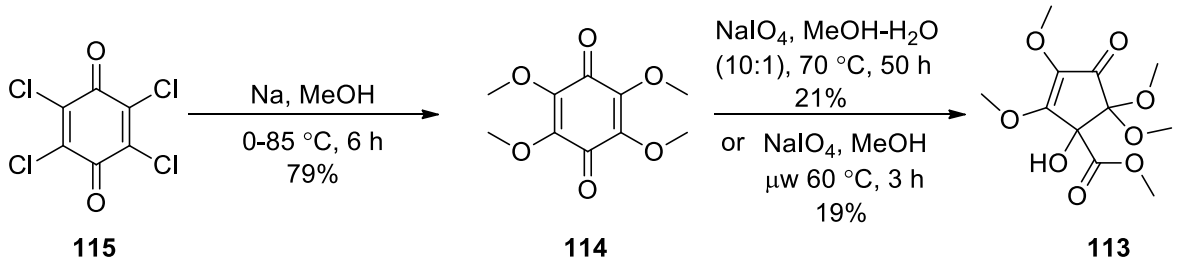
3.2.2. Approaches to synthesize limnophilaspiroketone

Firstly, 4-hydroxybenzaldehyde (**112**) must be protected and since limnophilaspiroketone (**2**) has two methoxy groups, the phenolic OH could be deprotected selectively at the end. Hence, the isopropyl group was used since it can be selectively removed by BCl_3. The reaction gave quantitative yield after 4 h with isopropyl bromide (Scheme 28). The 4-isopropoxybenzaldehyde (**111**) was treated with ethynylmagnesium bromide solution at -78 °C in THF affording the terminal alkyne **95** after 2 h in 98% yield.



Scheme 28: Grignard reaction to form the benzylic alcohol **109** with a terminal alkyne moiety.

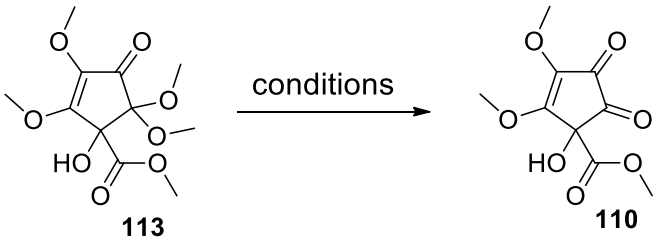
The second step was the preparation of the cyclopentenone skeleton **110**. Using the commercially available tetrachloro-1,4-benzoquinone (**115**) with four equivalents sodium methoxide (Scheme 29), orange crystals of tetramethoxy-1,4-benzoquinone (**114**) were obtained in 79% yield after 6 h. Then the six membered ring was contract to a five membered ring by ester rearrangement. Here, tetramethoxy-1,4-benzoquinone (**114**) was treated with sodium metaperiodate in a solvent mixture (MeOH-H_2O, 10:1) at 70 °C for 50 h. The reaction gave 21% yield, while doing the same reaction in the microwave at 60 °C in MeOH gave 19% yield after 3 h. It is worth noting that the second method is just useful on small scale, since larger scale (> 1 g) could not performed with the setting in use.



Scheme 29: Synthesis of compound **113** by ester rearrangement.

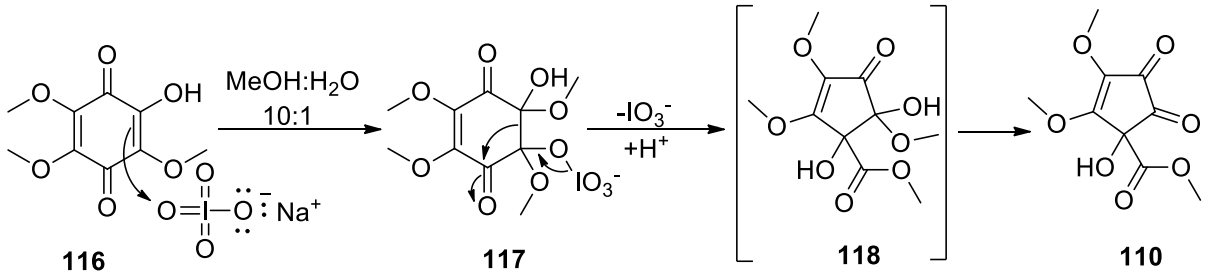
By having compound **113** in hand, the next step was the deprotection of the dimethylacetal in order to generate the carbonyl group which is needed in the next step. To deprotect the dimethylacetal different conditions were screened (Table 5). Herein, weak acids did not show any reaction even under reflux. Moreover, even when a stronger acid like TFA at 50 °C was used no reaction took place. The reaction with sulfuric acid at room temperature showed no conversion while heating to 40 °C caused degradation of the material without any product as seen in the mass spectrum. Similar results were obtained with BCl_3, I_2,[78] $Ce(OTF)_3$,[79] PCC,[80] $CuSO_4$,[81] and CAN[82] which are known as a dimethylacetal deprotection reagents.

Table 5: Attempted reactions to generate the carbonyl group in **113**.



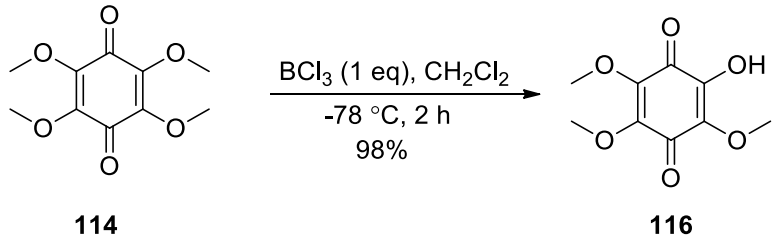
Reagent	Solvent	T (°C)	t (h)	Result
PTSA	acetone	reflux	36	no reaction
PTSA	H_2O	reflux	24	no reaction
AcOH	H_2O	r.t.	48	no reaction
AcOH	H_2O	reflux	24	no reaction
TFA	-	r.t.	24	no reaction
TFA	-	50	48	no reaction
1 M HCl	H_2O	r.t.	24	no reaction
H_2SO_4	CH_2Cl_2	r.t.	24	complex mixture
H_2SO_4	-	r.t.	24	no reaction
H_2SO_4	-	40	0.25	complex mixture
CAN	MeCN/H_2O (1:1)	70	24	no reaction
$Ce(OTf)_3$	CH_3NO_2	reflux	24	no reaction
$CuSO_4$/NaI	acetone	reflux	48	no reaction
I_2	acetone	reflux	48	no reaction
BCl_3	CH_2Cl_2	-78	5	complex mixture
PCC	-	60	24	no reaction

Since all the trials to deprotect the dimethylacetal failed, a new strategy was used in order to synthesize compound **110**. If one treats trimethoxy-1,4-benzoquinone **116** with sodium metaperiodate (Scheme 30), then the ester reareangment could take place to afford **118**. Finally, the hemiacetal in compound **118** will convert immediately to carbonyl group and afford **110**.



Scheme 30: Proposed mechanism to form cyclopentenone **110** from the 1,4-benzoquinone **116**.

Compound **116** was prepared by treating compound **114** with one equivalent BCl_3 (Scheme 31), which led to selective deprotection of one methoxy group in quantitative yield after 2 h at -78 °C.



Scheme 31: Selective deprotection of one methoxy group with BCl_3.

Some literature assumed that sodium metaperiodate forms an epoxide with a double bond followed by epoxide ring opening in the presence of water or alcohol. This behavior encouraging to screen different epoxide agents with compound **116** to produce the desired analogue **110**. Starting with $NaIO_4$ in MeOH no reaction was observed (Table 6, Entry 1) while a solvent mixture (MeOH-H_2O, 10:1) with $NaIO_4$ gave the undesired product **119** in 23% yield. ^{1}H-NMR (Figure 25) shows only 3 (OCH_3) groups and one CH, while mass spectrometry showed peak at 202.16 which belongs to the skeleton **119**.

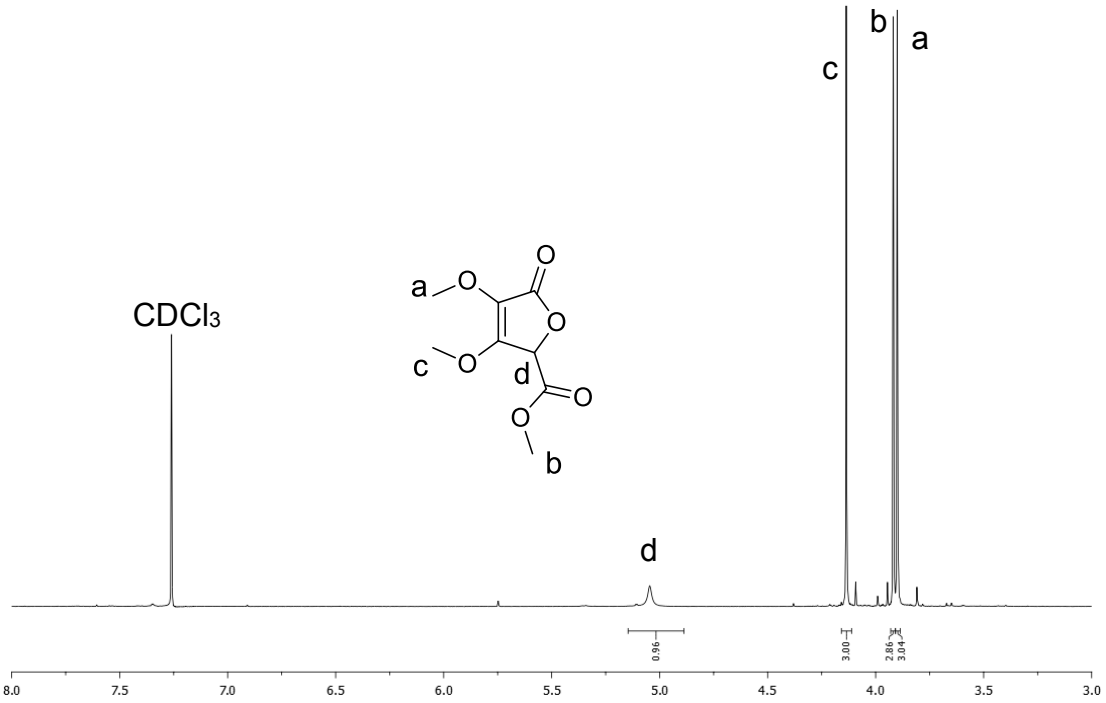
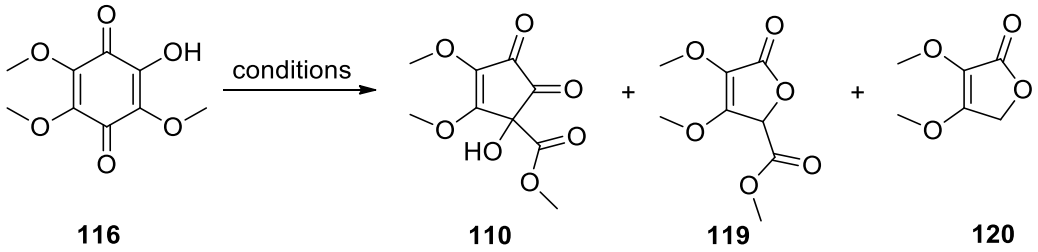


Figure 25: ^{1}H-NMR spectrum of compound **119**.

The same observation was obtained using oxone in dioxane-H_2O (3:1) which afforded **119** in 53% yield (Table 6, Entry 3), while using H_2O_2 in dioxane-H_2O (3:1) showed a new undesired side product **120** with 51% yield. In two other experiments *t*-BuOOH and DBU gave a mixture within just 10 min. While no reaction was observed by *m*CPBA after 20 h (Table 6, Entry 6).

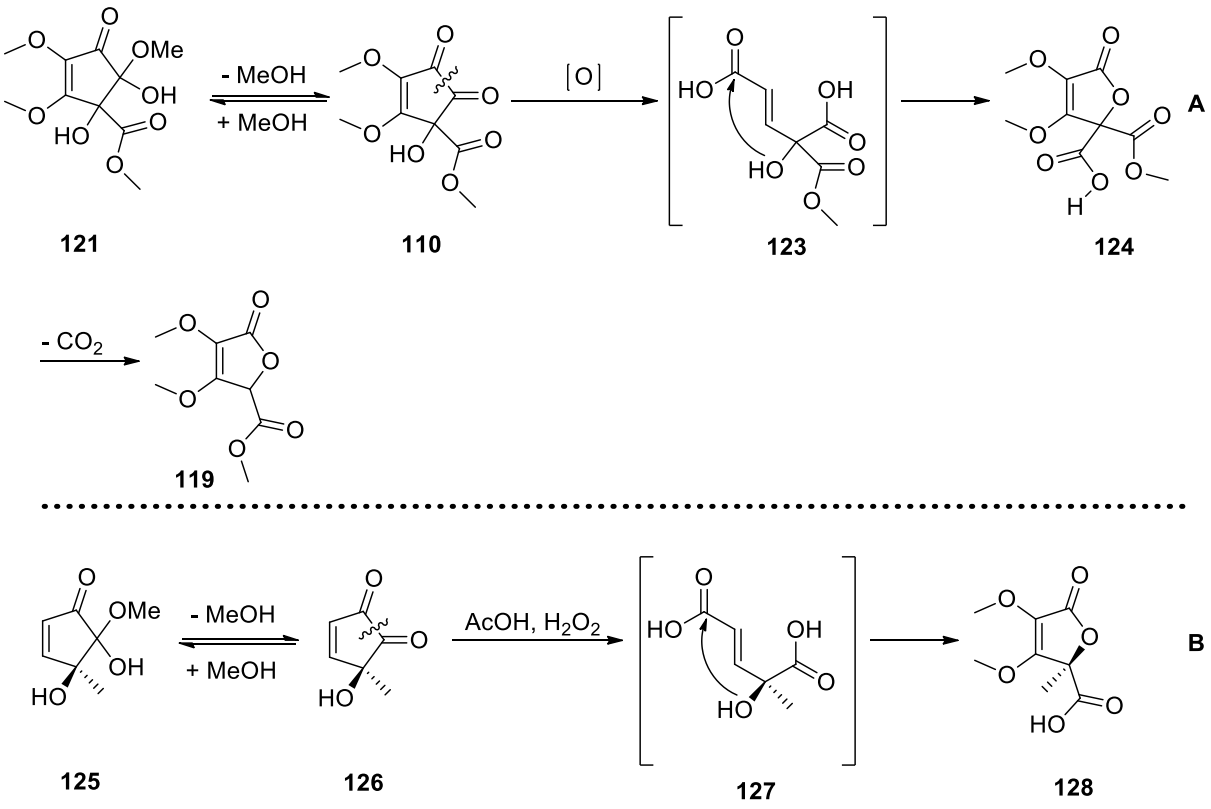
Table 6: Epoxidation reactions of *p*-quinone **116**.



116 **110** **119** **120**

Entry	Reagent	Solvent	T (°C)	t (h)	Product (Yield %)
1	$NaIO_4$	MeOH	70	50	no reaction
2	$NaIO_4$	MeOH:H_2O (10:1)	70	50	**119** (23)
3	Oxone	dioxane:H_2O (3:1)	60	48	**119** (53)
4	H_2O_2, K_2CO_3	dioxane:H_2O (3:1)	reflux	2	**120** (51)
5	*t*-BuOOH, DBU	CH_2Cl_2	r.t.	10 min	mixture
6	*m*CPBA	CH_2Cl_2	0-r.t.	20	no reaction

Scheme 32 (**A**) shows how compound **119** was formed, in this mechanism the 1,2-dicarbonyl cyclopentenone derivative **110** was produced from the hemiacetal **121**, and then the oxidizing agent cleaved the bond between the two carbonyl groups to afford dicarboxylic acid intermediate **123**. Intramolecular esterification took place to give γ-lactone **124**, finally, decarboxylation of compound **124** produced the desired product **119**. Paju *et al*.[83] described similar pathway (Scheme 32, **B**) in which 1,2-dicarbonyl cyclopentenone derivative **126** was treated with H_2O_2 in acetic acid. This causes ring cleavage and formation of dicarboxylic acid analogues **127**, followed by intramolecular cyclization to produce the γ-lactone **128**. The mechanism of the reaction clearly shows that the desired product **110** is formed during the reaction but it is not stable under the conditions with the screened oxidizing agents.



Scheme 32: **A**: Proposed mechanism to form the γ-lactone **119** by oxidation of 1,2-dicarbonyl compound **110**. **B**: Paju *et al*.[83] proposed mechanism to form the γ-lactone **128.**

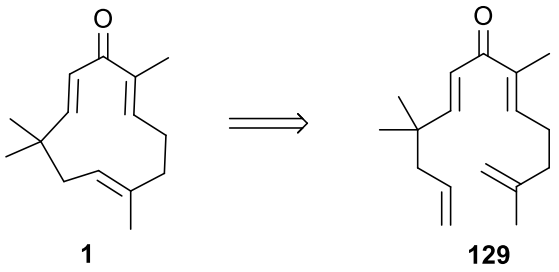
Different methods were failed to deprotect the carbonyl group in compound **113**, but there are still other methods available which could be tried in the future to prepare compound **110**.

3.3. Zerumbone

3.3.1. Retrosynthetic approach towards the total synthesis of zerumbone

Despite the fact that the natural product zerumbone (**1**) is known since 1960, there is still no efficient method to synthesize it on a large scale and the commercially available zerumbone (**1**) is still isolated from plant. Many factors make the synthesis of zerumbone (**1**) a big challenge such as low functionality, highly strained 11-membered ring, rigid part with divinyl carbonyl group and the stereochemistry with three *E*-configured double bonds.

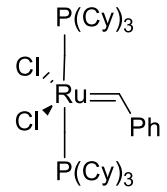
The retrosynthesis of zerumbone (**1**) (Scheme 33) started with breake the the non-conjugated double bond. Ring closing metathesis could be used to convert the precursor **129** to zerumbone (**1**) using ruthenium catalysts.



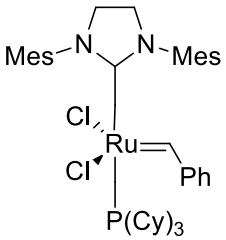
1 **129**

Scheme 33: Retrosynthesis of the last step in the zerumbone synthesis.

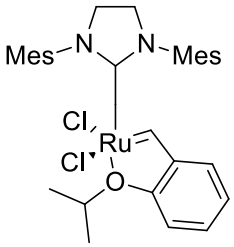
Examples from the literature show different types of catalysts which can be used depending on the ring size, functional groups in the skeleton, stereochemistry and the flexibility of the compound. Figure 26 shows the most common catalysts used in alkene ring closing metathesis. Ruthenium catalysts, including 1st (**130**) and 2nd (**131**) generation Grubbs catalysts have become mainstay of the modern ring closing metathesis. The percentage of the catalyst in these types of reactions is mostly 1-20%, with some exceptions (i.e. > 20%).[84] The Hovedya-Grubbs catalyst (HGC) (**132**) was used in some applications where the Grubbs catalysts were inefficient. Less common catalysts like those having Mo (Schrock's catalyst)[84] (**133**) and aluminum tris(2,6-diphenylphenoxide) ATPH (Yamamoto catalyst)[85] (**134**) are some times useful in special cases. Generally, in ring closing reactions, the concentration of the precursor plays an important role since high concentrations lead to dimer or polymer formation, while very low concentrations cause decrease in the reaction rate. Wide range of reaction conditions were used such as solvents (benzene, toluene and CH_2Cl_2) and temperature (room temperature to reflux in toluene).



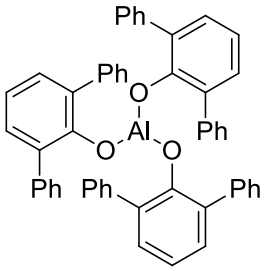
Grubbs catalyst
1st generation (**130**)



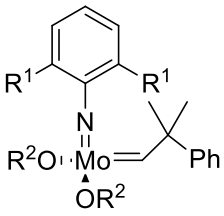
Grubbs catalyst
2nd generation (**131**)



Hoveyda-Grubbs
catalyst (**132**)

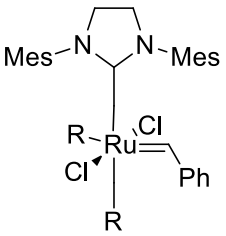


Yamamoto catalyst
(ATPH) (**133**)



R^1: isopropyl
R^2: $CCH_3(CF_3)_2$

Schrock's catalyst (**134**)

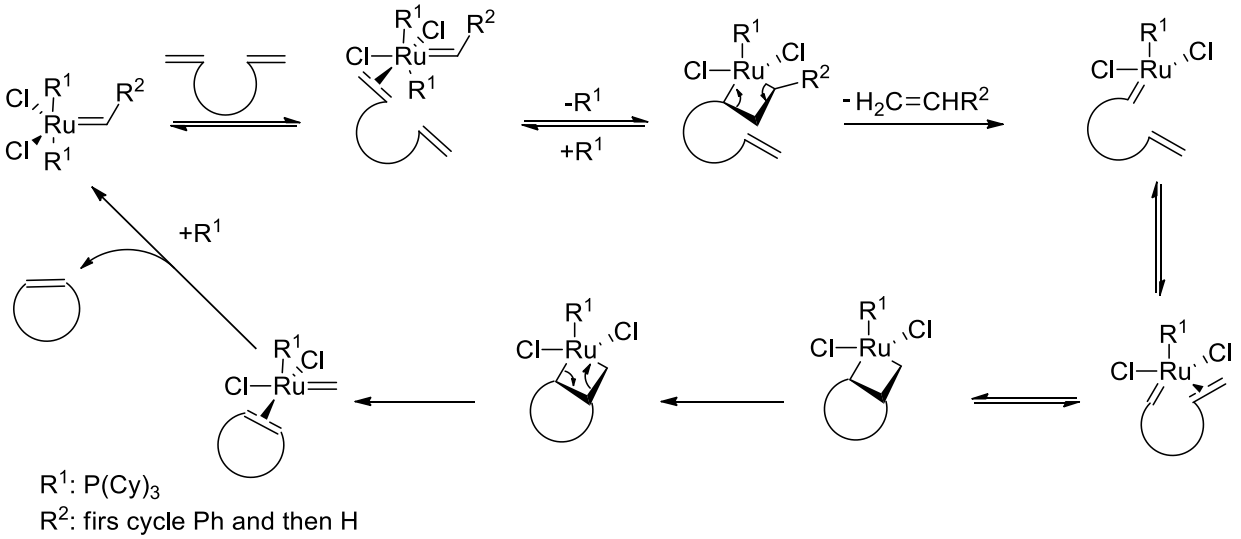


R: 3-bromopyridine

135

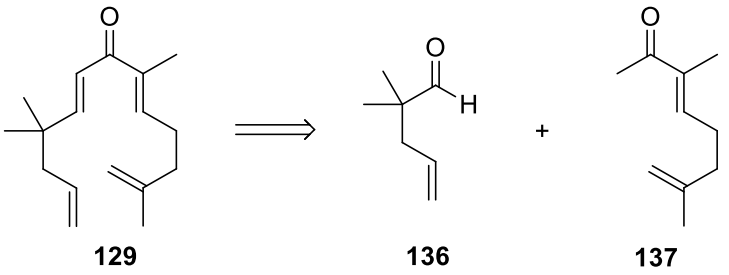
Figure 26: The most common catalysts used in the alkene ring closing metathesis.

The mechanism of the RCM (Scheme 34) using Grubbs I (**108**) starts with coordination between the Ru and the double bond followed by the elimination of $P(Cy)_3$ to form a four membered ring intermediate, then ring opening step occurs to produce the Ru=C bond and ethylene gas. The same sequence takes place with the second double bond. Finally, the ruthenium complex regenerates again to give the desired cyclic compound.



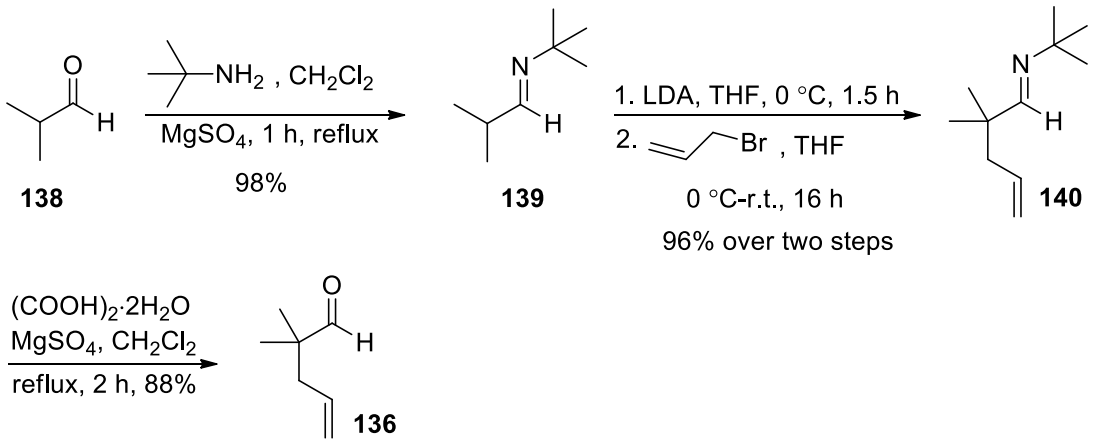
Scheme 34: Mechanism of alkene ring closing metathesis using Grubbs I catalyst.[86]

The precursor **129** (Scheme 35) can be synthesized using the Claisen-Schmidt condensation between the aldehyde **136** and the α,β-unsaturated ketone **137**.



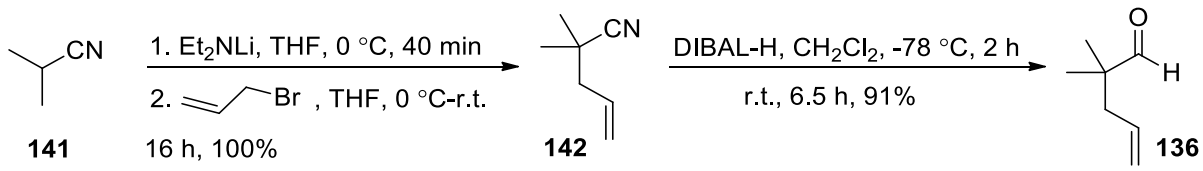
Scheme 35: Synthesis of precursor **129** by the Claisen-Schmidt condensation.

Aldehyde **136** was synthesized in the literature by different methods. De Kimpe *et al*.[87] used isobutyraldehyd (**138**) with *t*-BuNH$_2$ to form the imine (Scheme 36). The imine **139** was treated with LDA for 1 h at 0 °C, followed by the addition of allylbromide to afford the imine product **140**. Final hydrolyzation with oxalic acid led to the corresponding aldehyde **136** in total yield of 83%. Noack and Göttlich,[88] used a similar method with morpholine instead of *t*-BuNH$_2$.



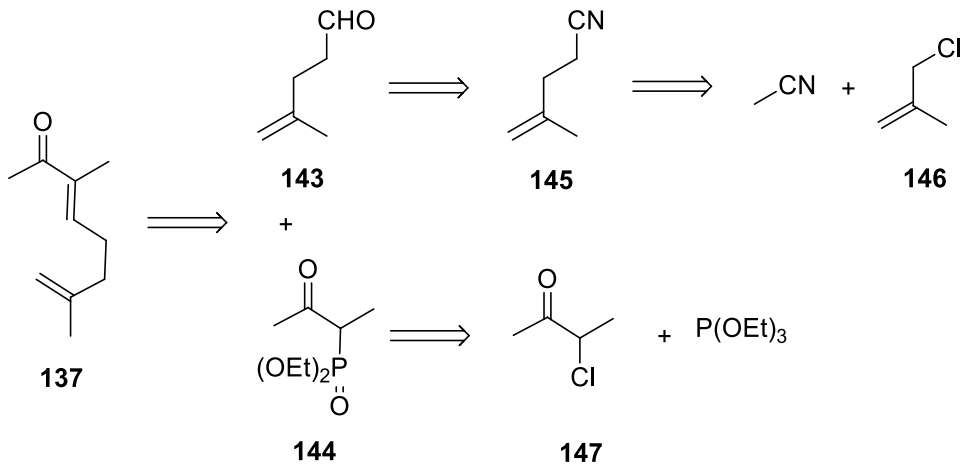
Scheme 36: Aldehyde **136** synthesized by De Kimpe *et al*.[87]

Cai *et al*.[89] prepared aldehyde **136** (Scheme 37) during the synthesis of the natural product buddledone A. In this method, isobutyronitrile (**141**) was deprotonated by lithium diethylamide followed by substitution reaction with allylbromide to produce the nitrile compound **142** which was reduced to the corresponding aldehyde **136** by DIBAL-H in total yield of 91% of aldehyde **136**. The same reduction procedure gave 98% yield of aldehyde **136** by Yang, T.[90]



Scheme 37: Aldehyde **136** synthesized by Cai *et al*.[89]

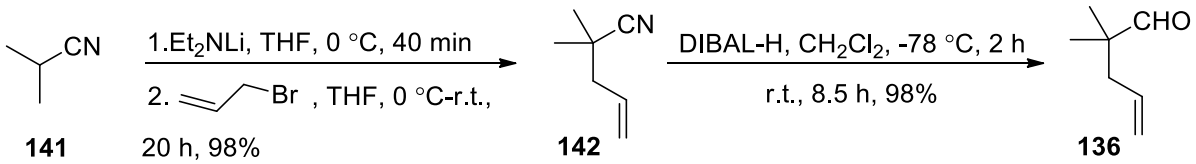
The α,β-unsaturated ketone **137** (Scheme 38) could be prepared using the Horner-Wadsworth-Emmons reaction between the aldehyde **143** and diethyl (3-oxobutan-2-yl)phosphonate **144**. Herein, aldehyde **143** could be generated by reduction of the corresponding nitrile **145**, which could be produced from the substitution reaction between acetonitrile and methyl allylchloride (**146**). The diethyl (3-oxobutan-2-yl)phosphonate (**144**) could be synthesized from the corresponding 3-chlorobutan-2-one (**147**) with triethylphosphite.



Scheme 38: Retrosynthesis planed of ketone **137**.

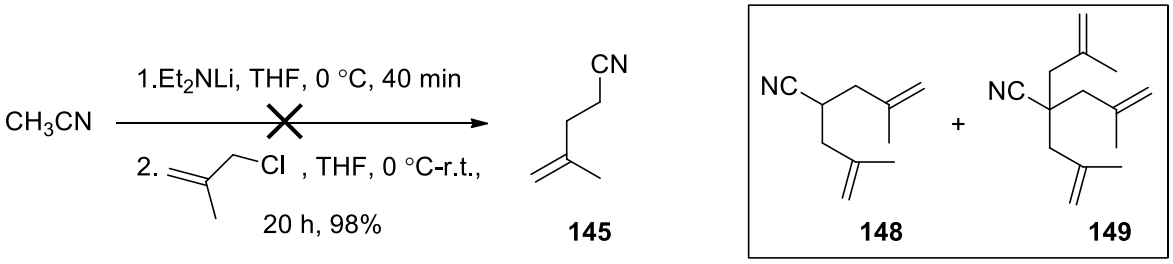
3.3.2. Synthetic approaches towards zerumbone

To synthesize aldehyde **113** (Scheme 39), isobutyronitril (**118**) was deprotonated by lithium diethylamide followed by a substitution reaction with allylbromide to produce the desired nitrile **142** in 98% yield. Nitrile **142** was reduced by DIBAL-H to the corresponding aldehyde **136** in 98% yield after 8.5 h.



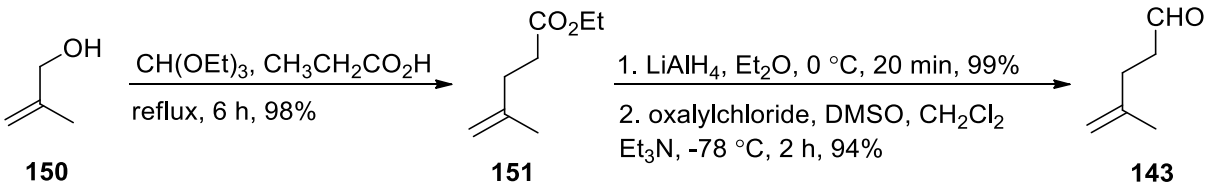
Scheme 39: Synthesis of aldehyde **136**.

The next step was the preparation of the aldehyde **143**. In this case acetonitrile was treated with lithium diethylamide (Scheme 40) followed by adding methyl allylchloride. The desired nitrile **145** was not observed and only amixture of di- and tri-alkylated acetonitrile **148** and **149** were observed.



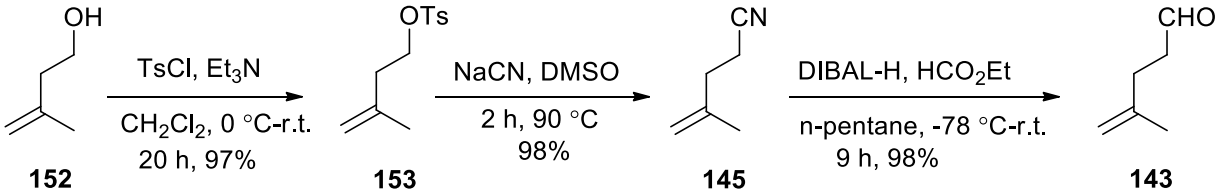
Scheme 40: Attempt to synthesize nitrile **145** from acetonitrile.

The reason is the acetonitrile has a high pK_a value 25 which means a strong conjugated base, this led to deprotonate the monoalkylated product **145** and formed the di- and tri-substituted acetonitrile **148** and **149**. Aldehyde **143** (Scheme **41**) was synthesized by Clarke *et al*.[91] fromed methyl allylalcohol (**150**) using Johnson-Claisen rearrangement to produce the ester **151**. The produced ester was reduced to the alcohol followed by oxidation to the corresponding aldehyde **143**. [91]



Scheme 41: Aldehyde **143** synthesized by Clarke *et al*.[91] using Johnson-Claisen rearrangement followed by oxidation-reduction reactions.

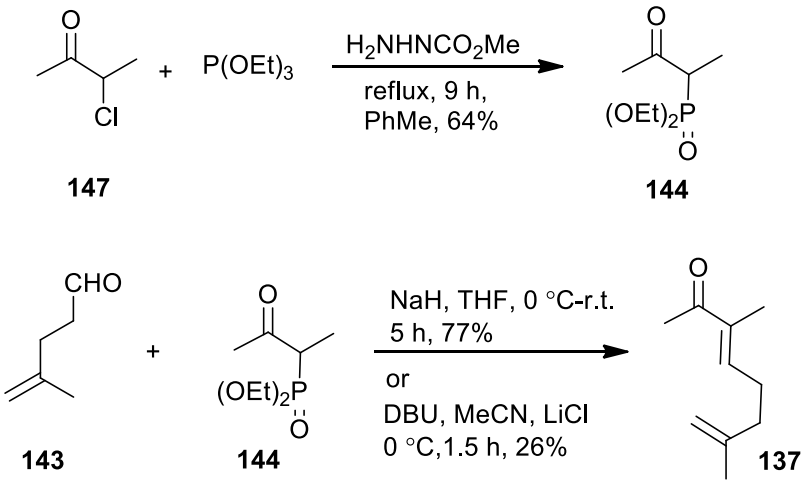
The starting material methyl allylalcohol is expensive, so instead of this method, a new method could be applied using very cheap starting material isoprenol (**152**). In this work isoprenol (**152**) (Scheme 42) was tosylated using Et_3N as a base,[92] to produce compound **153** in quantitative yield. Substitution reaction of the tosylate group in compound **153** with sodium cyanide in DMSO at 90 °C[92] produced the nitrile compound **145** in quantitative yield after 2 h. The nitrile **145** was reduced by DIBAL-H to the corresponding aldehyde **143** in excellent yield. This method gave an excellent yield over three steps without any special reagent.



Scheme 42: Synthesis of aldehyde **143** starting from isoprenol **152**.

Having aldehyde **143**, the next step was to prepare diethyl (3-oxobutan-2-yl)phosphonate (**144**) in order to apply the Horner-Wadsworth-Emmons reaction (Scheme 43). The method of Corbel *et al*.[93] was used to synthesize the phosphonate compound **144** by protection of the carbonyl group in 3-chlorobutan-2-one (**147**) with methyl hydrazinecarboxylate to prevent by-products and improve the yield. Then, a

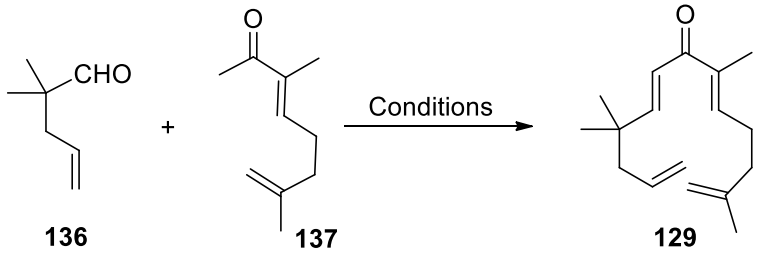
substitution reaction with triethylphosphite was performed by refluxing in toluene for 9 h to give 64% yield of compound **144**. The Horner-Wadsworth-Emmons reaction between compound **144** and aldehyde **143** took place using DBU or NaH as a base. The *E*-isomer was the only isolated product with 77% and 26% yield for NaH and DBU, respectively.



Scheme 43: Synthesis of the Horner-Wadsworth-Emmons phosphonate reagent.

The next step was forming the precursor **129**. The Claisen-Schmidt condensation is one of the most common methods to form enone unit. The results (Table 7) show that the hydroxide bases were not suitable since one equivalent (Table 7, Entry 1 and 3) from the base did not show any conversion in the starting material. The same observation when we tried three equivalents KOH at room temperature (Table 7, Entry 4), while using KOH in refluxed EtOH or MeOH (Table 7, Entry 5 and 7) led to complex mixture. Three equivalents of KOH at 50 °C caused decomposition of the aldehyde **136** (Table 7, Entry 6). Also DBU refluxing in MeOH,[94] did not show any reaction (Table 7, Entry 8), while NaH at 0 °C to room temperature,[95] after 1.5 h gave 9% yield of the product **129** with only *E*-isomer (Table 7, Entry 9). Since the NaH gave low yield, more investigations were done by *t*-BuOK in benzene at room temperature.[67] Here, the yield were around 11% as a mixtures of the *Z* and *E*-isomers were observed (Table 7, Entry 10 and 11). Both trifluoroacetic acid (TFA) (Table 7, Entry 12) and piperidinium trifluoroacetate salt[14] (Table 7, Entry 14) did not show any reaction, while LDA[89] (Table 7, Entry 13) produced a complex mixture even at -78 °C.

Table 7: Synthesis of precursor **129** by the Claisen-Schmidt condensation.



136 **137** **129**

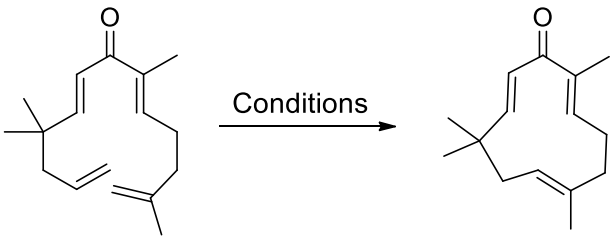
Entry	Base (eq)	Solvent	T (°C)	t (h)	Result
1	NaOH (1)	EtOH	60	48	no reaction
2	NaOH (2.5)	EtOH	60	48	complex mixture
3*	KOH (1)	EtOH	30	6	no reaction
4	KOH (3)	EtOH	rt	6	no reaction
5	KOH (3)	EtOH	reflux	2	complex mixture
6	KOH (3)	EtOH	50	3	decomposition of **136**
7	KOH (2)	MeOH	reflux	3	complex mixture
8	DBU (1)	MeOH	reflux	48	no reaction
9	NaH (1.2)	THF	0 - r.t.	1.5	9% of **129** (*E*)
10	*t*-BuOK (1.2)	benzene	r.t.	1	11% of **129** (*E* and *Z*)
11	*t*-BuOK (0.6)	benzene	r.t.	3	12% of **129** (*E* and *Z*)
12	TFA (0.05)	EtOH	reflux	48	no reaction
13	LDA (1.1)	THF	-78	2.5	complex mixture
14	piperidinium trifluoroacetate (0.2)	EtOH	reflux	48	no reaction

***** Ultrasound was used in the reaction.

The final step was trying to convert precursor **129** to the zerumbone (**1**) by ring closing metathesis using Ru catalysts. It was easy to decide whether we got the desired product or not by using zerumbone as a reference in the thin layer chromatography (TLC). The screening was started using 0.12 mM of precursor **129** with 20 mol% of Grubb's II catalyst (**131**). The reaction did not show the desired product in the TLC and the mass spectrum showed peak at $[M^+]$= 304.0. A new by-products were observed using Hovedya-Grubbs catalyst (**132**) at 80 °C in toluene or in refluxed CH_2Cl_2 (Table 8, Entry 2 and 3) the mass spectra showed peaks at

$[M+NH_4]^+$= 306.2. While the amount of Hovedya-Grubbs catalyst (**132**) was increased to 50 mol% a complex mixture was observed without any peak for the zerumbone (**1**) in the mass spectrum (Table 8, Entry 4). By using 10 mol% of Grubb's I catalyst (**130**) in refluxing CH_2Cl_2 isomerization reaction took place which means the same molecular weight as the starting material was observed in the mass spectrum ($[M+H]^+$ = 247.2) but different R_f value on TLC, the ^{1}H-NMR was not strong enough to characterize all the peaks but at least the terminal vinyl protons disappeared. In order to avoid this isomerization reaction *p*-benzoquinone was used as an additive to trap the ruthenium hydride which causes the isomerization reaction. The way how *p*-benzoquinone prevents the isomerization is not fully understood, but it could work either by prevention of the ruthenium hydride complex formation or by trapping the ruthenium hydride immediately during the reaction.[96] By using *p*-benzoquinone as an additive with Hovedya-Grubbs catalyst (Table 8, Entry 6) new by-product was observed with peak in the mass spectrum at $[M+H]^+$= 305.2.

Table 8: Approaches to zerumbone formation by ring closing metathesis.



129 **1**

Entry	Catalyst (mol%)	Solvent	T (°C)	t (h)	Result
1	Grubb's(II) (20)	toluene	60	20	$[M^+]$= 304.0
2	HGC (20)	toluene	80	20	$[M+NH_4]^+$= 306.2
3	HGC (20)	CH_2Cl_2	reflux	20	$[M+NH_4]^+$= 306.2
4	HGC (50)	CH_2Cl_2	reflux	20	complex mixture
5	Grubb's I (10)	CH_2Cl_2	reflux	20	isomerization $[M+H]^+$= 247.2
6[§]	HGC (20)	toluene	80	6	$[M+H]^+$= 305.2
7[§]*	HGC (20)	toluene	80	20	dimer

[§] *p*-benzoquinone was used as an additive. *****Using 0.5 mM precursor.

By increasing the concentration of the starting material to 0.5 mM (Table 8, Entry 7) the HRMS shows $[M+H]^+$ = 465.3722 which means that a dimer was formed. Also the ^{1}H-NMR spectrum (Figure 27) shows a dimer formation in which the two molecules were connected from the less steric hindered double bonds.

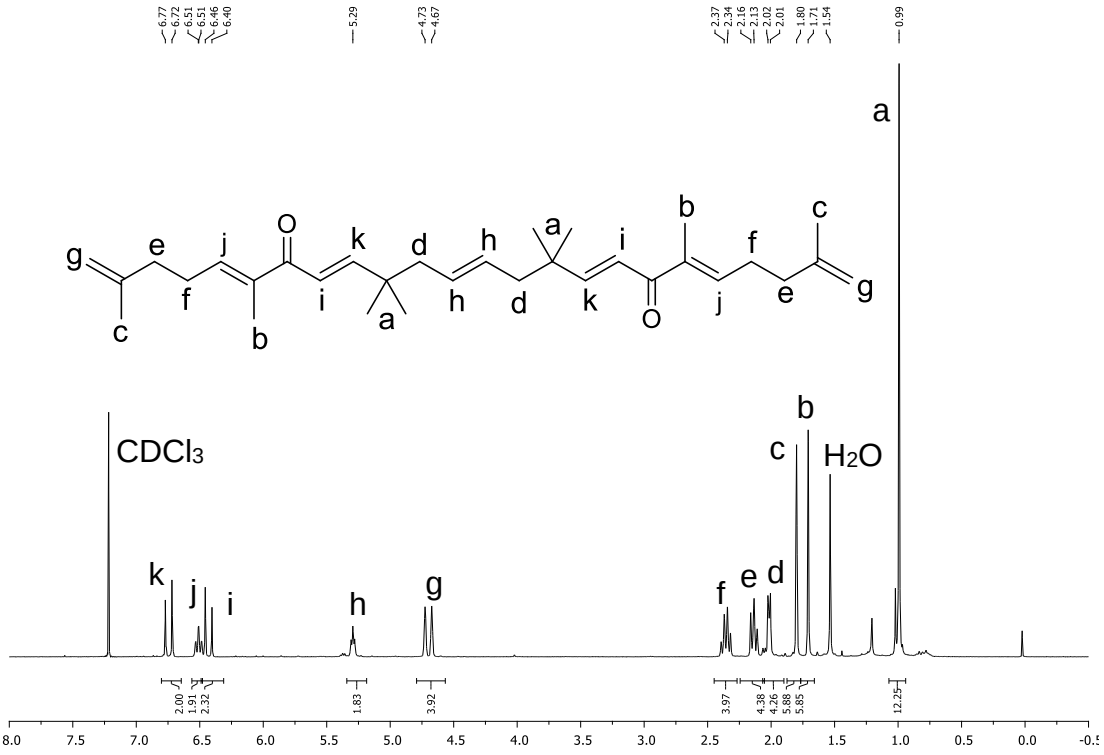


Figure 27: ^{1}H-NMR spectrum of the dimer.

The unsuccessful screening for the ring closing metathesis step could be related to the tow vinyl groups around the carbonyl group with *E*-configuration (Figure **28**), give some rigidity to the precursor **129**. This rigidity prevent joining the two terminal double bonds together.

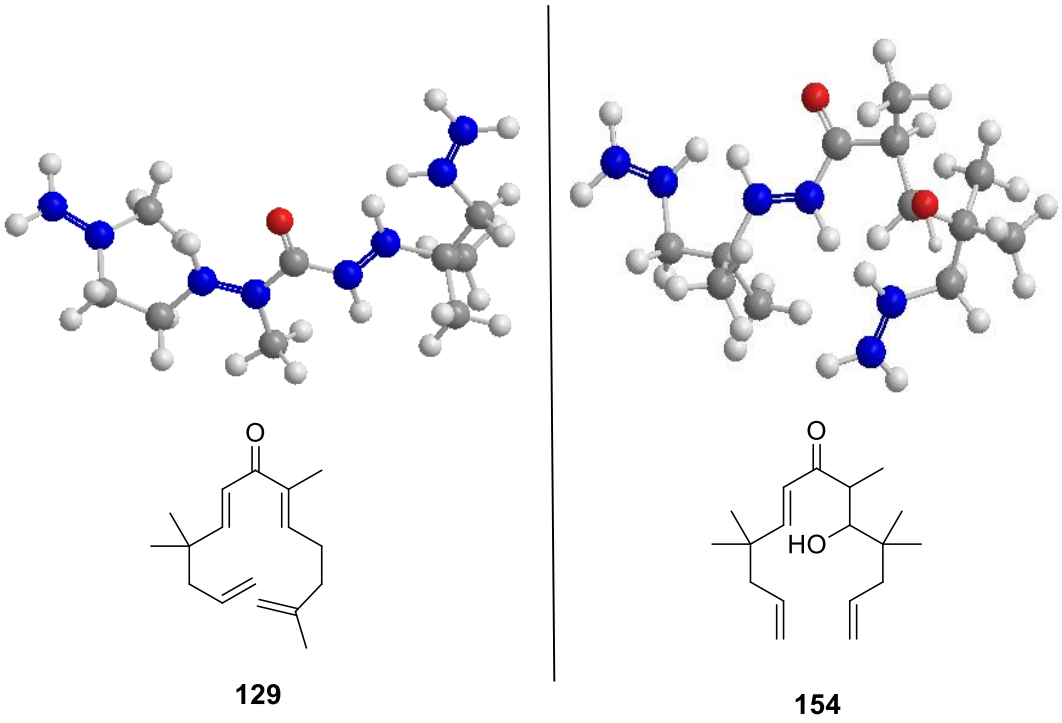
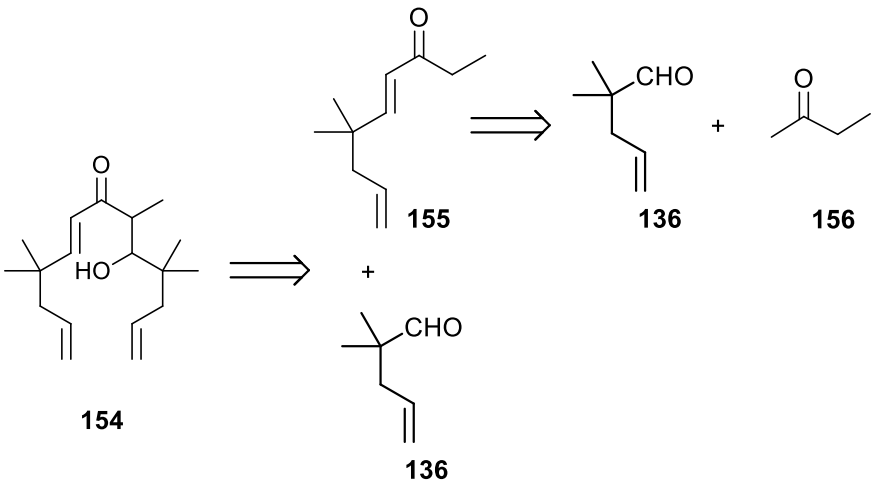


Figure 28: Three dimensional structure estimation of precursors **129** and **154**.

Therefore a new precursor with less number of double bonds **154** (Figure 28) could be more fixable, but a problem in the stability of the aldehyde **143** was observed since it was decomposed within 10 days even at -20 °C. To solve this problem, aldehyde **136** could be used instead of aldehyde **143** to optimize the conditions and after that aldehyde **143** could be used again to prepare zerumbone (**1**).

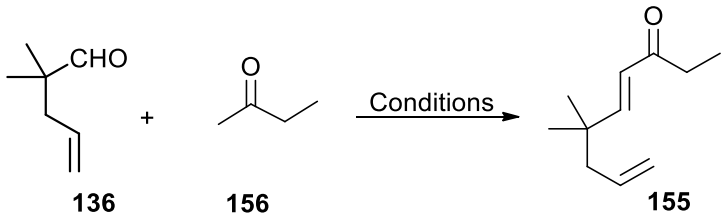
The new precursor **154** (Scheme 44) could be synthesized using two equivalents from aldehyde **136**. The advantage of this method is no need for phosphonate reagent which makes the sequence shorter.



Scheme 44: Retrosynthesis for the more flexible precursor **154**.

Ketone **155** can be synthesized by the Claisen-Schmidt condensation between aldehyde **136** and ethylmethylketone (**156**). In these reactions LDA/MsCl[89] gave a very good yield with 73% after 24 h (Table 9, Entry 1), while LDA/MOMCl[97] (Table 9, Entry 2) produced the desired product **155** in 58% yield after 10 h. Using morpholinium trifluoroacetate with ethylmethylketone (**156**) as a solvent 9% yield was observed after 48 h (Table 9, Entry 3). With *t*-BuOK reflux in benzene 22% yield of **155** was afforded after 20 h (Table 9, Entry 4). Further investigations using the Kelleher *et al*.[98] conditions with LiI reflux in Et_2O, here, 81% yield from the product **155** was afforded after 30 h using excess from the aldehyde **136** (ratio **136**/**156** = 1.4) (Table 9, Entry 5). By decreasing the ratio **136**/**156** to 0.9 no big different was observed and the desired ketone **155** was produced in 79% yield after 30 h (Table 9, Entry 6). When the solvent was changed to CH_2Cl_2.with 2 equivalents LiI no reaction took place after 48 h refluxed, since LiI is slightly soluble in Et_2O and it is not soluble in CH_2Cl_2.

Table 9: Synthesis of ketone **155**



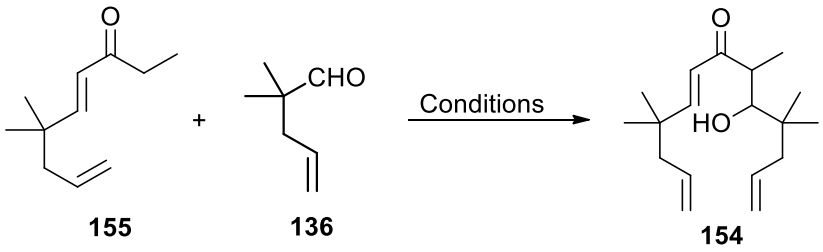
136 **156** **155**

Entry	Conditions	Ratio 136/156	Yield (%)
1	a. LDA (1.1 eq), THF, -78 °C, 3.5 h b. MsCl (2.4 eq), Et_3N, CH_2Cl_2, 0 °C-r.t., 20 h	1.1	73
2	a. LDA (1.1 eq), THF, -78 °C, 3.5 h b. MOMCl (2 eq), DIPEA, CH_2Cl_2, 0 °C-r.t., 6 h	0.9	58
3	Morpholinium trifluoroacetate (0.2 eq), 80 °C, 48 h	*	9
4	*t*-BuOK (1.1 eq), benzene, reflux, 20 h	1.1	22
5	LiI (2.5eq), Et_2O, reflux, 30 h	1.4	81
6	LiI (3 eq), Et_2O, reflux, 30 h	0.9	79
7	LiI (2 eq), CH_2Cl_2, reflux, 48 h	0.9	no reaction

* Ethylmethylketone was used as a solvent with 1 eq of **136**.

The precursor **154** could be synthesized by aldol reaction between ketone **155** and aldehyde **136**. Using LDA led to a complex mixture since mixing the ketone **155** first with LDA led to self-reaction (Table 10, Entry 1). While stabilizing the enolate with a TMS group by Mukaiyama aldol reaction followed by using $TiCl_4$ as a Lewis acid gave 13% yield product after 2 h and multiple by-products (Table 10, Entry 2). In contrast, LiI in Et_2O at 70 °C did not show any reaction in this case (Table 10, Entry 3).

Table 10: Synthesis of precursor **154**.

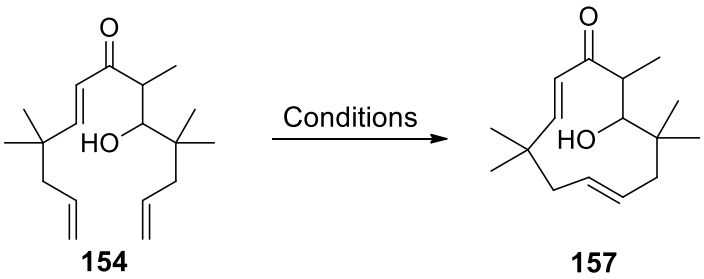


155 **136** **154**

Entry	Conditions*	Ratio 155/136	Result/ yield (%)
1	a. LDA (1.2 eq), THF, -78 °C, 2 h, b. NH_4Cl	1.5	complex mixture
2	a. LHMDS (2 eq), THF, -78 °C then Et_3N (3 eq), TMSCl (3 eq), 1.5 h. b. $TiCl_4$ (1 eq), CH_2Cl_2, -78 °C, 40 min	1	13
3	LiI (3 eq), Et_2O, 70 °C, 48 h, Sealed tube	1	no reaction

The precursor **154** has less double bonds which means it is more flexible than the first precursor **129**. Ring closing metathesis was applied using different ruthenium catalysts with 0.12 mM concentration of the starting material and 20 mol% of the catalyst. In these cases Grubbs I (**130**) (Table 11, Entry 1) and Grubbs II (**131**) (Table 11, Entry 2) did not show any reaction. When Hovedya-Grubbs catalyst (**132**) was used, a new product was observed on the TLC while the mass spectrometry shows the same molecular weight as starting material which means that an isomerization reaction was occurred (Table 11, Entry 3). Adding *p*-benzoquinone to the reaction as an additive caused side product formation (Table 11, Entry 4), while using catalyst **135** refluxed in toluene didn't show any reaction (Table 11, Entry 5).

Table 11: Attempted to ring closing metathesis of precursor **154**

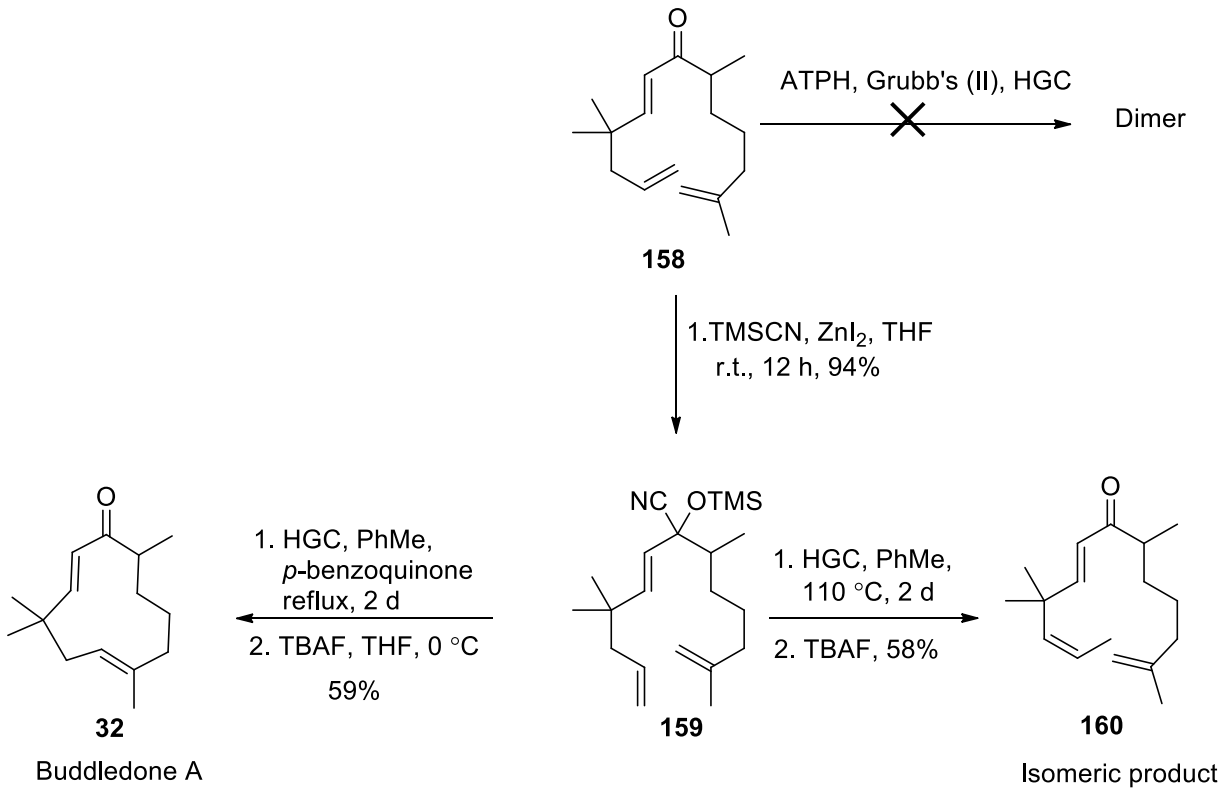


154 **157**

Entry	Catalyst (mol%)	Solvent	T (°C)	t (h)	Result
1	Grubb's(II) (20)	toluene	80	20	no reaction
2	Grubb's (I) (10)	toluene	reflux	48	no reaction
3	HGC (20)	toluene	80	6	isomerization reaction
4[§]	HGC (20)	toluene	80	6	unidentified product
5	**135**	toluene	reflux	4 d	no reaction

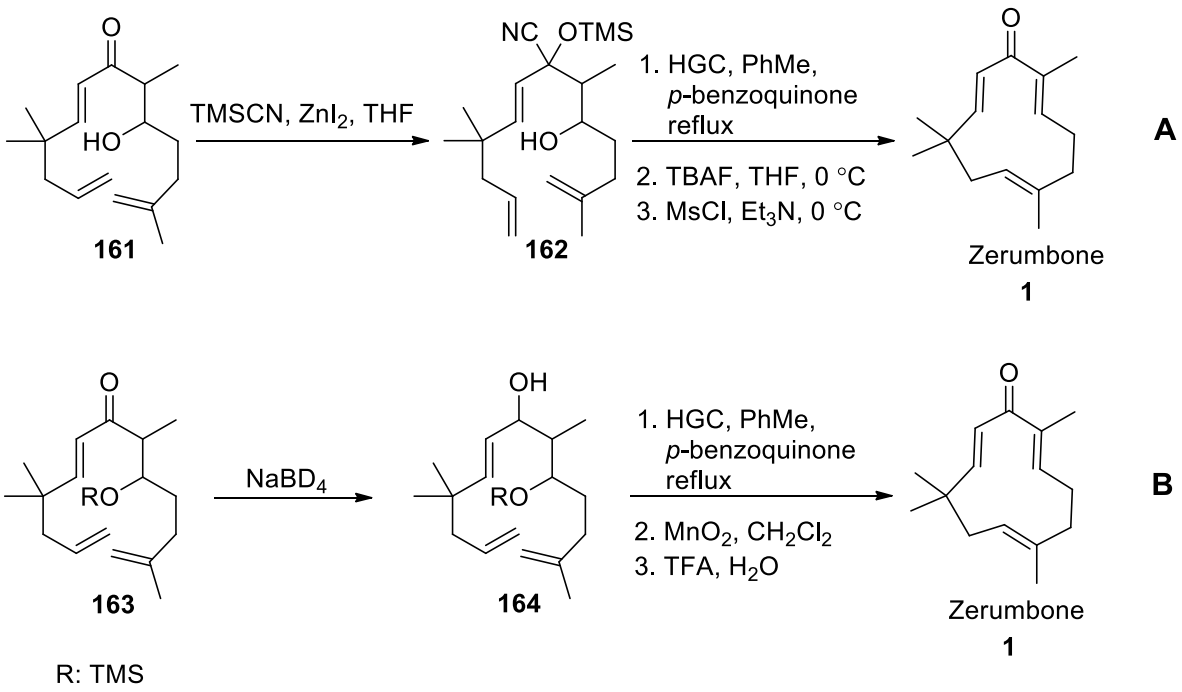
[§] *p*-Benzoquinone 0.2 equivalent was used as an additive.

All the attempts to produce the cyclic product were failed. By looking to similar skeletons in the literature many examples show problems when nine, ten and eleven membered rings need to be formed, since they all are highly strained. Cai *et al*.[89] prepared the 11-membered ring natural product buddledone A (**32**), all their trials to convert the precursor **156** to buddledone A (**32**) failed under different catalysts and conditions (Scheme 45). Then they decided to protect the carbonyl group, in this case the new carbon center became sp^3 hybridized which makes the precursor more flexible towards ring closing metathesis. TMSCN was used as a protection group for the carbonyl group to produce compound **159**, but approaches to form the ring by HGC in toluene for two days led to isomeric product **160**. To solve this problem, *p*-benzoquinone was used as an additive. Finally, the ring closing succeeded with HGC followed by regenerating the carbonyl group to produce buddledone A (**32**) in 59% yield over two steps.[89]



Scheme 45: Synthesis of buddldone A (**32**) by Cai *et al*.[89]

Herein, more investigations are need in the future to make the precursor more flexible, which could be achieved by using TMSCN to protect the carbonyl group (Scheme 46, **A**). An alternative method (Scheme 46, **B**) could be by reducing the carbonyl group to the corresponding alcohol to form sp^3 center and then the ring closing metathesis take place followed by regeneration the carbonyl group.



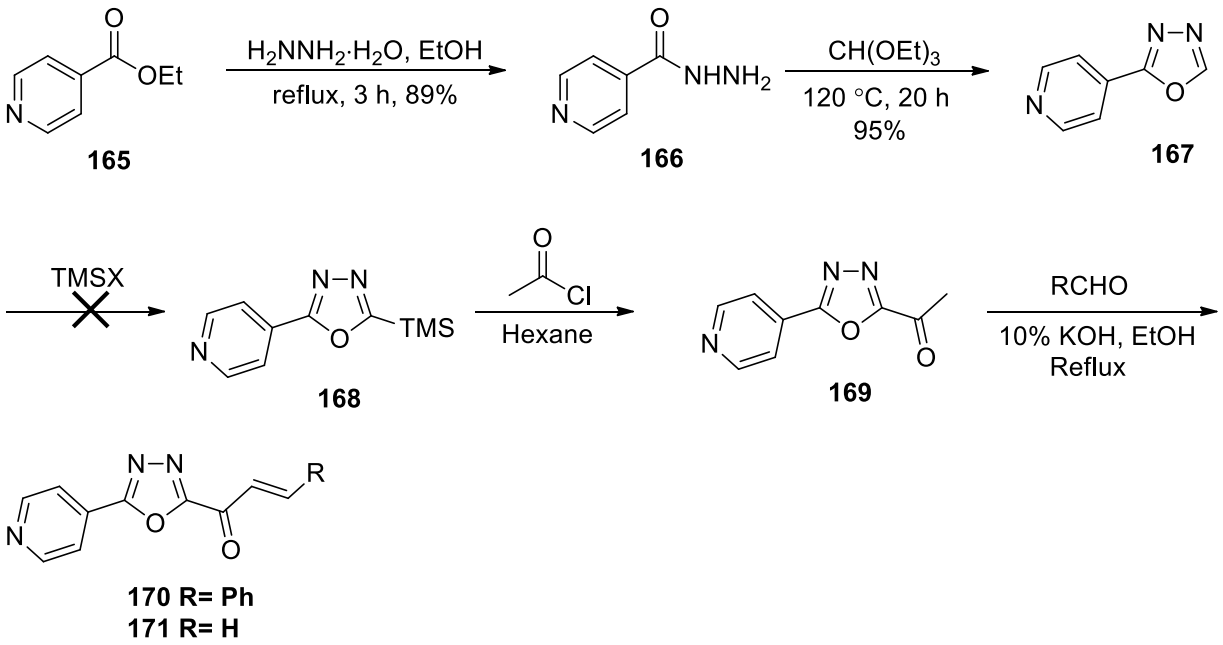
Scheme 46: Proposed synthesis for zerumbone (**1**) by convert the carbonyl group to sp^3 hybridized. **A**: Using TMSCN, **B**: by reduction to alcohol.

3.4. 1,3,4-Oxadiazole derivatives with an additional enone unit

All the previous sections were attempted to synthesize natural products with an enone system or tried to prepare derivatives from the natural products with some groups in the α-position of the conjugated system. The next idea was combined the enone unit with the synthetic biological active moiety oxadiazole.

3.4.1. Synthesis of 1,3,4-oxadiazoles with an additional enone unit in position two

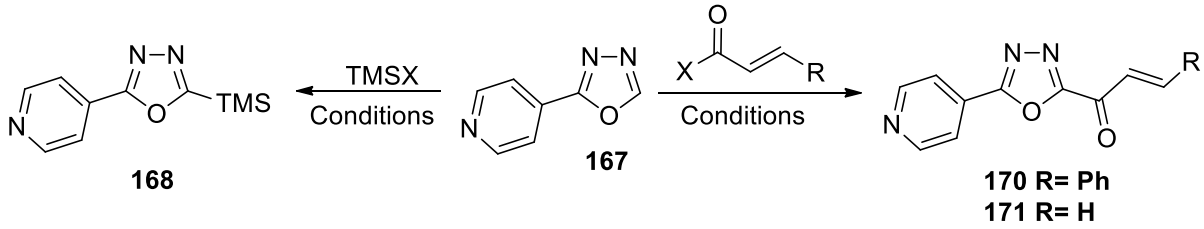
To combine 1,3,4-oxadiazole moiety with an enone unit in position 2 and a pyridinyl moiety in position 5 of the ring, isonicotinic ester **165** (Scheme 47) was treated with hydrazine monohydrate under refluxing in ethanol[63] this gave 89% yield of the hydrazide **166** after 3 h. Then the isonicotinic hydrazide **166** was treated with triethyl orthoformate at 120 °C to afford the oxadiazole ring with a pyridinyl moiety **167** to yield 95% after 20 h. The next step was attempted to introduce a TMS group in position two of the ring to get **168** using Zarudnitskii's[99] method. The TMS group could be substituted with an acetyl group to produce compound **169**. Finally, the Claisen-Schmidt condensation could be applied to get the desired derivatives **141**.



Scheme 47: Attempts to synthesize 1,3,4-oxadiazole ring with an additional α,β-unsaturated carbonyl unit in position 2

Different conditions were tried in order to have the compound **168** with a TMS group. Firstly, TMSCl and TMSBr were used with Et_3N (Table 12, Entry 1 and 2), no conversion was observed after 30 h and 48 h, respectively, even with increasing the temperature. The same result was observed with strong bases such as DIPEA and n-BuLi even with bromide as a leaving group (Table 12, Entry 3 and 4). Consequently, cinnamoyl chloride was used in order to directly insert an enone to the oxadiazole ring, different bases ranging from Et_3N as a weak base (Table 12, Entry 5) to strong bases like n-BuLi (Table 12, Entry 6) and NaH (Table 12, Entry 7) were used but no conversion at all was observed for the oxadiazole compound **167**. Then acryloyl chloride was used in a Friedel Crafts acylation using $AlCl_3$ as a Lewis acid, but still no conversion was observed (Table 12, Entry 8). The same observation was made when cinnamoyl chloride was treated with $TiCl_4$ at -78 °C (Table 12, Entry 9).

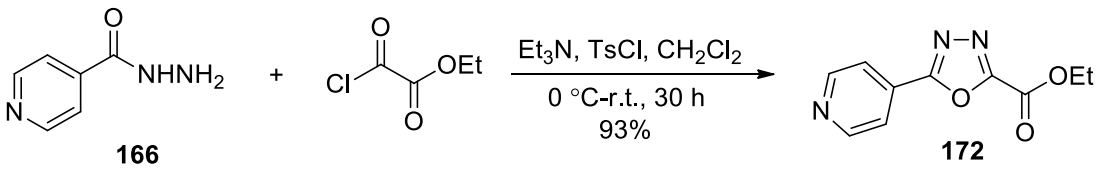
Table 12: Approaches to introduce TMS group or enone unit to 1,3,4-oxadiazole **167**.



Entry	R	X	Base/ Acid (eq)	Solvent	T (°C)	t (h)	Comment
1	-	Cl	Et_3N	Et_2O	r.t.-reflux	30	n.c.
2	-	Br	Et_3N	-	r.t.-60	48	n.c.
3	-	Br	DIPEA	toluene	r.t.-reflux	48	n.c.
4	-	Br	n-BuLi	THF	-78-r.t.	6	n.c.
5	Ph	Cl	Et_3N	THF	0-r.t.	24	n.c.
6	Ph	Cl	n-BuLi	THF	-78-r.t.	5	n.c.
7	Ph	Cl	NaH	THF	-10-r.t.	6	n.c.
8	H	Cl	$AlCl_3$	CH_2Cl_2	0-r.t.	48	n.c.
9	Ph	Cl	$TiCl_4$	CH_2Cl_2	-78-r.t.	2.5	n.c.

n.c.: no conversion was observed for the oxadiazole **167**.

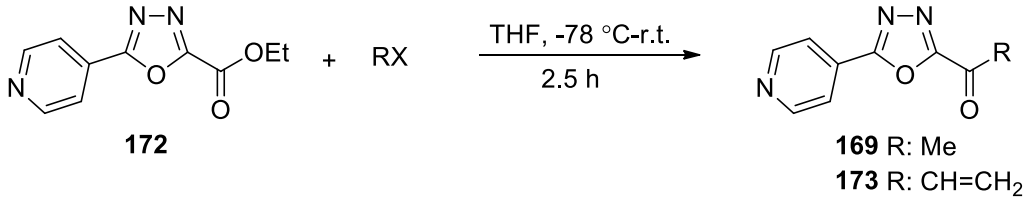
In all these trials, no conversion was observed for the oxadiazole compound **167**. The reason might be that pyridinyl moiety can react as a nucleophile using the nitrogen lone pair which means trap the TMS group and the acyl chloride during the reaction. While in Zarudnitskii *et al*.[99] case only a phenyl group was attached to the oxadiazole ring in all the examples. Since the methods to introduce the TMS or the enone unit to the oxadiazole ring failed, a new pathway was used. In this case an ester group in position 2 of **172** was prepared using Leung *et al*.[59] method. Isonicotinic hydrazide (**166**) was treated with ethyl oxalyl chloride, Et_3N and *p*-TsCl in CH_2Cl_2 at 0 °C-r.t. for 30 h to produce the desired ester **172** in 93% yield (Scheme 48).



Scheme 48: Synthesis of ethyl 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-carboxylate (**172**).

With having the oxadiazole ester **172** in hand, the next step was the substitution reaction to convert ester **172** to the ketone **169**. A complex mixture was observed using Grignard reagent MeMgBr (Table 13, Entry 1) or MeLi (Table 13, Entry 2), because the ketone product was more reactive than the ester starting material **172** which led to a consecutiveof reaction of the product. An excess of ester **172** led to traces from the product **169** (Table 13, Entry 3). Similarly, introducing vinyl moiety by the Grignard reaction gave complex mixture (Table 13, Entry 4).

Table 13: Attempts to convert the oxadiazole ester **172** to the corresponding ketone.

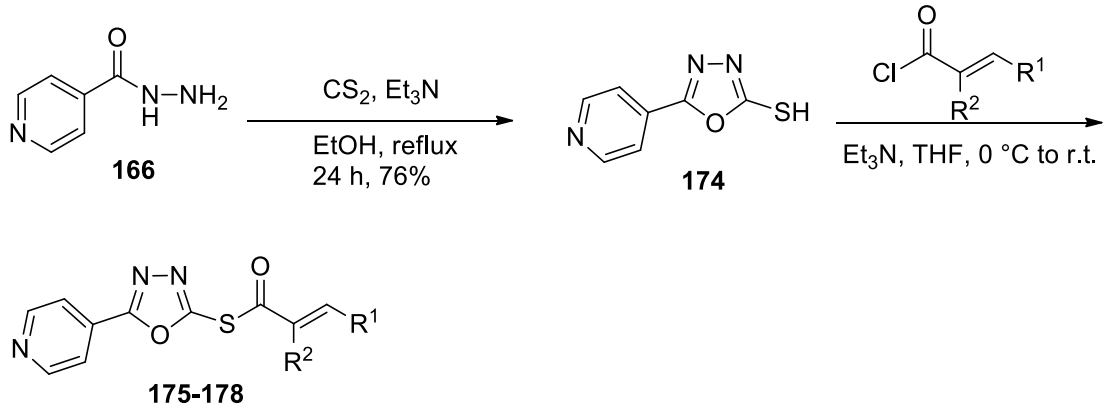


Entry	RX (eq)	Result
1	MeMgBr (1)	complex mixture
2	MeLi (1)	complex mixture
3	MeMgBr (0.5)	traces
4	CH_2=CHMgBr (1)	complex mixture

Since some oxadiazole ring with sulfur atom in position two show interesting biological activity, so the idea was to couple the oxadiazole ring with enone moiety using a sulfur atom at position 2 of the ring. The isonicotinic hydrazide (**166**) and carbondisulfide were refluxed in ethanol and triethylamine to produce 2-thiol-1,3,4-oxadiazole **174** with 78% yield after 24 h. The 2-thiol-1,3,4-oxadiazole **174** was combined with α,β-unsaturated acid chloride at 0 °C with triethylamine to generate

compounds **175** (Table 14, Entry 1) and **176** (Table 14, Entry 2) with 72% and 53% yield, respectively.

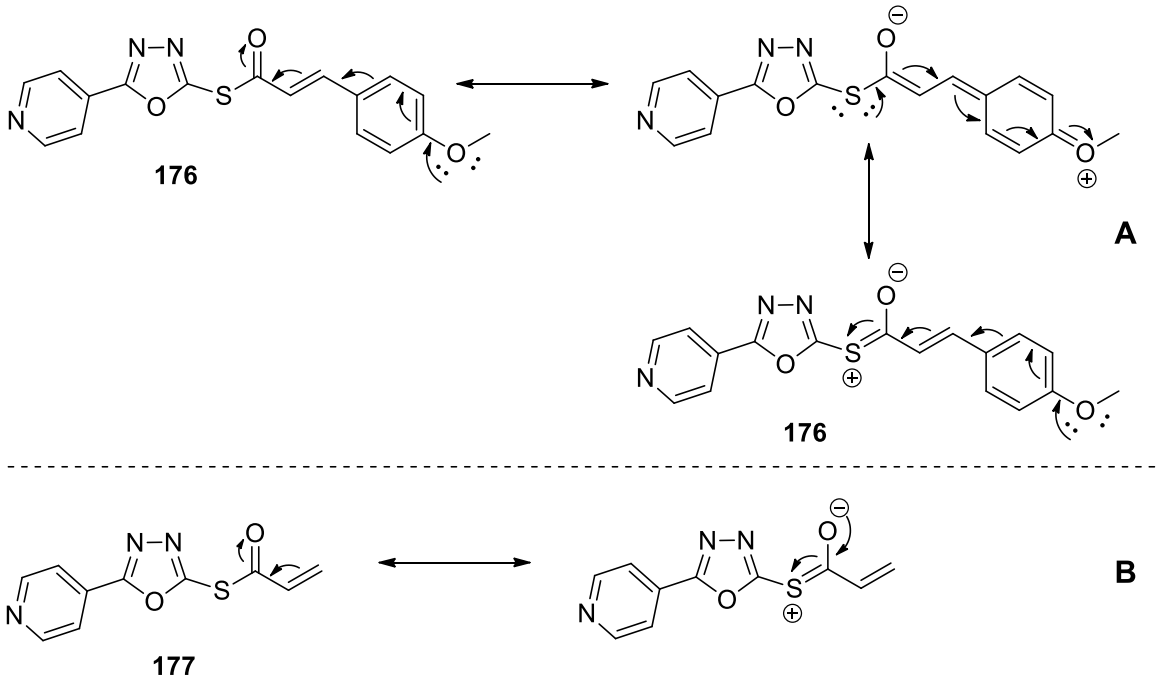
Table 14: Combine the oxadiazole **174** with enone unit using sulfur bridge.



Entry	Product	R^1	R^2	t (h)	Yield (%)
1	**175**	C_6H_4	H	3	72
2	**176**	*p*-MeO-C_6H_4	H	3	53
3	**177**	H	H	3	n.c
4	**178**	H	CH_3	4	n.c

n.c: no conversion was observed for the oxadiazole **174**.

Aliphatic α,β-unsaturated acid chloride (Table 14, Entry 3 and 4) did not show any conversion of the oxadiazole starting material **176**. Which seems to be the product unstable, because in the case of aromatic substituent in the β-position (Table 14, Entry 1 and 2) the product is stabilized by the conjugation (Scheme 49), while it is not the case with short conjugation in the aliphatic products (Table 14, Entry 3 and 4).

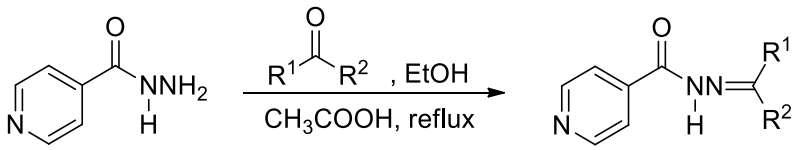


Scheme 49: **A**: long conjugation with β-aryl group stabilize the oxadiazole derivative **176**, **B**: short conjugation with unsubstituted enone system destabilize the oxadiazole derivative **177**.

3.4.2. Synthesis of 2,3-dihydro-1,3,4-oxadiazoles with an additional enone unit in position three

To prepare 1,3,4-oxadiazole with enone unit in position three and pyridinyl moiety in position, isonicotinic hydrazide (**166**) was treated with different aldehydes and ketones to have a set of hydrazones (**179**-**190**) (Table 15). In these examples monosubstituted, disubstituted, aliphatic, aromatic and cyclic carbonyl compounds were used to decorate the hydrazones **179**-**190** in order to study the structure activity relationship (SAR) of the resulting final compounds. Generally, the reaction produced hydrazones from moderate to high yield (41-98%) with catalytic amounts of glacial acetic acid. The differences are obvious in the reaction times, since the aldehydes react faster (3 h) compared with the ketones (24 h). This is related to the electrophilicity of the aldehydes which is higher than the ketones (Table 15).

Table 15: Synthesis of hydrazones **179-190**

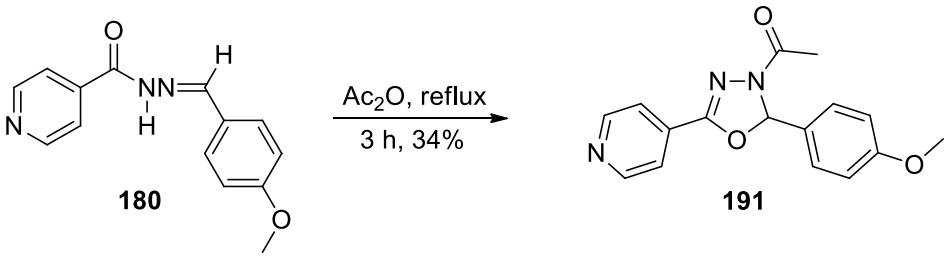


166 **179-190**

Product	R^1	R^2	t (h)	Yield (%)
179	C_6H_5	H	3	76
180	*p*-MeO-C_6H_4	H	3	96
181	*p*-NO_2-C_6H_4	H	3	98
182	C_6H_5	CH_3	24	95
183	-$CH_2(CH_2)_3CH_2$-		24	55
184	*p*-MeO-C_6H_4	CH_3	24	67
185	C_6H_5	CH_3	21	47
186	*p*-NO_2-C_6H_4	CH_3	24	41
187	*p*-CF_3-C_6H_4	CH_3	24	92
188	*p*-OCF_3-C_6H_4	CH_3	24	96
189	4-pyridinyl	CH_3	24	91
190	2-pyridinyl	CH_3	24	97

***** phenyl moiety in position 5 of the oxadiazole ring instead of the pyridinyl, compounds **186** and **187** were prepared by Maria Landa and **188** was prepared by Thomas Klein.

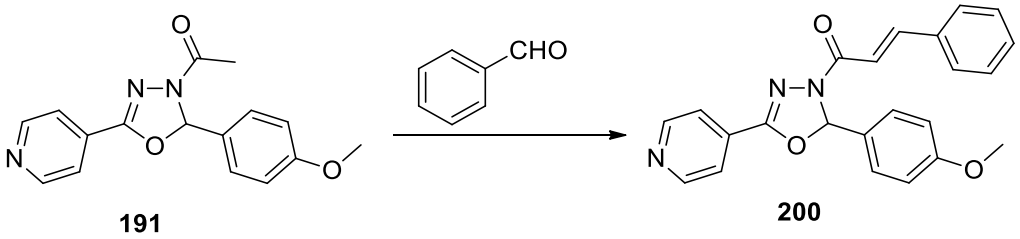
The next step was the formation of the 2,3-dihydro-1,3,4-oxadiazole ring from the corresponding hydrazone. To achieve this, hydrazone **180** was refluxed with acetic anhydride to produce the desired 2,3-dihydro-1,3,4-oxadiazole **191** with acetyl group in position 3 with only 34% yield after 3 h (Scheme 50).



Scheme 50: Synthesize 1,3,4-oxadiazole with acetyl group in position three **191**.

The acetyl group in position 3 in compound **191** was then available to create the α,β-unsaturated carbonyl unit. The oxadiazole **191** was treated with benzaldehyde under different conditions, by using the most common bases in the Claisen-Schmidt condensation (Table 16, Entry 1-3) under various conditions this caused hydrolysis of the oxadiazole starting material **191**, also with nonpolar solvent (Table 16, Entry 4). As a result, compound **191** was unstable in the basic medium since in all these cases complete conversion of the oxadiazole was observed, which means that this strategy is not useful to synthesize the desired derivatives.

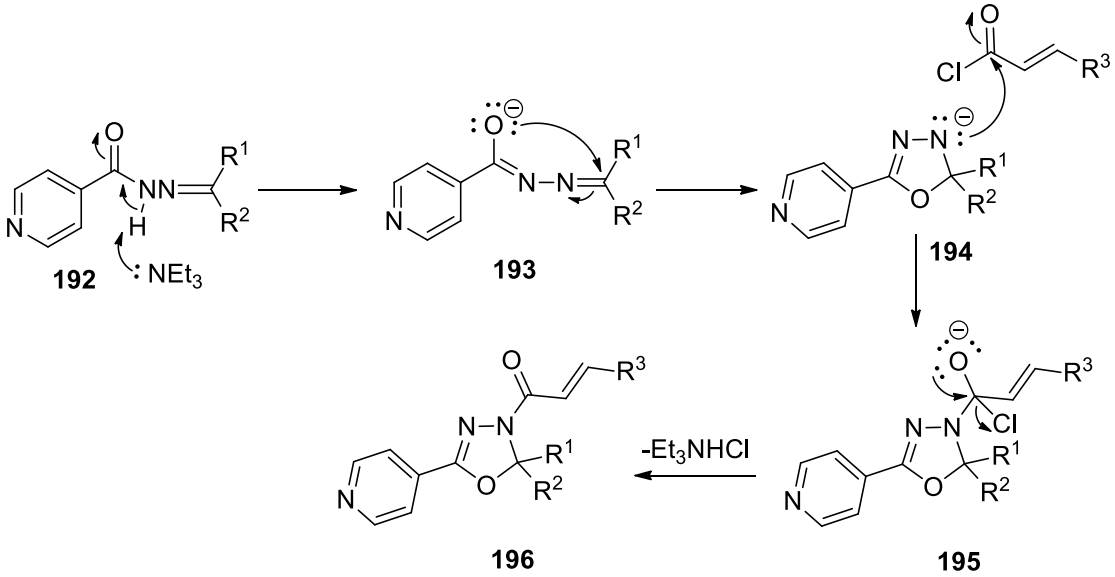
Table 16: Claisen-Schmidt condensation conditions to form the enone unit in position 3 of the oxadiazole ring.



Entry	Base	Solvent	T (°C)	t (h)	Result
1	$Ba(OH)_2 \cdot 8H_2O$	MeOH	reflux	0.5	hydrolysis of **191**
2	NaOH	EtOH	reflux	3	hydrolysis of **191**
3	NaOH	EtOH	r.t.	5	hydrolysis of **191**
4	piperidine	toluene	reflux	0.5	hydrolysis of **191**

An alternative pathway was necessary in order to prepare the desired products. Going back to the original mechanism of the 1,3,4-oxadiazole ring formation

(Scheme 8 in the introduction) a weak base (CH_3COO^-) was used to deprotonate the N-H and a carbonyl electrophile source (Ac_2O) was used to add carbonyl moiety to the position 3 of the oxadiazole ring. The new strategy used Et_3N as a base and α,β-unsaturated acid chloride as a carbonyl electrophile source. In this case the enone system was synthesized separately and then added to the ring during the formation of the heterocyclic. The proposed mechanism (Scheme 51) shows that Et_3N deprotonates the N-H in compound **192** to form the enolate **193**, then the enolate oxygen attacks the carbon in the C=N bond. The result is 1,3,4-oxadiazole ring with a nitrogen anion in position 3 in **194** which works as nucleophile and immediately attacks the excellent electrophile α,β-unsaturated acid chloride and looses chloride as a leaving group to form compound **196**. The advantages of this method are the following: mild conditions, formation of oxadiazole ring with the enone unit take place in one step and a wide range of aliphatic and aromatic α,β -unsaturated acid chloride can be used.

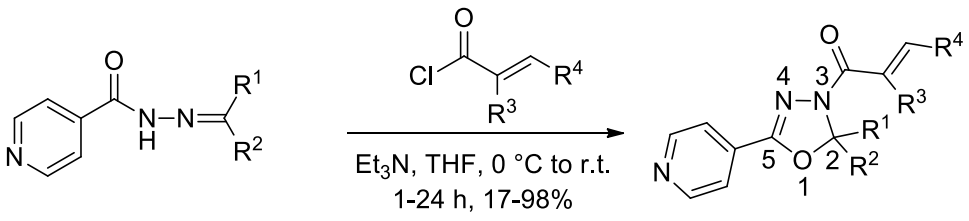


Scheme 51: Proposed mechanism for 2,3-dihydro-1,3,4-oxadiazole ring formation with an additional α,β-unsaturated carbonyl unit in position three.[100]

In this method, α,β-unsaturated acid chlorides were synthesized from the corresponding aldehyde as described by Pau *et al*.[101] the aliphatic α,β-unsaturated acid chloride are commercially available. Various acid chlorides were used in order to study the effect of the substituent around the enone unit on the biological activity of the compounds, the synthesis was done in parallel with testing. Reaction times,

temperatures and yields are summarized in Table 17. In general the reactions are fast and the aliphatic acid chlorides gave higher yields in comparison with the aromatic one since they are better electrophiles.

Table 17: Yields and reaction times for compounds **197-214**.



179-190 **197-214**

SM[a]	Product	R^1	R^2	R^3	R^4	t (h)	Yield (%)
179	**199**	C_6H_5	H	H	C_6H_5	4	69
180	**200**	*p*-MeO-C_6H_4	H	H	C_6H_5	5	72
181	**201**	*p*-NO_2-C_6H_4	H	H	C_6H_5	5	83
182	**204**	C_6H_5	CH_3	H	*p*-MeO-C_6H_4	5	25
182	**197**	C_6H_5	CH_3	H	C_6H_5	5	78
182	**206**	C_6H_5	CH_3	H	CH_3	4	86
182	**205**	C_6H_5	CH_3	H	H	4	98
182	**207**	C_6H_5	CH_3	CH_3	H	4	98
183	**203**	-$CH_2(CH_2)_3CH_2$-		H	C_6H_5	5	62
184	**202**	*p*-MeO-C_6H_4	CH_3	H	C_6H_5	4	57
185[b]	**198[b]**	C_6H_5	CH_3	H	C_6H_5	24	83
186	**209**	*p*-NO_2-C_6H_4	CH_3	H	H	3	27
187	**210**	*p*-CF_3-C_6H_4	CH_3	H	H	3	17
188	**211**	*p*-OCF_3-C_6H_4	CH_3	H	H	1.5	59
182	**208**	C_6H_5	CH_3	H	*p*-CF_3-C_6H_4	24	65

189	**212**	4-pyridinyl	CH_3	H	H	2	25
190	**213**	2-pyridinyl	CH_3	H	H	3	58
182	**214**[c]	C_6H_5	CH_3	H	H	1	54

[a]SM**,** starting material; [b]phenyl moiety in position 5 of the oxadiazole ring; [c]saturated carbonyl unit, compounds **209** and **210** were prepared by Maria Landa and **211** was prepared by Thomas Klein.

Twenty derivatives were synthesized in which the 1,3,4-oxadiazole ring, an α,β-unsaturated carbonyl unit and a pyridinyl moiety are combined together. All these moieties showed interesting biological activity as discussed before.

3.4.3. Antibacterial and antifungal activity of the 1,3,4-oxadiazole derivatives

This data was obtained by Galina Sergeev and Mark Brönstrup (Department of Chemical Biology, Helmholtz Center for Infection Research, Braunschweig, Germany). The synthesized compounds were tested against different gram-negative bacteria: *Acinetobacter baumannii* (DSM 30007, ATCC 19606), *Enterobacter cloacae* (DSM 26481, ATCC 23355), *Escherichia coli* (DSM 1116, ATCC 9637), *Klebsiella pneumoniae* (DSM 11678, ATCC 33495), *Pseudomonas aeruginosa* PA7 (DSM 24068), gram-positive bacteria: *Enterococcus faecium* (DSM 20477, ATCC 19434), *Staphylococcus aureus* MRSA (DSM 11822, ICB 25701), *Staphylococcus aureus* MRSA Nr.5 (RKI Nr. 11-02670) and also against the fungi *Candida albicans* (DSM 11225, ATCC 90028). All the derivatives were not active against the pathogens tested, which did not give any promising data for further investigations towards antimicrobial properties.

3.4.4. Antiproliferative activity of the 1,3,4-oxadiazole derivatives

This data was obtained by Galina Sergeev and Mark Brönstrup (Department of Chemical Biology, Helmholtz Center for Infection Research, Braunschweig, Germany). All the compounds **197**-**214** and **175-176** were screened for antiproliferative activity against L929 (mouse adipose tissue), MCF-7 (human breast adenocarcinoma) and KB-31 (human cervical carcinoma) cells. The derivatives were incubated for 5 days with the tested cell lines.

The results (Table 18) revealed that the pyridinyl moiety in position 5 of the 1,3,4-oxadiazole ring is necessary for the antiproliferative activity. This becomes clear by

comparing compound **197** with **198** (Table 18). The IC_{50} values are 15.7 µM against L929 for **197**, while compound **198** the same skeleton with phenyl moiety in position 5 of the ring instead of the pyridinyl was not active at all against the tested cells. The second step was focusing on position 2 of the oxadiazole ring. In this case a series of monosubstituted compounds including electron withdrawing and electron releasing groups **199**, **200** and **201** was prepared. These analogues did not show any activity against the tested cells. Consequently, a new set of disubstituted compounds in position 2 of the ring was synthesized. This time, an electron donating group in **202** reduced the activity, while the spirosystem in **203** showed week activity with IC_{50} values 20.9 µM against L929. The SAR showed that methyl and phenyl groups in position 2 of the ring are essential for the activity with pyridinyl moiety in position 5. Then the effect of the substituents around the enone unit was studied, so compounds **204**, **205**, **206**, **207** and **208** were synthesized. The unsubstituted enone compound **205** was the most active derivative with IC_{50} values 4.5 µM, 11.4 µM and 11.5 µM, against L929, KB-31 and MCF-7, respectively. At the same time electron donating groups decrease the activity, and an electron withdrawing group inhibits the activity. The SAR led us to compound **205** with promising activity. Further investigations were done in position 2 of the ring. In this case electron withdrawing groups were added to the phenyl moiety such as NO_2, CF_3 (**209** and **210,** respectively). These three compounds were very active and showed the promising results in comparison to the previous derivatives. In particular, compound **209** was the most potent derivative with IC_{50} values 3.2 µM, 6.0 µM and 6.3 µM against L929, KB-31 and MCF-7, respectively. Since the pyridinyl moiety in position 5 is important it was decided to introduce another pyridinyl moiety in position 2 of the ring as 4-pyridinyl **212** and 2-pyridinyl **213**. These compounds showed moderate activity with IC_{50} values around 10 µM. In order to proof the importance of the enone unit in the activity, a compound with saturated carbonyl group was synthesized **214** and the antiproliferative test showed no activity at all for this compound **214**. Indeed, the α,β-unsaturated carbonyl group is essential in the antiproliferative activity. Finally, the compounds containing the sulfur bridge between the oxadiazole ring and the enone unit **175** and **176** were tested and did not show any activity against the tested cell lines, so no further investigation was done in this skeleton.

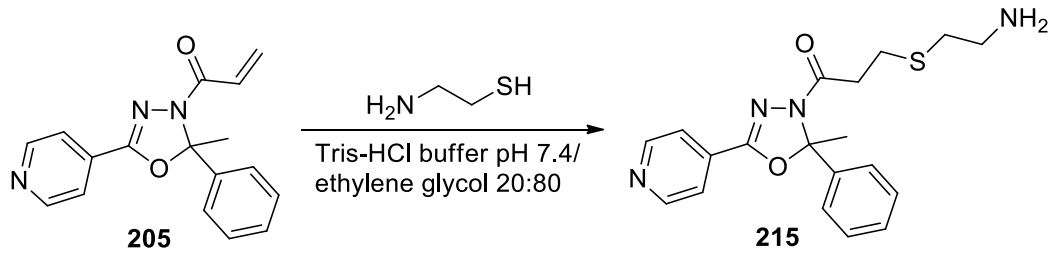
Table 18: Antiproliferative activity for compounds **197-214** and **175-176**.

	Cell line (IC_{50}, µM)		
Compound	**L929**	**KB-31**	**MCF-7**
197	15.6	n.a.	>25
198	n.a.	>25	>25
199	n.a.	n.a.	n.a.
200	n.a.	n.a.	n.a.
201	n.a.	n.a.	n.a.
202	>25	n.a.	>25
203	20.9	n.a.	>25
204	>25	n.a.	>25
205	4.5	11.4	11.5
206	n.a.	n.a.	n.a.
207	n.a.	n.a.	n.a.
208	n.a.	n.a.	n.a.
209	3.2	6.0	6.3
210	5.2	5.0	4.2
211	13.6	8.4	9.4
212	8.6	11.0	14.6
213	9.4	6.3	14.6
214	n.a.	n.a.	n.a.
175	n.a.	n.a.	n.a.
176	n.a.	n.a.	n.a.

IC_{50} values for antiproliferative activity with different cell lines (L929: mouse adipose tissue; KB-31: human cervical carcinoma; MCF-7: human breast adenocarcinoma) after incubation for 5 days. n.a.: not active. This data was obtained by Galina Sergeev and Mark Brönstrup (Department of Chemical Biology, Helmholtz Center for Infection Research, Braunschweig, Germany).

3.4.5. Michael acceptor activity of the oxadiazole derivatives

As the synthesized compounds contain Michael acceptor moieties, they are in principle able to react with biologically importance thiols, such as cysteine residues in proteins in a Michael addition reaction. This can lead to a great variety of biological activities. To evaluate the reactivity of this type of reaction, a thiol assay developed by Amslinger and Al-Rifai can be used.[8, 102] An UV/VIS monitoring of the thiol addition reaction for compound **205** as an example (Scheme 52) is possible because of a time-dependent decay of the absorption band of the enone system. Figure 29 shows that at λ_{max} = 330 nm the absorbance was dropped after the addition of cysteamine within only 1 min this shows fast reaction of the compound **205** as Michael acceptor. After 31 min the absorbance showed large decreasing while after 1 h there is no change in the absorbance, this indicates that the reaction reached the equilibrium. The reaction was performed in Tris-HCl buffer pH 7.4/ethylene glycol 20:80 and monitored at 330 nm. The second order rate constant (k_2) for the reaction of compound **205** with cysteamine is 1.40 $M^{-1}s^{-1}$. This value displays medium reactivity comparing to previously measured chalcones (k_2 = 0.0037-5800 $M^{-1}s^{-1}$) by Amslinger and Al-Rifai. This gave promising data to study the anti-inflammatory activity for the synthesized compounds. Further investigation of these compounds may lead to the development of new potent antiproliferative and anti-inflammatory analogues.



Scheme 52: Reaction of compound **205** as thia-Michael addition with cysteamine as model thiol.

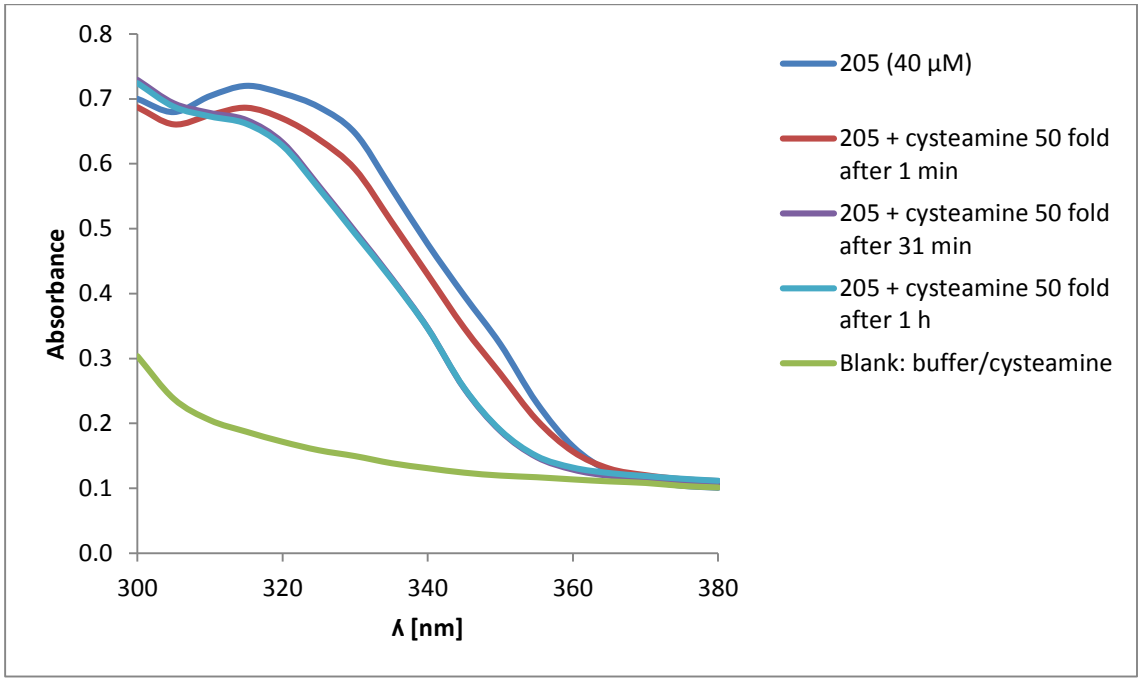


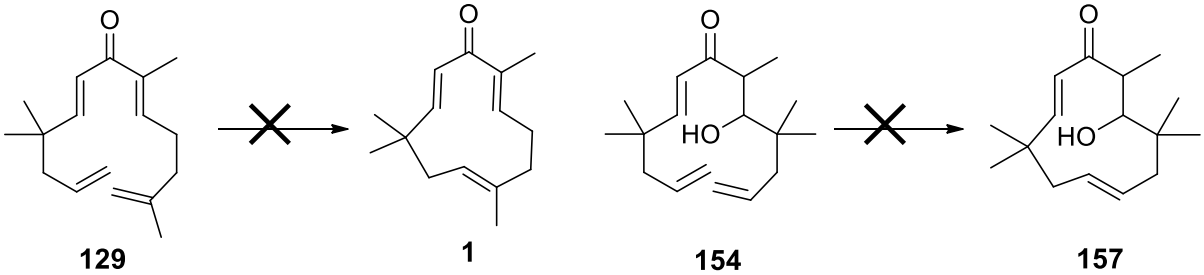
Figure 29: UV/VIS spectra for the reaction between compound **205** (40 µM) and cysteamine (50 fold) in Tris-HCl buffer pH 7.4/ethylene glycol 20:80 after different times. This data was obtained by Monika Enzinger and Sabine Amslinger.

4. Summary

In this work, a new derivative of the natural product limnophilaspiroketone called methylene-Limno-CP was synthesized, then different substituents were introduced to the α-position (Cl, Br, I, CN, Ph and *p*-NO_2-Ph) to modify the electrophilicity (Scheme 53). The key step of this synthetic sequence was the Heck reaction in order to form the enone system with an aryl group in the β-position. The synthesized derivatives did not show any reactivity as Michael acceptors with cysteamine as model thiol. Attempts to synthesize other derivatives of limnophilaspiroketone including S-Limno-CP and N-Limno-CP were not successful. Also the total synthesis of limnophilaspiroketone could not be accomplished.

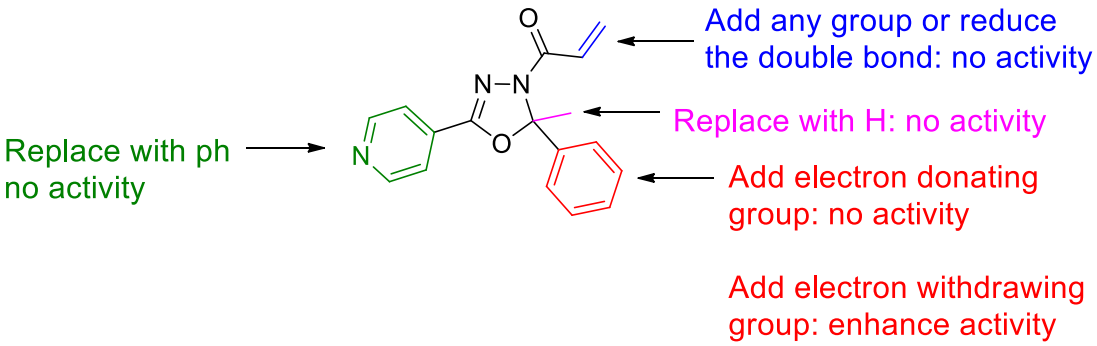
Scheme 53: Structures of α-X-methylene-Limno-CP derivatives.

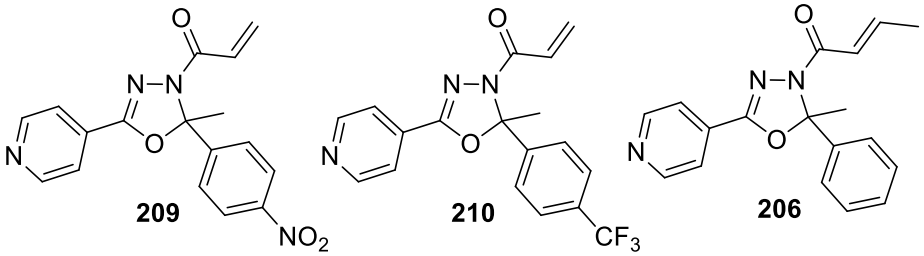
The biological activity of the natural product zerumbone (**1**) is encouraging, and therefore the ability to produce derivatives. The followed approval starts from the cheap starting material isoprenol. The key step in the sequence was transformation applying alkene ring closing metathesis (Scheme 54) to produce the cyclic compound **1**. Different ruthenium catalysts were used, but the precursor showed some rigidity, therefore the cyclization failed. A new precursor was prepared with smaller number double bonds **154** and the ring closing metathesis was tried again, but the ring closed product was not formed.



Scheme 54: Attempts to synthesize zerumbone (**1**) and zerumbone derivative **157** by ring closing metathesis.

The ability to produce derivatives such as α-X-modified enone derivatives is an important measure to get potentially biologically active electrophiles. Finally, twenty derivatives of 2,3-dihydro-1,3,4-oxadiazole and 1,3,4-oxadiazole in combination with an enone unit and a pyridinyl moiety were synthesized (Scheme 55). These derivatives were tested as antibacterial agents against different gram-positive and gram-negative bacteria, but they were not active against the tested cells. Also, the oxadiazole derivatives were screened as antiproliferative compounds against L929 (mouse adipose tissue), MCF-7 (human breast adenocarcinoma) and KB-31 (human cervical carcinoma) cells. The derivatives with an enone unit in position 2 did not show any activity, while some of the derivatives with enone unit in position 3 were active, and the most active compounds were **209** and **210**.





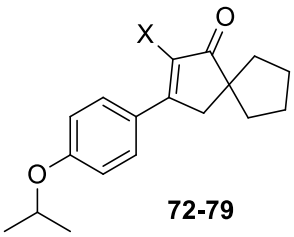
IC_{50} values for cell lines:				
	L929	3.2	5.2	n.a.
	KB-31	6.0	5.0	n.a.
	MCF-7	6.3	4.2	n.a.

Scheme 55: SAR for the 2,3-dihydro-1,3,4-oxadiazole derivatives as antiproliferative agents and the IC_{50} values (μM) against 3 cell lines for active compounds **209** and **210** and non active **206**.

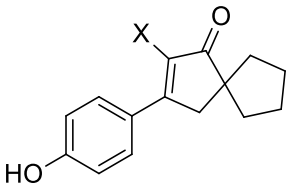
Further investigation is needed for the enone unit as a part of small molecules and its application in drug research.

Zusammenfassung

In dieser Arbeit wurde ein neues Derivat des Naturstoffes Limnophilaspiroketon, Methylen-Limnophilaspiroketon, synthetisiert, außerdem wurden verschiedene Substituenten (Cl, Br, I, Ph und *p*-NO_2-Ph) in die α-Position eingeführt um die Elektrophilie zu modifizieren (Schema 53). Der Schlüsselschritt dieser Synthesesequenz war die Heck-Reaktion um das Enonsystem mit einer Arylfunktion in β-Position aufzubauen. Die synthetisierten Derivate zeigten keine Reaktivität als Michael-Akzeptoren mit Cysteamin als Modell-Thiol. Auch konnte die Totalsynthese von Limnophilaspiroketon nicht vollendet werden.



72-79

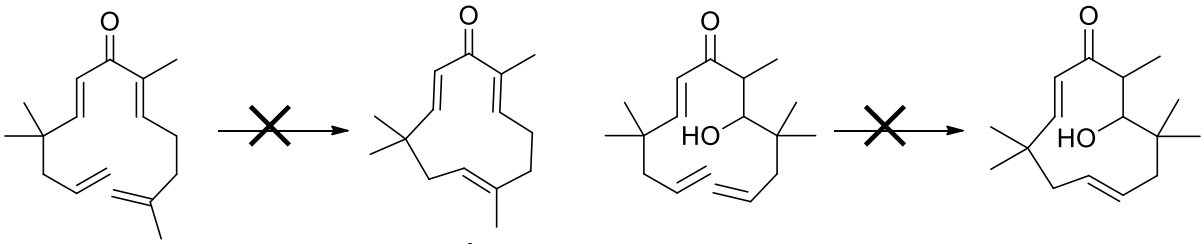


80-86

X= H, Cl, Br, I, CN, Ph and *p*-NO_2-Ph

Schema 53: Strukturen der α-X-Methylen-Limno-CP-Derivate.

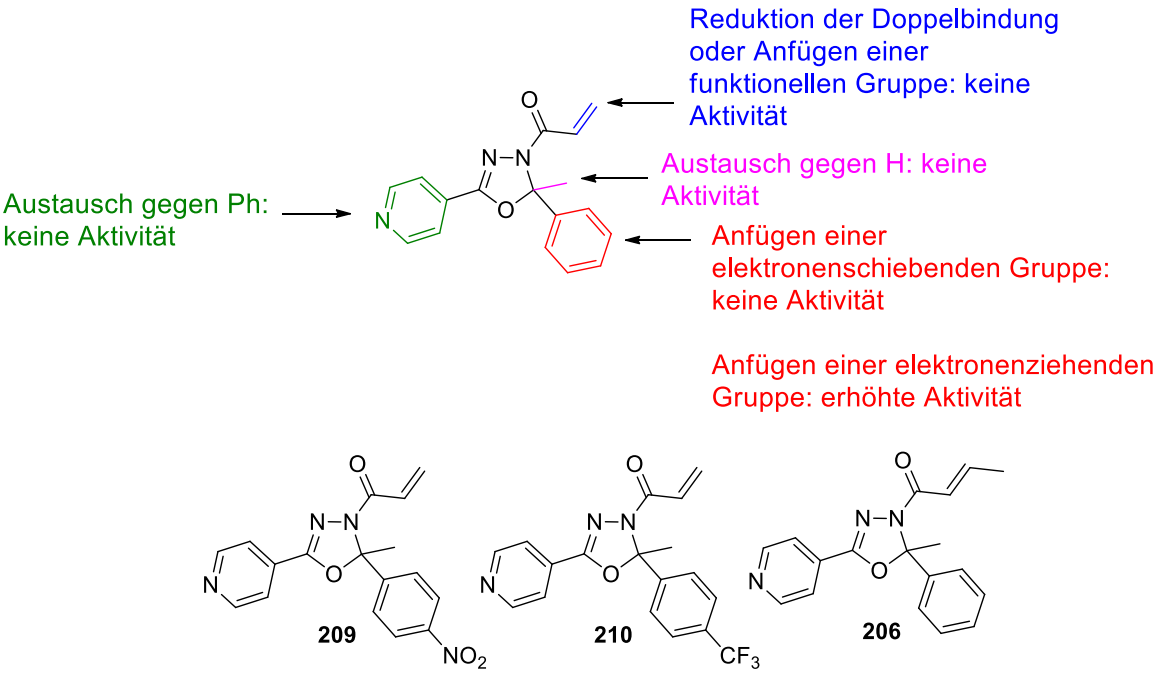
Die biologische Aktivität des Naturstoffes Zerumbon (**1**) ist vielversprechend und deshalb wurde auch die Möglichkeit angestrebt, **1** und dessen Derivate zu synthetisieren. Der verfolgte Ansatz zur Totalsynthese von Zerumbon begann mit dem kostengünstigen Ausgangsstoff Isoprenol. Der Schlüsselschritt in der Synthesesequenz war die Anwendung der Ringschlussmetathese (Schema 54) um die zyklische Verbindung **1** zu erhalten. Verschiedene Rutheniumkatalysatoren wurden verwendet, doch da die offenkettige Vorstufe **129** Starrheit im Molekül aufwies, misslang die Zyklisierung. Eine neue Vorstufe mit verringerter Anzahl an Doppelbindungen (**154**) wurde synthetisiert und ebenfalls mit Ringschlussmetathese umgesetzt, doch das zyklische Produkt **157** wurde nicht erhalten.



129 **1** **154** **157**

Schema 54: Versuche der Synthese von Zerumbon (**1**) und des Zerumbon-Derivates **157** mit Ringschlussmetathese.

Die Möglichkeit, Derivate wie zum Beispiel α-X-modifizierte Enone herzustellen, ist ein wichtiges Mittel um biologisch aktive Elektrophile zu erhalten. Im Hauptteil dieser Doktorarbeit wurden zwanzig Derivate von 2,3-Dihydro-1,3,4-Oxadiazol und 1,3,4-Oxadiazol in Kombination mit einer Enon-Einheit und einer Pyridinylgruppe synthetisiert (Schema 55). Diese Derivate wurden auf antibakterielle Aktivität gegen verschiedene gram-positive und gram-negative Bakterien getestet, allerdings konnte keine Aktivität gegen diese Zellen gefunden werden. Des Weiteren wurde die Oxadiazolderivate als antiproliferative Wirkstoffe gegen die Zelllinien L929 (Maus-Fettgewebe), MCF-7 (humanes Brust-Adenokarzinom) und KB-31 (humanes Zervixkarzinom) gescreent. Die Derivate mit der Enoneinheit in Position 2 zeigten keine Aktivität wohingegen einige Derivate mit der Enoneinheit in Position 3 aktiv waren. Die aktivsten Substanzen waren **209** und **210**.



IC_{50}-Werte für Zelllinien:	209	210	206
L929	3.2	5.2	n.a.
KB-31	6.0	5.0	n.a.
MCF-7	6.3	4.2	n.a.

Schema 55: Struktur-Wirkungsbeziehung für 2,3-Dihydro-1,3,4-Oxadiazolderivate als antiproliferative Wirkstoffe und die entsprechenden IC_{50}-Werte (µM) der aktiven Substanzen **209** und **210** sowie der inaktiven Substanz **206** gegen 3 Zellinien.

Weitere Untersuchungen der Enoneinheit als funktionelle Gruppe in niedermolekularen Verbindungen und deren Einsatz in der Wirkstoffforschung sind notwendig.

5. Experimental part

5.1. General methods and materials

All reagents were purchased from commercial sources and were used without further purification. Solvents were distilled before use. All reactions were carried out under nitrogen gas and the glassware was heated at 110 °C before use when dry conditions were necessary. The reactions were monitored by TLC on silica gel plates 60 F254 by MERCK (Darmstadt, Germany). Spots were detected under UV light (λ = 254 and 366 nm) or visualized by staining with vanillin reagent (15 g vanillin in 250 ml 95% ethanol and 2.5 ml conc. sulfuric acid). Column chromatography was performed on silica gel Geduran Si 60 (0.063-0.200 mm) by MERCK (Darmstadt, Germany), while preparative plates were prepared using silica gel 60 GF254 by MERCK (Darmstadt, Germany). Melting points were determined using BUCHI melting point B-545 (Switzerland) and were uncorrected.

^{1}H-NMR spectra were recorded on a Avance 300 (300 MHz) and Avance 400 (400 MHz) spectrometer. Chemical shifts δ are referenced to $CDCl_3$ (7.26 ppm), DMSO-d_6 (2.50 ppm) and acetone-d_6 (2.05 ppm). The NMR data were reported in ppm and the abbreviations were mentioned as: s = singlet, d = doublet, t = triplet, q = quartet, sept = septet, m = multiplet, dt = doublet of triplet, dd = doublet of doublet, coupling constants in Hz.

^{13}C NMR spectra were recorded on a Bruker Avance 300 (75 MHz) and Avance 400 (100 MHz) spectrometer and are given in ppm. Chemical shifts δ are referenced to $CDCl_3$ (77.0 ppm), DMSO-d_6 (39.4 ppm) and acetone-d_6 (29.8 ppm).

^{19}F-NMR spectra were recorded on a Bruker Avance 300 (282 MHz) spectrometer.

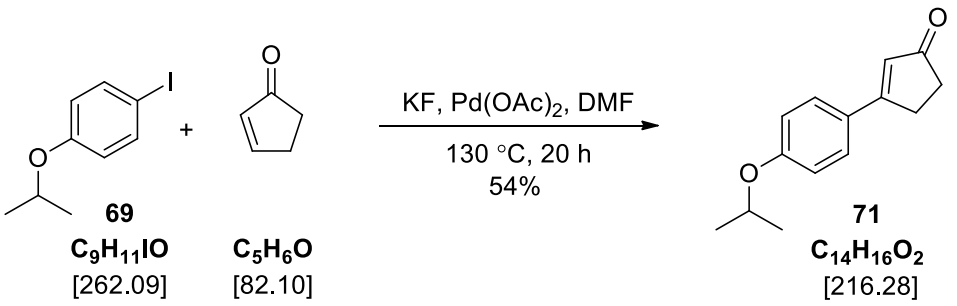
^{31}P-NMR spectra were recorded on a Bruker Avance 300 (121 MHz) spectrometer.

IR spectroscopy was carried out on a Specac Golden Gate Diamond Single Reflection ATR System Excalibur Series FTS3000MX by BIO-RAD (Munich, Germany) and samples were measured as neat compounds and the wave numbers are reported in cm^{-1}.

Mass data was obtained on Agilent Technologies 6540 UHD (Agilent, Santa Clara, USA), Finnigan MAT 95 or Thermo Quest Finnigan TSQ 7000 instruments (Bremen, Germany).

5.2. Experimental procedures

3-(4-Isopropoxyphenyl)cyclopent-2-enone (71)



69 $C_9H_{11}IO$ [262.09] + C_5H_6O [82.10] → **71** $C_{14}H_{16}O_2$ [216.28]

Aryl iodide **69** (30.0 g, 114 mmol, 1.0 eq), cyclopentenone (18.7 g, 228 mmol, 2.0 eq), KF (13.3 g, 229 mmol, 2.0 eq) and $Pd(OAc)_2$ (0.509 g, 2.27 mmol, 0.02 eq) were dissolved in 100 ml DMF under N_2. The reaction mixture was stirred at 130 °C for 20 h. Then the mixture was cold to room temperature and 50 ml H_2O was added and extracted with EtOAc (3 x 50 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel column chromatography (20 cm x 10 cm) (petroleum ether-EtOAc, 4:1) to produce aryl cyclopentenone **71** (13.4 g, 62.0 mmol, 54%) as an orange solid. $\mathbf{R_f}$ = 0.17 (SiO_2, petroleum ether-EtOAc, 4:1); $\mathbf{M_p}$ 87 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.60 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 6.92 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 6.46 (s, 1 H, C=CH), 4.63 (sept, *J* = 6.1 Hz, 1 H, CH, *i*Pr), 3.01 (m, 2 H, CH_2), 2.56 (m, 2 H, CH_2), 1.36 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

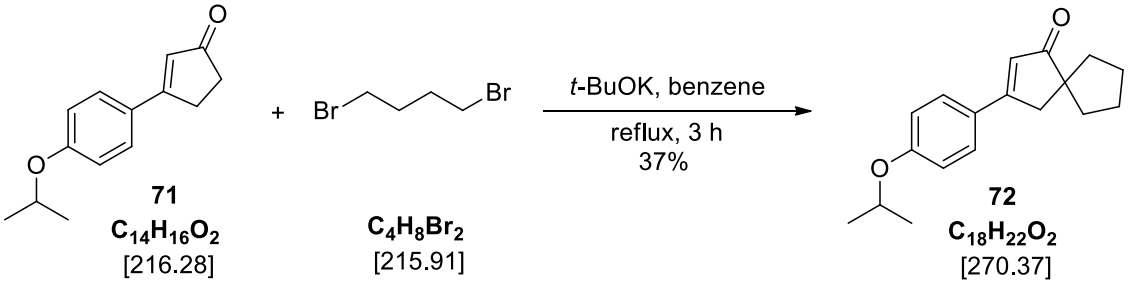
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 209.4, 173.8, 160.5, 128.6, 126.3, 125.2, 115.7, 70.0, 35.2, 28.5, 21.9 ppm.

IR (neat): 2978, 2932, 1668, 1592, 1565, 1506, 1260, 1183, 1139, 1120, 950 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 217.1 (100) $[M+H]^+$, 175.1 (5.6).

HRMS (EI-MS): *m/z* calcd for $C_{14}H_{16}O_2$: 216.1150 $[M^+]$; found 216.1151.

3-(4-Isopropoxyphenyl)spiro[4.4]non-2-en-1-one (72)



To a magnetically stirred solution of aryl cyclopentenone **71** (13.3 g, 61.4 mmol, 1.0 eq) and 1,4-dibromobutane (19.9 g, 92.1 mmol, 1.5 eq) in 80 ml of dry benzene was added *t*-BuOK (17.2 g, 153 mmol, 2.5 eq) in one portion. The mixture was refluxed for 3 h. Then the mixture was poured into 100 ml H_2O and extracted with EtOAc (3 x 50 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel column chromatography (20 cm x 5.5 cm) (petroleum ether-EtOAc, 4:1) to produce α-H-cyclopentenone **72** (6.21 g, 23.0 mmol, 37%) as a yellow solid. $\mathbf{R_f}$ = 0.44 (SiO_2, petroleum ether-EtOAc, 4:1); $\mathbf{M_p}$ 65 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.58 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 6.91 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 6.44 (s, 1 H, C=CH), 4.62 (sept, *J* = 6.1 Hz, 1 H, CH), 2.90 (d, *J* = 1.6 Hz, 2 H, CH_2), 1.98-1.84 (m, 4 H, CH_2), 1.79-165 (m, 2 H, CH_2), 1.60-1.50 (m, 2 H, CH_2), 1.36 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

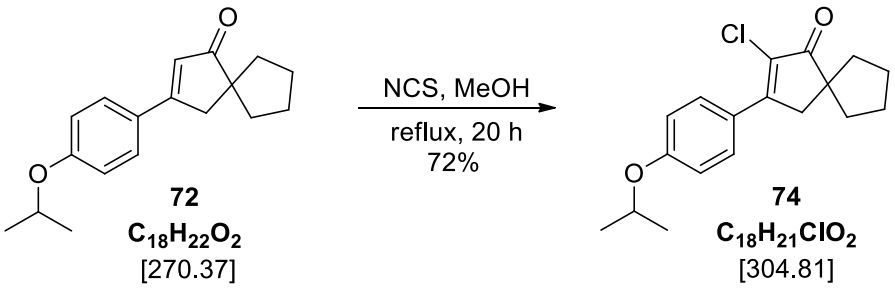
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 213.4, 170.8, 160.4, 128.6, 126.4, 123.9, 115.7, 70.0, 55.6, 45.7, 38.3, 25.5, 21.9 ppm.

IR (neat): 3065, 2974, 2932, 2869, 1675, 1587, 1506, 1421, 1338, 1311, 1258 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 271.2 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{18}H_{22}O_2$: 270.1620 $[M^+]$; found 270.1621.

2-Chloro-3-(4-isopropoxyphenyl)spiro[4.4]non-2-en-1-one (74)



72
$C_{18}H_{22}O_2$
[270.37]

74
$C_{18}H_{21}ClO_2$
[304.81]

A stirred solution of spiro compound **72** (0.102 g, 0.377 mmol, 1.0 eq) and N-chlorosuccinimide (64.5 mg, 0.485 mmol, 1.2 eq) in 20 ml MeOH was refluxed for 20 h. After removing the solvent 20 ml saturated $NaHCO_3$ solution was added and extracted with EtOAc (3 x 20 ml). The organic layer was washed with 10 ml brine solution and the combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by preparative thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 4:1) to give α-Cl-cyclopentenone **74** (82.4mg, 0.270 mmol, 72%) as a white solid. $\mathbf{R_f}$ = 0.60 (SiO_2, petroleum ether-EtOAc, 4:1); $\mathbf{M_p}$ 93 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.96 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 6.96 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 4.65 (sept, *J* = 6.1 Hz, 1 H, CH), 2.94 (s, 2 H, CH_2), 2.11-1.88 (m, 4 H, CH_2), 1.84-1.71 (m, 2 H, CH_2), 1.67-1.55 (m, 2 H, CH_2), 1.37 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

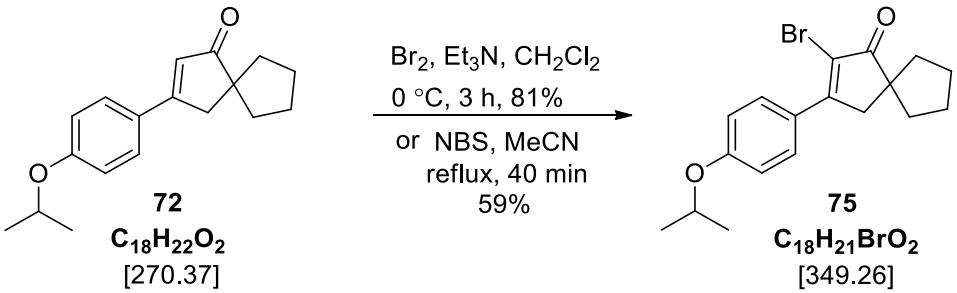
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 205.7, 160.3, 160.0, 130.1, 126.2, 125.3, 115.4, 70.0, 52.9, 45.6, 38.6, 25.4, 21.9 ppm.

IR (neat): 2968, 2937, 2877, 1702, 1601, 1562, 1506, 1259, 1190, 1128, 1108 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 305.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{18}H_{22}ClO_2$: 305.1308 $[M+H]^+$; found 305.1307.

2-Bromo-3-(4-isopropoxyphenyl)spiro[4.4]non-2-en-1-one (75)



Method A: A stirred solution of α-H-cyclopentenone **72** (0.116 g, 0.427 mmol, 1.0 eq) in 5 ml of CH_2Cl_2 was treated with a 1 M bromine solution (0.51 ml, 0.51 mmol, 1.2 eq) in CH_2Cl_2 at 0 °C. After 1 h a solution of Et_3N (84.3 mg, 0.835 mmol, 3.0 eq) in 5 ml CH_2Cl_2 was added dropwise. The solution was stirred 1 h at 0 °C and 1 h at room temperature before it was diluted with 10 ml of H_2O and extracted with CH_2Cl_2 (3 x 20 ml). The combined organic layers were washed with 10 ml of brine solution and dried over $MgSO_4$ followed by concentration in vacuo. The product was purified by preparative thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 4:1) and gave α-Br-cyclopentenone **75** (0.120 g, 0.344 mmol, 81%) as a yellow solid.

Method B: To a solution of α-H-cyclopentenone **72** (1.53 g, 5.67 mmol, 1.0 eq) in 20 ml of MeCN was added N-bromosuccinimide (1.11 g, 6.24 mmol, 1.1 eq) in portions at 0 °C. After 30 min saturated $NaHCO_3$ (20 ml) was added and the mixture was extracted with EtOAc (3 x 25 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel thin layer chromatography (petroleum ether-EtOAc, 4:1) to afford α-Br-cyclopentenone **75** (1.17 g, 3.35 mmol, 59%) as a yellow solid. $\mathbf{R_f}$ = 0.58 (SiO_2, petroleum ether-EtOAc, 4:1); $\mathbf{M_p}$ 107 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.98 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 6.96 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 4.65 (sept, *J* = 6.1 Hz, 1 H, CH), 2.96 (s, 2 H, CH_2), 2.09-1.86 (m, 4 H, CH_2), 1.81-1.67 (m, 2 H, CH_2), 1.64-1.55 (m, 2 H, CH_2), 1.38 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

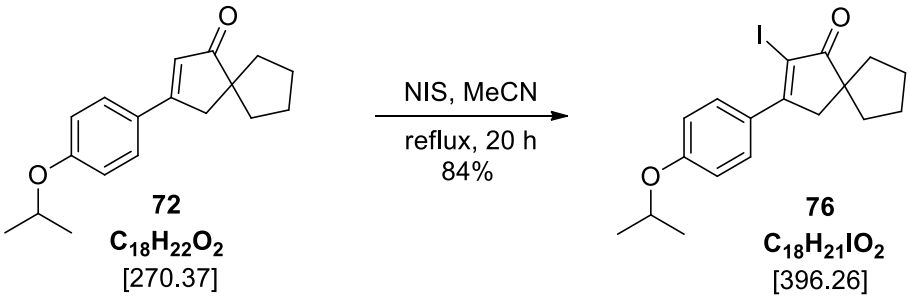
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 206.2, 163.8, 160.3, 130.0, 125.8, 117.6, 115.3, 70.1, 53.0, 47.5, 38.7, 25.4, 21.9 ppm.

IR (neat): 2969, 2934, 2875, 1700, 1600, 1561, 1504, 1258, 1188, 1106, 949 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 349.1 (100) $[M+H]^+$, 255.3 (3.2), 254.2 (16).

HRMS (EI-MS): *m/z* calcd for $C_{18}H_{22}BrO_2$: 349.0803 $[M+H]^+$; found 349.0802.

2-Iodo-3-(4-isopropoxyphenyl)spiro[4.4]non-2-en-1-one (76)



72
$C_{18}H_{22}O_2$
[270.37]

76
$C_{18}H_{21}IO_2$
[396.26]

A stirred solution of α-H-cyclopentenone **72** (0.300 g, 1.11 mmol, 1.0 eq) and N-iodosuccinimide (0.265 g, 1.18 mmol, 1.1 eq) in 25 ml of MeCN was refluxed for 20 h. Saturated $NaHCO_3$ solution 10 ml was added to the mixture and the aqueous phase was extracted with EtOAc (3 x 20 ml). The combined organic layers were washed with 20 ml of brine solution followed by drying over $MgSO_4$. The solvent was removed in vacuo and the product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 4:1) afforded α-I-cyclopentenone **76** (0.370 g, 0.934 mmol, 84%) as a yellow solid. $\mathbf{R_f}$ = 0.61 (SiO_2, petroleum ether-EtOAc, 4:1); $\mathbf{M_p}$ 124 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.93 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 6.96 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 4.65 (sept, *J* = 6.1 Hz, 1 H, CH), 3.05 (s, 2 H, CH_2), 2.08-1.85 (m, 4 H, CH_2), 1.81-1.67 (m, 2 H, CH_2), 1.66-1.51 (m, 2 H, CH_2), 1.38 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

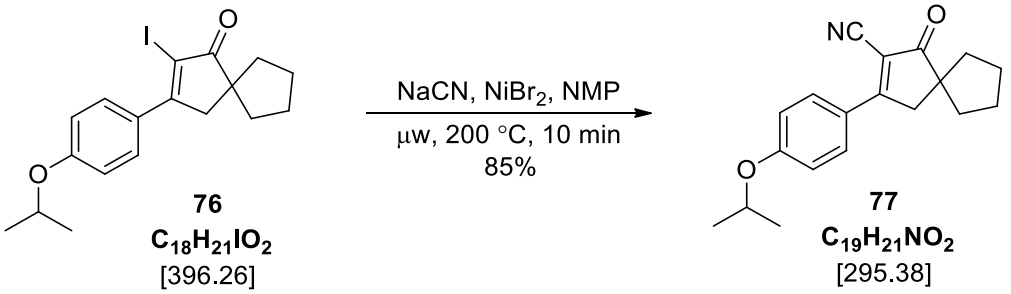
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 208.1, 170.2, 160.2, 129.7, 126.9, 115.2, 96.9, 70.0, 53.0, 49.8, 38.7, 25.3, 21.9 ppm.

IR (neat): 2954, 2911, 2860, 1689, 1598, 1549, 1503, 1419, 1253, 1185, 1107 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 397.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{18}H_{22}IO_2$: 397.0664 $[M+H]^+$; found 397.0660.

3-(4-Isopropoxyphenyl)-1-oxospiro[4.4]non-2-ene-2-carbonitrile (77)



76
$C_{18}H_{21}IO_2$
[396.26]

77
$C_{19}H_{21}NO_2$
[295.38]

A microwave glass tube with a magnetic stirring bar was placed α-I-cyclopentenone **76** (0.117 g, 0.317 mmol, 1.0 eq), sodium cyanide (31.1 mg, 0.635 mmol, 2.0 eq) and nickel(II) bromide (71.0 mg, 0.325 mmol, 1.0 eq), in 4 ml *N*-methyl-2-pyrrolidinone. The mixture was heated at 200 °C for 10 min, then the green mixture was cold to room temperature and diluted with 20 ml H_2O followed by extraction with EtOAc (3 x 20 ml). The combined organic layers were washed with 20 ml brine solution followed by drying over $MgSO_4$. The solvent was removed in vacuo and the product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 4:1) to afford α-CN-cyclopentenone **77** (73.7 mg, 0.250 mmol, 85%) as a yellow solid **R_f** = 0.42 (SiO_2, petroleum ether-EtOAc, 4:1); **Mp** 121 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.04 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 6.98 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 4.69 (sept, *J* = 6.1 Hz, 1 H, CH), 3.10 (s, 2 H, CH_2), 2.10-1.86 (m, 4 H, CH_2), 1.83-1.67 (m, 2 H, CH_2), 1.64-1.58 (m, 2 H, CH_2), 1.39 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

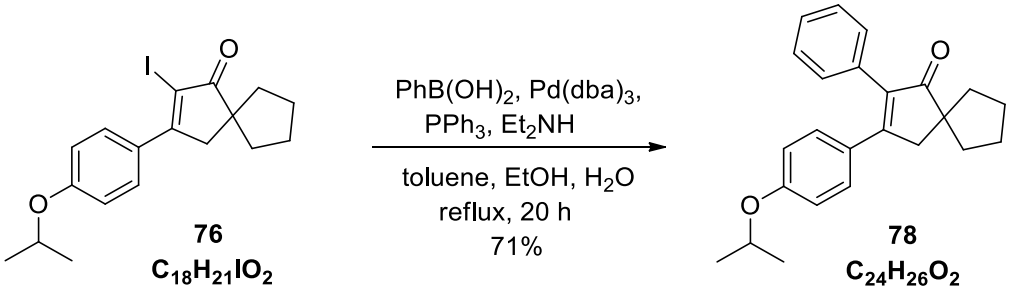
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 206.7, 176.6, 162.6, 130.7, 124.1, 115.9, 114.8, 108.1, 70.5, 54.9, 46.3, 38.5, 25.5, 21.8 ppm.

IR (neat): 2947, 2898, 2868, 2214, 1699, 1600, 1582, 1556, 1507, 1342, 1303 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 296.2 (100) $[M+H]^+$, 121.1 (9.7).

HRMS (EI-MS): *m/z* calcd for $C_{19}H_{21}NO_2$: 295.1572 $[M^+]$; found 295.1575.

3-(4-Isopropoxyphenyl)-2-phenylspiro[4.4]non-2-en-1-one (78)



76
$C_{18}H_{21}IO_2$
[396.26]

78
$C_{24}H_{26}O_2$
[346.46]

To a solution of α-I-cyclopentenone **76** (0.201 g, 0.507 mmol, 1.0 eq) in a 4 ml mixture of toluene-EtOH 3:1 were added $Pd_2(dba)_3 \cdot CHCl_3$ (30.0 mg, 0. 0290 mmol, 0.05 eq), triphenylphosphine (13.3 mg, 0.0500 mmol, 0.1 eq) and phenyl boronic acid (81.1 mg, 0.665 mmol, 1.2 eq). After stirring for 10 min 0.08 ml Et_2NH and 1 ml H_2O were added and the mixture was refluxed for 20 h. Then the mixture was extracted with EtOAc (3 x 10 ml). The combined organic layers were washed with 10 ml brine solution and dried over $MgSO_4$. The solvent was removed in vacuo and the product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 4:1) to give α-Ph-cyclopentenone **78** (0.125 g, 0.361 mmol, 71%) as an orange solid. **R_f** = 0.60 (SiO_2, petroleum ether-EtOAc, 4:1); **M_p** 95 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.39-7.22 (m, 7 H, CH, Ar), 6.75 (d, *J* = 8.9 Hz, 2H, CH, Ar), 4.55 (sept, *J* = 6.1 Hz, 1 H, CH), 2.95 (s, 2 H, CH_2), 2.09-2.01 (m, 2 H, CH_2), 1.97-1.88 (m, 2 H, CH_2), 1.86-1.74 (m, 2 H, CH_2), 1.70-1.58 (m, 2 H, CH_2), 1.33 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

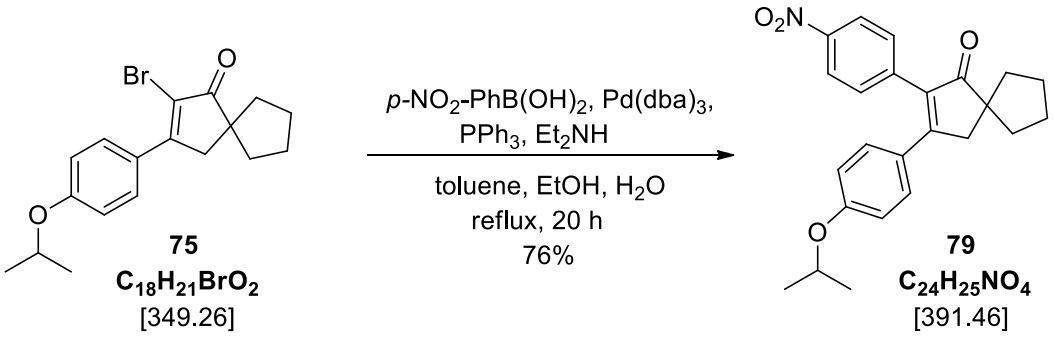
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 211.3, 164.4, 159.3, 136.7, 133.3, 129.9, 129.5, 128.4, 127.5, 127.5, 115.1, 69.8, 54.7, 46.8, 38.5, 25.6, 21.9 ppm.

IR (neat): 2978, 2934, 2869, 1678, 1601, 1563, 1508, 1490, 1350, 1293, 1250 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 347.2 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{24}H_{27}O_2$: 347.2011 $[M+H]^+$; found 347.2013.

3-(4-Isopropoxyphenyl)-2-(4-nitrophenyl)spiro[4.4]non-2-en-1-one (79)



To a stirred solution of α-Br-cyclopentenone **75** (0.206 g, 0.591 mmol, 1.0 eq) in a 4 ml mixture of toluene-EtOH 3:1 were added $Pd_2(dba)_3 \cdot CHCl_3$ (30.3 mg, 0.0293 mmol, 0.05 eq), triphenylphosphine (15.5 mg, 0.0590 mmol, 0.1 eq) and *p*-NO_2-phenyl boronic acid (0.118 g, 0.709 mmol, 1.2 eq). After stirring for 10 min 0.08 ml Et_2NH and 1 ml H_2O were added and the mixture was refluxed for 20 h. Then the mixture was extracted with EtOAc (3 x 10 ml). The combined organic layers were washed with 10 ml brine solution and dried over $MgSO_4$. The solvent was removed in vacuo and the product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 4:1) afforded α-(*p*-NO_2-C_6H_4)-cyclopentenone **79** (0.175 g, 0.447 mmol, 76%) as a yellow solid. $\mathbf{R_f}$ = 0.47 (SiO_2, petroleum ether-EtOAc, 4:1); $\mathbf{M_p}$ 99 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.20 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 7.47(d, *J* = 8.9 Hz, 2 H, CH, Ar), 7.24 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 6.78 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 4.56 (sept, *J* = 6.1 Hz, 1 H, CH), 2.99 (s, 2 H, CH_2), 2.11-1.87 (m, 4 H, CH_2), 1.85-1.74 (m, 2 H, CH_2), 1.70-1.57 (m, 2 H, CH_2), 1.34 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 210.5, 167.6, 159.9, 147.1, 140.4, 134.4, 130.8, 130.0, 126.6, 123.8, 115.4, 70.0, 55.3, 47.1, 38.7, 25.6, 21.9 ppm.

IR (neat): 3064, 2983, 2869, 1681, 1599, 1510, 1341, 1292, 1254, 1186, 1107 cm^{-1}.

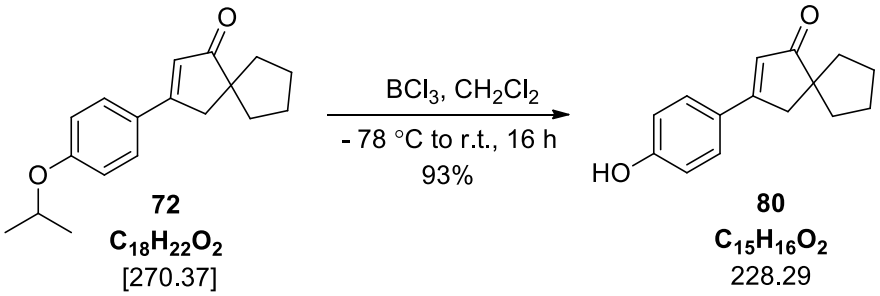
MS (EI, 70 eV): *m/z* (%) = 392.2 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{24}H_{26}NO_4$: 392.1856 $[M+H]^+$; found 392.1858.

General procedure for the deprotection of isopropyl ethers 80-86:

α-(X)-Cyclopentenones **72-79** (38.1-102 mg, 0.125 mmol-0.257 mmol, 1.0 eq) were dissolved in 1 ml CH_2Cl_2 and 6.0 eq of 1 M solution of BCl_3 in n-hexane were added slowly at -78 °C to the solution. The solution was warmed to room temperature and stirred until the reaction was complete (45 min to 16 h). Then THF (0.5 ml) and 1 M aqueous HCl (0.5 ml) were added and the solvent was removed before adding 10 ml of H_2O. The mixture was extracted with EtOAc (3 x 20 ml) and the combined organic layers were dried over $MgSO_4$. Then the solvent was removed in vacuo and the produced solid was washed with CH_2Cl_2 (2 x 2 ml) to produce the metyelen-Limno-CP products **80-86**.

3-(4-Hydroxyphenyl)spiro[4.4]non-2-en-1-one (80)



α-H-cyclopentenone **72** (56.4 mg, 0.209 mmol, 1.0 eq) gave after 16 h α-H-methylene-Limno-CP (**80**) (44.3 mg, 0.194 mmol, 93%) as a yellow solid. $\mathbf{R_f}$ = 0.19 (SiO_2, petroleum ether-EtOAc, 7:3); **Mp** 192 °C.

^{1}H-NMR (300 MHz, acetone-d_6): δ = 9.03 (s, 1 H, OH), 7.68 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 6.94 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 6.43 (s, 1 H, CH), 2.96 (s, 2 H, CH_2), 1.89-1.72 (m, 6 H, CH_2), 1.62-1.52 (m, 2 H, CH_2) ppm.

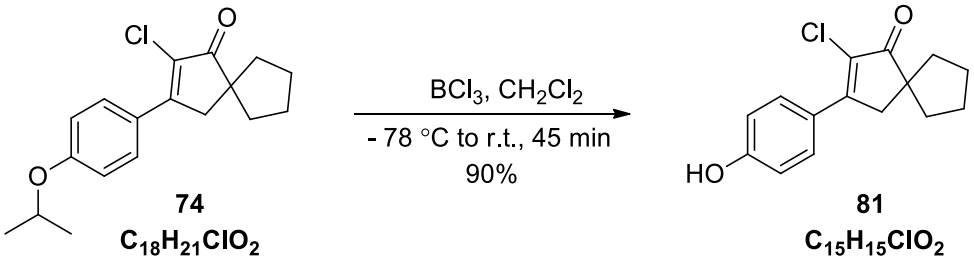
^{13}C-NMR (75 MHz, acetone-d_6): δ = 212.4, 171.6, 161.1, 129.8, 126.8, 123.8, 116.5, 56.0, 46.0, 38.8, 26.1 ppm.

IR (neat): 3151, 3031, 2949, 2917, 2864, 2828, 1659, 1602, 1571, 1510, 1335 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 229.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{15}H_{16}O_2$: 228.1150 $[M^+]$; found 228.1138.

2-Chloro-3-(4-hydroxyphenyl)spiro[4.4]non-2-en-1-one (81)



74
$C_{18}H_{21}ClO_2$
[304.81]

81
$C_{15}H_{15}ClO_2$
[262.73]

α-Cl-cyclopentenone **74** (38.1 mg, 0.125 mmol, 1.0 eq) gave after 45 min α-Cl-methylene-Limno-CP (**81**) (29.5 mg, 0.112 mmol, 90%) as a yellow solid. **R_f** = 0.38 (SiO_2, petrolum ether-EtOAc, 7:3); **M_p** 208 °C.

^{1}H-NMR (300 MHz, acetone-d_6): δ = 9.12 (s, 1 H, OH), 7.98 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 7.00 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 3.06 (s, 2 H, CH_2), 1.94-1.73 (m, 6 H, CH_2), 1.72-1.57 (m, 2 H, CH_2) ppm.

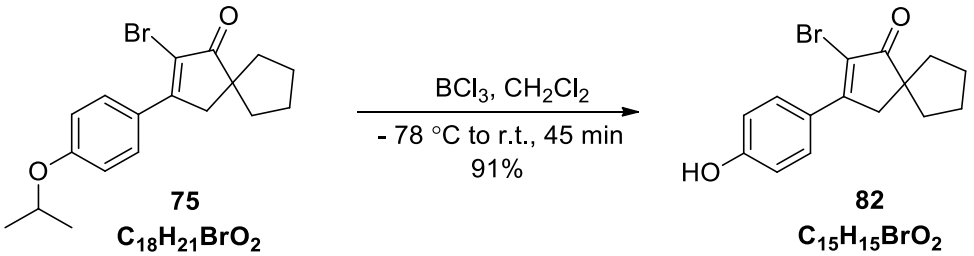
^{13}C-NMR (75 MHz, acetone-d_6): δ = 205.2, 161.6, 161.1, 131.2, 125.8, 125.7, 116.4, 53.5, 45.9, 38.9, 25.9 ppm.

IR (neat): 3210, 2946, 2924, 2868, 1679, 1607, 1561, 1508, 1266, 1229, 1181 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 263.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{15}H_{15}ClO_2$: 262.0761 $[M^+]$; found 262.0754.

2-Bromo-3-(4-hydroxyphenyl)spiro[4.4]non-2-en-1-one (82)



75
$C_{18}H_{21}BrO_2$
[349.26]

82
$C_{15}H_{15}BrO_2$
307.18

α-Br-cyclopentenone **75** (50.0 mg, 0.143 mmol, 1.0 eq) gave after 45 min α-Br-methylene-Limno-CP (**82**) (39.8 mg, 0.130 mmol, 91%) as a yellow solid. **R_f** = 0.38 (SiO_2, petroleum ether-EtOAc, 7:3); **M_p** 201 °C.

^{1}H-NMR (300 MHz, acetone-d_6): δ = 9.11 (s, 1 H, OH), 8.00 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 6.99 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 3.09 (s, 2 H, CH_2), 1.88-1.79 (m, 6 H, CH_2), 1.71-1.59 (m, 2 H, CH_2) ppm.

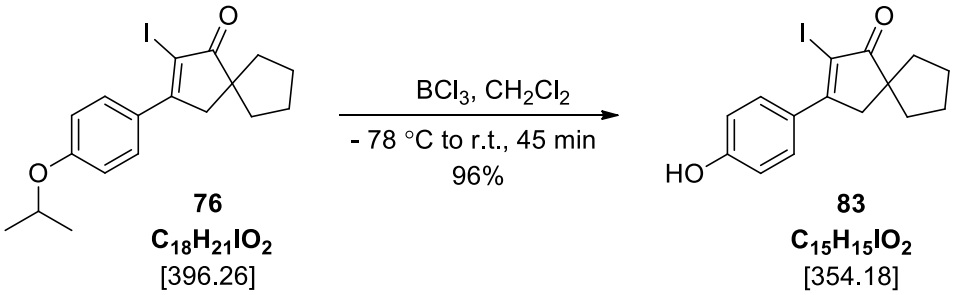
^{13}C-NMR (75 MHz, acetone-d_6): δ = 205.7, 165.4, 161.1, 131.2, 126.3, 117.3, 116.3, 53.6, 47.8, 39.0, 25.9 ppm.

IR (neat): 3226, 2946, 2924, 2868, 1678, 1604, 1556, 1504, 1452, 1263, 1226 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 307.0 (100) $[M+H]^+$, 223.0 (3.1), 215.0 (4.8), 214.0 (4.5), 121.1 (5.7).

HRMS (EI-MS): *m/z* calcd for $C_{15}H_{16}BrO_2$: 307.0334 $[M+H]^+$; found 307.0333.

3-(4-Hydroxyphenyl)-2-iodospiro[4.4]non-2-en-1-one (83)



76
$C_{18}H_{21}IO_2$
[396.26]

83
$C_{15}H_{15}IO_2$
[354.18]

α-I-cyclopentenone **76** (102 mg, 0.257 mmol, 1.0 eq) gave after 45 min α-I-methylene-Limno-CP (**83**) (87.2 mg, 0.246, 96%) as a yellow solid. **R_f** = 0.41 (SiO_2, petroleum ether-EtOAc, 7:3); **M_p** 198 °C.

^{1}H-NMR (300 MHz, acetone-d_6): δ = 9.08 (s, 1 H, OH), 7.96 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 6.99 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 3.17 (s, 2 H, CH_2), 1.89-1.79 (m, 6 H, CH_2), 1.68-1.58 (m, 2 H, CH_2) ppm.

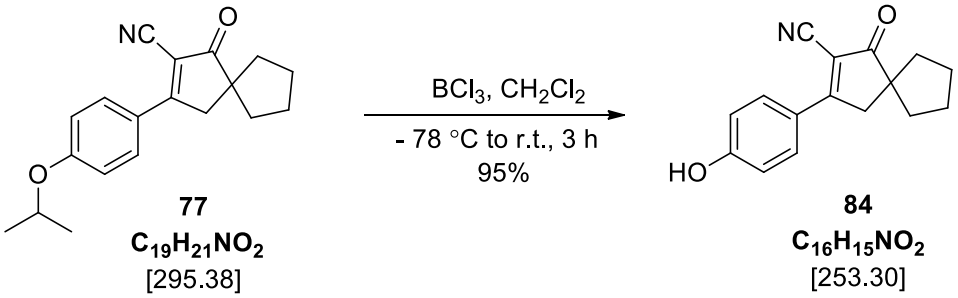
^{13}C-NMR (75 MHz, acetone-d_6): δ = 207.9, 171.8, 160.9, 130.9, 127.5, 116.1, 96.7, 53.6, 50.1, 39.1, 25.9 ppm.

IR (neat): 3232, 2948, 2922, 2867, 1673, 1603, 1548, 1499, 1451, 1261, 1224 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 355.0 (100) $[M+H]^+$, 246.0 (4.11), 238.0 (8.3), 121.1 (13.8).

HRMS (EI-MS): *m/z* calcd for $C_{15}H_{15}IO_2$: 354.0117 $[M^+]$; found 354.0112.

3-(4-Hydroxyphenyl)-1-oxospiro[4.4]non-2-ene-2-carbonitrile (84)



α-CN-cyclopentenone **77** (66.6 mg, 0.225 mmol, 1.0 eq) gave after 3 h α-CN-methylene-Limno-CP (**84**) (54.3 mg, 0.214 mmol, 95%) as a yellow solid. $\mathbf{R_f}$ = 0.15 (SiO_2, petroleum ether-EtOAc, 7:3); $\mathbf{M_p}$ 239 °C.

^{1}H-NMR (300 MHz, acetone-d_6): δ = 9.60 (s, 1 H, OH), 8.08 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 7.06 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 3.28 (s, 2 H, CH_2), 1.91-1.79 (m, 6 H, CH_2), 1.72-1.65 (m, 2 H, CH_2) ppm.

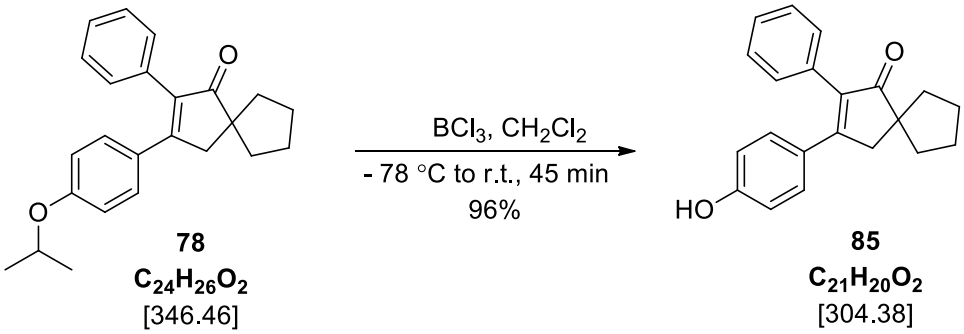
^{13}C-NMR (75 MHz, acetone-d_6): δ = 207.3, 178.8, 163.4, 131.9, 124.9, 117.0, 115.7, 107.9, 55.7, 46.6, 38.8, 26.0 ppm.

IR (neat): 3209, 2957, 2870, 2232, 1699, 1605, 1559, 1512, 1340, 1277, 1232 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 254.1 (100) $[M+H]^+$, 130.2 (4.4), 121.1 (3.9).

HRMS (EI-MS): *m/z* calcd for $C_{16}H_{15}NO_2$: 253.1103 [M^+]; found 253.1085.

3-(4-Hydroxyphenyl)-2-phenylspiro[4.4]non-2-en-1-one (85)



α-Ph-cyclopentenone **78** (38.9 mg, 0.112 mmol, 1.0 eq) gave after 45 min α-Ph-methylene-Limno-CP (**85**) (32.8 mg, 0.108 mmol, 96%) as a yellow solid. $\mathbf{R_f}$ = 0.38 (SiO_2, petroleum ether-EtOAc, 7:3); $\mathbf{M_p}$ 207 °C.

^{1}H-NMR (300 MHz, acetone-d_6): δ = 8.91(s, 1 H, OH), 7.37-7.15 (m, 7 H, CH, Ar), 6.75 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 3.02 (s, 2 H, CH_2), 1.96-1.77 (m, 6 H, CH_2), 1.69-1.59 (m, 2 H, CH_2) ppm.

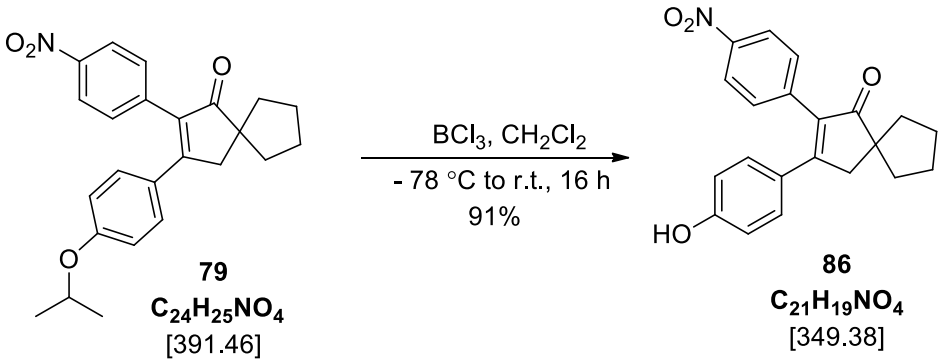
^{13}C-NMR (75 MHz, acetone-d_6): δ = 210.9, 165.7, 160.0, 137.0, 135.0, 131.0, 130.5, 129.0, 128.2, 127.8, 116.0, 55.3, 47.2, 39.0, 26.2 ppm.

IR (neat): 3236, 2950, 2861, 1661, 1601, 1568, 1515, 1485, 1349, 1284, 1226 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 305.2 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{21}H_{21}O_2$: 305.1542 $[M+H]^+$; found 305.1544.

3-(4-Hydroxyphenyl)-2-(4-nitrophenyl)spiro[4.4]non-2-en-1-one (86)



α-(*p*-NO_2-C_6H_4)-cyclopentenone **79** (64.1 mg, 0.164 mmol, 1.0 eq) gave after 16 h α-(*p*-NO_2-C_6H_4)-methylene-Limno-CP (**86**) (52.2 mg, 0.149 mmol, 91%) as a yellow solid. $\mathbf{R_f}$ = 0.72 (SiO_2, petroleum ether-EtOAc, 1:1); $\mathbf{M_p}$ 254 °C.

^{1}H-NMR (300 MHz, acetone-d_6): δ = 8.23 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 7.51 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 7.31 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 6.81 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 3.10 (s, 2 H, CH_2), 1.99-1.65 (m, 8 H, CH_2) ppm.

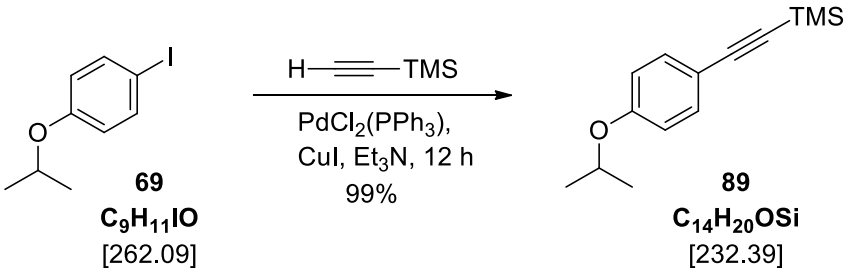
^{13}C-NMR (75 MHz, acetone-d_6): δ = 210.2, 168.4, 160.5, 147.9, 142.0, 135.0, 132.0, 131.3, 127.4, 124.3, 116.5, 62.9, 55.6, 47.5, 39.1, 26.2 ppm.

IR (neat): 3212, 2936, 2868, 1658, 1599, 1572, 1507, 1340, 1283, 1224, 1173 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 350.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{21}H_{19}NO_4$: 349.1309 $[M^+]$; found 349.1312.

((4-Isopropoxyphenyl)ethynyl)trimethylsilane (89)



69
$C_9H_{11}IO$
[262.09]

89
$C_{14}H_{20}OSi$
[232.39]

Aryl iodide **69** (9.02 g, 34.4 mmol, 1.0 eq), ethynyl trimethyl silane (7.53 g, 76.6 mmol, 2.2 eq), CuI (0.170 g, 894 mmol, 0.025 eq) and $PdCl_2(PPh_3)_2$ (0.303 g, 0.431 mmol, 0.012 eq) were added to 30 ml of dry Et_3N. N_2 gas was bubbled through the mixture at room temperature for 5 min and stirred for 12 h at the same conditions. After complete of the reaction the solvent was evaporated and 25 ml water was added followed by extraction with CH_2Cl_2 (3 x 20 ml). The combined organic layers were washed with 20 ml 1M aqueous HCl, then 20 ml brine solution. The organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel column chromatography (15 cm x 5.5 cm) using petroleum ether to afford alkyne **89** (7.93 g, 34.2 mmol, 99%) as a pale yellow oil. $\mathbf{R_f}$ = 0.63 (SiO_2, petroleum ether-EtOAc, 9:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.38 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 6.79 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 4.55 (sept, *J* = 6.1 Hz, 1 H, CH, *i*Pr), 1.32 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr), 0.23 (s, 9 H, $(CH_3)_3Si$) ppm.

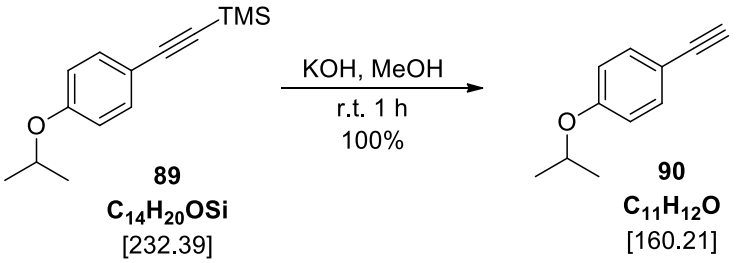
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 158.1, 133.4, 115.5, 114.8, 106.3, 92.2, 69.8, 21.9, 0.1 ppm.

IR (neat): 2960, 2151, 1604, 1504, 1287, 1246, 1181, 1118, 952, 830, 757 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 175.1 (100), 190.1 (22), 232.1 (19) [M^+].

HRMS (EI-MS): *m/z* calcd for $C_{14}H_{20}OSI$: 232.1283 [M^+]; found 232.1285.

1-Ethynyl-4-isopropoxybenzene (90)



89
$C_{14}H_{20}OSi$
[232.39]

90
$C_{11}H_{12}O$
[160.21]

Alkyne **89** (7.80 g, 33.6 mmol, 1.0 eq) was dissolved in 10 ml of MeOH, then a solution of KOH (3.05 g, 54.4 mmol, 1.6 eq) in 50 ml MeOH-CH_2Cl_2 (1:1) was added to the mixture which stirred for 1 h at room temperature. After that the solvent was evaporated in vacuo followed by adding 50 ml 1M aqueous HCl. The mixture was extracted with CH_2Cl_2 (3 x 25 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel column chromatography (5 cm x 5.5 cm) using petroleum ether to produce the terminal alkyne **90** (5.38 g, 33.6, 100%) as a yellow oil. $\mathbf{R_f}$ = 0.53 (SiO_2, petroleum ether-EtOAc, 9:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.41 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 6.82 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 4.56 (sept, *J* = 6.1 Hz, 1 H, CH, *i*Pr), 2.99 (s, 1 H, alkyne), 1.34 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 158.3, 133.6, 115.6, 113.7, 83.7, 75.6, 96.8, 21.9 ppm.

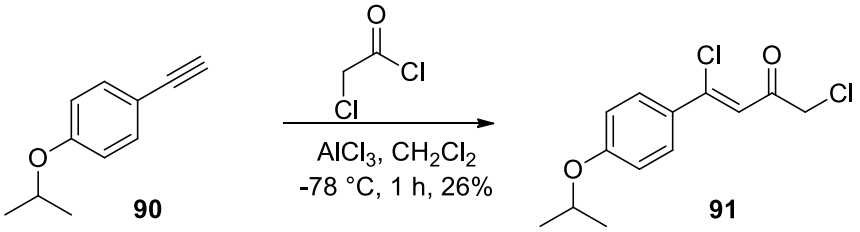
The ^{1}H NMR and ^{13}C NMR spectral data is in accordance with the literature.[103]

IR (neat): 3287, 2980, 1604, 1503, 1285, 1234, 1167, 1116, 951, 831 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 98.1 (22), 118.0 (100), 160.1 (37) [M^+].

HRMS (EI-MS): *m/z* calcd for $C_{11}H_{12}O$: 160.0888 [M^+]; found 160.0887.

1,4-Dichloro-4-(4-isopropoxyphenyl)but-3-en-2-one (91)



90
$C_{11}H_{12}O$
[160.21]

91
$C_{13}H_{14}Cl_2O_2$
[273.16]

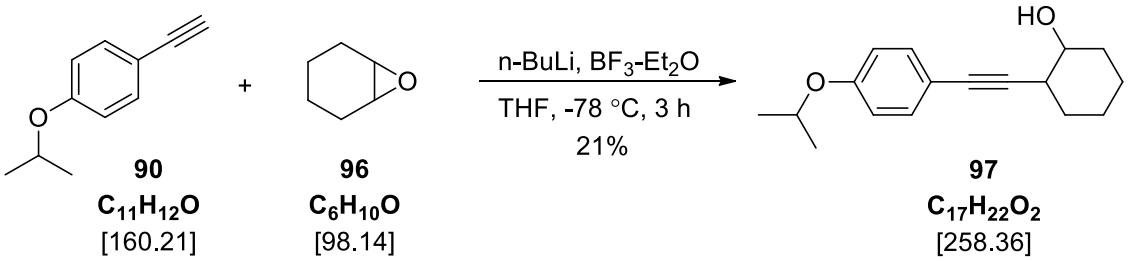
To a stirred suspension of $AlCl_3$ (40.1 mg, 0.301 mmol, 1.1 eq) in dry 2 ml CH_2Cl_2 at -78 °C, was added alkyne **90** (50.1 mg, 0.313 mmol, 1.0 eq) and chloroacetyl chloride (38.1 mg, 0.270 mmol, 1.0 eq) dropwise over 10 min under N_2. The mixture was warmed to room temperature and stirred for 1 h, followed by pouring it into 10 ml of ice cold brine solution and stirred for 2 h. After that the mixture was extracted with CH_2Cl_2 (3 x 10 ml). The product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 4:1) to give dichloro compound **91** (22.4 mg, 0.0820 mmol, 26%) as a yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.69 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 7.01 (s, 1 H, CH), 6.90 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 4.62 (sept, *J* = 6.1 Hz, 1 H, CH, *i*Pr), 4.32 (s, 2 H, CH_2Cl) 1.36 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 189.3, 160.8, 147.1, 129.3, 128.5, 117.4, 115.5, 70.2, 49.3, 21.9 ppm.

MS (EI, 70 eV): *m/z* (%) = 118.1 (13.4), 181.0 (100), 183.1(29), 272.0 [M^+].

2-((4-Isopropoxyphenyl)ethynyl)cyclohexanol (97)



90
$C_{11}H_{12}O$
[160.21]

96
$C_6H_{10}O$
[98.14]

97
$C_{17}H_{22}O_2$
[258.36]

To a stirred solution of alkyne **90** (59.5 mg, 0.371 mmol, 1.0 eq) in 15 ml of dry THF at -78 °C was added dropwise a solution of n-BuLi (302 µl, 1.6 M, 0.483 mmol, 1.3 eq). The reaction solution was stirred at -78 °C for 30 min, followed by treated

dropwise with BF_3-Et_2O (0.120 ml, 50%, 0.483 mmol, 1.3 eq). Then after 15 min at -78 °C, the epoxide solution **96** (43.7 mg, 0.445 mmol, 1.2 eq) in 5 ml of dry THF was added with stirrer at -78 °C for 2 h. The reaction was quenched with 10 ml saturated NH_4Cl solution and extracted with EtOAc (3 x 10 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 4:1) to give alcohol **97** (20.3 mg, 0.0786 mmol, 21%) as a colorless oil. $\mathbf{R_f}$ = 0.44 (SiO_2, petroleum ether-EtOAc, 4:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.32 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 6.79 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 4.53 (sept, *J* = 6.1 Hz, 1 H, CH, *i*Pr), 3.52 (m, 1 H, CH), 2.51-2.36 (m, 2 H, CH_2), 2.11-2.01 (m, 2 H, CH_2), 1.86-1.63 (m, 2 H, CH_2), 1.55-1.40 (m, 1 H, CH_2), 1.37-1.16 (m, 9 H, CH_3, *i*Pr and CH_2) ppm.

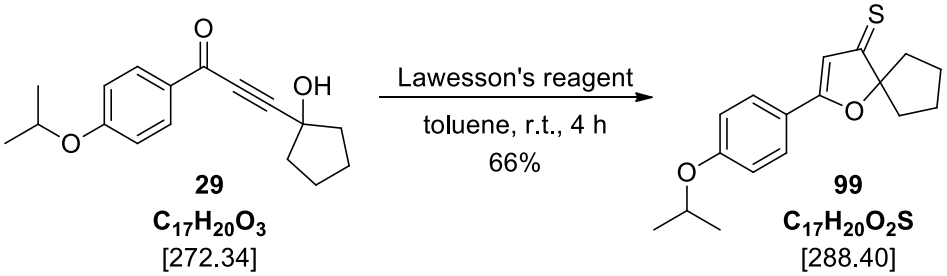
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 157.6, 133.0, 115.6, 115.0, 89.0, 82.5, 73.6, 69.8, 39.6, 33.0, 31.1, 24.9, 24.2, 21.9 ppm.

IR (neat): 3211, 2932, 2856, 1605, 1506, 1446, 1278, 1244, 1181, 1117, 1074 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 259.2 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{17}H_{23}O_2$: 259.1693 $[M+H]^+$; found 259.1696.

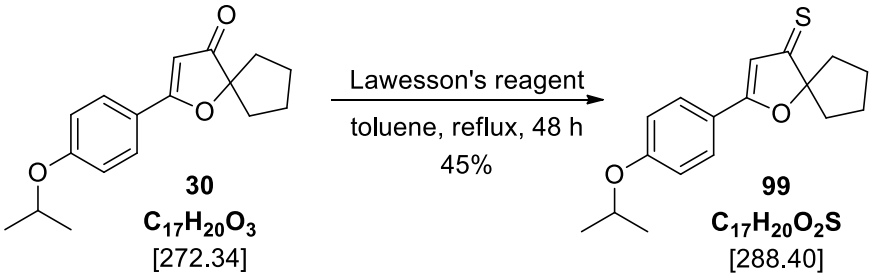
2-(4-Isopropoxyphenyl)-1-oxaspiro[4.4]non-2-ene-4-thione (99)



29
$C_{17}H_{20}O_3$
[272.34]

99
$C_{17}H_{20}O_2S$
[288.40]

Method A from alcohol 29: A solution of alcohol **29** (59.7 mg, 0.219 mmol, 1.0 eq) and Lawesson's reagent (177 mg, 0.438 mmol, 2.0 eq) in toluene (10 ml) was stirred at room temperature for 4 h. The solvent was removed and the product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 4:1) gave **99** (41.6 mg, 0.144 mmol, 66%) as an orange solid.



Method B from furanone 30: A solution of furanone **30** (55.1 mg, 0.202 mmol, 1.0 eq) and Lawesson's reagent (81.7 mg, 0.202 mmol, 1.0 eq) in toluene (10 ml) was refluxed for 48 h. The solvent was removed and the product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 4:1) to afford thiocarbonyl compound **99** (26.3 mg, 0.0912 mmol, 45%) as an orange solid. $\mathbf{R_f}$ = 0.56 (SiO_2, petroleum ether-EtOAc, 4:1). $\mathbf{M_p}$ 108 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.82 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 6.96 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 6.77 (s, 1 H, C=CH), 4.67 (sept, *J* = 6.1 Hz, 1 H, CH, *i*Pr), 2.39-2.17 (m, 2 H, CH_2), 2.13-1.92 (m, 6 H, CH_2), 1.38 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 230.6, 182.4, 162.4, 130.0, 120.2, 116.7, 115.9, 112.7, 70.3, 41.6, 25.8, 21.8 ppm.

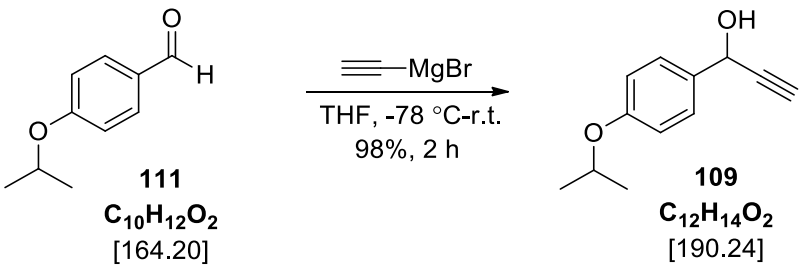
IR (neat): 3093, 2974, 2933, 2871, 1600, 1542, 1483, 1429, 1385, 1301, 1253 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 121.0 (26), 205.0 (100), 247.1 (91), 288.1 (59) [M^+].

HRMS (EI-MS): *m/z* calcd for $C_{17}H_{20}O_2S$: 288.1184 [M^+]; found 288.1185.

*Compounds **29** and **30** were prepared by Sabine Amslinger and Simon Lindner.[32]

1-(4-Isopropoxyphenyl)prop-2-yn-1-ol (109)



To aldehyde **111** (214 mg, 1.30 mmol, 1.0 eq) in 15 ml of dry THF at -78 °C was added dropwise ethynylmagnesium bromide (3.40 ml, 0.5 M in THF , 1.70 mmol, 1.3

eq). The reaction mixture was stirred at -78 °C for 30 min, then at room temperature for 1.5 h. After that the mixture was quenched with 20 ml saturated NH_4Cl solution and extracted with EtOAc (3 x 20 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel thin layer chromatography SiO_2 (petroleum ether-EtOAc, 4:1) to produce alcohol **109** (242 mg, 1.27 mmol, 98%) as a yellow oil. $\mathbf{R_f}$ = 0.43 (SiO_2, petroleum ether-EtOAc, 4:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.45 (d, *J* = 8.6 Hz, 2 H, CH, Ar), 6.98 (d, *J* = 8.6 Hz, 2 H, CH, Ar), 5.41 (s, 1 H, CH), 4.65 (sept, *J* = 6.1 Hz, 1 H, CH, *i*Pr), 2.67 (s, 1 H, alkyne), 2.52 (d, 1 H, *J* = 5.6 Hz, OH), 1.33 (d, *J* = 6.1 Hz, 6 H, CH_3, *i*Pr) ppm.

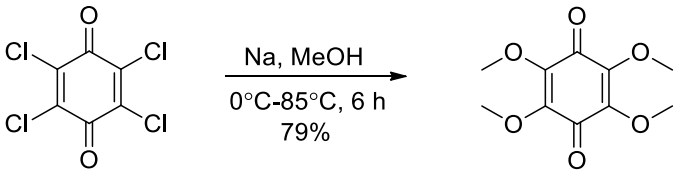
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 158.1, 132.0, 128.1, 115.8, 83.7, 74.6, 69.9, 64.0, 22.0 ppm.

IR (neat): 3446, 3270, 2984, 1605, 1505, 1383, 1300, 1250, 1177, 1121, 1015 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 95.1 (82), 102.1(35), 131.1 (71), 147.0 (100) [M^+].

HRMS (EI-MS): *m/z* calcd for $C_{12}H_{14}O_2$: 190.0994 [M^+]; found 190.0996.

2,3,5,6-Tetramethoxycyclohexa-2,5-diene-1,4-dione (114)



115
$C_6Cl_4O_2$
[245.88]

114
$C_{10}H_{12}O_6$
[228.20]

Sodium methoxide solution was prepared by adding sodium metal (1.31 g, 57.1 mmol, 4.0 eq) of under N_2 to dry methanol (50 ml) at 0 °C. After 2 h chloranil (**115**) (3.51 g, 14.3 mmol, 1.0 eq) was added. The mixture immediately turned red, which was heated at 85 °C for 6 h. The mixture was cold to 0 °C and the formed orange needles were filtrated and washed with water (3 x 10 ml) followed by washing with petroleum ether (3 x 10 ml) to produce *p*-quinone **114** (2.58 g, 11.3 mmol, 79%) as a pure orange needles. $\mathbf{R_f}$ = 0.50 (SiO_2, petroleum ether-EtOAc, 1:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.98 (s, 12 H, OCH_3) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 180.4, 142.7, 61.3 ppm.

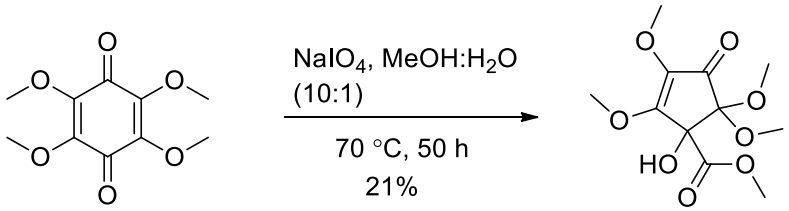
IR (neat): 2946, 1662, 1600, 1463, 1437, 1265, 1201, 1073, 1058, 965, 867 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 229.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{10}H_{13}O_6$: 229.0707 $[M+H]^+$; found 229.0709.

The ^{1}H NMR and ^{13}C NMR spectral data is in accordance with the literature.

Methyl 1-hydroxy-2,3,5,5-tetramethoxy-4-oxocyclopent-2-enecarboxylate (113)



114
$C_{10}H_{12}O_6$
[228.20]

113
$C_{11}H_{16}O_8$
[276.24]

To a solution of *p*-quinone **114** (91.6 mg, 0.401 mmol, 1.0 eq) in 30 ml MeOH was added sodium metaperiodate (343 mg, 1.61 mmol, 4.0 eq) and 3 ml water. The reaction mixture was refluxed for 50 h. Then the formed solid was filtrated off and the filtrate was evaporated and the product was purified using silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 1:1) afforded cyclopentenone **113** (23.7 mg, 0.0858 mmol, 21%) as a colorless oil. $\mathbf{R_f}$ = 0.33 (SiO_2, petroleum ether-EtOAc, 1:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.12 (s, 3 H, OCH_3, β-position), 3.88 (s, 3 H, OCH_3, α-position), 3.80 (s, 3 H, OCH_3, $COOCH_3$), 3.74 (s, 1 H, OH), 3.47 (s, 3 H, OCH_3, acetal), 3.39 (s, 3 H, OCH_3, acetal) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 189.4, 169.9, 164.0, 136.3, 97.3, 80.6, 59.9, 59.5, 53.7, 52.3, 51.9 ppm.

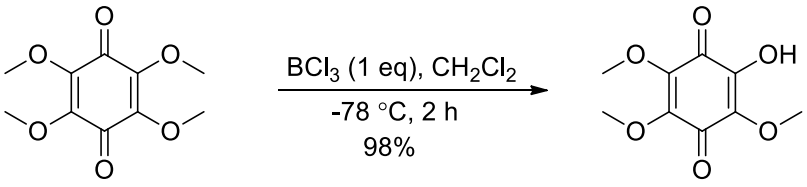
The ^{1}H NMR and ^{13}C NMR spectral data is in accordance with the literature.[77]

IR (neat): 3503, 2955, 1747, 1636, 1454, 1339, 1210, 1047, 994, 872, 732 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 185.0 (15), 213.0 (13), 245.1 (100).

HRMS (EI-MS): *m/z* calcd for $C_{11}H_{17}O_8$: 277.0918 $[M+H]^+$; found 277.0921.

2-Hydroxy-3,5,6-trimethoxycyclohexa-2,5-diene-1,4-dione (116)



114
$C_{10}H_{12}O_6$
[228.20]

116
$C_9H_{10}O_6$
[214.17]

p-Quinone **114** (1.00 g, 4.39 mmol, 1.0 eq) was dissolved in 30 ml of dry CH_2Cl_2 followed by additive of BCl_3 (4.83 ml, 1M solution in n-hexane), 4.83 mmol, 1.1 eq) dropwise at -78 °C. The solution was kept at this temperature for 2 h, then warmed to room temperature. 2 ml, 1 M aqueous HCl solution was added to the mixture followed by adding 20 ml brine solution. The mixture was extracted with CH_2Cl_2 (3 x 20 ml) and the combined organic layers were dried over $MgSO_4$ and evaporated to produce alcohol **116** (0.924 g, 4.31 mmol, 98%) as pure purple solid. $\mathbf{R_f}$ = 0.17 (SiO_2, petroleum ether-EtOAc, 1:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.56 (s, 1 H, OH), 4.04 (s, 3 H, OCH_3), 4.04 (s, 3 H, OCH_3), 2.96 (s, 3 H, OCH_3) ppm.

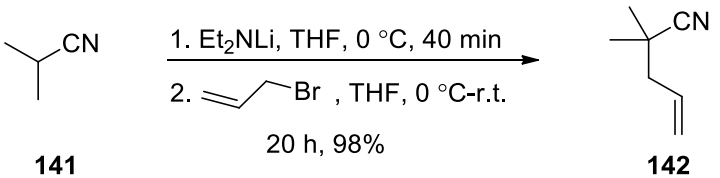
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 180.9, 179.7, 143.8, 140.7, 138.1, 135.3, 61.4, 60.4 ppm.

IR (neat): 3383, 3357, 1609, 1461, 1440, 1372, 1264, 1233, 1197, 1067 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 215.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_9H_{10}O_6$: 214.0472 $[M^+]$; found 214.0443.

2,2-Dimethylpent-4-enenitrile (142)



141
C_5H_7N
[69.11]

142
$C_7H_{11}N$
[109.17]

To a solution of diethyl amine (6.01 g, 82.1 mmol, 1.1 eq), in 100 ml of dry Et_2O at 0 °C under N_2 was added dropwise a solution of n-BuLi (51.3 ml, 1.6 M in hexanes, 82.1 mmol, 1.1 eq). The solution was stirred at 0 °C for 40 min followed by adding isobutyronitrile **141** (5.16 g, 74.7 mmol, 1.0 eq) dropwise at 0 °C. After 1 h a solution of allyl bromide (9.94 g, 82.1 mmol, 1.1 eq) in 20 ml of Et_2O was added dropwise at 0 °C, the reaction was warmed to room temperature and stirred overnight. Then the solution was cold to 0 °C, quenched with 20 ml of ice and then extracted with Et_2O (3 x 25 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo to give nitrile **142** (8.02 g, 73.5 mmol, 98%) as a colorless oil. $\mathbf{R_f}$ = 0.40 (SiO_2, petroleum ether-EtOAc, 9:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.99-5.69 (m, 1 H, CH), 5.26-5.06 (m, 2 H, vinyl), 2.23 (dt, *J* = 7.3, 1.2 Hz, 2 H, CH_2), 1.29 (s, 6 H, CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 132.0, 124.5, 119.7, 44.8, 31.9, 26.0 ppm.

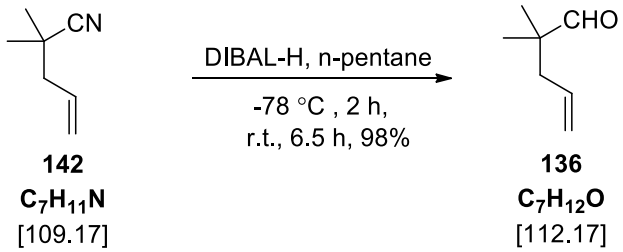
The ^{1}H NMR and ^{13}C NMR spectral data is in accordance with the literature.[90]

IR (neat): 2981, 2340, 2239, 1977, 1470, 997, 922, 406 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 41.2 (100), 68.2 (24), 109.2 (31) $[M]^+$.

HRMS (EI-MS): *m/z* calcd for $C_7H_{11}N$: 109.0891 $[M^+]$; found 109.0889.

2,2-Dimethylpent-4-enal (136)



142
$C_7H_{11}N$
[109.17]

136
$C_7H_{12}O$
[112.17]

To a solution of nitrile **136** (3.00 g, 27.5 mmol, 1.0 eq) in dry n-pentane (50 ml) at -78 °C under N_2 was added dropwise a solution of DIBAL-H (31.6 ml, 1.0 M in THF, 31.6 mmol, 1.1 eq). The reaction was kept 2 h at the same temperature followed by stirring at room temperature for 6.5 h then ethyl formate (0.611 g, 8.25 mmol, 0.3 eq) was added dropwise. After stirring for 1 h the reaction was quenched with 20 ml saturated NH_4Cl solution. The suspension was diluted with HCl (20 ml 10% in H_2O) and the organic layer was separated and diluted with H_2SO_4 (10 ml, 2.5 M in H_2O). The aqueous layer was extracted with n-pentane (3 x 20 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo to give aldehyde **136** (3.03 g, 27.0 mmol, 98%) as a colorless oil. $\mathbf{R_f}$ = 0.40 (SiO_2, petroleum ether-EtOAc, 9:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 9.44 (s, 1 H, CHO), 5.82-5.50 (m, 1 H, CH), 5.14-4.89 (m, 2 H, CH_2), 2.17 (d, *J* = 7.5 Hz, 2 H, CH_2), 1.01 (s, 6 H, CH_3) ppm.

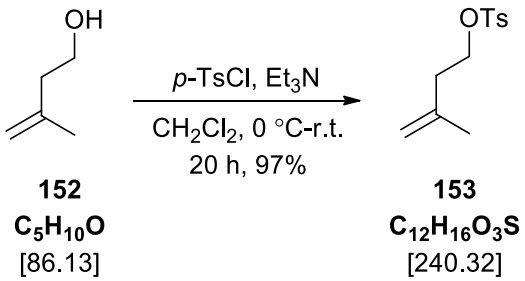
^{13}C-NMR (75 MHz, CDCl3): δ = 205.8, 133.0, 118.3, 45.6, 41.3, 21.0 ppm.

The ^{1}H NMR and ^{13}C NMR spectral data is in accordance with the literature.[88]

IR (neat): 2972, 2149, 1997, 1721, 1665, 1471, 1092, 996, 913 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 55.2 (100), 56.2 (25), 70.2 (22).

3-Methylbut-3-en-1-yl 4-methylbenzenesulfonate (153)



152
$C_5H_{10}O$
[86.13]

153
$C_{12}H_{16}O_3S$
[240.32]

To a solution of isoprenol (**152**) (10.0 g, 116 mmol, 1.0 eq) and *p*-TsCl (24.4 g, 127 mmol, 1.1 eq) in dry CH_2Cl_2 (100 ml) was added Et_3N (14.1 g, 11.8 mmol, 1.2 eq) at 0 °C. The reaction was stirred 2 h at 0 °C and at room temperature overnight. Then H_2O (100 ml) was added and the mixture was extracted with CH_2Cl_2 (3 x 25 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo to afford tosylate **153** (27.1 g, 112 mmol, 97%) as a colorless oil. $\mathbf{R_f}$ = 0.23 (SiO_2, petroleum ether-EtOAc, 9:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.74 (d, *J* = 7.4 Hz, 2 H, CH, Ar), 7.31 (d, *J* = 7.4 Hz, 2 H, CH, Ar), 4.74 (s, 1 H, CH), 4.63 (s, 1 H, CH), 4.07 (t, *J* = 6.7 Hz, 2 H, CH_2), 2.39 (s, 3 H, CH_3), 2.30 (t, *J* = 6.7 Hz, 2 H, CH_2), 1.60 (s, 3 H, CH_3) ppm.

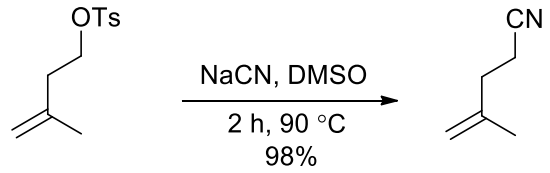
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 144.6, 139.9, 132.8, 129.6, 127.6, 112.9, 68.3, 36.5, 22.0, 21.4 ppm.

IR (neat): 1356, 1173, 1097, 961, 901, 814, 777, 660, 551, cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 258.1 (100) $[M+NH_4]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{12}H_{17}O_3S$: 241.0893 $[M+H]^+$; found 241.0894.

4-Methylpent-4-enenitrile (145)



153
$C_{12}H_{16}O_3S$
[240.32]

145
C_6H_9N
[95.14]

A solution of tosylate **153** (10.0 g, 41.6 mmol, 1.0 eq) and sodium cyanide (2.86 g, 58.3 mmol, 1.4 eq) in DMSO (50 ml) was heated at 90 °C for 2 h. The solution was cold to room temperature and poured into 100 ml H_2O followed by extraction with Et_2O (3 x 25 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo to produce nitrile **145** (3.87 g, 40.7 mmol, 98%) as a colorless oil. $\mathbf{R_f}$ = 0.23 (SiO_2, petroleum ether-EtOAc, 9:1).

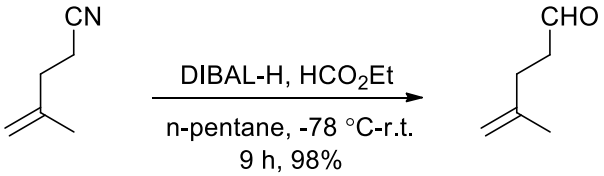
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.82 (s, 1 H, CH, vinyl), 4.74 (s, 1 H, CH, vinyl), 2.43 (t, *J* = 7.3 Hz, 2 H, CH_2), 2.28 (t, *J* = 7.3 Hz, 2 H, CH_2), 1.70 (s, 3 H, CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 141.4, 119.1, 112.0, 32.7, 21.7, 15.5 ppm.

IR (neat): 2906, 2245, 1652, 1437, 1379, 896 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 39.1 (31), 53.1 (20) 55.1 (100), 95.2 (32) [M^+].

4-methylpent-4-enal (143)



145
C_6H_9N
[95.14]

143
$C_6H_{10}O$
[98.14]

To a solution of nitrile **145** (3.01 g, 31.7 mmol, 1.0 eq) in dry n-pentane (50 ml) at -78 °C under N_2 was added dropwise a solution of DIBAL-H (36.4 ml, 1.0 M in THF, 36.4 mmol, 1.1 eq). The reaction was kept 2 h at the same temperature followed by stirring at room temperature for 7 h. Then ethyl formate (0.705 g, 9.52 mmol, 0.3 eq) was added dropwise. After stirring for 1 h the reaction was quenched with 20 ml

saturated NH_4Cl solution and the suspension was diluted with HCl (20 ml, 10%). The organic layer was separated and diluted with H_2SO_4 (10 ml, 2.5 M). The aqueous layer was extracted with n-pentane (3 x 20 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo to give aldehyde **143** (3.03 g, 27.0 mmol, 98%) as a colorless oil. $\mathbf{R_f}$ = 0.16 (SiO_2, petroleum ether-EtOAc, 9:1).

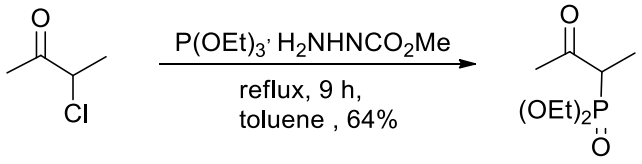
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 9.69 (m, 1 H, CHO), 4.69 (s, 1 H, CH), 4.61 (s, 1 H, CH), 2.50 (m, 2 H, CH_2), 2.27 (m, 2 H, CH_2), 1.67 (s, 3 H, CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 201.9, 143.5, 110.4, 41.5, 29.6, 22.3 ppm.

IR (neat): 2963, 2927, 1679, 1648, 1447, 1375, 884 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 39.1 (47), 41.1 (100), 55.1 (53), 56.1 (52), 69.2 (46), 70.2 (51), 98.1 (5) [M^+].

Diethyl (3-oxobutan-2-yl)phosphonate (144)



147
C_4H_7ClO
[106.55]

144
$C_8H_{17}O_4P$
[208.19]

Ketone **147** (5.00 g, 46.9 mmol, 1.0 eq) and methyl hydrazinecarboxylate (4.44 g, 49.3 mmol, 1.1 eq) was dissolved in 50 ml toluene under reflux condition with a Dean-Stark apparatus. After removing the H_2O (4 h) triethyl phosphite (8.58 g, 51.6 mmol, 1.1 eq) was added dropwise under reflux. The mixture was kept 2 h at the same conditions, then the solvent was removed and 10 ml acetone were added followed by HCl (10 ml, 3 N in H_2O). After 3 h at room temperature the acetone was removed and 25 ml H_2O were added and the mixture was extracted with CH_2Cl_2 (3 x 20 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel column chromatography (15 cm × 5.5 cm) using EtOAc to afford phosphonate **114** (6.26 g, 30.1 mmol, 64%) as a yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.11-3.89 (m, 4 H, CH_2), 3.22-2.95 (m, 1 H, CH), 2.28-2.11 (m, 3 H, CH_3), 1.28-1.11 (m, 9 H, CH_3) ppm.

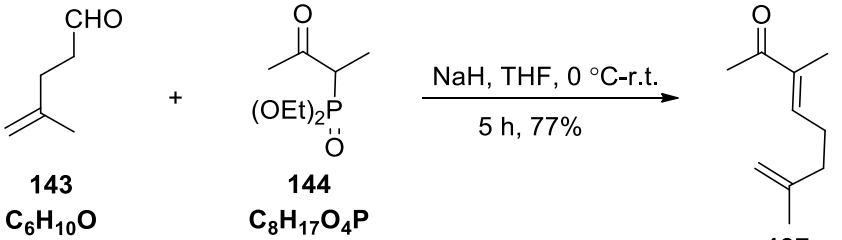
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 203.5, 62.3 (t, *J* = 7.6 Hz), 47.1 (d, *J* = 126.8 Hz), 30.1, 16.1 (d, *J* = 5.9 Hz), 10.6 (d, *J* = 6.4 Hz) ppm.

^{31}P-NMR (121 MHz, $CDCl_3$): δ = 24.0 ppm.

MS (EI, 70 eV): *m/z* (%) = 209.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_8H_{17}O_4P$: 208.0859 $[M^+]$; found 208.0873.

3,7-Dimethylocta-3,7-dien-2-one (137)



143
$C_6H_{10}O$
[98.14]

144
$C_8H_{17}O_4P$
[208.19]

137
$C_{10}H_{16}O$
[152.23]

To a suspension of NaH (49.0 mg (from 60% NaH suspension in paraffine oil), 1.26 mmol, 1.2 eq) in 5 ml of dry THF at 0 °C under N_2 was added ethyl phosphonate **144** (0.255 g, 1.23 mmol, 1.2 eq) and the mixture was stirred for 30 min at room temperature. Then the solution was cold to 0 °C followed by adding aldehyde **143** (0.100 g, 1.02 mmol, 1.0 eq). The reaction mixture was warmed to room temperature and stirred for 4 h followed by quenching with 20 ml brine solution and extracted with Et_2O (3 x 10 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel thin layer chromatography (petroleum ether-EtOAc, 95:5) to give ketone **137** (0.119 g, 0.782 mmol, 77%) as a yellow oil. $\mathbf{R_f}$ = 0.26 (SiO_2, petroleum ether-EtOAc, 9:1).

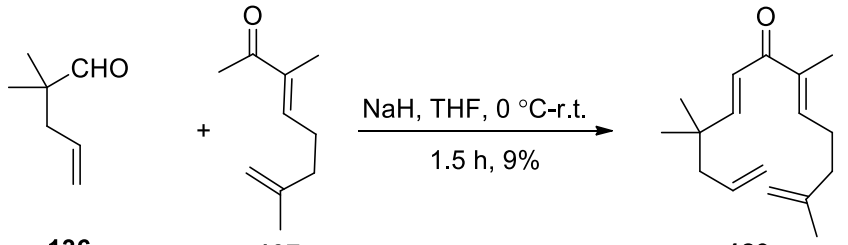
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 6.57 (m, 1 H, CH, vinyl), 4.72 (s, 1 H, CH, vinyl), 4.66 (s, 1 H, CH, vinyl), 2.41-2.28 (m, 2 H, CH_2), 2.24 (s, 3 H, CH_3), 2.13 (t, *J* = 7.5, 2 H, CH_2), 1.72 (s, 3 H, CH_3), 1.70 (s, 3 H, CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 199.7, 144.4, 143.0, 137.6, 110.6, 36.3, 27.0, 25.3, 22.2, 11.0 ppm.

MS (EI, 70 eV): *m/z* (%) = 153.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{10}H_{16}O$: 152.1196 $[M^+]$; found 152.1186.

2,6,10,10-Tetramethyltrideca-1,5,8,12-tetraen-7-one (129)



136
$C_7H_{12}O$
[112.17]

137
$C_{10}H_{16}O$
[152.23]

129
$C_{17}H_{26}O$
[246.39]

To a suspension of NaH (6.4 mg (from 60% NaH suspension in paraffine oil), 0.16 mmol, 1.2 eq) in dry THF (2 ml) at 0 °C under N_2 was added a solution of ketone **137** (20.2 mg, 0.133 mmol, 1.0 eq) in 1 ml of dry THF. The mixture was stirred for 30 min at 0 °C, followed by adding a solution of aldehyde **136** (14.9 mg, 0.133 mmol, 1.0 eq) in 1 ml of dry THF. The reaction mixture was stirred at 0 °C for 1 h followed by quenching with 20 ml brine solution and extracted with CH_2Cl_2 (3 x 5 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 95:5) gave ketone **129** (3.1 mg, 0.13 mmol, 9%) as a colorless oil. $\mathbf{R_f}$ = 0.67 (SiO_2, petroleum ether-EtOAc, 95:5).

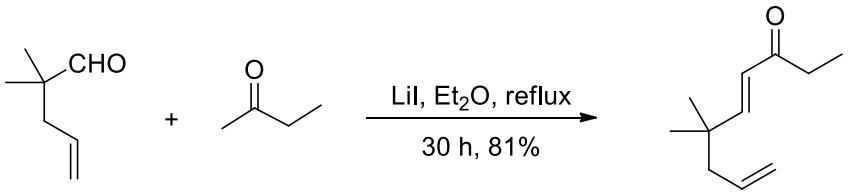
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 6.77 (d, *J* = 15.7 Hz, 1 H, CH), 6.54 (m, 1 H, CH), 7.24 (d, *J* = 15.7 Hz, 1 H, CH), 5.79-5.61 (m, 1 H, CH, vinyl), 5.07-4.97 (m, 2 H, CH, vinyl), 4.76 (s, 1 H, CH, vinyl), 4.71 (s, 1 H, CH, vinyl), 2.45-2.34 (m, 2 H, CH_2), 2.21-2.14 (m, 2 H, CH_2), 2.14-2.09 (m, 2 H, CH_2), 1.84 (s, 3 H, CH_3), 1.75 (s, 3 H, CH_3), 1.06 (s, 6 H, CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 193.0, 155.6, 144.6, 141.9, 137.9, 134.6, 121.6, 117.6, 110.7, 46.5, 36.8, 36.4, 27.1, 26.3, 22.3, 11.9 ppm.

MS (EI, 70 eV): *m/z* (%) = 247.2 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{17}H_{27}NO$: 247.2056 $[M+H]^+$; found 247.2060.

6,6-Dimethylnona-4,8-dien-3-one (155)



136 **156** **155**
$C_7H_{12}O$ **C_4H_8O** **$C_{11}H_{18}O$**
[112.17] [72.11] [166.26]

Ethyl methyl ketone (**156**) (9.1 mg, 0.13 mmol, 1.0 eq) was dissolved in 10 ml dry Et_2O. Then LiI (64.4 mg, 0.481 mmol, 3.8 eq) was added to the solution followed by adding aldehyde **136** (21.6 mg, 0.193 mmol, 1.5 eq) in 2 ml of dry Et_2O. The mixture was refluxed for 30 h, and then cold to room temperature and 10 ml H_2O was added. The aqueous phase was extracted with Et_2O (3 x 10 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 95:5) gave ketone **155** (17 mg, 0.10 mmol, 81%) as a colorless oil. $\mathbf{R_f}$ = 0.49 (SiO_2, petroleum ether-EtOAc, 9:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 6.78 (d, *J* = 16.2 Hz, 1 H, CH), 5.99 (d, *J* = 16.2 Hz, 1 H, CH), 5.82-5.58 (m, 1 H, CH, vinyl), 5.14-4.90 (m, 2 H, CH_2, vinyl), 2.57 (q, *J* = 7.3 Hz, 2 H, CH_2), 2.11 (dt, *J* = 7.5, 0.9 Hz, 2 H, CH_2), 1.09 (t, *J* = 7.3 Hz, 3 H, CH_3), 1.05 (s, 6 H, CH_3) ppm.

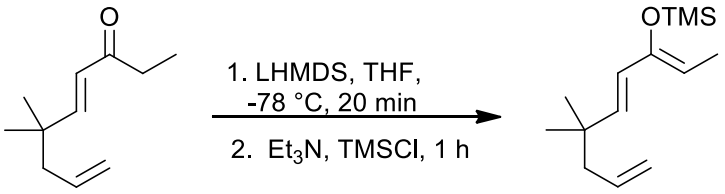
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 201.5, 155.3, 134.3, 126.3, 117.8, 46.5, 36.7, 33.4, 26.1, 8.2 ppm.

The ^{1}H NMR and ^{13}C NMR spectral data is in accordance with the literature.[89]

MS (EI, 70 eV): *m/z* (%) = 167.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{11}H_{18}O$: 166.1352 $[M^+]$; found 166.1350.

6,6-Dimethylnona-2,4,8-trien-3-yl)oxy)trimethylsilane

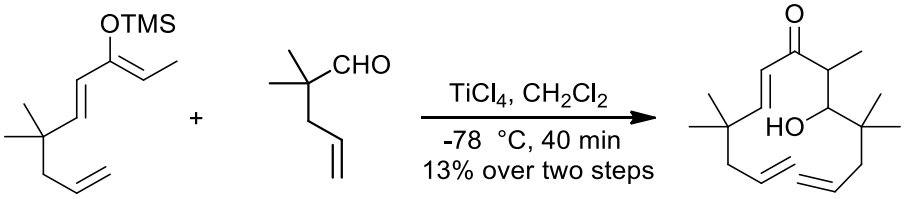


155
$C_{11}H_{18}O$
[166.26]

$C_{14}H_{26}OSi$
[238.44]

A solution of LHMDS (0.150 ml, 1M, 0.15 mmol, 2.0 eq) was added dropwise to a solution of ketone **155** (12.4 mg, 0.0746 mmol, 1.0 eq) in 10 ml of dry THF at -78 °C. The mixture was stirred at -78 °C for 20 min followed by addition of Et_3N (22.6 mg, 0.223 mmol, 3.0 eq) and TMSCl (28.4 µl, 0.224 mmol, 3.0 eq). The mixture was stirred at -78 °C for 30 min, followed by warming to room temperature for another 30 min. The mixture was concentrated in vacuo and the residue was washed with n-pentane (3 x 5 ml). The n-pentane was evaporated to give a yellow oil (17.9 mg) which was used in the next step without further purification.

9-Hydroxy-4,4,8,10,10-pentamethyltrideca-1,5,12-trien-7-one (154)



$C_{14}H_{26}OSi$
[238.44]

136
$C_7H_{12}O$
[112.17]

154
$C_{18}H_{30}O_2$
[278.43]

To aldehyde **136** (8.3 mg, 0.074 mmol, 1.0 eq) in 5 ml dry CH_2Cl_2 at -78 °C was added $TiCl_4$ (8.3 µl, 0.074 mmol, 1.0 eq). After 10 min silyl ether (17.7 mg, 0.0742 mmol, 1.0 eq) in 1 ml of dry CH_2Cl_2 was added to the solution at -78 °C and stirred for 30 min. Then the solution was quenched with 10 ml saturated aqueous $NaHCO_3$ solution. After warmed up to room temperature the mixture was extracted with Et_2O (3 x 10 ml) and the combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 90:10) afforded ketone **154** (2.6

mg, 0.0093 mmol, 13%) over two steps as a colorless oil. **R_f** = 0.12 (SiO_2, petroleum ether-EtOAc, 9:1).

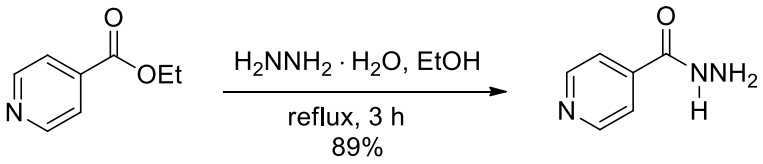
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 6.84 (d, *J* = 16.1 Hz, 1 H, CH), 6.00 (m, 1 H, CH), 5.93-5.57 (m, 2 H, CH, vinyl), 5.11-4.94 (m, 4 H, CH, vinyl), 3.68 (d, *J* = 3.1 Hz, 1 H, CH), 3.21-2.94 (m, 1 H, CH), 2.64-2.32 (br, 1 H, OH), 2.27-1.93 (m, 4 H, CH_2), 1.17 (d, *J* = 7.1 Hz, 3 H, CH_3), 1.07 (s, 6 H, CH_3), 0.91 (s, 3 H, CH_3), 0.88 (s, 3 H, CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 205.1, 156.9, 135.3, 134.1, 125.1, 117.9, 117.3, 75.6, 46.5, 44.6, 43.9, 38.4, 36.9, 26.1, 23.9, 23.5, 12.4 ppm.

MS (EI, 70 eV): *m/z* (%) = 167.1 (30), 179.6 (20), 279.2 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{18}H_{31}O_2$: 279.2319 $[M^+]$, found 279.2327.

Isonicotinohydrazide (166)



165
$C_8H_9NO_2$
[151.16]

166
$C_6H_7N_3O$
[137.14]

A solution of ethyl isonicotinate (**165**) (22.2 g, 147 mmol, 1.0 eq) and hydrazine monohydrate (14.7 g, 249 mmol, 2.0 eq) in ethanol (100 ml) were refluxed for 3 h, the solvent was evaporated approximately by half and the mixture was cold to -10 °C. The formed white precipitate was filtered and washed with cold EtOH (2 x 20 ml) and petroleum ether (2 x 20 ml) respectively, to give hydrazide **166** (18.0 g, 131 mmol, 89%) as a white crystals. **R_f** = 0.44 (SiO_2, CH_2Cl_2-MeOH, 5:1); **M_p** 172 °C, literature value 171-172 °C.[104]

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 10.11 (s, 1 H, NH), 8.70 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.72 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 4.62 (s, 2 H, NH_2) ppm.

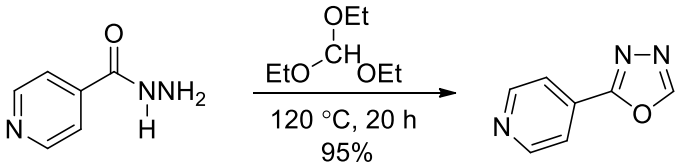
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 163.9, 150.2, 140.2, 121.0 ppm.

IR (neat): 3099, 3008, 1661, 1632, 1548, 1411, 1331, 1220, 1141, 994, 887 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 138.1 (100) $[M+H]^+$, 121.0 (7.7).

HRMS (EI-MS): *m/z* calcd for $C_6H_7N_3O$: 137.0584 $[M^+]$; found 137.0574.

2-(Pyridin-4-yl)-1,3,4-oxadiazole (167)



166
$C_6H_7N_3O$
[137.14]

167
$C_7H_5N_3O$
[147.13]

A solution of hydrazide **166** (2.00 g, 14.6 mmol, 1.0 eq) in 10 ml triethyl orthoformate was stirred at 120 °C for 20 h. Then the solvent was evaporated and the mixture was poured on 50 ml ice. The yellow precipitate was filtered off and washed with cold H_2O (2 x 10 ml) and petroleum ether (2 x 10 ml) respectively. Recrystallization from EtOH afforded oxadiazole **167** (2.03 g, 13.8 mmol, 95%) as a white solid. $\mathbf{R_f}$ = 0.34 (SiO_2, EtOAc); $\mathbf{M_p}$ 111 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.84 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 8.57 (s, 1 H, CH) 7.95 (d, *J* = 6.1 Hz, 2 H,CH, Ar), ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 163.0, 153.4, 150.9, 130.6, 120.5 ppm.

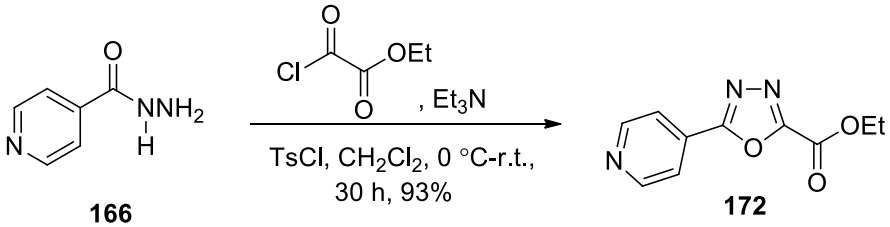
The ^{1}H NMR and ^{13}C NMR spectral data is in accordance with the literature.[105]

IR (neat): 3088, 2983, 1612, 1544, 1417, 1241, 1116, 1080, 1063, 993, 954 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 148.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_7H_6N_3O$: 148.0505 $[M^+]$; found 148.0504.

Ethyl 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-carboxylate (172)



166
$C_6H_7N_3O$
[137.14]

172
$C_{10}H_9N_3O_3$
[219.20]

Hydrazide **166** (0.434 g, 3.16 mmol, 1.0 eq) and Et_3N (0.960 g, 9.48 mmol, 3.0 eq) were dissolved in 100 ml CH_2Cl_2 at 0 °C and treated dropwise with ethyl oxalylchloride (0.432 g, 3.16 mmol, 1.0 eq). The solution was warmed to room temperature and stirred 6 h, and then tosyl chloride (0.603 g, 3.16 mmol, 1.0 eq) was added to the solution and stirred for 20 h. The reaction was diluted with 20 ml CH_2Cl_2 followed by washing with 20 ml of H_2O, 20 ml of saturated aqueous $NaHCO_3$ and 20 ml brine solution. The organic layer was dried over $MgSO_4$. The solvent was removed in vacuo and the product was purified by silica gel thin layer chromatography on SiO_2 (petroleum ether-EtOAc, 1:1) to afford ester oxadiazole **172** (0.642 g, 2.93 mmol, 93%) as a white solid. **R_f** = 0.58 (SiO_2, EtOAc); **M_p** 96 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.84 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.98 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 4.54 (q, *J* = 7.1 Hz, 2 H, CH_2), 1.46 (t, *J* = 7.1 Hz, 3 H, CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 164.5, 157.0, 154.0, 151.1, 129.8, 120.7, 63.8, 14 ppm.

IR (neat): 2990, 1736, 1572, 1536, 1413, 1325, 1284, 1220, 1194, 1163, 1019 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 220.1 (100) $[M+H]^+$.

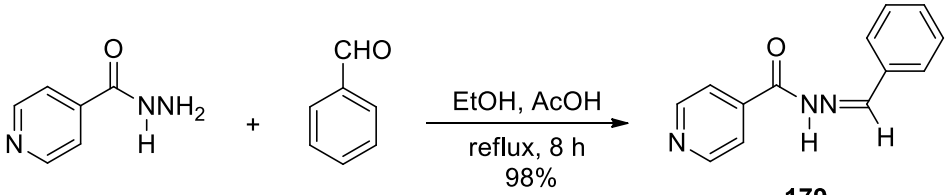
HRMS (EI-MS): *m/z* calcd for $C_{10}H_{10}N_3O_3$: 220.0717 $[M+H]^+$; found 220.0715.

General procedure for the synthesis of hydrazones 179-190[106]

Isonicotinohydrazide **166** (0.510 g-5.00 g, 3.72 mmol-36.5 mmol, 1.0 eq) and aldehyde or ketone (0.434 g-0.662 g, 4.09 mmol-4.01 mmol, 1.1eq) were dissolved in 50 ml EtOH then 0.1 ml glacial acetic acid was added and the mixture was refluxed until the reaction was completed. After cooling to room temperature the mixture was

poured into 20 ml H_2O and the solid was filtered. The precipitate was washed with H_2O (2 x 10 ml) and petroleum ether (2 x 10 ml) to afford the corresponding hydrazone **179-190**.

N'-Benzylideneisonicotinohydrazide (179)



166
$C_6H_7N_3O$
[137.14]

C_7H_6O
[106.12]

179
$C_{13}H_{11}N_3O$
[225.25]

Isonicotinohydrazide (**166**) (0.510 g, 3.72 mmol, 1.0 eq) gave after 8 h *N*'-benzylideneisonicotinohydrazide (**179**) (0.824 g, 3.66 mmol, 98%) as a white solid. **R_f** = 0.21 (SiO_2, EtOAc); **M_p** 199 °C, lit 205-207 °C.[107]

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 12.11 (s, 1 H, NH), 8.79 (d, *J* = 6.0 Hz, 2 H, CH, Ar), 8.48 (s, 1 H, CH. N=CH), 7.83 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.75 (m, 2 H, CH, Ar), 7.46 (m, 3 H, CH, Ar) ppm.

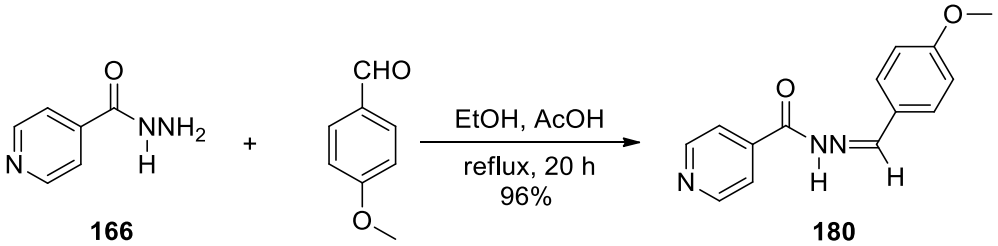
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 161.6, 150.3, 149.0, 140.4, 134.0, 130.4, 128.9, 127.2, 121.5 ppm.

IR (neat): 3200, 3020, 1682, 1562, 1412, 1282, 1228, 1148, 1057, 998, 994 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 226.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{13}H_{11}N_3O$: 225.0902 $[M^+]$; found 225.0905.

N'-(4-Methoxybenzylidene)isonicotinohydrazide (180)



166
$C_6H_7N_3O$
[137.14]

$C_8H_8O_2$
[136.15]

180
$C_{14}H_{13}N_3O_2$
[255.27]

Isonicotinohydrazide (**166**) (5.01 g, 36.5 mmol, 1.0 eq) gave after 20 h *N*'-(4-methoxybenzylidene)isonicotinohydrazide (**180**) (8.93 g, 35.0 mmol, 96%) as a white solid. **R_f** = 0.14 (SiO_2, EtOAc); **M_p** 163 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.69 (s, 1 H, NH), 8.78 (d, *J* = 6.0 Hz, 2 H, CH, Ar), 8.41 (s, 1 H, CH, N=CH), 7.82 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.70 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 7.04 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 3.81 (s, 3 H, OCH_3) ppm.

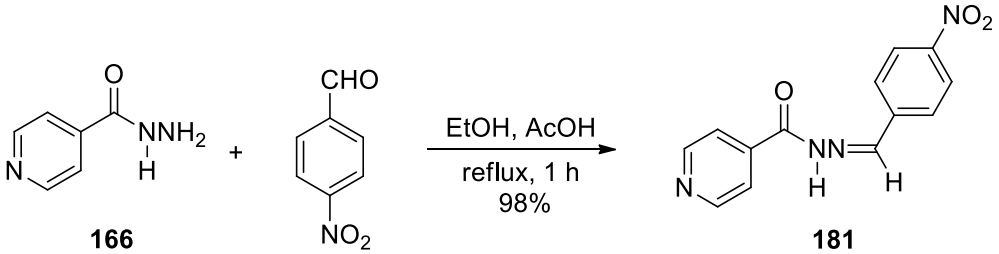
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 161.3, 161.0, 150.2, 148.8, 140.5, 128.8, 126.4, 121.4, 114.3, 55.2 ppm.

IR (neat): 3426, 3023, 1652, 1597, 1550, 1514, 1412, 1316, 1253, 1169, 1024 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 255.1 (11.7) [M^+], 134.1 (27.5), 133.1 (100.0), 106.1 (25.2), 79.1(10.1), 78.1(20.0), 51.1(12.6).

HRMS (EI-MS): *m/z* calcd for $C_{14}H_{14}N_3O_2$: 256.1081 $[M+H]^+$; found 256.1084.

N'-(4-Nitrobenzylidene)isonicotinohydrazide (181)



166
$C_6H_7N_3O$
[137.14]

$C_7H_5NO_3$
[151.12]

181
$C_{13}H_{10}N_4O_3$
[270.24]

Isonicotinohydrazide (**166**) (3.84 g, 28.0 mmol, 1.0 eq) gave after 1 h *N*'-(4-nitrobenzylidene)isonicotinohydrazide (**181**) (7.44 g, 27.5 mmol, 98%) as a yellow solid. **R_f** = 0.14 (SiO_2, EtOAc); **M_p** 277 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 12.40 (s, 1 H, NH), 8.81 (d, *J* = 6.0 Hz, 2 H, CH, Ar), 8.59 (s, 1 H, CH, N=CH), 8.31 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 8.02 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 7.85 (d, *J* = 6.0 Hz, 2 H, CH, Ar) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6): δ = 161.9, 150.3, 147.9, 146.4, 140.2, 140.0, 128.1, 124.0, 121.5 ppm.

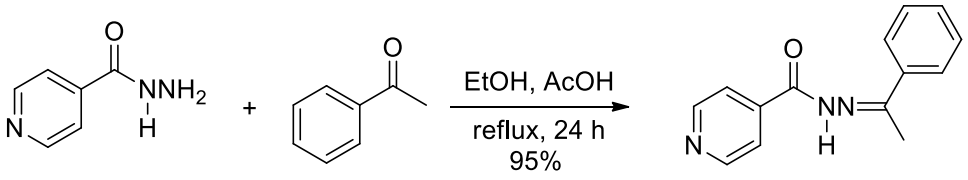
IR (neat): 3189, 2992, 2828, 1681, 1553, 1507, 1416, 1334, 1274, 1217, 1141 cm^{-1}.

The IR and ^{13}C NMR spectral data is in accordance with the literature.[106]

MS (EI, 70 eV): *m/z* (%) = 271.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{13}H_{11}N_4O_3$: 271.0826 $[M+H]^+$; found 271.0829.

N'-(1-Phenylethylidene)isonicotinohydrazide (182)



166
$C_6H_7N_3O$
[137.14]

C_8H_8O
[120.15]

182
$C_{14}H_{13}N_3O$
[239.27]

Isonicotinohydrazide (**166**) (0.508 g, 3.71 mmol, 1.0 eq) gave after 24 h *N*'-(4-nitrobenzylidene)isonicotinohydrazide (**182**) (0.843 g, 3.53 mmol, 95%) as a yellow solid. $\mathbf{R_f}$ = 0.26 (SiO_2, EtOAc); $\mathbf{M_p}$ 173 °C, lit 169-171 °C.[107]

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.06 (s, 1 H, NH), 8.77 (d, *J* = 5.2 Hz, 2 H, CH, Ar), 7.84 (m, 3 H, CH, Ar), 7.54 (m, 4 H, CH, Ar) 2.39 (s, 3 H, CH_3) ppm.

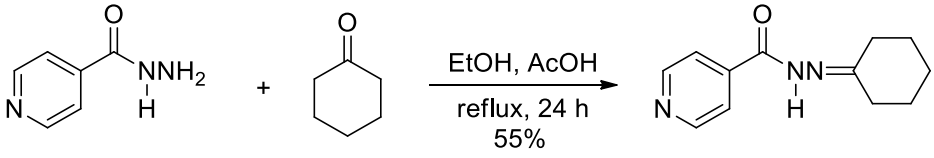
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 162.4, 156.8, 150.0, 149.3, 141.0, 137.7, 129.6, 128.3, 126.5, 125.9, 122.9, 121.8, 14.7 ppm.

IR (neat): 3176, 3061, 1651, 1596, 1549, 1384, 1298, 1153, 1118, 989, 843 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 240.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{14}H_{14}N_3O$: 240.1131 $[M+H]^+$; found 240.1136.

***N*'-Cyclohexylideneisonicotinohydrazide (183)**



166
$C_6H_7N_3O$
[137.14]

$C_6H_{10}O$
[98.14]

183
$C_{12}H_{15}N_3O$
[217.27]

Isonicotinohydrazide (**166**) (0.462 g, 3.37 mmol, 1.0 eq) gave after 24 h *N*'-(4-nitrobenzylidene)isonicotinohydrazide (**183**) (0.401 g, 1.85 mmol, 55%) as a yellow solid. $\mathbf{R_f}$ = 0.53 (SiO_2, CH_2Cl_2-MeOH, 5:1); $\mathbf{M_p}$ 165 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.74 (d, *J* = 4.2 Hz, 2 H, CH, Ar), 7.67 (d, *J* = 3.4 Hz, 2 H, CH, Ar), 2.67-2.11 (m, 4 H, CH_2), 2.00-1.38 (m, 6 H, CH_2) ppm.

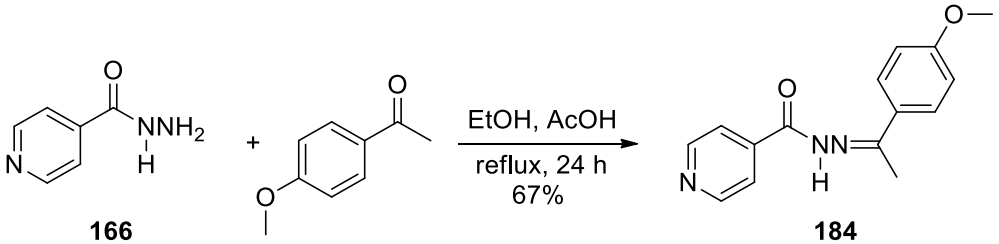
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 167.7, 161.6, 150.0, 141.0, 121.5, 35.0, 28.1, 26.8, 25.7, 24.9 ppm.

IR (neat): 3217, 3035, 2932, 2852, 1658, 1638, 1525, 1407, 1302, 1284, 1140 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 218.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{12}H_{16}N_3O$: 218.1288 $[M+H]^+$; found 218.1289.

N'-(1-(4-Methoxyphenyl)ethylidene)isonicotinohydrazide (184)



166
$C_6H_7N_3O$
[137.14]

$C_9H_{10}O_2$
[150.17]

184
$C_{15}H_{15}N_3O_2$
[269.30]

Isonicotinohydrazide (**166**) (0.482 g, 3.21 mmol, 1.0 eq) gave after 24 h *N*'-(1-(4-methoxyphenyl)ethylidene)isonicotinohydrazide (**184**) (0.528 g, 1.96 mmol, 67%) as white solid. **R_f** = 0.41 (SiO_2, EtOAc); **M_p** 196 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 10.98 (s, 1 H, NH), 8.76 (d, *J* = 5.7 Hz, 2 H, CH, Ar), 7.82 (m, 4 H, CH, Ar), 7.00 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 3.80 (s, 3 H, OCH_3), 2.35 (s, 3 H, CH_3) ppm.

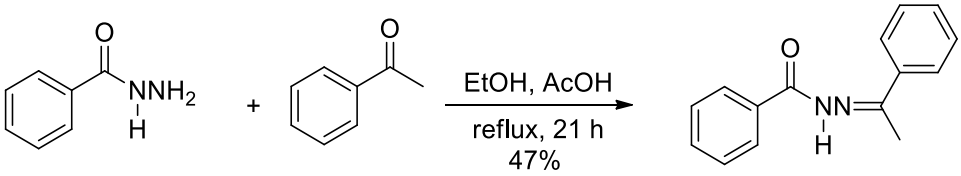
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 162.1, 160.5, 157.1, 150.0, 141.1, 130.0, 128.0, 121.7, 131.6, 55.1, 14.6 ppm.

IR (neat): 3170, 3057, 2900, 1656, 1599, 1384, 1302, 1247, 1179, 1113, 1031 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 270.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{15}H_{16}N_3O_2$: 270.1237 $[M+H]^+$; found 270.1238.

N'-(1-Phenylethylidene)benzohydrazide (185)



$C_7H_8N_2O$
[136.15]

C_8H_8O
[120.15]

185
$C_{15}H_{14}N_2O$
[238.28]

Benzohydrazide (0.150 g, 1.09 mmol, 1.0 eq) gave after 21 h *N*'-(1-phenylethylidene)benzohydrazide (**185**) (0.122 g, 0.512 mmol, 47%) as a white solid. **R_f** = 0.55 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 155 °C.

^{1}H-NMR (300 MHz, (DMSO-d_6): δ = 10.80 (s, 1 H, NH), 8.17-7.69 (m, 4 H, CH, Ar), 7.67-7.23 (m, 6 H, CH, Ar), 2.38 (s, 3 H, CH_3) ppm.

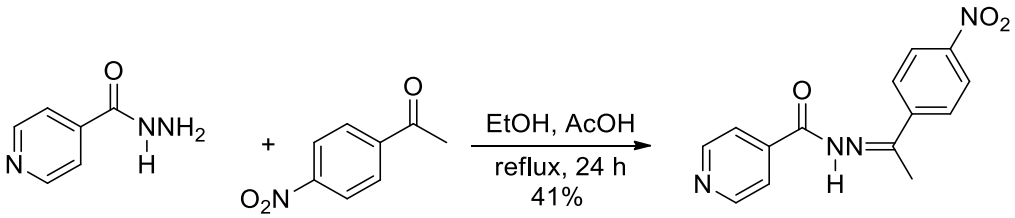
^{13}C-NMR (300 MHz, (DMSO-d_6): δ = 163.9, 155.4, 138.0, 134.0, 131.4, 129.3, 128.3, 127.8, 126.3, 14.5 ppm.

IR (neat): 3171, 3006, 1636, 1602, 1578, 1537, 1486, 1443, 1317, 1280, 1134 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 239.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{15}H_{15}N_2O$: 239.1179 $[M^+]$; found 239.1181.

***N*'-(1-(4-Nitrophenyl)ethylidene)isonicotinohydrazide (186)**



166
$C_6H_7N_3O$
[137.14]

$C_8H_7NO_3$
[165.15]

186
$C_{14}H_{12}N_4O_3$
[284.27]

Isonicotinohydrazide (**166**) (0.500 g, 3.65 mmol, 1.0 eq) gave after 24 h *N*'-(1-(4-nitrophenyl)ethylidene)isonicotinohydrazide (**186**) (0.471 g, 1.65 mmol, 41%) as a yellow solid. **R_f** = 023 (SiO_2, EtOAc); **M_p** 286 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.08 (s, 1 H, NH), 8.77 (d, *J* = 4.3 Hz, 2 H, CH, Ar), 8.25 (d, *J* = 8.5 Hz, 2 H, CH, Ar), 8.04 (br, 2 H, CH, Ar), 7.76 (s, 2 H, CH, Ar), 2.38 (s, 3 H, CH_3) ppm.

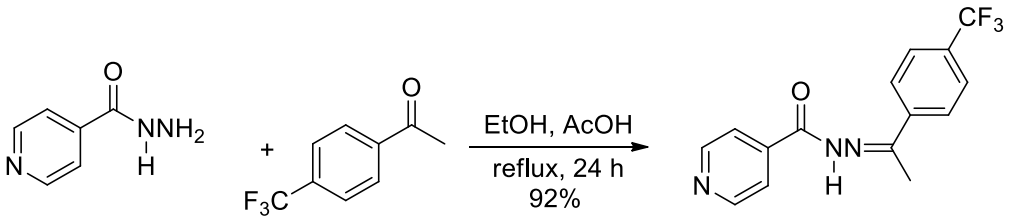
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 162.4, 149.5, 147.5, 143.6, 140.9, 139.1, 127.0, 123.0, 121.7, 13.9 ppm.

IR (neat): 3196, 3108, 1668, 1579, 1551, 1513, 1494, 1385, 1349, 1299, 1154 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 285.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{14}H_{13}N_4O_3$: 285.0982 $[M^+]$; found 285.0985.

N'-(1-(4-(Trifluoromethyl)phenyl)ethylidene)isonicotinohydrazide (187)



166
$C_6H_7N_3O$
[137.14]

$C_9H_7F_3O$
[188.15]

187
$C_{15}H_{12}F_3N_3O$
[307.27]

Isonicotinohydrazide (**166**) (0.500 g, 3.65 mmol, 1.0 eq) gave after 24 h *N*'-(1-(4-(trifluoromethyl)phenyl)ethylidene)isonicotinohydrazide (**187**) (1.03 g, 3.35 mmol, 92%) as white solid. **R_f** = 0.36 (SiO_2, EtOAc); **M_p** 224 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.16 (s, 1H, NH), 8.78 (d, *J* = 4.7 Hz, 2H, CH, Ar), 8.07 (d, *J* = 7.9 Hz, 2H, CH, Ar), 7.81 (m, 4H, CH, Ar), 2.43 (s, 3H, CH_3) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6): δ = 162.7, 154.5, 150.0, 141.6, 140.9, 127.2, 126.6, 125.2 (q, *J* = 3.8 Hz, *C*HCCF$_3$), 124.0 (q, *J* = 280.0 Hz, CF_3), 121.9, 14.6 ppm.

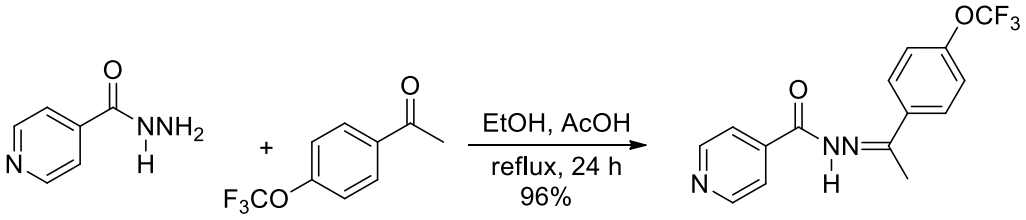
^{19}F-NMR (282 MHz, DMSO-d_6): δ = (-60.8, CF_3) ppm.

IR (neat): 3185, 3064, 1660, 1598, 1550, 1387, 1331, 1159, 1106, 1079, 1013 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 308.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{15}H_{13}F_3N_3O$: 308.1005 $[M^+]$; found 308.1004.

N'-(1-(4-(Trifluoromethoxy)phenyl)ethylidene)isonicotinohydrazide (188)



166
$C_6H_7N_3O$
[137.14]

$C_9H_7F_3O_2$
[204.15]

188
$C_{15}H_{12}F_3N_3O_2$
[323.27]

(0.501 g, 3.65 mmol, 1.0 eq) of isonicotinohydrazide (**166**) gave after 24 h *N*'-(1-(4-(trifluoromethoxy)phenyl)ethylidene)isonicotino hydrazide (**188**) (1.14 g, 3.51 mmol, 96%) as a white solid. **R_f** = 0.33 (SiO_2, EtOAc); **M_p** 203 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.17 (br, 1H, NH), 8.77 (s, 2H, CH, Ar), 8.14-7.20 (m, 6H, CH, Ar), 2.40 (s, 3H, CH_3) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6): δ = 162.5, 155.1, 150.0, 149.2, 140.9, 136.9, 128.5, 121.8, 120.7, 119.9 (q, *J* = 255.0, OCF_3).1, 14.7 ppm.

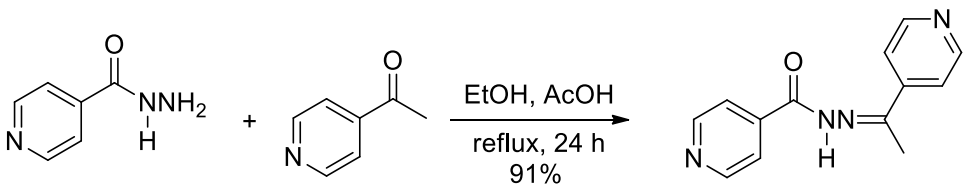
^{19}F-NMR (282 MHz, DMSO-d_6) δ = (-56.2, OCF_3) ppm.

IR (neat): 3188, 3080, 1668, 1387, 1280, 1201, 1151, 1113, 1070, 1017, 836 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 324.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{15}H_{13}F_3N_3O_2$: 324.0954 $[M^+]$; found 324.0958.

N'-(1-(Pyridin-4-yl)ethylidene)isonicotinohydrazide (189)



166
$C_6H_7N_3O$
[137.14]

C_7H_7NO
[121.14]

189
$C_{13}H_{12}N_4O$
[240.26]

Isonicotinohydrazide (**166**) (0.502 g, 3.66 mmol, 1.0 eq) afforded after 24 h *N*'-(1-(pyridin-4-yl)ethylidene)isonicotinohydrazide (**189**) (0.799 g, 3.33 mmol, 91%) as a white solid. **R_f** = 0.82 (SiO_2, CH_2Cl_2-MeOH, 4:1); **M_p** 186 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.19 (s, 1 H, NH), 8.77 (s, 2 H, CH, Ar), 8.66 (s, 2 H, CH, Ar), 7.80 (s, 4 H, CH, Ar), 2.40 (s, 3 H, CH_3) ppm.

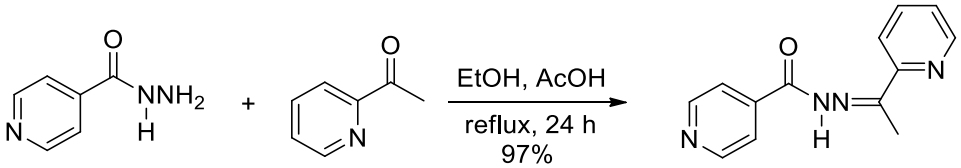
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 162.8, 153.6, 150.0, 150.0, 149.9, 144.8, 121.9, 120.5, 14.2 ppm.

IR (neat): 3519, 3185, 3038, 1672, 1591, 1539, 1410, 1387, 1275, 1141, 995 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 241.1(100) $[M+H]^+$, 121.1 (32.4).

HRMS (EI-MS): *m/z* calcd for $C_{13}H_{13}N_4O$: 241.1084 $[M+H]^+$; found 241.1082.

N'-(1-(Pyridin-2-yl)ethylidene)isonicotinohydrazide (190)



166
$C_6H_7N_3O$
[137.14]

C_7H_7NO
[121.14]

190
$C_{13}H_{12}N_4O$
[240.26]

(0.502 g, 3.65 mmol, 1.0 eq) of isonicotinohydrazide (**166**) gave after 24 h *N*'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide (**190**) (0.854 g, 3.55 mmol, 97%) as a white solid. **R_f** = 0.74 (SiO_2, CH_2Cl_2-MeOH, 4:1); **M_p** 168 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.12 (s, 1 H, NH), 8.78 (d, *J* = 4.1 Hz, 2 H, CH, Ar), 8.62 (m, 1 H, CH, Ar), 8.13 (d, *J* = 7.9 Hz, 1 H, CH, Ar), 7.91-7.28 (m, 4 H, CH, Ar), 2.48 (s, 3 H, CH_3) ppm.

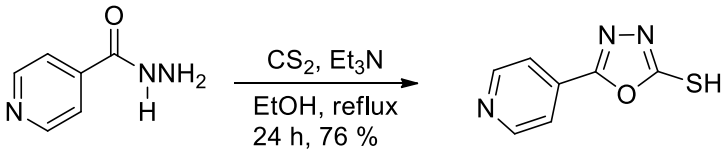
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 162.7, 156.1, 154.7, 150.0, 148.6, 140.9, 136.6, 124.3, 121.9, 120.5, 12.8 ppm.

IR (neat): 3181, 3100, 2367, 1666, 1624, 1581, 1409, 1375, 1302, 1218, 1151 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 241.1(100) $[M+H]^+$, 150.01 (7.4).

HRMS (EI-MS): *m/z* calcd for $C_{13}H_{13}N_4O$: 241.1084 $[M+H]^+$; found 241.1085.

5-(Pyridin-4-yl)-1,3,4-oxadiazole-2-thiol (174)



166
$C_6H_7N_3O$
[137.14]

174
$C_7H_5N_3OS$
[179.20]

To a solution of isonicotinohydrazide (**166**) (0.302 g, 2.20 mmol, 1.0 eq) in 5 ml EtOH was added carbon disulfide (0.502 g, 6.60 mmol, 3.0 eq) and Et_3N (0.267 g, 2.64 mmol, 1.2 eq). The solution was refluxed for 24 h then cold to room temperature and acidified with HCl (0.1 M in H_2O) and let stand for 4 h. The formed precipitate was filtered and washed with H_2O (2 x 10 ml) and petroleum ether (2 x 10 ml) to give oxadiazole-2-thiol **174** (0.296 g, 1.66 mmol, 76%) as a yellow solid. **R_f** = 0.27 (SiO_2, EtOAc); **M_p** 260 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 8.78 (s, 2 H, CH, Ar), 7.79 (s, 2 H, CH, Ar) ppm.

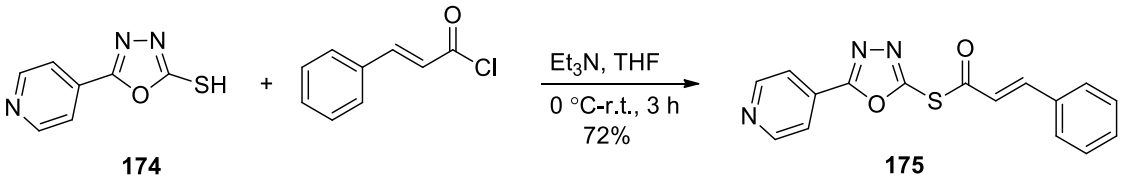
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 177.7, 158.7, 150.7, 129.7, 119.5 ppm.

IR (neat): 3090, 3034, 2281, 1860, 1596, 1550, 1533, 1494, 1358, 1329, 1234 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 180.0 (100) [M^+].

HRMS (EI-MS): *m/z* calcd for $C_7H_5N_3OS$: 179.0153 [M^+]; found 179.0157.

(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-yl) 3-phenylprop-2-enethioate (175)



174
$C_7H_5N_3OS$
[179.20]

C_9H_7ClO
[166.60]

175
$C_{16}H_{11}N_3O_2S$
[309.34]

To oxadiazole-2-thiol **174** (0.142 g, 0.793 mmol, 1.0 eq) in 10 ml of dry THF at 0 °C was added 0.5 ml Et_3N then cinnamoyl chloride (0.145 g, 0.872 mmol, 1.1 eq) was added dropwise to the solution at 0 °C. The reaction was warmed to room temperature and stirred for 3 h, then the solvent was evaporated and 10 ml saturated

$NaHCO_3$ solution was added. The precipitate was filtered and washed with H_2O (2 x 10 ml) and petroleum ether (2 x 10 ml). The product was recrystallized from EtOH to give oxadiazole **175** (0.176 g, 0.570 mmol, 72%) as a yellow solid. $\mathbf{R_f}$ = 0.45 (SiO_2, petroleum ether-EtOAc, 1:1); $\mathbf{M_p}$ 181 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.88 (d, *J* = 4.5 Hz, 2 H, CH, Ar), 8.08 (d, *J* = 15.7 Hz, 1 H, =CH), 7.89 (d, *J* = 5.6 Hz, 2 H, CH, Ar), 7.74 (d, *J* = 15.7 Hz, 1 H, =CH), 7.70-7.59 (m, 2 H, CH, Ar and CH), 7.65-7.37 (m, 3 H, CH, Ar) ppm.

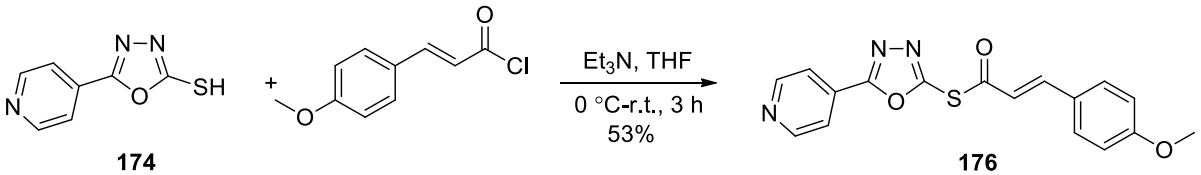
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 173.1, 161.6, 156.3, 151.0, 150.3, 133.9.0, 131.8, 129.1, 129.1, 129.0, 120.2, 115.2 ppm.

IR (neat): 3108, 3051, 1727, 1616, 1549, 1310, 1277, 1242, 1137, 1052, 984 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 310.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{16}H_{12}N_3O_2S$: 310.0645 $[M^+]$; found 310.0650.

(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-yl) 3-(4-methoxyphenyl)prop-2-enethioate (176)



174
$C_7H_5N_3OS$
[179.20]

$C_{10}H_8ClO_2$
[195.62]

176
$C_{17}H_{13}N_3O_3S$
[339.37]

To oxadiazole-2-thiol **174** (0.168 g, 0.947 mmol, 1.0 eq) in 10 ml of dry THF at 0 °C was added Et_3N (0.5 ml) then a dropwise addition of 4-methoxycinnamoyl chloride (0.105 g, 0.534 mmol, 1.1 eq) dropwise at 0 °C. The reaction was warmed to room temperature and stirred for 3 h, then the solvent was evaporated and 10 ml saturated aqueous $NaHCO_3$ solution was added. The precipitate was filtered and washed with H_2O (2 x 10 ml) and petroleum ether (2 x 10 ml). The product was recrystallized from EtOH to afford oxadiazole **176** (0.171 g, 0.503 mmol, 53%) as a yellow solid. $\mathbf{R_f}$ = 0.35 (SiO_2, petroleum ether-EtOAc, 1:1); $\mathbf{M_p}$ 214 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.88 (d, *J* = 4.9 Hz, 2 H, CH, Ar), 8.05 (d, *J* = 15.6 Hz, 1 H, CH), 7.90 (d, *J* = 5.9 Hz, 2 H, CH, Ar), 7.71-7.54 (m, 3 H, CH, Ar and CH), 6.97 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 3.88 (s, 3 H, OCH_3) ppm.

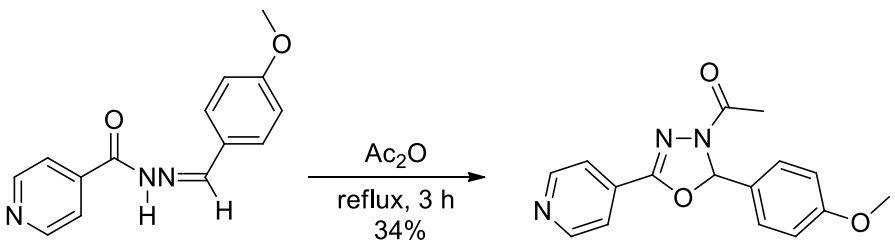
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 162.8, 161.8, 158.8, 156.2, 153.2, 151.1, 150.3, 131.2, 126.8, 120.2, 114.7, 112.5, 55.6 ppm.

IR (neat): 3036, 2999, 2937, 1117, 1592, 1571, 1514, 1314, 1285, 1238, 1176 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 340.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{17}H_{14}N_3O_3S$: 340.0750 $[M^+]$; found 340.0753.

1-(2-(4-Methoxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)ethanone (191)



180
$C_{14}H_{13}N_3O_2$
[255.27]

191
$C_{16}H_{15}N_3O_3$
[297.31]

Hydrazone **180** (3.50 g, 13.7 mmol, 1.0 eq), was dissolved in 30 ml acetic anhydride, the mixture was refluxed for 3 h. Then the solvent was evaporated and 20 ml H_2O was added, the mixture was extracted with EtOAc (3 x 20 ml), the organic layer dried over $MgSO_4$ and evaporated. The product was purified on column chromatography (SiO_2, petrolum ether-EtOAc, 1:1) to afford oxadiazole **191** (1.39 g, 4.68 mmol, 34%) as pale yellow solid. **R_f** = 0.45 (SiO_2, EtOAc); **M_p** 112 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.74 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.73 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.39 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 7.07 (s, 1 H, CH), 6.92 (d, *J* = 8.8 Hz, 2 H, CH, Ar), 3.81 (s, 3 H, OCH_3), 2.36 (s, 3 H, CH_3) ppm.

The ^{1}H NMR and spectral data is in accordance with the literature.[108]

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 167.9, 160.9, 153.6, 150.3, 132.2, 128.0, 120.4, 114.2, 93.1, 55.3, 21.4 ppm.

IR (neat): 3030, 2930, 2835, 1668, 1612, 1553, 1514, 1405, 1331, 1315, 1252 cm^{-1}.

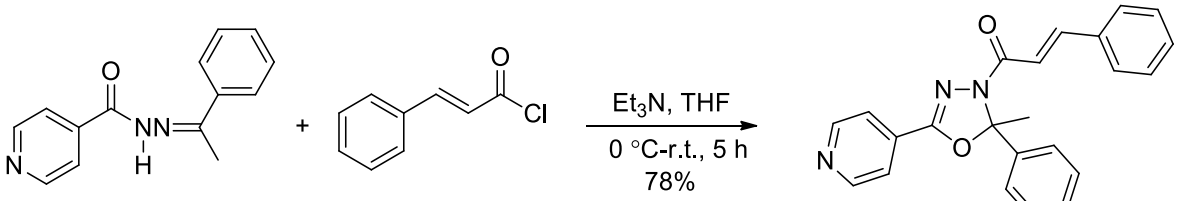
MS (EI, 70 eV): *m/z* (%) = 298.1 (100) $[M+H]^+$, (7.8).

HRMS (EI-MS): *m/z* calcd for $C_{16}H_{15}N_3O_3$: 297.1108 $[M^+]$; found 297.1098.

Synthesis of 2,3-dihydro-1,3,4-oxadiazole derivatives with an additional enone unit in position three 197-214:

To hydrazones **179-190** (0.0416 g-0.500 g, 0.163 mmol-2.22 mmol, 1.0 eq) in 10 ml of dry THF at 0 °C was added triethylamine (0.0198 g-0.270 g, 0.196 mmol-2.67 mmol, 1.2 eq). Then a solution of α,β-unsaturated acid chlorides (0.0299 g-0.407 g, 0.179 mmol-2.44 mmol, 1.1eq) in 5 ml THF was added dropwise to the mixture, the reaction was warmed to room temperature and stirred until it was finished (1-24 h). The solvent was evaporated and the residue was purified by TLC (SiO_2, petroleum ether-EtOAc, 1:1) to afford the desired 2,3-dihydro-1,3,4-oxadiazoles **197**-**214**.

1-(2-Methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)-3-phenylprop-2-en-1-one (197)



182
$C_{14}H_{13}N_3O$
[239.27]

C_9H_7ClO
[166.60]

197
$C_{23}H_{19}N_3O_2$
[369.42]

Hydrazone **182** (0.100 g, 0.420 mmol, 1.0 eq) gave after 5 h oxadiazole **197** (0.120 g, 0.325 mmol, 78%) as a yellow solid. **R_f** = 0.63 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 139 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.76 (d, *J* = 5.6 Hz, 2 H, CH, Ar), 7.78 (d, *J* = 6.0 Hz, 2 H, CH, Ar), 7.71 (d, *J* = 15.9 Hz, 1 H, =CH), 7.64-7.56 (m, 4 H, Ar), 7.46 (d, *J* = 15.9 Hz, 1 H, =CH), 7.43-7.33 (m, 6 H, Ar), 2.40 (s, 3 H, CH_3) ppm.

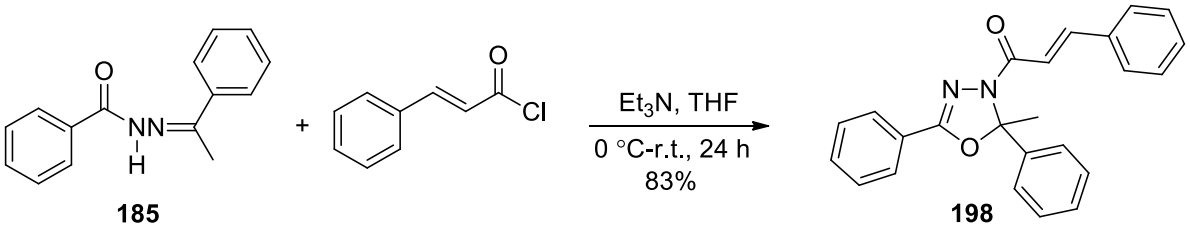
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 162.1, 152.1, 150.3, 143.3, 138.5, 134.9, 132.4, 130.0, 129.5, 128.8, 128.6, 128.2, 125.7, 120.4, 117.5, 102.0, 23 ppm.

IR (neat): 3061, 2940, 2860, 1653, 1612, 1596, 1407, 1343, 1263, 1229, 1140 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 370.1 (100) $[M+H]^+$, 240.1 (8.2).

HRMS (EI-MS): *m/z* calcd for $C_{23}H_{19}N_3O_2$: 369.1472 $[M^+]$; found 369.1473.

1-(2-Methyl-2,5-diphenyl-1,3,4-oxadiazol-3(2*H*)-yl)-3-phenylprop-2-en-1-one (198)



185
$C_{15}H_{14}N_2O$
[238.28]

C_9H_7ClO
[166.60]

198
$C_{24}H_{20}N_2O_2$
[368.43]

Hydrazone **185** (0.0985 g, 0.413 mmol, 1.0 eq) gave after 24 h oxadiazole **198** (0.126 g, 0.343 mmol, 83%) as a white solid. **R_f** = 0.84 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 105 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.01-7.90 (m, 2 H, CH, Ar), 7.70 (d, *J* = 15.9 Hz, 1 H, CH), 7.64-7.57 (m, 4 H, CH, Ar), 7.56-7.45 (m, 4 H, CH, Ar), 7.45-7.33 (m, 6 H, CH, Ar), 2.39 (s, 3 H, CH_3) ppm.

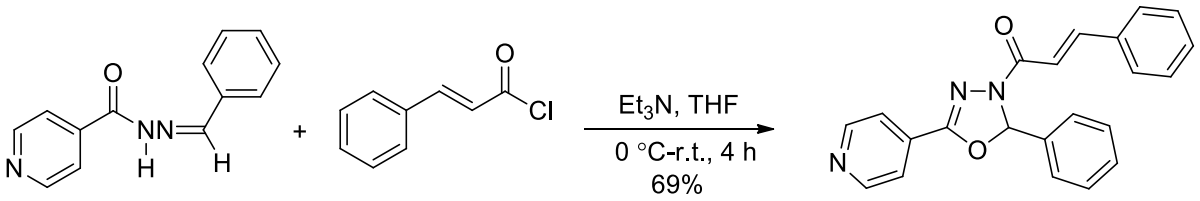
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 161.8, 161.8, 154.1, 154.1, 128.7, 128.7, 128.5, 128.5, 128.2, 128.2, 125.8, 125.8, 118.1, 118.1, 101.0, 101.0, 23.0 ppm.

IR (neat): 3061, 1653, 1619, 1449, 1411, 1378, 1333, 1287, 1258, 1197, 1058 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 369.2 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{24}H_{21}N_2O_2$: 369.1598 $[M+H]^+$; found 369.1605.

3-Phenyl-1-(2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (199)



179
$C_{13}H_{11}N_3O$
[225.25]

C_9H_7ClO
[166.60]

199
$C_{22}H_{17}N_3O_2$
[355.39]

Hydrazone **179** (0.500 g, 2.22 mmol, 1.0 eq) gave after 4 h oxadiazole **199** (0.597 g, 1.68 mmol, 69%) as a white solid. **R_f** = 0.53 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 194 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 8.78 (d, *J* = 4.4 Hz, 2 H, CH, Ar), 7.97-7.28 (m, 15 H, CH and CH, Ar) ppm.

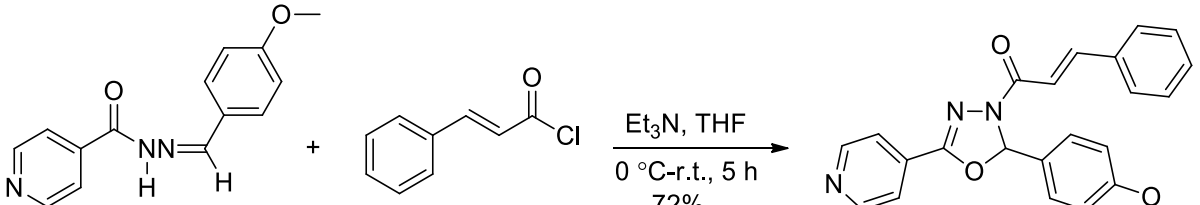
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 161.5, 155.9, 153.3, 150.3, 142.5, 134.2, 130.3, 130.0, 128.9, 128.8, 128.2, 126.7, 120.3, 117.0, 93.0 ppm.

IR (neat): 3056, 3033, 1660, 1620, 1598, 1411, 1340, 1239, 1212, 1077, 1010, 913, 825, 759, 698, 662 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 356.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{22}H_{18}N_3O_2$: 356.1394 $[M+H]^+$; found 356.1395.

1-(2-(4-Methoxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)-3-phenylprop-2-en-1-one (200)



180
$C_{14}H_{13}N_3O_2$
[255.27]

C_9H_7ClO
[166.60]

200
$C_{23}H_{19}N_3O_3$
[385.42]

Hydrazone **180** (41.6 mg, 0.163 mmol, 1.0 eq) gave after 5 h oxadiazole **200** (43.5 mg, 0.113 mmol, 72%) as a yellow solid. **R_f** = 0.44 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 156 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 8.77 (d, *J* = 5.9 Hz, 2 H, CH, Ar), 7.84 (d, *J* = 5.9 Hz, 2 H, CH, Ar), 7.75 (d, *J* = 3.8 Hz, 2 H, CH, Ar), 7.61 (d, *J* = 16.0 Hz, 1 H, =CH), 7.50 (m, 6 H, CH, Ar), 7.34 (s, 1 H, CH), 7.00 7.61 (d, *J* = 8.7 Hz, 2 H, CH, Ar), 3.77 (s, 3 H, OCH_3) ppm.

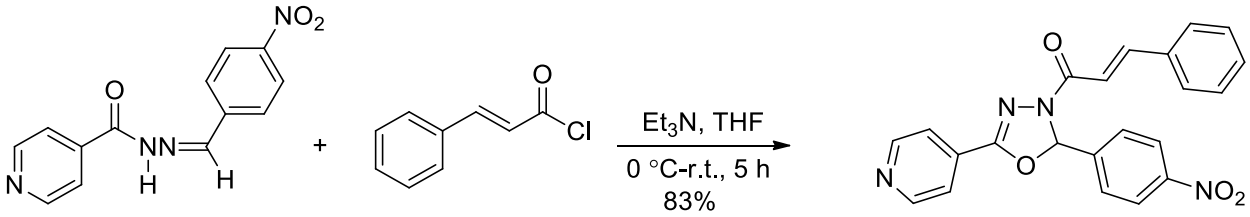
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 161.4, 160.4, 153.2, 150.5, 142.4, 134.3, 131.4, 130.3, 128.9, 128.2, 128.2, 128.1, 120.2, 117.1, 114.1, 92.9, 55.2 ppm.

IR (neat): 3061, 3023, 1651, 1609, 1517, 1434, 1338, 1297, 1253, 1239, 1181 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 386.2 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{23}H_{20}N_3O_3$: 386.1499 $[M+H]^+$; found 386.1501.

1-(2-(4-Nitrophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)-3-phenylprop-2-en-1-one (201)



181
$C_{13}H_{10}N_4O_3$
[270.24]

C_9H_7ClO
[166.60]

201
$C_{22}H_{16}N_4O_4$
[400.39]

Hydrazone **181** (0.300 g, 0.111 mmol, 1.0 eq) gave after 5 h oxadiazole **201** (0.371 g, 0.927 mmol, 83%) as a white solid. **R_f** = 0.41 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 212 °C.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 8.79 (d, *J* = 5.8 Hz, 2 H, CH, Ar), 8.30 (d, *J* = 8.6 Hz, 2 H, CH, Ar), 7.86 (d, *J* = 6.4 Hz, 4 H, CH, Ar), 7.79-7.71 (m, 2 H, CH, Ar), 7.65 (d, *J* = 16.0 Hz, 1 H, CH), 7.51 (d, *J* = 16.8 Hz, 1 H, CH),7.48-7.34 (m, 4 H, CH, Ar and CH) ppm.

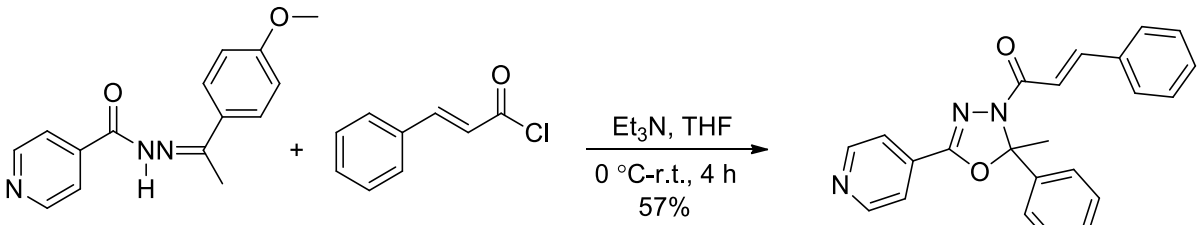
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 161.8, 153.4, 150.5, 148.4, 142.9, 142.2, 134.1, 131.0, 130.4, 128.9, 128.4, 128.3, 124.0, 122.0, 120.3, 116.6 ppm.

IR (neat): 3078, 2186, 1654, 1597, 1522, 1428, 1409, 1347, 1300, 1310, 1234 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 401.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{22}H_{17}N_4O_4$: 401.1244 $[M+H]^+$; found 401.1245.

1-(2-(4-Methoxyphenyl)-2-methyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)-3-phenylprop-2-en-1-one (202)



184
$C_{15}H_{15}N_3O_2$
[269.30]

C_9H_7ClO
[166.60]

202
$C_{24}H_{21}N_3O_3$
[399.44]

Hydrazone **184** (0.0850 g, 0.316 mmol, 1.0 eq) gave after 4 h oxadiazole **202** (0.0725 g, 0.181 mmol, 57%) as a white solid. **R_f** = 0.48 (SiO_2, petroleum ether: EtOAc, 1:1); **M_p** 203 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.75 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.77 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.70 (d, *J* = 15.9 Hz, 1 H, CH), 7.59 (m, 2 H, CH, Ar), 7.51 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 5.45 (d, *J* = 15.9 Hz, 1 H, CH), 7.42-7.38 (m, 3 H, CH, Ar), 6.92 (d, *J* = 8.9 Hz, 2 H, CH, Ar), 3.8 (s, 3 H, OCH_3), 2.36 (s, 3 H, CH_3) ppm.

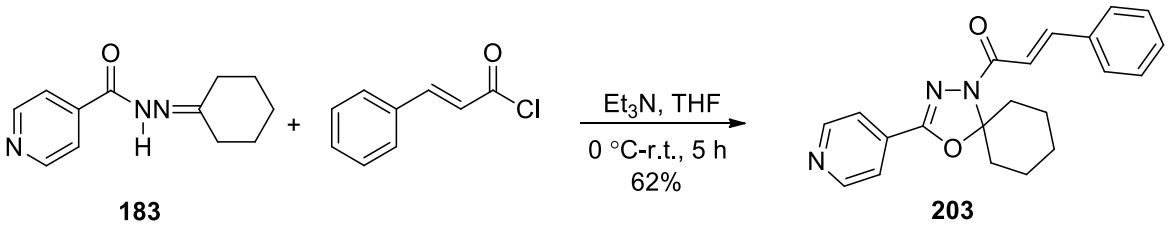
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 162.0, 160.3, 152.1, 150.4, 143.2, 135.0, 132.4, 130.7, 130.0, 128.8, 128.2, 127.3, 120.4, 117.6, 113.9, 102.0, 55.3, 23.0 ppm.

IR (neat): 3056, 3033, 1660, 1620, 1598, 1411, 1340, 1290, 1239, 1212, 1077 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 400.2 (100) $[M+H]^+$, 270.1 (6.4).

HRMS (EI-MS): *m/z* calcd for $C_{24}H_{21}N_3O_3$: 399.1577 $[M^+]$; found 399.1598.

3-Phenyl-1-(3-(pyridin-4-yl)-4-oxa-1,2-diazaspiro[4.5]dec-2-en-1-yl)prop-2-en-1-one (203)



183
$C_{12}H_{15}N_3O$
[217.27]

C_9H_7ClO
[166.60]

203
$C_{21}H_{21}N_3O_2$
[347.41]

Hydrazone **183** (0.114 g, 0.522 mmol, 1.0 eq) gave after 5 h oxadiazole **203** (0.112 g, 0.323 mmol, 62%) as a white solid. **R_f** = 0.50 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 143 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.74 (d, *J* = 4.1 Hz, 2 H, CH, Ar), 7.79-7.66 (m, 3 H, CH, Ar and CH), 7.64-7.54 (m, 2 H, CH, Ar), 7.47-7.31 (m, 4 H, Ar and CH), 2.89-2.59 (m, 2 H, CH_2), 2.07-1.92 (m, 2 H, CH_2), 1.86-1.56 (m, 5 H, CH_2), 1.53-1.29 (m, 1 H, CH_2) ppm.

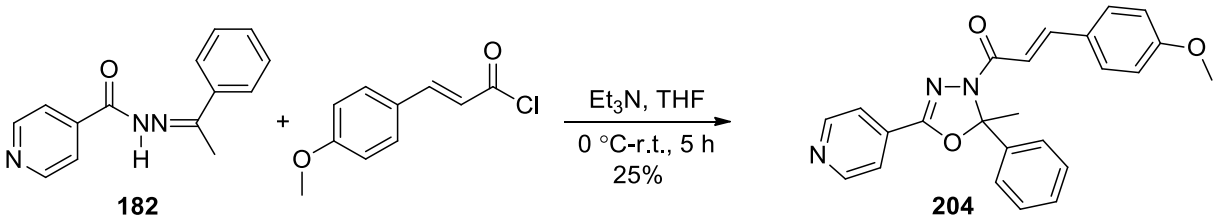
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 162.2, 152.0, 150.3, 142.5, 135.0, 132.7, 129.8, 128.7, 128.1, 120.3, 118.1, 103.7, 32.5, 24.2, 22.7 ppm.

IR (neat): 3067, 3036, 2996, 1655, 1599, 1422, 1406, 1336, 1315, 1241, 1193 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 348.2 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{21}H_{21}N_3O_2$: 347.1628 $[M^+]$; found 347.1631.

3-(4-Methoxyphenyl)-1-(2-methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (204)



182
$C_{14}H_{13}N_3O$
[239.27]

$C_{10}H_9ClO_2$
[196.63]

204
$C_{24}H_{21}N_3O_3$
[399.44]

Hydrazone **182** (0.200 g, 0.838 mmol, 1.0 eq) gave after 5 h oxadiazole **204** (0.0826 g, 0.207 mmol, 25%) as a yellow solid. **R_f** = 0.44 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 156 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.75 (d, *J* = 5.9 Hz, 2 H, CH, Ar), 7.77 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.60 (d, *J* = 11.9 Hz, 1H, =CH), 7.60 (m, 2 H, CH, Ar), 7.51 (d, *J* = 6.7 Hz, 1H, CH, Ar), 7.45 (d, *J* = 11.9 Hz, 2 H, =CH), 7.42-7.36 (m, 3 H, Ar), 6.92 (d, *J* = 8.8 Hz, 2H, CH, Ar), 3.84 (s, 3 H, CH_3), 2.38 (s, 3 H, CH_3) ppm.

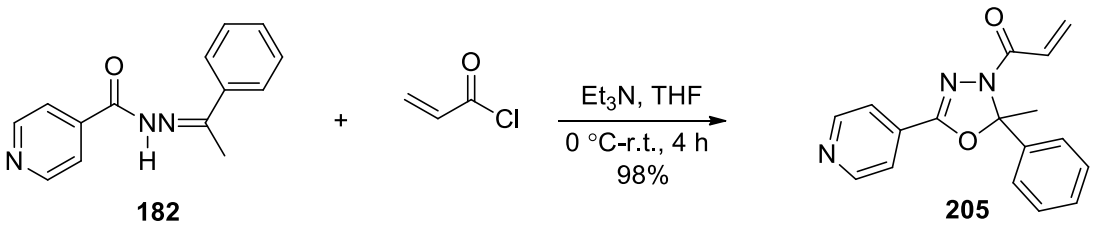
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 162.5, 161.2, 152.0, 150.4, 143.0, 138.6, 132.4, 129.9, 129.4, 128.6, 127.7, 125.7, 120.4, 115.0, 114.2, 102.0, 55.3, 23.0 ppm.

IR (neat): 3050, 2837, 1655, 1597, 1511, 1405, 1336, 1244, 1172, 1028, 987 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 400.2 (100) $[M+H]^+$, 240.1 (7.7).

HRMS (EI-MS): *m/z* calcd for $C_{24}H_{22}N_3O_3$: 400.1656 $[M+H]^+$; found 400.1655.

1-(2-Methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (205)



182
$C_{14}H_{13}N_3O$
[239.27]

C_3H_3ClO
[90.51]

205
$C_{17}H_{15}N_3O_2$
[293.32]

Hydrazone **182** (0.151 g, 0.632 mmol, 1.0 eq) gave after 4 h oxadiazole **205** (0.181 g, 0.618 mmol, 98%) as a yellow solid. **R_f** = 0.48 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 106 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.71 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.70 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.63-7.47 (m, 2 H, CH, Ar), 7.45-7.32 (m, 3 H, CH, Ar), 7.14 (dd, *J* = 17.2, 10.4 Hz, 1 H, CH), 6.41 (dd, *J* = 17.2, 1.8 Hz, 1 H, CH), 5.77 (dd, *J* = 10.4, 1.8 Hz, 1 H, CH), 2.34 (s, 3 H, CH_3) ppm.

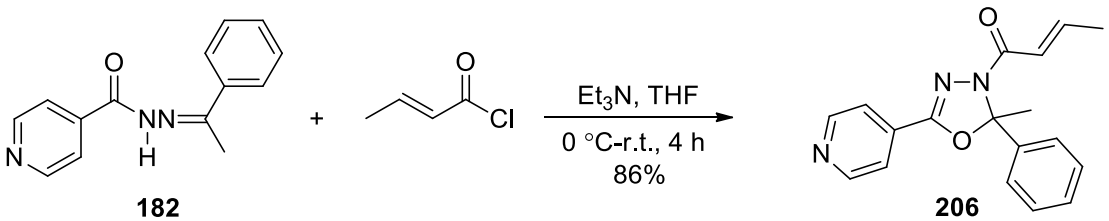
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 161.3, 152.1, 150.3, 138.2, 132.0, 129.3, 128.9, 128.4, 127.4, 125.6, 120.2, 101.7, 22.7 ppm.

IR (neat): 3080, 3033, 2991, 2934, 1660, 1626, 1597, 1427, 1412, 1377, 1332 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 294.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{17}H_{16}N_3O_2$: 294.1237 $[M+H]^+$; found 294.1239.

1-(2-Methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)but-2-en-1-one (206)



182
$C_{14}H_{13}N_3O$
[239.27]

C_4H_5ClO
[104.53]

206
$C_{18}H_{17}N_3O_2$
[307.35]

Hydrazone **182** (0.100 g, 0.295 mmol, 1.0 eq) gave after 4 h oxadiazole **206** (0.0781 g, 0.254 mmol, 86%) as a yellow solid. **R_f** = 0.52 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 116 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.73 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.73 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.55 (m, 2 H, CH, Ar), 7.39 (m, 3 H, CH, Ar), 6.97 (m, 1 H, CH), 6.85 (d, *J* = 15.5 Hz, 1 H, CH), 2.34 (s, 3 H, CH_3), 1.94 (d, *J* = 6.5 Hz, 3 H, CH_3) ppm.

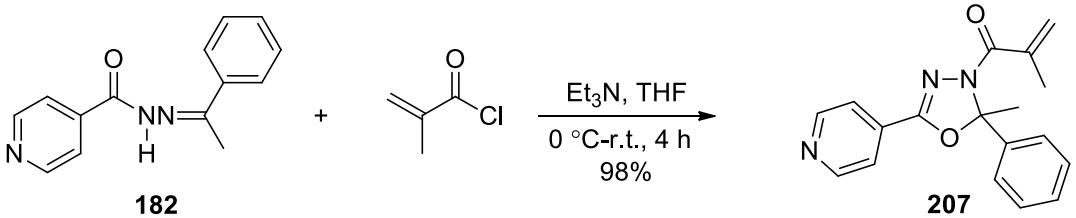
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 162.0, 151.9, 150.4, 143.1, 138.6, 132.4, 129.4, 128.5, 125.7, 121.9, 120.3, 101.8, 23.0, 18.2 ppm.

IR (neat): 3060, 3016, 2987, 2942, 1663, 1631, 1597, 1408, 1382, 1335, 1316 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 308.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{18}H_{18}N_3O_2$: 308.1394 $[M+H]^+$; found 308.1395.

2-Methyl-1-(2-methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (207)



182
$C_{14}H_{13}N_3O$
[239.27]

C_4H_5ClO
[104.53]

207
$C_{18}H_{17}N_3O_2$
[307.35]

Hydrazone **182** (0.200 g, 0.590 mmol, 1.0 eq) gave after 4 h oxadiazole **207** (0.179 g, 0.581 mmol, 98%) as a colorless oil. **R_f** = 0.35 (SiO_2, petroleum ether-EtOAc, 1:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.69 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.67 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.59-7.48 (m, 2 H, CH, Ar), 7.45-7.29 (m, 3 H, CH, Ar), 5.74 (t, 1 H, *J* = 0.9 Hz, CH), 5.49 (t, *J* = 1.5 Hz, 1 H, CH), 2.32 (s, 3 H, CH_3), 2.04 (t, *J* = 1.2 Hz, 3 H, CH_3) ppm.

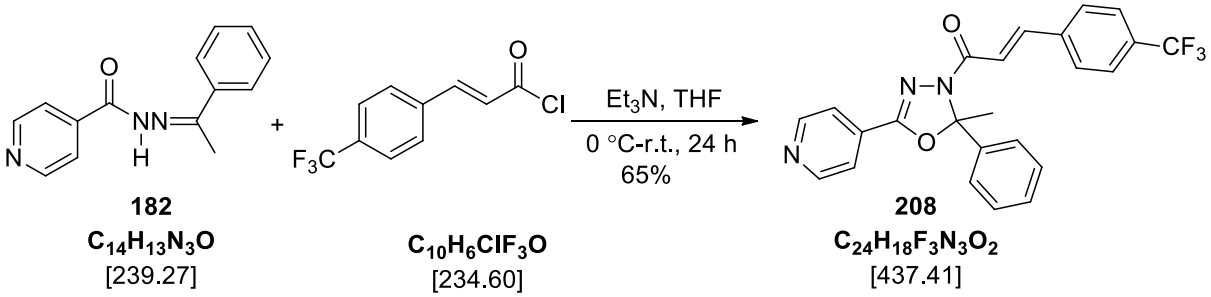
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 165.8, 151.9, 150.2, 139.1, 138.4, 132.2, 129.3, 128.4, 125.5, 121.7, 120.2, 102.0, 22.6, 19.9 ppm.

IR (neat): 2990, 1652, 1623, 1598, 1426, 1407, 1333, 1313, 1250, 1184, 1049 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 308.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{18}H_{17}N_3O_2$: 307.1315 $[M^+]$; found 307.1324.

1-(2-Methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)-3-(4-(trifluoromethyl) phenyl)prop-2-en-1-one (208)



182
$C_{14}H_{13}N_3O$
[239.27]

$C_{10}H_6ClF_3O$
[234.60]

208
$C_{24}H_{18}F_3N_3O_2$
[437.41]

Hydrazone **182** (22.0 mg, 0.089 mmol, 1.0 eq) gave after 24 h oxadiazole **208** (25.4 mg, 0.058 mmol, 65%) as a yellow solid. **R_f** = 0.58 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 189 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.77 (d, *J* = 4.8 Hz, 2 H, CH, Ar), 7.79 (d, *J* = 4.5 Hz, 2 H, CH, Ar), 7.72 (d, *J* = 6.9 Hz, 1 H, CH), 7.67 (m, 3 H, CH, Ar), 7.63 (d, *J* = 5.7 Hz, 1 H, CH), 7.60 (m, 1 H, CH, Ar), 7.58 (d, *J* = 1.5 Hz, 1 H, CH, Ar), 7.52 (d, *J* = 12.0 Hz, 1 H, CH), 7.42 (m, 3 H, CH, Ar), 2.40 (s, 3 H, CH_3) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 161.4, 152.4, 150.3, 141.4, 138.4, 132.4, 131.5 (q, *J* = 32.8 Hz, *C*CF_3), 129.6, 128.6, 128.3, 127.8, 125.8, 125.8, 123.9 (q, *J* = 269.0 Hz, CF_3), 120.5, 120.1, 102.2, 23.0 ppm.

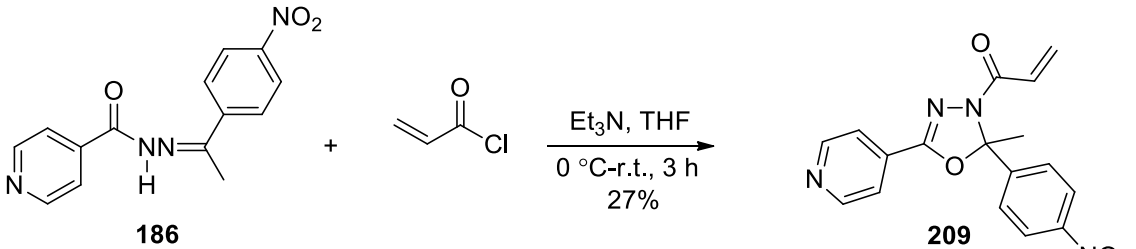
IR (neat): 3061, 1656, 1619, 1408, 1317, 1253, 1171, 1119, 1064, 984, 831 cm^{-1}.

^{19}F-NMR (282 MHz, $CDCl_3$): δ = (s, -62.7, CF_3) ppm.

MS (ESI, 120 V): *m/z* (%) = 438.1(100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{24}H_{19}F_3N_3O_2$: 438.1424 $[M+H]^+$; found: 438.1428.

1-(2-Methyl-2-(4-nitrophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (209)



186
$C_{14}H_{12}N_4O_3$
[284.27]

C_3H_3ClO
[90.51]

209
$C_{17}H_{14}N_4O_4$
[338.32]

Hydrazone **186** (0.101 g, 0.352 mmol, 1.0 eq) gave after 3 h oxadiazole **209** (32.3 mg, 0.095 mmol, 27%) as a yellow oil. **R_f** = 0.36 (SiO_2, petroleum ether-EtOAc, 1:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.77 (d, *J* = 5.6 Hz, 2 H, CH, Ar), 8.25 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 7.77 (d, *J* = 9.0 Hz, 2 H, CH, Ar), 7.73 (d, *J* = 6.1 Hz, 2 H, CH, Ar), 7.10 (dd, *J* = 17.2, 10.4 Hz, 1 H, =CH), 6.43 (dd, *J* = 17.2, 1.7 Hz, 1 H, =CH), 5.84 (dd, *J* = 10.4, 1.7 Hz, 1 H, =CH), 2.37 (s, 3 H, CH_3) ppm.

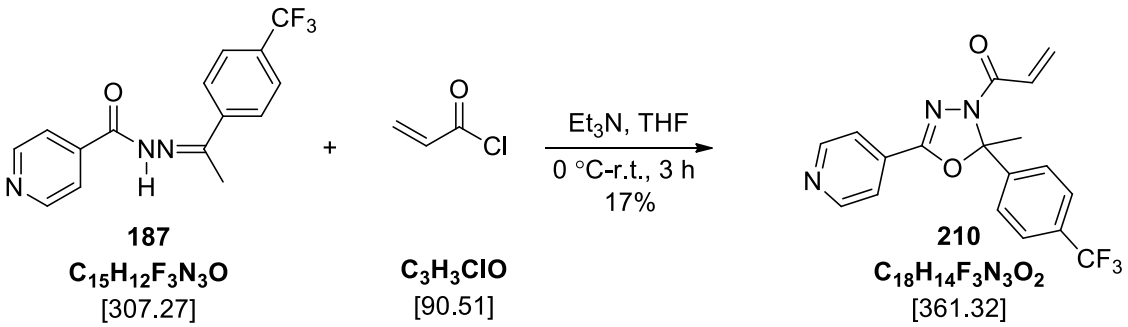
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 161.8, 152.2, 150.5, 148.4, 144.8, 131.8, 129.8, 127.2, 127.1, 123.8, 120.3, 100.7, 23.0 ppm.

IR (neat): 3051, 1660, 1622, 1598, 1521, 1427, 1410, 1348, 1330, 1310, 1256 cm^{-1}.

MS (ESI, 120 V): *m/z* (%) = 339.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{17}H_{15}N_4O_4$: 339.1088 $[M+H]^+$; found 339.1095.

1-(2-Methyl-5-(pyridin-4-yl)-2-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (210)



187
$C_{15}H_{12}F_3N_3O$
[307.27]

C_3H_3ClO
[90.51]

210
$C_{18}H_{14}F_3N_3O_2$
[361.32]

Hydrazone **187** (0.500 g, 1.63 mmol, 1.0 eq) gave after 3 h oxadiazole **210** (0.0986 g, 0.0273 mmol, 17%) as a yellow oil. R_f = 0.58 (SiO_2, petroleum ether-EtOAc, 1:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.75 (d, *J* = 5.1 Hz, 2 H, CH, Ar), 7.82-7.54 (m, 6 H, CH, Ar), 7.12 (dd, *J* =17.2, 10.4 Hz, 1 H, =CH), 6.43 (dd, *J* =17.2, 1.7 Hz, 1 H, =CH), 5.82 (dd, *J* =10.4, 1.7 Hz, 1 H, CH), 2.36 (s, 3 H, CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 161.7, 152.2, 150.4, 142.0, 131.9, 131.5 (q, *J* = 32.3 Hz, *C*CF_3), 129.5, 127.3, 126.3, 125.6 (q, *J* = 3.7 Hz, *C*H*C*CF_3), 123.7 (q, *J* = 270.8 Hz, CF_3), 120.3, 101.1, 22.9 ppm.

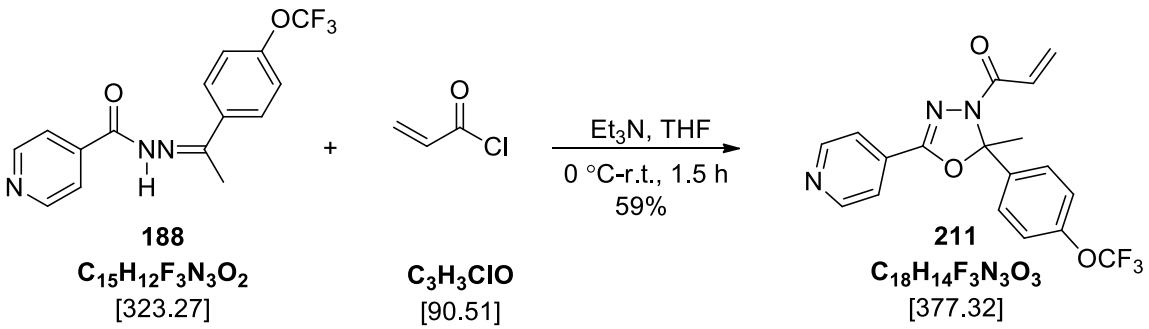
^{19}F-NMR (282 MHz, $CDCl_3$): δ = (-63.3, CF_3) ppm.

IR (neat): 1662, 1621, 1599, 1428, 1410, 1325, 1271, 1167, 1118, 1089, 1061 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 362.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{18}H_{15}F_3N_3O_2$: 362.1111 $[M+H]^+$; found 362.1113.

1-(2-Methyl-5-(pyridin-4-yl)-2-(4-(trifluoromethoxy)phenyl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (211)



188
$C_{15}H_{12}F_3N_3O_2$
[323.27]

C_3H_3ClO
[90.51]

211
$C_{18}H_{14}F_3N_3O_3$
[377.32]

Hydrazone **188** (0.100 g, 0.309 mmol, 1.0 eq) gave after 1.5 h oxadiazole **211** (0.0687 g, 0.182 mmol, 59%) as a yellow solid. **R_f** = 0.61 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 78 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.74 (dd, *J* = 4.6, 1.5 Hz, 2H, CH, Ar), 7.72 (dd, *J* = 4.5, 1.6 Hz, 2H, CH, Ar), 7.64 – 7.54 (m, 2H, CH, Ar), 7.23 (d, *J* = 8.1 Hz, 2H, CH, Ar), 7.12 (dd, *J* = 17.2, 10.4 Hz, 1H, =CH), 6.42 (dd, *J* = 17.2, 1.7 Hz, 1H, =CH), 5.80 (dd, *J* = 10.4, 1.7 Hz, 1H, =CH), 2.34 (s, 3H, CH_3) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 161.6, 152.1, 150.4, 149.8, 137.0, 132.0, 129.3, 127.6, 127.4, 124.2 (q, *J* = 263.1 Hz, CF_3), 120.7, 120.3, 119.0, 101.2, 23.0 ppm.

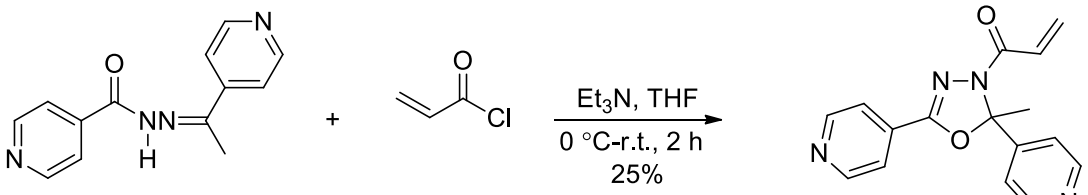
^{19}F-NMR (282 MHz, $CDCl_3$): δ = (s, -57.7, CF_3) ppm.

IR (neat): 1655, 1618, 1601, 1504, 1427, 1412, 1381, 1333, 1253, 1216, 1158 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 378.1 (100) $[M+H]^+$.

HRMS (EI-MS): *m/z* calcd for $C_{18}H_{15}F_3N_3O_3$: 378.1060 $[M+H]^+$; found 378.1066.

1-(2-Methyl-2,5-di(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (212)



189
$C_{13}H_{12}N_4O$
[240.26]

C_3H_3ClO
[90.51]

212
$C_{16}H_{14}N_4O_2$
[294.31]

Hydrazone **189** (0.200 g, 0.834 mmol, 1.0 eq) gave after 2 h oxadiazole **212** (0.0623 g, 0.212 mmol, 25%) as a white solid. R_f = 0.40 (SiO_2, EtOAc); **M_p** 128 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.73 (d, *J* = 6.1 Hz, 2H, CH, Ar), 8.65 (d, *J* = 6.2 Hz, 2H, CH, Ar), 7.69 (d, *J* = 6.2 Hz, 2H, CH, Ar), 7.44 (d, *J* = 6.2 Hz, 2H, CH, Ar), 7.08 (dd, *J* = 17.2, 10.4 Hz, 1H, =CH), 6.41 (dd, *J* = 17.2, 1.7 Hz, 1H, =CH), 5.80 (dd, *J* = 10.4, 1.8 Hz, 1H, =CH), 2.30 (s, 3H, CH_3) ppm.

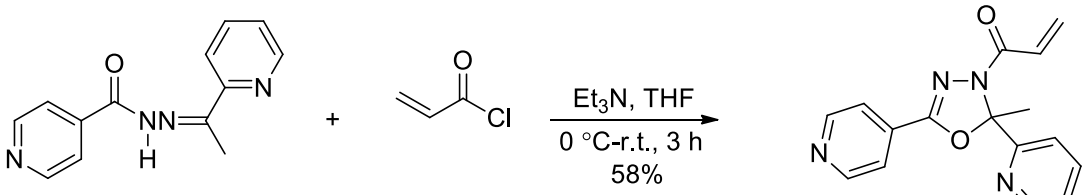
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 161.7, 152.2, 150.4, 150.3, 146.5, 131.6, 129.6, 127.1, 120.3, 120.2, 100.4, 22.5 ppm.

IR (neat): 3069, 2992, 1659, 1622, 1598, 1553, 1408, 1380, 1328, 1309, 1261 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 295.1 (100) $[M+H]^+$, 241.1 (10.7).

HRMS (EI-MS): *m/z* calcd for $C_{16}H_{15}N_4O_2$: 295.1190 $[M+H]^+$; found 295.1191.

1-(2-Methyl-2-(pyridin-2-yl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (213)



190
$C_{13}H_{12}N_4O$
[240.26]

C_3H_3ClO
[90.51]

213
$C_{16}H_{14}N_4O_2$
[294.31]

Hydrazone **190** (0.201 g, 0.835 mmol, 1.0 eq) gave after 3 h oxadiazole **213** (0.142 g, 0.481 mmol, 58%) as a colorless oil. R_f = 0.41 (SiO_2, EtOAc).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.70 (d, *J* = 6.2 Hz, 2H, CH, Ar), 8.63 (dd, *J* = 4.8, 0.8 Hz, 1H, CH, Ar), 7.76 (dd, *J* = 7.8, 1.8 Hz, 1H, CH, Ar), 7.71 (d, *J* = 6.1 Hz, 2H, CH, Ar), 7.54 (d, *J* = 8.0 Hz, 1H, CH, Ar), 7.29 (d, *J* = 7.6, 1H, CH, Ar), 7.17 (dd, *J* = 17.2, 10.4 Hz, 1H, =CH), 6.39 (dd, *J* = 17.2, 1.8 Hz, 1H, =CH), 5.80 (dd, *J* = 10.4, 1.8 Hz, 1H, =CH), 2.38 (s, 3H, CH_3) ppm.

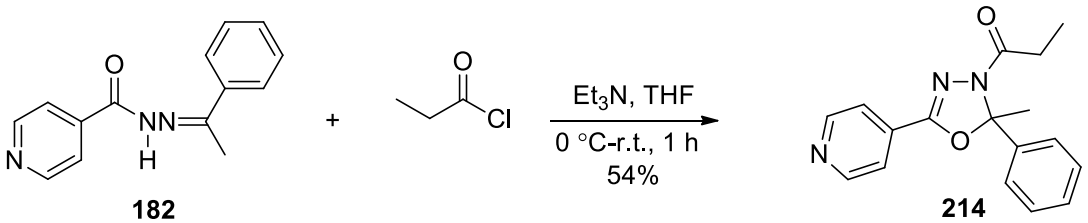
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 161.6, 155.7, 152.5, 150.3, 149.6, 136.9, 132.4, 129.2, 127.4, 124.3, 120.6, 120.5, 101.5, 21.5 ppm.

IR (neat): 3055, 1658, 1621, 1598, 1430, 1410, 1373, 1331, 1312, 1270, 1204 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 295.1 (100) $[M+H]^+$, 241.1 (8.4).

HRMS (EI-MS): *m/z* calcd for $C_{16}H_{15}N_4O_2$: 295.1190 $[M+H]^+$; found 295.1193.

1-(2-Methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)propan-1-one (214)



182
$C_{14}H_{13}N_3O$
[239.27]

C_3H_5ClO
[92.52]

214
$C_{17}H_{17}N_3O_2$
[295.34]

Hydrazone **182** (0.200 g, 0.837 mmol, 1.0 eq) gave after 1 h oxadiazole **214** (0.133 g, 0.451 mmol, 54%) as a brown solid. **R_f** = 0.57 (SiO_2, petroleum ether-EtOAc, 1:1); **M_p** 71 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.71 (d, *J* = 5.9 Hz, 2H, CH, Ar), 7.70 (d, *J* = 6.1 Hz, 2H, CH, Ar), 7.53 (dd, *J* = 7.8, 2.0 Hz, 2H, CH, Ar), 7.36 (m, 3H, CH, Ar), 2.71 (dq, *J* = 7.5, 3.3 Hz, 2H, CH_2), 2.29 (s, 3H, CH_3), 1.15 (t, *J* = 7.5 Hz, 3H, CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.5, 151.6, 150.2, 138.8, 132.4, 129.3, 128.4, 125.6, 120.2, 101.5, 27.7, 23.0, 8.4 ppm.

IR (neat): 3078, 2984, 2936, 1668, 1628, 1596, 1410, 1378, 1349, 1378, 1349 cm^{-1}.

MS (EI, 70 eV): *m/z* (%) = 296.1 (100) $[M+H]^+$, 240.1 (10.0).

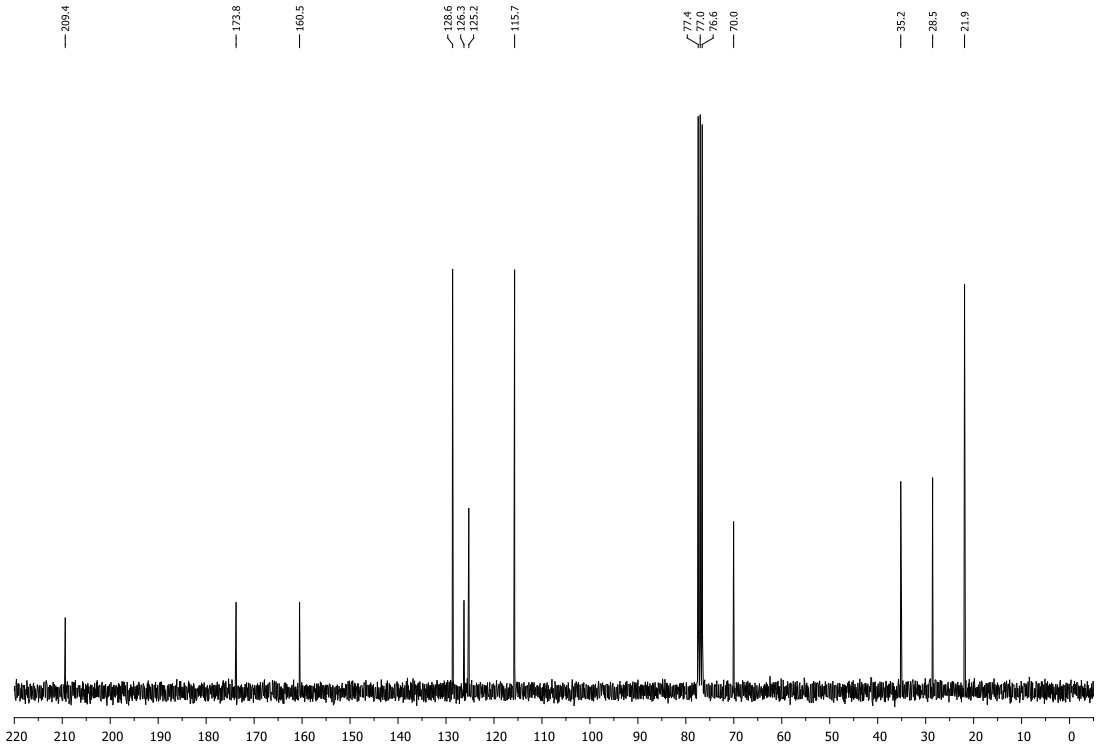
HRMS (EI-MS): *m/z* calcd for $C_{17}H_{18}N_3O_2$: 296.1394 $[M+H]^+$; found 296.1394.

6. Appendix (NMR spectra)

3-(4-Isopropoxyphenyl)cyclopent-2-enone (71) (in $CDCl_3$)

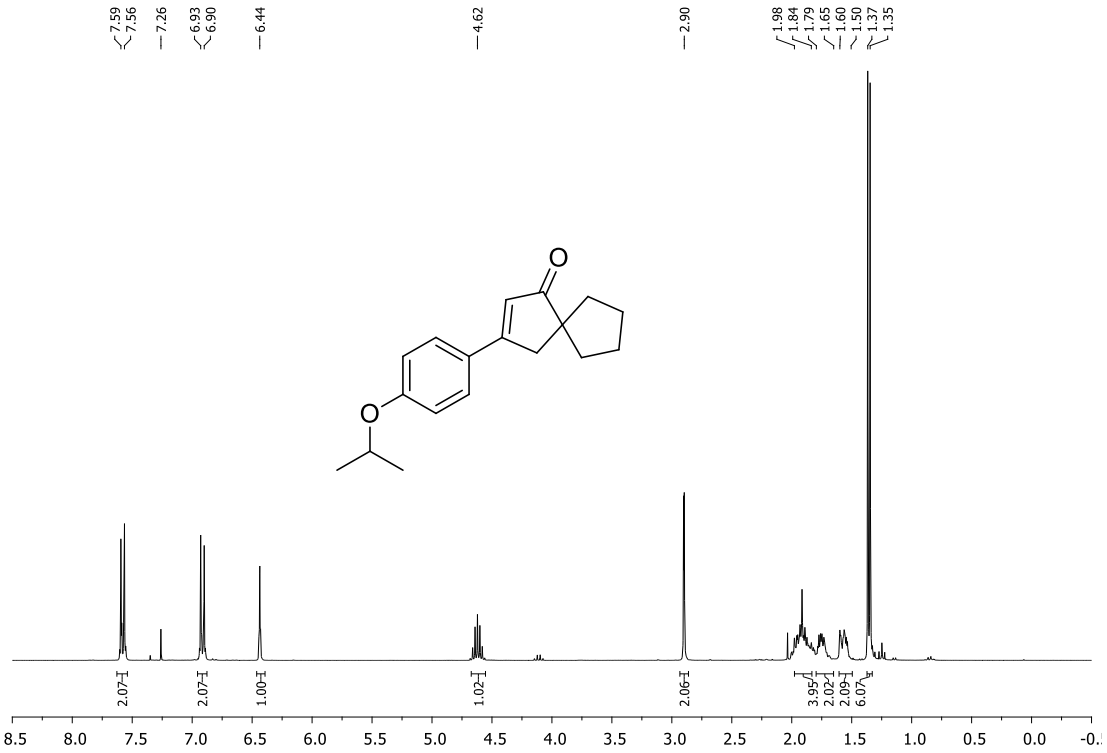
^{1}H NMR

^{13}C NMR

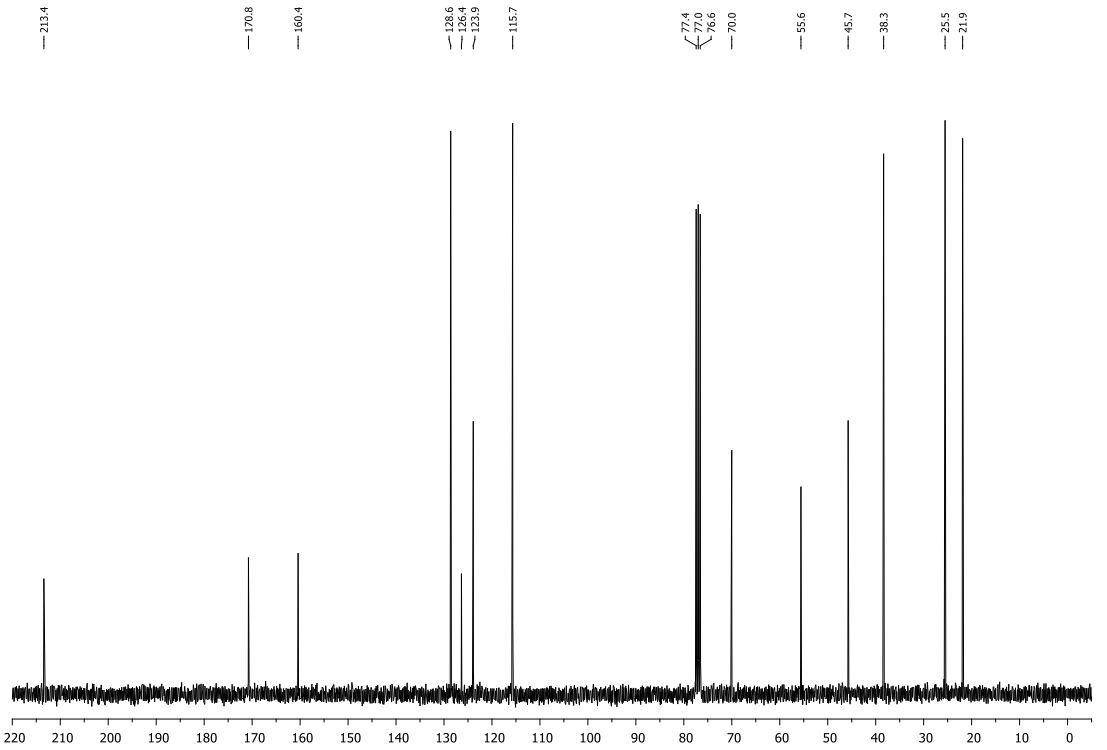


3-(4-Isopropoxyphenyl)spiro[4.4]non-2-en-1-one (72) (in $CDCl_3$)

^{1}H NMR

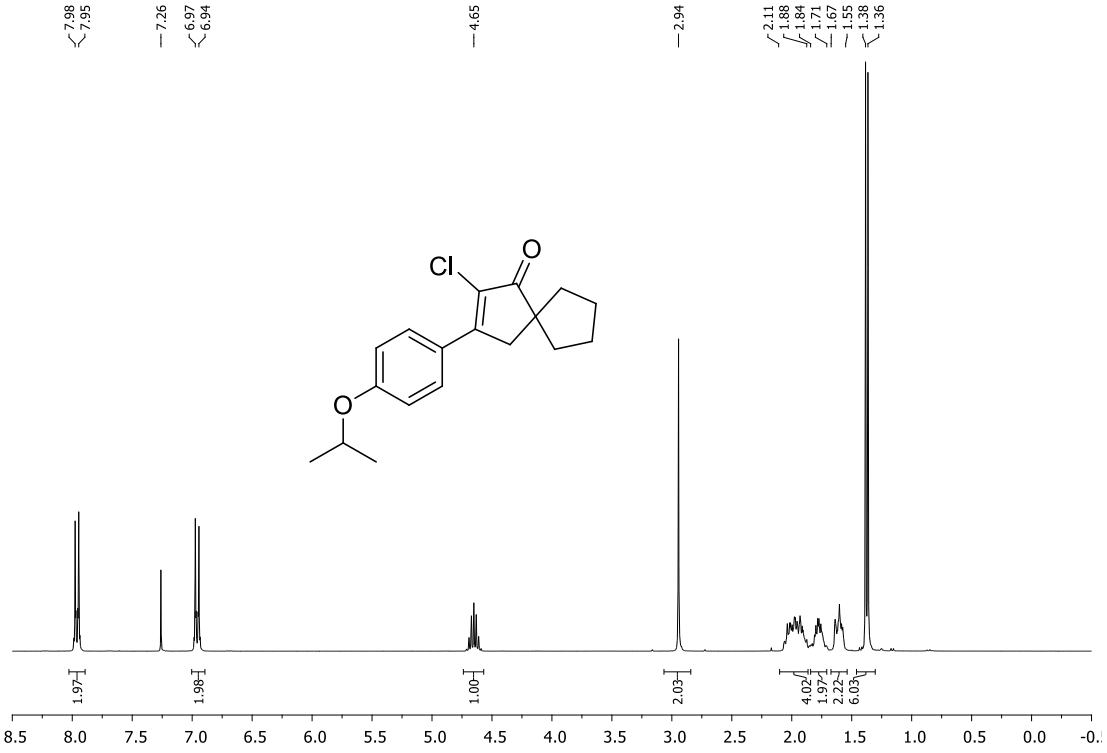


^{13}C NMR

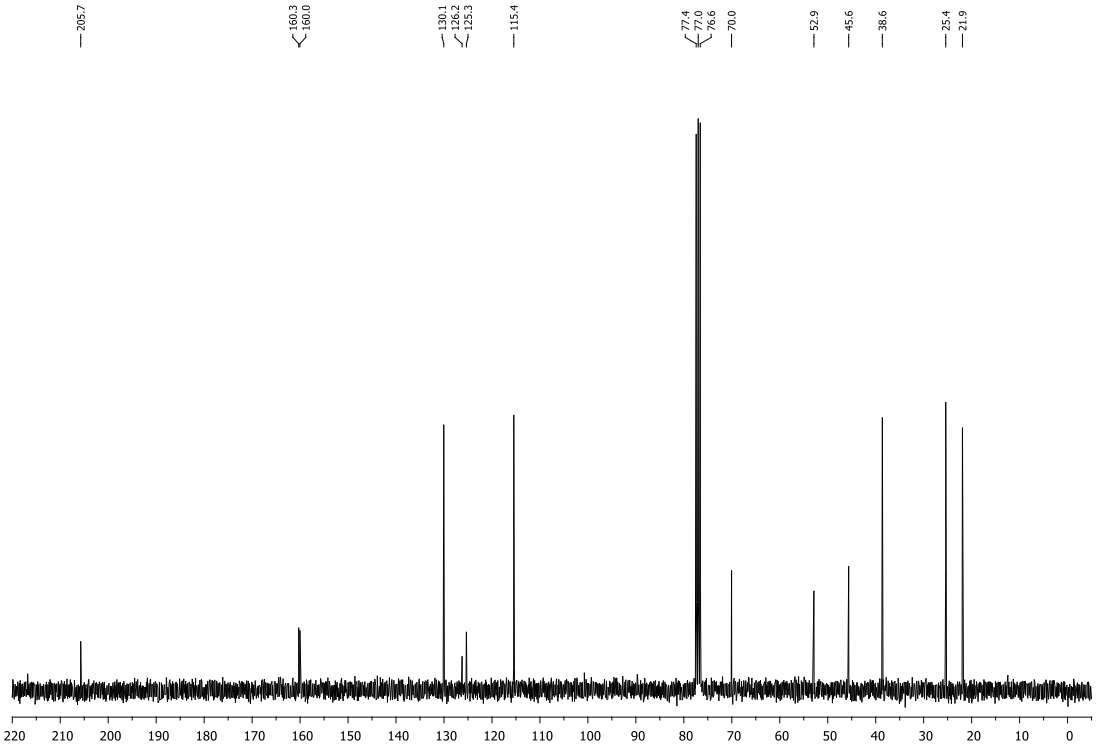


2-Chloro-3-(4-isopropoxyphenyl)spiro[4.4]non-2-en-1-one (74) (in $CDCl_3$)

^{1}H NMR

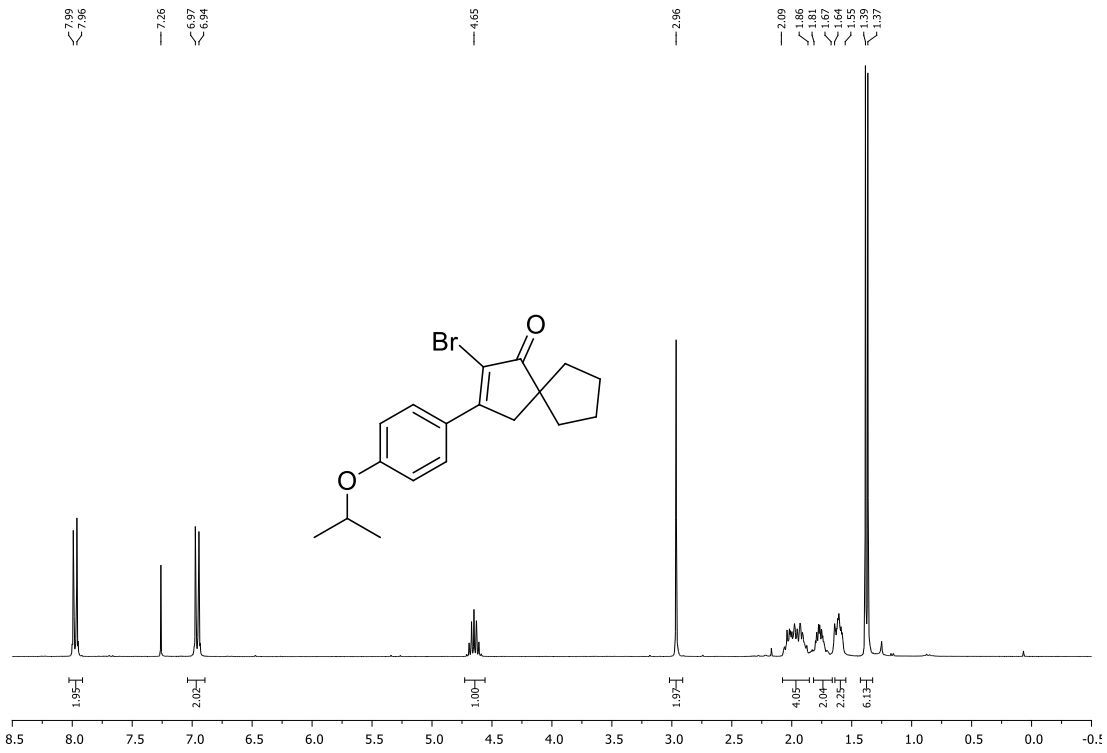


^{13}C NMR

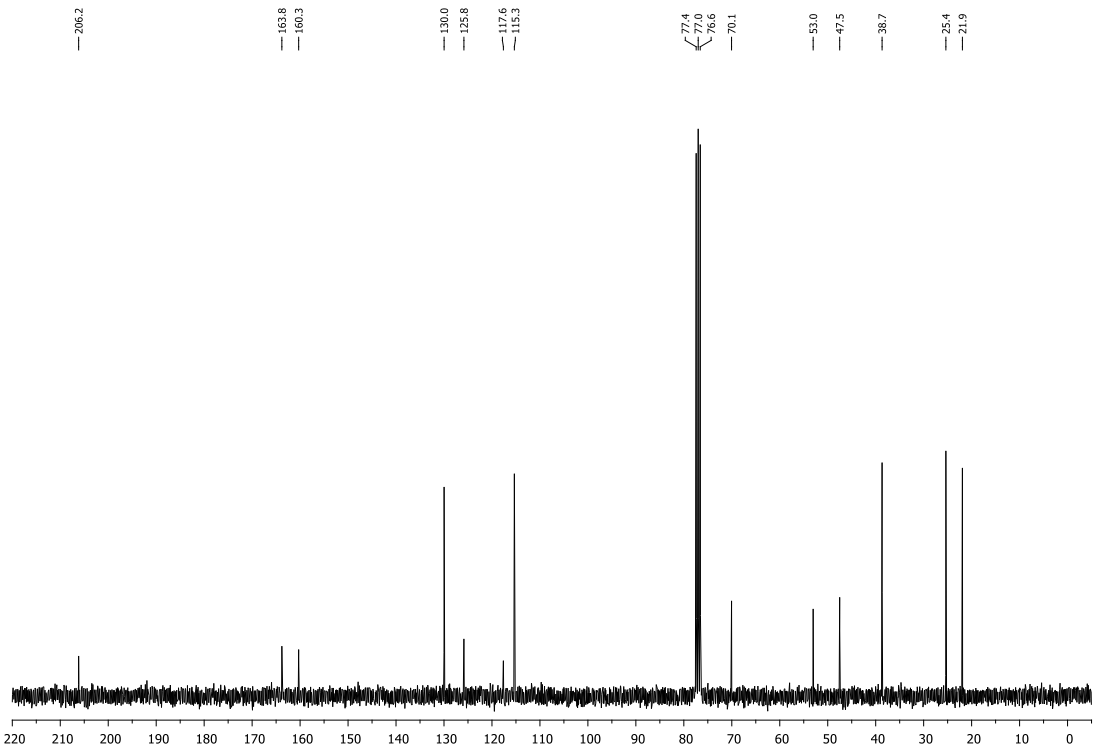


2-Bromo-3-(4-isopropoxyphenyl)spiro[4.4]non-2-en-1-one (75) (in $CDCl_3$)

^{1}H NMR



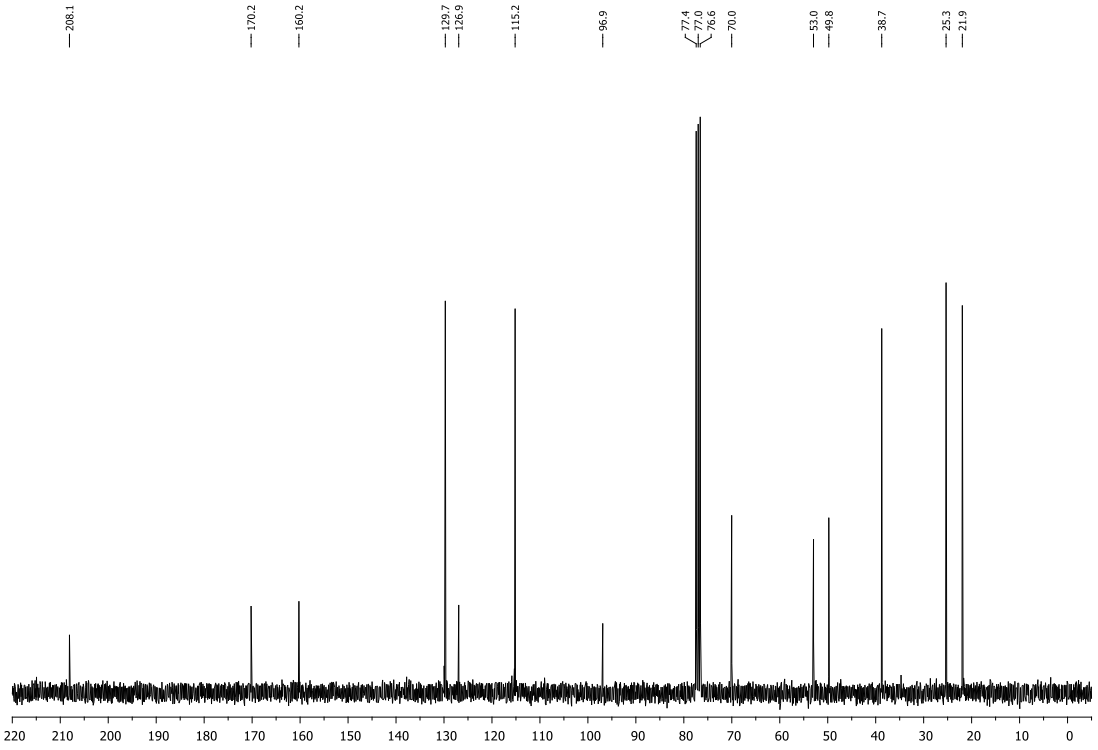
^{13}C NMR



2-Iodo-3-(4-isopropoxyphenyl)spiro[4.4]non-2-en-1-one (76) (in $CDCl_3$)

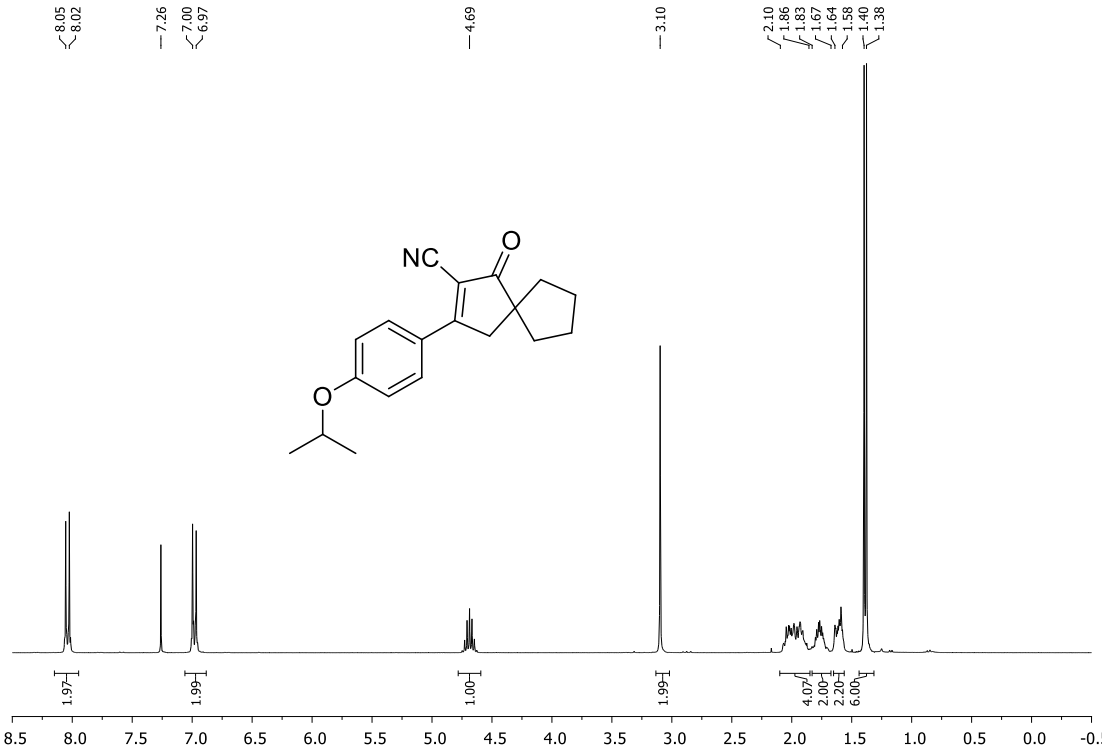
^{1}H NMR

^{13}C NMR

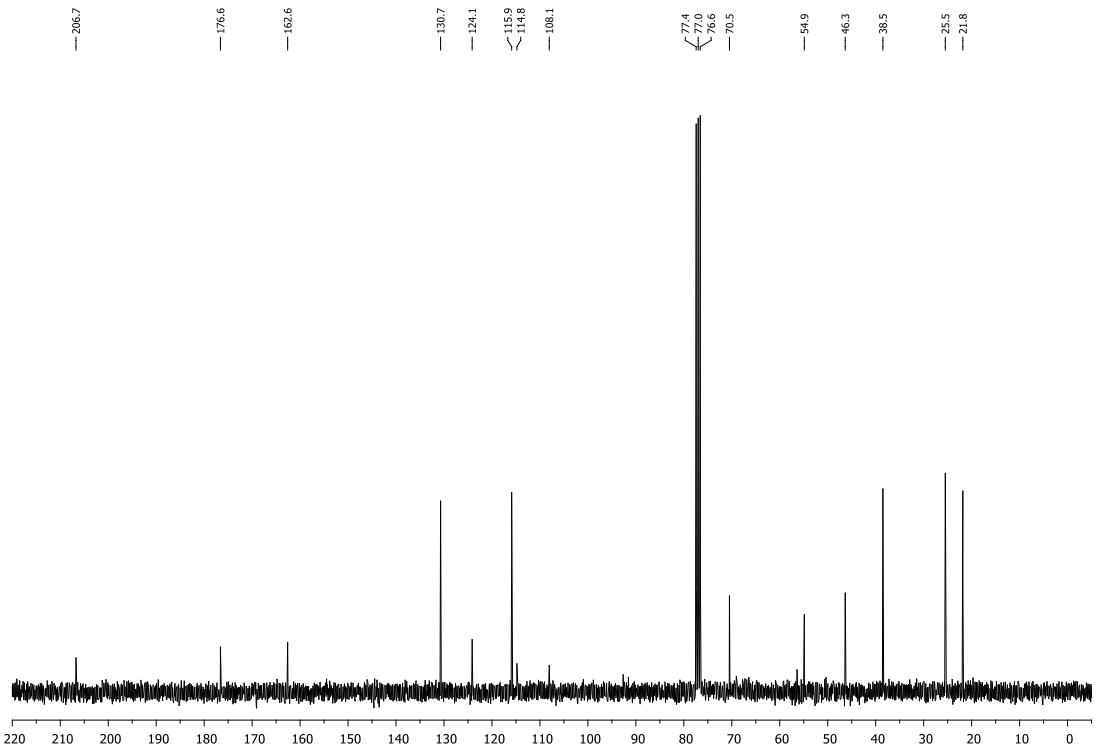


3-(4-Isopropoxyphenyl)-1-oxospiro[4.4]non-2-ene-2-carbonitrile (77) (in $CDCl_3$)

^{1}H NMR

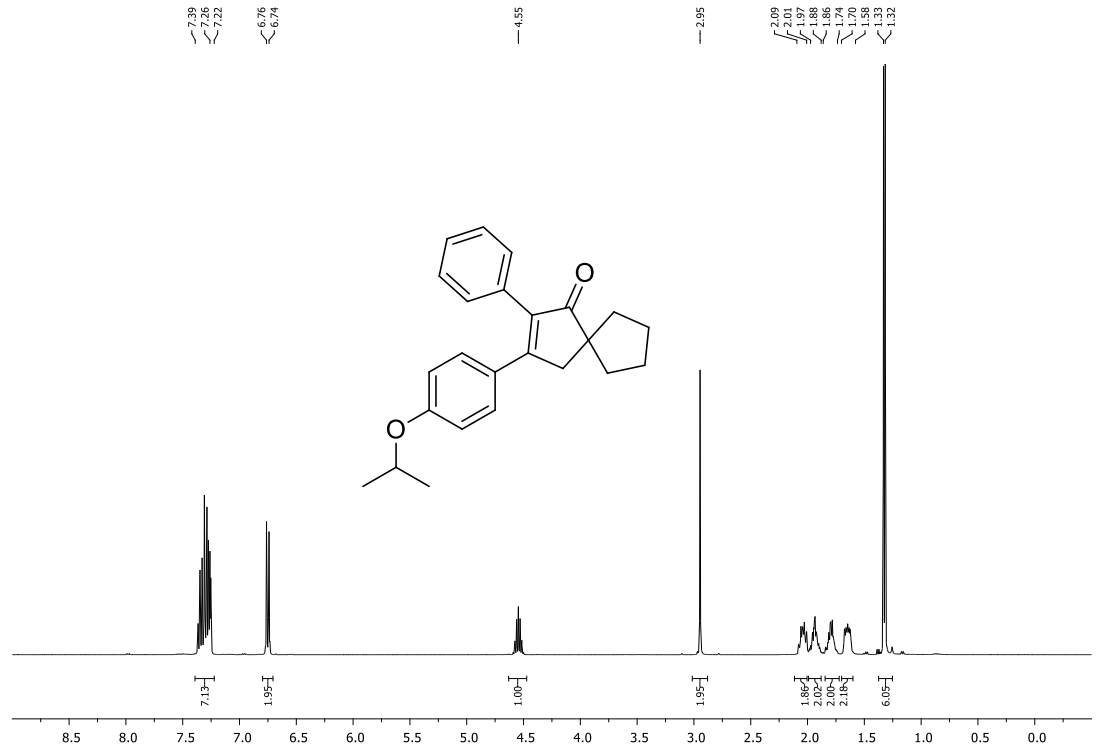


^{13}C NMR

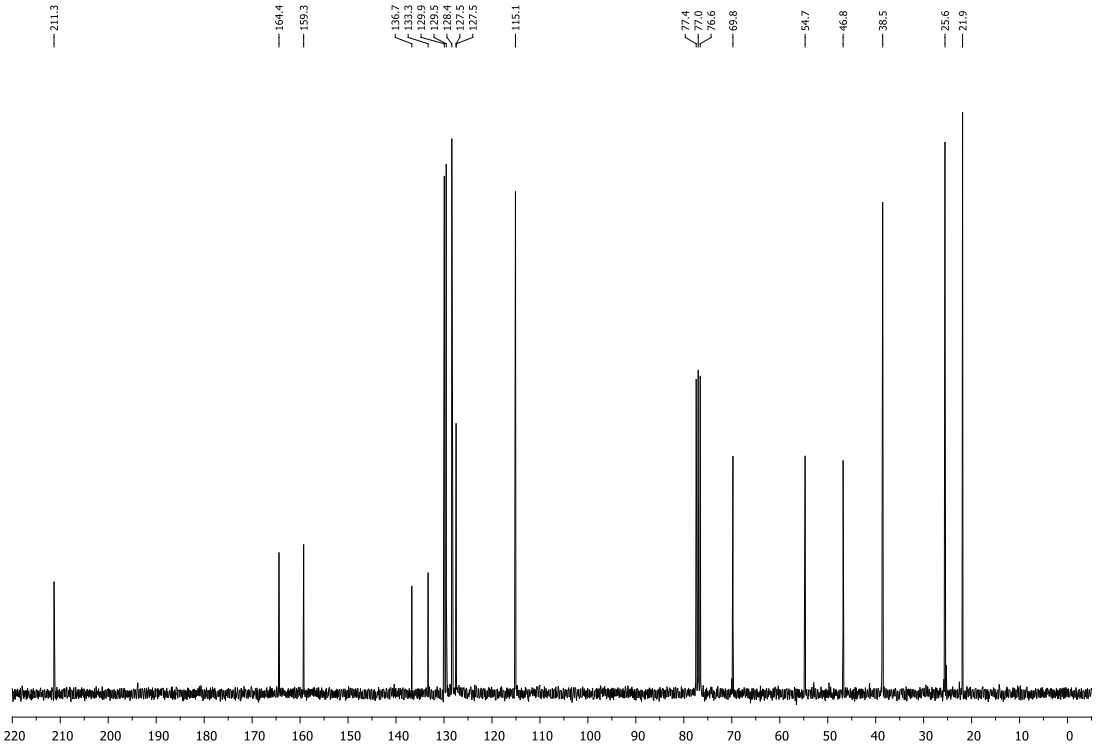


3-(4-Isopropoxyphenyl)-2-phenylspiro[4.4]non-2-en-1-one (78) (in $CDCl_3$)

^{1}H NMR

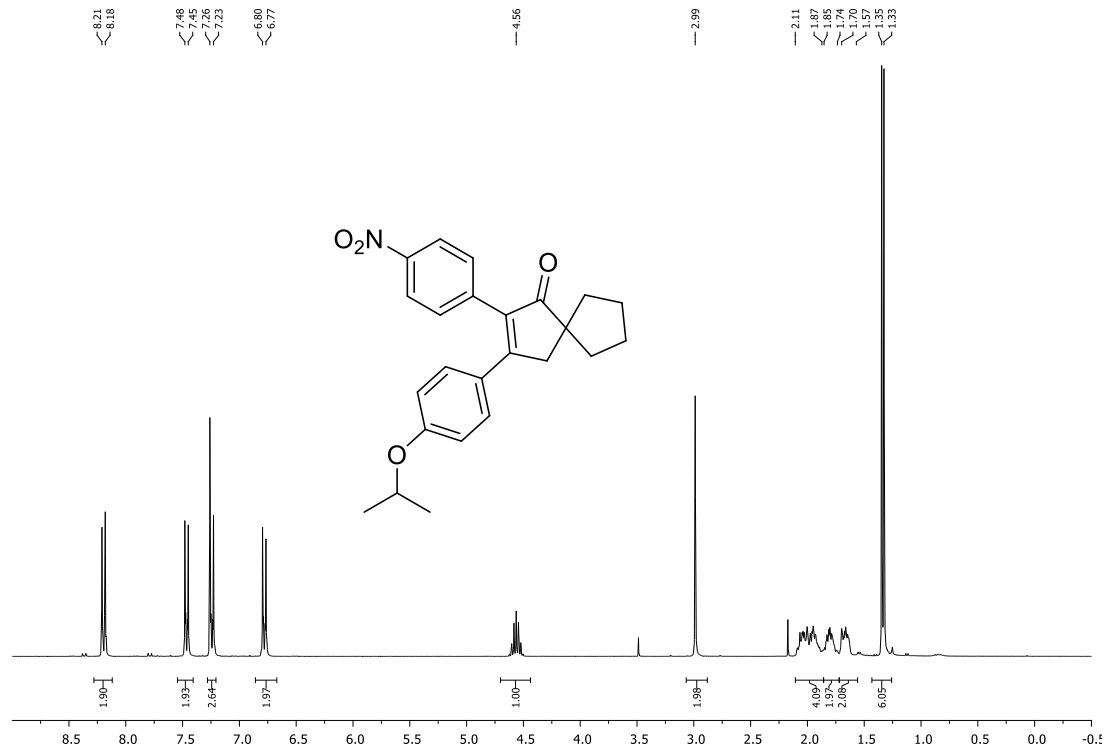


^{13}C NMR

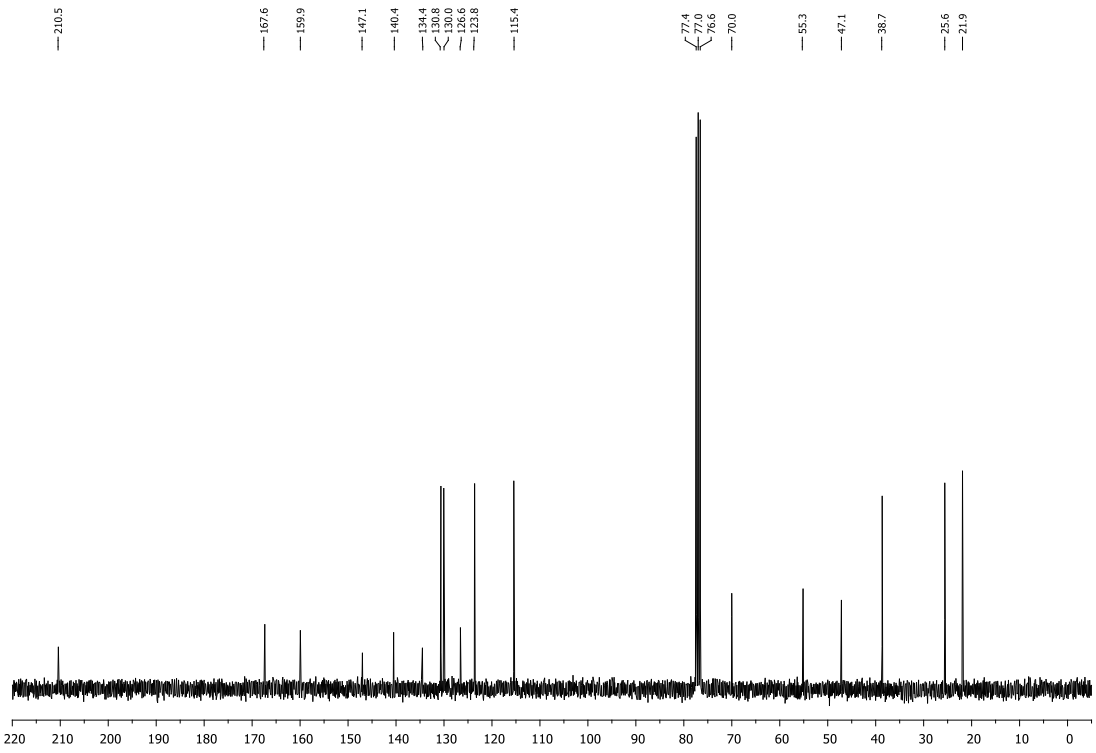


3-(4-isopropoxyphenyl)-2-(4-nitrophenyl)spiro[4.4]non-2-en-1-one (79) (in $CDCl_3$)

^{1}H NMR

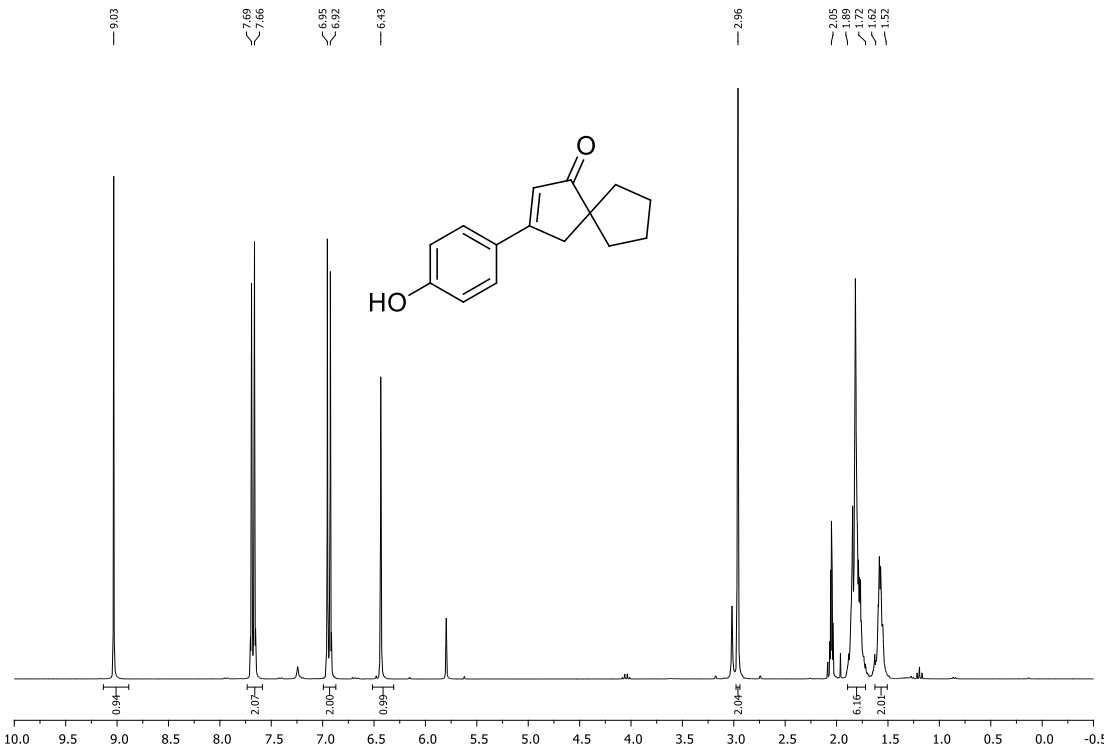


^{13}C NMR

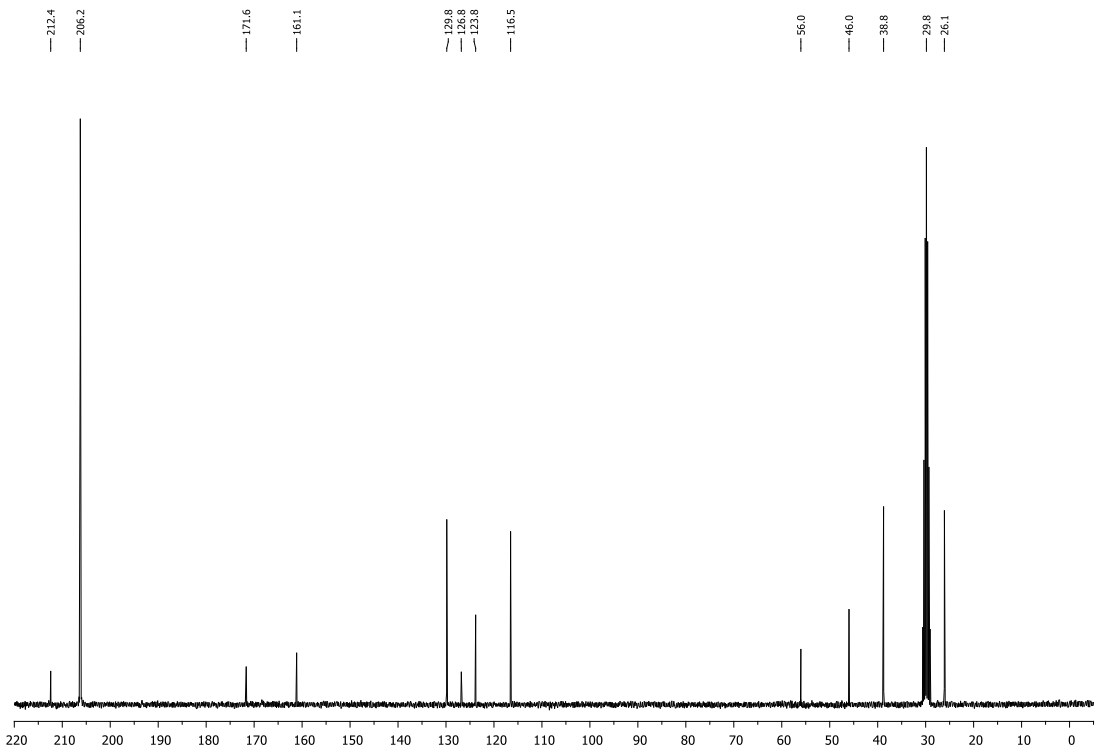


3-(4-Hydroxyphenyl)spiro[4.4]non-2-en-1-one (80) (in acetone-d_6)

^{1}H NMR

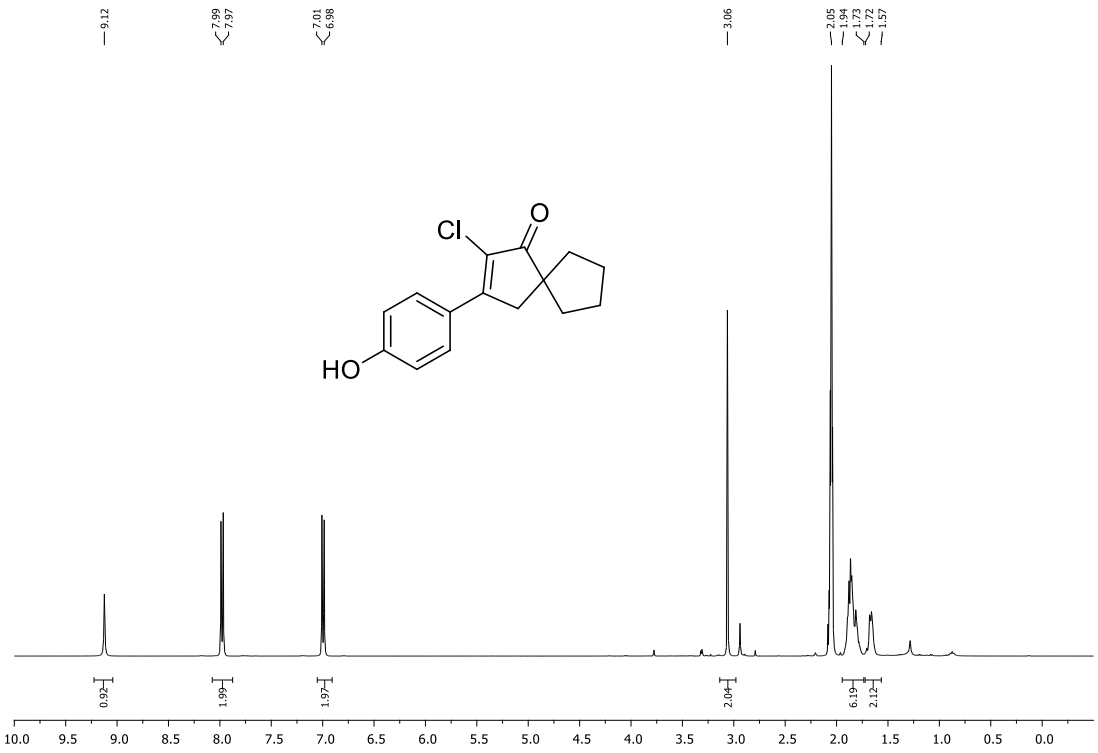


^{13}C NMR

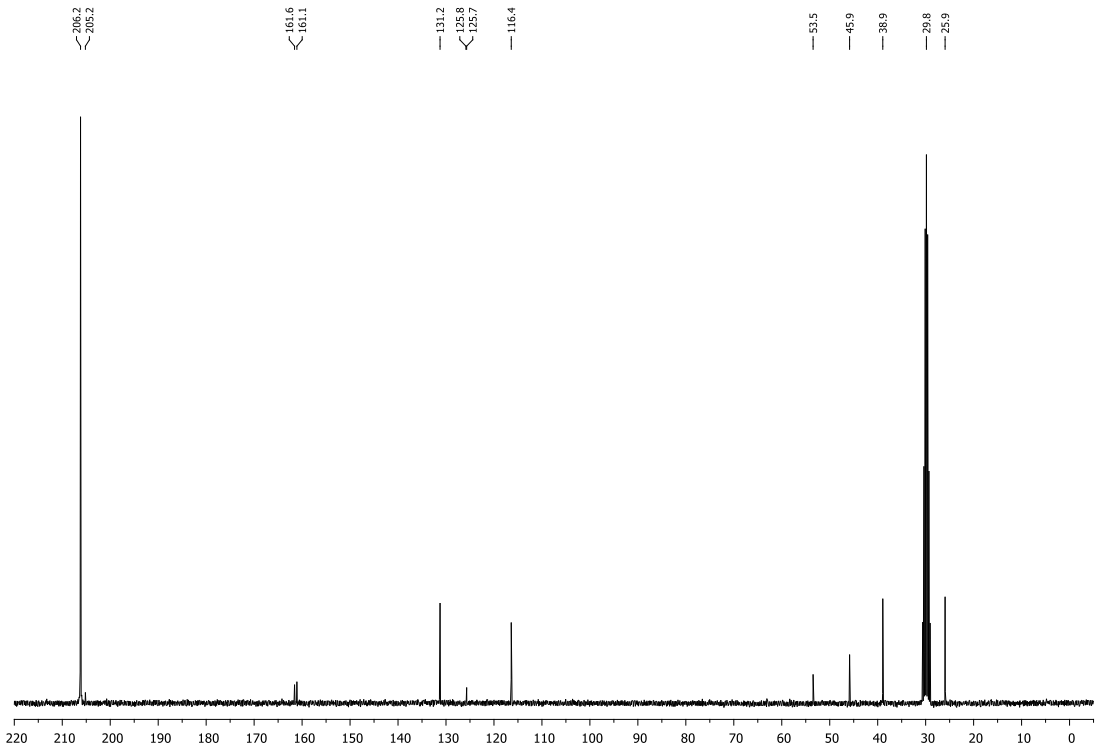


2-Chloro-3-(4-hydroxyphenyl)spiro[4.4]non-2-en-1-one (81) (in acetone-d_6)

^{1}H NMR

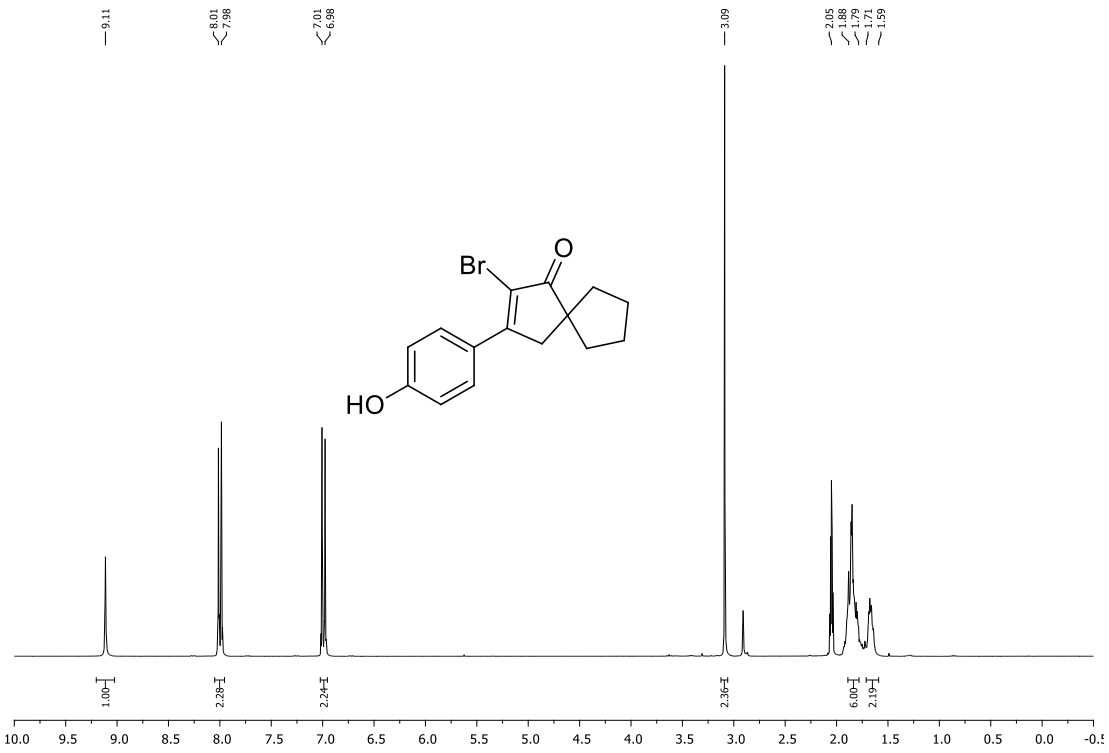


^{13}C NMR

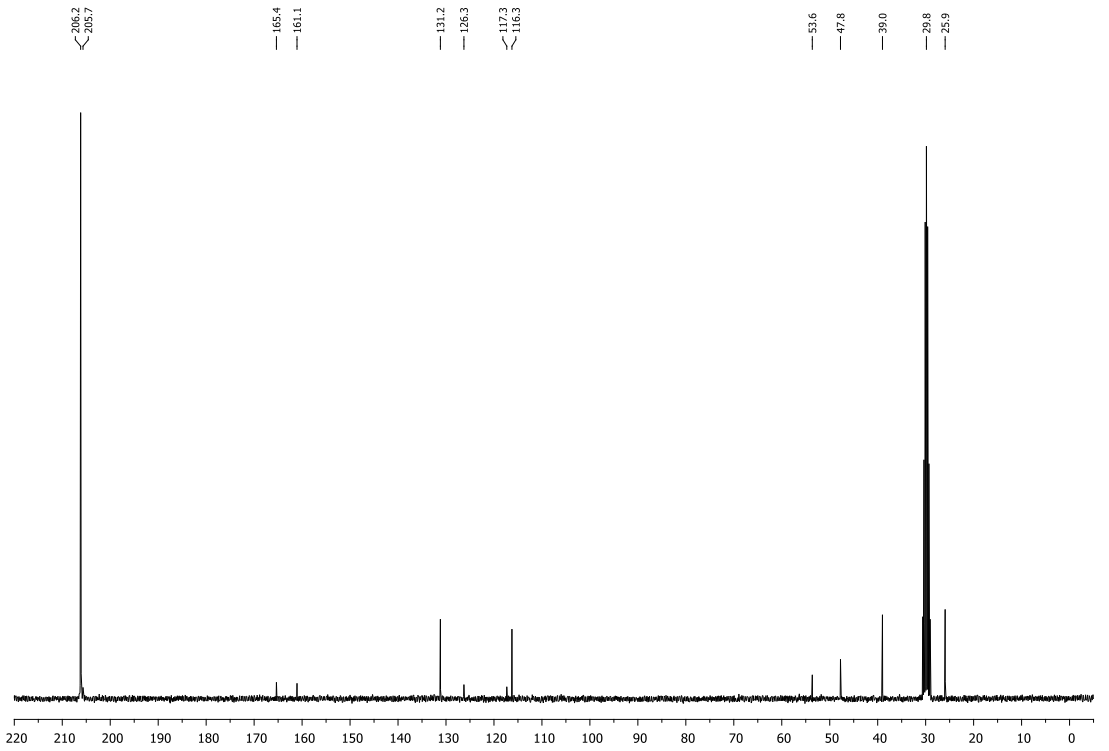


2-Bromo-3-(4-hydroxyphenyl)spiro[4.4]non-2-en-1-one (82) (in acetone-d_6)

^{1}H NMR

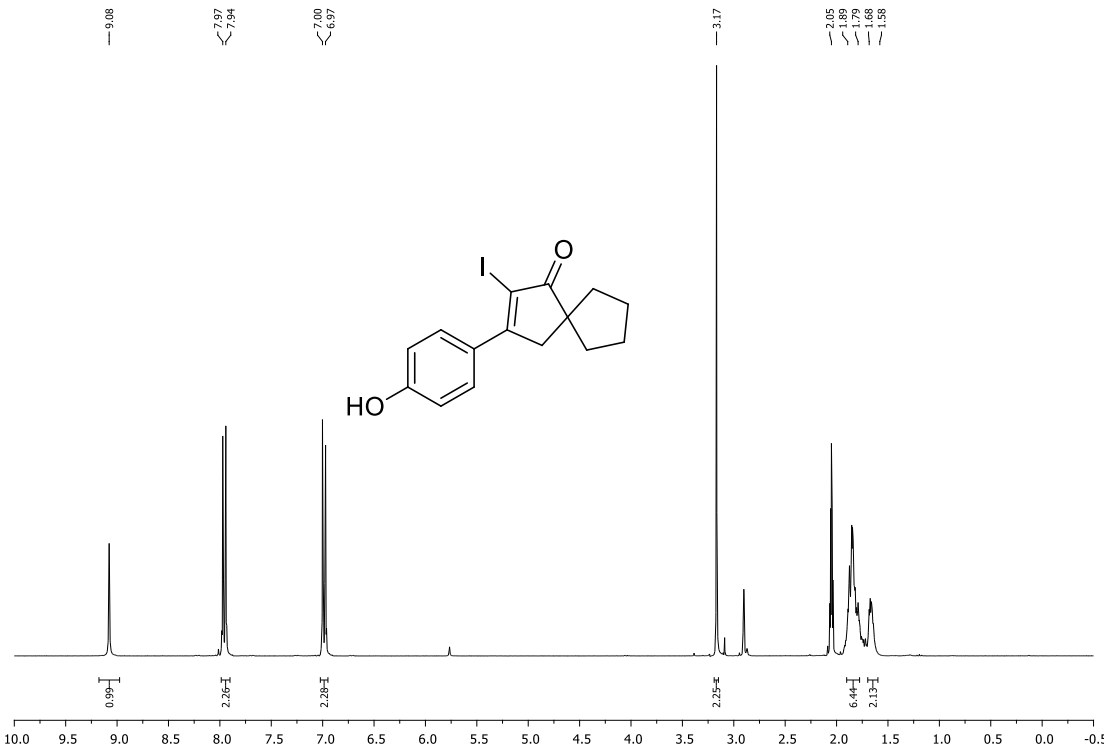


^{13}C NMR

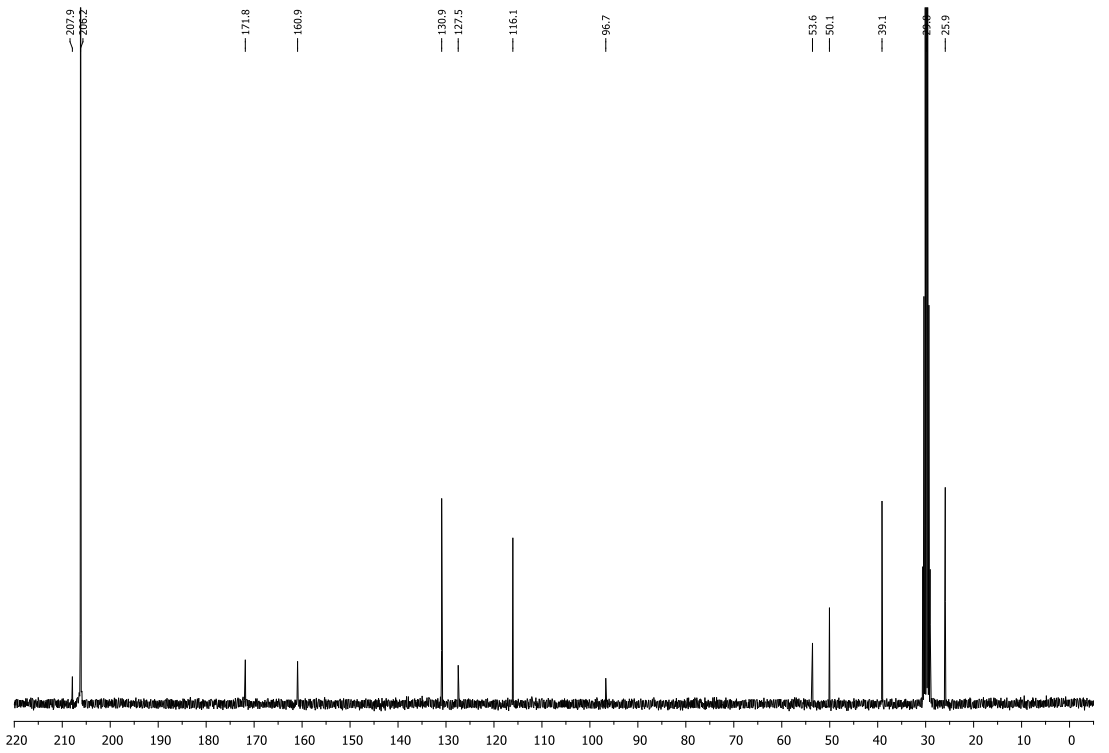


3-(4-Hydroxyphenyl)-2-iodospiro[4.4]non-2-en-1-one (83) (in acetone-d_6)

^{1}H NMR

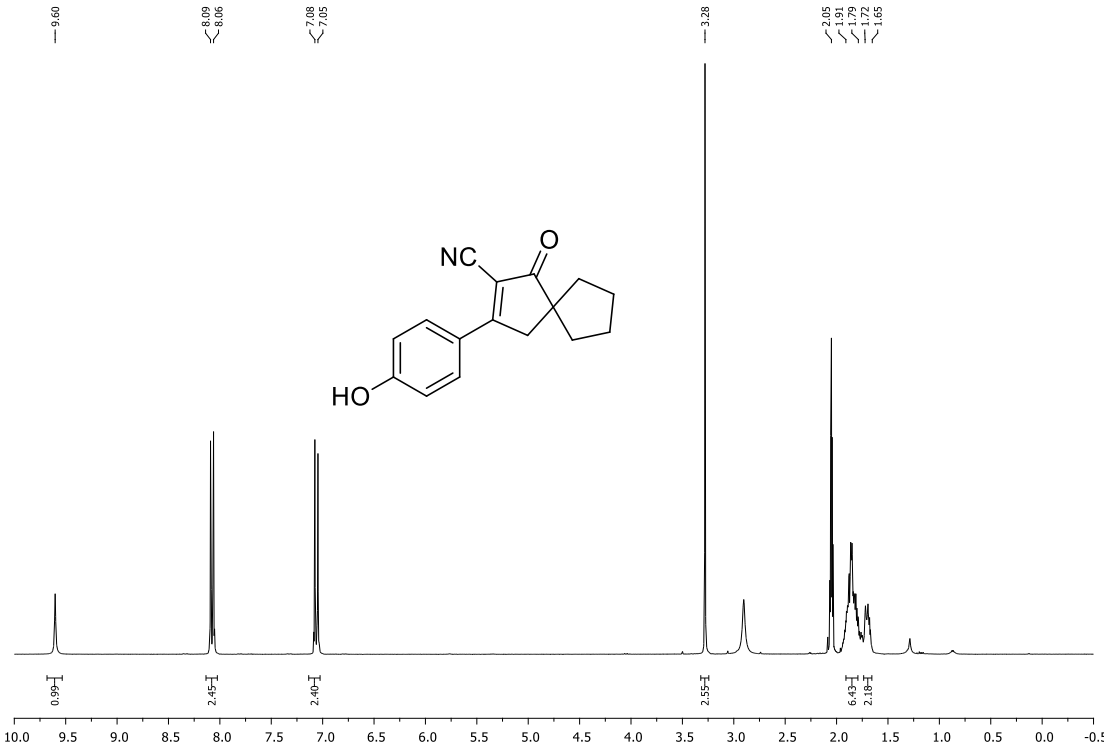


^{13}C NMR

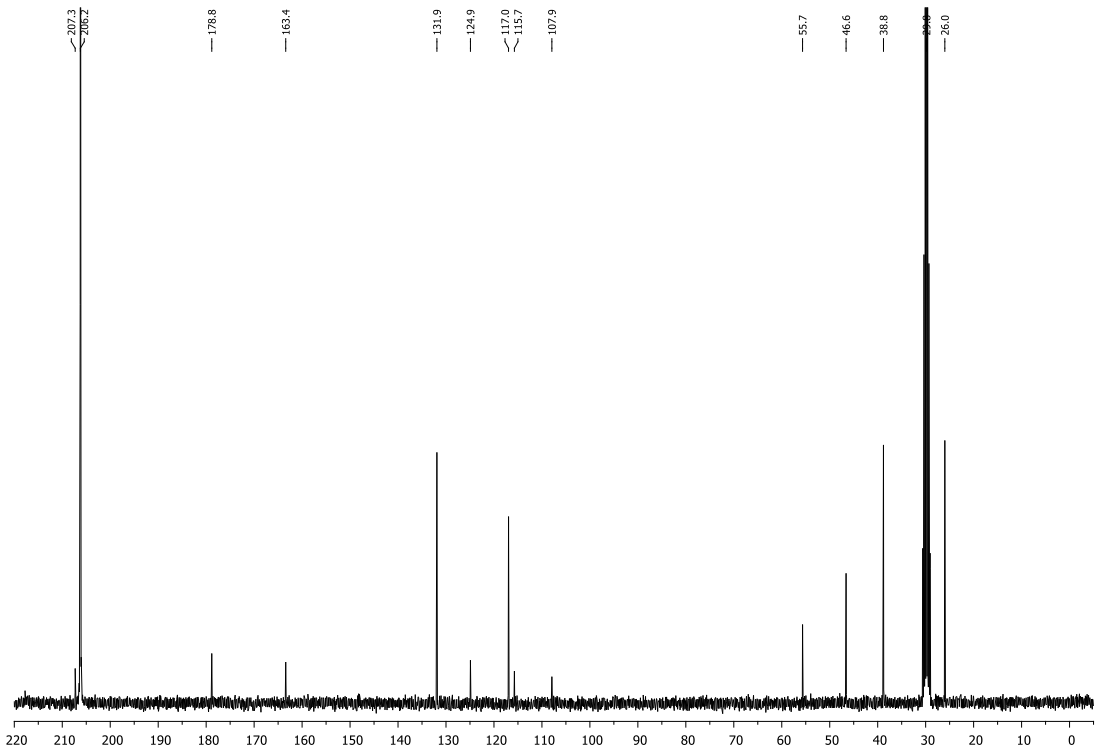


3-(4-Hydroxyphenyl)-1-oxospiro[4.4]non-2-ene-2-carbonitrile (84) (in acetone-d_6)

^{1}H NMR

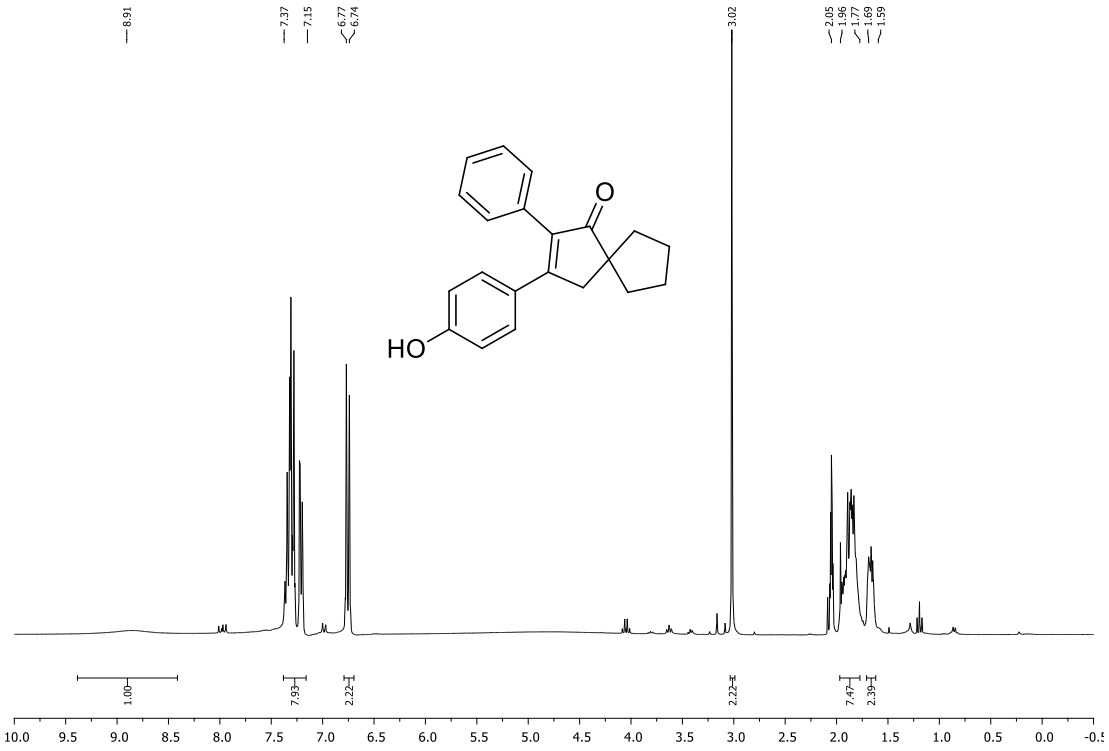


^{13}C NMR

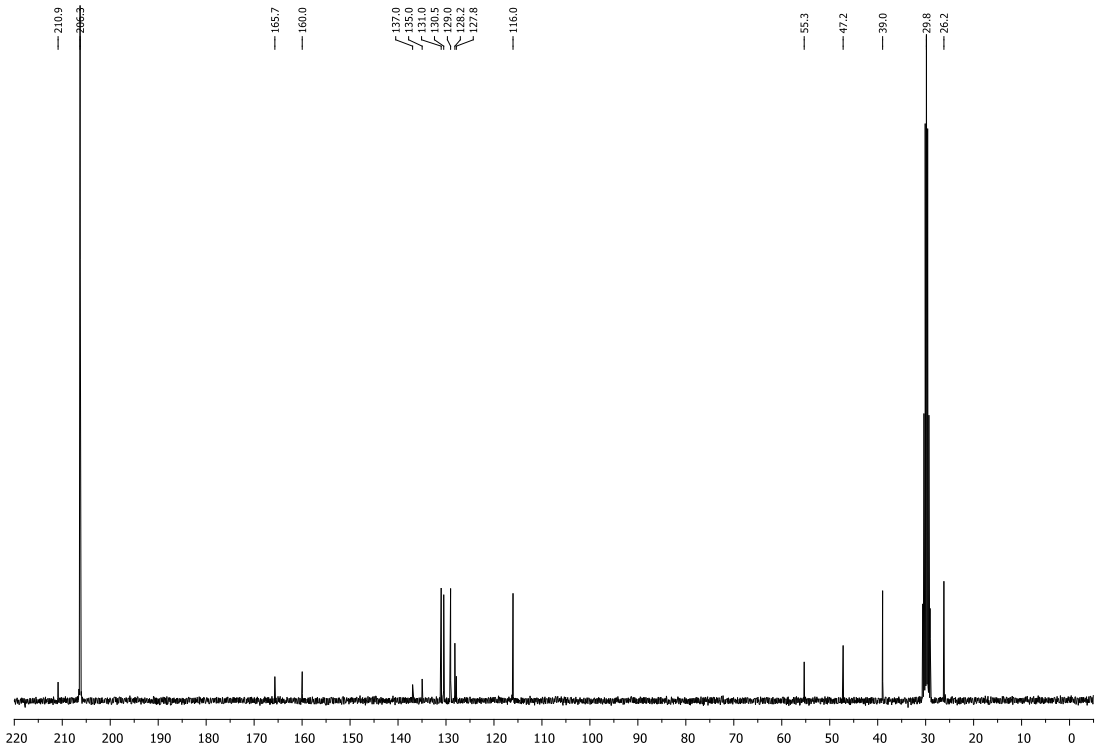


3-(4-Hydroxyphenyl)-2-phenylspiro[4.4]non-2-en-1-one (85) (in acetone-d_6)

^{1}H NMR

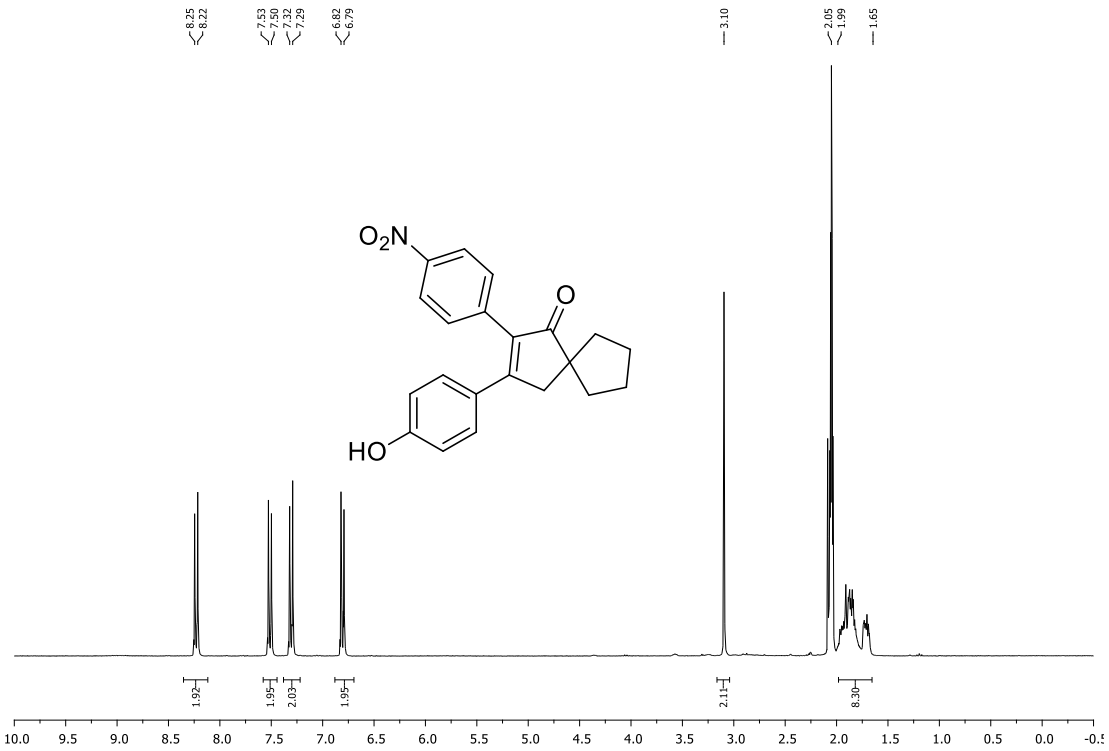


^{13}C NMR

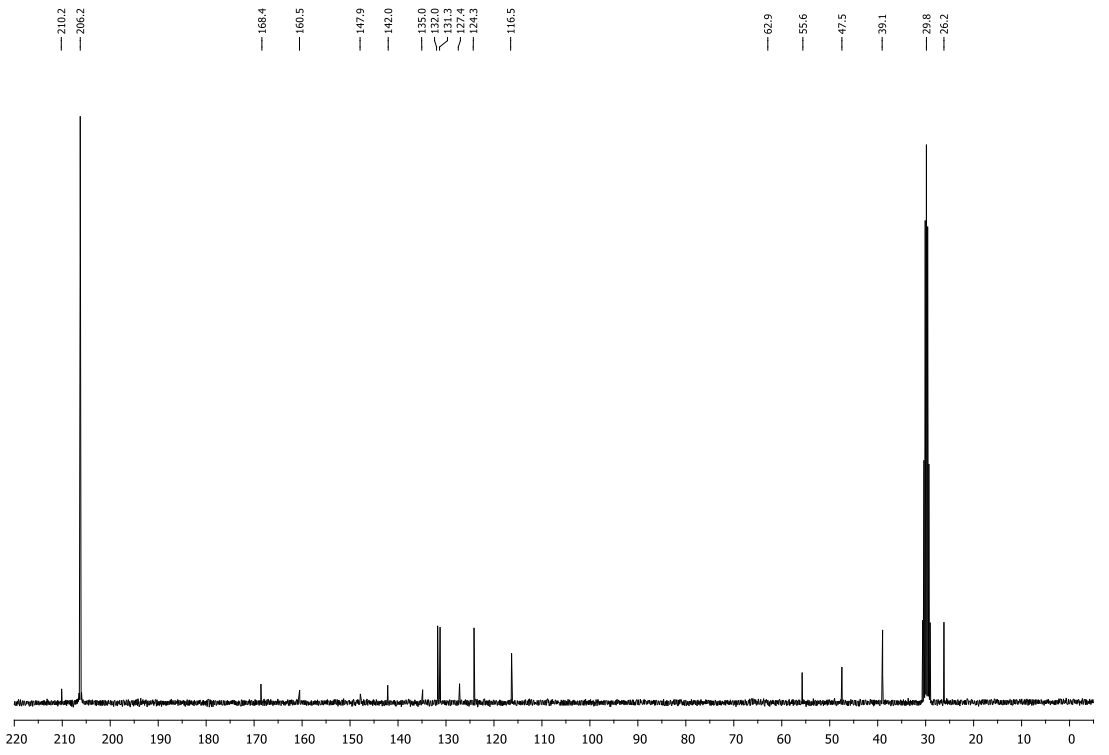


3-(4-Hydroxyphenyl)-2-(4-nitrophenyl)spiro[4.4]non-2-en-1-one (86) (in acetone-d_6)

^{1}H-NMR

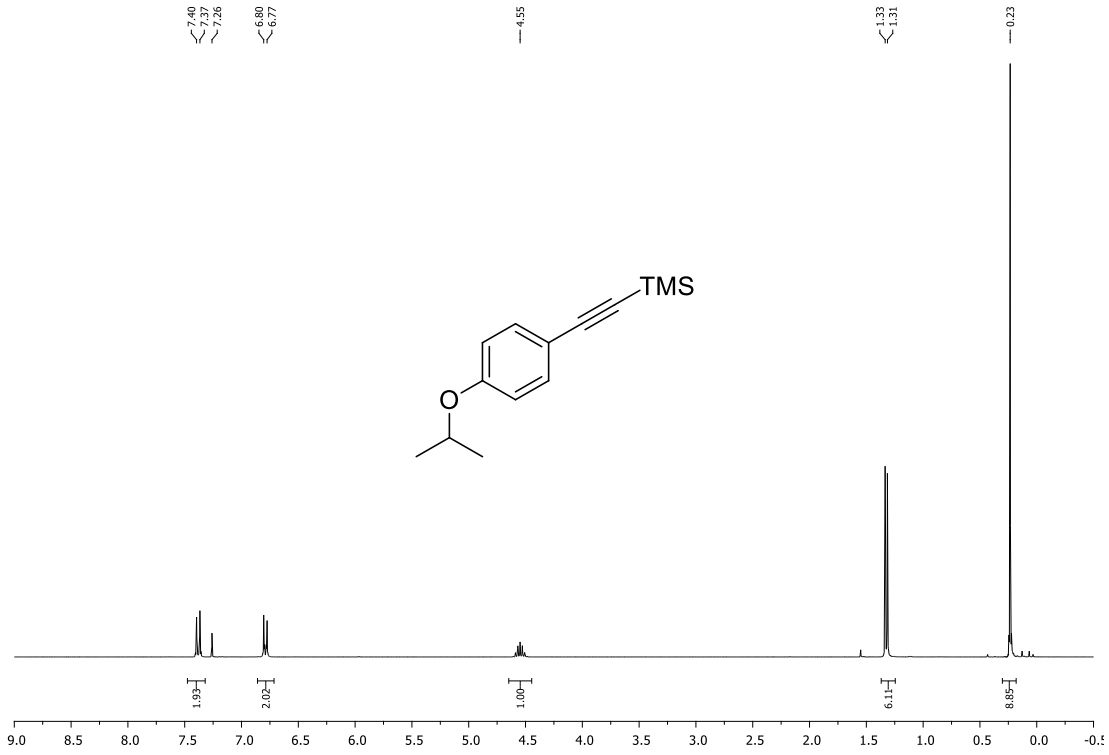


^{13}C-NMR

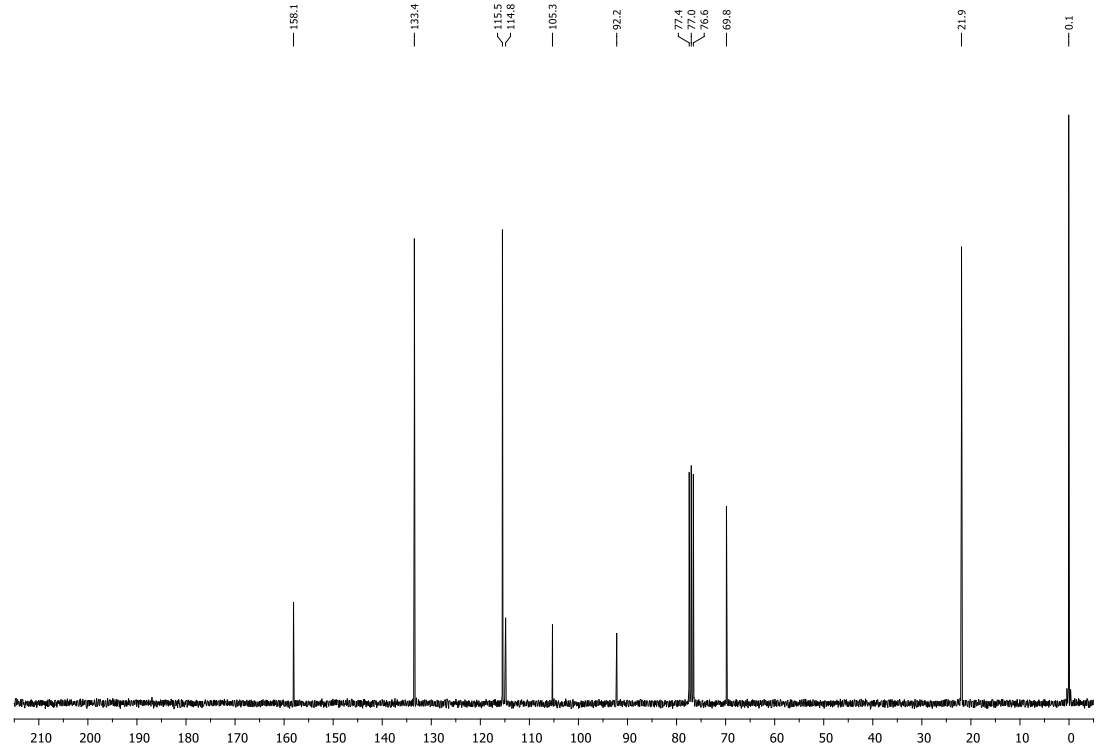


((4-Isopropoxyphenyl)ethynyl)trimethylsilane (89) (in $CDCl_3$)

^{1}H NMR

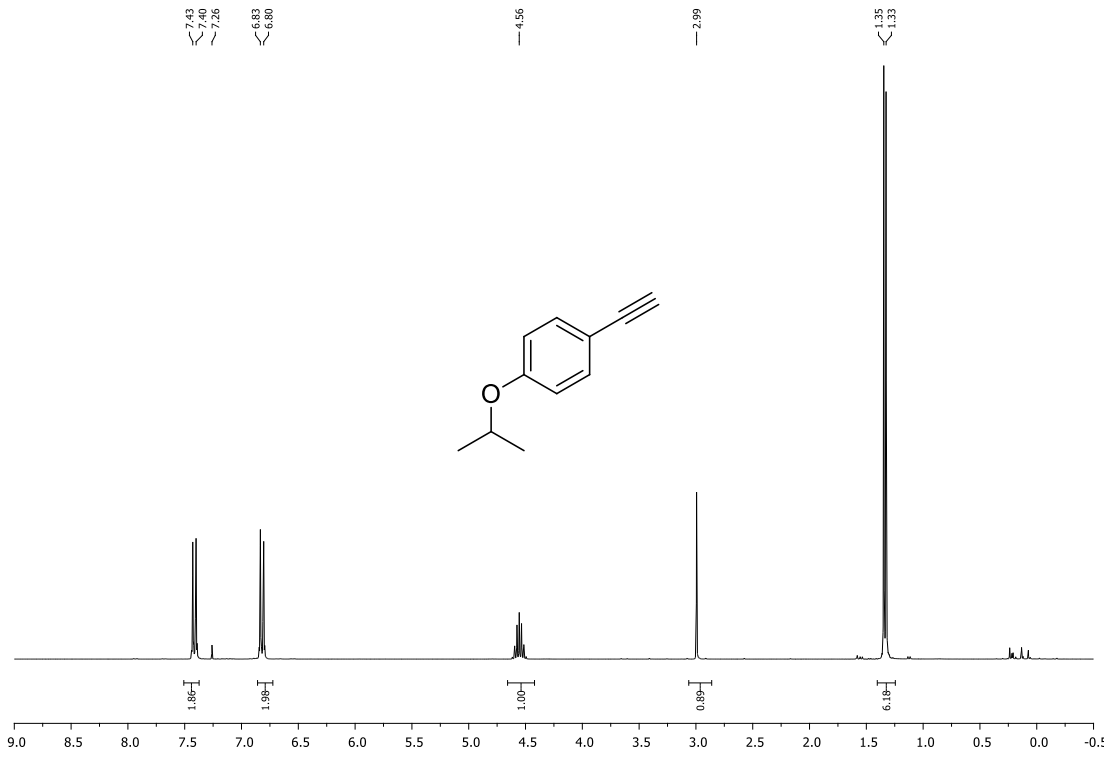


^{13}C NMR

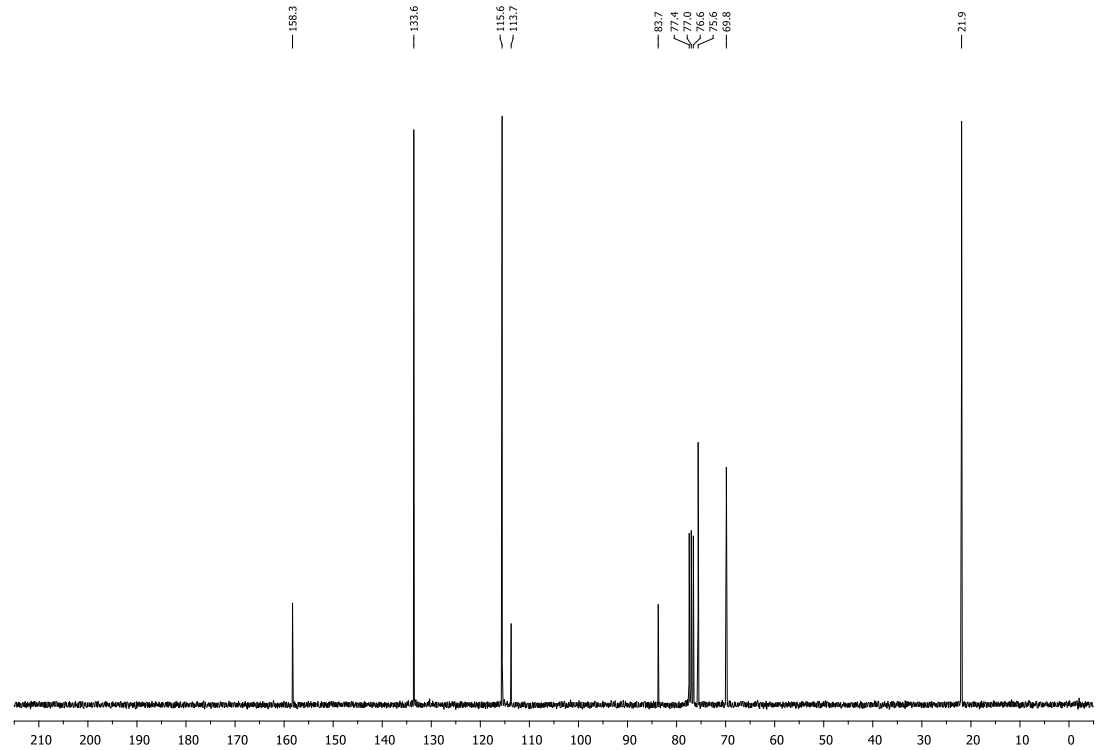


1-Ethynyl-4-isopropoxybenzene (90) (in $CDCl_3$)

^{1}H NMR



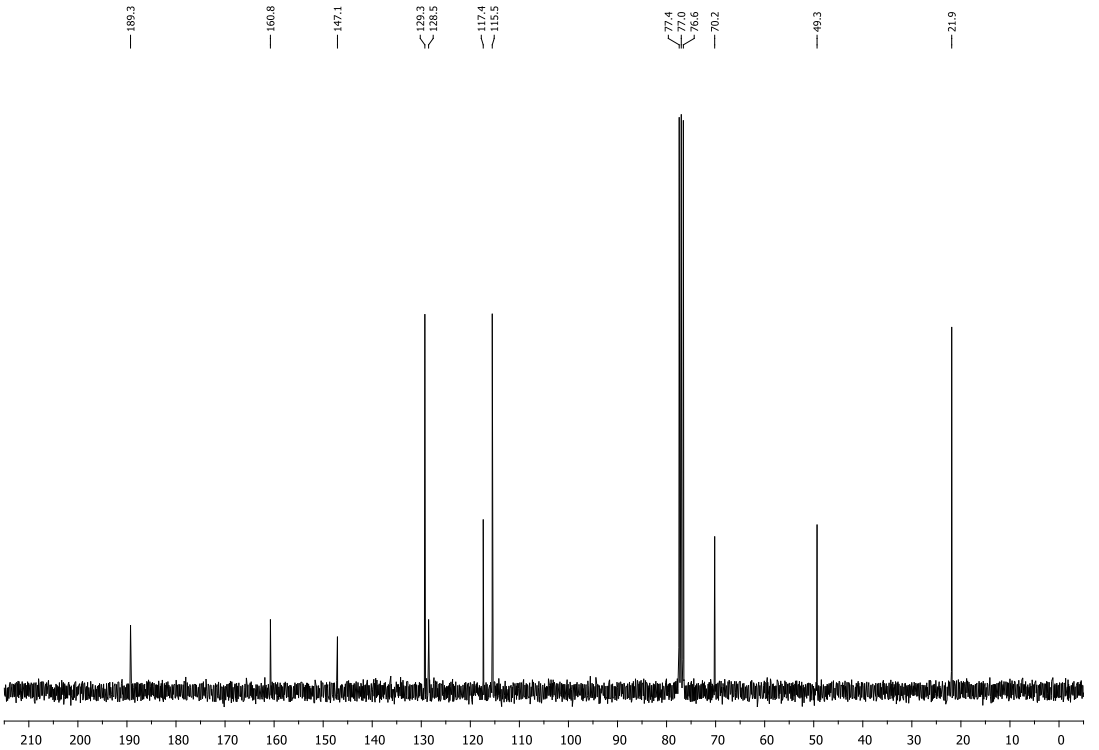
^{13}C NMR



1,4-Dichloro-4-(4-isopropoxyphenyl)but-3-en-2-one (91) (in $CDCl_3$)

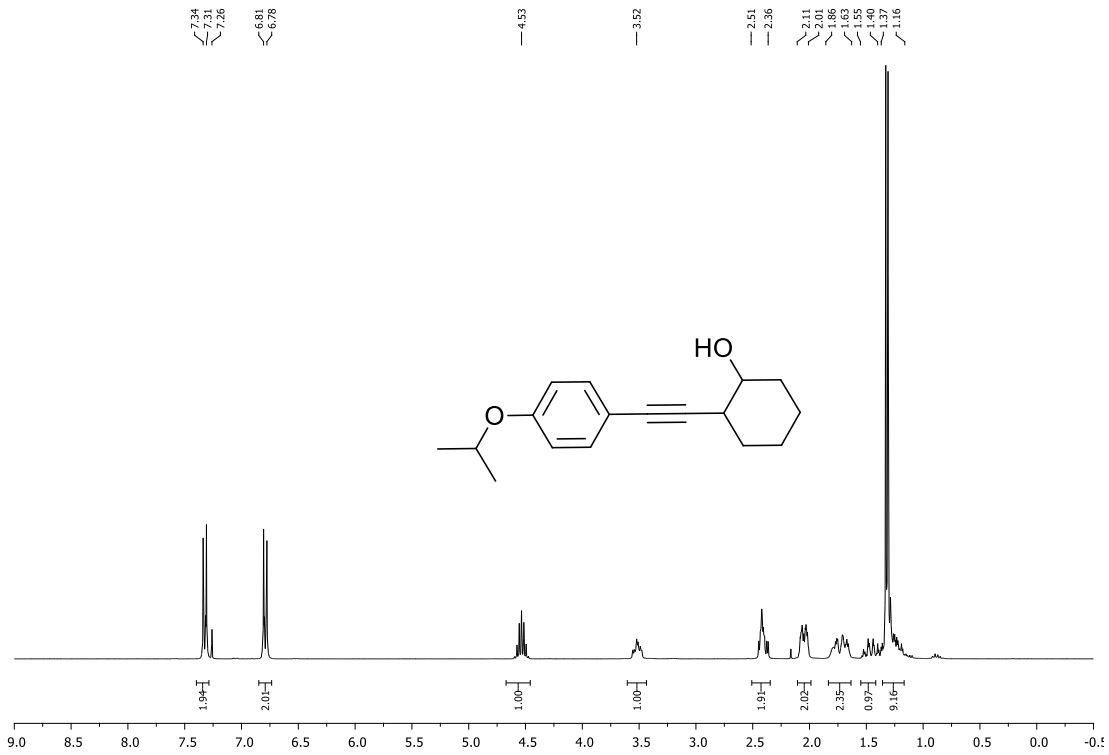
^{1}H NMR

^{13}C NMR

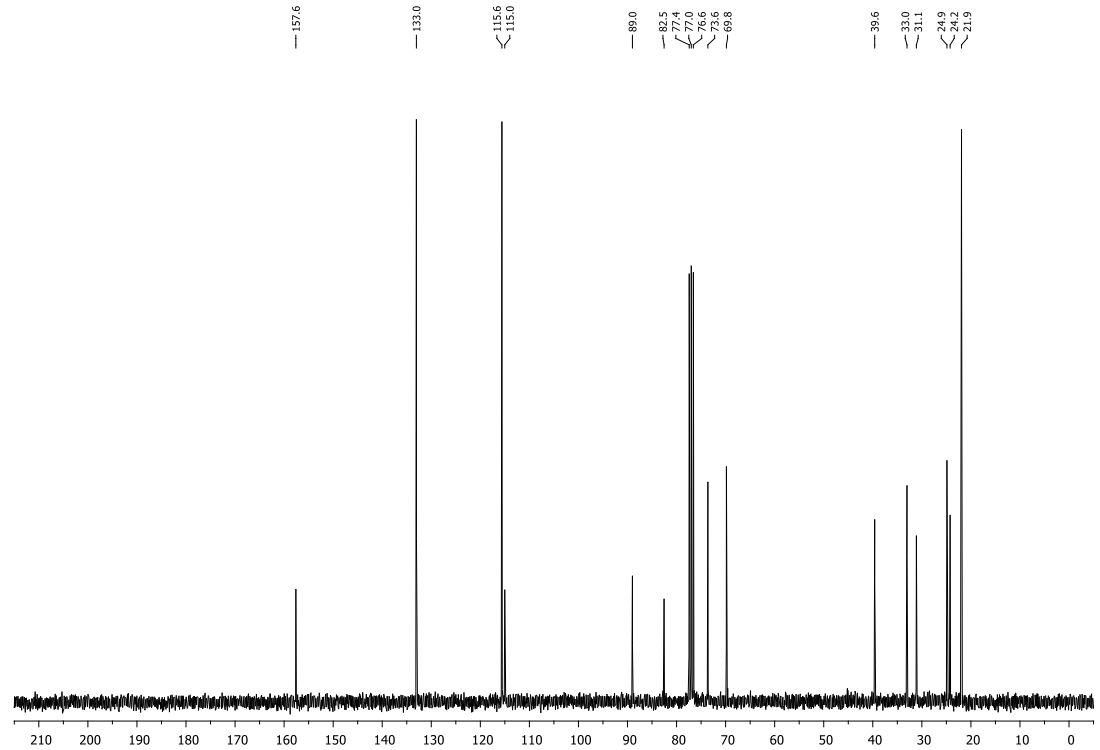


2-((4-Isopropoxyphenyl)ethynyl)cyclohexanol (97) (in $CDCl_3$)

^{1}H NMR

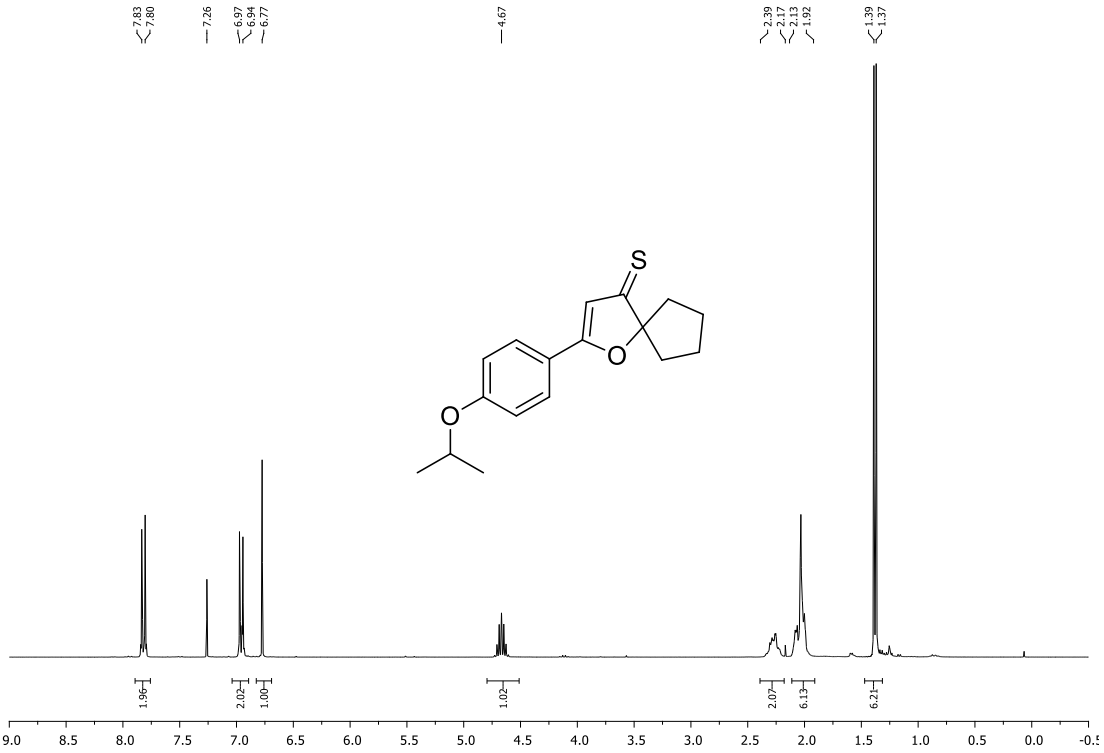


^{13}C NMR

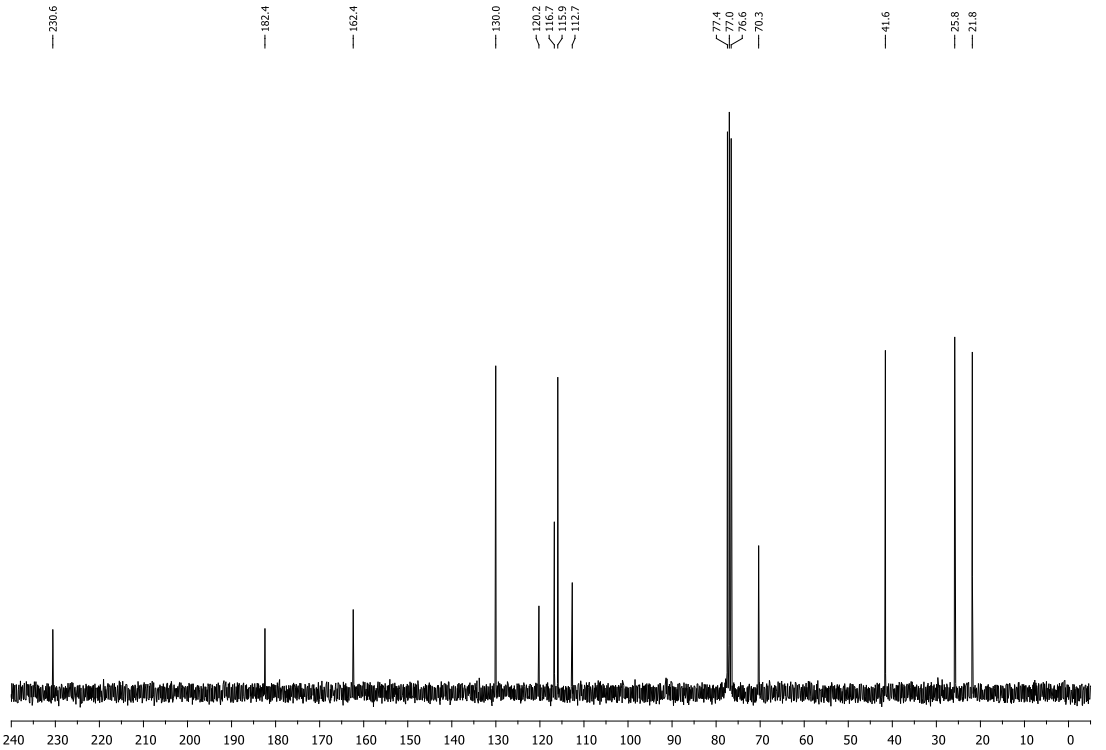


2-(4-Isopropoxyphenyl)-1-oxaspiro[4.4]non-2-ene-4-thione (99) (in $CDCl_3$)

^{1}H NMR

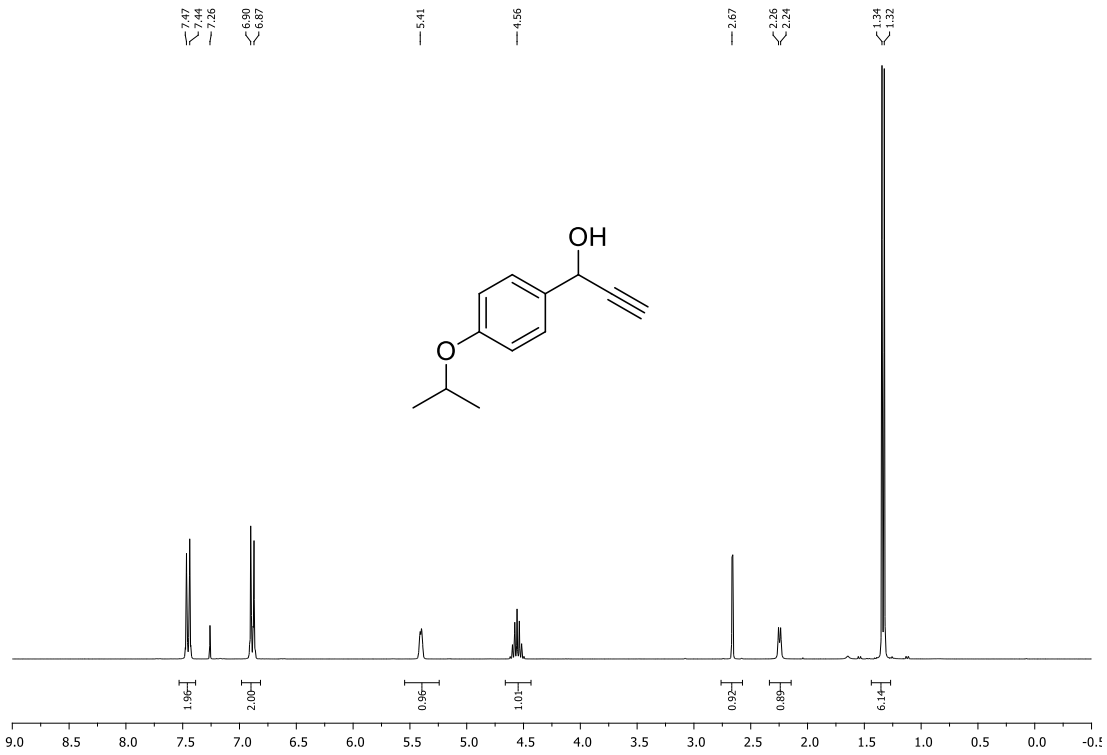


^{13}C NMR

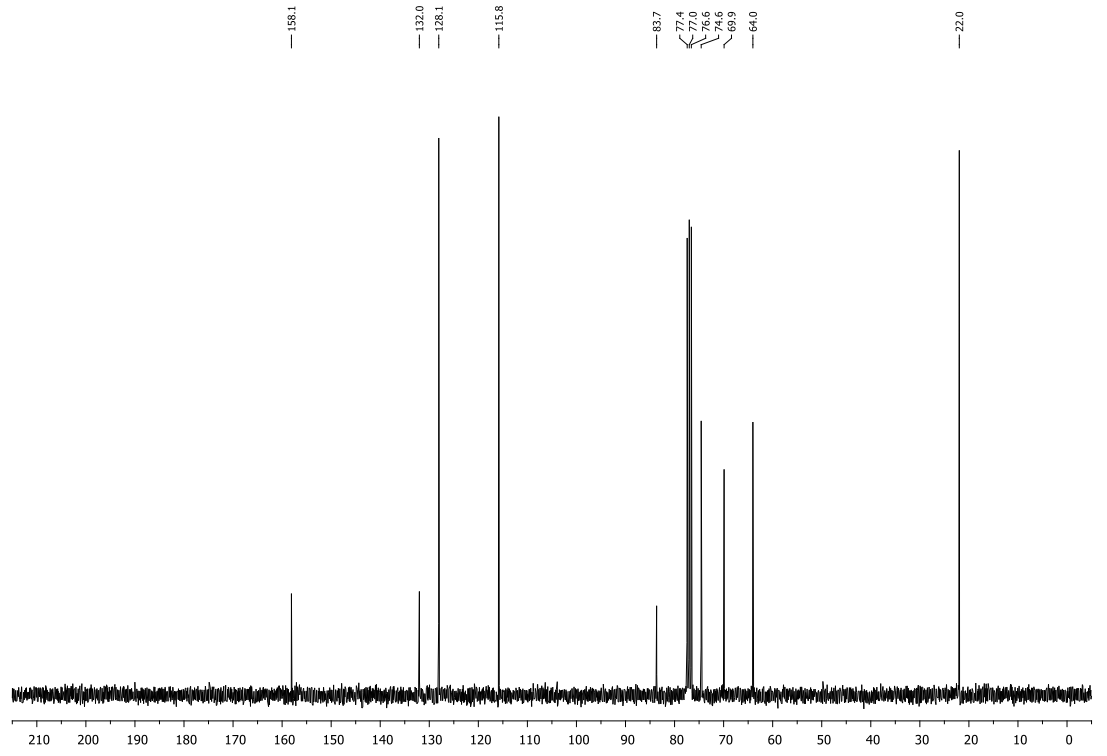


1-(4-Isopropoxyphenyl)prop-2-yn-1-ol (109) (in $CDCl_3$)

^{1}H NMR

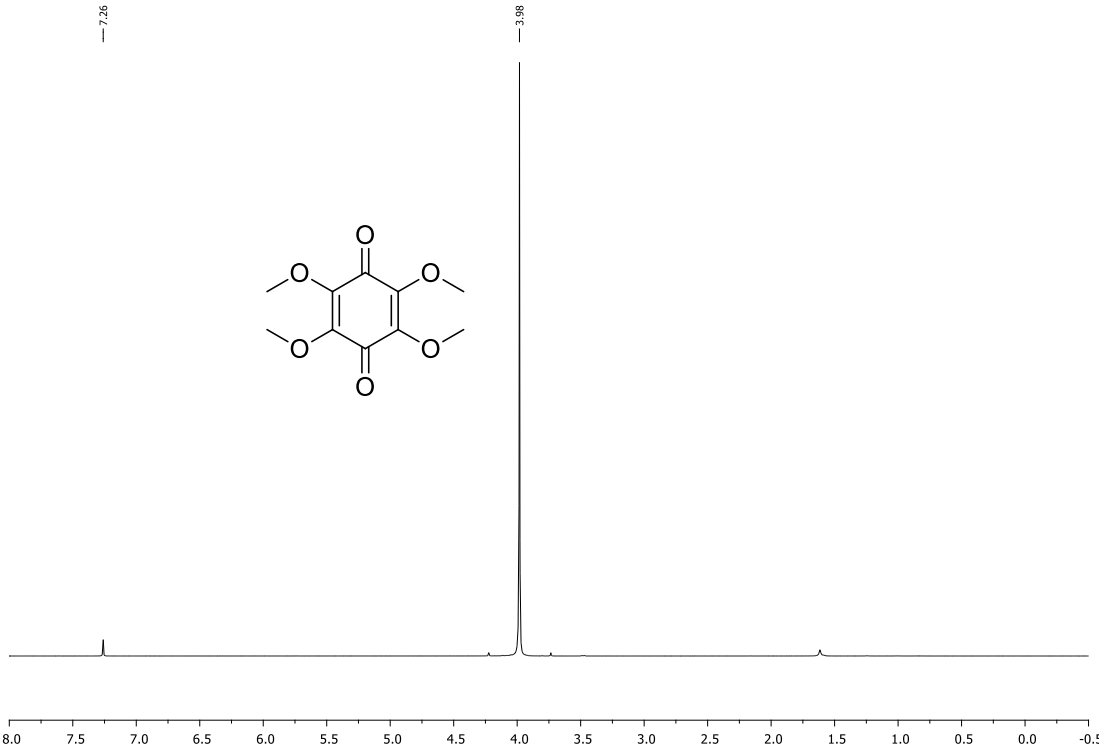


^{13}C NMR

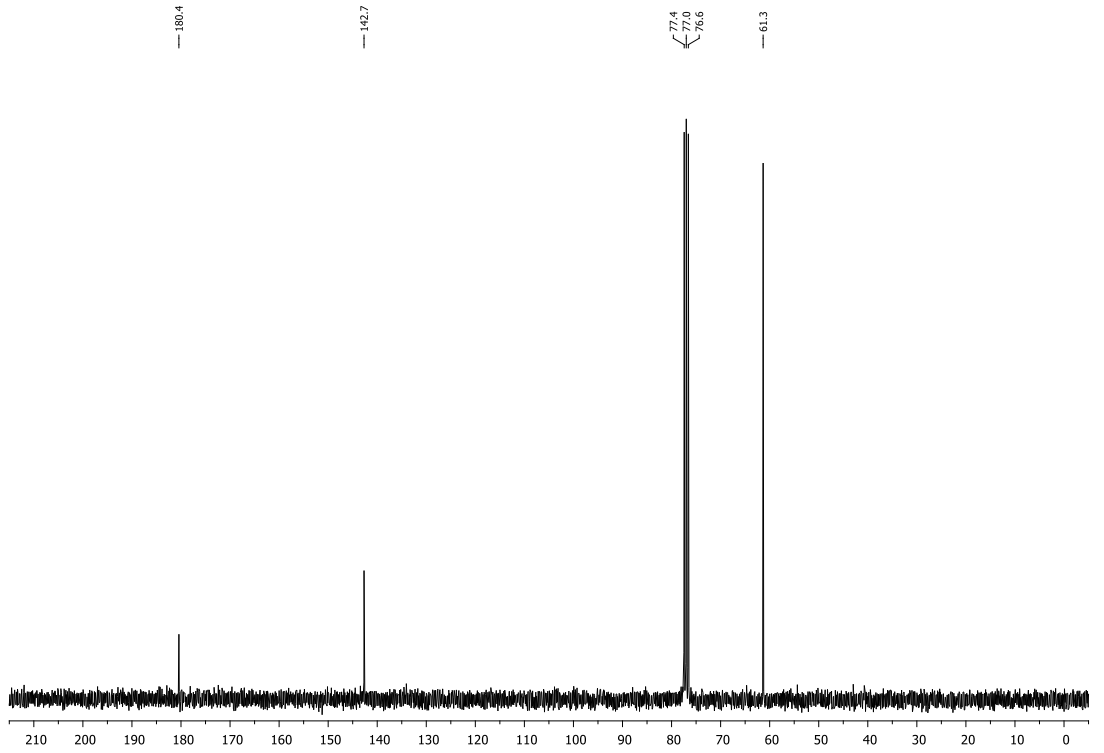


2,3,5,6-Tetramethoxycyclohexa-2,5-diene-1,4-dione (114) (in $CDCl_3$)

^{1}H NMR

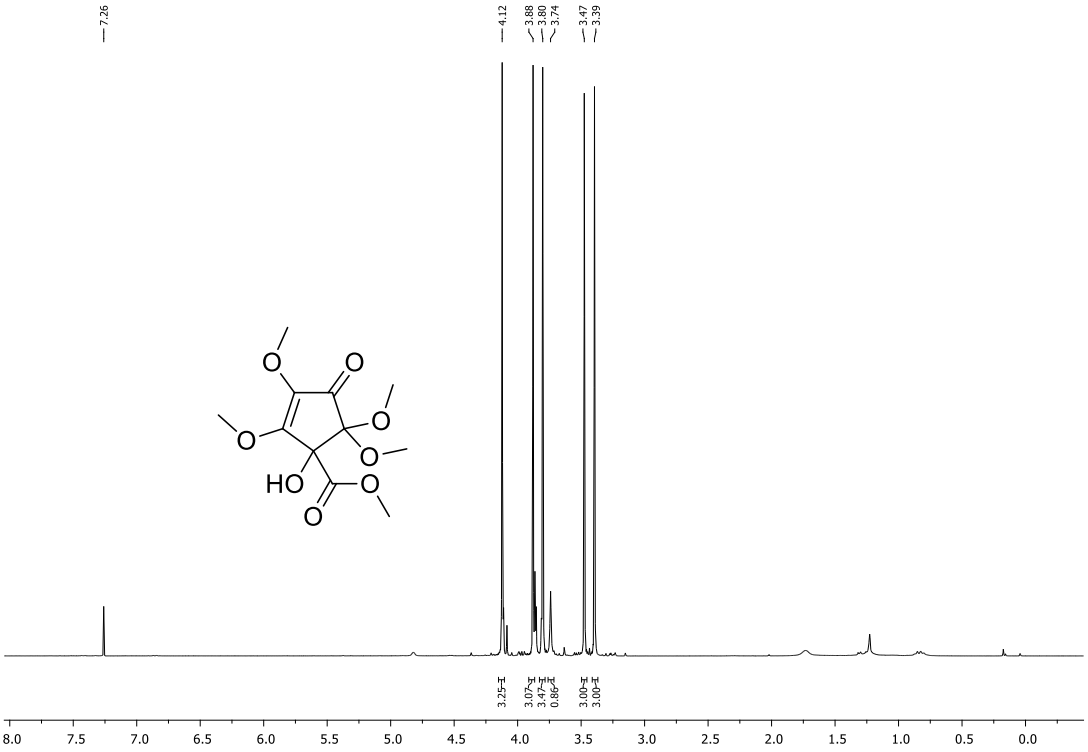


^{13}C NMR

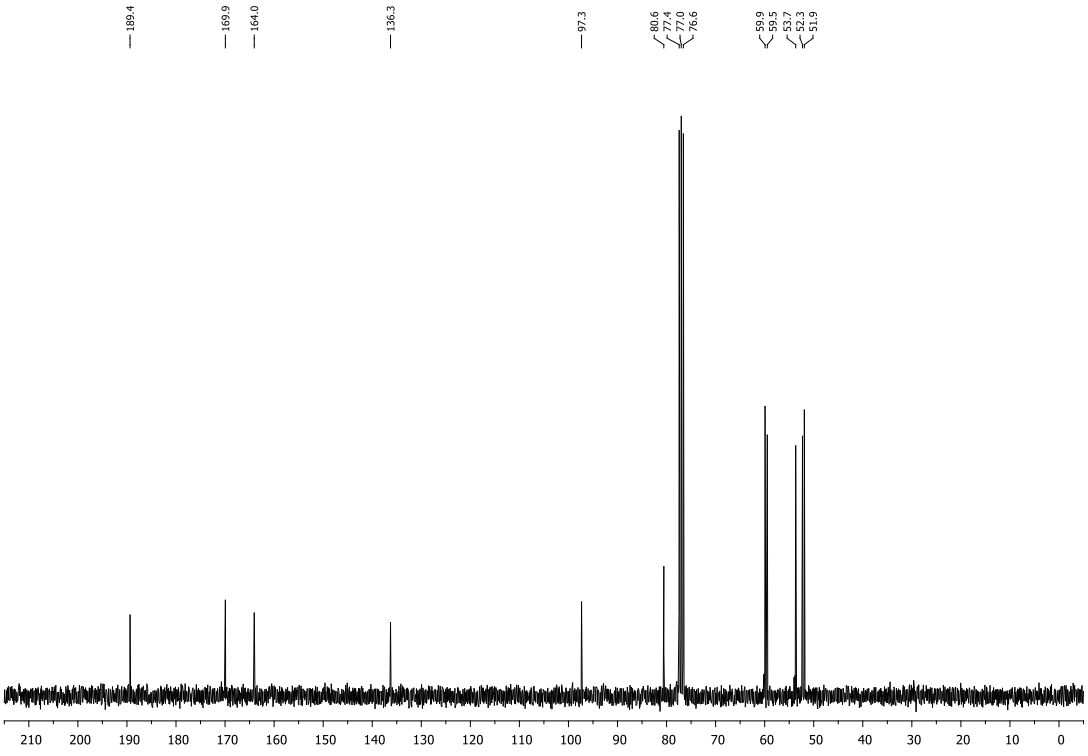


Methyl 1-hydroxy-2,3,5,5-tetramethoxy-4-oxocyclopent-2-enecarboxylate (113)
(in $CDCl_3$)

^{1}H NMR

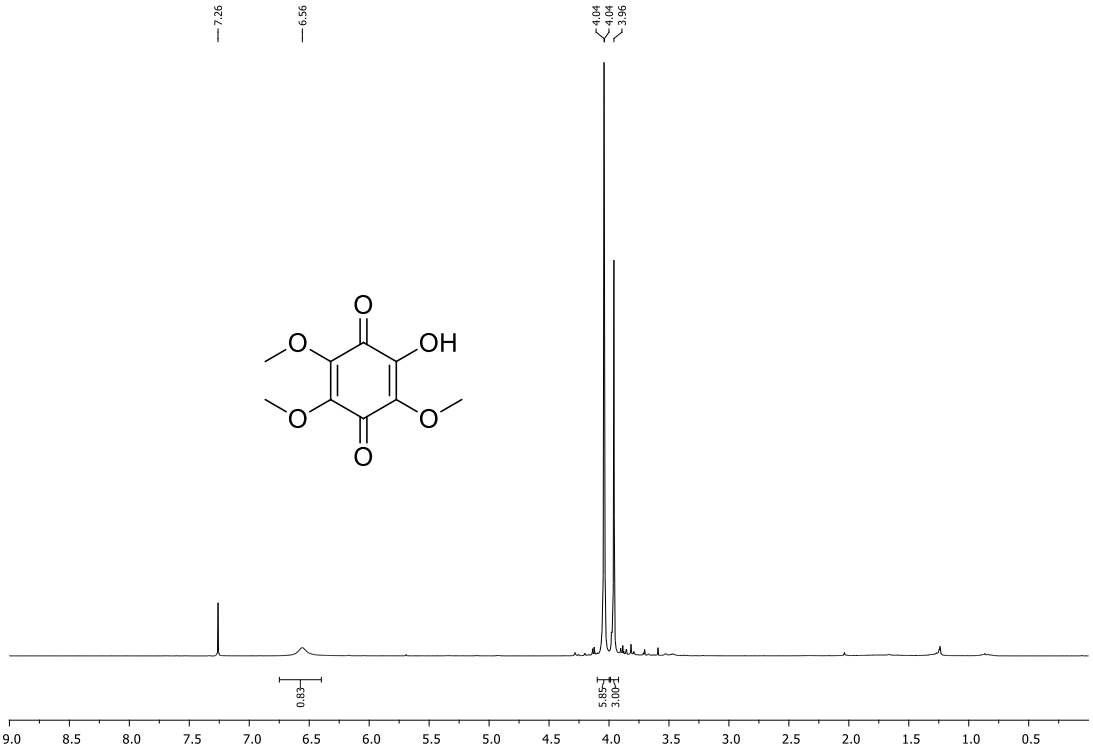


^{13}C NMR

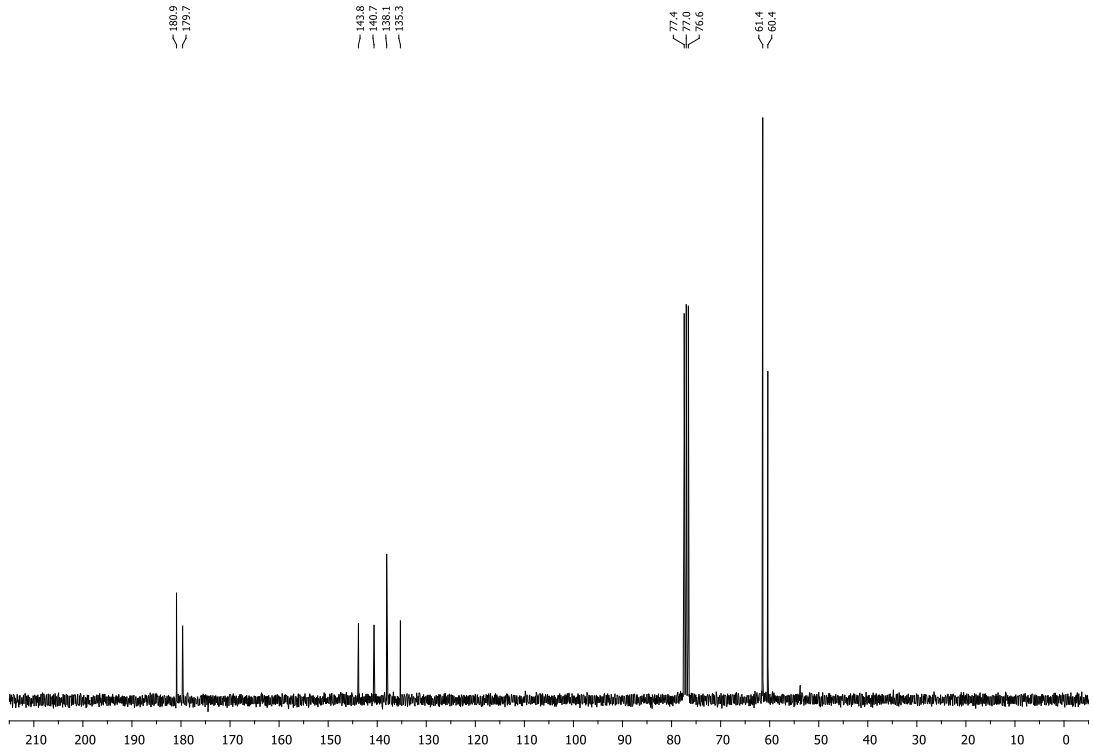


2-Hydroxy-3,5,6-trimethoxycyclohexa-2,5-diene-1,4-dione (116) (in $CDCl_3$)

^{1}H NMR

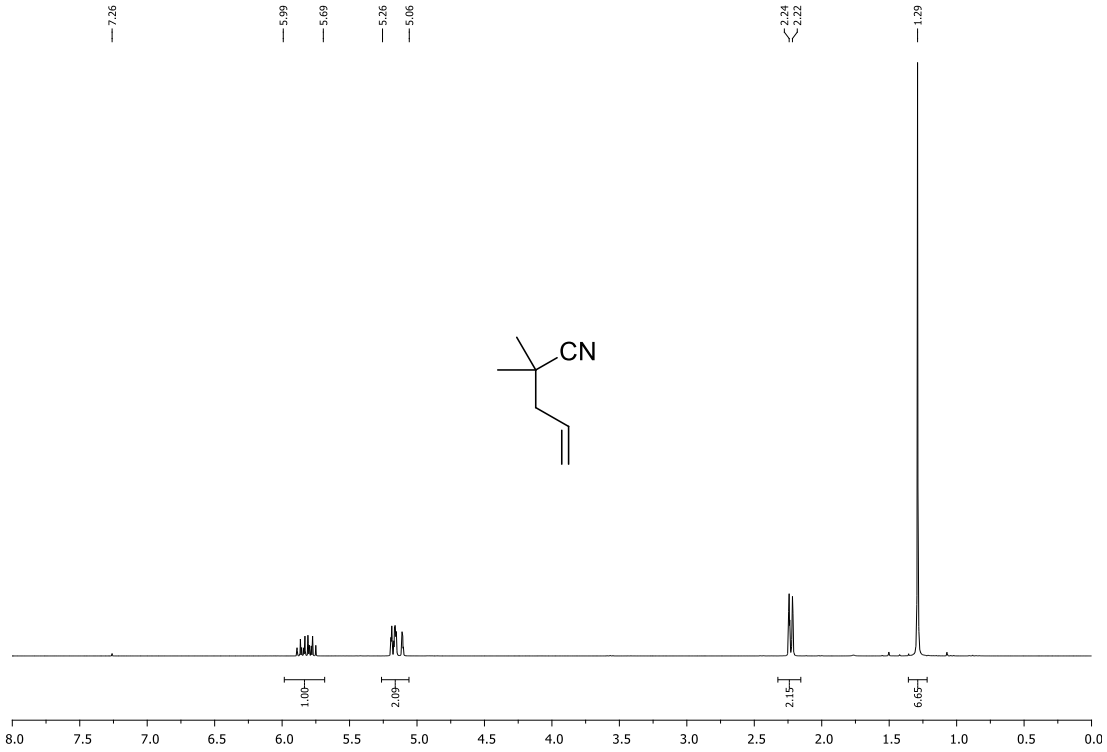


^{13}C NMR

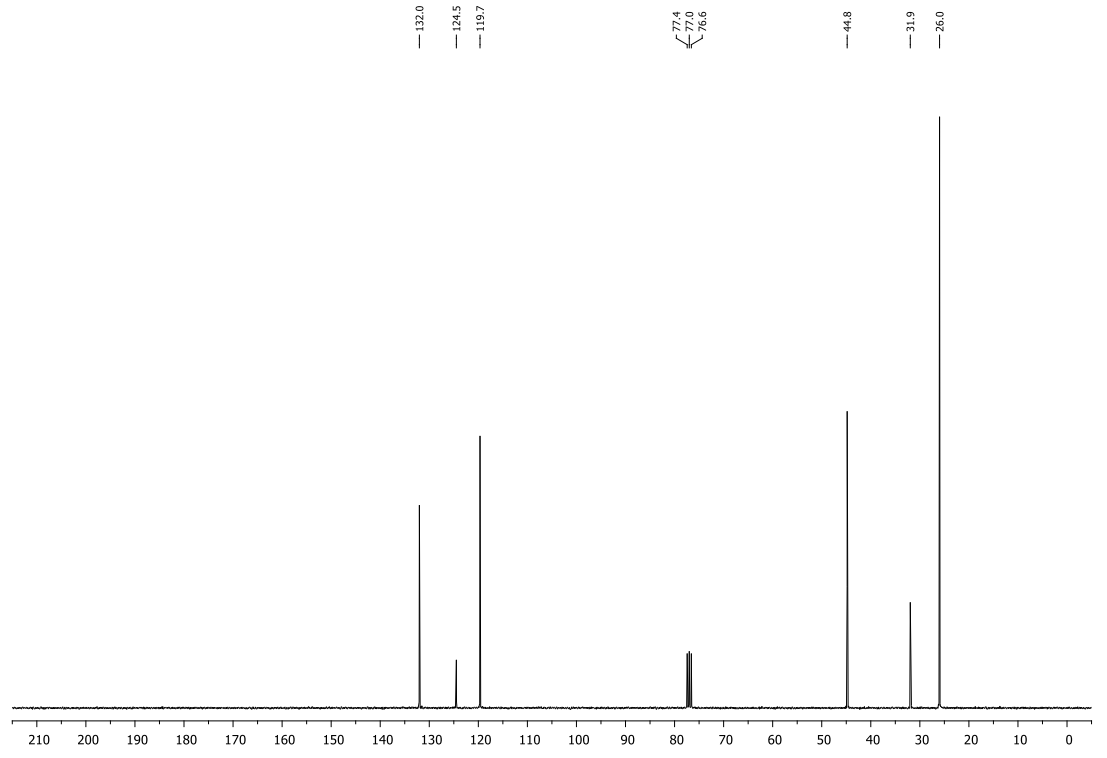


2,2-Dimethylpent-4-enenitrile (142) (in $CDCl_3$)

^{1}H NMR

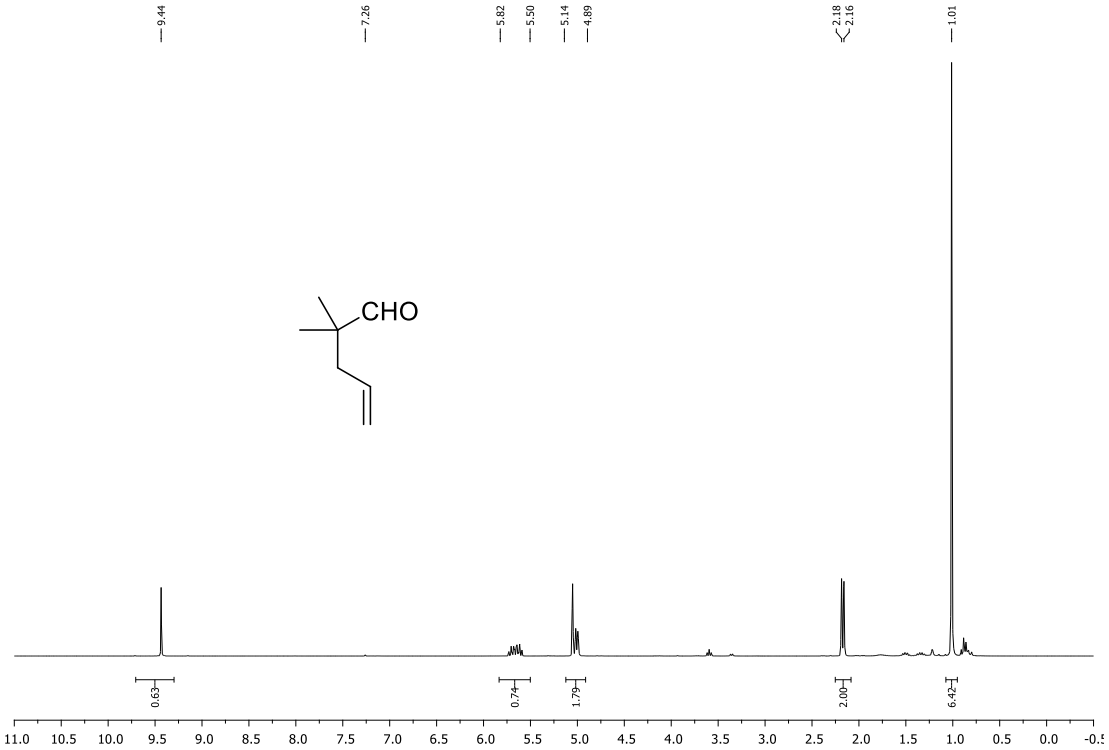


^{13}C NMR

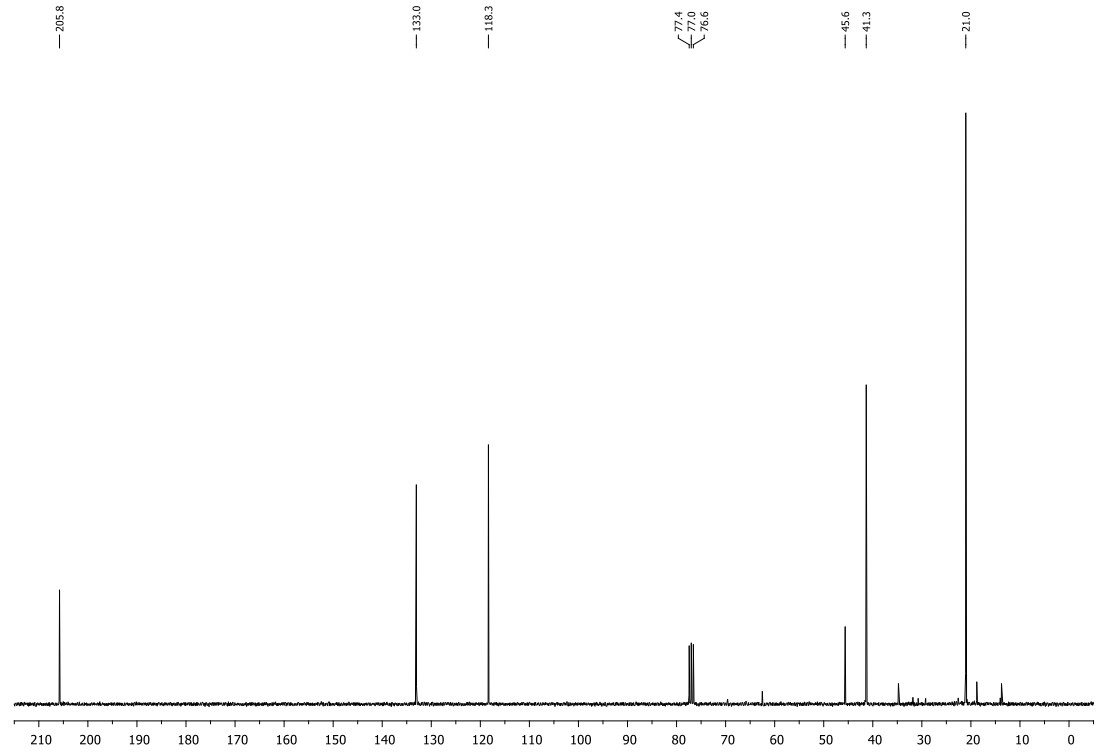


2,2-Dimethylpent-4-enal (136) (in $CDCl_3$)

^{1}H NMR

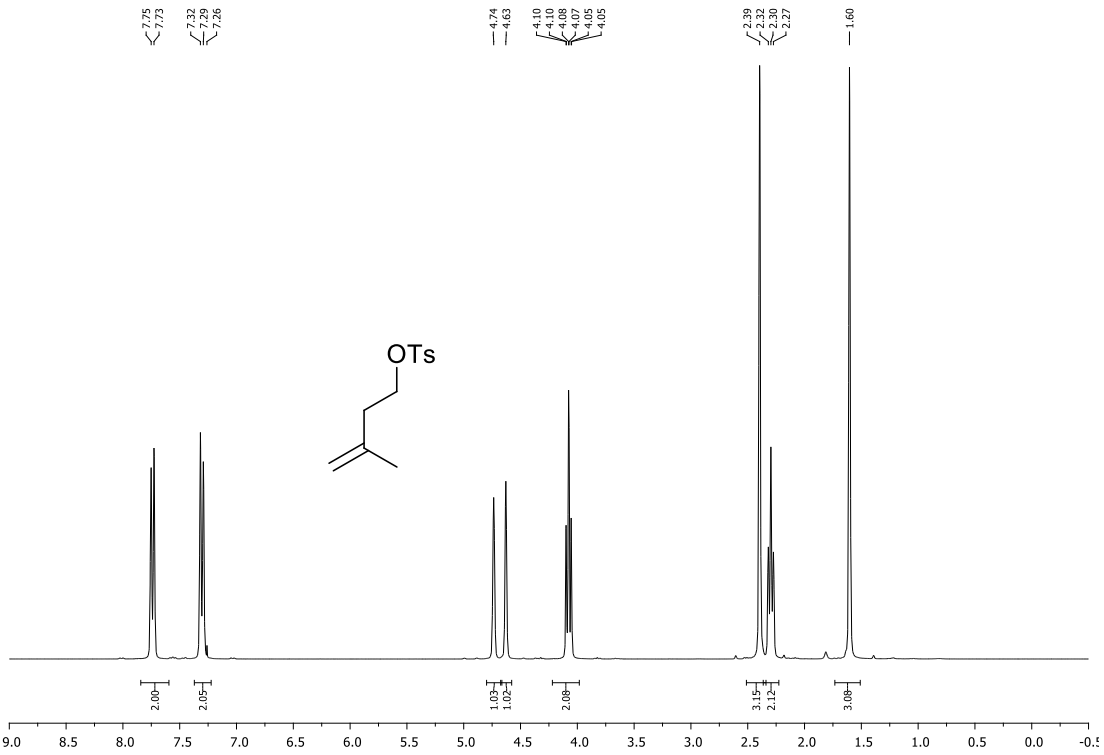


^{13}C NMR

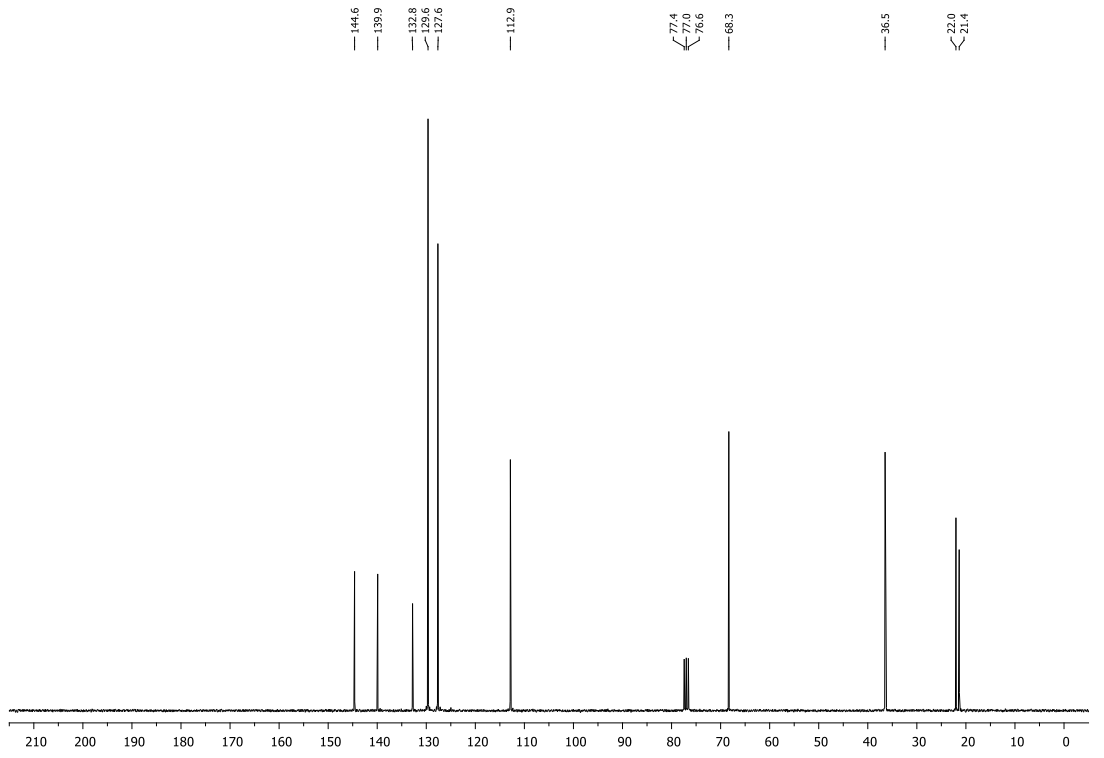


3-Methylbut-3-en-1-yl 4-methylbenzenesulfonate (153) (in $CDCl_3$)

^{1}H NMR

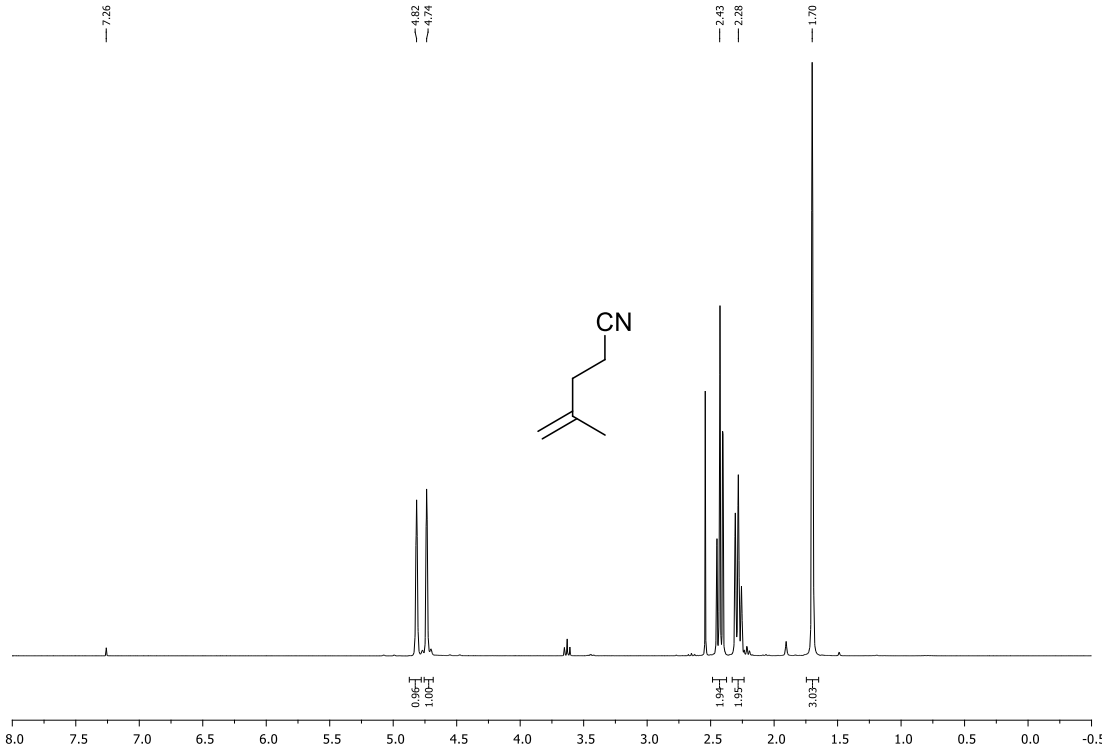


^{13}C NMR

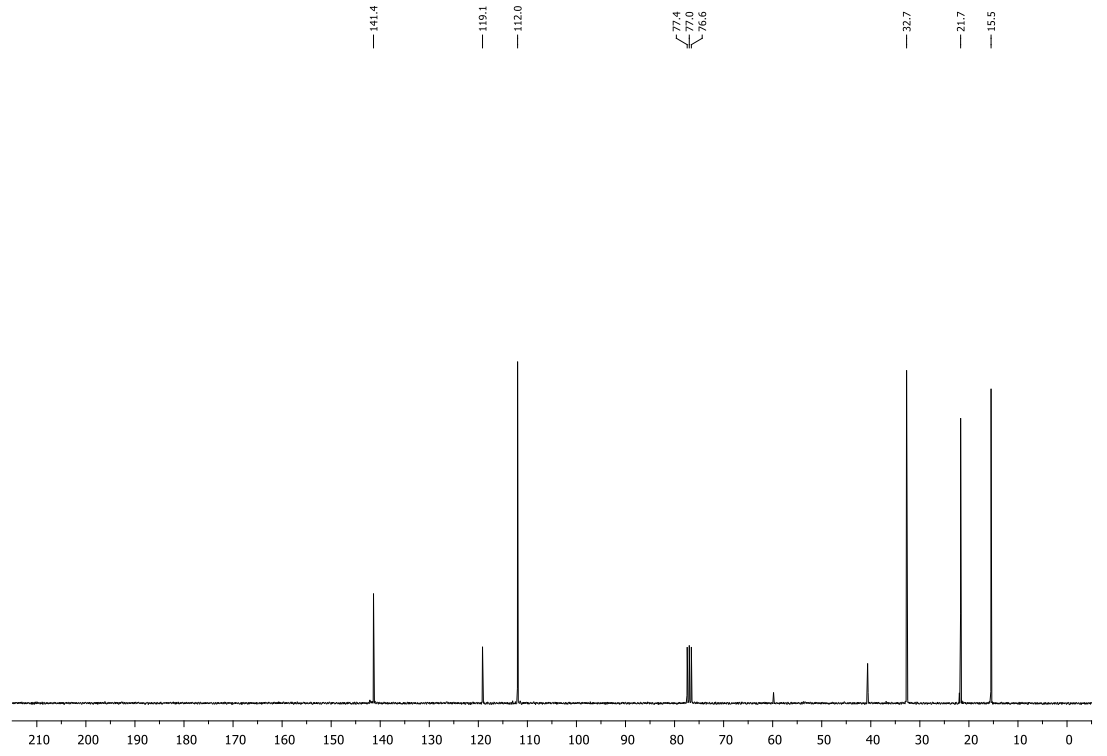


4-Methylpent-4-enenitrile (145) (in $CDCl_3$)

^{1}H NMR

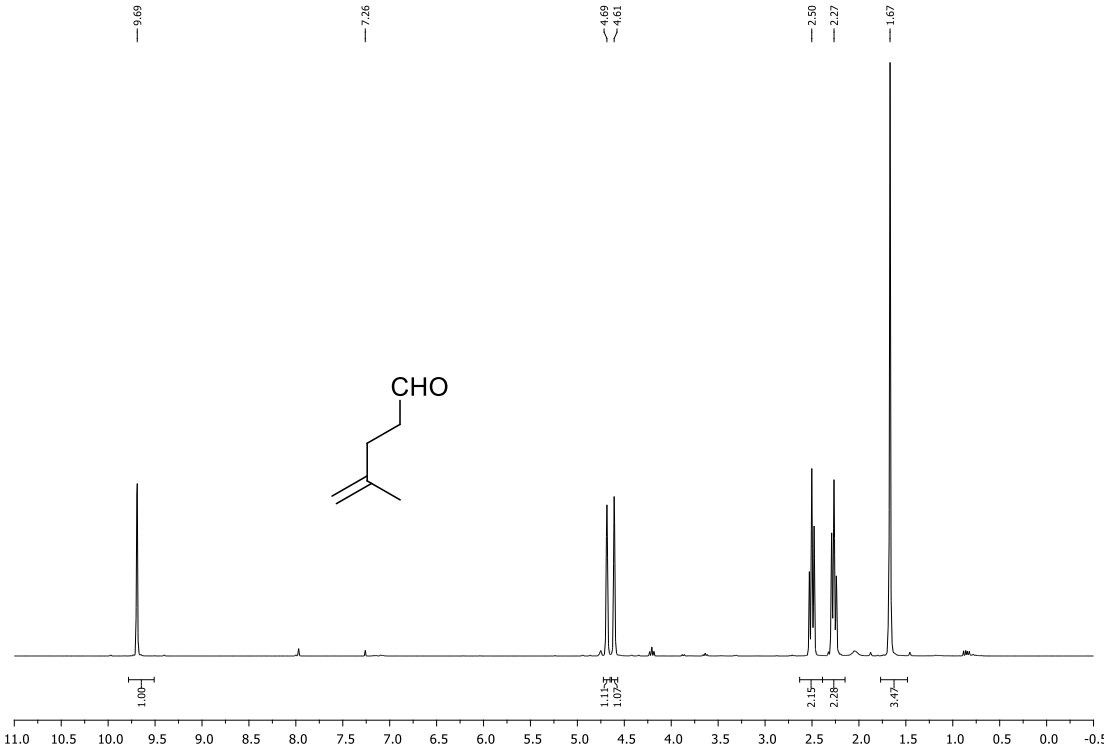


^{13}C NMR

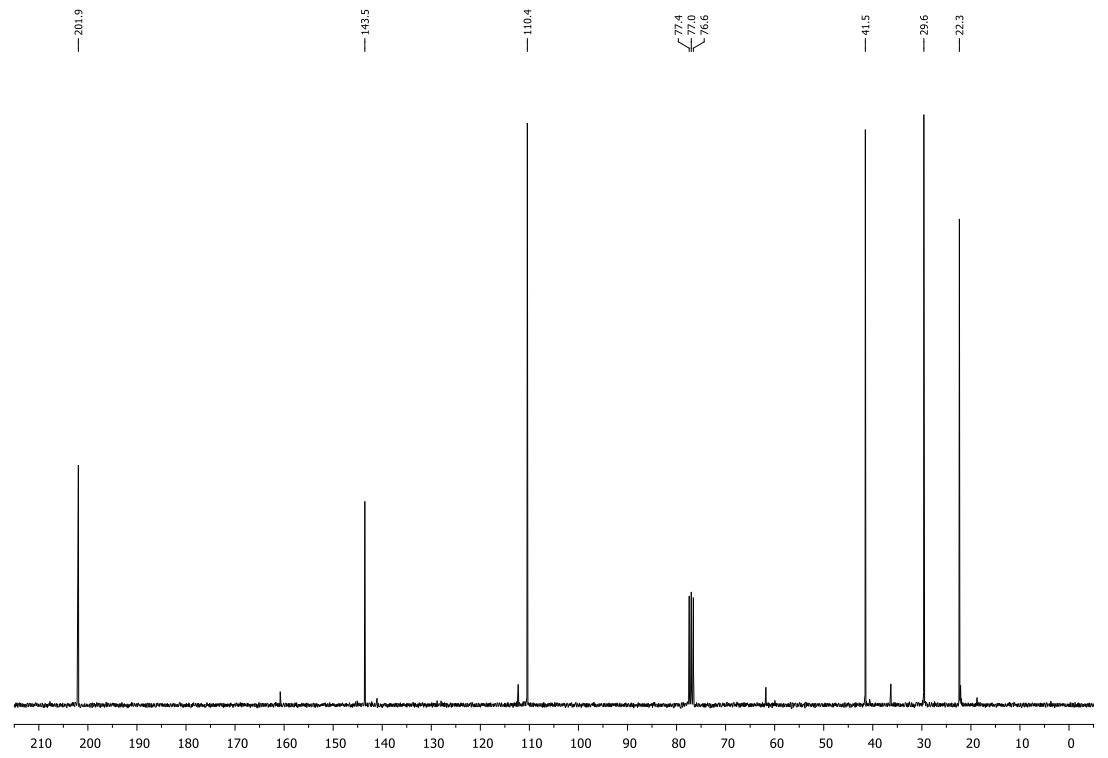


4-Methylpent-4-enal (143) (in $CDCl_3$)

^{1}H NMR

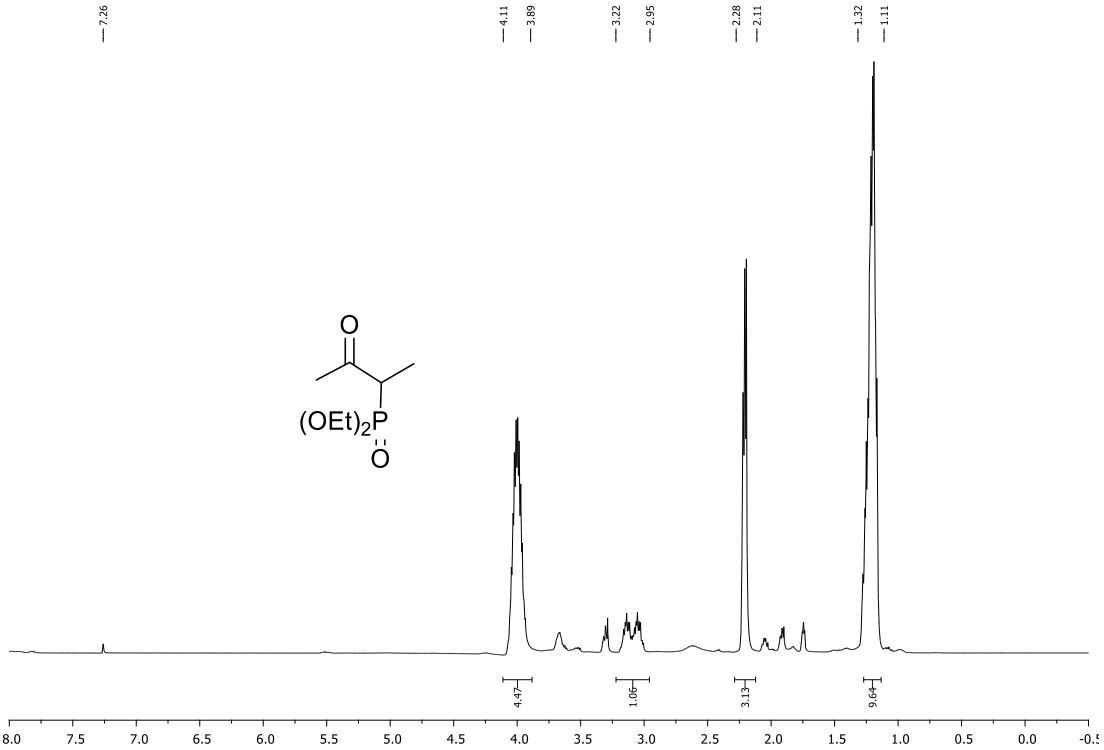


^{13}C NMR

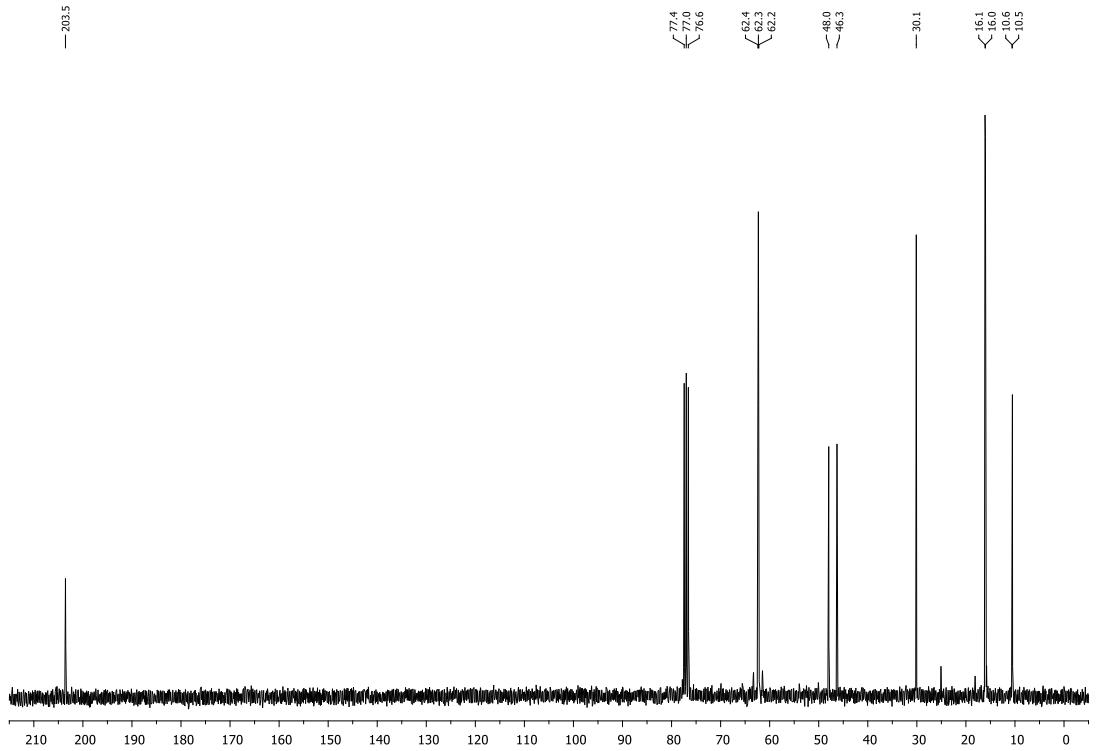


Diethyl (3-oxobutan-2-yl)phosphonate (144) (in $CDCl_3$)

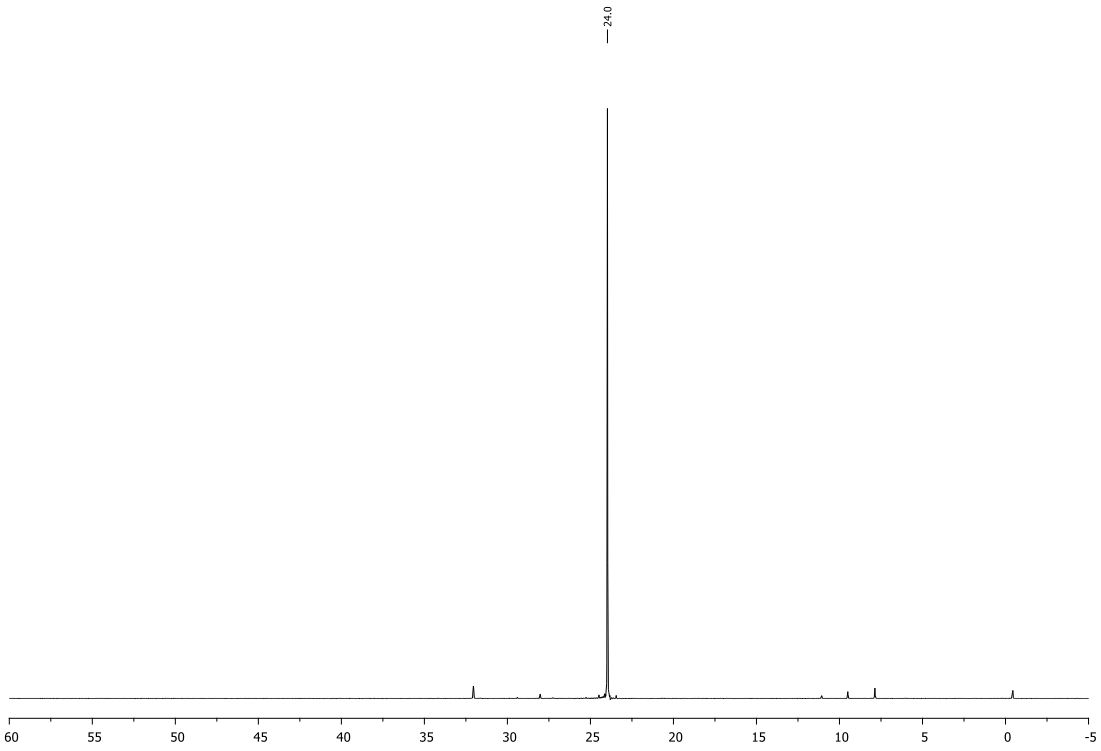
^{1}H NMR



^{13}C NMR

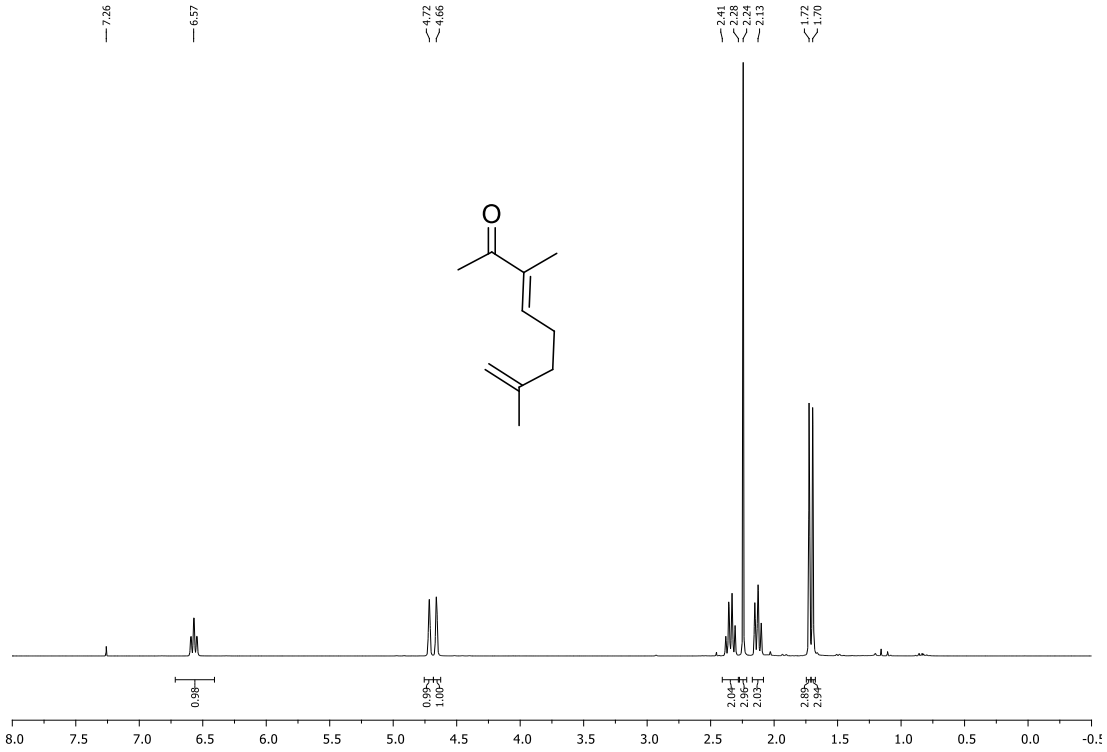


^{31}P NMR

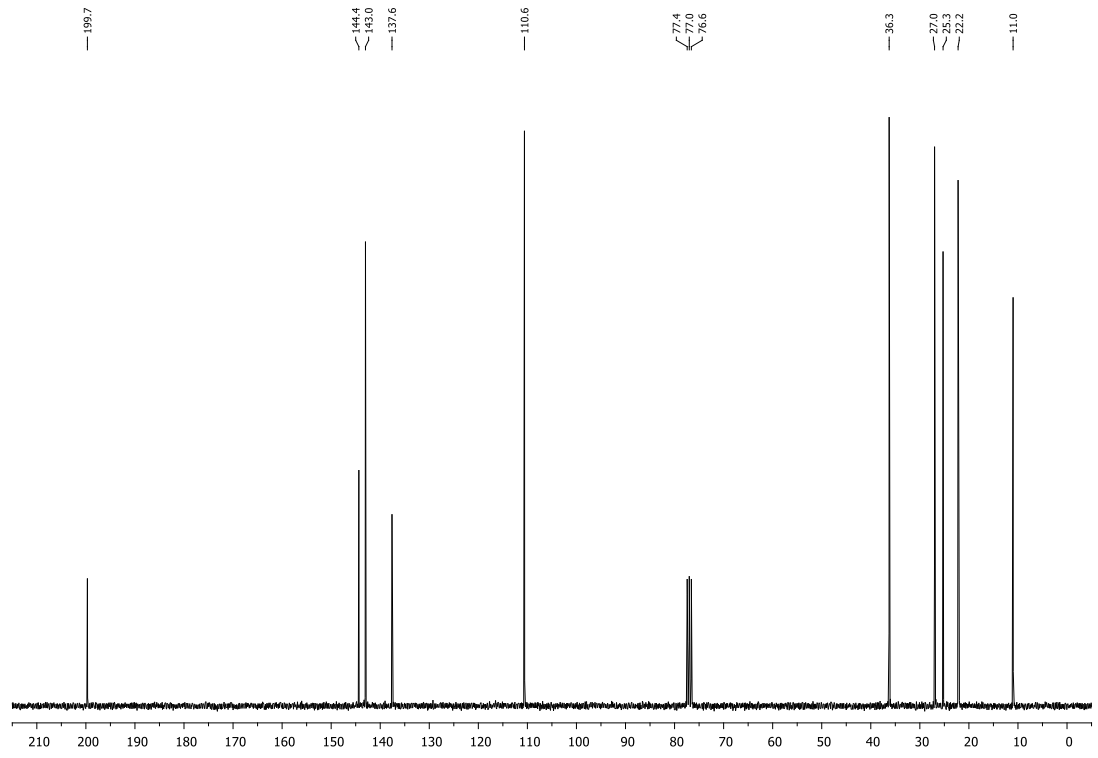


3,7-Dimethylocta-3,7-dien-2-one (137) (in $CDCl_3$)

^{1}H NMR

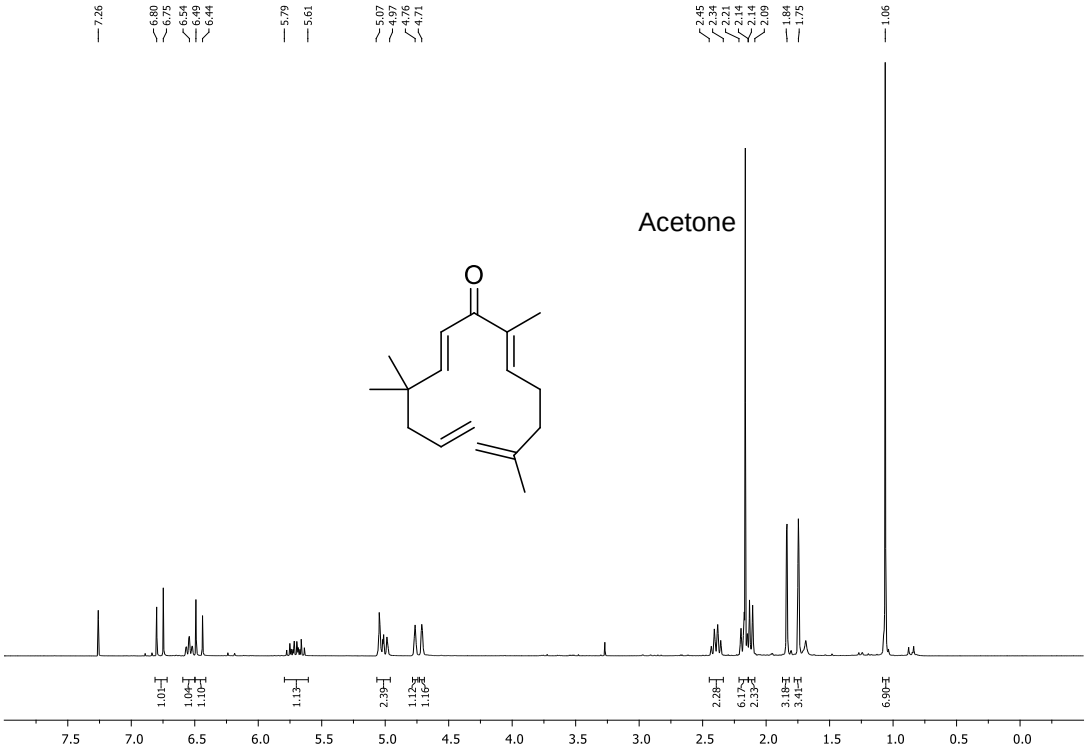


^{13}C NMR

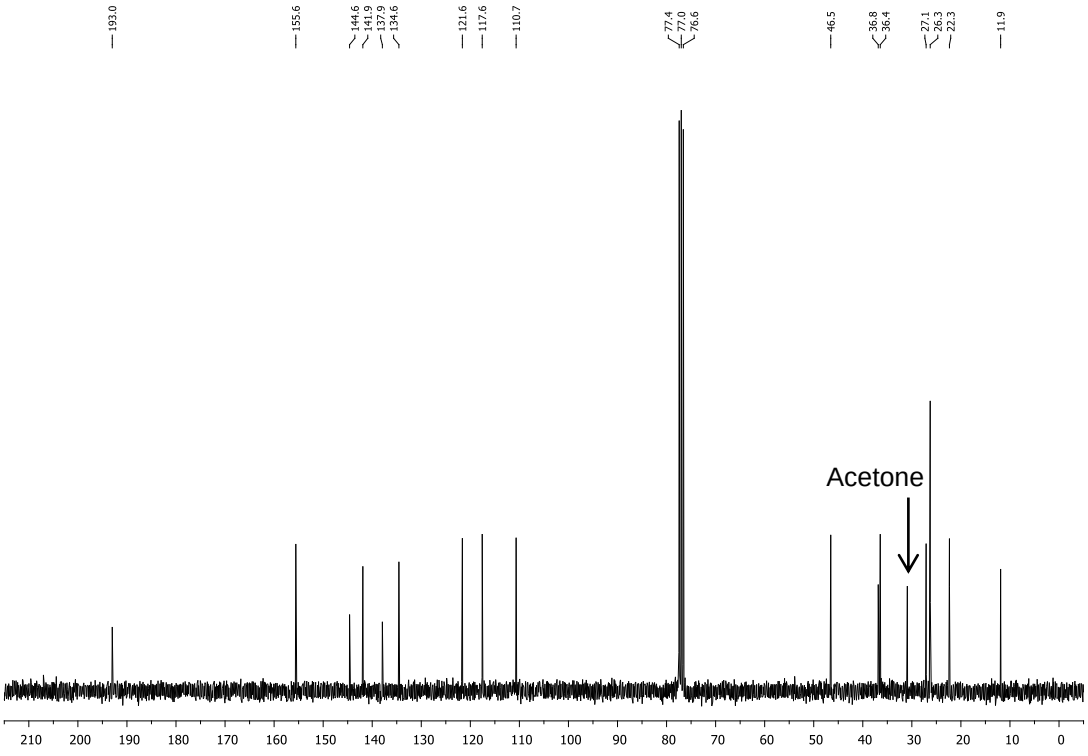


2,6,10,10-Tetramethyltrideca-1,5,8,12-tetraen-7-one (129) (in $CDCl_3$)

^{1}H NMR

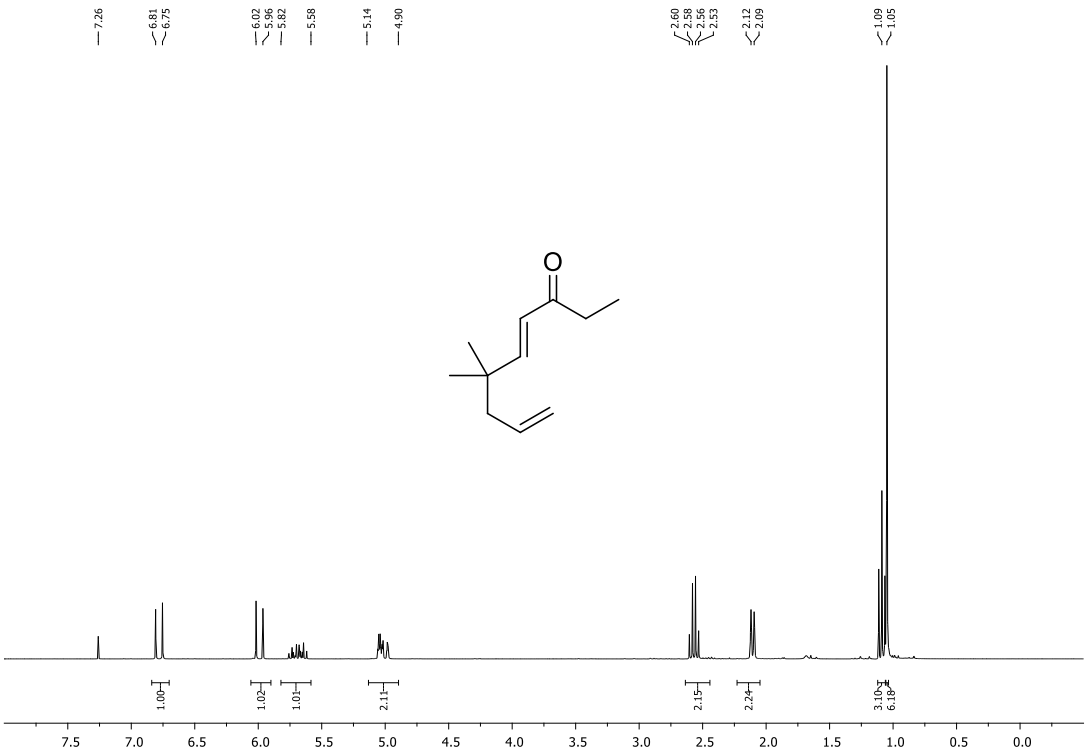


^{13}C NMR

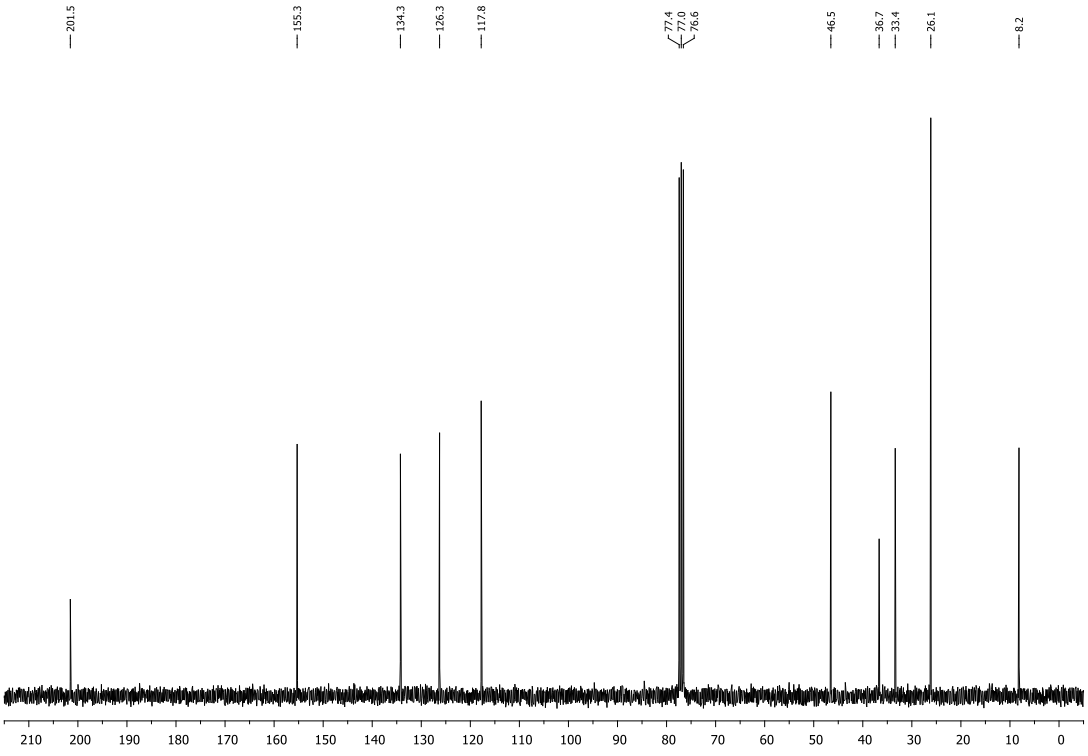


6,6-Dimethylnona-4,8-dien-3-one (155) (in $CDCl_3$)

^{1}H NMR

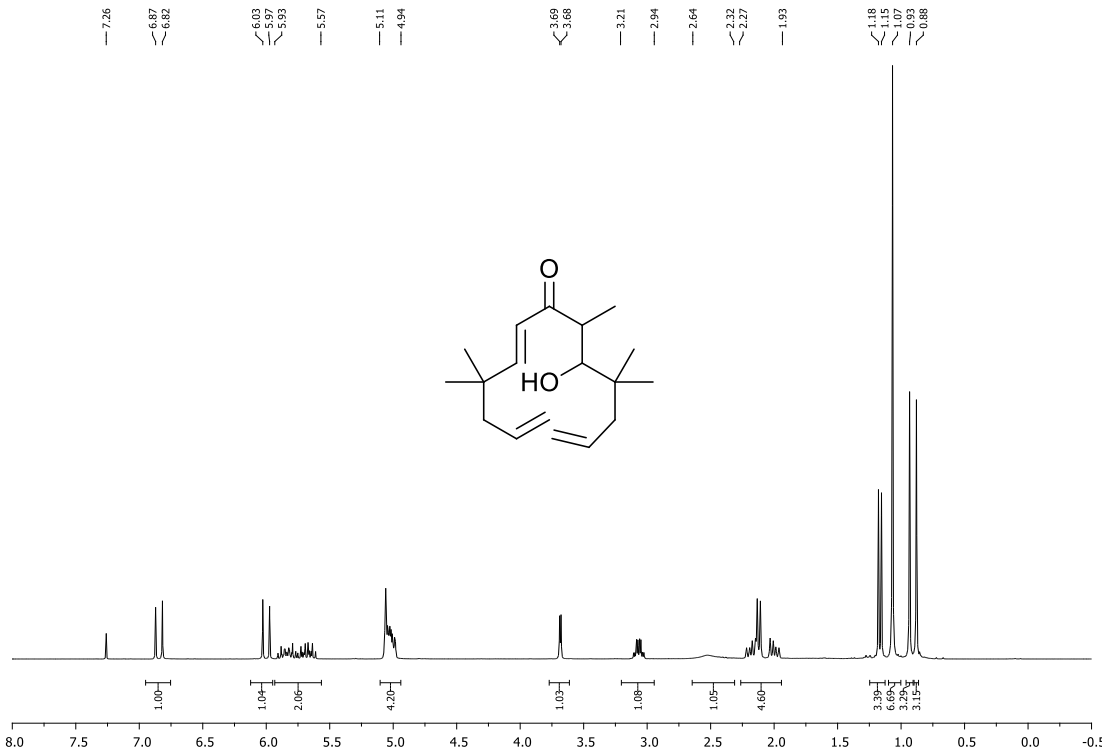


^{13}C NMR

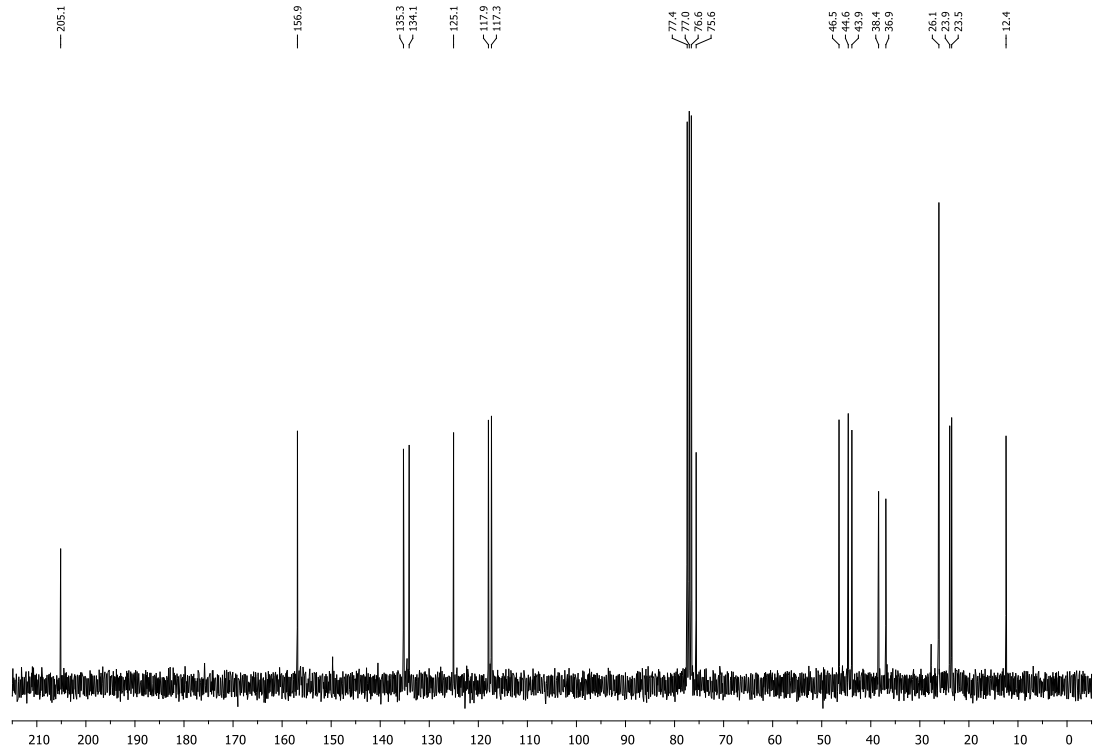


9-Hydroxy-4,4,8,10,10-pentamethyltrideca-1,5,12-trien-7-one (154) (in $CDCl_3$)

^{1}H NMR

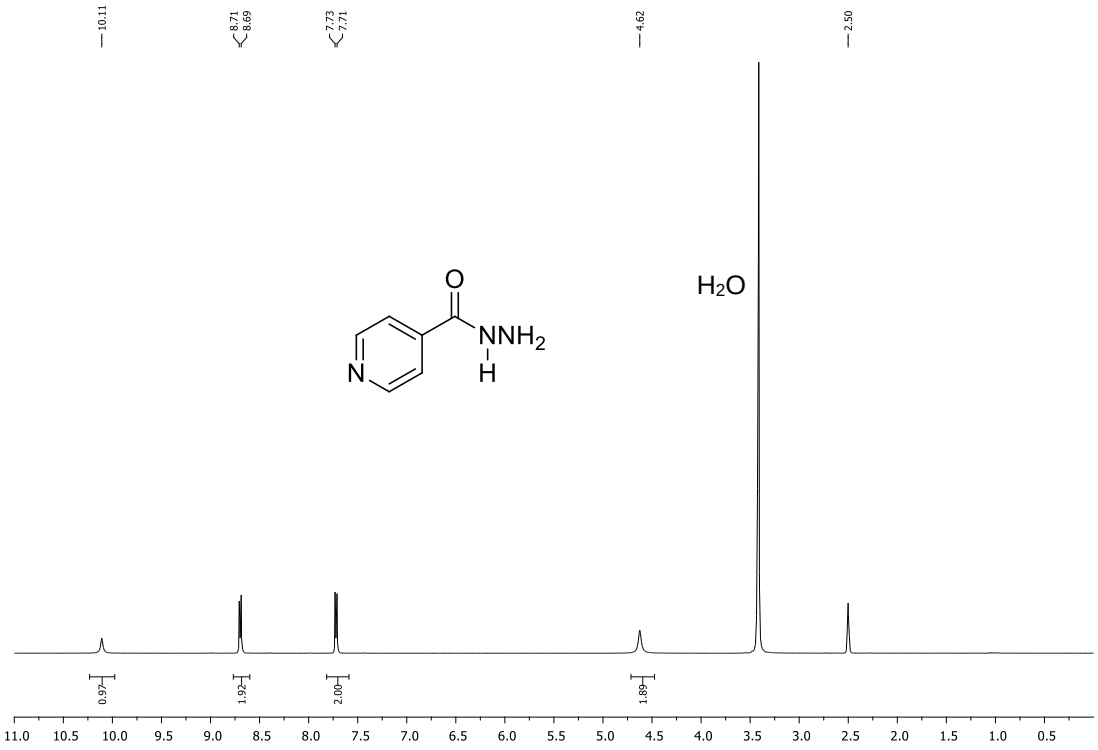


^{13}C NMR

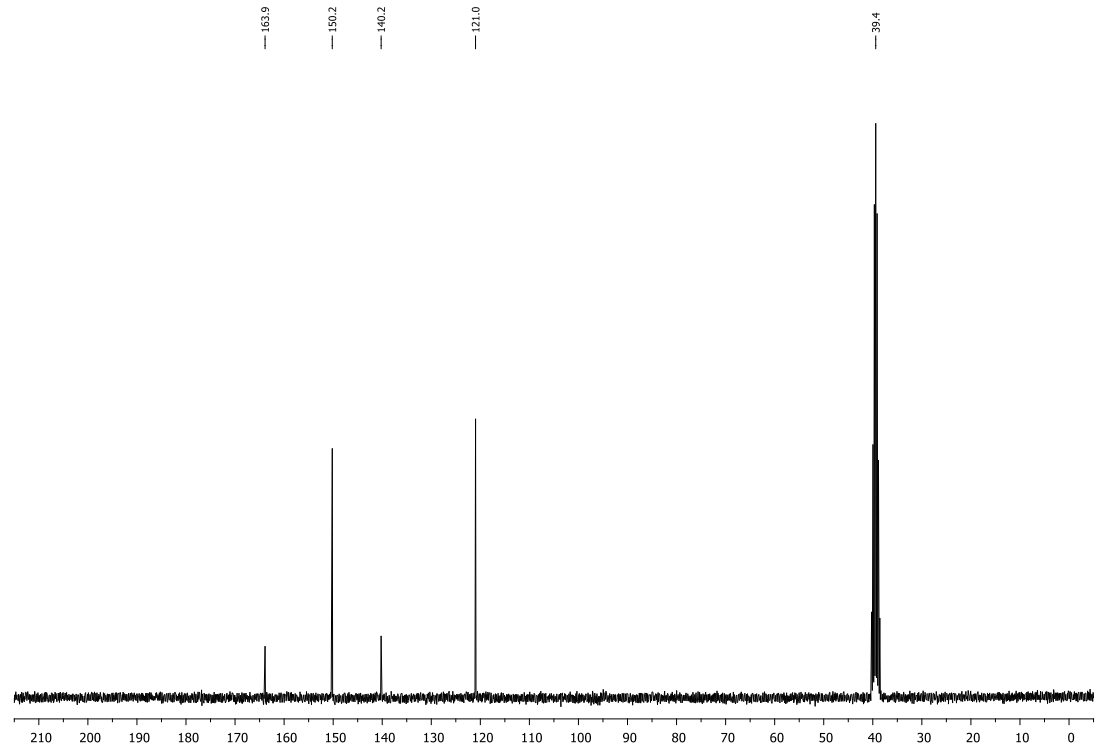


Isonicotinohydrazide (166) (in DMSO-d_6)

^{1}H NMR

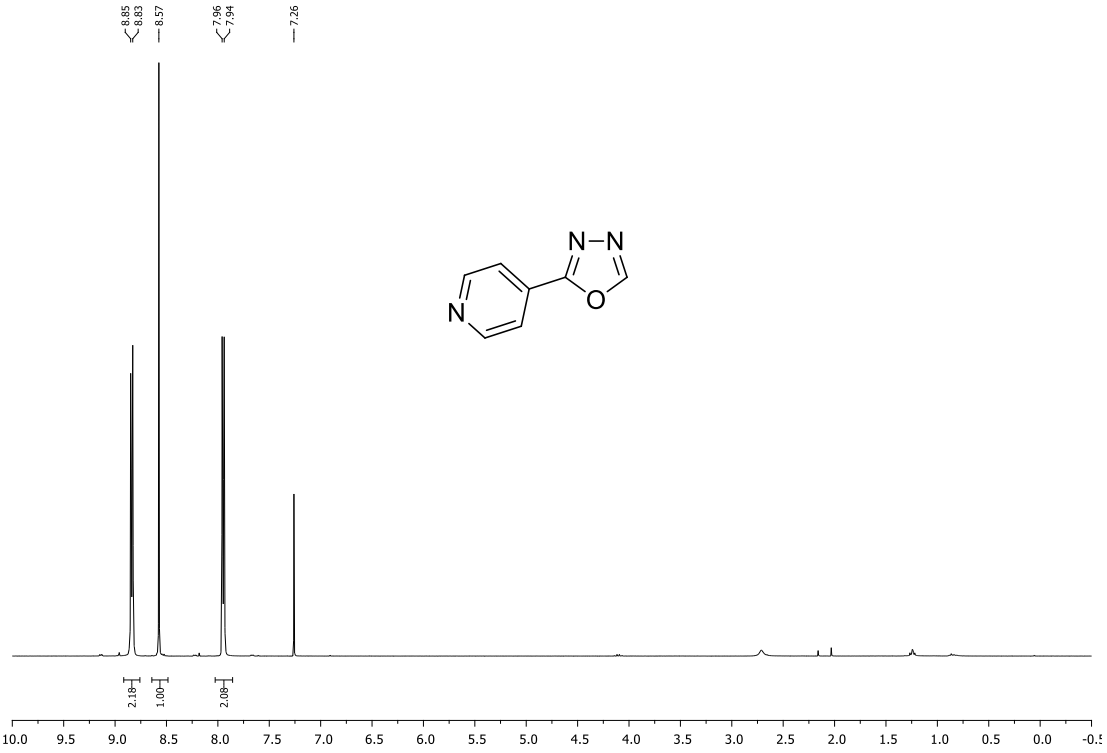


^{13}C NMR

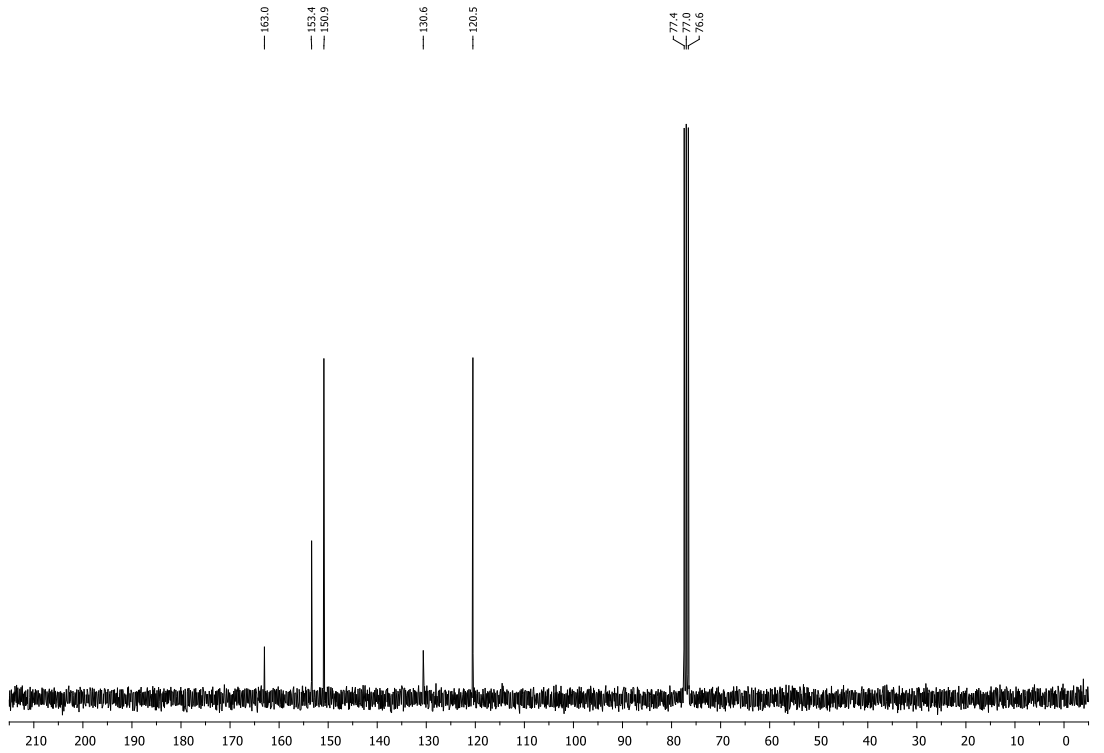


2-(Pyridin-4-yl)-1,3,4-oxadiazole (167) (in $CDCl_3$)

^{1}H NMR

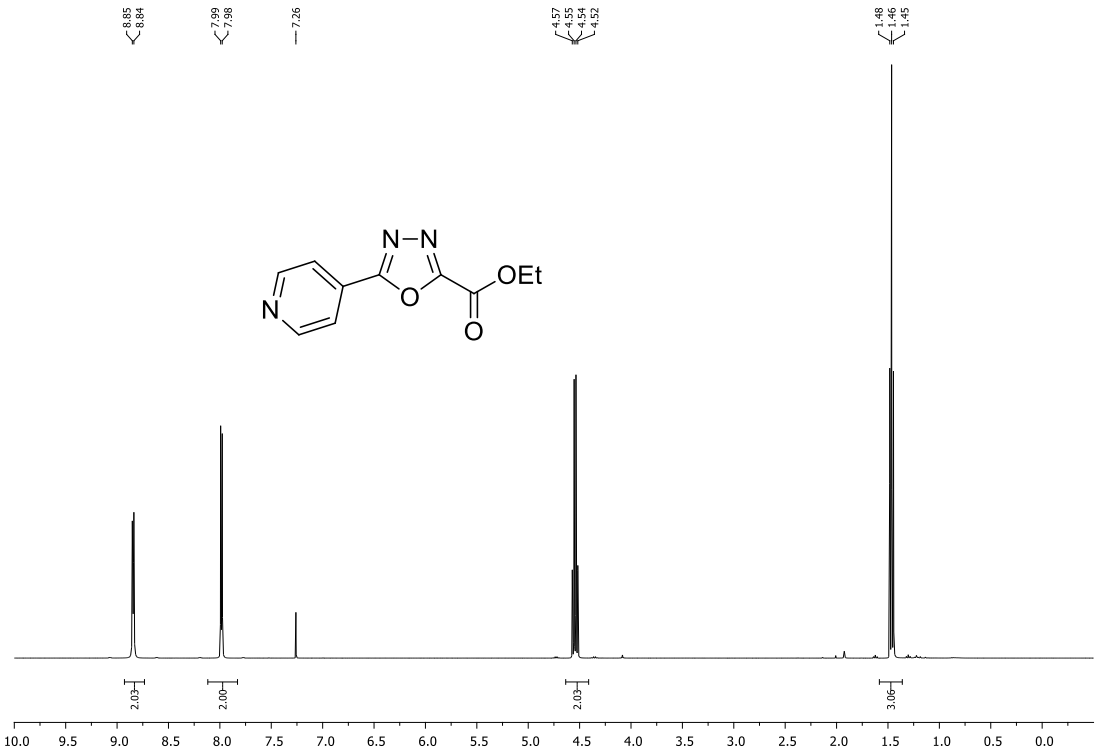


^{13}C NMR

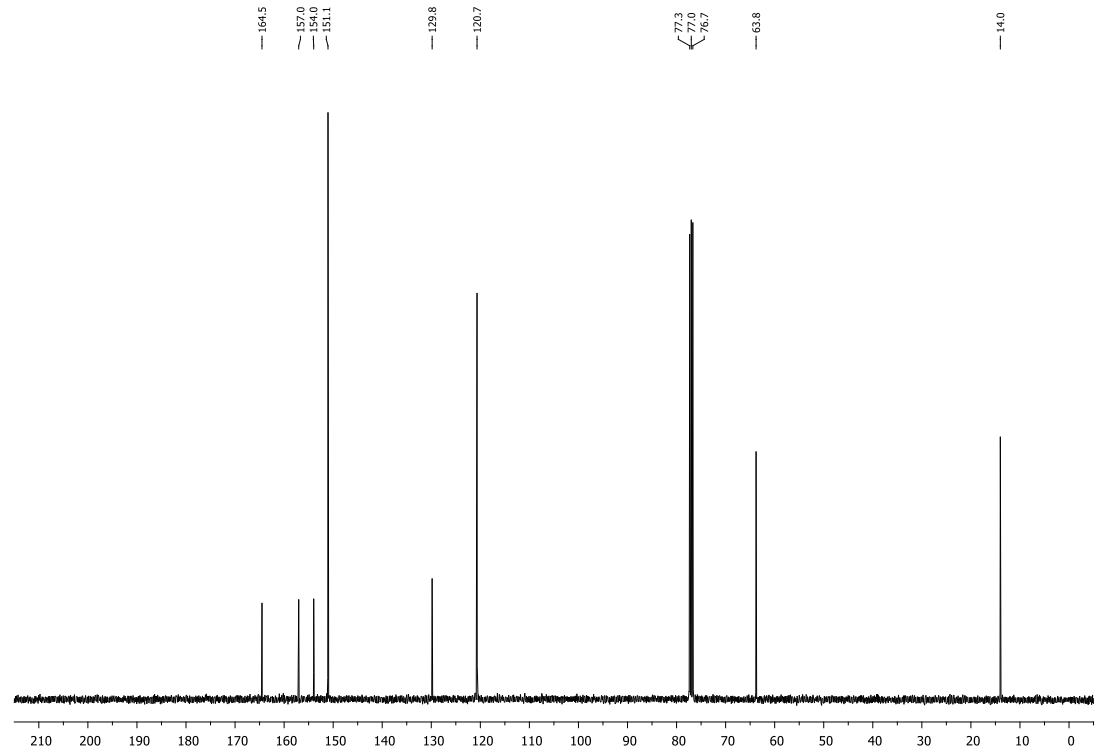


Ethyl 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-carboxylate (172) (in $CDCl_3$)

^{1}H NMR

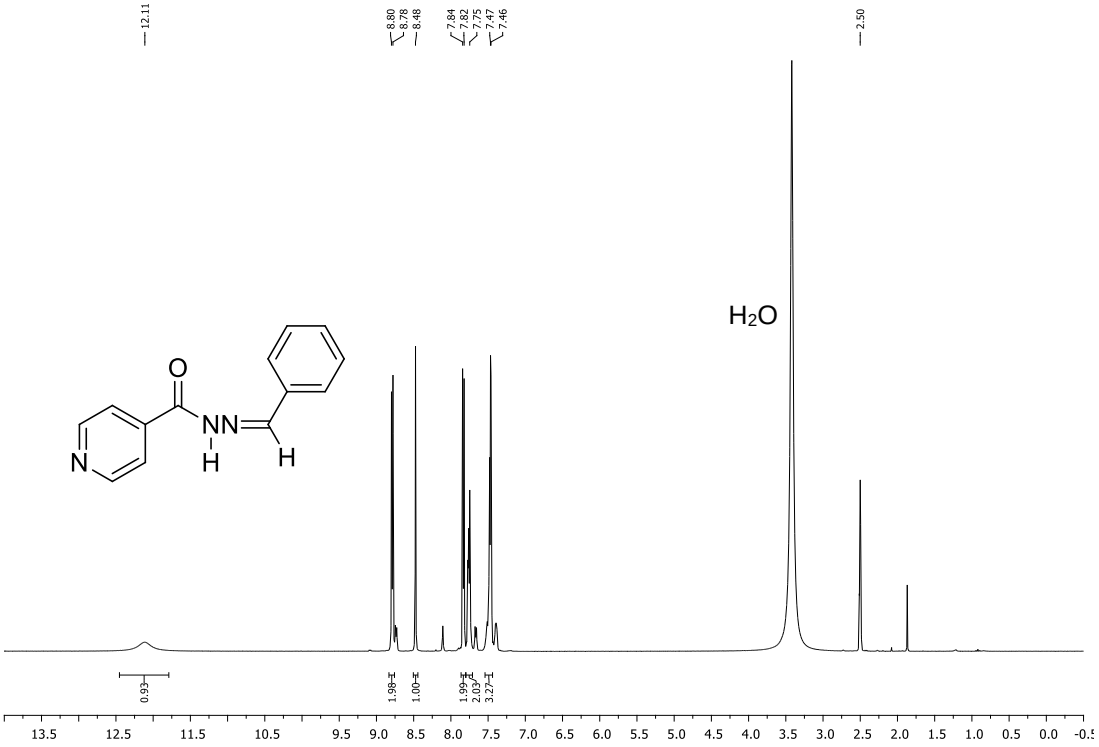


^{13}C NMR

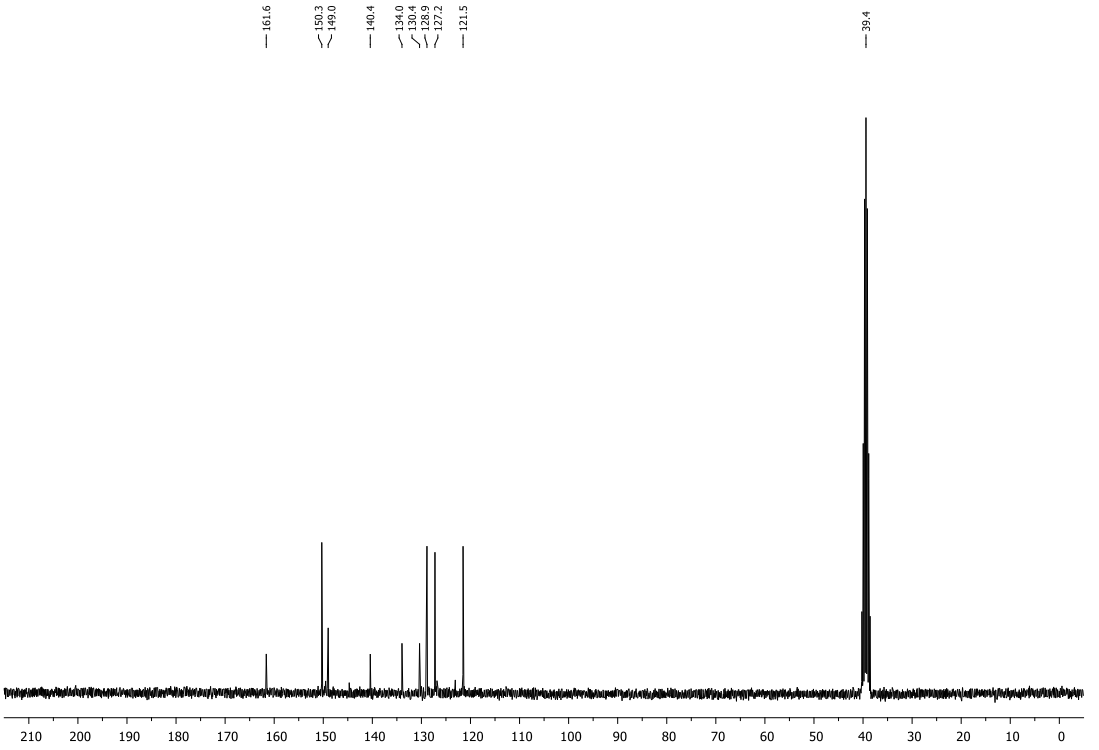


***N'*-Benzylideneisonicotinohydrazide (179)** (in DMSO-d_6)

^{1}H NMR

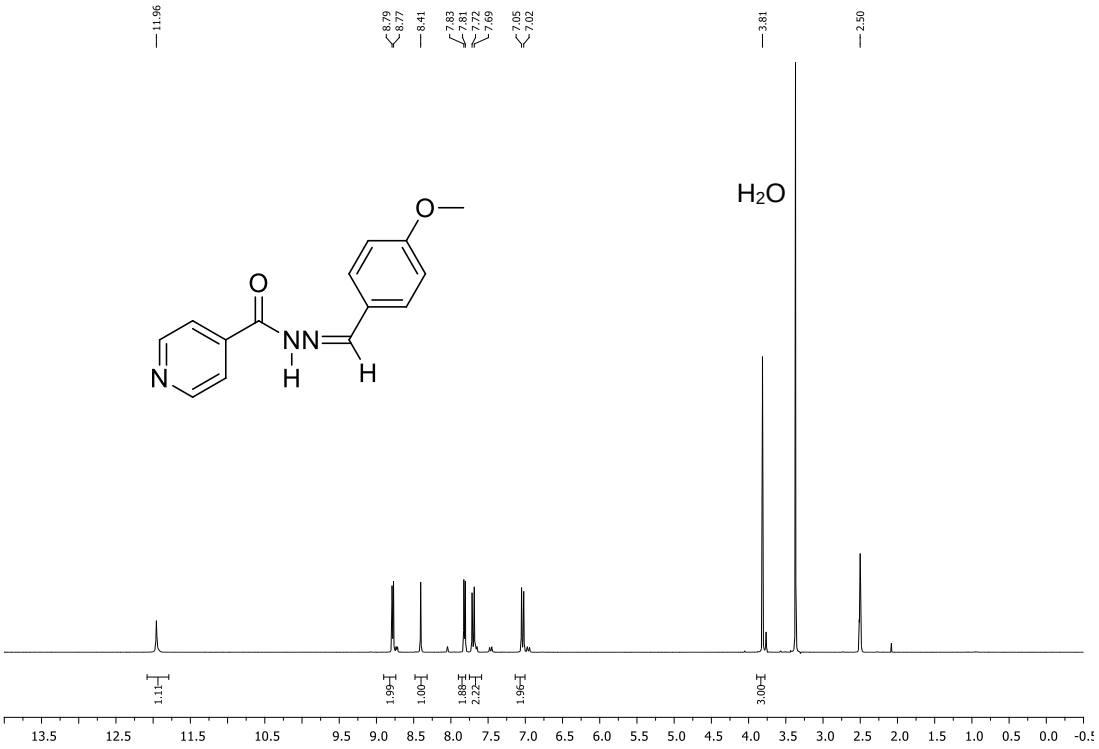


^{13}C NMR

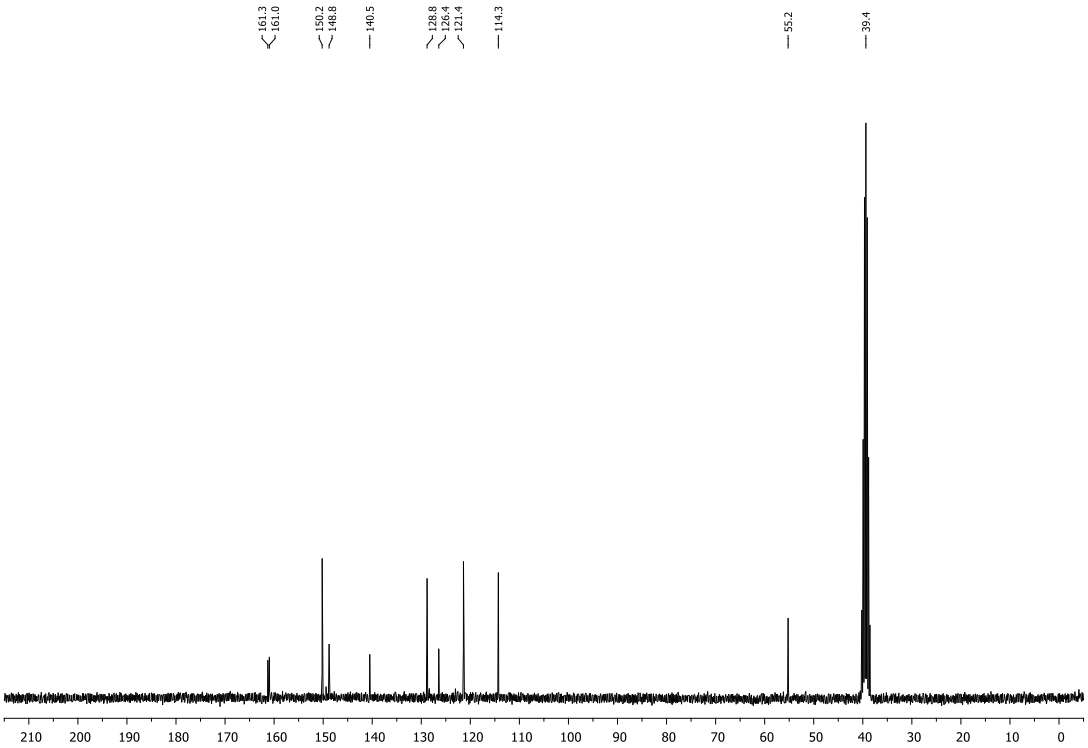


***N*'-(4-Methoxybenzylidene)isonicotinohydrazide (180)** (in DMSO-d_6)

^{1}H NMR

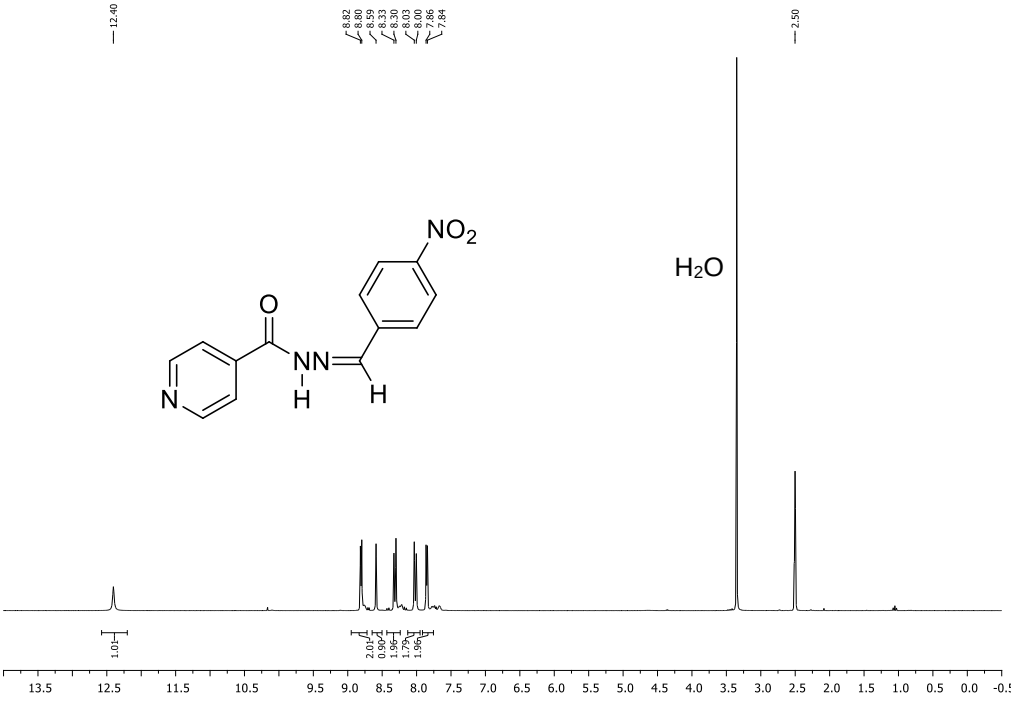


^{13}C NMR

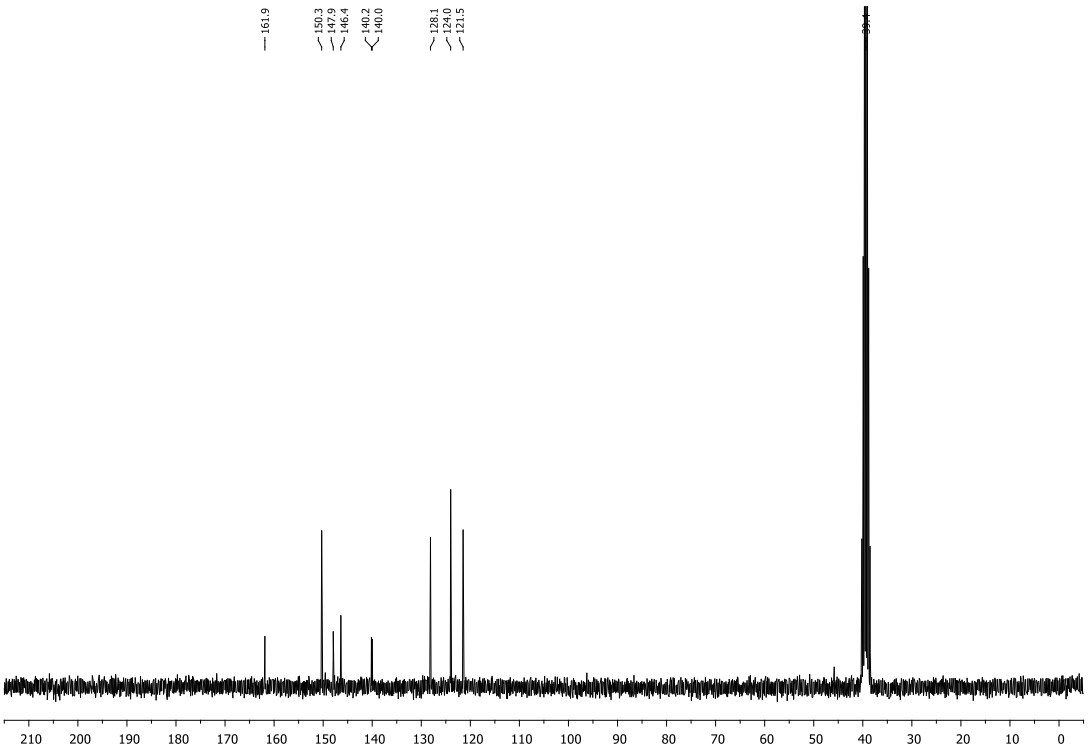


***N*'-(4-Nitrobenzylidene)isonicotinohydrazide (181)** (in DMSO-d_6)

^{1}H NMR

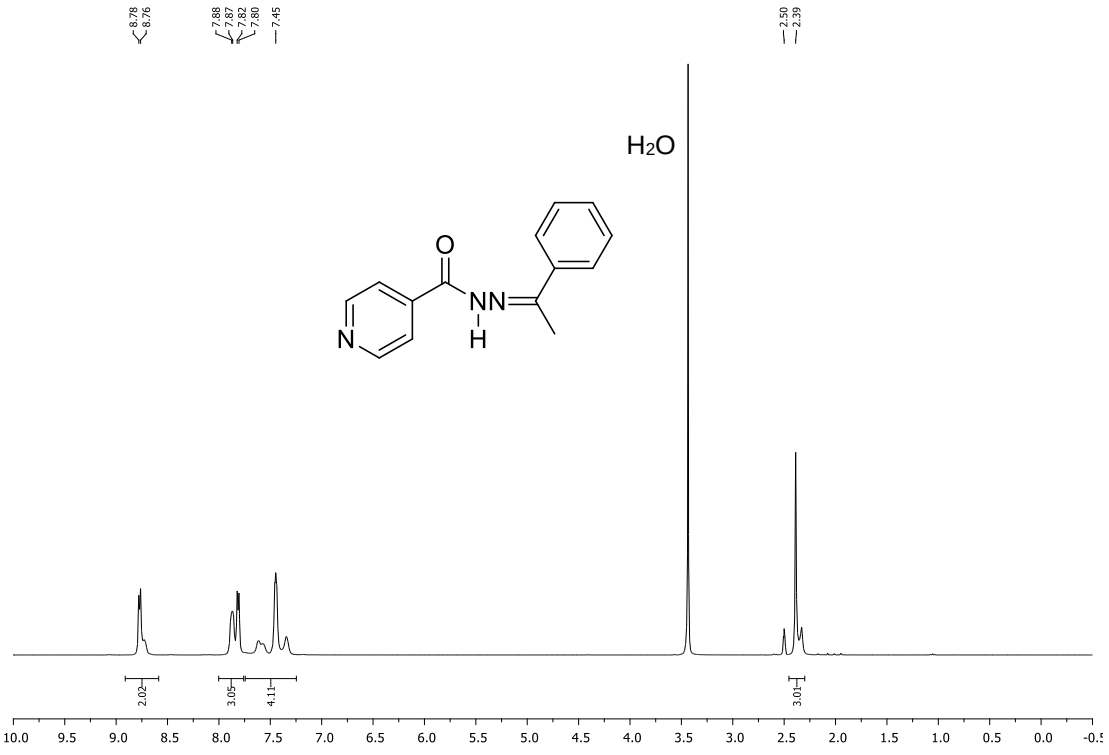


^{13}C NMR

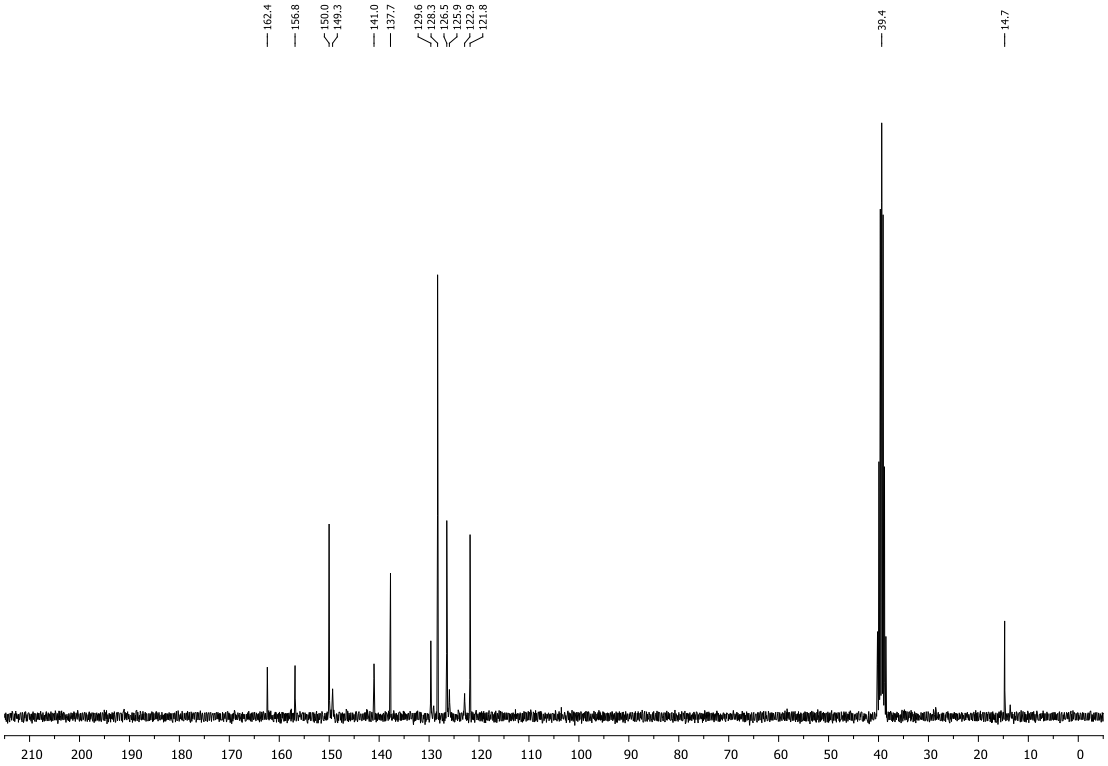


***N*'-(1-Phenylethylidene)isonicotinohydrazide (182)** (in DMSO-d_6)

^{1}H NMR

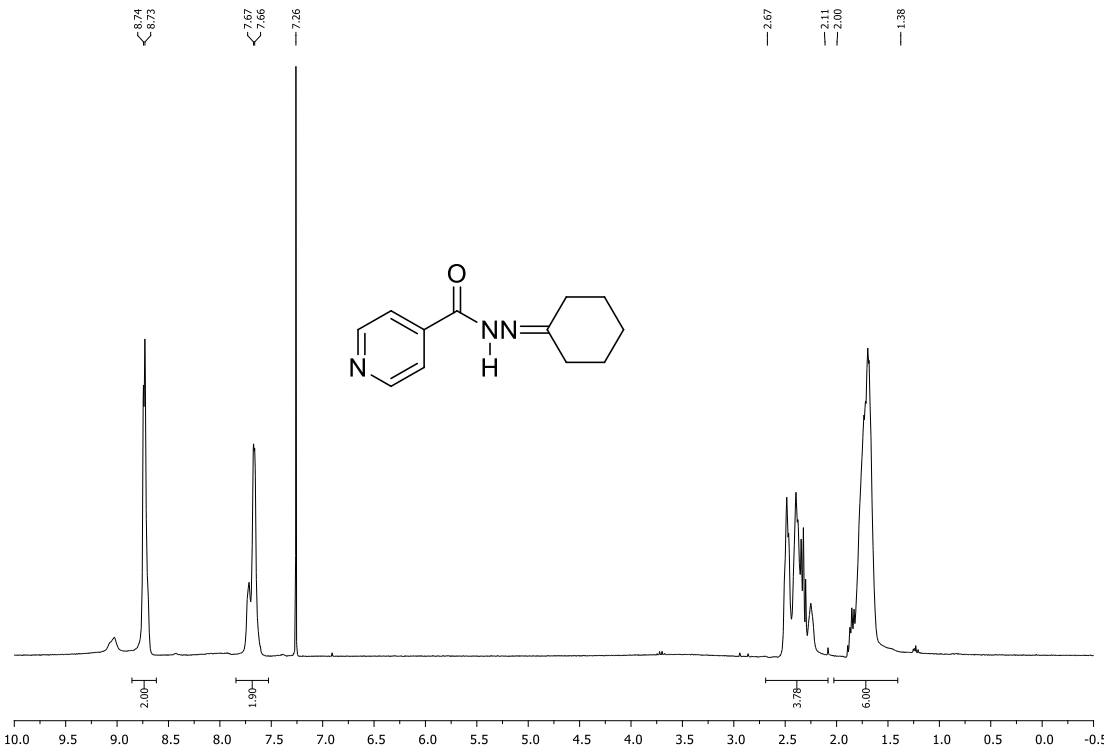


^{13}C NMR

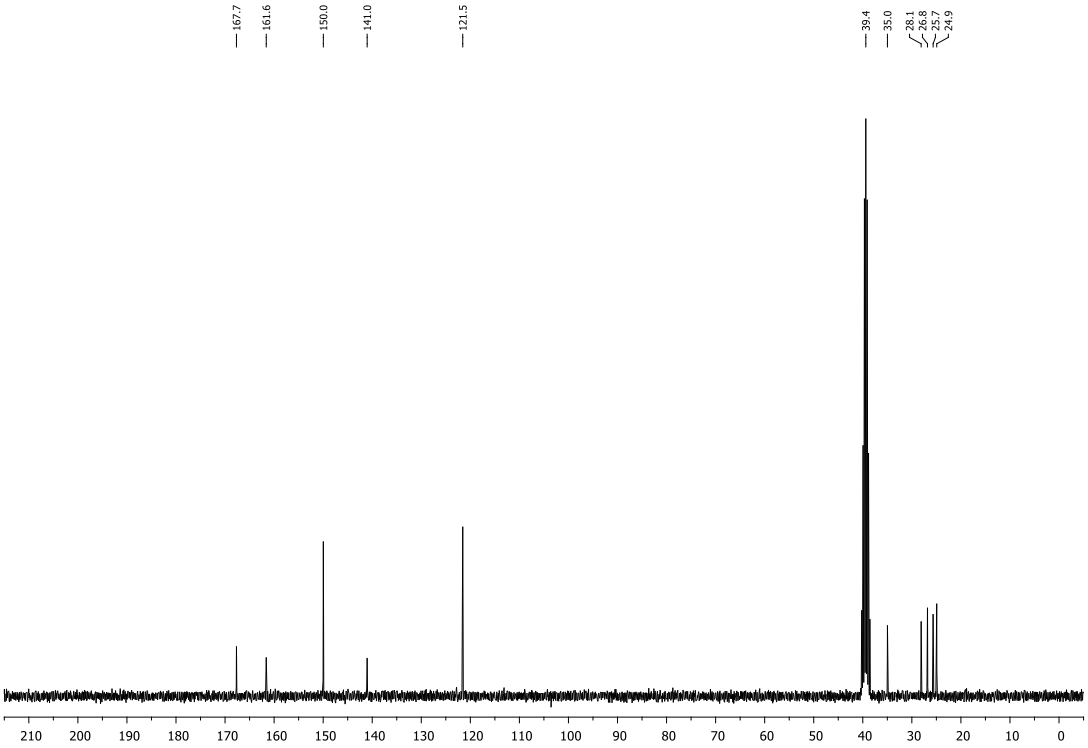


N'-Cyclohexylideneisonicotinohydrazide (183)

^{1}H NMR (in $CDCl_3$)

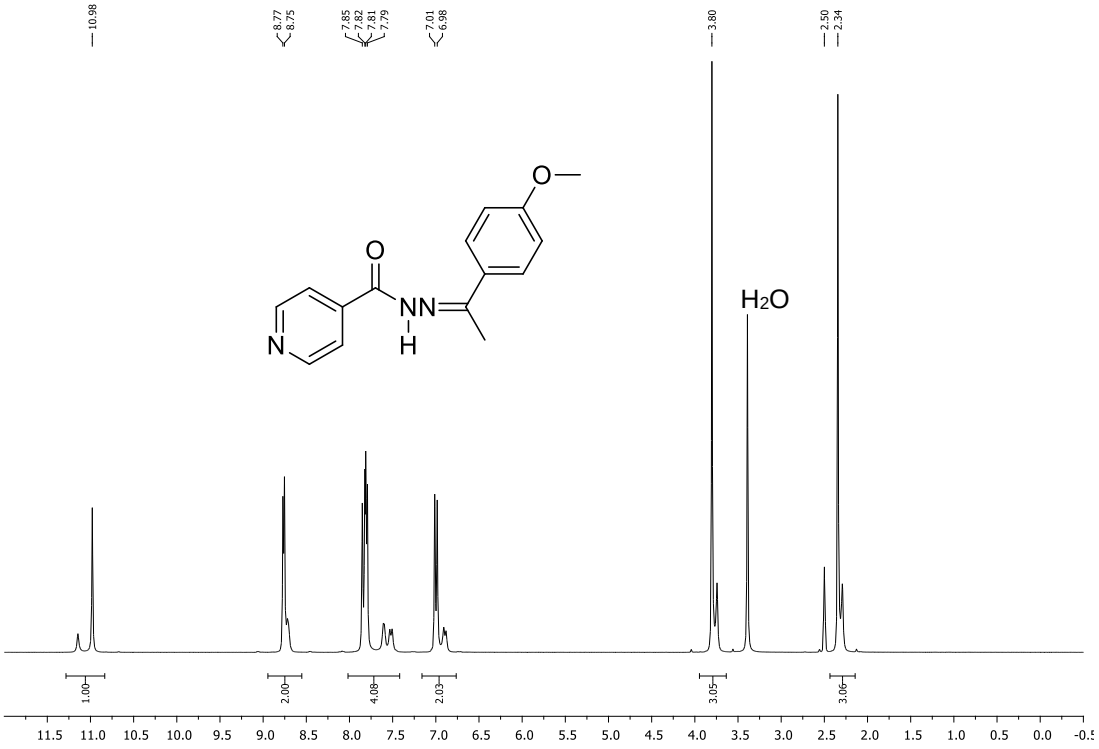


^{13}C NMR (in DMSO-d_6)

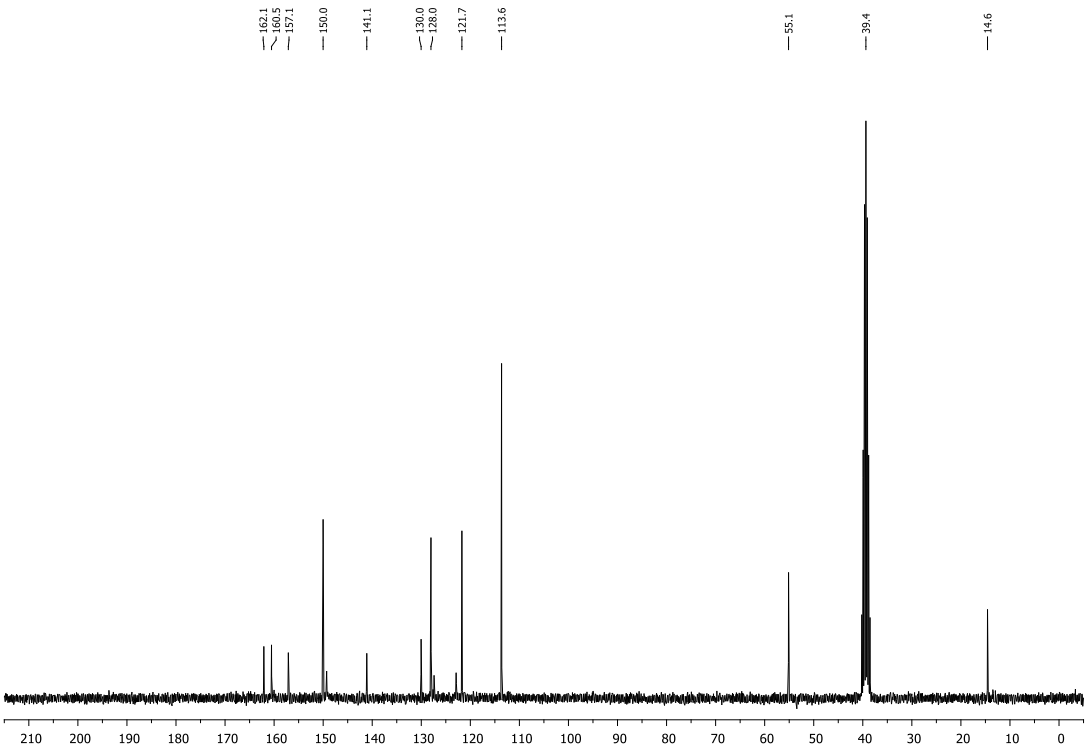


***N*'-(1-(4-Methoxyphenyl)ethylidene)isonicotinohydrazide (184)** (in DMSO-d_6)

^{1}H NMR

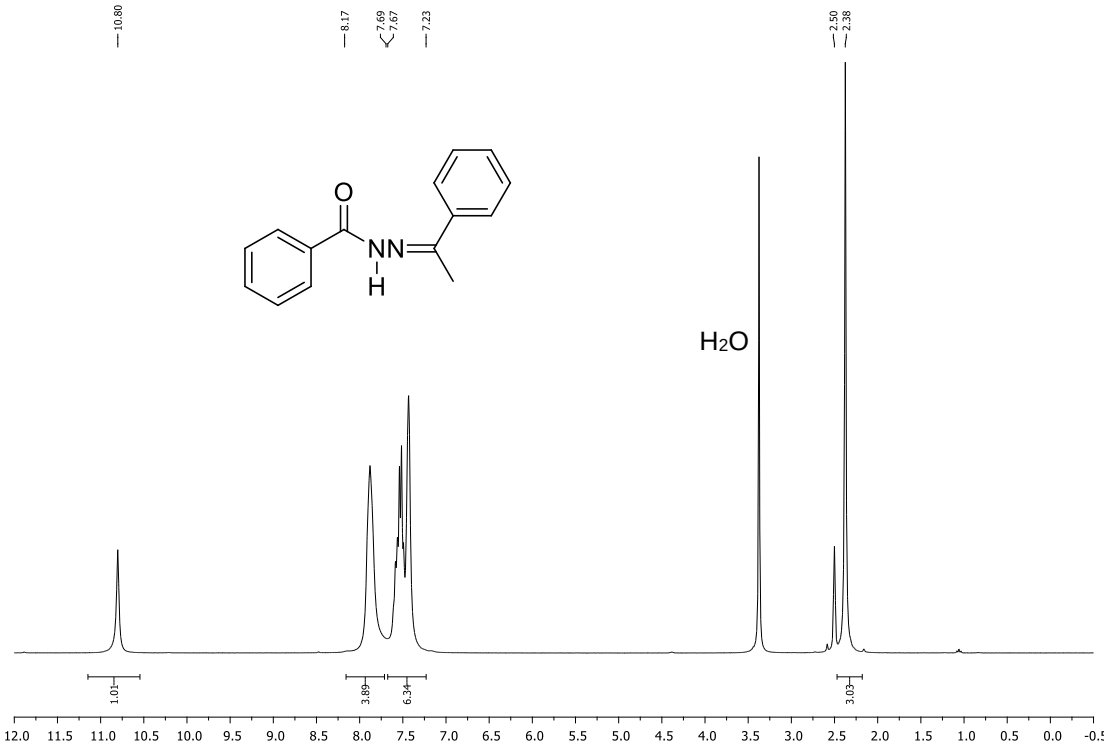


^{13}C NMR

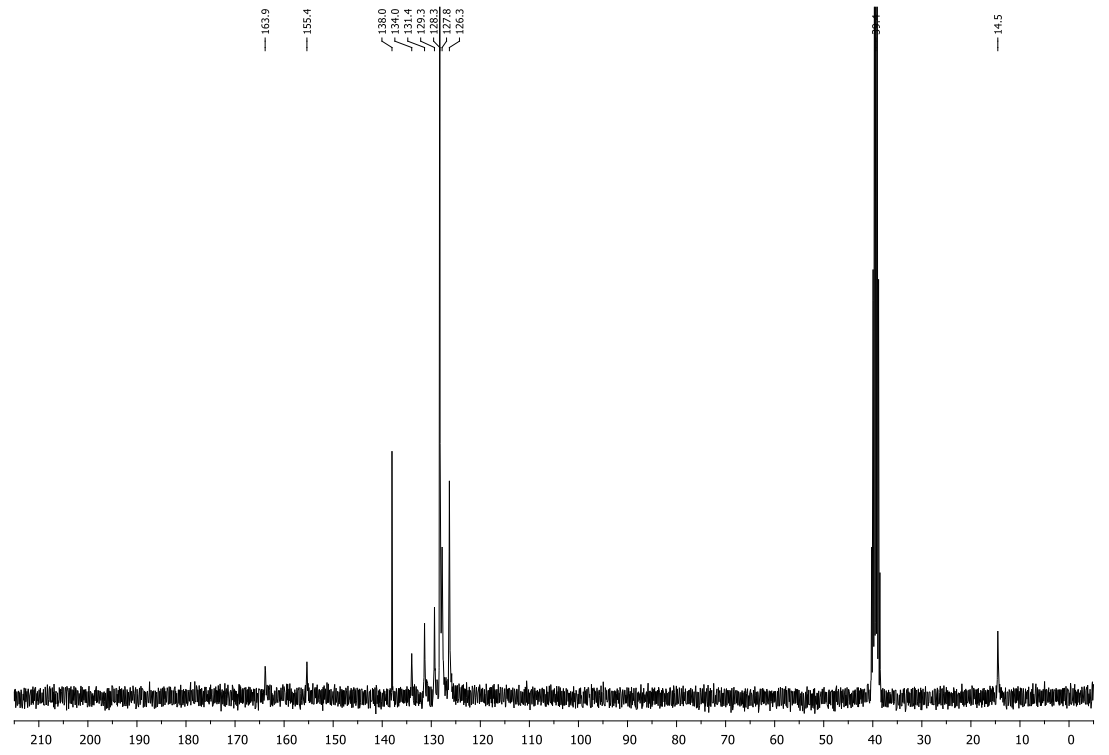


***N*'-(1-Phenylethylidene)benzohydrazide (185)** (in DMSO-d_6)

^{1}H NMR

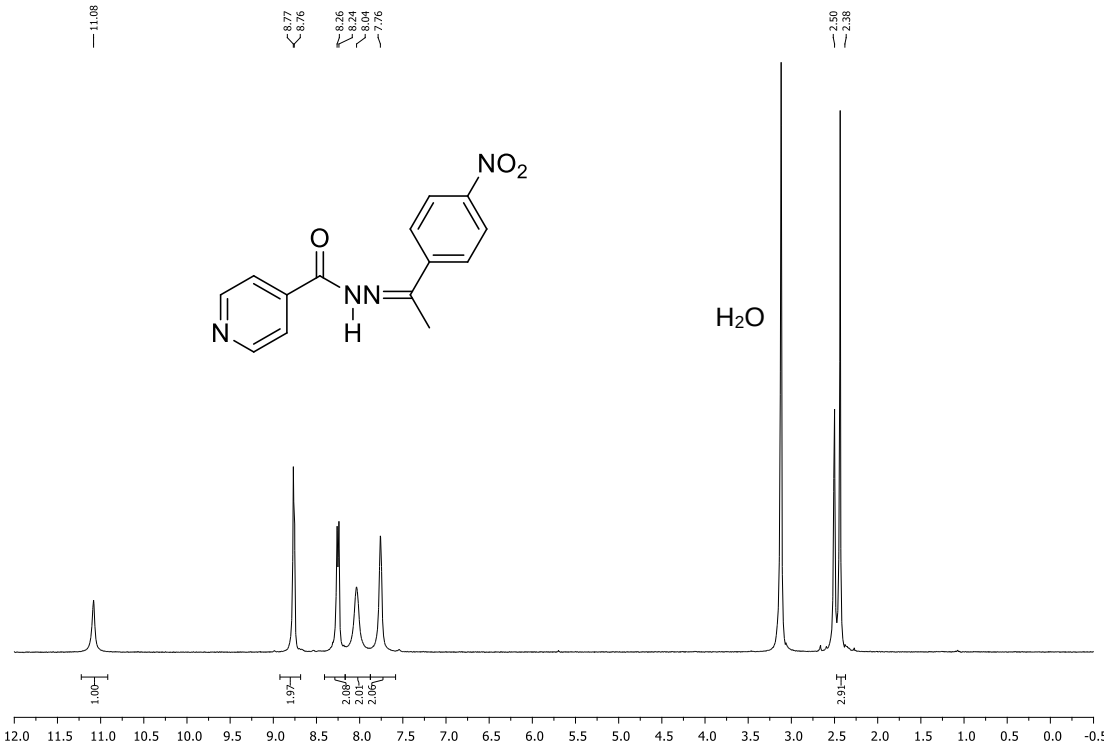


^{13}C NMR

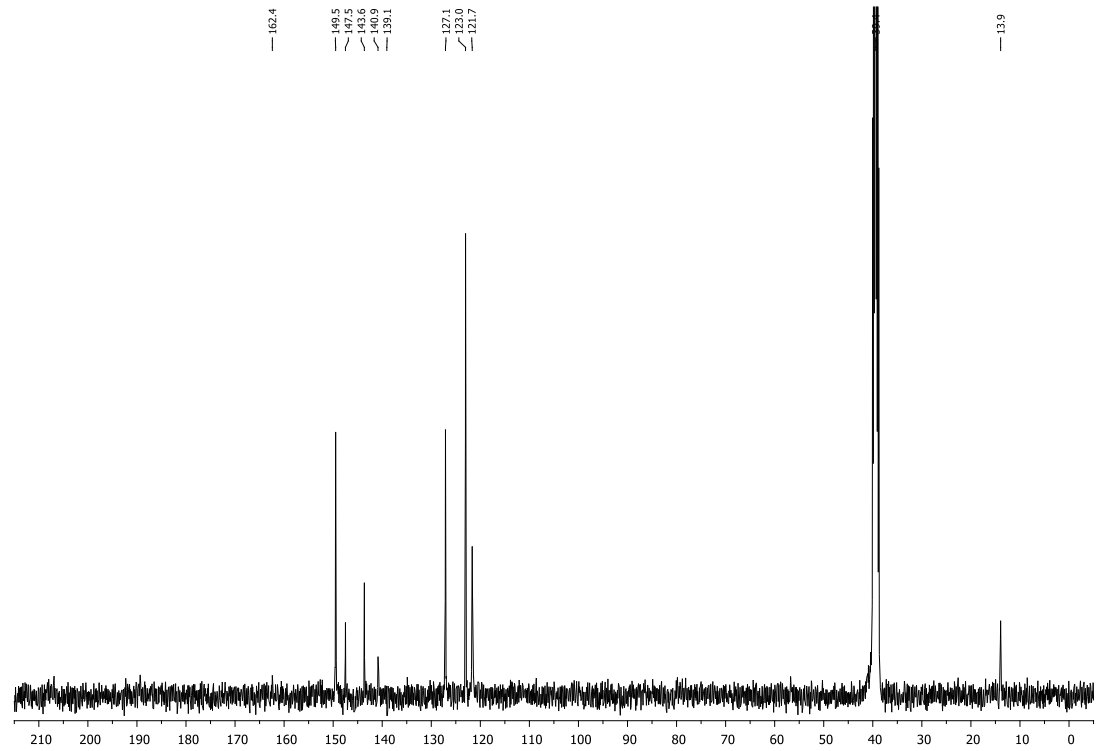


***N*'-(1-(4-Nitrophenyl)ethylidene)isonicotinohydrazide (186)** (in DMSO-d_6)

^{1}H NMR

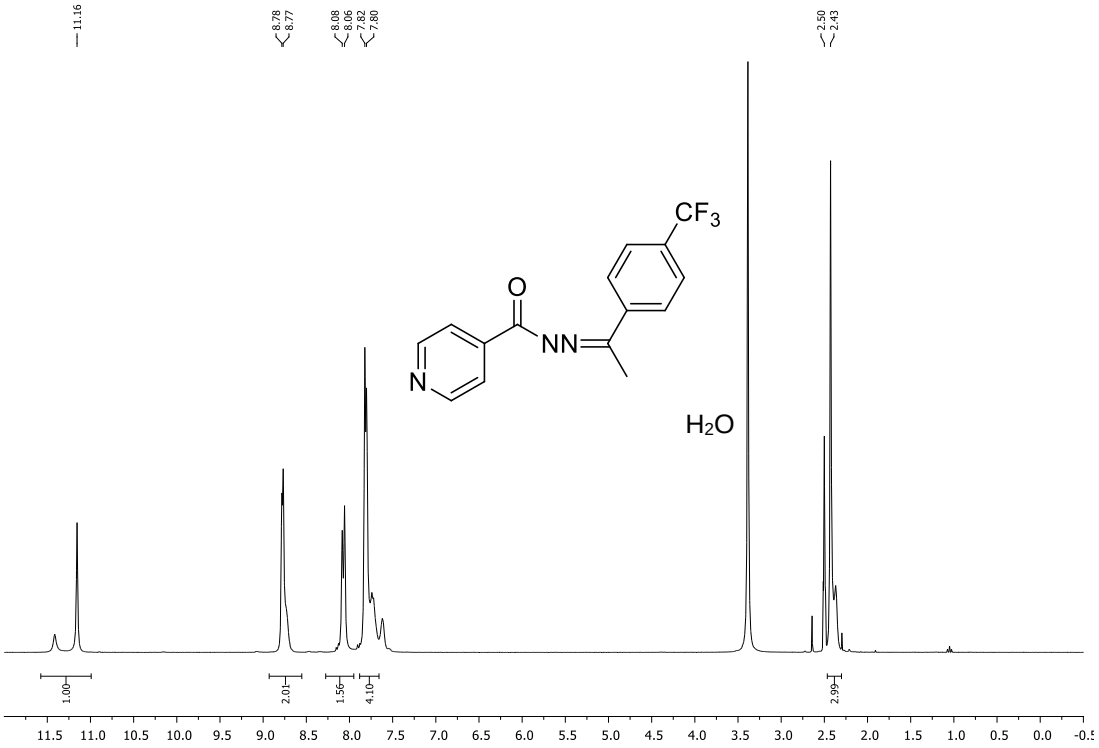


^{13}C NMR

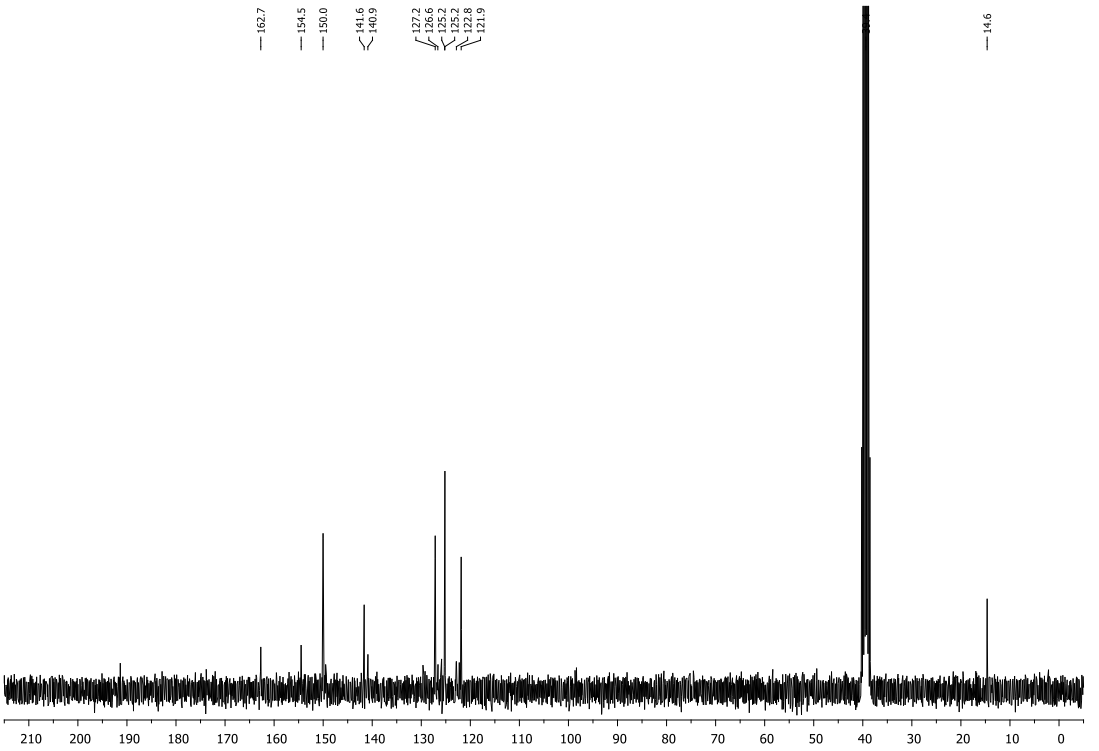


***N*'-(1-(4-(Trifluoromethyl)phenyl)ethylidene)isonicotinohydrazide (187)** (DMSO-d_6)

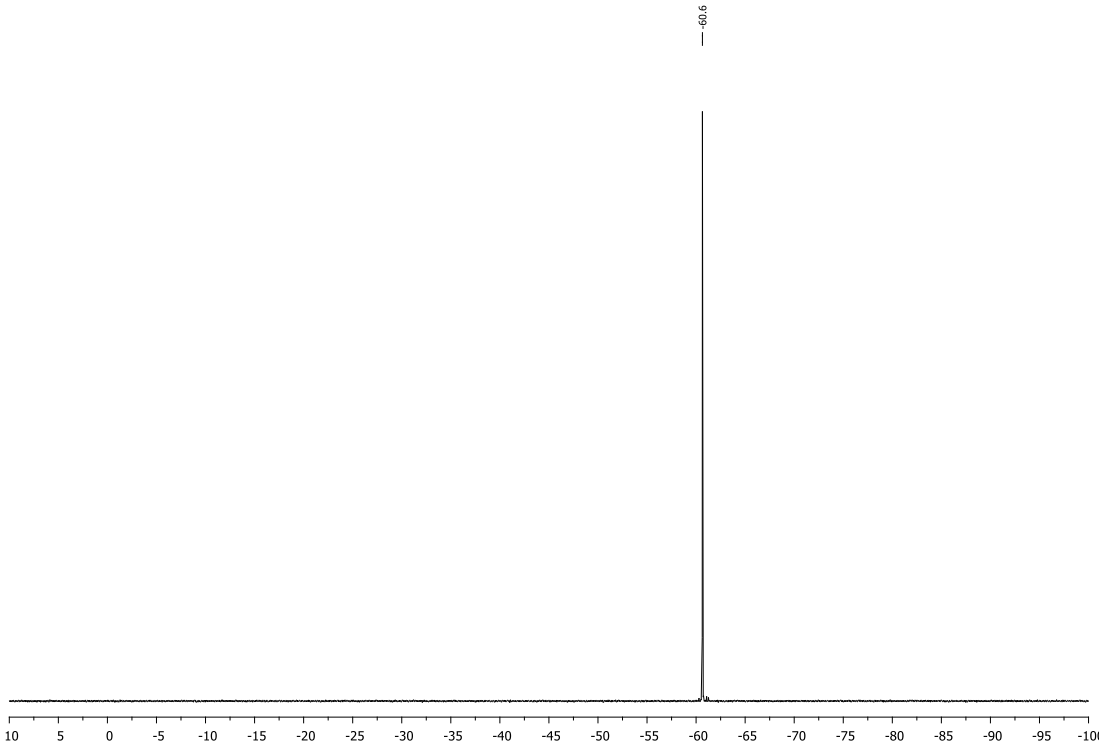
^{1}H NMR



^{13}C NMR

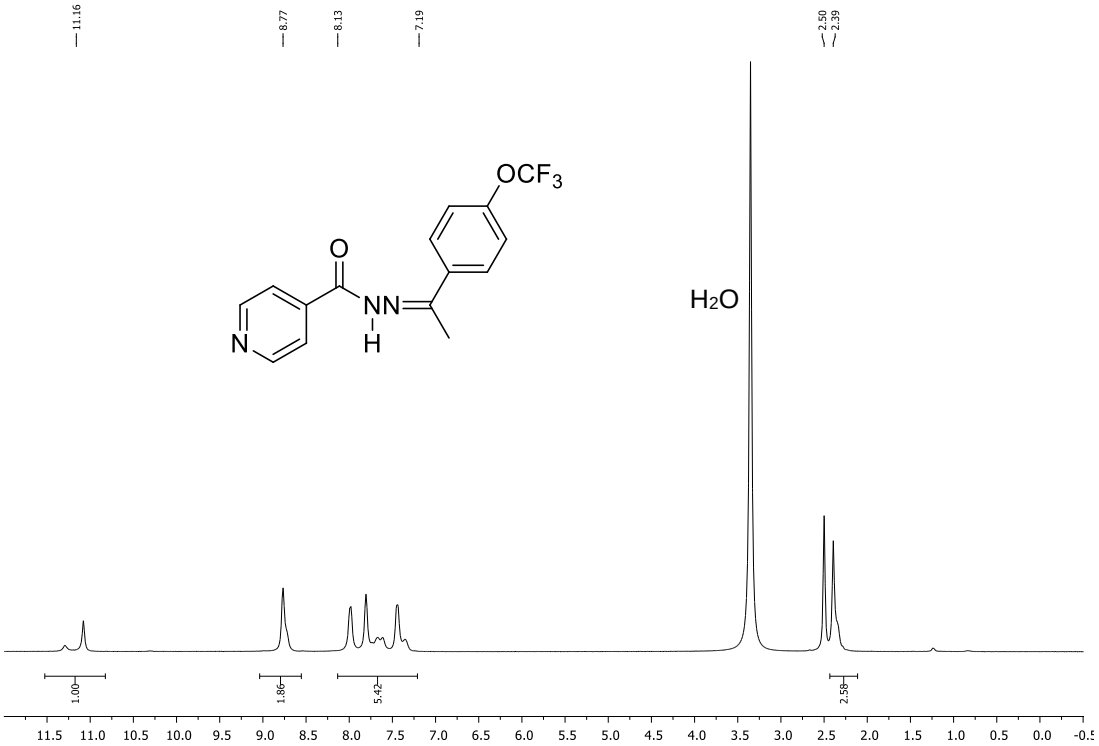


^{19}F NMR

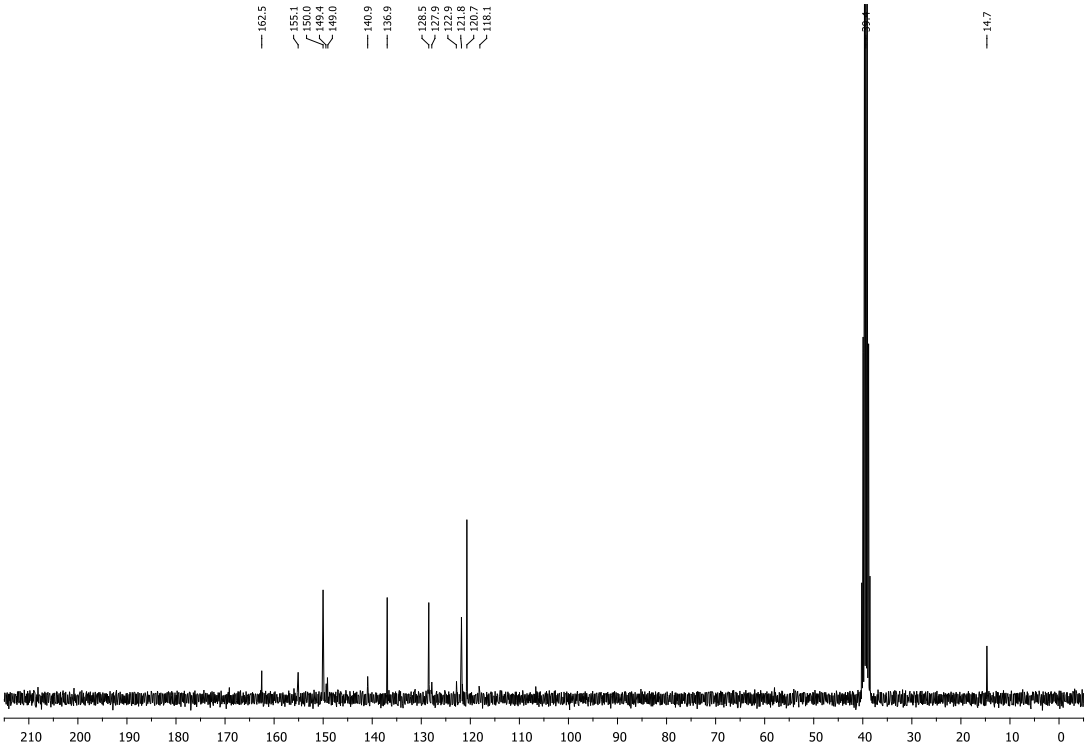


***N*'-(1-(4-(Trifluoromethoxy)phenyl)ethylidene)isonicotinohydrazide (188)** (in DMSO-d_6)

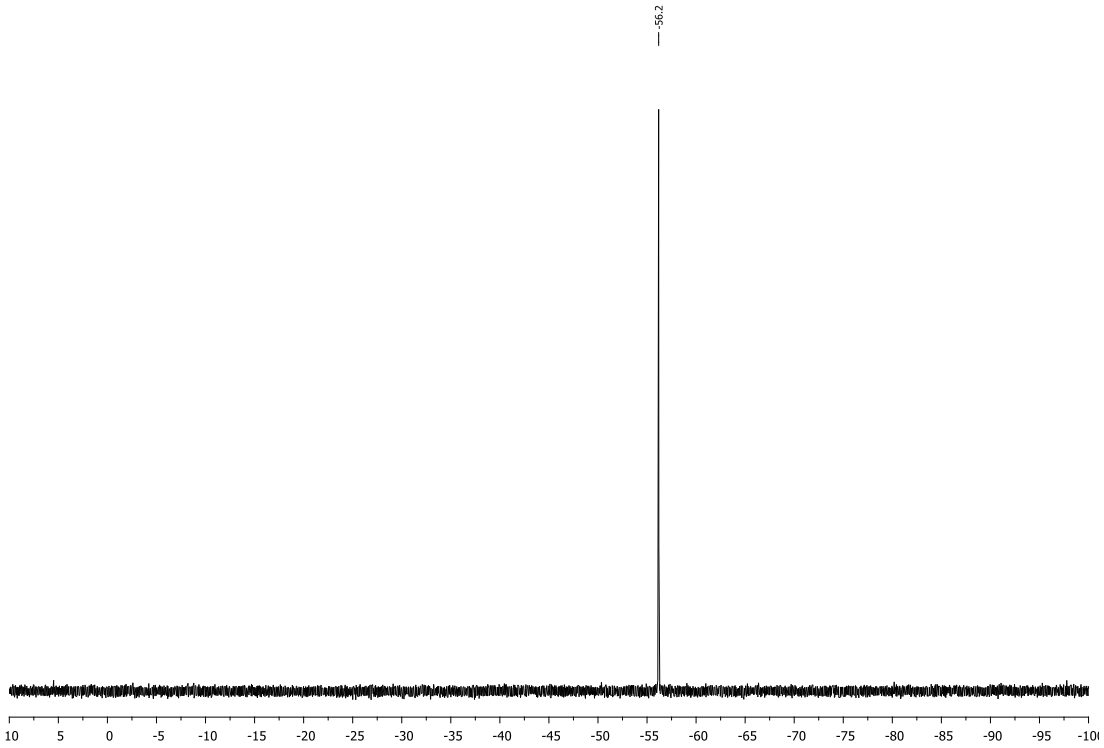
^{1}H NMR



^{13}C NMR

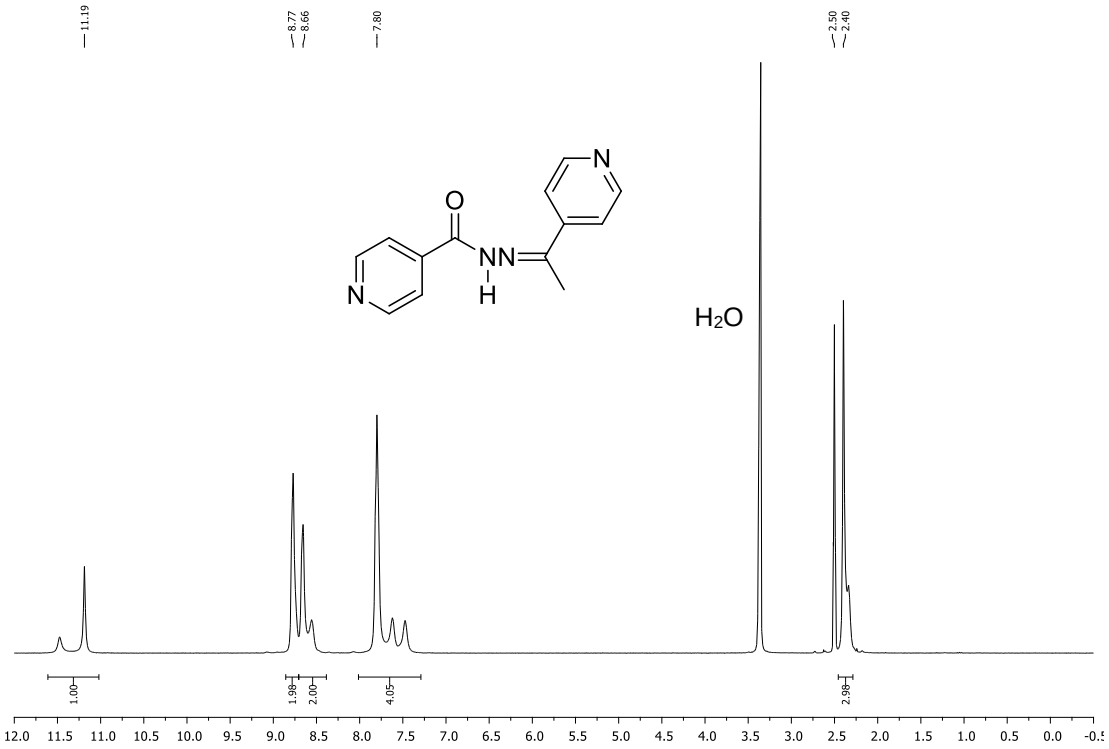


^{19}F NMR

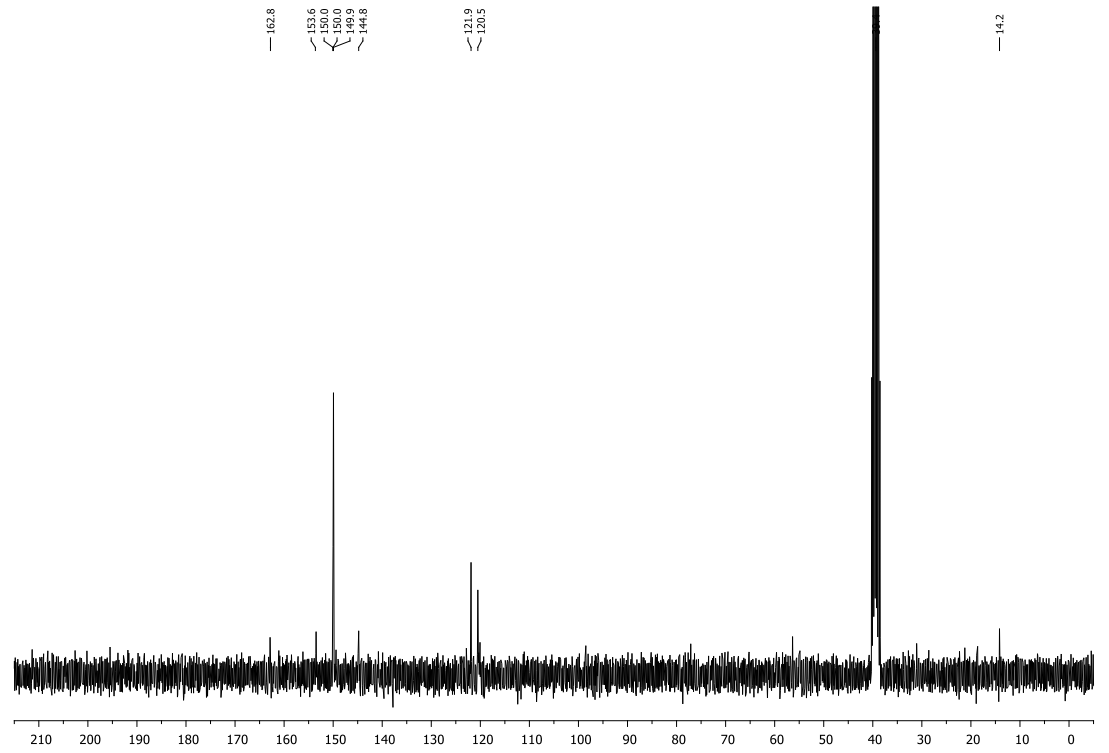


***N'*-(1-(Pyridin-4-yl)ethylidene)isonicotinohydrazide (189)** (in DMSO-d_6)

^{1}H NMR

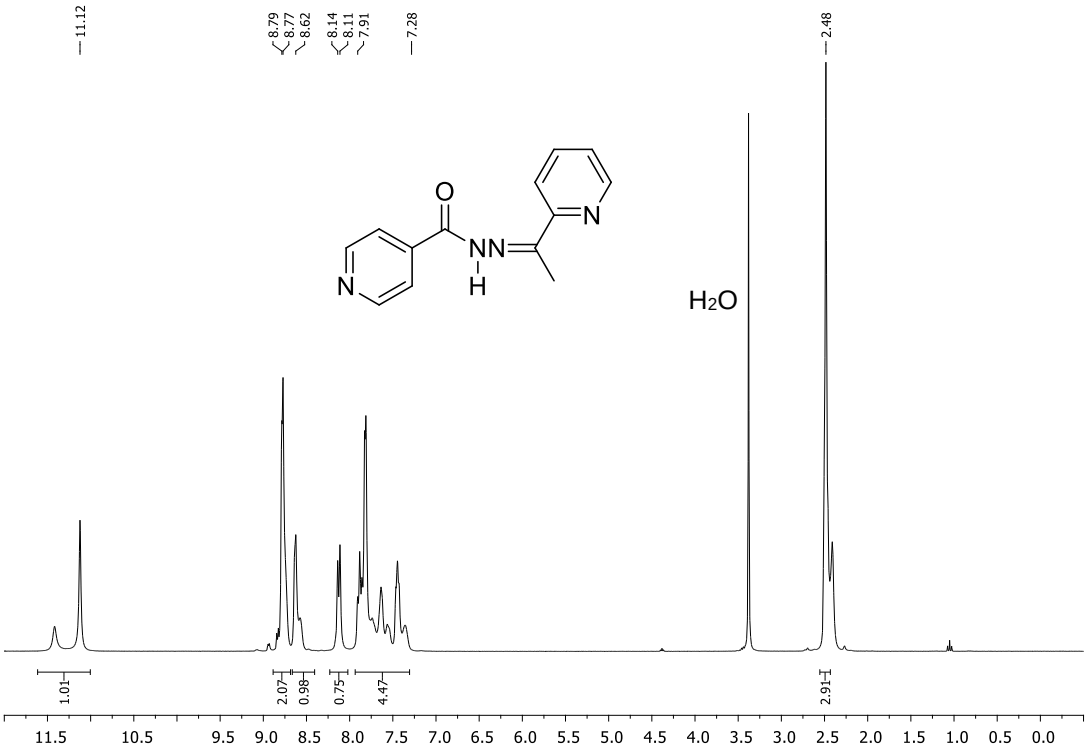


^{13}C NMR

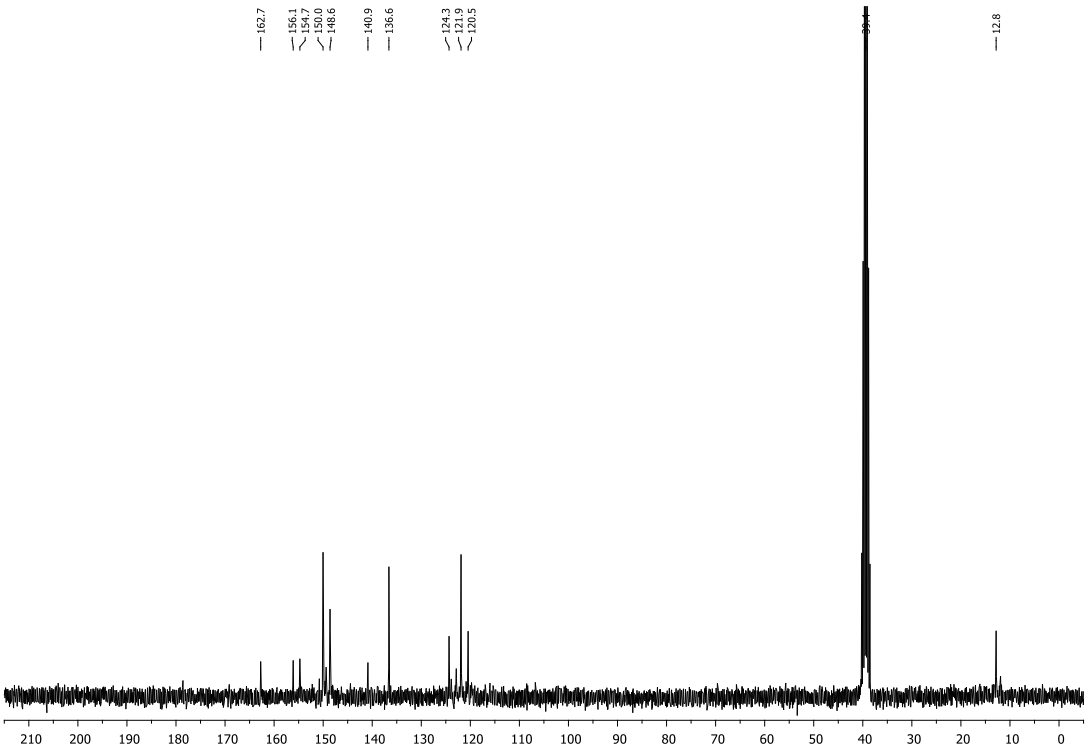


***N*'-(1-(Pyridin-2-yl)ethylidene)isonicotinohydrazide (190)** (in DMSO-d_6)

^{1}H NMR

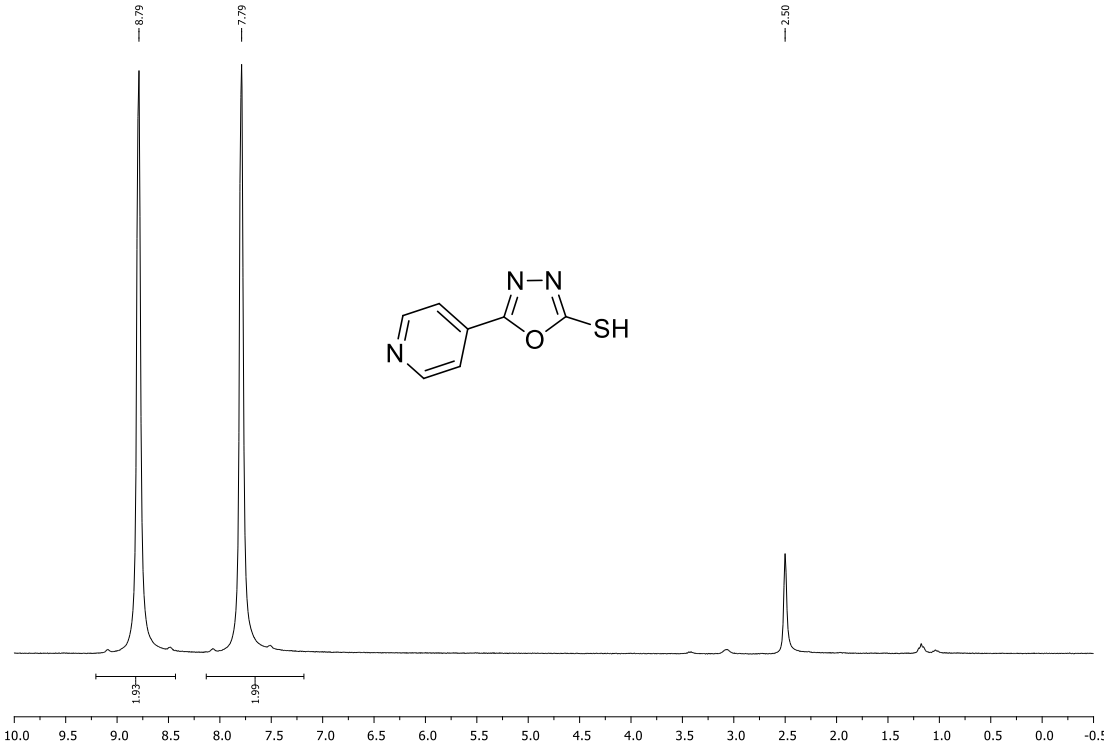


^{13}C NMR

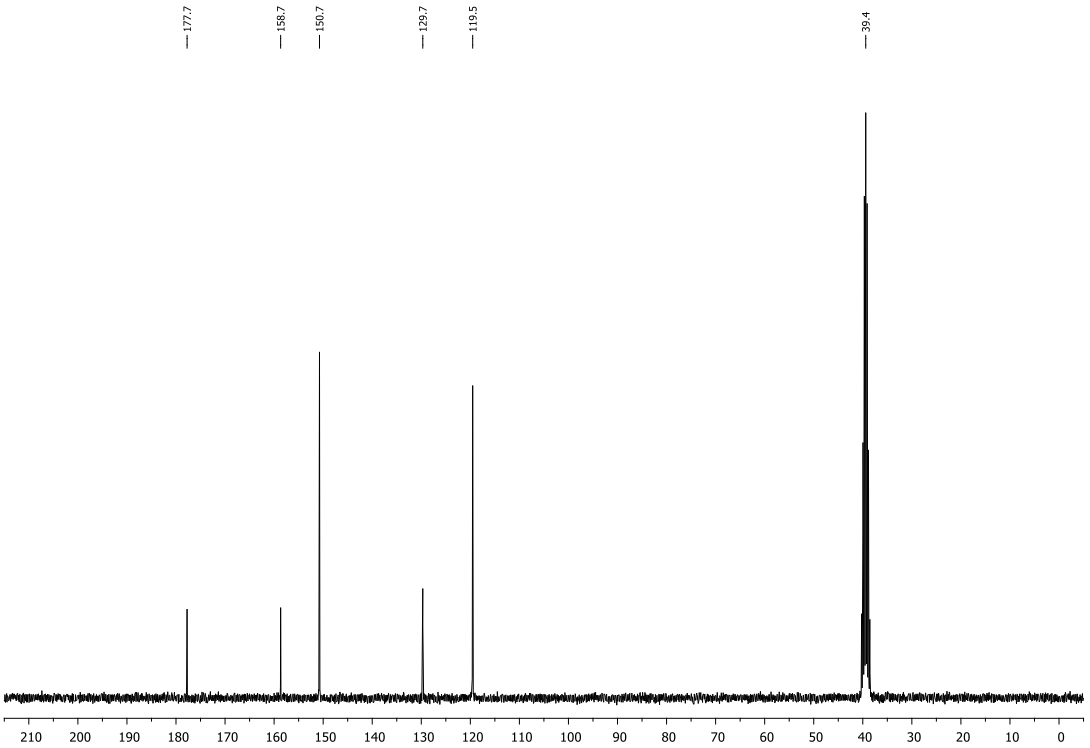


5-(Pyridin-4-yl)-1,3,4-oxadiazole-2-thiol (174) (in DMSO-d_6)

^{1}H NMR

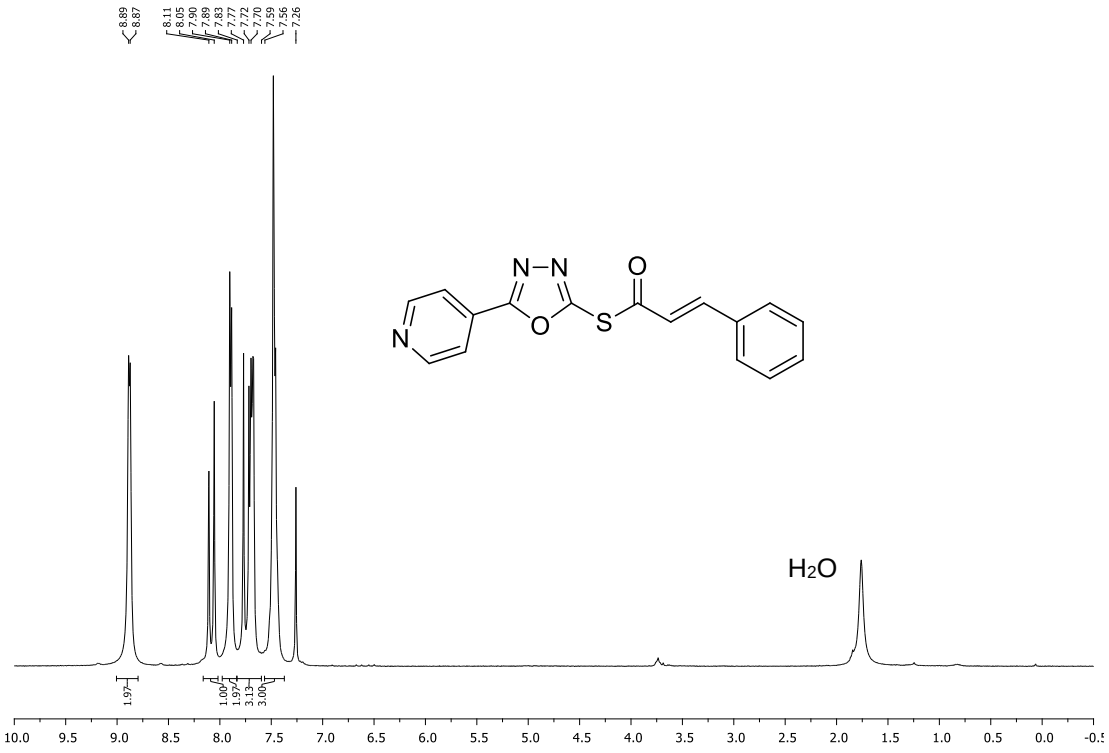


^{13}C NMR

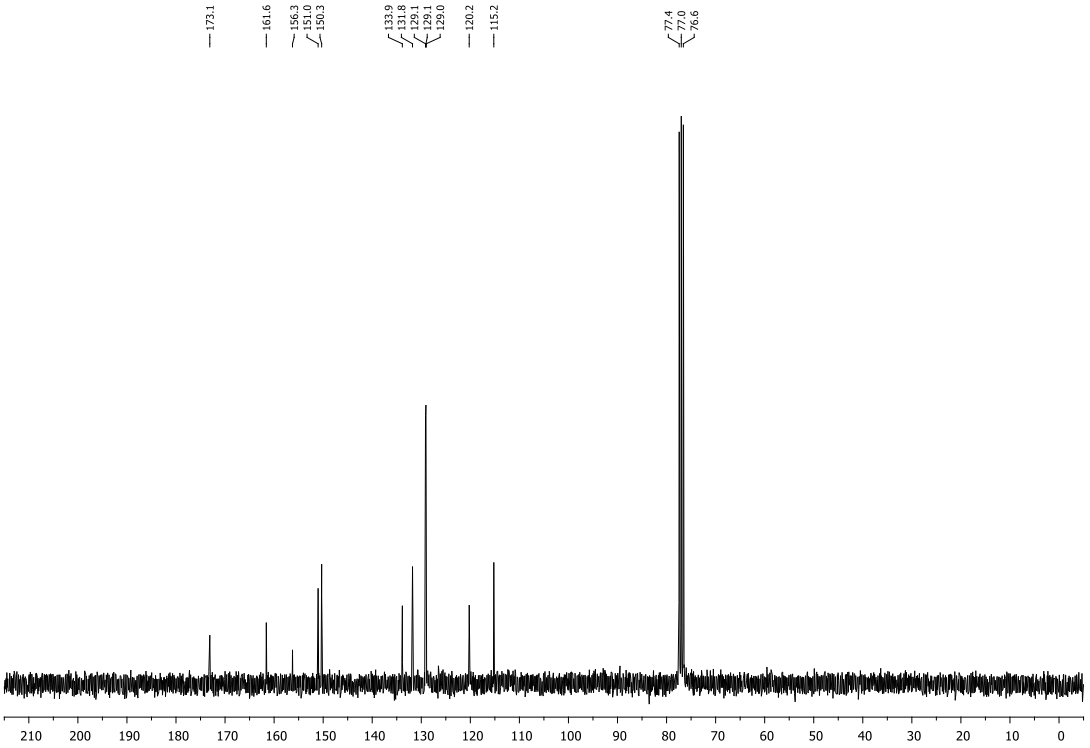


(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-yl) 3-phenylprop-2-enethioate (175) (in $CDCl_3$)

^1H NMR



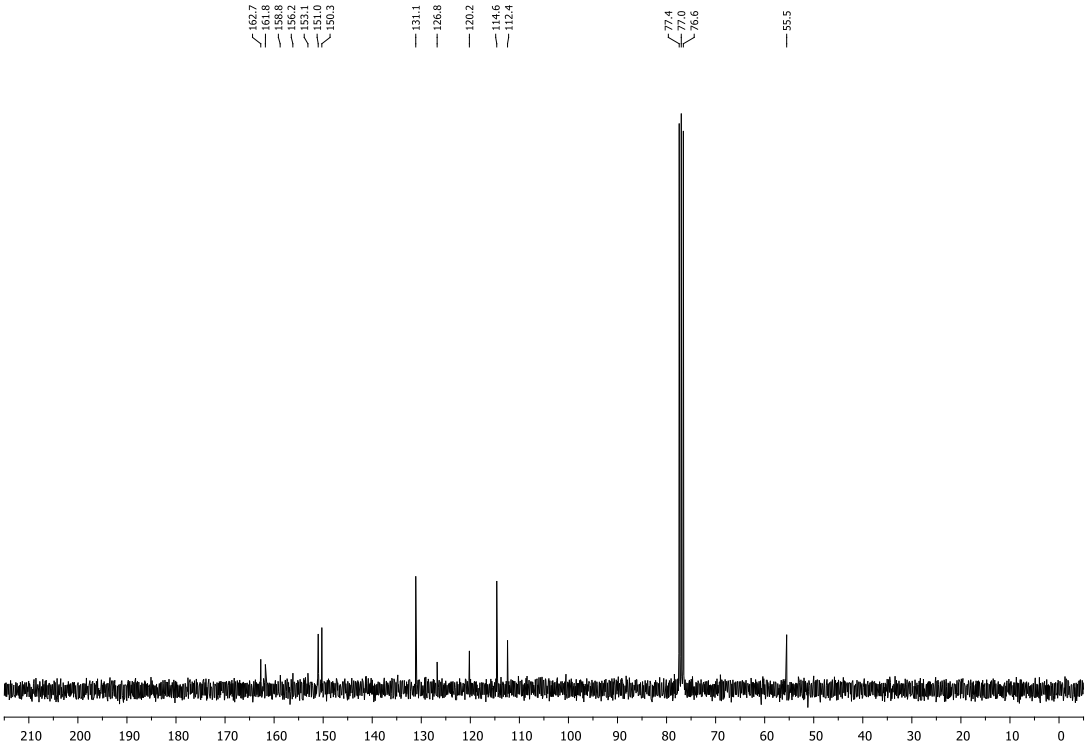
^{13}C NMR



(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-yl) 3-(4-methoxyphenyl)prop-2-enethioate (176) (in $CDCl_3$)

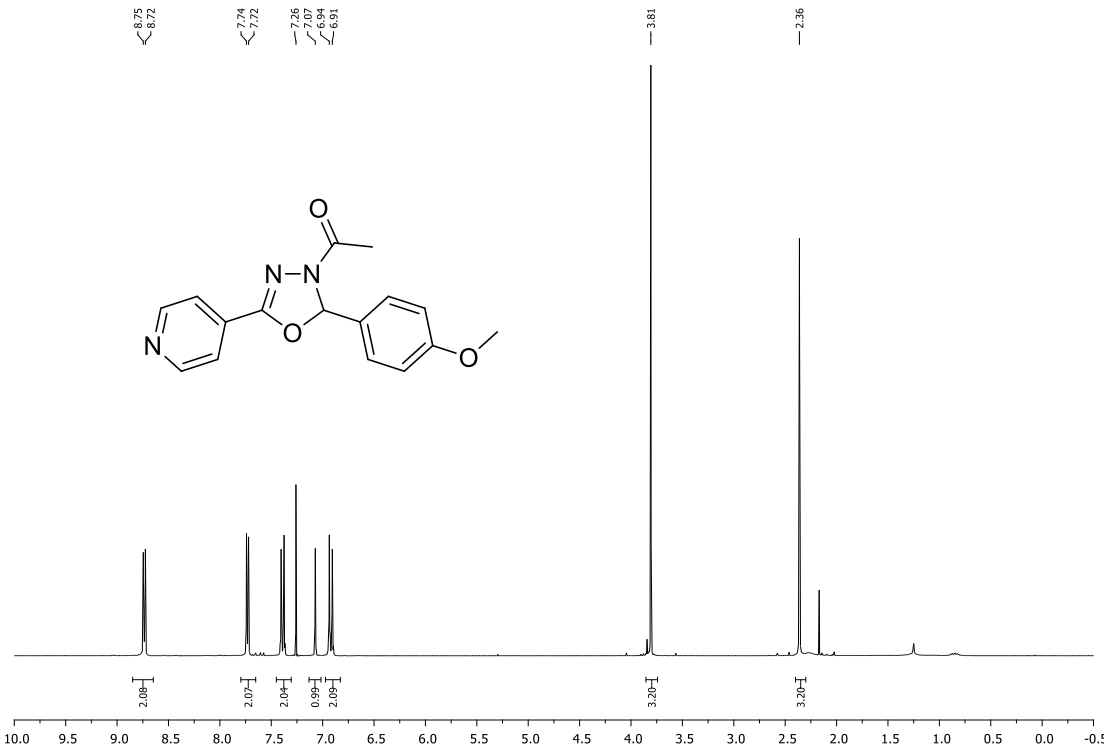
^{1}H NMR

^{13}C NMR

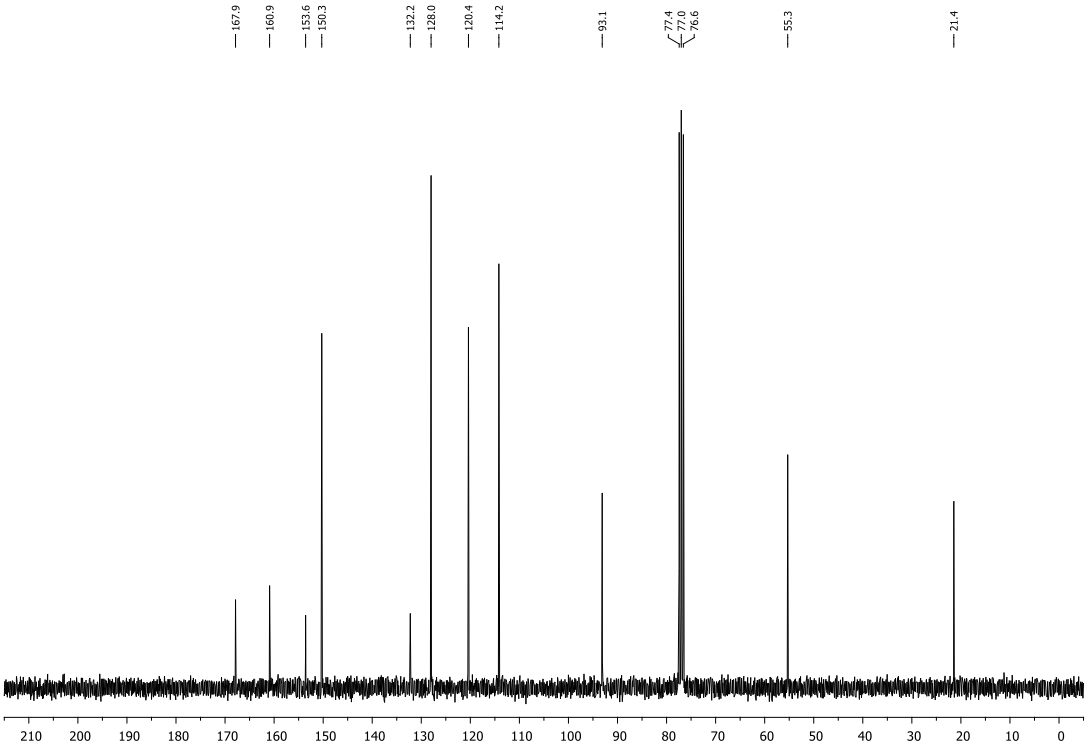


1-(2-(4-Methoxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)ethanone (191)
(in $CDCl_3$)

^{1}H NMR

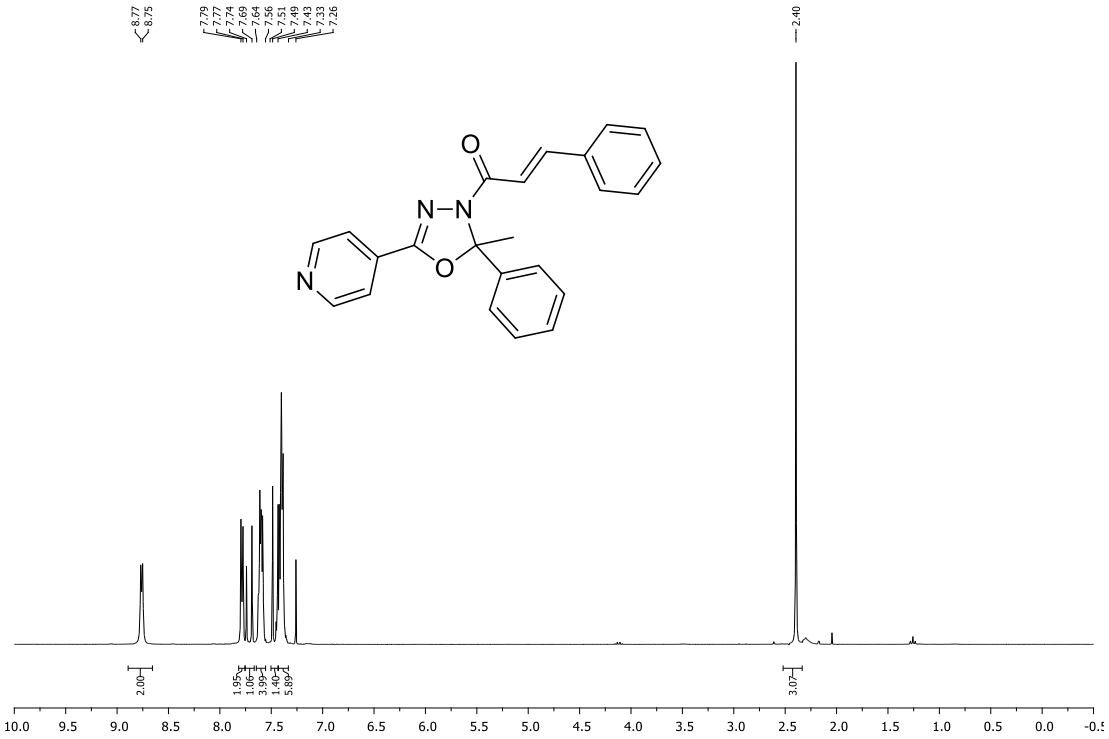


^{13}C NMR

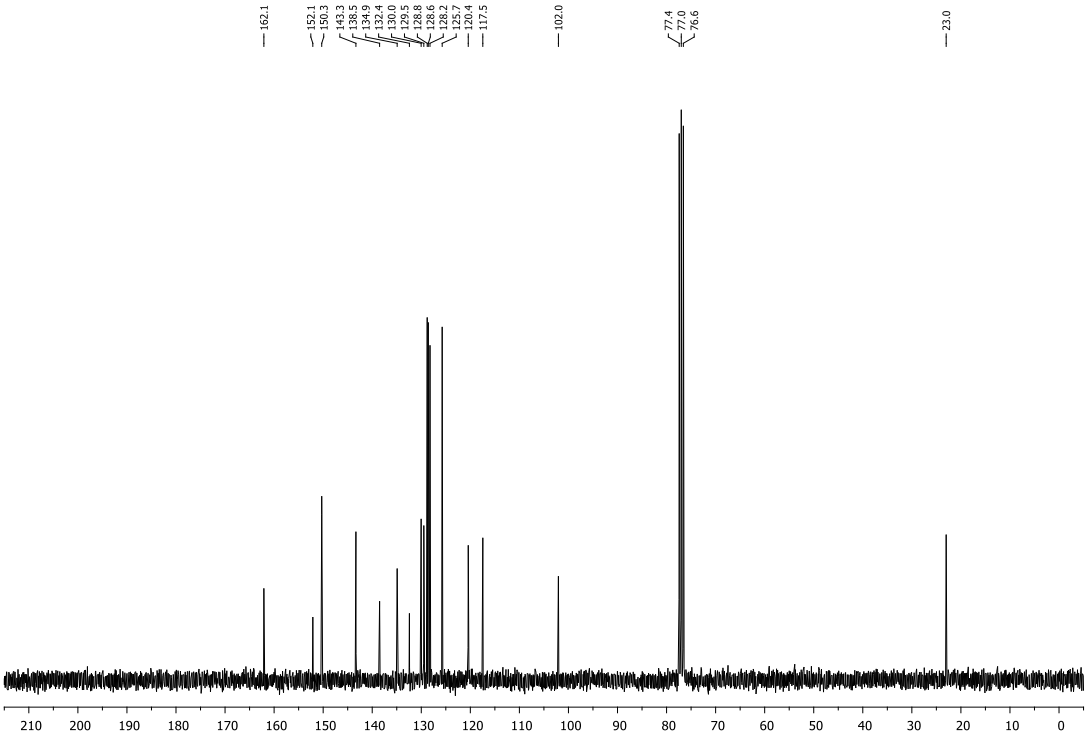


1-(2-Methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)-3-phenylprop-2-en-1-one (197) (in $CDCl_3$)

^{1}H NMR

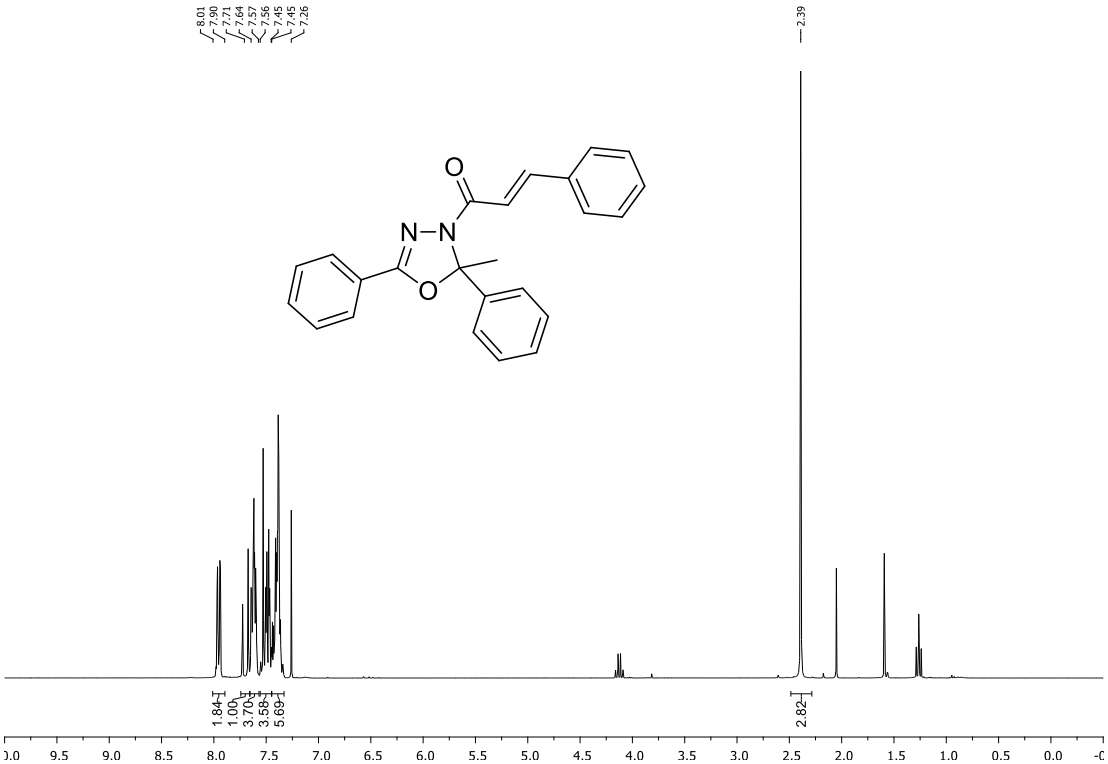


^{13}C NMR

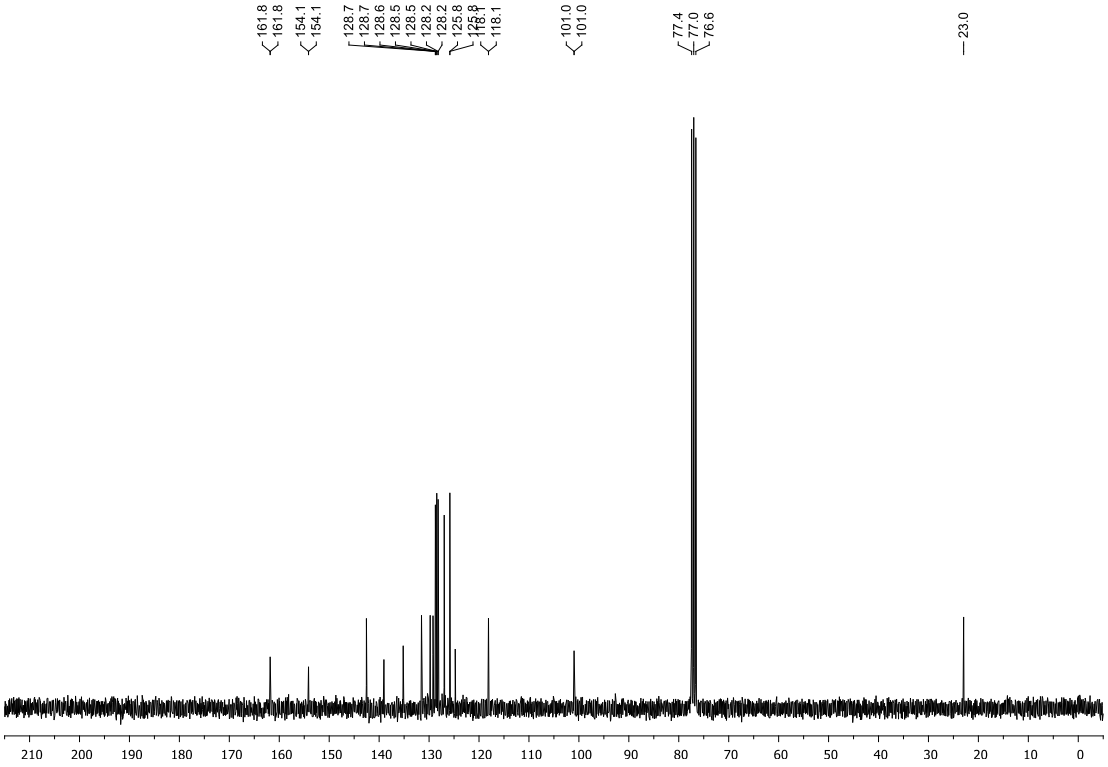


1-(2-Methyl-2,5-diphenyl-1,3,4-oxadiazol-3(2*H*)-yl)-3-phenylprop-2-en-1-one (198) ($CDCl_3$)

^{1}H NMR

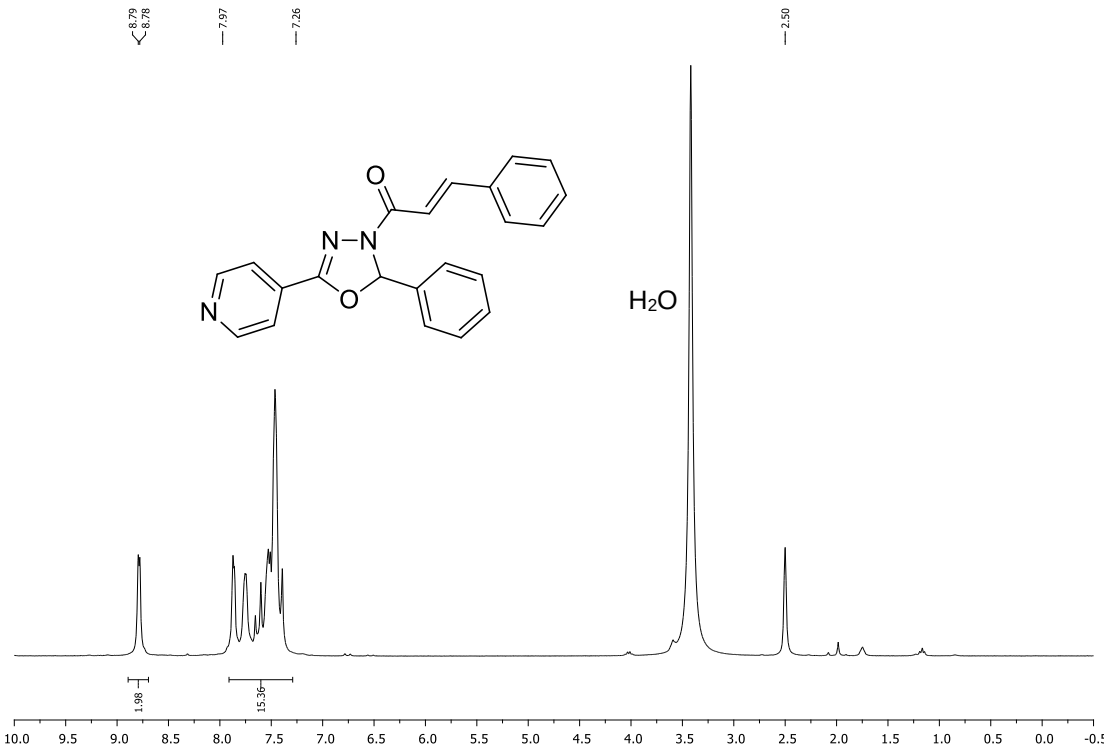


^{13}C NMR

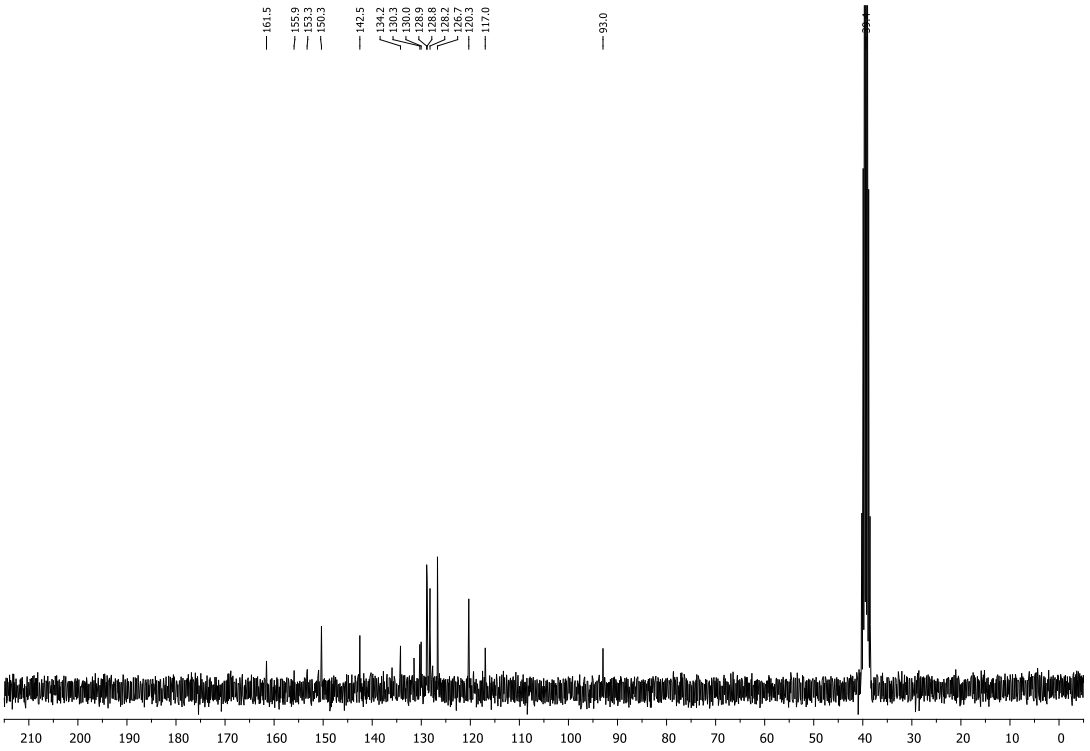


3-Phenyl-1-(2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (199) (in DMSO-d$_6$)

^{1}H NMR

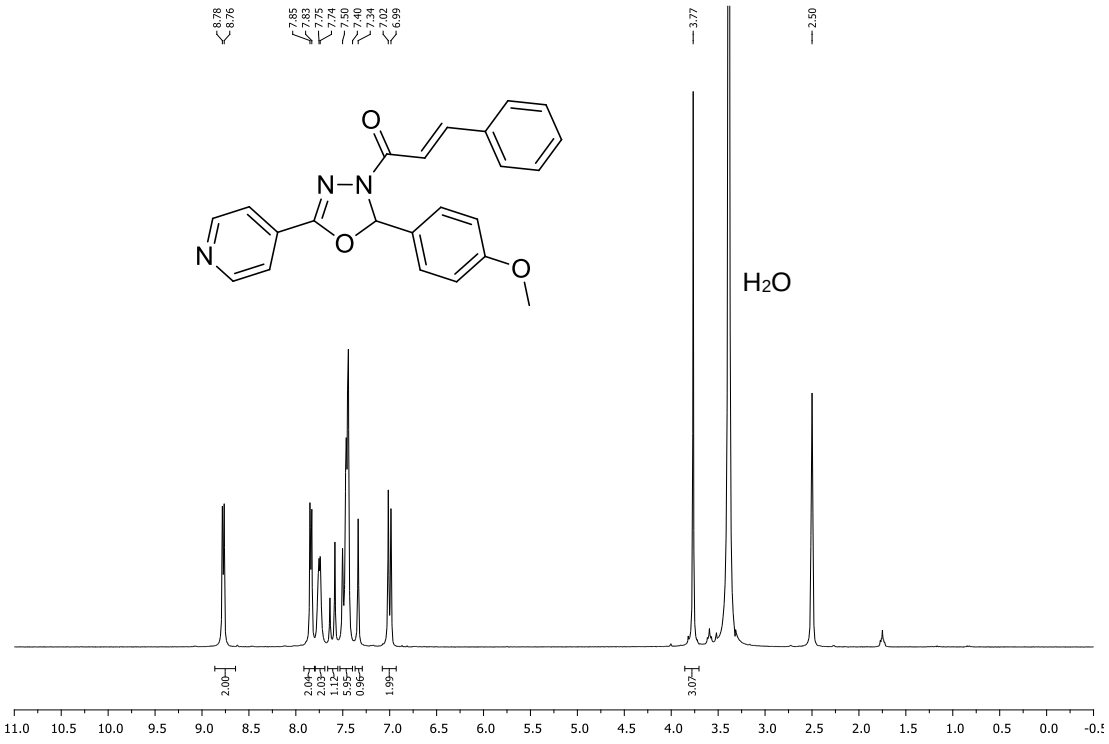


^{13}C NMR

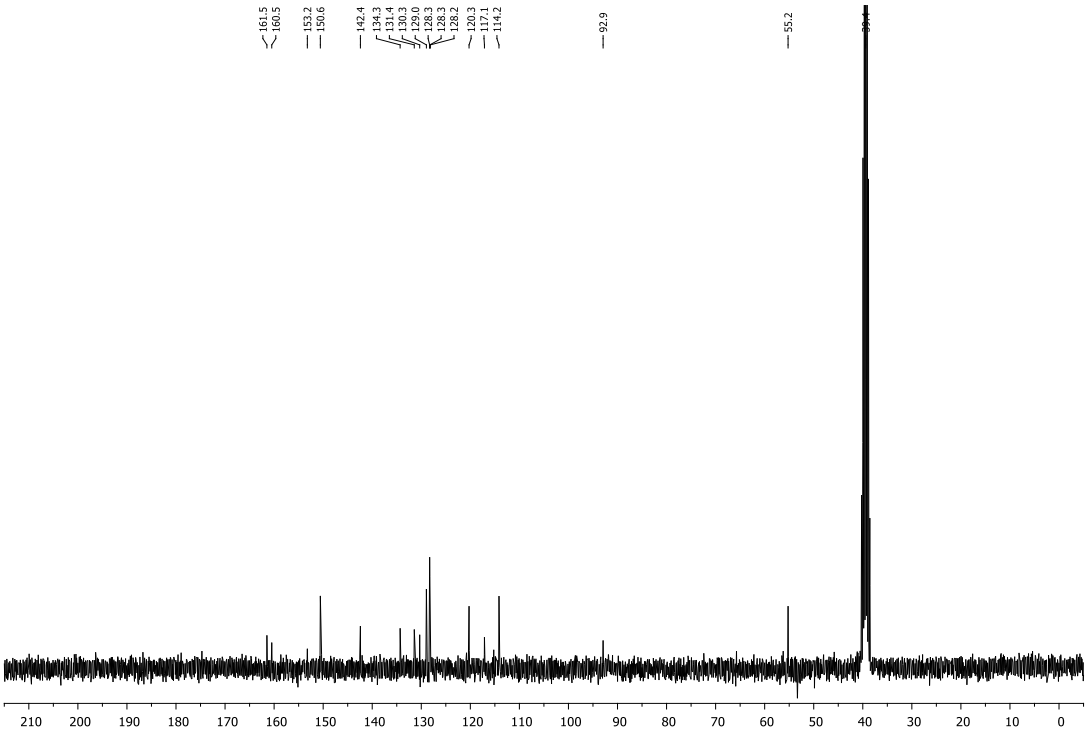


1-(2-(4-Methoxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)-3-phenylprop-2-en-1-one (200) (in DMSO-d_6)

^{1}H NMR

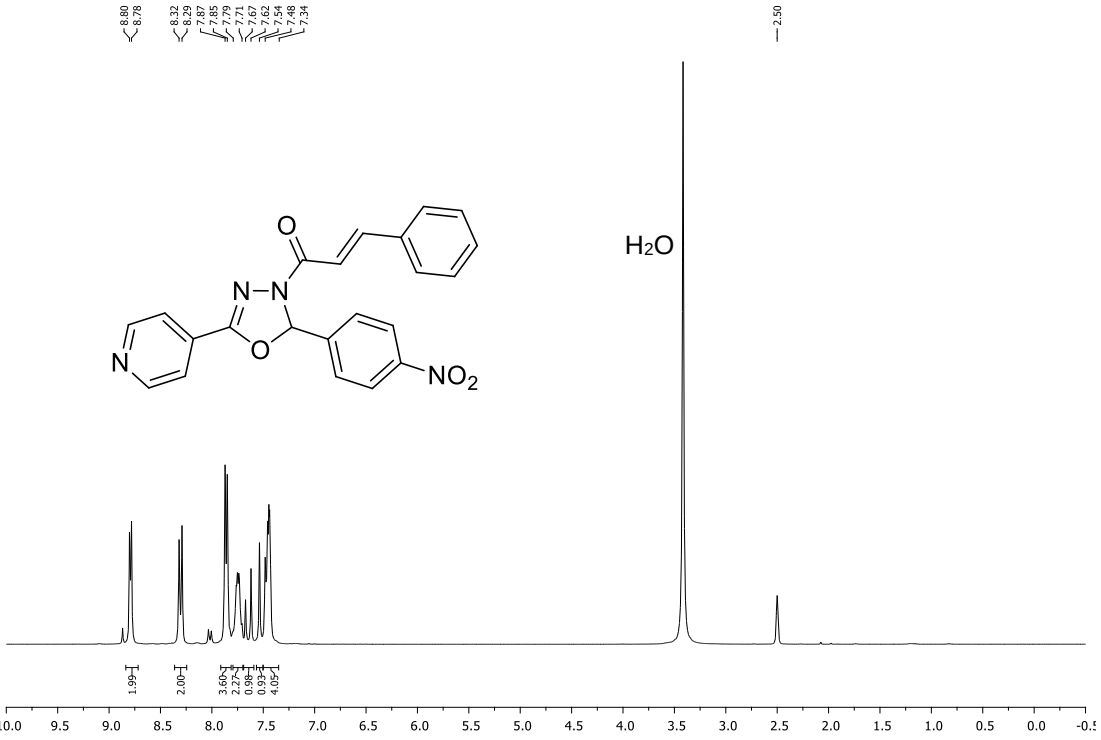


^{13}C NMR

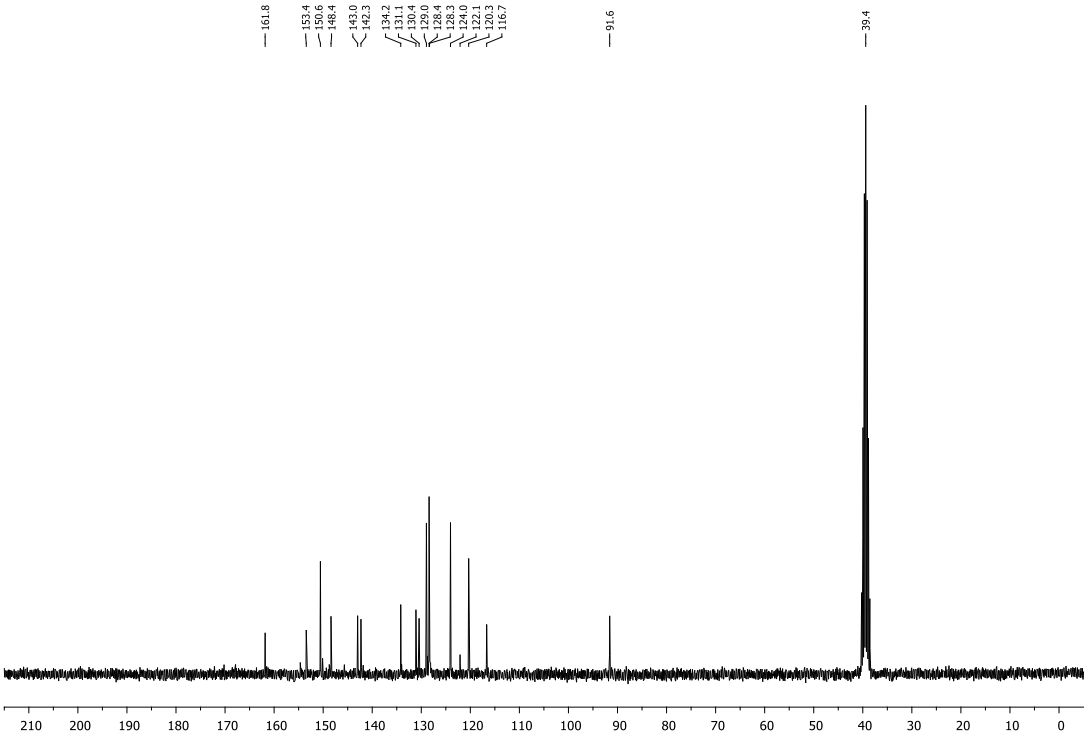


1-(2-(4-Nitrophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)-3-phenylprop-2-en-1-one (201) (in DMSO-d_6)

^{1}H NMR

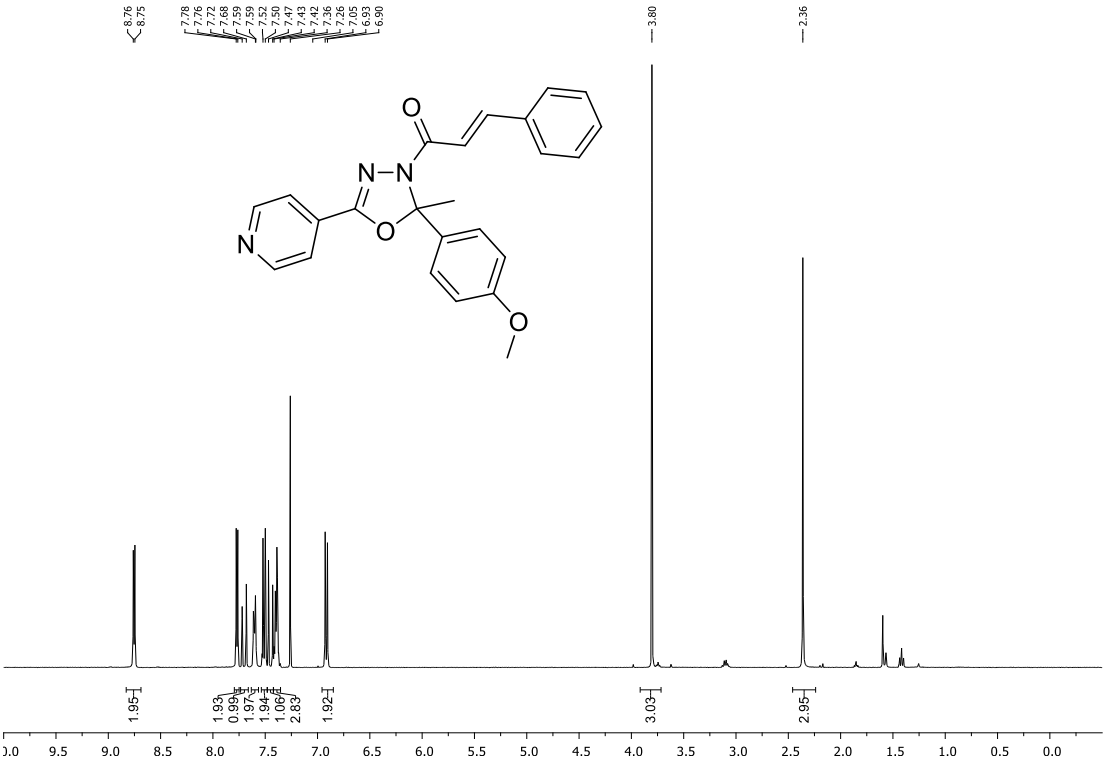


^{13}C NMR

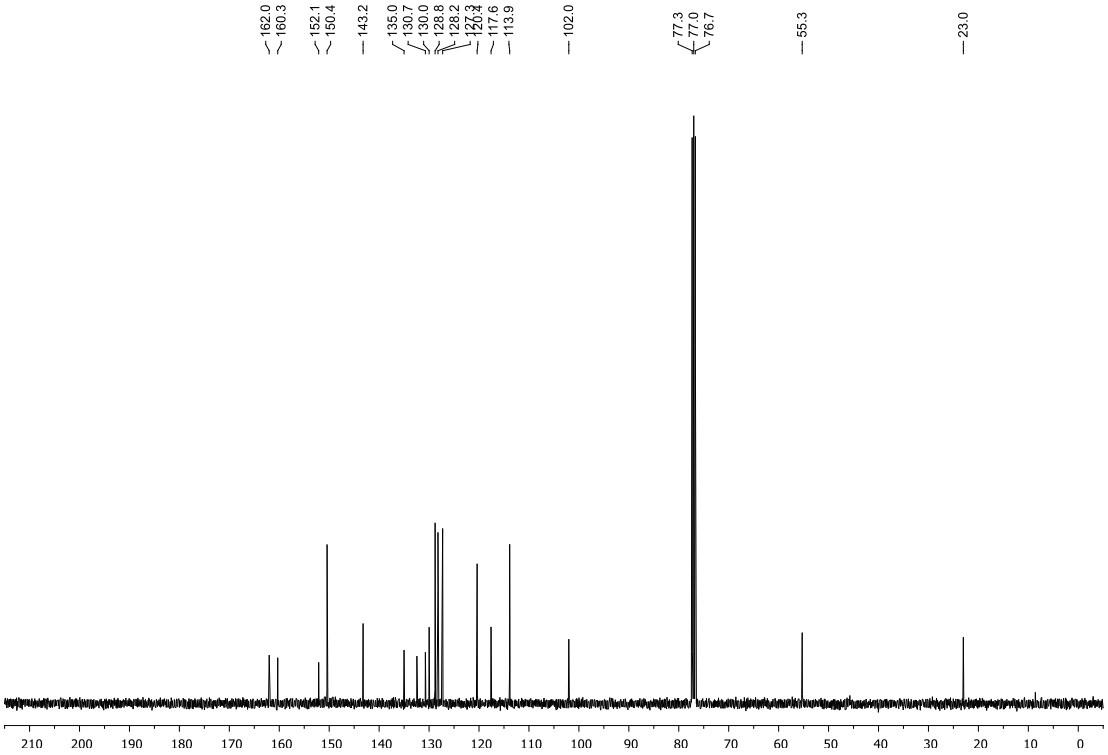


1-(2-(4-Methoxyphenyl)-2-methyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)-3-phenylprop-2-en-1-one (202) (in $CDCl_3$)

^{1}H NMR

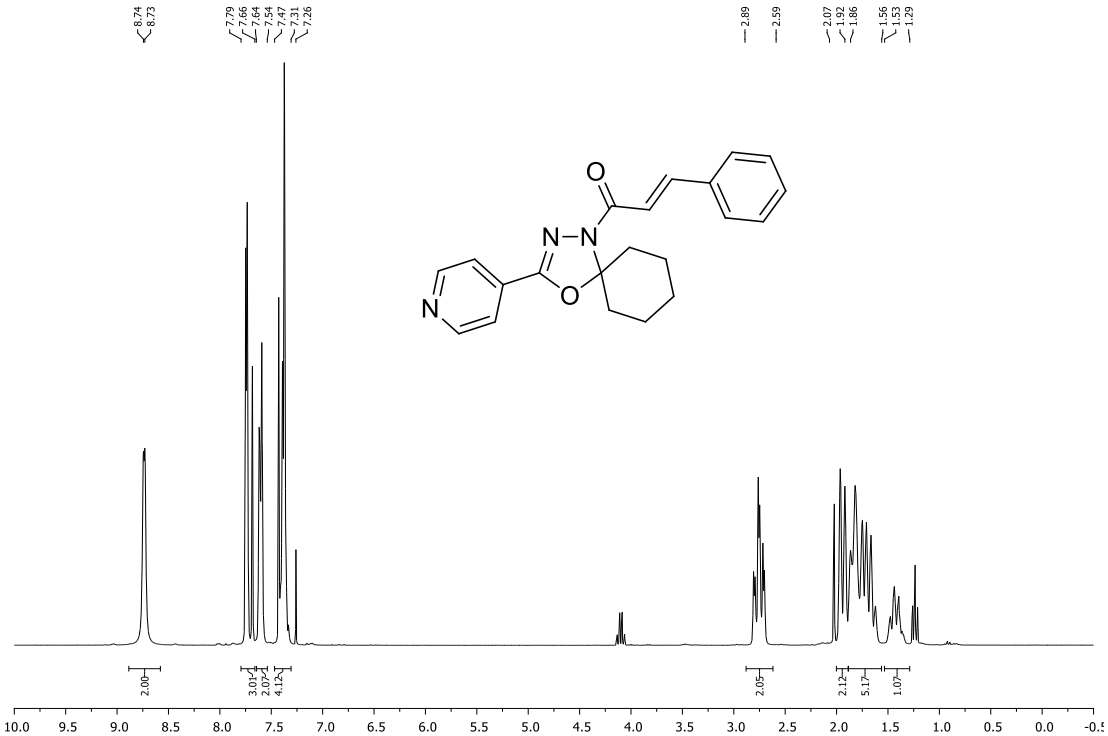


^{13}C NMR

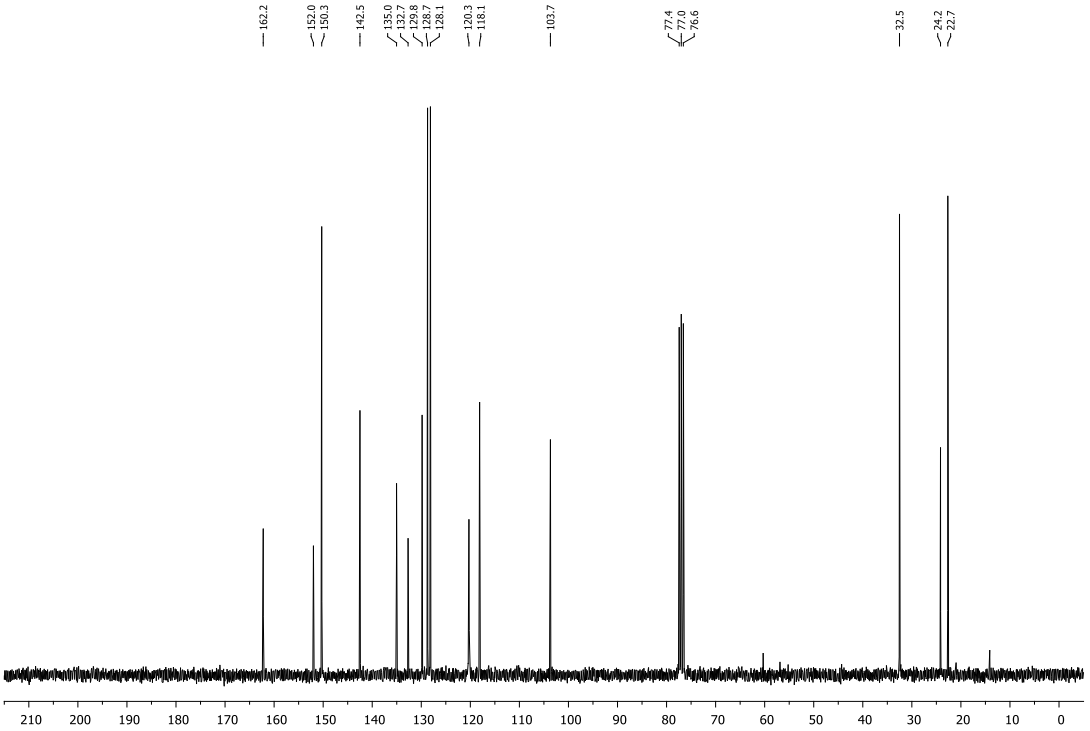


3-Phenyl-1-(3-(pyridin-4-yl)-4-oxa-1,2-diazaspiro[4.5]dec-2-en-1-yl)prop-2-en-1-one (203) (in $CDCl_3$)

^{1}H NMR

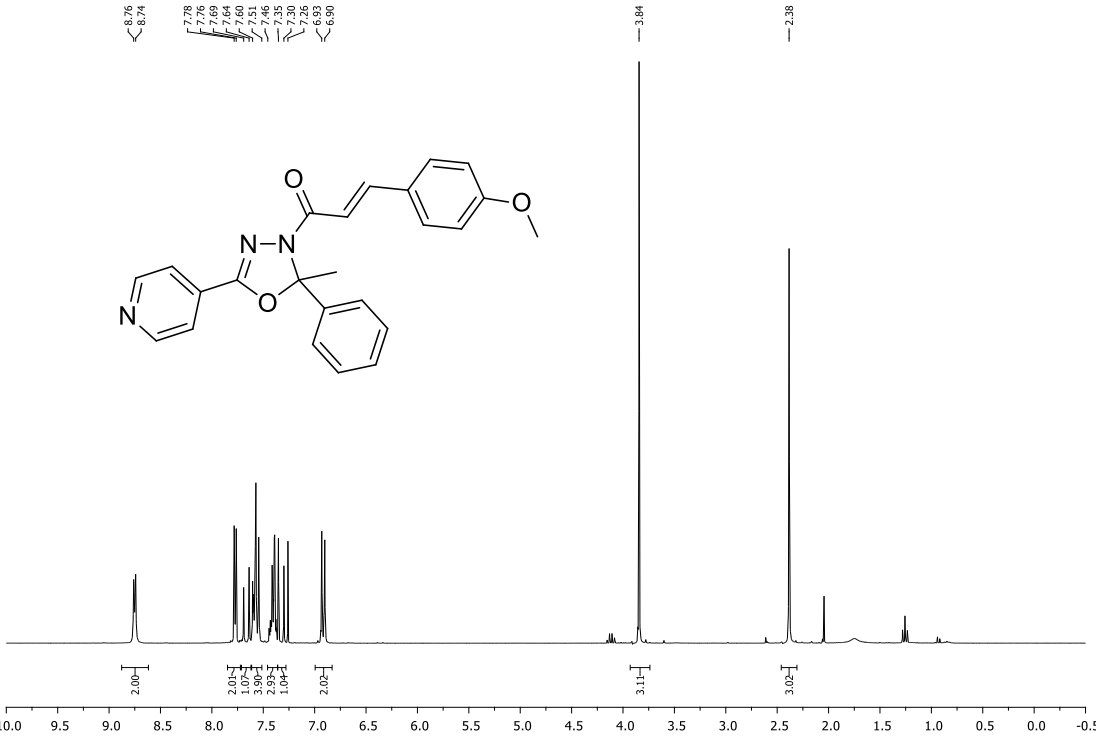


^{13}C NMR

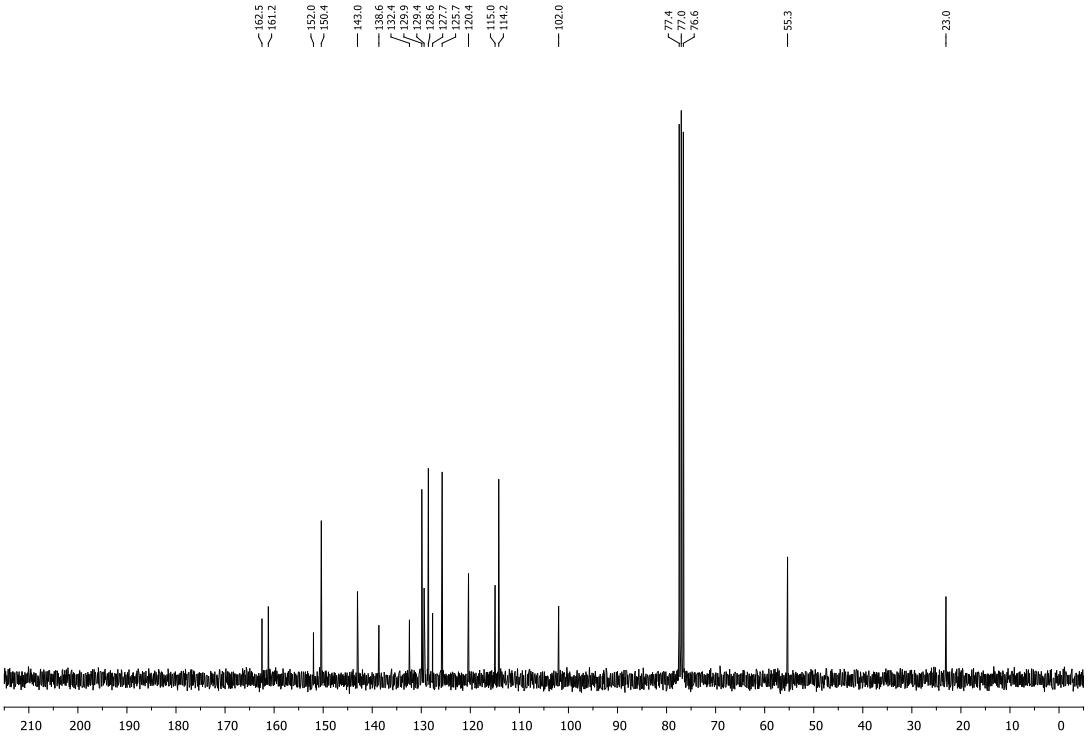


3-(4-Methoxyphenyl)-1-(2-methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (204) ($CDCl_3$)

^{1}H NMR

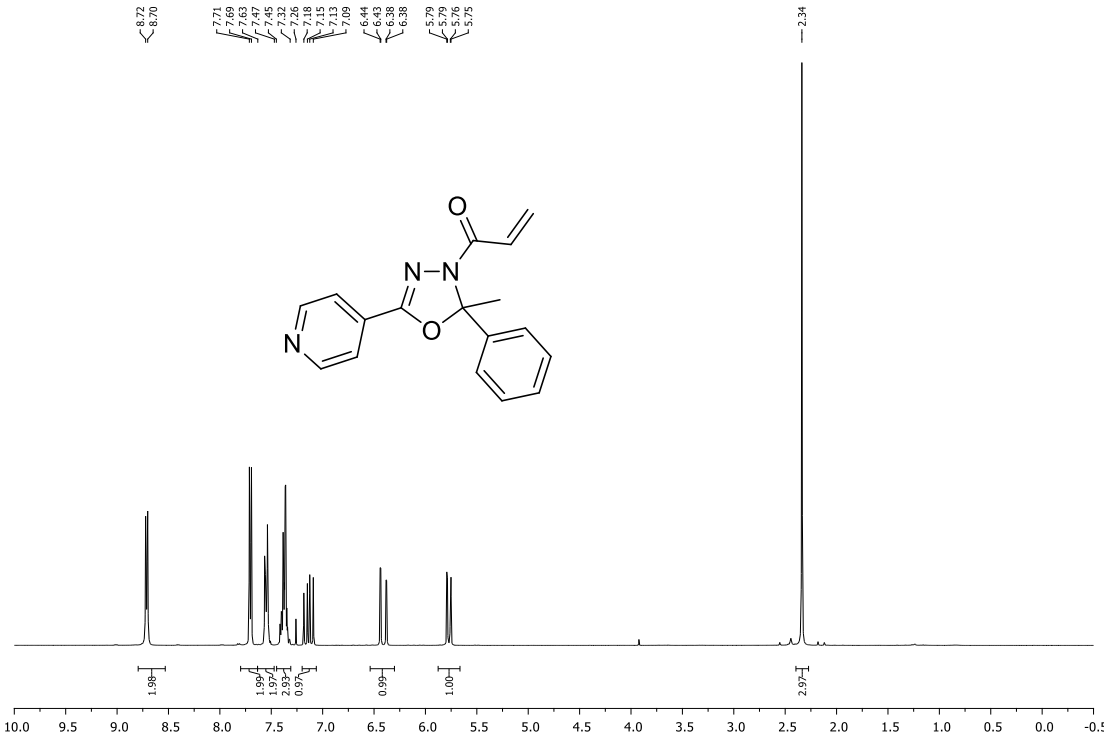


^{13}C NMR

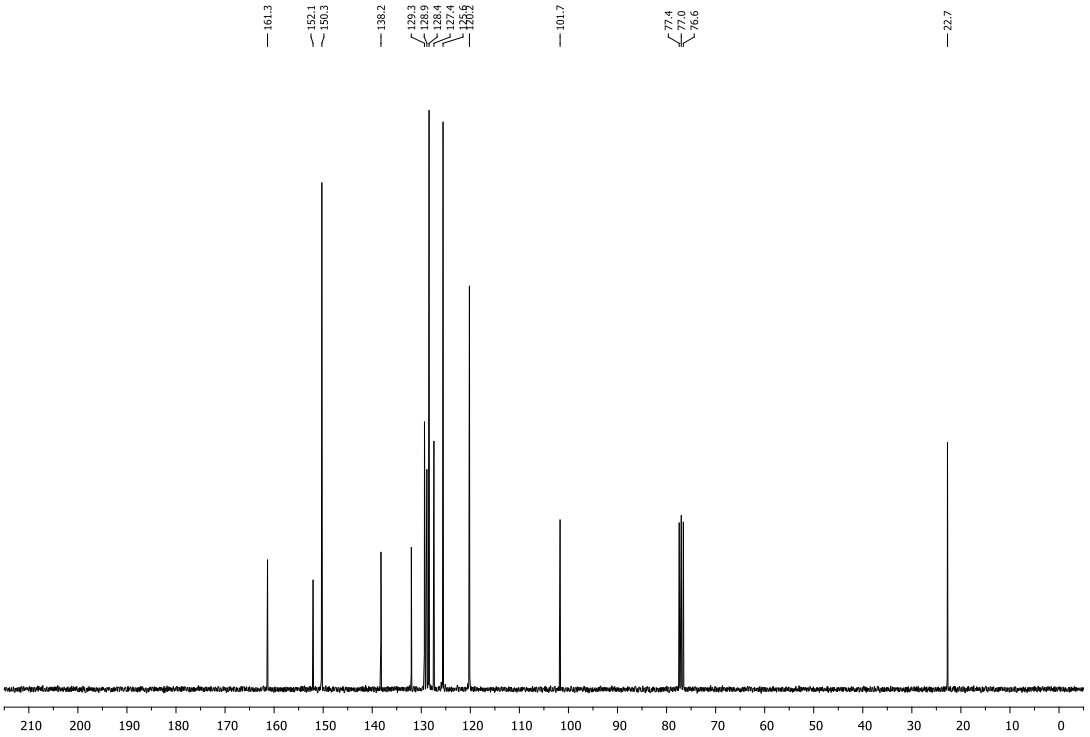


1-(2-Methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (205) ($CDCl_3$)

^{1}H NMR

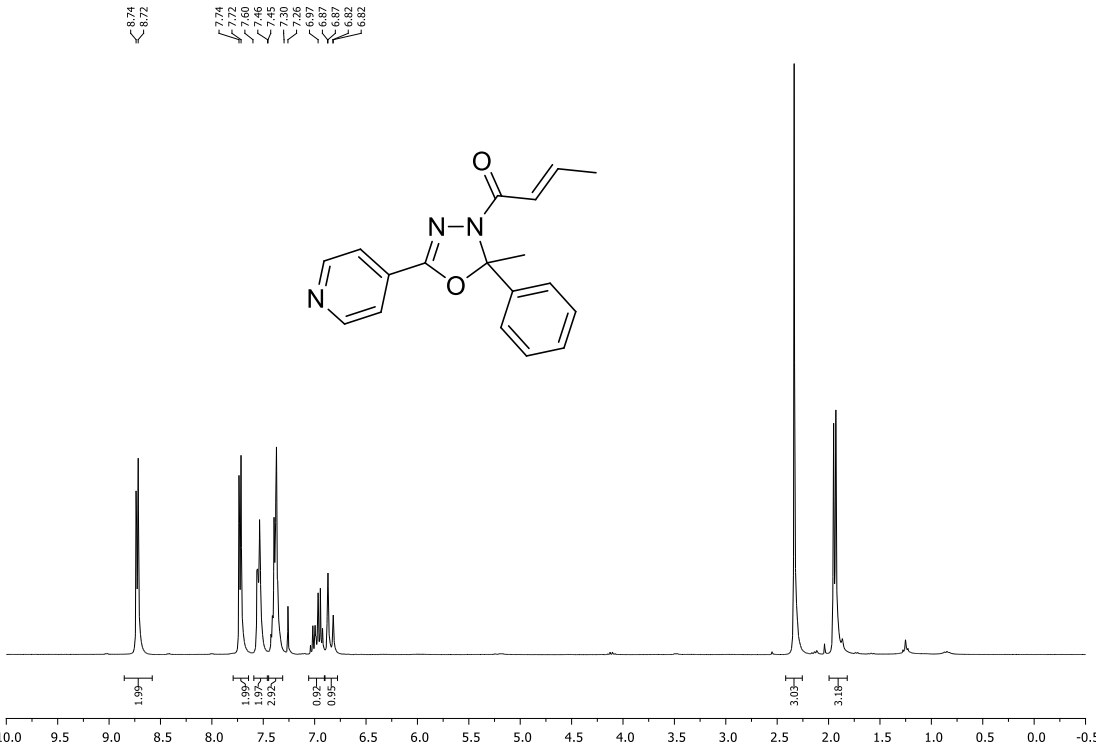


^{13}C NMR

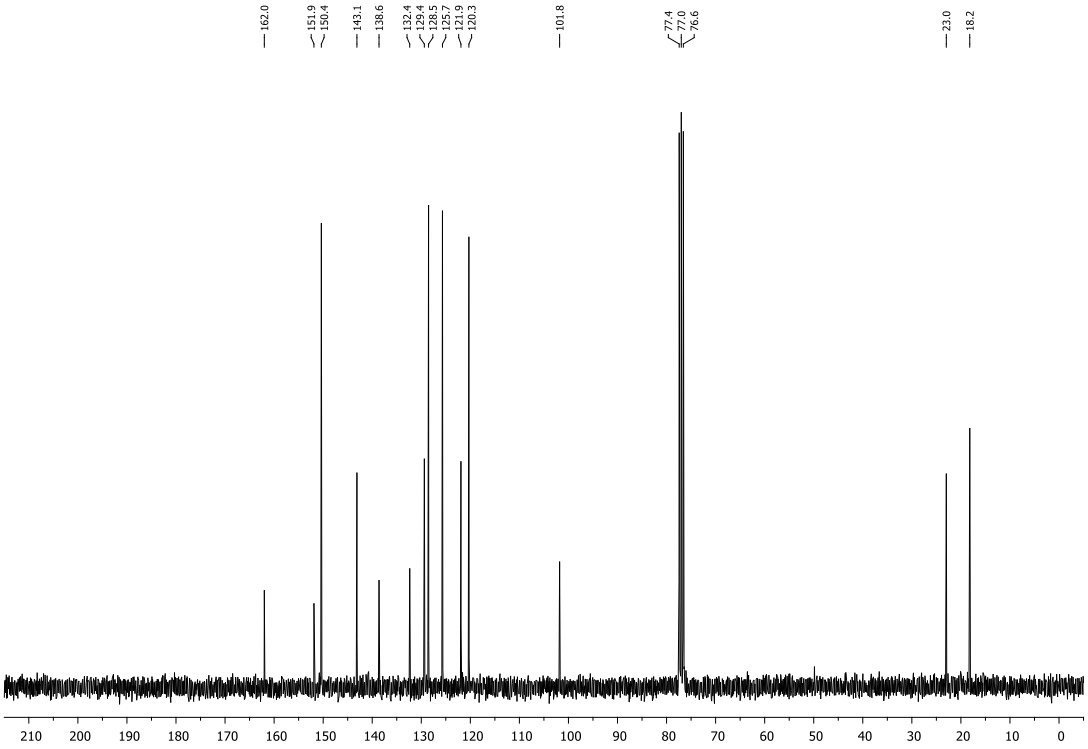


1-(2-Methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)but-2-en-1-one (206) ($CDCl_3$)

^{1}H NMR

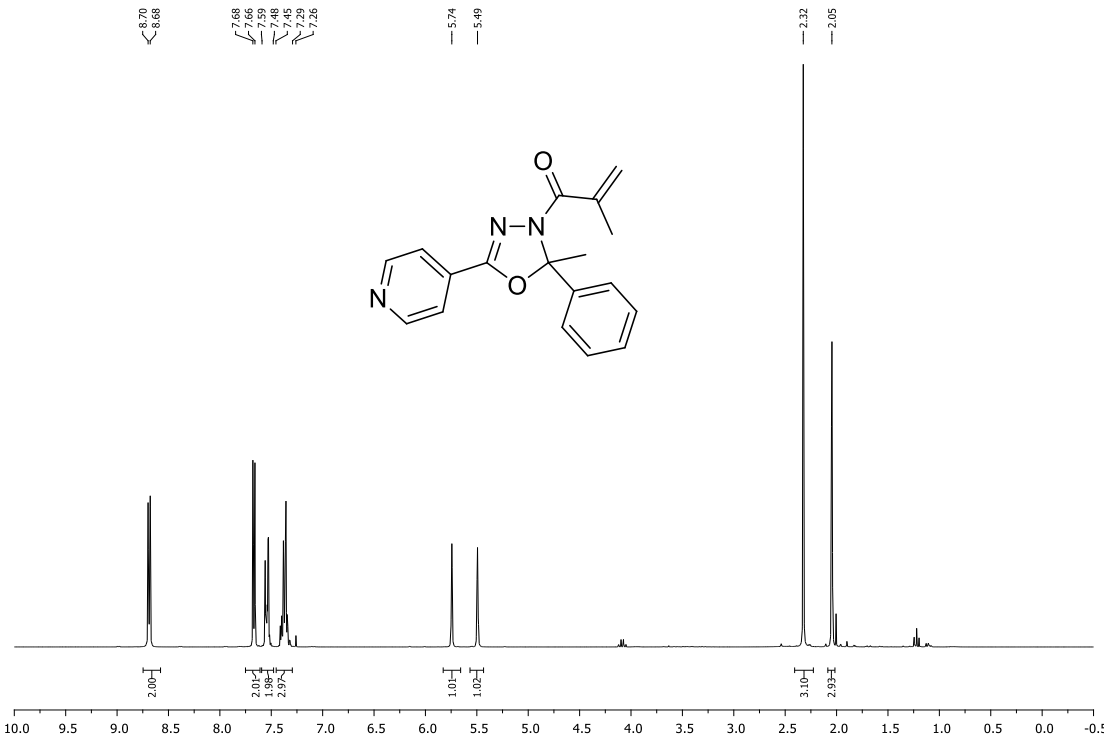


^{13}C NMR

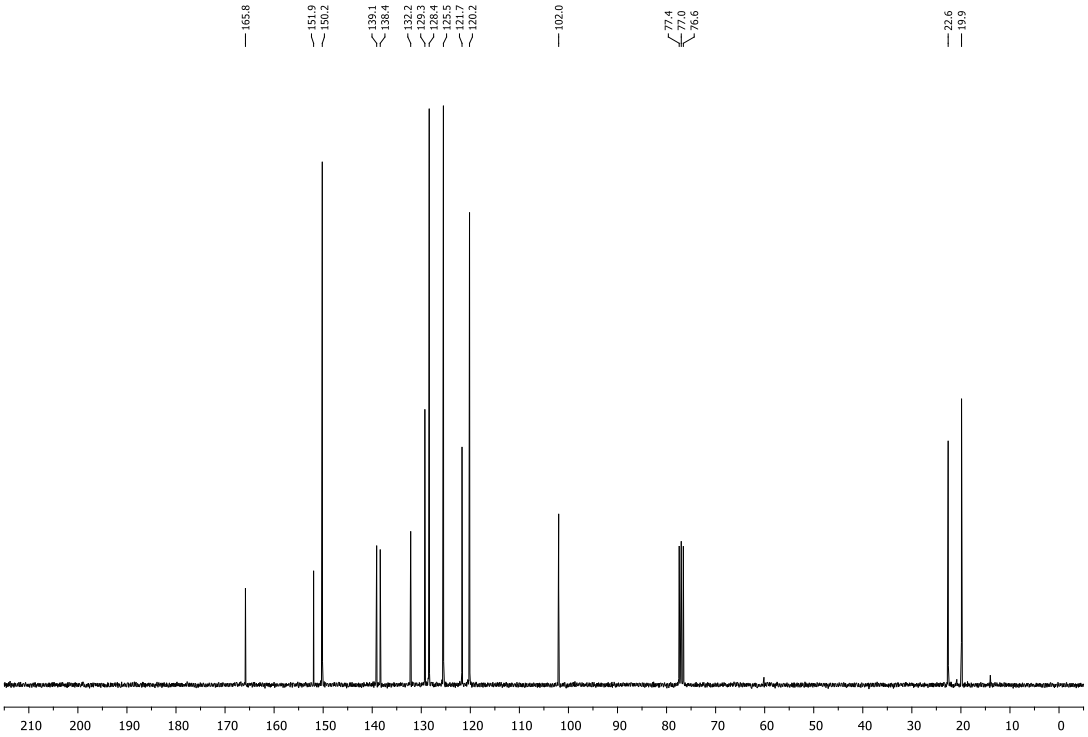


2-Methyl-1-(2-methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (207) (in $CDCl_3$)

^{1}H NMR

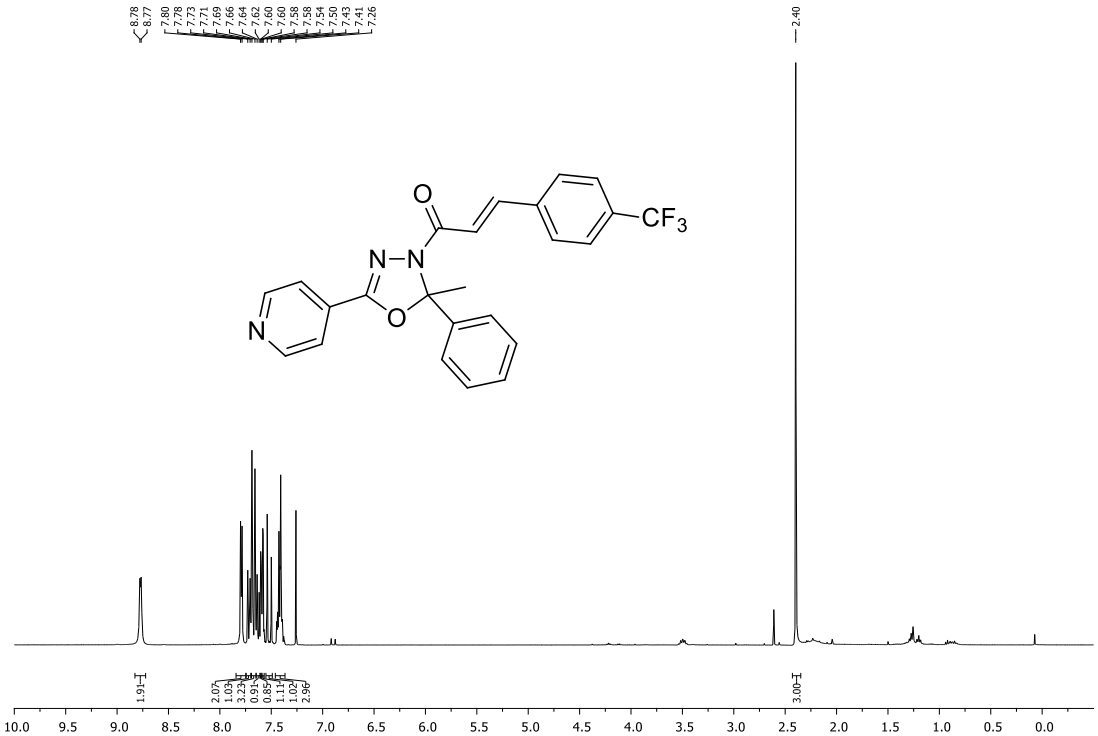


^{13}C NMR

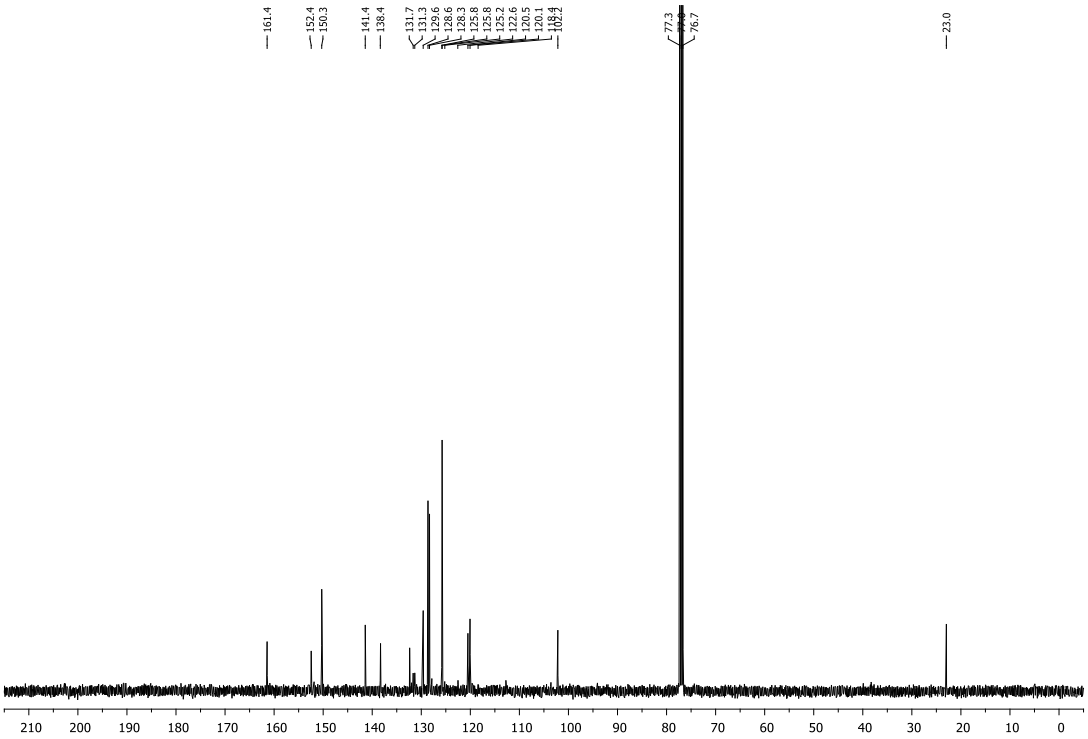


1-(2-Methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)-3-(4-(trifluoro methyl)phenyl)prop-2-en-1-one (208) (in $CDCl_3$)

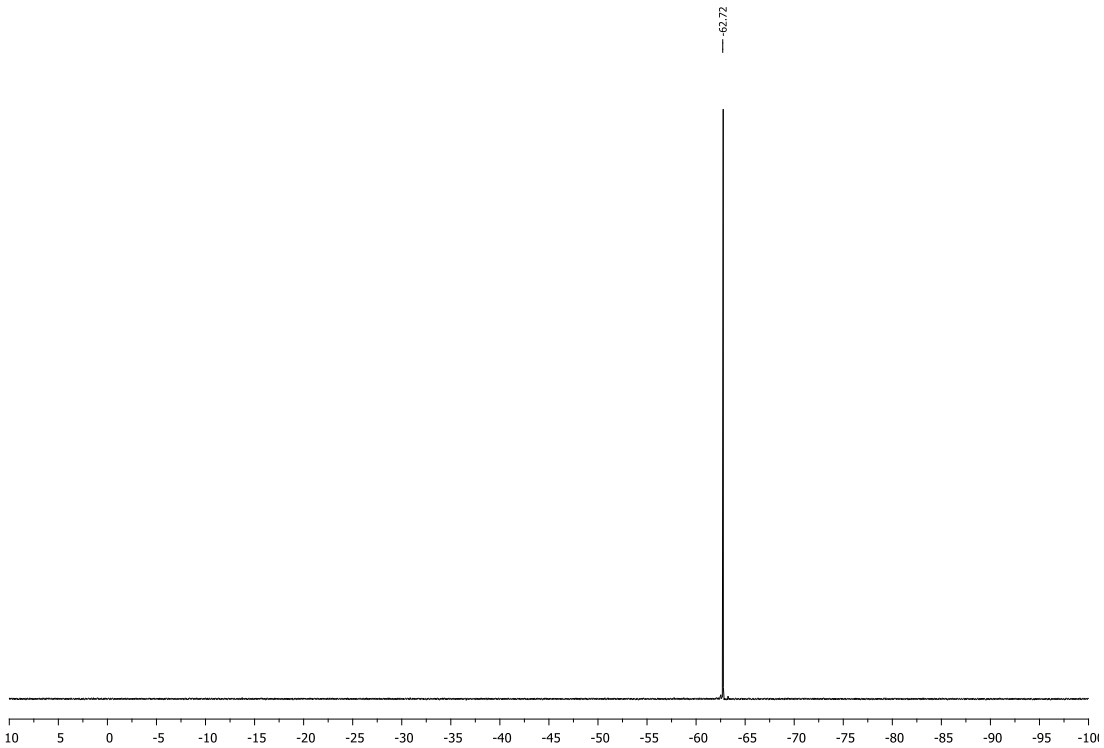
^{1}H NMR



^{13}C NMR

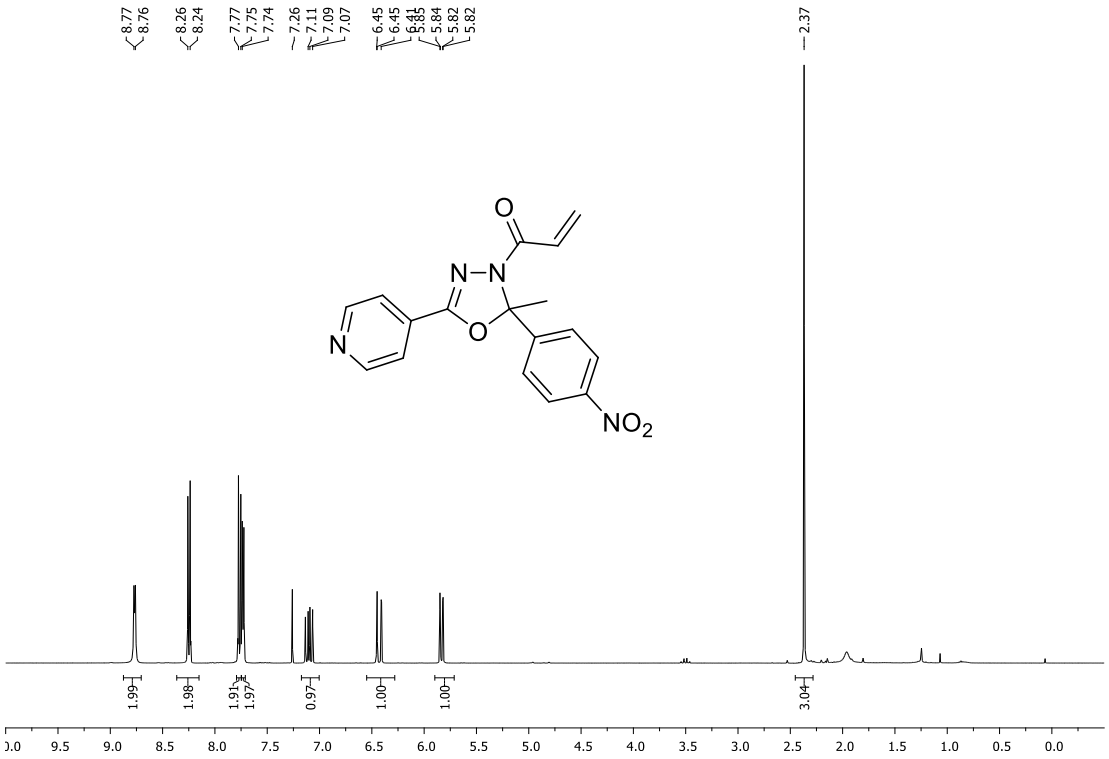


^{19}F NMR

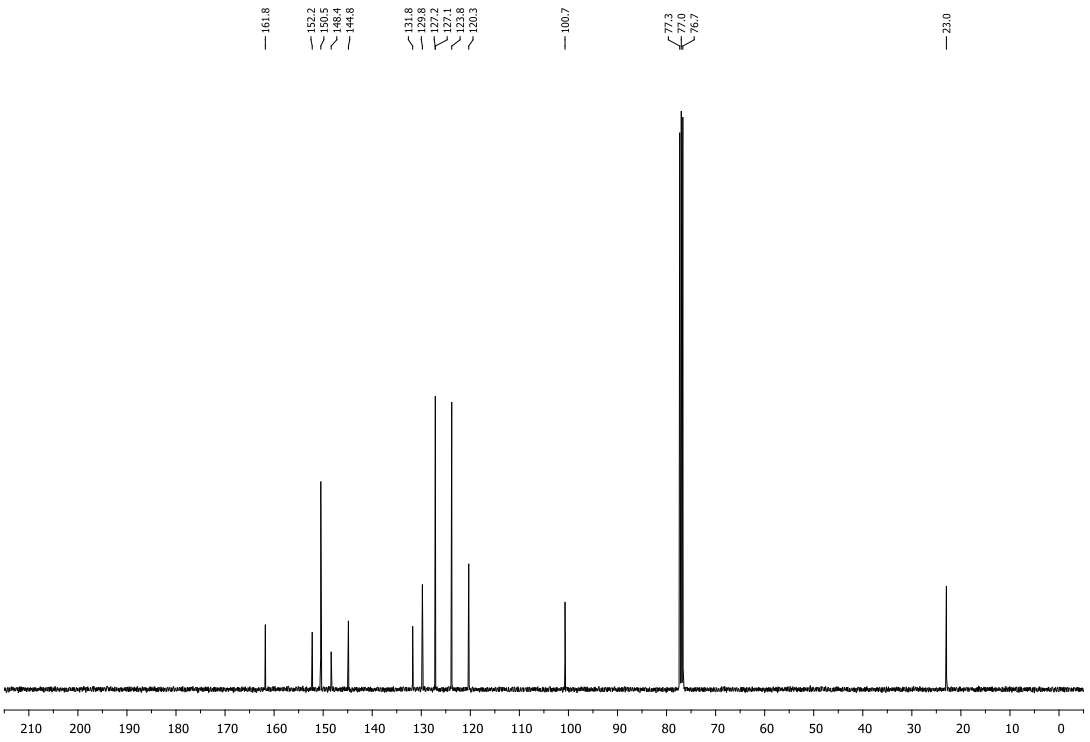


1-(2-Methyl-2-(4-nitrophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (209) (in $CDCl_3$)

^{1}H NMR

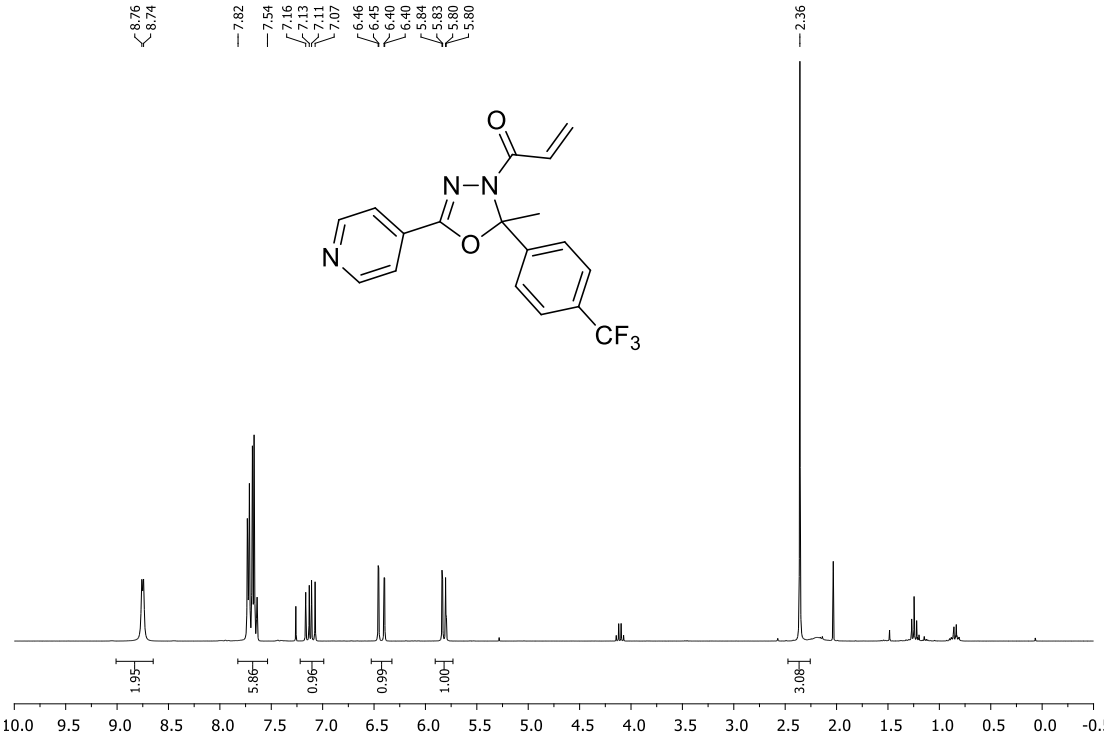


^{13}C NMR

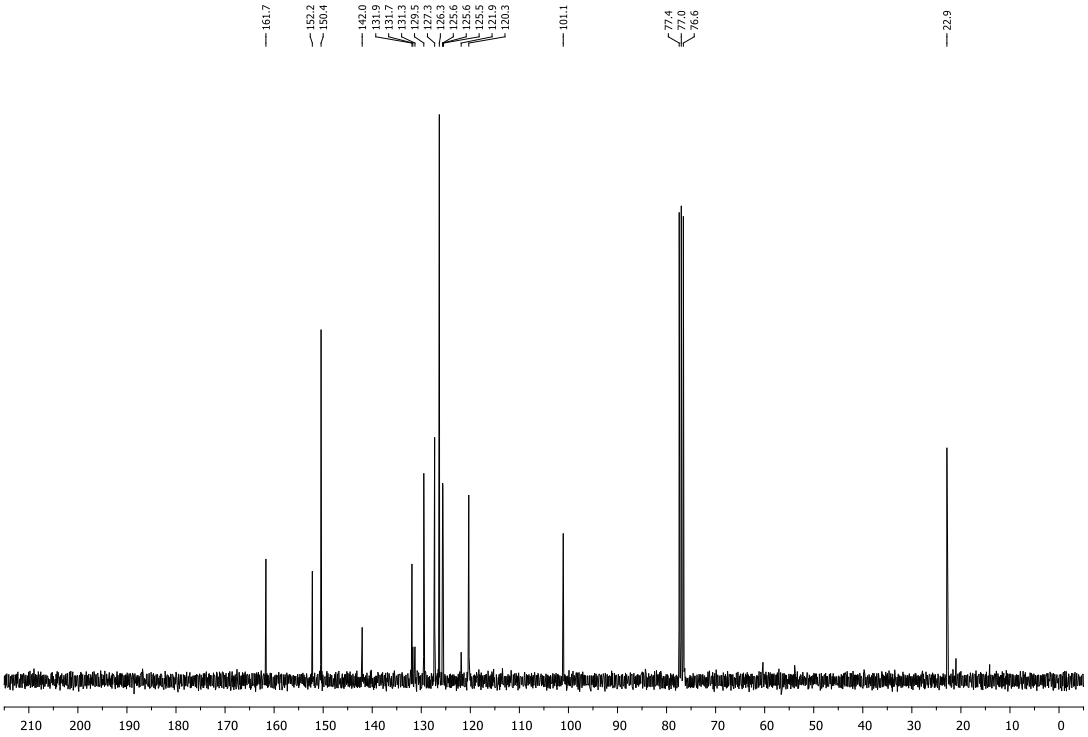


1-(2-Methyl-5-(pyridin-4-yl)-2-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (210) (in $CDCl_3$)

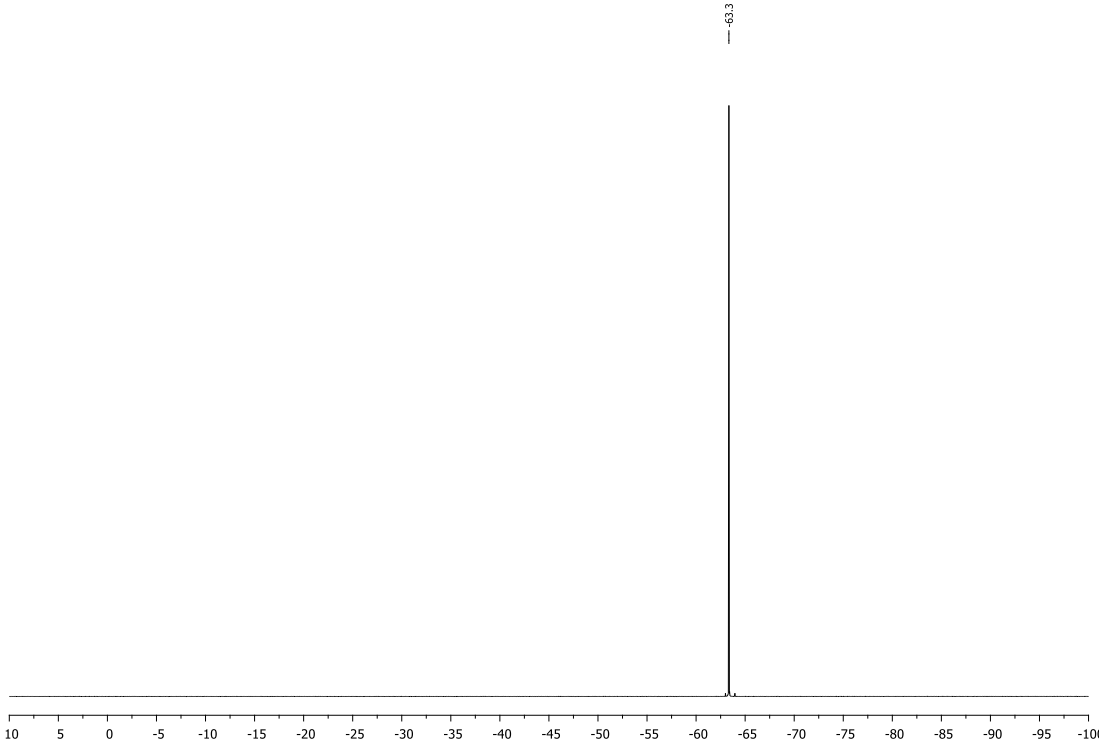
^{1}H NMR



^{13}C NMR

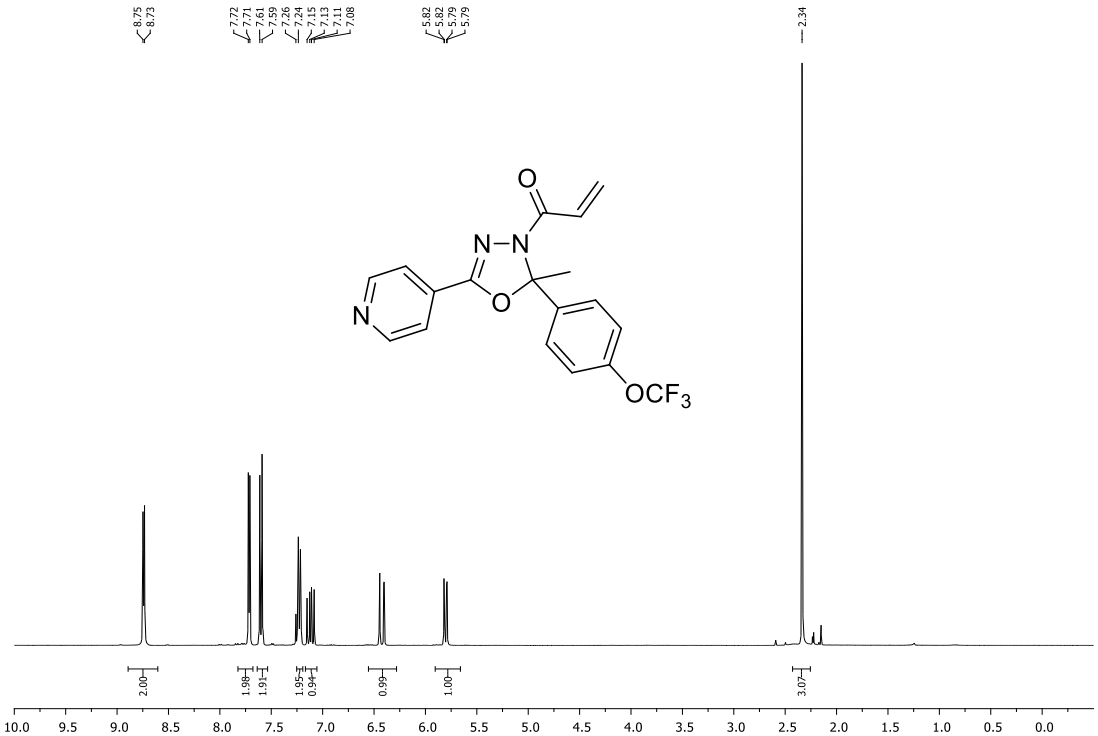


^{19}F NMR

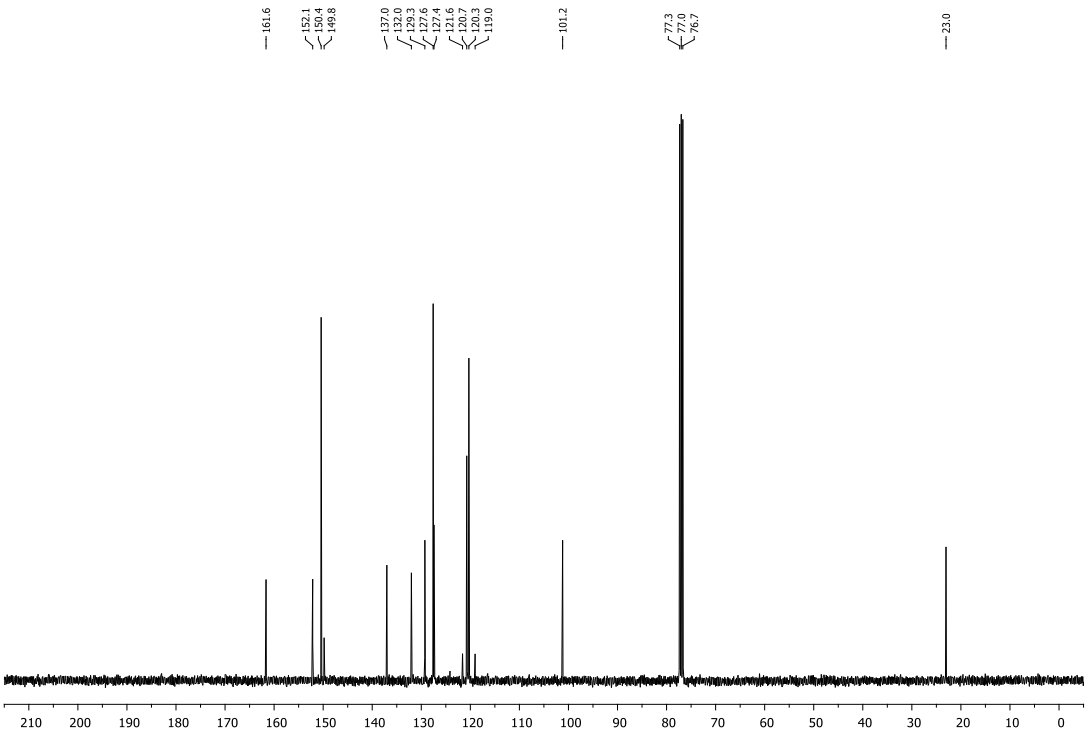


1-(2-Methyl-5-(pyridin-4-yl)-2-(4-(trifluoromethoxy)phenyl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (211) (in $CDCl_3$)

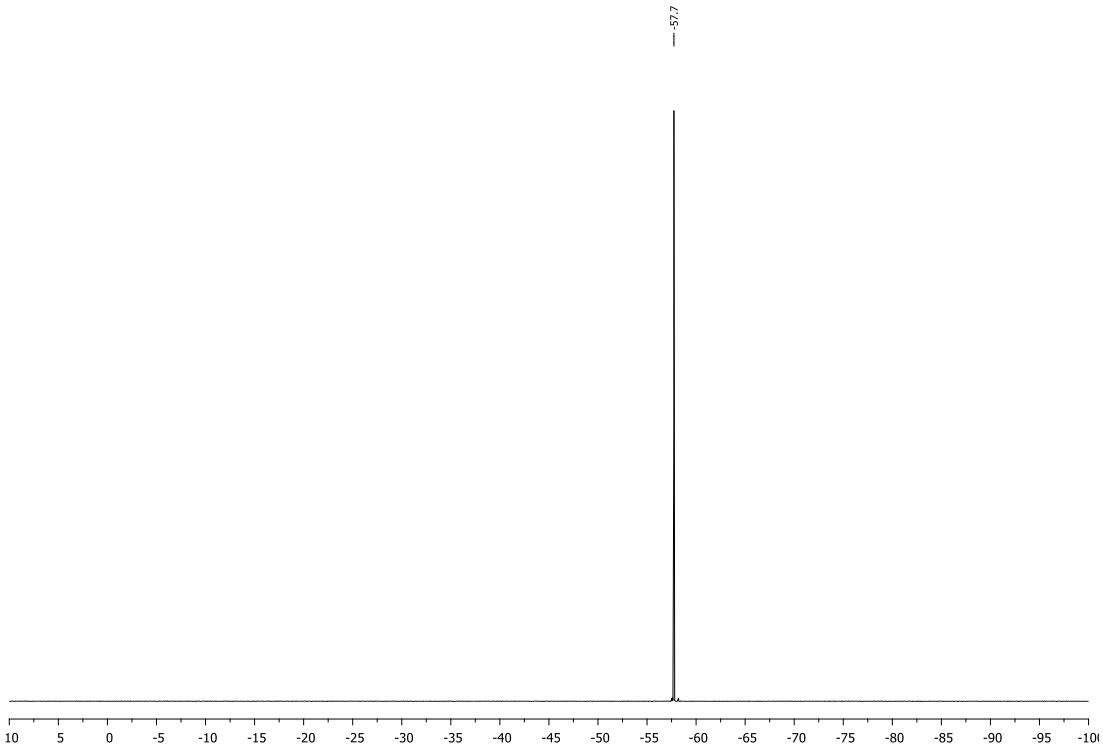
^{1}H NMR



^{13}C NMR

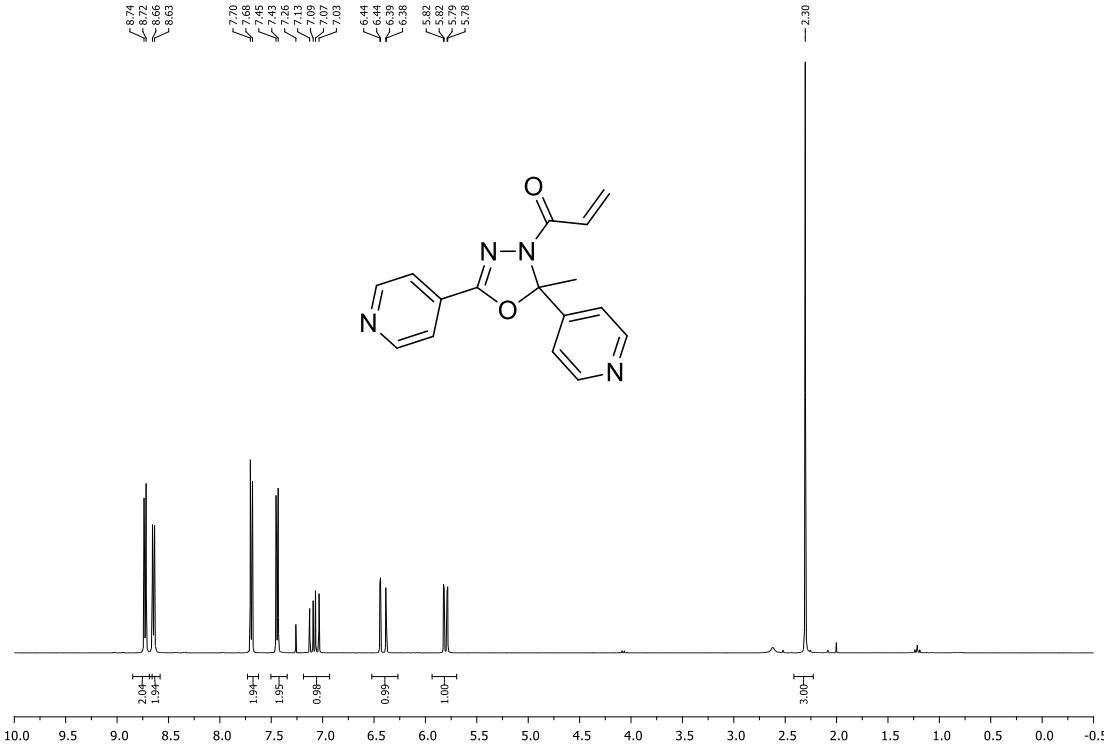

161.6
152.1
150.4
149.8
137.0
132.0
129.3
127.6
127.4
121.6
120.7
120.3
119.0
101.2
77.3
77.0
76.7
23.0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

^{19}F NMR

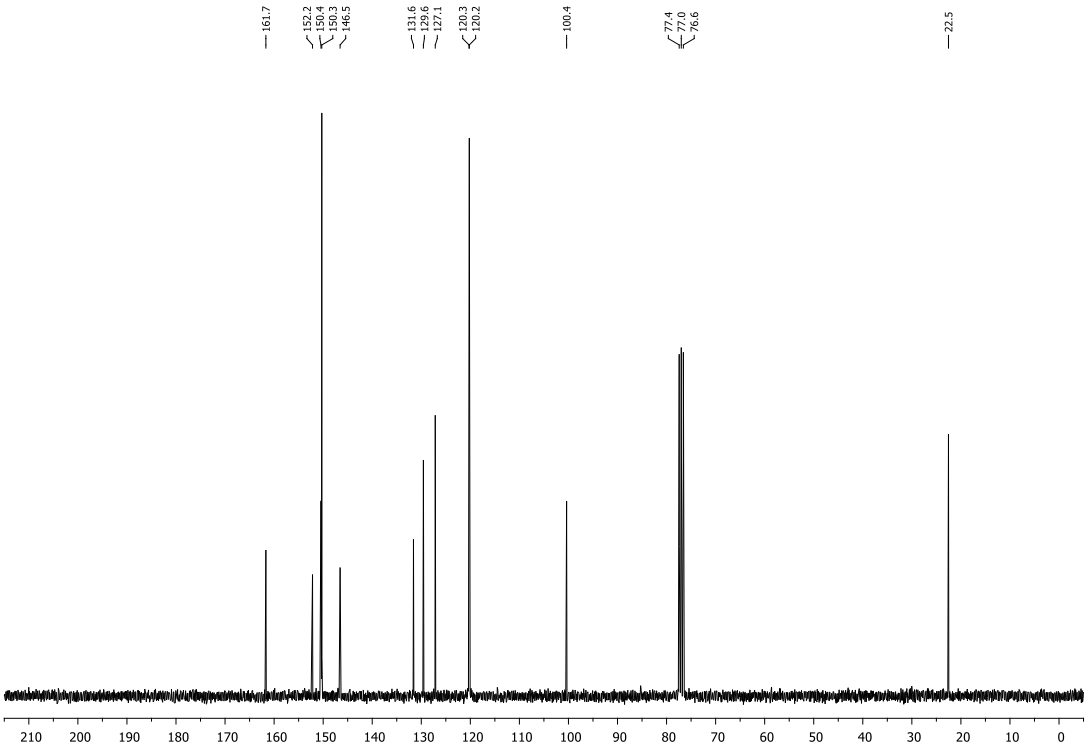

-57.7
10 5 0 -5 -10 -15 -20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -10

1-(2-Methyl-2,5-di(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (212) (in $CDCl_3$)

^{1}H NMR

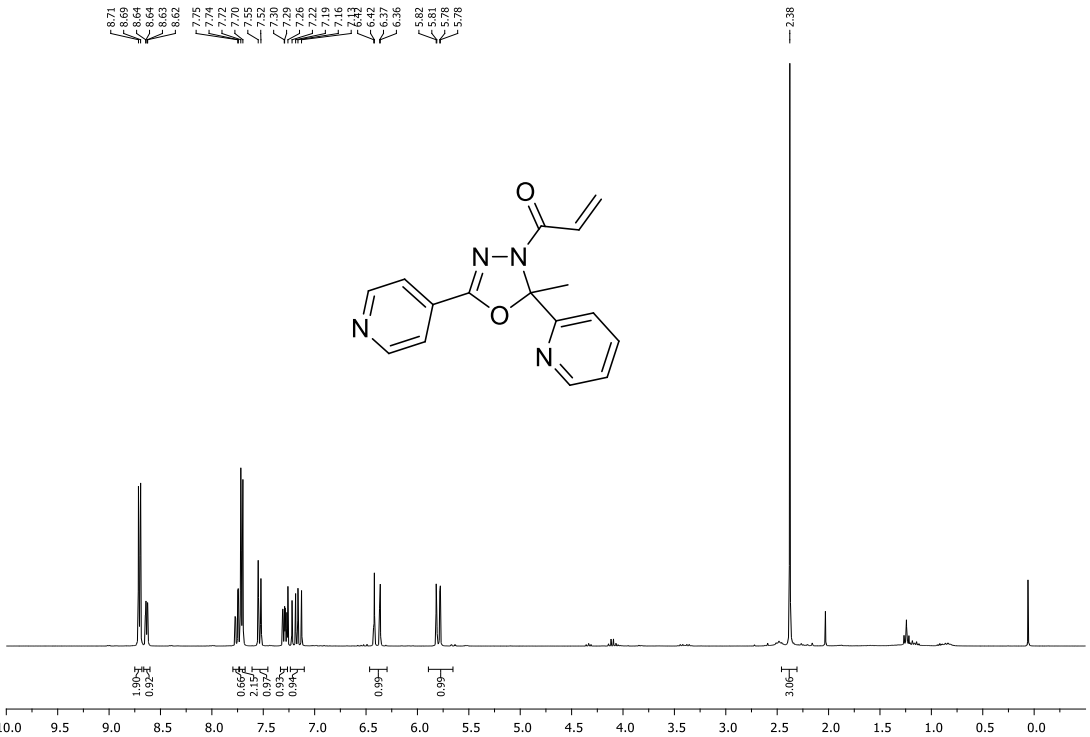


^{13}C NMR

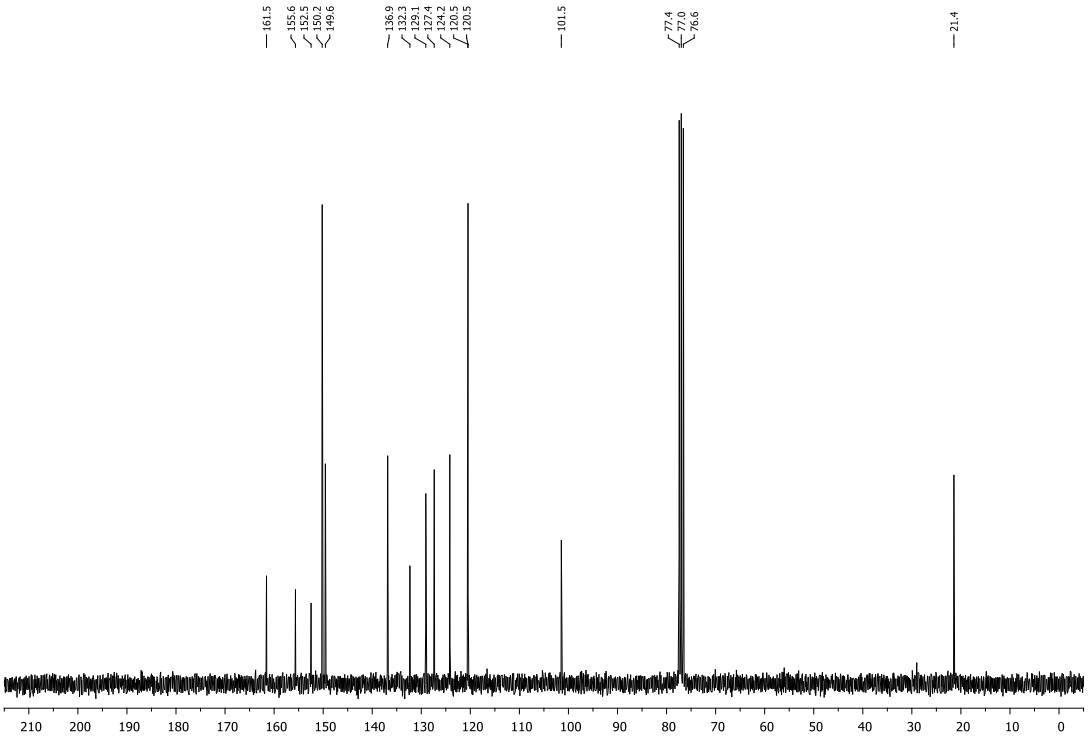


1-(2-Methyl-2-(pyridin-2-yl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)prop-2-en-1-one (213) (in $CDCl_3$)

^{1}H NMR

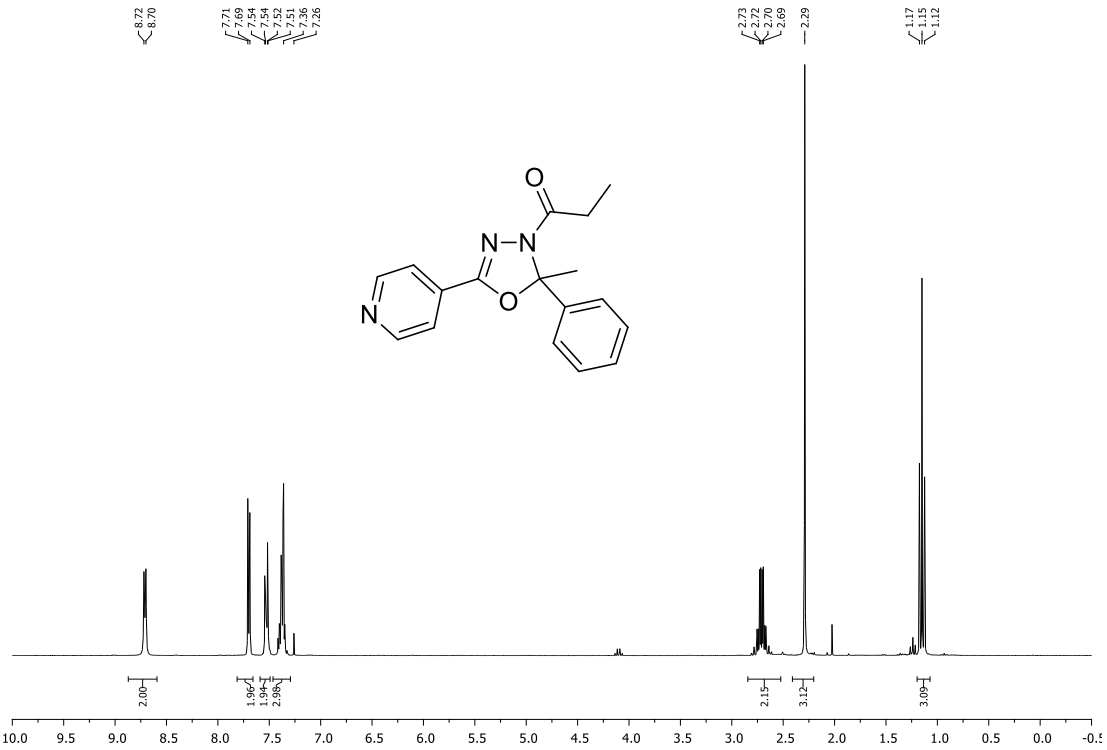


^{13}C NMR

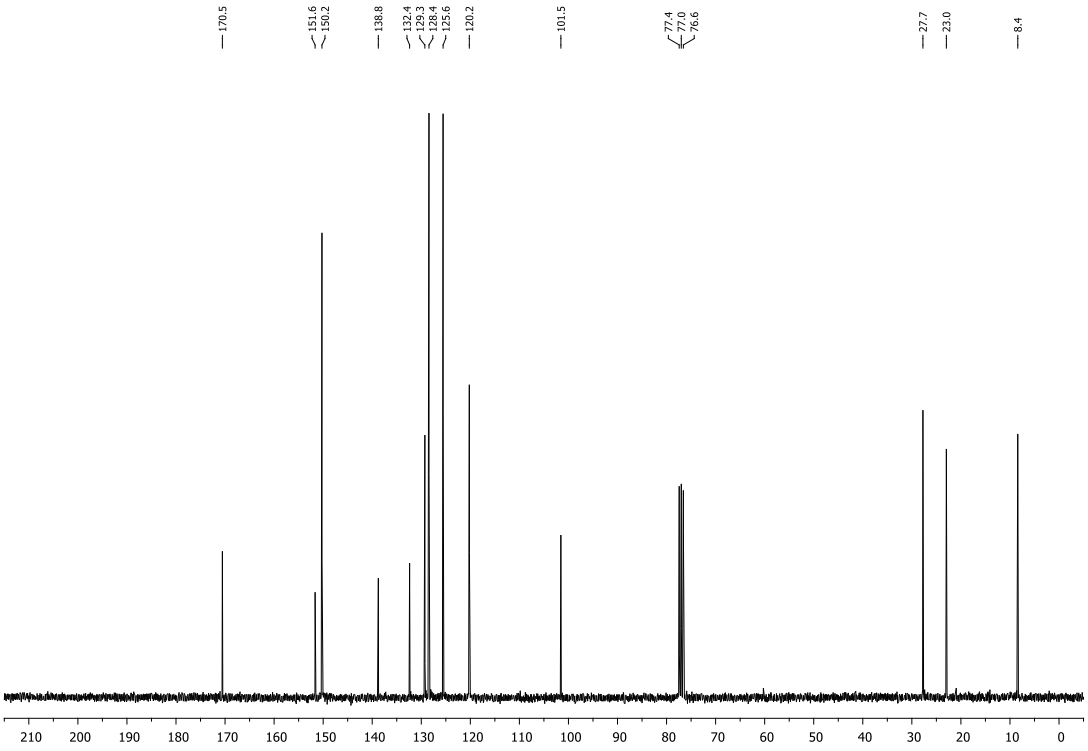


1-(2-Methyl-2-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)propan-1-one (214)
(in $CDCl_3$)

^{1}H NMR



^{13}C NMR


170.5
151.6
150.2
138.8
132.4
129.3
128.4
125.6
120.2
101.5
77.4
77.0
76.6
27.7
23.0
8.4
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

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Acknowledgements

First of all I want to thank Allah to help me during the PhD study and in all my life. Then I would like to thank PD. Dr. Sabine Amslinger very much for her supervision during my work in the PhD and for helping. My appreciation is also due to Prof. Dr. Burkhard König, Prof. Dr. Oliver Reiser, Prof. Dr. Jörg Heilmann and Prof. Dr. Wolfgang Wiegrebe.

I would also thank central analysis department at Univeristät Regensburg including: Josef Kiermaier, Wolfgang Söllner, Carola Prockl for the Mass Spectrometry, and Dr. Ilya Shenderovich, Fritz Kastner, Annette Schramm, Georgine Stühler for their efforts in NMR measurements.

Thank you very much to my colleagues: Nafisah Al-Rifai, Monika Enzinger, Hannelore Rücker, Petr Jirasek, Janina Ghonschor, Esther Heckel, Maria Landa, Andreas Bensch, Sonay Kasikci, Hermina Petkes, Katrin Winter, Gabriele Tepfenhart, Dita Fritsch.

My sincere gratitude to Bayerische Forschungsstiftung for my PhD scholarship, thank you very much to give me this opportunity to study in Germany and be with you for three years.

I want also to thank my friends Fouad Darras for helping and nice times, as well as Fady Abu-arar for helping and delicious food.

I would like to thank my parents for supporting me and also great thanks to my wife **Maha** to be patient and supporting me also my two sons **Malek** and **Omar**.