Synthesis of Analogs of a Potential Drug for Treatment of Epilepsy

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Master's degree in Chemistry

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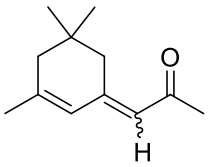
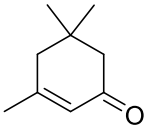
Abstract

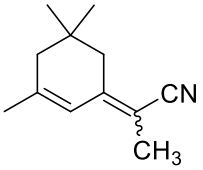
Prior work in the Durst group had generated more than forty analogs of the potent anticonvulsant isoxylitone isolated isolated from a medicinal plant *Delphinium denudatum Wall.* The nitrile designated as TD532 was the most potent compound generated by A. Saikaley. The starting material for the synthesis of TD532 is isophorone. The observation that TD532 showed considerable potential as an anticonvulsant suggested that other cyclohexenones might have have similar activity. During this project close to fifty derivatives of cyclohex-2-enone, focusing mainly on 3-arylcylohex-2-enones, were prepared. The synthesis of these compounds is described and structure activity relationships are discussed. Based on all the available structure activity data, we have designated the indicated portion of structure A as the pharmacophore for anticonvulsant and anti-epileptic activity.



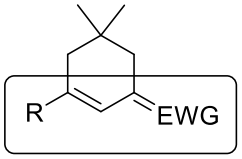
Isoxylitone, 1



Isophorone, 8

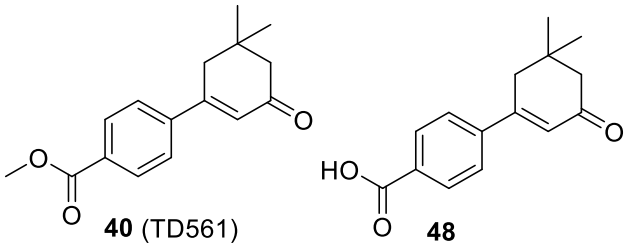


TD532



A

The ester designated as TD561 (**compound 40**) showed excellent potential in both in vitro and in vivo assays. It has been shown to be a pro-drug of the corresponding acid TD562 (**compound 48**). These two compounds and the sodium salt of TD562 are currently undergoing final pre-clinical studies at the Center for Drug Research and Development in Vancouver. Five analogs, including TD561 are also under investigation by the Epilepsy and Seizure Division of the US National Institutes of Health.



40 (TD561) **48**

The compounds **40**, **48** and the sodium salt of **48** are the centerpieces of a PCT patent applied for by OB Pharma (Toronto) in June 2017.

Acknowledgements

I would like to express my sincerest gratitude to Dr. Tony Durst, who was willing to give me a chance and take me on as an Honour's student during my undergraduate studies. His passion for chemistry was so contagious that I decided to pursue my graduate studies in his research lab, which was a great learning experience. His humanistic perspective on life and teaching has had a greatly positive impact on my attitude and outlook in general. His mentorship and unwavering support were greatly appreciated.

I would also like to thank my parents, Raymonde and Gérald, who helped me push myself through the challenging moments and helped me appreciate the good times. Their encouragement and assistance in many aspects of my life were very important to my motivation and ambition.

Whether they directly or indirectly lent a hand during my graduate studies, I have to acknowledge all of my fellow students that I met and spent time with in and out of the lab. Thank you for your friendly attitude and for contributing to a great positive learning and helping atmosphere. I will always cherish the good times we had as part of the Durst lab.

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°C	Degrees Celsius
^{13}C NMR	Carbon 13 NMR
^{1}H NMR	Proton NMR
AED	Anti Epileptic Drug
$CDCl_3$	Deuterated Chloroform
CDRD	Center for Drug Research and Development
DCM	Dichloromethane
EtOAc	Ethyl Acetate
ETSP	Epilepsy Therapy Screening Program
Eq.	Equivalents
g	Gram
GABA	γ-Aminobutyric acid
Hz	Hertz
LDA	Lithium Diisopropylamine
LogP	Octanol/Water partition coefficient
mg	milligram
m	Multiplet
M	Molar
mins	Minutes
mL	Millilitres
mmol	Millimoles
MW	Molecular Weight
nBuLi	nButyllithium
NINDS	National Institute of Neurological Disorders and Stroke
NMR	Nuclear Magnetic Resonance
p	Para substituted
Ph	Phenyl
PhMgBr	Phenylmagnesium bromide
ppm	Parts per million
SEM	Standard error of the mean

1 Introduction

1.1 Epilepsy

Epilepsy is one of the most common neurological diseases in the world.[1] There are approximately 50 million people worldwide affected by recurrent unprovoked seizures symptomatic of epilepsy.[1] Around 60% of affected people are believed to have idiopathic generalized epilepsy which has no clear identifiable cause but is presumed to have a strong underlying genetic basis and shows no structural brain malformations or defects.[1] Symptomatic epilepsy however is characterized by having known causes such as brain damage from problematic births, genetic conditions leading to brain malformations, limited oxygen supply to the brain related to a stroke, brain tumors and infection. For a patient to be considered to have epilepsy, they must suffer from at least one unprovoked seizure and present further predisposition determined by family history or the presence of epileptiform changes detected by electroencephalogram.[1] Whereas normal brain activity is characterized by asynchronous neuronal firing, these seizures are caused by an abnormal synchronous discharge of cortical neurons.[1] Here are the different types of seizures:

Table 1.1-1 Simplified classification of seizures by behavioral and electrophysiologic data

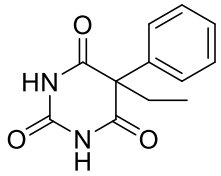
I. Partial (focal seizures)	II. Generalized seizures
A. Simple partial seizures with motor, sensory, psychic, or autonomic symptoms	A. Absence seizures
B. Complex partial seizures	B. Tonic-clonic seizures
C. Partial seizures with secondary generalization	C. Other (myoclonic, tonic, clonic, atonic)

Partial or focal seizures are caused by focal brain injury that are confined in one area of the brain and are preceded by diverse sensory experiences or auras.[2] These types of seizures are not always linked to loss of consciousness but can potentially lead to generalized seizures.[2] Generalized types of seizures always include loss of consciousness and can cause symptoms varying from short loss of mental focus and disorientation to falling and constant muscle contractions. Epilepsy and the associated unprovoked seizures can be successfully controlled through treatment with anti-epileptic drugs (AEDs) at a relatively low cost. However, there is evidence of medically resistant epileptic patients whose epileptic seizures persists after the use of two appropriate AED treatment trials.[2] There is also low availability of AED in low and middle-income countries, preventing appropriate treatment of people suffering from epilepsy. Therefore, there is still a need for more effective and affordable AED development.[1]

1.2 History of anti-epileptic drug development

Potassium bromide is considered to be the first drug with any documented value for treating epilepsy, first documented[3] by Sir Charles Locock of the Royal Medical and Chirurgical Society in May of 1857. He commented that he had successfully treated women with hysterical epilepsy and it was presumed that bromides could dampen sexual excitement which they believed was the cause of these seizures. The widespread of bromides for the treatment of epilepsy in conjunction with other agents such as zinc, digitalis and iron began after the publication of other reports of successful treatment trials. Despite the associated secondary effects such as dermatological conditions and more severe psychological symptoms potentially leading to psychosis, bromides remained the foundation of epilepsy treatment for many years.[3]

In 1912, barbiturates, already widely used as hypnotics and sedatives were given by clinical assistant Albert Hauptmann to epileptic patients as tranquilizers.[4] A reduction of frequency of seizures was observed, which led to subsequent studies on the subject. Bromides were then eventually replaced as the most widely used AED by phenobarbital because of its greater efficiency and the absence of severe secondary effects that were associated with bromides. Phenobarbital in combination with bromides were then used for treatment by some physicians. This treatment was discontinued after a few years because it was ineffective in most patients.[3]



Phenobarbital

Figure 1.2.1 Structure of the barbiturate AED, phenobarbital

The next advancement in AED development was marked with the beginning of testing of potential drugs on electrically induced epilepsy in animal models, notably cats, by Drs. Houston Merritt and Tracy Putnam.[5] The discovery of anti-seizure properties of phenytoin can be attributed to studies of their group using these methods in 1938.[5] Patients who were previously unaffected by the combination therapy of phenobarbital and bromides showed successful treatment using phenytoin and without the sedative effect of the previous AEDs. This not only demonstrated that animal models were a cost-effective approach to drug discovery, but also that the clinical trials on human patients could be reserved for compounds that were shown to provide the best results in these animal trials. Phenytoin was such a successful treatment associates with reduced neurological side effects that it was established as the best AED to be used for partial seizures and is still used in certain situations today. Subsequently, downsides of using phenytoin such as extensive drug interactions, chronic toxicities and serious delayed effects such as carcinogenicity and teratogenicity were discovered. More effective analogs of phenytoin were then rapidly discovered by altering certain functional groups on the basic chemical moiety, the five and six membered heterocyclic rings of initially described members of this family of AEDs.[3]

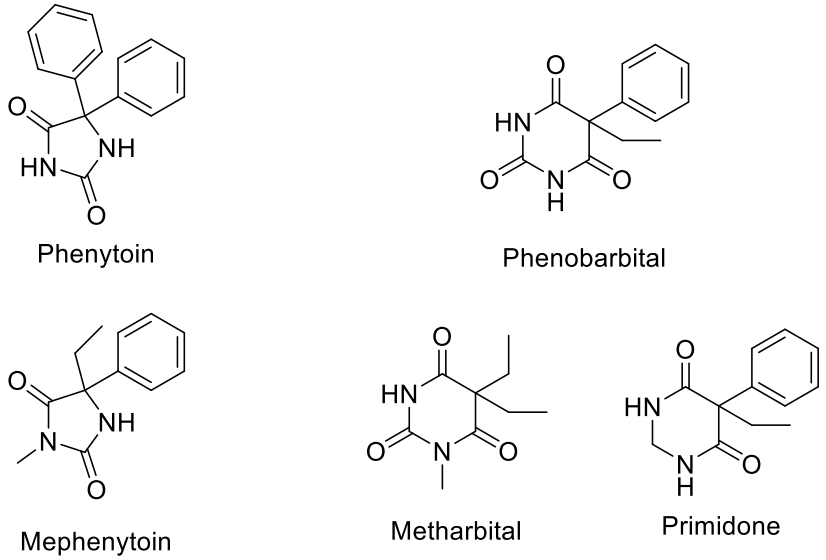
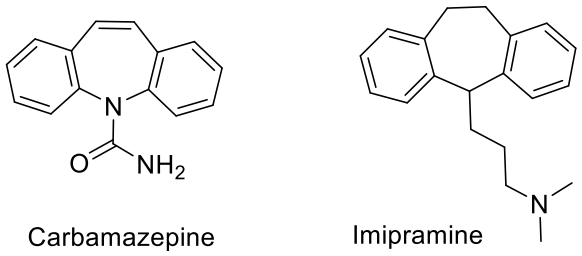


Figure 1.2.2 Structures of analogs and previously discovered AEDs used as their basis

The next major advancement in AED development was the drug carbamazepine which is still the standard treatment for partial onset epilepsy in Europe. During the development of the antidepressant drug imipramine in 1953, Walter Schindler discovered this tricyclic compound's anticonvulsant properties.[6] It was first marketed for treatment of another neurological disorder (antidepressant) but in the 1960s underwent subsequent trials leading to the identification and confirmation of its antiepileptic effects.[6] Despite reports of secondary effects such as rashes, hyponatremia, hepatic dysfunctions, haematological toxicity and other rare adverse effects, carbamazepine became by the mid 1980s the most prescribed AED in Europe and remains a recommended first-line treatment for partial seizures. However, studies conducted in the 1980s demonstrated that all readily available AEDs on the market were only capable of controlling seizures in one out of three patients and still had multiple common adverse effects associated with their use such as GI disturbance, sedation, ataxia, allergic rash, headache, blurred vision, nausea, drowsiness and insomnia.[3, 7]



Carbamazepine Imipramine

Figure 1.2.3 Structures of Carbamazepine and Imipramine

In 1975, the National Institute of Neurological Disorders and Strokes in the US established the Anticonvulsant Drug Development Program in order to stimulate the development of new AEDs. Following the screening of almost 30,000 new chemical compounds over the years, an increase of licensed AEDs has been observed. Suitable drug candidates were primarily found by systematic screening against a range of seizure animal models in rats, but also by structural modification of existing molecules to create new analogs and by target-oriented design. The deeper understanding of basic mechanisms of epilepsy has greatly contributed to progress in brain research (see section 1.3 below), which in turn led to discoveries of neurotransmitters of the brain and to further targeted drug development. These recent advances in AED development have improved the amount of medically controllable cases of seizures to about 70%.[3]

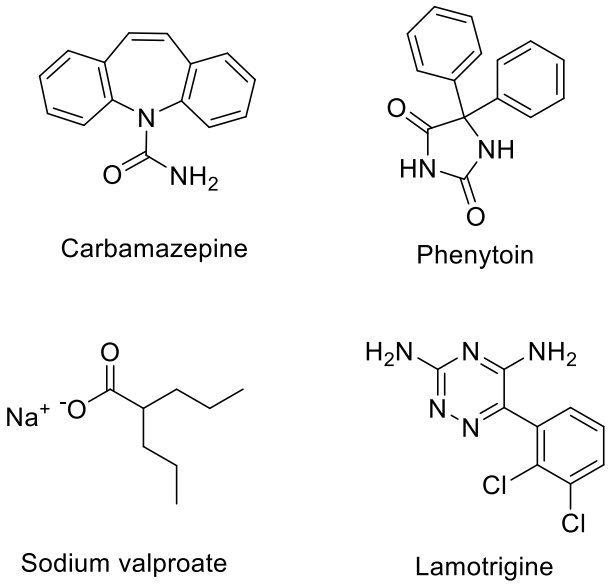


Figure 1.2.4 Common anticonvulsant drugs currently used on the market

Current treatment of seizures and epilepsy uses a variety of anticonvulsant drugs, some of which are combinations of several different types of drugs.[3] Many currently used AEDs share considerable structural similarities. With few exceptions, they contain a nitrogen heterocycle either fused to or carrying an additional aromatic ring (Figures 1.2.2 to 1.2.4).

Limitations of current AEDs

Other than the common, less severe side effects mentioned previously, AEDs can in rare cases have potentially life-threatening adverse effects such as systemic reactions resulting in multi-organ failure, epidermal necrolysis and thrombocytopenia.[8] Withdrawal from an AED medication program should be avoided because it can cause the resurgence of seizures and status epilepticus, which is a series of seizures without any time to recuperate between the events. [8]

It has also been reported that increase in risk of anxiety, depression and suicidality has been associated with the use of anticonvulsants. In a meta-analysis of clinical trials including 11 AEDs, the FDA reported that patients taking these drugs had twice the risk of suicidal thoughts and behaviours than those patients taking a placebo.[9]

AEDs are only currently used to treat the symptoms of epilepsy and not the cause of the disease itself. Only if the seizures occur or are expected to occur in a rate that is more disabling to the individual than the potential side effects of treatment is it advisable to proceed with treatment using AEDs. Even when blocking seizures, AEDs do not seem to affect the course of the underlying epilepsy, which is a serious limitation of current AED treatment.[10]

Importantly, it needs to be emphasized that fully 30% of epilepsy cases are resistant to currently available AEDs. (See section 1.4)

1.3 Mechanism of seizures on neuronal activity and the effect of AEDs

The action potential is the basic mechanism of neuronal activity and is mediated by a number or factors involving a network of neurotransmitters in the synaptic region and voltage-gated ion channels located along the neuron. Action potentials occur due to depolarization of the neuronal membrane, with membrane depolarization propagating down the axon to induce neurotransmitter release at the synapse between neurons. Common features of epileptic seizure activity are neuronal hyperexcitability (stimuli leads to larger increase in neuronal firing) and neuronal hypersynchronicity (abnormal synchronized neuronal firing leading to seizures) of multiple neurons. For a seizure to manifest, these features must be

present in large populations or networks of neurons. AEDs act on three different classes of molecular targets to limit epileptic activity. Efficient AED mechanisms affect either ionotropic glutamate receptors, γ-aminobutyric acid receptors ($GABA_A$ and $GABA_B$), voltage-gated sodium and calcium channel or a combination of these targets.[2, 11]

GABA is the primary inhibitory neurotransmitter in the brain. Inhibition mediated by GABA in the brain occurs because of release of GABA from the presynaptic neuron that acts on two postsynaptic receptors. $GABA_A$ receptor activation results in hyperpolarization of the neuron by increasing chloride transfer inside the neuron and causing rapid inhibitory effect. $GABA_B$ receptor activation results in decrease of calcium entry and causes a slow inhibitory effect. Epileptic central nervous systems were studied and found to have reduced GABA-mediated inhibition, which could lead to uncontrolled neuronal activation and subsequent seizures. Several GABA agonist drugs are potent AEDs and GABA antagonist drugs have been shown to induce seizures.[2, 12, 13]

Voltage-gated sodium channels are responsible for depolarisation of the nerve cell membrane and conduction of action potentials across the surface of neuronal cells. Inhibition of voltage-gated sodium channels resulting in blockage of sustained, high-frequency, repetitive firing of neurons has been observed in AEDs effective in the treatment of partial seizures and certain general seizures associated with epilepsy. The widely used AEDs phenytoin and carbamazepine inhibit voltage-gated sodium channels and this reduction of sodium current is thought to be the main mechanism of their therapeutic efficacy. These drugs produce a characteristic voltage and frequency-dependent reduction in channel conductance, resulting in

a limitation of repetitive neuronal firing, with little effect on the unaffected neurons with normal single action potentials.[2, 12, 14, 15]

1.4 The Current Epilepsy Drug Pipeline.

A recent review[16] by Kaur, Kumar and Medhi entitled "Antiepileptic drugs in development pipeline: A recent update" repeated the well known statistics that epilepsy affects as many as 70 million people in the world. In the United States a total of more than 1.5 million people of which more than 300,000 are younger than fourteen and more than 500,000 are older than sixty five suffer from various forms of this disease. Rates of epilepsy are even higher in developing countries. For example, the prevalence of epilepsy is reported at 6–10 per 1000 people which equates to between six and ten million out of a population that now exceeds one billion. It is therefore not surprising that considerable efforts are being made to develop new AEDs. Unfortunately many of these new drugs are modifications of older versions which are known to have rather serious side effects. Despite availability of a large number of AEDs, one-third of patients still have intolerable and untreatable conditions.[16]

Based on their analysis these authors suggest that there is an urgent need to create new opportunities and improve the existing drugs to relieve patients especially those not responding to the currently approved drugs. Their review covered AEDs that were under development and in clinical trials during the years 2015 to 2016 for treating onset seizures, refractory, partial seizures, generalized tonic clonic seizures, and resistant partial onset seizures.[14]

Most of the available AEDs have not shown efficacy in treatment of patients with refractory epilepsy. It is unlikely that analogs and modification of currently used AEDs will address this shortcoming. However the possibility exists that members of completely new families might offer such hope. Also, most currently used AEDs have significant adverse effects nausea, ataxia, drowsiness and other more rare life-threatening effects. It is plausible that novel families of AEDs might be efficacious in controlling seizures with more therapeutic efficacy and less adverse effects.[16]

The review article[16] describes twenty-two compounds that are currently in clinical trials and at various stages of development. The mechanism of action of most of the compounds under investigation has been identified. Nine compounds on the list target ion channel inhibition, mainly sodium or calcium, which is the most commonly recognized target for AEDs. Several others affect the GABA system. At least three quarters of the compounds on the list contain one or more nitrogen containing heterocycles a number of which are analogs of currently used AEDs. Interestingly a number of these compounds have other known application. Included in this group are Verapramil (hypertension, migraine), Buspirone (anxiolytic), Thalidomide (hypertension, migraines, leprosy), Pregabalin (neuropathic pain, restless leg syndrome) and Ganaxalone (anxiolytic).[16]

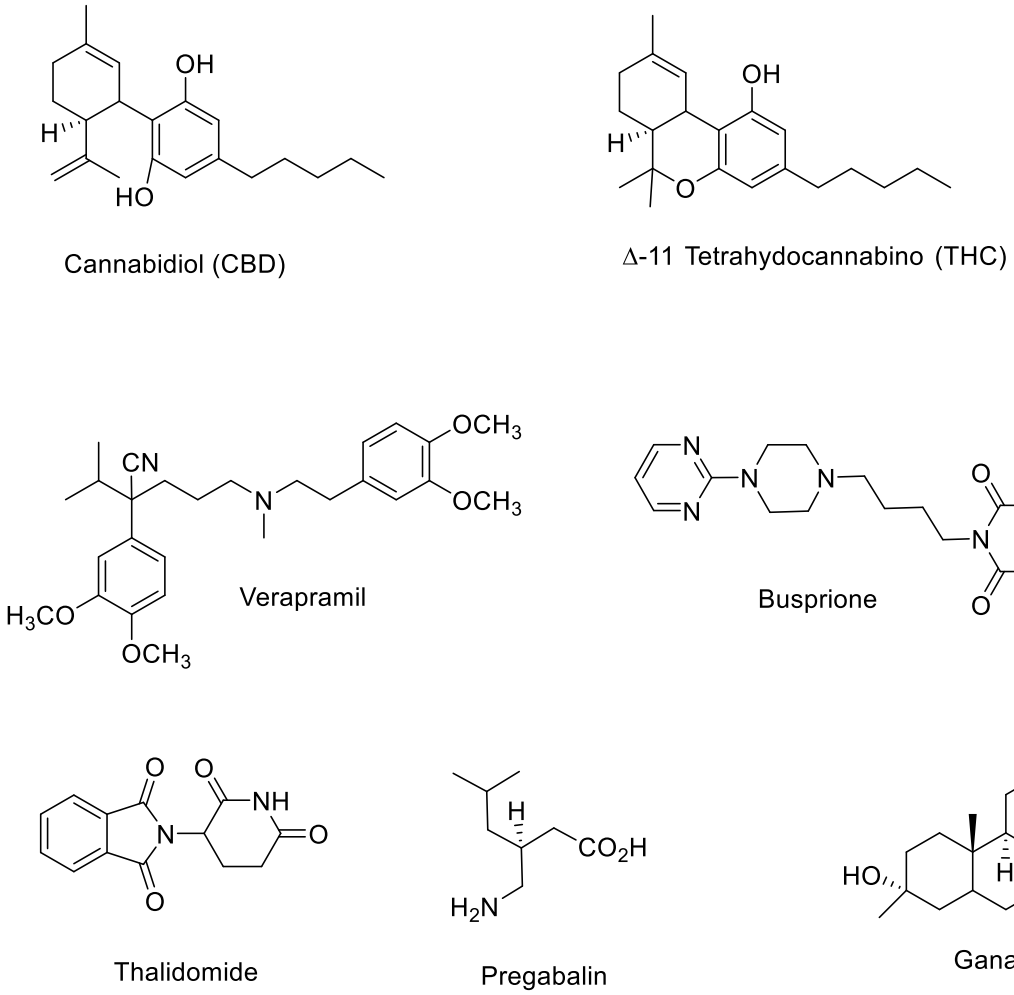
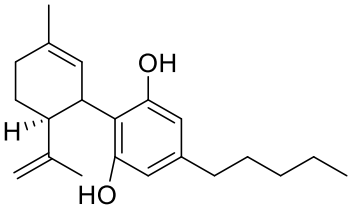


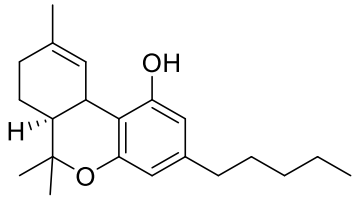
Figure 1.4.1 Structures of compounds currently in clinical trials for their potential as AEDs

Verapramil may be used for the prevention of migraines and cluster headaches. Buspirone is an anxiolytic agent and serotonin receptor agonist. Thalidomide, despite its terrible history for causing significant birth defect in the 1960s is currently used to treat leprosy. Pregabalin is a medication used to treat neuropathic pain, fibromyalgia, and generalized anxiety disorder. Its use for epilepsy is as an add-on therapy for partial seizures with or without secondary generalization in adults. Ganaxolone is an experimental drug which is under development for potential medical use as an anxiolytic and anticonvulsants.[16]

The U.S. Food and Drug Administration approved in late 2018 Epidiolex (cannabidiol) [CBD] oral solution for the treatment of seizures associated with two rare and severe forms of epilepsy, Lennox-Gastaut syndrome and Dravet syndrome, in patients two years of age and older. This is the first FDA-approved drug that contains a purified drug substance derived from marijuana.[17]



Cannabidiol (CBD)



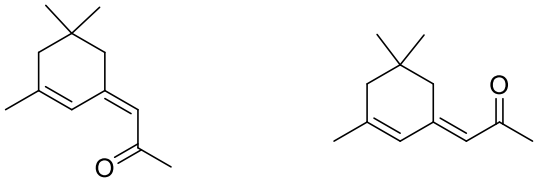
Δ-11 Tetrahydocannabino (THC)

It was recently reported by researchers at Toronto Sick Children's Hospital that a mixture of THC and CBD showed promise as a treatment for drug resistant epilepsy in children due to Dravet syndrome.[17] This was considered significant since despite advances in the treatment of epilepsy over the past twenty years no effective treatments have been available for children who suffer from this condition.[17]

1.5. Isoxylitones as potential AEDs

Rahman et al.[18] from the University of Karachi in Pakistan recently isolated and identified a potent anticonvulsant agent a medicinal plant *Delphinium denudatum Wall* growing in the Himalayan region of Pakistan. The crude ethanolic extracts from which alkaloids had been removed were used in epileptic animal model studies and shown to exhibit strong

anticonvulsant activity in vivo. This fraction was also used for in vitro studies and showed strong inhibition in epilepsy induced hippocampal neurons in cultured cells. It was further purified and an isomeric mixture of *E/Z* isoxylitones, **1**, was isolated and identified as the active compounds.[18, 19]

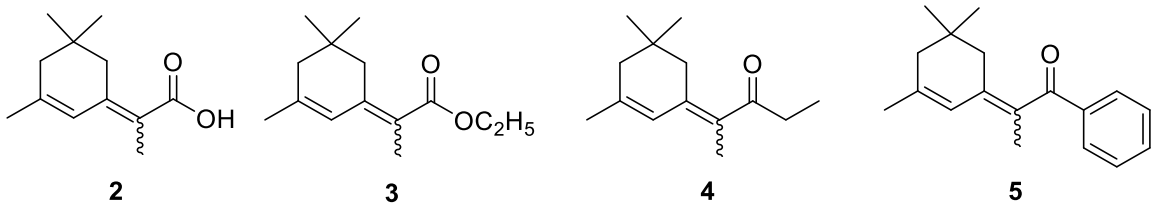


Z-isoxylitone **1a**

E-isoxylitone **1b**

Figure 1.5.1 Structures of *E/Z* isoxylitone isomers **1a** and **1b**

The first of the Raman group's two patents[18] described the isolation and in vitro and in vivo evaluation of the isoxylitones as a mixture of *E* and *Z* isomers. They also separated the two isomers and showed that they were easily interconverted by exposure to mild acid including stomach acidity. The second patent[19] reported a small group of analogs including a number of ketone, ester and acid analogs shown below. Their results showed that the natural product was more potent as determined by the bioassays described below than any of the derivatives that they had prepared; with only the acid, **2**, showing modest activity. It was clear from the results reported in the second patent[19] that replacing the methyl group in isoxylitone with larger groups such as ethyl, propyl and phenyl reduced potency; indeed the phenyl derivative, **5**, was essentially inactive. All compounds were bio-assayed as *E/Z* mixtures.



2 **3** **4** **5**

Figure 1.5.2 Isoxylitone analogs, **2**-**5** including the acid (**2**) reported by the Raman group

The bioassay tests that were performed on these compounds by the research group at the University of Karachi[18, 19] are the Maximal Electroshock Test (MEST), the subcutaneous pentylenetetrazol (scPTZ) test, and the electrical kindling model. These assays are commonly used models to evaluate potential new AEDs. The MEST and scPTZ tests are considered acute seizure models and are used to determine anticonvulsant activity of compounds whereas the electrical kindling model is considered a chronic seizure model and it used to identify more long-term antiepileptic activity. MEST consists of the application of an electric current via ear-clips or electrodes of fixed intensity and short duration (0.2s) to the animal resulting in tonic-clonic seizures. In this test, the AED is considered to have the ability to prevent the spread of seizures if the animal does not fully extend its hind limbs. In the scPTZ test, a convulsive dose of pentylenetetrazol is administered subcutaneously to induce a clonic seizure. The model is then observed for a post-injection period of 30 minutes. The MEST gives information about the potential activity against generalized tonic-clonic seizures whereas scPTZ tests identify the efficiency of compounds against myoclonic and absences seizures. The MEST and scPTZ tests are mainly used as preliminary screens for new AEDs because of the varying levels of predictability of efficacy compared to the more reliable electrical kindling tests.[18, 19]

The electrical kindling model is widely considered an excellent animal model for determination of antiepileptic efficacy of potential AEDs. This model can be used to study molecules that may interfere with the generation of epilepsy, which would normally progress to longer and more intense seizures. The kindling process consists of repeated induction of focal seizures by electrical discharge over a long period of time to produce a progressive and permanent increase of epileptic response. Once the rodent experiences an appropriate number of seizures of adequate intensity, the kindling process is considered completed and the animal is fully kindled.[20]

1.6 Objective

The Durst lab was approached by Professor Michael Poulter from the Department of Pharmacology at Western University in London, Ontario, to synthesize a library of analogs of isoxylitone. **1**. Dr. Poulter is a member of the Ontario Brain Institute; his group have been studying epilepsy for over 20 years. Poulter was intrigued by the level of activity of the natural product, its simplicity as a chemical structure and the novelty of these compounds as potential anti-epileptic compounds. All clinically used AEDs including the first used drug such as phentoin and the subsequently developed carbamazepine and imipramine and their analogs contain nitrogen heterocycles. These compounds are ineffective in treating almost 30% of epilepsy cases (refractory epilepsy). Additionally, significant unwanted side effects are associated with all of the classic anti-epilepsy drugs.

There was a distinct possibility that isoxylitone might become the lead structure of a new family of anti-epileptic compounds and that these would not have the unwanted side effects associated with typical AEDs. Dr. Poulter felt confident enough to start a small company which he named OB Pharma. The goal of this company was to not only investigate the potential of the isoxylitone molecule but also generate a library of analogs some of which would hopefully be more potent that isoxylitone itself and become potential drug candidates.

1.7 Previous work

A previous graduate student in the Durst lab, Amanda Saikaley, started working on this project during her M. Sc. prior to the publication of the second Raman group patent. The first part of her work consisted of synthesizing the isomeric mixture of the lead compound isoxylitone *E/Z* (**1a** and **1b)**. They chose not to follow the synthesis of the Pakistani researchers and developed the synthesis shown below in Fig 1.6.1. Reaction of ethyl acetoacetate **6**, first with sodium hydride and then nBuLi generated its highly reactive dianion **7** which was condensed with isophorone, **8**. The initial adduct **9** was dehydrated and then saponified to the beta-keto ester **10** which readily lost CO_2 to afford isoxylitone **1**. The success of this synthesis was confirmed by NMR analysis.[21]

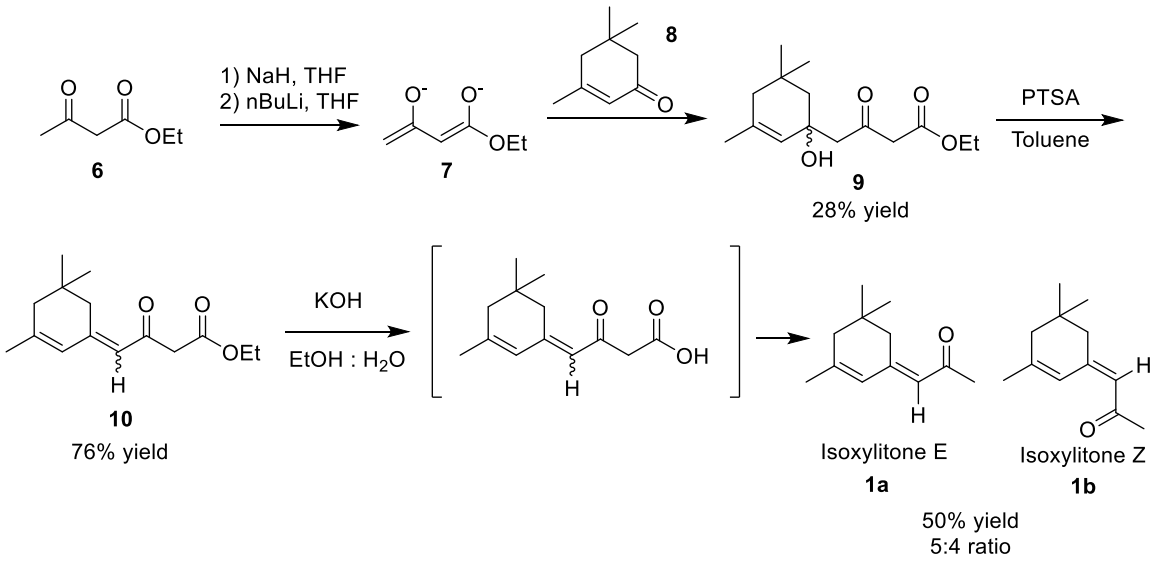


Figure 1.7.1 Synthesis of isoxylitone *E* and *Z*, **1a** and **1b**

Saikaley prepared more than thirty analogs in an effort to improve potency of the lead structure. A series of vinylic esters (**11-14**) closely resembling the structure of isoxilitone **1** were produced by condensing the anions derived from a number of acetates with isophorone **8**; these were also obtained as *E/Z* mixtures.[21]

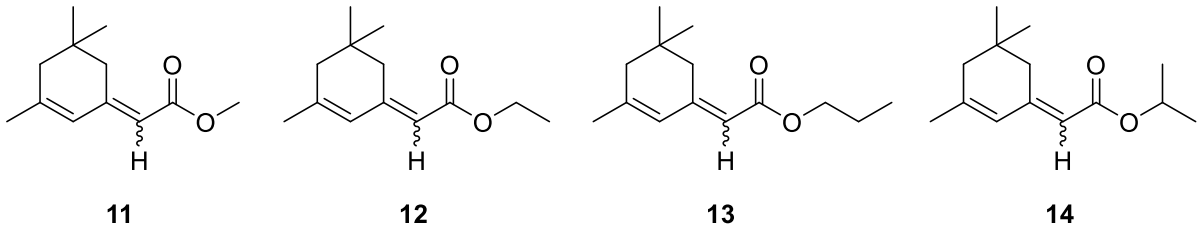


Figure 1.7.2 Series of ester analogs

The ethyl ester analogue **12** was proven to be equipotent to the parent isoxylitone based on the voltage sensitive dye imaging (VSDI) bioassay and on the kindling model assay carried out in the Poulter laboratories (see Section 2.1.1). It was possible to isolate a pure

sample of the *Z* acid (**2*Z***) and then esterify it to give pure *Z* ethyl ester (**12*Z***). The pure *Z* acid was shown to be less active than the mixture. While this was interesting, it was of relatively little importance since these compounds underwent ready isomerization between the *E* and the *Z* isomers even under mildly acidic conditions comparable to stomach acidity.

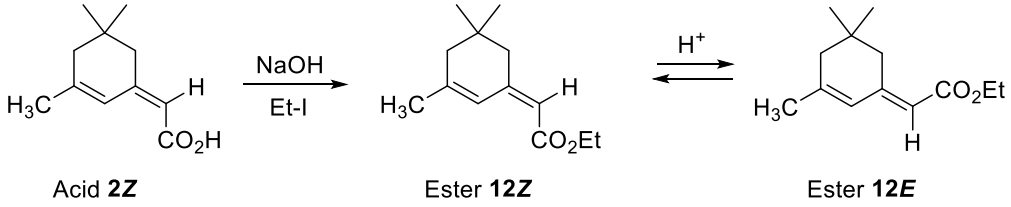


Figure 1.7.3 Synthesis of the ethyl ester analogs of isoxylitone.

It was also noted that the activity of isoxiltone type compounds was highly sensitive to steric effects of the substituents attached to the carbonyl carbon. Replacement of the methyl ketone by ethyl (**15**), propyl and phenyl (**16**) ketones successively reduced the desired biological activity with the latter compound being essentially inactive. This observation verified the conclusions arrived at by the Raman group in their second patent.[19] A similar observation was made in going from the methyl (**17**) to the phenyl (**18**) sulfoxide.

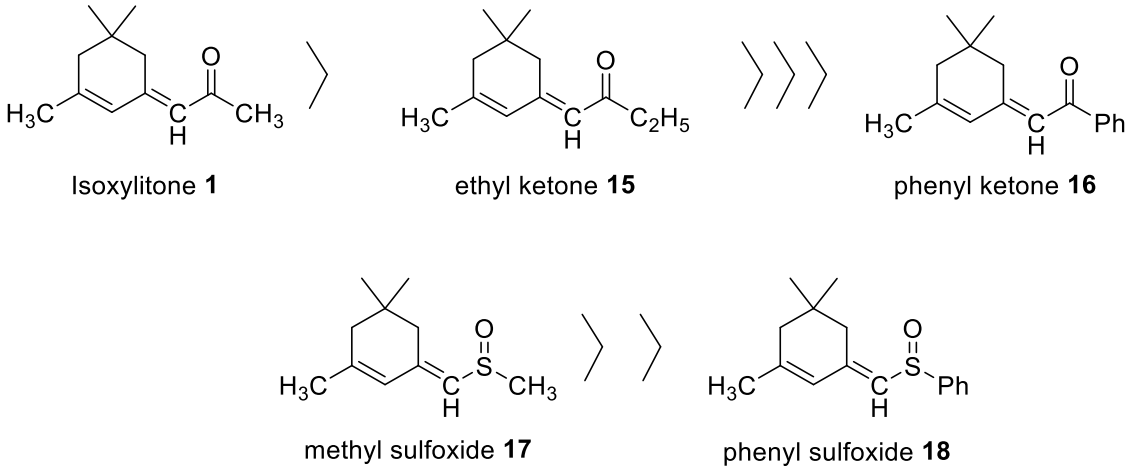


Figure 1.7.4 Biological activity depending on steric effects

The nitrile **19**, prepared by reaction of isophorone **8** with α-lithiopropionitrile **20** turned out to be the most potent compound reported in the Saikaley thesis. It too existed as a mixture of *E* and *Z* isomers. Indeed, initial dehydration of the adduct obtained from the condensation of α-lithiopropionitrile and isophorone yielded a mixture of five dienes including **19*E*** and **19*Z***. It was later discovered that heating of this mixture with *p*-toluene sulfonic acid in toluene resulted in the formation of essentially only *E* and *Z* **19** in an almost 1:1 ratio. This mixture was designated by OB Pharma as TD532.

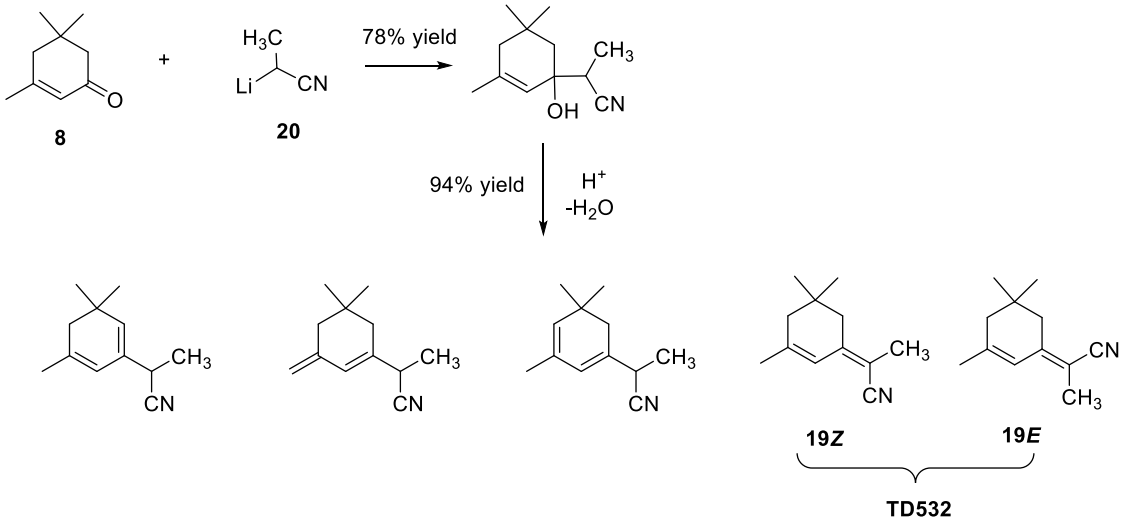


Figure 1.7.5. Synthesis of **19** (TD532)

Compound **19**, as a mixture of stereoisomers, showed remarkable biological activity. It was significantly superior to the lead structure isoxylitone. Saikaley produced a number of nitrile analogs (**21**-**24**) by condensing different α-lithionitriles with isophorone and also with other cyclohexenones to produce a set of compounds. A number of these are shown below.

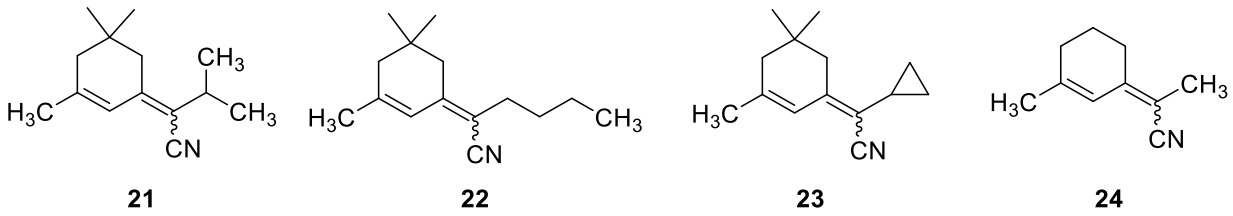
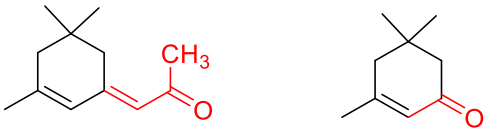


Figure 1.6.6. Analogs of **19** (TD532)

Almost all of the compounds produced showed some biological active, but none were superior to **19** (TD532). Saikaley concluded that replacement of the methyl group in **19** by larger substituents, even by the relatively small changes to cyclopropyl **23**, and isopropyl **21,**

resulted in the loss of potency. Surprisingly replacement of the α-methyl group in **19** by hydrogen also gave a less active compound. Removal of the 5,5-dimethyl group on the cyclohexenone ring also caused significant loss of the desired activity. The excellent activity of **19** both in the *in vitro* neuron firing and *in vivo* kindling assays carried out by the Poulter group were deemed sufficient to warrant the filing of a patent in which **19** became centerpiece of a new family of compounds in the AED field.[21]

The compounds described in the Saikaley thesis[21] and also in the Rahman patents[18, 19] indicated quite strongly that steric effects associated with the substituent on either the enone group in isoxylitone and the related sulfoxide or α to the nitrile group in **19** resulted in reduction of the desired activity. It was decided by our group to send isophorone **8** and compare its ability to reduce the firing of neurons in the bioassay as carried out by the Poulter group. The underlying question was: Could the $CH{=}CH\text{-}C(O)CH_3$ group in isoxylitone **1** be replaced by a simple carbonyl group as found in isophorone **8**? The observation that isophorone **8** reduced neuron firing became the basis of the investigations reported in Chapter 2 of this thesis.



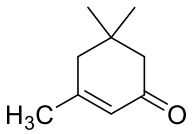
Isoxylitone **1** Isophorone **8**

Figure 1.7.6 Comparison of the structures of isoxylitone **1** and isophorone **8** and their functional group at C3

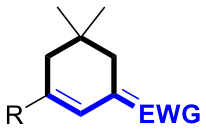
2 Discussion and Results

2.1 Introduction

The observation that isophorone **8**, produced a 20% reduction of neuronal firing at 1µM concentration stimulated by either 20 or 60 Hz voltage pulses examined by voltage sensitive dye imaging (VSDI) on rat brain slices *ex vivo* (methods in section 2.1.1) in a manner comparable to many of the compounds reported in the Saikaley M. Sc. thesis allowed us to propose a cyclohexene ring in which the alkene is conjugated to an electron withdrawing group (EWG) as the key pharmacophore.



Isophorone **8**



Pharmacophore for anti-epilepsy activity **25**

Figure 2.1.1 Structures of isophorone **8** and the key pharmacophore **25**.

The key active compounds known in this series at the beginning of this study were the natural product lead structure isoxylitone **1**, where R = CH_3 and the EWG = CH-C(O)CH_3 , the sulfoxide **17** (R = CH_3, EWG = CH-SOCH_3 and **19** (TD532), the most active compound reported in the Amanda Saikaley series where the EWG is =C(CH_3)CN. These compounds all contain the above suggested pharmacophore **25**.

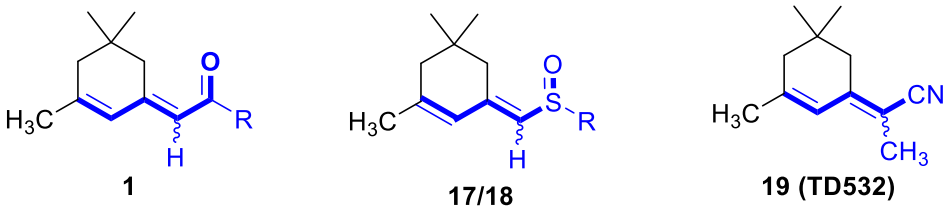


Figure 2.1.2 Structures of isoxylitone **1** (R=CH_3), sulfoxide analogs **17** (R=CH_3) **18** (R=Ph), and the active nitrile analogs **19** (TD532).

The question was: What structural changes could be made to isophorone **8** in order to enhance its potential for anti-epileptic activity. A number of possibilities are indicated on the structure below. It was clear from the work of Saikaley[21] and that of the Rahman et al.[18] that increasing the size of the EWG group reduced the desired activity of these compounds. For example, replacement of the methyl group joined to the carbonyl group in **1** or the methylsulfinyl group in **17** by larger substituents such as phenyl resulted in almost complete loss of potency.[21]

Table 2.1-1 Summary of reduction of neuron firing at 60Hz using in vitro bioassays on brain cell slices as part of Saikaley's work on analogs **17, 18** and **19**

Compound	Average reduction at 200 nM (%)	Average reduction at 1 µM (%)
17 (R= Methyl)	-50	Not tested
18 (R = Phenyl)	Inactive	Inactive
19 (TD532)	-50	Not tested

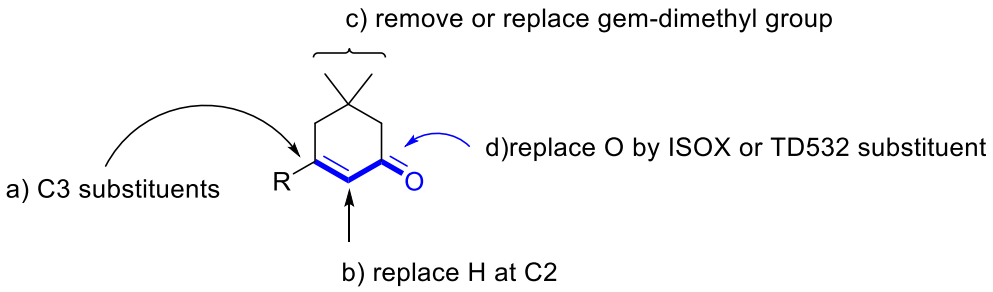


Figure 2.1.3 Proposed changes to the basic enone structure.

The most obvious and synthetically easiest changes that one could imagine would be to replace the R group (CH_3 in isophorone) by H or by groups that are sterically larger than methyl and have a variety of electronic characteristics. Most of the efforts described in this thesis were directed towards this goal. Several compounds were also prepared in which the changes in the substituent at C3 were also accompanied by changes at the other positions. The possibility of changing the ring size from cyclohexenone to cyclopentenone or cyclohexenone was not investigated.

2.1.1 Bioassays performed

The synthesized compounds were sent to OB Pharma and bioassays were performed to identify anti-epileptic drug potential.

To test for activity, a first assay was done using voltage sensitive dye imaging (VSDI) on isolated rat brain slices that were kept viable in artificial cerebral spinal fluid (ACSF). The neural assemblies were activated by electrical stimulation. A voltage sensitive dye, such as, di-4 ANEPPS, was incubated with a brain slice for 1 h in a suitable solution that enhances the dye penetration into the tissues. The dye reacted to the changes in voltage across the cell

membrane of the neurons in the brain slices. The synthesized compounds were added to the ACSF at known concentrations between 200 nM and 1 µM. The brain slices were then subjected to an electrical stimulus that activated the neurons in the slice. As the dye reacted to the change in voltage, the intensity of the dye can be observed and quantified. The degree of capability of the compound to dampen the activation of the brain was evaluated in this manner. A negative dye intensity value in relation to the control experiment can be interpreted as a reduction of neuronal activity related to voltage in the cells. This reduction can translate into potential anti-epileptic activity. This reduction is desired at the 60 Hz, which is the frequency at which neurons operate in the central nervous system. However, the inhibition of activity at 20Hz could cause unwanted side effects since essential neurons regulation base life functions such as in the heart operate at this frequency. Thus, compounds showing good inhibition of activity at 60 Hz but very low to no inhibition at 20 Hz were considered great potential candidates. Compounds showing this type of activity were investigated and further analogs were synthesized as future candidate compounds.

In a second assay, the inhibition of the compounds on the activity of voltage gated sodium channels was determined using patch clamp electrophysiology. A negative value in relation to the control can be interpreted as representing a statistically significant decrease of neuronal activity. This can translate to anti-epileptic activity caused using the compound being assayed. Compounds showing this type of activity were investigated and further analogs were synthesized as future candidate compounds. This analysis was done on cultured cortical neurons isolated from rats. A more in-depth description of the methods used by the Poulter group to identify compounds of interest is given below.

Slice Preparation and Staining

All animals used in these studies were adult male Sprague-Dawley rats aged 20–45 days. The preparation of brain slices and kindling methodology have been described in detail elsewhere.[22] Slices prepared from kindled rats were usually about 45 days old. Control rats for these experiments were age matched but no electrode was implanted. Brain slices were incubated for 30 min in a solution that contained 0.6 M Mofdyedi-4- ANEPPS (D-1199, Invitrogen Molecular Probes Inc., OR, USA). After washing for 10 min with ACSF slices were transferred to the recording chamber. During all recordings the slices were maintained at 32 ˚C and continuously perfused with ACSF bubbled with a mixture of 95:5 oxygen and carbon dioxide. The slices were stimulated with a platinum/iridium electrode (Micro Probes, Inc., MD, USA) with a tip diameter of 200–300 µm at the border of the lateral olfactory tract (LOT) and layer I of the PCtx. The stimulation of each slice was in the range of 160–200 µA, each square pulse was 2.0 ms in length. The electrode was connected to a stimulator (S88X dual output square pulse stimulator, Grass Technologies, AnAstro-Med, Inc., QC, Canada),which controlled the pulse frequency and train duration.

Patch Clamp Recording

The whole cell patch clamp recording technique used and the preparation of brain slices from adult rats have been both described in detail.[23,24] The internal solution used in these experiments was; K gluconate, 140 mM; $MgCl_2$, 2 mM; $CaCl_2$, 1 mM; MgATP, 2 mM; NaGTP, 0.2

mM; EGTA, 1.1 mM and HEPES, 10 mM. A multiclamp 700B amplifier was used to record from neurons located in layers II and III.

Optical Recording

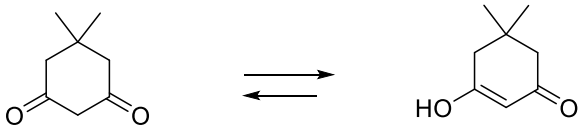
The composition of ACSF used for optical recordings was the same composition used in the patch clamp recordings. Each recording was about 20 s in length and consisted of two époques. The first was a 2 s recording of background activity before the stimulus followed by the stimulus application for 1 s with frequencies differing from 5 to 100 Hz. The acquisition rate was between 3 and 10 ms/frame. For each recording minimum camera saturation was set around 50% while the maximum was about 80%. Optical recording was conducted using a CMOS camera (Micam Ultima, BrainVision, Inc., Tokyo, Japan) mounted on top of an upright microscope (Fixed Stage Upright Microscope BX51WI, Olympus). The light from a 100 Whalogen lamp source (HLX 64625, Microlites Scientific, Corp.) passed through an excitation filter ($\lambda = 530 \pm 10$ nm). The fluorescent signals were collected and projected onto the CMOS sensor through a long pass emission filter ($\lambda > 590$ nm). A long-distance objective was used in these experiments (XLFluor4XN. A.0.28, Olympus). The movies were recorded and analyzed using Brain Vision Analyzer (Tokyo, Japan) software. The acquisition settings were: 100 × 100 pixels frame size, after magnification each represented 25 µm × 25 µm space on the brain slice. The dye signal intensity decreases as the membrane depolarizes. However, to better match conventional recordings the signals all have been converted so that the excitatory and inhibitory signals were shown as positive and negative values. As bleaching can strongly affect

the data, all recordings were corrected by subtracting the change in fluorescence that occurred in a region of the slice that was unresponsive to the stimulus. The fractional change in fluorescence signal relative to background signal (ΔF/F) was calculated for each frame of each recording. For all the recordings, we binned 3 × 3 pixels into one representative signal. As there was considerable variability in the magnitude of the responses from slice to slice due to differences in loading of the dye, we normalized the recordings by dividing all signals by the response to the 20 Hz stimuli. This permitted us to average the normalized responses between recordings. Thus, the input/output relationships shown are the normalized ΔF/F. The lag time was calculated by measuring the time between the stimulus on set and the time for the signal to be 20% above baseline. Instead of using pixels bins, we measured the ΔF/F along a "stripe" that could be precisely placed along a group of pixels before and after the cut. Each stripe consisted of 10 pixels and covered 250 μm length. The data derived from each stripe was the average ΔF/F of 10 pixels.

Bioassay were reported to the Durst group within a month or two of submitting the compounds. The data obtained allowed us to decide with relative confidence which analogs and target modifications to the pharmacophore structure **25** should be synthesized and performed. This process that lead to the synthesis and discovery of TD561 (compound **40**) as a potent potential anti-epileptic drug. A patent featuring this compound and analogs was submitted and published, showing the confidence of OB Pharma in the potential of this series of compounds.

2.2 Substituents at C3

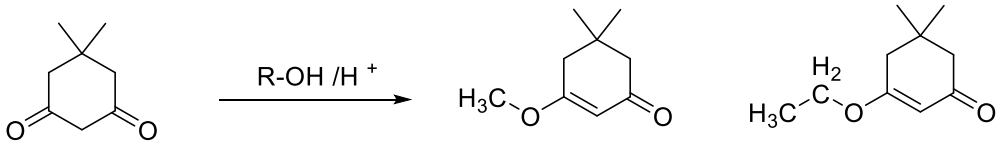
The simplest strategy for introducing different substituents at C3 utilizes the inexpensive and readily available dimedone **26**, as starting material. Dimedone has a 1,3-diketone structure and as such exists as a mixture of tautomers, the diketone **26** and the enol form **27**.



26 **27**

Figure 2.2.1 Structures of tautomers of dimedone

Reaction of dimedone with methanol or ethanol in the presence of a strong acid yields the enol ethers **28** and **29**, respectively.[25] The structures of these products are readily apparent from their ^{1}H NMRs which show the incorporation of a methoxy (OCH_3 at 3.66 ppm) or ethoxy (OCH_2 at 3.88 ppm) unit and the presence of a vinyl hydrogen.



dimedone **26** **28** **29**

Figure 2.2.2 Structure of enol ethers obtained from dimedone.

Reaction of above ethers with strong nucleophiles is known to cause replacement of the ether group by the nucleophile and result in the introduction of a new substituent at C3. This reaction sequence can be rationalized in one of two ways: a) Addition of the nucleophile at C3

followed by elimination of the alkoxy group, or b) addition of the nucleophile at the carbonyl carbon followed by regeneration of the carbonyl group at C3 during hydrolytic workup. The result of these two processes is the same in the case starting with dimedone due to its symmetrical nature.

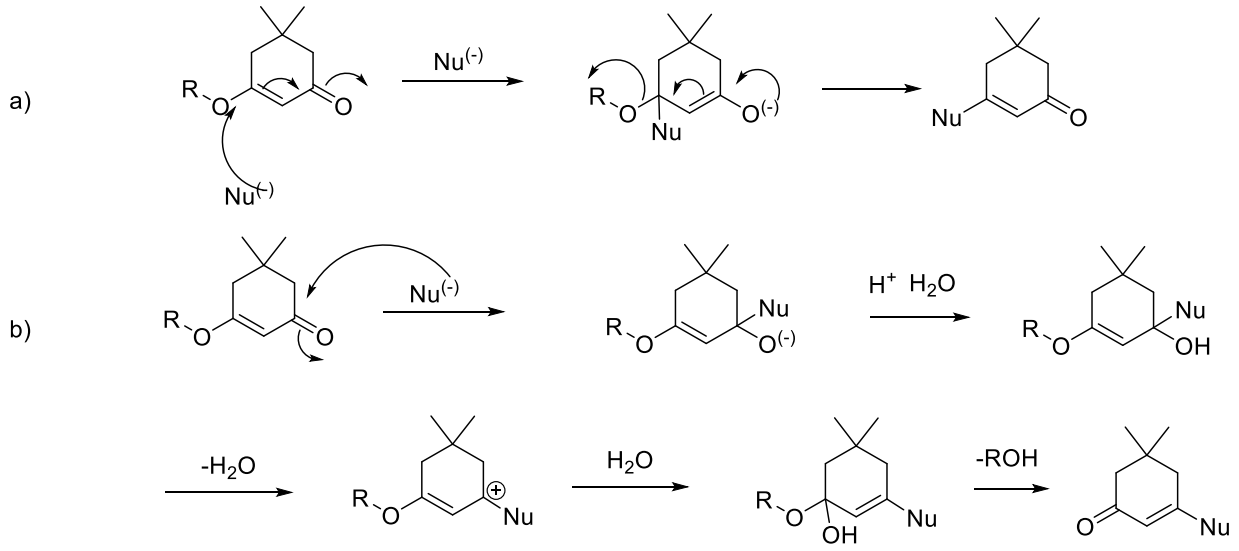


Figure 2.2.3 Mechanisms of introduction of substituents at C3 via nucleophilic attack.

The first nucleophiles chosen for these reactions were *n*-BuLi and PhMgBr. These reactions resulted in the formation of the products **30** and **31**, respectively.

Reaction of a THF solution of **29** with *n*-BuLi afforded 3-n-butyl-5,5-dimethyl cyclohex-2-enone, **30** in 32 % yield after purification by column chromatography. The ^{1}H and ^{13}C NMR spectra of the purified product showed the introduction of an *n*-Bu group with ^{1}H NMR peaks at 0.89, 1.32, 1.45 ppm and the retention of the enone hydrogen which absorbs at δ= 5.85 ppm.

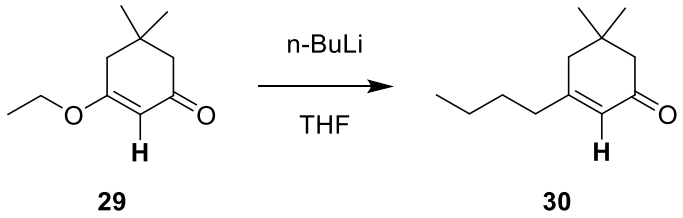


Figure 2.2.4 Synthesis of **30.**

Addition of **29** to an ether solution of phenylmagnesium bromide in diethyl ether afforded the 3-phenyl-cyclohexenone **31** in 40% yield after purification. The ^{1}H NMR spectrum of **31** showed the expected five aromatic hydrogens in addition to the other peaks due to the hydrogens on the cyclohexenone ring.

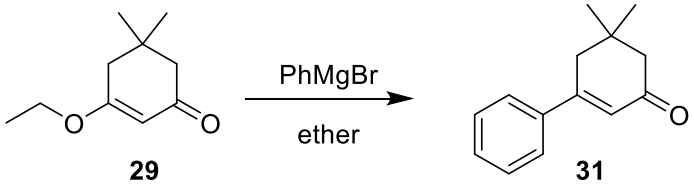


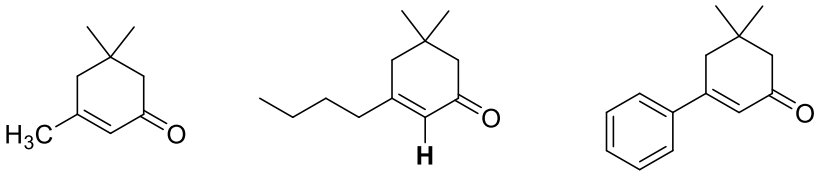
Figure 2.2.5 Synthesis of **31**.

The bioassay results for these two compounds and for isophorone **8**, are shown in Table 2.2.1. A reduction of neuronal activity of -39% and -36% was observed for compounds **30** and **31**, respectively, compared to a reduction of -22% for isophorone **8** when the VSDI determination was carried out at 200 nM. This indicated that the introduction of groups larger than methyl at C3 in the cylcohexenone ring results in more potent compounds. The difference in reducing uncontrolled neuronal activity by brain cells is similar for these compounds at the higher 1μM concentration, especially considering the uncertainty reported in standard error of

the mean in the measurements. These data indicated that it would be worthwhile to produce additional compounds with both different aryl and alkyl groups at this position.

Table 2.2-1 Summary of reduction of neuron firing at 60Hz using in vitro bioassays on brain cell slices of isoophorone **8**, and analogs containing larger carbon containing groups at C3

Compound	Average reduction at 200 nM (%)	SEM at 200nM (%)	Average reduction at 1 µM (%)	SEM at 1 µM (%)
8	-22	11	-46	9
30	-40	7	-51	7
31	-36	8	-46	8



Isophorone **8** **30** **31**

2.3 Analogs containing substituents at C3 and complex EWG at C1

Since our group had shown in the past that the compounds such as **19** (TD532) and to a lesser extent the related esters and acids **12** and **2** also had promising activity it was decided to generate a series of compounds **32-36** that incorporated both features, that is the nitrile or ester or acid function in **19**, **12** and **2** combined with sterically more demanding substituents at C3.

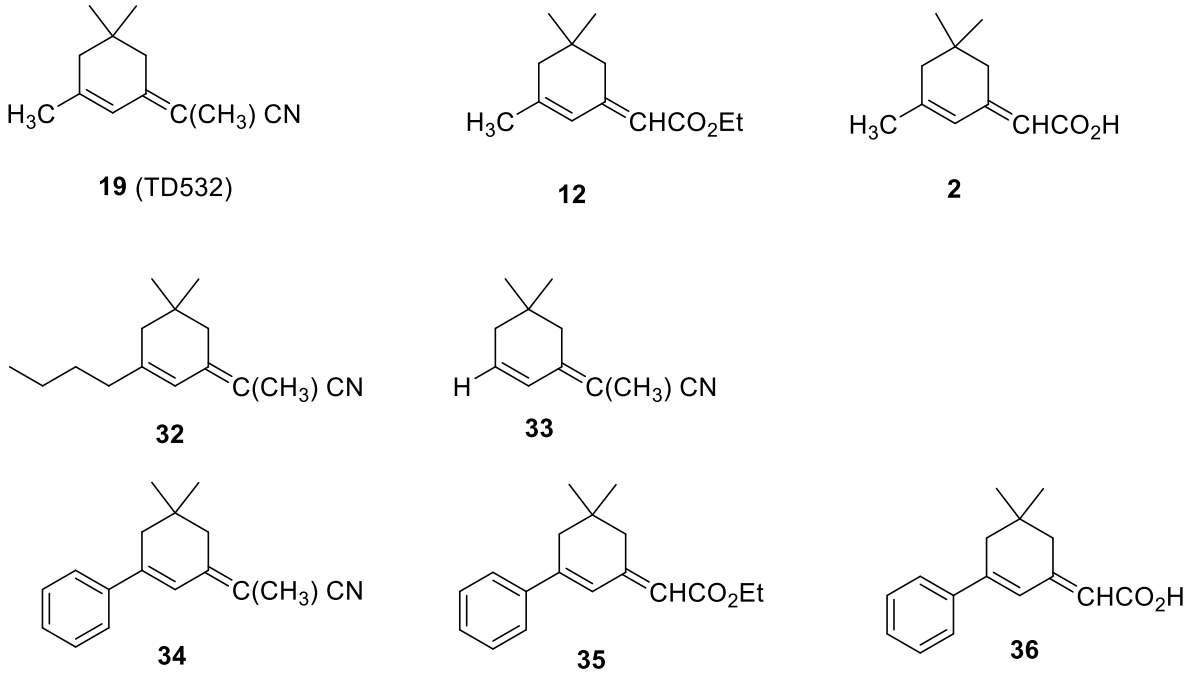


Figure 2.3.1 Structure of **19** (TD532) and related compounds.

The synthesis of these compounds commenced with the precursor cyclohexenones as shown below. Thus, reaction of the enone **30** with the lithium salt of propionitrile **20** yielded the expected addition product **37**. Dehydration of **37** gave **32** as a 1: 1 mixture of stereoisomers as determined by integrating the signal for C2 alkene hydrogens which occurred at δ= 6.17 and

6.44 ppm for the *E* and *Z* isomers, respectively. The overall yield for this two-step conversion was 38%. There was no evidence of isomers with both double bonds in the ring. No attempts were made to separate the isomers either here or for the related mixtures **33-34** below, since Saikaley had shown that they interconvert readily on exposure to mild acid.[21]

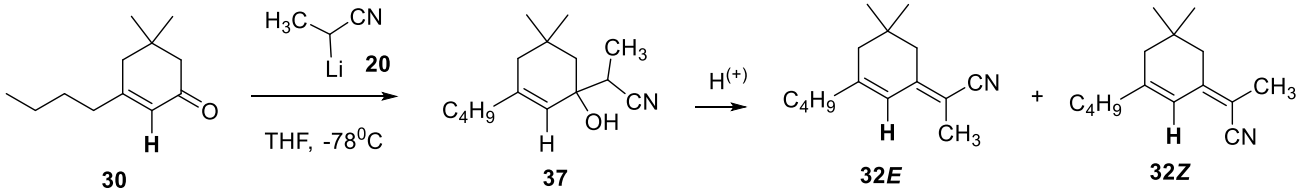


Figure 2.3.2 Synthesis of **32*E*** and **32*Z***.

A similar sequence of reactions was used to prepare the mixture of isomers of compound **34** from the ketone **31;** the *E/Z* ratio was determined to be 1:1.

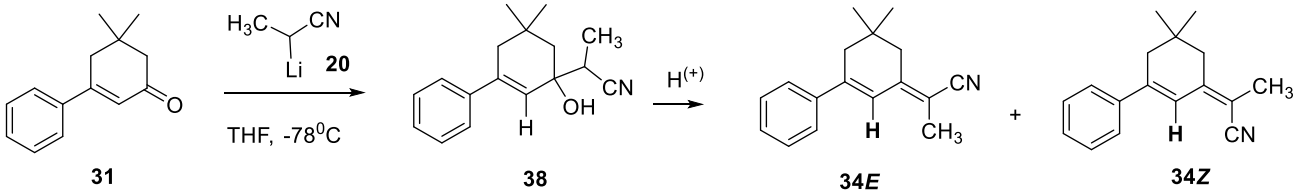


Figure 2.3.3 Synthesis of **34*E*** and **34*Z***.

The preparation of **33** required initial synthesis of the ketone **39**. This compound was prepared from dimedone **26** in two steps following the procedure by Zegarski[26]. Ethoxy dimedone, **29** was reduced to the allylic alcohol which upon treatment with aqueous acid afforded **39**[26]. Reaction of **39** with α-lithiopropionitrile **20**, followed by dehydration of the

intermediate product as described for the synthesis of **32** gave the desired compound **33** as a mixture of *E* and *Z* isomers in a ratio of 2:1.

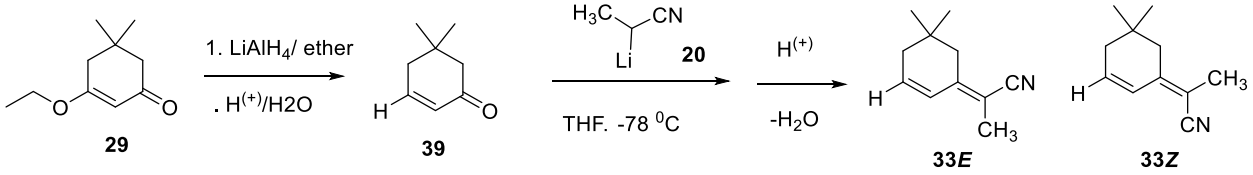


Figure 2.3.4 Synthesis of **33*E*** and **33*Z***

Finally, the unsaturated ester **35** was obtained from the enone **31** by a condensation with the lithium derivative of ethyl acetate and subsequent dehydration. The mixture of ester stereoisomers was hydrolyzed under basic conditions to give the acid **36**. The structures of these compounds were readily verified based on their ^{1}H NMR spectra. As above, the ratio of isomers was easily determined by integrating the signal for the remaining alkene hydrogen at C2.

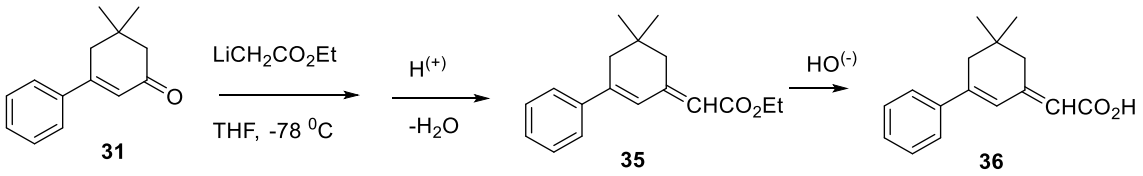


Figure 2.3.5 Synthesis of **35** and **36.**

At this stage we were able to compare the bioassay results for different series in order to decide whether the simple enones or the compounds with the more complex EWG at C1 were more effective. The relevant compound series together with the assay results indicating reduction of rat brain excitatory neuronal circuit activity stimulated at 60 Hz are shown below.

Table 2.3-1 Summary of comparisons of reduction of neuronal activity at 60Hz of three series of compounds to identify the effect of different sizes and complexities of EWG at C1

Compound	Average reduction at 200 nM (%)	SEM at 200nM (%)	Average reduction at 1 µM (%)	SEM at 1 µM (%)
30	-40	7	-51	7
32	-28	8	-51	6
31	-36	8	-46	8
34	33	7	-42	4
35	-40	5	-49	3
8	-22	11	-46	9
19	-33	7	-42	4
33	-35	8	-55	5

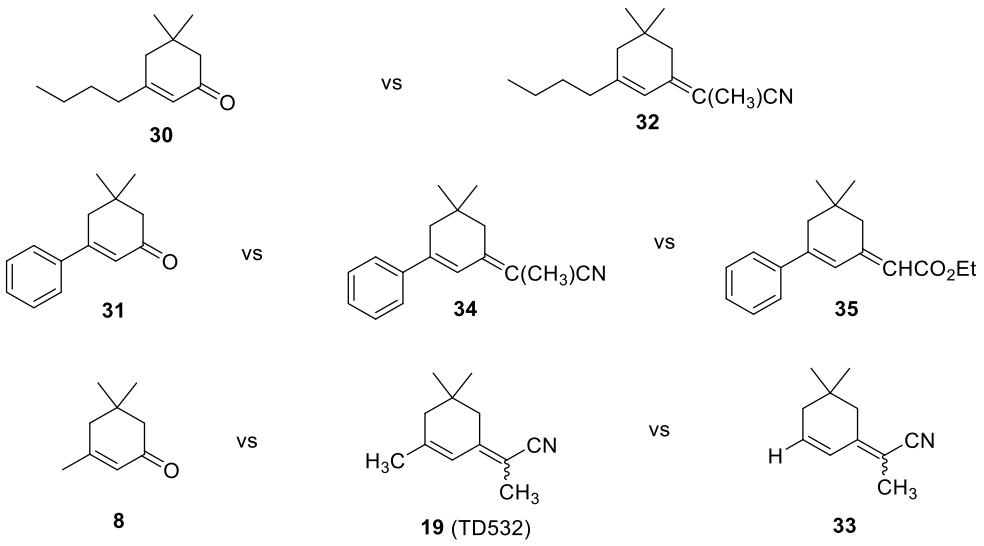
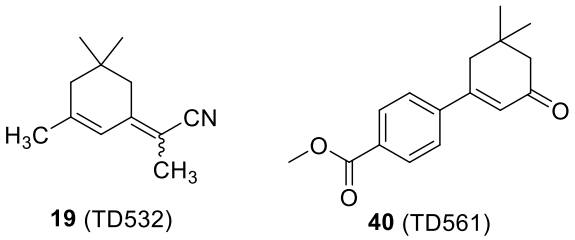


Figure 2.3.6. Compounds being compared using bioassay results separated in three series

Examination of the three sets of results does not give a clear indication that the additional effort to convert the enone structures to the extended conjugated nitriles and esters provides a significant advantage because of the lack of observed improvement in reduction of neuronal

activity. The reported measurement data in the VSDI bioassay do not allow us to make definitive conclusions regarding structure activity relationships. The data suggests that the size and nature of the substituent at either C1 and C3 within the variations studied do not affect the ability to reduce neuronal firing.

A disadvantage of the nitriles and esters is that the compounds are mixtures of isomers. It was shown by Amanda Saikaley[21] that these compounds isomerise easily under mildly acidic conditions between the *E* and the *Z* isomers. Also, compound **19** (TD532) and presumably also the other nitriles such as **32**, **33** and **34,** have much more limited thermal stability than ketones such as **31**. This was shown in a comparison of data following the World Health Organization Accelerated Stability Protocol[27] that was performed as part of this work. The ^{1}H NMR of **19** started showing the presence of many additional peaks upon storage for a period equivalent to two months at room temperature whereas that of the ester **40** whose synthesis is described below (section 2.4), remained unchanged for a period equivalent to more than six months.



19 (TD532) **40** (TD561)

Figure 2.3.7 Structures of compounds **19** and **40**, which were subjected to accelerated stability studies

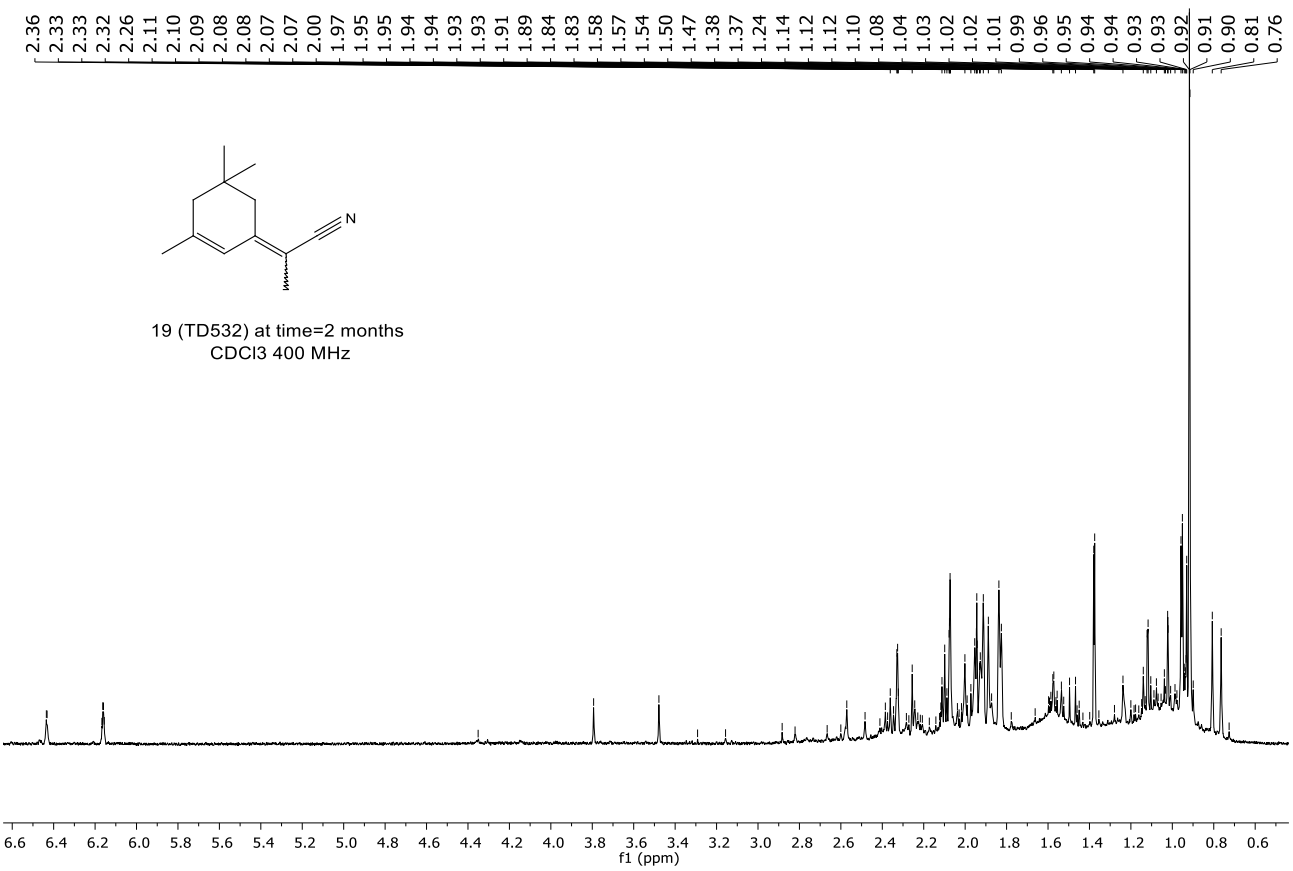


Figure 2.3.8 ^{1}H NMR spectrum of compound **19** showing degradation after and equivalent of two months of storage at room temperature

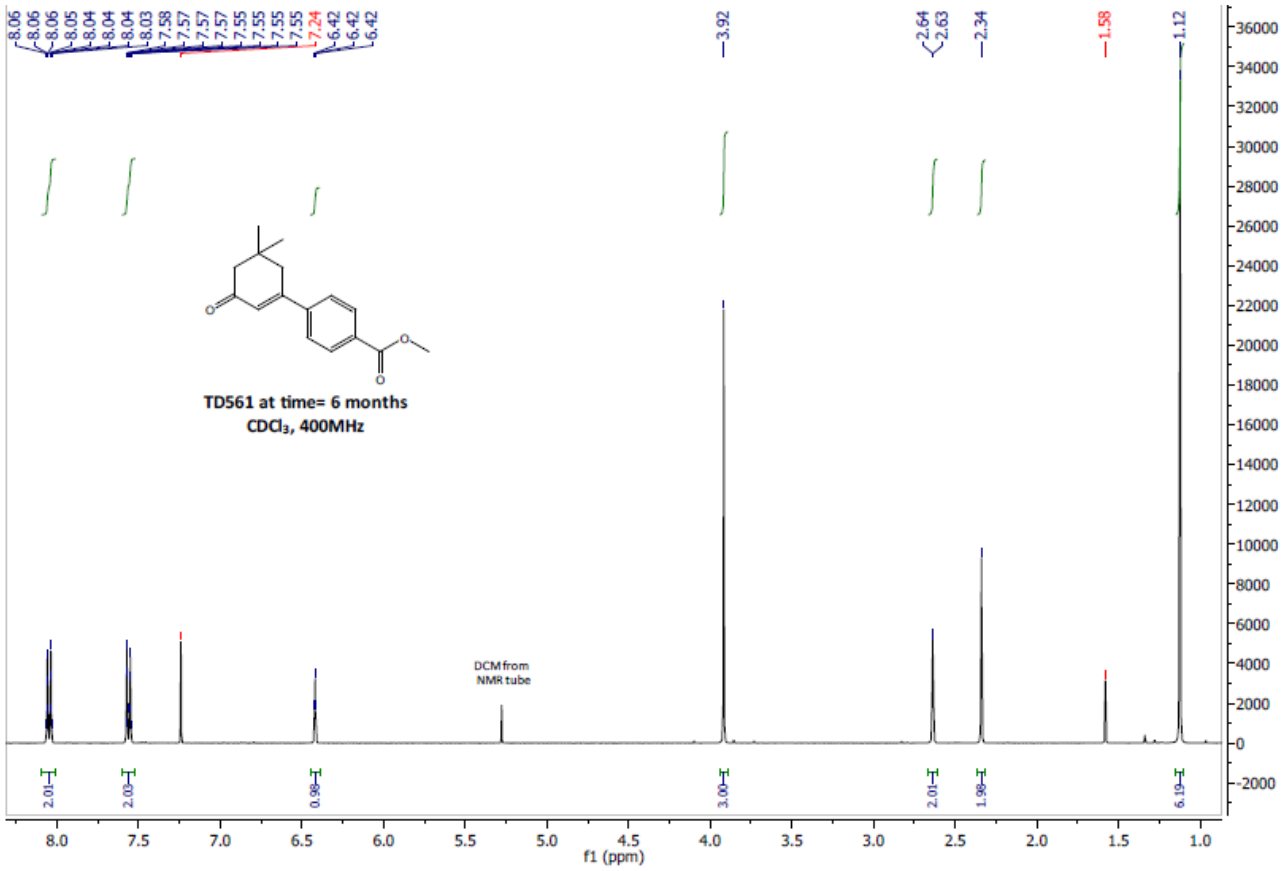


Figure 2.3.9 ^{1}H NMR spectrum of compound **40** showing stability after and equivalent of 6 months of storage at room temperature

Since there does not seem to be a clear advantage to the compounds containing nitriles, esters and acids at position C1, and that they also require additional steps in synthesis from the parent ketones, these types of compounds were not further synthesized and analysed. Furthermore, the poor stability of nitrile containing compounds could prove to be problematic during long-term storage. Therefore, it was decided to place focus and effort on the cyclohexenones, in particular those carrying aromatic residues at C3, as described in section 2.4 below.

2.4 Synthesis of 3-(4-carboxymethylphenyl)-5.5-dimethylcyclohex-2-en-1-one, 40(TD561)

The change in focus towards compounds containing aromatic residues at C3 resulted in the synthesis of compound **40**, also known as TD561, and related analogs.

The synthetic route described for the synthesis of the 3-phenyl derivative **31** is not applicable to the preparation of **40** since the ester substituent at the para position of the phenyl ring is not compatible with either the required lithio or Grignard intermediates. A search of the literature[28] indicated that **40** and similar substituted aromatic compounds should be accessible by coupling reaction between the boronic acid **41** and the enol tosylate **42**. This coupling is mediated by 3% tetrakistriphenylphosphine palladium (0).

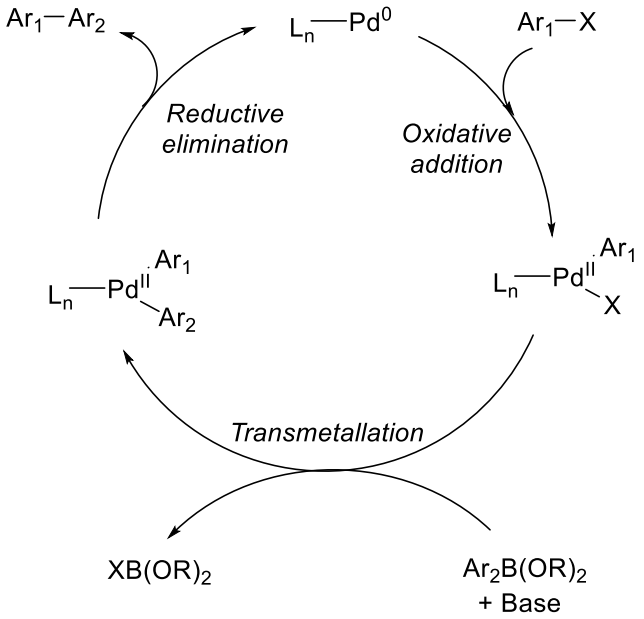


Figure 2.4.1 . General catalytic cycle for Suzuki-Miyaura couplings[28]

As seen below, the synthesis of **40** can be carried out conveniently in one pot following the literature protocol.[28] The dimedone tosylate **42** can also be prepared separately.

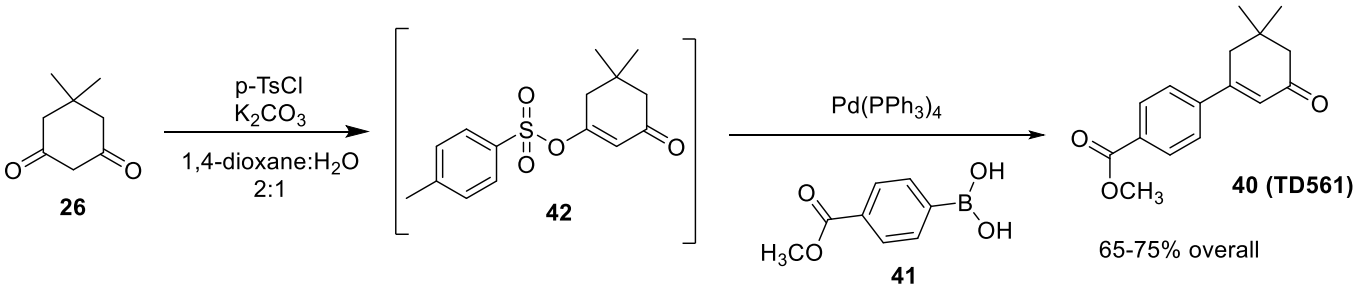


Figure 2.4.2. One pot, two-step, synthesis of **40** (TD561).

The major advantage to this procedure is that it is a simple two reaction – one-pot procedure requiring less than one day to set up and complete. The isolation of pure **40** was not trivial requiring often a minimum of two consecutive column chromatography separations.

The following procedure is representative. A mixture of dimedone (1.2 equiv) tosyl chloride (1.5 equiv) and potassium carbonate (3 equiv) are stirred at room temperature in a 2:1 mixture of dioxane and water for one hour; a longer time is not necessary but also not harmful. At that point 1 equivalent of the aryl boronic acid is added followed by 0.03 equiv of $Pd(PPh_3)_4$ and the mixture is refluxed for 90 min.

The structure assignment of **40** was consistent with the ^{1}H and ^{13}C NMR spectra. Assignments of individual H and C resonances, shown on the following pages.

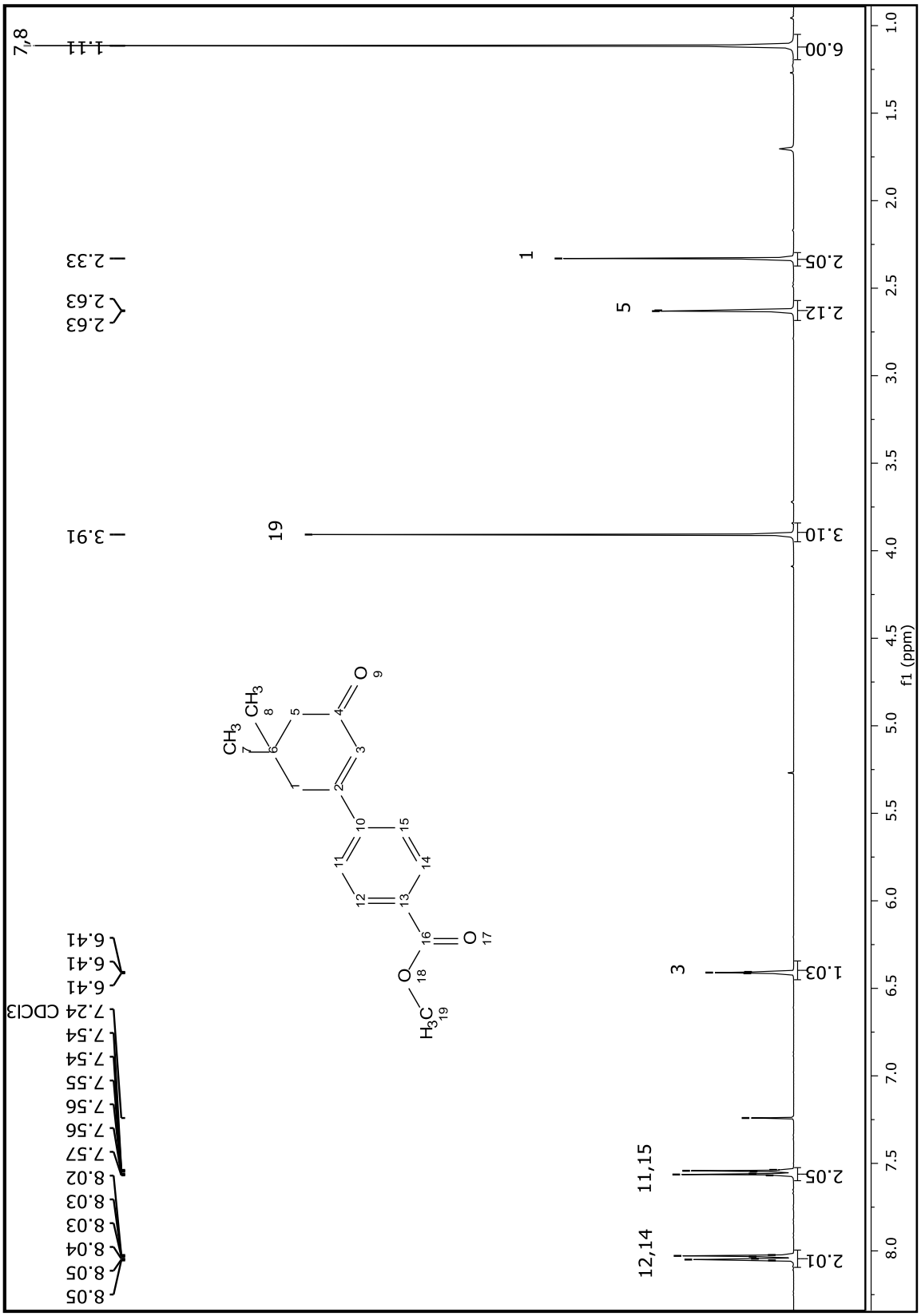


Figure 2.4.3 ^{1}H NMR spectrum of **40** (TD561) with assignments

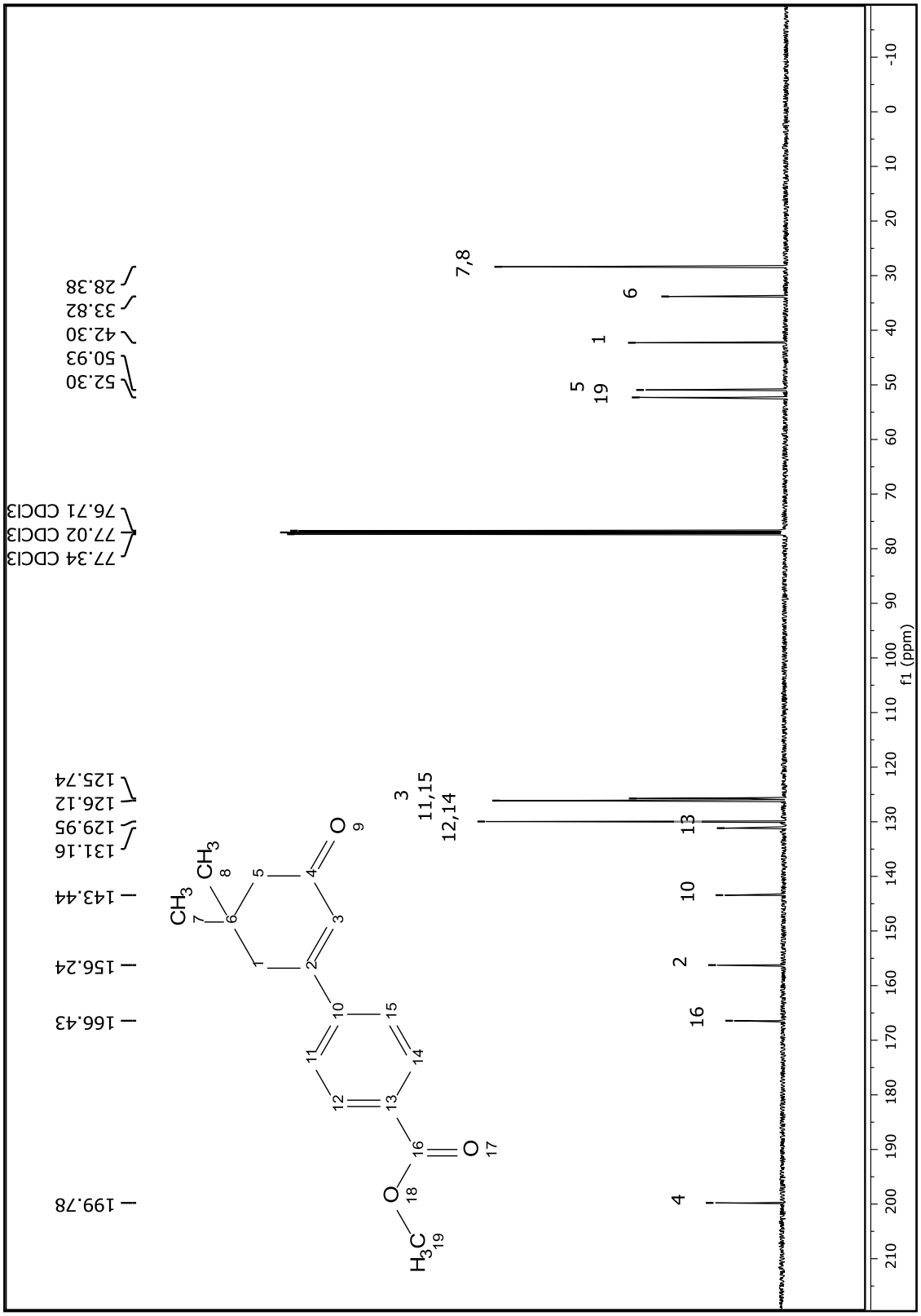
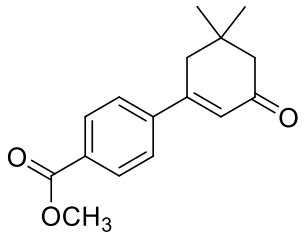


Figure 2.4.4 ^{13}C NMR spectrum of **40** (TD561) with peak assignments

Based on the initial screening results (shown below) by voltage sensitive dye imaging (VSDI), compound **40** was further investigated by the Poulter laboratory using the in vivo kindling assays. The promising results in the latter tests and the observation that rats treated orally with this compound showed no obvious side effects led to the decision by OB Pharmaceuticals, Inc. (London, ON) under the direction of Dr. Michael Poulter (CSO), to pursue it as a potential drug candidate. Most importantly, compound **40** when administered orally at 20 mg per kg prevented stage 1 epileptic seizures in kindled rats whereas those treated at the same level with AEDs such as Lamotrigine proceeded typically to stages 3 and 4.[29]

A considerable amount of biological data has been subsequently obtained for this compound. It is currently being evaluated in preclinical trials by scientists at the Center for Drug Research and Development (CDRD; Vancouver, BC). Compound **40** has also been sent to the Epilepsy Therapy Screening Program (ETSP) at the National Institute of Neurological Disorders and Stroke (NINDS) in Bethesda, MD, United States) for additional evaluation and verification of its activity *in vivo*.



40 (TD561)

Figure 2.4.5 Structure of **40** (TD561)

It is uncertain as to why **40** was chosen by OB Pharma over a number of other potential candidates but we can advance a hypothesis on the subject. The data for its effect on neuronal

firing at 60 Hz was very good but not significantly better, as will be seen later on in this chapter, than that of a number of other possible candidates. One important characteristic was that **40** showed significant differentiation between the 60 and 20 Hz stimulation, shown in table 2.4-1 below. At the lower 50 nM concentration this compound had essentially no effect on neuronal firing when stimulated at 20Hz. This difference was desirable since the higher frequency associated with gamma oscillations is present before and could be a likely cause for pathophysiological epileptiform activity whereas the lower frequency is representative of normal "basal" neuronal activity.[30]

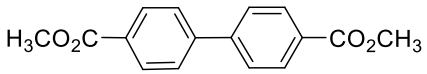
The physical properties of **40** may also have influenced the choice of this compound for further evaluation by the Poulter group. It is a colorless, nicely crystalline, stable solid with ready solubility in vegetable oils which were used as a vehicle for *in vivo* studies. The in vitro assays were performed using lower concentrations of compound in this case because of its relatively high potency. The lowest concentration in previous bioassays was 200 nM.

Table 2.4-1 Summary of reduction of neuron firing at 20 Hz and 60 Hz by **40** using in vitro bioassays on brain cell slices

Frequency (Hz)	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
20	1	5	-13	5
60	-29	5	-48	5

Because of the need for additional material for the more in-depth evaluation of this compound, this compound has been prepared at least six different times on scales ranging from 0.5 g to 5 g of **40**. Typical isolated yields were in the 65% range with the best yield being 75%.

A consistent by-product accompanying the formation of **40** was identified as the biphenyl derivative **43** based on comparison of its spectroscopic data with that reported in the literature.[31] It could not be separated from the desired product by crystallization but could be removed by careful silica gel chromatography using pure dichloromethane as eluent where it is much more fluorescent on analytical TLC and slightly less polar than **40**. The by-product **43** elutes first. Once this product has been eluted, the desired **40** is obtained quickly using 5% ethyl acetate: 95% DCM as eluent.



43

Figure 2.4.6 Structure of biphenyl by-product impurity

2.5 Potential alternate synthetic routes to 23.

Due to the purification problems associated with the preparation of multi-gram quantities of **40**, we considered alternate approaches to this compound. We investigated briefly the sequence in which the aryl to carbon bond is made via the protected Grignard reagent **45** which was prepared from THP protected 4-bromobenzyl alcohol **44**. (shown below)

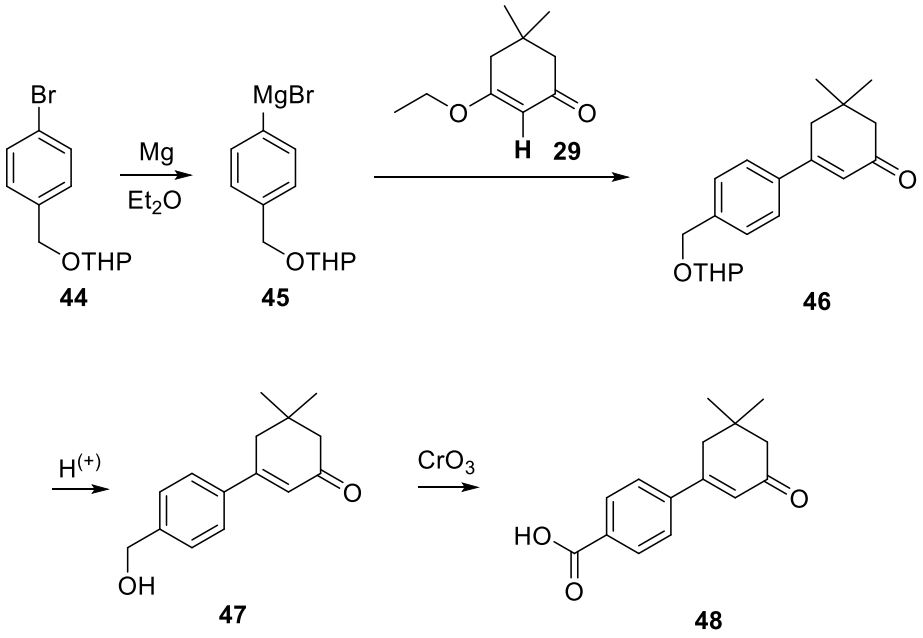


Figure 2.5.1 Scheme for an alternate synthetic route to **40**.

Reaction of the Grignard reagent **45** with ethoxy dimedone, **29**, afforded the adduct **46**. Removal of the protecting group gave the benzylic alcohol **47** which was oxidized with Jones' reagent to form directly the acid **48**. This transformation was carried out jointly with COOP student Michael Darnowski who carried out the first and last step in the synthesis. The isolation of **48** showed that this sequence worked in principle. All intermediates were appropriately characterized.

Upon further consideration it was decided not to optimize this sequence since it involved twice as many steps as the boronic acid approach. Additionally, it was realized that this would not be easily translated into a potential large-scale approach since it involved the use of the highly flammable ether as a solvent and the use of Cr^{+6} which has known toxicity as oxidizing agent.

A third approach based on a Heck coupling reaction is currently being pursued by another member of our group. A search of literature precedents led to an Organic Letters publication by the Andrew Myers group.[32] These authors pointed out the lack of reliable examples of classic Heck reaction conditions involving cyclohexenone as the electron poor carbonyl component. They presented a new approach involving decarboxylation of an aromatic acid and its coupling to 4-isoprpoylcyclohexanone.

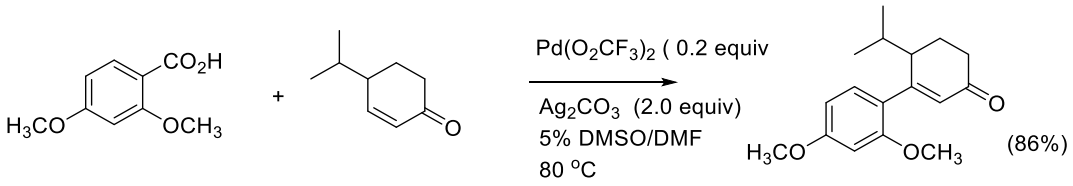


Figure 2.5.2 Decarboxylation of an aromatic acid and its coupling to 4-isoprpoylcyclohexanone

The authors pointed out that these reaction conditions worked best with ortho-substituted aromatic acids and thus such a sequence may not be useful for a synthesis of **40**. They did however also report application of the Jeffry reaction conditions[32] to the synthesis of **49.**

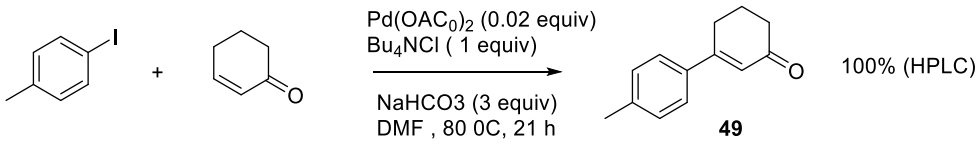


Figure 2.5.3 Application of Jeffrey conditions of Heck reaction as a potential route to **40**.

We believe that there is a reasonable possibility that this combination of reagents may lead to a process for the preparation of **40** that could be competitive with the boronic acid route both in terms of time and cost. (shown below)

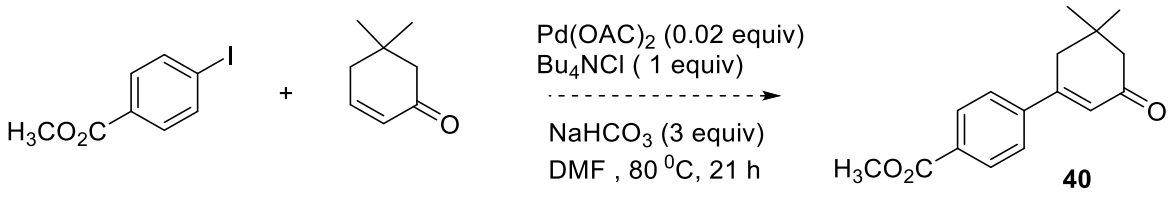


Figure 2.5.4 A potential approach to synthesis of **40** (TD561).

A modification on the initial boronic acid approach was attempted, wherein the tosylate of dimedone **42**, was isolated and the synthesis was carried out in two steps. This led to an easier separation and isolation via column chromatography and was, at the time, the best and simplest synthetic approach to obtain **40**. In order to prepare 15-20 g of **40** for preclinical toxicity and bioavailability studies, we used this two-step boronic acid approach involving the isolation of the intermediate. The tosylate **42** was obtained in essentially quantitative yield as a clear viscous oil which slowly crystallized into a white solid by reaction of tosyl chloride with dimedone in a 1,4-dioxane: water mixture (2:1 ratio) in the presence of potassium carbonate. Reaction of 1.5 equivalents of the isolated tosylate with 1 equiv of boronic acid **41**, catalyzed by $Pd(PPh_3)$ followed by extraction with DCM yielded a reddish color solid which upon recrystallization gave two batches of a tan solid which showed only traces of the by-product **43**. The mother liquors were chromatographed and additional **40** was obtained. The overall yield of **40** with greater than 98% purity according to ^{1}H NMR, starting with 4.5 g of the boronic acid **41**, was 85%. The necessary preclinical studies are being carried out at the CDRD and NINDS/ESTP.

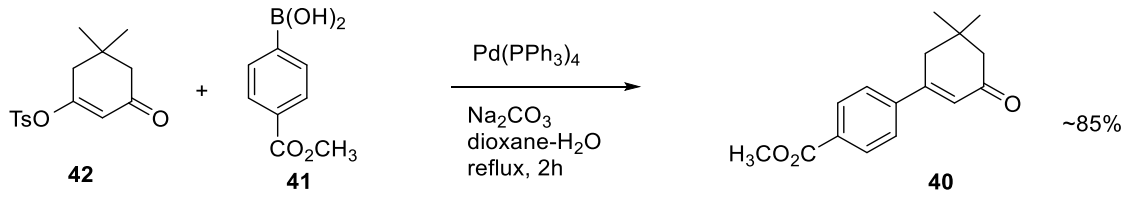


Figure 2.5.5 Second step of modified two pot boronic acid approach to **40** (TD561).

Recently, a COOP student in the group was asked to repeat the dimedone tosylate- boronic acid coupling reaction via the intermediate isolate route and at the same time reduce the tosylate to boronic acid ratio from 1.5: 1 to 1.25:1. It was found that the yield was lowered under the latter conditions.[33]

2.6 Synthesis of salt and isopropyl ester analogs of compound 40.

Since **40** is a methyl ester, it seemed reasonable to prepare and evaluate both the acid **48**, its sodium and the ammonium salt **50** and **51,** respectively. We considered that it was highly plausible that **40** was a pro-drug and of the acid **48** since the methyl ester **40** should be an excellent substrate for esterase enzymes. That this was indeed the case was shown by much later administering **40** orally to a rat. Examination of blood serum shortly after the administration showed only the presence the acid **48**.[29]

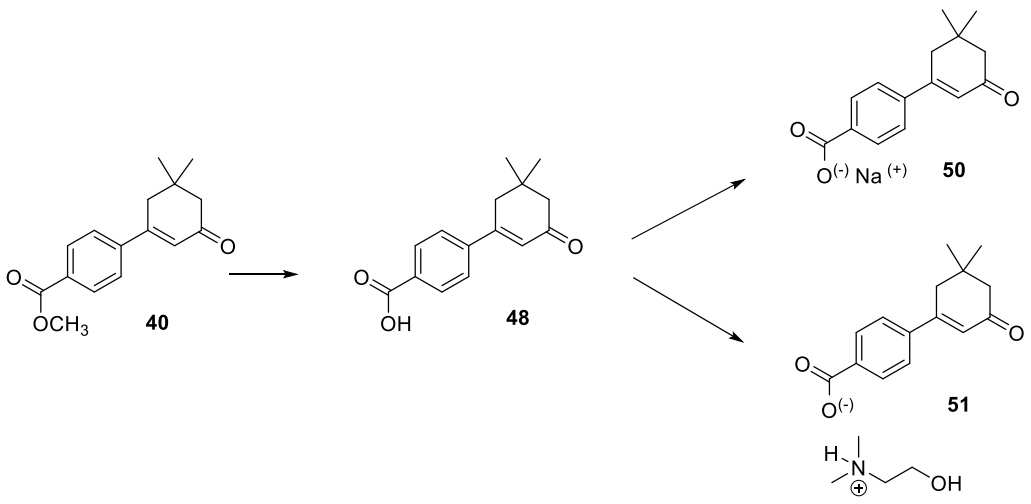


Figure 2.6.1 Synthesis of salt analogs of the acid **48**.

Finally, for this series we prepared the isopropyl ester **52**. We rationalized that **52** might be somewhat more resistant to esterase catalyzed hydrolysis than **40** due to the bulkier group surrounding the ester. Also, due to a greater hydrophobicity as shown by a comparison of the calculated Log P values (chemdraw), the isopropyl ester **52** might be more readily absorbed from the gut than the methyl ester or the acid **48** itself; a combination of these two factors could lead to greater bioavailability. The isopropyl ester **52** was prepared via two different classical routes: a) via the acid chloride and b) by heating a solution of the acid **48** in isopropanol in the presence of conc. sulfuric acid as shown in Figure 2.6.2.

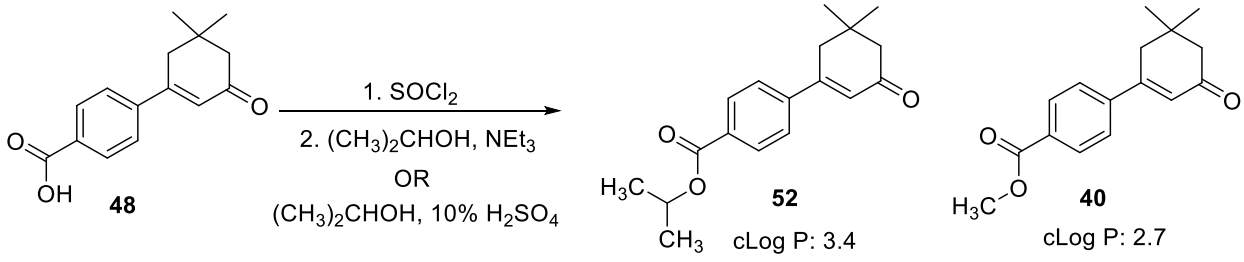


Figure 2.6.2 Preparation of the isopropyl ester **52**. Comparison of Log P values for **40** and **52.**

A comparison of the activity of these compounds using VSDI in rat brain tissue is given below. As a modification of the earlier assays, the two concentrations studied were reduced from 200 nM and 1 µM to 50 nM and 200 nM, respectively, and examined in slices that were stimulated at both 60 and 20 Hz. The two frequencies were used to display selectivity for compounds that preferentially reduced the higher gamma oscillation frequency believed to be associated with pathophysiological epileptiform neuronal activity versus the lower frequency over normal lower frequency "basal" neuronal activity.

Table 2.6-1 Summary of reduction of neuron firing at **60 Hz** by **40** and its salts and isopropyl ester using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
40	**-29**	**5**	**-48**	**5**
52	-31	12	-45	6
48	-25	6	-41	6
50	-33	6	-45	5
51	-13	8	-38	5

Table 2.6-2 Summary of reduction of neuron firing at **20 Hz** by **40** and its salts and isopropyl ester using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
40	**1**	**14**	**-13**	**13**
52	-7	11	-17	12
48	-20	19	-36	9
50	-6	12	-22	8
51	-10	25	-19	13

According to the standard error of the mean, there is no difference in the in vitro activity of the methyl vs the isopropyl ester, **40** vs **52**. The acid **48** and the sodium and ammonium salts **50** and **51** all show comparable activity at the 60 Hz frequency at the higher concentration. At the lowest concentration of 50 nM at 60 Hz, the ammonium salt **51** shows poor inhibition. Interestingly and somewhat surprisingly, the data at 20 Hz seems to indicate that the acid **48** shows the least amount of frequency selectivity. This would mean that it is susceptible to producing secondary effects and could affect non-epileptiform neuronal activity because of its strong inhibition at 20 Hz. A comparison of the in vivo activity of the methyl ester **40** and the isopropyl ester **52** is being carried out at Centre for Drug Research and Development (CDRD).

2.7 Synthesis of amide analogs of compound 40

The synthesis of amide derivatives of **40** was undertaken next. Such compounds should be metabolically somewhat more stable since amidases tend to cleave amide bonds more slowly when the amine part does not represent a natural amino acid.

Six amides with varying structural features were prepared: two aliphatic with one a secondary (**54**) and the other tertiary (**55**): one benzylic (**56**); and three aromatic, one tertiary (**57**) and two secondary (**58, 59**). They were synthesized by first converting the acid **48** into the its acid chloride **53** followed by reaction with the appropriate amine (Figure 2.7.1). Since all of these compounds represent novel structures, they were appropriately characterized (^{1}H and ^{13}C NMR, HRMS); the data is given in the Experimental Section.

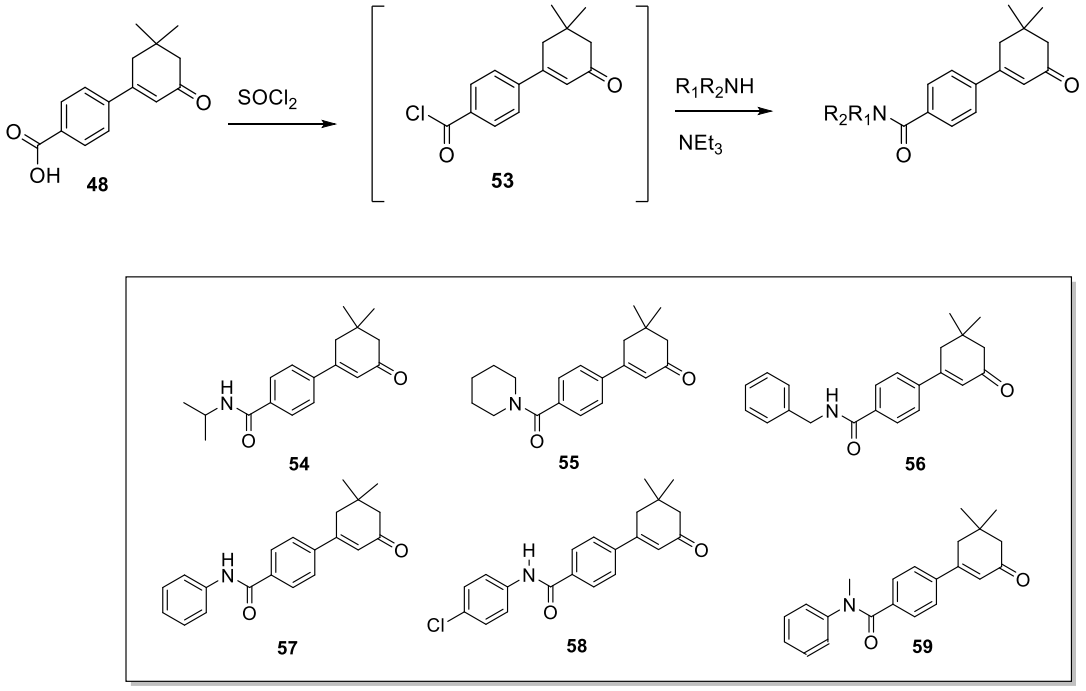


Figure 2.7.1 Amide analogs of **40**.

The bioassays for these six compounds are shown in the Table below; the data for **40** is also included for comparison. All of the above amides show rather similar activity when taking into account the standard error of the mean. The anilide **57** which caused the greatest reduction of neuronal firing at the 50 nM concentration was chosen as one of five compounds for submission to the NINDS/ESTP for further evaluation. This required an additional synthesis of **57** to produce the required quantity. Compound **58** carrying the *para* chloro group on the aromatic ring was considered a backup to **57** with the idea that **58**, if it had similar in vitro biological activity compared to **57,** would likely be more resistant to CYP3A4 metabolism due to the presence of the electronegative Cl substituent on the aromatic ring. The common metabolism of aromatic substrates by CYP enzymes including CYP3A4 results in the introduction of a hydroxyl group. The mechanism involves initial formation of an epoxide followed by a

rearrangement known as the NIH shift. The aromatic ring acts as a nucleophile for the epoxide formation. It has been observed that aromatic rings carrying strongly electron withdrawing groups such as F, CF_3, SO_2R and Cl are less prone to CYP induced hydroxylation.

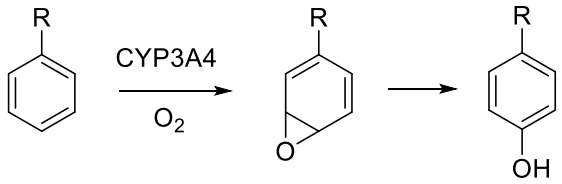


Figure 2.7.2 Hydroxylation by CYP3A4

A number of important drugs including Crestor were designed to be metabolised more slowly by having fluorine containing substituents in their aromatic rings.

Although the data had poor precision due to the large standard errors of the mean (SEM) the amides carrying an aromatic residue (**56**-**59**) appear to show considerable frequency selectivity. All, except **58**, show about a 40% reduction of VSDI activity at 200 nM when stimulated at 60 Hz. In contrast stimulation at 20 Hz appears to cause either an increase or have no effect on activity. Most surprisingly and unexpectedly, the positive stimulation seen with **58** and **59** appears stronger at the 50 nM as compared to the 200 nM concentration. This positive relative change could be explained by the relatively high standard error of the mean (SEM) and the low overall reduction compared to the control caused of the lower concentration of compound.

Table 2.7-1 Summary of reduction of neuron firing at 60 Hz by **40** and its amide analogs using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
40	**-29**	**5**	**-48**	**5**
54	-19	9	-29	10
55	-32	12	-45	6
56	-30	6	-39	6
57	-24	14	-42	7
58	-22	6	-16	10
59	-20	7	-34	7

Table 2.7-2 Summary of reduction of neuron firing at **20 Hz** by **40** and its amide analogs using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
40	**1**	**14**	**-13**	**13**
54	-1	31	-8	21
55	-17	15	-9	20
56	7	11	-1	21
57	25	16	47	33
58	30	25	-9	13
59	27	22	-5	13

2.8 Synthesis of analogs with different aromatic substituents

A number of other aromatic derivatives were prepared to map out the effect of substituents, both electron withdrawing and electron donating on the aromatic ring. Substituents such as F and SO_2R were included since these are known to reduce the rate of metabolism via hydroxylation in the aromatic ring via CYP enzymes.

These compounds are grouped below with the notion that they could be used to investigate the electronic effects of *para* substituents. For example, in the set of three compound **60**, **61**, and **62** the *para* nitro group makes the aromatic ring highly electron deficient.

The *p*-nitro derivative **60** was prepared with the help of Michael Darnowski as part of his Honours Project via the boronic acid coupling route. Reduction to the amino derivate **61** using H2 and Pd/C followed by methylation with methyl iodide resulted in an electron rich aromatic ring.

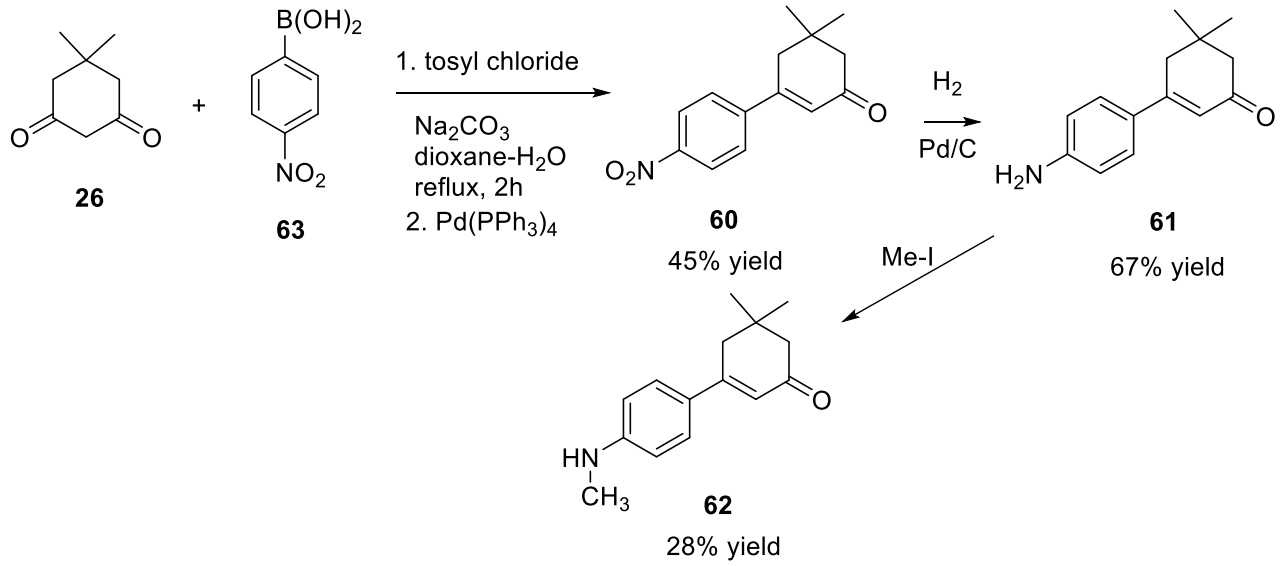


Figure 2.8.1 Synthesis of *p*-nitro and amine analogs via the boronic acid approach

Table 2.8-1 Summary of reduction of neuron firing at **60 Hz** by nitro and amine analogs using in vitro bioassays on brain cell slices

Compound (para substituent)	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
60 (Nitro)	-11	15	-22	17
61 (1° Amine)	-33	3	-33	7
62 (2° Amine)	-22	9	-35	9

Table 2.8-2 Summary of reduction of neuron firing at **20 Hz** by nitro and amine analogs using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
60 (Nitro)	-29	14	-11	15
61 (1° Amine)	-20	6	-32	10
62 (2° Amine)	-18	14	-33	14

The tentative conclusion is that the electron withdrawing *p*-nitro group (**60**) elicits a smaller response at 60 Hz compared to the electron donating *p*-amino groups. Also, surprisingly, **60** shows higher inhibition of -29% at low concentration compared to the -11% result at higher concentration at 20 Hz. This goes against all previous assays where a higher concentration leads to higher reduction. Unlike in the amides above, these three derivatives show little or no frequency selectivity. It should be pointed out that the *para* carboxymethyl group of **40** is, like a nitro group, also a potent electron withdrawing group, yet the activity of the latter is significantly superior to **60**.

In the next set of five compounds three have electron donating oxygen substituents in the *para* position. However, these compounds differ in several ways. The phenol **63** is both a strong hydrogen bond donor and an H bond acceptor while **64** to **66** are only H-bond acceptors. The methyl ether **65** is sterically small while the benzyl ether **66** adds considerable additional steric bulk to the para position. Would that cause an increase or decrease in the in vitro activity? The hydroxyl methyl substituent in **47** was added to this group to probe whether an OH or CH_2OH at the para position is more advantageous.

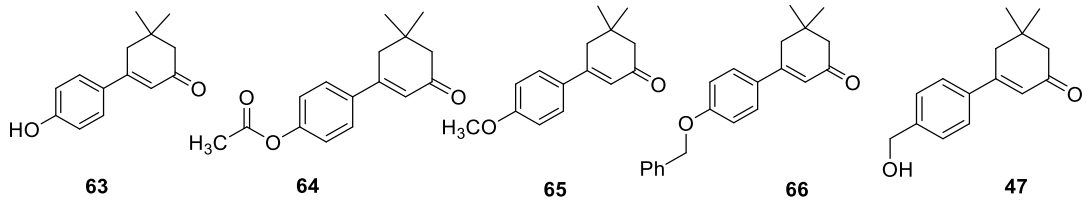


Figure 2.8.2 Structures of new series of analogs containing EWG on the benzene ring

The phenol **63** was prepared by first performing a metal-halogen exchange reaction on TBDMS protected *p*-bromophenol. Subsequent reaction with **29** yielded an intermediate which

was treated with acetyl chloride followed by acid/base wash to give the deprotected phenol **63**. Acetylation and benzylation of **63** afforded **64** and **66,** respectively.

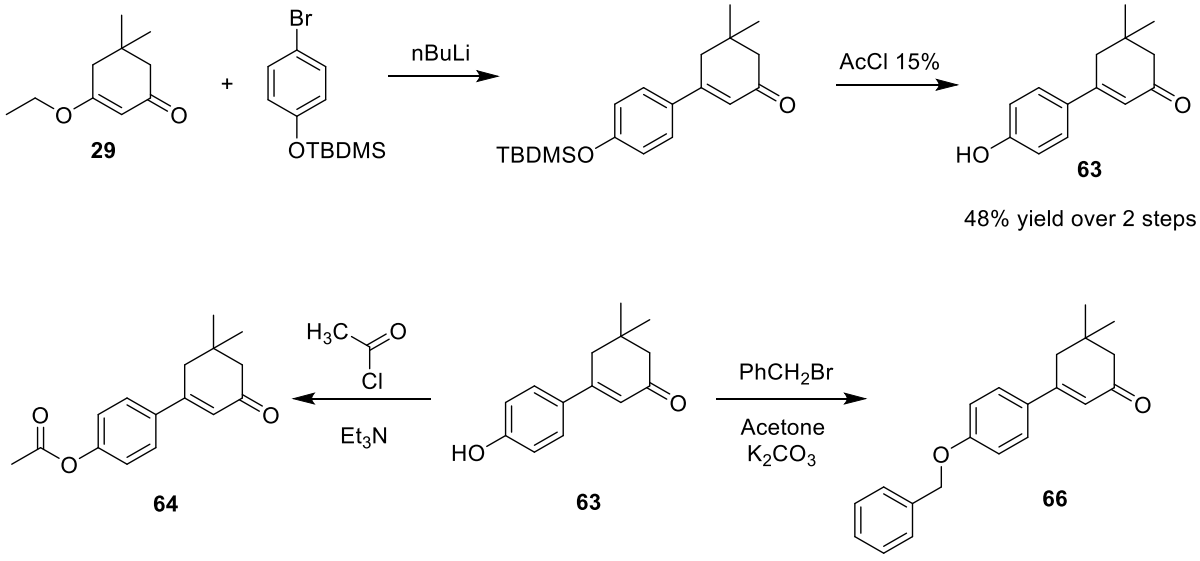


Figure 2.8.3 Synthesis of analogs **63**, **64** and **66**

Additional amounts of **66** were required for evaluation of this compound by NIH. This was accomplished via a somewhat shorter route by first benzylating 4-bromophenol to for the bromoether **67** and carrying out the bromine-lithium exchange reaction in THF followed by addition of ethoxydimedone. Treatment of the adduct **68** from this reaction with acid gave **66**.

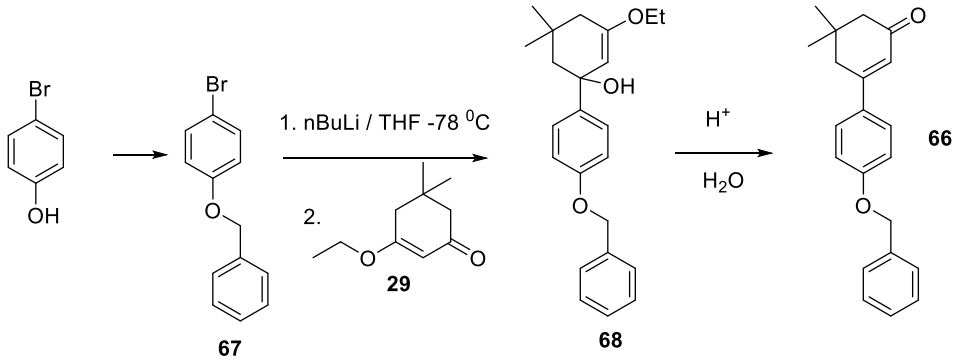


Figure 2.8.4. Alternate synthesis of **66**.

Bioassay data is available for these compounds. At the time compound **65** was submitted the concentrations used were the original of 200 nM and 1 µM and not the more recent 50 and 200 nM which became standard in to order differentiate the more potent compounds. Compound **47** was submitted much later at a time when the Poulter lab suspended bioassay determination in order to focus more on obtaining data for the potential drug candidate **40** therefore no data is available for this substance. All show significant reduction of activity at 60 Hz and almost no effect or a positive effect at the 20 Hz stimulation. Compound **66** was selected as one of the five compounds sent to the NIH. Overall, this set of compounds seems to be somewhat less effective at reducing firing when cells were stimulated at 60 Hz than **40**. The data obtained at 20 Hz is difficult to interpret with any certainty since the SEMs are quite large. The `best` conclusion appears to be that these compounds have little effect on activity when the cells were stimulated at 20 Hz.

Table 2.8-3 Summary of reduction of neuron firing at 6**0 Hz** by benzyl ether, acid and alcohol analogs using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
63	-25	5	-38	5
64	-21	8	-34	7
65	--	--	-46	11
66	-26	9	-42	6

Table 2.8-4 Summary of reduction of neuron firing at 2**0 Hz** by benzyl ether, acid and alcohol analogs using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
63	8	22	22	56
64	-4	13	-3	21
65	--	--	-4	18
66	-4	16	-8	17

The final group of substituted phenyl derivatives attached at C3 of the cyclohexenone ring focused on the introduction of fluorine substituents. A significant number of currently used drugs, for example one of the statins and the quinolone antibioitic ciprofloxacin, have one or more F atoms on an aromatic ring. The purpose is to increase the half-life of the drug by reducing CYP450 metabolism. The size of the fluorine atom is such that its introduction causes very little additional steric requirement compared to hydrogen.

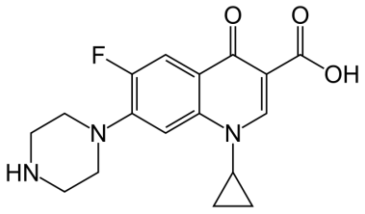


Figure 2.8.5 Structure of Ciprofloxacin

Thus, the two F-substituted aromatic compounds **69** and **70** were prepared using the respective boronic acid, tosyl chloride and dimedone. These compounds were submitted for bioassay in order to be able to compare them with the parent 3-phenyl-cyclohexenone **31**.

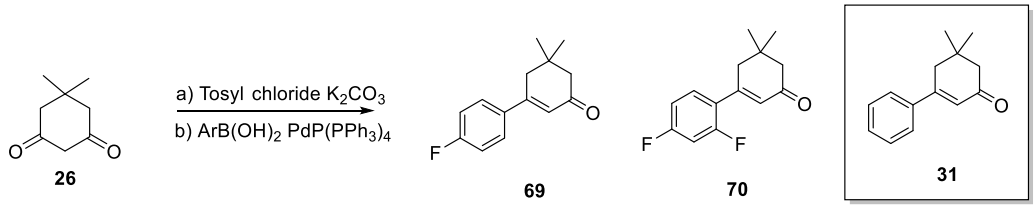


Figure 2.8.6 Synthesis of fluoro-substituted aromatic compounds

The data for **31** at 20 Hz seems to be an outlier. It shows a strong increase in activity at 20 Hz, but the large uncertainty makes it hard to make a clear conclusion. When **31** was tested via in vitro bioassay, 50 nM concentrations were not tested. Having this extra data would help make a clearer comparison with the fluoro substituted aromatic analogs. The introduction of the F atoms on the aromatic ring seems to have no significant effect on the in vitro activity when compared to the parent phenyl compound **31**.

Table 2.8-5 Summary of reduction of neuron firing at 6**0 Hz** by fluoro substituted aromatic and phenyl analogs using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
69	-36	5	-33	5
70	-28	12	-38	10
31	--	--	-36	7

Table 2.8-6 Summary of reduction of neuron firing at 2**0 Hz** by fluoro substituted aromatic and phenyl analogs using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
69	-10	9	-23	9
70	-2	12	-29	15
31	--	--	29	34

Sulfone substituents on aromatic rings often serve the same purpose as F in pharmaceuticals (CYP450 hydroxylation resistance). They are also sterically much more demanding. A comparison of the activity of our key compound, the potential drug candidate **40** which carries the CO_2CH_3 at the para position of the aryl ring with the p-trifluoromethyl derivative **71** and the p-methylsulfonyl compound **72** was also considered to be potentially highly informative. All three substituents are strong EWG groups but only **40** has a good hydrogen bond accepting group. Finally, these three compounds have significantly different log Ps with the sulfone **72** being most polar and the trifluoromethyl derivative **71** least polar amongst these three.

As above, **71** and **72** were prepared by the one-pot sequence using commercially available 4-trifluoromethyl and 4-sulfonylmethylboronic acid, respectively. They were purified via silica gel chromatography, followed by recrystallization from ethyl acetate-hexane mixtures. Each showed the required two sets of aromatic hydrogen doublets which in **71** were further split due to long range F-H coupling.

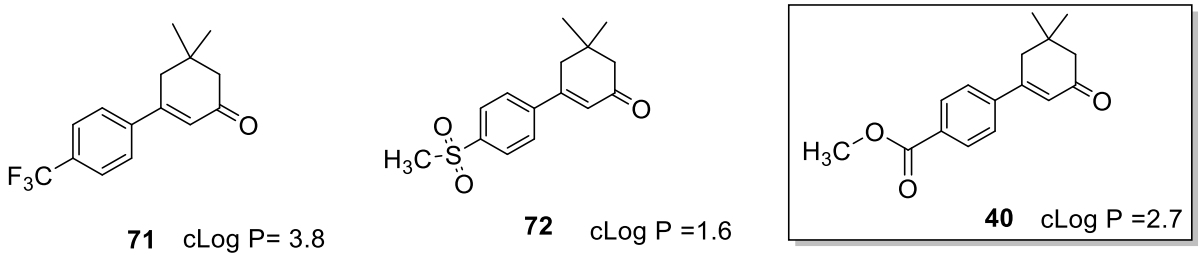


Figure 2.8.7 Structures and calculated LogP of trifluoromethyl and sulfone analogs compared to **40**

Table 2.8-7 Summary of reduction of neuron firing at 6**0 Hz** by trifluoro and sulfone substituted aromatics compared to **40** using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
71	-19	10	-26	11
72	-12	7	-46	7
40	-29	5	-48	5

Table 2.8-8 Summary of reduction of neuron firing at 2**0 Hz** by trifluoro and sulfone substituted aromatics compared to **40** using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
71	-22	12	-33	9
72	-14	8	-26	10
40	1	14	-13	13

The data for these three compounds indicates that reduction of neuronal firing when stimulated at 60 Hz is highest for **40**, especially at the lower, 50 nM concentration. More importantly, compound **40** shows essentially no activity at 50 nM when the cells were stimulated at 20 Hz and a 13% +|- 13 reduction at 200 nM. In contrast, the reduction of activity at the lower frequency is significant for both **71** and **72** at both concentrations. Based on these data compounds **71** and **72** will not be evaluated further.

Three heteroaromatic analogs 7**3-75** were prepared to investigate the possibility that a heterocyclic substituent at position 3 in the cyclohexanone ring might yield significantly more active compounds than the simple phenyl analog **31** due to the difference in reactivity of the nucleophilicity of phenyl versus thienyl versus pyridyl, with thienyl being the most and pyridyl being the least reactive towards electrophiles. The thiophene derivatives were prepared in

collaboration with COOP student Michael Darnowski via the one-pot procedure using the commercially available boronic acids.

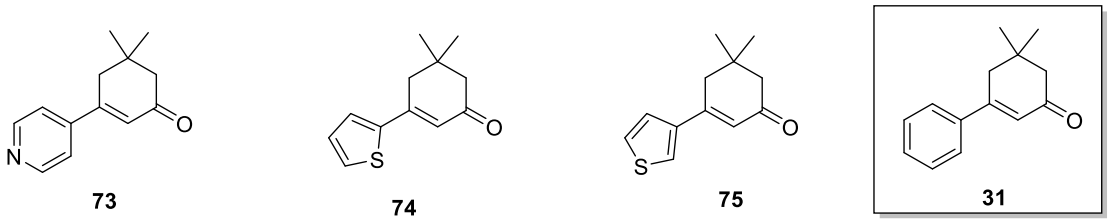


Figure 2.8.8 Structures of heteroaromatic analogs

Table 2.8-9 Summary of reduction of neuron firing at 6**0 Hz** by heteroaromatic and phenyl analogs using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
73	8	13	-8	13
74	-29	9	-49	9
75	-31	8	-38	9
31	--	--	-36	7

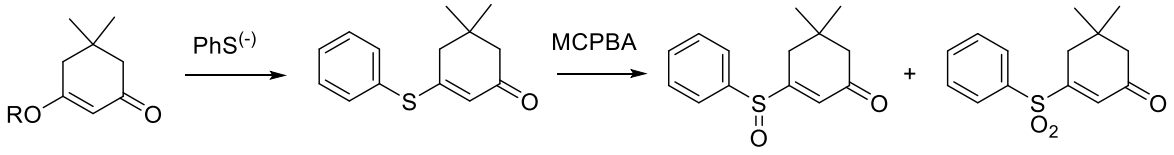
Table 2.8-10 Summary of reduction of neuron firing at 2**0 Hz** by heteroaromatic and phenyl analogs using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
73	-7	13	-6	14
74	-35	11	-27	7
75	-19	20	-32	14
31	--	--	29	34

From these data, it can be inferred that reduction of neuronal firing when stimulated at 60 Hz is high for both **74** and **75.** However, these compounds also reduce activity when exposed to the lower 20 Hz frequency, which could prove to be problematic. As previously explained, this would cause reduced firing in neurons responsible for functions crucial to life such as controlling heartrate. These compounds do not show much potential as anti-epileptic drugs because of their lack of selectivity between the two tested frequencies.

2.9 Sulfur and oxygen containing substituents at C3.

Two cyclohexenones carrying alkoxy substituent at C3, **28** and **29**, were available as intermediates in the synthesis of compounds such as **30** and **31**. These were submitted for bioassays. The results were sufficiently encouraging to suggest to us the preparation of the 3-thiophenyl analog **76** and from there the corresponding sulfoxide and sulfone **77** and **78**, respectively. The bio-assay data available for these compounds are shown below.

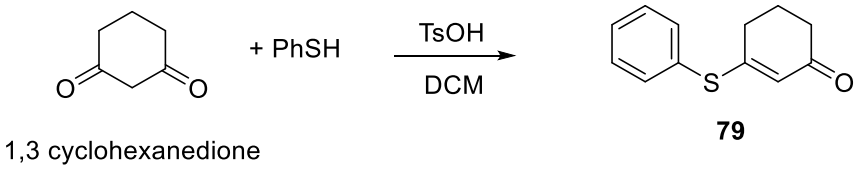


28, R = CH_3
29, R = CH_2CH_3

76 **77** **78**

Figure 2.9.1 Synthesis of sulfur containing analogs starting from dimedone intermediates

Compound **79**, the analog of **76** lacking the 5,5-dimethyl group was prepared directly from 1,3-cyclohexanedione by treatment with thiophenol in the presence of TsOH as the acid catalyst.

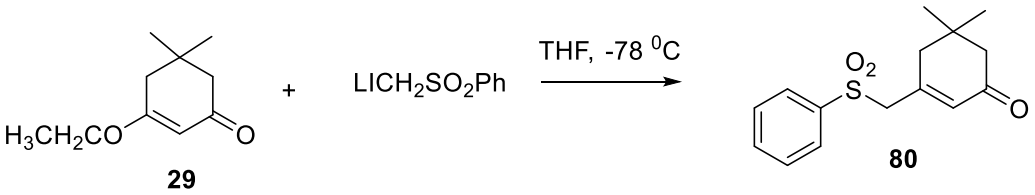


1,3 cyclohexanedione

79

Figure 2.9.2 Synthesis of sulfide analogue from 1,3-cyclohexanedione

Reaction of the lithio derivative of methyl phenyl sulfone with the ethoxy ether **29** afforded the sulfone derivative **80**.



29 **80**

Figure 2.9.3 Synthesis of sulfone analogue **80**

Several compounds, in particular the phenylthio analogs **76** and **79**, show reduction of neuronal firing at 60 Hz comparable to the **40**. Unlike **40**, these compounds seem to show lower frequency selectivity. It is unfortunate that data for sulfoxide and sulfone analogs **77** and **78** are not available at this time. The removal of gem-dimethyl group at C5 does not seem to have any effect on the activity when comparing **76** and **79**, as their VSDI bioassay data is almost identical.

Table 2.9-1 Summary of reduction of neuron firing at 6**0 Hz** by sulfur derivatives **76** and **79** compared to ethyl ether **29** using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
29	-20	7	-26	8
76	-32	5	-51	5
79	-33	5	-50	5

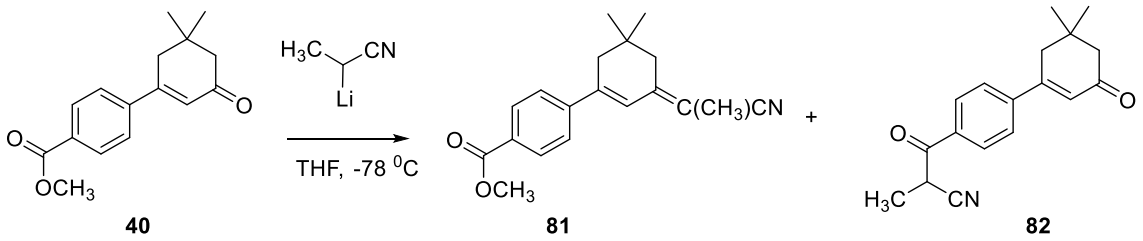
Table 2.9-2 Summary of reduction of neuron firing at 2**0 Hz** by sulfur derivatives **76** and **79** compared to ethyl ether **29** using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
29	-14	11	-23	13
76	-18	11	-25	12
79	-18	11	-25	12

2.10 Additional variations related to compound 40

Because of the importance of **40**, despite the conclusions reached earlier when comparing the activity of cylcohexenones **30** and **31** with the chain extended α-methyl nitriles, **32** and **34**, respectively, we reacted **40** with the lithium salt derived from proprionitrile. The crude reaction product was subsequently treated with acid to effect dehydration. This reaction sequence yielded the expected product **81** together with **82** which is the result of the attack of the lithio derivative on the methyl benzoate carbonyl in competition with attack at the enone carbonyl group. These compounds were separated by silica gel column chromatography using

a 9:1 mixture of hexanes and ethyl acetate. Unfortunately, the desired compound **81** was obtained in only minor quantities and never in a sufficiently pure state for a meaningful bioassay. The fact that both stereoisomers were present further complicated the purification process.



40 **81** **82**

Figure 2.10.1 Synthesis of propionitrile analogs of **40.**

Compound **82** showed two ketone carbonyl carbon absorptions at 190.0 and 199.7 ppm. The $CH(CH_3)CN$ portion in **82** was characterized by a ^1H quartet at 4.36 ppm and the methyl doublet at 1.65 ppm. Compound **82** showed a smaller reduction of neuron firing at 60 Hz than **40.**

2.11 Analogs derived from 1,3-cyclohexanedione

In order to assess the importance of the 5,5-dimethyl groups on the biological activity of the 3-substituted cyclohexenones, we prepared analogs of the most promising derivatives **40**, **48** and **19**. Starting from 1,3-cyclohexanedione, the same approach was used as for the isophorone (**8**) starting material. Conversion to the enol tosylate **83** and the one-pot procedure with the appropriate boronic acid led to the desired products.

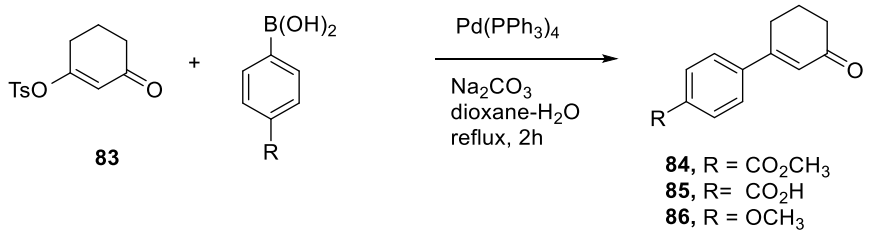


Figure 2.11.1 Synthesis of 1,3-cyclohexanedione analogs via boronic acid approach

We also prepared the corresponding chain-extended unsaturated nitriles to mimic the compound **19 (TD532)** by reacting both **84** and **86** with lithiated propionitrile**.** The bioassay results for these compounds were compared with those carrying the 5,5-dimethyl group. The results indicate quite clearly that removal of the 5,5-dimethyl substituents results in a significant decrease in the potency. The direct comparisons in this set of compounds are between the **40** and **84**, and the compounds **48** and **85**, which both show that the compound containing the 5,5-dimethyl have better activity at 60 Hz and better selectivity.

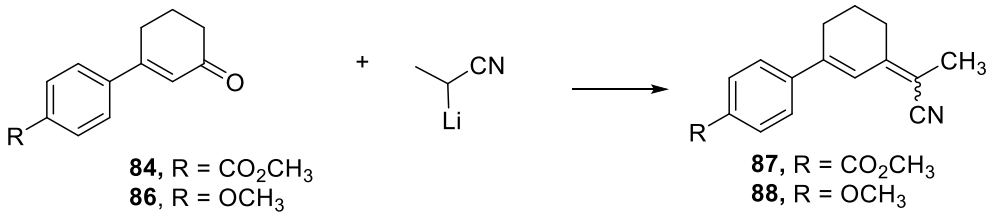


Figure 2.11.2 Synthesis of propionitrile analogs from 1,3-cyclohexanedione

Table 2.11-1 Summary of reduction of neuron firing at 6**0 Hz** by analogs derived from 1,3-cyclohexandione using in vitro bioassays on brain cell slices

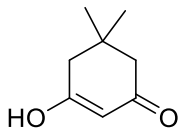
Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
40	-29	5	-48	5
84	-20	5	-29	4
48	-32	6	-41	6
85	--	--	-36	7
86	-24	10	-42	7
87	-12	8	-18	8

Table 2.11-2 Summary of reduction of neuron firing at 2**0 Hz** by analogs derived from 1,3-cyclohexandione using in vitro bioassays on brain cell slices

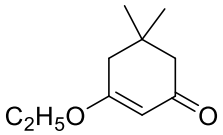
Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
40	1	14	-13	13
84	-2	16	-7	26
48	-24	19	-36	9
85	--	--	29	34
86	13	22	-15	20
87	-2	10	-4	11

2.12 Enol-thioether analogs

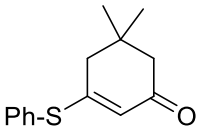
A final set of compounds comprise the simple dimedone, **26** draw in its enol form, the ethoxy enol ether **29**, and the enol thio compounds **76** and **79**. For each of the latter four compounds, most readily for the enol ethers, one can imagine a relatively simple acid catalysed hydrolysis back to dimedone.



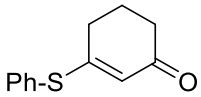
26



29



76



79

Table 2.12-1 Summary of reduction of neuron firing at 6**0 Hz** by enol-thio analog **76** compared to dimedone **26** and ethoxy enol ether **29** using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
26	-32	5	-39	5
29	-20	7	-26	8
76	-33	5	-50	5

Table 2.12-2 Summary of reduction of neuron firing at **20 Hz** by enol-thio analog **76** compared to dimedone **26** and ethoxy enol ether **29** using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
26	52	51	-12	14
29	-15	12	-26	15
76	-18	11	-25	12

The data for dimedone, **26**, itself shows surprisingly strong reduction of neuronal firing at the 60 Hz stimulation. It is difficult to interpret the results at 20 Hz due to the very high SEM. The suggestion is that there is little or no effect at this frequency. Since dimedone exists in the two tautomeric forms **26**, it is plausible that the erratic results are due to slight changes in the pH since the equilibrium between the enol and diketone form is highly pH dependent. The thiophenyl derivative **76** reduces neuronal firing when stimulated at 60 Hz at a level comparable to that of the potential clinical trial candidate **40**. The major difference is that **76**

also reduces significantly the firing at 20 Hz stimulation. Since this is a potential negative because of the potential interference with important functions in the body such as heartrate regulation, **76** has not been pursued further. The enol ether **29** shows no selectivity in its effect on neuronal firing at 20 vs 60 Hz; the activity level is not remarkable.

2.13 Additional comments concerning the bio-assay data.

While two frequency VSDI has the advantage of moderately rapid screening of effect across an entire neuronal circuit, it presented some difficulty in an accurate prediction of a clear structure-activity relationship (SAR). Typically, the SEs were significantly larger when the stimulation was carried out at 20 as compared to 60 Hz. Most all compounds in both the cyclohexenone and the extended conjugated nitrile series showed some activity, but none seemed by bioassay clearly superior to all the others. This made development of a more refined SAR beyond the core cyclohexenone structure difficult and currently hinders the development of more potent analogs. With regard to the present library of compounds, molecular variants were chosen partly based on a combination of their ease of synthesis and in an attempt to introduce a variety of functional groups. We have not yet succeeded to design a new structure and be confident that its SAR would unequivocally predict improved biological response. Based on the results thus far we conclude that the binding site for these molecules is capable of accommodating a significant structure variation at positions C3 and C5 within the cyclohexenone family.

The standard errors of the mean (SEM) in the measurements especially at the 20 Hz stimulation and the 50 nM concentrations even though the number of measurements were typically n=9-12 also make it difficult to extract meaningful data. For example, on neuron firing when stimulated at 20 Hz for the key compound **40** was reported as +1% at 50 nM and -13% at 200 nM with SEMs as 14 and 13%, respectively. This data could be interpreted as suggesting that 23 has no effect on neuron firing at these concentrations when the cells were stimulated at the 20 Hz frequency. Many other examples could have been chosen to illustrate this problem. A number of compounds appear to show an increase in neuron firing at 20 Hz. Unfortunately, in most of these examples the SEM is sufficiently large to make the conclusion questionable. Typically, the SEMs were significantly larger when the stimulation was carried out at 20 as compared to 60 Hz. We recognize that bio-assay results are inherently more difficult to reproduce than chemical reactions. Signal variance can be caused by such factors as tissue integrity, dye loading, unintentional quenching and the choice of field of interest for signal quantitation. Efforts are underway to better control some of these variables to make SAR interpretation. In the present case, we are simply pointing out that the bio-assay results were often difficult to interpret with a higher degree of confidence.

OB Pharmaceuticals chose **40** (TD561) as a potential drug candidate and develop further the profile of this compound *in vitro* and *in vivo*. Indeed, as was pointed out earlier in this chapter, this compound is now undergoing preclinical evaluation, including animal toxicity and pharmacokinetics studies with the hope that the data obtained would pave the way for clearance to carry out a Phase 1 clinical trial for safety. It is also one of five compounds selected jointly by the NINDS/ESTP and OB Pharmaceuticals for verification of activity and

examination in different epilepsy and seizure animal models. The *in vitro* data for these five compounds undergoing evaluation in the ESTP are shown below.

Table 2.13-1 Summary of reduction of neuron firing at **60 Hz** by compounds currently being studied by NINDS using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
40	-29	5	-48	5
51	-25	6	-41	7
65	-26	9	-42	6
57	-37	13	-44	10
Compound	**Average reduction at 200 nM (%)**	**SEM at 200 nM (%)**	**Average reduction at 1 µM (%)**	**SEM at 1 µM (%)**
64	-46	11	-57	6

Table 2.13-2 Summary of reduction of neuron firing at **20 Hz** by compounds currently being studied by NINDS using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
40	1	14	-13	13
51	-20	21	-36	9
65	-4	16	-8	17
57	1	1	1	12
Compound	**Average reduction at 200 nM (%)**	**SEM at 200 nM (%)**	**Average reduction at 1 µM (%)**	**SEM at 1 µM (%)**
64	-16	22	-4	18

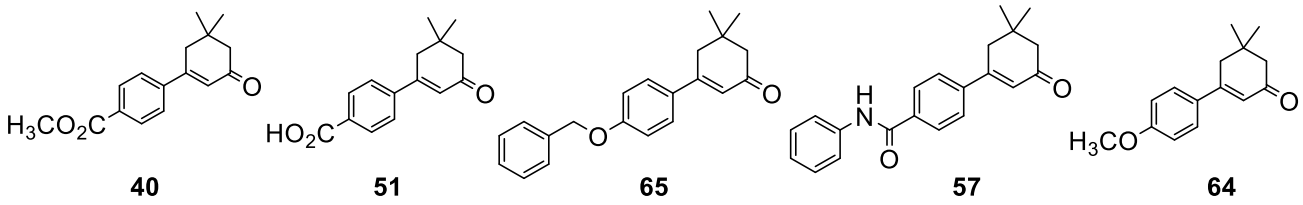


Figure 2.13.1 Structures of compounds currently being studied by NINDS

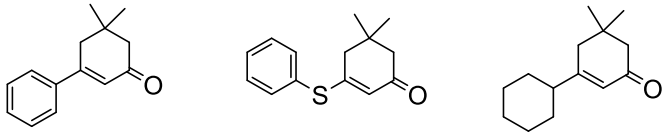
The available in vitro data presented below for the following three compounds are not substantially different from the above five, especially when one takes into account the standard errors associated with these measurements.

Table 2.13-3 Summary of reduction of neuron firing at **60 Hz** by other compounds with similar data compared to those currently being studied by NINDS using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
33	--	--	-41	6
76	-33	5	-50	5
89	-28	6	-39	5

Table 2.13-4 Summary of reduction of neuron firing at **20 Hz** by other compounds with similar data compared to those currently being studied by NINDS using in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
33	--	--	-9	22
76	-18	11	-25	12
89	6	21	37	9



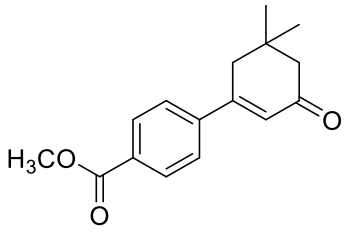
33 **76** **89**

Figure 2.13.2 Structure for certain analogs containing a variety of substituents

2.14 Biological evaluation of 40 (TD561): The path towards Phase 1 clinical trials and commercialization

In this section, we summarize the physicochemical and biological data that has been obtained in this lab and by OB Pharmaceuticals. In a number of cases indicated by [29], we are reduced to quoting results from our collaborators at OB Pharma since, for confidentiality reasons, they did not provide details. Nevertheless, we feel that it is worthwhile to quote these results since they illustrate the amount of effort that OB Pharma has put into this compound and the conviction that this compound may become a plausible clinical trial candidate.

Initially it should be reiterated that **40** is structurally different than any currently approved or pipeline anti-epileptic treatment. It is noteworthy that in subsequent *in vivo* studies in rats and mice conducted by OB Pharmaceuticals, **40** did not show any deleterious side effects (lethargy, motor impairment, feeding, etc.) at doses in excess of 25-fold its efficacious dose at suppressing seizures indicating some relevance to the differential frequency profile in the *ex vivo* VSDI bioassay.



40

Chemical Formula: $C_{16}H_{18}O_3$
Molecular Weight: 258.3170

cLogP = 2.7

Figure 2.14.1 Structural properties of **40** (TD561) and its calculated LogP value

Compound **40** is a white crystalline powder simple to produce in a one or two pot sequence from commercial starting materials. It is thermally stable as previously demonstrated by the accelerated stability studies.

It is chemically stable under neutral and somewhat acidic conditions but subject to basic hydrolysis to its acid. This was demonstrated by heating it for several hours in isopropanol in the presence of p-toluenesulfonic acid. No decomposition or reaction with isopropanol was observed by NMR. It is orally bioavailable with predictable pharmacokinetics.[29]

It meets the Lipinski rule describing drug-like structures. These rules give parameters such as MW, LogP, hydrogen bonding, which indicate predicted likely bioavailability. Compound **40** is also effective at or lower than currently used anti-epileptic drugs (AEDs).[29] It shows no behavioral side effects in treated animals.[29] Its molecular target is voltage gated sodium channels (VGSCs) and by mechanism of action different from current AEDs.[29] It has no physiologically meaningful effect on hERG channels and endogenous cardiomyocyte activity.[29] It is negative in the Ames test,[29] which is used to determine mutagenicity and carcinogenicity caused by chemicals.

The purity is easily monitored by TLC and ^{1}H NMR. A sample judged 'pure' by NMR was submitted for HPLC analysis as part of the metabolism study; only one peak was visible. This was reported by OB Pharma after analysis by the Centre for Drug Research and Development in Vancouver. The bioavailability was proven experimentally. Oral administration of **40** results in an ideal plasma profile with a half-life if 3.5 h. The only observable metabolite is the corresponding acid **48**.[29]

Compound **40** blocks 50 % of voltage gated sodium channels (VGSC) activity in single neurons *in vitro* at a concentration of 25 picoM only under conditions of higher frequency 16 ms (~60 Hz) depolarizations. In contrast, three currently used anti-epileptic drugs, Phenytoin, Lamotrigine and Carbamazepine require 40, 100 and 150 µM to reach the same effect.[29] The above observations were reported by the scientists who carried out many rat experiments involving **40** and compared their behavior to rats administered currently used AEDs.[29]

40 represents a new class of anti-seizure compounds that modulate VGSC function, by increasing the delay in the fast inactivation-reactivation cycle with a preference for high frequency-open (60 vs 20 Hz) opening channels thus attenuating epileptic seizures. Compound **40**, for example slows VGSC state recovery by approximately double the 13 milliseconds observed for control activity.[29]

In separate experiments, **40** given orally once daily (20 mg/kg) inhibits the development of kindling indicating its potential to prevent seizure onset (antiepileptogenic).[29]

Compound **40** inhibit sodium channel function. Unlike most of the current anti-epileptic drugs, it has little effect on cardiac sodium channels. Additionally, **40** shows no effect on cardiac mycocyte function when compared to the stimulant isoproterenol.[29]

Histopathological evaluation of tissues (heart liver, kidney) collected ant necropsy of kindled rats treated with **40** showed no evidence of toxicity.[29]

2.15 Conclusions and future work

This project resulted from the decision to send isophorone, **8**, the precursor to the parent lead structure **1**, for bio-assay and the discovery that **8** significantly reduced neuronal activity in rat brain tissue stimulated at 60 Hz using VSDI. This suggested that other 3-substituted cyclohex-2-en-1-ones should be evaluated. More than fifty such compounds have been prepared and indeed compound **40** shows considerable promise as a drug candidate.

Thus far we have only investigated only a small part of the potential chemical space surrounding the cyclohexenones. It appears that removal of the 5,5-dimethyl substituent reduces the potency of these compounds in the in vitro bioassay. The possibility of having substituents other than methyl at C5 or substituents at other positions in the cyclohexanone ring has not been addressed. Two compounds can be considered to begin such an investigation. One is the natural product 4-isopropylcyclohex-2-enone, **90** and the other is 5-phenyl-1,3-cyclohexanedione, **91**.

A comparison of the activity of isophorone, **8**, vs **90** (see below) suggests that the latter is more potent in inhibiting neuronal firing at 60 Hz with a 36% reduction for **90** at 200 nM compared to 22% for isophorone **8**.

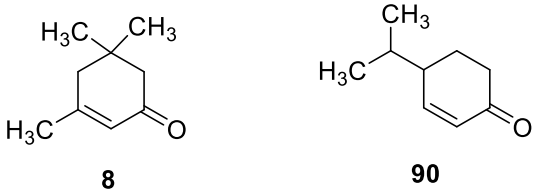


Figure 2.15.1 Structures of isophorone **8** and **90**

A plausible argument can be made that addition of a substituent such as the one found in **40** at C3 in **90** should result in increased potency. The preparation of several derivatives including **92** via a Heck coupling reaction is being planned.

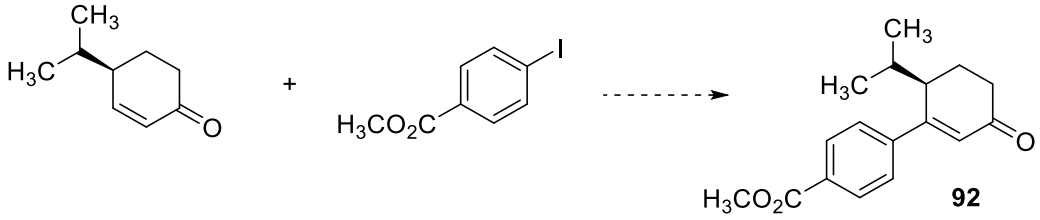


Figure 2.15.2 Potential synthesis of potential analogue **92**

Table 2.15-1 Summary of reduction of neuron firing at **60Hz** by isophorone **8** and the natural product **90** during in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
8	--	--	-22	13
90	-22	13	-36	7

Table 2.15-2 Summary of reduction of neuron firing at **20Hz** by isophorone **8** and the natural product **90** during in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
8	--	--	-15	12
90	5	26	-24	8

5-Phenylcyclphexan-1,3-dione, **91**, is commercially available. In view of the activity observed for 5,5-dimethyl-cycohexane-1,3-dione, (dimedone), **26**, it will be recommended that **91** itself should be tested and that at least four compounds, **93**-**96**, should be prepared in this series. All of these compounds should be available using the dione **91** as a starting material. Together, this group of compounds should indicate whether cyclohexenones carrying a 5-aryl substituent in place of the 5,5-methyl substituents common to most of the compounds described in this thesis are worth pursuing for their application to the treatment of epilepsy.

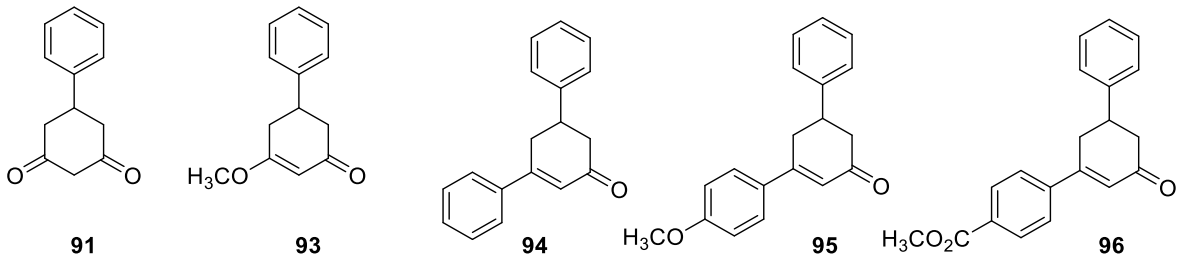
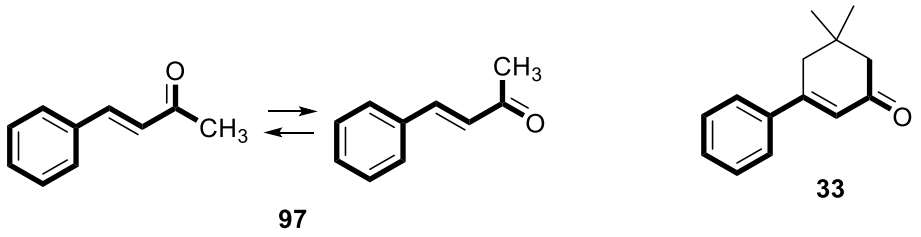


Figure 2.15.3 Structures of 5-aryl substituted analogs

Finally, we submitted the simple acyclic enone, 4-phenyl-but-3-en-2-one, **97**, for the relevant bioassays. This compound is compared structurally to 3-phenyl-5,5-dimethyl-cyclohex-2-en-1-one **33**, below. It is recognized that **97** could also be drawn as one of two rotamers, which only one overlaps readily with **33**.



97 **33**

Figure 2.15.4 Structure of open chain analogue **97** and comparison with **33**

Table 2.15-3 Summary of reduction of neuron firing at **60 Hz** by **97** and **33** during in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
97	-22	8	-26	5
33	--	--	-36	7

Table 2.15-4 Summary of reduction of neuron firing at **20 Hz** by **97** and **33** during in vitro bioassays on brain cell slices

Compound	Average reduction at 50 nM (%)	SEM at 50 nM (%)	Average reduction at 200 nM (%)	SEM at 200 nM (%)
97	-13	21	-20	12
33	--	--	29	34

The observation of a significant reduction of neuronal firing at both 60 and 20 Hz stimulation suggests that it might be worthwhile to consider exploring a number of open chain enone analogs of **97** for example compounds carrying substituents in the aromatic ring (**98**) which is related to **40 (TD 561)** or additional carbon substituents at both C1 and at C3 (**99**). The

additional substituent at C3 would decrease the potential of interaction with glutathione via electrophilic addition to the enone system.

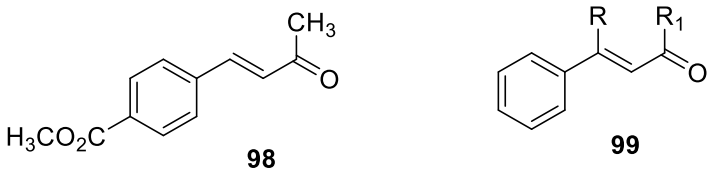


Figure 2.15.5 Structures of proposed of open chained next target analogs

3 Experimental data

Introduction

General:

All reagents were obtained from commercial sources and used without further purification. All solvents used in inert N_2 atmosphere reactions were dried prior to use. Other solvents were not dried before use. Most of the experimental details have been reported in the Owen-Barry Pharmaceuticals Inc. patent published in July 2018.[34]

NMR:

^{1}H NMR spectra were recorded in $CDCl_3$ or MeOD on a Bruker Avance II 300 MHz or Bruker Avance 400 MHz spectrometer. Chemical shifts are referenced to the residual solvent signal (δ = 7.26) for $CDCl_3$ or (δ = 3.31) for MeOD. ^{13}C NMR were recorded in $CDCl_3$ or MeOD on a Bruker Avance 400 MHz spectrometer. Chemical shifts are referenced to the residual solvent signal (δ = 77.16) for $CDCl_3$ or (δ = 49.0) for MeOD. The spectroscopic data for known compounds was compared with that reported in the literature; only the reference is supplied. Other compounds not found in the literature do not have an associated reference.

HRMS:

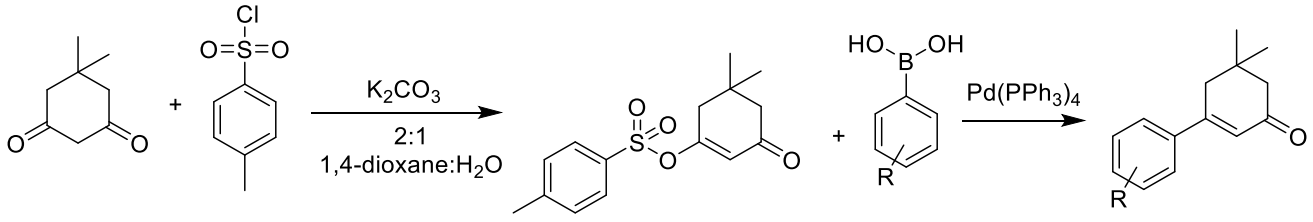
To obtain the high-resolution mass of compounds, the Kratos Concept - Magnetic sector Electron impact mass spectrometer, located in the John L. Holmes Mass Spectrometry Facility

at the University of Ottawa, was used. It was decided that HRMS data would only be obtained for compounds of interest to OB Pharma. The combination of 1H and 13 C NMR and the method of synthesis were considered sufficient evidence to support the structure assignments for patent purposes.

TLC:

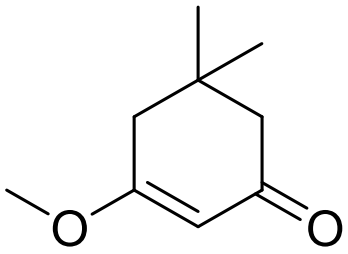
Thin layer chromatography was used to analyze the product formation during reactions and to assist with the isolation during column chromatography. Reaction mixtures and compound mixtures to isolate were deposited on thin layers silica attached to aluminum plates and eluted with the desired elution mixture of available solvents (DCM, EtOAc, hexanes). The eluted plates were visualized using UV light first to identify UV active compounds and secondly using the multi-purpose Hanessian's stain.

General Procedure #1



p-Toluenesulfonyl chloride (1.6 equivalents) was added to a mixture of dimedone (1.2 equivalents) and potassium carbonate (3 equivalents) in a 2:1 ratio of 1,4-dioxane and water. This mixture was stirred at room temperature for 2 hours. The appropriate phenyl boronic acid

(1 equivalent) and tetrakis(triphenylphosphine)palladium (0) (3% equivalents) were added and the mixture was heated under reflux for 4 hours or until completion. The resulting mixture was extracted with EtOAc. The extract was dried with $MgSO_4$, filtered and evaporate via rotary evaporator. The product was isolated via column chromatography. If further purification was required, a subsequent crystallization was performed to obtain white crystals.

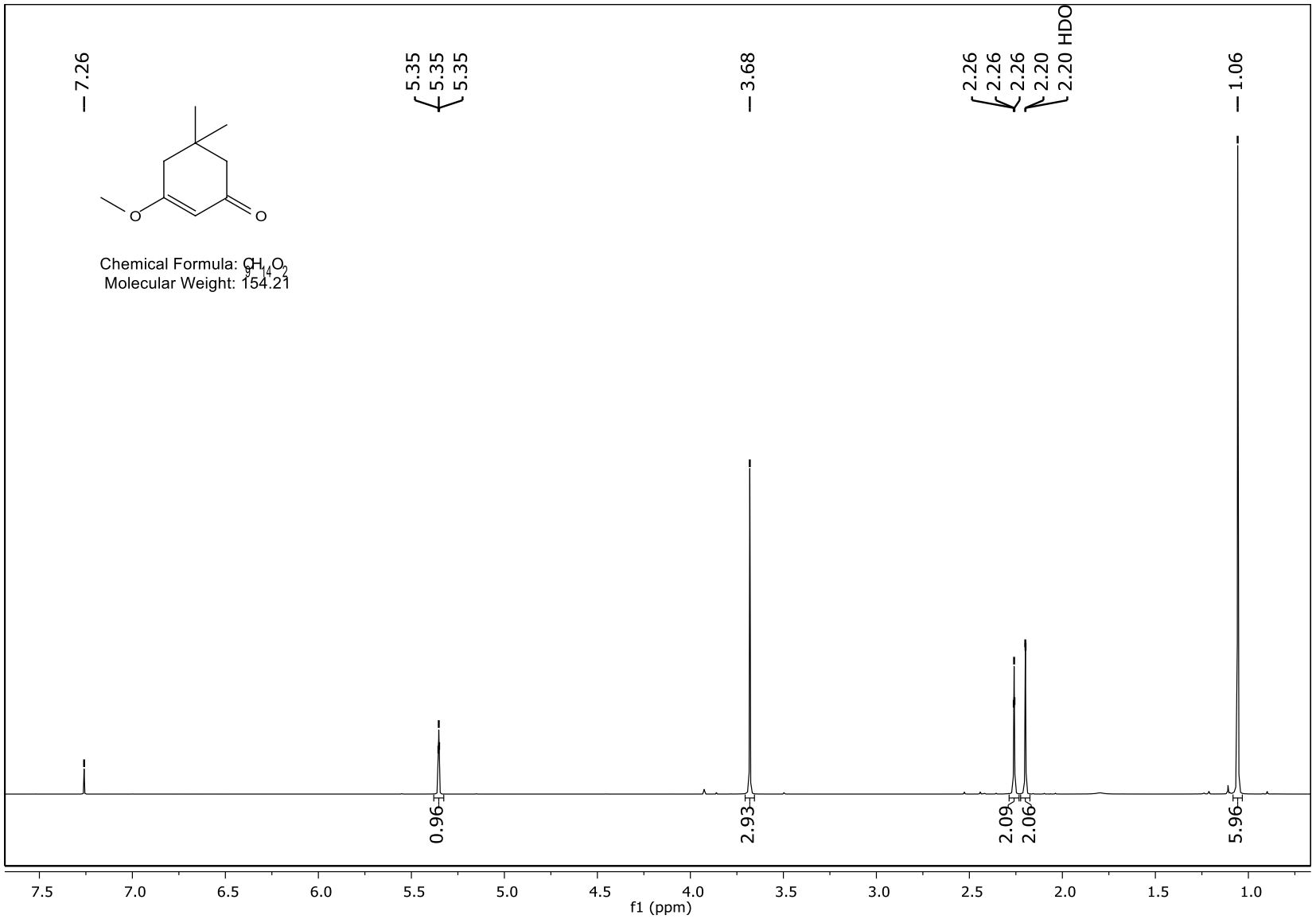


28

A mixture of dimedone (100 mg, 0.71 mmol), potassium carbonate (775 mg, 5.6 mmol) and CH_3I (0.18 mL, 2.80 mmol) in THF (15 mL) was stirred at room temperature for 24 hours. THF was evaporated via rotary evaporator and replaced with water. The mixture was extracted with EtOAc (3 x 10 mL), dried with $MgSO_4$, filtered and evaporated. The product **28** was isolated by column chromatography to obtain yellow crystals (20 mg, 18%) using a 35:65 EtOAc:hexanes eluent mixture.

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 5.33 (s, 1H), 3.66 (s, 3H), 2.24 (s, 2H), 2.18 (s, 2H), 1.04 (s, 6H)
^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.47, 176.97, 101.14, 55.66, 50.76, 42.69, 32.51, 28.27 (2C)

The ^{1}H and ^{13}C NMR data closely matches those reported in the literature[35].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5.35
5.35
5.35
3.68
2.26
2.26
2.26
2.20
2.20 HDO
1.06
O
O
Chemical Formula: C9H14O2
Molecular Weight: 154.21
0.96
2.93
2.09
2.06
5.96
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
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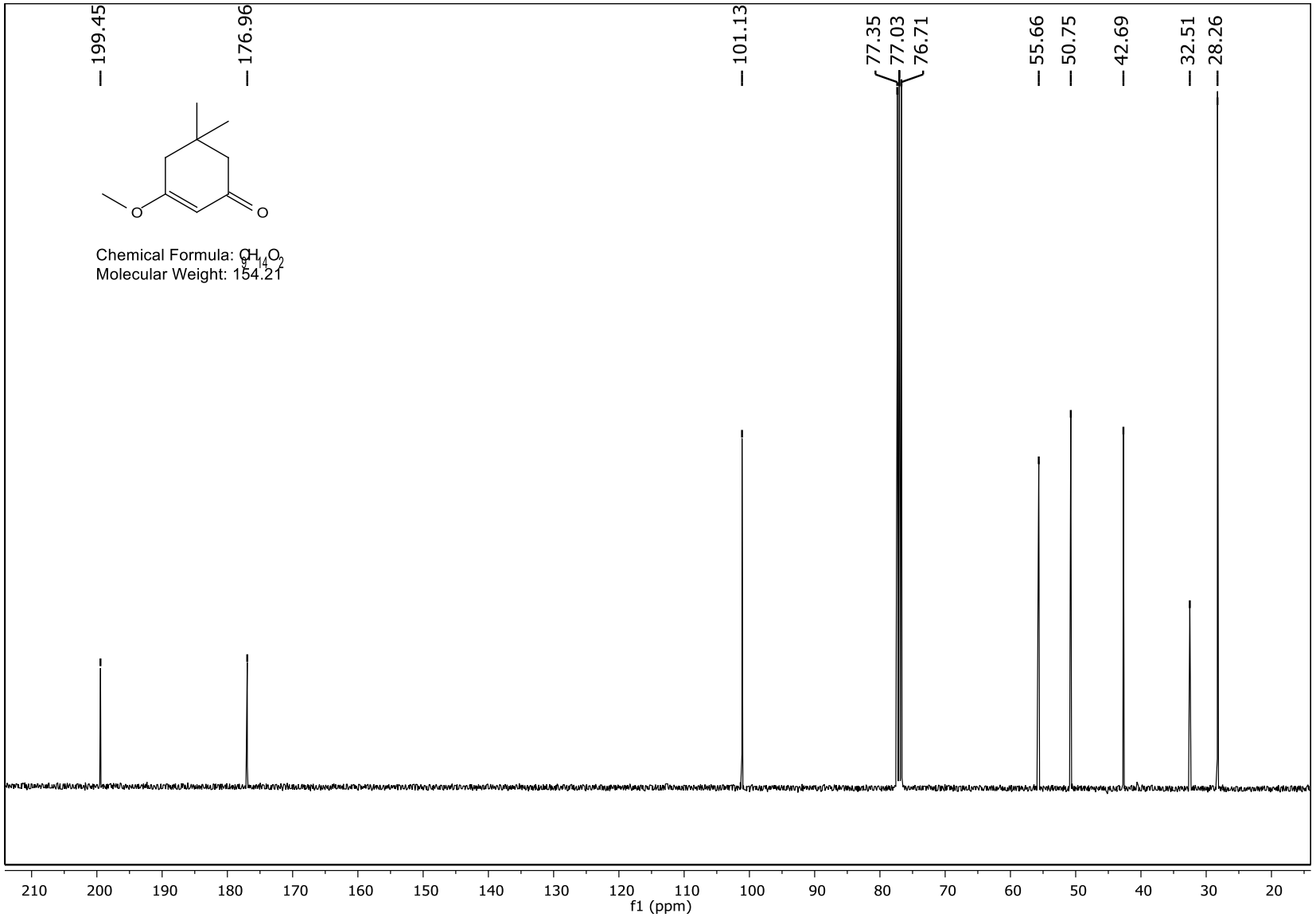
2.0
1.5
1.0
f1 (ppm)


199.45
176.96
101.13
77.35
77.03
76.71
55.66
50.75
42.69
32.51
28.26
O
O
Chemical Formula: C9H14O2
Molecular Weight: 154.21
210
200
190
180
170
160
150
140
130
120
110
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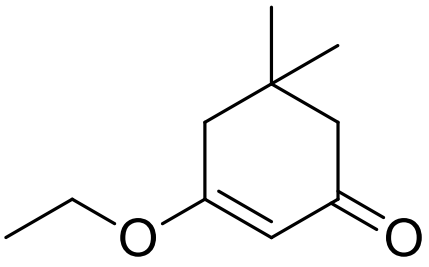
90
80
70
60
50
40
30
20
f1 (ppm)



29

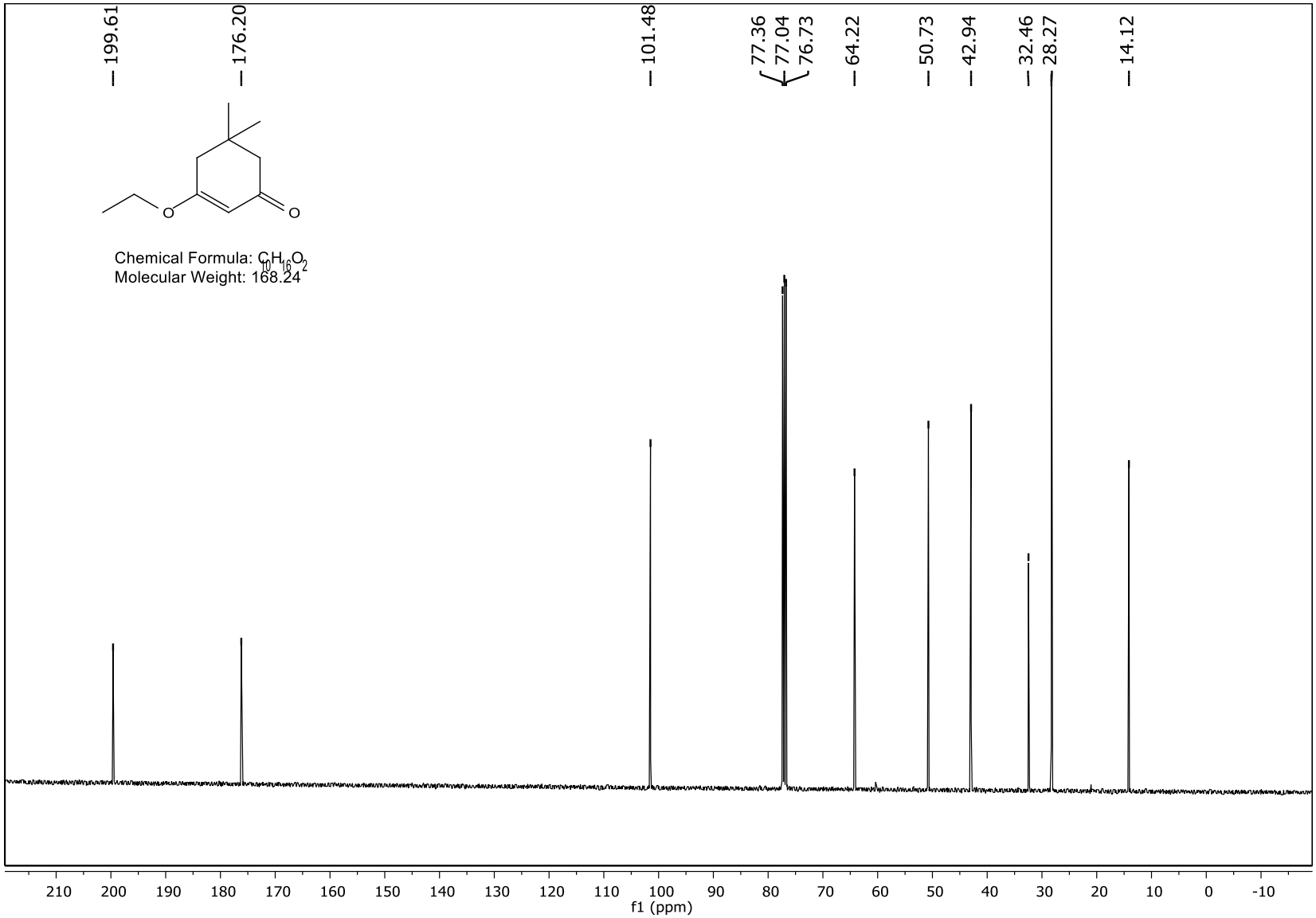
A mixture of dimedone (5.0 g, 35.67 mmol), absolute ethanol (6.7 mL, 114.93 mmol) and *p*-toluenesulfonic acid (200 mg, catalytic) in benzene was refluxed for 8 hours. The mixture was quenched using an aqueous $NaHCO_3$ solution and extracted with EtOAc (3 x 10 mL). The resulting extract was dried with $MgSO_4$, filtered and evaporated. The product **29** was isolated by column chromatography to obtain yellowish crystals (3.78g, 63%) using a 3:7 EtOAc : hexanes eluent mixture.

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 5.32 (s, 1H), 3.88 (q, *J* = 7.04 Hz, 2H), 2.25 (s, 2H), 2.19 (s, 2H), 1.34 (t, *J* = 7.04 Hz, 3H), 1.05 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.58, 176.16, 101.44, 64.17, 50.68, 42.90, 32.41, 28.22 (2C), 14.07

The ^{1}H and ^{13}C NMR data closely matches those reported in the literature[36].

7.26
5.32
3.91
3.89
3.87
3.85
2.25
2.19
2.18
1.36
1.34
1.32
1.05
0.96
2.10
2.00
2.06
3.12
5.76
Chemical Formula: C10H16O2
Molecular Weight: 168.24
f1 (ppm)


199.61
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101.48
77.36
77.04
76.73
64.22
50.73
42.94
32.46
28.27
14.12
O
O
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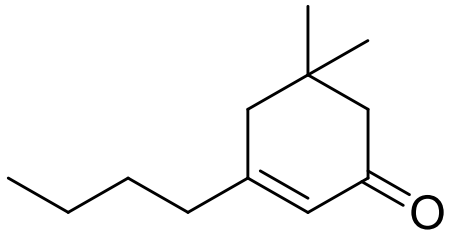
Molecular Weight: 168.24
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



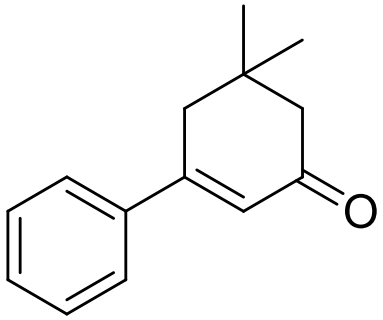
30

3-Methoxy-5,5-dimethylcyclohex-2-enone (**28**) (1.0 g, 6.48 mmol) was dissolved in dry THF at 0°C under nitrogen atmosphere. n-Butyllithium 2.5M (6.7 mL, 114 mmol) was slowly added and the solution was stirred for 20 minutes at 0°C. The mixture was quenched using an aqueous NH_4Cl solution and extracted with EtOAc (3 x 10 mL). The resulting extract was dried with $MgSO_4$, filtered and evaporated. Compound **30** was isolated by column chromatography (370 mg, 32%) using a 2.5% EtOAc in hexanes eluent mixture.

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 5.87 (s, 1H), 2.18 (m, 6H), 1.47 (m, 2H), 1.33 (qd, *J* = 14.36, 7.15 Hz, 2H), 1.02 (s, 6H), 0.91 (t, *J* = 7.28 Hz, 3H)

The ^{1}H NMR data closely matches those reported in the literature[37].

Chemical Formula: C12H20O
Molecular Weight: 180.29
f1 (ppm)

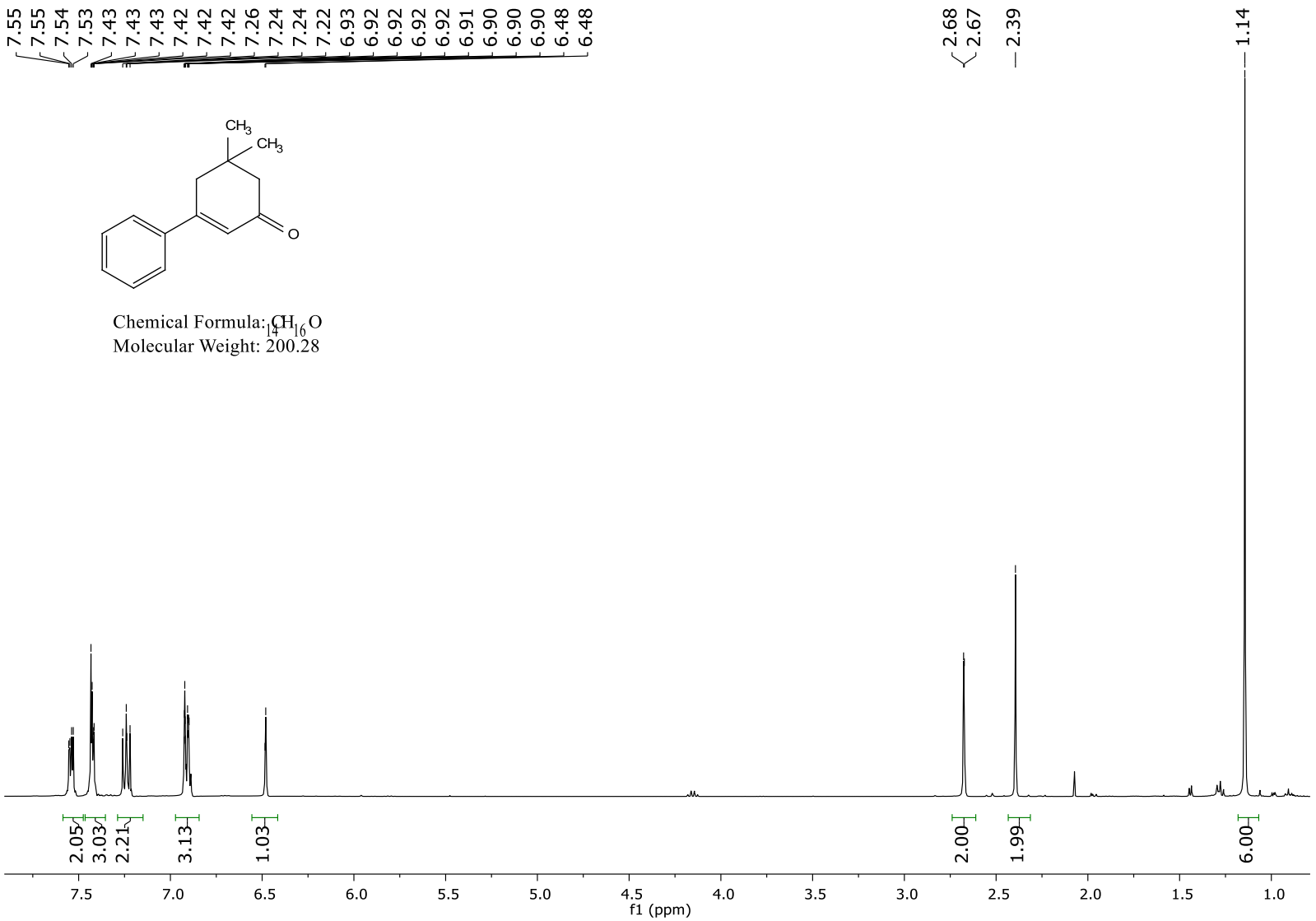


31

Magnesium turnings (0.30 g, 12.3 mmol) were added to a flask and put under nitrogen atmosphere. A solution of bromobenzene (1.50 g, 9.55 mmol) in dry diethyl ether (10 mL) was added and the mixture was lightly refluxed until all the magnesium turnings were consumed. A solution of 3-methoxy-5,5-dimethylcyclohex-2-enone (**28**) (0.50 g, 3.24 mmol) in diethyl ether (10 mL) was added at room temperature and the mixture was refluxed again for 30 minutes. The reaction was quenched using water and a saturated aqueous solution of NH_4Cl. The mixture was extracted with EtOAc, dried with $MgSO_4$, filtered and evaporated. The product **31** was isolated by column chromatography to obtain a white solid. (260 mg, 40 %) using a 5% EtOAc in hexanes eluent mixture.

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.54 (ddd, *J* = 4.48, 2.39, 1.40 Hz, 2H), 7.43-7.40 (m, 3H), 6.42 (t, *J* = 1.50, 1.50 Hz, 1H), 2.66 (d, *J* = 1.46 Hz, 2H), 2.35 (s, 2H), 1.14 (s, 6H)

The ^{1}H NMR data closely matches those reported in the literature[38].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7.43
7.42
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7.22
6.93
6.92
6.92
6.92
6.92
6.91
6.90
6.90
6.90
6.48
6.48
2.68
2.67
2.39
1.14
CH3
CH3
O
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Molecular Weight: 200.28
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3.03
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3.13
1.03
2.00
1.99
6.00
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7.0
6.5
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3.5
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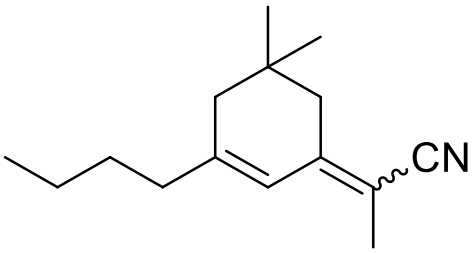
2.0
1.5
1.0
f1 (ppm)



32

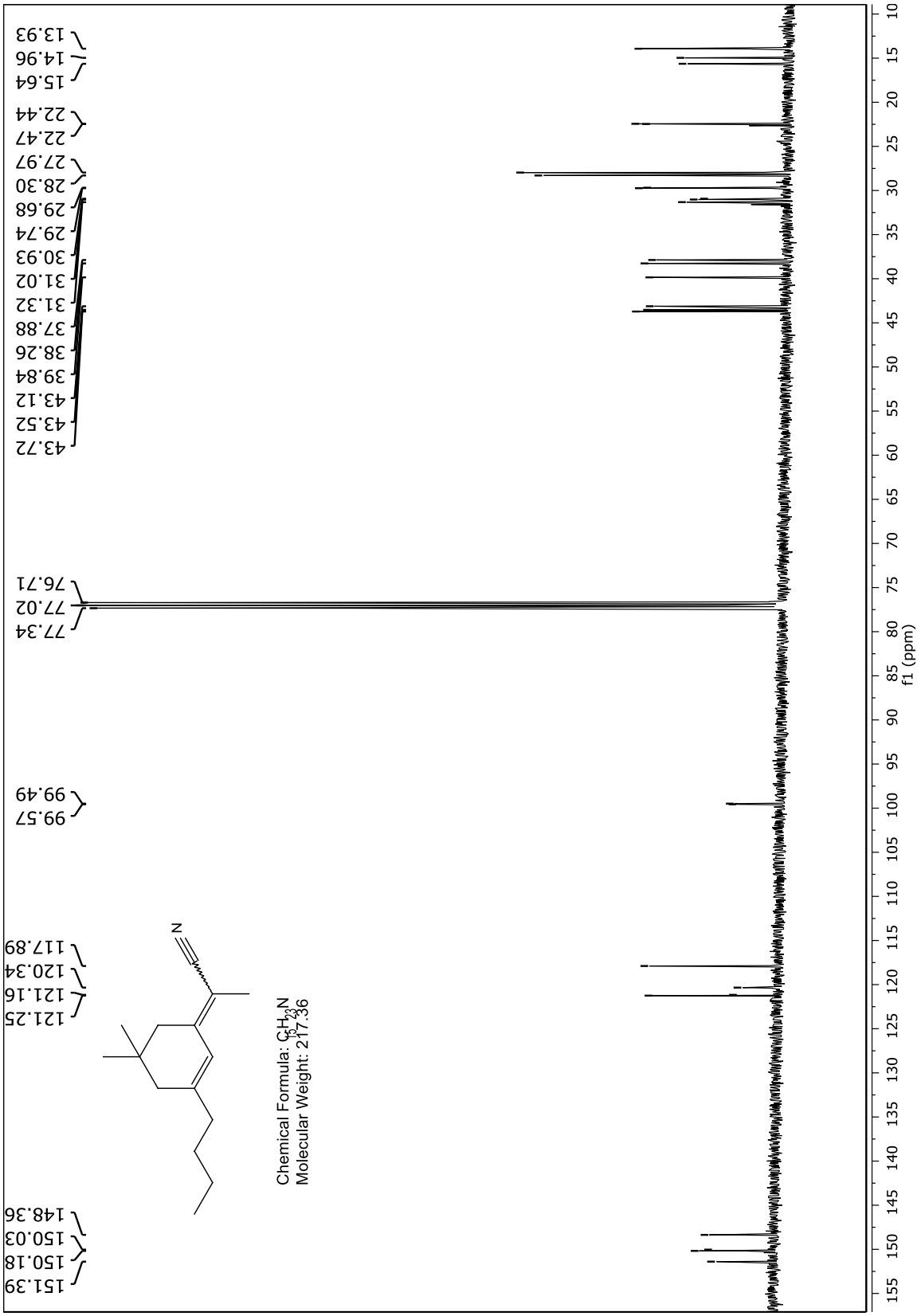
Lithium diisopropylamide 2M (2.05 mL, 4.10 mmol) was added to dry THF (15 mL) under nitrogen atmosphere at -78°C. Propionitrile (270 mg, 4.92 mmol) was added dropwise and the solution was stirred at -78°C for 15 minutes. 3-butyl-5,5-dimethylcyclohex-2-enone (**30**) (370 mg, 2.05 mmol) was added dropwise and the solution was stirred for 30 minutes. The mixture was quenched by transferring it to a beaker containing an aqueous NH_4Cl solution. The mixture was then warmed at room temperature and diluted with water followed by extraction with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and concentrated by rotary evaporator. The hydroxyl intermediate was isolated by column chromatography.

The hydroxyl intermediate was dissolved in toluene and *p*-toluenesulfonic acid (100 mg, catalytic) was added. The mixture was refluxed for 6 hours and was quenched by adding an aqueous $NaHCO_3$ solution. The mixture was extracted with EtOAc (3 x 10 mL) and the extract was dried with $MgSO_4$, filtered and evaporated by rotary evaporator. **32** was isolated by column chromatography (170 mg, 38%) using a 5% EtOAc in hexanes eluent mixutre.

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 6.44 (s, 1/2H), 6.17 (s, 1/2H), 2.35 (d, *J* = 1.35 Hz, 1H), 2.14-2.07 (m, 3H), 1.94 (m, 5H), 1.47-1.37 (m, 2H), 1.36-1.24 (m, 2H), 0.93 (s, 6H), 0.90 (m, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 151.38, 150.16, 150.02, 148.35, 121.23, 121.14, 120.32, 117.87, 99.55, 99.47, 43.69, 43.50, 43.09, 39.82, 38.24, 37.85, 31.29, 31.00, 30.90, 29.71, 29.65, 28.27 (2C), 27.94 (2C), 22.43, 15.61, 14.94, 13.99, 13.62

Chemical Formula: C16H23N
Molecular Weight: 217.36
N
f1 (ppm)


151.39
150.18
150.03
148.36
121.25
121.16
120.34
117.89
99.57
99.49
77.34
77.02
76.71
43.72
43.52
43.12
39.84
38.26
37.88
31.32
31.02
30.93
29.74
29.68
28.30
27.97
22.47
22.44
15.64
14.96
13.93
N
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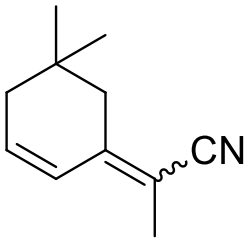
Molecular Weight: 217.36
f1 (ppm)



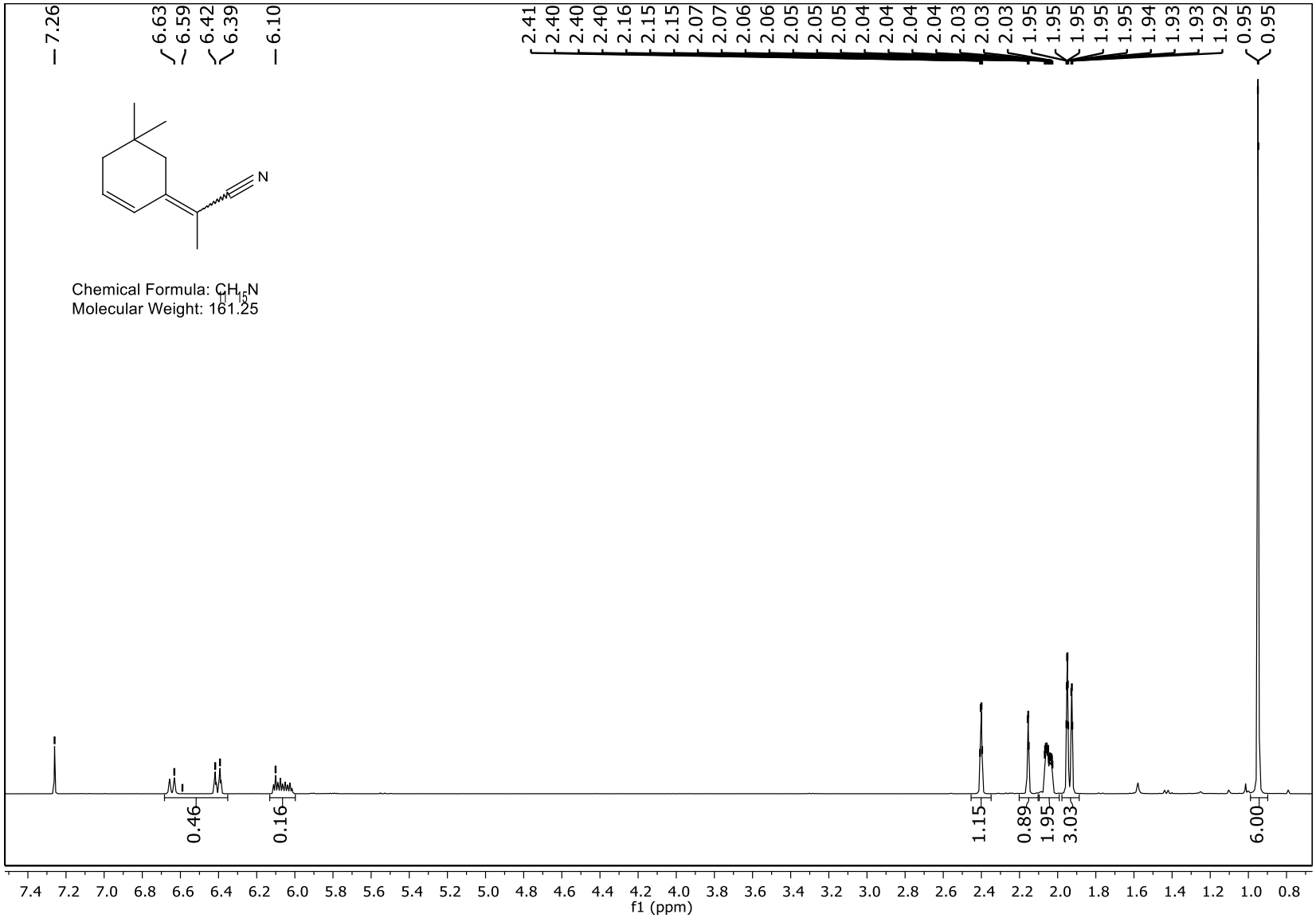
33

Lithium diisopropylamide 2M (3.22 mL, 6.44 mmol) was added to dry THF (15 mL) under nitrogen atmosphere at -78°C. Propionitrile (426 mg, 7.73 mmol) was added dropwise and the solution was stirred at -78°C for 15 minutes. 5,5-dimethylcyclohex-2-enone (400 mg, 3.22 mmol) was added dropwise and the solution was stirred for 30 minutes. The mixture was quenched by transferring it to a beaker containing an aqueous NH_4Cl solution. The mixture was then warmed at room temperature and diluted with water followed by extraction with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and concentrated by rotary evaporator. The hydroxyl intermediate was isolated by column chromatography.

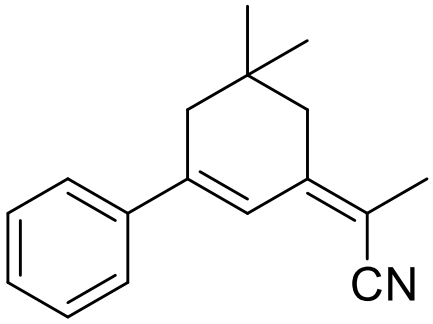
The intermediate alcohol was dissolved in toluene and *p*-toluenesulfonic acid (100 mg, catalytic) was added. The mixture was refluxed for 6 hours and was quenched by adding an aqueous $NaHCO_3$ solution. The mixture was extracted with EtOAc (3 x 10 mL) and the extract was dried with $MgSO_4$, filtered and evaporated by rotary evaporator. **33** was isolated by column chromatography (230 mg, 44%) using a 5:25:70 eluent mixture of EtOAc : DCM : hexanes.

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 6.52 (tdd, *J* = 96.04, 10.06, 2.06 Hz, 1H), 6.12-6.00 (m, 1H), 2.40 (d, *J* = 1.26 Hz, 1H), 2.15 (s, 1H), 2.08-2.02 (m, 2H), 1.94 (d, *J* = 8.98 Hz, 3H), 0.95 (s, 6H),

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 150.45, 149.17, 136.00, 134.28, 125.90, 122.43, 120.52, 119.74, 102.29, 102.13, 43.62, 40.14, 39.89, 39.51, 31.03, 30.68, 28.33 (2C), 28.00 (2C), 15.57, 15.06


7.26
6.63
6.59
6.42
6.39
6.10
2.41
2.40
2.40
2.40
2.16
2.15
2.15
2.07
2.07
2.06
2.06
2.05
2.05
2.05
2.04
2.04
2.04
2.04
2.03
2.03
2.03
1.95
1.95
1.95
1.95
1.95
1.94
1.93
1.93
1.92
0.95
0.95
N
Chemical Formula: C11H15N
Molecular Weight: 161.25
0.46
0.16
1.15
0.89
1.95
3.03
6.00
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8
f1 (ppm)

150.46
149.19
136.02
134.30
125.91
122.45
120.54
119.76
102.31
102.15
77.34
77.23
77.02
76.71
43.64
40.16
39.92
39.53
31.06
30.71
28.35
28.03
15.60
15.09
N
Chemical Formula: C11H15N
Molecular Weight: 161.25
f1 (ppm)



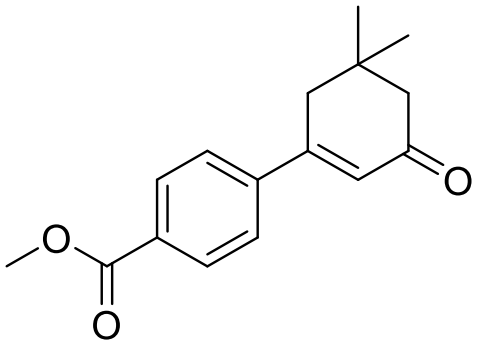
34

Lithium diisopropylamide 2M (0.8 mL, 1.5 mmol) was added to dry THF (15 mL) under nitrogen atmosphere at -78°C. Propionitrile (125 mg, 1.8 mmol) was added dropwise and the solution was stirred at -78°C for 15 minutes. **31** (150 mg, 0.75 mmol) was added dropwise and the solution was stirred for 30 minutes. The mixture was quenched by transferring it to a beaker containing an aqueous NH_4Cl solution. The mixture was then warmed at room temperature and diluted with water followed by extraction with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and concentrated by rotary evaporator. The hydroxyl intermediate was isolated by column chromatography.

The hydroxyl intermediate was dissolved in toluene and *p*-toluenesulfonic acid (100 mg, catalytic) was added. The mixture was refluxed for 6 hours and was quenched by adding an aqueous $NaHCO_3$ solution. The mixture was extracted with EtOAc (3 x 10 mL) and the extract was dried with $MgSO_4$, filtered and evaporated by rotary evaporator. **34** was isolated by column chromatography (80 mg, 45%) using a 10% EtOAc in hexanes eluent mixture.

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.51 (s, 3H), 7.37 (dd, *J* = 6.6, 5.1 Hz, 2H), 2.52 – 2.42 (m, 3H), 2.23 (d, *J* = 1.1 Hz, 1H), 2.05 (d, *J* = 3.2 Hz, 3H), 1.11 (s, 1H), 1.04 (d, *J* = 1.2 Hz, 6H)

7.51
7.49
7.49
7.45
7.45
7.37
7.36
7.36
7.35
7.35
7.34
7.33
7.33
7.32
7.24
4.11
4.09
2.47
2.47
2.45
2.43
2.42
2.03
2.03
1.98
1.02
1.02
CH3
CH3
CH3
N
Chemical Formula: C17H19N
Molecular Weight: 237.35
2.26
3.92
0.65
0.59
1.01
3.06
2.52
1.58
6.00
f1 (ppm)



40 (TD 561)

First synthesis (1 pot reaction):

p-Toluenesulfonyl chloride (1.65 g, 8.67 mmol) was added to a mixture of dimedone (935 mg, 6.67 mmol) and potassium carbonate (2.31 g, 16.7 mmol) in a 2:1 ratio of 1,4-dioxane (16 mL) and water (8 mL). This mixture was stirred at room temperature for 2 hours. 4-methoxycarbonylphenylboronic acid (1.35 g, 8.56 mmol) and tetrakis(triphenylphosphine)palladium (0) (231 mg, 0.20 mmol) were added and the mixture was heated under reflux for 4 hours or until completion. The resulting mixture was extracted with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and evaporated via rotary evaporator. The product **40** was isolated via column chromatography using a 5% EtOAc in DCM eluent mixture. A subsequent crystallization was performed to obtain light-yellow crystals. (0.71 g, 41 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.09-8.00 (m, 2H), 7.63-7.48 (m, 2H), 6.44 (t, *J* = 1.40 Hz, 1H), 3.93 (s, 3H), 2.66 (d, *J* = 1.40 Hz, 2H), 2.36 (s, 2H), 1.14 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.78, 166.43, 156.24, 143.44, 131.16, 129.95 (2C), 126.12 (2C), 125.74, 52.30, 50.93, 42.30, 33.82, 28.38 (2C).

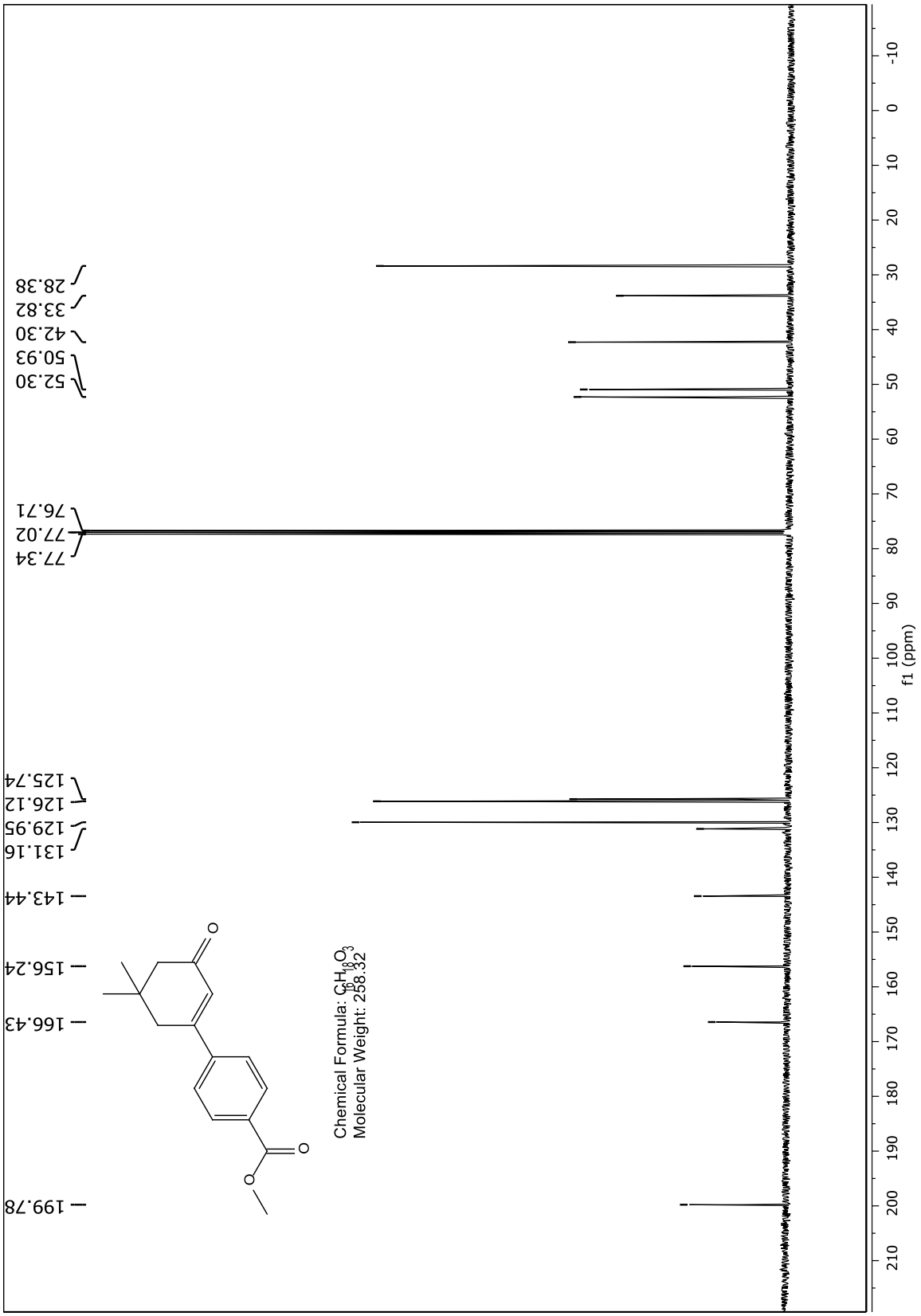
HRMS: Calculated for C16H18O3: 258.1256 **Found:** 258.1264

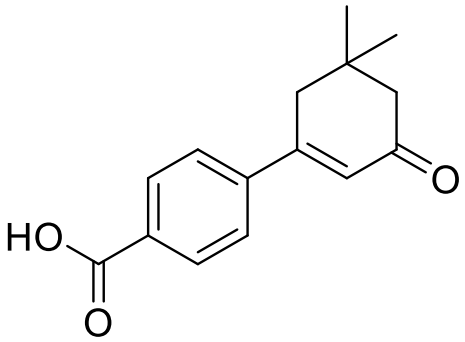
Second synthesis (2 step):

p-Toluenesulfonyl chloride (9.90 g, 52 mmol) was added to a mixture of dimedone (5.61 g, 40 mmol) and potassium carbonate (13.8 g, 100 mmol) in a 2:1 ratio of 1,4-dioxane (60 mL) and water (30 mL). This mixture was stirred at room temperature for 15 hours. The reaction mixture was diluted with 100 mL of water and extracted with 50 mL of DCM twice. The combined organic layer was washed with 50 mL of saturated NH_4Cl solution, dried with $MgSO_4$ and concentrated via rotary evaporator to isolate the tosylate intermediate (7.0 g, 23.8 mmol)

The tosylate intermediate (6.0 g, 20.4 mg), 4-methoxycarbonylphenylboronic acid (3.35 g, 18.6 mmol), potassium carbonate (5.64 g, 40.8 mmol) and tetrakis(triphenylphosphine)palladium (0) (707 mg, 0.612 mmol) were added and the mixture was heated under reflux for 3 hours or until completion. The resulting mixture was diluted with 50 mL of water and extracted with DCM (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and evaporated via rotary evaporator. The product **40** was isolated via crystallization. (2.7 g, 55%).

8.05
8.04
8.03
8.03
7.56
7.56
7.55
7.54
7.24
6.41
6.41
6.41
3.91
2.63
2.63
2.33
1.11
1.88
1.93
1.03
3.09
2.12
2.05
6.00
Chemical Formula: C16H18O3
Molecular Weight: 258.32
f1 (ppm)


199.78
166.43
156.24
143.44
131.16
129.95
126.12
125.74
77.34
77.02
76.71
52.30
50.93
42.30
33.82
28.38
Chemical Formula: C15H18O3
Molecular Weight: 258.32
f1 (ppm)



48

Methyl 3',3'-dimethyl-5'-oxo-2',3',4',5'-tetrahydro-[1,1'-biphenyl]-4-carboxylate (**40**) (50 mg, 0.19 mmol) was dissolved in methanol (4 mL) and a 5% sodium hydroxide aqueous solution was added (1 mL). This mixture was stirred at room temperature until disappearance of starting material was observed by TLC. When complete, half of the methanol solvent was evaporated using a rotary evaporator and water (5 mL) was added. The mixture was extracted with EtOAc (3 x 5 mL) and the organic extract was discarded. The aqueous phase was treated with a 5% hydrochloric acid aqueous solution (1 mL) and the mixture was extracted with EtOAc (3 x 5 mL). This second organic extract was dried with $MgSO_4$, filtered and evaporated to obtain **48** as a white solid. (40 mg, 86 %)

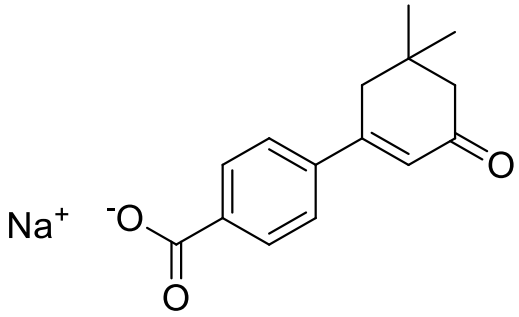
^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.15 (d, *J* = 8.49 Hz, 2H), 7.62 (d, *J* = 8.51 Hz, 2H), 6.47 (t, *J* = 1.31 Hz, 1H), 2.67 (d, *J* = 1.31 Hz, 2H), 2.38 (s, 2H), 1.15 (s, 6H)

^{13}C NMR (100 MHz, MeOD) δ, ppm: 201.14, 167.69, 158.32, 143.30, 131.80, 129.74 (2C), 126.06 (2C), 124.62, 50.28, 41.69, 33.33, 27.01 (2C).

HRMS: Calculated for C15H16O3: 244.1099 **Found:** 244.1095

8.16
8.13
7.63
7.61
7.61
7.26
6.46
2.68
2.67
2.38
1.16
1.60
1.52
0.62
2.10
2.11
6.00
Chemical Formula: C15H16O3
Molecular Weight: 244.29
O
O
OH
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

27.01
33.33
41.69
46.97
47.18
47.40
47.61
47.82
47.89
48.03
48.11
48.25
50.28
124.62
126.06
129.74
131.80
143.30
158.32
167.69
201.14
Chemical Formula: C13H16O3
Molecular Weight: 244.29
f1 (ppm)



50

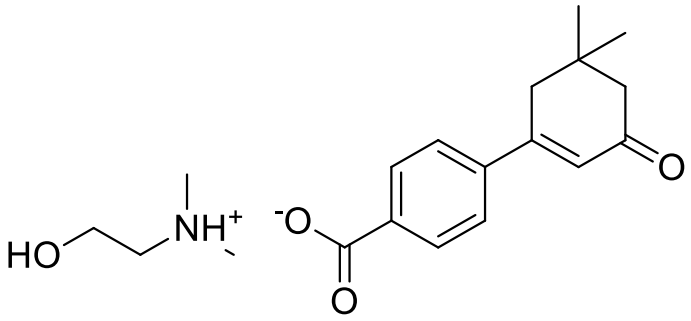
3',3'-dimethyl-5'-oxo-2',3',4',5'-tetrahydro-[1,1'-biphenyl]-4-carboxylic acid (**48**) (0.1 g, 0.41 mmol) was dissolved in dichloromethane (10 mL) at room temperature. Sodium bis(trimethylsilyl)amide 1M (0.41 mL, 0.41 mmol) was added dropwise and a white precipitate was immediately observed. All the solvent was evaporated using a rotary evaporator and diethyl ether (10 mL) was added to the mixture. The product **50** was obtained as a fine beige powder by filtering the suspension. (96.5 mg, 88 %)

^{1}H NMR (400 MHz, MeOH-D_4) δ, ppm: 7.97 (d, *J* = 8.19 Hz, 2H), 7.60 (d, *J* = 8.20 Hz, 2H), 6.39 (s, 1H), 2.73 (s, 2H), 2.33 (s, 2H), 1.11 (s, 6H)

^{13}C NMR (100 MHz, MeOH-D_4) δ, ppm: 202.85, 174.01, 160.76, 142.17, 140.15, 130.77 (2C), 126.96 (2C), 125.14, 51.76, 43.20, 34.75, 28.48 (2C)

8.01
7.99
7.64
7.62
6.42
2.76
2.76
2.36
1.14
2.00
1.98
0.48
2.10
2.08
6.00
Na+
O
O
O-
Chemical Formula: C15H13NaO3
Molecular Weight: 266.27
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

205.31
176.48
163.23
144.64
133.24
129.43
127.61
54.24
52.17
52.03
51.96
51.81
51.74
51.53
51.32
51.11
50.89
45.68
37.23
30.97
Chemical Formula: C15H15NaO3
Molecular Weight: 266.27
Na+
f1 (ppm)



51

3',3'-dimethyl-5'-oxo-2',3',4',5'-tetrahydro-[1,1'-biphenyl]-4-carboxylic acid (**48**) (100 mg, 0.41 mmol) was dissolved in dichloromethane (10 mL). 2-Dimethylaminoethanol (36.5 mg, 0.41 mmol) in a solution of dichloromethane was added and the mixture was stirred for 10 minutes. The solvent was completely evaporated and diethyl ether (10 mL) was added to the mixture. The observed precipitated salt was filtered to obtain **51** as a fine white powder. (103 mg, 75 %)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.08 (d, *J* = 8.41 Hz, 2H), 7.55 (d, *J* = 8.40 Hz, 2H), 6.44 (t, *J* = 1.23 Hz, 1H), 4.00-3.91 (m, 2H), 3.12-3.05 (m, 2H), 2.80 (s, 6H), 2.67 (d, *J* = 1.32 Hz, 2H), 2.35 (s, 2H), 1.14 (s, 6H)

^{13}C NMR (100 MHz, MeOD) δ, ppm: 201.35, 159.22, 140.87, 138.30, 129.33 (2C), 125.54 (2C), 123.74, 59.19, 55.62, 50.30, 42.42 (2C), 41.74, 33.29, 27.03 (2C).

8.09
8.07
7.56
7.54
7.26
6.44
3.97
3.96
3.96
3.95
3.95
3.09
3.08
3.07
2.80
2.67
2.66
2.35
1.14
Chemical Formula: C19H17NO4
Molecular Weight: 323.43
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1.46
5.81
2.05
2.02
6.00
8.0
7.5
7.0
6.5
6.0
5.5
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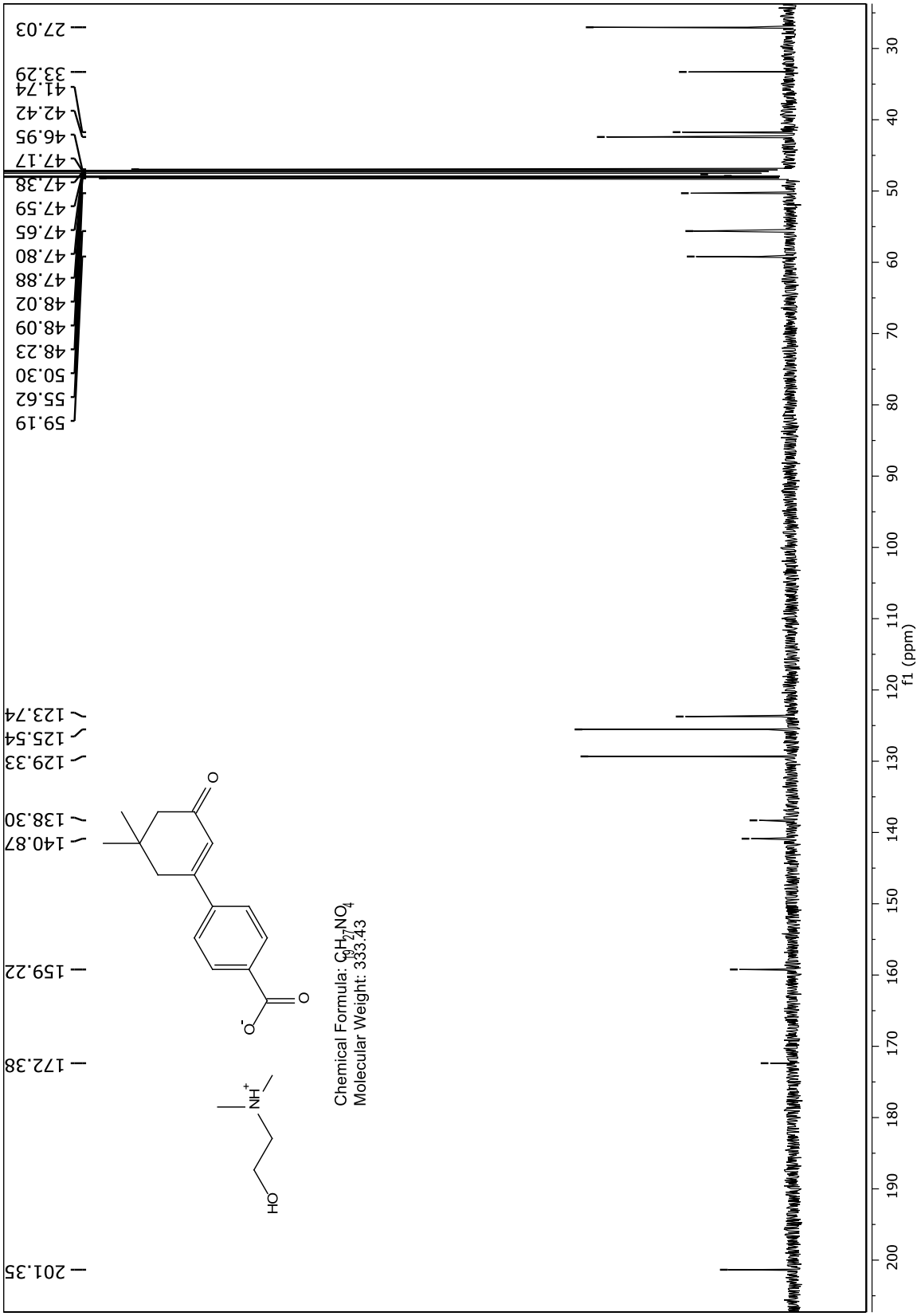
2.0
1.5
1.0
f1 (ppm)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47.88
48.02
48.09
48.23
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159.22
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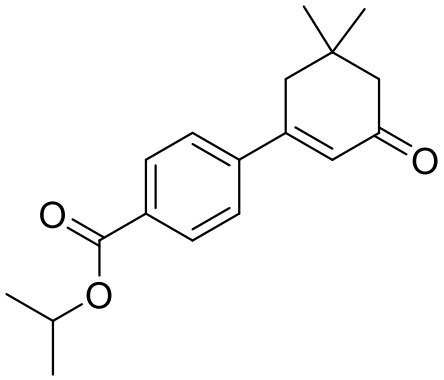
201.35
f1 (ppm)



52

48 (0.30 g, 1.22 mmol) was dissolved in a large excess of isopropyl alcohol (15mL). A catalytic amount of H_2SO_4 (1mL) was added and the mixture was refluxed for 6 hours. The reaction was quenched with $NaCO_3$ (20mL) and extracted with EtOAc. Column chromatography with 5% EtOAc in DCM followed by crystallization afforded **52** (120mg, 34%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.12 – 7.98 (m, 2H), 7.68 – 7.47 (m, 2H), 6.43 (t, *J* = 1.6 Hz, 1H), 5.26 (m, 1H), 2.65 (d, *J* = 1.6 Hz, 2H), 2.36 (s, 2H), 1.38 (d, *J* = 6.3 Hz, 6H), 1.14 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.79, 165.43, 156.36, 143.23, 131.96, 129.88 (2C), 126.02 (2C), 125.65, 68.71, 50.94, 42.31, 33.82, 28.39 (2C), 21.93 (2C).

HRMS: Calculated C18H22O3: 286.1569 **Found:** 286.1575

8.07
8.07
8.05
8.05
7.58
7.57
7.56
7.56
7.26
6.44
6.43
6.43
5.26
2.66
2.65
2.36
1.39
1.37
1.14
Chemical Formula: C18H22O3
Molecular Weight: 286.37
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2.04
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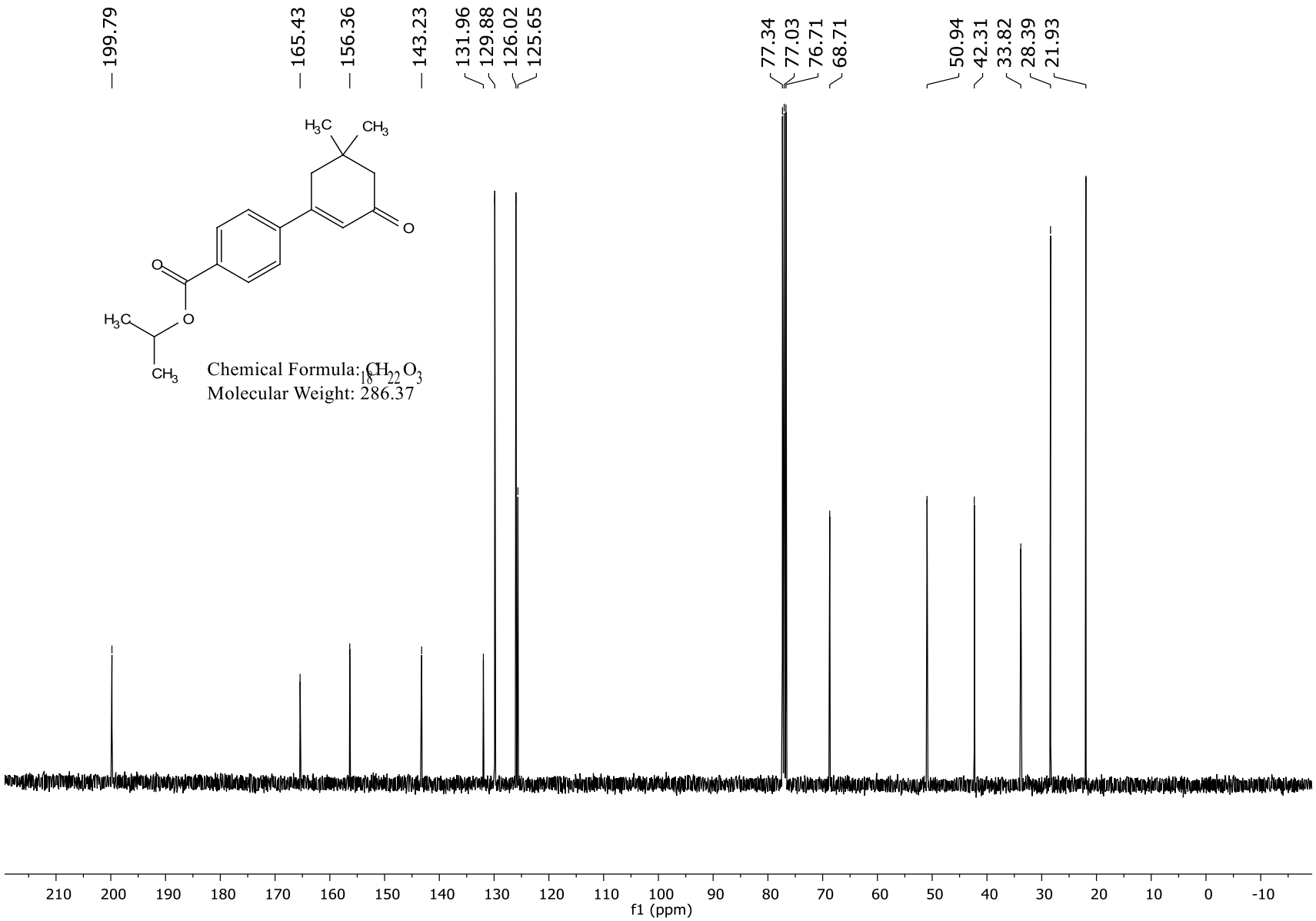
6.00
f1 (ppm)


199.79
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143.23
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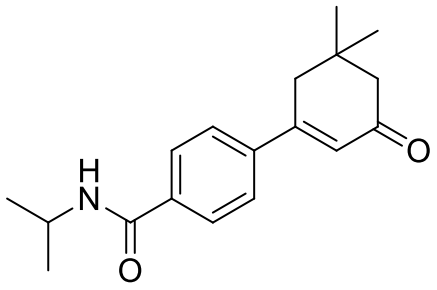
129.88
126.02
125.65
77.34
77.03
76.71
68.71
50.94
42.31
33.82
28.39
21.93
H3C
CH3
O
O
O
H3C
CH3
Chemical Formula: C18H22O3
Molecular Weight: 286.37
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

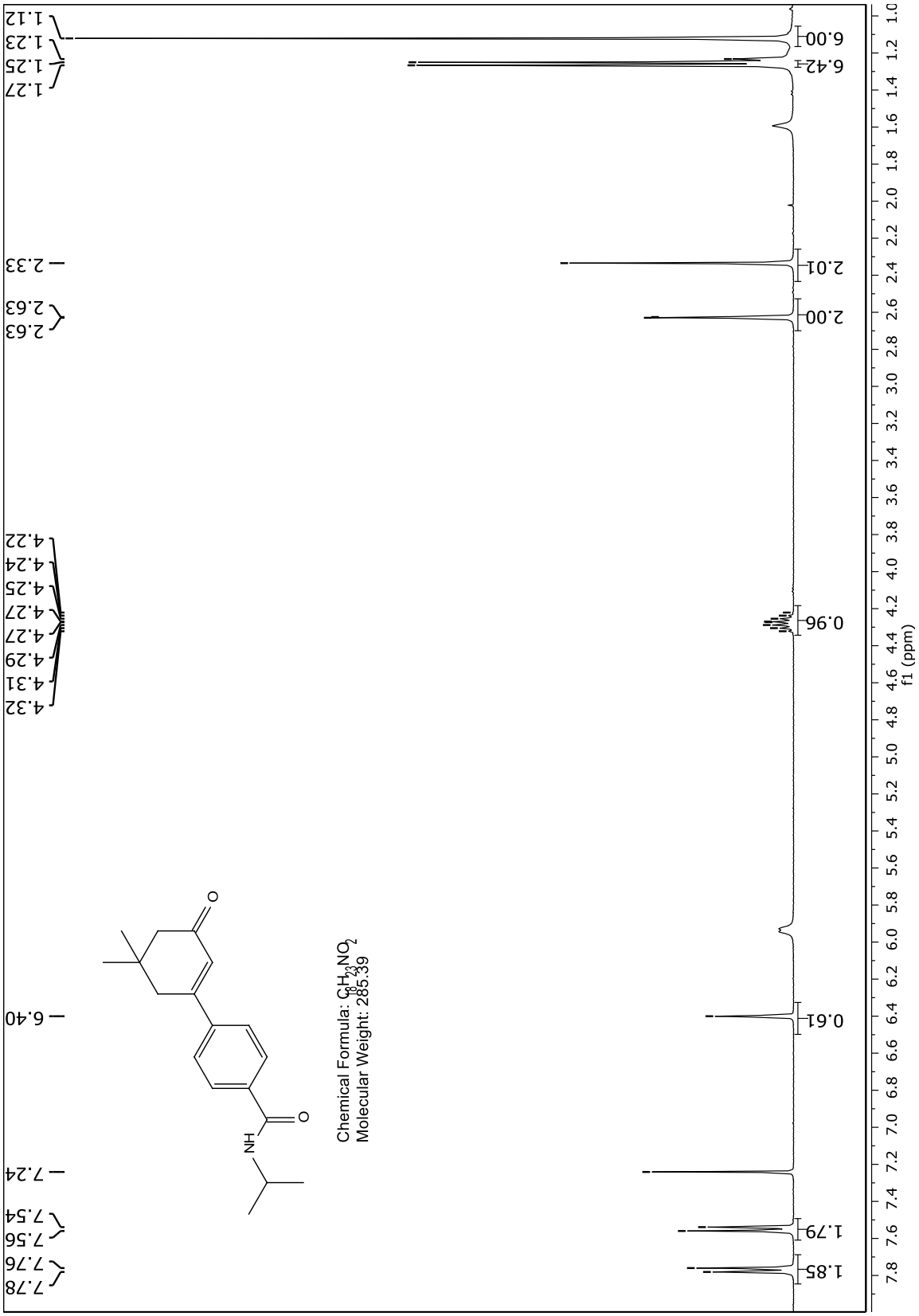


54

3',3'-dimethyl-5'-oxo-2',3',4',5'-tetrahydro-[1,1'-biphenyl]-4-carboxylic acid (**48**) (75 mg, 0.31 mmol) was added to dichloromethane (15 mL). Thionyl chloride (62.1 mg, 0.52 mmol) was added and the mixture was refluxed for 3 hours. The solvent and excess thionyl chloride were evaporated using a rotary evaporator. The flask was closed using a septum and the mixture was dissolved in benzene (15 mL) at 0^{o}C. Isopropylamine (72.7 mg, 1.23 mmol) and triethyl amine (124 mg, 1.23 mmol) were very slowly added simultaneously and the mixture was stirred for 5 minutes at 0^{o}C. The reaction was quenched with water and an aqueous solution of NH_4Cl. The mixture was extracted with EtOAc (3 x 10 mL), dried with $MgSO_4$ and filtered. The extract was treated with an aqueous solution of 5% sodium hydroxide. The product **54** was isolated by column chromatography with 30% EtOAc in hexanes to obtain a white fine powder. (50 mg, 57 %)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.79 (d, *J* = 8.37 Hz, 2H), 7.57 (d, *J* = 8.37 Hz, 2H), 6.42 (s, 1H), 5.95 (d, *J* = 6.25 Hz, 1H), 4.30 (qd, *J* = 13.30, 6.56 Hz, 1H), 2.65 (d, *J* = 1.15 Hz, 2H), 2.35 (s, 2H), 1.28 (d, *J* = 6.55 Hz, 6H), 1.14 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.84, 165.78, 156.34, 141.81, 136.00, 127.28 (2C), 126.24 (2C), 125.30, 50.89, 42.23, 42.03, 33.77, 28.36 (2C), 22.80 (2C)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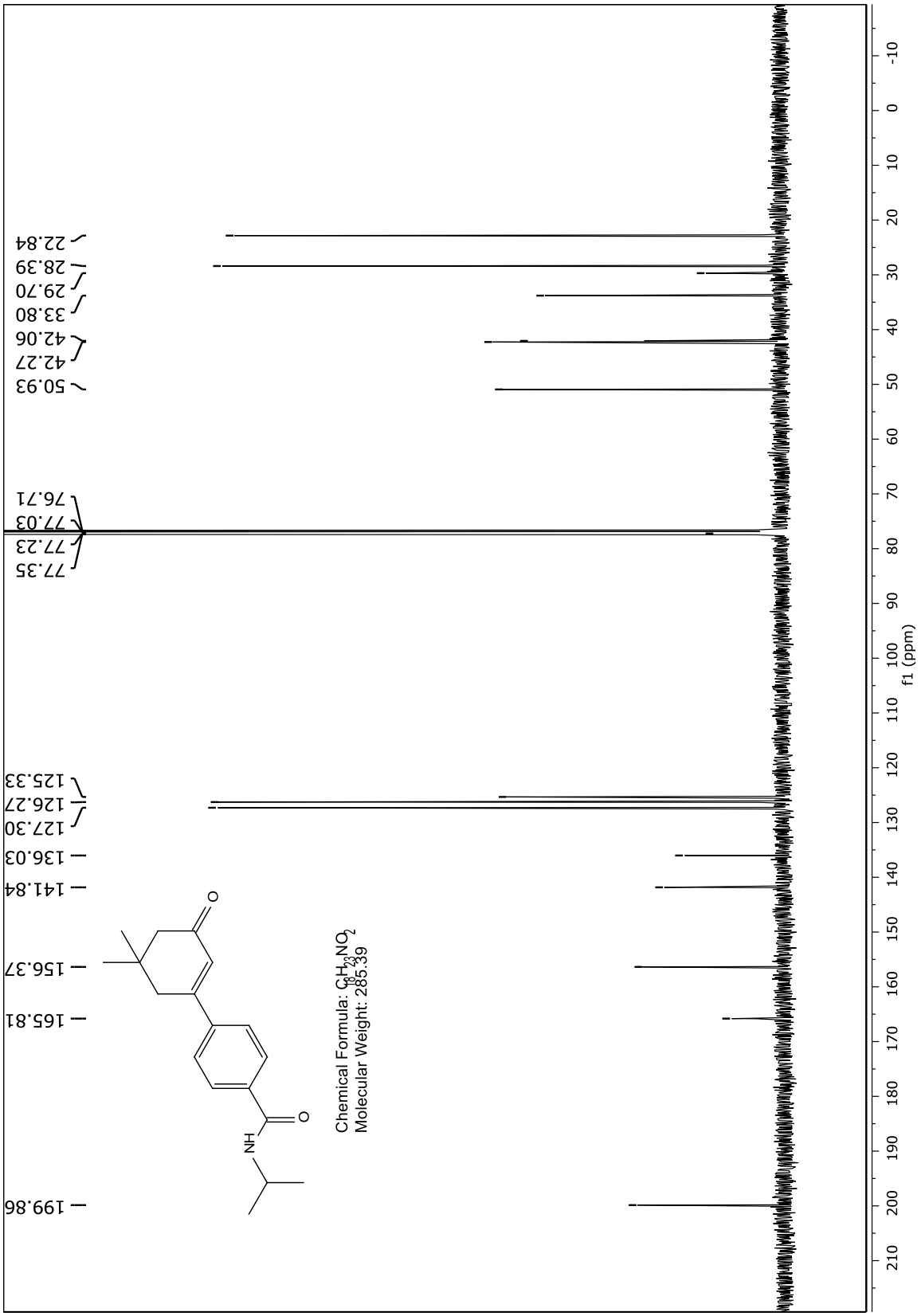
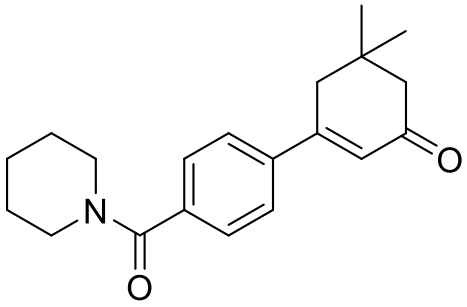
7.54
7.24
6.40
4.32
4.31
4.29
4.27
4.27
4.25
4.24
4.22
2.63
2.63
2.33
1.27
1.25
1.23
1.12
1.85
1.79
0.61
0.96
2.00
2.01
6.42
6.00
Chemical Formula: C18H23NO2
Molecular Weight: 285.39
f1 (ppm)


199.86
165.81
156.37
141.84
136.03
127.30
126.27
125.33
77.35
77.23
77.03
76.71
50.93
42.27
42.06
33.80
29.70
28.39
22.84
O
O
NH
Chemical Formula: C18H23NO2
Molecular Weight: 285.39
f1 (ppm)



55

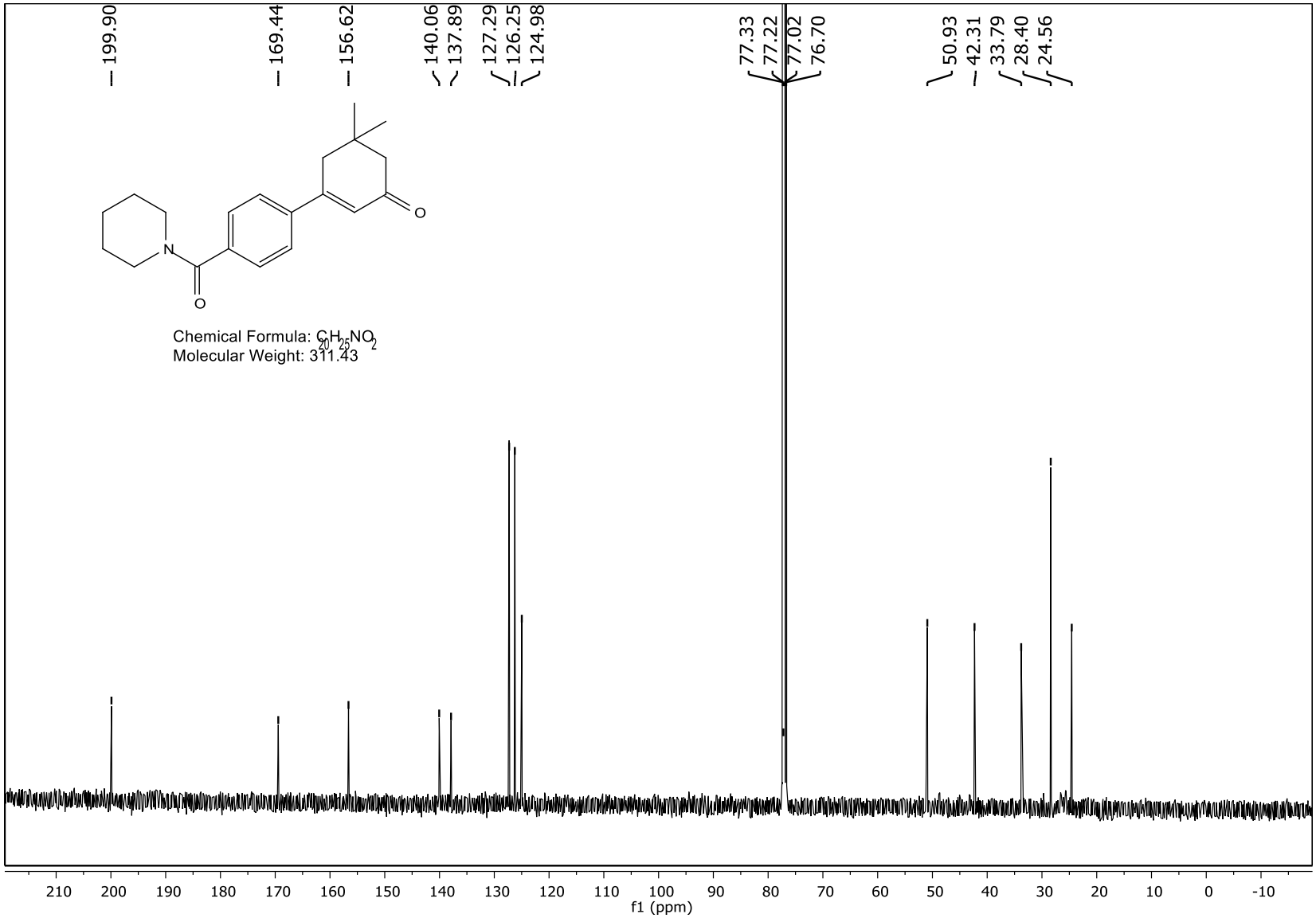
3',3'-Dimethyl-5'-oxo-2',3',4',5'-tetrahydro-[1,1'-biphenyl]-4-carboxylic acid (**48**) (0.1 g, 0.41 mmol) was added to dichloromethane (15 mL). Thionyl chloride (81.9 mg, 0.69 mmol) was added and the mixture was refluxed for 3 hours. The solvent and excess thionyl chloride were evaporated using a rotary evaporator. The flask was closed using a septum and the mixture was dissolved in benzene (15 mL) at 0°C. Piperidine (70.0 mg, 0.82 mmol) and triethyl amine (82.8 mg, 0.82 mmol) were very slowly added simultaneously and the mixture was stirred for 5 minutes at 0°C. The reaction was quenched with water and an aqueous solution of NH_4Cl. The mixture was extracted with EtOAc (3 x 10 mL), dried with $MgSO_4$ and filtered. The extract was treated with an aqueous solution of 5% sodium hydroxide. The product **55** was isolated by column chromatography with 35% EtOAc in hexanes to obtain a white fine powder. (90 mg, 70 %)

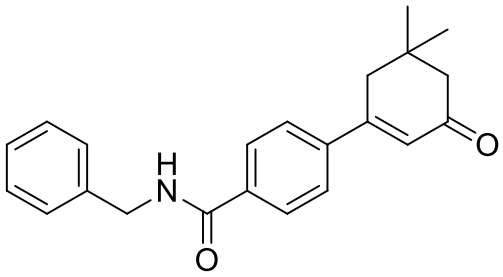
^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.59-7.52 (m, 2H), 7.46-7.41 (m, 2H), 6.42 (t, *J* = 1.45 Hz, 1H), 3.78-3.66 (br, 2H), 3.43-3.28 (br, 2H), 2.64 (d, *J* = 1.42 Hz, 2H), 2.35 (s, 2H), 1.72-1.61 (m, 4H), 1.57-1.49 (m, 2H), 1.13 (s, 6H)

^{13}C NMR (100 MHz, MeOD) δ, ppm: 199.90, 169.44, 156.62, 140.06, 137.89, 127.29 (2C), 126.25 (2C), 124.98, 50.93, 42.31, 33.79, 28.40 (2C), 24.56.

HRMS: Calculated for C20H25NO2: 311.1885 **Found:** 311.1889

7.56
7.54
7.44
7.42
7.26
6.42
3.71
3.35
2.64
2.64
2.35
1.69
1.53
1.13
Chemical Formula: C20H25NO2
Molecular Weight: 311.43
O
O
N
1.37
1.19
0.58
1.91
2.04
1.94
1.94
2.63
6.00
f1 (ppm)


199.90
169.44
156.62
140.06
137.89
127.29
126.25
124.98
77.33
77.22
77.02
76.70
50.93
42.31
33.79
28.40
24.56
O
N
O
Chemical Formula: C20H25NO2
Molecular Weight: 311.43
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

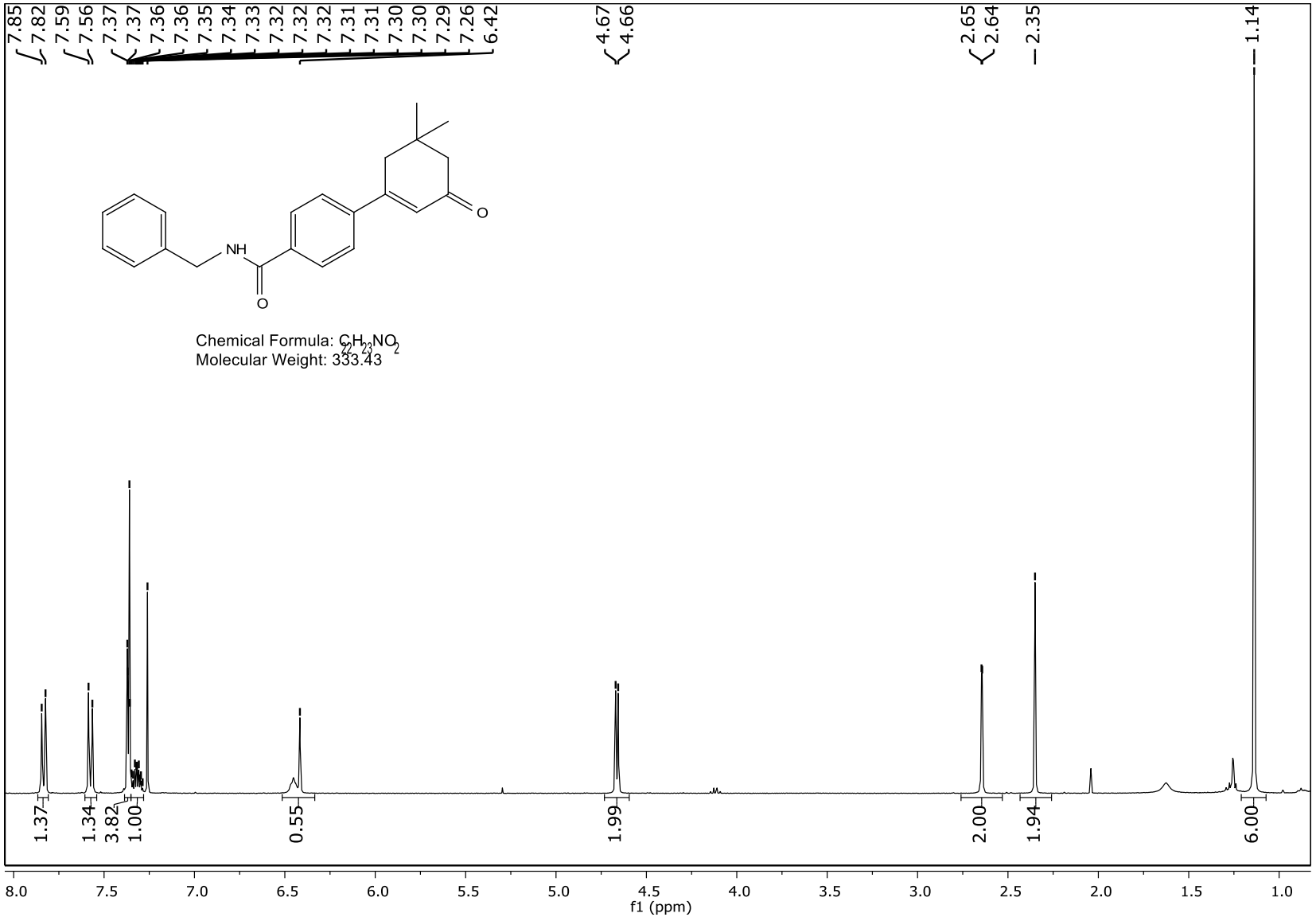


56

3',3'-Dimethyl-5'-oxo-2',3',4',5'-tetrahydro-[1,1'-biphenyl]-4-carboxylic acid (**48**) (100 mg, 0.41 mmol) was added to dichloromethane (15 mL). Thionyl chloride (82.7 mg, 0.70 mmol) was added and the mixture was refluxed for 3 hours. The solvent and excess thionyl chloride were evaporated using a rotary evaporator. The flask was closed using a septum and the mixture was dissolved in benzene (15 mL) at 0^{o}C. Benzyl amine (175 mg, 1.64 mmol) and triethyl amine (166 mg, 1.64 mmol) were very slowly added simultaneously and the mixture was stirred for 5 minutes at 0^{o}C. The reaction was quenched with water and an aqueous solution of NH_4Cl. The mixture was extracted with EtOAc (3 x 10 mL), dried with $MgSO_4$ and filtered. The extract was treated with an aqueous solution of 5% sodium hydroxide. The product **56** was isolated by column chromatography with 30% EtOAc in hexanes to obtain a beige fine powder. (70 mg, 51 %)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.85-7.81 (m, 2H), 7.60-7.55 (m, 2H), 7.38-7.28 (m, 5H), 6.49-6.43 (m, 1H), 6.42 (t, *J* = 1.44 Hz, 1H), 4.66 (d, *J* = 5.64 Hz, 2H), 2.64 (d, *J* = 1.43 Hz, 2H), 2.35 (s, 2H), 1.14 (s, 6H)

^{13}C NMR (101 MHz, $CDCl_3$) δ, **ppm:** 199.82, 166.43, 156.21, 142.19, 137.94, 135.36, 128.86 (2C), 127.98 (2C), 127.77, 127.41 (2C), 126.38 (2C), 125.47, 50.92, 44.28, 42.28, 33.81, 28.39 (2C).


7.85
7.82
7.59
7.56
7.37
7.37
7.36
7.36
7.35
7.34
7.33
7.32
7.32
7.32
7.31
7.31
7.30
7.30
7.29
7.26
6.42
4.67
4.66
2.65
2.64
2.35
1.14
NH
O
O
Chemical Formula: C22H23NO2
Molecular Weight: 333.43
1.37
1.34
3.82
1.00
0.55
1.99
2.00
1.94
6.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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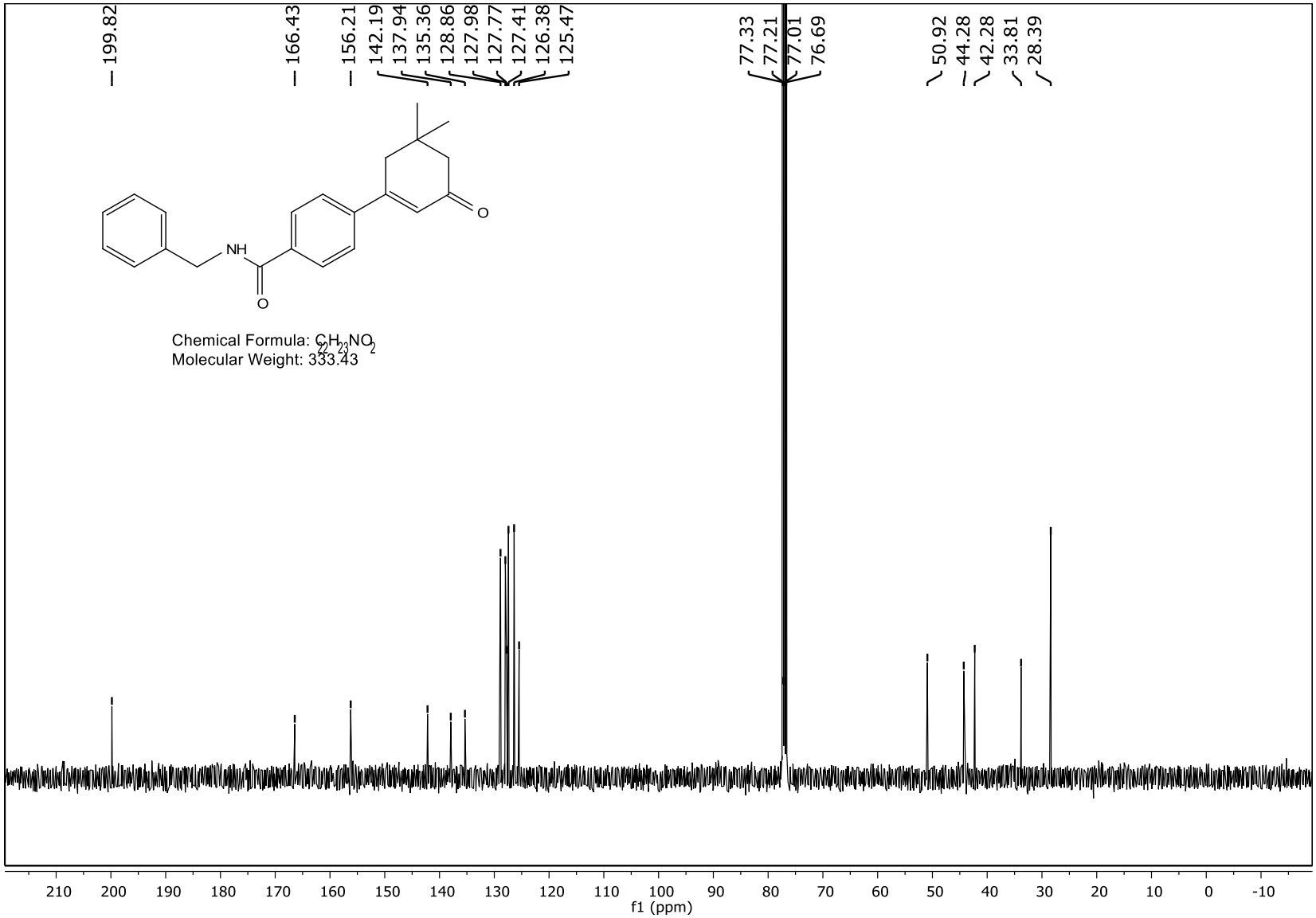
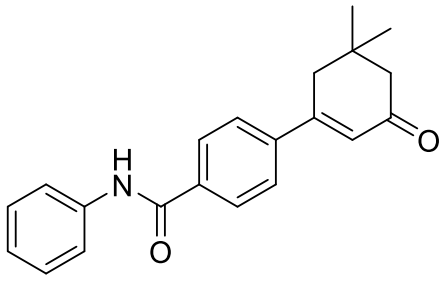
2.5
2.0
1.5
1.0
f1 (ppm)


199.82
166.43
156.21
142.19
137.94
135.36
128.86
127.98
127.77
127.41
126.38
125.47
77.33
77.21
77.01
76.69
50.92
44.28
42.28
33.81
28.39
NH
O
O
Chemical Formula: C22H23NO2
Molecular Weight: 333.43
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)



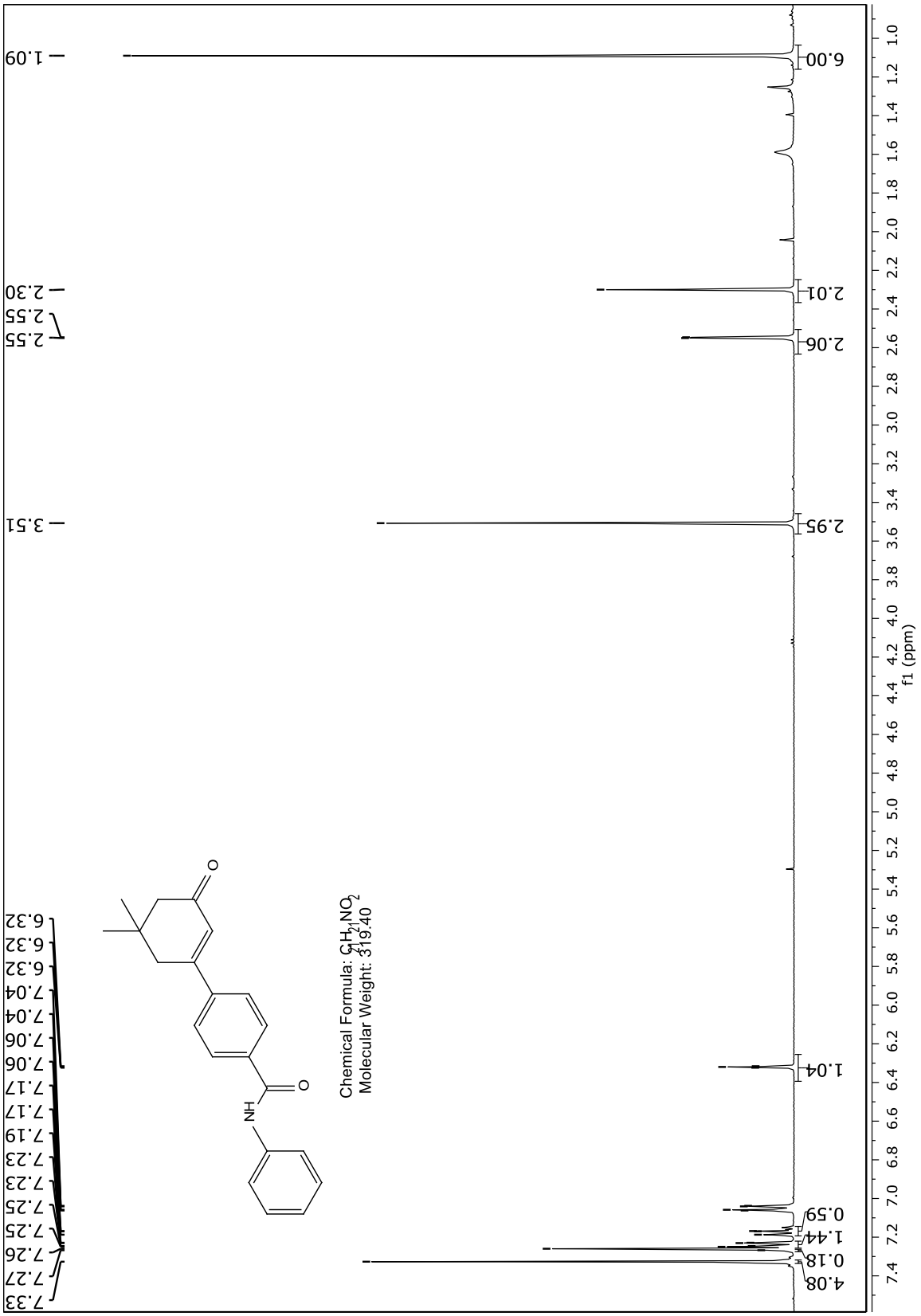
57

3',3'-dimethyl-5'-oxo-2',3',4',5'-tetrahydro-[1,1'-biphenyl]-4-carboxylic acid (**48**) (100 mg, 0.41 mmol) was added to dichloromethane (15 mL). Thionyl chloride (82.7 mg, 0.70 mmol) was added and the mixture was refluxed for 3 hours. The solvent and excess thionyl chloride were evaporated using a rotary evaporator. The flask was closed using a septum and the mixture was dissolved in benzene (15 mL) at 0^{o}C. Aniline (152 mg, 1.64 mmol) and triethyl amine (166 mg, 1.64 mmol) were very slowly added simultaneously and the mixture was stirred for 5 minutes at 0^{o}C. The reaction was quenched with water and an aqueous solution of NH_4Cl. The mixture was extracted with EtOAc (3 x 10 mL), dried with $MgSO_4$ and filtered. The extract was treated with an aqueous solution of 5% sodium hydroxide. The product **57** was isolated by column chromatography with 3% EtOAc in hexanes to obtain a white fine powder. (50 mg, 38 %)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.95-7.90 (m, 2H), 7.83 (br, 1H), 7.68-7.61 (m, 4H), 7.42-7.36 (m, 2H), 7.21-7.15 (m, 1H), 6.45 (t, *J* = 1.44 Hz, 1H), 2.67 (d, *J* = 1.45 Hz, 2H), 2.37 (s, 2H), 1.16 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.85, 164.81, 156.14, 142.47, 137.75, 135.99, 129.18 (2C), 127.52 (2C), 126.56 (2C), 125.61, 124.81, 120.24 (2C), 50.94, 42.29, 33.84, 28.41 (2C)

HRMS: Calculated for C21H21NO2: 319.1572 **Found:** 319.1554


Chemical Formula: C21H21NO2
Molecular Weight: 319.40
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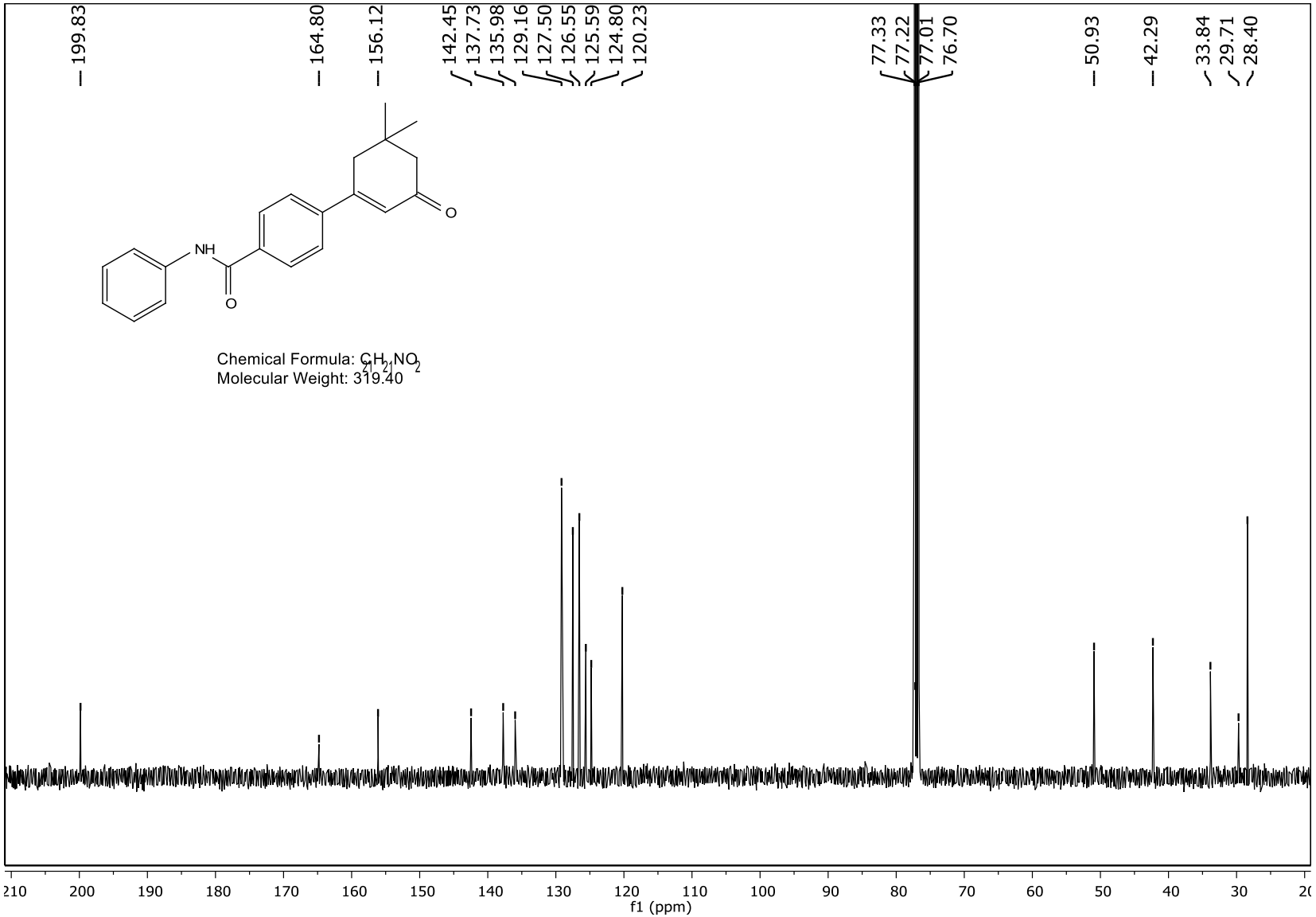
O
7.33
7.27
7.26
7.25
7.25
7.23
7.23
7.19
7.17
7.17
7.06
7.06
7.04
7.04
6.32
6.32
6.32
6.32
3.51
2.55
2.55
2.30
1.09
4.08
0.18
1.44
0.59
1.04
2.95
2.06
2.01
6.00
f1 (ppm)


199.83
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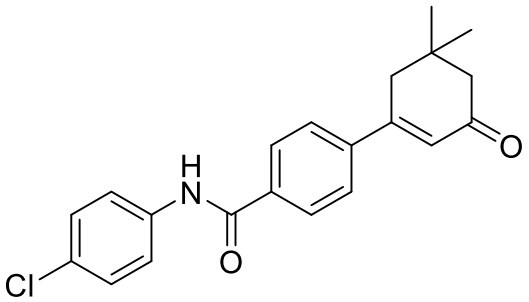
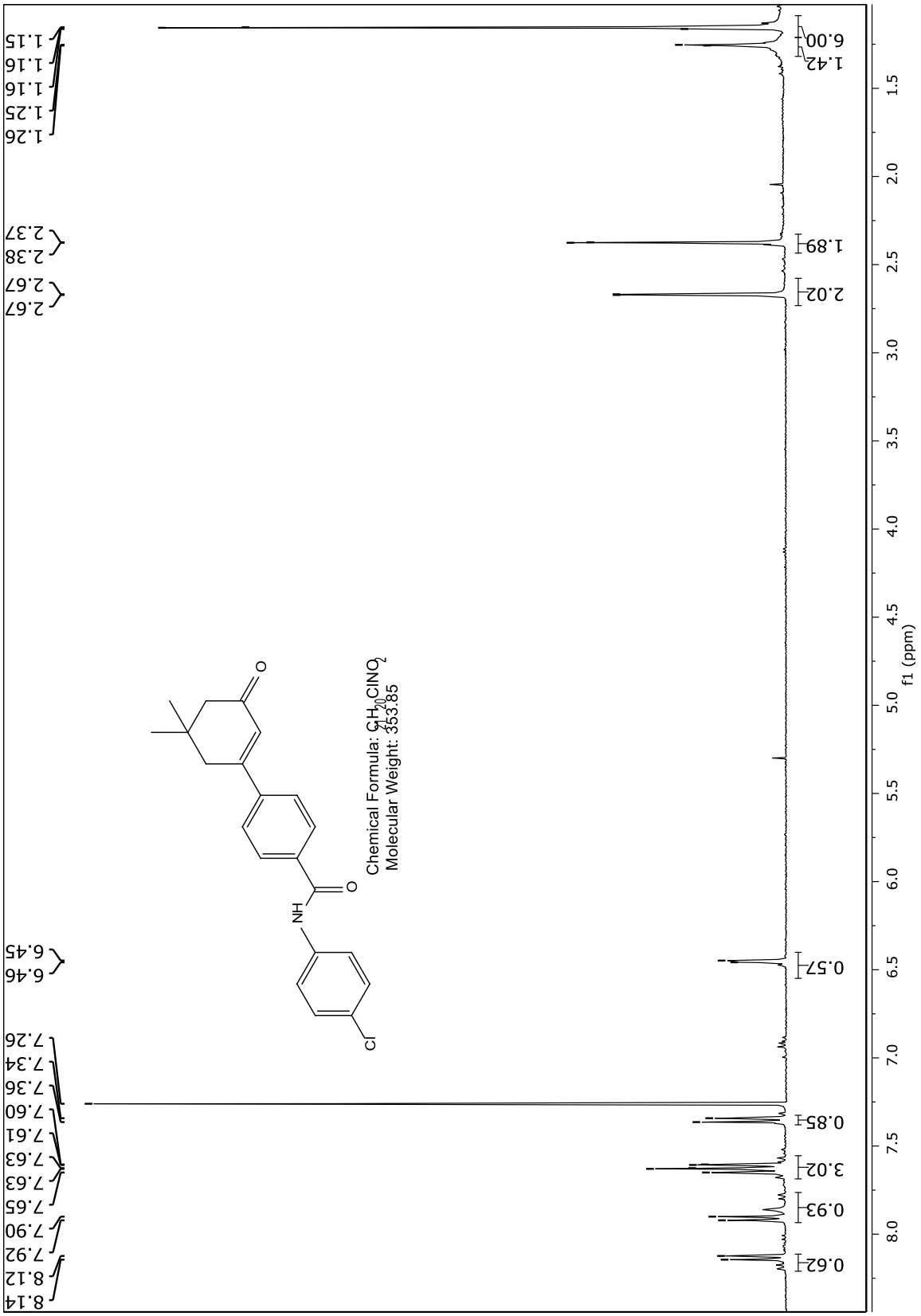
142.45
137.73
135.98
129.16
127.50
126.55
125.59
124.80
120.23
77.33
77.22
77.01
76.70
50.93
42.29
33.84
29.71
28.40
NH
O
O
Chemical Formula: C21H21NO2
Molecular Weight: 319.40
f1 (ppm)

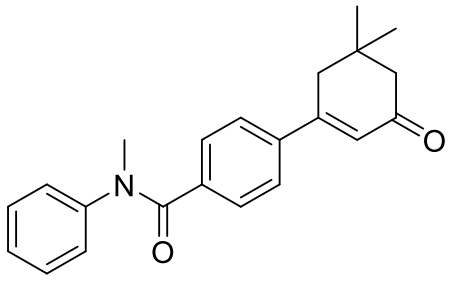


58

48 (0.10 g, 0.41 mmol) was dissolved in DCM (10 mL). Oxalyl chloride (0.10 mL, 1.13 mmol) was added with a few drops of DMF. The reaction was then left to bubble at room temperature for 15 minutes. The mixture is then concentrated by rotary evaporator. The oily/solid mixture is redissolved in in DCM and 4-chloroaniline (0.07 mL, 0.82 mmol) was added, followed by refluxing for 1 hour. The mixture is washed with a 5% HCl (10mL) solution, then with a 5% NaOH solution and extracted with DCM (10 mL). The organic phase is dried with $MgSO_4$, filtered and concentrated by rotary evaporator. The product **58** is obtained by column chromatography purification with 30% EtOAc in hexanes (0.04 g, 28%).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.13 (d, *J* = 8.5 Hz, 1H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.63 (dd, *J* = 9.4, 7.8 Hz, 3H), 7.35 (d, *J* = 8.8 Hz, 1H), 6.45 (d, *J* = 3.9 Hz, 1H), 2.67 (d, *J* = 1.6 Hz, 2H), 2.37 (d, *J* = 1.0 Hz, 2H), 1.15 (d, *J* = 1.4 Hz, 6H).


Chemical Formula: C17H20ClNO2
Molecular Weight: 305.80
f1 (ppm)

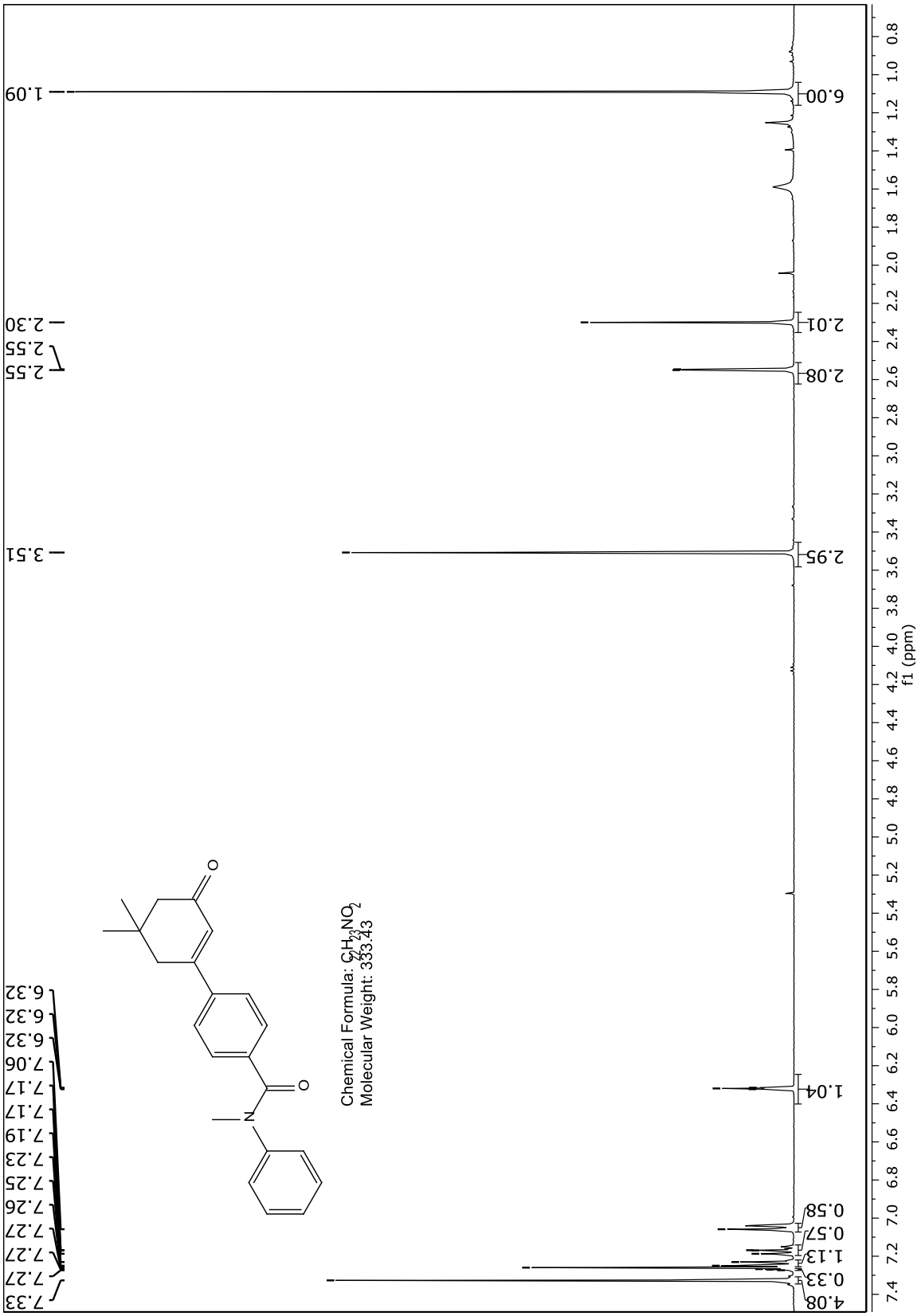


59

3',3'-dimethyl-5'-oxo-2',3',4',5'-tetrahydro-[1,1'-biphenyl]-4-carboxylic acid (**48**) (100 mg, 0.41 mmol) was added to dichloromethane (15 mL). Thionyl chloride (82.7 mg, 0.70 mmol) was added and the mixture was refluxed for 3 hours. The solvent and excess thionyl chloride were evaporated using a rotary evaporator. The flask was closed using a septum and the mixture was dissolved in benzene (15 mL) at 0^{o}C. N-methyl aniline (175 mg, 1.64 mmol) and triethyl amine (166 mg, 1.64 mmol) were very slowly added simultaneously and the mixture was stirred for 5 minutes at 0^{o}C. The reaction was quenched with water and an aqueous solution of NH_4Cl. The mixture was extracted with EtOAc (3 x 10 mL), dried with $MgSO_4$ and filtered. The extract was treated with an aqueous solution of 5% sodium hydroxide. The product **59** was isolated by column chromatography with 35% EtOAc in hexanes to obtain a beige fine powder. (60 mg, 44 %)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.33 (s, 4H), 7.28-7.22 (m, 2H), 7.19-7.14 (m, 1H), 7.07-7.03 (m, 2H), 6.32 (t, *J* = 1.48 Hz, 1H), 3.51 (s, 3H), 2.55 (d, *J* = 1.47 Hz, 2H), 2.30 (s, 2H), 1.09 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.83, 169.67, 156.34, 144.64, 140.02, 137.23, 129.32 (2C), 129.17 (2C), 126.89 (2C), 126.76, 125.49 (2C), 124.99, 50.88, 42.07, 38.46, 33.66, 28.36 (2C)


Chemical Formula: C23H23NO2
Molecular Weight: 333.43
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7.33
7.27
7.27
7.27
7.26
7.25
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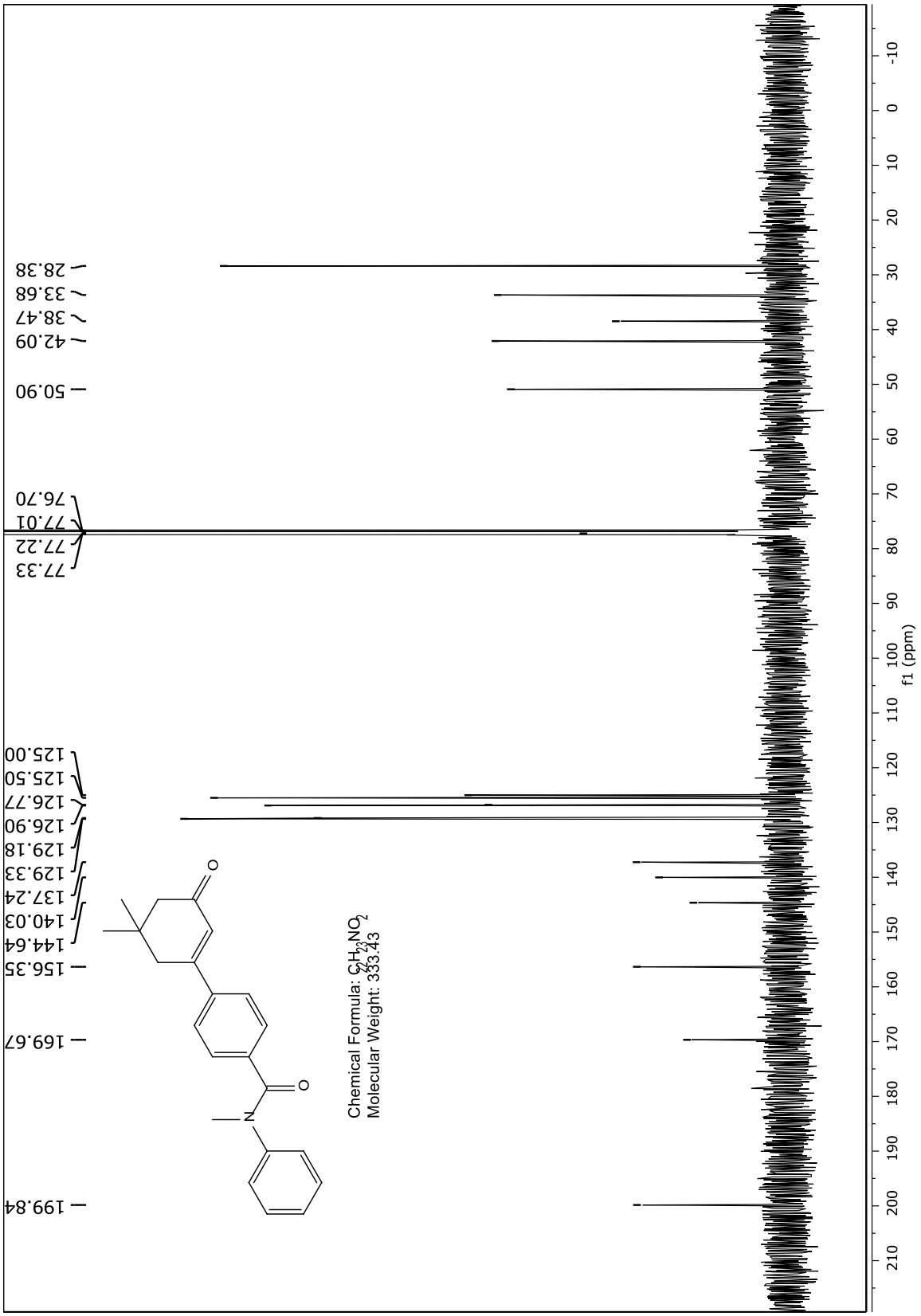
7.19
7.17
7.17
7.06
6.32
6.32
6.32
3.51
2.55
2.55
2.30
1.09
4.08
0.33
1.13
0.57
0.58
1.04
2.95
2.08
2.01
6.00
f1 (ppm)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140.03
137.24
129.33
129.18
126.90
126.77
125.50
125.00
77.33
77.22
77.01
76.70
50.90
42.09
38.47
33.68
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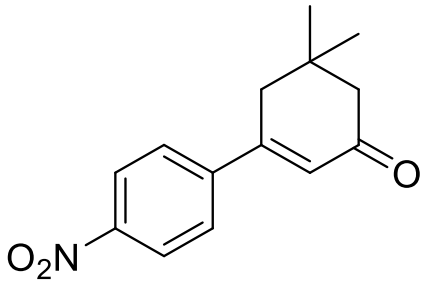
Chemical Formula: C24H25NO2
Molecular Weight: 383.43
f1 (ppm)



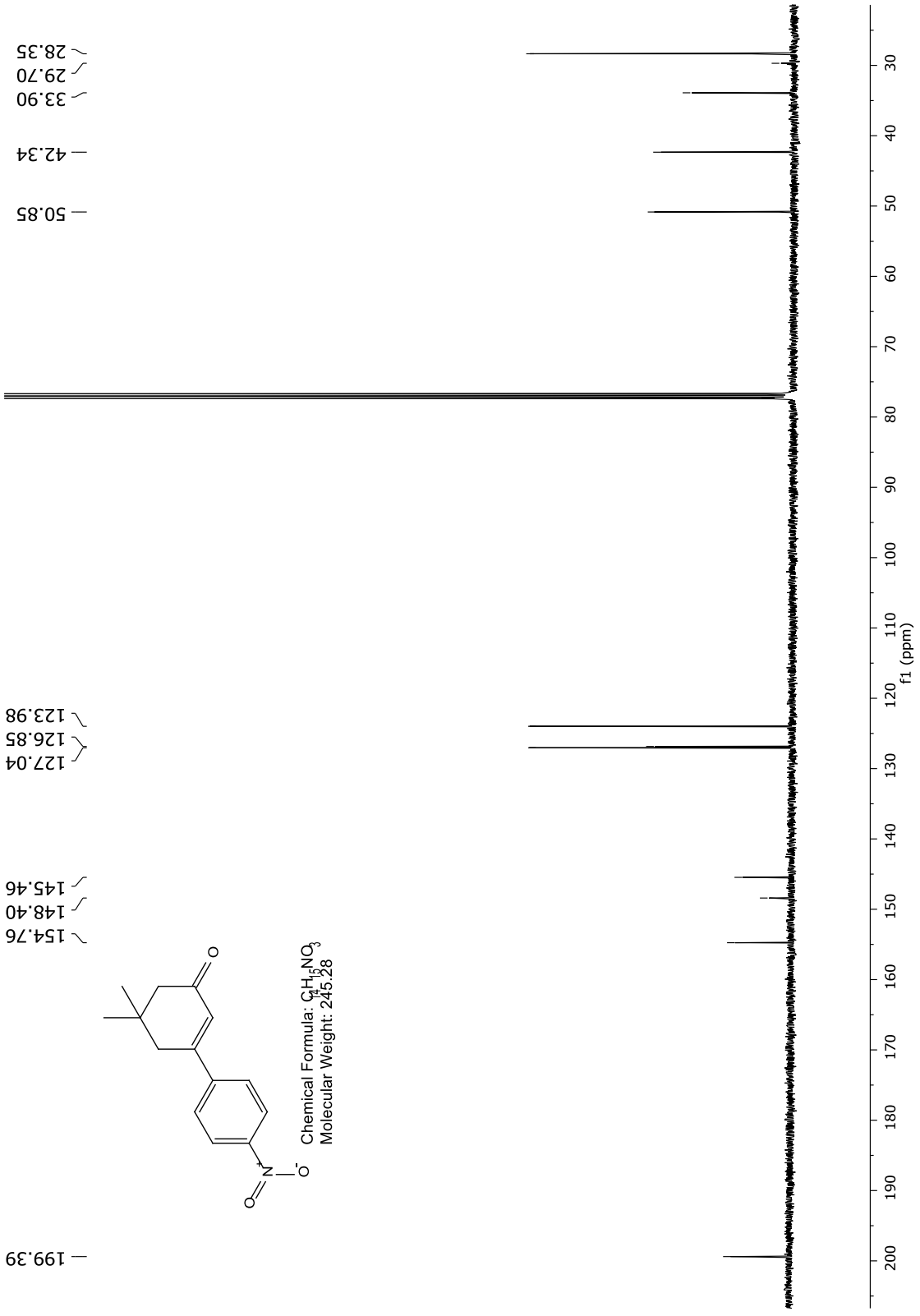
60

p-toluenesulfonyl chloride (2.30 g, 12.0 mmol) was added to a mixture of dimedone (2.02 g, 14.4 mmol) and potassium carbonate (2.35 g, 16.5 mmol) in a 2:1 ratio of 1,4-dioxane (26 mL) and water (13 mL). This mixture was stirred at room temperature for 2 hours. 4-nitrophenylboronic acid (2.0 g, 12.0 mmol) and tetrakis(triphenylphosphine)palladium (0) (416 mg, 0.36 mmol) were added and the mixture was heated under reflux for 4 hours or until completion. The resulting mixture was extracted with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and evaporate via rotary evaporator. The product **60** was isolated via column chromatography with 15% EtOAc in hexanes. (1.33 g, 45 %).

^{1}H NMR (400 MHz, CDCl3) δ, ppm: 8.27 (d, *J* = 9.0 Hz, 2H), 7.67 (d, *J* = 9.0 Hz, 2H), 6.45 (s, 1H), 2.66 (d, *J* = 1.6 Hz, 2H), 2.38 (s, 2H), 1.16 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: δ 199.39, 154.76, 148.40, 145.46, 127.04 (2C), 126.85, 123.98 (2C), 50.85, 42.34, 33.90, 29.70, 28.35 (2C).

8.28
8.26
7.68
7.66
7.26
6.45
2.66
2.66
2.38
1.16
2.12
2.26
1.16
6.00
2.35
2.19
Chemical Formula: $C_{14}H_{15}NO_3$
Molecular Weight: 245.28
f1 (ppm)


199.39
154.76
148.40
145.46
127.04
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123.98
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33.90
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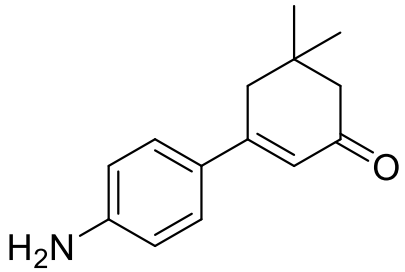
28.35
Chemical Formula: $C_{14}H_{15}NO_3$
Molecular Weight: 245.28
f1 (ppm)



61

60 (210 mg, 0.86 mmol) was dissolved in methanol (10 mL). Palladium on carbon (50 mg) was added and hydrogen gas pressure was added to the system and stirred for 2 hours. The mixture was then run through a celite column and then washed with 5% HCl solution followed by DCM extraction and a 10% NaOH wash. The organic phase was concentrated by rotary evaporator. The product **61** was obtained by column chromatography with 20% EtOAc in hexanes (125 mg, 67%)

^{1}H NMR (400 MHz, CDCl3) δ, ppm: 7.43 – 7.34 (m, 2H), 6.70 – 6.61 (m, 2H), 6.35 (d, *J* = 1.4 Hz, 1H), 2.57 (d, *J* = 1.4 Hz, 2H), 2.28 (s, 2H), 1.08 (s, 6H).

7.40
7.38
7.24
6.67
6.64
6.35
2.58
2.57
2.28
1.08
0.32
0.32
0.16
0.32
0.33
0.33
1.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
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1.0
f1 (ppm)
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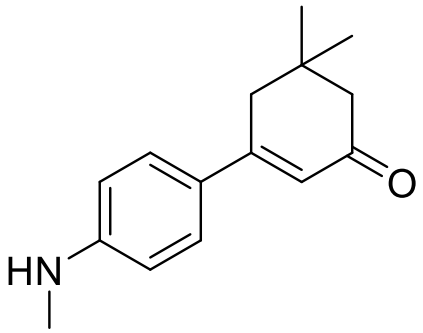
Chemical Formula: C14H17NO
Molecular Weight: 215.30



62

61 (100 mg, 0.46 mmol) was dissolved in DCM (10 mL). K_2CO_3 (80 mg, 1.3 mmol) was added followed by iodomethane (98 mg, 0.7 mmol) and the reaction was stirred for 1 hour. The mixture was quenched with a solution of NH_4Cl and the organic phase was dried over $MgSO_4$ and concentrated by rotary evaporator. The product **62** was isolated by column chromatography with 15% EtOAc in hexanes. (30 mg, 28%)

^{1}H NMR (400 MHz, CDCl3) δ, ppm: 7.47 (d, *J* = 8.8 Hz, 2H), 6.61 (dd, *J* = 8.9, 1.2 Hz, 2H), 6.39 (t, *J* = 1.4 Hz, 1H), 2.89 (s, 3H), 2.61 (d, *J* = 1.4 Hz, 2H), 2.31 (s, 2H), 1.11 (s, 6H).

7.48
7.45
7.26
6.63
6.62
6.61
6.60
6.60
6.39
6.39
6.39
2.89
2.61
2.61
2.31
1.11
1.98
1.01
1.98
1.98
2.88
1.94
1.98
6.00
Chemical Formula: C15H19NO
Molecular Weight: 229.32
HN
O
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6.5
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5.5
5.0
4.5
4.0
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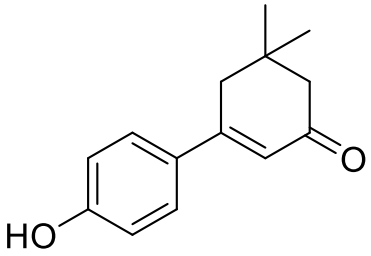
3.0
2.5
2.0
1.5
1.0
f1 (ppm)



63

4-Bromophenol (4.33 g, 25 mmol) and imidazole (2.21 g, 32.5 mmol) were dissolved in a 1:1 ratio mixture of tetrahydrofuran (15 mL) and dimethylformamide (15 mL). *tert*-Butyldimethylsilyl chloride (4.15 g, 27.5 mmol) and 4-dimethylaminopyridine (trace amount) were added and the reaction was stirred for 12 hours at room temperature. The mixture was diluted with water (30 mL) and ether (40 mL) and then extracted with EtOAc (3 x 20 mL). The organic extracts were dried with $MgSO_4$, filtered and evaporated using a rotary evaporator. The silyl protected intermediate was isolated by column chromatography. (5.63 g, 68%)

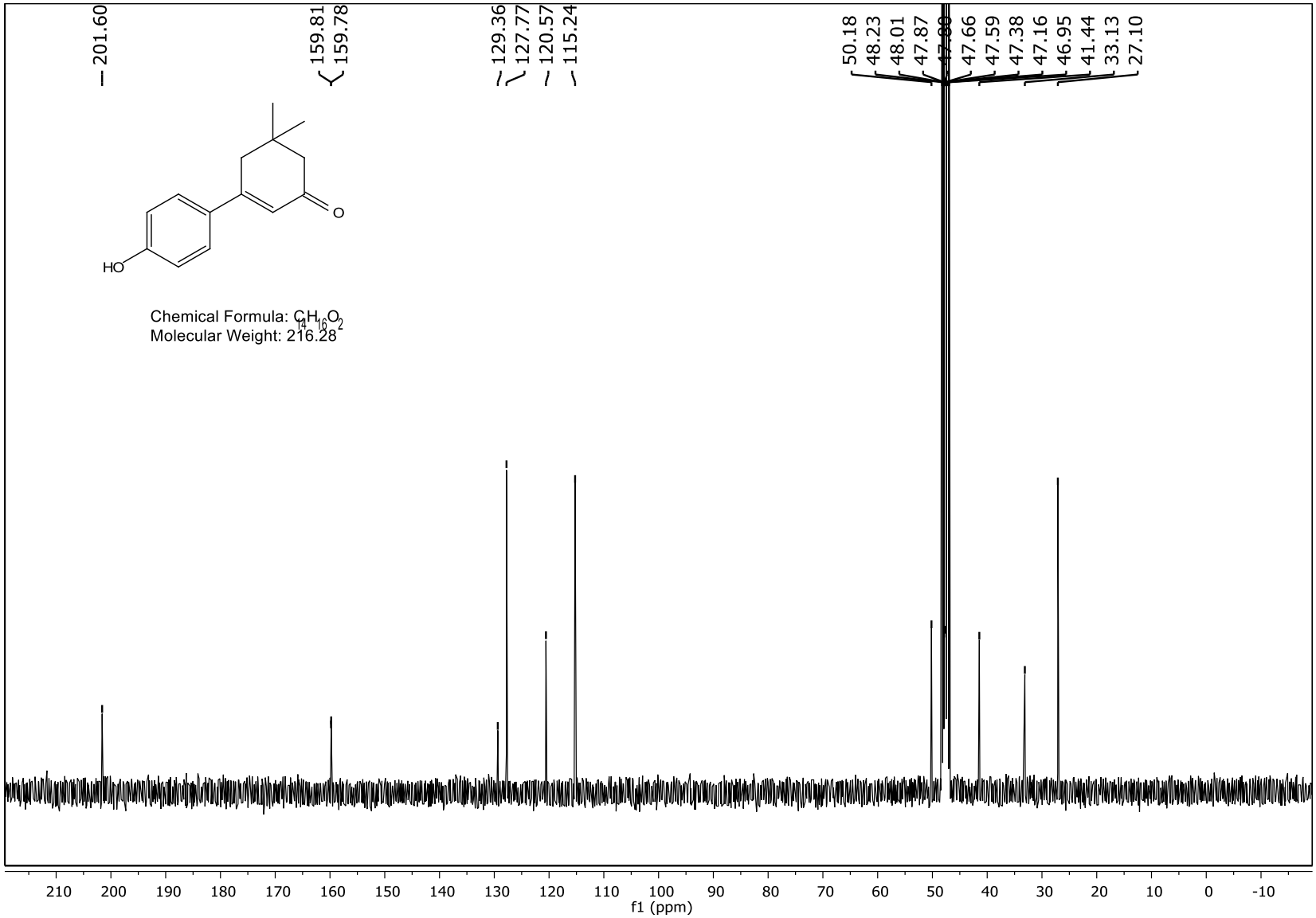
The silyl protected intermediate (320 mg, 0.97 mmol) was dissolved in EtOAc (25 mL) and an aqueous 5% sodium hydroxide solution (25 mL) was added. The aqueous phase was kept and treated with an aqueous 10% hydrochloric acid solution (15 mL). This mixture was extracted with EtOAc (3 x 10 mL) and this organic extract was dried with $MgSO_4$, filtered and evaporated to obtain **63** as a beige solid. (150 mg, 71%)

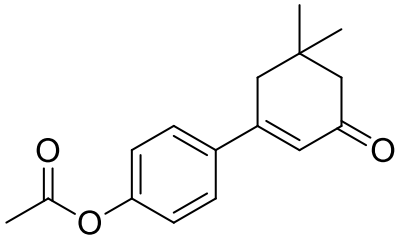
Total synthesis yield = 48%

^{1}H NMR (300 MHz, MeOH-D_4) δ, ppm: 7.57-7.48 (m, 2H), 6.94-6.73 (m, 2H), 6.34 (t, *J* = 1.39 Hz, 1H), 2.70 (d, *J* = 1.36 Hz, 2H), 2.32 (s, 2H), 1.12 (s, 6H)

^{13}C NMR (101 MHz, MeOD) δ, ppm: 201.60, 159.81, 159.78, 129.36, 127.77 (2C), 120.57, 115.24 (2C), 50.18, 41.44, 33.13, 27.10 (2C).**HRMS: Calculated for C14H16O2:** 216.1150 **Found:** 216.1174

7.55
7.54
7.54
7.54
7.53
7.52
7.51
7.51
7.50
6.86
6.85
6.85
6.84
6.84
6.83
6.82
6.82
6.81
6.35
6.34
6.34
6.34
4.85
3.32
3.31
3.31
3.30
3.30
2.70
2.70
2.70
2.70
2.32
2.32
2.15
2.15
2.01
1.98
1.98
1.12
1.12
1.12
1.11
6.00
1.86
1.83
0.92
1.74
1.85
O
OH
Chemical Formula: C16H16O2
Molecular Weight: 216.28
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


201.60
159.81
159.78
129.36
127.77
120.57
115.24
50.18
48.23
48.01
47.87
47.66
47.59
47.38
47.16
46.95
41.44
33.13
27.10
HO
O
Chemical Formula: C14H16O2
Molecular Weight: 216.28
f1 (ppm)



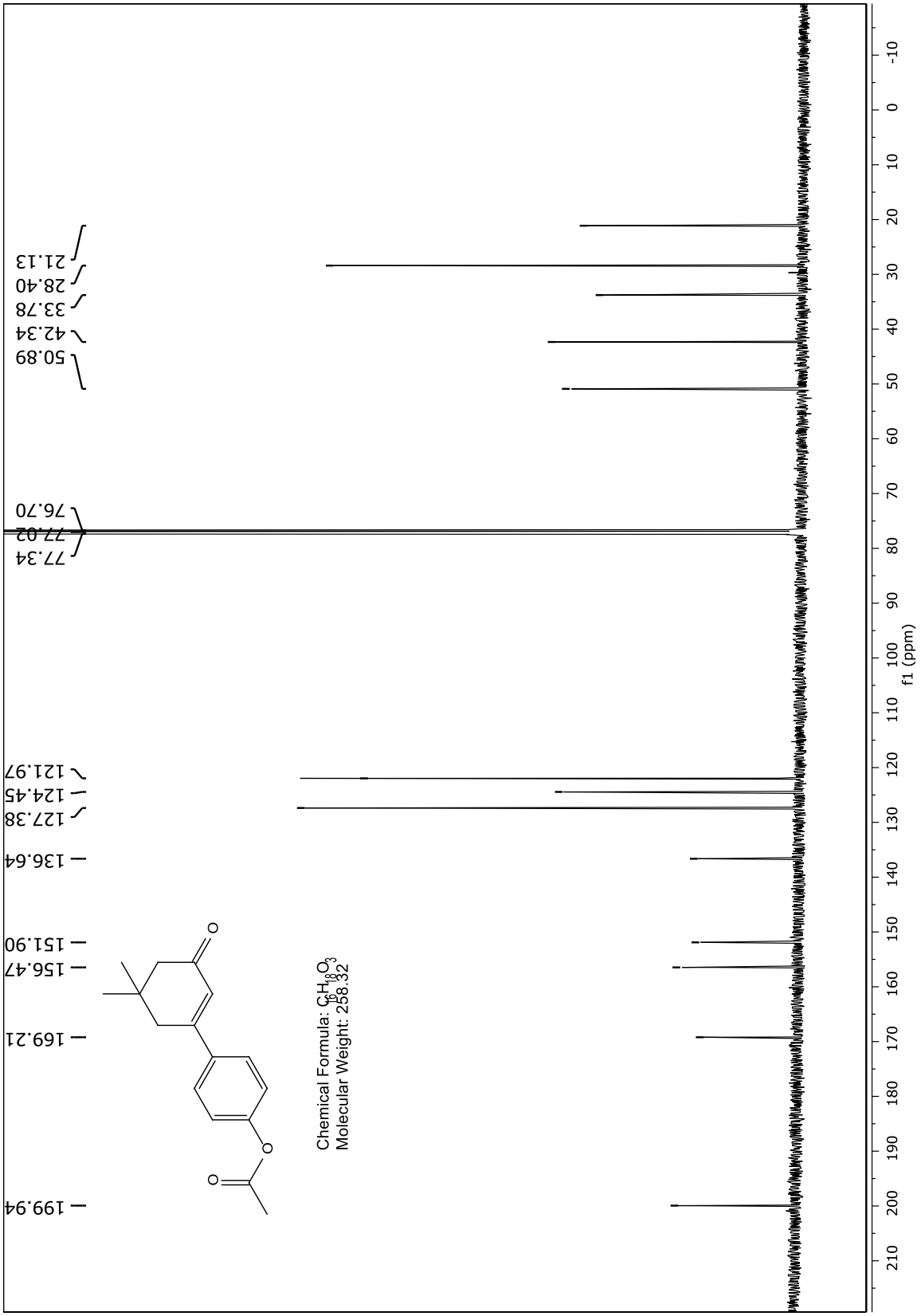
64

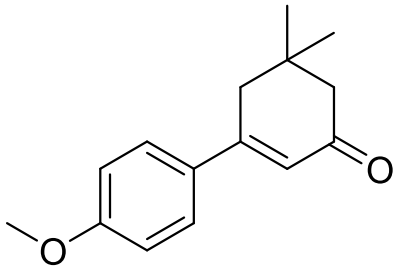
4'-hydroxy-5,5-dimethyl-5,6-dihydro-[1,1'-biphenyl]-3(4H)-one (**63**) (50 mg, 0.23 mmol) was dissolved in dichloromethane (10 mL) and 4-dimethylaminopyridine (trace amounts) was added. Acetic anhydride (27.7 mg, 0.46 mmol) and triethyl amine (70.1 mg, 0.69 mmol) were added and the solution was stirred for 30 minutes at room temperature. The reaction mixture was then poured into a flask containing a saturated aqueous solution of $NaHCO_3$ and extracted with dichloromethane (3 x 10 mL). The organic extract was dried with $MgSO_4$, filtered and evaporated. The product **64** was isolated by column chromatography with 10% EtOAc in hexanes to obtain a light-beige solid. (25 mg, 42%)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.59-7.50 (m, 2H), 7.20-7.06 (m, 2H), 6.38 (s, 1H), 2.62 (d, *J* = 1.25 Hz, 2H), 2.33 (s, 2H), 2.31 (s, 3H), 1.12 (s, 6H)

^{13}C NMR (101 MHz, $CDCl_3$) δ, ppm: 199.94, 169.21, 156.47, 151.90, 136.64, 127.38 (2C), 124.45, 121.97 (2C), 50.89, 42.34, 33.78, 28.40 (2C), 21.13.

7.56
7.53
7.26
7.16
7.15
7.12
6.39
6.38
2.62
2.62
2.33
2.31
1.12
6.00
3.00
2.15
2.09
0.81
1.68
1.48
Chemical Formula: C15H18O3
Molecular Weight: 258.32
f1 (ppm)


199.94
169.21
156.47
151.90
136.64
127.38
124.45
121.97
77.34
77.02
76.70
50.89
42.34
33.78
28.40
21.13
Chemical Formula: C16H18O3
Molecular Weight: 258.32
f1 (ppm)



65

p-toluenesulfonyl chloride (247.8 mg, 1.3 mmol) was added to a mixture of dimedone (140.2 mg, 1.0 mmol) and potassium carbonate (345 mg, 2.5 mmol) in a 2:1 ratio of 1,4-dioxane (4 mL) and water (2 mL). This mixture was stirred at room temperature for 1 hour. 4-methylphenyl boronic acid (182.4 mg, 1.2 mmol) and tetrakis(triphenylphosphine)palladium (0) (34.7 mg, 0.03 mmol) were added and the mixture was heated under reflux for 2 hours or until completion. The resulting mixture was extracted with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and evaporate via rotary evaporator. The product **65** was isolated via column chromatography with 5% EtOAc in hexanes followed by a wash with an aqueous 10% NaOH solution to obtain white crystals (130 mg, 56%).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.51-7.40 (m, 2H), 6.89-6.84 (m, 2H), 6.32 (s, 1H), 3.77 (s, 3H), 2.55 (d, *J* = 1.25 Hz, 2H), 2.25 (s, 2H), 1.06 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.94, 161.03, 156.92, 130.72, 127.48 (2C), 122.24, 113.93 (2C), 55.14, 50.59, 41.79, 33.38, 28.21 (2C)

HRMS: Calculated for C15H18O2: 230.1307 **Found:** 230.1323

7.48
7.48
7.46
7.45
7.26
6.89
6.89
6.87
6.87
6.35
6.34
3.79
3.78
2.58
2.57
2.57
2.27
2.27
1.07
1.07
O
O
Chemical Formula: C15H18O2
Molecular Weight: 230.31
2.01
2.14
1.06
3.06
2.24
2.15
6.00
7.5
7.0
6.5
6.0
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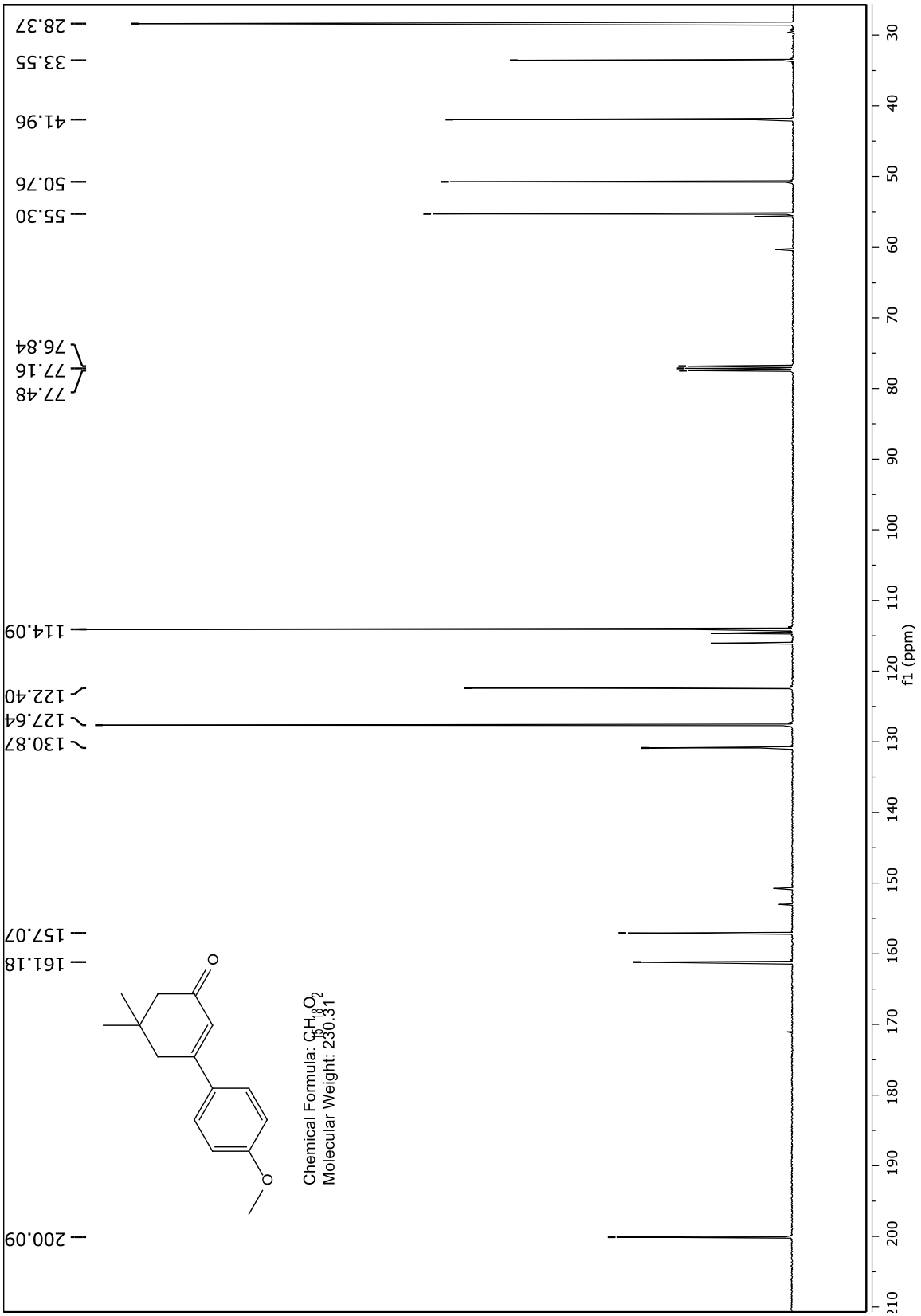
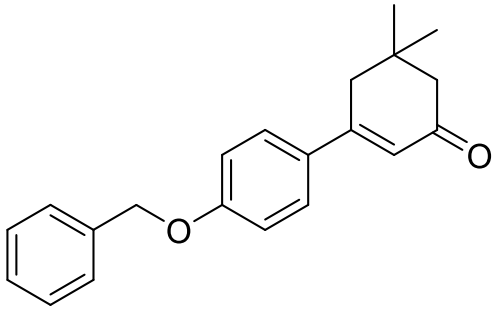
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


200.09
161.18
157.07
130.87
127.64
122.40
114.09
77.48
77.16
76.84
55.30
50.76
41.96
33.55
28.37
Chemical Formula: C15H18O2
Molecular Weight: 230.31
f1 (ppm)



66

First synthesis (via compound **63**):

4'-hydroxy-5,5-dimethyl-5,6-dihydro-[1,1'-biphenyl]-3(4H)-one (**63**) (50 mg, 0.23 mmol) was dissolved in dry acetone (15 mL). Potassium carbonate (73.5 mg, 0.53 mmol) and benzyl bromide (43.4 mg, 0.25 mmol) were added and the reaction mixture was refluxed for 3 hours. The mixture was filtered to get rid of the excess potassium carbonate and water was added to the remaining solution. This mixture was extracted with EtOAc (3 x 10 mL), dried with $MgSO_4$, filtered and evaporated. The product **66** was isolated by column chromatography with 7.5% EtOAc in hexanes to obtain a light-yellow solid. (48 mg, 71%)

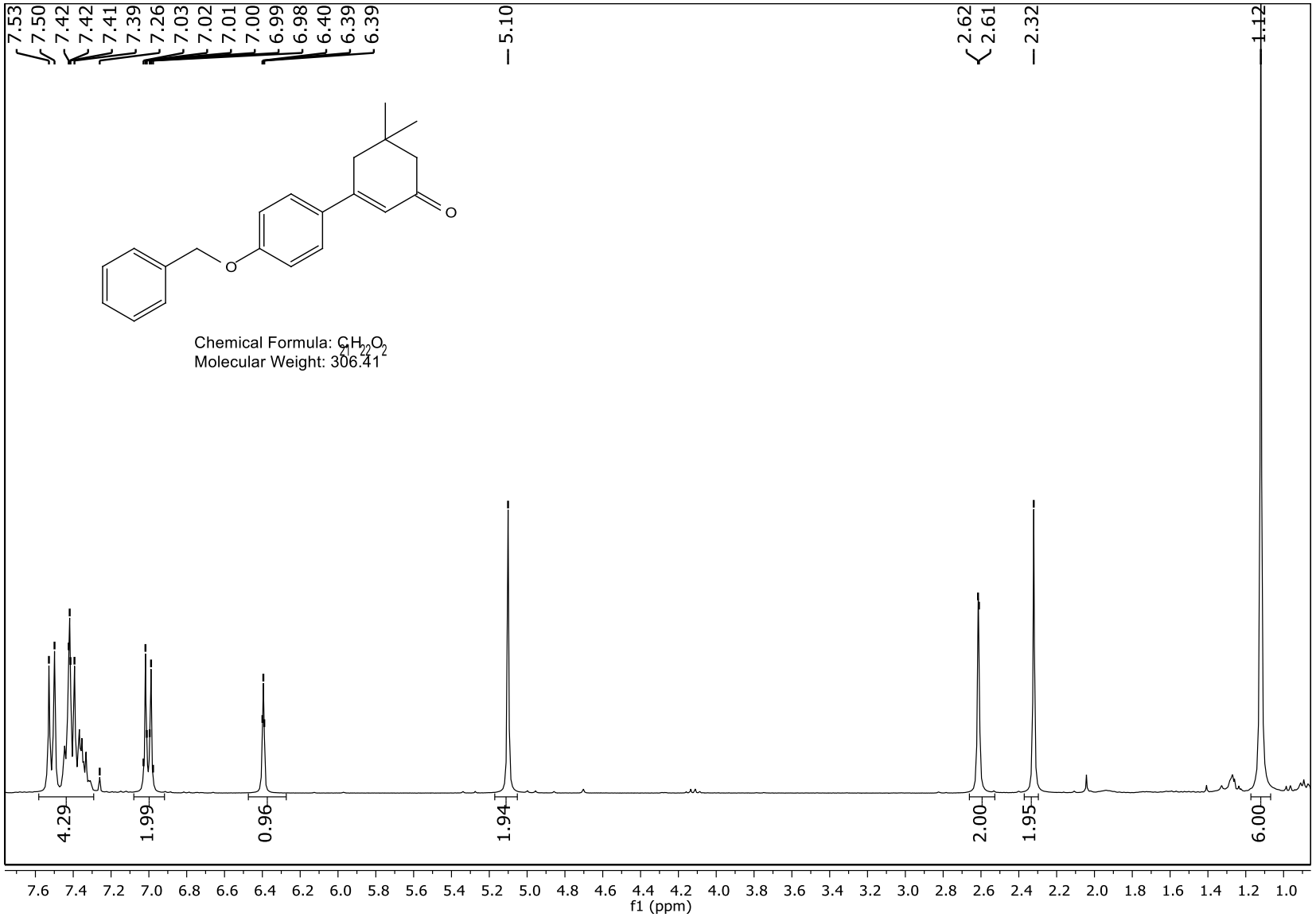
^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.55-7.30 (m, 7H), 7.00 (d, *J* = 8.86 Hz, 2H), 6.39 (s, 1H), 5.10 (s, 2H), 2.61 (d, *J* = 0.80 Hz, 2H), 2.32 (s, 2H), 1.12 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 200.09, 160.37, 156.86, 136.50, 131.34, 128.69 (2C), 128.17, 127.73 (2C), 127.44 (2C), 122.75, 115.06 (2C), 70.11, 50.90, 42.11, 33.67, 28.48 (2C)

HRMS: Calculated for C21H22O2: 306.1620 **Found:** 306.1587

Second synthesis (via compound **29**):

A round bottom flask was heated dry and an inert N_2 was created within and placed in a -78^{o}C acetone/dry ice bath. Compound **29** (1.02 g, 6.1 mmol) was dissolved in dry THF (10 mL) and added to the flask. N-BuLi 2.5M (3.04 mL, 7.6 mmol) was added to the mixture and stirred for 5 minutes. 1,4-Bromophenyl benzyl ether was dissolved in dry THF (10 mL) and added to the reaction mixture dropwise. After 15 minutes, the reaction mixture was quenched with water (15 mL) and extracted with DCM (10 mL x 2). The organic layer was dried with $MgSO_4$, filtered and concentrated via rotary evaporator. The product **66** was isolated by column chromatography with 7.5% EtOAc in hexanes. (1.3 g, 72%)


7.53
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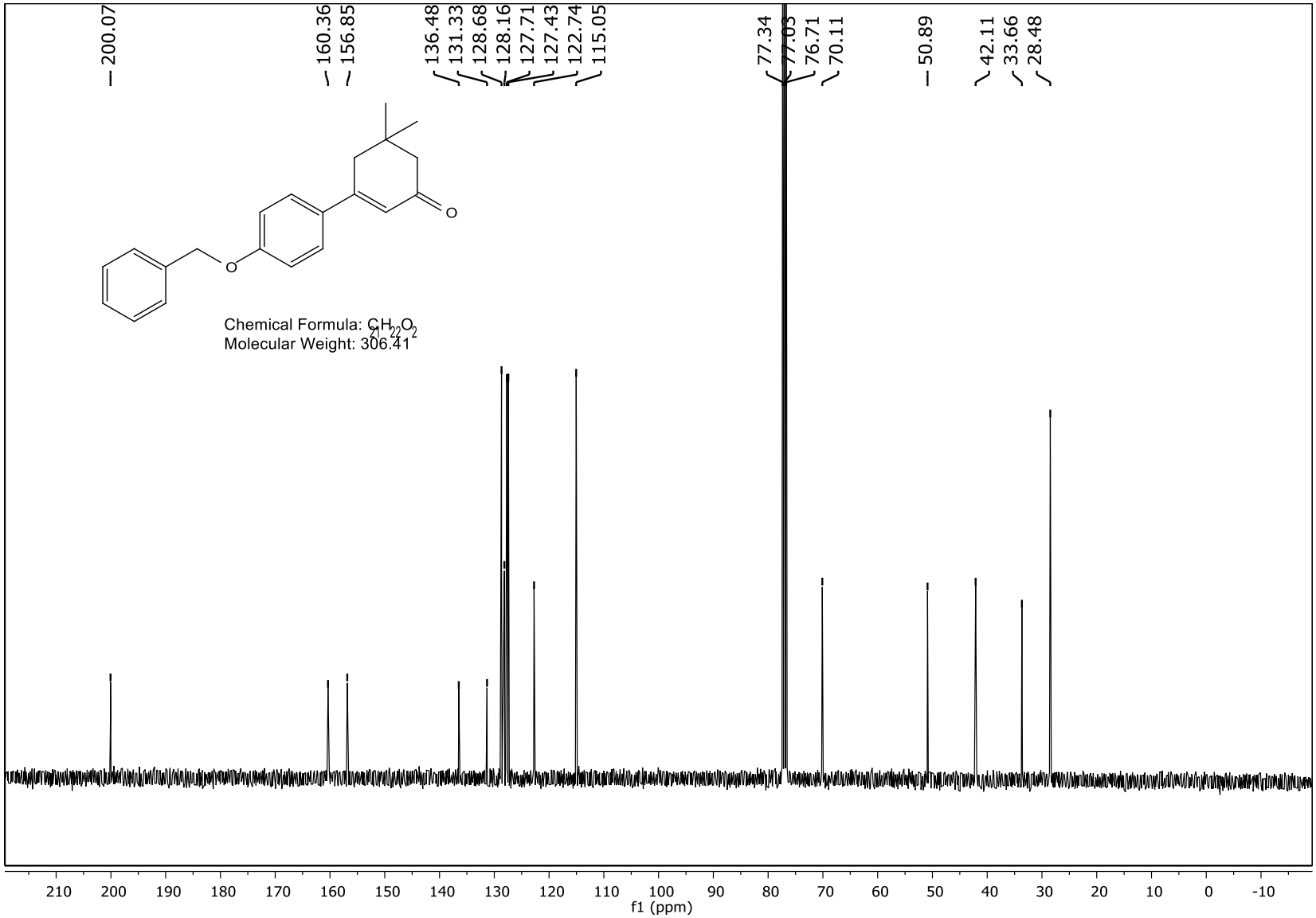
7.42
7.42
7.41
7.39
7.26
7.03
7.02
7.01
7.00
6.99
6.98
6.40
6.39
6.39
5.10
2.62
2.61
2.32
1.12
O
O
Chemical Formula: C21H22O2
Molecular Weight: 306.41
4.29
1.99
0.96
1.94
2.00
1.95
6.00
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0
f1 (ppm)


200.07
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131.33
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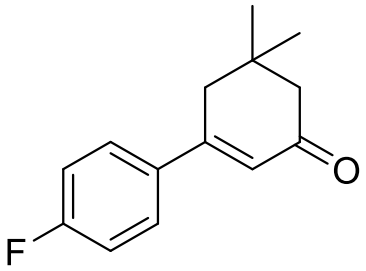
128.16
127.71
127.43
122.74
115.05
77.34
77.03
76.71
70.11
50.89
42.11
33.66
28.48
O
O
Chemical Formula: C21H22O2
Molecular Weight: 306.41
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

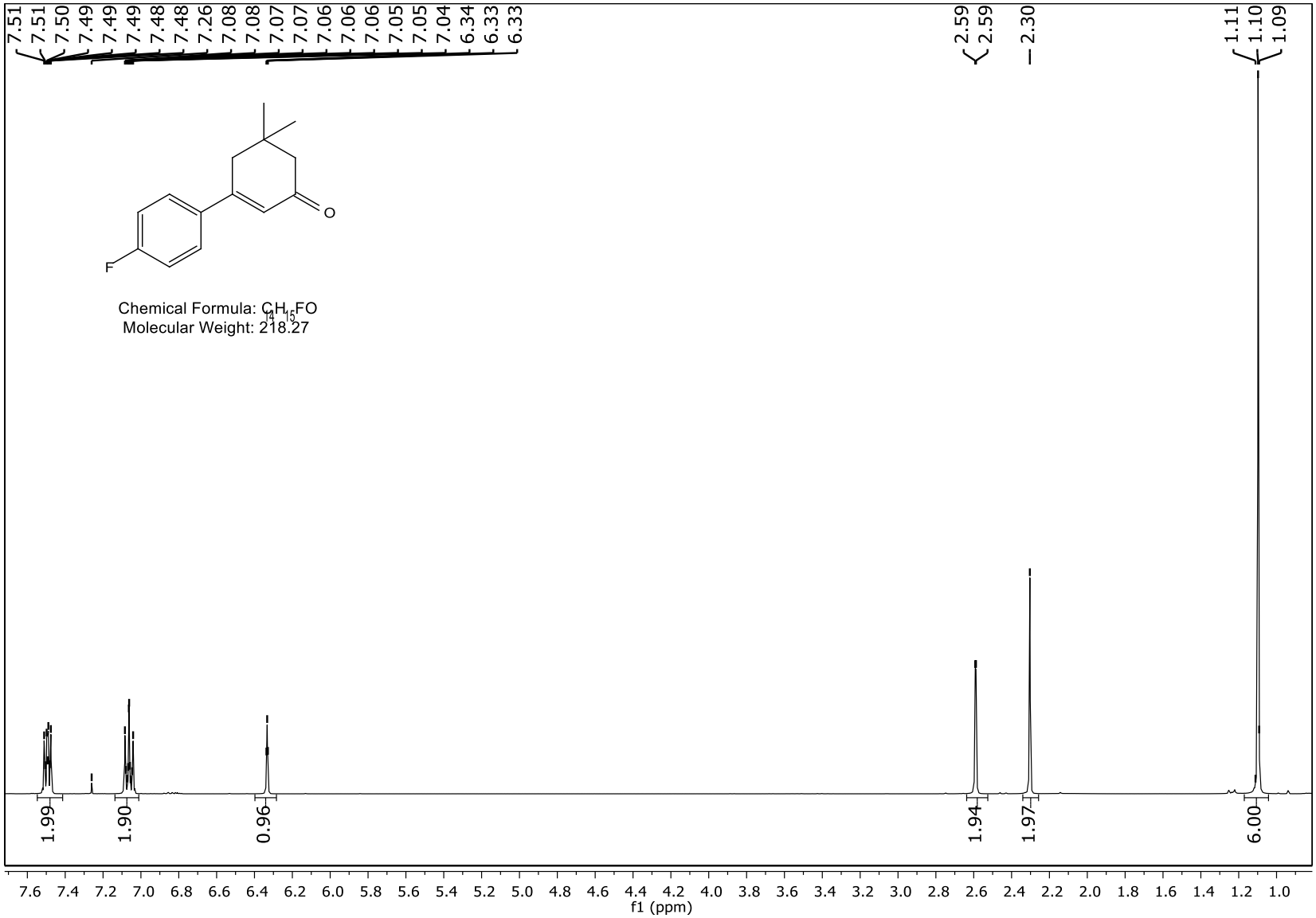


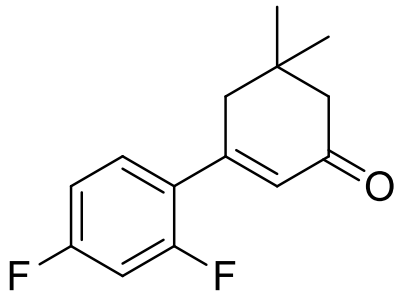
69

p-toluenesulfonyl chloride (1.42 g, 7.42 mmol) was added to a mixture of dimedone (800 mg, 5.71 mmol) and potassium carbonate (1.97 g, 14.3 mmol) in a 2:1 ratio of 1,4-dioxane (12 mL) and water (6 mL). This mixture was stirred at room temperature for 2 hours. 4-fluorophenylboronic acid (1.08 g, 6.85 mmol) and tetrakis(triphenylphosphine)palladium(0) (197 mg, 0.17 mmol) were added and the mixture was heated under reflux for 3 hours or until completion. The resulting mixture was extracted with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and evaporate via rotary evaporator. The product **69** was isolated via column chromatography with 15% EtOAc in hexanes to obtain a light-brown solid. (0.71 g, 57 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.56-7.43 (m, 2H), 7.13-6.97 (m, 2H), 6.33 (t, *J* = 1.39 Hz, 1H), 2.59 (d, *J* = 1.45 Hz, 2H), 2.30 (s, 2H), 1.10 (s, 6H)

HRMS: Calculated for C14H15FO: 218.1107 **Found:** 218.1091


7.51
7.51
7.50
7.49
7.49
7.49
7.48
7.48
7.26
7.08
7.08
7.07
7.07
7.06
7.06
7.06
7.05
7.05
7.04
6.34
6.33
6.33
2.59
2.59
2.30
1.11
1.10
1.09
O
F
Chemical Formula: C14H15FO
Molecular Weight: 218.27
1.99
1.90
0.96
1.94
1.97
6.00
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0
f1 (ppm)

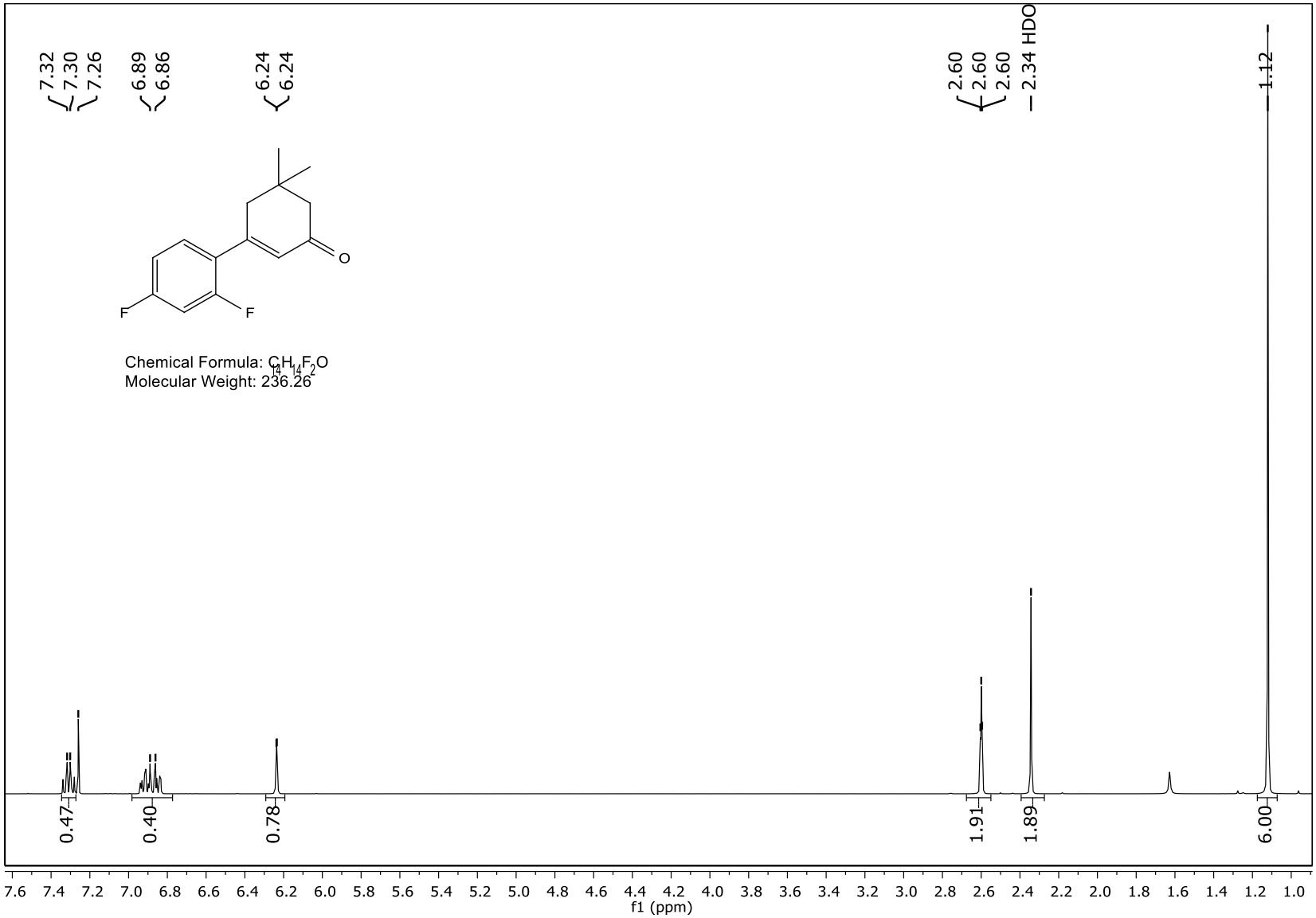


70

p-toluenesulfonyl chloride (1.77 g, 9.27 mmol) was added to a mixture of dimedone (1.00 g, 7.13 mmol) and potassium carbonate (2.46 g, 17.8 mmol) in a 2:1 ratio of 1,4-dioxane (14 mL) and water (7 mL). This mixture was stirred at room temperature for 2 hours. 2,4-difluorophenylboronic acid (1.35 g, 8.56 mmol) and tetrakis(triphenylphosphine)palladium(0) (247 mg, 0.21 mmol) were added and the mixture was heated under reflux for 3 hours or until completion. The resulting mixture was extracted with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and evaporate via rotary evaporator. The product **70** was isolated via column chromatography with 15% EtOAc in hexanes to obtain a beige solid. (0.73 g, 43%).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.31 (dt, *J* = 8.62, 6.36 Hz, 1H), 6.96-6.82 (m, 2H), 6.24 (s, 1H), 2.60 (t, *J* = 1.61 Hz, 2H), 2.34 (s, 2H), 1.12 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.58, 163.40, 160.15, 153.86, 129.92, 127.89, 124.29, 111.81, 104.79, 51.00, 43.67, 34.15, 28.17 (2C)


7.32
7.30
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6.89
6.86
6.24
6.24
2.60
2.60
2.60
2.34 HDO
1.12
O
F
F
Chemical Formula: C14H14F2O
Molecular Weight: 236.26
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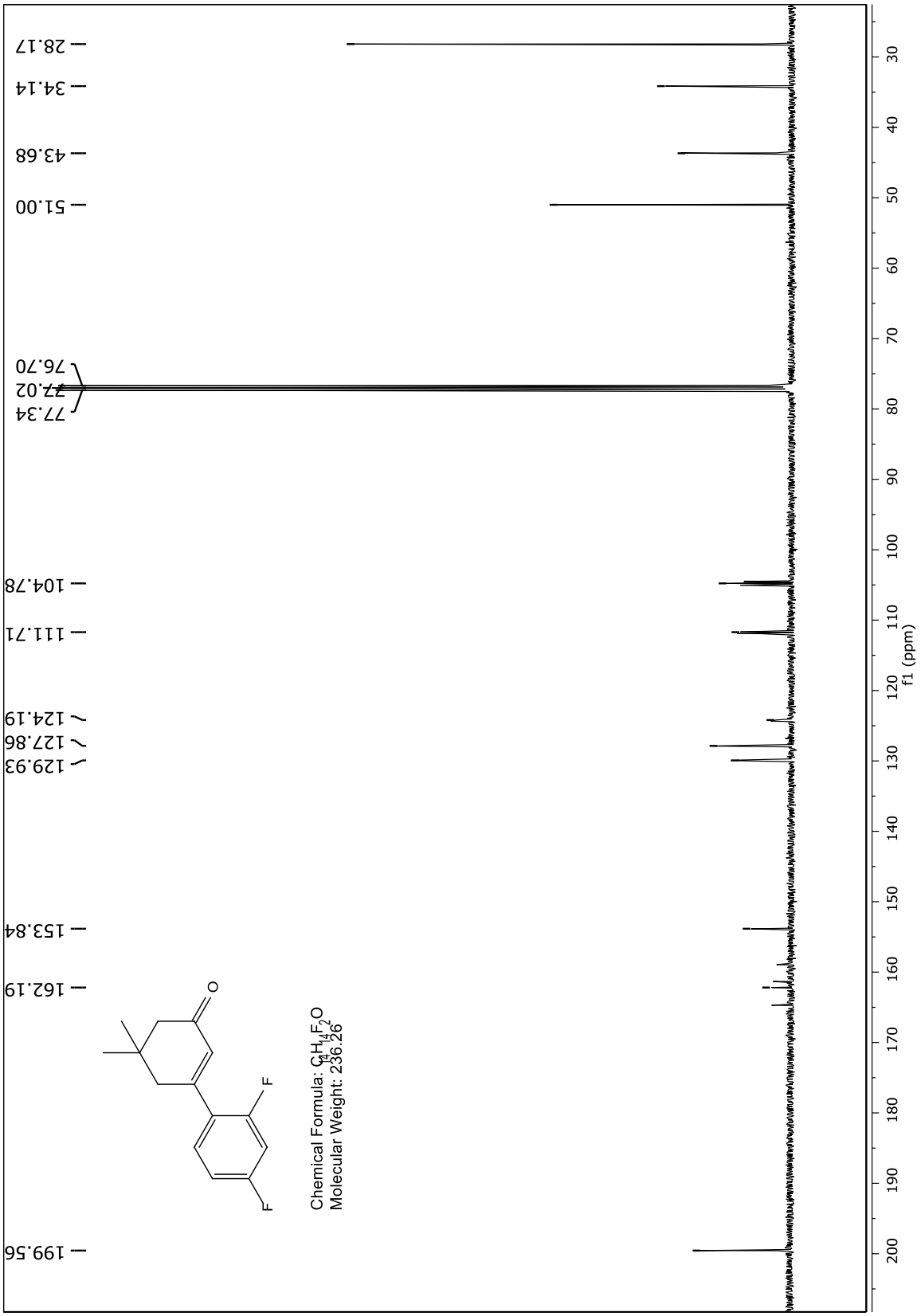
0.40
0.78
1.91
1.89
6.00
f1 (ppm)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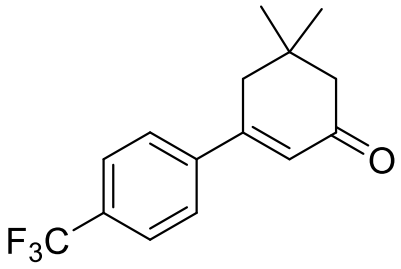
127.86
124.19
111.71
104.78
77.34
77.02
76.70
51.00
43.68
34.14
28.17
Chemical Formula: C13H14F2O
Molecular Weight: 236.26
F
F
O
f1 (ppm)

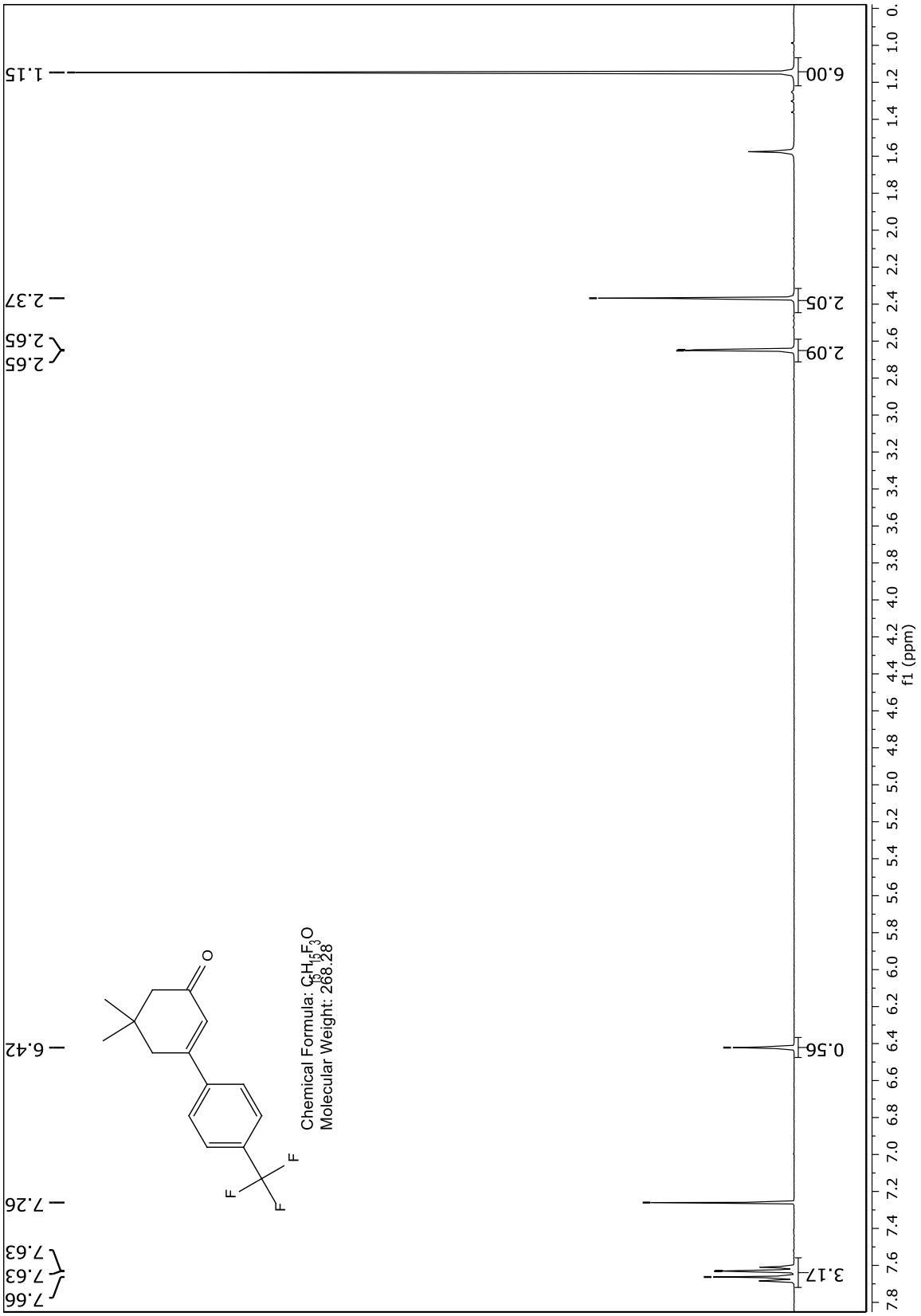


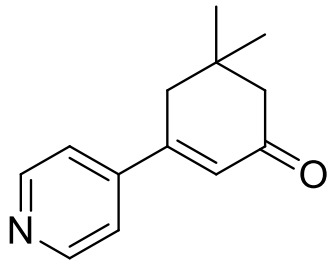
71

p-toluenesulfonyl chloride (0.78 g, 4.10 mmol) was added to a mixture of dimedone (0.37 g, 3.16 mmol) and potassium carbonate (1.09 g, 7.89 mmol) in a 2:1 ratio of 1,4-dioxane (14 mL) and water (7 mL). This mixture was stirred at room temperature for 2 hours. 4(trifluoromethyl)phenylboronic acid (0.5 g, 2.63 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.09 mg, 0.08 mmol) were added and the mixture was heated under reflux for 3 hours or until completion. The resulting mixture was extracted with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and evaporate via rotary evaporator. The product **71** was isolated via column chromatography with 30% EtOAc in hexanes followed by recrystallization to obtain a white solid. (0.35 g, 41%).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.72 – 7.56 (m, 3H), 6.42 (s, 1H), 2.65 (d, *J* = 1.6 Hz, 2H), 2.37 (s, 2H), 1.15 (s, 6H).

HRMS: Calculated for C15H15F3O: 268.1075 **Found:** 268.1082

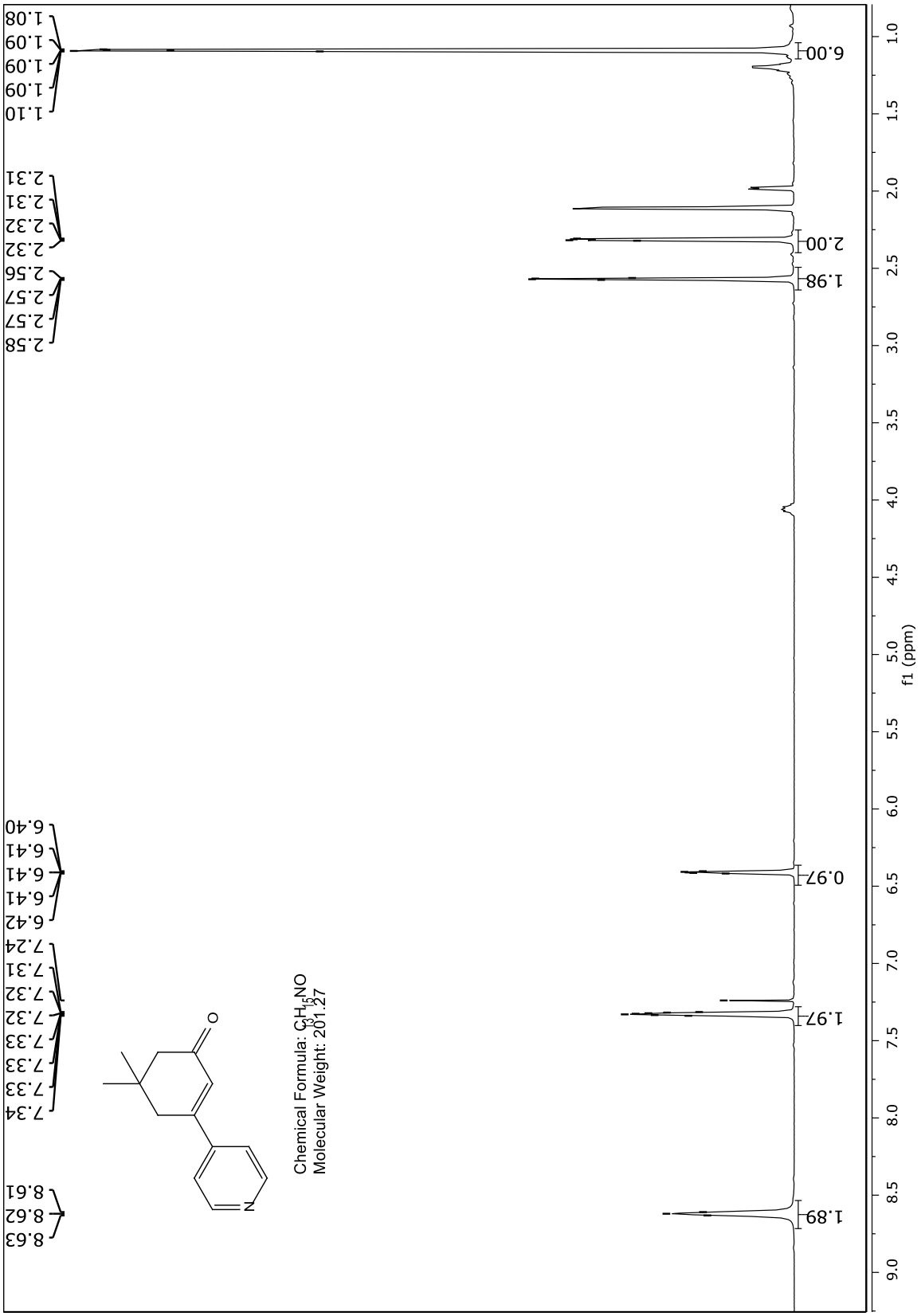

7.66
7.63
7.63
7.26
6.42
2.65
2.65
2.37
1.15
6.00
2.05
2.09
0.56
3.17
Chemical Formula: C15H15F3O
Molecular Weight: 268.28
O
F
F
F
f1 (ppm)



73

p-toluenesulfonyl chloride (0.97 g, 5.1 mmol) was added to a mixture of dimedone (0.55 g, 3.9 mmol) and potassium carbonate (1.35 g, 9.8 mmol) in a 2:1 ratio of 1,4-dioxane (8 mL) and water (4 mL). This mixture was stirred at room temperature for 1 hour. 4-pyridinyl boronic acid (0.4 g, 3.3 mmol) and tetrakis(triphenylphosphine)palladium (0) (0.11 g, 0.1 mmol) were added and the mixture was heated under reflux for 2 hours or until completion. The resulting mixture was extracted with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and evaporate via rotary evaporator. The product **73** was isolated via column chromatography with 60% EtOAc in hexanes followed by a wash with a 5% HCl solution then with an aqueous 10% NaOH solution to obtain white crystals (0.32 g, 31%).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.62 (t, *J* = 4.4 Hz, 2H), 7.33 (dq, *J* = 4.5, 1.6 Hz, 2H), 6.41 (dq, *J* = 3.3, 1.6 Hz, 1H), 2.57 (q, *J* = 1.7 Hz, 2H), 2.35 – 2.28 (m, 2H), 1.12 – 1.06 (m, 6H).


Chemical Formula: C13H15NO
Molecular Weight: 201.27
O
N
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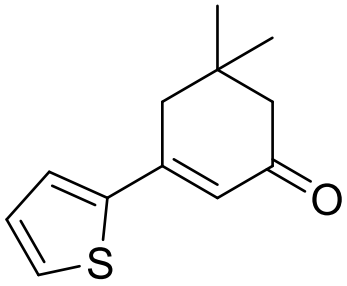
8.62
8.61
7.34
7.33
7.33
7.33
7.32
7.32
7.32
7.31
7.24
6.42
6.41
6.41
6.41
6.40
2.58
2.57
2.57
2.56
2.32
2.32
2.31
2.31
1.10
1.09
1.09
1.09
1.08
1.89
0.97
1.97
6.00
1.98
2.00
f1 (ppm)



74

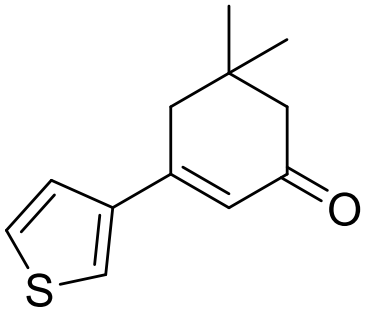
p-toluenesulfonyl chloride (1.16 g, 6.1 mmol) was added to a mixture of dimedone (0.65 g, 4.7 mmol) and potassium carbonate (1.62 g, 11.7 mmol) in a 2:1 ratio of 1,4-dioxane (12 mL) and water (6 mL). This mixture was stirred at room temperature for 1 hour. 2-thienylboronic acid (0.5 g, 3.9 mmol) and tetrakis(triphenylphosphine)palladium (0) (0.135 g, 0.12 mmol) were added and the mixture was heated under reflux for 2 hours or until completion. The resulting mixture was extracted with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and evaporate via rotary evaporator. The product **74** was isolated via column chromatography with 10% EtOAc in hexanes. (0.427 g, 53%).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.35 – 7.24 (m, 2H), 6.98 (dd, *J* = 5.1, 3.8 Hz, 1H), 6.30 (t, *J* = 1.5 Hz, 1H), 2.53 (d, *J* = 1.5 Hz, 2H), 2.19 (s, 2H), 1.01 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.41, 150.21, 142.97, 128.70, 128.26, 127.29, 121.59, 50.94, 41.97, 33.51, 28.39 (2C).

7.33
7.32
7.31
7.31
7.28
7.28
7.27
7.27
7.24 CDCl3
6.99
6.98
6.98
6.97
6.31
6.30
6.30
Chemical Formula: C14H14OS
Molecular Weight: 206.30
O
S
2.53
2.53
2.32
2.26
2.19
1.15
1.14
1.01
0.96
0.96
6.00
2.01
2.01
0.94
0.97
1.96
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

Chemical Formula: C12H14OS
Molecular Weight: 206.30
199.42
199.41
199.39
150.21
142.97
135.34
130.61
128.70
128.26
127.29
121.59
77.47 CDCl3
77.16
77.14 CDCl3
76.83
50.94
41.97
33.51
30.90
29.68
28.39
28.03
27.96
f1 (ppm)



75

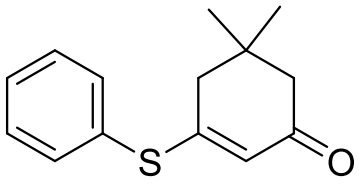
p-Toluenesulfonyl chloride (1.16 g, 6.1 mmol) was added to a mixture of dimedone (0.65 g, 4.7 mmol) and potassium carbonate (1.62 g, 11.7 mmol) in a 2:1 ratio of 1,4-dioxane (12 mL) and water (6 mL). This mixture was stirred at room temperature for 1 hour. 3-thienylboronic acid (0.5 g, 3.9 mmol) and tetrakis(triphenylphosphine)palladium (0) (0.135 g, 0.12 mmol) were added and the mixture was heated under reflux for 2 hours or until completion. The resulting mixture was extracted with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and evaporate via rotary evaporator. The product **75** was isolated via column chromatography with 10% EtOAc in hexanes. (0.367 g, 46%).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.48 (dd, *J* = 2.8, 1.4 Hz, 1H), 7.26 (dd, *J* = 5.1, 2.2 Hz, 2H), 6.31 (t, *J* = 1.5 Hz, 1H), 2.53 (d, *J* = 1.5 Hz, 2H), 2.22 (s, 2H), 1.03 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 200.31, 151.22, 140.90, 126.71, 125.23, 124.98, 122.86, 51.03, 41.96, 33.51, 28.50 (2C).

7.49
7.48
7.48
7.47
7.27
7.26
7.25
7.25
6.31
6.31
6.31
2.53
2.53
2.22
1.03
0.98
1.98
0.97
2.01
2.04
6.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)
O
S
Chemical Formula: C12H14OS
Molecular Weight: 206.30

200.31
151.22
140.90
126.71
125.23
124.98
122.86
77.33 CDCl3
77.02 CDCl3
76.70 CDCl3
51.03
41.96
33.51
28.50
O
S
Chemical Formula: C13H14OS
Molecular Weight: 206.30
f1 (ppm)



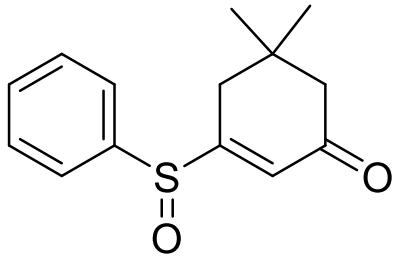
76

Thiophenol (3.77 g, 34.2 mmol), 5,5-dimethylcyclohexane-1,3-dione (4.0 g, 28.5 mmol) and anhydrous ferrous chloride (0.93 g, 5.71 mmol) were added to a flask and stirred at room temperature for 5 hours. The mixture was dissolved in dichloromethane (30 mL) and washed with a 10% sodium hydroxide solution in an extraction funnel. The aqueous phase was treated with bleach to get rid of the strong thiophenol smell. The organic phase was dried with $MgSO_4$, filtered and concentrated via rotary evaporator. The product **76** was isolated via column chromatography with 20% EtOAc in hexanes (1.47 g, 22%).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.45 (tdt, *J* = 3.93, 2.51, 1.89 Hz, 5H), 5.48 (s, 1H), 2.40 (s, 2H), 2.23 (s, 2H), 1.08 (s, 6H)\

HRMS: Calculated for C14H16OS: 232.0922 **Found:** 232.0932

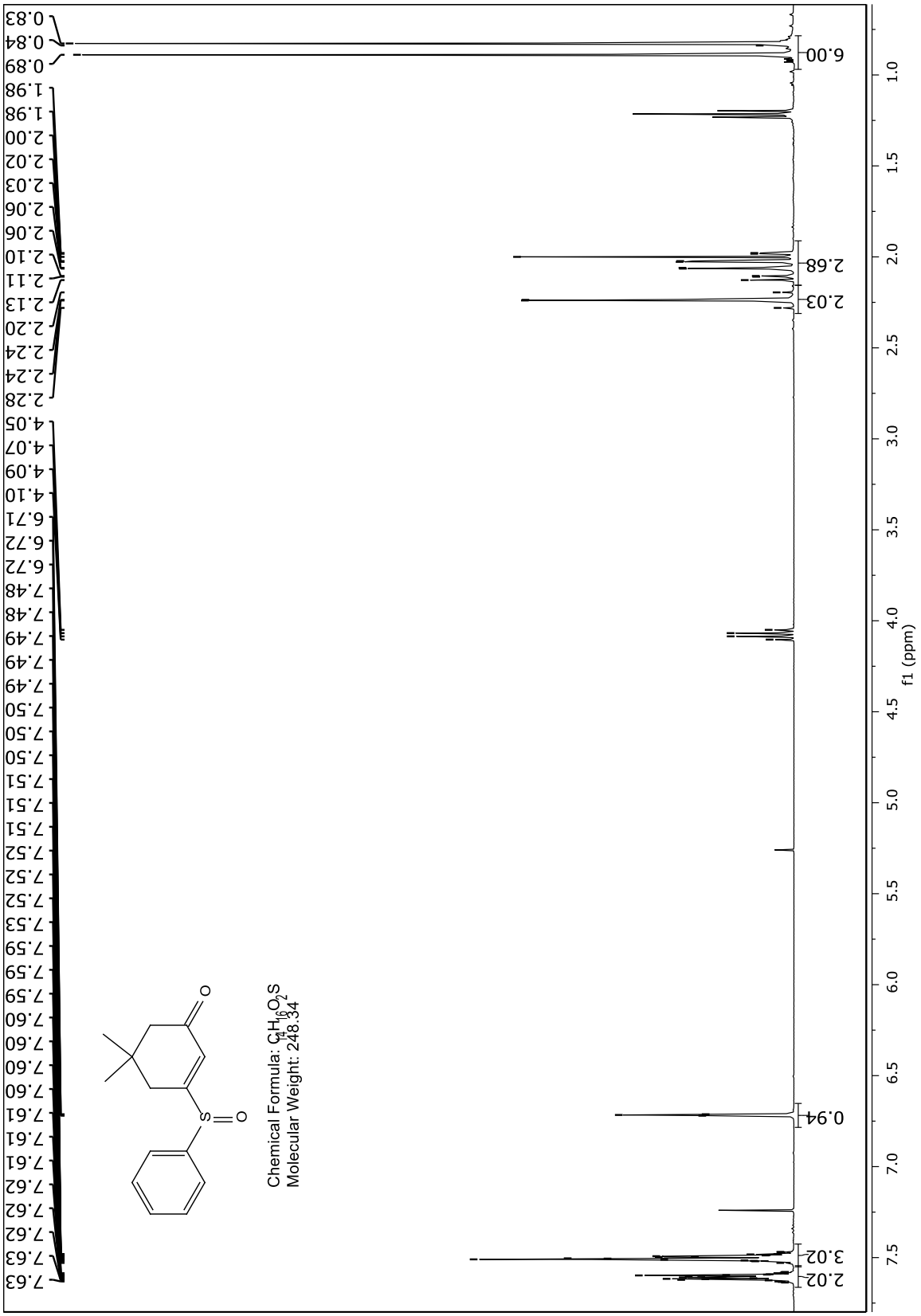
7.47
7.44
7.43
7.43
7.26
5.48
2.40
2.40
2.23
1.08
2.20
0.61
1.98
1.99
6.00
Chemical Formula: C14H16OS
Molecular Weight: 232.34
O
S
f1 (ppm)

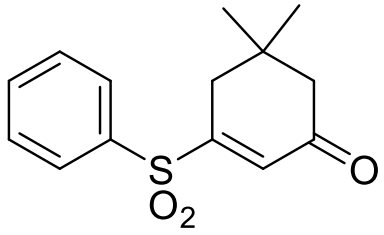


77

5,5-Dimethyl-3-(phenylthio)cyclohex-2-enone (**76**) (1.09g, 4.69 mmol) was dissolved in dichloromethane (15 mL) and the solution was cooled to 0°C. mCPBA was dissolved in a small amount of dichloromethane and added dropwise. The mixture was stirred over 30 mins at 0°C, then warmed to room temperature and stirred for an additional 1 hour. The reaction was quenched with a 10% aqueous solution of sodium carbonate. The mixture was extracted with dichloromethane (2 x 10 mL) and washed a last time with a solution of brine (20 mL). The organic extract was dried with $MgSO_4$, filtered and concentrated via rotary evaporator. The sulfoxide **77** was isolated using column chromatography with 20% EtOAc in hexanes to obtain a clear yellow oil (550 mg, 47%)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.66 – 7.54 (m, 2H), 7.55 – 7.43 (m, 3H), 6.72 (t, *J* = 1.6 Hz, 1H), 2.24 (d, *J* = 1.4 Hz, 2H), 2.14 – 2.01 (m, 2H), 0.86 (d, *J* = 24.9 Hz, 6H).


Chemical Formula: C14H16O2S
Molecular Weight: 248.34
f1 (ppm)
6.00
2.68
2.03
0.94
3.02
2.02

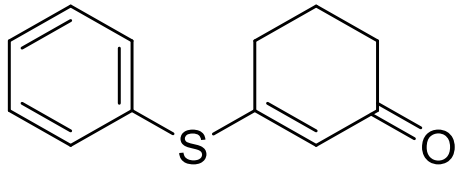


78

5,5-DImethyl-3-(phenylthio)cyclohex-2-enone (**76**) (1.09g, 4.69 mmol) was dissolved in dichloromethane (15 mL) and the solution was cooled to 0°C. mCPBA was dissolved in a small amount of dichloromethane and added dropwise. The mixture was stirred over 30 minutes at 0°C, then warmed to room temperature and stirred for an additional 1 hour. The reaction was quenched with a 10% aqueous solution of sodium carbonate. The mixture was extracted with dichloromethane (2 x 10 mL) and washed a last time with a solution of brine (20 mL). The organic extract was dried with $MgSO_4$, filtered and concentrated via rotary evaporator. The sulfone 7**8** was isolated using column chromatography with 20% EtOAc in hexanes to obtain a clear yellow oil (300 mg, 24%)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.90 (ddd, *J* = 7.22, 2.93, 1.67 Hz, 2H), 7.70 (m, 1H), 7.62-7.57 (m, 2H), 6.72 (t, *J* = 1.73 Hz, 1H), 2.39 (d, *J* = 1.75 Hz, 2H), 2.27 (s, 2H), 0.96 (s, 6H)

7.89
7.89
7.87
7.87
7.68
7.60
7.60
7.58
7.58
7.24
6.70
2.37
2.36
2.26
2.15
0.94
0.94
6.00
1.96
1.97
0.52
1.63
0.41
1.42
Chemical Formula: C14H16O3S
Molecular Weight: 264.34
f1 (ppm)

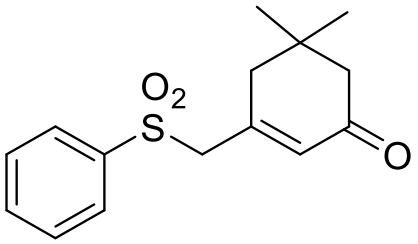


79

Thiophenol (4.7 g, 42.8 mmol), cyclohexane-1,3-dione (4.0 g, 35.7 mmol) and anhydrous ferrous chloride (0.93 g, 5.71 mmol) were added to a flask and stirred at room temperature for 5 hours. The mixture was dissolved in dichloromethane (30 mL) and washed with a 10% sodium hydroxide solution in an extraction funnel. The aqueous phase was treated with bleach to get rid of the strong thiophenol smell. The organic phase was dried with $MgSO_4$, filtered and concentrated via rotary evaporator. The product **79** was isolated via column chromatography with 20% EtOAc in hexanes (1.54 g, 22%).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.55 – 7.35 (m, 5H), 5.55 – 5.42 (m, 1H), 2.52 (ddd, *J* = 6.4, 5.6, 1.2 Hz, 2H), 2.36 (dd, *J* = 7.3, 5.9 Hz, 2H), 2.14 – 1.95 (m, 2H).

7.49
7.48
7.46
7.46
7.46
7.45
7.43
7.43
7.42
7.42
7.42
7.41
7.40
7.40
7.40
7.40
5.47
5.46
5.46
2.54
2.54
2.52
2.52
2.51
2.50
2.50
2.38
2.36
2.36
2.34
2.06
2.04
2.04
2.03
2.01
4.73
1.00
2.31
2.07
1.58
O
S
Chemical Formula: C12H12OS
Molecular Weight: 204.29
f1 (ppm)



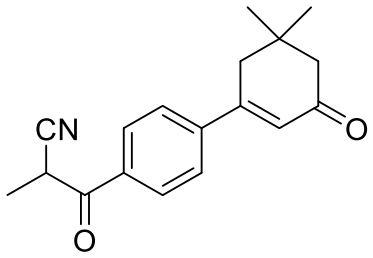
80

nBuLi 2.5M (0.48 mL, 1.19 mmol) was added to dry THF (10 mL) under N_2 atmospthere to a flask cooled to -78°C. Phenyl methyl sulfone (0.19 g, 1.19 mmol) was dissolved in dry THF (5 mL) and added dropwise. Ethoxydimedone **29** (0.2 g, 1.19 mmol) was dissolved in dry THF (5 mL) and added dropwise. The reaction was quenched with a solution of NH_4Cl (10 mL) and extracted with H_2O and EtOAc (10 mL x 2). The organic phase was dried over $MgSO_4$ and concentrated by rotary evaporator. The product **80** was isolated by column chromatography with 20% EtOAc in hexanes to obtain a clear yellow oil (0.03 g, 10%)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.92 – 7.75 (m, 2H), 7.65 (d, *J* = 7.5 Hz, 1H), 7.55 (dd, *J* = 8.3, 7.0 Hz, 2H), 5.56 (t, *J* = 0.9 Hz, 1H), 3.90 (d, *J* = 0.8 Hz, 2H), 2.41 (d, *J* = 1.8 Hz, 2H), 2.17 (s, 2H), 1.02 (s, 6H).

HRMS: Calculated for C15H18O3S: 278.0977 **Found:** 278.0962

7.85
7.85
7.85
7.83
7.83
7.66
7.64
7.57
7.55
7.55
7.53
7.24
5.56
5.56
5.55
3.90
3.89
2.42
2.41
2.17
1.02
2.00
0.62
1.91
0.70
2.11
2.10
2.08
6.00
Chemical Formula: C17H18O3S
Molecular Weight: 278.37
f1 (ppm)



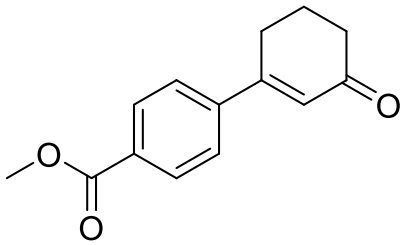
82

Lithium Diisopropylamide 2M (3.87 mL, 7.74 mmol) was added to dry THF (25 mL) under nitrogen atmosphere at -78°C. Propionitrile (0.06 mL, 7.74 mmol) was added dropwise and the solution was stirred at -78°C for 15 minutes. 3',3'-dimethyl-5'-oxo-2',3',4',5'-tetrahydro-[1,1'-biphenyl]-4-carboxylic acid (**40**) (100 mg, 0.387 mmol) was added dropwise and the solution was stirred for 30 minutes. The mixture was quenched by transferring it to a beaker containing an aqueous NH_4Cl solution. The mixture was then warmed at room temperature and diluted with water followed by extraction with EtOAc (3 x 10 mL). The extract was dried with $MgSO_4$, filtered and concentrated by rotary evaporator. The hydroxyl intermediate was isolated by column chromatography.

The hydroxyl intermediate was dissolved in toluene and *p*-toluenesulfonic acid (50 mg, catalytic) was added. The mixture was refluxed for 3 hours and was quenched by adding an aqueous $NaHCO_3$ solution. The mixture was extracted with EtOAc (3 x 10 mL) and the extract was dried with $MgSO_4$, filtered and evaporated by rotary evaporator. The product **82** was isolated by column chromatography with 40% EtOAc in hexanes (30 mg, 28%).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.07-7.98 (m, 2H), 7.71-7.61 (m, 2H), 6.45 (s, 1H), 4.36 (q, *J* = 7.18 Hz, 1H), 2.65 (d, *J* = 1.53 Hz, 2H), 2.36 (d, *J* = 5.96 Hz, 2H), 1.65 (d, *J* = 7.14 Hz, 3H), 1.14 (s, 6H)

8.05
8.04
8.02
8.02
7.68
7.68
7.65
7.65
6.45
4.41
4.39
2.67
2.66
2.37
1.67
1.65
1.15
1.14
1.58
1.61
0.82
0.79
1.77
1.86
2.55
6.00
f1 (ppm)
Chemical Formula: C18H19NO2
Molecular Weight: 281.36



84

p-Toluenesulfonyl chloride (371 mg, 1.95 mmol) was added to a mixture of 1,3-cyclohexanedione (168 mg, 1.5 mmol) and potassium carbonate (432 mg, 3.0 mmol) in a 2:1 ratio of 1,4-dioxane (10 mL) and water (5 mL). This mixture was stirred at room temperature for 1 hour. 4-Methoxycarbonylphenylboronic acid (225 mg, 1.25 mmol) and tetrakis(triphenylphosphine)palladium (0) (7.22 mg, 0.006 mmol) were added and the mixture was heated under reflux for 1.5 hours or until completion. The resulting mixture was extracted with EtOAc (2 x 25 mL). The extract was dried with $MgSO_4$, filtered and evaporated via rotary evaporator. The product **84** was isolated via column chromatography with DCM. (0.09 g, 26%).

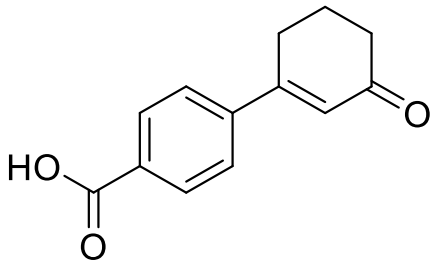
^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.09 – 8.01 (m, 2H), 7.61 – 7.53 (m, 2H), 6.43 (t, *J* = 1.5 Hz, 1H), 3.92 (s, 3H), 2.77 (td, *J* = 6.0, 1.5 Hz, 2H), 2.49 (dd, *J* = 7.5, 5.9 Hz, 2H), 2.22 – 2.10 (m, 2H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.55, 166.44, 158.39, 143.21, 131.20, 129.96 (2C), 126.79, 126.05 (2C), 52.30, 37.25, 28.10, 22.75.

HRMS: Calculated for $C_{14}H_{14}O_3$: 230.0943 **Found:** 230.0975

8.07
8.06
8.06
8.06
8.06
8.04
8.04
8.04
7.58
7.58
7.58
7.57
7.56
7.56
7.55
7.24 CDCl3
6.43
6.43
6.42
3.92
2.77
2.76
2.50
2.49
2.48
2.47
2.16
2.16
2.14
Chemical Formula: C14H14O3
Molecular Weight: 230.26
0.98
1.01
0.49
1.49
1.01
1.05
1.00
f1 (ppm)

199.55
166.44
158.39
143.21
131.20
129.96
126.79
126.05
77.33 CDCl3
77.01 CDCl3
76.70 CDCl3
52.30
37.25
28.10
22.75
0.28
0.25
0.18
0.78
0.81
0.69
0.66
0.22
1.17
0.60
1.10
0.28
Chemical Formula: C14H14O3
Molecular Weight: 230.26
f1 (ppm)



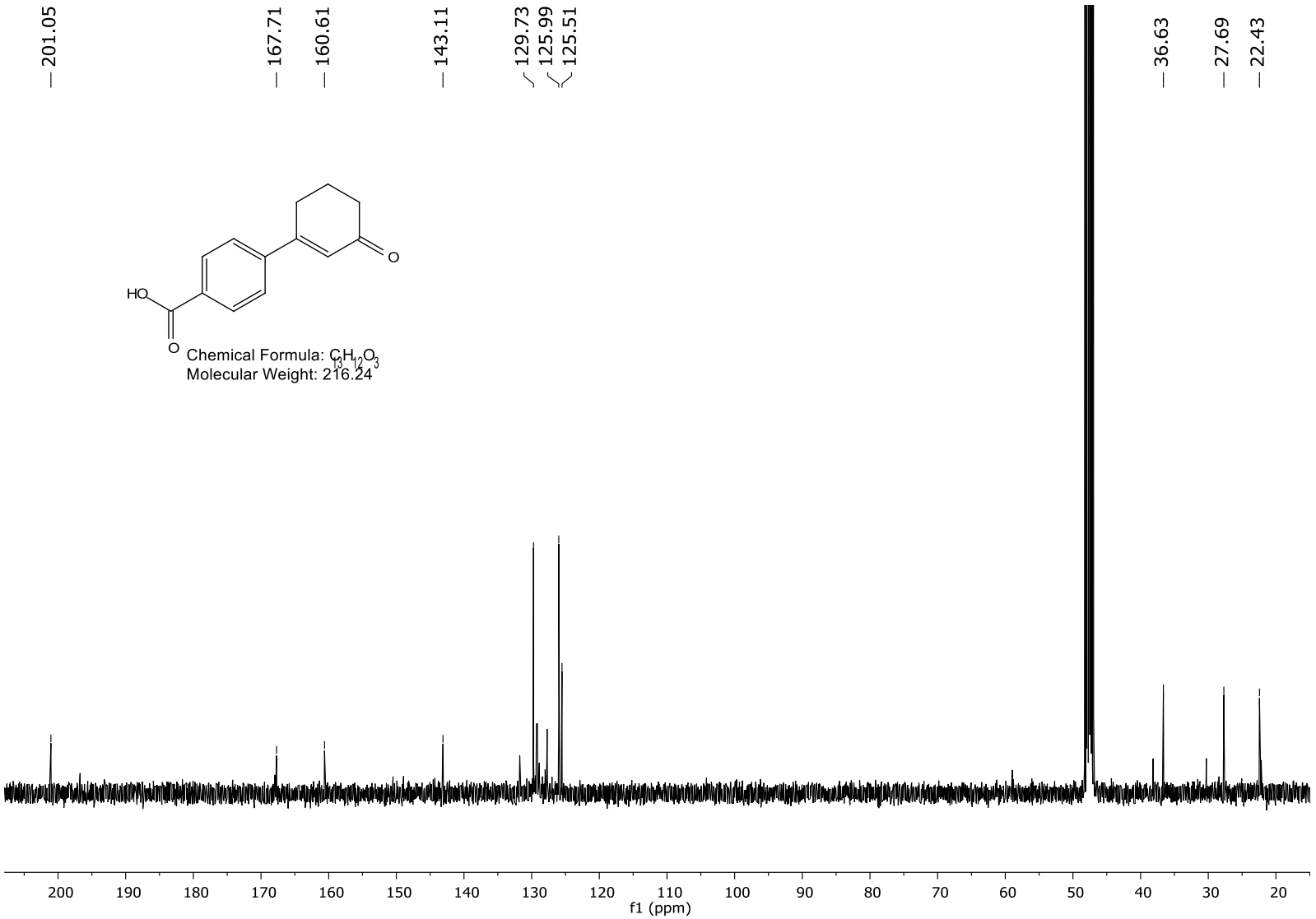
85

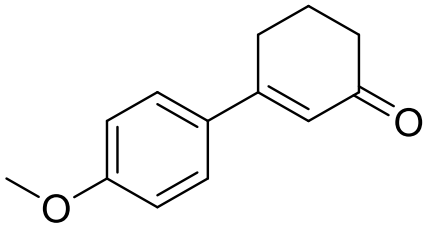
84 (20 mg, 0.09 mmol) was added to a 5% solution of NaOH in water (2 mL) and methanol (8 mL) and stirred at room temperature for 12 hours. The reaction mixture was washed with EtOAc (20 mL). The aqueous phase was treated with a 5% HCl solution (10 mL) followed by an extraction with EtOAc (20 mL). The resulting organic phase was dried over $MgSO_4$ and concentrated by rotary evaporator to obtain **85**. (10 mg, 51%)

^{1}H NMR (400 MHz, MeOD) δ, ppm: 8.07 (d, *J* = 8.5 Hz, 2H), 7.72 (d, *J* = 8.5 Hz, 2H), 6.44 (s, 1H), 2.96 – 2.75 (m, 2H), 2.50 (dd, *J* = 7.5, 6.0 Hz, 2H), 2.27 – 2.06 (m, 2H).

^{13}C NMR (100 MHz, MeOD) δ, ppm: 201.05, 167.71, 160.61, 143.11, 131.76, 129.73 (2C), 125.99 (2C), 125.51, 36.63, 27.69, 22.43.

8.09
8.06
7.73
7.71
6.44
4.86
3.31
2.88
2.87
2.86
2.85
2.85
2.52
2.50
2.50
2.48
2.19
2.18
2.16
2.16
2.31
1.87
0.92
1.88
1.57
2.00
Chemical Formula: C13H12O3
Molecular Weight: 216.24
OH
O
O
f1 (ppm)


201.05
167.71
160.61
143.11
129.73
125.99
125.51
36.63
27.69
22.43
HO
O
O
Chemical Formula: $C_{13}H_{12}O_3$
Molecular Weight: 216.24
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)



86

p-Toluenesulfonyl chloride (440 mg, 2.31 mmol) was added to a mixture of 1,3-cyclohexanedione (200 mg, 1.78 mmol) and potassium carbonate (511 mg, 3.7 mmol) in a 2:1 ratio of 1,4-dioxane (10 mL) and water (5 mL). This mixture was stirred at room temperature for 1 hour. 4-Methoxyphenylboronic acid (225 mg, 1.48 mmol) and tetrakis(triphenylphosphine)palladium (0) (0.135 g, 0.12 mmol) were added and the mixture was heated under reflux for 1.5 hours or until completion. The resulting mixture was extracted with EtOAc (2 x 25 mL). The extract was dried with $MgSO_4$, filtered and evaporated via rotary evaporator. The product **86** was isolated via column chromatography with 5% EtOAc in hexanes. (0.2 g, 38%).

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.54 – 7.46 (m, 2H), 6.95 – 6.87 (m, 2H), 6.38 (t, *J* = 1.4 Hz, 1H), 3.83 (s, 3H), 2.73 (td, *J* = 6.1, 1.4 Hz, 2H), 2.45 (dd, *J* = 7.4, 6.0 Hz, 2H), 2.18 – 2.06 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 199.90, 161.23, 159.09, 130.83, 127.63(2C), 123.72, 114.15 (2C), 55.40, 37.21, 27.88, 22.79.

HRMS: Calculated for $C_{13}H_{14}O_2$: 202.0994 **Found:** 202.0997

7.51
7.51
7.49
7.49
7.24 CDCl3
6.93
6.92
6.92
6.90
6.90
6.89
6.38
6.38
6.37
5.86
3.99
3.83
3.73
2.75
2.75
2.73
2.73
2.72
2.71
2.46
2.46
2.45
2.45
2.44
2.43
2.29
2.15
2.14
2.14
2.12
2.12
2.12
2.11
2.10
2.09
2.02
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Molecular Weight: 202.25
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1.88
1.02
2.88
2.12
2.00
2.10
f1 (ppm)

199.90
161.23
159.09
130.83
127.63
123.72
114.15
77.34 CDCl3
77.03 CDCl3
76.71 CDCl3
55.40
37.21
27.88
22.79
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Molecular Weight: 202.25
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0.19
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0.55
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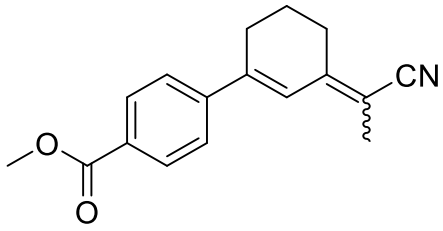
0.63
0.58
0.60
f1 (ppm)



87

To dry THF (12 mL) under N_2 atmosphere at -78°C is added LDA 2M (0.434 mL, 0.868 mmol) dropwise. Propionitrile (0.07 mL, 1.04 mmol) was added dropwise, followed by a solution of **85** (0.1 g, 0.434 mmol) in dry THF (5 mL). The reaction was stirred during 15 min at -78°C. The reaction was quenched with NH_4Cl at room temperature and extracted with EtOAc (20 mL). The organic phase was washed with water (20 mL), dried over $MgSO_4$ and concentrated by rotary evaporator. The hydroxyl intermediate was isolated by column chromatography.

The hydroxyl intermediate (10 mg) was dissolved in toluene (10 mL) and PTSA (30 mg, 0.174 mmol) was added and the reaction was refluxed for 2 hours. The reaction was quenched by a saturated $NaCO_3$ solution. The mixture was extracted with EtOAc (10 mL) and the organic phase was washed with water (10 mL). The resulting organic phase was dried over $MgSO_4$, filtered and concentrated by rotary evaporator. The isomeric mixture **87** was obtained by column chromatography with 10% EtOAc in hexanes. (0.01 g, 9%)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.11 – 7.95 (m, 2H), 7.61 – 7.46 (m, 2H), 7.15 (d, *J* = 1.6 Hz, 1/2H), 6.85 (t, *J* = 1.6 Hz, 1/2H), 3.91 (d, *J* = 1.0 Hz, 3H), 2.74 – 2.54 (m, 3H), 2.50 – 2.38 (m, 1H), 2.07 – 1.90 (m, 4H), 1.95 – 1.82 (m, 2H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 166.74, 166.65, 150.64, 149.33, 145.59, 144.85, 144.52, 144.12, 129.92, 129.86, 129.81, 129.74 (2C), 129.48 (2C), 125.56 (2C), 125.47 (2C), 125.04, 121.67, 120.51, 119.76, 103.03, 102.45, 52.21, 52.16, 29.63, 28.19, 27.49, 26.02, 22.97, 22.38, 21.87, 16.13, 15.16.

8.03
8.03
8.02
8.02
8.02
8.02
8.01
8.01
8.00
7.99
7.99
7.57
7.56
7.55
7.54
7.53
7.53
7.51
7.50
7.24 CDCl3
7.16
7.15
7.15
6.86
6.85
6.85
3.91
3.91
2.69
2.68
2.68
2.67
2.66
2.66
2.64
2.63
2.62
2.60
2.58
2.46
2.46
2.44
2.42
2.42
2.04
2.03
2.03
1.98
1.98
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1.91
1.89
1.87
Chemical Formula: C17H17NO2
Molecular Weight: 267.33
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3.65
1.79
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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f1 (ppm)

166.74
166.65
150.64
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145.59
144.85
144.52
144.12
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129.86
129.81
129.74
129.48
125.56
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125.04
121.67
120.51
119.76
103.03
102.45
77.34 CDCl3
77.02 CDCl3
76.70 CDCl3
52.21
52.16
29.63
28.19
27.49
26.02
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Chemical Formula: C17H17NO2
Molecular Weight: 267.33
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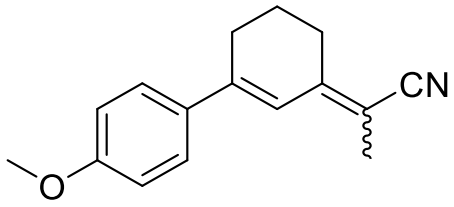
O
O
f1 (ppm)



88

To dry THF (12 mL) under N_2 atmosphere at -78°C is added LDA 2M (0.434 mL, 0.868 mmol) dropwise. Propionitrile (0.07 mL, 1.04 mmol) was added dropwise, followed by a solution of **86** (0.1 g, 0.434 mmol) in dry THF (5 mL). The reaction was stirred during 15 min at -78°C. The reaction was quenched with NH_4Cl at room temperature and extracted with EtOAc (20 mL). The organic phase was washed with water (20 mL), dried over $MgSO_4$ and concentrated by rotary evaporator. The hydroxyl intermediate was isolated by column chromatography.

The hydroxyl intermediate (10 mg) was dissolved in toluene (10 mL) and PTSA (30 mg, 0.174 mmol) was added and the reaction was refluxed for 2 hours. The reaction was quenched by a saturated $NaCO_3$ solution. The mixture was extracted with EtOAc (10 mL) and the organic phase was washed with water (10 mL). The resulting organic phase was dried over $MgSO_4$, filtered and concentrated by rotary evaporator. The product **88** was obtained by column chromatography with 10% EtOAc in hexanes. (0.01 g, 9%)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.49 (dd, *J* = 10.6, 8.8 Hz, 2H), 7.14 – 6.67 (m, 3H), 3.85 (d, *J* = 1.3 Hz, 3H), 2.81 – 2.38 (m, 4H), 2.09 – 1.84 (m, 5H)

^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 160.10, 159.96, 151.44, 150.15, 146.19, 144.70, 132.86, 132.54, 126.92, 126.85, 121.81, 121.04, 120.26, 118.53, 113.98, 113.92, 100.44, 100.01, 55.37, 55.35, 29.74, 28.19, 27.50, 26.12, 22.51, 22.01, 15.95, 15.00.

7.52
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7.06
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6.90
6.75
3.85
3.85
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2.62
2.60
2.44
2.04
1.99
1.94
1.92
1.90
1.88
1.59
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Molecular Weight: 239.32
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7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

Chemical Formula: C16H17NO
Molecular Weight: 239.32
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159.96
151.44
150.15
146.19
144.70
132.86
132.54
126.92
126.85
121.81
121.04
120.26
118.53
113.98
113.92
100.44
100.01
77.34 CDCl3
77.02 CDCl3
76.70 CDCl3
55.37
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29.74
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27.50
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22.51
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15.95
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0.12
0.18
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0.16
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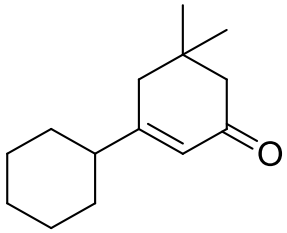
f1 (ppm)



89

Magnesium turnings (0.43 g, 17.9 mmol) were added to a flask and put under nitrogen atmosphere. A solution of bromocyclohexane (2.91 g, 17.9 mmol) in dry diethyl ether (10 mL) was added and the mixture was lightly refluxed until all the magnesium turnings are consumed. A solution of 3-ethoxy-5,5-dimethylcyclohex-2-enone (**29**) (1.0 g, 5.95 mmol) in diethyl ether (10 mL) was added at room temperature and the mixture is refluxed again for 30 minutes. The reaction was quenched using water and an aqueous solution of NH_4Cl. The mixture was extracted with EtOAc, dried with $MgSO_4$, filtered and evaporated **89** was isolated by column chromatography with 20% EtOAc in hexanes to obtain a white solid. (290 mg, 24 %)

^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 5.84 (d, *J* = 0.69 Hz, 1H), 2.19 (s, 2H), 2.17 (d, *J* = 0.86 Hz, 2H), 2.05-1.94 (m, 1H), 1.84-1.65 (m, 5H), 1.35-1.11 (m, 5H), 1.00 (s, 6H)

7.26
5.84
5.84
2.19
2.17
2.17
1.99
1.81
1.80
1.79
1.78
1.77
1.76
1.74
1.74
1.73
1.73
1.73
1.27
1.25
1.24
1.22
1.21
1.19
1.19
1.18
1.00
0.89
6.00
3.72
3.82
0.34
4.02
Chemical Formula: C14H22O
Molecular Weight: 206.33
f1 (ppm)

References:

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