Inês de Sousa Fino

Licenciada em Bioquímica

Synthesis of metabolites from the 5-APB and 6-APB drugs of abuse

Dissertação para obtenção do Grau de Mestre em
Bioquímica para a Saúde

Orientadora: Paula Sério Branco, Professora Auxiliar com Agregação, FCT/UNL

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Novembro, 2020

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

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Part of the results of this work was included in the following scientific communication:

Poster:

I. Fino, C. Cardoso, Luísa M. Ferreira and Paula S. Branco, “Synthesis of drug metabolites of abuse of Benzo Fury´s”, in the “13º Encontro Nacional de Química Orgânica e 6º Encontro Nacional de Química Terapêutica”, Universidade de Aveiro, Aveiro, Portugal, 15- 17 of January of 2020.

Abstract

Benzofurans, also known as "Benzo Fury´s", are synthetic phenethylamines that belong to a growing group of designer drugs so-called novel psychoactive substances (NPS). Benzofurans act preferentially by disturbing the functioning of serotonergic circuits as a serotonin-norepinephrine-dopamine reuptake inhibitor. Their ability to induce entactogenic and stimulant effects, similar to classic illicit psychostimulants but with higher potency, lead to an increase in the recreational consumed. Therefore, the number of benzo *fury*-related deaths has been increasing, and some recent research work put in evidence that the abuse of these NPS is an alarming threat for public health. The first benzofurans to appear on the drug scene, in 2010, were 5-(2-aminopropyl)benzofuran (5-APB) and 6-(2-aminopropyl)benzofuran (6-APB). These compounds have previously been patented in 2006 as potential therapeutic drugs for eating disorders and seizures. To date, there is still a lack of toxicological and clinical information regarding these compound and their metabolites. For this reason, it is essential to develop a synthesis for benzofurans metabolites and determine their *in vitro* toxicity.

The main goal of this thesis was to develop a synthesis for 5-APB and 6-APB oxidized metabolites for toxicological studies. For the preparation of 5-APB metabolites, four different pathways were outlined. Both synthetic ways started with the methylation of 2-hydroxyphenyl acetic acid. The acid metabolite follows a general procedure: methylation, Rieche formylation, aldol reaction with nitroethane, reduction by catalytic hydrogenation. For the alcohol metabolite there were some differences, the methylation reaction is followed by a reduction reaction and alcohol protection before the formylation reaction, and besides the catalytic hydrogenation, another reduction using aluminium lithium hydride was performed. For the preparation of 6-APB metabolites, only one pathway was outlined and only one metabolite was a target of this work. The synthetic way started with the reduction of 2-(4-bromo-2-methoxyphenyl) acetic acid, followed by an alcohol protection reaction and formylation reaction. As envisaged for the alcohol metabolite of 5-APB, two different reductions were performed, catalytic hydrogenation and a reduction using aluminium lithium hydride.

Only one metabolite was attained and previous synthetic stable intermediates were achieved. All compounds were chemically characterized and can be used in upcoming toxicological studies.

Keywords: Drugs of abuse, *Benzofury*, 5-APB metabolites, 6-APB metabolites

Resumo

Os **benzofuranos**, também conhecidos como "Benzo Fury's", são fenetilaminas sintéticas que pertencem a um grupo de “designer drugs em crescimento” chamadas novas substâncias psicoativas (NPS). Os benzofuranos perturbam preferencialmente o funcionamento dos circuitos serotonérgicos na forma de inibidor da recaptação da serotonina-norepinefrina-dopamina. A sua capacidade de induzir efeitos entactogênicos e estimulantes, semelhantes aos psicoestimulantes ilícitos clássicos mas com maior potência, levou a um aumento no consumo recreativo. Subsequentemente, o número de mortes relacionadas com benzofuranos tem aumentado, e alguns trabalhos de pesquisa recentes evidenciam que o uso abusivo das NPS se tornou uma ameaça alarmante para a saúde pública. Os primeiros benzofuranos a serem identificados foram 5-(2-aminopropil) benzofurano (5-APB) e 6-(2-aminopropil) benzofurano (6-APB) em 2010. Estes compostos foram patenteados em 2006 como drogas terapêuticas com potencial para tratar distúrbios alimentares e convulsões. Até o momento, as informações toxicológicas e clínicas sobre estes compostos e os seus metabolitos é bastante reduzida. Por esse motivo, é essencial desenvolver uma via simtética para os seus metabolitos e determinar a sua toxicidade *in vitro*.

O objetivo principal desta tese foi desenvolver uma síntese para metabolitos oxidados do 5-APB e do 6-APB para estudos toxicológicos. Para a preparação dos metabolitos do 5-APB, quatro vias diferentes foram delineadas. Ambas as vias sintéticas começaram com a metilação do ácido 2-hidroxifenilacético. O metabolito ácido seguiu um procedimento geral: metilação, formilação de Rieche, reação aldólica com nitroetano e redução por hidrogenação catalítica. Para o metabolito álcool surgiram algumas diferenças, a reação de metilação é seguida por uma reação de redução e proteção do álcool antes da reação de formilação e, além da hidrogenação catalítica, foi realizada outra redução com hidreto de alumínio lítio. Para a preparação de um dos metabolitos do 6-APB apenas uma via foi delineada. A via sintética começou com a redução do ácido 2- (4-bromo-2-metoxifenil) acético, seguida por uma reação de proteção do álcool e reação de formilação. Conforme realizado para o metabólito álcool do 5-APB, diferentes reduções foram realizadas, hidrogenação catalítica e uma redução utilizando hidreto de alumínio lítio.

Apenas um metabolito foi sintetizado e outros intermediários estáveis foram obtidos. Estes novos compostos foram caracterizados quimicamente e poderão ser usados futuramente em estudos toxicológicos.

Palavras-chave: Drogas de abuso, benzofuranos, metabolitos do 5-APB, metabolitos do 6-APB

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Abbreviations

^{13}C NMR	Carbon-13 nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
5-APB	5-(2-aminopropyl)benzofuran
5-APDB	5-(2-aminopropyl)-2,3-dihydrobenzofuran
5-HT	5-hydroxytryptamine
6-APB	6-(2-aminopropyl)benzofuran
6-APDB	6-(2-aminopropyl)-2,3-dihydrobenzofuran
ADHD	Attention deficit hyperactivity disorder
Ar	Aromatic
C18	Octadecylsilane
CNS	Central nervous system
d	Doublet
DA	Dopamine
DAT	Dopamine receptor
dd	Doublet of doublets
DMF	Dimethylformamide
DNP	2,4-Dinitrophenylhydrazine
eq.	Equivalents
FTIR-ATR	Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy
g	gram
GC-MS	Gas chromatography coupled with mass spectrometry
h	Hour
HIV	human immunodeficiency virus
HPLC	High-performance liquid chromatography
HPLC-HRMS	High-performance liquid chromatography-high resolution mass spectrometry
HPLC-MS	High-performance liquid chromatography-mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
LSD	Lysergic acid diethylamide
m	multiplet
MDA	3,4-Methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
mg	milligram
MS	Mass spectrometry
MXE	Methoxamine
NA	Noradrenaline
NET	Noradrenaline receptor
NPS	Novel psychoactive substances

PEA	phenylethylamine
PNS	Peripheral nervous system
rf	retention factor
s	Singlet
SERT	Serotonin receptor
t	triplet
TAAR	Trace amine-associated receptor
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
UHPLC-MS/MS	Ultra-performance liquid chromatography-tandem mass spectrometer
UNODC	United Nations Office on Drugs and Crime
UV	Ultraviolet
VT	vesicular transporter

1 Introduction

1.1 Drug Use

Substance abuse can be defined as harmful and hazardous use of psychoactive substances, like alcohol and illicit drugs. These substances can cause dependence and withdrawal syndromes, such as behavioural, cognitive and/or physiological effects.[1] These phenomena are associated with an increase in the consuming desire, a higher difficulty in controlling the substance-taking behaviour, a persistence on taking it despite its harmful consequence and, subsequently progressive neglect of alternative pleasures or interests. Their toxicological effects are the main reason behind their drug group classification, being the most common illicit drugs of abuse the psychostimulants, depressants and hallucinogens.[2] These toxicological effects can affect a variety of body systems, leading to symptoms of organ dysfunction such as:

- Central Nervous System (CNS) symptoms, including headaches, altered mental state and coma;
- Cardiovascular alterations as changes in blood pressure and heart rate;
- Hepatic damage, that includes severe hepatotoxicity and liver failure;
- Reproductive consequences, for instance, decrease of infertility and teratogenesis, neonatal syndromes and Attention deficit hyperactivity disorder (ADHD);
- Infections complications from intravenous drug use, that can be divided into two types, viral (e.g. HIV) and bacterial (e.g. osteomyelitis).

The toxicology of illicit drugs depends on the administration route, which affects bioavailability, biodistribution and metabolism. The biotransformation of a specific drug can lead to dysfunction, destruction or a generation of a new toxic compound. As expected, from all these, may result in cellular dysfunction and cellular death.[3]

Drug use disorders are becoming an alarming problem in our society as a result of the increasing prevalence and overdose rates. Facing the worldwide statistics of the prevalence of drug use disorders (excluding alcohol) revealed that, in 2017, 0.9% of the world population had drug use disorder. In the same year, the country with the highest numbers was the United States with 3.45%, focusing on Europe, it fluctuates between 0.68% and 1.13% depending on the region (**Figure 1.1**). These drug use disorders often lead to death, directly, in case of overdose, or indirectly, as suicide. Statistics show that around 166000 died directly from drug use disorders in 2017, the United States had a higher amount of deceased, 18.75 in 100000 individuals.[4] Although, the numbers of indirect deaths are higher.

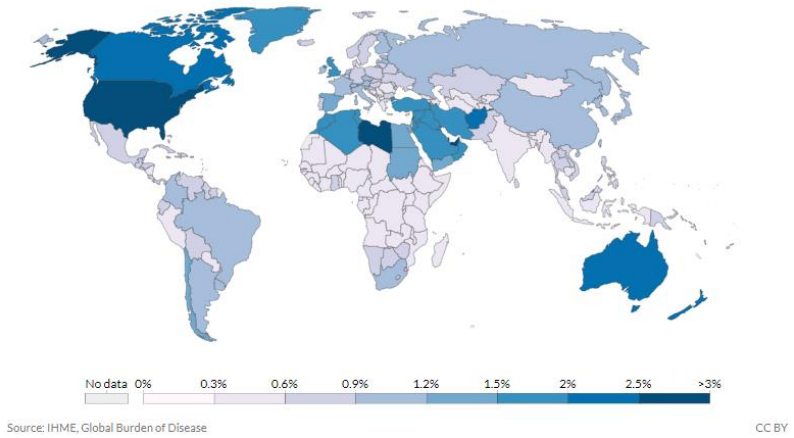


Figure 1.1: Worldwide prevalence of drug use disorders in 2017.[5]

The prevalence of drug use disorders can depend on multiple factors, such as age, gender, mental health, along with others. Focalising on the individual's age, studies show that people in their twenties have a higher drug use prevalence, and this can be linked to behavioural and social factors. At this age, young generations start to explore and self-identify, the social pressure raises, a lot of questions appear along with all this, overall it contributes to the potential development of drug use. As the chart (**Figure 1.2**) shows, 20-24 years old have a higher number of drug use disorder in central Europe. This tends to fade as individuals age and new priorities in life, such as marriage and parenthood, gain ground.

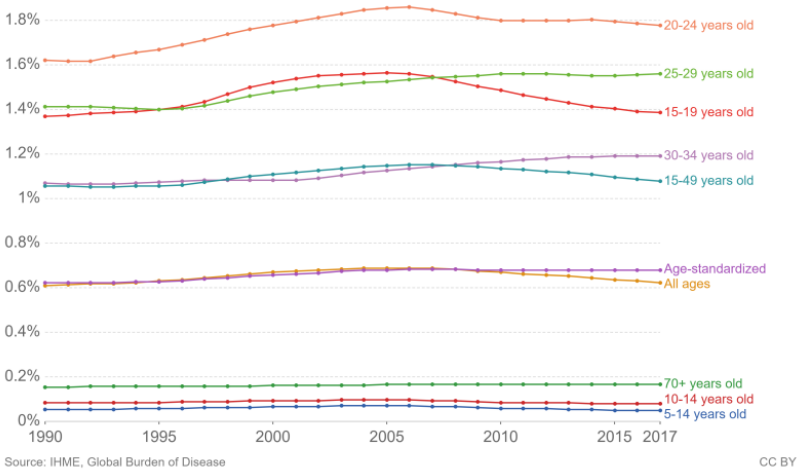


Figure 1.2: Prevalence of drug use disorders by age in Central Europe, from 1990 to 2017.[4]

Mental health and substance use disorders can be closely related. A group of scientists followed, during 10 years, 5000 individuals with and without mental health disorder (and without a drug use disorder) to detect if any nicotine, alcohol or illicit drug dependence was developed. The results in the chart (**Figure 1.3**) show the increased risk of developing an illicit drug use disorder in individuals with a mental health disorder, compared with those without. Focusing on ADHD, a value of 5.2 indicates that an individual with this kind of disorder would be 5 times more likely to develop a drug use disorder.[4] Overall, individuals with mental health disorders have an increased risk of developing a drug use disorder.

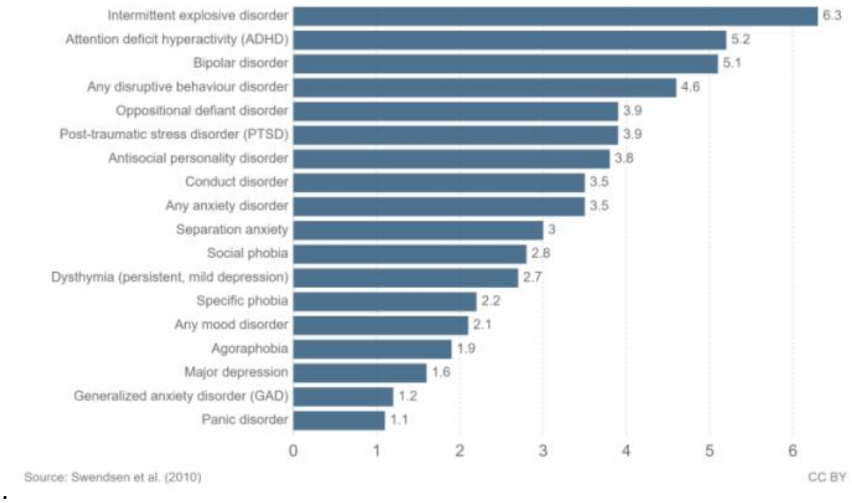


Figure 1.3: Mental health as a risk factor for illicit drug dependency or abuse.[4]

1.2 Novel psychoactive substances

New psychoactive substances (NPS) are defined as “substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat”.[6] The term "new" does not refer to newly synthesised drugs, only to new NPS that became available recently in the drug market. Since 2009, NPS has captured the attention of the international community and changed the landscape of the global synthetic drug market. The unprecedented proliferation of these substances had implications in the monetarization, understanding and control of these drugs, along with their precursor chemicals. A change in a precursor chemical leads to new accountable substances with different chemistries and new synthetic routes, manly an adaptation of the ones known (**Figure 1.4**).[6,7]

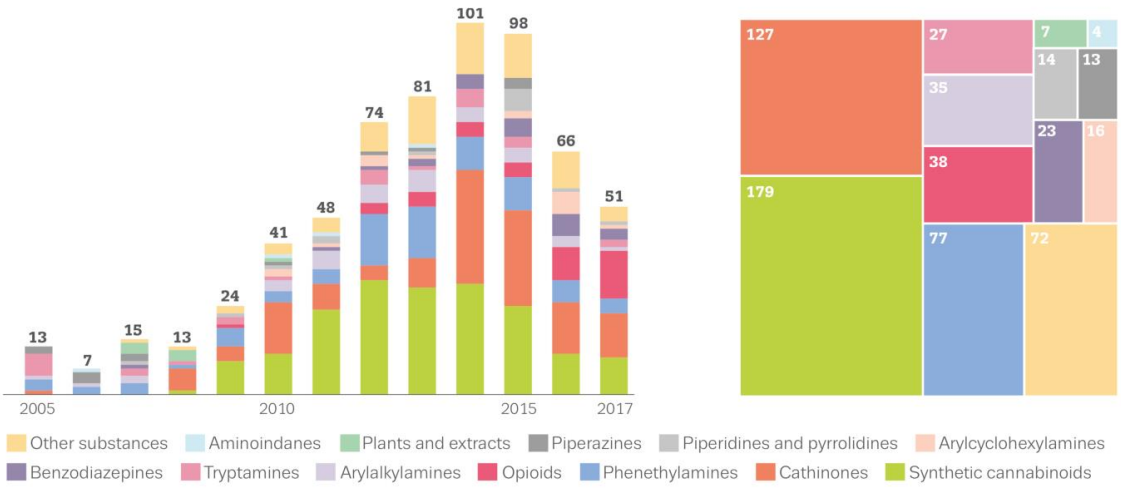


Figure 1.4: New psychoactive substances notified to the EU Early Warning System between 2005-2017: number per year (left) and total number per category (right).[8]

Originally, NPS were divided into the same six classes of psychoactive effects known from traditional drugs under international control. In 2012, the increment of NPS that did not fit within previous methods demanded a new drug wheel design (**Annexe 1**). UK DrugWatch designed a wheel

that combined the drug effects, two different types of drug control and legal examples of each category. The category "Other", that included drugs such as 5-APB and Methoxamine (MXE), was divided into three new categories: cannabinoids, empathogens and dissociatives.[9]

The majority of recreational drugs are synthesised in East Europe or Asian countries and are advertised online as “baths salts” and “plant food”, for example. These compounds are not labelled for human consumption so that under the Medicines Act 1968, their manufacturers were not legally required to list the ingredients. The simplified access to this NPS via the Internet has become an alarming problem in our society, as a result of the difficulty in controlling the drug scene. Additionally, the toxicological and pharmacological effects and the health risk associated with these drugs are still unknown, whereas the “traditional” illegal drugs have been monitored for years. Individuals reports and internet forums are the main sources of information about these NPS. Furthermore, there are many reasons for the increment of NPS consumption, such as price, strength, availability and better quality, including quicker onset and duration of action and improved side-effect profile. [10,11]All these elements together contributed for their use as “drug clubs”.[12]

1.3 Phenylethylamines

Phenylethylamine, also known as PEA, is the simplest organic compound of the phenethylamines class and a wide range of species produces it, like algae, fungi, bacteria and a variety of different plants. This small molecule is chemically related to amphetamines, being the only difference between both the lacks of a methyl group. Minor alterations of this apparently simple structure lead to the appearance of a vast number of NPS with a variety of psychoactive and stimulant effects. The class of phenethylamines include ring-substituted substances such as benzofurans (e.g. 6- APB), benzodifurans (e.g. Bromodragon FLY) and MDMA derivatives (**Figure 1.5**). Both phenethylamines and amphetamines are members of the trace amines, amines that are chemically and functionally related to biogenic amines and neurotransmitters.[13]

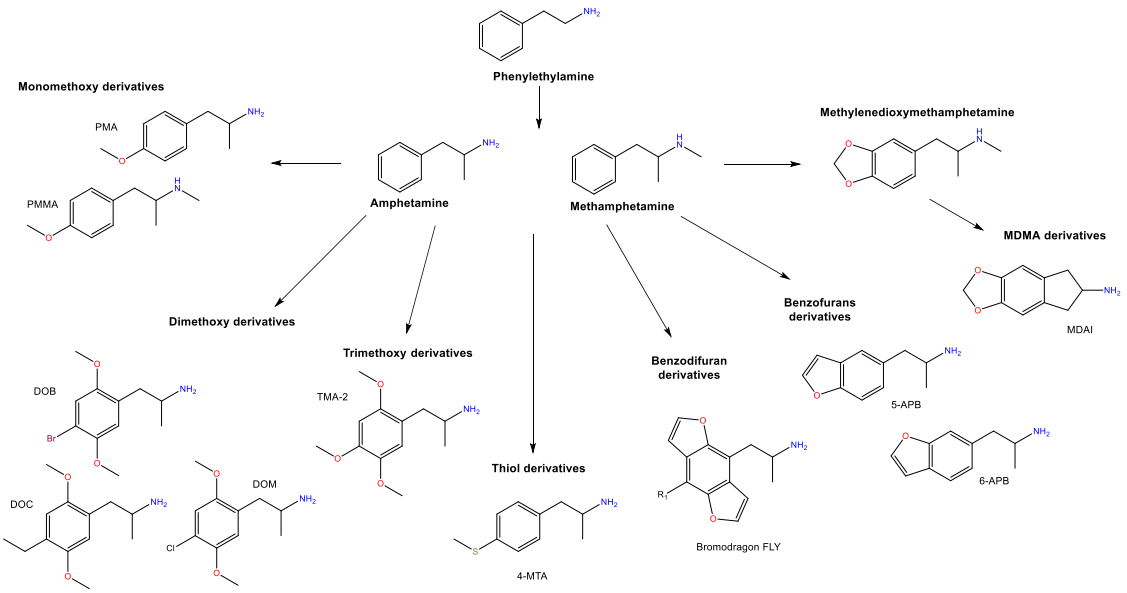


Figure 1.5: Connection between phenethylamines and their analogues. Adapted from [14]

PEA as referred previously is an organic compound synthesised naturally by phenylalanine decarboxylation. This compound can also be synthesised in a laboratory by reducing nitrostyrene with lithium aluminium hydride (**Figure 1.6**) or by catalytic hydrogenation under palladium.[13]

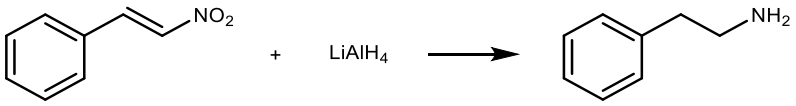


Figure 1.6: PEA chemical synthesis.

Phenethylamines display a pharmacological profile that is not yet well understood due to the interaction between the molecule and TAAR1. The hypothesis formed affirms that when PEA binds to TAAR1 an alteration on the monoamine transporter functions leads to the inhibition of the reuptake of dopamine, serotonin, and norepinephrine, and an increase of concentration of these neurotransmitters at the synapses. The increase of the synaptic concentrations of dopamine occurs by the blocking of the dopamine transporter directly. The mechanism of methylphenidate is shown in the figure as an example of a class of drugs that can perform this blockage (**Figure 1.7**).[13]

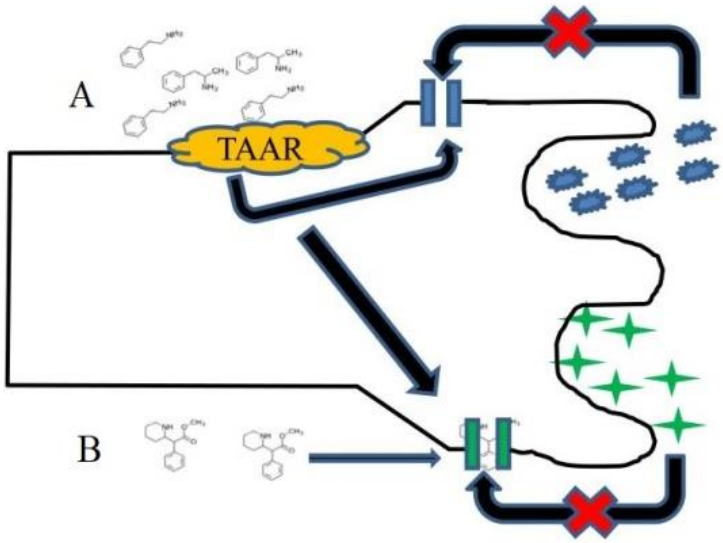
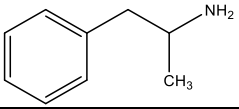
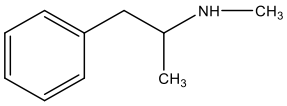
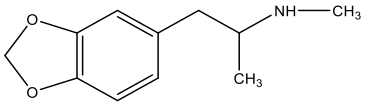


Figure 1.7: Mechanisms of action of PEA and amphetamine (A) and Methylphenidate (B).[13]

1.4 Amphetamines

Amphetamine is a small molecule member of amphetamines class with a low molecular weight. Minor alterations of its structure lead to the appearance of analogues, such as methamphetamine and MDMA, being methamphetamine the most simple and powerful analogue of them all. The chemical properties of amphetamines and their isomers are summarized in **Table 1.1**. All these compounds are members of the trace amine, as so, this molecule and its analogue, methamphetamine, show structural similarities with dopamine and noradrenaline, responsible for the sympathomimetic effects of these psychostimulants. Equally, MDMA (also known as “ecstasy”) displays closer structural similarities with serotonin, justifying the peculiar pharmacological profile of this drug.[15]

Table 1.1: Characterization of amphetamine and their analogues, methamphetamine and MDMA. [16–18]

	Amphetamine	Methamphetamine	MDMA
Structural formula			
IUPAC name	1-phenylpropan-2-amine	(2*S*)-*N*-methyl-1-phenylpropan-2-amine	1-(1,3-benzodioxol-5-yl)-*N*-methylpropan-2-amine
Molecular formula	$C_9H_{13}N$	$C_{10}H_{15}N$	$C_{11}H_{15}NO_2$
Molecular weight	135.21 g/mol	149.23 g/mol	193.24 g/mol

Amphetamine was firstly synthesised in the university of Berlin in 1887 by Lazar Edeleanu, however, its stimulant properties were not discovered until the 1930s, when it started to be used to treat nasal congestions. Nowadays, this drug is used in medical practice to treat ADHD and narcolepsy, and in some situations, used to treat depression. MDMA appeared years later in 1912 and was synthesised and patented by the pharmaceutical company Merck under the name "methylsafrylamin", to be used as a precursor for therapeutically active compounds. It started being used in 1976 in psychiatric treatment and, in the early 1980s, MDMA was classified as a schedule one drug use due to its high abuse potential, lack of safety and, possible, being neurotoxic. Afterwards, MDMA and methamphetamine became popular on the streets as recreational drugs, essentially as "club drugs". As every drug it can be consumed in different ways, for methamphetamine, it can be intranasal or orally used and for MDMA is almost exclusively orally used in form of coloured tablets. For MDMA, the typical pattern use varies from 1 to 2 tablets in a single-use being equivalent to 75-125 mg for dose. The data regarding the onset and duration of the effects of these drugs can be found in online forums and this information was compiled in **Table 1.2**.

Table 1.2: Commonly ingested doses and average trip time for amphetamines. [3,19–21]

		Methamphetamine	MDMA
Route		Oral	Oral
Dose (mg) necessary to produce effect	Threshold	5	30
	Light	5-15	40-75
	Common	10-30	75-125
	Strong	20-60	150-200
	Heavy	40-150	>200
Onset (min)		20-70	20-70
Duration (h)		3-5	3-5
Alter Effects (h)		Up to 24	Up to 24
Biological half-life (h)		4-5	6-9
Lethal dose in human (mg)		140-1650	150-250

Amphetamines are rapidly absorbed after oral ingestion and concentrate in the liver, kidney, lungs, cerebrospinal fluid, and brain, due to their high lipid solubility, capability on crossing the blood-brain barrier and high volume of distribution The major metabolic pathway for amphetamine and methamphetamine is the aromatic hydroxylation to produce the p-hydroxy derivatives. Also, demethylation can occur in the N-methyl derivatives with the metabolization of methamphetamine to amphetamine. This compound can also be deaminated to phenylacetone by CYP2C and,

subsequently, is oxidized to benzoic acid and excreted as glucuronide or glycine conjugate. The analogue of amphetamine, MDMA, is also a substrate for CYP2D6 and the major metabolic pathway is characterized by N-demethylation, O-demethylation and deamination. MDMA is converted, mainly by CYP2D6, to the catechol, 3,4-dihydroxymethamphetamine and the N-demethylated psychoactive product, 3,4-methylenedioxyamphetamine, also known as MDA. The metabolic pathways of these drugs are presented in the following figure (**Figure 1.8**).[22]

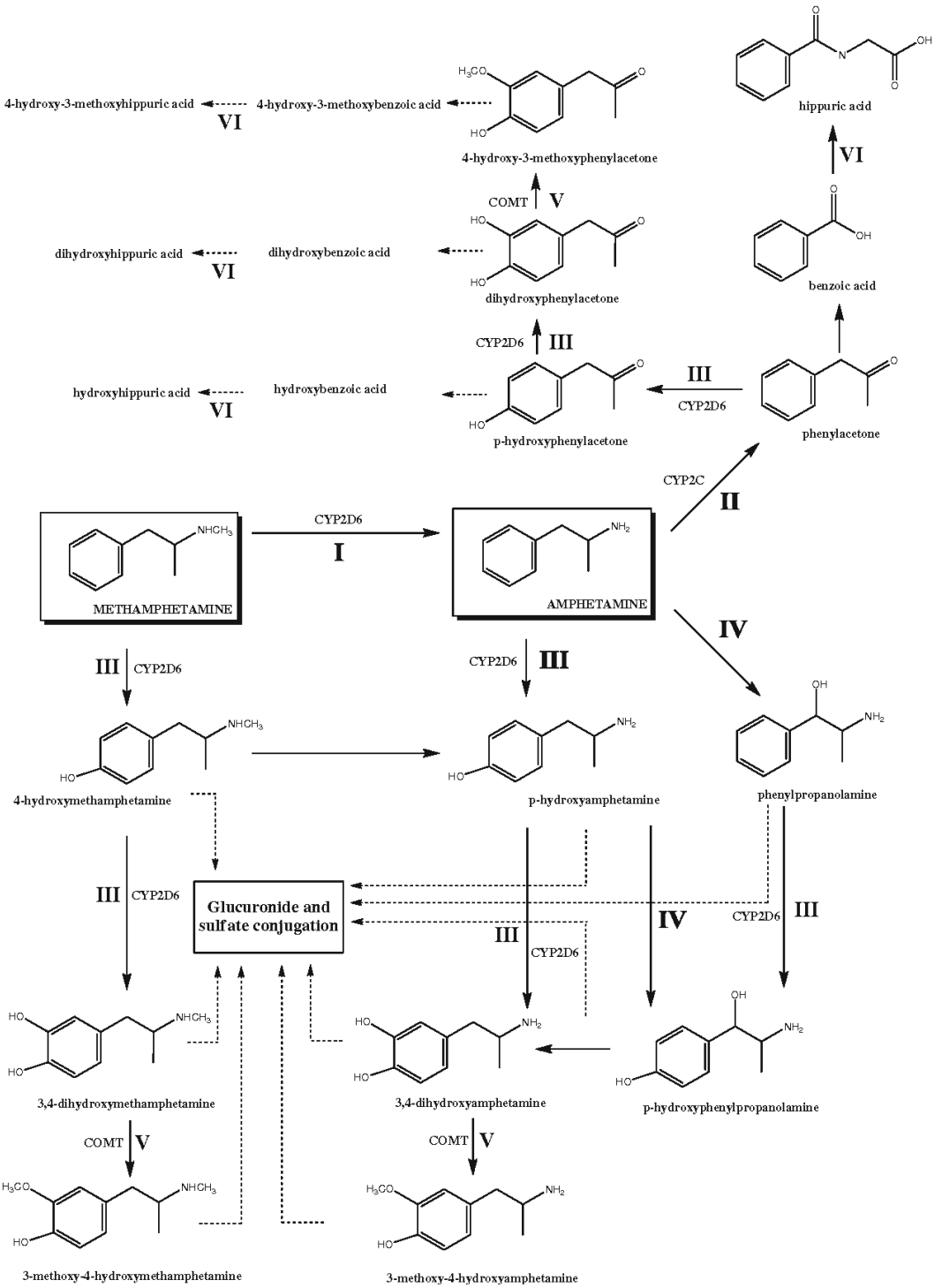


Figure 1.8: Metabolic pathway of amphetamine and methamphetamine.[22]

Amphetamines derivatives display a pharmacological profile which closely resembles the parent compound (e.g., methamphetamine), while others have atypical pharmacological properties (e.g. MDMA). Nevertheless, amphetamines as a class of drug are characterized by a common mechanism of action, the possession of addictive properties, and the ability to elicit toxicity at both the central and peripheral level. The effects detected in the users can be divided into categories: single-dose and long-term consumption. Euphoria, endurance and sense of energy, improved task performance and greater sociability are the main single-dose effects of amphetamines. The long-term consumption has neurotoxic effects, such as psychotic symptoms and serotonin syndrome, and toxic effects in the liver and the cardiovascular system. Many of the biological effects caused by amphetamines overlap the endogenous amines previously presented. Amphetamines can directly penetrate the presynaptic terminals or be taken up by the membrane transporters for dopamine (DAT) or norepinephrine (NET). Once inside the presynaptic terminals, vesicular transporter (VT) pumped amphetamine to the inside of the intracellular vesicles. Afterwards, amphetamine relocates monoamines from vesicles, causing a dramatic increase in their cytoplasmic concentrations and promoting a massive outflow of monoamines through the membrane transporters. The monoamine extracellular concentrations increase leading to their bind to postsynaptic receptors, hence producing the pharmacological effects of these molecules. (**Figure 1.9**). Despite this MDMA has significantly less CNS stimulant properties, has a high affinity for 5-HT_2 receptors and may cause acute depletion of presynaptic 5-HT, depression of 5-HT synthesis, and retrograde destruction of 5-HT neurons. This molecule can easily diffuse across the cell membranes and accumulated inside serotonergic neurons through the serotonin transporter (SERT), which also increases dopamine and norepinephrine concentrations. The stimulation of 5-HT_{2A}-receptors is related with the hallucinogenic properties of MDMA. The increase in synaptic dopamine in the brain leads to an increase in its oxidative metabolism, which generates free radicals that may induce cytotoxicity. This neurotransmitter is responsible for locomotor stimulation, psychosis, and perception disturbances, whereas changes in norepinephrine concentration can be associated with alerting, anorectic, locomotor, and sympathomimetic effects and 5-HT is responsible for delusions and psychosis. The toxicity of methamphetamine often leads to renal and liver failure, hyperthermia, cardiac arrhythmias, heart attack, cerebrovascular haemorrhages, stroke, seizures, and death. [3,15,23]

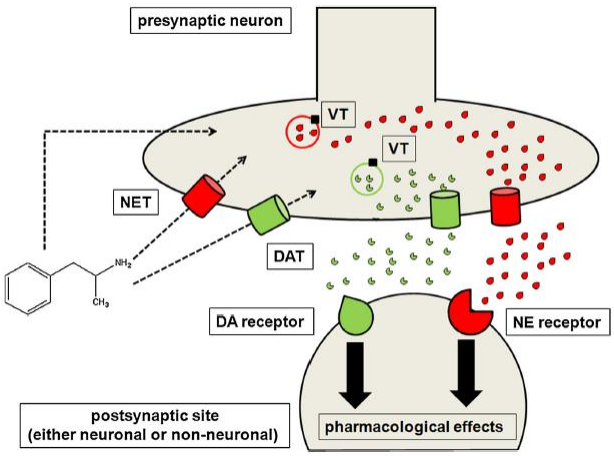


Figure 1.9: Mechanisms of action of amphetamines.[15]

1.5 Benzofurans

Benzofurans, also known as "Benzo Fury´s", are part of a growing group of designer drugs (NPS) that appeared on the drug scene in 2010-11. Due to their structural and functional similarity with amphetamines and phenylethylamines, these drugs are classified as synthetic phenethylamines. These compounds combine a benzene ring and, one or more, attached heterocyclic furan rings. They can be seen as if in the MDA, the 3,4-methylenedioxyphenyl ring was replaced by a benzofuran ring. 5-(2-aminopropyl)benzofuran (5-APB) and 6-(2-aminopropyl)benzofuran (6-APB) were the first isomers to be identified in the drug market, although 6-APB is the main compound marked as "Benzo Fury". While 5-APB (**1**) and 6-APB (**2**) are the subjects of most published investigations other benzofuran analogues have also been marketed as “legal highs”, such as 5-APDB (**3**) and 6-APDB (**4**). The structural differences between these derivatives can be seen in the following figure (**Figure 1.10**).

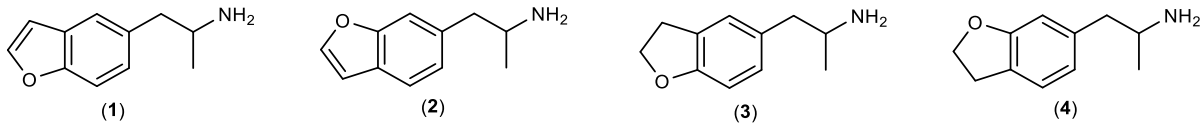
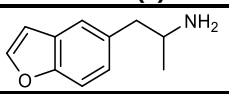
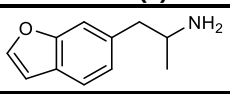
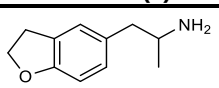
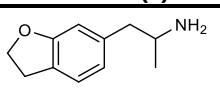


Figure 1.10: Benzofurans and benzodifurans derivatives: 5- APB (**1**), 6- APB (**2**), 5-APDB (**3**) and 6-APDB (**4**).

The chemical properties of these benzofurans and benzodifurans analogues were summarized in Table **1.3**.

Table 1.3: Characterization of benzofurans isomers, 5-APB (**1**) and 6-APB (**2**), and benzodifurans isomers, 5-APBD (**3**) and 6-APDB (**4**).[24–27]

	5-APB (1)	6-APB (2)	5-APDB (3)	6-APDB (4)
Structural formula				
IUPAC name	1-(1-benzofuran-5-yl)propan-2-amine	1-(1-benzofuran-6-yl)propan-2-amine	1-(2,3-dihydro-1-benzofuran-5-yl)propan-2-amine	1-(2,3-dihydro-1-benzofuran-6-yl)propan-2-amine
Molecular formula	$C_{11}H_{13}NO$		$C_{11}H_{15}NO$	
Molecular weight	175.23 g/mol		177.24 g/mol	

In 1993, a group of investigators at Purdue University who examined the role of the MDA dioxide ring structure in interactions with serotonergic and catecholamine uptake carriers came across with benzofurans analogues, 5-APDB and 6-APDB. For a long time, these benzofurans were mistaken with their analogues, 5-APB and 6-APB. Before used as a drug of abuse these benzofurans were firstly mentioned in 2006 in a patent for the synthesis of serotonergic aminoalkylbenzofuran of a US pharmaceutical company.[21,28] In 2012, a synthesis for these compounds based on this patent was published, the exact experimental details were not provided due to the journal policy. In **Figure 1.11**, the synthesis of 6-APB (**2**) and 4-APB (**5**) is presented. The first step of this synthesis was to reflux bromophenol with bromoacetaldehyde and NaH to obtain the diethyl acetyl, which was heated with polyphosphoric acid to cyclize to the furan ring (step 1). The mixture of bromobenzofurans obtained was separated via silica gel column chromatography, catalytically converted to their respective 2-propanone (step 2) and then reductively aminated (step 3) to 6-APB (**2**) and 4-APB (**5**). For 5-APB, in

Figure 1.12, the benzofuran carbaldehydes were converted to their respective benzonitrostyrenes (step 1) and followed by LAH reduction (step 2) to the amines 5-APB (**1**) and 7-APB (**6**). All the synthesised benzofurans were converted to their HCl salts.[29,30]

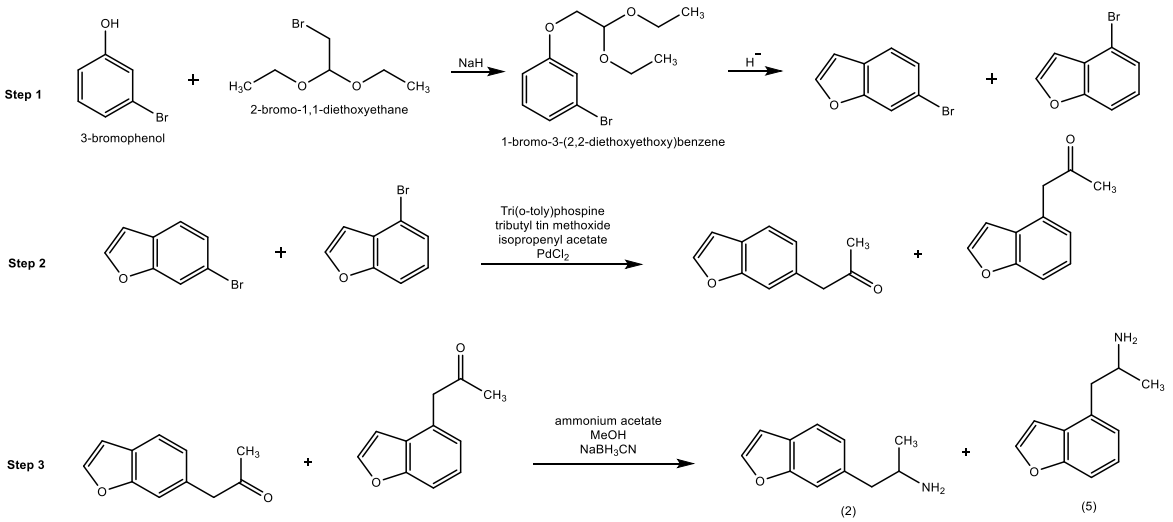


Figure 1.11: Synthetic scheme for 4-(2-aminopropyl)benzofuran (**5**) and 6-(2-aminopropyl)benzofuran (**2**).[29]

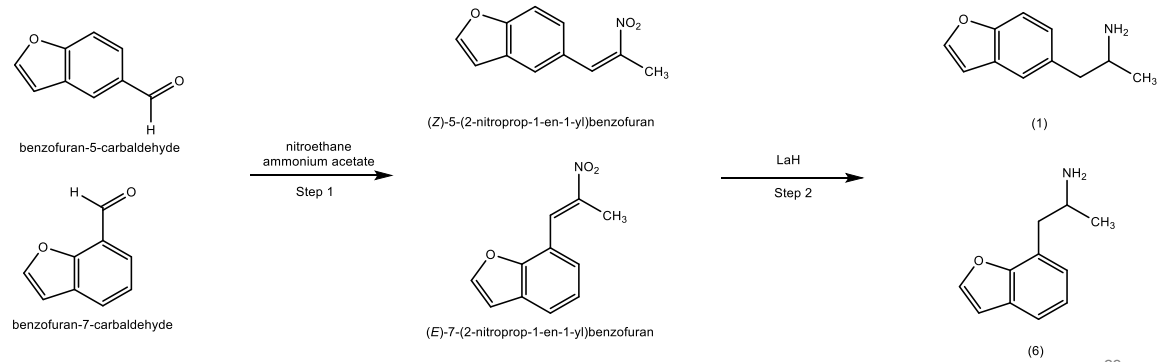


Figure 1.12: Synthetic scheme for 5-(2-aminopropyl)benzofuran (**1**) and 7-(2-aminopropyl)benzofuran (**6**).[29]

These drugs started being abuse due to their ability to induce entactogenic and stimulant effects, similar to MDMA but with higher potency. Reportedly effects of these benzofurans can be easily found at online forums, users mention an increase in music appreciation and empathy, mood-lifting effects and psychedelic properties. 5-APB and 6-APB are mainly taken orally and there is variability in dose-response, doses lower than 75 mg is thought to have less effect. The data regarding the onset and duration of these effects can also be found in online forums and this information was compiled in **Table 1.4** to better understand the reasons behind the drug abuse. Comparing with MDMA and methamphetamine (**Table 1.2**), benzofurans have a higher onset and duration of effects using lower doses.

Table 1.4: Commonly ingested doses and average trip time for 5-APB and 6-APB. [21,31]

		5-APB	6-APB	5-APDB	6-APDB
Route		Oral	Oral	Oral	Oral
Dose (mg) necessary to produce effect	Threshold	15-25	20-40	Not available	20-30
	Light	40-80	40-60	5-75	30-70
	Common	80-100	60-80	75-125	70-100
	Strong	80-100	80-100	Not available	100-130
	Heavy	>100	>100	125 - >200mg	>130
Onset (min)		30-60	20-60	20-60	30-60
Duration (h)		6-9	3-8	4-8	6-8
Alter Effects (h)		6-48	6-40	-	-
Biological half-life (h)		Not available			

As stated before, 5-APB and 6-APB are new NPS, as so not very pharmacodynamics and toxicokinetics information is available. These drugs have a similar effect to MDMA, inhibiting monoamine transporters, inducing transporter-mediated monoamine and interacting with the trace amine-associated receptor-1 (TAAR-1). NET and SERT are inhibited at submicromolar concentrations and DAT have a weaker inhibition comparing with the previous ones. 5-APB has a higher potential of inhibition of SERT that benzofurans and amphetamines, this can be explained by the para-position of the oxygen increasing the absolute and relative potency. The affinity of these molecules to the TAAR-1 is higher than MDMA affinity and is thought to be responsible for the locomotion and the neurochemical stimulant effects of these drugs. In comparison with MDMA and methamphetamines, benzofurans have a higher power of stimulation in adrenoreceptors and 5-HT receptors. The affinity to the adrenergic receptors is quite different for these two molecules, 6-APB is the only one to show submicromolar affinity to α-2 receptor. This higher affinity causes an intense release of norepinephrine that can explain the sympathomimetic effects of these drugs. Lastly, benzofurans show submicromolar affinity and act as low-potency partial agonists at the serotoninergic receptors. The interaction of 5-APB with the 5-HT_{2A} receptors induces vasoconstriction and is associated with its hallucinogenic properties. The interaction with 5-HT_{2B} receptors can induce cardiotoxicity by promoting the proliferation of cardiac cells of the interstitial valve, that subsequently causes heart valve fibrosis.[32,33]

1.6 Metabolites of 5-APB and 6-APB

The metabolism of benzofurans is far from being fully elucidated, only a few analytical data is available for the two benzofurans analogues. In 2014-2015, two studies developed in German identified 5-APB (**1**), 5-MAPB (**5**), 6-APB (**3**) and 6-MAPB (**6**) metabolites in urine and plasma using GC-MS and LC-(HR)-MS^n. In these studies, the phase I metabolites were separated and identified after acetylation by GC-MS and/or LC-HR-MS^n and the phase II metabolites by LC-HR-MS^n.[34,35]

The hydroxylation of 5-APB (**1**) is the initial step (**7**), followed by the ring cleavage (**8**) and reduction of the resulting unsaturated aldehyde (**9**). The corresponding aldehyde was either oxidized, resulting in a carboxylic acid (**10**), or reduce to the alcohol (**11**). The obtained metabolites are the ones studied in this thesis, **metabolite 1** and **metabolite 3** (**Figure 1.13**). The evaluation of the initial CYP activity screening for the 5-APB metabolism was not possible due to the very low metabolite formation

rate. For 5-MAPB, the enzymes involved in the N-demethylation were CYP1A2, CYP2B6, CYP2C19, and CYP2D6.

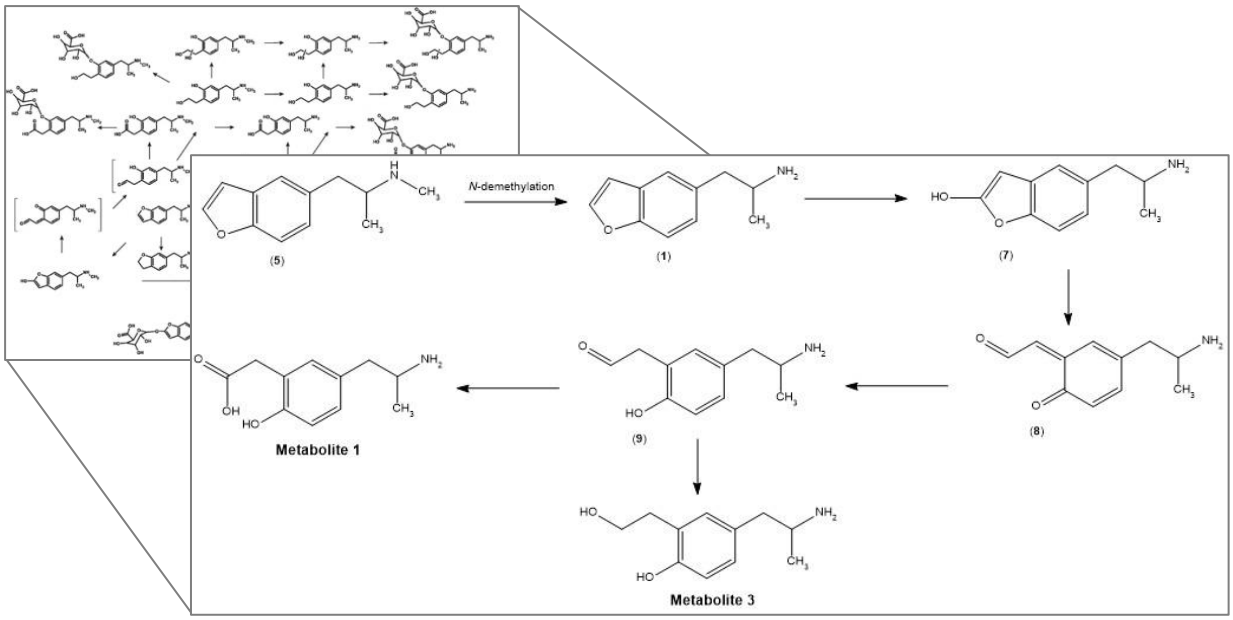


Figure 1.13- Proposed metabolic pathways for 5-APB (**1**) and 5-MAPB (**5**) metabolites in rats. Adapted from [35]

The metabolic pathway of 6-APB is very similar to the one described for the 5-APB. Both started with the hydroxylation of the benzofuran ring and, end up, with the studied metabolite, **metabolite 2** and **metabolite 4** (**Figure 1.14**). The evaluation of the initial CYP activity screening for the 6-APB metabolism was not possible due to the very low metabolite formation rate. For 6-MAPB, the enzymes involved in the N- demethylation were CYP1A2, CYP2D6 and CYP3A4.

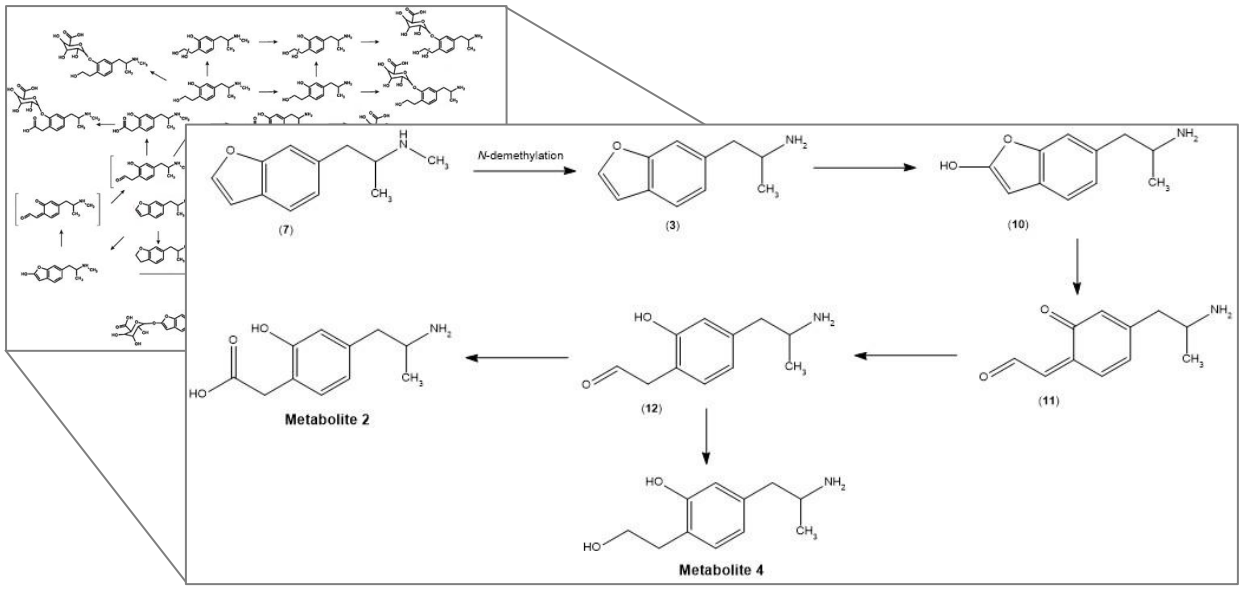


Figure 1.14: Proposed metabolic pathways for 6-APB (**3**) and 6-MAPB (**6**) metabolites in rats. Adapted from [34**]**

2 Results and discussion analysis

This thesis aimed to synthesise 5-APB and 6-APB oxidized metabolites (**1-4**) for toxicological studies using as starting material the salicylic acid derivatives, 2-hydroxyphenyl acetic acid (**5**) and 2-(4-bromo-2-methoxyphenyl) acetic acid (**6**) (**Figure 2.1**). This chapter will be divided into the different synthetic pathway envisaged for each metabolite. Synthesis of metabolite **1** and **3** will be discussed in sections **2.1.1** and **2.1.2**. As for metabolite **4,** its synthesis will be presented and discussed in section **2.2.1**. The synthesis of metabolite 2 was not a target of this work.

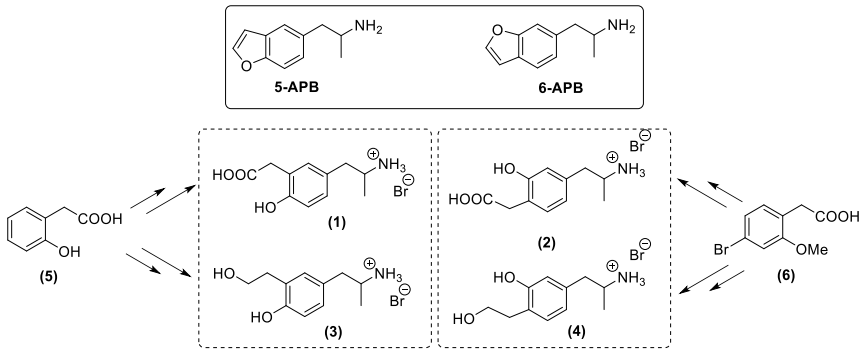


Figure 2.1: Benzo Fury´s drugs, their oxidized metabolite 1-4 and the respective starting materials (5 and 6).

2.1 Synthesise of 5-APB metabolites

2.1.1 Synthesis of 2- (5-(2-aminopropyl)-2-hydroxyphenyl) acetic acid (1)

The reaction scheme for the preparation of metabolite **1**, the 2-(5-(2-aminopropyl)-2-hydroxyphenyl) acetic acid initially proposed is represented in **Figure 2.2** and will be reviewed afterwards. It is proposed a synthetic methodology involving methylation reactions, Rieche formylation, aldol type condensation and reduction. The compound **9** was also used to synthesise metabolite **3** as a result of a reduction reaction using a lithium aluminium hydride that will be described in **section 2.1.2**.

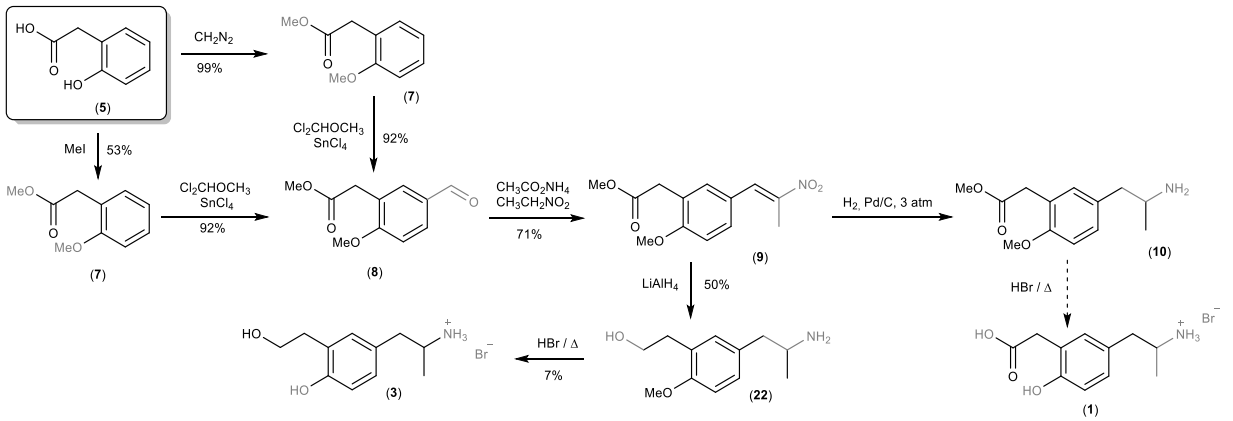


Figure 2.2: Envisaged scheme for the synthesis of 2-(5-(2-Aminopropyl)-2-hydroxyphenyl) acetic acid (**Metabolite 1**) and 4-(2-aminopropyl)-2-(2-hydroxyethyl)phenol (**Metabolite 3**).

To synthesised compound methyl 2-(2-methoxyphenyl) acetate (**7**) was necessary to protect the phenol and the carboxyl groups of 2-hydroxyphenyl acetic acid (**5**). To accomplished these two different procedures were tested, one using diazomethane and the other using iodomethane and potassium carbonate.

Before starting the first procedure, CH_2N_2 was synthesised in the laboratory using Vogel's 1987 procedure[36], described in chapter **3.1**. For the protection reaction, diazomethane reacted with **5** at room temperature in an inert atmosphere. The mechanism of this reaction is presented in **Figure 2.3**. Diazomethane is frequently used to methylate carboxylic acids due to the excellent yields on small scale. However, this compound is a toxic and highly explosive gas with a low boiling point. The reactivity of CH_2N_2 is related to the existence of an N_2 group, a good leaving group, that when it leaves it is substituted by the carboxylate anion on an S_N2 reaction as shown in the mechanism below.[37]

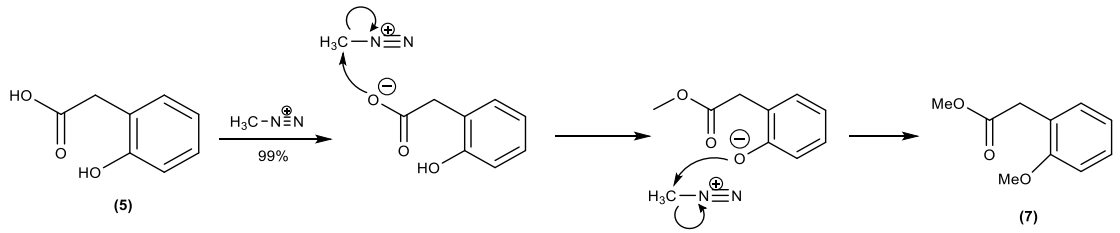
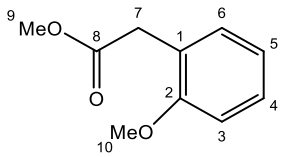


Figure 2.3: Synthesis mechanism of methyl 2-(2-methoxyphenyl) acetate (**7**) using Diazomethane.

Thin Layer Chromatography (TLC) in silica gel plates was used to follow the progress of the reaction. The consumption of starting material was completed in 140h and a new compound was synthesised, which was identified with the spray reagent phosphomolybdic acid. To identify the compound formed as compound **7** some spectral techniques were used. FTIR-ATR allowed us to identify at 2952 cm^{-1} the band corresponding to the C-H bond of sp^3 carbons and at 1734 cm^{-1} the band corresponding to the C=O bond characteristic of an ester group. Comparing the spectra of starting material and the compound synthesised, the hydroxyl group band disappeared as expected and the C=C bands at 1495 cm^{-1} of the aromatic ring are present in both spectra.[38]

Using NMR was possible to identify the compound synthesised. Through ^1H NMR, the aromatic protons were identified as four multiplets, at 7.28 ppm for H4, 6.95 for H5, and 7.20 and 6.91 for H6 and H3 resulting from ortho and meta couplings. The protons for both CH_3 (C9 and C10) appear as a singlet, respectively, at 3.84 and 3.71. Lastly, the signal corresponding to the methylene (7) was identified as a singlet at 3.67.[39] Through ^{13}C NMR, the aromatic carbons were identified between 157.66 and 110.65 (C1 to C6). The carbon of the ester can be found at 172.49 and the carbon chemical shift corresponding to the two methyl groups can be found at 55.62 and 52.06. Lastly, at 35.90 was possible to identify the carbon signal corresponding to the methylene group C7.[40] For the carbon characterization, there was used a chemical shift table[41,42] and the "Predict ^{13}C NMR shifts" tool provided by **ChemDraw®**. In **Table 2.1** is presented a resume of the spectroscopic data. This reaction presents a yield of 99% and considering the purity of the compound it can be classified as a very efficient reaction.

Table 2.1: Chemical characterization of methyl 2-(2-methoxyphenyl) acetate (**7**).



^{1}H-RMN (400 MHz, $CDCl_3$): δ 7.28 (m, 1H, H4-Ar), 7.20 (m, 1H, H6-Ar), 6.96 (m, 1H, H5-Ar), 6.61 (m, 1H, H3-Ar), 3.84 (s, 3H, CH_3-10), 3.71 (s, 3H, CH_3-9), 3.67 (s, 2H, CH_2-7)

^{13}C-RMN (400 MHz, $CDCl_3$): 172.49 (C8), 157.66 (C2), 131.01 (C6), 128.72 (C4), 123.15 (C5), 120.66 (C1), 110.65 (C3), 55.62 (C10), 52.06 (C9), 35.90 (C7)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2952 (C-H sp^3), 1734 (O-C=O), 1495 (C=C aromatic)

Alternatively, a procedure using iodomethane and potassium carbonate was used for the methylation reaction. In one case the solvent used was acetone at room temperature and the second one used acetonitrile at refluxed, both in an inert atmosphere.[43,44] The methylation using acetone as a solvent at room temperature did not occur as expected, the room temperature was quite high and lead to the evaporation of the iodomethane to the ballon with argon (to keep an inert atmosphere) since no adapter for refrigeration was used. This reaction follows the same principle as the methylation with diazomethane being Iodomethane an excellent substrate for SN_2 substitution reactions. The base (K_2CO_3) is used to remove the acidic proton to form the carboxylate anion, which serves as the nucleophile in this type of reactions. The mechanism of this reaction is presented in **Figure 2.4**.

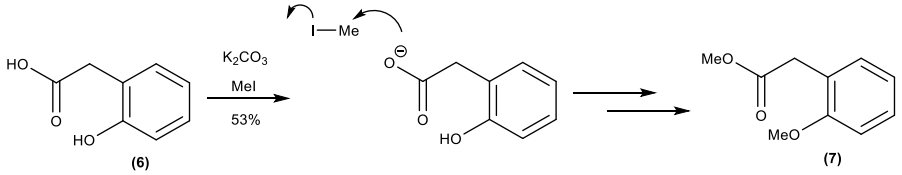


Figure 2.4: Synthesis mechanism of methyl 2-(2-methoxyphenyl) acetate using Iodomethane.

The progress of the reaction was verified by TLC using the spray reagent phosphomolybdic acid. The consumption of starting material was completed overnight but the reaction was not clean and some collateral products were formed, compound **7** was the major one to be formed although in lower yield (53%) than the previous reaction with diazomethane. To sum up, it is possible to conclude that the methylation using diazomethane was better in this type of compound, even when taking into account the reaction time.

To synthesise the methyl 2-(5-formyl-2-methoxyphenyl) acetate (**8**), was necessary to proceed to the formylation of the aromatic group of methyl 2-(2-methoxyphenyl) acetate (**7**). The Rieche formylation with dichloromethyl methyl ether in the presence of a Lewis acid catalyst ($SnCl_4$) was applied to **7** at an inert atmosphere and low temperature, 0º. The mechanism of this reaction is presented in **Figure 2.5**.[45] This formylation is similar to the Gattermann-Koch reaction but it has significant advantages, such as the use of a milder acid and the short reaction time.

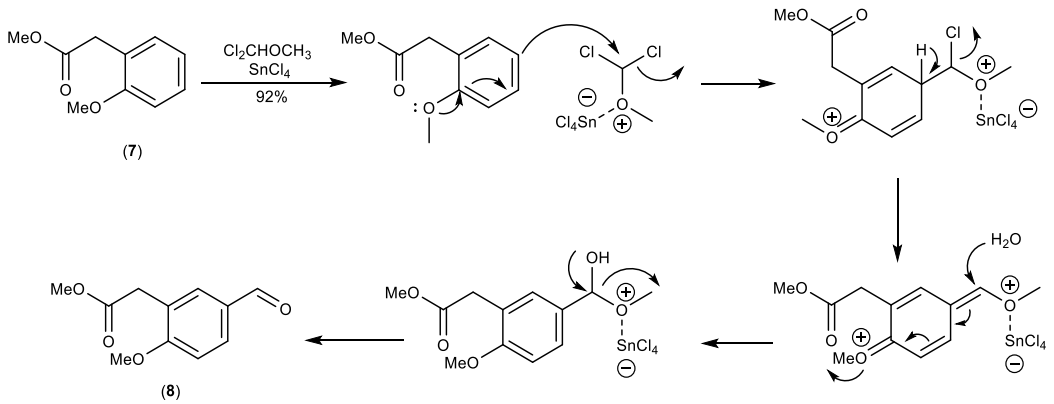
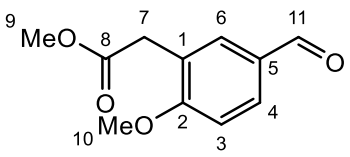


Figure 2.5: Mechanism of the Rieche formylation for the preparation of methyl 2-(5-formyl-2-methoxyphenyl) acetate (**8**).

The progress of the reaction was verified by TLC. The consumption of starting material was completed in 2 hours and a new compound was formed, which was identified with the spray reagent of 2,4-dinitrophenylhydrazine (DNP) frequently used for the identification of ketone and aldehyde groups. The identification of compound **8** was confirmed using spectroscopic methods. FTIR-ATR allowed us to identify the presence of aldehyde by the bands at 2843 and 2739 cm^{-1} for H-CO group and at 1683 cm^{-1} the C=O bond. All other main bands are similar to the bands presented by compound **7**.

The NMR spectra also confirmed the presence of the compound synthesised. Through ^{1}H NMR, the most important signal was identified at 9.88 ppm as a singlet and confirmed the formation of the aldehyde. The disappearance of the triplet corresponding to the protons of C-5 reinforced the hypothesis of a successful synthesis. Through ^{13}C NMR, it was possible to identify the aldehyde carbon at 190.91 ppm and, at 171.66 ppm the ester carbonyl group as described in the literature. In both spectra, the remaining signals stayed similar to the starting material (**7**). The NMR characterization of the aldehyde presented in **Table 2.2** is similar to the one described in the literature.[40] This reaction presents a yield of 92% and it can be classified as a very efficient reaction, short reaction time conjugated with a pure compound.

Table 2.2: Chemical characterization of methyl 2-(5-formyl-2-methoxyphenyl) acetate (**8**).



^{1}H NMR (400 MHz, $CDCl_3$): δ 9.88 (s, 1H, H11), 7.82 (d, J = 8.4 Hz, 1H, H3-Ar), 7.75 (s, 1H, H6-Ar), 6.99 (d, J = 8.1 Hz, 1H, H4-Ar), 3.94 – 3.88 (s, 3H, CH_3-10), 3.70 (s, 5H, CH_2-7 and CH_3-9)

^{13}C-RMN (400 MHz, $CDCl_3$): 190.91 (C11), 171.66 (C8), 162.88 (C2), 132.39 (C6), 132.02 (C5), 129.80 (C4), 124.16 (C1), 110.55 (C3), 56.07 (C10), 52.20 (C9), 35.71 (s, C7)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2952 (C-H sp^3), 2843 (H-C aldehyde),1735 (O-C=O), 1683 (H-C=O), 1500 (C=C aromatic), 1020 (C-O ether)

For the synthesis of methyl (*E*)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl) phenyl) acetate **(9)** it was necessary to perform an Aldol type reaction to convert the aromatic aldehyde into the α,β-unsaturated nitro group. The Knoevenagel condensation with nitroethane and ammonium acetate was applied to **8** at reflux in an inert atmosphere. The mechanism of this reaction is presented in **Figure 2.6**.

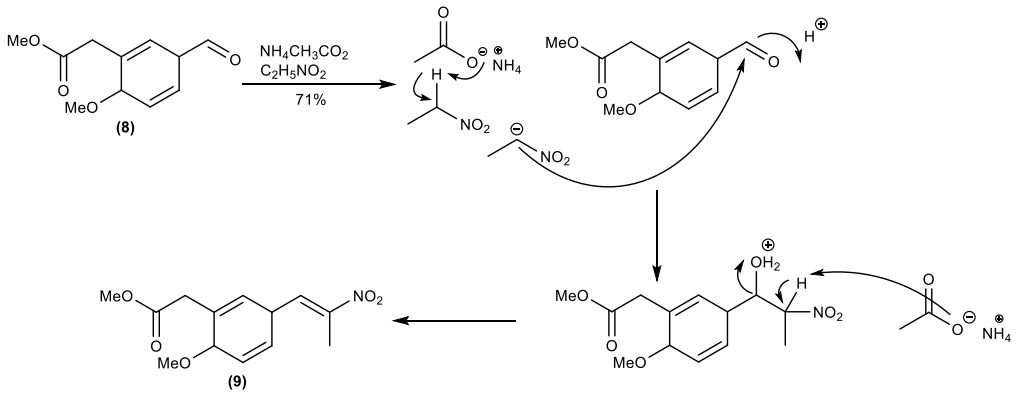
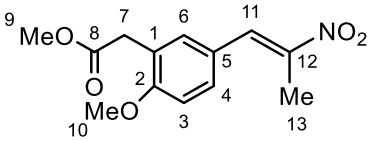


Figure 2.6: Synthesis mechanism of methyl (*E*)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl) phenyl) acetate (**9**).

The progress of the reaction was verified by TLC. The consumption of starting material was completed overnight and a new compound was formed, spray reagent DNP was used to follow the consumption of the starting material. The identification of compound **9** was confirmed using spectroscopic methods. Comparing the FTIR-ATR spectra of starting material and the compound synthesised, the nitro band (N-O) appeared at 1342 cm^{-1} as expected. All other main bands are similar to the bands presented by compound **8**.

The NMR spectra also confirmed the presence of the compound synthesised. Through ^{1}H NMR, the most important signal was identified at low field, at 8.02 ppm as a singlet, the β-H, and confirmed the formation of the α,β-unsaturated nitro group. The appearance of the singlet corresponding to the protons of C13 reinforced the hypothesis of a successful synthesis. Through ^{13}C NMR, it was possible to identify the new methyl carbon (C13) at 14.24 ppm. This reaction presents a yield of 71%. In **Table 2.3** is presented a resume of the spectroscopic data. This compound is not described in the literature therefore, ESI-MS was performed to determine the exact mass and complete the chemical characterization. The mass spectrum is presented in **annexe 2 –Compound 9**.

Table 2.3: Chemical characterization of methyl (*E*)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl) phenyl) acetate (**9**).



^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (s, 1H, H-11), 7.38 (dd, *J* = 8.5, 2.3 Hz, 1H, H4-Ar), 7.30 (d, *J* = 2.3 Hz, 1H, H3-Ar), 6.92 (d, *J* = 8.6 Hz, 1H, H6-Ar), 3.85 (s, 3H, CH_3-10), 3.68 (s, 3H, CH_3-9), 3.63 (s, 2H, CH_2-7), 2.44 (s, 3H, CH_3-13)

^{13}C-RMN (400 MHz, $CDCl_3$): δ 171.82 (C8), 159.13 (C2), 145.98 (C11 e C12), 133.60 (C6), 133.23 (C5), 131.44 (C4), 123.94 (C1) 110.90 (C3), 55.86 (C10), 52.18 (C9), 35.58 (C7), 14.24 (C13).

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2924 (C-H sp^3), 1504 (C=C aromatic), 1342 (N-O)

For the synthesis of 2-(5-(2-aminopropyl)-2-methoxyphenyl) acetate (**10**), it was necessary to reduce the α,β-unsaturated nitro group; for this, catalytic hydrogenation using palladium on activated charcoal (Pd/C) as the catalyst was tested. This catalytic hydrogenation took place on the surface of the palladium catalyst. The reduction of the nitro compound to the amine has different intermediated compounds resulting in the hydrogen consumption and water molecules elimination as presented in the following mechanisms, **Figure 2.7**.

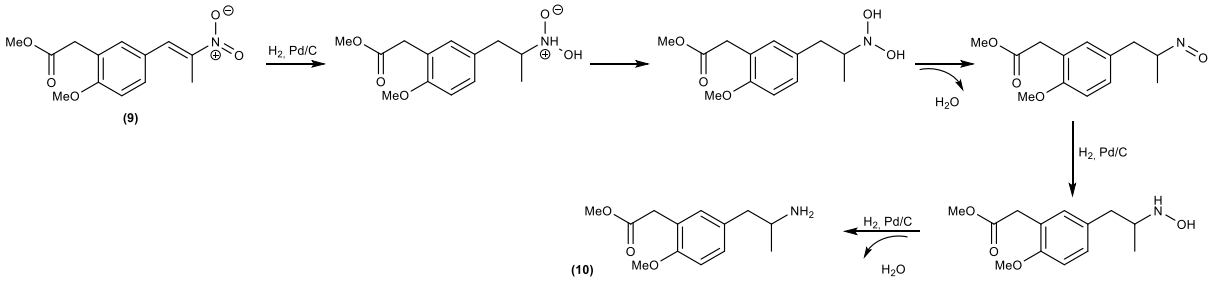


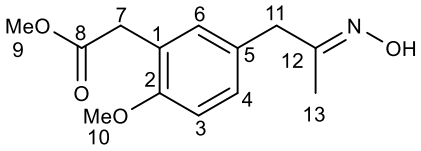
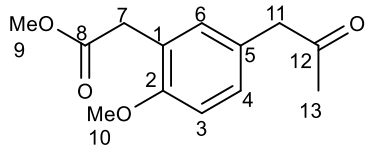
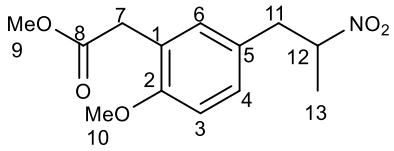
Figure 2.7: Synthesis mechanism of methyl 2- (5- (2-aminopropyl) -2-methoxyphenyl) acetate (**10**).

The procedure used for this reaction was the one described in chapter **3.9**, the compound (**9**) was dissolved in anhydrous ethanol and Pd/C was added. In the first procedure envisaged, the starting material solution was not acidified and the reaction took place for 24h hours at approximately 3 atmospheres. The progress of the reaction was verified by TLC. The consumption of starting material was completed, but two different new compounds were formed. When analysing the NMR spectra, it was not possible to identify the signal corresponding to the methyl group (C13) from the expected compound (**10**) but two different signals were identified in the reactional mixture, one at 1.84 ppm and other at 2.17 ppm. The appearance of these signals leads to suppose that the reaction was not finished and a ketone (**12**) and an oxime (**11**) were obtained. The structural elucidation of these compounds will be presented further on. To circumvent this drawback the reduction was next performed in acidic conditions. Two different conditions were used to acidify the solution, in one procedure was used acetyl chloride and methanol to *in situ* generate HCl and, in the other, TFA.

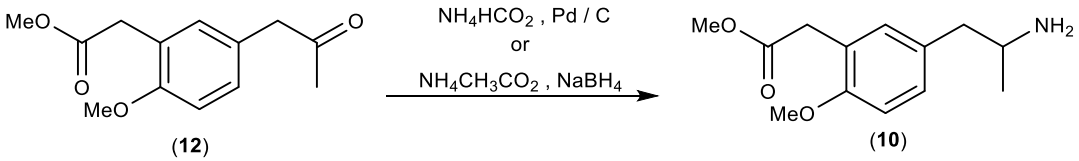
In the second procedure envisaged, to the starting material solution was added acetyl chloride in ethanol to form HCl *in situ*. The reaction took place for 24h hours at 3 atmospheres. The progress of the reaction was verified by TLC. The consumption of starting material was completed even so the expected product was not identified in the NMR of the reactional mixture.

In the third procedure envisaged, the starting material solution in ethanol was acidified with TFA and proceed in the same way as described previously. The progress of the reaction was verified by TLC. The consumption of starting material was completed, but three different new compounds were synthesised. The mixture was purified using a silica flash column eluted with DCM/MeOH 1% and it was possible to attain three pure fractions which were analysed using FTIR-ATR and ^{1}H and ^{13}C-NMR. It was possible to identify all of them, being one the methyl (*E*)-2-(5-(2-(hydroxyimino)propyl)-2-methoxyphenyl)acetate (**11**), the other one, the methyl 2-(2-methoxy-5-(2-oxopropyl)phenyl)acetate (**12**) and, the last one, the methyl 2-(2-methoxy-5-(2-nitropropyl)phenyl)acetate (**13**). The disappearance in both ^{1}H-NMR spectra of the signal of the double bond at 8.01 ppm reinforced the hypothesis of the consumption of the starting material. When analysing each one, it was possible to identify the methylene protons (C11) and the methyl proton (C13) for the oxime (**11**) at 3.61 ppm and 2.14 ppm. The signal at 3.61 ppm integrates for 4H (**Table 2.4**). The compounds **11** and **12** were identified previously in the research group by the work of bachelor degree colleague.[46] The compound **13** was also analysed by ESI-MS to ensure that the compound was the one predicted from the NMR, the mass spectrum is presented in **annexe 2- compound 13**.

Table 2.4: Chemical characterization of methyl (*E*)-2-(5-(2-(hydroxyimino)propyl)-2-methoxyphenyl)acetate (**11**), methyl 2-(2-methoxy-5-(2-oxopropyl)phenyl)acetate (**12**) and methyl 2-(2-methoxy-5-(2-nitropropyl)phenyl)acetate (**13**).

 **(11)**	**^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.08 (d, *J* = 8.4, 1.7 Hz, 1H, H4-Ar), 7.01 (s, 1H, H6-Ar), 6.83 (d, *J* = 8.4 Hz, 1H, H3-Ar), 3.80 (s, 3H, CH_3-10), 3.68 (s, 3H, CH_3-9), 3.61 (d, *J* = 3.0 Hz, 4H, CH_2-7 and CH_2-11), 2.14 (s, 3H, CH_3-13). **^{13}C-RMN (400 MHz, $CDCl_3$):** 170.67 (C8), 162.11 (C11), 156.83 (C2), 133.30 (C5), 131.92 (C6), 129.56 (C1), 121.52 (C4), 110.16 (C3), 55.96 (C9), 50.21 (C10), 35.78 (C7), 14.25 (C13). **IR (*ATR*) $V_{máx}$ (cm^{-1}):** 2953 (C-H sp^3), 1505 (C=C aromatic), 1342 (N-O)
 **(12)**	**^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.10 (d, *J* = 8.3, 4.3 Hz, 1H, H4-Ar), 7.03 (s, 1H, H6-Ar), 6.80 (d, *J* = 8.4, 4.7 Hz, 1H, H3-Ar), 3.79 (s, 3H, CH_3-10), 3.68 (s, 3H, CH_3-9), 3.61 (s, 2H, CH_2-7), 3.43 (s, 2H, CH_2-11), 1.81 (d, *J* = 4.6 Hz, 3H, CH_3-13). **^{13}C-RMN (400 MHz, $CDCl_3$):** δ 206.99 (C12), 172.32 (C8), 156.82 (C2), 132.10 (C5), 129.56 (C6), 126.25 (C4), 123.48 (C1), 110.94 (C3), 55.73 (C10), 52.07 (C9), 50.21 (C11), 35.78 (C7), 29.34 (C13) **IR (*ATR*) $V_{máx}$ (cm^{-1}):** 2953 (C-H sp^3), 1505 (C=C aromatic), 1296 (C-O ether)
 **(13)**	**^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.05 (d, 1H, H4-Ar), 6.98 (s, 1H, H6-Ar), 6.80 (d, *J* = 8.3 Hz, 1H, H3-Ar), 3.80 (d, *J* = 10.5 Hz, 3H, CH_3-10), 3.69 (s, 3H, CH_3-9), 3.59 (d, *J* = 7.7 Hz, 2H, CH_2-7), 2.36 – 2.20 (m, 2H, CH_2-11), 1.28 (s, 3H, CH_3-13). **^{13}C-RMN (400 MHz, $CDCl_3$):** δ 172.13 (C8), 156.86 (C2), 131.55 (C6), 129.05 (C5), 127.34 (C4), 123.39 (C1), 110.76 (C3), 84.62 (C12), 55.58 (C10), 51.97 (C9), 35.57 (C7), 18.76 (C13) **IR (*ATR*) $V_{máx}$ (cm^{-1}):** 2926 (C-H sp^3), 1505 (C=C aromatic), 1339 (N-O)

After the identification of these three compounds, a new procedure was envisaged to transform the methyl 2-(2-methoxy-5-(2-oxopropyl)phenyl)acetate (**12**) into methyl 2-(5-(2-aminopropyl) -2-methoxyphenyl) acetate (**10**). For this, a reductive amination to form the primary amine was envisaged. Two different procedures (**Figure 2.8**) were tested, one, using ammonium formate and palladium on activated charcoal, and the other, using ammonium acetate and sodium borohydride.



(**12**) (**10**)

Figure 2.8: Reductive amination of methyl 2-(2-methoxy-5-(2-oxopropyl)phenyl)acetate.

The procedure using ammonium formate and palladium on activated charcoal did not occur as expected, the starting material was not consumed. So another procedure was envisaged using ammonium acetate and sodium borohydride. The progress of the reaction was verified by TLC and a complexed mixture was obtained. The expected compound was identified using spray reagent Drangendorff, commonly used to identify the formation of the amine group. To purify the reaction mixture a C18 Reversed-Phase Silica Gel Flash Chromatography, using as eluents water and methanol, was performed. The fractions were separated according to their UV analysis and residual masses were obtained. Therefore, the NMR analysis of these fractions was inconclusive.

Overall, this synthesis did not go as envisaged and **Metabolite 1** was not attained, being the main problem, the catalytic hydrogenation reaction. However, it was possible to develop a new synthesis for **Metabolite 3** using as base the first synthetic steps of this pathway.

2.1.2 Synthesis of 4-(2-aminopropyl)-2-(2-hydroxyethyl)phenol (3)

The overall reaction scheme for the preparation of metabolite 4-(2-aminopropyl)-2-(2-hydroxyethyl)phenol **(3)**, is represented in **Figure 2.9** and reports all the attempts developed for attaining this metabolite.

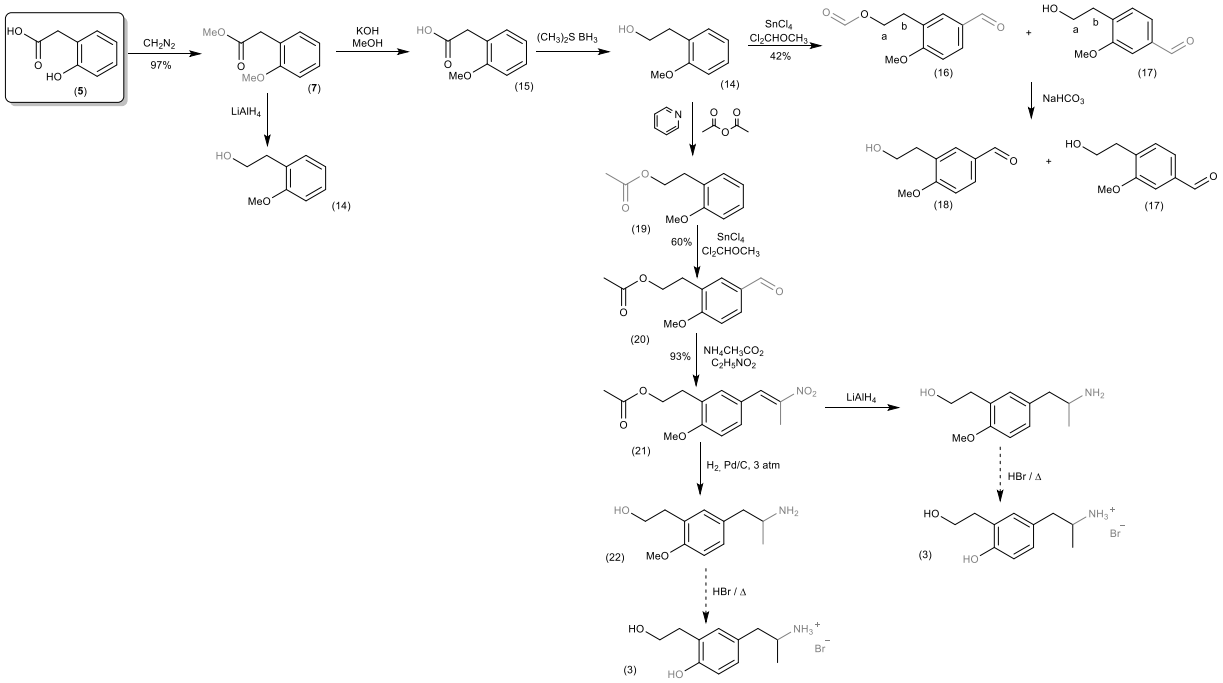


Figure 2.9: Envisaged scheme to the synthesis of 4-(2-aminopropyl)-2-(2-hydroxyethyl)phenol synthesis – **Metabolite 3**.

To attain metabolite **3** it was proposed a methodology involving a reduction of compound **7** (for that two procedure were envisaged) as described in **Figure 2.10** followed by a Rieche formylation.

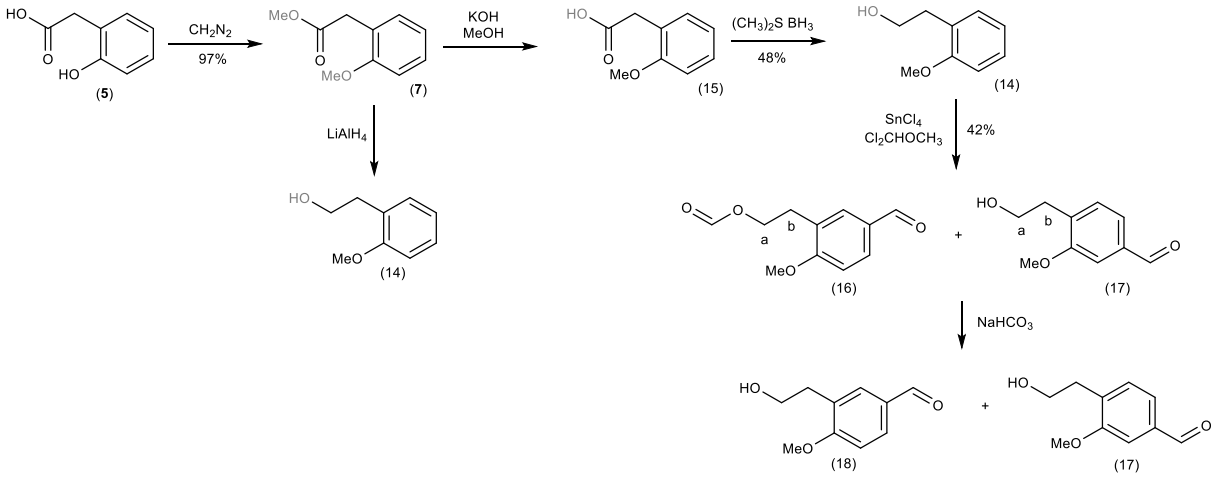


Figure 2.10: First attempt to synthesise 4-(2-aminopropyl)-2-(2-hydroxyethyl)phenol (**Metabolite 3**).

The reduction of the carbonyl group of methyl 2-(2-methoxyphenyl) acetate, compound **7**, using lithium aluminium hydride was aspected to allow the synthesis of **14** following the mechanism presented in **Figure 2.11**. For this, two different solvents were tested, THF and dioxane, and both reactions were put to reflux in an oil bath for 3h at an inert atmosphere. Lithium aluminium hydride is one of the most powerful reducing agents and can be used to reduce the carbonyl group of an ester. The reduction starts with the attack of $LiAlH_4$ to the carbonyl group resulting in a tetrahedral intermediate, this geometry collapses an aldehyde is obtained.[37,47] The compound formed is more reactive than the starting material, so a second reaction takes place and the wanted alcohol **14** is synthesised.

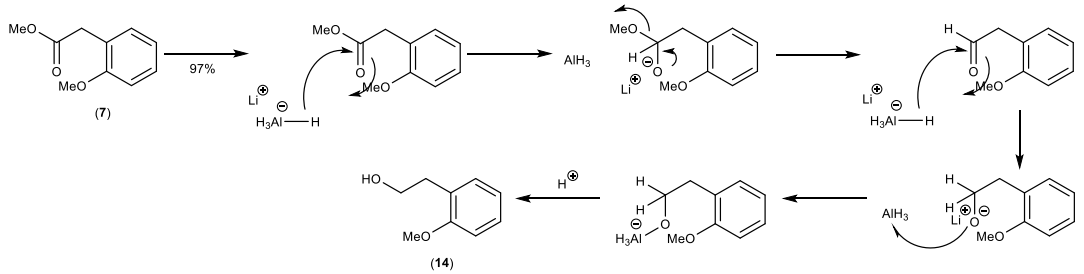


Figure 2.11: Synthesis mechanism of 2-(2-methoxyphenyl) ethan-1-ol (**14**) using lithium aluminium hydride.

TLC allowed us to observe that there wasn't the consumption of the starting material. To confirm that the alcohol was not synthesised a spectral technique was used. FTIR-ATR showed no formation of the alcohol band and, comparing the spectra of starting material and the final product was possible to comprised that the compound is the same. For this result could account the fact that $LiAlH_4$ was not in the best condition.

Next, it was envisaged the hydrolysis of **7** to the corresponding carboxylic acid (**15**) further reduced using borane dimethylsulfide to **14**. One of the best reagents for the reduction of carboxylic acids to alcohols is borane, BH_3. Borane is a gas and it can be "tamed" as a liquid by complexing with different molecules, in this case, a complex with dimethyl sulfide. BH_3 has only three pairs of bonding electrons, meaning that it can be described as sp^2 hybridized with an empty p orbital. The overlap of the sp^2 orbital with the hydrogen 1s orbital results in the B-H bond, leaving the p orbital empty. In reaction with the carboxyl group, the empty p orbital of BH_3 is attacked by the oxygen lone pair of electrons forming the O-B bond. Following this, acylborates are formed and hydrogen gas is released. This process is repeated three times and the triacylborates are reduced until the compound **14** is formed. The mechanism of this reaction follows is presented in **Figure 2.12**.

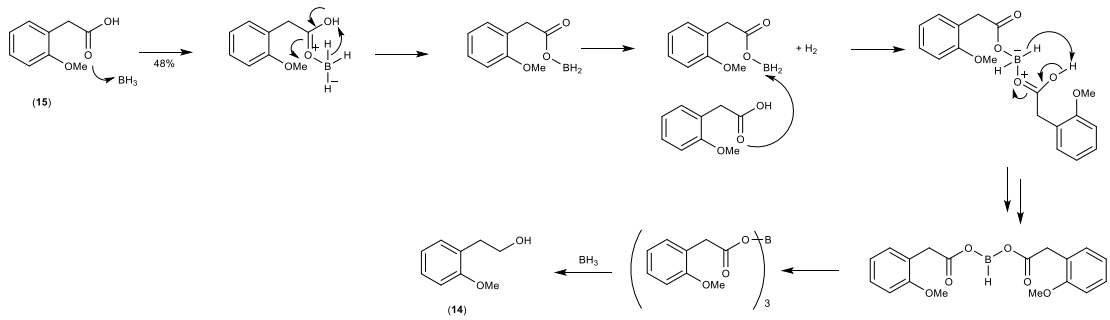


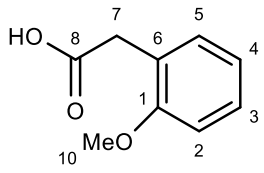
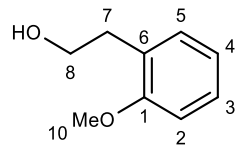
Figure 2.12: Synthesis mechanism of 2-(2-methoxyphenyl) ethan-1-ol (**14**) using borane dimethyl sulfide complex.

Focusing on compound **15**, FTIR-ATR allowed us to identify the hydroxyl group at 3282 cm^{-1} and the carboxylic acid carbonyl band appeared at 1710 cm^{-1}, the remaining bands stayed similar to the bands presented by compound **7**.

. Using NMR was possible to identify the compound synthesised. Through ^{1}H NMR, was possible to confirm the disappearance of the singlet at 3.71 ppm corresponding to the methyl protons of C9, as expected. Through ^{13}C NMR, it was possible to confirm the disappearance of methyl C9 signal at 52.06 ppm, as it was observed in the proton NMR. In both spectra, the remaining signals stayed similar to the starting material (**7**), reinforcing the hypothesis of a successful synthesis. This reaction presents a quantitative yield. Considering the purity of the compound and the short time reaction, it can be classified as a very efficient reaction.

Focusing on compound **14**, FTIR-ATR allowed us to identify the hydroxyl group at 3331 cm^{-1} and, at 2937 cm^{-1}, the band corresponding to the C-H bond of sp^3 carbons. Comparing the spectra of starting material and the compound synthesised, the band at 1710 cm^{-1} corresponding to the acid carboxylic disappeared, the remaining bands stayed similar. Using NMR was possible to identify the compound synthesised. Through ^{1}H NMR, the most important signal was identified at 3.83 ppm as a multiplet which integrated for 5 hydrogens which is due to the hydrogens of C8 and C10, reinforcing the hypothesis of a successful synthesis. The methylene protons (C8) and the methyl protons (C10) appear in the same signal due to the chemical environment. Through ^{13}C NMR, it was possible to identify the methylene carbon (C8) at 63.00 ppm. In both spectra, the remaining signals stayed similar to the starting material (**15**). In table **2.5** is presented a resume of the spectroscopic data. This reaction presents a yield of 48%.

Table 2.5: Chemical characterization of 2-(2-methoxyphenyl) ethan-1-ol (**14**) and 2-(2-methoxyphenyl)acetic acid (**15**).

 **(15)**	**^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.30 (d, *J* = 8.0 Hz, 1H, H5-Ar), 7.21 (d, *J* = 7.6 Hz, 1H, H4-Ar), 6.95 (d, *J* = 7.5 Hz, 1H, H2-Ar), 6.91 (t, 1H, H3-Ar), 3.86 (s, 3H, CH_3-10), 3.69 (s, 2H, CH_2-7). **^{13}C-RMN (400 MHz, $CDCl_3$):** δ 157.47 (C1), 131.17 (C5), 129.08 (C6), 122.46 (C3), 120.92 (C4), 110.74 (C2), 55.68 (C10), 35.88 (C7). **IR (*ATR*) $V_{máx}$ (cm^{-1}):** 3282 (O-H band), 2970 (C-H sp^3), 1710 (O-C=O), 1496 (C=C aromatic), 1248 (C-O alcohols)
 **(14)**	**^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.22 (t, J = 7.9 Hz, 1H, H5-Ar), 7.17 (d, J = 7.4 Hz, 1H, H4-Ar), 6.92 (d, J = 7.4 Hz, 1H, H2-Ar), 6.88 (d, 1H, H3-Ar), 3.83 (t, 5H, CH_2-8 and CH_3-10), 2.91 (t, J = 6.4 Hz, 2H, CH_2-7). **^{13}C-RMN (400 MHz, $CDCl_3$):** 157.76 (C1), 131.04 (C5), 127.97 (C6), 127.16 (C3), 120.81 (C4), 110.57 (C2), 63.00 (C8), 55.45 (C10), 34.22 (C7) **IR (*ATR*) $V_{máx}$ (cm^{-1}):** 3331 (O-H band), 2937 (C-H sp^3), 1494 (C=C aromatic), 1242 (C-O alcohols)

The formylation of the aromatic group of 2-(2-methoxyphenyl) ethan-1-ol (**14**) using dichloromethyl methyl ether and Tin(IV) chloride was performed as reported before in chapter **2.1.1** for compound **8**. TLC was used to follow the progress of the reaction, the starting material was consumed and a new compound was synthesised, being identified with the spray reagent DNP. Through NMR was possible to identify the formation of two products. A purposed mechanism of this reaction is

presented in **Figure 2.13**. The singlet at 8.02 ppm and the two singlets at 9.88 ppm and 9.87 ppm corresponding to the aldehyde groups indicate a mixture of compound **16** and **17**. The triplet at the low field at 4.39 ppm indicates the presence of methylene protons from compound **16** "**a**" due to the deshielding induced by the formate group, these protons can also be found in compound **17** at 3.73 ppm marked as "**c**". Also, the triplets "**b**" and "**d**" appear in different deviations in both compounds, at 3.13 and 3.04 (Table **2.6**). Through FTIR-ATR, was also possible to identify the formate group at 1720 cm^{-1} and the aldehyde band at 1683 cm^{-1} and a tenuous band corresponding to the OH group. To obtain the wanted aldehyde a hydrolysis reaction using sodium hydrogen carbonate was performed. When analysing the hydrolysis NMR, two compounds were identified (**18** and **17**). Through FTIR-ATR, was also possible to identify the band corresponding to the OH group at 3454 cm^{-1} and the aldehyde band at 1683 cm^{-1} (**Table 2-6**).

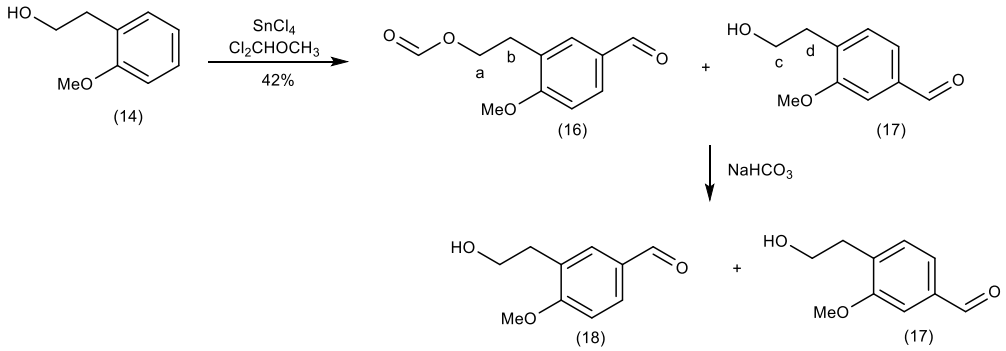
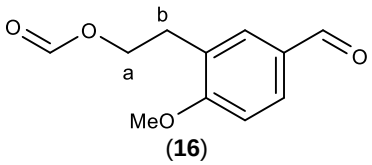
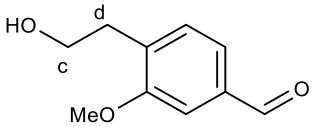
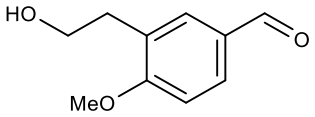
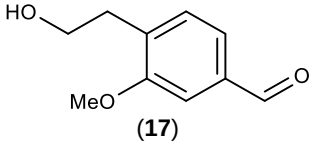


Figure 2.13: Richie formylation of compound 13 and compounds synthesised **(15-17).**

Table 2.6: Chemical characterization of the mixture of 5-formyl-2-methoxyphenethyl formate (**16**) and 4-(2-hydroxyethyl)-3-methoxybenzaldehyde (**17**), and the mixture of 3-(2-hydroxyethyl)-4-methoxybenzaldehyde (**18**) and 4-(2-hydroxyethyl)-3-methoxybenzaldehyde (**17**).

 (**16**)  (**17**)	**^{1}H NMR (400 MHz, $CDCl_3$):** δ 9.88 (s, 1H, H12), 9.87 (s, 1H, H11), 8.02 (s, 1H, H11), 7.79 (dd, *J* = 8.4, 4.2, 2.1 Hz, 2H, H-Ar), 7.72 (s, 2H, H-Ar), 6.98 (d, *J* = 8.4 Hz, 2H, H-Ar), 4.39 (t, *J* = 6.8 Hz, 2H, **a**), 3.93 (s, 6H, H-Me), 3.73 (t, *J* = 7.3 Hz, 2H, **c**) 3.13 (t, *J* = 7.3 Hz, 2H, **b**), 3.04 (t, *J* = 6.8 Hz, 2H, **d**). **IR (*ATR*) $V_{máx}$ (cm^{-1}):** 2930 (C-H sp^3), 1720 (C=O), 1683 (H-C=O), 1498 (C=C aromatic), 1255 (C-O).
 (**18**)  (**17**)	**^{1}H NMR (400 MHz, $CDCl_3$):** δ 9.88 (d, *J* = 1.5 Hz, 2H), 7.78 (dd, *J* = 8.5, 2.2 Hz, 2H), 7.73 (dd, *J* = 4.1, 2.2 Hz, 2H), 6.98 (s, 1H), 4.12 (q, *J* = 7.1 Hz, 4H), 3.93 (s, 6H), 3.87 (t, *J* = 6.5 Hz, 2H), 3.73 (t, *J* = 7.2 Hz, 2H), 3.13 (t, *J* = 7.2 Hz, 2H), 2.96 (t, *J* = 6.5 Hz, 2H). **IR (*ATR*) $V_{máx}$ (cm^{-1}):** 3454 (OH band), 2929 (C-H sp^3), 1723 (C=O), 1683 (H-C=O), 1498 (C=C aromatic), 1255 (C-O).

Due to the mixture of compounds and similarity of the rf which precluded the separation and isolation of the compounds, it was decided to envisaged another synthetic methodology involving the protection of the alcohol group before proceeding to the Rieche formylation. To finish the synthesis, an aldol type condensation and catalytic hydrogenation were performed. The following scheme shows the envisaged synthesis, **Figure 2.14**.

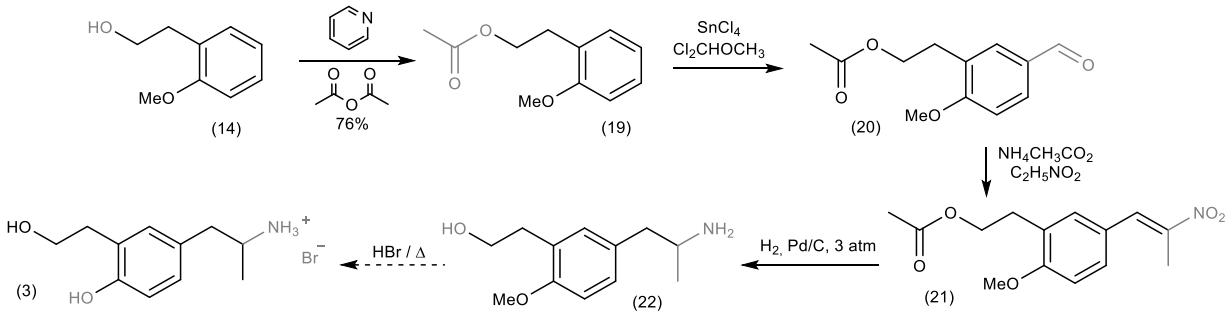


Figure 2.14: Second attempt to synthesise 4-(2-aminopropyl)-2-(2-hydroxyethyl)phenol (**Metabolite 3**).

To protect the alcohol **14** an acetylation reaction, using pyridine and acetic anhydride, was executed. In this reaction, pyridine acted as the basic catalyst. Furthermore, pyridine is a good nucleophile due to the lone pair of electrons on the nitrogen. The combination of both compounds usually promotes a smooth reaction. The mechanism of this reaction is presented in **Figure 2.15**. [48,49]

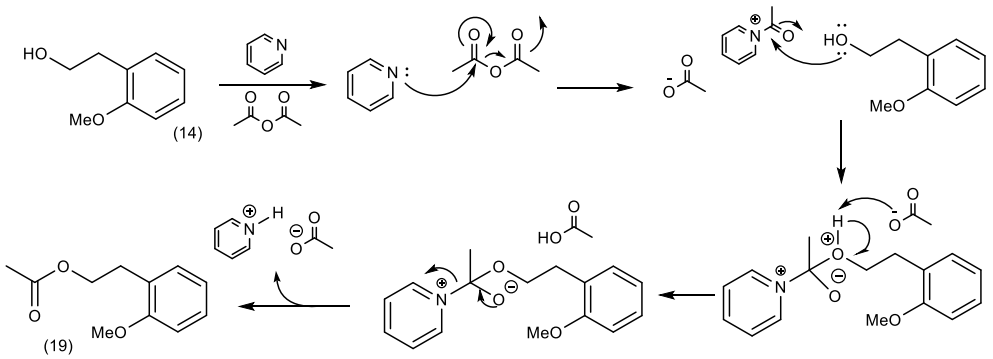


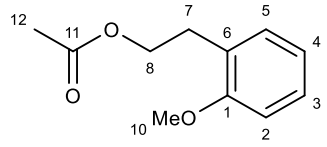
Figure 2.15: Synthesis mechanism of 2-methoxyphenethyl acetate (**19**).[37,50]

The progress of the reaction was verified by TLC using the spray reagent phosphomolybdic acid. The consumption of starting material was completed overnight and a new compound was synthesised. Comparing the FTIR-ATR spectra of starting material and the compound synthesised, the band corresponding to the C=O bond appeared at 1735 cm^{-1} and the OH band at 3331 cm^{-1} disappeared, as expected. All other main bands are similar to the bands presented by compound **14**.

Using NMR was possible to identify the compound synthesised. Through ^{1}H NMR, the most important signal was identified at 2.02 ppm corresponding to the hydrogens of the methyl group (C12) of the acetyl group. Through ^{13}C NMR, two new carbon signals were identified, one at 171.30 ppm C11 and the other at 21.16 ppm for the methyl carbon (C12). Comparing both NMR spectra from starting material and the product, the remaining signals stayed similar on both, reinforcing the hypothesis of a successful synthesis. In **Table 2.7** is presented a resume of the spectroscopic data. This compound is

not described in the literature therefore, ESI-MS was performed to determine the exact mass and complete the chemical characterization. The mass spectrum is presented in **Annexe 2 – Compound 19**.

Table 2.7: Chemical characterization of 2-methoxyphenethyl acetate (**19**).

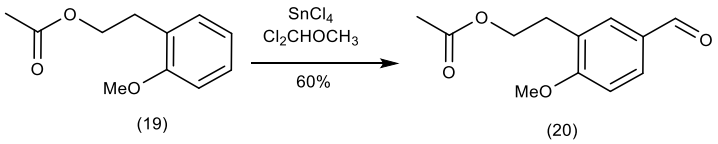


^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (t, *J* = 8.4, 6.8 Hz, 1H, H5-Ar), 7.14 (d, *J* = 7.4 Hz, 51H, H4-Ar), 6.90 (d, *J* = 7.6 Hz, 1H, H2-Ar), 6.85 (d, *J* = 8.6 Hz, 1H, H3-Ar), 4.26 (t, *J* = 7.1 Hz, 2H, CH_2- 8), 3.82 (s, 3H, CH_3-10), 2.95 (t, *J* = 7.1 Hz, 2H, CH_2-7), 2.02 (s, 3H, CH_3-12).

^{13}C-RMN (400 MHz, $CDCl_3$): 171.30 (C11), 157.79 (C1), 130.80 (C5), 128.06 (C6), 126.16 (C3), 120.53 (C4). 110.40 C2), 64.03 (C8), 55.37 (C10), 30.02 (C7), 21.16 (C12)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2957 (C-H sp^3), 1735 (O-C=O), 1494 (C=C aromatic), 1237 (C-O), 1033 (C-O ether), 753 (C-H alkene)

The formylation of the aromatic group of 2-methoxyphenethyl acetate (**19**) using dichloromethyl methyl ether and Tin(IV) chloride was performed as reported before in chapter **2.1.1** for compound **8,** and compound **16/17** and compound **20** was obtained using the same conditions (Figure **2.16**).



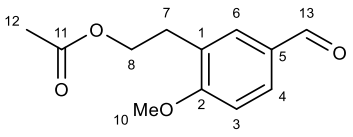
(19) (20)

Figure 2.16: Reaction scheme for the preparation of 3-(2-hydroxyethyl)-4-methoxybenzaldehyde (**20**).

FTIR-ATR allowed us to identify at 2750 cm^{-1} the band corresponding to the aldehyde group.

Using NMR was possible to identify the compound synthesised. Through ^{1}H NMR, the most important signal was identified at 9.87 ppm corresponding to the aldehyde hydrogen (C13). Through ^{13}C NMR, the aldehyde carbon (C13) was identified at 191.07 ppm. Comparing the starting material and the product the remaining signals stayed similar on both, reinforcing the hypothesis of a successful synthesis. In **Table 2.8** is presented a resume of the spectroscopic data. This reaction presents a yield of 60%. Comparing to the previously Rieche formylation of compound **16/17** here, in case of compound **20,** it was not observed the formation of two isomers due probably to a more electron-deficient ring that favour the formylation at the *para* position in relation to the methoxyl group.

Table 2.8: Chemical characterization of 3-(2-hydroxyethyl)-4-methoxybenzaldehyde (**20**).



^{1}H NMR (400 MHz, $CDCl_3$): δ 9.87 (s, 1H, CH-13), 7.77 (dd, *J* = 8.4, 2.1 Hz, 1H, H2-Ar), 7.70 (dd, 1H, H5-Ar), 6.97 (dd, *J* = 8.4 Hz, 1H, H3-Ar), 4.28 (t, *J* = 6.9 Hz, 2H, CH_2-8), 3.92 (s, 3H, CH_3-10), 3.00 (t, *J* = 6.9 Hz, 2H, CH_2-7), 2.02 (s, 3H, CH_3-12).

^{13}C-RMN (400 MHz, $CDCl_3$): 191.07 (C13), 171.17 (C11), 162.81 (C1), 131.79 (C4), 131.65 (C5), 129.70 (C3), 127.28 (C6), 110.33 (C2), 63.31 (C8), 55.87 (C10), 29.88 (C7), 21.05 (C12)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2955 (C-H sp^3), 2750 (C-H aldehyde) 1735 (O-C=O), 1500 (C=C aromatic), 1257 (C-O), 1024 (C-O ether)

The Knoevenagel condensation of 3-(2-hydroxyethyl)-4-methoxybenzaldehyde (**20**) using nitroethane and ammonium acetate (**Figure 2.17)** was performed as reported before in chapter **2.1.1** for compound **9.**

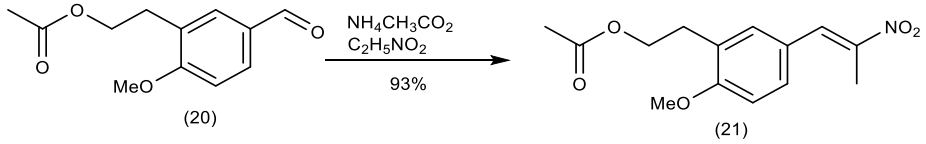
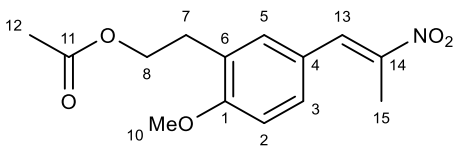


Figure 2.17: Reaction scheme for the preparation of (E)-2-methoxy-5-(2-nitroprop-1-en-1-yl)phenethyl acetate (**21**).

FTIR-ATR of compound **21** allowed to identify at 1313 cm^{-1} the band corresponding to the N-O stretching and as so the presence of the nitro group.

Using NMR was possible to identify the compound synthesised. Through ^{1}H NMR, the most important signals were identified at 8.06 ppm corresponding to the C13 hydrogen and at 2.48 ppm corresponding to the methyl group (C15). Through ^{13}C NMR, the C13 was identified at 133.82 ppm and, at 14.29, the methyl C15. Comparing the spectra from the starting material and the compound synthesised, the remaining signals stayed similar on both, reinforcing the hypothesis of a successful synthesis. In **Table 2.9** is presented a resume of the spectroscopic data. This reaction presents a yield of 93%. This compound is not described in the literature therefore, ESI-MS was performed to determine the exact mass and complete the chemical characterization. The mass spectrum is presented in **Annexe 2 –Compound 21**.

Table 2.9: Chemical characterization of (*E*)-2-methoxy-5-(2-nitroprop-1-en-1-yl)phenethyl acetate **(21).**



^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (s, 1H, CH-13), 7.36 (d, *J* = 8.8 Hz, 1H, H2-Ar), 7.27 (s, 1H, H5-Ar), 6.93 (d, *J* = 8.6 Hz, 1H, H3-Ar), 4.27 (t, *J* = 6.8 Hz, 2H, CH_2-8), 3.89 (s, 3H, CH_3-10), 2.97 (t, *J* = 6.9 Hz, 2H, CH_2-7), 2.48 (s, 3H, CH_3-15), 2.02 (s, 3H, CH_3-12)

^{13}C-RMN (400 MHz, $CDCl_3$): 171.20 (C11) , 158.48 (C1), 145.85 (C14), 133.82 (C13), 133.03 (C5), 131.05 (C3), 128.64 (C4), 125.91 (C6), 110.41 (C2), 63.61 (C8), 55.54 (C10), 29.84 (C7), 21.13 (C12), 14.29 (C15).

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2964 (C-H sp^3), 1736 (O-C=O), 1503 (C=C aromatic), 1313 (N-O), 1258 (C-O), 1030 (C-O ether)

For the synthesis of 2-(5-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (**22**), it was necessary to reduce the α,β-unsaturated nitro group of **21**, for this a catalytic hydrogenation using palladium on activated charcoal as the catalyst was performed as described before in chapter **2.1.1** (**Figure 2.18)**. But the results observed on the crude mixture by NMR didn't indicate the presence of the reduction product.

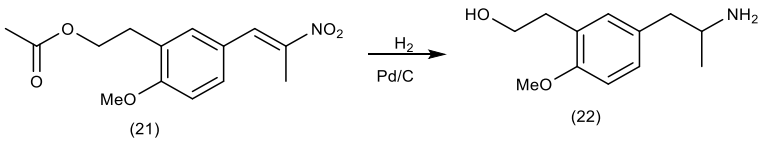
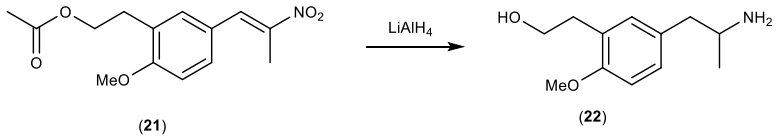


Figure 2.18: Reaction scheme for the preparation of 2-(5-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (**22**).

Since the hydrogenation reaction did not occur as expected, another reaction was tested to reduce the double bond and the α,β-unsaturated nitro group of methyl (*E*)-2-methoxy-5-(2-nitroprop-1-en-1-yl)phenethyl acetate (**21**) using lithium aluminium hydride (**Figure 2.19**).



(**21**) (**22**)

Figure 2.19: Reaction scheme for the preparation of 2-(5-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (**22**) using compound **21** as starting material.

The progress of the reaction was verified by TLC. The consumption of starting material was completed overnight and a mixture of compounds was formed, spray reagent Drangendorff was used to identify the formation of the amine group. By ^{1}H NMR (**Figure 2.20**) it was possible to identify a major compound formed. The doublet a 1.11 ppm corresponding to the protons of the methyl group (H15) and also, this structure presents a characteristic double duplet at 2.65 and 2.45 ppm corresponding to the prochiral methylene protons (H13) coupled with the H14 proton. When analysing the NMR, a characteristic singlet of the oxime appears at 2.14 ppm, confirming the hypothesis of an incomplete reduction reaction.

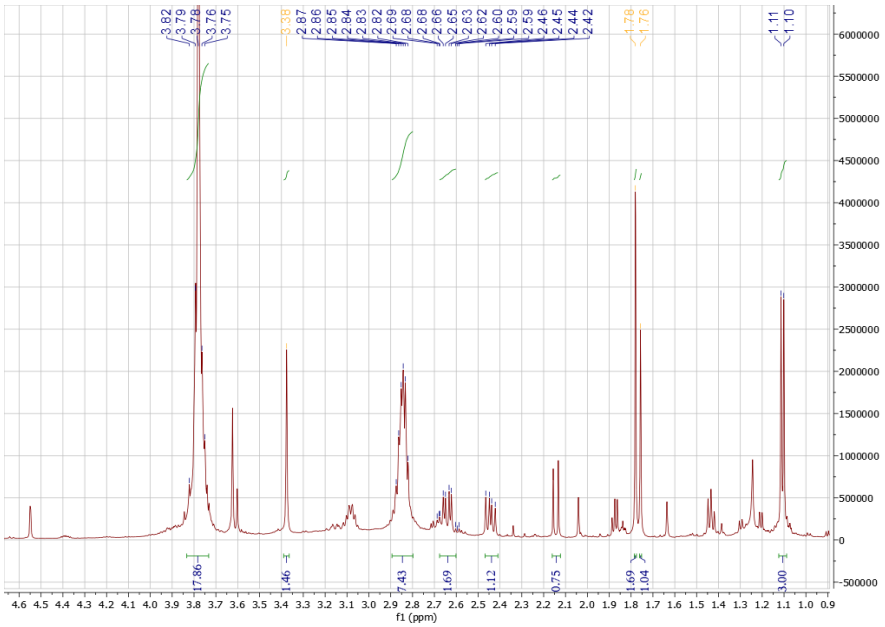


Figure 2.20: Proton NMR of the reaction mixture obtained from the reduction of compound **21**.

The reaction mixture was analysed by LC-MS (**Figure 2.21**) and the following compounds were identified at the corresponding retention time (**Figure 2.22**). The major compound (**22**) was identified at 25.129 min (**Figure 2.23**), the intermediated compound with acetylation protection (**A**) at 28.663 min (**Figure 2.24**), the *E* and *Z* forms of the oxime (**B**) at 29.245 min and 29.910 min (**Figure 2.25**) and, at 30.458 min, the compound **C** (**Figure 2.26**).

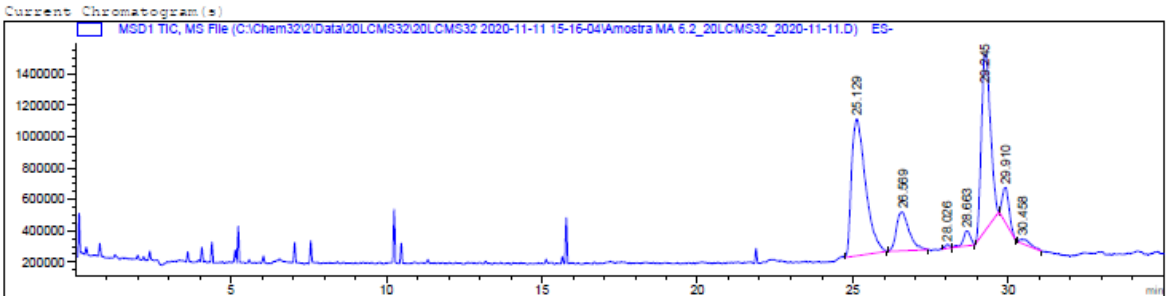


Figure 2.21: LC-MS spectrum of the reaction mixture with the identification of the compounds **22**, **A**, **B** and **C**.

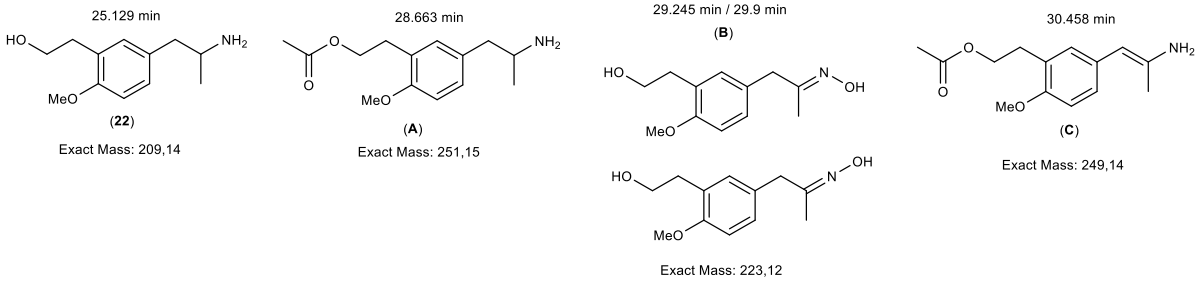


Figure 2.22: Compounds identified in the reaction mixture (**22, A, B and C**) with the respective retention time.

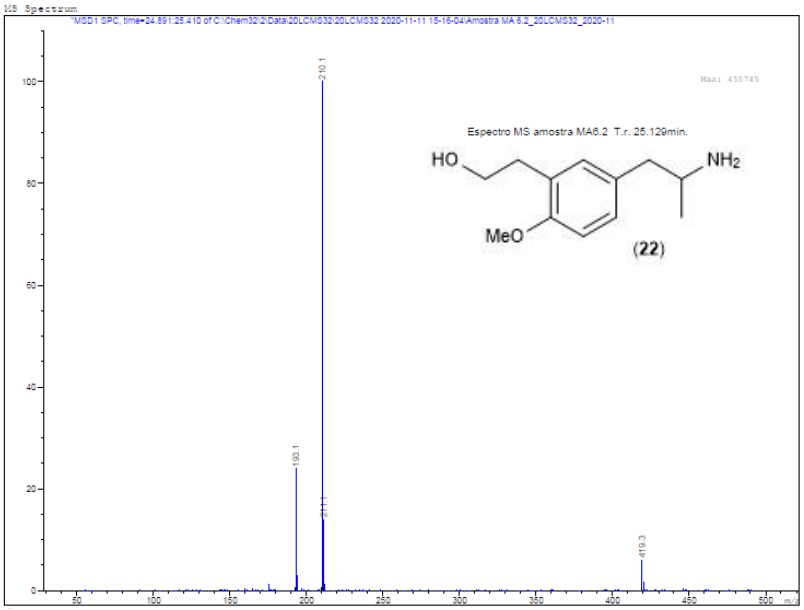


Figure 2.23: MS spectrum of compound **22** with rt 25.129 min.

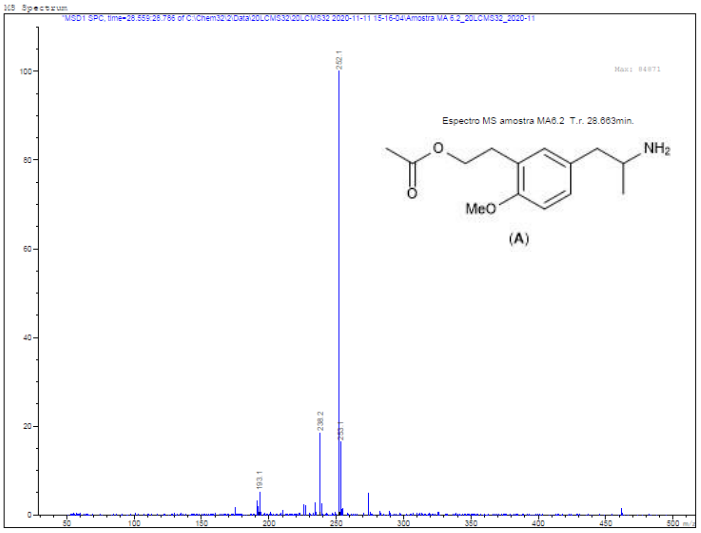


Figure 2.24: MS spectrum of compound A with rt 28.663 min.

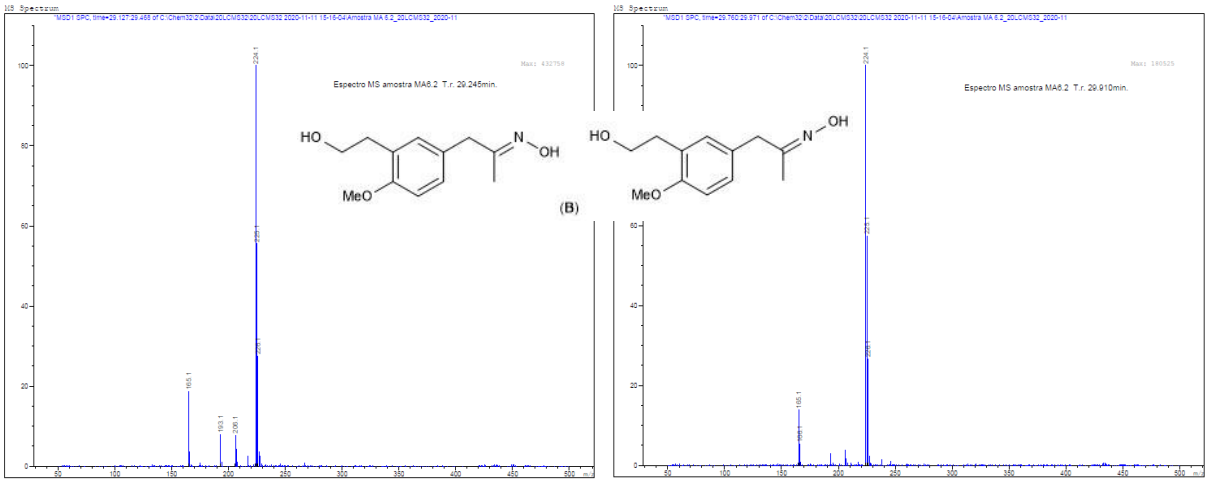


Figure 2.25: MS spectrum of the compound **B**, *E* and *Z* forms, with rt 29.910 and 29.2465 min.

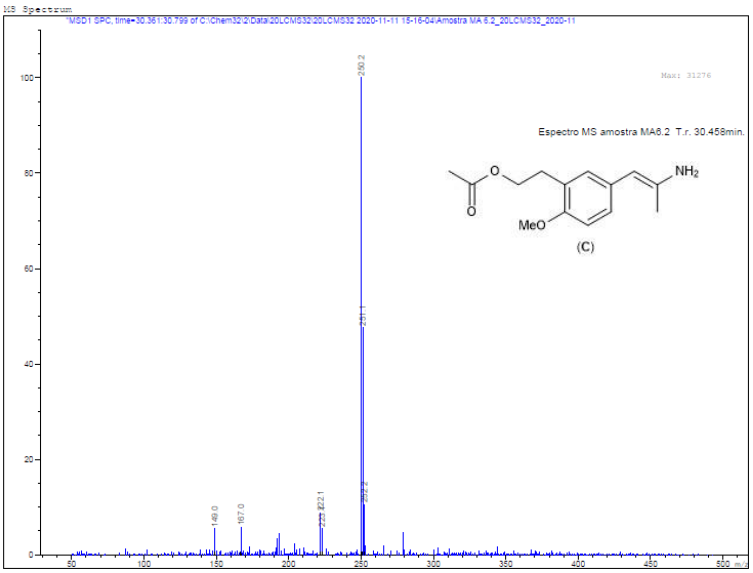
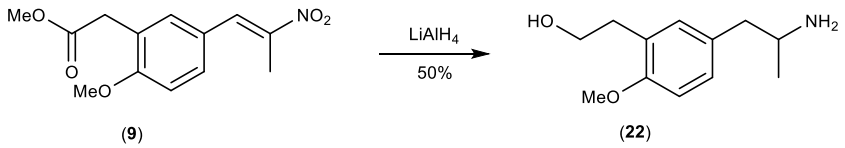


Figure 2.26: MS spectrum of compound **C** with rt 30.458 min.

Succeeding the results obtained in this reaction, another reduction using lithium aluminium hydride was envisaged. This reaction was performed using as starting material compound **9** (**chapter 2.1.1**) and reducing the ester group, the double bond and the α,β-unsaturated nitro group to obtain compound **22** (**Figure 2.27**).

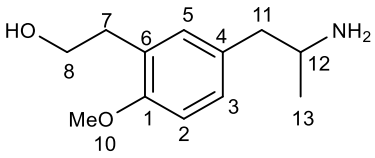


(**9**) (**22**)

Figure 2.27: Reaction scheme for the preparation of 2-(5-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (**22**) using compound **9** as the starting material.

The consumption of starting material was completed in 96h and one compound was formed and identified using spray reagent Drangendorff. FTIR-ATR of compound **22** allowed identifying at 3297 cm-1 the band corresponding to the OH group and as so confirming the reduction of the carboxylic group. By ^{1}H NMR it was possible to identify the doublet a 1.10 ppm corresponding to the protons of the methyl group (H13) and also, this structure presents a characteristic double duplet at 2.64 corresponding to the methylene protons (H11) and 2.44 ppm H12 proton. The disappearance of the methyl protons (H9) confirmed the successful reduction of the carboxylic group. The ^{13}C-NMR reconfirmed the hypothesis of a successful synthesis. In **Table 2.10** is presented a resume of the spectroscopic data. This reaction presented a lower yield (50%) comparing to the other synthetic steps.

Table 2.10: Chemical characterization of 2-(4-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (**22**).

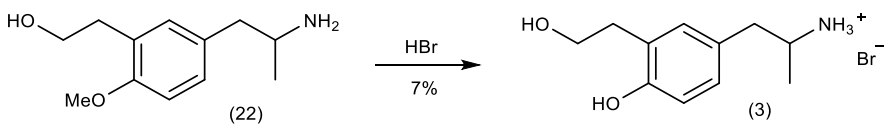


^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (d, *J* = 11.2 Hz, 2H, H2-Ar and H3-Ar), 6.79 (d, *J* = 10.1, 5.7 Hz, 1H, H5-Ar), 3.80 (s, 7H, CH_2-8, CH_3-10 and CH_2-7), 2.64 (dd, *J* = 13.6, 6.6 Hz, 2H, CH_2-11), 2.44 (dd, *J* = 13.5, 8.2 Hz, 1H, H12), 1.10 (d, *J* = 9.7 Hz, 3H, CH_3-13).

^{13}C-RMN (400 MHz, $CDCl_3$): δ 156.35 (C1), 132.08 (C4), 128.38 (C5), 127.23 (C3), 126.86 (C6), 110.54 (C2), 62.71 (C8), 55.51 (C10), 48.78 (C11), 34.17 (C7), 22.76 (C13).

IR (*ATR*) $V_{máx}$ (cm^{-1}): 3297 (OH band), 2925 (C-H sp^3), 1501 (C=C aromatic), 1249 (C-O), 1029 (C-O ether)

To attain **metabolite 3**, hydrolysis of the methoxyl group 10 using a strong acid media was envisaged. This reaction was performed using as starting material compound **22** and hydrogen bromide to synthesise **metabolite 3** (**Figure 2.28**) as it is described in **chapter 3.12**.



(22) (3)

Figure 2.28: Reaction scheme for the preparation of 4-(2-aminopropyl)-2-(2-hydroxyethyl)phenol (**Metabolite 3**).

In this reaction was not possible to follow the progress of the reaction, and so, this reaction was refluxed for 2 hours and purified using C18 Reversed-Phase sep-pak using as eluent water and

methanol. The fractions were separated individually, some presented residual masses but some of them had enough mass to be analysed through NMR.

Through the NMR and MS (ESI) analysis of the fractions, was possible to identify traces of the starting material (**22**) and most importantly, the **Metabolite 3**. By ^{1}H NMR (**Figure 2-29**) it was possible to reidentify the doublet a 1.30 ppm corresponding to the protons of the methyl group (H13) and the other excepted signals at 3.58 (H12) and 2.85 (H7 and H11). The diminishing in the integration of the signal at 3.83 confirmed the successful reduction of the methoxy group C10. In **Table 2.11** is presented a resume of the spectroscopic data.

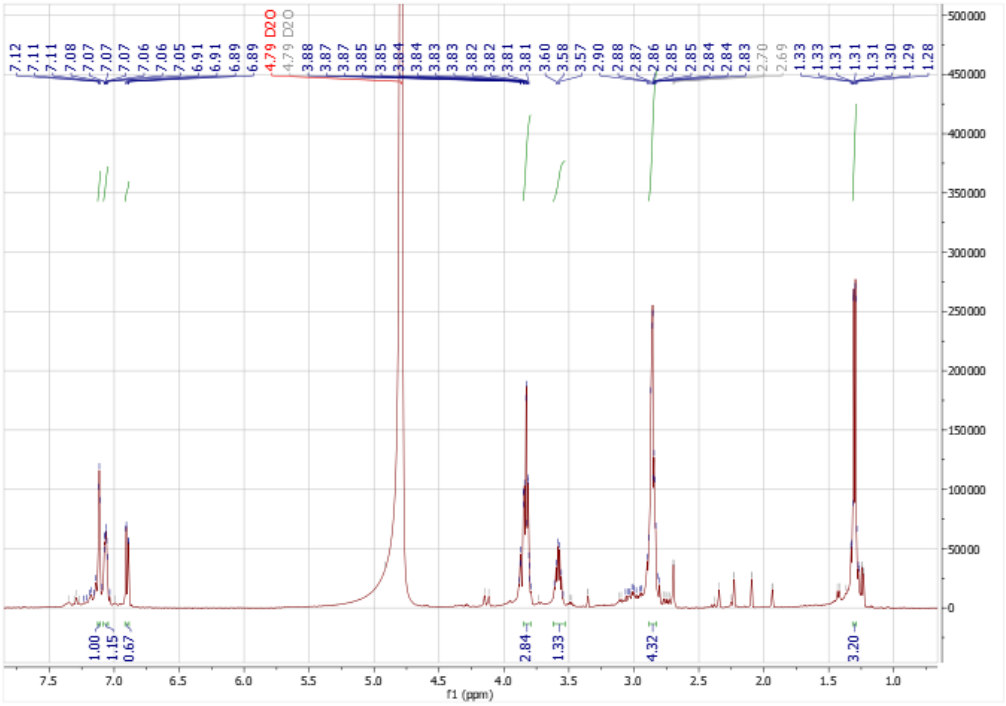
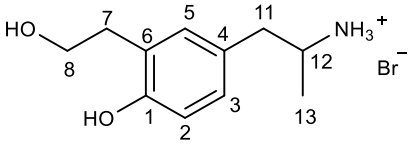


Figure 2.29: ^{1}H NMR of 4-(2-aminopropyl)-2-(2-hydroxyethyl)phenol (**Metabolite 3**).

Table 2.11: Chemical characterization of 4-(2-aminopropyl)-2-(2-hydroxyethyl)phenol (**Metabolite 3**).



^{1}H NMR (400 MHz, Deuterium oxide): δ 7.11 (s, 1H, H2-Ar), 7.07 (s, 1H, H3-Ar), 6.90 (d, *J* = 8.2, 1.3 Hz, 1H, H5-Ar), 3.83 (dd, *J* = 10.0, 6.9, 2.4 Hz, 2H, CH_2-8), 3.58 (q, *J* = 6.7 Hz, 1H, CH-12), 2.85 (t, 4H, CH_2-7 and CH_2-11), 1.30 (d, *J* = 6.6, 1.3 Hz, 3H, CH_3-13).

IR (*ATR*) $V_{máx}$ (cm^{-1}): 3410 (OH band), 2926 (C-H sp^3),1259 (C-O)

The reaction mixture was analysed by LC-MS (**Figure 2.30**) and **metabolite 3** was identified at 26.568 min (**Figure 2.31**) and the not protonated form of metabolite 3 was identified at 9.193 min (**Figure 2.32**).

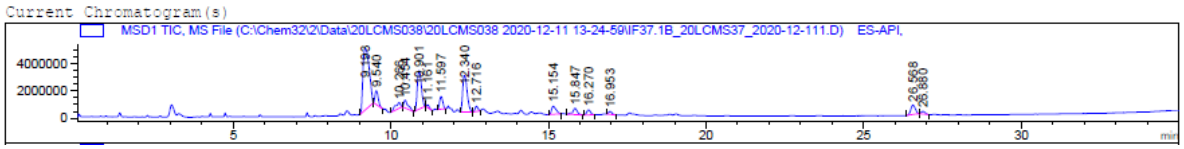


Figure 2.30: LC-MS spectrum of the reaction mixture.

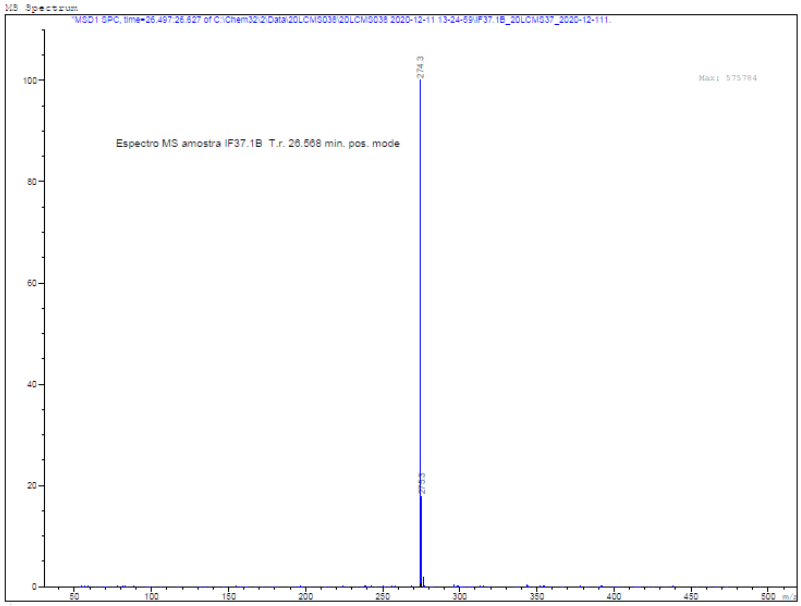


Figure 2.31: MS spectrum of **Metabolite 3** with rt 26.568 min.

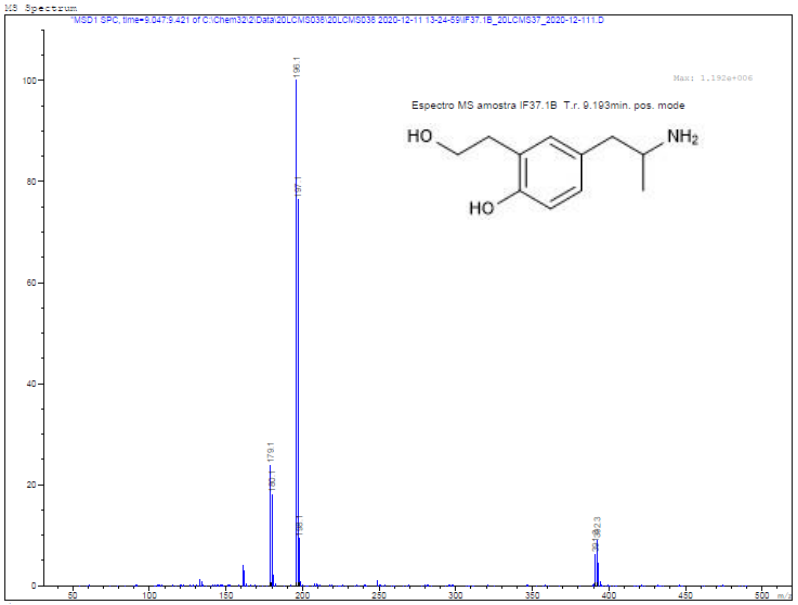


Figure 2.32: MS spectrum of the not protonated form of **Metabolite 3** with rt 9.193 min.

The envisaged synthesis proved to be too long, increasing the difficulty to attain **Metabolite 3**. In this synthesis, the main problem stayed the same, the catalytic hydrogenation reaction. However, it was possible to develop a new synthesis for **Metabolite 3** using as a base the first synthetic steps of **Metabolite 1** and testing a different reduction reaction with lithium aluminium hydride. Additionally to the importance of **Metabolite 3**, compound **22** can also be a target of toxicological studies.

2.2 Synthesise of 6-APB metabolite

The major challenge of the synthesis of this metabolite resides in the *meta* relationship between the hydroxyl substituent and the ethylamine chain. As starting material it was decided to use the 2-(4-bromo-2-methoxyphenyl) acetic acid (**6**). The Bromo substituent could be further derivatized to include an aldehyde group crucial for the Knoevenagel reaction with nitroethane following the previous procedures used for the synthesis of the 5-APB metabolites.

2.2.1 6-(2-aminopropyl)-2-(2-hydroxyethyl)phenol (4)

The reaction scheme for the preparation of metabolite **4**, the 6-(2-aminopropyl)-2-(2-hydroxyethyl)phenol envisaged is represented in **Figure 2.33**.

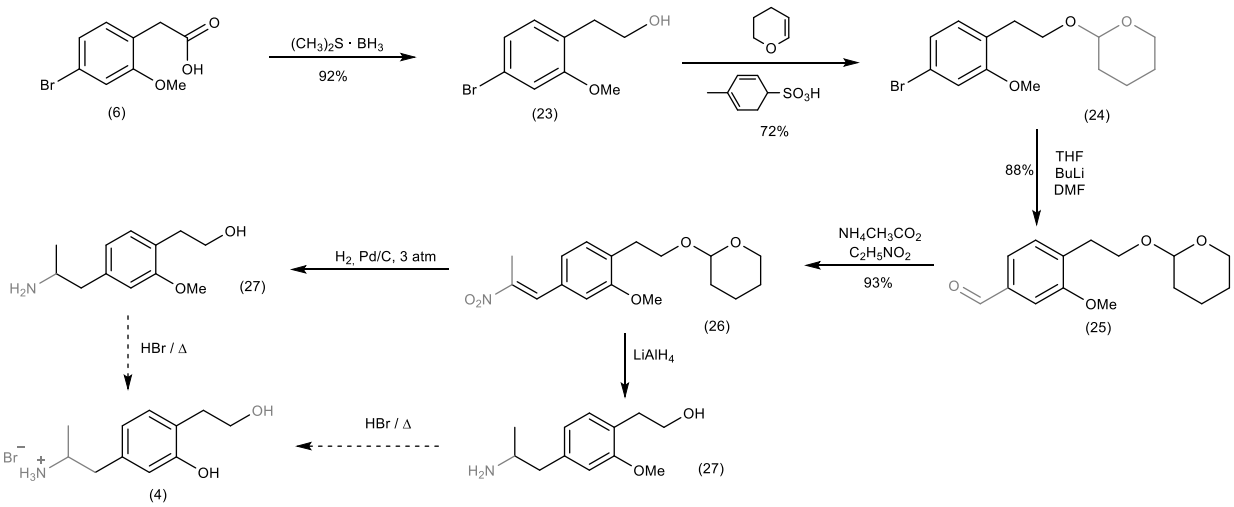


Figure 2.33: 6-(2-aminopropyl)-2-(2-hydroxyethyl)phenol synthesis – **Metabolite (4)**.

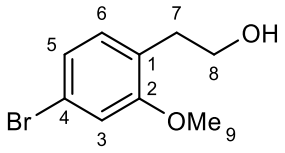
To synthesize the 2-(4-bromo-2-methoxyphenyl)ethan-1-ol, compound **23**, was necessary to reduce the carboxyl group of **6**. Here again and since we are in the presence of a carboxylic acid, the borane dimethylsulfide was used as reduced agent. The reaction conditions were the same used for the reduction of compound **14** in chapter **2.1.2**.

The progress of the reaction was verified by TLC using the spray reagent phosphomolybdic acid. The consumption of starting material was completed in 2 hours and a new compound was formed. FTIR-ATR spectra allowed to identify the hydroxyl group band at 3217 cm^{-1} as it was expected.

Through ^{1}H NMR, the aromatic protons were identified as one doublet at 7.01 ppm. The signal corresponding to the methyl protons of the methoxyl group are grouped with the protons from the CH_2 (C8), this signal appears at 3.81 in the form of a triplet. Lastly, the signal corresponding to the CH_2 (7) was identified as a triplet at 2.85. Through ^{13}C NMR, the aromatic carbons were identified between 158.38 and 114.17 ppm (C1 to C6). The chemical shift in the ^{13}C NMR corresponding to the two methylene groups can be found at 62.52 and 33.72 ppm. Lastly, at 55.74 ppm was possible to

identify the carbon chemical shift corresponding to the methyl group C9. In **Table 2.12** is presented a resume of the spectroscopic data. This compound is not described in the literature. This reaction presents a yield of 92%. Considering the purity of the compound and the short time reaction, it can be classified as a very efficient reaction.

Table 2.12: Chemical characterization of 2-(4-bromo-2-methoxyphenyl)ethan-1-ol (**23**).



^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (d, *J* = 18.5 Hz, 3H, H3-Ar, H5-Ar and H6-Ar), 3.81 (t, *J* = 7.5 Hz, 5H, CH_2-8 and CH_3-9), 2.85 (t, *J* = 6.4 Hz, 2H, CH_2-7).

^{13}C-RMN (400 MHz, $CDCl_3$): 158.38 (C2), 132.06 (C1), 126.20 (C6), 123.68 (C5), 120.88 (C4), 114.17 (C3), 62.52 (C8), 55.74 (C9), 33.72 (C7)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 3217 (O-H), 2940 (CH sp^3), 1592 (C=C aromatic), 1239 (C-O ether), 662 (C-Br)

To further continue the synthesis and before the metallation reaction, it was necessary to proceed to the protection of the hydroxyl group of **23**. It was decided to protect with 3-4-dihydro-2H-piran to prepare the 2-(4-bromo-2-methoxyphenethoxy)tetrahydro-2H-pyran, compound **24**. 3-4-Dihydro-2H-piran was chosen due to several advantages, such as being low cost and ease of introduction. The mechanism of this reaction is presented in **Figure 2.34**. This vinyl ether reacts with alcohols under mild acid catalysis, in this case, p-toluenesulfonic acid, and is susceptible to hydrolysis under aqueous acidic conditions.

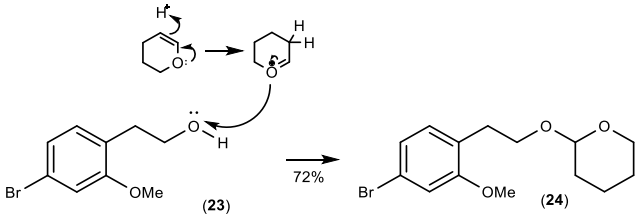


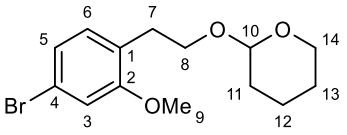
Figure 2.34: Synthesis mechanism of 2-(4-bromo-2-methoxyphenethoxy)tetrahydro-2H-pyran (**24**).

The progress of the reaction was verified by TLC using the spray reagent phosphomolybdic acid. The consumption of starting material was completed in 3 hours and several compounds were formed. The reaction mixture was purified using a silica flash gel chromatography column eluted with DCM/Hex 9/1.

FTIR-ATR analysis of the major compound isolated allowed us to identify at 2939 cm^{-1} the band corresponding to the C-H bond of sp^3 carbons, the C-O band of ether at 1027 cm^{-1} and at 630 cm^{-1} the band corresponding to the C-Br bond. Comparing the spectra of starting material and the compound synthesised, the band corresponding to the hydroxyl group disappeared as expected and the aromatic C=C bands at 1590 cm^{-1} stayed similar on both spectra. Through ^{1}H NMR, the most important signals were identified at 4.58 ppm for H-10, a multiplet between 3.61 and 3.42 for CH_2-14, and finally, CH_2-11, CH_2-12 and CH_2-13 as a multiplet between 1.61 and 1.45. The result accounts for the success of the protection of the hydroxyl group with 3-4-dihydro-2H-Piran. Through ^{13}C NMR, it was also possible to identify the pyran carbons at 98.64 for C10, at 62.25 for C14, at 30.37 for C11, at 25.51 for C13 and lastly, at 19.55 for C12. In **Table 2.13** is presented a resume of the spectroscopic

data. This reaction presents a yield of 72%. Considering the purity of the compound and the short time of reaction, it can be classified as a very efficient reaction.

Table 2.13: Chemical characterization of 2-(4-bromo-2-methoxyphenethoxy)tetrahydro-2H-pyran (**24**).



^{1}H NMR (400 MHz, $CDCl_3$): δ 7.07 – 6.98 (m, 2H, H5-Ar and H6-Ar), 6.96 (s, 1H, H3-Ar), 4.58 (t, *J* = 3.5 Hz, 1H, H-10), 3.83 – 3.73 (m, 5H, CH_3-9 and CH_2-8), 3.61 – 3.42 (m, 2H, CH_2-14), 2.87 (t, *J* = 7.3 Hz, 2H, CH_2-7), 1.61 – 1.45 (m, 6H, $7CH_2$-7, CH_2-11, CH_2-12 and CH_2-13)

^{13}C-RMN (400 MHz, $CDCl_3$): 158.27 (C2), 131.93 (C1), 126.46 (C6), 123.26 (C5), 120.40 (C4), 113.79 (C3), 98.64 (C10), 66.51 (C8), 62.25 (C14), 55.59 (C9), 30.71 (C7), 30.37 (C11), 25.51 (C13), 19.55 (C12)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2939 (CH sp^3), 1590 (C=C aromatic), 1027 (C-O ether), 630 (C-Br)

After the protection of the compound, it was possible to proceed with the metalation reaction that would allow us to do the substitution of the Bromo to an aldehyde group. For the synthesis of 3-methoxy-4-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethyl)benzaldehyde (**25**), it was necessary to do a lithium-halogen exchange with n-butyllithium followed by an electrophilic quench with DMF. This reaction was conducted at low temperature, -78ºC, using an acetone bath cooled to this temperature by adding liquid nitrogen. The mechanism of this reaction is presented in **Figure 2.35**.

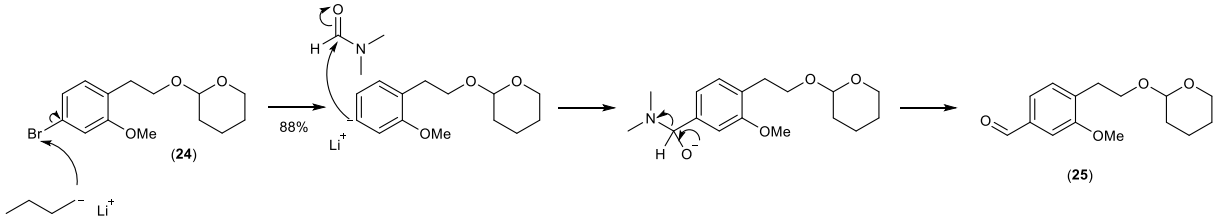


Figure 2.35: Synthesis mechanism of 3-methoxy-4-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethyl)benzaldehyde (**25**).

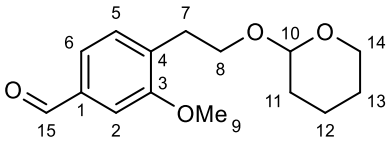
The progress of the reaction was verified by TLC. The consumption of starting material was completed in 3 hours and a new compound was formed which was identified with the spray reagent DNP.

Comparing the spectra of starting material and the compound synthesised, the band of C-Br bond at 630 cm^{-1} disappeared and the band corresponding to the carbonyl group of the aldehyde (H-C=O) appeared at 1687 cm^{-1} as expected. All other main bands are similar to the bands presented by compound **24**.

Through ^{1}H NMR, the most important signal was identified at 9.95 ppm as a singlet and it corresponds to the hydrogen of the aldehyde group. The appearance of this hydrogen chemical signal reinforced the hypothesis of a successful synthesis. Through ^{13}C NMR, it was also possible to identify the aldehyde carbon, C15, at 192.17. Also comparing the starting material spectrum (**24**) and the one obtained for **25**, the remaining signals stayed similar in both spectra confirming a successful synthesis. In **Table 2.14** is presented a resume of the spectroscopic data. This reaction presents a yield of 88%. Considering the purity of the compound and the short time reaction, it can be classified as a very efficient reaction. This compound is not described in the literature therefore, ESI-MS was performed to

determine the exact mass and complete the chemical characterization. The mass spectrum is presented in **Annexe 2 – Compound 25**.

Table 2.14: Chemical characterization of 3-methoxy-4-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethyl)benzaldehyde (**25**).

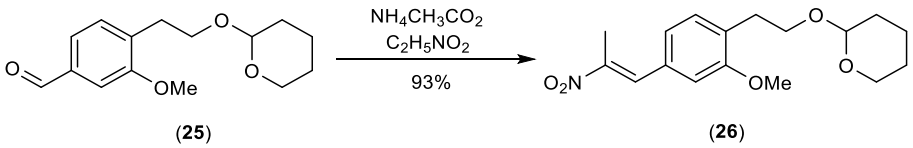


^{1}H NMR (400 MHz, $CDCl_3$): δ 9.95 (s, 1H, H-15), 7.42 – 7.35 (m, 3H, H-Ar), 4.61 (td, *J* = 4.8, 4.3, 2.9 Hz, 1H, H-10), 3.99 – 3.79 (m, 7H, CH_3-9, CH_2-8 and CH_2-14), 3.01 (t, *J* = 7.0 Hz, 2H, CH_2-7), 1.63 – 1.46 (m, 6H, CH_2-11, CH_2-12 and CH_2-13)

^{13}C-RMN (400 MHz, $CDCl_3$): 192.17 (C7), 158.31 (C3), 136.31 (C1), 135.55 (C4), 131.17 (C5), 124.47 (C6), 108.40 (C2), 98.78 (C10), 66.28 (C8), 62.31 (C14), 55.63 (C9), 34.42 (C7), 31.23 (C11), 25.56 (C13),19.60 (C12)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2940 (C-H sp^3), 1687 (H-C=O), 1028 (C-O ether)

The Knoevenagel condensation of 3-(2-hydroxyethyl)-4-methoxybenzaldehyde (**25**) using nitroethane and ammonium acetate was performed as reported before and presented in **Figure 2.36.**



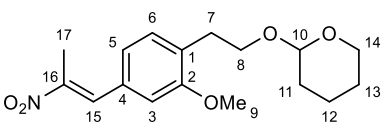
(**25**) (**26**)

Figure 2.36: Reaction scheme for the preparation of (*E*)-2-(2-methoxy-4-(2-nitroprop-1-en-1-yl)phenethoxy)tetrahydro-2H-pyran (**26**).

The progress of the reaction was verified by TLC. The consumption of starting material was completed overnight and a new compound was formed, spray reagent DNP was used to follow the consumption of the starting material. FTIR-ATR allowed identifying, at 1320 cm^{-1}, the band corresponding to the presence of the nitro group. All other main bands are similar to the bands presented by compound **26**.

Using NMR was possible to identify the compound synthesised. Through ^{1}H NMR, the most important signals were identified at 8.04 ppm corresponding to the C15 protons and at 2.44 ppm corresponding to the methyl group (C17). Through ^{13}C NMR, the C15 was identified at 133.77 and, at 14.30 the methyl C17. Comparing the spectra from the starting material and the compound synthesised the remaining signals stayed similar on both, reinforcing the hypothesis of a successful synthesis. In **Table 2.15** is presented a resume of the spectroscopic data This reaction presents a yield of 93%. This compound is not described in the literature therefore, ESI-MS was performed to determine the exact mass and complete the chemical characterization. The mass spectrum is presented in **Annexe 2 – Compound 26**.

Table 2.15: Chemical characterization of (*E*)-2-(2-methoxy-4-(2-nitroprop-1-en-1-yl)phenethoxy)tetrahydro-2H-pyran (**26**).



^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (s, 1H, H-15), 7.24 (d, *J* = 3.1 Hz, 1H, H6-Ar), 6.96 (s, 1H, H5-Ar), 6.84 (d, *J* = 1.5 Hz, 1H, H3-Ar), 4.58 (t, *J* = 4.4, 2.9 Hz, 1H, H-10), 3.84 – 3.78 (m, 7H, CH_3-9, CH_2-8 and CH_2-14), 2.96 – 2.90 (m, 2H, CH_2-7), 2.44 (s, 3H, CH_3-17), 1.55 – 1.43 (m, 6H, CH_2-11, CH_2-12 and CH_2-13).

^{13}C-RMN (400 MHz, $CDCl_3$): 157.90 (C2), 147.34 (C16), 134.02 (C4), 133.77 (C15), 131.18 (C1), 130.25 (C6), 122.29 (C5), 111.84 (C3), 98.81 (C10), 66.55 (C8), 62.36 (C14), 55.53 (C9), 34.05 (C7), 30.81 (C11), 25.58 (C13), 19.66 (C12), 14.30 (C17)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2939 (C-H), 1463 (C=C aromatic), 1320 (N-O), 1255 (C-N), 1027 (C-O ether)

For the synthesis of 2-(4-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (**27**), it was necessary to reduce the α,β-unsaturated nitro group of the starting material, for this a catalytic hydrogenation using palladium on activated charcoal as the catalyst was performed as described before in chapter **2.1.1** and **2.1.2** (**Figure 2.37**)**.**

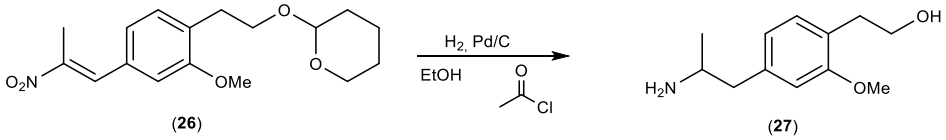


Figure 2.37: Reaction scheme for the preparation of 2-(4-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (**27**).

It was decided to generate HCl *in situ* by the reaction of acetyl chloride and ethanol. The progress of the reaction was verified by TLC. The consumption of starting material was completed but two different new compounds were synthesised. To purify the reaction mixture a C18 Reversed-Phase Silica Gel Flash Chromatography, using as eluents water and methanol, was performed. The fractions were separated according to their UV analysis and residual masses were obtained. Therefore, the NMR analysis of these fractions was inconclusive.

Since the catalytic hydrogenation reaction did not occur as expected, another reaction was tested to reduce the double bond and the nitro group of (*E*)-2-(2-methoxy-4-(2-nitroprop-1-en-1-yl)phenethoxy)tetrahydro-2H-pyran (**27**) using lithium aluminium hydride (**Figure 2.38**).

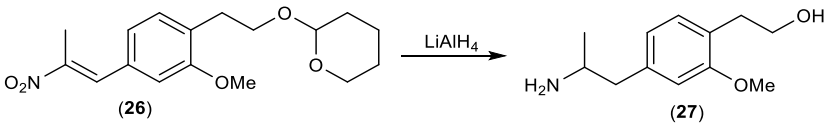


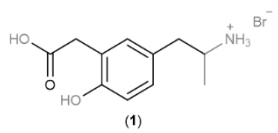
Figure 2.38: Reaction scheme for the preparation of 2-(4-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (**27**).

The progress of the reaction was verified by TLC. The consumption of starting material was completed overnight and spray reagent Drangendorff was used to identify the formation of the amine group. However, the NMR analysis was inconclusive.

Overall, this synthesis did not go as envisaged and **Metabolite 4** was not attained, being the main problem again, the reduction of the α,β-unsaturated nitro group. The reduction using lithium aluminium hydride should be repeated with higher mass in order to confirm the synthesis of **compound 27**.

3 Conclusion and future perspectives

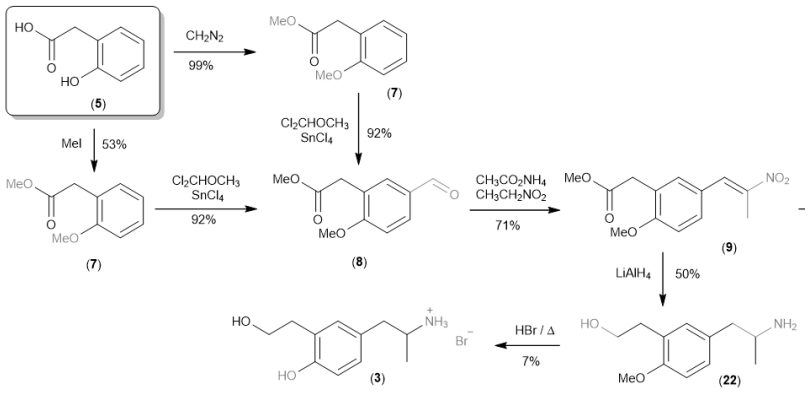
This thesis had as goal the synthesis of three different oxidized metabolites from the 5-APB and 6-APB drugs of abuse using two different starting material. For the **Metabolites 1 and 3**, was used the 2-hydroxyphenyl acetic acid (**5**) and, for **Metabolite 4**, the 2-(4-bromo-2-methoxyphenyl) acetic acid (**6**).

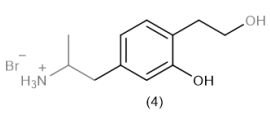


(**1**)

In the synthesis of **Metabolite 1**, all reactions presented a good yield, however the catalytic hydrogenation reaction did not allow the synthesis of compound **10**. Another new procedure to attain this compound was tested, a reductive amination reaction, but the NMR analysis proved to be inconclusive. Therefore, this synthesis was not completed and **Metabolite 1** was not attained.

For the synthesis of **Metabolite 3**, two different pathways were tested. In the first synthetic pathway, the formylation reaction of compound **14** did not go as expected and two different compounds with similar rf were attained, precluding the separation and isolation of compound **17**. In the second synthetic pathway, the problem stayed the same, the catalytic hydrogenation reaction did not allow continuing the synthesis. So another reduction procedure with lithium aluminium hydride was tested and a reactional mixture was attained and analysed using LC-MS, identifying four different intermediated compounds and compound **22**. Succeeding the results of these synthetic pathways, the reduction using lithium aluminium hydride was tested in compound **9**. And, with this reaction, was possible to attain compound **22** and proceed with the envisaged synthesis and achieve the **Metabolite 3**. To sum up, the synthesis envisaged for **Metabolite 1** allowed the synthesis of **Metabolite 3**.





(4)

In the synthesis of **Metabolite 4**, all reactions also presented a good yield, however the catalytic hydrogenation reaction and the reduction reaction using lithium aluminium hydride did not go as envisaged and ,in both reactions, the NMR analysis was inconclusive. Therefore, this synthesis was not and **Metabolite 4** was not attained.

In a future work, a new reaction should be devised to replace the catalytic hydrogenation in the synthesis of **Metabolite 1** and **Metabolite 4** and, in the case of **metabolite 4**, the reduction reaction using lithium aluminium hydride should be repeated. Additionally, **Metabolite 3** and compound **22** should be a target of toxicological studies using a differentiated human neuroblastoma cell line (SH-SY5Y).

4 Materials and methods

All reagents and solvents used in the experimental procedures of this thesis were acquired from commercial sources, specifically *Sigma-Aldrich, LaborSpirit and Alfa-Aesar*. The solvents were used without further purification except, when necessary, dried according to standardized methods.

All the reactions were followed using Analysis *Merck* TLC Silica gel 60 F_{254} Aluminium sheets with 22 µm of layer thickness. The visualization of the *TLC* was made in a UV lamp (254 nm) followed by the staining of the plate using different methods, mainly DNP, Dragendorff Stain and phosphomolybdic acid stain. In the purification of the compounds, two different types of chromatography were used. The Flash chromatography was performed on *Merck* Silica Gel (200-400 mesh) and the reverse phase chromatography using *Merck* LiChroprep RP-18 (40-63 µm).

The chemical characterization of the compounds was performed by using IR spectroscopy, ^{1}H and ^{13}C NMR spectroscopy and UV absorption spectra.

The IR-ATR spectra were acquired between 4000-400 cm^{-1} in a *Perkin Elmer Spectrum Two* spectrometer equipped with an attenuated total reflectance module.

The ^{1}H NMR and ^{13}C NMR spectra were recorded in a *Brucker ARX400* at 400 MHz. The ^{1}H NMR spectra data was presented by the following order: deuterated solvent used, chemical shift, spin multiplicity (s, singlet; d, doublet; dd, double doublet t, triplet; m, multiplet) and, if possible, the molecular assignment. The ^{13}C NMR spectra data was presented by the following order: deuterated solvent used, chemical shift and, if possible, the molecular assignment. The chemical shifts of both spectra are referenced to the solvent used.

The UV absorption spectra were recorded in a *Thermo Corporation Helius γ* using a 10 mm path length quartz cuvette between the spectral ranges of 190 to 300 cm^{-1}.

The HRMS spectra were recorded in a HPLC *Agilent 1100* coupled with *a QSTAR® XL Hybrid LC/MS/MS.*

The ESI spectra were recorded in a LC *Agilent 1200* Series with Binary pump / MS *Agilent 6130B* Single Quadrupole with an ESI source.

4.1 Diazomethane preparation [36]

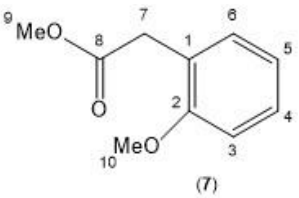
To a solution of 0.4g of potassium hydroxide in 10 mL of ethanol was added a previously prepared solution of 2.14g of *N*-methyl-*N*-nitroso-*p*-toluene sulphonamide in 30 mL of ethyl ether. The reaction mixture was placed in a bath of water and ice, for 5 minutes, and distilled off. A solution of diazomethane in ethyl ether was collected in an ice-cold conical flask.

4.2 Preparation of methyl 2-(2-methoxyphenyl)acetate (7)

4.2.1 Using diazomethane as a methylating reagent

To a 50 mL round bottom flask equipped with a stir bar was added a solution of 2-hydroxyphenyl acetic acid (**5**) (0.513 g; 3.37 mmol) in ethyl ether (20 mL) and methanol (2mL). Next 7 mL of diazomethane ethyl ether solution was added and the solution was kept in an inert atmosphere for 140h. The reaction was followed by TLC and more diazomethane was added until the consumption of the starting material. In total, 12 mL of diazomethane ethyl ether solution were used. The reaction was concentrated under reduced pressure and methyl 2-(2-methoxyphenyl) acetate (**7**) was obtained as a pale yellow oil in 99% (0,601 g).

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2952 (C-H sp^3), 1734 (O-C=O), 1495 (C=C aromatic); **^{1}H-RMN (400 MHz, $CDCl_3$)**: δ 7.28 (t, J = 7.6, 4.4 Hz, 1H, H4-Ar), 7.20 (d, 1H, H6-Ar), 6.96 (t, 1H, H5-Ar), 6.61 (d, 1H, H3-Ar), 3.84 (s, 3H, CH_3-10), 3.71 (s, 3H, CH_3-9), 3.67 (s, 2H, CH_2-7); **^{13}C-RMN (400 MHz, $CDCl_3$):** 172.49 (C8), 157.66 (C2), 131.01 (C6), 128.72 (C4), 123.15 (C1), 120.66 (C5), 110.65 (C3), 55.62 (C10), 52.06 (C9), 35.90 (C7).



(7)

4.2.2 Using iodomethane as a methylating reagent

The methylation of **5** using iodomethane was tested with two different procedures. The reagents used are the same, iodomethane and potassium carbonate, but the solvent used and the conditions of both reactions were altered due to the ambient conditions.

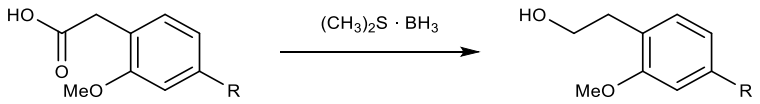
I. To a 50 mL round bottom flask equipped with a stir bar, 2-hydroxyphenyl acetic acid (**5**) (0.200 g; 1.31mmol) was dissolved in acetone (10 mL). To the solution under nitrogen atmosphere, it was added K_2CO_3 (0.545 g; 3.94 mmol; 2.5 eq.) and MeI (0.2 mL; 3.27 mmol; 3 eq.). The mixture was kept at room temperature overnight and the purity of the compound was monitored by TLC (Eluent: DCM/Hex 1/1). The acetone was removed under reduced pressure and water (20 mL) was added to the mixture and extracted with ethyl acetate (2x30 mL). The organic phase was washed with water (2x20 mL), brine (20 mL), dried using anhydrous sodium sulfate, filtered and concentrated under reduced pressure. A yellow oil (0.032 g; 14%) was obtained but was not possible to identify by NMR.

II. In a 50 mL round bottom flask equipped with a stir bar, a mixture of 2-hydroxyphenyl acetic (**5**) acid (0.200 g; 1.31 mmol) and K_2CO_3 (0.635 g; 4.60 mmol; 3,5 eq.) was dissolved in acetonitrile (2 mL). In another round bottom flask under a nitrogen atmosphere, a solution of acetonitrile (5 mL) and

MeI (0.2 mL: 3.31mmol; 2.5 eq.) was prepared. The prepared solution was carefully added to the 50 mL round bottom flask under a nitrogen atmosphere. The reactional mixture was refluxed overnight and the purity of the compound was followed by TLC (Eluent: DCM/Hex 1/1). The formed precipitated was filtered and, the obtained solution was centrifuged twice for 5 min at 4500 rpm. Between centrifugations, the supernatant solution was removed and more acetone was added. The obtained solution was concentrated under reduced pressure. The compound was purified by flash chromatography (DCM/n-Hex 1/1) and a yellow oil (0.1257 g; 53%) was obtained whose spectroscopic data is in accordance with those obtained previously and described in 3.1.

4.3 Reduction using borane dimethylsulfide

To a 50 mL round bottom flask equipped with a stir bar was added a solution of starting material (0.15M) and anhydrous THF. The solution under nitrogen atmosphere was cooled to 0°C and borane dimethylsulfide (6.2 eq.) was added dropwise. The reactional mixture was warmed to 23 °C, and the purity of the compound was followed through TLC (Eluent: DCM). After 3h, the reaction was cooled to 0 °C, and methanol (2 mL) and water (5 mL) were added to quench it. The reaction was concentrated under reduced pressure and the residue was dissolved in water (20 mL). The reaction mixture was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ (3 x 20 mL) and saturated aqueous NaCl (20 mL). This layer was dried with sodium sulfate and concentrated under reduced pressure.[51]

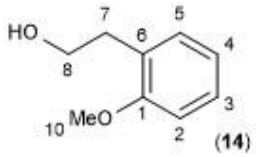


With R being H or Br

4.3.1 2-(2-methoxyphenyl)ethan-1-ol (14)

Following the general procedure, and stating with 2-(2-methoxyphenyl) acetic acid (**15**) (0.1573g; 946.59 µmol) in THF (1.1mL) was added borane dimethylsulfide (0.58 mL; 5.87 mmol, 6.2 eq.). 2-(2-methoxyphenyl)ethan-1-ol (**14**) was obtained as a white oil in 120% (0.1715 g).[51]

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2957 (OH band), 1494 (C=C aromatic), 1243 (C-O alcohols); **^{1}H-RMN (400 MHz, $CDCl_3$):** δ 7.22 (t, J = 7.9 Hz, 1H, H5-Ar), 7.17 (d, J = 7.4 Hz, 1H, H4-Ar), 6.92 (d, J = 7.4 Hz, 1H, H2-Ar), 6.88 (d, 1H, H3-Ar), 3.83 (t, 5H, CH_2-8 and CH_3-10), 2.91 (t, J = 6.4 Hz, 2H, CH_2-7); **^{13}C-RMN (400 MHz, $CDCl_3$):** 157.76 (C1), 131.04 (C5), 127.97 (C6), 127.16 (C3), 120.81 (C4), 110.57 (C2), 63.00 (C8), 55.45 (C10), 34.22 (C7).

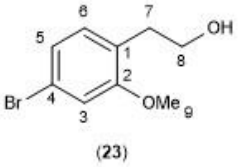


(14)

4.3.2 2-(4-bromo-2-methoxyphenyl)ethan-1-ol (23)

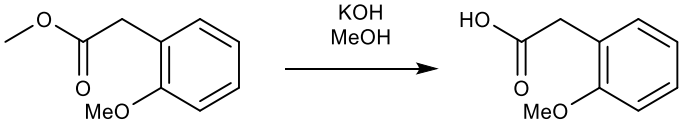
Following the general procedure, and stating with 2-(2-methoxyphenyl) acetic acid (**6**) (0.5 g; 2.04 mmol) in THF (5 mL) was added borane dimethylsulfide (1.01 mL; 10.10 mmol; 6.2 eq). 2-(4-bromo-2-methoxyphenyl)ethan-1-ol (**23**) was obtained as a white oil in 98% (0.4598 g).[51]

IR (*ATR*) $V_{máx}$ (cm^{-1}): 3217 (O-H), 2940 (CH sp^3), 1592 (C=C aromatic), 1239 (C-O ether), 662 (C-Br); **^{1}H-RMN (400 MHz, $CDCl_3$):** δ 7.01 (d, J = 18.5 Hz, 3H, H3-Ar, H5-Ar and H6-Ar), 3.81 (d, J = 7.5 Hz, 5H, CH_2-8 and CH3-9), 2.85 (t, J = 6.4 Hz, 2H, CH_2-7); **^{13}C-RMN (400 MHz, $CDCl_3$):** 158.38 (C2), 132.06 (C1), 126.20 (C6), 123.68 (C5), 120.88 (C4), 114.17 (C3), 62.52 (C8), 55.74 (C9), 33.72 (C7).



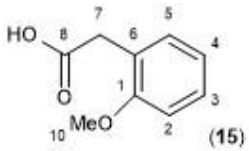
(23)

4.4 Hydrolysis using Methanol and KOH – 2-(2-methoxyphenyl) acetic acid (15)



(7) (15)

To a 50 mL round bottom flask equipped with a stir bar was added the methyl 2-(2-methoxyphenyl) acetate (**7**) (0.131g; 726,96µmol), methanol (5 mL) and KOH (0.081g, 1,45mmol, 2 eq.). The solution was kept under a nitrogen atmosphere and the purity of the compound was followed through TLC (Eluent: DCM). The reactional mixture was quenched with 50mL of water and was extracted with ethyl acetate (2 × 20 mL). The aqueous layer was acidified with HCl 1M and extracted again with ethyl acetate (3 × 20 mL). This layer was dried with sodium sulfate and concentrated under reduced pressure. 2-(2-methoxyphenyl)acetic acid (**15**) was obtained as a crude dark orange oil (0.157 g).[52]



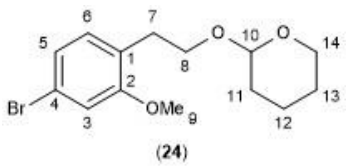
(15)

IR (*ATR*) $V_{máx}$ (cm^{-1}) : 3282 (O-H band), 2970 (C-H), 1710 (O-C=O), 1496 (C=C aromatic), 1248 (C-O); **^{1}H-RMN (400 MHz, $CDCl_3$):** δ 7.30 (d, *J* = 8.0 Hz, 1H, H5-Ar), 7.21 (d, *J* = 7.6 Hz, 1H, H4-Ar), 6.95 (d, *J* = 7.5 Hz, 1H, H2-Ar), 6.91 (t, 1H, H3-Ar), 3.86 (s, 3H, CH_3-10), 3.69 (s, 2H, CH_2-7); **^{13}C-RMN (400 MHz, $CDCl_3$):** δ 157.47 (C1), 131.17 (C5), 129.08 (C6), 122.46 (C3), 120.92 (C4), 110.74 (C2), 55.68 (C10), 35.88 (C7).

4.5 Protection reaction

4.5.1 2-(4-bromo-2-methoxyphenethoxy)tetrahydro-2H-pyran (24)

To a 50 mL round bottom flask equipped with a stir bar was added 2-(4-bromo-2-methoxyphenyl) ethan-1-ol (**23**) (0.3408 g; 1.47 mmol), dichloromethane (2 mL) and 3-4dihydro-2H-piran (0.14 ml; 1.47 mmol; 1 eq.). The solution under nitrogen atmosphere was cooled to 0°C and p-Toluenesulfonic acid (0.25 g; 147.48 µmol; 0.1 eq) was added dropwise. The reactional mixture was warmed to RT, and the purity of the compound was followed through TLC (Eluent: DCM/Hex 9/1). After 1h, the reactional mixture was washed with saturated aqueous $NaHCO_3$ and, the organic phase was extracted with dichloromethane (3 × 20 mL). The organic layers were combined and dried with sodium sulfate and concentrated under reduced pressure.[53] The crude was purified using flash chromatography (Eluent: DCM/Hex 9/1) and 2-(4-Bromo-2-methoxyphenethoxy)tetrahydro-2H-pyran (**24**) was obtained as a yellow oil in 72% (0.3364 g).



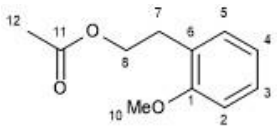
(24)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2939 (CH sp^3), 1590 (C=C aromatic), 1027 (C-O ether), 630 (C-Br); **^{1}H-RMN (400 MHz, $CDCl_3$):** δ 7.07 – 6.98 (m, 2H, H5-Ar and H6-Ar), 6.96 (s, 1H, H3-Ar), 4.58 (t, J = 3.5 Hz, 1H, H-10), 3. 83 – 3.73 (m, 5H,

CH_3-9 and CH_2-8), 3.61 – 3.42 (m, 2H, CH_2-14), 2.87 (t, J = 7.3 Hz, 2H, CH_2-7) , 1.61 – 1.45 (m, 6H, CH_2-7, CH_2-11, CH_2-12 and CH_2-13); **^{13}C-RMN (400 MHz, $CDCl_3$):** 158.27 (C2), 131.93 (C1), 126.46 (C6), 123.26 (C5), 120.40 (C4), 113.79 (C3), 98.64 (C10), 66.51 (C8), 62.25 (C14), 55.59 (C9), 30.71 (C7), 30.37 (C11), 25.51 (C13), 19.55 (C12).

4.5.2 2-methoxyphenethyl acetate (19)

To a 50 mL round bottom flask equipped with a stir bar was added 2-(2-methoxyphenyl) ethan-1-ol (**13**) (0.2283 g; 1.5 mmol), pyridine (1 mL; 12.41 mmol; 8.28 eq.) and acetic anhydride (1 mL; 10.58 mmol; 7.05 eq.). The solution under nitrogen atmosphere was left overnight. The purity of the compound was monitored by TLC (Eluent: DCM). The reaction mixture was carefully added to a beaker with water and extracted with ethyl ether. The organic phase was dried using anhydrous sodium sulfate, filtered and concentrated under reduced pressure. 2-methoxyphenethyl acetate (**19**) was obtained as a pale yellow oil in 76% (0.2219 g).



(19)

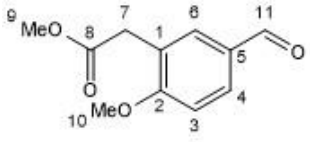
IR (*ATR*) $V_{máx}$ (cm^{-1}): 2957 (C-H), 1735 (C=O), 1494 (C=C aromatic), 1237 (C-O), 1033 (C-O ether), 753 (C-H alkene); **^{1}H-RMN (400 MHz, $CDCl_3$):** δ 7.22 (t, *J* = 8.4, 6.8 Hz, 1H, H5-Ar), 7.14 (d, *J* = 7.4 Hz, 51H, H4-Ar), 6.90 (d, *J* = 7.6 Hz, 1H, H2-Ar), 6.85 (d, *J* = 8.6 Hz, 1H, H3-Ar), 4.26 (t, *J* = 7.1 Hz, 2H, CH_2- 8), 3.82 (s, 3H, CH_3-10), 2.95 (t, *J* = 7.1 Hz, 2H, CH_2-7), 2.02 (s, 3H, CH_3-12); **^{13}C-RMN (400 MHz, $CDCl_3$):** 171.30 (C11), 157.79 (C1), 130.80 (C5), 128.06 (C6), 126.16 (C3), 120.53 (C4). 110.40 C2), 64.03 (C8), 55.37 (C10), 30.02 (C7), 21.16 (C12). **MS (ESI) *m/z*** calculated for $C_{11}H_{14}O_3Na$ $[MNa]^+$: 217.07; Found: 217.1.

4.6 Rieche formylation using dichloromethyl methyl ether and Tin (IV) chloride – general procedure

To a 50 mL round bottom flask equipped with a stir bar to a 0.3M solution of starting material in anhydrous dichloromethane under a nitrogen atmosphere was added dichloromethyl methyl ether (2 eq.) and Tin (IV) chloride (1.43 eq.). The reactional mixture was cooled to 0 °C, and the progress of the reaction was monitored by TLC (Eluent: DCM). After 2h, the reaction mixture was carefully added to a beaker with water and ice and extracted with DCM. The organic phase was extracted with brine (20 mL), dried using anhydrous sodium sulfate, filtered and concentrated under reduced pressure.

4.6.1 Methyl 2-(5-formyl-2-methoxyphenyl)acetate (8)

Following the general procedure, and starting with methyl 2-(2-methoxyphenyl) acetate (**7**) (0.6 g; 3.33 mmol) in anhydrous dichloromethane (9 mL) was added dichloromethyl methyl ether (0.6 mL; 6.67 mmol; 2 eq.) and Tin (IV) chloride (0.57 mL; 4.77 mmol; 1.43 eq.). Methyl 2-(5-formyl-2-methoxyphenyl)acetate (**8**) was obtained as a yellow oil in 92% (0.6365 g).



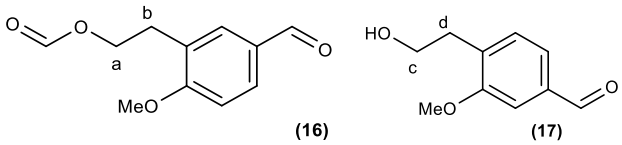
(8)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2952 (C-H sp^3), 1735 (O-C=O), 1683 (H-C=O), 1500 (C=C aromatic), 1020 (C-O ether); **^{1}H NMR (400 MHz, $CDCl_3$)**: δ 9.88 (s, J = 2.6 Hz, 1H, H11), 7.82 (d, J = 8.4 Hz, 1H, H3-Ar), 7.75 (s, 1H, H6-Ar), 6.99 (d, J = 8.1 Hz, 1H, H4-Ar), 3.94 – 3.88 (s, 3H, CH_3-10), 3.70 (s, 5H, CH_2-7 e CH_3-9); **^{13}C-**

RMN (400 MHz, $CDCl_3$): 190,91 (C11), 171.66 (C8), 162.88 (C2), 132.39 (C6), 132.02 (C5), 129.80 (C4), 124.16 (C1), 110,55 (C3), 56,07 (C10), 52,20 (C9), 35,71 (C7).

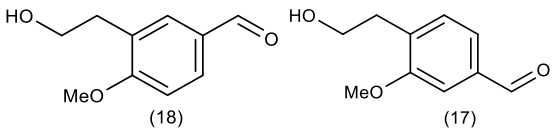
4.6.2 Mixture of 5-formyl-2-methoxyphenethyl formate (16) and 4-(2-hydroxyethyl)-3-methoxybenzaldehyde (17), and the mixture of 3-(2-hydroxyethyl)-4-methoxybenzaldehyde (18) and 4-(2-hydroxyethyl)-3-methoxybenzaldehyde (17)

Following the general procedure, and starting with 2-(2-methoxyphenyl) ethan-1-ol (0.1715 g; 1.13mmol) in anhydrous dichloromethane (4mL) was added dichloromethyl methyl ether (0.408mL; 4.51mmol; 4 eq.) and Tin (IV) chloride (0.394 mL; 3.38 mmol; 3 eq.). The solution was kept in an ice bath at an inert atmosphere for 2h, the organic phase was concentrated under reduced pressure. A purple oil that changed colour to red (0.085g; 42%) when in contact with the air was obtained. Through the NMR spectra was not possible to identify the compound but was possible to ideate a hypothesis. To the synthesised compound was added THF (5mL) and a solution of sodium bicarbonate aqueous concentrate. The reactional mixture was purged with argon, and the purity of the compound was monitored by TLC (Eluent: DCM). After 24h, the reactional mixture was neutralized using HCl 1M and extracted with ethyl acetate. The organic phase was dried using anhydrous sodium sulfate, filtered and concentrated under reduced pressure. An orange oil (0.060g) was obtained but was not possible to identify by NMR. When preparing the NMR tube in chloroform the tube changed colour from light orange to light pink.



(16) **(17)**

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2930 (C-H sp^3), 1720 (C=O), 1683 (H-C=O), 1498 (C=C aromatic), 1255 (C-O). **^{1}H NMR (400 MHz, $CDCl_3$)**: δ 9.88 (s, 1H, H12), 9.87 (s, 1H, H11), 8.02 (s, 1H, H11), 7.79 (dd, *J* = 8.4, 4.2, 2.1 Hz, 2H, H-Ar), 7.72 (s, 2H, H-Ar), 6.98 (d, *J* = 8.4 Hz, 2H, H-Ar), 4.39 (t, *J* = 6.8 Hz, 2H, **a**), 3.93 (s, 6H, H-Me), 3.73 (t, *J* = 7.3 Hz, 2H, c) 3.13 (t, *J* = 7.3 Hz, 2H, b), 3.04 (t, *J* = 6.8 Hz, 2H, d).

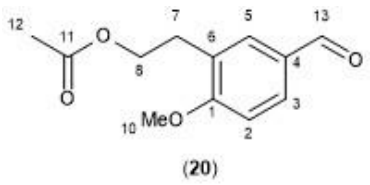


(18) (17)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 3454 (OH band), 2929 (C-H sp^3), 1723 (C=O), 1683 (H-C=O), 1498 (C=C aromatic), 1255 (C-O). **^{1}H NMR (400 MHz, $CDCl_3$)**: δ 9.88 (d, *J* = 1.5 Hz, 2H), 7.78 (dd, *J* = 8.5, 2.2 Hz, 2H), 7.73 (dd, *J* = 4.1, 2.2 Hz, 2H), 6.98 (s, 1H), 4.12 (q, *J* = 7.1 Hz, 4H), 3.93 (s, 6H), 3.87 (t, *J* = 6.5 Hz, 2H), 3.73 (t, *J* = 7.2 Hz, 2H), 3.13 (t, *J* = 7.2 Hz, 2H), 2.96 (t, *J* = 6.5 Hz, 2H).

4.6.3 5-formyl-2-methoxyphenethyl acetate (20)

Following the general procedure, and starting with 2-methoxyphenethyl acetate (**19**) (0.1662 g; 855.69 µmol) in anhydrous dichloromethane (4 mL) was added dichloromethyl methyl ether (0.31 mL; 3.42 mmol; 4 eq.) and Tin (IV) chloride (0.29 mL; 2.57 mmol; 3 eq.). 5-formyl-2-methoxyphenethyl acetate (**20**) was obtained as a dark yellow oil in 60% (0.1145 g).

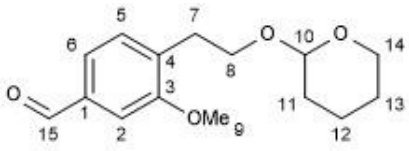


(20)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2955 (C-H sp^3), 1735 (C=O), 1500 (C=C aromatic), 1257 (C-O), 1024 (C-O ether); **^{1}H-RMN (400 MHz, $CDCl_3$):** δ 9.87 (s, 1H, CH-13), 7.77 (dd, *J* = 8.4, 2.1 Hz, 1H, H2-Ar), 7.70 (s, 1H, H5-Ar), 6.97 (dd, *J* = 8.4 Hz, 1H, H3-Ar), 4.28 (t, *J* = 6.9 Hz, 2H, CH_2-8), 3.92 (s, 3H, CH_3-10), 3.00 (t, *J* = 6.9 Hz, 2H, CH_2-7), 2.02 (s, 3H, CH_3-12); **^{13}C-RMN (400 MHz, $CDCl_3$):** 191.07 (C13), 171.17 (C11), 162.81 (C1), 131.79 (C4), 131.65 (C5), 129.70 (C3), 127.28 (C6), 110.33 (C2), 63.31 (C8), 55.87 (C10), 29.88 (C7), 21.05 (C12).

4.7 Formylation using BuLi and DMF - 3-methoxy-4-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethyl)benzaldehyde (25)

To a 50 mL round bottom flask equipped with a stir bar was added 2-(4-bromo-2-methoxyphenethoxy)tetrahydro-2H-pyran (**24**) (0.118g; 377.21µmol) and THF (2 mL). The solution under nitrogen atmosphere was cooled to -78°C and BuLi (0.29 mL; 414.93µmol; 1.1 eq.) was added. After 30 minutes, DMF (0.032 mL; 414.93µmol; 1.1 eq.) was added. The reactional mixture was warmed to RT, and the purity of the compound was followed through TLC (Eluent: DCM/MeOH 0.5%). The reaction was concentrated under reduced pressure to evaporate the THF and water (5mL) was added. The reaction mixture was extracted with dichloromethane (4 × 20 mL). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ (3 x 20 mL) and saturated aqueous NaCl (20 mL). This layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. 3-methoxy-4-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethyl)benzaldehyde (**25**) was obtained in 88% yield.

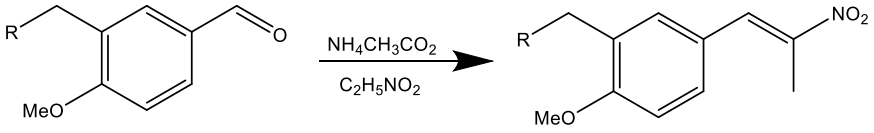


(25)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2940 (C-H sp^3), 1687 (H-C=O), 1028 (C-O ether); **^{1}H-RMN (400 MHz, $CDCl_3$):** δ 9.95 (s, 1H, H-15), 7.42 – 7.35 (m, 3H, H-Ar), 4.61 (td, J = 4.8, 4.3, 2.9 Hz, 1H, H-10), 3.99 – 3.79 (m, 7H, CH_3-9, CH_2-8 and CH_2-14), 3.01 (t, J = 7.0 Hz, 2H, CH_2-7), 1.63 – 1.46 (m, 6H, CH_2-11, CH_2-12 and CH_2-13); **^{13}C-RMN (400 MHz, $CDCl_3$):** 192.17 (C15), 158.31 (C3), 136.31 (C1), 135.55 (C4), 131.17 (C5), 124.47 (C6), 108.40 (C2), 98.78 (C10), 66.28 (C8), 62.31 (C14), 55.63 (C9), 34.42 (C7), 31.23 (C11), 25.56 (C13), 19.60 (C12). **HRMS (ESI) *m/z*** calculated for $C_{15}H_{20}O_4Na$ $[MNa]^+$: 287.1253; Found: 287.1257.

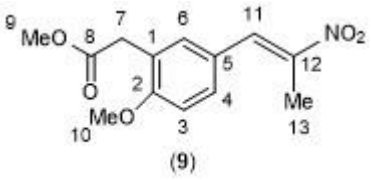
4.8 Aldol type reaction - general procedure

To a 50 mL round bottom flask equipped with a stir bar was added the starting material, nitroethane (37 eq.) and ammonium acetate (2.1 eq.). The solution was refluxed under a nitrogen atmosphere. The progress of the reaction was followed by TLC (Eluent: DCM). After 2h, the reaction mixture was concentrated under reduced pressure to evaporate the nitroethane. The flask was washed with ethyl acetate and added to a decanting ampoule with water and extracted with more ethyl acetate (3x20 mL). The organic phase was brine, dried using anhydrous sodium sulfate, filtered and concentrated under reduced pressure.



4.8.1 Methyl (*E*)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl)phenyl)acetate (9)

Following the general procedure, and starting with methyl 2-(5-formyl-2-methoxyphenyl)acetate (**8**) (0.6365g; 3.06mmol) nitroethane (8.13 mL; 113.11mmol; 37 eq.) and ammonium acetate (0.4948g; 6.42mmol; 2.1 eq.) were added. Methyl (*E*)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl)phenyl)acetate (**9**) was obtained as a brown oil in 71% (0.5755g).

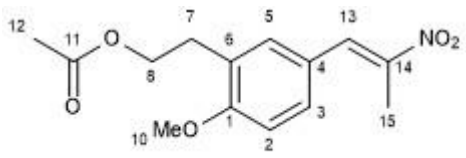


(9)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2923 (C-H sp^3), 1505 (C=C aromatic), 1261 (C-N), 1066 (C-O ether); **^{1}H-RMN (400 MHz, $CDCl_3$):** δ 8.02 (s, 1H, H-11), 7.38 (dd, J = 8.5, 2.3 Hz, 1H, H4-Ar) , 7.30 (d, J = 2.3 Hz, 1H, H3-Ar), 6.92 (d, J = 8.6 Hz, 1H, H6-Ar), 3.85 (s, 3H, CH_3-10), 3.68 (s, 3H, CH_3-9), 3.63 (s, 2H, CH_2-7), 2.44 (s, 3H, CH_3-13); **^{13}C-RMN (400 MHz, $CDCl_3$):** δ 171.82 (C8), 159.13 (C2), 145.98 (C11 e C12), 133.60 (C6), 133.23 (C5), 131.44 (C4), 123.94 (C1) 110.90 (C3), 55.86 (C10), 52.18 (C9), 35.58 (C7), 14.24 (C13). **MS (ESI) *m/z*** calculated for $C_{13}H_{15}NO_5Na$ $[MNa]^+$: 288.08; Found: 288.1.

4.8.2 (*E*)-2-methoxy-5-(2-nitroprop-1-en-1-yl)phenethyl acetate (21)

Following the general procedure, and starting with 5-formyl-2-methoxyphenethyl acetate (**20**) (0.1145 g; 515.21 µmol) nitroethane (1.38 mL; 19.06 mmol; 37 eq.) and ammonium acetate (0.834 g; 1.08 mmol; 2.1 eq.) were added. (*E*)-2-methoxy-5-(2-nitroprop-1-en-1-yl)phenethyl acetate (**21**) was obtained as a crude brown oil (0.1658g).

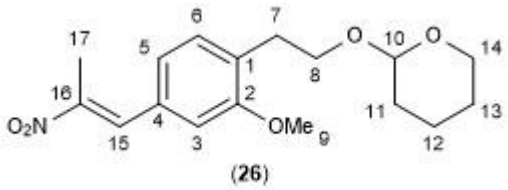


(21)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2964 (C-H), 1736 (C=O), 1503 (C=C aromatic), 1313 (N-O), 1258 (C-O), 1030 (C-O ester); **^{1}H-RMN (400 MHz, $CDCl_3$):** δ 8.06 (s, 1H, CH-12), 7.36 (d, *J* = 8.8 Hz, 1H, H2-Ar), 7.27 (s, 1H, H5-Ar), 6.93 (d, *J* = 8.6 Hz, 1H, H3-Ar), 4.27 (t, *J* = 6.8 Hz, 2H, CH_2-8), 3.89 (s, 3H, CH_3-9), 2.97 (t, *J* = 6.9 Hz, 2H, CH_2-7), 2.48 (s, 3H, CH_3-14), 2.02 (s, 3H, CH_3-11); **^{13}C-RMN (400 MHz, $CDCl_3$):** 171.20 (C11) , 158.48 (C1), 145.85 (C14), 133.82 (C13), 133.03 (C5), 131.05 (C3), 128.64 (C4), 125.91 (C6), 110.41 (C2), 63.61 (C8), 55.54 (C10), 29.84 (C7), 21.13 (C12), 14.29 (C15). **HRMS (ESI) *m/z*** calculated for $C_{14}H_{17}NO_5Na$ $[MNa]^+$: 302.0998; Found: 302.0995.

4.8.3 (*E*)-2-(2-methoxy-4-(2-nitroprop-1-en-1-yl)phenethoxy)tetrahydro-2H-pyran (26)

Following the general procedure, and starting with 3-methoxy-4-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethyl)benzaldehyde (**25**) (0.086 g; 325.36 µmol) was added nitroethane (0.9 mL; 12.04 mmol; 37 eq.) and ammonium acetate (0.049 g; 650.72 µmol; 2.1 eq.). (E)-2-(2-methoxy-4-(2-nitroprop-1-en-1-yl)phenethoxy)tetrahydro-2H-pyran (**26**) was obtained as a brown oil in 93% (0.0968g).



(26)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2939 (C-H sp^3), 1517(N-O), 1463 (C=C aromatic), 1255 (C-N), 1027 (C-O ether); **^{1}H-RMN (400 MHz, $CDCl_3$):** δ 8.04 (s, 1H, H-15), 7.24 (d, J = 3.1 Hz, 1H, H6-Ar), 6.96 (s, 1H, H5-Ar), 6.84 (d, J = 1.5 Hz, 1H, H3-Ar), 4.58 (t, J =

4.4, 2.9 Hz, 1H, H-10), 3.84 – 3.78 (m, 7H, CH_3-9, CH_2-8 and CH_2-14), 2.96 – 2.90 (m, 2H, CH_2-7), 2.44 (s, 3H, CH_3-17), 1.55 – 1.43 (m, 6H, CH_2-11, CH_2-12 and CH_2-13); **^{13}C-RMN (400 MHz, $CDCl_3$):** 157.90 (C2), 147.34 (C16), 134.02 (C4), 133.77 (C15), 131.18 (C1), 130.25 (C6), 122.29 (C5), 111.84 (C3), 98.81 (C10), 66.55 (C8), 62.36 (C14), 55.53 (C9), 34.05 (C7), 30.81 (C11), 25.58 (C13), 19.66 (C12), 14.30 (C17). **HRMS (ESI) *m/z*** calculated for $C_{17}H_{23}NO_5Na$ $[MNa]^+$: 344.1468; Found: 344.1461.

4.9 Catalytic hydrogenation

4.9.1 Reduction of methyl (*E*)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl)phenyl)acetate (9) in anhydrous ethanol

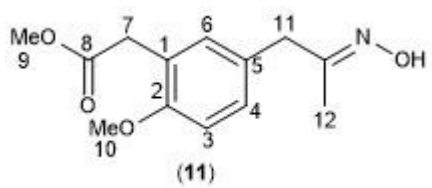
To a borosilicate glass vessel was added the methyl (*E*)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl)phenyl)acetate (**8**) (0.5755g, 2.17mmol) dissolved in anhydrous ethanol (15 mL) and palladium on charcoal (0.05g). The reactional mixture was purged with hydrogen in the hydrogenator for 24 hours at 3 atm. The progress of the reaction was followed by TLC (eluent: DCM/ MeOH 5%). The reactional mixture was filtered and concentrated under reduced pressure. A green oil was obtained but could not be identified by ^{1}H NMR spectrum.

4.9.2 Reduction of methyl (*E*)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl)phenyl)acetate (9) with *in situ* generated HCl

To a Borosilicate Glass vessel was added the methyl (*E*)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl)phenyl)acetate (**8**) (0.100 g; 376.98 µmol) dissolved in anhydrous ethanol (5 mL) and palladium on charcoal (0.011g). acetyl chloride (0.5 mL; 753.96 µmol; 2 eq.) was dissolved in anhydrous ethanol (15 mL) to form HCl *in situ* and added to the vessel. The reactional mixture was purged with hydrogen in the hydrogenator for 24 hours at 3 atm. The progress of the reaction was followed by TLC (eluent: DCM: 5% MeOH). The reactional mixture was filtered and concentrated under reduced pressure. A green oil was obtained but also it was not possible to identify any major compound on the reaction mixture.

4.9.3 Reduction of methyl (*E*)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl)phenyl)acetate (9) with anhydrous ethanol and TFA

To a Borosilicate Glass vessel was added the solution of methyl (E)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl)phenyl)acetate (0.100 g; 376.98 µmol) in anhydrous ethanol (15 mL) was added palladium on charcoal (0.011 g) and TFA (0,12 mL). The reactional mixture was purged with hydrogen in the hydrogenator for 24 hours at 3 atm. The progress of the reaction was followed by TLC (eluent: DCM: 5% MeOH). The mixture was purified using a silica flash column eluted with DCM/MeOH 1% and 5 fractions were obtained, 3 of them were pure and were analysed using FTIR-*ATR* and ^{1}H and ^{13}C-NMR. It was possible to identify three of them, being one the methyl (E)-2-(5-(2-(hydroxyimino)propyl)-2-methoxyphenyl)acetate (**11**), the other one, the methyl 2-(2-methoxy-5-(2-oxopropyl)phenyl)acetate (**12**) and finally methyl 2-(2-methoxy-5-(2-nitropropyl)phenyl)acetate (**13**).

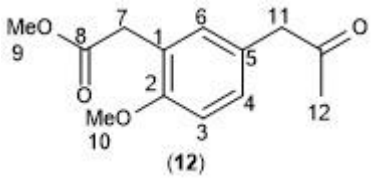


(**11**)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2953 (C-H sp^3), 1505 (C=C aromatic), 1342 (N-O); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.08 (d, *J* = 8.4, 1.7 Hz, 1H, H4-Ar),

7.01 (s, 1H, H6-Ar), 6.83 (d, *J* = 8.4 Hz, 1H, H3-Ar), 3.80 (s, 3H, CH_3-10), 3.68 (s, 3H, CH_3-9), 3.61 (d, *J* = 3.0 Hz, 4H, CH_2-7 and CH_2-11), 2.14 (s, 3H, CH_3-12); **^{13}C-RMN (400 MHz, $CDCl_3$):** 170.67 (C8), 162.11 (C11), 156.83 (C2), 133.30 (C5), 131.92 (C6), 129.56 (C1), 121.52 (C4), 110.16 (C3), 55.96 (C9), 50.21 (C10), 35.78 (C7), 14.25 (C13).

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2953 (C-H sp^3), 1505 (C=C aromatic), 1342 (N-O); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.10 (d, *J* = 8.3, 4.3 Hz, 1H, H4-Ar), 7.03 (s, 1H, H6-Ar), 6.80 (d, *J* = 8.4, 4.7 Hz, 1H, H3-Ar), 3.79 (s, 3H, CH_3-10), 3.68 (s, 3H, CH_3-9), 3.61 (s, 2H, CH_2-7), 3.43 (s, 2H, CH_2-11), 1.81 (d, *J* = 4.6 Hz, 3H, CH_3-12). **^{13}C-RMN (400 MHz, $CDCl_3$):** δ 206.99 (C12), 172.32 (C8), 156.82 (C2), 132.10 (C5), 129.56 (C6), 126.25 (C4), 123.48 (C1), 110.94 (C3), 55.73 (C10), 52.07 (C9), 50.21 (C11), 35.78 (C7), 29.34 (C13).



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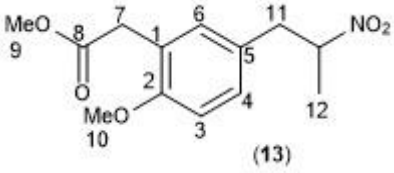


(13)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 2926 (C-H sp^3), 1505 (C=C aromatic), 1339 (N-O); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.05 (d, 1H, H4-Ar), 6.98 (s, 1H, H6-Ar), 6.80 (d, J = 8.3 Hz, 1H, H3-Ar), 3.80 (d, J = 10.5 Hz, 3H, CH3-10), 3.69 (s, 3H, CH3-9), 3.59 (d, J = 7.7 Hz, 2H, CH2-7), 2.36 – 2.20 (m, 2H, CH2-11), 1.28 (s, 3H, CH3-12). **^{13}C-RMN (400 MHz, $CDCl_3$):** δ 172.13 (C8), 156.86 (C2), 131.55 (C6), 129.05 (C5), 127.34 (C4), 123.39 (C1), 110.76 (C3), 84.62 (C12), 55.58 (C10), 51.97 (C9), 35.57 (C7), 18.76 (C13). **HRMS (ESI) *m/z*** calculated for $C_{13}H_{17}NO_5Na$ $[MNa]^+$:290.0998; Found: 290.0995.

4.9.4 Reduction of (*E*)-2-methoxy-5-(2-nitroprop-1-en-1-yl)phenethyl acetate (21) with anhydrous ethanol and TFA

To a Borosilicate Glass vessel was added the solution of (E)-2-methoxy-5-(2-nitroprop-1-en-1-yl)phenethyl acetate (0.1658 g; 593.64µmol) in anhydrous ethanol (10 mL) was added palladium on charcoal (0.016 g) and TFA (0,12 mL). The reactional mixture was purged with hydrogen in the hydrogenator for 24 hours at 3 atm. The progress of the reaction was followed by TLC (eluent: DCM: 5% MeOH). The reactional mixture was filtered and concentrated under reduced pressure. A green oil was obtained but also it was not possible to identify any major compound on the reaction mixture.

4.9.5 Reduction of (*E*)-2-methoxy-5-(2-nitroprop-1-en-1-yl)phenethyl acetate (26) with *in situ* generated HCl

To a borosilicate glass vessel was added the methyl (E)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl)phenyl)acetate (26) (0.0109 g; 33.92 µmol) dissolved in anhydrous ethanol (10 mL) and palladium on charcoal (0.002g). Acetyl chloride (0.1 mL; 1.19mmol; 2 eq.) was dissolved in anhydrous ethanol (15 mL) to form HCl in situ and added to the vessel. The reactional mixture was subject to a hydrogen atmosphere in the hydrogenator for 48 hours at 5 atm. The progress of the reaction was followed by TLC (eluent: DCM: 5% MeOH). The reactional mixture was filtered and concentrated under reduced pressure. A green oil was obtained but could not be identified.

4.10 Reduction using lithium aluminium hydride

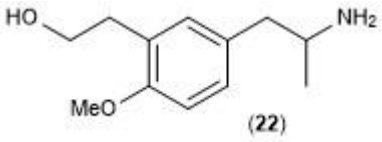
4.10.1 2-(2-methoxyphenyl)ethan-1-ol (14)

To a 50 mL flask equipped with a stir bar, a condenser and an isobaric dropping funnel were added $LiAlH_4$ (0.141 g, 3.73mmol, 3 eq.) and anhydrous THF/ Dioxane (2 mL) in a nitrogen atmosphere. In the funnel, was mixed methyl 2-(2-methoxyphenyl)acetate (**7**) (0.225 g, 1.24mmol) and THF/ Dioxane (2 mL) and added dropwise to the flask. The reactional mixture was refluxed in an oil bath for 3h under nitrogen atmosphere, and the purity of the compound was followed through TLC (Eluent: DCM). The excess of $LiAlH_4$ was eliminated using water and a solution of NaOH 15%. The reaction was concentrated under reduced pressure and the compound was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ (3 x 20 mL) and saturated aqueous NaCl (20 mL). This layer was dried with sodium sulfate and concentrated under reduced pressure. A clear oil was obtained but was not possible to identify by NMR.

4.10.2 2-(5-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (22)

To a 50 mL flask equipped with a stir bar, a condenser and an isobaric dropping funnel was added LiAlH4 (0.028g, 753,96µmol, 4 eq.) and anhydrous THF (2 mL) in a nitrogen atmosphere. In the funnel, was prepared a solution of methyl (E)-2-(2-methoxy-5-(2-nitroprop-1-en-1-yl)phenyl)acetate (**9**) (0.050g, 188,49µmol) in anhydrous THF (2 mL) and added dropwise to the flask. The reactional mixture was refluxed overnight in an oil bath under a nitrogen atmosphere, and the purity of the compound was followed through TLC (Eluent: DCM). The excess of LiAlH4 was eliminated using water and a solution of NaOH 15%. The reaction was concentrated under reduced pressure and the compound was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined and washed with saturated aqueous NaHCO3 (3 x 20 mL) and saturated aqueous NaCl (20 mL). This layer was dried with sodium sulfate and concentrated under reduced pressure. 2-(5-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (10) was obtained as a clear oil in 50% (0,019g).

IR (*ATR*) $V_{máx}$ (cm^{-1}): 3297 (OH band), 2925 (C-H sp^3), 1501 (C=C aromatic), 1249 (C-O), 1029 (C-O ether); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.01 (d, *J* = 11.2 Hz, 2H, H2-Ar and H3-Ar), 6.79 (d, *J* = 10.1, 5.7 Hz, 1H, H5-Ar), 3.80 (s, 7H, CH_2-8, CH_3-10 and CH_2-7), 2.64 (dd, *J* = 13.6, 6.6 Hz, 2H, CH_2-11), 2.44 (dd, *J* = 13.5, 8.2 Hz, 1H, H12), 1.10 (d, *J* = 9.7 Hz, 3H, CH_3-13); **^{13}C-RMN (400 MHz, $CDCl_3$):** δ 156.35 (C1), 132.08 (C4), 128.38 (C5), 127.23 (C3), 126.86 (C6), 110.54 (C2), 62.71 (C8), 55.51 (C10), 48.78 (C11), 34.17 (C7), 22.76 (C13).



(22)

4.10.3 2-(4-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (27)

To a 50 mL flask equipped with a stir bar, a condenser and an isobaric dropping funnel was added LiAlH4 (0.03833 g, 10.10mmol, 19.37 eq.) and anhydrous THF (2 mL) in a nitrogen atmosphere. In the funnel, was prepared a solution of (E)-2-(2-methoxy-4-(2-nitroprop-1-en-1-yl)phenethoxy)tetrahydro-2H-pyran (**26**) (0.1676 g, 521.51µmol) in anhydrous THF and added dropwise to the flask. The reactional mixture was refluxed in an oil bath for 3h under nitrogen atmosphere, and the purity of the compound was followed through TLC (Eluent: DCM). The excess of LiAlH4 was eliminated using

water and a solution of NaOH 15%. The reaction was concentrated under reduced pressure and the compound was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ (3 x 20 mL) and saturated aqueous NaCl (20 mL). This layer was dried with sodium sulfate and concentrated under reduced pressure. A clear oil was obtained but was not possible to identify by NMR.

4.11 Reductive amination - methyl 2-(5-(2-aminopropyl)-2-methoxyphenyl)acetate (10)

(**12**) (**10**)

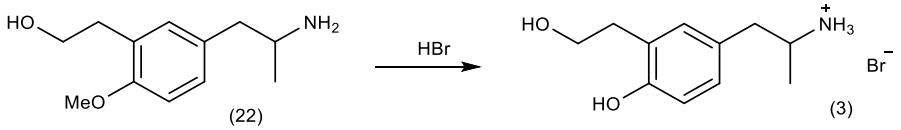
4.11.1 Using ammonium formate and palladium on activated charcoal

Ammonium formate (0.088 g, 1.4 mmol) was dissolved in a mixture of water (0.1 mL) and MeOH (0.5 mL). Methyl 2-(2-methoxy-5-(2-oxopropyl)phenyl)acetate (**12**) (0.030 g, 128.04 µmol) and palladium (0.008 g) on activated charcoal (10 w/w%) were added. The resulting mixture was stirred overnight at room temperature under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite to remove the palladium catalyst. The Celite was washed with MeOH, the filtrate was evaporated and concentrated HCl (14 mL) was added to the residue. The organic layers were combined, brined (20 mL), dried with sodium sulfate and concentrated under reduced pressure.[54] A yellow oil was obtained but was not possible to identify by NMR.

4.11.2 Using ammonium acetate and sodium cyanoborohydride

To a solution of ammonium acetate (0,098 g, 1.26mmol, 18.58 eq.) in methanol (1 mL) was added methyl 2-(2-methoxy-5-(2-oxopropyl)phenyl)acetate (**12**) (0.016 g, 67.72µmol) and then sodium cyanoborohydride (0.015 g, 238.69µmol, 3.52 eq.). The mixture was stirred for 36 h at room temperature after which water (5 mL) containing concentrated HCl (10 mL) was added. The unreacted starting material was extracted with DCM (3x20 mL). The aqueous layer was basified to pH 12 with dilute NaOH solution (1 M) and extracted with DCM, (3x25 mL). The organic layers were combined, brined (20 mL), dried with sodium sulfate and concentrated under reduced pressure.[55] A yellow oil was obtained but was not possible to identify by NMR.

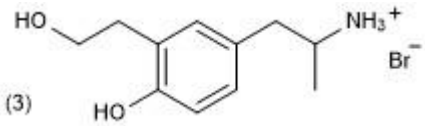
4.12 Strong acid media reaction – Metabolite 3



(22) (3)

To a 25 mL round bottom flask equipped with a stir bar was added 2-(5-(2-aminopropyl)-2-methoxyphenyl)ethan-1-ol (**22**) (0.0278 g, 132.83µmol) and hydrobromic acid (1 mL, 18.42mmol, 139 eq.). The solution was refluxed in an oil bath for 2 hours under a nitrogen atmosphere. The reactional

mixture was concentrated under reduced pressure and was purified using an RP18 column. The fractions were analysed using UV absorption (190- 300 nm). Compound 3 was obtained as a dark orange oil in 7% (0.002g).



(3)

IR (*ATR*) $V_{máx}$ (cm^{-1}): 3410 (OH band), 2926 (C-H sp^3),1259 (C-O); **^{1}H NMR (400 MHz, Deuterium oxide):** δ 7.11 (s, 1H, H2-Ar), 7.07 (s, 1H, H3-Ar), 6.90 (d, *J* = 8.2, 1.3 Hz, 1H, H5-Ar), 3.83 (dd, *J* = 10.0, 6.9, 2.4 Hz, 2H, CH_2-8), 3.58 (q, *J* = 6.7 Hz, 1H, CH-12), 2.85 (t, 4H, CH_2-7 and CH_2-11), 1.30 (d, *J* = 6.6, 1.3 Hz, 3H, CH_3-13); **MS (ESI) *m/z*** calculated for $C_{11}H_{18}BrNO_2$ $[MH]^+$: 275.05; Found: 274.1. **MS (ESI) *m/z*** calculated for $C_{11}H_{18}NO_2$ $[MH]^+$: 196.13; Found: 196.1.

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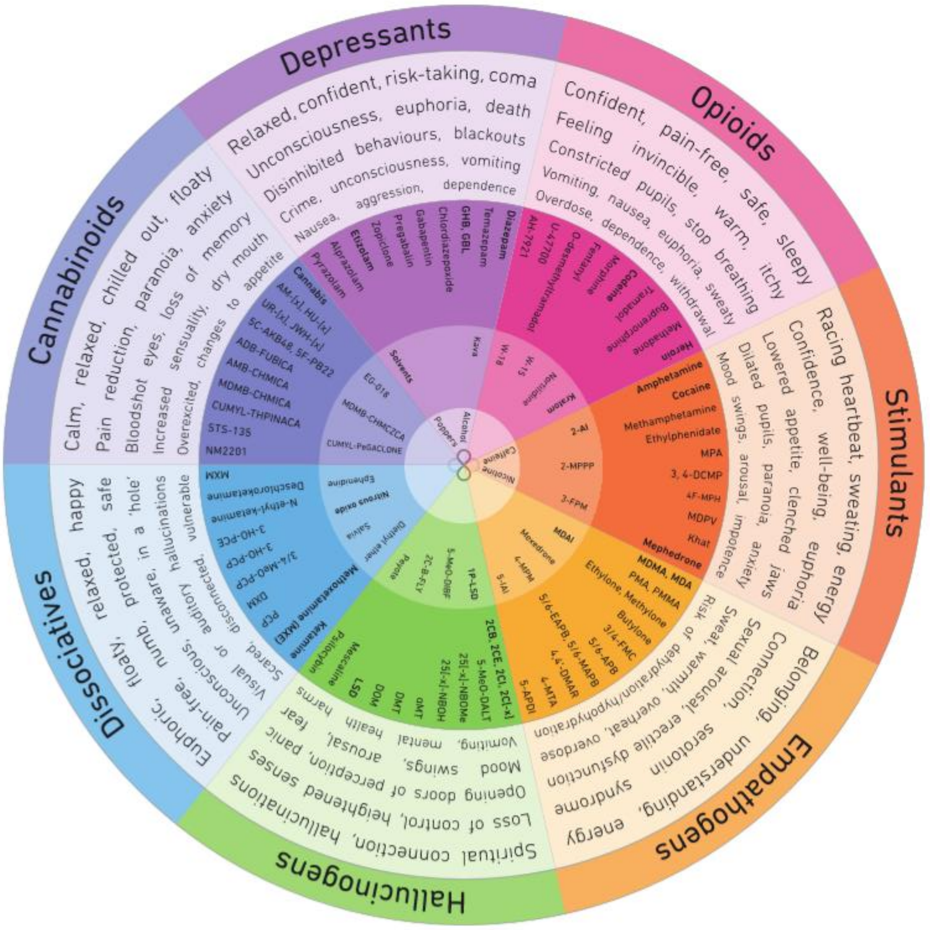
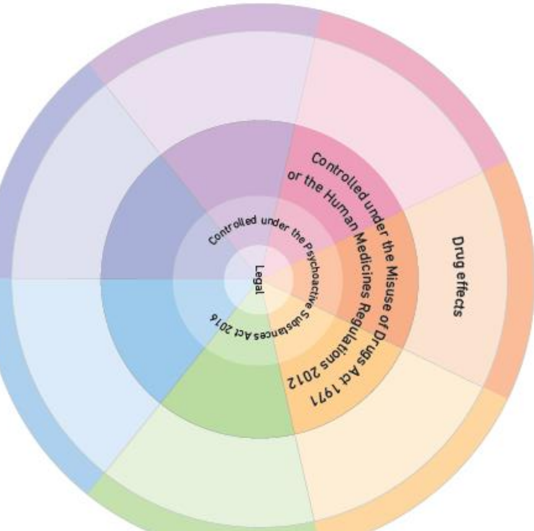
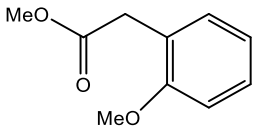
Annexe 1

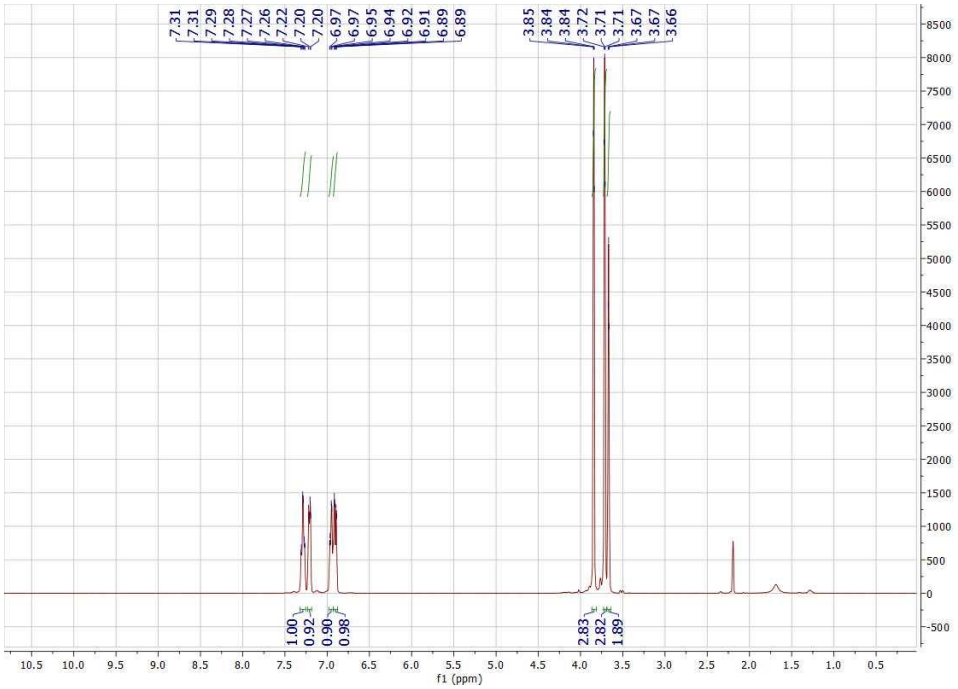

Depressants
Relaxed, confident, risk-taking, coma
Unconsciousness, euphoria, death
Disinhibited behaviours, blackouts
Crime, unconsciousness, vomiting
Nausea, aggression, dependence
Opioids
Confident, pain-free, safe, sleepy
Feeling invincible, warm, itchy
Constricted pupils, stop breathing
Vomiting, nausea, euphoria, sweaty
Overdose, dependence, withdrawal
Stimulants
Racing heartbeat, sweating, energy
Confidence, well-being, euphoria
Lowered appetite, clenched jaws
Dilated pupils, paranoia, anxiety
Mood swings, arousal, impotence
Empathogens
Belonging, understanding, energy
Connection, serotonin syndrome
Sexual arousal, erectile dysfunction
Sweat, warmth, overheat, overdose
Risk of dehydration/hypohydration
Hallucinogens
Spiritual connection, hallucinations
Loss of control, heightened senses
Opening doors of perception, panic
Mood swings, arousal, fear
Vomiting, mental health harms
Dissociatives
Euphoric, floaty, relaxed, happy
Pain-free, numb, protected, safe
Unconscious, unaware, in a 'hole'
Visual or auditory hallucinations
Scared, disconnected, vulnerable
Cannabinoids
Calm, relaxed, chilled out, floaty
Pain reduction, paranoia, anxiety
Bloodshot eyes, loss of memory
Increased sensuality, dry mouth
Overexcited, changes to appetite
Diazepam
Temazepam
GHB, GBL
Chlordiazepoxide
Gabapentin
Pregabalin
Zopiclone
Etizolam
Alprazolam
Pyrazolam
Heroin
Methadone
Buprenorphine
Tramadol
Codeine
Morphine
Fentanyl
O-desmethyltramadol
U-47700
AH-7921
Amphetamine
Cocaine
Methamphetamine
Ethylphenidate
MPA
3, 4-DCMP
4F-MPH
MDPV
Khat
Mephedrone
MDMA, MDA
PMA, PMMA
Ethylone, Methylone
Butylone
3/4-FMC
5/6-APB
4,4'-DMAR
4-MTA
5-APDI
2CB, 2CE, 2CI, 2CI-[x]
5-MeO-DALT
25I-[x]-NBOMe
25I-[x]-NBOH
αMT
DMT
DOM
LSD
Mescaline
Psilocybin
Ketamine
Methoxetamine (MXE)
PCP
DXM
3/4-MeO-PCP
3-HO-PCP
3-HO-PCE
N-ethyl-ketamine
Deschloroketamine
MXM
Cannabis
AM-[x], HU-[x]
UR-[x], JWH-[x]
5C-AKB48, 5F-PB22
ADB-FUBICA
AMB-CHMICA
MDMB-CHMICA
CUMYL-THPINACA
STS-135
NM2201
Solvents
EG-018
MDMB-CHMCZCA
CUMYL-PeGACLONE
Kava
W-18
W-15
Nortilidine
Kratom
2-AI
2-MPPP
3-FPM
MDAI
Mexedrone
4-MPM
5-IAI
1P-LSD
5-MeO-DIBF
2C-B-FLY
Peyote
Diethyl ether
Salvia
Nitrous oxide
Ephenidine
Alcohol
Poppers
Caffeine
Nicotine


Controlled under the Misuse of Drugs Act 1971
or the Human Medicines Regulations 2012
Controlled under the Psychoactive Substances Act 2016
Legal
Drug effects

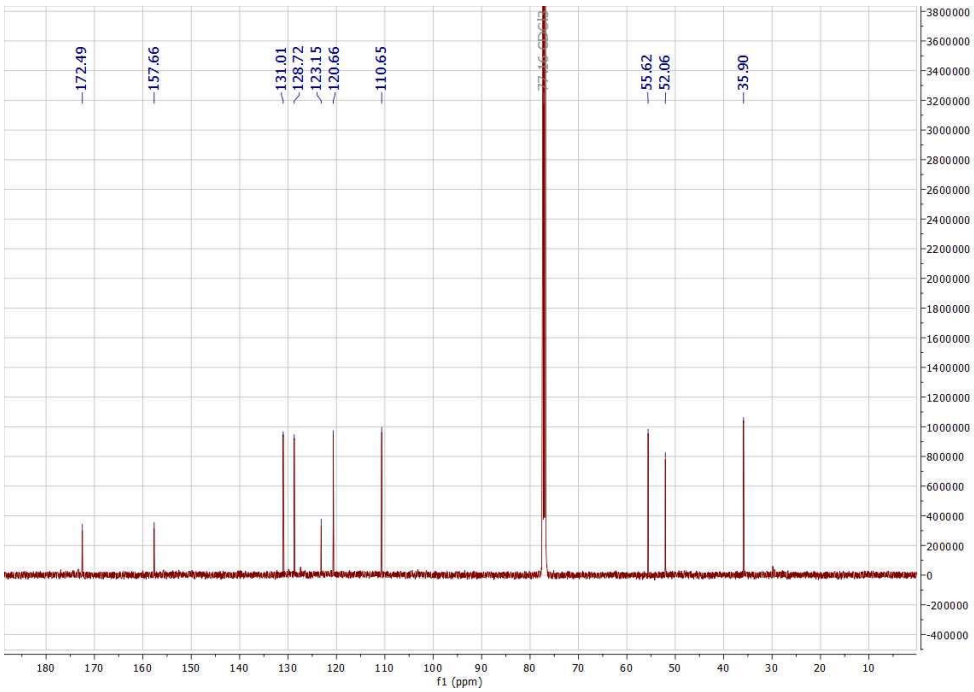
Compound 7



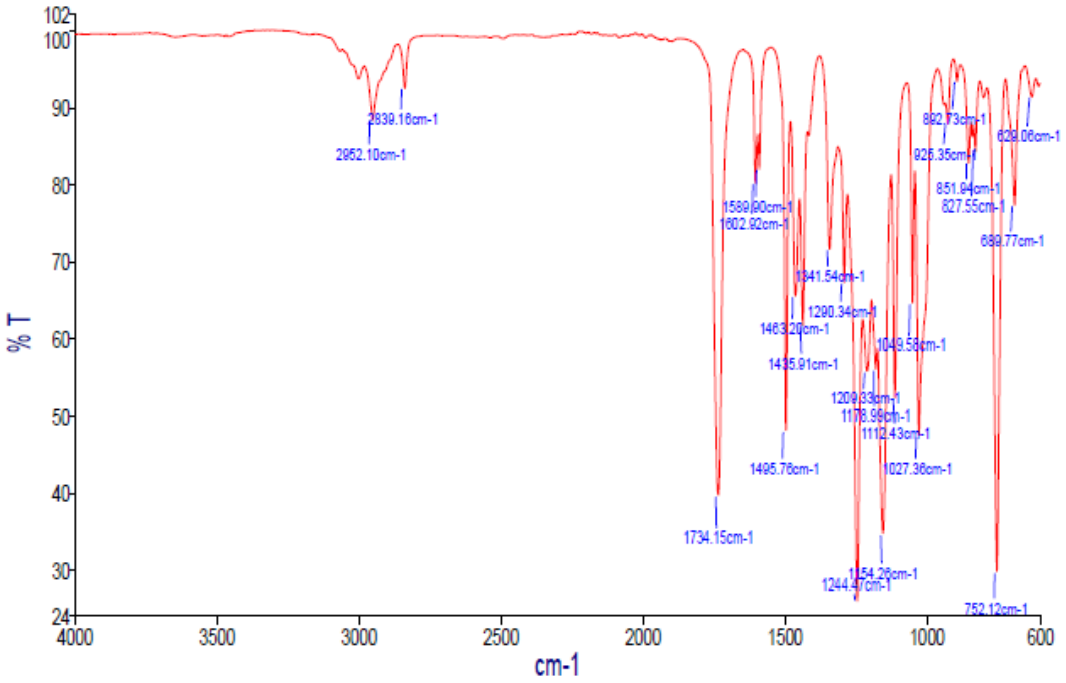
^{1}H-NMR



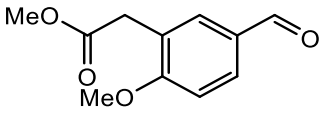
^{13}C-NMR



IR (*ATR*) $V_{máx}$ (cm^{-1}):



Compound 8



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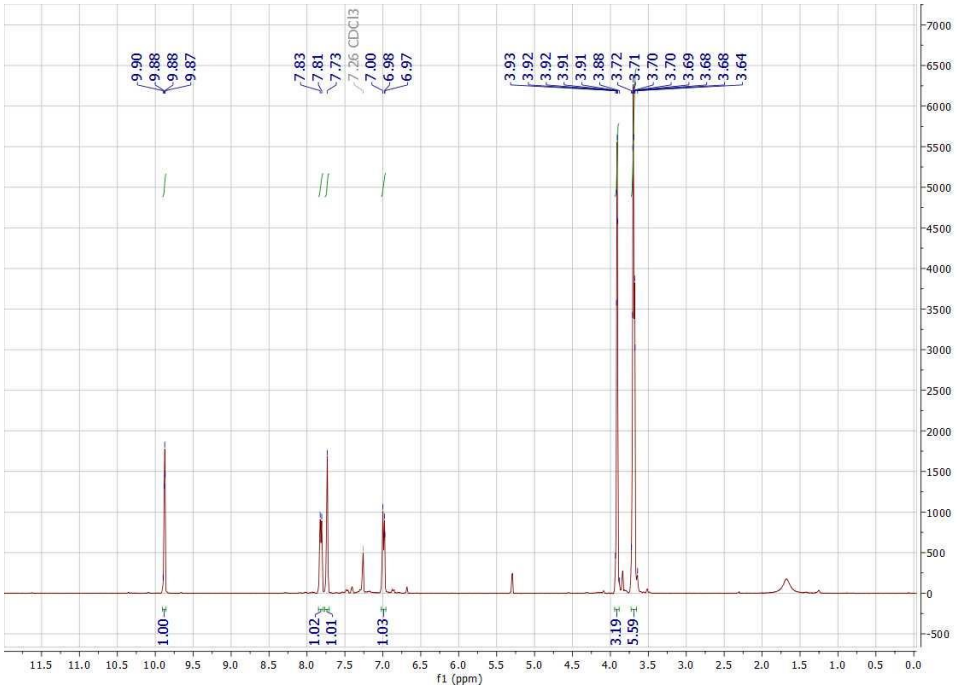

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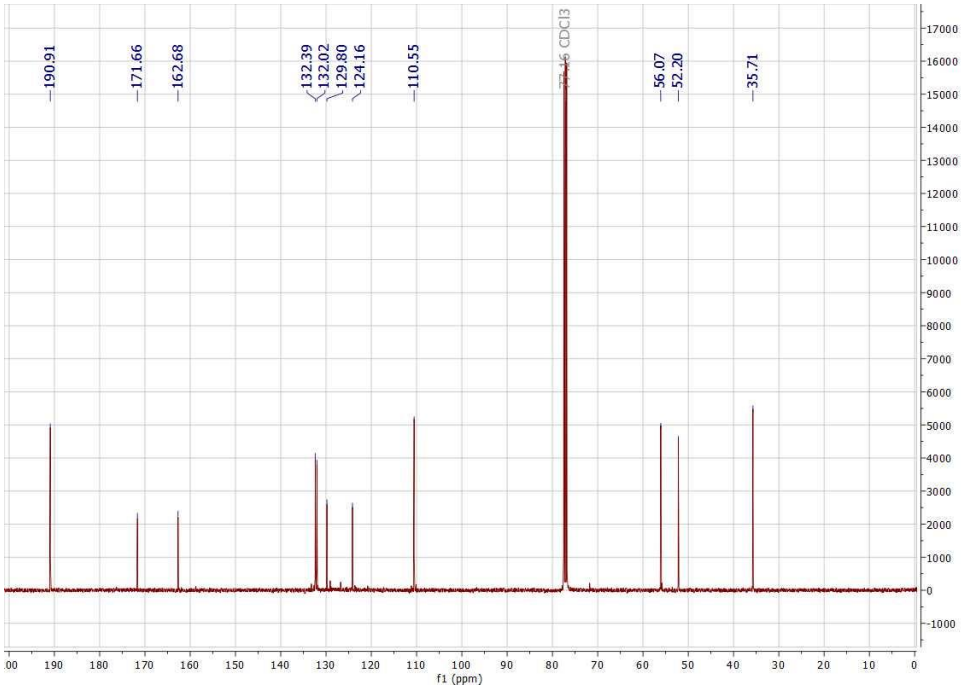
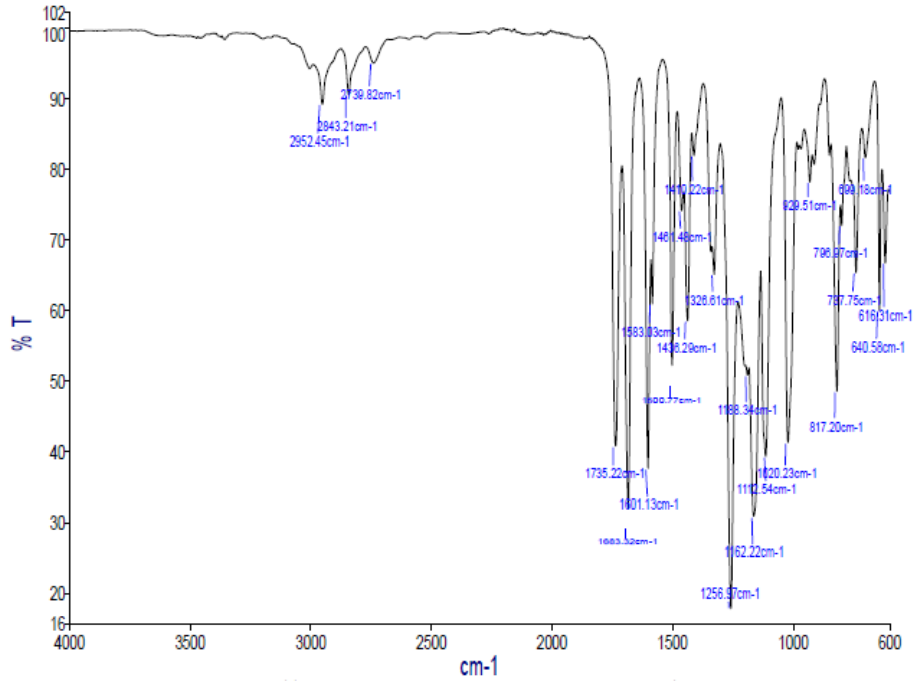
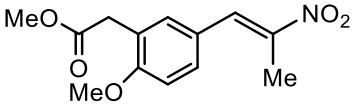


IR (*ATR*) $V_{máx}$ (cm^{-1})



Compound 9



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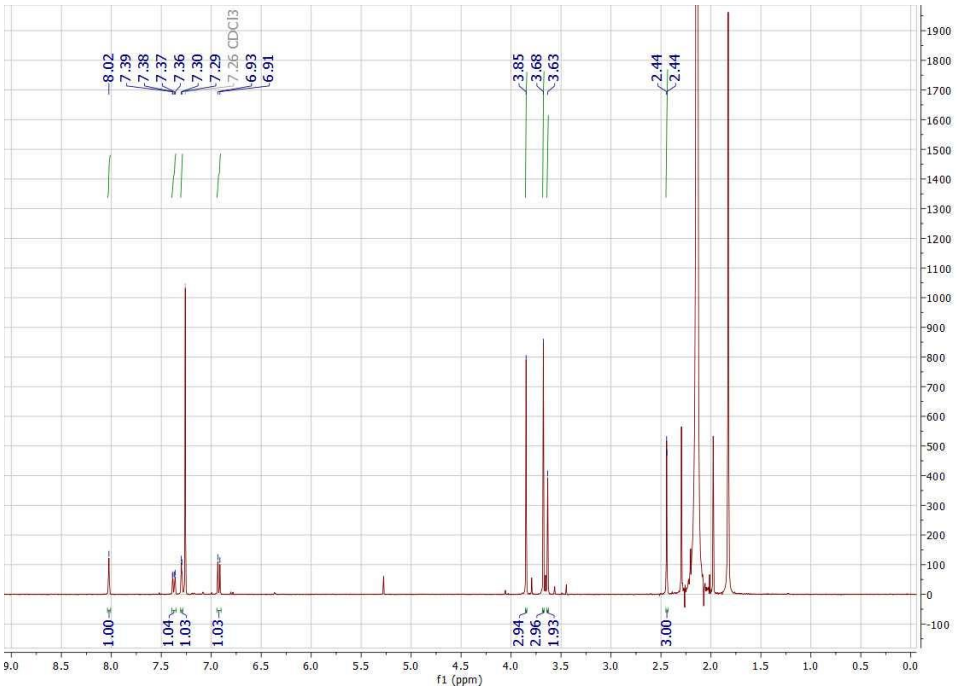

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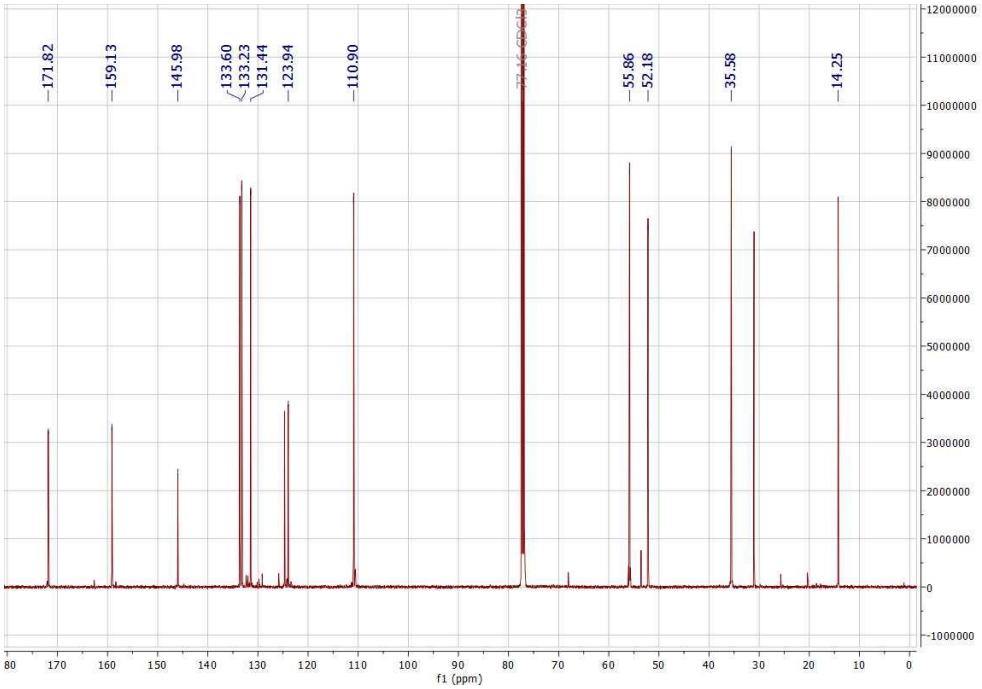
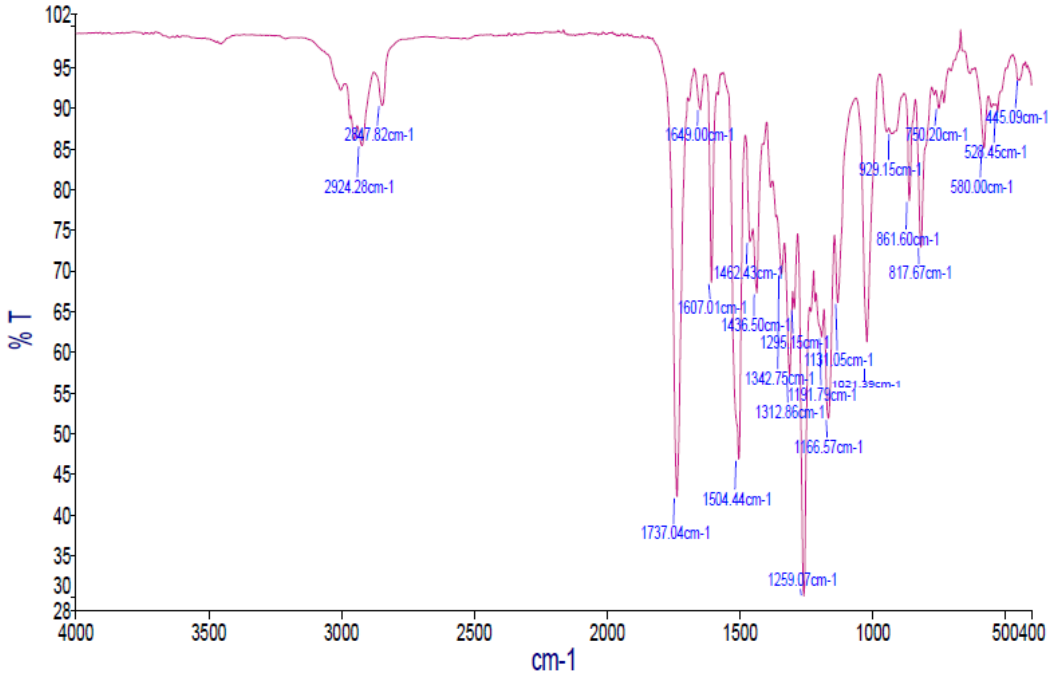
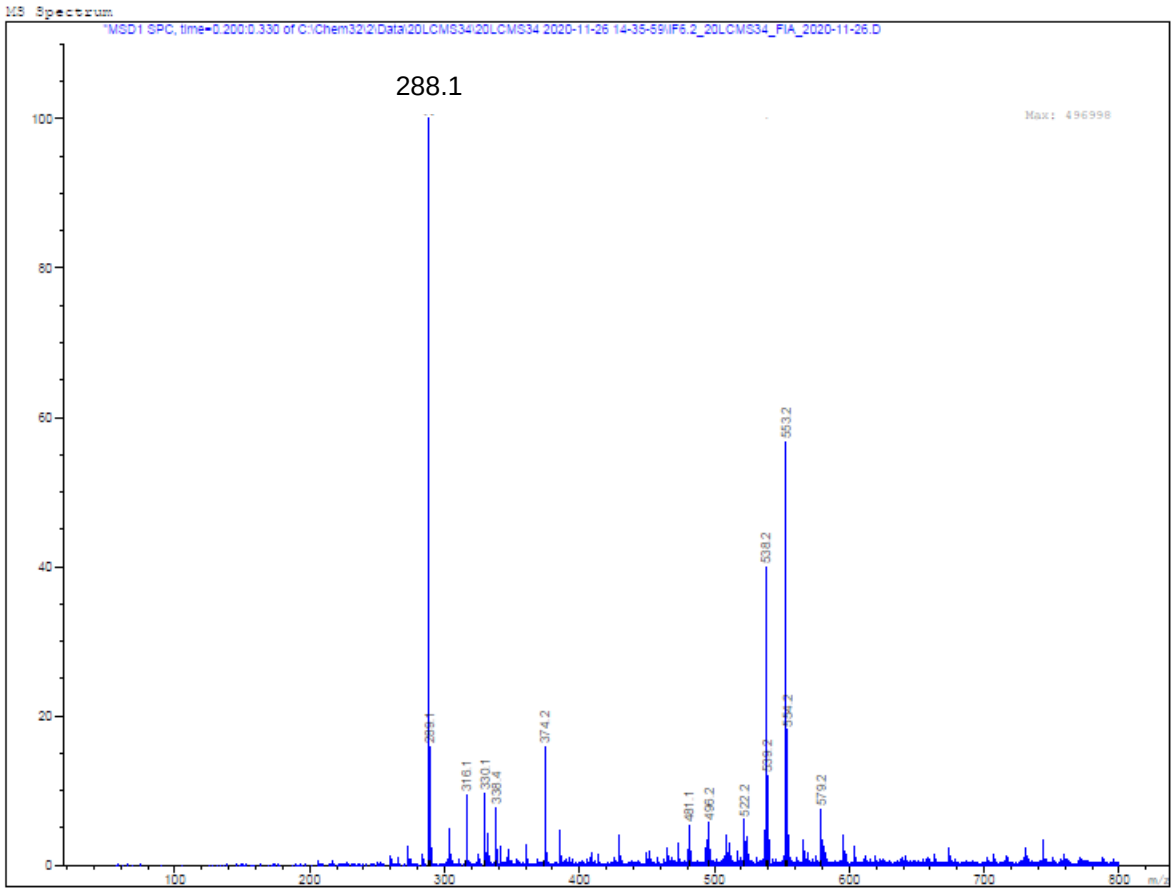


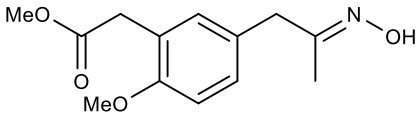
IR (*ATR*) $V_{máx}$ (cm^{-1})



MS (ESI)



Compound 11



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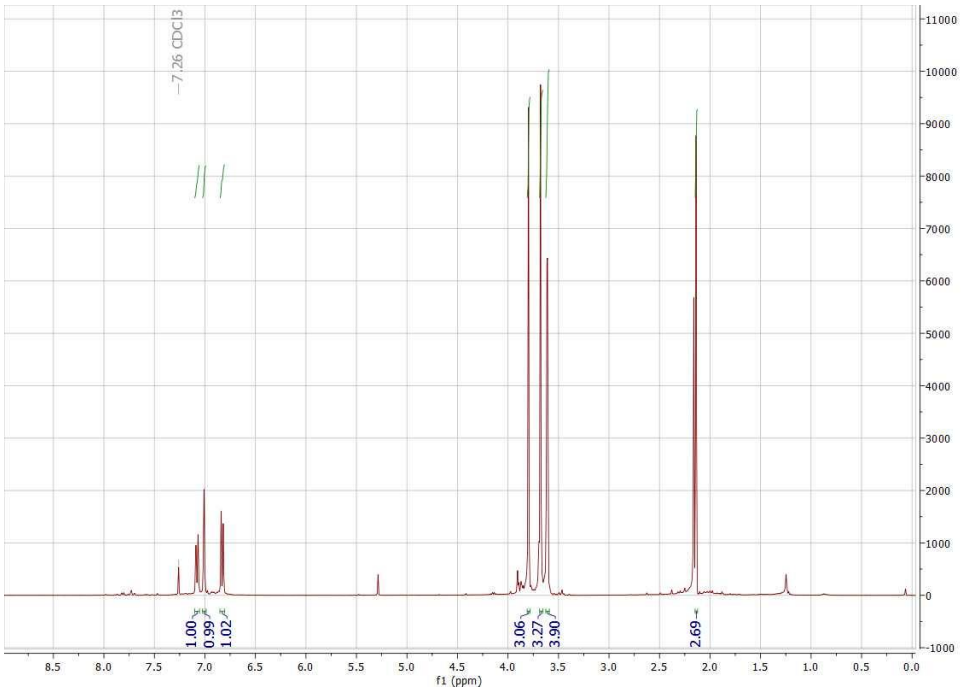
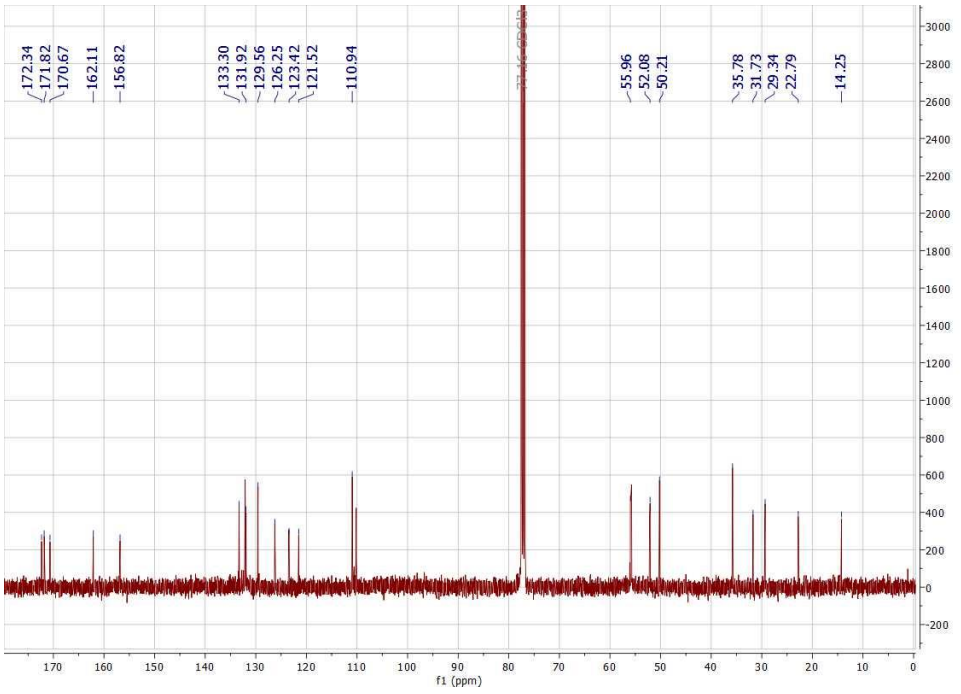
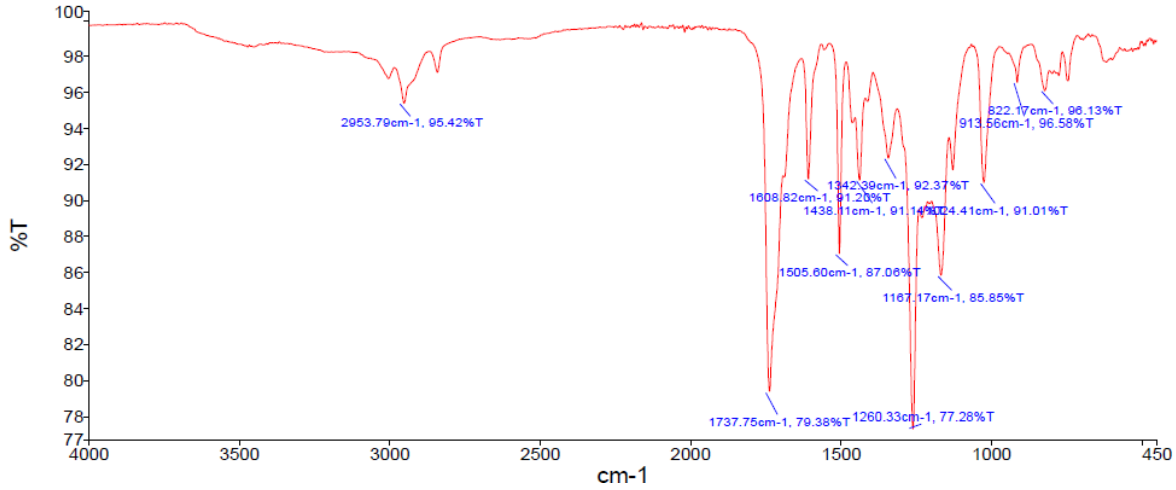


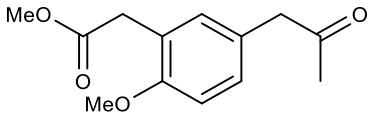
^{13}C-NMR



IR (*ATR*) $V_{máx}$ (cm^{-1})



Compound 12



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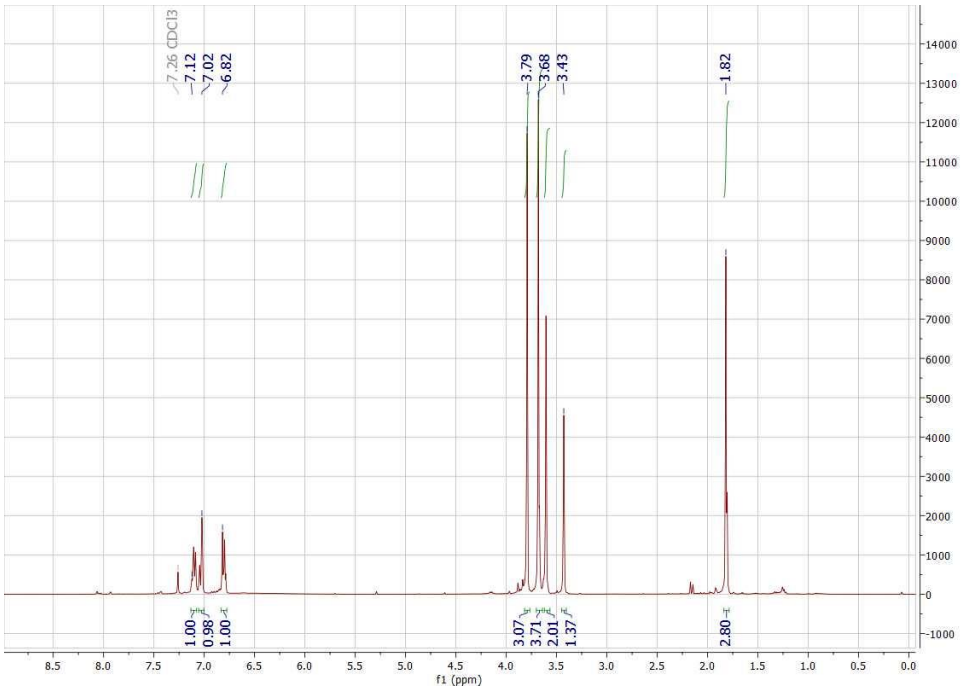

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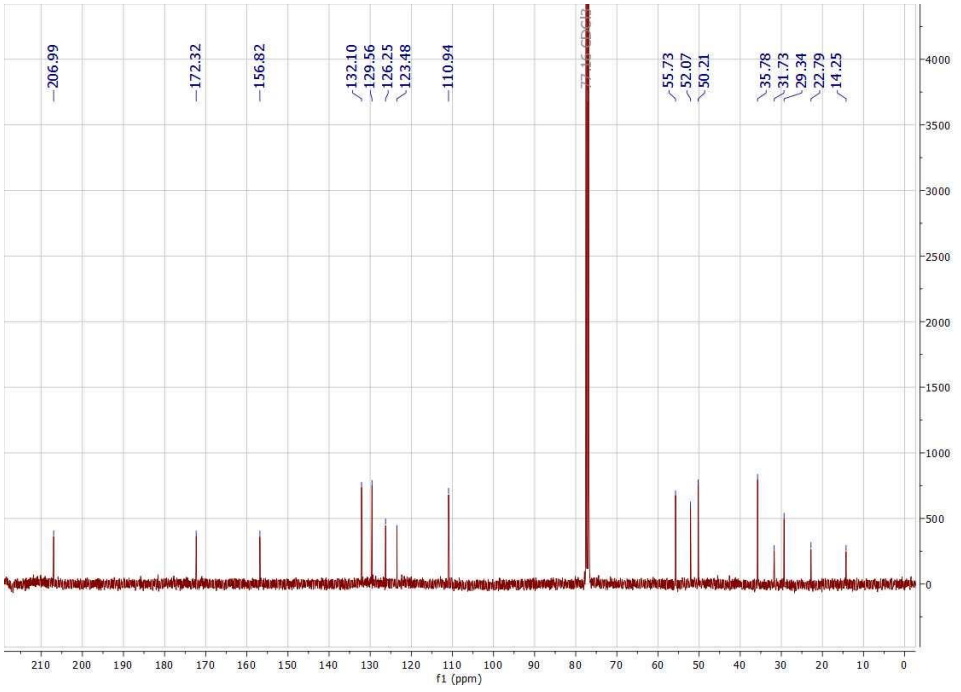
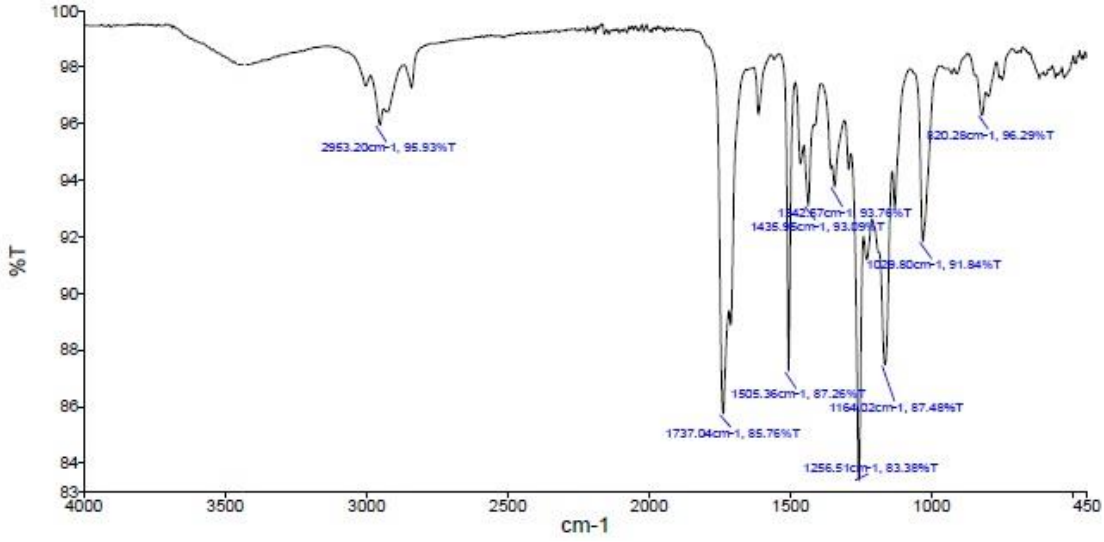
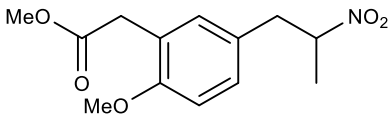


IR (*ATR*) $V_{máx}$ (cm^{-1})



Compound 13



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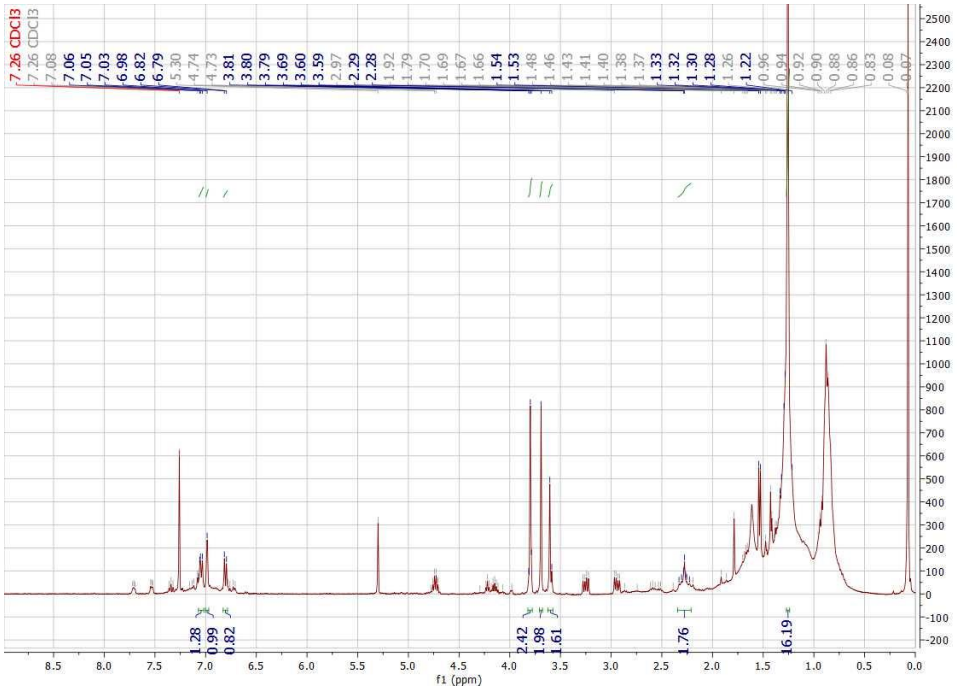

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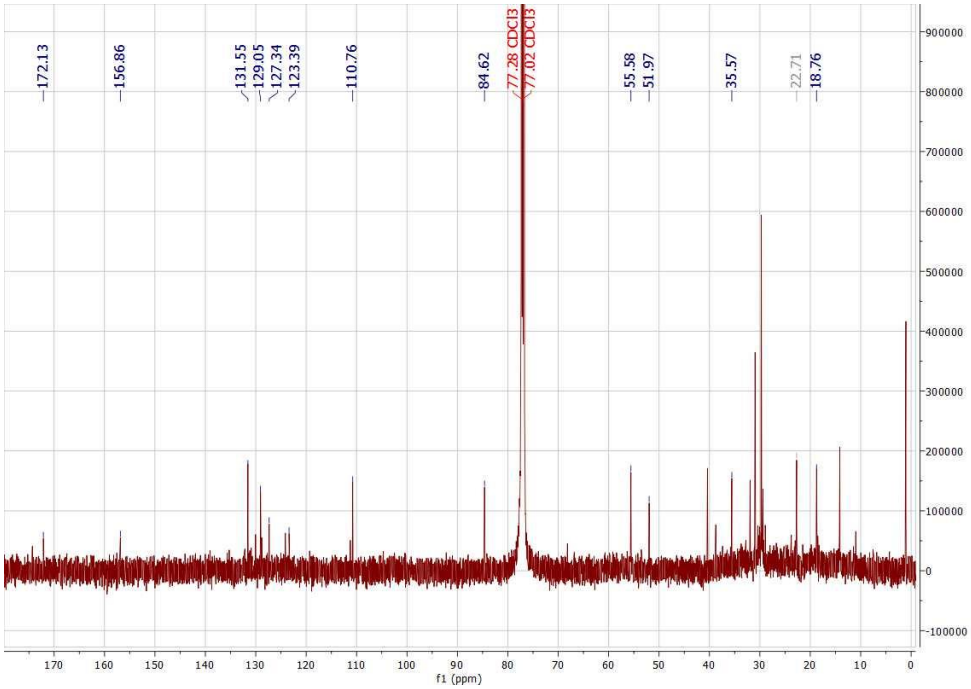
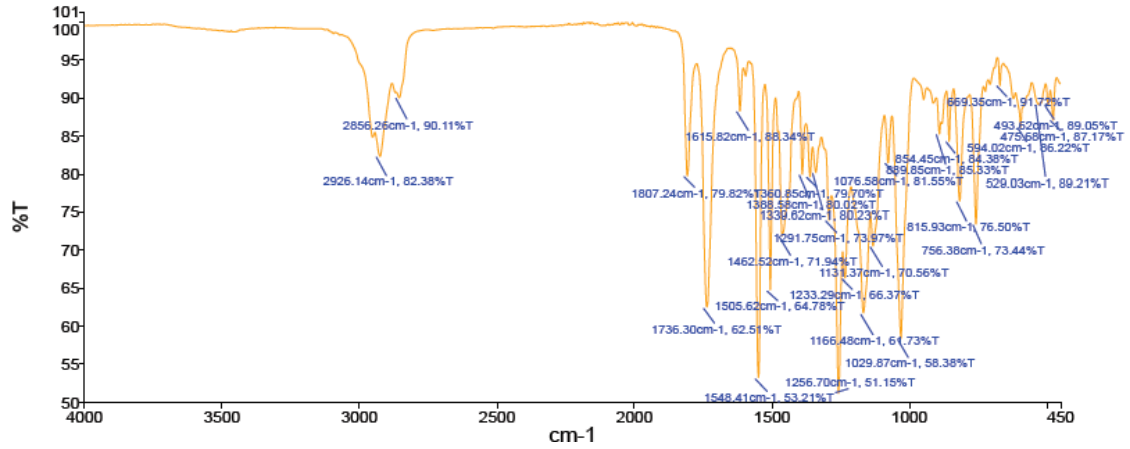
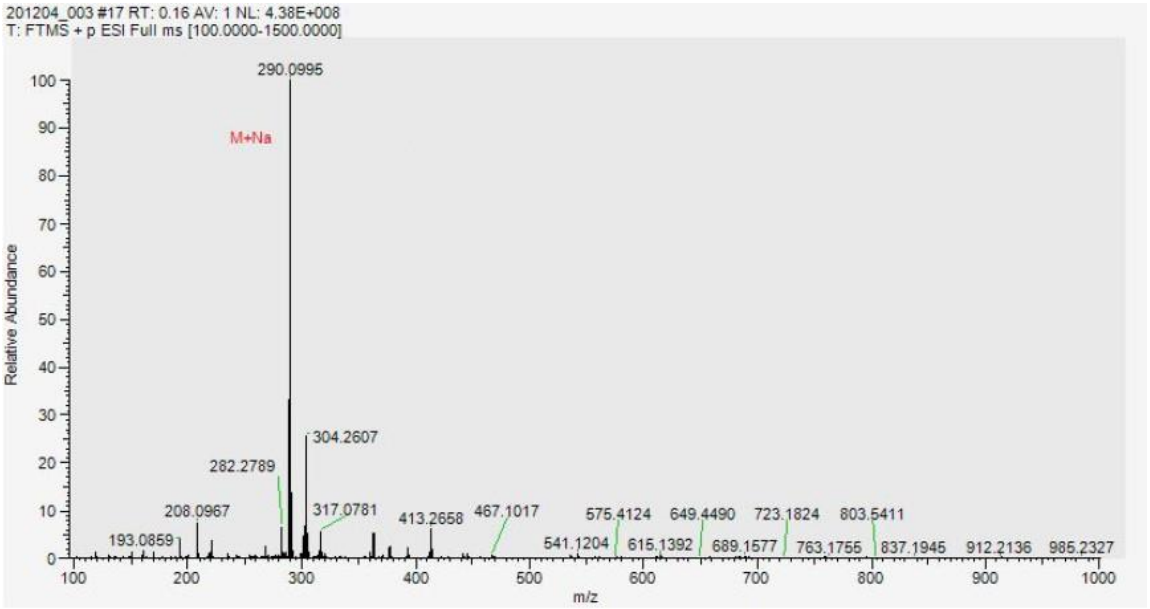


FTIR-*ATR*

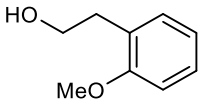


HRMS (ESI)

Display Formula	S Fit	RDB	Delta [ppm]	Theo. mass	Combined Score	# Matched Iso.
$C_{13}H_{17}O_5N^{23}Na$	70,01	5,5	-1,51	290,09989	97,74	4
$C_{11}H_{12}O_3N_7$	26,71	9,5	-0,54	290,09961	94,25	3
$C_{11}H_{20}O_2N_3{}^{32}S_2$	16,54	3,5	1,08	290,09914	93,72	3
$C_{14}H_{21}N^{23}Na^{32}S_2$	10,57	4,5	-4,5	290,10076	93,4	2
$C_9H_{21}O_7N^{35}Cl$	18,65	-0,5	-2,24	290,10011	83,66	4
$C_{10}H_{16}O_7N_3$	8,742	4,5	4,07	290,09828	81,94	2
$C_{19}H_{16}N^{32}S$	8,462	12,5	-1,17	290,0998	81,92	3
$C_4H_{20}N_9{}^{32}S_3$	7,163	-0,5	-1,29	290,09983	81,85	1
$C_3H_{16}O_5N_9{}^{32}S$	6,97	0,5	1,71	290,09896	81,84	1
$C_8H_{18}O_3N_5{}^{35}Cl^{23}Na$	6,181	1,5	1,44	290,09904	81,8	3



Compound 14



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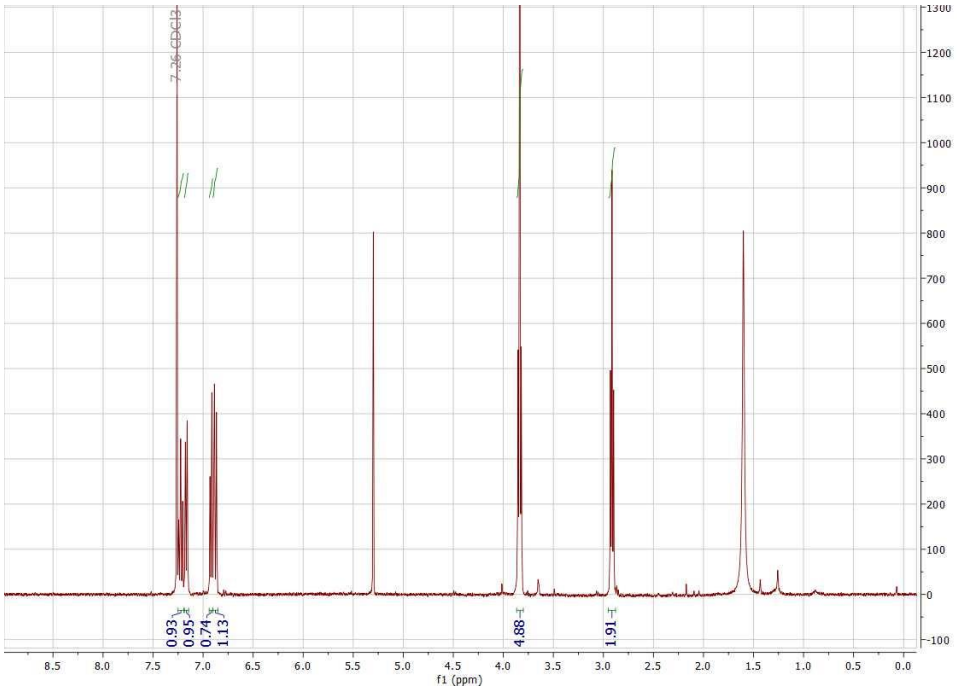

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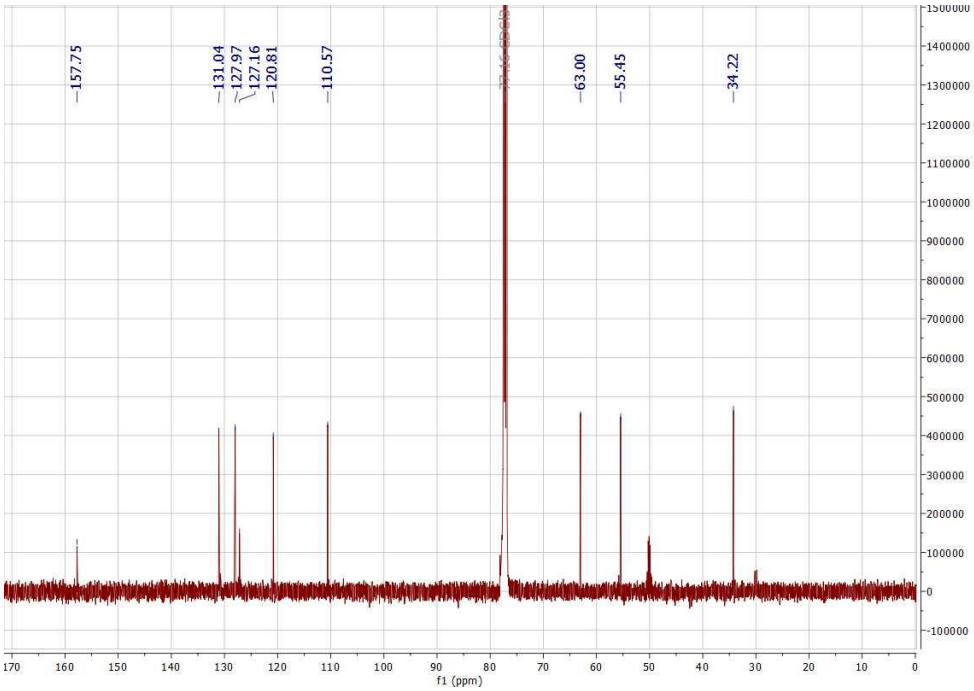
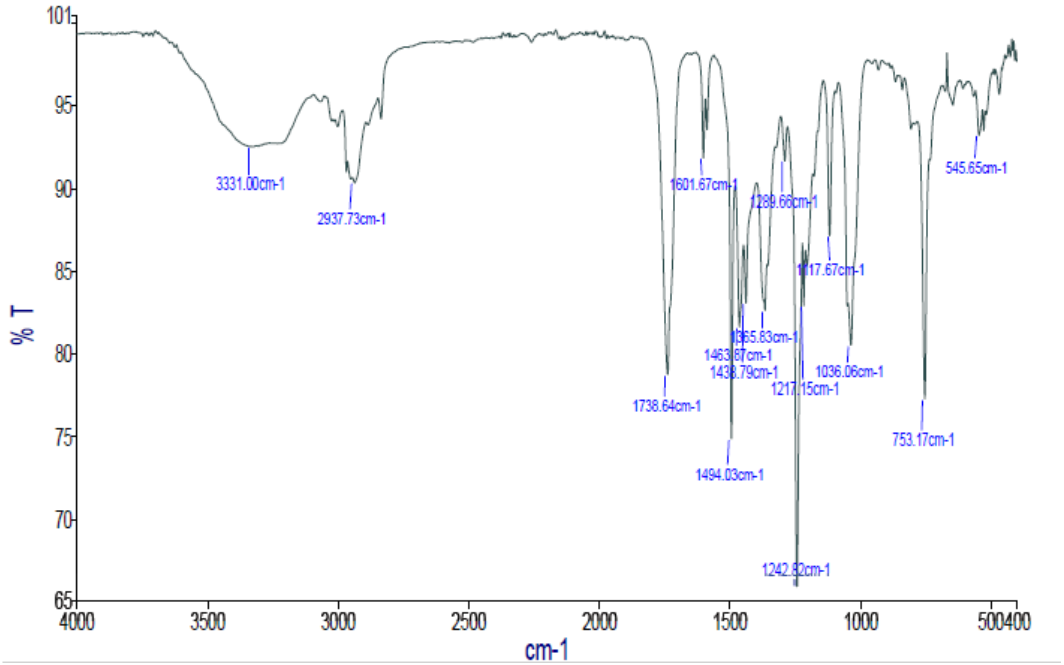
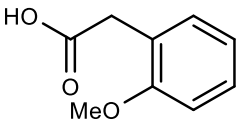


IR (*ATR*) $V_{máx}$ (cm^{-1})



Compound 15



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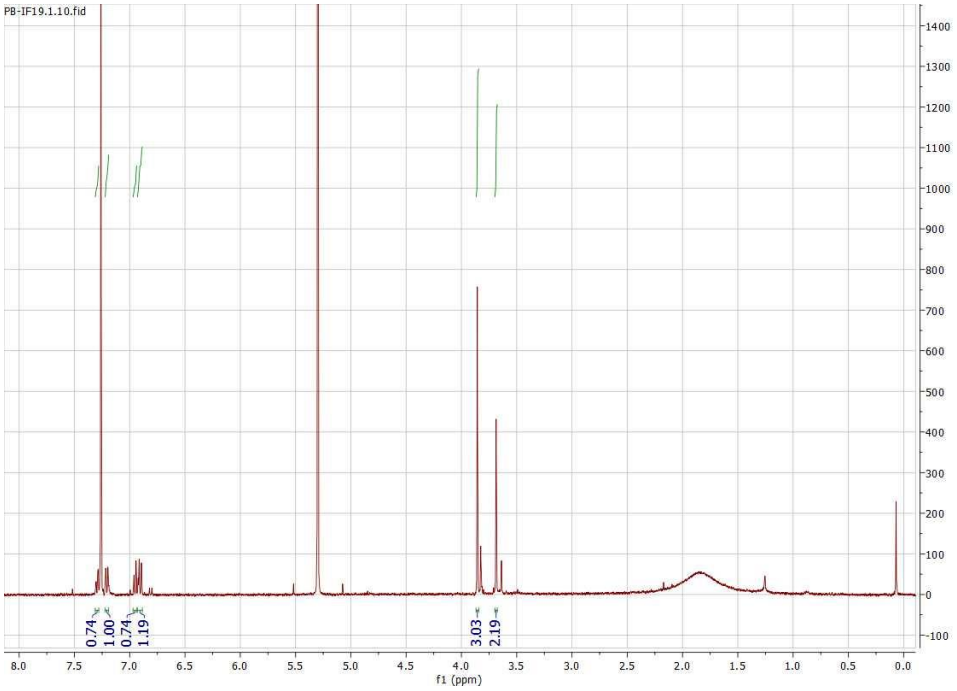
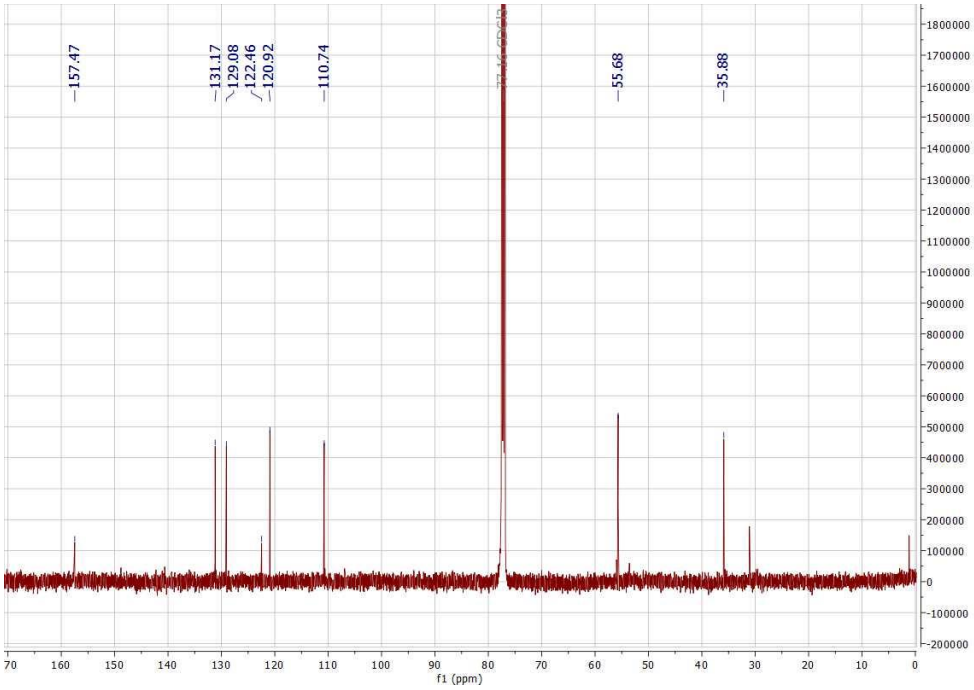
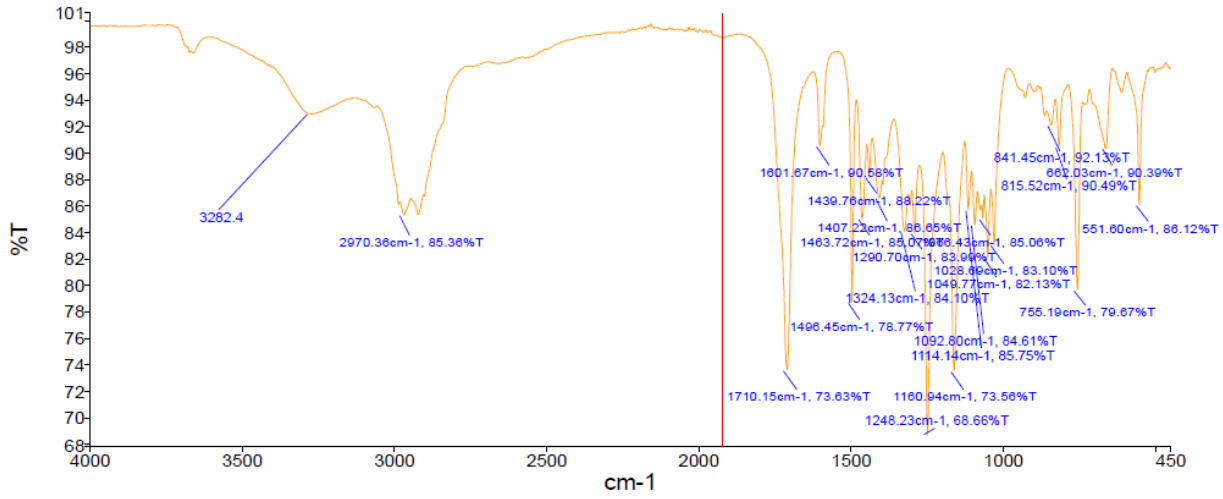


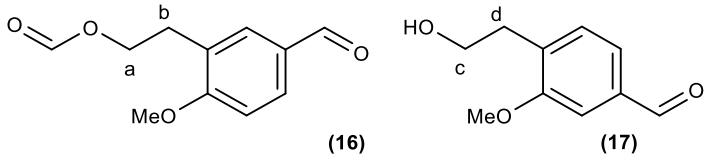
^{13}C-NMR



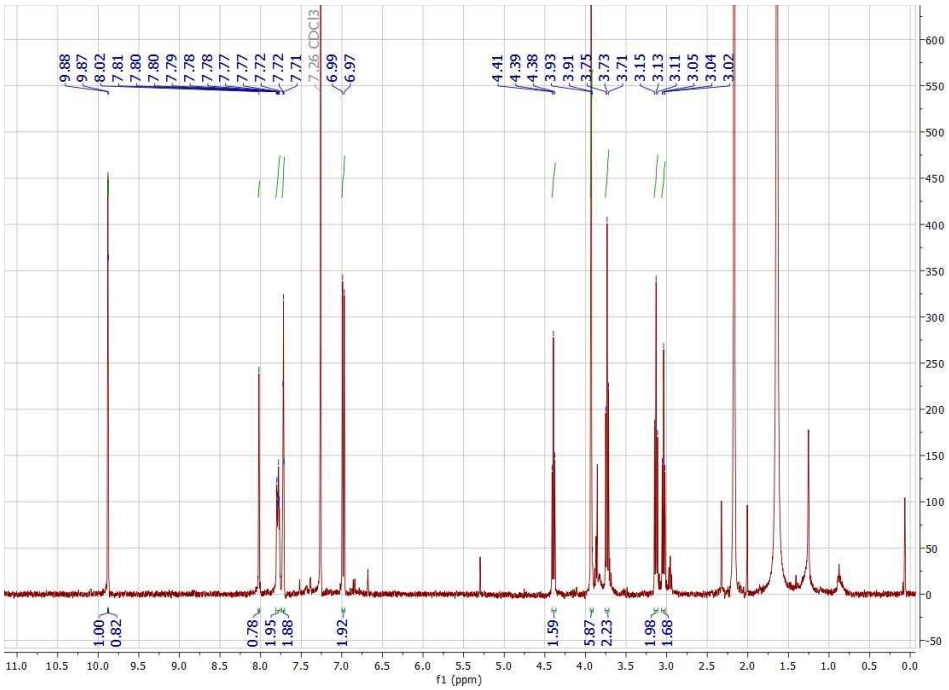
IR (*ATR*) $V_{máx}$ (cm^{-1})



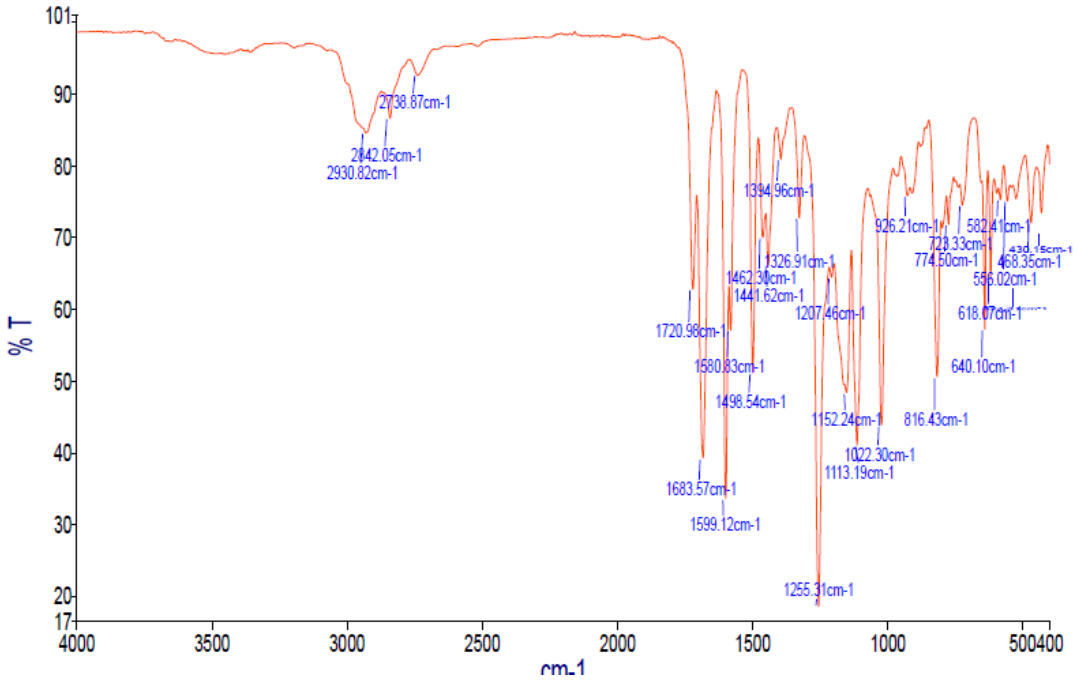
Mixture of Compound 16 e Compound 17



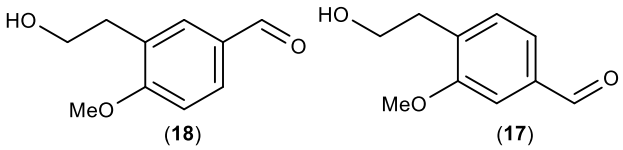
^{1}H-NMR



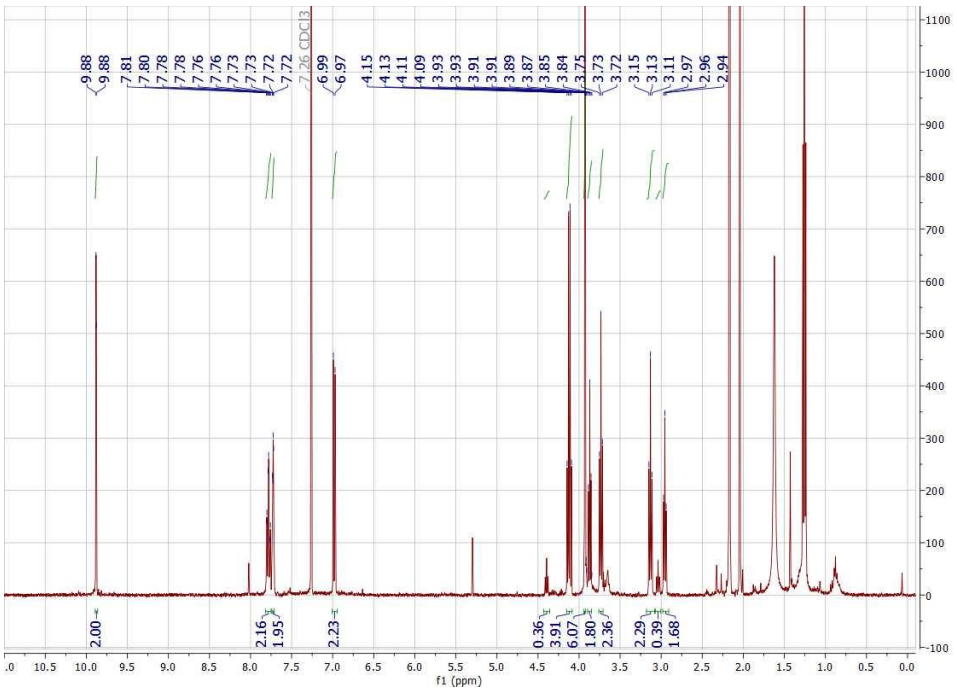
IR (*ATR*) $V_{máx}$ (cm^{-1})



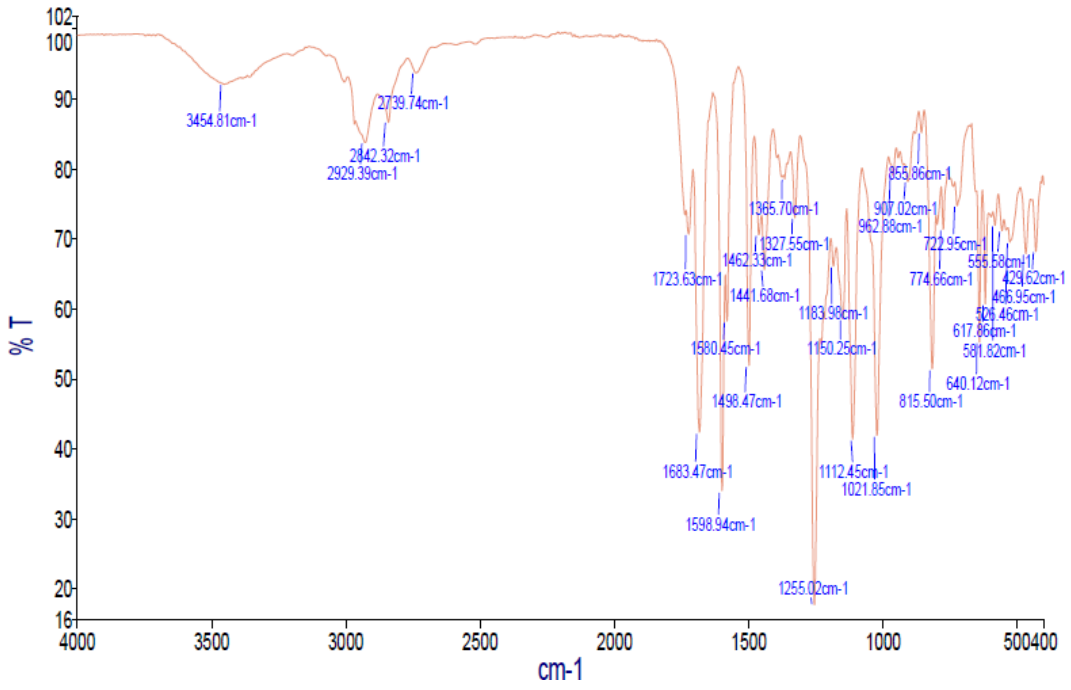
Mixture of Compound 18 e Compound 17



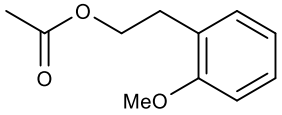
^{1}H-NMR



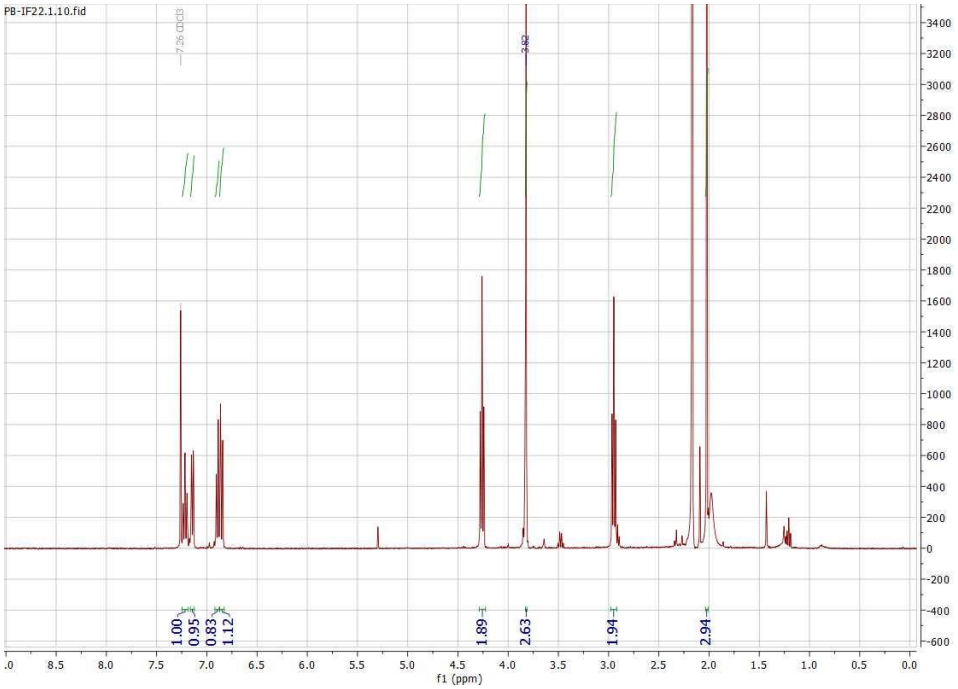
IR (*ATR*) $V_{máx}$ (cm^{-1})



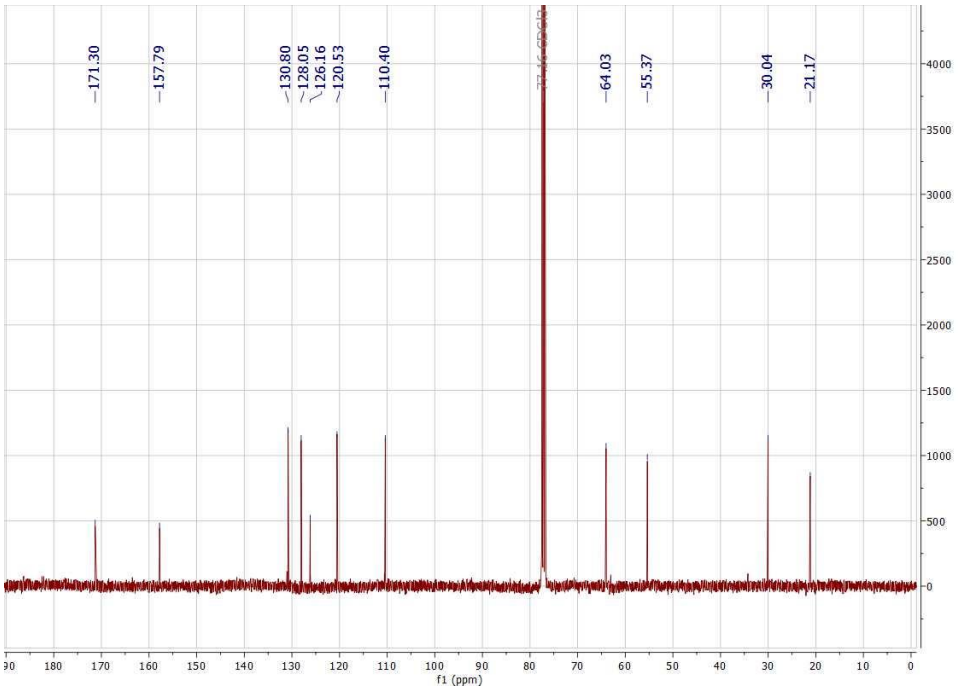
Compound 19



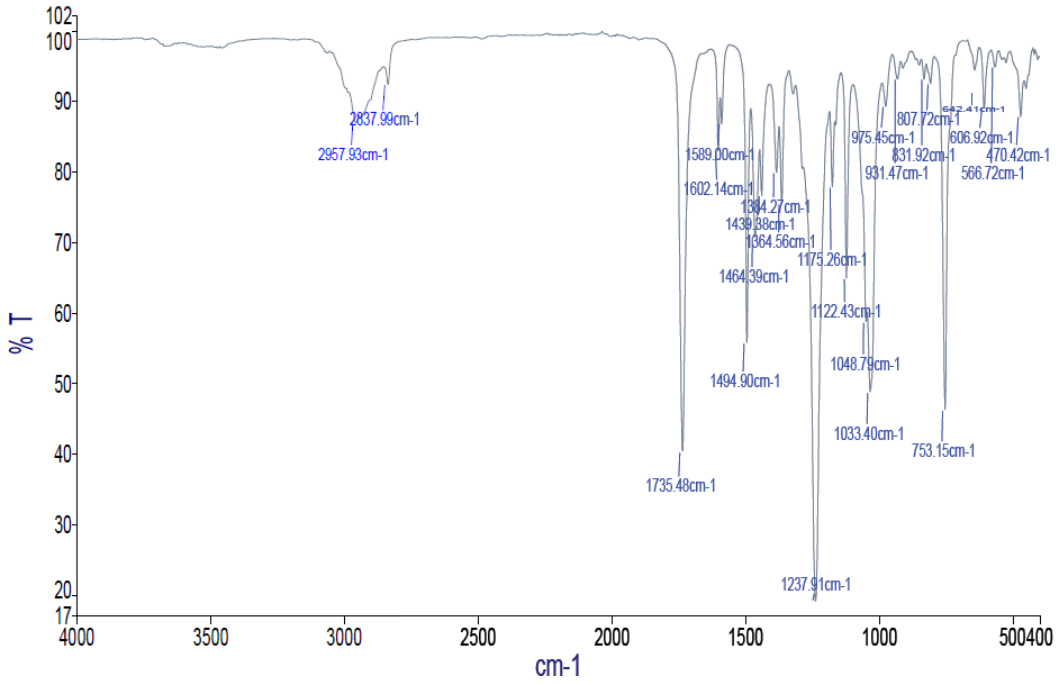
^{1}H-NMR



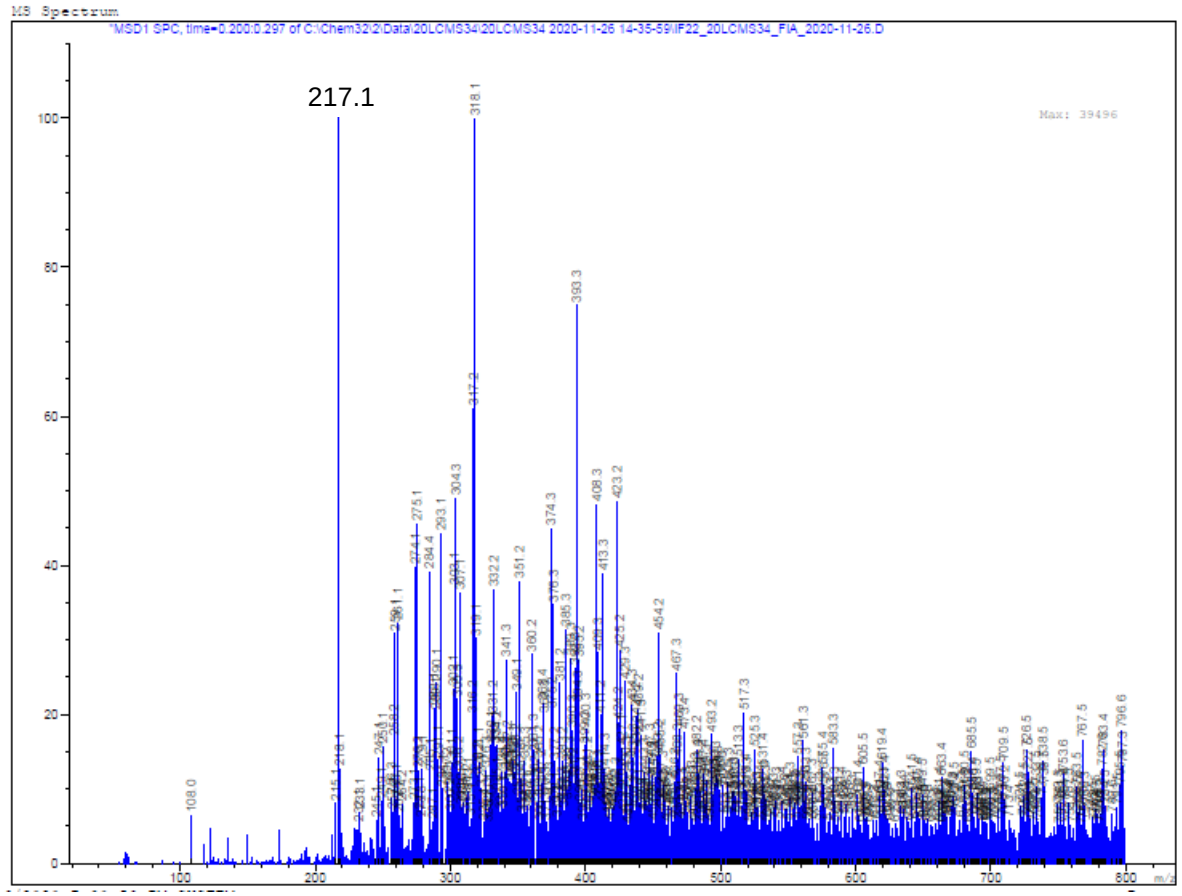
^{13}C-NMR



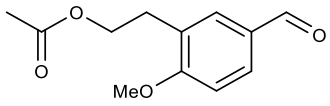
IR (*ATR*) $V_{máx}$ (cm^{-1})



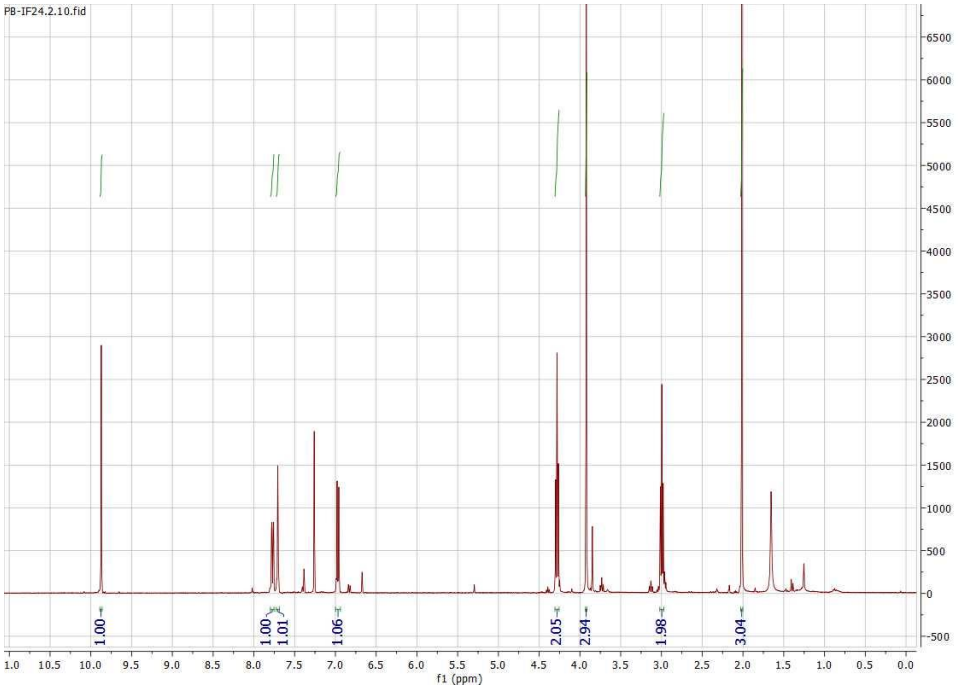
MS (ESI)



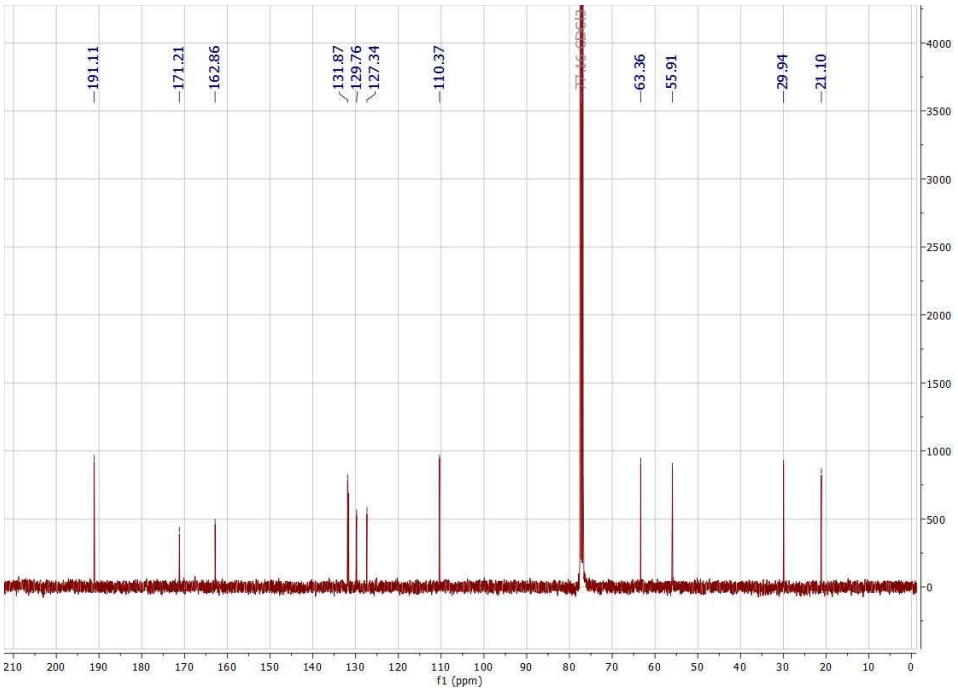
Compound 20



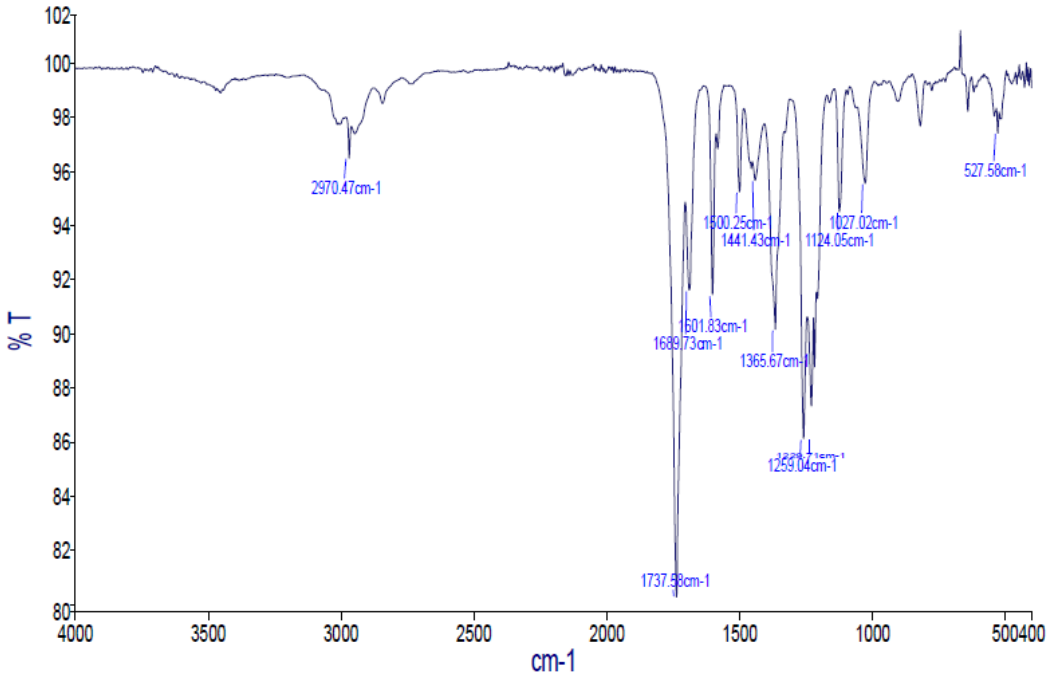
^{1}H-NMR



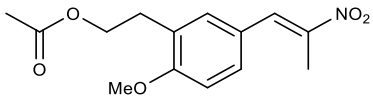
^{13}C-NMR



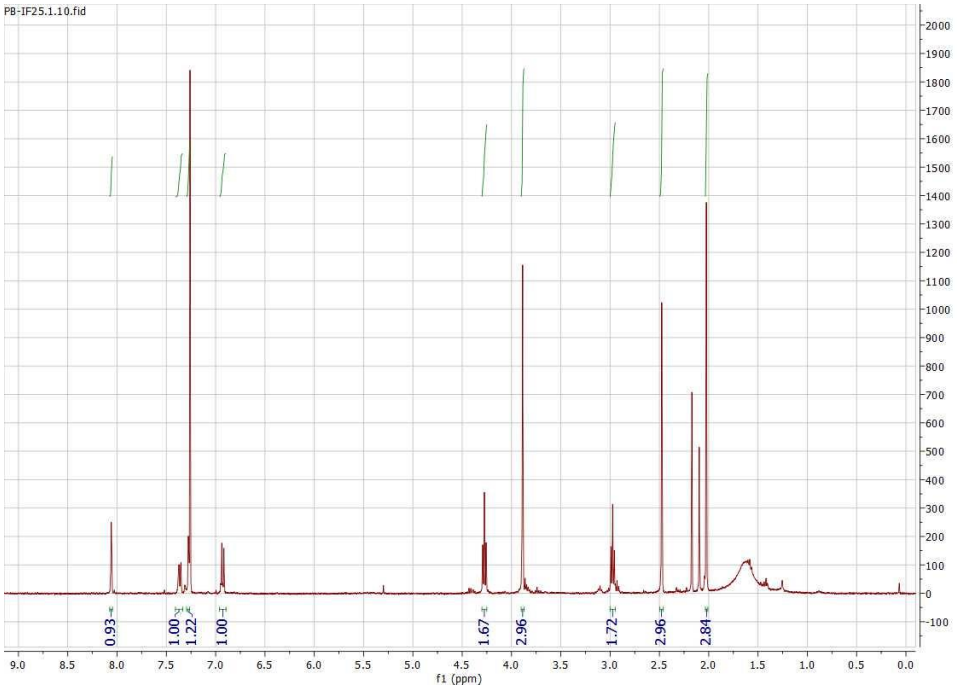
IR (*ATR*) $V_{máx}$ (cm^{-1})



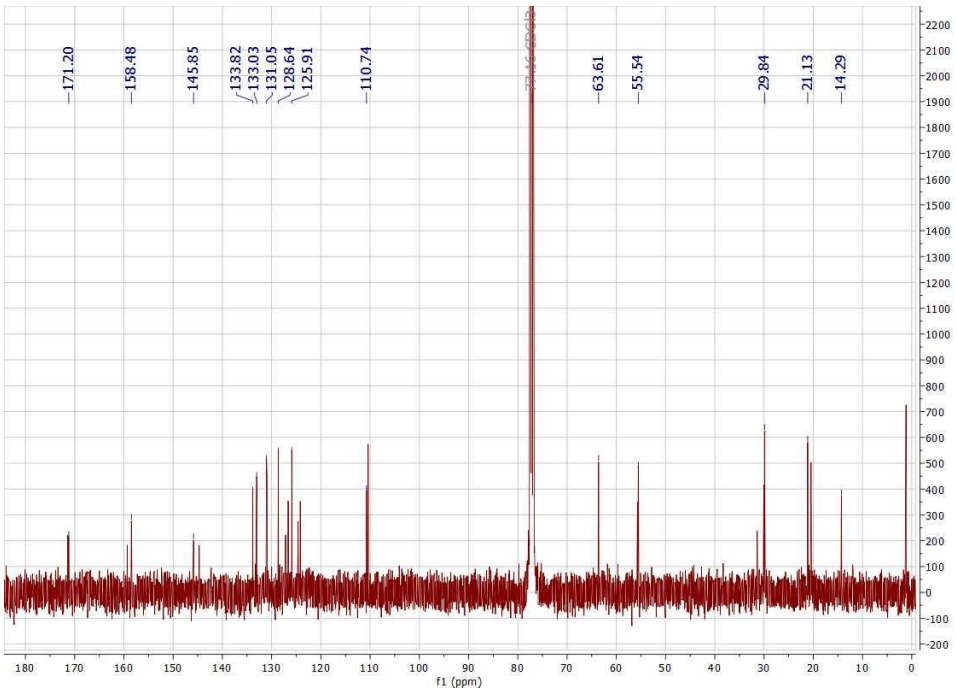
Compound 21



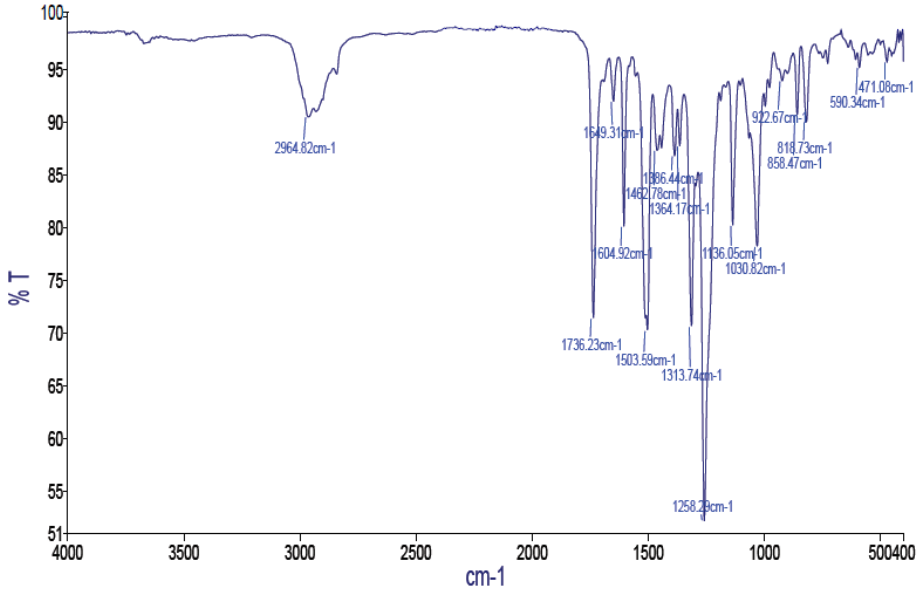
^{1}H-NMR



^{13}C-NMR

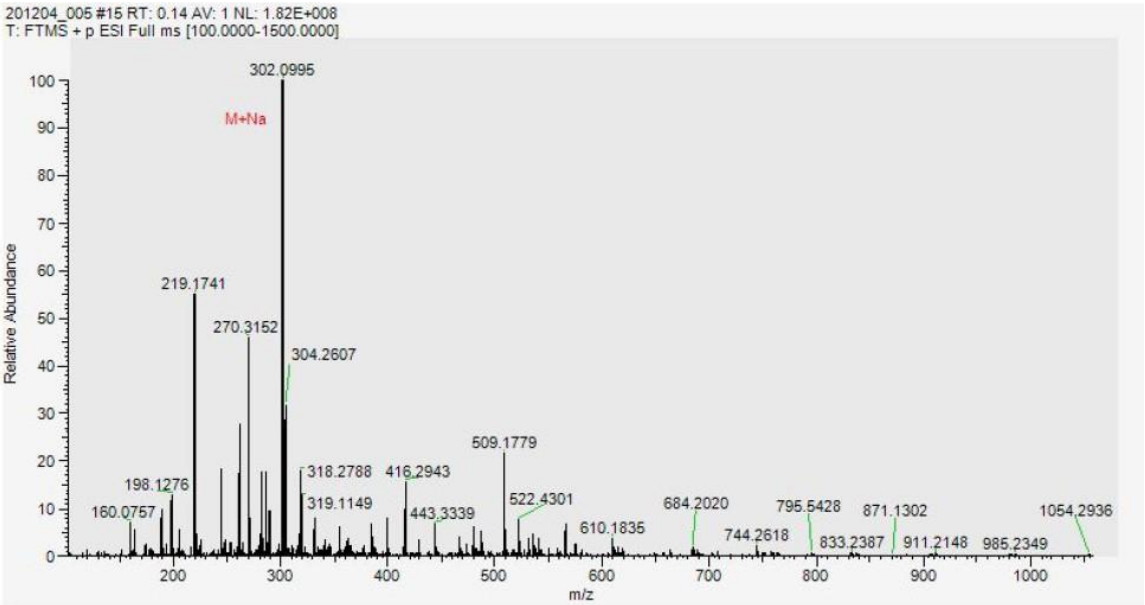


IR (*ATR*) $V_{máx}$ (cm^{-1})

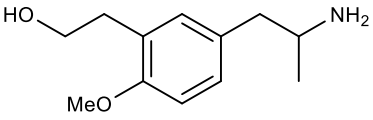


HRMS (ESI)

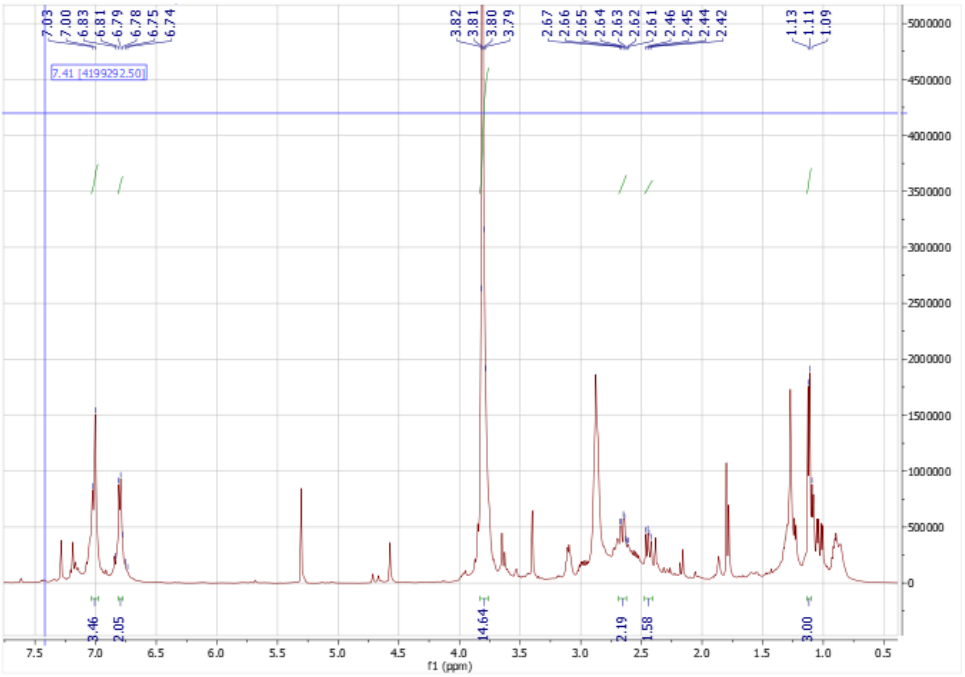
Display Formula	S Fit	RDB	Delta [ppm]	Theo. mass	Combined Score	# Matched Iso.
$C_{14}H_{17}O_5N^{23}Na$	79,93	6,5	-1,14	302,09989	64,53	4
$C_{12}H_{12}O_3N_7$	28,56	10,5	-0,22	302,09961	60,86	3
$C_{12}H_{20}O_2N_3{}^{32}S_2$	17,19	4,5	1,34	302,09914	60,27	4
$C_{15}H_{21}N^{23}Na^{32}S_2$	9,698	5,5	-4,02	302,10076	59,87	3
$C_{10}H_{21}O_7N^{35}Cl$	16,33	0,5	-1,85	302,10011	53,67	4
$C_{11}H_{16}O_7N_3$	16,09	5,5	4,21	302,09828	52,9	3
$C_7H_{17}O_3N_7{}^{23}Na^{32}S$	12	2,5	-3,41	302,10058	52,79	4
$C_{20}H_{16}N^{32}S$	7,314	13,5	-0,82	302,0998	52,34	4
$C_5H_{20}N_9{}^{32}S_3$	7,284	0,5	-0,93	302,09983	52,34	2
$C_9H_{18}O_3N_5{}^{35}Cl^{23}Na$	6,979	2,5	1,69	302,09904	52,32	3



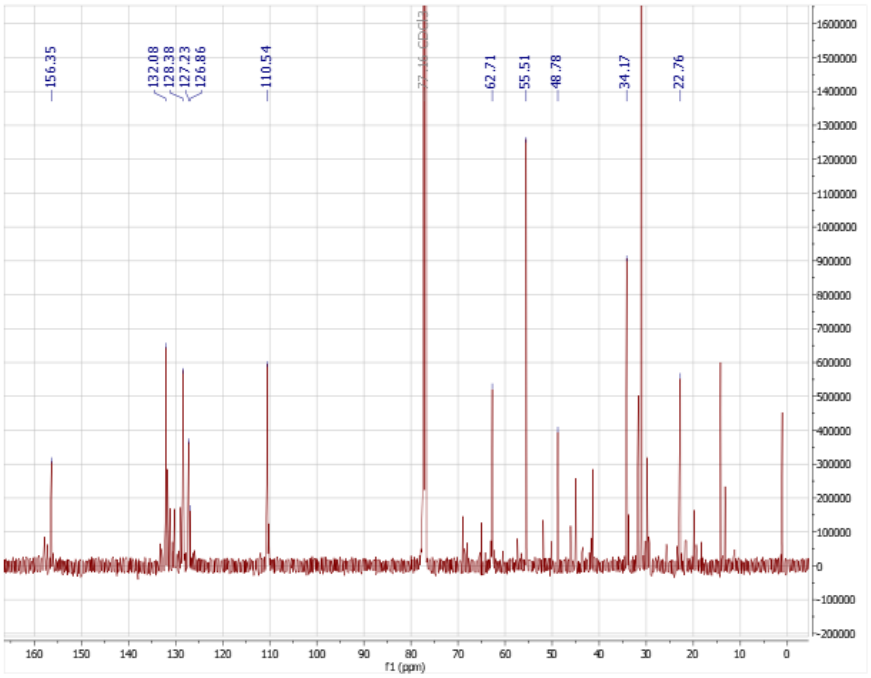
Compound 22



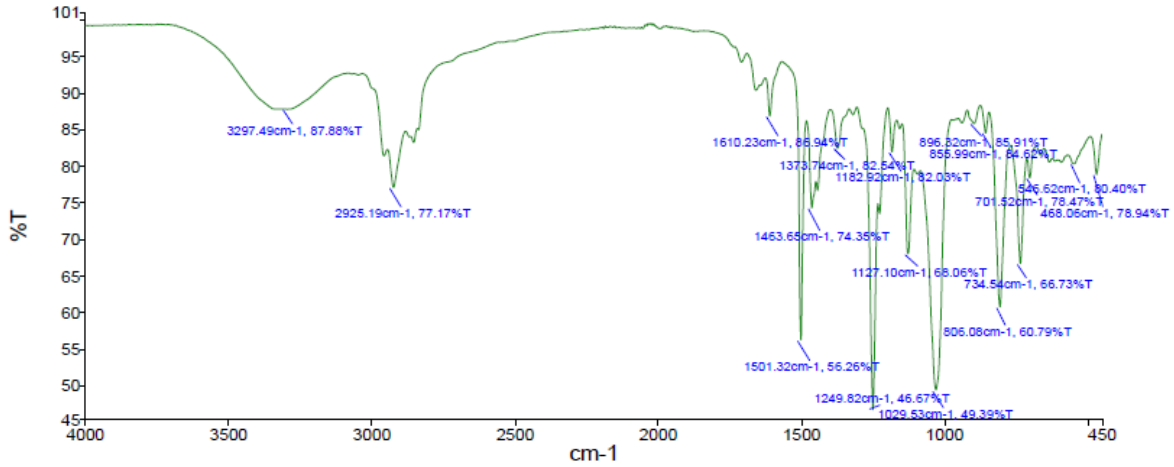
^{1}H-NMR



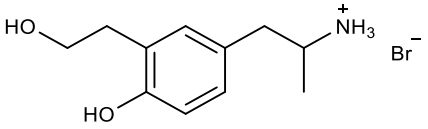
^{13}C-NMR



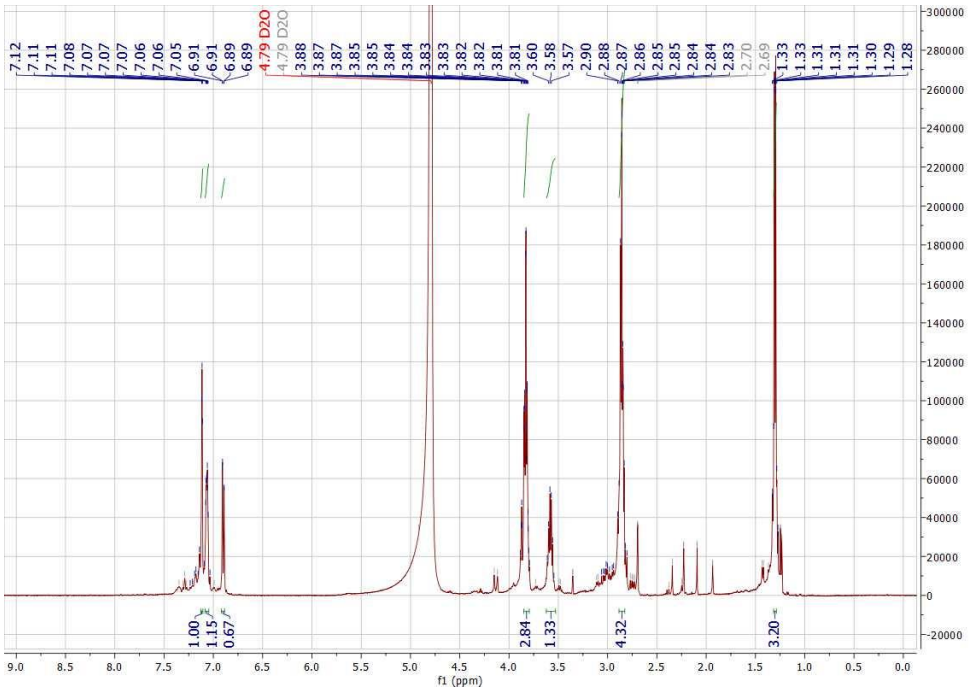
IR (*ATR*) $V_{máx}$ (cm^{-1})



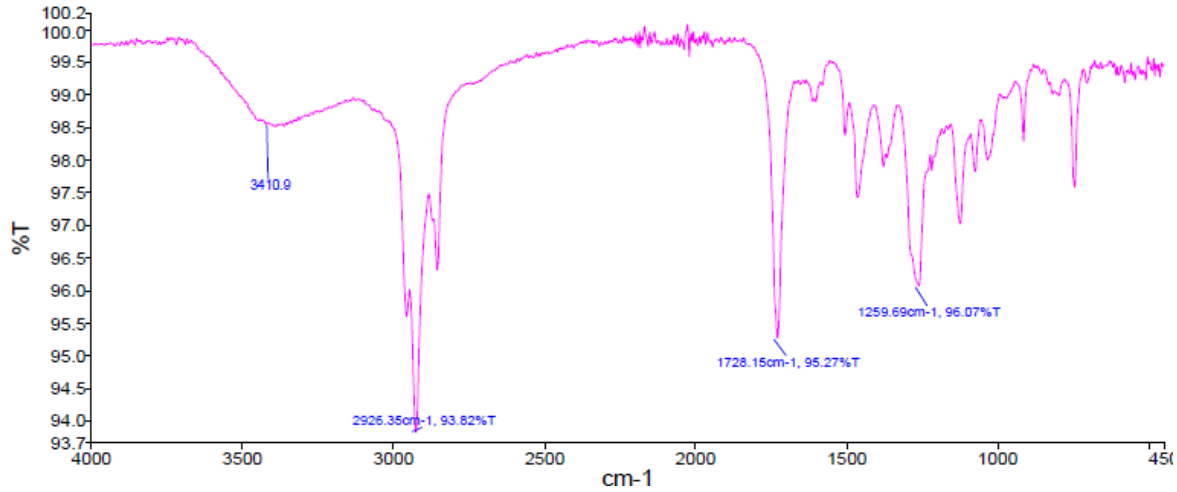
Metabolite 3



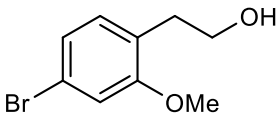
^{1}H-NMR



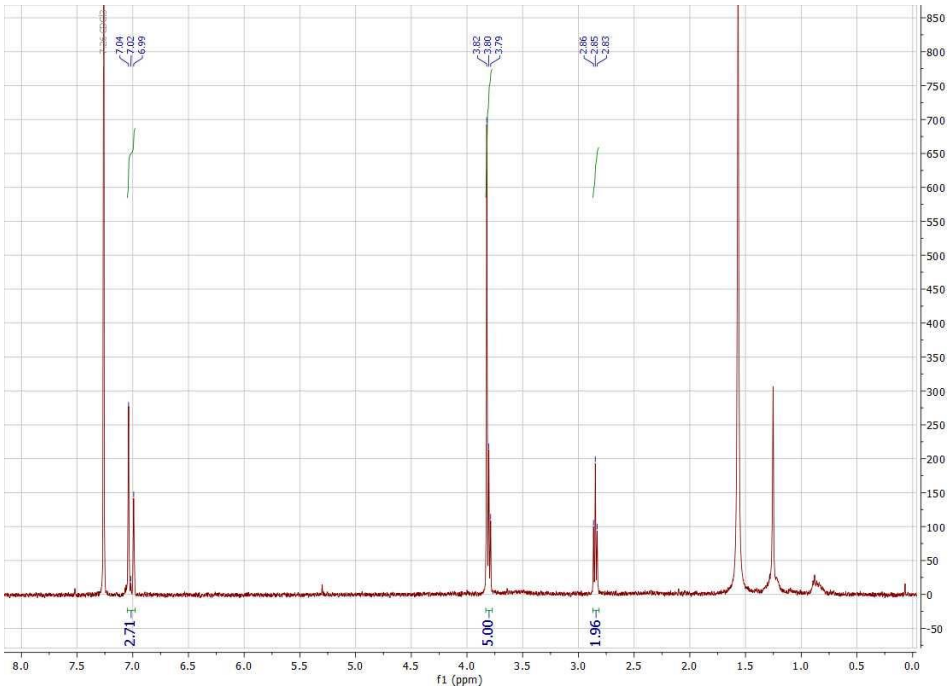
IR (*ATR*) $V_{máx}$ (cm^{-1})



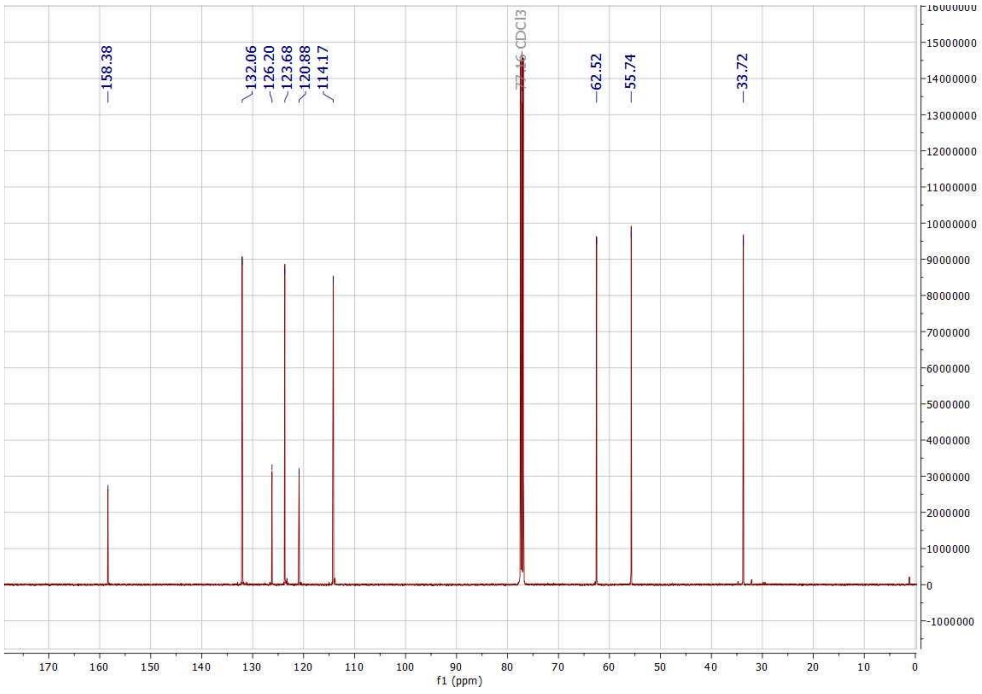
Compound 23



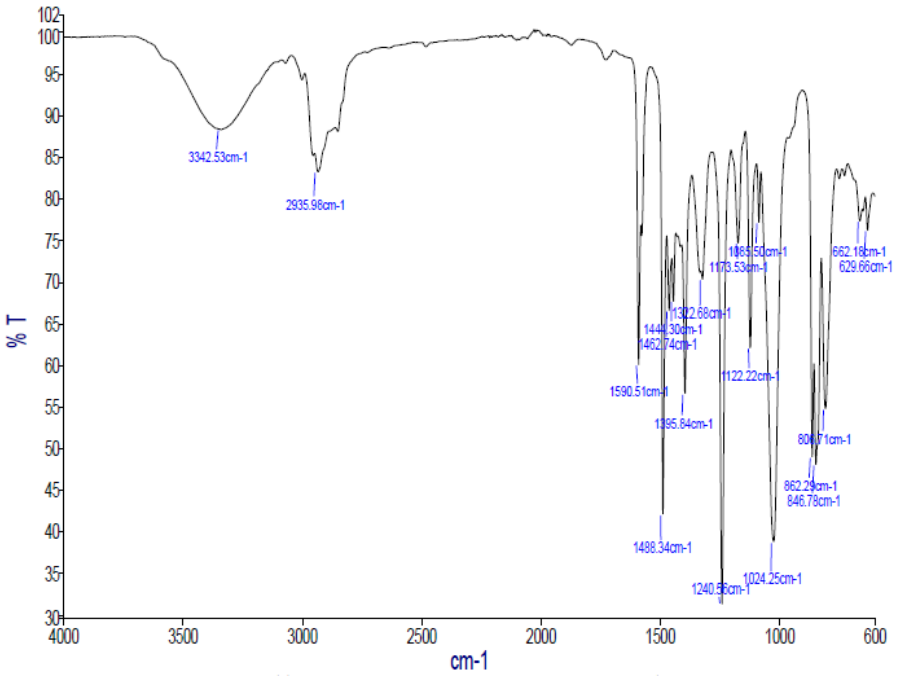
^{1}H-NMR



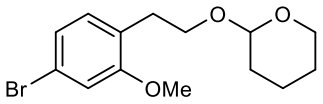
^{13}C-NMR



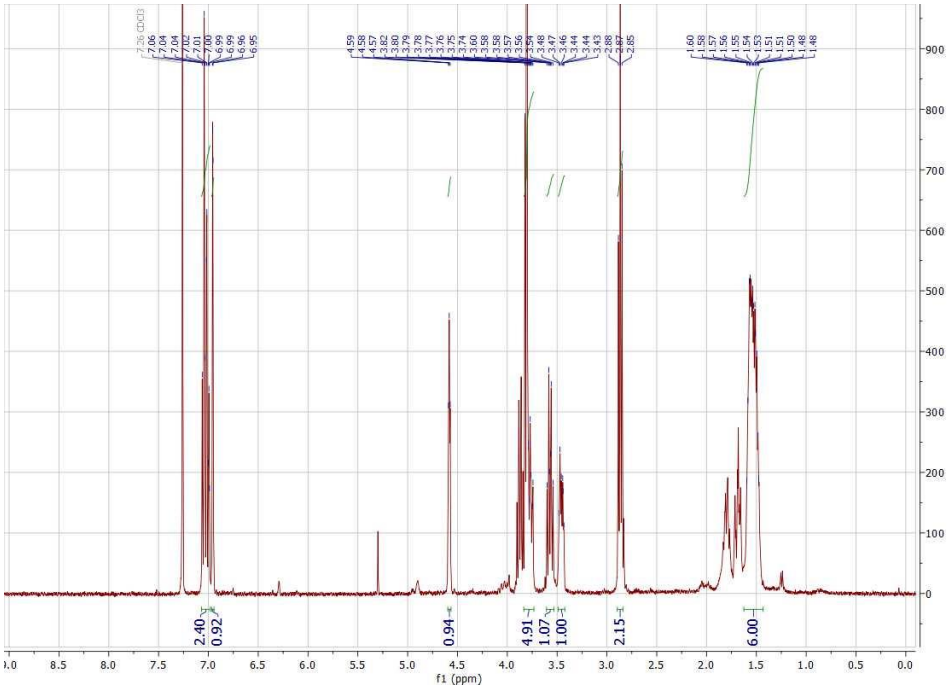
IR (*ATR*) $V_{máx}$ (cm^{-1})



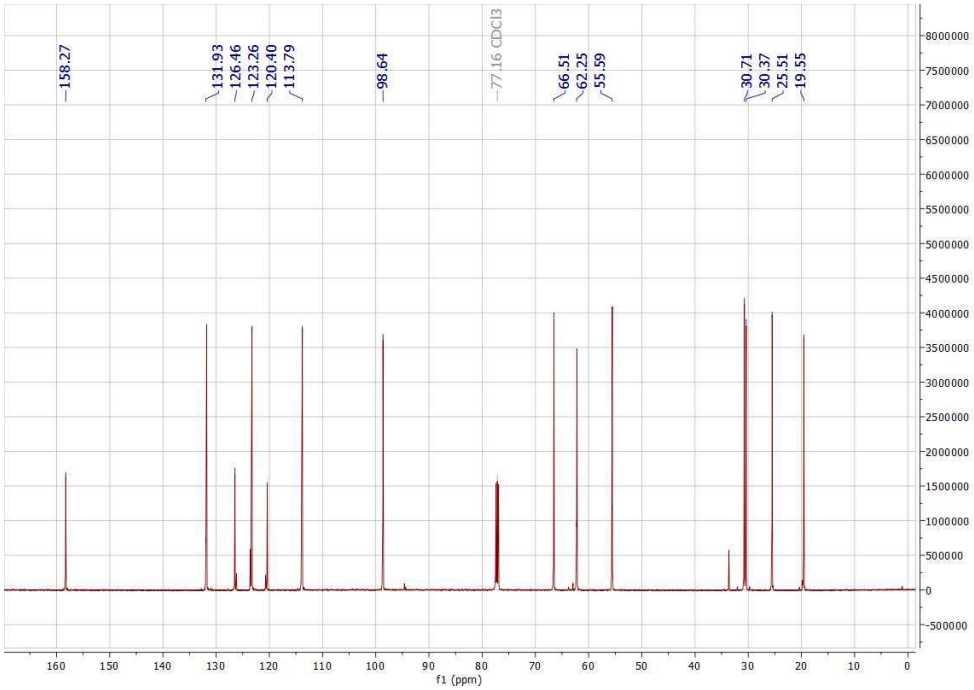
Compound 24



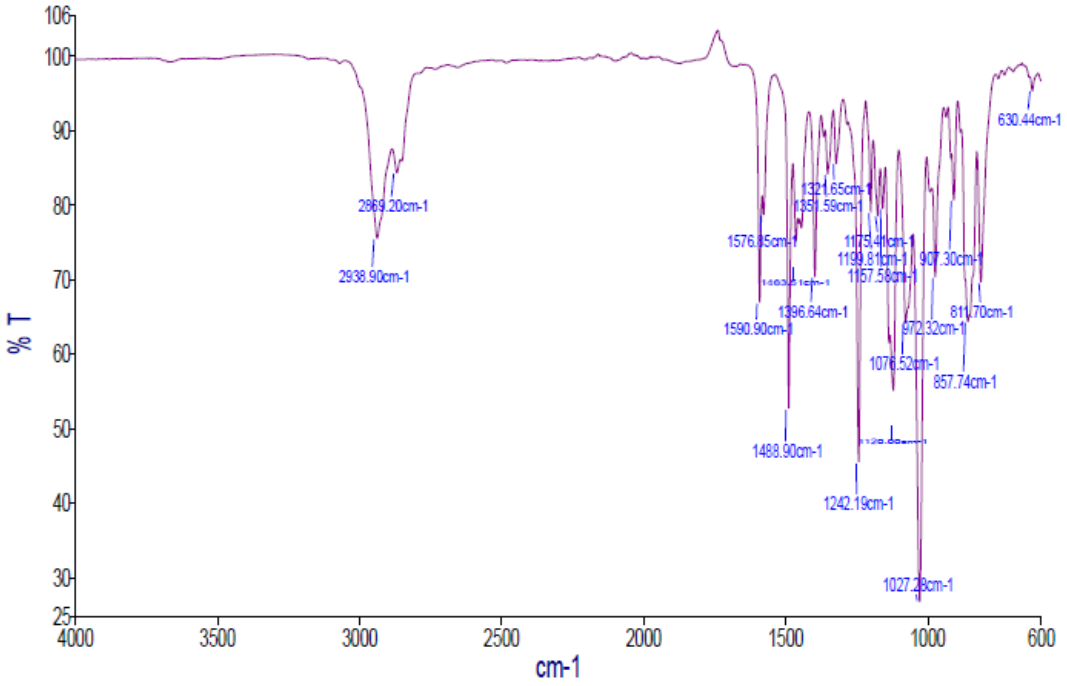
^{1}H-NMR



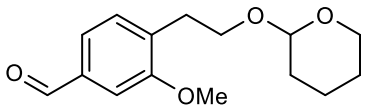
^{13}C-NMR



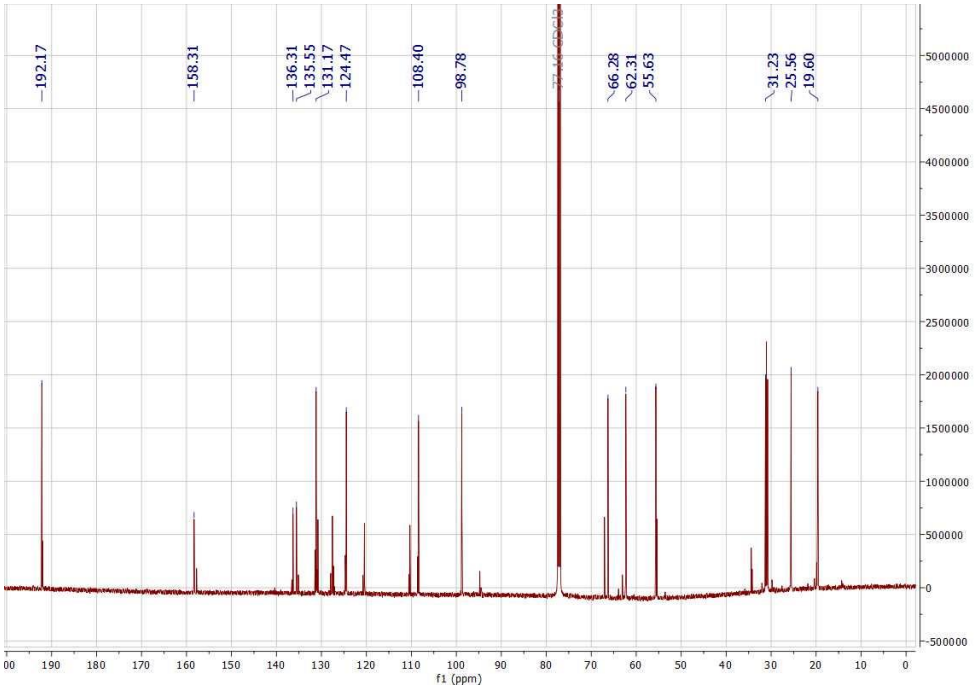
IR (*ATR*) $V_{máx}$ (cm^{-1})



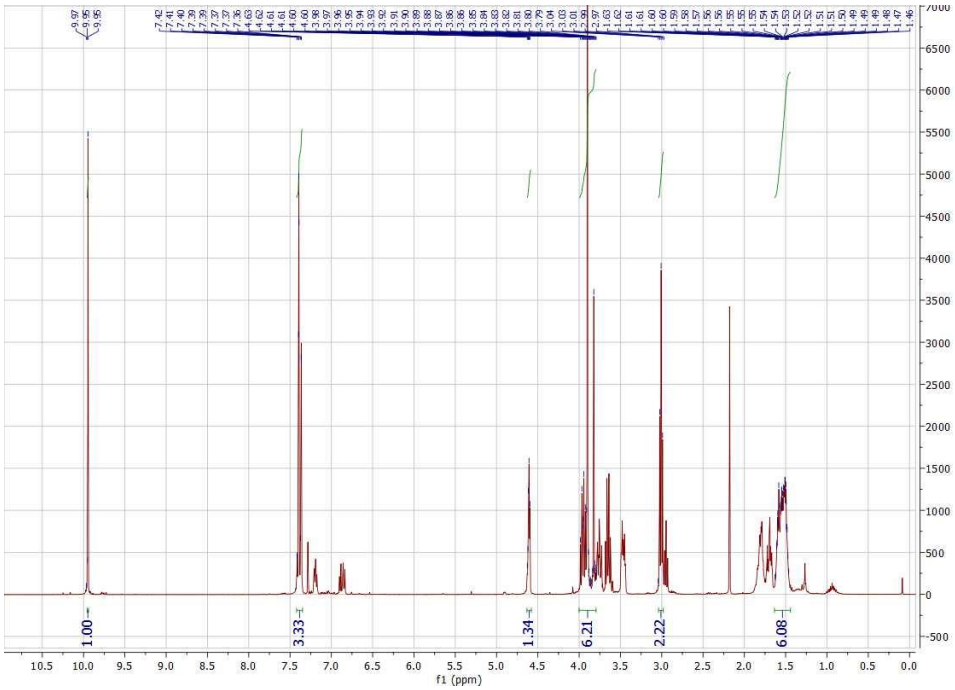
Compound 25



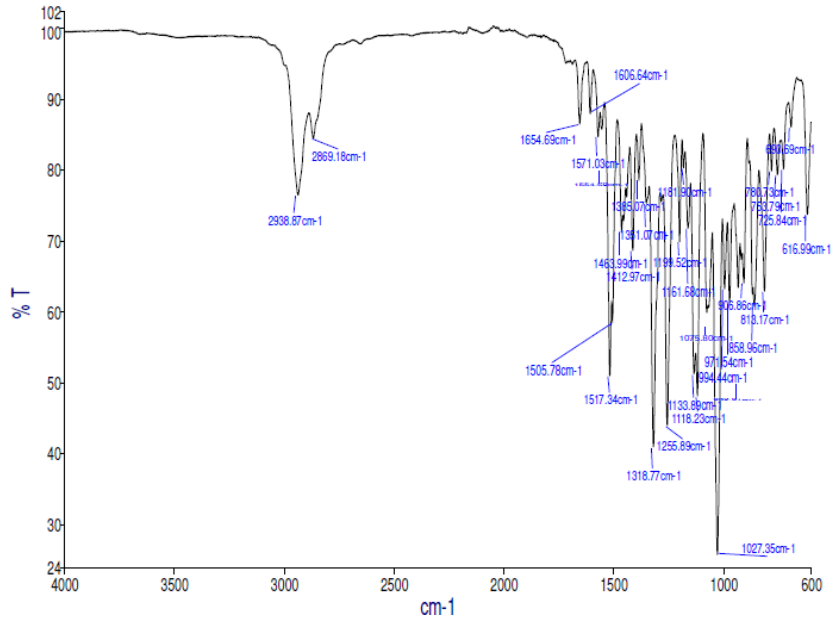
^{1}H-NMR



^{13}C-NMR

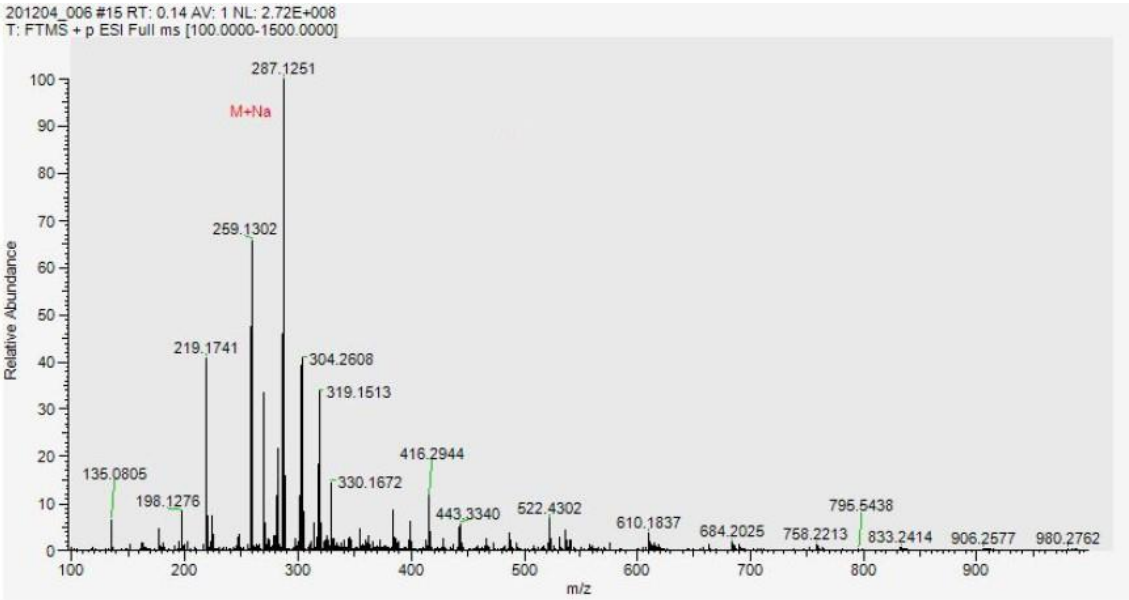


IR (*ATR*) $V_{máx}$ (cm^{-1})

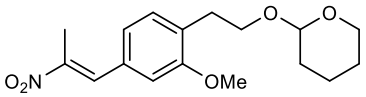


HRMS (ESI)

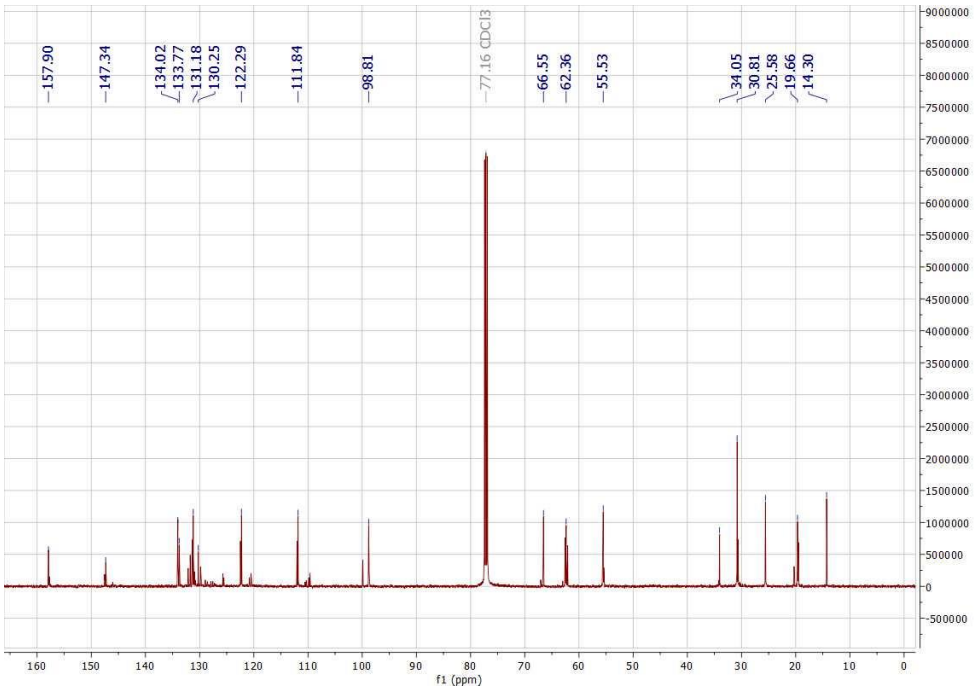
Display Formula	S Fit	RDB	Delta [ppm]	Theo. mass	Combined Score	# Matched Iso.
$C_{13}H_{15}O_2N_6$	45,89	9,5	-0,14	287,1251	92,77	3
$C_{15}H_{20}O_4{}^{23}Na$	46,39	5,5	-1,11	287,12538	92,1	4
$C_{13}H_{23}ON_2{}^{32}S_2$	26,36	3,5	1,5	287,12463	91,74	3
$C_{11}H_{24}O_6{}^{35}Cl$	11,66	-0,5	-1,85	287,12559	77,54	4
$C_{12}H_{19}O_6N_2$	7,684	4,5	4,52	287,12376	77,21	2
$C_5H_{19}O_4N_8{}^{32}S$	6,718	0,5	2,13	287,12445	77,16	1
$C_{10}H_{21}O_2N_4{}^{35}Cl^{23}Na$	5,994	1,5	1,87	287,12452	77,12	3
$C_8H_{20}O_2N_6{}^{23}Na^{32}S$	5,547	1,5	-3,5	287,12607	77,1	3
$C_8H_{16}N_{10}{}^{35}Cl$	4,771	5,5	2,84	287,12424	77,06	2
$C_7H_{21}N_8{}^{35}Cl_2$	3,473	0,5	-3,53	287,12607	76,99	3



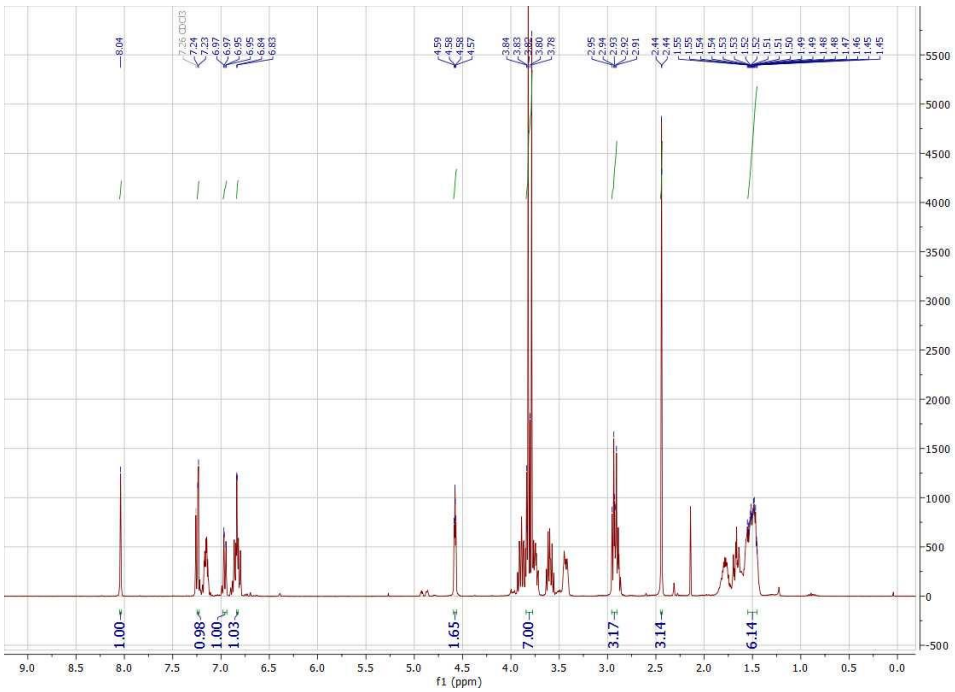
Compound 26



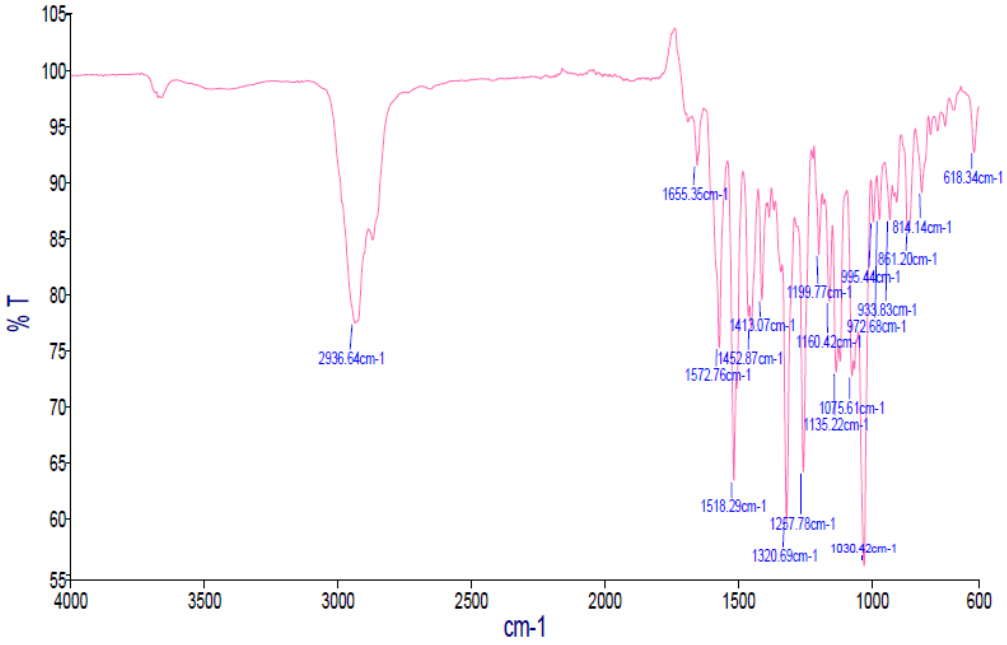
^{1}H-NMR



^{13}C-NMR



IR (*ATR*) $V_{máx}$ (cm^{-1})



HRMS (ESI)

Display Formula	S Fit	RDB	Delta [ppm]	Theo. mass	Combined Score	# Matched Iso.
$C_{15}H_{18}O_3N_7$	43,24	10,5	-1,3	344,14656	96,67	4
$C_{17}H_{23}O_5N^{23}Na$	42,51	6,5	-2,11	344,14684	96,63	4
$C_{15}H_{26}O_2N_3{}^{32}S_2$	18,31	4,5	0,07	344,14609	95,36	4
$C_{17}H_{27}O_2N^{35}Cl^{32}S$	10,88	4,5	4,55	344,14455	94,97	4
$C_{14}H_{22}O_7N_3$	21,76	5,5	2,59	344,14523	93,97	2
$C_{23}H_{22}N^{32}S$	16,41	13,5	-1,83	344,14675	93,83	5
$C_{13}H_{27}O_7N^{35}Cl$	14,61	0,5	-2,73	344,14706	93,59	2
$C_{18}H_{27}N^{23}Na^{32}S_2$	7,652	5,5	-4,63	344,14771	93,23	4
$C_{12}H_{24}O_3N_5{}^{35}Cl^{23}Na$	10,53	2,5	0,38	344,14599	79,1	3
$C_7H_{22}O_5N_9{}^{32}S$	10,48	1,5	0,6	344,14591	79,1	2