

**GC-MS Studies on Methoxymethylene- Substituted Phenethylamines
Related to MDA, MDMA, MDEA and MDMMA**

by

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Abstract

This thesis mainly focused on synthesis and analysis approaches of eight methoxymethylene phenethylamine compounds associated with MDMA and MBDB series structures. Gas chromatography- mass spectrometry (GC-MS) methods were used to separate the target compounds and important intermediates with their homologues or regioisomeric compounds. Gas chromatography- time of flight detector (GC-TOF) was used for the exact mass analysis of characteristic MS fragments.

Unlike the ethoxy phenethylamines, the studied methoxymethylene phenethylamines have a unique fragment of m/z 104 under EI mass spectrometry. Possible mechanism for this m/z 104 fragment is proposed and supportive analytical studies were carried out in this thesis. The eight target compounds are divided into two groups: 4-methoxymethylene amphetamine series and 4-methoxymethylene butanamine series. Underivatized compounds in each group were nicely separated by GC. Heptafluorobutyramide derivatives of 6 derivatizable target compounds were synthesized, and the mass spectrum of the each derivatized compound reveals unique structural information for identification purposes.

Previous studies have revealed the structure-activity relationships and separation approaches for direct, indirect regioisomeric and some isobarics related to MDMA. Especially, the most recent study of MDMA related compounds reveals a unique fragment m/z 107 generated by EI MS of ethoxy ring substituted phenethylamines. The

proposed mechanism for the generation of the m/z 107 involves the engagement of ethoxy oxygen, and we are interested to find out what will happen to this characteristic m/z 107 fragment if the ester oxygen is moved one carbon away toward the side chain end. Thus, it seems necessary to initiate a study of the methoxymethylene phenethylamine series of compounds in order to be compared with the ethoxy phenethylamine homologues, and to further complete the separation study of MDMA with its regioisomeric and isobaric compounds.

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List of Abbreviations

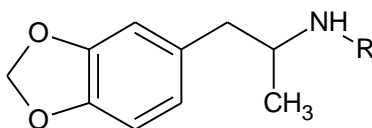
°C	Degree centigrade
5-HT	Serotonin
ACh	Acetylcholine
COMT	Catechol-O-methyltransferase
CSA	Controlled Substances Act
DA	Dopamine
DEA	Drug Enforcement Administration
EI	Electronic ionization
eV	Electron volt
GC	Gas chromatography
GC- TOF	Gas chromatography- time of flight detector
GC-IRD	Gas chromatography coupled to infrared detection
GC-MS	Gas chromatography– mass spectrometry
HFBA	Heptafluorobutyramide
HHA	Dihydroxyamphetamine
HHMA	Dihydroxymethamphetamine
HMA	4-hydroxy-3-methoxy-amphetamine
HMMA	4-hydroxy-3-methoxy methamphetamine
M	Mole per liter

MDA	Methylenedioxyamphetamine
MDEA	Methylenedioxy ethylamphetamine
MDMA	Methylenedioxyphenethylamine
MDMMA	Methylenedioxy-N,N-dimethylamphetamine
min	Minute
mL	Milliliter
mm	Millimeter
mmol	Micro Mole
NA	Noradrenaline
PCC	Pyridinium chlorochromate
PFPA	Pentafluoropropionamide
ppm	Part per million
Red Al	Sodium bis(2-methoxyethoxy) aluminum hydride
μL	Micro liter
μm	Micro meter

1. Literature Review

1.1 Introduction

Methylenedioxyphenethylamines such as 3, 4- methylenedioxyamphetamine (MDA), 3, 4- methylenedioxymethamphetamine (MDMA) and 3, 4- methylenedioxyethylamphetamine (MDEA), are psychoactive compounds with structural similarities to both amphetamine and mescaline. The methylenedioxy-derivatives of amphetamine and methamphetamine represent a large group of designer drugs, and they are popular controlled drugs in Europe and North America. The structures of those compounds are shown in Figure 1.



MDA: R= H

MDMA: R= CH₃

MDEA: R= C₂H₅

Figure 1 Structures of methylenedioxyphenethylamine controlled drugs MDA, MDMA and MDEA

MDMA is the most commonly used derivative of this series and is known by the street names “Ecstasy” or “XTC”. It has both stimulant and hallucinogenic effects in humans and has become one of the major drugs of abuse in recent times. On the street, people call it the “party pill”. It was not an illegal drug until 1985, when its addictive nature of causing hallucination and being neurotoxic was widely discussed. MDMA was moved to the Schedule I controlled drug list by the drug enforcement administration (DEA) of U.S. in 1986 (Lawn, J.C. 1986).

The most common way to administer MDMA is orally, usually in tablet or capsule form, and its effects last approximately four to six hours. Users of the drug say that it produces profoundly positive feelings, empathy for others, elimination of anxiety, and extreme relaxation.

The goal of clandestine manufacturers is to prepare substances with pharmacological profiles that are sought after by the user population. Clandestine manufacturers are also driven by the desire to create substances that fall outside national and/or international control regimes in order to circumvent existing laws and to avoid prosecution. In the USA this has resulted in legislation (Controlled Substances Analog Act) to upgrade the penalties associated with clandestine use of all of these compounds. In Europe, because of the substance-by-substance scheduling method, the appearance of new substances cannot be immediately considered as illicit drugs. This offers room for clandestine experimentation into individual substances within a class of drugs with similar pharmacological profiles. This phenomenon is not only used to bypass the legal regulations but to produce even more potent substances from non-controlled precursors. This has created the continued designer-drug exploration and especially within the MDA series. Thus, identification of new designer drug derivatives is essential and a highly challenging task for forensic laboratories.

MDA, which is a derivative of phenethylamines, has similar pharmacological effects as MDMA. MDA was first synthesized by Mannich and Jacobsohn in 1910 (Mannich and Jacobsohn, 1910). It did not become a popular drug until 1960s and was put on the Schedule I list by the enforcement of Controlled Substances Act (CSA) in 1970s (The green list, 2003).

MDMA was first synthesized by Merck Company, which filed a patent for MDMA in German in 1912. The synthetic procedure for the production of MDMA was first published by Yakugaku Zasshi as a part of antispasmodics research program (Yutaka Kasuya, 1985). Around 1970s, Alexander Shulgin and his colleges studied MDMA's pharmacological effects on human beings, which were first published in 1978. Alexander Shulgin himself, who has been called "the father of MDMA", described the effect of MDMA as bringing "altered state of consciousness with emotional and sensual overtones" to users (Shulgin and Nichols, 1978). Since then and until its schedule I control, MDMA's usage as a psychotherapy drug was adopted by many scientists in Europe and North America.

MDEA is another popular phenethylamine derivative used on the street and now a controlled drug. MDEA has very similar effects in humans as MDMA requiring a slightly higher dosage. Given the high popularity of MDMA and MDA over decades, clandestine labs have every incentive to search for another similar structure which will generate similar pharmacological effects as an analogue or substitution for those two drugs. In 1993, MDEA's drug effects were reported by Tehan and his colleges (Tehan *et al.*, 1993).

The phenethylamine drugs are still among the most popular drugs of abuse today, especially MDMA. Clandestine labs have continued to search for a substitution for MDMA in order to avoid the legal control on this specific molecule. It is very important to work on analytical

methods especially identification and discrimination procedures for the phenethylamine related structures to provide reliable and solid data/evidence for forensic use.

1.2 Pharmacology

MDA, MDMA and MDEA have all been reported to produce very similar central and peripheral effects in humans differing only in potency, time of onset and duration of action. Studies have reported that MDMA is a potent releaser of serotonin (5-HT), dopamine (DA), noradrenaline (NA) and acetylcholine (ACh). More importantly, MDMA can act as a 5-HT uptake inhibitor. The combined “unique behavioral effects of MDMA” results in an increase of extracellular monoamine concentration (Cole J.C and Sumnall H.R, 2003). Although MDMA’s structure is similar to amphetamine, studies reported that they have different pharmacological paths. Unlike amphetamine, which achieves its effect via dopamine (DA) release, drug users experience a new state of consciousness complied with altered mood and reinforced perception of emotions due to high extracellular 5-HT and DA level (Maldonado E. and Navarro J.F, 2000).

Clinic reports show panic attacks, depression, flashbacks and psychosis, indicating MDMA’s effect of changing neurotransmitter level inside the brain is not a temporary effect (White S.R. *et al.*, 1996). Lab animal studies show that after the last injection of MDMA, changes in 5-HT and DA neurotransmission in the central nervous system (i.e. brain) will last as long as two weeks. The study suggested that frequent users of MDMA are likely to experience relatively more harmful effects (White S.R. *et al.*, 1996).

1.3 Negative effects

Although MDMA was put on schedule I controlled list by DEA in 1986, it is still widely used. Some researchers and clinician believe that the ban on MDMA was only based on animal

studies. The following few years, many studies focused on MDMA's effects on human beings and were carried out all over the world.

It was reported the immediate physical and psychological effects attributed to MDMA use by humans include (Richard S.C., 1995): euphoria, increased energy, sexual arousal, paranoia, anxiety, depression, papillary dilation, bruxism, lower back pain, and nausea. Long term residual effects attributed to MDMA use in humans include (Richard S.C., 1995): depersonalization, insomnia, depression, flashbacks, lower back pain, neck hyper tonicity, joint stiffness, acne/skin rash, frequent headaches, and frequent stomach cramps.

Other common effects that have been reported are trismus and vomiting in recreational users (Greer and Toibert, 1986); hallucination and papillary dilation (Brown and Osterloh, 1987). Other long lasting residual effects reported are blurred vision and muscle hyper tonicity (Hayner and Mc Kinney, 1986).

1.4 Neurotoxicity

Until early this century, the mechanism of MDMA's neurotoxicity in humans had not been directly demonstrated and proven. It is believed the mechanism is related to oxidative stress, hyperthermia and increased extracellular concentration of dopamine (Sanchez *et al.*, 2001). Further studies revealed that MDMA's neurotoxicity is related to MDMA's ability to reduce the uptake of both synaptosomal and vesicular serotonin and dopamine depending on the dosage, while glutamate and γ -aminobutyric acid (GABA) uptake process remains unaffected (Bogen *et al.*, 2003). The serotonergic neurotoxicity is the most accepted mechanism to explain and predict MDMA's long lasting negative effects on the neurosystem. Yet, the answer to the concern over

how the animal toxicity study results relate to the human condition is still not clear (Lyles J. and Cadet J. L., 2003).

1.5 Metabolism

The metabolism of MDMA involves two major processes: O-demethylation generates the major metabolite 3, 4-dihydroxymethamphetamine (HHMA); while N-demethylation generates methylenedioxyamphetamine (MDA). Further O-demethylation of MDA results in 3, 4-dihydroxyamphetamine (HHA). Metabolism of HHMA and HHA by catechol-O-methyltransferase (COMT) will generate 4-hydroxy-3-methoxy methamphetamine (HMMA) and 4-hydroxy-3-methoxy-amphetamine (HMA) respectively (Lyles J. and Cadet J. L., 2003). The formation of HHA and HHMA did not produce neurotoxicity (McKenna D. J. and Peroutka S. J., 1990). The HHMA is metabolized to quinone-like structures which were thought to contribute to MDMA's neurotoxicity (Hiramatsu M. *et al.*, 1990).

1.6 Analytical methods used to identify and separate phenylethylamine regioisomers and isobars related to MDMA, MDA and MDEA

Regioisomeric relationships are the result of different positions of attachment of functional groups in compounds that possess the same molecular formula (elemental composition). Isobaric substances are of the same nominal mass but different elemental compositions. There are mainly three types of regioisomeric and isobaric compounds of MDMA: (1) direct regioisomers of MDMA include side chain and methylenedioxy substitution pattern; (2) indirect regioisomers include methoxymethcathinones; and (3) isobaric substances include four major types of substitution patterns which are (i) methoxymethyl ring substitution patterns; (ii) ethoxy ring

substitution pattern; (iii) methoxy group on the ring and methyl group on the side chain pattern; and (iv) methoxymethylene ring substitution pattern.

The direct regioisomers include five arrangement possibilities of the side chain all yielding an m/z 58 cation fragment in their EI mass spectrum, shown in Figure 2. Those 5 regioisomeric structures will all yield the base peak m/z 58 under mass spectrometry; the structures of those m/z 58 peaks are shown in Figure 3.

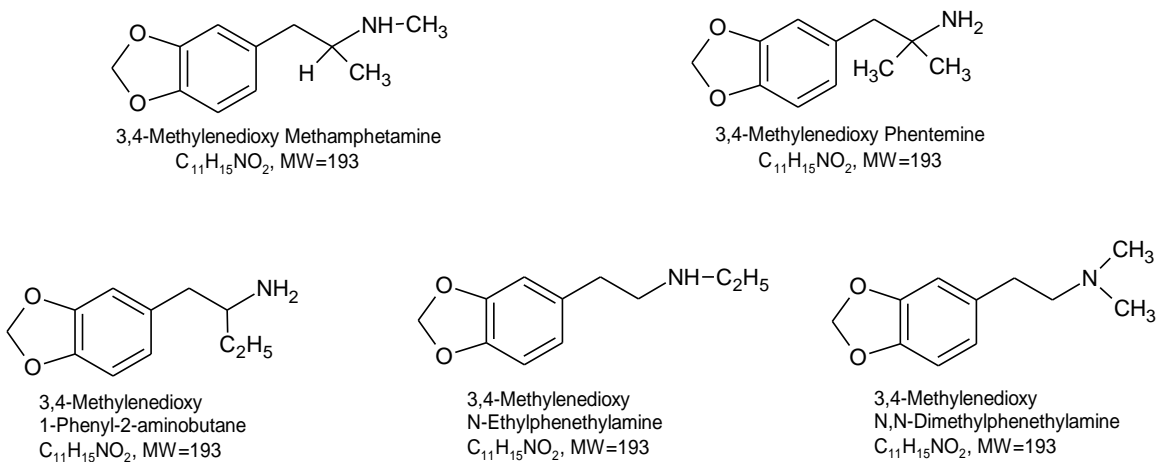


Figure 2 Regioisomeric side chains patterns yielding m/z 58 cation in the 3, 4-methylenedioxy methamphetamine series

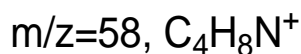
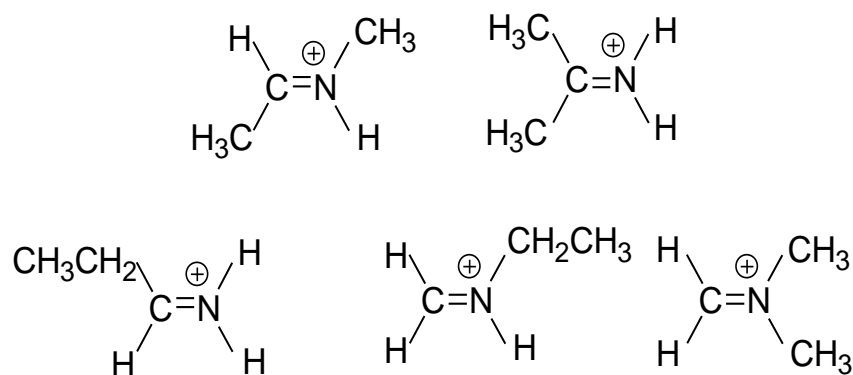


Figure 3 Ion structures of regioisomeric m/z 58 generated by side chain regioisomers of MDMA under EI mass spectrometry

With the mass 58 side chain arrangement possibilities abbreviated as 58, the regioisomeric and isobaric compound structures related to MDMA discussed above are shown in Figure 4. The first structure shows direct regioisomers of MDMA, with 10 possible different compounds; the second structure shows indirect regioisomers of MDMA, with 15 possible different compounds; and the last four structures stands for the four most likely types of isobaric compounds related to MDMA, with a total of 95 possible different compounds. Thus the total number of compounds represented by the general structures in Figure 4 is 120 compounds of nominal molecular weight 193 and an EI base peak of m/z 58.

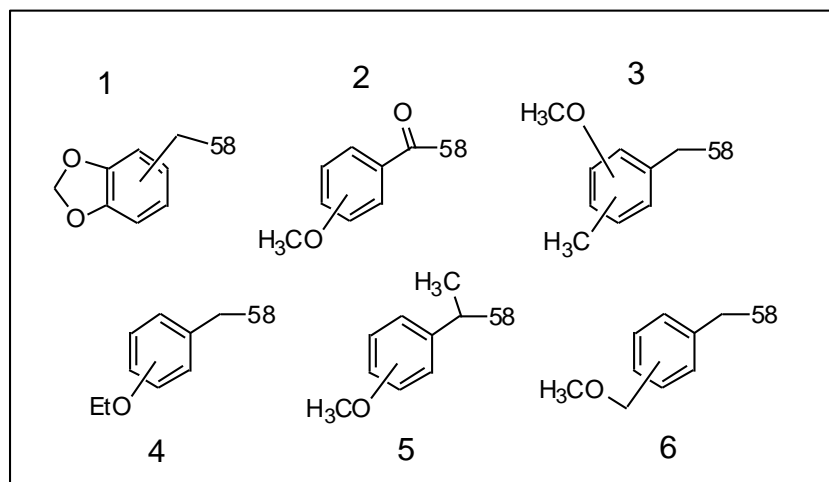


Figure 4 Regioisomeric and isobaric compound structures of MDMA

Among the 120 regioisomeric and isobaric compounds related to MDMA, previous studies show identification and separation analytical procedures on direct regioisomer methylenedioxy phenethylamines, indirect regioisomer methoxymethcathinones, and isobaric substances such as methoxymethyl ring and side chain substitutive phenethylamine as well as ethoxy ring substitutive phenethylamine isobarics. The first published study used mass spectrometry to separate 3, 4-MDMA from other phenethylamines in 1988 by Noggle *et al.* Following studies mainly focused on GC-MS separations of derivatized phenethylamines in order to overcome the limitation of EI mass spectrometry. GC-IRD analysis on derivatized or underivatized phenethylamines is another widely discussed topic studied by many forensic scientists.

1.6.1 Analytical studies of direct regioisomers of MDMA

Effects of the side chain on compounds was studied by the separation of methamphetamine and its four other regioisomers (Figure 5) in 2009 (Awad T. *et al.*, 2009). The studied five mass equivalent compounds have the same molecular weight of 149 with a base peak of m/z 58 under EI mass spectrometry.

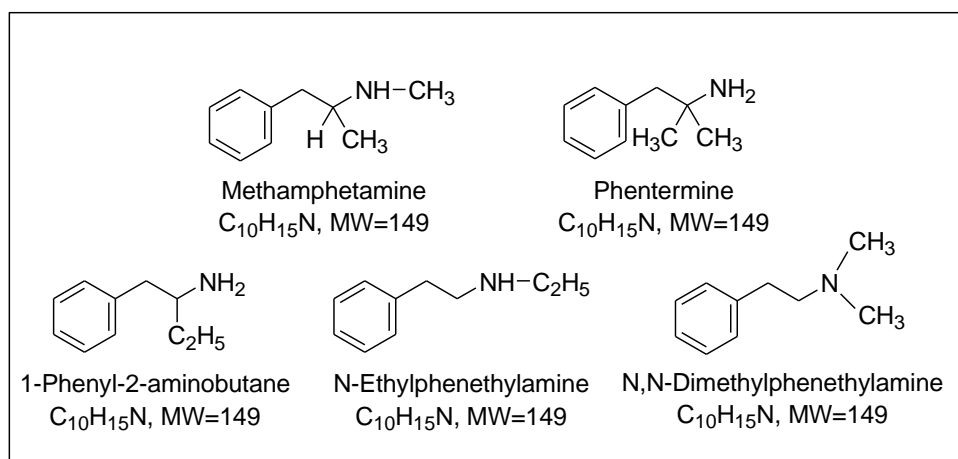


Figure 5 Structures of the five side chain regioisomeric phenethylamines

The study reported that trifluoroacetyl derivatives of the primary and secondary amines yield unique fragment ions for identification purposes. The underivatized compounds can be nicely separated by gas chromatography and show unique vapor phase IR spectra.

The ten direct regioisomers of MDMA generated from side chain and methylenedioxy substitution patterns shown in Figure 6 were reported being separated by GC, the ultimate separation results were obtained using the polar stationary phase DB-35 MS (Laura A., *et al*, 2004).

Those 10 compounds were also separated by the study of their perfluoroacyl derivatives under GC-MS. After being converted into their perfluoroacyl derivatives, the ten direct regioisomers of MDMA show elution differences under GC using nonpolar stationary phases, such as Rtx-1 and Rtx-5. The results of mass spectra of these ten compounds are significantly individualized, thus specific side-chain identification is possible based on unique fragment ions (Awad T., DeRuiter J. and Clark C. R., 2005). Previous studies also show that the perfluoroacylated 3, 4-MDMA will yield some specific fragments that can be specifically

identified (Belal T. *et al.*, 2009). The compound structures reported being separated are shown in Figure 6.

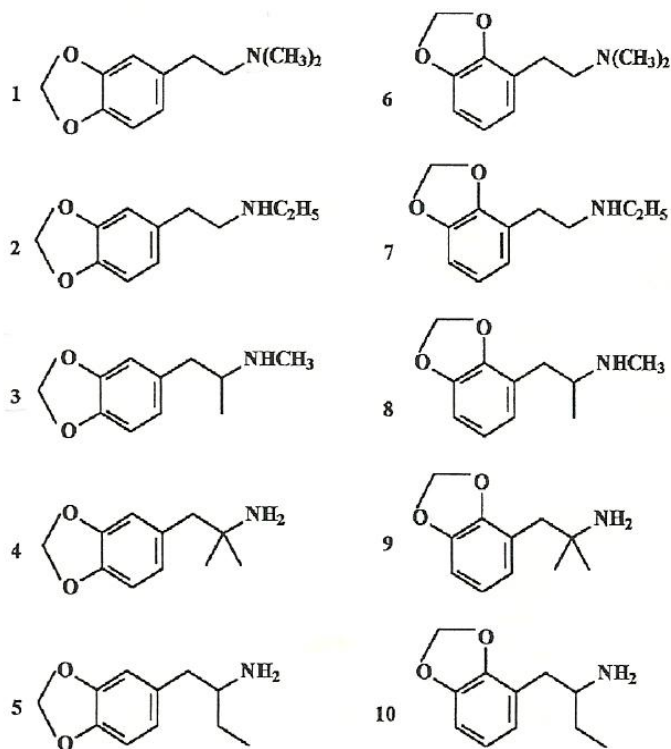


Figure 6 Structures of the ten direct regioisomers related to MDMA

1.6.2 Analytical studies of indirect regioisomers of MDMA

The separation of three methoxymethcathinones (with the same side chain arrangement pattern as MDMA) from 3, 4-MDMA and 2, 3-MDMA was reported by Belal T. *et al* in 2009. The structures being separated are shown in Figure 7. While mass spectrometry is unable to differentiate methoxymethcathinones from 3, 4-MDMA and 2, 3-MDMA since they are of mass spectra equivalence (both methoxymethcathinones and MDMA have molecular weight at 193 and the only significant fragments of those compounds under mass spectrometry are the m/z 58 and m/z 135 or 136 ions), the study adopted GC-IRD and successfully separated target

compounds. The methoxymethcathinones can be identified without chemical derivatization based on the fact that the carbonyl group of methoxymethcathinones shows unique infrared absorption bands in the 1690-1700 cm^{-1} range. Moreover, the study also indicates that the three methoxymethcathinones can also be separated from 3, 4-MDMA and 2, 3-MDMA by GC using Rxi-50 as a stationary phase (Belal T. *et al.*, 2009).

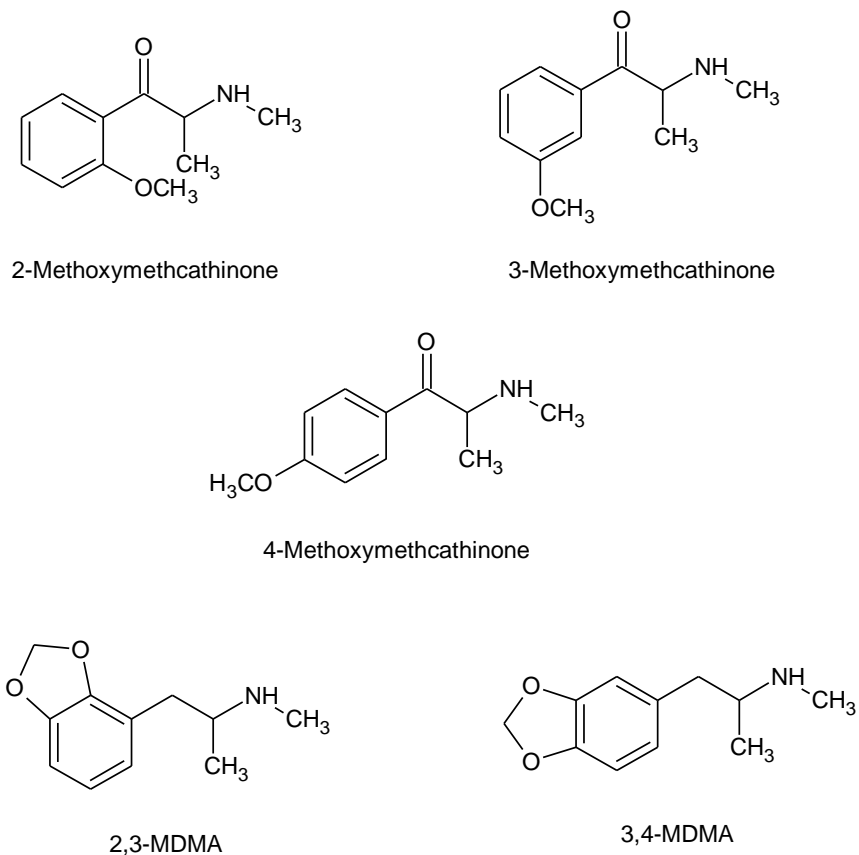


Figure 7 Methoxymethcathinone and MDMA structures separated by Belal T. *et al.*, 2009

1.6.3 Analytical studies of methoxymethyl ring substitutive compounds related to MDMA

Among the fifty methoxy methyl ring substituted isobarics related to MDMA, the most thoroughly studied group of compounds are the methoxy methyl methamphetamines, which have the same side chain arrangement pattern as MDMA and also have the mass spectra equivalent to

MDMA (both methoxy methyl methamphetamines and MDMA have molecular weight at 193 and the only significant fragments of those compounds under mass spectrometry are the m/z 58 and m/z 135 or 136 ions). A previous study showed that perfluoroacyl derivatives, such as pentafluoropropionamides (PFPA) and heptafluorobutyramides (HFBA), of methoxy methyl methamphetamines with methoxy group at 2- or 4- position will yield unique ions under mass spectrometry and can be identified from related MDMA. It is also reported that methoxy methyl methamphetamines can be successfully separated from 2, 3-MDMA and 3, 4-MDMA in the PFPA and HFBA derivative forms by GC with non-polar stationary phases (Awad T., DeRuiter J. and Clark C.R., 2007). Structures studied are shown in Figure 8 .

Further studies of 3-methoxy-4methyl- and 4-methoxy-3methyl-phenethylamines show the results that trifluoroacetyl derivatives provide unique fragment ions for identification purposes. These derivatives also show excellent resolution on GC with a non-polar stationary phase, such as Rtx-1 (Belal T. *et al.*, 2008).

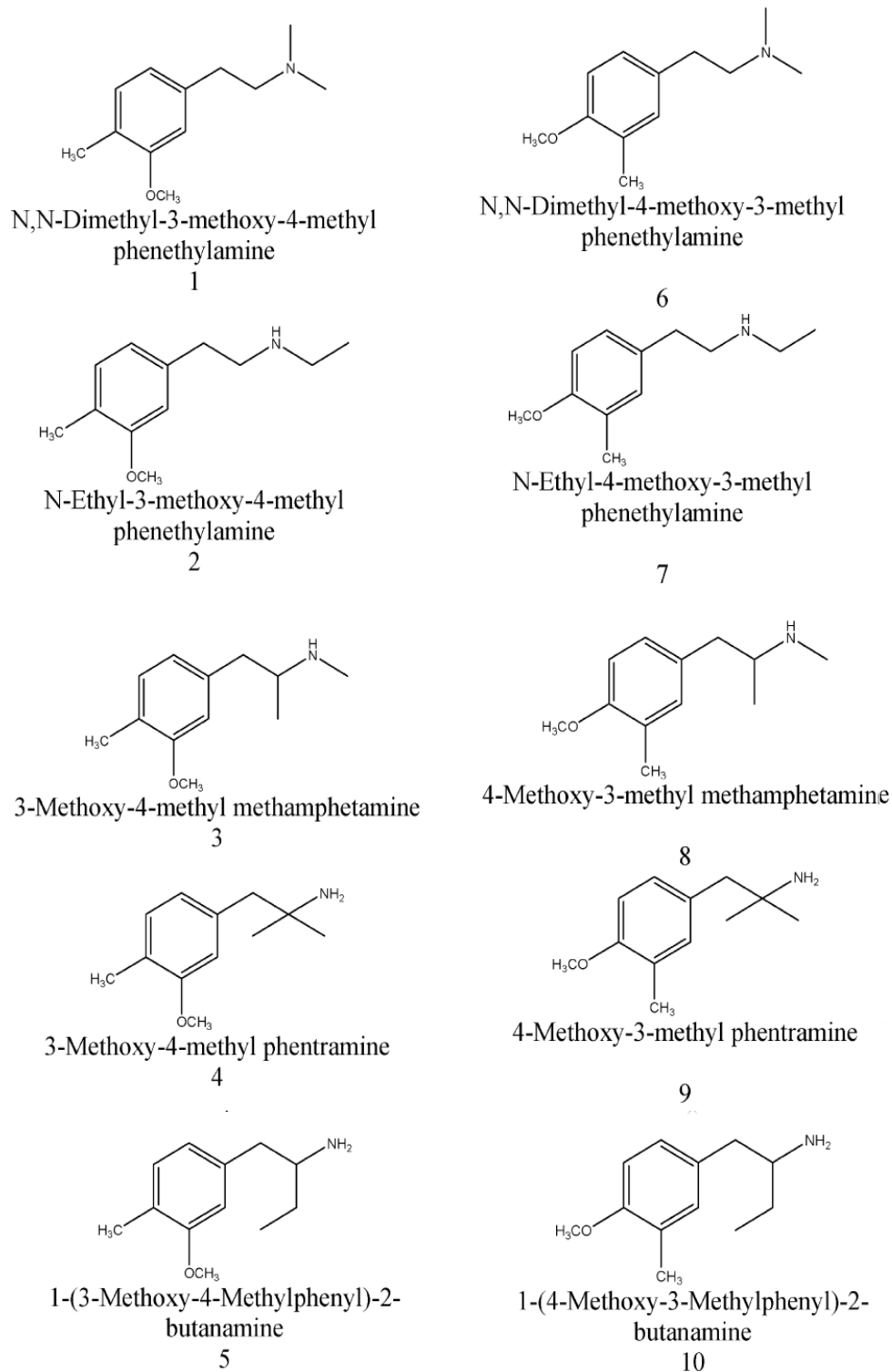


Figure 8 Methoxy methyl methamphetamines structures studied by Awad T., DeRuiter J. and Clark C.R., 2007

1.6.4 Analytical studies of ethoxy ring substitutive compounds related to MDMA

GC-IRD used to separate ethoxy ring substituted isobarics related to MDMA from 3, 4-MDMA and 2, 3-MDMA was reported (Belal T. *et al.*, 2009). It was also reported that capillary GC using the stationary phase Rxi-50 will give satisfactory separation between the side chain regioisomers and the ethoxy substituted methamphetamines.

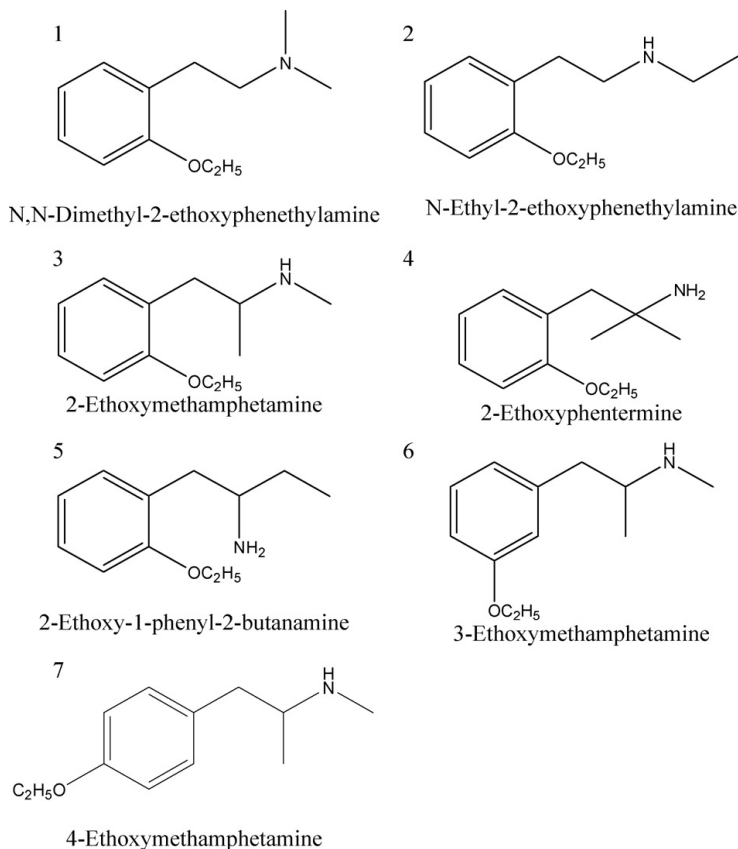


Figure 9 Structures of ethoxy ring substituted isobarics related to MDMA studied by Belal T. *et al.*, 2009

Abdullah M. A. *et al.* reported a unique m/z 107 cation generated by perfluoroacyl derivatives of the ring substituted ethoxy methamphetamines under EI mass spectrometry (Al-Hossaini A. M. *et al.*, 2010). The existence of the m/z 107 fragment is an indicator of the ethoxy

phenethylamine structure. A proposed mechanism of this unique m/z 107 fragment is shown in Figure 10.

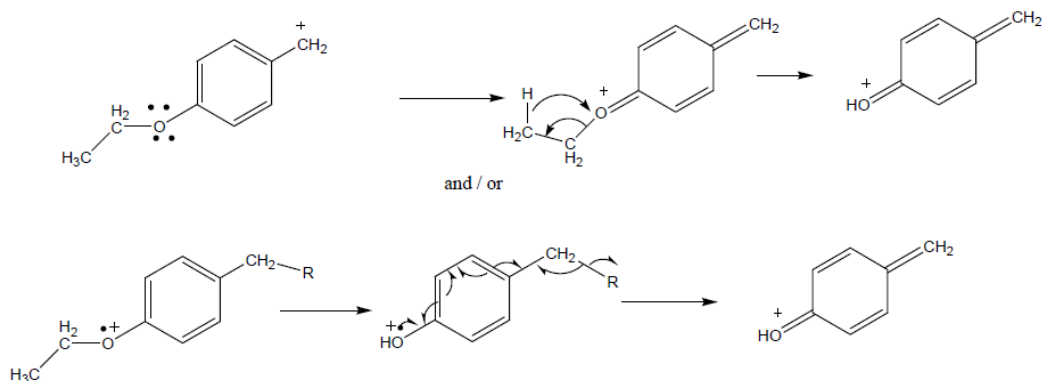


Figure 10 Mechanism of mass spectral fragment m/z 107 cation generated by ethoxy phenethylamines

This proposed mechanism yielding the m/z 107 fragment involves the ethoxy oxygen. It is necessary to find out what if the ethoxy group is substituted with regioisomeric methoxymethylene group and the key oxygen is one more carbon away? In order to answer this question, this thesis is based on a study of a series of compounds with the methoxymethylene ring substitution pattern.

1.7 Statement of research purposes

As mentioned in the previous discussion, the three major types of regioisomeric and isobaric compounds, a total 120 different structures (shown in Figure 4), are of mass spectra equivalence and not identifiable under mass spectrometry alone. They all have the same molecular weight 193, the only significant fragments of those compounds under mass spectrometry are the m/z 58 and m/z 135 or 136 ions. Figure 11 shows the major fragment ions of some regioisomeric ring

substituted methamphetamines under EI mass spectrometry. Since the majority of forensic labs use MS information as the predominant data set for identification purposes, it is a huge challenge for them to identify controlled ring substituted methamphetamines from uncontrolled regioisomers or isobarics with a similar structure.

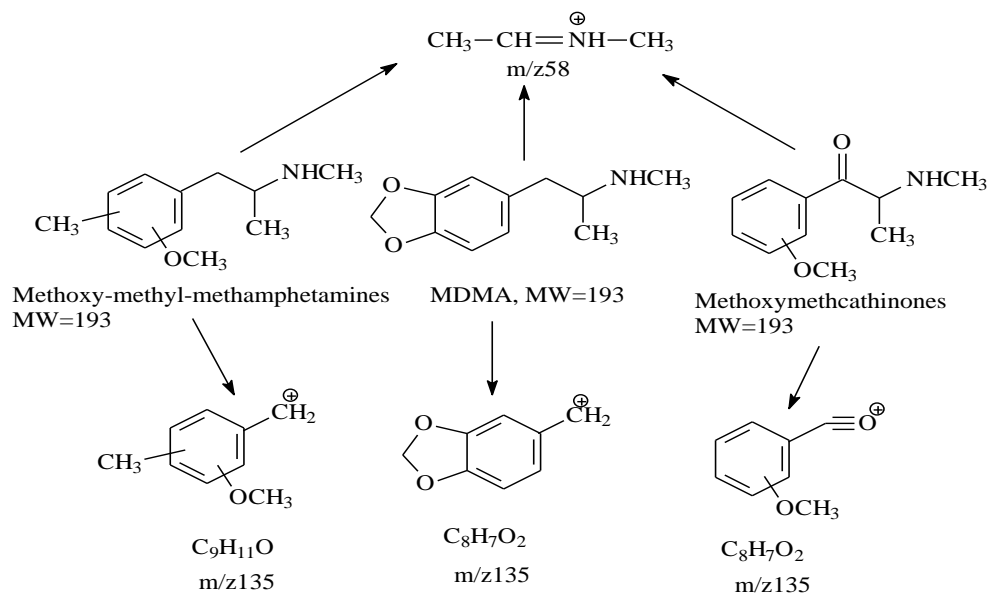


Figure 11 Major fragment ions for ring substituted methamphetamines

Previous studies discussed earlier show that the MDMA separation/identification hardship generated by the existence of the possibility of direct regioisomer phenethylamines, indirect regioisomer methoxymethcathinones, and methoxymethyl ring substitutive phenethylamine isobarics and ethoxy ring substituted phenethylamine isobarics have been successfully solved by the adoption of acylation, perfluoroacyl derivatives and GC-IRD. Now it is necessary to study the properties of methylene methoxy ring substitutive phenethylamine isobarics and establish identification approaches for them from MDMA; there are several reasons for this:

- 1) Successful identification approaches for methylene methoxy ring substituted phenethylamine isobarics from MDMA will contribute another portion to the file of

identification of MDMA from its mass spectra equivalent structures. This is essential for a complete set of data on the forensic chemistry of these compounds.

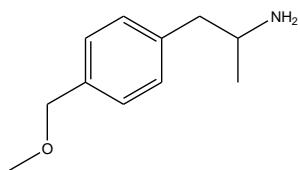
- 2) For structural analysis purposes, it is meaningful to find out how will the methoxymethylene oxygen affect compounds' fragmentation pattern under mass spectrometry compare to ethoxy ring substituted phenethylamines.
- 3) It is also useful to study how the methoxymethylene oxygen will affect the fragmentation pattern of the synthetic intermediates, such as benzaldehyde and phenylacetone, under mass spectrometry compare to those synthesis intermediates of ethoxy ring substituted phenethylamines.

This thesis is mainly focused on eight compounds related to MDMA and MBDB series. The structures of the target compounds in this study are shown in Figure 12 .

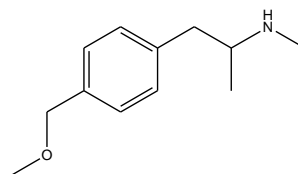
This thesis will focus on the following goals:

- 1) Chemical synthesis of the eight methoxymethylene substituted phenethylamines related to MDMA, MDA and MDEA.
- 2) Create analytical profiles for each compound and some of the related intermediates using the following analytical techniques: GC-MS and GC-TOF.
- 3) Design isotope labeling and regioisomer comparison procedures to confirm or rationalize fragment ion structures under mass spectrometry.
- 4) Establish effective separation approaches of the eight methoxymethylene substituted phenethylamines; document the unique GC-MS analytical information for each compound.

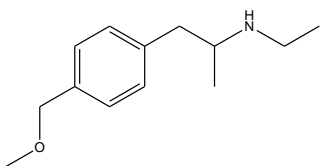
4-Methoxymethylene amphetamine series



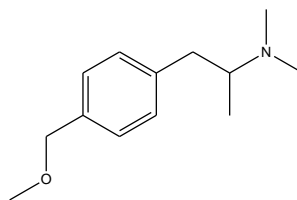
4-Methoxymethylene Amphetamine



4-Methoxymethylene Methamphetamine

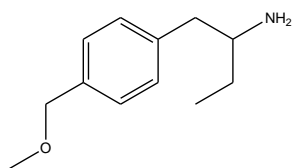


4-Methoxymethylene N-Ethylamphetamine

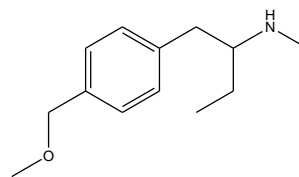


4-Methoxymethylene
N,N-Dimethylamphetamine

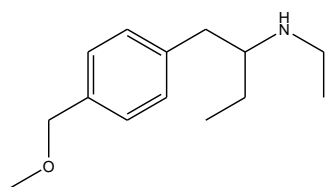
4-Methoxymethylene Butanamine series



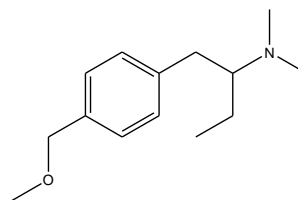
4-Methoxymethylene 2-Butanamine



4-Methoxymethylene N-methyl 2-Butanamine



4-Methoxymethylene N-ethyl 2-Butanamine



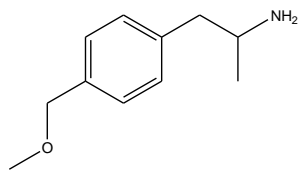
4-Methoxymethylene
N,N-dimethyl 2-Butanamine

Figure 12 Compound structures involved in this thesis

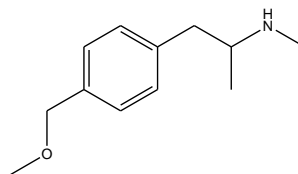
2. Synthesis of regioisomeric and isobaric methoxymethylene substituted phenethylamines

3, 4-Methylenedioxyamphetamine (3, 4-MDMA) is a schedule I controlled drug according to the U.S. Drug Enforcement Administration (DEA). Forensic scientists must specifically identify 3, 4-MDMA in forensic evidence in legal matters including drug issues. This level of identification standard includes the elimination of possible regioisomeric and isobaric compounds as interfering substances. Methylenedioxyamphetamine (MDA), methylenedioxyamphetamine (MDMA) series, methylenedioxyethylamphetamine (MDEA) and methylenedioxy-N,N-dimethylamphetamine (MDMMA) series of compounds and their regioisomers and isobarics create extreme difficulties for the discrimination of 3, 4-MDMA. Those compounds will yield similar gas chromatography-mass spectrum results as that for 3, 4-MDMA. This research mainly focuses on eight methoxymethylene substituted phenethylamines related to MDA, MDMA, MDEA and MDMMA. These eight compounds include four of the 4-methoxymethylene amphetamine series: 4-methoxymethylene amphetamine, 4-methoxymethylene methamphetamine, 4-methoxymethylene ethylamphetamine and 4-methoxymethylene dimethylamphetamine, and four of 4-methoxymethylene butanamine series: 4-methoxymethylene butanamine, 4-methoxymethylene N-methyl butanamine, 4-methoxymethylene N-ethyl butanamine and 4-methoxymethylene N, N-dimethyl butanamine. The structures of those eight compounds are shown in Figure 13.

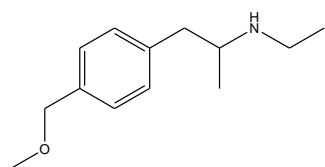
4-Methoxymethylene amphetamine series



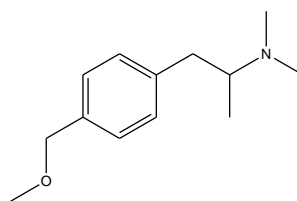
4-Methoxymethylene Amphetamine



4-Methoxymethylene Methamphetamine

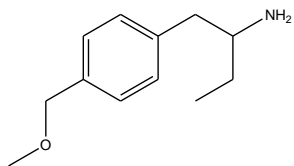


4-Methoxymethylene N-Ethylamphetamine

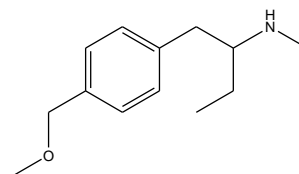


4-Methoxymethylene
N,N-Dimethylamphetamine

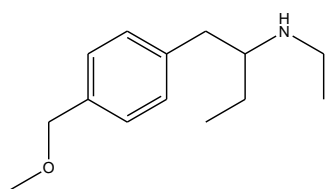
4-Methoxymethylene Butanamine series



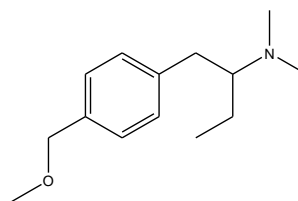
4-Methoxymethylene 2-Butanamine



4-Methoxymethylene N-methyl 2-Butanamine



4-Methoxymethylene N-ethyl 2-Butanamine



4-Methoxymethylene
N,N-dimethyl 2-Butanamine

Figure 13 Structures of the eight target compounds studied in the research

Studying analytical characteristics of these eight compounds is critical because they are expected to share similar cleavage patterns to MDA, MDMA, MDEA and MDMMA compounds under EI mass spectral conditions. Previous studies show that the two most significant peaks the

MDA compounds will yield under mass spectrum are m/z 44 and m/z 135, the MDMA compounds will yield under mass spectrum m/z 58 and m/z 135, MDEA and the MDMMA compounds will yield under mass spectrum m/z 72 and m/z 135. There are two model compounds that serve as comparison points for this project. The first are the MDA-type compounds and the target methoxymethyl compounds have an isobaric relationship to the MDA compounds. The second set of model compounds for comparison are the ethoxy substituted phenethylamines and the methoxymethyl series are regioisomeric based on the position of the oxygen in the side chain group. In this study each of the eight target regioisomeric or isobaric compounds related to MDA, MDMA, MDEA and MDMMA were synthesized in order to study their analytical properties and find an efficient approach to differentiate them from the model compounds. In this chapter, synthetic approaches of these eight compounds are described, while their analytical properties and separation results are discussed later in this thesis.

2.1 Synthesis of 4-methoxymethylene benzaldehyde

4-Methoxymethylene benzaldehyde is a key intermediate in this project. It is the central precursor substance for the synthesis of all the target compounds.

Commercially available 4-chloromethylbenzoyl chloride was treated with methanol and solid sodium metal as catalyst to yield methyl 4-methoxymethyl benzoate. Based on our experience with this reaction, the methyl ester formed by methanol displacement of the chloride of the acid chloride functionally occurs just by dissolving the substrate material in methanol. Thus the intermediate 4-chloromethyl substituted methylbenzoate is the first product formed and without the addition of sodium metal is often present as a major product along with the desired 4-methoxymethylene substituted methylbenzoate. The addition of sodium metal to form the

methoxide anion allows for the complete displacement of the benzylchloride and the complete conversion of the starting material to the desired product.

The strong reducing agent sodium bis (2-methoxyethoxy) aluminum hydride solution (Red-Al) in toluene can reduce the ester functional group in 4-methoxymethyl benzoate methyl ester to give the alcohol and yield 4-methoxymethyl benzyl alcohol. The primary alcohol group was then converted into an aldehyde and this requires a special oxidant which can stop the oxidization at the aldehyde stage with no further oxidation occurs. Treat 4-methoxymethyl benzyl alcohol with fresh PCC, converted the 4-methoxymethyl benzyl alcohol into 4-methoxymethylene benzaldehyde. The entire reaction sequence for getting the desired precursor aldehyde, 4-methoxymethylene benzaldehyde, is outlined in Figure 14.

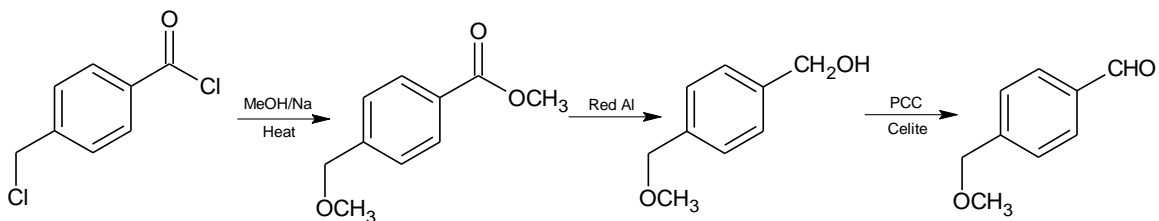


Figure 14 Synthesis procedures for 4-methoxymethylene benzaldehyde

2.2 Synthesis of 4-methoxymethylene phenylacetone and 4-methoxymethylene phenyl-2-butanone

The synthesis of the two homologous ketones, 4-methoxy methylene phenylacetone and 4-methoxymethylene phenyl-2-butanone, is a three step sequence including formation of the imine adduct between the precursor aldehyde and butylamine. This step is followed by condensation of the imine with nitroethane or 1-nitropropane to give the corresponding nitroalkene. The

nitroalkenes were then subjected to reductive hydrolysis. The complete reaction sequence is outlined in Figure 15.

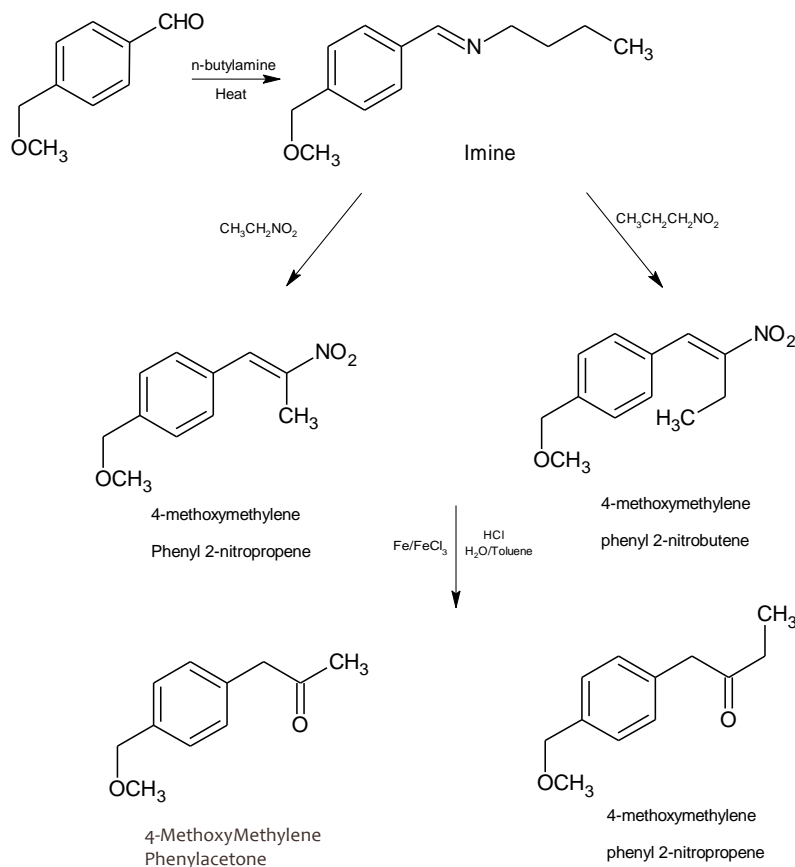


Figure 15 Synthesis of 4-methoxymethylene phenylacetone and 4-methoxymethylene phenyl-2-butanone

The first step of the reaction sequence is the nucleophilic addition of n-butylamine and 4-methoxymethylene benzaldehyde, electrons on the nitrogen will attack the aldehyde carbon and yield the carbinolamine intermediate. This is followed by dehydration under heating, to give the imine. This mechanism is shown in Figure 16. Since the nucleophilic addition reaction is an equilibrium reaction, it is important to remove the water generated to keep the reaction moving

in the forward direction toward the desired imine. In the research, we used a Dean Stark trap to remove the water formed in the equilibrium reaction.

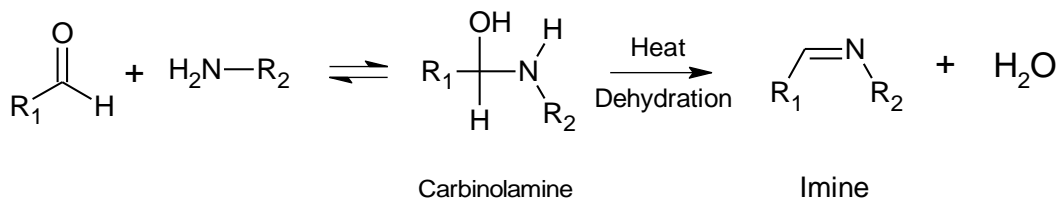


Figure 16 Mechanisms for imine formation from an aldehyde and a primary amine

The next step is to get the corresponding nitroalkene from the reaction between nitroalkane and imine intermediate. The α -carbon of nitroalkane attacks the electrophilic carbon of the imine, followed by elimination of the amine to yield the nitroalkene product, the mechanism is shown in Figure 17. In the reaction, we used an organic acid, glacial acetic acid, as a catalyst to accelerate the reaction.

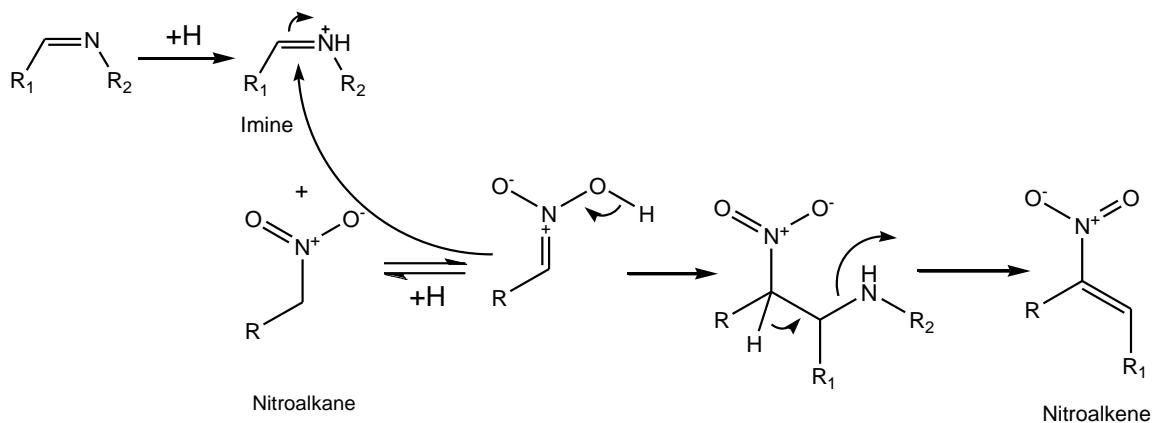


Figure 17 Nitroalkene formation mechanism

Note that we designed to have the imine as an intermediate in order to activate the aldehyde carbon, but it's not a required step for the reaction between the aldehyde and nitroalkane to yield the nitroalkene. As shown in Figure 18, the aldehyde and the nitroalkane can go through a nitroaldol reaction under base catalyst and yield the nitroalkene product. In our research, we did not choose this approach. Instead we designed an approach to activate the aldehyde carbon reaction center and make it more reactive when exposed to nucleophilic attack. Research results show that under our approach, the reaction can be finished in one hour and the average yield is around 90%.

After the imine intermediate is generated from the first step, the following reaction with nitroethane will yield 4-methoxymethylene phenyl 2-nitropropene, while the reaction with nitropropane will yield 4-methoxymethylene phenyl-2-butene.

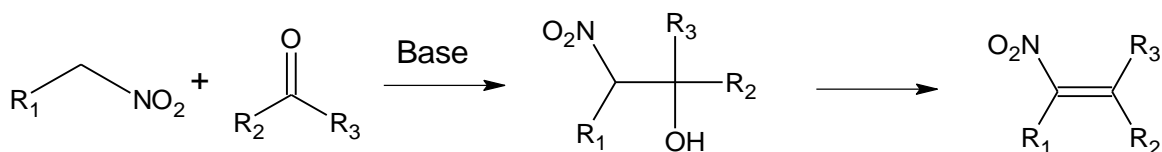


Figure 18 Reaction outline of nitroaldol reaction

The reductive hydrolysis of the nitroalkene is a two phase reaction involving a solvent mixture of equal parts water and toluene. The reaction mixture also consists of iron powder and ferric chloride for the reduction of the nitro group and hydrolysis of the resulting enamine intermediate to yield the corresponding ketone. Reduction of 4-methoxymethylene phenyl 2-nitropropene will yield 4-methoxymethylene phenylacetone, while a similar reaction starting with 4-methoxymethylene phenyl-2-butene will yield 4-methoxymethylene phenyl-2-butanone, shown in Figure 19.

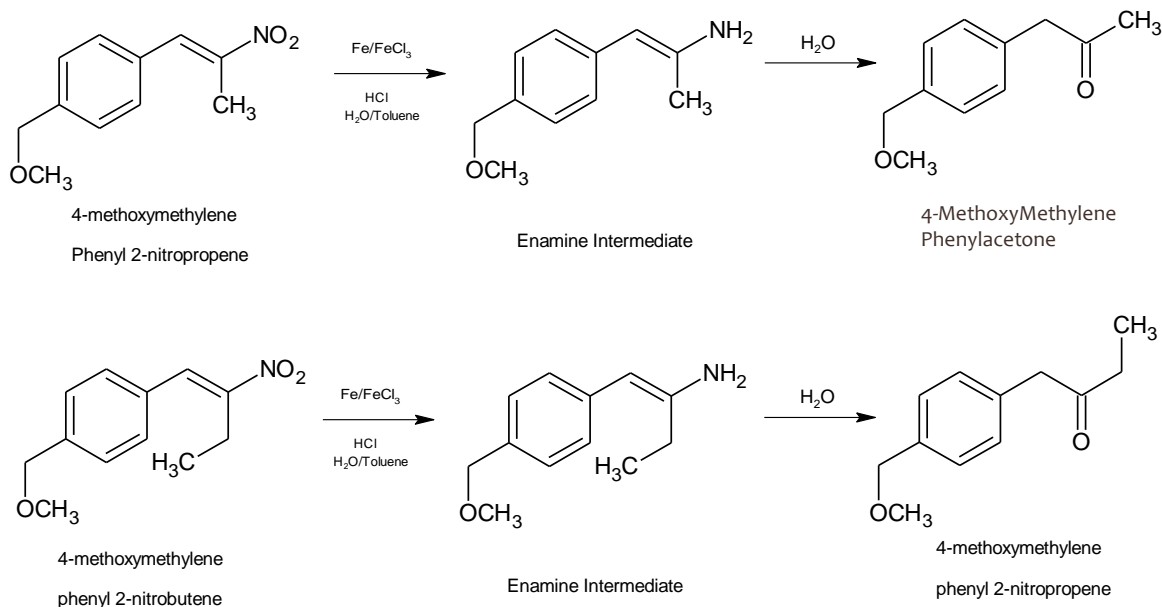


Figure 19 Reaction scheme of reductive hydrolysis of nitroalkenes

2.3 Synthesis of 4-methoxymethylene phenethylamines (Compound 1-8)

The last step to get the target compounds involves converting the ketone carbonyl group into an amine group; this approach is known as reductive amination. The process uses one equivalent of 4-methoxymethylene phenylacetone in methanol, ten equivalents of sodium cyanoborohydride and ten equivalents of the required amine. The mixture is stirred under room temperature and the system maintained at pH 7 for three days for the reaction to go to completion. Products were purified by solvent extraction to yield compounds 1-4. The reaction scheme is shown in Figure 20.

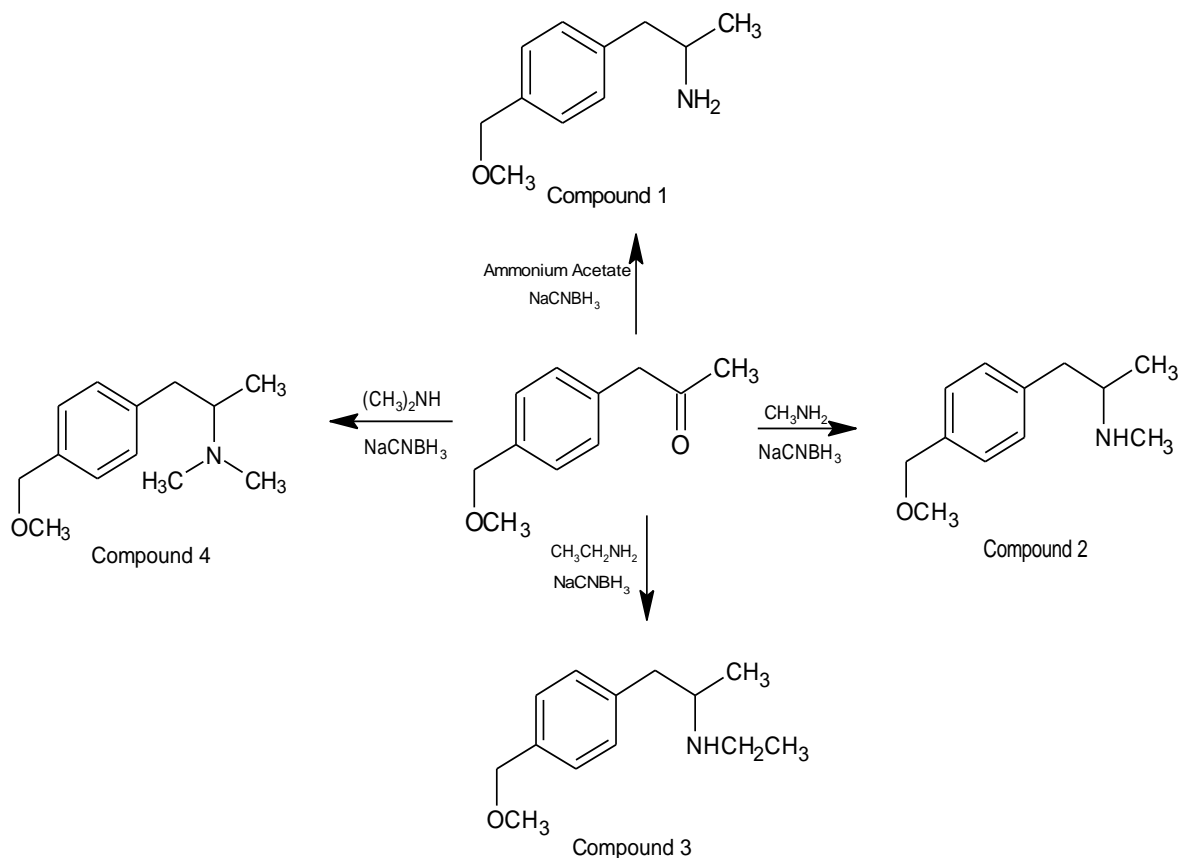


Figure 20 Synthesis of compound 1-4 starting from 4-methoxymethylene phenylacetone

The mechanism of this reductive amination is shown in Figure 21. The lone pair of electrons on the amine nitrogen attack the carbonyl carbon, the generated intermediate grabs H^+ ion and goes through a dehydration process to yield the imine intermediate. At this point, the original carbonyl carbon is activated and more subjective to nucleophilic attack. Then the electrons on sodium cyanoborohydride attack the activated imine carbon center, lose H_2BCN and yield the amine product. Since this reaction consumes H^+ ion, it is important to check pH from time to time, and add concentrated hydrochloride to maintain pH at 7.

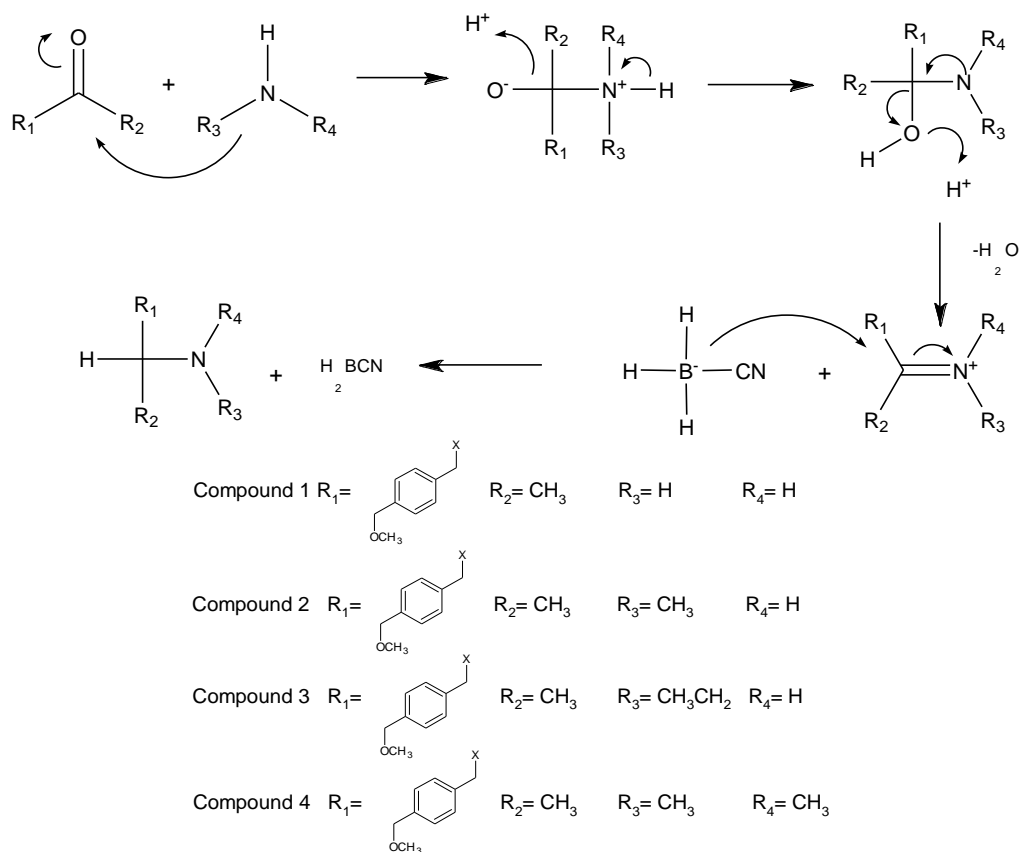


Figure 21 Mechanism of reductive amination that yields compound 1-4

A similar reaction process starting with 4-methoxymethylene phenyl-2-butanone will generate compound 5 through 8 as shown in Figure 22.

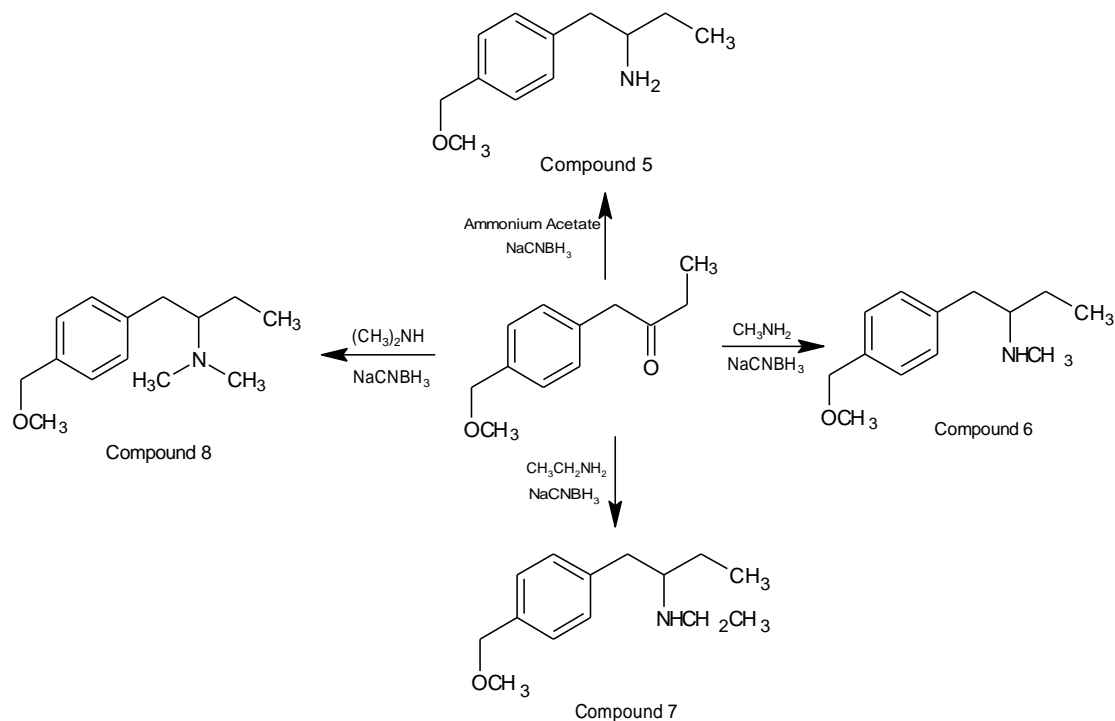


Figure 22 Synthesis of compound 5-8 starting from 4-methoxymethylene phenyl-2-butanone

2.4 Synthesis of 4-methoxymethylene benzaldehyde and 4-methoxymethylene phenyl acetone with methoxy methyl group labeled with deuterium

2.4.1 Synthesis of deuterium labeled 4-methoxymethylene benzaldehyde

4-Methoxymethylene benzaldehyde is a key and very important intermediate in this project. It is the central precursor substance for the synthesis of all the target compounds.

Commercially available 4-chloromethylbenzoyl chloride was treated with CD_3 -labeled methanol and solid sodium metal as catalyst to yield D_6 -labeled methyl 4-methoxymethyl benzoate. Based on our experience with this reaction, the labeled methyl ester formed by D_3 -labeled methanol displacement of the chloride of the acid chloride functionally occurs just by dissolving the substrate material in D-labeled methanol. Thus the intermediate D-labeled 4-

chloromethyl substituted methylbenzoate is the first product formed and without the addition of sodium metal is often present as a major product along with the desired D-labeled 4-methoxymethylene substituted methylbenzoate. The addition of sodium metal to form the D-labeled methoxide anion allows for the complete displacement of the benzylchloride and the complete conversion of the starting material to the desired product.

The strong reducing agent sodium bis (2-methoxyethoxy) aluminum hydride solution (Red-Al) in toluene can reduce the ester functional group in D-labeled 4-methoxymethyl benzoate methyl ester into the alcohol group and yield D₃-labeled 4-methoxymethyl benzyl alcohol. The primary alcohol group was then converted into an aldehyde and this requires a special oxidant which can stop the oxidization at the aldehyde stage without further oxidation. The treatment of the D-labeled 4-methoxymethyl benzyl alcohol with the fresh oxidation agent PCC, the D₃-labeled 4-methoxymethyl benzyl alcohol was selectively oxidized into D-labeled 4-methoxymethylene benzaldehyde. The entire reaction sequence for getting the desired precursor aldehyde, D₃-labeled 4-methoxymethylene benzaldehyde is shown in Figure 23.

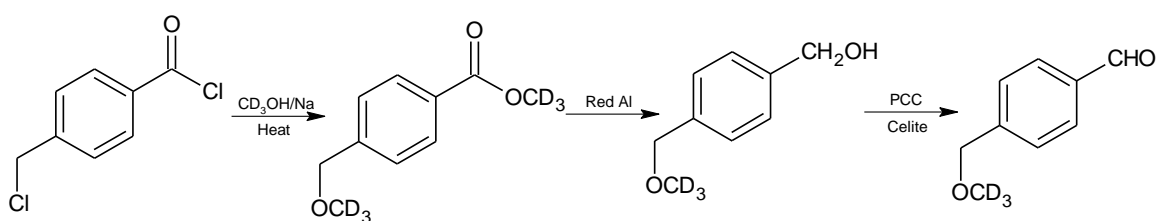


Figure 23 The synthesis procedure for deuterium labeled 4-methoxymethylene benzaldehyde

2.4.2 Synthesis of 4-methoxymethylene phenylacetone with methoxy methyl group labeled with deuterium

The synthesis of D₃ methyl-labeled 4-methoxymethylene phenylacetone is a three step sequence including formation of the imine adduct between the D₃-labeled precursor aldehyde and butylamine. This step is followed by condensation of the D-labeled imine with (nitroethane or 1-nitropropane) to give the corresponding D-labeled nitroalkene. The D-labeled nitroalkene were then subjected to reductive hydrolysis. The complete reaction sequence is outlined in Figure 24. The first step of the reaction sequence is the nucleophilic addition of n-butylamine and D-labeled 4-methoxymethylene benzaldehyde; electrons on the nitrogen will attack the aldehyde carbon and yield the carbinolamine intermediate. This is followed by dehydration under heating, to give the D-labeled imine intermediate. Since the nucleophilic addition reaction is an equilibrium reaction, it is important to remove the water generated to keep the reaction moving in the forward direction toward the desired imine. In the research, we used a Dean Stark trap to remove the water formed in the equilibrium reaction.

The next step is to get D-labeled nitroalkene from the reaction between nitroalkane and D-labeled imine intermediate. The α -carbon of nitroalkane attacks the electrophilic carbon of the imine, followed by loss of the amine to yield the D-labeled nitroalkene product. In the reaction, we used an organic acid, glacial acetic acid, as a catalyst to accelerate the reaction.

After the D-labeled imine intermediate was generated from the first step, the following reaction with nitroethane will yield D-labeled 4-methoxymethylene phenyl 2-nitropropene. The reductive hydrolysis of the D-labeled nitroalkene is a two phase reaction involving a solvent mixture of equal parts water and toluene. The reaction mixture also consists of iron powder and

ferric chloride for the reduction of the nitro group and hydrolysis of the resulting D-labeled enamine intermediate to yield the corresponding D-labeled ketone.

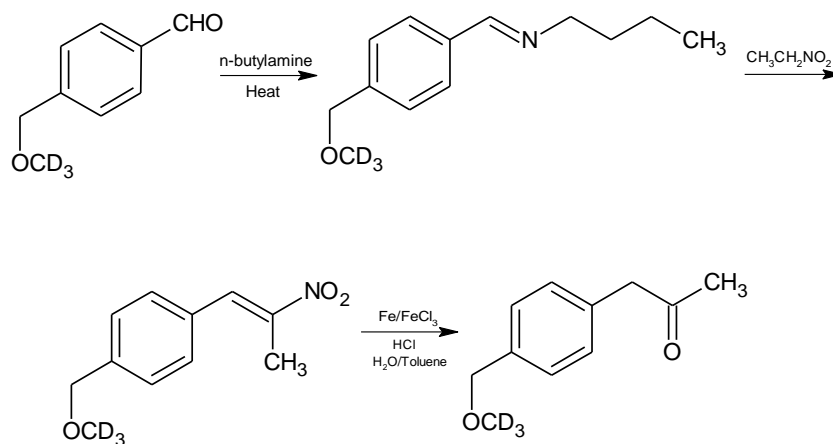


Figure 24 The synthesis procedure for deuterium labeled 4-methoxymethylene phenylacetone

3. Analytical studies on the methoxymethylene- substituted phenethylamines related to MDA, MDMA, MDEA and MDMMA and their related intermediates

Gas chromatographic separation coupled with mass spectrometry detect ionization (GC-MS) is the most widely adopted analytical method in forensic labs because of its relatively low cost, fast analyzing time and relatively low requirement on the purity of samples. A mass spectrum is considered a “finger print” of a compound that forensic scientists can use for a confirmation level of identification. As discussed in the introductory part of this thesis, large numbers of MDMA’s regioisomers and isobarics will yield similar mass spectral information. This section of the thesis will discuss the analysis methods used to identify the methoxymethylene- substituted phenethylamines and synthetic precursors, and their differentiation from other isobaric and regioisomeric phenethylamines having equivalent mass spectrometry properties.

Gas chromatography is based on different compounds having different interacting forces with column coating materials (stationary phase liquids) and these different interacting forces will result in different elution times that can be used to separate and identify compounds. Gas chromatography is most effective in separation when the number of compounds to be separated is somehow small. However, in the case of identifying MDMA from other potential structures, co-eluting compounds pose a huge challenge for forensic scientists.

After eluting from the GC columns, the compounds will enter the mass spectrometer detector. The substances will be ionized and form fragments and those fragments are captured and displayed based on their mass to charge ratio (m/z). This research project used the electron ionization (EI) method with an energy of 70eV. Since the fragmentation is based on molecular structure, MS is very powerful in revealing the structure by putting all the fragments back together, like a “jigsaw puzzle”. Yet MS has its drawbacks, it does not show the actual structure of the compound, not even a calculated elemental composition. Those regioisomers and isobarics of MDMA that will yield similar MS information are significant obstacles for MDMA identification.

Gas Chromatography coupled with Time-Of-Flight mass spectrometry detector (GC-TOF) is an analytical method which can generate a high resolution exact mass molecular formula for fragments and the molecular ion. This can then be compared to a calculated exact mass to determine the elemental composition of fragment ions. In the detector, ions are accelerated and will travel through a certain distance; their time of flight is captured. The time of flight is dependent on ion's m/z value. GC-TOF is an advance over GC-MS in acquiring high precision compound structure information; however, its usage is limited because of high instrument cost and maintenance.

Nuclear magnetic resonance (NMR) is an analytical method that is based on the specific resonance frequency of atoms. NMR is by far the most accurate and effective method in revealing unknown compound's structure information. Its usage in forensic industry is limited because it requires high quality pure samples, while in most cases pure samples will not be available in forensic labs. High cost is another hindrance for wide use of NMR in forensic labs. In this research, we used NMR as a supportive method in confirming synthesis intermediates.

In this section, we will discuss different kinds of analysis and isotope labeling used to confirm certain important intermediates of the synthesis procedures. We will also discuss the comparison of methoxymethylene- substituted intermediates and their ethoxy- substituted counterparts, as well as the separation of methoxymethylene- substituted phenethylamines.

3.1 Analytical studies on the comparison of synthesis intermediate 4-methoxymethylene benzaldehyde with its regioisomeric compound 4-ethoxymethylene benzaldehyde

4-Methoxymethylene benzaldehyde and 4- ethoxy benzaldehyde are regioisomers based on the position of the oxygen in the ether side chain. Studying their analytical properties will help understand the different impacts of the methoxymethylene group and ethoxy group of regioisomers on gas chromatography and mass spectra.

3.1.1 Gas chromatography separation of 4- methoxymethylene benzaldehyde and 4- ethoxy benzaldehyde

Gas chromatography separation of 4- methoxymethylene benzaldehyde and 4- ethoxy benzaldehyde was carried out on several columns, and only the best separation result is shown in Figure 25. The Rtx-1 column has a length of 30 meters, internal dimension of 0.25 mm and a stationary phase film thickness of 0.25 μm . The stationary phase of the Rtx-1 column is 100% dimethyl polysiloxane. The temperature program used for separation was to set the injector temperature at 250 $^{\circ}\text{C}$, detector temperature at 280 $^{\circ}\text{C}$. The column is started at 70 $^{\circ}\text{C}$, held at that temperature for 1 minute then the temperature was ramped up to 250 $^{\circ}\text{C}$ at a rate of 30 $^{\circ}\text{C}$ per minute and set at 250 $^{\circ}\text{C}$ for 5 minutes. The finish time is 20 minutes.

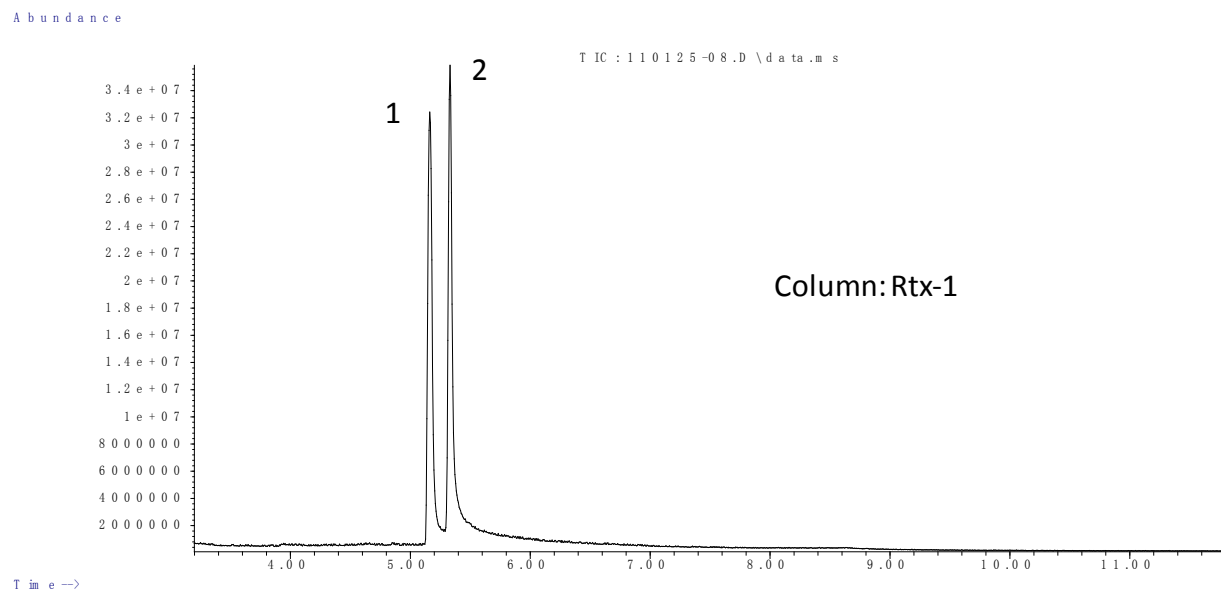


Figure 25 Gas chromatography separation of (1): 4- methoxymethylene benzaldehyde and (2): 4- ethoxy benzaldehyde on column Rtx-1

The two regioisomeric compounds have an elution time around five to six minutes; while the ethoxy ring substituted compound has a slightly higher retention time. The retention time of 4- methoxymethylene benzaldehyde is 5.175 min and the retention time of 4- ethoxy benzaldehyde is 5.337 min, the two regioisomers are nicely separated with essentially baseline resolution by the stationary phase Rtx-1 as shown in Figure 25.

3.1.2 Mass spectra studies of 4- methoxymethylene benzaldehyde and 4- ethoxy benzaldehyde

During the course of this research project, 4- methoxymethylene benzaldehyde and 4- ethoxy benzaldehyde were available for comparison as two regioisomeric substituted benzaldehydes containing an ether functionality at the 4- position and side chain elemental composition of C_2H_5O . The mass spectra for these two aldehydes are shown in Figure 26.

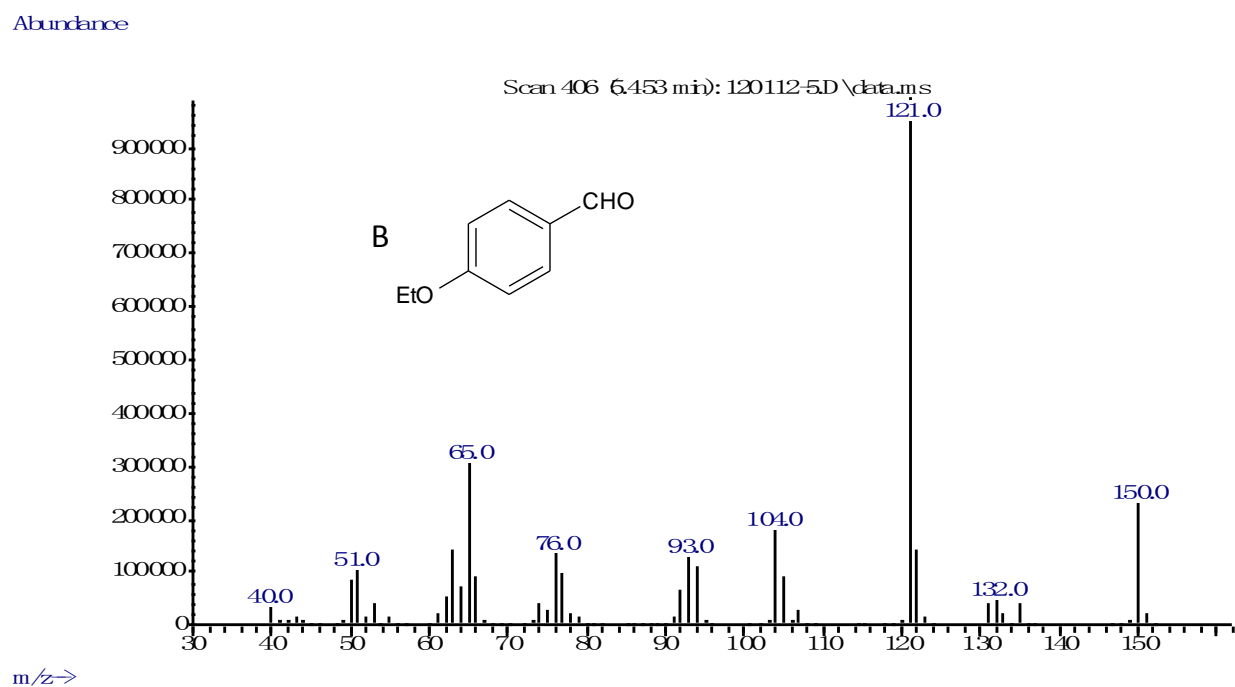
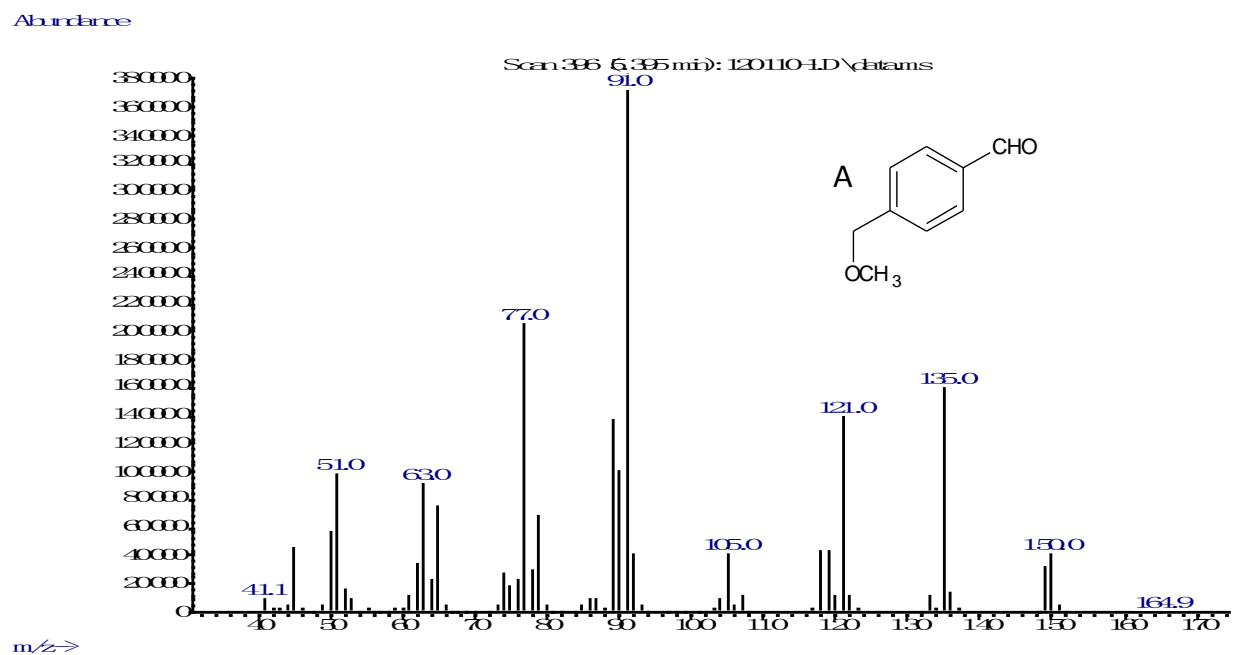


Figure 26 EI Mass spectra of (A) 4- methoxymethylene benzaldehyde and (B) 4- ethoxy benzaldehyde

Both compounds show the molecular ion m/z 150. 4- Methoxymethylene benzaldehyde shows a base peak at m/z 91 for the benzylic carbocation and significant fragment peaks at m/z 135 and m/z 121. The proposed structures for these ions are shown in Figure 27. The 4- ethoxy benzaldehyde regioisomer shows a base peak at m/z 121 which corresponds to the loss of 29 mass units, possibly the aldehyde group or the ethyl group.

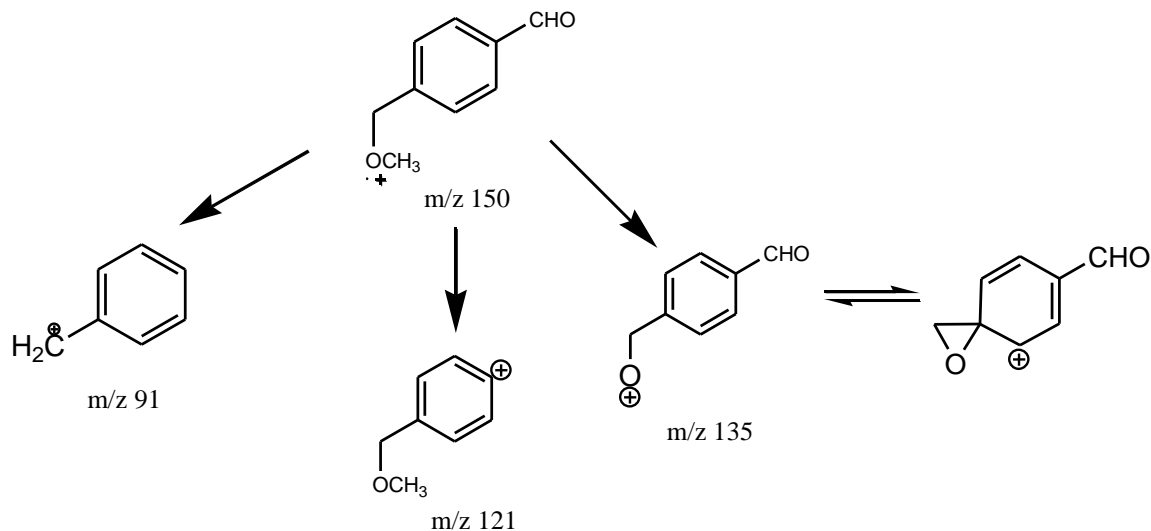


Figure 27 Mass spectrum fragmentation pattern of 4- methoxymethylene benzaldehyde

Since the 4-ethoxymethylenebenzaldehyde mass spectrum also showed an m/z 121 ion as a major fragment, exact mass analysis of this ion was done in order to confirm its structure. While the loss of the aldehyde group or an ethyl group would account for the m/z 121 in 4-ethoxybenzaldehyde, the loss of CHO is the only obvious method for formation of this ion in 4-methoxymethylenebenzaldehyde. Exact mass analysis of the m/z 121 ion in each compound was used to confirm the elemental composition of this fragment from each aldehyde.

3.1.3 GC-TOF analysis of 4-methoxymethylene benzaldehyde on certain characteristic fragments

GC-TOF is an analytical method that can provide elemental composition information for fragments in mass spectrometry, thus it is an important approach for confirming synthesis results. Figure 28 shows the TOF mass spectrum of 4-methoxymethylene benzaldehyde. These TOF mass spectra were generated under GC-MS conditions with 70eV electron ionization, thus, these fragments are identical to those generated in the previous nominal mass experiments.

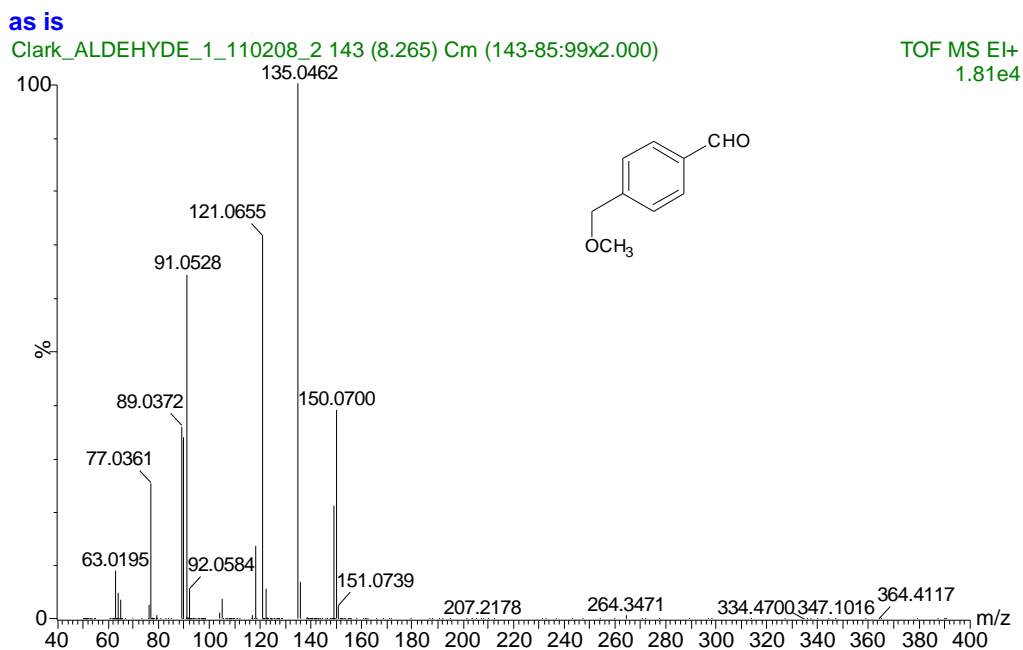
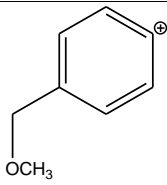


Figure 28 TOF mass spectra of 4-methoxymethylene benzaldehyde

The mass spectrum of 4-methoxymethylene benzaldehyde shows that the base peak is m/z 135 and other significant peaks are at m/z 150, m/z 121 and m/z 91. The m/z 150 peak is the molecular ion peak, based on previous experience analyzing substituted benzenes; m/z 91 is

suggesting the existence of a benzyl group. The structure studies of m/z 121 and m/z 135 are shown in Table 1 and Table 2.

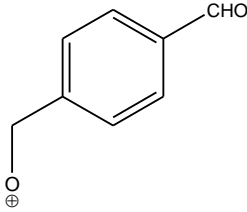
Table 1 TOF data on m/z 121 fragment of 4-methoxymethylene benzaldehyde

Measured Mass	121.0655
Calculated Mass	121.0653
Deviation (in PPM)	1.7
Fragment Formula	C_8H_9O
Best Fit Ion Structure	

Deviation in absolute value between the calculated masses and the measured masses of the fragment ions within 5 ppm is acceptable in this study. The calculated and experimentally measured exact masses all fall in that acceptable deviation range so we accept the suggested fragment formula proposed for the m/z 121 fragment (C_8H_9O). The m/z 121 fragment is a loss of 29 mass units from 4-methoxymethylene benzaldehyde and it has a suggested formula of C_8H_9O which is different with the molecular formula by CHO. It is most likely that aldehyde oxygen was ionized and followed by a heterolysis loss of the aldehyde functional group.

Additionally, the structure of the fragment m/z 135 was also studied and the results obtained are summarized in Table 2.

Table 2 TOF data on m/z 135 fragment of 4-methoxymethylene benzaldehyde

Measured Mass	135.0442
Calculated Mass	135.0446
Deviation (in PPM)	-3.0
Fragment Formula	$C_8H_7O_2$
Best Fit Ion Structure	

The m/z 135 fragment is a loss of 15 mass from the molecular ion for 4-methoxymethylene benzaldehyde, and it has a suggested formula of $C_8H_7O_2$ which is different with the molecular formula by CH_3 . The most likely fragmentation is the loss of the methyl group from the side chain methoxy methyl. A proposed structure for this ion is shown in Table 2 and Figure 27.

3.1.4 GC-TOF analysis on 4-ethoxy benzaldehyde m/z 121 fragment

GC-TOF is an analytical method that can provide elemental composition information for fragments in mass spectrometry, thus it is an important approach for confirming synthesis results and structural analysis. Figure 29 shows the TOF mass spectrum of 4-ethoxy benzaldehyde.

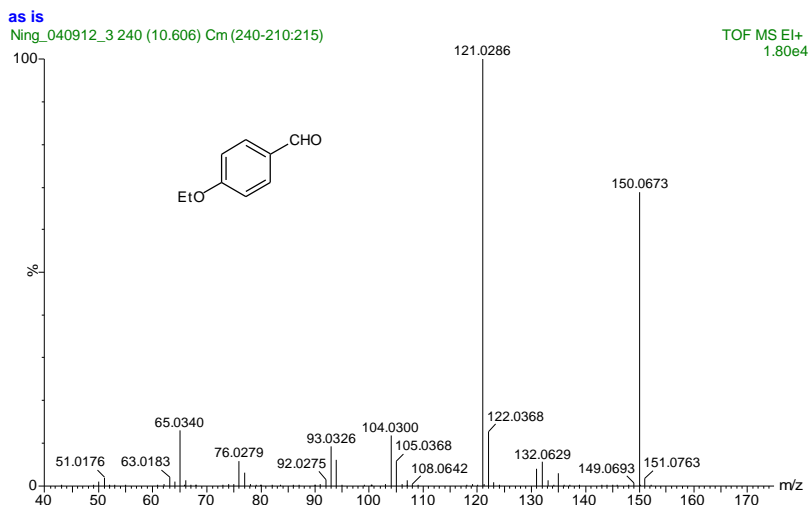


Figure 29 TOF mass spectra of 4-ethoxy benzaldehyde

Mass spectrum of 4-ethoxy benzaldehyde shows that the base peak is the m/z 121 fragment, and the other significant peak is the molecular ion m/z 150. The structure study of m/z 121 is shown in Table 3.

Table 3 TOF data on m/z 121 fragment of 4-ethoxy benzaldehyde

Measured Mass	121.0286
Calculated Mass	121.0290
Deviation (in PPM)	-3.3
Fragment Formula	$C_7H_5O_2$
Best Fit Ion Structure	

Deviation of 5 ppm in absolute value between calculated and observed masses is acceptable in this study and a 3.3 ppm is in the acceptable deviation range so we accept the suggested fragment formula proposed for the m/z 121 fragment. The m/z 121 fragment is a loss of 29 mass units from 4-ethoxy benzaldehyde molecular ion and it has a suggested formula of $C_7H_5O_2$ which is different with the molecular formula by C_2H_5 . It is most likely that the ethoxy oxygen was ionized followed by the loss of the ethyl group to yield the base peak at m/z 121.

These exact mass studies suggest that the characteristic m/z 121 fragments of the EI mass spectra of 4-methoxymethylene benzaldehyde and 4-ethoxy benzaldehyde are generated from two different mechanisms. The m/z 121 fragment of 4-methoxymethylene benzaldehyde is derived from the loss of the aldehyde group, while the m/z 121 fragment of 4-ethoxy benzaldehyde is a result of losing the ethyl ester group, shown in Figure 30.

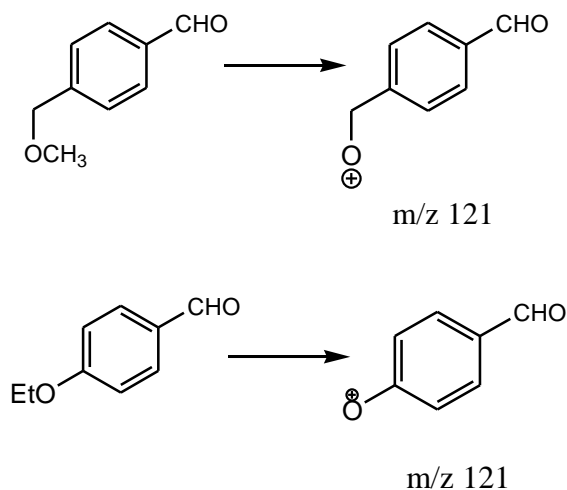


Figure 30 The generation of m/z 121 fragments of 4-methoxymethylene benzaldehyde and 4-ethoxy benzaldehyde under EI mass spectrometry suggested by TOF studies

3.1.5 GC-MS studies on isotopic labeled 4-methoxymethylene benzaldehyde

In order to further confirm the significant fragments shown in the mass spectrum of 4-methoxymethylene benzaldehyde, i.e. the m/z 121 and m/z 135 fragments, analysis work of deuterium labeled 4-methoxymethylene benzaldehyde was carried out during this research. The isotopic labeling technique often provides valuable insights into the processes of fragmentation in mass spectrometry. In this project, the methoxy methyl group hydrogens of 4-methoxymethylene benzaldehyde were labeled with deuterium in order to further demonstrate that the methyl position of the methoxy methyl group is actually lost to generate the m/z 135 ion. If the previous interpretation for the m/z 135 peak is true, then the methoxy methyl group is lost under electron ionization. Thus, an m/z 135 peak is expected in the mass spectra of deuterium labeled 4-methoxymethylene benzaldehyde. If this methyl group remains a part of the fragment in question the mass would be shifted by the number of the labeled deuterium atoms (m/z 138 in this case).

Gas chromatography and mass spectra of isotopic labeled 4-methoxymethylene benzaldehyde is shown in Figure 31 and Figure 32 below.

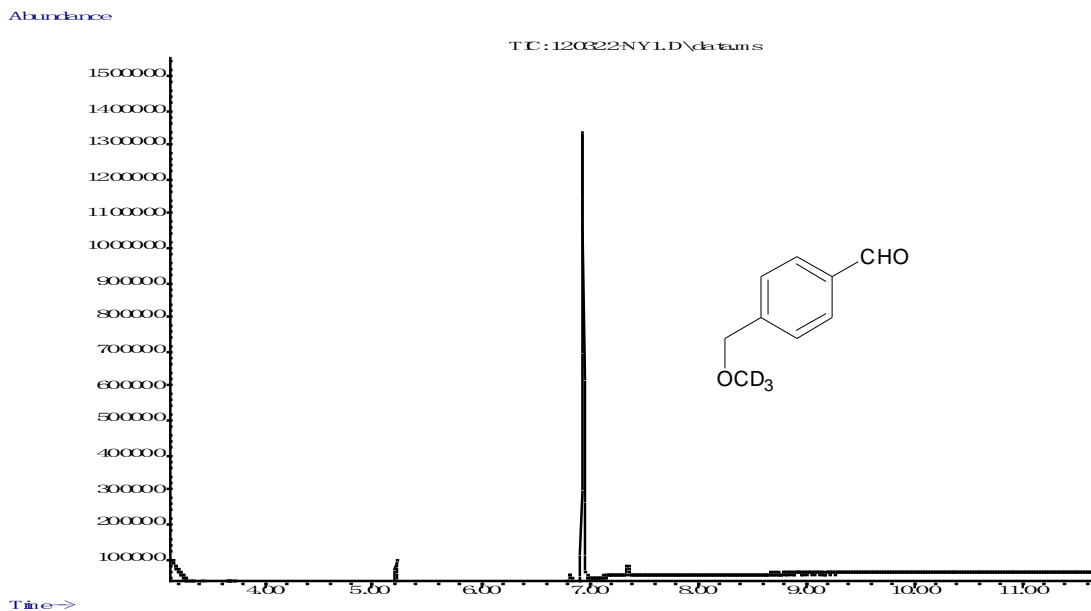


Figure 31 Gas chromatography of isotopic labeled 4-methoxymethylene benzaldehyde. Column: Rtx-35

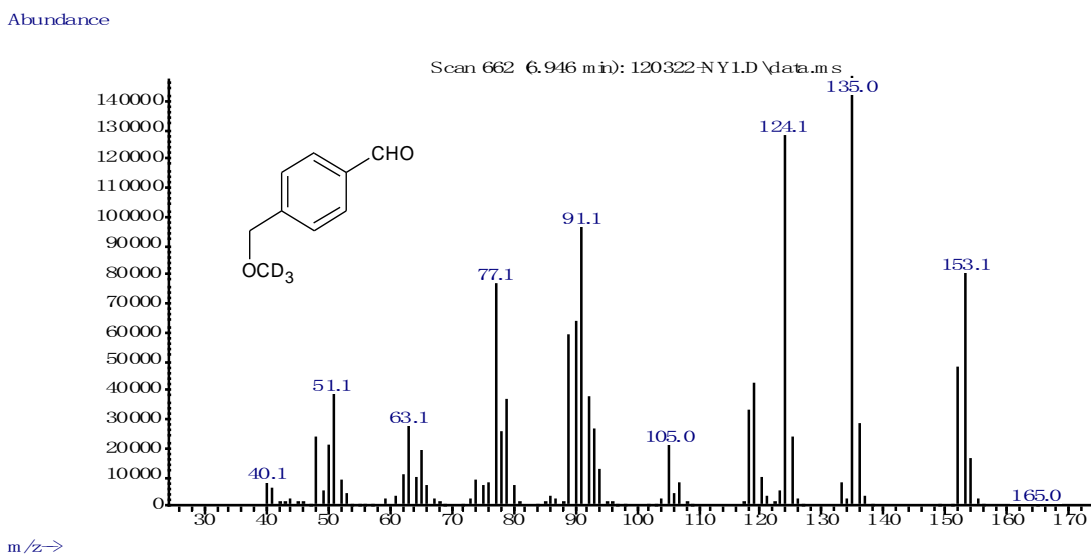


Figure 32 EI mass spectra of isotopic D_3 -methyl labeled 4-methoxymethylene benzaldehyde

The mass spectrum in Figure 32 shows the base peak unchanged at m/z 135. The m/z 153 is the molecular ion peak and other peaks are consistent with unlabeled 4-methoxymethylene benzaldehyde. As expected and illustrated above, the m/z 135 peak of deuterium labeled 4-methoxymethylene benzaldehyde provides confirmation information suggesting that this peak is derived from the loss of the methoxy methyl group. The m/z 124 ion in Figure 32 also confirms the structure of the previously described m/z 121 ion as the loss of CHO from the molecular ion. This test result is consistent with the proposed structure for the ion m/z 121 and the ion m/z 135 shown in the 4-methoxymethylene benzaldehyde mass spectrum.

3.2 Analytical studies on the comparison of synthesis intermediate 4-methoxymethylene phenylacetone with its homologue 4-methoxymethylene phenyl-2-butanone

4-Methoxymethylene phenylacetone and 4-methoxymethylene phenyl-2-butanone are homologues with 4-methoxymethylene phenyl-2-butanone having one more methyl group at the alkyl end of the ketone side chain. Studying their analytical properties will help in understanding the different impacts of side chain structures on compounds' gas chromatography and mass spectra properties.

3.2.1 Gas chromatography separation of 4- methoxymethylene phenylacetone and 4-methoxymethylene phenyl-2-butanone

Gas chromatography separation of 4- methoxymethylene phenylacetone and 4-methoxymethylene phenyl-2-butanone was carried out on a Rtx-5 Sil column. The column has a length of 30 meters, an internal dimension of 0.25 mm and a stationary phase film thickness of 0.25 μm . The stationary phase is 5% diphenyl- 95% dimethyl Polysiloxane. The temperature program used for separation is to set the injector temperature at 250 $^{\circ}\text{C}$, and detector temperature

at 280 °C. The column is started at 70 °C, held at that temperature for 1 minute then the temperature was ramped up to 250 °C at a rate of 30 °C per minute and set at 250 °C for 5 minutes. The finish time is 20 minutes. GC results are shown in Figure 33.

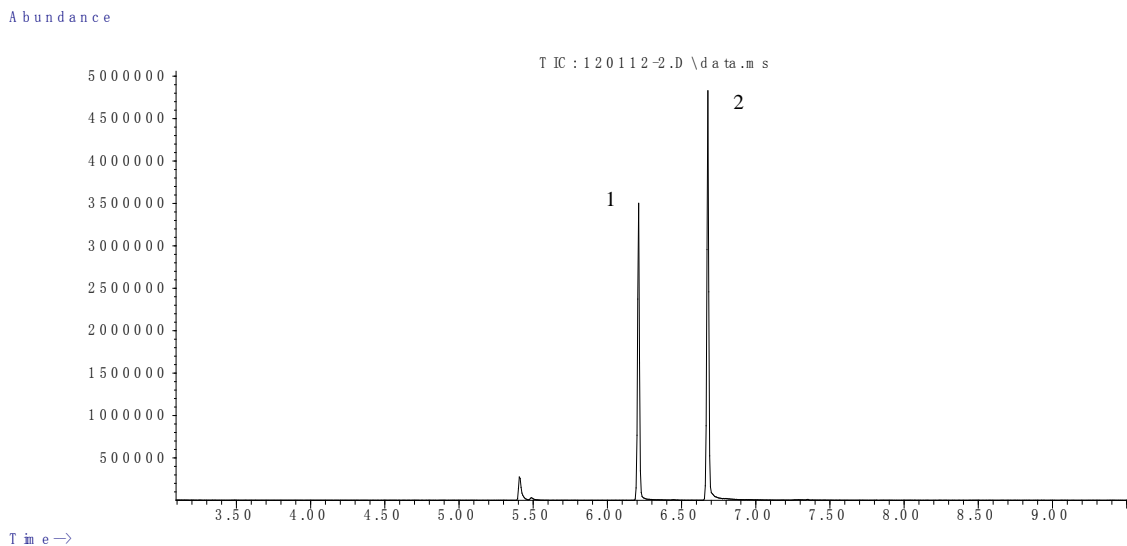
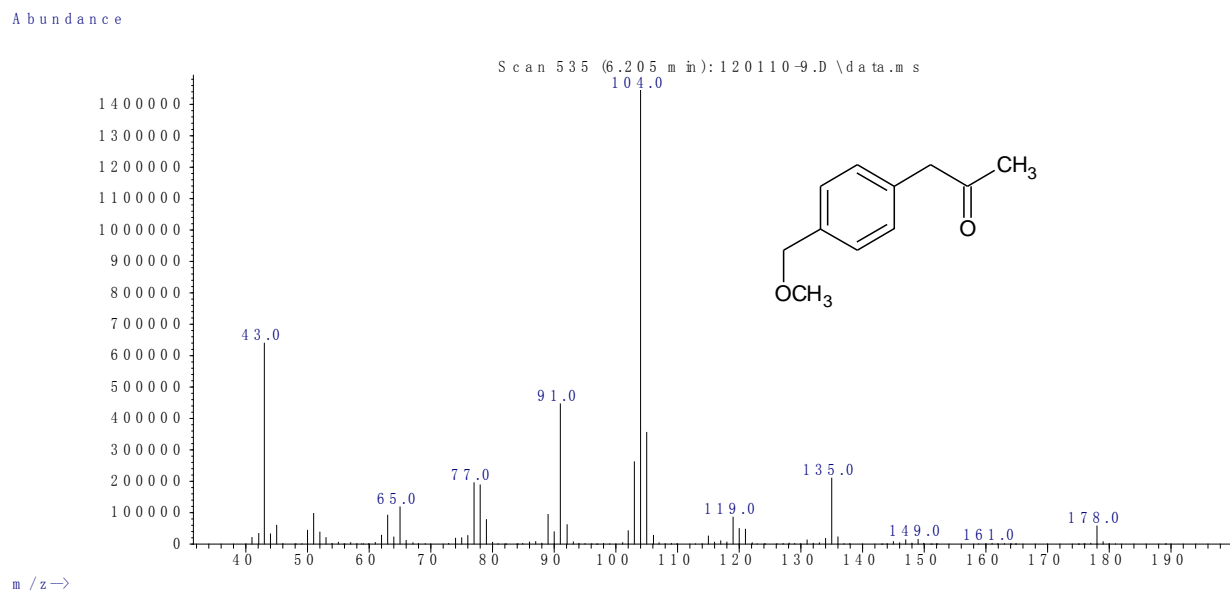


Figure 33 Gas chromatography separation of (1): 4- methoxymethylene phenylacetone and (2): 4- methoxymethylene phenyl-2-butanone on column Rtx-5 Sil

The retention time of 4- methoxymethylene phenylacetone is 6.205 min, and the retention time of 4- methoxymethylene phenyl-2-butanone is 6.671 min. These compounds are well resolved in this system with the higher chain homologue butanone having the greater retention time. The two compounds can be nicely separated on an Rtx-5 Sil stationary phase showing baseline resolution with an analysis time in the six to seven minute range.

3.2.2 Mass spectra studies of 4- methoxymethylene phenylacetone and 4-methoxymethylene phenyl-2-butanone

This research studied the mass spectra of 4- methoxymethylene phenylacetone and 4-methoxymethylene phenyl-2-butanone for comparison and separation purposes under 70eV electronic ionization, the result is shown in Figure 34.



Abundance

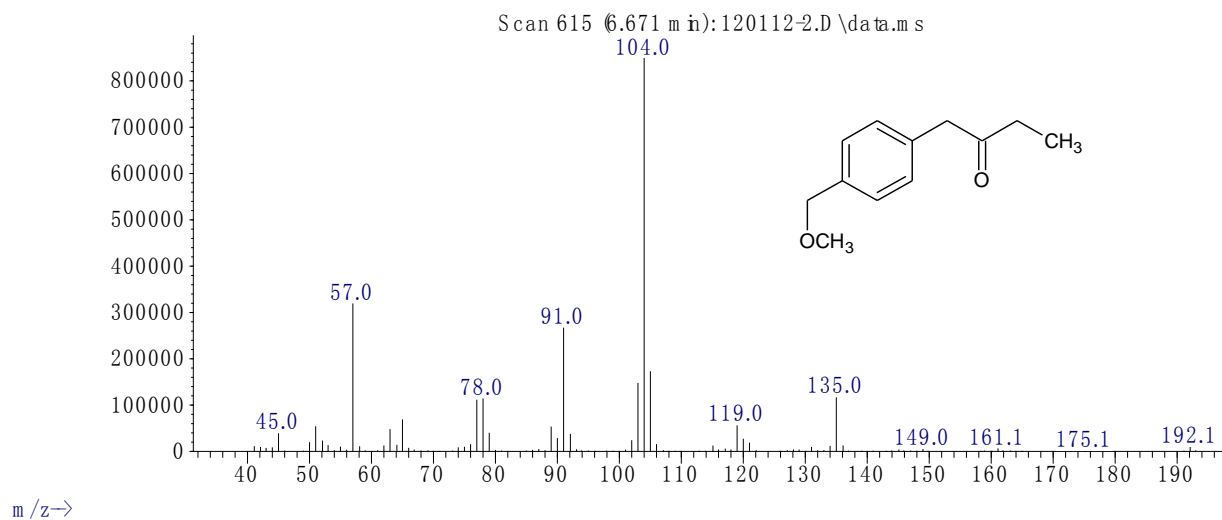


Figure 34 EI Mass spectra of 4- methoxymethylene phenylacetone and 4- methoxymethylene phenyl-2-butanone

Both compounds show a predominant unique base peak at m/z 104. Exact mass analysis of the m/z 104 ion in each compound was used to analyze the elemental composition of this fragment from each ketone. Other significant peaks in common are m/z 135 and m/z 91. The unique peaks for 4- methoxymethylene phenylacetone are m/z 178 and m/z 43, corresponding to the molecular ion and the acetyl ion. Unique peaks for 4- methoxymethylene phenyl butanone are m/z 192 and m/z 57, corresponding to molecular ion and propionyl ion respectively. Thus, the additional methyl group on the carbonyl side chain can bring small differences to mass spectra, but will not significantly change the fragmentation pattern.

3.2.3 GC-TOF analysis on 4-methoxymethylene phenylacetone

GC-TOF is an analytical method that can provide elemental composition information for fragments under the mass spectrum, thus it is an important approach for confirming analytical results. Figure 35 below shows the TOF mass spectra of 4-methoxymethylene phenylacetone.

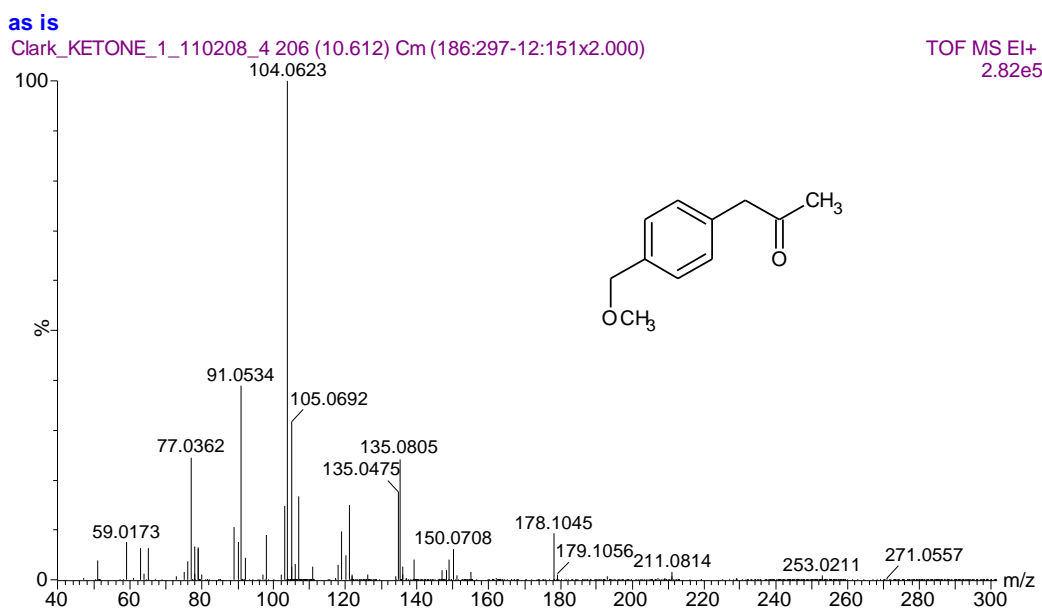
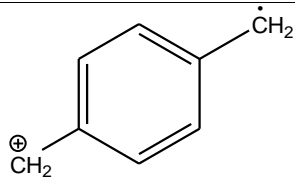


Figure 35 TOF mass spectra of 4-methoxymethylene phenylacetone

Mass spectra of 4-methoxymethylene phenylacetone shows that the base peak is m/z 104, and other significant peaks are m/z 178, m/z 135, m/z 91, and m/z 77. The m/z 178 peak is the molecular ion peak and based on experience dealing with substituted benzene ring analysis, m/z 91 and m/z 77 and fragmentation series suggest the presence of the benzyl group. The m/z 135 fragment is 4-methoxymethylene benzyl cation derived from the loss of the acetyl group. The TOF-MS structure study of m/z 104 is shown in Table 4.

Table 4 TOF data on m/z 104 fragment of 4-methoxymethylene phenylacetone

Measured Mass	104.0623
Calculated Mass	104.0626
Deviation (in PPM)	-2.9
Fragment Formula	C_8H_8
Best Fit Ion Structure	

Deviation of 5 ppm in absolute value of the measured mass and the calculated masses is acceptable in this study. Deviation of the m/z 104 piece falls in that acceptable deviation range thus we accept the suggested fragment formula. The m/z 104 fragment is a loss of 74 mass from 4-methoxymethylene phenylacetone, and it has a suggested formula of C_8H_8 which is different with the molecular formula by $C_3H_6O_2$. Since there is no oxygen present in the m/z 104 piece, it is highly likely that both the carbonyl side chain and the methoxy side chain are lost during fragmentation. One possible pathway of fragmentation is shown in Figure 36.

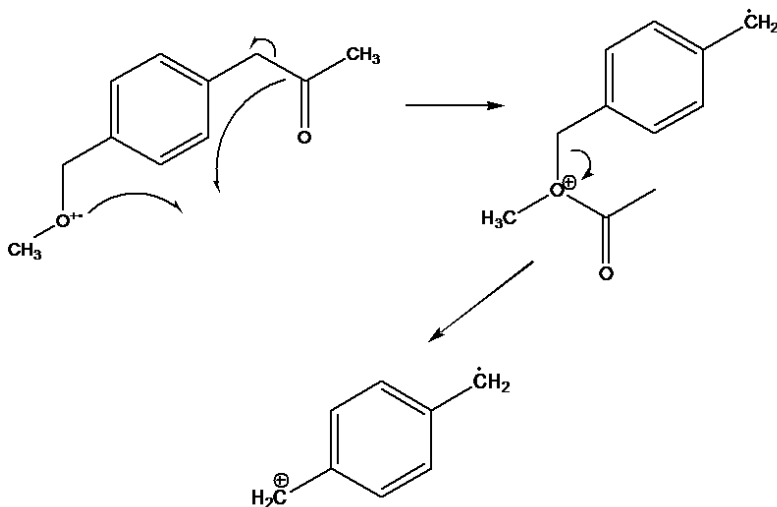


Figure 36 Possible mechanisms for the formation of mass 104 fragment

In this fragmentation mechanism, the methoxy oxygen is ionized first. The following homolytic cleavage completes a rearrangement by transferring the carbonyl side chain to the methoxy oxygen. The next step is heterolytic cleavage, losing the rearranged methyl acetate side chain and leaving the m/z 104 piece.

The previous section shows that both 4-methoxymethylene phenylacetone and 4-methoxymethylene phenyl-2-butanone show a base peak at m/z 104 and this observation contributes additional support to the proposed mechanism of formation of this unique ion. This support is based on the change in structure of the alkyl side of the ketone making no difference in the mass of the base peak fragment.

In order to further confirm this fragmentation and mechanism, this research designed an isotope labeling procedure similar to that previously described for the corresponding aldehydes.

3.2.4 GC-MS studies on isotopic labeled 4-methoxymethylene phenylacetone

The isotopic labeling technique often provides valuable insights into the processes of fragmentation in mass spectrometry. In this project, the methyl group hydrogens of 4-methoxymethylene phenylacetone were labeled with deuterium in order to confirm whether that the methyl of methoxy methyl group is actually lost to generate the m/z 104 ion. If the previous interpretation for the m/z 104 peak is true, then the methoxy methyl group is lost under electron ionization. Thus, the equivalent m/z 104 peak is expected to retain in the mass spectra of deuterium labeled 4-methoxymethylene phenylacetone since the generation of m/z 104 requires the loss of the methyl of the methoxy group. The gas chromatography and mass spectra analysis of isotopic labeled 4-methoxymethylene phenylacetone is shown in Figure 37 and Figure 38.

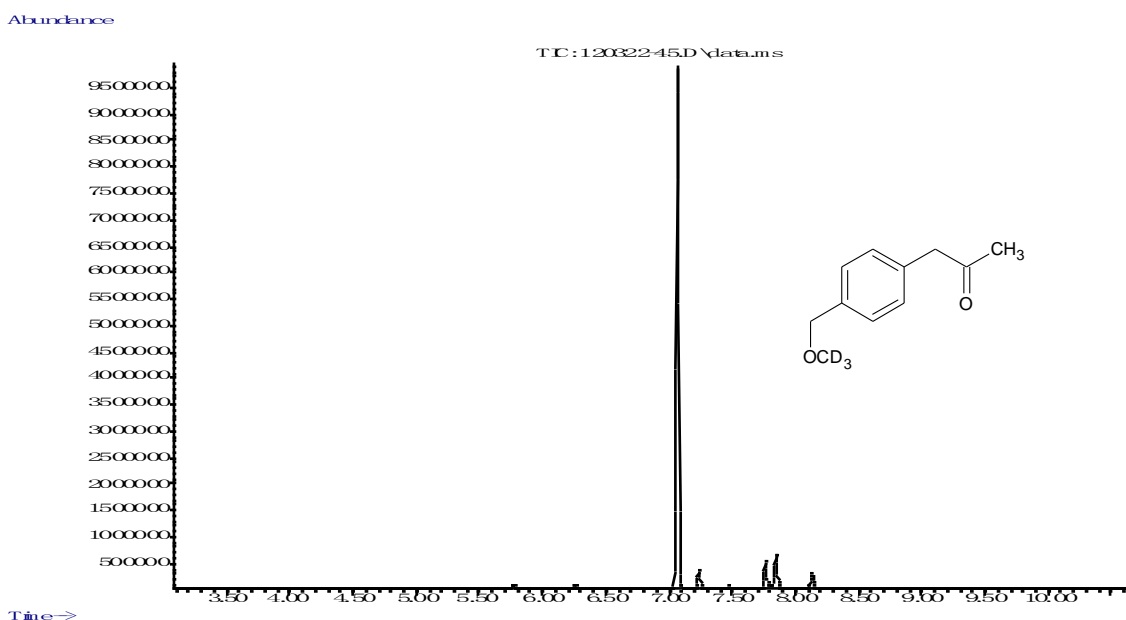


Figure 37 Gas chromatography of isotopic labeled 4-methoxymethylene phenylacetone. Column:

Rxi-50

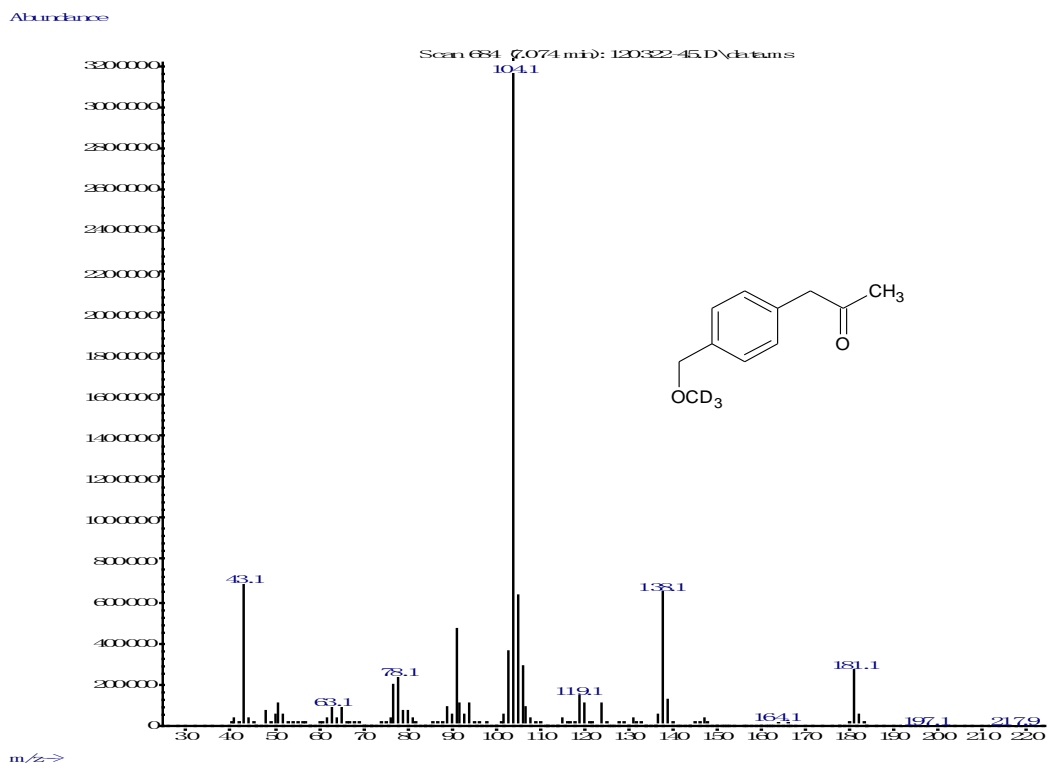


Figure 38 EI mass spectra of isotopic labeled 4-methoxymethylene phenylacetone

In the mass spectra, the base peak remained m/z 104 while the molecular ion peak is at m/z 181. 4-methoxymethylene benzyl cation with labeled CD_3 group is at m/z 138 from the loss of the acetyl group. These two significant peaks are consistent with the explanation provided for their counterpart peaks of unlabeled 4-methoxymethylene phenylacetone. Most importantly, the base peak m/z 104 of labeled 4-methoxymethylene phenylacetone proved that the methyl of the methoxy methyl group was lost in the process of producing this fragment, since there is no mass shift caused by the existence of isotope deuterium compared to the counterpart fragment m/z 104 of the unlabeled 4-methoxymethylene phenylacetone. The isotope labeling study further supports the provided mechanism of the m/z 104 peak.

Since the proposed mechanism of the m/z 104 fragment involves the methoxymethylene oxygen, we would like to study how the fragmentation pattern would be affected if the oxygen

position is moved one carbon away. Thus we designed an analytical procedure comparing 4-methoxymethylene phenylacetone with its regioisomer, 4-ethoxy phenylacetone.

3.2.5 Analytical studies comparing 4-methoxymethylene phenylacetone with its regioisomer 4-ethoxy phenylacetone

4- Methoxymethylene phenylacetone and 4- ethoxy phenylacetone are regioisomeric substances based on the position of the ether oxygen in the side chain. A comparison of their mass spectral properties will help understand the different impacts of the methoxymethylene group and ethoxy group on the structure property relationships in these closely related compounds, particularly on the mechanism of the generation of the unique m/z 104 fragment shown in the mass spectrum of 4- methoxymethylene phenylacetone.

Gas chromatography separation of 4- methoxymethylene phenylacetone and 4- ethoxy phenylacetone was carried out on two different columns, Rtx-1 and Rtx-5 Sil. The GC results show that these two compounds have very similar retention times on both the Rtx-1 column and Rtx-5 Sil column, thus they are not well resolved in this study. Study results are shown in Table 5 below.

Table 5 GC study results of 4- methoxymethylene phenylacetone and 4- ethoxy phenylacetone under column Rtx-1 and Rtx-5 Sil

Compound	Column	Retention Time
4- methoxymethylene phenylacetone	Rtx-1	5.925 min
	Rtx-5 Sil	6.205 min
4- ethoxy phenylacetone	Rtx-1	6.059 min
	Rtx-5 Sil	6.193 min

Mass spectrometry is a key analytical tool providing unique information of drug and precursor substances for identification in forensic labs. This research compared of the mass spectra of 4- methoxymethylene phenylacetone and 4- ethoxy phenylacetone for mass spectral differentiation purposes under 70eV standard electronic ionization, the result is shown in Figure 39.

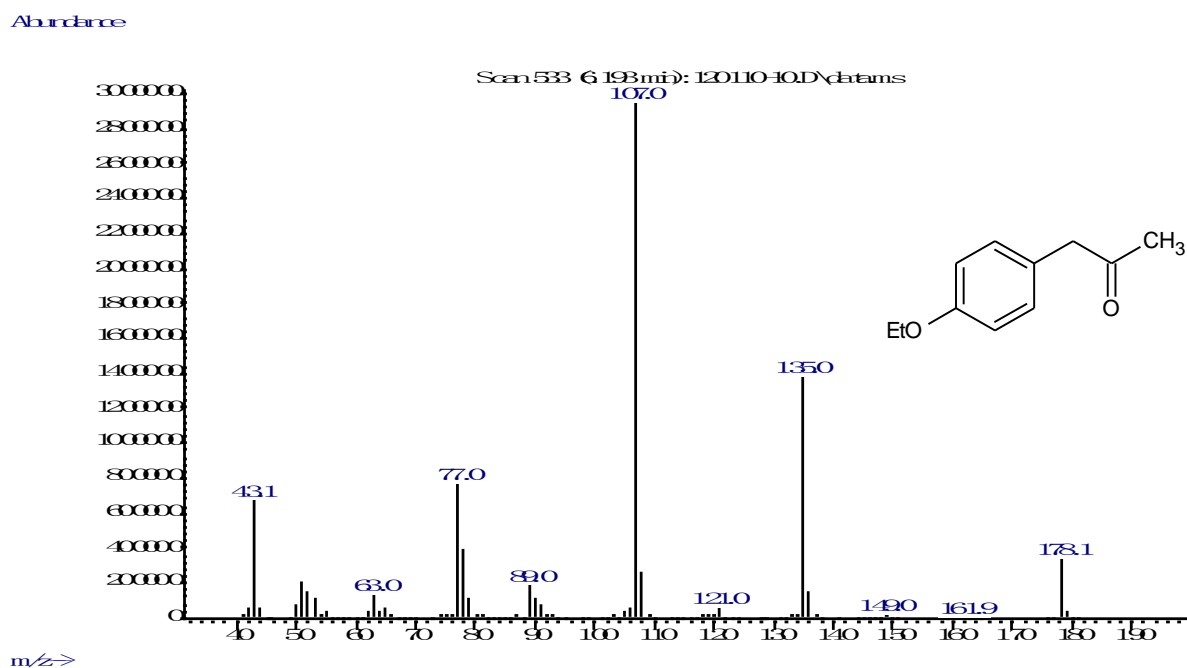
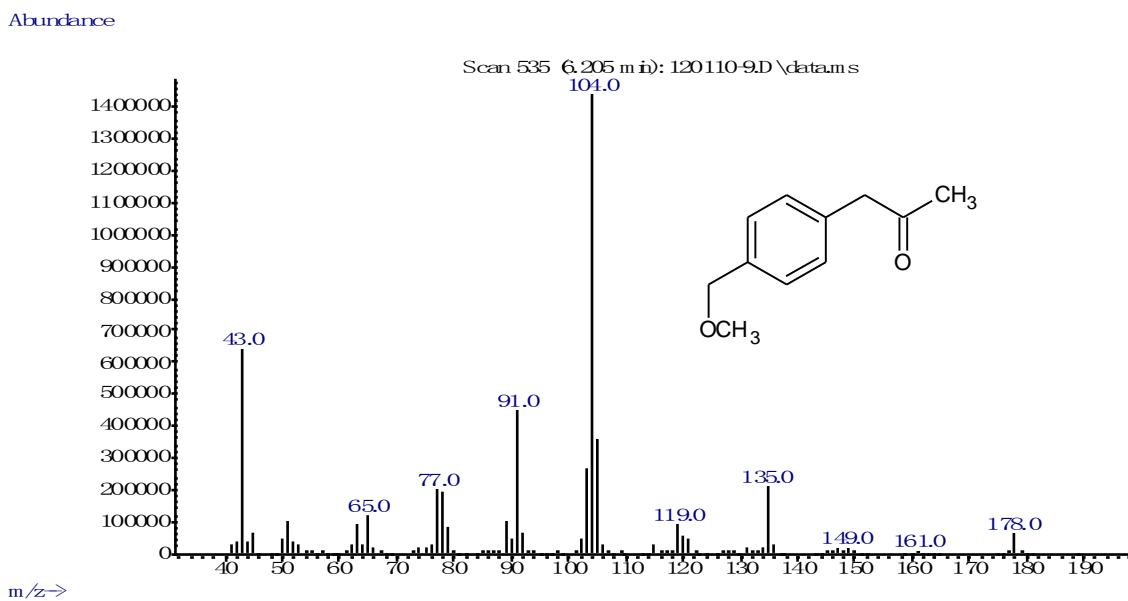


Figure 39 EI mass spectra of 4- methoxymethylene phenylacetone and 4- ethoxy phenylacetone

Both compounds show significant ions of m/z 178, m/z 135 and m/z 43 corresponding to the molecular ion, methoxymethyl/ethoxy benzyl ion and acetyl ion, respectively. The most significant difference between the two mass spectra is the mass of the base peaks, the

methoxymethylene substituted phenylacetone at m/z 104 while the ethoxy substituted ketone at m/z 107. The base peak mass spectral difference is caused by substitution pattern variance in the position of the ether oxygen in the substituent at the 4- position. This placement of the oxygen allows for two quite different rearrangements to occur in these regioisomeric ketones. This study further supports the mechanism of the generation of the fragment m/z 104, Figure 36, and the fragment m/z 107, Figure 10. The support is based on the fact that as the positions of the ester side chain oxygen are different, the EI mass spectra ions generated are different. The fact suggests that the procedure for generating the m/z 104 and the m/z 107 involves the ester side chain of oxygen.

3.2.6 GC-TOF analysis on 4-ethoxy phenylacetone

GC-TOF is an analytical method that can provide elemental composition information for fragments under mass spectrum, thus it is an important approach for confirming synthesis result purposes. Figure 40 shows the TOF mass spectrum of 4-ethoxy phenylacetone, a regioisomer of methoxy methylenephenylacetone based on oxygen positioning in the side chain.

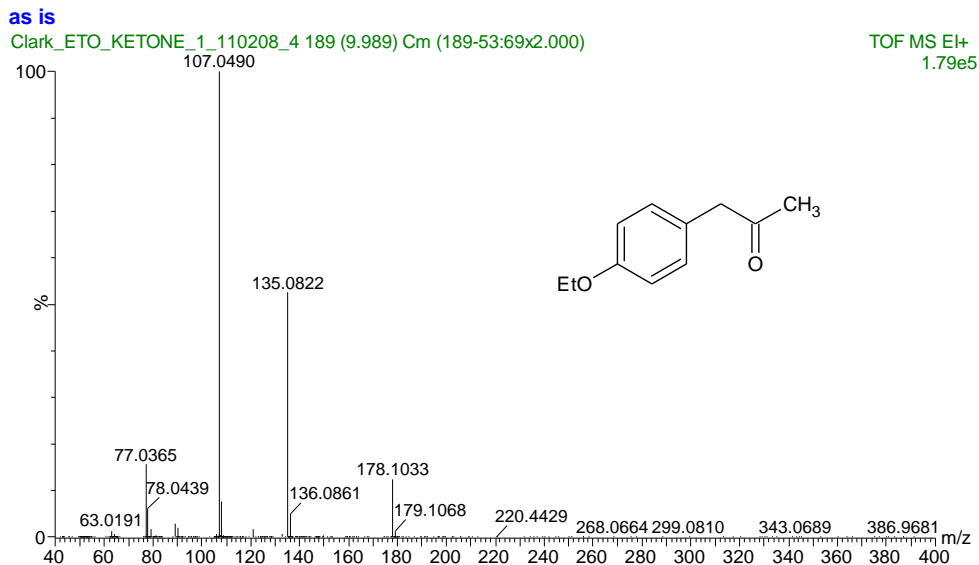


Figure 40 TOF mass spectra of 4-ethoxy phenylacetone

The mass spectrum of 4-ethoxy phenylacetone shows that the base peak is m/z 107, other significant peaks are the molecular ion peak at m/z 178 and the m/z 135 ion. The m/z 135 fragment is the 4-ethoxy benzyl ion derived from the loss of the acetyl group (CH_3CO). The structure study of m/z 107 is shown in Table 6.

Table 6 TOF data on m/z 107 fragment of 4-ethoxy phenylacetone

Measured Mass	107.0490
Calculated Mass	107.0497
Deviation (in PPM)	-6.5
Fragment Formula	$\text{C}_7\text{H}_7\text{O}$
Best Fit Ion Structure	

Deviation of 5 ppm in absolute value is acceptable in this study, calculated masses of the m/z 107 piece is somehow near that acceptable deviation range and is by far the nearest statistical fit of any possible combination of atoms, we accept the suggested fragment formula. The m/z 107 fragment is a loss of 71 mass from 4-ethoxy phenylacetone, and it has a suggested formula of C_7H_7O which is different with the molecular formula by C_4H_7O . The previous cleavage mechanism works well in interpreting this 107 piece also, shown in Figure 41.

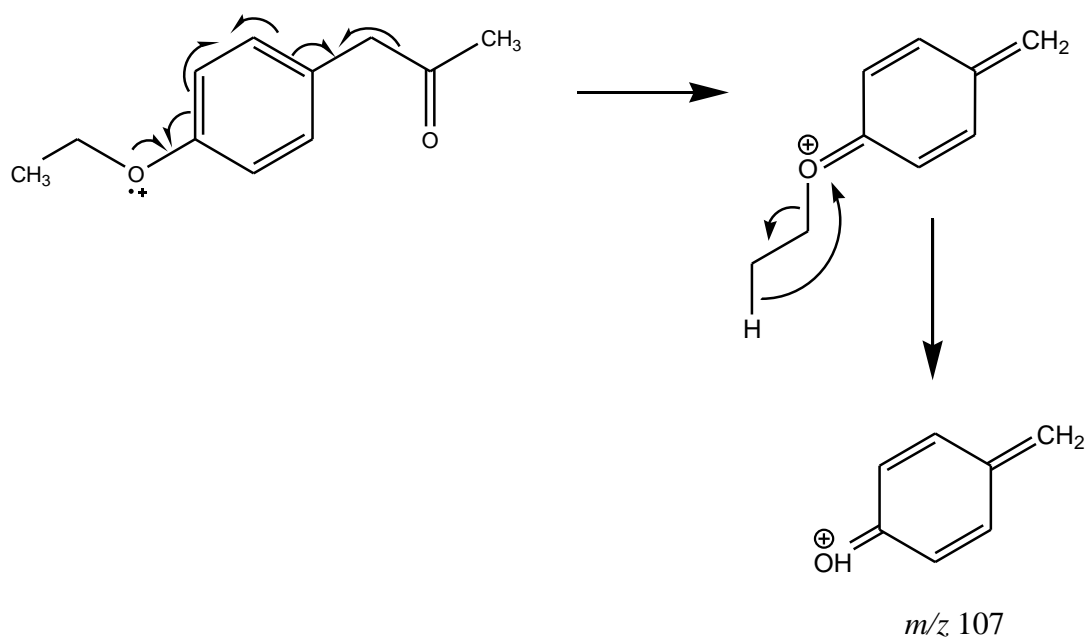


Figure 41 Possible mechanisms for the formation of mass 107 piece

In this fragmentation mechanism, the methoxy oxygen is ionized first. The following homolytic cleavage triggers the loss of the acetyl group. Finally, a charge initiated four membered ring hydrogen rearrangement generates the m/z 107 fragment. The analytical result is consistent with previously published study (Al-Hossaini A. M. *et al.*, 2010), and the GC-TOF exact mass confirms the mechanism of the m/z 107 generation involves the engagement of the

ester oxygen. This mechanism is very different from the generation of the m/z 104 fragment of the methoxymethylene ring substituted phenethylamines

3.3 GC-MS studies of the 4-methoxymethylene amphetamine series compounds

3.3.1 GC separation of the 4-methoxymethylene amphetamine series compounds

Gas chromatography separation of the 4-methoxymethylene amphetamine series of compounds was carried out on an Rtx-1 column. The column had a length of 30 meters, an internal dimension of 0.25 mm and a stationary phase film thickness of 0.25 μm . The stationary phase was 100% dimethyl polysiloxane. A temperature program was used for separation with the injector temperature set at 250 $^{\circ}\text{C}$, and detector temperature set at 280 $^{\circ}\text{C}$. The column was started at 70 $^{\circ}\text{C}$, held at that temperature for 1 minute then the temperature was ramped up to 250 $^{\circ}\text{C}$ at a rate of 30 $^{\circ}\text{C}$ per minute and set at 250 $^{\circ}\text{C}$ for 5 minutes. The finish time is 20 minutes. GC results are shown in Figure 42.

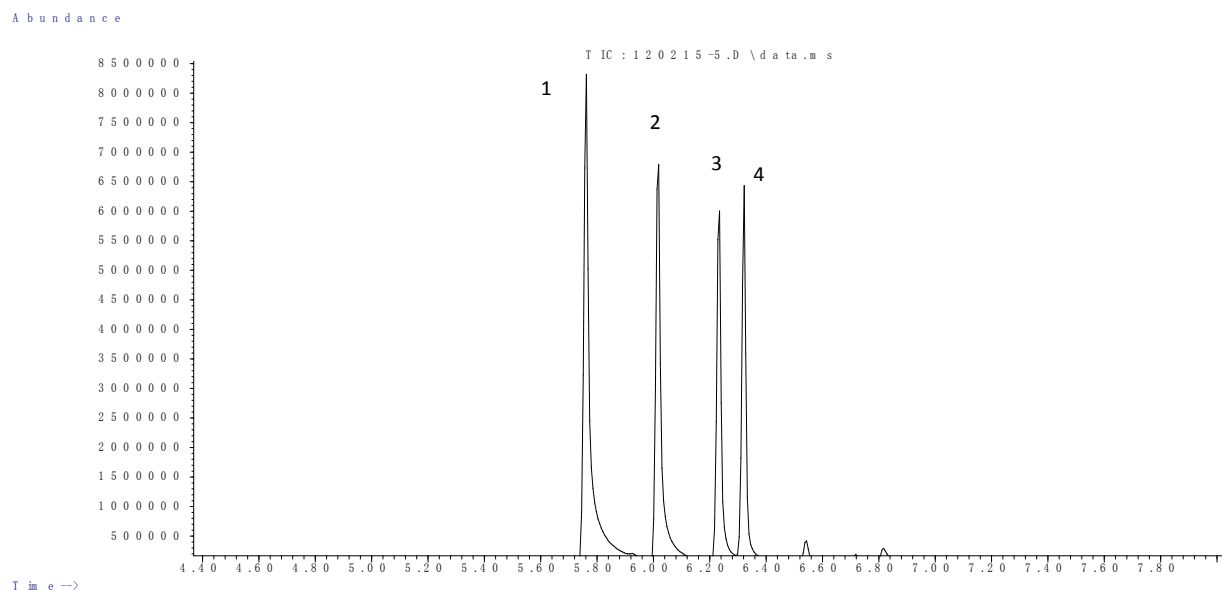


Figure 42 Gas chromatography separation of (1) 4-methoxymethylene amphetamine (2) 4-methoxymethylene methamphetamine (3) N-ethyl-4-methoxymethylene amphetamine (4) N, N-dimethyl-4-methoxymethylene amphetamine on an Rtx-1 column

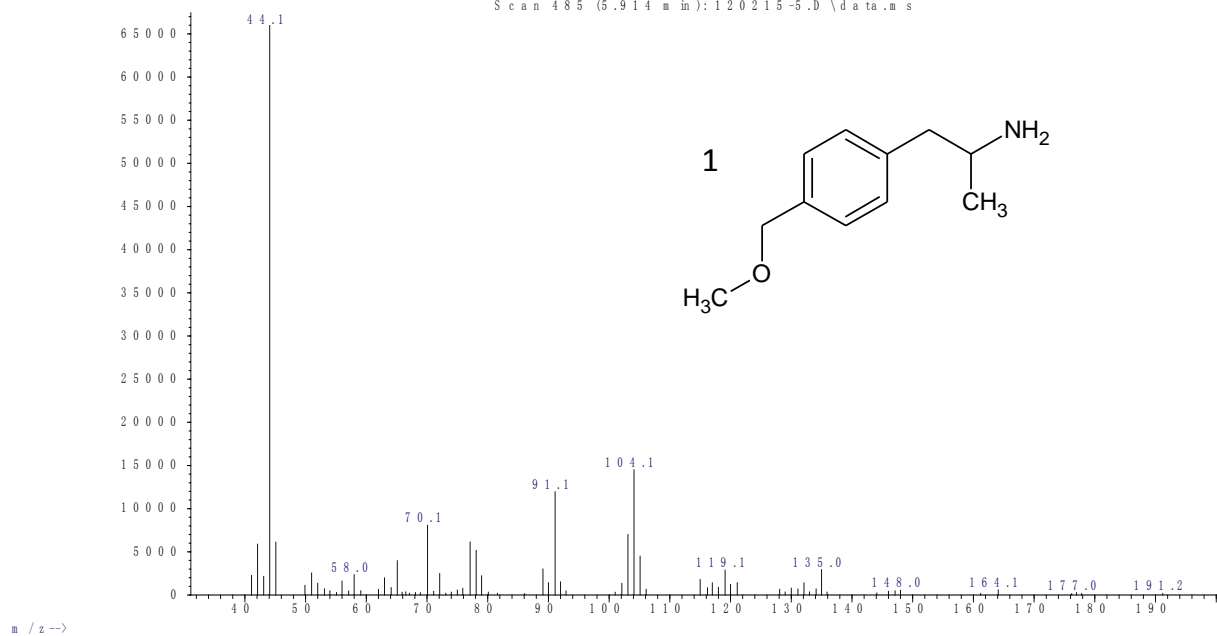
The retention time of 4-methoxymethylene amphetamine is 5.762 minute, the retention time of 4-methoxymethylene methamphetamine is 6.019 minute, the retention time of 4-methoxymethylene ethylamphetamine is 6.234 minute and that of 4-methoxymethylene dimethylamphetamine is 6.322 minute. Thus, the 4-methoxymethylene amphetamine series compounds can be nicely separated on column Rtx-1.

3.3.2 Mass spectra studies of the 4-methoxymethylene amphetamine series

This research studied the mass spectra of the 4-methoxymethylene amphetamine series compounds for comparison and separation purposes under 70eV electronic ionization, the results are shown in Figure 43.

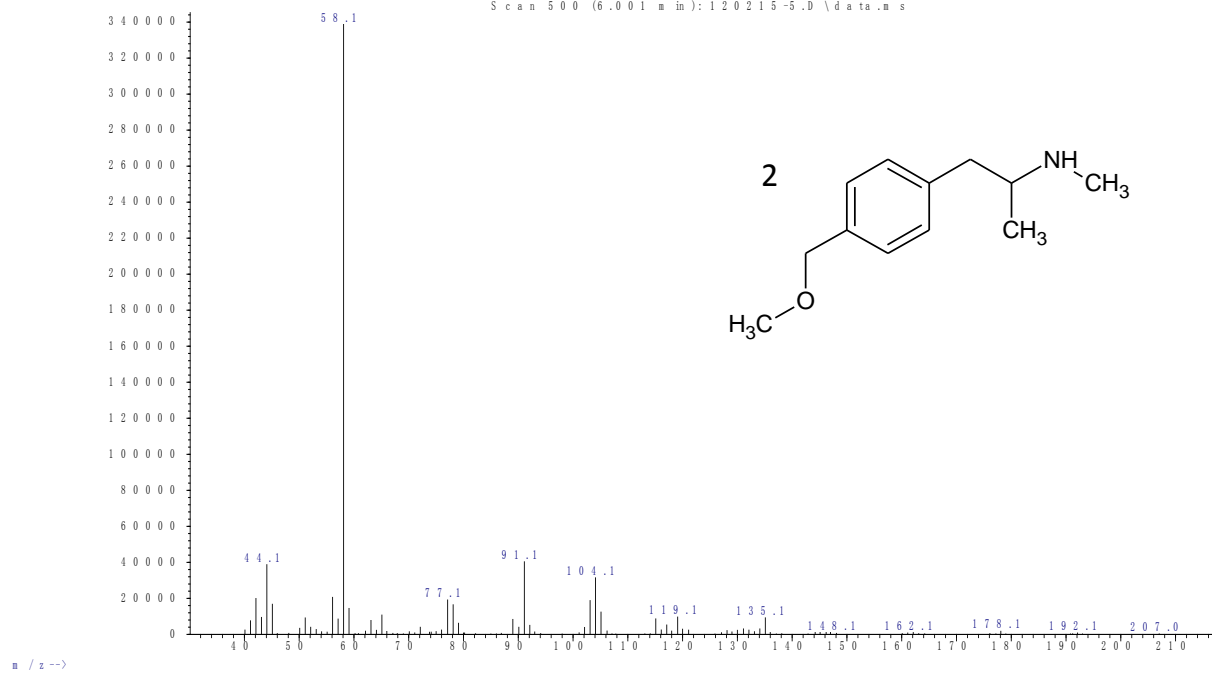
Abundance

Scan 485 (5.914 min): 120215-5.D\data.ms



Abundance

Scan 500 (6.001 min): 120215-5.D\data.ms



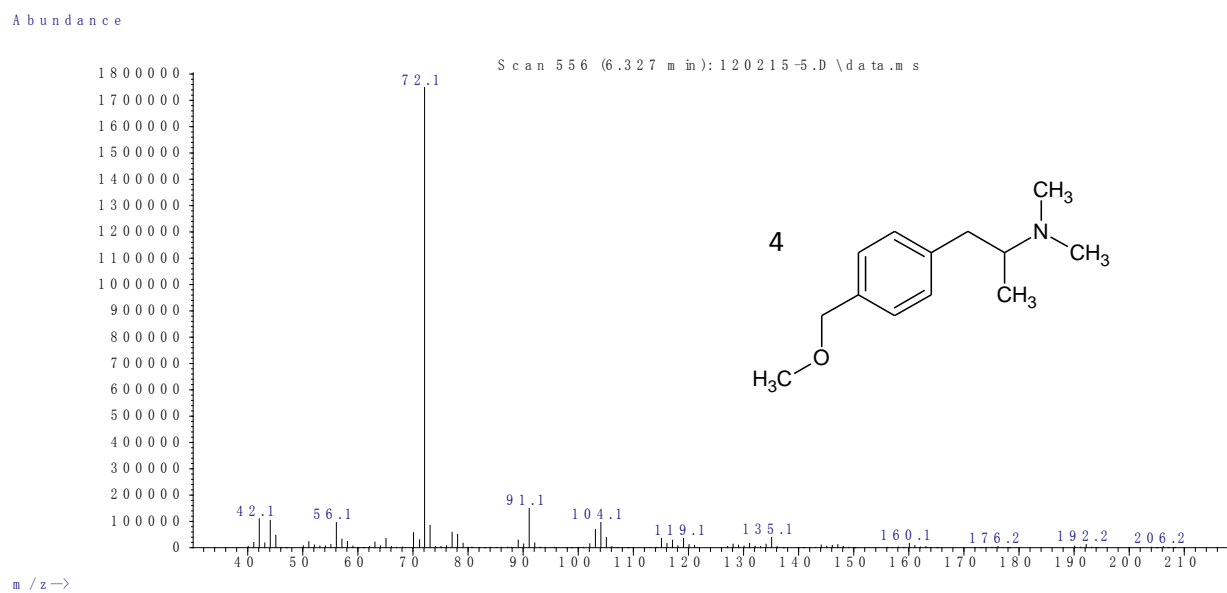
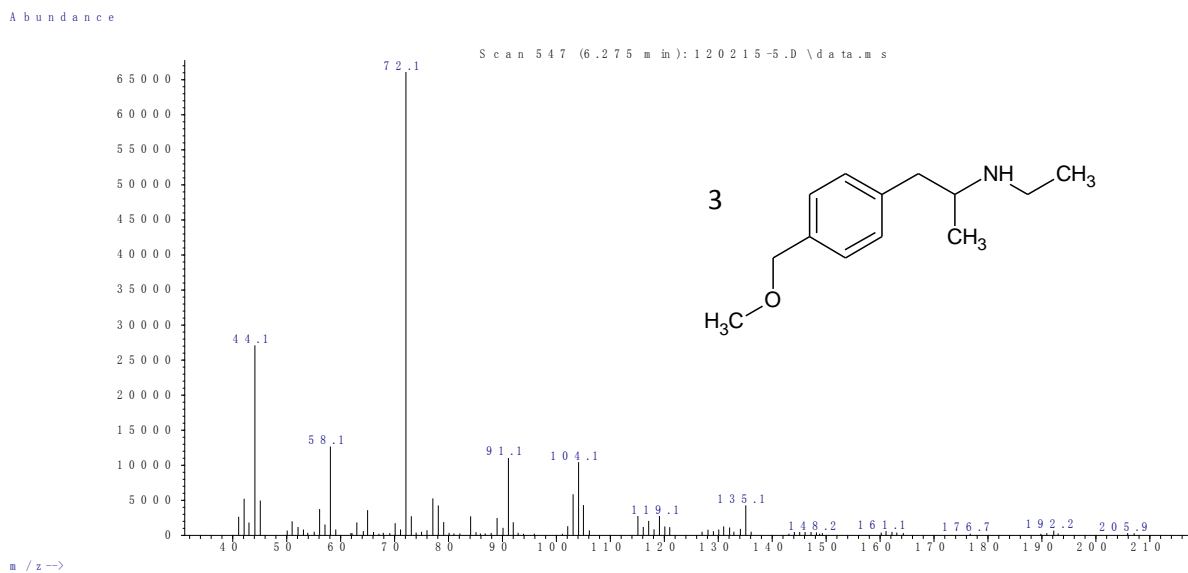


Figure 43 EI mass spectra of the 4-methoxymethylene amphetamine series

The base peak of each one of the mass spectra is derived from the ionization of nitrogen followed by α -cleavage. The base peak fragment structures of these four are shown in Figure 44.

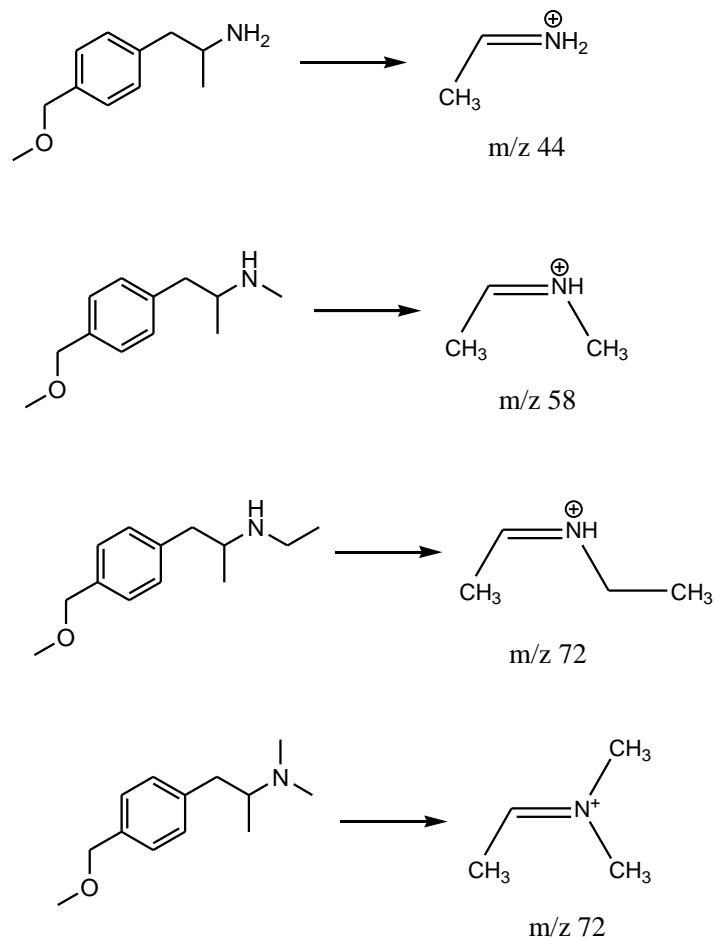


Figure 44 Base peak fragment structures of the 4-methoxymethylene amphetamine series under EI mass spectrometry

The mass spectrum of 4-methoxymethylene N-ethylamphetamine show a certain abundance of m/z 44, while the mass spectrum of 4-methoxymethylene N, N-dimethylamphetamine barely shows any m/z 44 peak. The m/z 44 peak is generated from a 4-membered ring H-rearrangement from the previous m/z 72 fragment, the most likely mechanism of this rearrangement is shown in Figure 45. The difference of the m/z 44 peak abundance in these two compounds is because only the N-ethylamine m/z 72 precursor has available β -hydrogen, which is required for the occurrence of the 4-membered ring H-rearrangement.

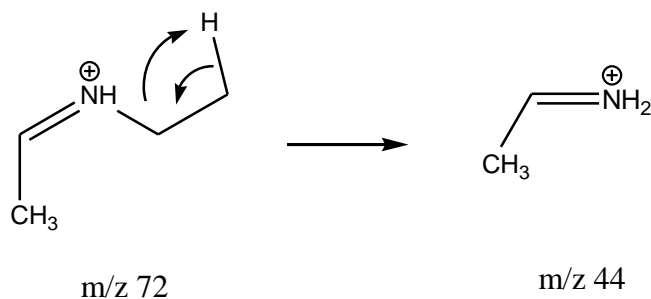


Figure 45 Mechanism proposed for the generation of m/z 44 fragment of 4-methoxymethylene N-ethylamphetamine under mass spectrometry

Another characteristic of the mass spectra of the 4-methoxymethylene amphetamine series compounds is that each spectrum shows the existence of the m/z 104 fragment. This is a predominant difference from the mass spectra of the 4-ethoxy amphetamine series compounds studied in previous work. The key characteristic of the mass spectra of the 4-ethoxy amphetamine series compounds is the existence of the m/z 107 fragment, which is generated by the mechanism shown in Figure 10.

Other than the structure information revealed by EI mass spectrometry discussed above, there is not much other information shown. In fact, this is a prevalent characteristic of mass spectra of regioisomer and isobaric amines related to MDA, MDMA, MDEA and MDMMA, generating huge challenges for identification work.

3.4 GC-MS studies of the 4-methoxymethylene butanamine series compounds

3.4.1 GC separation of the 4-methoxymethylene butanamine series compounds

Gas chromatography separation of the 4-methoxymethylene butanamine series compounds was carried out on an Rxi-50column. The column had a length of 30 meters, an internal dimension of 0.25 mm and a stationary phase film thickness of 0.25 μm . The stationary phase

was 50% phenyl- 50% methyl polysiloxane. The temperature program used for separation is to set the injector temperature at 250 °C, and the detector temperature at 280 °C. The column was started at 100 °C, held at that temperature for 1 min then the temperature was ramped up to 180 °C at a rate of 7.5 °C per minute and set at 180 °C for 2 minutes. Then the temperature was ramped up to 200 °C at a rate of 10 °C per minute. The finish time is 60 minutes. GC results are shown in Figure 46.

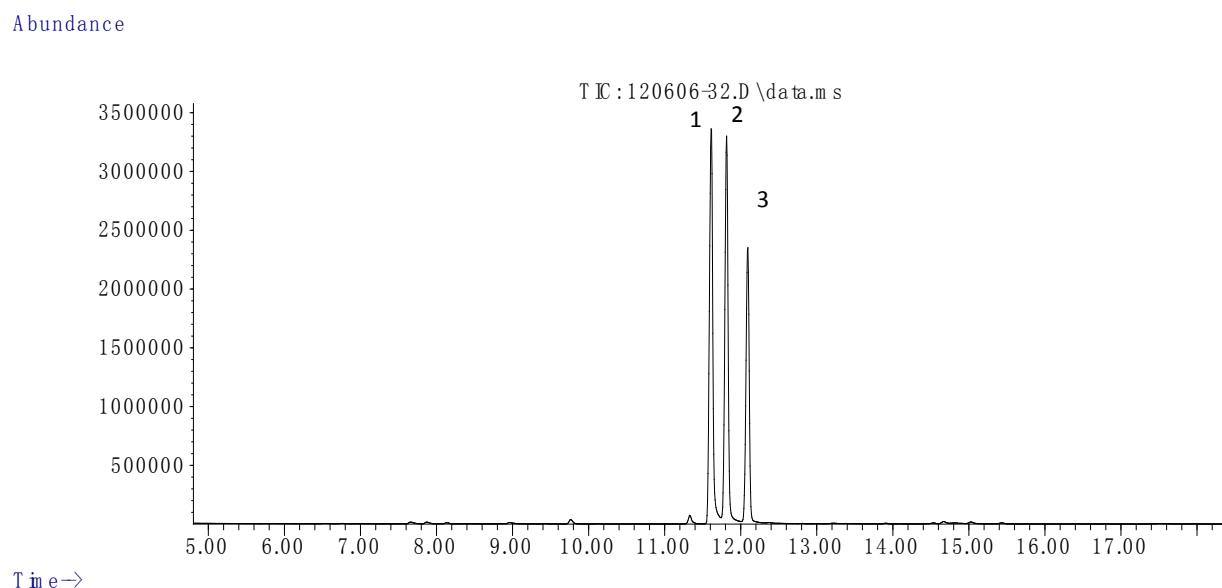


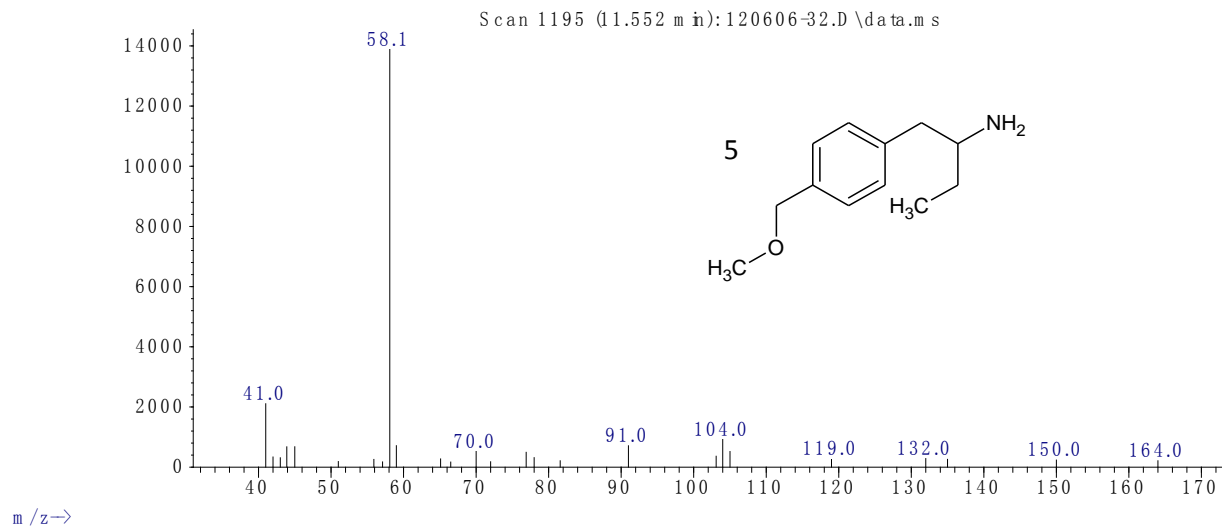
Figure 46 Gas chromatography separation of (1) 4-methoxymethylene 2-butanamine (2) 4-methoxymethylene N-methyl 2-butanamine (3) 4-methoxymethylene N-ethyl 2-butanamine on columns Rxi-50

The retention time of 4-methoxymethylene 2-butanamine was 11.610 minute, the retention time of 4-methoxymethylene N-methyl 2-butanamine was 11.820 minute and the retention time of 4-methoxymethylene N-ethyl 2-butanamine was 12.094 minute. The 4-methoxymethylene butanamine series compounds can be nicely separated on an Rxi-50 column.

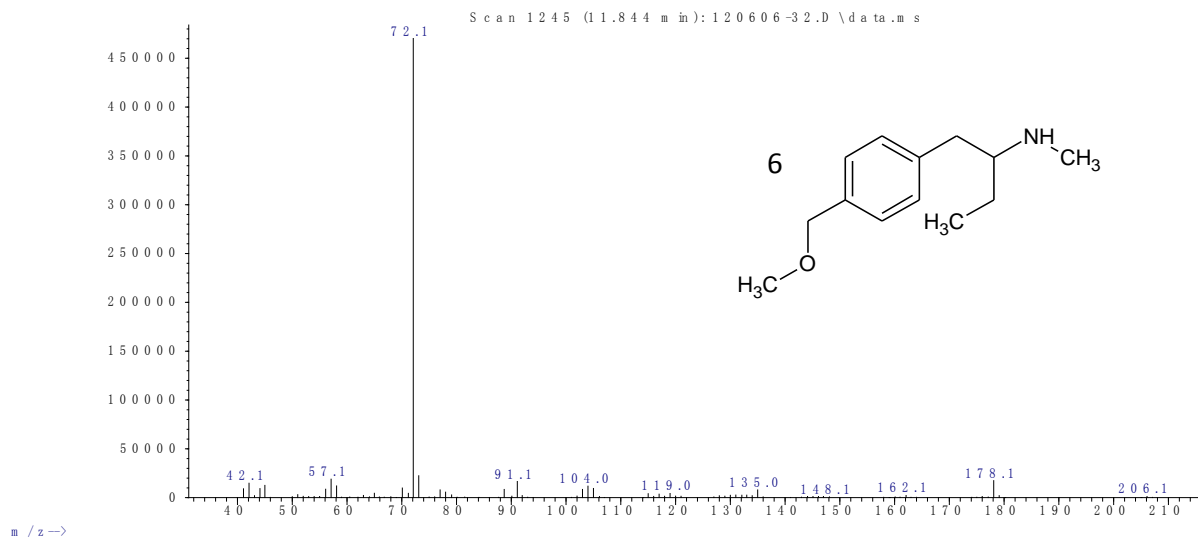
3.4.2 Mass spectra studies of the 4-methoxymethylene butanamine series compounds

This research studied the mass spectra of the 4-methoxymethylene butanamine series compounds for comparison and separation purposes under 70eV electronic ionization, the results are shown in Figure 47.

Abundance



Abundance



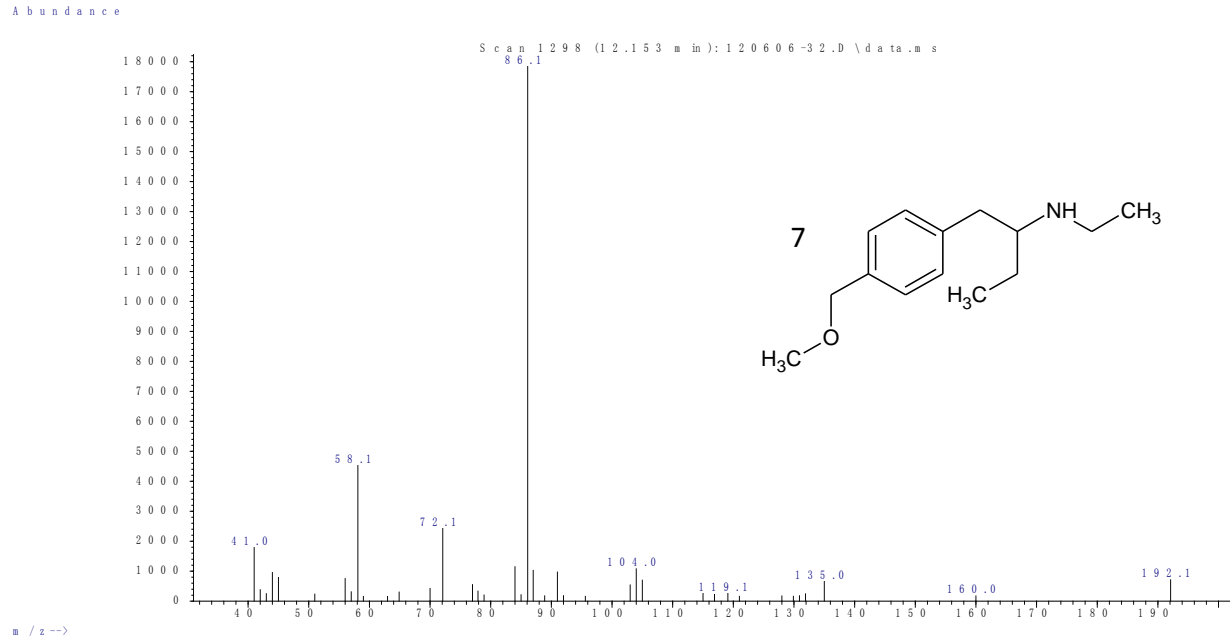


Figure 47 Mass spectra of the 4-methoxymethylene butanamine series compounds

The base peak of each one of the mass spectra is derived from the ionization of nitrogen followed by α -cleavage. The base peak fragment structures of these four compounds are shown in Figure 48.

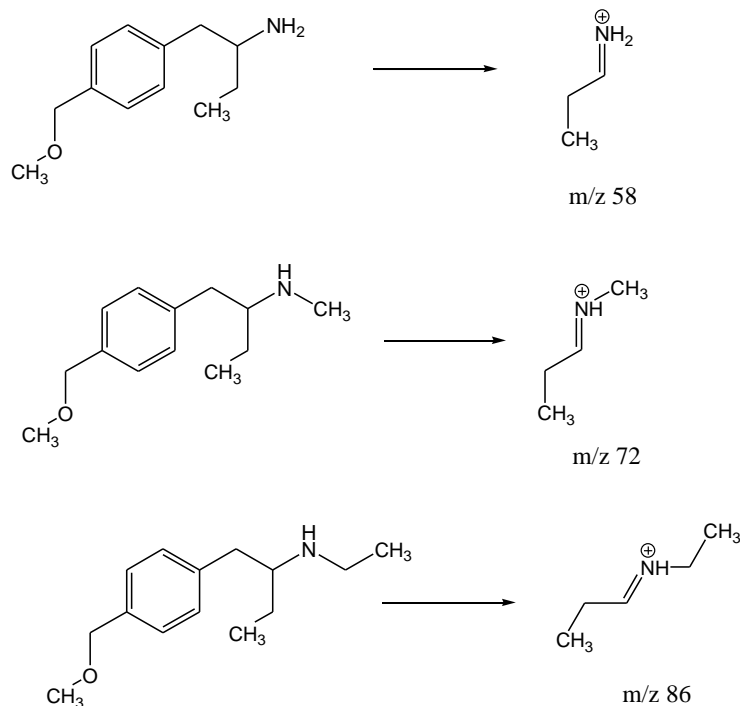


Figure 48 Base peak fragment structures of the 4-methoxymethylene butanamine series compounds under EI mass spectrometry

A major characteristic of the mass spectra of the 4-methoxymethylene butanamine series compounds is that each spectrum shows the existence of the m/z 104 fragment. This is a predominant difference from the mass spectra of the 4-ethoxy butanamine series compounds studied in previous work. The key characteristic of the mass spectra of the 4-ethoxy amphetamine series of compounds is the existence of an m/z 107 fragment, which is generated by the mechanism shown in Figure 10.

Other than the structure information revealed by EI mass spectrometry discussed above, there is not much other information shown. In fact, this is a prevalent characteristic of mass spectra of regioisomer and isobaric amines related to MDA, MDMA, MDEA and MDMA, generating huge challenges for identification work.

From the study results, the major character of the mass spectra of the methoxymethylene ring substituted phenethylamines is that each compound shows a certain amount of the m/z 104 fragment. This is very different from the unique m/z 107 peak we saw in the previous ethoxy counterpart compounds. The difference reveals that the generation of both the m/z 104 fragment and the m/z 107 fragment involves the oxygen on the ester side chain.

3.5 GC-MS analysis on HFBA derivatized 4-methoxymethylene phenethylamines: compound 1-3 and 5-7

3.5.1 GC separation of the HFBA derivatized 4-methoxymethylene amphetamine series and 4-methoxymethylene butanamine series

Gas chromatography separation of the 4-methoxymethylene amphetamine series of compounds and the 4-methoxymethylene butanamine series of compounds were carried out separately on Rtx-5 amine columns. The column has a length of 30 meters, an internal dimension of 0.25 mm and a stationary phase film thickness of 0.25 μm . The stationary phase is 5% diphenyl- 95% dimethyl polysiloxane. The temperature program used for separation is to set the injector temperature at 250 $^{\circ}\text{C}$, and the detector temperature at 280 $^{\circ}\text{C}$. The column is started at 70 $^{\circ}\text{C}$, held at that temperature for 1 minute then the temperature was ramped up to 250 $^{\circ}\text{C}$ at a rate of 30 $^{\circ}\text{C}$ per minute and set at 250 $^{\circ}\text{C}$ for 5 minutes. The finish time is 20 minutes. GC results are shown in Figure 49 and Figure 50.

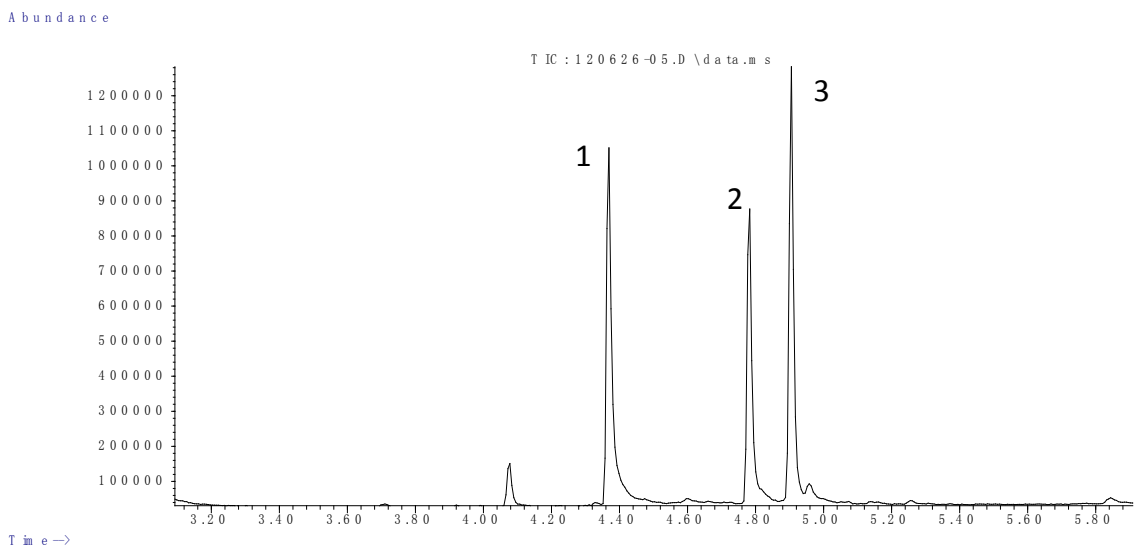


Figure 49 GC separation of the HFBA derivatized 4-methoxymethylene amphetamine series: HFBA derivatized compound 1, 2 and 3 on an Rtx-5 amine column

The retention time of HFBA derivatized 4-methoxymethylene amphetamine is 4.369 minute, the retention time of HFBA derivatized 4-methoxymethylene methamphetamine is 4.783 minute, and the retention time of HFBA derivatized 4-methoxymethylene ethylamphetamine is 4.905 minute. Thus, the 4-methoxymethylene amphetamine series compounds can be nicely separated on Rtx-5 amine.

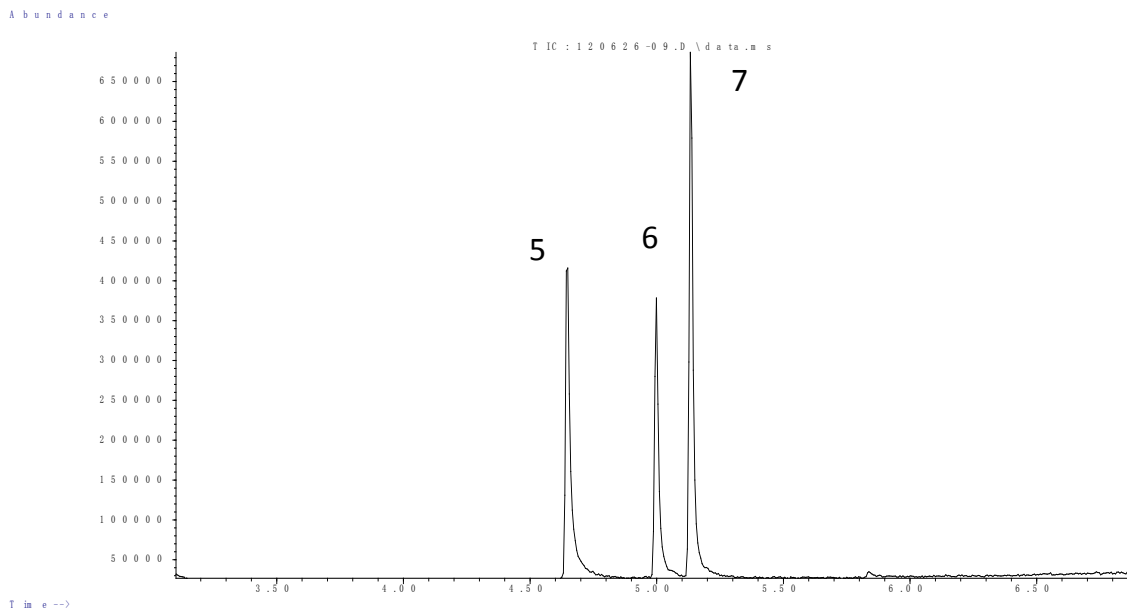


Figure 50 GC separation of the HFBA derivatized 4-methoxymethylene butanamine series: compound 5, 6 and 7 on an Rtx-5 amine column

The retention time of HFBA derivatized 4-methoxymethylene 2-butanamine is 4.643 minute, the retention time of HFBA derivatized 4-methoxymethylene N-methyl 2-butanamine is 4.999 minute and the retention time of HFBA derivatized 4-methoxymethylene N-ethyl 2-butanamine is 5.139 minute. The HFBA derivatized 4-methoxymethylene butanamine series compounds can be nicely separated on column Rtx-5 amine.

3.5.2 Mass spectra studies of the HFBA derivatized 4-methoxymethylene amphetamine series and 4-methoxymethylene butanamine series

This research studied the mass spectra of the HFBA derivatized 4-methoxymethylene amphetamine series compounds and the HFBA derivatized 4-methoxymethylene butanamine series for comparison and separation purposes under 70eV electronic ionization, the results are shown in Figure 51 and Figure 55.

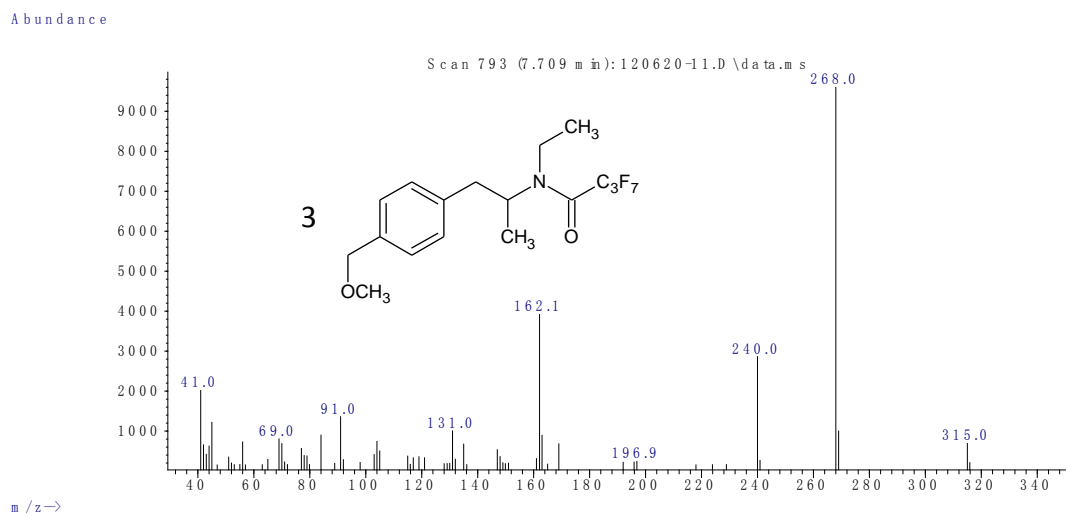
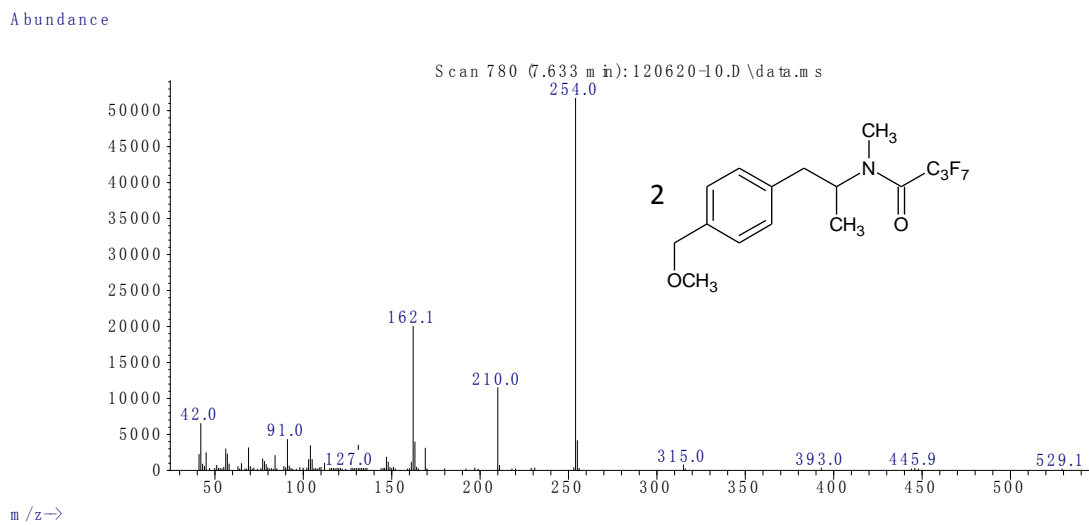
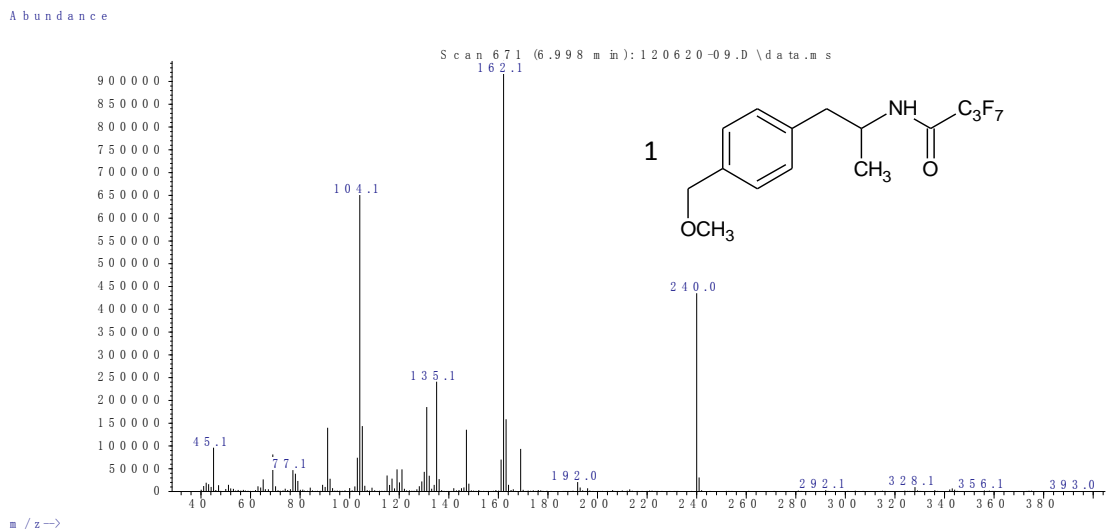


Figure 51 Mass spectra of the HFBA derivatives of compounds 1-3

The mass spectra of the HFBA derivatives of the 4-methoxymethylene amphetamine series show several characteristic fragments in each compound that allow for identification. The HFBA derivatized 4-methoxymethylene amphetamine (compound 1) shows unique peaks such as m/z 240, m/z 162 (base peak) and m/z 104. The formation of the m/z 240 fragment is an α -cleavage followed by the ionization of the amine nitrogen, shown in Figure 52. The formation of the base fragment m/z 162 is from the ionization of the acyl oxygen, followed by 6 membered ring hydrogen rearrangement. Charge initiated hetero bond cleavage will generate the m/z 162 fragment, shown in Figure 53. The mechanism of the formation of the m/z 104 fragment was discussed previously.

The HFBA derivatized 4-methoxymethylene methylamphetamine (compound 2) shows unique peaks such as m/z 254 (base peak), m/z 162 and m/z 210. The formation of base peak m/z 254 fragment is α -cleavage followed by the ionization of the amine nitrogen, shown in Figure 52. Further 4 membered ring rearrangement of the fragment m/z 254 will yield the fragment m/z 210, shown in Figure 54. The formation of m/z 162 is from the ionization of the acyl oxygen, followed by 6 membered ring hydrogen rearrangement. Charge initiated hetero bond cleavage will generate the m/z 162 fragment, shown in Figure 53.

The HFBA derivatized N-ethyl 4-methoxymethylene amphetamine (compound 3) shows unique peaks such as m/z 268 (base peak), m/z 162 and m/z 240. The formation of base peak m/z 268 fragment is α -cleavage followed by the ionization of the amine nitrogen, shown in Figure 52. Since the ionized nitrogen center has available β -hydrogens, further 4-membered ring rearrangement of the m/z 268 fragment will induce a loss of 28 mass units (C_2H_4) and generate the m/z 240 fragment. The formation of m/z 162 is from the ionization of the acyl oxygen,

followed by 6 membered ring hydrogen rearrangement. Charge initiated hetero bond cleavage will generate the m/z 162 fragment, shown in Figure 53.

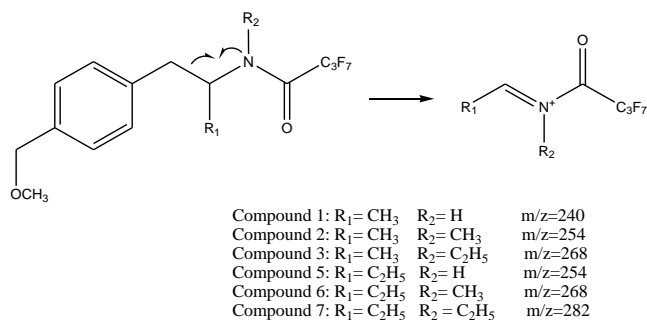


Figure 52 Formation of m/z 240, m/z 254, m/z 268 and m/z 282 fragments from perfluoroacyl derivatives of compound 1-3 and compound 5-7

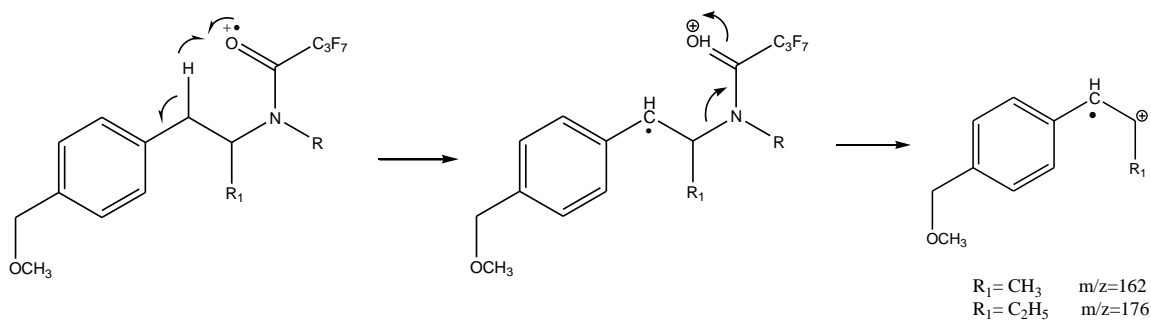
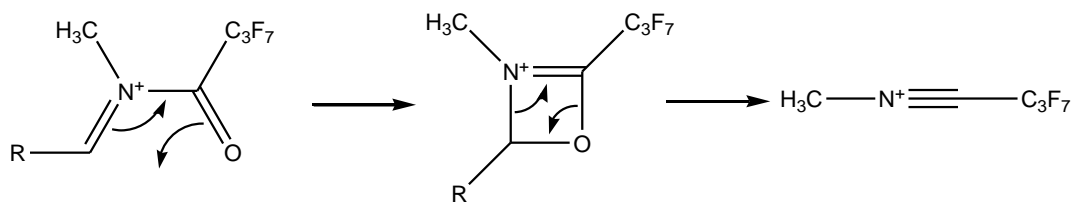


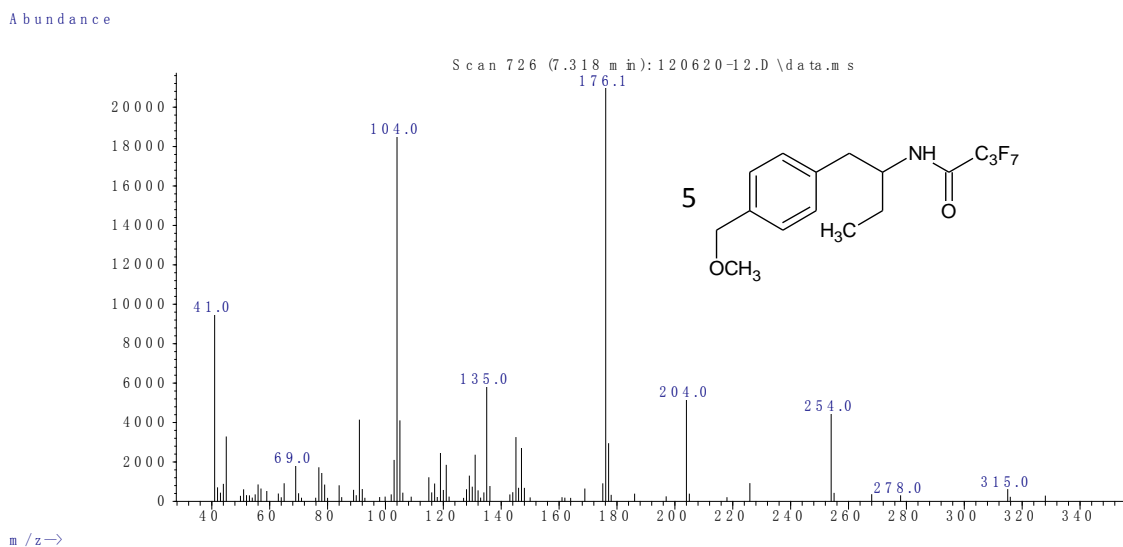
Figure 53 Formation of m/z 162 from perfluoroacyl derivative of 4-methoxymethylene amphetamine series and m/z 176 from perfluoroacyl derivative of 4-methoxymethylene butanamine series



$m/z = 210$

Compound 2: R=CH₃ $m/z=254$
 Compound 6: R=C₂H₅ $m/z=268$

Figure 54 Formation of m/z 210 fragments from m/z 254 of N-methyl compound 2 and m/z 268 of N-methyl compound 6



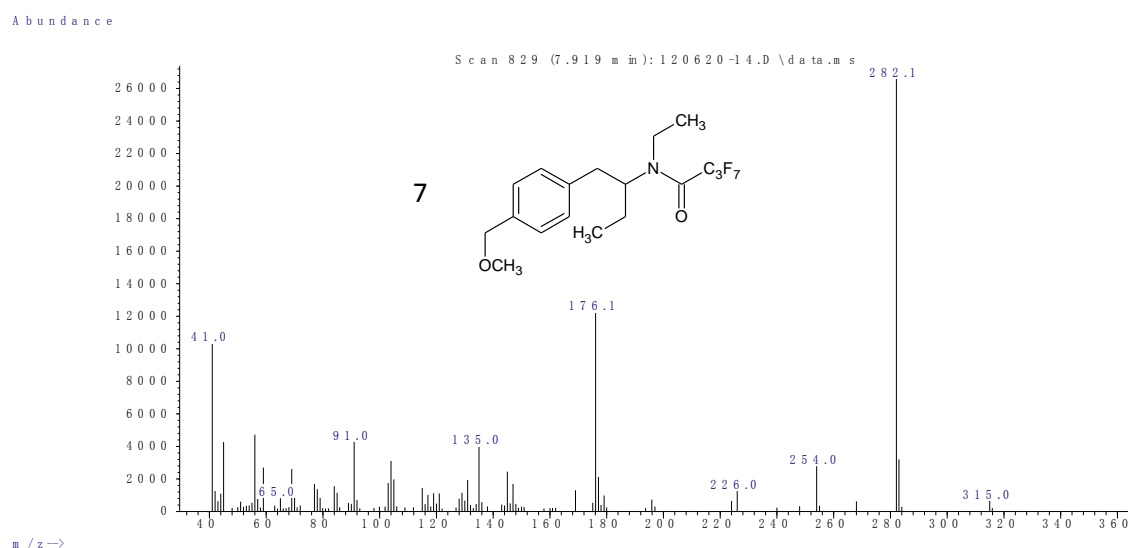
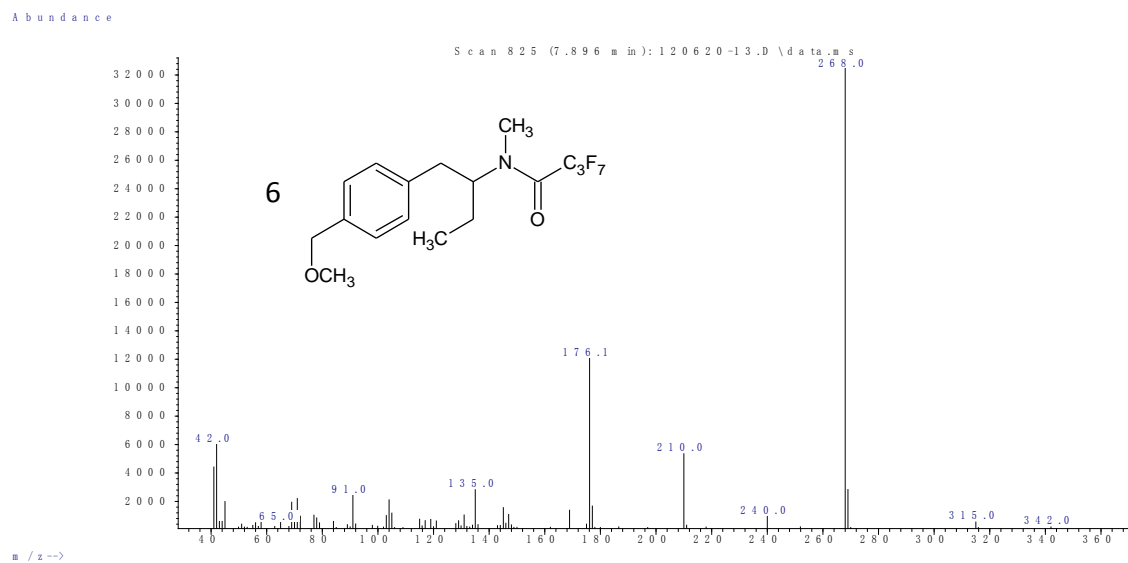


Figure 55 Mass spectra of the HFBA derivatives of compounds 5-7

The HFBA derivatized 4-methoxymethylene 2-butanamine (compound 5) shows unique peaks such as m/z 176 (base peak), m/z 104 and m/z 254. The formation of the base fragment m/z 176 is from the ionization of the acyl oxygen, followed by 6 membered ring hydrogen rearrangement. Charge initiated hetero bond cleavage will generate the m/z 176 fragment, shown in Figure 53. The formation of m/z 254 fragment is α -cleavage followed by the ionization

of the amine nitrogen, shown in Figure 52. The mechanism of the formation of the m/z 104 fragment was discussed previously.

The HFBA derivatized 4-methoxymethylene N-methyl 2-butanamine (compound 6) shows unique peaks such as m/z 176, m/z 268 (base peak) and m/z 210. The formation of the base peak m/z 268 fragment is due to α -cleavage followed by the ionization of the amine nitrogen, shown in Figure 52. Further 4 membered ring rearrangement of the fragment m/z 268 will yield the fragment m/z 210, shown in Figure 54. The formation of m/z 176 is from the ionization of the acyl oxygen, followed by 6 membered ring hydrogen rearrangement. Charge initiated hetero bond cleavage will generate the m/z 176 fragment, shown in Figure 53.

The HFBA derivatized 4-methoxymethylene N-ethyl 2-butanamine (compound 3) shows unique peaks such as m/z 282 (base peak), m/z 176 and m/z 254. The formation of base peak m/z 282 fragment is due to α -cleavage followed by the ionization of the amine nitrogen, shown in Figure 52. Since the ionized nitrogen center has available β -hydrogens, further 4-membered ring rearrangement of the m/z 282 fragment will induce a loss of 28 mass units (C_2H_4) and generate the m/z 254 fragment. The formation of m/z 176 is from the ionization of the acyl oxygen, followed by 6 membered ring hydrogen rearrangement. Charge initiated hetero bond cleavage will generate the m/z 176 fragment, shown in Figure 53.

4. Experimental

4.1 Materials, Instruments, GC-Columns and Temperature Programs

4.1.1 Materials

Analysis comparison compound 4-ethoxybenzaldehyde as well as most of the synthesis materials, such as Red-Al sodium bis (2-methoxyethoxy) aluminum hydride solution in toluene, Nitroethane, 1-nitropropane, methylamine hydrochloride, ethylamine hydrochloride, dimethylamine hydrochloride, chromium (VI) oxide, sodium stick, iron powder and sodium cyanoborohydride, were purchased from Aldrich chemical company (Milwaukee, WI, USA).

4-Chloromethylbenzoyl chloride was purchased from TCI America (Portland, OR, USA). Pyridine and sodium sulfate (anhydrous) were purchased from EMD chemicals, USA (Gibbstown, NJ, USA). Potassium hydroxide pellets was purchased from Acros Organics (Morris Plains, NJ, USA). Iron (III) chloride anhydrous and ammonium acetate were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Celite was purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Organic solvents such as HPLC grade acetonitrile, methylene chloride, methanol, and toluene were purchased from Fisher Scientific Company (Atlanta, GA, USA). Benzene was purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Silica gel was purchased from Natland International Corporation (Research Triangle Park, NC).

4.1.2 Instruments

GC-MS analysis of targets compounds and related intermediates were performed using a 7890A gas chromatograph with an auto injector 7683B, coupled with a mass selective detector 5975C VL, purchased from Agilent Technologies (Santa Clara, CA). The GC was operated in splitless mode under a constant helium (grade 5) flow with a rate at 0.7 mL/min. The GC column head pressure is 10 psi. The GC injector was maintained at 250 °C and the transfer temperature is 280 °C. The MS detector operated in the electron impact (EI) mode with an ionization voltage of 70 eV and a source temperature of 230 °C. The MS has a scan rate at 2.86 scans/s.

GC-TOF analysis was performed by a gas chromatograph with an auto injector 7683B, coupled with a Waters GCT Premier benchtop orthogonal acceleration time-of flight (oa-TOF) mass spectrometer, located in the Mass Spectrometry Center, Auburn University. The GC-TOF was purchased from Agilent Technologies (Santa Clara, CA). An accurate mass method was used to confirm the elemental composition of target fragments. The TOF detector compares exact mass of calculated model and the measured mass, the theoretical acceptable deviation is 5 ppm. Isotope marking was also adopted to confirm the elemental composition of specific fragments.

4.1.3 GC-Columns

GC-Columns used for analytical separations related to this research are listed in Table 7. All GC-Columns were purchased from Restek Corporation (Bellefonte, PA, USA). All the columns

are 30 meters long with 0.25 mm internal dimension and coated with 0.25 μm silicone complex film.

Table 7 List of GC-Columns and their stationary phase composition

Column Name	Column Composition
Rtx-1	100% Dimethyl Polysiloxane
Rtx-35	35% Diphenyl- 65% Dimethyl Polysiloxane
Rtx-50	50% Phenyl- 10% Methyl Polysiloxane
Rxi-50	50% Phenyl- 50% Methyl Polysiloxane
Rtx-200	100% Trifluoropropyl Methyl Polysiloxane
Rtx-5 Sil	5% Diphenyl- 95% Dimethyl Polysiloxane
Rtx-5 Amine	5% Diphenyl- 95% Dimethyl Polysiloxane

4.1.4 Temperature Programs

To get the best analytical and separation results, the following temperature programs were used during this research period.

Temperature program 1: injector temperature at 250 $^{\circ}\text{C}$, detector temperature at 280 $^{\circ}\text{C}$. Initial temperature for column is 70 $^{\circ}\text{C}$, hold that temperature for 1 minute then the temperature was ramped up to 250 $^{\circ}\text{C}$ at a rate of 30 $^{\circ}\text{C}$ per minute and set at 250 $^{\circ}\text{C}$ for 5 minutes. The finish time is 20 minutes.

Temperature program 2: injector temperature at 250 $^{\circ}\text{C}$, detector temperature at 280 $^{\circ}\text{C}$. Column is started at 100 $^{\circ}\text{C}$, hold that temperature for 1 min then the temperature was ramped up

to 180 °C at a rate of 7.5 °C per minute and set at 180 °C for 2 minutes. Then the temperature was ramped up to 200 °C at a rate of 10 °C per minute. The finish time is 60 minutes.

4.2 Synthesis of 4-methoxymethylene benzaldehyde

Sodium metal weighing 1.83 g (80 mmol) was cut into small pieces and placed in a 3 neck flask, and then methanol was added under nitrogen. 4-Chloromethylbenzyl chloride (5 g, 26.4 mmol) dissolved in methanol was added drop wise into that 3 neck flask. The resulting mixture was refluxed for 3 hours then the mixture was cooled to room temperature. The mixture was further cooled in an ice bath and 6 mL 12 M aqueous hydrochloride (80 mmol) was added drop wise. The methanol was evaporated using a rotary evaporator and the resulting aqueous residue was extracted with methylene chloride. The organic layer was dried with sodium sulfate and then evaporated under reduced pressure to remove methylene chloride. The product methyl 4-methoxymethyl benzoate was obtained as a light yellow oil.

A solution of 5 g methyl 4-(methoxymethyl) benzoate (0.028 mol) in 80 ml benzene was added to a 500 ml three neck flask and stirred at room temperature under nitrogen. A syringe was used to inject 10 mL of sodium bis (2-methoxyethoxy) aluminum hydride (Red-Al) solution in toluene (65% wt) slowly into the reaction solution through a rubber septum fitted to one neck of the flask. The resulting solution was stirred overnight at room temperature.

The excess Red-Al was relinquished by the consecutive addition of 14 mL 2M sodium hydroxide solution, 28 mL water and 42 mL 2M sodium hydroxide solution. The resulting precipitated aluminum salts were removed by filtration and the filtrate was evaporated under vacuum to remove the benzene. The remaining solution was extracted with methylene chloride and the organic layer was dried with sodium sulfate and then evaporated under reduced pressure

to remove methylene chloride. The target product 4-methoxymethyl benzylalcohol was obtained as light yellow oil.

4.3 Synthesis of 4-methoxymethylene benzyl aldehyde

Chromium trioxide (50 g, 0.5 mol) was added rapidly with stirring to 92 mL of 6 M hydrochloric acid solution. After 5 minute, the homogenous solution was cooled to 0 °C and 35g of pyridine (0.5 mol) was carefully added over 10 minutes. Cooling the mixture to 0 °C over an ice bath again resulted in the formation of a yellow orange solid precipitate. The solid was collected by filtration and dried over vacuum for one hour to give dry pyridinium chlorochromate (PCC).

A solution of 5 g of 4-methoxymethyl benzyl alcohol (0.033 mol) was dissolved in 100 mL of methylene chloride in a 250 mL round bottle flask. To the stirring solution was added 10.80 g of freshly made pyridinium chlorochromate (0.05 mol) and 10.80 g celite. The resulting mixture was stirred at room temperature for 3 hours.

The reaction mixture was filtered through a bed of silica gel in a Buchner funnel and then the filtrate was evaporated under reduced pressure to get a yellow liquid, 4-methoxymethylene benzaldehyde as a crude product. Kugelrohr distillation of the crude product gave a purified yellow oil, 4-methoxymethylene benzaldehyde, molecular formula: $C_9H_{10}O_2$, molecular mass 150 g/mol.

4.4 Synthesis of 4-methoxymethylene phenylacetone

4.4.1 Synthesis of 4-methoxymethylene phenyl 2-nitropropene

4-Methoxymethylene benzaldehyde (5.0 g, 0.033 mol) was dissolved in 80 mL benzene in a 250 mL round bottom flask, followed by the addition of 8 mL of n-butylamine (0.11 mol). The reaction mixture was refluxed overnight using a Dean Stark trap to remove water. Cooling the reaction mixture to room temperature and the solvent was evaporated under reduced pressure to give the crude imine intermediate yellow oil. The imine intermediate was dissolved in 20 mL of glacial acetic acid in a 250 mL round bottom flask, and 8 mL of nitroethane (0.11 mol) was added drop wise. The resulting reaction mixture was refluxed for one hour. During reflux, yellow green crystals of 4-methoxymethylene phenyl 2-nitropropene were formed in the reaction mixture. After the reaction, another 25 mL of glacial acetic acid was added to the reaction mixture which was then cooled down to room temperature on an ice bath. The reaction mixture was poured over crushed ice and was acidified to pH 1 using concentrated hydrochloric acid. Yellow green crystals of 4-methoxymethylene phenyl 2-nitropropene were isolated by vacuum filtration, washed with water, air dried for further use.

4.4.2 Synthesis of 4-methoxymethylene phenylacetone

4-Methoxymethylene phenyl 2-nitropropene (3.0 g, 0.014 mol) was dissolved in 15 mL toluene and 15 mL water in a 250 mL round bottom flask. The resulting solution was mixed with 4.5 g powdered iron (0.08 mol); 0.9 g ferric chloride (0.006 mol) and 6 mL concentrated hydrochloric acid. The mixture was stirred vigorously and refluxed over a day. After cooled to room temperature, 30 mL toluene and 30 mL water was added and the mixture was filtered under reduced pressure. The precipitate was washed with additional toluene and water. The toluene

layer was separated, and washed with 6M hydrochloric acid, water and saturated sodium bicarbonate solution. The organic layer was dried over sodium sulfate, filtered and the solvent was evaporated to give dark brown crude product 4-methoxymethylene phenylacetone. Kugelrohr distillation of the crude product gave purified yellow oil 4-methoxymethylene phenylacetone, molecular formula: $C_{11}H_{14}O_2$, molecular mass 178 g/mol.

4.5 Synthesis of the 4-methoxymethylene amphetamine series compounds

4.5.1 Synthesis of 4-methoxymethylene amphetamine

4-Methoxymethylene phenylacetone (0.5 g, 0.0028 mol) was dissolved in 25 mL methanol, and then 2.165 g of ammonium acetate (0.028 mol) and 1.765 g of sodium cyanoborohydride (0.028 mol) were added to the stirring solution. The reaction mixture was stirred at room temperature for three days and the mixture pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was then evaporated under reduced pressure to yield a white solid residue. The residue was suspended in 40 mL cold water, and slowly acidified by the addition of concentrated hydrochloric acid. The resulting solution mixture was stirred under room temperature over night. After that the aqueous acidic solution was washed with methylene chloride. The aqueous layer was separated and alkalized by the addition of potassium hydroxide pellets. The aqueous basic suspension was extracted with methylene chloride and the organic layer was dried with sodium sulfate. The organic layer was evaporated under reduced pressure to give 4-methoxymethylene amphetamine as light yellow oil. The product has a molecular formula of $C_{11}H_{17}NO$ and a molecular mass of 179 g/mol.

4.5.2 Synthesis of 4-methoxymethylene methamphetamine

4-Methoxymethylene phenylacetone (0.5 g, 0.0028 mol) was dissolved in 25 mL methanol, and then 1.875 g of methylamine hydrochloride (0.028 mol) and 1.765 g of sodium cyanoborohydride (0.028 mol) were added to the stirring solution. The reaction mixture was stirred at room temperature for three days and the mixture pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was then evaporated under reduced pressure to yield a white solid residue. The residue was suspended in 40 mL cold water, and slowly acidified by the addition of concentrated hydrochloric acid. The resulting solution mixture was stirred under room temperature over night. After that the aqueous acidic solution was washed with methylene chloride. The aqueous layer was separated and alkalized by the addition of potassium hydroxide pellets. The aqueous basic suspension was extracted with methylene chloride and the organic layer was dried with sodium sulfate. The organic layer was evaporated under reduced pressure to give 4-methoxymethylene methamphetamine as light yellow oil. The product has a molecular formula of $C_{12}H_{19}NO$ and a molecular mass of 193 g/mol.

4.5.3 Synthesis of 4-methoxymethylene ethylamphetamine

4-Methoxymethylene phenylacetone (0.5 g, 0.0028 mol) was dissolved in 25 mL methanol, and then 2.29 g of ethylamine hydrochloride (0.028 mol) and 1.765 g of sodium cyanoborohydride (0.028 mol) were added to the stirring solution. The reaction mixture was stirred at room temperature for three days and the mixture pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was then evaporated under reduced pressure to yield a white solid residue. The residue was suspended in 40 mL cold water, and slowly acidified by the addition of concentrated hydrochloric acid. The resulting solution

mixture was stirred under room temperature over night. After that the aqueous acidic solution was washed with methylene chloride. The aqueous layer was separated and alkalized by the addition of potassium hydroxide pellets. The aqueous basic suspension was extracted with methylene chloride and the organic layer was dried with sodium sulfate. The organic layer was evaporated under reduced pressure to give 4-methoxymethylene ethylamphetamine as light yellow oil. The product has a molecular formula of $C_{13}H_{21}NO$ and a molecular mass of 207g/mol.

4.5.4 Synthesis of 4-methoxymethylene dimethylamphetamine

4-Methoxymethylene phenylacetone (0.5 g, 0.0028 mol) was dissolved in 25 mL methanol, and then 2.29 g of dimethylamine hydrochloride (0.028 mol) and 1.765 g of sodium cyanoborohydride (0.028 mol) were added to the stirring solution. The reaction mixture was stirred at room temperature for three days and the mixture pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was then evaporated under reduced pressure to yield a white solid residue. The residue was suspended in 40 mL cold water, and slowly acidified by the addition of concentrated hydrochloric acid. The resulting solution mixture was stirred under room temperature over night. After that the aqueous acidic solution was washed with methylene chloride. The aqueous layer was separated and alkalized by the addition of potassium hydroxide pellets. The aqueous basic suspension was extracted with methylene chloride and the organic layer was dried with sodium sulfate. The organic layer was evaporated under reduced pressure to give 4-methoxymethylene dimethylamphetamine as light yellow oil. The product has a molecular formula of $C_{13}H_{21}NO$ and a molecular mass of 207 g/mol.

4.6 Synthesis of 4-methoxymethylene phenyl-2-butanone

4.6.1 Synthesis of 4-methoxymethylene phenyl 2-nitrobutene

4-Methoxymethylene benzaldehyde (5.0 g, 0.033 mol) was dissolved in 80 mL benzene in a 250 mL round bottom flask, followed by the addition of 8 mL of n-butylamine (0.11 mol). The reaction mixture was refluxed overnight using a Dean Stark trap to remove water. Cooling the reaction mixture to room temperature and the solvent was evaporated under reduced pressure to give the crude imine intermediate yellow oil. The imine intermediate was dissolved in 20ml of glacial acetic acid in a 250 mL round bottom flask, and 10 mL nitropropane (0.11 mol) was added drop wise. The resulting reaction mixture was refluxed for one hour. During reflux, yellow green oil of 4-methoxymethylene phenyl 2-nitrobutene were formed in the reaction mixture. After the reaction, another 25ml of glacial acetic acid was added to the reaction mixture which was then cooled down to room temperature on an ice bath. The reaction mixture was poured over crushed ice and was acidify the mixture to pH 1 using concentrated hydrochloric acid. The resulting solution was extracted with methylene chloride and the organic layer was dried with sodium sulfate. The methylene chloride was removed under vacuum evaporator to give green oil of 4-methoxymethylene phenyl 2-nitrobutene product.

4.6.2 Synthesis of 4-methoxymethylene phenyl-2-butanone

4-Methoxymethylene phenyl 2-nitrobutene (3.0 g, 0.014 mol) was dissolved in 15 mL toluene and 15 mL water in a 250 mL round bottle flask. The resulting solution was mixed with 4.5 g powdered iron (0.08 mol); 0.9g ferric chloride (0.006 mol) and 6 mL concentrated hydrochloric acid. The mixture was stirred vigorously and refluxed over a day. After cooled to room temperature, 30 mL toluene and 30 mL water was added and the mixture was filtered under

reduced pressure. The precipitate was washed with additional toluene and water. The toluene layer was separated, and washed with 6 M hydrochloric acid, water and saturated sodium bicarbonate solution. The organic layer was dried over sodium sulfate, filtered and the solvent was evaporated to give dark brown crude product 4-methoxymethylene phenyl-2-butanone. Kugelrohr distillation of the crude product gave purified yellow oil 4-methoxymethylene phenyl-2-butanone, molecular formula: $C_{12}H_{16}O_2$, molecular mass 192 g/mol.

4.7 Synthesis of the 4-methoxymethylene butanamine series compounds

4.7.1 Synthesis of 4-methoxymethylene butanamine

4-Methoxymethylene phenyl-2-butanone (0.5 g, 0.0028 mol) was dissolved in 25 mL methanol, and then 2.007 g of ammonium acetate (0.026 mol) and 1.765g of sodium cyanoborohydride (0.028 mol) were added to the stirring solution. The reaction mixture was stirred at room temperature for three days and the mixture pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was then evaporated under reduced pressure to yield a white solid residue. The residue was suspended in 40 mL cold water, and slowly acidified by the addition of concentrated hydrochloric acid. The resulting solution mixture was stirred under room temperature over night. After that the aqueous acidic solution was washed with methylene chloride. The aqueous layer was separated and alkalized by the addition of potassium hydroxide pellets. The aqueous basic suspension was extracted with methylene chloride and the organic layer was dried with sodium sulfate. The organic layer was evaporated under reduced pressure to give 4-methoxymethylene butanamine as light yellow oil. The product has a molecular formula of $C_{12}H_{19}NO$ and a molecular mass of 193 g/mol.

4.7.2 Synthesis of 4-methoxymethylene N-methyl butanamine

4-Methoxymethylene phenyl-2-butanone (0.5 g, 0.0028 mol) was dissolved in 25 mL methanol, and then 1.738g of methylamine hydrochloride (0.026 mol) and 1.765g of sodium cyanoborohydride (0.028 mol) were added to the stirring solution. The reaction mixture was stirred at room temperature for three days and the mixture pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was then evaporated under reduced pressure to yield a white solid residue. The residue was suspended in 40 mL cold water, and slowly acidified by the addition of concentrated hydrochloric acid. The resulting solution mixture was stirred under room temperature over night. After that the aqueous acidic solution was washed with methylene chloride. The aqueous layer was separated and alkalized by the addition of potassium hydroxide pellets. The aqueous basic suspension was extracted with methylene chloride and the organic layer was dried with sodium sulfate. The organic layer was evaporated under reduced pressure to give 4-methoxymethylene N-methyl butanamine as light yellow oil. The product has a molecular formula of $C_{13}H_{21}NO$ and a molecular mass of 207 g/mol.

4.7.3 Synthesis of 4-methoxymethylene N-ethyl butanamine

4-Methoxymethylene phenyl-2-butanone (0.5 g, 0.0028 mol) was dissolved in 25 mL methanol, and then 2.124 g of ethylamine hydrochloride (0.026 mol) and 1.765g of sodium cyanoborohydride (0.028 mol) were added to the stirring solution. The reaction mixture was stirred at room temperature for three days and the mixture pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was then evaporated under reduced pressure to yield a white solid residue. The residue was suspended in 40 mL cold water, and slowly acidified by the addition of concentrated hydrochloric acid. The resulting solution

mixture was stirred under room temperature over night. After that the aqueous acidic solution was washed with methylene chloride. The aqueous layer was separated and alkalized by the addition of potassium hydroxide pellets. The aqueous basic suspension was extracted with methylene chloride and the organic layer was dried with sodium sulfate. The organic layer was evaporated under reduced pressure to give 4-methoxymethylene N-ethyl butanamine as light yellow oil. The product has a molecular formula of $C_{14}H_{23}NO$ and a molecular mass of 221 g/mol.

4.7.4 Synthesis of 4-methoxymethylene N, N-dimethyl butanamine

4-Methoxymethylene phenyl-2-butanone (0.5 g, 0.0028 mol) was dissolved in 25 mL methanol, and then 2.124 g dimethylamine hydrochloride (0.026 mol) and 1.765 g of sodium cyanoborohydride (0.028 mol) were added to the stirring solution. The reaction mixture was stirred at room temperature for three days and the mixture pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was then evaporated under reduced pressure to yield a white solid residue. The residue was suspended in 40 mL cold water, and slowly acidified by the addition of concentrated hydrochloric acid. The resulting solution mixture was stirred under room temperature over night. After that the aqueous acidic solution was washed with methylene chloride. The aqueous layer was separated and alkalized by the addition of potassium hydroxide pellets. The aqueous basic suspension was extracted with methylene chloride and the organic layer was dried with sodium sulfate. The organic layer was evaporated under reduced pressure to give 4-methoxymethylene N, N-dimethyl butanamine as light yellow oil. The product has a molecular formula of $C_{14}H_{23}NO$ and a molecular mass of 221 g/mol.

4.8 Synthesis of deuterium labeled 4-methoxymethylene benzyl aldehyde

4.8.1 Synthesis of deuterium labeled methyl 4-methoxymethyl benzoate

Sodium metal weighing 1.83 g (80 mmol) was cut into small pieces and placed in a 3 neck flask, and then deuterated methanol was added under nitrogen. 5 g (26.4 mmol) of 4-chloromethylbenzyl chloride was dissolved in deuterated methanol was added drop wise into that 3 neck flask. The resulting mixture was refluxed for 3 hours then the mixture was cooled to room temperature. The mixture was further cooled in an ice bath and 6 mL of aqueous hydrochloride (80 mmol) was added drop wise. The deuterated methanol was evaporated using a rotary evaporator and the resulting aqueous residue was extracted with methylene chloride. The organic layer was dried with sodium sulfate and then evaporated under reduced pressure to remove methylene chloride. The product methyl 4-methoxymethyl benzoate with two methyl group labeled with deuterium was obtained as light yellow oil.

4.8.2 Synthesis of deuterium labeled 4-methoxymethyl benzyl alcohol

A solution of 5 g D-labeled methyl 4-(methoxymethyl) benzoate (0.028 mol) in 80ml benzene was added to a 500 ml three neck flask and stirred at room temperature under nitrogen. A syringe was used to inject 10 mL of sodium bis (2-methoxyethoxy) aluminum hydride (Red-Al) solution in toluene (65% wt) slowly into the reaction solution through a rubber septum fitted to one neck of the flask. The resulting solution was stirred overnight at room temperature.

The excess Red-Al was relinquished by the consecutive addition of 14 mL 2 M sodium hydroxide solution, 28 mL water and 42 mL 2 M sodium hydroxide solution. The resulting precipitated aluminum salts were removed by filtration and the filtrate was evaporated under vacuum remove the benzene. The remaining solution was extracted with methylene chloride and

the organic layer was dried with sodium sulfate and then evaporated under reduced pressure to remove methylene chloride. The target product D-labeled 4-methoxymethyl benzylalcohol was obtained as light yellow oil.

4.8.3 Synthesis of deuterium labeled 4-methoxymethylene benzyl aldehyde

Chromium trioxide (50 g, 0.5 mol) was added rapidly with stirring to 92 mL of 6 M hydrochloric acid solution. After 5 minute, the homogenous solution was cooled to 0 °C and 35g of pyridine (0.5 mol) was carefully added over 10 minutes. Cooling the mixture to 0 °C over an ice bath again resulted in the formation of a yellow orange solid precipitate. The solid was collected by filtration and dried over vacuum for one hour to give dry pyridinium chlorochromate (PCC).

4.8.4 Synthesis of deuterium labeled 4-methoxymethylene benzaldehyde

A solution of 5 g of D-labeled 4-methoxymethyl benzyl alcohol (0.033 mol) was dissolved in 100 mL of methylene chloride in a 250 mL round bottle flask. To the stirring solution was added 10.80 g of freshly made pyridinium chlorochromate (0.05 mol) and 10.80 g celite. The resulting mixture was stirred at room temperature for 3 hours.

The reaction mixture was filtered through a bed of silica gel in a Buchner funnel and then the filtrate was evaporated under reduced pressure to get a yellow liquid, labeled 4-methoxymethylene benzaldehyde as a crude product. Kugelrohr distillation of the crude product gave purified yellow oil, labeled 4-methoxymethylene benzaldehyde, molecular formula: $C_9H_7D_3O_2$, molecular mass 153 g/mol.

4.8.5 Synthesis of deuterium labeled 4-methoxymethylene phenylacetone

4.8.5.1 Synthesis of deuterium labeled 4-methoxymethylene phenyl 2-nitropropene

D-labeled 4-methoxymethylene benzaldehyde (5.0 g, 0.033 mol) was dissolved in 80 mL benzene in a 250 mL round bottom flask, followed by the addition of 8 mL of n-butylamine (0.11 mol). The reaction mixture was refluxed overnight using a Dean Stark trap to remove water. Cooling the reaction mixture to room temperature and the solvent was evaporated under reduced pressure to give the crude imine intermediate yellow oil. The imine intermediate was dissolved in 20 mL of glacial acetic acid in a 250 mL round bottom flask, and 8 mL of nitroethane (0.11 mol) was added drop wise. The resulting reaction mixture was refluxed for one hour. During reflux, yellow green crystals of D-labeled 4-methoxymethylene phenyl 2-nitropropene were formed in the reaction mixture. After the reaction, another 25 mL of glacial acetic acid was added to the reaction mixture which was then cooled down to room temperature on an ice bath. The reaction mixture was poured over crushed ice and was acidify the mixture to pH 1 using concentrated hydrochloric acid. Yellow green crystals of D-labeled 4-methoxymethylene phenyl 2-nitropropene were isolated by vacuum filtration, washed with water, air dried for further use.

4.8.5.2 Synthesis of deuterium labeled 4-methoxymethylene phenylacetone

D-labeled 4-methoxymethylene phenyl 2-nitropropene (3.0 g, 0.014 mol) was dissolved in 15 mL toluene and 15 mL water in a 250 mL round bottle flask. The resulting solution was mixed with 4.5g powdered iron (0.08 mol); 0.9 g ferric chloride (0.006 mol) and 6 mL concentrated hydrochloric acid. The mixture was stirred vigorously and refluxed over a day. After cooled to room temperature, 30 mL toluene and 30 mL water was added and the mixture was filtered under reduced pressure. The precipitate was washed with additional toluene and water. The toluene

layer was separated, and washed with 6 M hydrochloric acid, water and saturated sodium bicarbonate solution. The organic layer was dried over sodium sulfate, filtered and the solvent was evaporated to give dark brown crude product D-labeled 4-methoxymethylene phenylacetone. Kugelrohr distillation of the crude product gave purified yellow oil D-labeled 4-methoxymethylene phenylacetone, molecular formula: $C_{11}H_{11}D_3O_2$, molecular mass 181 g/mol.

4.8.6 Synthesis of HFBA derivatized 4-methoxymethylene phenethylamines compound 1-3 and 5-7

The product amine 0.3 mg and 50 μ L ethyl acetate was added to the glass test tube, then 250 μ L derivatization agent heptafluorobutyramide (HFBA) was added in the reaction solution. The reaction solution was incubated in capped tubes at 70 $^{\circ}$ C for 20 min, and then the solvent was evaporated under stream of air at 55 $^{\circ}$ C. After that, 200 μ L ethyl acetate and 50 μ L pyridine was added to the glass tube for reconstitute. Dilute 50 μ L of the above solution with 200 μ L HPLC grade acetonitrile for GC analysis.

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