# UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

**Escuela de Ciencias Químicas e Ingeniería**

## TÍTULO: Synthesis of new fatty acid synthase inhibitors with cytotoxic properties

Trabajo de integración curricular presentado como requisito para la obtención del título de Químico

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Urcuquí, febrero 2020

UNIVERSIDAD YACHAY TECH

Urcuquí, 27 de febrero de 2020

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**ESCUELA DE CIENCIAS QUÍMICAS E INGENIERÍA**
**CARRERA DE QUÍMICA**
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**Acknowledgments**

I want to express my deeply gratitude to every single person that contribute to this achievement. First of all, special thanks to my dad Mariano and my mom Janeth, for their guidance, love and constant motivational and economic support throughout the entire career. Thanks to my brothers and sister, for their wise advices and constant support during my university experience.

Thanks to Prof. Dr. Jordi García and Dr. Xavier Ariza of the Barcelona University for the internship opportunity. I worked in their lab, in a project focus on the synthesis of FAS inhibitors for cancer therapy to obtain the results of this degree work. It was an amazing experience in the scientific field, and very enriching for my chemistry career. It helped me a lot to improve my laboratory skills and also to increase my knowledge in organic synthesis, and methods of purification and characterization in this field.

Special thanks to my mentor PhD. Kamil Makowski for his constant guidance and motivational support throughout the development of this degree work. I consider you a great friend and colleague.

Thanks to all my chemistry professors, specially Kamil, Marta, Juan Pablo, Hortensia, Solmar, Gottfried and Ruth, for inspiring me to follow their steps, and teach me how beautiful and functional the chemistry can be.

Thanks to all my friends that I made in these five years of career, for their incredible help, motivation, support and for making this experience so unique and full of colors. I will always carry you in my heart: Pepa, Adrian, Diego, Josecín, Rafaela.

Nicolás Alejandro Montesdeoca Solórzano

**Resumen**

El cáncer es una de las enfermedades con mayor tasa de mortalidad, normalmente su tratamiento es extremadamente doloroso y lleno de efectos secundarios no deseados. Una encima que comúnmente es sobre-expresada en muchas células cancerígenas, es la ácido grasa sintasa (FAS por sus siglas en inglés). FAS es considerada como un blanco para el tratamiento del cáncer; muchos inhibidores de FAS han mostrado un potente efecto citotóxico con daños mínimos en células normales. El C75 es un muy conocido inhibidor de FAS que tiene fuertes efectos citotóxicos en ratones, pero también tiene efectos anoréxicos secundarios. Estudios posteriores han mostrado que el enantiómero (-)-C75 tiene fuertes efectos citotóxicos sin afectar la ingesta alimenticia y el peso corporal. Por lo tanto, se desarrolló un inhibidor basado (-)-C75 con mejores efectos citotóxicos en varias líneas celulares, el compuesto (-)-UB006. Este inhibidor ha mostrado ser muy potente en rangos micro-molares, pero es necesario desarrollar nuevos análogos con efectos citotóxicos mas fuertes en rangos nano-molares. En el presente trabajo, se sintetizaron precursores del (±)-UB006 y un análogo nitrogenado en altas cantidades mediante síntesis orgánica convencional. Se llevo a cabo una nueva metodología para la obtención del análogo nitrogenado del (±)-UB006, la cual resultó ser muy problemática y por consiguiente fallida. Además, el escalamiento de la síntesis global del análogo nitrogenado del (±)-UB006 mostró que tanto el rendimiento de la reacción como la pureza de compuesto final fueron afectados negativamente por el incremento en la cantidad de crudo a purificar. Finalmente, los compuestos obtenidos en este trabajo serán utilizados en pasos sintéticos posteriores para la obtención de nuevos inhibidores de FAS.

**Palabras Clave:**

cáncer, lípidos, ácidos grasos, encima FAS, inhibidor de FAS, propiedades citotóxicas, C75, síntesis orgánica, escalado

**Abstract**

Cancer is one of the diseases with the highest rates of mortality, its treatment is normally extremely painful and full of unwanted side-effects. One enzyme that is commonly overexpressed in many cancer cells, is the fatty acid synthase (FAS). FAS is considered a target for cancer treatment; many FAS inhibitors have shown a potent cytotoxic effect with minimal damage to normal cells. C75 is a well-known FAS inhibitor that has strong cytotoxic effects in mice but also has anorexic side-effects. Further studies determined that the (-)-C75 enantiomer has strong cytotoxic effects without affecting the food intake and body weight. Therefore, it was developed a (-)-C75-based inhibitor with better cytotoxic effects in several cell lines, the compound (-)-UB006. This inhibitor showed to be very potent in micromolar ranges, but it is necessary to develop new analogs with stronger cytotoxic effects in nanomolar ranges. In the present work, precursors of the (±)-UB006 and a nitrogenated analog were synthesized in high amounts by conventional organic synthesis. A new methodology to obtain the nitrogenated analog of (±)-UB006 was performed, which turned out to be very problematic and therefore unsuccessful. Moreover, the scaling-up of the overall synthesis of nitrogenated analog of (±)-UB006 showed that both reaction yield and purity were negatively affected by the increase in the amount of crude to be purified. Finally, the compounds obtained in this work will be used in further synthetic steps for the obtention of new FAS inhibitors.

**Key Words:**

cancer, lipids, fatty acids, FAS enzyme, FAS inhibitor, cytotoxic properties, C75, organic synthesis, scale-up

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# CHAPTER 1. INTRODUCTION - JUSTIFICATION

## 1.1 General Introduction

Many types of cancer are detected each year around the globe and according to World Health Organization is the second cause of death, bringing the disturbing number of 9.6 million deaths for 2018 [1–5]. Cancer is a disorder of cell growth and proliferation that requires high amounts of energy and cellular building blocks including nucleic acids, proteins, and lipids [6–9]. Lipids comprise a wide group of biomolecules make-up of FAs of different chain length, number and location of double bonds, and backbone structure [8]. Lipid metabolism is of special interest in cancer therapy because lipids are involved in multiple biochemical processes during cancer initiation and development [9]. Lipids participate in the growth, energy and redox homeostasis of cancer cells. Moreover, they have structural roles as passive components of cell membranes, as cholesterol and sphingolipids that are important components of membrane rafts [9,10]. Furthermore, they initiate some signal transduction cascade processes and also can be broken down into bioactive lipid mediators that regulate cancer cell growth, migration, and metastasis formation [9–13].

### *1.1.1 Importance of Lipids in Cancer Cells*

The main building blocks of cell membranes are phospholipids (PLs), sterols, sphingolipids and also lyso-PLs; all of these are derived from acetyl-CoA and many contain FAs [8]. The FAs structure consists of a terminal carboxyl group and a hydrocarbon chain (usually with an even number of carbons) that can be saturated or unsaturated [8]. FAs can be used to generate many different types of lipids including diacylglycerides (DAGs) and triacylglycerides (TGs); this last one is mainly used for energy storage in the form of lipid droplets (LDs) [8–10,13,17–19]. Moreover, DAGs and TGs are synthesized via glycerol phosphate pathway, which uses the glycolytic intermediate glycerol-3-phosphate to form the glycerol backbone of these lipids; the intermediates in this process can be converted into different phosphoglycerides that are the major structural components of biological membranes [8,13].

The cancer cells can obtain FAs either from exogenous sources and from *de novo* lipogenesis (DNL) [8,9,20,21]. In the presence of oxygen and abundant extracellular nutrients, most cancer cells synthesize FAs *de novo*; but under conditions of metabolic stress, they collect extracellular lipids as an adaptation to survive [7,8,22–24]. This adaptation implies a reduction of the carbon supply and power for the FAs synthetic pathway [7]. Moreover, FAs can be used as an energy source when mitochondrial oxidation (β-oxidation) occurs; they produce more than the double of ATP per mol when compared to the glucose or aminoacids oxidation [7–10,13]. Consequently,

some cancer cells prefer to use FAs as an energy source even under nutrient-replete conditions [7,25].

Another important biosynthetic process within lipid metabolism is the mevalonate pathway, which facilitates the synthesis of cholesterol [9,10,13]. Cholesterol is one of the main components of biological membranes, as it modulates the fluidity of the lipid bilayer, and also forms detergent-resistant microdomains called lipid rafts, that coordinate the activation of some signal transduction pathways [9,10,13,26]. In cancer cells, many signaling proteins as protein kinase B (AKT), and receptors regulating prooncogenic and apoptotic pathways reside in lipids rafts [9,27]. Moreover, the activation of oncogenic signaling pathways only depends on the lipid rafts integrity, therefore, by disrupting them, the activation of the anchored-lipid raft AKT protein is inhibited and the tumor cell proliferation is reduced [9,28–33].

Some of the already mentioned characteristics of cancer cells, improve their proliferation and resistance to chemotherapy. TGs and cholesteryl esters are stored in LDs, which are highly ordered intracellular structures formed in the endoplasmic reticulum [9,10,17]. LDs are typically found in some aggressive cancers as well as high levels of saturated FAs in some aggressive breast cancers [9,13,18,19,34–36]. Cancer cells have higher amounts of LDs compared with normal tissue, which enhances their resistance to chemotherapy [9,10,34,37–39]. Moreover, the high levels of saturated FAs increase the levels of saturated phospholipids in cancer cells, reducing the membrane fluidity and protecting cancer cells from oxidative damage [11,13,36].

Lipids are also important signaling molecules; for example, phosphoinositides, are a family of second messengers that transmit signals from activated growth factor receptors to the cellular machinery [13,40,41]. Besides, the phosphoinositides act as specific binding sites for the coupling of effector proteins into specific membrane sites [13]. Other lipids that act as second messengers are lysophosphatidic acid, phosphatidic acid and DAG [13]. Moreover, sphingolipids are other important signaling molecules; the simplest of them is ceramide [13,40]. In cancer cells, ceramide mediates growth inhibitory signals and is involved in the initiation of the apoptotic process and growth arrest [13]. Furthermore, the enzymes involved in the sphingolipid metabolism pathway are normally deregulated in cancer cells, producing low ceramide levels and the consequently increased resistance to chemotherapy [13,42].

Besides the already mentioned lipids employment in cancer cells, lipids have an important role in post-translational modification of proteins [13]. Palmitate and myristate are saturated acyl chains that are normally (covalently) coupled to proteins and improve the protein interaction with membrane rafts [13,43]. Lipid metabolism is also involved in the autophagic process, which is a mechanism of self-degradation required for the removal of defective proteins and

organelles [13]. Moreover, the autophagic process is favored under conditions of nutrient scarcity and enhances the survival of cancer cells by contributing to the maintenance of energy supply during tumorigenesis [13,44–46].

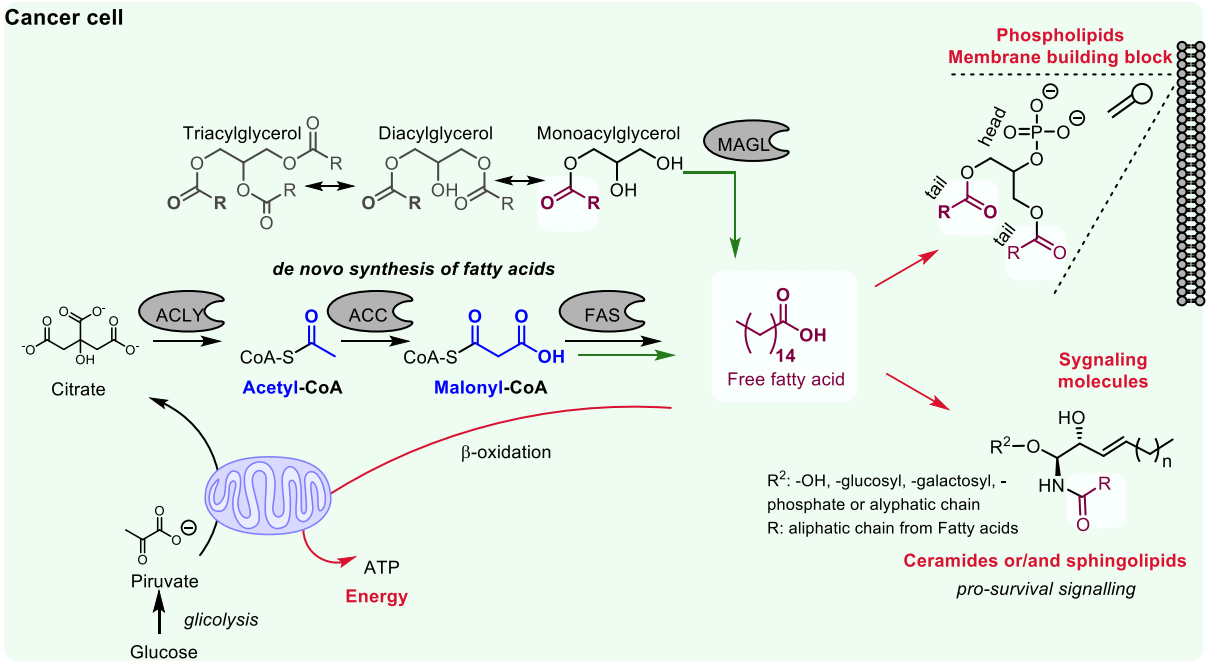


***Figure 1. Overview of lipogenesis in cancer and four target enzymes responsible for fatty acid synthesis.***

Apart from the importance of lipids in cancer cells proliferation and survival, they are also implicated in other more complex processes as cell migration, invasion, tumor angiogenesis and metastasis formation [13]. Finally, it is important to mention that the overexpression of lipogenic enzymes, such as ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and monoacylglycerol lipase (MAGL), represent a nearly-universal phenotypic alteration in most tumors and cancer cells (Figure 1) [7–10,13].

## 1.2 Fatty Acid Synthase

### *1.2.1 Function and Description*

The human fatty acid synthase (hFAS) is a complex homodimeric cytosolic enzyme of 552 kDa, that catalyzes the formation of palmitate ($C_{16}$) from acetyl-CoA and malonyl-CoA in the presence of NADPH (Figure 1) [47–49]. FAS has seven catalytic domains, which are (in linear order from the carboxy terminus): thioesterase (TE), acyl-carrier protein (ACP), β-ketoacyl reductase (KR), enoyl reductase (ER), β-hydroxyacyl dehydratase (DH), malonyl/acetyl transferase (MAT) and β-ketoacyl synthase (KS). Moreover, there are two additional nonenzymatic domains, the pseudoketoreductase (ΨKR) and the peripheral pseudomethyltransferase (ΨME) [50].

In the FA biosynthesis, the final step is catalyzed by the FAS enzyme (Figure 1). It starts with a load of acetyl (from acetyl-CoA) onto the terminal thiol of the phosphopantetheine cofactor of the ACP, this process is performed by the MAT [48]. The ACP passes the acetyl moiety over the active site cysteine of the KS. Subsequently, the MAT transfers the malonyl group of malonyl-CoA to the ACP, and the KS catalyzes the decarboxylative condensation of the acetyl and malonyl moieties to an ACP-bound β-ketoacyl intermediate [48]. Then, the β-carbon position is modified by the NADPH-dependent KR, DH, and NADPH-dependent ER domains to finally generate a saturated acyl group product with two extra carbon units. This molecule is the starting substrate for the next reactions of elongation until a fatty acid of 16 to 18 carbon atoms of length is obtained. Finally, the products are released from ACP as free FAs by the TE domain [48].

### *1.2.2 Expression and Regulation in Cancer Cells*

In cancer cells, the FAS overexpression is one of the most frequent phenotypic alterations, moreover, it is related to a higher risk of cancer recurrence and death [51]. FAS overexpression has been evidenced in many human cancer cell lines including breast, colorectum, prostate, bladder, ovary, esophagus, stomach, lung, oral tongue, oral cavity, head and neck, thyroid and endometrium, among others [24,51–56]. Normally, FAS is mainly regulated by nutritional signals and is expressed in hormone-sensitive cells and cells with high lipid metabolism [57,58]. In contrast, FAS regulation in cancer cells implicates the activity of several transcriptional and post-translational factors (growth factors, hormones, and their receptors), in parallel with microenvironmental effects [50]. Two well-studied pathways involved in the FAS regulation, are the MAPK and the PI3K/AKT pathways [59]. The HER2 and EGF growth factor receptors are involved in the downstream of PI3K/AKT and MAPK signaling pathways, which subsequently activates FAS expression transcriptionally [60]. Moreover, FAS expression can be amplified by the crosstalk between sex hormones, growth factors, and their receptors [61]. Both AKT and MAPK transduction pathways regulate FAS by the same mechanism; they regulate the expression of sterol regulatory element-binding protein (SREBP)-1c which interacts with regulatory elements in the FAS promoter [50]. Also, SREBP-1c is regulated directly by the proto-oncogene FBI-1 (Pokemon) through its DNA-binding domain, and thus synergistically activates the FAS transcription [62]. Another transcription factor that is also regulated as (SREBP)-1c and is highly implicated in the FAS expression, is the carbohydrate-activated transcription factor response element-binding protein (ChREBP) [63]. There are also other factors

as NAC1, the acetyltransferase P300, and some microRNAs that regulate FAS expression in tumor cells [64–67].

In breast cancer, FAS mediates the overexpression of S14, a lipogenesis-related nuclear protein that is regulated by SREBP-1c, supporting cell growth and survival [68]. Moreover, in SK-BR-3 and BT-474 breast cancer cell lines, FAS might be regulated by another mechanism, via mTOR-mediated translational induction [69]. By this mechanism, HER2 is overexpressed and with-it higher levels of FAS are observed [50]. In prostate cancer, the ubiquitin-specific protease 2a (USP2a) is overexpressed and plays a critical role in cell survival, it may interact with FAS to stabilize it through the removal of ubiquitin [70]. USP2a is regulated by androgen and its inactivation results in the FAS protein decrease and enhanced apoptosis [50].

Microenvironmental effects as hypoxia and acidity have important roles in the regulation of FAS [50]. It has been evidenced that under hypoxic conditions in human breast cancer cell lines, FAS in upregulated [71]. Moreover, Furuta *et al.* found that SREBP-1c is also upregulated as an effect of the phosphorylation of AKT with the subsequent activation of HIF1. Finally, excessive extracellular acid conditions could result in changes in the transcriptional activation of the FAS gene in breast cancer cells [72].

### *1.2.3 Inhibitors and Cytotoxic Effect.*

Since the discovery of FAS as an oncogenic target, many inhibitors have been developed and proved against several cancer cell lines. Moreover, it has been evidenced that the FAS inhibition, stops the proliferation and induce the apoptosis of cancer cells, with minimal effects on normal cells [64,73]. Most of the FAS inhibitors have been previously reviewed [50,74,75], but it is worth to mention the most important, promising and potent inhibitors that are found in the literature. First, the semisynthetic compound **C75** (Figure 2), a weak irreversible FAS inhibitor with an $IC_{50}$ between 200 - 500 μM [75–77]. C75 interacts with FAS in different domains, specifically with the KS, TE, and ER domains [78]; showing anticancer activity in several cancer cell lines and xenografts models [79–86]. However, *in vivo* studies showed that C75 has a negative side effect; it reduces food intake and induces body weight loss [87–89]. Further studies showed that the (-)-C75 enantiomer is capable of inhibiting FAS *in vitro*, producing a cytotoxic effect in several cancer cell lines without affecting food consumption [90]. In the other hand, it was evidenced that the (+)-C75 enantiomer inhibit CPT1 and produce anorexic effects. With these results, Makowski *et al.* developed a series of C75-based inhibitors taking into account the enantiomeric selectivity of FAS [77,91]. They found that the elongation of the aliphatic chain or the introduction of larger groups in the β-position of the lactone causes a decrease in the

inhibitory activity of FAS [91]. This reduced structure-activity relationship led to the development of a better FAS inhibitor, the **(-)-UB006** (Figure 2) with an $IC_{50}$ of 220 μM for hFAS [77]. *In vitro* studies showed that (-)-UB006 is more cytotoxic than C75 (racemic mixture) against several cancer cells; but specifically, against the OVCAR3 cell line, it showed to be 40 times more cytotoxic than C75. Furthermore, *in vivo* administration of (-)-UB006, evidenced that it does not affect the food intake and body weight.

**Orlistat** (Figure 2) is a potent FAS inhibitor that was initially designed for obesity treatment, moreover, it is an FDA-approved pancreatic lipase inhibitor [74]. Orlistat forms a covalent adduct with the serine of the TE domain and has an $IC_{50}$ of 0.9 μM [75,92,93]. It has shown tumor growth inhibition in xenograft models of prostate cancer and melanoma, and also reduced proliferation and enhanced apoptosis in breast cancer that overexpresses HER2 [94–97]. However, Orlistat has poor oral bioavailability and metabolic stability, and thus it is difficult to use for cancer treatment [74]. Moreover, the use of a drug-delivery system based on nanoparticles for Orlistat can improve its bioavailability, water-solubility and even its cytotoxic effect on aggressive breast cancer models [98–101].

In 2014, GlaxoSmithKline pharmaceutical company developed a highly potent, reversible and specific inhibitor of the KR domain of hFAS [102]. The compound **GSK2194069** (Figure 2), has an $IC_{50}$ of 7.7 nM for hFAS and showed acceptable solubility and permeability. The authors demonstrated that GSK2194069 decrease the DNL, producing a potent inhibition in the cancer cell growth and proliferation in gastric and non-small-cell lung cancer cell lines. Moreover, they identified that GSK2194069 interacts specifically with the KR domain and works as a competitive inhibitor. Further studies evidenced that treatment with GSK2194069 in prostate cancer C42b cell xenografts inhibited the tumor growth with no apparent side effects [103].

Sagimet Biosciences (previously 3-V Biosciences) developed a new generation of highly potent, reversible, imidazopyridine-based FAS inhibitors [104–107]. One of them is **TVB-3166** (Figure 2), that has an $IC_{50}$ of 0.042 μM for FAS (from rabbit). It is capable of stopping the FA synthesis and disrupt the lipid raft structure, affecting all the membrane-associated molecules and signaling pathways as Ras, AKT-mTOR, and Wnt-β-catenin [107]. *In vivo* studies showed that a single daily dose can inhibit FAS for 10-12 h each day, inducing xenograft tumor growth inhibition in lung, ovarian and pancreatic tumor models [107]. These results showed that an irreversible inhibitor is not necessary to stop the tumor growth *in vivo*. Moreover, TVB-3166 does not have any apparent negative side effects.

Another compound developed by Sagimet Biosciences is **TVB-2640** (Figure 2), which is the first and only FAS inhibitor that has reached the clinical trials until date [74]. TVB-2640 is described as a highly potent, selective and reversible FAS inhibitor that acts in the KR domain and has an $IC_{50}$ of 0.05 μM [108]. In 2017, the phase 1 clinical trial of TVB-2640 in patients with solid tumors was finished, demonstrating its antitumor activity in monotherapy and co-treatment with paclitaxel [109]. Some common negative side effects were observed including alopecia, palmar-plantar erythrodysesthesia, decreased appetite, among others. Nowadays, the phase 2 of clinical trials of TVB-2640 (monotherapy and/or co-treatment) is underway, including the treatment of lung, colon, breast, and astrocytoma cancer (NCT03808558, NCT02980029, NCT03179904, and NCT03032484). Moreover, partial results of the phase 2 trial of TVB-2640 in combination with Bevacizumab in patients with the first relapse of high-grade astrocytoma, showed that the co-treatment is well tolerated in humans [110].

In 2016, Alwarawrah *et al.* discover a potent thiophenopyrimidine-based FAS inhibitor with broad antitumor activity against various non-tumorigenic and aggressive tumor-forming breast cancer cell lines [111]. **Fasnall** (Figure 2) with an $IC_{50}$ of 3.71 μM for hFAS, can produce a significative change in the global cellular lipid profiles. Its mechanism of action includes the increase of intracellular levels of ceramide (also in DAGs and unsaturated FA) which increases the apoptosis of cancer cells. Moreover, Fasnall inhibits the formation of phospholipids with saturated acyl chains and promotes the uptake of unsaturated FAs, affecting critically the lipid raft structure and functioning [112,113]. Fasnall treatment has no apparent negative side effects and its combination with other chemotherapeutic agents as carboplatin augments the tumor volumes reduction and survival *in vivo* studies [111]. All these characteristics and the ease of adaptability of the Fasnall synthetic route, suggest that it can be further optimized to developed new derivatives with better pharmacological properties.

In 2018, Lu *et al.* developed a series of spirocyclic imidazolinone FAS inhibitors; one of them showed high FAS inhibitory activity with good cellular activity and oral bioavailability [114]. The compound **JNJ-54302833** (Figure 2) has an $IC_{50}$ of 28 nM for hFAS and effectively inhibits the proliferation of several cancer cell lines including ovarian, prostate, lymphoma, leukemia, lung and breast. The authors found that one compound of the series of spirocyclic imidazolinone FAS inhibitors (not exactly JNJ-54302833) binds to the KR domain by H-bonds and also hydrophobic interactions occurred with the KR and non-catalytic domains of FAS [114]. However, specific mechanistic information about JNJ-54302833 is not described.

In 2019, Infinity Pharmaceuticals published the discovery of a potent and irreversible inhibitor of hFAS, the **IPI-9119** (Figure 2) [115]. It has an $IC_{50}$ of 0.3 nM for hFAS and inhibits the TE domain by promoting acylation of the catalytic serine. The authors evidenced that IPI-9119 significantly reduced prostate cancer cell growth, and induced cell cycle arrest and apoptosis in PCa cells. Moreover, FAS inhibition generated an entire lipid homeostasis change, including the accumulation of polyunsaturated FAs produced by the uptake and use of exogenous FAs. Also, the cholesterol synthesis was increased as a type of redirection of the unused acetyl-CoA. Therefore, it is evidenced that PCa cells tried to compensate for the DNL deficiency by up-regulating genes encoding enzymes and transcription factors involved in lipid synthesis [115]. Further investigations are necessary to understand these anomalies caused by IPI-9119 treatment.

FORMA Therapeutics developed a series of novel piperazine derivative FAS inhibitors; one of them is the compound **FT113** (Figure 2) with an $IC_{50}$ of 0.213 μM for hFAS [116]. The authors reported that several H-bond interactions occurred between the hydroxyl and carbonyl of the hydroxy-cyclopropyl amide and the active site residues of the KR domain. These observations were determined by the X-ray co-crystal structure of FT113 bound to a ΨME-ΨKR-KR tridomain FAS construct [116]. FT113 was the compound with the best balance between physicochemical and pharmacokinetic properties and potency. Moreover, FT113 showed anti-proliferative activity against prostate (PC3), breast (BT474) and leukemia (MV-411) cancer cells. After 16 days of treatment with FT113, it was evidenced an increase in malonyl-CoA levels in the tumors as well as a tumor growth inhibition of 32% and 50%, by treatment with 25 and 50 mg/kg respectively, compared to the vehicle.

It has been evidenced that FAS is a viable target for the inhibition of FA biosynthesis, as many compounds showed high FAS inhibitory activity and cytotoxic effect against several cell lines, with no apparent side effects. Moreover, there is one compound (**TVB-2640**) that is currently been tested in humans, suggesting that FAS targeting has a great potential for anticancer therapy. It is important to mention that nowadays, we have new tools for the discovery of FAS inhibitors, like the computational screening [117]. With new tools of this kind, there is more ease of discovery, and optimization of new compounds with lower expenses and time in the process.

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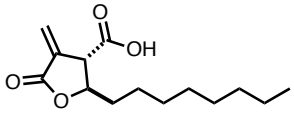
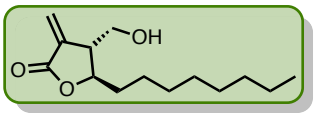
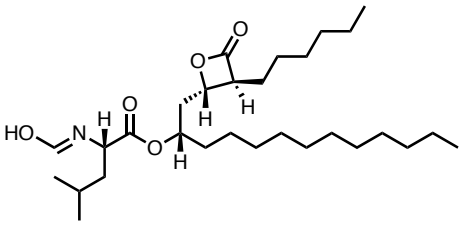
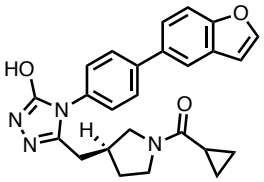
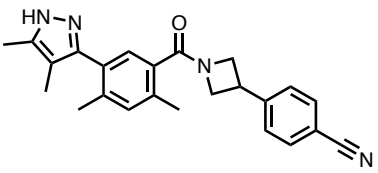
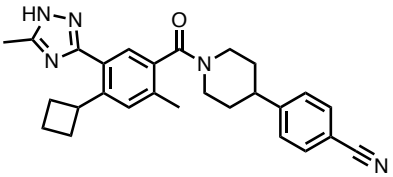
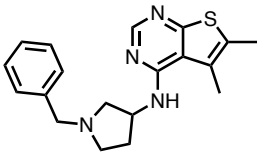
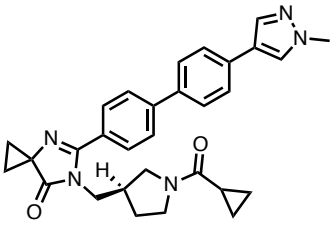
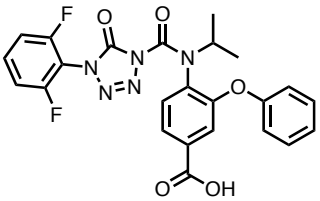
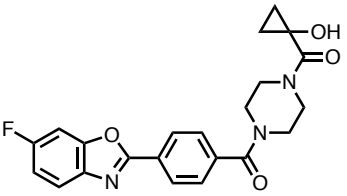
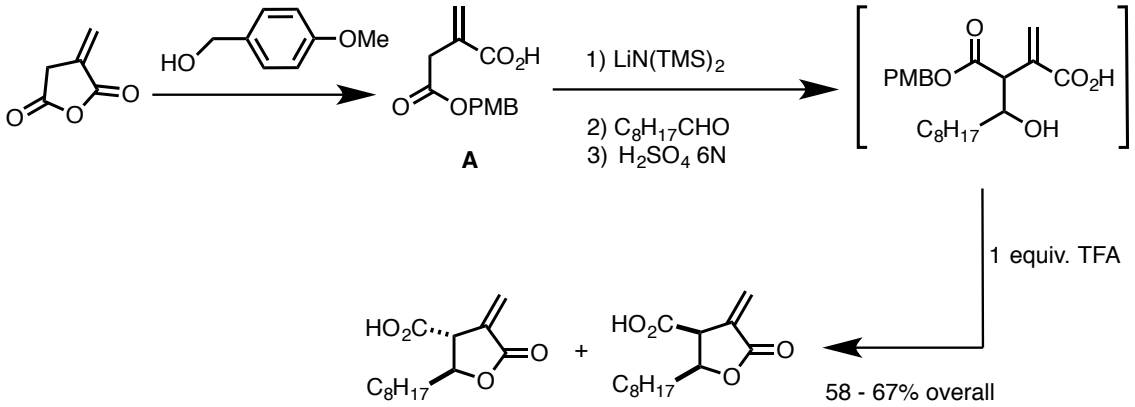
| |  |  |
| Compound | **C75** | **(-)-UB006** |
| FAS $IC_{50}$ | 200-500 μM FAS (variable) | 220 μM FAS (rat liver) |
| Cytotoxicity $IC_{50}$ | 1.6 μM (RWPE1) | 0.5 μM (OVCAR3) |
| |  |  |
| Compound | **Orlistat** | **GSK2194091** |
| FAS $IC_{50}$ | 0.9 μM FAS (not specified) | 7.7 nM hFAS |
| Cytotoxicity $IC_{50}$ | 7.5 μM (SKBR3) | - |
| |  |  |
| Compound | **TVB-3166** | **TVB-2640** |
| FAS $IC_{50}$ | 0.042 μM FAS (rabbit) | 0.05 μM FAS (not specified) |
| Cytotoxicity $IC_{50}$ | - | - |
| |  |  |
| Compound | **Fasnall** | **JNJ-54302833** |
| FAS $IC_{50}$ | 3.71 μM hFAS | 28 nM hFAS |
| Cytotoxicity $IC_{50}$ | - | 13 nM (A2780) |
| |  |  |
| Compound | **IPI-9119** | **FT113** |
| FAS $IC_{50}$ | 0.3 nM hFAS | 0.213 μM hFAS |
| Cytotoxicity $IC_{50}$ | - | 0.026 μM (MV411) |

***Figure 2. Structure and half-maximal inhibitory concentration ($IC_{50}$) of selected FAS inhibitors. In the green frame is located the FAS inhibitor that is of special interest in the present work. The organism from which FAS was extracted and the cell line of the cytotoxicity studies are indicated in parenthesis.***

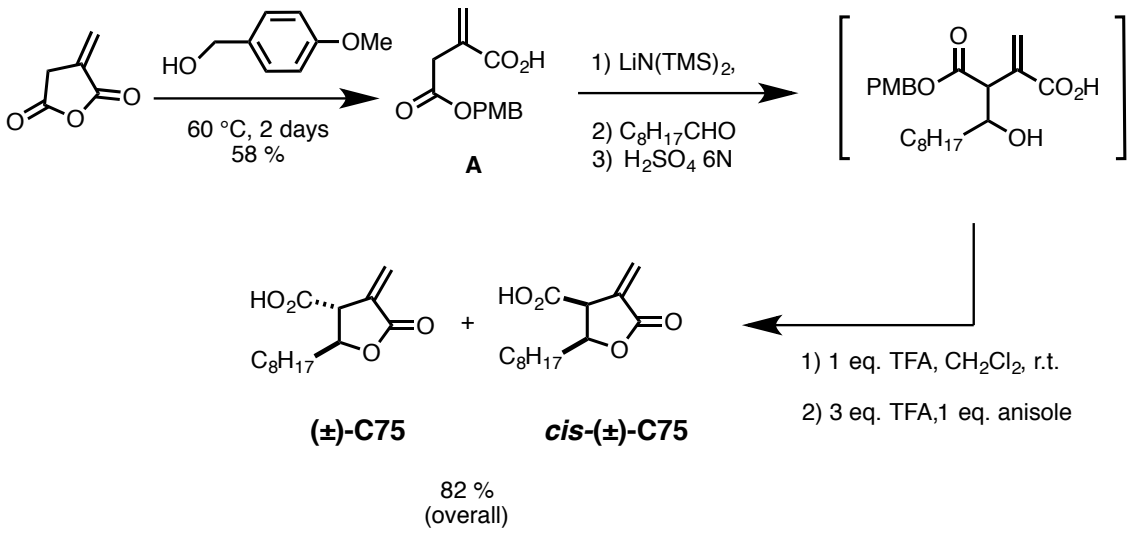
## 1.3 Synthetic Methodologies of C75

The synthesis of **C75** was published for the first time in 1997 by the research group of Dr. Kuhajda [118]. The authors use a procedure described before by Carlson and Oyler; it was based on the use of the anhydride of the itaconic acid [119]. The mention anhydride is reacted with p-methoxybenzyl alcohol and the formed adduct is condensate with *n*-nonanal, previous transformation into the corresponding dianion. A further acid treatment results in a mixture of lactones *cis* and *trans* in a 1:1 approximated proportion (Scheme 1). The authors indicate that the diastereomers, in a racemic mixture, can be separated by CC.



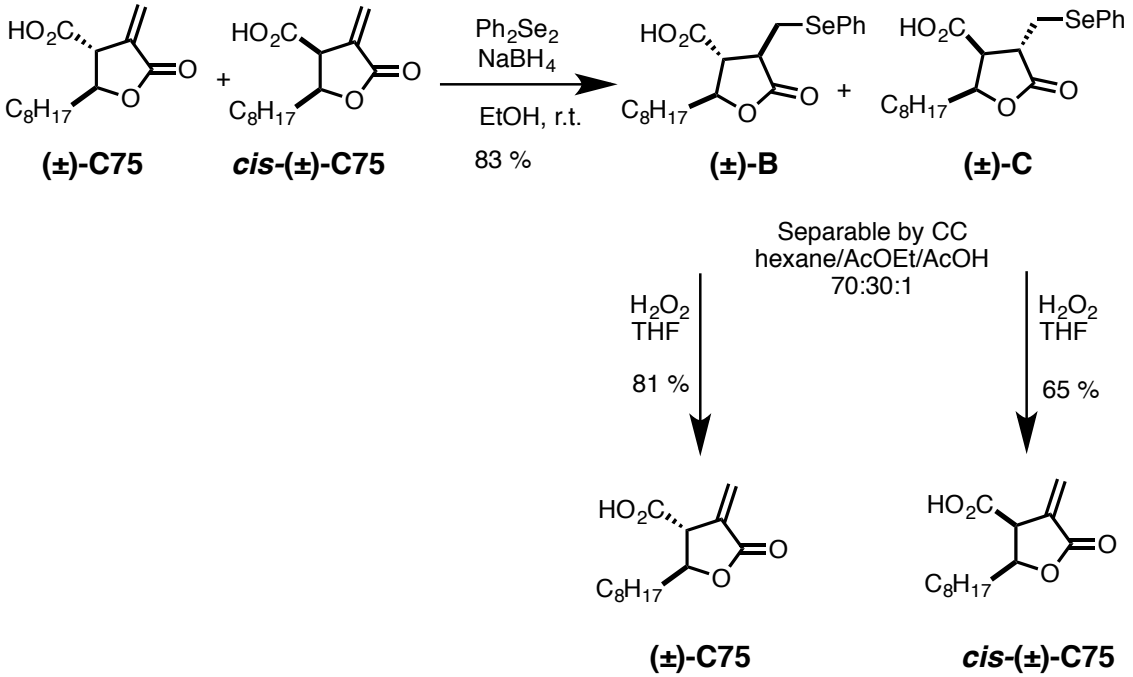

***Scheme 1. Synthesis of C75***

Further studies showed some improvements in the previously mentioned **C75** synthetic pathway increasing the yield to 82% [120]. The used methodology (Scheme 2) was initiated with the regioselective opening of the itaconic anhydride with p-methoxybenzyl alcohol at 60 °C for 2 days. Then, the obtained monoester **A** was enolized using lithium bis(trimethylsilyl)amide at -78 °C and the resulting enolate was condensed with *n*-nonanal. The extraction of the obtained aldol was performed with $CH_2Cl_2$. The cycle formation and deprotection were performed in a single step using the γ-hydroxy acid without purification. Then, the crude was treated with four equivalents of TFA and one equivalent of anisole as cations scavenger overnight. The mixture of lactones (*cis*/*trans*, ~1:1) was isolated by acid/base extraction, using $CH_2Cl_2$.




***Scheme 2. Improved synthesis of C75***

To separate the diastereomeric mixture of lactones it was developed protection for the exocyclic double bond as a selenoether. The resulting mixture of **(±)-B y (±)-C** was chromatographically separable with much less effort (Scheme 3). Finally, the double bond was regenerated by the β-elimination of the selenoether in oxidative conditions.




***Scheme 3. Protection and recovery of the exocyclic double bond of C75.***

### 1.4 Problem Statement

The development of new small molecules with anti-cancer properties is very important. On one hand cancer in many cases remain a deadly disease and on the other hand, the new therapeutics may give new insight for the understanding of cancer cell metabolism that could lead to the development of a new safe and efficient drug. Specifically, FAS inhibitors have shown to be

very promising and efficient in the disruption of cancer development. One FAS inhibitor has already entered the clinic and it is currently been tested against several cancers in humans. Therefore, it important to keep investigating new FAS inhibitors that could have stronger cytotoxic effects and lower side-effects. Since the discovery of C75, several derivatives have been developed but none of them has entered the clinic. One very promising FAS inhibitor is (-)-UB006, a C75 derivative with strong cytotoxic effects and no anorexic side-effects in micromolar ranges. However, (-)-UB006 lacks efficacy and is necessary to develop a new analog with strong cytotoxic effects in nanomolar ranges. In this way, further research is necessary for the development of new (-)-UB006 analogs with strong cytotoxic effects in nanomolar ranges.

## 1.5 General and Specific Objectives

### *1.5.1 General Objective*

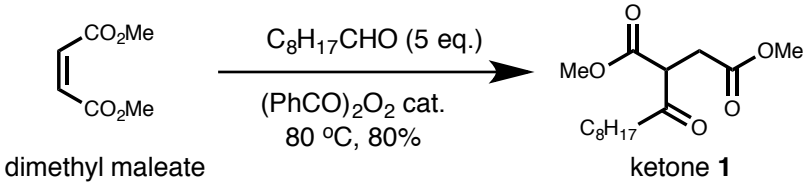
Synthesize in high amounts precursors of the FAS inhibitor (±)-UB006 and the nitrogenated analog using established methodologies and try to develop a new methodology for the obtention of the nitrogenated analog of (±)-UB006.

### *1.5.2 Specific Objectives*

- Synthesize precursors of (±)-UB006 and the nitrogenated analog, using dimethyl maleate as the starting material.
- Scale-up the synthesis of (±)-UB006 nitrogenated analog precursor.
- Try a new methodology of oxime formation for the synthesis of the nitrogenated analog of (±)-UB006.
- Characterize the obtained products by TLC, $^{1}$H-NMR, $^{13}$C-NMR, FTIR, and HRMS.

## CHAPTER 2. RESULTS AND DISCUSSION

In previous works performed by the investigation group of Prof. Dr. Jordi Garcia Gomez, new C75 synthetic routes were discovered by Ph.D. Kamil Makowski [120]. One of those is used in thesis work. The first step consists of the radical *n*-nonanal addition to the dimethyl maleate using benzoyl peroxide as a radical initiator to afford the ketone **1** with a good yield (Scheme 4). After several attempts, I was able to improve the yield of the reaction and ketone **1** was obtained with an 80% yield and high purity (in previous studies it was obtained with 48% [120] and 76 % [121] reaction yields).

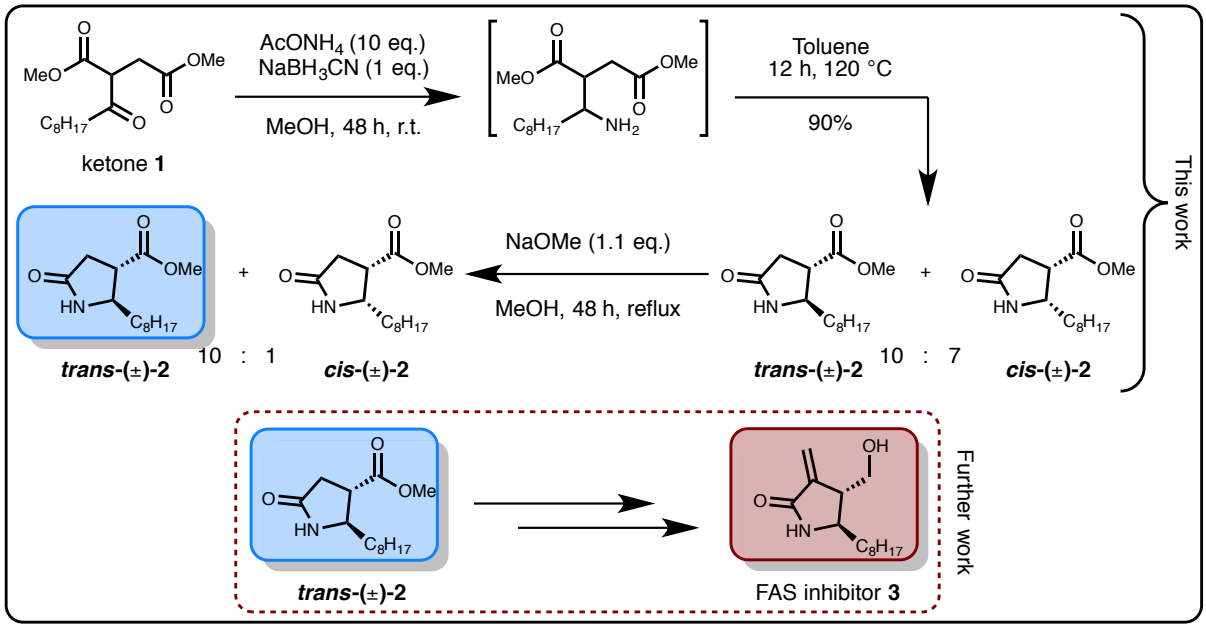



***Scheme 4. Synthesis of ketone 1***

The obtained ketone **1** was used as the starting material for the synthesis of precursors of (±)-UB006 and the nitrogenated analog, following previously performed methods [120,121]. However, it is important to mention that after some trials we decided to not purify ketone **1** (further used in section 2.1.3 ).

### 2.1 Synthesis of the Nitrogenated Analog Precursor of (±)-UB006

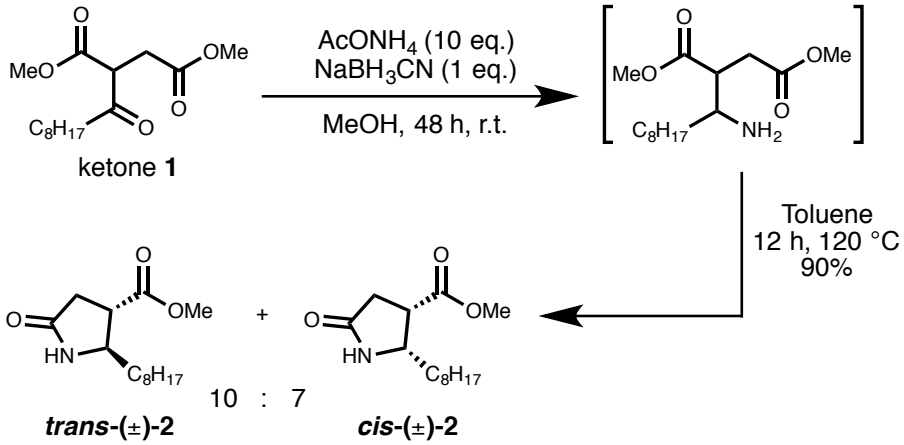
The present work consisted in the synthesis of some precursors of the nitrogenated analog of (±)-UB006 (Scheme 5), that will be used in further synthetic steps to finally obtain a desired final α-methylenated lactam, the compound **3**. In a previous study [121], it was obtained an *N*-substituted lactam that showed a interesting cytotoxic activity against the OVCAR3 cell line (ovarian cancer). It was tried to obtain compound **3** but the used synthetic pathways were unsuccessful. Therefore, the importance of the present work born from the necessity of trying new synthetic pathways to obtain the FAS inhibitor **3**.



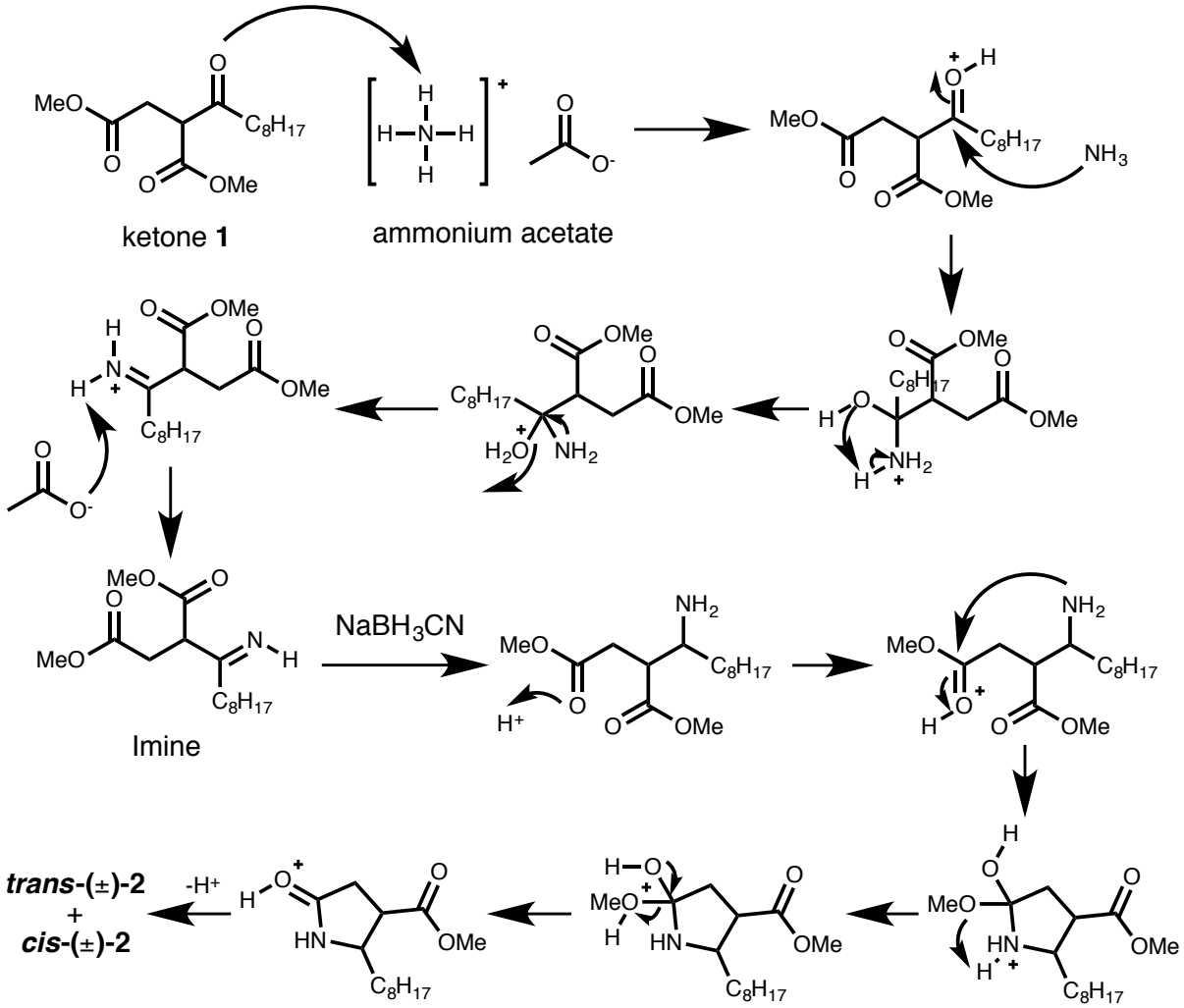

***Scheme 5. Overall synthetic pathway of the nitrogenated analog of (±)-UB006. The compound in the blue box is the product obtained in the present work. The red dashed frame delimits the synthetic steps that are not part of the present work. The compound in the red box is the final product that will be obtained in further studies (doctoral thesis work).***

### *2.1.1 Reductive Amination and Cycle Formation*

The reductive amination of the ketone **1** was carried out "one-pot". In the first step, the imine formation was achieved using $AcONH_4$ as an ammonia source (Scheme 6) and reduction using mild reducing agent $NaBH_3CN$ that allowed the C=N double bond reduction without reducing carbonyl from starting material. The formed amine was treated with toluene at high temperature for 12 hours, here the amine attacks the carbonyl group, forming a five-member ring amide instead of a four-member one due to stability. Finally, under these conditions, it is obtained a mixture of lactams in a 10:7 proportion in favor of *trans* diastereomer (observed by $^1$H-NMR) with a 90% yield.




***Scheme 6. Synthesis of lactam 2 diastereomers.***



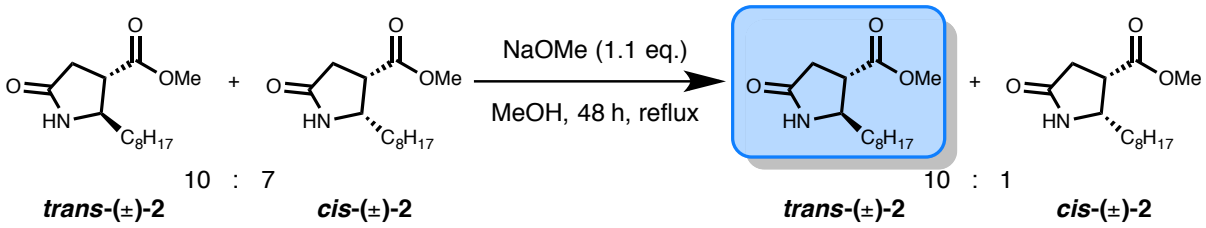

***Scheme 7. Mechanism of reductive amination and cycle formation of lactam 2 diastereomers.***

In Scheme 7 is observed the reaction mechanism of the lactam **2** diastereomers formation. First, the ketone **1** is transformed into its imine form by $AcONH_4$ with the corresponding loss of water. Then, the imine is reduced selectively by the $NaBH_3CN$ after the iminium ion formation (not shown). Finally, the formed amine attacks the carbonyl to form the most stable ring, and by intramolecular rearrangement the lactam diastereomers ***trans*-(±)-2** and ***cis*-(±)-2** are obtained.

### *2.1.2 Isomerization of Lactams Diastereomeric Mixture*

Only one of the previously formed diastereomers is desired for the synthesis of FAS inhibitor **3**, therefore, an isomerization was performed to enrich the diastereomeric mixture. It was favored the formation of the most thermodynamically stable diastereomer, the lactam ***trans*-(±)-2** (Scheme 8). The isomerization was carried out using 1.1 equivalent of MeONa as a base in MeOH heating under reflux for 48 hours. Finally, it was obtained a mixture of diastereomers

with a *trans*:*cis* relation of 10:1 (observed by $^{1}$H-NMR). The mixture was separable by CC and lactam ***trans*-(±)-2** was isolated with a 21% overall yield.




***trans*-(±)-2** ***cis*-(±)-2** ***trans*-(±)-2** ***cis*-(±)-2**

***Scheme 8. Isomerization of lactam 2 diastereomers.***

A previous study [121], determined that this method is the best for the isomerization of lactam **2** diastereomers, with a 92% of conversion in favor of the *trans* diastereomer. As can be observed in Figure 3, there is an evident change in the $^{1}$H-NMR spectrum of the lactam **2** diastereomers mixture after the isomerization step. Almost all the ***cis*-(±)-2** diastereomer was transformed into the thermodynamically stable ***trans*-(±)-2** diastereomer. Moreover, the specific proton signals that were conclusive in the determination of the diastereomers proportion are shown in Figure 3.

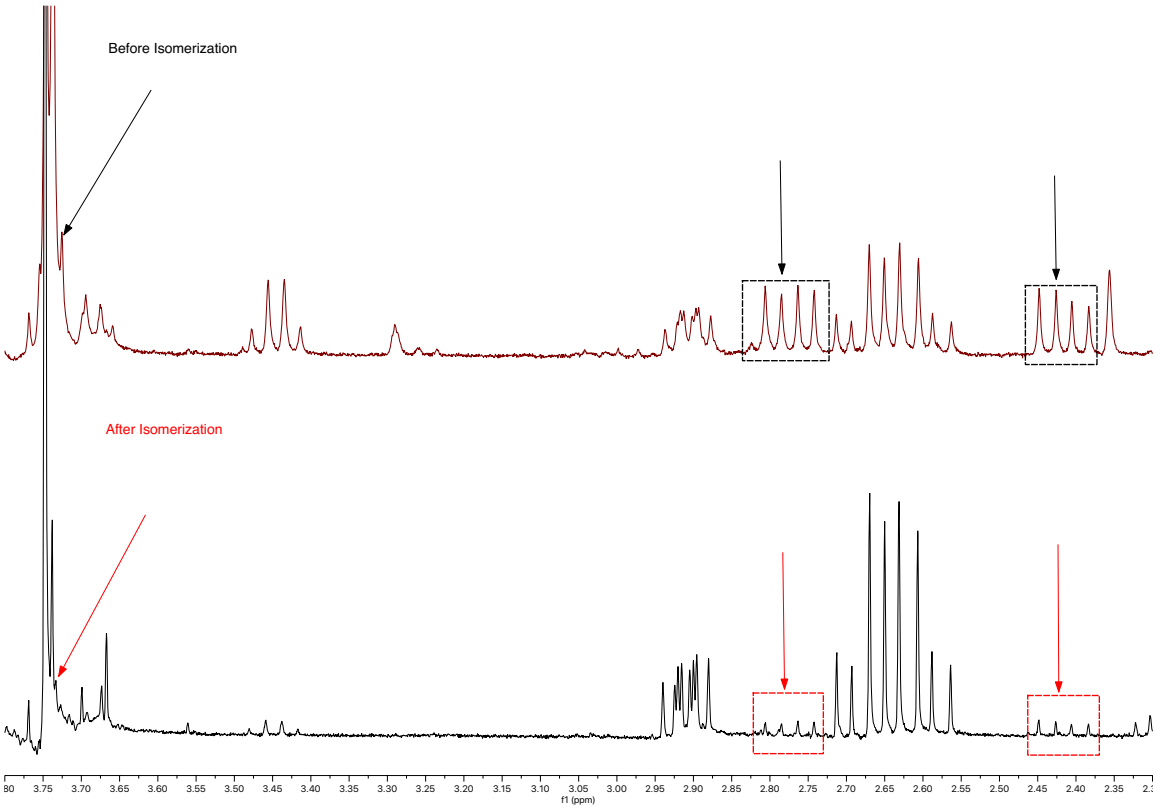



***Figure 3. Part of the $^{1}$H-NMR spectra of the lactam diastereomers before (top) and after (bottom) isomerization. The most evident changes in the spectra are indicated with dashed squares and pointed by arrows. They indicate the decrease of cis diastereomer.***

### *2.1.3 Scale-up of the Reaction*

The ***trans*-(±)-2** lactam was synthesized several times, changing the initial quantities of ketone **1** to determine the ease of scaling-up the reaction. During this process, it was observed that with increasing quantities of lactam **2** diastereomers, the difficulty of separation also increases. Therefore, the obtained quantities of ***trans*-(±)-2** lactam were not entirely pure as indicated in Table 1. The main observed impurities were nonanoic acid and ***cis*-(±)-2**, the first one was easily removed by washing with a $NaHCO_3$. Finally, it was evidenced that the scaling-up process reduces the overall yield of the reaction, however avoiding chromatographic separation is highly desirable when the process is carried out in the industry and would be an advantage if some day scale-up to kilograms or higher scale .

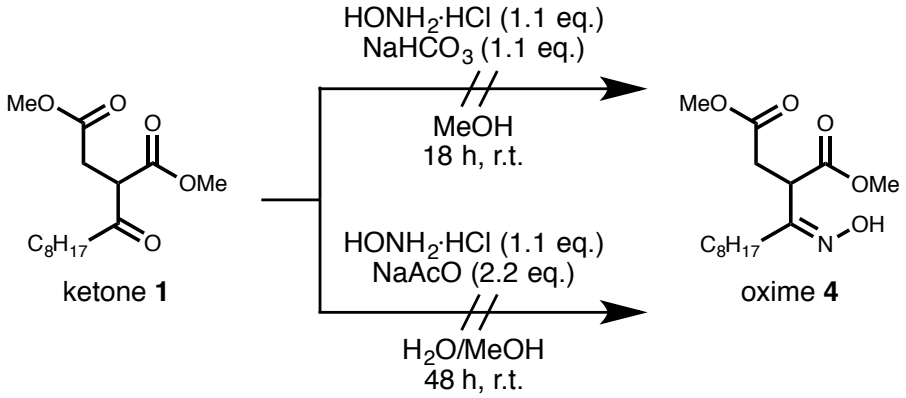
***Table 1. Yields of the scaling-up reactions***

| Ketone 1 [g] | Lactam *trans*-(±)-2 [g] | Yield [%] | Purity |
|---|---|---|---|
| 0.5 (purified by CC) * | 0.167 * | 33 | Very High * |
| 5.0 g (not purified by CC) | 0.933 | 19 | High |
| 10.0 g (not purified by CC) | 2.474 | 25 | Medium |

**Values taken from* [121]

### *2.1.4 Attempt of New Methodology: Oxime Formation*

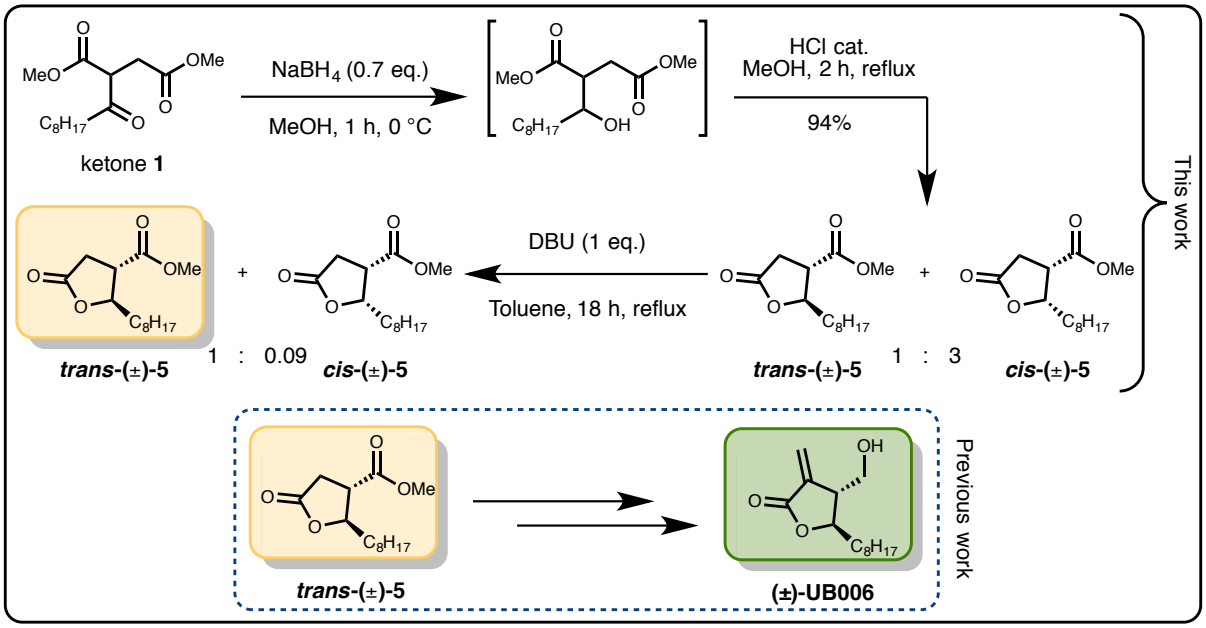
A new methodology to form the lactam ring was tried, starting with the oxime formation from ketone **1** in neutral and basic media (Scheme 9). In the first attempt, it was used the same equivalents of hydroxylammonium chloride (acid) and sodium bicarbonate (base), using methanol as solvent. After 18 h of reaction, oxime **4** was possibly formed as observed in $^{1}$H-NMR, but further analysis of $^{13}$C-NMR could not conclude our expectation because of the complexity of the crude mixture. It was tried to separate the oxime **4** from the impurities by column chromatography, but it was not successful. Further attempts were performed by using the ketone **1** previously purified by CC, however, the final result was the same as before. Next, we tried to obtain the oxime **4** in basic media, using 2.2 equivalents of sodium acetate (base), 1.1 equivalents of $HONH_2 \cdot HCl$ and a 1:1 mixture of $H_2O$ and MeOH as the solvent. After 48h, the reaction was stopped but the $^{1}$H-NMR showed no change in the signals, thus, it was evidenced that the reaction did not work. Altogether, regarding many attempts and effort the new methodology to obtain the lactam ring was not successful as this initial step did not work as expected probably due to the high steric effects in ketone **1**.




*Scheme 9. Attempts of oxime formation.*

## 2.2 Synthesis of (±)-UB006 Precursor

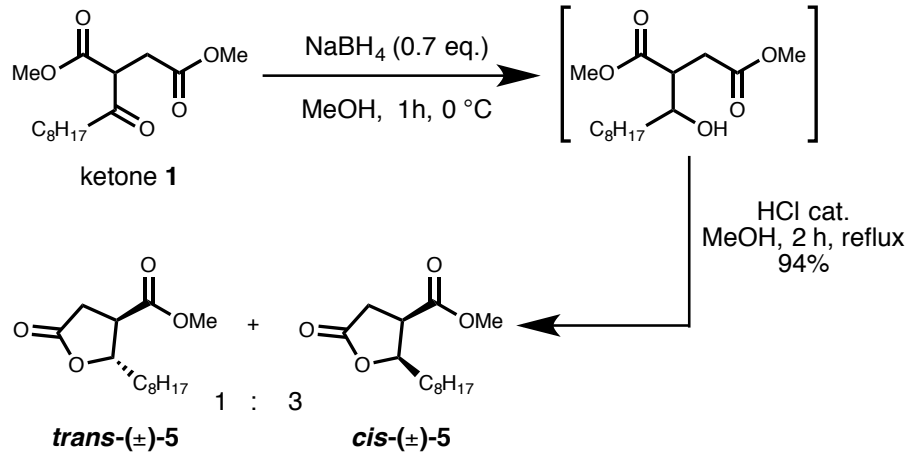
The (±)-UB006 is a C75 derivative with high cytotoxic activity, it was developed by Kamil Makowski in his doctoral thesis [120]. In this synthesis, the ketone **1** without CC purification was used as the starting material of the (±)-UB006 synthetic pathway (Scheme 10).



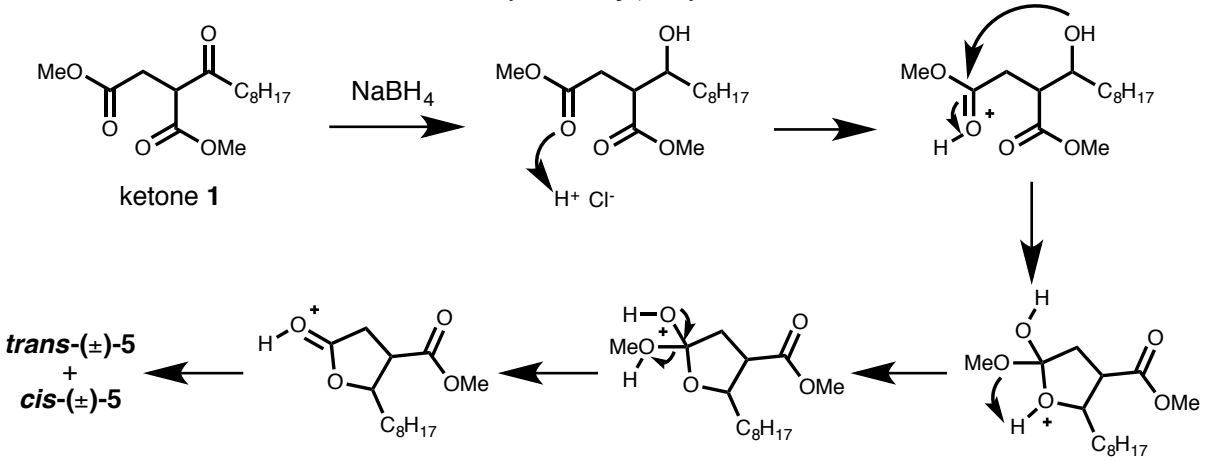

*Scheme 10. Overall synthetic pathway of (±)-UB006. The compound in the yellow box is the product obtained in the present work. The blue dashed frame delimits the synthetic steps that are not part of the present work. The compound in the green box is (±)-UB006 that has been studied in previous works.*

### *2.2.1 Reduction of Ketone and Cycle Formation*

The ketone **1** was reduced with $NaBH_4$ to its correspondent alcohol that was directly cyclized in acid media (Scheme 11). A mixture of lactones was obtained in a 1:3 proportion in favor of lactone ***cis*-(±)-5**.




*Scheme 11. Synthesis of γ-butyrolactones 5*

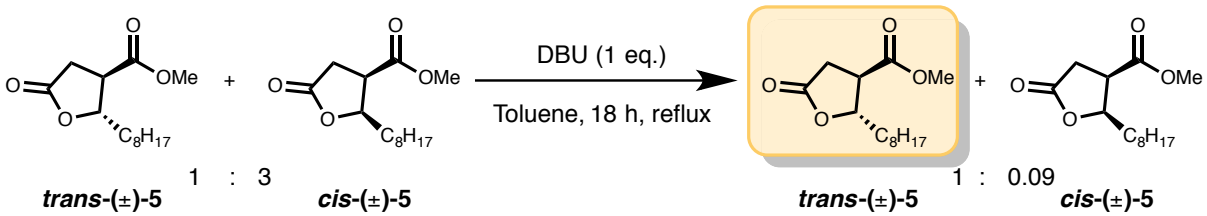



*Scheme 12. Mechanism of ketone 1 reduction and cycle formation of lactone 5 diastereomers.*

In Scheme 12, is observed the reaction mechanism of the lactone **5** diastereomers formation. First, the ketone **1** is reduced by the $NaBH_4$ (not shown). Finally, with the help of concentrated HCl, the formed alcohol attacks the electrophile carbonyl to form the most stable ring, and by intramolecular rearrangements, the lactone diastereomers ***trans*-(±)-5** and ***cis*-(±)-5** were obtained.

### *2.2.2 Isomerization of Lactones Diastereomeric Mixture*

Similar to lactam preparation, only one diastereomer was required for the next steps; therefore, the diastereomeric mixture of lactones **5** was isomerized (Scheme 13). The proton of the neighboring carbon next to the methyl esters is acid, and in presence of a strong base is possible to achieve the isomerization in favor of the most thermodynamically stable product, the lactone ***trans*-(±)-5**. The mixture of lactones was heated under toluene reflux for 18 h, using 1 equivalent of DBU as the isomerizing agent. Finally, the diastereomeric mixture of lactones **5** was obtained in a *trans:cis* relation of 1:0.09 (observed by $^1$H-NMR), and after CC separation it was obtained the lactone ***trans*-(±)-5** with a 37% yield.




**trans-(±)-5** **cis-(±)-5** **trans-(±)-5** **cis-(±)-5**

*Scheme 13. Isomerization of γ-butyrolactones 5*

As can be observed in Figure 4, there is an evident change in the $^{1}$H-NMR spectrum of the lactone **5** diastereomers mixture after the isomerization step. Almost all the ***cis*-(±)-5** diastereomer was transformed into the thermodynamically stable ***trans*-(±)-5** diastereomer.

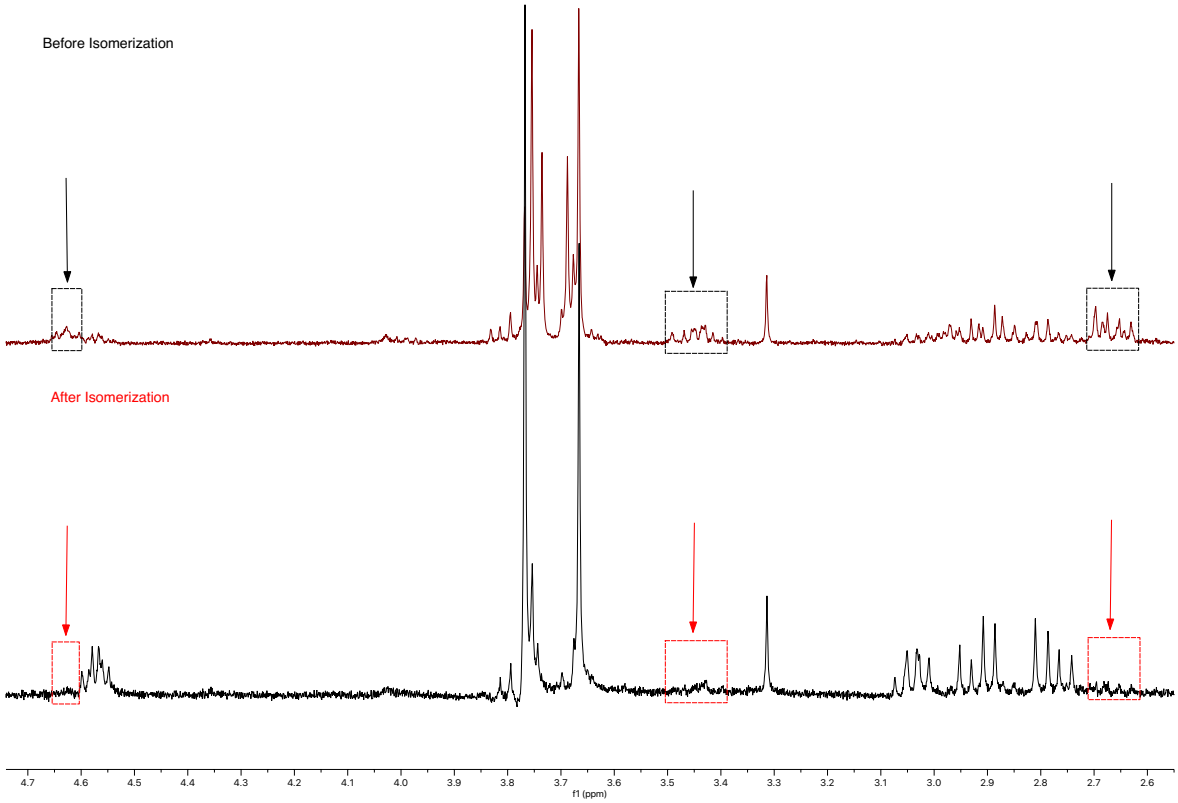



***Figure 4. Part of the $^{1}$H-NMR spectra of the lactone diastereomers before (top) and after (bottom) isomerization. The most evident changes in the spectra are indicated with dashed squares and pointed by arrows. They indicate the decrease of cis diastereomer.***

To sum up, precursors of the (±)-UB006 (***trans*-(±)-5**) and the nitrogenated analog (***trans*-(±)-2**) were synthesized with high purity. Moreover, the synthesis of the lactam ***trans*-(±)-2** was scaled-up, but some difficulties were observed in the purification of the diastereomer that reduce the overall yield of the reaction. Finally, a new methodology was tried for the synthesis of lactam ***trans*-(±)-2**, but unfortunately, the first step (oxime **4** formation) did not work as expected. All the obtained products were characterized by TLC, $^{1}$H-NMR, $^{13}$C-NMR, FTIR, and HRMS (spectra in section 6.2 ).

# CHAPTER 3. METHODOLOGY

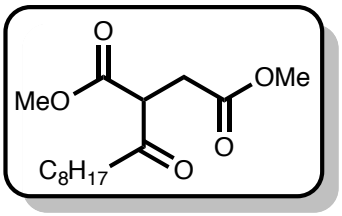
## 3.1 Reagents and Equipment

All reagents were purchased from commercial suppliers and used as received without further purification. Some solvents as MeOH and *n*-nonanal were distilled before their use. Thin-layer chromatography (TLC) was carried out using silica gel analytical plates of 0.20 mm thick ($F_{254}$ Merk), and the used solvent is indicated in each case. To visualize the TLCs, it was only used phosphomolybdic acid as stain, and in some cases UV light (259 nm). The retention factors ($R_f$) are approximated values. Column Chromatography was carried out using the flash technique on silica gel with 0.040-0.063 mm particle size and the used solvent is specified in each case. The melting point (MP) was determined on a Gallenkamp instrument.

The $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on a Mercury 400 MHz and 101 MHz, respectively. Only $CDCl_3$ was used as solvent and tetramethylsilane (TMS) as the reference. The coupling constant (*J*) are in Hz, the chemical shift in parts per million (ppm), and the multiplicities of the signals are indicated with the following abbreviations: s (singlet), d (doublet), t (triplet), c (quartet), q (quintet), m (multiplet), dd (doublet of doublets), td (triplet of doublets), ddd (doublet of doublet of doublets). Infrared spectra were recorded on a Nicolet 6700 FTIR spectrometer, for the characterization of the compounds the most relevant absorptions are indicated in $cm^{-1}$. The high-resolution mass spectrometry (HRMS) analysis was recorded on Agilent LC/MSD-TOF mass spectrometer.

## 3.2 Synthesis of dimethyl 2-nonanoylsuccinate

A mixture of dimethyl maleate (7.72 mL, 61.7 mmol) and *n*-nonanal (54 mL, 310 mmol) was prepared under $N_2$ atmosphere. Benzoyl peroxide (0.603 g, 1.9 mmol) was added and the mixture was stirred at 80 °C. After 18 h an additional portion of benzoyl peroxide (0.107 g, 0.55 mmol) was added and the mixture was heated at 80 °C for 3 h. After the mixture was cooled to r.t., AcOEt (30 mL) and $NaHCO_3$ (15 mL) were added and stirred for 10 min. The organic layer was separated and washed with water (2 x 15 mL) and aqueous saturated solution of NaCl (15 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The organic phase was concentrated under reduced pressure. The *n*-nonanal excess was eliminated by reduced pressure distillation, obtaining 19.507 g of ketone **1** crude. Column chromatography was not necessary for the purposes of this work, but it was performed with a small quantity obtaining ketone **1** with an 80% yield.



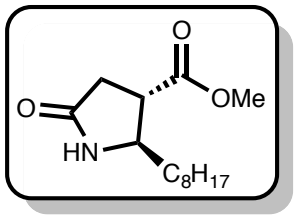

**Compound 1**: colorless oil; $\mathbf{R_f}$ = 0.53 ($CH_2Cl_2$); **$^1$H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (3H, t, *J* = 6.8 Hz, $CH_3$), 1.27 - 1.64 (12H, m, $CH_2$), 2.57 - 2.75 (2H, m, $CH_2$-C*H*$_2$-CO), 2.84 (1H, dd, *J* = 6.3, 17.6 Hz, C*H*H'-CO-$CH_3$), 2.98 (1H, dd, *J* = 8.2, 17.6 Hz, CH*H*'-CO-$CH_3$), 3.68 (3H, s, $CH_2$-CO-OC*H*$_3$), 3.74 (3H, s, CHR-CO-OC*H*$_3$), 3.99 (1H, dd, *J* = 6.3, 8.2 Hz, C*H*R-CO-$OCH_3$); **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): δ 14.3, 22.8, 23.6, 29.2, 29.3, 29.5, 32.0, 32.4, 43.0, 52.2, 52.9, 54.0, 169.2, 172.0, 204.1; **FTIR** (ATR): 669, 724, 872, 1007, 1160, 1195, 1258, 1365, 1408, 1436, 1718, 1736, 2854, 2925; **HRMS** (ESI-) calculated for $C_{15}H_{25}O_5$ $[M-H]^-$ = 285.1702, found = 285.2.

### 3.3 Synthesis of methyl *trans*-2-octyl-5-oxopyrrolidine-3-carboxylate

Ammonium acetate (13.49 g, 174.8 mmol) and $NaBH_3CN$ (1.11 g, 17.5 mmol) were added to a solution of ketone **1** (5.00 g, 17.5 mmol) in distilled MeOH (80 mL) and the mixture was stirred for 48 h at r.t. The mixture was concentrated under reduced pressure and the residue was dissolved in toluene (80 mL) heated to reflux for 12 h. Toluene was evaporated under reduced pressure and the residue was dissolved in $CH_2Cl_2$ (150 mL). The organic layer was washed with HCl 0.01 N (150 mL) and brine (200 mL), then it was dried over anhydrous $MgSO_4$. $CH_2Cl_2$ was evaporated under reduced pressure, obtaining a mixture of diastereomers (4.509 g, 90%).

The crude residue (4.509 g, 17.6 mmol) was isomerized with NaOMe (0.994 g, 18.4 mmol) and distilled MeOH (90 mL). The mixture was heated to reflux for 48h and concentrated under reduced pressure. The residue was dissolved in $CH_2Cl_2$ (150 mL). The organic layer was washed with HCl 2N (150 mL) and dried over $MgSO_4$. $CH_2Cl_2$ was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel ($CH_2Cl_2$/MeOH 95:5). Finally, the residue was washed with a saturated solution of $NaHCO_3$ (150 mL) to afford lactam ***trans*-(±)-2**. (0.933g, 21%, *trans/cis* 1:0.1).

This reaction was performed several times, to obtain high amounts of lactam ***trans*-(±)-2**. The final wash with the saturated solution of $NaHCO_3$ was performed to remove some acid impurities from the *n*-nonanal. Specifically, it was observed by $^1$H-NMR a triplet at δ 2.3 that corresponds to the acid proton of nonanoic acid.



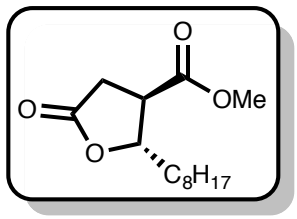

**Compound *trans*-(±)-2**: white crystalline solid; **MP** = 55-58 °C; **$R_f$** = 0.48 ($CH_2Cl_2$/MeOH 95:5); **$^1$H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (3H, t, *J* = 6.8 Hz, $CH_3$), 1.24 - 1.35 (12H, m, $CH_2$), 1.57 - 1.67 (2H, m, $CH_2$-C*H*$_2$-CH), 2.56 - 2.71 (2H, m, $CH_2$-CO), 2.88 - 2.94 (1H, m, C*H*-$CO_2$Me), 3.75 (3H, s, $OCH_3$), 3.80 - 3.86 (1H, dd, *J* = 13.0, 5.9 Hz, C*H*-NH); **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): δ 14.0, 22.6, 25.6, 29.1, 29.3, 29.5, 31.8, 33.8, 36.4, 45.0, 52.3, 57.2, 173.2, 175.6; **FTIR** (ATR): 550, 670, 721, 748, 762, 788, 804, 864, 889, 943, 1007, 1096, 1159, 1176, 1193, 1208, 1236, 1260, 1292, 1348, 1391, 1436, 1466, 1541, 1558, 1697, 1744, 2341, 2360, 2850, 2915, 2955, 3093, 3177; **HRMS** (ESI+) calculated for $C_{14}H_{26}NO_3$ $[M+H]^+$ = 256.1913, found = 256.1; (ESI-) calculated for $C_{14}H_{24}NO_3$ $[M-H]^-$ = 254.1756, found = 254.2

* ***cis*** most important signals: δ 2.37 (1H, m, CH*H*CO), 2.77 (1H, m, C*H*HCO), 3.71 (3H, s, $OCH_3$).

### 3.4 Synthesis of methyl *trans*-2-octyl-5-oxotetrahydrofuran-3-carboxylate

In a round bottom flask, a solution of ketone **1** (2.250 g, 7.9 mmol) in MeOH (60 mL) was prepared. Subsequently, at 0 °C and with magnetic stirring, $NaBH_4$ (0.216 g, 5.7 mmol) was added little by little. The mixture was left under stirring for 2 h at r.t. Then, HCl conc. (25 drops, *p*H = 2-3) was added, the mixture was stirred for 1h under reflux. The mixture was concentrated under reduced pressure, and the crude was dissolved with $CH_2Cl_2$ (100 mL) and $NaHCO_3$ (100 mL). The organic phase was washed with $H_2O$ (100 mL), dried over anhydrous $MgSO_4$, and the solvent was eliminated under reduced pressure, obtaining a mixture of lactone diastereomers (2.105 g, 94%).

The crude residue (2.105 g, 12.7 mmol) was dissolved in toluene (100 mL), DBU (1.09 mL, 12.7 mmol) was added and the mixture was heated for 18 h at reflux. The resulting mixture was washed with HCl 0.02 N (100 mL) and aqueous saturated solution of NaCl (100 mL). The organic phase was dried over anhydrous $MgSO_4$ and after filtration, it was concentrated under reduced pressure. It was obtained an isomerized mixture of lactones *cis/trans* (0.09:1, $^1$H-NMR). The mixture of lactones was separated by column chromatography in silica gel ($CH_2Cl_2$), obtaining 0.774 g of lactone ***trans*-(±)-5** (37%).




**Compound *trans*-(±)-5**: colorless oil; $\mathbf{R_f}$ = 0.46 (hexane/AcOEt, 7:3); **$^1$H-NMR** ($CDCl_3$, 400 MHz): δ 0.84 - 0. 93 (3H, m, $CH_3$), 1.18 - 1. 59 (m, 12H, $CH_2$), 1. 64 - 1.84 (2H, m, $CH_2$-C*H*$_2$-CH), 2.77 (1H, dd, *J* = 17.6, 9.5 Hz, C*H*H'-CO), 2.92 (1H, dd, *J* = 17.6, 8.7, CH*H*'-CO), 3.04 (1H, ddd, *J* = 9.5, 8.7, 7.1, C*H*-$CO_2$Me), 3.77 (3H, s, $OCH_3$), 4.57 (1H, td, *J* = 7.3, 5.0, CHO); **$^{13}$C-NMR** ($CDCl_3$, 100.25 MHz): δ 14, 22.6, 25.11, 29.0, 29.1, 29.3, 31.7, 32.1, 35.2, 45.6, 52.6, 74.3, 81.9, 171.5; **FTIR** (ATR): 722, 766, 858, 937, 978, 1171, 1197, 1364, 1437, 1458, 1736, 1781, 2854, 2924; **HRMS** (ESI+) calculated for $C_{14}H_{25}O_4$ $[M+H]^+$ = 257.1753, found = 256.1.

* ***cis*** most important signals: δ 2.66 (1H, dd, *J* = 17.6, 8.7, $CH_2$-CO), 3.44 (1H, ddd, *J* = 8.7, 7.4, 5.7, C*H*-COOMe), 4.62 (1H, td, *J* = 7.4, 6.3, CHO).

# CHAPTER 4. CONCLUSION AND RECOMENDATIONS

## 4.1 Conclusions

In the present work, high amounts of the nitrogenated analog precursor of (±)-UB006 were synthesized and purified by CC and will be used as an advanced starting material in further research by the doctorate student Roberto Gómez. The isomerization of the lactam diastereomeric mixture was observed by $^{1}$H-NMR, evidencing the efficiency of the used method. The scaling-up of the overall synthesis of lactam ***trans*-(±)-2**, showed that both reaction yield and purity are negatively affected. The new methodology of oxime formation showed to be problematic due to the high steric effects of ketone **1**. Also, high amounts of the (±)-UB006 precursor were synthesized and purified by CC and will serve as a starting material for further synthesis of FAS inhibitors.

## 4.2 Recommendations

- Use the ketone **1** purified by CC or perform a great vacuum distillation to remove all the *n*-nonanal impurities and avoid possible problems.
- Use thin and long columns for the CC purification and when there is too much crude to purify, divide it into parts previously (do not do it at once).
- Try to perform the α-methylenation as the continuation of FAS inhibitor **3** synthesis.

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# CHAPTER 6. ANNEXES

## 6.1 Abbreviations and Acronyms

| | |
|---|---|
| FA | Fatty acid |
| PL | Phospholipid |
| DAG | Diacylglyceride |
| TG | Triacylglyceride |
| LD | Lipid droplets |
| ATP | Adenosine triphosphate |
| DNL | *De novo* lipogenesis |
| AKT | Protein kinase B |
| Co-A | Coenzyme A |
| ACLY | ATP-citrate lyase |
| ACC | Acetyl-CoA carboxylase |
| FAS | Fatty acid synthase |
| MAGL | Monoacylglycerol lipase |
| hFAS | Human FAS |
| TE | Thioesterase domain |
| ACP | Acyl-carrier protein domain |
| KR | β-ketoacyl reductase domain |
| ER | Enoyl reductase domain |
| DH | β-hydroxyacyl dehydratase |
| MAT | Malonyl/acetyl transferase |
| KS | β-ketoacyl synthase |
| YKR | pseudoketoreductase domain |
| YME | pseudomethyltransferase domain |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| MAPK | Mitogen-activated protein kinase |
| PI3K | Phosphoinositide 3-kinase |
| HER2 | Receptor tyrosine-protein kinase erbB-2 |
| EGF | Epidermal growth factor |
| SREBP-1c | Sterol regulatory element-binding protein 1 |
| ChREBP | Carbohydrate-responsive element-binding protein |
| USP2a | Ubiquitin-specific cysteine protease 2a |
| C75 | (*2R,3S*)-4-methylidene-2-octyl-5-oxooxolane-3-carboxylic acid |
| $IC_{50}$ | Half-maximal inhibitory concentration |
| CPT1 | Carnitine palmitoyltransferase I |
| UB006 | *trans*-4-(hydroxymethyl)-3-methylene-5-octyldihydrofuran-2(3H)-one |
| CC | Column chromatography |
| TLC | Thin layer chromatography |

| | |
|---|---|
| r.t. | Room temperature |
| TFA | Trifluoroacetic acid |
| $^{1}$H-NMR | Nuclear magnetic resonance of proton |
| $^{13}$C-NMR | Nuclear magnetic resonance of carbon |
| FTIR | Fourier-transform infrared spectroscopy |
| HRMS | High resolution mass spectrometry |
| UV | Ultraviolet |
| $R_f$ | Retention factor |
| MP | Melting point |
| TMS | Tetramethylsilane |

## 6.2 Spectra of the Obtained Products

### *6.2.1 Ketone 1 Spectra*

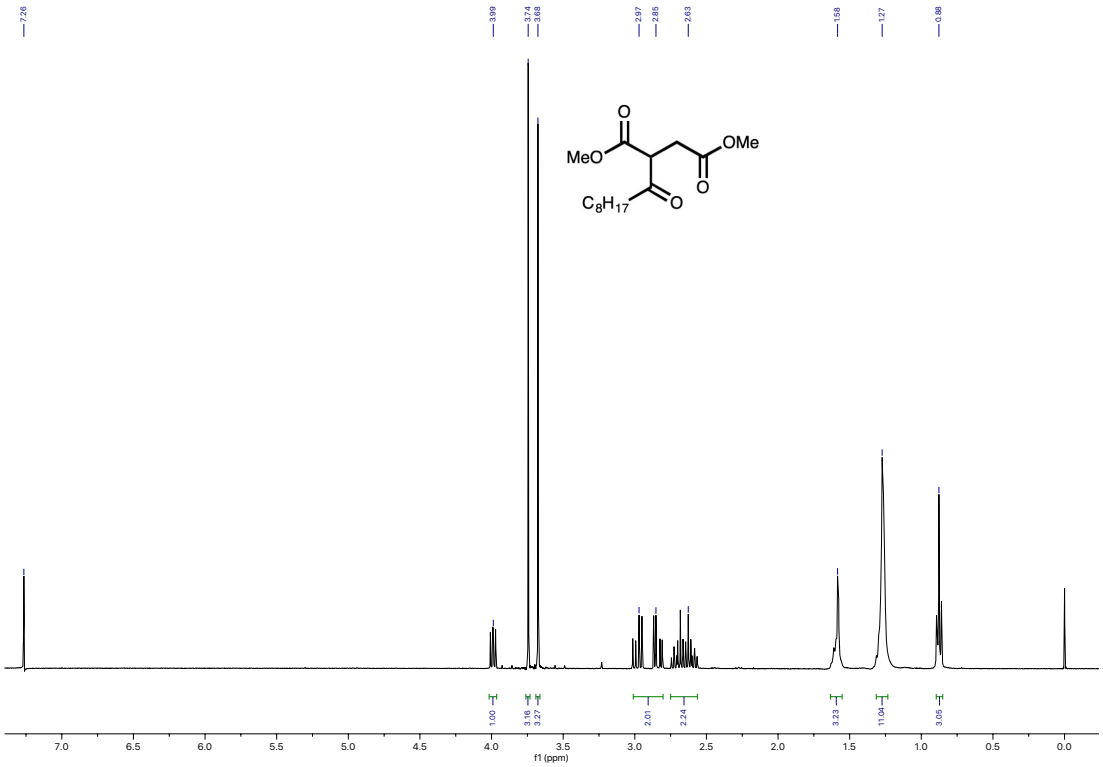


*Figure 5. [1]H-NMR spectrum of ketone 1.*

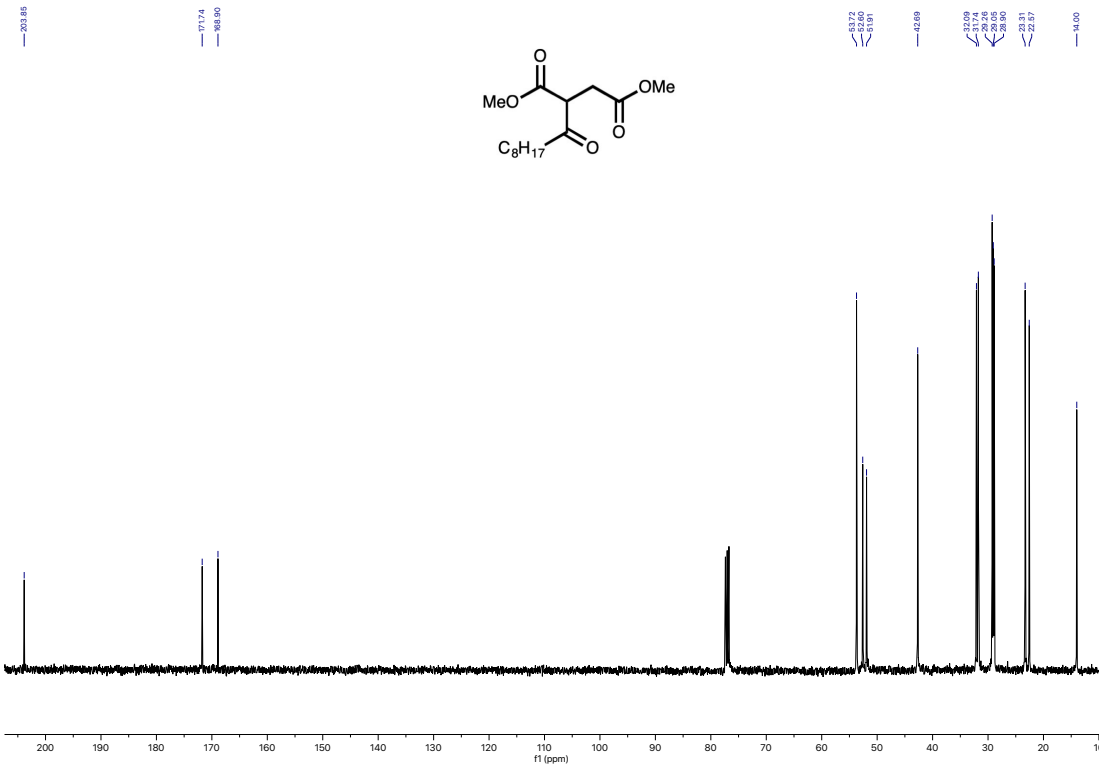


*Figure 6. [13]C-NMR spectrum of ketone 1.*

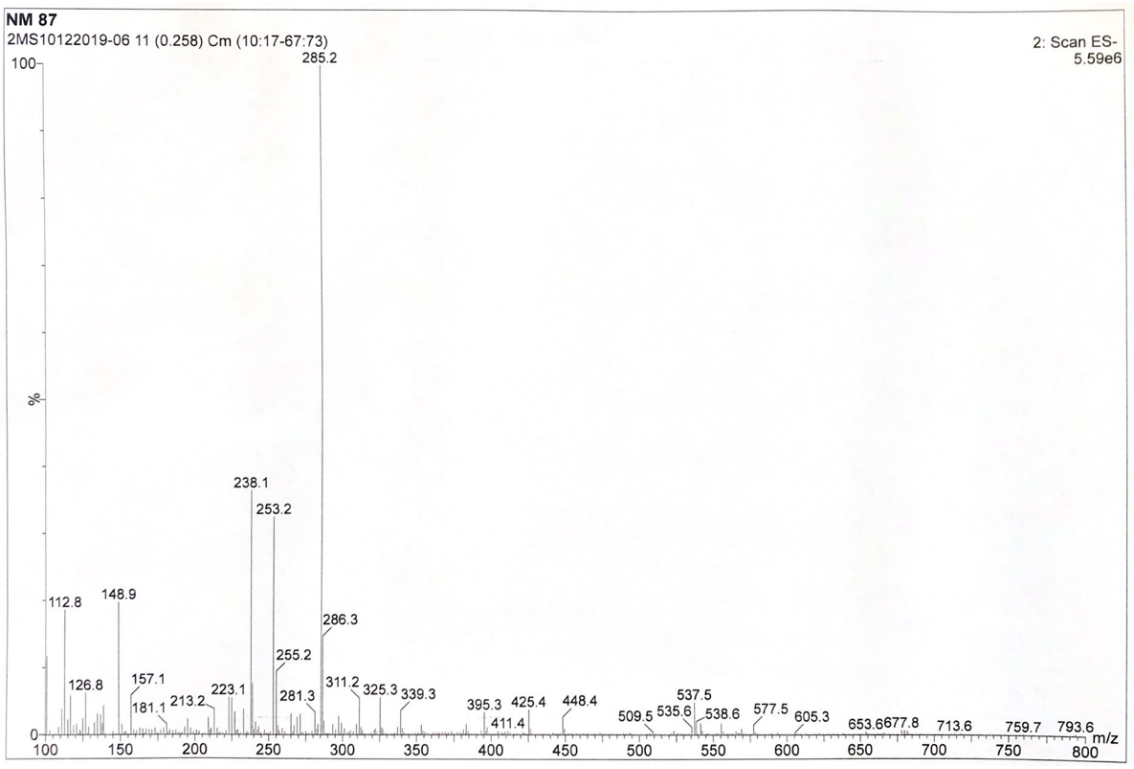


*Figure 7. HRMS spectrum (ES-) of ketone 1.*

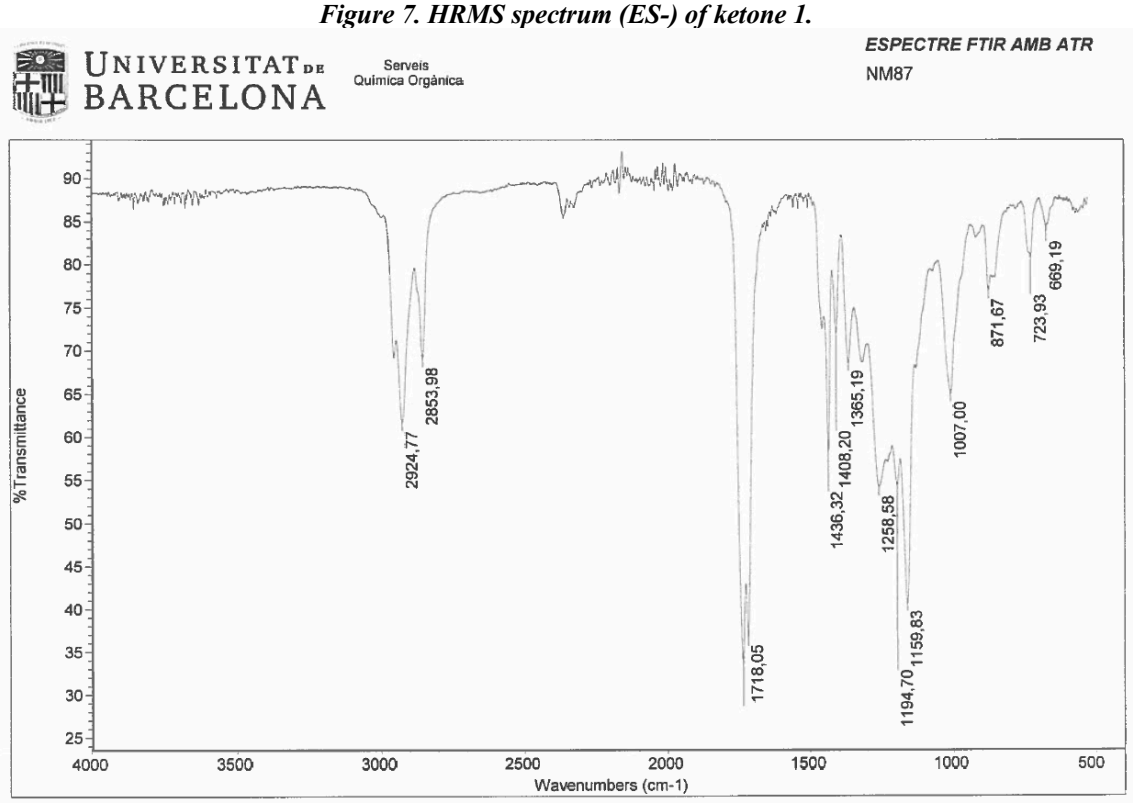


*Figure 8. FTIR spectrum of ketone 1.*

### 6.2.2 Lactam trans-(±)-2 Spectra

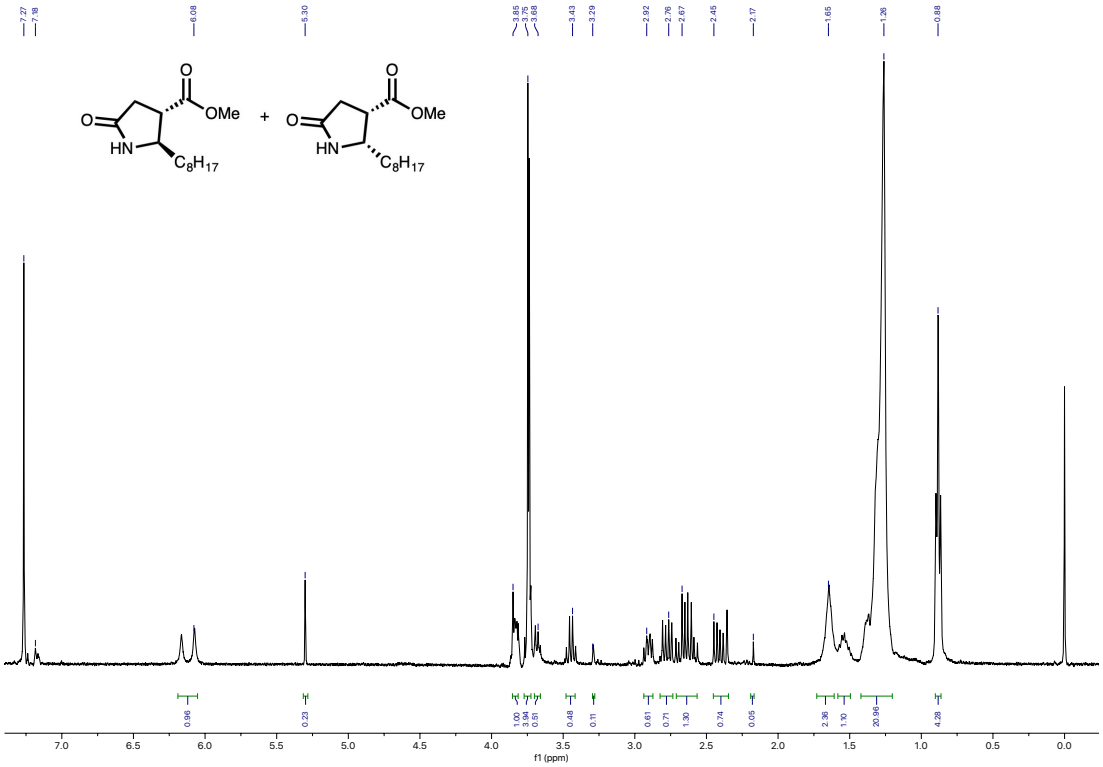


*Figure 9. $^1$H-NMR spectrum of lactam 2 (10:7) mixture in favor of trans diastereomer.*

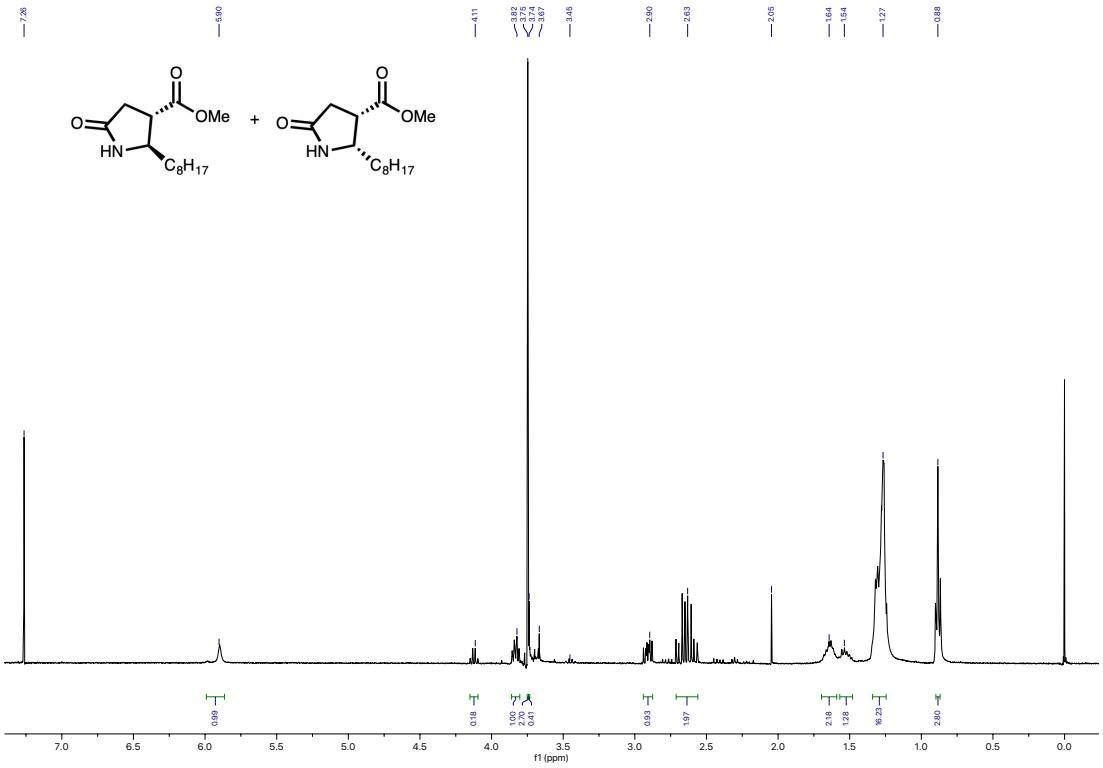


*Figure 10. $^1$H-NMR spectrum of lactam 2 (10:1) mixture in favor of trans diastereomer.*

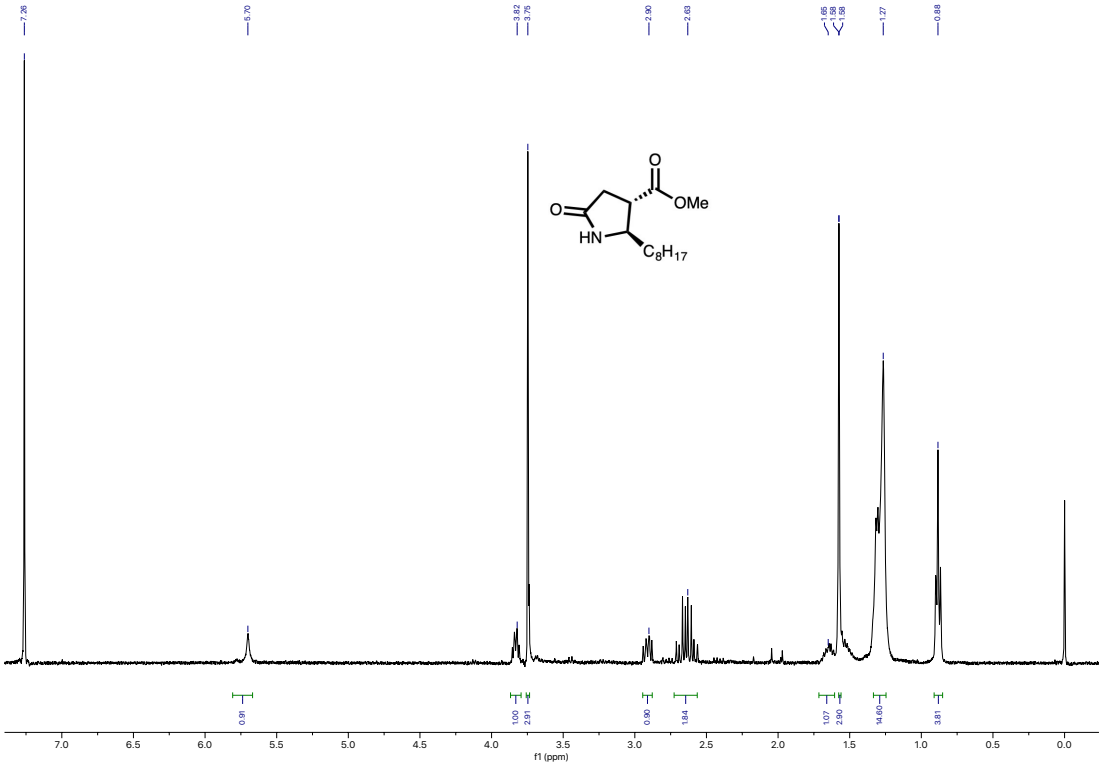


*Figure 11. $^{1}$H-NMR spectrum of lactam trans-(±)-2.*

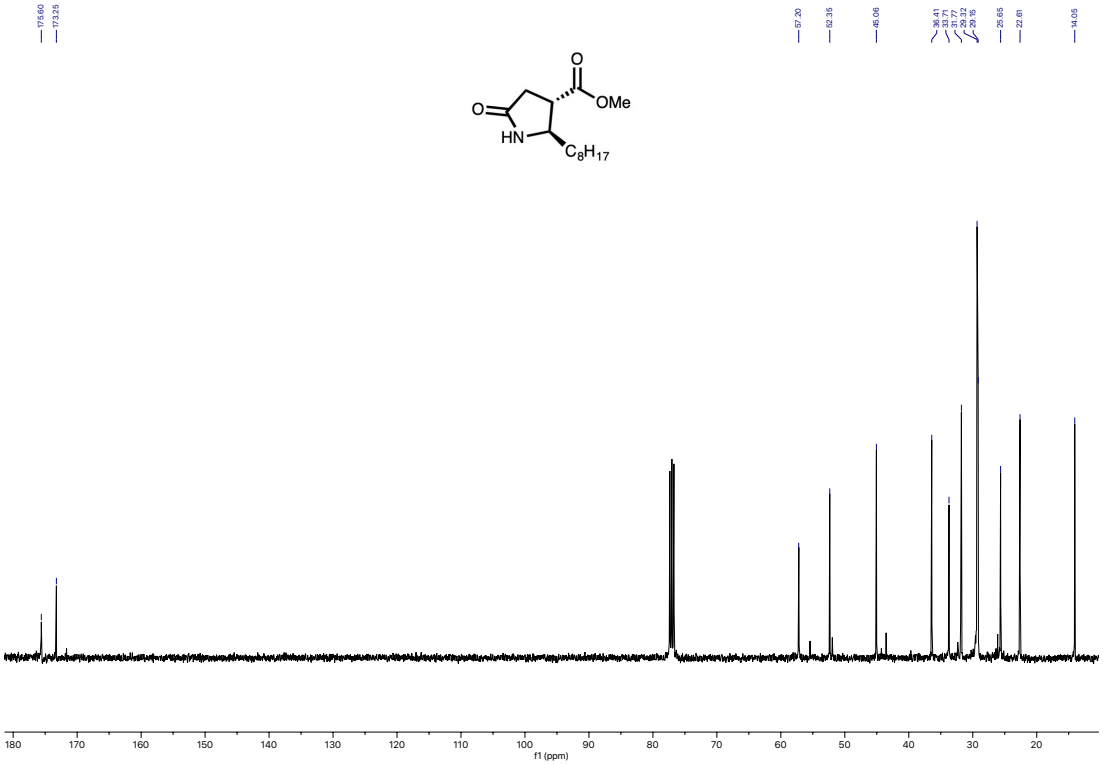


*Figure 12. $^{13}$C-NMR spectrum of lactam trans-(±)-2.*

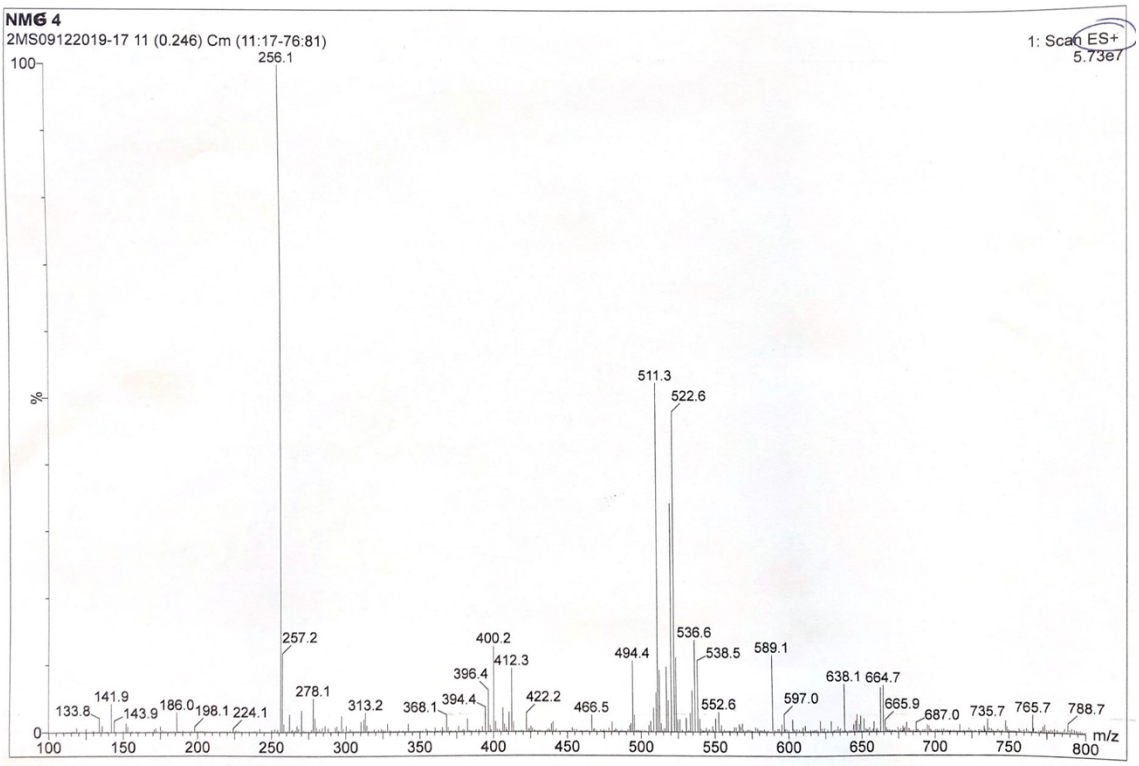


*Figure 13. HRMS spectrum (ES+) of lactam trans-(±)-2.*

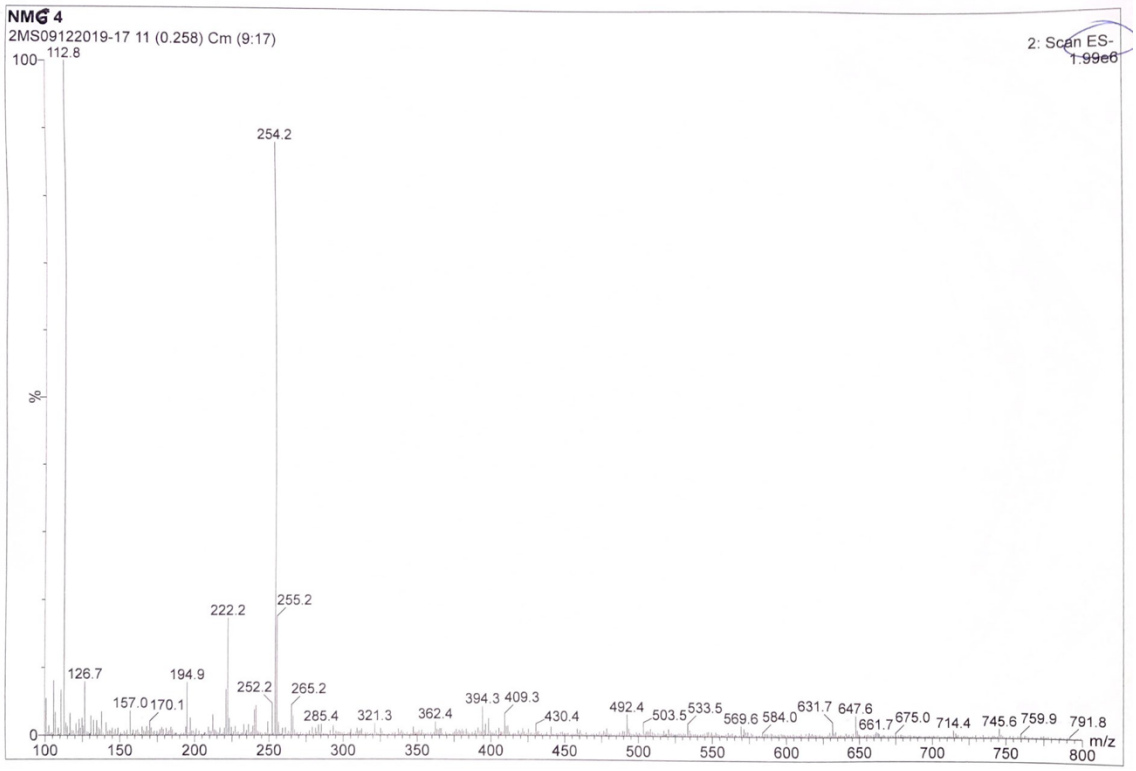


*Figure 14. HRMS spectrum (ES-) of lactam trans-(±)-2.*

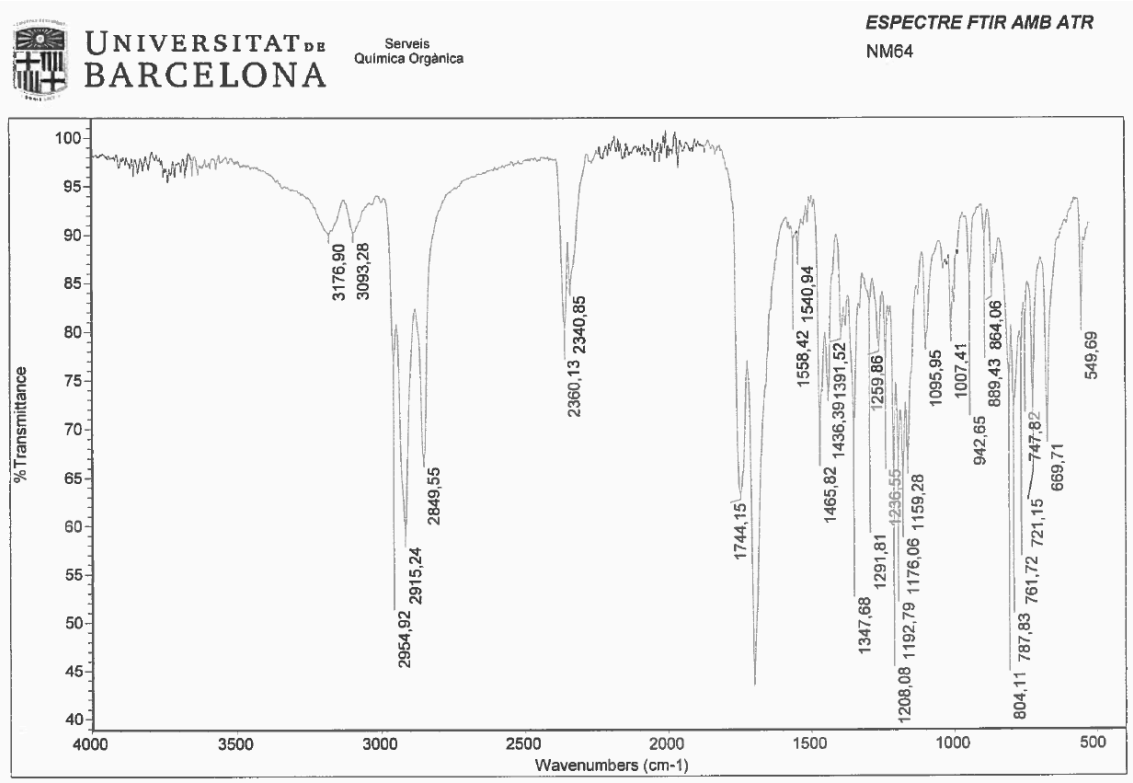


*Figure 15. FTIR spectrum of lactam trans-(±)-2.*

### *6.2.3 Lactone trans-(±)-5 Spectra*

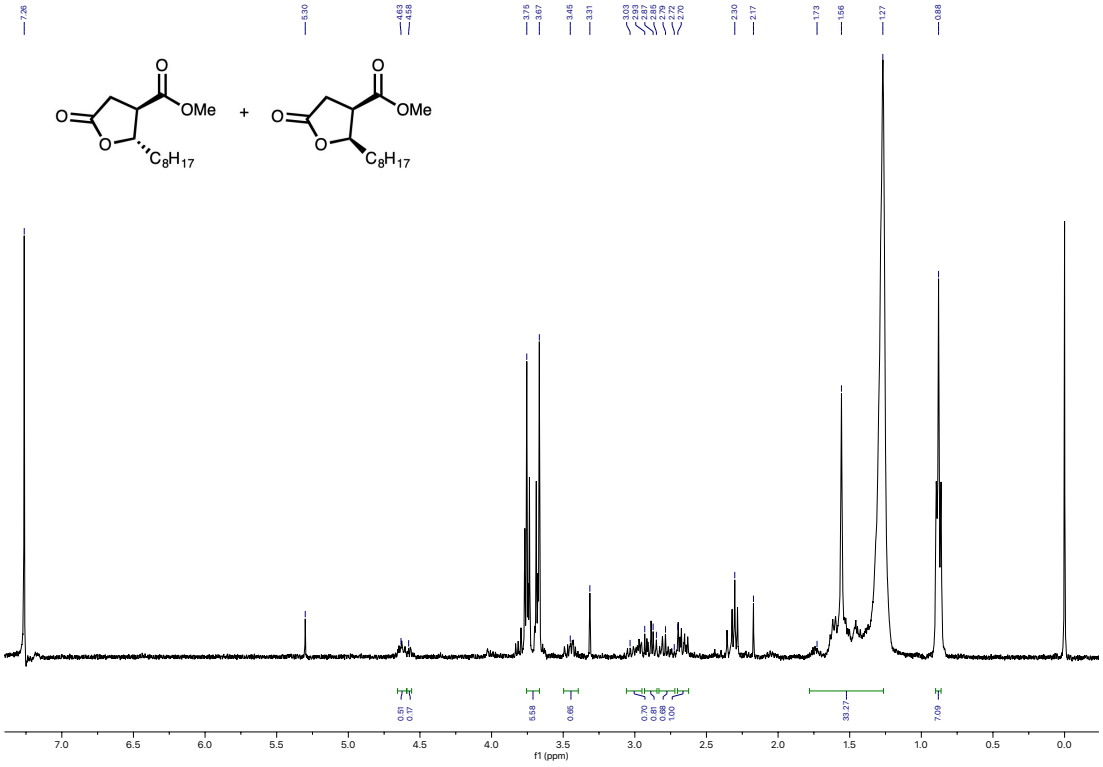


*Figure 16. [1]H-NMR spectrum of lactone 5 (1:3) mixture in favor of cis diastereomer.*

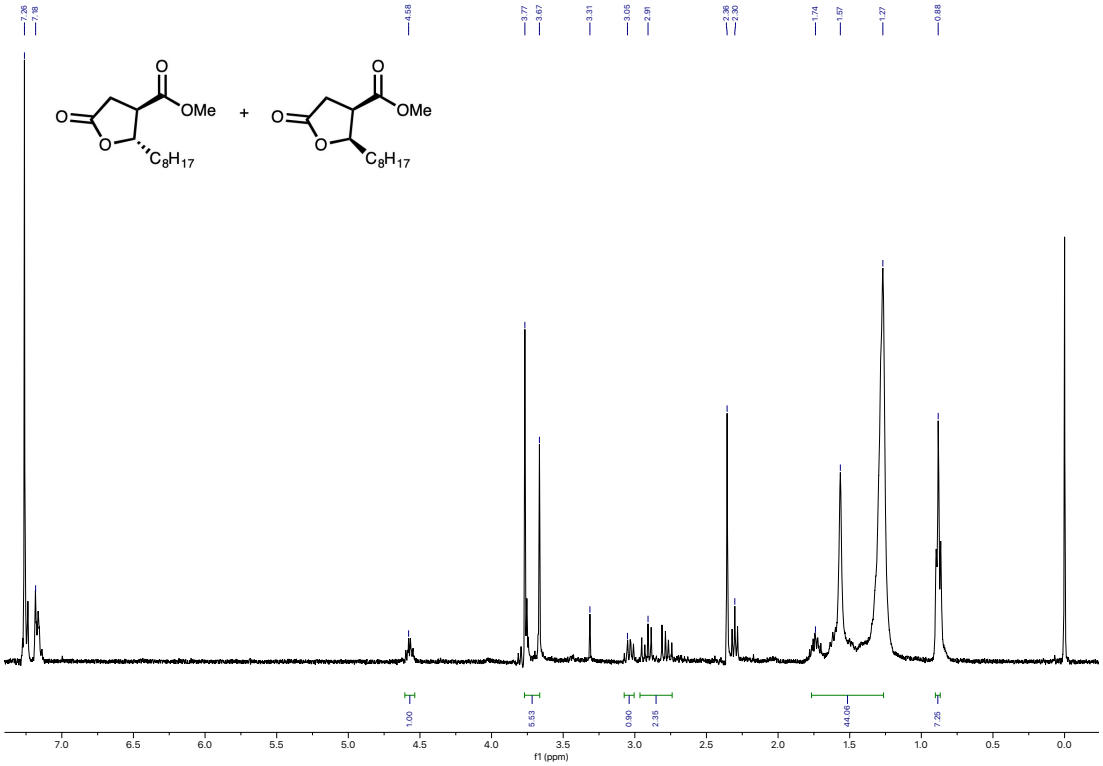


*Figure 17. [1]H-NMR spectrum of lactone 5 (1:0.09) mixture in favor of trans diastereomer.*

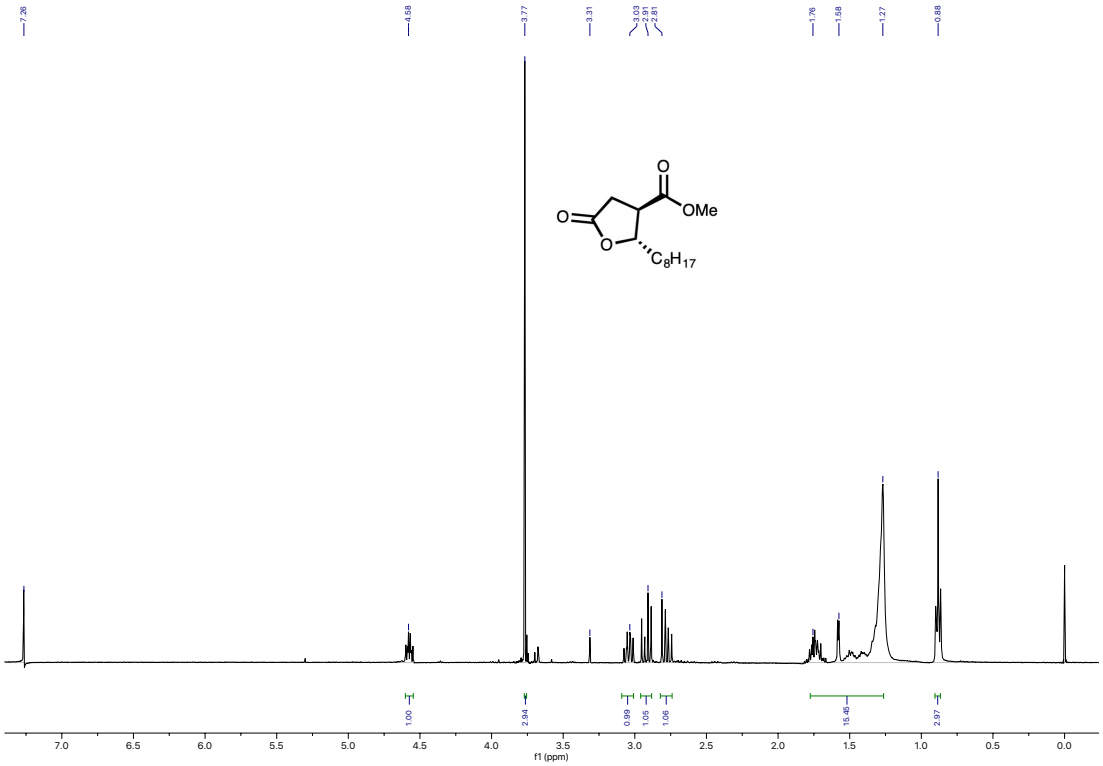


*Figure 18. [1]H-NMR spectrum of lactone trans-(±)-5.*

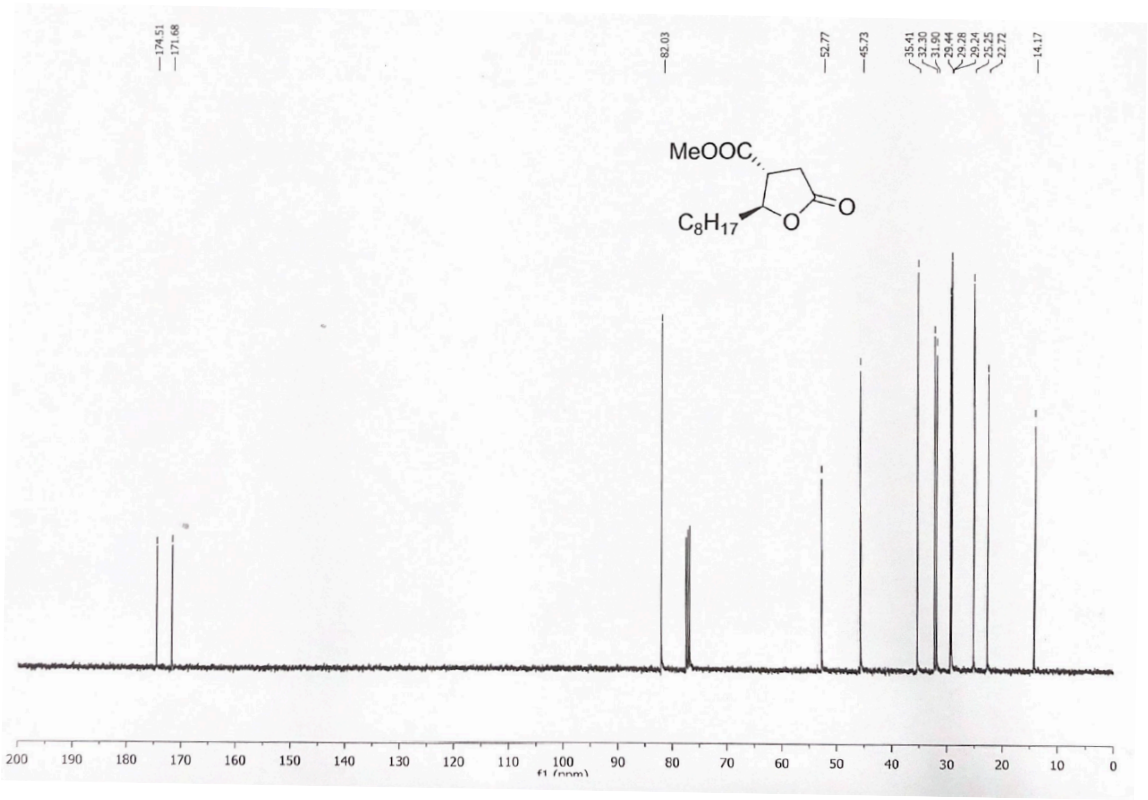


***Figure 19. $^{13}$C-NMR spectrum of lactone trans-(±)-5.***

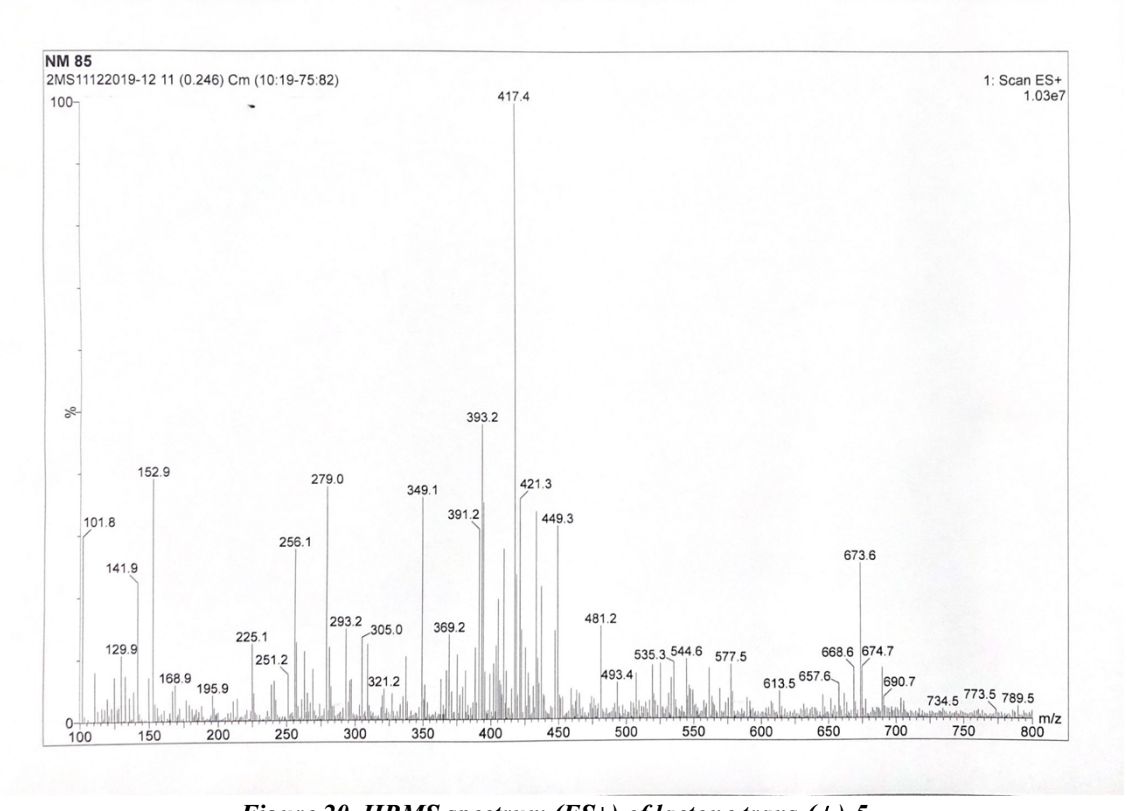


***Figure 20. HRMS spectrum (ES+) of lactone trans-(±)-5.***

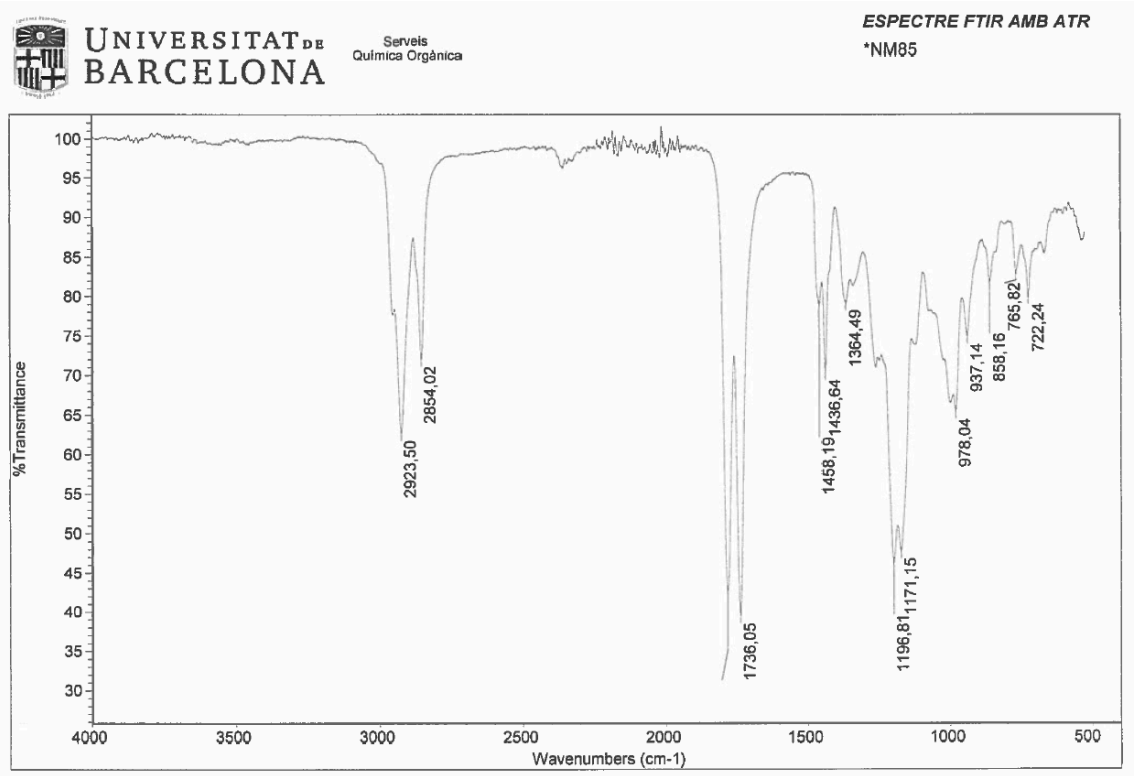


***Figure 21. FTIR spectrum of lactone trans-(±)-5.***