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Grau de Química

Treball Final de Grau

Preparation of the enzymatic inhibitor UB-207.
Preparación del inhibidor enzimático UB-207.

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B:KC Barcelona Knowledge Campus
Campus d'Excel·lència Internacional

One's mind, once stretched by a new idea, never regains its original dimensions.

Oliver Wendell Holmes Sr.

Ante todo, me gustaría agradecer al Dr. Xavier Ariza por haberme guiado, aconsejado y apoyado a lo largo de estos meses de trabajo. Agradezco por su ayuda y compañerismo a todos los miembros del grupo, especialmente a Héctor, Miquel, Wouter y Alejandra con los cuales he compartido momentos inolvidables trabajando codo a codo en el laboratorio.

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Dedico este trabajo a mis padres y mi hermana. Por haber creído en mí y haberme dado su confianza y cariño.

REPORT

CONTENTS

1. SUMMARY

Obesity has become a priority health issue for many governments and organisations such as the WHO, it has gained the status of epidemic and according to the past years trends, the forecasts are not hopeful. The research for anti-obesity drugs has grown in the past years as the percentage of obese people grows globally. A relatively new anti-cancer drug (C75) was tested as anti-cancer drug to rodents, and it was observed that the specimens were showing an anorexigenic behaviour. The molecule was interacting in the hypothalamus reducing the food intake. Since this side effect was discovered many have been the attempts to find derivatives and analogues of C75 focusing on its food intake modulator basal.

In this project, we have focused on the synthesis of C75 analogue called (±)-UB-207 that was first reported in 2012. The novelty of the present work consists in modifying the initial synthetic route in order to simplify the obtainment of the pure derivative.

Keywords: anti-obesity, food intake, hypothalamus, C75.

2. RESUMEN

La obesidad se ha convertido en un asunto sanitario de alta prioridad en los objetivos de gobiernos y organizaciones tales como la OMS, ha ganado el estatus de epidemia y las previsiones no son alentadoras. La investigación para hallar fármacos anti-obesidad ha crecido en los últimos años a medida que el porcentaje de obesos aumenta globalmente. Un fármaco (C75) relativamente reciente fue probado como antitumoral en roedores, y se observó que los especímenes mostraban un comportamiento anorexigénico. La molécula estaba interactuando en el hipotálamo reduciendo la ingesta de alimentos. Desde que este efecto secundario fue descubierto muchos han sido los intentos para hallar derivados y análogos del C75 enfocándose en su vertiente como regulador de la ingesta.

En este trabajo nos hemos centrado en la síntesis de un análogo del C75 llamado (±)-UB-207, cuya primera síntesis fue descrita en 2012. La novedad de este trabajo consiste en la modificación de la ruta sintética con el objetivo de facilitar su obtención.

Palabras clave: anti-obesidad, ingesta de alimento, hipotálamo, C75.

3. INTRODUCTION

3.1. OBESITY AS A PUBLIC HEALTH CHALLENGE

Obesity is one of the greatest public health challenges of the 21st century. Defined as abnormal or excessive fat accumulation that presents a risk to health, obesity is globally considered an epidemic disease by the World Health Organisation (WHO). According to the WHO obesity causes at least 2.8 million deaths each year either by direct consequence or by any of its non-communicable diseases (NCDs), including cardiovascular diseases, diabetes and certain types of cancer. [1] In addition, obesity and overweight lead to adverse metabolic effects on cholesterol levels, blood pressure and insulin resistance. As our body mass index (BMI) increases so do the risks of coronary disease, ischemic stroke and type 2 diabetes. A high BMI increases risk of cancer of the breast, colon, prostate, endometrium, kidney and gall bladder. As a result, obesity also strongly affects economic and social development. Adult obesity and overweight are responsible for up to 6 % of health care expenditure in the European Region; moreover, they impose indirect costs (due to the loss of lives, productivity and related income) that are at least two times higher. [2] Once considered a problem only in high income countries, overweight and obesity are now dramatically on the rise in low- and middle-income countries, particularly in urban settings.

In 2016, 39 % of both adult men and women were overweight (BMI) ≥ 25 kg/m^2) and 11 % of men and 15 % of women were obese (BMI ≥30 kg/m^2). Thus, nearly 2 billion adults worldwide were overweight and, of these, more than 500 million were obese. Both overweight and obesity have shown a marked increase over the past 4 decades. Obesity rates have risen from around 3 % in men and 6 % in women in 1975 wherein overweight has risen over this same period from 20 % in men and 23 % in women. Its prevalence has tripled in many countries of the WHO European Region since the 1980s, and the numbers of those affected continue to rise at an alarming rate (Figure 1). [3]

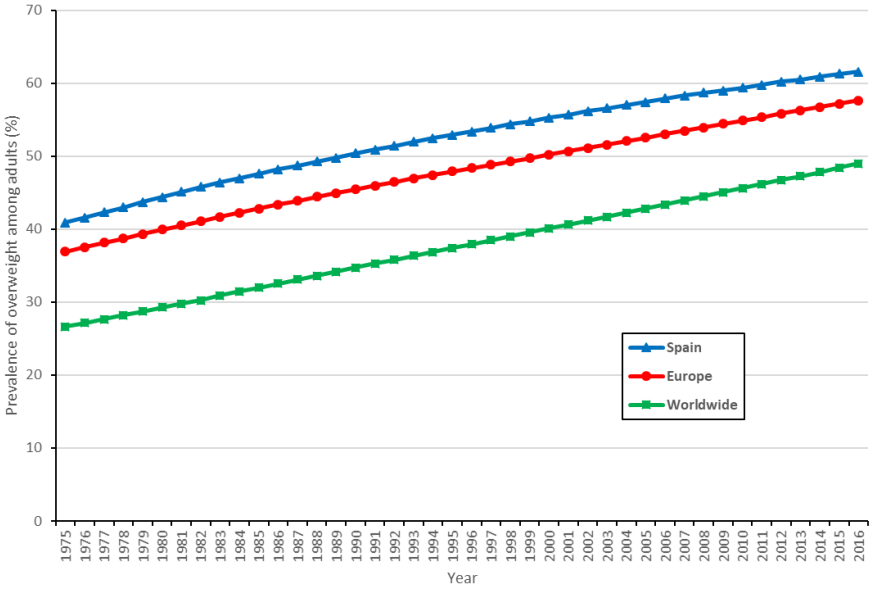


Figure 1. Graphic showing the percentage of prevalence of obesity among adults over the years in Spain, Europe and Worldwide. [2]

One may be lead to the conclusion that after all these years of increasing and desolating tendencies, governments and societies would have already found the way to solve this public health issue. However, it is not just that we have not achieve a solution, but the future seems to follow the exact same tendency, as the prevalence of overweight and obesity among children and adolescents (aged 5-19) has risen dramatically from 4 % in 1975 to just over 18 % in 2016 (18 % of girls and 19 % of boys were overweight). While just under 1 % of children and adolescents were obese in 1975, nearly 7 % were obese by 2016. [1]

Fortunately, in the past years, a drug intended to be used as a treatment for certain types of cancer has shown a side effect that can lead us to a potential application against obesity as it lowers the food intake. [4]

3.2. STARVING CANCER

C75 was one of the molecules synthesized in 1997 by Kuhadja *et al*. [5]. It was designed as an inhibitor of the fatty acid synthase (FAS) enzyme which is responsible for the biosynthesis of palmitate from malonyl-CoA and acetyl-CoA. [6]

Since tumour cells present a typical phenotype of abnormally elevated FAS activity, targeting the enzyme has been aim of many studies. It has been proved that C75 is capable of inhibit the FAS by binding to the enzyme and stopping its activity, reducing the synthesis of lipids and leading to an eventual increase of cell apoptosis. [8]

The drug was tested on cell lines and animal models, in these last, the specimens presented a reduction on their food intake which meant that the molecule was interfering somewhere else apart of the FAS. [5]

3.3. STARVING THE BRAIN

The hypothalamus is responsible for the regulation of thirst, blood pressure, body temperature and appetite. Thus, hypothalamus plays a key role on the energy homeostasis of our body and the control of food intake. Following a complex pathway, neurons sense the fatty acid (FA) concentration in blood to start a chain neuron response. Depending on the concentration of FA that response will cause hyperphagia (when FA concentration is low) or hypophagia (when FA concentration is high). [7] Particularly, it has been proved that the levels of malonyl-CoA and long chain fatty acids-CoA (LCFA-CoA) were higher before reduced food intake behaviour. [7] Hence, C75 may be interfering the FA metabolism.

3.4. FATTY ACID OXIDATION (FAO)

Fatty acid oxidation (FAO) is one of the most important cellular energy sources. It is carried out in the mitochondria of the eukaryotes to generate acetyl-CoA which participates in a large list of biochemical processes, from the citric acid cycle to the melatonin synthesis. FAO machinery involves numerous steps and processes, but probably the first step, which is the entrance of the FA to the mitochondria, might be the key one due to our capability to modulate it.

The membrane transport of the FA to the mitochondria matrix will be different depending of the size of its chain:

a) A short chain FA will react with ATP thanks to acyl-CoA synthase giving its CoA ester, now the fatty acyl-CoA is small enough to diffuse from the outer mitochondrial membrane all the way to the inner mitochondrial membrane and finally arrive to the mitochondria matrix.

b) When a LCFA arrives to the outer membrane it is first converted to its CoA ester, but the mitochondrial membrane is not permeable to LCFA (over 12C) so the acyl-CoA will be converted to acylcarnitine derivative by the enzyme carnitine palmitoyltransferase 1 (CPT1). Acylcarnitine is now a substrate for the shuttle-transporter carnitine acylcarnitine translocase (CACT), which allows the transit of acylcarnitine from cytosol to the mitochondria matrix. Once inside, the enzyme carnitine palmitoyltransferase 2 (CPT2) reconverts the acylcarnitine to acyl-CoA which is now ready to enter the FAO cycle (Figure 2). [4]

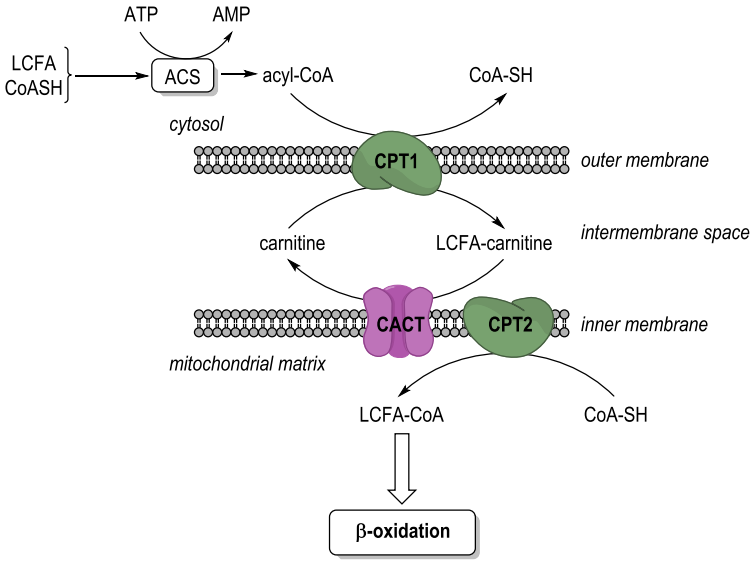


Figure 2. Long chain fatty acids are first converted to their CoA esters thanks to acetyl-CoA synthase enzyme. Enzyme CPT1 transports the LCFA-CoA to the intermembrane space by making the LCFA-carnitine. The transport is continued by CACT which takes the molecule to the inner membrane, there the CPT2 reconverts the LCFA-carnitine to LCFA-CoA which is now ready to start the β-oxidation process.

Since the mechanism of the LCFA transport into the mitochondria and the function of CPT1 was elucidated in 1983 by Fritz and Yue [11] and McGarry and Foster [12] many have been the attempts to design small molecules to modulate the function of the enzyme. Most of the modulators consist of inhibitors of the CPT1, wherein many of these molecules could be divided between oxirane carboxylic acids and derivatives and/or analogues of carnitine. Our research focuses on synthesizing derivatives of C75, placing more emphasis on its anti-obesity basal.

3.5. C75

C75 or *trans*-4-methylene-2-octyl-5-oxotetrahydrofuran-3-carboxylic acid is a trisubstituted α-methylene-γ-butyrolactone similar to the natural products family of paraconic acids. As explained above, C75 presents bioactivity since it affects both the biosynthesis of lipids (by inhibiting FAS) and the FAO (inhibiting CPT1). [4]

Previous work in the group carried out by K. Makowski showed that each enantiomer was responsible for a particular biological activity, being the (–)-C75 the inhibitor of the FAS and the (+)-C75 the CPT1 inhibitor (Figure 3). [10]

Furthermore, it was proved that the (+)-C75 was not directly inhibiting CPT1 but its ester with coenzyme A which was really exhibiting the anorexigenic properties. Nonetheless, the adduct (+)-C75-CoA is naturally formed *in vivo* and it does not require the acetyl-CoA enzyme to be formed. [4]

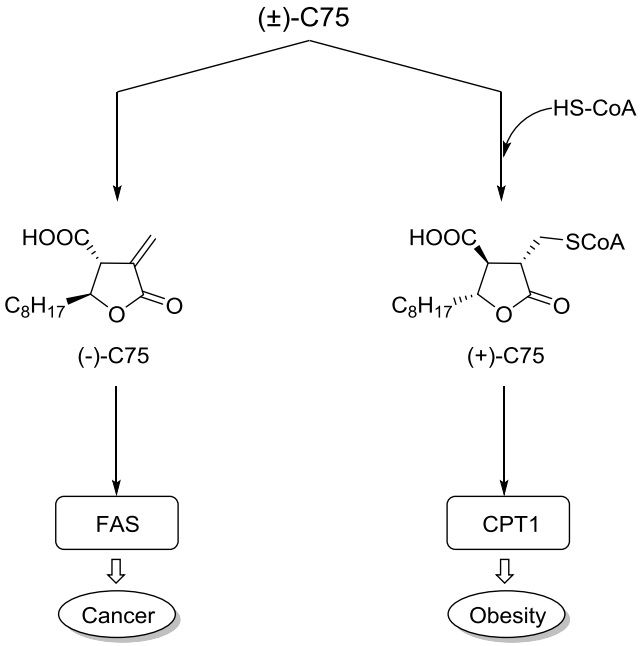
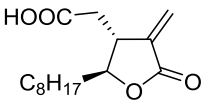
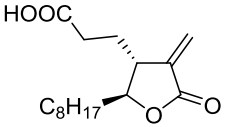


Figure 3. Scheme showing the double edged-sword behaviour of the C75 enantiomers.

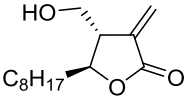
These are some examples of C75 analogues synthesized in the research group in previous years.



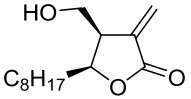
(±)-UB-207



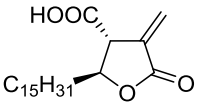
(±)-UB-244



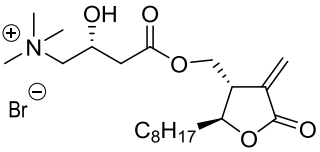
(±)-UB-006



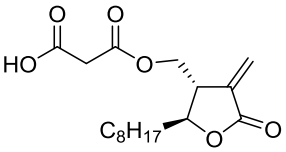
cis-(±)-UB-006



(±)-UB-183



(±)-UB-079



(±)-UB-001

Figure 4. Analogues of C75.

4. OBJECTIVES

On the basis of these precedents, in this project we focused on keep developing and studying new C75 analogues.

Two main goals were pursued, one of them being the synthesis of analogue (±)-UB-207 following a modified route that could give us access to new regioisomer intermediaries that were easier to separate, thus, eventually leading us to improve the synthesis of the target molecule.

On the other hand, by making early modifications on the synthetic route we wanted not just exploring a new way to synthesize the same analogue but to get to new derivatives of C75 for further studies.

The specific objectives of this work were:

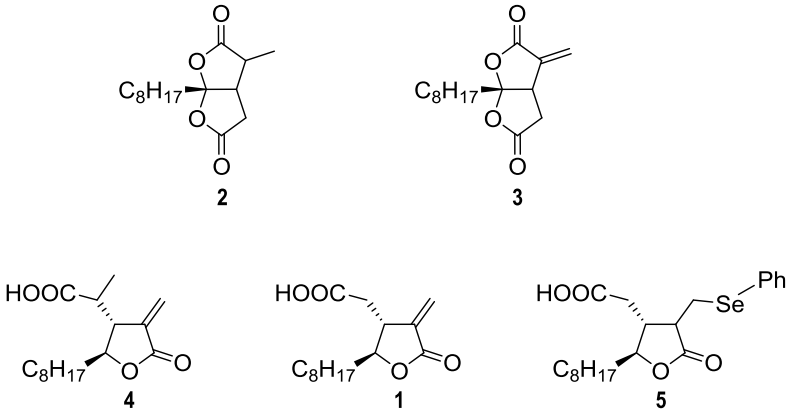
i) Synthesis of (±)-UB-207 **1**

ii) Synthesis of 3-methyl-6*a*-octyldihydrofuro[2,3-*b*]furan-2,5(3*H*,4*H*)-dione (**2**)

iii) Synthesis of 3-methylene-6*a*-octyldihydrofuro[2,3-*b*]furan-2,5(3*H*,4*H*)-dione (**3**)

iv) Synthesis of (*S*)-2-((2*S*,3*R*)-4-methylene-2-octyl-5-oxotetrahydrofuran-3-yl)propanoic acid (**4**)

v) Synthesis of 2-((2*S*,3*R*)-2-octyl-5-oxo-4-((phenylselanyl)methyl)tetrahydrofuran-3-yl)acetic acid (**5**)



2 **3** **4** **1** **5**

Figure 5. Objective molecules.

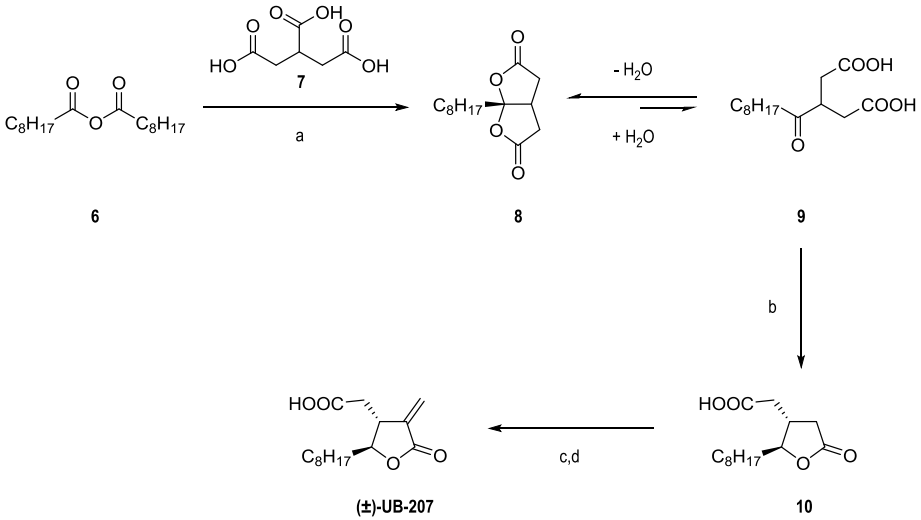
5. RESULTS AND DISCUSSION

This section has been divided in two parts. One will explain the synthesis of the enzymatic inhibitor and the other will explain the different attempts to achieve new intermediates.

5.1. SYNTHESIS OF (±)-UB-207

5.1.1. Background

The target molecule was first synthetized by K. Makowski in 2012 during his PhD thesis. [10] In his thesis, Makowski followed the procedure reported by Parker [13] in 1973 to form the γ-butyrolactone.

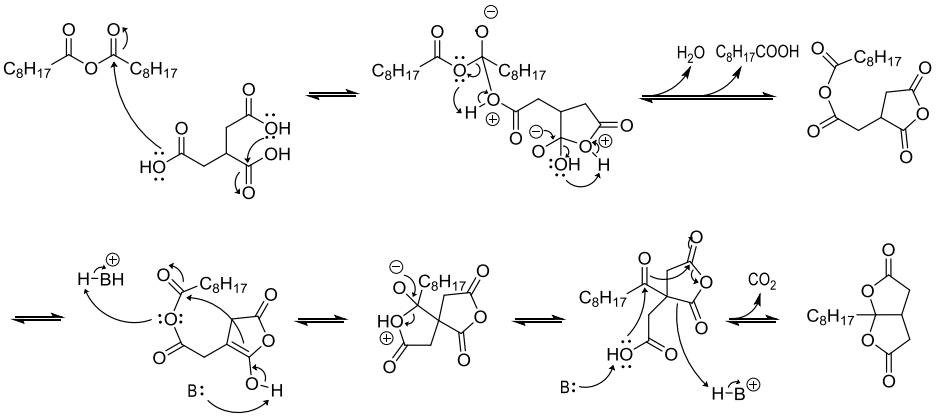


Scheme 1. a) N_2-atm,185 ºC, DMAP (cat), then **7** in three portions (1 h between each portion), 5 h at 185 ºC, 77%; b) KOH, 85 ºC, then $NaBH_4$ at 85 ºC for 5 h, r.t. and HCl to pH 1, 97%; c) N_2-atm, LiHMDS, -78 ºC then **10** dissolved in THF (anh), 1 h; r.t. and $CF_3CO_2CH_2CF_3$ for 1 h; d) work-up and K_2CO_3, 18-*crown*-6, paraformaldehyde, 80 ºC, 2 h, 100 ºC, 4 h, work-up.

In this case, the α-methylenation was carried out following the procedure reported by Colby [14] in 2011 which was first tested by Rosa Diego in a similar molecular model during her MSc final project, leading Oscar Benito to finally apply the method in the UB-207 synthesis during his MSc final project.

5.1.2. Synthesis of 6*a*-octyldihydrofuro[2,3-*b*]furan-2,5(3*H*,4*H*)-dione

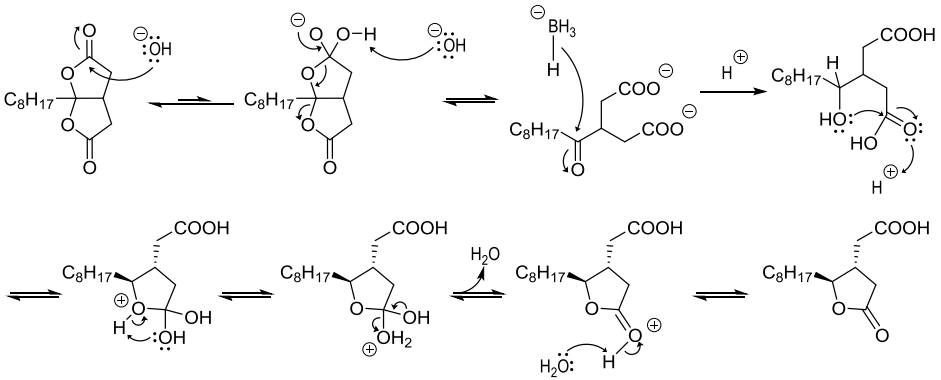
The bis-lactone preparation consists of an acylative decarboxylation of tricarballylic acid by an acid anhydride. Although the reaction was studied by Lawson [15] we followed the procedure described by Parker. [13] On average the yield obtained by the group until now was 44 % but thanks to the addition of DMAP, which acts as a catalyst, the yield has been almost doubled to reach 77 %, the increasing of the first step yield means an important improvement to the synthesis. As reported before in the group, no further purification was needed for the product. The purity of bis-lactone **8** was checked by ^{1}H-NMR and it could be directly used in next steps of the synthesis.



Scheme 2. Proposed mechanism for the bis-lactone formation.

5.1.3. Ketone reduction and lactonization

Next step of the synthesis is also based on the procedure reported by Parker [13] which consists in the opening of the bis-lactone **8** in basic media, followed by the reduction of the ketone and finally a lactonization in acidic media. Only one diastereoisomer was formed thermodynamically as is less strained than the other one. The yield obtained (97 %) was similar as reported by the literature and the reaction crude needed no purification for further reactions, as shown in the ^{1}H-NMR spectra.



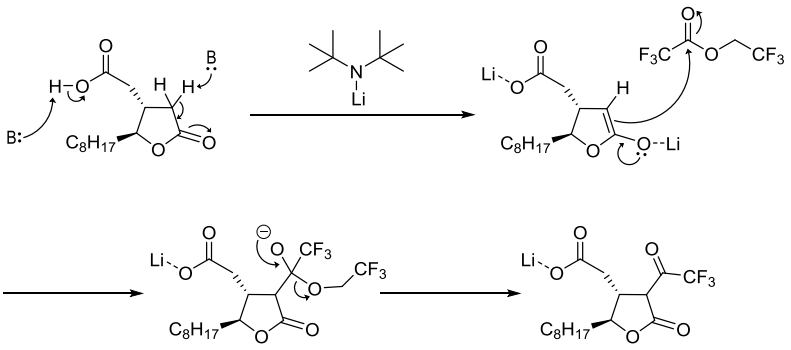
Scheme 3. Proposed mechanism for the reduction and further lactonization of the bis-lactone.

5.1.4. α-Methylenation

It is required to introduce an exocyclic methylene in the α position of the carbonyl group to finally obtain UB-207. In order to do that, we followed the procedure reported by Colby [14] which uses the easy elimination of a trifluoroacetate group during the formation of the olefin.

5.1.4.1. Trifluoroacetylation

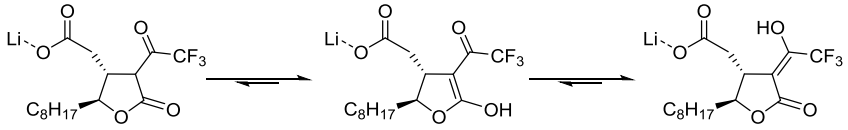
The first step of the methylenation consists in the incorporation of a trifluoroacetyl group, it was carried out following the Danheiser's protocol using LiHMDS and $CF_3CO_2CH_2CF_3$. [16]



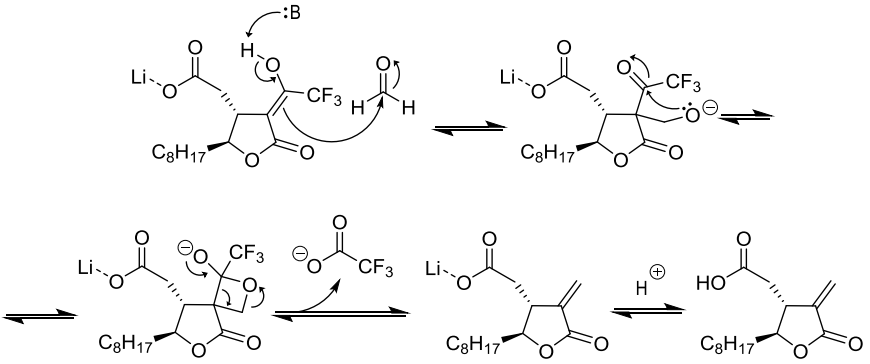
Scheme 4. Proposed mechanism for the trifluoroacetylation.

5.1.4.2. Trifluoroacetyl group elimination

Next, the release of trifluoroacetyl was carried out using a milder base such as K_2CO_3 which promoted an easier formaldehyde addition due to the enhanced acidity of the enolic proton.

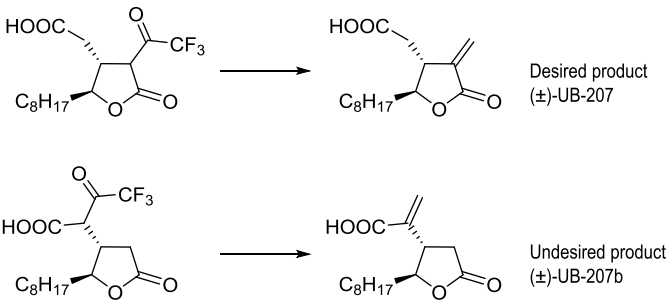


Scheme 5. System equilibrium favouring the enol tautomer.



Scheme 6. Proposed mechanism for the trifluoroacetylate release and olefin formation.

The main issue with this method is the formation of an undesired regioisomer due to the carbonyl group of the carboxylic acid. Furthermore, it was studied before by O. Benito that modifying the experimental conditions could hardly enhance the original ratio of the desired/undesired product of 70:30.



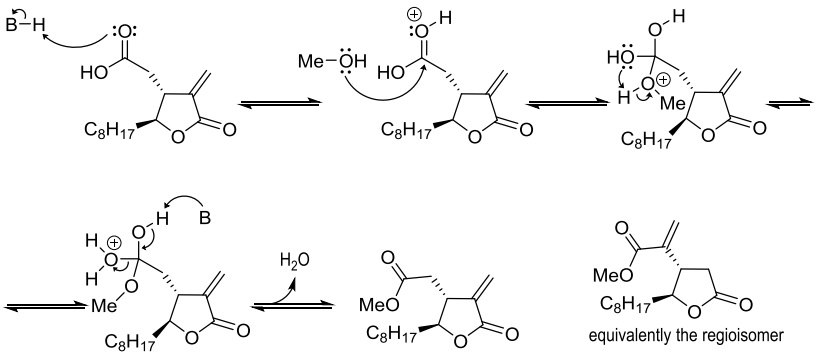
Scheme 7. Appearance of regioisomers after the trifluoacetylation.

Since the separation of the two carboxylic acids by column chromatography was not possible, two different derivatives where synthesized in order to enhance the purification of the desire product.

5.1.5. Synthesis of the methyl esters

First, we were decide to mask the acid group as it was probably the reason of the poor separation between regioisomers. A simple esterification was done using TMSCl and MeOH [17] giving the methyl ester and not needing further purification.

However, the separation was not fully completed. Besides, the deprotection was not complete and the lactone seemed to be opening under the conditions used, generating more isomers and making it harder to separate. Probably an interesting test to run should be the esterification using a different kind of ester which could be released under orthogonal conditions with the lactone.

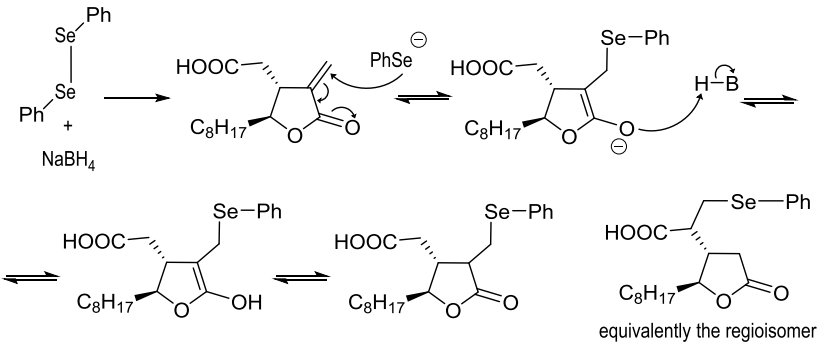


Scheme 8. Proposed mechanism for the methyl ester formation.

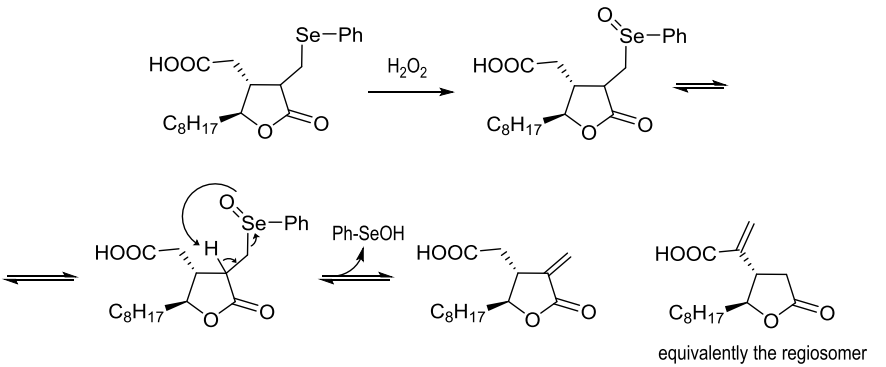
5.1.6. Synthesis of the selenide ethers

Alternatively, we synthesized selenide ethers to protect the olefins. Makowski had tried this method mainly to protect the double bond from reactions conditions and to profit its stability over the olefin for a better storage. [10] Using a diphenyl selenide and a hydrogen source the reaction was conducted to a complete conversion. Then, after oxidation with H_2O_2 and thanks to the low stability of the selenoxide the elimination can be easily achieved if there is a hydrogen in β-position, [10] thus, recovering the olefin. It is worth saying that the ether oxidation occurred in mere contact with the atmosphere.

The regioisomers could be separated by column chromatography (80:20 CH_2Cl_2:hexanes).



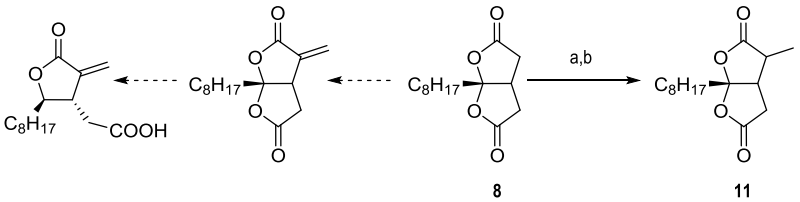
Scheme 9. Proposed mechanism for the selenide ether formation.



Scheme 10. Proposed mechanism for the selenide ether oxidation.

5.2. SYNTHESIS OF 3-METHYL-6*A*-OCTYLDIHYDROFURO[2,3-*B*]FURAN-2,5(3*H*,4*H*)-DIONE

An interesting derivative to synthesize previous to the reduction and lactonization of the bis-lactone was the α-methylated product. In case the methylation was achieved a hypothetic α-methylenation directly on the bis-lactone could be tested, thus, avoiding the appearance of regioisomers, reducing the efforts on separating the desire product.



8 11

Scheme 11. a) A mix of 8 and a base, under N_2 atm, - 78 ºC for 2h; b) Heat to r.t., add MeI, stir for 2h.

A series of experiments were run modulating the conditions to test the reactivity of the bis-lactone in front of a enolization.

Base	Mole equivalents	Temperature [ºC]	Time [h]
LiHMDS	1	- 78	2
LiHMDS	2	- 78	2
LDA	1	- 78	2
LDA	2	- 78	2
^{t}BuLi	1	- 78	2
^{t}BuLi	2	- 78	2
^{t}BuLi	2	- 78	24
NaH	1	60	2
NaH	1	60	15

Table 1. The mole equivalents are referring to the mole number in respect of the bis-lactone, the quantity of bis-lactone in each case could be different. Time shown in the table is the reaction time between the base and the bis-lactone, in all the cases the reaction time with de MeI was 2 hours. All the reactions were carried out using a CO_2-acetone bath and were isolated from the atmosphere cannulating the solutions.

Unluckily, no methylation attempt conducted to the desired product. Due to the lack of literature exploring enolization on similar models to our bis-lactone no further test was run. With the few data collected one may be led to the conclusion that somehow the bis-lactone system was favouring a nucleophilic attack from the base to the carbonyl instead of the enolization. Simulating the potential surface of the molecule could be interesting to check if there's really an increase of the electrophilic behaviour in comparison to the γ-butyrolactone.

6. EXPERIMENTAL SECTION

6.1. MATERIALS AND METHODS

All reactions were done under atmospheric pressure and the non-aqueous ones also under a nitrogen atmosphere, with magnetic stirring.

6.1.1. Reagents and solvents

All reagents were commercially available and used without further purification. Tetrahydrofuran (THF) was distilled from sodium and benzophenone immediately prior to use while other solvents used were dried following the conventional techniques.

6.1.2. Nuclear magnetic resonance spectroscopy (NMR)

The NMR spectra were recorded at 25 ºC on a Varian Mercury 400 MHz, using $CDCl_3$ as solvent containing tetramethyilsilane (TMS) as internal standard. Coupling constants (J) are given in Hz, chemical shifts in parts per million (ppm) and the signals multiplicities are indicated with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), ddd (doublet of doublets of doublets), m (multiplet).

6.1.3. Chromatographic techniques

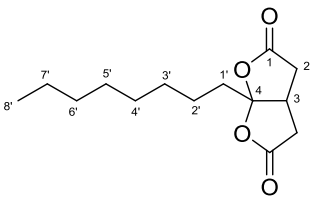
Analytical TLC was performed on aluminium plates pre-coated with silica gel, 0.20 nm thick (F_{254} Merck), solvents used are indicated in each case. TLC were visualised directly under UV lamp (254 nm and 365 nm) or dipping the TLC plate into stain solutions of phosphomolybdic acid or potassium permanganate.

Column chromatography was carried out using a middle pressure technique (flash), with silica gel Chromatogel 60 Å (35-70 μm), and the solvents used are indicated in each case.

6.2. SYNTHETIC PROCEDURES

6.2.1. Synthesis of 6*a*-octyldihydrofuro[2,3-*b*]furan-2,5(3*H*,4*H*)-dione (8)

In a round bottom flask, nonanoic anhydride (10 mL, 0.028 mol) with DMAP (0.12 g, 0.98 mmol) were heated to 185 ºC, then tricarballylic acid (1.83 g, 0.010 mol) was added in three portions at 1 hour intervals. The mixture was heated at 185 ºC for further 5 hours. After cooling down to room temperature, 30 mL of hexane at 0 ºC were added to the brown solid obtained and the resulting suspension was filtered and the solid was washed with hexanes at 0 ºC. Then the solvent was removed to afford 2.05 g (77 % yield) of **8** as a colourless solid.

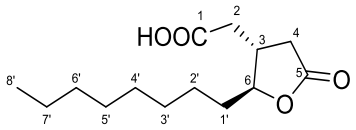


Product **8**

Colourless solid. **Rf** = 0.43 (hexanes/EtOAc, 6:4). m.p. = 201–202 ºC **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 3.16 – 3.06 (m, 1H, H-3), 2.99 (dd, J = 18.3, 9.7 Hz, 1H, H-2a), 2.54 (dd, J = 18.4, 4.3 Hz, 1H, H-2b), 2.05 – 1.91 (m, 1H, CH_2-1'), 1.52 – 1.41 (m, 2H, CH_2-2'), 1.40 – 1.27 (m, 10H, CH_2-3' CH_2-4' CH_2-5' CH_2-6' CH_2-7'), 0.90 (t, J = 13.6 Hz, 3H, CH_3-8'). **^{13}C NMR** (CD_3OD, 101 MHz): δ = 175.5 (C, C-1), 116.8 (C, C-4), 38.5 (CH, C-3), 38.1 (CH_2, C-1'), 36.2 (CH_2, C-2), 33.0 (CH_2, C-7'), 30.5 (CH_2,C-3'), 30.4 (CH_2, C-4'), 30.3 (CH_2, C-5'), 23.9 (CH_2, C-2'), 23.7 (CH_2, C-6'), 14.4 (CH_3, C-8')

6.2.2. Synthesis of (±)-2-((2*S*,3*R*)-2-octyl-5-oxotetrahydrofuran-3-yl)acetic acid (10)

The dilactone **8** (0.97 g, 3.8 mmol) was dissolved in 15 mL of a 1M KOH solution and then heated to 65 ºC. Once the dilactone is totally dissolved, sodium borohydride (0.37 mg, 9.5 mmol) was added over 10 minutes while stirring, the mixture was then heated to 85 ºC for 5 hours. After cooling down to room temperature it was acidified with concentrated hydrochloric acid to pH 1, then the solution was extracted with CH_2Cl_2 (3 x 10 mL). The collected organic phases were dried over anhydrous $MgSO_4$, and the solvent was removed, hexanes (10 mL) were added to the oil and stirred for another 10 minutes. The solvent was again removed giving 0.96 g (97% yield) of **10** as a colourless solid.



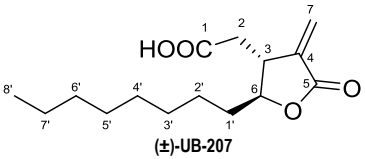
Product **10**

Colourless solid. **Rf** = 0.25 (hexanes/EtOAc/AcOH 8:2:0.1). mp = 57-58.5 ºC. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.18 (ddd, J = 7.9, 6.1, 4.5 Hz, 1H, H-6), 2.85 (dd, J = 17.7, 8.3 Hz, 1H, H-2a), 2.70 – 2.55 (m, 2H, H-3 H-4a), 2.46 (dd, J = 15.3, 7.5 Hz, 1H, H-4b), 2.32 (dd, J = 17.6, 7.4 Hz, 1H, H-2b), 1.74 – 1.62 (m, 2H, CH_2-1'), 1.57 – 1.37 (m, 2H, CH_2-2'), 1.35 – 1.22 (m, 10H, CH_2-3' CH_2-4' CH_2-5' CH_2-6' CH_2-7'), 0.87 (t, J = 13.6 Hz, 3H, CH_3-8'). **^{13}C NMR** ($CDCl_3$, 101 MHz): δ = 176.8 (C, C-1), 176.1 (C, C-5), 85.0 (CH, C-6), 37.1 (CH_2, C-4), 37.0 (CH, C-3), 34.9 (CH_2, C-2), 34.5 (CH_2,C-1'), 31.9 (CH_2, C-7'), 29.5 (CH_2, C-3'), 29.4 (CH_2, C-4'), 29.3 (CH_2, C-5'), 25.6 (CH_2, C-2'), 22.8 (CH_2, C-6'), 14.2 (CH_3, C-8')

6.2.3. Synthesis of (±)-UB-207 and (±)-UB-207b (±)-2-((2*S*,3*R*)-2-octyl-5-oxotetrahydrofuran-3-yl)acetic acid, (±)-2-((2*S*,3*S*)-2-octyl-5-oxotetrahydrofuran-3-yl)acrylic acid

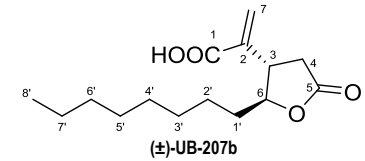
The lactone **10** (0.2 g, 0.78 mmol) was dissolved in THF anhydrous (2 mL) then cooled at 0 ºC and added to a pear-shaped flask containing LiHMDS (3.7 mL, 3.7 mmol) cooled at -78 ºC. The reaction mixture was stirred for 1 hour and allowed to warm to rt over 20 minutes. 2,2,2-trifluoroethyl trifluoroacetate (0.24 mL, 1.76 mmol) was added to the flask and stirred for 1 hour. Saturated solution of NH_4Cl (20 mL) was added and the mixture was stirred for 20 minutes, then concentrated HCl was added until pH 1. The solution was extracted with EtOAc (3 x 15 mL), dried over $MgSO_4$ and the solvent was removed with the rotatory evaporator giving a reddish oil (418 mg).

Without further purification the reddish oil was solved in toluene (20 mL), the solution was treated with K_2CO_3 (324 mg, 2.3 mmol), 18-*crown*-6 (52 mg, 0.2 mmol) and paraformaldehyde (0.98 mg, 0.03 mmol). After adding the reagents, the suspension was heated at 80 ºC for 2 hours followed by 4 hours at 100 ºC. After cooling to down rt, saturated NH_4Cl was added and the mixture was extracted with EtOAc (3 x 15 mL), combined organic phases were washed with brine and dried over $MgSO_4$ and the solvent was removed under reduced pressure giving a brown oil (236 mg) that was the mixture of two regioisomers, (±)-UB-207 and (±)-UB-207b



(±)-UB-207

Rf = 0.35 (DCM/MeOH 97:3). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.32 (d, J = 2.5 Hz, 1H, H-7a), 5.70 (d, J = 2.5 Hz, 1H, H-7b), 4.26 (dt, J = 8.5, 4.6 Hz, 1H, H-6), 3.15 – 3.08 (m, 1H, H-3), 2.65 – 2.63 (m, 2H, CH_2-2), 1.77 – 1.60 (m, 2H, CH_2-1'). 1.54 – 1.34 (m, 2H, CH_2-2'), 1.33 – 1.20 (m, 10H, CH_2-3' CH_2-4' CH_2-5' CH_2-6' CH_2-7'), 0.88 (t, J = 6.9 Hz, 3H, CH_3-8'). **^{13}C NMR** ($CDCl_3$, 101 MHz): δ = 176.2 (C, C-1), 169.9 (C, C-5), 138.1 (C, C-4), 123.5 (CH_2, C-7), 83.1 (CH, C-6), 40.6 (CH, C-3), 38.2 (CH_2, C-2), 35.8 (CH_2, C-1'), 31.9 (CH_2, C-7'), 29.5 (CH_2, C-3'), 29.4 (CH_2, C-4'), 29.3 (CH_2, C-5'), 25.0 (CH_2, C-2'), 22.7 (CH_3, C-6'), 14.2 (CH_3, C-8').

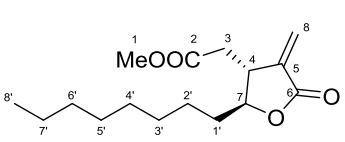


(±)-UB-207b

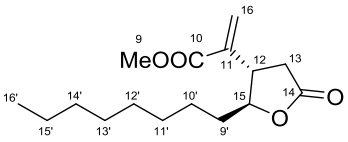
Rf = 0.35 (DCM/MeOH 97:3). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.49 (s, 1H, H-7a), 5.84 (s, 1H, H-7b), 4.46 (dt, J = 6.5, 6.5 Hz, 1H, H-6), 3.19 (ddd, J = 9.2, 7.1, 6.5 Hz, 1H, H-3), 2.83 (dd, J = 17.7, 9.2 Hz, 1H, H-4a), 2.66 (dd, J = 17.7, 7.1 Hz, 1H, H-4b), 1.77 – 1.60 (m, 2H, CH_2-1'), 1.54 – 1.34 (m, 2H, CH_2-2'), 1.33 – 1.20 (m, 10H, CH_2-3' CH_2-4' CH_2-5' CH_2-6' CH_2-7'), 0.88 (t, J = 6.9 Hz, 3H,CH_3-8'). **^{13}C NMR** ($CDCl_3$, 101 MHz): δ = 176.2 (C, C-1), 170.6 (C, C-5), 138.2 (C, C-2), 129.3 (CH_2, C-7), 84.8 (CH, C-6), 43.2 (CH, C-3), 38.9 (CH_2, C-4), 38.8 (CH_2, C-1'), 31.9 (CH_2, C-7'), 29.5 (CH_2, C-3'), 29.4 (CH_2, C-4'), 29.3 (CH_2, C-5'), 25.6 (CH_2, C-2'), 22.7 (CH_3, C-6'), 14.2 (CH_3, C-8').

6.2.4. Synthesis of (±)-methyl 2-((2*S*,3*R*)-4-methylene-2-octyl-5-oxotetrahydrofuran-3-yl)acetate (12) and (±)-methyl 2-((2*S*,3*S*)-2-octyl-5-oxotetrahydrofuran-3-yl)acrylate (13)

A part of the mixture of regioisomers **UB-207** and **UB-207b** (71 mg, 0.26 mmol) previously obtained was dissolved in MeOH (4 mL) and TMSCl (70 µL, 0.53 mmol) was added. The solution was stirred for 48 hours at room temperature. The reaction was followed by TLC, when the starting material was consumed, the solvent was removed under reduced pressure giving a brown oil (69 mg) of **12** and **13**.



Product **12**

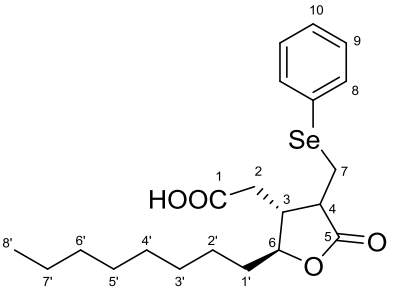


Product **13**

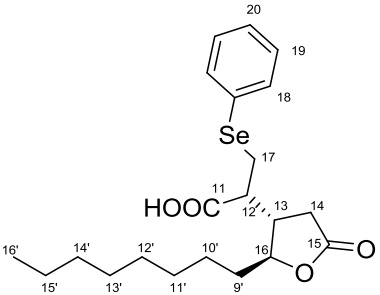
Mixture of **12** & **13**:**Rf** = 0.65 (DCM/MeOH) **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.34 (s, 0.3H, H-16a), 6.30 (s, 0.7H, H-8a), 5.71 (s, 0.3H, H-16b), 5.65 (s, 0.7H, H-8b), 4.44 (dt, J = 6.5, 6.5 Hz, 0.3H, H-15), 4.21 (dt, J = 6.5, 6.5 Hz, 0.7H, H-7), 3.80 (s, 0.9H, H-9), 3.71 (s, 2.1H, H-1), 3.19 (ddd, J = 9.2, 7.1, 6.5 Hz, 0.7H, H-12), 3.15-3.08 (m, 0.7H, H-4), 2.83 (dd, J = 17.7, 9.2 Hz, 0.3H, H-13a), 2.66 (dd, J = 17.7, 7.1 Hz, 0.3H, H-13b), 2.60 – 2.58 (m, 1.4H, CH_2-3), 1.77 – 1.60 (m, 2H, CH_2-1' CH_2-9'), 1.54 – 1.34 (m, 2H, CH_2-2' CH_2-10'), 1.33 – 1.20 (m, 10H, CH_2-3' CH_2-4' CH_2-5' CH_2-6' CH_2-7' CH_2-11' CH_2-12' CH_2-13' CH_2-14' CH_2-15'), 0.88 (t, J = 6.9 Hz, 3H, CH_3-8' CH_3-16').

6.2.5. Synthesis of (±)-2-((2*S*,3*R*)-2-octyl-5-oxo-4-((phenylselanyl)methyl)tetrahydrofuran-3-yl)acetic acid (5) and (±)-(*S*)-2-((2*S*,3*S*)-2-octyl-5-oxotetrahydrofuran-3-yl)-3-(phenylselanyl)propanoic acid (14)

A solution of the two regioisomers **(±)-UB-207** and **(±)-UB-207b** (165 mg, 0.61 mmols).in anhydrous EtOH (2 mL) was prepared under N_2 atmosphere and room temperature. Another solution of Ph_2Se_2 (118 mg, 0.61 mmol) and $NaBH_4$ (18 mg, 0.38 mmol) in anhydrous EtOH (2 mL) under N_2 and room temperature was added to the first solution. The mixture was stirred for 3.5 hours at room temperature. Then the reaction was stopped by adding HCl (2 N) until reaching pH = 1. The solvent was removed by rotatory evaporation. The oil obtained was diluted in H_2O (5 mL) and extractions were performed with DCM (3 x 8 mL). The combined organic phases were washed with NH_4Cl 20% (2 x 5mL), saturated NaCl (2 x 5 mL) and H_2O (5 mL). Finally, the organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure giving a brown oil which contained the mixture of both regioisomers which was purified by column chromatography (Hexanes/DCM 80:20) giving a yellow oil (20 mg).



Product **5**



Product **14**

Mixture of **5** & **14**: **Rf** = 0.35 (DCM/MeOH) **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.60 (m, 1.5H, H-18 H-19 H-20) 7.54 (m, 3.5H, H-8 H-9 H-10), 4.44 (dt, J = 6.5, 6.5 Hz, 0.3H, H-16), 4.21 (dt, J = 6.5, 6.5 Hz, 0.7H, H-6), 3.19 (ddd, J = 9.2, 7.1, 6.5 Hz, 0.7H, H-13), 3.15-3.08 (m, 0.7H, H-3), 2.83 (dd, J = 17.7, 9.2 Hz, 0.3H, H-14a), 2.66 (dd, J = 17.7, 7.1 Hz, 0.3H, H-14b), 2.60 – 2.58 (m, 1.4H, CH_2-2), 1.77 – 1.60 (m, 2H, CH_2-1' CH_2-9'), 1.54 – 1.34 (m, 2H, CH_2-2' CH_2-10'), 1.33 – 1.20 (m, 10H, CH_2-3' CH_2-4' CH_2-5' CH_2-6' CH_2-7' CH_2-11' CH_2-12' CH_2-13' CH_2-14' CH_2-15'), 0.88 (t, J = 6.9 Hz, 3H, CH_3-8' CH_3-16').

6.2.6. Deprotection of (±)-2-((2*S*,3*R*)-2-octyl-5-oxo-4-((phenylselanyl)methyl)tetrahydrofuran-3-yl)acetic acid

The selenide ether **5** (20 mg, 0.047 mmol) was dissolved in THF anhydrous (0.4 mL) then H_2O_2 35% (20 µL) were added, the reaction was stirring at room temperature for 3.5 hours. Then, CH_2Cl_2 (5 mL) and saturated $NaHCO_3$ (2 mL) were added and the mixture was stirred for 5 minutes. After removing the organic phase, the aqueous phase was washed with CH_2Cl_2 (3 x 3 mL) and then acidified to pH = 1 with concentrated HCl. Then the extractions were done with CH_2Cl_2 (4 x 3 mL) and the combined organic phase was washed with saturated NaCl solution and dried over $MgSO_4$. The solvent was removed under reduced pressure giving the product (±)-UB-207 (10 mg).

(±)-UB-207 characterisation data as described previously.

7. CONCLUSIONS

Although the synthetic route to (±)-UB-207 was stablished by K. Makowski in his PhD thesis, in this project we have achieved to separate the regioisomers obtained after the α-methylenation. We could get rid of the undesired regiosiomer covering the methylene via a selenium ether, the ether formation was as easy as its latter deprotection which encourages us to keep studying the process to enhance its yield.

On the other hand, it has been proved that the addition of DMAP as a catalyst on the bis-lactone formation increased the yield remarkably (almost doubled it) without the need of purifying the reaction crude, thus, optimizing the synthetic route.

Finally, after many attempts to methylate the bis-lactone (**8**) with different bases and conditions the methylation could not be achieved. In the absence of carrying out the appropriate tests, the bis-lactone seemed to be reacting as an electrophile, favouring the attack of the base to the carbonyl.

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12. ACRONYMS

ACS: acetyl-CoA synthetase

ATP: adenosine triphosphate

CACT: carnitine acylcarnitine translocase

CPT 1: carnitine palmitoyltransferase 1

CPT 2: carnitine palmitoyltransferase 2

DCM: dichloromethane

DMAP: 4-dimethylaminopyridine

EtOAc: ethyl acetate

EtOH: ethanol

FA: fatty acid

FAO: fatty acid oxidation

FAS: fatty acid synthase

LCFA: long chain fatty acid

LDA: lithium diisopropylamide

LiHMDS: lithium bis(trimethylsilyl)amide

MeOH: methanol

NMR: nuclear magnetic resonance

Rf: retention factor

^{t}BuLi: tert-butyllithium

THF: tetrahydrofuran

TLC: thin layer chromatography

TMSCl: trimethylsilyl chloride

TMS: tetramethylsilane

Mp: melting point