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CARBOXYXANTHONE DERIVATIVES: SYNTHESIS AND STRUCTURE ELUCIDATION

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ABSTRACT

Xanthone derivatives (XDs) represent a broad group of compounds obtained from natural sources as well as by synthetic procedures. Total synthesis is a crucial strategy allowing the access to structures that otherwise could not be reached within the natural products; through this way many compounds can be obtained and used as useful chemical substrates for molecular modification. Among the great diversity of XDs, carboxyxanthones (XCar) stand out taking in account that carboxylic group is associated with interesting biological activities and also because chemically allows easily the acquisition of diverse derivatives.

In this study, the total synthesis of two isomeric XCars by a multi-step pathway was planned. The synthesis involved several chemical reactions including Fisher esterifications, Ullmann aryl ether synthesis, alkaline hydrolysis and intramolecular acylation. The structure elucidation of XCars, as well as all the intermediates, was established by spectroscopic methods (^{1}H NMR, ^{13}C NMR, and IR).

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1. **Introduction**

1.1 Xanthone derivatives: chemistry and importance in Medicinal Chemistry

Xanthones (9*H*-xanthen-9-ones) are oxygenated heterocyclic compounds with a dibenzo-γ-pyrone frame work, whose scaffold can be seen in **Figure 1** [1].

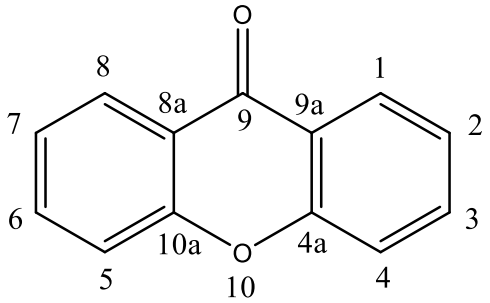


Figure 1: Xanthone scaffold and numbering.

Xanthone derivatives (XDs) are a class of natural compounds that can be found in higher plants, fungi and lichens as well as from marine sources [6, 7]. Natural xanthones can have different substituents in its dibenzo-γ-pyrone scaffold and, based on this characteristic, these compounds can be subdivided into: simple oxygenated xanthones, glycosylated xanthones, prenylated xanthones and their derivatives, xanthone dimers, xanthonolignoids and miscellaneous [8]. Natural products have always been used as a source of inspiration for drug discovery and xanthones are no exception since they can interact with several different targets being an interesting starting point for the discovery of new potential drug candidates. They are known, among other properties, for their physiological and pharmacological activities, such as antimicrobial, anti-inflammatory, antioxidant and antitumor [15]. The bioactivities can change, depending on the type and position of the different substituents. In addition, most of these compounds exhibit fluorescence, which can be an advantage since they can be used as fluorescence probes [9, 10].

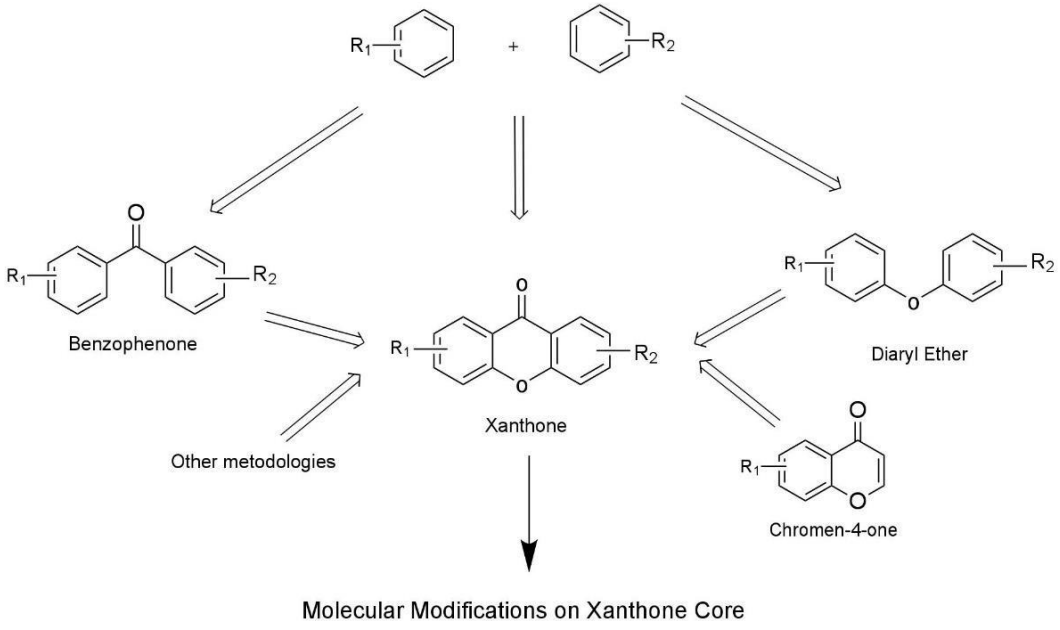
The total synthesis of XDs can be an added value to the pharmaceutical sector, allowing the access to structures that otherwise could not be reached if it was only used the natural product as a launching platform for the molecular modification. In addition, the natural product field

usually does not allow the acquisition of a great quantity of product and the synthetic methods can be a convenient way to fix this problem [11]. In the case of chiral derivatives of xanthones (CDXs), synthetic procedures are also an advantage since they may allow the preparation of both enantiomers to explore the enantioselectivity in biological screening assays [12].

Synthetic XDs can have simple groups on the xanthone scaffold, such as hydroxyl, methoxyl, methyl, carboxyl, as well as more complex substituents such as epoxide, azole, methylidenebutyrolactone, amino alcohol, sulfamoyl, methylthiocarboxylicacid, and dihydropyridine [13].

1.2 Different approaches to synthesize xanthone derivatives

In **Scheme 1**, a representation of different classic approaches that can be used to obtain XDs are presented.



Scheme 1: Different approaches to obtain xanthone derivatives.

Concerning to the classical methods, XDs can be obtained through three different routes: via benzophenone intermediate, via diaryl ether intermediate, and via Grover, Shah, and Shah (GSS) method. The synthesis of XDs starting from chromen-4-one is a recent approach [1, 11].

1.2.1. Grover, Shah and Shah (GSS) method

The GSS method consists in heating a salicylic acid derivative together with a suitable phenol. The reaction must be catalyzed by strong acids like zinc chloride and phosphoryl chloride that can be also used as solvent. This method allows the acquisition of xanthones in only one step as long as the benzophenone intermediate has at least three hydroxyl groups at 2, 2', 6 or 6' positions. Otherwise, a benzophenone will be obtained instead of the xanthone and it will be necessary to proceed an additional cyclization reaction [11].

1.2.2. Synthesis *via* benzophenone intermediate

The benzophenone route allows the acquisition of a high yield of XD and consists in a multi-step approach that can take place through different methodologies. The most common is the Friedel-Crafts acylation between a substituted benzoyl chloride and a phenolic derivative, usually catalyzed by aluminum, followed by a cyclization step. It take place through an electrophilic aromatic substituition between an acyl chloride and a phenol or protected phenol derivative, usually catalized by aluminium, and subsequent cyclization. This reaction lacks of regioselectivity since the acylation can happen both on *ortho* and *para* positions. Moreover, some functional groups are sensitive to the strong acid conditions [1,11].

1.2.3. Synthesis *via* diaryl ether intermediate

One of the most common approaches is the diaryl ether intermediate route, which involves a reaction between an aryl halide and a phenol. Most of the examples described in literature use copper catalyzed Ullmann-ether synthesis for the formation of an ether bond. This approach has been reported with significantly low yields and harsh experimental conditions due to the high temperatures and the long reaction times needed. However, this method is more selective when compared to others, for example the Friedel-Crafts acylation. The bond formed between a phenol and an aryl halide through this method occurs only at a certain position of the aromatic ring, which may allow the acquisition of just one product. Thus, Ullmann-ether coupling has seen significant improvements, allowing the acquirement of diaryl ethers in high yields using milder experimental conditions [11].

1.3. Carboxylated xanthone derivatives (XCars)

XDs include a broad range of different compounds comprising a diversity of substituents. However, xanthones containing a carboxylic group - carboxylated xanthone derivatives (XCars) - have shown great significance in medicinal chemistry since they are an important chemical substrate for molecular modifications allowing to obtain more diverse and complex compounds that can be useful for biological activity and structure-activity relationship (SAR) studies [12,13].

For example, XCars possess a suitable functional group that enables the coupling with enantiomerically pure units such as amino alcohols, amino acids, amino esters and amines to obtain chiral derivatives of xanthones (CDXs). These chiral compounds can also be very useful for the development of chiral stationary phases (CSPs) for liquid chromatography (LC) [21-24].

Moreover, XCars are described as promising for the preparation of fluorescence probes [25, 26] since most of these compounds exhibit fluorescence.

Concerning to the biological activities shown by these compounds, there are many examples referred in literature such as antitumor [15], antibacterial [16], antiallergic [17, 18], anti- inflammatory [28, 29], diuretic and uricosuric [27], inhibitory activity against α- glucosidase [19], phospholipase C [20] and aldolase reductase [30], among others. These compounds have also shown effective results as antagonists of leukotriene B4 receptor [31-33].

The next subchapters will be focused on two XCars that have proven to have a very promising biological activity: DMXAA [12, 13, 34] and 9-oxo-9*H*-xanthene-2-carboxylic acid and analogues [13].

1.3.1 DMXAA (5,6-dimethyl-9-oxo-9*H*-xanthene-4-acetic acid)

5,6-Dimethyl-9-oxo-9*H*-xanthene-4-acetic acid (DMXAA, Vadimezan) (**Figure 2**) is a remarkable example of the great potential of XCars from a pharmacological point of view [13]. This compound was discovered in 1991 in a SAR study that used diverse xanthenone-4- acetic acids analogues of a flavone acetic acid drug [35]. In the following years,

DMXAA synthesis was improved and optimized by different techniques allowing the acquisition of higher yields and short reaction times [36, 37].

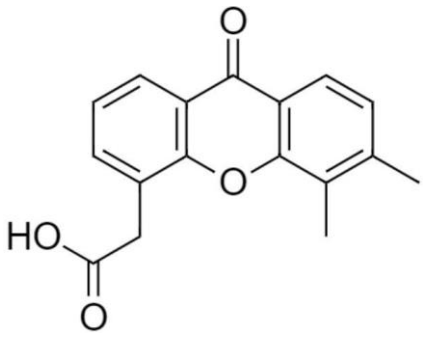


Figure 2: Structure of 5,6-dimethyl-9-oxo-9*H*-xanthene-4-acetic acid.

This compound has several biological activities already described in literature such as antiviral [38], antiplatelet and antithrombotic activity [39]. However, the most remarkable it is the antitumor activity of DMXAA since this compound is capable of induce apoptosis in tumor vascular endothelial cells by attacking tumor's blood vessels in a selective way [40, 42]. Moreover, DMXAA is involved in the release of different compounds that cause hemorrhagic tumor necrosis by interacting with tumor endothelial cells. Among these compounds are some vasoactive factors, cytokines and chemokines [42-44]. DMXAA has already showed promising results in phase I/II clinical trials for the treatment of non-small-cell lung cancer when in combination with other anticancer agents. However, it failed to increase their efficacy in phase III clinical trials [45, 46].

DMXAA scaffold has been used for the development of other bioactive analogues and several derivatives have been investigated over the years, mainly for their potential cytotoxic activity [13].

1.3.2 9-oxo-9*H*-Xanthene-2-carboxylic acid and derivatives

9-oxo-9*H*-Xanthene-2-carboxylic acid as well as some derivatives are a group of compounds that have been studied for its potential antiallergic activity, inhibitory activity against aldolase reductase and as antagonists of leukotriene B4 receptor [13].

Furthermore, other derivatives of 9-oxo-9*H*-xanthene-2-carboxylic acid were tested as blockers of the sciatic nerve conduction [11].

The usual local anesthetics block the peripheral nerve conduction by inhibiting voltage gated Na+ channels and its action mechanism can be differentiated whether they are hydrophilic or lipophilic molecules. Due to the structure similarity of these compounds (**Figure 3**) to some local anesthetics, they might share with them the same pharmacophore. In a study conducted by LQOF/FFUP (*Laboratório de Química Orgânica e Farmacêutica, Faculdade de Farmácia da Universidade do Porto*) it was proved that these three CXDs can block the sciatic nerve conduction in rats through a mechanism that is correlated with the ability to act as modulators of Na+ influx from a selective interference with Na+ ionic currents [11].

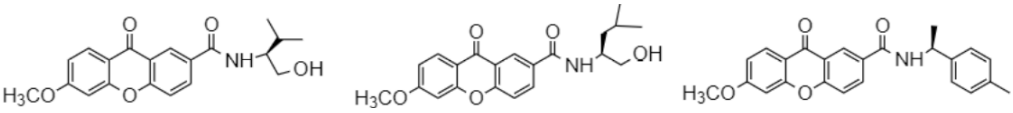
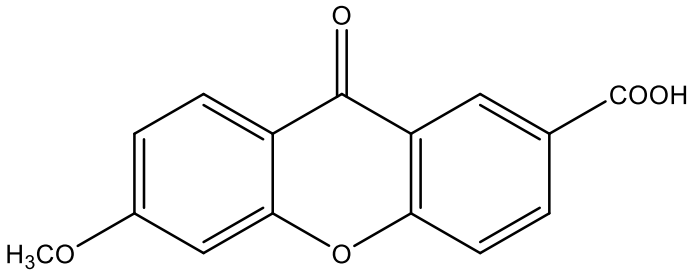


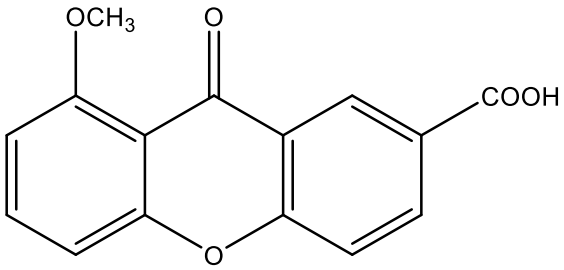
Figure 3: Derivatives of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (**XCar 2**) tested as blockers of the sciatic nerve conduction.

2. Aims

The main objective of this project was to synthesize the carboxylated xanthone derivative (XCar) 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar 2) (**Figure 4**), and its isomer 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar 3) (**Figure 4**) by a multi-step pathway. The chosen method to synthesize these compounds was based on one of the classical methods to obtain XDs, already mentioned in the introduction of this report, via diaryl method.



XCar 2



XCar 3

Figure 4: Structure of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar 2)** and of 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar 3)**.

XCar 2 and XCar 3 were prepared according to procedures previously established in the research group of LQOF(FFUP)/CIIMAR [11]. Their structure elucidation, as well as all the intermediates of the synthesis, was established by spectroscopic methods, such as ^{1}H NMR, ^{13}C NMR and IR.

As mentioned before, XCars present a suitable functional group for the introduction of molecular modifications that can lead to a diverse range of new compounds for biological activity evaluation [13]. Moreover, when coupled with enantiomerically pure units, XCars can also be used as interesting chiral selectors for stationary phases [21-24].

3. Result and Discussion

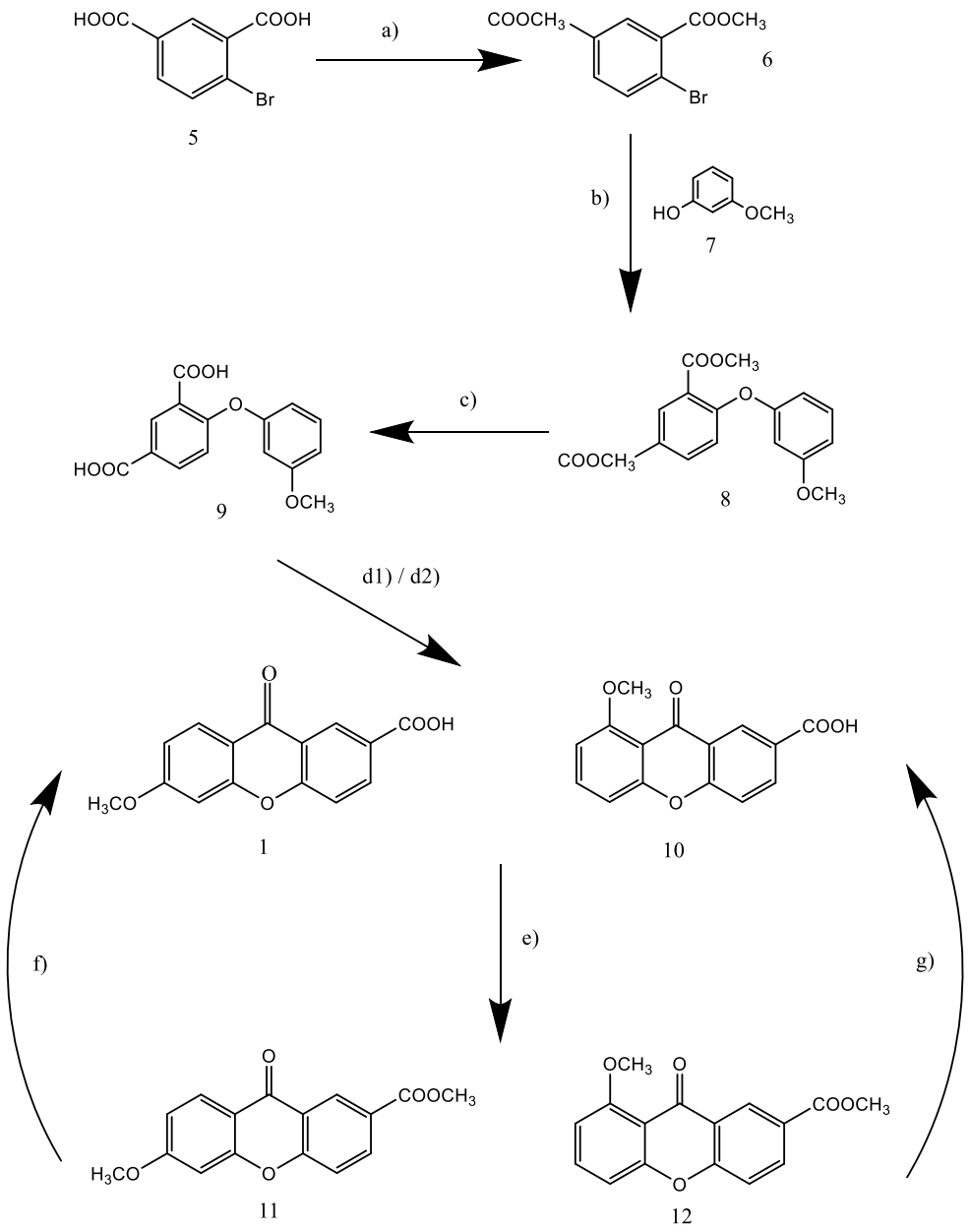
3.1 Synthesis

In **Scheme 2** is shown the multistep pathway for the total synthesis of 6-methoxy-9-oxo-9*H*- xanthene-2-carboxylic acid (**XCar2, 1**) and its isomer (**XCar3, 10**).

The synthesis starts with a Fisher esterification of 4-bromoisophthalic acid **(5)**, obtaining dimethyl 4-bromoisophthalate **(6)**. This reaction was followed by an Ullmann aryl ether synthesis using 3-methoxyphenol **(7)** that allowed the acquisition of dimethyl 4-(3'-methoxyphenoxy)isophthalate **(8)**. The resulting product went through an alkaline hydrolysis giving rise to 4-(3'-methoxyphenoxy)isophthalic acid **(9)** that has undergone an intramolecular acylation affording 6-methoxy-9-oxo-9*H*-xanthene-2- carboxylic acid **(XCar2, 1)** and 8-methoxy-9-oxo-9-*H*-xanthene-2-carboxylic acid **(XCar3, 10)**. Since it is difficult to separate the isomers by classical purification method such chromatography, crystallization or extraction, an esterification was performed and the resulting products were purified by filtration and flash chromatography allowing to obtain methyl 6- methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(11)** and methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(12)**. Finally, these compounds were submitted separately to an alkaline hydrolysis resulting in **XCar2 (1)** and **XCar3 (10)**.

The reactions products and the efficiency of the purification methods were controlled by TLC with appropriate mobile phases and using as standards samples of the expected products that were previously synthesized in LQOF(FFUP)/CIIMAR group.

In the following subchapters, the reaction mechanisms involved in the multi-step pathway to afford **XCar2 (1)** and **XCar3 (10)** will be discussed in detail as well as the structure elucidation of each of the compounds.

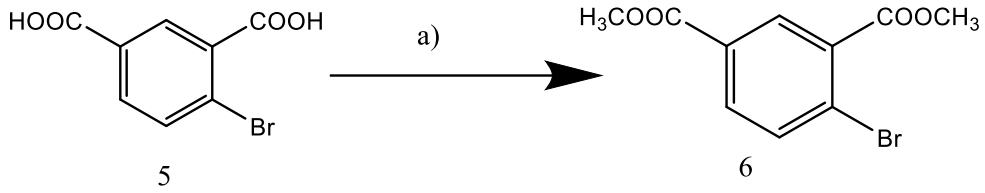


a) Methanol, H_2SO_4, reflux, 17h; b) CuI, Cs_2CO_3, N,N-Dimethyl glycine, Dioxane, N_2, 90°C, 14h; c) Methanol/Tetrahydrofuran (1:1 v/v), 5M NaOH, room temp., 18h; d1) P_2O_5, CH_3SO_3H, room temp., 22h; d2) Polyphosphoric acid, 80°C, 2h; e) Methanol, H_2SO_4, reflux, 19h; f) Methanol/Dichloromethane (1:1 v/v), 5M NaOH, room temp., 22h; g) Methanol/Dichloromethane (1:1 v/v), 5M NaOH, room temp., 22h.

Scheme 2: Total synthesis of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar2, 1)** and of 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar3, 10).**

3.1.1. Synthesis of dimethyl 4-bromoisophthalate (6)

In **Scheme 3**, is presented the reaction synthesis of dimethyl 4-bromoisophthalate (**6**).

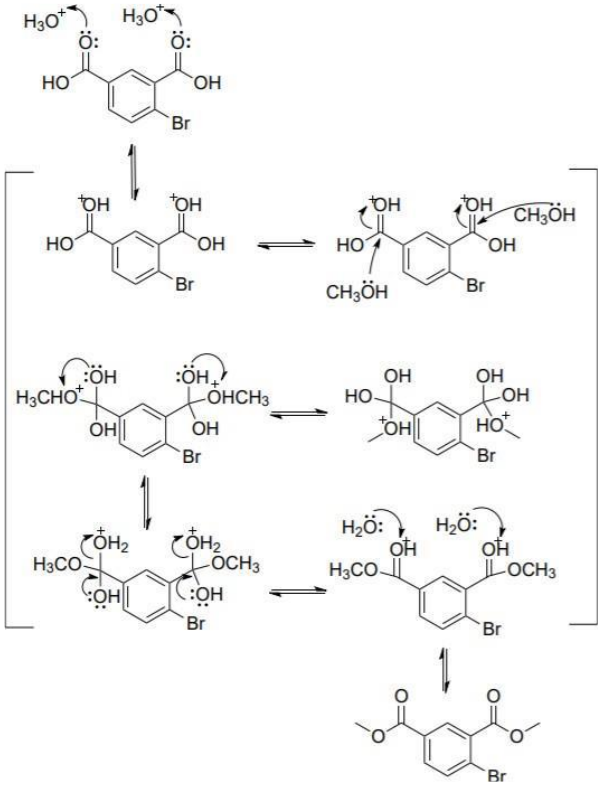


5 6

a) Methanol, H_2SO_4, reflux, 17h.

Scheme 3: Synthesis of dimethyl 4-bromoisophthalate **(6)**.

To synthesize dimethyl 4-bromoisophthalate **(6)** a Fisher esterification is performed, starting from the corresponding carboxilic acid **(5)**. In **Scheme 4** is represented the mechanism of the reaction.

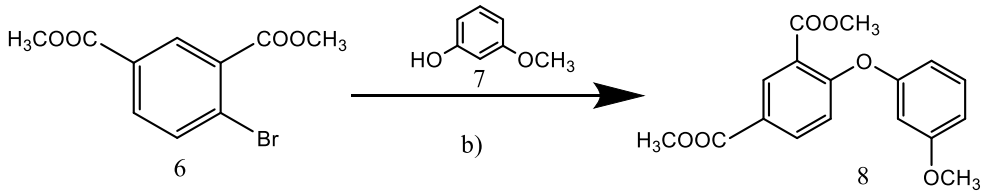


Scheme 4: Reaction mechanism of dimethyl 4-bromoisophthalate **(6)** synthesis.

In Fischer esterification the sulfuric acid act as the catalyst, that protons the carbonyl group of the carboxylic acid. The carbon of these groups now it is more susceptible to the nucleophilic attack. The methanol works as the nucleophile, and there is the formation of a water molecule that is the abandoning group.

3.1.2 Synthesis of dimethyl 4-(3-methoxyphenoxy) isophthalate (8)

Scheme 5 represents the synthesis of dimethyl 4-(3-methoxyphenoxy) isophthalate **(8).**

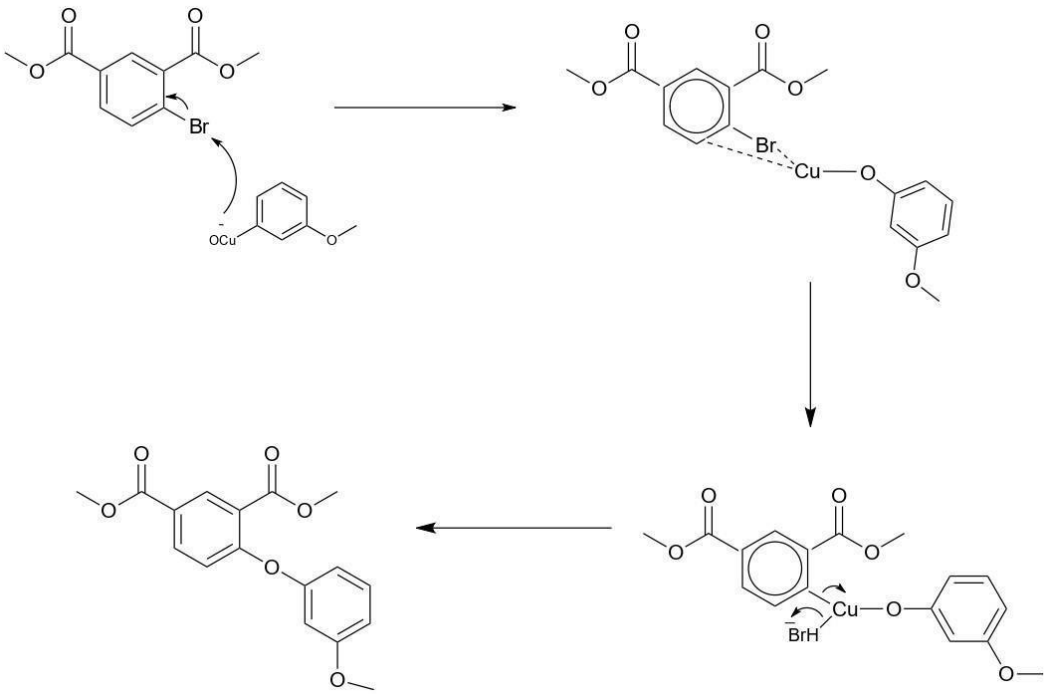


b) CuI, Cs_2CO_3, *N*,*N*-Dimethyl glycine, Dioxane, N_2, 90°C, 14h.

Scheme 5: Synthesis of dimethyl 4-(3-methoxyphenoxy) isophthalate **(8)**.

Dimethyl 4-(3-methoxyphenoxy) isophthalate **(8)** was synthesize with Ullmann aryl ether reaction.

The proposed reaction mechanism is shown in **Scheme 6**. The Ullmann reaction refers to a copper-mediated (stechiometric or catalytic) reaction between an aryl halide and an amine, phenol or thiophenol to synthesize the corresponding aryl -amine, -ether or -thioether compounds, respectively. Fritz Ullmann and Irma Goldberg have developed this copper-mediated aromatic nucleophilic substitution reaction, which required stechiometric amounts of copper and very high reaction temperatures [49]. It was recently found that the addition of relatively cheap ligands (diamines, amino alcohols, diketones, diols) made these reactions truly catalytic, with catalyst amounts as low as 1 mol% or even lower [49].

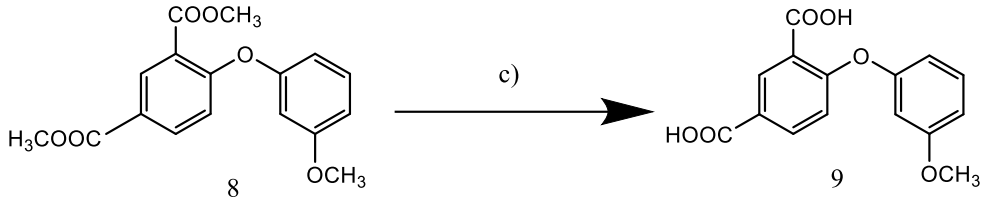


Scheme 6: Reaction mechanism of dimethyl 4-(3-methoxyphenoxy) isophthalate **(8)** synthesis.

The successful development of improved catalytic versions of this grand old chemistry has caused a great revolution of what is now known as the 'modified Ullmann reaction. The key of the 'modified Ullmann' procedure lies in the addition of ligands to the copper catalyst in order to improve the solubility of the copper precursors, leading to the use of milder reaction conditions [49]. Still does not exist a consensus about the reaction mechanism. However, some aspects are common to several theories [49].
The nucleophilic substitution that takes place in Ullmann reaction occurs in two steps. The first step is the nucleophilic attack to the aromatic ring leading to a carbanion formation. This is limiting step of the reaction. In the second step, the carbanion from the halon ion leaves the molecule forming the intended product. The intermediate carbanion is a hybrid structure, but it is still considered as a real compound due to its stability, since the negative charge is distributed around all ring [47].

3.1.3. Synthesis of 4-(3-methoxyphenoxy) isophthalic acid (9)

Scheme 7 represents the synthesis of 4-(3-methoxyphenoxy)isophthalic acid **(9)**.

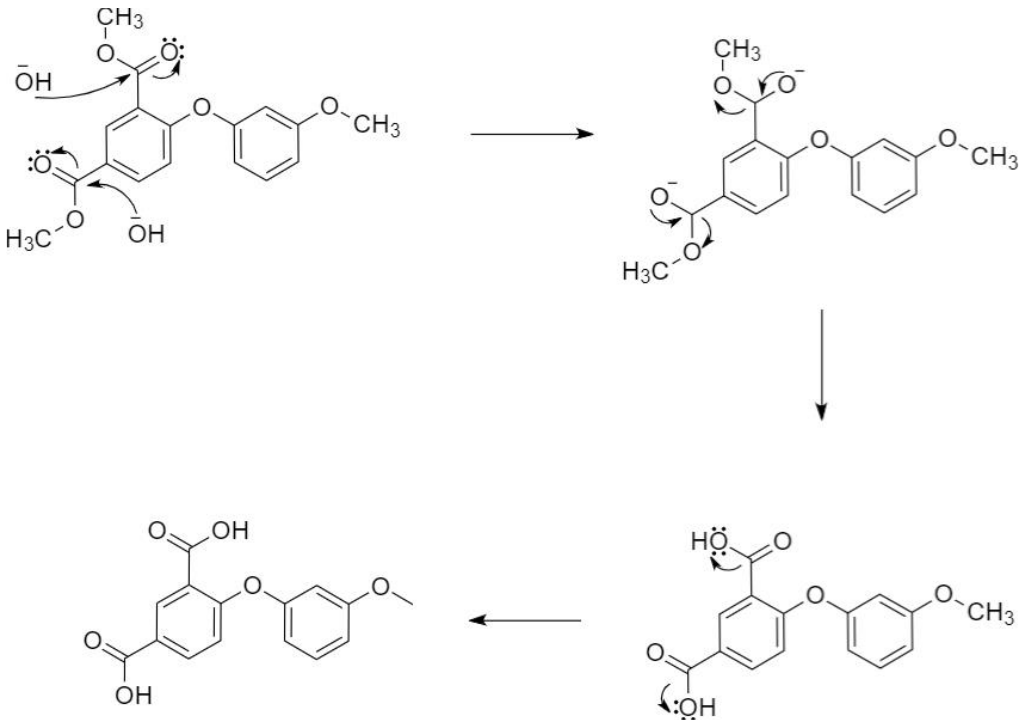


c) Methanol/Tetrahydrofuran (1:1 v/v), 5M NaOH, room temp., 18h.

Scheme 7: Synthesis of 4-(3-methoxyphenoxy)isophthalic acid **(9)**.

In this step an alkaline hydrolysis is performed to obtain 4-(3-methoxyphenoxy)isophthalic acid **(9)** from 4-(3-methoxyphenoxy) isophthalate **(8)**. The ester hydrolysis in a basic medium can also be called saponification. It is a reaction that occurs through a typical nucleophilic acyl substitution pathway in which the hydroxide ion acts as the nucleophile that attacks the ester carbonyl group forming a tetrahedral intermediate. This is followed by the loss of the alkoxide ion and the carboxylic acid is formed. However, the alkoxide ion abstracts the acidic proton of the carboxylic acid forming a carboxylate ion. The carboxylic acid can be regenerated through addition of an inorganic acid that will protonate the carboxylate ion [48]. This reaction is irreversible since the carboxylate ion is stabilized by resonance effect and consequently is little reactive with the alcohol [47].

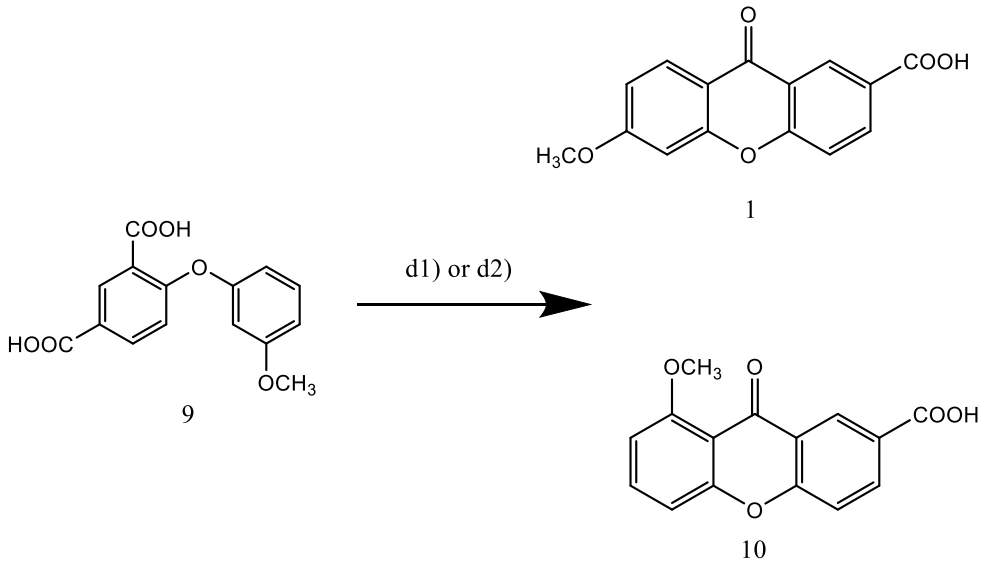
The reaction mechanism is represented in the **Scheme 8**. In this case, the final protonation of the carboxylated ion is mediated by the CH_3OH.



Scheme 8: Reaction mechanism of 4-(3-methoxyphenoxy) isophthalic acid **(9)** synthesis.

3.1.4. Synthesis of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar2, 1) and 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar3, 10).

Scheme 9 represent the synthesis of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar2, 1**) and 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar3, 10)** carried out with two different reaction conditions.

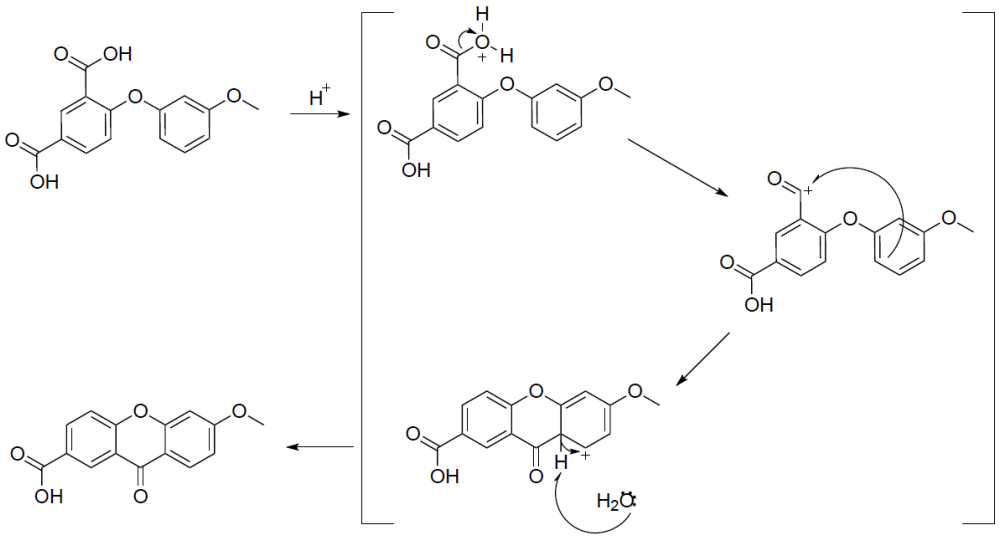


d1)P_2O_5, CH_3SO_3H, room temp., 22h.
d2)Polyphosphoric acid, 80°C, 2h.

Scheme 9: Synthesis of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar2, 1)** and 8- methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar3, 10)**.

In both reactions, an intramolecular acylation of diaryl ether 4-(3-methoxyphenoxy) isophthalic acid **(9)**, using phosphorus pentoxide and methane sulfonic acid at room temperature (Reaction d1) or polyphosphoric acid at 80°C(Reaction d2) was performed, affording two xanthones, **XCar2 (1)** and **XCar3 (10)**.

Reaction d1: This intramolecular acylation is a Friedel-Crafts acylation. This type of reaction essentially consists of the production of an aromatic ketone by reacting an aromatic substrate with an acyl component in the presence of a catalyst. The electrophilic acylations are catalyzed by Lewis acids or strong protic acids [8]. In this case, the acid utilized was methane sulfonic acid and the carbonyl group acts as a Lewis base forming an electrophile in the acyl group. The reaction mechanism concerning to the synthesis of **XCar 2 (1)** is represented in **Scheme 10**.

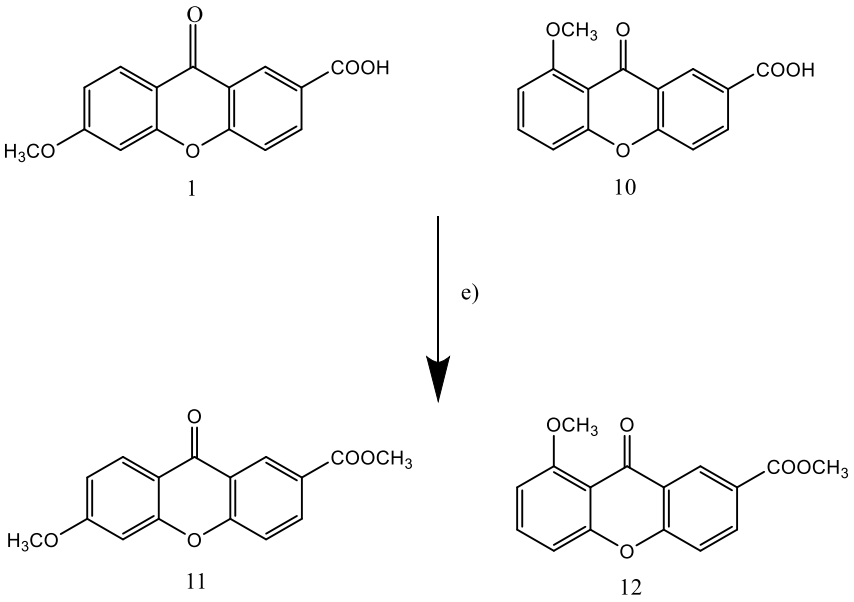


Scheme 10: Reaction mechanism of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar2, 1)** synthesis.

Reaction d2: This reaction is also a Friedel-Crafts intramolecular acylation. The polyphosphoric acid acts as catalyst in order to obtain an aromatic ketone [50]. The reaction mechanism is the same as shown in **Scheme 10**.

3.1.4.1. Synthesis of methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (11) and methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (12)

Scheme 11 represents the synthesis of methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(11)** and methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(12)**.



e) Methanol, H_2SO_4, reflux, 19h.

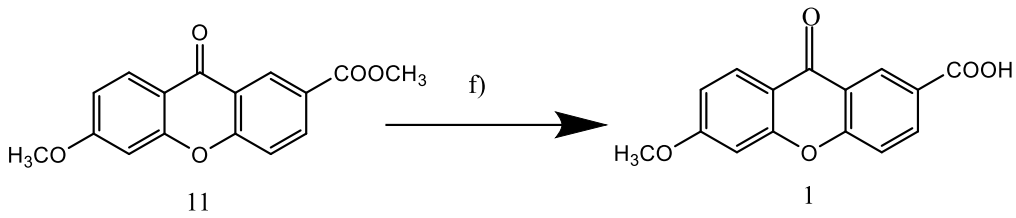
Scheme 11: Synthesis of methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(11)** and methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(12)**

After obtaining **XCar 2 (1)** and **XCar 3 (10)**, it was necessary to convert these compounds in the respective esters through a Fisher esterification affording methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(11)** and methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(12)**, respectively. In this way, it was possible to isolate methyl 6-methoxy-9-oxo-9H-xanthene-2-carboxylate **(11)** by filtration, and flash column chromatography.

3.1.4.2. Recovery of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar2, 1)

After separation of methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(11)**, it was hydrolyzed in an alkaline medium using 5M NaOH

at room temperature for 22h, yielding the 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar2, 1) (Scheme 12)** [4].

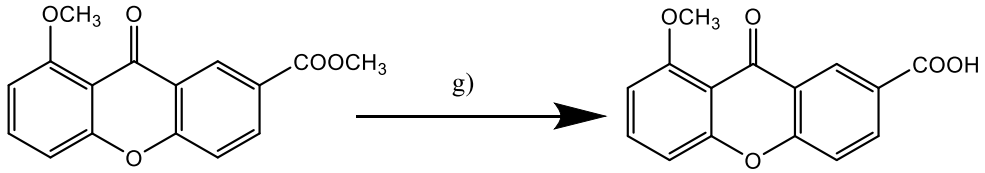


f) Methanol/Dichloromethane (1:1 v/v), 5M NaOH, room temp., 22h.

Scheme 12: Synthesis of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(Xcar2, 1)**.

3.1.4.3. Recovery 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (Xcar3, 10)

After separation of methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(12)**, it was hydrolyzed in an alkaline medium using 5M NaOH at room temperature for 22h, yielding the xanthone derivative 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(Xcar3, 10)**, as shown in **Scheme 13** [4].



g) Methanol/Dichloromethane (1:1 v/v), 5M NaOH, room temp., 22h.

Scheme 13: Synthesis of isolated 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar3, 10)**

3.2 Structure Elucidation

The structure elucidation of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar2, 1)**, 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar3, 10)**, and all the intermediates, was established by spectroscopic methods, such as ^{1}H NMR, ^{13}C NMR and IR, and the data are shown in **Figures 5-18** and **Tables 1-4**.

3.2.1. Structure elucidation of dimethyl 4-bromoisophthalate (6)

Dimethyl 4-bromoisophtalate **(6) (Figure 5)** was obtained through Fisher esterification of 4-bromoisophtalic acid **(5)**.

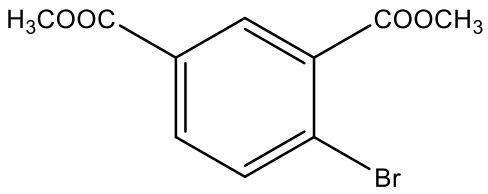


Figure 5: Dimethyl 4-bromoisophthalate **(6)**.

The ^{1}H NMR, ^{13}C NMR and IR data allowed the structure elucidation of compound **6**.

IR data obtained after spectrum's analysis of this compound is represented in **Table 1.** It is important to highlight the presence of one characteristic band at 1687 cm-1, corresponding to C=O bond of the carbonyl group, which confirmed the success of the synthesis.

Table 1: IR data of compound **6**.

Bond	***λ max* (cm^{-1})**
C=C Aromatic	930.0
C=O Ester	1687.2
C-O Ester	1255.0 and 1296.7
O-CH$_3$ Ester	1409.3

Concerning the ^{1}H NMR spectrum, it is essential to highlight the presence of two distinct singlets, with integration for three protons each,

with chemical shifts at δ 3.93 and δ 3.96 ppm corresponding to the protons of the methyl groups of the two esters **(Figure 6)**.

In ^{13}C NMR spectrum is essential to point out the presence of the two signals with chemical shifts at δ 52.5 and δ 52.6 ppm, corresponding to the carbons of the methyl groups. The chemical shifts of the carbonyl group of the esters are placed at δ 165.5 and δ 165.7 ppm **(Figure 6)**.

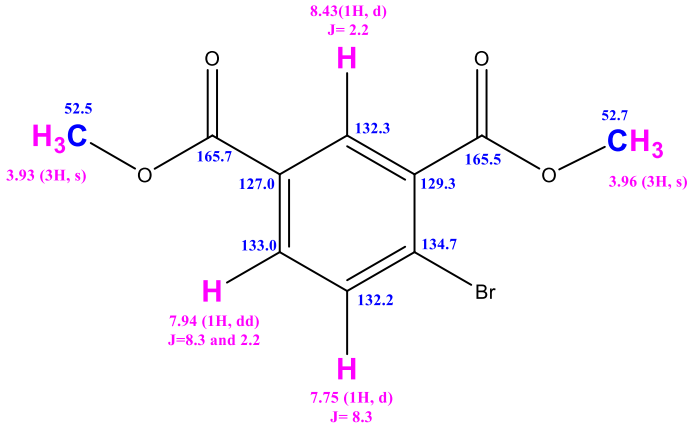


Figure 6: ^{1}H NMR and ^{13}C NMR data of compound **6**(chemical shift values, δ, in ppm, and J, in Hz).

The ^{1}H NMR, ^{13}C NMR and IR data of compound 6 **(Figure 5)** is compatible with the proposed structure for this compound and confirmed the success of the Fisher esterification.

3.2.2. Structure elucidation of dimethyl 4-(3-methoxyphenoxy)isophthalate (8) and 4-(3-methoxyphenoxy)isophthalic acid (9)

Dimethyl 4-(3-methoxyphenoxy)isophthalate **(8) (Figure 7)** resulted from Ullmann diaryl ether coupling reaction of dimethyl 4-bromoisophthalate **(6)** and 3-methoxphenol **(7)**.

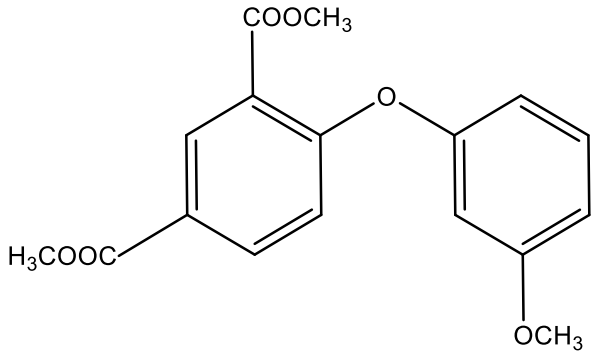


Figure 7: Dimethyl 4-(3-methoxyphenoxy)isophthalate **(8)**.

4-(3-Methoxyphenoxy)isophthalic acid **(9) (Figure 8)** resulted from an hydrolysis of the methyl esters of compound **8 (Figure 7)**.

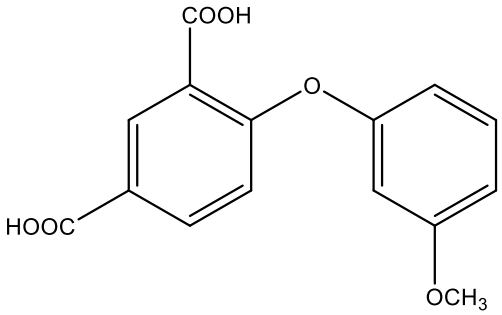


Figure 8: 4-(3-Methoxyphenoxy)isophthalic acid **(9)**.

The ^{1}H NMR, ^{13}C NMR and IR data allowed the structure elucidation of compounds **8** and **9**.

The comparison between the IR data obtained after spectrum's analysis of the compound **9** with the spectrum's analysis of its precursor **8**, showed in **Table 2**, revealed the presence of absorption bands corresponding to the C=O bond and to the aromatic ring bond C=C in both

spectra. It is important to highlight the presence of the absorption band C-O-C corresponding to the ether bond at 1153.4 cm^{-1} **(8)** and 1135.5 cm^{-1} **(9)** in the spectra of both compounds.

The main difference to be emphasized is the presence of a broad absorption band at 2907.0 cm^{-1} corresponding to the O-H carboxylic acid bond on spectrum of compound **9** confirmed the accomplishment of the reaction. Besides, the modification of the C=O band at 1735 cm^{-1} (C=O of $COOCH_3$) to 1680 cm^{-1} (C=O of COOH) confirmed the conversion from $COOCH_3$ to COOH and the success of the reaction.

Table 2: IR data of compound **8** and **9**

Bond	**Compound 8** ***λ max*** **(cm^{-1})**	**Compound 9** ***λ max*** **(cm^{-1})**
C=O	1735.0	1680.0
C=C (Aromatic)	1599.7 and 1613.4	1601.4
C-O-C (Ether)	1153.4	1135.5
O-H	-	2907.0

The ^{1}H NMR and ^{13}C NMR data obtained for **8** and **9** presented δ values consistent to their proposed structures (**Figure 9 and 10**).

Compounds **8** and **9** showed similar ^{1}H NMR profiles for the aromatic protons with equivalent chemical shifts and coupling constants (*J)*. The major difference in the ^{1}H NMR data is the absence of two singlets with the integration of three protons (at δ 3.93 ppm and δ 3.89 ppm) in compound **9** spectrum and the presence of those protons (of the two COOC**H**3 methyl groups) in compound **8 (Figure 7).** This modification confirmed the accomplishment of the desired hydrolysis.

Concerning the ^{13}C NMR data of compounds **8** and **9** (**Figures 9 and 10**) there are two main differences that should be highlighted. Firstly, in **8** spectrum the carbons of the two carbonyl of ester groups **(C**$OOCH_3$) are present at δ 165.3 ppm and δ 165.7 ppm, whereas in **9** spectrum the two **C**OOH groups are both at δ 179.3 ppm**.** Secondly, the absence of two signals at δ 52.2 ppm and δ 52.3 ppm in the spectrum of compound **9**, correspondent to the carbons of COO**C**H_3 methyl groups present in

compound **8**. These differences confirmed the accomplishment of the desired chemical modification.

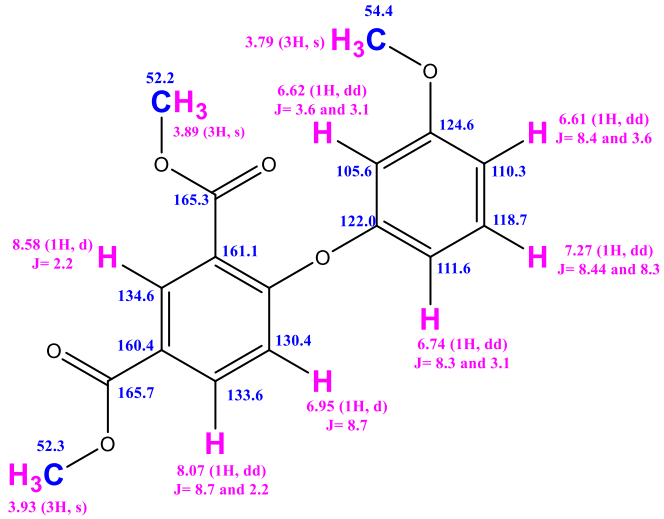


Figure 9: ^{1}H NMR and ^{13}C NMR data of compound **8**(chemical shift values, δ, in ppm, and J, in Hz).

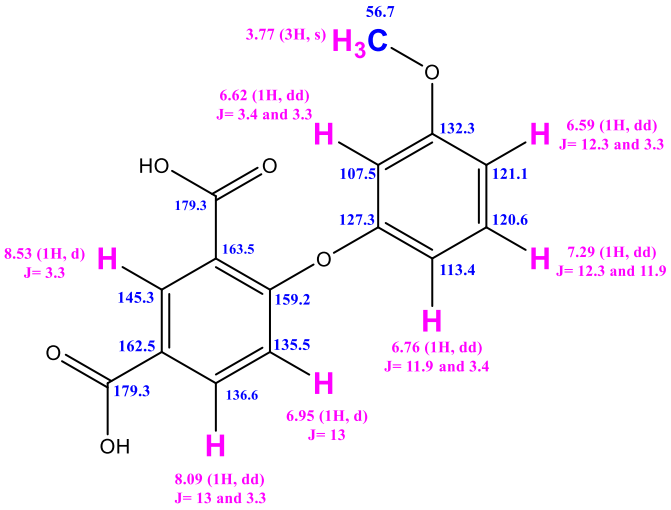


Figure 10: ^{1}H NMR and ^{13}C NMR data of compound **9**(chemical shift values, δ, in ppm, and J, in Hz).

3.2.3. Structure elucidation of methyl 6-methoxy-9-oxo-9*H*-xanthene-2- carboxylate (11) and 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (Xcar2, 1)

6-Methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar2, 1) (Figure 11)** was obtained through intramolecular acylation of diaryl ether, 4-(3-methoxyphenoxy) isophthalic acid **(9)**. After that it was necessary to convert this compound into methyl 6-methoxy-9-oxo-9*H*- xanthene-2-carboxylate **(11) (Figure 12)** in order to be possible to isolate **XCar2 (1)** from its isomer.

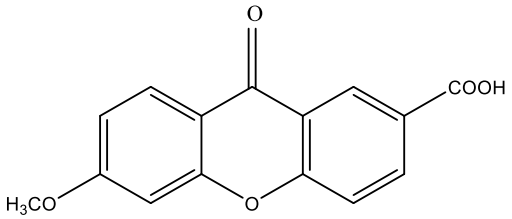


Figure 11: 6-Methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar2, 1)**.

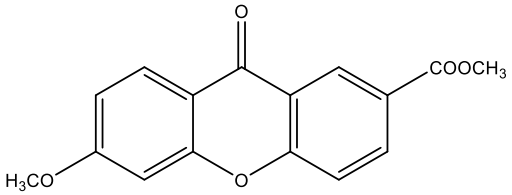


Figure 12: Methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(11)**.

The ^{1}H NMR, ^{13}C NMR and IR data allowed the structure elucidation of compounds **11** and **1.**

The comparison between the IR data obtained after spectrum's analysis of the compound **1** with the spectrum's analysis of its carboxylate (compound **11**), showed in **Table 3**, revealed the presence of absorption bands corresponding to the C=O bond of the functional group ketone, the aromatic ring bond C=C and the ether C-O-C bond in both spectra. For the mentioned bands the IR frequencies are not substantially distinctive. The main difference is the presence of the absorption band at 2964.0 and 3411.4 cm^{-1} corresponding to the O-H carboxylic acid bond on compound **1**. This same band is absent in its precursor, compound **11**, confirming the accomplishment of the reaction.

Table 3: IR data of compounds **11** and **1**

Bond	**Compound 11** ***λ max*** **(cm^{-1})**	**Compound 1** ***λ max*** **(cm^{-1})**
C=O (Ketone)	1663.3	1687.4
C=O (Acid/Ester)	1730.2	1610.0
C-O-C (Ether)	1116.7	1159.0
C=C (Aromatic)	1438.4	1432.8
O-H	-	2964.0 and 3411.4

The ^{1}H NMR, ^{13}C NMR and IR data obtained for **11** and **1** presented δ values consistent to their proposed structures (**Figure 13 and 14**).

Compounds **11** and **1** showed similar ^{1}H NMR profiles with equivalent chemical shifts and coupling constants (*J*). The major difference in the ^{1}H NMR data is the absence of one singlet with an integration of three protons at δ 3.95 ppm in compound **1** spectrum and the presence of those protons corresponding to the methyl group (COOC**H**$_3$) in compound **11 (Figure 13)**. This modification confirmed the accomplishment of the desired hydrolysis.

Concerning the ^{13}C NMR data of compounds **11** and **1** (**Figures 13 and 14**) the main difference is the absence of the signal at δ 52.3 ppm in the spectrum of compound **1**. This signal corresponds to the COO**C**H$_3$ that was hydrolyzed from compound **11**. This difference confirms the accomplishment of the desired chemical modification. All other signals are similar between both compounds.

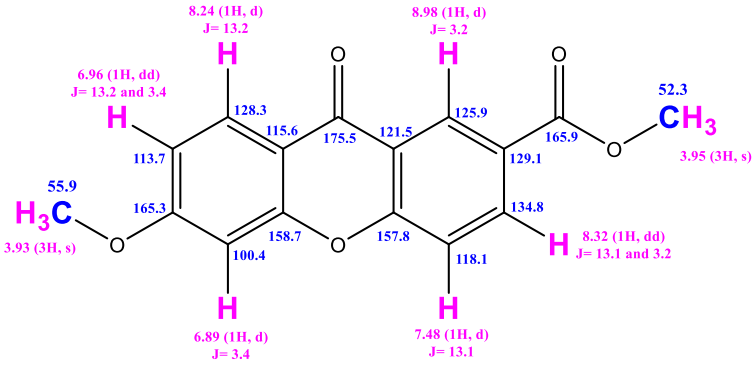


Figure 13: ^{1}H NMR and ^{13}C NMR data of compound **11** (chemical shift values, δ, in ppm, and J, in Hz).

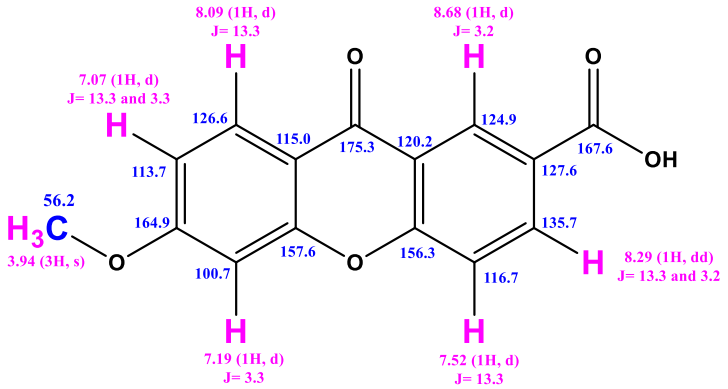


Figure 14: ^{1}H NMR and ^{13}C NMR data of compound **1**(chemical shift values, δ, in ppm, and J, in Hz).

3.2.4. Structure elucidation of methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (12) and 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar3, 10)

8-Methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar3, 10)** **(Figure 15)** was obtained through intramolecular acylation of diaryl ether, 4-(3-methoxyphenoxy) isophthalic acid **(9)**. After that it was necessary to convert this compound into methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(12)** **(Figure 16)** in order to be possible to isolate **XCar3 (10)** from its isomer.

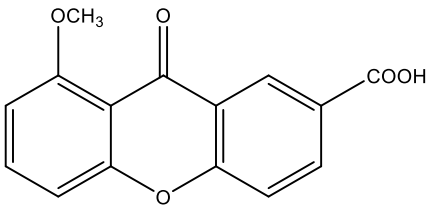


Figure 15: 8-Methoxy-9-oxo-9*H*-xanthene-2- carboxylic acid **(XCar3, 10)**.

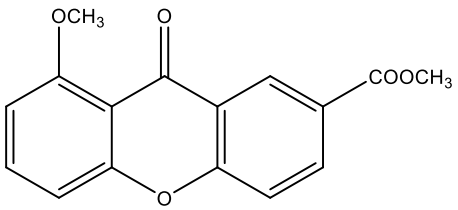


Figure 16: Methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(12)**.

The ^{1}H NMR, ^{13}C NMR and IR data allowed the structure elucidation of compounds **12** and **10.**

The comparison between the IR data obtained after spectrum's analysis of the compound **10** with the spectrum's analysis of its carboxylate (compound **12**) showed in **Table 4** revealed the presence of absorption bands corresponding to the C=O bond of the functional group ketone, the aromatic ring bond C=C and the ether C-O-C bond in both spectra. For the mentioned bands the IR frequencies are not substantially distinctive. The main difference is the presence of the absorption band at 3460 cm^{-1} corresponding to the O-H carboxylic acid bond on compound **10**. This same band is absent in its precursor, compound **12**, confirming the accomplishment of the reaction.

Table 4: IR data of compounds **12** and **10**

Bond	**Compound 12** ***λ max*** **(cm^{-1})**	**Compound 10** ***λ max*** **(cm^{-1})**
C=O (Ketone)	1668	1687
C=O (Acid/Ester)	1726	1603
C-O-C (Ether)	1079	1266
C=C (Aromatic)	1431	1420
O-H	-	3460

Compounds **12** and **10** showed similar ^{1}H NMR profiles with equivalent chemical shifts and coupling constants (*J)*. The major difference in the ^{1}H NMR data is the absence of one singlet with an integration of three protons at δ 4.05 ppm in compound **10** spectrum and the presence of those protons corresponding to the methyl group (COOC$\mathbf{H}_3$) in compound **12 (Figure 17)**. This modification confirmed the accomplishment of the desired hydrolysis.

Concerning the ^{13}C NMR data of compounds **12** and **10** (**Figures 17 and 18**) the main difference is the absence of the signal at δ 52.4 ppm in the spectrum of compound **10**. This signal corresponds to the COO**C**H_3 that was hydrolyzed from compound **12**. This difference confirms the accomplishment of the desired chemical modification. All other signals are similar between both compounds.

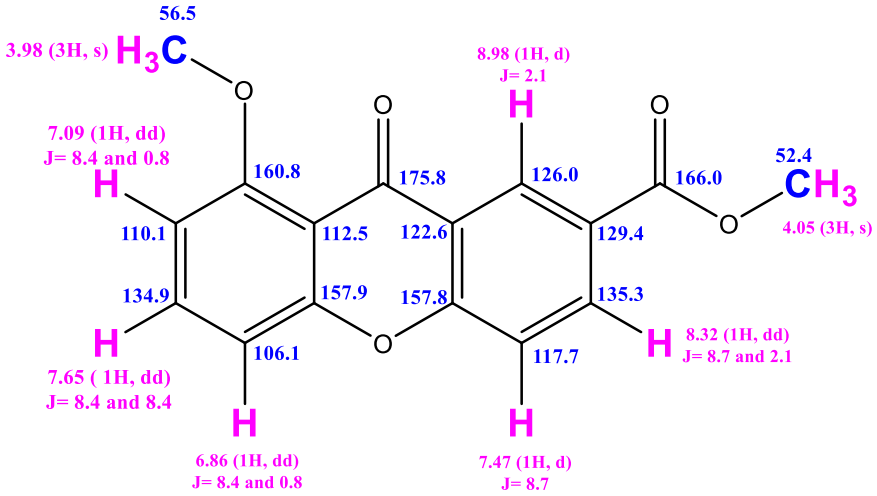


Figure 17: ^{1}H NMR and ^{13}C NMR data of compound **12** (chemical shift values, δ, in ppm, and J, in Hz).

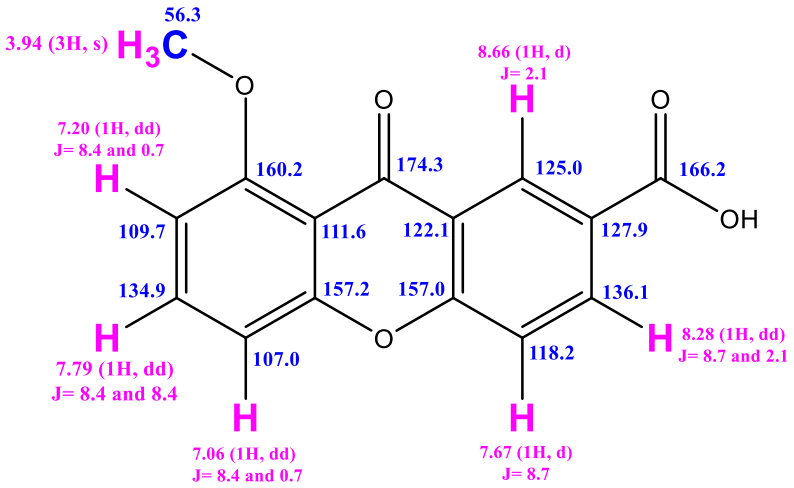


Figure 18: ^{1}H NMR and ^{13}C NMR data of compound **10** (chemical shift values, δ, in ppm, and J, in Hz).

4. Experimental procedure

4.1 General Methods

The solvents were evaporated on a rotary evaporator under reduced pressure (rotative evaporator Büchi).

The reactions and purification methodologies were controlled by thin-layer chromatography (TLC) (silica gel, 60 GF254 Merck) with appropriate mobile phases, and UV detection at 245 and 365 nm.

^{1}H NMR and ^{13}C NMR spectra were performed in the Department of Chemistry of the University of Aveiro, and were taken on Brücker AC-200, DRX-300 or DRX-500 spectrometers (300.13 MHz for 1H and 75.47 MHz for 13C), using DMSO-d_6 and $CDCl_3$ as solvents.

IR spectra were recorded in a KBr microplate in a FTIR spectrometer Nicolet iS10 from Thermo Scientific (Waltham, MA, USA.) with Smart OMNI-Transmission accessory (Software 188 OMNIC 8.3).

4.2 Esterification of 4-bromoisophthalic acid (5). Formation of dimethyl 4- bromoisophthalate (6)

To a solution of 4-bromoisophthalic acid **(2)** (9.96 g, 40.80 mmol) in methanol (600 mL) 7.31 mL of concentrated H_2SO_4 was added. Then, the reaction mixture was refluxed for 20h. After evaporation of the methanol, water (100 mL) was added and the crude product was extracted with diethyl ether (3 x 100 mL). The organic layer was washed with water (2x100 mL), saturated $NaHCO_3$ solution (3x100 mL) and again with water (2x100 mL), successively. The solvent was evaporated under reduced pressure. During overnight at room temperature the dimethyl 4-bromoisophthalate **(3)** appeared as a white solid.

Yield: 94.4%; ^{1}H NMR, ^{13}C NMR and IR data are shown in **Figure 6** and **Table 1**.

4.3 Ullmann diaryl ether coupling forming dimethyl 4-(3 methoxyphenoxy)isophthalate (8)

A mixture of dimethyl 4-bromoisophthalate **(6)** (10.52 g, 38.50 mmol), 3-methoxyphenol **(7)** (6.35 mL, 57.75 mmol), CuI (0.73 g, 3.85 mmol), Cs_2CO_3 (25.3 g, 77 mmol) and *N*,*N*-dimethyl glycine (1.19 g, 11.55 mmol) and dioxane (75 mL) was transferred to a sealed flask, and heated at 90°C under nitrogen atmosphere for 14h.

After cooling water was added (100 mL) and the crude product was extracted with ethyl acetate (100 mL). The organic layer was separated, and the acqueous layer was extracted with ethyl acetate (2x100 mL). The combined organic layer was washed with brine, and the solvent was evaporated, providing dimethyl 4-(3-methoxyphenoxy) isophthalate **(8)**.

^{1}H NMR, ^{13}C NMR and IR data are shown in **Figure 9** and **Table 2**.

4.4 Hydrolysis of dimethyl 4-(3-methoxyphenoxy)isophthalate (8) forming 4-(3-methoxyphenoxy)isophthalic acid (9)

Dimethyl 4-(3-methoxyphenoxy)isophthalate **(5)** (13.14 g , 41.5 mmol) was dissolved in methanol/tetrahydrofuran (1:1 v/v) and stirred at room temperature with 5M NaOH solution (45.2 mL) for 18 h. After the evaporation of the organic solvents, water was added (150 mL) and the crude product was washed with dichloromethane (2x200 mL). The organic layer was washed with water two more times (2x150 mL). The aqueous layer was acidified with 5M HCL solution; since there is no precipitation, the acqueous layer was washed with ethyl acetate (2x200 mL), then the organic layer was evaporated under reduced pressure to provide 4-(3-methoxyphenoxy)isophthalic acid **(9)** as a yellow oil.

Yield: 33.7%; ^{1}H NMR, ^{13}C NMR and IR data are shown in **Figure 10** and **Table 2**.

4.5 Intramolecular acylation to obtain methyl 6-methoxy-9-oxo-9*H*-xanthene-2- carboxylate (11) and methyl 8- methoxy-9-oxo-9*H*-xanthene-2-carboxylate (12)

4.5.1 Intramolecular acylation with methane sulfonic acid and phosphorous pentoxide

To a solution of 4-(3´-methoxyphenoxy)isophthalic acid **(9)** (4.03 g, 13.98 mmol) in methane sulfonic acid (55 mL) was added phosphorus pentoxide (6.34 g, 44.73 mmol) and the reaction mixture was stirred at room temperature for 22h. The mixture was poured over ice, resulting in the formation of a cream-coloured solid that was collected by filtration under reduced pressure and dried at room temperature. The crude product was dissolved in methanol (500 mL) and H_2SO_4 (16 mL) was added. The mixture was refluxed for approximately 19h. The crude product was filtered under reduced pressure. The expected products are methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(11)** and methyl 8-methoxy- 9-oxo-9*H*-xanthene-2-carboxylate **(12)**, but this reaction was unsuccessful and the desired products were not obtained.

4.5.2 Intramolecular acylation with polyphosphoric acid

A solution of 4-(3´-methoxyphenoxy)isophthalic acid **(9)** (3.5 g, 12.14 mmol) in polyphosphoric acid (12 mL) was heated up to 80°C and stirred for 4h. The solution was poured over ice resulting in a dark solid, and let precipitate overnight. The solid was collected by filtration under reduced pressure and dried at room temperature.

The crude product was dissolved in methanol (500 mL) and H_2SO_4 (27 mL) was added. The mixture was refluxed for approximately 19h. The crude product was filtered under reduced pressure. The resulting products are methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(11)** and methyl 8-methoxy- 9-oxo-9*H*-xanthene-2-carboxylate **(12)**. To isolate methyl 6-methoxy-9-oxo-9*H*-xanthene-2- carboxylate **(11)** a filtration with ethanol under reduced pressure was performed.

^{1}H NMR, ^{13}C NMR and IR data are shown in **Figure 13** and **Table 3**.

4.6 Hydrolysis of methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (11) to obtain 6-methoxy-9-oxo-9*H*-xanthene- 2-carboxylic acid (Xcar2, 1)

The methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(11)** (0.231 g, 0.813 mmol) was dissolved in methanol/dichloromethane (100 mL) and 5M NaOH solution (3.2 mL) was added. The mixture was stirred at room temperature for 26 h. After evaporation of the organic solvents, water was added (100 mL) and the solution was acidified with 5M HCL solution resulting in the formation of a white precipitate. The suspension was filtered under reduced pressure and the white solid was washed with water, to afford 6-methoxy-9-oxo-9*H*- xanthene-2-carboxylic acid **(Xcar2, 1)**.
Yield: 93.3%; ^{1}H NMR, ^{13}C NMR and IR data are shown in **Figure 14** and **Table 3**.

4.7 Hydrolysis of methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (12) to obtain 8-methoxy-9-oxo-9*H*-xanthene- 2-carboxylic acid (Xcar3, 10)

The methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate **(12)** (0.361 g, 1.27 mmol) was dissolved in methanol/dichloromethane (100 mL) and 5M NaOH solution (4.95 mL) was added. The mixture was stirred at room temperature for 22 h. After evaporation of the organic solvents, water was added (100 mL) and the solution was acidified with 5M HCL solution resulting in the formation of a yellow precipitate. The suspension was filtered under reduced pressure and the yellow solid was washed with water, to afford 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(Xcar3, 10)**.

Yield: 10.5%; ^{1}H NMR, ^{13}C NMR and IR data are shown in **Figure 18** and **Table 4**.

5. Conclusion

In this project the total synthesis of the XCar 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid, **XCar 2 (1)**, and its isomer 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid, **XCar 3 (10)**, was successfully accomplished by a multistep pathway *via* diaryl ether intermediate.

The structure of **XCar 2 (1), XCar 3 (10)** and all the intermediates, was successfully elucidated by spectroscopic methods, specifically by ^{1}H NMR, ^{13}C NMR and IR.

These compounds have already proved to be a promising chemical substrates to afford bioactive derivatives, thus, they could be used in the future as suitable building block to synthesize new compounds, such as chiral derivatives of xanthones by coupling reactions with pure enantiomeric units, namely amino acids, amino alcohols, amino esters and chiral amines.

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