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Synthesis of new carboxylic chiral derivatives of xanthones inspired on marine xanthones

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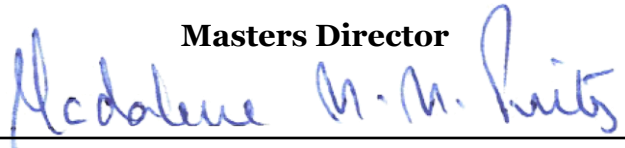

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„The diversity of the phenomena of nature is so great and the treasures hidden in the heavens so rich precisely in order that the human mind shall never be lacking in fresh nourishment."

Johannes Kepler

Resumo

Nas últimas décadas, a quiralidade tem sido um tema que provou ser de consideração obrigatória no campo da química medicinal e farmacêutica e no desenvolvimento de novos fármacos devido às diferentes atividades que os enantiómeros podem apresentar, principalmente em sistemas biológicos.

As xantonas são compostos carbonílicos que pertencem à classe dos heterocíclicos de oxigénio. O núcleo xantónico é simétrico, apresenta uma estrutura 3D "quase-planar" e propriedades eletrónicas peculiares, podendo originar a uma grande diversidade de derivados de acordo com a natureza e posição dos diversos substituintes. As xantonas carboxiladas podem ser de origem natural e encontradas em diferentes ambientes, como em ambientes marinhos, apresentando atividades biológicas interessantes, como anti-inflamatória e antimicrobiana. Além disso, estes compostos são excelentes substratos químicos para a obtenção de uma variedade de derivados, incluindo derivados xantónicos quirais (DXQ).

Neste trabalho, apresenta-se a metodologia de síntese de uma pequena biblioteca de DXQs, bem como a sua caracterização, estudos de excesso enantiomérico e ensaios preliminares de atividade antimicrobiana. Um total de 20 DXQ foram obtidos recorrendo à metodologia de *chiral pool*. Os derivados foram obtidos a partir de um bloco xantónico carboxilado, o ácido 6-metoxi-9-oxo-9H-xanteno-2-carboxílico (XCar 2), e posteriores reações de acoplamento usando dois reagentes de acoplamento diferentes: Hexafluorofosfato de 1-[(1-(Ciano-2-etoxi-2-oxoetilidenoaminooxi) dimetilaminomorfolino)] urónio (COMU) e tetrafluorato de *O*-(benzotriazol-1-il)-*N,N,N',N'*-tetrametilurónico (TBTU). O substrato químico XCar 2 foi obtido por um método já bem estabelecido no nosso grupo de pesquisa anteriormente. Os DXQs foram preparados com base em duas reações principais: acoplamento de ambos os enantiómeros do aminoéster quiral comercial da alanina, fenilalanina, fenilglicina, serina, tirosina e triptofano. Quando obtidos em quantidades suficientes, os ésteres foram submetidos a uma reação de hidrólise em condições alcalinas para obtenção de novos derivados com o ácido carboxílico livre. Foram obtidos 10 novos compostos derivados de amino ester e 8 DXQ resultantes de hidrólise.

Todos os compostos foram caracterizados por espectroscopia de infravermelho (IV), ressonância magnética nuclear (RMN) de ^{1}H e ^{13}C. A pureza enantiomérica dos novos DXQ foi avaliada por cromatografia líquida de alta eficiência (CLAE/*HPLC*), recorrendo a uma fase estacionária quiral comercial, Lux® 3 μm celulose-2, em condição de eluição no modo normal. Após a análise dos cromatogramas obtidos por HPLC verificou-se que os DXQ apresentavam excesso enantiomérico (ee) acima dos 89% e uma compostição enantiomérica (e.c.) acima de 90%, exceto para os derivados de amino ácidos dos enantiomeros da fenilglicina.

Após a elucidação estrutural e a avaliação da pureza enantiomérica, o potencial poder de inibição de crescimento de alguns dos compostos em microrganismos (*Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* e *Candida albicans)* foi avaliado. Os resultados preliminares revelaram que ambos os enantiómeros dos derivados do amino ester da serina têm alguma capacidade de inibir o crescimento de *S. epidermidis,* enquanto os DXQ dos amino ésteres da *L*-serina e da *D*-tirosina, demonstraram capacidade para inibir a *S. aureus*.

Palavras-chave: quiralidade; xantonas, derivados xantónicos quirais, chiral pool, enantioseletividade.

Abstract

In the last decades, chirality has been a topic that has proven to be of mandatory consideration in the field of medicinal and pharmaceutical chemistry and in the development of new drugs due to the different effects that enantiomers can present, especially in biological systems.

Xanthones are carbonyl compounds belonging to the class of *O*-heterocyclics. The xanthonic nucleus is symmetric and has a quasi-planar 3D structure with peculiar electronic properties that may correspond to a wide variety of derivatives, according to the nature and position of the various substituents. Carboxylated xanthones can be found in nature in differentecossistems, such as in marine environments, and have already presented interesting biological activities, such as anti-inflammatory and antimicrobial. In addition, these compounds are excellent chemical substrates for the preparation of a variety of derivatives, including chiral xanthone derivatives (CDXs).

In this work, the methodology of synthesis of a small library of CDXs, as well as its characterization, studies of enantiomeric excess and preliminary tests of antimicrobial activity are presented. A total of 20 CDXs were obtained using the chiral pool methodology. The derivatives were obtained from a carboxylated xanthonic block, 6-methoxy-9-oxo-9H-xanthene-2-carboxylic acid (XCar 2), and subsequent coupling reactions with two different coupling reagents: COMU ((1-Cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-morpholino-carbenium hexafluorophosphate) and TBTU (*O*-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate). The XCar 2 chemical substrate was obtained by a method already well established in our research group previously. The CDXs were prepared based on two main reactions: coupling of a commercial chiral amino ester of alanine, phenylalanine, phenylglycine, serine, tyrosine and tryptophan, and when obtained in sufficient quantities, the esters were subjected to a hydrolysis reaction under alkaline conditions for new derivatives with the free carboxylic acid. Ten new CDXs of amino esters were synthesized and nine CDXs of amino acid.

All compounds were characterized by infrared (IR) spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance (NMR). The enantiomeric purity of the novel CDX was evaluated by high

performance liquid chromatography (HPLC) using a commercial chiral stationary phase, Lux™ 3 μm cellulose-2, under normal mode elution conditions. After analyzing the chromatograms obtained by HPLC, it was found that the CDXs had enantiomeric excess (ee) above 89% and an enantiomeric composition (ec) above 90% except for the amino acid derivatives of the phenylglycine.

After structural elucidation and evaluation of enantiomeric purity, four of the compounds obtained were tested in microorganisms (*Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* e *Candida albicans)* Preliminary results appear to show that both the enantiomers of the serine amino ester derivatives have some ability to inhibit S. epidermidis growth, whereas the CDXs of the amino esters of *L*-serine and *D*-tyrosine have demonstrated the ability to inhibit *Staphylococcus aureus*.

Keywords: chirality; xanthones, chiral xanthonic derivatives, chiral pool, enantioselectivity.

Abbreviations

^{13}C NMR	Carbon nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
3D	Three-dimensional
Ar	Aromatic
CDX	Chiral Derivatives of Xanthones
COMU	(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-morpholino-carbenium hexafluorophosphate
CSP	Chiral stationary phase
DXQ	Derivados xantónicos quirais
ee	Enantiomeric excess
ec	Enantiomeric composition
ESI	Electron spray ionization
EtOH	Ethanol
FTIR	Fourier-transformation infrared
HMBC	Heteronuclear multiple bond correlation
HPLC	High Pressure Liquid Chromatography
HRMS	High-Resolution mass spectometry
HSQC	Heteronuclear single quantum coherence
IR	Infrared
J	Coupling constant
LC	Liquid Chtomatography
MHz	Mega hertz
MS	Mass spectometry
PPA	Polyphosphoric acid
TBTU	*O*-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate
TEA	Triethylamine
TFA	Trifluoroascetic acid
THF	Tetrahydrofuran
XCar 2	6-methoxy-9-oxo-9H-xanthene-2-carboxylic acid
δ	Chemicalshift
ν	Frequency

Aims

- Synthesis a library of new chiral xanthone derivatives from proteinogenic amino acids, in its enantiomerically pure form.
- Characterization and structure elucidation for the derivatives synthesized.
- Evaluation of the enantiomeric purity for the new chiral derivatives.
- Bioactivity studies regarding the potencial bacterial growth inihibition of the compounds obtained.

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Outline of dissertation

This dissertation is organized into 6 chapters.

In chapter 1 is presented a brief introduction on the importance of chirality and what has been developed in the field of chiral derivatives of xanthones. The synthetic pathways towards obtaining the xanthone scaffold are also presented.

Chapter 2 presents the results obtained throughout this project and its discussion concerning the synthesis of the compounds obtained, along with their structure elucidation, enantiomeric purity studies and antimicrobial activity tests.

In chapter 3 it is described the reagents, solvents, instruments, methodologies and techniques used in the synthesis of the chiral derivatives of xanthones and its correspondent procedures.

In chapter 4 presents the final considerations about the work and the summarized results.

Chapter 5 contains the references cited throughout the dissertation as well as the information search engines used.

Annex 1 contains a table presenting the structures and chemical names that correspond to the code names used for the final products obtained.

CHAPTER 1: INTRODUCTION

1. Introduction

1.1. Chirality

We live in a chiral Universe[1]. Nature has a right and left, and it can tell the difference between the two. The origin from the word chiral comes from the Greek word *cheir*, which means hand, and just like our hands, many of the molecules that surround us are not superimposable with their mirror image (Figure 1), so they are designated as chiral[2].

French and Dutch chemists, Joseph Le Bell and Jacobus van't Hoff, discovered molecular chirality when working independently, simultaneously proposed that the carbon atom was the basis for molecular asymmetry. They suggested that in this atom, if the four substituents that surround it are different and the spatial arrangement is not planar, two non-superimposable forms can occur[3].

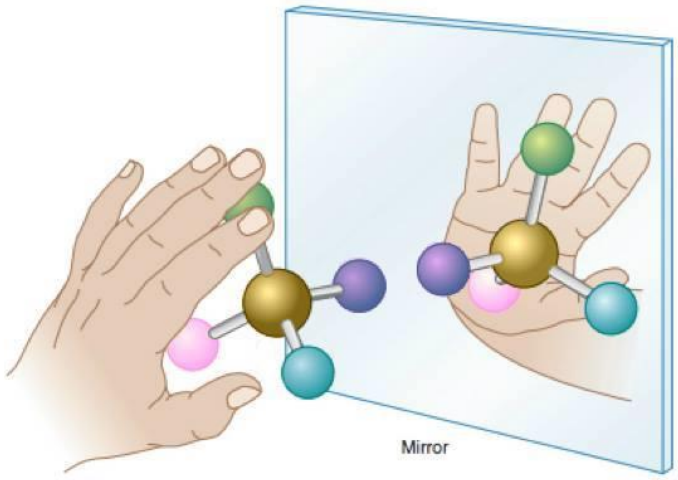


Figure 1. Tetrahedral carbon atom and its mirror image (adapted from ref. 4).

It is important to say that although the most familiar type of stereogenic centre in a molecule is the carbon atom with four different substituents, there are also chiral compounds with stereogenic heteroatoms such as tetravalent nitrogen (ammonium) and phosphorous

(phosphorium) ions. Also, phosphine oxides are tetrahedral and chiral if its three substituents (besides oxygen) are different. Some phosphorous compounds, together with trivalent sulphur are not so evident cases, as the structure is approximately tetrahedral, with an electron pair filling one of the tetrahedral positions (Figure 2).

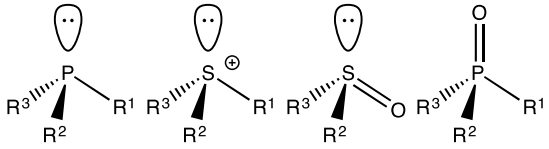


Figure 2. Structures of phosphine, sulfonium ion, sulfoxide and phosphine oxide, respectively.

Trivalent nitrogen (Figure 3) molecules are also approximately tetrahedral, but they suffer inversion very fast under normal conditions due to the low energetic level inversion barrier, and therefore, it is not possible to isolate the enantiomers separately under normal conditions[5].

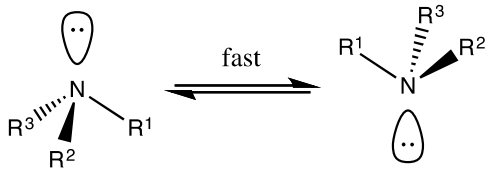


Figure 3. Inversion of trivalent nitrogen molecules.

In addition to this sp^3-hybridized atom type of chirality, others features that confers chirality in molecules were observed, giving place to other kinds of chirality. These can be listed in four categories[6]:

1. *Central chirality* – molecules with tetravalent atoms differently substituted; tetrahedral molecules with trivalent atoms differently substituted and a pair of electrons acting as the fourth substituent.
2. *Axial chirality and atropisomers* – atoms are positioned in two perpendicular planes and cannot rotate freely.
3. *Planar chirality* – with loss of symmetry plane.
4. *Helical chirality* – molecules that have a helicoidal structure.

The two non-superimposable mirror-image arrangements of chiral molecules that have different spacial arrangements but identical physical and chemical properties, are named enantiomers by stereochemistry terminology and the equimolar mixture of the two enantiomers is denominated racemate or racemic mixture[7].

Enantiomers can be recognized by the way to rotate the polarized light. When rotation happens for the right (clockwise), they are called dextrorotatory, (d) or (+), and when for the left (counter-clockwise), they are denominated levorotatory, (l) or (-). Configuration can be specified by the Cahn-Ingold-Prelog system, which assigns the configurations as (*R*) and (*S*) (from the Latin words *rectus* and *sinister* which means right and left, respectively) through a set of priority rules[8].

1.2. Chirality in drug development

Chirality is an intrinsic property of the "building blocks of life" due to chiral structures of their essential subunits such as amino acids and carbohydrates, which form proteins, glycoproteins and nucleic acids. For these structures, evolution has forced the use of a single spatial arrangement. For example, all the amino acids in our body are in the same enantiomeric *L*- configuration and all the sugars are present in de *D*- form, and this specificity is transported to the macromolecules that they constitute. As a result, metabolic and regulatory processes mediated by biological systems are sensitive to stereochemistry and different activities can frequently be observed when analyzing a response to each pair of enantiomers[9].

The human body is a complex chiral environment and the interactions of small molecules with receptors will determine the biological response it will induce. Drugs can interact with the receptors through five kinds of chemical forces: covalent, electrostatic, hydrogen bonds, hydrophobic and π-π interactions. Enantiomers can have different biological responses, depending on how well they "fit" the receptor (Figure 4). When this happens, the most active enantiomer is called eutomer, while the weaker is called distomer[10].

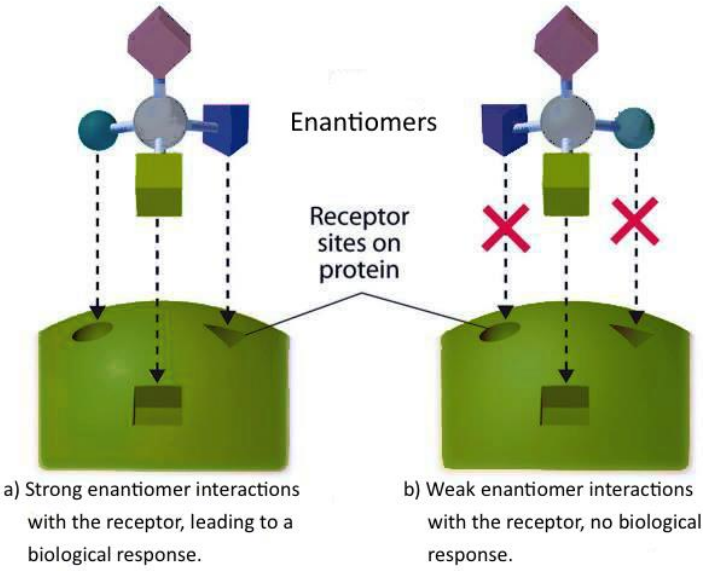


Figure 4. Enantioselective molecular recognition (Adapted from Ref. [11])

Drug chirality has not only implications on pharmacodynamics, but also through the pharmacokinetic spectrum.

Absorption is the process by which a drug reaches the bloodstream from the site where it is administrated. This process usually happens by passive diffusion or with the help of active transporters. The passive diffusion phenomenon is ruled by lipophilicity, molecular weight and the extent of neutral form of the molecules at physiological pH. It is an energy-free process that happens spontaneously from areas where the substance concentration is high, to lower concentration areas. Therefore, this type of molecular crossing through membranes is almost or totally enantiomerically non-selective. On the other hand, active transportation through membranes is mediated by a receptor that may be stereoselective and it requires energy to occur. This means that the amount and rate of absorption of different enantiomers can be different and if so, it can affect their biological activities[12].

Once a drug enters systemic circulation, it is distributed to interstitial and intracellular fluids of organs and tissues. Plasma proteins, mostly albumin and glycoprotein, are responsible for this transport through the blood and stereoselectivity in drug distribution occurs as a consequence of the binding to both plasma or tissue proteins due its chiral nature.

Once the drug is bind to plasma proteins, the free fraction of it is important for tissue distribution and variations in this drug availability in the free form between enantiomers can be

responsible for the differences in their potency[13].

Enzyme systems depend on receptor-substrate interactions and these are susceptible to strereochemical influences just like other systems that are interaction-dependent. In this case, stereoselectivity is also associated to catalysis because of differential orientation and reactivity of the target group's catalytic site. Stereoselectivity in metabolism may be related to the substrate, when the transformation of one enantiomer is preferred, to the product, when the reaction is oriented mainly to the formation of a specific stereoisomer of the metabolite, and to both, in which a substrate enantiomer is preferred and the metabolic reaction is extended to the formation of a particular molecular configuration. Given this, metabolic pathways can be grouped into five categories[14]:

1. *Prochiral to chiral transformations* – the prochiral centre or an enantiotopic group in the molecule suffers metabolism.
2. *Chiral to chiral transformations* – each enantiomer undergoes metabolism at a place in the molecule where there is no alteration in the configuration.
3. *Chiral to diastereoisomer transformations* – another chiral centre is introduced in the molecule through reaction at a prochiral centre or by coupling with a chiral conjugate.
4. *Chiral to achiral transformations* – the molecule suffers a modification in the chiral centre, resulting in a loss of asymmetry.
5. *Chiral inversion* – one enantiomer is transformed into its pair with no structural consequences.

These phenomenons can have toxicological implications, as the eudismic ratio (ratio between eutomer and distomer) can be altered, and is important to know that nefarious effects may come from the isomeric metabolites and not from the parent molecule.

Concerning clearance, essentially three passive and active processes govern renal excretion: glomerular filtration, passive and active tubular reabsorption and active secretion. Glomerular filtration is dependent on the rate of plasma water filtration and the unbound plasma concentration of the drug, so the stereoselectivity present here is related with the interactions that each enantiomer establishes with plasma proteins. Active tubular secretion and

reabsorption involve the action of transport proteins and are thought to be responsible for the differences in the clearance of enantiomers[15].

Nowadays, it is widely recognized that chirality is a fundamental variable on the effects and properties of a substance and the importance of chirality in drug development has been on the rise in the past fifty years. Some years ago, the commercialization of enantiopure drugs could only be encouraged by regulatory entities as a desirable challenge to be overcome by the pharmaceutical industry, with many practical obstacles. Today, this situation has completely changed, as the field of organic chemistry has suffered a major revolution in the past decades, with the development of highly selective methods to obtain enantiomerically pure substances, and so, many enantiopure drugs have reached the market. Given this, in 1992, health and regulatory agencies like US Food and Drugs Administration (FDA), were forced to issue formal regulatory guidance for the approval of new drugs, where it states that separation and characterization has to be carried out for each enantiomer, if possible, as well as bioactivity, pharmacokinetic, pharmacodynamic, toxicological evaluation of each isomer. This policy was followed by Europe in 1994[16]. Furthermore, drugs that were marketed and patent protected as racemates can extend their years of exclusivity by applying for a "chiral switch", which is a change for a single enantiomer version of a drug[17]. In the last two decades, the number of single-enantiomer drugs has been increasing steadily to nearly two thirds of all active principle ingredients and this number is expected to increase even more in the next years (Figure 5).[18,19]

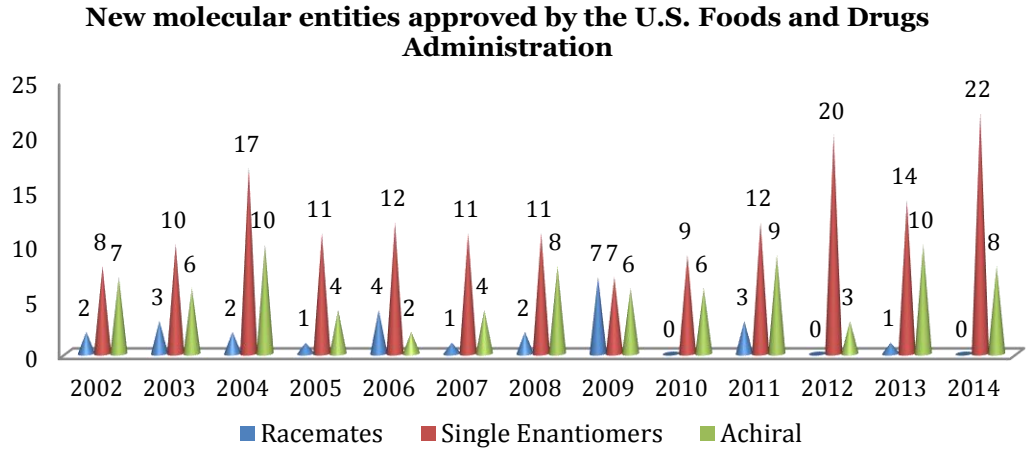


Figure 5. Chirality in new molecular entities. (Adapted from Ref. [18] and [19])

Given the verified importance of chirality in the biological response and the demand from the regulatory authorities, the industry and researchers are being pushed to develop new techniques to obtain chiral compounds. There are two ways to approach the making of an enantiopure drug: the "chiral approach", based on the development of an asymmetric synthesis of only one of the enantiomers, and the "racemic approach", that focuses on the synthesis of both enantiomers and separation of the racemic mixture. The following diagram (Figure 6) represents the possible pathways towards obtaining an enantiopure compound[20].

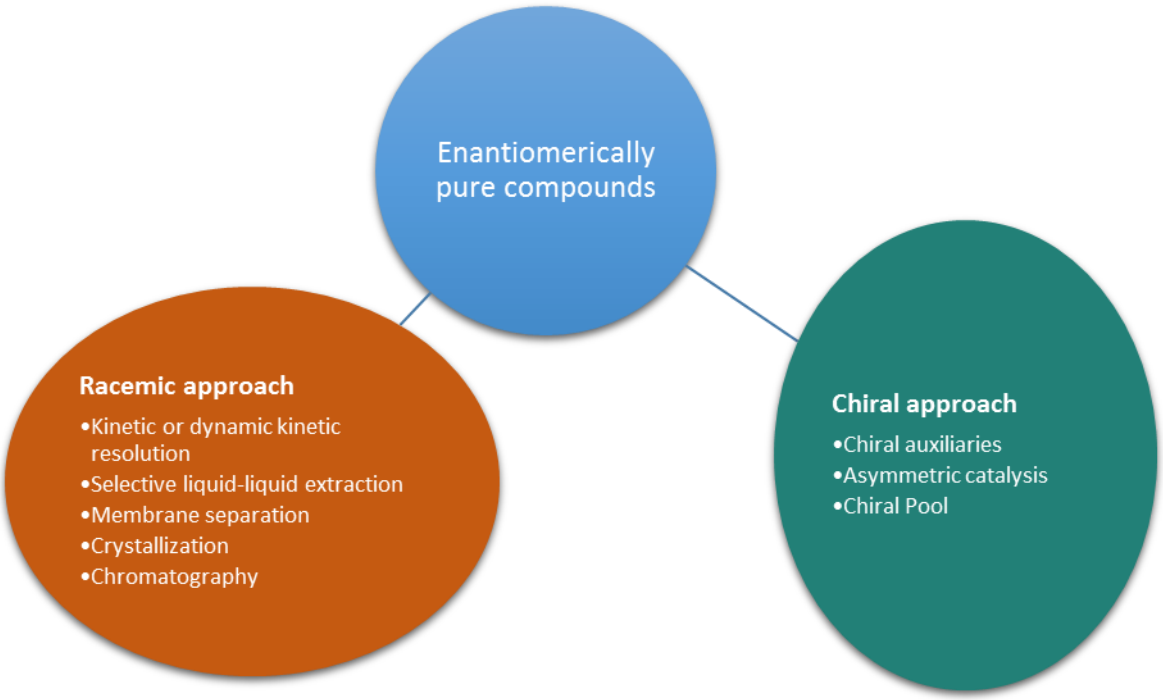


Figure 6. Approaches to obtain enantiopure compounds.

1.3. Preparation of pure enantiomers with chiral approach: Chiral Pool

Nature has provided a wide range variety of enantiomerically pure substances that go from amino acids to carbohydrates, terpenes, and some are found in great abundance. The chiral pool methodology makes use nature's own chiral pool and incorporates these abundant,

economical and optically pure materials in the synthesis of complex substrates, transporting its chirality into to the final product. With the advances in synthetic methods, new chiral substrates and derivatives of existing ones are being added to this chiral pool.

Although this method is very effective for obtaining enantiomerically pure compounds, it is limited to the availability of these chiral starting materials[21]. On the other hand, the easiness to operate and the inexpensiveness of these materials makes them very attractive for industrial use, like *L*-aspartic acid, whose pure enantiomeric material is even cheaper than the racemate.

In our research group, this strategy was considered the one that best suited the purpose of the synthesis of CDX for further studies of its biological activity[22].

1.4. Xanthone Derivatives

Xanthones (9H-xanthon-9-one) are a family of *O*-heterocycle symmetrical compounds with a dibenzo-ϒ-pyrone scaffold (Figure 7), where the carbons are usually numbered according to a biosynthetic convention. Hence, in the case of plants, carbons 1-4 are given to the acetate-derived ring A, while carbons 5-8 to the shikimate-derived ring B.

The xanthone derivatives are very attractive in drug development, being considered privileged structures, due the endless of different patterns of substitution that can generate a diversity of library of compounds able to interact with several biological targets[23],[24],[25]. Xanthone derivatives were found to have antioxidant[26], antithrombotic[27], vasorelaxant[28], antiulcer[29], anti-inflammatory[30], antiallergic[31], diuretic[24], MAO inhibitory[32], cytotoxic[33], antimicrobial[34], antiviral[35] and antifungal activities[36], among others[25].

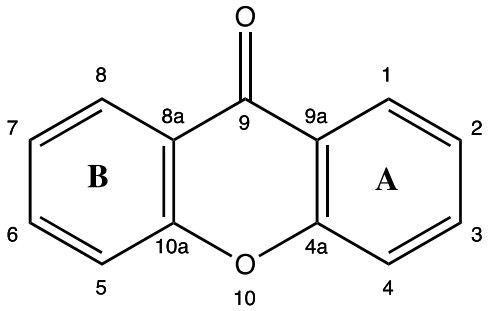


Figure 7. Structure of the xanthone scaffold.

Xanthone derivatives can be found all over nature as metabolites produced by plants, fungi, bacteria and lichens[37,38]. They even have been found in fossil fuel[39], suggesting an excellent stability of these structures. This class of compounds is biosynthesized through different pathways in fungi and plants (Figure 8 and Figure 9).

In higher plants, the xanthone is of diverse biosynthetic provenance. While ring A originates from the acetate pathway, ring B is shikimic acid-derived. 3-hydroxybenzoic acids, from phenylalanine ancestry, are bonded to a 3 acetate equivalents polyketide to suffer cyclization. The bond rotation allows the formation of two different products because of the two oxidative phenolic coupling possibilities.

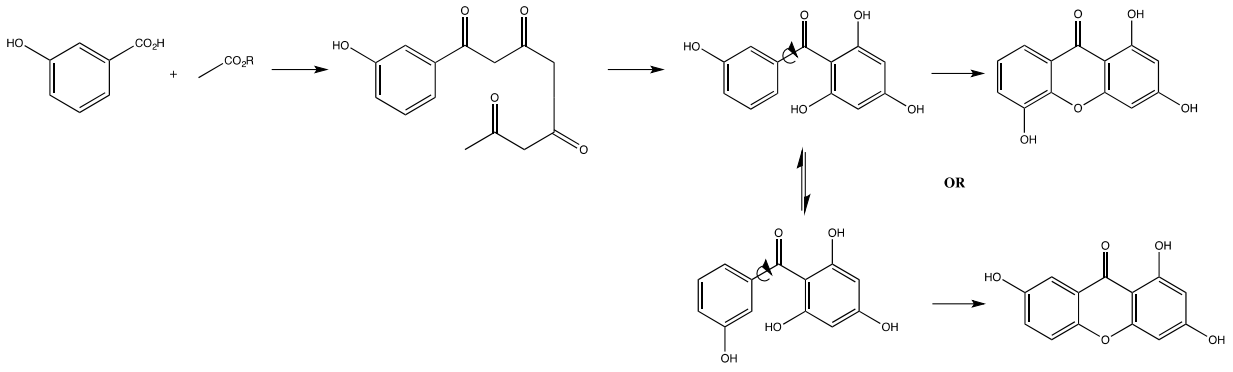


Figure 8. Biosynthesis of xanthones in plants.

In fungi, the xanthone structure is derived completely from polyketide unit that goes under cyclization and consequent oxidative cleavage (pathway 1). Depending on the specie that is synthesizing the xanthone, the pathway may be different, and in some cases the cyclization happens by allylic rearrangement and further elimination (pathway 2).

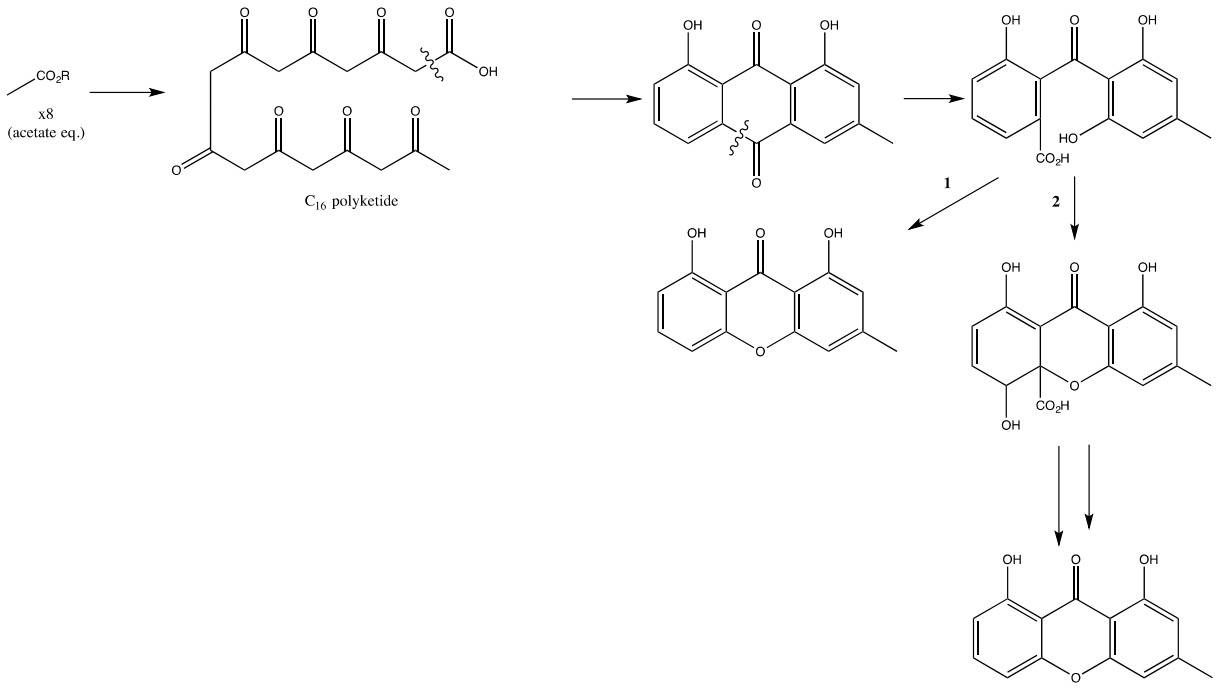


Figure 9. Biosynthesis of xanthones in fungi.

Xanthones isolated from natural sources have diverse substitution patterns and therefore constitute a vast collection of compounds. Still, its biosynthesis is restricted to the presence of specific moieties in well-defined positions of the scaffold. Thus, total synthesis is not only an interesting strategy to obtain molecular arrangements that could not be achieved by transforming natural occurring xanthone substrates, but also a way to achieve these compounds in higher quantities[40]. The following scheme (Figure 10) represents the three classical methods to attain the xanthone scaffold.

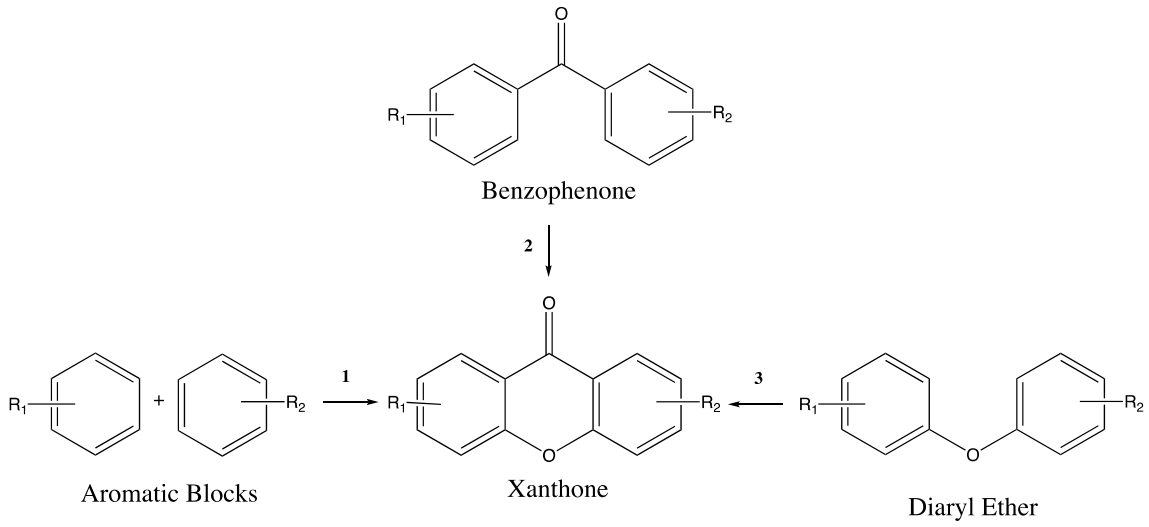


Figure 10. Classical methods towards obtaining the xanthone scaffold..

Methodology 1 makes use of salicylic acids/esters/aldehydes and another aromatic building block in order to obtain the xanthone scaffolds in one step by condensation of the two aryl building blocks (Figure 11). Route 1.1, which was named Grover, Shah and Shah reaction leads to the xanthone scaffold by the reaction of salicylic acids or salicylic esters with polyphenolic compounds, catalyzed by strong acids[41]. The second route 1.2, which was reported for the first time in 2005, uses also salicylate esters but instead of a polyphenolic compound, it is used an aryne intermediate or *o*-halobenzoic acids[42]. 1.3 uses palladium as catalyst to promote an annulation between salicylaldehyde and 1,2-dibromoarene derivatives[43].

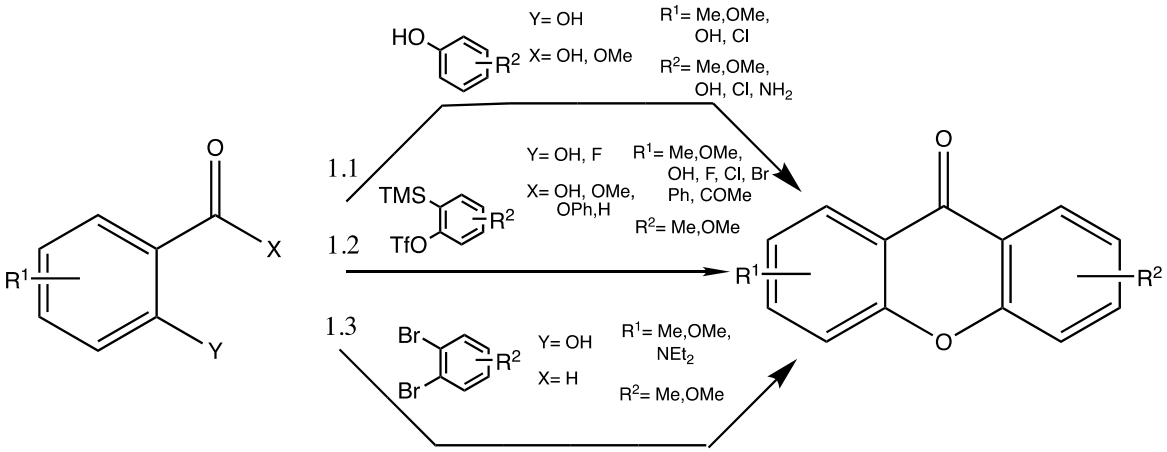


Figure 11. Syntesis of xanthones via aromatic building blocks.

The synthesis of xanthones *via* benzophenone is the most used of the methodologies referred herein for the synthesis of the xanthone scaffold because of the high yields obtained[44]. It is essentially constituted by two steps: the synthesis of a benzophenone and cyclization of it (Figure 12).

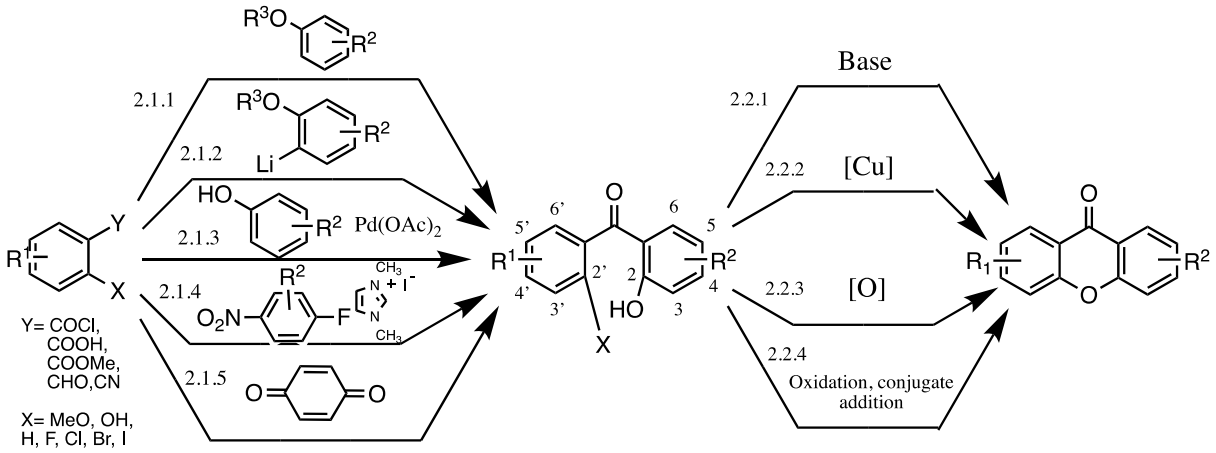


Figure 12. Synthesis of xanthones via benzophenone.

The synthesis of benzophenones is most commonly done by Friedel-Crafts acylations by the electrophilic aromatic substitution mechanism. In route 2.1.1, this reaction occurs in the presence of an acyl and a phenolic derivative (R3 = OH) or a protected phenol (R3 = alkyl), usually catalyzed by aluminum chloride[45]. However, this reaction has some limitations due to lack of regioselectivity, since both ortho and para positions can undergo acylation.

Another well-known strategy for the synthesis of benzophenones is that by the addition of an aryl intermediate of lithium to a carbonyl, pathway 2.1.2[46]. This is initiated by the formation of the strong nucleophile intermediate by the direct introduction of lithium into the benzene ring, or by exchange with a halogen. This nucleophile then reacts with a carbonyl group to give the benzophenone which will determine whether it occurs in one step or two. In the case of aldehydes, the formation of a secondary alcohol which requires further oxidation to form the benzophenone occurs first, whereas esters or acyl chlorides do so in one step.

Richard Larock obtained several benzophenones through reactions catalyzed by palladium, via route 2.1.3. The addition of C-H from arenes to nitriles leads to the formation of ketimines, which, through treatments that cause hydrolysis, give rise to benzophenones[47].

The nucleophilic aroylation catalyzed by imidazolidenyl carbenes, via 2.1.4, allows the formation of benzophenones from two electron-deficient construct blocks, which enables the introduction of aroyl groups at positions that are inaccessible by normal Friedel-Crafts acylations[48].

Pathway 2.1.5 describes the formation of benzophenones from photoacylation between a benzaldehyde and a β-quinone, which may serve as a viable alternative to Friedel-Crafts acylation[49].

The next step in the synthesis of xanthone is the cyclization of benzophenone through formation of an ether bridge between the positions adjacent to the carbonyl. The presence of at least one hydroxyl in one of these positions is necessary for the success of the reaction. The substituent present in the same position of the other ring will determine the mechanism followed by this stage of xanthone formation.

Cyclization can occur through nucleophilic aromatic substitution, intramolecularly catalyzed by a base, via 2.2.1., through the group hydroxyl referred in the previous paragraph and a functional group which behaves as a leaving group, on the other ring[50].

Another mode of cyclization is by oxidative coupling, via 2.2.2. Although not being as common as the above, it is very useful for the cyclization of 2-hydroxybenzophenone having a hydroxyl or methoxy in the 3' position, but that do not have a leaving group at the 2' or 6' position. This can occur in two ways: *p*-coupling which always corresponds to the major product, and the *o*-coupling, which is occasionally observed[51].

2.2.3 demonstrates that cyclization is possible through a copper-catalyzed intramolecular O-arylation. Unlike route 2.2.1, this allows for optimum yields of benzophenones having bromine or iodine as leaving groups through an Ullmann condensation, and is a green method as it is carried out using water as a solvent and where the catalyst can be recycled.[52]

Finally, in pathway 2.2.4, the xanthone is obtained by the oxidation of 2,5-dihydroxybenzophenone derivatives with the 3' position substituted by a free or protected hydroxyl group, followed by an intramolecular conjugated addition. This pathway is useful in the preparation of xanthones with hydroquinone groups[53].

The pathway towards xanthones via biphenyl ether is also done in two steps. The ether bond formation between the two aryl rings is first step of this methodology, followed by the cyclization of this intermediate (Figure 13). Compared to previous methodologies, this has lowest yields[44].

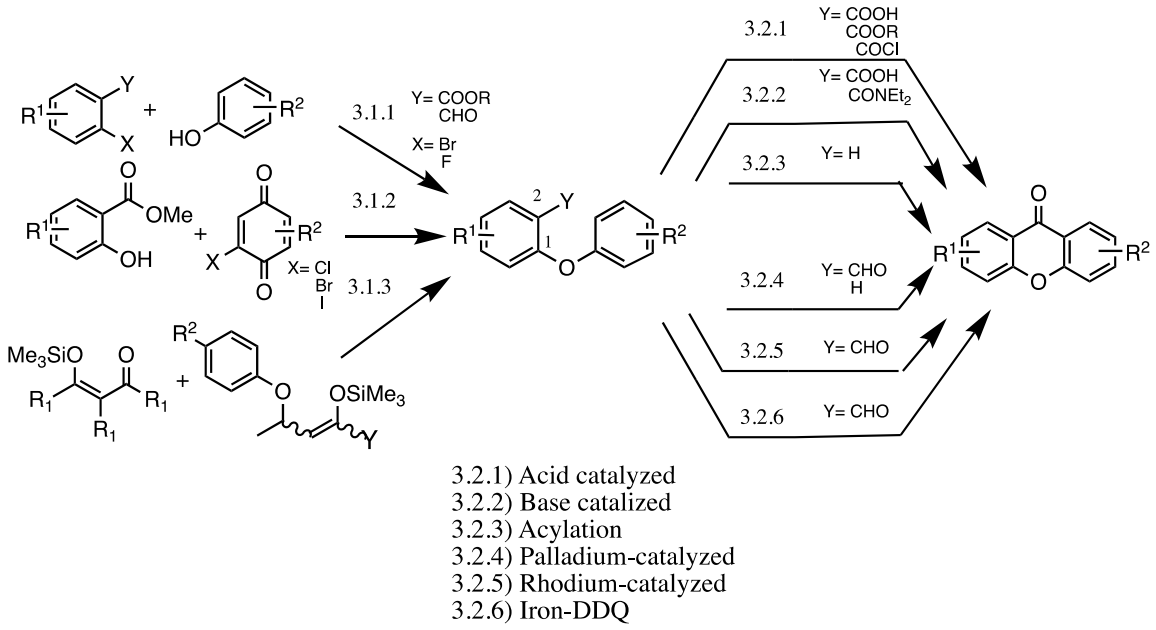


Figure 13. Syntesis of xanthones via biphenyl ether.

The ether bridge can be obtained through many different routes. Pathway 3.1.1 uses copper-catalyzed aryl halides and phenolic derivatives in most of the published articles[54,55,56], through Ullmann condensations, as previously mentioned in route 2.2.1, but in an intramolecular manner, since it is applied to a cyclization.

3.1.2 is an excellent way to obtain 1,4-dihydroxyxanthones or 1,4-dimethoxyxanthones[57], using the same approach used in intramolecular cyclization of benzophenones to xanthones in 2.2.4.

The formal cyclocondensation [22b], joins two arene groups through a C-C coupling, via 3.1.3.[58]. This route is especially useful when biphenyl ethers are hindered by their substituents. The yields for this are moderate but it is possible to obtain several substitution patterns.

The cyclization of the biphenyl ethers occurs through the formation of a carbonyl bridge between the two aryl rings. The strategy used for this purpose is highly dependent on the functionality of the 2' position and in the presence of a carboxylic acid a carboxylic acid derivative. In these cases, two situations can be considered: an electrophilic cycloacylation catalyzed by an acid[59], via 3.2.1 the most frequent; or an anionic Friedel-Crafts acylation, 3.2.2[60]. If there is no substituent in position two, it is necessary to introduce a carbonyl at that position through an intermolecular acylation and then a intramolecular cyclization may occur, via 3.2.3, or a palldium-catalyzed reaction may also be applied without the substituent[61], via 3.2.4. In the hypotheses of cyclization where position 2' is bearing an aldehyde, palladium, rhodium[62] (via 3.2.5) and iron catalysts[63] (via 3.3.6) are used.

1.5. Chiral Derivatives of Xanthones

The fact that biological systems have highly selectivity towards chirality and given the broad spectrum biological activities reported for xanthone derivatives, the research concerning chiral derivatives of xanthones (CDXs) and their pharmacological/biological properties constitute a very interesting and promising field. In our group of research, when using 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar 2) as chemical substrate for the synthesis of CDXs, bioactivity studies have shown inhibition of the growth of human tumour cell lines as well as enantioselectivity[22a]. CDXs of XCar 2 were also considered effective in the blocking of nerve conduction in the sciatic nerve of rats[22b]. Marona et al. has also given a great contribution to the field of CDXs, with reports where these structures were evaluated for activities such as anticonvulsant[64], local anaesthetic[64], cardiovascular[65], antifungal and antibacterial[66], antiarrhythmic[67], anti-platelet aggregation[68], their affinity with different adrenergic receptors[69], and more recently for antioxidant[70] and anticancer potential[71].

Studies of CDX within our group of research have also shown the viability in the use of these compounds as Pirkle-type chiral selectors in chiral stationary phases (CSP). Six new xanthonic chiral stationary phases (XCSPs) were developed using the well-established synthetic methodology, in just a few simple steps of reactions, like the chiral pool methodology mentioned before, with good yields[72]. The XCSPs were covalently bonded to fully porous silica, which guarantee long lasting columns, and show high stability, versatility in the selection of the mobile phase composition, and reproducibility. It was found that, in general, they show enantioselectivity in the separation of CDXs and other chiral compounds.

The literature has pointed out the antimicrobial activities of carboxylated xanthone derivatives, like the ones found in marine environments, yicathin B and C[73]. Also, Antimicrobial peptides (AMPs), also termed as host-defense peptides, have been extensively studied and are recognized to be the most promising choice for next-generation anti-infective agents because the probability of resistance emerging against AMPs is very low[74]. The merging of the two areas has lead to recent studies of amino acid modified xanthone derivatives that act as membrane targeting antimicrobials. In these, the xanthone scaffold was used as template for the amino acid derivatizations with cationic moieties and lipophilic chains, showing excellent results[75], but enantiantioselectivity was not taken into account.

This project follows this methodology in search for new antimicrobial agents. The well-known chemical subtract XCar 2 (Figure 14), a chemical analogue of yicathins, was coupled to a series of amino ester and further hydrolyzed to achieve a library of new CDXs for antimicrobial activity screening.

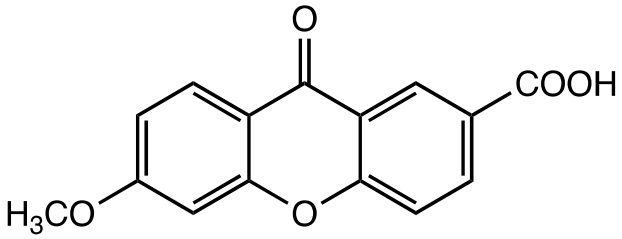


Figure 14. Chemical structure of **XCar 2**.

CHAPTER 2: RESULTS AND DISCUSSION

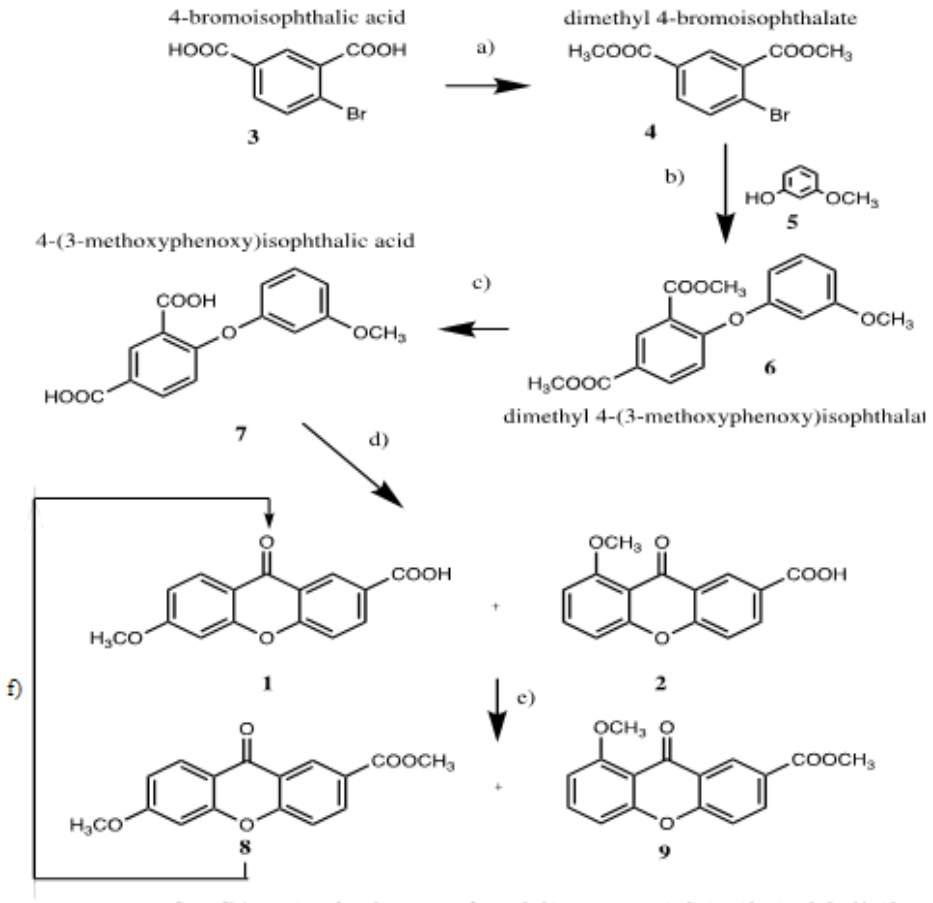
2. Results and Discussion

2.1. Synthesis and characterization of carboxyxanthone derivatives

Carboxyxanthone derivatives (XCars) are not only interesting bioactive compounds by itself but they can also be used as templates to obtain new chiral derivatives using the chiral pool strategy. Throughout this project, the total synthesis XCars 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar **2**, **1**) and 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar 3, **2**) was carried out by a multi-step pathway.

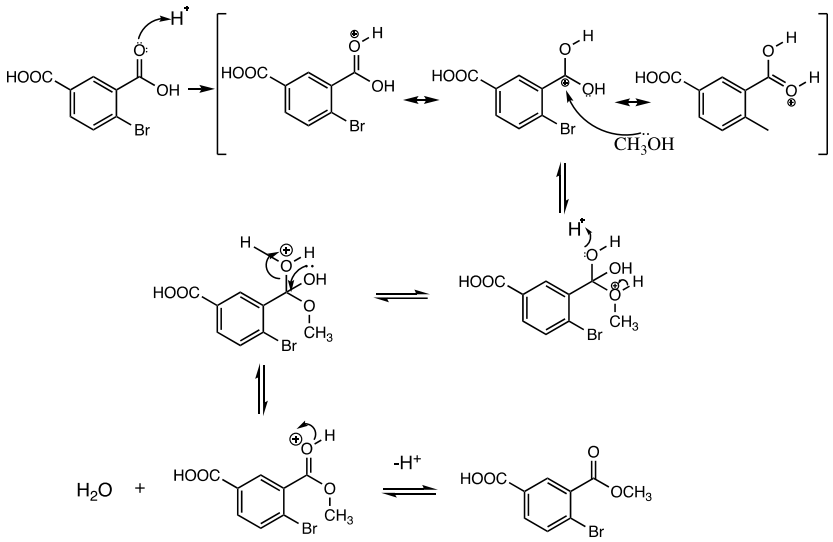
2.1.1. Synthesis of 6-methoxy-9-oxo-9H-xanthene-2-carboxylic acid (XCar 2) and 8-methoxy-9-oxo-9H-xanthene-2-catboxylic acid (XCar 3)

The multi-step pathway for synthesis of XCar 2 and XCar 3 is illustrated on the following Scheme 1. This process has already been described and used in our group of research[22a, 22b]. This synthetic pathway was followed as described, and a new experiment using polyphosphoric acid instead of Eaton's reagent in the intramolecular acylation step (**d**) was tried, in order to see if we could avoid the formation of both isomers.



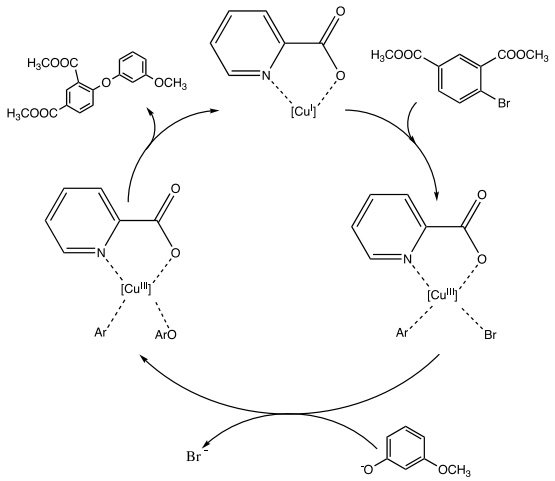
Scheme 1. Total synthesis of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (**XCar 2**, **1**) and 8-methoxy-9-oxo- 9*H*-xanthene-2-carboxylic acid (**XCar 3**, **2**).

The two carboxyxanthones, XCar 2 (**1**) and XCar 3 (**2**), were synthesized *via* Ullmann reaction, with the formation of the diaryl ether intermediate **6** from dimethyl 4-bromoisophthalate (**4**) and 3-methoxyphenol (**5**). The aryl bromide (**4**) was previously prepared from the corresponding carboxylic acid (**3**) by Fisher esterification (Scheme 2).



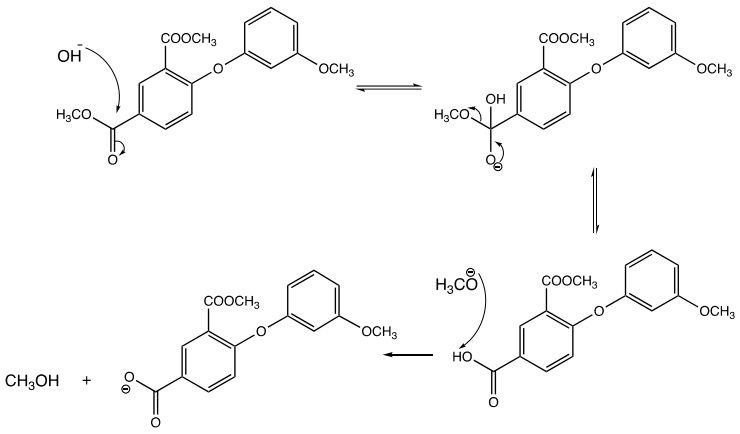
Scheme 2. Synthesis of dimethyl 4-bromoisophthalate by Fischer esterification of 4-bromoisophthalic acid.

The Ullmann condensation (Scheme 3) between **4** and **5** under the catalytic action of CuI and picolinic acid in combination with K_3PO_4 in dimethyl sulfoxide (DMSO) provided the diaryl ether **6**. This coupling reaction was carried out at 80 °C under nitrogen atmosphere for 28 h to give the compound **6**. The K_3PO_4/DMSO was used as a base/solvent combination and picolinic acid as a ligand for copper.



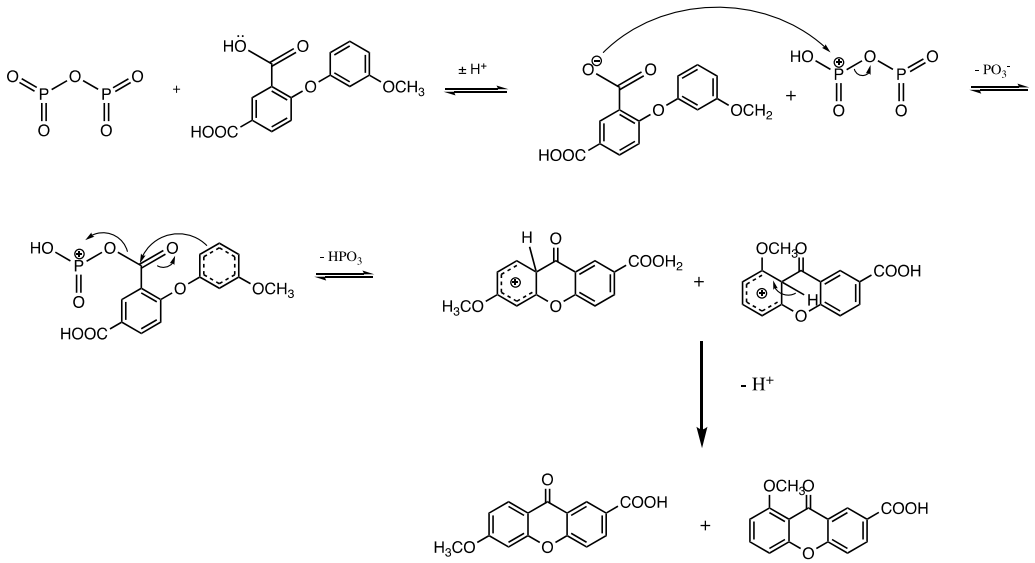
Scheme 3. Proposed mechanism for Cu-catalyzed Ullman reaction.

The diaryl ether **6** was dissolved in a mixture of tetrahydrofuran/methanol (1:1) and a 5M NaOH solution to hydrolyze the methyl esters (Scheme 4), affording the compound **7**.



Scheme 4. Mechanism for the hydrolysis of dimethyl 4-(3-methoxyphenoxy)isophthalate.

An intramolecular acylation of diaryl ether 6 (Scheme 5), using phosphorus pentoxide and methane sulfonic acid (Eaton's reagent) at room temperature resulted in two isomers, XCar 2 (**1**) and XCar 3 (**2**) in different yields. This was a consequence of the different positions for the electrophilic substitution, directed by the ring's substituents, and the steric hindrance between the moieties of the reaction products. For this step, it was tested the use of polyphosphoric acid (PPA) instead of Eaton's reagent, in hope that the bulkiness of PPA would avoid the formation of compound **3**. As result, both isomers were still formed, but it was observed a decrease in reaction time from 22h to 2 h, which constitutes a significant advance in the synthesis the chemical substrate **2**.



Scheme 5. Mechanism of intramolecular acylation reaction of 4-(3-methoxyphenoxy)isophthalic acid.

The separation of the two isomers was achieved through a Fischer esterification (reaction **e,** Scheme 1) followed by flash column chromatography or fractional crystallization, when feasible. After separation, the methyl esters of compounds **8** and **9** were hydrolyzed (reactions **f** and **g**, Scheme 1, respectively) in alkaline medium yielding the carboxyxanthones XCar 2 (**1**) and XCar 3 (**2**), respectively.

2.1.2. Structure elucidation of carboxyxanthone derivatives and their intermediates

IR spectroscopy, ^{1}H and ^{13}C NMR, and mass spectrometry were the techniques used to characterize the carboxyxanthone derivatives XCar 2 and XCar 3, along with their intermediates.

2.1.2.1. Structure elucidation of dimethyl 4-bromoisophthalate (4)

The compound dimethyl 4-bromoisophtalate (**4**) resulted from the esterification of 4-bromoisophthalic acid (**3**).

By comparing the IR data from both compounds (Table 1), we can conclude that the esterification reaction was successfully accomplished. For the acid compound (**3**), we can observe two broad bands at 2625 and 2880 cm^{-1}, corresponding to O-H bond of carboxylic acids, which are missing in the spectrum of the ester compound (**4**). Moreover, the shift of the band at 1687 cm^{-1} (C=O bond of carboxylic acid) to 1754 cm^{-1} (C=O bond of ester) confirmed that the conversion from COOH to $COOCH_3$ occurred.

Table 1. IR data of 4-bromoisophthalic acid (**3**) and dimethyl 4-bromoisophthalate (**4**).

Assignments	ν (cm^{-1})	
	(**3**)	(**4**)
C=O	1687	1754
C-O	1255	1309 and 1253
O-H	2880 (broad band)	--
C=C (aromatic)	930	929

Considering the ^{1}H NMR spectrum of compound **4** (Figure 15) it is important to highlight the presence of two singlets, at 3.95 ppm and 3.93 ppm, with integration for three protons each, that correspond to the protons of two methyl groups from the ester moieties. In the ^{13}C NMR spectrum of compound **4** it is possible to observe the presence of two signals at 52.7 and 52.5 ppm corresponding to the carbons from methyl group of the ester.

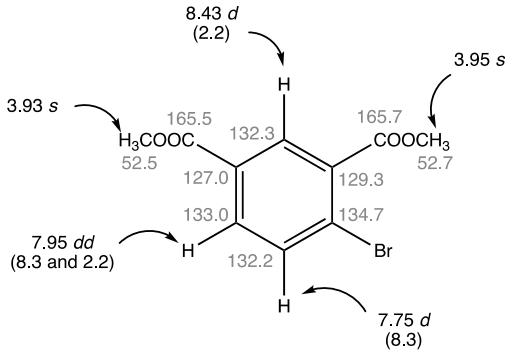


Figure 15. ^{1}H NMR (CDCl3, 300.13 MHz) and ^{13}C NMR (CDCl3, 75.47 MHz) of dimethyl 4-bromoisopthalate (**4**).

MS spectra of compound **4** showed the following peaks and *m/z* values: 273 $[M]^{+}$ (100), 256 (9), 240 (13), 221 (6), 209 (8), 203 (11). The presence of the respective molecular ion peak at the expected m/z values for this compound, confirmed the success of the reaction.

2.1.2.2. Structure elucidation of dimethyl 4-(3'-methoxyphenoxy) isophthalate (6) and 4-(3'-methoxyphenoxy) isophthalic acid (7)

Dimethyl 4-(3'-methoxyphenoxy) isophthalate resulted from the Ullmann diaryl ether coupling of dimethyl 4-bromoisophthalate (**4**) and 3-methoxyphenol (**5**). The compound 4-(3'-methoxyphenoxy) isophthalic acid (**7**) resulted from the consequent hydrolysis of the methyl esters from the ether product **6** of the Ullmann reaction. The IR, ^{1}H and ^{13}C NMR, and MS allowed the structure elucidation of compounds **6** and **7**.

Comparing the IR spectrum of compound **6** (Table 2) with the spectrum of its precursor **4** (Table 1), main difference important to refer is the presence of an extra absorption band C-O-C corresponding to the ether bond (1229 cm^{-1}) the IR spectra the ether product.

Comparing the IR spectra of compounds **6** and **7** (Table 2), it can be seen the presence of the absorption bands corresponding to C-O-C ether bond in both spectra, with IR frequencies not significantly different. The presence of a broad absorption band at 3411 cm^{-1}, corresponding to the O-H carboxylic acid bond on the spectrum of compound **7** confirmed that the reaction occurred successfully. Moreover, the shift of the band at 1719 cm^{-1} and 1724 cm^{-1} (C=O of $COOCH_3$) to 1680 cm^{-1} and 1694cm^{-1} (C=O of COOH) confirmed that occurred transformation from the ester to a carboxylic acid functional group.

Table 2. IR data of dimethyl 4-(3'-methoxyphenoxy)isophthalate (**6**) and 4-(3'-methoxyphenoxy)isophthalic acid (**7**).

Assignments	ν (cm^{-1})	
	(**6**)	(**7**)
C=C (aromatic)	1613 and 1488	1601 and 1486
C=O	1724 and 1719	1694 and 1680
O-H	--	2907 (Broad band)
C-O (ester)	1153	--
Ar-OCH_3	1274	1271
C-O-C	1229	1265

The ^{1}H NMR spectrum of compound **6** (Figure 16), compared to the spectrum of its precursor (**4**) (Figure 15), presented new signals such as one singlet at δ 3.79 ppm, with integration for three protons, corresponding to metoxy group, which confirmed the desired transformation.

Compounds **6** and **7** showed similar NMR profiles (Figure 16 Figure 17) with respect to the aromatic protons with similar chemical shifts and coupling constants. The most significant difference in the ^{1}H NMR spectra is to the absence in compound **7** spectrum of two singlets at δ 3.89 ppm and δ 3.93 ppm, with the integration for three protons each, corresponding to the protons of the two methyl groups from the ether moieties of compound **6**. This difference confirmed the success of the desired transformation.

The ^{13}C NMR spectrum of compound **6** compared to the spectrum of its precursor (**4**), presented a signal at δ 55.4 ppm, corresponding to the carbon of the metoxy group, and associated to the signals of aromatic carbons, confirmed the desired transformation.

The most significant differences between the ^{13}C NMR spectra of compounds **6** and **7** are: the shift of carbons signals from δ 165.3 and 165.7 ppm (ester) to δ 179.3 ppm (carboxylic acid), and the absence, in the spectrum corresponding to compound **7**, of two signals at δ 52.2 and 52.3 ppm, corresponding to the carbons of the two $COOCH_3$ methyl groups.

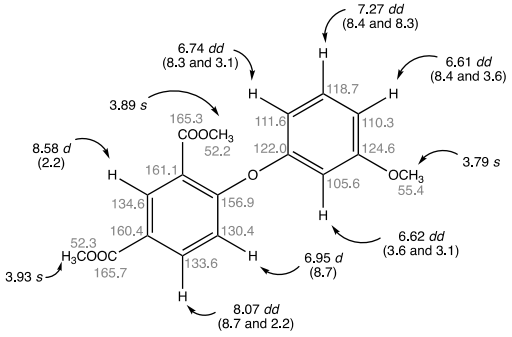


Figure 16. ^{1}H NMR ($CDCl_3$, 300.13 MHz) and ^{13}C NMR ($CDCl_3$, 75.47 MHz) of dimethyl 4-(3'- methoxyphenoxy)isophthalate (**6**).

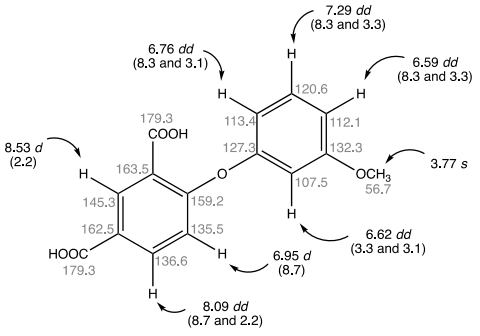


Figure 17. ^{1}H NMR ($CDCl_3$, 300.13 MHz) and ^{13}C NMR ($CDCl_3$, 75.47 MHz) of dimethyl 4-(3'- methoxyphenoxy)isophthalic acid (**7**).

MS spectra of compound **6** showed the following peaks and *m/z* values: 316 $[M]^+$ (100), 285 $[M\text{-}OCH_3]^+$ (55), 253 (54), 242 (10), 225 (16), 213 (12), 198 (25), 138 (15), 127 (10), 92 (15), 83 (14), 64 (11). The intense signal at 316 corresponding to the peak of the molecular ion, the base peak is consistent with the proposed structure.

MS spectra of compound **7** showed the following peaks and *m/z* values: 288 $[M]^+$ (100), 271 (10), 257 $[M\text{-}OCH]^+$ (12), 227 (11), 199 (23), 165 (61), 124 (63), 92 (23), 77 (20), 64 (19). The molecular ion peak at 288, which also corresponded to the most intense peak of the spectrum is consistent with the proposed structure.

2.1.2.3. Structure elucidation of XCar 2 (1) and methyl 6-methoxy-9-oxo-9H-xanthene-2-carboxylate (8)

The 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar 2, **1**) resulted from an intramolecular acylation reaction of the 4-(3'-methoxyphenoxy)isophthalic acid (**7**). Methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**8**) resulted from the esterification of XCar 2. The IR, ^1H and ^{13}C NMR, and MS allowed the structure elucidation of compounds **1** and **8**.

The IR spectrum from compound **1** shows the C=O carbonyl bands from the ketone and carboxylic acid overlapping at 1687 cm^{-1}. After esterification and IR analysis to compounds **8,** we can observe a shift of the band at 1687 cm^{-1} from the carboxylic acid carbonyl to 1730 cm^{-1} (C=O of $COOCH_3$), and the presence of the ketone band at 1663 cm^{-1}, an indication that cyclization was accomplished. In addition, the absence of a broad band at 3411 cm^{-1} (OH of COOH) in the IR spectrum of compound **8** is another proof that transformation from COOH to $COOCH_3$ was successful.

Table 3. IR data of XCar 2 (**1**) and methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**8**)

Assignments	ν (cm^{-1})	
	(**1**)	(**8**)
O-H	3411	--
C=O (ester)	--	1730
C=O (ketone)	1687	1663
C=O (carboxylic acid)	1687	--
C=C (aromatic)	1575, 1500 and 1433	1581,1467 and 1438
Ar-OCH_3	1271	1270
C-O (ester)	--	1117
C-H (aromatic)	766	764

The ^1H and ^{13}C NMR spectra data of the xanthones **1** (Figure 18) and 8 (Figure 19) are consistent to the proposed structure for these compounds, and allowed not only to confirm the success of the transformation, but also to structurally elucidate them.

The analysis of 1H NMR data of compounds **1** and **8** revealed the presence in of a singlet at δ 3.95 ppm in compound **8**, with the integration for three protons, corresponding to the $COOCH_3$ protons, which are absent in compound **1**.

The most significant differences between the ^{13}C NMR spectra of compounds **1** and **8** were associated to the signals of the carbons of the groups COOH and $COOCH_3$. The presence in the spectrum of compound **8** of the signal at δ 52.3 ppm corresponding to the carbon atom from the methyl group of the ester, confirmed the desired transformation from COOH to $COOCH_3$.

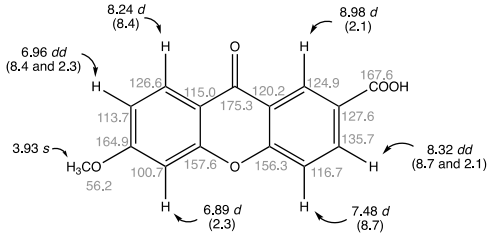


Figure 18. ^1H NMR (DMSO-D_6, 300.13 MHz) and ^{13}C NMR (DMSO-D_6, 75.47 MHz) of 6-methoxy- 9-oxo-9*H*-xanthene-2-carboxylic acid (XCar 2, **1**).

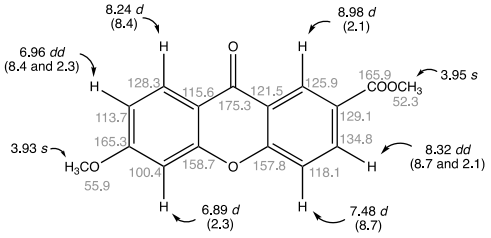


Figure 19. ^1H NMR ($CDCl_3$, 300.13 MHz) and ^{13}C NMR ($CDCl_3$, 75.47 MHz) of methyl 6-methoxy- 9-oxo-9*H*-xanthene-2-carboxylate (**8**).

The MS spectra of the compound **1** showed the following peaks and *m/z* values: 270 $[M]^+$. (100), 253 $[M\text{-}OH]^+$. (26), 226 (27), 199 (15), 182 (15), 169 (8), 154 (7), 139 (7), 126 (17), 115 (10), 63 (16). The strong signal at 284, corresponding to the peak of the molecular ion obtained is consistent with the proposed structure.

The MS spectra of the compound **8** showed the following peaks and *m/z* values: 284 $[M]^+$. (89), 253 $[M\text{-}OCH_3]^+$. (100), 225 (32), 197 (14), 182 (30), 169 (17), 154 (16), 142 (14), 126 (28), 111 (12), 75 (12), 63 (14). The intense signal at 270, corresponding to the peak of the molecular ion obtained is consistent with the proposed structure.

2.1.2.4. Structure elucidation of XCar 3 (2) and methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (9)

The 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar 3, **2**) resulted from an intramolecular acylation reaction of the 4-(3'-methoxyphenoxy) isophthalic acid (7). Methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**9**) resulted from the esterification of XCar 3 (**2**). The IR, ^1H and ^{13}C NMR, and MS allowed the structure elucidation of compounds **3** and **9**.

Like discussed in the previous section, it is possible to observe from the IR data of compounds **2** and **9** (Table 4) a decrease in the values of the C=O bands, from 1694 and 1680 cm^{-1} in 4-(3'-methoxyphenoxy)isophthalic acid, to 1687 and 1663 cm^{-1} in XCar 3. The value 1663 cm-1 is considered too low for a C=O from a carboxylic acid, but acceptable for a xanthone derivative ketone, indicating that the cyclization was successful.

In the IR spectra of compounds **2** and **9** the main changes are: the shift of the band at 1687 cm-1 (C=O of COOH) to 1726 cm-1 (C=O of $COOCH_3$), as well as the absence of a broad band at 3460 cm-1 (OH of COOH) in IR spectrum of compound **9**. From this information, we can consider that the transformation from COOH to $COOCH_3$ occurred.

Table 4. IR data of XCar 3 (**2**) and methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**9**).

Assignments	ν (cm^{-1})	
	(**2**)	(**9**)
O-H	3460	--
C=O (ester)	--	1726
C=O (ketone)	1663	1668
C=O (carboxylic acid)	1687	--
C=C (aromatic)	1420, 1469 and 1603	1431, 1480 and 1611
Ar-OCH_3	1266	1264
C-O (ester)	--	1079
C-H (aromatic)	763	760

The ^{1}H and ^{13}C NMR spectra data are in agreement with the proposed structure for compounds **2** and **9** (Figure 20 and 21).

The analysis of 1H NRM data of compounds **2** and **9** revealed that both compounds presented a singlet at δ 3.94 and 3.98 ppm, respectively, with integration for three protons, corresponding to the group metoxy group protons. Moreover, it is also important to note the presence in compound **9** spectrum of a singlet at δ 4.05 ppm, with the integration for three protons, corresponding to the group $COOCH_3$ protons, which are absent in xanthone **2**.

The most significant difference between the ^{13}C NMR spectra of compounds **3** and **9** was related to the signals of the carbons of the groups COOH and $COOCH_3$. The presence in the spectrum of compound **9**, an extra signal at δ 52.4 ppm corresponding to the methyl group from the ester, indicates that the desired transformation was successful.

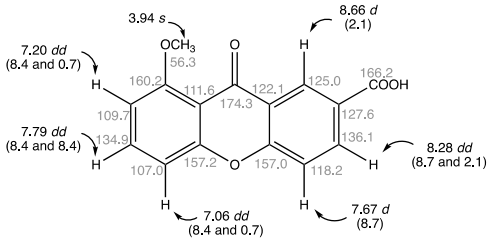


Figure 20. ^{1}H NMR (DMSO-D_6, 300.13 MHz) and ^{13}C NMR (DMSO-D_6, 75.47 MHz) of 8-methoxy- 9-oxo-9*H*-xanthene-2-carboxylic acid (XCar 3, **2**).

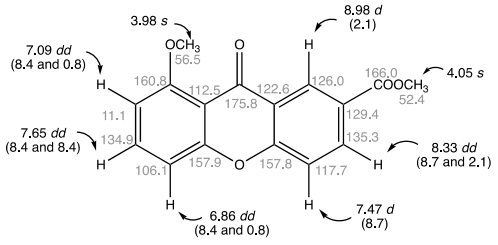


Figure 21. ^{1}H NMR ($CDCl_3$, 300.13 MHz) and ^{13}C NMR ($CDCl_3$, 75.47 MHz) of methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**9**).

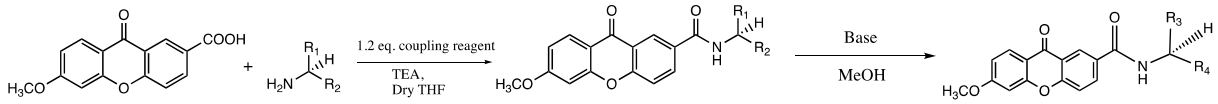
The xanthone 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (**3**) showed the following values for HRMS (ESI) m/z: calcd for ($C_{15}H_{10}O_5$ + H): 271.16994, found: 271.06010.

The MS spectra of the compound **9** showed the following peaks and *m/z* values: 284 ($[M]^+$. (100), 255 (56), 253 (30), 197 (14), 238 (56), 223 (47), 195 (27), 139 (33), 126 (20), 111 (18), 70 (15), 63 (**12**).

2.2. Synthesis of chiral derivatives of xanthones

Twelve new chiral derivatives of xanthones (CDXs) were synthesized by coupling reaction of the carboxyxanthones 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar 2, **1**) with a variety of commercially available, enantiomerically pure, amino esters from proteinogenic amino acid building blocks, through an amide bond formation (Scheme 6).

For the second step of the scheme below, nine CDXs were obtained by hydrolysis of the ester CXDs.

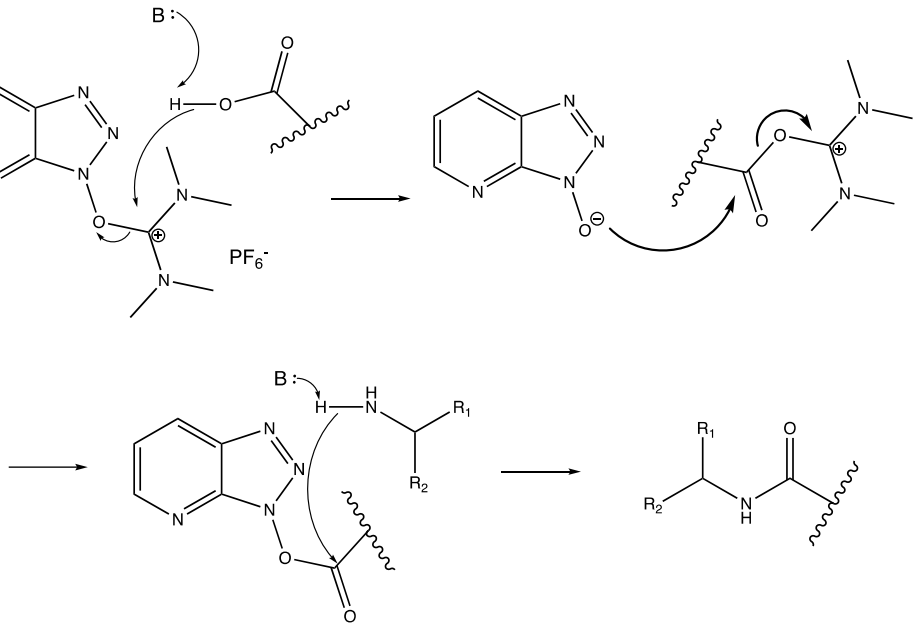


Scheme 6. Synthesis of CDXs.

2.2.1. Synthesis of chiral xanthone derivatives from amino esters

In these coupling reactions, the activation of the carboxylic acid group attached to the xanthonic scaffold in chemical substrate (**1**) was carried out using *O*-(benzotriazol-1-yl-)-*N*,*N*,*N*',*N*'-tetramethylluronium tetrafluoroborate (TBTU), in a methodology well established in our research group[22b,22a,] and (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-

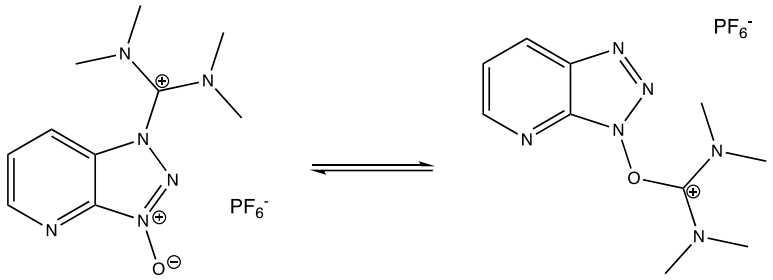
morpholino-carbenium hexafluorophosphate (COMU), as coupling reagent. Catalytic amount of a base, triethylamine (TEA), in dry tetrahydrofuran (THF) were used in the reaction conditions (Scheme 6). The coupling reaction mechanism with TBTU is represented in Scheme 7.



Scheme 7. Mechanism for the coupling reaction using TBTU.

TBTU is one of the most used coupling reagents due to its higher efficiency and lower tendency towards racemization. COMU, like TBTU, is part of the Aminium/Uronium-Imonium coupling reagents category, but in the first, the dimethylamino moiety is replaced with a more polar morpholino group, which has proved to enhance stability, solubility, and reactivity of the reagent[76].

TBTU, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), and similar peptide coupling reagents based on benzotriazoles predominantly exist in the less reactive guanidinium or N-form, which is less reactive than the uronium or O-form[77] (Scheme 8).



Scheme 8. Conversion of TBTU in its guanidinium or N-form and uronium or O-form.

Notably, COMU (Figure 22) only appears to exist as the more reactive uronium structure[78]. In addition to its high and fast coupling efficiency, it shows very low or non-existent tendencies for racemization and by-products formed by COMU are water-soluble and can be separated by simple extraction. Also, in some coupling reactions, a color change throughout it allows visual or colorimetric reaction monitoring (Figure 23).

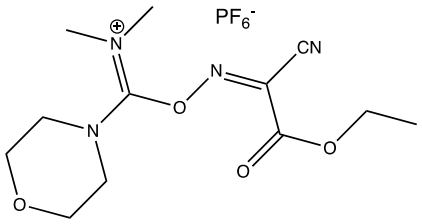
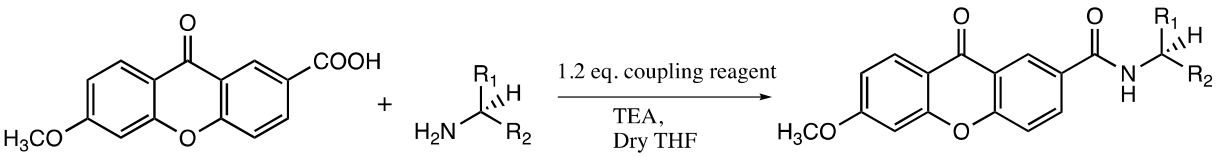


Figure 22. Chemical structure of COMU.

Figure 23. Colorimetric coupling reaction with COMU.

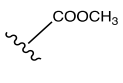
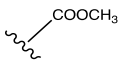
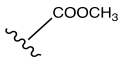
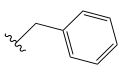
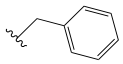
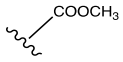
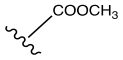
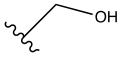
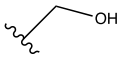
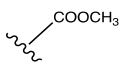
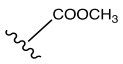
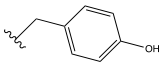
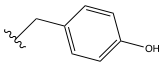
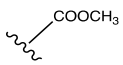
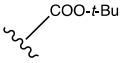
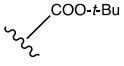
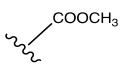
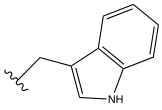
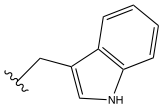
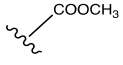
The coupling reactions (Scheme 9) performed throughout this project, as demonstrated in Table 5 showed poor to fair yields and different reaction times (50 min – 24 h), depending on the coupling reagent used. Comparing the couplings with COMU and TBTU, we can conclude that although yields were not significantly different, the reaction times changed from a 11 to 22 hour range with TBTU to 1h to 1h and 30 min for COMU. On the other hand, reaction with COMU always originated side-products, which was not observed with TBTU. The purification procedures, involving acid-base liquid-liquid extraction and fractional crystallization, were mostly hard and a setback. In most cases the starting material XCar 2 was not completely

consumed and extractable with liquid-liquid acid-base extraction. Isolation of the CDXs was achieved through fractional crystallization, using methanol and dichloromethane as solvents. In **Annex 1**, a table with the corresponding chemical synonyms and structures for the code names of the following compounds obtained is available.



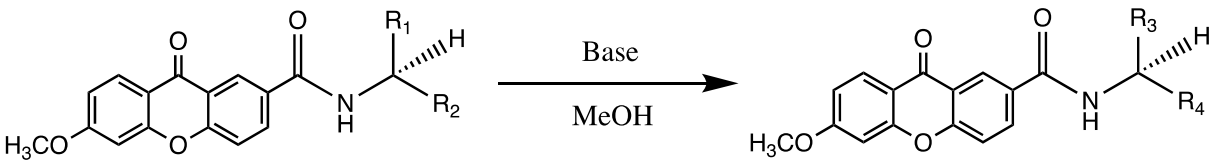
Scheme 9. General synthesis of CDXs from amino esters.

Table 5. Results obtained for the synthesis of CDXs from amino esters.

CDX	R_1	R_2	Coupling Reagent	Mass (g)	Yield (%)	Reaction Time	Crystalization
X2AEDPG-Me (**10**)			COMU	0.0373	38	1h 30min	MeOH
X2AELPG-Me (**11**)			COMU	0.0556	53	1h 30min	MeOH
X2AEDPA-Me (**12**)			COMU	0.0931	59	50min	MeOH
X2AELPA-Me (**13**)			TBTU	0.0048	6	16h	MeOH
X2AEDS-Me (**14**)			TBTU	0.0279	60	15h	DCM
X2AELS-Me (**15**)			COMU TBTU	0.0103 0.0114	26 25	1h 11h	DCM
X2AEDT-Me (**16**)			COMU	0.0063	13	1h	MeOH
X2AELT-Me (**17**)			TBTU	0.0299	64	22h	MeOH
X2AEDA-TB (**18**)			TBTU	0.4477	89	21h	MeOH
X2AELA-TB (**19**)			TBTU	0.1728	91	21	MeOH
X2AEDTrp-Me (**20**)			TBTU	0.0054	4	12h	MeOH
X2AELTrp-Me (**21**)			COMU	0.04617	51	1h	MeOH

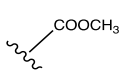
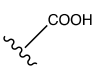
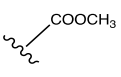
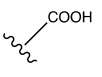
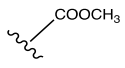
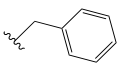
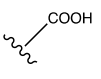
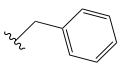
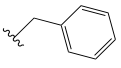
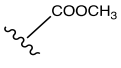
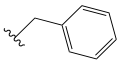
2.2.2. Synthesis of CDXs from amino acids

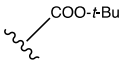
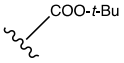
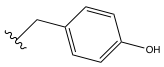
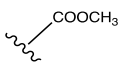
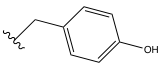
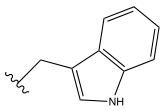
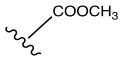
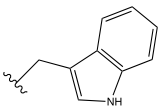
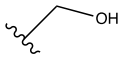
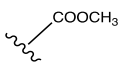
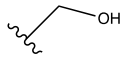
After the coupling reactions and the purifications of its products, some of the derivatives were hydrolyzed under basic conditions, in order to afford the correspondent amino acid derivatives (Scheme 10). This procedure was only done for the amino ester derivatives that were possible to obtain in good quantities. The reaction follows the typical basic hydrolysis mechanism, described in scheme 4 specifically for the intermediate of XCar 2 and 3. In the table 6, it is possible to see the results obtained for these reactions, as well as the alkaline conditions used in each case. In order to avoid hydrolysis of the amide bond and also racemization, mild alkali conditions were a priority, but for the alanine derivatives that were protected with a bulky *tert*-butyl group, this forced the use of a 6M NaOH solution and longer reaction times, compared with the 1 hour and 30 minutes and 0,25M NaOH in the methyl esters hydrolysis. These reaction where obtained with yields that range from 37% for X2AALA, to 95% for X2AADA.



Scheme 10. Hydrolysis reaction of CDXs from amino esters (CDX 10-21).

Table 6. Results obtained for the hydrolysis of the amino ester derivatives.

CDX	R_1	R_2	R_3	R_4	Base	Reaction Time	Yield (%)
X2AADPG (**22**)					NaOH 0,25M	1h 30min	48
X2AALPG (**23**)					NaOH 0,25M	1h 30min	66
X2AADPA (**24**)					NaOH 0,25M	1h 30min	60
X2AALPA (**25**)					NaOH 0,25M	1h 30min	47

X2AADA (**26**)					NaOH 6M	24	95
X2AALA (**27**)					NaOH 6M	24	36
X2AALT (**28**)					NaOH 0,25M	1h 30min	66
X2AALTrp (**29**)					NaOH 0,25M	1h 30min	70
X2AADS (**30**)					NaOH 0,25M	1h 30min	53

2.2.3. Structure elucidation of CDXs

The chiral derivatives of xanthone obtained were submitted to techniques such as IR, ^{1}H and ^{13}C NMR for its chemical structure analysis.

The table below shows the values of IR frequencies registered for the expected products of the CDXs synthesis, which gave valuable information to confirm the presence of the expected functional groups for these compounds.

Table 7. IR data from CDXs.

CDX	ν assignments (cm^{-1})									
	C=O (ketone)	C=O (ester)	C=O (carboxylic acid)	C=O (amide)	N-H	O-H	C-N	Ar-OCH_3	C-H (aromatic)	C=C (aromatic)
X2AEDPG-Me (**10**)	1660	1739	--	1620	3295	--	1542	1274	673, 698, 728, 758, 780 and 832	1442, 1478, 1496, and 1589
X2AELPG-Me (**11**)	1661	1740	--	1621	3293	--	1542	1282	674, 698, 729, 756, 779 and 834	1442, 1478, 1497, and 1589
X2AEDPA-Me (**12**)	1660	1744	--	1618	3315	--	1536	1274	669, 700, 758 and 834	1443, 1478 and 1588
X2AELPA-Me (**13**)	1671	1731	--	1622	3413	--	1566	1273	670, 687, 763 and 837	1443, 1483 and 1590
X2AEDS-Me (**14**)	1666	1752	--	1622	3317	Broad Band 3405	1546	1215	663, 701, 780, 831 and 849	1445, 1480
X2AELS-Me (**15**)	1663	1752	--	1620	3316	Broad Band 3411	1546	1284	674, 701, 767 and 852	1444, 1480

X2AEDT-Me (16)	1646	1741	--	1615	3368	Broad Band 3405	1541	1272	669, 759 and 836	1445, 1479, 1516 and 1588
X2AELT-Me (17)	1644	1741	--	1615	3347	Broad Band 3347	1541	1276	668, 758 and 836	1446, 1478, 1516 and 1587
X2AEDA-TB (18)	1657	1733	--	1620	3309	--	1540	1274	670, 760, 780 and 833	1445, 1479, 1566 and 1589
X2AELA-TB (19	1655	1735	--	1620	3311	--	1541	1275	670,760, 780 and 834	1446, 1479, 1567 and 1590
X2AEDTrp-Me (20)	1653	1735	--	1621	3311	--	1539	1274	669, 690, 745 and 835	1445, 1479, 1537, 1565 and 1588
X2AELTrp-Me (21)	1651	1740	--	1616	3312	--	1539	1275	668, 689, 745 and 835	1444, 1477, 1565 and 1588
X2AADPG (22)	1652	--	1734	1615	3365	Broad Band 3385	1520	1271	673, 696, 711, 724, 761, 784 and 835	1440, 1449, 1474, 1521 and 1582
X2AALPG (23)	1653	--	1732	1614	3366	Broad Band 3385	1520	1270	669, 696, 723, 761, 783 and 836	1441, 1448, 1474 and 1582
X2AALA (27)	1664	--	1734	1613	3414	Broad Band 3341	1539	1283	674, 756, 779, 805, 836 and 852	1452, 1477, and 1578
X2AADA (26)	1662	--	1732	1614	3411	Broad Band 3343	1539	1281	674, 756, 779, 805, 836 and 852	1452, 1477, and 1578
X2AADPA (24)	1647	--	1733	1616	3342	Broad Band 3342	1540	1276	670, 701, 759, 780 and 834	1446, 1478, 1497, 1564 and 1586
X2AALTrp (29)	1649	--	1730	1615	3404	Broad Band 3404	1538	1276	670, 744, 779 and 835	1445, 1477, 1566 and 1586
X2AALT (28)	1646	--	1729	1615	3383	Broad Band 3383	1540	1277	671, 761, 780 and 836	1446, 1478, 1516, 1564, 1586
X2AADS (30)	1648	--	1730	1623	3422	Broad Band 3442	1566	1272	662, 735, 758, 781 and 835	1448, 1481, and 1590

Through analysis of Table 7, it was verified that the coupling reactions were accomplished. The presence of the two stretching bands correspondent to the amide N-H bond (3300-3500 cm^{-1}), the amide carbonyl C=O stretching (1680-1620 cm^{-1}) and the C-N stretch band (~1550 cm^{-1}) indicate that an amide bond was formed.

The ketone carbonyl band (1700-1640 cm^{-1} for conjugated ketones) is present in all of the spectrums, and sometimes it appears as a doublet because of Fermi resonance effect, which causes the splitting of the band[79].

It can also be noticeable a slight overall decrease in the frequencies from the ester to the carboxylic acid carbonyl that belong the amino ester and the amino acid derivatives, respectively. In the spectrums of the amino acid derivatives we can observe a broad band between 3300 and 3400 cm^{-1}, which corresponds to the O-H bond of carboxylic acids in the deprotected derivatives, that indicates that the hydrolysis was successful. Moreover, the strong band of the N-H from the amide moiety at ~3300 cm^{-1} was still present after hydrolysis, showing that the amide bond remained intact.

All compounds obtained were analyzed through ^{1}H and ^{13}C NMR spectroscopy, in order to verify if the chemical structure was the one expected for each CDX. The following figures represent the chemical structure and signal assignments of protons and carbons, for one of the enantiomers from the different amino ester and amino acid derivatives.

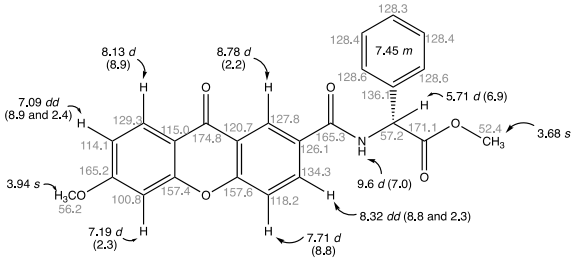


Figure 24. ^{1}H NMR (DMSO-D_6, 300.13 MHz) and ^{13}C NMR (DMSO-D_6, 75.47 MHz) of methyl (*R*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-2-phenylacetate (X2AEDPG-Me).

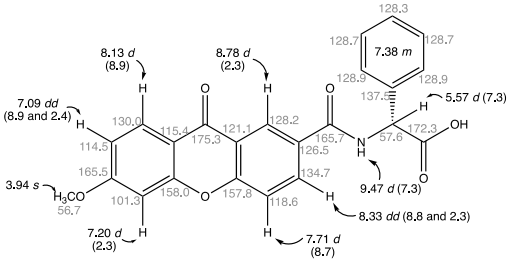


Figure 25. ^{1}H NMR (DMSO-D_6, 300.13 MHz) and ^{13}C NMR (DMSO-D_6, 75.47 MHz) of (*R*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-2-phenylacetic acid (X2AADPG).

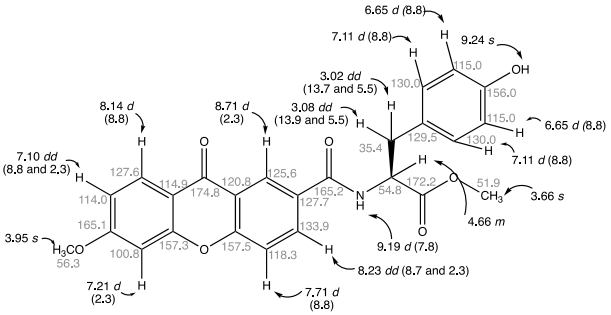


Figure 26. ^{1}H NMR (DMSO-D_6, 300.13 MHz) and ^{13}C NMR (DMSO-D_6, 75.47 MHz) of methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tyrosinate (X2AELT-Me).

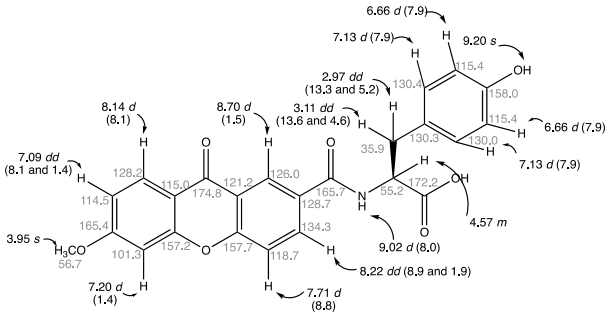


Figure 27. ^{1}H NMR (DMSO-D_6, 300.13 MHz) and ^{13}C NMR (DMSO-D6, 75.47 MHz) of 6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tyrosinate (X2AALT.)

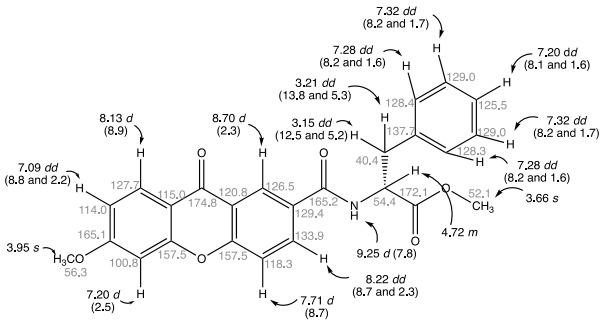


Figure 28. ^{1}H NMR (DMSO-D$_6$, 300.13 MHz) and ^{13}C NMR (DMSO-D$_6$, 75.47 MHz) of methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-phenylalaninate (X2AEDPA-Me).

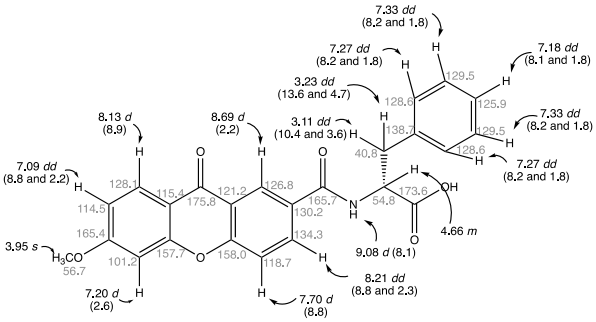


Figure 29. ^{1}H NMR (DMSO-D6, 300.13 MHz) and ^{13}C NMR (DMSO-D$_6$, 75.47 MHz) of (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-phenylalanine (X2AADPA).

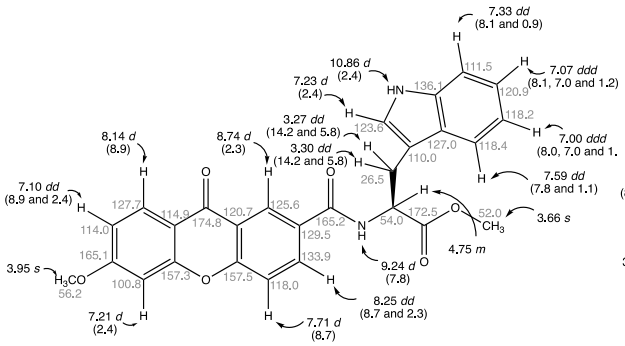


Figure 30. ^{1}H NMR (DMSO-D$_6$, 300.13 MHz) and ^{13}C NMR (DMSO-D$_6$, 75.47 MHz) of methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tryptophanate (X2AELTrp-Me).

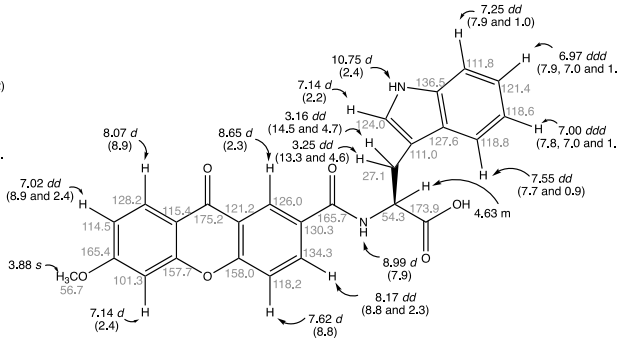


Figure 31. ^{1}H NMR (DMSO-D$_6$, 300.13 MHz) and ^{13}C NMR (DMSO-D$_6$, 75.47 MHz) of (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tryptophan (X2AALTrp).

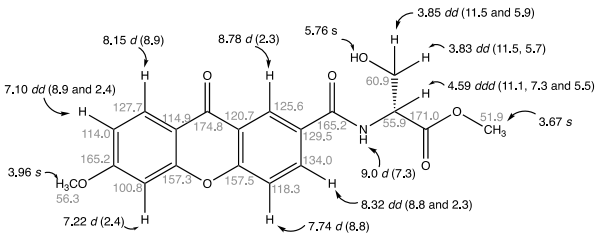


Figure 32. ^{1}H NMR (DMSO-D6, 300.13 MHz) and ^{13}C NMR (DMSO-D$_6$, 75.47 MHz) of methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-serinate (X2AEDS-Me)

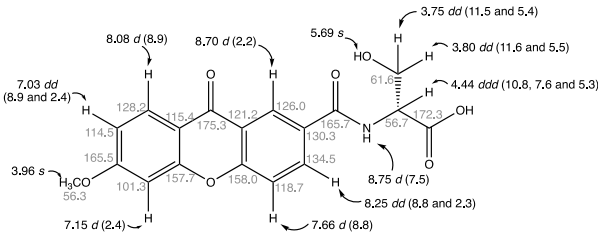


Figure 33. ^{1}H NMR (DMSO-D$_6$, 300.13 MHz) and ^{13}C NMR (DMSO-D$_6$, 75.47 MHz) of (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-serine (X2AADS).

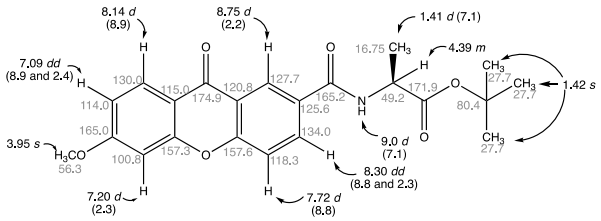


Figure 36. ^{1}H NMR (DMSO-D$_6$, 300.13 MHz) and ^{13}C NMR (DMSO-D$_6$, 75.47 MHz) of *tert*-butyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-alaninate X2AELA-TB.

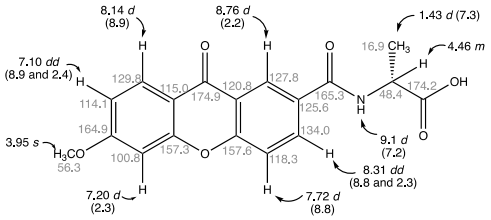


Figure 37. ^{1}H NMR (DMSO-D$_6$, 300.13 MHz) and ^{13}C NMR (DMSO-D$_6$, 75.47 MHz) of (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-alanine (X2AALA).

The ^{1}H NMR and the ^{13}C NMR spectra of all CDXs showed chemical shifts corresponding to the aromatic protons and carbons of the xanthonic scaffold. The most significant differences of ^{1}H NMR and the ^{13}C NMR of the CDXs, comparing with the spectrum of its precursor, XCar 2 (**1**), correspond to the presence of new spectral signals from the amino ester and correspondent amino acid, which confirms the success of the coupling reactions. From these it is possible also possible to notice an increase in most of the chemical shifts from the amino acids, comparing with those from the correspondent amino ester derivative. Furthermore, the suppression o the signal that corresponds to the protecting groups of the ester indicates that the hydrolysis was successful. It is also important to point out the presence in ^{1}H NMR spectra of the chemical shift between 9.25 - 8.75 ppm corresponding to the proton of amide group, the presence multiplet signal from the proton bonded to the Cα at around 5.50 ppm, and the presence in ^{13}C NMR spectra of the chemical shift around 165.5 ppm corresponding to the carbonyl of amide group in all CDX spectrums.

2.3. Enantiomeric purity

Liquid chromatography (LC) has proven to be one of the best methods for the separation and analysis of enantiomers and the use of chiral stationary phase (CSPs) is the most helpful between the currently analytical methods to determine the enantiomeric purity[80,81,81]

The chiral separation method should take only a short analysis time and preferably use inexpensive and "green" solvents. Herein, we explored the LC enantioseparation of the synthesized CDXs using a commercially available CSP Lux™ 3 µm cellulose-2, which demonstrated a very good enantioresolution performance for all the CDXs, with excellent separation (α) values and resolution (Rs) factors above baseline resolution, except for CDXs of from serine (Table 8). The mobile phase was optimized with 0.01% TFA : ethanol with 0.01% TFA and *n*-hexane (50:50 *v/v*) at a flow rate of 1.0 mL min^{-1} in isocratic mode and the diode array detection at 264 nm.

Table 8. HPLC separation values for CDXs enantiomeric mixtures.

Enantiomeric mixtures of CDXs	k' 1	k' 2	α	Rs
X2AEA-TB	1.12	1.08	1.25	3.71
X2AEPG-Me	4.5	5.39	1.2	2.11
X2AEPA-Me	3.57	4.07	1.14	1.89
X2AES-Me		No separation		
X2AET-Me	2.98	3.56	1.2	2.32
X2AETrp-Me	4.62	5.36	1.16	1.79
X2AAA	1.47	2.33	1.59	3.77

The chromatographic parameters obtained in the enantiomeric separation method using Lux™ 3 chiral column allowed evaluating the enantiomeric purity of the CDX synthesized.

Table 9. HPLC data values for enantiomeric purity studies.

Compound	Major Peak Area	Minor Peak Area	Peak Purity	tR (min)	ee(%)	ec(%)
X2AELA-TB	1100576	23121	1.000	23.87	95.9	97.9
X2AEDA-TB	13093404	0	1.000	29.68	>99.9	>99.9
X2AEDPA-Me	19517555	270988	1.000	9.04	97.3	98.6
X2AELPA-Me	13059376	466105	1.000	8.21	93.1	96.6
X2AELPG-Me (11)	21162292	662889	0.999	8.37	93.9	97.0
X2AEDPG-Me (10)	12387395	265546	1.000	9.84	95.8	97.9
X2AEDS-Me (14)	16636040	No separation	1.000	12.27	No separation	
X2AELS-Me (15)	8863264		1.000	12.16		
X2AEDT-Me (16)	29379004	114135	1.000	8.27	99.2	99.6
X2AELT-Me (17)	10886420	0	1.000	7.12	>99.9	>99.9
X2AEDTrp-Me (20)	4968681	17995	1.000	11.36	99.3	99.6
X2AELTrp-Me (21)	20204880	0	1.000	10.21	>99.9	>99.9
X2AADPA (24)	11641868	174337	1.000	6.45	97.1	98.5
X2AADPG (22)	8310889	6517952	0.999	10.61	12.1	56.0
X2AALPG (23)	11175438	8205380	0.999	6.75	15.3	57.7
X2AADS (30)	2751601	1006130	0.999	13.76	46.5	73.2
X2AALT (28)	14401998	30273	1.000	5.28	99.6	99.1
X2AALTrp (21)	10687161	161752	1.000	7.41	97.0	98.5
X2AALA (27)	5413290	295750	1.000	4.72	89.6	94.8
X2AADA (26)	5753610	293701	1.000	6.37	90.3	95.1

The results for enantiomeric purity (Table 9) showed excellent values of enantiomeric excess for the amino ester derivatives, with values of ee and ec above 90%, and some derivatives, like X2AELT-Me and X2AELTrp-Me reached approximately 100%, considering the methodology used (Figure 40). The amino acids derivatives obtained were also submitted to enantiomeric excess evaluation, in which they exhibited very good results too, with exception for both the enantiomers of phenylglycine. For phenylglycine, we can be sure that racemization occurred because separation of the amino ester racemic mixture was successfully done with excellent chromatographic parameters, and the single enantiomers showed to be enatiomerically pure before going under hydrolysis. This phenomenon is thought to happen because the chiral center of these serine and phenylglycine are bonded to an hydroxymethyl and a phenyl group, respectively, which make the chiral proton acidic, allowing it to leave the structure and interconverting the molecule in its enantiomer, under basic conditions. The values for D-serine

were also unsatisfactory, but with no separation of the amino esters, we can't conclude that this was because of racemization, although its side chain also turn the alpha proton more acidic.

Furthermore, from analysis of the chromatographic retention times and peak purities of the CDXs, we can verify that the *L*- enantiomer was always the first one to elute and all peaks showed to be pure. In the next figures, the chromatograms of phenylglycine amino esters and amino acids which show the presence of two peaks. The chromatograms for tyrosine amino ester separations, for the hydrolyzed final product X2AALT and starting material X2AELT-Me are shown, as an exemple of a successful synthesis of enantiomerically pure compounds.

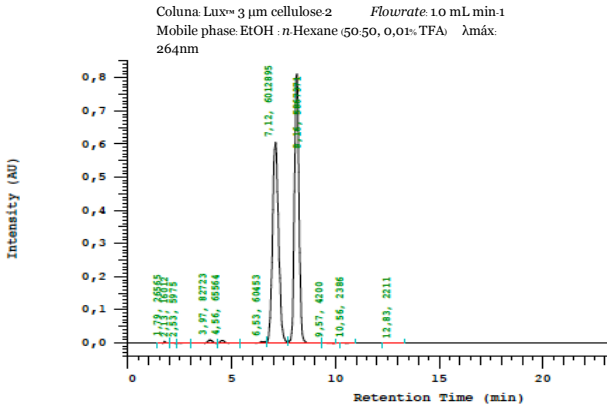


Figure 34.Chromatogram from the HPLC analysis of X2AELT-Me+X2AEDT-Me mixture.

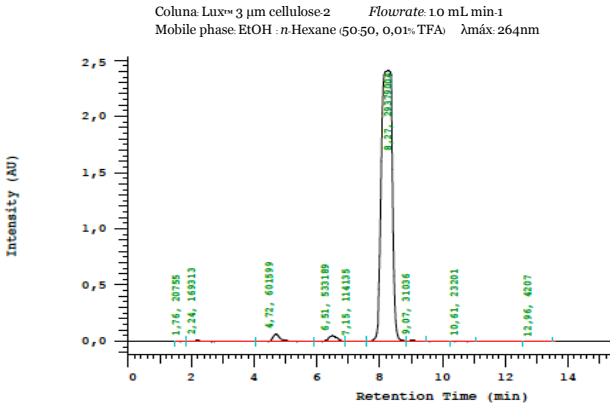


Figure 35. Chromatogram from the HPLC analysis of X2AEDT-Me.

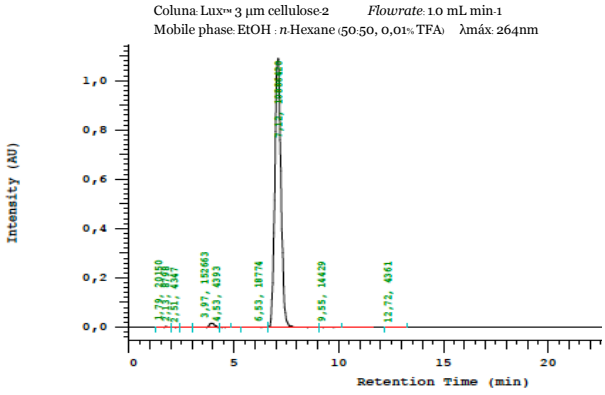


Figure 36. Chromatogram from the HPLC analysis of X2AELT-Me.

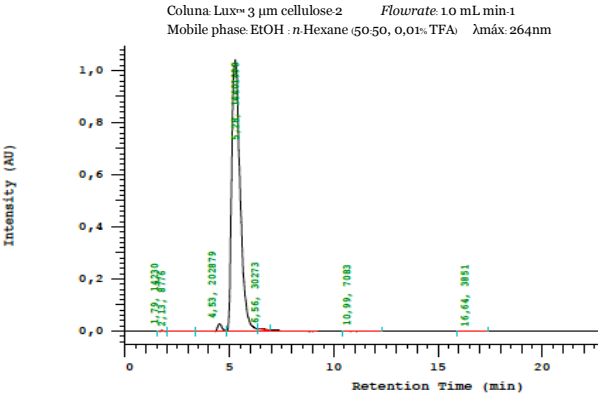


Figure 37.Chromatogram from the HPLC analysis of X2AAT.

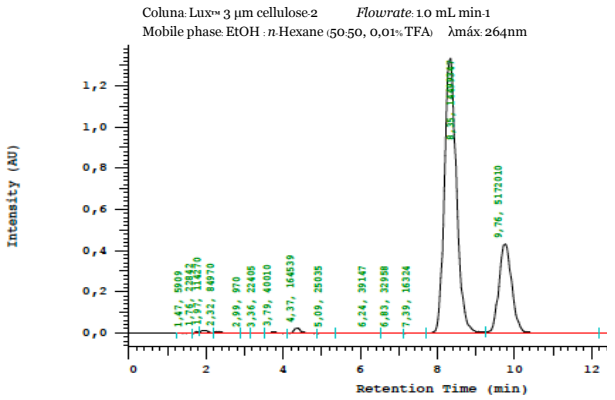


Figure 38.Chromatogram from the HPLC analysis of X2AELPG-Me+X2AEDPG-Me mixture.

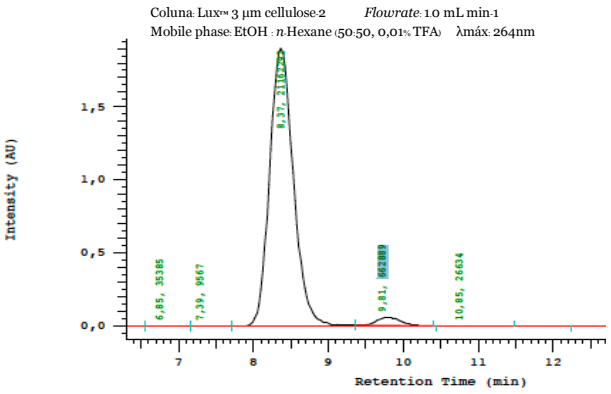


Figure 39.Chromatogram from the HPLC analysis of X2AELPG-Me.

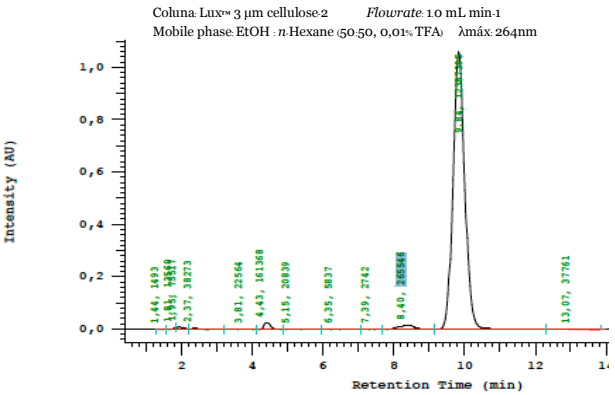


Figure 40.Chromatogram from the HPLC analysis of X2AEDPG-Me.

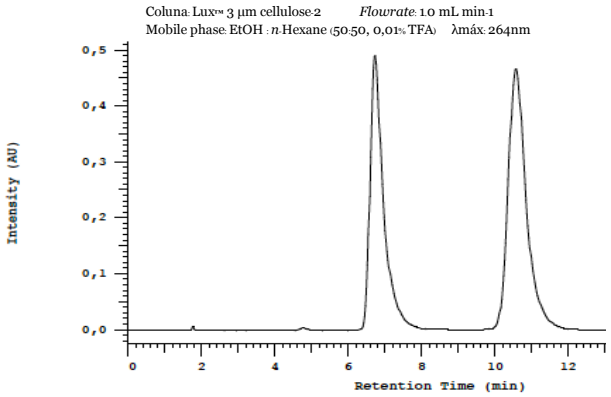


Figure 41. Chromatogram from the HPLC analysis of X2AADPG.

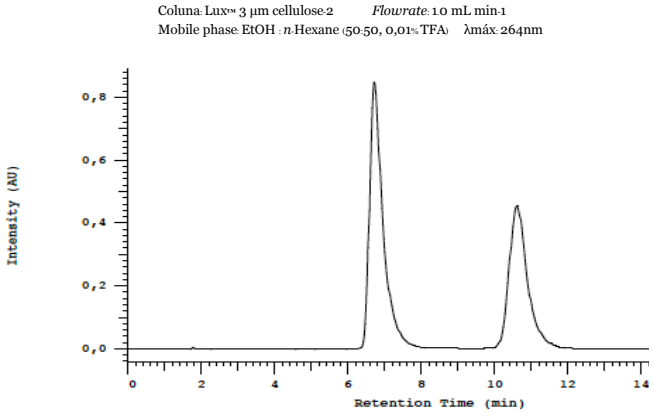


Figure 42. Chromatogram from the HPLC analysis of X2AADPG.

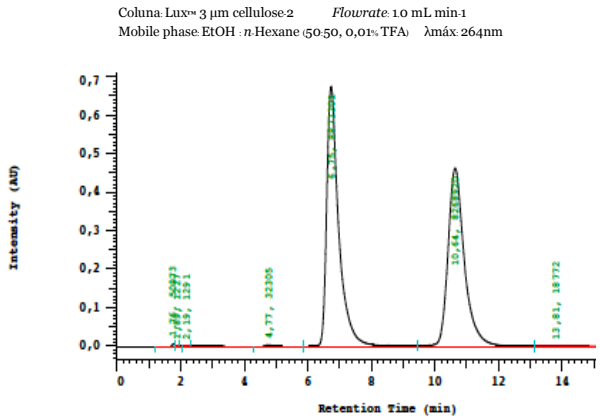


Figure 43. Chromatogram from the HPLC analysis of X2AALPG+X2AADPG mixture.

2.4. Antimicrobial activity

The studies about the potential activity of the synthesized compounds against bacterial growth were performed by measuring the optical density variations during proliferation. These studies are still ongoing, but the compounds analyzed so far in preliminary tests (Table 10), X2AELT-Me, X2AEDT-Me, X2AELS-Me and X2AEDS-Me, showed promising results in the inhibition of bacterial growth of *Staphylococcus aureus (S.a.)* and *Staphylococcus epidermidis (S.e.)*, showing enantioselectivity at inhibiting the first bacterial strain. On the other hand, some compounds were found to stimulate bacterial growth in the cases of *Candida albicans (C.a.) and Pseudomonas aeruginosa (P.a.),* in which the measured optical density was greater. Also, the ability of these strains in forming biofilms was tested when subjected to the CDXs, with X2AELT-Me showing decreased biofilm formation for *P.a.*. The method applied in these assays is described in the experimental section.

Table 10. Antimicrobial activity results in preliminary tests.

Compound	Bacterial growth		Biofilm formation	
X2AELT-Me (4,58 mg : 100 µL)	*S.aureus*	-	*S.aureus*	-
	S.epidermidis	-	*S.epidermidis*	*
	C.albicans	-	*C.albicans*	*
	P.aeruginosa	*	*P.aeruginosa*	+
X2AEDT-Me (0,76 mg : 100 µL)	*S.aureus*	+	*S.aureus*	-
	S.epidermidis	-	*S.epidermidis*	*
	C.albicans	*	*C.albicans*	*
	P.aeruginosa	*	*P.aeruginosa*	-
X2AELS-Me (0,80 mg : 100 µL)	*S.aureus*	+	*S.aureus*	-
	S.epidermidis	+	*S.epidermidis*	-
	C.albicans	*	*C.albicans*	-
	P.aeruginosa	*	*P.aeruginosa*	-
X2AEDS-Me (0,58 mg : 100 µL)	*S.aureus*	-	*S.aureus*	-
	S.epidermidis	+	*S.epidermidis*	*
	C.albicans	-	*C.albicans*	-
	P.aeruginosa	*	*P.aeruginosa*	-

(+)inhibition, (-) no inhibition, (*) increased OD

CHAPTER 3: EXPERIMENTAL

3. Experimental

3.1. General Methods

The synthesis and purification of the xanthone substrates XCar 2 and XCar 3 was performed at the Laboratory of Organic and Medicinal Chemistry from the Faculty of Pharmacy of the University of Porto, while the coupling reactions and consequent hydrolysis, as well as the final product purifications and enantiomeric excess studies, were done in the Laboratory of Applied Chemistry of Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde IINFACTS from Cooperativa de Ensino Superior Politécnico Universitário – CESPU.

The commercial available reagents and solvents were purchased from Sigma Aldrich Co, and were used without purification.

All the reactions were monitored by thin-layer chromatography (Merck silica gel, 60 (GF254 plates), with appropriate mobile phases, and UV detection at 245 and 365 nm.

Purifications of compounds were carried out by flash chromatography using Macherey-Nagel silica gel 60 (0.04-0.063 mm), liquid-liquid extraction and crystallization.

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

Melting points were obtained in a Köfler microscope and are incorrect.

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

^{1}H and ^{13}C NMR spectra were performed in the Department of Chemistry of the University of Aveiro and were taken using $CDCl_3$ or DMSO as solvent at room temperature, on Bruker Avance 300 and 500 instruments (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C). Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) used as an internal reference; ^{13}C NMR assignments were made by 2D (HSQC and HMBC) NMR experiments (long-range ^{13}C-^{1}H coupling constants were optimized to 7 Hz).

3.2. Synthesis of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar 2, 1) and 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar 3, 2)

3.2.1. Esterification of 4-bromoisophthalic acid (3). Dimethyl 4-bromoisophthalate (4)

To a solution of 4-bromoisophthalic acid (3) (19.78 g, 79.62 mmol) in methanol (600 mL) was added 12 mL of concentrated H_2SO_4. Then, the reaction mixture was refluxed for 20 h. After evaporation of the methanol, water (65 mL) was added and the crude product was extracted with diethyl ether (3 x 70 mL). The organic layer was washed with water (100 mL), saturated NaHCO3 solution (3 x 120 mL) and water (2 x 100 mL), successively. After drying with anhydrous sodium sulfate and filtered, the solvent was evaporated under reduced pressure. During overnight at room temperature the dimethyl 4-bromoisophthalate (4) appeared as a white solid. Yield: 92.3%; m.p. 56-58oC; IR ν max (cm-1) (KBr): 1754, 1309, 1253, 929, 565; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 8.43 (1H, *d*, *J*= 2.2 Hz, H-2), 7.95 (^{1}H, *dd*, *J*= 8.3 and 2.2 Hz, H-6), 7.75 (^{1}H, *d*, *J*= 8.3 Hz, H-5), 3.95 (3H, *s*, C(1'')$OOCH_3$), 3.93 (3H,*s*, C(1')$OOCH_3$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ: 165.7 (C-1''), 165.5 (C-1'), 134.7 (C-4), 133.0 (C-6), 132.3 (C-2), 132.2 (C-5), 129.3 (C-3), 127.0 (C-1), 52.7 (C(1'')$OOCH_3$), 52.5 (C(1')$OOCH_3$); MS (El) *m/z* (%): 273 $[M]^+$. (100), 256 (9), 240 (13), 221 (6), 209 (8), 203 (11).

3.2.2. Ullmann diaryl ether coupling. Dimethyl 4-(3-methoxyphenoxy) isophthalate (6)

A mixture of dimethyl 4-bromoisophthalate (4) (7.03 g, 25.73 mmol), 3-methoxyphenol (5) (4.2 mL, 38.55 mmol), CuI (0.25 g, 1.30 mmol), K_3PO_4 (10.98 g, 51.71 mmol) and picolinic acid (0.31 g, 2.55 mmol) was prepared in a flask that was sealed and then evacuated and backfilled with

nitrogen. The evacuation/backfill sequence was repeated two additional times. Under nitrogen atmosphere, the remaining liquid reagent dimethyl sulfoxide (51 mL) was added.

The sealed flask was placed in a preheated oil bath at 80 °C and the reaction mixture was stirred vigorously with a magnetic stirrer for 28h. The reaction mixture was cooled to room temperature, filtered and extract with ethyl acetate (300 mL) and water (30 mL). The organic layer was washed with water (30 mL) and separated. The aqueous layer was extract twice more with ethyl acetate (20 mL). The combined organic layer were washed with brine dried with anhydrous sodium sulfate, evaporated and filtered under reduce pressure to provide brown solid of dimethyl 4-(3-methoxyphenoxy) isophthalate (6). Yield: 25%; m.p. 88-90 °C; IR ν max (cm-1) (KBr): 1724, 1719, 1613, 1488, 1274, 1229, 1153, 948, 763; 1H NMR ($CDCl_3$, 300MHz) δ: 8.58 (1H, *d*, *J*= 2.2 Hz, H-2), 8.07 (1H, *dd*, *J*=8.7 and 2.2 Hz, H-6), 7.27 (1H, *dd*, *J*= 8.4 and 8.3 Hz, H-5'), 6.95 (1H, *d*, *J*=8.7 Hz, H-5), 6.74 (1H, *dd*, *J*= 8.3 and 3.1 Hz, H-6'), 6.62 (1H, *dd*, *J*= 3.6 and 3.1 Hz, H-2'), 6.61 (1H, *dd*, *J*= 8.4 and 3.6 Hz, H-4'), 3.93 (3H, *s*, C(1')$OOCH_3$), 3.89 (3H, *s*, C(1'')$OOCH_3$), 3.79 (3H, *s*, Ar-OCH_3); 13C NMR ($CDCl_3$, 75.47 MHz) δ: 165.7 (C-1'), 165.3 (C-1''), 161.1 (C-3), 160.4 (C-1), 156.9 (C-4), 134.6 (C-2), 133.6 (C-6), 130.4 (C-5), 124.6 (C-3'), 122.0 (C-1'), 118.7 (C-5'), 111.6 (C-6'), 110.3 (C-4'), 105.6 (C-2'), 55.4 (Ar-OCH_3), 52.3 (C(1')$OOCH_3$), 52.2 (C(1')$OOCH_3$); MS (El) *m/z* (%): 316 [M]+. (100), 285 [M-OCH_3]+. (55), 253 (54), 242 (10), 225 (16), 213 (12), 198 (25), 138 (15), 127 (10), 92 (15), 83 (14), 64 (11).

3.2.3. Hydrolysis of dimethyl ester. 4-(3-Methoxyphenoxy) isophthalic acid (7)

Dimethyl 4-(3-methoxyphenoxy)isophthalate (6) (2.00 g, 6.32 mmol) was dissolved in methanol/tetrahydrofuran (1:1 *v/v*) and stirred at room temperature with 5M NaOH solution (10 mL) for 18 h. After evaporation of the organic solvents, water was added (40 mL) and the crude product was washed with ethyl acetate (2 x 20 mL). The organic layer was extracted with water (2 x 15 mL). The aqueous layer was acidified with 5M HCl solution resulting in the formation of a precipitate that was collected by filtration under reduced pressure and washed with cooled

water, to provide 4-(3-methoxyphenoxy)isophthalic acid (7) as a light brown solid. Yield: 85% m.p. 232-234°C; IR ν max (cm-1) (KBr): 2907, 1694, 1680, 1601, 1486,1271,1265, 911, 758; 1HNMR ($CDCl_3$, 300.13 MHz) δ: 8.53 (1H, *d*, *J*= 2.2 Hz, H-2), 8.09 (1H, *dd*, *J*= 8.7 and 2.2 Hz, H-6), 7.29 (1H, *dd*, *J*= 8.3 and 8.4 Hz, H-5'), 6.95 (1H, *d*, *J*= 8.7 Hz, H-5), 6.76 (1H, *dd*, *J*= 3.1 and 8.3 Hz, H-6'), 6.62 (1H, *dd*, *J*= 3.1 and 3.3 Hz, H-2'), 6.59 (1H, *dd*, *J*= 3.3 and 8.3 Hz, H-4'), 3.77 (3H, *s*, Ar-OCH_3); 13C NMR ($CDCl_3$, 75.47 MHz) δ: 179.3 (C-1''), 179.3 (C-1'), 163.5 (C-3), 162.5 (C-1), 159.2 (C-4), 145.3 (C-2), 136.6 (C-6), 135.5 (C-5), 132.3 (C-3'), 127.3 (C-1'), 120.6 (C-5'), 113.4 (C-6'), 112.1 (C-4'), 107.5 (C-2'), 56.7(Ar-OCH_3); MS (EI) *m/z* (%): 288[M]+. (100), 271 (10), 257[M-OCH_3]+. (12), 227 (11), 199 (23), 165 (61), 124 (63), 92 (23), 77 (20), 64 (19).

3.2.4. Intramolecular acylation. Xanthone formation

3.2.4.1. Intramolecular acylation with Eaton's Reagent

To a solution of 4-(3'-methoxypenthoxy)isophthalic acid (7) (1.60 g, 5.06 mmol) in methane sulfonic acid (22.9 mL) was added phosphorus pentoxide (2.56 g, 9.03 mmol) and the reaction mixture was stirred at room temperature for 22 h. 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 (330 mL) and H_2SO_4 (7 mL) was added. The mixture was refluxed for approximately 19h. The products methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (8) and methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (9) were separated by flash column chromatography (silica gel, petrolium ether:ethyl acetate 8:2). After that, compound 8 (1.04 g) was dissolved in methanol/dichloromethane (177 mL, 1:1 *v/v*) and 5M NaOH solution (14 mL) was added. The mixture was stirred at room temperature for 22 h. After evaporation of the organic solvents, water was added (25 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 (1). Yield: 76%. The same procedure was followed to hydrolyse the methyl 8-methoxy-9-oxo-9H-xanthene-2-carboxylate (9) (0.06 mg, 0.21 mmol) to afford 8-methoxy-9-oxo-9H-xanthene-2-carboxylic acid (2). Yield: 3%

3.2.4.2. Intramolecular acylation with PPA

A solution of 4-(3'-methoxypenthoxy)isophthalic acid (7) (0.71 g, 2.45 mmol) in polyphosphoric acid (4.86 mL) was heated up to 80°C and stirred for 2h. The solution was poured over ice resulting a dark solid, collected by filtration under reduced pressure and dried at room temperature. The product was dissolved in methanol (300 mL) and 15 mL of H_2SO_4 was added. The mixture was refluxed for approximately 19h and the products were separated by flash column chromatography (silica gel, petrolium ether:ethyl acetate 8:2). After that, compound 8 (0.42 g) was dissolved in methanol/dichloromethane (60 mL, 1:1 *v/v*) and 5M NaOH solution (6 mL) was added. The mixture was stirred at room temperature for 22 h.

After evaporation of the organic solvents, water was added (14 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 (1). Yield: 60%.

3.2.5. Methyl 6-methoxy-9-oxo-9H-xanthene-2-carboxylate (8)

m.p.: 176-178 °C; IR ν max (cm^{-1}) (KBr): 1730, 1663, 1581, 1467, 1438, 1270, 1117, 764; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.98 (1H, *d*, *J*= 2.1 Hz, H-1), 8.32 (1H, *dd*, *J*= 2.1 and 8.7 Hz, H-3), 8.24 (1H, *d*, *J*= 8.4 Hz, H-8), 7.48 (1H, *d*, *J*= 8.7 Hz, H-4), 6.96 (1H, *dd*, *J*= 2.3 and 8.4 Hz, H-7), 6.89 (1H, *d*, *J*= 2.3 Hz, H-5), 3.95 (3H, *s*, $COOCH_3$), 3.93 (3H, *s*, Ar-OCH_3); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ:

175.5 (C-9), 165.9 ($COOCH_3$), 165.3 (C-6), 158.7 (C-10a), 157.8 (C-4a), 134.8 (C-3), 129.1 (C-2), 128.3 (C-8), 125.9 (C-1), 121.5 (C-9a), 118.1 (C-4), 115.6 (C-8a), 113.7 (C-7), 100.4 (C-5), 55.9 ($Ar\text{-}OCH_3$), 52.3 ($COOCH_3$); MS (EI) *m/z* (%): 284 $[M]^{+\cdot}$ (89), 253 $[M\text{-}OCH_3]^{+}$ (100), 225 (32), 197 (14), 182 (30), 169 (17), 154 (16), 142 (14), 126 (28), 111 (12), 75 (12), 63 (14).

3.2.6. Methyl 8-methoxy-9-oxo-9H-xanthene-2-carboxylate (9)

m.p.: 206-207 °C; IR ν max (cm^{-1}) (KBr): 1726, 1668, 1611, 1480, 1431, 1264, 1079, 760; ^1H NMR ($CDCl_3$, 300.13 MHz) δ: 8.98 (1H, *d*, *J*= 2.1 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.1 and 8.7 Hz, H-3), 7.65 (1H, *dd*, *J*= 8.4 and 8.4 Hz, H-6), 7.47 (1H, *d*, *J*= 8.7 Hz, H-4), 7.09 (1H, *dd*, *J*= 0.8 and 8.4 Hz, H-7), 6.86 (1H, *dd*, *J*= 0.8 and 8.4 Hz, H-5), 4.05 (3H, *s*, $COOCH_3$), 3.98 (3H, *s*, $Ar\text{-}OCH_3$); ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 175.8 (C-9),166.0 ($COOCH_3$), 160.8 (C-8), 157.9 (C-10a), 157.8 (C-4a), 135.3 (C-3), 134.9 (C-6), 129.4 (C- 2), 126.0 (C-1), 122.6 (C-9a), 117.7 (C-4), 112.5 (C-8a), 110.1 (C-7), 106.1 (C-5), 56.5 ($Ar\text{-}OCH_3$), 52.4 ($COOCH_3$); MS (EI) *m/z* (%): 284 $[M]^{+\cdot}$ (100), 255 (56), 253 (30), 238 (56), 223 (47), 195 (27), 139 (33), 126 (20), 112 (18), 70 (15), 63 (12).

3.2.7. 6-Methoxy-9-oxo-9H-xanthene-2-carboxylic acid (1)

m.p.: >300 °C; IR ν max (cm^{-1}) (KBr): 3411, 1687, 1610, 1575, 1500, 1433,1271, 766; ^1H NMR (DMSO-d_6, 300.13 MHz) δ: 8.68 (1H, *d*, *J*= 2.1 Hz, H-1), 8.29 (1H, *dd*, *J*= 2.1 and 8.7 Hz, H-3), 8.09 (1H, *d*, *J*= 8.4 Hz, H-8), 7.69 (1H, *d*, *J*= 8.7 Hz, H-4), 7.19 (1H, *d*, *J*= 2.3 Hz, H-5), 7.07 (1H, *dd*, *J*= 2.3 and 8.4 Hz, H-7), 3.93 (3H, *s*, $Ar\text{-}OCH_3$); ^{13}C NMR (DMSO-d_6, 75.47 MHz) δ: 175.3 (C-9), 167.6 (COOH), 164.9 (C-6), 157.6 (C-10a), 156.3 (C-4a), 135.7 (C-3), 127.6 (C-2), 126.6 (C-8), 124.9 (C-1), 120.2 (C-9a), 116.7 (C-4), 115.0 (C-8a), 113.7 (C-7), 100.7 (C-5), 56.2 ($Ar\text{-}OCH_3$); MS (EI) *m/z* (%): 270 $[M]^{+\cdot}$ (100), 253 $[M\text{-}OH]^{+}$ (26), 226 (27), 199 (15), 182 (15), 169 (8), 154 (7), 139 (7), 126 (17), 115 (10), 63 (16).

3.2.8. 8-Methoxy-9-oxo-9H-xanthene-2-carboxylic acid (2)

m.p.: 268-270 °C; IR ν max (cm^{-1}) (KBr): 3460, 1687, 1663, 1603, 1469, 1420, 1266, 763; ^{1}H NMR (DMSO-d_6, 300.13MHz) δ: 8.66 (1H, *d*, *J*= 2.1 Hz, H-1), 8.28 (1H, *dd*, *J*= 2.1 and 8.7 Hz, H-3), 7.79 (1H, *dd*, *J*= 8.4 and 8.4 Hz, H-6), 7.67 (1H, *d*, *J*= 8.7 Hz, H-4), 7.20 (1H, *dd*, *J*= 0.7 and 8.4 Hz, H-7), 7.06 (1H, *dd*, *J*= 0.7 and 8.4 Hz, H-5), 3.94 (3H, *s*, Ar-OCH_3); ^{13}C NMR (DMSO-d_6, 125.77 MHz) δ: 174.3 (C-9), 166.2 (COOH), 160.2 (C-8), 157.2 (C-10a), 157.0 (C-4a), 136.1 (C-3), 134.9 (C-6), 127.9 (C-2), 125.0 (C-1), 122.1 (C-9a), 118.2 (C-4), 111.6 (C-8a), 109.7 (C-7), 107.0 (C-5), 56.3 (Ar-OCH_3); HRMS (ESI) *m/z*: calcd for ($C_{15}H_{10}O_5$ + H): 271.16994, found: e

3.3. General procedure for the synthesis of chiral derivatives of xanthones

The 6-methoxy-9-oxo-9H-xanthene-2-carboxylic acid – XCar 2 (**1**) (50 mg, 0.19 mmol) was dissolved in dry THF (10 mL) and TEA (52 μL, 0.37 mmol) was added. Following, the coupling reagent (1.2 eq. mmol) was added and the solution stirred for about 15 min before the addition of an appropriate chiral reagent (1.7 eq.). The mixture was stirred at room temperature for 1h up to 24 h. The reaction was followed by thin layer chromatography using ethyl acetate:*n*-hexane (7:3) as mobile phase, and after completion its completion, the solvent was evaporated under reduced pressure at 40 °C and the crude product was dissolved in dichloromethane. This solution was washed with a 5% HCl solution (2 X 13 mL), 5% solution of $NaHCO_3$ (2 X 15 mL) and water (3 X 25 mL). The organic layer was dried with anhydrous sodium sulphate, filtered and the solvent was evaporated under reduced pressure. The product was crystallized from methanol or dichloromethane at low temperatures (5 - -15°C) and filtered under vaccum to afford the CDXs.

3.3.1. Methyl (*S*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-2-phenylacetate (X2AELPG-Me) (11)

m.p.: 145-147 °C (MeOH); IR ν max (cm^{-1}) (KBr): 3298, 1740, 1661, 1621, 1542, 1282,3411, 1687, 1610, 1589, 1575, 1500,1478, 1496, 1442, 1433, 1271, 834, 756, 729 698 and 674;
^{1}H NMR (300 MHz, DMSO-d_6): δ 8.96 (1H, d, J = 7.4 Hz NH), 8.78 (1H, d, J = 2.3 Hz, H-1), 8.32 (1H, dd, J = 8.8 and 2.3 Hz, H-3), 8.12 (d, J = 8.9 Hz, H-8), 7.70 (1H, d, J = 8.8 Hz, H-4), 7.45 (5H, m, H 2"-6"), 7.19 (1H, d, J = 2.3 Hz, H-5), 7.10 (1H, dd, J = 8.8 and 2.4 Hz, H-7), 5.71 (1H, d, J = 7.4 Hz, H-2'), 3.94 (3H, s, Ar-OCH_3), 3.68 ppm (3H, s, COO-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.8 (C-9), 171.1 (C=O ester), 165.3 (C=O amide), 165.2 (C-6), 157.6 (C-4a), 157.4 (C-10a), 136.1 (C-2') 134.3 (C-3), 129.3 (C-2), 128.6 (C-2" and C-6"), 128.4 (C-3" and C-5"), 128.3 (C-4"), 127.8 (C-8), 126.1 (C-1), 120.7 (C-9a), 118.2 (C-4), 115.0 (C-8a), 114.1 (C-7), 100.8 (C-5), 57.2 (C-1'), 56.3 (Ar-OCH_3), 52.4 ppm (COO-$\underline{C}H_3$).

3.3.2. (*R*)-Methyl-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-2-phenylacetate (X2AEDPG-Me) (10)

m.p.: 154-156 °C (MeOH); IR ν max (cm^{-1}) (KBr): 3295 ,1739, 1660, 1620, 1589, 1542, 1496, 1478, 1442, 1274, 832, 780, 758, 728, 698, 673;
^{1}H NMR (300 MHz, DMSO-d_6): δ 8.96 (1H, d, J = 7.4 Hz NH), 8.78 (1H, d, J = 2.2 Hz, H-1), 8.32 (1H, dd, J = 8.8 and 2.3 Hz, H-3), 8.12 (d, J = 8.9 Hz, H-8), 7.70 (1H, d, J = 8.8 Hz, H-4), 7.45 (5H, m, H 2"-6"), 7.19 (1H, d, J = 2.3 Hz, H-5), 7.09 (1H, dd, J = 8.9 and 2.4 Hz, H-7), 5.71 (1H, d, J = 7.3 Hz, H-2'), 3.94 (3H, s, Ar-OCH_3), 3.68 ppm (3H, s, COO-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.8 (C-9), 171.1 (C=O ester), 165.3 (C=O amide), 165.2 (C-6), 157.6 (C-4a), 157.4 (C-10a), 136.1 (C-2") 134.3 (C-3), 129.3 (C-2), 128.6 (C-2" and C-6"), 128.4 (C-3" and C-5"), 128.3 (C-4"), 127.8 (C-8), 126.1 (C-1), 120.7 (C-9a), 118.2 (C-4), 115.0 (C-8a), 114.1 (C-7), 100.8 (C-5), 57.2 (C-1'), 56.3 (Ar-OCH_3), 52.4 ppm (COO-$\underline{C}H_3$).

3.3.3. (*S*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-2-phenylacetic acid (X2AALPG) (23)

m.p.: 229-231 °C (H2O); IR ν max (cm^{-1}) (KBr): 3385, 3366, 1732, 1653, 1614, 1582, 1520, 1474, 1448, 1441, 1270, 836, 783, 761, 723, 696, 669;
^{1}H NMR (300 MHz, DMSO-d_6): δ 9.40 (1H, d, J = 7.4 Hz NH), 8.71 (1H, d, J = 2.2 Hz, H-1), 8.26 (1H, dd, J = 8.8 and 2.3 Hz, H-3), 8.06 (d, J = 8.9 Hz, H-8), 7.64 (1H, d, J = 8.8 Hz, H-4), 7.46 (2H, m, H-2" and 6"), 7.32 (3H, m, H-3"-5"), 7.13 (1H, d, J = 2.4 Hz, H-5), 7.02 (1H, dd, J = 8.9 and 2.4 Hz, H-7), 5.57 (1H, d, J = 7.3 Hz, H-2'), 3.88 ppm (3H, s, Ar-OCH$_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.2 (C-9), 172.3 (C=O carboxylic acid), 165.7 (C=O amide), 165.5 (C-6), 158.0 (C-4a), 157.8 (C-10a), 137.5 (C-1") 134.7 (C-3), 130.0 (C-2), 128.9 (C-2" and C-6"), 128.7 (C-3" and C-5"), 128.2 (C-4"), 128.2 (C-8), 126.5 (C-1), 121.1 (C-9a), 118.6 (C-4), 115.4 (C-8a), 114.5 (C-7), 101.3 (C-5), 57.5 (C-2'), 56.7 ppm (Ar-OCH$_3$).

3.3.4. (*R*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-2-phenylacetic acid (X2AADPG) (22)

m.p.: 229-231 °C (H2O); IR ν max (cm^{-1}) (KBr): 3385, 3365, 1734, 1652, 1615, 1582, 152, 1474, 1449, 1440, 1270, 836, 783, 761, 724, 711;
^{1}H NMR (300 MHz, DMSO-d_6): δ 9.40 (1H, d, J = 7.4 Hz NH), 8.70 (1H, d, J = 2.3 Hz, H-1), 8.26 (1H, dd, J = 8.8 and 2.3 Hz, H-3), 8.06 (d, J = 8.9 Hz, H-8), 7.64 (1H, d, J = 8.8 Hz, H-4), 7.46 (2H, m, H-2" and 6"), 7.31 (3H, m, H-3"-5"), 7.13 (1H, d, J = 2.2 Hz, H-5), 7.02 (1H, dd, J = 8.9 and 2.4 Hz, H-7), 5.57 (1H, d, J = 7.3 Hz, H-2'), 3.88 ppm (3H, s, Ar-OCH$_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.2 (C-9), 172.1 (C=O carboxylic acid), 165.7 (C=O amide), 165.5 (C-6), 158.0 (C-4a), 157.8 (C-10a), 137.5 (C-1") 134.7 (C-3), 130.0 (C-2), 128.9 (C-2" and C-6"), 128.7 (C-3" and C-5"), 128.2 (C-4"), 128.2 (C-8), 126.5 (C-1), 121.1 (C-9a), 118.5 (C-4), 115.4 (C-8a), 114.5 (C-7), 101.3 (C-5), 57.5 (C-2'), 56.7 ppm (Ar-OCH$_3$).

3.3.5. *tert*-butyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-alaninate (X2AELA-TB) (19)

m.p.: 170-172 ^{o}C (MeOH); IR ν max (cm^{-1}) (KBr): 3311, 1735, 1655, 1620 ,1590, 1567, 1541, 1479, 1446, 834, 780, 760, 670;
^{1}H NMR (300 MHz, DMSO-d_6): δ 9.0 (1H, d, J = 7.1 Hz NH), 8.75 (1H, d, J = 2.2 Hz, H-1), 8.30 (1H, dd, J = 8.8 and 2.3 Hz, H-3), 8.14 (d, J = 8.9 Hz, H-8), 7.72 (1H, d, J = 8.8 Hz, H-4), 7.20 (1H, d, J = 2.3 Hz, H-5), 7.09 (1H, dd, J = 8.9 and 2.4 Hz, H-7), 4.39 (1H, quint, J = 7.1 Hz, H-2'), 3.95 (3H, s, Ar-OCH_3), 1.41 (3H, d, J = 7.1 Hz, H-1''), 1.35 ppm (9H, s, $COOC(CH_3)_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.9 (C-9), 171.9 (C=O ester), 165.2 (C=O amide), 165.0 (C-6), 157.6 (C-4a), 157.3 (C-10a), 134.0 (C-3), 130.0 (C-2), 127.7 (C-8), 125.6 (C-1), 120.7 (C-9a), 118.3 (C-4), 115.0 (C-8a), 114.0 (C-7), 100.8 (C-5), 56.3 (Ar-OCH_3), 80.4 ($\underline{C}(CH_3)_3$), 49.2 (C-2'), 27.7 ($C(CH_3)_3$), 16.8 ppm (C-1'').

3.3.6. *tert*-Butyl(6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-alaninate (X2AEDA-TB) (18)

m.p.: 168-170 ^{o}C (MeOH); IR ν max (cm^{-1}) (KBr): 3309, 1733, 1657, 1620, 1589, 1540, 1479, 1445, 1274, 833, 780, 760, 670;
^{1}H NMR (300 MHz, DMSO-d_6): δ 9.0 (1H, d, J = 7.0 Hz NH), 8.75 (1H, d, J = 2.3 Hz, H-1), 8.30 (1H, dd, J = 8.8 and 2.3 Hz, H-3), 8.13 (d, J = 8.9 Hz, H-8), 7.72 (1H, d, J = 8.8 Hz, H-4), 7.19 (1H, d, J = 2.3 Hz, H-5), 7.09 (1H, dd, J = 8.9 and 2.4 Hz, H-7), 4.39 (1H, quint, J = 7.2 Hz, H-2'), 3.94 (3H, s, Ar-OCH_3), 1.41 (3H, d, J = 7.1 Hz, H-1''), 1.35 ppm (9H, s, $COOC(CH_3)_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.9 (C-9), 171.9 (C=O ester), 165.2 (C=O amide), 165.0 (C-6), 157.7 (C-4a), 157.3 (C-10a), 134.0 (C-3), 129.8 (C-2), 127.8 (C-8), 125.6 (C-1), 120.8 (C-9a), 118.3 (C-4), 115.0 (C-8a), 114.0 (C-7), 100.8 (C-5), 56.3 (Ar-OCH_3), 80.4 ($\underline{C}(CH_3)_3$), 49.2 (C-2'), 27.7 ($C(CH_3)_3$), 16.8 ppm (C-1'').

3.3.7. (6-Methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-alanine (X2AALA) (27)

m.p.: 226-228 ^{o}C (H2O); IR ν max (cm^{-1}) (KBr): 3414, 3341, 1734, 1664, 1613, 1578, 1539, 1477, 1452, 1283, 852, 836, 805, 779, 756, 674;
^{1}H NMR ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.1 (1H, d, *J* = 7.2 Hz NH), 8.76 (1H, d, *J* = 2.2 Hz, H-1), 8.31 (1H, dd, *J* = 8.8 and 2.3 Hz, H-3), 8.14 (d, *J* = 8.9 Hz, H-8), 7.72 (1H, d, *J* = 8.8 Hz, H-4), 7.20 (1H, d, *J* = 2.3 Hz, H-5), 7.10 (1H, dd, *J* = 8.9 and 2.4 Hz, H-7), 4.46 (1H, quint, *J* = 7.3 Hz, H-2'), 3.95 (3H, s, Ar-OCH_3), 1.43 ppm (3H, d, *J* = 7.3 Hz, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.9 (C-9), 174.2 (C=O carboxylic acid), 165.3 (C=O amide), 164.9 (C-6), 157.6 (C-4a), 157.3 (C-10a), 134.0 (C-3), 129.8 (C-2), 127.8 (C-8), 125.6 (C-1), 120.8 (C-9a), 118.3 (C-4), 115.0 (C-8a), 114.1 (C-7), 100.8 (C-5), 56.3 (Ar-OCH_3), 48.4 (C-2'),16.9 ppm (C-1").

3.3.8. (6-Methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-alanine (X2AADA) (26)

m.p.: 230-233 ^{o}C (H2O); IR ν max (cm^{-1}) (KBr): 3411, 3343, 1732, 1662, 1614, 1578, 1539, 1477, 1452, 1281, 852, 836, 805, 779, 756, 674;
^{1}H NMR ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.0 (1H, d, *J* = 6.9 Hz NH), 8.76 (1H, d, *J* = 2.2 Hz, H-1), 8.31 (1H, dd, *J* = 8.8 and 2.3 Hz, H-3), 8.14 (d, *J* = 8.9 Hz, H-8), 7.72 (1H, d, *J* = 8.7 Hz, H-4), 7.20 (1H, d, *J* = 2.3 Hz, H-5), 7.09 (1H, dd, *J* = 8.9 and 2.4 Hz, H-7), 4.45 (1H, quint, *J* = 7.1 Hz, H-1'), 3.95 (3H, s, Ar-OCH_3), 1.43 ppm (3H, d, *J* = 6.9 Hz, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.9 (C-9), 174.2 (C=O carboxylic acid), 165.3 (C=O amide), 164.8 (C-6), 157.6 (C-4a), 157.3 (C-10a), 134.0 (C-3), 129.8 (C-2), 127.8 (C-8), 125.6 (C-1), 120.8 (C-9a), 118.3 (C-4), 115.0 (C-8a), 114.0 (C-7), 100.8 (C-5), 56.3 (Ar-OCH_3), 48.4 (C-2'), 16.9 ppm (C-1").

3.3.9. Methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-phenylalaninate (X2AELPA-Me) (13)

m.p.: 54-55 ^{o}C (MeOH); IR ν max (cm^{-1}) (KBr): 3413, 1731, 1671, 1622, 1590, 1566, 1483, 1443, 1273, 837 , 763 , 687, 670;
^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25 (1H, d, *J* = 7.8 Hz NH), 8.70 (1H, d, *J* = 2.3 Hz, H-1), 8.22 (1H, dd, *J* = 8.7 and 2.3 Hz, H-3), 8.13 (d, *J* = 8.9 Hz, H-8), 7.71 (1H, d, *J* = 8.7 Hz, H-4), 7.32 (2H, dd, *J* = (8.2 and 1.7), H-4"-6"), 7.28 (2H, dd, *J* = (8.2 and 1.6), H-3"-7"), 7.20 (1H, dd, *J* =8.1 and 1.6 Hz, H-4"), 7.20 (1H, d, *J* = 2.5 Hz, H-5), 7.09 (1H, dd, *J* = 8.8 and 2.2 Hz, H-7), 4.72 (1H, m, H-2'), 3.95 (3H, s, Ar-OCH_3), 3.66 (3H, s, COO-CH_3), 3.21 (1H, dd, *J* = 13.8 and 5.3, H-1"), 3.15 ppm (1H, dd, *J* = 12.5 and 5.2, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.8 (C-9), 172.1 (C=O ester), 165.2 (C=O amide), 165.1 (C-6), 157.5 (C-4a), 157.5 (C-10a), 137.7 (C-2") 133.9 (C-3), 129.4 (C-2), 129.0 (C-4" and C-6"), 128.4 (C-3" and C-7"), 127.7 (C-8), 126.5 (C-5"), 125.5 (C-1), 120.8 (C-9a), 118.3 (C-4), 115.0 (C-8a), 114.0 (C-7), 100.8 (C-5), 56.3 (Ar-OCH_3), 54.4 (C-2'), 52.1 ppm (COO$\underline{C}H_3$), 40.4 ppm (C-1")

3.3.10. Methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-phenylalaninate (X2AEDPA-Me) (12)

m.p.: 71-73 ^{o}C (MeOH); IR ν max (cm^{-1}) (KBr): 3315, 1744, 1660 1618, 1588, 1536, 1478, 1443, 1274, 834, 758, 700, 669;
^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25 (1H, d, *J* = 7.8 Hz NH), 8.70 (1H, d, *J* = 2.3 Hz, H-1), 8.22 (1H, dd, *J* = 8.7 and 2.3 Hz, H-3), 8.13 (d, *J* = 8.9 Hz, H-8), 7.71 (1H, d, *J* = 8.7 Hz, H-4), 7.32 (2H, dd, *J* = (8.2 and 1.7), H-4"-6"), 7.28 (2H, dd, *J* = (8.1 and 1.6), H-3"-7"), 7.20 (1H, dd, *J* =8.1 and 1.6 Hz, H-4"), 7.20 (1H, d, *J* = 2.4 Hz, H-5), 7.10 (1H, dd, *J* = 8.8 and 2.4 Hz, H-7), 4.72 (1H, m, H-2'), 3.95 (3H, s, Ar-OCH_3), 3.66 (3H, s, COO-CH_3), 3.21 (1H, dd, *J* = 13.8 and 5.3, H-1"), 3.15 ppm (1H, dd, *J* = 12.5 and 5.2, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.7 (C-9), 172.1 (C=O ester), 165.3 (C=O amide), 165.1 (C-6), 157.5 (C-4a), 157.5 (C-10a), 137.7 (C-2") 133.8 (C-3), 129.4 (C-2), 129.0 (C-4" and C-6"), 128.4 (C-3" and C-7"), 127.7 (C-8), 126.5 (C-5"), 125.5 (C-1), 120.8 (C-9a), 118.3 (C-4), 115.0 (C-8a), 114.0 (C-7), 100.8 (C-5), 56.3 (Ar-OCH_3), 54.4 (C-2'), 52.1 ppm (COO$\underline{C}H_3$), 40.4 ppm (C-1")

3.3.11. (6-Methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-phenylalanine (X2AADPA) (24)

m.p.:111-113 ^{o}C (H2O); IR ν max (cm^{-1}) (KBr): 3342, 1733, 1647, 1616, 1586, 1540, 1564, 1497, 1478, 1446, 1276, 834, 780, 759, 701, 670;

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (1H, d, *J* = 8.1 Hz NH), 8.69 (1H, d, *J* = 2.2 Hz, H-1), 8.21 (1H, dd, *J* = 8.8 and 2.3 Hz, H-3), 8.13 (d, *J* = 8.9 Hz, H-8), 7.70 (1H, d, *J* = 8.8 Hz, H-4), 7.33 (2H, dd, *J* = (8.2 and 1.8), H-4"-6"), 7.27 (2H, dd, *J* = (8.2 and 1.8), H-3"-7"), 7.18 (1H, dd, *J* =8.1 and 1.8 Hz, H-4"), 7.20 (1H, d, *J* = 2.6 Hz, H-5), 7.09 (1H, dd, *J* = 8.8 and 2.2 Hz, H-7), 4.66 (1H, m, H-2'), 3.95 (3H, s, Ar-OCH_3), 3.23 (1H, dd, *J* = 13.6 and 4.7, H-1"), 3.11 ppm (1H, dd, *J* = 10.4 and 3.6, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.8 (C-9), 173.6 (C=O ester), 165.7 (C=O amide), 165.5 (C-6), 158.0 (C-4a), 157.7 (C-10a), 138.7 (C-2") 134.3 (C-3), 130.2 (C-2), 129.5 (C-4" and C-6"), 128.6 (C-3" and C-7"), 128.1 (C-8), 126.8 (C-5"), 125.9 (C-1), 121.2 (C-9a), 118.3 (C-4), 115.4 (C-8a), 114.5 (C-7), 101.2 (C-5), 56.7 (Ar-OCH_3), 54.8 (C-2'), 40.8 ppm (C-1").

3.3.12. Methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-serinate (X2AELS-Me) (15)

m.p.: 110-112 ^{o}C (MeOH); IR ν max (cm^{-1}) (KBr): 3411, 3316, 1752, 1663, 1620, 1546, 1480 , 1444, 1284, 852, 767, 701, 674;

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.01 (1H, d, *J* = 7.3 Hz NH), 8.78 (1H, d, *J* = 2.3 Hz, H-1), 8.32 (1H, dd, *J* = 8.8 and 2.3 Hz, H-3), 8.15 (d, *J* = 8.9 Hz, H-8), 7.74 (1H, d, *J* = 8.8 Hz, H-4), 7.22 (1H, d, *J* = 2.4 Hz, H-5), 7.10 (1H, dd, *J* = 8.9 and 2.4 Hz, H-7), 5.76 (1H, s, OH), 4.59 (1H, m, H-2'), 3.96 (3H, s, Ar-OCH_3), 3.85 (1H, dd, *J* = 11.5 and 5.9, H-1"), 3.83 (1H, dd, *J* = 11.5 and 5.7, H-1"), 3.66 ppm (3H, s, COO-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.8 (C-9), 171.0 (C=O ester), 165.2 (C=O amide), 165.2 (C-6), 157.5 (C-4a), 157.3 (C-10a), 134.0 (C-3), 129.5 (C-2), 127.7 (C-8), 125.6 (C-1), 120.7 (C-9a), 118.3 (C-4), 114.9 (C-8a), 114.0 (C-7), 100.8 (C-5), 60.9 (C-1") 56.3 (Ar-OCH_3), 55.9 (C-2"), 51.9 ppm (COO$\underline{C}H_3$).

3.3.13. Methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-serinate (X2AEDS-Me) (14)

m.p.: 115-117 ^{o}C (MeOH); IR ν max (cm^{-1}) (KBr): 3405, 3317, 1752, 1666, 1622, 1546, 1480 ,1445, 1215 ,849, 831, 780, 701, 663;
^{1}H NMR (300 MHz, DMSO-d_6): δ 9.01 (1H, d, J = 7.3 Hz NH), 8.78 (1H, d, J = 2.3 Hz, H-1), 8.31 (1H, dd, J = 8.8 and 2.3 Hz, H-3), 8.14 (d, J = 8.9 Hz, H-8), 7.73 (1H, d, J = 8.9 Hz, H-4), 7.22 (1H, d, J = 2.4 Hz, H-5), 7.10 (1H, dd, J = 8.9 and 2.4 Hz, H-7), 5.76 (1H, s, OH), 4.59 (1H, m, H-2'), 3.96 (3H, s, Ar-OCH_3), 3.84 (1H, dd, J = 11.5 and 5.9, H-1"), 3.83 (1H, dd, J = 11.5 and 5.7, H-1"), 3.66 ppm (3H, s, COO-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.8 (C-9), 171.2 (C=O ester), 165.1 (C=O amide), 165.2 (C-6), 157.5 (C-4a), 157.3 (C-10a), 134.0 (C-3), 129.5 (C-2), 127.7 (C-8), 125.6 (C-1), 120.7 (C-9a), 118.3 (C-4), 114.9 (C-8a), 114.0 (C-7), 100.8 (C-5), 60.9 (C-1") 56.3 (Ar-OCH_3), 55.9 (C-2'), 51.9 ppm ($\underline{C}OOCH_3$).

3.3.14. (6-Methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-serine (X2AADS) (30)

m.p.: 206-209 ^{o}C (H_2O); IR ν max (cm^{-1}) (KBr): 3422, 1730, 1648, 1623 ,1590, 1566, 1481, 1448, 1272, 835, 781, 758, 735, 662;
^{1}H NMR (300 MHz, DMSO-d_6): δ 8.75 (1H, d, J = 7.5 Hz NH), 8.70 (1H, d, J = 2.2 Hz, H-1), 8.25 (1H, dd, J = 8.8 and 2.3 Hz, H-3), 8.08 (d, J = 8.9 Hz, H-8), 7.66 (1H, d, J = 8.8 Hz, H-4), 7.15 (1H, d, J = 2.4 Hz, H-5), 7.03 (1H, dd, J = 8.9 and 2.4 Hz, H-7), 5.59 (1H, s, OH), 4.44 (1H, m, H-2'), 3.96 (3H, s, Ar-OCH_3), 3.80 (1H, dd, J = 11.6 and 5.5, H-1"), 3.75 ppm (1H, dd, J = 11.5 and 5.5, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.3 (C-9), 172.3 (C=O ester), 165.7 (C=O amide), 165.5 (C-6), 158.0 (C-4a), 157.7 (C-10a), 134.05(C-3), 130.3 (C-2), 128.2 (C-8), 126.0 (C-1), 121.2 (C-9a), 118.7 (C-4), 115.4 (C-8a), 114.5 (C-7), 101.3 (C-5), 61.6 (C-1") 56.3 (Ar-OCH_3), 56.7 ppm (C-2')

3.3.15. Methyl (6-Methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tyrosinate (X2AELT-Me) (17)

m.p.: 85-87 ºC (MeOH); IR ν max (cm^{-1}) (KBr): 3347, 1741, 1644, 1615, 1587, 1541, 1516, 1478, 1446, 1276, 836, 758, 668;
^{1}H NMR (300 MHz, DMSO-d_6): δ 9.24 (1H, s, Ar-OH) 9.19 (1H, d, J = 7.8 Hz NH), 8.71 (1H, d, J = 2.3 Hz, H-1), 8.23 (1H, dd, J = 8.7 and 2.3 Hz, H-3), 8.14 (d, J = 8.8 Hz, H-8), 7.71 (1H, d, J = 8.8 Hz, H-4), 7.11 (2H, d, J = 8.8, H-3" and 7"), 7.20 (1H, d, J = 2.3 Hz, H-5), 7.09 (1H, dd, J = 8.8 and 2.3 Hz, H-7), 6.65 (2H, d, J = 8.8 Hz, H-4" and H6"), 4.66 (1H, m, H-2'), 3.95 (3H, s, Ar-OCH_3), 3.66 (3H, s, COO-CH_3), 3.08 (1H, dd, J = 13.9 and 5.5, H-1"), 3.02 ppm (1H, dd, J = 13.7 and 5.5, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.8 (C-9), 172.2 (C=O ester), 165.2 (C=O amide), 165.1 (C-6), 157.5 (C-4a), 157.3 (C-10a), 156.0 (C-5"), 133.9 (C-3), 130.0 (C-3" and C-7"), 127.7 (C-2), 129.5 (C-2"), 127.6 (C-8), 125.6 (C-1), 120.8 (C-9a), 118.3 (C-4), 115.0 (C-4" and C-6"), 114.9 (C-8a), 114.0 (C-7), 100.8 (C-5), 56.3 (Ar-OCH_3), 54.8 (C-2'), 51.9 (COO$\underline{C}H_3$), 35.4 ppm (C-1").

3.3.16. Methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-tyrosinate (X2AEDT-Me) (16)

m.p.: 69-70 ºC (MeOH); IR ν max (cm^{-1}) (KBr): 3405, 3368, 1741, 1646, 1615, 1588, 1541, 1516, 1479, 1445, 1272, 836, 759, 669:
^{1}H NMR (300 MHz, DMSO-d_6): δ 9.24 (1H, s, Ar-OH) 9.20 (1H, d, J = 7.9 Hz NH), 8.71 (1H, d, J = 2.3 Hz, H-1), 8.23 (1H, dd, J = 8.8 and 2.3 Hz, H-3), 8.13 (d, J = 8.8 Hz, H-8), 7.71 (1H, d, J = 8.8 Hz, H-4), 7.10 (2H, d, J = 8.9, H-3" and 7"), 7.20 (1H, d, J = 2.4 Hz, H-5), 7.09 (1H, dd, J = 8.8 and 2.3 Hz, H-7), 6.65 (2H, dd, J = 8.9 Hz, H-4" and H6"), 4.66 (1H, m, H-2'), 3.95 (3H, s, Ar-OCH_3), 3.66 (3H, s, COO-CH_3), 3.08 (1H, dd, J = 13.9 and 5.5, H-1"), 3.02 ppm (1H, dd, J = 13.7 and 5.5, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.8 (C-9), 172.2 (C=O ester), 165.2 (C=O amide), 165.1 (C-6), 157.5 (C-4a), 157.3 (C-10a), 156.0 (C-5"), 133.9 (C-3), 130.0 (C-3" and C-7"), 127.7 (C-2), 129.5 (C-2"), 127.6 (C-8), 125.6 (C-1), 120.8 (C-9a), 118.3 (C-4), 115.0 (C-4" and C-6"), 114.9 (C-8a), 114.0 (C-7), 100.8 (C-5), 56.3 (Ar-OCH_3), 54.8 (C-2'), 51.9 (COO$\underline{C}H_3$), 35.5 ppm (C-1").

3.3.17. (6-Methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tyrosine (X2AALT) (28)

m.p.: 145-146 ^{o}C (H2O); IR ν max (cm^{-1}) (KBr): 3383, 1729, 1646, 1615, 1586, 1540, 1564, 1516, 1478, 1446, 1277, 836, 780, 761, 671;
^{1}H NMR (300 MHz, DMSO-d_6): δ 9.20 (1H, s, Ar-OH) 9.02 (1H, d, *J* = 8.0 Hz NH), 8.70 (1H, d, *J* = 1.5 Hz, H-1), 8.22 (1H, dd, *J* = 8.9 and 1.9 Hz, H-3), 8.14 (d, *J* = 8.1 Hz, H-8), 7.71 (1H, d, *J* = 8.8 Hz, H-4), 7.20 (1H, d, *J* = 1.4 Hz, H-5), 7.13 (2H, d, *J* = 7.9, H-3" and 7"), 7.09 (1H, dd, *J* = 8.1 and 1.4 Hz, H-7), 6.66 (2H, dd, *J* = 7.9 Hz, H-4" and H6"), 4.57 (1H, m, H-2'), 3.95 (3H, s, Ar-OCH_3), 3.11 (1H, dd, *J* = 13.6 and 4.6, H-1"), 2.97 ppm (1H, dd, *J* = 13.3 and 5.2, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.8 (C-9), 172.2 (C=O ester), 165.7 (C=O amide), 165.4 (C-6), 157.7 (C-4a), 157.2 (C-10a), 158.0 (C-5"), 134.3 (C-3), 130.4 (C-3" and C-7"), 130.3 (C-2"), 128.7 (C-2), 128.2 (C-8), 126.0 (C-1), 121.2 (C-9a), 118.7 (C-4), 115.4 (C-4" and C-6"), 115.0 (C-8a), 114.5 (C-7), 101.3 (C-5), 56.7 (Ar-OCH_3), 55.2 (C-2'), 35.9 ppm (C-1").

3.3.18. Methyl(6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tryptophanate (X2AELTrp-Me) (29)

m.p.: 95-97 ^{o}C (MeOH); IR ν max (cm^{-1}) (KBr): 3312, 1740, 1651, 1616, 1588, 1565, 1539, 1477, 1444, 1275, 835, 745, 689, 668;
^{1}H NMR (300 MHz, DMSO-d_6): δ 10.86 (1H, d, J = 2.4 Hz, NH'), 9.24 (1H, d, *J* = 7.8 Hz NH), 8.74 (1H, d, *J* = 2.3 Hz, H-1), 8.25 (1H, dd, *J* = 8.7 and 2.3 Hz, H-3), 8.14 (d, *J* = 8.9 Hz, H-8), 7.71 (1H, d, *J* = 8.7 Hz, H-4), 7.59 (1H, dd, *J* = 7.8 and 1.1 Hz, H-8"), 7.33 (1H, dd, *J* = 8.1 and 0.9 Hz, H-5"), 7.23 (1H, d, *J* = 2.4 Hz, H-3"), 7.21 (1H, d, *J* = 2.4 Hz, H-5), 7.10 (1H, dd, *J* = 8.9 and 2.4 Hz, H-7), 7.07 (1H,ddd, *J* = 8.1, 7.0 and 1.2, H-6"), 7.00 (1H, ddd, *J* = 8.0, 7.0 and 1.1, H-7"), 4.57 (1H, m, H-2'), 3.95 (3H, s, Ar-OCH_3), 3.30 (1H, dd, *J* = 14.2 and 5.8, H-1"), 3.27 ppm (1H, dd, *J* = 14.2 and 5.8, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.8 (C-9), 172.5 (C=O ester), 165.2 (C=O amide), 165.1 (C-6), 157.5 (C-4a), 157.3 (C-10a), 136.1 (C-4"), 133.9 (C-3), 129.5 (C-2) 127.7 (C-8), 127.1 (C-9"), 125.6 (C-1), 123.6 (C-3"), 120.9 (C-6"), 120.7 (C-9a), 118.4 (C-8"), 118,2 (C-7") 118.0 (C-4), 114.9 (C-8a), 111.5 (C-5"), 114.0 (C-7), 110.0 (C-2"), 100.8 (C-5), 56.2 (Ar-OCH_3), 54.0 (C-2'), 52.0 (COO$\underline{C}H_3$), 26.5 ppm (C-1").

3.3.19. Methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-tryptophanate (X2AEDTrp-Me) (20)

m.p.: 94-96 ^{o}C (MeOH); IR ν max (cm^{-1}) (KBr): 3311, 1735, 1653, 1621, 1588, 1565 ,1539, 1537, 1479, 1445, 1274, 835, 745 , 690, 669;
^{1}H NMR (300 MHz, DMSO-d_6): δ 10.86 (1H, d, J = 2.4 Hz, NH'), 9.23 (1H, d, *J* = 7.7 Hz NH), 8.74 (1H, d, *J* = 2.3 Hz, H-1), 8.25 (1H, dd, *J* = 8.7 and 2.3 Hz, H-3), 8.13 (d, *J* = 8.9 Hz, H-8), 7.70 (1H, d, *J* = 8.8 Hz, H-4), 7.58 (1H, dd, *J* = 7.8 and 1.1 Hz, H-8"), 7.33 (1H, dd, *J* = 8.1 and 0.9 Hz, H-5"), 7.23 (1H, d, *J* = 2.4 Hz, H-3"), 7.21 (1H, d, *J* = 2.4 Hz, H-5), 7.10 (1H, dd, *J* = 8.9 and 2.4 Hz, H-7), 7.07 (1H,ddd, *J* = 8.0, 7.0 and 1.1, H-6"), 7.00 (1H, ddd, *J* = 7.0, 7.0 and 1.1, H-7"), 4.57 (1H, m, H-2'), 3.95 (3H, 0, Ar-OCH_3), 3.30 (1H, dd, *J* = 14.2 and 5.8, H-1"), 3.27 ppm (1H, dd, *J* = 14.2 and 5.8, H-1"). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.8 (C-9), 172.5 (C=O ester), 165.2 (C=O amide), 165.1 (C-6), 157.5 (C-4a), 157.3 (C-10a), 136.2 (C-5"), 133.9 (C-3), 129.5 (C-2) 127.8 (C-8), 127.9 (C-9"), 125.6 (C-1), 123.8 (C-3"), 120.9 (C-6"), 120.8 (C-9a), 118.4 (C-8"), 118.1 (C-7") 118.0 (C-4), 111.5 (C-5"), 114.9 (C-8a), 114.0 (C-7), 110.0 (C-2"), 100.8 (C-5), 56.2 (Ar-OCH_3), 54.0 (C-2'), 52.1 (COO$\underline{C}H_3$), 26.5 ppm (C-1").

3.3.20. (6-Methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tryptophan (X2AALTrp) (29)

m.p.: 152-155 ^{o}C (H2O); IR ν max (cm^{-1}) (KBr): 3404, 1730, 1649, 1615, 1586, 1566, 1538, 1477, 1445, 1276, 835, 779, 744, 670;
^{1}H NMR (300 MHz, DMSO-d_6): δ 10.75 (1H, d, J = 2.4 Hz, NH'), 8.99 (1H, d, *J* = 7.9 Hz NH), 8.65 (1H, d, *J* = 2.3 Hz, H-1), 8.17 (1H, dd, *J* = 8.8 and 2.3 Hz, H-3), 8.07 (d, *J* = 8.9 Hz, H-8), 7.62 (1H, d, *J* = 8.5 Hz, H-4), 7.55 (1H, dd, *J* = 7.7 and 0.9 Hz, H-8"), 7.25 (1H, dd, *J* = 7.9 and 1.0 Hz, H-5"), 7.14 (1H, d, *J* = 2.2 Hz, H-3"), 7.14 (1H, d, *J* = 2.4 Hz, H-5), 7.02 (1H, dd, *J* = 8.9 and 2.4 Hz, H-7), 7.00 (1H,ddd, *J* = 7.8, 7.0 and 1.2, H-7"), 6.97 (1H, ddd, *J* = 7.9, 7.0 and 1.2, H-6"), 4.63 (1H, m, H-2'), 3.95 (3H, s, Ar-OCH_3), 3.25 (1H, dd, *J* = 13.3 and 4.6, H-1"), 3.16 ppm (1H, dd, *J* = 14.2 and 4.7, H-1"). ^{13}C NMR (75

MHz, DMSO-d_6): δ 175.2 (C-9), 173.9 (C=O ester), 165.7 (C=O amide), 165.4 (C-6), 158.0 (C-4a), 157.7 (C-10a), 136.5 (C-4"), 134.3 (C-3), 130.3 (C-2) 128.2 (C-8), 127.6 (C-9"), 126.0 (C-1), 124.0 (C-3"), 121.4 (C-6"), 121.2 (C-9a), 118.8 (C-8"), 118,6 (C-7") 118.2 (C-4), 115.4 (C-8a), 114.5 (C-7), 111.8 (C-5"), 110.0 (C-2"), 101.3 (C-5), 56.7 (Ar-OCH_3), 54.3 (C-2'), 27.1 ppm (C-1").

3.4. Enantiomeric Purity

3.4.1. Instrumentation and chromatographic conditions

Liquid chromatography analysis was performed in Laboratory of Applied Chemistry of Cooperativa De Ensino Superior Politécnico Universitário – CESPU. Analytical HPLC analyses were performed on a LaChrom Merck Hitachi HPLC, equipped with an L-7100 pump, an L-7200 auto-injector, an L-7455 diode arrangement detector and a D-7000 interface. The stationary phase used was Lux ™ 3 μm celullose-2 from Phenomenex and data analysis was performed using HPLC System Manager HSMD-7000 software, version 3.0. Ethanol (EtOH) and *n*-Hexane for HPLC were purchased from Sigma-Aldrich Co.

The normal-phase mode LC evaluation was carried out using a mixture of EtOH and *n*-Hexane as mobile phase, prepared in a volume/volume (50:50 *v/v*) relation with 0.01% TFA. The flow rate used was 1 mL/min and the chromatograms were monitored by UV detection at a wavelength of 254 nm. The sample injections (10 μL) were carried out in duplicate. The dead time (t_0) was considered to be equal to the peak of the solvent front and was taken from each particular run. The stock solutions of CDXs in EtOH at the concentration of 1 mg/mL were prepared and working solutions of enantiomeric mixtures of CDXs were prepared mixing equal aliquots of each enantiomer. The analyses were performed at room temperature.

The retention factor (k) was calculated using the equation ($k = [t_R - t_0] / t_0$). The separation factor (α) was calculated as ($\alpha = k_2/k_1$). The resolution factor (Rs) was calculated using the equation

(Rs = 1.18 [t_{R2} - t_{R1}]/[$\frac{1}{2}W_1 + \frac{1}{2}W_2$]) where t_{R1} and t_{R2} are the retention times of the first and second enantiomers, respectively, and $12W_1$ and $12W_2$ are the corresponding peak width measured on half height. rThe methematica formulas used for the calculation of the percentage of enantiomeric excess and enantiomeric composition were the following:

$$\%\ Enantiomeric\ excess = \frac{[E1]-[E2]}{[E1]+[E2]} \times 100$$

$$\%\ Enantiomeric\ composition = \frac{[E1]}{[E1]+[E2]} \times 100$$

3.5. Antimicrobial activity

Microbiological activity tests were carried out for the strains *Staphylococcus aureus* (Sa) (ATCC 29213), *Staphylococcus epidermidis* (Se) (this strain was supplied by the microbiology research laboratory), *Candida albicans* (Ca) (ATCC 10231) and *Pseudomonas aeruginosa* (Pa) (ATCC 27853). The bioactivity assays were iniciated by testing the medium in which the strains grew best, evaluating the multiwell plate and determing where there was more biofilm formation. Also, it was also necessary to determine the incubation time. Therefore, the growth of the strains in Trypic Soy Broth (TSB), nutritive broth and Brain Heart Infusion (BHI) in two types of plates of different brands: Orange and VWR. The plates were allowed to incubate for 24h and 48h. After these tests, it was concluded that the best conditions were on the VWR plate, with TSB medium, for 24h. The wells were inoculated with 100 µl of TSB medium, in each well one of the 4 strains (5 µl of suspension of each strain in sterile saline was placed to give an initial optical density (OD) 600 nm of about 0.1) and 1 µl of compound dissolved in DMSO. The OD was read at 600 nm. Negative control wells (culture medium + 5 µL DMSO) and positive controls (inoculated culture medium + 5 µL DMSO, but no compounds) were also inoculated into each plate. The assay

with each strain was always replicated 4 to 10 times (wells) per plate, according to the scheme of each plate. After the growth time, the OD at 600 nm was measured again and the difference from the initial value of each well is used as indicator of the proliferation of the strain. To quantify the biofilm, the suspension was discarded, the cells adhered to the well were stained with 0.1% crystal violet , each well was washed with water and 200 µl of absolute ethanol was placed in each well. 100 µl of this solvent was taken from each well and placed in a new 96 well plate, and then read at 595 nm. Differences between negative, positive and test controls were analyzed using a t-test.

CHAPTER 4: CONCLUSIONS

4. Conclusions

This dissertation described a methodology towards obtaining new chiral derivatives from a carboxylated xanthone subtract, XCar 2. This was accomplished through a chiral pool synthesis, in which commercially available chiral amino esters were coupled to the xanthone by its amine group, forming an amide bond. In this step, two different coupling reagents were used, COMU and TBTU, and both their reactivity and stability were observed. Although the yields obtained could give any clue for a more suitable choice, reaction times for COMU were much shorter that for TBTU, with times around 1 hour and 30 min for the first against times that reached 22h for the latter. On the other hand, the stability of the reactions with COMU were worse that those with TBTU, forming side products. Regarding this, TBTU showed much cleaner couplings, originating only one product.

In the next step, the amino ester derivatives were submitted to a hydrolysis under mild alkaline conditions, to give the correspondent derivative with a free carboxylic acid. The protecting *tert*-butyl demanded the use of more aggressive bases for a longer time, which could constitute a threat to the stability of the amide bond and also for racemization.

The enantiomeric purity studies, using chiral chromatography, showed very good enantiomeric excess values, except for the amino acid derivatives of both enantiomers from phenilglycine, showing that racemization occurred during hydrolysis.

All of the compounds ^{1}H, ^{13}C and IR spectrums were obtained in order to characterize the CDXs, along with their melting points. The analysis showed that the performed reactions were accomplished, and their purification was effective.

The compounds submitted to preliminary biological assays obtained promising results in the inhibition of bacterial growth, constituting an exciting clue that these compounds could give a contribute to the discovery of new antibiotics.

In the future, this project intends to purse with its objective, to build a complete library of CDX from all the proteinogenic amino acids. The possibility of polyamino acid chain derivatives is also something to have into account.

CHAPTER 5: REFERENCES

5. References

The search for the references used in the present dissertation was made using the following search engines (last access in August 2017):

http://www.webofknowledge.com
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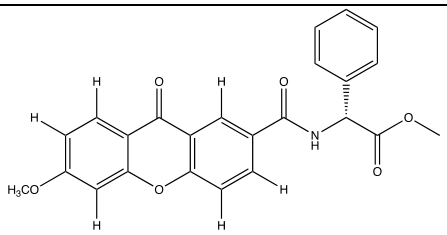
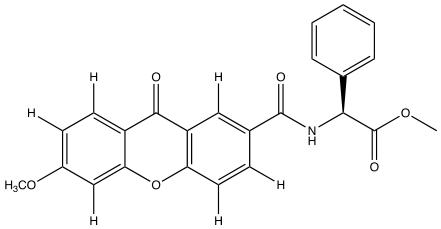
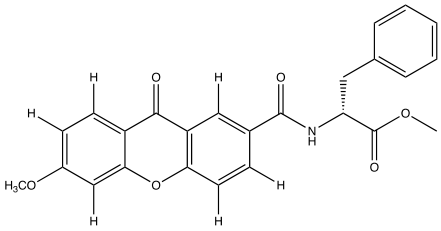
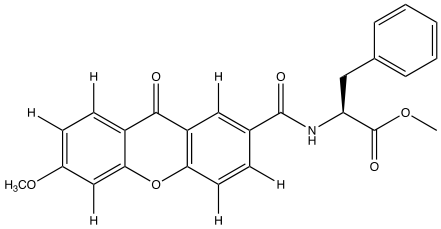
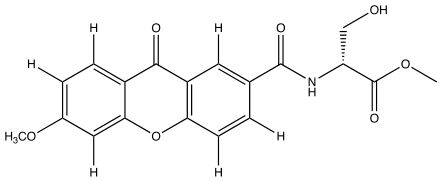
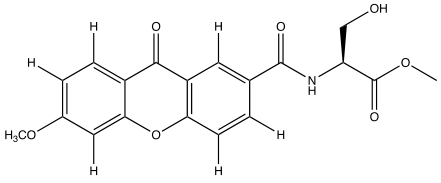
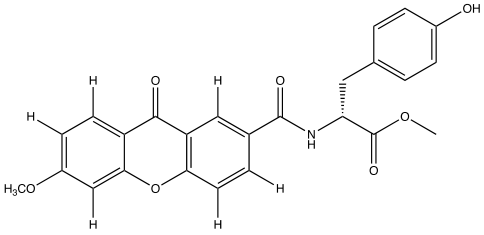
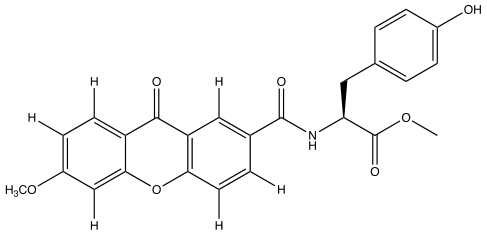
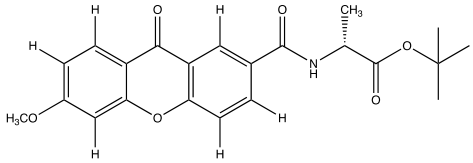
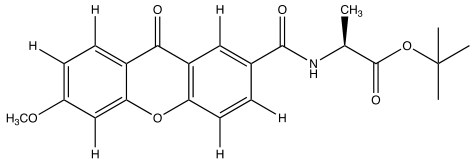
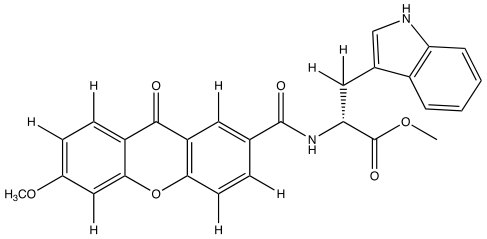
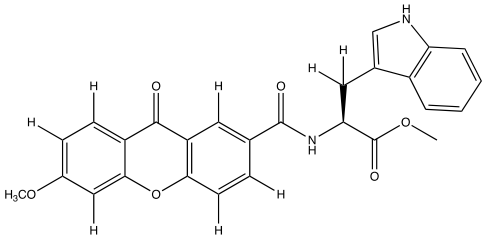
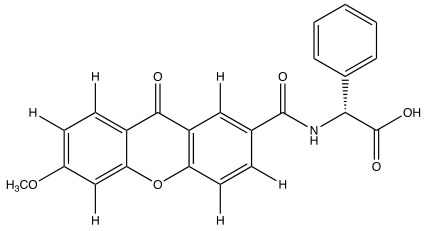
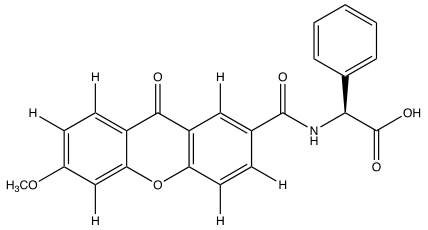
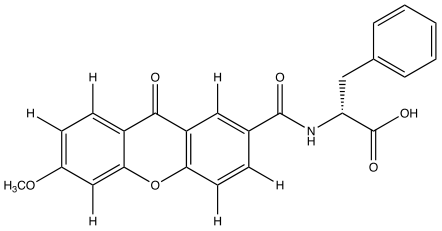
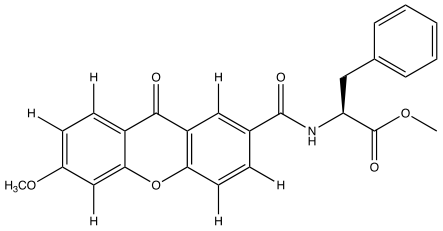
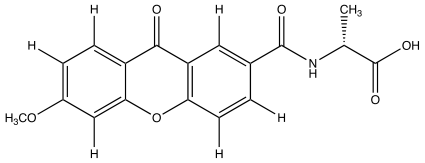
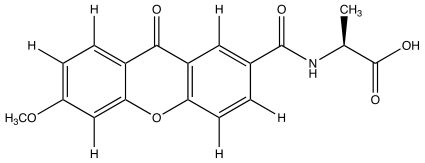
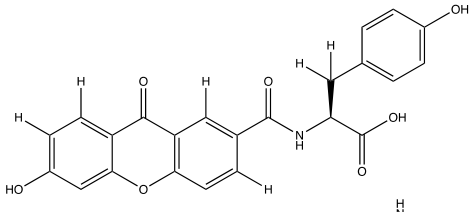
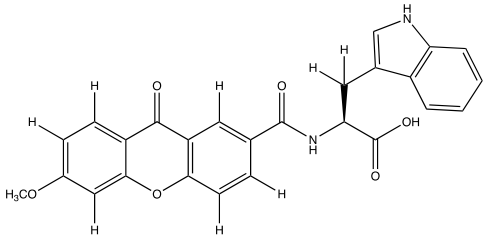
Annex 1

Annex 1

Table 11. Code names, structural names and chemical structures.

Compound code	Chemical name	Chemical Structure
X2AEDPG-Me (**10**)	methyl (*R*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-2-phenylacetate	
X2AELPG-Me (**11**)	methyl (*S*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-2-phenylacetate	
X2AEDPA-Me (**12**)	methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-phenylalaninate	
X2AELPA-Me (**13**)	methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-phenylalaninate	
X2AEDS-Me (**14**)	methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-serinate	
X2AELS-ME (**15**)	methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-serinate	
X2AEDT-Me (**16**)	methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-tyrosinate	

X2AELT-Me (**17**)	methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tyrosinate	
X2AEDA-TB (**18**)	*tert*-butyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-alaninate	
X2AELA-TB (**19**)	*tert*-butyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-alaninate	
X2AEDTrp-Me (**20**)	methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-tryptophanate	
X2AELTrp-Me (**21**)	methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tryptophanate	
X2AADPG (**22**)	(*R*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-2-phenylacetic acid	
X2AALPG (**23**)	(*S*)-2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido)-2-phenylacetic acid	
X2AADPA (**24**)	(6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-phenylalanine	

X2AALPA (**25**)	methyl (6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-phenylalaninate	
X2AADA (**26**)	(6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-alanine	
X2AALA (**27**)	(6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-alanine	
X2AALT (**28**)	(6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tyrosine	
X2AALTrp (**29**)	(6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*L*-tryptophan	
X2AADS (**30**)	(6-methoxy-9-oxo-9*H*-xanthene-2-carbonyl)-*D*-serine