Universidade Federal de Minas Gerais – UFMG
Instituto de Ciências Exatas – ICEx
Departamento de Química

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# SYNTHESIS OF NATURAL BUTENOLIDES AND ANALOGS

Belo Horizonte

*2018*

UFMG/ICEx/DQ. 1302ª

T. 589ª

Jaime Alejandro Martínez Acosta

# SYNTHESIS OF NATURAL BUTENOLIDES AND ANALOGS

Thesis presented to the Chemistry Department of the Institute of Exact Sciences of the Federal University of Minas Gerais, as a partial requirement to obtain a PhD in Chemistry - Organic Chemistry.

Belo Horizonte

2018

Ficha Catalográfica

M375s
2018
T

Martínez Acosta, Jaime Alejandro
Synthesis of natural butenolides and analogs [manuscrito] / Jaime Alejandro Martínez Acosta. 2018.
[xvi], 251 f. : il.

Orientador: Luiz Cláudio de Almeida Barbosa.

Tese (doutorado) – Universidade Federal de Minas Gerais – Departamento de Química.
Inclui bibliografia.

1. Química orgânica - Teses 2. Lactonas - Teses 3. Produtos naturais - Teses 4. Aldeídos – Teses 5. Herbicidas - Teses 6. Furanos – Teses 7. Produtos de ação antimicrobiana – Teses 8. Reações de adição – Teses 9. Mecanismos de reações orgânicas - Teses I. Barbosa, Luiz Cláudio de Almeida, Orientador II. Título.

CDU 043

Elaborada pela Biblioteca do Departamento de Química - UFMG

UFMG
Programa de Pós-Graduação em Química
Departamento de Química - ICEx

"Synthesis of Natural Butenolides and Analogs"

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Belo Horizonte, 24 de setembro de 2018.

*The nitrogen in our DNA, the calcium in our teeth, the iron in our blood, the carbon in our apple pies were made in the interiors of collapsing stars. We are made of starstuff."*

*— Carl Sagan, Cosmos*

*To my loved father:*

*Jaime Martínez Betancourt*
*(1953-2013)*

*Who taught me that the dedication and the love for learning is the one of the most important conditions to be a better man…as he was.*

## AKNOWLEDGMENT

Initially I want to thank God for allowing me to successfully cross this new stage of my life.

I want to thank all the members of my family which were a very important support. With all my love I want to dedicate this work to all of them, mainly my mom Rosa Acosta, my sister María Martínez, my grandmother Zulay Acosta and my girlfriend Edna Gómez, that support me in several aspects during this PhD.

I want to give infinite thanks to my supervisor, prof. Dr. Luiz Cláudio de Almeida Barbosa, his trust, advice, wisdom and teaching were always a reason of pride and motivation for me.

I want to thank the members of the internal and external evaluators, the PhDs. Ricardo Alves (UFMG), Diogo Vidal (UFMG), Julio Pastre (UNICAMP) and Sergio Pinheiro (UFF), whose corrections and advices will give a new perspective that will surely improve the written and the quality of my work.

I want to thank the CNPq support and funding, it was the agency that provide me with my MSc and PhD scholarships, and, together with CAPES and FAPEMIG, financing our projects.

I want to thank the UFMG and to the entire chemistry department for allowing me an excellent education as a doctor and as a person. To all its educational, administrative and technical staff which were always of great help to advance in my project, as well as my academic and personal development.

I want to thank Prof. Dr. John Boukouvalas who welcomed me in his laboratory at the Université Laval, himself was very helpful in the development of many of our projects in Brazil, furthermore, his synthesis project of basidalin and enhygrolides was excellent and allowed me to improve my skills in chemistry, I consider him an excellent person, a comprehensive supervisor and an excellent chemist. I also wanted to thank all the members of his lab, Ramesh, Mark, Charles and Raphael.

I want to thank Prof. Dr. Martin Wills, who received me in his laboratory at the University of Warwick, his project of enantioselective catalysis allowed me to approach an area that I did not know, besides his great charisma and his knowledge, he served as a great inspiration for my career. I also wanted to thank the members of his lab, Jonathan and Sam.

I want to thank the universities of Laval and Warwick and all their administrative and technical staff. They always proved to be excellent professionals and welcomed me in a great way. Besides I want to thank for the financial help that they offered me through the ELAP and Warwick's scholarship.

I would like to thank Dr. Milandip Karak (UFV) and Ramesh Muddala (ULaval) with whom I developed my doctorate projects, besides being excellent people they are outstanding professionals and several things that I learned and I know in chemistry is thanks to them.

I would like to thank our collaborators Drs. Giusseppe Forlani (biological activities), Ariel Sarotti (computational calculations), Felipe Martins (X-Ray) and Guy Clarkson (X-Ray) for their significant contribution to the projects developed during my PhD.

I want to thank the members of the laboratory 220 of the department of chemistry of the UFMG: Amalyn, Isabel, Thais, Milandip, Almodevar, Diego, Muhammad and Thiago. They were a small family and whose help, both personal and professional, was vital to obtain my PhD. I consider them great friends and chemists and I will always be proud to have shared this space with them.

I would like to thank Brazil, all its wonderful places and people that I met at this time.

I would like to thank all my friends in Colombia and Brazil who at all times were aware of me and always were very important emotional support.

I would like to thank the UTP and UFV and all its staff. They represented all my bases in chemistry.

# INDEX

## LIST OF ABBREVIATIONS AND SYMBOLS

| | | | |
|---|---|---|---|
| BINOL | 1,1′-binaphthalene-2,2′-diol | *ee* | enantiomeric excess |
| DBU | 1,8-diazabicyclo[5.4.0]undec-7-ene | eq | equivalent |
| IBX | 2-iodoxybenzoic acid | AcOEt | ethyl acetate |
| Ac | acetyl group | Et | ethyl group |
| $\delta$ | angular deformation (in IR) | FT-IR | Fourier transform - infrared spectroscopy |
| ArES | aromatic electrophilic substitution | GC | gas chromatography |
| Ar | aryl group | $\sigma$ | Hammett parameter |
| ATH | asymmetric transfer hydrogenation | Hz | hertz |
| atm | atmosphere | HMPA | hexamethyl phosphoramide |
| $\rho$ | balance angular deformation out of plane (in IR) | HRMS | high resolution mass spectroscopy |
| B | base | HPLC | high pressure liquid chromatography |
| Bn | benzyl group | IR | infrared |
| Bz | benzoyl group | MS | mass spectroscopy |
| Bp | boiling point | *m/z* | mass to charge ratio |
| Bu | butyl group | RDA | Retro Diels-Alder |
| Cat | catalyst | DCM | dichloromethane |
| E1cb | conjugated base-unimolecular elimination mechanism | Mp | melting point |
| DMP | Dess-Martin periodinane | $\mu$ | dipole moment |
| dr | diastereomeric ratio | M.W. | micro wave |
| DMAP | dimethylamino pyridine | THF | tetrahydrofuran |
| DMF | dimethylformamide | TLC | thin layer chromatography |
| DMSO | Dimethyl sulfoxide | M | molar |
| $\mu$M | micromolar | DIPEA | *N,N*-diisopropylethylamine |
| MIC | minimum inhibitory concentration | NBS | *N*-bromosuccinimide |
| EDG | electron donating group | NMP | *N*-Methyl-2-pyrrolidone |
| EWG | electron withdrawing group | N.R. | no reaction |
| ESI | electrospray ionization | N.D. | not determinated |
| Ph | phenyl group | TMS | trimethylsilane |

| | |
|---|---|
| Pv | pivalate group |
| Pr | propyl group |
| PTSA | *p*-toluenesulfonic acid |
| Py | pyridine |
| PCC | pyridinium chlorochromate |
| PDC | pyridinium dichromate |
| MsCl | mesyl chloride |
| r.t. | room temperature, retention time (in HPLC) |
| T | temperature |
| TBDMSOTf | *tert*-butyldimethylsilyl trifluoromethanesulfonate |
| Teth | tethered |
| DA | Diels-Alder |
| OMe | methoxy group |
| TBAB | tetra-*n*-butylammonium bromide |
| t | time |
| TsCl | tosyl chloride |
| Ts | tosyl group |
| TEA | triethylamine |
| Tf | triflate group |
| TFA | trifluoroacetic acid |
| TIPSOTf | triisopropylsilyl trifluoromethanesulfonate |
| VAR | vinylogous aldol reaction |
| VMAR | vinylogous Mukaiyama's aldol reaction |
| v/v | volume to volume |
| W | watts |
| $\bar{\upsilon}$ | wave number |

*NMR symbols and abbreviations*

| | |
|---|---|
| brd | broad |
| $\delta$ | chemical shift |
| COSY | correlation spectroscopy |
| *J* | coupling constant |
| DEPT | distortionless enhancement by polarization transfer |
| d | doublet |
| dd | doublet of doublets |
| MHz | megahertz |
| m | multiplet |
| NOESY | nuclear Overhauser effect spectroscopy |
| ppm | part(s) per million |
| q | quartet |
| sept | septet |
| sext | sextet |
| s | singlet |
| t | triplet |

## ABSTRACT

ACOSTA, Jaime Alejandro Martínez, Ph.D. Universidade Federal de Minas Gerais, September, 2018. **Synthesis of natural butenolides and analogs**. Supervisor: Luiz Cláudio de Almeida Barbosa.

Butenolides represent a large family of γ-lactones, having a common furan-2(5*H*)-one subunit that is highly diversified in natural products. These lactones are found in several naturally-occurring sources and are associated with a wide range of biological activities, including, antibiotic, anti-inflammatory, anticancer, phytotoxic, insecticidal, among other properties. In addition to this, these compounds are employed as models for synthetic materials displaying pharmacological and industrial potential.

Rubrolides are a butenolide sub-family of natural products occurring in marine species. These compounds are constituted by a polysubstituited 4-aryl-5-benzylidenefuran-2(5*H*)-one structure. Numerous total synthesis and biological activity studies have been described in literature, including cytotoxic, bactericidal, and photosynthesis inhibition reports. The first part of this work presents a concise and efficient synthesis of two of these natural products, rubrolides B and K. The main steps include: (i) synthesis of 4-arylfuran-2(5*H*)-one via selective Suzuki-Miyaura cross-coupling; (ii) vinylogous aldol condensation between silyloxyfuran derivatives and aldehydes; and (iii) regioselective bromination/demethylation, leading to the functionalization of the aromatic rings. The methodology developed allowed the total synthesis of rubrolides B and K in three to four steps with 41% and 37% global yield, respectively.

Additionally, another type of butenolide, known as tetronamides, has attracted much synthetic interest. Its structure is characterized by a 4-aminofuran-2(5*H*)-one moiety and was used as a model in the development of agrochemicals and wide-range antibacterials.

Basidalin is the first and only natural tetronamide reported, this compound of fungal origin is a derivate of (*Z*)-4-amino-5-(formylmethylene)furan-2(5*H*)-one and has antibiotic and antitumor activities. Its structure is simple, but richly functionalized, leading to various synthetic approaches, but so far with little successful synthesis. Therefore, the second part of this work describes the first

successful total synthesis of basidalin. The main steps of synthesis include: (i) regio and stereocontrolled vinylogous aldol condensation forming key intermediate (*Z*)-4-bromo-5-((1,3-dithiane-2-yl)methylene)butenolide starting with 2-formyl-1,3-dithiane and 4-bromo-2-triisopropylsilyloxyfuran, (ii) addition of the 4-amino group using an Aza-Michael addition/elimination reaction, and (iii) thioacetal removal as the last step. Basidalin synthesis was completed in five steps with 39% global yield.

Enhygrolides are a class of novel, highly substituted antibiotic butenolides found in the obligate marine myxobacterium, *Enhygromyxa salina*, their structure is related to the biologically-active compounds, nostoclides and cadiolides; whose synthesis were amply described in literature. The third chapter of the present work will describe the first synthesis of scarce enhygrolide A. The key steps include (i) addition of α-benzyl group through organocatalytic reductive alkylation, (ii) introduction of β-alkyl substituent by efficient and green iron-catalyzed $sp^2$–$sp^3$ cross-coupling with Grignard reagents, and (iii) typical stereoselective vinylogous aldol condensation reaction to stablish characteristic (*Z*)-γ-benzylidenefuran-2(5*H*)-one scaffold. Aside from its brevity and reliance on environmentally sustainable processes, the synthesis demonstrates the serviceability of butenolide pivalates in cross-coupling reactions. This process was achieved in five steps and 54% overall yield.

Natural and synthetic rubrolides and tetronamides are a relevant class of highly biologically-active butenolides, giving rise to the development of diverse derivatives with important properties ranging from herbicidal to anticancer. In the last chapter of this work, the procedures involved in the synthesis and study of herbicide and cyanobactericide activities of β-azarubrolide analogs or γ-alkylidenetetronamides, are described. The main steps of the synthesis include: (i) *syn* diastereospecific aldol condensation protocol yielding aldolic addition tetronamide products, starting from *N*-arylbutenolides and aromatic aldehydes and (ii) optimized dehydration reaction to get the γ-alkylidene derivatives. However only three γ-benzylidene derivatives with no substituted and with simple aromatic substituents as 2-Cl and 3-$NO_2$ yielded satisfactory result. Nevertheless, cyanobacterial and photosynthesis inhibition assays of all precursors and the final unsaturated derivatives were developed. γ-Alkylidene molecules did not render

good results giving $IC_{50}$ >180 μM and >150 μM, respectively. On the contrary, its precursors showed more promising results, mainly when possess biphenyl group, showed a moderate $IC_{50}$ = 58.4 μM for herbicidal activity and an important cyanobatericidal activity, with $IC_{50}$ = 2.8 μM. In this manner, a total of 20 4-amino-5-(hydroxy(aryl)methyl)furan-2(5*H*)-one analogs were produced in 51%-100% yield and dr(*syn*:*anti*) from 56:44 to >99:1. Later, on these compounds for the same activities were tested. Herbicide activity were moderate, differently to the cyanobactericide, where 5 biphenyl analogs gave the best activity with $IC_{50}$ = 1.3 - 5.1 μM.

Since aldolic addition products are obtained as racemic mixture, an enantioselective synthetic approach for these tetronamides was described. This was done by means of two steps: (i) oxidation of addition aldolic products and (ii) optimized asymmetric hydrogen transfer protocol with Ru tethered catalyst (Ru-teth-cat). Initial *N*-aryltetronamide analog tested for the oxidation showed to be an instable product. Then, more robust β-pyrrolidinebutenolides were employed. This, in turn, rendered excellent yields for the production of specific tetronamide *syn*-enantiomers with yield of 96% and *ee* = 87% using (*R*,*R*)-Ru-teth-cat and yield of 89% and *ee* = 80% using (*S*,*S*)-Ru-teth-cat for the dextrorotatory and levorotatory species, respectively.

## RESUMO

ACOSTA, Jaime Alejandro Martínez, Ph.D. Universidade Federal de Minas Gerais, setembro, 2018. **Síntese de butenolídeos naturais e análogos**. Orientador: Luiz Cláudio de Almeida Barbosa.

Os butenolídeos representam uma grande família de γ-lactonas, as quais têm uma subunidade comum conhecida como furan-2(5*H*)-ona a qual é muito diversificada em produtos naturais. Essas lactonas são encontradas em muitas fontes naturais e estão associadas com uma grande variedade de atividades biológicas, incluindo antibiótica, anti-inflamatória, anticâncer, fitotóxica, inseticida, entre outras. Além disso, esses compostos são muito usados como modelos de outros materiais sintéticos apresentando potencial farmacológico e industrial.

Os rubrolídeos são uma subfamília dos butenolídeos de origem marinha. Esses compostos estão constituídos por uma estrutura 4-aril-5-benzilidenofuran-2(5*H*)-ona poli substituída. Diversas sínteses totais e estudos de atividade biológica têm sido descritos na literatura, incluindo trabalhos sobre rubrolídeos citotóxicos, bactericidas e inibidores da fotossíntese. A primeira parte desse trabalho apresenta a síntese concisa e eficiente de dois desses produtos naturais, os rubrolídeos B e K. As principais etapas da síntese incluem: (i) síntese do 4-arilfuran-2(5*H*)-ona por meio de um acoplamento cruzado de Suzuki-Miyaura; (ii) condensação aldólica viníloga entre os derivados sililoxifuranos e aldeídos; e (iii) bromação/desmetilação regiosseletiva, levando à funcionalização dos anéis aromáticos. A metodologia desenvolvida permitiu a síntese dos rubrolídeos B e K em três a quatro etapas com 41% e 37% de rendimento global, respetivamente.

Adicionalmente, outro tipo de butenolídeo, conhecido como tetronamida, tem adquirido muito interesse sintético. Sua estrutura central caracterizada pela 4-aminofuran-2(5*H*)-ona, e esta é muito usada como modelo no desenvolvimento de agroquímicos e antibacterianos de amplo espectro.

A basidalina é a primeira e a única tetronamidas de origem natural reportada. Esse composto de origem fúngico é derivado do (*Z*)-4-amino-5-(formilmetilen)furan-2(5*H*)-ona, e possui atividades antitumorais e antibióticas. Essa estrutura é simples, mas muito funcionalizada, o que tem levado a diversas tentativas de síntese, mas até entaõ, nenhuma havia obtido total sucesso.

Portanto, a segunda parte desse trabalho descreve a primeira síntese total da basidalina. A principais etapas incluem: (i) condensação aldólica vinílogа régio e estéreocontrolada, formando o intermediário chave (*Z*)-4-bromo-5-((1,3-ditiano-2-il)metilen)butenolídeo, e iniciando do 2-formil-1,3-ditiano e do 4-bromo-2-triisopropolsililoxifurano; (ii) adição do grupo 4-amino por meio da reação de adição/eliminação aza-Michael; e (iii) remoção do grupo tioacetal como etapa final. A síntese da basidalina foi concluída em cinco etapas com 39% de rendimento global.

Os enhygrolídeos são uma nova classe de butenolídeos poli-funcionalizados, encontrados na mixobactéria marinha *Enhygromyxa salina*. Sua estrutura está muito relacionada com os compostos biologicamente ativos, nostoclídeos e cadiolídeos, cujas sínteses foram largamente reportadas. A terceira parte desse trabalho mostra a primeira síntese do escasso enhygrolídeo A. As principais etapas incluem (i) adição do grupo $\alpha$-benzila por meio de alquilação redutiva organocatalítica, (ii) introdução do substituinte $\beta$-alquila através de uma metodologia eficiente e verde de acoplamento cruzado $sp^2-sp^3$ catalisada por ferro junto com reagentes de Grignard; e (iii) condensação aldólica vinílogа para obter a característica estrutura (*Z*)-$\gamma$-benzilidenfuran-2(5*H*)-ona. Além da brevidade e a confiabilidade desse processo ambientalmente seguro, essa síntese demonstrou a capacidade dos pivalatos de butenolídeos em reações de acoplamento cruzado. Essa síntese foi realizada em cinco etapas e 54% de rendimento global.

Rubrolídeos e tetronamidas naturais e sintéticas são uma classe relevante de butenolídeos biologicamente ativos, permitindo o desenvolvimento de diversos derivados com propriedades importantes incluindo herbicida e anticâncer. No capítulo final desse trabalho são descritos os procedimentos que envolvem a síntese e o estudo das atividades herbicidas e cianobactericidas de análogos $\beta$-azarubrolídeos ou $\gamma$-alquilidentetronamidas. As etapas mais importantes incluem: (i) protocolo de reação aldólica *syn*-diasteroespecífica levando a tetronamidas de adição aldólica, iniciando de *N*-arilbutenolídeos e aldeídos aromáticos; e (ii) reação de desidratação otimizada para obter os derivados $\gamma$-alquilidenbutenolídeos. Contudo, somente três derivados $\gamma$-benzilidenos não substituídos e com substituição simples no anel aromático tais

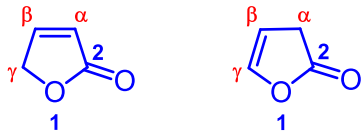
como 2-Cl e 3-$NO_2$ forneceram resultados satisfatórios. Apesar disso, os ensaios de inibição da fotossíntese e do crescimento de cianobactéria de todos os produtos finais e precursores foram realizados. Os derivados insaturados não apresentaram bons resultados, obtendo $IC_{50}$ >180 μM e >150 μM, respetivamente. Todo o contrário com os seus precursores, os quais mostraram resultados mais promissores, principalmente quando possuíam grupos bifenilas na sua estrutura, mostrando um moderado $IC_{50}$ = 58.4 μM para a atividade herbicida e uma importante atividade cianobactericida com $IC_{50}$ = 2.8 μM. Por conseguinte, um total de 20 análogos de 4-amino-5-(hidroxi(aril)metil)furan-2(5*H*)-onas foram produzidos em 51%-100% de rendimento com dr(*syn*:*anti*) de 56:44 até >99:1. Posteriormente, esses foram testados para as mesmas atividades. A atividade herbicida foi moderada, diferentemente da cianobactericida que mostrou que 5 derivados das bifenilas possuíam excelente $IC_{50}$ = 1.3 - 5.1 μM.

Uma vez que os produtos de adição aldólica são obtidos como misturas racêmicas, foi realizado uma abordagem sintética enantioseletiva para esse tipo de tetronamidas. Esse foi realizado mediante dois etapas: (i) oxidação dos produtos de adição; e (ii) um protocolo de transferência de hidrogênio assimétrica usando um catalisador de rutênio “atado” (Ru-teth-cat). Inicialmente, o análogo *N*-arilbutenolídeo testado na reação de oxidação não ofereceu um produto estável. Por sua vez, o uso de β-pirrolidinbutenolídeos mais robustos renderam melhores resultados, produzindo os enantiômeros *syn* de tetronamidas aldólicas com rendimentos de 96% e *ee* = 87% usando o (*R*,*R*)-Ru-teth-cat e 89% e *ee* = 80% usando o (*S*,*S*)-Ru-teth-cat, para as espécies dexorotatórias e levorotatórias, respetivamente.

# 1. INTRODUCTION

## 1.1. Subunit furan-2(5*H*)-one and derivatives.

The subunit furan-2(5*H*)-one (**1**) (**Fig. 1.1**), represented by an α,β-unsaturated γ-lactone is the most distinctive structure of the butenolide motif, being one of the most diversified and widespread nuclei found in nature. It was previously known as crotonolactone, and along with its isomer, furan-2(3*H*)-one (**2**) (isocrotonolatone), it was found as one of many complex natural products and its synthetic derivatives.




(**1**) (**2**)

**Figure 1.1:** Butenolide core structure.

For several years butenolides had attracted the attention of researchers pursuing numerous studies in an attempt to accomplish its extraction, determine its activities, and succeed in its synthesis. These fascinating compounds are found in a variety of natural sources, including microorganisms, insects, plants and vertebrates. These compounds also have a wide range of biological activities such as antimicrobial, anti-inflammatory, anticancer, antiviral (HIV-1), phytotoxic, cardiotonic, insecticidal, among others.[1,2]

In that regard, this lactone shows an important application in medical and agricultural fields, acting as precursor for a variety of synthetic products. The most prominent examples of these synthetic compounds are represented by antiepileptic and anti-inflammatory drugs, pesticides, and even some precursors in the synthesis of antibodies.[3,4]

Quite a few bioactive natural products belong to the butenolide class. Perhaps, the most renowned of these is ascorbic acid (vitamin C) (**Fig. 1.2**; **3**) whose properties have been greatly explored. In that respect, two classical terpenoid butenolides may be discussed. Firstly, the cardenolides (**4**), a class of glycosides present in plants of *Asclepias* spp. (milkweed), displaying powerful activity over the cardiac muscle in animals. Secondly, the furanocembranolides,

represented by pukalide (**5**) and its analogs. These compounds are diterpenoids with a cembrane skeleton and were extracted from *Sinularia abrupta*, a soft coral, in 1975, and showed an organoleptic defense mechanism against predators.[5-7]

Other brief examples are: 4-benzyl-3-phenylfuran-2(5*H*)-one (**Fig. 1.2**; **6**), isolated from *Malbranchea filamentosa*, with vasodilating activity.[8] Compounds **7** and **8**, obtained from the fungus, *Malbranchea aurantiaca*, and the plant, *Siphonochilus aethiopicus*, showed phytotoxic and antimalarial activities, respectively.[9,10] The unsaturated molecule **9,** found in the asymptomatic endophytic fungus E99297, symbiont of the plant *Cistus salviifolius*, is also worth noting and it exhibits anticandidal activity as well as cytotoxicity against the Hep G2 (human hepatocarcinoma) and Colo 320 (adenocarcinoma) cell lines.[11] The marine compound Lissoclinolide (**10**), present in the ascidian *Lissoclinum patella,* showed antibiotic and antitumoral activities.[12,13] Finally, the isomers ellipsoidone A and B (**11**-**12**), isolated from the plant *Hemsleya ellipsoidea*, possess moderate cytotoxicity against human cancer cell lines.[14]

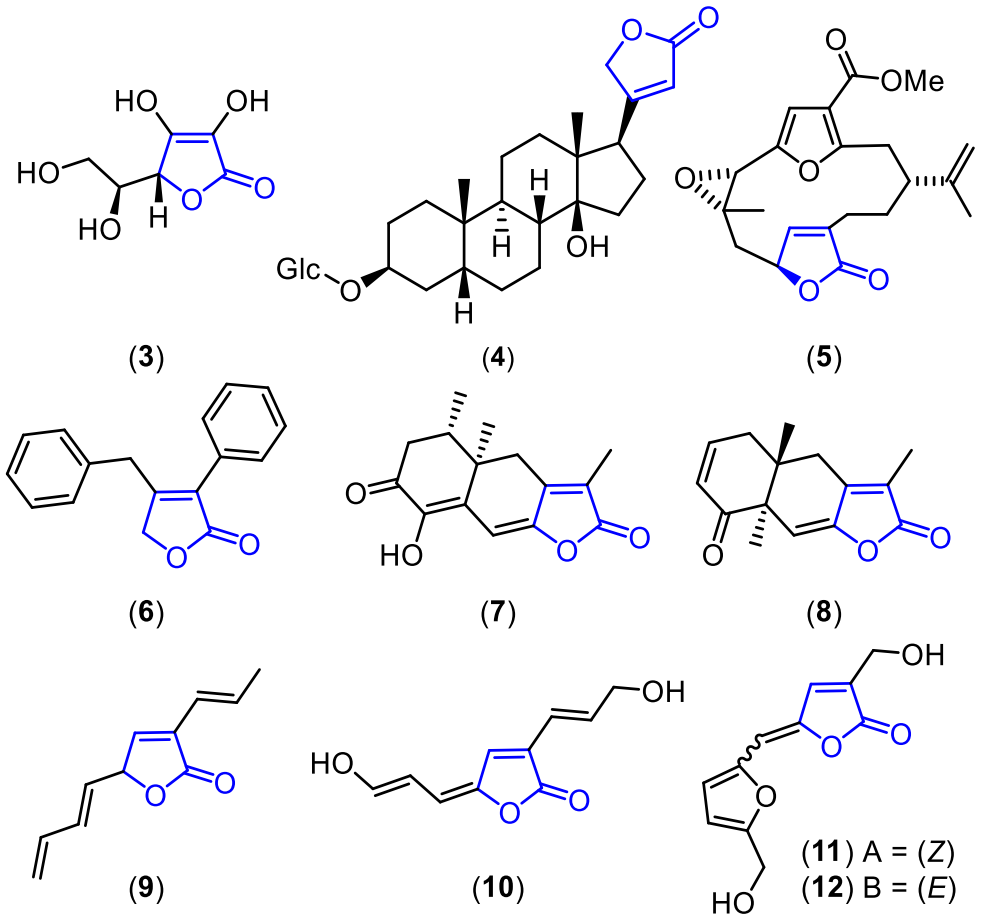
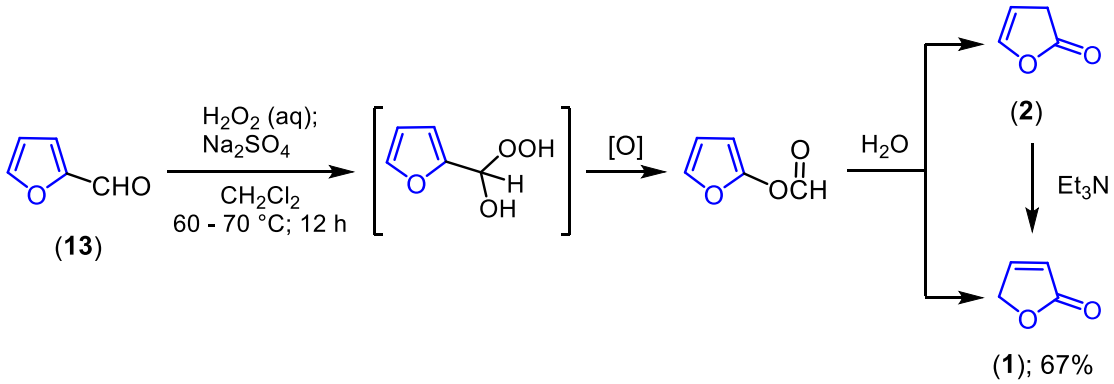



**Figure 1.2:** Natural bioactive butenolide examples.

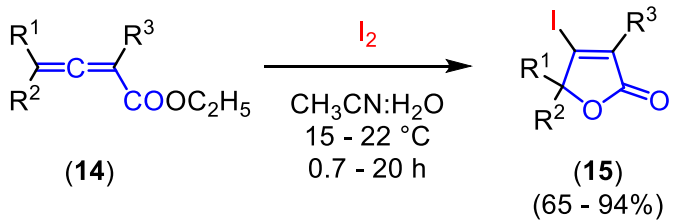
Several synthetic methodologies have been developed for the construction of biologically-active butenolide derivatives, both synthetic and natural. Moreover, these approaches include the synthesis of a γ-lactonic ring (*de novo* synthesis), as well as the use of starting materials with furan-2(5*H*)-one building blocks.

As for the assembly of five-membered lactone, Lui and co-workers, described the synthesis of the simple furan-2(5*H*)-one (**2**). The reaction occurs through the oxidation of furfural (**13**), done under reflux with $H_2O_2$ and $Na_2SO_4$ in a biphasic solution of $H_2O$:$CH_2Cl_2$, producing an isomeric mixture of furanones **1** and **2**. This mixture, when further treated with $Et_3N$, results in the isomerization of **2** to **1** producing a 67% yield of **1** (**Scheme 1.1**).[15]




**Scheme 1.1:** Furan-2(5*H*)-one (**1**) synthesis starting from furfural.[15]

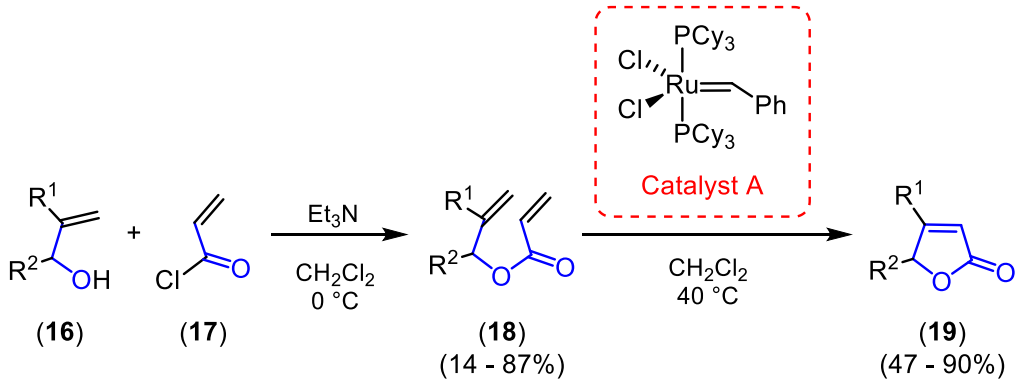
Later, Fu and co-workers, reported a synthesis of 4-iodofuran-2(5*H*)-one derivates (**15**) through lactonization of allenolates **14** using molecular iodine (**Scheme 1.2**).[16]




**Scheme 1.2**: Synthesis of 4-iodofuran-2(5*H*)-ones.[16]

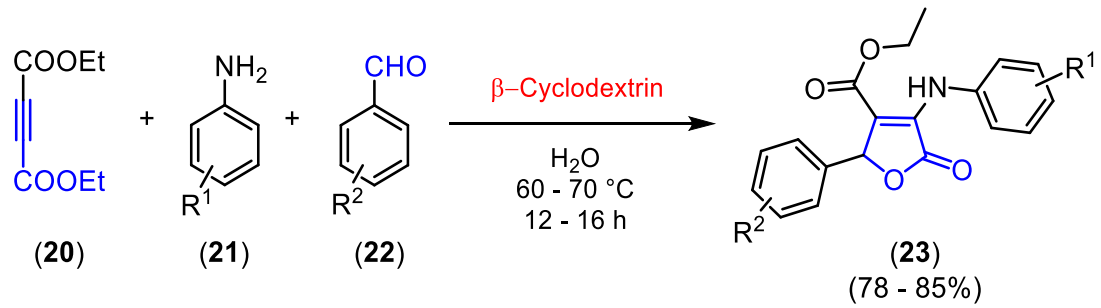
In 2005, Bassetti and co-workers, developed a synthetic methodology for 4,5-dialkylfuran-2(5*H*)-one analogues (**19**), starting from secondary allylic alcohols (**16**) and acryloyl chloride (**17**). Firstly, the intermediate acrylates **18**

were formed, then they were submitted to ring closing metathesis through first generation Grubbs catalyst (Cat. A), which yielded lactone **19** (**Scheme 1.3**).[17]



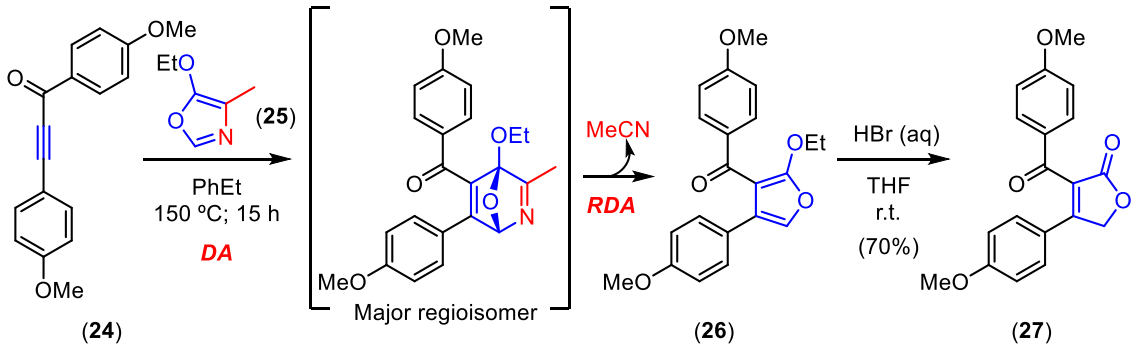

**Scheme 1.3:** Synthesis of furan-2(5*H*)-ones using ring closing metathesis.[17]

Finally, Natayana and co-workers, developed a new methodology to obtain 3,4,5-trisubstitutedfuran-2(5*H*)-ones (**23**), by using β-cyclodextrin as supramolecular catalyst in an aqueous medium using ethyl acetylendicarboxylate (**20**), anilines (**21**), and aromatic aldehydes (**22**) as starting materials (**Scheme 1.4**).[2]




**Scheme 1.4:** Synthesis of 3,4,5-trisubstituted furan-2(5*H*)-ones with β-cyclodextrin supramolecular catalyst.[2]

Another recent concise synthetic approach, including pericyclic reactions, has been reported by Boukouvalas and co-workers.[18] They illustrate a remarkable synthesis of butenolide intermediates throughout sequential Diels-Alder (***DA***) and retro Diels-Alder (***RDA***) reactions with excellent regioselectivity, using alkyne **24** and oxazole **25** as starting materials, producing furan **26** which, through hydrolysis treatment with HBr, yielded the 3-ketofuran-2(5*H*)-one **27** (**Scheme 1.5**). Some other electrocyclic ring closing/opening methodologies will be discussed further in this work.




**Scheme 1.5:** Synthesis of 3-ketofuran-2(5*H*)-one via DA/RDA.[18]

The most common synthetic approach to butenolides includes the use of furan-2(5*H*)-one motifs. For this purpose, some versatile, commercial compounds are employed as starting materials. Some examples include tetronic acid (**28**) and the muchohalic acids (**29**) (**Fig. 1.3**).[1,19-23] Some of their most important characteristics and reactivity will be explained further.

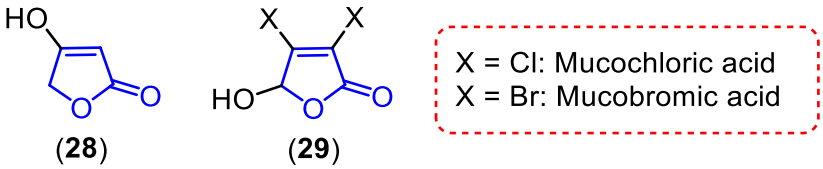



**Figure 1.3:** tetronic and mucohalic acids structures.

Within the butenolide family, the 5- or γ-substituited furan-2(5*H*)-ones, represent approximately 13,000 natural derivatives.[1] Among these compounds, the γ-alkylidenebutenolides (**Fig. 1.4**; **30**) and specially their *Z*-isomers, stand out as the most important furan-2(5*H*)-one subclass, comprising numerous structural forms and several biological activities.[24] Among these molecules are the rubrolides[25], tetronamides[26], prunolides[27], furanocembranolides[7], enhygrolides[28], cadiolides[29], nostoclides[30,31], aporpinones.[32]

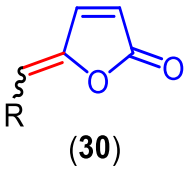



**Figure 1.4:** General structure of γ-alkylidenbutenolides.

Nowadays, the need for the development of new compounds with pharmacological, agroecological, and industrial value inspired our group to

explore different synthetic procedures of many of these natural and synthetic products.

Recently, we have researched several methodologies for the synthesis of butenolides, since these appear to be very promising synthetic targets, besides exhibiting several activities. In chapter 1 of this work, the synthesis of rubrolides B and K will be described, where the key stages are palladium catalyzed coupling, vinyl aldol condensation and bromination of benzene rings.

In Chapter 2, the main focus will be the preparation of the tetronamide basidalin, of which, the most important steps include aldol condensation, elimination, addition of amine group, and deprotection of thioacetals. Chapter 3 will describe the first synthesis of the scarce myxobacterial *E*/*Z* isomers, enhygrolide A and B, respectively. Their syntheses include organocatalytic reductive alkylation, a novel Iron catalyzed cross-coupling with Grignard reagents using robust pivalate substrate, vinylogous aldol condensation and photochemical isomerization. These two syntheses were developed in collaboration with Prof. Dr. John Boukouvalas, from Laval University.

Lastly, in chapter 4, herbicidal and cyanobactericidal evaluation of synthetic γ-alkylidenetetronamides, will be described. These products were produced by typical dehydration reaction of *N*-aryltetronamide analogs, synthetized by means of a stereospecific aldolic reaction protocol previously developed by our group. Finally, we will also discuss a novel enantioselective design in tetronamides synthesis, by means of an asymmetric hydrogen transfer protocol with Ru-arene tethered catalyst, developed in collaboration with Prof. Dr. Martin Wills from Warwick University.

# CHAPTER 1

# TOTAL SYNTHESIS OF RUBROLIDES B AND K

## 1.1. INTRODUCTION

### 1.1.1. Rubrolides

Rubrolides are compounds that possess a polysubstituted 4-aryl-5-benzylidenefuran-2(5*H*)-one structure (**Fig. 1.5**). They are naturally-occurring butenolides extracted from marine organisms, being more recurrent in ascidians (tunicates)[25] and fungi.[33] These molecules present a wide range of biological activities, representing excellent models in research of pharmaceuticals and agrochemicals.

In 1991, Miao and co-workers, reported the first isolation of this kind of compounds, the rubrolides A-I, from ascidian *Ritterella rubra* (**Fig. 1.5**; **31**-**37**; **46** and **47**). They showed potent antibiotic activity *in vitro* and moderate but selective inhibition of phosphatases.[25]

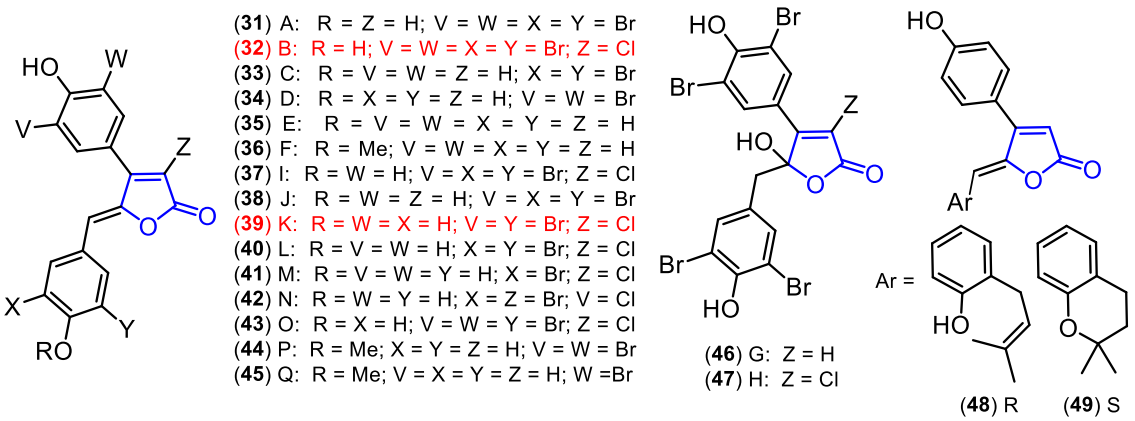


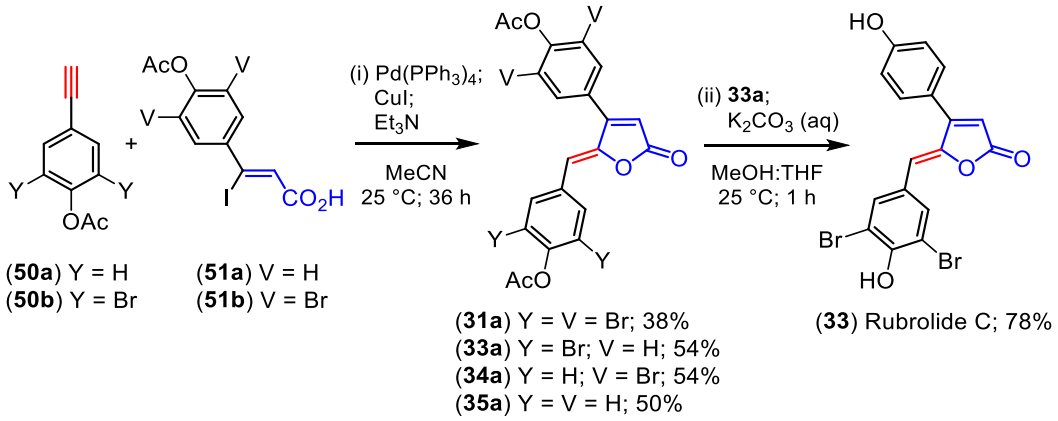

**Figure 1.5:** Natural rubrolide structures.

Rubrolide A (**31**) was isolated from ascidian of the *Botryllus* genus and *Synoicum prunum*.[27,29] Rubrolides I-N (**37**-**42**) were found in ascidians *Synoicum blochmanni*, from which the extracted compounds I (**37**), K (**39**), L (**40**), and M (**41**) showed significant cytotoxicity against HT-294 (human colon carcinoma), P-388 (leukemia), A-549 (lung) e MEL-28 (melanoma) tumor cell lines.[34] Rubrolide N (**42**) and analogs, exhibited cytotoxicity against MCF7 (breast), NCI-H460 (lung) and SF-268 (epidermis) cancer cell lines.[35]

Further studies presented the isolation of rubrolides I and O (**43**) from ascidian *Synoicum sp.*, the latter presented anti-inflammatory activity.[36] Rubrolides P (**44**) and Q (**45**), meanwhile, were found in tunicates,

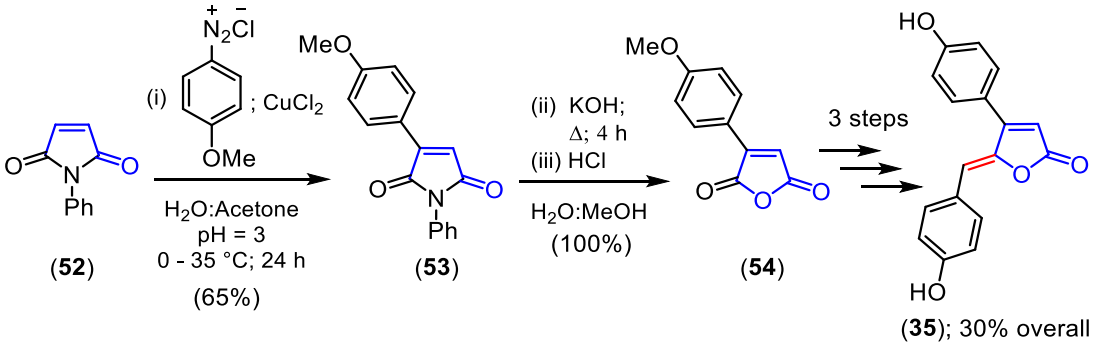
*Pseudodistoma antinboja* and *Synoicum globosum*, and exhibited antibiotic activity against gram-positive bacteria.[28,29] Lastly, Zhu and co-workers, isolated the rubrolides R (**48**) and S (**49**) form marine fungi *Aspergillus terreus*. This compound showed antioxidant and antiviral (H1N1) activities as well as cytotoxicity against K562 (leukemia) tumor cell lines.[33]

The first reported synthesis of rubrolides was developed by Negishi and co-workers. The method developed involved a tandem lactonization through Sonogashira cross-coupling with arylalkynes (**50**) and β-iodocinnamic acids (**51**) resulting in diacetylated derivatives of rubrolides A (**31a**), C (**33a**), D (**34a**), and E (**35a**), with moderate yields. Finally, basic hydrolysis of **33a**, yielded the naturally-occurring product, rubrolide C (**33**) (**Scheme 1.6**).[24]

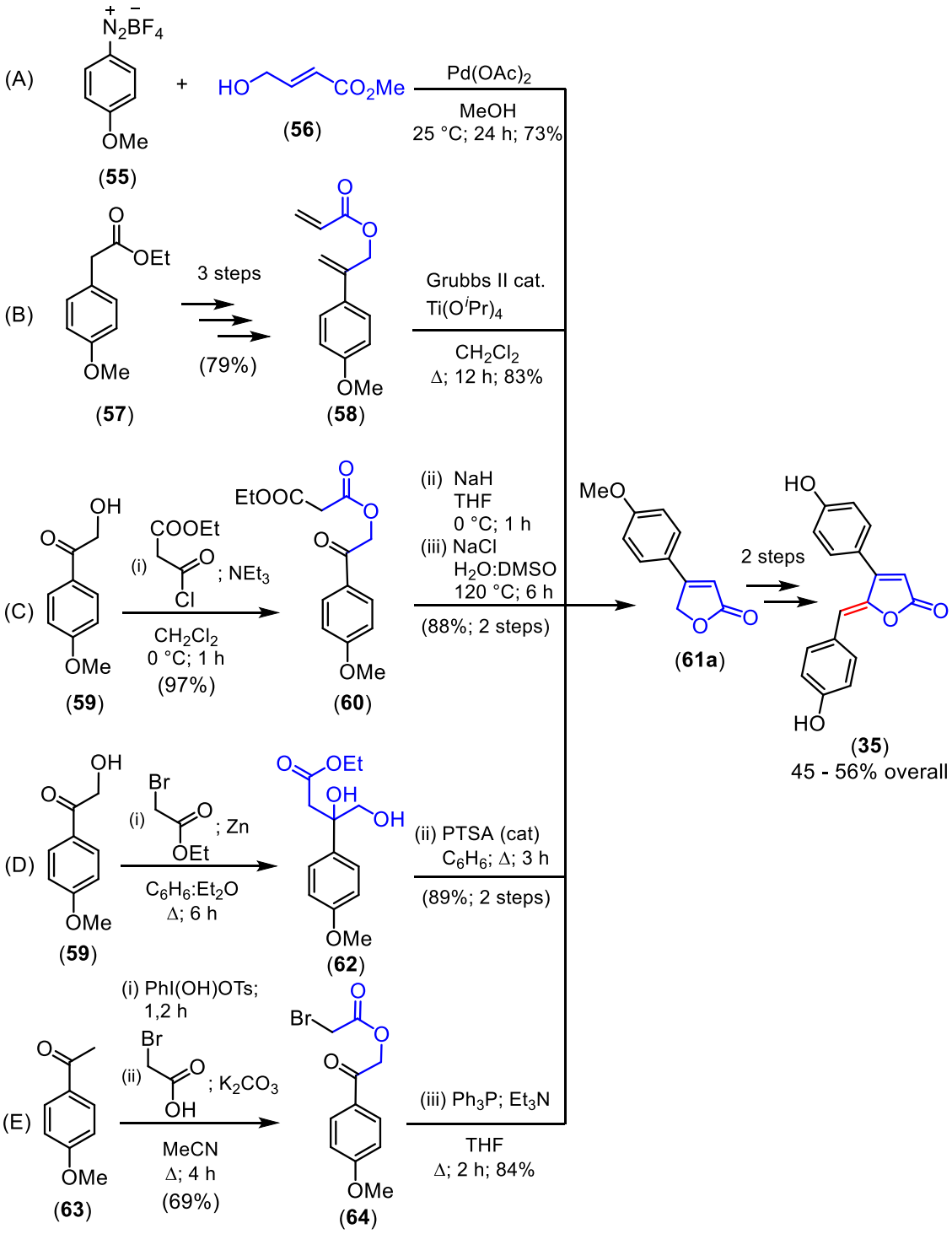



**Scheme 1.6:** First synthesis of rubrolides via iodo lactonization.[24]

Some time after the previous procedure, another syntheses were reported for rubrolide E (**35**) (**Scheme 1.7-8**). Within these methodologies, we may consider Meerwein cross-coupling (**Scheme 1.7**);[37] Heck cross-coupling (**Scheme 1.8**; **A**);[38] ring close metathesis with Grubbs catalyst; Knoevenagel condensation; Reformatsky reaction (**B**-**D**)[39] and intramolecular Wittig reactions (**E**).[40]




**Scheme 1.7:** Rubrolide E (**35**) synthesis using Meerwein coupling.[37]




**Scheme 1.8:** Reported syntheses of Rubrolide E (**35**).[38-40]

Unlike the aforementioned rubrolides, their α-halogenated congeners present a synthetic challenge. To overcome this drawback, the α,β-substituted butenolide building blocks emerge as suitable starting materials. Therefore, commercial and inexpensive products such as tetronic (**28**) and mucohalic acids (**29**) can be submitted to simple transformations (*e.g.* protection reactions (**A**) and

reductions (**B**)) to produce more versatile furan-2(5*H*)-ones (**65**-**66**) (**Fig. 1.6**) and expedite the synthesis of these natural butenolides and analogs.[30-41]

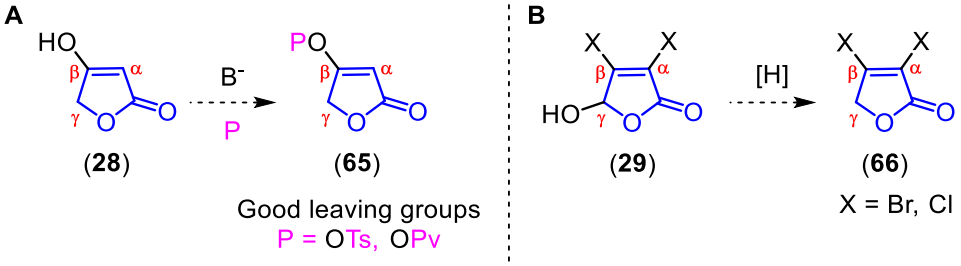



**Figure 1.6:** Transformations of commercial simple furanone starting materials in furan-2(5*H*)-ones, **A**) protection of β-hydroxy group and **B**) reduction of γ-hydroxy group.

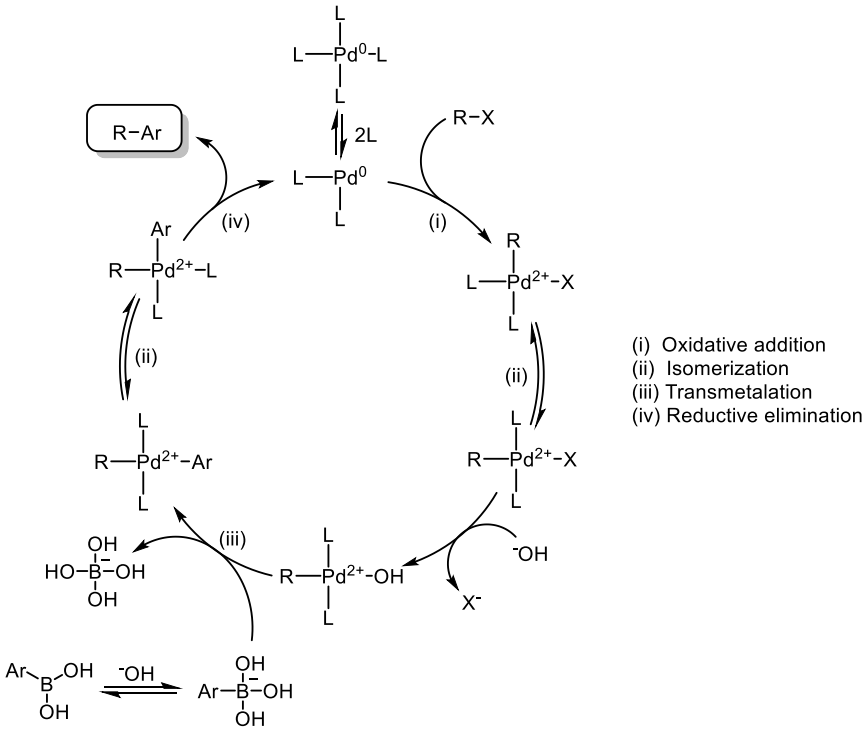
These furan-2(5*H*)-ones are highly functionalized, being ideal for construction of butenolide's skeleton. Thereby, the presence of good leaving groups in appropriate β-position permits C-C bond formation via organometallic cross-coupling reactions.[1] Likewise, γ-position acts as a remarkable synthetic aim thanks to its pro-nucleophilic center (as a result of higher acidity of H-5 protons), which allows the addition of different groups through aldolic condensation reactions.[30] These important factors represent the two key methodologies for the synthesis of bioactive butenolides and analogs, hence, they will be detailed in our synthetic design.

### 1.1.2. Rubrolide synthesis studies using Suzuki-Miyaura cross-coupling and vinylogous aldol condensation (VAC).

Only scarce examples of *de novo* synthesis of furan-2(5*H*)-one exist, even less so when considering rubrolide's motif. In fact, the most common methodology oriented at the construction of these natural products is employing polysubstituted γ-lactone starting materials, *e.g.* α,β-dihalofuran-2(5*H*)-ones (**66**; **Fig. 1.6**). In this manner, addition of the distinctive β and γ aromatic groups in rubrolides can be generally accomplished by cross-coupling reactions and vinylogous aldol condensation (VAC), respectively.

Firstly, transition metal catalyst cross-coupling reactions are an interesting and efficient method for the formation of C-C bonds in specific starting materials. In the case of rubrolides, Pd catalyzed Suzuki-Miyaura cross-coupling (**Scheme**

**1.9**) represents the best choice to form β-arylfuran-2(5*H*)-one intermediates from tetronic acid derivatives β-substituted with good leaving groups.[42]




**Scheme 1.9:** Suzuki-Miyaura cross coupling reaction mechanism.

Suzuki-Miyaura cross-coupling represents another important method for presenting some important characteristics: (i) tolerance to various functional groups, (ii) resistance of organoboronic acids to heating, water, and air, (iii) straightforward separation of boron-containing byproducts, and (iv) simple degradation into environmentally-safe products.[42-44]

Optimized condition of Suzuki-Miyaura cross-coupling reaction in synthesis of 4-aryl-3-halofuran-2(5*H*)-ones have already been reported; according to these studies, the choice of bases, catalyst, ligands, and solvents are dependent on the starting materials used in the reaction.[19,35,45,46]

In Suzuki-Miyaura's coupling, a base presents a very important role increasing the nucleophilicity of the organic group bonded to the boron atom.[47] In addition, it can activate the Pd complex obtained after oxidative addition reaction, due to the substitution of a halogen ligand (**Scheme 1.9**, R-Pd($L_2$)-X to R-Pd($L_2$)-OH).[48] The most commonly used bases are: $Na_2CO_3$, $Ba(OH)_2$, $K_3PO_4$, $Cs_2CO_3$, $K_2CO_3$, KF, and NaOH. Nevertheless, selection of the base is empirical and conditional to starting materials.[42] In case of furan-2(5*H*)-ones, the use of bases is mediated by their susceptibility to interference when in the presence of a

stronger alkaline medium that can induce ring opening, nucleophilic attacks, or decomposition reactions.[21]

The Pd compounds used as catalysts in these reactions are readily accessible, easy to prepare and manipulate. The Pd chemistry consists of two oxidation states: the zero-valence state ($Pd^0$) and the +2 oxidized form ($Pd^{2+}$). Each oxidation state reacts differently, thus, $Pd^0$ complexes are electron-rich nucleophilic species, and are prone to oxidation, ligand dissociation, insertion, and oxidative coupling reactions. Conversely, $Pd^{2+}$ complexes are electrophilic and undergo ligand association and reductive coupling reactions.[42,48]

Lastly, the nature of the ligand plays an important function in the coupling reactions. For example, phosphine and arsine compounds provide a high electron density to the complexed metal. In this case, Pd becomes a better nucleophile, favoring oxidative addition (reaction-determining step) with suitable substrates. In addition, the high electron density in the metal benefits the dissociation of the ligands to an unsaturated coordination complex.[42,48]

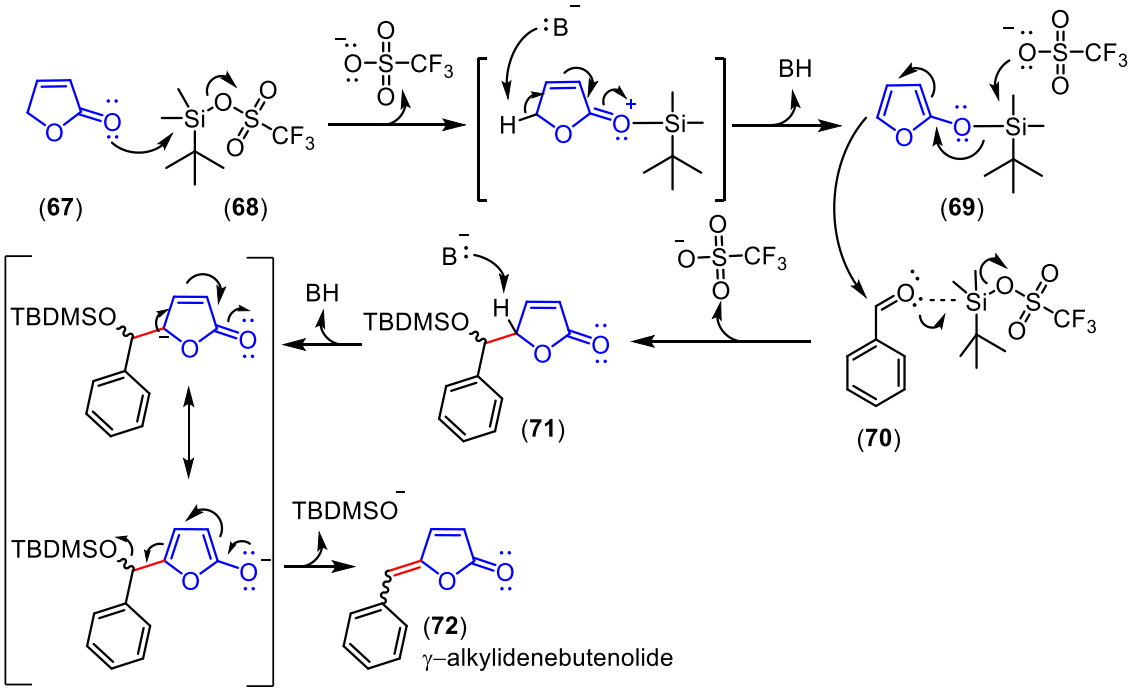
The aldol condensation reaction is considered one of the most important reactions to C-C bond formation. This occurs as a result of the presence of relatively acidic hydrogen atoms in $\alpha$-position to a group such as nitrile, sulfonyl, and especially carbonyl, or in a related conjugated systems (*e.g.* $\alpha,\beta$-unsaturated structures).[48] The acidity of the C-H bond in these compounds is due to a combination of the electron withdrawing and inductive effects, and the formed carbanion resonance stabilization. Once these nucleophiles are formed, they can undergo functional group addition, including condensation reactions with other carbonyl derivatives.[48]

Aldol condensation in $\gamma$-lactones represents a stereoselective access to $\gamma$-substituted butenolide derivatives, this occurrence has been theorized through the concept of vinylogy, which involves the carbon–carbon formation with an appropriate electrophile at the 5-position of furan-2(5*H*)-ones.[26,30,49] Following this line, vinylogous aldol condensation reaction (VAC), is favored in $\gamma$-lactone building blocks thanks to an H-5 acidic proton (pKa ~ 17 in DMSO)[50] once its conjugated base is stabilized by the adjacent $\alpha,\beta$-unsaturated carbonyl system.

Numerous classical VAC approaches for butenolides synthesis have been explored (*e.g.* piperidine/MeOH or piperidine/Py methods).[51] Unfortunately some

of them have been inefficient. In response to this, an adaptation using silylated furanolates (known as Mukaiyama's variant; VMAC) gained great importance, representing one of the most broadly-investigated selective and effective methods for mounting a γ−carbon substituent. [1,30,35,49,52-55]

One-pot VMAC mechanism (**Scheme 1.10**) consists in the *in situ* formation of a silyloxyfuran intermediate (**69**), through a nucleophilic attack of the furan-2(5*H*)-one starting material (**67**) to a silylation agent **68** (*tert*-butyldimethylsilyl triflate).




**Scheme 1.10:** VMAC reaction mechanism, using a furan-2(5*H*)-one, an aromatic aldehyde and TBDMSOTf.

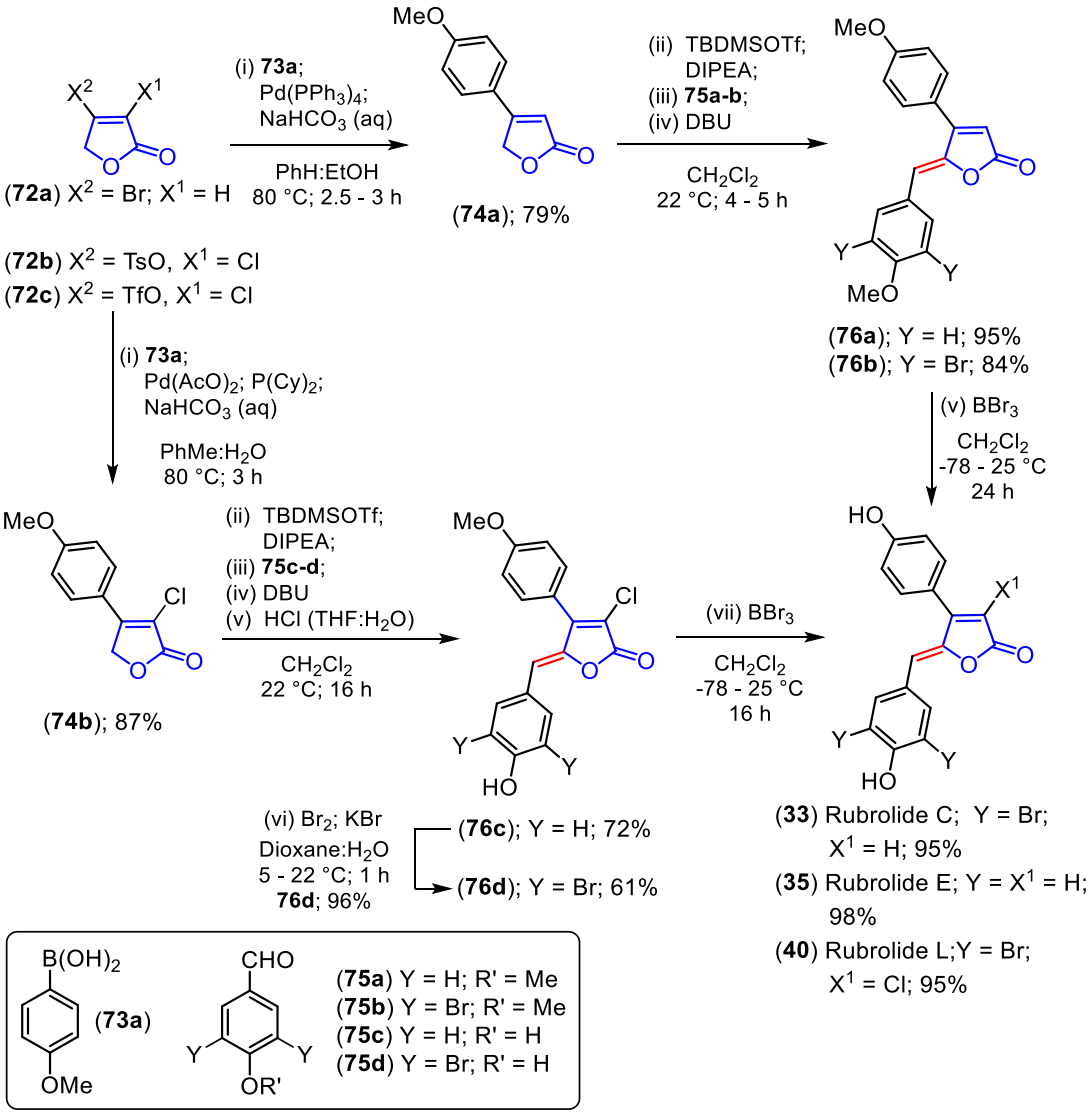
A base then captures one of the acidic hydrogens of the γ-carbon of the recently-formed intermediate resulting in **69**. In the next step, **69** attacks the carbonyl group in aldehyde **70**, whose nucleophilicity is increased by coordination with another molecule of the aforementioned silyl group, forming a mixture of diastereoisomers of a silylated alcohol (**71**).

In the final step, a stronger base captures the acidic hydrogen of **71**, resulting in a unimolecular conjugate base elimination reaction (E1cb) with formation of an exocyclic double bond, producing γ-alkylidenebutenolide **72**.[30,54,56]

Despite the fact that in rubrolide synthesis the main goal is the obtaining of γ-alkylidene motif, much effort has been dedicated in recent years in controlling

the relative and absolute stereochemistry of the formed stereogenic centers in γ-substituted lactone-related compounds (*e.g.* **71** in **Scheme 1.10**).[52,55,57-66] This important fact will be discussed in detail further.

Rubrolides syntheses employing these two methodologies were investigated by Boukouvalas and co-workers (**Scheme 1.11**).[54,56]

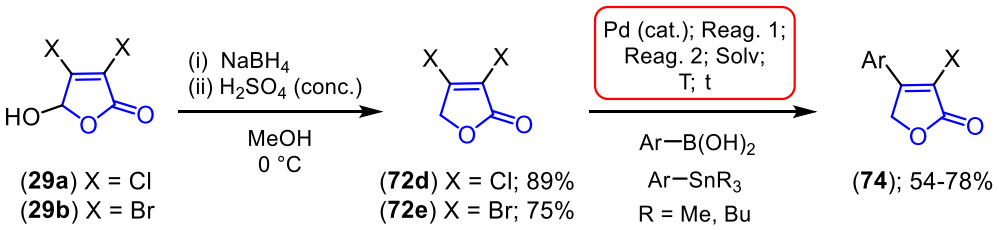



**Scheme 1.11:** Synthesis of the rubrolides C, E and L.[54,56]

These authors have developed a simple procedure based on the use of a Suzuki-Miyaura coupling using β-substituted derivatives of tetronic acid (**72**) together with *p*-methoxyphenylboronic acid (**73**), providing 4-(*p*-methoxyphenyl)furan-2(5*H*)-one (**74**). Subsequently, the formed products were reacted with the respective aldehydes (**75**) using the VMAC, obtaining intermediate **76**. Afterward, demethylation and/or bromination reactions provided rubrolides C (**33**), E (**35**), and L (**40**).

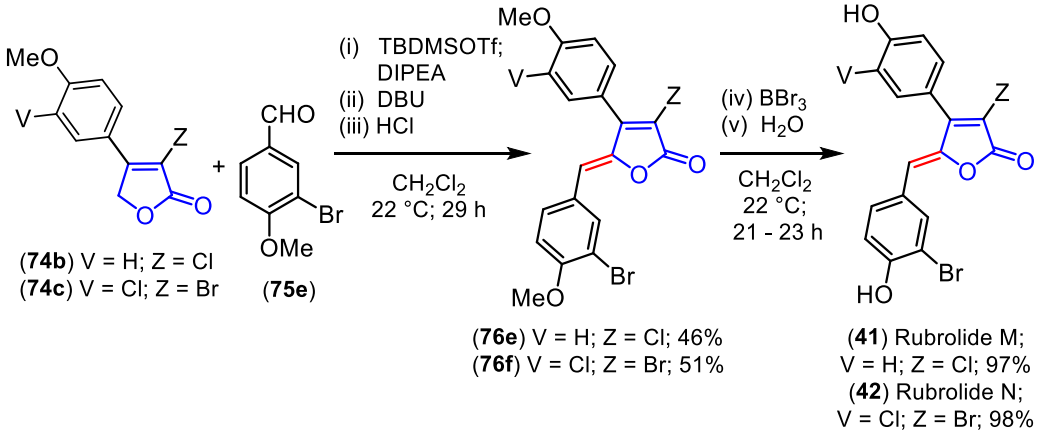
In 2000, Bellina and co-workers, produced two new natural butenolides and some analogs; the rubrolide M (**41**), the first α-chlorinated congener, and a structure believed to be the rubrolide N (**42**)[a].[19,35,66] In this approach, numerous reactions of Suzuki and Stille couplings (using organostannic compounds) were optimized using both mucohalic acids (**29**) as starting materials. Consequently, various α-aryl-β-halofuran-2(5*H*)-ones (**74**) were produced (**Table 1.1**).[19,35,67]

**Table 1.1:** Sn/Pd-catalyzed coupling reactions optimization conditions for the synthesis of rubrolides M and N [19,35,67]




| Conditions | Suzuki | Stille | Suzuki/Stille | Suzuki |
|---|---|---|---|---|
| Pd (cat.) | $PdCl_2(MeCN)_2$ | $PdCl_2(MeCN)_2/Pd_2(dba)_3$ | $Pd_2(dba)_3$ | $PdCl_2(PPh_3)_2$ |
| Reag. 1 | $Ag_2O$ | - | KF | CsF |
| Reag. 2 | $AsPh_3$ | $AsPh_3$ | P(*o*-PhMe)$_3$ | $BnEt_3N^+Cl^-$ |
| Solv. | THF | NMP | PhMe | PhMe:Agua |
| t (h) | 18-26 | 93-250 | 21-27 | 168-192 |
| T (°C) | 65 | 20 | 85 | 22 |

Next step includes VMAC between the intermediate coupling products **74c-d** and the 3-bromo-4-anisaldehyde (**75e**), providing (*Z*)-β-aril-γ-alkylidenbutenolides **76e-f**. To conclude, demethylation reactions of the latter intermediates produced the rubrolides M and N (**Scheme 1.12**; **41**-**42**).[19,35,67]

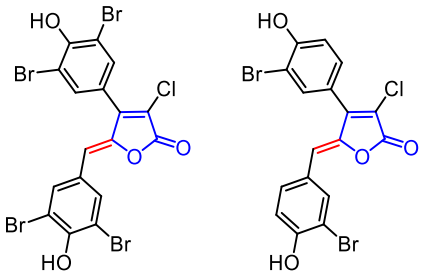



**Scheme 1.12:** Synthesis of the rubrolides M and N.[19,35,67]

[a]$^1$H NMR and $^{13}$C NMR data are not fully consistant with the isolated natural product.[34,66]

### 1.1.3. Rubrolides B and K.

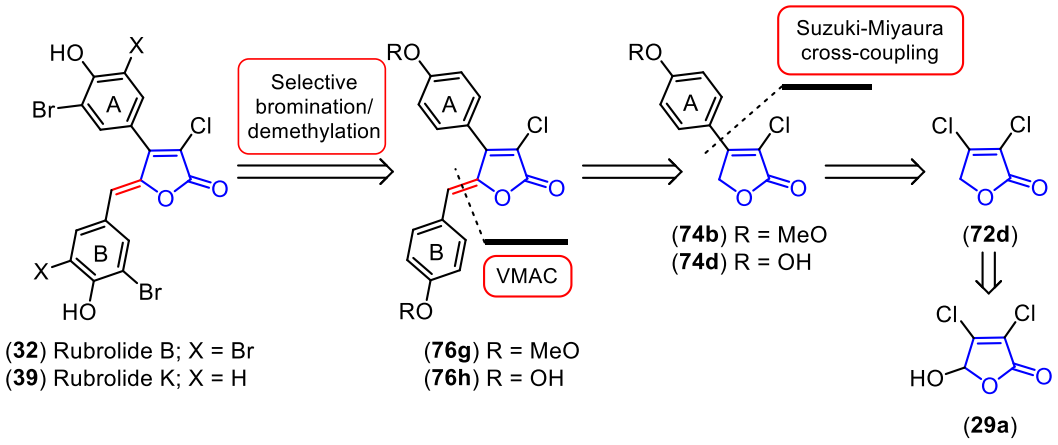
The interest in the synthesis of rubrolides B (**32**) and K (**39**) (**Fig. 1.7**) exists as a result of their interesting bioactivities. Notably, rubrolide K shows cytotoxicity against human colon and lung carcinoma, melanoma and leukemia in levels of $ED_{50}$ = 1.2-5 μg $mL^{-1}$.[34] Likewise, rubrolide B presented antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* (MIC = 2 μg/disc).[25]




(**32**) (**39**)

**Figure 1.7:** Rubrolides B (**32**) and K (**39**) structures.

Synthesis of these promising natural products is also relevant since only a small number of α-halogenated natural rubrolides and their analogs have been successfully produced. This limited success rate is mostly attributed to scarcely-available and complex synthetic strategies. Likewise, α-halogenated intermediates present some additional difficulties associated with moderate yields in VAC steps, mainly when used with polysubstituted aromatic aldehydes (*e.g.* **76e-f**, 46-51%; **Scheme 1.12**).[19,35,67] The present work will propose an efficient synthesis of rubrolides B and K, based on the retrosynthetic analysis in **Scheme. 1.13**.




(**32**) Rubrolide B; X = Br
(**39**) Rubrolide K; X = H

(**76g**) R = MeO
(**76h**) R = OH

(**74b**) R = MeO
(**74d**) R = OH

(**72d**)

(**29a**)

**Scheme 1.13**: Rubrolides B (**32**) and K (**39**) retrosynthetic analysis.

As a relevant point in this design, the regio-selective late-stage electrophilic aromatic bromination will be the determining step to obtain the final two products.[53,54] As can be expected, when bromination reaction occurs in intermediate **76** the *p*-hydroxy funtional group should achieve higher activating effect than the *p*-methoxy. Consequently, bromination procedure with more reactive intermediate **76h** will lead to polybrominated products (in this case rubrolide B; **32**). On the other hand, -OMe group in intermediate **76g** will result a less reactive species, producing the less bromitated compounds (rubrolide K; **39**). Therefore, at this point, an adequate demethylation/bromination reaction sequence will be essential.

The formation of key intermediate **76** will be completed with a pivotal sequence of Suzuki-Miyaura cross-coupling followed by VMAC. Initially, α,β-dichlorofuran-2(5*H*)-one (**72d**) will be specifically coupled in β-position with respective boronic acid, leading to the formation of 4-aryl-3-chlorofuran-2(5*H*)-one (**74**). Upon obtaining compound **74**, it will be submitted to a *Z*-selective VMAC, favored by bulky 4-aryl stereocontrol group. Finally, intermediate **72d** will be prepared by reduction of the commercially-available starting material, mucochloric acid (**29a**).[23,26,40,52,54,56,68]

## 1.2. OBJETIVES AND STRATEGIES

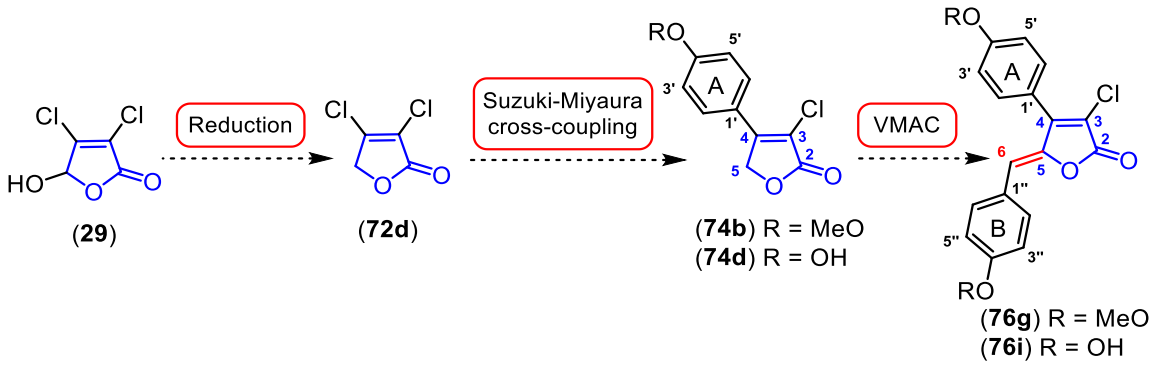
In this chapter we aim to develop a simple, short, and efficient methodology to accomplish the first total synthesis of two biologically-active natural products, rubrolides B and K. The main steps to be evaluated include cross-coupling, vinylogous Mukaiyama's aldol condensation, bromination, and demethylation reactions. The last two procedures in the sequence will be determinant to the final natural product to be obtained.

Additionally, we aim to study the late-stage bromination step of the common intermediates, determining the importance and the effects of the different groups in the β-aromatic ring attached to furan-2(5*H*)-one motif, leading to a promising reactivity-structure analysis.

## 1.3. RESULTS AND DISCUSSIONS

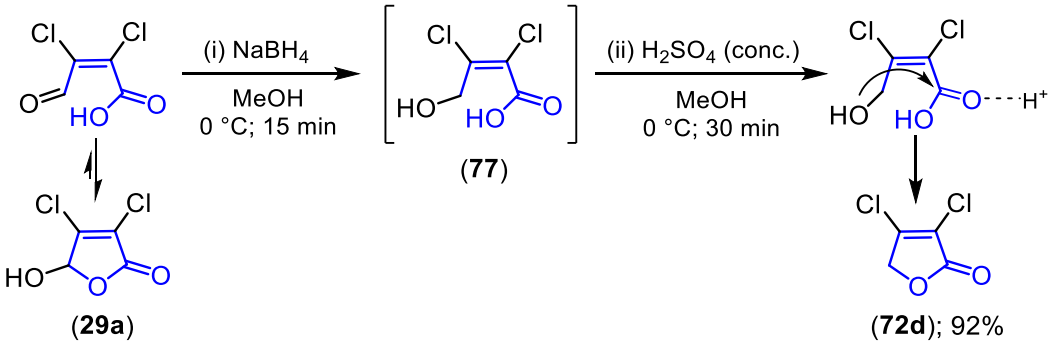
### 1.3.1. Synthesis of key intermediate the γ-alkylidenebutenolide 76g.

The synthesis of the rubrolides starts with the production of the key precursor **76g**, that could be accomplished following the procedure in **Scheme 1.14**.




**Scheme 1.14:** Synthetic procedure for the key precursor **76g**.

Firstly, the starting material, mucochloric acid (**29a**), was submitted to an acidic reduction method with $H_2SO_4$/$NaBH_4$. This reaction reduces selectively the γ–aldehyde group present in open chain form of tautomeric lactonic equilibrium in **29a** (**Scheme 1.15**).[69] Afterwards, acid addition will favor an intramolecular cyclization of the γ-hydroxy carboxylic acid formed *in situ* (**77**), resulting in lactone **72d**.

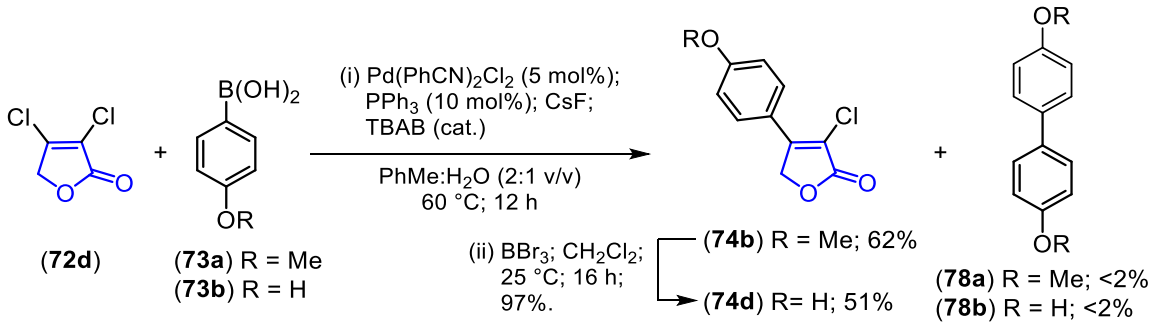



**Scheme 1.15:** Reaction conditions for the reduction of mucochloric acid (**29a**).

Compound **72d** was identified by NMR analysis. In the $^{1}H$ NMR spectrum, may be observed the presence of a singlet at $\delta$ = 4.87 related to the H-5 methylene hydrogens (see annexes, **Fig. A 1.1**). Next, in the $^{13}C$ NMR spectrum, only four signals are observed. The carbonyl signal is observed at $\delta$ = 166.8. The

signals of C-4 and C-3 are observed at $\delta$ = 143.7 and 114.8, respectively. Finally, the C-5 carbon is at $\delta$ = 74.4 (**Fig. A 1.2**). These data coincide with those found in the literature.[19,41,46]

Compound **72d** was submitted to Suzuki-Miyaura cross-coupling reaction, to afford the β-aryl substituted intermediates **74b**, and its hydroxylated derivative **74d**. Hereupon, boronic acids **73a-b** were used in the reaction resulting in the selective synthesis of **74b** and **74d** in 62% and 51% yield, respectively (**Scheme 1.16**). Furthermore, the use of an excess of three equivalents of boronic acid **73a**, particularly, led to an increase in the yield of the respective reaction to 76%. Finally, it is worth mentioning that hydroxylated compound **74d** was obtained also as a result of demethylation reaction of **74b** in 97% yield.




**Scheme 1.16:** Reaction conditions for the synthesis of the intermediates **74b** and **74d**.

A number of synthetic methods for β-arylbutenolide intermediates production, have already been described using the Suzuki-Miyaura coupling, including our own.[19,23,35,41,49,67,70] These methodologies served as the basis for the selection of conditions used in this study. Accordingly, the best results were obtained by using catalytic precursors $PdCl_2(PhCN)_2$ and $PPh_3$.[40,56] Likewise, CsF was used as the base, which offers slightly alkaline reaction conditions, avoiding undesired reactions such as γ-lactone hydrolysis and ring opening.[23,69] Furthermore, the solvent system and additional employment of the tetrabutylammonium bromide (TBAB) phase transfer catalyst, provided a biphasic reaction system, which seeks to favor the insertion of palladium (in the organic layer) and minimize undesired side reactions (in the aqueous phase). Nevertheless, the formation of homocoupling byproducts (**78**) were minimized though they were obtained as minor components in the reactions (**Scheme 1.16**). Additionally, there is the possibility of coupling in the α-carbon in lactonic ring,

resulting in α,β-biarylfuran-2(5*H*)-ones, nonetheless, these were not obtained. The absence of these compounds is attributed to the selectivity given by 4,5-dichlorofuran-2(5*H*)-one molecule, whose 4-position is highly-reactive thanks to its high electron deficiency, established by the electron attraction on the conjugated α,β-unsaturated carbonyl system.[40,56,59]

Products **74b** and **74d** were identified using infrared (IR) spectrometry, $^{1}$H NMR, $^{13}$C NMR, and high-resolution mass spectrometry (HRMS) techniques. 4-aryl-3-chlorofuran-2(5*H*)-ones (**74**) showed great similarity in spectral data, exhibiting variations only in the characteristic signals of the aromatic ring substituents.

IR spectrum of compound **74b** shows the stretching bands of α,β-unsaturated carbonyl bond at 1749 cm$^{-1}$, C=C bond at 1603 cm$^{-1}$ and 1444 cm$^{-1}$, and finally, the C-O bond, at 1254 cm$^{-1}$. The spectrum of the demethylated product **74d** shows similar signals, differences are noticeable in the characteristic broad absorption of the O-H bond at 3271 cm$^{-1}$, as well as a shift in the carbonyl band, which appears at 1726 cm$^{-1}$, decreasing by 23 units because of the resonant electron donor effect of the hydroxyl group that slightly weakens the C=O bond.

Initially, in the $^{1}$H NMR spectrum of **74b** (**Fig. 1.8**) two singlet signals may be observed, one in 3.88 ppm for a methoxy group, and other at 5.19 ppm for the two methylene hydrogens of the H-5 lactonic ring. Clearly, the difference with compound **74d** is the absence of the OMe group signal, and the presence of the phenolic hydroxyl signal at $\delta$ = 10.38 (**Fig. 1.9**). Continuing, two doublets, each one integrating for two hydrogens, belong to the disubstituted aromatic ring, whose symmetry allows similar chemical environments for the H-2'-6' and H-3'-5' hydrogens. These signals are in $\delta$ = 7.79 and 7.01 (*J* = 8.4 Hz) for compound **74b** and in $\delta$ = 7.76 and 6.93 (*J* = 8.0 Hz) for compound **74d**.

Lastly, in $^{13}$C NMR spectrum of **74b**, the methoxy and C-5 group signals, were initially assigned at $\delta$ = 55.5 and 70.0, respectively (**Fig. 1.10**). Likewise, in the $^{1}$H NMR spectrum, the absences of OMe signals are observed in the spectrum of compound **74d** (**Fig. 1.11**). Two signals of greater intensity can be verified, corresponding to two carbons with the same chemical environment. These signals represent the unsubstituted carbons of the symmetrical aromatic

ring, with chemical shift of 129.0 ppm to C-2'-6' and 114.6 ppm to C-3'-5', and then signals of C-1' (114.8 ppm) and C-3 (121.2 ppm) are assigned. The C-4 carbon signals (151.3 ppm) located at the β-position in α,β-unsaturated carbonyl system and the C-4' carbon signal (162.2 ppm) of the aromatic ring connected to oxygen may also be seen, as well as the carbonyl signal, C-2 (169.5 ppm). Similar assignments were made for hydroxy compound **74d**. All these data were compared with those described in the literature.[19]

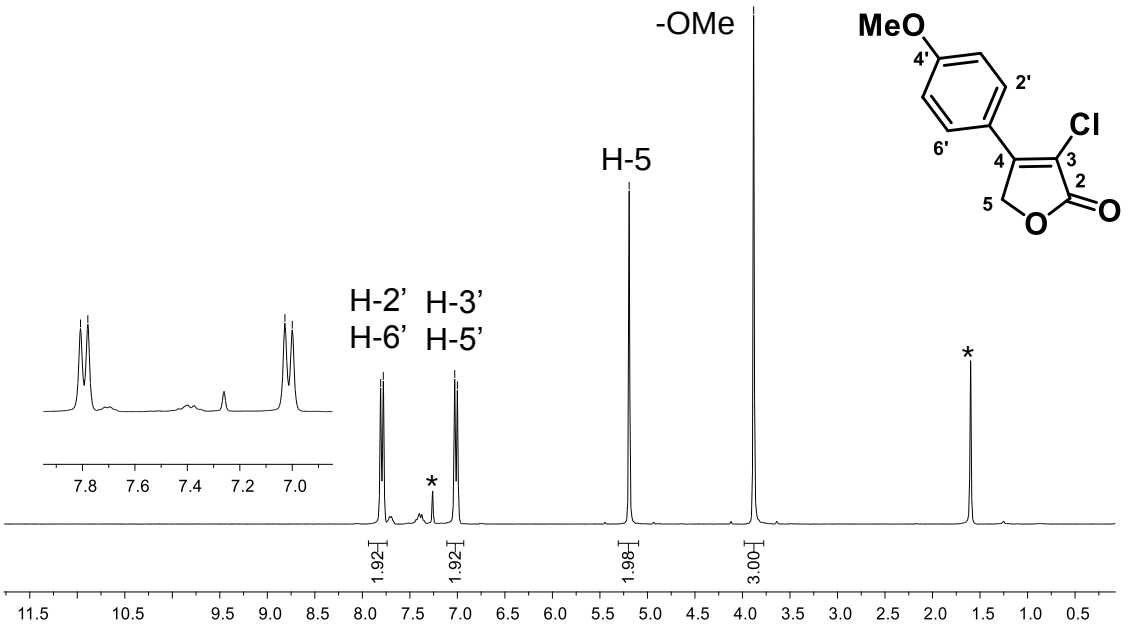



**Figure 1.8:** $^{1}H$ NMR ($CDCl_3$, 400 MHz) of compound **74b**. *Solvent residues and water signals.

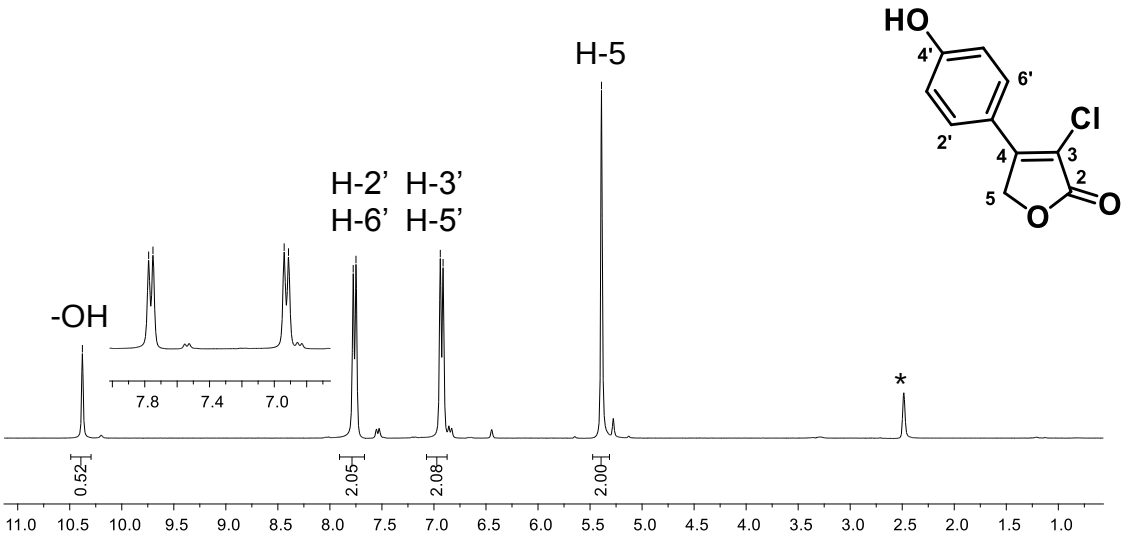



**Figure 1.9:** $^{1}H$ NMR ($(CD_3)_2SO$, 400 MHz) of compound **74d**. *Solvent residues signals.

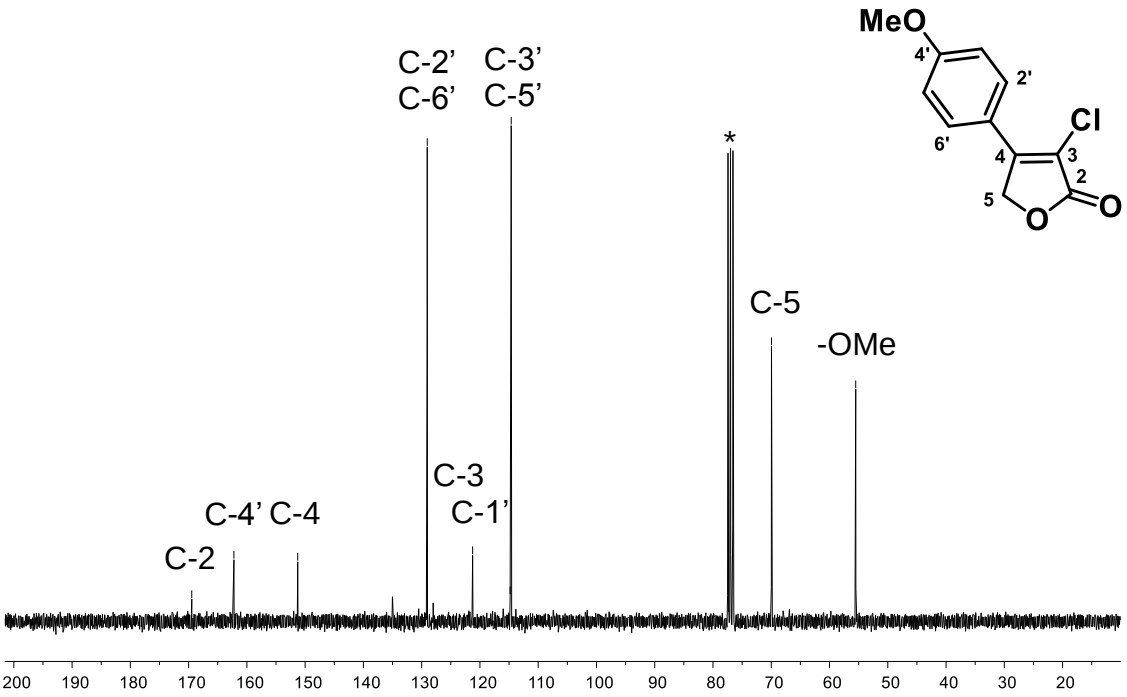



**Figure 1.10:** $^{13}C$ NMR ($CDCl_3$, 100 MHz) of compound **74b**. *Solvent residues signals.

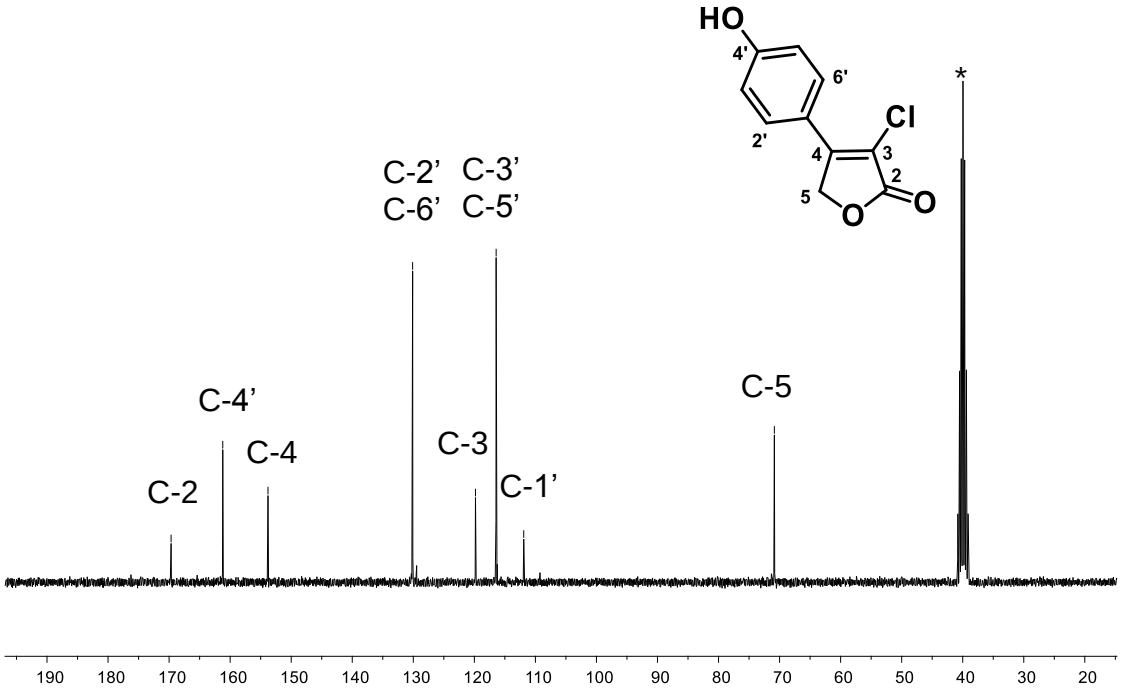
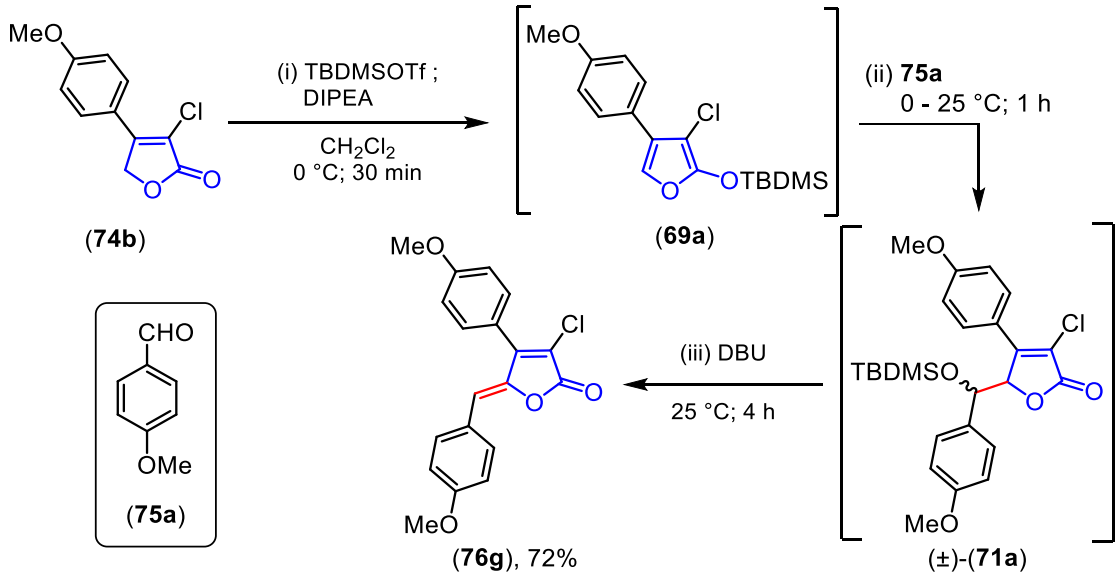



**Figure 1.11:** $^{13}C$ NMR ($(CD_3)_2SO$, 100 MHz) of compound **74d**. *Solvent residues signals.

Lastly, the key intermediate **76g** was synthesized using the one-pot VMAC procedure on product **74b** with *p*-anisaldehyde (**75a**). For this reaction the silylating agent was *tert*-butyldimethylsilyl triflate (TBDMSOTf) and *N*,*N*-diisopropylethylamine (DIPEA) was used as the base (**Scheme 1.17**).




**Scheme 1.17:** Reaction conditions for one-pot synthesis of intermediate **76g**.

Based in the VMAC mechanism, we can expect the *in situ* formation of the silyloxyfuran intermediate **69a**. Then, the aldol addition of the aromatic aldehyde would form, presumably, racemic *syn* TBDMS protected alcohol **71a** as major product (this intermediate was not separated). Later, adding the DBU base, will facilitates elimination at the 6-position, yielding selective *Z*-isomer of the product **76g** in one pot with 72% yield (**Scheme 1.17**).

Concerning the stereoselectivity observed in the Mukaiyama's aldol condensation reaction, an important highlight can be made: the major isomer produced in situ is *syn* protected alcohol **71a**.[16,21,47-50,51,59,60,61] The elimination reaction then leads preferably to the formation of the *Z* product, due to bulky stereocontrol substituent at the β-position in butenolide precursor, all this following an E1cb mechanism.[21,49-51] The reason for this *syn*-isomer selectivity is probably due to the manner with which the silyloxyfurane compound approaches the electrophile, producing in each case a different transition state favored by stereo-electronic factors (**Fig. 1.12**).[52]

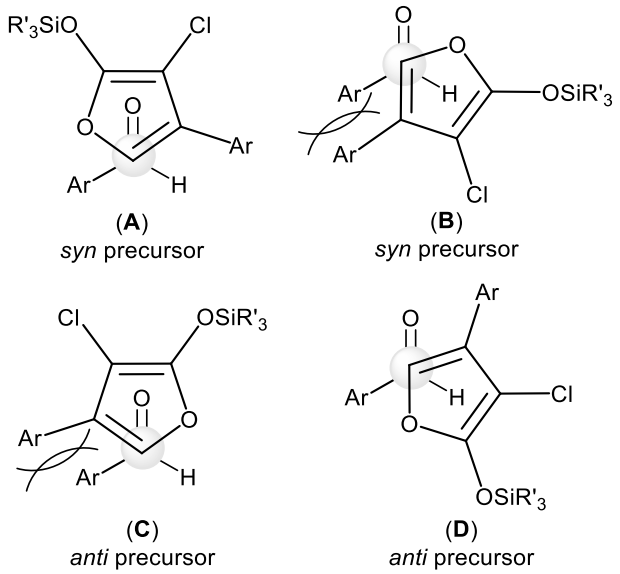
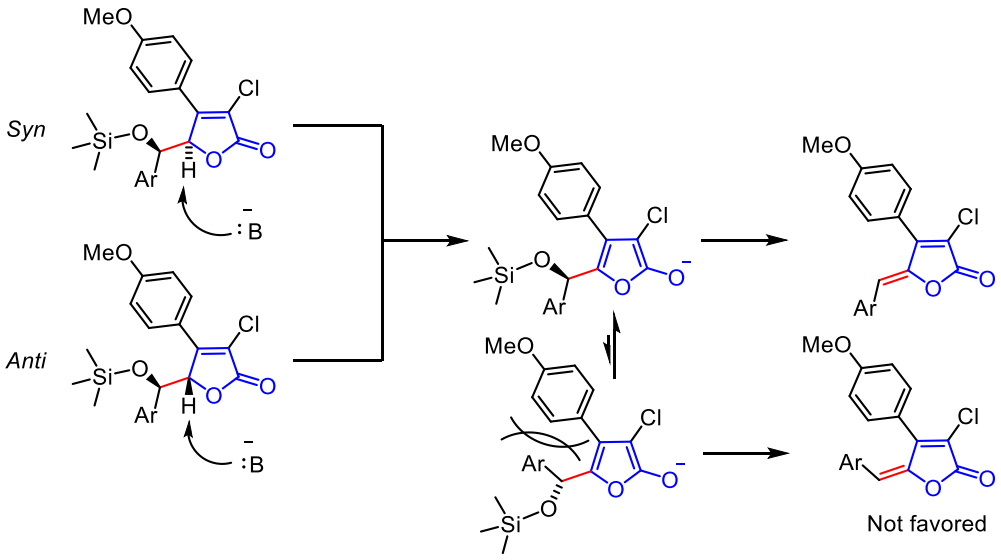



**Figure 1.12:** Spatial approach of the silyloxyfuran ring to the aldehyde in the Mukaiyama's aldol condensation.[58,71]

The transition state shows how the approach of both molecules on the same surface (Si, Si), favors the production of the *syn* isomer. The main reason is the superposition of $\pi$ orbitals between the silyloxyfuran ring and the aldehyde's carbonyl double bond, similar to a Diels-Alder reaction (**Fig. 1.12**; **A**).[47,60] In the case of the antiperplanar approach **B**, which could also produce the *syn* isomer, the spatial hindrance would disfavor the reaction. Furthermore, the other transition states (**C**-**D**), that will lead to the formation of the *anti*-isomer, do not have an adequate stereo-electronic effect that favors their spatial approach in the reaction. Nevertheless, although one of them contains a Diels-Alder type arrangement (**C**), its hindrance clearly affects the selectivity.[52,53,72]

After the formation of intermediate **71a**, the E1cb elimination occurs. In this reaction, a particularly stable conjugated base is formed by the abstraction of the $\alpha$-proton adjacent to the group to be eliminated. The resulting negative charge is stabilized either by electron resonance promoted by conjugation or the presence of electron-withdrawing groups. In the case of the furanolate derivatives, this charge is distributed in the electron cloud formed by the $\alpha,\beta$-unsaturated carbonyl system. The intermediates formed in this mechanism after the proton abstraction are characterized by a single bond in the $\gamma,\delta$–position, which can rotate freely, allowing the two isomers (*syn* and *anti*) to lead selectively to an elimination *Z*-product, thanks to the bulky $\beta$ group in the butenolide reactant (**Scheme 1.18**).




**Scheme 1.18**: Determination of the selectivity according to the elimination mechanism E1cb.[55]

Compound **76g** was identified by spectroscopic analysis, IR, $^{1}$H NMR, $^{13}$C NMR and HRMS. The IR spectrum shows C=O stretching at 1769 cm$^{-1}$, characteristic for α,β-unsaturated carbonyl. Next, C=C stretching at 1603 cm$^{-1}$ and C-O single bonds at 1257 cm$^{-1}$.

$^{1}$H NMR spectrum showed two singlets of the two methoxy groups at $\delta$ = 3.92 and 3.86. A singlet may also be observed at $\delta$ = 6.13, referring to the hydrogen H-6 of the recently-formed double bond. Meanwhile, in the aromatic region, signals of the new aromatic group added to the molecule may be clearly observed. Signals corresponding to the hydrogens H-2''-H-6'' and H-3''-H-5'', with similar chemical environments of the benzylidene ring, may be recognized in 7.09 and 7.75 ppm ($J$ = 8.8 Hz), respectively. Likewise, the hydrogens of the β-aryl ring are present in 6.92 and 7.51 ppm ($J$ = 8.7 Hz) (**Fig. 1.13**).

In the $^{13}$C NMR spectrum, the carbon signals of the methoxy groups are likely to be at $\delta$= 55.5 and 55.6. Four signals of higher intensity may also be seen, which clearly corresponds to the unsubstituted carbons of the aromatic ring, showing two very close signals with chemical shift of 114.5 ppm, and another two in 130.8 and 132.5. Signals of carbons C-6 ($\delta$= 114.7) and C-5 ($\delta$ = 145.0), corresponding to the instauration formed by the elimination reaction are also noticeable, as are the signals of C-1' (120.5 ppm), C-3 116.7 ppm) and C-1'' (125.6 ppm). The β-carbonyl C-4 signal at 149.8 ppm, C-4'' ($\delta$= 160.8) and C-4' ($\delta$ = 161.4) which are the aromatic carbons bonded to the methoxy groups are

also easily discernible. Finally, the carbonyl C-2 is apparent at $\delta$ = 165.0 (**Fig. 1.14**). All these spectroscopic data were also compared with those described in the literature.[19]

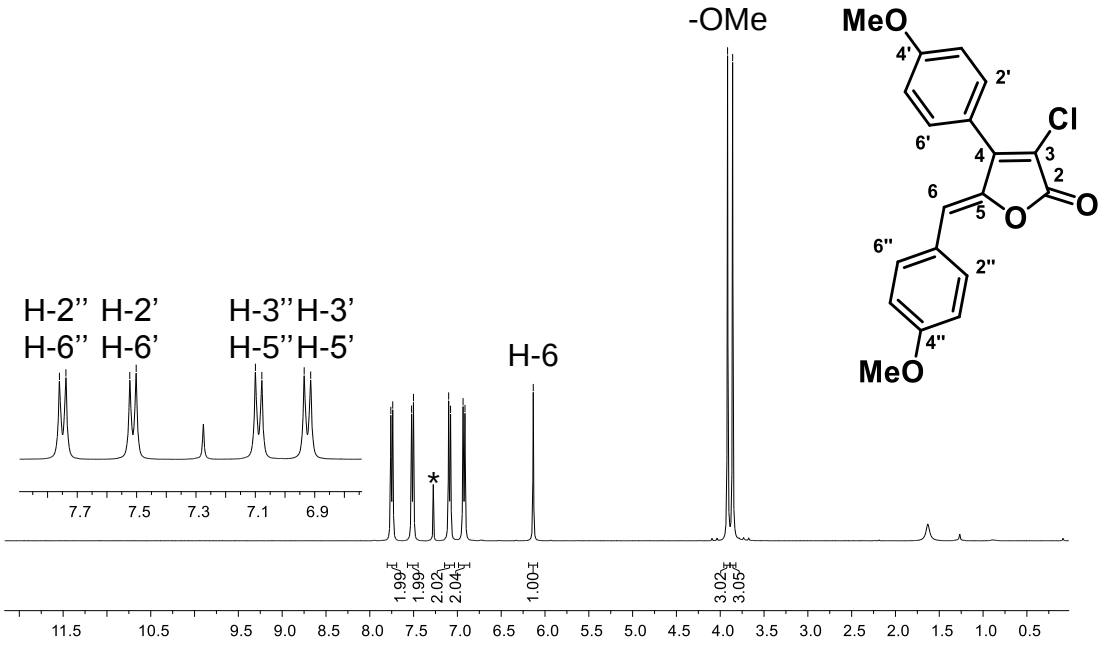



**Figure 1.13**: $^{1}$H NMR ($CDCl_3$, 400 MHz) of compound **76g**. *Solvent residues signals.

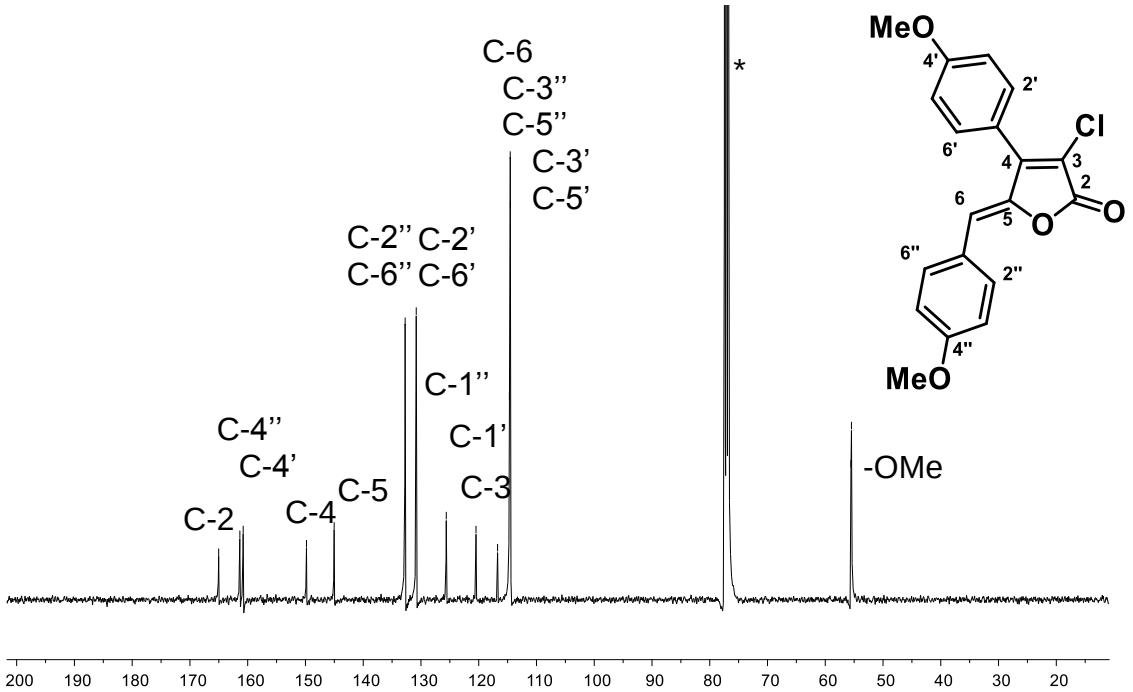



**Figure 1.14**: $^{13}$C NMR ($CDCl_3$, 100 MHz) of compound **76g**. *Solvent residues signals.

### 1.3.2. Scope of the electrophilic aromatic bromination.

The main objective of this study was to explore the reactivity and the regioselectivity of electrophilic aromatic bromination reaction in the benzene rings of the 5-benzylidene-4-phenyl-3-chlorofuran-2(5*H*)-one skeleton, substituted with *p*-hydroxy or *p*-methoxy groups in order to apply this protocol to the synthesis of rubrolides. In this manner, two different butenolide motifs (**Fig. 1.15**; **74** and **79**) containing the benzene rings susceptible to the bromine attack (ring A and ring B) were selected. As follows, the optimization parameters for the aromatic electrophilic substitution (ArES) reaction such as the solvent, the bromine source (type and concentration), catalysts, and reaction times were varied.

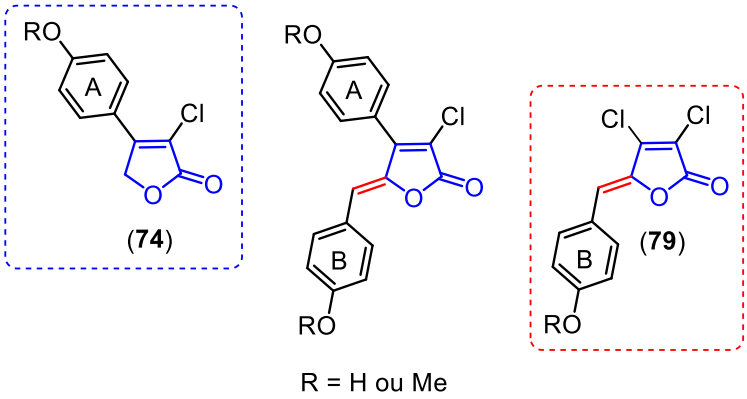



**Figure 1.15:** Butenolide motifs **74** and **79** for studing the scope of bromination reaction.

In first instance, for this experiment, the Suzuki coupling reaction products **74b** and **74d** were used as models for reactions in ring A (**Fig. 1.15**). Both intermediates represent *para* di-substituted aromatic compounds containing a donor group (OMe and OH respectively) and an electron-withdrawing group (furan-2(5*H*)-one).

The reactivity of aromatic rings to ArES reactions is owed to its electronic richness, which, consequently, in the presence of substituents whose electron donating characteristic is greater, will manifest in more reactive compounds.[73-75] The methods of evaluating this capacity are through the vector of dipole moment ($\mu$) and Hammett's constant ($\sigma$)[76,77] (**Fig. 1.16**). Both values determine the influence of electronic effects for different substituents on the ring, in which case the hydroxyl group is the most powerful activator. Activating groups tend to

selectively produce substitutions in their *ortho* and *para* positions. In our case, the use of the products **74b** and **74d** would keep the latter blocked, offering only *ortho* substituted compounds. Accordingly, it is expected that the hydroxyl group will lead to the *ortho* di-bromination of the benzene ring, related to the activating group through its greater electron-donating capacity (greater $\mu$ and smaller $\sigma$). Similarly, the methoxy group, being a less efficient donor than the OH group (lower $\mu$ and greater $\sigma$), would essentially lead to monobrominated derivatives.[73-75]

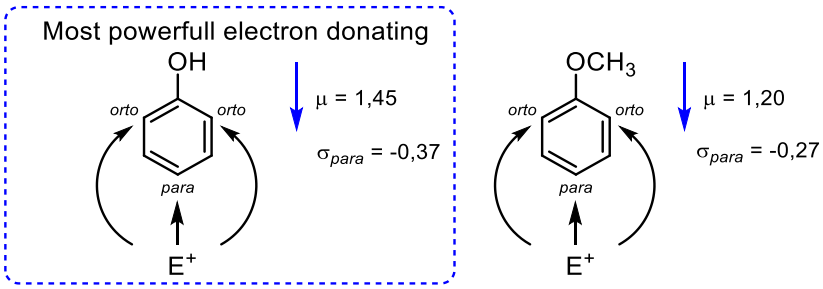
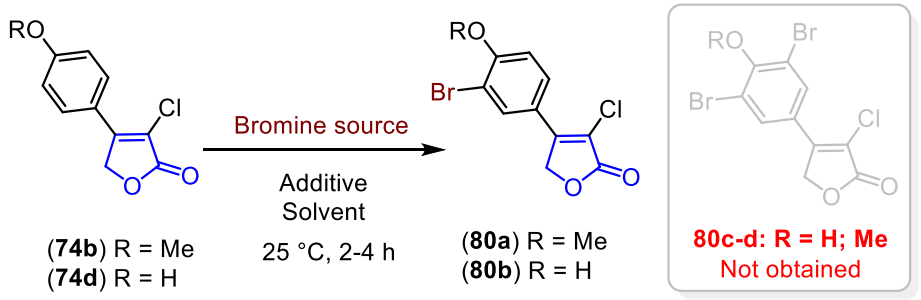



**Figure 1.16:** Hammett constant ($\sigma$) and dipole moments ($\mu$) for the hydroxy and methoxy groups in the benzene.

The scope of bromination starts with the reaction of the methoxylated intermediate **74b** with 2.0 eq of $Br_2$, using dichloromethane (DCM) as solvent at 25 °C for 2 h, obtaining exclusively the monobrominatied product **80a** in 56% yield (**Table 1.2**; **entry 1**). Based on analysis of the crude product by gas chromatography coupled to mass spectrometry (GC-MS) (data not shown), no dibromo compound (**80c**) was detected. As mentioned previously, the OMe group is a less efficient activator, and this result was therefore expected. Other reaction variables were then explored.

Addition of KBr as a catalyst significantly improved the yield of **80a** in a yield of 68% (**Table 1.2**; **entry 2**). Further improvement was observed, yielding 72%, with the use of an excess of $Br_2$ and a longer reaction time (**entry 3**). However, even using a large excess of $Br_2$ (5.0 eq), the dibromo compound **80c** still was not formed. Subsequently, acetic acid was used as an additive, additionally with an increase in reaction temperature to 60 °C, resulting in only 59% yield of **80a** (**entry 4**). The source of bromine was then changed to NBS (**entries 5-6**), resulting in a lower yield and still no dibromo product.

**Table 1.2:** Optimization conditions for the bromination reaction in intermediates **74b** and **74d**.




(**74b**) R = Me
(**74d**) R = H

(**80a**) R = Me
(**80b**) R = H

**80c-d: R = H; Me**
Not obtained

| Entry | Starting material | Bromine source (eq) | Additive (eq) | Solvent | Product (%) |
|---|---|---|---|---|---|
| 1 | **74b** | $Br_2$ (2.0) | - | DCM | **80a** (56) |
| 2 | **74b** | $Br_2$ (2.0) | KBr (0.1) | DCM | **80a** (68) |
| 3[a] | **74b** | $Br_2$ (5.0) | KBr (0.1) | DCM | **80a** (72) |
| 4[b] | **74b** | $Br_2$ (5.0) | AcOH[c] | DCM | **80a** (59) |
| 5 | **74b** | NBS (2.0) | - | DCM | **80a** (30) |
| 6 | **74b** | NBS (2.0) | KBr (0.1) | DCM | **80a** (41) |
| 7 | **74d** | $Br_2$ (2.0) | KBr (0.1) | DCM | **80b** (72) |
| 8 | **74d** | NBS (2.0) | KBr (0.1) | DCM | **80b** (46) |
| 9 | **74b** | $Br_2$ (2.0) | KBr (0.1) | $Et_2O$ | NR[d] |
| 10 | **74b** | $Br_2$ (2.0) | KBr (0.1) | THF | NR[d] |
| 11 | **74b** | $Br_2$ (2.0) | KBr (0.1) | $H_2O$ | **80a** (21) |
| 12 | **74b** | $Br_2$ (2.0) | KBr (0.1) | MeCN | **80a** (62) |
| 13 | **74b** | $Br_2$ (2.0) | KBr (0.1) | DMF | **80a** (67) |

[a] t = 12 h; [b] T = 60 ˚C; [c] DCM:AcOH 1:1 (v/v); [d] NR= no reaction t = 12 h, no monobrominated product detected

Unsuccessful results in obtaining the dibromo product using intermediate **74b**, led to the investigation of a reaction using the *p*-hydroxy derivative, due to its stronger activating potential. Unfortunately, after several attempts using $Br_2$ and NBS with KBr as catalyst, only the monobrominated product **80b** was observed (**entries 7-8**).

Having established efficient conditions for the bromination of **74b** and **74d** (**entry 2**), the effects of different solvents (**entries 9-13**) were investigated. The use of tetrahydrofuran (THF) or diethyl ether ($Et_2O$) resulted in no reaction (**entries 9-10**). Next, the use of water, yielded only 21% of **80a** (entry 11). In

addition, better yields were obtained using acetonitrile (MeCN) (**entry 12**; 62%) and dimethylformamide (DMF) (**entry 13**; 67%). In the latter case, although DMF was as efficient as DCM, it was decided to use DCM as solvent for new experiments because of its practicality.

As was observed, still having an excellent activator group such as the hydroxyl group, the dibromo product was not obtained. One plausible explanation is the fact that furan-2(5*H*)-one is a good deactivating group. Therefore, the electron withdrawing effect by resonance decreases the electron density in the benzene ring (**Fig. 1.17**). This effect deceases the reactivity of the aromatic ring, enough to disfavor, in all cases, the formation of the polybrominated intermediate.[78]

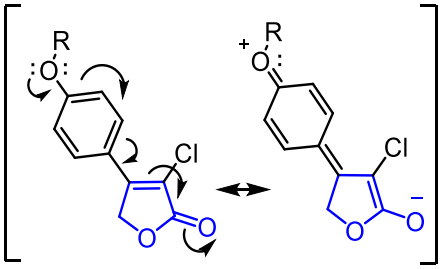
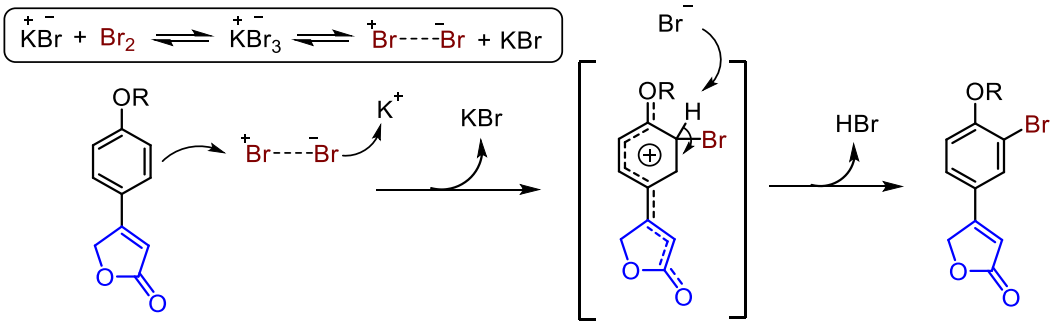



**Figure 1.17:** Deactivating resonance effect on 4-arylfuran-2(5*H*)-one.

From the results described above, it can also be concluded that the yields of the monobrominated products **80a-b**, had a significant increase with the use of the additive KBr, due to the *in situ* formation of $KBr_3$,[79] a very reactive intermediate towards ArES (**Scheme 1.19**).




**Scheme 1.19:** Bromination reaction mechanism propose using KBr as Catalyst in *p*-oxy-substituted 4-arylfuran-2(5*H*)-one.

The structures of compounds **80a-b** were confirmed by spectroscopic data IR, NMR and HRMS. Initially, it is interesting to note that the absorption of the carbonyl in the IR occurs at a greater wave number for compound **80a**

(1758 $cm^{-1}$) relative to the starting material **74b** (1749 $cm^{-1}$). This increase is attributable to the electron withdrawing effect of the bromine atom that slightly strengthens the C=O bond.

HRMS spectrum of **80a** showed a signal in *m/z* = 302.9425, corresponding to the formula $C_{10}H_9BrClO_3$, referring to the $[M+H]^+$ ion.

The $^1H$ NMR spectrum showed a singlet at 5.15 ppm, corresponding to the methylene group of the lactone. In the aromatic region, only three hydrogen signals were observed. The singlet at 7.99 ppm corresponds to H-6', while the doublets at $\delta$ = 7.80 and 7.01 (*J* = 8.5 Hz) refer to H-3' and H-2' hydrogens (**Fig. 1.18**).

The $^{13}C$ NMR spectrum, showed six signals in the aromatic region, clearly indicating that this ring is not symmetrical, in agreement with the structure of the expected product. In this way, three intense signals are observed for the hydrogenated carbons C-2' ($\delta$ = 111.95), C-3' ($\delta$ = 128.09) and C-6' ($\delta$ = 132.13). Likewise, we detected the signals of the non-hydrogenated carbon atoms C-1' ($\delta$ = 122.44), C-4 '($\delta$ = 116.10), and C-5' ($\delta$ = 122.63) (**Fig. 1.19**). A similar analysis was done for the assignment of signals in compound **80b** (**Fig. A 1.11-12**).

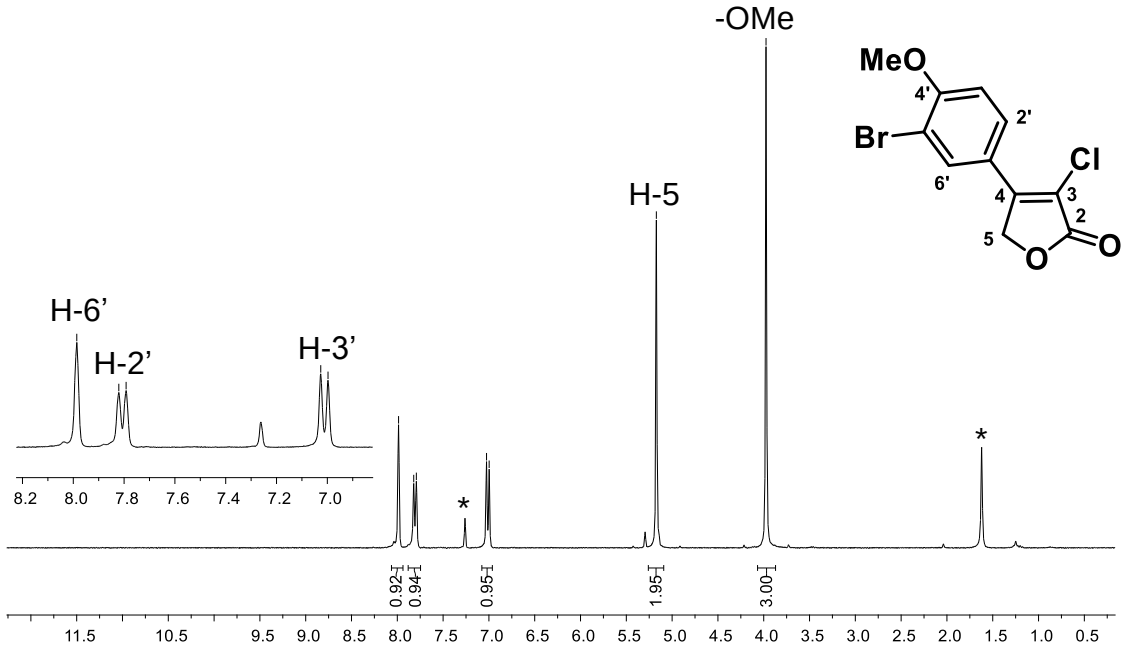



**Figure 1.18:** $^1H$ NMR ($CDCl_3$, 400 MHz) of compound **80a**.*Solvent residues and water signals.

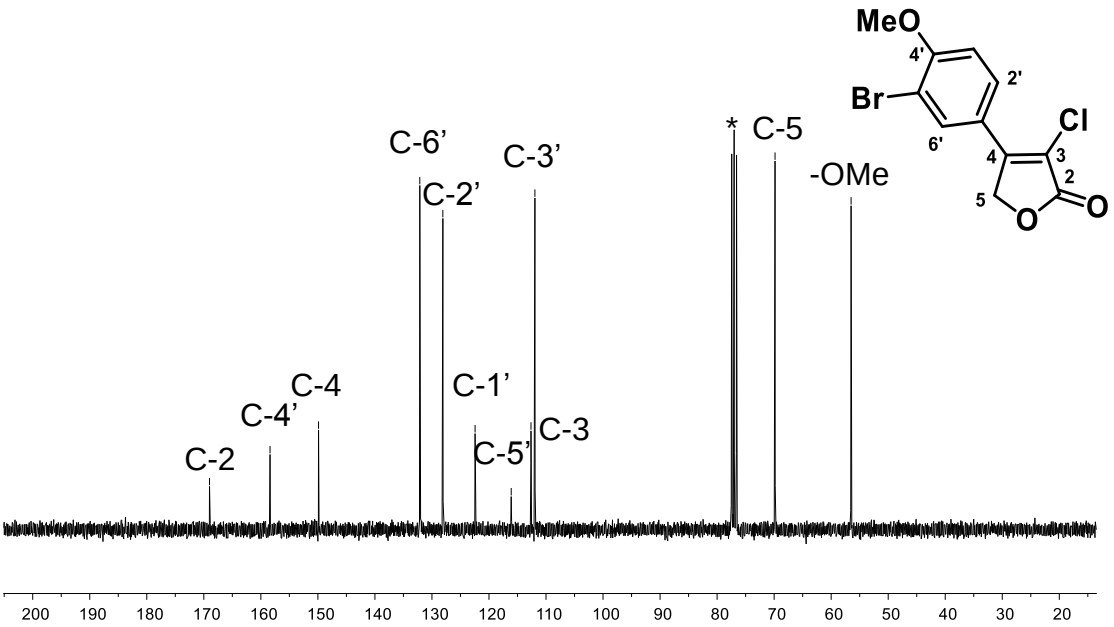
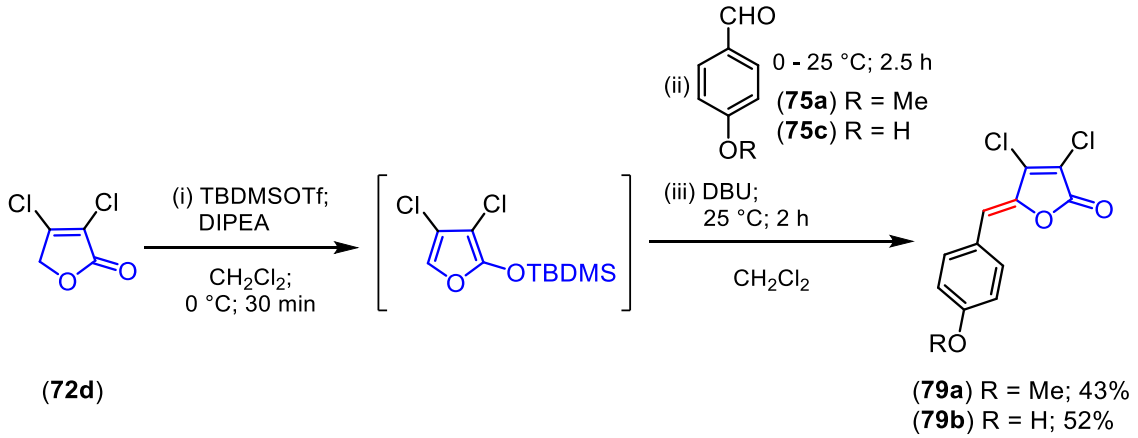



**Figure 1.19:** $^{13}C$ NMR ($CDCl_3$, 100 MHz) of compound **80a**. *Solvent residues signals.

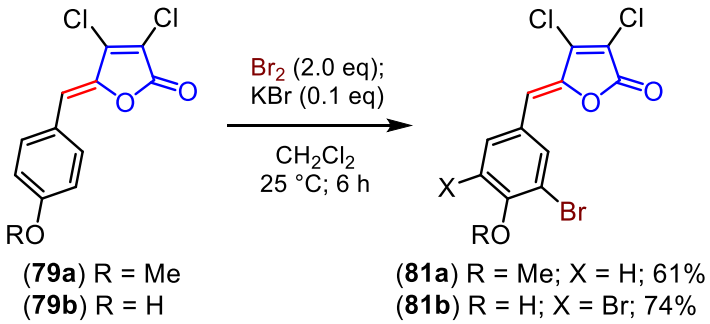
We then turned our attention to investigating bromination reaction in the γ-benzylidenebutenolide ring (ring B). Initially, the model molecules **79a-b** were synthesized using VMAC from lactone **72d** and aldehydes **75a** and **75c** (**Scheme 1.20**). Previous studies with condensation reactions with this same starting material have already been performed and presented encouraging results.[31,60] Compounds **79a-b** were obtained in 43% and 52% yield, respectively. These were identified by NMR spectroscopic analysis (**Fig. A.1.5-8**).




**Scheme 1.20:** Synthesis conditions for the compounds **79a-b**.

Thereafter, the bromination reactions were performed using the best previous conditions found for ring A. In this manner, compound **79a** was treated

with 2.0 eq of bromine using KBr as catalyst, producing the monobromo derivative **81a** in a yield of 61%. Using similar reaction conditions, the hydroxyl compound **79b** produced the dibromo derivative **81b** in 74% yield as sole product (**Scheme 1.21**).




**Scheme 1.21:** Synthesis conditions for the compounds **81a-b**.

In this case, it is important to disclose that even with the addition of a large excess of bromine, 5.0 eq, to the methoxylated compound **79a**, the dibromo product was only formed in small quantities (<10% by GC-MS analysis of the crude mixture). In contrast, reacting compound **79b** with only 1.0 eq of $Br_2$ under the same conditions resulted in a complex mixture of mono and dibrominated products as well as the starting material, as shown by GC-MS analysis of the crude mixture. These results clearly demonstrate that the γ-benzylidene group (ring B) is more reactive towards the bromination reaction than the aromatic ring at position β (ring A). One possible explanation would be that the conjugation between the α,β-unsaturated carbonyl system and β-aryl substituent results in a more efficient electron-withdrawing effect. Therefore, it is a stronger deactivator. In this manner, the formation of a single monobrominated product occurs, even in the presence of strong activating groups such as hydroxyl. Comparatively, when reaction is performed in the γ-benzylidene region in presence of the weaker activator (methoxy in compound **79a**), it leads to the production of monobrominated product and, when the strongest activating group (hydroxyl in compound **79b**) is used, the dibrominated product is obtained, despite having the same type of effect produced by conjugation with the lactone. Consequently, the deactivating effect is clearly reduced, probably due to greater conjugation system, making this ring relatively richer in electrons and, therefore, more prone to dibromation (**Fig. 1.20**).

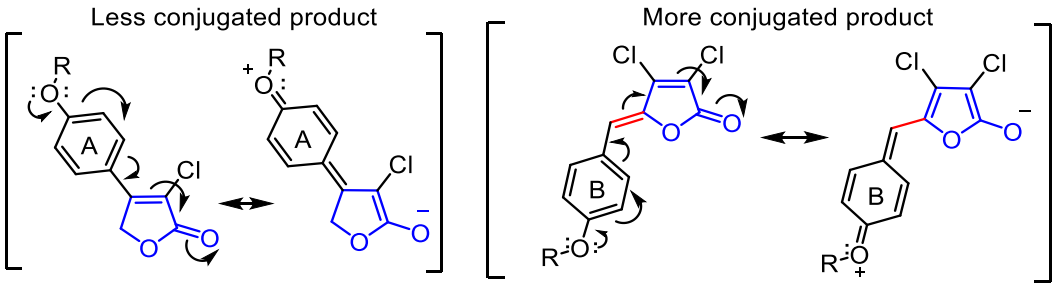



**Figure 1.20:** Comparison between the effects of electron attraction by resonance between the two rings of the rubrolide skeleton.

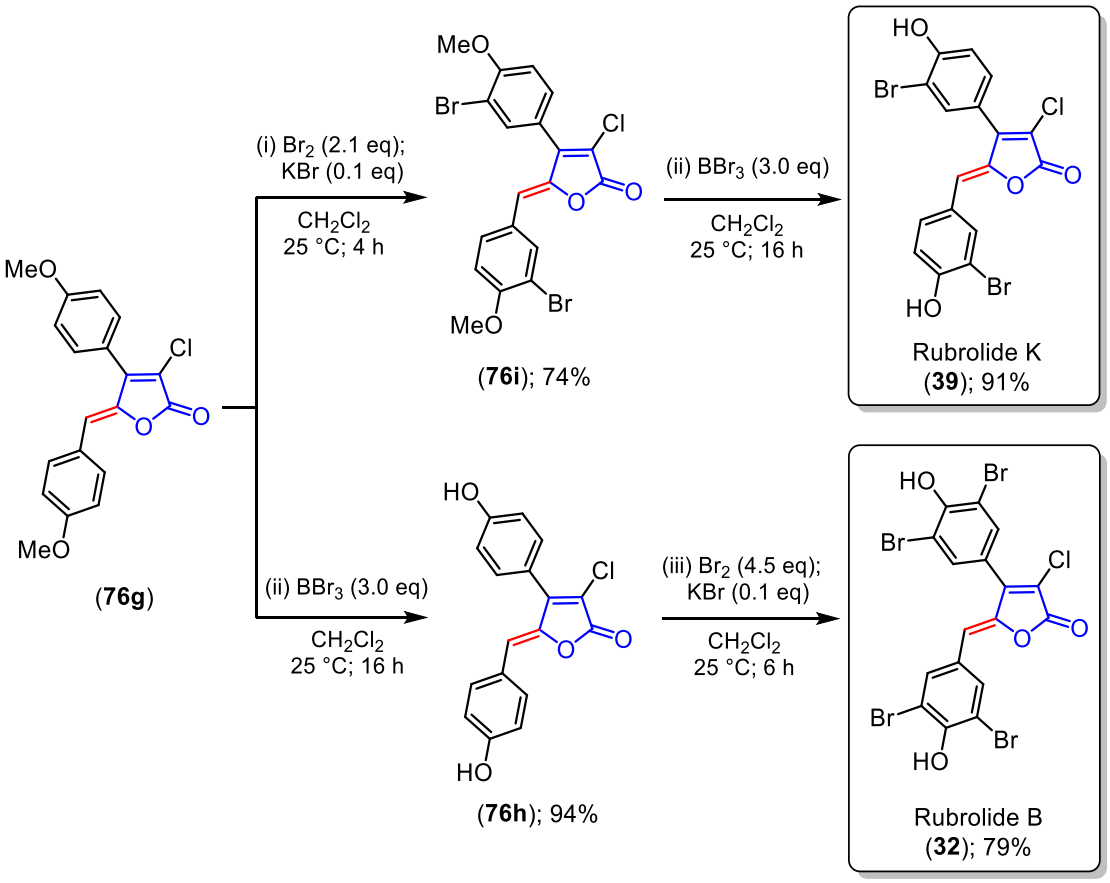
The structures of compounds **79a-b** and **81a-b** were identified by NMR spectroscopic analysis (**Fig. A 1.13-20**) as discussed for the bromination products **80a-b** and the aldol condensation product **76g**.

### 1.3.3. Final stages of the synthesis of rubrolides B and K

In this final stage, the rubrolides B and K were finally obtained with the synthesis strategy. Due to the studied scope of bromination and an appropriated bromination/demethylation reaction sequence, we can initially expect that the less brominated natural product, rubrolide K (**39**), can be produced as a result of an adequate bromine addition method on the slightly reactive dimethylated intermediate **76g**. Keeping in mind that, individually, either methoxylated benzene rings A and B in rubrolides are prone to yield only monobrominated products. On the other hand, the minor reactivity observed in ring A will lead to some complications in rubrolide B synthesis. Nevertheless, as were discussed in retrosynthetic analysis, demethylation of intermediate **76g** will hopefully yield a more reactive dihydroxylated compound **76h** that, followed by a suitable bromination procedure, will provide the naturally-occurring polybrominated product, rubrolide B (**32**).

To begin the reaction, compound **76g** was treated with 2.1 eq of $Br_2$ on the optimized bromination conditions (**Table 1.2**, **entry 2**) generating **76i**, as expected, with a yield of 74% (**Scheme 1.22;** (i)). Then, treatment of **76i** with $BBr_3$, leaves to the removal of all of the methyl groups with a yield of 91%. Therefore, leading to the production of rubrolide K in five steps (**Scheme 1.22**; **39**; 37% overall yield).

Expecting an increasing in the aforementioned product yield, the same procedure was performed with dihydroxylated product **76h**, which was obtained after demethylation of **76g** (94% yield), though production of rubrolide K was not achieved. To our delight and surprise, a mixture of other products was obtained, including a small amount of the desired polybrominated rubrolide B (**32**, 20% yield). With this new outcome, the amount of bromine used was increased in order to improve the yield. With this new approach, sequential treatments with 3.0 eq of $Br_2$ followed by 4.5 eq, yielded 25% and 79% of rubrolide B, respectively (**Scheme 1.22**; 41% overall yield).




**Scheme 1.22:** Reaction conditions for the synthesis of rubrolides B and K.

As discussed throughout the scope of the bromination reaction, butenolide ring A is less reactive than ring B separately, due to the differences in electronic density caused by their respective conjugation systems (**Fig. 1.21**; (**I**) and (**II**)). Nonetheless, when the reaction is performing with a structure containing the two rings (ring A + ring B; *e.g* compound **76**), ring A becomes more susceptible to bromination. Therefore, this reactivity increase is attributable to the additional conjugation provided by the 4-aryl-5-benzylidenebutenolide motif, leading to

reduction of the deactivating effect of α,β-unsaturated carbonyl system by interfering in the electrons withdrawing effect over the β-aryl group, resulting in polybromination (**Fig. 1.21**, (**III**)).

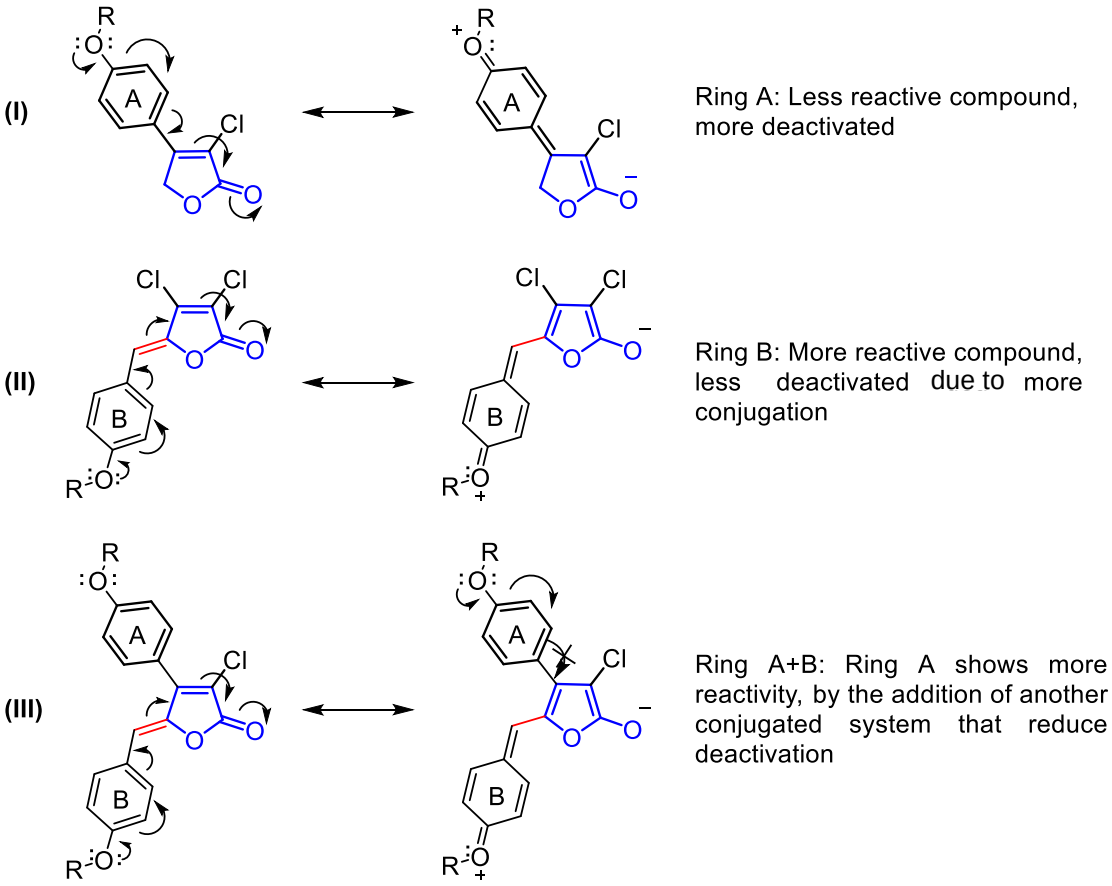
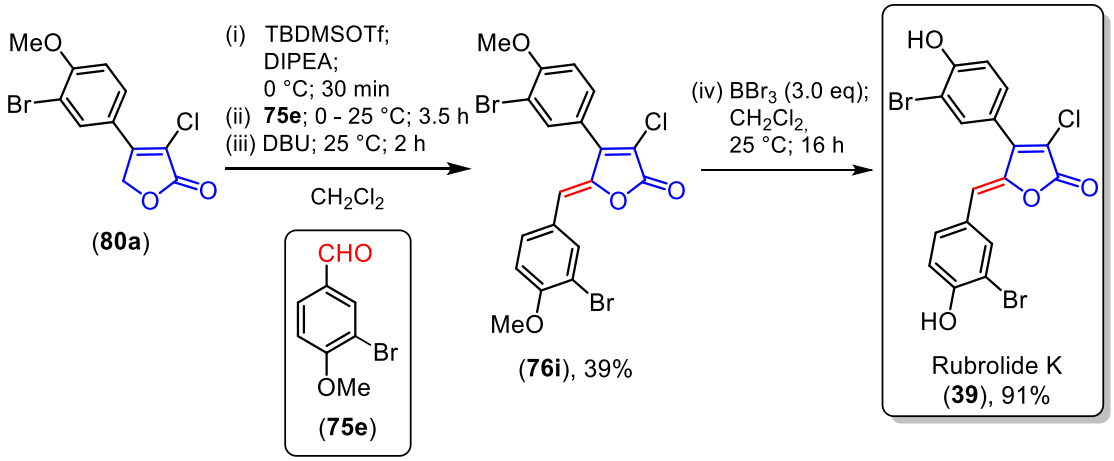


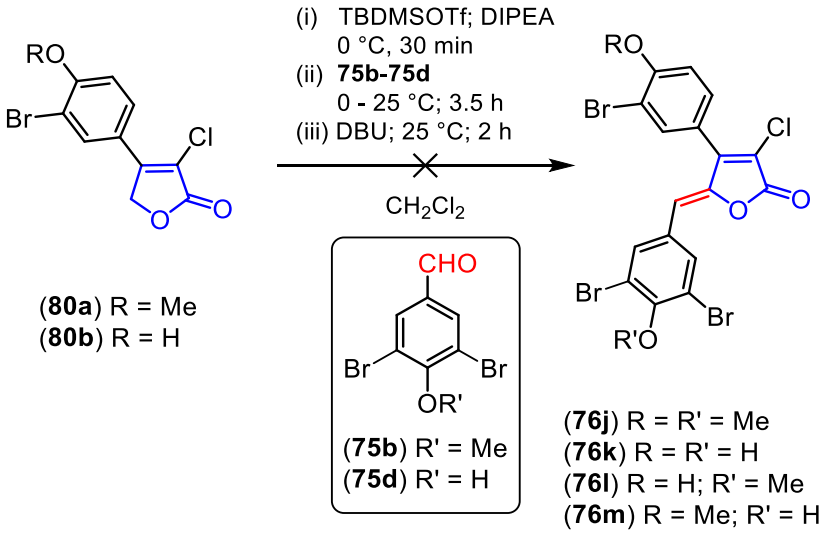

**Figure 1.21:** Comparison between electron withdrawing effects by resonance between the two regions of the rubrolides skeleton.

To conclude and as an alternative to the synthesis of the rubrolides B and K, a VMAC reactions were developed between the β-arylfuran-2(5*H*)-one products obtained in the bromination optimization reactions **80a-b**, against the aldehydes **75b**, **75d** and **75e** to produce the γ-benzylidene-β-arylfuran-2(5*H*)-ones intermediates **76i-m**. At that point, the aldol condensation of **80a** and aldehyde **75e** was performed, resulting in precursor **76i** in 39% yield, thereafter, by demethylation of this intermediate, the rubrolide K (**39**) was obtained. This methodology was performed in five steps with an overall yield of 20% of the natural product (**Scheme 1.23**).




**Scheme 1.23:** Reaction conditions for the synthesis of the rubrolidide K (**39**) using aldol condensation in compounds **80a** and **75e**.

The same procedure was used to produce the **76j-m** precursors for the synthesis of rubrolide B (**32**). However, the reactions of the intermediates **80a-b** with the aldehydes **75b** and **75d** to produce **76j-m** were inefficient (**Scheme 1.24**). Analysis of crude GC-MS reaction mixtures showed only minimal amounts of the starting aldehydes and degradation of **80a-b** after addition of DBU. This low reactivity of the highly-substituted aldehydes (**75b** and **75d**) when dealing with an aldol condensation reaction has been reported in other findings.[56,67]




**Scheme 1.24:** Reaction conditions for direct synthesis using aldol condensation of rubrolide B (**32**).

The naturally-occurring products obtained were analyzed using spectroscopic techniques IV, HRMS, $^{1}$H NMR, and $^{13}$C NMR. The characteristic

signals of the latter coinciding perfectly with those reported for previously isolated products (**Tables 1.3-4**)

**Table 1.3:** Comparative $^{1}H$ and $^{13}C$ NMR data between the synthesized and the isolate rubrolide B (**32**)

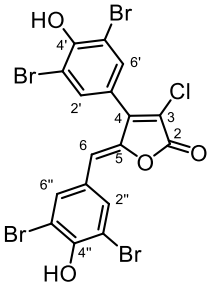
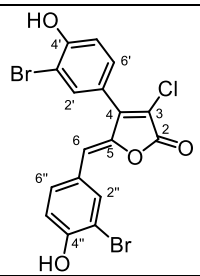
|  | | Synthetized natural product | Isolated natural product[25] |
|---|---|---|---|
| | | 400 MHz | 100 MHz |
| $^{1}H$ NMR ($(CD_3)_2SO$) | -OH | - | 10.6 (br, 2H) |
| | H-2″ e H-6″ | 8.04 (s, 2H) | 8.05 (s, 2H) |
| | H-2′ e H-6′ | 7.74 (s, 2H) | 7.73 (s, 2H) |
| | H-6 | 6.32 (s, 1H) | 6.32 (s, 1H) |
| | | 100 MHz | 25.2 MHz |
| $^{13}C$ NMR ($(CD_3)_2SO$) | C-2 | 163.4 | 163.4 |
| | C-4′ | 153.1 | 152.8 |
| | C-4″ | 152.7 | 151.9 |
| | C-4 | 147.2 | 147.3 |
| | C-5 | 144.7 | 145.2 |
| | C-2″/C-6″ | 134.3 (2C) | 134.3 (2C) |
| | C-2′/C-6′ | 132.8 (2C) | 132.9 (2C) |
| | C-1″ | 126.1 | 127.0 |
| | C-1′ | 120.6 | 121.0 |
| | C-3 | 117.1 | 118.0 |
| | C-3′/C-5′ | 112.2 (2C) | 112.1 (2C) |
| | C-3″/C-5″ | 112.1 (2C) | 112.0 (2C) |
| | C-6 | 111.7 | 111.4 |

**Table 1.4:** Comparative $^{1}H$ and $^{13}C$ NMR data between the synthesized and the isolate rubrolide K (**39**)

|  | | Synthetized natural product | Isolated natural product[34] |
|---|---|---|---|
| | | 400 MHz | 100 MHz |
| $^{1}H$ NMR ($(CD_3)_2CO$) | -OH | 9.57 (br, 2H) | 9.57 (br, 2H) |
| | H-2″ | 8.05 (d, *J* = 2.0 Hz, 1H) | 8.04 (d, *J* = 2.0 Hz, 1H) |
| | H-2′ | 7.80 (d, *J* = 2.0 Hz, 1H) | 7.78 (d, *J* = 2.0 Hz, 1H) |
| | H-6″ | 7.74 (dd, *J* = 2.0, 8.6 Hz, 1H) | 7.72 (dd, *J* = 2.0, 8.8 Hz, 1H) |
| | H-6′ | 7.51 (dd, *J* = 2.0, 8.4 Hz, 1H) | 7.48 (dd, *J* = 2.0, 8.4 Hz, 1H) |
| | H-5′ | 7.23 (d, *J* = 8.4 Hz, 1H) | 7.22 (d, *J* = 8.4 Hz, 1H) |
| | H-5 | 7.09 (d, *J* = 8.6 Hz, 1H) | 7.08 (d, *J* = 8.8 Hz, 1H) |
| | H-6 | 6.31 (s, 1H) | 6.29 (s, 1H) |
| | | 100 MHz | 25.2 MHz |
| $^{13}C$ NMR ($(CD_3)_2CO$) | C-2 | 164.5 | 164.5 |
| | C-4′ | 156.8 | 156.8 |
| | C-4″ | 156.0 | 156.0 |
| | C-4 | 149.5 | 149.6 |
| | C-5 | 146.1 | 146.1 |
| | C-2″ | 136.3 | 136.3 |
| | C-2′ | 134.7 | 134.7 |
| | C-6″ | 132.4 | 132.5 |
| | C-6′ | 130.9 | 130.9 |
| | C-1″ | 127.3 | 127.2 |
| | C-1′ | 121.5 | 121.3 |
| | C-3 | 117.9 | 117.8 |
| | C-5′/ C-5″ | 117.5 (2C) | 117.5 (2C) |
| | C-6 | 113.5 | 113.6 |
| | C-3′/ C-3″ | 110.8 (2C) | 110.9 (2C) |

## 1.4. CONCLUSIONS AND PERSPECTIVES

In this work, the first synthesis of rubrolides B (**32**) and K (**39**) was presented through the bromination reaction from easily-produced intermediates (**76g-h**), with overall yields of 41% and 37%, respectively. In addition, **39** was produced through another alternative pathway, presenting 5 steps and 20% overall yield, through the coupling reactions with palladium, condensation, bromination and demethylation.

Likewise, a versatile strategy has been developed using the aromatic bromination reaction in different stages of the rubrolide synthesis. Therefore, this is applicable to the preparation of other rubrolides as well as several non-natural analogues with potential bioactivity. The optimization of the bromination reaction, allowed us to study how the products with different levels of conjugation can increase or decrease the electrophilicity in the aromatic rings against the ArES by altering its electronic distribution.

The vinylogous aldol condensation reaction proves to be the most suitable reaction for formation of γ-alkylidenbutenolide skeleton. Variations in their conditions allows for access to a wide variety of natural products such as rubrolides, as well as tetronamides, enhygrolides, and its derivatives, as will be discussed further on.

As a perspective, this synthesis will allow the production of several derivatives of rubrolides endowed with diverse biological activities. These will be evaluated for the purpose of searching for new substances with potential use as pharmaceuticals and/or agrochemicals.

## 1.5. METHODOLOGIES

### 1.5.1. General experimental techniques.

The following experimental procedures were used according to the methodologies reported in the literature. All reactions were performed using analytical-grade solvents. Reagents and solvents were purified, when necessary. All reactions were carried out under dry nitrogen. Moisture sensitive reactions were performed on oven dried glass and sealed with rubber septum. Moisture sensitive liquids, solutions and anhydrous solvents were transferred via the syringe or cannula through rubber septa.

Analytical thin layer chromatography analyses were conducted on aluminum-backed pre-coated silica gel plates Polygram-UV254 0.20 mm, Macherey-Nagel (20x20 cm) observed under UV light ($\lambda$ = 254 nm; 365 nm). Column chromatography was performed on silica gel (230–400 mesh).

Melting points are uncorrected and were obtained using an MQAPF-301 melting point apparatus (Microquimica, Brazil).

### 1.5.2. General spectroscopic techniques.

$^{1}$H and $^{13}$C NMR spectroscopic data were recorded at 400 and 100 MHz, respectively, with a Bruker NMR spectrometer with $CDCl_3$ ($\delta_H$ 7.26; $\delta_C$ 77.16), DMSO-$d_6$ ($\delta_H$ 2.50; $\delta_C$ 39.52) or acetone-$d_6$ ($\delta_H$ 2.05; $\delta_C$ 29.84, 206.26) as solvent and in some cases tetramethylsilane (TMS) as internal standard ($\delta$ = 0 ppm). Chemical shifts of $^{1}$H and $^{13}$C NMR spectra are reported in ppm and related to solvent signals. All coupling constants (*J* values) are expressed in Hertz (Hz). Multiplicities are reported as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), multiplet (m) and broad (brd). Infrared spectrum was recorded with a Varian 660-IR instrument, equipped with GladiATR scanning from 4000 to 500 $cm^{-1}$. High-resolution mass spectrum was recorded with a Bruker MicroTof (resolution = 10000 FWHM) using electrospray ionization (ESI) and are given to four decimal places.

### 1.5.3. General purification techniques.

#### *1.5.3.1. Drying of tetrahydrofuran (THF).*

To a 500 mL flask were added 300 mL of THF and 12 g of calcium hydride. The system was allowed to reflux for 2 hours. The THF was then distilled and transferred to another 500 mL metal sodium-containing flask. The mixture was allowed to reflux for one hour. Next, benzophenone was added to the flask containing THF, leaving the system under reflux until the reaction mixture became blue. After the change in staining, the anhydrous THF was distilled, which was stored on a 4Å molecular sieve in a sealed amber glass vial under a nitrogen atmosphere.[80]

#### *1.5.3.2. Drying of dichloromethane (DCM) and methanol (MeOH).*

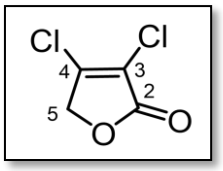
To a 500 mL flask were added 300 mL of dichloromethane or methanol and 3 g of calcium hydride. The system was allowed to reflux for three hours. The anhydrous dichloromethane and methanol were then distilled off, which was stored on a 4Å molecular sieve in a sealed amber glass vial under a nitrogen atmosphere.[80]

#### *1.5.3.3. Drying of N,N-*diisopropylethylamine *(DIPEA).*

To a 500 mL flask was added 300 mL of diisopropylethylamine on potassium hydroxide lentils. The mixture was refluxed for two hours. After this time, the diisopropylethylamine was distilled and stored on potassium hydroxide lentils.[80]

### 1.5.4. Synthetic procedures for the preparation of reaction intermediates corresponding to the Suzuki-Miyaura cross-coupling steps.

*1.5.4.1. 3,4-dichorofuran-2(5H)-one (**72d**).*




In a 250 mL round-bottomed flask containing mucochloric acid (**29**) (21.51 g, 83.4 mmol, 1.0 eq) and methanol (120 mL) under magnetic stirring at 0 °C was added $NaBH_4$ (4.74 g, 125.1 mmol, 1.5 eq) in small portions. The resulting mixture was stirred for 15 minutes at 0 °C. Thereafter, a solution of concentrated sulfuric acid (4.5 mL, 83.4 mmol, 1.0 eq) in methanol (42 mL) was added at 0 °C, the resulting mixture being kept under stirring for 15 minutes. At the end of this period, the mixture was diluted with diethyl ether (1125 mL) and washed with saturated aqueous NaCl solution (4 x 150 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The solid formed was recrystallized from a 1:1 (v/v) hexane:$Et_2O$ mixture, giving the lactone (**72d**) (16.67 g, 69.2 mmol, 83% yield).

**Characteristics**: White crystals.

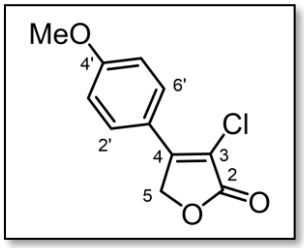
**Mp**: 89.6-90.3 °C.

**FTIR (KBr; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 2925; 2853; 1758; 1605; 1431; 1339; 1217; 1023; 992; 869; 749; 718.

**$^1$H NMR (400 MHz. $CDCl_3$)** $\delta$**:** 4.86 (s; 2H; H-5).

**$^{13}$C NMR (100 MHz. $CDCl_3$)** $\delta$**:** 74.4 (C-5); 114.7 (C-3); 143.7 (C-4); 166.8 (C-2).

*1.5.4.2. 3-chloro-4-(4-metoxyphenyl)furan-2(5H)-one (**74b**).*




In a 100 mL round-bottomed flask fitted with a reflux condenser, the lactone (**72d**) (2.0 g, 13.1 mmol, 1.0 eq) was added along with 4-methoxyphenylboronic acid (**73a**) (2.4 g, 19.6 mmol, 1.5 eq), cesium fluoride (5.96 g, 39.3 mmol, 3.0 eq), $Pd(PhCN)_2Cl_2$ (0.25 g, 0.66 mmol, 0.05 eq), $PPh_3$ (0.35 g, 1.31 mmol, 0.1 eq) and $Bu_4NBr$ (0.26 g, 1.31 mmol, 0.1

eq). Under $N_2$ atmosphere, 24 mL of toluene/water (2:1) was added. The reaction mixture was stirred and degassed for 10 min under $N_2$ flow. Then, it was heated up at 60 °C for 12 h under $N_2$ atmosphere. After the reaction was complete, the mixture was vacuum filtered through Celite by washing with a little AcOEt. The filtrate was extracted with EtOAc (3 x 50 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and concentrated in vacuo. The crude product was purified by silica gel column chromatography (10% EtOAc:hexane) to give compound **74b** (1.82 g, 8.12 mmol, 62% yield).

**Characteristics:** White solid.

**Mp:** 174.4-175.3 ºC.

**FTIR (KBr; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3033; 2937; 1749; 1603; 1510; 1444; 1254; 1201; 1184; 1022; 751; 734.

**$^1$H NMR (400 MHz. $CDCl_3$)** $\delta$**:** 7.79 (d; *J* = 8.4 Hz; 2H; H-2' e H-6'); 7.01 (d; *J* = 8.4 Hz; 2H; H-3'e H-5'); 5.19 (s; 2H; H-5); 3.88 (s; 3H; -OMe).

**$^{13}$C NMR (100 MHz. $CDCl_3$)** $\delta$**:** 169.5 (C-2); 162.2 (C-4'); 151.3 (C-4); 129.0 (2C; C-2' e C-6'); 121.2 (C-3); 114.8 (C-1'); 114.6 (2C; C-3'e C-5'); 70.0 (C-5); 55.5 (-$OCH_3$).

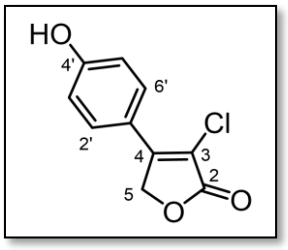
**HRMS (ESI-TOF)** ***m/z*** **[M+H]$^+$:** Calculated for $C_{11}H_{10}ClO_3$ 225.0313; found 225.0317.

*1.5.4.3. 3-chloro-4-(4-hydroxyphenyl)furan-2(5H)-one (**74d**).*

*1.5.4.3.1. By Suzuki-Miyaura cross coupling.*

Compound **74d** was synthesized using a method similar to that used for the production of compound **74b**, starting from the lactone (**72d**) (2.0 g, 13.1 mmol, 1.0 eq) along with 4-hydroxyphenylboronic acid (**73b**) (2.4 g, 19.6 mmol, 1.5 eq). The crude product was purified by silica gel column chromatography (20% EtOAc:hexane) to provide **74d** in 51% yield (1.4 g, 6.68 mmol).

*1.5.4.3.2. By demethylation reaction.*




In a 25 mL round bottom flask, compound **74b** (500 mg, 2.23 mmol, 1.0 eq) was diluted with 15 mL of anhydrous $CH_2Cl_2$. It was then allowed to stir and cooled in an ice bath at 0 °C. Then, 1 mL of $BBr_3$ solution diluted in anhydrous $CH_2Cl_2$ (32%$^{v/v}$, 3.35 mmol, 2.0 eq) was added dropwise. Thereafter, the resulting mixture was allowed to warm to room temperature and stirred for an additional 16 h under $N_2$ atmosphere. At the end, the reaction was quenched with 20 mL of saturated aqueous $NH_4Cl$ and then the $CH_2Cl_2$ was removed in vacuo. Thereafter, the aqueous phase was extracted with EtOAc (3 x 20 mL) and the organic layer was dried ($Na_2SO_4$), filtered and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (20% EtOAc:hexane) to provide **74d** (97%, 456 mg, 2.16 mmol).

**Characteristics:** White solid.

**Mp:** 254.8-255.2 ºC.

**FTIR (KBr; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3271; 1725; 1604; 1580; 1510; 1441; 1330; 1274; 1181; 751.

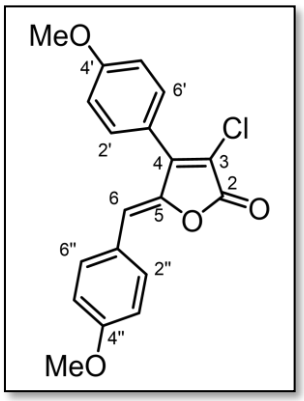
**$^1$H NMR (400 MHz. $(CD_3)_2SO$) $\delta$:** 10.38 (s; 1H; -OH); 7.76 (d; *J* = 8.0 Hz; 2H; H-2' e H-6'); 6.93 (d; *J* = 8.0 Hz; 2H; H-3' e H-5'); 5.39 (s; 2H; H-5).

**$^{13}$C NMR (100 MHz. $(CD_3)_2SO$) $\delta$:** 169.7 (C-2); 161.2 (C-4'); 153.8 (C-4); 130.1 (2C; C-2' e C-6'); 119.8 (C-3); 116.4 (2C; C-3' e C-5'); 111.9 (C-1'); 70.9 (C-5).

**HRMS (ESI-TOF) *m/z* $[M+H]^+$:** Calculated for $C_{10}H_8ClO_3$ 211.0156; Found 211.0167.

### 1.5.5. General procedure for the preparation of reaction intermediates for the steps of Mukaiyama's vinylougous condensation.

*1.5.5.1. (Z)-3-chloro-5-(4-metoxybenzylydene)-4-(4-metoxyphenyl)furan-2(5H)-one (**76g**).*




In a 25 mL round-bottomed flask containing a solution of the lactone **74b** (200 mg, 0.89 mmol, 1.0 eq) in 5 mL of anhydrous $CH_2Cl_2$, cooled in an ice bath to 0 °C, were added successively: DIPEA (0.46 mL, 2.67 mmol, 3.0 eq) and TBDMSOTf (0.4 mL, 1.78 mmol, 2.0 eq). The solution is allowed to stir under $N_2$ atmosphere for 30 min. Then *p*-anisaldehyde (**75a**) (0.12 mL, 1.07 mmol, 1.2 eq) was added stirring at 0 °C for a further 1 h. The solution is then warmed to ambient temperature and stirred for a further 3 h. Then DBU (0.26 mL, 1.78 mmol, 2.0 eq) was added. The resulting solution was allowed to stir for another 4 h before being neutralized with 10 mL of 1 M aqueous HCl solution. Then $CH_2Cl_2$ was removed in vacuo and the aqueous phase was extracted with EtOAc (3 x 15 mL), the combined organic layers were washed with saturated aqueous NaCl, the extract dried ($Na_2SO_4$), filtered and concentrated under reduced pressure. The crude extract from the reaction was purified by silica gel column chromatography (15% EtOAc:hexane) to afford the product **76g** in 72% yield (256mg, 0.64mmol).

**Characteristics:** Yellow solid.

**Mp:** 170.5-171.8 ºC.

**FTIR (KBr; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3021; 2939; 2842; 1767; 1640; 1602; 1565; 1503; 1429; 1348; 1306; 1256; 1179; 1015; 824; 751.

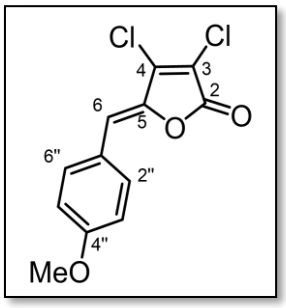
**$^1$H NMR (400 MHz. $CDCl_3$) $\delta$:** 7.75 (d; *J* = 8.8 Hz; 2H; H-2'' e H-6''); 7.51 (d; *J* = 8.7 Hz; 2H; H-2' e H-6'); 7.09 (d; *J* = 8.8 Hz; 2H; H-3'' e H-5''); 6.92 (d; *J* = 8.7 Hz; 2H; H-3' e H-5'); 6.13 (s; 1H; H-6); 3.92 (s; 3H; -$OCH_3$); 3.86 (s; 3H; -$OCH_3$).

**$^{13}$C NMR (100 MHz. $CDCl_3$) $\delta$:** 165.0 (C-2); 161.4 (C-4'); 160.8 (C-4''); 149.8 (C-4); 145.0 (C-5); 132.7 (2C; C-2'' e C-6''); 130.8 (2C; C-2' e C-6'); 125.6 (C-1'');

120.5 (C-1'); 116.7 (C-3); 114.7 (C-6); 114.5 (2C; C-3'' e C-5''); 114.5 (2C; C-3' e C-5'); 55.6; 55.5 (-$OCH_3$).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{19}H_{16}ClO_4$ 343.0732; found 343.0726.

*1.5.5.2. (Z)-3,4-dichloro-5-(4-hydroxybenzylydene)furan-2(5H)-one (**81a**).*




Compound **81a** was synthesized using a method similar to that used for the production of compound **76g** from lactone **72d** (200 mg, 1.3 mmol, 1.0 eq) and aldehyde **75a**. Compound **81a** was purified by silica gel column chromatography (10% EtOAc:hexane) in 43% yield (152 mg, 0.56 mmol)

**Characteristics:** Yellow solid.

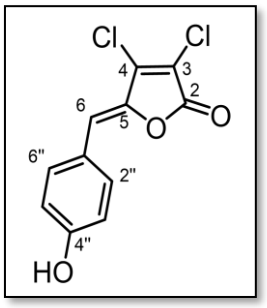
**Mp:** 111.3-112.8 °C.

**$^1$H NMR (400 MHz. $CDCl_3$) $\delta$:** 7.72 (d; *J* = 8.5 Hz; 2H; H-2'' e H-6''); 6.92 (d; *J* = 8.5 Hz; 2H; H-3'' and H-5''); 6.32 (s; 1H; H-6); 3.84 (s; 3H; -$OCH_3$).

**$^{13}$C NMR (100 MHz. $CDCl_3$) $\delta$:** 162.5 (C-1); 161.3 (C-4''); 142.6 (C-4); 141.7 (C-5); 133.00 (2C; C-2'' e C-6''); 124.4 (C-1''); 118.0 (C-3); 114.6 (2C; C-3'' e C-5''); 112.8 (C-6); 55.4 (-$OCH_3$).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{12}H_9Cl_2O_3$ 270.9923; found 270.9929.

*1.5.5.3. (Z)-3,4-dichloro-5-(4-hydroxybenzylydene)furan-2(5H)-one (**81b**).*




Compound **81b** was synthesized using a method similar to that used for the production of compound **76g** from lactone **72d** (200 mg, 1.3 mmol, 1.0 eq) and aldehyde **75c**. Compound **81b** was purified by silica gel column chromatography (20% EtOAc:hexane), isolated in 52% yield (174 mg, 0.68 mmol).

**Characteristics:** Yellow solid.

**Mp:** 178.1-179.7 °C.

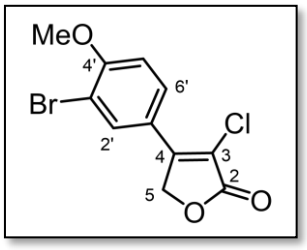
**$^{1}$H NMR (400 MHz. $(CD_3)_2CO$) $\delta$:** 9.06 (s; 1H; -OH); 7.74 (d; *J* = 7.5 Hz; 2H; H-2'' e H-6''); 6.96 (d; *J* = 7.5 Hz; 2H; H-3'' e H-5''); 6.52 (s; 1H; H-6).

**$^{13}$C NMR (100 MHz. $(CD_3)_2CO$) $\delta$:** 161.8 (C-2); 159.5 (C-4''); 142.3 (C-4); 141.4 (C-5); 133.2 (2C; C-2'' e C-6''); 123.8 (C-1''); 117.4 (C-3); 116.1 (2C; C-3'' e C-5''); 112.7 (C-6).

**HRMS (ESI-TOF) *m/z* $[M+H]^+$:** Calculated for $C_{11}H_7Cl_2O_3$ 256.9767; Found 256.9756.

### 1.5.6. General procedure for the preparation of reaction intermediates for the optimization steps of aromatic bromination.

*1.5.6.1. 4-(3-bromo-4-metoxyphenyl)-3-chlorofuran-2(5H)-one (**80a**).*




In 25 mL round bottom flask containing a solution of compound **74b** (100 mg, 0.45 mmol, 1.0 eq) and KBr (5 mg, 0.04 mmol, 0.1 eq) in anhydrous $CH_2Cl_2$ ( 5 mL) was slowly added $Br_2$ (0.04 mL, 0.89 mmol, 2.0 eq). The resulting mixture was stirred at room temperature under $N_2$ atmosphere for 6 h. Then, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ and $CH_2Cl_2$ was removed in vacuo. Then, the aqueous phase was extracted with EtOAc (3 x 15 mL) and the organic layer was dried ($Na_2SO_4$), filtered and evaporated under reduced pressure. The crude extract from the reaction showed only a solid corresponding to **80a** in 68% yield (92 mg, 0.31 mmol).

**Characteristics:** White solid.

**Mp:** 152.9-153.4 ºC.

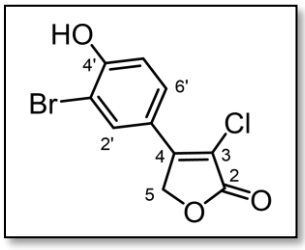
**FTIR (KBr; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3091; 2946; 2858; 1758; 1616; 1594; 1503; 1444; 1326; 1261; 1202; 1034; 1005; 828; 752; 678; 599.

**$^{1}$H NMR (400 MHz. $CDCl_3$) $\delta$:** 7.99 (s; 1H; H-2'); 7.81 (d; *J* = 8.5 Hz; 1H; H-6'); 7.01 (d; *J* = 8.5 Hz; 1H; H-5'); 5.17 (s; 2H; H-5); 3.97 (s; 3H; $-OCH_3$).

**$^{13}$C NMR (100 MHz. $CDCl_3$) $\delta$:** 169.0 (C-2); 158.4 (C-4'); 149.9 (C-4); 132.1 (C-2'); 128.1 (C-6'); 122.4 (C-1'); 116.1 (C-3); 112.6 (C-3'); 111.9 (C-5'); 69.8 (C-5); 56.51 ($-OCH_3$).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{10}H_9BrClO_3$ 302.9418; found 302.9425.

*1.5.6.2. 4-(3-bromo-4-hydroxyphenyl)-3-chlorofuran-2(5H)-one (**80b**).*




Compound **80b** was synthesized using a method similar to that used for the production of compound **80a** from intermediate **74d** (136.7 mg, 0.47 mmol, 1.0 eq). Compound **80b** was obtained in the crude extract from the reaction as a white solid in 72% yield (99 mg, 0.34 mmol).

**Characteristics:** White solid.

**Mp:** 213.5-214.4 °C.

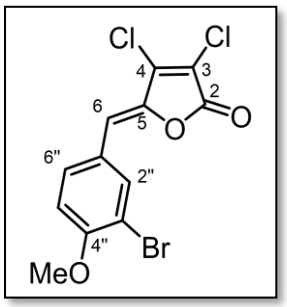
**FTIR (KBr; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3287; 1748; 1595; 1562; 1504; 1445; 1423; 1295; 1199; 1156; 1055; 1013; 814; 741; 627; 591.

**$^1$H NMR (400 MHz. $(CD_3)_2SO$) $\delta$:** 7.99 (s; 1H; H-2′); 7.79 (d; *J* = 8.6 Hz; 1H; H-6′); 7.10 (d; *J* = 8.6 Hz; 1H; H-5′); 5.40 (s; 2H; H-5).

**$^{13}$C NMR (100 MHz. $(CD_3)_2SO$) $\delta$:** 169.4 (C-2); 157.7 (C-4′); 152.6 (C-4); 132.7 (C-2′); 129.1 (C-6′); 121.3 (C-1′); 117.0 (C-3); 113.3 (C-3′); 110.5 (C-5′); 70.9 (C-5).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{10}H_7BrClO_3$ 288.9262; found 288.9263.

*1.5.6.3. (Z)-5-(3-bromo-4-metoxybenzylydene)-3,4-dichlorofuran-2(5H)-one (**81a**).*




Compound **81a** was synthesized using a method similar to that used for the production of compound **80a** from intermediate **79a** (126.2 mg, 0.37 mmol, 1.0 eq). Compound 59a was purified by silica gel column chromatography (5% EtOAc:hexane) in 61% yield (79 mg, 0.23 mmol).

**Characteristics:** Yellow solid.

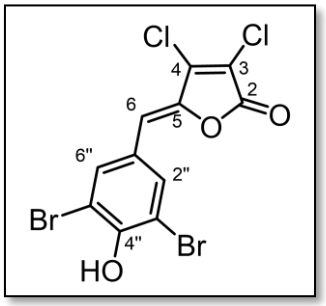
**Mp:** 143.4-144.2 °C.

**$^{1}$H NMR (400 MHz. $CDCl_3$) $\delta$:** 7.78 (s; 1H; H-2″); 7.57 (d; *J* = 8.6 Hz; 1H; H-6″); 6.93 (d; *J* = 8.6 Hz; 1H; H-5″); 6.34 (s; 1H; H-6); 3.95 (s; 3H; -$OCH_3$).

**$^{13}$C NMR (100 MHz. $CDCl_3$) $\delta$:** 161.3 (C-2); 157.0 (C-4″); 154.0 (C-4); 134.8 (C-5); 130.6 (C-2″); 128.2 (C-6″); 123.1 (C-1″); 117.3 (C-3); 111.6 (C-5″); 111.6 (C-3″); 111.1 (C-6); 56.4 (-$OCH_3$).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{12}H_8BrCl_2O_3$ 348.9034; found 348.9021.

*1.5.6.4. (Z)-3,4-dichloro-5-(3,5-dibromo-4-hydroxybenzylydene)furan-2(5H)-one (**81b**).*




Compound **81b** was synthesized using a method similar to that used for the production of compound **80a** from intermediate **79b** (162.6 mg, 0.39 mmol, 1.0 eq). Compound **81b** was purified by silica gel column chromatography (20% EtOAc:hexane) in 74% yield (119 mg, 0.29 mmol).

**Characteristics:** Yellow solid.

**Mp:** 211.5-212.9 °C.

**$^{1}$H NMR (400 MHz. $(CD_3)_2CO$) $\delta$:** 8.08 (s; 2H; H-2″ e H-6″); 6.58 (s; 1H; H-6).

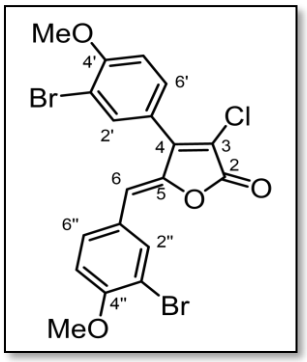
**$^{13}$C NMR (100 MHz. $(CD_3)_2CO$) $\delta$:** 161.4 (C-2); 152.1 (C-4″); 143.3 (C-4); 137.9 (C-5); 134.6 (2C; C-2″ e C-6″); 126.8 (C-1″); 119.3 (C-3); 111.0 (C-6); 109.0 (2C; C-3″ e C-5″).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{11}H_5Br_2Cl_2O_3$ 412.7977; found 412.7963.

### 1.5.7. General procedure for the synthesis of rubrolide K (39).

*1.5.7.1. (Z)-5-(3-bromo-4-metoxybenzylydene)-4-(3-bromo-4-metoxy phenyl)-3-chlorofuran-2(5H)-one (**76i**).*

*1.5.7.1.1. By VMAC.*




Compound **76i** was synthesized using the Mukaiyama's vinylogous aldol condensation methodology, similar to that used for the production of compound **76g**, from intermediate **80a** (50 mg, 0.16 mmol, 1.0 eq) with aldehyde **75e** (35 mg, 0.2 mmol, 1.2 eq) in the presence of TBDMSOTf (0.08 mL, 0.32 mmol, 2.0 eq) and DIPEA (0.08 mL, 0.48 mmol, 3.0 eq) followed by in situ elimination with DBU (0.05 mL, 0.32 mmol, 2.0 eq) yielding intermediate **76i**, which was purified using silica gel column chromatography (15% EtOAc:hexane) in a yield of 39% (31 mg, 0.06 mmol).

*1.5.7.1.2. By selective aromatic bromination reaction.*

Alternatively, compound **76i** was synthesized using the bromination methodology, similar to that used for the production of compound **80a**, starting from compound **76g** (50 mg, 0.15 mmol, 1.0 eq) with $Br_2$ (0.02 mL, 31 mmol, 2.1 eq) and KBr (2 mg, 0.015 mmol, 0.1 eq) in 5 mL of anhydrous $CH_2Cl_2$, providing compound **76i**. This was purified using silica gel column chromatography (15% EtOAc:hexane) in 74% yield (56 mg, 0.11 mmol).

**Characteristics:** Yellow solid.

**Mp:** 200.3-201.4 °C.

**FTIR (KBr; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 2946; 2842; 1761; 1592; 1489; 1453; 1437; 1291; 1259; 1231; 1163; 1160; 1052; 1013; 896; 810; 693; 677; 618.

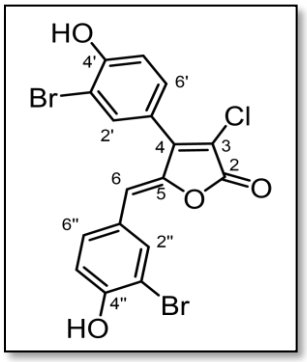
**$^1$H NMR (400 MHz. $CDCl_3$) $\delta$:** 7.94 (d; *J* = 1.6 Hz; 1H; H-2″); 7.79 (dd; *J* = 1.6; 8.5 Hz; 1H; H-6″); 7.74 (d; *J* = 2.0 Hz; 1H; H-2′); 7.49 (dd; *J* = 2.0; 8.4 Hz; 1H; H-6′);

7.09 (d; *J* = 8.4 Hz; 1H; H-5′); 6.93 (d; *J* = 8.5 Hz; 1H; H-5″); 6.03 (s; 1H; H-6); 4.01 (s; 3H; -$OCH_3$); 3.96 (s; 3H; -$OCH_3$).

**$^{13}$C NMR (100 MHz. $CDCl_3$) $\delta$:** 165.8 (C-2); 157.6 (C-4′); 156.9 (C-4″); 148.9 (C-4); 145.4 (C-5); 135.6 (C-2″); 133.8 (C-2′); 131.5 (C-6″); 129.7 (C-6′); 121.3 (C-1″); 121.1 (C-1′); 118.2 (C-3); 112.9 (C-6); 112.7 (C-5″); 112.4 (C-5′); 112.0 (C-3″); 111.6 (C-3′); 56.6 (-$OCH_3$); 56.4 (-$OCH_3$).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{19}H_{14}Br_2ClO_4$ 498.8942; found 498.8929.

*1.5.7.2. Synthesis of rubrolide K (**39**)*




The rubrolide K (**39**) was synthesized using the demethylation method, similar to that used for the production of compound **74d**, starting from intermediate **76i** (70 mg, 0.14 mmol, 1.0 eq) using $BBr_3$ (0.04 mL, 0.42 mmol, 3.0 eq), removing all methyl groups. No colum separation was necessary, obtaining the natural product rubrolide K (**39**) as an orange solid in 91% yield (60 mg, 0.13 mmol).

**Characteristics:** Amorphous Orange solid.

**Mp:** 214.3-215.6 °C.

**FTIR; (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3332; 3205; 1745; 1644; 1598; 1494; 1406; 1324; 1296; 1275; 1188; 1159; 1136; 1023; 910; 824; 808; 752; 701; 666; 570; 492.

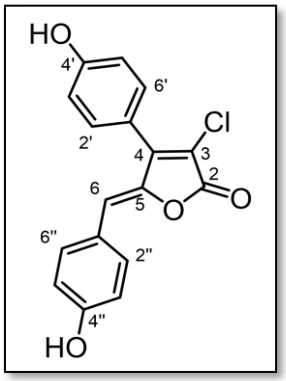
**$^1$H NMR (400 MHz. $(CD_3)_2CO$) $\delta$:** 9.57 (brd; 2H; -OH); 8.05 (d; *J* = 2.0 Hz; 1H; H-2″); 7.80 (d; *J* = 2.0 Hz; 1H; H-2′); 7.74 (dd; *J* = 2.0; 8.6 Hz; 1H; H-6″) 7.51 (dd; *J* = 2.0; 8.4 Hz; 1H; H-6′); 7.23 (d; *J* = 8.4 Hz; 1H; H-5′); 7.09 (d; *J* = 8.6 Hz; 1H; H-5″); 6.31 (s; 1H; H-6).

**$^{13}$C NMR (100 MHz. $(CD_3)_2CO$) $\delta$:** 164.5 (C-2); 156.8 (C-4′); 156.0 (C-4″); 149.5 (C-4); 146.1 (C-5); 136.3 (C-2″); 134.7 (C-2′); 132.4 (C-6″); 130.9 (C-6′); 127.3 (C-1″); 121.5 (C-1′); 117.9 (C-3); 117.5 (2C; C-5′ e C-5″); 113.5 (C-6); 110.8 (2C; C-3′ e C-3″).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{17}H_{10}Br_2ClO_4$ 470.8629; found 470.8624.

### 1.5.8. General procedure for the synthesis of rubrolide B (32).

*1.5.8.1. (Z)-3-chloro-5-(4-hydroxybenzylydene)-4-(4-hydroxyphenyl)furan-2(5H)-one (**76h**).*




Compound **76h** was synthesized using a demethylation method similar to that used for the production of compound **74d** from intermediate **76g** (100 mg, 0.29 mmol, 1.0 eq) using $BBr_3$ (0.08 mL, 88 mmol, 3.0 eq), removing all methyl groups. Intermediate **76h** was obtained in the crude extract from the reaction as a yellow solid in 94% yield (86 mg, 0.27 mmol).

**Characteristics:** Yellow solid.

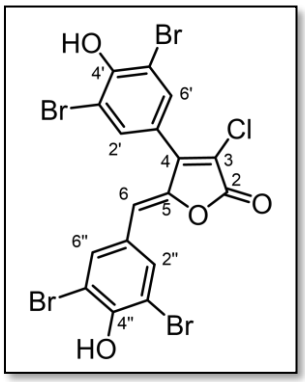
**Mp:** 234.1-234.8 °C.

**$^1$H NMR (400 MHz. $(CD_3)_2CO$) $\delta$:** 7.73 (d; *J* = 8.8 Hz; 2H; H-2″ e H-6″); 7.51 (d; *J* = 8.7 Hz; 2H; H-2′ e H-6′); 7.07 (d; *J* = 8.8 Hz; 2H; H-3″ e H-5″); 6.93 (d; *J* = 8.7 Hz; 2H; H-3′ e H-5′); 6.27 (s; 1H; H-6).

**$^{13}$C NMR (100 MHz. $(CD_3)_2CO$) $\delta$:** 164.2 (C-2); 159.6 (C-4″); 159.0 (C-4′); 150.3 (C-4); 144.7 (C-5); 132.9 (2C; C-2″ e C-6″); 131.1 (2C; C-2′ e C-6′); 124.9 (C-1″); 120.0 (C-1′); 116.0 (2C; C-3″ e C-5″); 115.9 (2C; C-3′ e C-5′); 115.4 (C-3); 114.5 (C-6).

**HRMS (ESI-TOF) *m/z* $[M+H]^+$:** Calculated for $C_{17}H_{12}ClO_4$ 315.0424; found 315.0421.

*1.5.8.2. Synthesis of rubrolide B (**32**)*




Rubrolide B (**32**) was synthesized using a bromination method similar to that used for the production of compound **80a** from intermediate **76h** (40 mg, 0.13 mmol, 1.0 eq) using $Br_2$ (0.03 mL) and KBr (2 mg, 0.013 mmol, 0.1 eq) in 5 mL of dry $CH_2Cl_2$. Obtaining the crude natural product as a yellow solid in 79% yield (65 mg, 0.10 mmol).

**Characteristics:** Amorphous Yellow Solid, in MeOH solutions and water has a yellow color in solutions of acetone or DMSO has a red color.

**Mp:** 256.7-257.6 °C.

**FTIR (KBr; cm$^{-1}$)** $\bar{\upsilon}_{max}$ 3491; 3266; 3066; 1739; 1641; 1541; 1470; 1288; 1234; 1146; 1025; 880; 741; 725; 654; 582; 493.

**$^{1}$H NMR (400 MHz. $(CD_3)_2SO$) $\delta$:** 8.04 (s; 2H; H-2″ e H-6″); 7.74 (s; 2H; H-2′ e H-6′); 6.32 (s; 1H; H-6).

**$^{13}$C NMR (100 MHz. $(CD_3)_2SO$) $\delta$:** 163.4 (C-2); 153.1 (C-4′); 152.7 (C-4″); 147.2 (C-4); 144.7 (C-5); 134.3 (2C; C-2″ e C-6″); 132.8 (2C; C-2′ e C-6′); 126.1 (C-1″); 120.6 (C-1′); 117.1 (C-3); 112.2 (2C; C-3′ e C-5′); 112.1 (2C; C-3″ e C-5″); 111.7 (C-6).

**HRMS (ESI-TOF) *m/z* [M+H]$^{+}$:** Calculated for $C_{17}H_8Br_4ClO_4$ 626.6839; found 626.6806.

## 1.6. REFERENCES


[1] DE SOUZA, M. V. N. The furan-2(5*H*)-ones: Recent synthetic methodologies and its application in total synthesis of natural products. *Mini-Reviews in Organic Chemistry* **2005**, *2*, 139.

[2] NARAYANA, S.; MADHAV, B.; VIJAY, A.; RAMA, K.; NAGESWAR, Y. V. D. Facile and efficient synthesis of 3,4,5-substituted furan-2(5*H*)-ones by using β-cyclodextrin as reusable catalyst. *Tetrahedron* **2009**, *65*, 5251.

[3] KITAZUME, T.; TAKEDA, M. A Cyclization reaction catalysed by antibodies. *Journal of the Chemical Society, Chemical Communications* **1995**, 39.

[4] BAULAC, M.; KLEMENT, S. Efficacy and safety of losigamone in partial seizures: a randomized double-blind study. *Epilepsy Research* **2003**, *55*, 177.

[5] RAO, Y. S. Chemistry of butenolides. *Chemical Reviews* **1964**, *64*, 353.

[6] HAYNES, L. J.; PLIMMER, J. R. Tetronic acids. *Quarterly Reviews, Chemical Society* **1960**, *14*, 292.

[7] MISSAKIAN, M. G.; BURRESON, B. J.; SCHEUER, P. J. Pukalide, a furanocembranolide from the soft coral *Sinularia abrupta*. *Tetrahedron* **1975**, *31*, 2513.

[8] HOSOE, T.; IIZUKA, T.; KOMAI, S.-I.; WAKANA, D.; ITABASHI, T.; NOZAWA, K.; FUKUSHIMA, K.; KAWAI, K.-I. 4-Benzyl-3-phenyl-5H-furan-2-one, a vasodilator isolated from *Malbranchea filamentosa* IFM 41300. *Phytochemistry* **2005**, *66*, 2776.

[9] MARTÍNEZ-LUIS, S.; GONZÁLEZ, M. C.; ULLOA, M.; MATA, R. Phytotoxins from the fungus *Malbranchea aurantiaca*. *Phytochemistry* **2005**, *66*, 1012.

[10] LATEGAN, C. A.; CAMPBELL, W. E.; SEAMAN, T.; SMITH, P. J. The bioactivity of novel furanoterpenoids isolated from *Siphonochilus aethiopicus*. *Journal of Ethnopharmacology* **2009**, *121*, 92.

[11] WEBER, R. W. S.; KAPPE, R.; PAULULAT, T.; MÖSKER, E.; ANKE, H. Anti-Candida metabolites from endophytic fungi. *Phytochemistry* **2007**, *68*, 886.

[12] DAVIDSON, B. S.; IRELAND, C. M. Lissoclinolide, the First Non-Nitrogenous Metabolite from a Lissoclinum tunicate. *Journal of Natural Products* **1990**, *53*, 1036.

[13] RICHARDSON, A. D.; IRELAND, C. M. A profile of the *in vitro* antitumor activity of lissoclinolide. *Toxicology and Applied Pharmacology* **2004**, *195*, 55.

[14] GOGOI, S.; ARGADE, N. P. A facile chemoenzymatic approach to natural cytotoxic ellipsoidone A and natural ellipsoidone B. *Tetrahedron* **2006**, *62*, 2715.

[15] CAO, R.; LIU, C.; LIU, L. A Convenient Synthesis of 2(5*H*)-Furanone. *Organic Preparations and Procedures International* **1996**, *28*, 215.

[16] FU, C.; MA, S. Efficient Preparation of 4-Iodofuran-2(5*H*)-ones by Iodolactonisation of 2,3-allenoates with $I_2$. *European Journal of Organic Chemistry* **2005**, 3942.

[17] BASSETTI, M.; D'ANNIBALE, A.; FANFONI, A.; MINISSI, F. Synthesis of α,β-unsaturated 4,5-disubstituted γ-Lactones via ring-closing metathesis catalyzed by the first-generation Grubbs' catalyst. *Organic Letters* **2005**, *7*, 1805.

[18] BOUKOUVALAS, J.; THIBAULT, C. Step-economical synthesis of the marine ascidian antibiotics cadiolide A, B, and D. *The Journal of Organic Chemistry* **2015**, *80*, 681.

[19] BELLINA, F.; ANSELMI, C.; MARTINA, F.; ROSSI, R. Mucochloric Acid: A useful synthon for the selective synthesis of 4-aryl-3-chloro-2(5*H*)-furanones, (Z)-4-aryl-5-[1-(aryl)methylidene]-3-chloro-2(5*H*)-furanones and 3,4-diaryl-2(5*H*)-furanones. *European Journal of Organic Chemistry* **2003**, 2290.

[20] BLAZECKA, P. G.; BELMONT, D.; CURRAN, T.; PFLUM, D.; ZHANG, J. Further utilization of mucohalic acids: palladium-free, regioselective etherification and amination of α,β-dihalo γ-methoxycarbonyloxy and γ-acetoxy butenolides. *Organic letters* **2003**, *5*, 5015.

[21] CUNHA, S.; OLIVEIRA, C. C. Aplicações sintéticas do ácido mucobrômico e da 3,4-dibromofuran-2(5*H*)-ona. *Quim. Nova* **2011**, *34*.

[22] DAS SARMA, K.; ZHANG, J.; CURRAN, T. T. Novel synthons from mucochloric acid: The first use of α,β-dichloro-γ-butenolides and γ-butyrolactams for direct vinylogous aldol addition. *J. Org. Chem.* **2007**, *72*.

[23] ZHANG, J.; BLAZECKA , P. G.; BELMONT , D.; DAVIDSON, J. G. Reinvestigation of mucohalic acids, versatile and useful building blocks for highly functionalized α,β-unsaturated γ-butyrolactones. *Org. Lett.* **2002**, *4*, 4559.

[24] KOTORA, M.; NEGISHI, E. Highly efficient and selective procedures for the synthesis of γ-alkylidenbutenolides via palladium-catalyzed ene-yne coupling and palladium- or silver-catalyzed lactonization in (*Z*)-2-en-4-ynoic acids. Synthesis of rubrolides A, C, D and E. *Synthesis* **1997**, *1*, 121.

[25] MIAO, S.; ANDERSEN, R. J. Rubrolides A-H, metabolites of the colonial tunicate *Ritterella rubra*. *The Journal of Organic Chemistry* **1991**, *56*, 6275.

[26] KARAK, M.; BARBOSA, L. C. A.; ACOSTA, J. A. M.; SAROTTI, A. M.; BOUKOUVALAS, J. Thermodynamically driven, *syn*-selective vinylogous aldol reaction of tetronamides. *Organic & Biomolecular Chemistry* **2016**, *14*.

[27] CARROLL, A. R.; HEALY, P. C.; QUINN, R. J.; TRANTER, C. J. Prunolides A, B, and C: Novel tetraphenolic bis-spiroketals from the australian ascidian *Synoicum prunum*. *The Journal of Organic Chemistry* **1999**, *64*, 2680.

[28] FELDER, S.; KEHRAUS, S.; NEU, E.; BIERBAUM, G.; SCHÄBERLE, T. F.; KÖNIG, G. M. Salimyxins and enhygrolides: antibiotic, sponge-related metabolites from the obligate marine myxobacterium *Enhygromyxa salina*. *ChemBioChem* **2013**, *14*, 1363.

[29] SMITH, C. J.; HETTICH, R. L.; JOMPA, J.; TAHIR, A.; BUCHANAN, M. V.; IRELAND, C. M. Cadiolides A and B, New metabolites from an ascidian of the genus *Botryllus*. *The Journal of Organic Chemistry* **1998**, *63*, 4147.

[30] BOUKOUVALAS, J.; MALTAIS, F.; LACHANCE, N. Furanolate-based strategy for sequential 2,3,4-trisubstitution of butenolide: Total synthesis of nostoclides I and II. *Tetrahedron Letters* **1994**, *35*, 7897.

[31] BARBOSA, L. C.; MALTHA, C. R.; DEMUNER, A. J.; PINHEIRO, P. F.; VAREJÃO, J. O.; MONTANARI, R. M.; ANDRADE, N. J. Síntese e avaliação da atividade antimicrobiana de furanonas halogenadas e de compostos análogos aos nostoclídeos. *Quim. Nova* **2010**, *33*, 2020.

[32] LEVY, L. M.; CABRERA, G. M.; WRIGHT, J. E.; SELDES, A. M. 5*H*-furan-2-ones from fungal cultures of *Aporpium caryae*. *Phytochemistry* **2003**, *62*, 239.

[33] ZHU, T.; CHEN, Z.; LIU, P.; WANG, Y.; XIN, Z.; ZHU, W. New rubrolides from the marine-derived fungus *Aspergillus terreus* OUCMDZ-1925. *J Antibiot* **2014**, *67*, 315.

[34] ORTEGA, M. A. J.; ZUBÍA, E.; OCAÑA, J. M.; NARANJO, S.; SALVÁ, J. New rubrolides from the ascidian *Synoicum blochmanni. Tetrahedron* **2000**, *56*, 3963.

[35] BELLINA, F.; ANSELMI, C.; VIEL, S.; MANNINA, L.; ROSSI, R. Selective synthesis of (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-bromo-2(5*H*)-furanones. *Tetrahedron* **2001**, *57*, 9997.

[36] PEARCE, A. N.; CHIA, E. W.; BERRIDGE, M. V.; MAAS, E. W.; PAGE, M. J.; WEBB, V. L.; HARPER, J. L.; COPP, B. R. *E*/*Z*-Rubrolide O, an anti-inflammatory halogenated furanone from the New Zealand ascidian *Synoicum n.* sp. *Journal of Natural Products* **2007**, *70*, 111.

[37] KAR, A.; ARGADE, N. P. A Facile synthesis of rubrolide E. *Synthesis* **2005**, *14*, 2284.

[38] TALE, N. P.; SHELKE, A. V.; TIWARI, G. B.; THORAT, P. B.; KARADE, N. N. New concise and efficient synthesis of rubrolides C and E via Intramolecular Wittig Reaction. *Helvetica Chimica Acta* **2012**, *95*, 852.

[39] CACCHI, S.; FABRIZI, G.; GOGGIAMANI, A.; SFERRAZZA, A. Palladium-catalyzed reaction of arenediazonium tetrafluoroborates with methyl 4-hydroxy-2-butenoate: An approach to 4-aryl butenolides and an expeditious synthesis of rubrolide E. *Synlett* **2009**, *8*, 1277.

[40] CHAVAN, S. P.; PATHAK, A. B.; PANDEY, A.; KALKOTE, U. R. Short and efficient synthesis of rubrolide E. *Synthetic Communications* **2007**, *37*, 4253.

[41] PEREIRA, U. A.; MOREIRA, T. A.; BARBOSA, L. C. A.; MALTHA, C. R. A.; BOMFIM, I. S.; MARANHAO, S. S.; MORAES, M. O.; PESSOA, C.; BARROS-NEPOMUCENO, F. W. A. Rubrolide analogues and their derived lactams as potential anticancer agents. *MedChemComm* **2016**, *7*, 345.

[42] KOTHA, S.; LAHIRI, K.; KASHINATH, D. Recent applications of the Suzuki–Miyaura cross-coupling reaction in organic synthesis. *Tetrahedron* **2002**, *58*, 9633.

[43] SUZUKI, A. Carbon-carbon bonding made easy. *Chemical Communications* **2005**, *0*.

[44] SUZUKI, A. Cross-coupling reactions of organoboranes: an easy way to construct C-C bonds (Nobel Lecture). *Angewandte Chemie International Edition* **2011**, *50*, 6722.

[45] YAO, M.-L.; DENG, M.-Z. Facile Approach to 4-Substituted 2(5*H*)-Furanones. *The Journal of Organic Chemistry* **2000**, *65*, 5034.

[46] ZHANG, J.; BLAZECKA, P. G.; BELMONT, D.; DAVIDSON, J. G. Reinvestigation of mucohalic acids, versatile and useful building blocks for highly functionalized $\alpha,\beta$-unsaturated $\gamma$-butyrolactones. *Organic letters* **2002**, *4*, 4559.

[47] MIYAURA, N.; SUZUKI, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chemical Reviews* **1995**, *95*, 2457.

[48] NANTZ, M.; ZWEIFEL, G. *Modern Organic Synthesis*; Macmillan Higher Education, 2007; Vol. 1.

[49] BARBOSA, L. C. A.; TEIXEIRA, R. R.; AMARANTE, G. W. Synthetic strategies for the preparation of butenolides and their transformation into other derivatives. *Current Organic Synthesis* **2015**, *12*, 746.

[50] AKIYAMA, T.; ARAKAWA, Y.; CHEN, Y. C.; CUI, H. L.; DENG, L. *Science of synthesis: Asymmetric organocatalysis vol. 2: Bronsted base and acid catalysts, and additional topics*; Thieme, 2014.

[51] KAKLYUGINAA, T. Y.; BADOVSKAYAA, L. A.; SOROTSKAYAA, L. N.; KOZHINAA, N. D.; JURÁŠEKB, A.; KADAB, R.; KOVÁČB, J.; KULNEVICHA, V. G. Reaction of 2-butenolide and 4-bromo-2-butenolide with 5-aryl-2-furaldehydes and thiolates. *Collect. Czech. Chem. Commun.* **1986**, *51*, 2181.

[52] JEFFORD, C. W.; JAGGI, D.; BOUKOUVALAS, J. Diastereoselectivity in the directed aldol condensation of 2-trimethylsiloxyfuran with aldehydes. A stereodivergent route to threo and erythro δ-hydroxy-γ-lactones. *Tetrahedron letters* **1987**, *28*, 4037.

[53] PLEWE, M.; SCHMIDT, R. R. (±)-erythro-γ,δ-dihydroxycarboxylic acid lactones from a β-lithiopropionate equivalent and α-chloroaldehydes. *Synthesis* **1989**, *1989*, 534.

[54] BOUKOUVALAS, J.; LACHANCE, N.; OUELLET, M.; TRUDEAU, M. Facile access to 4-aryl-2(5*H*)-furanones by Suzuki cross coupling: Efficient synthesis of rubrolides C and E. *Tetrahedron Letters* **1998**, *39*, 7665.

[55] BOUKOUVALAS, J.; BELTRAN, P. P.; LACHANCE, N.; COTE, S.; MALTAIS, F.; POULIOT, M. A new, highly stereoselective synthesis of β-unsubstituted (*Z*)-γ-alkylidene-butenolides using bromine as a removable stereocontrol element. *Synlett* **2007**, 219.

[56] BOUKOUVALAS, J.; MCCANN, L. C. Synthesis of the human aldose reductase inhibitor rubrolide L. *Tetrahedron Letters* **2010**, *51*, 4636.

[57] BROWN, D. W.; CAMPBELL, M. M.; TAYLOR, A. P.; ZHANG, X.-A. Regio- and diastereoselectivity in aldol reactions of cyclopent-2-enone, 2-(5*H*)-furanone and their derived trimethylsilyloxydienes. *Tetrahedron Letters* **1987**, *28*, 985.

[58] JEFFORD, C. W.; JAGGI, D.; BERNARDINELLI, G.; BOUKOUVALAS, J. The synthesis of (±)-cavernosine. *Tetrahedron Letters* **1987**, *28*, 4041.

[59] BOUKOUVALAS, J.; MALTAIS, F. An efficient total synthesis of neopatulin. *Tetrahedron Letters* **1994**, *35*, 5769.

[60] LIU, G.-Y.; GUO, B.-Q.; CHEN, W.-N.; CHENG, C.; ZHANG, Q.-L.; DAI, M.-B.; SUN, J.-R.; SUN, P.-H.; CHEN, W.-M. Synthesis, molecular docking, and biofilm formation inhibitory activity of 5-substituted 3,4-dihalo-5*H*-furan-2-one derivatives on *Pseudomonas aeruginosa*. *Chemical Biology & Drug Design* **2012**, *79*, 628.

[61] DAVID, J. G.; BAI, W.-J.; WEAVER, M. G.; PETTUS, T. R. R. A General diastereoselective catalytic vinylogous aldol reaction among tetramic acid-derived pyrroles. *Organic Letters* **2014**, *16*, 4384.

[62] KABESHOV, M. A.; KYSILKA, O.; RULÍŠEK, L.; SULEIMANOV, Y. V.; BELLA, M.; MALKOV, A. V.; KOČOVSKÝ, P. Cross-aldol reaction of isatin with acetone catalyzed by leucinol: A mechanistic investigation. *Chemistry-A European Journal* **2015**, *21*, 12026.

[63] FRINGS, M.; ATODIRESEI, I.; WANG, Y.; RUNSINK, J.; RAABE, G.; BOLM, C. C1 - symmetric aminosulfoximines in copper - catalyzed asymmetric vinylogous mukaiyama aldol reactions. *Chemistry-A European Journal* **2010**, *16*, 4577.

[64] YANG, Y.; ZHENG, K.; ZHAO, J.; SHI, J.; LIN, L.; LIU, X.; FENG, X. Asymmetric direct vinylogous aldol reaction of unactivated γ-butenolide to aldehydes. *The Journal of Organic Chemistry* **2010**, *75*, 5382.

[65] HITOSHI, U.; NAOKI, S.; MASAHIRO, T. Asymmetric direct vinylogous aldol reaction of furanone derivatives catalyzed by an axially chiral guanidine base. *Angewandte Chemie International Edition* **2010**, *49*, 1858.

[66] JIE, L.; HAIFEI, W.; XIAO, H.; LI - WEN, X.; JACEK, K.; KUO - WEI, H.; YIXIN, L. The direct asymmetric vinylogous aldol reaction of furanones with α-ketoesters: access to chiral γ-butenolides and glycerol derivatives. *Angewandte Chemie International Edition* **2011**, *50*, 1861.

[67] BELLINA, F.; ANSELMI, C.; ROSSI, R. Total synthesis of rubrolide M and some of its unnatural congeners. *Tetrahedron Letters* **2002**, *43*, 2023.

[68] BISWAS, K.; GHOLAP, R.; SRINIVAS, P.; KANYALA, S.; DAS SARMA, K. β-Substituted γ-butyrolactams from mucochloric acid: synthesis of (±)-baclofen and other γ-aminobutyric acids and useful building blocks. *RSC Adv.* **2014**, *4*, 2538.

[69] PEREIRA, U. A. Doutorado em Agroquímica, UFV, 2014.

[70] PEREIRA, U. A.; BARBOSA, L. C. A.; MALTHA, C. R. A.; DEMUNER, A. J.; MASOOD, M. A.; PIMENTA, A. L. Inhibition of *Enterococcus faecalis* biofilm formation by highly active lactones and lactams analogues of rubrolides. *European Journal of Medicinal Chemistry* **2014**, *82*, 127.

[71] MARTIN, S. F.; CORBETT, J. W. The stereochemical course of nucleophilic additions of 2-trialkylsiloxyfurans to cyclic *N*-acyliminium ions. *Synthesis* **1992**, 55.

[72] YAMAMOTO, Y.; KOMATSU, T.; MARUYAMA, K. Diastereofacial selectivity in the reaction of allylic organometallic compounds with imines. Stereoelectronic effect of imine group. *The Journal of Organic Chemistry* **1985**, *50*, 3115.

[73] BOEHLOW, T. R.; HARBURN, J. J.; SPILLING, C. D. Approaches to the synthesis of some tyrosine-derived marine sponge metabolites: Synthesis of verongamine and purealidin N. *The Journal of Organic Chemistry* **2001**, *66*, 3111.

[74] AITKEN, H. R. M.; FURKERT, D. P.; HUBERT, J. G.; WOOD, J. M.; BRIMBLE, M. A. Enantioselective access to benzannulated spiroketals using a chiral sulfoxide auxiliary. *Organic & Biomolecular Chemistry* **2013**, *11*, 5147.

[75] MISHRA, A. K.; NAGARAJAIAH, H.; MOORTHY, J. N. Trihaloisocyanuric acids as atom-economic reagents for halogenation of aromatics and carbonyl compounds in the solid state by ball milling. *European Journal of Organic Chemistry* **2015**, *2015*, 2733.

[76] HANSCH, C.; LEO, A.; TAFT, R. W. A survey of Hammett substituent constants and resonance and field parameters. *Chemical Reviews* **1991**, *91*, 165.

[77] MCDANIEL, D. H.; BROWN, H. C. An extended table of hammett substitutent constants based on the ionization of substituted benzoic acids. *The Journal of Organic Chemistry* **1958**, *23*, 420.

[78] HOU, J.; LI, Z.; JIA, X.-D.; LIU, Z.-Q. Bromination of arenes using $I_2O_5$-KBr in water. *Synthetic Communications* **2014**, *44*, 181.

[79] KUMAR, L.; SHARMA, V.; MAHAJAN, T.; AGARWAL, D. D. Instantaneous, facile and selective synthesis of tetrabromobisphenol a using potassium tribromide: an efficient and renewable brominating agent. *Organic Process Research & Development* **2010**, *14*, 174.

[80] PERRIN, D. D.; ARMAREGO, W. L. F. *Purification of laboratory chemicals*; 5 ed ed.; Bodmin: Butterworth-Heinemann Ltd., 2003.

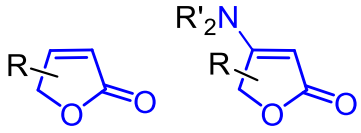
# CHAPTER 2

# TOTAL SYNTHESIS OF BASIDALIN

## 2.1. INTRODUCTION

### 2.1.1. Tetronamides

Tetronamides are an important class of β-aminosubstituted butenolides (**Fig. 2.1**; **B**), which have recently attracted attention from synthetic and medicinal chemists.[1-6] These compounds are not easily found in nature, unlike their γ-lactone derivatives (furan-2(5*H*)-ones) (**Fig. 2.1**; **A**) that have been extensively studied.[7-9] Some 4-aminobutenolides may exhibit important biological activities. The most representative example is the first isolated tetronamide, and fungical antitumor antibiotic, basidalin (**1a**).[10]




(**A**) (**B**)

**Figure 2.1:** Basic structure of butenolide derivatives (**A**) and tetronamides (**B**).

The immense majority of tetronamides are non-natural. Many of them have already been patented as herbicides and pesticides (**Fig. 2.2**, **2**-**4**).[1] Some important examples include flupiradifurone (Sivanto®, **5**) with insecticidal activity,[11,12] the 4-azapodophyllotoxin derivatives (**6**-**7**) showing potent antimitotic and of tubulin-destabilizing activity,[13,14] as well as 5-hydroxy-4-amino-2(5*H*)-furanones (**8**-**9**) compounds that are broad-spectrum antibacterials.[15] Many other tetronamides have been used as precursors of more complex biologically-active natural and synthetic products.[15-18]

Many synthetic methodologies had been developed for the preparation of 4-aminofuran-2(5*H*)-ones. These comprise a *de novo* synthesis of the lactone ring, as well as the use of starting materials containing butanolide motif, as mentioned previously. In first instance, Hiyama and co-workers (1987), used protected derivatives of cyanohydrins as starting materials (**10**). These were reacted with *tert*-butyl, acetyl and propionyl magnesium enolates (**11**). Accordingly, adduct **12** was produced and its acid hydrolysis afforded β-aminofuranones (**13**) (**Scheme 2.1**).[19]

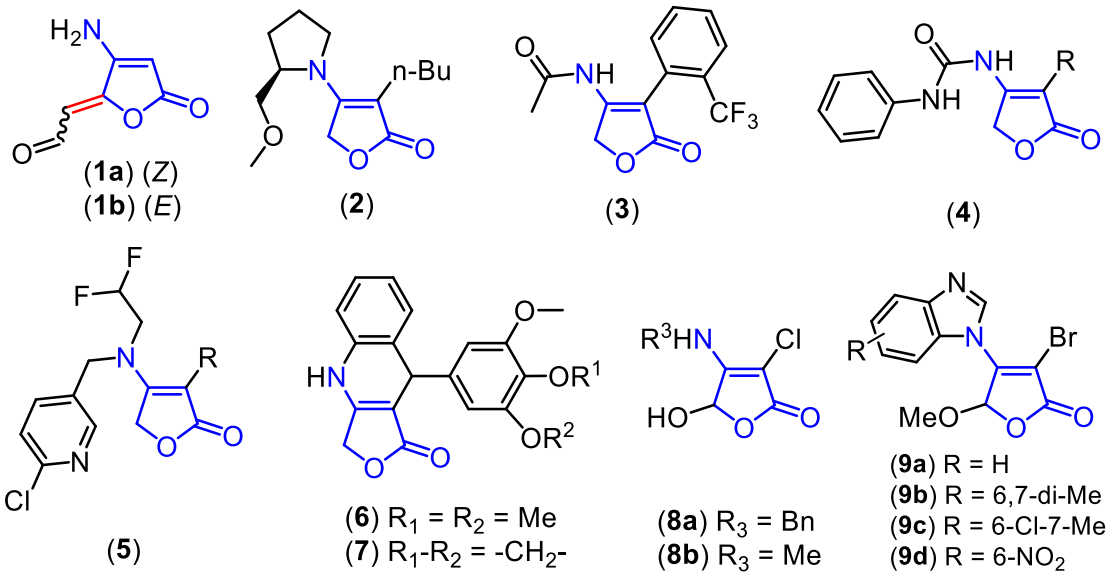
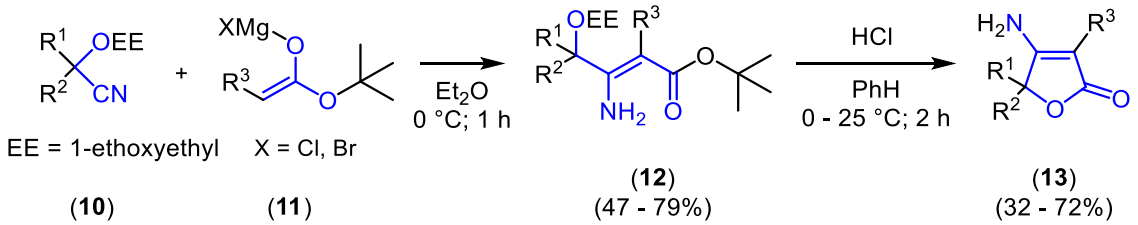
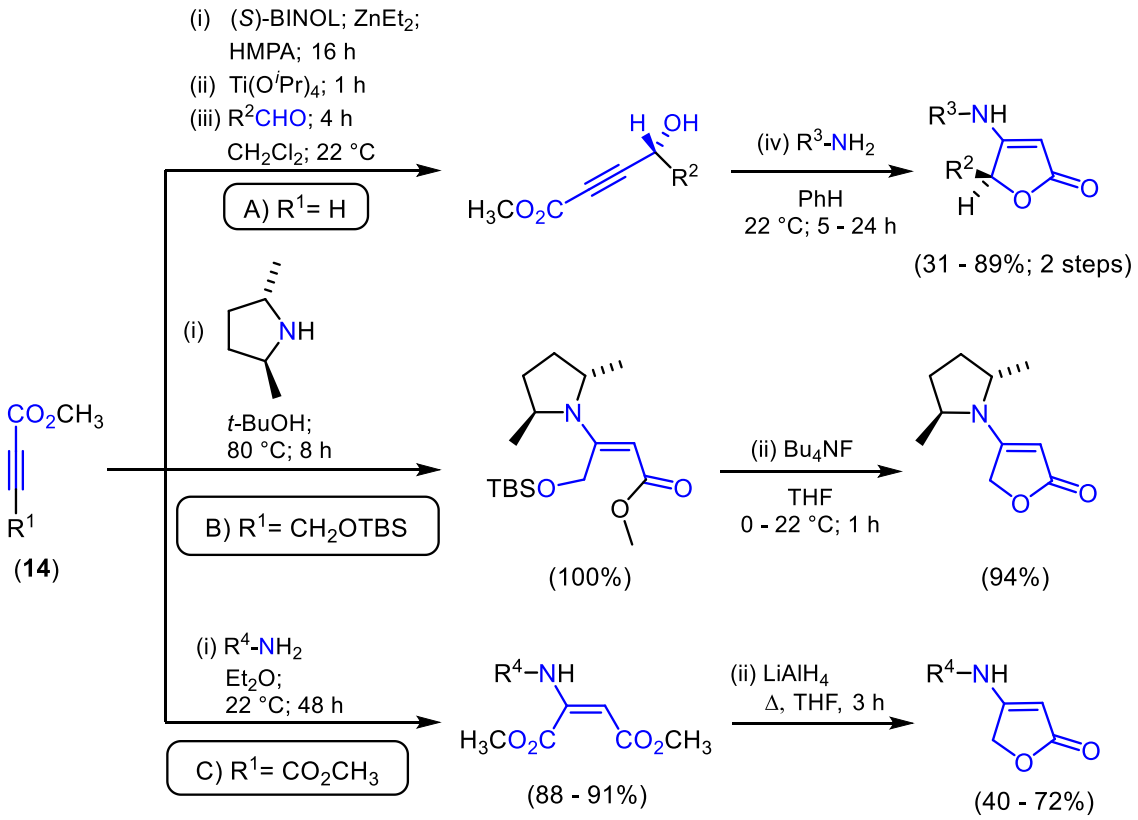



**Figure 2.2**: Examples of synthetic and natural tetronamides.



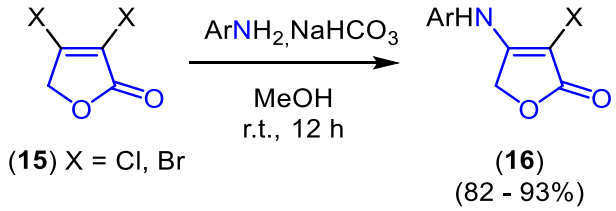

**Scheme 2.1:** Synthesis of 4-aminofuran-2(5*H*)-ones used protected derivatives of cyanohydrins.[19]

Another examples includes methodologies proposed by Zhou's,[2] Schlessinger's[20] and Greenhill's[21] groups, these includes sequential amino addition reactions in acetylenecarboxylates (**14**) followed by intramolecular cyclization, leaving the formation of α-unsubstituted 4-aminofuran-2(5*H*)-ones (**Scheme 2.2**).



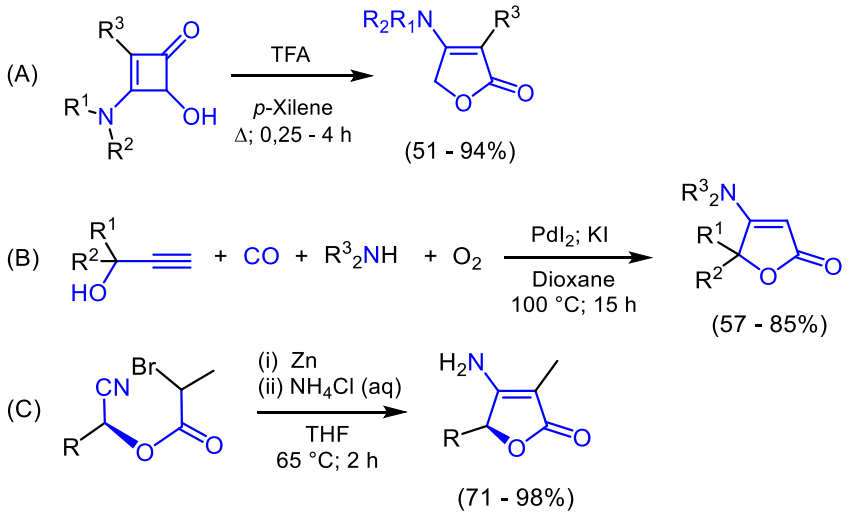

**Scheme 2.2:** 4-Aminofuran-2(5*H*)-one syntheses proposed by the groups Zhou (**A**);[2] Schlessinger (**B**);[20] and Greenhill (**C**).[21]

Most common synthetic methodologies of tetronamides have been developed using β-substituited tetronic acid derivatives. These mainly involve aza-Michael addition/elimination reactions, favored by the presence of good leaving groups in β-position, leading to 4-aminobutenolides. Recently our group has used a simple and effective synthesis for preparing α-halotetronamides (**16**) starting from α,β-dihalofuran-2(5*H*)-one (**15**) and anilines (**Scheme 2.3**), based on the work of Cunha and co-workers.[22,23] Although many of these procedures are very accessible,[5,14,17,22,24-26] this method has been limited due to the probability of secondary reactions; most common of these being ring opening resulting in the formation of pyrrole-2(5*H*)-ones.[24-27]




**Scheme 2.3:** Preparation of β-(*N*-aryl)-α-halotetronamides.

Other methods for the preparation of tetronamides include: electrocyclic ring opening reaction of 4-hydroxy-3-aminocyclobutenones (**Scheme 2.4**; **A**);[1] Pd-catalyzed oxidative carbonylation, conjugated addition and lactonization tandem reactions of 3-hydroxyalkines (**B**);[28] intermolecular Blaise reaction of *O*-(α-bromoacyl)-cyanohydrins (**C**),[3] among others.




**Scheme 2.4:** Synthesis of tetronamides using electrocyclic ring opening reaction (**A**);[1] Pd-catalyzed oxidative carbonylation (**B**)[28] and intermolecular Blaise reaction (**C**).[3]

### 2.1.2. Basidalin

Basidalin (**1a**) is considered the only naturally occurring tetronamide. Its structure (*Z*)-β-amino-γ-formylmethylidenebutenolide is relatively small but highly functionalized (**Fig. 2.3**). **1a** was first isolated in 1983 from the fungus *Leucoagaricus naucina*,[10] and later in 1994 from *Leucoagaricus cameifolia*.[29] Its activities include antibiotic and antitumor properties. The *E*-isomer (**1b**) was obtained as a minor component of the extraction, perhaps as an artifact, and likely why its natural origin is still questioned.[b,10] Moreover, latter has been already synthesized in some studies.[30]

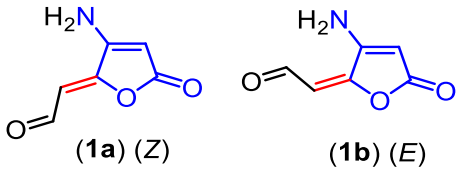
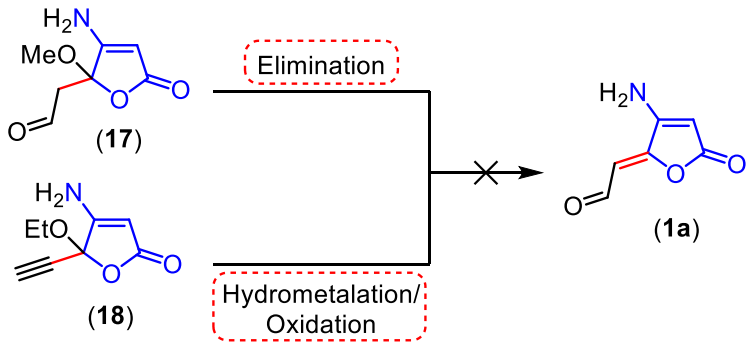



**Figure 2.3:** (*Z*)/(*E*) basidalin structures.

[b] Its origin may be due to photochemical or thermal isomerization.[10]

Basidalin showed antibacterial activity against *Aeromonas salmonicida*, *Vibrio anguillarum* (MIC 100 μg/mL),[10] *Bacillus brevis* (MIC 50 μg/mL), *Sarcina lutea*, *Streptomyces* sp. and *Botrytis cinerea* (MIC 100 μg/mL).[29] In addition to that, this tetronamide inhibits protein, RNA and DNA synthesis in L1210 cells lines (leukemia) ($IC_{50}$=0.4-0.6 μg $mL^{-1}$). The same study has shown that intraperitoneal administration of **1a** in L1210 inoculated in mice prolongs their longevity.[10] These characteristics have stimulated considerable interest in the synthesis of basidalin, as well as a variety of analogues.[19,31,32-35] In this manner, some derivatives have already been synthesized. However, the synthesis of basidalin itself has not yet shown positive results.[19,32,35]

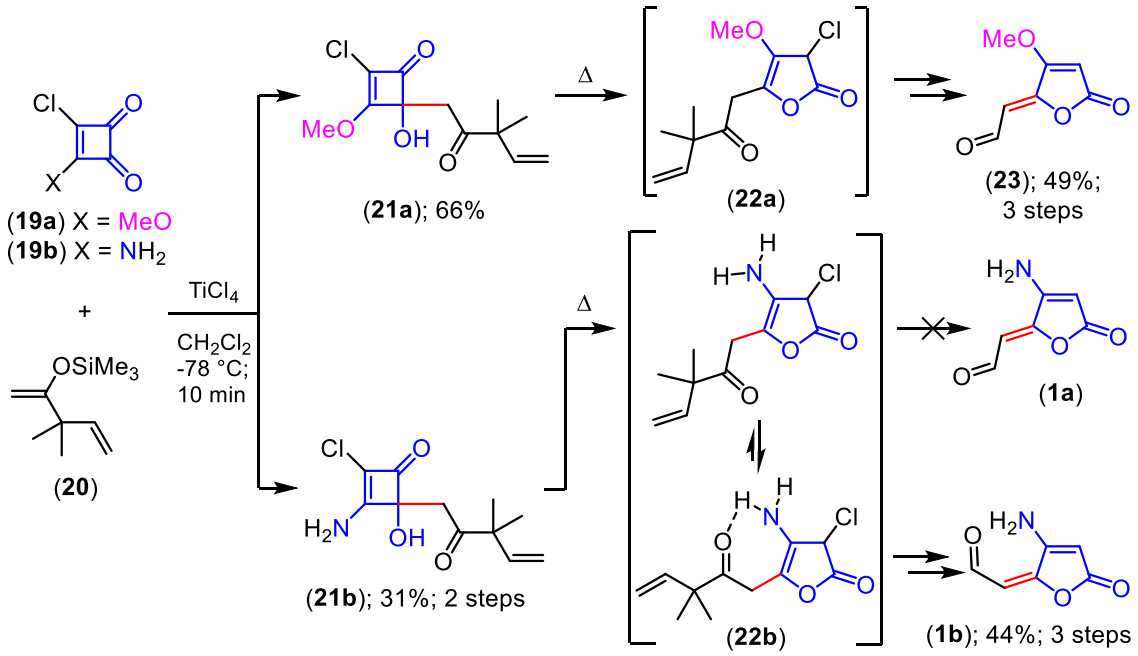
Previously, the synthetic methodology used by Hiyama and co-workers was discussed. Herein, 4-aminofuran-2(5*H*)-ones were produced using the intermolecular nitrile-enolester addition reaction (**Scheme 2.1**).[19] In this same work, basidalin synthesis was proposed. Consequently, the 4-aminobutenolide derivatives **17** and **18** were produced, as very promising basidalin precursors (**Scheme 2.5**). Nevertheless, their respective final steps aimed at the natural product were not achieved to satisfaction. In this instance, compound **17** was subjected to multiple attempts for MeOH elimination, but basic, acidic and neutral conditions, only managed to achieve starting material decomposition. In like manner, compound **18**, was submitted to unsuccessfully hydrometallation and oxidation reactions.




**Scheme 2.5:** Transformation attempts of intermediates **17** and **18** for the synthesis of basidalina (**1a**).[19]

In 1994, Yamamoto and co-workers. employing squaric acid derivatives (**19**) and silylenolates (**20**), found an unexpected thermal rearrangement that provided (*Z*)-β-methoxy-γ-formylmethylidenebutenolides (**23**) when the reaction was

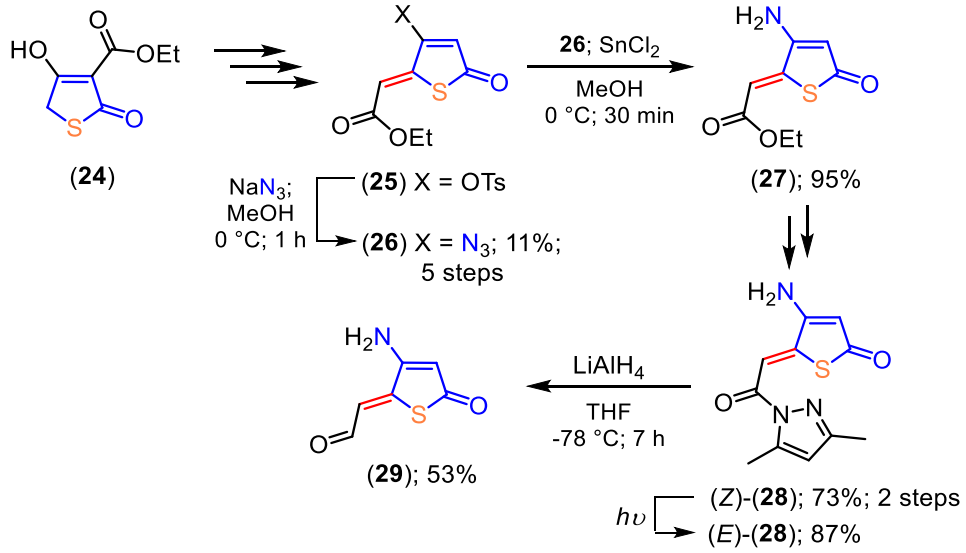
conducted with 3-methoxy-α,β-unsaturated butanone intermediates (**21a**). Nevertheless, attempts made with the respective amino derivatives (**19b**) to obtain basidalin (**1a**) were unsuccessful, obtaining the *E*-isomer (**1b**) instead. This unanticipated product was a result of a conformational change in intermediate **22b**, which led to a more stable conformer favored by an intramolecular hydrogen bond (**Scheme 2.6**).[30]




**Scheme 2.6:** Synthesis of γ-formylmethylidenebutenolides.[30]

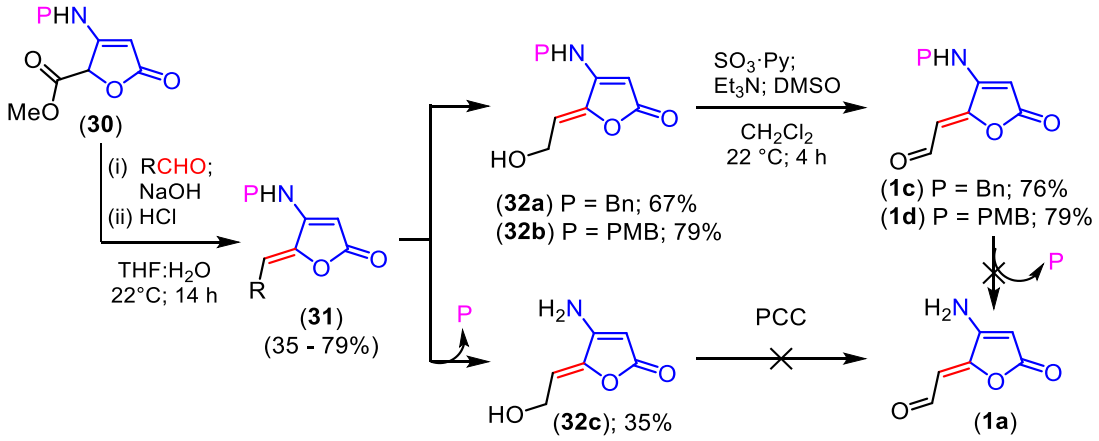
In 1998, Schachtner and co-workers. inspired by basidialin structure, succeeded in the synthesis of a sulphur derivative known as thiobasidalin (**29**). Through a nine-step reaction sequence from large amounts of a thiotetronic acid derivative (**24**) (**Scheme 2.7**). Although product **29** is not basidalin itself, its synthesis presented interesting methodology. Initially, compound **24** was transformed into a γ-alkylidenebutenolide product via alkylidenation reaction. Then, followed by an aza-Michael addition/elimination reaction resulting in the formation of a vinylazide (**26**). Accordingly, reaction of tosylated derivative (**25**) with sodium azide provided intermediate **26**, which was readily submited to reduction conditions and yielded the β-aminothiotetronate **27**. Finally, the (*Z*)-3,5-dimethylpyrazole derivative **28** appeared as the most favorable alternative for the reduction and formation of thiobasidalin (**29**). Additionally, an experiment was carried out with the latter precursor, the intermediate (*Z*)-**28**, showing a

photochemical isomerization for (*E*)-**28**, thus becoming in a clear alternative for the synthesis of (*E*)-thiobasidalin and derivatives.[32]




**Scheme 2.7:** Thiobasidalin synthesis.[32]

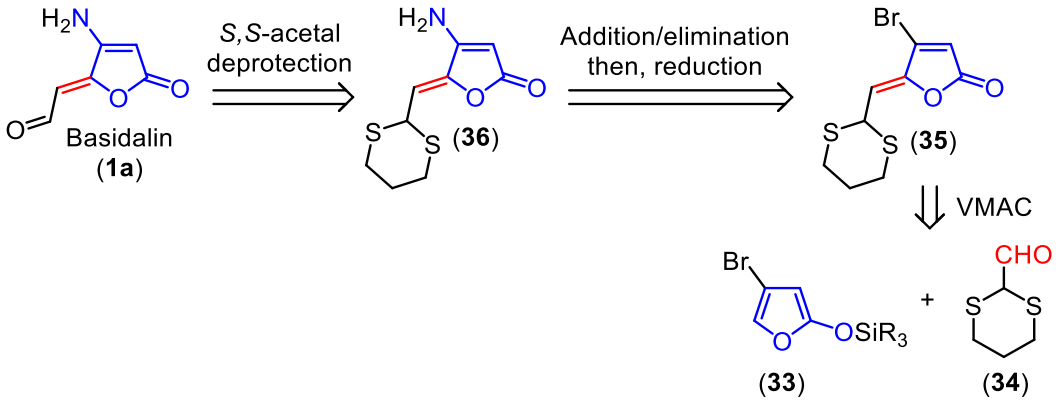
Recently, Ear and co-workers. attempted to synthesize basidalin (**1a**) using decarboxylated Knoevenagel-Doebner condensation reactions starting from *N*-protected γ-acyltetronamides (**30**). Thereby, some (*Z*)-γ-yliden-β-amino-2(5*H*)-furanones derivatives have been obtained with good results and under relatively mild and practical conditions. Unexpectedly, the developed deprotection and oxidation treatments of precursors **1c-d** and **23c**, respectively, did not conduce to the formation of basidalin (**1a**). These reactions have mainly resulted in very complicated decomposition products or mixtures (**Scheme 2.8**).[33]




**Scheme 2.8:** Attempts of basidalin synthesis using decarboxylative Knoevenagel-Doebner condensation.[33]

In the present work the first successful synthetic approach to basidalin (**1a**), using a new strategy for pivotal construction of (*Z*)-γ-alkylidenetetronamides will

be presented. Thereby, considering the afore mentioned difficulties for the synthesis of **1a** from suitable precursors **1c-d**, **17**, **18** and **21b**, and the exploration of methodologies for the construction of other butenolides such as rubrolides and derivatives, a new retrosynthetic analysis was proposed (**Scheme 2.9**). Therefore, an intermediate containing the thioacetal protecting group (**36**) will represent the final precursor of **1a**. this reactions, facilitated by the availability of highly-specific and mild conditions for the cleavage of the thioacetal group,[36,37] will be ideal in greatly simplifying the task of obtaining the aldehyde group in basidalin.




**Scheme 2.9:** Retrosynthetic analysis for Basidalin.

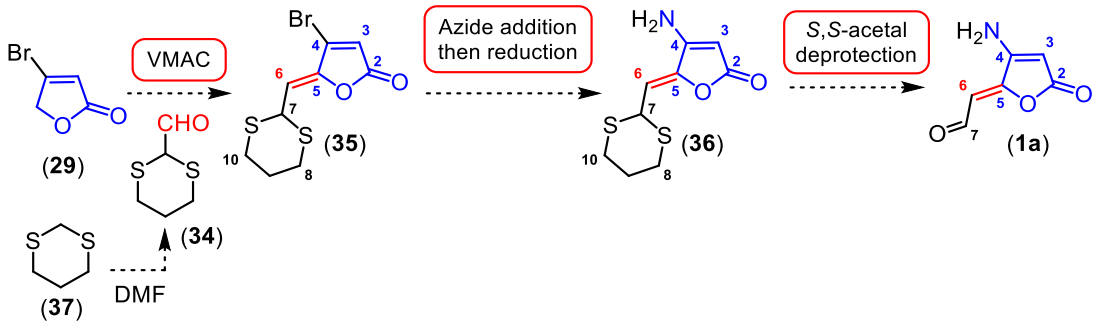
Proceeding, the formation of tetronamide **36** will be carried out from β-bromo-γ-alkylidenebutenolide **35**, using an aza-Michael addition/elimination reaction, to replace the halogen with an azide group, which will subsequently be reduced.[32] Compound **35** will result from a vinylogous Mukaiyama's aldol condensation (VMAC) from aldehyde **34** with sililoxyfurane **33**, as described in chapter 1 in the synthesis of rubrolides.[22,34-40] This reaction will allow a *Z*-isomer-specific production through stereocontrol directed by a bulky group such as Br in β-position in the furan-2(5*H*)-one motif. All the while, the bromine atom will favor the addition/elimination reaction owing to its leaving character.[29,41,44-46]

## 2.2. OBJECTIVES AND STRATEGIES

The aim of the present chapter is to develop the first synthesis of antibiotic and antitumor basidalin, starting from β-bromofuran-2(5*H*)-one and 1,3-dithiane-2-carbaldehyde. Hereby, the stereo-controlled vinylogous Mukaiyama´s aldol condensation reaction will be used as the crucial step to obtain the desired key *Z*-isomer intermediate. Afterwards, we will use a variation in the aza-Michael addition/elimination reaction, followed by reduction of the adduct formed, to obtain the characteristic β-amino group of the tetronamides. Finally, by deprotection of the thioacetal group, the final structure of basidalin will be obtained.

## 2.3. RESULTS AND DISCUSSIONS.

Basidalin synthesis begins with the production of the key γ-alkylidenebutenolide precursor **35**, which could be accomplish following the VMAC procedure showed in **Scheme 1.13**. Subsequently reactions aimed at the addition of β-amino group will occur and finally, a *S*,*S*-acetal deprotection will produce the characteristic aldehyde in the basidalin structure.



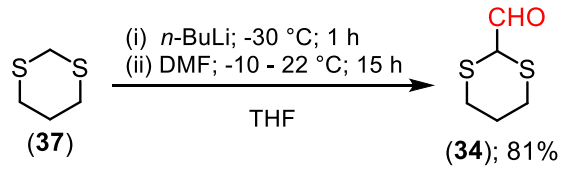

**Scheme 2.10:** Synthetic procedure for basidalin (**1a**).

### 2.3.1. Synthesis of γ-alkylidenebutenolide intermediate 35.

*2.3.1.1. Vinylogous Mukaiyama´s aldol condensation procedure.*

The starting materials for the aldol condensation reaction were initially prepared from respective commercially-available precursors. Firstly, aldehyde **34**

was synthetized using a methodology with 1,3-dithiane and DMF (**Scheme 2.11**).[41]




(**37**) (**34**); 81%

**Scheme 2.11:** Reaction conditions for the synthesis of 1,3-dithiane-2-carbaldehyde (**34**).[41]

The starting aldehyde preparation consists mainly of the removal of the slightly acidic H-2 hydrogen from the 1,3-dithiane using a strong base such as *n*-BuLi. Next, a carbanion is formed whose stability is increased by the presence of two adjacent sulfur atoms. Thereby the carbanion attacks the DMF to form compound **34**, as an off-white oil. The oil was separated by vacuum distillation and stored at -20 °C to prevent its dimerization.[41] The separated aldehyde was identified with 2,4-dinitrophenylhydrazine on TLC plate, in addition to its IR, $^{1}$H NMR, $^{13}$C NMR and HRMS spectra. These data coincided with those reported in the literature.[41]

The IR spectrum of compound **34** essentially shows the aldehyde bands at 2827 and 2703 $cm^{-1}$ as well as the carbonyl band at 1715 $cm^{-1}$.

The $^{1}$H NMR spectrum showed the aldehyde hydrogen signal as a singlet at 9.52 ppm. Furthermore, is observed hydrogen H-2 at 4.10 ppm. Lastly, the signals of the remaining hydrogen pairs of the ring appear as multiplets in the region between 3.00-2.10 ppm, corresponding to H-3 and H-5, by their proximity to the most electronegative S atom (**Fig. 2.4**).

The $^{13}$C NMR spectrum presented the characteristic carbonyl signal for aldehyde at 188.1 ppm. In the high field region, the signals of C-2 located between the sulphur atoms at 47.6 ppm. Lastly, the aliphatic carbons were detected at 25.4 ppm (2C; C-5 e C-3) and 24.8 ppm (C-4) (**Fig. 2.5**).

The VMAC one-pot protocol was then performed, as discussed in chapter 1. In this case, β-bromofuran-2(5*H*)-one (**38**) was reacted with 1,3-dithiane-2-carboxaldehyde **34** using TBDMSOTf as a silylating agent as well as DIPEA and DBU as bases.[42] However, the reaction resulted in a very complex mixture without providing the desired product. Similar results with other aliphatic aldehydes have been already reported.[37] Therefore, as an alternative, we

decided to perform the reaction in three different steps: first, formation of the silyloxyfuran, followed by condensation, then elimination, according to prior research of Boukouvalas' group.[37]

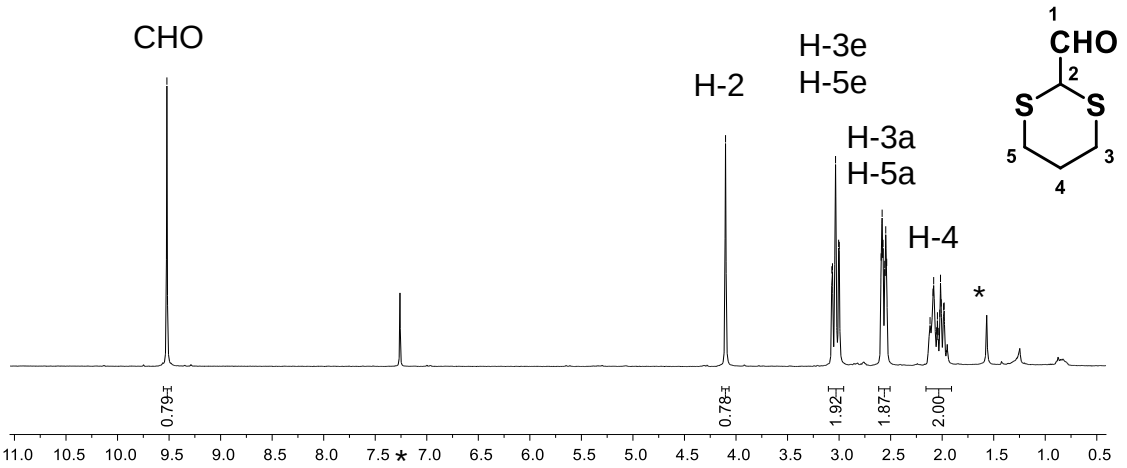



**Figure 2.4:** $^{1}H$ NMR ($CDCl_3$, 400 MHz) of aldehyde **34**. *Solvent residues and water signals.

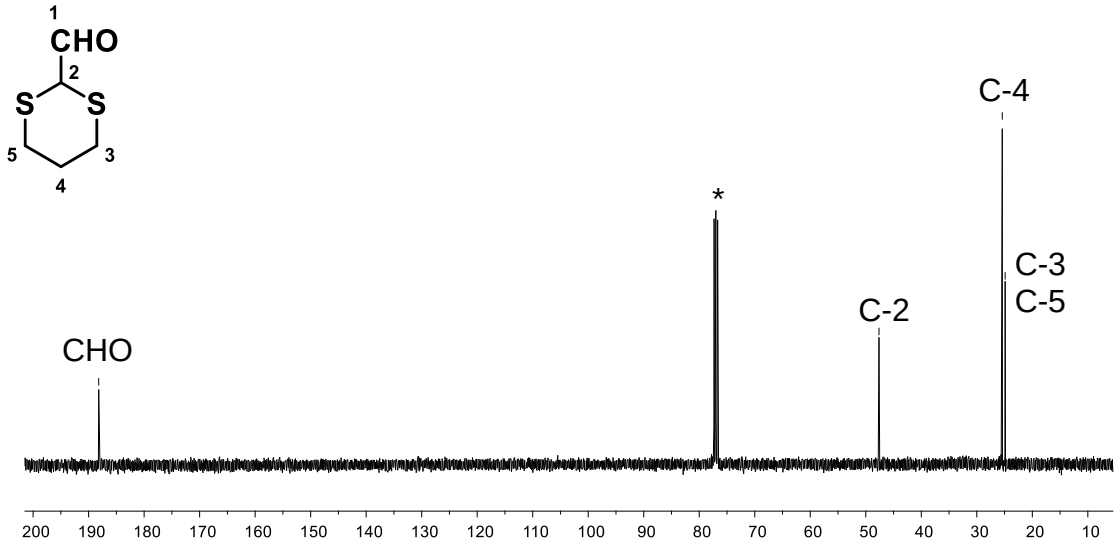
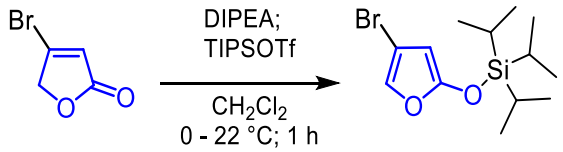



**Figure 2.5:** $^{13}C$ NMR ($CDCl_3$, 100 MHz) of aldehyde **34**. *Solvent residues signals.

Initially butenolide **38** was silylated with TBDMSOTf and DIPEA but in this case, the product seemed unstable. Nonetheless, simple modification with a more robust silylated reagent such as triisopropilsilyloxy triflate (TIPSOTf) yielded the stable silyloxyfuran derivative **33** (**Scheme 2.12**). The obtained silyloxyfuran was separated using of a neutral alumina column, resulting in a colorless oil, which was maintained at -20 °C to avoid degradation. Identification of the compound was performed through spectroscopic analyzes.




(**38**) (**33**); 92%

**Scheme 2.12:** Synthesis conditions for silyloxyfuran (**33**).

The IR spectrum of silyloxyfuran **33** showed the characteristic furan ring absorptions at 1611, 1464 and 1356 $cm^{-1}$, as well as the bands for the Si-O-C asymmetric stretch at 1099 $cm^{-1}$ and angular deformation $\delta_{C\text{-}Si}$ at 1269 and balance-like angular deformation $\rho_{C\text{-}Si}$ at 823 $cm^{-1}$.

The $^{1}H$ NMR spectrum of compound **33** showed the characteristic signals of alkylsilyl groups, revealing a doublet at $\delta$ = 1.09, corresponding to the methyl groups of the isopropyl. It is also possible to observe a septet at $\delta$ = 1.25, corresponding to the methylenes attached to the Si. Finally, the doublets corresponding to the hydrogen H-3 at $\delta$ = 5.24 and H-5 at $\delta$ = 6.83 ($J$ = 1.2 Hz) (**Fig. 2.6**).

$^{13}C$ NMR spectrum, showed signals for the carbons near the silicon, in the range of 10-20 ppm. Additionally, another signal corresponding to C-2 ($\delta$ = 156.6) that is a part of the enolsilyl ether system formed after the reaction with the protecting group. Next, the unsaturated C-5 carbon at $\delta$ = 130.0, bound directly to oxygen in the ring, C-3 at $\delta$ = 100.0 and finally C-4 at $\delta$ = 86.7, bound to Br, were also revealed (**Fig. 2.7**).

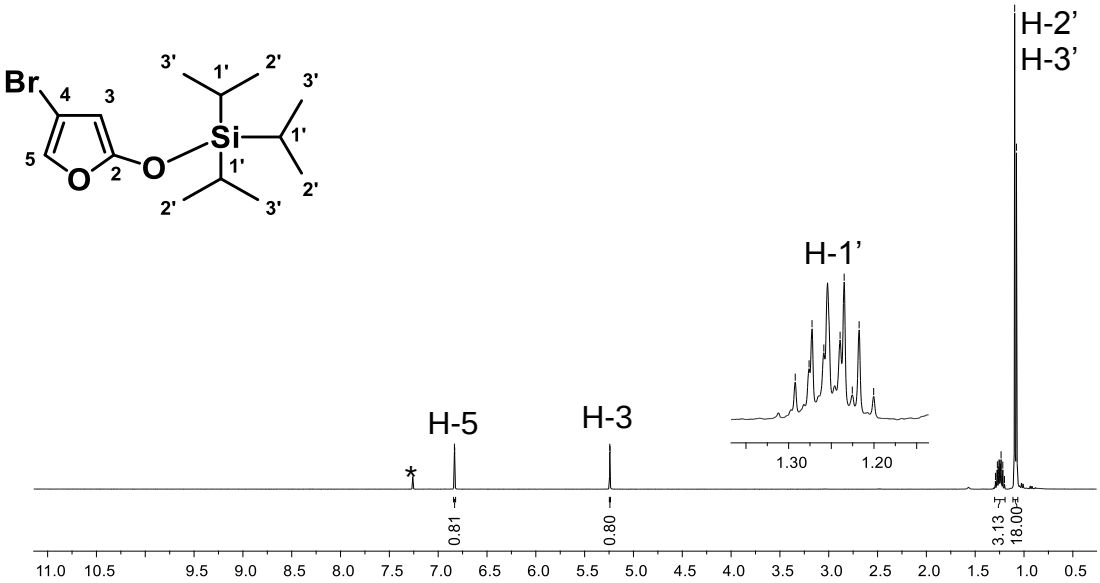



**Figure 2.6:** $^{1}H$ NMR ($CDCl_3$, 400 MHz) of silyloxyfuran **33**. *Solvent residues signals.

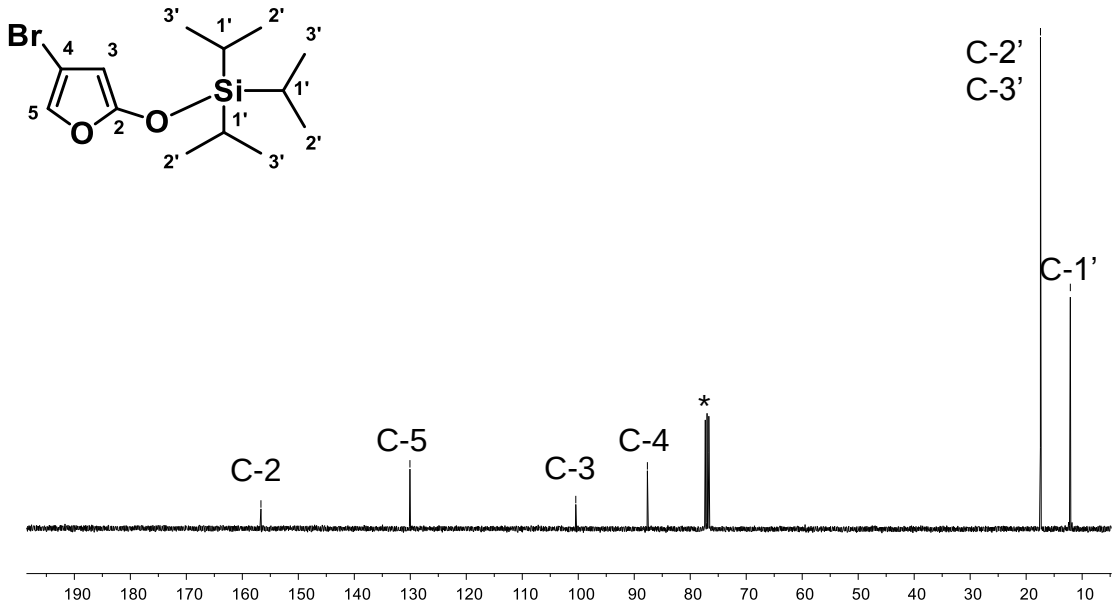
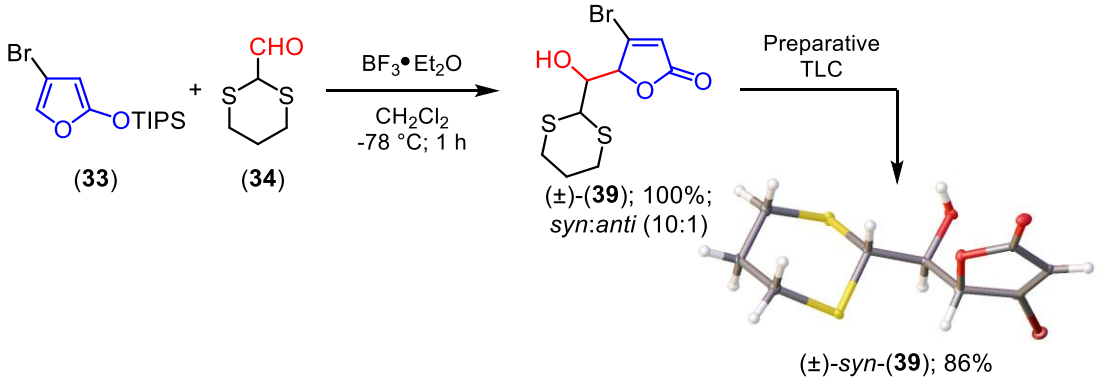



**Figure 2.7:** $^{13}C$ NMR ($CDCl_3$, 100 MHz) of silyloxyfuran **33**. *Solvent residues signals.

The aldol condensation reaction was developed using intermediates **33** and **34**. Thus, they were reacted with $BF_3$•$Et_2O$, generating the mixture of alcohols (±)-**39**, in essentially quantitative yield (**Scheme 2.13**). The products formed are apparently racemic isomers with a *syn*:*anti* ratio of 10:1 according to $^1H$ NMR analysis (see amplification of the region at 3.50-6.00 ppm in **Fig. 2.8**).




**Scheme 2.13:** Reaction conditions for the synthesis of alcohol **39** using $BF_3$-mediated aldol condensation.

Previously, it was discussed how the condensation reactions in furanones produce the *syn* isomer as main component favored by stereo-electronic factors.[43,44] In this case, we have a similar situation. Proceeding to confirm this fact, the isomer mixture was separated by preparative TLC using DCM as the mobile phase. Moreover, the structure of the fully-purified (±)-*syn* isomer was

determined by X-ray (See **Fig. 2.12**, and annexes **Fig. A 2.2**), in like manner, some other analyses were performed, such as IR, $^{1}$H NMR, $^{13}$C NMR and HRMS.

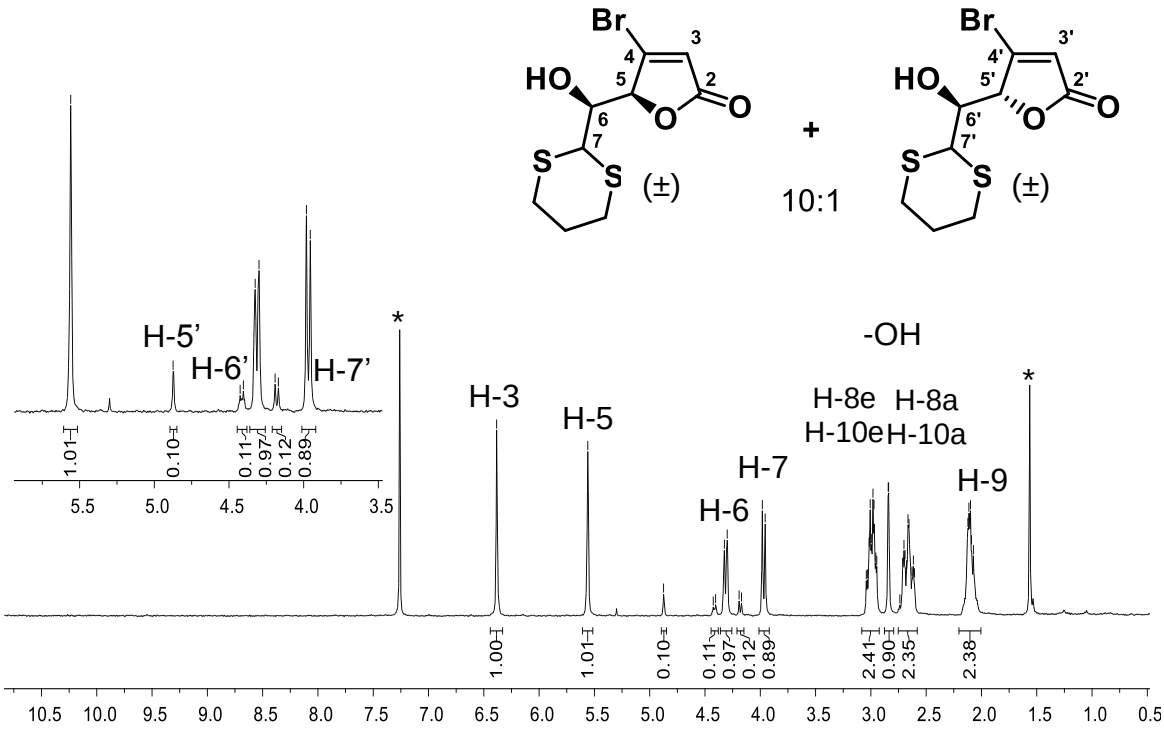



**Figure 2.8:** $^{1}$H NMR ($CDCl_3$, 400 MHz) of isomers (±)-***syn*-39** e (±)-***anti*-39** (10:1). *Solvent residues and water signals.

The $^{1}$H NMR spectrum initially shows the characteristic thioacetal ring signals as multiplets at $\delta$ = 2.05-3.05. Nearby, the -OH group signal can be observed at 2.86 ppm. The signal corresponding to H-7 is seen at 3.97 ppm, and H-6 at 4.31 ppm, these two hydrogens couple with $J$ = 10.0 Hz. The signal of H-5 at $\delta$ = 5.56 ppm can be seen as a singlet, and finally the signal of H-3 corresponds $\delta$ = 6.38 (**Fig. 2.8**; for pure *syn* isomer see annex, **Fig. A 2.3**).

COSY spectrum allow us to identify some hydrogen signals, which shows the coupling between H-5 and H-6, as well as coupling of H-5 with the hydrogen of OH and with H-3. Similarly, the coupling signals of H-6 with its respective OH, H-5 and H-7 neighbors may also noted (**Fig. 2.9**).

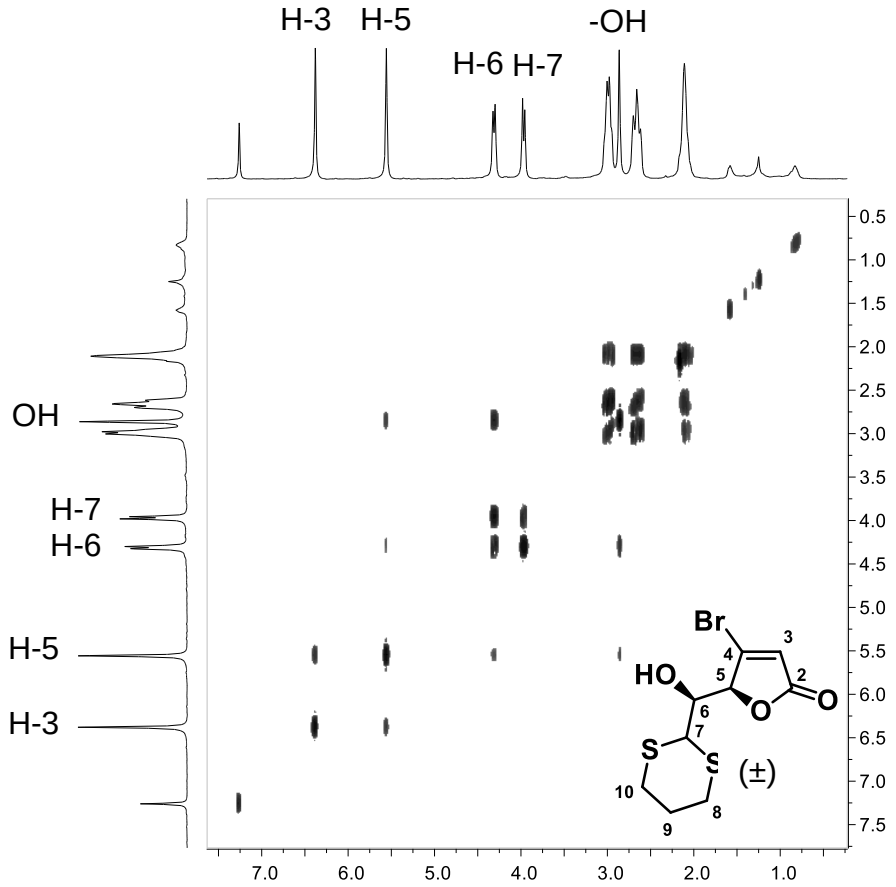



**Figure 2.9:** COSY Spectrum ($CDCl_3$, 400 MHz) of alcohol ***syn*-39**.

The $^{13}C$ NMR spectrum showed the characteristic signals of the thioacetal ($\delta$ = 24-26). Subsequently, the oxygen-bonded carbons, C-5 ($\delta$ = 83.6) and C-6 ($\delta$ = 67.6) were observed. The unsaturated carbons C-4 ($\delta$ = 148.2) located at β-position and α-carbon C-3 ($\delta$ = 122.93) are also present in the spectrum as is the carbonyl C-2 at $\delta$ = 170.1 ppm (**Fig. 2.10**).

*2.3.1.2. Elimination reaction of aldolic product **39**.*

Concerning elimination mechanism of *in situ* vinylogous Mukaiyama's aldol condensation, it was previously described that the δ-silyloxy-γ-alkylbutenolide intermediate is selectively conducted to the respective *Z*-alkene by an E1cb mechanism favored by the spatial hindrance of the substituent group at position β (in this case bromine).[38-40] Therefore, the same mechanism may occur for elimination reaction of alcohol **39**. Consequently, it was considered that both *syn* and *anti*-**39** isomers are capable of undergoing selective *Z*-elimination. Whereby,

the obtained mixture was used without purification. This situation was confirmed by a model reaction consisting of the condensation of silyloxyfurane **33** with isobutyraldehyde (**40**), the product of which was subjected to the elimination reaction (**Scheme 2.14**).

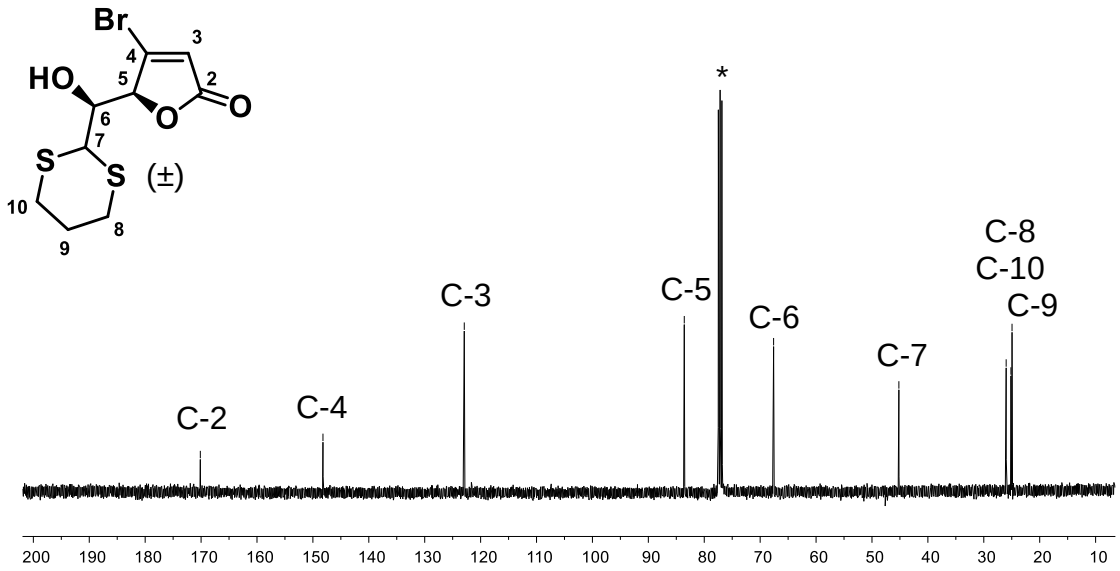
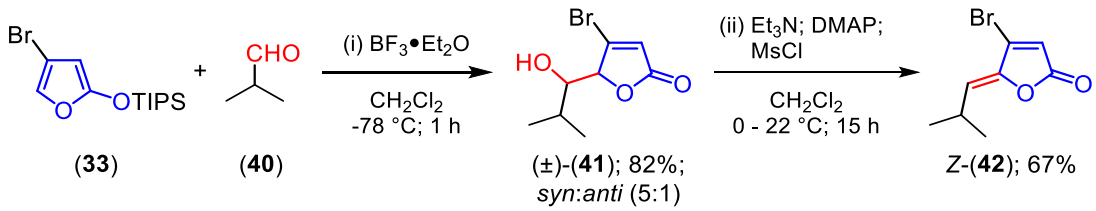
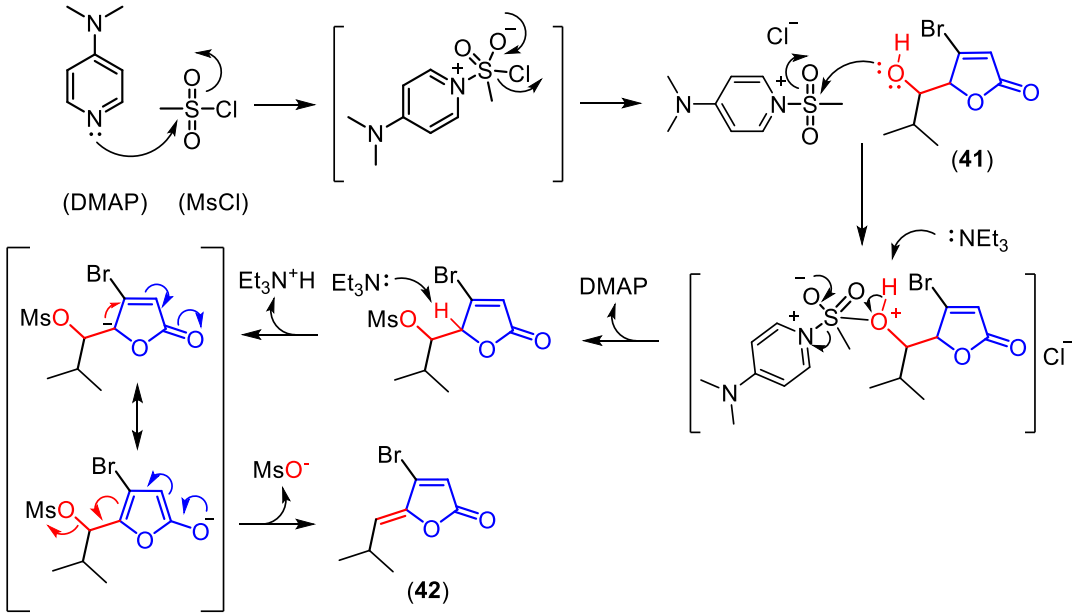



**Figure 2.10:** $^{13}C$ NMR ($CDCl_3$, 100 MHz) of alcohol (±)-***syn*-39**. *Solvent residues signals.




**Scheme 2.14:** Model reaction conditions for the ***Z*-42** synthesis.

In this manner, the mixture of (±)-***syn*-41** and (±)-***anti*-41** intermediate alcohols (5:1) was obtained. The *syn* isomer was readily separated by column chromatography for spectroscopic analysis. The isomer mixture was submitted to elimination reaction, using a methodology based on the works of Boukouvalas and co-workers,[37] where an organic base such as triethylamine (TEA), a 4-(*N*,*N*-dimethylamino)pyridine (DMAP) as the catalyst and MsCl substitution group were used to acquire a better leaving group (**Scheme 2.15**). The reaction provided ***Z*-42** as the single isomer in 67% yield. These compounds were identified by $^1H$ NMR (see annexes; **Fig. A 2.4-5**), whose signals coincide perfectly with those reported in the literature.[37,45]

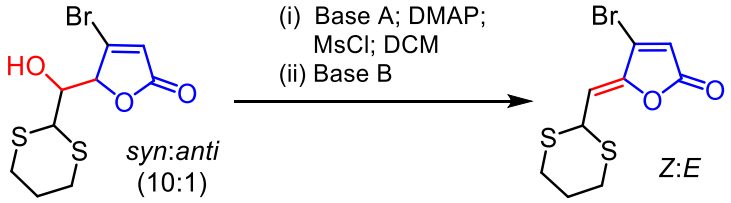



**Scheme 2.15:** Elimination reaction mechanism of **41** using DMAP and MsCl.

Thereafter, the alcohol mixture **39** was subjected to the elimination reaction, using the same methodology. Although reactions with aldehydes containing only a single carbon chain proved to be satisfactory, initial attempts with the thioacetal group-containing molecule resulted in degradation of the starting material and by-products (based on TLC and $^{1}$H NMR analysis of crude reaction). However, it was decided to improve the reaction conditions, varying the type and quantity of the base, additives, concentration, time and temperature (**Table 2.1**).

Initially, treatment of the mixture of alcohols **39** with TEA, MsCl and DMAP under the conditions shown did not provide any reaction. Instead several spots on TLC plate, suggesting degradation of the materials, could be seen (**Table 2.1**, **entry 1**). Likewise, changes in reagent quantity and reaction time did not confer better results (**Entries 2-3**). Shorter reaction times and temperatures ultimately exhibited elimination product but presented low yields, conversions and a *Z*:*E* ratio of 10:1 (determined by $^{1}$H NMR of the crude reaction extract; see annexes; **Fig. A 2.6**) (**Entries 4-5**).

**Table 2.1:** Optimization of the elimination reaction for the synthesis of the compound **35**




(**39**) (**35**)

| Entry | Base A (eq) | Base B (eq)[a] | Eq DMAP | Eq MsCl | t (h) | T (°C) | *Z*:*E* Ratio[b] | Yield (%) | Conv. (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | TEA (3) | - | 1 | 1.6 | 15 | 0 – 22 | - | NR[c] | - |
| 2 | TEA (3) | - | 2 | 2 | 15 | 0 – 22 | - | NR | - |
| 3 | TEA (3) | - | 2 | 2 | 8 | 0 – 22 | - | NR | - |
| 4 | TEA (3) | - | 2 | 2 | 5 | 0 – 22 | (10:1) | 19 | 50 |
| 5 | TEA (3) | - | 2 | 2 | 5 | -10 | (10:1) | 25 | 60 |
| 6 | TEA (2) | TEA (4) | 2 | 2 | 5 | -10 | - | NR | - |
| 7 | TEA (2) | TEA (2) | 2 | 2 | 5 | -10 | (10:1) | 21 | 70 |
| 8 | TEA (3) | *t*-BuOK (3) | 2 | 2 | 5 | -10 | (10:1) | 24 | 74 |
| 9 | *t*-BuOK (3) | - | 2 | 2 | 5 | -10 | - | NR | - |
| 10 | DBU (3) | - | 2 | 2 | 5 | -10 | - | NR | - |
| 11 | DIPEA (2) | DIPEA (2) | 2 | 2 | 5 | -10 | (10:1) | 56 | 100 |
| 12 | DIPEA (2) | DIPEA (2) | 2 | 2 | 8 | -10 | (10:1) | 38 | 100 |
| 13 | DIPEA (2) | DIPEA (2) | 1 | 2 | 8 | -10 | (20:1) | 33 | 100 |
| 14 | DIPEA (2) | DIPEA (2) | 0,6 | 2 | 4 | -10 | (20:1) | 39 | 100 |
| 15 | DIPEA (1) | DIPEA (3) | 0,6 | 1 | 4 | -10 | (20:1) | 37 | 100 |
| 16 | DIPEA (1) | DIPEA (5) | 0,6 | 1 | 4 | -10 | (20:1) | 35 | 100 |
| 17 | Py (4) | - | 0.0 | 1.8 | 6 | -10 – 22 | (100:0) | 57 | 78 |
| 18 | Py (2) | Py (2) | 0.0 | 1.8 | 15 | -10 – 22 | (100:0) | 81 | 100 |

[a] The addition of a second portion of base was carried out according to TLC, where after 2-4 h the consumption of starting material was not observed. [b] The ratio of the *Z*:*E* isomers was determined by the respective $^{1}$H NMR integration [c] NR = no reaction; after t = 24 h no elimination product was detected, the starting material was degraded.

Addition of an excess of base (6 eq) did not show reaction (**Entry 6**). In an attempt to improve the conversion of the starting material, the base was added in two portions, since monitoring by TLC showed that the starting material was

consumed efficiently with this sequential addition. However, the conversion was slightly improved with the addition of two portions of 2 eq of base (**Entry 7**). In other experiments *tert*-BuOK and DBU bases were also used, but still with unsatisfactory results (**Entries 8-10**). As may be noted, the use of relatively strong bases such as TEA ($pKa_H$ = 10.75), *tert*-BuOK ($pKa_H$ = 17.00)[46] or DBU ($pKa_H$ = 12.00)[46] did not provide good results.

Afterwards, a treatment with DIPEA ($pKa_H$ = 10.75)[46] was performed, giving up to 56% of the required product and a total conversion of the starting material (**Entries 11-12**). Subsequently, the amount of DMAP ($pKa_H$ = 9.2)[46] (**Entries 13-16**) was decreased, resulting in a better selectivity with the ratio of *Z*:*E* isomers raised to 20:1. Lastly, the use of pyridine (Py; $pKa_H$ = 5.21)[46] (**Entries 17-18**) resulted in an increment of the yield and selectivity, leading to production of only the *Z* isomer in 82% yield (**Entry 18**).

The results shown that the use of weaker bases improves the elimination reaction of the alcohol mixture **39**. On the other hand, when a stronger base is used undesired reactions occur. This can be expected since the acidity of allylic H, present in the unsaturated product **35** is quite high, due to the electronic attraction of the γ-alkylidenebutenolide backbone conjugated system and its location between the two sulfur atoms. Both factors stabilize the formed carbanion and improves the reactivity of the respective conjugate base (**Fig. 2.11**). This circumstance can be confirmed since some degradation products were observed by the TLC. Some studies on secondary reactions due to the thioacetal group have been already reported.[42,43] In addition, the electron withdrawing effect of the γ-alkylidenfuran-2(5*H*)-one system was discussed in Chapter 1.

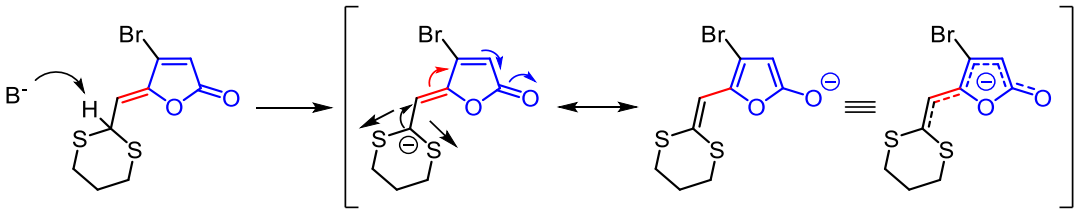



**Figure 2.11:** Stabilization of the anion formed by the removal of acidic H from the product **35**.

Confirmation of the structure of **35** was done through spectroscopic analysis of IR, NMR and HRMS (**Fig. 2.12-13**). The $^1$H NMR spectrum showed the signals of the thioacetal group between $\delta$ = 2.20-1.85. In $\delta$ = 2.90-2.96 a multiplet corresponding to H-8 and H-10 is observed. Then there are two doublets, the first

at $\delta$ = 5.19 of the H-7 located between the sulfur, and the second at $\delta$ = 5.68 characteristic of the double bond, both couple with *J* = 11.0 Hz. A simplet associated to of H-3 is located at $\delta$ = 6.45 (**Fig. 2.12**).

Likewise, the $^{13}$C NMR spectrum shows the carbons belonging to the thioacetal ring in the region of $\delta$ = 24-30. The C-7 carbon located between the sulfur at $\delta$ = 40.2, the signal of C-3 is observed at $\delta$ = 110.7, followed by C-6 at $\delta$ = 121.6. C-4 bound to bromine in β-position to carbonyl may be noticed at $\delta$ = 137.3 and finally, the signals for C-5 and the C-2 carbonyl appear at $\delta$ = 147.9 and 165.8, respectively (**Fig. 2.13**).

Attempts to separate the pure ***E-35*** isomer were unsatisfactory, due to its instability during Si-gel chromatographic separation. Thus, attempts to obtain a clean NMR were unsuccessful. Nevertheless some $^{1}$H NMR signals can be observed and attributed to ***E*-35**. The signals of the hydrogens H-6 and H-7 were observed at $\delta$ = 6.07 and $\delta$ = 5.72 (*J* = 12.0 Hz), respectively. These signals are clearly more shifted to greater $\delta$ when compared to its *Z*-isomer ($\delta$ = 5.68 and $\delta$ = 5.19 for the ***Z*-35**) (**Fig. 2.14**). Furthermore, the isomers had the same exact mass.

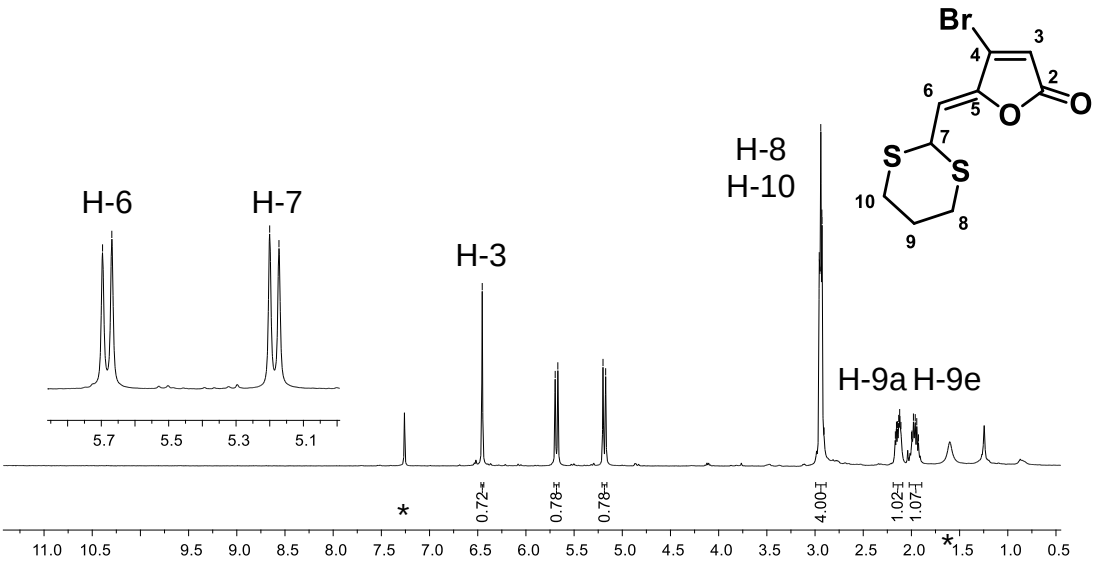



**Figure 2.12:** $^{1}$H NMR ($CDCl_3$, 400 MHz) of compound ***Z*-35**. *Solvent residues and water signals.

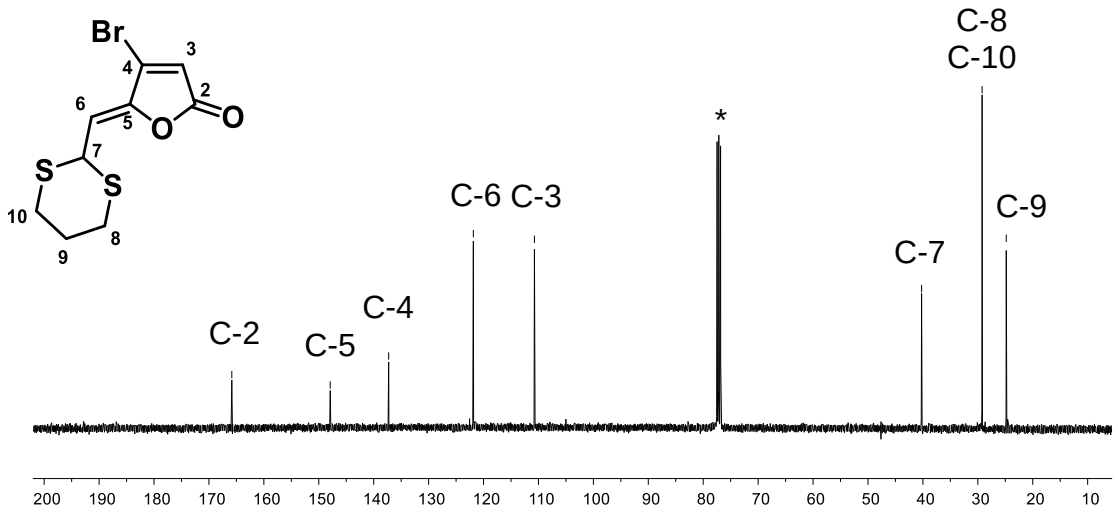



**Figure 2.13:** $^{13}C$ NMR ($CDCl_3$, 100 MHz) of compound ***Z*-35**. *Solvent residues signals.

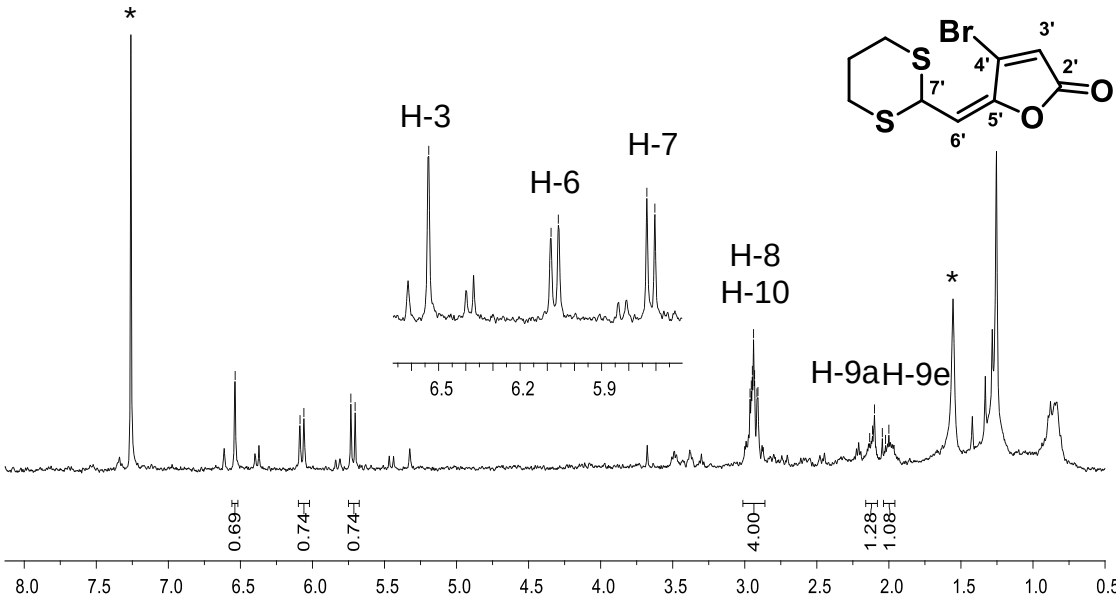
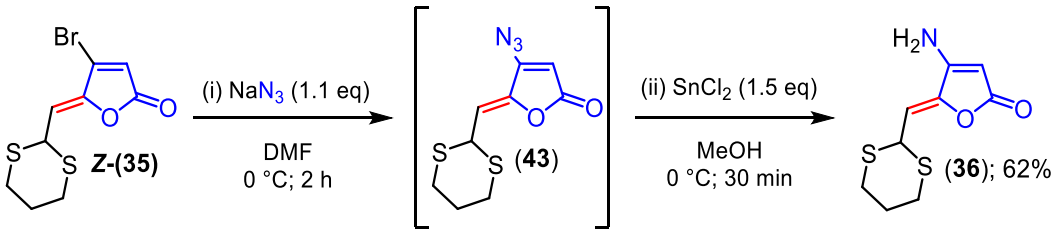



**Figure 2.14:** $^{1}H$ NMR ($CDCl_3$, 400 MHz) of compound ***E*-35**. *Solvent residues and water signals.

### 2.3.2. Final steps for the synthesis of basidalin.

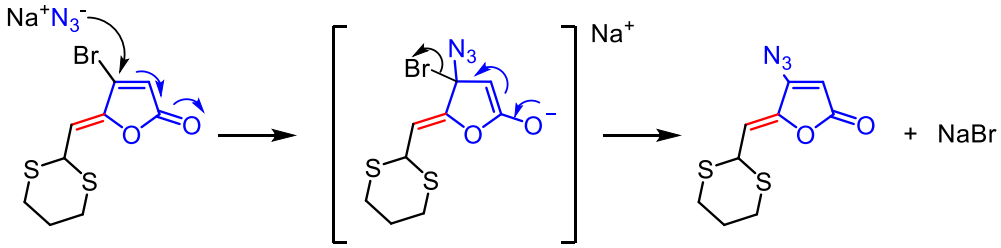
With the acquisition of elimination product ***Z*-35**, we proceed with the aza-Michael addition/elimination reaction to introduce the amino group into the β-position. The procedure was done in two steps via a vinylazide intermediate.[47,48] Thus, exposure of the ***Z*-35** with sodium azide in DMF at 0 °C easily led to the addition/elimination β-azide product,[47] (**Scheme 2.16**; (i)). Azide **43** proved to be unstable at room temperature and therefore it was immediately reduced with

$SnCl_2$/MeOH at 0 °C[49] to afford tetronamide **36** (**Scheme 2.16**; (ii)) in 62% yield in two steps.




**Scheme 2.16:** Reaction conditions for the synthesis of tetronamide **36**.

The mechanism of the addition/elimination reaction consists of a nucleophilic attack on the β-carbon of an α,β-unsaturated carbonyl compound, showing an intermediate state stabilized by resonance. Finally, the recovery of the conjugated system was facilitated by the elimination of the leaving group $Br^-$ (**Scheme 2.17**).




**Scheme 2.17:** Michael's Addition/Elimination Reaction Mechanism.

Structures of compounds **43** and **36** were confirmed by their respective IR, $^1$H NMR, $^{13}$C NMR and HRMS spectra (see annexes; **Fig. A 2.8-11**).

The IR spectrum of compound **43** shows the characteristic bands of the azide substituent at 2150 and 2126 $cm^{-1}$, this band is absent in the IR spectrum in tetronamide **36**. Furthermore, in compound **36** the band of the amino group is observed at 3197 $cm^{-1}$. It is also important to mention that there is a difference in the characteristic band of carbonyl. In the case of azide **43** the band is at 1768 $cm^{-1}$ and for the amine **36** it is at 1716 $cm^{-1}$. This smaller value is due to the electron donor effect of the amino group.

The transformation of the group was also confirmed by $^1$H NMR. In this spectrum, one may observe a clear variation of the H-3 hydrogen signal located on the α-carbonyl. Meanwhile, in the azide **43** this is at $\delta$ = 5.85, while in the case of amine **36**, it is located at $\delta$ = 4.88, due to its electron donor effect. In the latter,

the signal of the hydrogens of the amino group can be observed as a broad signal at $\delta$ = 6.67 (**Fig. 2.15**).

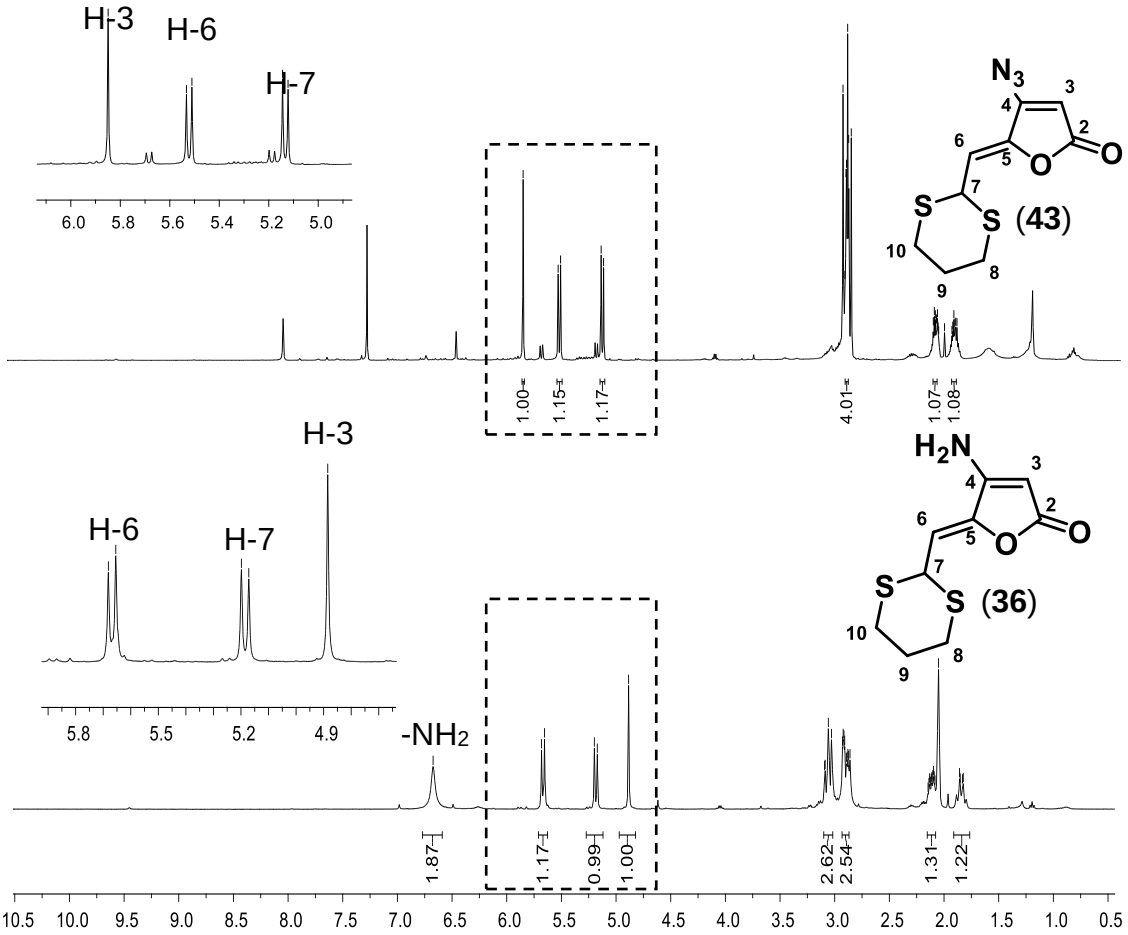
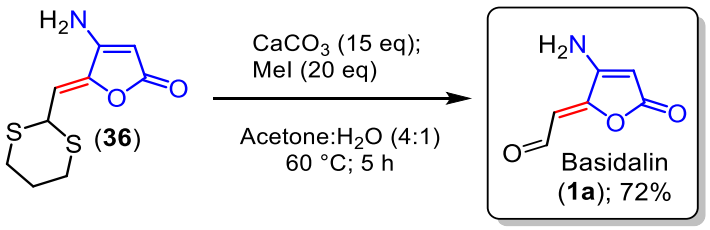



**Figure 2.15:** Comparison of the $^{1}$H NMR Spectrum of azide **43** ($CDCl_3$, 400 MHz) and tetronamide **36** ($(CD_3)_2CO$, 400 MHz).

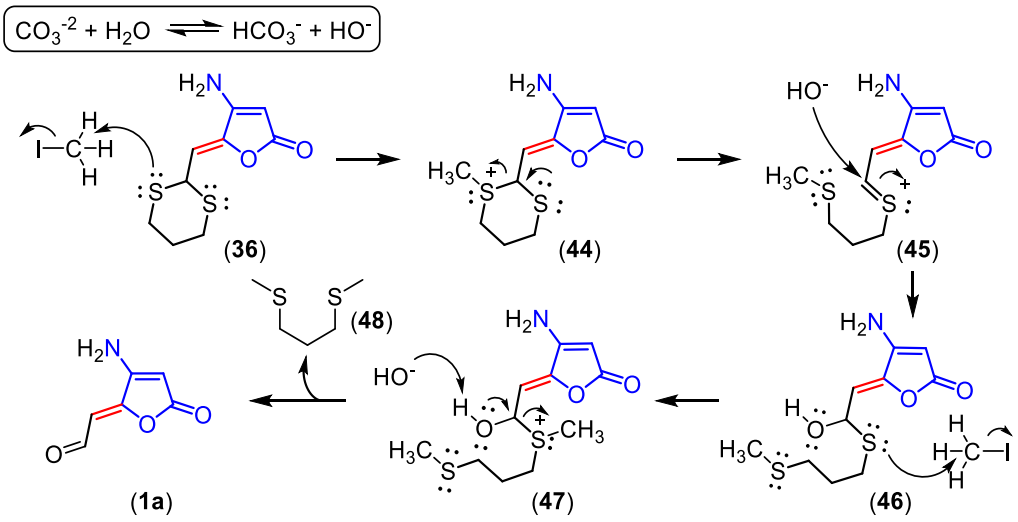
With successful acquisition of tetronamide **36**, the hydrolysis of the dithiane group under mild conditions, using Mel/$CaCO_3$ as proposed by Fetizon and co-workers,[50,51] was performed (**Scheme 2.18**). Thus, basidalin was satisfactorily obtained in 72% yield (**1a**, overall yield 39%).




**Scheme 2.18:** Reaction conditions for elimination of thioacetal for the formation of basidalin (**1a**).

The mechanism of alkylative deprotection of thioacetal initially consists in the methylation of one of the sulfur atoms by the use of MeI through an $A_ND_N$ mechanis[c,52] forming the positive charged species **44**. Subsequently, the free electron pair of the non-methylated sulfur migrates, forming the open chain intermediate **45** obtaining a C=S electrophilic center. This step is driven by the recovery of the free electron pair of the previously methylated positive sulfur atom. The C=S group in **45** is then attacked by an *in situ* formed hydroxyl anion (produced by the reaction of the water with $CaCO_3$) leading to adduct **46**. Subsequently, the second sulfur at **46** is also methylated, yielding intermediate **47**. Thus, free sulfur electrons are recovered by the migration of the electronic oxygen pair, which permits the formation of the final aldehyde in basidalin (**1a**) and the release of the dithiane by-product **48** (**Scheme 2.19**).

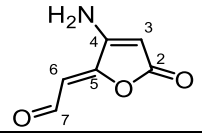
The obtained natural product was analyzed by spectroscopic techniques IR, NMR and HRMS. The basidalin signals in the $^1H$ NMR and $^{13}C$ NMR spectrum perfectly matched those reported for the previously isolated compound (**Table 2.2**; see annexes **Fig. A 2.12-13**).[10]




**Scheme 2.19:** Mechanism of reaction for the alkylative deprotection of thioacetal for the formation of basidalin (**1a**).

[c] $A_ND_N$ is the recommended symbolism for the synchronized reaction that involves **a**ttachment to the **n**ucleophile and **d**etachment with the **n**ucleofuge, also symbolized as $S_N2$.[52]

**Table 2.2:** Comparative $^{1}H$ and $^{13}C$ NMR data between baseline synthesized and isolated

|  | | Synthetized product | Natural Product[10] |
|---|---|---|---|
| | | 400 MHz | 100 MHz |
| $^{1}H$ NMR ($(CD_3)_2SO$) | CHO | 10.01 (1H, d, *J* = 7.8 Hz) | 10.03 (1H, d, *J* = 8 Hz) |
| | $NH_2$ | 7.73 (2H, brd) | 7.68 (2H, brd) |
| | H-6 | 6.11 (1H, d, *J* = 7.8 Hz) | 6.12 (1H d, *J* = 8 Hz) |
| | H-3 | 4.96 (1H, s) | 4.97 (1H, s) |
| | | 100 MHz | 25.2 MHz |
| $^{13}C$ NMR ($(CD_3)_2SO$) | CHO | 189.3 | 189.2 |
| | C-2 | 168.2 | 168.2 |
| | C-4 | 159.5 | 159.5 |
| | C-5 | 158.5 | 158.6 |
| | C-6 | 102.9 | 102.8 |
| | C-3 | 81.8 | 81.9 |

In order to confirm the stereochemistry of the γ-alkylidene double bound, a NOESY-1D experiment was carried out (**Fig. 2.16**). The spectrum clearly showed that the amino group couples with the H-6 and H-3 hydrogens by spatial proximity, corroboring that the obtained structure is the *Z* isomer, since in *E*-isomer the coupling with H-6 is clearly less probable (**Fig. 2.17**).

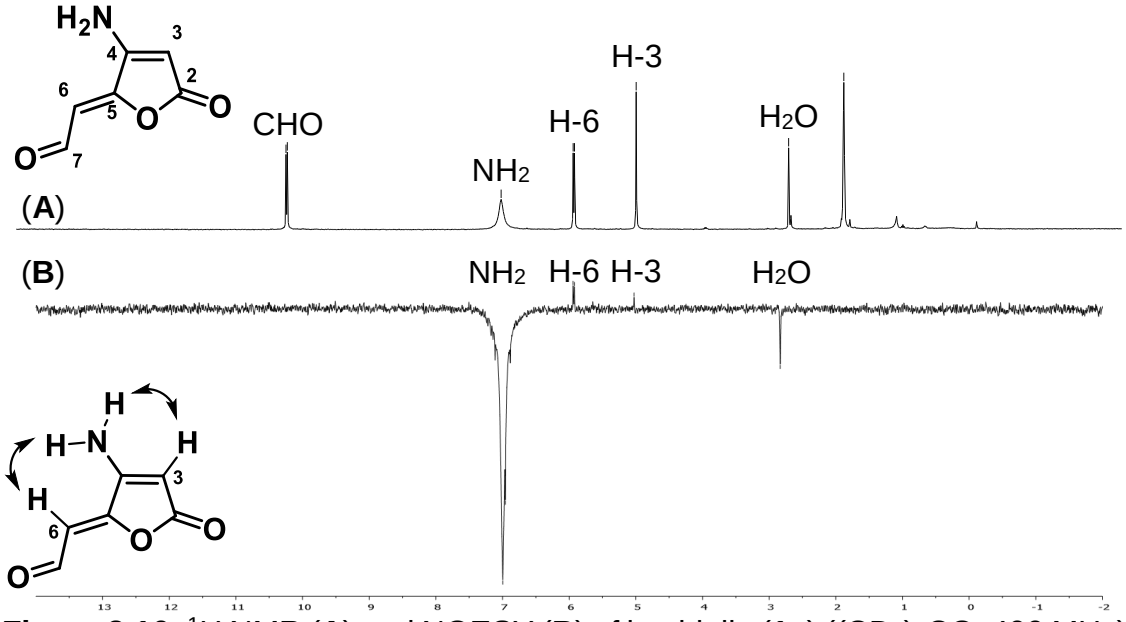
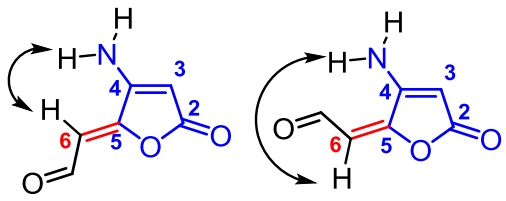



**Figure 2.16:** $^{1}H$ NMR (**A**) and NOESY (**B**) of basidalin (**1a**) ($(CD_3)_2CO$, 400 MHz).




Short distance
Coupling
(**1a**) (*Z*)

Long distance
No coupling
(**1b**) (*E*)

**Figure 2.17:** Comparison of distance for the coupling between H-6 and $NH_2$ in (*Z*)/(*E*) basidalin structures.

## 2.4. CONCLUSIONS AND PERSPECTIVES

In summary, the first synthesis of the tetronamide basidalin was achieved in 5 steps with 39% overall yield from commercially-available compounds. In addition to providing access to the naturally-product for further biological evaluation, the synthesis demonstrates the usefulness of the vinyl aldol condensation reaction and subsequent elimination for rapid regio and stereoselectivity of (*Z*)-γ-ylidenbutenolides and functionalized tetronamides.

The final steps of the reaction were simple and selective. The use of mild conditions for the deprotection of thioacetals made possible obtention of the characteristic aldehyde group of basidalin.

As a perspective, different basidalin analogs could be synthesized, exploring the possibility of some modification in the 4-amine and the 5-formylidene group. Another type of transformation could include photochemical isomerization reaction to obtain *E*-basidalin.

## 2.5. METHODOLOGIES

### 2.5.1. General experimental techniques

All commercial reagents were used as received. Moisture sensitive reactions were carried out on flame dried glass material sealed in dry air atmosphere. The solvents used were either analytical grade or purified. Moisture sensitive liquids, solutions and anhydrous solvents were transferred via the syringe or cannula through rubber septa. When necessary the reactions were carried out under a dry Air atmosphere. Light-sensitive reactions were made in the dark involving the vials in aluminum foil.

TLC analysis was performed on 0.20 mm Polygram-UV254 aluminum support silica gel plates, Macherey-Nagel (20 x 20 cm). After elution, the plates were observed under ultraviolet light ($\lambda$ = 254 nm) and developed with solutions of 2,4-dinitrophenylhydrazine and $KMnO_4$. Column chromatographic separations were performed by flash chromatography on an automated system (UV-VIS detector) using silica gel 60 70-230 mesh or 230-400 mesh, as stationary phase.

Uncorrected melting temperatures were obtained from the 301-MQAPF apparatus.

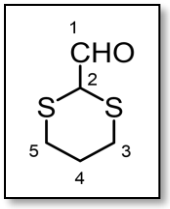
Purification of triethylamine (TEA) and pyridine (py) were performed as described in the section 5.3.3 in chapter 1.

### 2.5.2. General spectroscopic techniques.

$^{1}H$ and $^{13}C$ NMR spectroscopic data were recorded at 300 or 500 and 75 or 125 MHz, respectively, with a Bruker NMR spectrometer with $CDCl_3$ ($\delta_H$ 7.26; $\delta_C$ 77.16), DMSO-$d_6$ ($\delta_H$ 2.50; $\delta_C$ 39.52) or acetone-$d_6$ ($\delta_H$ 2.05; $\delta_C$ 29.84, 206.26) as solvent. Chemical shifts of $^{1}H$ and $^{13}C$ NMR spectra are reported in ppm and related to solvent signals. All coupling constants (*J* values) are expressed in Hertz (Hz). Multiplicities are reported as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), sextet (sext), septet (sept), multiplet (m), and broad (brd). Infrared spectrum was recorded with a Varian 660-IR instrument, equipped with GladiATR scanning from 4000 to 500 $cm^{-1}$. High-resolution mass spectrum was

recorded with a Varian using electrospray ionization (ESI) and are given to four decimal places.

### 2.5.3. Synthetic procedures for the synthesis of 1,3-dithiane-2-carbaldehyde (34).




In a 100 mL round-bottom flask, a solution of 1,3-dithiane (**37**) (1.0 g, 8.3 mmol, 1.0 eq) in 15 mL of anhydrous THF was prepared, maintained at argon atmosphere. This was then cooled to -30 °C with *i*-PrOH/dry ice bath. Then, *n*-BuLi (3.33 mL, 8.3 mmol, 2.5 M in hexane, 1.0 eq) was added dropwise and allowed to stir for 1 h. After that, the solution was transferred through a cannula to another 50 mL flask containing DMF (2.45 mL) under argon and pre-cooled to -10 °C using ice/NaCl bath. The mixture was allowed to stir for a further 2 h at -10 °C and then left overnight at room temperature. The resulting suspension was poured into 100 mL of iced water and the mixture was extracted 4 times with hexane. The aqueous layer was neutralized with 1N aqueous HCl solution. This was then extracted several times with ethyl ether (4x20 mL). The ether extracts were dried ($MgSO_4$) and concentrated in vacuo to give a viscous oil. This was distilled under reduced pressure (0.45 mmHg). Obtaining aldehyde **34** in 81% yield (1.0 g, 6.72 mmol). The product was stored at -20 °C to avoid dimerization, which occurs slowly at room temperature.

**Characteristics:** Colorless liquid.

**Bp:** 83-85 °C (0,45 mmHg).

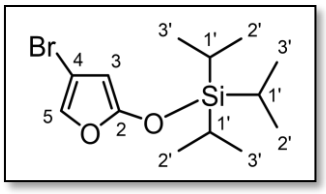
**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 2903; 2826; 2703; 1715; 1423; 1277; 1244; 1014; 1000; 644.

**$^1$H NMR (400 MHz. $CDCl_3$) $\delta$:** 9.52 (s; 1H; -CHO); 4.10 (s; 1H; H-2); 2.99-3.07 (m; 2H; H-3); 2.53-2.59 (m; 2H; H-5); 1.98-2.11 (m; 2H; H-4).

**$^{13}$C NMR (100 MHz. $CDCl_3$) $\delta$:** 188.1 (-CHO); 47.6 (C-2); 25.4 (2C; C-5 e C-3); 24.8 (C-4).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_5H_9OS_2$ 149.0089; found 149.0097.

### 2.5.4. Synthesis of ((4-bromofuran-2-yl)oxy)triisopropylsilane (33)




A solution of 4-bromofuran-2(5*H*)-one (38) (400 mg, 2.5 mmol, 1.0 eq) in anhydrous $CH_2Cl_2$ (6 mL) was prepared in a 50 mL round-bottom flask, which was maintained in argon atmosphere. This was cooled to 0 °C with an ice bath. Then, DIPEA (0.64 mL, 3.7 mmol, 1.5 eq) and TIPSOTf (0.85 mL, 3.2 mmol, 1.3 eq) were added dropwise sequentially. After 1 h, the mixture was poured into 20 mL of a saturated aqueous $NaHCO_3$ solution. This was extracted with $CH_2Cl_2$ (3 x 20 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was separated by filtration with neutral alumina and repeatedly washing with *n*-hexane to give silyloxyfurane **33** (750 mg, 2.3 mmol, 92%).

**Characteristics:** Colorless liquid.

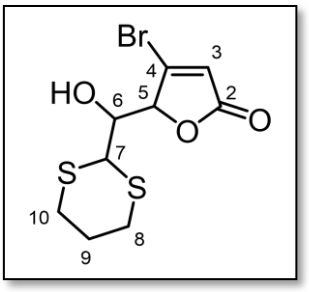
**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3160; 3140; 2946; 2893; 2869; 1611; 1536; 1464; 1356; 1269; 1189; 1099; 957; 919; 882; 823.

**$^1$H NMR (400 MHz. $CDCl_3$) $\delta$:** 6.83 (d; *J* = 1.2 Hz; 1H; H-5); 5.24 (d; *J* = 1.2 Hz; 1H; H-3); 1.25 (sept; *J* = 8 Hz; 3H; H-1'); 1.09 (d; *J* = 7.3 Hz; 18H; H-2' e H-3').

**$^{13}$C NMR (100 MHz. $CDCl_3$) $\delta$:** 156.6 (C-2); 130.0 (C-5); 100.4 (C-3); 87.6 (C-4); 17.4 (3C; C-1'); 12.1 (6C; C-2' e C-3').

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{13}H_{24}BrO_2Si$ 319.0723; Found 319.0722.

### 2.5.5. Synthesis of (±)-5-((1,3-dithian-2-yl)(hydroxy)methyl)-4-bromofuran-2 (5*H*)-one (±)-(39)




In a 50 mL round-bottom flask, a solution of silyloxyfurane (**33**) (300 mg, 0.94 mmol, 1.0 eq) in 6 mL dry $CH_2Cl_2$, maintained under argon atmosphere is prepared. Then, this is cooled to -78 °C with acetone/dry ice bath. Then, 1,3-dithiane-2-carbaldehyde (**34**) (120 µL, 1.03 mmol, 1.1 eq) and $BF_3$•$Et_2O$ complex (140 µL, 1.13 mmol; 1.2 eq). After 1 h, the mixture was washed with 20 mL of a saturated aqueous $NaHCO_3$ solution. Then, the aqueous phase

was extracted with $CH_2Cl_2$ (3 x 15 mL) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo to afford **39** (292 mg, 0.94 mmol, 100%) as a white product. The crude extract was used in a next step. The ratio of the *syn* and *anti* isomers (dr = 10:1) was determined by integration of the $^1$H NMR signals: $\delta_{major}$ = 5.56; 4.31; 3.97; $\delta_{minor}$ = 4.87; 4.41; 4.18 ppm; and as comparison $^{13}$C NMR signals were also designed: $\delta_{major}$ = 148.2; 122.9; 83.6; 67.7; 26.0; 25.2; 24.9; $\delta_{minor}$ = 146.5; 123.8; 84.5; 71.2; 27.0; 25.9; 25.0.

Subsequently, the major (±)-***syn*-39** isomer was purified by preparative TLC (100% $CH_2Cl_2$) with 86% of yield. Then, suitable crystals for X-ray crystallography were obtained by recrystallization from hot absolute ethanol.

**Characteristics:** White solid.

**Mp:** 106-112 °C.

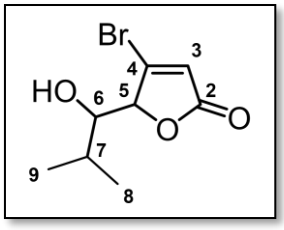
**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3436; 3100; 2895; 1755; 1603; 1422; 1281; 1159; 1102; 1023; 994; 907; 823; 775.

**$^1$H NMR (400 MHz. $CDCl_3$) $\delta$:** 6.38 (s; 1H; H-3); 5.56 (s; 1H; H-5); 4.31 (d; *J* = 10.0 Hz; 1H; H-6); 3.97 (d; *J* = 10.0 Hz; 1H; H-7); 2.93-3.05 (m; 2H; H-1; H-8$_{ax}$ e H-10$_{ax}$); 2.86 (d; *J* = 1.6 Hz; 1H; -OH); 2.58-2.73 (m; 2H; H-8$_{eq}$ e H-10$_{eq}$); 2.05-2.15 (m; 2H; H-9).

**$^{13}$C NMR (100 MHz. $CDCl_3$) $\delta$:** 170.1 (C-2); 148.2 (C-4); 122.9 (C-3); 83.6 (C-5); 67.6 (C-6); 45.2 (C-7); 26.0 (C-8); 25.1 (C-10); 24.9 (C-9).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_9H_{12}BrO_3S_2$ 310.9406; Found 310.9405.

### 2.5.6. Synthesis of (±)-4-bromo-5-(1-hydroxy-2-methylpropyl)furan-2(5*H*)-one (41)



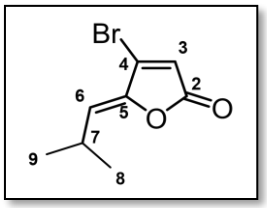

Compound **41** was synthesized using the aldol condensation method similar to that used for the production of compound **39** from silyloxyfurane **33** (30 mg, 0.09 mmol, 1.0 eq), isobutyraldehyde **40** (10 μL, 0.10 mmol, 1.1 eq). Compound **41** was purified by silica gel column chromatography (6% EtOAc:hexane), isolated in 82% yield (17.4 mg, 0.074 mmol).

**Characteristics:** Yellow liquid.

**$^1$H NMR (400 MHz. $(CD_3)_2CO$) $\delta$:** 6.33 (s; 1H; H-3); 5.05 (s; 1H; H-5); 3.60 (d; *J* = 8.0 Hz; H-6); 1.99 (sext; 1H; H-7); 1.09 (d; *J* = 8.0 Hz; H-8). 1.03 (d; *J* = 8.0 Hz; H-9).

**HRMS (ESI-TOF) *m/z* $[M+H]^+$:** Calculated for $C_8H_{11}BrO_3$ 233.9892; Found 233.9793.

### 2.5.7. Synthesis of (*Z*)-4-bromo-5-(2-methylpropylidene)furan-2(5*H*)-one (42)



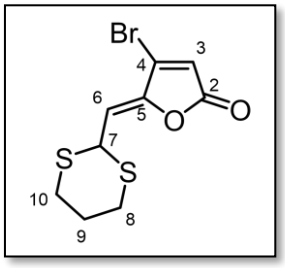

In a 25 mL round-bottom flask, product **41** (20 mg, 0.09 mmol, 1.0 eq) was dissolved in 2 mL $CH_2Cl_2$. This solution was maintained under argon atmosphere and cooled to 0 °C using an ice bath. Then, dry triethylamine (4 µL, 0.19 mmol, 3.0 eq) was added, then a solution of DMAP (20.66 mg, 0.17 mmol, 1.8 eq) in 1 mL of $CH_2Cl_2$ and finally, methanesulfonyl chloride dropwise (10 µL, 0.19 mmol, 2.0 eq). The solution was allowed to stir at the same temperature for 1 h and left at room temperature while stirring overnight. After 15 h, the solution was washed with 10 mL of 1N aqueous HCl solution. The aqueous phase was then extracted with $CH_2Cl_2$ (3 x 10 mL). Then, the combined organic phases were dried ($MgSO_4$), filtered and concentrated in vacuo. The product was purified by flash column chromatography (5% EtOAc:hexane) to give the ***Z*-42** isomer (13.0 mg, 0.06 mmol, 67%).

**Characteristics:** Yellow liquid.

**NMR de $^1$H (400 MHz. $CDCl_3$) $\delta$:** 6.34 (s; 1H; H-3); 5.46 (d; *J* = 8.0 Hz; 1H; H-6); 2.90-2.96 (m; 1H; H-7); 1.13 (s; 3H; H-8); 1.11 (s; 3H; H-9).

**HRMS (ESI-TOF) *m/z* $[M+H]^+$:** Calculated for $C_8H_9BrO_2$ 215.9786; Found 215.9476.

### 2.5.8. Synthesis of (*Z*)-5-((1,3-dithian-2-yl)methylene)-4-bromofuran-2(5*H*)-one (35)




In a 25 mL round-bottom flask, the crude product **39** (dr = 10:1) (170 mg, 0.55 mmol, 1.0 eq) was dissolved in 4 mL $CH_2Cl_2$. This solution was maintained under argon atmosphere and cooled to -10 °C with an ice/NaCl bath. Thereafter, was added dry pyridine (90 μL, 1.09 mmol, 2.0 eq) and then methanesulfonyl chloride dropwise (80 μL, 0.98 mmol, 1.8 eq). The solution was allowed to stir at the same temperature for 1 h. Then another 90 μL of pyridine was added and the solution was slowly left at room temperature while stirring overnight. After 15 h, the solution was washed with 15 mL of 1 N HCl aqueous solution. The aqueous phase was then extracted with $CH_2Cl_2$ (3 x 15 mL). Then, the combined organic phases were dried ($MgSO_4$), filtered and concentrated in vacuo. The product was purified by flash column chromatography (5% EtOAc:hexane) to give the ***Z*-35** isomer (135 mg, 0.45 mmol, 82%) and a mixture of several compounds containing the *E*-**35** isomer.

**Characteristics, *Z*-35:** Solid pale brown.

**Mp, *Z*-35:** 97-100 °C.

**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3133; 2901; 1781; 1670; 1559; 1422; 1293; 1000; 967; 905; 660.

**$^1$H NMR (*Z*-35. 400 MHz. $CDCl_3$) $\delta$:** 6.45 (s; 1H; H-3); 5.68 (d; *J* = 11.0 Hz; 1H; H-6); 5.19 (d; *J* = 11.0 Hz; 1H; H-7); 2.90-2.96 (m; 4H; H-8 e H-9); 2.04-2.20 (m; 1H; H-9$_{ax}$); 1.85-2.03 (m; 1H; H-9$_{eq}$).

**$^1$H NMR (*E*-35. 400 MHz. $CDCl_3$) $\delta$:** 6.54 (s; 1H; H-3); 6.07 (d; *J* = 12.0 Hz; 1H; H-6); 5.72 (d; *J* = 12.0 Hz; 1H; H-7); 2.86-3.02 (m; 4H; H-8 e H-9); 2.08-2.16 (m; 1H; H-9$_{ax}$); 1.96-2.04 (m; 1H; H-9$_{eq}$).

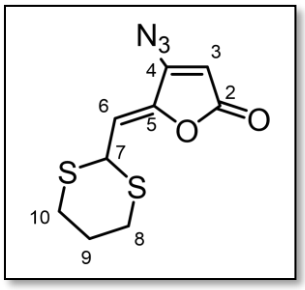
**$^{13}$C NMR (*Z*-35. 100 MHz. $CDCl_3$) $\delta$:** 165.8 (C-2); 147.9 (C-5); 137.3 (C-4); 121.8 (C-6); 110.7 (C-3); 40.2 (C-7); 29.2 (2C; C-8 e C-10); 24.8 (C-9).

**HRMS (ESI-TOF) *Z*-35. *m/z* [M+H]$^+$:** Calculated for $C_9H_{10}BrO_2S_2$ 292.9300; found 292.9286.

**HRMS (ESI-TOF) *E*-35. *m/z* [M+H]$^+$:** Calculated for $C_9H_{10}BrO_2S_2$ 292.9300; found 292.9313.

**Elemental analysis. *Z*-35:** Calculated for $C_9H_9BrO_2S_2$: C: 36.87; H: 3.09; Found: C: 36.47. H: 2.77.

### 2.5.9. Synthesis of (*Z*)-5-((1,3-dithian-2-yl)methylene)-4-azidofuran-2(5*H*)-one (43)




Into a 25 mL round-bottom flask was added a mixture of ***Z*-35** (120 mg, 0.41 mmol, 1.0 eq) and sodium azide (29.3 mg, 0.45 mmol, 1.1 eq) in 0.4 mL of DMF. This was maintained under argon atmosphere and allowed to stir at 0 °C in an ice bath for 2 h. The obtained mixture was diluted with 30 mL of ethyl acetate and washed successively with two portions of 10 mL of cold water and 15 mL of saturated NaCl solution. The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude extract containing the azide **43** (102 mg) was used for the next step without purification.

**Characteristics:** Mixture of the crude extract, oil color reddish yellow.

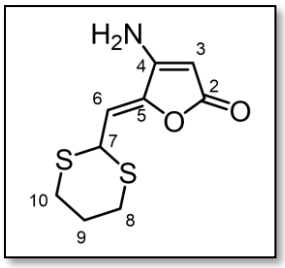
**FTIR (NaCl film; $cm^{-1}$):** $\bar{\upsilon}_{max}$ 2927; 2361; 2342; 2149; 2126; 1768; 1675; 1422; 1329; 1231; 1168; 1.010; 847.

**$^1$H NMR (300 MHz. $CDCl_3$) $\delta$:** 5.85 (s; 1H; H-3); 5.52 (d; *J* = 11.1 Hz; 1H; H-6); 5.13 (d; J = 11.1 Hz; 1H; H-7); 2.89-2.92 (m; 2H; H-8); 2.07-2.18 (m; 2H; H-10); 1.87-2.01 (m; 2H; H-9).

**$^{13}$C NMR (75 MHz. $CDCl_3$) $\delta$:** 165.9 (C-2); 155.6 (C-5); 143.8 (C-4); 107.3 (C-6); 102.3 (C-3); 40.0 (C-7); 29.2 (2C; C-8 e C-10); 24.9 (C-9).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_9H_{10}N_3O_2S_2$ 256.0209; Found 256.0197.

### 2.5.10. Synthesis of (*Z*)-5-((1,3-dithian-2-yl)methylene)-4-aminofuran-2(5*H*)-one (36)




In a 25 mL round-bottom flask, a suspension of stannous chloride (113.6 mg, 0.61 mmol, 1.5 eq) in 0.5 mL MeOH was prepared. This was kept under stirring and cooled to 0 °C in an ice bath under argon. Thereafter, a solution of azide **43** (102 mg) in 5 mL MeOH was added dropwise. The reaction is exothermic and there is production of $N_2$ gas. After addition, the reaction mixture was stirred at 0 °C for a total period of 0.5 h. At the end of the reaction the methanol was removed in vacuo, the residue was diluted with 10 mL of cold water and basified dropwise with aqueous 2N NaOH solution. Then, 20 mL AcOEt was added and the layers were separated. The aqueous phase was saturated by adding 5 g of NaCl and reextracted with EtOAc (2 x 10 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (50% EtOAc:hexane) to afford amine **36** (58 mg, 0.25 mmol, 62% for 2 steps).

**Characteristics:** Yellow Solid.

**Mp:** 150-153 °C.

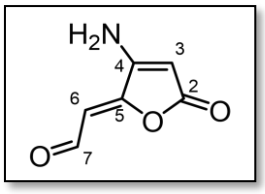
**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3197; 1716; 1642; 1584; 1568; 1355; 937; 771; 664.

**$^1$H NMR (400 MHz. $(CD_3)_2CO$) $\delta$:** 6.67 (s brd; 2H; -$NH_2$); 5.67 (d; *J* = 10.7 Hz; 1H; H-6); 5.19 (d; *J* = 10.7 Hz; 1H; H-7); 4.88 (s; 1H; H-3); 2.01-3.10 (m; 2H; H-8); 2.85-2.94 (m; 2H; H-10); 2.07-2.15 (m; 1H; H-$9_{ax}$); 1.78-1.92 (m; 1H; H-$9_{eq}$).

**$^{13}$C NMR (100 MHz. $(CD_3)_2CO$) $\delta$:** 169.4 (C-2); 159.6 (C-4); 146.3 (C-5); 103.5 (C-6); 85.0 (C-3); 41.0 (2C; C-8 e C-10); 25.8 (C-9).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_9H_{12}NO_2S_2$ 230.0304; Found 230.0292.

### 2.5.11. Final step of alkylative deprotection of *S*,*S*-thioacetal: Synthesis of basidalin (1a)




Into a 25 mL round-bottom flask fitted with a reflux condenser, a mixture of amine **27** (50 mg, 0.22 mmol, 1.0 eq) and $CaCO_3$ (327.3 mg, 3.27 mmol; 15.0 eq) were suspended with 5 mL of acetone:$H_2O$ (4:1). This was allowed to stir and maintained under argon. Then, MeI (0.27 mL, 4.36 mmol, 20.0 eq) was added dropwise. Next, the reaction mixture was heated at 60 °C for 5 h. At the end of the reaction, the system was cooled to room temperature. The acetone was then evaporated under reduced pressure and the resulting mixture was diluted with 10 mL of AcOEt, then washed with 10 mL of water and the layers were separated. The organic phase was dried ($Na_2SO_4$), filtered and concentrated in vacuo. The extract was purified by flash column chromatography (40% EtOAc:hexane) to give **1a** (22 mg, 0.16 mmol, 72%).

**Characteristics:** Yellow Solid.

**Mp:** 138-142 °C.

**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3300; 2200; 1754; 1671; 1584; 1416; 1400; 1320; 1184; 1028; 926; 835; 786.

**$^1$H NMR (500 MHz. $(CD_3)_2SO$) $\delta$:** 10.01 (d; *J* = 7.8 Hz; 1H; -CHO); 7.73 (s brd; 2H; -$NH_2$); 6.11 (d; *J* = 7.8 Hz; 1H; H-6); 4.96 (s; 1H; H-3).

**$^1$H NMR (400 MHz. $(CD_3)_2CO$) $\delta$:** 10.11 (d; *J* = 7.7 Hz; 1H; -CHO); 7.01 (s brd; 2H; -$NH_2$); 5.96 (d; *J* = 7.7 Hz; 1H; H-6); 5.05 (s; 1H; H-3).

**$^{13}$C NMR (125 MHz. $(CD_3)_2SO$) $\delta$:** 189.3 (-CHO); 168.2 (C-2); 159.5 (C-5); 158.5 (C-4); 102.9 (C-6); 81.8 (C-3).

**$^{13}$C NMR (100 MHz. $(CD_3)_2CO$) $\delta$:** 189.2 (-CHO); 168.6 (C-2); 160.0 (C-4); 159.6 (C-5); 103.4 (C-6); 84.7 (C-3).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_6H_6NO_3$ 140.0342; Found 140.0346.

## 2.6. REFERENCES


[1] WANG, J.; JIANG, X.; CHEN, M.; GE, Z.; HU, Y.; HU, H. TFA-catalyzed ring transformation of 4-hydroxycyclobutenone: A simple and general route for preparation of 3-substituted 4-aminofuran-2(5*H*)-ones. *Journal of the Chemical Society, Perkin Transactions 1* **2001**, 66.

[2] ZHOU, L.-H.; YU, X.-Q.; PU, L. Reactivity of γ-hydroxy-α,β-acetylenic esters with amines: Facile synthesis of the optically active 4-amino-2(5*H*)-furanones. *The Journal of organic chemistry* **2009**, *74*, 2013.

[3] HERTZBERG, R.; MOBERG, C. One-step preparation of *O*-(α-bromoacyl) cyanohydrins by minor enantiomer recycling: Synthesis of 4-amino-2(5*H*)-furanones. *The Journal of organic chemistry* **2013**, *78*, 9174.

[4] AILLERIE, A.; TALANCÉ, V. L. D.; MONCOMBLE, A.; BOUSQUET, T.; PÉLINSKI, L. Enantioselective organocatalytic partial transfer hydrogenation of lactone-fused quinolines. *Organic Letters* **2014**, *16*, 2982.

[5] XIAO, Z.-P.; MA, T.-W.; LIAO, M.-L.; FENG, Y.-T.; PENG, X.-C.; LI, J.-L.; LI, Z.-P.; WU, Y.; LUO, Q.; DENG, Y.; LIANG, X.; ZHU, H.-L. Tyrosyl-tRNA synthetase inhibitors as antibacterial agents: Synthesis, molecular docking and structure–activity relationship analysis of 3-aryl-4-arylaminofuran-2(5*H*)-ones. *European Journal of Medicinal Chemistry* **2011**, *46*, 4904.

[6] KAMAL, A.; SRINIVASA REDDY, T.; POLEPALLI, S.; PAIDAKULA, S.; SRINIVASULU, V.; GANGA REDDY, V.; JAIN, N.; SHANKARAIAH, N. Synthesis and biological evaluation of 4-aza-2,3-dihydropyridophenanthrolines as tubulin polymerization inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2014**, *24*, 3356.

[7] DE SOUZA, M. V. N. The furan-2(5*H*)-ones: Recent synthetic methodologies and its application in total synthesis of natural products. *Mini-Reviews in Organic Chemistry* **2005**, *2*, 139.

[8] ZOGRAFOS, A. L.; GEORGIADIS, D. Synthetic strategies towards naturally occurring tetronic acids. *Synthesis* **2006**, *2006*, 3157.

[9] SCHOBERT, R.; SCHLENK, A. Tetramic and tetronic acids: an update on new derivatives and biological aspects. *Bioorganic & medicinal chemistry* **2008**, *16*, 4203.

[10] IINUMA, H.; NAKAMURA, H.; NAGANAWI, H.; MASUDA, T.; TAKANO, S.; TAKEUCHI, T.; UMEZAWA, H.; IITAKA, Y.; OBAYASHI, A. Basidalin, a new antibiotic from basidiomycetes. *The Journal of antibiotics* **1983**, *36*, 448.

[11] JESCHKE, P.; NAUEN, R.; GUTBROD, O.; BECK, M. E.; MATTHIESEN, S.; HAAS, M.; VELTEN, R. Flupyradifurone (Sivanto™) and its novel butenolide pharmacophore: Structural considerations. *Pesticide Biochemistry and Physiology* **2015**, *121*, 31.

[12] NAUEN, R.; JESCHKE, P.; VELTEN, R.; BECK, M. E.; EBBINGHAUS-KINTSCHER, U.; THIELERT, W.; WÖLFEL, K.; HAAS, M.; KUNZ, K.; RAUPACH, G. Flupyradifurone: a brief profile of a new butenolide insecticide. *Pest Management Science* **2015**, *71*, 850.

[13] SEMENOVA, M. N.; KISELYOV, A. S.; TSYGANOV, D. V.; KONYUSHKIN, L. D.; FIRGANG, S. I.; SEMENOV, R. V.; MALYSHEV, O. R.; RAIHSTAT, M. M.; FUCHS, F.; STIELOW, A.; LANTOW, M.; PHILCHENKOV, A. A.; ZAVELEVICH, M. P.; ZEFIROV, N. S.; KUZNETSOV, S. A.; SEMENOV, V. V. Polyalkoxybenzenes from plants. 5. Parsley seed extract in synthesis of azapodophyllotoxins featuring strong tubulin destabilizing activity in the sea urchin embryo and cell culture assays. *Journal of Medicinal Chemistry* **2011**, *54*, 7138.

[14] MADEC, D.; MINGOIA, F.; PRESTAT, G.; POLI, G. *N*-Substituted tetronamides as ambident nucleophilic building blocks for the synthesis of new 4-aza-2,3-didehydropodophyllotoxins. *Synlett* **2008**, *2008*, 1475.

[15] LATTMANN, E.; DUNN, S.; NIAMSANIT, S.; SATTAYASAI, N. Synthesis and antibacterial activities of 5-hydroxy-4-amino-2(5*H*)-furanones. *Bioorganic & Medicinal Chemistry Letters* **2005**, *15*, 919.

[16] JIANG, B.; FENG, B. M.; WANG, S. L.; TU, S. J.; LI, G. Domino constructions of pentacyclic indeno [2, 1 ‐ c] quinolines and pyrano [4, 3 ‐ b] oxepines by [4+1]/[3+2+1]/[5+1] and [4+3] multiple cyclizations. *Chemistry–A European Journal* **2012**, *18*, 9823.

[17] GHAHREMANZADEH, R.; RASHID, Z.; ZARNANI, A.-H.; NAEIMI, H. Manganese ferrite nanoparticle catalyzed tandem and green synthesis of spirooxindoles. *RSC Advances* **2014**, *4*, 43661.

[18] YANG, Z.; HAO, W.-J.; XU, H.-W.; WANG, S.-L.; JIANG, B.; LI, G.; TU, S.-J. Base-promoted transannulation of heterocyclic enamines and 2,3-epoxypropan-1-ones: regio- and stereoselective synthesis of fused pyridines and pyrroles. *The Journal of Organic Chemistry* **2015**, *80*, 2781.

[19] HIYAMA, T.; OISHI, H.; SUETSUGU, Y.; NISHIDE, K.; SAIMOTO, H. Synthesis of 4-amino-2(5*h*)-furanones through intra- and intermolecular nitrile addition of ester enolates. construction of carbon framework of an antitumor antibiotic basidalin. *Bulletin of the Chemical Society of Japan* **1987**, *60*, 2139.

[20] SCHLESSINGER, R. H.; IWANOWICZ, E. J.; SPRINGER, J. P. Highly diastereoselective alkylation reactions of vinylogous urethanes derived from simple tetronic acids. *Tetrahedron Letters* **1988**, *29*, 1489.

[21] GREENHILL, J. V.; RAMLI, M.; TOMASSINI, T. Reduction of enaminones in the preparation of 3-aminocyclohexanols; a novel preparation of tetronic acid. *Journal of the Chemical Society, Perkin Transactions 1* **1975**, 588.

[22] KARAK, M.; BARBOSA, L. C. A.; ACOSTA, J. A. M.; SAROTTI, A. M.; BOUKOUVALAS, J. Thermodynamically driven, *syn*-selective vinylogous aldol reaction of tetronamides. *Organic & Biomolecular Chemistry* **2016**, *14*.

[23] CUNHA, S.; OLIVEIRA, C. C. Aplicações sintéticas do ácido mucobrômico e da 3,4-dibromofuran-2(5*H*)-ona. *Quim. Nova* **2011**, *34*.

[24] BLAZECKA, P. G.; BELMONT, D.; CURRAN, T.; PFLUM, D.; ZHANG, J. Further utilization of mucohalic acids: palladium-free, regioselective etherification and amination of α,β-dihalo-γ-methoxycarbonyloxy and γ-acetoxy butenolides. *Organic letters* **2003**, *5*, 5015.

[25] YAMADA, K.; TOGAWA, Y.; KATO, T.; HIRATA, Y. A convenient method for the preparation of γ-arylidene-α,β-unsaturated γ-lactones: Application to the synthesis of the thiophene lactone obtained from *Chamaemelum nobile* L. *Tetrahedron* **1971**, *27*, 5445.

[26] PARK, B. R.; LIM, C. H.; LIM, J. W.; KIM, J. N. Facile synthesis of 5-alkylidene-1,5-dihydropyrrol-2-ones from Morita-Baylis-Hillman adducts. *Bull. Korean Chem. Soc.* **2012**, *33*, 1337.

[27] PEREIRA, U. A.; MOREIRA, T. A.; BARBOSA, L. C. A.; MALTHA, C. R. A.; BOMFIM, I. S.; MARANHAO, S. S.; MORAES, M. O.; PESSOA, C.; BARROS-NEPOMUCENO, F. W. A. Rubrolide analogues and their derived lactams as potential anticancer agents. *MedChemComm* **2016**, *7*, 345.

[28] GABRIELE, B.; SALERNO, G.; PLASTINA, P.; COSTA, M.; CRISPINI, A. Expedient synthesis of 4-dialkylamino-5*H*-furan-2-ones by one-pot sequential Pd-catalyzed oxidative carbonylation of 2-yn-1-ols–conjugate addition-lactonization. *Advanced Synthesis & Catalysis* **2004**, *346*, 351.

[29] HUFF, T.; KUBALL, H.-G.; ANKE, T. 7-Chloro-4,6-dimethoxy-1(3*H*)-isobenzofurane and basidalin: Antibiotic secondary metabolites from *Leucoagaricus carneifolia* Gillet (basidiomycetes). *Zeitschrift für Naturforschung C* **1994**, *49*, 407.

[30] YAMAMOTO, Y.; OHNO, M.; EGUCHI, S. Ring transformation of 4-acylmethyl-2-chloro-4-hydroxy-2-cyclobutenone to γ-acylmethylenetetronate by thermal rearrangement: New synthetic aspect of squaric acid as a C4-synthon. *Tetrahedron* **1994**, *50*, 7783.

[31] ACOSTA, J. A. M.; MUDDALA, R.; BARBOSA, L. C. A.; BOUKOUVALAS, J. Total synthesis of the antitumor antibiotic basidalin. *The Journal of Organic Chemistry* **2016**, *81*, 6883.

[32] SCHACHTNER, J. E.; STACHEL, H.-D.; POLBORN, K.; ANKE, T. Synthesis and biological activity of thiobasidalin. *European journal of medicinal chemistry* **1998**, *33*, 309.

[33] EAR, A.; TOUM, V.; THORIMBERT, S.; DECHOUX, L. Decarboxylative Knoevenagel-type reactions on tetronamides: synthesis of 4-amino-5-ylidene-2(5*H*)-Furanones. *Synlett* **2014**, *25*, 1713.

[34] BELLINA, F.; ANSELMI, C.; MARTINA, F.; ROSSI, R. Mucochloric acid: a useful synthon for the selective synthesis of 4-aryl-3-chloro-2(5*H*)-furanones, (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-chloro-2(5*H*)-furanones and 3,4-diaryl-2(5*H*)-furanones. *European Journal of Organic Chemistry* **2003**, 2290.

[35] BELLINA, F.; ANSELMI, C.; ROSSI, R. Total synthesis of rubrolide M and some of its unnatural congeners. *Tetrahedron Letters* **2002**, *43*, 2023.

[36] BELLINA, F.; ANSELMI, C.; VIEL, S.; MANNINA, L.; ROSSI, R. Selective synthesis of (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-bromo-2(5*H*)-furanones. *Tetrahedron* **2001**, *57*, 9997.

[37] BOUKOUVALAS, J.; BELTRAN, P. P.; LACHANCE, N.; COTE, S.; MALTAIS, F.; POULIOT, M. A new, highly stereoselective synthesis of β-unsubstituted (*Z*)- γ-alkylidenebutenolides using bromine as a removable stereocontrol element. *Synlett* **2007**, 219.

[38] BOUKOUVALAS, J.; LACHANCE, N.; OUELLET, M.; TRUDEAU, M. Facile access to 4-aryl-2(5*H*)-furanones by Suzuki cross coupling: Efficient synthesis of rubrolides C and E. *Tetrahedron Letters* **1998**, *39*, 7665.

[39] BOUKOUVALAS, J.; MALTAIS, F.; LACHANCE, N. Furanolate-based strategy for sequential 2,3,4-trisubstitution of butenolide: Total synthesis of nostoclides I and II. *Tetrahedron Letters* **1994**, *35*, 7897.

[40] BOUKOUVALAS, J.; MCCANN, L. C. Synthesis of the human aldose reductase inhibitor rubrolide L. *Tetrahedron Letters* **2010**, *51*, 4636.

[41] MEYERS, A.; STRICKLAND, R. C. Synthesis and properties of 2-(2-cyanoethylidene)-1,3-dithiane and its isomeric ketene thioacetal. *The Journal of Organic Chemistry* **1972**, *37*, 2579.

[42] MILANDIP, K.; M., A. J. A.; A., B. L. C.; JOHN, B. Late-Stage Bromination Enables the Synthesis of Rubrolides B, I, K, and O. *European Journal of Organic Chemistry* **2016**, *2016*, 3780.

[43] JEFFORD, C. W.; JAGGI, D.; BOUKOUVALAS, J. Diastereoselectivity in the directed aldol condensation of 2-trimethylsiloxyfuran with aldehydes. A stereodivergent route to threo and erythro δ-hydroxy-γ-lactones. *Tetrahedron letters* **1987**, *28*, 4037.

[44] MARTIN, S. F.; CORBETT, J. W. The stereochemical course of nucleophilic additions of 2-trialkylsiloxyfurans to cyclic *N*-acyliminium ions. *Synthesis* **1992**, 55.

[45] ZHANG, R.; CHAN, D.; JESSICA, S.; ISKANDER, G.; BLACK, D. S.; KUMAR, N. Synthesis of new aryl substituted 5-alkylidenefuran-2(5*H*)-ones. *Arkivoc* **2009**, *102*, 115.

[46] RIPIN, D.; EVANS, D. pKa Table. **2005**, *2016*.

[47] MOORE, H. W.; HERNANDEZ, L.; KUNERT, D. M.; MERCER, F.; SING, A. A new synthetic route to 2-azetidinones. Ring contraction of 4-azido-2-pyrrolinones to 3-cyano-2-azetidinones. *Journal of the American Chemical Society* **1981**, *103*, 1769.

[48] BECCALLI, E. M.; ERBA, E.; TRIMARCO, P. 4-Azidotetronic acids: a new class of azido derivatives. *Synthetic Communications* **2000**, *30*, 629.

[49] MAITI, S. N.; SINGH, M. P.; MICETICH, R. G. Facile conversion of azides to amines. *Tetrahedron letters* **1986**, *27*, 1423.

[50] EGE, S. N.; TIEN, C. J.; DLESK, A.; POTTER, B. E.; EAGLESON, B. K. Photochemical oxidation of pyrazolidinones. *Journal of the Chemical Society, Chemical Communications* **1972**, 682.

[51] KREIER, A.; FRÖHLICH, R.; WEGELIUS, E.; HOPPE, D. Asymmetric synthesis of stereodefined 7-(alk-1-enyl)-5,6,7,8-tetrahydronaphthalene-2-carboxylic acids and their precursors, bearing a polar group in the 8-position, by the 3-sulfonyl-1,3-oxazolidine Method. *Synthesis* **2000**, *2000*, 1391.

[52] BARBOSA, L. C.; VELOSO, D. P. Sistema para a representação simbólica de mecanismos de reação (Recomendações 1988). *Quim. Nova* **1994**, *17*.

# CHAPTER 3

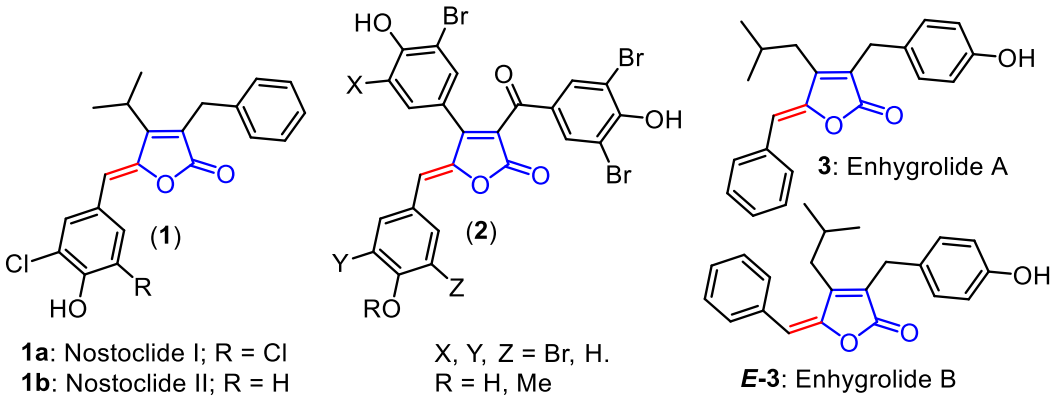
# TOTAL SYNTHESIS OF ENHYGROLIDE A

## 3.1. INTRODUCTION

### 3.1.1. Biologically active α,β-dialkyl-γ-ylidenebutenolides

In the present thesis it has been already mentioned the interesting faculty of furan-2(5*H*)-one ring to adopt several substituents. In that regard, a densely-substituted (*Z*)-γ-ylidenebutenolide motif arises as one of the most diverse and active in nature. Besides this, some examples were exposed in the rubrolide synthesis chapter 1, in which we clearly exemplify the most important biological characteristics of butenolides.

Since 1990, an important subclass of butenolides with α,β-dialkyl-γ-ylidenebutenolide skeleton have been isolated from diverse sources. The most representative examples are the nostoclides (**1a-b**),[1] cadiolides (**2**),[2-7] and the novel enhygrolides (**3 and *E*-3**) (**Fig. 3.1**)[8].




**1a**: Nostoclide I; R = Cl
**1b**: Nostoclide II; R = H

X, Y, Z = Br, H.
R = H, Me

***E*-3**: Enhygrolide B

**Figure 3.1:** Examples of α,β-dialkyl-γ-ylidenebutenolide structures.

Initially, in 1993 nostoclides I and II (**1a-b**) were obtained from symbiotic cyanobacteria, *Nostoc sp*., of the Lichen *Peltigera canina*, by Shimizu and co-workers.[1] These two chlorine-containing metabolites, presented moderate cytotoxicity ($LC_{50}$ = 10 μg $mL^{-1}$) against the cell lines Neuro-2a CCL 131 (mouse neuroblastoma) and KB CCL17 (human papilloma).[1] Additionally, nostoclides seem to be allelopathic agents owing to the peculiar fact that *Nostoc sp*. was first noticed to sustain unusually clean, contamination-free culture.[1] This observation is also reinforced by other allelopathic cyanobacteria metabolites, including the nostoclide homologous lactone, cyanobacterin (**4**).[9,10] However, no more studies about **1a-b** bioactivities are reported, instead, some analogs were synthetized by

our group and exhibited promising results in antibacterial activity[11] and moderate cytotoxicity[12] (**Fig. 3.2**; **5**). Similarly, β-unsubstituted derivatives (**6**) showed moderate photosynthesis inhibition activities with $IC_{50}$<10.0 μg $mL^{-1}$.[13,14]

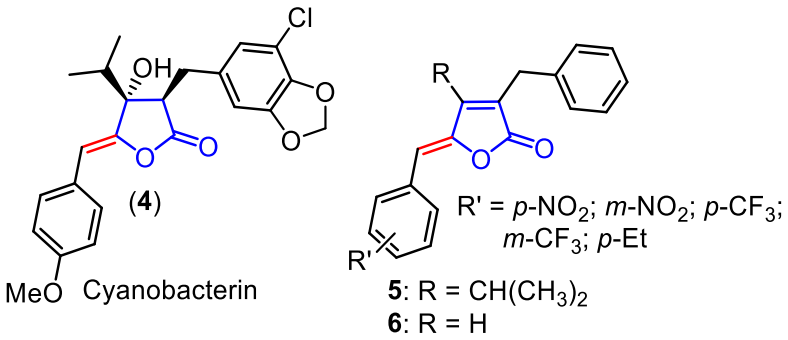
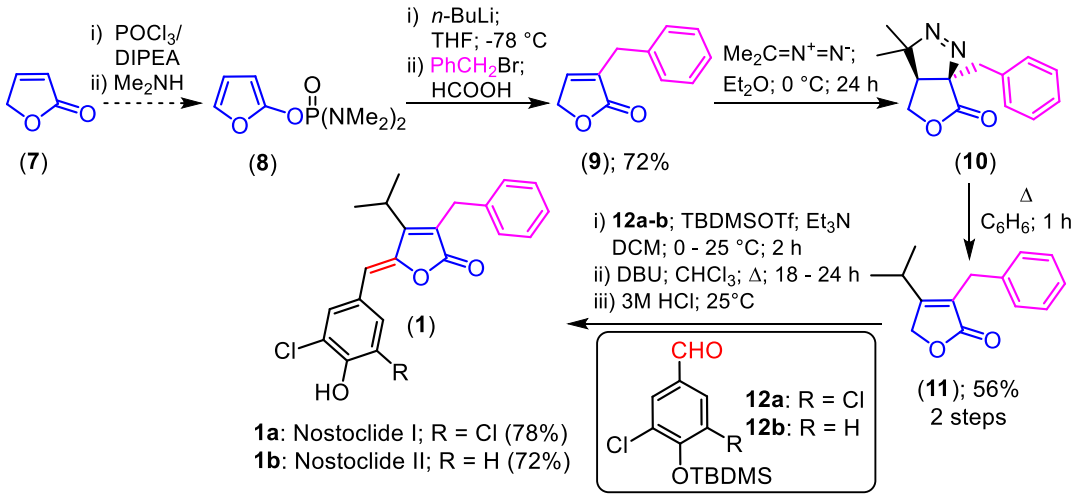



**Figure 3.2:** Nostoclide related bioactive analogues.

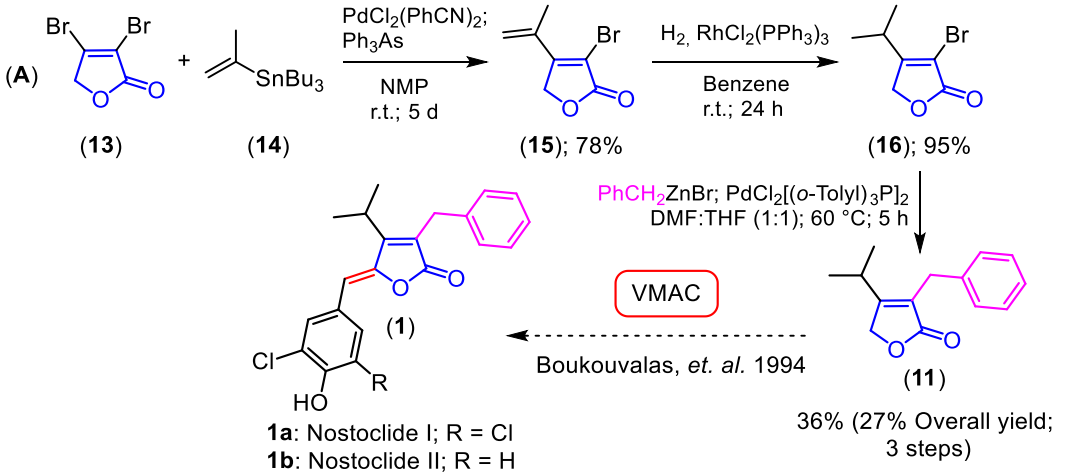
Few examples of total synthesis of nostoclide I, II and analogs have been been reported.[15-18] In these syntheses the most representative procedures include typical vinylogous Mukaiyama's aldol condensation (VMAC) for stereoselective formation of (*Z*)-γ-alkylidenebutenolide. The first total synthesis of nostoclides was develop in 1994 by Boukouvalas and co-workers.[15] In this approach a versatile protocol of furanolate chemistry, starting with a suitable furan-2(5*H*)-one species was used, which provides the ideal scaffold for the formation of the final poly-substituted butenolide.[15]

The synthesis was developed with the commercially available 2-furyl *N*,*N*,*N*',*N*'-tetramethyldiamidophosphate (**8**) (can also be easily prepared from butenolide **7** via addition of $POCl_3$/DIPEA followed by $Me_2NH$)[19] which was transformed into α-benzylbutenolide (**9**), followed by directed *ortho*-lithiation and benzylation using Nasman's procedure (**Scheme 3.1**).[19] Introduction of an β-isopropyl group in **9** was carried out with a C(3)-regioselective 1,3-dipolar cycloaddition with 2-diazopropane followed by thermolysis of the resulting pyrazolinolactone (**10**) to deliver 2-benzyl-3-isopropyl-2-butenolide (**11**). The final steps include VMAC via silylation of lactone **10** with TBDMSOTf and triethylamine provided 3-benzyl-2-(*tert*-butyldimethylsilyloxy)-4-isopropylfuran. The latter was treated with an excess of DBU and a subsequent quenching with aqueous HCl, leading to nostoclides I and II as the sole products (**Scheme 3.1**).[15]



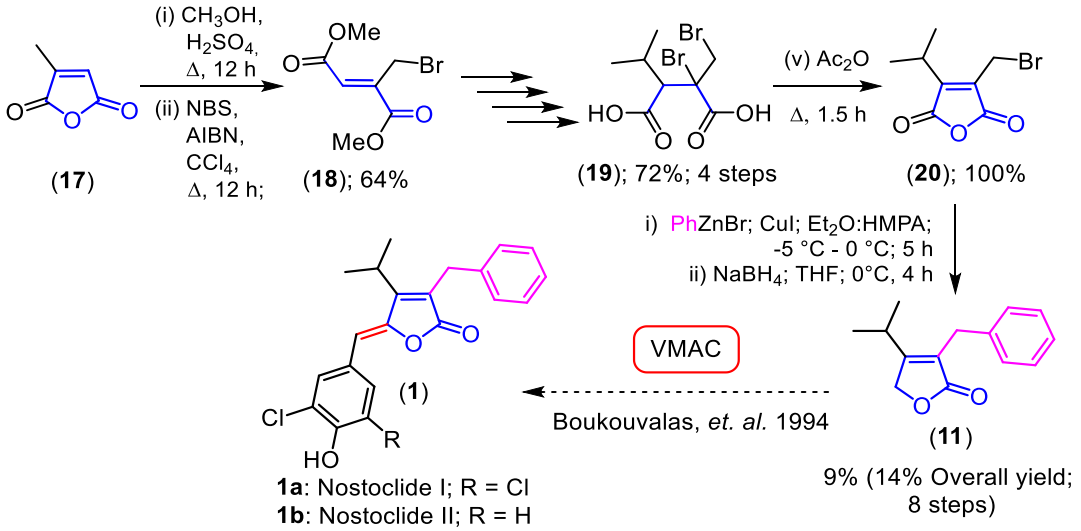

**Scheme 3.1:** First total synthesis of nostoclides I and II.[15]

Two more formal syntheses of nostoclides were reported. The first among them was developed by Bellina and co-workers in 2002.[16] In this case the authors used a furanone-containing starting material, the versatile 3,4-dibromofuran-2(5*H*)-one (**13**), whose properties were described in chapter 1. Similarly to rubrolides, the nostoclides are readily accessible via metallic cross-coupling reactions using this kind of halogenated starting material. With this in mind, **13** was submitted to Still cross-coupling with isopropenyltributyltin (**14**) in the presence of catalytic precursors $PdCl_2(PhCN)_2$/$Ph_3As$, obtaining intermediate **15**. The intermediate was then hydrogenated using Rh(I) as a catalyst, yielding the compound 4-isopropyl-3-bromofuran-2(5*H*)-one (**16**). As a last step, **16** was coupled with benzylzinc bromide via Pd-catalyzed benzylation to finally obtain nostoclide's precursor **11** in three steps with 27% global yield. (**Scheme 3.2**).




**Scheme 3.2:** Formal nostoclides synthesis starting with 3,4-dibromofura-2(5*H*)-one (**13**).[16]

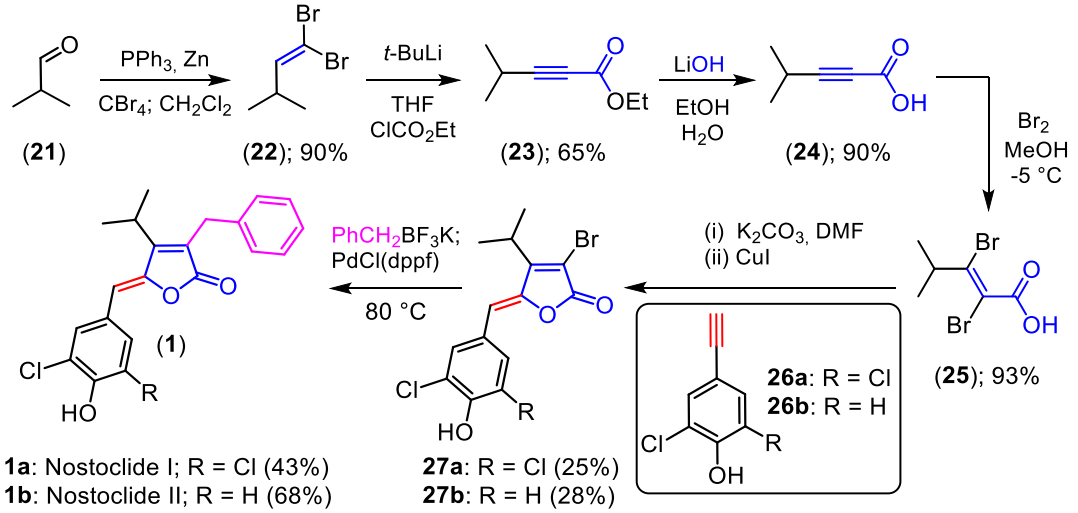
The second example was developed by Argade and co-workers in 2005, who reported the formal synthesis of nostoclide I.[20] Their synthetic process started with citraconic anhydride (**17**) that was converted to bromodiester **18** in 2-steps with 64% overall yield by using an optimized Argarde's procedure that includes allylic bromination and isomerization of the carbon-carbon double bond.[17] Compound **18** was then transformed into the key intermediate, the anhydride **20** by a series of reactions such as $A_ND_N$[21]/Grignard coupling, hydrolysis, bromination and *in situ* dehydration followed by dehydrobromination. Proceeding, allylic substitution in **20** following by $NaBH_4$ reduction, yielded a mixture of regioisomers including **11**, in 8 steps and 14% global yield. The obtained isomers were impossible to separate in the resulting mixture. The authors suggested Boukouvalas optimized VMAC procedure[15] to convert **11** to nostoclide I (**1a**) (**Scheme 3.3**).




**Scheme 3.3:** Formal nostoclides synthesis starting with citraconic anhydride (**17**).[17]

In 2013, a second total synthesis of nostoclides was reported by Ngi and co-workers.[18] The new methodology includes: initial formation of β-isopropyl-γ-benzylidenefuran-2(5*H*)-one motif (**27**) by employing copper-catalyzed domino reaction and an α-benzylidene ring was added by metallic coupling reactions (**Scheme 3.4**). The synthesis starts with isobutyraldehyde (**21**), which was subjected to bromination, elimination and hydrolysis followed by bromination, which afforded the key intermediate (**25**). The reaction between the dibromo compound **25** and substituted acetylene **26** was carried out by using copper-

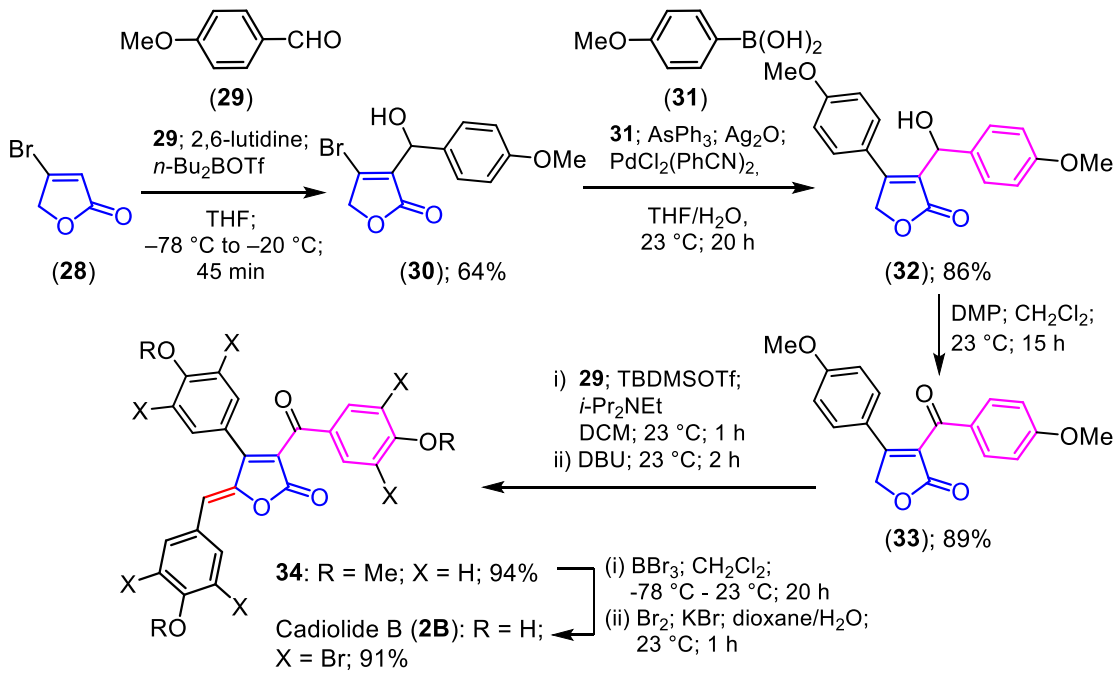
catalyzed domino reaction to afford trisubstituted lactone **27**. In the final stage, palladium catalyzed a cross-coupling reaction between bromolactone **27** with phenyl borane, which produced the desired nostoclides I and II (**1a-b**).[18]




**Scheme 3.4:** Total synthesis of nostoclides I and II, via copper-catalyzed domino route.[18]

The cadiolides (**2**) belong to the butenolide family and are also derived from marine ascidians. Similarly to the rubrolides, the cadiolides have the 4-aryl-5-benzylidenefuran-2(5*H*)-one moiety (**Fig. 3.1**; **2**). The cadiolides A and B were the first to be isolated, together with the rubrolide A. These compounds were found in ascidians of the genus *Botryllus*.[2] The cadiolides B-I were isolated more recently from the ascidian *Pseudodistoma antinboja*[6] and from the genus *Synoicum.*[4,5,7] Lastly, cadiolides J-M were found very recently in tunicate *Pseudodistoma antinboja*.[3] Cadiolide's biological activities have been readily explored. For example, cadiolides B-F and J-M present significant activity against strains of methicillin-resistant *Staphylococcus aureus* (MRSA), with MIC of <0.13-2 μg $mL^{-1}$.[3,6] In addition to the antibacterial activity, another recent publication reveals that the cadiolides E, H and I strongly inhibit the enzyme isocitrate lyase of *Candida albicans* with $IC_{50}$ of 7.62-17.16 μM.[4] Similarly, cadiolide B has been reported to inhibit the Japanese encephalitis virus at a concentration of 1 μg $mL^{-1}$.[5]

The first synthesis of cadiolides were reported in 2005 by Boukouvalas and Pouliot.[22] In this work, cadiolide B was synthesized from lactone 4-bromofuran-2(5*H*)-one (**28**) in 6 steps and 42% overall yield (**Scheme 3.5**).




**Scheme 3.5:** First total synthesis of cadiolide B (**2B**).[22]

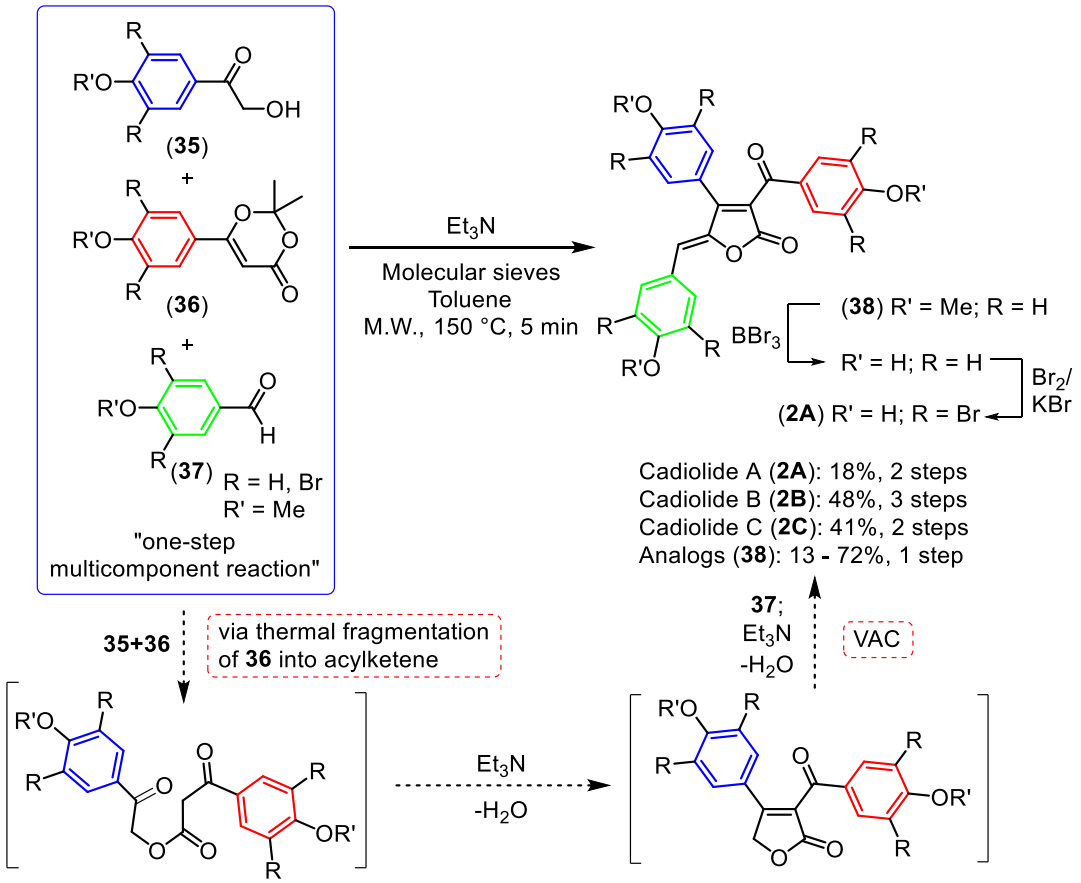
Initially, commercially available furanone (**28**) was reacted with *n*-$Bu_2$BOTf in the presence of 2,6-lutidine, obtaining the corresponding dibutylboron-2-furanolate. This furanolate was further reacted with *p*-anisaldehyde **29** to afford the alkylated product **30** in 64% yield. Then butenolide **30** was submitted to a Suzuki-Miyaura cross-coupling reaction with the respective 4-methoxyphenylboronic acid (**31**) to acquire β-aryl derivative **32**. The characteristic α-benzylic ketone was obtained by Dess-Martin oxidation with 89% yield. *Z*-stereoselective VMAC reaction was subsequentially employed to afford γ-alkylidenebutenolide **34**. Lastly, sequential reactions of demethylation and bromination yielded the polybrominated cadiolide B (**2B**).

More recently, the synthesis of cadiolides A (**2A**), B (**2B**) and C (**2C**) as well as several analogs were reported by the research group of Franck and Leleu[23,24] in only 1-3 steps, with 13-72% overall yield from a multicomponent reaction with α-hydroxyketone (**35**), dioxynone (**36**) and aromatic aldehyde (**37**) (**Scheme 3.6**).

In this procedure a multicomponent microwave reaction (300 W), using reagents **35**-**37**, triethylamine and toluene, was performed. Unbrominated butenolide **38** was obtained in 77% yield. In the special case of cadiolide B its synthesis involved sequential demethylation with $BBr_3$ (94% yield) and bromination with $Br_2$/KBr (67% yield) (**Scheme 3.6**). Correspondingly, cadiolides

A and C were obtained with this strategy using their corresponding mono and di-brominated starting materials **35**-**37**, affording in 2 steps, in 18 and 37% of overall yield, respectively, including a demethylation procedure. Finally, some analogs were produced with 13-72% yield.[24]

The mechanism of this multicomponent reaction includes: one-pot consecutive thermal fragmentation of dioxinone **35** in acylketene following by an attack of the hydroxyketone **36** in alkaline medium. An intramolecular condensation was then performed and, lastly, a simple vinylogous aldol condensation reaction with the respective aromatic aldehyde (**37**). In Franck and Leleu's procedure, the use of aliphatic substrates was not shown, but the authors revealed that the yields were moderate.[23,24]

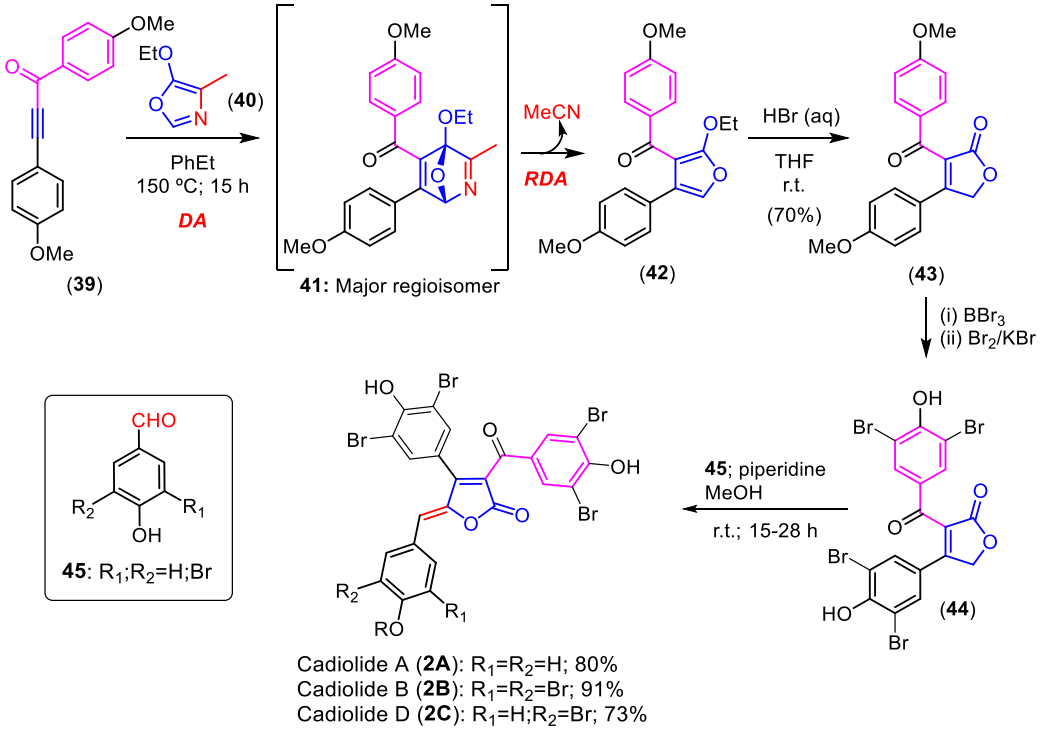



**Scheme 3.6:** Synthetic methodology for synthesis of cadiolides A (**2A**), B (**2B**), C (**2C**) and analogs.[23,24]

Is important to mention that the antibacterial activities of these compounds, as well as their methoxylated precursors were evaluated against strains of Gram-positive and Gram-negative bacteria. The methoxylated compounds were

inactive at the assessed concentration (125 μg $mL^{-1}$), while the hydroxylated compounds showed moderate to significant activity. The presence of bromine atoms and their position influenced activity.[24] The most active compounds showed MIC of 1.95 μg $mL^{-1}$ against *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella typhi* and *Escherichia coli* 405, while the standard antibiotic tetracycline presented MIC of 3.90, 0.24, 0.48, 7.81 and 7.81 μg $mL^{-1}$, in regard to each pathogen. Among the natural cadiolides, cadiolide C was the most active, with MIC of 3.90 μg $mL^{-1}$.[24]

In the same year, Boukouvalas and Thibault described the total synthesis of cadiolides A, B and D using a methodology shown previously in chapter 1.[25] This procedure includes the *de novo* synthesis of α,β-diakylatedbutenolide (**43**) in one-pot Diel-Alder/retro Diels-Alder (DA/RDA) using oxazole/inone, followed by hydrolysis reaction. Then, a Knoevenagel-like VAC reaction resulting in the formation of cadiolides A, B and D was performed (**Scheme 3.7**).



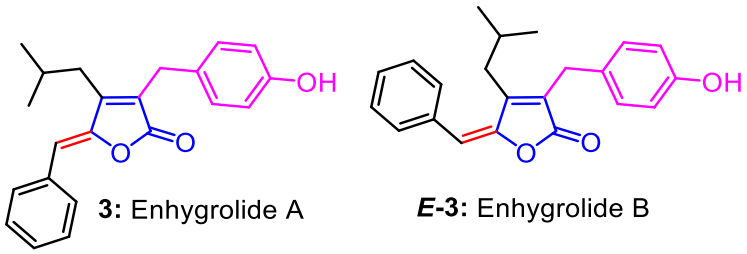

**Scheme 3.7:** Synthesis of cadiolides via DA/RDA procedure.[25]

In this synthesis, first step consists in the reaction of inone **39**, obtained by the Sonogashira coupling reaction between 4-methoxybenzoyl chloride and 1-ethynyl-4-methoxybenzene, with oxazole **40**, leading to *in situ* formation of adduct **41** through DA reaction. The RDA reaction occurs with loss of acetonitrile, forming

compound **42** which was submitted to hydrolysis using hydrobromic acid, yielding lactone **43** at 70%. One-pot demethylation/bromination reactions of **43** produced **44** with 84% yield. Finally, the reaction of Knoevenagel condensation furnished cadiolides A, B and D in yields of 80%, 65% and 73%, respectively (**Scheme 3.7**).

### 3.1.2. Enhygrolides

In 2013, König and her group reported the discovery of three new antibiotics from the hitherto unexplored marine myxobacterium *Enhygromyxa salina*.[8,26] The most potent of them, enhygrolide A (**3**), exhibited an MIC of 4 μg $mL^{-1}$ against *Arthrobacter crystallopoietes*.[8] As was mentioned, structurally, enhygrolide A features a densely-substituted (*Z*)-α-alkyl-γ-ylidenebutenolide motif reminiscent of the cyanobacterial metabolites nostoclides I and II (**1a-b**).[1,11,13,15-18] It is important to mention that, unlike the nostoclides, enhygrolide A was obtained along with its *E*-isomer, enhygrolide B (***E*-3**), although the latter could not be isolated in pure form due to conversion into the more stable *Z*-isomer **3**.[8]




**3:** Enhygrolide A ***E*-3:** Enhygrolide B

**Figure 3.3:** Enhygrolide A and B structures.

The secondary metabolites of myxobacteria, specially *Enhygromyxa salina*, are extremely scarce, so much so, that in the case of compound **3**, a mere 0.7 mg was obtained from 64 L of culture,[8] that situation, unfortunately precluded more profound biological studies. In addition to this, these marine bacteria are difficult to isolate and cultivate, leading to, only a handful of their secondary metabolites having been identified to date.[8,26-28] Following this line, enhygrolide appears as a new polysubstituted butenolide target for synthesis and biological studies.

As mentioned, some total[15,18] and formal[16,20] syntheses of α-benzyl-γ-ylidenebutenolides have been reported. These methods fall short of ideal results for several reasons including (i) modest efficiency of cross-coupling regimens for

installing the α-benzyl moiety[16,18,20] (ii) reliance on expensive or inaccessible starting materials and reagents, notably 2-furyl *N*,*N*,*N*',*N*'-tetramethyldiamidophosphate[15] and benzyl trifluoroboronates[18] and (iii) moderate yield with aliphatic substrates.[23,24] Besides these complications, these approaches can be helpful to establish a new synthetic pathway to the new butenolide targets as will be discussed further.

### 3.1.3. Antibiotic compounds discovered in marine microorganisms: myxobacteria as important source of biologically active compounds

The last half century has superbly established the importance of terrestrial bacteria and fungi as sources of valuable bioactive metabolites. Consequently, over 120 of the most important medicines in use today (penicillin, cyclosporin A, adriamycin, etc.) are obtained from these microorganisms.[29] The marine environment also represents a largely unexplored source for isolation of new microbes (bacteria, fungi, actinomycetes, microalgae-cyanobacteria and diatoms) that are potent producers of bioactive secondary metabolites.[30] The enormous expected biodiversity of marine microorganisms might have been the primary reason for the interest in their study. To date a number of biologically-active compounds with varying degrees of action, such as antitumor,[31] anticancer,[32,33] antimicrotubule,[34] photo-protective,[35] as well as antibiotic[2,8] and antifouling properties,[36,37] have been isolated from marine sources.[30]

In the past decades, studies on marine natural products have been focused mainly on macro-organisms, *i.e.* sponges, corals, ascidians and other marine invertebrates, and, to a lesser extent, on micro-organisms.[38] An example are the hitherto explored marine myxobacteria, that appear as an unique Gram-negative organisms that possess larger genomes than other taxa of bacteria and as such are capable of producing a variety of bioactive metabolites that are not found in actinomycetes or fungi.[27,28] While myxobacteria dwell in both terrestrial and marine habitats, these microbes were largely unrecognized in the world's oceans until recently. This is mainly due to the difficulties that are encountered during isolation and cultivation.[8] Therefore, to date, the pool of secondary metabolites isolated from organisms of this kind is very low, when compared to that of their

terrestrial counterparts.[8] However, herein we intend to show that these organisms are a great research niche, which offer the opportunity to uncover novel bioactive compounds with the potential to become drug leads.[27] Some examples include the macrocyclic peptidic-polyketide moiety called miuraenamide A-F from *Paraliomyxa miuraensis*. From them, a remarkable inhibitory effect towards the fungal phytopathogen, *Phytophthora capsici* NBRC 8386 (MIC: 0.4 mg $mL^{-1}$) and *Candida rugosa* AJ 14513 (MIC: 12.5 mg $mL^{-1}$), has been demonstrated.[27] Other examples include the bioactive polyketides, haliangicin (**46**)[39] and haliamide (**47**)[40] from *Haliangium ochraceum*. Haliangicin exhibited activity against the fungal organisms *Aspergillus niger* with MIC = 12.5 μg $mL^{-1}$ and *Fusarium sp*. MIC = 6.3 μg $mL^{-1}$, comparable values with known antifungal compounds, such as amphotericin B and nystatin against the same fungi (MIC = 3.1 μg $mL^{-1}$ for both compounds).[39] Furthermore, haliamide was shown to have cytotoxic effects towards HeLa-S3 cells (cervix adenocarcinoma) ($IC_{50}$ = 12 μM).[40]

With respect to *Enhygromyxa* sp., only five structures of polyketide, shikimate and terpenoid origin have been described and classified: salimabromide (**Fig. 3.4**; **48**)[26], produced by *E. salina* SWB007; the salimyxins A and B (**49**-**50**) and enhygrolides A and B (**3** and ***E*-3**) both classes produced by *E. salina* SWB005.[8] Salimabromide exhibited inhibitory activity against the bacterium *Arthrobacter crystallopoietes* with MIC = 16 μg $mL^{-1}$. Further bioactivity assays were impossible to carry out due to the minute amounts in which this metabolite is produced.[26] Consequently, a synthetic approach has been utilized in order to overcome this problem, although, the complete natural product has not yet been synthesized.[41]

The salimyxins belongs to a subgroup of terpenoids named incisterols, which were first isolated from the sponge *Dictyonella incisa*.[42] Their biosynthesis presumably involves oxidative degradation of a sterol, leading to the tricyclic core structure.[27] Compounds **49** and **50** have shown inhibitory activity towards *A. crystallopoietes* (MIC = 8 and 4 μg $mL^{-1}$, respectively).[8]

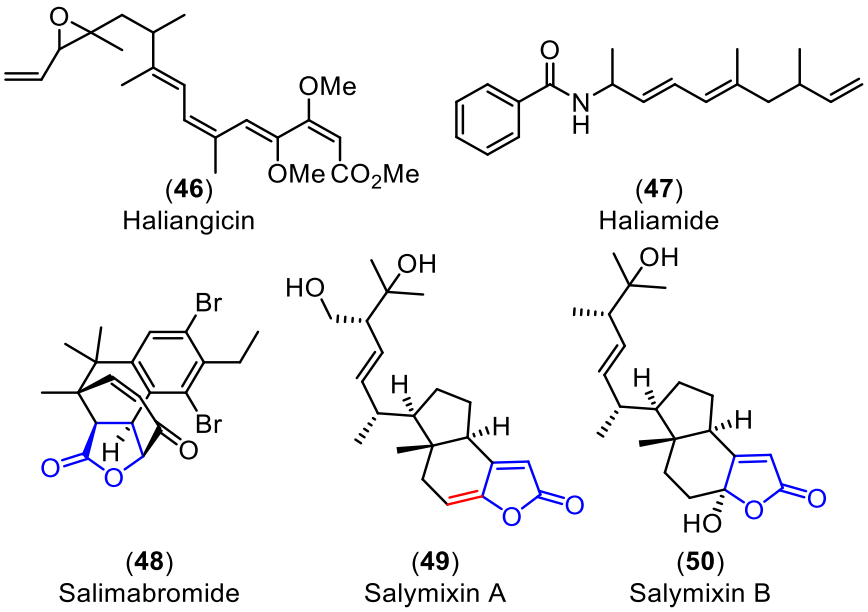
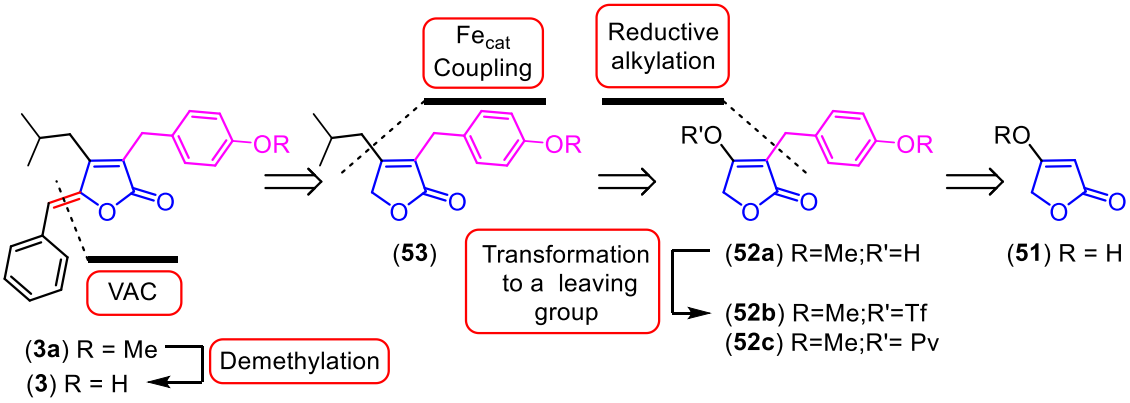



**Figure 3.4:** Examples of marine secondary metabolites.

The relentless rise in antibiotic resistance has created an urgent need for new antimicrobials.[43] Nature's small, genetically-encoded molecules have traditionally been, and still are, the best starting point for antibiotic development.[44,45] Currently, over 60% of all clinically used antibiotics come from Gram-positive, cultivable soil bacteria of the order actinomycetes.[46,47] Although these microbes continue to be studied today,[48-51] the search for new metabolites has been met with diminished returns, thus all too often leading to the rediscovery of known compounds.[44,45,48] As a result, growing attention has been drawn to new reservoirs of biodiversity, particularly the microbial communities of marine ecosystems. Clearly, extraction of secondary metabolites from marine organisms normally involves low yields and issues with isolation, as such, synthetic approaches appear as a viable alternative for the development of new concise studies with these new pharmacological targets.

### 3.1.4. Synthesis proposal

Enhygrolide A is a promising biologically-active compound and, together with its unprecedented carbon skeleton and its scarcity in natural sources, prompted us to undertake its synthesis based on the retrosynthetic analysis show in **scheme 3.8**.




**Scheme 3.8:** Retrosynthetic analysis for Enhygrolide A (**3**).

Our synthetic design starts with tetronic acid (**51**), an inexpensive source of the butenolide nucleus, onto which the appropriate benzyl, alkyl, and benzylidene substituents would be appended sequentially. In first instance, α-benzyl group is obtained by means reductive alkylation based in an organocatalytic method developed by Ramachary and co-workers,[52,53]. Later, an iron-catalyzed cross-coupling with Grignard reagents in derivatives **52b-c,** with good leaving groups will offer an appealing alternative to the Suzuki reaction for introducing the isobutyl substituent, thus obtaining β-alkylbutenolide **53**.[54] Following, vinylogous aldol condensation (VAC) methodology will allow for a stereoselective recourse for pivotal (*Z*)-γ-alkylidenebutenolide motif of enhygrolide A precursor (**3a**). finally, **3a** will be submitted to demethylation reaction resulting in the desired natural product **3**. Besides the butenolide ring, all carbon substituents would derive from simple, readily available chemicals (*cf.* benzaldehydes and RMgX). In the course of our work, we also explored the feasibility of converting **3** to enhygrolide B (***E*-3**) by means of photoisomerization. Such a process has reportedly enabled good conversion of a similarly substituted (*Z*)-γ-ylidenebutenolide to its *E*-isomer.[55]

## 3.2. OBJECTIVE AND STRATEGIES

The aim of the present chapter is to develop an efficient, cheap and green methodology for the first synthesis of enhygrolide A, starting from commercial tetronic acid. The key steps to be evaluated will include: organocatalytic reductive alkylation; iron-catalyzed cross-coupling with Grignard reagents and stereo-

controlled vinylogous aldol condensation reaction as the crucial step to obtain the desired *Z*-isomer intermediate.

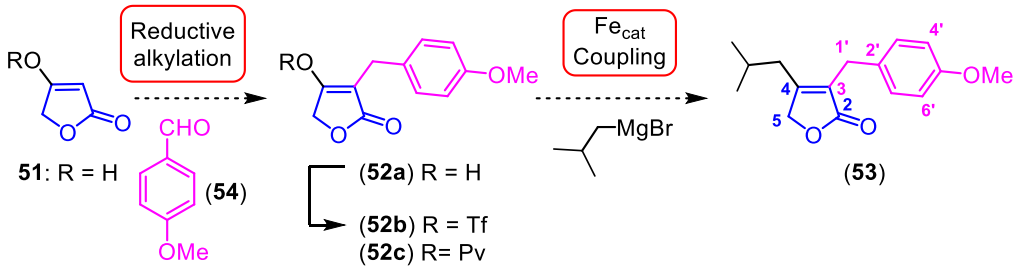
We also aim to obtain enhygrolide B (*E*-isomer) by means photochemical isomerization reaction of enhygrolide A (*Z*-isomer).

## 3.3. RESULTS AND DISCUSSIONS.

### 3.3.1. Synthesis of intermediate 53.

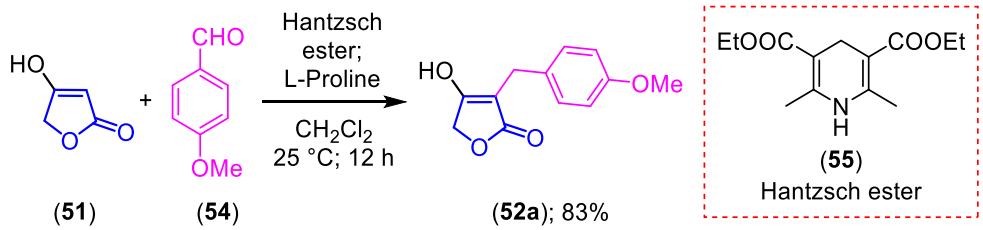
#### 3.3.1.1. *Reductive alkylation reaction for production of β-benzylbutenolide **52**.*

Enhygrolide A (**3**) synthesis begins with the production of its key precursor, α-alkylbutenolide **53**, this could be accomplished by the procedure showed in **scheme 3.9**.



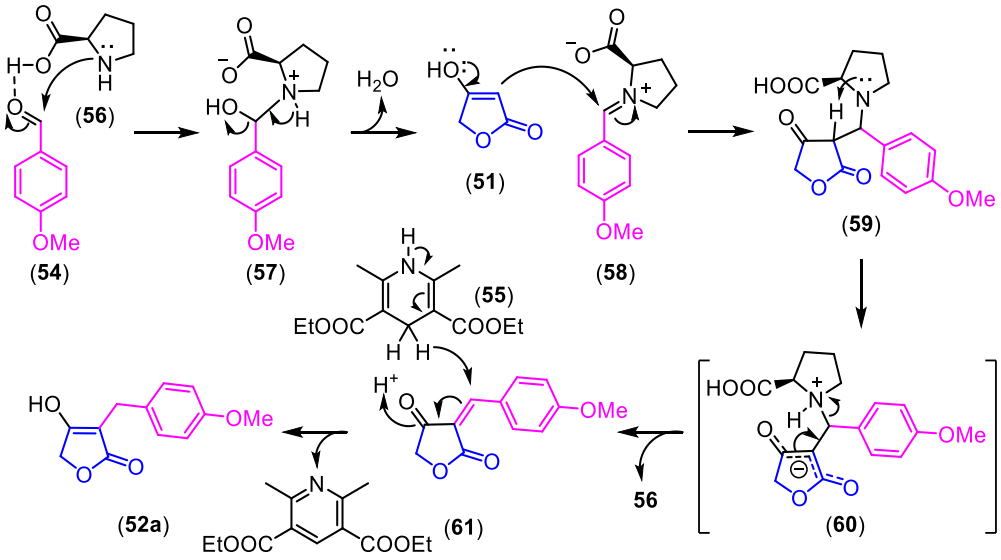

**Scheme 3.9:** Synthetic procedure for the γ-alkylbutenolide intermediate **53**.

Firstly, commercial tetronic acid (**51**) was subjected to reductive alkylation with *p*-methoxybenzaldehyde **54** using an organocatalytic method developed by Ramachary and co-workers.[52,53] This method consists in an organocatalytic chemoselective direct tandem Knoevenagel/hydrogenation/alkylation. In the sequence, the butenolide substrate tetronic acid reacts in a Knoevenagel condensation catalyzed by L-proline (5 mol%) on its activated methylene leading to *in situ* α,β-unsaturated product. Then, Hantzsch esters as the hydride source will selectively reduce the recently formed double bond. In doing so, gram quantities of the known lactone **52a** were obtained in a single operation with substantially higher yield than the current two-step procedure (83% vs 55− 63%) (**Scheme 3.10**).[56,57]




**Scheme 3.10:** Synthesis conditions for compound **52a**.

The mechanism of the reaction is described in **scheme 3.11**.[53] The first step in this mechanism shows how catalytic L-proline (**56**) activates the *p*-anisaldehyde (**54**), producing the highly reactive intermediate, enamine **58**. Recently, amino acid catalysis has emerged as a powerful green synthetic tool for the development of both achiral and chiral catalysis of condensations with many electrophiles.[53] Next, condensation occurs between tetronic acid **51** and intermediate **58**, leading to an α,β-unsaturated product (**61**), which is susceptible to the sequential reduction carried out by the hydride source, in this case Hantzsch ester (**55**). Then, desired α-benzylbutenolide (**52a**) is formed. This ester is a biomimetic NAD(P)H model that is very used in reductions of α,β-unsaturated aldehydes and ketones.[58,59]




**Scheme 3.11:** Reductive alkylation mechanism.

The structure of compound **52a** was determined through spectroscopic analyzes. IR spectrum shows the characteristic bands of alkyl C-H and O-H stretch combined in a broad band around 2984 $cm^{-1}$. The typical α,β-unsaturated carbonyl bond is seen at 1771 $cm^{-1}$, the CO bond stretch at 1248 $cm^{-1}$ and 1098 $cm^{-1}$.

The $^{1}$H NMR spectrum analysis of **52a** (**Fig. 3.6**) shows one small broad signal corresponding to the OH group at $\delta$ = 3.18. The methylene of $\alpha$-benzylic group is identified at $\delta$ = 3.43. Next, two singlet signals are observed, one at $\delta$ = 3.74 for methoxy group, and another at $\delta$ = 4.63 for the two methylene hydrogens of the H-5 lactonic ring. Two doublets may also be seen, each one corresponding to the two hydrogens of the di-substituted aromatic ring are located at $\delta$ = 7.17 and 6.81 ($J$ = 8.6 Hz).

Lastly, the $^{13}$C NMR spectrum of **52a** (**Fig. 3.7**), shows the benzylic methylene at $\delta$ = 26.8. Two signals at $\delta$ = 55.4 and 67.2, corresponding to the methoxy and C-5 carbon signals, respectively. Later, C-3 signal at $\delta$ = 101.4. Then, two intense signals were observed, corresponding to two carbons with the same chemical environment with chemical shift of 130.1 ppm for C-3'-7' and 114.4 ppm for C-4'-6'. The signals of aromatic C-2' and C-5’ may be placed at $\delta$ = 132.3 and $\delta$ = 159.0, respectively. Lastly, the carbonyl signal appears at $\delta$ = 173.1 and the $\beta$-position C-4 signal is located at $\delta$ = 175.3.

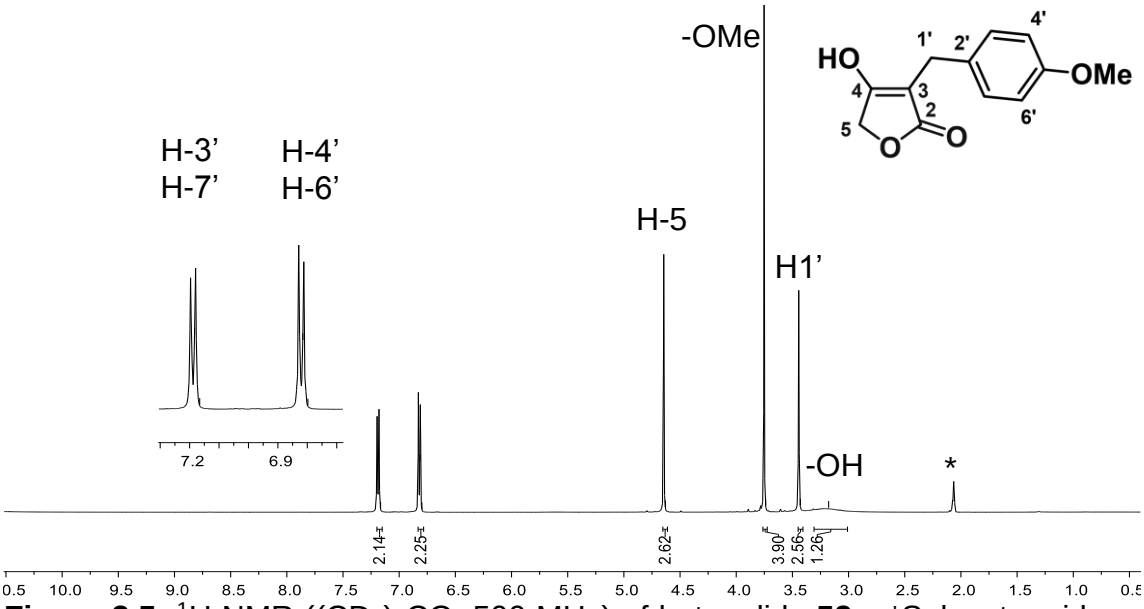



**Figure 3.5:** $^{1}$H NMR ($(CD_3)_2CO$, 500 MHz) of butenolide **52a**. *Solvent residues signal.

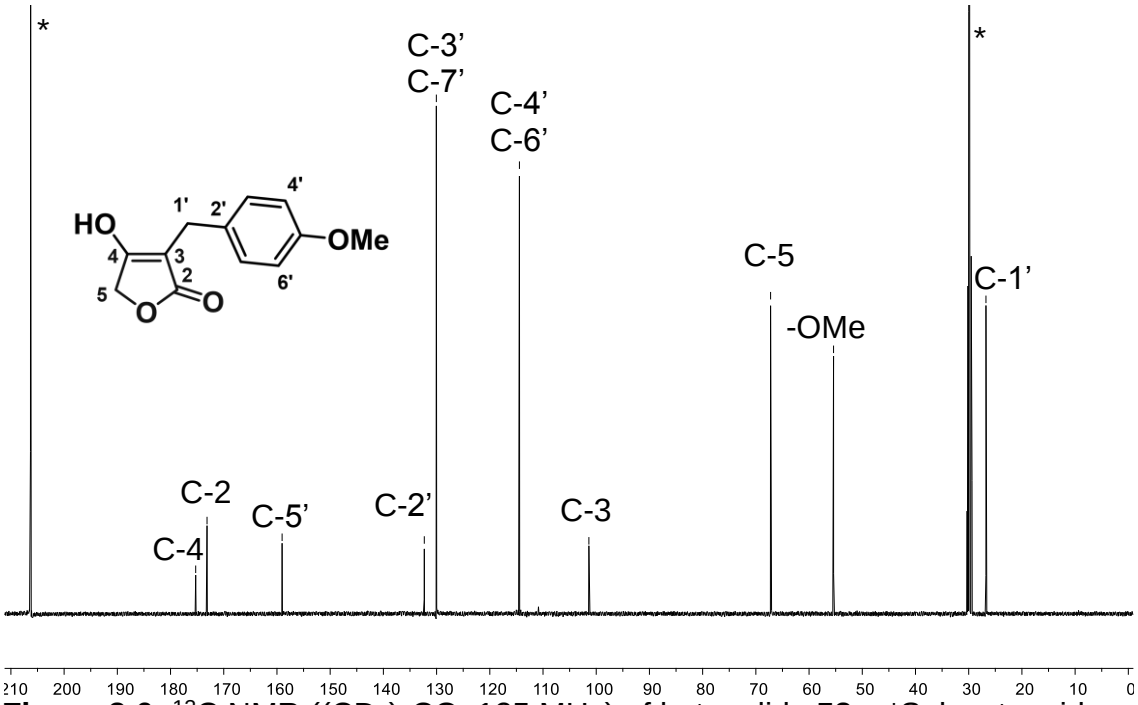


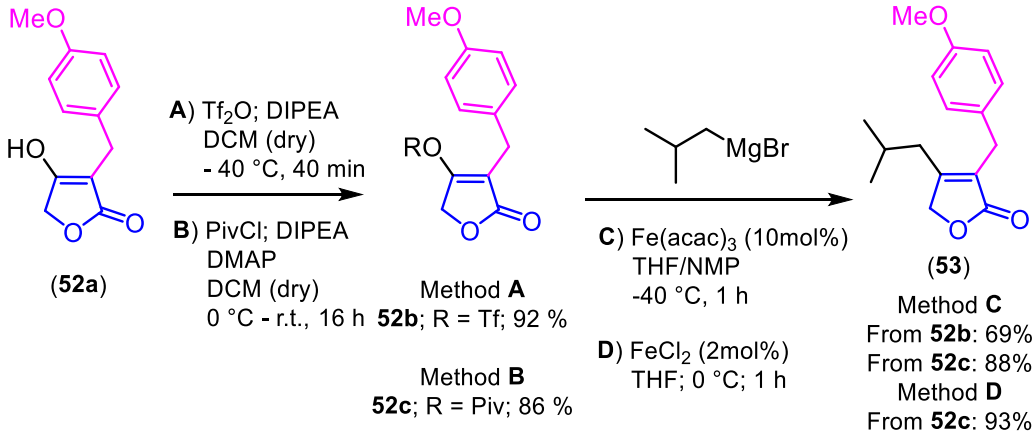

**Figure 3.6:** $^{13}C$ NMR ($(CD_3)_2CO$, 125 MHz) of butenolide **52a**. *Solvent residues signal.

3.3.1.2. *Fe catalyst/Grignard reagent cross-coupling reaction for production of compound **53**.*

The next step includes the addition of the β-alkyl group, which will be accomplished through the use of iron catalyst cross-coupling reaction. Our synthetic plan includes the transformation of the β-hydroxy group in butenolide **52a** to a good leaving group, leading to an adequate coupling substrate **52b-c** (**Scheme 3.12**). Prior research by Boukouvalas group suggests that this type of coupling in butenolide triflates using Grignard reagents offers an appealing alternative to the Suzuki reaction for introducing the isobutyl substituent.[54] In this manner, α-benzylbutenolide **52a** was converted to triflate **52b** and pivalate **52c**. In the present instance, reaction of **52b-c** with *i*-BuMgBr under slightly modified Fürstner conditions,[60,61] (10%mol $Fe(acac)_3$, *N*-methylpyrrolidone/ tetrahydrofuran (NMP/THF), −40 °C, 1 h) provided butenolide **53** in a respectable yield of 69% and 88% respectively (**Scheme 3.12**).

While other iron catalysts and conditions were somewhat less effective (data not shown), we can appreciate that switching to the more robust pivalate leaving group proved highly rewarding. At that point, employing optimal reaction

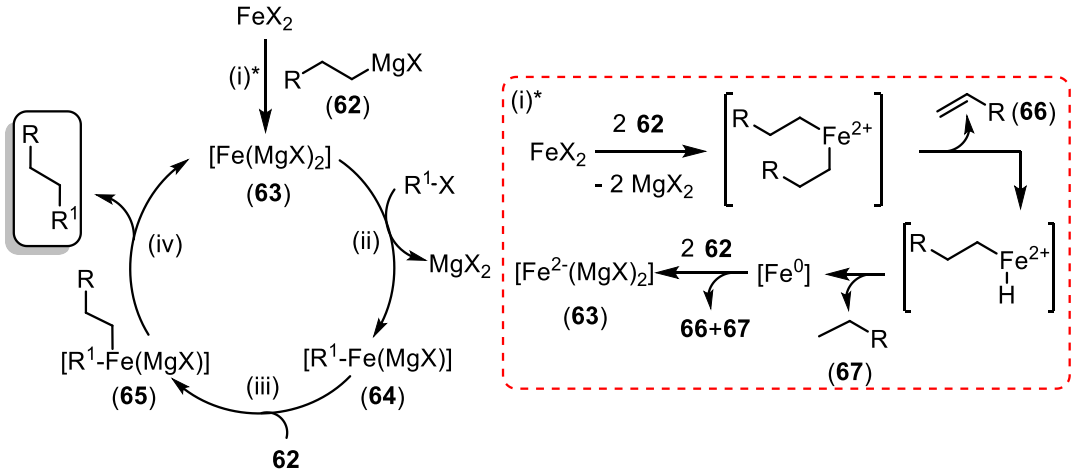
conditions as lower ligand-free catalyst loading (2 mol% $FeCl_2$, THF, 0 °C, 1 h), the best results were obtained.




**Scheme 3.12:** Synthesis conditions for α,β-dialkylbutenolide **53**.

The coupling of pivalate **52c** with *i*-BuMgBr was remarkably clean and afforded **53** in 93% of yield after flash column chromatography. This is the first cross-coupling reaction of a butenolide pivalate. Additionally, it allows us to obtain the desired compound in better yields than in achieved with a leaving group such as triflate. Similarly, the use of low catalyst loading of readily-available more accessible iron compound, such as $FeCl_2$, allow us to establish a new alternative for this type of coupling.

This Iron catalyst coupling method is a cheap, mild, readily-available, air stable, nontoxic, and environmentally benign alternative to other classes of metallic couplings.[61] Its mechanism is described in the **Scheme 3.13.** In this case, Grignard species EtMgBr and longer chain homologues with an ideal iron pre-catalyst produce low-valent iron cluster species (such formation can be appreciated in the reaction with a dark brown color) (**Scheme 3.13**; (i); **63**). These species will then activate aryl halides or related substrates.[60] Following, the reaction of **63** with an organic halide (or triflate/pivalate **52b-c** in our case) constitutes a σ-bond metathesis rather than a “conventional” oxidative insertion (ii).[60] Because such a process does not generate an organoiron halide, the subsequent reaction with RMgX (**62**) must occur by alkylation rather than by regular transmetalation of the intermediate primarily formed (iii). The resulting diorganoiron species then undergoes reductive elimination (iv) to afford the product and regenerate the catalyst.[60]




**Scheme 3.13:** Fe catalyst/Grignard reagent cross coupling reaction mechanism; (i) Low-valent iron cluster production, (ii) σ-bond metathesis, (iii) Alkylation, (iv) Reductive elimination.

Structural confirmation of compounds **52b-c** and **53** were determined by spectroscopic analysis. Initially IR spectrum of both derivatives (**52b-c**) exhibited the absence of typical -OH stretch, evidencing the chemical modification. For triflate **52b**, bands of S=O stretch were present at 1250 and 1136 $cm^{-1}$. For pivalate **52c** the band for C-F bond at 1046 $cm^{-1}$ as well as a second carbonyl signal of nonconjugate ester could be seen at 1766 $cm^{-1}$.

In comparison, the $^{1}H$ NMR spectrum of the triflate **52b** have not noteworthy difference with the starting material **52a**. The $^{1}H$ NMR of pivalate **52c** just shows characteristic signals of newly -$CH_3$ of -OTf group as a singlet at $\delta = 1.27$. Lastly, the $^{1}H$ NMR of coupling product **53** presented the recently added *i*-Bu group, firstly as a -$CH_2$ doublet at $\delta = 2.31$ ($J = 7.5$ Hz), then a septet for the -CH can be observed at $\delta = 1.79$ ($J = 6.9$ Hz) and lastly, another doublet signal for methyl groups at $\delta = 0.90$ ($J = 6.6$ Hz) (**Fig. 3.7-9**).

Finally, spectrum of compound **52b** shows the signals corresponding to the triflate group at 119.8 and 118.4 ppm (q, $^{1}J_{C-F} = 321.2$ Hz; -$CF_3$). The $^{13}C$ NMR of pivalate **52c** have the signals present at 39.5 ppm for quaternary C (-$CCH_3$) and 26.9 ppm for the corresponding methyl groups. To conclude, the key intermediate α-isobutyl butenolide **53**, was revealed its signals at $\delta = 36.4$ (-$CH_2$), 27.8 (-CH), 22.7 (-$CH_3$) (**Fig. 3.10-12**).

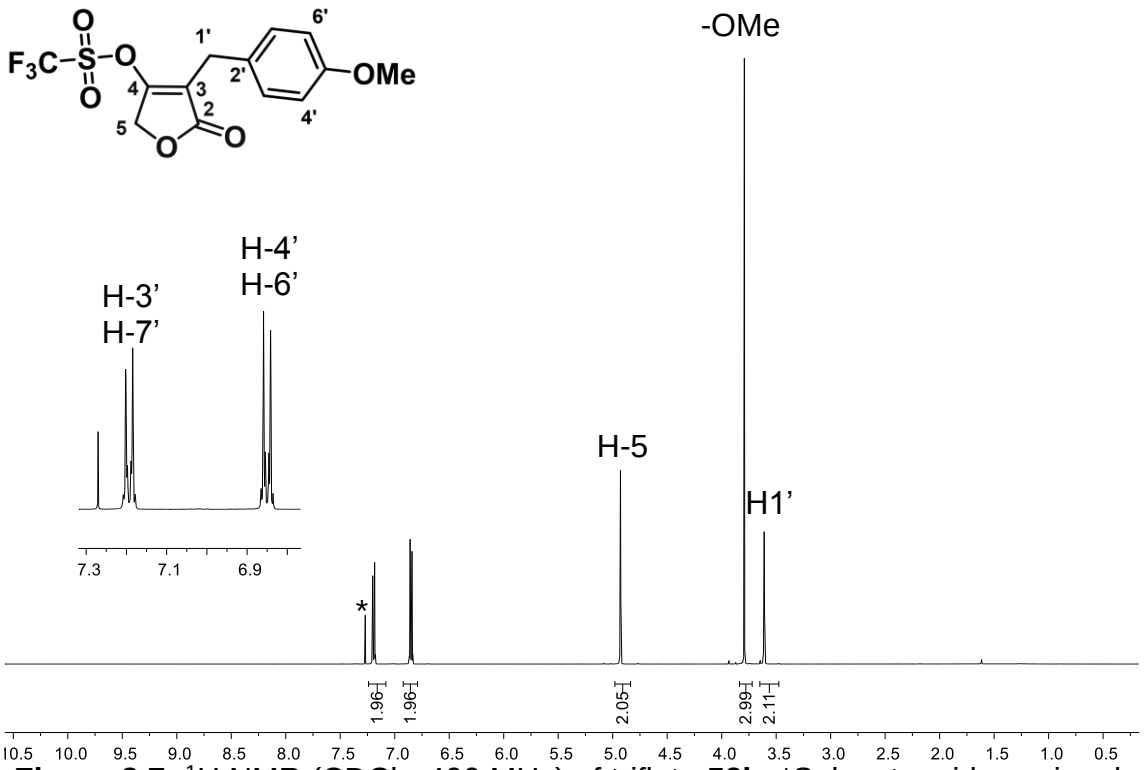



**Figure 3.7:** $^{1}$H NMR ($CDCl_3$, 400 MHz) of triflate **52b**. *Solvent residues signal.

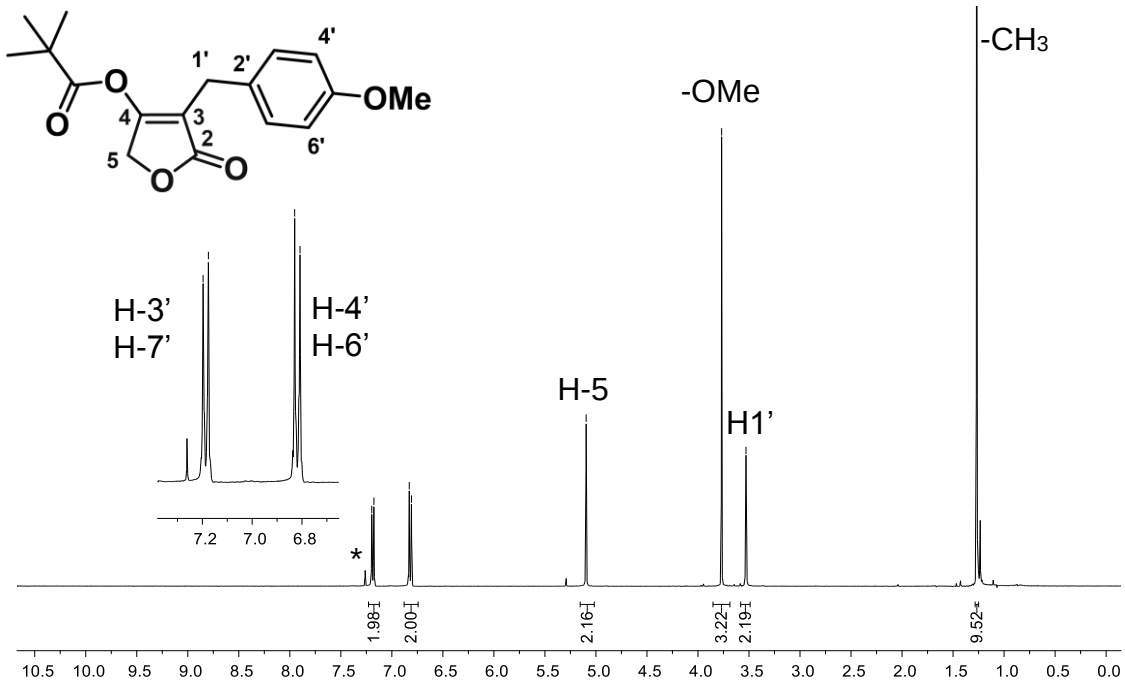



**Figure 3.8:** $^{1}$H NMR ($CDCl_3$, 400 MHz) of pivalate **52c**. *Solvent residues signal.

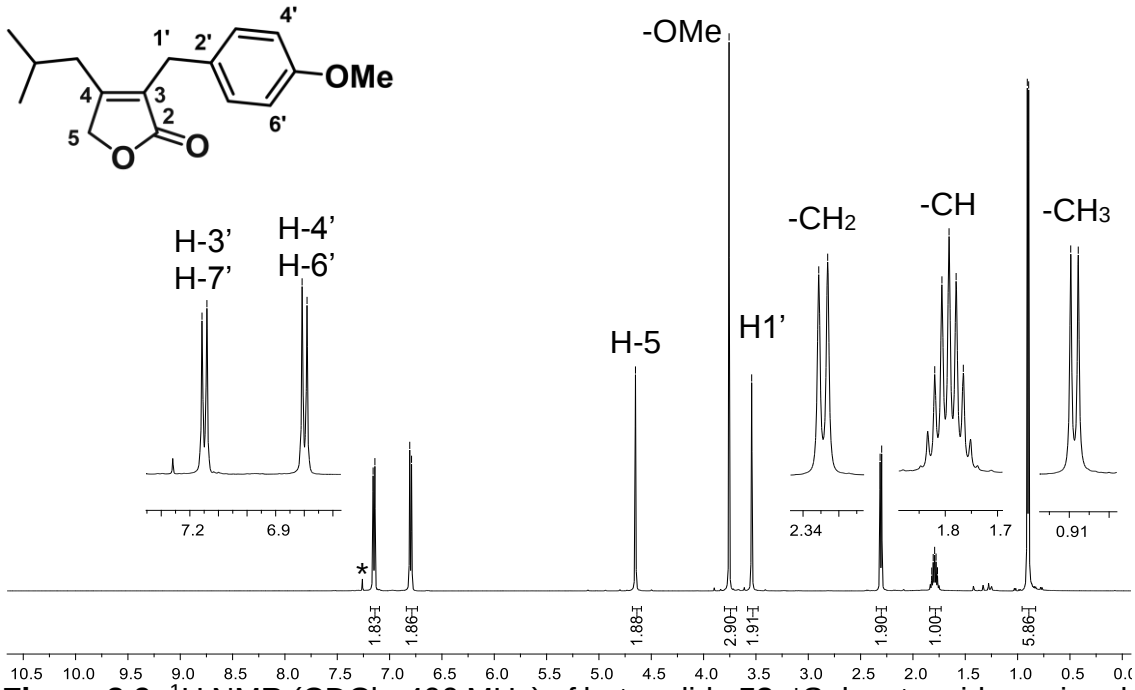



**Figure 3.9:** $^{1}H$ NMR ($CDCl_3$, 400 MHz) of butenolide **53**. *Solvent residues signal.

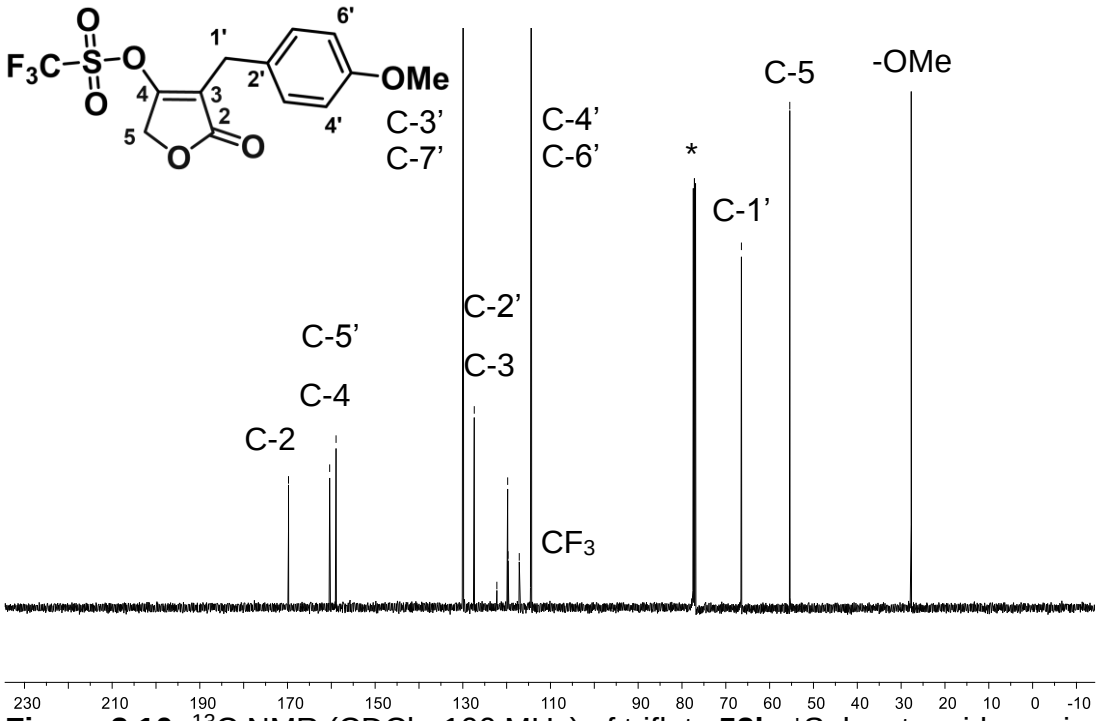



**Figure 3.10:** $^{13}C$ NMR ($CDCl_3$, 100 MHz) of triflate **52b**. *Solvent residues signal.

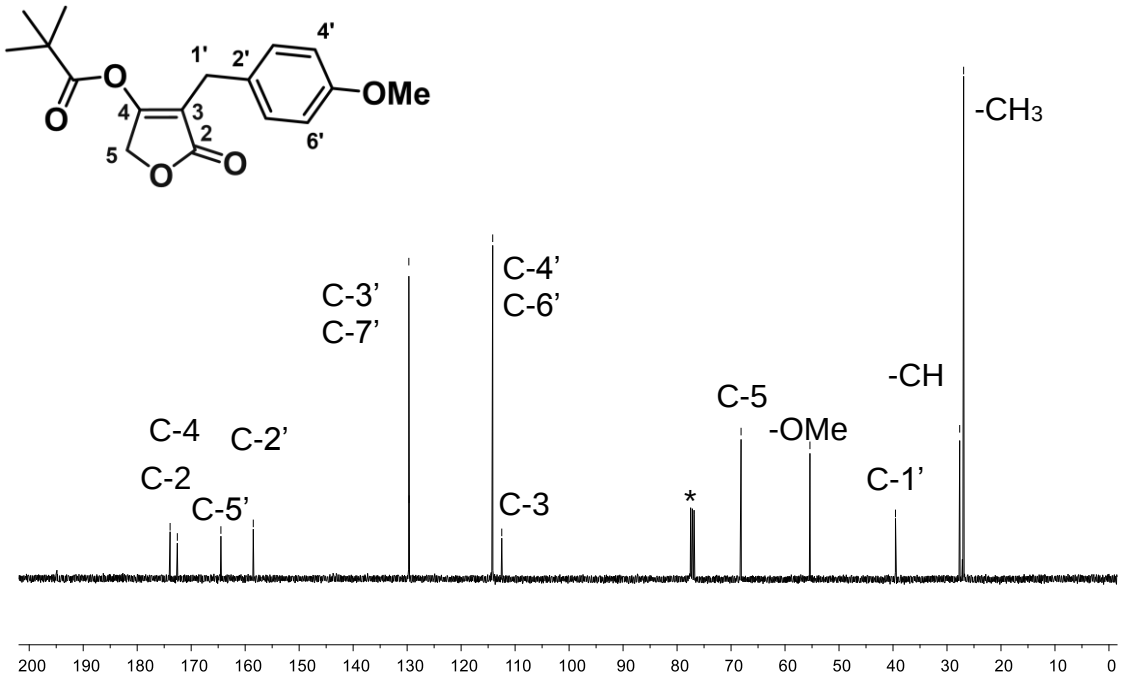



**Figure 3.11:** $^{13}C$ NMR ($CDCl_3$, 100 MHz) of pivalate **52c**. *Solvent residues signal.

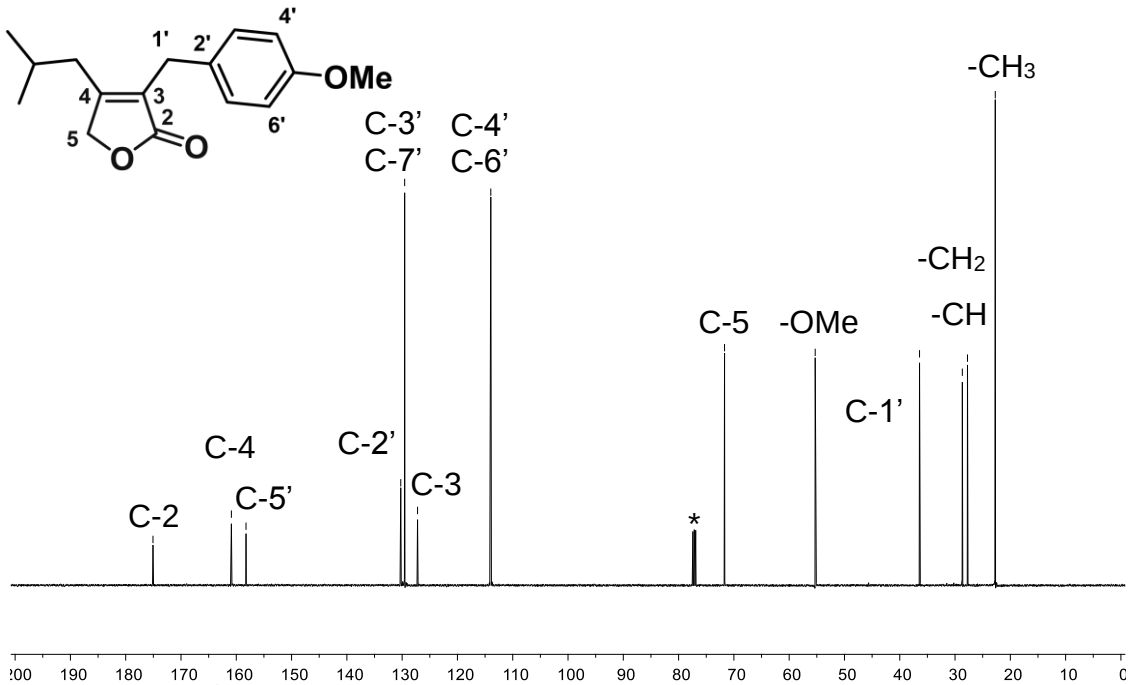
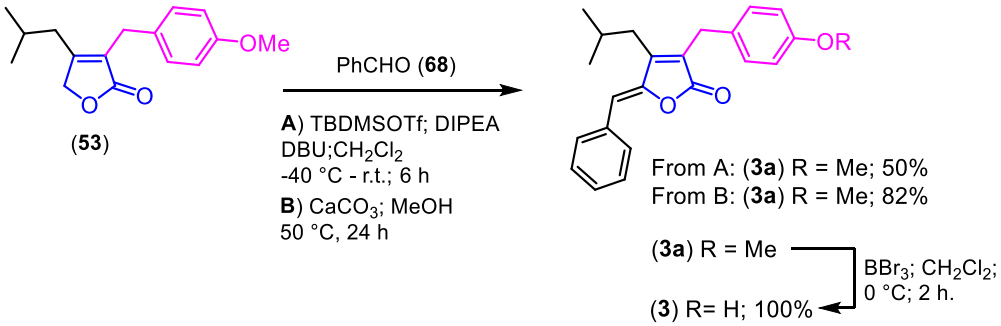



**Figure 3.12:** $^{13}C$ NMR ($CDCl_3$, 100 MHz) of butenolide **53**. *Solvent residues signal.

### 3.3.2. Synthesis of Enhygrolide A (3).

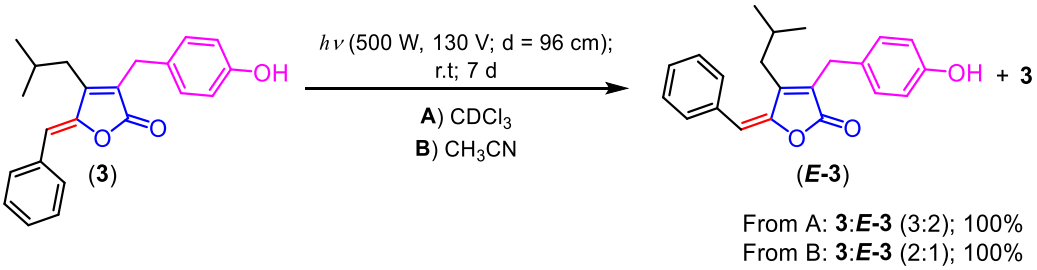
After obtaining **53**, the *Z*-configured benzylidene moiety could be installed in order to generate **3a** (**Scheme 3.14**). As described in this work, VAC reactions of butenolides having a bulky β-substituent, such as Ar, *i*-Pr, or Br, generally lead to *Z*-products, whereas those bearing small substituents (*e.g.*, Me) provide *Z*/*E* mixtures.[62,63] Since the isobutyl group in **53** falls in between these categories, we were pleased to find that the desired VAC led exclusively to the *Z*-isomer, regardless of the method employed (TBSOTf/DIPEA/DBU/$CH_2Cl_2$,[64] or sodium carbonate[62] in MeOH). Furthermore, the latter procedure[62] proved responsive to optimization (50 °C, 24 h), affording **3a** in a reproducible 82% yield. Finally, methyl ether cleavage with boron tribromide straightforwardly led to enhygrolide A (**3**, **Scheme 3.14**), whose $^1H$ and $^{13}C$ NMR spectra were in excellent agreement with those reported in the literature as can be confirmed in **Table 3.1**.[8]




**Scheme 3.14:** Synthesis conditions for enhygrolide A (**3**).

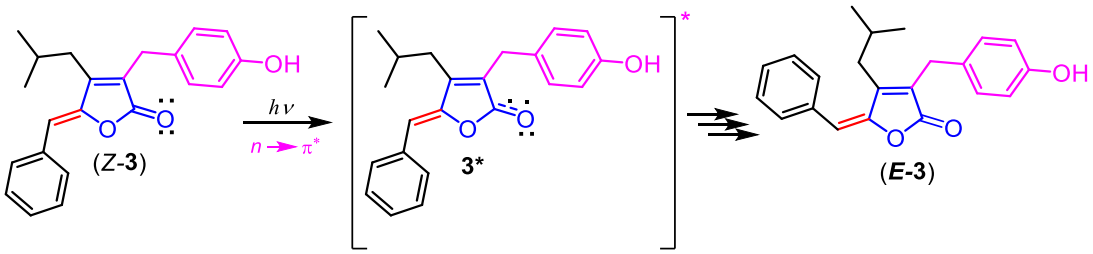
### 3.3.3. Photoisomerization for the synthesis of enhygrolide B (*E*-3).

In the course of our work, we also explored the feasibility of converting **3** to enhygrolide B (***E*-3**) by means of photoisomerization. Such a process has reportedly enabled 72% conversion of a similarly substituted (*Z*)-γ-ylidenebutenolide to its *E*-isomer.[10] In this instance, exposure of a $CDCl_3$ solution of **3** to light for 6−8 days at room temperature afforded a 3:2 mixture of the *Z*/*E*-isomers, essentially free from by-products. Likewise, clean photoequilibration could be achieved in acetonitrile, albeit still in favor of the *Z*-isomer (**3**/***E*-3** ca. 2:1). The $^1H$ and $^{13}C$ NMR spectra were in excellent agreement with those reported in the literature as can be confirmed in **Table 3.2**.[8]




**Scheme 3.15:** Photoisomerization conditions for the synthesis of Enhygrolide B (***E*-3**).

Photochemical reaction is an important phenomenon in this class of molecules, owing to its rich carbonyl conjugation system. Conjugated carbonyls are often good sensitizers, thanks to the efficiency of intersystem crossing from the $n \rightarrow \pi^*$ singlet to the triplet (**3***). In our case it may be expected that the formation of an excited radical species of carbonyl oxygen will drive to the resonance states leading to isomerization process when a partial single bond is formed in γ-position (**Scheme 3.14**).




**Scheme 3.14:** Proposal photochemical isomerization.

**Table 3.1:** Comparative $^{1}H$ and $^{13}C$ NMR data between enhygrolide A synthesized and isolated.

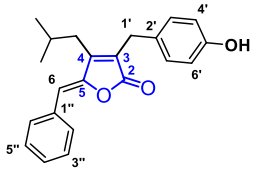
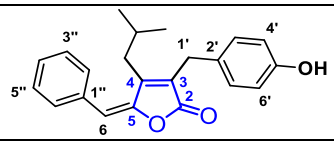
|  | | Synthetic Product | Natural Product[8] |
|---|---|---|---|
| | | 500 MHz | 500 MHz |
| $^{1}H$ NMR (**$CD_3OD$**) | H-2'' and H-6'' | 7.80 (d, *J* = 7.6 Hz, 2H) | 7.85 (d, *J* = 7.6 Hz, 2H) |
| | H-3'' and H-5'' | 7.39 (t, *J* = 7.6 Hz, 2H) | 7.44 (t, *J* = 7.6 Hz, 2H) |
| | H-4'' | 7.31 (t, *J* = 7.6 Hz, 1H) | 7.37 (t, *J* = 7.6 Hz, 1H) |
| | H-3' and H-7' | 7.06 (d; *J* = 8.5 Hz; 2H) | 7.11 (d; *J* = 8.8 Hz; 2H) |
| | H-2' and 6' | 6.71 (d; *J* = 8.5 Hz; 2H) | 6.74 (d; *J* = 8.8 Hz; 2H) |
| | H-6 | 6.25 (s, 1H) | 6.31 (s, 1H) |
| | H-1' | 3.65 (s; 2H) | 3.69 (s; 2H) |
| | -$CH_2$ | 2.50 (d; *J* = 7.5 Hz; 2H) | 2.56 (d; *J* = 7.6 Hz; 2H) |
| | -CH | 2.00–1.84 (m, 1H) | 1.97 (m, 1H) |
| | -$CH_3$ | 0.96 (d; *J* = 6.6 Hz; 6H) | 1.01 (d; *J* = 6.6 Hz; 6H) |
| | | 125 MHz | 125 MHz |
| $^{13}C$ NMR (**$CD_3OD$**) | C-2 | 172.0 | 172.3 |
| | C-5' | 157.0 | 157.2 |
| | C-4 | 154.2 | 154.4 |
| | C-5 | 149.8 | 150.1 |
| | C-1'' | 134.6 | 134.8 |
| | C-2'' and C-6'' | 131.4 (2C) | 131.5 (2C) |
| | C-3' and C-7' | 130.3 (2C) | 130.5 (2C) |
| | C-2' | 129.9 | 130.1 |
| | C-3'' and C-5'' | 129.6 (2C) | 129.8 (2C) |
| | C-4'' | 128.3 | 129.8 |
| | C-3' and C-7' | 116.2 (2C) | 116.4 (2C) |
| | C-6 | 110.9 | 111.0 |
| | -$CH_2$ | 34.2 | 34.3 |
| | -CH | 30.3 | 30.7 |
| | C-1' | 29.5 | 29.7 |
| | -$CH_3$ | 22.8 | 23.0 |

**Table 3.2:** Comparative $^{1}H$ and $^{13}C$ NMR data between Enhygrolide B synthesized and isolated.

|  | | Synthetic Product | Natural Product[8] |
|---|---|---|---|
| | | 500 MHz | 500 MHz |
| $^{1}H$ NMR (**$CD_3OD$**) | H-2'' and H-6'' | 7.80 (d, *J* = 7.6 Hz, 2H) | 7.83 (d, *J* = 7.6 Hz, 2H) |
| | H-3'' and H-5'' | 7.39 (t, *J* = 7.6 Hz, 2H) | 7.43 (t, *J* = 7.6 Hz, 2H) |
| | H-4'' | 7.31 (t, *J* = 7.6 Hz, 1H) | 7.37 (t, *J* = 7.6 Hz, 1H) |
| | H-3' and H-7' | 7.06 (d; *J* = 8.8 Hz; 2H) | 7.08 (d; *J* = 8.8 Hz; 2H) |
| | H-6 | 6.89 (s, 1H) | 6.95 (s, 1H) |
| | H-2' and 6' | 6.71 (d; *J* = 8.8 Hz; 2H) | 6.73 (d; *J* = 8.8 Hz; 2H) |
| | H-1' | 3.59 (s; 2H) | 3.64 (s; 2H) |
| | $-CH_2$ | 2.30 (d; *J* = 7.6 Hz; 2H) | 2.35 (d; *J* = 7.6 Hz; 2H) |
| | -CH | 1.24-1.15 (m, 1H) | 1.24 (m, 1H) |
| | $-CH_2$ | 0.45 (d; *J* = 6.6 Hz; 6H) | 0.50 (d; *J* = 6.6 Hz; 6H) |
| | | 125 MHz | 125 MHz |
| $^{13}C$ NMR (**$CD_3OD$**) | C-2 | 171.7 | 171.8 |
| | C-5' | 157.0 | 157.2 |
| | C-4 | 151.6 | 151.8 |
| | C-5 | 149.8 | 150.1 |
| | C-1'' | 134.6 | 134.8 |
| | C-2'' and C-6'' | 131.4 (2C) | 130.8 (2C) |
| | C-3' and C-7' | 130.3 (2C) | 130.4 (2C) |
| | C-2' | 129.9 | 130.1 |
| | C-3'' and C-5'' | 129.6 (2C) | 129.8 (2C) |
| | C-4'' | 129.3 | 129.8 |
| | C-6 | 116.4 | 116.4 |
| | C-3' and C-7' | 116.2 (2C) | 116.4 (2C) |
| | $-CH_2$ | 34.2 | 35.7 |
| | C-1' | 30.3 | 29.7 |
| | -CH | 29.5 | 29.4 |
| | $-CH_3$ | 22.8 | 22.2 |

## 3.4. CONCLUSIONS AND PERSPECTIVES

Herein the first synthesis of the scarce naturally-occurring antibiotic, Enhygrolide A originating from the obligate marine myxobacteria *E. salina*, was described. This synthesis was accomplished in five steps with 54% overall yield from commercially-available reagents, such as tetronic acid, Grignard reagents, $FeCl_2$ catalyst and aldehydes. The route is efficient and scalable, relies on green processes and inexpensive reagents, while demonstrating, for the first time, the exceptional performance of a butenolide pivaloate in an iron-catalyzed cross-coupling.

Again, a stereoselective vinylogous aldol condensation is shown to be a key step and applicability in the synthesis of γ-substituted butenolides rendered excellent results.

Efficient, economical and environmentally friendly production of this class of rare compounds are an optimal alternative to their standard and efficient acquisition, owing to its normal scarce in nature. Therefore, the possibility for the development of novel biological studies can be achieved.

## 3.5. METHODOLOGIES

### 3.5.1. General experimental techniques

All commercial reagents were used as received. Moisture sensitive reactions were carried out on flame dried glass material sealed in dry air atmosphere. The solvents used were either analytical grade or purified. Moisture sensitive liquids, solutions and anhydrous solvents were transferred via the syringe or cannula through rubber septa. When necessary the reactions were carried out under a dry Air atmosphere. Light-sensitive reactions were made in the dark involving the vials in aluminum foil.

TLC analysis was performed on 0.20 mm Polygram-UV254 aluminum support silica gel plates, Macherey-Nagel (20 x 20 cm). After elution, the plates were observed under ultraviolet light ($\lambda$ = 254 nm) and developed with solutions of $KMnO_4$. Flash column chromatography was performed on a Teledyne Isco Combi-flash Rf-200 UV−vis using Silicycle silica gel 60 (230−400 mesh) as stationary phase.

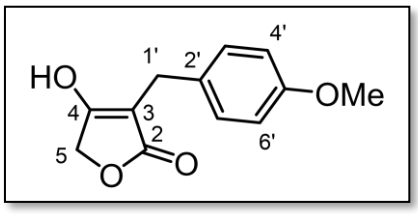
Uncorrected melting temperatures were obtained from the Barnstead Electrothermal Mel-Temp 1201D apparatus in open capillary tubes. Purification of Hüning's base (DIPEA), $CH_2Cl_2$ and THF were performed as described in the section 5.3.3 in chapter I.

### 3.5.2. General spectroscopic techniques.

$^1H$ and $^{13}C$ NMR spectroscopic data were recorded at 500 or 400 and 125 or 100 MHz, respectively, with an Agilent DD2 spectrometer with $CDCl_3$ ($\delta_H$ 7.26; $\delta_C$ 77.16) and CD3OH ($\delta_H$ 3.31; $\delta_C$ 49.00) as solvent. Chemical shifts of $^1H$ and $^{13}C$ NMR spectra were reported in ppm and related to solvent signals. All coupling constants (*J* values) are expressed in Hertz (Hz). Multiplicities are reported as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), sextet (sext), septet (sept), multiplet (m), and broad (brd). Infrared spectrum was recorded on a Bomem Arid Zone MB-series FTIR instrument, with samples prepared on single NaCl plates, scanning from 4000 to 500 $cm^{-1}$. Electrospray ionization (ESI) high-

resolution mass spectrum was recorded on a time-of-flight Agilent 6210 TOF LC/MS instrument.

### 3.5.3. Reductive alkylation procedures for the synthesis of 4-hydroxy-3-(4-methoxybenzyl)furan-2(5*H*)-one (52a).




In a 50 mL round-bottom flask, a solution of tetronic acid (**51**) (502 mg, 5.02 mmol, 1.0 eq), *p*-anisaldehyde (**54**) (2.05 g, 15.06 mmol, 3.00 eq), and Hantzsch ester **55** (1.27 g, 5.02 mmol, 1.0 eq) in dry $CH_2Cl_2$ (15 mL) and L-proline (28.9 mg, 0.25 mmol, 0.05 eq) was added, and the reaction mixture was stirred for 12 h at rt. The volatiles were evaporated under reduced pressure, and the residue was purified by flash chromatography (5% MeOH:$CH_2Cl_2$) to give final $\alpha$-benzyl-butenolide (**52a**) (920 mg, 83%) as a yellow solid.

**Characteristics:** Yellow Solid.

**Mp:** 161−162 °C.

**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 2984, 2691, 1771, 1456, 1361, 1248, 1098, 1027, 789.

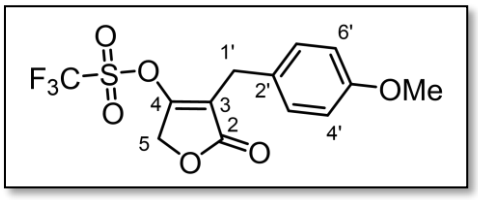
**$^1$H NMR (500 MHz; $(CD_3)_2CO$) $\delta$:** 7.17 (d; *J* = 8.6 Hz; 2H; H-3’ and H-7’), 6.81 (d, *J* = 8.6 Hz, 2H; H-4’ and H-6’), 4.63 (s, 2H; H-5), 3.74 (s, 3H; -OMe), 3.43 (s, 2H; H-1’), 3.18 (brd, 1H; -OH).

**$^{13}$C NMR (125 MHz. $CDCl_3$) $\delta$:** 175.3 (C-4), 173.1 (C-2), 159.0 (C-5’), 132.3 (C-2’), 130.1 (2C; C-3’ and C-7’), 114.4 (2C; C-4’ and C-6’), 101.4 (C-3), 67.2 (C-5), 55.4 (-OMe), 26.8 (C-1’).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{12}H_{13}O_4$ 221.0808; found 221.0807.

**Elemental analysis:** calculated for $C_{12}H_{12}O_4$, C, 65.45; H, 5.49; Found: C: 65.54; H: 5.67.

### 3.5.4. Synthesis of 4-(4-methoxybenzyl)-5-oxo-2,5-dihydrofuran-3-yl trifluoromethanesulfonate (52b)




In a 100 mL round-bottom flask, a solution of α-benzylfuran-2(5*H*)-one (**52a**) (630 mg, 2.86 mmol, 1.0 eq) and Hünig's base (1.0 mL, 5.72 mmol, 2.0 eq) in dry $CH_2Cl_2$ (40 mL) was cooled to -40 °C. Trifluoromethanesulfonic anhydride (0.63 mL, 3.72 mmol, 1.3 eq) was added dropwise over 10 min. After stirring for 50 min at -40 °C, TLC indicated complete consumption of starting material, at which point $H_2O$ (100 mL) was added and the temperature allowed to rise to room temperature. Once all the ice had melted, the organic layer was separated and the aqueous layer was extracted with $CH_2Cl_2$ (3 x 200 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated in vacuo. Purification by flash column chromatography (10% AcOEt:hexane) furnished **52b** (932 mg, 92%).

**Characteristics:** Bright orange oil.

**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 2840, 1773, 1514, 1250, 1222, 1136, 1046, 811, 609.

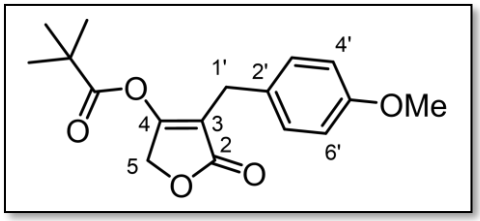
**$^1$H NMR (500 MHz. $CDCl_3$)** $\delta$**:** 7.18 (d; *J* = 8.7 Hz; 2H; H-3' and H-7'), 6.84 (d; *J* = 8.7 Hz; 2H; H-2' and 6'), 4.92 (s; 2H; H-5), 3.78 (s; 3H; -OMe), 3.60 (s; 2H; H-1').

**$^{13}$C NMR (125 MHz. $CDCl_3$)** $\delta$**:** 169.8 (C-4), 160.4 (C-2), 158.9 (C-5'), 129.9 (2C; C-3' and C-7'), 127.4 (C-2'), 119.8 (-$CF_3$), 119.7 (2C; C-4' and C-6'), 118.4 (q, 1*J*C−F = 321.2 Hz; -$CF_3$), 114.4 (C-3), 66.4 (C-5), 55.4 (-OMe), 27.7 (C-1').

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{13}H_{12}F_3O_6S$ 353.0301; Found 353.0290.

**Elemental analysis:** calculated for $C_{13}H_{11}F_3O_6S$, C, 44.32; H, 3.15; found C, 44.44; H, 3.11.

### 3.5.5. Synthesis of 4-(4-methoxybenzyl)-5-oxo-2,5-dihydrofuran-3-yl trifluoromethanesulfonate (52c)




In a 25 mL round-bottomed flask, with a stirred solution of **52a** (195.1 mg, 0.89 mmol, 1.0 eq), 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (5.4 mg, 0.04 mmol, 0.05 eq), and Hünig's base (0.16 mL, 0.93 mmol, 1.05 eq) in $CH_2Cl_2$ (2 mL) at 0 °C with the help of an ice bath was added neat pivaloyl chloride (0.11 mL, 0.93 mmol, 1.05 eq) dropwise, and the mixture was allowed to warm to rt. After stirring for 16 h, the mixture was concentrated in vacuo to give an amber oil. The oil was dissolved in $CH_2Cl_2$ (30 mL) and washed with $H_2O$ (20 mL). The aqueous layer was extracted with $CH_2Cl_2$ (2 x 50 mL), and the combined organic layers were dried ($MgSO_4$), concentrated in vacuo, and purified by flash column chromatography (20% EtOAc:hexanes) to provide **52c** (233.0 mg, 86%).

**Characteristics:** Colorless Crystals.

**Mp:** 39−41 °C.

**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 2977, 2837, 1766, 1686, 1512, 1248, 1102, 1071, 855, 788, 752, 683.

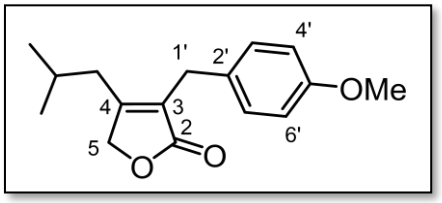
**$^1$H NMR (400 MHz. $CDCl_3$) $\delta$:** 7.19 (d; *J* = 8.6 Hz; 2H; H-3' and H-7'), 6.82 (d; *J* = 8.7 Hz; 2H; H-2' and 6'), 5.10 (s; 2H; H-5), 3.77 (s; 3H; -OMe), 3.53 (s; 2H; H-1'), 1.27 (s; 9H; $-CH_3$).

**$^{13}$C NMR (100 MHz. $CDCl_3$) $\delta$:** 173.9 (COO), 172.6 (C-4), 164.5 (C-2), 158.5 (C-5'), 129.7 (C-2'), 129.6 (2C; C-4' and C-6'), 114.2 (2C; C-3' and C-7'), 112.5 (C-3), 68.2 (C-5), 55.4 (-OMe), 39.5 ($-CCH_3$), 27.7 (C-1'), 26.9 ($-CH_3$).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{17}H_{21}O_5$ 305.1383; Found 305.1390.

### 3.5.6. Fe catalyst cross-coupling for the synthesis 4-isopropyl-3-(4-methoxybenzyl)furan-2(5*H*)-one (53)

*Procedure a:*




To a 25 mL round-bottomed flask with dry and degassed THF (3 mL), were added pivalate **52c** (105.8 mg, 0.35 mmol, 1.0 eq) and $FeCl_2$ (0.88 mg, 0.007 mmol, 2 mol%) and the reaction mixture was cooled to 0 °C in an ice bath. The mixture was stirred for 10 min at this temperature, and isobutylmagnesium bromide (0.42 mL of a 2 M in $Et_2O$, 0.84 mmol, 2.4 eq) was rapidly added. There was an immediate color change from light brown to black-brown. The mixture was left stirring for 50 min at 0 °C and then quenched with saturated $NH_4Cl$ (aq) (4 mL). The mixture was partitioned between $H_2O$ (20 mL) and $Et_2O$ (20 mL) and then extracted with $Et_2O$ (3 x 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to give a brown residue. Purification by flash column chromatography (20% EtOAc:hexanes) gave **53** (84.9 mg, 93%).

*Procedure b:*

To a 25 mL round-bottomed flask with dry and degassed THF (3 mL), were prepared a solution of triflate **52b** (50 mg, 0.128 mmol, 1.0 eq), $Fe(acac)_3$ (2.1 mg, 0.0064 mmol, 0.05 eq) 1-methyl-2-pyrrolidinone (NMP, 0.1 mL) and were cooled to -30 °C with a *i*-PrOH/Dry ice bath and stirred for 5 min. Then a solution of isobutylmagnesium bromide (2 M in THF, 300 mL, 0.154 mmol, 1.2 eq) was rapidly added and the solution showed an immediate color change from orange-red to brown/black. The mixture was left stirring for 45 min at this temperature and then quenched with saturated $NH_4Cl$ (aq) (4 mL). The mixture was partitioned between $H_2O$ (20 mL) and $Et_2O$ (20 mL) and then extracted with $Et_2O$ (3 x 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to give a brown residue. Purification by flash column chromatography (20% EtOAc:hexanes) gave **53** (84.9 mg, 69%).

**Characteristics:** Colorless Oil.

**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 2957, 1749, 1558, 1512, 1464, 1248, 793.

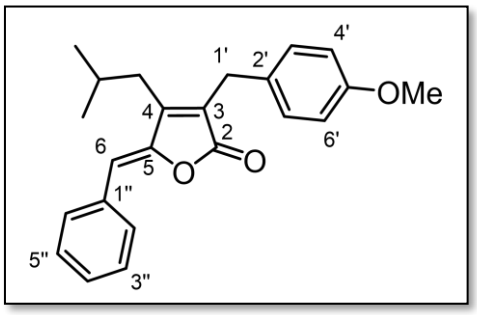
**$^{1}$H NMR (500 MHz. $CDCl_3$)** $\delta$**:** 7.15 (d; *J* = 8.6 Hz; 2H; H-3' and H-7'), 6.80 (d; *J* = 8.7 Hz; 2H; H-2' and 6'), 4.65 (s; 2H; H-5), 3.76 (s; 3H; -OMe), 3.54 (s; 2H; H-1'), 2.31 (d; *J* = 7.5 Hz; 2H; -$CH_2$), 1.79 (sept; *J* = 6.9 Hz; 1H; -CH), 0.90 (d; *J* = 6.6 Hz; 6H; -$CH_3$).

**$^{13}$C NMR (125 MHz. $CDCl_3$)** $\delta$**:** 175.1 (C-4), 160.9 (C-2), 158.2 (C-5'), 130.3 (C-2'), 129.5 (2C; C-4' and C-6'), 127.2 (C-3), 114.0 (2C; C-3' and C-7'), 71.7 (C-5), 55.3 (-OMe), 36.4 (-$CH_2$), 28.7 (C-1'), 27.8 (-CH), 22.7 (-$CH_3$).

**HRMS (ESI-TOF)** ***m/z*** **[M+H]$^{+}$:** Calculated for $C_{16}H_{21}O_3$ 261.1485; Found 261.1480.

### 3.5.7. VAC reaction for the synthesis of (*Z*)-5-benzylidene-4-isobutyl-3-(4-methoxybenzyl)furan-2(5*H*)-one (3a)

*Procedure a: Vinylogous aldol condensation*




Butenolide **53** (102.4 mg, 0.39 mmol, 1.0 eq) and anhydrous $Na_2CO_3$ (41.3 mg, 0.39 mmol, 1.0 eq) were dissolved in MeOH (4.0 mL), and then benzaldehyde (62.6 mg, 0.59 mmol, 1.5 eq) was added and the mixture was stirred at 50 °C for 24 h. The mixture was concentrated under reduced pressure and purified by flash column chromatography (10% EtOAc:hexanes) to afford **3a** (111.8 mg, 82%).

*Procedure b: Vinylogous Mukaiyama's aldol condensation*

In a 25 mL round-bottomed flask containing a solution of the lactone **53** (200 mg, 0.89 mmol, 1.0 eq) in 5 mL of anhydrous $CH_2Cl_2$, cooled in an *i*-PrOH/Dry ice bath to -40 °C, were added successively: DIPEA (0.46 mL, 2.67 mmol, 3.0 eq) and TBDMSOTf (0.4 mL, 1.78 mmol, 2.0 eq). The solution is allowed to stir under argon atmosphere for 1 h. Then benzaldehyde (**68**) (0.12 mL, 1.07 mmol, 1.0 eq)

was added stirring at 0 °C for a further 1 h. The solution is then warmed to ambient temperature and stirred for a further 2 h. Then DBU (0.26 mL, 1.78 mmol, 2.0 eq) was added. The resulting solution was allowed to stir for another 2 h before being neutralized with 10 mL of 1 M aqueous HCl solution. Then $CH_2Cl_2$ was removed in vacuo and the aqueous phase was extracted with AcOEt (3 x 15 mL), the combined organic layers were washed with saturated aqueous NaCl, the extract dried ($Na_2SO_4$), filtered and concentrated under reduced pressure. The crude extract from the reaction was purified by silica gel column chromatography (10% EtOAc:hexane) to afford the product **3a** in 50% yield (155 mg, 0.445 mmol).

**Characteristics:** White Solid.

**Mp:** 86−88 °C.

**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 2957, 1770, 1558, 1508, 1457, 1247, 970, 691.

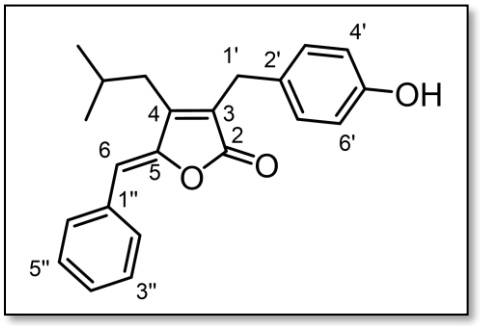
**$^1$H NMR (500 MHz. $CDCl_3$)** $\delta$**:** 7.79 (d, *J* = 7.7 Hz, 2H), 7.38 (t, *J* = 7.6 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 1H), 7.18 (d; *J* = 8.2 Hz; 2H; H-3' and H-7'), 6.83 (d; *J* = 8.2 Hz; 2H; H-2' and 6'), 6.01 (s, 1H; H-6), 3.78 (s; 3H; -OMe), 3.69 (s; 2H; H-1'), 2.43 (d; *J* = 7.5 Hz; 2H; -$CH_2$), 2.01–1.89 (m, 1H; -CH), 0.98 (d; *J* = 6.6 Hz; 6H; -$CH_2$).

**$^{13}$C NMR (125 MHz. $CDCl_3$)** $\delta$**:** 170.6 (C-2), 158.4 (C-5'), 152.5 (C-4), 148.8 (C-5), 133.2 (C-1''), 130.5 (2C; C-2'' and C-6''), 129.8 (2C; C-3' and C-7'), 129.6 (C-2'), 128.8 (2C; C-3'' and C-5''), 128.8 (C-4''), 127.3, 114.1 (2C; C-3' and C-7') 109.6 (C-6), 55.3 (-OMe), 33.9 (-$CH_2$), 29.4 (C-1'), 29.0 (-CH), 22.9 (-$CH_3$).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{23}H_{25}O_3$ 349.1798; Found 349.1808.

**Elemental analysis:** calculated for $C_{23}H_{24}O_3$, C, 79.28; H, 6.94; found C, 79.60; H, 7.23.

### 3.5.8. Demethylation reaction for the synthesis of enhygrolide A (3)




To a solution of **3a** (78.5 mg, 0.23 mmol, 1.0 eq) in dry $CH_2Cl_2$ (4 mL) was added $BBr_3$ (0.34 mL of a 1 M in $CH_2Cl_2$, 0.34 mmol, 1.5 eq) at 0 °C in an ice bath. The mixture was stirred for 2 h at that temperature, quenched with saturated $NaHCO_3$(aq) (5 mL), and then extracted with $CH_2Cl_2$ (3 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (30% EtOAc:hexanes) to give **3** (76.9 mg, 100%).

**Characteristics:** Greenish, Amorphous solid.

**FTIR (NaCl film; cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3384, 2928, 2869, 1770, 1558, 1449, 1264, 971, 675.

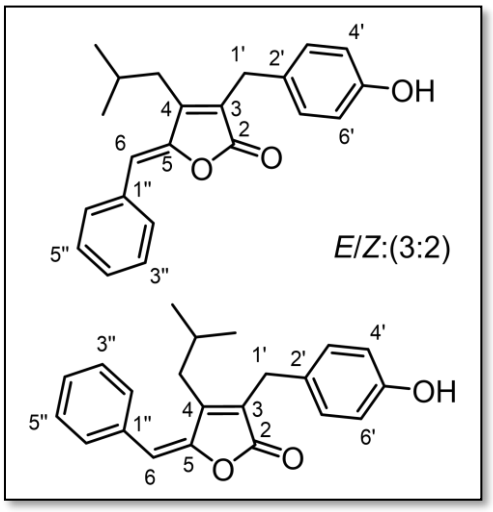
**$^1$H NMR (500 MHz. $CD_3OD$) $\delta$:** 7.80 (d, *J* = 7.6 Hz, 2H; H-2'' and H-6''), 7.39 (t, *J* = 7.6 Hz, 2H; H-3'' and H-5''), 7.31 (t, *J* = 7.6 Hz, 1H; H-4''), 7.06 (d; *J* = 8.5 Hz; 2H; H-3' and H-7'), 6.71 (d; *J* = 8.5 Hz; 2H; H-2' and 6'), 6.25 (s, 1H; H-6), 3.65 (s; 2H; H-1'), 2.50 (d; *J* = 7.5 Hz; 2H; -$CH_2$), 2.00–1.84 (m, 1H; -CH), 0.96 (d; *J* = 6.6 Hz; 6H; -$CH_3$).

**$^{13}$C NMR (125 MHz. $CD_3OD$) $\delta$:** 172.0 (C-2), 157.0 (C-5'), 154.2 (C-4), 149.8 (C-5), 134.6 (C-1''), 131.4 (2C; C-2'' and C-6''), 130.3 (2C; C-3' and C-7'), 129.9 (C-2'), 129.6 (2C; C-3'' and C-5''), 128.3 (C-4''), 116.2 (2C; C-3' and C-7'), 110.9 (C-6), 34.2 (-$CH_2$), 30.3 (-CH), 29.5 (C-1'), 22.8 (-$CH_3$).

**HRMS (ESI-TOF) *m/z* [M+H]$^+$:** Calculated for $C_{22}H_{23}O_3$ 335.1641; Found 335.1631.

**Elemental analysis:** calculated for $C_{22}H_{22}O_3$, C, 79.02; H, 6.63; found C, 79.37; H, 6.89.

### 3.5.9. Photoisomerization of enhygrolide A (3)




A solution of **3** (23.0 mg, 0.07 mmol) in $CDCl_3$ (3.0 mL) was irradiated with a halogen light bulb (500 W, 130 V; placed at a distance of 96 cm) under stirring for 7 days at rt. The solvent was evaporated under reduced pressure to give a 3:2 mixture of 3.1 and ***E*-3** (23.0 mg, 100%). Their respective identities were established by integration of diagnostic NMR signals ($^1$H NMR (500 MHz, $CD_3OD$) $\delta_Z$ = 6.24, 3.64, 2.50, 2.00-1.84; 0.95; $\delta_E$ = 6.89, 3.59, 2.30, 1.24-1.15; 0.45; $^{13}$C NMR (125 MHz, $CD_3OD$) $\delta_Z$ = 172.0, 154.2, 129.6, 111.0; $\delta_E$ = 171.7, 151.6, 133.4, 116.4 ppm) and comparison with the reported values for naturally derived samples.[8] When the same procedure was performed using MeCN as a solvent, a 2:1 mixture of **3** and ***E*-3** (100%) was obtained.

**$^1$H NMR (500 MHz. $CD_3OD$) $\delta$:** 7.80 (d, *J* = 7.6 Hz, 2H; H-2'' and H-6''), 7.39 (t, *J* = 7.6 Hz, 2H; H-3'' and H-5''), 7.31 (t, *J* = 7.6 Hz, 1H; H-4''), 7.06 (d; *J* = 8.5 Hz; 2H; H-3' and H-7'), 6.71 (d; *J* = 8.5 Hz; 2H; H-2' and 6'), 6.25 (s, 1H; H-6), 3.65 (s; 2H; H-1'), 2.50 (d; *J* = 7.5 Hz; 2H; -$CH_2$), 2.00–1.84 (m, 1H; -CH), 0.96 (d; *J* = 6.6 Hz; 6H; -$CH_3$).

**$^{13}$C NMR (125 MHz. $CD_3OD$) $\delta$:** 172.0 (C-2), 157.0 (C-5'), 154.2 (C-4), 149.8 (C-5), 134.6 (C-1''), 131.4 (2C; C-2'' and C-6''), 130.3 (2C; C-3' and C-7'), 129.9 (C-2'), 129.6 (2C; C-3'' and C-5''), 129.3 (C-4''), 116.4 (C-6),116.2 (2C; C-3' and C-7'), 34.2 (-$CH_2$), 30.3 (C-1'), 29.5 (-CH), 22.8 (-$CH_3$).

## 3.6. REFERENCES


[1] YANG, X.; SHIMIZU, Y.; STEINER, J. R.; CLARDY, J. Nostoclide I and II, extracellular metabolites from a symbiotic cyanobacterium, *Nostoc sp.*, from the lichen *Peltigera canina*. *Tetrahedron Letters* **1993**, *34*, 761.

[2] SMITH, C. J.; HETTICH, R. L.; JOMPA, J.; TAHIR, A.; BUCHANAN, M. V.; IRELAND, C. M. Cadiolides A and B, new metabolites from an ascidian of the genus *Botryllus*. *The Journal of Organic Chemistry* **1998**, *63*, 4147.

[3] WANG, W.; KIM, H.; PATIL, R. S.; GIRI, A. G.; WON, D. H.; HAHN, D.; SUNG, Y.; LEE, J.; CHOI, H.; NAM, S.-J.; KANG, H. Cadiolides J–M, antibacterial polyphenyl butenolides from the Korean tunicate *Pseudodistoma antinboja*. *Bioorganic & Medicinal Chemistry Letters* **2017**, *27*, 574.

[4] AHN, C.-H.; WON, T. H.; KIM, H.; SHIN, J.; OH, K.-B. Inhibition of *Candida albicans* isocitrate lyase activity by cadiolides and synoilides from the ascidian *Synoicum* sp. *Bioorganic & Medicinal Chemistry Letters* **2013**, *23*, 4099.

[5] SMITHA, D.; KUMAR, M. M. K.; RAMANA, H.; RAO, D. V. Rubrolide R: a new furanone metabolite from the ascidian *Synoicum* of the Indian Ocean. *Natural Product Research* **2014**, *28*, 12.

[6] WANG, W.; KIM, H.; NAM, S.-J.; RHO, B. J.; KANG, H. Antibacterial Butenolides from the Korean Tunicate *Pseudodistoma antinboja*. *Journal of Natural Products* **2012**, *75*, 2049.

[7] WON, T. H.; JEON, J.-E.; KIM, S.-H.; LEE, S.-H.; RHO, B. J.; OH, D.-C.; OH, K.-B.; SHIN, J. Brominated aromatic furanones and related esters from the ascidian *Synoicum* sp. *Journal of Natural Products* **2012**, *75*, 2055.

[8] FELDER, S.; KEHRAUS, S.; NEU, E.; BIERBAUM, G.; SCHÄBERLE, T. F.; KÖNIG, G. M. Salimyxins and enhygrolides: antibiotic, sponge-related metabolites from the obligate marine myxobacterium *Enhygromyxa salina*. *ChemBioChem* **2013**, *14*, 1363.

[9] MASON, C.; EDWARDS, K.; CARLSON, R.; PIGNATELLO, J.; GLEASON, F.; WOOD, J. Isolation of chlorine-containing antibiotic from the freshwater cyanobacterium *Scytonema hofmanni*. *Science* **1982**, *215*, 400.

[10] PIGNATELLO, J. J.; PORWOLL, J.; CARLSON, R. E.; XAVIER, A.; GLEASON, F. K.; WOOD, J. M. Structure of the antibiotic cyanobacterin, a chlorine-containing γ-lactone from the freshwater cyanobacterium *Scytonema hofmanni*. *The Journal of Organic Chemistry* **1983**, *48*, 4035.

[11] BARBOSA, L. C.; MALTHA, C. R.; DEMUNER, A. J.; PINHEIRO, P. F.; VAREJÃO, J. O.; MONTANARI, R. M.; ANDRADE, N. J. Síntese e avaliação da atividade antimicrobiana de furanonas halogenadas e de compostos análogos aos nostoclídeos. *Quim. Nova* **2010**, *33*, 2020.

[12] TEIXEIRA, R. R.; BARBOSA, L. C.; MALTHA, C. R. A.; ROCHA, M. E.; BEZERRA, D. P.; COSTA-LOTUF, L. V.; PESSOA, C.; MORAES, M. O. Synthesis and cytotoxic activity of some 3-benzyl-5-arylidenefuran-2(5*H*)-ones. *Molecules* **2007**, *12*.

[13] BARBOSA, L. C. A.; VAREJÃO, J. O. S.; PETROLLINO, D.; PINHEIRO, P. F.; DEMUNER, A. J.; MALTHA, C. R. A.; FORLANI, G. Tailoring nostoclide structure to target the chloroplastic electron transport chain. *ARKIVOC* **2012**, *2012*, 15.

[14] TEIXEIRA, R. R.; PINHEIRO, P. F.; BARBOSA, L. C. D. A.; CARNEIRO, J. W. D. M.; FORLANI, G. QSAR modeling of photosynthesis-inhibiting nostoclide derivatives. *Pest Management Science* **2010**, *66*, 196.

[15] BOUKOUVALAS, J.; MALTAIS, F.; LACHANCE, N. Furanolate-based strategy for sequential 2,3,4-trisubstitution of butenolide: Total synthesis of nostoclides I and II. *Tetrahedron Letters* **1994**, *35*, 7897.

[16] BELLINA, F.; ROSSI, R. Synthetic Applications of 3,4-dihalo-2(5*H*)-furanones: A formal total synthesis of nostoclides I and II. *Synthesis* **2002**, *2002*, 2729.

[17] KAR, A.; ARGADE, N. P. A Facile synthesis of natural products chaetomellic acid a and 1,7-(*Z*)-nonadecadiene-2,3-dicarboxylic acid. *The Journal of Organic Chemistry* **2002**, *67*, 7131.

[18] INACK, N. S.; JULIEN, P.; ROMAIN, D.; MOSTAFA, E. H. E.; MOHAMED, A.; ALAIN, D.; JÉRÔME, T. Copper-catalyzed domino route to natural nostoclides and analogues: A total synthesis of nostoclides I and II. *Advanced Synthesis & Catalysis* **2013**, *355*, 2936.

[19] NÄSMAN, J. H. 3-Methyl-2(5*H*)-Furanone. *Org. Synth.* **1989**, *68*, 162.

[20] KAR, A.; GOGOI, S.; ARGADE, N. P. Synthesis of naturally occurring bioactive butyrolactones: maculalactones A–C and nostoclide I. *Tetrahedron* **2005**, *61*, 5297.

[21] BARBOSA, L. C.; VELOSO, D. P. Sistema para a representação simbólica de mecanismos de reação (Recomendações 1988). *Quim. Nova* **1994**, *17*.

[22] BOUKOUVALAS, J.; POULIOT, M. Short and efficient synthesis of cadiolide B. *Synlett* **2005**, *2005*, 343.

[23] PEIXOTO, P. A.; BOULANGÉ, A.; LELEU, S.; FRANCK, X. Versatile synthesis of acylfuranones by reaction of acylketenes with α-hydroxy ketones: Application to the one-step multicomponent synthesis of cadiolide B and its analogues. *European Journal of Organic Chemistry* **2013**, *2013*, 3316.

[24] BOULANGÉ, A.; PARRAGA, J.; GALÁN, A.; CABEDO, N.; LELEU, S.; SANZ, M. J.; CORTES, D.; FRANCK, X. Synthesis and antibacterial activities of cadiolides A, B and C and analogues. *Bioorganic & Medicinal Chemistry* **2015**, *23*, 3618.

[25] BOUKOUVALAS, J.; THIBAULT, C. Step-economical synthesis of the marine ascidian antibiotics cadiolide A, B, and D. *The Journal of Organic Chemistry* **2015**, *80*, 681.

[26] FELDER, S.; DREISIGACKER, S.; KEHRAUS, S.; NEU, E.; BIERBAUM, G.; WRIGHT, P. R.; MENCHE, D.; SCHÄBERLE, T. F.; KÖNIG, G. M. Salimabromide: Unexpected chemistry from the obligate marine myxobacterium *Enhygromxya salina*. *Chemistry - A European Journal* **2013**, *19*, 9319.

[27] DÁVILA-CÉSPEDES, A.; HUFENDIEK, P.; CRÜSEMANN, M.; SCHÄBERLE, T. F.; KÖNIG, G. M. Marine-derived myxobacteria of the suborder *Nannocystineae*: An underexplored source of structurally intriguing and biologically active metabolites. *Beilstein J. Org. Chem.* **2016**, *12*.

[28] SUN, Y.; FENG, Z.; TOMURA, T.; SUZUKI, A.; MIYANO, S.; TSUGE, T.; MORI, H.; SUH, J.-W.; IIZUKA, T.; FUDOU, R.; OJIKA, M. Heterologous production of the marine myxobacterial antibiotic haliangicin and its unnatural analogues generated by engineering of the biochemical pathway. *Scientific Reports* **2016**, *6*, 22091.

[29] KELECOM, A. Secondary metabolites from marine microorganisms. *Anais da Academia Brasileira de Ciências* **2002**, *74*.

[30] BHATNAGAR, I.; KIM, S.-K. Immense essence of excellence: Marine microbial bioactive compounds. *Marine Drugs* **2010**, *8*, 2673.

[31] LIU, G.-Z.; XU, H.-W.; WANG, P.; LIN, Z.-T.; DUAN, Y.-C.; ZHENG, J.-X.; LIU, H.-M. Stereoselective synthesis and anti-proliferative effects on prostate cancer evaluation of 5-substituted-3,4-diphenylfuran-2-ones. *European Journal of Medicinal Chemistry* **2013**, *65*, 323.

[32] ORTEGA, M. A. J.; ZUBÍA, E.; OCAÑA, J. M.; NARANJO, S.; SALVÁ, J. New Rubrolides from the Ascidian *Synoicum blochmanni*. *Tetrahedron* **2000**, *56*, 3963.

[33] ASOLKAR, R. N.; JENSEN, P. R.; KAUFFMAN, C. A.; FENICAL, W. Daryamides A−C, Weakly cytotoxic polyketides from a marine-derived actinomycete of the genus *Streptomyces* strain CNQ-085. *J. Nat. Prod.* **2006**, *69*.

[34] TAN, L. T. Bioactive natural products from marine cyanobacteria for drug discovery. *Phytochemistry* **2007**, *68*, 954.

[35] MARTINS, A.; TENREIRO, T.; ANDRADE, G.; GADANHO, M.; CHAVES, S.; ABRANTES, M.; CALADO, P.; TENREIRO, R.; VIEIRA, H. Photoprotective bioactivity present in a unique marine bacteria collection from portuguese deep sea hydrothermal vents. *Marine Drugs* **2013**, *11*, 1506.

[36] PALANICHAMY, S.; SUBRAMANIAN, G. Antifouling properties of marine bacteriocin incorporated epoxy based paint. *Progress in Organic Coatings* **2017**, *103*, 33.

[37] KWONG, T. F. N.; MIAO, L.; LI, X.; QIAN, P. Y. Novel antifouling and antimicrobial compound from a marine-derived fungus *Ampelomyces* sp. *Marine Biotechnology* **2006**, *8*, 634.

[38] MARTINS, A.; VIEIRA, H.; GASPAR, H.; SANTOS, S. Marketed marine natural products in the pharmaceutical and cosmeceutical industries: Tips for success. *Marine Drugs* **2014**, *12*, 1066.

[39] FUDOU, R.; IIZUKA, T.; YAMANAKA, S. Haliangicin, a novel antifungal metabolite produced by a marine myxobacterium. *The Jour. of Antibiotics* **2001**, *54*.

[40] SUN, Y.; TOMURA, T.; SATO, J.; IIZUKA, T.; FUDOU, R.; OJIKA, M. Isolation and biosynthetic analysis of haliamide, a new pks-nrps hybrid metabolite from the marine myxobacterium *Haliangium ochraceum*. *Molecules* **2016**, *21*, 59.

[41] SCHMALZBAUER, B.; MENCHE, D. Concise Synthesis of the tricyclic core of salimabromide. *Organic Letters* **2015**, *17*, 2956.

[42] CIMINIELLO, P.; FATTORUSSO, E.; MAGNO, S.; MANGONI, A.; PANSINI, M. Incisterols, a new class of highly degraded sterols from the marine sponge *Dictyonella incisa*. *Journal of the American Chemical Society* **1990**, *112*, 3505.

[43] BROWN, E. D.; WRIGHT, G. D. Antibacterial drug discovery in the resistance era. *Nature* **2016**, *529*, 336.

[44] LING, L. L.; SCHNEIDER, T.; PEOPLES, A. J.; SPOERING, A. L.; ENGELS, I.; CONLON, B. P.; MUELLER, A.; SCHÄBERLE, T. F.; HUGHES, D. E.; EPSTEIN, S.; JONES, M.; LAZARIDES, L.; STEADMAN, V. A.; COHEN, D. R.; FELIX, C. R.; FETTERMAN, K. A.; MILLETT, W. P.; NITTI, A. G.; ZULLO, A. M.; CHEN, C.; LEWIS, K. A new antibiotic kills pathogens without detectable resistance. *Nature* **2015**, *517*, 455.

[45] KOLTER, R.; VAN WEZEL, G. P. Goodbye to brute force in antibiotic discovery? *Nature Microbiology* **2016**, *1*, 15020.

[46] BARKA, E. A.; VATSA, P.; SANCHEZ, L.; GAVEAU-VAILLANT, N.; JACQUARD, C.; KLENK, H.-P.; CLÉMENT, C.; OUHDOUCH, Y.; VAN WEZEL, G. P. Taxonomy, physiology, and natural products of actinobacteria. *Microbiology and Molecular Biology Reviews* **2016**, *80*, 1.

[47] BOUKOUVALAS, J.; LOACH, R. P. Concise total synthesis of (-)-auxofuran by a click Diels–Alder strategy. *Organic Letters* **2013**, *15*.

[48] LU, S.; NISHIMURA, S.; ITO, M.; TSUCHIDA, T.; KAKEYA, H. Isolation and structure elucidation of cytotoxic saccharothriolides d to f from a rare actinomycete *Saccharothrix* sp. and their structure-activity relationship. *Journal of Natural Products* **2016**, *79*, 1891.

[49] SHENG, Y.; LAM, P. W.; SHAHAB, S.; SANTOSA, D. A.; PROTEAU, P. J.; ZABRISKIE, T. M.; MAHMUD, T. Identification of elaiophylin skeletal variants from the indonesian *Streptomyces* sp. ICBB 9297. *Journal of Natural Products* **2015**, *78*, 2768.

[50] PESIC, A.; STEINHAUS, B.; KEMPER, S.; NACHTIGALL, J.; KUTZNER, H. J.; HÖFLE, G.; SÜSSMUTH, R. D. Isolation and structure elucidation of the nucleoside antibiotic strepturidin from *Streptomyces albus* DSM 40763. *The Journal Of Antibiotics* **2014**, *67*, 471.

[51] SHIN, B.; KIM, B.-Y.; CHO, E.; OH, K.-B.; SHIN, J.; GOODFELLOW, M.; OH, D.-C. actinomadurol, an antibacterial norditerpenoid from a rare actinomycete, *Actinomadura* sp. KC 191. *Journal of Natural Products* **2016**, *79*, 1886.

[52] RAMACHARY, D. B.; KISHOR, M. Direct catalytic asymmetric synthesis of highly functionalized tetronic acids/tetrahydro-isobenzofuran-1,5-diones via combination of cascade three-component reductive alkylations and Michael-aldol reactions. *Organic & Biomolecular Chemistry* **2010**, *8*, 2859.

[53] RAMACHARY, D. B.; KISHOR, M.; REDDY, G. B. Development of drug intermediates by using direct organocatalytic multi-component reactions. *Organic & Biomolecular Chemistry* **2006**, *4*, 1641.

[54] BOUKOUVALAS, J.; ALBERT, V.; LOACH, R. P.; LAFLEUR-LAMBERT, R. Unified route to asymmetrically substituted butenolide, maleic anhydride, and maleimide constituents of *Antrodia camphorata*. *Tetrahedron* **2012**, *68*, 9592.

[55] SCHACHTNER, J. E.; STACHEL, H.-D.; POLBORN, K.; ANKE, T. Synthesis and biological activity of thiobasidalin. *European journal of medicinal chemistry* **1998**, *33*, 309.

[56] PASHKOVSKII, F. S.; SHCHUKINA, E. M.; GRIBOVSKII, M. G.; LAKHVICH, F. A. Heterocyclic analogs of prostaglandines: IV. Synthesis of 3,7-interphenylene 3,10(11)-dioxa-13-azaprostanoids and 9-oxa-7-azaprostanoids based on tetronic acid and aromatic aldehydes. *Russian Journal of Organic Chemistry* **2008**, *44*, 657.

[57] SCHRÖDER, P.; BAUER, J. O.; STROHMANN, C.; KUMAR, K.; WALDMANN, H. Synthesis of an iridoid-inspired compound collection and discovery of autophagy inhibitors. *The Journal of Organic Chemistry* **2016**, *81*, 10242.

[58] PANDIT, U. K.; CABRÉ, F. R. M.; GASE, R. A.; NIE-SARINK, M. J. Biomimetic stereospecific reduction of α,β-unsaturated iminium salts. *J. Chem. Soc., Chem. Commun.* **1974**, *0*.

[59] NIE-SARINK, M. J.; PANDIT, U. K. Chemioselectivity of the reduction of phenyl-substituted α,β-unsaturated iminium salts by an nadh model. *Tetrahedron Letters* **1979**, *20*.

[60] FÜRSTNER, A.; MARTIN, R.; KRAUSE, H.; SEIDEL, G.; GODDARD, R.; LEHMANN, C. W. Preparation, Structure, and Reactivity of Nonstabilized Organoiron Compounds. Implications for iron-catalyzed cross coupling reactions. *Journal of the American Chemical Society* **2008**, *130*, 8773.

[61] SCHEIPER, B.; BONNEKESSEL, M.; KRAUSE, H.; FÜRSTNER, A. Selective iron-catalyzed cross-coupling reactions of grignard reagents with enol triflates, acid chlorides, and dichloroarenes. *The Journal of Organic Chemistry* **2004**, *69*, 3943.

[62] YU, B.; ZHANG, E.; FANG, Y.; SUN, X.-N.; REN, J.-L.; YU, D.-Q.; LIU, H.-M. The new convenient synthesis of novel γ-alkylidenebutenolides from 6-aminopenicillanic acid. *Heterocycles* **2013**, *87*.

[63] THOMBAL, R. S.; JADHAV, V. H. Total synthesis of the natural product EBC-329. *Organic & Biomolecular Chemistry* **2015**, *13*, 9485.

[64] BOUKOUVALAS, J.; BELTRAN, P. P.; LACHANCE, N.; COTE, S.; MALTAIS, F.; POULIOT, M. A new, highly stereoselective synthesis of β-unsubstituted (*Z*)-γ-alkylidene-butenolides using bromine as a removable stereocontrol element. *Synlett* **2007**, 219.

# CHAPTER 4

# SYNTHESIS OF TETRONAMIDE ANALOGS WITH CYANOBACTERICIDAL ACTIVITY

## 4.1. INTRODUCTION

### 4.1.1. Tetronamides as promising bioactive scaffolds

The biological importance of tetronamides has been discussed with basidalin synthesis in chapter 2. Therefore, regardless of its scarcity in nature (recently synthetized basidalin is the uniquely found in nature[1,2]), several synthetic congeners appeared as newly accessible and potently active compounds. Its activities can include antibiotic,[1,3] anticancer,[1] insecticidal,[4,5] antimitotic,[6,7] pesticidal and herbicidal[8] properties. Clearly, the structural diversity of natural butenolides and mainly their (*Z*)-γ-alkylidene derivatives represent an outstanding starting point for the design of new bioactive compounds.[9,10] Unexpectedly, in literature, we have few examples of γ-unsaturated tetronamide derivatives,[2] contrary to their non β-nitrogenated congeners, such as rubrolides.[11-15]

In the present instance 4-aminobutenolides are of interest for our group. Consequently we developed an interesting methodology for the selective synthesis of aldolic *N*-aryl tetronamides (**Fig. 4.1**; **3**).[16] These alcoholic derivatives (**3**) were the result of optimal vinylogous aldol reaction (VAR) on simple tetronamides (**1**) with aromatic aldehydes (**2**). Considering the biological potential of the γ-alkylidenefuran-2(5*H*)-ones, we may regard them as excellent precursor compounds for γ-alkylidene tetronamides (**4**), due to the fact that, only dehydration reaction on **4**, or one-pot vinylogous Mukaiyama's aldol condensation (VMAC) on **1**, may yield the respective unsaturated derivatives (**Fig. 4.1**). Likewise, both compounds **3** and **4** are not reported to show biological activities to this date, making them a new target for study.

Aldolic tetronamide derivatives (**3**) are also an interesting class of β-aminobutenolides whose properties have only recently explored.[16] As was discussed in this work, aldolic reactions, represents one of the most widely explored avenues for installing a γ-carbon substituent,[17-21] including our own work.[16] Surprisingly, little is known concerning the serviceability of tetronamides.

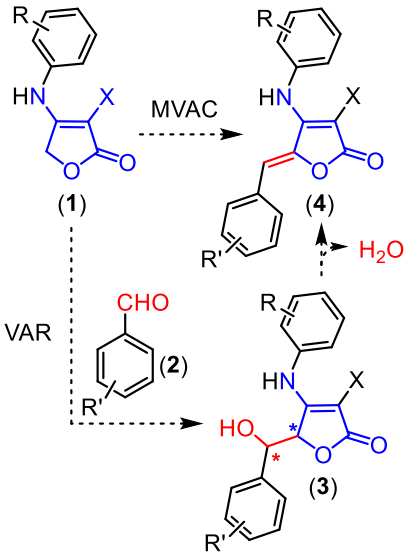
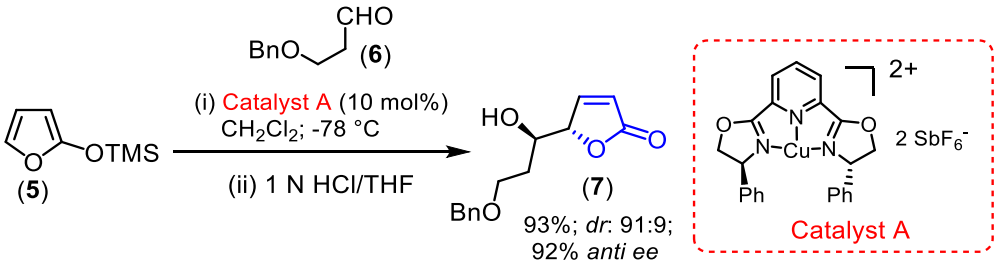



**Figure 4.1:** Ideally approach to γ-alkylidenetetronamides (**4**) via aldolic intermediates (**3**) and *N*-aryltetronamides (**1**).

### 4.1.2. Aldol reactions in butenolides

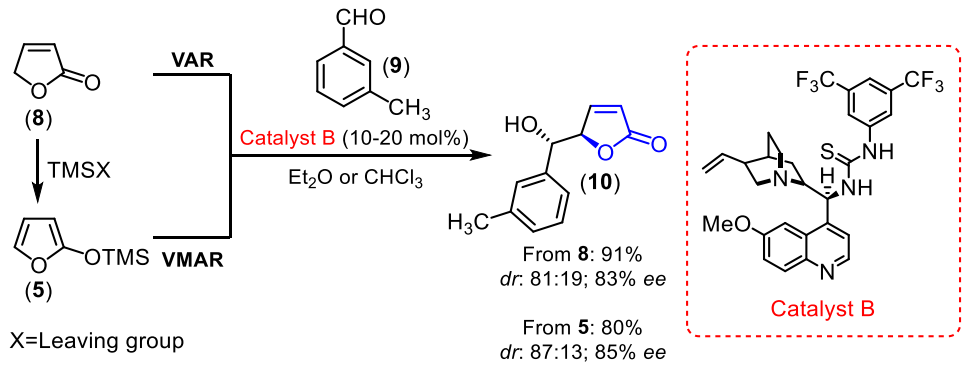
Controlling the relative and absolute configuration of the newly formed stereogenic centers is one of the main effort in aldolic reactions in butenolides.[22-35] In this way, several butenolides have been utilized as substrates.[26-29,34,35]

In 2000, Szlosek and Figadère reported the first catalytic asymmetric aldol reaction between trimethylsilyloxyfuran and achiral aldehydes in the presence of a BINOL/Ti(*i*-PrO)$_4$ complex with good to excellent yields as well as enantiomeric excess.[36] Subsequently, varius asymmetric aldolic reactions were developed for 2-silyloxyfurans and γ-butenolides using different organic and metallic catalysts. For example, there are only two examples of anti-selective, highly enantioselective vinylogous aldol reactions of silyloxyfurans and aliphatic aldehydes, which are achieved through a chiral Cu-bisoxazoline complex (**Scheme 4.1**)[37] and chiral phase transfer catalyst,[38] respectively.




**Scheme 4.1:** Enantioselective vinylogous aldol reaction of silyloxyfurans catalyzed by Cu-bisoxazoline complex.[37]

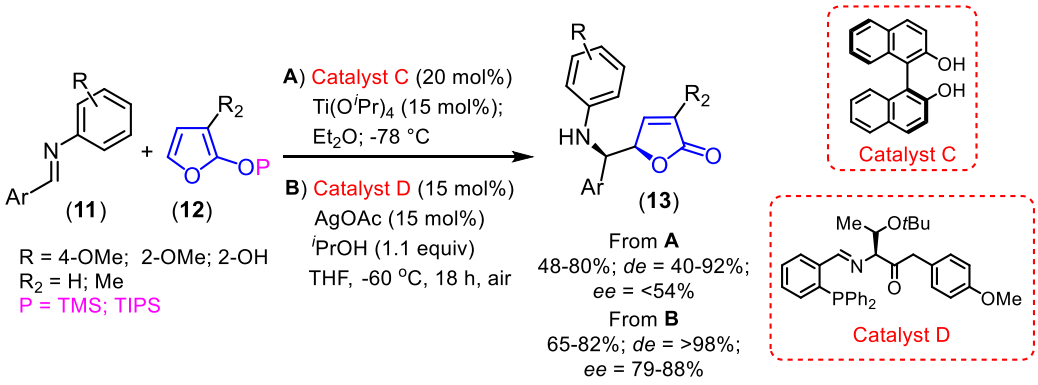
Other examples include the use of mono and bifunctional alkaloid (quinine)[39] and thiourea organocatalyst[40] (*e.g.* Catalyst B) for vinylogous Mukaiyama's aldol reaction (VMAR) of 2-(trimethylsilyloxy)furan[41] (**5**) and unactivated γ-butenolides (**8**)[33] with the aldehyde **9**, which were developed with high enantioselectivity and anti-selectivity (**Scheme 4.2**).




**Scheme 4.2:** Enantioselective vinylogous aldol reaction of butenolides.[40]

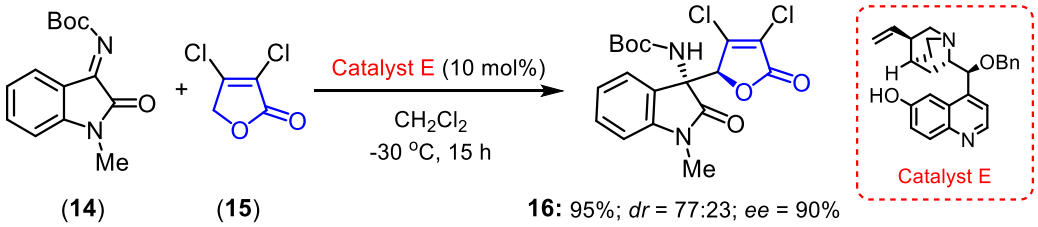
As of late, several new approaches have been developed for stereoselective VMAR and VAR of 2-silyloxyfurans and γ-butenolides catalyzed by a simple bases and Lewis acids, *e.g.* trimethylamine,[28] $BF_3$•$OEt_2$,[26] $Bi(OTf)_3$•$4H_2O$.[42] In addition to those, the reaction may be executed by organocatalysts, *e.g.* an axially chiral guanidine base,[34] tryptophan derived bifunctional organocatalyst,[35] bifunctional aminothiourea and aminosquaramide organocatalysts .[43]

Several examples of stereoselective vinylogous Mannich reactions have been reported. Terada and co-workers have outlined an enantioselective (up to 97% *ee*) protocol for Brønsted acid-catalyzed Friedel-Crafts reactions of *N*-Boc aldimines with 2-methoxyfuran. Enantiomerically enriched furan-2-yl-amines may be oxidized to afford alkylamine-substituted γ-butenolides via a two-step sequence that generates the carbinol stereogenic center with moderate diastereoselectivity (70% *de*).[44] In 1999, Martin and Lopez reported a method (Ti-catalyzed) for addition of silyloxyfurans and aldimines with Ti(*i*-PrO)$_4$/(*S*)-BINOL complex. The reactions proceeded in 40–92% *de* and up to 54% *ee* (**Scheme 4.3; A**).[45] Subsequently, a high diastereo- and enantioselective protocol for catalytic asymmetric vinylogous Mannich reaction have been reported via Ag-catalyst and a chiral phosphine ligand (**Scheme 4.3**; **B**).[46]




**Scheme 4.3:** Ti and Ag-catalyzed asymmetric vinylogous Mannich reactions with silyloxyfurans.[45,46]

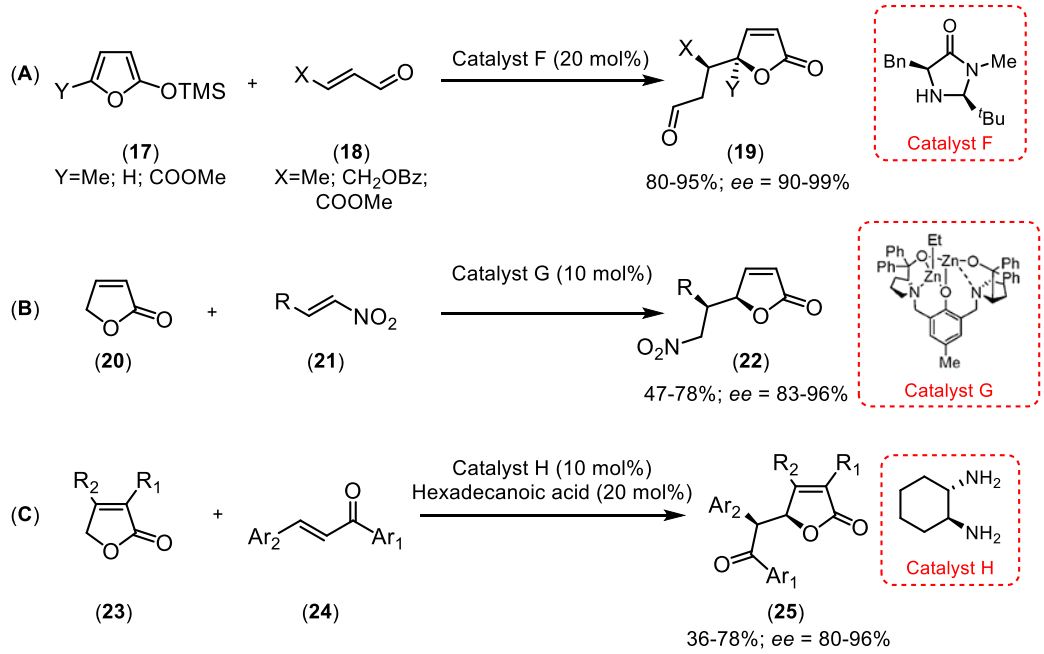
The direct asymmetric vinylogous Mannich reaction of butenolide derivatives has recently managed to draw enough attention of researches. Shibasaki's group reported the first direct asymmetric Mannich-type reactions of γ-butenolides catalyzed by a chiral lanthanumpyridine bisoxazolinecomplex.[47] More recently, the same group developed another direct catalytic asymmetric vinylogous Mannich-type reaction of ketimines. Activation of the *N*-thiophosphinoyl group by a soft Lewis acid was the key to achieve the enantioselective addition of the *in situ* dienolate to unreactive ketimine substrates. Recently, the first direct asymmetric vinylogous Mannich reaction of α,β-dihalo butenolides with isatin-derived ketimines was developed through a bifunctional quinidine-derived catalyst (Catalyst E) with a broad substrate scope and high enantioselectivity (**Scheme 4.4**).[48]




**Scheme 4.4:** Direct asymmetric vinylogous Mannich reaction of γ-butenolides.[48]

Following this line, the Michael addition is also one of the most powerful C-C bond forming procedure in aldolic transformations. The first organocatalytic asymmetric Mukaiyama–Michael reaction of 2-silyloxyfurans was reported by MacMillan (**Scheme 4.5**; **A**).[49] Trost and co-workers later disclosed the first direct use of butenolides in Michael reactions with nitroalkenes through zinc catalysis (**Scheme 4.5**; **B**),[50] which avoided the formation of 2-silyloxyfurans from γ-

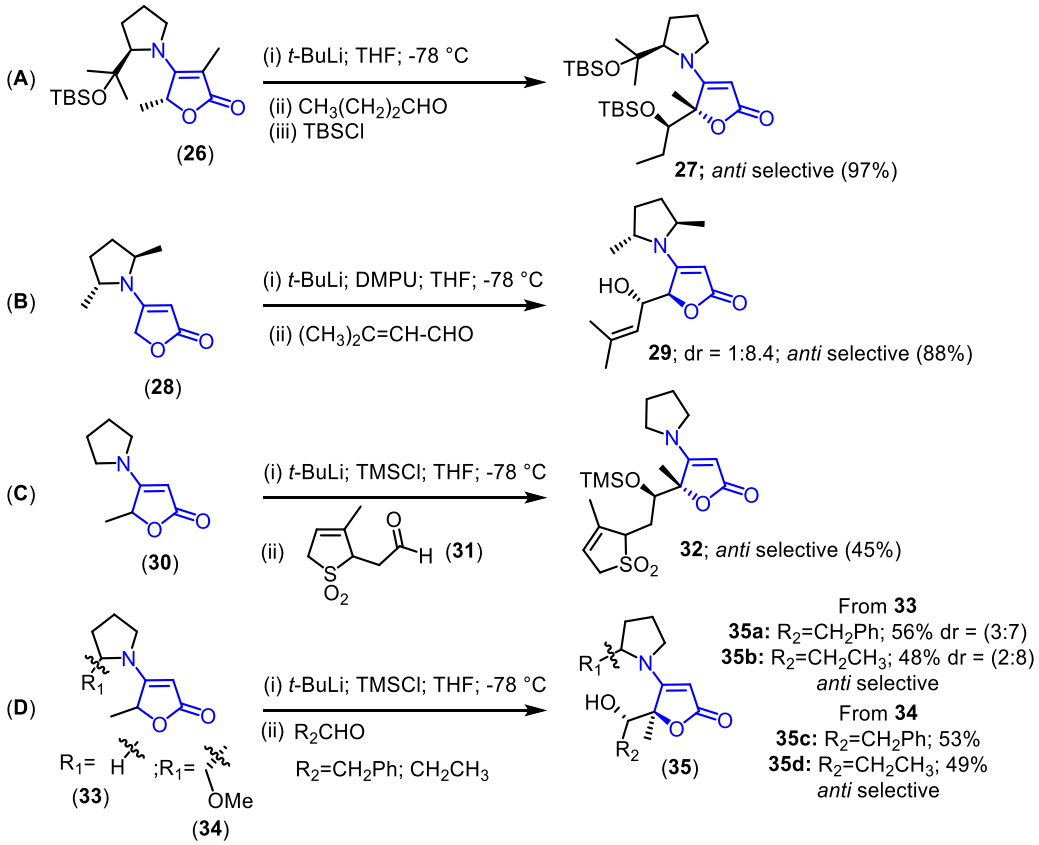
butenolides. Lately, in 2010, the first direct organocatalytic asymmetric vinylogous Michael addition reaction of γ-butenolides catalyzed by vicinal primarydiamine salts to produce chalcones in good yields, diastereo- and enatioselectively (**Scheme 4.5**; **C**) has been reported.[51]




**Scheme 4.5:** Asymmetric Michael addition reaction for the synthesis of chiral γ-butenolides.

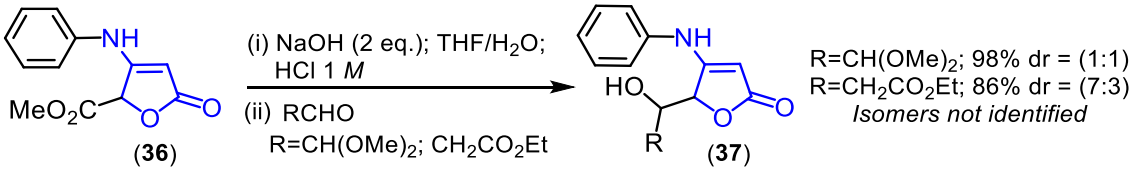
In relation to aldol reaction of tetronamides, few pertinent examples have invariably employed β-pyrrolidine tetronamide derivatives in conjunction with a strong base (*tert*-BuLi, −78 °C), which led mainly to *anti*-aldolate adducts (**Scheme 4.6; A-D**).[52-55] The first study of these reactions was developed by Schlessinger and co-workers.[52] In their approach, the intermediate ***anti*-27** is obtained as the sole diastereomer owing to the influence of the bulky substituent in pyrrolidine moiety (**A**). Later, Dankwardt and co-workers, synthetized the intermediate **29** starting from 4-(2,5-dimethylpyrrolidin-1-yl)furan-2(5*H*)-one (**28**), with 88% yield and *anti*-selective dr = 1:8.4. In this case, the stereoselectivity was determined by more accessible *anti*-approaching in transition state.[53] Finally, two investigations were reported by Bruyère and co-workers. In these the use of 5-methyl-4-(pyrrolidin-1-yl)furan-2(5*H*)-ones (**30** and **33**) with different aldehydes afforded *anti*-aldolic products **32** and **35** with moderate yields. Product **32** proved to be unstable, causing lower yields and only one diastereomer after chromatographic separation.[55] Similarly, the reaction of same starting material

with simple aldehydes lead to moderate diastereoselectivity (**35a-b**; dr = (3:7)-(2:8)), and, the addition of a chiral group adjacent to N in the pyrrolidine ring (compound **34**) allowed for the exclusive formation of the *anti*-isomer (**35c-d**).[54]




**Scheme 4.6:** VAC approaches in pyrrolidine tetronamides derivatives.

To date, only two *N*-monosubstituted tetronamide-derived aldolates have been described in the literature by Dechoux and co-workers in regard to their synthetic approach to basidalin synthesis.[56] In this case, using a decarboxylative Knoevenagel-Doebner-type reaction on γ-carboxymethyl tetronamides with aldehydes, resulted in a mixture of undentified diastereoisomers (dr = 1:1 to 2:1) in excellent yields (**Scheme 4.7**).[56]

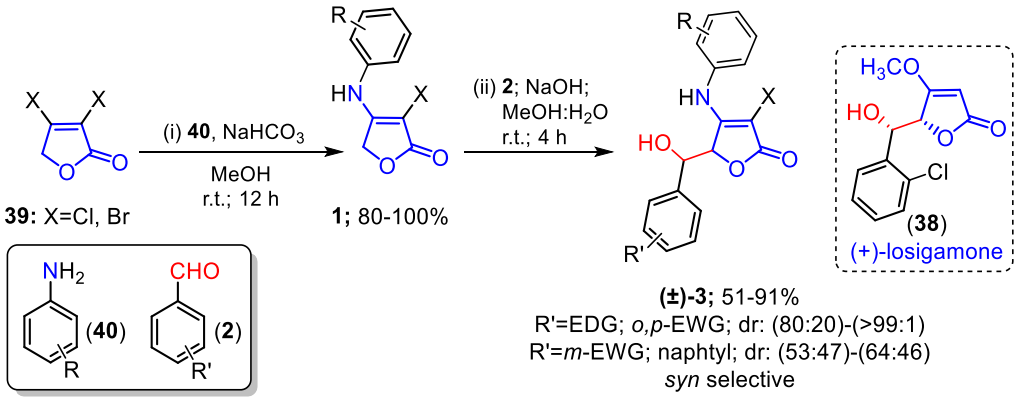



**Scheme 4.7:** Decarboxylative Knoevenagel-type reaction of 5-carboxymethyl tetronamides.[56]

Based on the most recently described stereoselective approaches and through the inspiration by the tetronate *syn*-aldol losigamone (**38**), an

experimental drug undergoing phase III clinical trials for the treatment of epilepsy,[57] our group recently developed a stereoselective vinylogous aldol reaction of *N*-aryl tetronamides with diverse aldehydes. The procedure is simple, scalable and affords mainly the corresponding *syn*-aldol adducts. In many cases, these products were obtained essentially free of their *anti*-isomers (dr > 99:1) in high yields (70–90%). Experimental and computational studies suggest that the observed diastereoselectivity arises through *anti-syn* isomer interconversion, enabled by the aldol/retro-aldol mechanism.[16]

Our synthetic method consists in formation of simple tetronamide intermediates through aza-Michael addition/elimination reaction based on a methodology developed by Cunha and co-workers.[58] Accessible and commercially available α,β-dihalobutenolides namely mucochloric and mucobromic acids (**39**), and different anilines (**40**) afforded β-*N*-aryl-α-halofuran-2(5*H*)-ones (**1**) with yields between 80-100%. Efficient *syn*-diastereoselective VAR treatment (NaOH/LiOH; MeOH:$H_2O$; r.t. open air) was then performed, which resulted in racemic aldolic tetronamides (±)-**3**, leading to several analogs. In this reagard, moderate yields and selectivity were obtained when *orto* and *meta* electron withdrawing groups (EWG) were used. On the contrary, excellent results were finded when electron donating groups (EDG) were employed (**Scheme 4.8**).




**Scheme 4.8:** General synthetic methodology for production of *N*-aryl-γ-substituted tetronamides.[16]

This chapter will describe the approach to obtaining γ-alkylidene tetronamides from these aldolic derivatives **3** and following that; both compounds will be evaluated for their herbicidal and cyanobactericidal activities.

### 4.1.3. Photosynthesis and cyanobacteria inhibition activities in described butenolides.

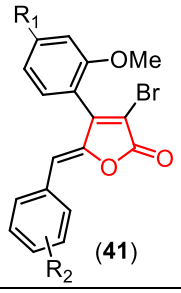
Recently, increasing agricultural productivity and quality has been a necessary and challenging task. Therefore, the reduction of losses to agricultural pests becomes a great concern.[59] Because weeds are the major pests causing agricultural loss of crops worldwide, the maintenance of high agricultural standards is highly dependent on the chemical and biological control of such invasive plants.[60,61] However, the continuous application of herbicides (Glyphosate as most known example) has resulted in selective pressure in the pest, leading to the replacement of sensitive biotypes by herbicide-resistant biotypes, thus making weed management an even more difficult task.[62-64] In addition to this, no novel herbicides with innovative mechanism of action have been introduced into the market in the last two decades.[65] By virtue of this scenario, the discovery of new herbicide molecules that are capable of acting through novel mechanisms of action and additionally present benign environmental and toxicological profiles is imperative. To meet this demand, academic and industrial studies have focused on the search for natural products to serve as models for the development of novel herbicides.[66-68] Many compounds have been developed, as a result, several of them were found to inhibit the light-driven reduction of ferricyanide in isolated spinach chloroplasts. This photo-driven reduction is known as, the Hill reaction, suggesting an inhibition of photosynthetic electron transport chain.[69,70]

Cyanobacteria, also called blue-green algae, are the only prokaryotes able to perform oxygenic photosynthesis.[71] These microorganisms are broadly distributed in the planet and play a major role in both carbon and nitrogen cycling.[72] In favorable conditions, cyanobacteria are capable of blooming rapidly and forming a dense surface scum on the water. This often leads to the establishment of anoxic conditions, altering the ecological structure and contributing to the ecosystem's decline.[73,74] Moreover, the majority of cyanobacteria produce a variety of potentially harmful toxins, targeting either the liver, the gastrointestinal apparatus, or the nervous system,[75] as well as impacting the overall quality of freshwater. Therefore, blooms may affect aquaculture and fisheries, thus potentially causing serious economic damage.[76] Since the

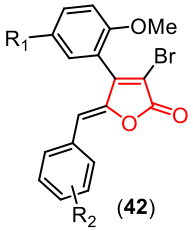
frequency of cyanobacterial blooms is increasing as a consequence of both pollution and climate change,[77] the development of efficient methods for bloom control is urgently required. With this aim, physical[78] and chemical[79] strategies have been exploited. However, since the former are too costly to be applied on a large scale and the latter can cause further pollution, a biological approach to the problem would possibly offer a better alternative.[80] Despite the large number of organisms that produce compounds with algicidal activity, the development of effective biological strategies for bloom control requires isolation, characterization, and inexpensive chemical synthesis of these active compounds.[81,82]

Following this tendency, our research group has synthesized and investigated the phytotoxic and cyanobactericidal activity of a wide range of analogues of naturally-occurring γ-alkylidenebutenolides.[59,83-90] From this immense class of compounds a subclass called rubrolides exhibited interesting results for herbicidal and cyanobactericidal activity.[59,84,90] Thus, the capacity of inhibition of the Hill reaction were investigated, delivering some rubrolide analogs with $IC_{50}$= 1.0-6.0 μM (**Table 4.1**; **41**).[90] The study of effects on the photoautotrophic growth of the cyanobacterium *Synechococcus elongatus* delivered moderate results, with up to 20% inhibition at 50 μM (**42**).[91] Although these results may be considered good to moderate, the synthetic diversity and the biological potential of furan-2(5*H*)-ones, led us to think of new β-*N*-substituited γ-alkylidenebutenolides derivatives based in rubrolide and tetronamide skeletons. With that on mind, we can access to these β-azarubrolide analogs via our recent versatile and stereoselective method to obtain *syn*-aldolate tetronamides. These, in turn, can be transformed into γ-alkylidene products by means of a dehydration reaction, and finally they may be tested as inhibitors of photosynthesis and cyanobacteria.

**Table 4.1:** Rubrolide analogs with herbicide and cyanobatericidal activities



(**41**)



(**42**)

| Comp. | $R_1$ | $R_2$ | $IC_{50}$ (μM) Assay 1[a] | $IC_{50}$(μM) Assay 2[b] | Comp. | $R_1$ | $R_2$ | %Inhibition Assay 1[a,d] | %Inhibition Assay 2[b,e] |
|---|---|---|---|---|---|---|---|---|---|
| 41a | F | *p*-$NO_2$ | 1.1±0.2 | N.D.[c] | 42a | Cl | *p*-$NO_2$ | 62.2±2.9 | 17.6±2.5 |
| 41b | H | *p*-$NO_2$ | 1.3±0.3 | N.D. | 42b | Br | *p*-F | 25.3±1.5 | 9.9±3.0 |
| 41c | F | *p*-$CF_3$ | 3.5±0.4 | N.D. | 42c | Br | *p*-$CF_3$ | 25.3±1.5 | 1.1±2.7 |
| 41d | F | *o*-OH | 5.9±0.9 | N.D. | 42d | Br | *m*-OMe | 6.3±0.7 | 19.4±3.0 |
| DIURON | - | - | 0.27 | N.D. | DIURON | - | - | 98.3±1.7 | 100±0.0 |

[a]**Assay 1:** *In vitro* evaluation of inhibitory activities on ferricyanide reduction by chloroplasts isolated from *S. oleracea* leaves. [b]**Assay 2:** Effects on the photoautotrophic growth of the cyanobacterium *S. elongatus* PCC 6301. [c]N.D.= Not determined; [d]Effect at 10 μM; [e]Effect at 50 μM;

## 4.2. OBJECTIVE AND STRATEGIES

The aim of this chapter is to develop a protocol to carry out the dehydration of aldolic addition products obtained by means of an optimized aldol reaction methodology previously developed by our group. This method consists in the production of *N*-aryltetronamides using aza-Michael addition/elimination performed on α,β-halobutenolide starting materials followed by a diastereoselective vinylogous aldol reaction with aromatic aldehydes.

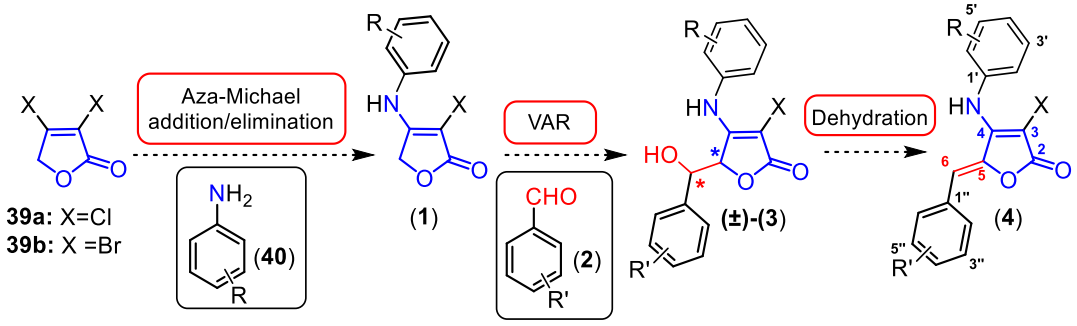
We also aim to evaluate both of the *N*-aryltetronamide precursors and respective unsaturated final products for their photosynthesis and cyanobacteria growth inhibition potential. These activities were selected based on the biological potential of rubrolide and tetronamide connegers.

Lastly, we aim to apply a stereoselective approach for the synthesis of tetronamides using an established protocol for asymmetric hydrogen transfer using Ru catalyst with the oxidized aldolic addition tetronamide congeners.

## 4.3. RESULTS AND DISCUSSIONS.

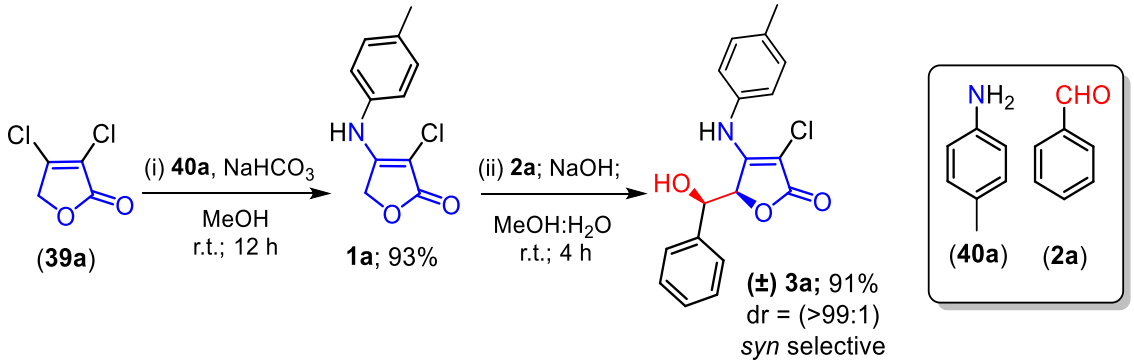
### 4.3.1. Synthesis of γ-alkylidenetetronamides

Our study starts with the synthesis of the γ-alkylidenetetronamides (**4**) this will be accomplished by the dehydration of racemic aldolic precursors (±)-(**3**), in turn, (±)-**3** will be produced by vinylogous aldol reaction (VAR) protocol developed by Karak and co-workers (**Scheme 4.9)**.[16]




**Scheme 4.9:** Synthetic procedure for the γ-alkylidenetetronamides (**4**).

Firstly, aldolic precursor (±)-**3a** was synthetized. In this way, an aza-Michael addition/elimination reaction between mucochloric acid (**39a**) and *p*-toluidine (**40a**) with $NaHCO_3$ as a base, were employed, obtaining tetronamide **1a** in 93% yield (**Scheme 4.10**). Following, **1a** were submitted to optimized VAR using benzaldehyde and NaOH as a base, yielding the racemic tetronamide analog (±)-**3a** in 91% as an essentially *syn* isomer (**Scheme 4.10**).




**Scheme 4.10:** Synthesis of tetronamide analog (±)-**3a**.

The aldolic product (±)-***syn*-3a** structure was confirmed via $^1H$ NMR spectroscopic analysis (**Fig. 4.2**). The singlet signal of -NH at $\delta$= 7.83 may initially be observed followed by multiplet signal corresponding to the γ-benzyl group at $\delta$

= 7.32–7.18. Two doublets from the *N*-aryl signals are seen at 7.07 ppm for H-3'-H-5' and 6.89 ppm for H-2'-H-6' with *J* = 8.3 Hz, as was expected for a *p*-disubstituted benzene ring. Proceeding, the signals of the two newly-formed two chiral centers H-5 and H-6, are viewed at 5.15 ppm and 5.03 ppm, respectively, with *J* = 3.6 Hz. Their chemical shift and coupling constant are coincidental with their *syn*-isomer reported in the literature.[16] Lastly, the *p*-methyl group of *N*-benzene ring may be observed at 2.27 ppm.

The $^{13}$C NMR of compound (±)-***syn*-3a** (**Fig. 4.3**), initially shows the signals of carbonyl C-2 at 169.74 ppm and the β-carbon C-4 at 156.24 ppm. The aromatic signals of C-1'' and C-4'' from the γ-benzylidene ring and C-1' and C-4 from the *N*-aryl group are found at 138.85 ppm, 127.78 ppm, 135.28 ppm and 134.24, respectively. In addition to these, four large signals corresponding to two carbons belonging to benzene rings are observed. These carbons appear at $\delta$ = 129.10 for C-3' and C-5', $\delta$ = 127.89 for C-3'' and C-5'', $\delta$ = 126.40 for C-2'' and C-6'' and $\delta$ = 123.96 for C-2' and C-6'. The α-carbon C-3 is seen at 88.62 and the chiral centers' signals C-5 and C-6 are at 79.40 ppm and 71.26 ppm, respectively. Lastly, the *p*-methyl signal is found at 20.80 ppm. These data corroborates with available literature data.[16]

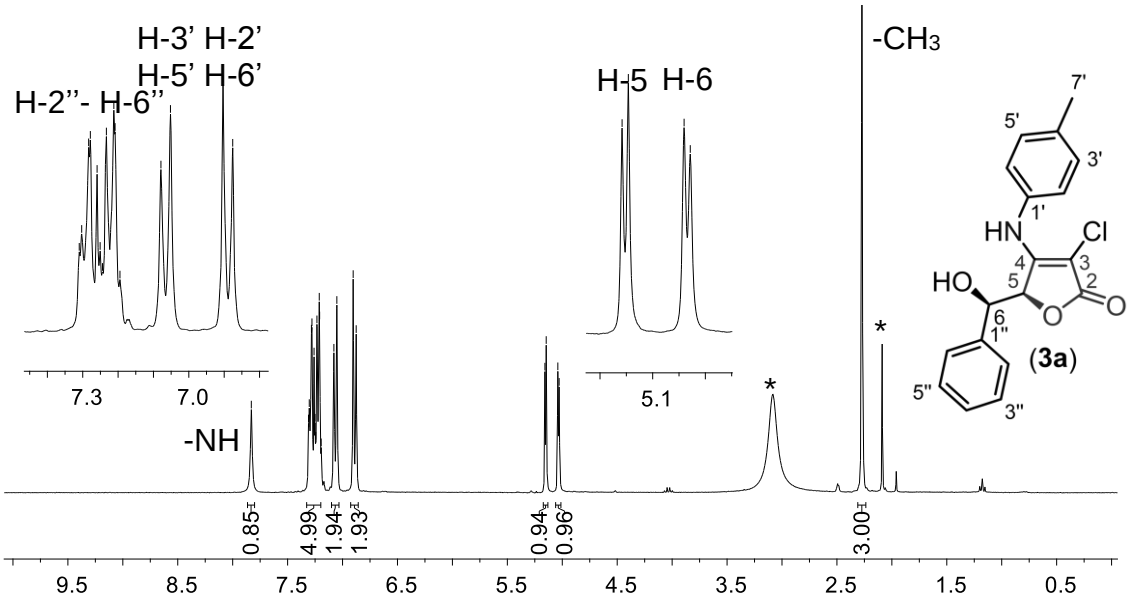



**Figure 4.2:** $^{1}$H NMR (300 MHz, $CDCl_3$:$(CD_3)_2SO$ (9:1)) of aldolic tetronamide (**3a**). *Solvent residues and water signals.

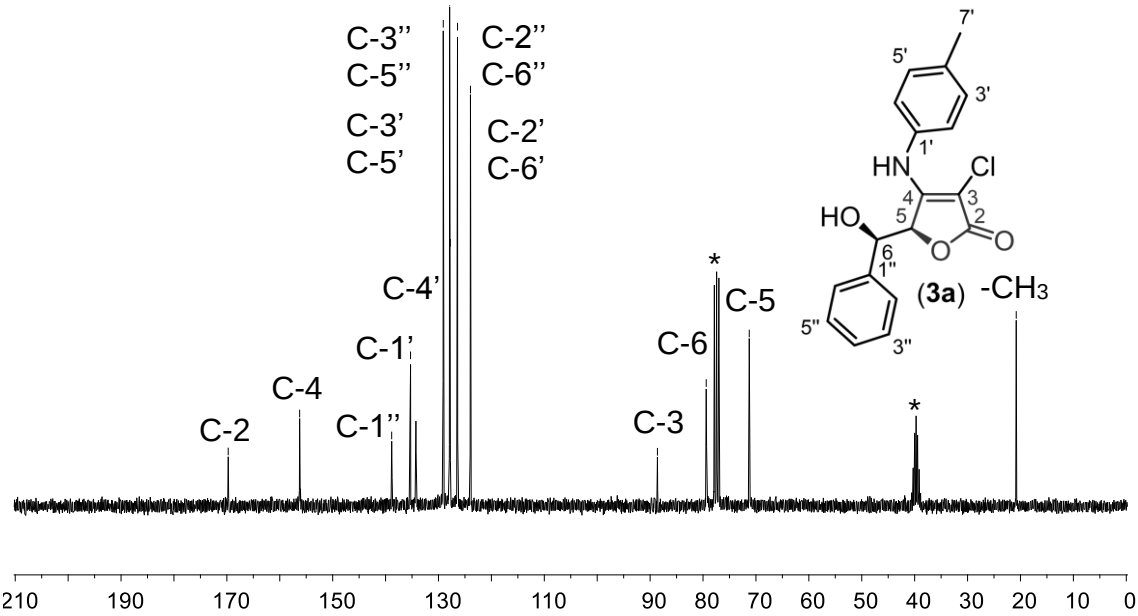
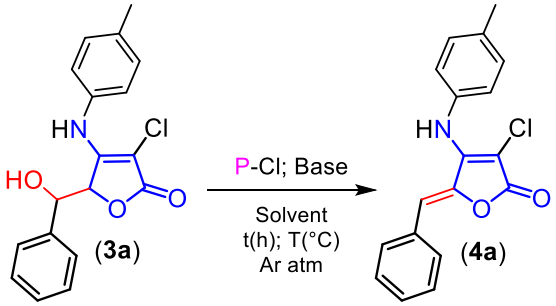



**Figure 4.3:** $^{13}C$ NMR (75 MHz, $CDCl_3$:$(CD_3)_2SO$ (9:1)) of aldolic tetronamide (**3a**). *Solvent residues signals.

With precursor (±)-**3a** in hands, we proceeded to the acquisition of desired γ-alkylidenetetronamide **4a** by means of an adaptation to a dehydration protocol reaction that optimization was already described in chapter 2 for the basidalin synthesis.[2] In this regard, compound (**3a**) was treated with pyridine as a base and mesyl chloride as a pro-leaving group. Unfortunately, this substrate did not promote reaction (**Table 4.2; entry 1**). Therefore, it was decided to optimize the procedure as shown in **table 4.2**.

It was then observed that, the use of the mesyl leaving group did not yield any product mainly due to the degradation of the starting materials, as confirmed by TLC analysis (**Table 4.2; entries 1-4**). This is attributed to the lability of the $MsO^-$ group or to the instability of the *in situ* mesylated intermediate. The MsCl were switched for the more robust TsCl. At first sight, the reaction apparently had gone well, exhibiting one yellow product in TLC analysis. However, after column chromatography separation we discerned a considerably low yield and conversion (**entry 5**). It is important to mention that product was not stable when common silica gel chromatography was applied. In this case, neutral alumina was selected as the stationary phase. Following, a problem with the poor solubility of the starting material in DCM was also identified. Consequently, THF (**entry 6**) and MeCN (**entry 7**) were used, and the latter rendered better results by increasing the to 26% yield.

**Table 4.2:** Optimization of dehydration reaction of aldol product (**3a**)




| Entry | Leaving group (P) (eq) | Base (eq) | Solvent[d] | T (°C) | t (h) | %Rec. | %Yield |
|---|---|---|---|---|---|---|---|
| 1 | MsCl (1)[a] | Py (1)[c] | DCM | r.t. | 4 | 0 | N.R.[e] |
| 2 | MsCl (2) | Py (2) | DCM | r.t. | 8 | 0 | N.R. |
| 3 | MsCl (2) | Py (2) | DCM | 60 | 8 | 0 | N.R. |
| 4 | MsCl (2) | DIPEA (2) | THF | 60 | 8 | 0 | N.R. |
| 5 | TsCl (1)[b] | Py (1) | DCM | r.t. | 4 | 67 | 8 |
| 6 | TsCl (1) | DIPEA (1) | THF | r.t. | 8 | 57 | 16 |
| 7 | TsCl (1) | DIPEA (1) | MeCN | r.t. | 2 | 40 | 26 |
| 8 | TsCl (1) | DIPEA (1) | MeCN | r.t. | 6 | 55 | 34 |
| 9 | TsCl (2) | DIPEA (2) | MeCN | r.t. | 8 | 30 | 48 |
| 10 | TsCl (2) | DBU (2) | MeCN | r.t. | 6 | 0 | 57 |
| 11 | TsCl (2) | DBU (2) | MeCN | 60 | 8 | 0 | 62 |
| **12** | **TsCl** (2) | **DBU** (2) | **MeCN** | **60** | **12** | **0** | **87** |
| 13 | TsCl (2) | DBU (2) | MeCN | 60 | 16 | 0 | 74 |
| 14 | TsCl (2) | DBU (2) | MeCN | 110 | 12 | 0 | N.R. |

[a] MsCl = Mesyl chloride; [b] TsCl = Tosyl chloride; [c] Py = Pyridine; [d] All the solvents were drying; [e] N.R. Don't show any reaction and show consumption of starting material and/or product

Afterwards, the base was changed for the stronger DIPEA, which showed an increment to the yield and the conventions (**Entries 8-9**). As final result, the use of a stronger base, such as DBU, completely consumes the starting material and exhibited 57% yield (**entry 10**). In the same conditions, an increase of the temperature also shows good results, increasing the 62% yield (**entry 11**). To conclude, an adjust in the reaction time to 12 h was attempted which resulted in excellent 87% yield (**entry 13**). It is important to mention that high temperatures decomposed the final product (**entry 14**).

As we can observe in the reaction optimization, the use of polar solvents is adequate for this type of substrate in terms of solubility. Additionally, the use of relatively less efficient leaving group, such as tosyl (pKa (MsOH) = -1.9 Vs pKa (TsOH) = -2.8),[92] may produce more stable reaction conditions. As a final point,

it is clear that the deprotonation of the γ-butenolide proton H-5 in the elimination reaction, requires a stronger base, such as DBU, as was confirmed with the total conversion of starting materials when this base was used. It is also important to mention that only one unsaturated isomer was observed in the reaction mixture and this was expected for the *Z*-isomer that is favored by the bulky β-substituent.[2,27]

The γ-alkylidenetetronamide **4a** structure were confirmed by NMR analysis. The $^{1}$H NMR (**Fig. 4.4**) revealed a singlet belonging to the -NH at $\delta$ = 8.74. The signals of the γ-alkylidene benzene ring were also shown, firstly, a doublet for H-2'' and H-6'' at 7.73 ppm (*J* = 7.2 Hz), secondly, a triplet signal for H-3'' and H-5'' at 7.44 ppm (*J* = 7.0 Hz), and finally, the doublet signal for the H-4'' at 7.37 ppm (*J* = 6.8 Hz). The signals for the *p*-disubstituted *N*-aryl ring are also shown at 7.22 ppm for H-3' and H-5' and 7.22 ppm for H-2' and H-6' (*J* = 7.6 Hz). An important singlet signal was found at 6.60 ppm which that is attributed to H-6, implying that a new instauration was formed. Lastly, the signal of the *p*-methyl group may be observed at $\delta$ = 2.34.

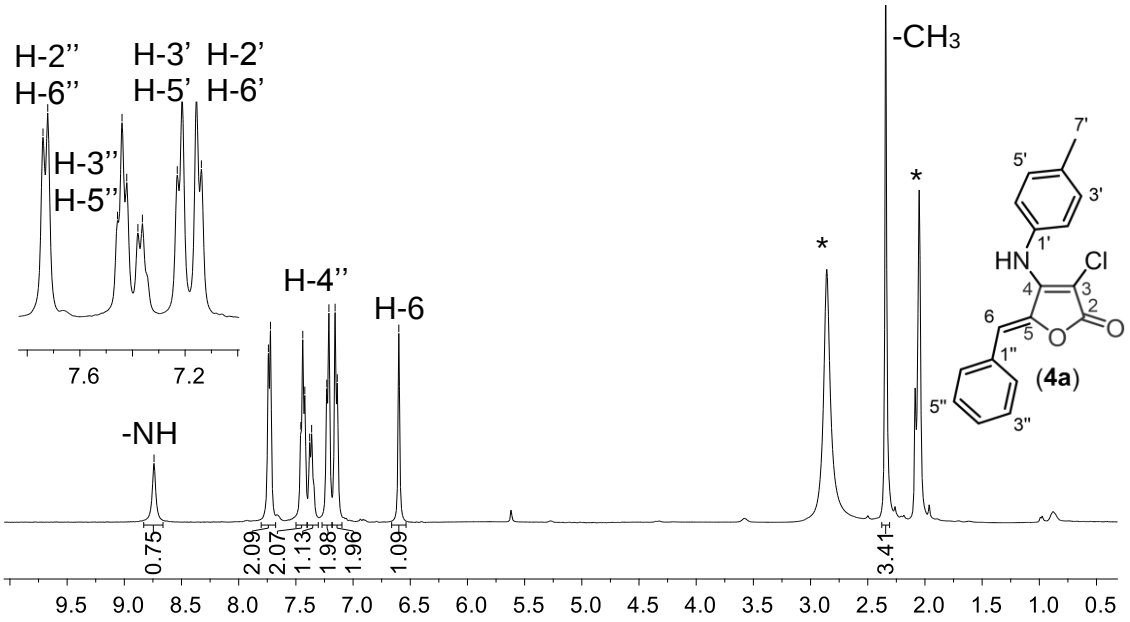



**Figure 4.4:** $^{1}$H NMR (400 MHz, $(CD_3)_2CO$) of γ-alkylidenetetronamide **4a**. *Solvent residues and water signals.

The $^{13}$C NMR of compound **4a** (**Fig. 4.5**), showed the signals of C-2 and C-4 at 165.62 ppm and 149.35 ppm, respectively. The signal of the unsaturated C-5 at 143.29 ppm is also evident, which also indicates that the reaction was successful. The signals for aromatic region may be assigned at 136.00 ppm (C-

1'), 135.94 ppm (C-1''), 133.83 (C-4') ppm, 131.11 ppm (2C, C-3' and C-5'), 130.06 ppm (2C, C-3'' and C-5''), 129.74 ppm (2C, C-2'' and C-6''), 129.69 ppm (C-4'') and 124.93 ppm (2C, C-2' and C-6'). Finally, the double bond signal of C-6 was determined at 108.56 ppm, as well as C-3 at 91.78 ppm, and lastly, the signal of the aromatic-bonded methyl group was at 20.95 ppm.

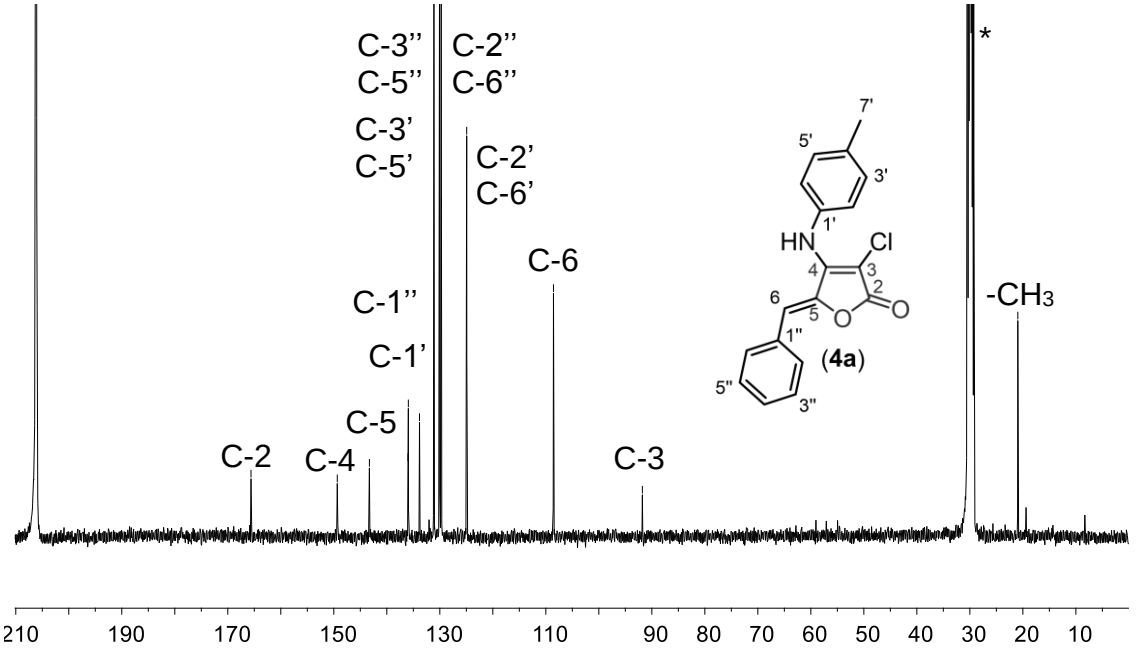



**Figure 4.5:** $^{13}C$ NMR (100 MHz, $(CD_3)_2CO$) of γ-alkylidenetetronamide **4a**. *Solvent residues signals.

With the optimized dehydration procedure, we proceeded to synthetize other unsaturated derivatives. For reasons of time and availability, we attempted to synthesize only another 4 compounds (**Fig. 4.6**). Initially, aldol precursor (±)-**3b** were obtained in 61% yield mostly as *syn* isomer. Its dehydration furnished compound **4b** in 97% yield. The nitro derivative (±)-**3c** was produced in 54% yield with no stereoselectivity (dr around 1:1). This result was expected since Karak and co-workers reported that the use of aldehydes with electron withdrawing groups in *meta*-position decreases the selectivity.[16] The dehydration procedure was done with an isomer mixture of *syn*:*anti* (±)-**3c**, since both isomers are susceptible to this elimination, thus affording the γ-alkylidenetetronamide **4c** with a moderate yield of 52%.

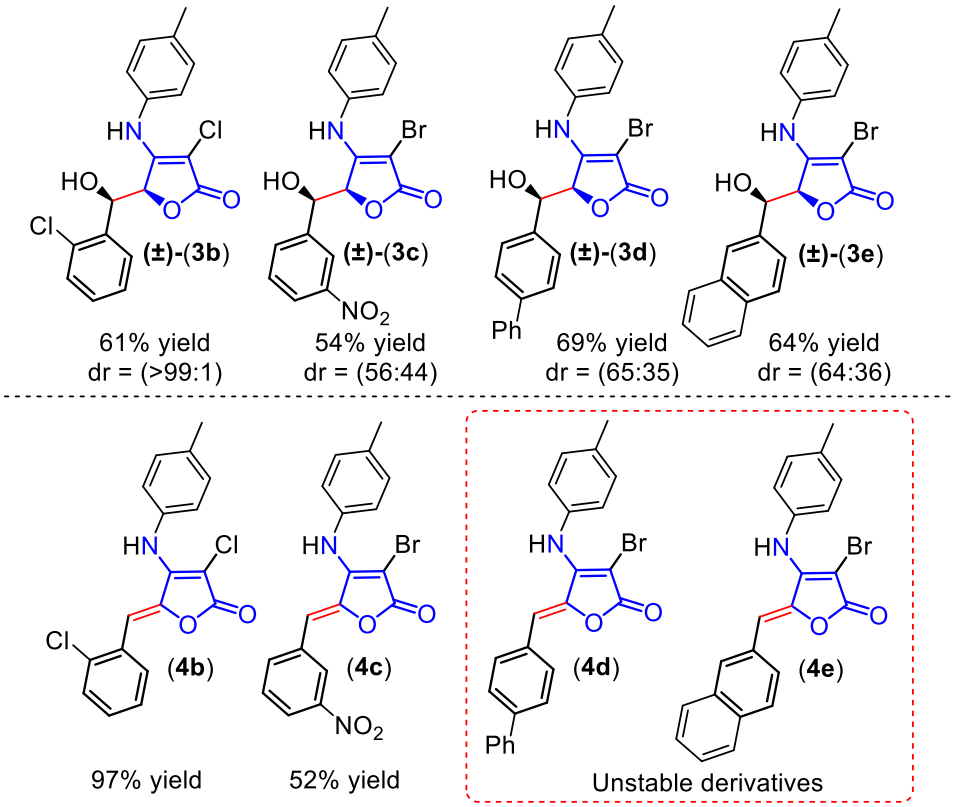



**Figure 4.6:** Synthetic γ–alkylidenetetronamides (**4**) analogs and precursos (**3**). *Major diasteroisomer represented.

Proceeding, in the same conditions, the polyaromatic aldolic compounds (±)-**3d** and (±)-**3e** were obtained in 97% and 64% yield and dr = (>99:1) and (64:36), respectively. Unfortunately, when these tetronamides were submitted to elimination reaction unstable compounds were obtained (**4d-e**), which, was confirmed by TLC analysis, as well as some separation difficulties and considerably complex NMR spectra when the supposedly pure compound was separated. In spite of this unfortunate event, we decide to explore the biological activities of both, alcoholic and unsaturated tetronamides to determinate the real influence of the structural modification in the herbicidal and cyanobactericidal tests.

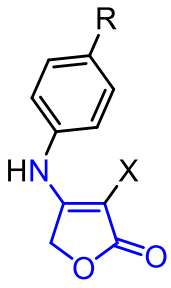
### 4.3.2. Biological test

In this part of our study a structure-activity investigation of some analogs of *N*-aryltetronamides (**1**), its racemic aldolic derivatives ((±)-**3**) and final γ-alkylidenetetronamides (**4**) was develop by means of two biological assays. One of these essays was the evaluation of photosynthesis inhibition (Hill's reaction

inhibition) for the establishment of herbicidal activity and the growth inhibition of the cyanobacterium *S. elongatus*. These assays were based on the results obtained by our group concerning the activity of some congeners, such as rubrolides.[59,84,90,91] This analysis was developed by professor Dr. Giuseppe Forlani from the Department of Life Science and Biotechnology in Ferrara University, Italy. His team developed two assays: an *in vitro* evaluation of the inhibitory activities on ferricyanide reduction in chloroplasts isolated from *Spinacia oleracea* leaves (spinach) (**Assay 1**) and a study of the effects on the photoautotrophic growth of the *Synechococcus elongatus* PCC 6301 cyanobacterium (**Assay 2**).

Initially, some simple tetronamide starting materials **1a-h** were synthetized via the aza-Michael addition/elimination procedure. Additionally, the α-dehalogenated compound **1i** was synthetized via palladium-catalyzed hydrodehalogenation of butenolides, starting from **1a**, following a procedure reported by Karak and co-workers.[93] Following their synthesis, these compouds were tested using both mentioned assays as shown in **Table 4.3**.

**Table 4.3:** Herbicidal and cyanobatericidal biological assays on simple tetronamides analogs (**1**).

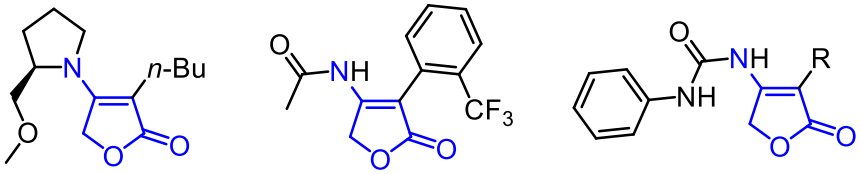


(**1**)

| Entry | Comp. (%Yield) | X | R | $IC_{50}$ (μM) assay 1[a] | $IC_{50}$ (μM) assay 2[b] |
|---|---|---|---|---|---|
| 1 | 1a (93) | Cl | Me | 314.0±77.0 | 253.0±92.0 |
| 2 | 1b (100) | Cl | H | >1000 | >1000 |
| 3 | 1c (99) | Cl | OMe | >1000 | 332.0±130.0 |
| **4** | **1d (89)** | **Cl** | **Br** | **>1000** | **41.0±4.0** |
| 5 | 1e (82) | Br | Me | 434.0±170.0 | 297.0±123.0 |
| 6 | 1f (96) | Br | H | >1000 | >1000 |
| 7 | 1g (84) | Br | OMe | >1000 | 180.0±26.0 |
| **8** | **1h (89)** | **Br** | **Br** | **>1000** | **40.0±5.0** |
| 9 | 1i[c] (66) | H | Me | >1000 | 882.0±636.0 |

[a]**Assay 1:** *In vitro* evaluation of inhibitory activities on ferricyanide reduction in chloroplasts isolated from *S. oleracea* leaves. Positive control, DIURON® $IC_{50}$=0.27 μM. [b]**Assay 2:** Effects on the photoautotrophic growth of the cyanobacterium *S. elongatus* pcc 6301. [c]compound **1i** were obtained by dehalogenation reaction from **1a**.[93]

As can be observed, the results for the herbicidal assay are not promising for simple tetronamides, exhibiting an $IC_{50}$>300 μM. This result is surprising considering that some small patented 4-azobutenolides (**43**-**45**) and their analogs presents outstanding herbicidal activity (**Fig. 4.7**).[8] On the other hand, some moderate results were found in the cyanobactericidal assays; in this case poly-halogenated tetronamides **1d** and **1h** showed some efficacy in inhibiting the growth of *S. elongatus* with an $IC_{50}$ around 40 μM.



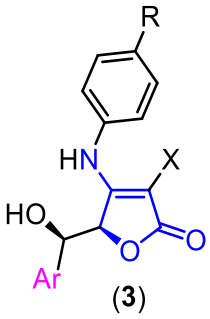

(**43**) (**44**) (**45**)

**Figure 4.7:** Tetronamides with herbicidal activities.

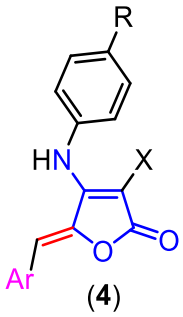
The aldolic adducts **3** and the γ-alkylidene tetronamides (**4**) that were synthetized before were then tested, just to assess the influence of the new double bond in the tetronamide moiety (**Table 4.4**). It is important to mention that, when possible to separate, only the *syn* isomer of intermediates **3** were biologically evaluated.

The results initially show that the tested γ-alkylidene tetronamides **4** are not good herbicides nor cyanobatericidals agents, on account of the high $IC_{50}$ values in Hill's reaction and blue-green algae growth inhibition (>1000 μM and >180 μM respectively). On the contrary, very interesting results were found when adducts **3** were tested. The addition of the alcoholic groups in C-5 promote an increase in herbicidal activities, 3 or 4 times greater when compared to aldolic compounds **3** with its respective simple tetronamides precursors **1** (*e.g.* compounds **1a** $IC_{50}$ = 314.0 vs **3a** $IC_{50}$ = 72.8 μM and compounds **1e** $IC_{50}$ = 434.0 vs **3c** $IC_{50}$ = 152.0 μM). Moreover, the most exciting result came from the cyanobactericidal activity when biphenyl adducts **3d** were used, showing an excellent $IC_{50}$ = 2.8 μM (**Table 4.4, entry 7**).

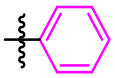
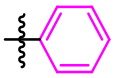
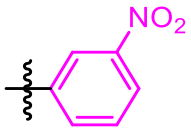
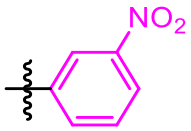
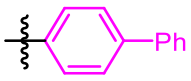
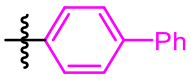
**Table 4.4:** Herbicidal and cyanobatericidal biological assays of aldol products (**3**) and γ-benzylidenetetronamides (**4**).



(**3**)



(**4**)

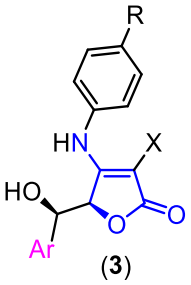
| Entry | Comp. (%Yield) dr: [*syn:anti*] | X | R | Ar | $IC_{50}$ (μM) Assay 1[a] | $IC_{50}$ (μM) Assay 2[b] |
|---|---|---|---|---|---|---|
| 1 | 3a[c] (91) [99:1] | Cl | Me |  | 72.8±11.0 | 198.0±79.0 |
| 2 | 4a[d] (87) | Cl | Me |  | >1000 | 285.0±120.0 |
| 3 | 3b (61) [>99:1] | Cl | Me |  | >1000 | >1000 |
| 4 | 4b (97) | Cl | Me |  | >1000 | 180.0±3 |
| 5 | 3c (54) [56:44] | Br | Me |  | 152.0±40.0 | 89.6±10.8 |
| 6 | 4c (52) | Br | Me |  | >1000 | 433.0±162.0 |
| **7** | **3d (69) [65:35]** | **Br** | **Me** |  | **58.4±53.7** | **2.8±0.3** |
| 8 | 4d | Br | Me |  | N.D.[e] | N.D. |

[a]**Assay 1:** *In vitro* evaluation of inhibitory activities on ferricyanide reduction in chloroplasts isolated from *S. oleracea* leaves. Positive control, DIURON® $IC_{50}$=0.27 μM. [b]**Assay 2:** Effects on the photoautotrophic growth of the cyanobacterium *S. elongatus* pcc 6301. [c]yield and dr obtained from VAC reaction, just *syn* isomer were biologically tested. [d]yield obtained from dehydration reaction. [e]N.D. Not determined.

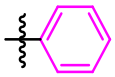
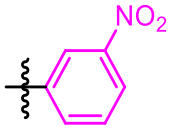
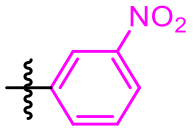
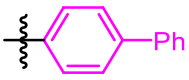
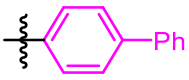
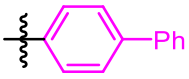
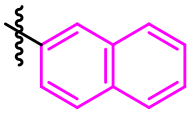
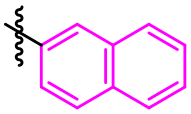
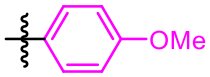
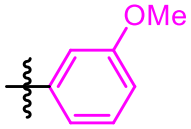
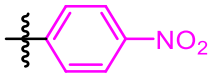
Therefore, in spite of the weak inhibitory effects of the desired γ-alkylidenetetronamides and their instability, we decided to focus only on the new more promising aldol intermediates **3**. Their potential was evaluated through a variation of the aromatic γ-substituents.

In this regard, some other analogs were sinthesized using a vinylogous aldol condensation reaction between simple tetronamdies (**1**) and many aromatic aldehydes. The reaction's selectivities and yields corroborated with other reported VAC reactions (**Table 4.5**).[16] These compounds were then tested for their herbicidal and cyanobatericidal activities as shown in **Table 4.5**.

**Table 4.5:** Herbicidal and cyanobatericidal biological assays of aldol tetronamide products (**3**)



(**3**)

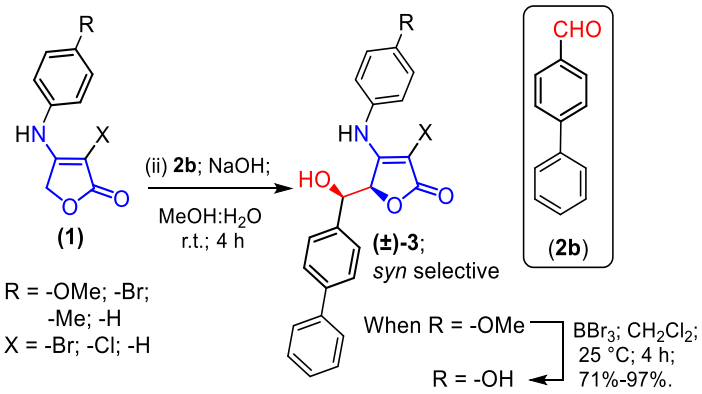
| Entry | Comp. (%Yield) dr [*syn:anti*][a] | X | R | Ar | $IC_{50}$ (μM) Assay 1[b] | $IC_{50}$ (μM) Assay 2[c] |
|---|---|---|---|---|---|---|
| 1 | 3a (91) [>99:1] | Cl | Me |  | 72.8±11.0 | 198.0±79.0 |
| 2 | 3e* (51) [53:47] | Cl | Me |  | 86.8±35.1 | 238.0±34.0 |
| 3 | 3c (54) [56:44] | Br | Me |  | 152.0±40.0 | 89.6±10.8 |
| **4** | **3d (69) [65:35]** | **Br** | **Me** |  | **58.4±53.7** | **2.8±0.3** |
| **5** | **3f (93) [90:10]** | **Cl** | **Me** |  | **>1000** | **4.4±0.1** |
| **6** | **3g* (72) [66:33]** | **Cl** | **Br** |  | **186.0±308.0** | **1.3±0.1** |
| 7 | 3h (67) [64:36] | Br | Me |  | 28.8±5.7 | 50.7±5.2 |
| 8 | 3i* (59) [70:30] | Cl | Br |  | 23.8±7.4 | 16.1±1.8 |
| 9 | 3j* (75) [98:2] | Cl | Br |  | 106.0±78.0 | 499.0±146.0 |
| 10 | 3k* (68) [>99:1] | Cl | Br |  | 104.0±50.0 | 485.0±249.0 |
| 11 | 3l* (81) [93:7] | Cl | Br |  | 23.7±4.9 | 32.0±2.4 |

[a]yield and dr obtained from VAC reaction, just *syn* isomers were biologically tested. [b]**Assay 1:** *In vitro* evaluation of inhibitory activities on ferricyanide reduction in chloroplasts isolated from *S. oleracea* leaves. Positive control, DIURON® $IC_{50}$=0.27 μM. [c]**Assay 2:** Effects on the photoautotrophic growth of the cyanobacterium *S. elongatus* pcc 6301. *These compounds were previously synthetized by Karak and co-workers.[16]

The importance of the presence of bipheny groups in the compounds tested for growth inhibition of cyanobacteria is clear. Thus, excellent results were obtained in this singular case, with $IC_{50}$=1.3-4.4 μM (**Table 4.5, entries 4-6**). Another important annotation is the fact that the presence of Br atoms in the α and the *para*-position in the β-*N*-ring (*i.e.* R and/or X) improves the

cyanobactericidal activity giving the lowest concentration results when Cl is changed by Br in other molecules such as **3e** and **3c** ($IC_{50}$=238 μM vs $IC_{50}$=89.6 μM; **entries 2 and 3**) or when a molecule possesses only halogen atoms as showed for compounds **3h** with **3i** ($IC_{50}$=50.7 μM vs $IC_{50}$=16.1 μM; **entries 7 and 8**). Another interesting result is the moderate cyanobactericidal activity of naphthalene and *p*-$NO_2$ derivatives in the order of $IC_{50}$=16.0-50.0 μM as well as its moderate herbicidal activities with $IC_{50}$=23.0-29.0 μM (**entries 7,8 and 11**).

Finally, the activity of some different biphenyl derivatives were studied and some analogs were sinthesized with a structural modification on β-*N*-aryl ring, by means of a vinylogous aldol condensation between simple tetronamides **1a-i** with [1,1'-biphenyl]-4-carbaldehyde (**2b**). Additionally a demethylation reaction on *N*-*p*-OMe-aryl derivatives was applied to acquire the respective hydroxylated products. (**Scheme 4.11**). The yields, diastereoselectivity and biological activities of such analogs are presented in **Table 4.6**.

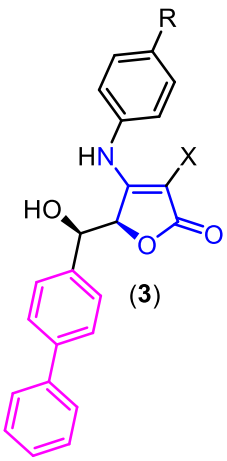



**Scheme 4.11:** Synthesis of biphenyl tetronamide analogs **3**.

In first instance, it may be observed that the herbicidal activities of the biphenyl analogs did not show significant results. On the other hand, the cyanobactericidal test was more promising. Initially, the α-bromine derivatives showed the best activities, enhancing the importance of the presence of this halogen in these molecules (**Table 4.6**; **entries 6-9**). Secondly, it is noteworthy that the dehalogenation on the α-carbon in the lactone ring reduces the activity, as can be seen in compounds **3d** and **3f** when compared with **3t** ($IC_{50}$ = 2.8 and 4.4 μM vs $IC_{50}$ = 31 μM; **entries 1, 6 and 11**). We may also confirm that the *p*-hydroxylated homologues **3o** and **3s** showed less activity when compared with respective precursors compounds **3n** and **3r**, giving a reduction of $IC_{50}$ from 275.0

μM to 444.0 μM (**entries 4 and 5**) and $IC_{50}$ from 5.1 μM to 116.0 μM (**entries 9 and 10)**, respectively. Nonetheless only *p*-OMe with α-bromine (**3r**) exhibited excellent cyanobactericidal activities.

**Table 4.6:** Herbicidal and cyanobatericidal biological assays of byphenil aldolic analogs (**3**).



(**3**)

| Entry | Comp. (%Yield) dr [*syn:anti*][a] | X | R | $IC_{50}$ (μM) Assay 1[b] | $IC_{50}$ (μM) Assay 2[c] |
|---|---|---|---|---|---|
| **1** | **3f (93) [90:10]** | **Cl** | **Me** | **>1000** | **4.4±0.1** |
| **2** | **3g (72) [66:33]** | **Cl** | **Br** | **186.0±308.0** | **1.3±0.1** |
| 3 | 3m (100) [2:1] | Cl | H | >1000 | >1000 |
| 4 | 3n (91) [90:10] | Cl | OMe | >1000 | 275.0±55 |
| 5 | 3o[d] (97) | Cl | OH | 313.0±115.0 | 444.0±310 |
| **6** | **3d (69) [65:35]** | **Br** | **Me** | **58.4±53.7** | **2.8±0.3** |
| **7** | **3p (61) [>99:1]** | **Br** | **Br** | **77.0±15.0** | **5.0±0.6** |
| 8 | 3q (91) [94:6] | Br | H | >1000 | 20.0±3.0 |
| **9** | **3r (78) [>99:1]** | **Br** | **OMe** | **>1000** | **5.1±0.5** |
| 10 | 3s[d] (71) | Br | OH | 335.0±120.0 | 116.0±16.0 |
| 11 | 3t (73) [66:33] | H | Me | >1000 | 31.0±4.0 |

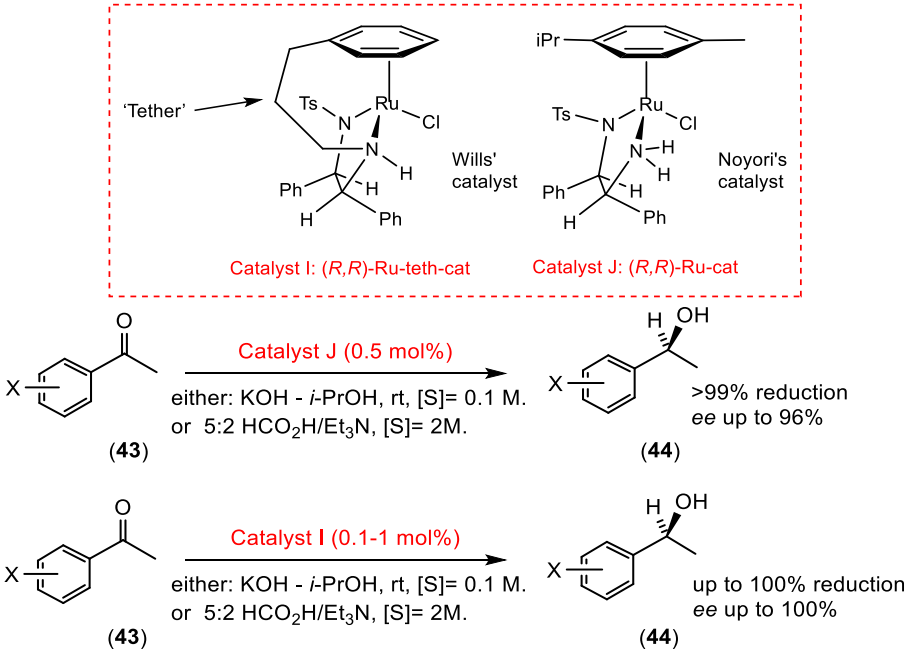
[a]yield and dr obtained from VAC reaction, just *syn* isomer were biologically tested. [b]**Assay 1:** *In vitro* evaluation of inhibitory activities on ferricyanide reduction in chloroplasts isolated from *S. oleracea* leaves. Positive control, DIURON® $IC_{50}$=0.27 μM. [c]**Assay 2:** Effects on the photoautotrophic growth of the cyanobacterium *S. elongatus* pcc 6301. [d]yield obtained from demethylation reaction.

As a conclusion, compounds **3d** and **3g** exhibited the best activities with $IC_{50}$ = 1.3 and 2.8 μM. They are therefore promising candidates for cyanobacterial control. As important result, the presence of halogens in the molecules, evidently

improve their activity, thus making these types of biphenyl analogs very promising biological models.

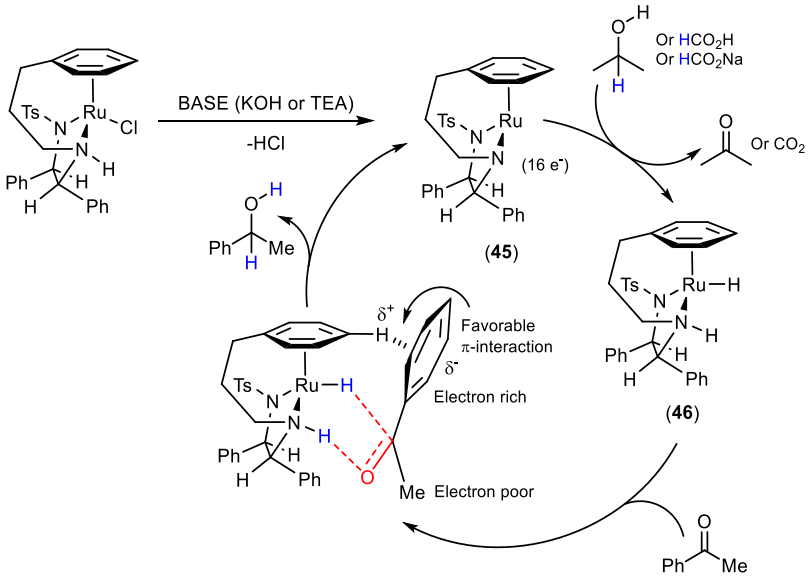
### 4.3.3. Enantioselective approach for the synthesis of aldolic tetronamides.

As was discussed before, some tetronamide analogs were synthetized by means of vinylogous aldol condensation reaction and some showed excellent cyanobactericidal activities. Nevertheless, all the compounds were obtained as racemic mixtures of diastereoselective *syn*-products.[16] Ideally, biological studies requires an enantiopure chiral compounds to correctly determine their properties. Therefore, to date several different stereo selective protocols and catalysts have been developed, as were shown for the aldol reactions.[36-51] In accordance to this tendency, Wills and co-workers designed an optimal asymmetric transfer hydrogenation (ATH) reaction with ruthenium(II) catalyst for the enantioselective reduction of ketones. Such stability, efficience and performance were previously proved (**Scheme 4.12**).[94-96] This catalyst was based on the homologous Noyori's catalyst,[97,98] exhibiting its main difference in the adaptation of a lateral carbon chain producing a more versatile tethered catalyst system (**Scheme 4.12**). As a result, η6-aryl cannot rotate and dissociation of the ligand should be reduced due to multipoint binding.[94]



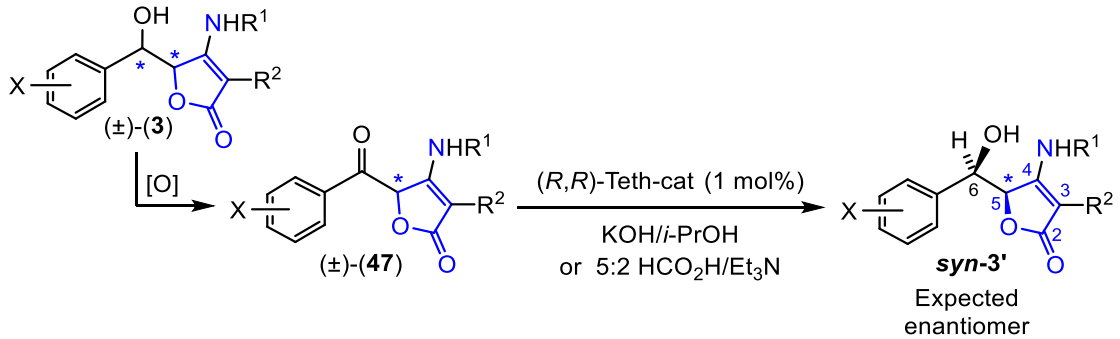

**Scheme 4.12:** Comparation between Noyori's catalyst and Wills' catalyst.

The ATH mechanism with the Ru(II)-catalyst and ketone substrates initially shows a Brønsted acid-base activation, resulting in an electron-deficient catalyst form **45** (**Scheme 4.13**). This activation makes **45** susceptible to bond with the *in situ* hydrogen formed by the mixtures of $HCO_2H/Et_3N$ (5:2) or *i*-PrOH/KOH. Lastly, the hydrogenated catalyst (**46**) reacts stereoselectively with the substrate, restoring activated catalyst **45**. This highly-specific interaction with the substrate is determined by the approach of the most electron-rich region in the ketone with the η6-arene group due to a strong π-interaction (**Scheme 4.13**).



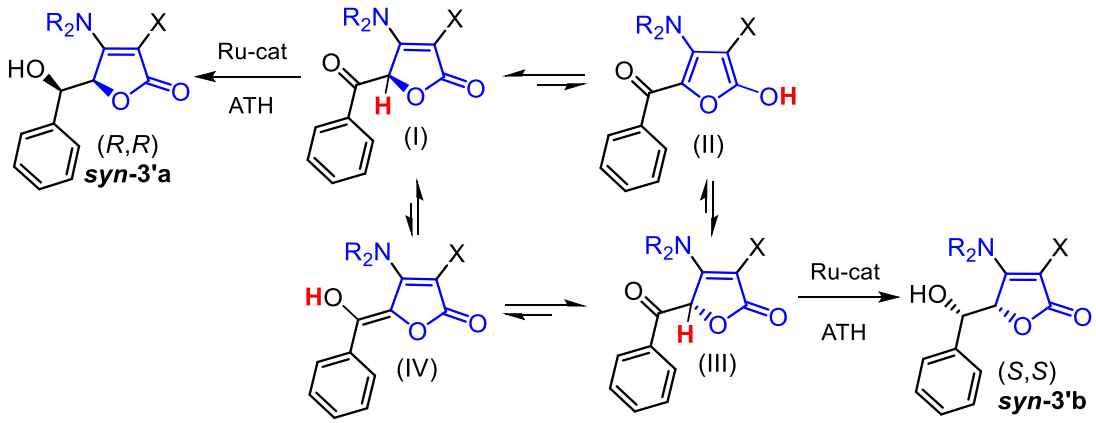

**Scheme 4.13:** Asymmetric hydrogen transfer hydrogenation (ATH) mechanism.

Based on this information, we can adapt the specific ATH methodology to our tetronamide racemic analogs (±)-**3**. Hereafter, taking advantage of the oxidation susceptible benzylic alcohol moiety in our products, some oxidized aldolic tetronamides (±)-**47** can be produced and finally, these products may be submitted to optimal catalytic ATH methodology using (*R,R*)-Ru-Teth-cat (**Scheme 4.14**).



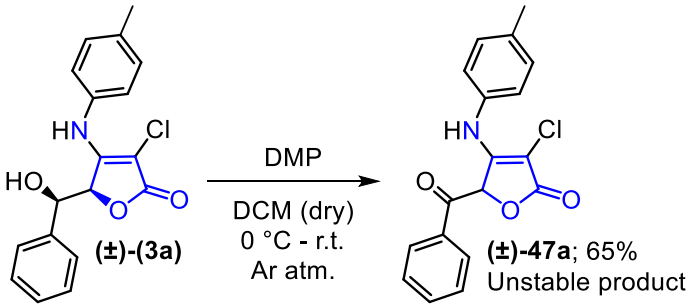

**Scheme 4.14:** Synthetic approach to ATH for oxidated tetronamides.

The correct stereochemistry in reduced product ***syn*-3'** can be assumed considering that *syn* diastereomer of the aldolic tetronamides (**3**) is thermodynamically more stable, as previously dicussed.[16] Indeed, we have to contemplate that oxidized precursor (±)-**47** is obtained as a racemic mixture, as well as, it can exist in an keto-enolic equilibrium due to the higher acidity of its γ-hydrogen (**I-IV**; **Scheme 5.15**). In this context, the selective production of one of the *syn* enantiomers **syn-3'a** (*R*,*R)* or **syn-3'b** (*S*,*S*) will depend of which equilibrium species reacts with selected catalyst, in this case, the precursors **I** or **III** are suitable to accomplish the asymmetric reduction. Once the reaction takes place, the equilibrium, through species **II** or **IV**, is displaced in favor to the consumed species, resulting in complete transformation of starting material.



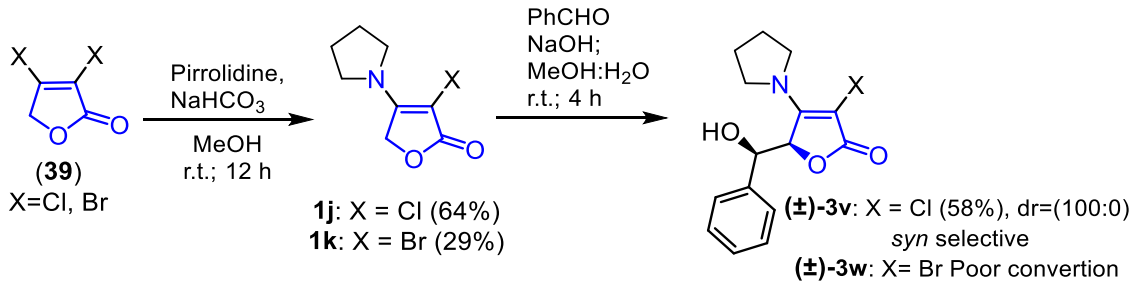

**Scheme 4.15:** Oxidized tetronamides (±)-**47** equilibrium in AHT reaction.

Our strategy is then performed by submitting compound (±)-**3a** to different oxidation methods (PCC, PDC, $MnO_2$, IBX and DMP) Unfortunately, no successful results were obtained. In all methodologies the starting material were consumed but degradation compounds were obtained as confirmed by TLC. Only the Dess-Martin oxidation method resulted in some apparent oxidation product (±)-**47a** yielding 65% of a dark brown oil (**Scheme 4.16**). However, further TLC and NMR analysis confirmed that (±)-**47a** was unstable. Since this instability may be caused by the sensitive N-H group in these *N*-aryltetronamides, an alternative to use a non-*N*-hydrogenated tetronamide derivative was proposed, in order to achieve more robust tetronamide derivatives. Thus, a secondary amine was used in the initial Michael addition/elimination step.




**Scheme 4.16:** Oxidation of the tetronamide (±)-**3a**.

With that on mind, pyrrolidine derivatives were used as new substrates and synthetized using the same optimized VAR methodology (**Scheme 4.17**). In this case the reaction produced chlorinated compound (±)-**3v** in 58% yield with a very high selectivity for the *syn-isomer*, as well as its *N*-aromatic congeners. Curiously, when reaction was performed with 3,4-dibromofuran-2(5*H*)-one in both steps presented low yields and very poor conversions. Specially, in VAR step where after 12 h of reaction just little apparent product and no consumed starting material was confirmed by TLC analysis, making it an unreliable substrate.




**Scheme 4.17:** Synthesis of pyrrolidine derivatives of aldol tetronamides.

The tetronamide (±)-**3v** structure were confirmed by IR and NMR analysis. Initially, the IR spectrum showed characteristic bands of -OH group at 3310 $cm^{-1}$ and C=O stretch band at 1715 $cm^{-1}$.

$^{1}$H NMR spectrum (**Fig. 4.8**) showed the aromatic signals, firstly a doublet at 7.44 ppm (*J* = 7.5 Hz) for H-2'' and H-6''. Secondly, a triplet is seen at 7.36 ppm (*J* = 7.3 Hz) corresponding to H-3'' and H-5'' and lastly, a doublet is found at 7.44 ppm (*J* = 7.1 Hz) for H-4''. The doubet signal for H-5 is found at 5.72 ppm (*J* = 5.5 Hz), while the -OH signal is at 5.32 and the other chiral center H-6 at 5.12 ppm (*J* = 5.6 Hz). Finally, the signals of the pyrrolidine ring are at 5.73 ppm and 5.72 ppm for H-1' and H-4' for the methylene bonded to the nitrogen, and the final signals are at 5.13 ppm and 5.12 for H-2' and H-3'.

The DEPT spectrum of tetronamide (±)-**3v** (**Fig. 4.9**) showed the signals of carbonyl C-2 at $\delta$= 169.39 and C-4 $\delta$= 158.03. We may also observe the aromatic signals at 141.27 ppm for C-1'' and at 127.17 ppm for C-4''. Two large signals are present at 127.77 ppm for C-3'' and C-5'' and 126.42 ppm for C-2'' and C-6''. The signal of the $\alpha$-carbon C-3 is at $\delta$= 84.15, and the signals of the chiral centers C-5 and C-6 may be assigned at $\delta$= 80.61 and $\delta$= 69.03, respectively. Lastly, the signals of the pyrrolidine ring are found at 40.36 ppm for C-1' and C-4' and 24.81 ppm for C-2' and C-3'.

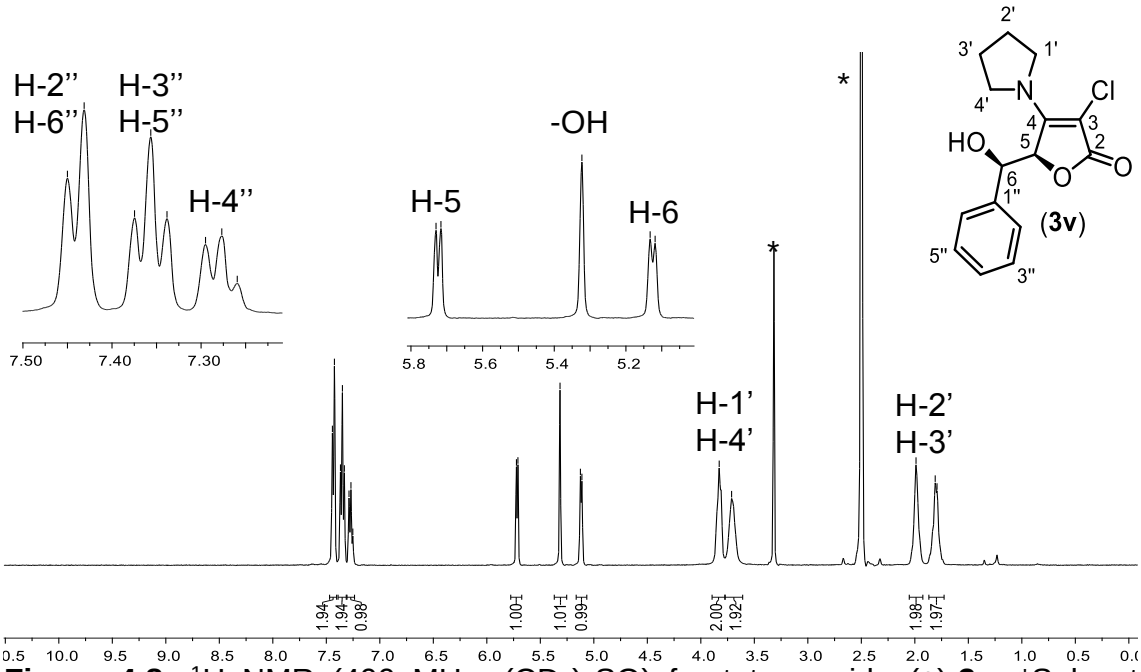



**Figure 4.8:** $^{1}$H NMR (400 MHz; $(CD_3)_2SO$) for tetronamide (±)-**3v**. *Solvent residues solvent and water signals.

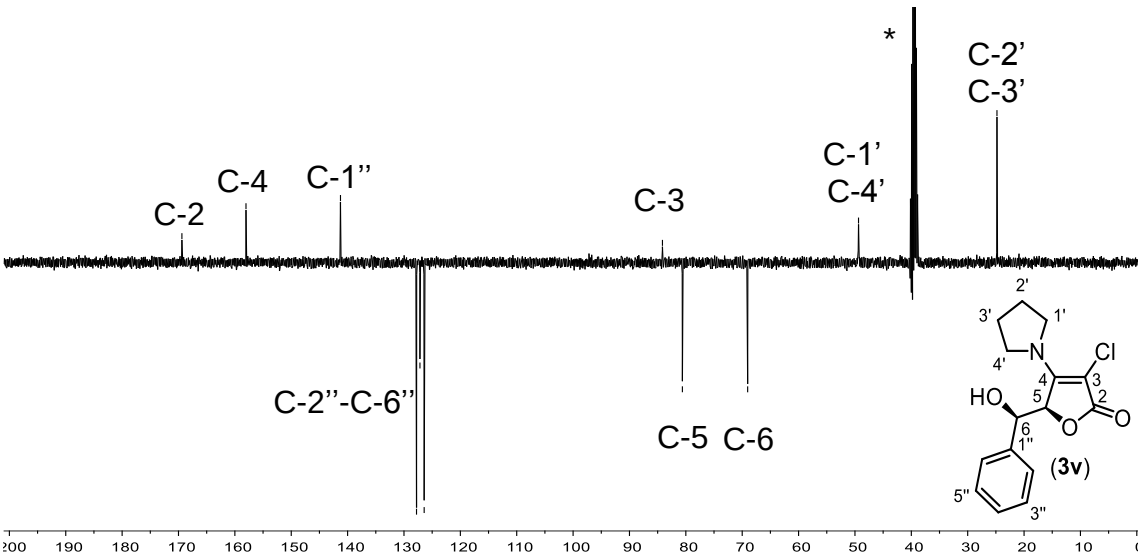



**Figure 4.9:** DEPT135 NMR (100 MHz, $(CD_3)_2SO$) for tetronamide (±)-**3v**. *Solvent residues solvent signal.

Compound (±)-**3v** was then submitted to oxidation with PCC giving an excellent 80% yield and a completely stable ketone intermediate (±)-**47b** (**Scheme 4.18**). Oxidation of the compound was confirmed by IR and NMR analysis of the product. When comparing only with tetronamide (±)-**3v** in IR spectrum of (±)-**47b** can be detected a second carbonyl band at 1692 $cm^{-1}$ and the absent of the typical -OH band. $^{1}H$ NMR analysis only revealed the signal of H-5 at 6.79 ppm, which is shown to have shifted due to the new adjacent carbonyl group, and no observable signals of -OH and H-6, confirming oxidation (**Fig. 4.10**). In the same regard, DEPT shows the new ketone signal C-6 at 191.24 ppm (**Fig. 4.11**). The other signals were attributed similarly to tetronamide (±)-**3v.**

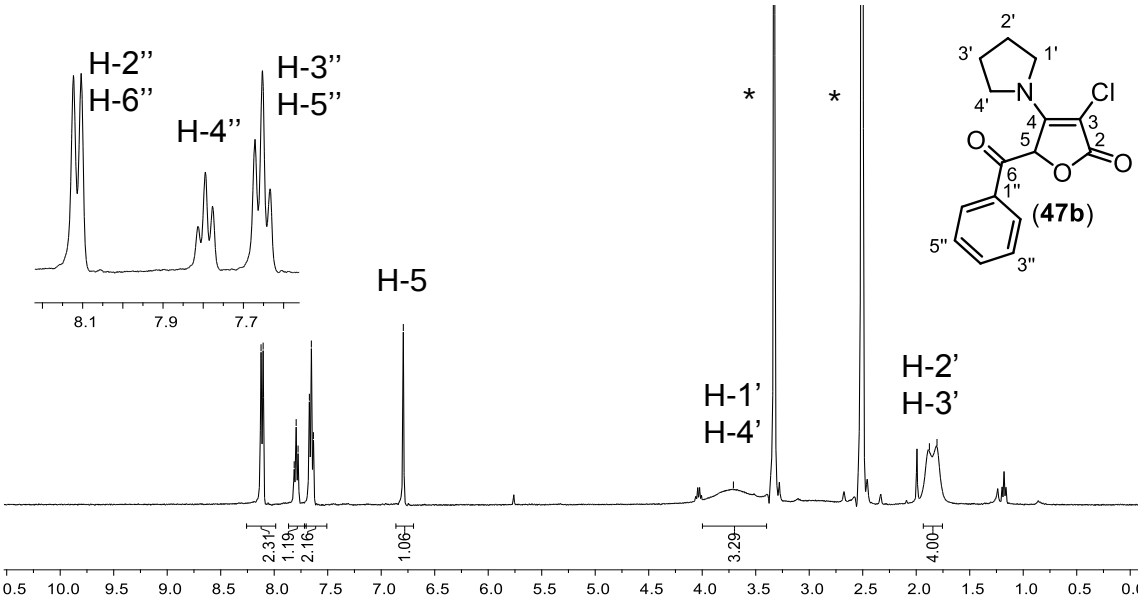



**Figure 4.10:** $^{1}H$ NMR (400 MHz, $(CD_3)_2SO$) for oxidized tetronamide (±)-**47b**. *Solvent residues and water solvent signal.

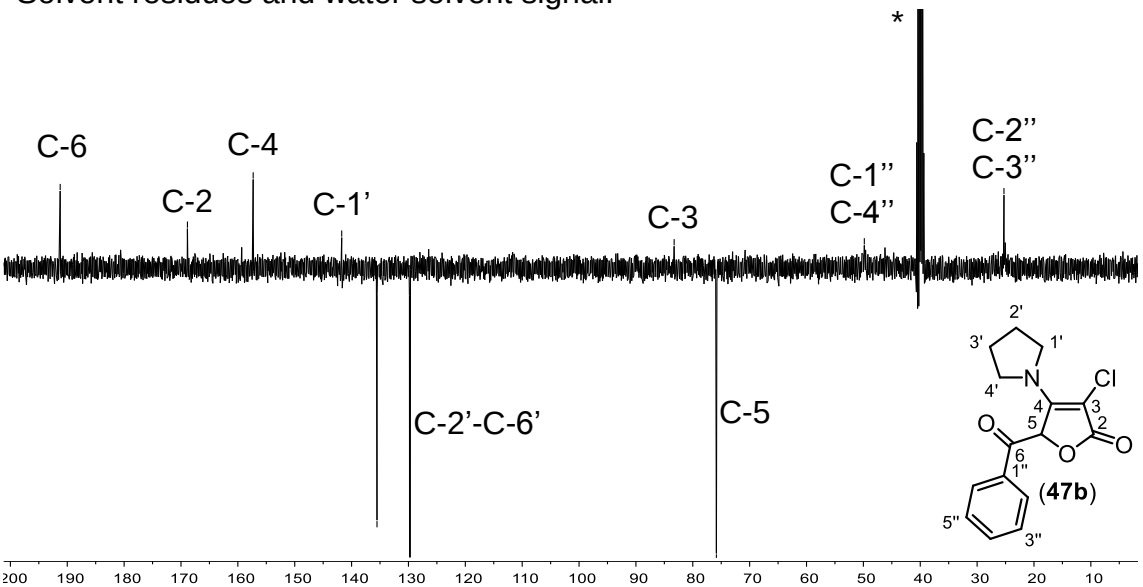
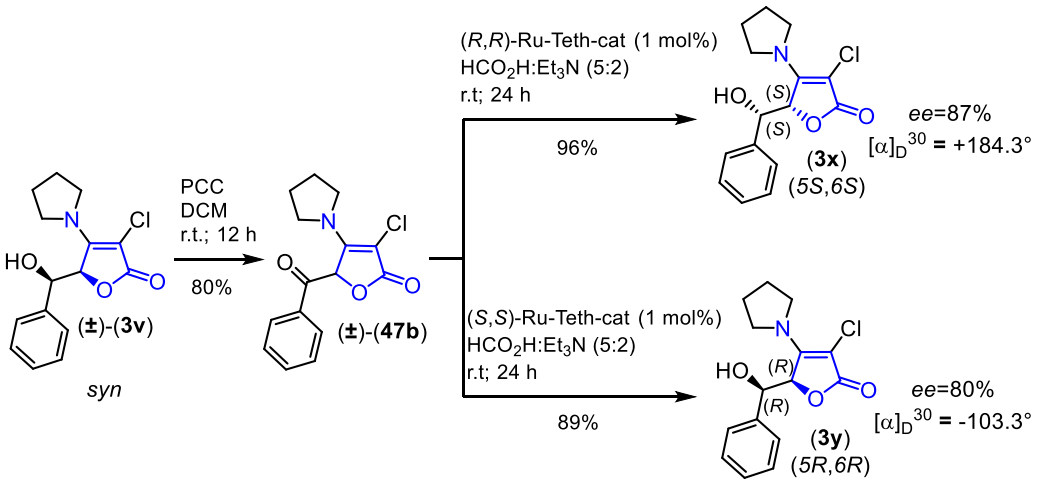



**Figure 4.11:** DEPT135 NMR (100 MHz, $(CD_3)_2SO$)) for oxidized tetronamide (±)-**47b.** *Solvent residues signal.

Lastly, the ketone (±)-**47b** was reacted by the optimized AHT protocol,[94-96] employing both enantiomers of the Wills catalyst (*R*,*R* and *S*,*S*-Ru-Teth-cat) and the azeotropic mixture of $HCO_2H$:$Et_3N$ (5:2) as a source of hydrogen (**Scheme 4.18**).



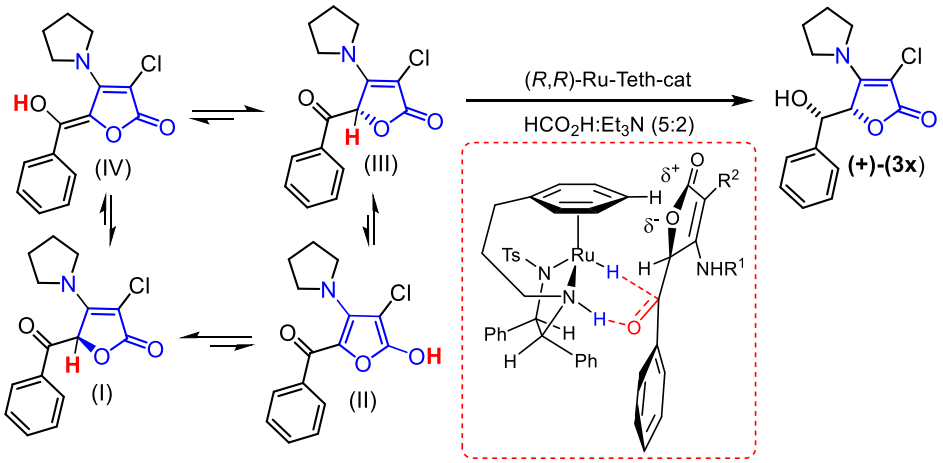

**Scheme 4.18:** AHT reaction of compound (±)-**47b** with both enantiomer of Wills' catalyst.

In the first instance, a successful AHT reaction in tetronamides was achieved with excellent conversion, yield and enantioselectivity for both catalysts. The corresponding chirality and optical rotation were determined by X-ray and polarimetry analysis, confirming compounds (+)-**(*5S*,*6S*)-3x** and (-)-**(*5R*,*6R*)-3y**. Additionally, as was discussed, only the more stable *syn* enantiomer was produced in both reactions.[16]

The specific production of *syn* enantiomer (+)-**3x** can be explained using the equilibrium of its precursor (±)-**47b** (**Scheme 4.19**), as previously analyzed. In our case, when (*R*,*R*)-Ru-teth-cat was used, the keto form **III** is favorably reduced. But, contrary as we expected, the selectivity is given by the π-interaction between catalyst's arene and the substrate's furanone (see reactants approach in **scheme 4.19**), suggesting more electronic density in lactone nucleus. The same explanation can be applied to the production of the other enantiomer (-)-**3y** with the *S*,*S*-Ru-Teth-cat.

This complete procedure was monitored by chiral HPLC (**Fig. 4.12; A-D**). Initially we have the results of the running of the racemic tetronamide (±)-**3v**, showing two peaks with r.t = 57.1 min and 64.9 min with the same areas, indicating a racemic mixture of one diastereomer (in this case *syn*) (**A**). The oxidized compound (±)-**47b** revealed a considerably broad peak, suggesting a

mixture of compounds, confirming the keto-enolic equilibrium (**B**). Finally, the compounds (+)-**3x** (**C**) exhibited a major peak with r.t = 53.7 min, corresponding to the first enantiomer and (-)-**3y** (**D**) exhibiting a major peak with r.t. = 65.7 min, corresponding to the second enantiomer, thus confirming the enantioselective reaction.




**Scheme 4.19:** Compound (±)-**47b** equilibrium in AHT reaction.

HPLC analysis also allowed us to calculate the enantiomeric excess (*ee*) by means of peak area comparison. Is important to mention that some variations in retention time (r.t.) were observed when compared to the analysis of a racemic mixture with enantiopure compounds. This may be attributed to a change in room temperature and concentration variables since the peaks exhibited low resolution due to its broad form.

Further specificity of reaction was determinate by means of NMR spectroscopy. The $^{1}$H NMR and $^{13}$C NMR analysis of the racemic mixture and the enantiomeric products was exactly the same (**Fig. 4.13-14**). IR and HRMS was also identical. Lastly X-ray analysis was performed. A suitable crystal was then obtained with DCM leading crystallographic structure determination. Fortunately, the X-ray enantiopure structures were confirmed (**Fig. 4.15**).

Herein, an interesting and promising result for the enantioselective production of novel potential biologically-active tetronamides was obtained. This protocol must be optimized for the *N*-aryl tetronamides. Such unstable oxidized products represent a serious difficulty in this procedure. However, as could be observed with more robust pyrrolidine homologues, this problem can be solved.

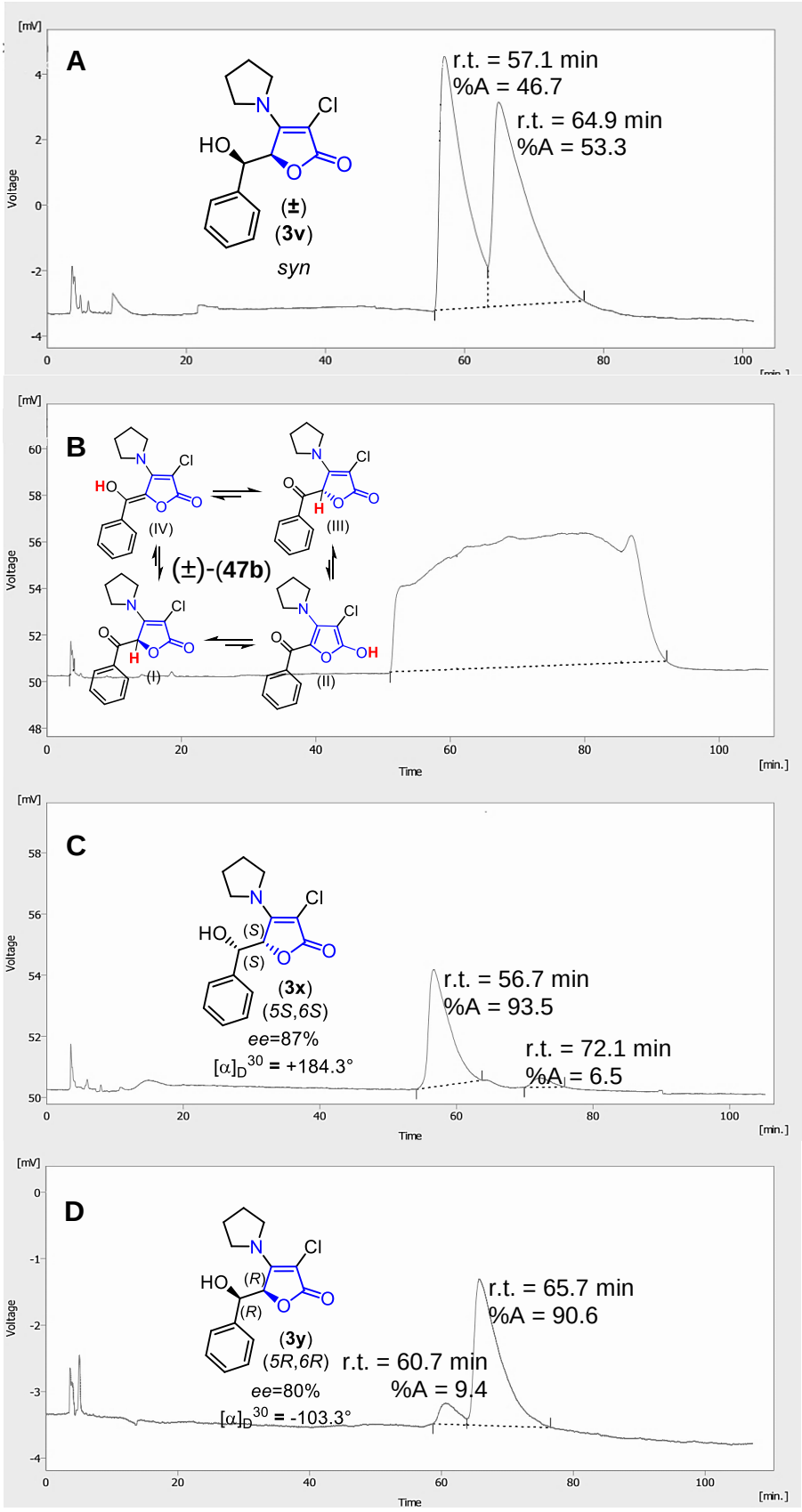



**Figure 4.12:** HPLC analysis of ATH of compound **3v**; Method: Column: Chiral Cel-ODH; 0.5 mLmin-1; *i*-PrOH:Hex (5:95).

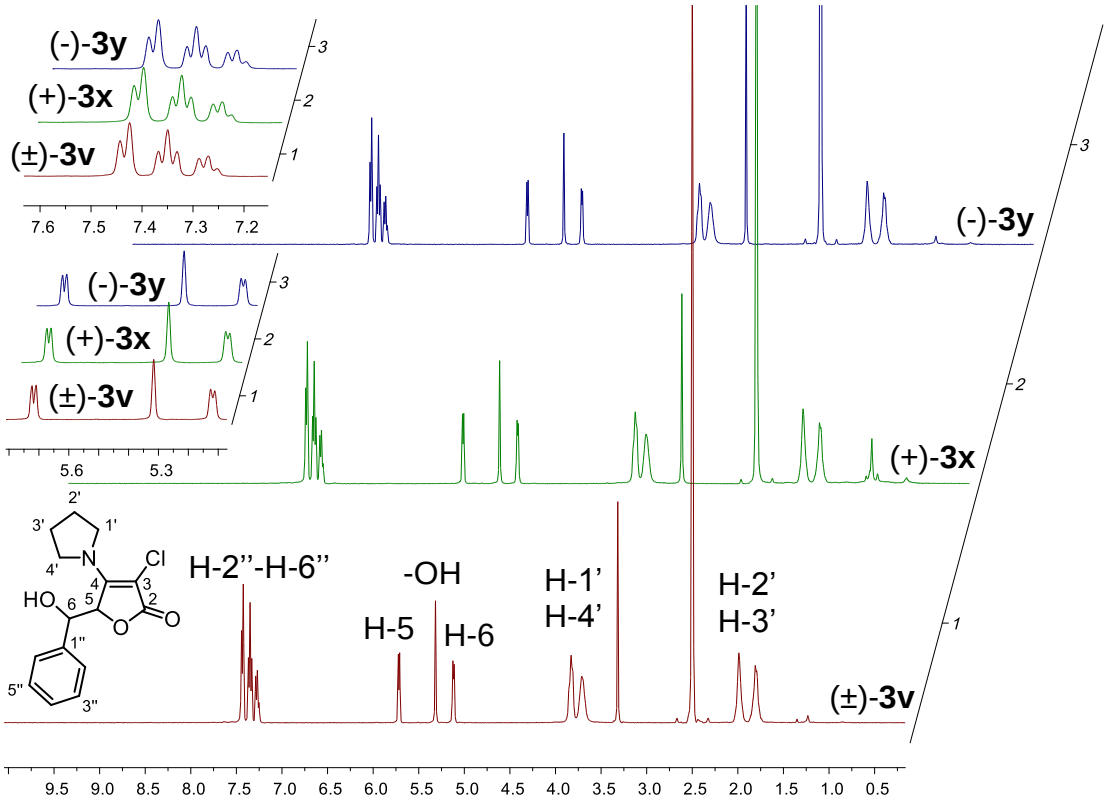



**Figure 4.13:** $^{1}$H NMR (400 MHz; $(CD_3)_2SO$) comparison of compounds (±)-**3v**, (+)-**3x** and (-)-**3y**.

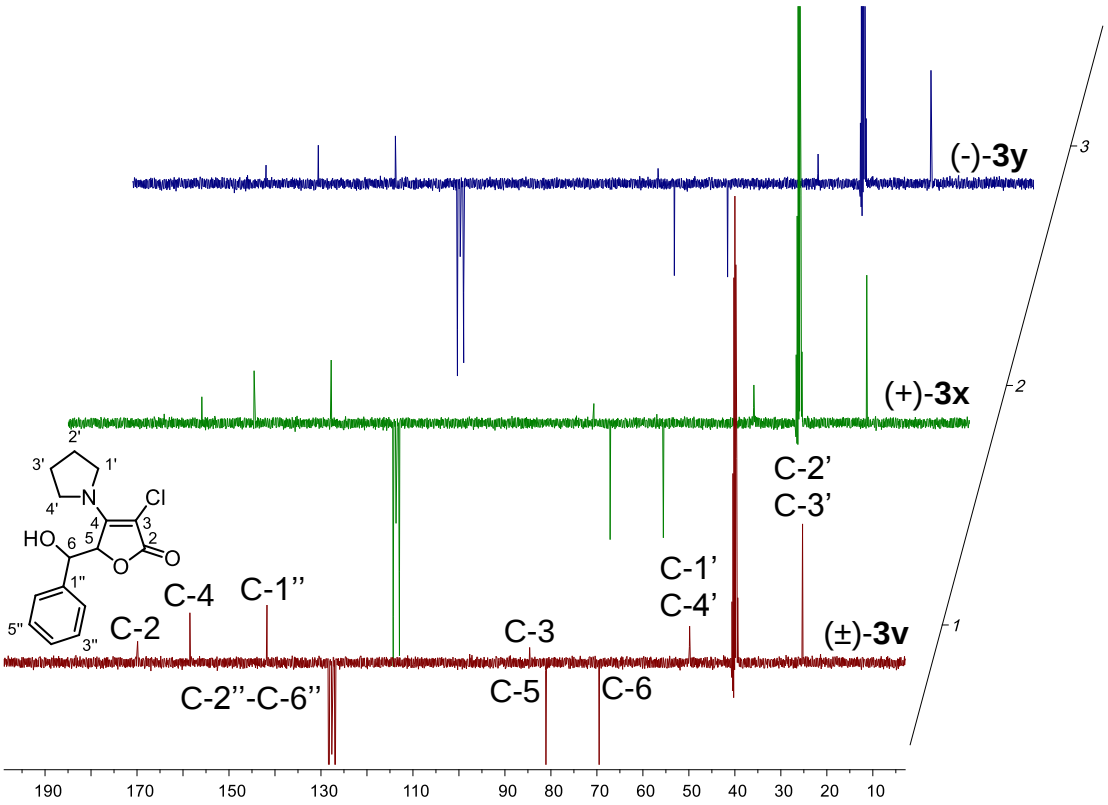



**Figure 4.14:** DEPT135 NMR (100 MHz; $(CD_3)_2SO$) comparison of compounds (±)-**3v**, (+)-**3x** and (-)-**3y**.

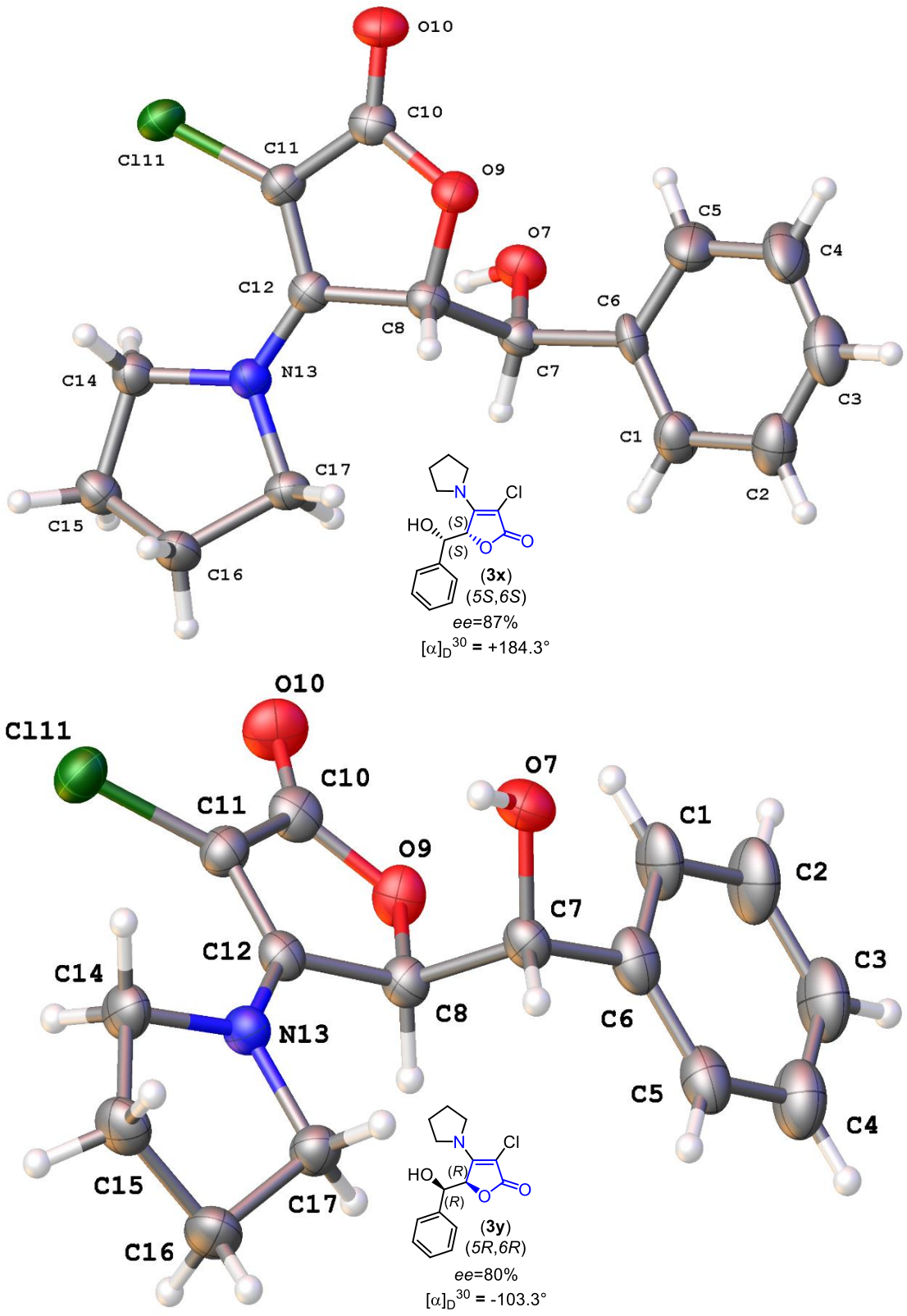



**Figure 4.15:** X-Ray analyses of compound (+)-3x and (-)-3y.

## 4.4. CONCLUSIONS AND PERSPECTIVES

This study determined the synthetic and biological importance of tetronamides scaffold. Initially, an optimized protocol for the dehydration of aldolic adducts was obtained just for simple derivatives. However, this procedure was not applicable to all of our analogs. This does, nevertheless, represent the first successful approach to synthesize γ-alkylidene tetronamides.

The biological importance of synthetic tetronamides was also determined. An excellent inhibition of the *S. elongatus* cyanobacterium was confirmed for the aldolic biphenyl products ($IC_{50}$ = 1.3-5.1 μM). This study must be continued with some other cyanobacteria species as well as some other tests to verify the activities against another microorganisms. Some moderate results were obtained for the inhibition of Hill's reaction. In this case, some structure modification may be attempted to achieve more promising herbicidal compounds.

Finally, a new form to access enantioselective tetronamides was achieved. An optimized protocol of AHT using Ru-Teth-cat, resulted in a new methodology and application for the synthesis of tetronamides. By applying such a protocol, the two enantiomers of *syn*-pyrrolidine aldol tetronamides were obtained with excellent yields (96% and 89%) and an outstanding enantiomeric excess (87% and 80%), respectively. Considering this results, other related catalysts and pyrrolidine congeners should be tested along with their respective activities. This procedure, however, was not satisfactory for *N*-aryl analogs due to issues pertaining to oxidation problems. Nevertheless, other oxidation protocols or perhaps protected derivatives should be used to evaluate their value in the synthesis of these new derivatives and, once enantiomerically pure, test their respective biological activities.

## 4.5. METHODOLOGIES

### 4.5.1. General experimental techniques.

All reactions were performed using analytical-grade solvents. Reagents and solvents were purified, when necessary. Sensitive reactions were carried out under dry nitrogen. Moisture sensitive reactions were performed on oven dried glass and sealed with rubber septum. Moisture sensitive liquids, solutions and anhydrous solvents were transferred via the syringe or cannula through rubber septa.

Analytical thin layer chromatography analyses were conducted on aluminum-backed pre-coated silica gel plates Polygram-UV254 0.20 mm, Macherey-Nagel (20x20 cm) observed under UV light (λ = 254 nm; 365 nm). Column chromatography was performed on silica gel (230–400 mesh).

Melting points are uncorrected and were obtained using an MQAPF-301 melting point apparatus (Microquimica, Brazil). Optical rotations of optically active alcohols were measured in the specified solution using a 2 dm cell with an Optical Activity Ltd. AA-1000 polarimeter. Chiral HPLC was performed on a Hewlett Packard 1050 HPLC machine incorporating a Diacel CHIRALCEL OD-H column.

### 4.5.2. General spectroscopic techniques.

$^{1}$H NMR spectroscopic data were recorded at 500, 400 and 300, and $^{13}$C NMR at 125, 100 and 75 MHz, with a Bruker NMR spectrometer and with $CDCl_3$ ($\delta_H$ 7.26; $\delta_C$ 77.16), DMSO-$d_6$ ($\delta_H$ 2.50; $\delta_C$ 39.52) or acetone-$d_6$ ($\delta_H$ 2.05; $\delta_C$ 29.84, 206.26) as solvent and in some cases tetramethylsilane (TMS) as internal standard ($\delta$ = 0 ppm). Chemical shifts of $^{1}$H and $^{13}$C NMR spectra are reported in ppm and related to solvent signals. All coupling constants (*J* values) are expressed in Hertz (Hz). Multiplicities are reported as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), multiplet (m) and broad (br). Infrared spectrum was recorded with a Varian 660-IR instrument, equipped with GladiATR scanning from 4000 to 500 $cm^{-1}$. High-resolution mass spectrum was recorded with a Bruker MicroTof (resolution = 10000 FWHM) using electrospray ionization (ESI) and are given to four decimal places. For X-ray analysis, a suitable crystal

was selected and mounted on a glass fibre with Fomblin oil and placed on an Xcalibur Gemini diffractometer with a Ruby CCD area detector. The crystal was kept at 150(2) K during data collection. Using Olex2 [1], the structure was solved with the ShelXT [2] structure solution program using Intrinsic Phasing and refined with the ShelXL [3] refinement package using Least Squares minimisation.

### 4.5.3. Synthetic procedures for the synthesis of simple tetronamides (1).

#### *4.5.3.1. General procedure for the preparation of α-halogenated compounds **1a–h**.*

In 100 mL round bottomed flask, were dissolved compound mucochloric and mucobromic acid (**39**) (300 mg, 1.0 eq) in 5 mL MeOH, then, was added $NaHCO_3$ (0.6 eq) and the different *p*-substituted anilines (1.1 eq). The reaction mixture was stirred at room temperature for 12 h. After the consumption of the starting butenolide 8a-b, the reaction mixture was quenched by addition of aqueous HCl solution (1 M, 10 mL). The methanol was then removed under reduced pressure and the aqueous mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtrated and the solvent evaporated in vacuo. The crude residue was purified by silica gel column chromatography, eluted with hexane/ethyl acetate mixture to afford compounds **1a-h**.

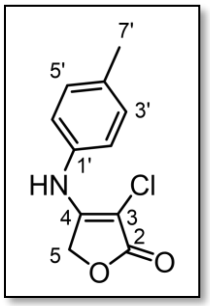
#### *4.5.3.2. General procedure for the preparation of the dehalogented compound **1i**.*

To a 25 mL two-necked round-bottom flask charged with compound **1a** (1.0 g, 3.73 mmol) were added $Pd(MeCN)_2Cl_2$ (24.13 mg, 2.5 mol %), $Pd(OAc)_2$ (21.00 mg, 2.5 mol %), $PPh_3$ (48.87 mg, 5 mol%), DIPEA (3.25 mL, 18.7 mmol), formic acid (0.71 mL, 18.7 mmol) and acetonitrile (without further purification, 10 mL). The reaction mixture was then degassed for 10 min under flow of nitrogen and then stirred at 100 °C for 12 h before it was filtered through a celite pad. The filtrate was extracted with ethyl acetate (3×30 mL) and the combined organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel eluted

with hexane/ethyl acetate (6:4 v/v) to afford compound **14i** as a beige solid in 66% yield (465.8 mg, 2.46 mmol).

*4.5.3.3. Simple tetronamides **1** data*

*3-chloro-4-(p-tolylamino)furan-2(5H)-one (**1a**).*




**Yield:** 93%.

**Characteristics**: White solid.

**Eluent (ethyl acetate/hexane (v/v)):** 1:1.

**Mp**: 213.8 - 215.1 °C.

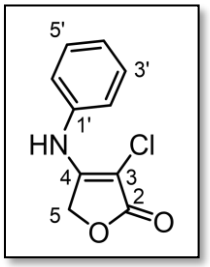
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3234, 3068, 1741, 1629, 1052, 982, 899, 741, 531.

**$^{1}$H NMR (300 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$; (9:1))** $\delta$**:** 9.06 (s, 1H, -NH), 7.19 (s, 4H, H-2', H-3', H-5' and H-6'), 4.99 (s, 2H, H-5), 2.31 (s, 3H, H-7').

**$^{13}$C NMR (75 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$; (9:1))** $\delta$*:* 168.76 (C-2), 158.51 (C-4), 135.95 (C-1'), 134.85 (C-4'), 129.78 (2C, C-3' and C-5'), 122.48 (2C, C-2' and C-6'), 87.21 (C-3), 66.07 (C-5), 20.01 (C-7').

**HRMS (ESI) *m/z* [M-H]$^-$:** calculated for $C_{11}H_9ClNO_2$, 222.0322; found, 222.0326.

*3-chloro-4-(phenylamino)furan-2(5H)-one (**1b**).*




**Yield:** 100%

**Characteristics**: Beige crystals

**Mp**: 182.4 – 185.5 °C.

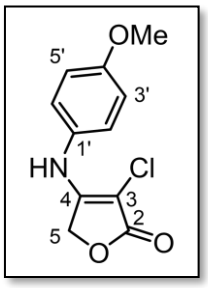
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3205, 3118, 3059, 2990, 2938, 1731, 1619, 1595, 1584, 1487, 1434, 1397, 1349, 1339, 1311, 1264, 1189, 1011, 731.

**$^{1}$H NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** $\delta$**:** 8.67 (s; 1H; -NH), .7.11 (t, *J* = 7.86 Hz, 2H; H-3' and H-5'), 6.99-6.85 (m, 3H, H-2', H-4' and H-6'), 4.64 (s, 2H, H-5).

**$^{13}$C NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** $\delta$**:** 169.20 (C-2), 157.35 (C-4), 137.51 (C-1'), 129.01 (2C, C-2' and C-6'), 125.09 (C-4'), 121.91 (2C, C-3' and C-5'), 87.99 (C-3), 65.89 (C-5).

**LRMS (ESI) *m/z* [M-H]$^-$:** calculated for $C_{10}H_8ClNO_2$: 208.02, founded: 208.00.

*3-chloro-4-((4-methoxyphenyl)amino)furan-2(5H)-one (**1c**).*




**Yield:** 99%.

**Characteristics**: Dark purple solid.

**Mp**: 122.7 – 124.6 °C.

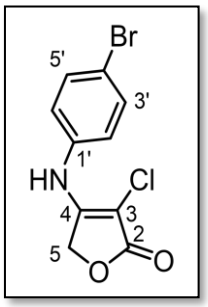
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3334, 3229, 2937, 1754, 1725, 1638, 1614, 1583, 1513, 1437, 1396, 1342, 1301, 1248, 1193, 1012, 989, 827, 740.

**$^1$H NMR (400 MHz, $CDCl_3$):** $\delta$ 7.07 (d; *J* = 8.9 Hz, 2H; H-2' and H-6'), 6.90 (d; *J* = 8.9 Hz 2H; H-3' and H-5'), 6.80 (brd, 1H, -NH), 4.75 (s, 2H, H-5), 3.81 (s, 3H, -OMe)

**$^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$:** 169.50 (C-2), 158.69 (C-4), 158.53 (C-4') 129.82 (C-1'), 125.10 (2C, C-2' and C-6'), 115.18 (2C, C-3' and C-5'), 89.01 (C-3), 65.99 (C-5), 55.70 (-OMe).

**LRMS (ESI) *m/z* [M-H]$^-$:** calculated for $C_{11}H_9ClNO_3$: 238.03, founded: 238.00.

*4-((4-bromophenyl)amino)-3-chlorofuran-2(5H)-one (**1d**).*




**Yield:** 89%.

**Characteristics**: White solid

**Eluent (ethyl acetate/hexane (v/v)):** 32:68.

**Mp**: 221.3 - 222.6 °C.

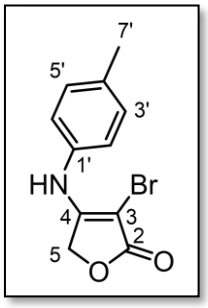
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3235, 3061, 1749, 1632, 1054, 977, 891, 739, 515.

**$^1$H NMR (300 MHz, $(CD_3)_2CO$):** $\delta$ 8.80 (s, 1H, -NH), 7.56 (d, *J* = 8.9 Hz, 2H, H-3' and H-5'), 7.27 (d, *J* = 8.9 Hz, 2H, H-2' and H-6'), 5.12 (s, 2H, H-5).

**$^{13}$C NMR (75 MHz, $(CD_3)_2CO$):** $\delta$: 168.38 (C-2), 157.65 (C-4), 137.90 (C-1'), 132.31 (2C, C-3' and C-5'), 123.69 (C-2'), 123.58 (C-6'), 117.36 (C-4'), 89.13 (C-3), 66.15 (C-5).

**HRMS (ESI) *m/z* [M-H]$^-$:** calculated for $C_{10}H_6BrClNO_2$, 285.9270; found, 285.9273.

*3-bromo-4-(p-tolylamino)furan-2(5H)-one (**1e**).*




**Yield:** 82%.

**Characteristics**: Orange solid.

**Eluent (ethyl acetate/hexane (v/v)):** 3:7

**Mp**: 225.2–226.8°C.

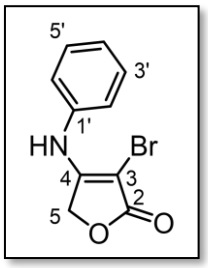
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3230, 3074, 1729, 1628, 1050, 985, 896, 740, 520.

**$^{1}$H NMR (300 MHz, $(CD_3)_2SO$)** *δ:* 9.45 (s, 1H, -NH), 7.15 (s, 4H, H-2', H-3', H-5' and H-6'), 4.99 (s, 2H, H-5), 2.27 (s, 3H, H-7').

**$^{13}$C NMR (75 MHz, $(CD_3)_2SO$)** *δ:* 170.09 (C-2), 162.19 (C-4), 135.91 (C-1'), 135.05 (C-4'), 130.09 (2C, C-3' and C-5'), 123.21 (2C, C-2' and C-6'), 73.82 (C-3), 67.66 (C-5), 20.87 (C-7').

**HRMS (ESI) *m/z* [M-H]$^-$:** calculated for $C_{11}H_9BrNO_2$, 265.9817; found, 265.9832.

*3-bromo-4-(phenylamino)furan-2(5H)-one (**1f**).*




**Yield:** 96%

**Characteristics**: Light brown solid

**Mp**: 151.0 – 152.3 °C (decomp.).

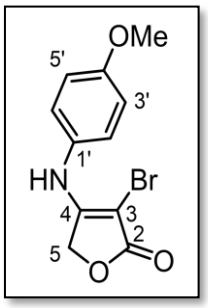
**FTIR (KBr) (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3200, 3110, 2982, 2933, 1731, 1615, 1553, 1582, 1485, 1432, 1391, 1337, 1309, 1258, 1109, 1047, 980, 724.

**$^{1}$H NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** *δ:* 8.59 (s; 1H; -NH), 7.01 (t, *J* = 7.72 Hz, 2H; H-3' and H-5'), 6.88-6.78 (m, 3H, H-2', H-4' and H-6'), 4.53 (s, 2H, H-5).

**$^{13}$C NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** *δ:* 169.40 (C-2), 160.26 (C-4), 137.17 (C-1'), 128.76 (2C, C-2' and C-6'), 125.10 (C-4'), 122.20 (2C, C-3' and C-5'), 74.68 (C-3), 66.73 (C-5).

**LRMS (ESI) *m/z* [M+Na]$^+$:** calculated for $C_{10}H_9BrNNaO_2$: 275.96, founded: 276.10.

*3-bromo-4-((4-methoxyphenyl)amino)furan-2(5H)-one (**1g**).*




**Yield:** 84%.

**Characteristics**: Brown solid.

**Eluent (ethyl acetate/hexane (v/v)): 3:7**

**Mp**: 129.3 – 130.8 °C.

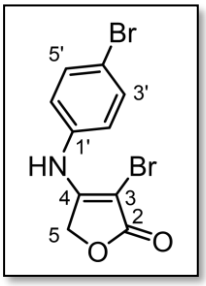
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3172, 3068, 2979, 2935, 1722, 1619, 1603, 1584, 1506, 1444, 1339, 1242, 1032, 978, 825.

**$^{1}$H NMR (400 MHz, $CDCl_3$)** *δ:* 7.06 (d; *J* = 8.9 Hz, 2H; H-2' and H-6'), 6.92 (d; *J* = 8.9 Hz 2H; H-3' and H-5'), 6.61 (brd, 1H, -NH), 4.74 (s, 2H, H-5), 3.82 (s, 3H, -OMe)

**$^{13}$C NMR (100 MHz, $CDCl_3$)** *δ*: 169.64 (C-2), 161.49 (C-4), 158.74 (C-4') 129.69 (C-1'), 125.33 (2C, C-2' and C-6'), 115.30 (2C, C-3' and C-5'), 76.60 (C-3), 66.96 (C-5), 55.73 (-OMe).

**LRMS (ESI) *m/z* [M-H]$^{-}$:** calculated for $C_{11}H_9BrNO_3$: 281.98, founded: 282.00.

*4-((4-bromophenyl)amino)-3-bromofuran-2(5H)-one (**1h**).*




**Yield:** 89%.

**Characteristics**: Dark yellow solid

**Eluent (ethyl acetate/hexane (v/v)):** 4:6.

**Mp**: 152.5 – 154.0 °C.

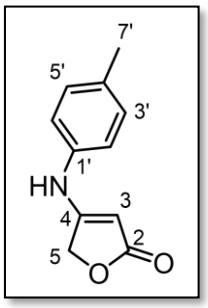
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3213, 3094, 2929, 1726, 1608, 1575, 1448, 1408, 1379, 1333, 1248, 1186, 1044, 977, 816, 737.

**$^{1}$H NMR (400 MHz, $CDCl_3$)** *δ:* 7.52 (d; *J* = 8.7 Hz, 2H; H-2' and H-6'), 6.98 (d; *J* = 8.7 Hz 2H; H-3' and H-5'), 6.78 (brd, 1H, -NH), 4.91 (s, 2H, H-5).

**$^{13}$C NMR (100 MHz, $CDCl_3$)** *δ*: 169.17 (C-2), 159.71 (C-4), 136.46 (C-1'), 133.29 (2C, C-2' and C-6'), 123.40 (2C, C-3' and C-5'), 119.56 (C-4'), 79.11 (C-3), 67.23 (C-5).

**LRMS (ESI) *m/z* [M-H]$^{-}$:** calculated for $C_{10}H_6Br_2NO_2$, 331.88, found, 331.90.

*4-(p-tolylamino)furan-2(5H)-one (**1i**).*




**Yield:** 66%.

**Characteristics**: Beige solid.

**Eluent (ethyl acetate/hexane (v/v)):** 4:6

**Mp**: 204.9 – 205.9 °C.

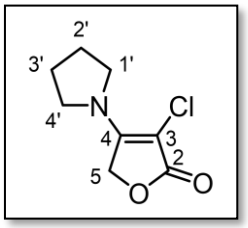
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3264, 3198, 3077, 1695, 1622, 1589, 1557, 1513, 1433, 1323, 1264, 1157, 1046, 900, 812, 777.

**$^1$H NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** *δ:* 8.83 (s; 1H; -NH), .6.83 (d, *J* = 8.4 Hz, 2H; H-3' and H-5' ), 6.72 (d, *J* = 8.4 Hz, 2H; H-2' and H-6' ), 4.82 (s, 2H, H-3),.4.48 (s, 2H, H-5), 2.00 (s, 2H, H-7').

**$^{13}$C NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** *δ*: 175.52 (C-2), 163.12 (C-4), 136.98 (C-1'), 132.78 (C-4'), 129.29 (2C, C-2' and C-6'), 118.96 (2C, C-3' and C-5'), 82.79 (C-3), 67.84 (C-5), 20.15 (C-7').

**LRMS (ESI) *m/z* [M+Na$^+$]$^+$:** calculated for $C_{11}H_{11}NNaO_2$, 212.07; found, 212.20.

*3-chloro-4-(pyrrolidin-1-yl)furan-2(5H)-one (**1j**).*




**Yield:** 64%

**Characteristics**: White crystals

**Mp**: 126.7 – 128.2 °C.

**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 2976, 1953, 2875, 1753, 1636, 1612, 1450, 1346, 1337, 1243, 1036, 989, 871, 764.

**$^1$H NMR (400 MHz, $CDCl_3$)** *δ*: 4.64 (s; 2H; H-5), 3.88 (brd; 2H, H-1' and H-4'), 3.33 (brd; 2H, H-1' and H-4'), 1.98 (s; 4H; H-2' and H-3')

**$^{13}$C NMR (100 MHz, $CDCl_3$)** *δ*: 171.12 (C-2), 157.02 (C-4), 91.54 (C-5), 84.33 (C-3), 66.26 (2C, C-1' and C-4'), 48.58 (2C, C-2' and C-3').

**LRMS (ESI) *m/z* [M+Na$^+$]$^+$:** calculated for $C_8H_{10}ClNNaO_2$ : 210.03, founded: 210.10.

### 4.5.4. General procedure for synthesis of aldolic products (±)-(3a-u).

To a 25 mL one neck round bottomed flask were added the tetronamides **1** (200 mg, 1.0 eq), a mixture of MeOH and $H_2O$ (4 and 2 mL, v/v), followed by NaOH (1.2 eq). After stirring the reaction mixture for 5 min at room temperature,

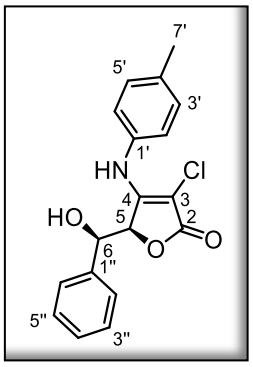
the aromatic aldehydes (1.0 eq) were added slowly. The reaction mixture was stirred at room temperature until TLC analysis revealed total consumption of starting material. The reaction was then quenched by addition of an aqueous solution of HCl (1 M, 10 mL). The methanol was removed under reduced pressure and the aqueous mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtrated and the solvent evaporated. The crude residue was purified by silica gel column chromatography eluting with hexane/ethyl acetate to afford racemic aldolic tetronamides (±)-**3**.

*4.5.4.1. General procedure for synthesis of aldolic products ((±)-**3p** and (±)-**3t**) by demethylation reaction.*

In a 25 mL round bottom flask, methoxylated aldolic tetronamides (±)-**3o** or (±)-**3s** (100 mg, 0.24 mmol and 0.22, 1.0 eq) was diluted with 5 mL of anhydrous $CH_2Cl_2$. It was then allowed to stir and cooled in an ice bath at 0 °C. Then, 1 mL of $BBr_3$ solution diluted in anhydrous $CH_2Cl_2$ (32%$^{v/v}$, 0.44 mmol, 2.0 eq) was added dropwise. Thereafter, the resulting mixture was allowed to warm to room temperature and stirred for an additional 4 h under $N_2$ atmosphere. At the end, the reaction was quenched with 5 mL of saturated aqueous $NH_4Cl$ and then the $CH_2Cl_2$ was removed in vacuo. Thereafter, the aqueous phase was extracted with EtOAc (3 x 10 mL) and the organic layer was dried ($Na_2SO_4$), filtered and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography to provide hydroxylated aldol tetronamides (±)-**3p** and (±)-**3t**.

*4.5.4.2. Racemic aldolic tetronamides 3 data*

*(±)-3-chloro-5-(hydroxy(phenyl)methyl)-4-(p-tolylamino)furan-2(5H)-one (±)-(**3a**).*




**Yield:** 91%

**dr:** >99:1

**Characteristics**: White solid.

**Eluent (ethyl acetate/hexane (v/v)):** 2:8.

**Mp**: 192.3–194.6 °C.

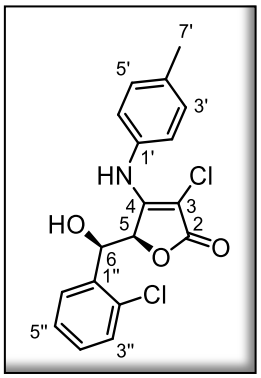
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3386, 3282, 3228, 3070, 3037, 3002, 2971, 1754, 1635, 1197, 1029, 647.

**$^{1}$H NMR (300 MHz, $CDCl_3$:$(CD_3)_2SO$ (9:1)) $\delta$:** 7.83 (s, 1H, –NH), 7.32–7.18 (m, 5H, H-2'' to H-6''), 7.07 (d, *J* = 8.3 Hz, 2H, H-3' and H-5'), 6.89 (d, *J* = 8.3 Hz, 2H, H-2' and H-6'), 5.15 (d, *J* = 3.6 Hz, 1H, H-5), 5.03 (d, *J* = 3.6 Hz, 1H, H-6), 2.27 (s, 3H, H-7').

**$^{13}$C NMR (75 MHz, $CDCl_3$:$(CD_3)_2SO$ (9:1)) $\delta$:** 169.74 (C-2), 156.24 (C-4), 138.85 (C-1''), 135.28 (C-1'), 134.24 (C-4'), 129.10 (2C, C-3' and C-5'), 127.89 (2C, C-3'' and C-5''), 127.78 (C-4''), 126.40 (2C, C-2'' and C-6''), 123.96 (2C, C-2' and C-6'), 88.62 (C-3), 79.40 (C-5), 71.26 (C-6), 20.80 (C-7').

**HRMS (ESI-TOF) *m/z* [M−H]$^-$:** calculated for $C_{18}H_{15}ClNO_3$, 328.0740; found, 328.0732.

*(±)-3-chloro-5-((2-chlorophenyl)(hydroxy)methyl)-4-(p-tolylamino)furan-2(5H)-one (±)-(**3b**).*




**Yield:** 61%

**dr:** >99:1

**Characteristics**: White solid

**Eluent (ethyl acetate/hexane (v/v)):** 3:7

**Mp**: 228.5 – 229.3 °C.

**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3398, 3238, 3100, 3033, 2925, 1752, 1633, 1611, 1513, 1391, 1092, 1030, 1013, 815, 740.

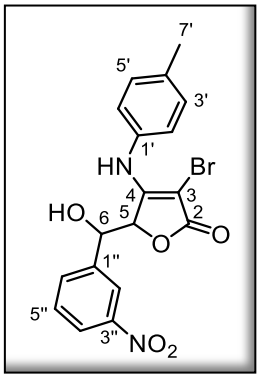
**$^{1}$H NMR (300 MHz, $(CD_3)_2SO$) $\delta$:** 9.51 (s, -NH), 7.56 (d, *J* = 7.0 Hz, 1H, H-3''), 7.32 (t, *J* = 7.0 Hz, H-4''-H-6''), 7.21 (s, 4H, H-2', H-3', H-5' and

H-6'), 5.94 (d, *J* = 5.4 Hz, 1H, H-6), 5.45 (s, -OH), 4.99 (d, *J* = 3.8 Hz, 1H, H-5), 2.30 (s, H-7').

**$^{13}$C NMR (75 MHz, $(CD_3)_2CO$) $\delta$:** 169.03 (C-2), 157.10 (C-4), 137.34 (C-1'), 135.09 (C-3''), 134.77 (C-1''), 130.48 (C-4'), 129.25 (C-4''), 129.17 (2C, C-3' and C-5'), 128.34 (C-5''), 128.17 (C-6''), 126.17 (C-5''), 124.07 (2C, C-2' and C-6'), 121.99, 89.30 (C-3), 77.89 (C-5), 66.43 (C-6), 20.39 (C-7').

**HRMS (ESI) *m/z* [M−H]$^-$:** calculated for $C_{18}H_{14}Cl_2NO_3$, 362.0356; found, 362.0352.

*(±)-3-bromo-5-(hydroxy(3-nitrophenyl)methyl)-4-(p-tolylamino)furan-2(5H)-one (±)-(**3c**).*




**Yield:** 54%

**dr:** 56:44

**Characteristics**: Yellow solid

**Eluent (ethyl acetate/hexane (v/v)):** 2:8

**Mp**: 103.2 - 105.9 °C.

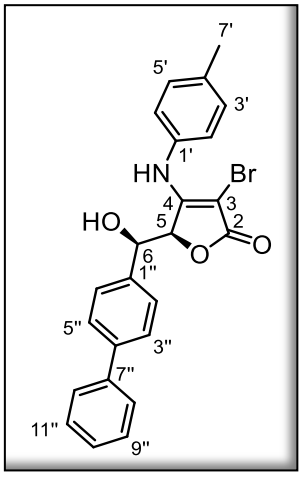
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3212, 3058, 3029, 3004, 1727, 1621, 1600, 1523, 1346, 1191, 1046, 997, 727.

**$^1$H NMR (300 MHz, $(CD_3)_2CO$) $\delta$:** 8.62 and 8.53 (s, -NH), 8.27 - 8.11 (m, 2H, H-5'' and H-6''), 7.82 (d, *J* = 7.8 Hz, 0.56H, H-2'') 7.76 (d, *J* = 7.8 Hz, 0.44H, H-2''), 7.65 (t, *J* = 5.9 Hz, 0.56H, H-3''), 7.60 (t, *J* = 5.9 Hz, 0.44H, H-3''), 7.32 - 7.16 (m, 4H, H-2', H-3', H-5' and H-6'), 5.70 (d, *J* = 3.5 Hz, 0.44H, H-5), 5.59 (d, *J* = 1.9 Hz, 0.56H, H-5), 5.49 (br, -OH), 5.12 (br, 0.56H, H-6) and 5.08 (d, *J* = 3.5 Hz, 0.44H, H-6), 2.36 and 2.34 (s, 3H, H-7').

**$^{13}$C NMR (75 MHz, $(CD_3)_2CO$) $\delta$:** 168.60, 168.04 (C-2), 159.26, 159.11 (C-4), 148.17, 147.81 (C-5''), 143.05, 140.62 (C-1''), 135.71, 135.47 (C-1'), 135.07, 134.46 (C-2''), 133.54, 132.67 (C-3''), 129.76, 129.57 (C-3' and C-5'), 129.40, 129.11 (C-4'), 124.43, 124.40, 123.77, 123.74 (C-2' and C-6'), 122.80,122.43 (C-6''), 121.98, 121.12 (C-4''), 80.75, 80.70 (C-5), 76.75, 76.68 (C-3), 71.93, 69.61 (C-6), 20.06, 20.02 (C-7').

**HRMS (ESI-TOF) *m/z* [M−H]$^-$:** calculated for $C_{18}H_{14}BrN_2O_5$, 417.0086; found, 417.0075.

*(±)-5-([1,1'-biphenyl]-4-yl(hydroxy)methyl)-3-bromo-4-(p-tolylamino)furan-2(5H)-one (±)-(**3d**).*




**Yield:** 40%

**dr:** 65:35

**Characteristics**: White solid.

**Eluent (ethyl acetate/hexane (v/v)):** 3:7

**Mp**: 189.0 - 190.9°C.

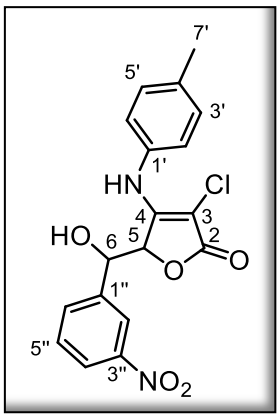
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3241, 3054, 3029, 2969, 1722, 1619, 1592, 1517, 1315, 1191, 1006, 744, 696.

**$^{1}$H NMR (300 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1))** $\delta$**:** 9.29 (s, 1H, -NH), 7.72-7.58 (m, 4H, H-8'', H-9'', H-11'' and H-12''), 7.53-7.40 (m, 4H, H-2'', H-3'', H-5'' and H-6''), 7.35 (t, *J* =7.3 Hz, 1H, H-10''), 7.25 (brd, 4H, H-2', H-3', H-5' and H-6'), 5.72 (d, *J* = 6.0, 1H, H-6), 5.44 (d, *J* = 1.6 Hz, 1H, -OH), 5.01 (d, *J* = 5.1 Hz, 1H, H-5), 2.35 (s, 3H, H-7').

**$^{13}$C NMR (75 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1))** $\delta$**:** 169.59 (C-2), 159.99 (C-4), [140.94, 140.67, 139.84, 135.60, 134.96, 129.37 (2C), 128.96 (2C), 127.37, 127.12 (2C), 126.87 (2C), 126.46 (2C), 124.49 (2C)] (C-1'' to C-12'' and C-1' to C-6'), 81.97 (C-5), 75.96 (C-3), 69.95 (C-6), 20.33 (C-7').

**HRMS (ESI-TOF) *m/z* [M−H]$^{-}$:** calculated for $C_{24}H_{19}BrNO_3$, 448.0548; found, 448.0542

*(±)-3-chloro-5-(hydroxy(3-nitrophenyl)methyl)-4-(p-tolylamino)furan-2(5H)-one (±)- (**3e**).*




**Yield:** 51%

**dr:** 53:47

**Characteristics**: Yellow solid

**Eluent (ethyl acetate/hexane (v/v)):** 2:8

**Mp**: 224.5 - 226.8 °C.

**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3261, 3060, 3029, 3006, 1724, 1627, 1604, 1523, 1348, 1020, 811, 680.

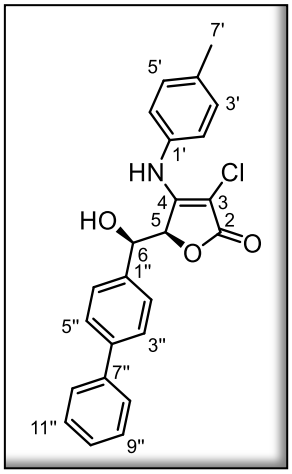
**$^{1}$H NMR (300 MHz, $(CD_3)_2CO$)** $\delta$**:** 8.68 and 8.59 (s, -NH), 8.31 - 8.11 (m, H-2'' and H-6''), 7.82 (t, *J* = 8.8 Hz, H-3''), 7.71 - 7.56 (m, H-4''), 7.30 - 7.10 (m, 4H, H-2' to H-6'), 5.65 (d, *J* = 3.8 Hz, 0.47H, H-5 and -OH), 5.55

(d, *J* = 3.1 Hz, 0.53H, H-5 and -OH), 5.22 (brd, 0.53H, H-6), 5.15 (d, *J* = 3.8 Hz, 0.47H, H-6), 2.33 and 2.35 (s, H-7').

**$^{13}$C NMR (75 MHz, $(CD_3)_2CO$) $\delta$:** 168.35, 167.78 (C-2), 156.24, 156.24(C-4), 148.17, 147.86 (C-5''), 142.95, 140.77 (C-1''), 135.42, 135.38 (C-1'), 135.24, 135.20 (C-2''), 133.59, 132.75 (C-3''), 129.63, 129.45 (2C, C-3' and C-5'), 129.41, 129.18 (C-4'), 124.00, 123.44 (2C, C-2' and C-6'), 122.86, 122.48 (C-6''), 121.99, 121.16 (C-4''), 90.16, 89.52 (C-3), 79.72, 79.60 (C-5), 72.32, 69.95 (C-6), 20.06 and 20.01 (C-7').

**HRMS (ESI-TOF) *m/z* [M−H]$^-$:** calculated for $C_{18}H_{14}ClN_2O_5$, 373.0591; found, 373.0598.

*(±)-5-([1,1'-biphenyl]-4-yl(hydroxy)methyl)-3-chloro-4-(p-tolylamino)furan-2(5H)-one (±)-(**3f**).*




**Yield:** 93%

**dr:** 90:10

**Characteristics**: White solid.

**Eluent (ethyl acetate/hexane (v/v)):** 35:65.

**Mp**: 164.4 – 165.1 °C (decomp.).

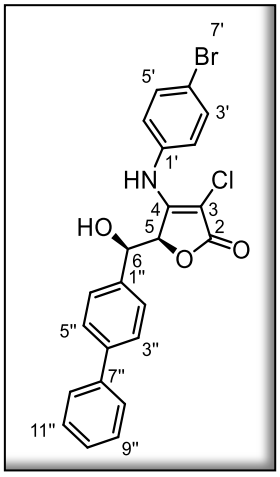
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3299, 3028, 2910, 2874, 1726, 1624, 1603, 1524, 1291, 1189, 1029, 1020, 771.

**$^1$H NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (8:2)) $\delta$:** 8.60 (s, 1H, -NH), 7.58 (s, 0.3H, -OH), 7.55-7.46 (m, 4H, H-8'', H-9'', H-11'' and H-12''), 7.42-7.33 (m, 4H, H-2'', H-3'', H-5'' and H-6''), 7.27 (t, *J* =7.3 Hz, 1H, H-10'), 7.09 (d, *J* = 8.2 Hz, 2H, H-3' and H-5'), 6.95 (d, *J* = 8.2 Hz, 2H, H-2' and H-6'), 5.22 (d, *J* = 5.1 Hz, 1H, H-6), 4.86 (d, *J* = 5.1 Hz, 1H, H-5), 2.28 (s, 3H, H-7').

**$^{13}$C NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (8:2)) $\delta$:** 168.67 (C-2), 155.77 (C-4), [139.94, 139.59, 137.14, 134.25, 133.91, 128.52 (2C), 128.06 (2C), 127.15 (2C), 126.64, 126.07 (2C), 125.73 (2C), 122.83 (2C)] (C-1'' to C-12'' and C-2' to C-6'), 88.13 (C-5), 79.14 (C-3), 72.96 (C-6), 20.13 (C-7').

**HRMS (ESI) *m/z* [M+Na$^+$]$^+$:** calculated for $C_{24}H_{20}ClNNaO_3$, 428.1024, found, 428.1023.

*(±)-5-([1,1'-biphenyl]-4-yl(hydroxy)methyl)-4-((4-bromophenyl)amino)-3-chloro furan-2(5H)-one (±)-(**3g**).*




**Yield:** 72%

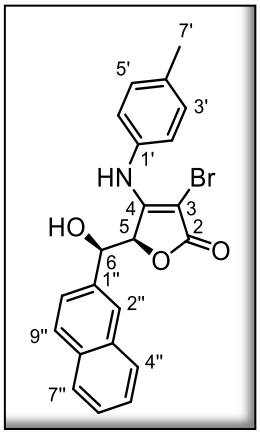
**Physic state and color**: White solid.

**Eluent (ethyl acetate/hexane (v/v)):** 3:7.

**$^1$H NMR (300 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1))** $\delta$**:** 9.15 (s, 1H, -NH), 7.67-7.50 (m, 8H, H-2''-H-12''), 7.48 (d, *J* = 8.7 Hz, 2H, H-2' and H-6'), 7.15 (d, *J* = 8.7 Hz, 2H, H-2' and H-6'), 5.79 (brd, -OH) 5.52 (d, *J* = 4.2 Hz, 1H, H-6), 5.07 (d, *J* = 4.10 Hz, 1H, H-5).

**$^{13}$C NMR (300 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1))** $\delta$**:** 169.01 (C-2), 158.45 (C-4), [141.27, 140.84, 138.73, 138.24, 132.47 (2C), 129.64 (2C), 128.82 (2C), 128.14, 127.59(2C), 127.01 (2C), 125.71 (2C), 117.90] (C-1'' to C-12'' and C-2' to C-6'), 91.72 (C-5), 81.07 (C-3), 74.28 (C-6).

*(±)-3-bromo-5-(hydroxy(naphthalen-2-yl)methyl)-4-(p-tolylamino)furan-2(5H)-one (±)-(**3h**).*




**Yield:** 67%

**dr:** 64:36

**Characteristics**: White solid.

**Eluent (ethyl acetate/hexane (v/v)):** 24:76.

**Mp**: 170.7-173.0 °C.

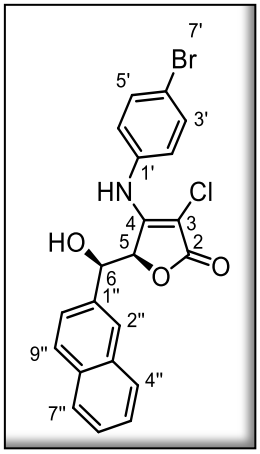
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3235, 3216, 3120, 3037, 2913, 2852, 1720, 1619, 1594, 1525, 1510, 1319, 1191, 1106, 1006, 806, 738

**$^1$H NMR (300 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1))** $\delta$**:** 8.94 (s, 1H, -NH), 7.92-7.78 (m, 4H, H-2'', H-4'', H-7'' and H-9''), 7.55-7.44 (m, 3H, H-5'', H-6'' and H-10''), 7.26 (s, 4H, H-2', H-3', H-5' and H- 6'), 5.57 (d, *J* = 5.7 Hz, 1H, -OH), 5.53 (d, *J* = 1.6 Hz, 1H, H-5), 5.14 (br, 1H, H-6), 2.36 (s, 1H, H-7').

**$^{13}$C NMR (75 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1))** $\delta$**:** 169.13 (C-2), 159.83 (C-4), [138.73, 135.49, 135.21, 133.14, 133.01, 129.40 (2C), 127.89, 127.60, 127.49, 126.04, 125.78, 125.19, 124.68, 124.44 (2C)] (C-1'' to C-10'' and C-1' to C-6'), 81.51 (C-5), 76.33 (C-3), 70.50 (C-6), 20.14 (C-7').

**HRMS (ESI-TOF) *m/z* [M−H]⁻:** calculated for $C_{22}H_{17}BrNO_3$, 422.0392; found, 422.0331.

*(±)-4-((4-bromophenyl)amino)-3-chloro-5-(hydroxy(naphthalen-2-yl)methyl) furan-2(5H)-one (±)-(**3i**).*




**Yield:** 59%

**dr:** 7:3

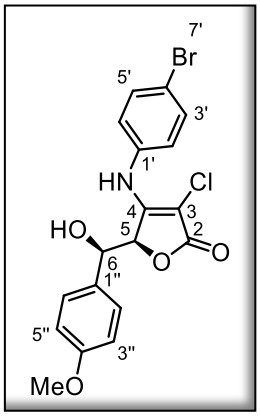
**Characteristics**: White solid.

**Eluent (ethyl acetate/hexane (v/v)):** 25:75.

**$^1$H NMR (300 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1)) $\delta$:** 8.76 (brd, 1H, -NH), 7.95-7.81 (m, 4H, H-2'', H-4'', H-7'' and H-9''), 7.63-7.45 (m, 5H, H-5'', H-6'', H-10'', H-2' and H- 6'), 7.25 (d, *J* =8.9 Hz, 2H, H-3' and H-5'), 5.58 (d, *J* = 2.2 Hz, 1H, H-6), 5.31 (d, *J* = 1.7 Hz, 1H, H-5).

**$^{13}$C NMR (75 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1)) $\delta$:** 168.23 (C-2), 155.71 (C-4), [137.73, 137.37, 133.13, 133.11, 131.77(2C), 127.89, 127.59 (2C), 126.11, 125.92, 125.34, 125.28 (2C), 124.55, 117.53] (C-1'' to C-10'' and C-1' to C-6'), 91.09 (C-5), 80.20 (C-3), 71.24 (C-6).

*(±)-4-((4-bromophenyl)amino)-3-chloro-5-(hydroxy(4-methoxyphenyl)methyl) furan-2(5H)-one (±)-(**3j**).*




**Yield:** 75%

**dr:** 98:2

**Characteristics**: White solid

**Eluent (ethyl acetate/hexane (v/v)):** 23:77.

**Mp**: 170.3-172.8 °C.

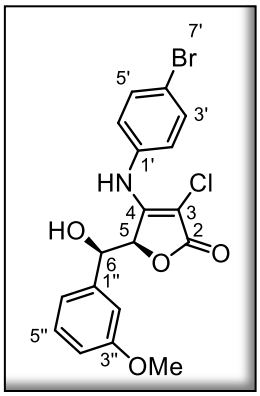
**FTIR ($cm^{-1}$):** $\bar{\upsilon}_{max}$ 3212, 3174, 3031, 3010, 2989, 2929, 2886, 1714, 1614, 1581, 1511, 1251, 1191, 1170, 1022, 744.

**$^1$H NMR (300 MHz, $(CD_3)_2CO$) $\delta$:** 8.88 (s, 1H, -NH), 7.56 (d, *J* = 8.7, 2H, H-3' and H-5'), 7.34 (d, *J* = 8.7 Hz, 2H, H-2'' and H-6''), 7.20 (d, *J* = 8.7 Hz, 2H, H-3'' and H-5''), 6.89 (d, *J* = 8.7 Hz, 2H, H-2' and H-6'), 5.35 (d, *J* = 2.3 Hz, 2H, H-5), 5.29 (d, *J* = 4.9 Hz, 1H, -OH), 5.06 (brd, 1H, H-6), 3.78 (s, 3H, -OMe).

**$^{13}$C NMR (75 MHz, $(CD_3)_2CO$):** $\delta$ 168.50 (C-2), 159.33 (C-4''), 155.78 (C-4), 137.44 (C-1'), 132.22 (C-1''), 131.62 (2C, C-3' and C-5'), 127.74 (2C, C-2'' and C-6''), 125.26 (2C, C-2' and C-6'), 117.26 (C-4'), 113.28 (2C, C-3'' and C-5''), 90.69 (C-3), 80.39 (C-5), 70.81 (C-6), 54.63 (-OMe).

**HRMS (ESI-TOF) *m/z* [M−H]$^-$:** calculated for $C_{18}H_{14}BrClNO_4$, 421.9795; found, 421.9804.

*(±)-4-((4-bromophenyl)amino)-3-chloro-5-(hydroxy(3-methoxyphenyl)methyl) furan-2(5H)-one (±)-(**3k**).*




**Yield:** 68%

**dr:** >99:2

**Characteristics**: White solid

**Eluent (ethyl acetate/hexane (v/v)):** 24:76.

**Mp**: 170.3-172.8 °C.

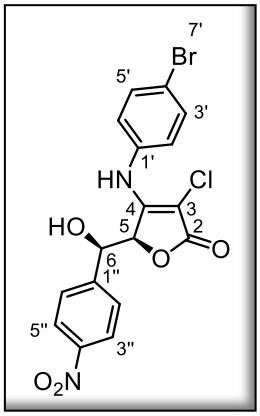
**FTIR (KBr) (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3347, 3193, 3083, 3018, 2996, 2950, 2929, 1752, 1656, 1635, 1587, 1486, 1029, 703.

**$^1$H NMR (300 MHz, $CDCl_3$:$(CD_3)_2SO$; (3:2)) $\delta$:** 9.09 (s, 1H, -NH), 7.38 (d, *J* = 8.6 Hz, 2H, H-3' and H-5'), 7.15 (t, *J* = 8.0 Hz, 1H, H-5''), 6.97 (d, *J* = 8.6 Hz, 2H, H-2' and H-6'), 6.89 (d, *J* = 7.6 Hz, 2H, H-2'' and H-6''), 6.73 (dd, *J* = 1.7, 8.6 Hz, 1H, H-4''), 5.14 (d, *J* = 1.6 Hz, 1H, H-5), 4.95 (brd, 1H, H-6), 3.69 (s, 3H, -OMe).

**$^{13}$C NMR (75 MHz, $(CD_3)_2CO$) $\delta$:** 169.62 (C-2), 159.39 (C-3''), 155.77 (C-4), 141.97 (C-1''), 136.88 (C-1'), 131.50 (2C, C-3' and C-5'), 129.11 (C-9), 125.26 (2C, C-2' and C-6'), 118.70 (C-8), 117.68 (C-4'), 113.01 (C-4''), 112.37 (C-2''), 90.08 (C-3), 80.62 (C-5), 70.94 (C-6), 55.16 (-OMe).

**HRMS (ESI-TOF) *m/z* [M−H]$^-$:** calculated for $C_{18}H_{14}BrClNO_4$, 421.9795; found, 421.9789.

*(±)-4-((4-bromophenyl)amino)-3-chloro-5-(hydroxy(4-nitrophenyl)methyl)furan-2(5H)-one (±)-(**3l**).*




**Yield:** 79%

**dr:** 93:7

**Characteristics**: Yellow solid

**Eluent (ethyl acetate/hexane (v/v)):** 21:79.

**Mp**: 127.2-129.3 °C.

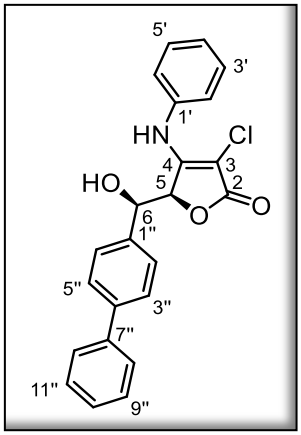
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3241, 3187, 3145, 3052, 2969, 2805, 1718, 1619, 1585, 1515, 1486, 1344, 1191, 1031, 1010, 821, 703.

**$^{1}$H NMR (300 MHz, $(CD_3)_2CO$) $\delta$:** 8.85 (s, 0.93H, -NH) and 8.70 (s, 0.07H, -NH), 8.22 (d, *J* = 8.8 Hz, 0.93H, H-3'' and H-5''), 8.17 (d, *J* = 8.6 Hz, 0.07H, H-3'' and H-5''), 7.71 (d, *J* = 8.8 Hz, 0.93H, H-2'' and H-6''), 7.7 (d, *J* = 8.6 Hz, 0.07H, H-2'' and H-6''), 7.58 (d, *J* = 8.6 Hz, 0.93H, H-3' and H-5'),7.53 (d, *J* = 8.8 Hz, 0.07H, H-3' and H-5'), 7.25 (d, *J* = 8.6 Hz, 0.93H, H-2' and H-6'), 7.19 (d, *J* = 8.8 Hz, 0.07H, H-2' and H-6'), 5.62 (d, *J* = 5.3 Hz, 0.93H, -OH), 5.57 (d, *J* = 1.8 Hz, 0.93H, H-5) and 5.32 (d, *J* = 1.8 Hz, 0.93H, H-6).

**$^{13}$C NMR (75 MHz, $(CD_3)_2CO$) $\delta$:** 168.32 (C-2), 155.42 (C-4), 147.82 (C-4''), 147.56 (C-1''), 137.16 (C-1'), 131.82 (2C, C-3' and C-5'), 127.59 (2C, C-2'' and C-6''), 125.34 (2C, C-2' and C-6'), 123.09 (2C, C-3'' and C-5''), 117.74 (C-4'), 91.02 (C-3), 79.92 (C-5), 70.32 (C-6).

**HRMS (ESI-TOF) *m/z* $[M-H]^-$:** calculated for $C_{17}H_{11}BrClN_2O_5$, 436.9540; found, 436.9519.

*(±)-5-([1,1'-biphenyl]-4-yl(hydroxy)methyl)-3-chloro-4-(phenylamino)furan-2(5H)-one (±)-(**3m**).*




**Yield:** 100%

**dr:** 2:1

**Characteristics**: Beige solid.

**Eluent (ethyl acetate/hexane (v/v)):** 4:6.

**Mp**: 201.5 – 203.8 °C (decomp.).

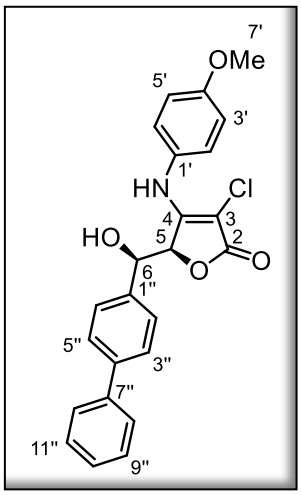
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3216, 3036, 2925, 1715, 1614, 1588, 1544, 1450, 1321, 1195, 1030, 750, 695.

**$^1$H NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** ***δ*****:** 8.98 (s, 1H, -NH), 7.54-7.46 (m, 4H, H-8'', H-9'', H-11'' and H-12''), 7.42-7.33 (m, 4H, H-2'', H-3'', H-5'' and H-6''), 7.33-7.23 (m, 3H, H-2'', H-3'', H-5'' and H-6''), 7.33-7.23 (m, 3H, H-2', H-6' and H-10''), 5.21 (d, *J* = 1.9 Hz, 1H, H-6), 4.99 (d, *J* = 1.8 Hz, 1H, H-5).

**$^{13}$C NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** ***δ*****:** 168.75 (C-2), 155.35 (C-4), [139.51, 139.23, 138.52, 136.53, 127.88 (2C), 127.75 (2C), 126.39, 126.13 (2C), 125.87 (2C), 125.58 (2C), 124.33, 122.83 (2C)] (C-1'' to C-12'' and C-2' to C-6'), 88.48 (C-5), 79.46 (C-3), 69.65(C-6).

**HRMS (ESI) *m/z* [M+Na$^+$]$^+$:** calculated for $C_{23}H_{18}ClNNaO_3$, 414.0867; found, 414.0869.

*(±)-5-([1,1'-biphenyl]-4-yl(hydroxy)methyl)-3-bromo-4-((4-methoxyphenyl) amino) furan-2(5H)-one (±)-(**3n**).*




**Yield:** 91%

**dr:** 10:1

**Characteristics**: Pale purple solid.

**Eluent (ethyl acetate/hexane (v/v)):** 35:65.

**Mp**: 237.7 – 238.4 °C.

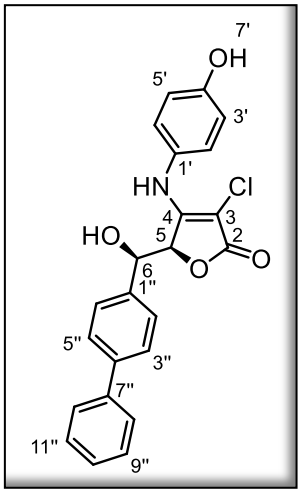
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3572, 3224, 3047, 1713, 1615, 1601, 1542, 1508, 1245, 1031, 831, 746.

**$^1$H NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (7:3))** ***δ:*** 8.58 (s, 1H, -NH), 7.55 (s, 0.56H, -OH) 7.53-7.46 (m, 4H, H-8'', H-9'', H-11'' and H-12''), 7.42-7.32 (m, 4H, H-2'', H-3'', H-5'' and H-6''), 7.26j (t, *J* =7.3 Hz, 1H, H-10''), ), 7.05 (d, *J* = 8.8, 1H, H-3' and H-5'), 6.82 (d, *J* = 8.8 Hz, 1H, H-2' and H-6'), 5.14 (d, *J* = 2.5, 1H, H-6), 5.01 (d, *J* = 2.1 Hz, 1H, H-5), 3.75 (s, 3H, -OMe).

**$^{13}$C NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (7:3))** ***δ:*** 169.09 (C-2), 156.99 (C-4), [156.21, 139.78, 139.62, 138.20, 129.42, 128.10 (2C), 126.63, 126.39 (2C), 126.16 (2C), 125.84 (2C), 125.46 (2C), 113.17 (2C)] (C-1'' to C-12'' and C-1' to C-6'), 87.40 (C-5), 79.14 (C-3), 70.29 (C-6), 54.73 (-OMe).

**HRMS (ESI) *m/z* [M−H]$^-$:** calculated for $C_{24}H_{19}ClNO_4$, 420.1008, found: 420.1007.

*(±)-5-([1,1'-biphenyl]-4-yl(hydroxy)methyl)-3-chloro-4-((4-hydroxyphenyl) amino) furan-2(5H)-one (**3o**).*




**Yield:** 97%

**Physic state and color**: Beige solid.

**Mp**: 145.2 – 145.8 °C.

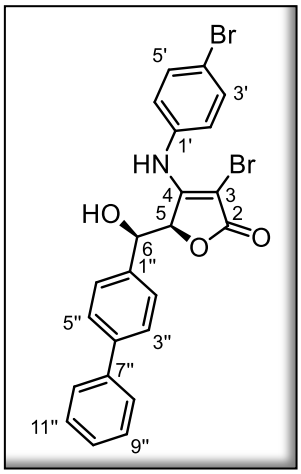
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3611, 3293, 3036, 1724, 1617, 1608, 1510, 1239, 1192, 1033, 828, 749.

**$^{1}$H RMN (400 MHz, $(CD_3)_2SO$)** $\delta$*:* 9.56 (s, 1H, -NH), 9.37 (s, 1H, -OH), 7.69-7.61 (m, 4H, H-3'', H-5'', H-8'' and H-12''), 7.50-7.42 (m, 2H, H-2'' and H-6''), 7.40 (m, 3H, H-9'', H-10'' and H-11''), 7.13 (d, *J* = 8.4, 2H, H-3' and H-5'), 6.80 (d, *J* = 8.4 Hz, 2H, H-2' and H-6'), 5.82 (d, *J* = 5.7, 1H, H-6), 5.38 (s, 1H, H-5), 4.87 (brd, 1H, -OH).

**$^{13}$C RMN (100 MHz, $(CD_3)_2SO$)** $\delta$*:* 169.33 (C-2), 157.88 (C-4), [155.64, 140.73, 140.00, 139.22, 128.99 (2C), 127.45, 126.78 (2C), 126.68 (2C), 126.33 (2C), 126.10 (2C), 115.33 (2C)] (C-1'' to C-12'' and C-1' to C-6'), 86.44 (C-5), 80.49 (C-3), 69.14 (C-6).

**HRMS (ESI) *m/z* [M+Na$^+$]$^+$:** calculated for $C_{23}H_{18}ClNNaO_4$, 430.0817, found, 430.0817.

*(±)-5-([1,1'-biphenyl]-4-yl(hydroxy)methyl)-3-bromo-4-((4-bromophenyl) amino) furan-2(5H)-one (±)-(**3p**).*




**Yield:** 61%

**dr:** >99:1

**Characteristics**: Pale brown solid.

**Eluent (ethyl acetate/hexane (v/v)):** 40:60.

**Mp**: 183.1 – 186.3 °C (decomp.).

**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3294, 3025, 2911, 1725, 1613, 1582, 1516, 1485, 1408, 1290, 1186, 1009, 823, 772, 753.

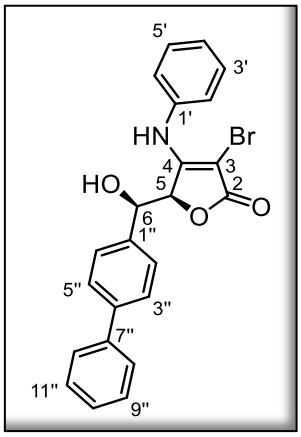
**$^{1}$H NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** $\delta$**:** 9.40 (s, 1H, -NH), 7.56 (d, *J* = 7.5 Hz, 2H, H-8'' and H-12''), 7.49 (d, *J* = 8.1 Hz, 2H, H-9'' and H-11''), 7.46-7.35 (m, 4H, H-2'', H-3'', H-5'' and H-6''),

7.35-7.24 (m, 3H, H-3', H-5' and H-10''), 7.02 (d, *J* = 8.6 Hz, 2H, H-2' and H-6'), 6.05 (brd, -OH) 5.45 (d, *J* = 3.3 Hz, 1H, H-6), 4.9 (brd, 1H, H-5).

**$^{13}$C NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** ***δ*****:** 169.03 (C-2), 158.22 (C-4), [139.75, 139.72, 137.05, 136.52, 131.26 (2C), 128.55 (2C), 127.60 (2C), 127.09, 126.39 (2C), 125.73 (2C), 124.90 (2C), 117.14] (C-1'' to C-12'' and C-2' to C-6'), 81.28 (C-5), 77.26 (C-3), 72.53 (C-6).

**HRMS (ESI-TOF) *m/z* [M+Na$^+$]$^+$:** calculated for $C_{23}H_{17}Br_2NNaO_3$, 535.9467, found, 535.9467.

*(±)-5-([1,1'-biphenyl]-4-yl(hydroxy)methyl)-3-bromo-4-(phenylamino)furan-2(5H)-one (±)-(**3q**).*




**Yield:** 91%

**dr:** 94:6

**Characteristics**: Yellowish solid.

**Eluent (ethyl acetate/hexane (v/v)):** 35:75.

**Mp**: 163.4 – 165.6 °C (decomp.).

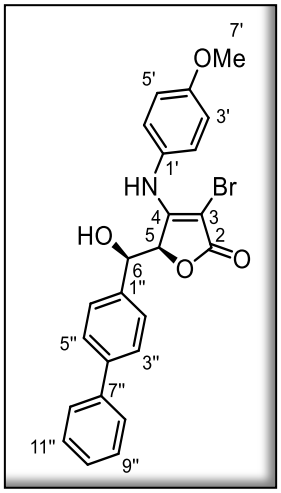
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3293, 3026, 2911, 1726, 1615, 1590, 1530, 1485, 1449, 1294, 1186, 1040, 1015, 773, 734.

**$^1$H NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** ***δ:*** 9.23 (s, 1H, -NH), 7.94 (s, 0.3H, -OH), 7.55 (d, *J* = 7.5 Hz, 2H, H-8'' and H-12''), 7.49 (d, *J* = 8.1 Hz, 2H, H-9'' and H-11''), 7.42-7.23 (m, 7H, H-2'', H-3'', H-5'' H-6'', H-3', H-5' and H-6'), 7.19-7.09 (m, 3H, H-2', H-6' and H-10''), 5.44 (d, *J* = 3.4 Hz, 1H, H-6), 4.87 (d, *J* = 3.4 Hz, 1H, H-5).

**$^{13}$C NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** ***δ:*** 168.02 (C-2), 157.57 (C-4), [138.69 (2C), 136.03, 127.42 (2C), 127.35 (2C), 126.54 (2C), 125.95, 125.27 (2C), 124.62 (2C), 123.80, 122.30 (2C)] (C-1'' to C-12'' and C-2' to C-6'), 80.05 (C-5), 75.19 (C-3), 71.40 (C-6).

**HRMS (ESI) *m/z* [M+Na$^+$]$^+$:** calculated for $C_{23}H_{18}BrNaNO_3$, 458.0362; found, 458.0360.

*(±)-5-([1,1'-biphenyl]-4-yl(hydroxy)methyl)-3-bromo-4-((4-methoxyphenyl) amino) furan-2(5H)-one (±)-(**3r**).*




**Yield:** 78%

**dr:** 94:6

**Characteristics**: Yellowish solid.

**Eluent (ethyl acetate/hexane (v/v)):** 35:65.

**Mp**: 223.6 – 224.6 °C.

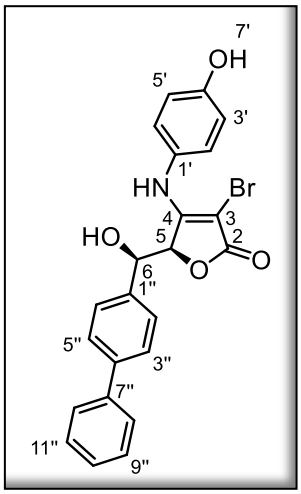
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3355, 3221, 3057, 2958, 1740, 1627, 1508, 1486, 1243, 1197, 1024, 990, 833, 736.

**$^{1}$H NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** *δ:* 8.28 (s, 1H, -NH), 7.54-7.41 (m, 5H, H-8'', H-9'', H-11'', H-12'' and -OH), 7.40-7.32 (m, 4H, H-2'', H-3'', H-5'' and H-6''), 7.26 (t, *J* = 7.3 Hz, 1H, H-10''), 7.06 (d, *J* = 8.7, 1H, H-3' and H-5'), 6.82 (d, *J* = 8.7 Hz, 1H, H-2' and H-6'), 5.16 (d, *J* = 2.4, 1H, H-6), 4.98 (brd, 1H, H-5), 3.75 (s, 3H, -OMe).

**$^{13}$C NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** *δ:* 169.68 (C-2), 156.41 (C-4), [157.58, 140.08, 140.02, 138.03, 129.27, 128.33 (2C), 126.88, 126.60 (2C), 126.45 (2C), 126.41 (2C), 126.16 (2C), 113.49 (2C)] (C-1'' to C-12'' and C-1' to C-6'), 80.26 (C-5), 74.56 (C-3), 70.64 (C-6), 55.01 (-OMe).

**HRMS (ESI) *m/z* [M−H]$^-$:** calculated for $C_{24}H_{19}ClNO_4$, 464.0503, found, 464.0500.

*(±)-5-([1,1'-biphenyl]-4-yl(hydroxy)methyl)-3-bromo-4-((4-hydroxyphenyl) amino) furan-2(5H)-one (±)-(**3s**).*




**Yield:** 71%

**dr:** 94:6

**Characteristics**: Yellowish solid.

**Mp**: 131.6 – 132.3 °C.

**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3317, 1722, 1615, 1512, 1467, 1336, 1261, 1193, 998, 833, 749.

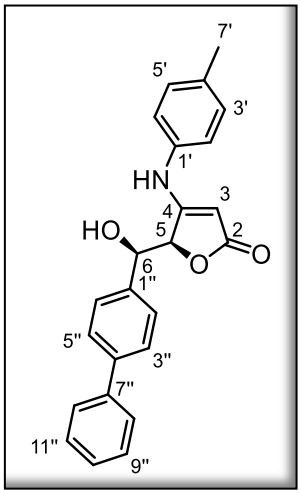
**$^{1}$H RMN (400 MHz, $(CD_3)_2SO$)** *δ:* 9.58 (s, 1H, -NH), 9.33 (s, 1H, -OH), 7.71-7.59 (m, 4H, H-3'', H-5'', H-8'' and H-12''), 7.50-7.42 (m, 2H, H-2'' and H-6''), 7.40-7.29 (m, 3H, H-9'', H-10'' and H-11''), 7.16 (d, *J* = 8.0, 2H, H-3' and H-5'), 6.81 (d, *J* = 8.0 Hz,

2H, H-2’ and H-6’), 5.80 (d, *J* = 5.7, 1H, H-6), 5.38 (s, 1H, H-5), 4.81 (brd, 1H, -OH).

**$^{13}$C RMN (100 MHz, $(CD_3)_2SO$)** $\delta$*:* 169.81 (C-2), 160.80 (C-4), [155.64, 140.78, 140.02, 139.23, 129.01 (2C), 127.47, 126.78 (2C), 126.70 (2C), 126.61 (2C), 126.36 (2C), 115.40 (2C)] (C-1’’ to C-12’’ and C-1’ to C-6’), 81.59 (C-5), 73.78 (C-3), 69.02 (C-6).

**HRMS (ESI) *m/z* [M−H]$^-$:** calculated for $C_{23}H_{17}BrNO_4$, 450.0346; found, 450.0346.

*(±)-5-([1,1'-biphenyl]-4-yl(hydroxy)methyl)-4-(p-tolylamino)furan-2(5H)-one (±)-(**3t**).*




**Yield:** 73%

**dr:** 66:33

**Characteristics**: Yellowish solid.

**Eluent (ethyl acetate/hexane (v/v)):** 45:55.

**Mp**: 199.6 – 201.3 °C.

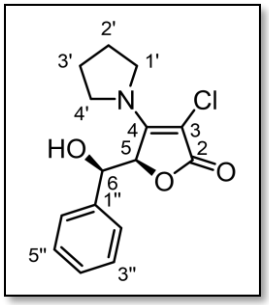
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3561, 3270, 3132, 3075, 2918, 1714, 1622, 1598, 1548, 1513, 1327, 1252, 1166, 1042, 771, 761.

**$^1$H RMN (400 MHz, $(CD_3)_2SO$)** $\delta$**:** 9.39 (s, 1H, -NH), 7.70-7.64 (m, 4H, H-8’’, H-9’’, H-11’’ and H-12’’), 7.58-7.44 (m, 4H, H-2’’, H-3’’, H-5’’ and H-6’’), 7.36 (t, *J* =7.3 Hz, 1H, H-10’’), 7.20 (d, *J* = 8.5 Hz, 2H, H-3’ and H-5’), 7.15 (d, *J* = 8.5 Hz, 2H, H-2’ and H-6’), 5.75 (d, *J* = 5.5 Hz, 1H, H-6), 5.23 (d, *J* = 5.5 Hz, 1H, H-5), 5.20 (s, 1H, H-3), 4.89 (d, *J* = 1.2 Hz, 1H, -OH).

**$^{13}$C RMN (400 MHz, $(CD_3)_2SO$)** $\delta$**:** 174.36 (C-2), 162.91 (C-4), [141.08, 140.08, 138.05, 137.91, 132.42, 129.87 (2C), 129.00 (2C), 127.42, 127.08 (2C), 126.67 (2C), 126.21 (2C), 119.29 (2C)] (C-1’’ to C-12’’ and C-2’ to C-6’), 84.99 (C-3), 81.97 (C-5), 70.22 (C-6).

**HRMS (ESI) *m/z* [M−H]$^-$:** calculated for $C_{24}H_{19}BrNO_3$, 370.1449, found, 370.1444.

*(±)-3-chloro-5-(hydroxy(phenyl)methyl)-4-(pyrrolidin-1-yl)furan-2(5H)-one (**3v**)*




**Yield:** 58%

**dr:** >99:1

**Characteristics**: White solid.

**Eluent (ethyl acetate/hexane (v/v)):** 35:65.

**Mp**: 225.4 – 225.9°C.

**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3301, 2978, 2921, 2882, 1715, 1599, 1452, 1414, 1341, 1245, 1066, 1011, 869, 802, 743, 706.

**$^{1}$H NMR (400 MHz, $(CD_3)_2SO$)** *δ:* 7.44 (d, *J* = 7.5 Hz, 2H, H-2' and H-6'), 7.36 (t, *J* = 7.3 Hz, 2H, H-3' and H-5'), 7.44 (d, *J* = 7.1 Hz, 1H, H-4'), 5.72 (d, *J* = 5.5 Hz, 1H, H-5), 5.32 (s, 1H, -OH), 5.12 (d, *J* = 5.6 Hz, 1H, H-6), 5.73 (brd, 2H, H-1'' - H-4''), 5.72 (brd, 2H, H-1'' - H-4''), 5.13 (brd, 2H, H-2'' - H-3''), 5.12 (brd, 2H, H-2'' - H-3'').

**$^{13}$C NMR (100 MHz, $(CD_3)_2SO$)** *δ:* 169.39 (C-2), 158.03 (C-4), 141.27 (C-1'), 127.77 (2C, C-3' and C-5'), 127.17 (C-4'), 126.42 (2C, C-2' and C-6'), 84.15 (C-3), 80.61 (C-5), 69.03 (C-6), 40.36 (2C, C-1'' and C-4''), 24.81 (2C, C-2'' and C-3'').

**HRMS (ESI) *m/z* [M+Na$^+$]$^+$:** calculated for $C_{15}H_{16}ClNNaO_3$, 316.0711; found, 316.0710.

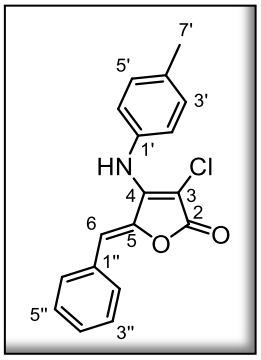
### 4.5.5. General procedure for synthesis of 4-Amino-3-halo-5-benzylidene-furan-2-(5*H*)-one (4a-e).

General procedure for the preparation of compound **4b–e** consists in: To a 25 mL two neck round bottomed flask were added aldol products (±)-**3b-3** (1.0 eq), and dissolved with dry acetonitrile, then *p*-Toluenesulfonyl chloride (2.0 eq) was added and leaving stirring under argon atmosphere for 5 min, thereafter, DBU (2.0 eq) was added dropwise and the reaction mixture was stirred for 12 h at 60 °C. When the reaction was finish, was quenched by addition of an aqueous solution of HCl (1 M). The acetonitrile was removed under reduced pressure and the aqueous mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtrated and the solvent

evaporated. The crude residue was purified by neutral alumina; and eluted with hexane/ethyl acetate to afford pure dehydration product (**4**).

*4.5.5.1. γ-Alkylidenetetronamides 4 data:*

*(Z)-5-benzylidene-3-chloro-4-(p-tolylamino)furan-2(5H)-one (**4a**).*




**Yield:** 87%

**Physic state and color**: Yellow solid.

**Eluent (ethyl acetate/hexane (v/v)):** 1:9.

**Mp**: 227.5 – 228.1 °C.

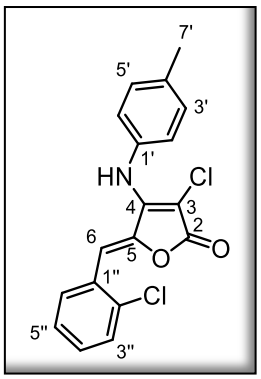
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3380, 3230, 3070, 3002, 2971, 1731, 1595, 1535, 1248, 1006, 996, 750, 685, 679.

**$^{1}$H NMR (400 MHz, $(CD_3)_2CO$)** *δ:* 8.74 (s, 1H, –NH), 7.73 (d, *J* = 7.2 Hz, 2H, H-2'' and H-6''), 7.44 (t, *J* = 7.0 Hz, 2H, H-3'' and H-5''), 7.37 (d, *J* = 6.8 Hz, 1H, H-4''), 7.22 (d, *J* = 7.5 Hz, 2H, H-3' and H-5'), 7.22 (d, *J* = 7.5 Hz, 2H, H-2' and H-6'), 6.60 (s, 1H, H-6), 2.34 (s, 3H, H-7').

**$^{13}$C NMR (100 MHz, $(CD_3)_2CO$)** *δ:* 165.62 (C-2), 149.35 (C-4), 143.29 (C-5), 136.00 (C-1'), 135.94 (C-1''), 133.83 (C-4'), 131.11 (2C, C-3' and C-5'), 130.06 (2C, C-3'' and C-5''), 129.74 (2C, C-2'' and C-6''), 129.69 (C-4''), 124.93 (2C, C-2' and C-6'), 108.56 (C-6), 91.78 (C-3), 20.95 (C-7').

**HRMS (ESI) *m/z* [M−H]$^{-}$:** calculated for $C_{18}H_{13}ClNO_3$, 310.0640; found, 310.0642.

*(Z)-3-chloro-5-(2-chlorobenzylidene)-4-(p-tolylamino)furan-2(5H)-one (**4b**).*




**Yield:** 97%

**Physic state and color**: Yellow solid.

**Eluent (ethyl acetate/hexane (v/v)):** 1:9.

**Mp**: 137.8 – 140.1 °C (decomp.).

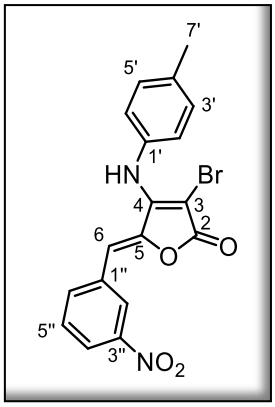
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3330, 2923, 2853, 1746, 1619, 1593, 1522, 1440, 1235, 973, 756.

**$^{1}$H NMR (400 MHz, $(CD_3)_2CO$)** *δ:* 10.04 (s, 1H, –NH), 8.82 (d, *J* = 7.4 Hz, 1H, H-3''), 8.22 – 7.98 (m, 3H, H-4''- H-6''), 7.90 (s, 4H, H-2', H-3', H-5' and H-6'), 7.64 (s, 1H, H-6), 2.70 (s, 3H, H-7').

**$^{13}$C NMR (100 MHz, $(CD_3)_2CO$)** *δ:* 165.38 (C-2), 149.45 (C-4), 144.72 (C-5), 135.99 (C-1'), 134.65 (C-2''), 132.56 (C-1''), 131.73 (C-4'), 130.82 (C-4''), 130.52 (C-6''), 130.04 (2C, C-3' and C-5'), 128.15 (C-1''), 125.20 (2C, C-2' and C-6'), 121.82 (C-5''), 104.43 (C-6), 91.88 (C-3), 20.96 (C-7').

**HRMS (ESI) *m/z* [M+Na$^+$]$^+$:** calculated for $C_{18}H_{13}Cl_2NNaO_3$, 368.0216; found, 368.0216.

*(Z)-3-bromo-5-(3-nitrobenzylidene)-4-(p-tolylamino)furan-2(5H)-one (**4c**).*




**Yield:** 52%

**Physic state and color**: Dark yellow solid.

**Eluent (ethyl acetate/hexane (v/v)):** 1:9.

**Mp**: 217.6 – 218.0 °C.

**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3308, 3036, 2860, 1732, 1588, 1525, 1346, 1235, 981, 964, 906, 739, 665.

**$^1$H NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** *δ:* 9.31 (s, 1H, –NH), 8.42 (s, 1H, H-2''), 8.06 (d, *J* = 7.4 Hz, 1H, H-6''), 7.99 (d, *J* = 7.7 Hz, 1H, H-4''), 7.54 (t, *J* = 8.0 Hz, 1H, H-5''), 7.11 (d, *J* = 8.2 Hz, 2H, H-3' and H-5'), 7.01 (d, *J* = 8.2 Hz, 2H, H-2' and H-6'), 6.75 (s, 1H, H-6), 2.30 (s, 3H, H-7').

**$^{13}$C NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1))** *δ:* 164.77 (C-2), 150.05 (C-4), 147.38 (C-5), 144.02 (C-3''), 134.81 (C-1'), 134.51 (C-6''), 133.59 (C-1''), 133.14 (C-4'), 128.92 (C-5'), 128.11 (2C, C-3' and C-5'), 123.99 (2C, C-2' and C-6'), 123.26 (C-4''), 121.82 (C-2''), 104.31 (C-6), 75.78 (C-3), 19.99 (C-7').

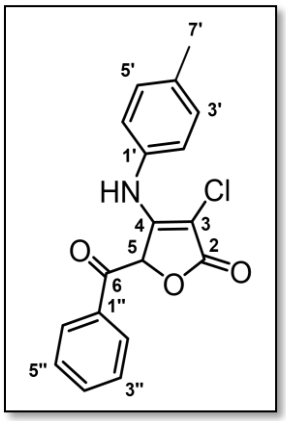
**HRMS (ESI) *m/z* [M+Na$^+$]$^+$:**calculated for $C_{18}H_{13}BrN_2NaO_4$, 422.9951; found, 422.9953.

### 4.5.6. General procedure for the oxidation of tetronamide (±)-3a

In a 50 mL two neck round bottom flask was prepared a solution of 440 mg (1.33 mmol; 1.0 eq) of tetronamide (±)-**3a** in 5 mL of dry DCM. The solution was stirred, and cooled at 0 °C on ice/salt bath at inert atmosphere with Ar flux. When achieve desired temperature, 678.62 mg (1.60 mmol; 1.2 eq) of DMP was added portion wise. Then the reaction mixture was stirred for 2 h under Ar and leave

until room temperature. When reaction finish, it was diluted with 15 mL of DCM, then poured in 20 mL of $Na_2S_2O_3$ (sat) aqueous solution, and extracted, then the aqueous phase was washed with DCM (15 mL X 2). Organic phase was combined and dried with $Na_2SO_4$ (anh) and solvent evaporated in vacuo. Crude mixture was separated by silica gel column chromatography.

*(±)-5-benzoyl-3-chloro-4-(p-tolylamino)furan-2(5H)-one (±)-(**47a**)*




**Yield:** 65%

**Characteristics**: Dark brown oil.

**Eluent (ethyl acetate/hexane (v/v)):** 2:8.

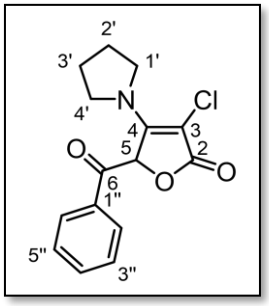
**$^1$H NMR (400 MHz, $CDCl_3$)** *δ:* 7.88 (d, *J* = 7.8 Hz, 2H, H-2'' and H-6''), 7.66 (t, *J* = 7.3 Hz, 1H, H-4''), 7.49 (t, *J* = 7.7 Hz, 2H, H-3'' and H-5''), 7.06 (d, *J* = 8.0 Hz, 2H, H-2' and H-6'), 6.85 (d, *J* = 8.0 Hz, 2H, H-3' and H-5'), 6.47 (s,1H, H-5), 5.92 (brd, 1H, -NH), 2.33 (s, 1H, $-CH_3$).

**$^{13}$C NMR (100 MHz, $CDCl_3$)** *δ:* 191.01 (C-6), 167.30 (C-2), 156.01 (C-4), 137.40 (C-1'), 134.97 (2C, C-1' and C-1''), 132.92 (C-4'), 132.11 (C-4''), 129.70 (2C, C-3' and C-5'), 129.51 (2C, C-3'' and C-5''), 129.16 (2C, C-2'' and C-6''), 125.55 (2C, C-2' and C-6'), 98.70 (C-5), 91.23 (C-3), 21.14 (C-7').

### 4.5.7. General procedure for oxidation of tetronamide (±)-3v.

To a nitrogen flushed Schlenk tube was prepared a suspension of PCC (55 mg, 0.26 mmol, 1.5 eq) in 5 mL of $CH_2Cl_2$ anh., the solution was leaved stirring for 10 min at r.t and then a solution of aldolic tetronamide (±)-**3v** (50 mg, 0.17 mmol, 1.0 eq) in 1 mL of $CH_2Cl_2$ (anh). Was added in one portion. After 2 h of reaction 5 mL of diethylether was added and a dark solid was formed. This solid was sequentially washed with 2 X 5 mL of diethylether. The solid was filtered in a Si-gel flash column and the solvent evapored in vacuo. Reaction mixture was then purified by Si gel column with AcOEt:Hex (3:7) as eluent to furnish compound (±)-**47b** as a white solid with 80% yield.

*(±)-5-benzoyl-3-chloro-4-(pyrrolidin-1-yl)furan-2(5H)-one (±)-(**47b**)*




**Yield:** 80%

**Characteristics**: White solid.

**Eluent (ethyl acetate/hexane (v/v)):** 2:8.

**Mp**: 162.1 – 163.3 °C.

**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3064, 2971, 2925, 2877, 1737, 1692, 1632, 1612, 1477, 1101, 1072, 917, 669, 610.

**$^{1}$H NMR (400 MHz, $(CD_3)_2SO$)** *δ:* 8.11 (d, *J* = 7.8 Hz, 2H, H-2' and H-6'), 7.65 (t, *J* = 7.5 Hz, 1H, H-4'), 7.79 (t, *J* = 7.3 Hz, 2H, H-3' and H-5'), 6.79 (s,1H, H-5), 3.71 (brd, 4H, H-1'' - H-4''), 1.88 (brd, 2H, H-2'' - H-3''), 1.81 (brd, 2H, H-2'' - H-3'').

**$^{13}$C NMR (100 MHz, $(CD_3)_2SO$)** *δ:* 191.24 (C-6), 168.87 (C-2), 157.30 (C-4), 141.74 (C-1'), 135.53 (2C, C-3' and C-5'), 129.74 (3C, C-2', C-4'and C-6'), 83.28 (C-3), 75.85 (C-5), 49.84 (2C, C-1'' and C-4''), 25.28 (2C, C-2'' and C-3'').

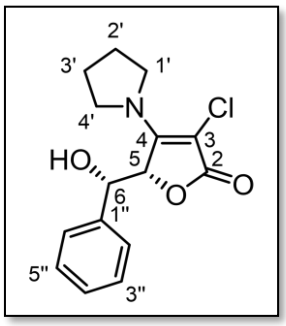
**HRMS (ESI) *m/z* $[M+Na^+]^+$:** calculated for $C_{15}H_{14}ClNNaO_3$, 314.0554; found, 314.0554.

### 4.5.8. General procedure for enantioselective synthesis of chiral aldolic tetronamides (+)-3x and (-)-3y.

To a nitrogen flushed Schlenk tube was charged with catalyst (*R,R*)-Ru-Teth-cat or (*S,S*)-Ru-Teth-cat (1 mol%) before the addition of equivalent volumes of chloroform and 5:2 formic acid/triethylamine azeotropic solution such that the total concentration of the ketone is 1M, then activation of the catalyst was leaved for 30 min at r.t.. Next a solution of oxidized tetronamide (±)-**47b** (20 mg, 0.069 mmol, 1.0 eq.) in 0.1 mL of $CH_2Cl_2$ and Reaction is allowed to stir overnight (15 h) at room temperature. Before quenching with excess saturated sodium bicarbonate solution and subsequently extracting the mixture with ethyl acetate (2 x 3 mL). The combined organic layers were concentrated then purified by flash chromatography on silica with AcOEt:Hex (1:1) to afford the desired chiral alcohols (+)-**3x** and (-)-**3y**. The compounds were recrystallized with $CH_2Cl_2$ to send to X-ray analysis.

*4.5.8.1. Chiral tetronamides **3** data:*

*(+)-(S)-3-chloro-5-((S)-hydroxy(phenyl)methyl)-4-(pyrrolidin-1-yl)furan-2(5H)-one (+)-(**3x**)*




**Yield:** 96%

***ee*:** 87%

**Characteristics**: White solid.

**Eluent (ethyl acetate/hexane (v/v)):** 35:65.

**Mp**: 207.4 – 208.9 °C.

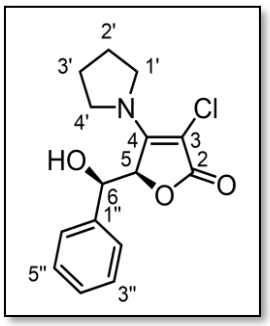
**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3310, 2956, 2924, 2883, 1713, 1598, 1454, 1409, 1341, 1240, 1065, 1015, 868, 802, 744, 706.

**$^{1}$H NMR (400 MHz, $(CD_3)_2SO$)** *δ:* 7.44 (d, *J* = 7.5 Hz, 2H, H-2' and H-6'), 7.36 (t, *J* = 7.3 Hz, 2H, H-3' and H-5'), 7.44 (d, *J* = 7.1 Hz, 1H, H-4'), 5.72 (d, *J* = 5.5 Hz, 1H, H-5), 5.32 (s, 1H, -OH), 5.12 (d, *J* = 5.6 Hz, 1H, H-6), 5.73 (brd, 2H, H-1'' - H-4''), 5.72 (brd, 2H, H-1'' - H-4''), 5.13 (brd, 2H, H-2'' - H-3''), 5.12 (brd, 2H, H-2'' - H-3'').

**$^{13}$C NMR (100 MHz, $(CD_3)_2SO$)** *δ:* 169.39 (C-2), 158.03 (C-4), 141.27 (C-1'), 127.77 (2C, C-3' and C-5'), 127.17 (C-4'), 126.42 (2C, C-2' and C-6'), 84.15 (C-3), 80.61 (C-5), 69.03 (C-6), 40.36 (2C, C-1'' and C-4''), 24.81 (2C, C-2'' and C-3'').

**HRMS (ESI) *m/z* [M+Na$^+$]$^+$:** calculated for $C_{15}H_{14}ClNNaO_3$, 314.0554; found, 314.0554.

*(-)-(R)-3-chloro-5-((R)-hydroxy(phenyl)methyl)-4-(pyrrolidin-1-yl)furan-2(5H)-one (-)-(**3y**)*




**Yield:** 89%

***ee*:** 80%

**Characteristics**: White solid.

**Eluent (ethyl acetate/hexane (v/v)):** 35:65.

**Mp**: 208.7 – 209.8 °C.

**FTIR (cm$^{-1}$):** $\bar{\upsilon}_{max}$ 3316, 2955, 2925, 2883, 1712, 1596, 1452, 1408, 1341, 1240, 1065, 1016, 869, 803, 744, 707.

**$^{1}$H NMR (400 MHz, $(CD_3)_2SO$):** *δ:* 7.44 (d, *J* = 7.5 Hz, 2H, H-2' and H-6'), 7.36 (t, *J* = 7.3 Hz, 2H, H-3' and H-5'), 7.44 (d, *J* = 7.1 Hz, 1H, H-4'), 5.72 (d, *J* = 5.5 Hz, 1H, H-5), 5.32 (s, 1H, -OH), 5.12 (d, *J* = 5.6 Hz, 1H, H-6), 5.73 (brd, 2H, H-1'' - H-4''), 5.72 (brd, 2H, H-1'' - H-4''), 5.13 (brd, 2H, H-2'' - H-3''), 5.12 (brd, 2H, H-2'' - H-3'').

**$^{13}$C NMR (100 MHz, $(CD_3)_2SO$)** *δ:* 169.39 (C-2), 158.03 (C-4), 141.27 (C-1'), 127.77 (2C, C-3' and C-5'), 127.17 (C-4'), 126.42 (2C, C-2' and C-6'), 84.15 (C-3), 80.61 (C-5), 69.03 (C-6), 40.36 (2C, C-1'' and C-4''), 24.81 (2C, C-2'' and C-3'').

**HRMS (ESI) *m/z* $[M+Na^+]^+$:** calculated for $C_{15}H_{14}ClNNaO_3$, 314.0554; found, 314.0554.

## 4.6. REFERENCES


[1] IINUMA, H.; NAKAMURA, H.; NAGANAWI, H.; MASUDA, T.; TAKANO, S.; TAKEUCHI, T.; UMEZAWA, H.; IITAKA, Y.; OBAYASHI, A. Basidalin, a new antibiotic from basidiomycetes. *The Journal of antibiotics* **1983**, *36*, 448.

[2] ACOSTA, J. A. M.; MUDDALA, R.; BARBOSA, L. C. A.; BOUKOUVALAS, J. Total synthesis of the antitumor antibiotic basidalin. *The Journal of Organic Chemistry* **2016**, *81*, 6883.

[3] LATTMANN, E.; DUNN, S.; NIAMSANIT, S.; SATTAYASAI, N. Synthesis and antibacterial activities of 5-hydroxy-4-amino-2(5*H*)-furanones. *Bioorganic & Medicinal Chemistry Letters* **2005**, *15*, 919.

[4] JESCHKE, P.; NAUEN, R.; GUTBROD, O.; BECK, M. E.; MATTHIESEN, S.; HAAS, M.; VELTEN, R. Flupyradifurone (Sivanto™) and its novel butenolide pharmacophore: Structural considerations. *Pesticide Biochemistry and Physiology* **2015**, *121*, 31.

[5] NAUEN, R.; JESCHKE, P.; VELTEN, R.; BECK, M. E.; EBBINGHAUS-KINTSCHER, U.; THIELERT, W.; WÖLFEL, K.; HAAS, M.; KUNZ, K.; RAUPACH, G. Flupyradifurone: a brief profile of a new butenolide insecticide. *Pest Management Science* **2015**, *71*, 850.

[6] SEMENOVA, M. N.; KISELYOV, A. S.; TSYGANOV, D. V.; KONYUSHKIN, L. D.; FIRGANG, S. I.; SEMENOV, R. V.; MALYSHEV, O. R.; RAIHSTAT, M. M.; FUCHS, F.; STIELOW, A.; LANTOW, M.; PHILCHENKOV, A. A.; ZAVELEVICH, M. P.; ZEFIROV, N. S.; KUZNETSOV, S. A.; SEMENOV, V. V. Polyalkoxybenzenes from plants. 5. Parsley seed extract in synthesis of azapodophyllotoxins featuring strong tubulin destabilizing activity in the sea urchin embryo and cell culture assays. *Journal of Medicinal Chemistry* **2011**, *54*, 7138.

[7] MADEC, D.; MINGOIA, F.; PRESTAT, G.; POLI, G. *N*-Substituted tetronamides as ambident nucleophilic building blocks for the synthesis of new 4-aza-2,3-didehydropodophyllotoxins. *Synlett* **2008**, *2008*, 1475.

[8] WANG, J.; JIANG, X.; CHEN, M.; GE, Z.; HU, Y.; HU, H. TFA-catalyzed ring transformation of 4-hydroxycyclobutenone: A simple and general route for preparation of 3-substituted 4-aminofuran-2(5*H*)-ones. *Journal of the Chemical Society, Perkin Transactions 1* **2001**, 66.

[9] DE SOUZA, M. V. N. The furan-2(5*H*)-ones: Recent synthetic methodologies and its application in total synthesis of natural products. *Mini-Reviews in Organic Chemistry* **2005**, *2*, 139.

[10] KOTORA, M.; NEGISHI, E.-I. Highly efficient and selective procedures for the synthesis of γ-alkylidenbutenolides via palladium-catalyzed ene-yne coupling and palladium- or silver-catalyzed lactonization if (*Z*)-2-en-4-ynoic acids. Synthesis of rubrolides A, C, D and E. *Synthesis* **1997**, *1*, 121.

[11] MIAO, S.; ANDERSEN, R. J. Rubrolides A-H, metabolites of the colonial tunicate *Ritterella rubra*. *The Journal of Organic Chemistry* **1991**, *56*, 6275.

[12] ORTEGA, M. A. J.; ZUBÍA, E.; OCAÑA, J. M.; NARANJO, S.; SALVÁ, J. New Rubrolides from the Ascidian *Synoicum blochmanni*. *Tetrahedron* **2000**, *56*, 3963.

[13] PEARCE, A. N.; CHIA, E. W.; BERRIDGE, M. V.; MAAS, E. W.; PAGE, M. J.; WEBB, V. L.; HARPER, J. L.; COPP, B. R. *E*/*Z*-Rubrolide O, an anti-inflammatory halogenated furanone from the new zealand ascidian *Synoicum n.* sp. *Journal of Natural Products* **2007**, *70*, 111.

[14] SIKORSKA, J.; PARKER-NANCE, S.; DAVIES-COLEMAN, M. T.; VINING, O. B.; SIKORA, A. E.; MCPHAIL, K. L. Antimicrobial rubrolides from a south african species of *Synoicum* tunicate. *Journal of Natural Products* **2012**, *75*, 1824.

[15] SMITHA, D.; KUMAR, M. M. K.; RAMANA, H.; RAO, D. V. Rubrolide R: a new furanone metabolite from the ascidian *Synoicum* of the Indian Ocean. *Natural Product Research* **2014**, *28*, 12.

[16] KARAK, M.; BARBOSA, L. C. A.; ACOSTA, J. A. M.; SAROTTI, A. M.; BOUKOUVALAS, J. Thermodynamically driven, *syn*-selective vinylogous aldol reaction of tetronamides. *Organic & Biomolecular Chemistry* **2016**, *14*.

[17] CASIRAGHI, G.; ZANARDI, F. The vinylogous aldol reaction: A valuable, yet understated carbon−carbon bond-forming maneuver. *Chem. Rev.* **2000**, *100*.

[18] CASIRAGHI, G.; BATTISTINI, L.; CURTI, C.; RASSU, G.; ZANARDI, F. The vinylogous aldol and related addition reactions: Ten years of progress. *Chemical Reviews* **2011**, *111*, 3076.

[19] PANSARE, S. V.; PAUL, E. K. The organocatalytic vinylogous aldol reaction: recent advances. *Chemistry-A European Journal* **2011**, *17*, 8770.

[20] BISAI, V. Organocatalytic asymmetric vinylogous aldol reactions. *Synthesis* **2012**, *44*, 1453.

[21] BARBOSA, L. C. A.; TEIXEIRA, R., R. ; AMARANTE, G., W. . Synthetic strategies for the preparation of butenolides and their transformation into other derivatives. *Current Organic Synthesis* **2015**, *12*, 746.

[22] BROWN, D. W.; CAMPBELL, M. M.; TAYLOR, A. P.; ZHANG, X.-A. Regio- and diastereoselectivity in aldol reactions of cyclopent-2-enone, 2-(5*H*)-furanone and their derived trimethylsilyloxydienes. *Tetrahedron Letters* **1987**, *28*, 985.

[23] JEFFORD, C. W.; JAGGI, D.; BERNARDINELLI, G.; BOUKOUVALAS, J. The synthesis of (±)-cavernosine. *Tetrahedron Letters* **1987**, *28*, 4041.

[24] JEFFORD, C. W.; JAGGI, D.; BOUKOUVALAS, J. Diastereoselectivity in the directed aldol condensation of 2-trimethylsiloxyfuran with aldehydes. A stereodivergent route to threo and erythro δ-hydroxy-γ-lactones. *Tetrahedron letters* **1987**, *28*, 4037.

[25] BOUKOUVALAS, J.; MALTAIS, F. An efficient total synthesis of neopatulin. *Tetrahedron Letters* **1994**, *35*, 5769.

[26] LÓPEZ, C. S.; ÁLVAREZ, R.; VAZ, B.; FAZA, O. N.; DE LERA, Á. R. Simple diastereoselectivity of the $BF_3{\cdot}OEt_2$-catalyzed vinylogous mukaiyama aldol reaction of 2-(trimethylsiloxy)furans with aldehydes. *The Journal of Organic Chemistry* **2005**, *70*, 3654.

[27] BOUKOUVALAS, J.; BELTRAN, P. P.; LACHANCE, N.; COTE, S.; MALTAIS, F.; POULIOT, M. A new, highly stereoselective synthesis of β-unsubstituted (*Z*)-γ-alkylidenebutenolides using bromine as a removable stereocontrol element. *Synlett* **2007**, 219.

[28] DAS SARMA, K.; ZHANG, J.; CURRAN, T. T. Novel synthons from mucochloric acid: The first use of α,β-dichloro-γ-butenolides and γ-butyrolactams for direct vinylogous aldol addition. *J. Org. Chem.* **2007**, *72*.

[29] LIU, G.-Y.; GUO, B.-Q.; CHEN, W.-N.; CHENG, C.; ZHANG, Q.-L.; DAI, M.-B.; SUN, J.-R.; SUN, P.-H.; CHEN, W.-M. Synthesis, molecular docking, and biofilm formation inhibitory activity of 5-substituted 3,4-dihalo-5*H*-furan-2-one derivatives on *Pseudomonas aeruginosa*. *Chemical Biology & Drug Design* **2012**, *79*, 628.

[30] DAVID, J. G.; BAI, W.-J.; WEAVER, M. G.; PETTUS, T. R. R. A general diastereoselective catalytic vinylogous aldol reaction among tetramic acid-derived pyrroles. *Organic Letters* **2014**, *16*, 4384.

[31] KABESHOV, M. A.; KYSILKA, O.; RULÍŠEK, L.; SULEIMANOV, Y. V.; BELLA, M.; MALKOV, A. V.; KOČOVSKÝ, P. Cross-aldol reaction of isatin with acetone catalyzed by leucinol: A mechanistic investigation. *Chemistry-A European Journal* **2015**, *21*, 12026.

[32] FRINGS, M.; ATODIRESEI, I.; WANG, Y.; RUNSINK, J.; RAABE, G.; BOLM, C. C1-symmetric aminosulfoximines in copper-catalyzed asymmetric vinylogous mukaiyama aldol reactions. *Chemistry-A European Journal* **2010**, *16*, 4577.

[33] YANG, Y.; ZHENG, K.; ZHAO, J.; SHI, J.; LIN, L.; LIU, X.; FENG, X. Asymmetric direct vinylogous aldol reaction of unactivated γ-butenolide to aldehydes. *J. Org. Chem.* **2010**, *75*

[34] HITOSHI, U.; NAOKI, S.; MASAHIRO, T. Asymmetric direct vinylogous aldol reaction of furanone derivatives catalyzed by an axially chiral guanidine base. *Angewandte Chemie International Edition* **2010**, *49*, 1858.

[35] JIE, L.; HAIFEI, W.; XIAO, H.; LI-WEN, X.; JACEK, K.; KUO-WEI, H.; YIXIN, L. The direct asymmetric vinylogous aldol reaction of furanones with α-ketoesters: access to chiral γ-butenolides and glycerol derivatives. *Angewandte Chemie International Edition* **2011**, *50*, 1861.

[36] SZLOSEK, M.; FIGADÈRE, B. Highly enantioselective aldol reaction with 2-trimethylsilyloxyfuran: the first catalytic asymmetric autoinductive aldol reaction. *Angewandte Chemie International Edition* **2000**, *39*, 1799.

[37] EVANS, D. A.; KOZLOWSKI, M. C.; MURRY, J. A.; BURGEY, C. S.; CAMPOS, K. R.; CONNELL, B. T.; STAPLES, R. J. C2-symmetric copper(II) complexes as chiral lewis acids. Scope and mechanism of catalytic enantioselective aldol additions of enolsilanes to (benzyloxy)acetaldehyde. *Journal of the American Chemical Society* **1999**, *121*, 669.

[38] PALOMBI, L.; ACOCELLA, M. R.; CELENTA, N.; MASSA, A.; VILLANO, R.; SCETTRI, A. Highly enantioselective vinylogous addition of 2-trimethylsilyloxyfuran to aldehydes promoted by the $SiCl_4$/chiral Lewis base system. *Tetrahedron: Asymmetry* **2006**, *17*, 3300.

[39] UTPAL, D.; YI-RU, C.; YI-LING, T.; WENWEI, L. Organocatalytic enantioselective direct vinylogous michael addition of γ-substituted butenolides to 3-aroyl acrylates and 1,2-diaroylethylenes. *Chemistry-A European Journal* **2013**, *19*, 7713.

[40] SINGH, R. P.; FOXMAN, B. M.; DENG, L. Asymmetric vinylogous aldol reaction of silyloxy furans with a chiral organic salt. *Journal of the American Chemical Society* **2010**, *132*, 9558.

[41] NING, Z.; BAO-CHUN, M.; YONG, Z.; WEI, W. Organocatalyzed highly enantioselective and *anti*-selective construction of γ-butenolides through vinylogous mukaiyama aldol reaction. *Advanced Synthesis & Catalysis* **2010**, *352*, 1291.

[42] OLLEVIER, T.; BOUCHARD, J.-E.; DESYROY, V. Diastereoselective mukaiyama aldol reaction of 2-(trimethylsilyloxy)furan catalyzed by bismuth triflate. *The Journal of Organic Chemistry* **2008**, *73*, 331.

[43] PANSARE, S. V.; PAULA, E. K. Organocatalytic asymmetric direct vinylogous aldol reactions of γ-crotonolactone with aromatic aldehydes. *ChemComm* **2010**, *47*.

[44] URAGUCHI, D.; SORIMACHI, K.; TERADA, M. Organocatalytic asymmetric aza-Friedel-Crafts alkylation of furan. *J. Am. Chem. Soc.* **2004**, *126*

[45] MARTIN, S. F.; LOPEZ, O. D. Vinylogous Mannich reactions. Catalytic, asymmetric additions of triisopropylsilyloxyfurans to aldimines. *Tetrahedron Letters* **1999**, *40*, 8949.

[46] CARSWELL, E. L.; SNAPPER, M. L.; HOVEYDA, A. H. A Highly efficient and practical method for catalytic asymmetric vinylogous Mannich (AVM) reactions. *Angewandte Chemie International Edition* **2006**, *45*, 7230.

[47] YAMAGUCHI, A.; MATSUNAGA, S.; SHIBASAKI, M. Direct catalytic asymmetric Mannich-type reactions of γ-butenolides: Effectiveness of Brønsted acid in chiral metal catalysis. *Org. Lett.* **2008**, *10*

[48] GUO, Y.; ZHANG, Y.; QI, L.; TIANA, F.; WANG, L. Organocatalytic direct asymmetric vinylogous Mannich reaction of γ-butenolides with isatin-derived ketimines. *RSC Adv.* **2014**, *4*.

[49] BROWN, S. P.; GOODWIN, N. C.; MACMILLAN, D. W. C. The first enantioselective organocatalytic Mukaiyama-Michael reaction: A direct method for the synthesis of enantioenriched γ-butenolide architecture. *J. Am. Chem. Soc.* **2003**, *125*.

[50] TROST, B. M.; HITCE, J. Direct asymmetric michael addition to nitroalkenes: Vinylogous nucleophilicity under dinuclear zinc catalysis. *Journal of the American Chemical Society* **2009**, *131*, 4572.

[51] WANG, J.; QI, C.; GE, Z.; CHENGA, T.; LI, R. Efficient direct asymmetric vinylogous Michael addition reactions of γ-butenolides to chalcones catalyzed by vicinal primary-diamine salts. *Chem. Commum.* **2010**, *46*.

[52] SCHLESSINGER, R. H.; MJALLI, A. M. M.; ADAMS, A. D.; SPRINGER, J. P.; HOOGSTEEN, K. An approach to erythronolide A seco acid via a simple tetronic acid. *The Journal of Organic Chemistry* **1992**, *57*, 2992.

[53] DANKWARDT, J. W.; DANKWARDT, S. M.; SCHLESSINGER, R. H. Synthesis of the C19 through C27 segment of okadaic acid using vinylogous urethane aldol chemistry: Part III. *Tetrahedron Letters* **1998**, *39*, 4979.

[54] BRUYÈRE, H.; BALLEREAU, S.; SELKTI, M.; ROYER, J. Asymmetric synthesis of 5-(1-hydroxyalkyl)-5-methyl-5*H*-furan-2-ones. *Tetrahedron* **2003**, *59*, 5879.

[55] BRUYÈRE, H.; DOS REIS, C.; SAMARITANI, S.; BALLEREAU, S.; ROYER, J. Approach to the eleutherobin core: Synthesis of a key intermediate by intramolecular-Diels-Alder cycloaddition. *Synthesis* **2006**, *2006*, 1673.

[56] EAR, A.; TOUM, V.; THORIMBERT, S.; DECHOUX, L. Decarboxylative Knoevenagel-type reactions on tetronamides: Synthesis of 5-ylidene-4-amino-2(5*H*)-furanones. *Synlett* **2014**, *25*, 1713.

[57] BAULAC, M.; KLEMENT, S. Efficacy and safety of Losigamone in partial seizures: A randomized double-blind study. *Epilepsy Research* **2003**, *55*, 177.

[58] CUNHA, S.; OLIVEIRA, C. C.; SABINO, J. R. Synthesis of 3-bromotetronamides via amination of 3,4-dibromofuran-2(5*H*)-one. *Journal of the Brazilian Chemical Society* **2011**, *22*, 598.

[59] VAREJÃO, J. O. S.; BARBOSA, L. C. A.; RAMOS, G. Á.; VAREJÃO, E. V. V.; KING-DÍAZ, B.; LOTINA-HENNSEN, B. New rubrolide analogues as inhibitors of photosynthesis light reactions. *Journal of Photochemistry and Photobiology B: Biology* **2015**, *145*, 11.

[60] OERKE, E. C. Crop losses to pests. *The Journal of Agricultural Science* **2005**, *144*, 31.

[61] PAUL, N.; MARTIN, V.-A.; FABRICE, R. Evolutionary-thinking in agricultural weed management. *New Phytologist* **2009**, *184*, 783.

[62] POWLES, S. B. Evolved glyphosate-resistant weeds around the world: Lessons to be learnt. *Pest Management Science* **2008**, *64*, 360.

[63] BECKIE, H. J. Herbicide-resistant weed management: Focus on glyphosate. *Pest Management Science* **2011**, *67*, 1037.

[64] DUKE, S. O.; POWLES, S. B. Glyphosate-resistant crops and weeds: Now and in the future. *AgBioForum* **2009**, *12*.

[65] DUKE, S. O. Why have no new herbicide modes of action appeared in recent years? *Pest Management Science* **2012**, *68*, 505.

[66] DAYAN, F. E.; CANTRELL, C. L.; DUKE, S. O. Natural products in crop protection. *Bioorganic & Medicinal Chemistry* **2009**, *17*, 4022.

[67] CANTRELL, C. L.; DAYAN, F. E.; DUKE, S. O. Natural products as sources for new pesticides. *Journal of Natural Products* **2012**, *75*, 1231.

[68] CLARK, R. D. A perspective on the role of quantitative structure-activity and structure-property relationships in herbicide discovery. *Pest Management Science* **2012**, *68*, 513.

[69] GOOD, N. E. Inhibitors of the Hill reaction. *Plant Physiology* **1961**, *36*, 788.

[70] DICKS, J. W. Inhibition of the Hill reaction of isolated chloroplasts by herbicidal phenylureas. *Pesticide Science* **1978**, *9*, 59.

[71] STANIER, R. Y.; BAZINE, G. C. Phototrophic prokaryotes: The cyanobacteria. *Annual Review of Microbiology* **1977**, *31*, 225.

[72] TSYGANKOV, A. A. Nitrogen-fixing cyanobacteria: A review. *Applied Biochemistry and Microbiology* **2007**, *43*, 250.

[73] PAERL, H. W.; OTTEN, T. G. Harmful cyanobacterial blooms: Causes, consequences, and controls. *Microbial Ecology* **2013**, *65*, 995.

[74] Havens, K. E. In *Cyanobacterial harmful algal blooms: State of the science and research needs*; Hudnell, H. K., Ed.; Springer New York: New York, NY, 2008, p 733.

[75] MEREL, S.; WALKER, D.; CHICANA, R.; SNYDER, S.; BAURÈS, E.; THOMAS, O. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environment International* **2013**, *59*, 303.

[76] CHEUNG, M. Y.; LIANG, S.; LEE, J. Toxin-producing cyanobacteria in freshwater: A review of the problems, impact on drinking water safety, and efforts for protecting public health. *Journal of Microbiology* **2013**, *51*, 1.

[77] O'NEIL, J. M.; DAVIS, T. W.; BURFORD, M. A.; GOBLER, C. J. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae* **2012**, *14*, 313.

[78] RAJASEKHAR, P.; FAN, L.; NGUYEN, T.; RODDICK, F. A. A review of the use of sonication to control cyanobacterial blooms. *Water Research* **2012**, *46*, 4319.

[79] JANČULA, D.; MARŠÁLEK, B. Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms. *Chemosphere* **2011**, *85*, 1415.

[80] RASTOGI, R. P.; MADAMWAR, D.; INCHAROENSAKDI, A. Bloom dynamics of cyanobacteria and their toxins: Environmental health impacts and mitigation strategies. *Frontiers in Microbiology* **2015**, *6*.

[81] SHAO, J.; LI, R.; LEPO, J. E.; GU, J.-D. Potential for control of harmful cyanobacterial blooms using biologically derived substances: Problems and prospects. *Journal of Environmental Management* **2013**, *125*, 149.

[82] NAIN-PEREZ, A.; BARBOSA, L. C. A.; MALTHA, C. R. Á.; FORLANI, G. Natural abenquines and their synthetic analogues exert algicidal activity against bloom-forming cyanobacteria. *Journal of Natural Products* **2017**, *80*, 813.

[83] BARBOSA, L. C. A.; DEMUNER, A. J.; BORGES, E. E. L.; MANN, J. Synthesis and evaluation of the plant growth regulatory activity of 8-oxabicyclo[3.2.1]oct-6-en-3-one derivatives. *Journal of the Brazilian Chemical Society* **1997**, *8*, 19.

[84] VAREJAO, J. O. S.; BARBOSA, L. C. A.; MALTHA, C. R. A.; LAGE, M. R.; LANZNASTER, M.; CARNEIRO, J. W. M.; FORLANI, G. Voltammetric and theoretical study of the redox properties of rubrolide analogues. *Electrochimica Acta* **2014**, *120*, 334.

[85] BARBOSA, L. C. A.; VAREJÃO, J. O. S.; PETROLLINO, D.; PINHEIRO, P. F.; DEMUNER, A. J.; MALTHA, C. R. A.; FORLANI, G. Tailoring nostoclide structure to target the chloroplastic electron transport chain. *ARKIVOC* **2012**, *2012*, 15.

[86] TEIXEIRA, R. R.; PINHEIRO, P. F.; BARBOSA, L. C.; CARNEIRO, J. W.; FORLANI, G. QSAR modeling of photosynthesis-inhibiting nostoclide derivatives. *Pest. Manag. Sci.* **2010**, *66*.

[87] BARBOSA, L. C. A.; ROCHA, M. E.; TEIXEIRA, R. R.; MALTHA, C. R. A.; FORLANI, G. Synthesis of 3-(4-bromobenzyl)-5-(arylmethylene)-5*H*-furan-2-ones and their activity as inhibitors of the photosynthetic electron transport chain. *Journal of Agricultural and Food Chemistry* **2007**, *55*, 8562.

[88] TEIXEIRA, R. R.; BARBOSA, L. C. A.; FORLANI, G.; PILÓ-VELOSO, D.; WALKIMAR DE MESQUITA CARNEIRO, J. Synthesis of photosynthesis-inhibiting nostoclide analogues. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 2321.

[89] BARBOSA, L. C.; DEMUNER, A. J.; DE ALVARENGA, E. S.; OLIVEIRA, A.; KING-DIAZ, B.; LOTINA-HENNSEN, B. Phytogrowth- and photosynthesis-inhibiting properties of nostoclide analogues. *Pest Management Science* **2006**, *62*, 214.

[90] BARBOSA, L. C. A.; MALTHA, C. R. A.; LAGE, M. R.; BARCELOS, R. C.; DONÀ, A.; CARNEIRO, J. W. M.; FORLANI, G. Synthesis of rubrolide analogues as new inhibitors of the photosynthetic electron transport chain. *Journal of Agricultural and Food Chemistry* **2012**, *60*, 10555.

[91] PEREIRA, U. A.; BARBOSA, L. C. A.; DEMUNER, A. J.; SILVA, A. A.; BERTAZZINI, M.; FORLANI, G. Rubrolides as model for the development of new lactones and their aza analogs as potential photosynthesis inhibitors. *Chemistry & Biodiversity* **2015**, *12*, 987.

[92] GUTHRIE, J. P. Hydrolysis of esters of oxy acids: pKa values for strong acids; Brønsted relationship for attack of water at methyl; free energies of hydrolysis of esters of oxy acids; and a linear relationship between free energy of hydrolysis and pKa holding over a range of 20 pK units. *Canadian Journal of Chemistry* **1978**, *56*, 2342.

[93] KARAK, M.; BARBOSA, L. C. A.; MALTHA, C. R. A.; SILVA, T. M.; BOUKOUVALAS, J. Palladium-catalyzed hydrodehalogenation of butenolides: An efficient and sustainable access to β-arylbutenolides. *Tetrahedron Letters* **2017**, *58*.

[94] HAYES, A. M.; MORRIS, D. J.; CLARKSON, G. J.; WILLS, M. A class of ruthenium(II) catalyst for asymmetric transfer hydrogenations of ketones. *Journal of the American Chemical Society* **2005**, *127*, 7318.

[95] MORRIS, D. J.; HAYES, A. M.; WILLS, M. The “Reverse-tethered” ruthenium (II) catalyst for asymmetric transfer hydrogenation: Further applications. *The Journal of Organic Chemistry* **2006**, *71*, 7035.

[96] CHEUNG, F. K.; LIN, C.; MINISSI, F.; CRIVILLÉ, A. L.; GRAHAM, M. A.; FOX, D. J.; WILLS, M. An investigation into the tether length and substitution pattern of arene-substituted complexes for asymmetric transfer hydrogenation of ketones. *Organic Letters* **2007**, *9*, 4659.

[97] HASHIGUCHI, S.; FUJII, A.; TAKEHARA, J.; IKARIYA, T.; NOYORI, R. Asymmetric transfer hydrogenation of aromatic ketones catalyzed by chiral ruthenium(II) complexes. *Journal of the American Chemical Society* **1995**, *117*, 7562.

[98] FUJII, A.; HASHIGUCHI, S.; UEMATSU, N.; IKARIYA, T.; NOYORI, R. Ruthenium(II)-catalyzed asymmetric transfer hydrogenation of ketones using a formic acid−triethylamine Mixture. *J. Am. Chem. Soc.* **1996**, *118*.

## ANNEX

# 1. CHAPTER 1: Total synthesis of rubrolídes B e K.

## 1.1. Spectra for the synthesis of the intermediate 76g.

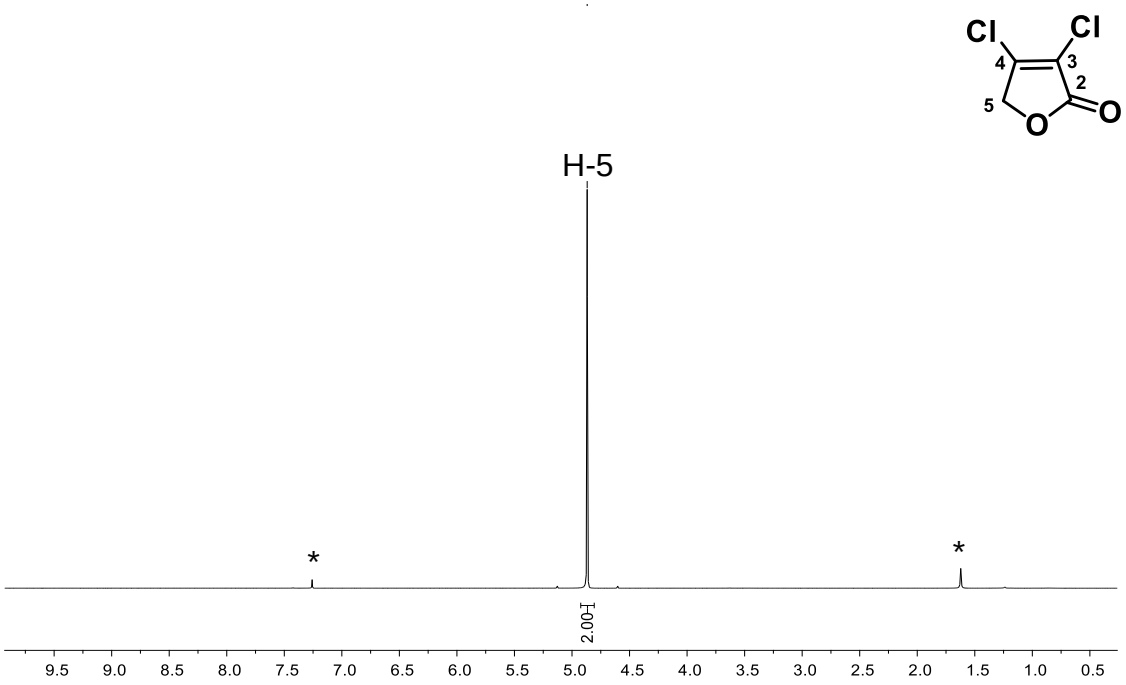



**Figure A 1.1:** $^{1}H$ NMR ($CDCl_3$; 400 MHz) of lactone **72d**. *Solvent residues and water signals.

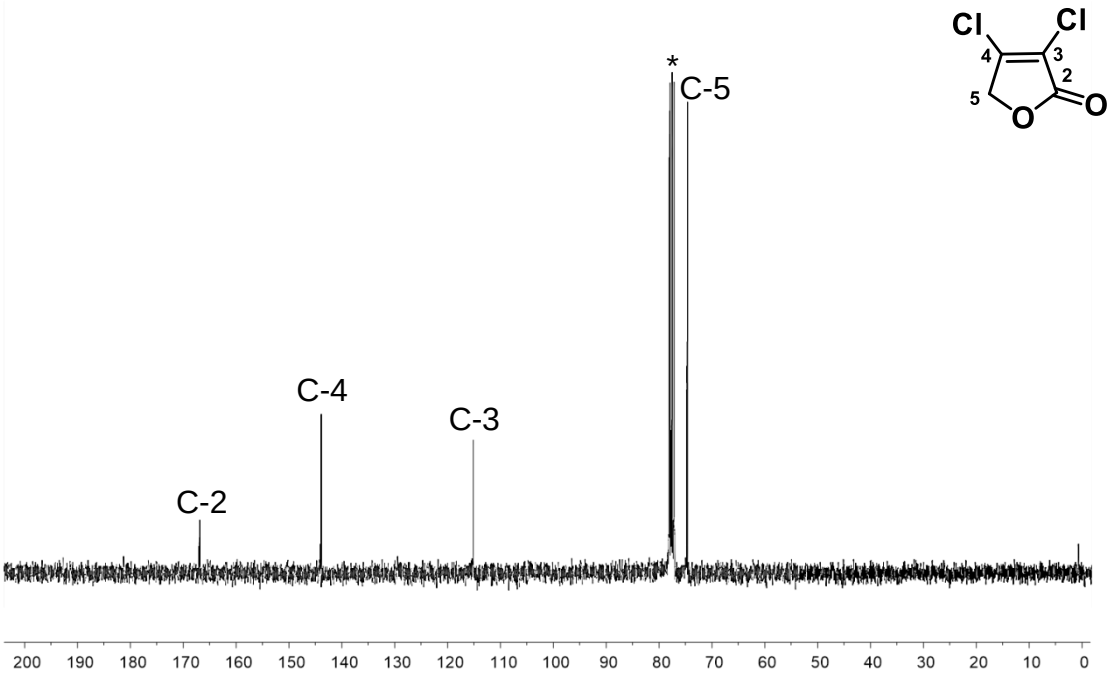



**Figure A 1.2:** $^{13}C$ NMR ($CDCl_3$; 100 MHz) of lactone **72d**. *Solvent residues signals.

## 1.2. Spectra of the optimization of the bromination reaction.

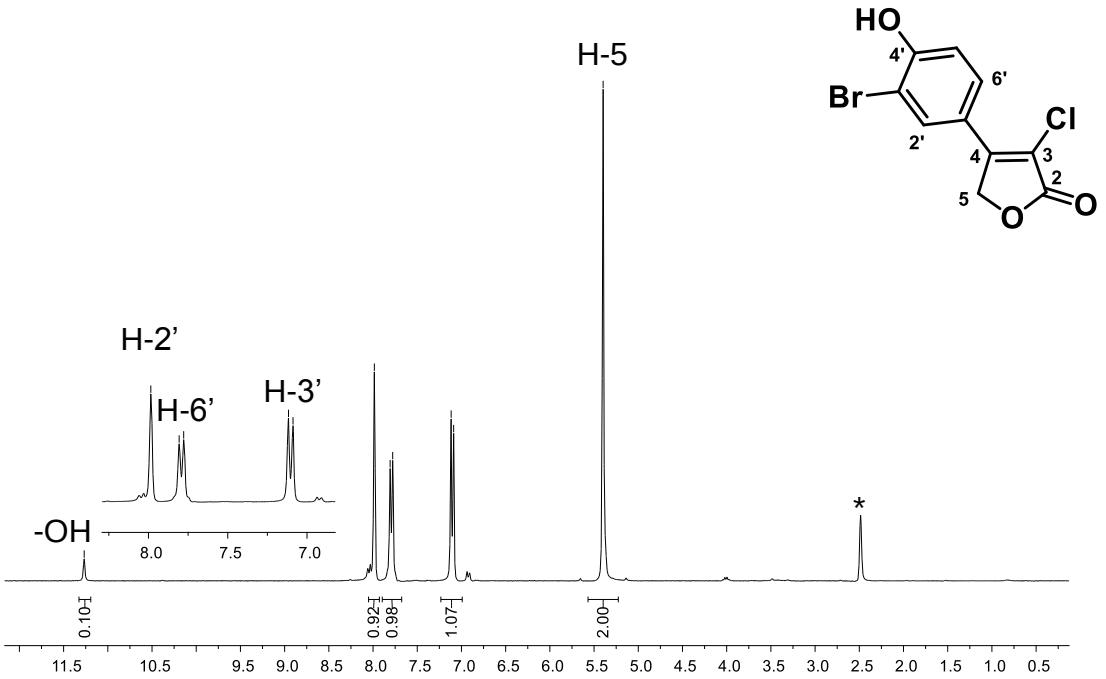



**Figure A 1.3**: $^{1}H$ NMR ($(CD_3)_2SO$, 400 MHz) of compound **80b.** *Solvent residues signals.

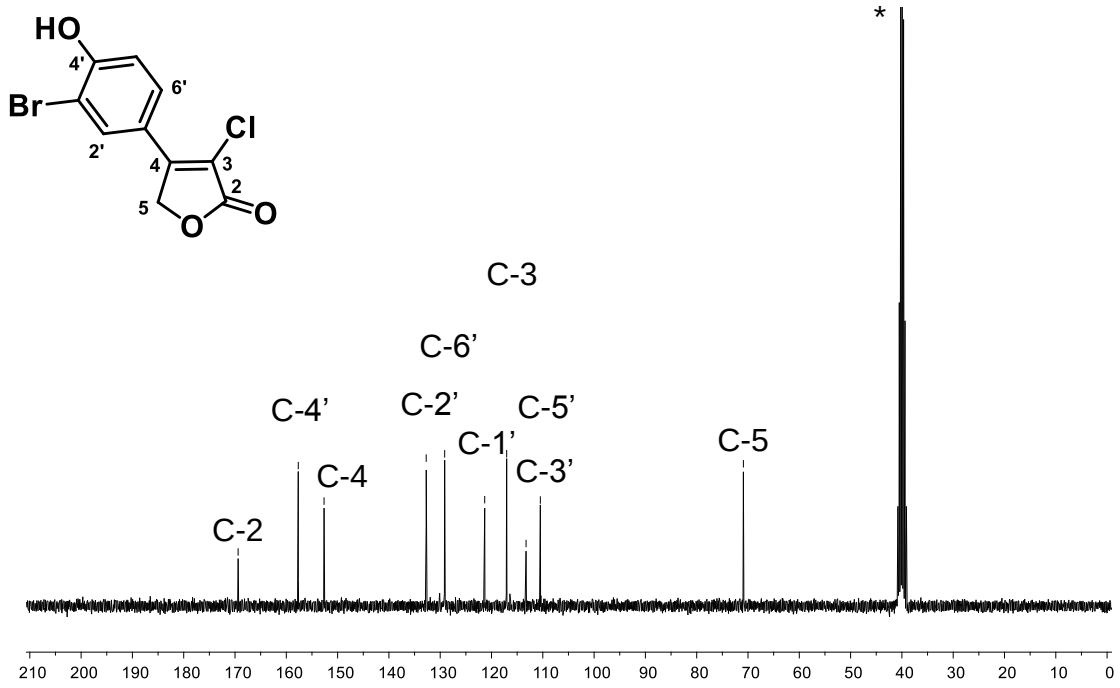



**Figure A 1.4**: $^{13}C$ NMR ($(CD_3)_2SO$, 100 MHz) of compound **80b**. *Solvent residues signals.

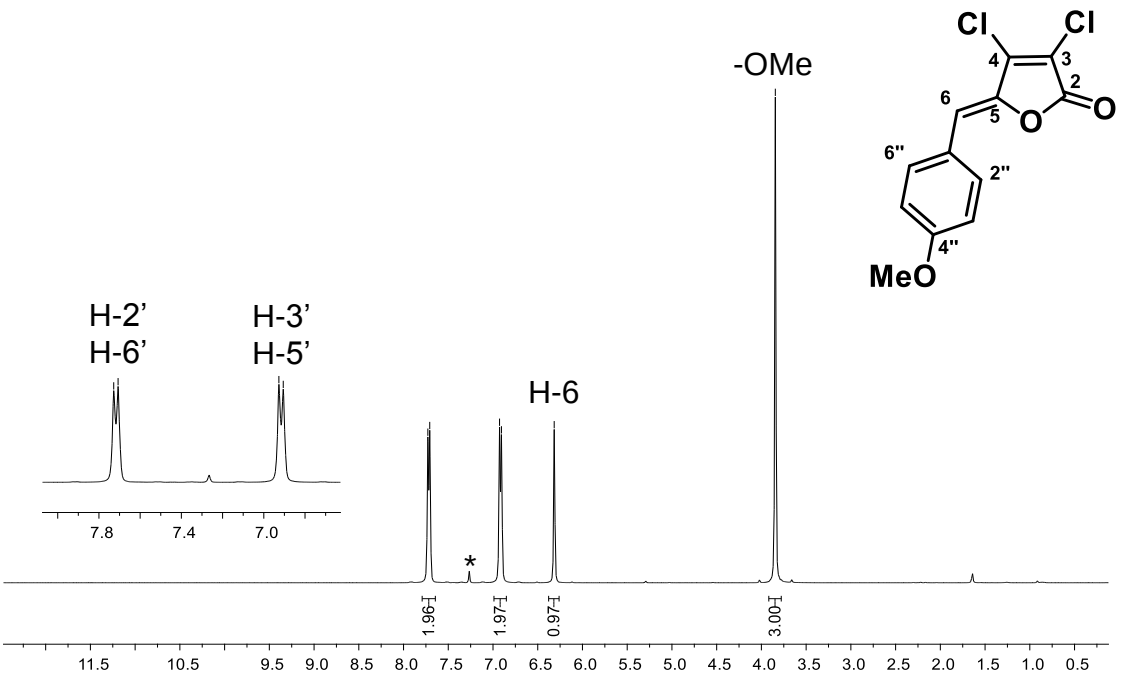



**Figure A 1.5**: $^{1}H$ NMR ($CDCl_3$, 400 MHz) of compound **79a.** *Solvent residues signals.

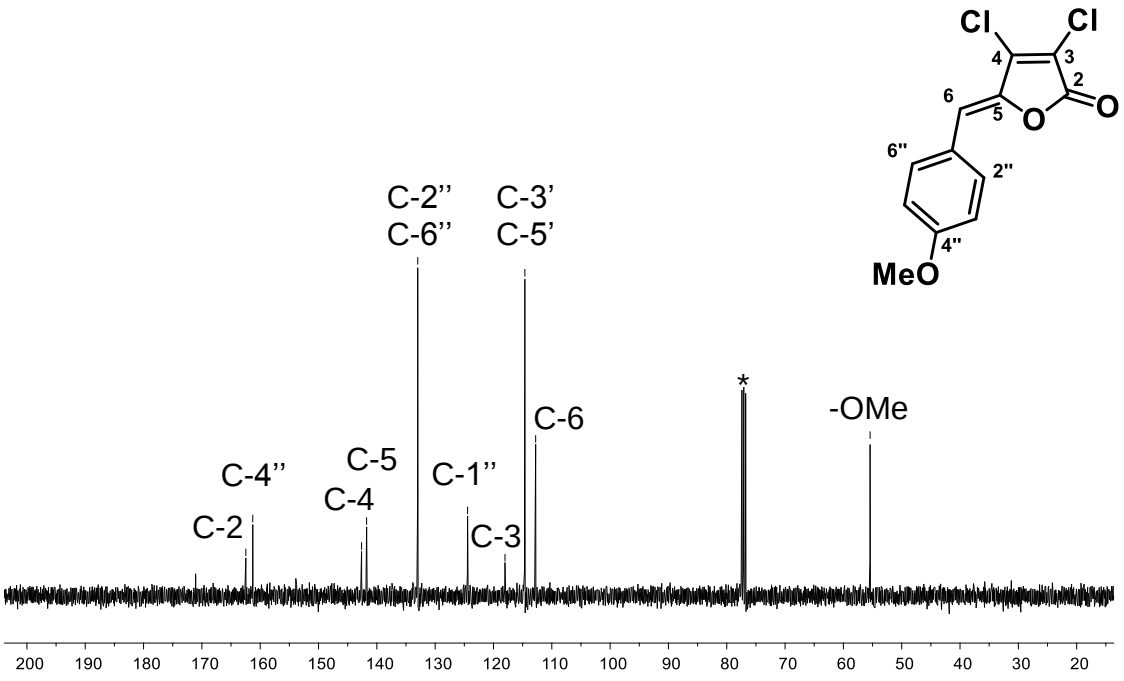



**Figure A 1.6**: $^{13}C$ NMR ($CDCl_3$, 100 MHz) of compound **79a.** *Solvent residues signals.

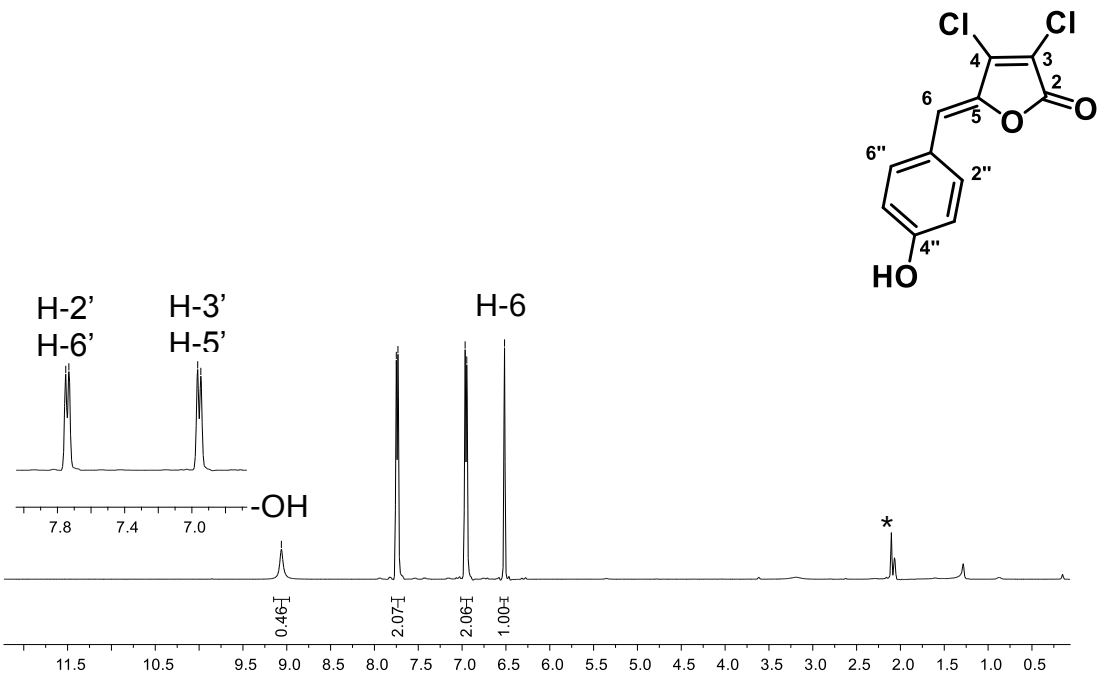



**Figure A 1.7**: $^{1}H$ NMR ($(CD_3)_2CO$, 400 MHz) of compound **79b.** *Solvent residues signals.

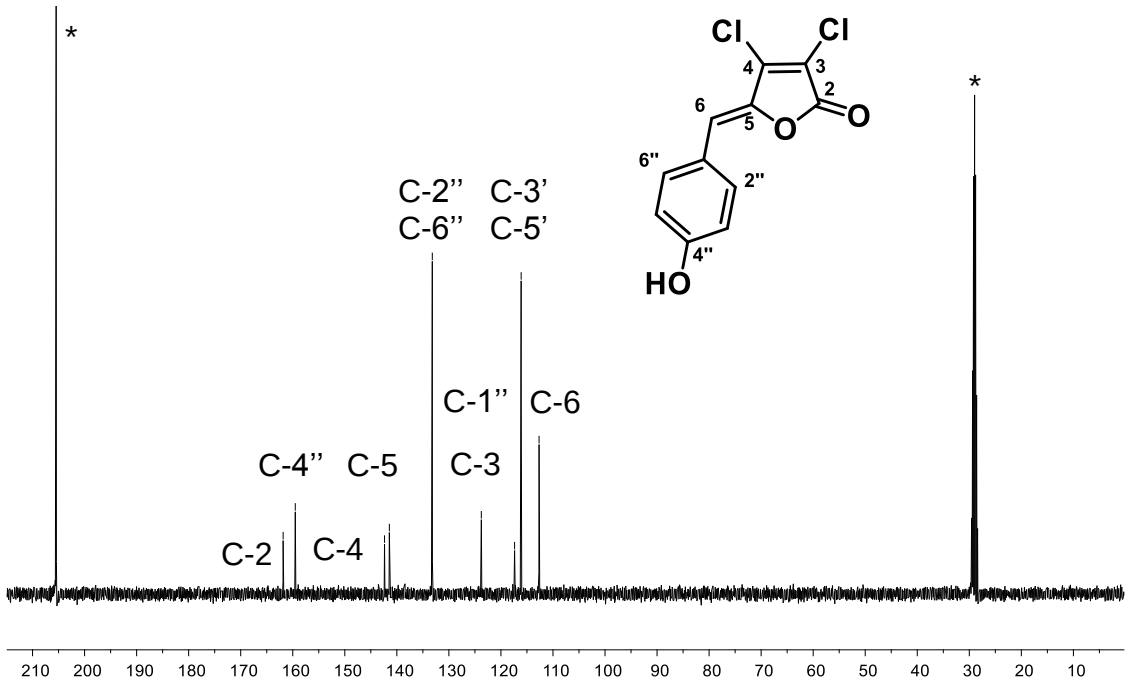



**Figure A 1.8**: $^{13}C$ NMR ($(CD_3)_2CO$, 100 MHz) of compound **79b.** *Solvent residues signals.

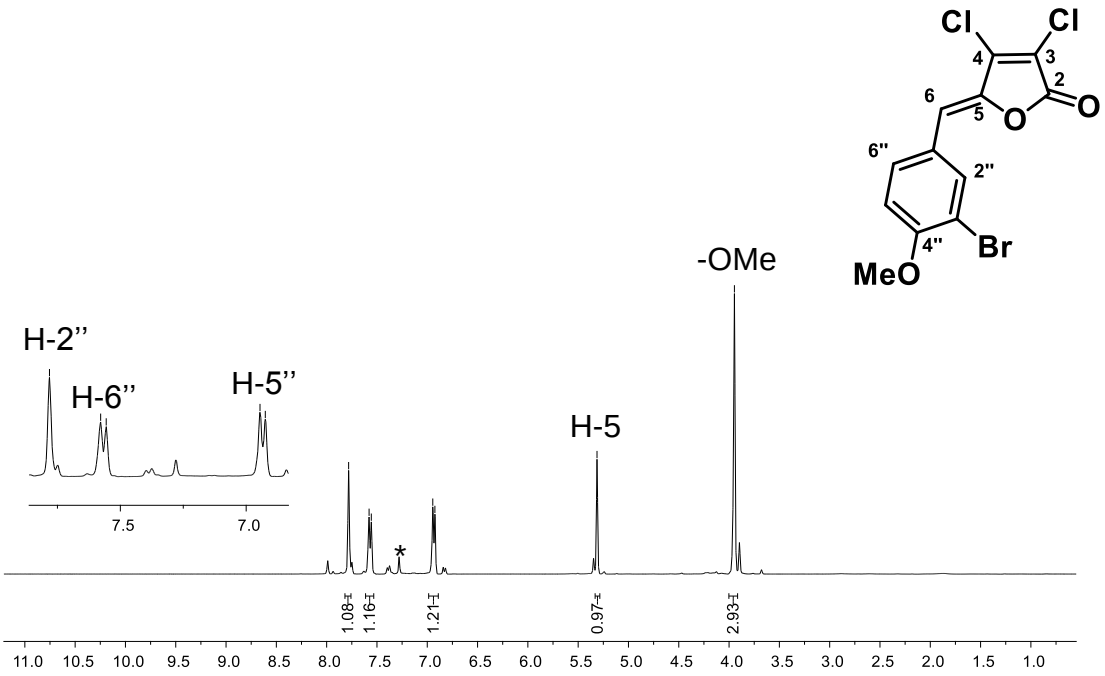



**Figure A 1.9**: $^{1}H$ NMR ($CDCl_3$, 400 MHz) of compound **81a.** *Solvent residues signals.

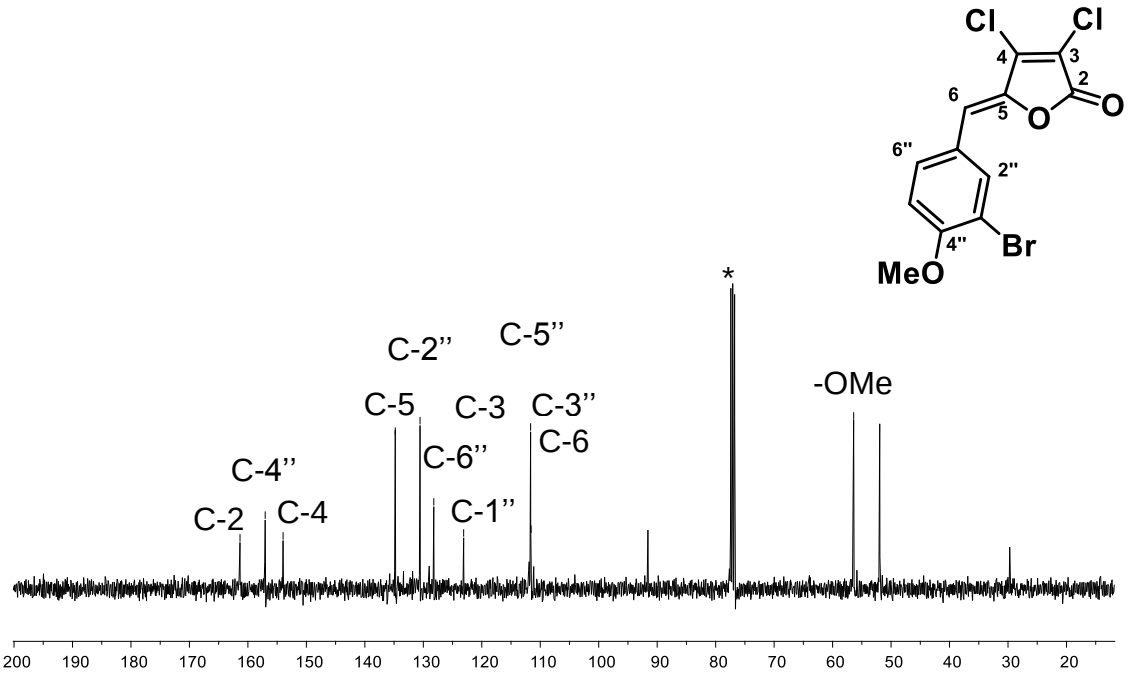



**Figure A 1.10**: $^{13}C$ NMR ($CDCl_3$, 100 MHz) of compound **81a**. *Solvent residues signals.

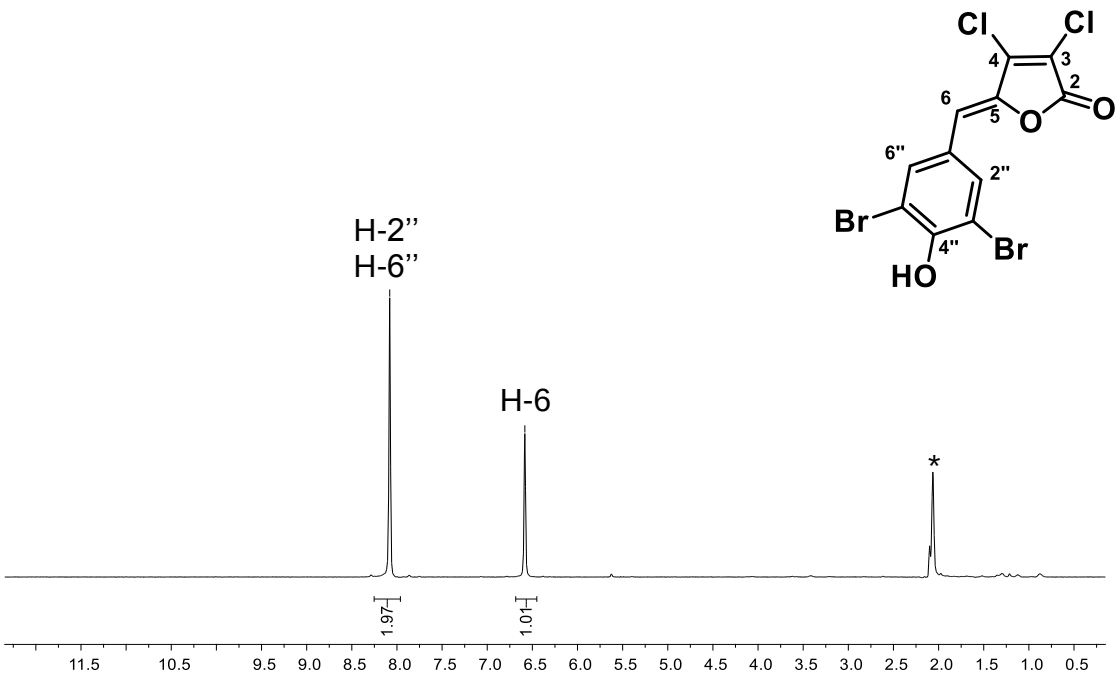



**Figure A 1.11**: $^{1}H$ NMR ($(CD_3)_2CO$, 400 MHz) of compound **81b.** *Solvent residues signals.

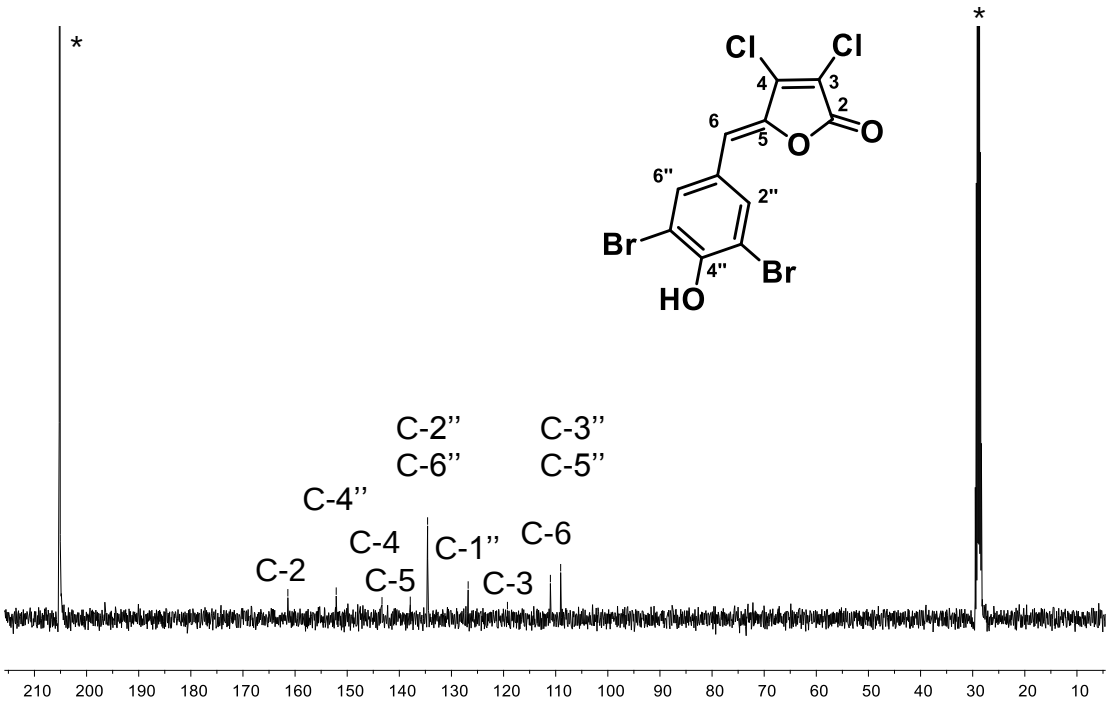



**Figure A 1.12**: $^{13}C$ NMR ($(CD_3)_2CO$, 100 MHz) of compound **81b.** *Solvent residues signals.

## 1.3. Spectra for the last steps for the synthesis of rubrolides B e K.

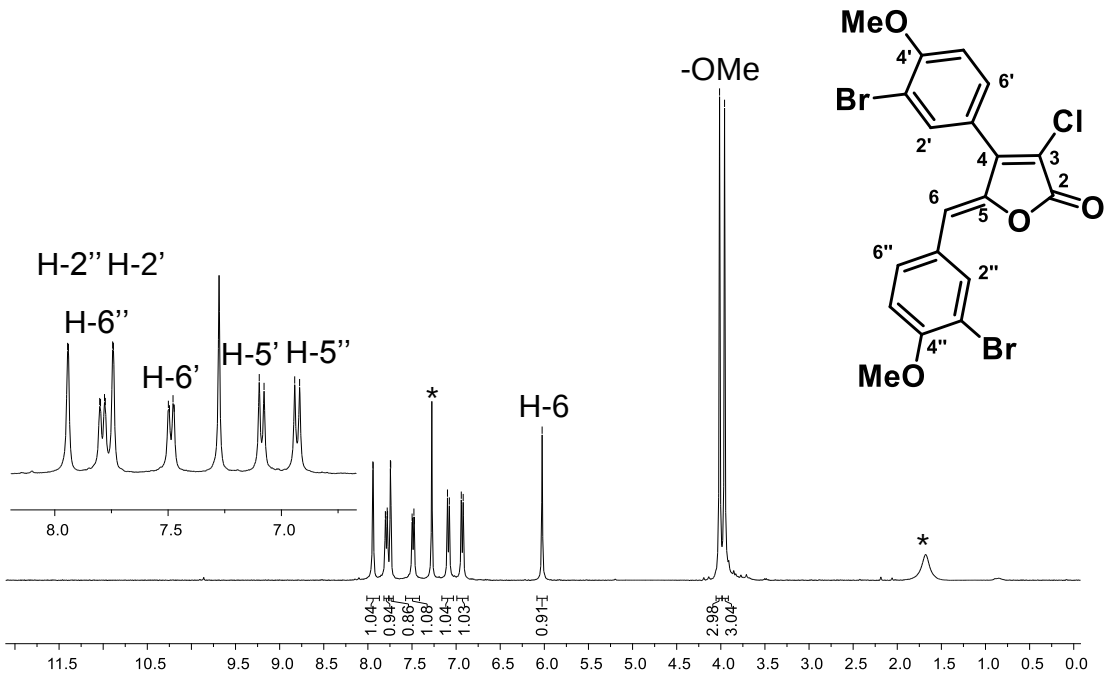



**Figure A 1.13**: $^{1}H$ NMR ($CDCl_3$, 400 MHz) of compound **76h**. *Solvent residues signals.

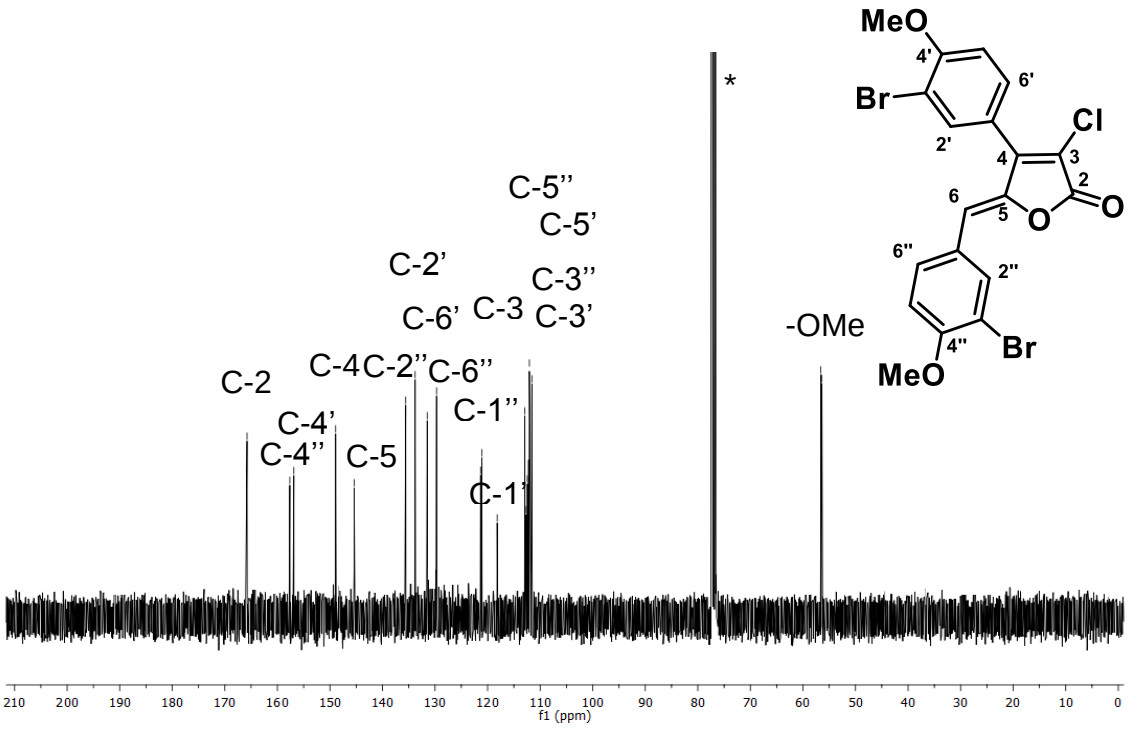



**Figure A 1.14**: $^{13}C$ NMR ($CDCl_3$, 100 MHz) of compound **76h**. *Solvent residues signals.

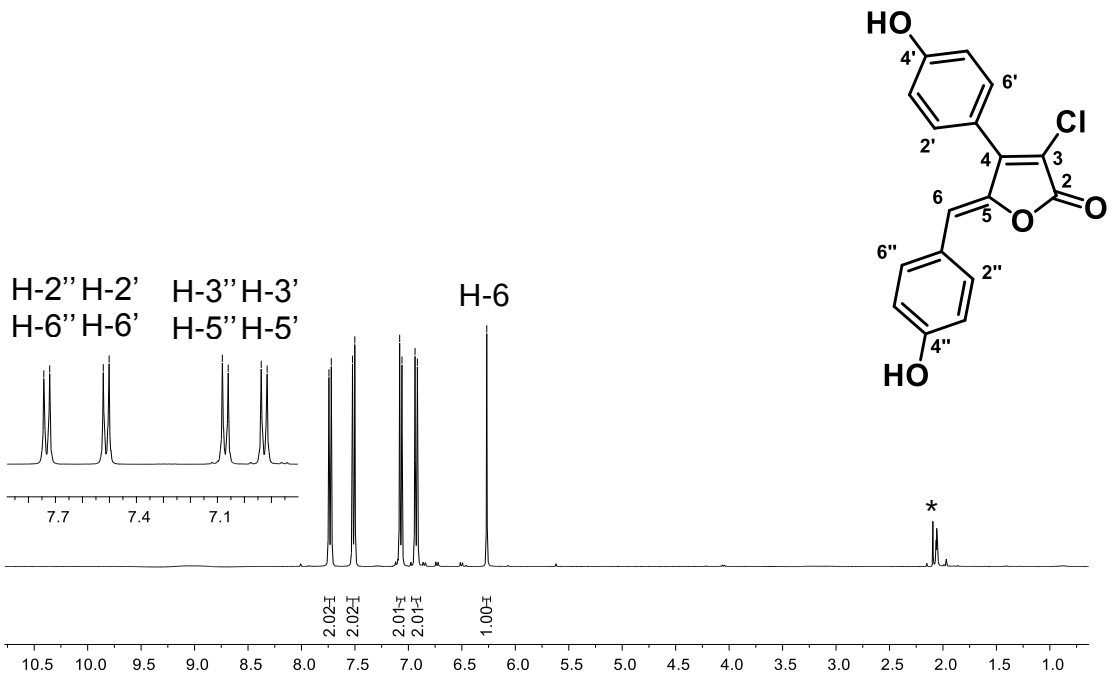



**Figure A 1.15**: $^{1}H$ NMR ($(CD_3)_2CO$, 400 MHz) of compound **76i.** *Solvent residues signals.

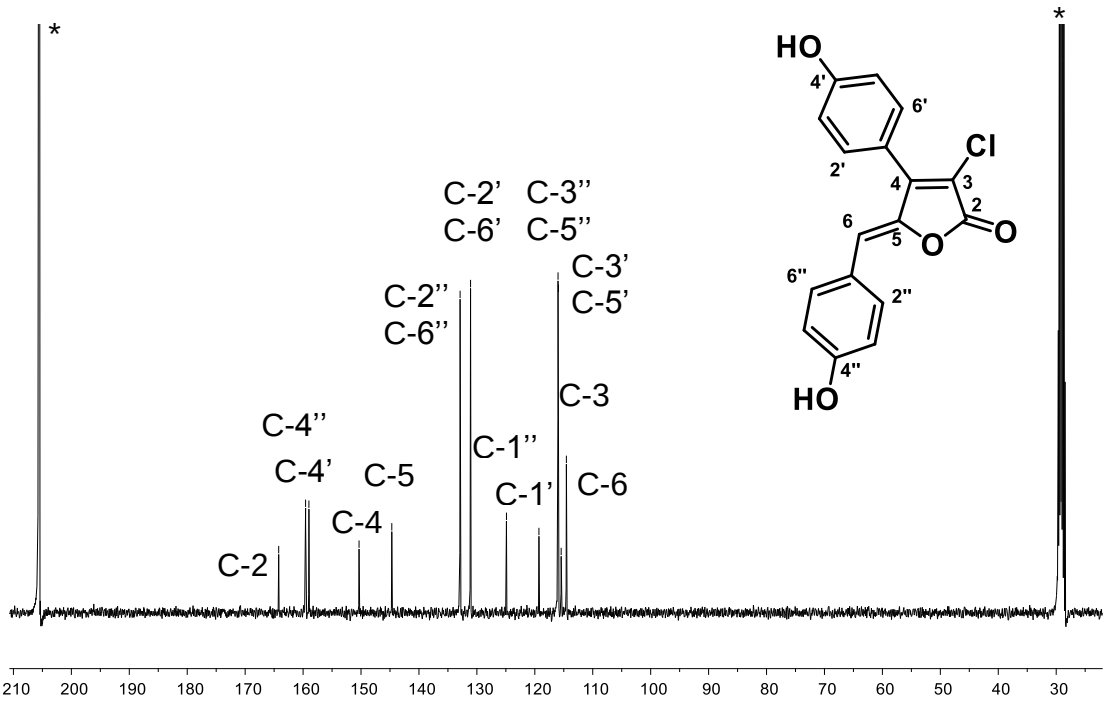



**Figure A 1.16**: $^{13}C$ NMR ($(CD_3)_2CO$, 100 MHz) of compound **76i.** *Solvent residues signals.

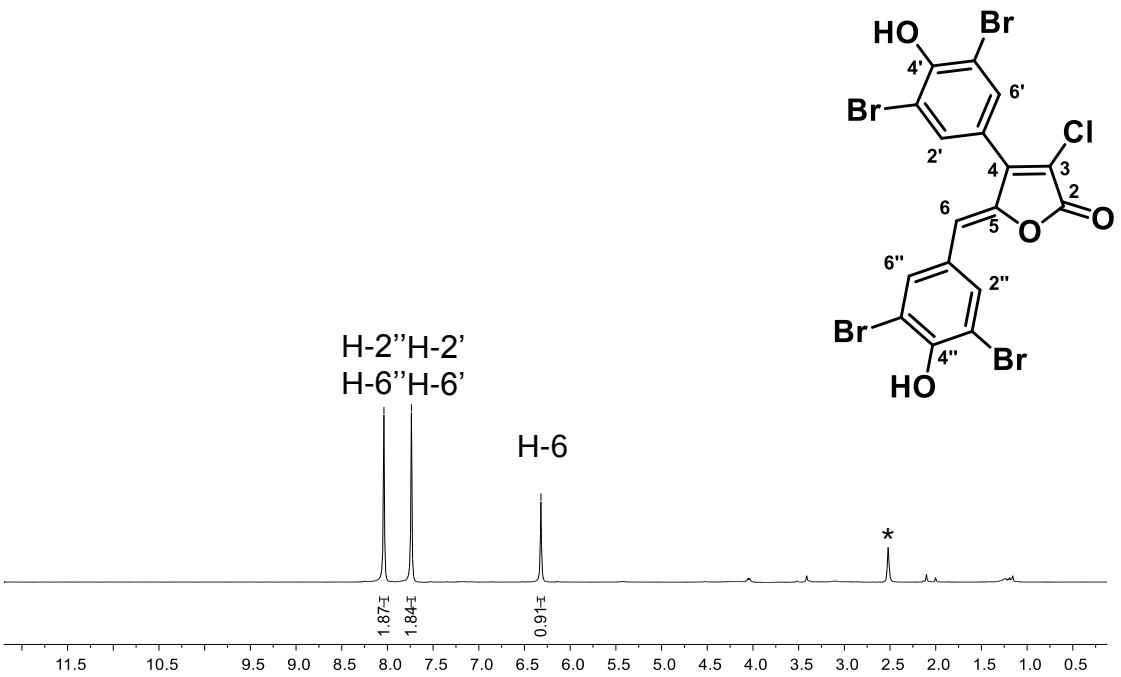



**Figure A 1.17**: $^{1}H$ NMR ($(CD_3)_2SO$, 400 MHz) of compound rubrolide B (**32**). *Solvent residues signals.

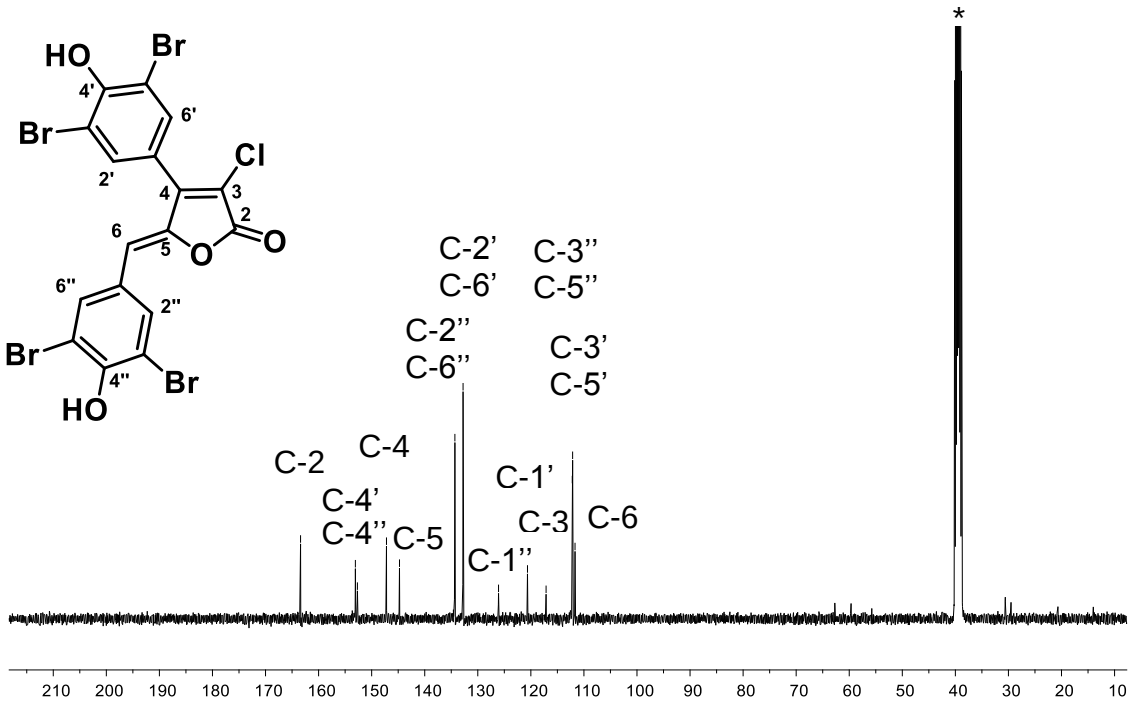



**Figure A 1.18**: $^{13}C$ NMR ($(CD_3)_2SO$, 100 MHz) of compound rubrolide B (**32**). *Solvent residues signals.

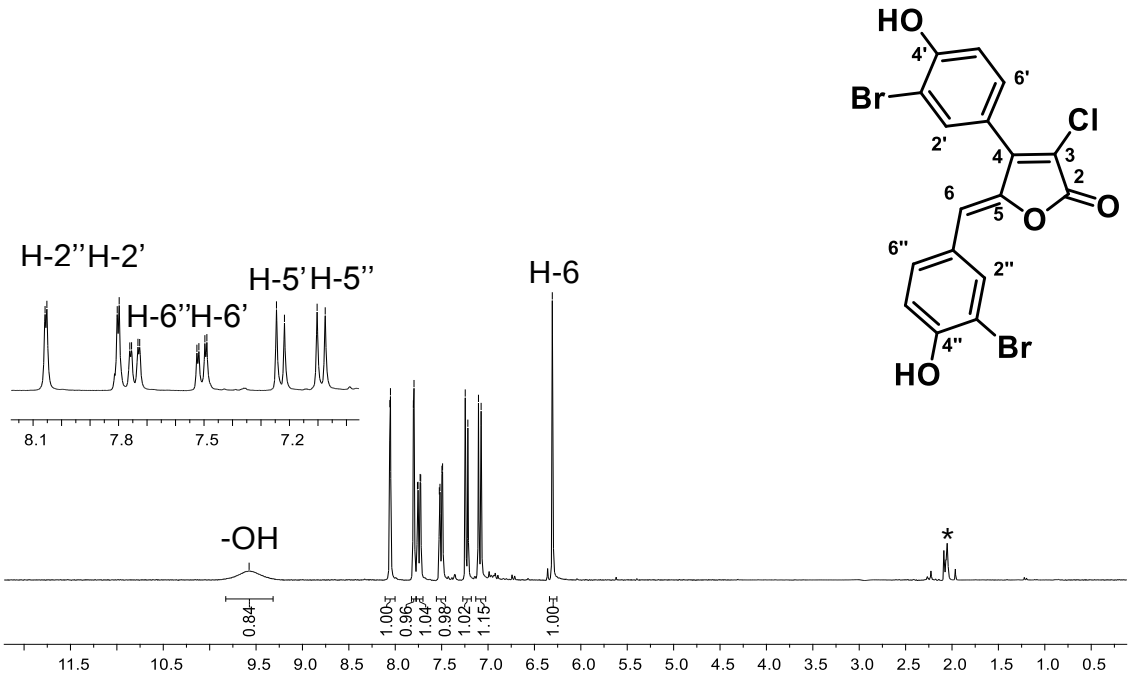



**Figure A 1.19**: $^{1}H$ NMR ($(CD_3)_2CO$, 400 MHz) of compound rubrolide K (**39**). *Solvent residues signals.

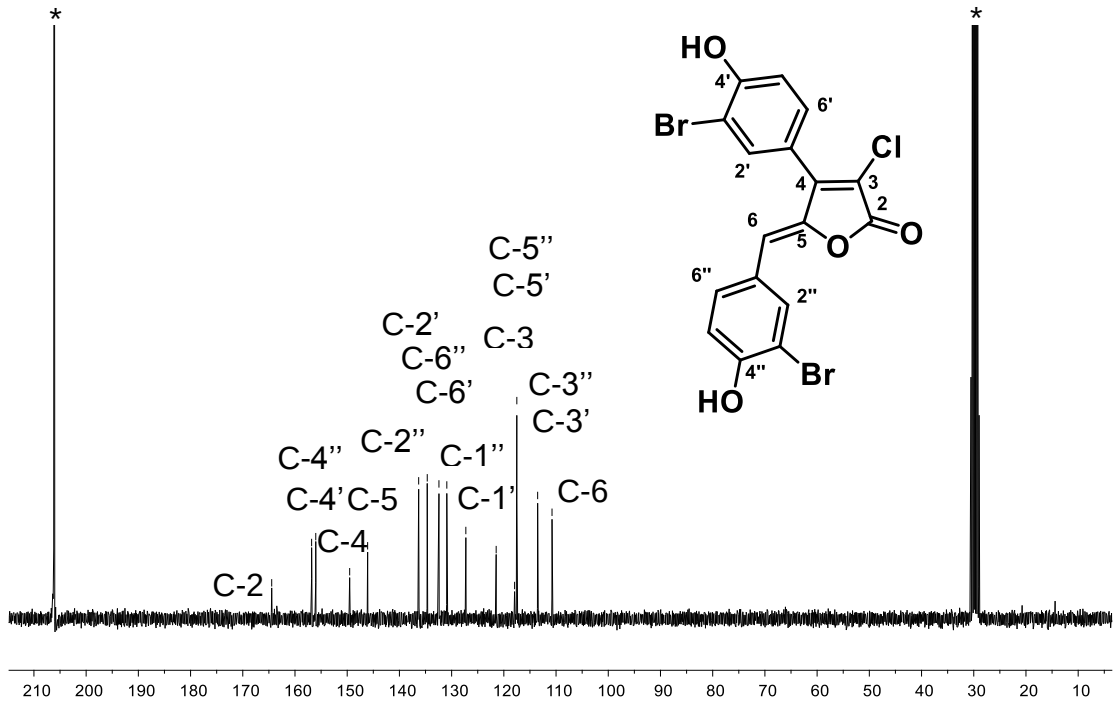



**Figure A 1.20**: $^{13}C$ NMR ($(CD_3)_2CO$, 100 MHz) of compound rubrolide K (**39**). *Solvent residues signals.

## 2. CHAPTER 2: Total Synthesis of Basidalin.

### 2.1. Spectra for the synthesis of intermediate γ-alkylidene butenolides 35.

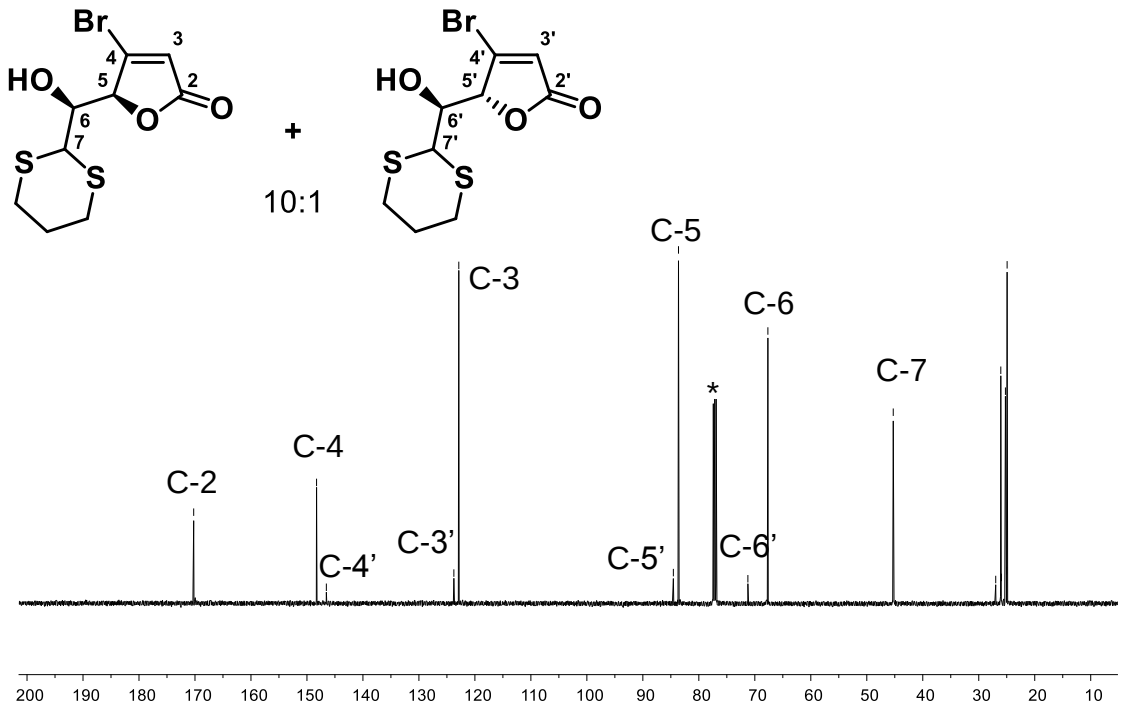
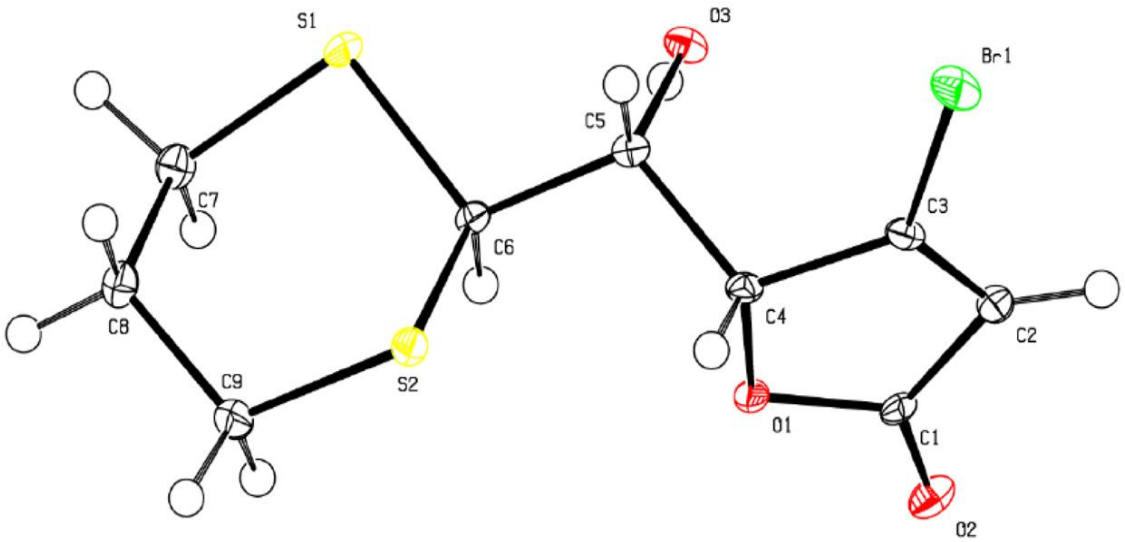



**Figure A 2.1**: $^{13}C$ NMR ($CDCl_3$, 100 MHz) of isomers ***syn*-39** e ***anti*-39** (10:1). *Solvent residues signal.




Crystallographic data for $C_9H_{11}BrO_3S_2$, MW = 311.21: monoclinic, Spatial group = $P2_1/c$, a = 13.1126(11), b = 6.5684(6), c = 13.1785(11) Å, β = 90.335(2)°, V = 1135.03(17) $Å^3$, Z = 4, T = 100 K, μ = 5.562 $mm^{-1}$, ρ = 1.821 $g/cm^3$, Collected reflections = 21320, unique = 2536, $R_1$ = 0.0272, $wR_2$ = 0.0700.

**Figure A 2.2**: Structural determination for the crystal of compound ***syn*-39**. ORTEP diagram, ellipsoids determined at the probability level of 50%.

**Tabela A 2.1**: Crystallographic daofs and crystal structure refinement for ***syn*-39**

| | |
|---|---|
| Empiric formula | $C_9H_{11}BrO_3S_2$ |
| Temperature (K) | 100(2) |
| Wave lenght (Å) | 1,34139 |
| Crystallin system | Monoclinic |
| Spatial group | $P2_1/c$ |
| Unit Cell Dimensions (Å) | a = 13.1126(11); $\alpha$ = 90°; b = 6.5684(6); β = 90.335(2)°; c = 13.1785(11); $\gamma$ = 90° |
| Volume $Å^3$ | 1135,03(17) |
| Z | 4 |
| Density (calculada) ($g/cm^3$) | 1,821 |
| Absorption coefficient ($mm^{-1}$) | 5,562 |
| F (000) | 624,0 |
| Crystal size ($mm^3$) | 0,36 × 0,33 × 0,21 |
| 2θ range for data collection | 11.684 - 121.236° |
| Index ranges | -17 ≤ h ≤ 17; -8 ≤ k ≤ 8; -16 ≤ l ≤ 17 |
| Reflections collected | 21320 |
| Independent reflections | 2536 [R(int) = 0,0450; R(sigma) = 0,0283] |
| Transmitions max. and min. | 0,752 e 0,511 |
| Data / restrictions / parameters | 2536/0/140 |
| Adjustment quality on F2 | 1,193 |
| Final index R [I ≥ 2σ(I)] | $R_1$ = 0,0272; $wR_2$ = 0,0700 |
| Greater diff. peak and aperture.($e.Å^{-3}$) | 0,59 e -0.82 |

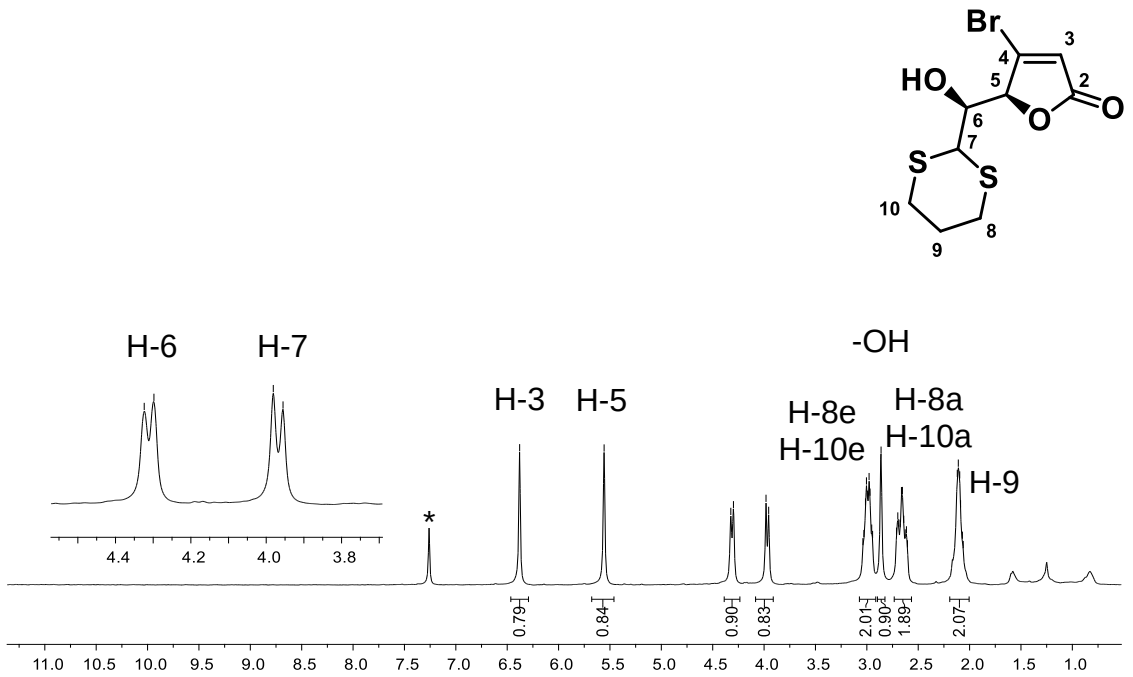



**Figure A 2.3:** $^{1}$H NMR ($CDCl_3$, 400 MHz) of alcohol ***syn*-39**. *Solvent residues signal.

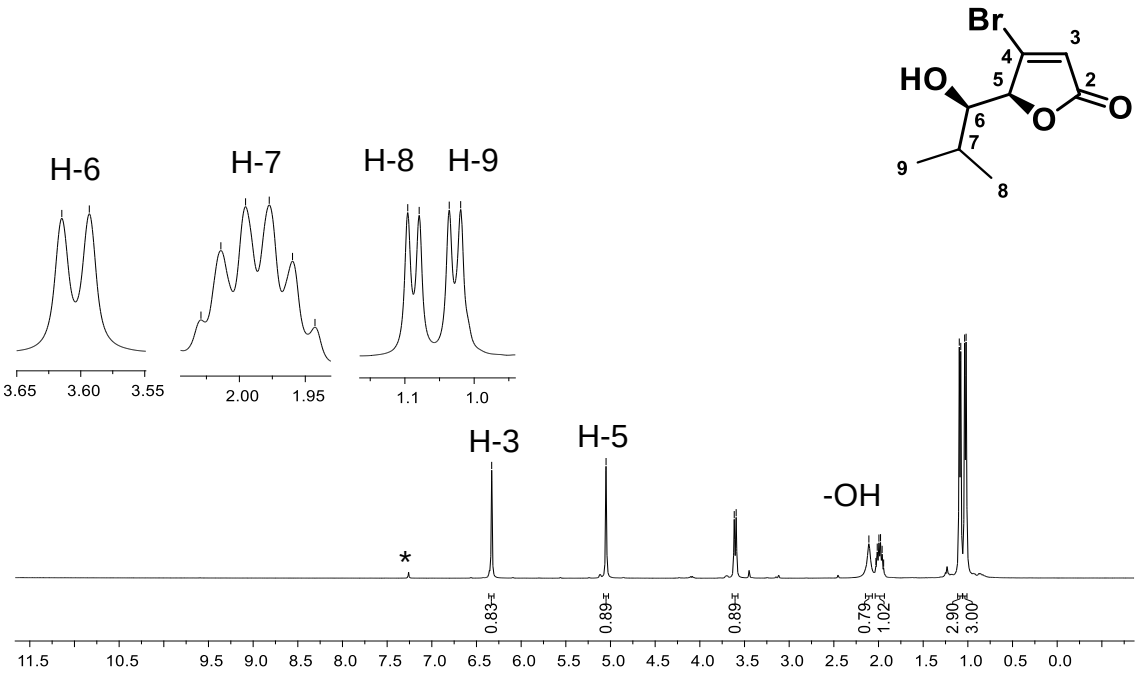



**Figure A 2.4:** $^{1}$H NMR ($CDCl_3$, 400 MHz) of alcohol ***syn*-41**. *Solvent residues signal.

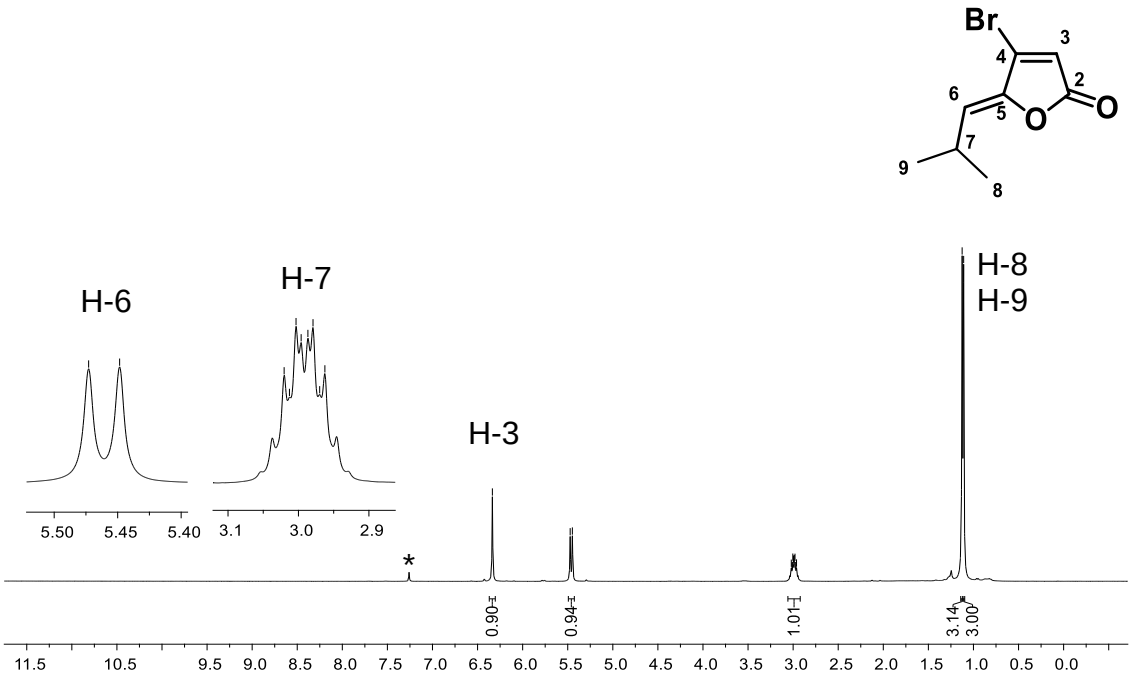



**Figure A 2.5:** $^{1}H$ NMR ($CDCl_3$, 400 MHz) of compound ***Z*-42.** *Solvent residues signal.

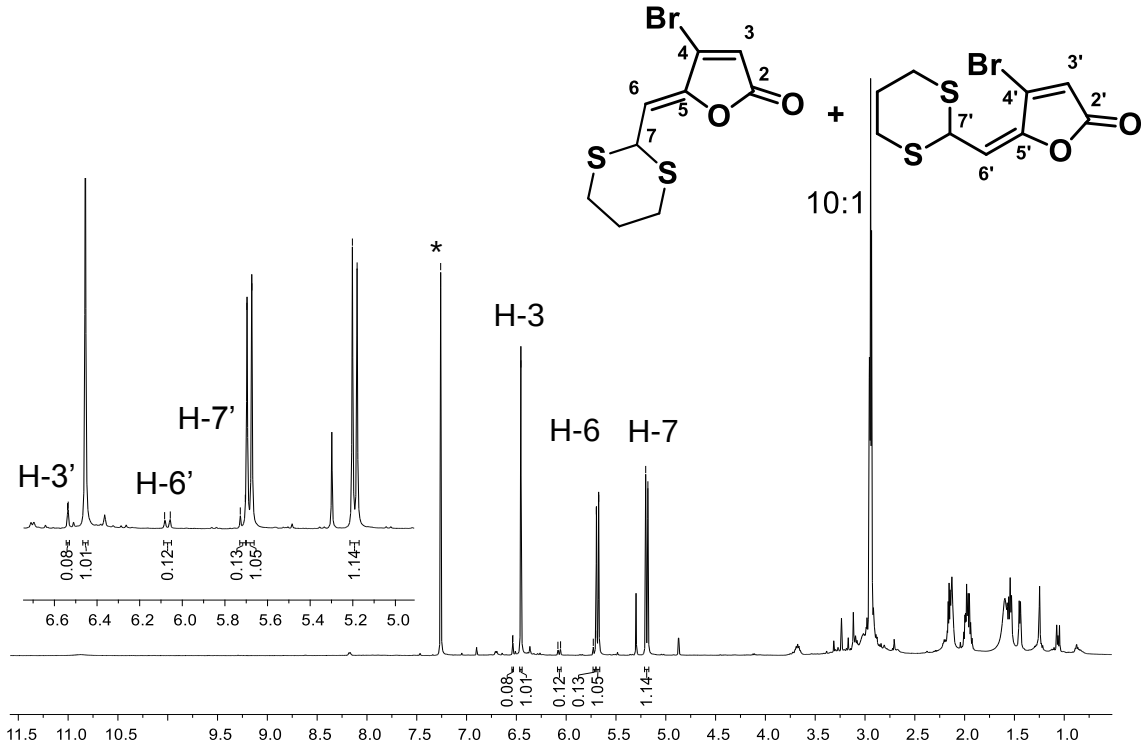



**Figure A 2.6:** $^{1}H$ NMR ($CDCl_3$, 400 MHz) of isomers ***Z*-35** e ***E*-35** (10:1). *Solvent residues signal.

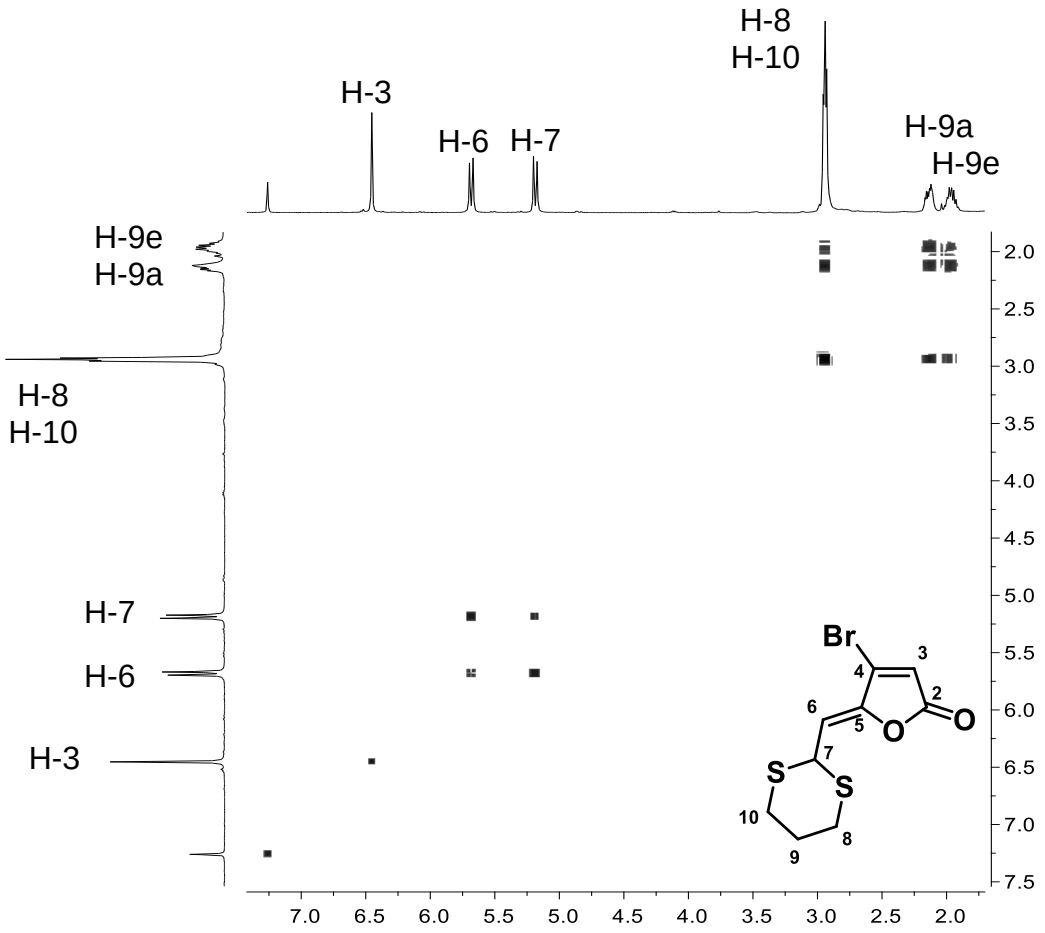



**Figure A 2.7**: COSY ($CDCl_3$, 400 MHz) of compound ***Z*-35**.

## 2.2. Spectra corresponding to the final stages of basidalin synthesis.

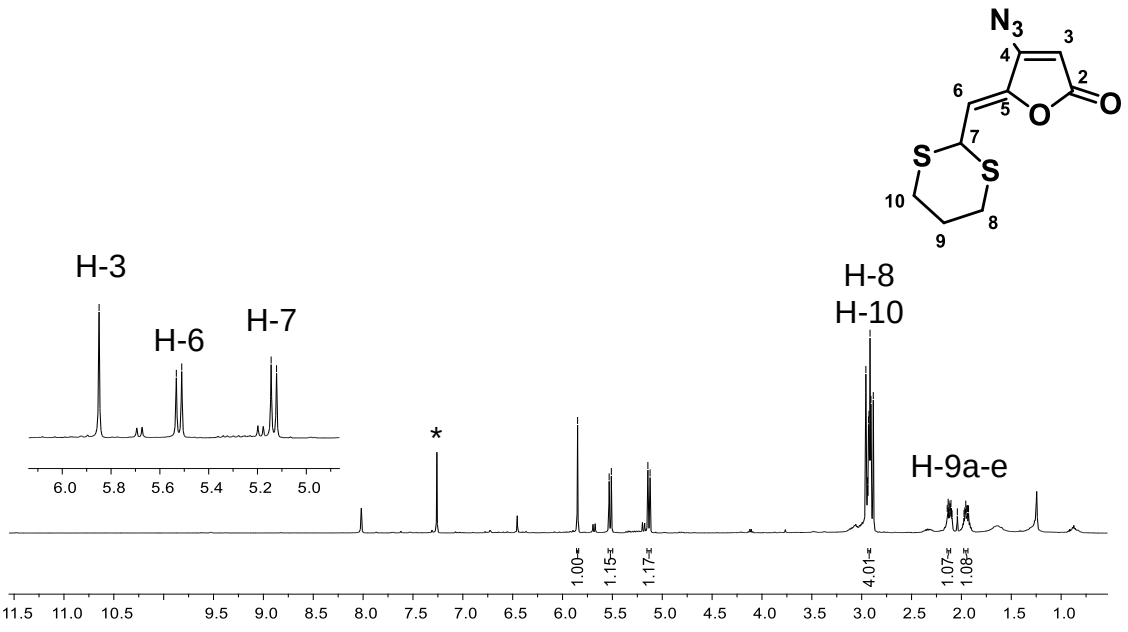



**Figure A 2.8:** $^1H$ NMR ($CDCl_3$, 400 MHz) of crude azide **43**. *Solvent residues signal.

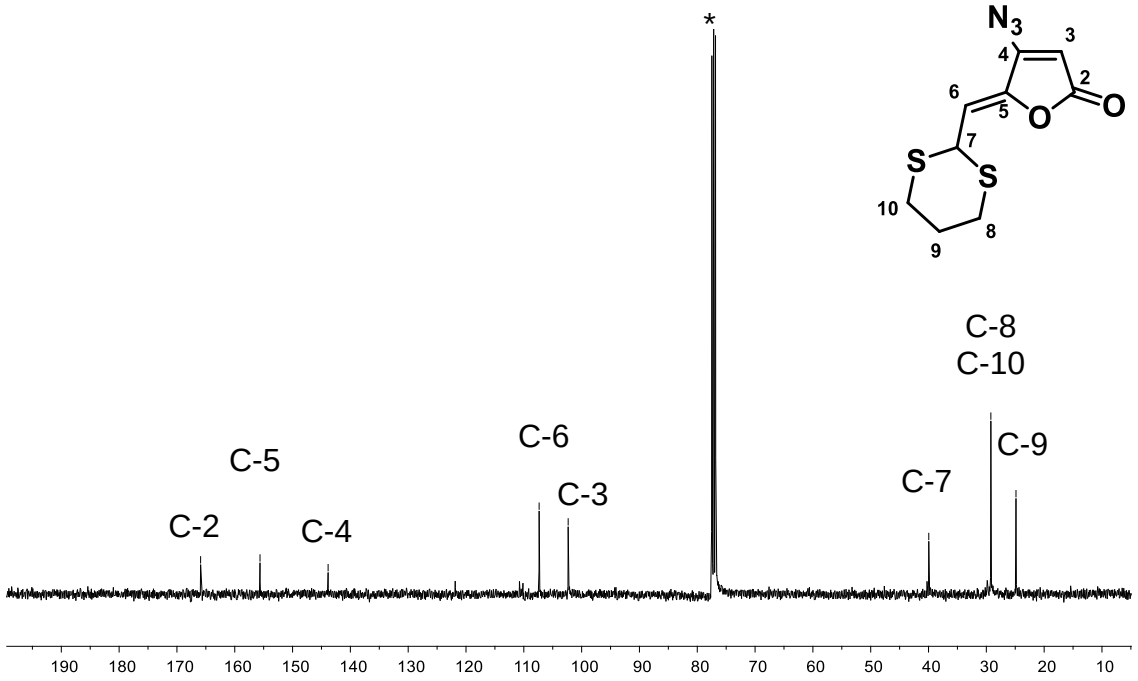



**Figure A 2.9:** $^{13}C$ NMR ($CDCl_3$, 125 MHz) of crude azide **43**. *Solvent residues signal.

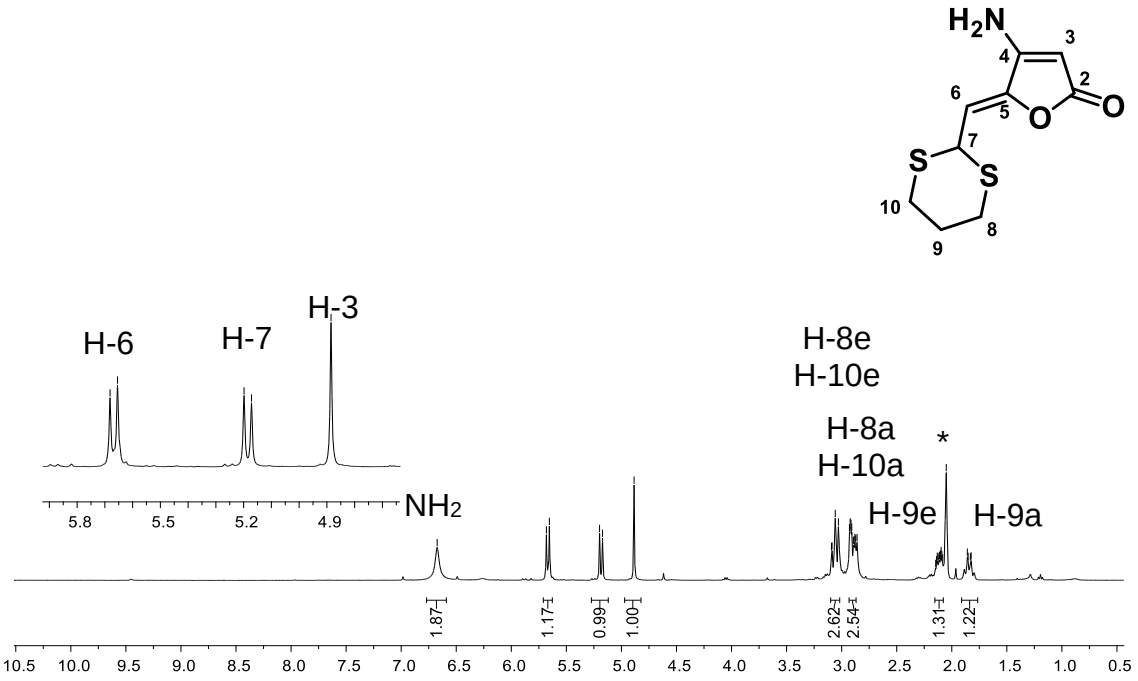



**Figure A 2.10:** $^{1}H$ NMR ($(CD_3)_2CO$, 400 MHz) of tetronamide **36**. *Solvent residues signal.

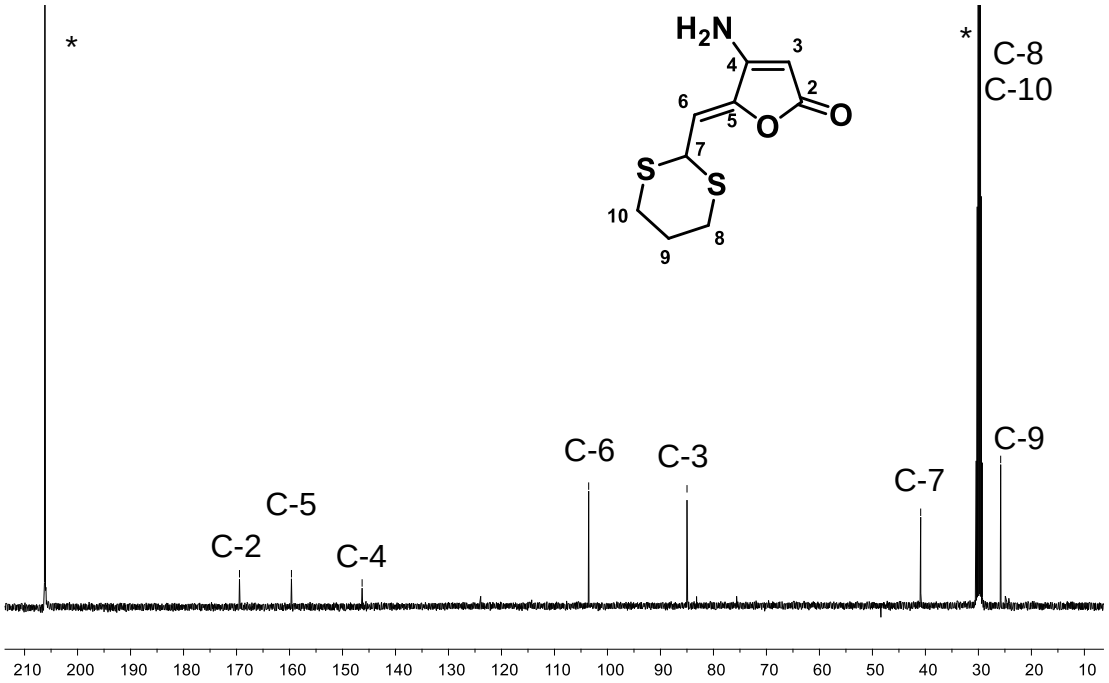



**Figure A 2.11:** $^{13}C$ NMR ($(CD_3)_2CO$, 100 MHz) of tetronamide **36**. *Solvent residues signal.

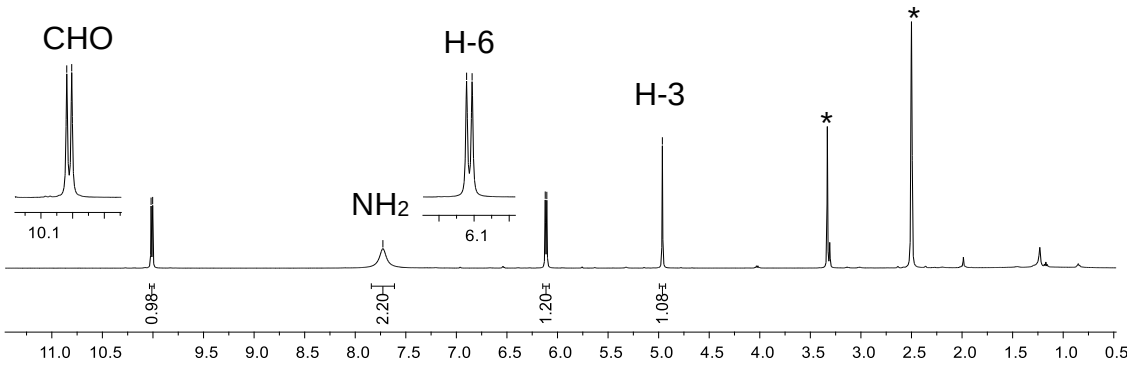



**Figure A 2.12**: $^{1}H$ NMR ($(CD_3)_2SO$, 500 MHz) of basidalin (**1a**)**.** *Solvent and water residues signal.

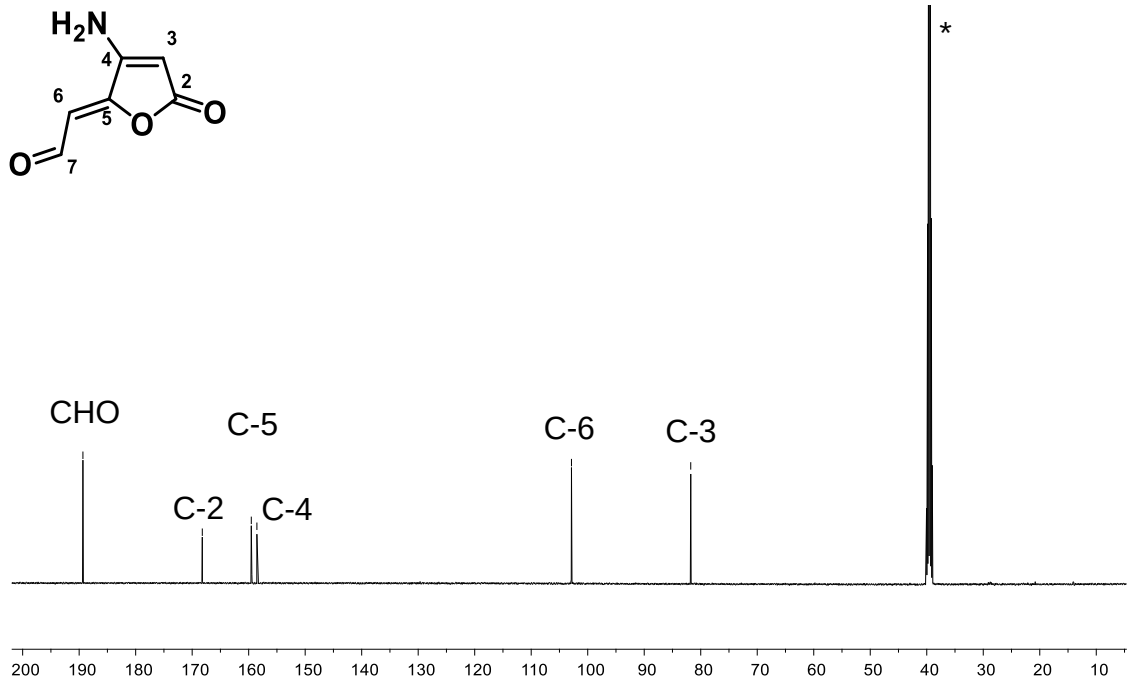



**Figure A 2.13**: $^{13}C$ NMR ($(CD_3)_2SO$, 125 MHz) of basidalin (**1a**). *Solvent residues signal.

# 3. CHAPTER 3: Total synthesis of enhygrolide A.

## 3.1. Spectra for the final steps of synthesis of enhygrolide A and B.

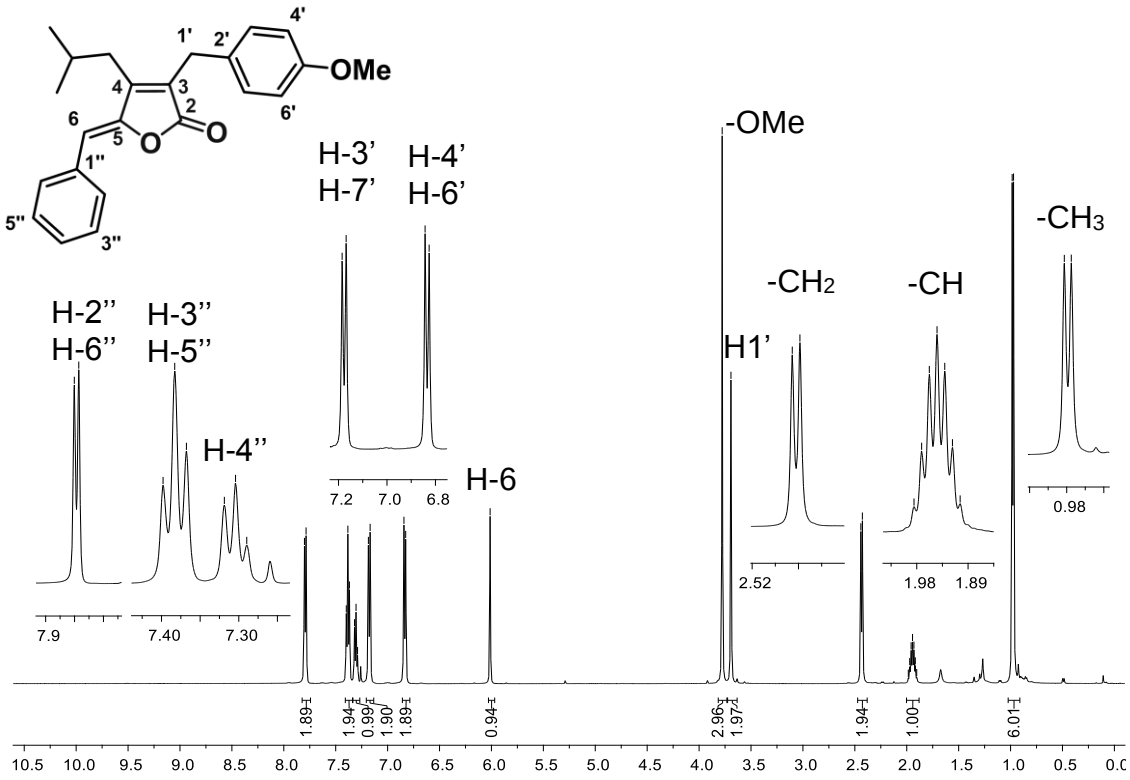



**Figure A 3.1:** $^{1}H$ NMR ($CDCl_3$, 400 MHz) of butenolide **3a**.

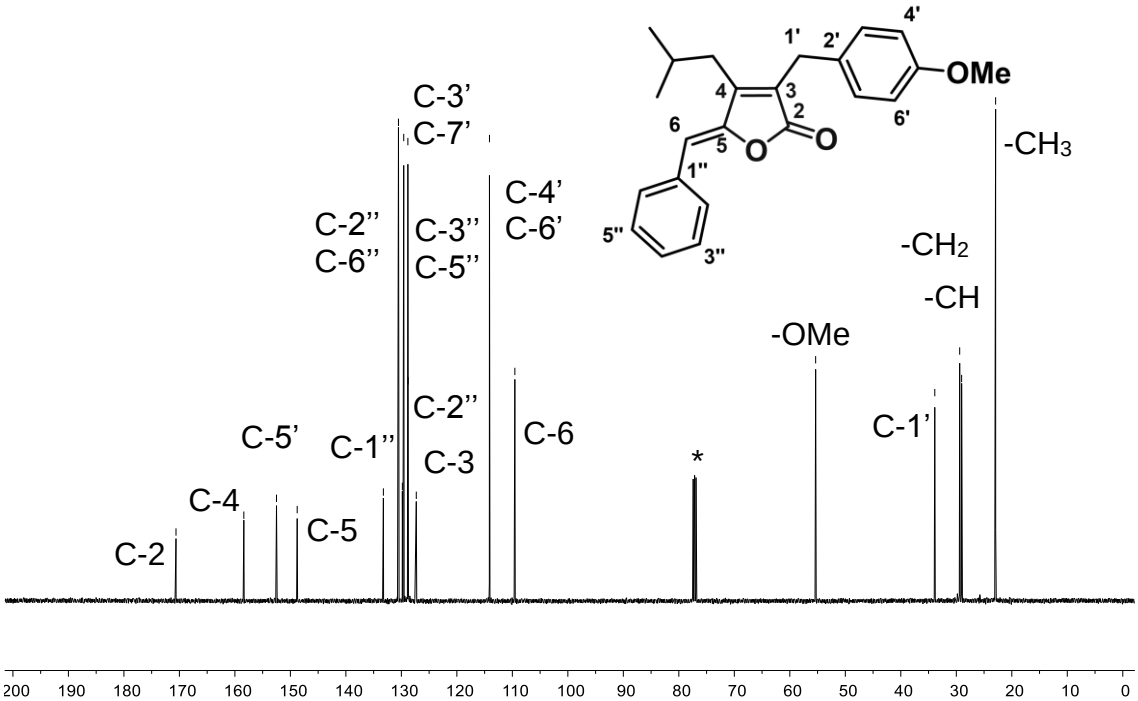



**Figure A 3.2:** $^{13}C$ NMR ($CDCl_3$, 100 MHz) of butenolide **3a**. *Solvent residues signal.

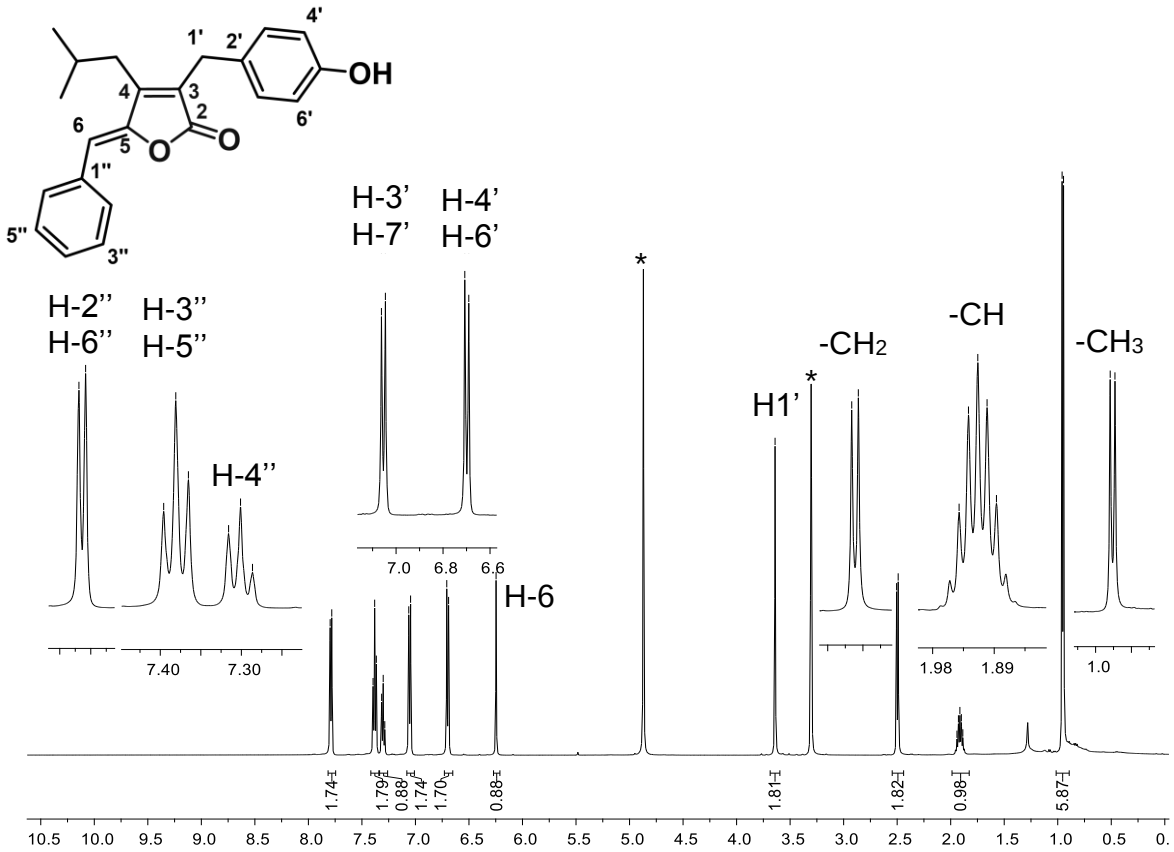



**Figure A 3.3:** $^{1}H$ NMR ($CD_3OD$, 400 MHz) of enhygrolide A **3**. *Solvent residues and water signals.

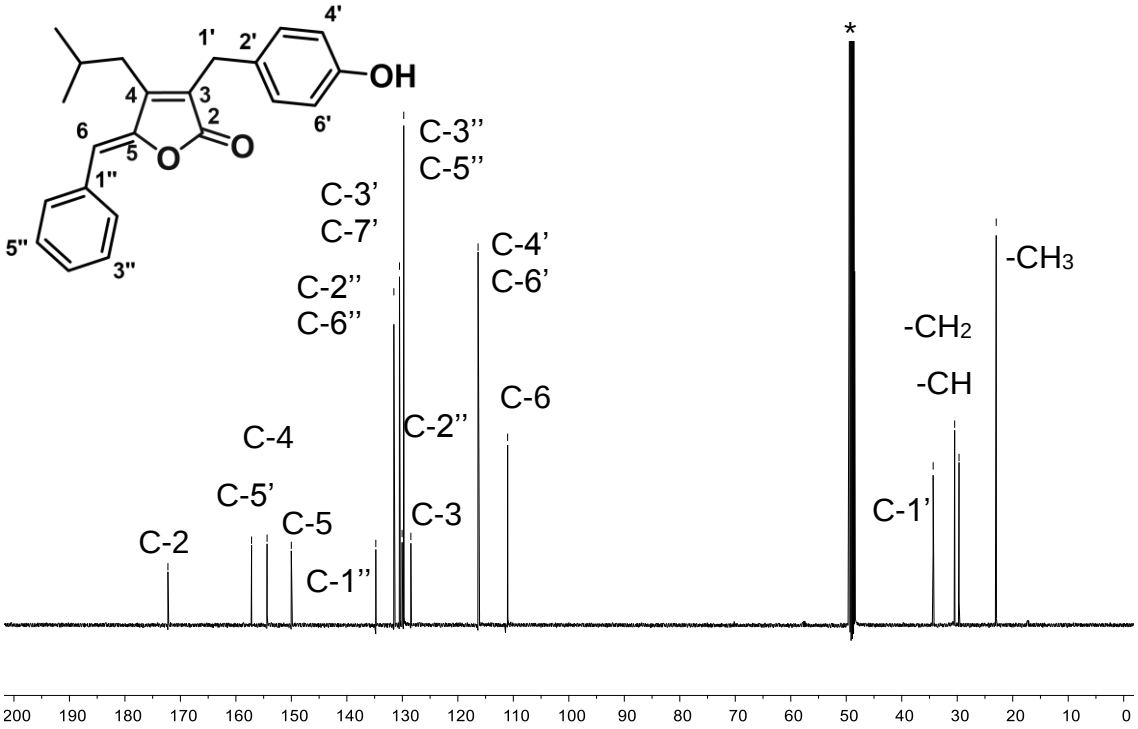



**Figure A 3.4:** $^{13}C$ NMR ($CD_3OD$, 100 MHz) of enhygrolide A **3**. *Solvent residues and water signals.

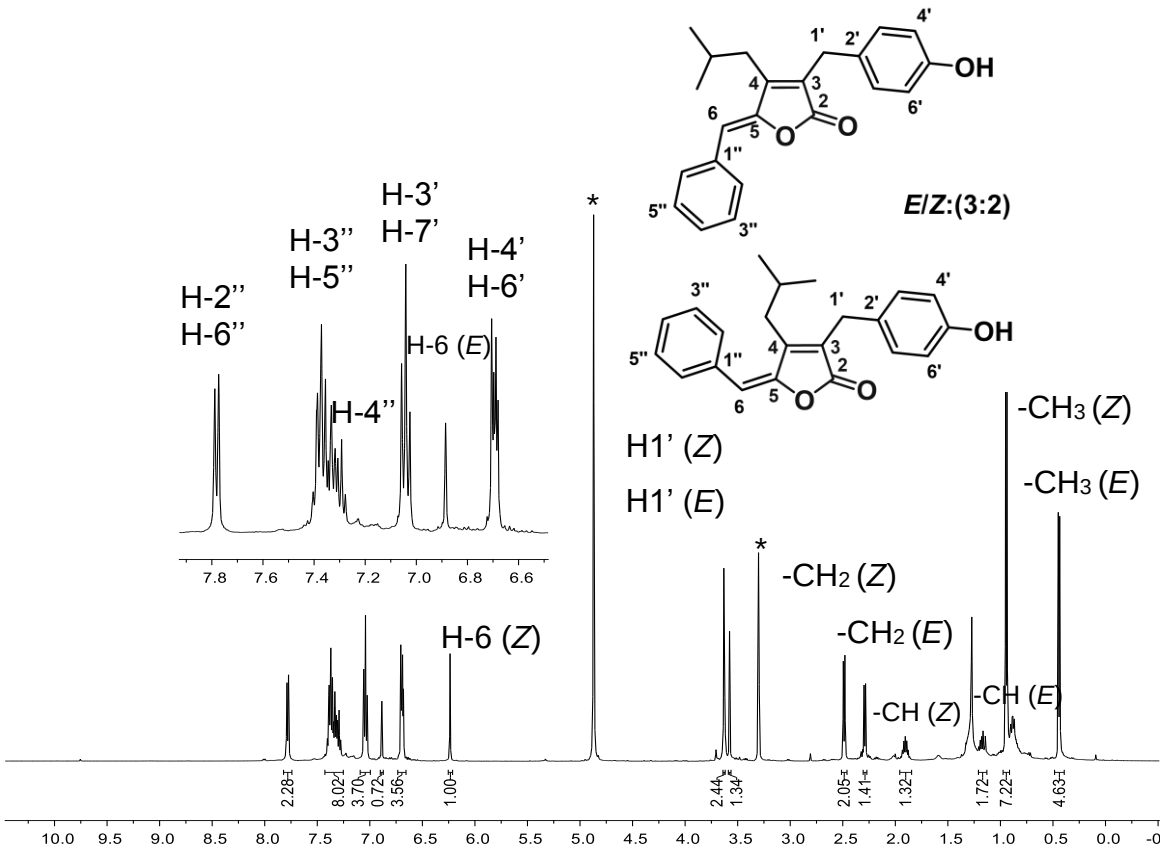



**Figure A 3.5:** $^{1}H$ NMR ($CD_3OD$, 400 MHz) of enhygrolide A and B (3:2). *Solvent residues and water signals.

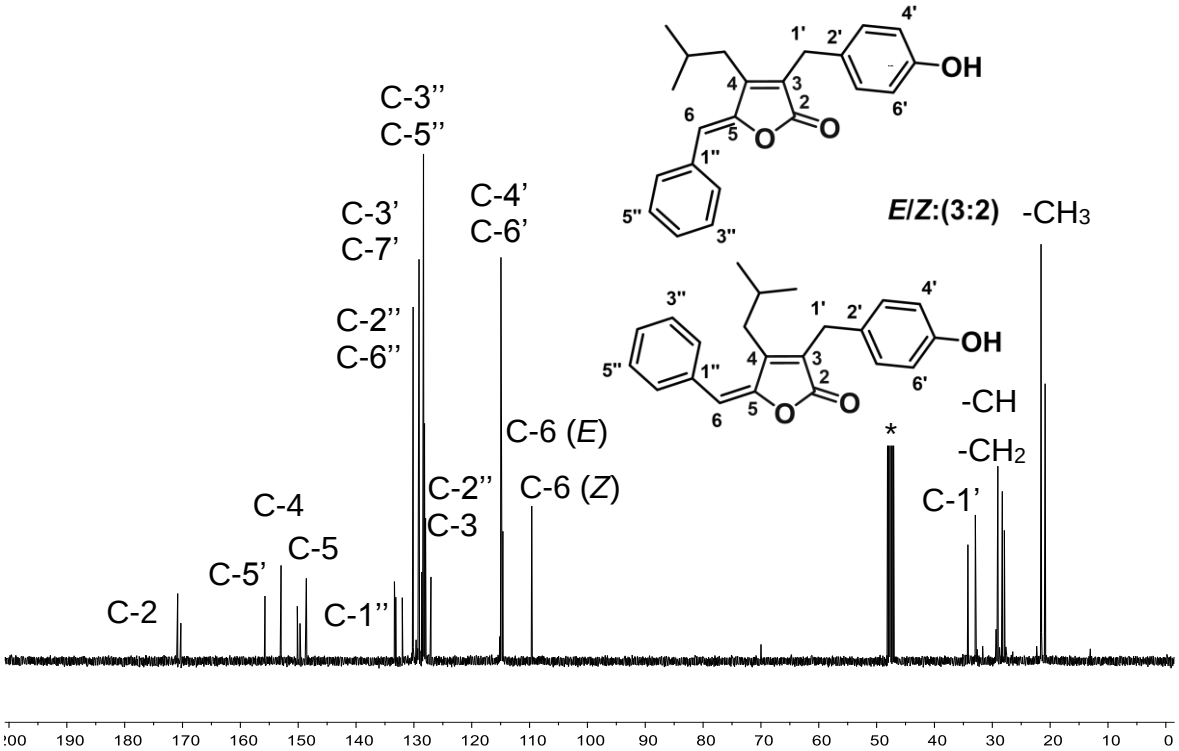



**Figure A 3.6:** $^{13}C$ NMR ($CD_3OD$, 100 MHz) of enhygrolide A and B (3:2). *Solvent residues signal.

# 4. CHAPTER 4: Synthesis of tetronamide analogs with cyanobactericide activity.

## 4.1. Selected spectra for the synthesis of basic tetronamides 1.

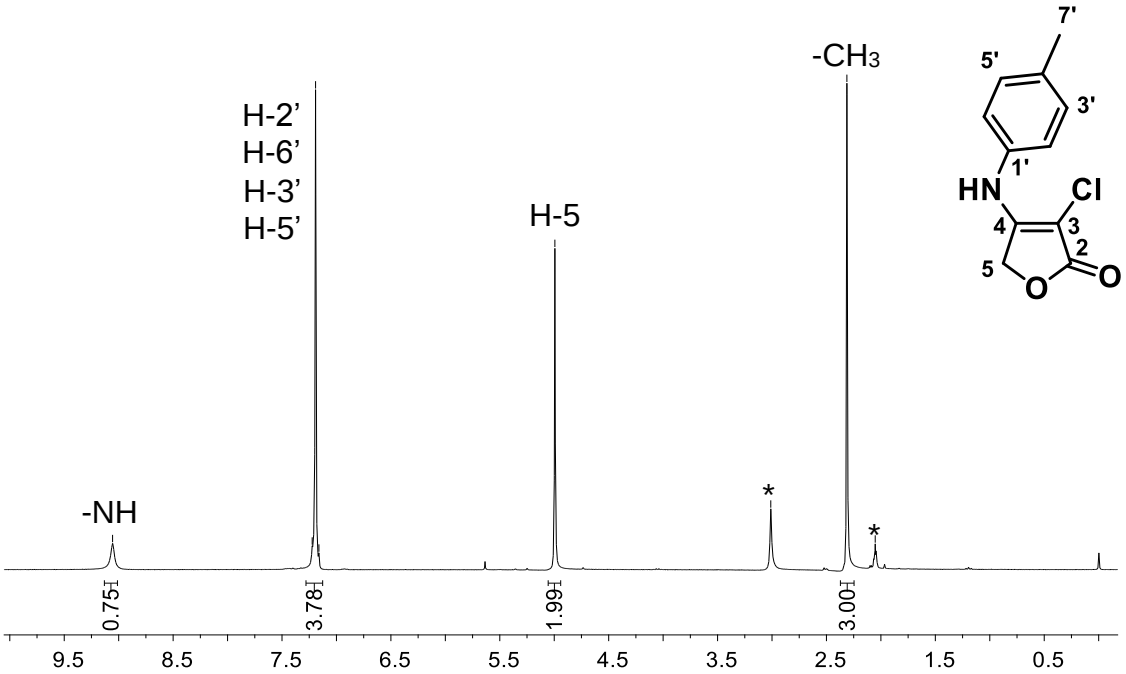



**Figure A 4.1:** $^{1}$H NMR (300 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$; (9:1)) of compound **1a**. *Solvent residues and water signals.

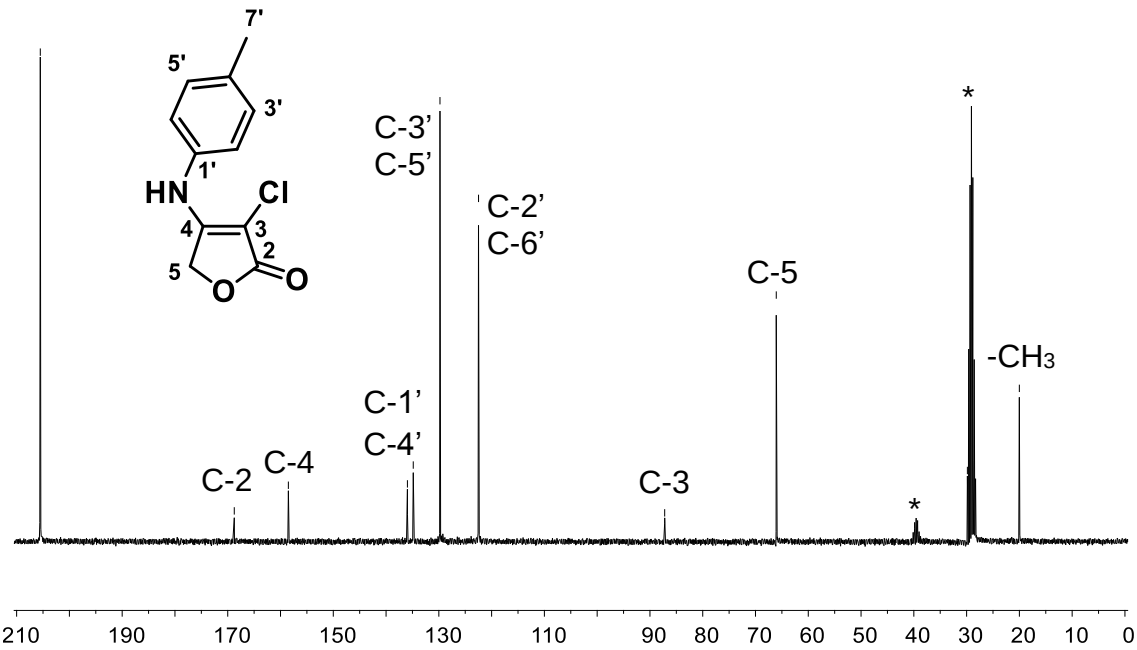



**Figure A 4.2:** $^{13}$C NMR (100 MHz, $(CD_3)_2CO$:$(CD_3)_2SO$; (9:1)) of compound **1a**. *Solvent residues signals.

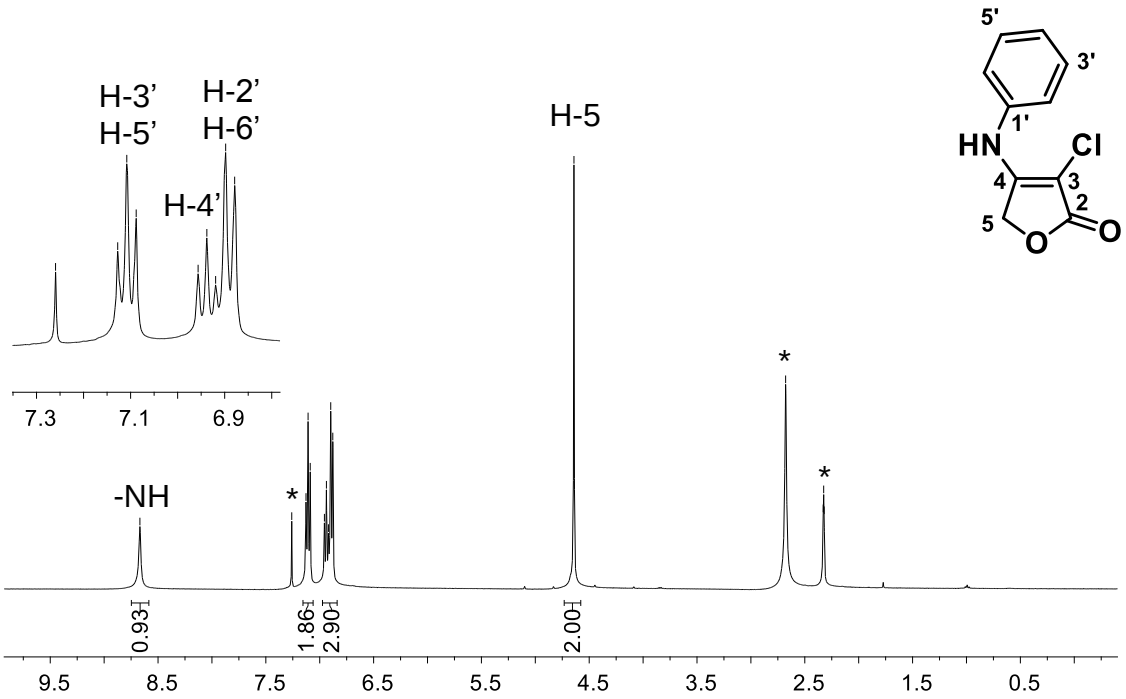



**Figure A 4.3:** $^{1}H$ NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1)) of compound **1b**. *Solvent residues and water signals.

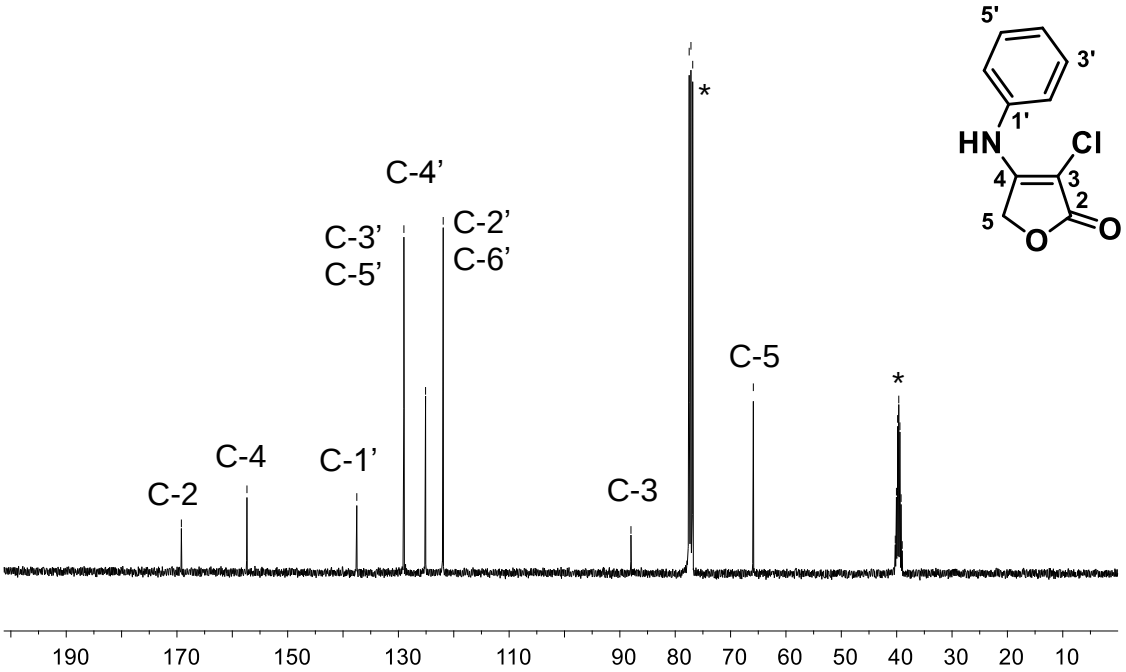



**Figure A 4.4:** $^{13}C$ NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1)) of compound **1b**. *Solvent residues signals.

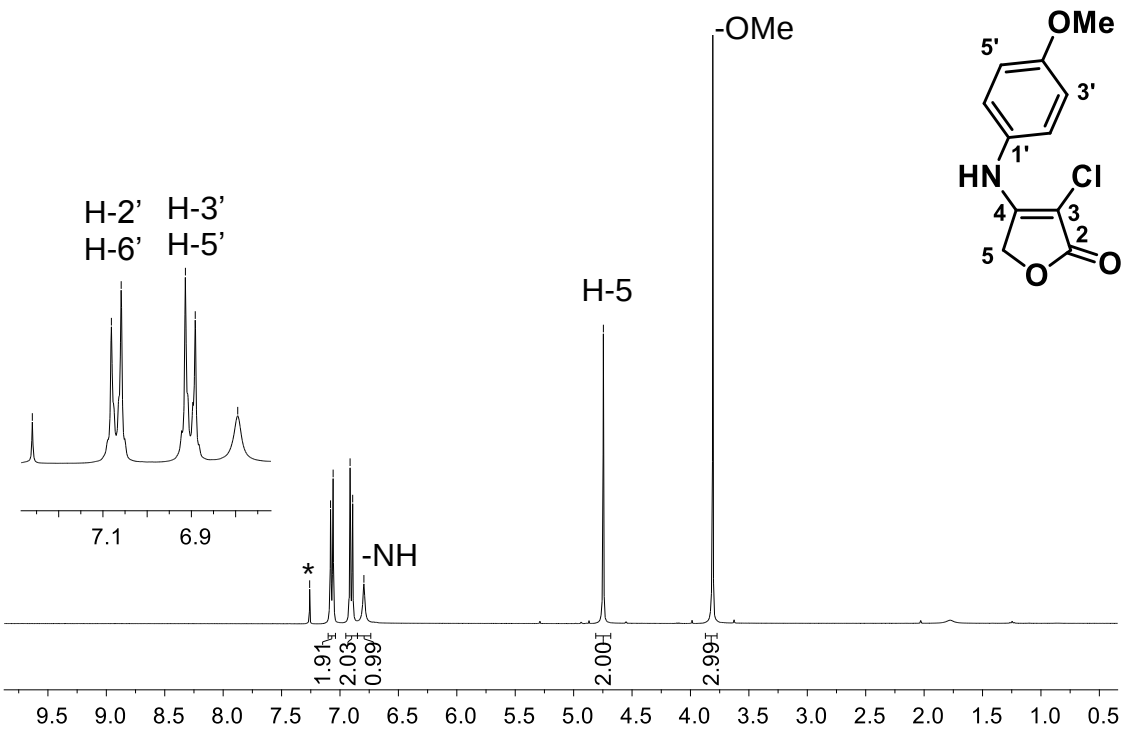



**Figure A 4.5:** $^{1}H$ NMR (400 MHz, $CDCl_3$) of compound **1c**. *Solvent residues and water signals.

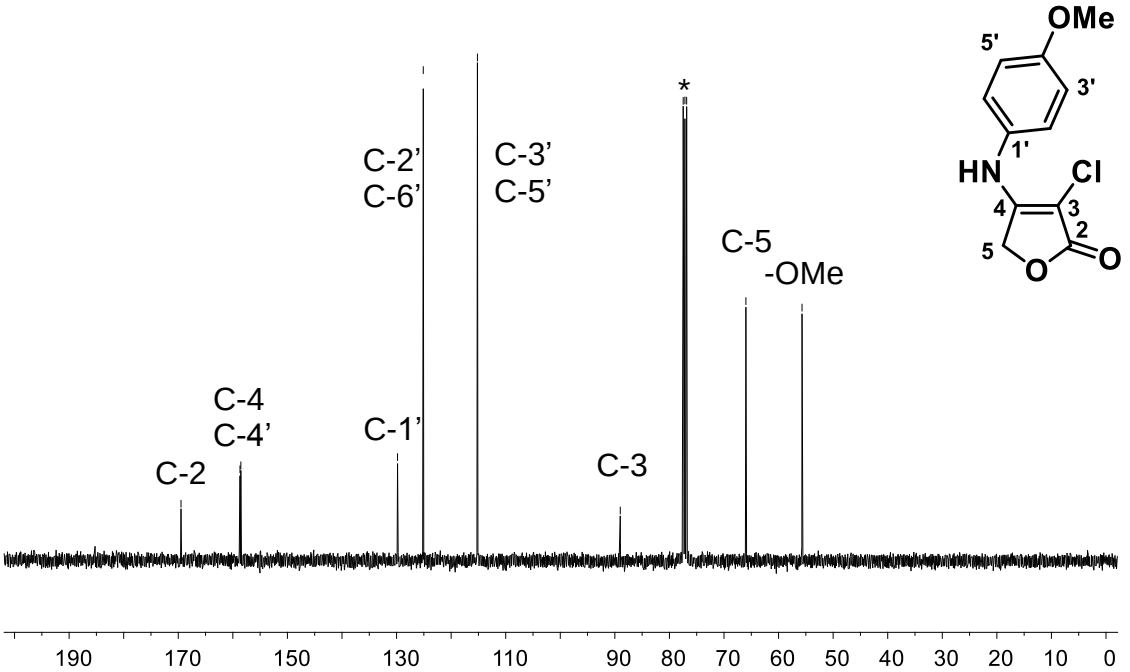



**Figure A 4.6:** $^{13}C$ NMR (100 MHz, $CDCl_3$) of compound **1c**. *Solvent residues signals.

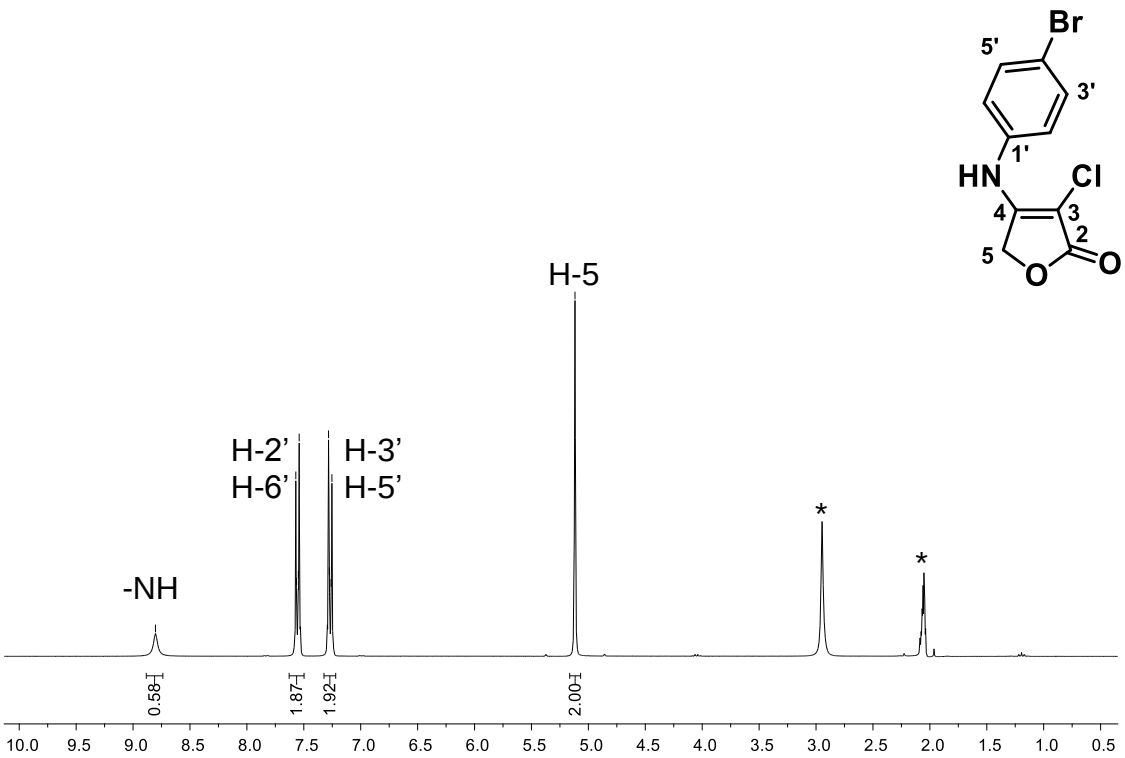



**Figure A 4.7:** $^{1}$H NMR (400 MHz, $(CD_3)_2SO$) of compound **1d**. *Solvent residues and water signals.

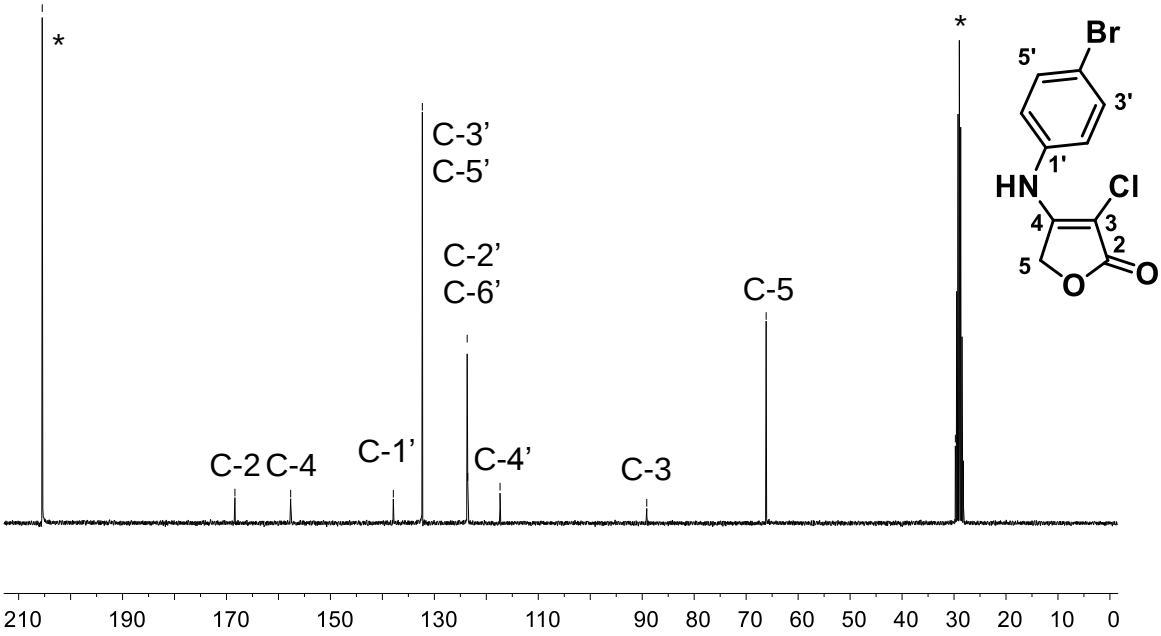



**Figure A 4.8:** $^{13}$C NMR (100 MHz, $(CD_3)_2SO$) of compound **1d**. *Solvent residues signals.

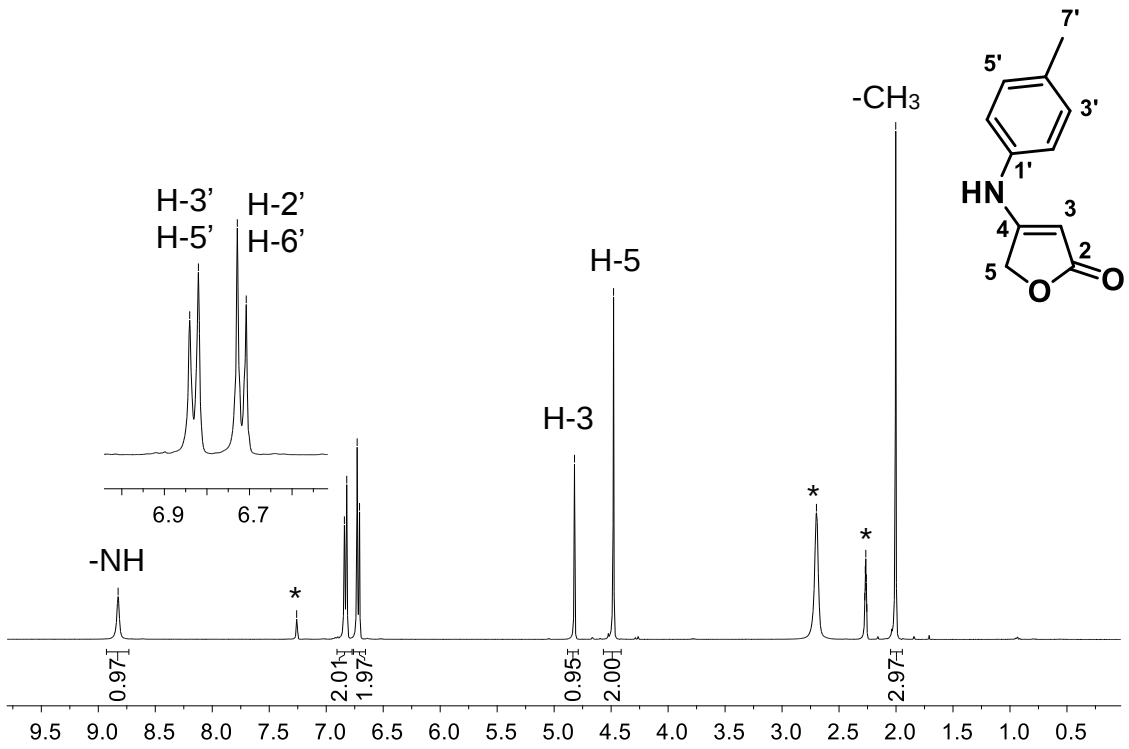



**Figure A 4.9:** $^{1}H$ NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1)) of compound **1i**. *Solvent residues and water signals.

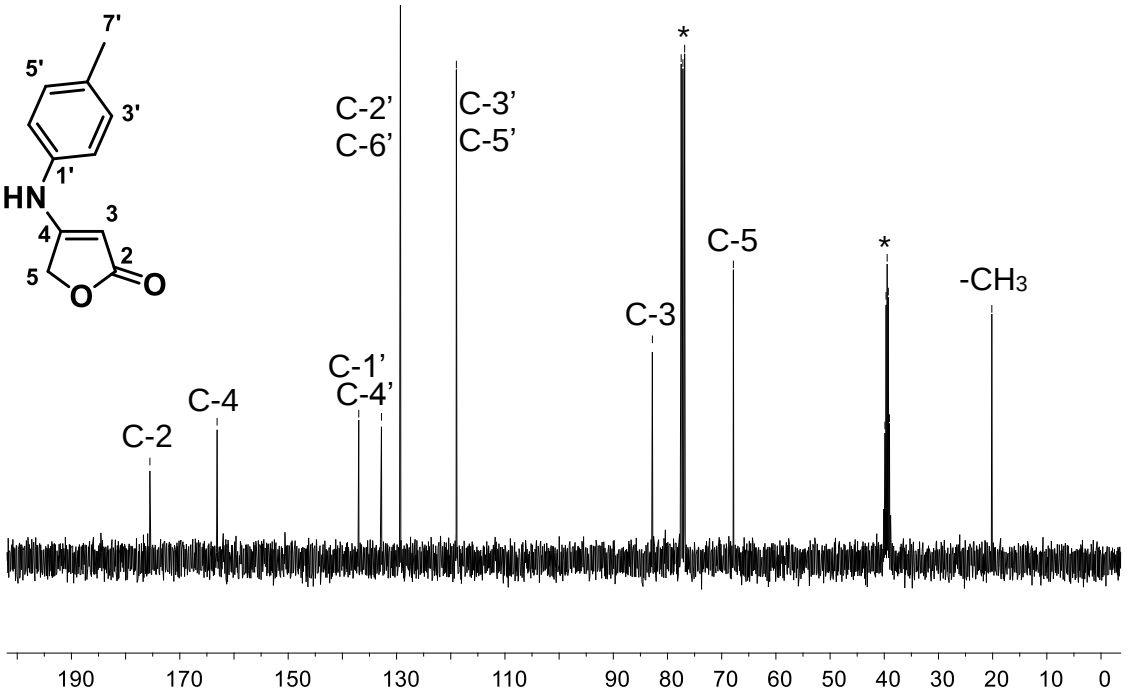



**Figure A 4.10:** $^{13}C$ NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1)) of compound **1i**. *Solvent residues signals.

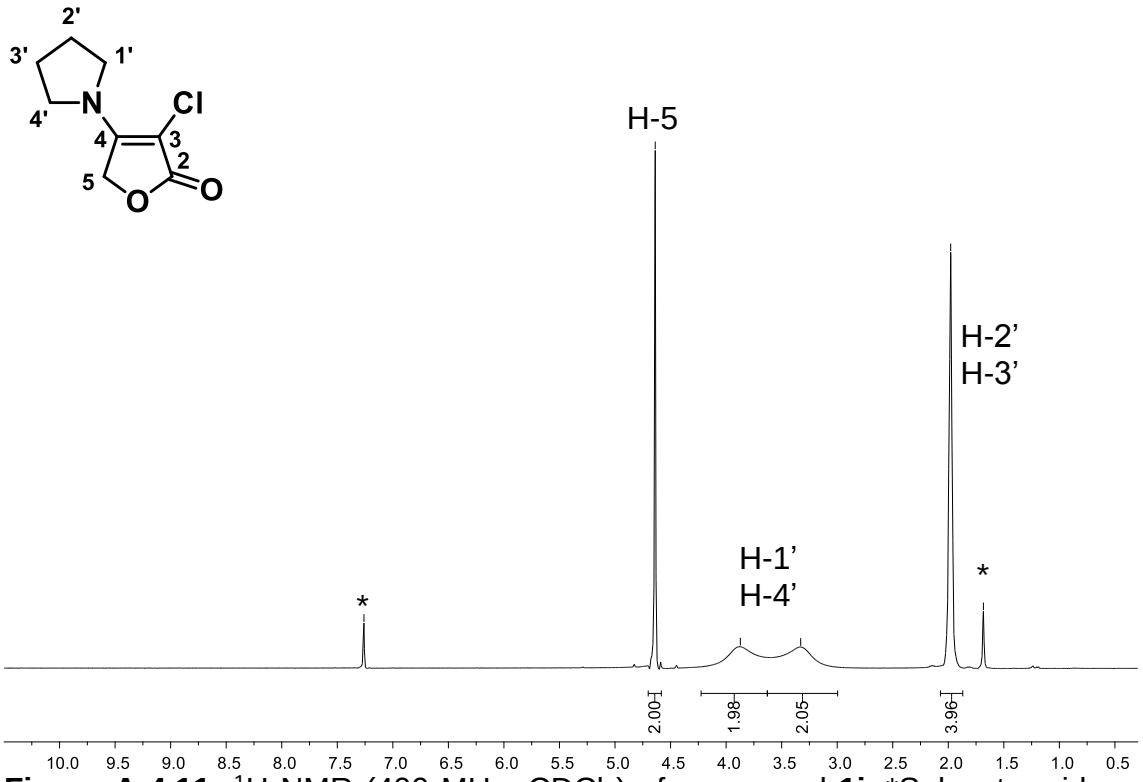



**Figure A 4.11:** $^{1}H$ NMR (400 MHz, $CDCl_3$) of compound **1j**. *Solvent residues and water signals.

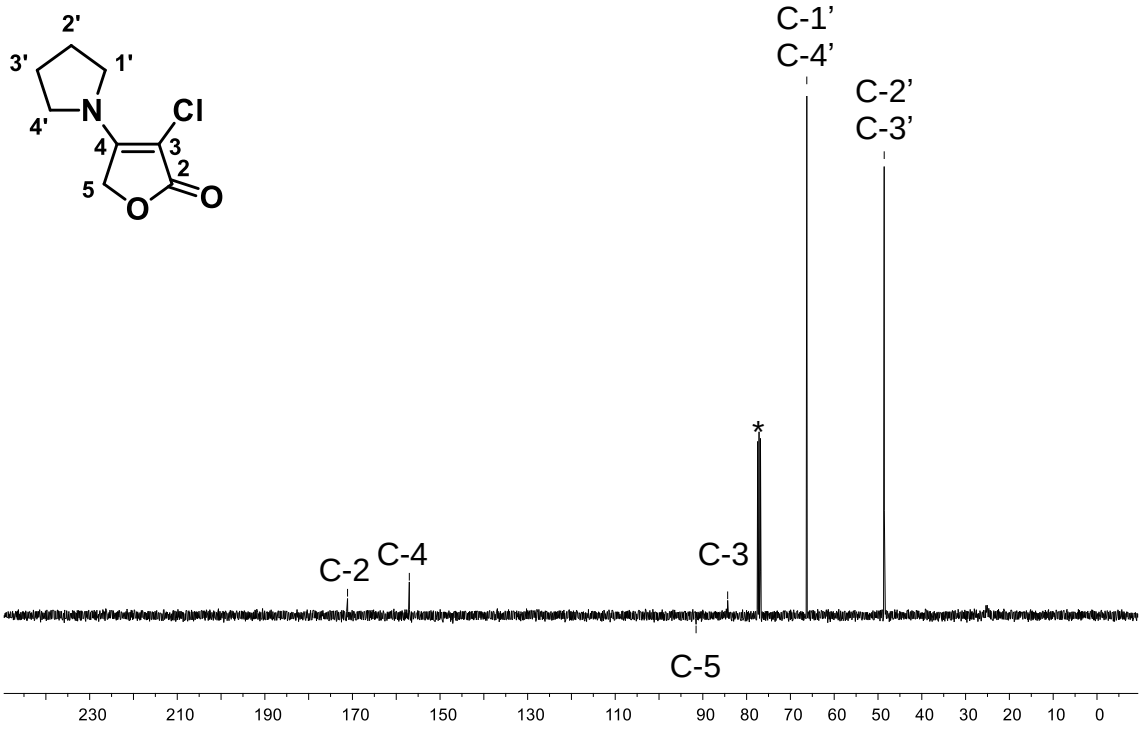



**Figure A 4.12:** DEPT135 (100 MHz, $CDCl_3$) of compound **1i**. *Solvent residues signals.

## 4.2. Selected spectra for the synthesis of aldolic tetronamides 3.

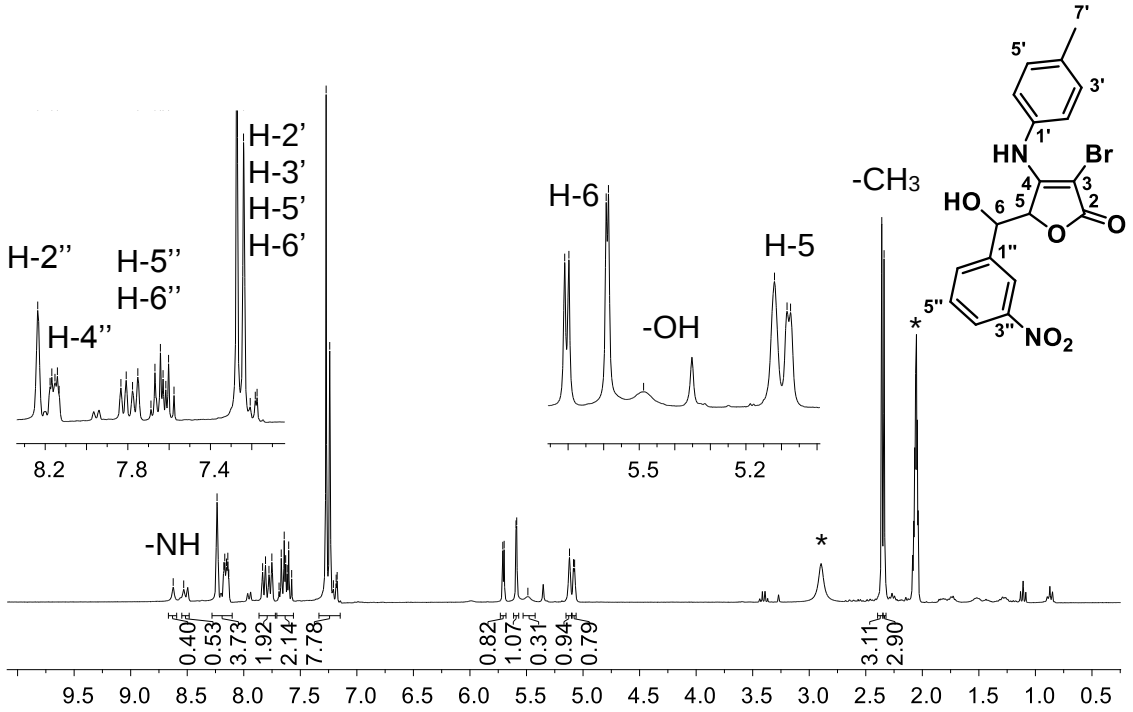



**Figure A 4.13:** $^{1}H$ NMR (300 MHz, $(CD_3)_2CO$) of compound **3c** (56:44). *Solvent residues and water signals.

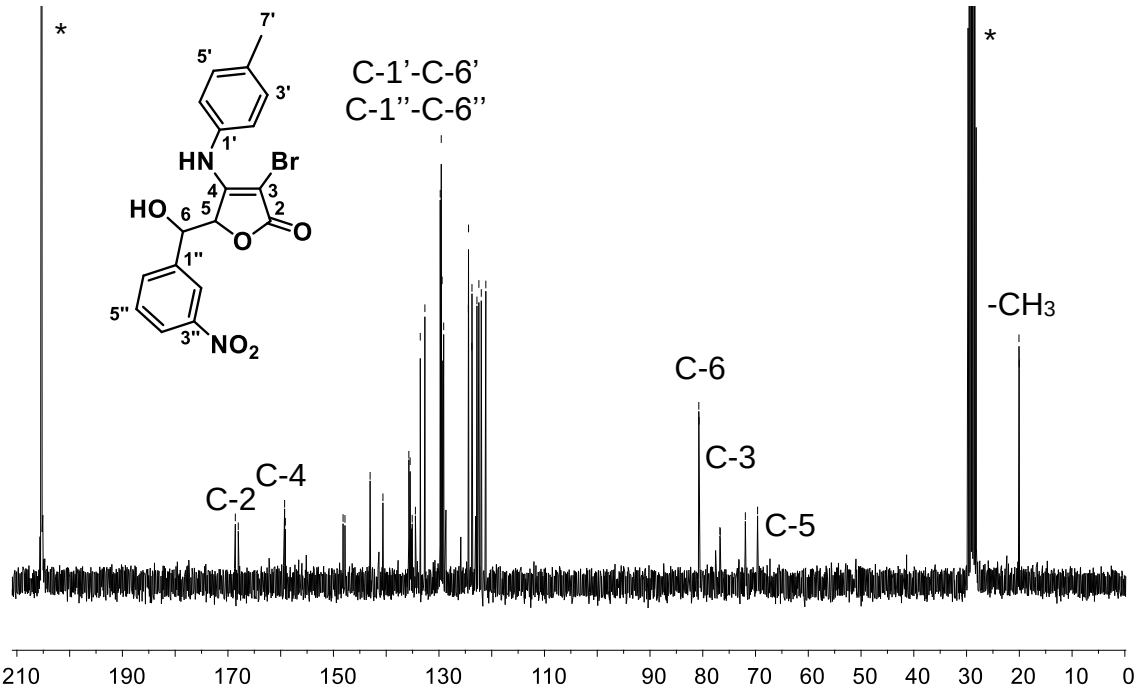



**Figure A 4.14:** $^{13}C$ NMR (75 MHz, $(CD_3)_2CO$) of compound **3c** (56:44). *Solvent residues signals.

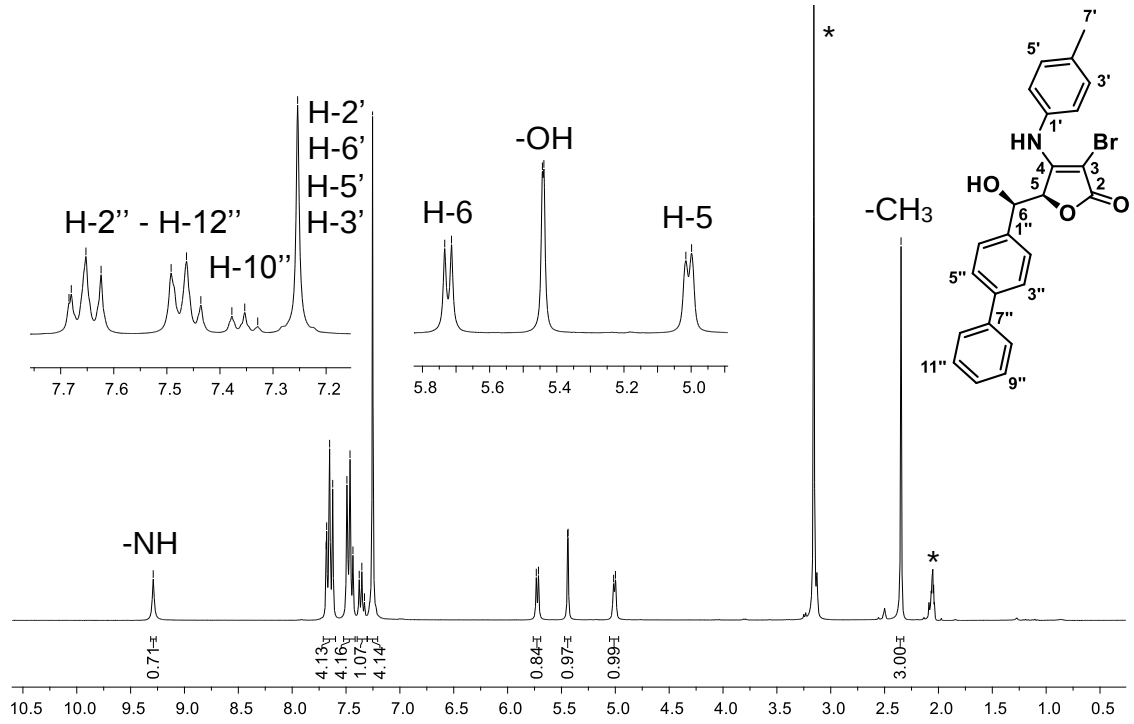



**Figure A 4.15:** $^{1}H$ NMR (300 MHz; $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1)) of compound **3d**. *Solvent residues and water signals.

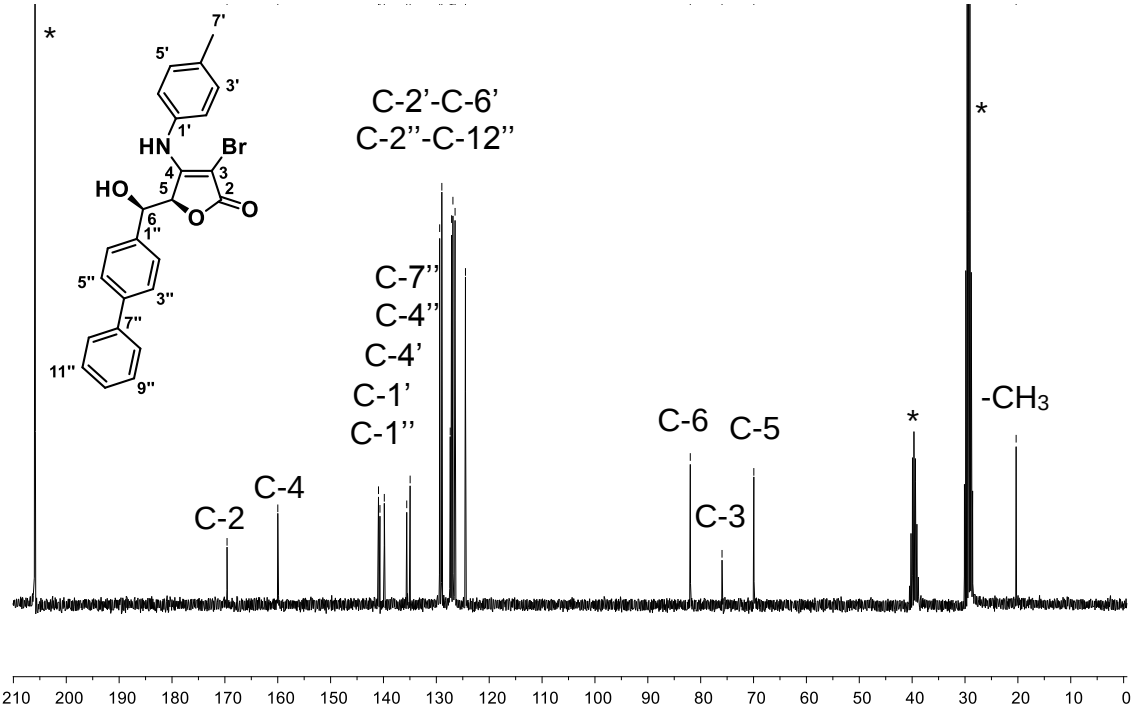



**Figure A 4.16:** $^{13}C$ NMR (75 MHz; $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1)) of compound **3d**. *Solvent residues signals.

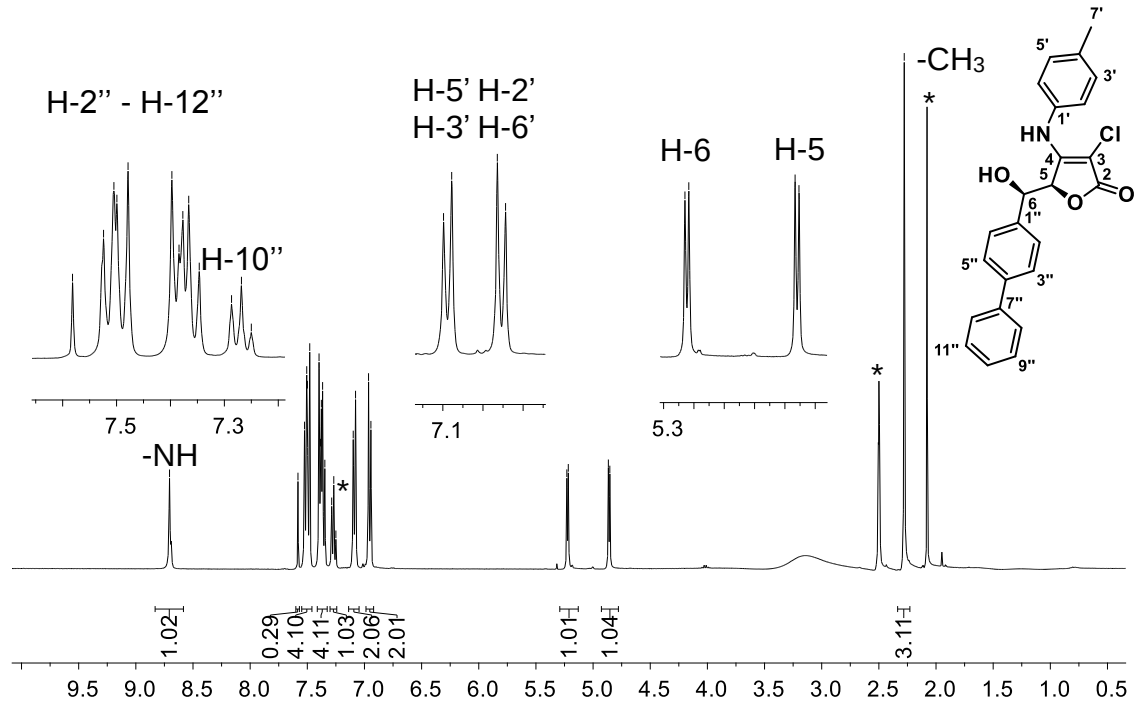



**Figure A 4.17:** $^{1}H$ NMR (400 MHz; $CDCl_3$:$(CD_3)_2SO$ (8:2)) of compound **3f**. *Solvent residues and water signals.

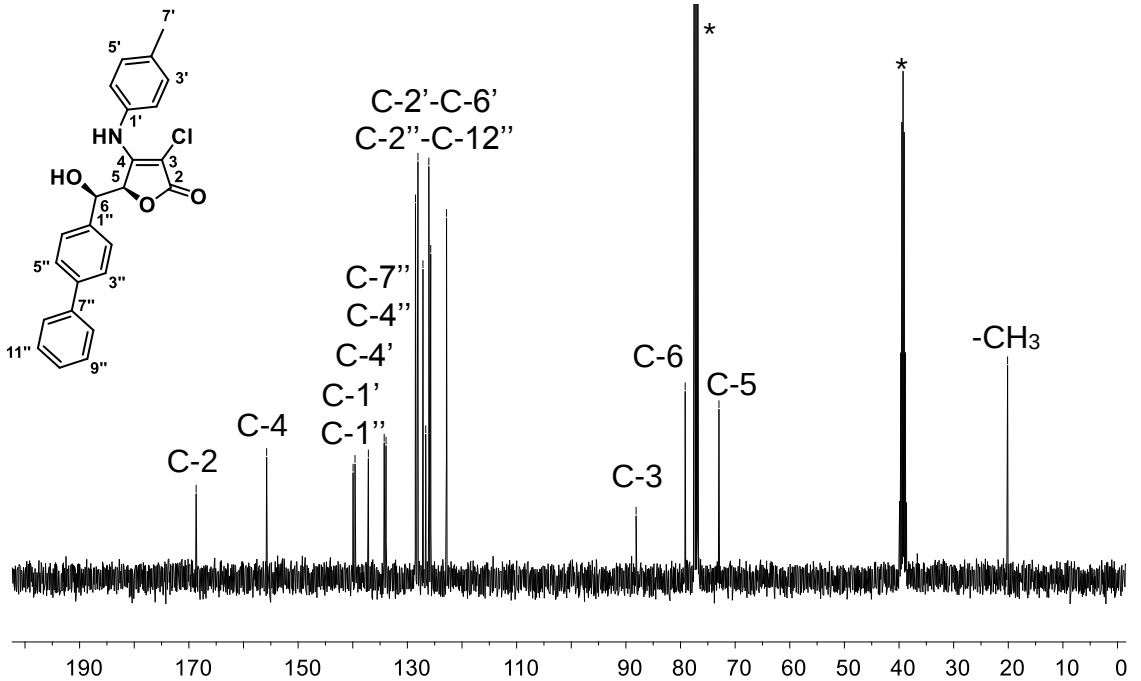



**Figure A 4.18:** $^{13}C$ NMR (100 MHz; $CDCl_3$:$(CD_3)_2SO$ (8:2)) of compound **3f**. *Solvent residues signals.

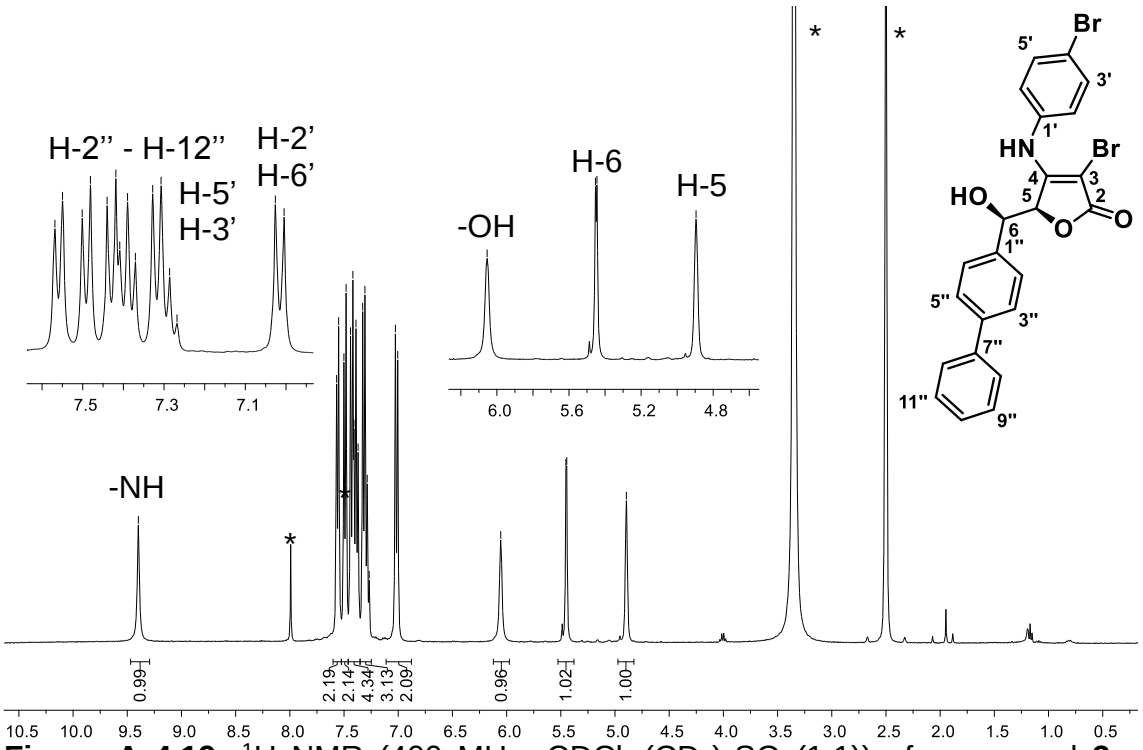



**Figure A 4.19:** $^{1}H$ NMR (400 MHz; $CDCl_3$:$(CD_3)_2SO$ (1:1)) of compound **3q**. *Solvent residues and water signals.

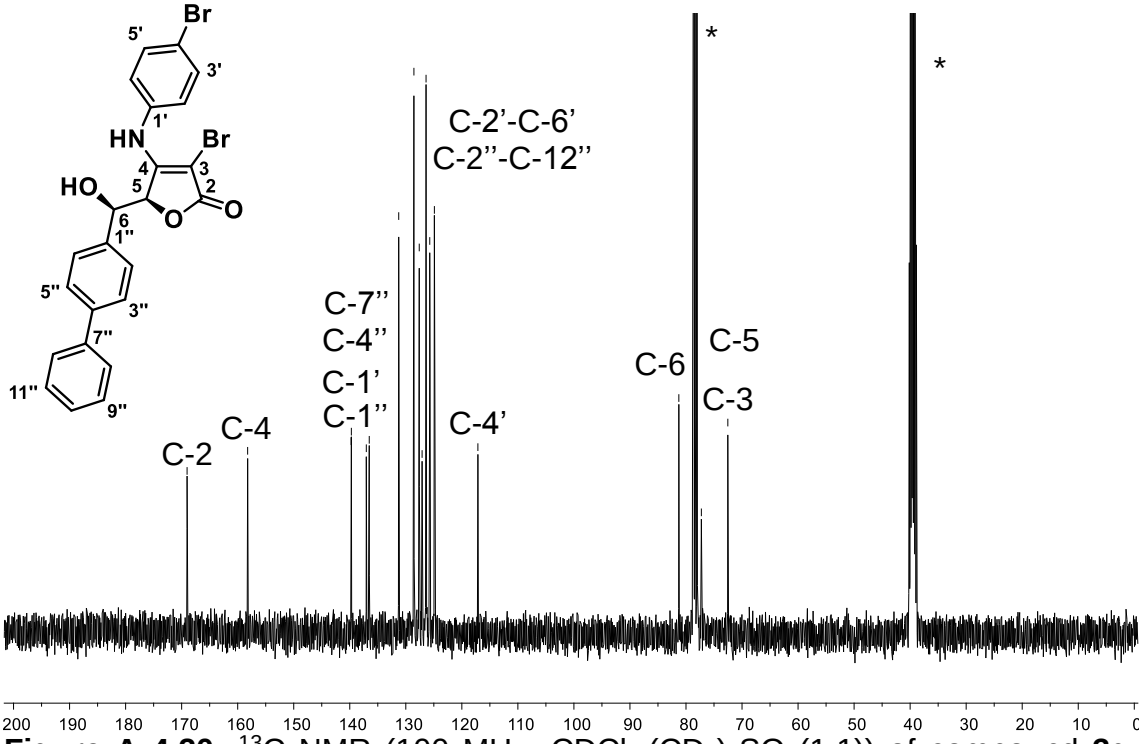



**Figure A 4.20:** $^{13}C$ NMR (100 MHz; $CDCl_3$:$(CD_3)_2SO$ (1:1)) of compound **3q**. *Solvent residues signals.

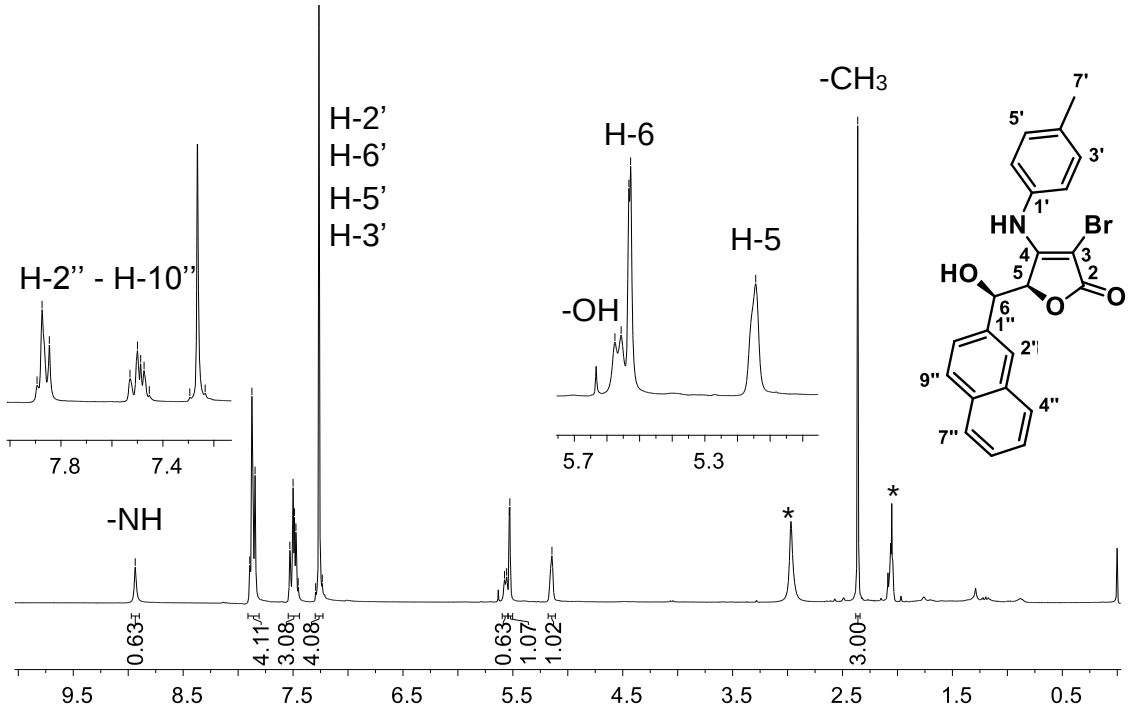



**Figure A 4.21:** $^{1}H$ NMR (300 MHz; $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1)) of compound **3i**. *Solvent residues and water signals.

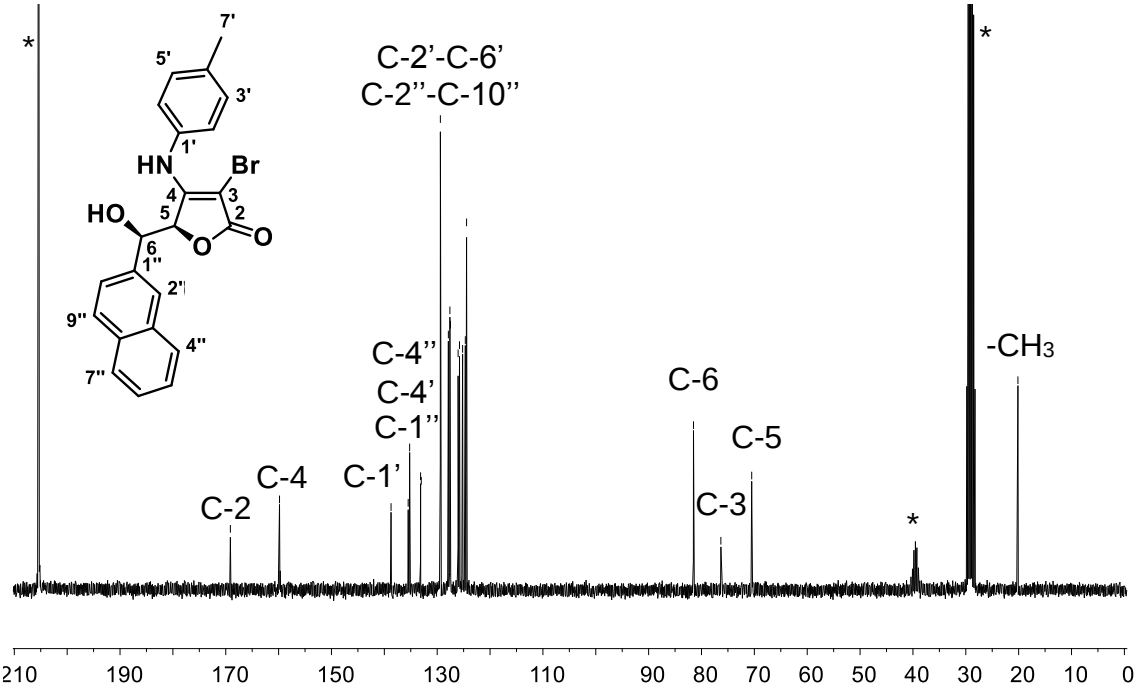



**Figure A 4.22:** $^{13}C$ NMR (100 MHz; $(CD_3)_2CO$:$(CD_3)_2SO$ (9:1)) of compound **3i**. *Solvent residues signals.

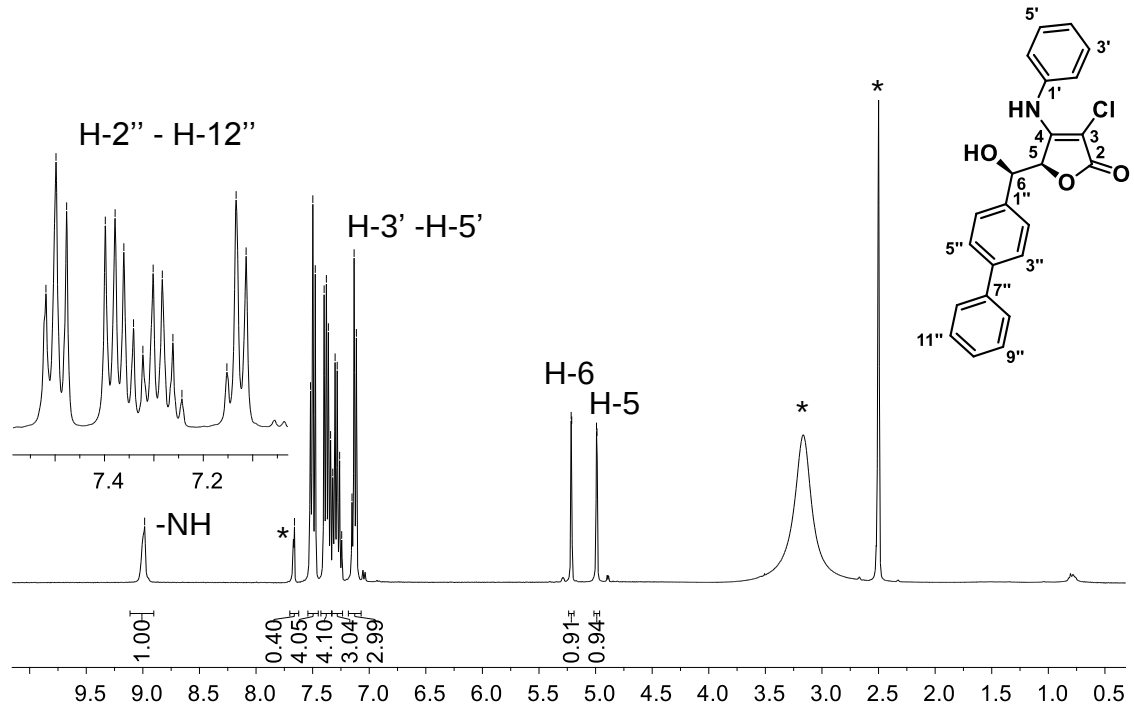



**Figure A 4.23:** $^{1}H$ NMR (400 MHz; $CDCl_3$:$(CD_3)_2SO$ (1:1)) of compound **3n**. *Solvent residues and water signals.

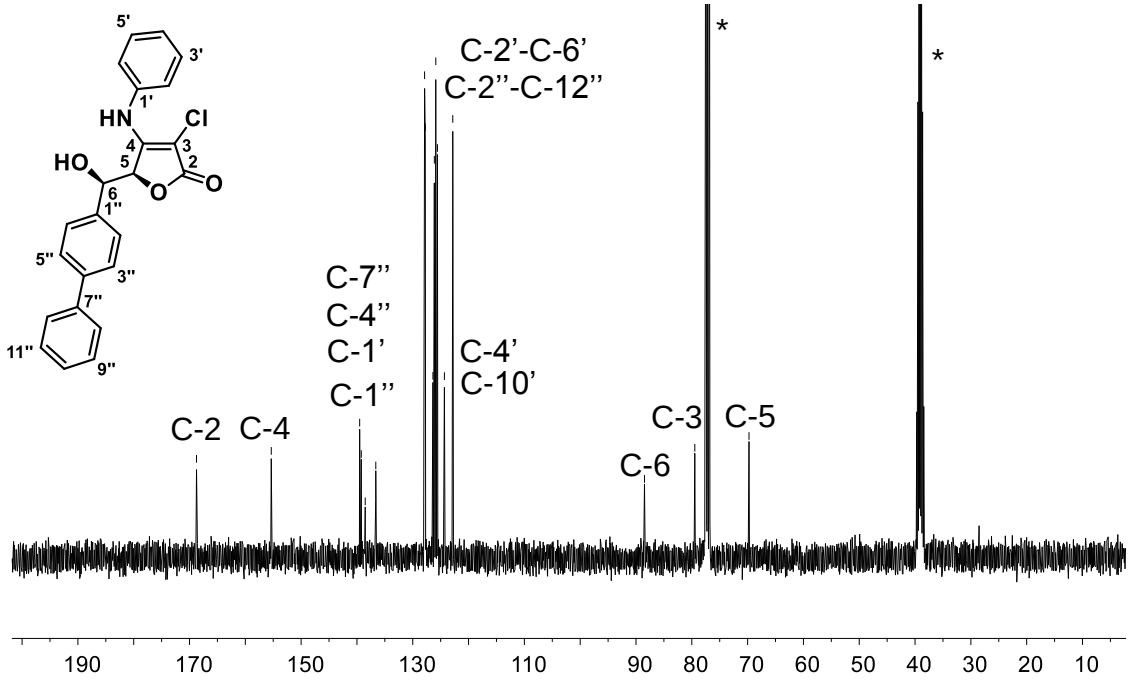



**Figure A 4.24:** $^{13}C$ NMR (100 MHz; $CDCl_3$:$(CD_3)_2SO$ (1:1)) of compound **3n**. *Solvent residues signals.

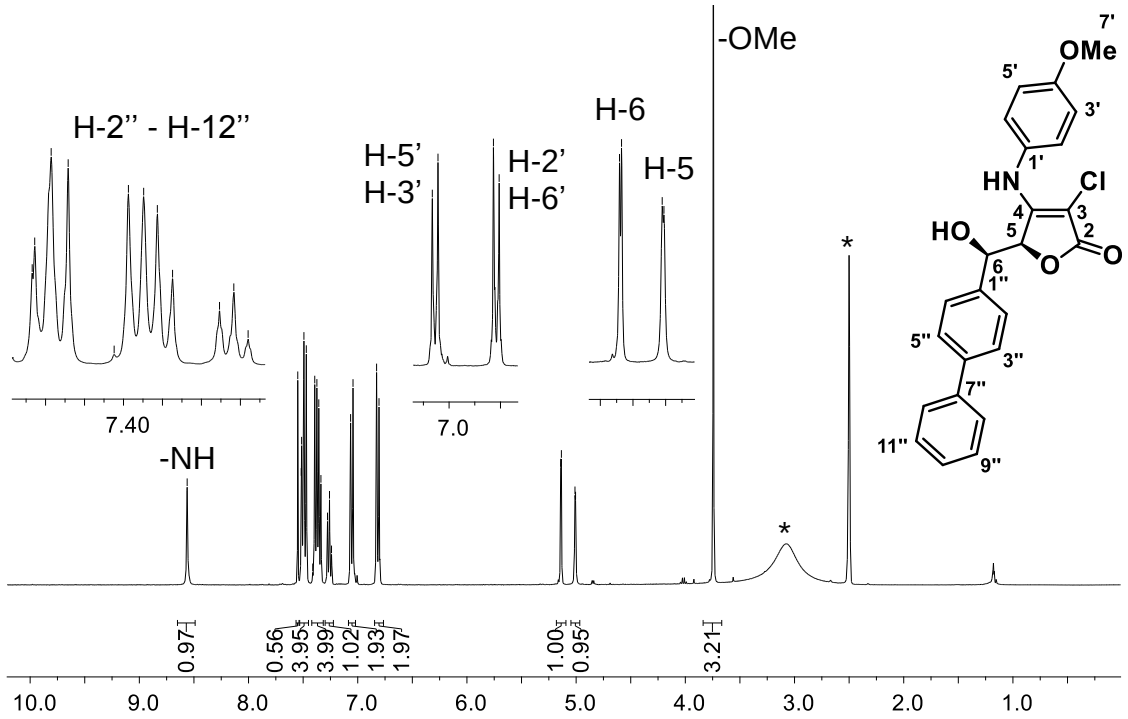



**Figure A 4.25:** $^{1}H$ NMR (400 MHz; $CDCl_3$:$(CD_3)_2SO$ (7:3)) of compound **3o**. *Solvent residues and water signals.

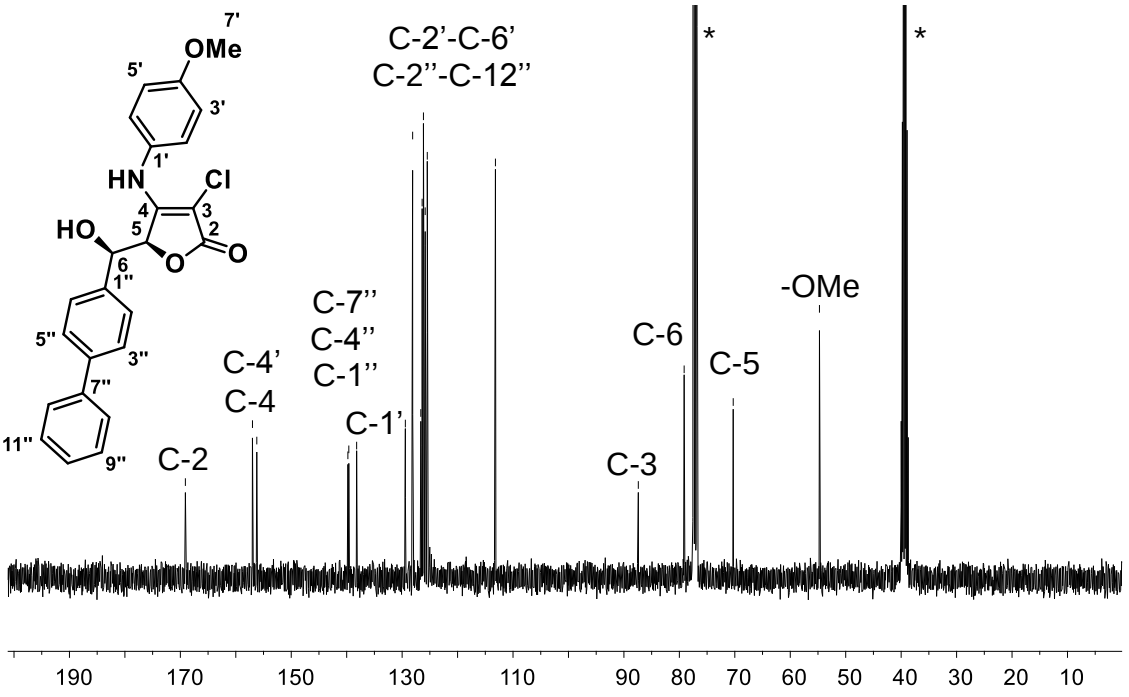



**Figure A 4.26:** $^{13}C$ NMR (100 MHz; $CDCl_3$:$(CD_3)_2SO$ (7:3)) of compound **3o**. *Solvent residues signals.

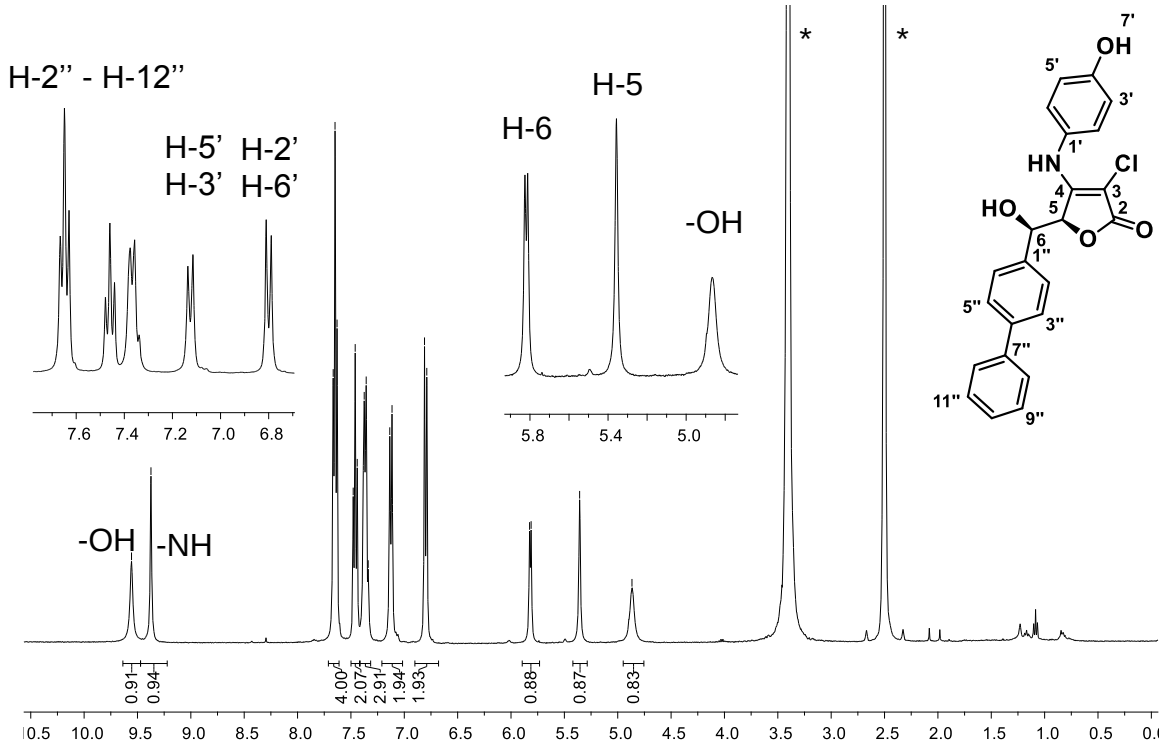



**Figure A 4.27:** $^{1}H$ NMR (400 MHz; $(CD_3)_2SO$) of compound **3p**. *Solvent residues and water signals.

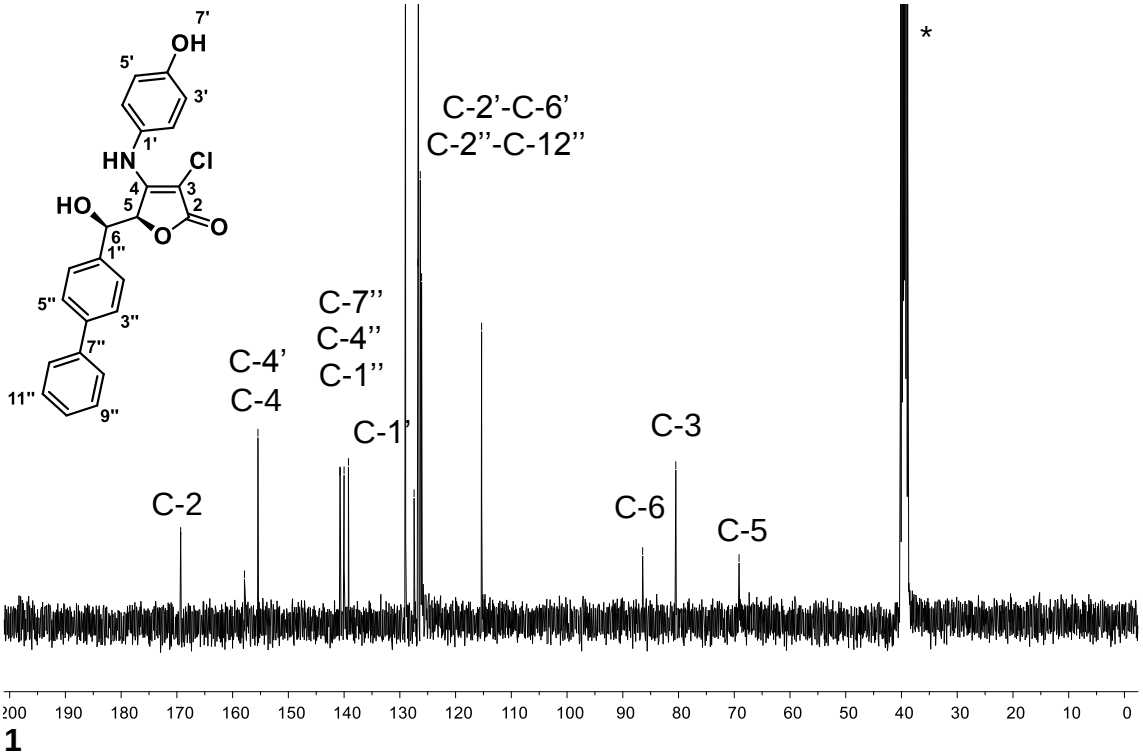



**Figure A 4.28:** $^{13}C$ NMR (100 MHz; $(CD_3)_2SO$) of compound **3p**. *Solvent residues signals.

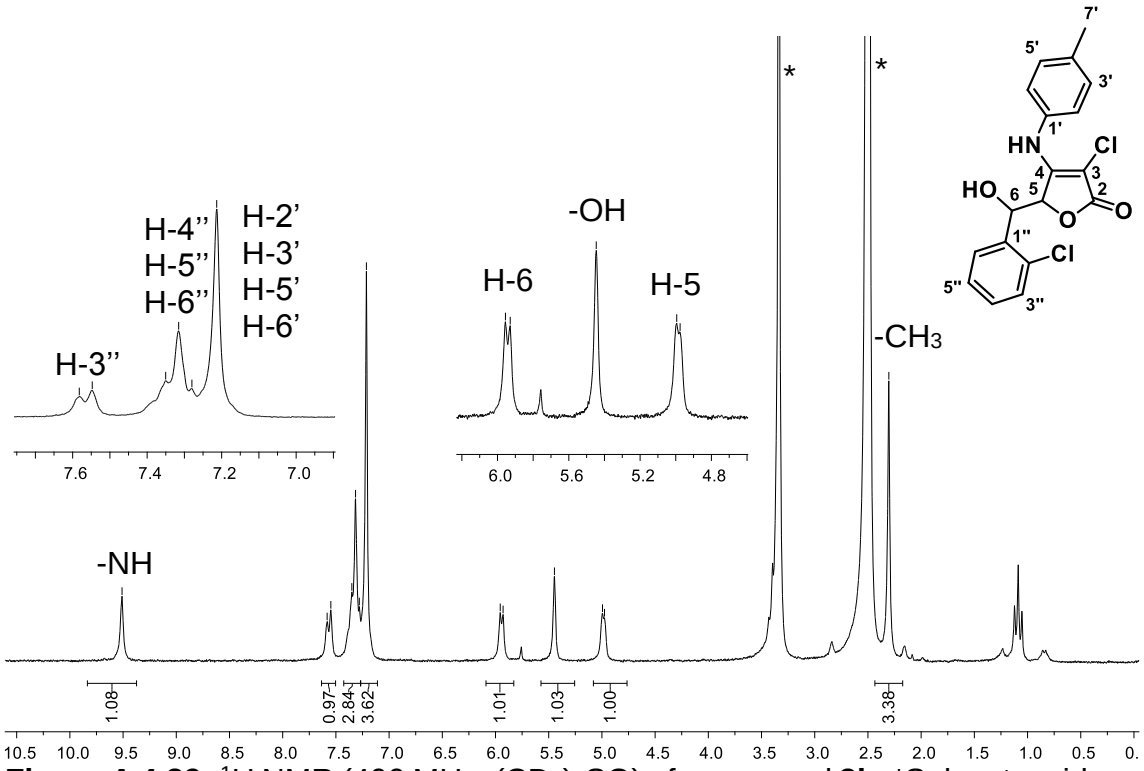



**Figure A 4.29:** $^{1}H$ NMR (400 MHz, $(CD_3)_2SO$) of compound **3b**. *Solvent residues and water signals.

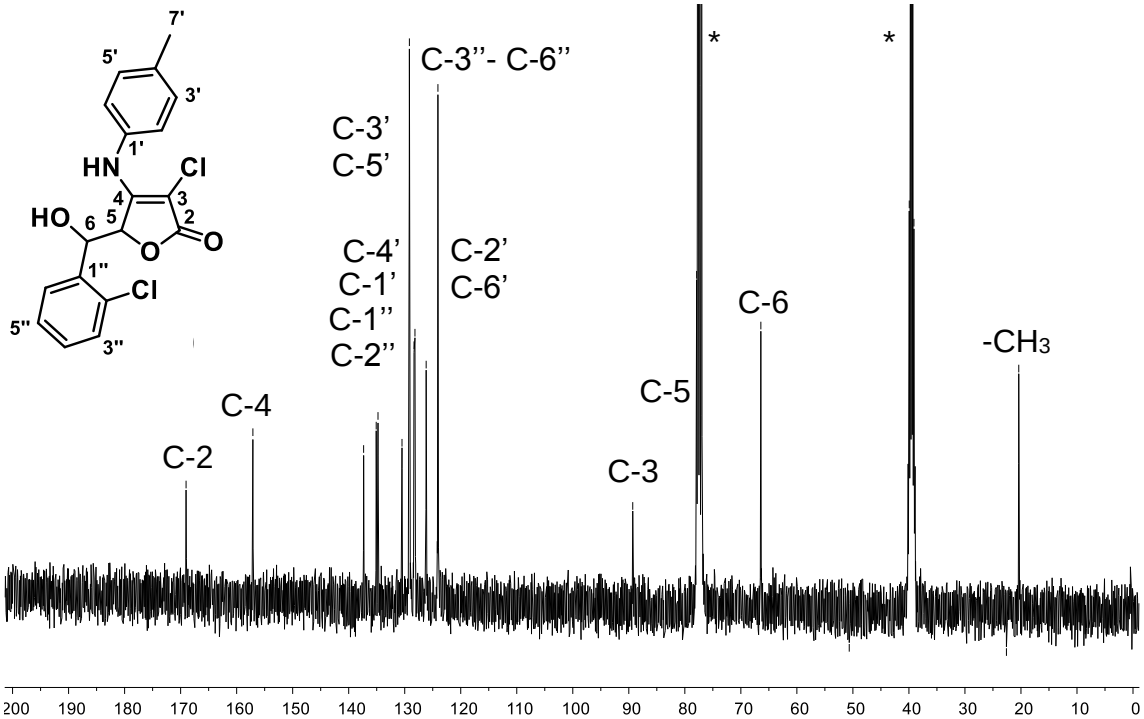



**Figure A 4.30:** $^{13}C$ NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (7:3)) of compound **3b** (56:44). *Solvent residues signals.

## 4.3. Selected spectra for the synthesis of γ-alkylidene tetronamides 4.

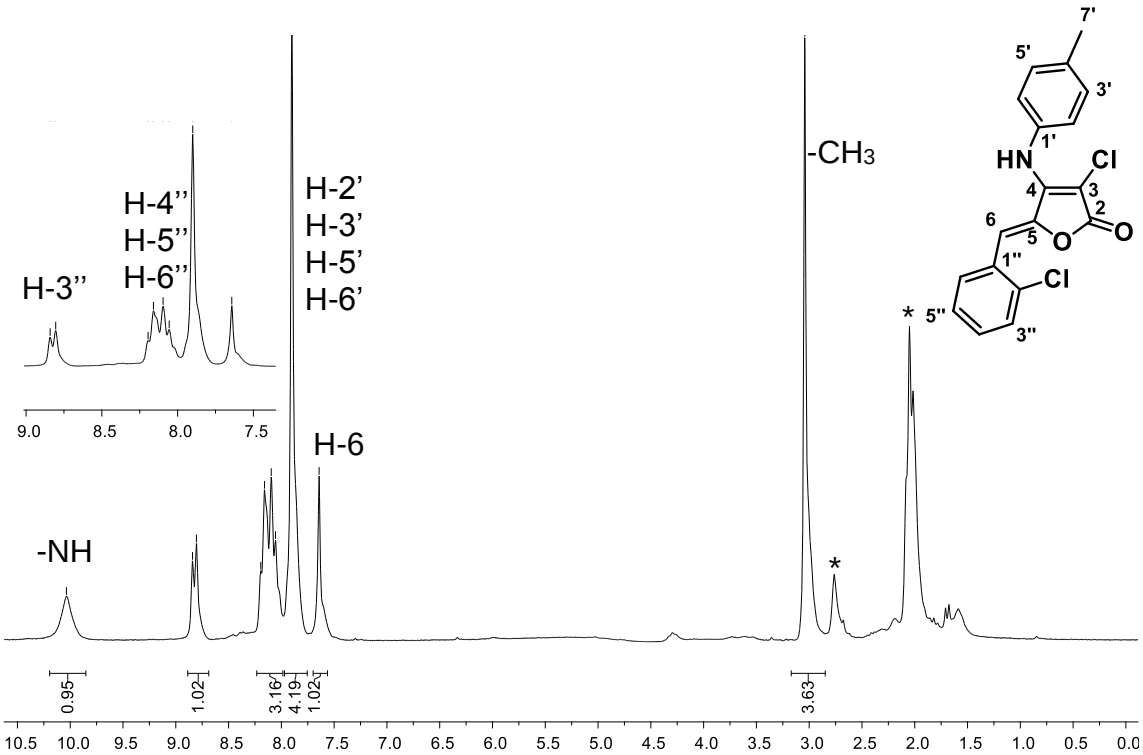



**Figure A 4.31:** $^{1}H$ NMR (400 MHz, $(CD_3)_2CO$) of compound **4b**. *Solvent residues and water signals.

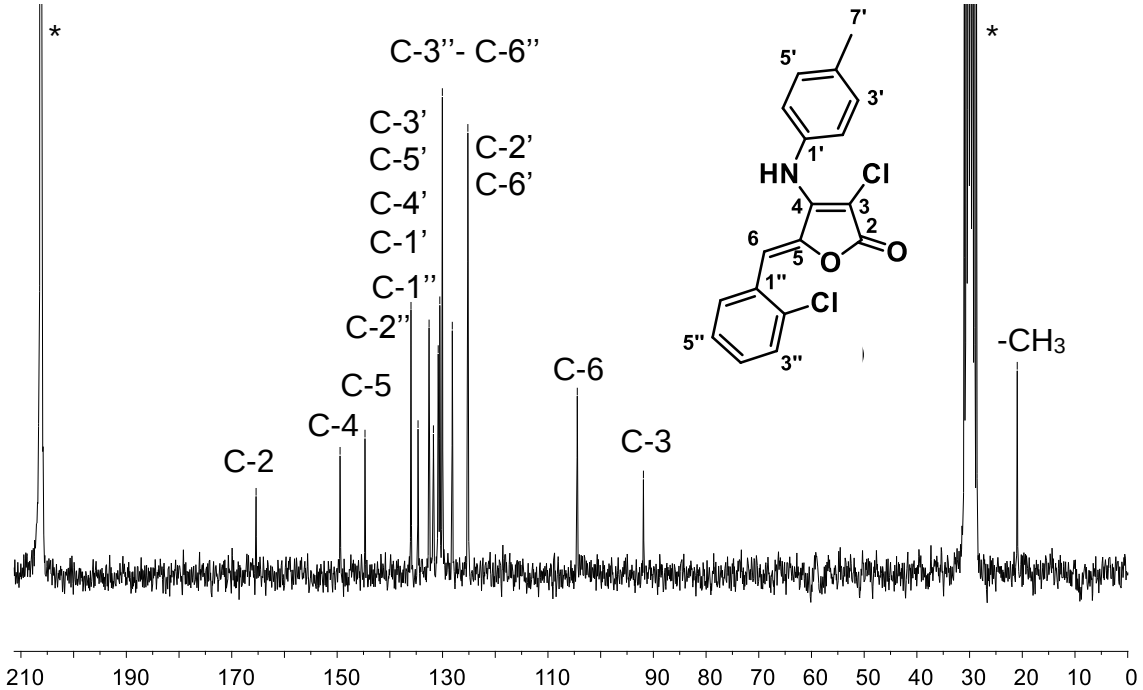



**Figure A 4.32:** $^{13}C$ NMR (100 MHz, $(CD_3)_2CO$) of compound **4b**. *Solvent residues signals.

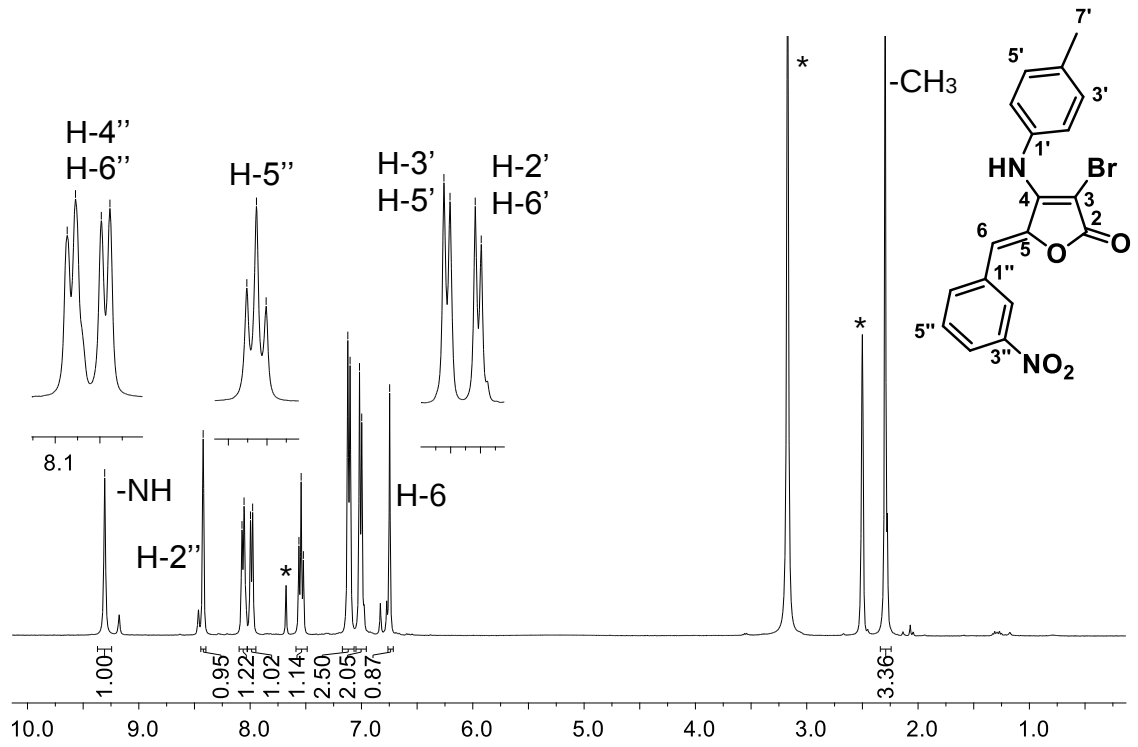



**Figure A 4.33:** $^{1}H$ NMR (400 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1)) of compound **4c**. *Solvent residues and water signals.

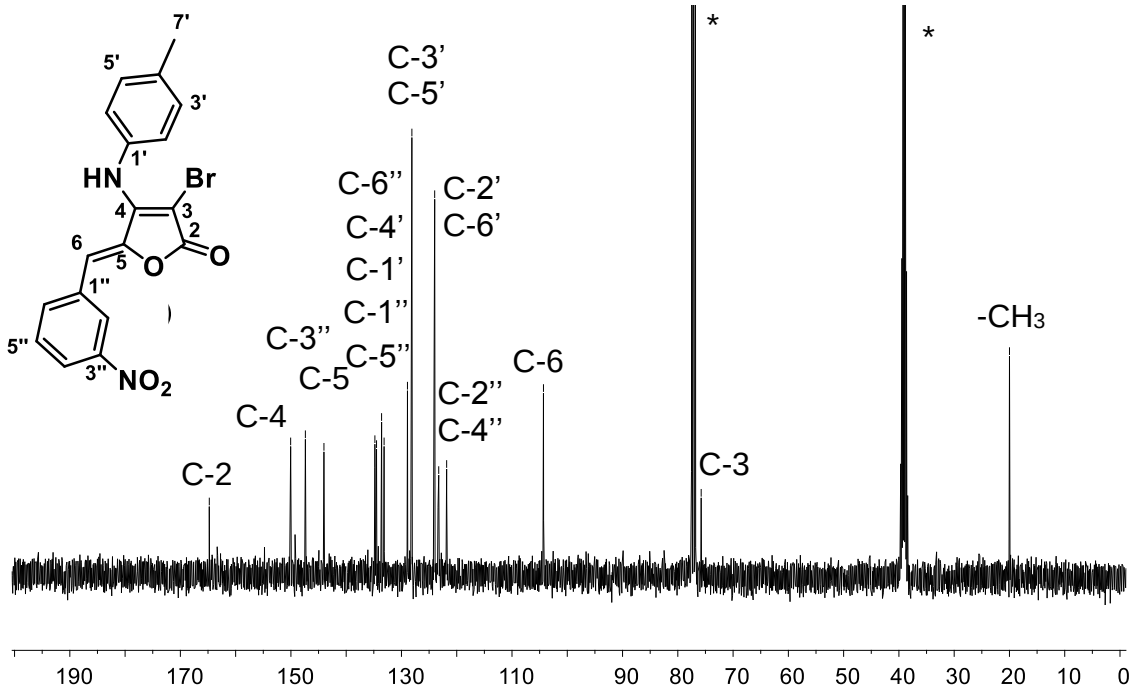



**Figure A 4.34:** $^{13}C$ NMR (100 MHz, $CDCl_3$:$(CD_3)_2SO$ (1:1)) of compound **4c**. *Solvent residues signals.

**Tabela A 2.1**: Crystallographic daofs and crystal structure refinement for chiral tetronamide **3x**

| | |
|---|---|
| Empirical formula | $C_{15}H_{16}ClNO_3$ |
| Formula weight | 293.74 |
| Temperature/K | 150(2) |
| Crystal system | monoclinic |
| Space group | $P2_1$ |
| a/Å | 5.49795(6) |
| b/Å | 12.14363(9) |
| c/Å | 10.52109(8) |
| α/° | 90 |
| β/° | 98.9701(9) |
| γ/° | 90 |
| Volume/Å$^3$ | 693.851(11) |
| Z | 2 |
| $\rho_{calc}$g/cm$^3$ | 1.406 |
| μ/mm$^{-1}$ | 2.503 |
| F(000) | 308.0 |
| Crystal size/mm$^3$ | 0.38 × 0.2 × 0.02 colourless plate |
| Radiation | CuKα (λ = 1.54184) |
| 2Θ range for data collection/° | 8.508 to 156.122 |
| Index ranges | -6 ≤ h ≤ 6, -15 ≤ k ≤ 15, -13 ≤ l ≤ 13 |
| Reflections collected | 17730 |
| Independent reflections | 2919 [$R_{int}$ = 0.0344, $R_{sigma}$ = 0.0186] |
| Data/restraints/parameters | 2919/73/213 |
| Goodness-of-fit on $F^2$ | 1.053 |
| Final R indexes [I>=2σ (I)] | $R_1$ = 0.0278, $wR_2$ = 0.0744 |
| Final R indexes [all data] | $R_1$ = 0.0280, $wR_2$ = 0.0746 |
| Largest diff. peak/hole / e Å$^{-3}$ | 0.23/-0.14 |
| Flack parameter | -0.006(4) |

**Tabela A 2.2**: Crystallographic daofs and crystal structure refinement for chiral tetronamide **3y**

| | |
|---|---|
| Empirical formula | $C_{15}H_{16}ClNO_3$ |
| Formula weight | 293.74 |
| Temperature/K | 150(2) |
| Crystal system | monoclinic |
| Space group | $P2_1$ |
| a/Å | 5.52008(5) |
| b/Å | 12.20933(12) |
| c/Å | 10.49998(8) |
| α/° | 90 |
| β/° | 98.4789(8) |
| γ/° | 90 |
| Volume/Å$^3$ | 699.927(11) |
| Z | 2 |
| $\rho_{calc}$g/cm$^3$ | 1.394 |
| μ/mm$^{-1}$ | 2.481 |
| F(000) | 308.0 |
| Crystal size/mm$^3$ | 0.3 × 0.2 × 0.02 colourless plate |
| Radiation | CuKα (λ = 1.54184) |
| 2Θ range for data collection/° | 8.514 to 147.326 |
| Index ranges | -6 ≤ h ≤ 6, -15 ≤ k ≤ 15, -13 ≤ l ≤ 13 |
| Reflections collected | 21839 |
| Independent reflections | 2787 [$R_{int}$ = 0.0611, $R_{sigma}$ = 0.0254] |
| Data/restraints/parameters | 2787/73/225 |
| Goodness-of-fit on $F^2$ | 1.039 |
| Final R indexes [I>=2σ (I)] | $R_1$ = 0.0278, $wR_2$ = 0.0734 |
| Final R indexes [all data] | $R_1$ = 0.0280, $wR_2$ = 0.0736 |
| Largest diff. peak/hole / e Å$^{-3}$ | 0.26/-0.14 |
| Flack parameter | -0.006(8) |