UNIVERSITÀ DEGLI STUDI DI MILANO

Ph.D. Programme in Food Systems

Department of Food, Environmental and Nutritional Sciences (DeFENS)
Division of Chemistry and Biochemistry

XXXI Cycle

New approaches in the discovery of natural product-based agrochemicals

[CHIM/06]

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2017/2018

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ABBREVIATIONS

$(Boc)_2O$	di-*tert*-butyl dicarbonate
Å	Ångström
abs.	absolute
AIBN	2,2azobisisobutyronitrile
Ala	alanine
Arg	arginine
ATP	adenosine triphosphate
Boc	*tert*-butyloxycarbonyl
$CDCl_3$	deuterochloroform
CH_2Cl_2	dichloromethane
$CHCl_3$	chloroform
COSY	correlation spectroscopy
Cyt *b*	cytochrome *b*
Cyt *c*	cytochrome *c*
DBH_2	reduced decylubiquinone
DCPIP	2,6-dichlorophenolindophenol
de	diastereomeric excess
DIBAL	diisobutylaluminium hydride
DIPEA	*N*,*N*-diisopropylethylamine (Hünig's base)
DMAP	4-dimethylaminopyridine
DMSO	dimethyl sulfoxide
dr	diastereoisomeric ratio
E	entgegen
EDC·HCl	*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
ee	enantiomeric excess
eq.	equivalent
Et_2O	diethyl ether
Et_3N	triethylamine
EtOH	ethanol
FRAC	Fungicide Resistance Action Committee
HETCOR	heteronuclear correlation spectroscopy
His	histidine
HMBC	heteronuclear multiple bond correlation

HMDS	bis(trimethylsilyl)amine
HMPA	hexamethylphosphoramide
HOBt	1-hydroxybenzotriazole
HSQC	heteronuclear single quantum correlation
IC_{50}	half maximal inhibitory concentration
Ile	isoleucine
Leu	leucine
MA	malt extract agar
MeOH	methanol
Met	methionine
min.	minute
mp	melting point
MS	molecular sieves
MTPA	α-methoxy-α-trifluoromethylphenylacetic acid
MTPACl	α-methoxy-α-trifluoromethyl-phenylacetyl chloride
NADH	nicotinamide adenine dinucleotide
NBS	*N*-bromosuccinimide
*n*BuLi	*n*-butyllithium
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
Phe	phenylalanine
PPTS	pyridinium p-toluenesulfonate
Pro	proline
Q	quinone ‘outer’ binding site
R	Rectus
rt	room temperature
S	Sinister
SDH	succinate dehydrogenase
SDHA	succinate dehydrogenase complex flavoprotein subunit A
SDHB	succinate dehydrogenase complex iron sulfur subunit B
SDHC	succinate dehydrogenase complex subunit C
SDHD	succinate dehydrogenase complex subunit D
SDHI	succinate dehydrogenase inhibitor

TBAI	tetrabutylammonium iodide
THF	tetrahydrofuran
TPAP	tetrapropylammonium perruthenate
Trp	tryptophan
Tyr	tyrosine
Val	valine
X	undefined halide
Z	zussamen

ABSTRACT

Currently, the increase of food demand of a steadily growing human population is not balanced by a global agricultural supply. As a consequence, crop protection plays a crucial role in maximising crop productivity and preventing losses caused by biological and not biological agents. Biological agents, collectively named as pests, are responsible for quantitative losses ranging from 20 % to 40 %, and qualitative losses, thus reducing the value of crops. For these reasons, new methods to control pests must be developed.The discovery of new agrochemicals is necessary to face the problem of the emergence of resistances and the increasing of stringent regulatory standards, which are leading to the ban of many products no longer considered acceptable. All these factors are changing the research of new pesticides that is becoming a more expensive and time-consuming process. Thus, new methods to improve and speed up the discovery of new agrochemicals must be developed. In this context, the aim of this Ph.D. thesis was to explore new approaches to the synthesis of agrochemicals. Our attention was focused on the naturally derived compound-based molecules, due to the proved value of these compounds in development of agrochemicals and more in general of biologically active compounds.

The first part of this Ph.D. thesis is focused on the development of dual-target fungicides obtained by combination of the naturally derived strobilurin fungicides with succinate dehydrogenase inhibitors. The development of dual-target molecules is a well-known approach in pharmaceutic research, while it is still underexplored in the field of agrochemicals. The research work was organized following a cycle of design, synthesis, *in vitro* tests, and evaluation of the mechanism of action. The dual-target compounds were designed on the basis of the reported structure-activity relationship for both the classes of fungicides. The designed compounds were synthetized, and the antifungal activity was screened *in vitro* by measurement of the mycelium growth inhibition. Finally, the mechanism of action was evaluated *in silico* by docking studies and *in vitro* in a cell free system.

The second part of this dissertation was focused on the development of versatile synthetic approaches in the synthesis of biologically active natural compounds. SmI_2 chemistry was employed in light of the remarkable synthetic power of this reagent. First, a SmI_2-mediated Reformatsky reaction was developed as a complementary approach to Evans aldol reaction for the synthesis of precursors of cembranoids. Then, a SmI_2-mediated pinacol coupling was investigated as a key step for the synthesis of the natural herbicide auscalitoxin aglycone.

RIASSUNTO

La domanda di cibo della popolazione umana in costanta crescita non è attualmente soddisfatta dalla produzione agricola globale. Come conseguenza la protezione delle colture gioca un ruolo di vitale importanza nel massimizzare le rese produttive e nel prevenire le perdite provocate da fattori sia abiotici che biotici. Gli agenti biologici sono causa di perdite quantitative, comprese tra il 20 % e il 40 %, ma possono provocare anche perdite qualitative riducendo il valore dei raccolti. Per questi motivi è importante lo sviluppo di nuovi metodi di controllo, tra cui anche nuovi agrofarmaci. Lo sviluppo di nuovi agrofarmaci è indispensabile anche per affrontare il problema dell'insorgenza di resistenze e per compensare la messa al bando di prodotti in uso a causa dell'aumentata pressione regolatoria in termini di salute umana e ambientale. Questi ultimi fattori hanno come conseguenza l'aumento dei tempi e dei costi di ricerca e sviluppo di nuovi prodotti e, di conseguenza, diventa importante trovare nuovi metodi per accelerare e aumentare l'efficienza dei processi di ricercar e sviluppo.

In questo contesto lo scopo di questa tesi di dottorato è l'esplorazione di nuovi potenziali approcci alla ricerca di nuovi agrofarmaci. La nostra attenzione è stata focalizzata in particolare verso molecule naturali o derivate da composti naturali per via della loro riconosciuta importanza nei processi di ricercar e sviluppo di nuovi agrofarmaci e, più in generale di nuove molecule biologicamente attive.

La prima parte di questa tesi tratta lo sviluppo di fungicidi dual-target ottenuti combinando le strutture delle strobilurine con gli inibitori della succinato deidrogenasi, in quanto nonostante lo sviluppo di composti dual-target sia un approccio ben conosciuto in ambito farmaceutico nel campo degli agrofarmaci è quasi sconosciuto. Il lavoro di ricercar è stato organizzato secondo un ciclo di design, sintesi, test *in vitro* e valutazione dell'attività. I composti progettati sulla base delle relazioni struttura-attività di entrambe le classis ono stati sintetizzati e poi testati *in vitro* per la loro capacità di inibire la crescita del micelio. Infine, la loro attività è stata valutata *in silico* tramiti studi di docking e *in vitro* su frazioni mitocondriali isolate.

La seconda parte della tesi tratta lo sviluppo di approcci sintetici alternativi nella sintesi di composti naturali. In particolare, l'attenzione è stata posta nei confronti della chimica del SmI_2 per la dimostrata utilità e versatilità sintetica di questo reagente. Per prima è stata sviluppata una metodologia per la sintesi di precursori dei cembranoidi basata sull'utilizzo della reazione di Reformatsky mediate da SmI_2. In seguito, è stato studiate un coupling pinacolico mediato da SmI_2 come passaggio chiave per la sintesi dell'aglicone dell'auscalitossina.

1 INTRODUCTION

Despite the world growth rates is declining, the human population is steadily growing, and it is estimated to reach 9.73 billion by 2050 and 11.2 billion by 2100. The growth of population is generally accompanied by increasing urbanization and this will lead to changes in dietary habits, that will eventually have a strong influence on the agriculture and the food systems.[1]
Nowadays, the increase of food demand of a growing human population is not balanced by the increase of the global agricultural supply. This situation has dramatically worsened in the recent decades culminating in the 2007-8 and 2010-12 world food prices crisis.[2,3] Crop protection has a pivotal role in meeting this increasing food demand. Animals, weeds and plant pathologies are altogether responsible for crop losses ranging from 20 % to 40 %. Food security can be divided into different components including food availability (production, import, storing), physical and economic access to food, and food utilisation (e.g. nutritional value, safety). Crop losses directly affect the first component of food security, but they can also affect directly or indirectly the others. Crop losses are not only related to yield reductions but also to post harvest quality losses and accumulation of toxins. The scope of crop protection is to maximise crop productivity preventing losses caused by biological and no biological agents. Currently, this is a still challenging problems, but new levels of complexity have arisen because of the reduction of natural resources (e.g. arable land, water, fertilizers, biodiversity) available for the agriculture.[4]

1.1 Crop losses and pest control

Crop losses can be caused by abiotic as well as by biotic factors. Abiotic factors include inadequate temperature, irradiation, water and nutrients supply, and, except for nutrient supply, only a limited control can be done. Biotic factors comprise animal organisms (insects, mites and other arthropods, nematodes, rodents, slugs and snails, birds), plant pathogens (fungi, bacteria, viruses) and weeds. All of these are collectively named as pests.
Pests can cause crop losses in different ways and classified in different categories: stand reducers (damping-off pathogens), photosynthetic rate reducers (fungi, bacteria, viruses), leaf senescence accelerators (pathogens), light stealers (weeds, some pathogens), assimilate sappers (nematodes, pathogens, sucking arthropods), and tissue consumers (chewing animals, necrotrophic pathogens). Crop losses can be quantitative and/or qualitative: quantitative losses are the direct result of reduced productivity, conversely, qualitative losses are linked to the reduction of the value of crop related to a reduced content of valuable ingredients, reduced

market quality, reduced storage characteristics, or to the contamination of the harvested product with toxic products (e.g. mycotoxins).[5]

Crop protection against pest can be obtained by applying physical and agronomical methods (e.g. weeding, crop rotation, improving growing conditions etc.), biological methods (e.g. resistant varieties, biological agents etc.) and/or the action of chemicals (pesticides).

1.2 Pesticides

In the Manual on development and use of FAO and WHO specifications for pesticides defines pesticides as: "Any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit. It is also used for substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport."[6]

Pesticides are characterized by different structures with different physical and chemical properties; thus, different system of classification are adopted depending on the needs. The three most popular methods of classification currently in use were proposed by Drum.[7] The three pesticides classification methods are based on the mode of entry, on the pesticide function and the pest organism they kill, and on the chemical composition. Classification based on the mode of entry considers the pathways used by pesticides to enter in contact or enter in the target pest organism. Thus, this method distinguishes systemic, non-systemic (contact), stomach poisonings and stomach toxicant, fumigant, and repellent pesticides. The second method considers the kind of pest controlled. The name of a group of pesticide usually, but not necessarily, derives from the name of the target pest with the suffix *-cide* (e.g. insecticides, fungicides, herbicides etc.).

Classification based on the chemical composition is the most common method used. This method provides the largest number of information on the physicochemical properties, which are useful in determining the rates, modes, risks of application. Classification based on the chemical nature is complex. Pesticides are generally organic compounds, both synthetic or of natural origin, however also inorganic compounds are currently in use.

Other less used classification methods exist which are based on mode of action, sources of origin, on range of target (broad spectrum and selective pesticides) and on the type of formulation.

The World Health Organization (WHO) classification method is based on the toxicity and the risk related with pesticides. Considering the oral and dermal median lethal dose (LD_{50}) on rat, pesticides are classified in different categories with increasing toxicity.[8] (**Table 1.1**).

Table 1.1 WHO classifications of pesticides[9]

WHO Class		**LD50 for the rat (mg/kg body weight)**	
		Oral	Dermal
Ia	Extremely hazardous	<5	<50
Ib	Highly hazardous	5-50	50-200
II	Moderately hazardous	50-2000	200-2000
III	Slightly hazardous	Over 2000	Over 2000
U	Unlikely to present acute hazard	5000 or higher	

1.2.1 Development of new pesticides

In the past, pesticides had a key role in the green revolution providing increasing crop yields. Nowadays, they still have a key role in controlling a wide array of pests, thus providing the desired crop yields. The agronomical systems are characterized by an extreme dynamicity due to the onset of resistance in pest populations, appearance of new pests, changes in in the cropping systems, introduction of genetically modified crops and increasing regulatory pressure in terms of health and environmental safety. To face all these changes, it is necessary to develop new methods for control of pests and this includes the discovery and development of new agrochemicals.

Today, worldwide emergence of resistance in pest population represents a serious problem in crop protection. The situation is particularly critical for insecticides with about 15000 unique cases of resistances observed. Resistance in weeds and plant pathogens seem to be less critical but an increasing number of unique cases of resistance, now close to 500 each, has been reported. Thus, the overcoming of resistance is an important driving force to the development of new pesticides along with an increased attention to resistance management. Particularly valuable is the discovery of agents with different mechanism of action, e.g. compounds able to interact in different ways with a known molecular target in the pest organism or able to

bind new or underexploited molecular targets. New mechanisms of action are important in resistance control since they facilitate the management schemes, in particular those based on the rotation of compounds with different mechanisms of action.

Regulatory standards are in continuous evolution and move towards a more favourable toxicological and ecological profile. Considering the increase of regulatory pressure, the use of many old products has been banned since they no longer meet the standards. The more stringent regulatory standards have the effect to make the discovery of new pesticides more challenging and time-consuming. Furthermore, the need to fully characterize the new pesticides and to complete all the required regulatory studies contribute to the increase of the costs of the development.[10]

1.2.2 Evolution of modern discovery process of pesticides

The emergence of resistance and the increasing of stringent regulatory standards with consequent increased costs of research and development represent the main challenges faced by companies in development of new agrochemicals. These challenges are forcing the agrochemical companies in finding new ways to improve and speed up the process of discovery and development of new pesticides.

Modern agrochemicals and pharmaceuticals share several features. Both agrochemicals and pharmaceuticals act by interaction with the target (generally and enzyme or receptor) with the same molecular mechanisms. Bioavailability, with some variation, is another important aspect for both the classes of molecules. Thus, the process of discovery and development of pesticides is evolving from a "chemistry first" approach to a process incorporating elements and techniques derived from the pharmaceutical industry.

In the development process several chemical sources (e.g. natural compounds, intermediates form other projects, competitor-inspired chemicals, combinatorial chemistry libraries etc.) can be used to obtain lead candidates for the development. Then, the selected compounds are subjected to different steps of design-synthesis-test-analysis cycle (**Figure 1.1**).

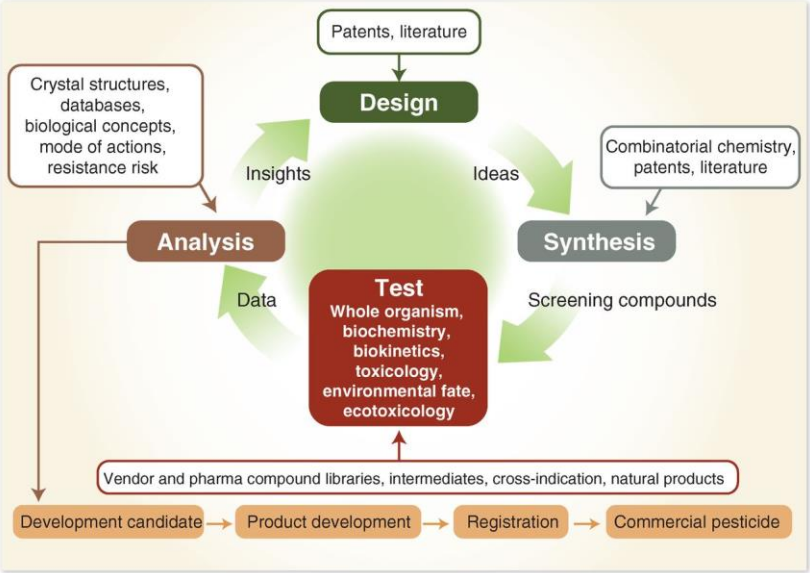

Figure 1.1 Development process of agrochemicals[12]

Structure-based design is a well-known approach to the development of active substances in pharmaceutical companies. Conversely, this is relatively new in agrochemical industries. However, in recent years, this approach demonstrated its usefulness. The development of scytalone dehydratase inhibitors as fungicide represent on the most detailed examples of application of structure-based design in crop protection.[11] Other techniques like fragment-based design, virtual screening and gene sequencing developed in pharmaceutical industry could be exploited also by agrochemical industry to improve and to speed up the process of discovery of new lead candidates.[12]

1.2.3 Natural compounds in development of pesticides

Natural products have been, and still are, valuable tools either in pharmaceutical and agrochemical research and development. In particular, their importance is related to their role as model compounds in the discovery of new active molecules. A good example of the importance of natural compounds in agrochemicals discovery is represented by the development of strobilurin fungicides from natural products produced by fungi. [13]

The value of natural compounds in the agrochemicals discovery arises from the combination of useful properties. These compounds are the result of a coevolution of the producing organism with the biotic component of its environment. Thus, this continuous adaptation has led to products with physico-chemical properties particularly suitable to perform *in vivo*, although the environmental half life of natural compounds is generally shorter than half life of synthetic compounds and this might reduce the environmental impact. Furthermore, natural

compounds are often featured by structural diversity and complexity greater than the structures generated by chemists, allowing a greater exploration of the chemical space and increasing the possibility to obtain lead structure.[14]

Whereas the chemical complexity represents a useful source of chemical diversity, on the other hand, it represents a limitation to a natural products-based discovery and development process. Indeed, the chemical structure of natural products is generally complex, often with multiple stereogenic centres, which make their synthesis particularly challenging. This complexity reduces the "druggability" of natural-derived compounds, complicating the conversion of many compounds in products suitable for the use as pesticides.

Many natural compounds possess a short environmental half-life. Despite the advantages linked to a reduction of environmental impact, this also represents an important limitation to the direct use as pesticides. Furthermore, many natural products often do not have the right physicochemical, stability, activity, and toxicity properties, and present issues related to the production. For these reasons, natural compounds are generally used as models and subjected to several steps of chemical modifications to obtain compounds with optimized structures, activities, and properties.[13]

Quite often, the amount of easily available natural compounds is very low, and this is an important limitation both for their use as such and for the discovery and development studies. Thus, the development of appropriate synthetic protocols is pivotal to fully exploit the potential of natural products.

1.2.4 Dual-target pesticides

Traditionally, drug discovery was oriented on the design of agents targeting a single biological structure with high selectivity to avoid unwanted effect arising by binding with other biological entities. However, the complexity of diseases like cancer, metabolic disorder and central nervous system pathologies demonstrated that a single agent treatment is inadequate to obtain significant therapeutic effects. This has led the concept of dual(multi)-target drugs, first enunciated at the beginning of 2000, to become a well-known strategy to the development of new active substances. Simultaneous intervention on two or more targets has demonstrated to improve the therapeutic efficacy. Dual-target drugs can modulate two receptors, inhibit two enzymes, act on an enzyme and a receptor, or affect an ion channel and a transporter.

Multi-target compounds can be designed by combination of different structures into a new chemical entity. Thus, multi-target compounds derive by the linkage of pharmacophores of

distinct molecules. Pharmacophores with similar structure could be fused or merged depending on the overlapping degree. Conversely, pharmacophores with great structural diversity could be conjugates with a cleavable or non-cleavable linker, although this strategy often leads to compounds with poor drug-likeness properties. The essential requirement of multi-target compound is the retain of the ability to interact with the different targets. Thus, it is pivotal to take into account the structure-activity relationship that underpin the interactions of the parent compounds with their specific target. [15]

The concept of dual(multi)-target agents could be exploited for the discovery and development of new pesticides. A dual(multi)-target agrochemical might express a synergistic activity then an increased efficacy and consequent reduction of the rates of application can be envisaged. Furthermore, activity on two or more target might reduce the risk of resistance. Despite of the possible advantage, this approach is almost unexploited in the field of agrochemicals. Indeed, up to now only one synthetic study directed to the synthesis of a dual-target insecticides is reported in scientific literature.[16]

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2 AIM OF THE THESIS

The aim of the present Ph.D. thesis was the discovery and development of new agrochemicals. In particular, the research activity was focused on natural and natural derived molecules because of the demonstrated importance of natural compounds as starting material in the process of research and development of agrochemicals and other bioactive molecules.
The research project has been divided into two part, as described below.

Part I

The first part of this study was focused on the development of dual-target fungicides obtained combining the natural-derived fungicides of the class of strobilurins with compounds of the class of succinate dehydrogenase inhibitors. With this work we intend to demonstrate that the design of dual-target active compounds is a valuable approach to the discovery and development of new agrochemicals. In this context, five different activities were scheduled:

I. Design of hybrid compounds based on the reported structure-activity relationship of both classes of fungicides;
II. Development of a synthetic strategy for the preparation of the designed compounds;
III. Evaluation of the *in vitro* antifungal activity of the synthetized compounds;
IV. Investigation of the mechanism of action *in silico* and in cell-free *in vitro* systems;
V. Modification of the structure, according to the results of points III and IV, to improve the activity and subsequent assessment of the activity of the newly synthetized compounds.

Part II

In the second part different application of samarium (II) iodide were studied in order to develop novel alternatives for the synthesis of bioactive compounds. In this context, two different activities were scheduled:

I. Development of a Sm(II) iodide-mediated Reformatsky reaction of Evans's *N*-acyl-oxazolidinones with terpene aldehydes as key step for the synthesis of cembranoids
II. Development of a synthetic approach towards the total synthesis of the natural herbicide auscalitoxin aglycone involving a Sm(II) iodide-mediated asymmetric pinacol coupling.

The first of these activities was carried out under the supervision of professor Sabine Laschat at the Institut für Organische Chemie, Universität Stuttgart (Stuttgart, Germany).

PART I: SYNTHESIS OF DUAL-TARGET AGROCHEMICALS

3 DESIGN AND DEVELOPMENT OF SDHI-STROBILURIN AS DUAL TARGET FUGICIDES

3.1 Introduction

As discussed in **Chapter 1**, the design of hybrid bifunctional compounds could represent a promising alternative for the discovery and the development of new agrochemicals.

In this Chapter are reported the results of our efforts to develop hybrid fungicides combining the pharmacophoric features of strobilurins and those of SDH inhibitors. The main reason behind the choice of these two classes of fungicides is related to their mechanism of action. Indeed, both classes act at the level of mitochondria, and in particular by inhibition of enzymes involved in the mitochondrial electron transport chain. More importantly, both the classes interfere with the reduction or the oxidation of ubiquinone. Thus, the design of this hybrid compound should be easier since the two classes share the same sub-cellular site of action and act as competitive antagonists for the same substrate.

3.1.1 Strobilurins

Strobilurins are a class of natural substances with fungicidal activity, mainly isolated from saprophytic basidiomycete fungi such as *Strobilurus tenacellus* (Pers. ex Fr.) Singer and *Oudemansiella mucida* (Schrad. ex Fr.) Hohn. Strobilurin A **3.1** was the first isolated compound of this class. All compounds contain a methyl (*E*)-β-methoxyacrylate group, generally linked to a 5-phenylpenta-2,4-dienyl chain with different substitutions on the phenyl ring at the positions 3 and 4.[1,2] These molecules show a very high photolability, due to the extended double bonds conjugation, which strongly limits their application as fungicides. In order to solve this problem, the first research was focused on the replacement of the dienyl system. The modification of the double bond *proximal* to the methyl β-methoxyacrylate group led to the active stilbene derivative **3.2**. Replacement of the other double bond of **3.2** led to the active naphthalene derivative **3.3**. The *distal* double bond can be replaced as well, leading to the antifungal diphenyl **3.4**.

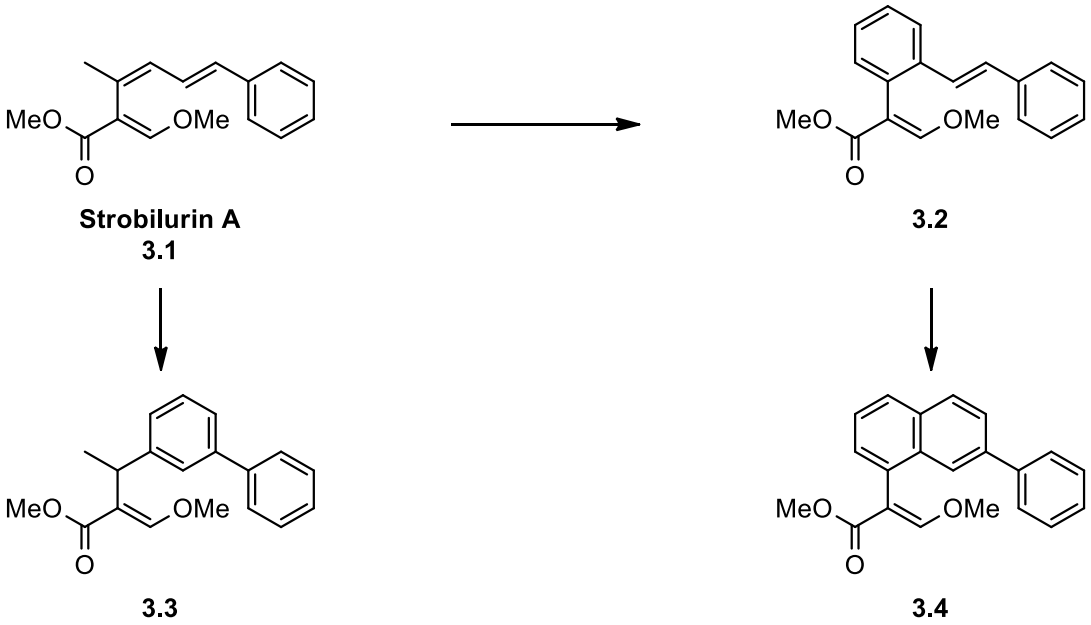
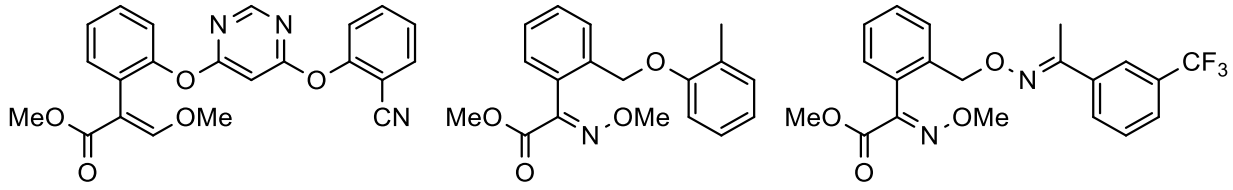


Figure 3.1 Modification of strobilurin A by double bond replacement

Since also the stilbene derivative **3.2** has been found to be not photostable, further optimization of the structure was made to replace the styryl group with different aryl ethers side chains. The result of these efforts was the discovery of azoxystrobin **3.5**, that became first strobilurin fungicide launsched on the market. Further studies demonstrated that strobilurin fungicides tolerate a great variety of different side chain and the most common used in the commercial compounds are aryl ethers, aryl alkyl ethers and oxime ethers.

Many efforts in the optimization works were made in finding moieties that could replace the methyl β-methoxyacrylate group. It was found that the conversion into methoxyiminoacetate (as in **3.6** and **3.7**) by bioisosteric replacement of the β CH with a nitrogen atom is compatible with the activity. Further replacement of the methyl β-methoxyacrylate group with other moieties such as methoxyiminoacetamide, and methoxycarbamate pharmacophores has also been uncovered. It was demonstrated that the side chain should be linked at the *ortho* position of the phenyl ring bearing the methyl β-methoxyacrylate group as the presence of the side chain in *meta* or *para* position generally leads to the loss of activity.[3] The ortho-substitution pattern seems to be essential for twisting the pharmacophoric group outside the plane of the phenyl ring due to steric hindrance, as demonstrated by X-ray crystallographic mesauremets.[4]



Azoxystrobin **3.5** | **Kresoxim-methyl** **3.6** | **Trifloxystrobin** **3.7**

Figure 3.2 Examples of commercially available strobilurin fungicides

Strobilurins, pyribencarb, and the commercially fungicides famoxadone and fenamidone are classified in the same resistance group (FRAC group 11) by the Fungicide Resistance Action Committee (FRAC).[5] Despite the structural differences between the strobilurins and the other compounds, all of them are able to bind the quinone outer (Q_o) binding site of cytochrome bc_1 complex (complex III) in the inner mitochondrial membrane. Strobilurins and the other fungicides of this group bind the ubiquinol oxidation site of cytochrome *b* located on the outer part of the mitochondrial inner membrane. The occupation of the Q_o site does not hamper the binding of the ubiquinol but prevents the oxidation of the quinol and the transfer of the electrons to the cytochrome c_1. The block of the electron transfer leads to the block of the oxidative phosphorylation and the production of ATP.

Strobilurins are broad-spectrum fungicides with protectant activity against several plant pathogenic fungi and oomycetes. Some of strobilurins possess systemic, translaminar and protectant activity.[6]

Unfortunately, strobilurins and the other Qo inhibitors are prone to the onset of resistance. The main mechanism of resistance to these fungicides involves the mutation of the target. The most common mutations observed are the substitution from glycine to alanine at position 143 (G143A), from phenylalanine to leucine at position 129 (F129L), and from glycine to arginine at position 137 (G137R). It is noteworthy that expression of G143A mutated protein is correlated to total expression of resistance. Other mechanisms of resistance to strobilurins, such as expression of alternative respiration or expression of efflux transporters, were observed.[2]

3.1.2 SDH Inhibitors

The class of succinate dehydrogenase (SDH) inhibitors includes compounds originally called carboxamides since the presence of an amide moiety is pivotal for the activity. The first compound of this class, carboxin **3.8**, was launched on the market in 1966 and was characterized by a narrow-spectrum of activity and limited applications, mainly as seed

treatment. Further developments have led to compounds such as flutolanil **3.9** and mepronil **3.10** with slightly broader-spectrum of action, but it was boscalid **3.11**, launched in 2003, the first compound of this class with a true broad-spectrum of action. More recently, the research trends were focused on the development of pyrazole-4carboxamides. In particular, compounds such as fluxapyroxad **3.12**, containing a difluoromethyl group in position 3 of the pyrazole ring, have gained a considerable attention for their broad-spectrum of activity.[7] Fluopyram **3.13** is the only SDH inhibitor belonging to the subclass of the pyridinyl-ethyl benzamides. With the discovery of this compound it was demonstrated that the rigid anilide moiety is not essential for the activity. Notwithstanding the lack of activity against Oomycetes and Basidiomycetes, it shows an outstanding activity against a great number of Ascomycetes species with lower application rates than other SDH inhibitors.[8]

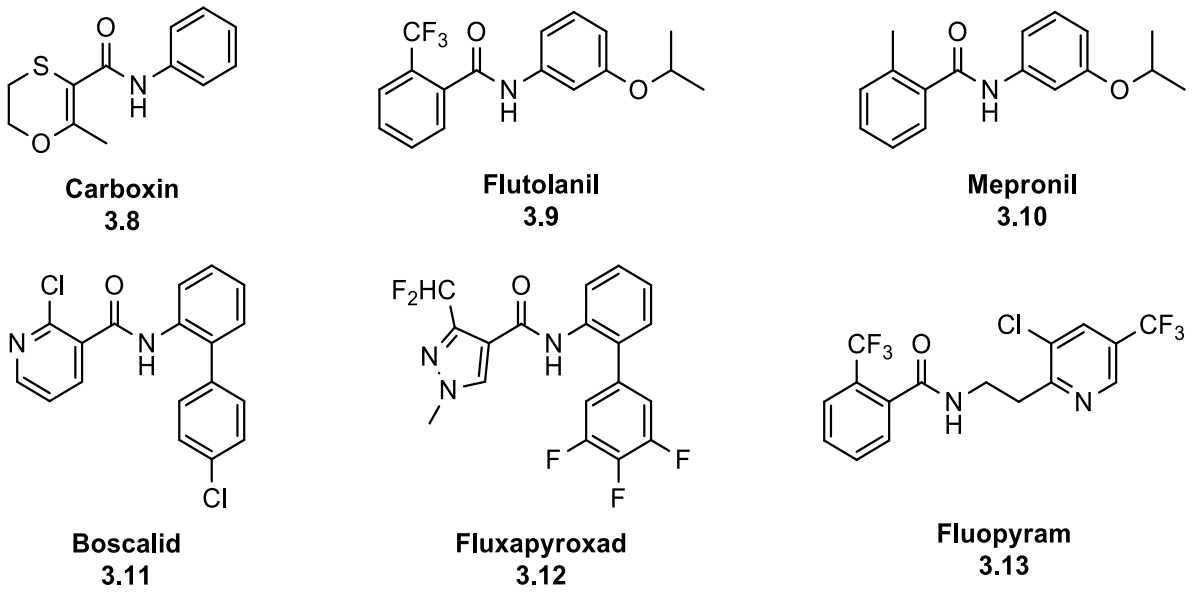


Figure 3.3 Examples of commercially available SDH inhibitors

Despite the chemical diversity exhibited, all SDH inhibitors share a similar chemical structure, characterized by the presence of the central amide group. As highlighted in **Figure 3.4**, a five- or six-membered ring is usually linked to the carbonyl side of the amide group. This system is essential for the binding to the target and for *in vivo* potency. On the amino side of the amide group, it is possible to distinguish two portions: the linker and the hydrophobic rest. The linker is often represented by an *ortho*-substituted phenyl ring, but the replacement with an heteroaromatic ring or an ethyl bridge is possible. The main function of the linker is to maintain the right geometry between the amide group and the hydrophobic

rest, that is partially exposed at the surface of the enzyme, having an important role in order to determine the potency of action and the spectrum of activity.[9]

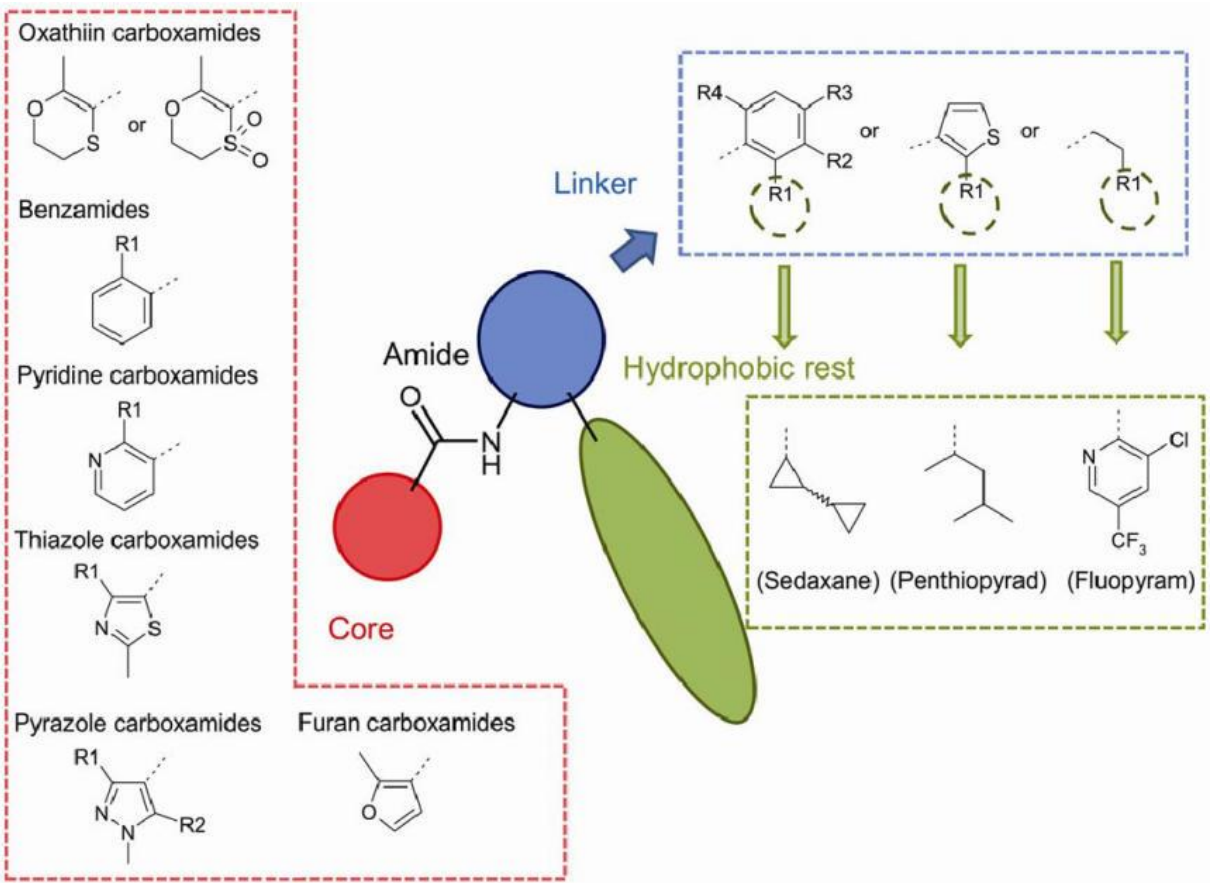


Figure 3.4 Schematic overview of chemical structures of SDH inhibitors[9]

SDH enzyme, also called complex II, represent the contact point between the Krebs cycle and the mitochondrial respiration as it couples the oxidation of succinate to fumarate with the reduction of ubiquinone derived from the pool of the respiratory chain.[10] All compounds belonging to the class if SDHI bind the ubiquinone-binding site hampering the access of ubiquinone. The presence of the inhibitor does not permit to ubiquinone to act as electron acceptor preventing further oxidation of the succinate. The binding mode of the SDH inhibitors is depicted in **Figure 3.5**. H-bond mediated interactions between the amide group and the strictly conserved Trp124 and Tyr130 characterize the binding of most of SDH inhibitors.[9]

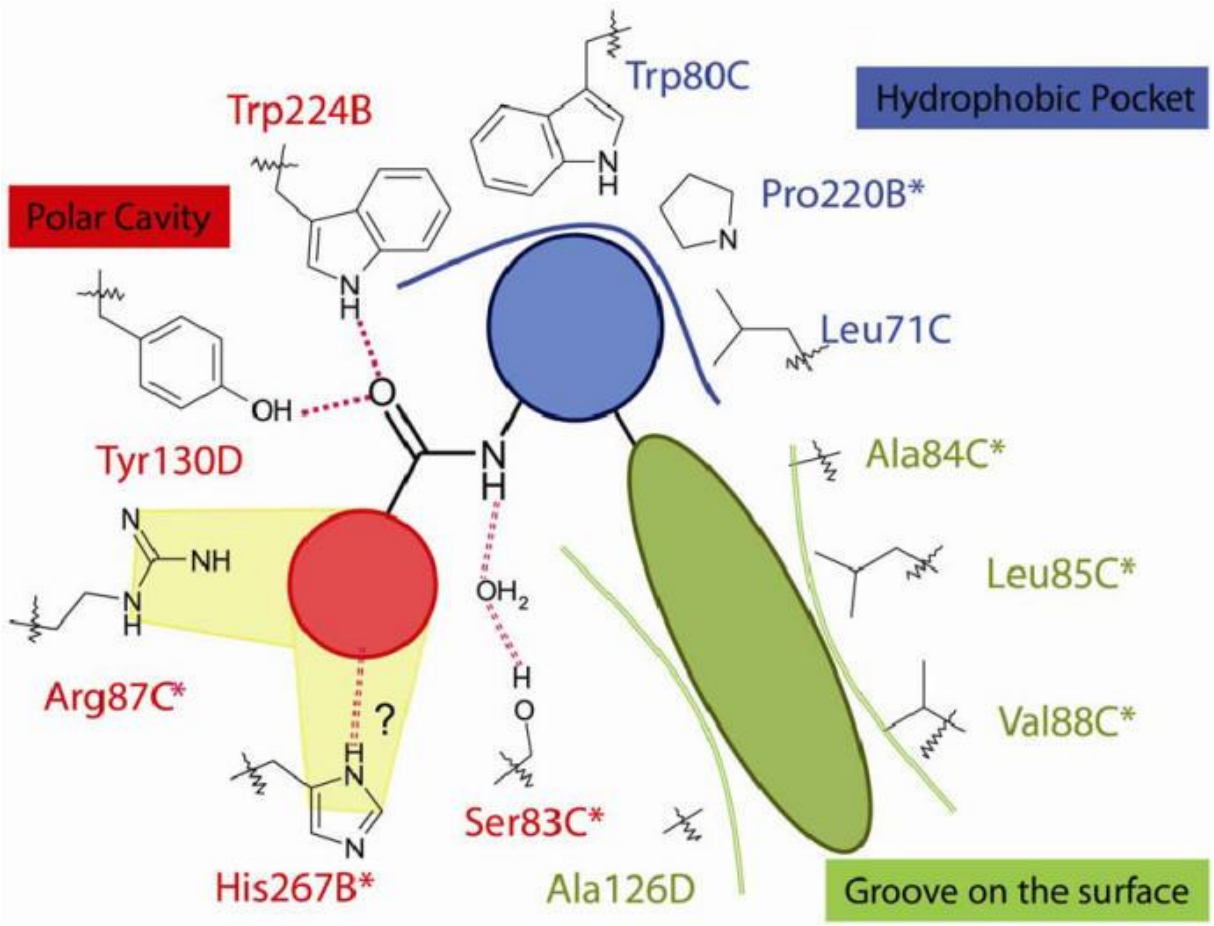


Figure 3.5 Schematic view of binding mode and intermolecular interaction of SDH inhibitors inside *Mycosphaerella graminicola* ubiquinonebinding site[9]

3.2 Results and discussion

3.2.1 Design of hybrids

The dual target fungicides were designed taking into account the structure activity relationships discussed above for both classes of fungicides. The strobilurin trifloxystrobin **3.7** and the SDH inhibitor fluopyram **3.13**, as highlighted in **Figure 3.6**, were chosen as model compounds for the design of the hybrids. The methyl β-methoxyacrylate moiety of strobilurins was incorporated into a substituted benzamide, which is considered the pharmacophore of SDHI. The phenyl ring directly connected to the methyl β-methoxy acrylate to exert the desired activity tolerates only one substitution in *ortho* position. This substituent acts as a linker group, and we perceived it as a flexibility element to obtain a better adaptation in binding both the enzymes. Since the identification of the outdistancing between the two pharmacophores is a crucial step in the design, initially we chose a spacer group of four atoms (including the carboxamide group) between the aromatic ring of SDH inhibitors and the phenyl ring bearing the β-methoxy acrylate. In this way, the distance between the aromatic rings are comparable to the distance of the aromatic rings in the two model compounds.

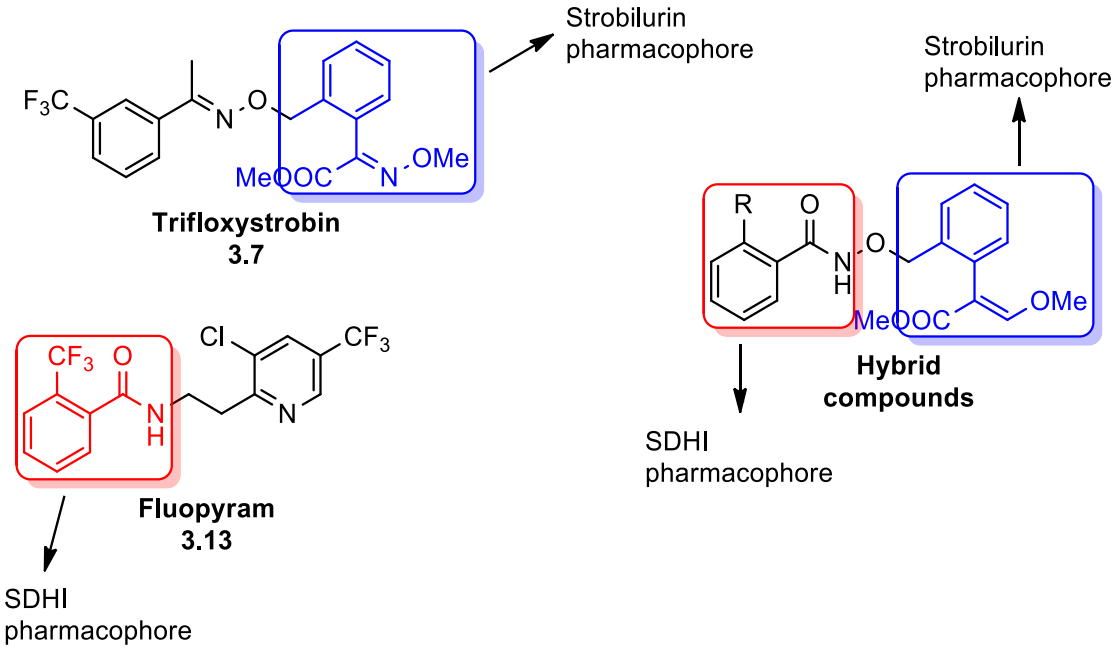


Figure 3.6 Design of hybrid compounds

The oxygen atom in the linker group of strobilurins was maintained and incorporated into the spacer group of the hybrids. Consequently, the hybrid compounds were characterized by the

presence of an *ortho* substituted arylhydroxamate moiety linked by a methylene group to an aromatic ring bearing the methyl β-methoxy acrylate.

SDH inhibitors show a great variability in terms of aromatic and heteroaromatic rings directly linked to the carboxamide moiety. Thus, the design was limited to the development of three hybrids compound incorporating the aromatic rings of the commercial fungicides mepronil **3.10**, boscalid **3.11**, and fluopyram **3.13**.

3.2.2 Synthesis of hybrids 3.18 and 3.19a-c

Our synthetic strategy for the preparation of compounds was thought to be a divergent pathway, starting from the pharmacophore of the strobilurins and introducing the variable aromatic rings of SDH inhibitors in the end.

The pharmacophoric moiety of strobilurins was prepared starting from the commercially available methyl β-methoxy acrylate **3.14**, which was converted in good yield into the α-iodo-β-methoxy acrylate **3.15** following the procedure reported by Coleman and Lua in 2006.[11] Then, the methyl β-methoxy acrylate group was linked to the *ortho*-methyl phenyl ring by a Suzuki-Miyaura cross-coupling with the commercially available 2-methyl-1-phenylboronic acid **3.20**, in presence of 4 mol% of $Pd(PPh_3)_4$ and K_3PO_4 as base.[12] Despite the good yields obtained, the amount of catalyst used made this protocol non-suitable for the scale up of the reaction on gram scale. The reaction was repeated using 250 mg of compound **3.15** with decreasing amounts of catalysts to test if a further optimization of the reaction condition was possible. We observed that the catalyst can be reduced up to 0.5 mol% without significant variation in yield and reaction time. Further reduction of the loading of catalyst led to prolonged reaction time and incomplete conversion of the starting materials. With these results in hand, the optimized conditions were successfully applied for the scale up of the reaction. The benzyl bromide **3.17** was prepared by radical bromination starting from compound **3.16** in presence of NBS and AIBN.[13] *N*-Hydroxyphthalimide was used as hydroxylamine equivalent for the synthesis of the hydroxamate moiety of the hybrid compound. The nucleophilic substitution of benzyl bromide **3.17** with *N*-hydroxyphthalimide was achieved in presence of K_2CO_3 as a base.[14,15] Compound **3.18** was obtained in unsatisfactory yield (40 %), thus a screening of different bases was performed to increase the yield of the reaction. Organic bases, like triethylamine and Hünig's base, performed similarly to K_2CO_3 affording compound **3.18** in 38 % and 35 % yield, respectively. The use of sodium and potassium hydroxides has led to poor yield because of the competing hydrolysis of the methyl ester on the methoxy acrylate. The replacement of K_2CO_3 with Na_2CO_3 or Cs_2CO_3 has

not led to significant changes in the reaction outcome. Furthermore, no changes were observed after addition of potassium iodide or TBAI in catalytic amount (0.1 eq.).

The phthalimido-derivative **3.18** was deprotected by hydrazinolysis and the unstable *O*-benzyl hydroxylamine was used in the acylation reactions without purification. The reactions of acylation were performed in presence of pyridine with the acyl chlorides **3.21a-c**.[15] The three acyl chlorides **3.21a-c** were previously prepared from the respective acids following reported procedures.[16] The three hybrid compounds **3.19a-c** were obtained in 26 %, 32 % and 24 % yield, respectively. The yields of the last three reaction were poor because of the instability of the *O*-benzyl hydroxylamine. Indeed, the main products of the reactions was the cyclic hydroxamate derived by nucleophilic acyl substitution on the ester of β-methoxy acrylate.

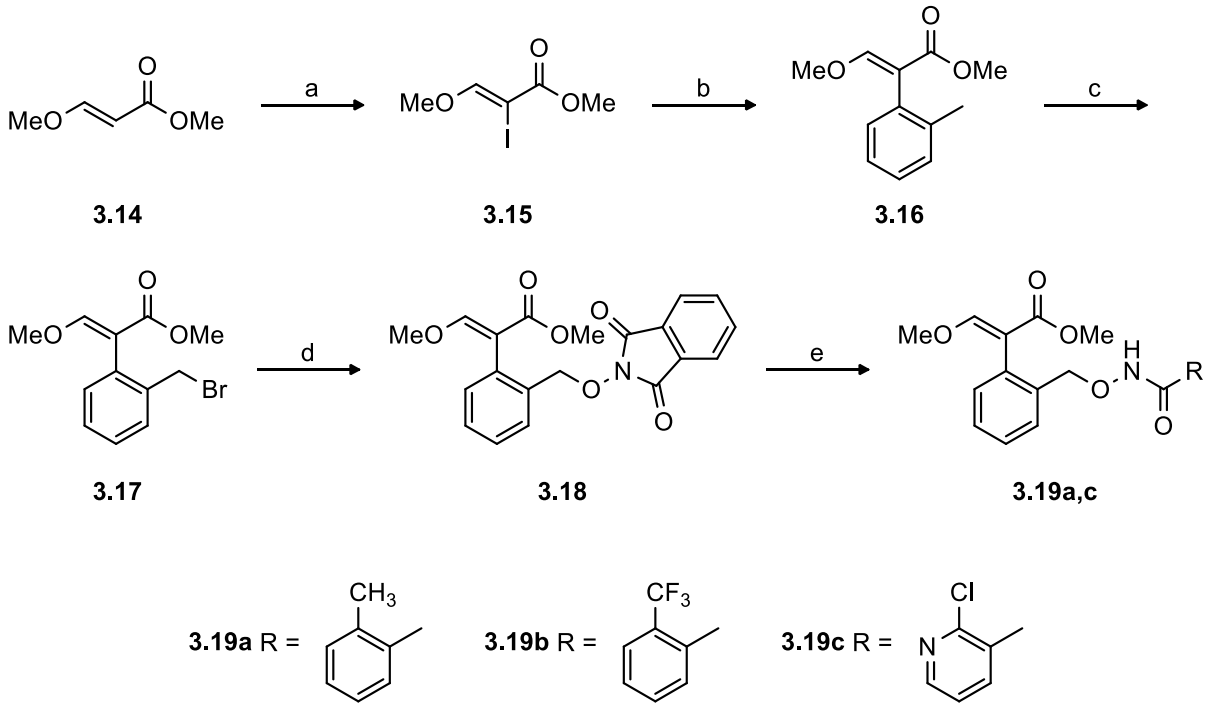


Figure 3.7 Synthesis of compounds **3.18** and **3.19a-c**. (a) i. NIS, AcOH, DCM, rt; ii. Et_3N, CH_2Cl_2, rt, 70 %; (b) 2-methyl-1-phenylboronic acid (**3.20**), $Pd(PPh_3)_4$, K_3PO_4, dioxane/H_2O, 90 °C, 83 %; (c) NBS, AIBN, CCl_4, 78 %; (d) N-hydroxyphthalimide, NaOH, DMF, rt, 40 %; (e) $NH_2NH_2{\cdot}H_2O$, EtOH, rt; (f) **3.21a-c**, py, CH_2Cl_2, 0 °C to rt, 24-39 %.

3.2.3 In vitro screening of antifungal activity of 3.18 and 3.19a-c

The antifungal activity of compounds **3.19a-c** was evaluated *in vitro* by inhibition of mycelium growth. Their direct precursor **3.18** could be considered a conformationally blocked analogue, because of the presence of the phthalimido moiety, and was included in the test.

The tested compounds were dissolved in acetone (concentration of the stock solution was 2500 ppm) due to their poor solubility in water.[17] Therefore, malt agar (MA) medium supplement with 1 % acetone was included as control together with untreated MA medium

control. The radial growth of the mycelium was evaluated daily up to seven days after inoculum (dai), except the fast-growing fungus *Sclerotinia sclerotiorum*, whose growth was evaluated only for two days. Results are highlighted in **Figure 3.8**

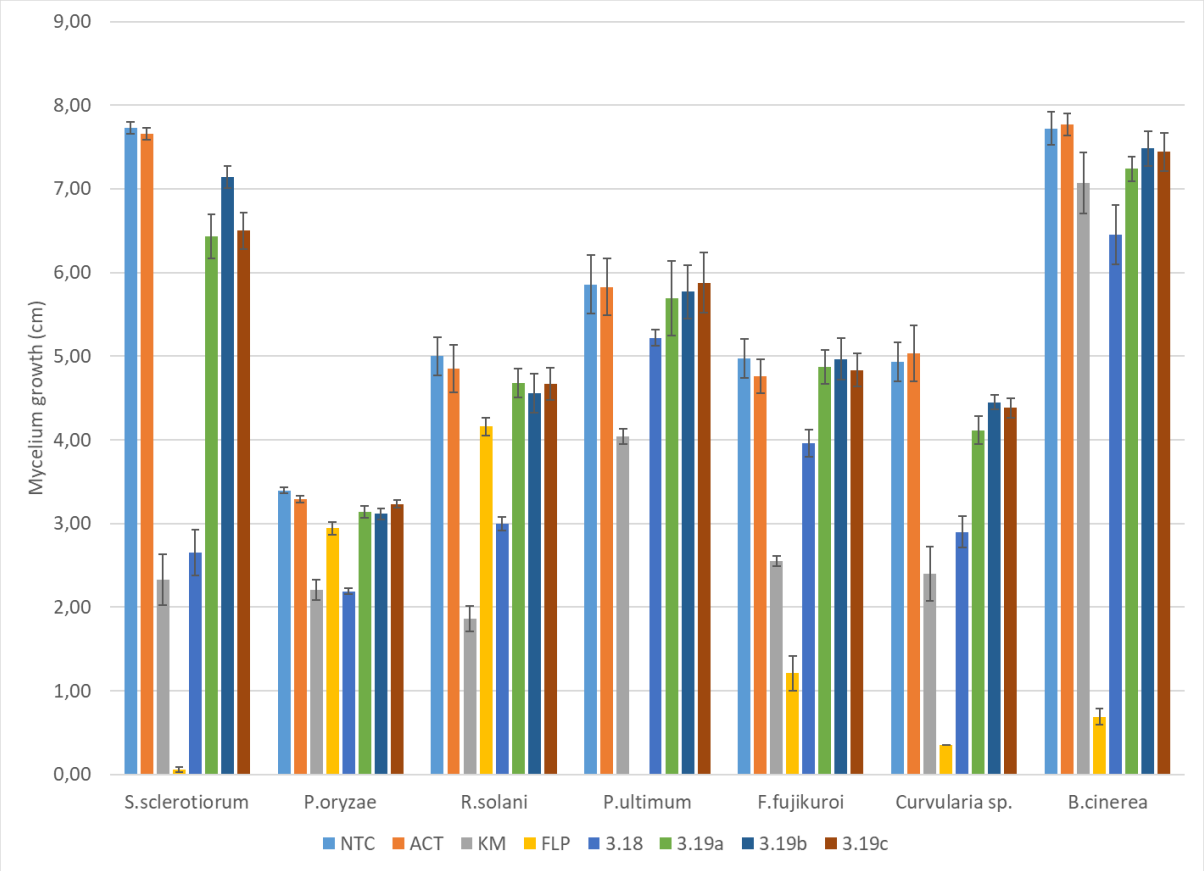


Figure 3.8 Mycelium growth of six fungal species on control media (NTC – non-treated control, ACT – 1 % acetone) and media supplemented with commercial fungicides (KM – kresoxim-methyl **3.6**, FLP – fluopyram **3.13** at concentrations 25 mg/L) and compounds **3.18** and **3.19a-c**, at concentrations 25 mg/L. The measurements were done at 7 dai. The error bars represent standard error (N = 3).

Compound **3.18** showed the highest inhibitory activity, while compound **3.19a-c** showed no or very low activity. In particular, the growth of *Pyricularia oryzae* was inhibited by compound **3.18** by ca. 35 %, and its activity was comparable to the commercial fungicide kresoxim-methyl **3.6**. Moreover, **3.18** inhibited *S. sclerotiorum* and *Curvularia* sp. to the same extent of kresoxim-methyl **3.6**, but less than fluopyram **3.13**.

The rice blast fungus *P. oryzae* is the causal agent of one of the most serious disease of cultivated rice.[18] Each year rice blast is responsible for 10-30 % yield loss of rice at harvest, the equivalent of feeding 60 million people. Furthermore, the fungus is part of the species complex that cause disease on a wide range of economically important crops including barley,

oats, rye grass, millets and, most recently, wheat.[18,19] Thus, the activity of compound **3.18** against this fungus was further investigated using three different concentrations against four strains of *P. oryzae*. The activity of four commercial fungicides, two strobilurins (azoxystrobin **3.5** and kresoxim-methyl **3.6**) and two SDH inhibitors (fluxapyroxad **3.12** and fluopyram **3.13**) was also investigated at two concentrations, 2.5 and 25 mg/mL, respectively. Azoxystrobin **3.5** showed the highest inhibitory activity, while the activity of compound **3.18** at 25 mg/L was better than fluopyram **3.13** and comparable to fluxapyroxad **3.12** at 2.5 mg/L. Results are highlighted in **Figure 3.9**.

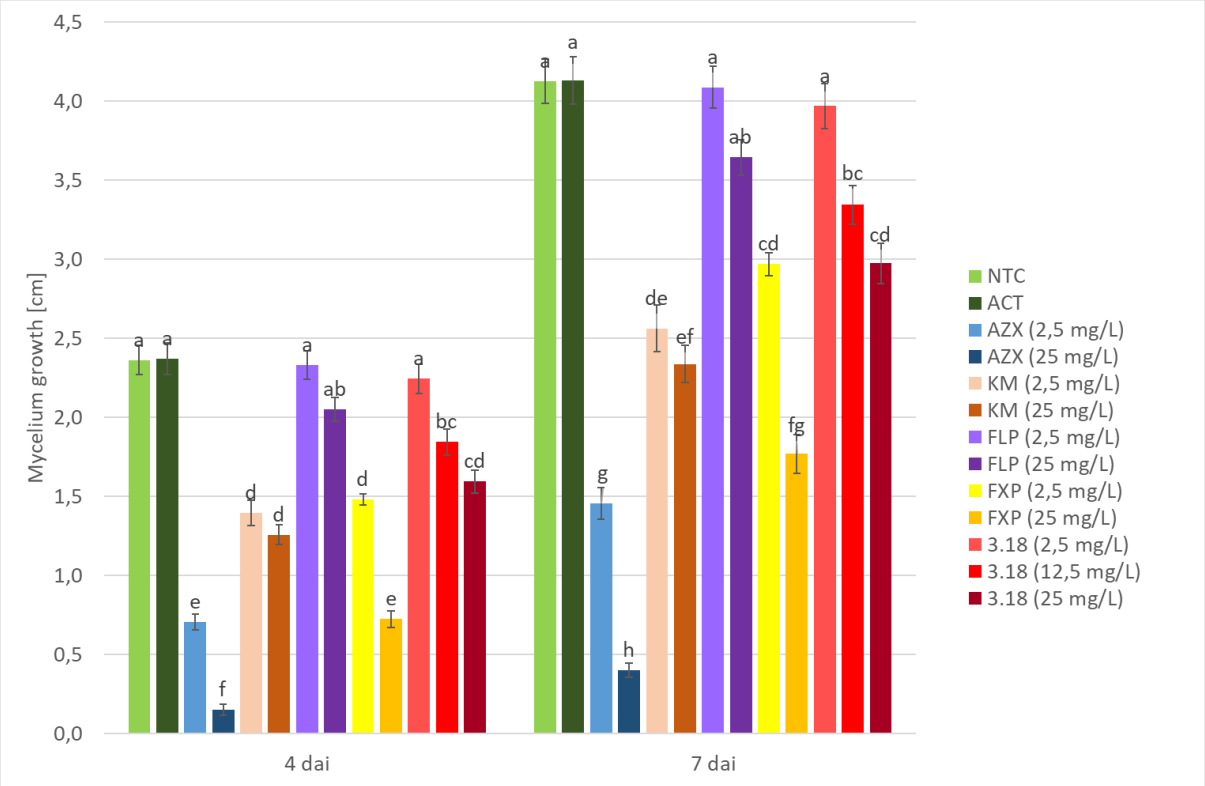


Figure 3.9 Mycelium growth of four *P. oryzae* strains on control media (NTC – non-treated control, ACT – 1 % acetone) and media supplemented with commercial fungicides (AZX – azoxystrobin, KM – kresoxim-methyl, FLP – fluopyram, FXP – fluxapyroxad, at concentrations 2.5 or 25 mg/L) and compound **3.18**, at concentrations 2.5, 12.5 or 25 mg/L. The mycelium growth was measured at 4 and 7 days after inoculation (dai). The error bars represent standard error (N = 8). The mean values with the same letter at each dai measurement are not significantly different (P = 0.05) according to Tukey post-hoc test.

3.2.4 Docking studies

Docking simulations were performed to understand the mechanism of action of compound **3.18**, to explain the lack of activity of compounds **3.19a-c**, and to explore the binding mode of

the new synthesized molecules to both Cyt *b* subunit and succinate dehydrogenase. For both the enzymes the models were built by homology modelling techniques and the docking results were analysed by comparison with known ligands. Considering the importance of *P. oryzae* in crop protection and the promising results obtained with compound **3.18** in the *in vitro* screening for the antifungal activity, a study of the binding mode to Cyt *b* was planned. The available sequence for Cyt *b* of *P. oryzae* is unsuitable to model the binding pocket of the enzyme. Indeed, the sequence comprises a fragment with only 170 residues. The available sequence and the Cyt *b* of *P. grisea* are substantially identical in the common region, suggesting a high identity degree. Therefore, the homology model was based on the primary sequence of the Cyt *b* of *P. grisea* (Uniprot code Q85KP9). The three-dimensional structure of Cyt *b* of *P. grisea* was modelled using as a template the resolved structure of the complex of Cyt *b* with the inhibitor stigmatellin A from *S. cerevisiae* (PDB Id: 3CX5). In the docking studies, all simulated compounds **3.18** and **3.19a-c** showed a binding mode and an interaction pattern similar to that observed for azoxystrobin **3.5**, which was included in the simulation for comparison.

Figure 3.10 compares the poses as computed for **3.18** and azoxystrobin **3.5** and reveals that both complexes are almost exclusively stabilized by hydrophobic contacts. In detail, the (*E*)-methyl β-methoxyacrylate moiety of both molecules is inserted within a highly hydrophobic subpocket, where it interacts with Phe75, Val92, Leu96, Pro218, Leu222, Tyr226, and Leu229 side chains. The phenyl ring elicits π-π stacking with Phe225 and S/π interactions with Met242. The phthalimido ring of **3.18** and the aromatic side chain of azoxystrobin **3.5** are similarly accommodated inside an apolar subpocket and interact with Met71, Ala72, Phe75, Leu76, Ile93, and Phe243. In good agreement with what observed for the resolved complex with stigmatellin A, the computed complexes are completely devoid of polar interactions. On these grounds, the difference in bioactivity seen for the tested hybrids compounds can be mostly ascribed to the capacity of the variable portion to stabilize π-π contacts with aromatic residues. Therefore, the marked activity of **3.18** can be explained by considering the enriched stacking interactions mediated by the phthalimido moiety with Phe243.[20] In the same way, the reduced activity of **3.19b** could be explained by the hampering of the arrangement of Phe75 caused by the steric hindrance of trifluoromethyl group. Furthermore, for compound **3.19c** the loss of activity could be explained by the less effectiveness of pyridine ring in stabilizing T-shaped π-π stacking with Phe75 and to minor extent with Phe125.

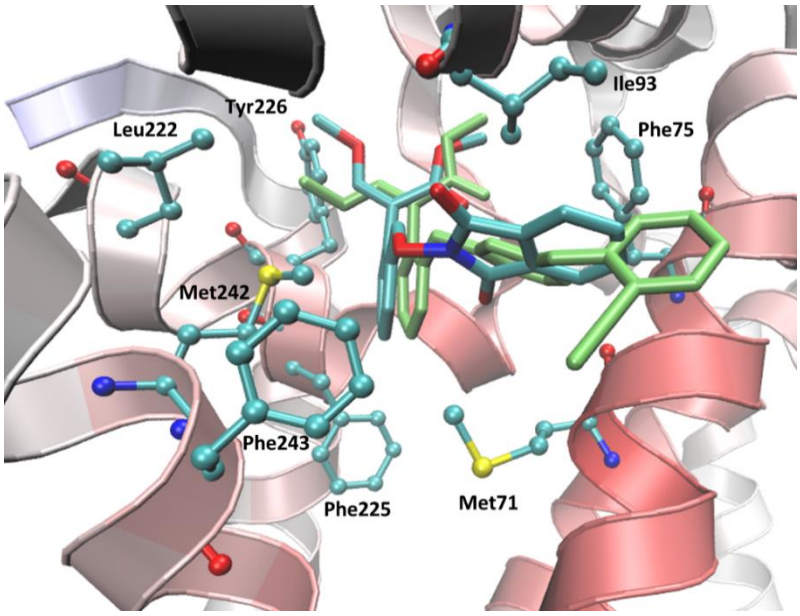


Figure 3.10 Comparison of the putative poses as computed for **3.18** and azoxystrobin **3.5** (colored in light green) within the Cyt *b* binding site

The SDH enzyme is a tetrameric complex composed of four subunit which must be modelled together since the ubiquinone-binding site is located at the interface between the subunits SDHB, SDHC, and SDHD.[21] Since the primary sequence of SDHD from *P. oryzae* was unavailable, the model was based on the primary sequences of SDHA, SDHB, and SDHC from *P. oryzae* (SDHA: G4NE44; SDHB: L7JQS7; SDHC: G4NP5), while the primary sequence of SDHD was obtained from *P. grisea* (Q5G5B2). The three-dimensional structure of the protein was modelled using as template the resolved structure the porcine enzyme in complex with *N*-[(4-tert-butylphenyl)-methyl]-2-(trifluoromethyl)-benzamide (E23).

Figure 3.11 compares the poses as computed for **3.18** and for mepronil **3.10** and reveals a different binding mode for the two molecules. In both cases a charge transfer interaction was observed between a phenyl ring and Arg83, and the presence of the phthalimido ring should reinforce this interaction in the case of compound **3.18**. The other aromatic ring is inserted in a lateral subpocket, and, in the case of mepronil **3.10**, it elicits Trp201 and Trp202 π-π stacking interaction. Conversely, for compound **3.18** the steric hindrance due to the presence of (*E*)-methyl β-methoxyacrylate group determines a significative shift of the phenyl ring to a more lateral position, where it contacts Tyr71 and elicits hydrophobic interactions with Leu76 and Val80. The substituents on the phenyl ring stabilize different interactions because of the different arrangement of the phenyl ring itself. In the case of the mepronil **3.10**, the isopropyloxy group stabilizes a H-bond with His65 while the (*E*)-methyl β-methoxyacrylate group is moved to a more lateral position, where it can interact with Tyr123 and Trp202.

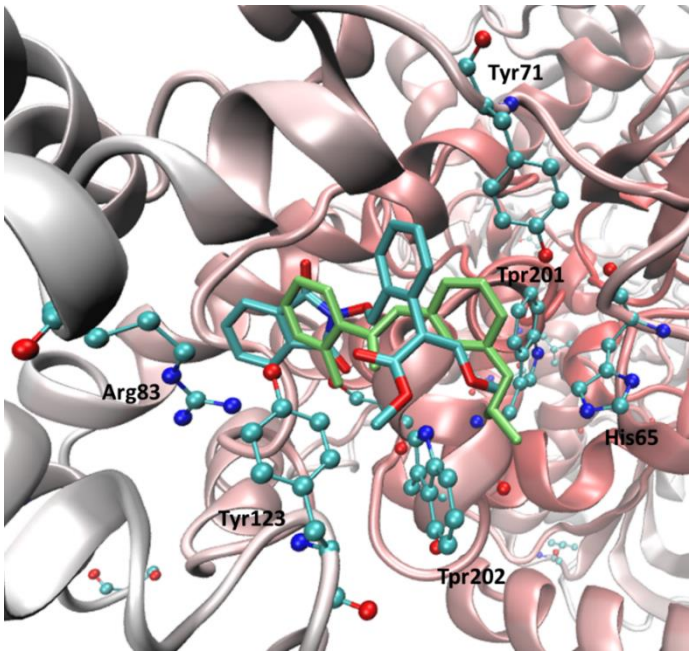


Figure 3.11 Comparison of the putative poses as computed for **3.18** and mepronil **3.10** (colored in light green) within the SDH binding site

The main and most important difference between the two molecules can be seen for the contacts stabilized by the central link. Indeed, the carbonyl function of the amido group of mepronil **3.10** stabilizes two H-bonds with the highly conserved Tyr123 and Trp202, and these interactions characterize the binding mode of most SDHI. Conversely, in the case of compound **3.18**, Tyr123 and Trp202 are shielded by (*E*)-methyl β-methoxyacrylate moiety and cannot conveniently approach the central link. Notably and regardless of the specific arrangement of the phenyl ring bearing the (*E*)-methyl β-methoxyacrylate moiety, the poor contacts stabilized by the central link are observed in all proposed ligands.

3.2.5 Enzymatic inhibition assessment in vitro cell-free system of 3.18 and 3.19a-c

Compound **3.18** was tested for the ability to affect the NADH-driven respiration. For this test, the consumption rate of NADH mediated by the submitochondrial fraction of *P. oryzae* A2.5.2 was measured. The NADH-consumption rate in presence of compound **3.18** (200 μM) was 67.2 ± 7.2 % lower than that measured in the control, containing DMSO (used as cosolvent) without the tested compound. A weaker reduction of the NADH-consumption rate (33.3 ± 6.0 %) for compound **3.18** was still observable reducing the concentration to 20 μM. Azoxystrobin **3.5**, used as positive control, (20 μM) and in the same assay conditions showed a higher inhibition of the NADH-consumption rate (80.7 ± 5.4 %) than compound **3.18**. The ability of compound **3.18** to inhibit the NADH-driven respiration in a cell-free system

supports the hypothesis that the target of this compound is located in the mitochondrial respiratory chain. Results are highlighted in **Figure 3.12**.

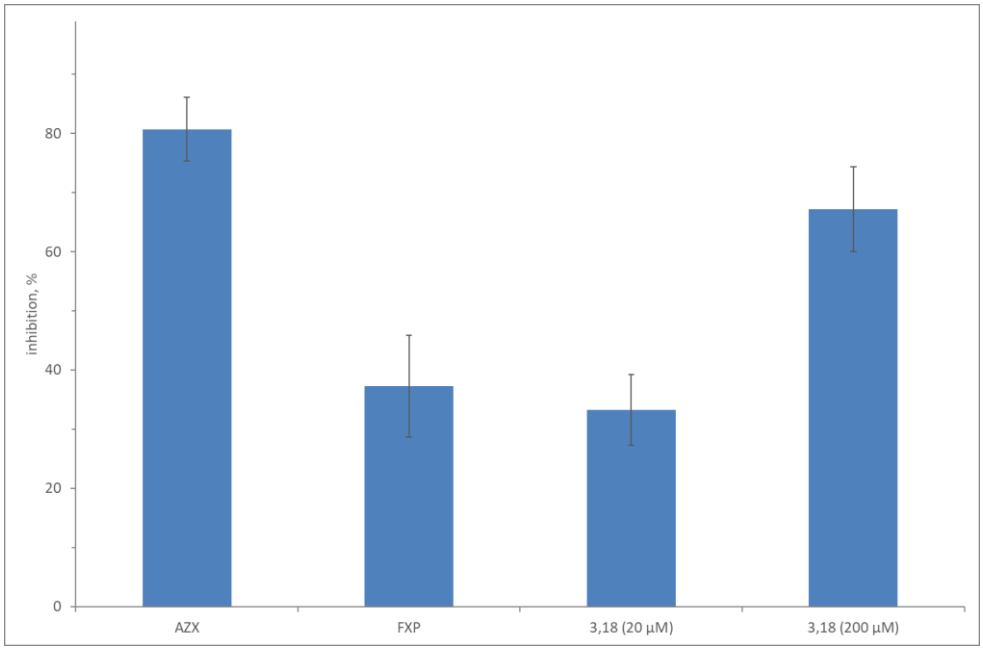


Figure 3.12 Inhibition of the *P. oryzae* A2.5.2 mitochondrial NADH-mediated respiration. Rate of NADH-consumption mediated by the submitochondrial fraction was measured at 30°C (λ = 340 nm) in the presence of AZX – azoxystrobin (20 µM), FXP – fluxapyroxad (200 µM), or **3.18** (20 and 200 µM).

Two specific sets of experiments were designed to assess the expression of the strobilurin-like activity and the ability to inhibit the SDH enzyme of compound **3.18**. The same *in vitro* cell-free system was used for both tests.

The assessment of the DBH_2-Cyt *c* oxidoreductase activity (i.e. the cytochrome *bc*1 complex activity) was used to evaluate the strobilurin-like activity of compounds **3.18**. For this test the reduction rate of Cyt *c* mediated by the submitochondrial fraction of *P. oryzae* A2.5.2 was measured. The Cyt *c*-reduction rate in presence of compound **3.18** (20 µM) was 68.2 ± 13.4 % lower than that measured in the control, containing DMSO without the tested compound. Compound **3.18** at 100 µM tend to reah saturation of the inhibition (83.1 ± 10.9 %). Azoxystrobin **3.5**, used as positive control, (20 µM) and in the same assay conditions showed an inhibition of the Cyt *c*-reduction rate (76.7 ± 11.2 %) similar to that of compound **3.18**. Fluxapyroxad **3.12**, used as negative control, (20 µM) and in the same assay conditions showed an almost not detectable inhibition of the Cyt *c*-reduction rate (6.7 ± 15 %). The obtained results for compound **3.18** support the hypothesis that this compound is able to express a strobilurin-like activity. Results are highlighted in **Figure 3.13**.

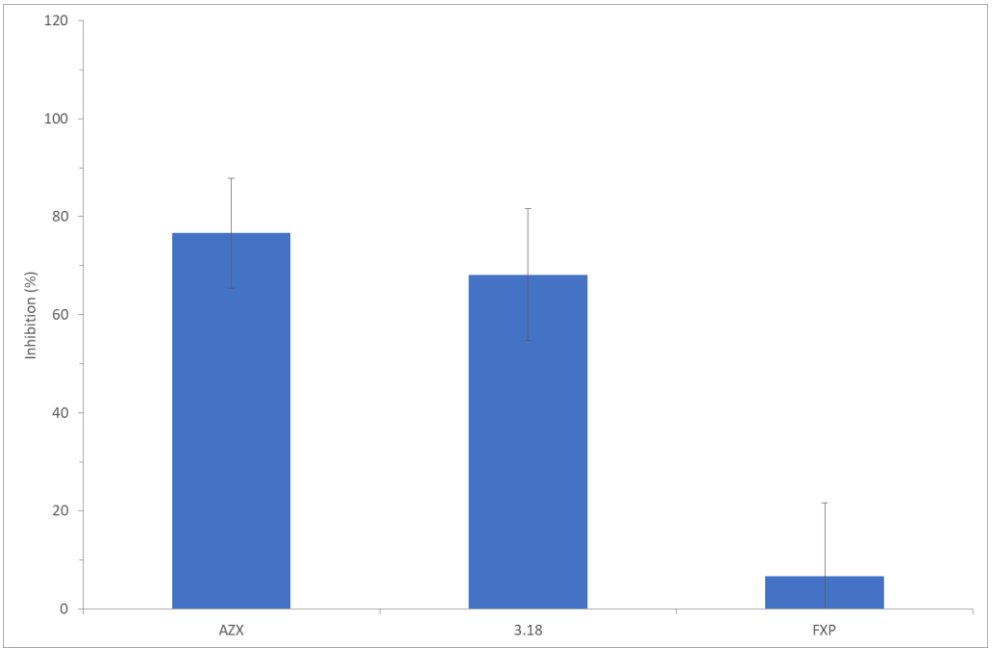


Figure 3.13 Inhibition of the *P. oryzae* A2.5.2 mitochondrial DBH2-Cyt *c* oxidoreductase activity. Rate of Cyt *c*-reduction mediated by the submitochondrial fraction was measured at 30°C (λ = 550 nm) in the presence of AZX – azoxystrobin (20 µM), FXP – fluxapyroxad (20 µM), or **3.18** (20 µM).

Compound **3.18** was tested for the ability to inhibit the SDH activity. For this test, the reduction rate of 2,6-dichlorophenolindophenol (DCPIP) mediated by the submitochondrial fraction of *P. oryzae* A2.5.2 was measured by means of a colorimetric method. Inhibition of SDH activity (65.7 ± 17.4 %) was observed for fluxapyroxad **3.12** (20 µM). Conversely, no inhibition was observed for compound **3.18** and azoxystrobin **3.5**. Results are highlighted in **Figure 3.14**.

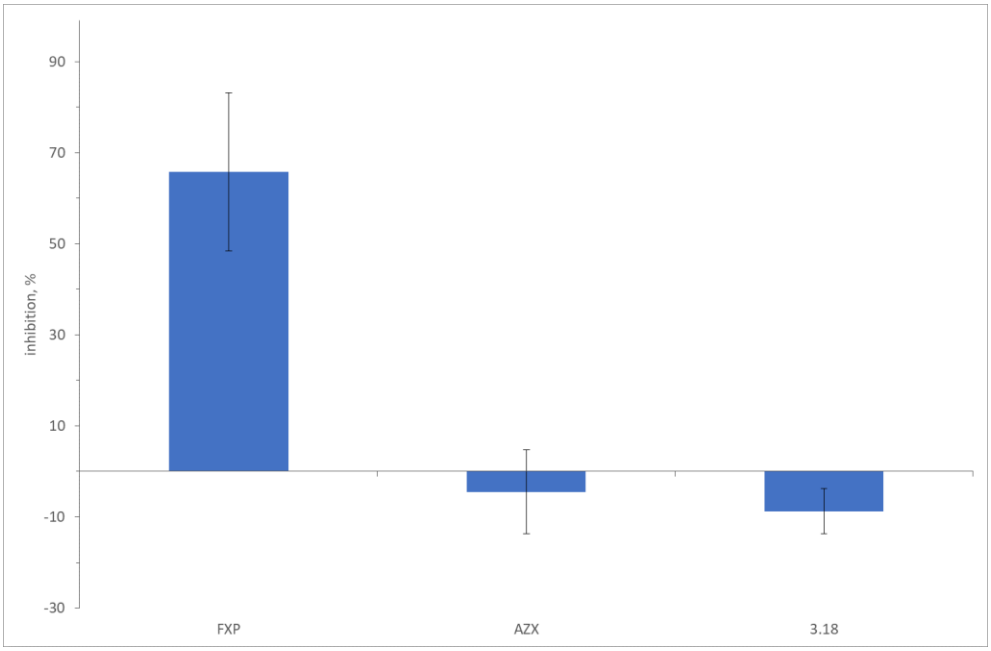


Figure 3.14 Inhibition of the *P. oryzae* A2.5.2 mitochondrial DBH2-Cyt *c* oxidoreductase activity. Rate of DCPIP-reduction mediated by the submitochondrial fraction was measured at 30°C (λ = 595 nm) in the presence of AZX – azoxystrobin (20 µM), FXP – fluxapyroxad (20 µM), or **3.18** (20 µM).

3.2.6 Optimization of the activity

As resulted from the in vitro cell-free enzymatic assay, compound **3.18** seems to exhibit only a strobilurin-like activity. Since the scope of this work was the design and synthesis of dual-target fungicides, the structure of compound **3.18** was modified in an effort to improve the affinity and the activity on SDH enzyme.

The design of the new molecules was based on the information obtained from the docking studies. As discussed above, the binding mode of compound **3.18** to the SDH enzyme differs significantly from that of mepronil **3.10**. The sterically demanding methyl β-methoxyacrylate group seems to interfere with the binding, hindering the hydrogen bonding between the carbonyl of the amide (or the phthalimide ring) with Tyr123 and Trp202.

Initially, we planned to increase the distance between the two pharmacophores to reduce the steric hindrance. Thus, the hydroxamate group was replaced and a phenylene group was inserted between the nitrogen and the oxygen both in compound **3.18** and in compounds **3.19a-c**, as highlighted in **Figure 3.15**.

We observed that most strobilurins, i.e. azoxystrobin **3.5** and kresoxim-methyl **3.6**, possess an aromatic ring linked by a short spacer to the phenyl ring bearing the methyl β-methoxyacrylate. In trifloxystrobin **3.7**, this aromatic ring is replaced by a linker containing a methyleneaminoxy methyl moiety. It was reported that the methyleneaminoxy methyl moiety

could be used as bioisoster of aryl and other aromatic groups.[22] Compound **3.19a-c** were designed using the structure of trifloxystrobin **3.7** as model, however replacing the methyleneaminoxy methyl moiety with a hydroxamate moiety, and this could be the reason for the loss of the strobilurin-like activity. The insertion of a phenylene group could be useful to solve this problem. The resulting compounds **3.23a-c**, as highlighted in **Figure 3.13**, would assume a three rings-based structure similar to that of azoxystrobin **3.5**. In order to select the most promising structures, the new proposed compounds were evaluated in a docking experiment. Compound **3.23d** was designed as flexible analogue of **3.22** where the *ortho*-substituted aromatic ring was replaced by an unsubstituted phenyl ring to exclude every possible interference and limit the differences between the two structure to the change of conformational mobility. No SDH inhibitors with unsubstituted phenyl ring have been launched in the market; it is likely that small substituent linked to the *ortho* position are useful to stabilize the best conformation for the activity. For this reason, compound **3.23d** was not synthetized. Compounds **3.22** and **3.23d** were evaluated in a docking experiments using the computational model of the SDH enzyme model discussed above.

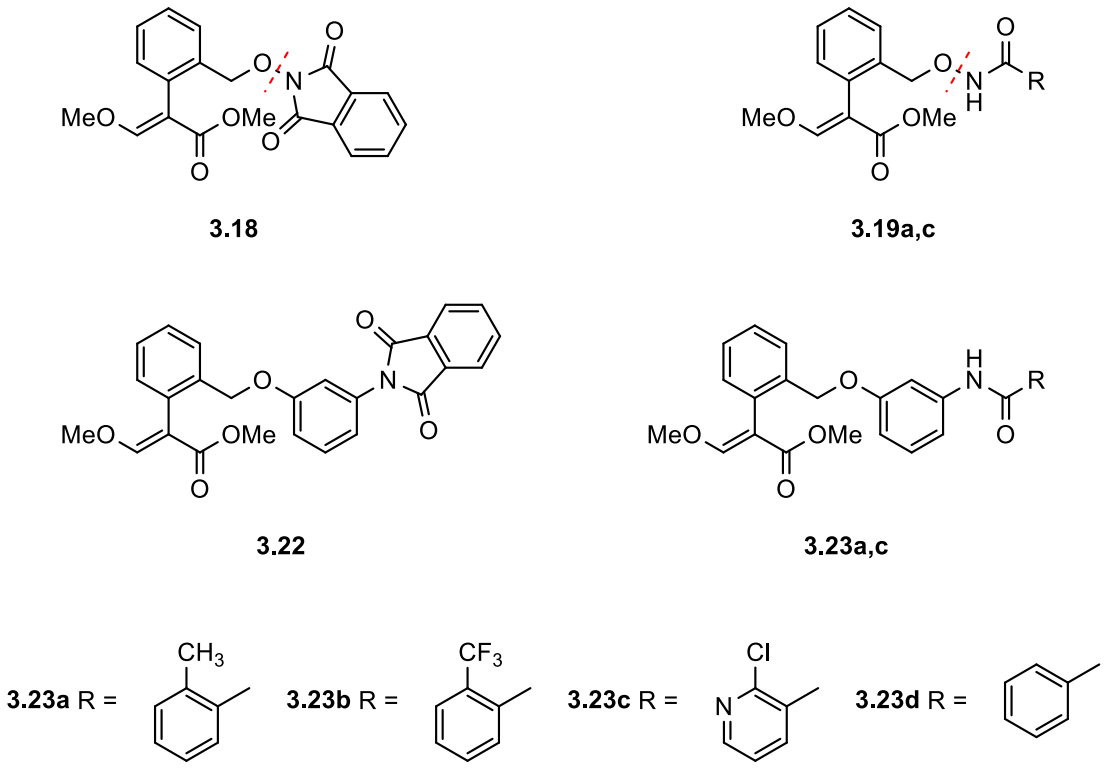


Figure 3.15 Design of hybrid compounds

The structures of compounds **3.23a-c** provided a better score of docking than compound **3.22**. Compounds **3.23a-c** reveal a π-cation interaction between the phenyl ring and Arg83, and its

central link is stabilized by hydrogen bonds with Tyr123 and Trp202. Furthermore, the aromatic ring connected with the (*E*)-β-methoxyacrylate moiety is able to establish π-π interactions with Tyr123, thanks to the addition of the central phenyl ring which acts as a spacer which decreases the (*E*)-β-methoxyacrylate steric hindrance.

In contrast, **3.22** shows only the hydrogen bonds involving the central link as mentioned above and lacks the interaction of the aromatic rings with Arg83 and Tyr123. These differences are probably due to the lower flexibility of **3.22**, which prevents the right fitting within the pocket. The results of docking were reported in **Figure 3.16**.

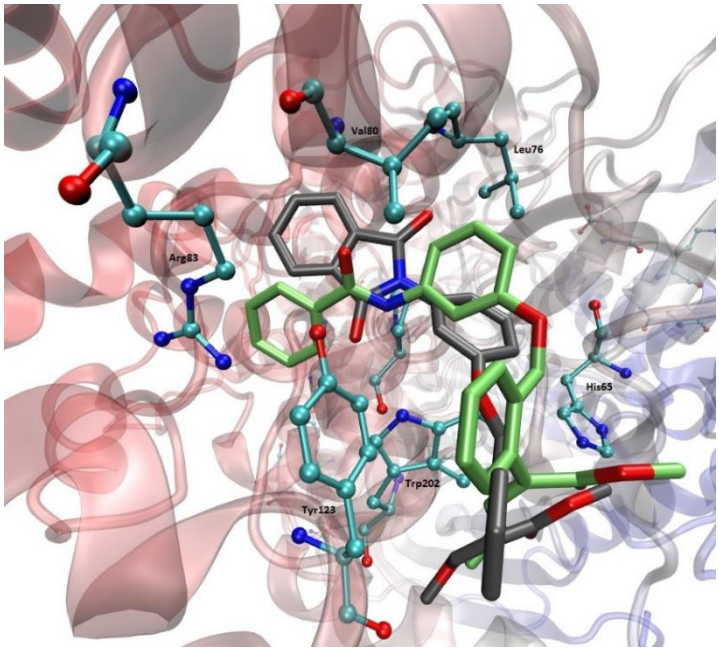


Figure 3.16 Comparison of the putative poses as computed for **3.22** (colored in grey)and for **3.23d** (colored in light green) within the SDH binding site

3.2.7 Synthesis of hybrids 3.23a-c

For the synthesis of compounds **3.23a-c** we followed a divergent strategy similar to that used for compound **3.19a-c**. The synthetic strategy is highlighted in **Figure 3.17**.

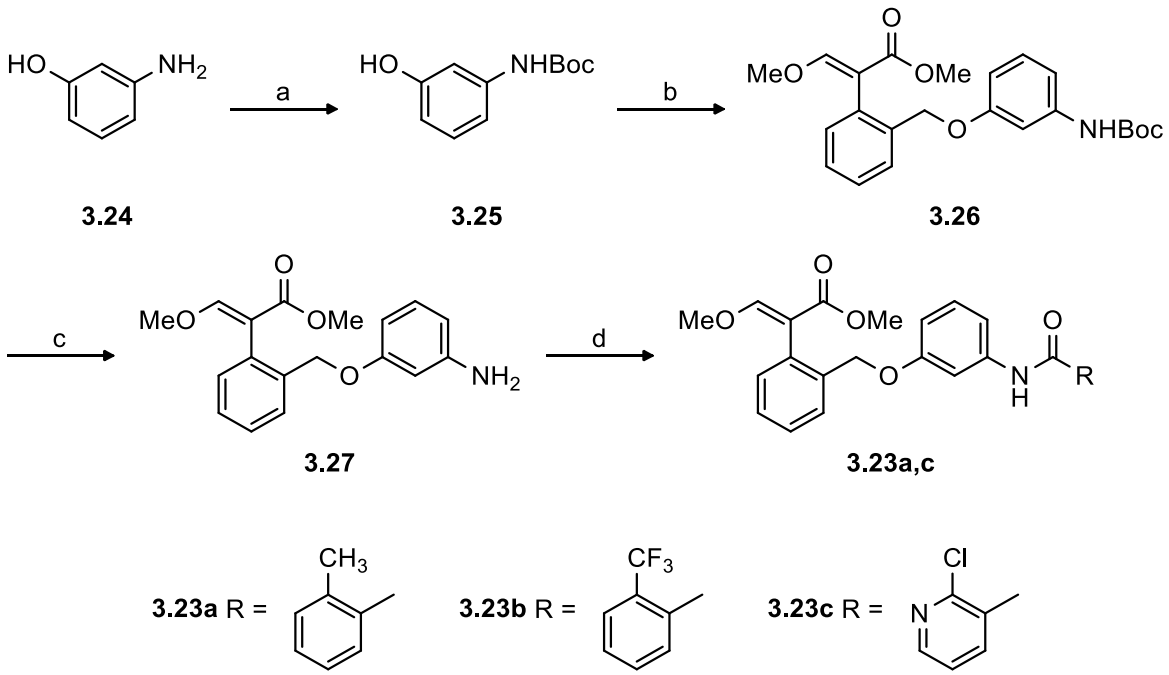


Figure 3.17 Synthesis of compounds **3.23a-c**. (a) $(Boc)_2O$, NaI, CH_2Cl_2, rt; 90 %; (b) **3.17**, K_2CO_3, acetone, reflux, 65 %; (c) 10 % v/v trifluoroacetic acid, CH_2Cl_2, 0 °C, 90 %; (d) **3.28a-c**, EDC·HCl, HOBt, DIPEA, CH_2Cl_2, 0 °C to rt, 65-86 %.

Protection of the commercially available 3-aminophenol **3.24** was performed with $(Boc)_2O$ following a reported procedure. Protected aminophenol **3.25** was obtained in 90 % yield. As described in the literature, the presence of NaI resulted in a dramatic reduction of the reaction time; indeed, complete conversion was obtained after only one hour.[23] Then, the *N*-Boc protected aminophenol **3.25** was alkylated with the benzyl bromide **3.17** in presence of K_2CO_3 as a base, affording compound **3.26** in 65 % yield. Boc deprotection was easily performed with 10 % v/v trifluoroacetic acid in anhydrous dichloromethane. The reaction afforded the trifluoroacetic salt that was converted to the free amine **3.27** during the work up by treatment with $NaHCO_3$ saturated solution. Finally, the amine **3.27** was coupled with the three acids **3.28a-c** providing the desired compounds **3.23a-c**. The coupling was performed with EDC·HCl and HOBt as coupling reagents and Hünig's base. The reaction required an excess of both acid and of coupling reagents to reah completion, affording the three compounds **3.23a-c** in good yields.

3.2.8 In vitro screening of antifungal activity of 3.23a-c

Antifungal activity of compounds **3.23a-c** were evaluated *in vitro* following the same protocol used for compound **3.18** and **3.19a-c**. The tested compounds were dissolved in acetone because of their poor solubility in water. Therefore, MA medium supplement with 1 %

acetone and untreated MA medium were included as controls. Commercially fungicides azoxystrobin **3.5** and fluxapyroxad **3.12** were included together with kresoxim-methyl **3.6** and fluopyram **3.13** as positive controls. The radial growth of the mycelium was evaluated daily up to seven days, except the fast-growing fungus *Sclerotinia sclerotiorum*, whose growing was evaluated after three days. Results are highlighted in **Figure 3.18**.

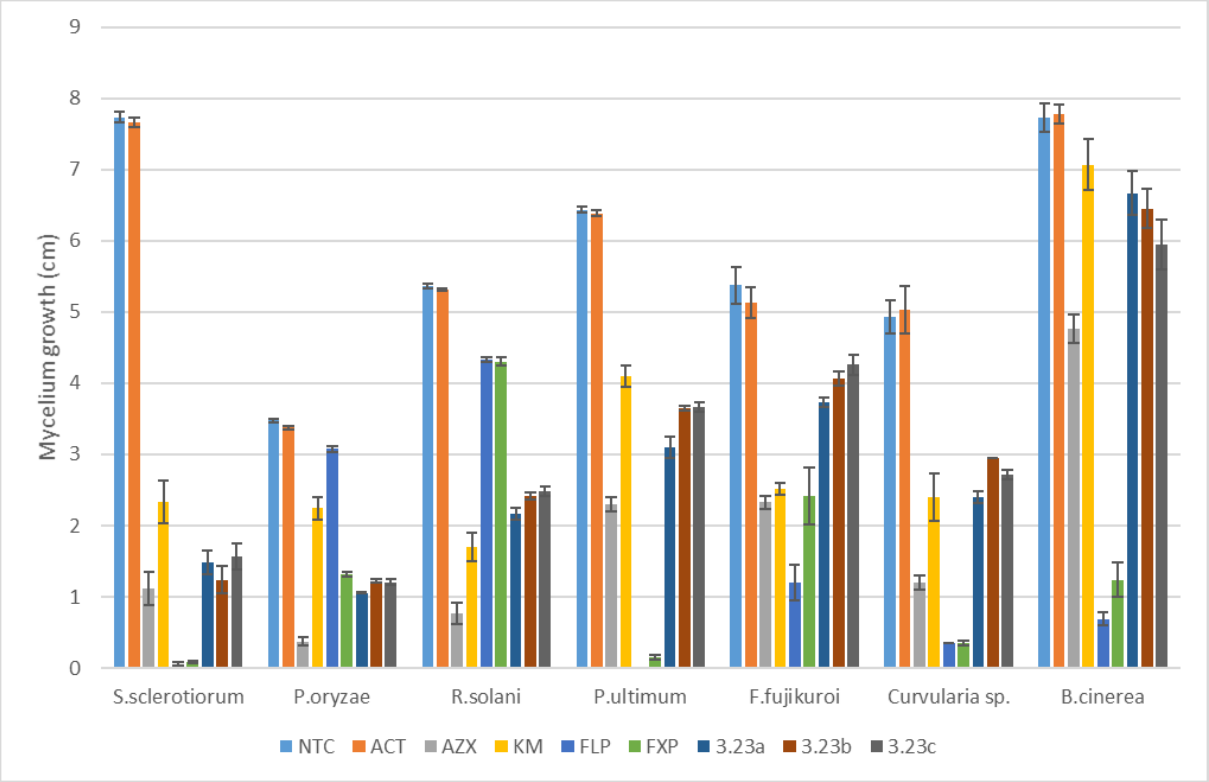


Figure 3.18 Mycelium growth of seven fungal species on control media (NTC – non-treated control, ACT – 1 % acetone) and media supplemented with commercial fungicides (AZX – azoxystrobin, KM – kresoxim-methyl, FLP – fluopyram, FXP – fluxapyroxad, at concentrations 25 mg/L) and compounds **3.23a-c**, at concentrations 25 mg/L. The measurements were done at 7 dai, except for *S. sclerotiorum*, which were done at 3 dai. The error bars represent standard error (N = 3).

Moderate inhibitory activity was observed for **3.23a-c** against *Curvularia* sp., *P. ultimum*, and *R. solani*, while poor activity was observed for *F. fujikuroi* and *B. cinerea*. Conversely, good inhibitory activity was observed for all compounds **3.23a-c** against *P. oryzae* and *S. sclerotiorum*. In particular, **3.23a-c** exhibited inhibitory activities against *P. oryzae* for **3.23a-c** in a range of ca. 60-70 % and their activity was better than that of kresoxim-methyl **3.6** and fluopyram **3.13** and comparable to that of fluxapyroxad **3.12**. Azoxystrobin **3.5** exhibited the best inhibitory activity against *P. oryzae*.

Compounds **3.23a-c** showed inhibitory activities against *S. sclerotiorum* in a range of ca. 70-80 %. The activities were comparable to that of azoxystrobin **3.5**, but inferior to that of fluxapyroxad **3.12** and fluopyram **3.13**.
In all the cases compounds **3.23a-c** showed comparable inhibitory activity.

3.2.9 Enzymatic inhibition assessment in cell-free in vitro system of 3.23a-c

The assessment of the DBH_2-Cyt *c* oxidoreductase activity was used to evaluate the strobilurin-like activity of compounds **3.23a-c**. The test was performed as described for compound **3.18**. Inhibition of Cyt *c* reduction was observed with the three target compounds. Compounds **3.23a,b** (20 µM) showed a complete inhibition, while for compound **3.23c** lower activity (54.6 ± 13.7 %) was measured. Azoxystrobin **3.5**, used as positive control, (20 µM) and under the same experimental conditions showed an inhibition of the Cyt *c*-reduction rate (76.7 ± 11.2 %), whereas fluxapyroxad **3.12**, used as negative control, (20 µM) showed no inhibition of the Cyt *c*-consumption rate (6.7 ± 15.0 %; inhibition is not distinguishable from zero with this value of error). The ability of compounds **3.23a,b** and, even if in minor extend, of compound **3.23c** to inhibit the DBH_2-Cyt *c* oxidoreductase activity in a cell-free system supports the hypothesis that these compounds are able to express a strobilurin-like activity. Results are highlighted in **Figure 3.19**.

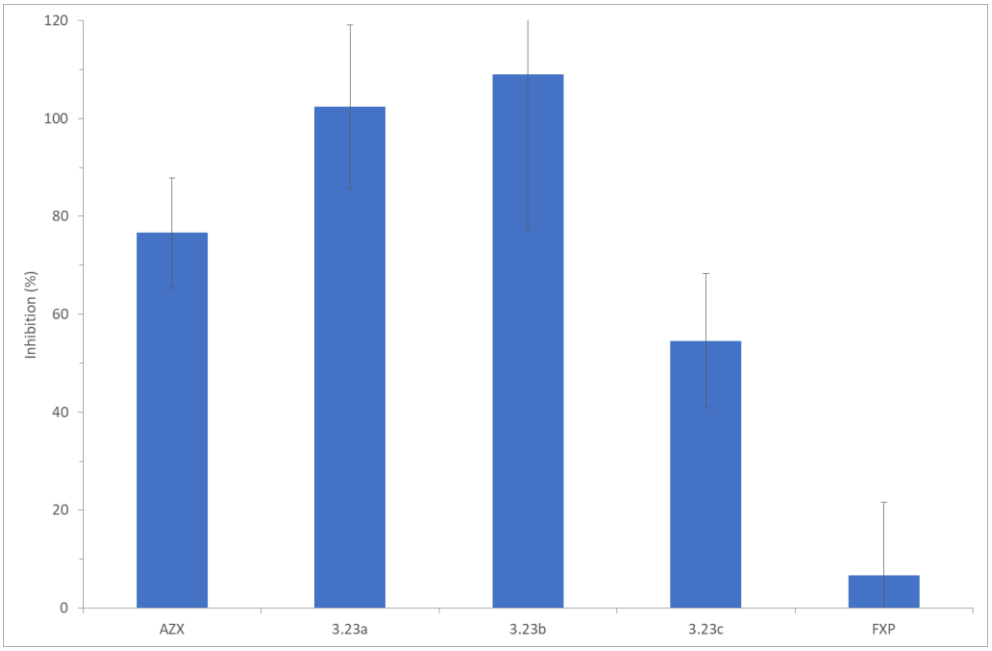


Figure 3.19 Inhibition of the *P. oryzae* A2.5.2 mitochondrial DBH2-Cyt *c* oxidoreductase activity. Rate of Cyt *c*-reduction mediated by the submitochondrial fraction was measured at 30°C (λ = 550 nm) in the presence of AZX – azoxystrobin (20 µM), FXP – fluxapyroxad (20 µM), or **3.23a,c** (20 µM).

Compounds **3.23a,c** were tested for the ability to inhibit the SDH activity. The test was performed as described for compound **3.18**. Inhibition of the SDH activity was observed for all the tested compounds at 80 µM concentration. Out of three tested compounds, **3.23b** showed the highest inhibitory activity (47.6 ± 16.6), followed by **3.23a** (34.7 ± 14.2) and **3.23c** (19.0 ± 8.6). For all three tested compounds the inhibition was minor than that of **3.12** 50 µM and in the same assay conditions (65.7 ± 17.4). No inhibition was observed for azoxystrobin **3.5**. These results suggest support the hypothesis that compound **3.23a-c** are able to inhibit SDH activity. Results are highlighted in **Figure 3.20**.

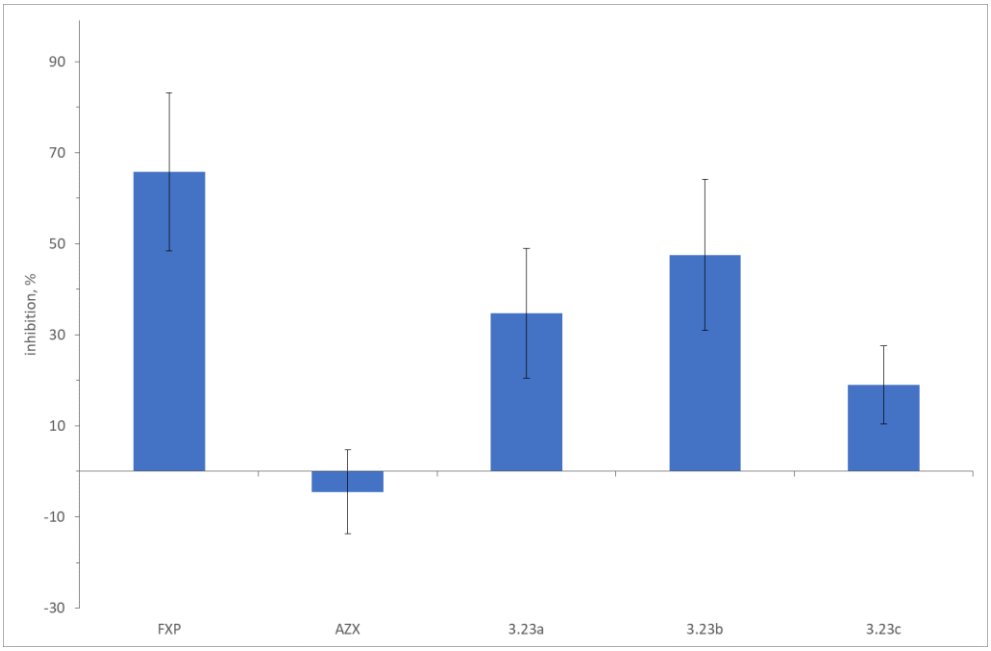


Figure 3.20 Inhibition of the *P. oryzae* A2.5.2 mitochondrial DBH2-Cyt *c* oxidoreductase activity. Rate of DCPIP-reduction mediated by the submitochondrial fraction was measured at 30°C (λ = 340 nm) in the presence of AZX – azoxystrobin (50 µM), FXP – fluxapyroxad (50 µM), or **3.23a-c** (80 µM).

3.3 Summary

In this Chapter the results of the design and development of dual-target fungicides are reported. The efforts were focussed on the development of hybrid molecules obtained by the combination of the SDH inhibitors with the strobilurin fungicides.

Compounds **3.18** and **3.19a-c** were designed taking into account the known SAR of both the classes of fungicides. The phenyl ring bearing the methyl (*E*)-β-methoxy acrylate of strobilurins was incorporate in the structure of a substituted benzamide, which is considered the pharmacophore of SDH inhibitors. The substituent linked in *ortho* position of phenyl ring was exploited as linker group. A four atoms spacer group (including the carboxamide group) was used to link the aromatic ring of SDH inhibitors and the phenyl ring bearing the β-methoxy acrylate. The oxygen atom in the linker group of strobilurins was maintained and incorporated into the spacer group. Thus, the amide group was converted into a hydroxamate group.

A synthetic strategy for the preparation of compounds **3.18** and **3.19a-c** was developed. Optimization of the reaction conditions for the Suzuki-Miyaura reaction used to synthetize compound **3.16** was successful. In particular, it was demonstrated that the reduction of the amount of catalyst up to 0.5 mol% is possible without effect on the yields and on reaction time. Compound **3.18** was obtained in low yield and no improvements seemed to be possible. Compounds **3.19a-c** were obtained with poor yields because of the instability of their direct precursor formed during the deprotection of **3.18**.

The antifungal activity of compounds **3.18** and **3.19a-c** was tested *in vitro* by evaluation of the inhibition of the mycelium growth. Compounds **3.19a-c** showed poor or no activity against all the tested fungal species, while compound **3.18** expressed an inhibitory activity against *P. oryzae* that was comparable to that of the commercial fungicide kresoxim-methyl **3.6**.

The binding mode of **3.18** to both the target enzymes was investigated *in silico* through docking studies. The computational models of SDH enzyme and Cyt *b* were built by homology. The docking of compound **3.18** with the computational model of Cyt *b* showed a binding mode similar to that of the commercial fungicide azoxystrobin **3.5**. Conversely, a binding mode different from the commercial fungicide mepronil **3.10** was observed for **3.18** in the active site of SDH enzyme. The observed differences were attributed to the steric hindrance of the (*E*)-β-methoxy acrylate group.

The results of docking studies were confirmed by assessment of the enzymatic inhibition in a *in vitro* cell-free system. Tests were performed on isolated submitochondrial fractions of *P.*

oryzae A2.5.2. Compound **3.18** was tested to assess the ability to inhibit the mitochondrial NADH-mediated respiration, the activity of the DBH_2-Cyt *c* oxidoreductase cytochrome *bc*1 complex-mediated, and the activity of SDH enzyme. Inhibition was observed for the NADH-mediated respiration and for the Cyt *c* reduction, while no inhibitory activity was observed on SDH enzyme. These results support the hypothesis that compound **3.18** is able to express only a strobilurin-like activity.

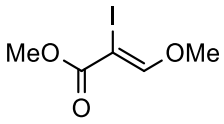
Thus, a second series of compounds with increased distance between the two pharmacophoric moieties was designed. The results of docking studies were taken in account for the design of compounds **3.23a-c**. A synthetic strategy was successfully developed and compounds **3.23a-c** were obtained in good yields.

The antifungal activity of **3.23a-c** was assessed *in vitro* by evaluation of the mycelium growth inhibition. All the tested compounds resulted to be active. In particular, good activities were expressed against *P. oryzae* and *S. sclerotiorum*.

Enzymatic inhibition was evaluated in the same *in vitro* cell-free system used for **3.18**. Inhibition of the reduction of Cyt *c* was observed for all the tested compounds. A complete inhibition was observed for compounds **3.23a,b**, while **3.23c** expressed a lower activity. More importantly, a moderate to weak inhibitory activity of SDH enzyme was observed for all the tested compounds. These results support the hypothesis that compounds **3.23a-c** can act as dual target fungicides expressing the activity of SDH inhibitors and strobilurins.

3.4 Experimental part

Methyl (*Z*)-2-Iodo-3-methoxypropenoate (3.15)



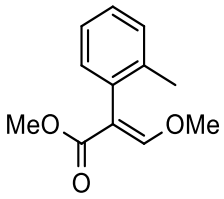
$C_5H_7IO_3$
242,01 g/mol

To a solution of methyl (*E*)-3-methoxypropenoate **3.14** (3.48 g, 30.0 mmol) in CH_2Cl_2 (60 mL), N-iodosuccinimide (8.10 g, 36.0 mmol) and glacial acetic acid (4.5 mL, 60 mmol) were added at room temperature and the resulting solution was stirred at the same temperature for 24 h. Et_3N (12.6 mL, 90.0 mmol) was added, and the solution was stirred for 12 h at room temperature. Water (90 mL) was added, the layers were separated, and the aqueous layers was further extracted with Et_2O (3×60 mL). The combined organic extracts were washed with a saturated solution of $Na_2S_2O_3$ (3×60 mL), saturated solution of $NaHCO_3$ (3×60 mL), and water (3×60 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed at reduced pressure. The oily residue was purified by flash chromatography (SiO_2, 5:1 petroleum ether/ethyl acetate) to afford **3.15** (5.09 g, 70 %) as a white solid: mp 50-52 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.68 (s, 1H,), 4.01 (s, 3H), 3.81 (s, 3H,) ppm.

Spectroscopic data were in accordance with the literature.[11]

Methyl (*E*)-2-(2-methylphenyl)-3-methoxyacrylate (3.16)



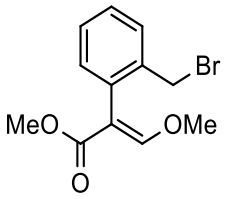
$C_{12}H_{14}O_3$
206,24 g/mol

To a solution of methyl (*Z*)-2-Iodo-3-methoxypropenoate **3.15** (2.50 g, 10.3 mmol) in a 5:1 dioxane/water mixture (52 mL), 2-methyl-1-phenylboronic acid **3.20** (1.68 g, 12.4 mmol,) and K_3PO_4 (6.58 g, 31.0 mmol) were added at room temperature and the resulting mixture was degassed by bubbling nitrogen through the solution for 20 min. Then $Pd(PPh_3)_4$ (59.7 mg, 0.5 mol%) was added, and the reaction mixture was stirred in the dark at 90 °C for 6 h. After cooling to room temperature, the mixture was diluted with ethyl acetate (50 mL) and the organic layer was washed with water (1×50 mL) and with a saturated solution of NaCl (1×50 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed at reduced pressure. The dark oily residue was purified by flash chromatography (SiO_2, 7:1 petroleum ether/ethyl acetate) to afford **3.16** (1.76 g, 83%) as colourless oil

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.52 (s, 1H), 7.39-6.97 (m, 4H), 3.79 (s, 3H), 3.68 (s, 3H), 2.24 (s, 3H) ppm.

Spectroscopic data were in accordance with the literature.[12]

Methyl (*E*)-2-(2-(bromomethyl) phenyl)-3-methoxyacrylate (3.17)



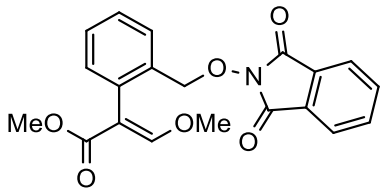
$C_{12}H_{13}BrO_3$
285,13 g/mol

To a solution of methyl (E)-2-(2-methylphenyl)-3-methoxyacrylate **3.16** (2.09 g, 10.1 mmol) in abs. carbon tetrachloride (50 mL), N-bromosuccinimide (1.98 g, 11.1 mmol) and 2,2'-azo-bisisobutyronitrile (332.2 mg, 2.02 mmol) were added and the resulting suspension was stirred at reflux in the dark for 6 h. After cooling to room temperature, the mixture was filtered to remove the insoluble succinimide and the solid residue was washed with a small volume of carbon tetrachloride. The filtrated was concentrated at reduced pressure and the oily residue was purified by flash chromatography (SiO_2, 8:1 petroleum ether/ethyl acetate) to afford **3.17** (2.25 g, 78%) as a white solid: mp 64-65 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.65 (s, 1H), 7.10-7.64 (m, 4H), 4.40 (s, 2H), 3.69 (s, 3H), 3.38 (s, 3H) ppm.

Spectroscopic data were in accordance with the literature.[13]

Methyl (*E*)-2-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yloxymethyl)-phenyl]-3-methoxy-acrylate (3.18)



$C_{20}H_{17}NO_6$
367,35 g/mol

To a solution of *N*-hydroxyphthalimide (630 mg, 3.86 mmol) in abs. *N*,*N*-dimethylformamide (35 mL), K_2CO_3 (728 mg, 5.26 mmol) was added and the resulting red suspension was stirred at room temperature for 20 min. After that, methyl (*E*)-2-(2-(bromomethyl) phenyl)-3-methoxyacrylate **3.17** (1000 mg, 3.50 mmol) was added and the reaction mixture was stirred at 40 °C overnight. The reaction mixture was poured in cold water and stirred for 1 h. The obtained solid was collected by in vacuo filtration and washed with water. The solid was dissolved in CH_2Cl_2 (20 mL) and washed with water (1×20 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed under reduced pressure. The residue was recrystallized from 1:4 CH_2Cl_2/hexane to afford **3.18** (568 mg, 40%) as a white solid: mp 161-163 °C.

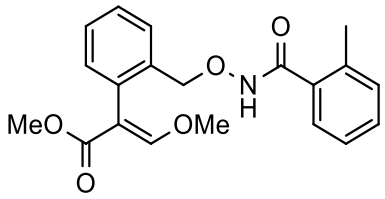
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.88-7.67 (m, 5H), 7.63 (s, 1H), 7.47-7.31 (m, 2H), 7.20-7.09 (m, 1H), 5.12 (s, 2H), 3.75 (s, 3H), 3.61 (s, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.9, 163.5, 160.7, 134.4, 133.3, 133.2, 131.1, 130.6, 129.2, 129.0, 128.1, 123.4, 77.5, 62.0, 51.7 ppm.

General procedure for the synthesis of 3.19a-c.

To a solution of compound **3.18** (100 mg, 0.27mmol) in methanol (2 mL), hydrazine hydrate (15 μL, 0.30 mmol) was added dropwise and the resulting solution was stirred at room temperature for 4 h. The solution was then cooled to 0 °C and the white solid was removed by vacuum filtration. The methanolic solution was evaporated to dryness and the residue was suspended with a small volume of Et_2O. The white solid was removed by vacuum filtration, the ethereal solution was dried with anhydrous $CaCl_2$ and used for the next reaction without further purification. The ethereal solution was dried until dryness with a stream of nitrogen in a two necked round bottom flask. The residue was dissolved in abs. CH_2Cl_2 (2 mL) and the resulting solution was cooled to 0 °C. A solution of the suitable acyl chloride **3.21a-c** (0.32 mmol) in abs. CH_2Cl_2 (2 mL) and abs. pyridine (66 μL, 0.81 mmol) were added dropwise at the same temperature and the resulting solution was stirred at room temperature overnight. The three acyl chlorides **3.21a-c** were previously prepared from the respective acids following reported procedures.[16] The reaction mixture was diluted with CH_2Cl_2 (6 mL), washed with a saturated solution $NaHCO_3$ (10 mL×1) and with a saturated solution of NaCl (10 mL×1). The organic phase was dried with Na_2SO_4, the solvent was removed at reduced pressure, and the raw material was purified by flash chromatography.

Methyl (*E*)-2-[2-(2-methyl-benzoylaminooxymethyl)-phenyl]-3-methoxy-acrylate (3.19a)



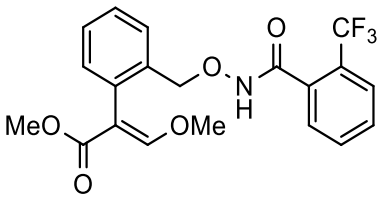
$C_{20}H_{21}NO_5$
355,38 g/mol

Obtained according to the general procedure from **3.18** and **3.21a** after flash chromatography (SiO_2, 2:1 hexane/ethyl acetate) (29 mg, 30%) as white waxy solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 8.46 (s, 1H), 7.54 (s, 1H), 7.49-7.42 (m, 1H), 7.42-7.30 (m, 2H), 7.30-7.11 (m, 9H), 5.00 (s, 2H), 3.77 (s, 3H), 3.66 (s, 3H), 2.38 (s, 3H) ppm.

^{13}C NMR ($CDCl_3$, 75 MHz) δ = 171.6, 168.5, 159.7, 137.0, 136.9, 134.8, 134.1, 133.5, 133.1, 130.7, 130.4, 129.0, 128.8, 128.3, 127.3, 126.0, 125.9, 125.7, 110.8, 76.4, 62.0, 51.8, 19.0 ppm.

Methyl (*E*)-2-[2-(2-trifluoromethyl-benzoylaminooxymethyl)-phenyl]-3-methoxy-acrylate (3.19b)



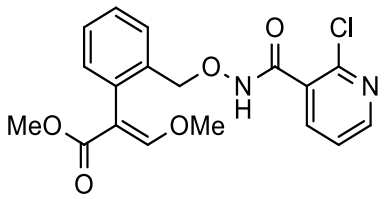
$C_{20}H_{18}F_3NO_5$
409,36 g/mol

Obtained according to the general procedure from **3.18** and **3.21b** after flash chromatography (SiO_2, 2:1 hexane/ethyl acetate) (44 mg, 39 %) as white waxy solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 8.63 (s, 1H), 7.73-7.59 (m, 1H), 7.54 (s, 1H), 7.54-7.41 (m, 4H), 7.41-7.30 (m, 4H), 7.30-7.23 (m, 2H), 7.19-7.12 (m, 1H) 4.99 (s, 2H), 3.79 (s, 3H), 3.67 (s, 3H) ppm.

^{13}C NMR ($CDCl_3$, 75 MHz) δ = 168.8, 164.9, 159.8, 134.6, 133.5, 132.5, 132.0, 131.4, 130.7, 130.4, 129.1, 129.0, 128.2, 126.6, 110.6, 76.6, 62.1, 51.8 ppm.

Methyl (*E*)-2-{2-[(2-Chloro-pyridine-3-carbonyl)-aminooxymethyl]-phenyl}-3-methoxy-acrylate (3.19c)



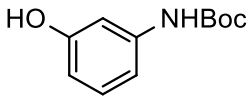
$C_{18}H_{17}ClN_2O_5$
376,79 g/mol

Obtained according to the general procedure from **3.18** and **3.21c** after flash chromatography (SiO_2, 1:1 hexane/ethyl acetate) (10.2 mg, 24 %) as yellow waxy solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 9.12 (s, 1H), 8.45-8.36 (m, 1H), 7.81-7.71 (m, 1H), 7.55 (s, 1H), 7.49-7.41 (m, 2H), 7.42-7.29 (m, 4H), 7.26-7.20 (m, 2H), 7.19-7.12 (m, 1H), 5.01 (s, 2H), 3.80 (s, 3H), 3.68 (s, 3H) ppm.

^{13}C NMR ($CDCl_3$, 75 MHz) δ = 168.7, 159.9, 151.2, 150.1, 139.3, 137.0, 134.4, 133.6, 131.6, 130.8, 129.2, 128.3, 122.8, 122.5, 110.6, 76.6, 62.1, 51.9 ppm.

1,1-Dimethylethyl *N*-(3-hydroxyphenyl)carbamate (3.25)



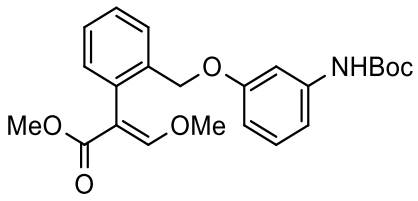
$C_{11}H_{15}NO_3$
209,24 g/mol

To a solution of 3-aminophenol **3.24** (500 mg, 4.58 mmol) and NaI (686.7 mg, 4.58 mmol) in abs. THF (10 mL), $(Boc)_2O$ (1.26 mL, 5.50 mmol) was added dropwise at 0 °C and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with ethyl acetate (20 mL), washed with a saturated solution of $Na_2S_2O_3$ (1×20 mL) and with a saturated solution of $NaHCO_3$ (1×20 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed under reduced pressure. The oily residue was crystallized by scraping the oil in the presence of a mixture of 10 % ethyl acetate in hexane recovering **3.25** (862 mg, 90 %) as a white solid: mp 130-132 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.19-7.04 (m, 2H), 6.71 (ddd, 1 H, *J* = 8.1, 2.0, 0.9 Hz), 6.53 (ddd, 1H, *J* = 8.1, 2.4, 0.9 Hz), 6.50 (bs, 1H), 5.51 (s, 1H), 1.51 (s, 9H) ppm.

Spectroscopic data were in accordance with the literature.[20]

Methyl (*E*)-2-[2-(3-*tert*-butoxycarbonylamino-phenoxymethyl)-phenyl-3-methoxy-acrylate (3.26)



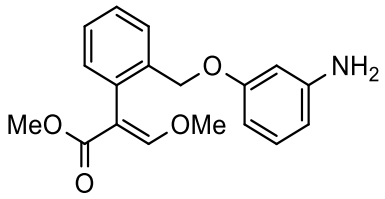
$C_{23}H_{27}NO_6$
413,46 g/mol

Solid **3.25** (388 mg, 1.85 mmol) was added at room temperature to a suspension of anhydrous K_2CO_3 (308 mg, 2.23 mmol) in abs. acetone (11.6 mL) and the resulting mixture was stirred at reflux for 1 h. After that, solid benzyl bromide **3.17** (480 mg, 1.68 mmol) was added and the reaction mixture was stirred at reflux for 9 h. The suspended K_2CO_3 was removed by vacuum filtration and the filtrate was removed under reduced pressure. The residue was purified by flash chromatography (SiO_2, 3:1 petroleum ether/ethyl acetate) to obtain compound **3.26** (452 mg, 65 %) as white waxy solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.59 (s, 1H), 7.57-7.50 (m, 1H), 7.37-7.27 (m, 2H), 7.20-7.08 (m, 2H), 7.00-6.94 (m, 1H), 6.94-6.90 (m, 1H), 6.57 (ddd, 1H, *J* = 8.2, 2.5, 0.9 Hz), 6.50 (bs, 1H), 4.94 (s, 2H), 3.83 (s, 3H), 3.72 (s, 3H), 1.50 (s, 9H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.1, 160.3, 159.6, 152.7, 139.7, 136.3, 131.3, 131.1, 129.8, 128.2, 127.7, 127.6, 111.0, 110.2, 109.7, 105.3, 80.5, 68.1, 62.1, 51.8, 28.5 ppm.

Methyl (*E*)-2-[2-(3-amino-phenoxymethyl)-phenyl]-3-methoxy-acrylate (3.27)



$C_{18}H_{19}NO_4$
313,35 g/mol

To a solution of **3.26** (202 mg, 0.49 mmol) in abs. CH_2Cl_2 (4.9 mL), trifluoroacetic acid (0.49 mL) was added dropwise at 0 °C. The reaction mixture was stirred at the same temperature for 3 h. The solvent was removed under reduced pressure, stripping with toluene (2×2 mL) and Et_2O (2×2 mL) to remove the excess of trifluoroacetic acid. The residue was dissolved in CH_2Cl_2 (15 mL) and washed with saturated solution of $NaHCO_3$ (1×15 mL), the layers were separated and the aqueous layer was extracted with ethyl acetate (2×15 mL). The combined organic layers were dried with Na_2SO_4 and the solvent was removed in vacuo. Compound **3.27** (139,0 mg, 90 %) was obtained as white waxy solid and used for the next synthetic steps without further purification

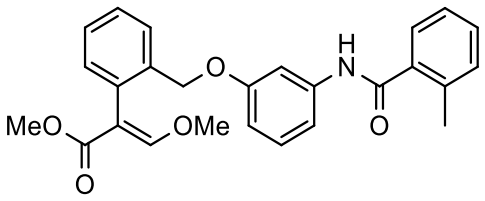
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.59 (s, 1H), 7.57-7.52 (m, 1H), 7.38-7.27 (m, 2H), 7.19-7.13 (m, 1H), 7.05-6.98 (m, 1H), 6.36-6.22 (m, 3H), 4.91 (s, 2H), 3.82 (s, 3H), 3.71 (s, 3H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ = 167.8, 159.6, 150.2, 147.7, 138.6, 133.7, 133.6, 130.3, 130.0, 126.7, 126.6, 109.4, 107.4, 104.1, 100.7, 71.7, 60.1, 50.8 ppm.

General procedure for the synthesis of compound 3.23a-c

To a solution of the suitable arylic acid **3.28a-c** (0.18 mmol) in abs. CH_2Cl_2 (1 mL) at 0 °C under N_2 atmosphere, EDC·HCl (0.19 mmol) and HOBt (0.19 mmol) were added. The reaction mixture was stirred at 0 °C for 1 h. After that, a solution of compound **3.27** (0.16 mmol) in abs. CH_2Cl_2 (0.7 mL) was added dropwise at 0 °C. Then, DIPEA (0.32 mmol, 56 μl) was added dropwise at the same temperature and the reaction mixture was stirred at room temperature for 8 h. The arylic acid (0.18 mmol), EDC·HCl (0.19 mmol), HOBt (0.19 mmol), and DIPEA (0.32 mmol, 56 μl) were again added at 0 °C after 24 and 48 h of stirring, then the reaction mixture was stirred for further 24 h. The reaction mixture was diluted with ethyl acetate (15 mL) and washed with a saturated solution of NH_4Cl (3×20 mL), saturated solution of $NaHCO_3$ (1×20 mL) and saturated solution of NaCl (1×20 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed in vacuo The residue was purified by flash chromatography to give the corresponding compound **3.23a-c**.

Methyl (*E*)-2-{2-[3-(2-methyl-benzoylamino)-phenoxymethyl]-phenyl}-3-methoxy-acrylate (3.23a)



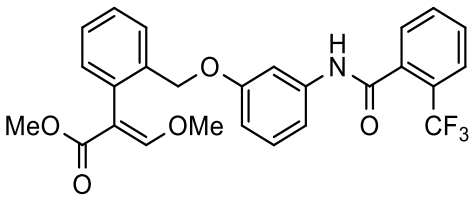
$C_{26}H_{25}NO_5$
431,48 g/mol

Prepared according to general procedure from **3.27** and **3.28a** after flash chromatography (SiO_2, 5:1 hexane/acetone) (59.4 mg, 86 %) as a white waxy solid.

^{1}H NMR (300 MHz, CDCl3): δ = 7.59 (s, 1H), 7.58-7.54 (m, 2H), 7.52-7.45 (m, 1H), 7.39-7.30 (m, 3H), 7.30-7.20 (m, 4H), 7.20-7.13 (m, 2H), 6.73-6.66 (m, 1H), 4.98 (s, 2H), 3.83 (s, 3H), 3.64 (s, 3H), 2.49 (s, 3H) ppm.

^{13}C NMR (75 MHz, CDCl3): δ = 168.2, 168.1, 160.4, 159.5, 139.4, 136.7, 136.6, 136.2, 131.3, 131.1, 130.3, 129.9, 128.3, 127.7, 126.8, 126.0, 112.3, 111.5, 110.2, 106.5, 68.1, 62.2, 51.8 ppm.

Methyl (*E*)-2-{2-[3-(2-trifluoromethyl-benzoylamino)-phenoxymethyl]-phenyl}-3-methoxy-acrylate (3.23b)



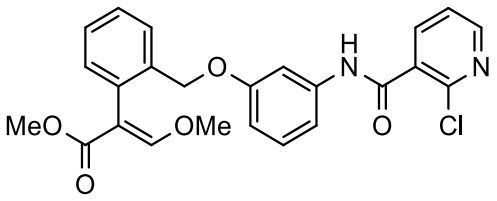
$C_{26}H_{22}F_3NO_5$
485,45 g/mol

Prepared according to general procedure from **3.27** and **3.28b** after flash chromatography (SiO_2, 3:1 hexane/ethyl acetate) (62.2 mg, 80 %) as a white waxy solid.

^{1}H NMR (300 MHz, CDCl3): δ = 7.78-7.71 (m, 1H), 7.68-7.52 (m, 4H), 7.58 (s, 1H), 7.38-7.28 (m, 2H), 7.25-7.19 (m, 2H), 7.19-7.13 (m, 2H), 6.76-6.68 (m, 1H), 4.98 (s, 2H), 3.82 (s, 3H), 3.64 (s, 3H) ppm.

^{13}C NMR (100 MHz, CDCl3): δ = 168.2, 165.7, 160.4, 159.5, 138.8, 163.1, 132.3, 131.4, 131.1, 130.2, 129.9, 128.7, 128.3, 127.8, 126.6, 125.5, 121.9, 112.5, 111.8, 110.1, 106.8, 68.2, 62.2, 51.7 ppm.

Methyl (*E*)-2-(2-{3-[(2-chloro-pyridine-3-carbonyl)-amino]-phenoxymethyl}-phenyl)-3-methoxy-acrylate (3.23c)



$C_{24}H_{21}ClN_2O_5$
452,89 g/mol

Prepared according to general procedure from **3.27** and **3.28c** after flash chromatography (SiO_2, 1:1 hexane/acetone) (47.3 mg, 65 %) as a white waxy solid.

^{1}H NMR (300 MHz, CDCl3): δ = 8.50 (dd, *J* = 4.8, 1.9 Hz, 1H), 8.22-8.16 (m, 1H), 8.12 (dd, *J* = 7.6, 1.9 Hz, 1H), 7.59 (s, 1H), 7.58-7.52 (m, 2H), 7.42-7.30 (m, 2H), 7.30-7.20 (m, 2H), 7.20-7.13 (m, 1H), 6.77-6.70 (m, 1H), 4.99 (s, 1H), 3.84 (s, 3H), 3.67 (s, 3H) ppm.

^{13}C NMR (75 MHz, CDCl3): δ = 168.2, 165.2, 162.2, 154.8, 148.9, 147.2, 140.3, 139.2, 138.6, 133.7, 130.3, 129.7, 127.9, 128.0, 127.3, 127.2, 112.7, 110.0, 109.7, 105.9, 72.3, 54.0, 51.2 ppm.

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PART II: SAMARIUM CHEMISTRY IN SYNTHESIS OF NATURAL PRODUCTS

4 SmI_2-MEDIATED REFORMATSKY REACTION OF α-HALO *N*-ACYL-OXAZOLIDINONE WITH TERPENE ALDEHYDES

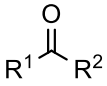
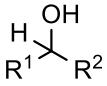
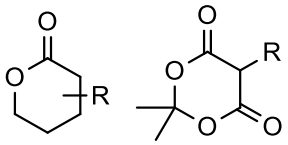
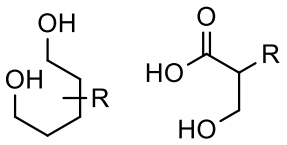
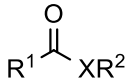
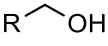
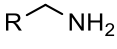
4.1 Introduction

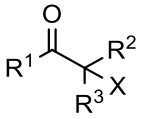
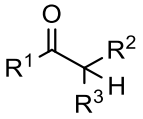
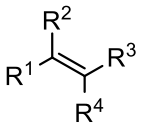
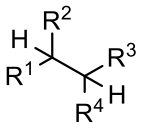
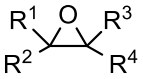
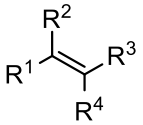
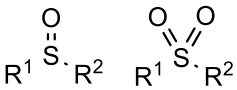
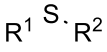
Samarium(II) iodide (SmI_2, Kagan's reagent) is a single-electron transfer reagent that can mediate both radical and ionic processes; it was first introduced in organic synthesis in 1977 by Kagan who described the SmI_2-mediated reduction of alkyl halides in refluxing THF.[1,2] Since its discovery, the number of applications in organic synthesis of SmI_2 has been greatly expanded and SmI_2 turned out to be one of the most important reducing agents available. This success comes from a valuable combination of high versatility, high power and a remarkable chemoselectivity. SmI_2 can mediate many reactions including reduction, reductive coupling, and cascade reactions, with high tolerance for functional groups. SmI_2 is an oxophilic reagent, and the ability to coordinate oxygenated centres can be exploited to perform reactions with high degree of stereoselectivity. Furthermore, its reactivity and selectivity can be manipulated and finely tuned by adding various additives and cosolvents (Lewis bases, proton donors, and inorganic additives) leading to control of reaction rate and chemo- and stereoselectivity of the reaction.[3]

Despite their large varieties, SmI_2-mediated reactions can be mainly classified in two large classes: (i) functional groups reductions and (ii) carbon-carbon bond-forming reactions.

Many SmI_2-mediated reductions of functional groups have been described in literature and the main conversions are listed in **Table 4.1**.[4]

Table 4.1 Functional groups reduced by SmI_2

Entry	Functional group	Product	Condition (SmI_2 + additives)
1	R–X X = I, Br, Cl, F	R–H	HMPA, ROH, hν, temp. (X = I, Br, Cl) $Sm(HMDS)_2$ (X = F)
2			ROH, EG, HMPA, amine-H_2O
3			H_2O
4	 X = O, N; R^2 = H, alkyl, aryl; R^1 = alkyl, aryl		H_2O, amine-H_2O
5	R–CN		ROH-hν, amine-H_2O

6			MeOH, EG, HMPA
7			H_2O, amine-H_2O ROH if R_4 = EWG
8			SmI_2-THF
9	 X = OR, NR, SO_2R		SmI_2-THF, MeOH, amine-H_2O, HMPA
10			SmI_2-THF, HMPA

EG = ethylene glycol; EWG = electron-withdrawing group

The high versatility in functional groups reduction can be explained by coordination and complexation of SmI_2 and Sm(III) salts by-products. SmI_2 Lewis acid nature allows the coordination with functional groups making easier the electron transfer. Furthermore, Sm(III) salts can form complexes with functional groups enhancing the leaving ability of such groups.[3] As mentioned above, the use of additives and cosolvents permits a fine tuning of SmI_2 reactivity and selectivity. Particularly, this can be exploited in many cases to perform selective reductions in presence of different reducible groups.[5-7]

The SmI_2-mediated reduction of functional groups underlies carbon-carbon bonds formation by reductive coupling. Radicals and organo-samarium species formed by reductions can be quenched with a proton source to obtain the reduced products or can be exploited to perform carbon-carbon bond-forming reactions. Reduction of alkyl halides generates both radicals, useful for cyclization reactions with alkenes,[8,9] and organo-samarium intermediates that can be used for both intramolecular and intermolecular Barbier and Grignard reactions.[10] Reduction of carbonyl compounds generates ketyl radical anion intermediates used in pinacol coupling reactions[11] and in carbonyl-alkene coupling reactions. [12-14]

Reduction of α-heteroatom-substituted carbonyl compounds is one of the most common use of SmI_2.[15] As reported by Molander in 1986, α-oxygenated, α-halo, α-sulfanyl, α-sulfinyl and α-sulfonyl ketones can be easily reduced with SmI_2.[16] Reduction of α-heteroatom-substituted carbonyl compounds is not limited to ketones, as it was also described on α-oxygenated esters

and α-heteroatom-substituted amides.[17,18] SmI_2-reduction of α-heteroatom-substituted carbonyl compounds proceeds through the formation of intermediate Sm(III) enolates that are usually quenched with a proton source to obtain the parent carbonyl compounds.[16] Furthermore, Sm(III) enolates that can be used for the formation of new carbon-carbon bonds in aldol reactions with aldehydes and ketones.[3]

4.1.2 SmI_2-promoted Reformatsky reactions

As mentioned above, Sm(III) enolate can be prepared starting from a variety of α-heteroatom-substituted carbonyl compounds. When the starting material is an α-halo carbonyl compound the reaction is generally indicated as SmI_2-promoted Reformatsky reaction. This Reformatsky reaction occurs under mild, homogeneous, cryogenic conditions and in the absence of additives, allowing the presence of functional groups susceptible of SmI_2-mediated reduction in other conditions.[4]

The first example of SmI_2-Reformatsky was described by Kagan in 1980. In this work the authors describe the reaction of methyl α-bromopropionate **4.1** with cyclohexanone **4.2** in presence of SmI_2 to obtain the corresponding coupling products **4.3** in high yields as diastereomeric mixture (**Figure 4.1**).[19]

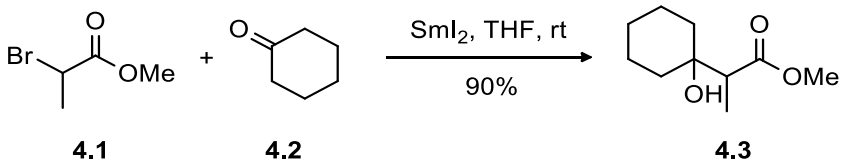


Figure 4.1 SmI2-mediated Reformatsky reaction of methyl 2-bromopropionate with cyclohexanone

Since this first results, the scope of the reaction was greatly expanded and several examples of intramolecular and intermolecular SmI_2-Reformatsky reactions were reported in scientific literature. The largest number of applications of SmI_2-Reformatsky is in its intramolecular version; this reaction has proven to be successful on a large number of substrates that can be classified into four large classes: α-halo esters, α-halo amides, α-halo ketones, and α-halo nitriles. Conversely, the application of the intermolecular version of this reaction has proven to be more challenging because of competition with side reactions, such as carbonyl reduction, pinacol coupling, self-condensation of α-halo carbonyls, and Evans-Tishchenko-type reaction. Despite these problems, several examples of intermolecular SmI_2-Reformatsky with various classes of substrates have been reported in literature in the past decades.[4]

One of the most interesting advancement in SmI_2-promoted Reformatsky reaction is the development of the asymmetric version with the use of chiral auxiliaries. The first examples were reported by Fukuzawa in 2000 (**Figure 4.2**). It was demonstrated that chiral α-bromo *N*-acetyl-oxazolidinones **4.4a** can react with aliphatic and aromatic aldehydes in SmI_2-Reformatsky reaction. The reaction occurs with good to excellent yields and high diastereomeric excess, particularly in case of aldehydes with hindered side chain. As suggested by the authors, samarium(III) ions seem to play a pivotal role in the transition state, and the stereochemistry of the reaction is determined by chelation control and coordination of the incoming aldehydes.[20]

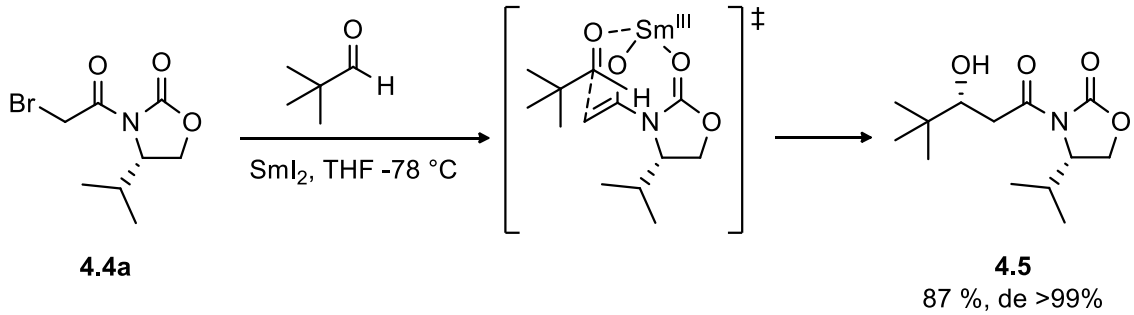


Figure 4.2 Asymmetric SmI2-mediated Reformatsky reaction of α-bromo *N*-acetyl-oxazolidinones with 2,2-dimethylpropanal.

An interesting application of asymmetric SmI_2-Reformatsky reaction was described by Burke in 2012. In this work, the authors describe the reaction of samarium(III) enolates derived from α-chloro *N*-acetyl Evans' oxazolidinone **4.4b** with chiral α-amino aldehydes **4.6**, providing β-hydroxy-γ-amino acids with good yields and high diastereoselectivity (**Figure 4.3**).[21]

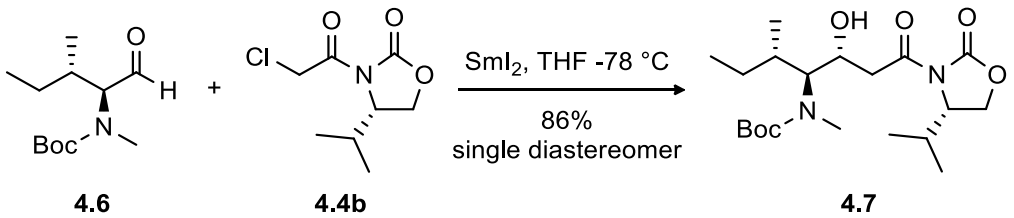


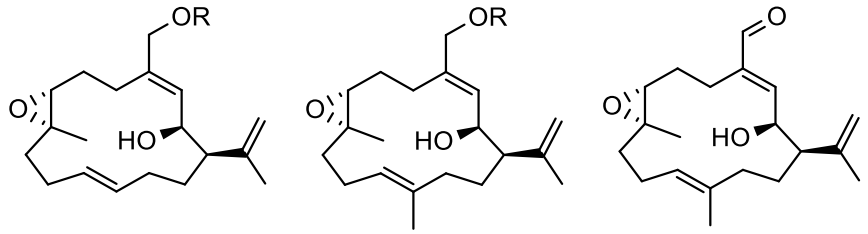
Figure 4.3 Asymmetric SmI2-mediated Reformatsky reaction of α-chloro *N*-acetyl oxazolidinone **4.4b** with chiral α-amino aldehydes **4.6**

Despite the good results obtained and the promising application, the use of chiral SmI_2-Reformatsky is still an underexplored area. In the examples previously discussed, the applications were limited to alkyl and aromatic aldehydes and no evidences are reported in the

use of enals and terpene-derived aldehydes, that can be exploited as precursors in the synthesis of natural products like cembranoids.[22]

4.1.3 Cembranoids

Cembranoids are a class of 14-membered macrocyclic diterpenoids derived mainly from marine organism.[23] This class of secondary metabolites exhibits a wide range of biological activities, thus cembranoids are potentially candidates as lead compounds for pharmaceutical development.[24-26] Some examples of cembranoids are shown in **Figure 4.5**.



4.8a R = H
4.8b R = Ac

4.9a R = H
4.9b R = Ac

4.10

Figure 4.5 Asperdiol (**4.8a**), its analogue knightol (**4.9a**), their acetates**4.8b**, **4.9b** and knightal (**4.10**)

Asperdiol **4.8a** and its acetate **4.8b** are two members of this class which have been isolated from soft corals *Eunicea asperula* and *E. tourneforti* by Weinheimer in 1977.[24] Asperdiol and its closely related analogues knightol **4.9a**, knightol acetate **4.9b**, and knightal **4.10**, which were isolated from *Eunicea knighti*,[25] have received increasing interest due to their promising cytotoxic and antimicrobial activity.

The main source of cembranoids is still the extraction from natural sources, but this is not an ecologically sustainable strategy because of the large amount of biomass requested to extract small amounts of compound.[27] Thus, a synthetic approach for the preparation of cembranoids was investigated.

Five total syntheses of asperdiol have been reported by Still,[28] Marshall,[29] and Tius[30] utilizing a Nozaki-Hiyami-Kishi reaction,[28] sulfone alkylation,[29b] [2,3]-Witting rearrangements[29a] or Horner-Wadsworth-Emmons olefinations[30] as key steps for the ring closure.

In 2014, Kriening proposed the use of asymmetric aldol reaction of *N*-acyloxazolidinones with terpene-derived enals as key step for the synthesis of asperdiol precursor. Despite the high diastereoselectivity, the synthesis turned out to be *syn*-selective, leading to a product with opposite stereochemistry at alcohol moiety (**Figure 4.6**). The stereochemistry of alcohol could not be inverted and any attempts to obtain *anti*-aldol reactions was unsuccessfully.[22]

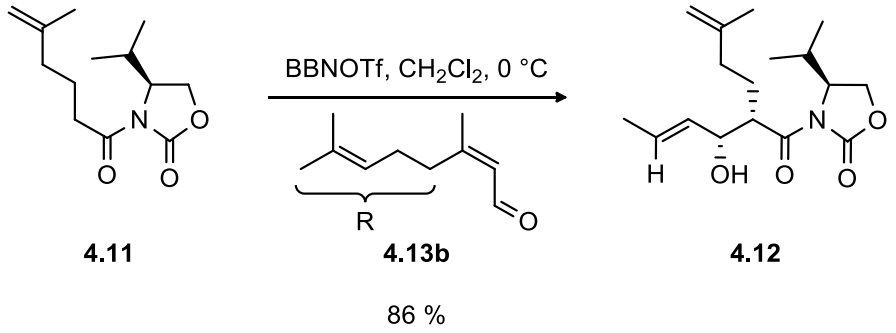


Figure 4.6 Asymmetric aldol reactions of *N*-acyloxazolidinones **4.11** with neral **4.13b**

In this Chapter is described the development of a SmI_2-mediated Reformatsky reaction of Evans' *N*-acyloxazolidinones with terpene-derived aldehydes as key step for the synthesis of asperdiol precursor. We intend to develop a complementar method to the Evans aldol reaction-based approach to asperdiol precursors.

The investigation was based on the results previously reported by Fukuzawa[20] and then by Burke.[21] As shown in **Figure 4.7**, the reactions of readily accessible *N*-acyloxazolidinones **4.4a-d** with simple terpene-derived aldehydes **4.13a,b** were performed as a simplified model to investigate which factors influence the outcome of the reaction in terms of yield and diastereoselectivity. As highlighted in **Figure 4.7**, the project was focused on the investigation of the effect *E-Z* configuration of the double bond of the aldehyde, the role of the halide on the *N*-acyloxazolidinones, the effect of the temperature, and the presence and the configuration of stereocenters on the *N*-acyloxazolidinones.

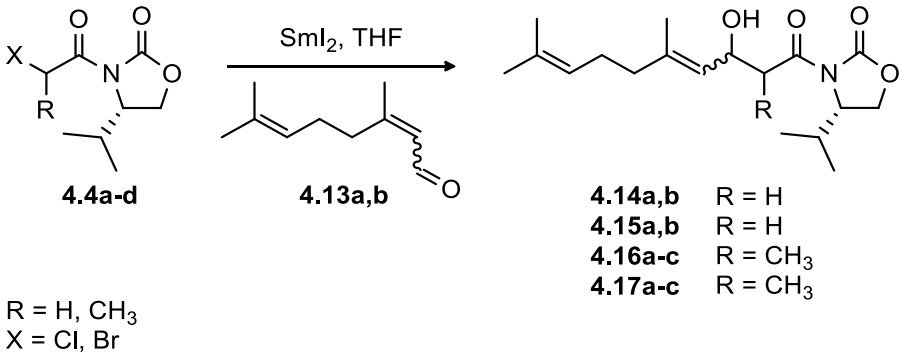


Figure 4.7 SmI_2-mediated Reformatsky reaction *N*-acyloxazolidinones **4.4a-d** with enals **4.13a,b**

4.2 Results and discussion

4.2.1 Synthesis of precursors Evans' N-acyloxazolidinones 4.4a-d

N-Acetyloxazolidinones **4.4a** and **4.4b** were prepared starting from Evans' oxazolidinone **4.18** and obtained after chromatographic separation in 74 % and 85 % yield, respectively (**Figure 4.8**).[31]

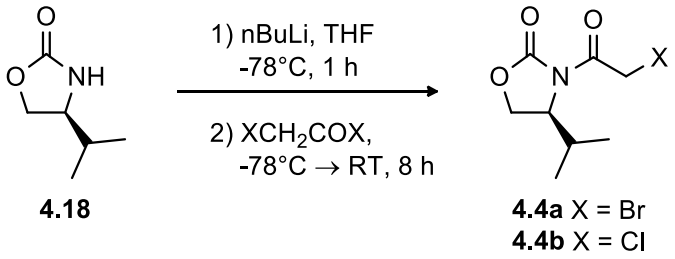


Figure 4.8 Synthesis of *N*-Acetyloxazolidinones **4.4a,b**

α-Bromo *N*-propionyloxazolidinones **4.4c,d** were prepared with the same procedure used for compound **4.4a,b** starting from Evans' oxazolidinone **4.18** and freshly distilled racemic bromopropionyl bromide (**Figure 4.9**). The products **4.4c,d** were obtained in 53 % yield as approximately 1:2 mixture of diastereomers.[32] Separation of the two diastereomers has been accomplished by flash cromatography, but any attempts to assign the absolute configuration was unsuccessful because of the rapid epimerization of the single diastereomers. Complete epimerization was observed in samples stored at -22 °C in less than a week.

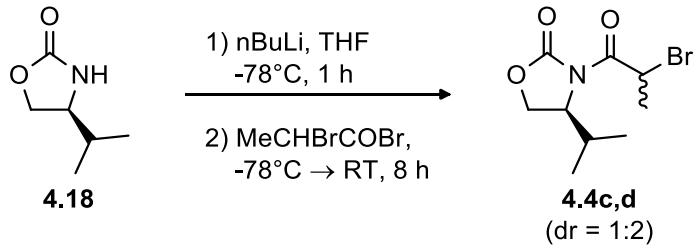


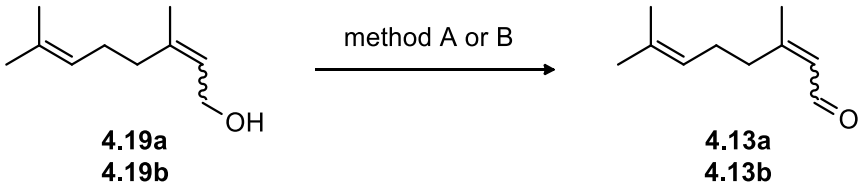
Figure 4.9 Synthesis of α-Bromo *N*-propionyloxazolidinones **4.4c,d**

4.2.2 Synthesis of precursors terpene aldehydes 4.13a,b

Firstly, geranial **4.13a** and neral **4.13b** were prepared by oxidation of geraniol **4.19a** and nerol **4.19b** with Dess-Martin periodinane (**Figure 4.10, method A**).[33] The oxidation with Dess-Martin periodinane has proved to be a good strategy for small scale oxidation of terpene alcohols providing the desired terpene aldehydes in good yield with user-friendly and

operationally simple reaction conditions. Conversely, this strategy turned out to be not suitable for scale-up because of the high cost of the reagent and its poor atom economy.

Ley-Griffith oxidantion[34] was chosen as alternative strategy for terpene alcohols oxidation (**Figure 4.10, method B**). Therefore, the starting terpene alcohol **4.19a** or **4.19b** was dissolved in a stirred suspension of grinded 4 Å molecular sieves and *N*-methylmorpholine-*N*-oxide before the Ley-Griffith reagent (tetrapropylammonium perruthenate, TPAP) was added in catalityc amount. The desired terpene aldehydes **4.13a** and **4.13b** were obtained in 81 % and 78 % yield, respectively. It should be noted that complete removal of any residues of the catalyst turned out to be crucial to avoid isomerization of the double bound.



Method A: 1.2 equiv. Dess-Martin Periodinane, CH_2Cl_2, RT, 2 h
Method B: 1.5 equiv. NMO, 0.1 mol% TPAP, 4Å MS, CH_2Cl_2, RT, 8 h

4.13a 89 % (via method A); 81 % (via method B)
4.13b 83 % (via method A); 78 % (via method B)

Figure 4.10 Synthesis of terpene aldehydes **4.13a,b**

4.2.3 SmI_2-mediate Reformatsky reaction

Initially α-bromo *N*-acetyloxazolidinone **4.15a** was coupled with geranial **4.13a** and neral **4.13b** in SmI_2-mediated Reformatsky reaction following the procedure reported in ref [18] (**Figure 4.11**). A solution of SmI_2 (0.1 M in THF) was cooled to -78 °C before simultaneous addition of α-bromo *N*-acetyloxazolidinone **4.15a** and aldehyde **4.13a** or **4.13b** dissolved in THF. In these conditions reaction proceeded sluggish and the consumption of starting α-bromo *N*-acetyloxazolidinone **4.15a** was incomplete, affording compounds **4.14b** [(*E*)-derivative] and **4.15b** [(*Z*)-derivative] in only 32 % and 23 % yield, respectively. Moreover, the formation of a large number of side products was observed thus the isolation of the pure minor diastereomers was hampered.

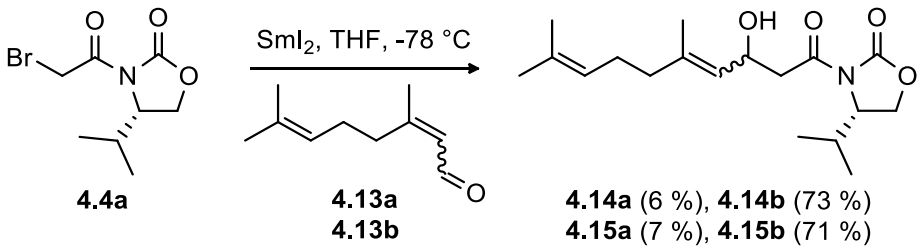


Figure 4.11 SmI_2-mediated Reformatsky reaction of **4.15a** with **4.13a,b**

The ability of SmI_2 to carry out the reduction of carbonyl compounds and to promote carbonyl-alkene coupling is well known.[2] In agreement with this evidence, reduction of terpene aldehydes could compete with the SmI_2-mediate Reformatsky reaction leading to consumption of SmI_2 and formation of side products. Hence, a solution of SmI_2 0.1 M in THF was cooled to -78 °C before addition of a solution of α-bromo *N*-acetyloxazolidinone **4.15a** in THF. The reaction mixture was stirred in the dark for 5 minutes to permit the formation of Sm(III) enolate. After that, a solution of geranial **4.13a** was added dropwise at -78 °C and the reaction mixture was stirred at the same temperature in the dark. In these conditions the reaction was complete after 0.5 h, affording diastereomers **4.14a** and **4.14b** with a ratio **4.14b**/**4.14a** 92:8 (**Table 4.1; entry 1**). The reaction was carried out in the same condition using neral **4.13b**, affording after chromatographic separation diastereomers **4.15a** and **4.15b** after chromatographic separation with a ratio **4.15b**/**4.15a** 91:9 (**Table 4.1; entry 2**).

4.2.4 Role of the halide and effect of the temperature

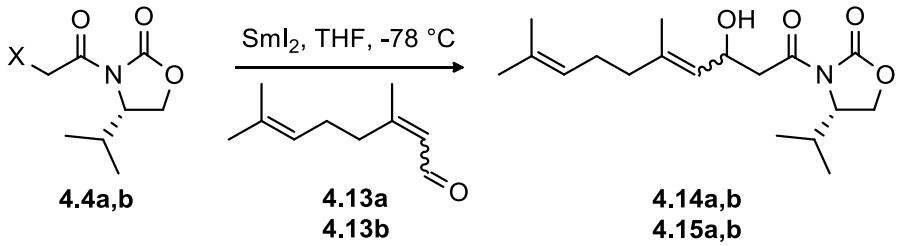
To investigate the role of the halide and the effect of the temperature on the SmI_2-mediate Reformatsky reaction, a series of screening reactions were undertaken; the results are summarized in **Table 4.2**. All the reactions were carried with the optimized procedure described above. Reaction of α-bromo *N*-acetyloxazolidinone **4.4a** with geranial **4.13a** at -100 °C provided products **4.14a** and **4.14b** in ratio **4.14b**/**4.14a** 90:10 (**Table 4.2; entry 3**). The yield of the reaction is lower compared to the same reaction performed at -78 °C. Sm(III) enolate formed as intermediate is poorly soluble at -100 °C and this slows down the reaction with the terpene aldehyde, favouring the formation of side products. It should be noted that the lowering the temperature did not significantly influence the diastereomeric ratio. Analogous results were obtained from the same reaction with neral **4.13b** (**Table 4.2; entry 4**).

Reactions of α-chloro *N*-acetyloxazolidinone **4.4b** with geranial **4.13a** at -78 °C provided products **4.14a** and **4.14b** with lower yield compared to α-bromo *N*-acetyloxazolidinone **4.4a**.

Despite a slightly increase of the yields could be obtained lowering the temperature of the reaction to -100 °C, the yields of the reaction with α-chloro *N*-acetyloxazolidinone **4.4b** were still lower compared to the reaction with α-bromo *N*-acetyloxazolidinone **4.4a** at -78 °C.

The diastereomeric ratios were not significantly different, suggesting that the diastereoselectivity of the reactions was mainly controlled by the chiral auxiliary in the samarium(III) enolate (**Table 4.2; entries 5, 7**). Analogous results were obtained from the same reactions with neral **4.13b** (**Table 4.2; entries 6, 8**).

Table 4.2 Determination of the effect of temperature and halide on yield and diastereoselectivity



4.4a,b **4.13a** **4.13b** **4.14a,b** **4.15a,b**

Entry	Enal	X	T	Products		Yield (**b**)	Ratio **b**/**a**
1	**4.13a**	Br	-78 °C	**4.14°**	**4.14b**	73 %	92:8
2	**4.13b**	Br	-78 °C	**4.15°**	**4.15b**	71 %	91:9
3	**4.13a**	Br	-100 °C	**4.14°**	**4.14b**	59 %	90:10
4	**4.13b**	Br	-100 °C	**4.15°**	**4.15b**	54 %	90:10
5	**4.13a**	Cl	-78 °C	**4.14°**	**4.14b**	48 %	89:11
6	**4.13b**	Cl	-78 °C	**4.15°**	**4.15b**	43 %	90:10
7	**4.13a**	Cl	-100 °C	**4.14°**	**4.14b**	56 %	90:10
8	**4.13b**	Cl	-100 °C	**4.15°**	**4.15b**	50 %	91:9

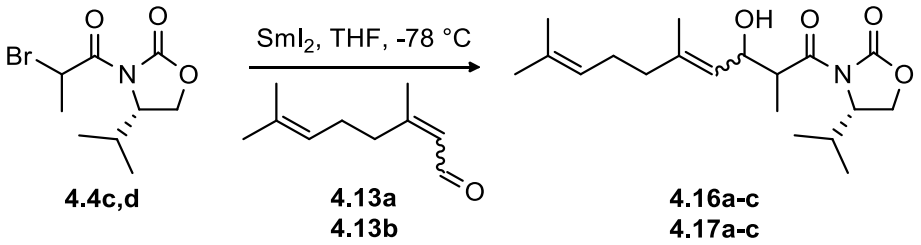
1.2 equiv. of enal, 2.2 equiv. SmI_2, stirring 30 min. in dark

4.2.5 Role of stereocenters on the N-acyloxazolidinones

To investigate the reactivity of secondary halides on the acyl chain and the effect of chirality on the reaction outcomes, a series of screening reactions on α-bromo *N*-propionyloxazolidinones **4.4c** and **4.4d** were undertaken. Reactions were performed as

described above both on diastereomeric mixture and on single diastereomers. Because of the rapid epimerization of the α-bromo *N*-propionyloxazolidinones,[32] the pure single diastereomers were obtained by chromatographic separation and immediately used for the reactions. Results are summarized in **Table 4.3**. The yields of reactions of the diastereomeric mixture with geranial **4.13a** and neral **4.13b** were good, suggesting that the reactivity of α-bromo *N*-propionyloxazolidinones **4.4c** and **4.4d** doesn't differ significantly from α-bromo *N*-acetyloxazolidinone **4.4a**. Diastereomers **4.16a-c** and **4.17a-c** were isolated after chromatographic separation. Reaction of single diastereomers **4.4c** and **4.4d** with geranial and neral **4.13a** or **4.13b** provided the diastereomers with no significant differences in terms of yields and diastereomeric ratios, suggesting that the configuration of the stereocenter on α-bromo *N*-propionyl group had no influence on the outcome of the reaction. In agreement with the proposed mechanism,[1] the first step of samarium(II) iodide-mediated Reformatsky reaction should involve the reduction of the α-bromopropionyl group to give the samarium(III) enolate with loss of chirality. Therefore, the configuration of the α-bromopropionyl group should not influence the outcome of the reaction and the ratio of diastereomers.

Table 4.3 Determination of the effect of the stereocenter on the acyl group



4.4c,d **4.13a** **4.13b** **4.16a-c** **4.17a-c**

Entry	N-acyloxazolidinone	Enal	Products	Yield (**b**)	Ratio **a**/**b**/**c**
1	**4.4c,d** (1:2 mixture)	**4.13a**	**4.16a-c**	55 %	16:63:21
2	**4.4c,d** (1:2 mixture)	**4.13b**	**4.17a-c**	59 %	9:71:20
4	**4.4c**	**4.13a**	**4.16a-c**	52 %	15:63:22
5	**4.4c**	**4.13b**	**4.17a-c**	59 %	11:70:19
7	**4.4d**	**4.13a**	**4.16a-c**	53 %	16:62:22
8	**4.4d**	**4.13b**	**4.17a-c**	57 %	10:71:19

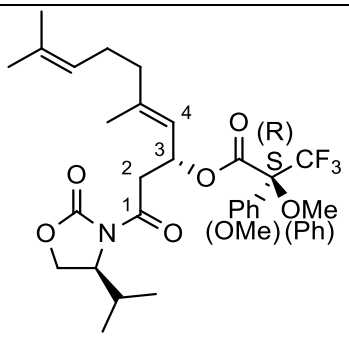
1.2 equiv. of enal, 2.2 equiv. SmI2, stirring 30 min. in dark

4.2.6 Assigment of absolute configuration at C-2/C-3

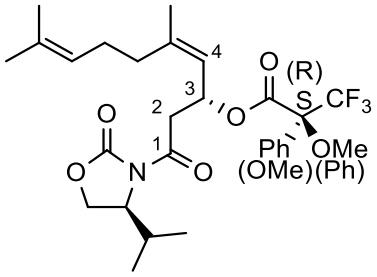
Mosher's method[35] was applied to determine the absolute configuration at C-3. Therefore, product **4.14b** was esterified with Mosher acyl chloride (*R*)- and (*S*)-MTPA to obtain the corresponding (*S*)- and (*R*)-MTPA esters **(*S*)-4.20** and **(*R*)-4.20** in 85 and 84 % yield, respectively. The procedure was adapted from the procedure repoted in ref.[22] (*S*)- and (*R*)-MTPA esters **(*S*)-4.21** and **(*R*)-4.21** were prepared from **4.15b** following the same procedure, in 70 % and 80 % yield, respectively.

Analysis of the ^{1}H NMR differences in the chemical shifts[35] indicated the absolute configuration for both products **4.14b** and **4.15b** at C-3 as (*R*). (**Table 4.4**)

Table 4.4 Determination of the configuration of **4.14b** and **4.15b** by Mosher's method



(S)-4.20 (85 %) **(R)-4.20** (84 %)



(S)-4.21 (70 %) **(R)-4.21** (80 %)

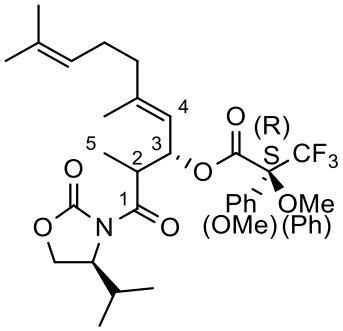
	Compound 4.14b			**Compound 4.15b**		
	(*S*)-4.20	**(*R*)-4.20**	$\Delta\delta = \delta_{(S)}-\delta_{(R)}$	**(*S*)-4.21**	**(*R*)-4.21**	$\Delta\delta = \delta_{(S)}-\delta_{(R)}$
Proton	δ =	δ =	(ppm)	δ =	δ =	(ppm)
2-H_a	3.45	3.53	-0.08	3.47	3.54	-0.07
2-H_b	3.16	3.03	+0.13	3.07	2.94	+0.13
4-H	5.07	5.23	-0.16	5.08	5.25	-0.17

1.3 equiv. (*R*/*S*)-MTPACl, 2.2 equiv. pyridine, 0.5 equiv. DMAP, 0 °C to RT, 12 h

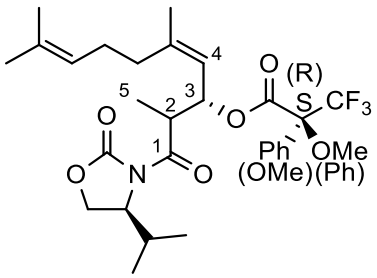
(*S*)- and (*R*)-MTPA esters **(*S*)-4.22** and **(*R*)-4.22** were prepared by the same procedures starting from products **4.16b**, while esters **(*S*)-4.23** and **(*R*)-4.23** were prepared from **4.17b**.

Analysis of the ^{1}H NMR differences in the chemical shifts[35] indicated the absolute configuration for both products **4.16b** and **4.17b** at C-3 as (2*S*). (**Table 4.5**)

Table 4.5 Determination of the configuration of **4.16b** and **4.17b** by Mosher's method



(S)-22b (R)-22b
(88 %) (87 %)



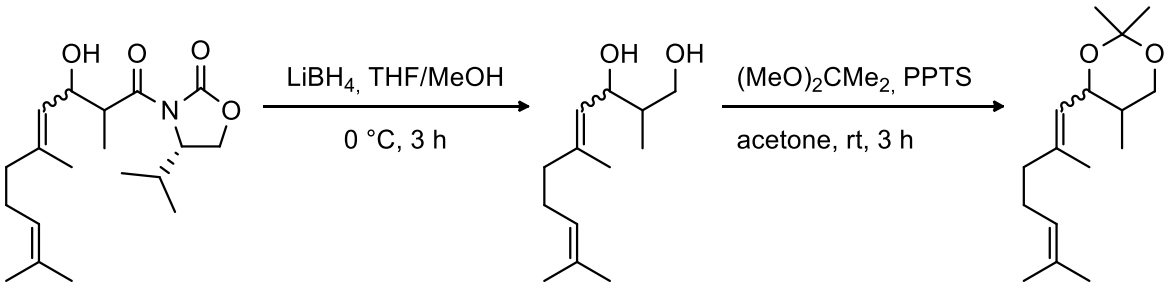
(S)-23b (R)-23b
(85 %) (88 %)

	Compound 4.16b			**Compound 4.17b**		
	(*S*)-4.22	**(*R*)-4.23**	$\Delta\delta = \delta_{(S)}-\delta_{(R)}$	**(*S*)-4.23**	**(*R*)-4.23**	$\Delta\delta = \delta_{(S)}-\delta_{(R)}$
Proton	δ =	δ =	(ppm)	δ =	Δ =	(ppm)
5-Me	1.23	1.08	+0.15	1.23	1.07	+0.16
4-H	5.09	5.25	-0.16	5.08	5.27	-0.19

1.3 equiv. (R/S)-MTPACl, 2.2 equiv. pyridine, 0.5 equiv. DMAP, 0 °C to RT, 12 h

To assign the configuration at C-2, products **4.16b** and **4.17b** were converted into the conformational blocked cyclic acetal **4.26** and **4.27** (**Figure 4.10**). To remove the chiral auxiliary, **4.16b** was treated a 0 °C with $LiBH_4$ in abs. THF/abs. MeOH 100:1. Diol **4.24** was obtained in 64 %.[22] Diol **4.25** was obtained following the same procedure in 60 % yield.

To synthesize the cyclic acetal, diol **4.24** was treated with 2,2-dimethoxypropane and PPTS at room temperature. Acetal **4.26** was obtained in 90 % yield.[22] Acetal **4.27** was obtained following the same procedure in 88 % yield.



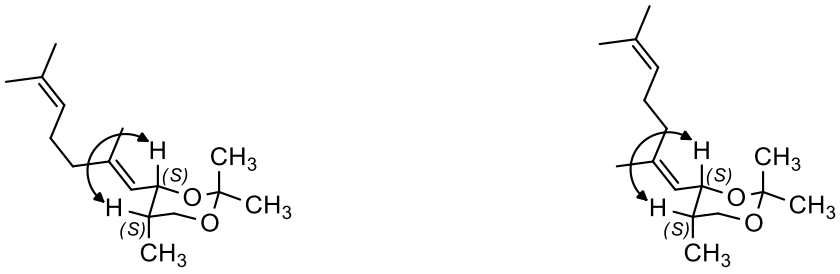
4.16b
4.17b

4.24 (64 %)
4.25 (60 %)

4.26 (90 %)
4.27 (88 %)

Figure 4.12 Synthesis of conformationally blocked acetals **4.26** and **4.27**

NOESY correlations were used to assign the relative configuration at C-2/C-3.[22] A strong NOE of H-2 with H-3 was observed for acetal **4.26**, confirming a *syn*-configuration of the stereogenic centers C-2/C-3. Consequently, a (2*S*,3*S*)-configuration for acetal **4.26** and (2*R*,3*S*)-configuration for **4.16b** were assumed (**Figure 4.11**). Analogous results were obtained for acetal **4.27**, confirming a *syn*-configuration of the stereogenic centers C-2/C-3. Consequently, a (2*S*,3*S*)-configuration for acetal **4.27** and (2*R*,3*S*)-configuration for **4.17b** were assumed (**Figure 4.11**)



4.26 **4.27**

Figure 4.13 Observed NOE for **4.26** and **4.27**

Because of the very limited amount of products obtained, the procedure described above could not be used for minor diastereomers **4.16a-c** and **4.17a-c**. Hence, the absolute and relative configuration at C-2/C-3 for these products has been hypothesized considering the structure of the chelated transition state described in ref.[20] and the possible presence of an equilibrium between (*Z*) and (*E*)-enolate. The formation of the three diastereomers may be the consequence of an equilibrium between the (*Z*) and the less sterically favoured (*E*)-enolate. As described by Fukuzawa,[20] asymmetric samarium(II) iodide-promoted Reformatsky reactions should occur with the formation of a chelated intermediate state. Samarium

coordinates the oxygen of the incoming aldehyde favouring the *re* face attack because of the isopropyl group of the Evans' oxazolidinone is oriented in a less sterically hindered environment. Thus, (*Z*)-enolate can lead to the formation of two *syn*-diastereomers deriving by favoured *re* face and disfavoured *si* face attack. (**Figure 4.12**)

The stereochemistry of the adduct derived by *re* face attack is in agreement with the absolute and relative configuration determined experimentally for the main diastereomers **4.16b** and **4.17b**.

The less sterically favoured (*E*)-enolate may potentially lead to the formation of two diastereomers, but probably the presence of the pseudo-equatorial methyl group in the chelated transition state further disfavours the *si* face attack, leading to the formation of a single *anti*-diastereomer rather than two. (**Figure 4.12**)

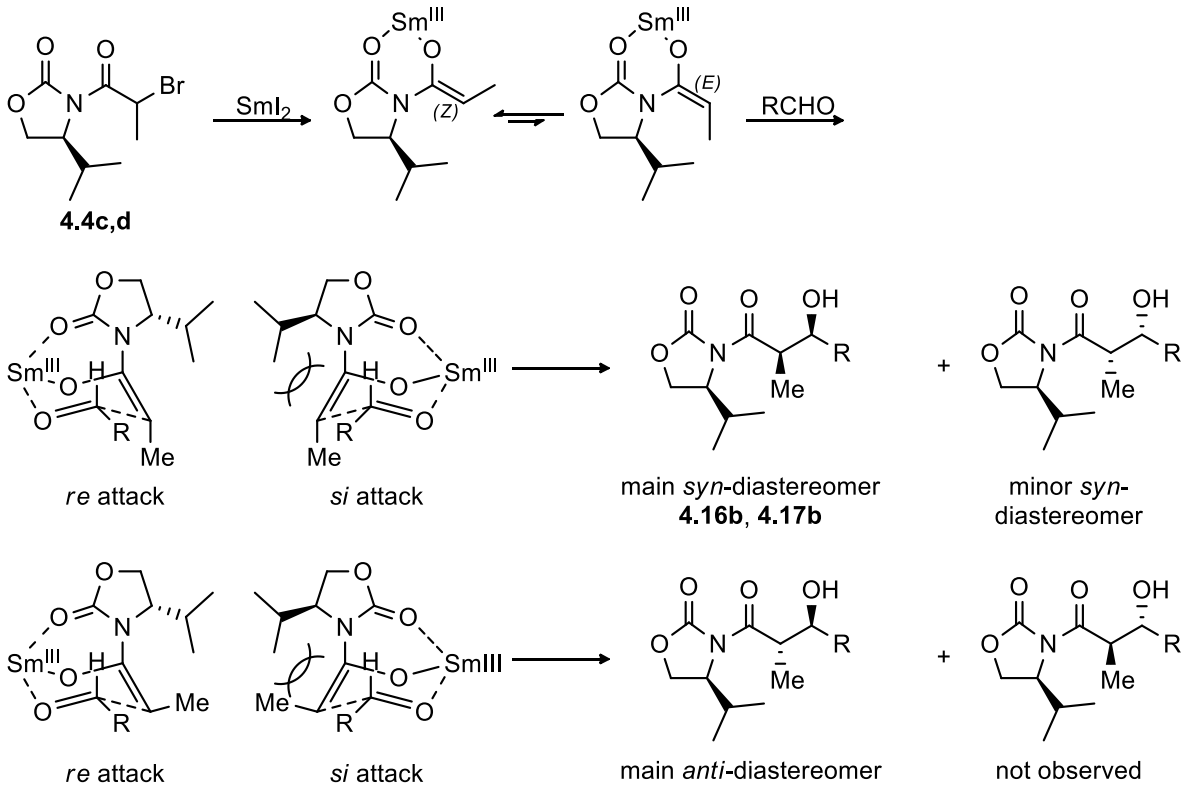


Figure 4.14 Speculation on the configurations at C-2/C-3 for **4.16a,c** and **4.17a,c**

4.3 Summary

In this Chapter are reported the results for the investigation of the use of enals in samarium(II) iodide-promoted Reformatsky reaction. The efforts were focused on the use of terpene-derived aldehydes geranial **4.13a** and neral **4.13b**, to investigate the potential of this reaction for the synthesis of cembranoids.

Samarium(II) iodide-promoted Reformatsky reaction of α-halo *N*-acyl-oxazolidinones **4.4a-d** with terpene-derived aldehydes **4.13a,b** was successful. The reaction provided the coupling product **4.14b**, **4.15b**, **4.16b**, and **4.17b** with good yields and high diastereomer ratio.

The role of the halide, the effect of the temperature, and influence of stereochemistry on the *N*-acyl group were investigated. Replacing the halogen atom on the *N*-acyl group and lowering the temperature of the reaction turned out to be detrimental for the yield of the reaction. Conversely, it was demonstrated that the temperature and the halide have little or no effect on the diastereoselectivity of the reaction. The stereochemistry on the *N*-acyl group turned out to have no effects on the outcome of the reaction and on the diastereomeric ratio.

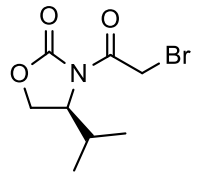
The absolute configuration at the C-3 for products **4.14b**, **4.15b**, **4.16b**, and **4.17b** was successful determined by application of the Mosher's method. It was demostrated that the absolute configuration at C-3 is (*R*) for compounds **4.14b** and **4.15b** and (*S*) for compounds **4.16b** and **4.17b**.

The absolute configuration at the C-2 for products **4.16b** and **4.17b** was successful determined by conversion into the conformational blocked cyclic acetal **4.26** and **4.27** and analisy of NOESY correlation. It was demostrated a (2*R*,3*S*)-configuration for both the products **4.16b** and **4.17b**.

Experimental determination of the absolute configuration at C-2/C-3 for products **4.16a,c** and **4.17a,c** was not possible. A conjecture on the stereochemistry was made considering the chelated transition state of the reaction.

4.4 Experimental part

(4*S*)-3-(bromoacetyl)-4-(1-methylethyl)-oxazolidin-2-one (4.4a)



$C_8H_{12}BrNO_3$
250,09 g/mol

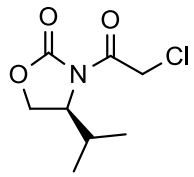
To a solution of (*S*)-4-(1-methylethyl)-oxazolidin-2-one **4.18** (1.0 g, 7.74 mmol) in abs. THF (30 mL) a solution of *n*BuLi (2.5 M in hexane, 3.4 mL, 8.52 mmol) was added dropwise at -78 °C, and the reaction mixture was stirred at the same temperature. After 60 min. a solution of bromoacetyl bromide (810 µL, 9.29 mmol) in abs. THF (8 mL) was added dropwise at -78 °C. The mixture was kept for 30 min. at -78 °C, then slowly warmed to room temperature and stirred overnight. A saturated solution of $NaHCO_3$ (50 mL) was added and the layers were separated. The aqueous layer was extracted with ethyl acetate (3×50 mL), the combined organic layers were dried with $MgSO_4$ and the solvent was removed in vacuo. The residue was purified by flash chromatography (SiO_2, 5:1 petroleum ether/ethyl acetate) to **4.4a** (1.45g, 75 %) as a white solid: mp 54-56 °C. $[\alpha]^{20}_D$ = +83.0 (c 1.0; $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.61-4.25 (m, 5H), 2.47-2.36 (m, 1H), 0.94 (dd, *J* = 7.0 Hz, 3H), 0.88 (dd, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.9, 153.5, 63.9, 58.6, 28.1, 17.7, 14.6 ppm.

Spectroscopic data were in accordance with the literature.[32]

(4*S*)-3-(chloroacetyl)-4-(1-methylethyl)-oxazolidin-2-one (4.4b)



$C_8H_{12}ClNO_3$
205,64 g/mol

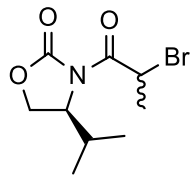
Prepared as described for **4.4a** from **4.18** (1.5 g, 11.61 mmol) and freshly distilled chloroacety chloride (1.11 mL, 13.93 mmol) to give **4.4b** (2.03 g, 85 %) after flash chromatography (SiO_2, 5:1 petroleum ether/ethyl acetate) as a yellow oil. $[\alpha]^{20}_D$ = +97.9 (c 1.0; $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.81-4.63 (m, 2H), 4.50-4.41 (m, 1H), 4.39-4.31 (m, 1H), 4.28 (dd, *J* = 9.1, 3.2 Hz, 1H), 2.50-2.34 (m, 1H), 0.94 (d, *J* = 7.0 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.2, 153.9, 64.3, 58.9, 43.8, 28.3, 18.0, 14.7 ppm.

Spectroscopic data were in accordance with the literature.[32]

(4*S*)-3-(2-bromopropanoyl)-4-(1-methylethyl)-oxazolidin-2-one (4.4c,d)



$C_9H_{14}BrNO_3$
264,12 g/mol

To a solution of (*S*)-4-(1-methylethyl)-oxazolidin-2-one **4.18** (1.0 g, 7.74 mmol) in abs. THF (30 mL) a solution of *n*BuLi (2.5 M in hexane, 3.72 mL, 9.29 mmol) was added dropwise at -78 °C and the reaction mixture was stirred at the same temperature. After 60 min. a solution of (2*RS*)-bromopropanoyl bromide (1.05 mL, 10.06 mmol) in abs. THF (10 mL) was added dropwise at -78 °C. The mixture was kept for 60 min. at -78 °C, then slowly warmed to room temperature and stirred overnight. The reaction mixture was diluted with CH_2Cl_2 (40 mL) and washed with saturated solution of $NaHCO_3$ (1×40 mL); afterwards the aqueous layer was extracted with ethyl acetate (3×40 mL). The combined organic layers were washed with brine (1×120 mL), dried with $MgSO_4$ and the solvent was removed in vacuo. The residue was purified by flash chromatography (SiO_2, 5:1 petroleum ether/ethyl acetate) to give products **4.4c,d** in 1:2 diastereomeric mixture (1.06g, 52 %) as a white waxy solid.

4.4c

Obtained as a white waxy solid (354 mg, 17 %). $[\alpha]^{20}_D$ = + 53.3 (c 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.75 (q, *J* = 6.8 Hz, 1H), 4.43 (ddd, *J* = 8.0, 3.7, 2.7 Hz, 1H), 4.34 (t, *J* = 8.6 Hz, 1H), 4.25 (dd, *J* = 9.0, 2.5 Hz, 1H), 1.85 (d, *J* = 6.8 Hz, 3H), 0.91 (dd, *J* = 19.8, 7.0 Hz, 6H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.9, 153.5, 63.9, 58.6, 28.1, 17.7, 14.6 ppm.

4.4d

Obtained as a white solid (708 mg, 35 %): mp 54-56 °C. $[\alpha]^{20}_D$ = +72.2 (c 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.75 (q, *J* = 6.8 Hz, 1H), 4.43 (ddd, *J* = 8.0, 3.7, 2.7 Hz, 1H), 4.34 (t, *J* = 8.6 Hz, 1H), 4.25 (dd, *J* = 9.0, 2.5 Hz, 1H), 1.85 (d, *J* = 6.8 Hz, 3H), 0.91 (dd, *J* = 19.8, 7.0 Hz, 6H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.9, 153.5, 63.9, 58.6, 28.1, 17.7, 14.6 ppm.

Spectroscopic data were in accordance with the literature.[32]

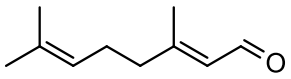
General procedure for the synthesis of (*E*/*Z*)-3,7-dimethylocta-2,6-dienal) 4.13a and 4.13b with Dess-Martin Periodinane (Method A)

To a solution of the appropriate terpenic alcohol (300 mg, 1.94 mmol) in abs. CH_2Cl_2 (15 mL) Dess-Martin Periodinane (987.4 mg, 2.33 mmol) was added at room temperature. The reaction mixture was stirred at the same temperature for 2 h. Then a saturated solution of $Na_2S_2O_3$ was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were washed with brine (1×45 mL), dried with $MgSO_4$ and the solvent was removed in vacuo. The residue was purified by flash chromatography (SiO_2, 25:1 petroleum ether/ethyl acetate) to give the corresponding enal.

General procedure for the synthesis of (*E*/*Z*)-3,7-dimethylocta-2,6-dienal) 4.13a and 4.13b with Ley-Griffith Reagent (tetrapropylammonium perruthenate, TPAP) (Method B)

In a Schlenk flask, grinded 4 Å molecular sieves (6.5 g) were activated by prolonged heating under vacuo. After cooling under N_2 atmosphere, N-methylmorpholine-N-oxide (2,28 g) was added and the flask was three times evacuated and then filled with N_2. The powder mixture was suspended with abs. CH_2Cl_2 (26 ml) and the appropriate terpenic alcohol (2,0 g) was added at room temperature under vigorous stirring. TPAP (44mg, 0.1 mol%) was quickly added and the reaction mixture was stirred overnight. The reaction mixture was filtered on a short plug of silica and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 25:1 petroleum ether/ethyl acetate) to give the corresponding enal.

(2*E*)-3,7-dimethylocta-2,6-dienal (4.13a, Geranial)



$C_{10}H_{16}O$
152,23 g/mol

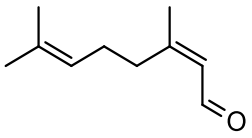
Prepared according to method A (263 mg, 89 %) as a colourless oil.
Prepared according to method B (1.64 g, 83 %) as a colourless oil.

^{1}H NMR (400 MHz, CDCl3): δ = 10.00 (d, *J* = 8.0 Hz, 1H), 5.90-5.87 (m, 1H), 5.09-5.05 (m, 1H), 2.26-2.19 (m, 2H), 2.17 (s, 3H), 1.69 (s, 3H), 2.61 (s, 3H) ppm.

^{13}C NMR (100 MHz, CDCl3): δ = 191.4, 163.9, 133.1, 127.5, 122.7, 40.7, 25.9, 25.8, 17.8, 17.7 ppm.

Spectroscopic data are in good agreement with ref.[33]

(2*Z*)-3,7-dimethylocta-2,6-dienal (4.13b, Neral)



$C_{10}H_{16}O$
152,23 g/mol

Prepared according to method A (239 mg, 81 %) as a colourless oil.
Prepared according to method B (1.54 g, 78 %) as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.88 (d, *J* = 8.3 Hz, 1H), 5.89-5.87 (m, 1H), 5.10-5.06 (m, 1H), 2.58-2.22 (m, 2H), 1.98 (s, 3H), 1.68 (s, 3H), 1.59 (s, 3H) ppm.

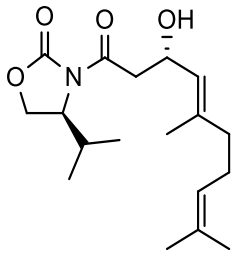
^{13}C NMR (100 MHz, CDCl3): δ = 190.9, 163.9, 133.8, 128.8, 122.4, 32.7, 27.2, 25.8, 25.2, 17.9 ppm.

Spectroscopic data are in agreement with ref.[33]

General Procedure for the Samarium(II) iodide mediated-Reformatsky Reaction of *N*-Acyloxazolidinones (4.4a-d) with enals (4.13a, 4.13b)

To a solution of samarium(II) iodide (0.1M in THF, 22 mL) a solution of *N*-acyloxazolidinone (1.0 mmol) in abs. THF (3 mL) was added dropwise at -78 °C in the dark. The reaction mixture was stirred for 5 min. before a solution of the enal (1.2 mmol) in abs. THF (3 mL) was added dropwise. The reaction was stirred at -78 °C in the dark for 30 min. Then a saturated solution of NH_4Cl (1×20 mL) was added at -78 °C, and the reaction mixture was warmed to room temperature. The layers were separated, and the aqueous fraction was extracted with ethyl acetate (3×15 mL). The combined organic layers were washed with a saturated solution $Na_2S_2O_3$ (1×45, dried with $MgSO_4$ and concentrated under vacuum. The residue was purified by flash chromatography (SiO_2, 5:1→1:1 petroleum ether/ethyl acetate).

(*S*)-3-((*S*,*E*)-3-hydroxy-5,9-dimethyldeca-4,8-dienoyl)-4-isopropyloxazolidin-2-one (4.14a)



$C_{18}H_{29}NO_4$
323,43 g/mol

Prepared according to general procedure from **4.4a** and **4.13a** after flash chromatography (20 mg, 6 %) as a colourless oil.

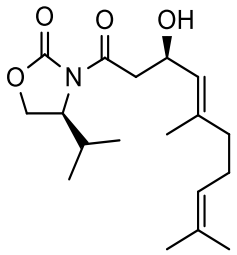
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.29-5.24 (m, 1H), 5.12-5.05 (m, 1H), 4.88-4.83 (m, 1H), 4.48-4.44 (m, 1H), 4.31-4.21 (m, 2H), 3.22 (dd, *J* = 17.1, 9.1 Hz, 1H), 3.05 (dd, *J* = 17.1, 3.3 Hz, 1H), 2.46-2.30 (m, 1H), 2.12-1.99 (m, 4H) 1.70 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H), 0.94 (d, *J* = 7.0 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.8, 154.2, 141.1, 132.1, 125.4, 124.1, 70.9, 63.8, 58.8, 44.7, 39.9, 28.9, 26.6, 18.2, 18.0, 17.1, 15.1, 14.9 ppm.

MS (ESI): *m/z* = calcd for[$C_{18}H_{29}NO_4^+$]: 346.1995, found 346.1983.

FT-IR (ATR): ν = 3524 (w), 2963 (m), 2923 (m), 2855 (m), 1782 (vs), 1699 (s), 1486 (w), 1448 (w), 1387 (s), 1374 (s), 1303 (m), 1205 (s), 1142 (w), 1120 (w), 1105 (w), 1061 (w), 1021 (w), 973 (w).

(*S*)-3-((*R*,*E*)-3-hydroxy-5,9-dimethyldeca-4,8-dienoyl)-4-isopropyloxazolidin-2-one (4.14b)



$C_{18}H_{29}NO_4$
323,43 g/mol

Prepared according to general procedure from **4.4a** and **4.13a** after flash chromatography (236 mg, 73 %) as a colourless oil. $[\alpha]^{20}_D$ = +33.5 (c 1.2, CH_2Cl_2)

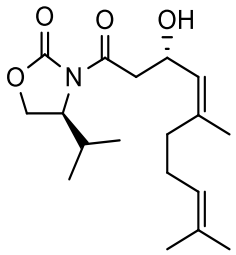
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.25 (dd, *J* = 8.5, 1.1 Hz, 1H), 5.11-5.04 (m, 1H), 4.91-4.85 (m, 1H), 4.48-4.39 (m, 1H), 4.30-4.20 (m, 2H), 3.16-3.06 (m, 2H), 2.43-2.32 (m, 1H), 2.14-1.95 (m, 4H) 1.71 (s, 3H), 1.67 (s, 3H), 1.59 (s, 3H), 0.92 (d, *J* = 7.0 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.3, 154.1, 139.5, 131.9, 125.5, 124.0, 65.2, 63.8, 58.6, 43.0, 39.6, 28.6, 26.5, 25.8, 18.1, 17.8, 16.8, 14.9 ppm.

MS (ESI): *m/z* = calcd for[$C_{18}H_{29}NO_4^+$]: 346.1995, found 346.1993.

FT-IR (ATR): ν =3485 (w), 2964 (m), 2920 (m), 1777 (vs), 1696 (s), 1486 (w), 1441 (w), 1372 (s), 1301 (m), 1202 (s), 1141 (m), 1120 (m), 1104 (m), 1058 (s), 1020 (s), 928 (w), 814 (w), 774 (m), 753 (m), 713 (m), 640 (m), 590 (w), 526 (w).

(*S*)-3-((*S*,*Z*)-3-hydroxy-5,9-dimethyldeca-4,8-dienoyl)-4-isopropyloxazolidin-2-one (4.15a)



$C_{18}H_{29}NO_4$
323,43 g/mol

Prepared according to the general procedure from **4.4a** and **4.13b** after flash chromatography (21 mg, 7 %) as a colourless oil.

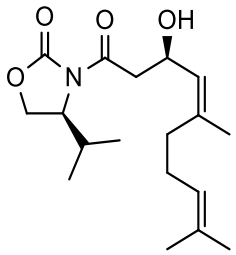
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.28 (d, *J* = 8.9 Hz, 1H), 5.15-5.07 (m, 1H), 4.92-4.83 (m, 1H), 4.48 -4.41 (m, 1H), 4.30-4.20 (m, 2H), 3.16-3.06 (m, 2H), 2.43-2.32 (m, 1H), 2.14-1.95 (m, 4H) 1.71 (s, 3H), 1.67 (s, 3H), 1.59 (s, 3H), 0.93 (d, *J* = 7.0 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.3, 154.1, 139.5, 131.9, 125.5, 124.0, 65.2, 63.8, 58.6, 43.0, 39.6, 28.6, 26.5, 25.8, 18.1, 17.8, 16.8, 14.9 ppm.

MS (ESI): *m/z* = calcd for[$C_{18}H_{29}NO_4^+$]: 346.1995, found 346.2004.

FT-IR (ATR): ν =3495 (w), 2964 (w), 2925 (w), 2976 (w), 1778 (s), 16996 (m), 1447 (w), 1386 (s), 1373 (s), 1302 (m), 1204 (s), 1179 (m), 1143 (w), 1120 (w), 1102 (w), 1058 (m), 1020 (m), 972 (w), 845 (w), 775 (w), 754 (w), 713 (w), 640 (w), 587 (w), 527 (w), 454 (w).

(*S*)-3-((*R*,*Z*)-3-hydroxy-5,9-dimethyldeca-4,8-dienoyl)-4-isopropyloxazolidin-2-one (4.15b)



$C_{18}H_{29}NO_4$
323,43 g/mol

Prepared according to the general procedure from **4.4a** and **4.13b** after flash chromatography (230 mg, 71 %) as a colourless oil. $[\alpha]^{20}_D$ = +38.7 (c 1.0, CH_2Cl_2).

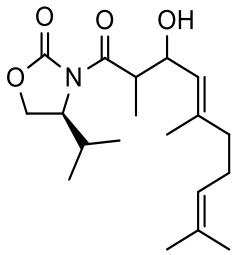
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.28 (d, *J* = 8.9 Hz, 1H), 5.15-5.08 (m, 1H), 4.90-4.85 (m, 1H), 4.49-4.41 (m, 1H), 4.32-4.19 (m, 2H), 3.14-3.09 (m, 2H), 2.44-2.33 (m, 1H), 2.20-2.03 (m, 4H) 1.73 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H), 0.92 (d, *J* = 7.0 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.3, 154.1, 139.5, 131.9, 125.5, 124.0, 65.2, 63.8, 58.6, 43.0, 39.6, 28.6, 26.5, 25.8, 18.1, 17.8, 16.8, 14.9 ppm.

MS (ESI): *m/z* = calcd for[$C_{18}H_{29}NO_4^+$]: 346.1995, found 346.1980.

FT-IR (ATR): ν =3495 (w), 2964 (w), 2925 (w), 2976 (w), 1778 (s), 1699 (m), 1447 (w), 1386 (s), 1373 (s), 1302 (m), 1204 (s), 1179 (m), 1143 (w), 1120 (w), 1102 (w), 1058 (m), 1020 (m), 972 (w), 845 (w), 775 (w), 754 (w), 713 (w), 640 (w), 587 (w), 527 (w), 454 (w)

(4*S*)-3-((*E*)-3-hydroxy-2,5,9-trimethyldeca-4,8-dienoyl)-4-isopropyloxazolidin-2-one (4.16a)



$C_{19}H_{31}NO_4$
337,45 g/mol

Prepared according to the general procedure from **4.4c,d** and **4.13a** after flash chromatography (46 mg, 14 %) as colourless oil. $[\alpha]^{20}_D$ = +42.7 (c 1.0, CH_2Cl_2).

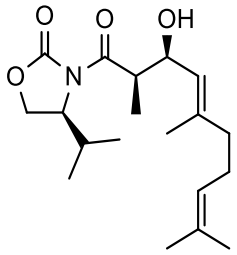
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.19 (d, *J* = 8.3 Hz), 5.12-5.01 (m, 1H), 4.51-4.40 (m, 1H), 4.32-4.16 (m, 1H), 4.32-4.19 (m, 2H), 3.91 (dq, *J* = 13.8, 6.9 Hz), 2.50-2.34 (m, 1H), 2.16-1.95 (m, 4H) 1.70 (s, 3H), 1.67 (s, 3H), 1.60 (s, 3H), 1.08 (d, *J* = 6.9 Hz, 3H), 0.96 (d, *J* = 7.0 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.7, 154.5, 140.9, 132.0, 125.7, 124.0, 71.9, 63.4, 59.1, 44.2, 39.8, 28.6, 26.5, 25.8, 18.1, 17.8, 17.0, 14.7, 14.2 ppm.

MS (ESI): *m/z* = calcd for[$C_{18}H_{29}NO_4^+$]: 337.2253, found 360.2145.

FT-IR (ATR): ν = 3501 (w), 2966 (m), 2924 (m), 2878 (w), 1775 (vs), 1698 (s), 1487 (w), 1454 (m), 1383 (vs), 1301 (m), 1251 (m), 1202 (vs), 1142 (m), 1121 (m), 1107 (m), 1081 (m), 1054 (m), 990 (s), 954 (m), 901 (w), 817 (w), 775 (w), 760 (w), 709 (w), 636 (w), 530 (w), 462 (w).

(*S*)-3-((2*R*,3*S*,*E*)-3-hydroxy-2,5,9-trimethyldeca-4,8-dienoyl)-4-isopropyloxazolidin-2-one (4.16b)



$C_{19}H_{31}NO_4$
337,45 g/mol

Prepared according to the general procedure from **4.4c,d** and **4.13a** after flash chromatography (184 mg, 55 %) as a colourless oil. $[\alpha]^{20}_D$ = +43.6 (c 1.0, CH_2Cl_2).

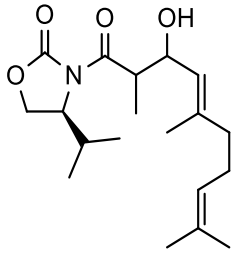
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.22 (dd, *J* = 8.7, 1.1 Hz, 1H), 5.11-5.01 (m, 1H), 4.49-4.42 (m, 1H), 4.32-4.16 (m, 1H), 4.31-4.17 (m, 2H), 4.04-3.94 (m, 1H), 2.42-2.26 (m, 1H), 2.14-1.96 (m, 4H) 1.70 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H), 1.19 (d, *J* = 6.9 Hz, 3H), 0.91 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 7.0 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.9, 154.3, 140.4, 131.9, 125.7, 124.0, 69.8, 63.4, 58.8, 43.1, 39.9, 28.7, 26.5, 25.8, 18.1, 17.8, 17.0, 14.8, 11.9 ppm.

MS (ESI): *m/z* = calcd for[$C_{18}H_{29}NO_4^+$]: 360.2151, found 360.2145.

FT-IR (ATR): ν = 3496 (w), 2965 (m), 2926 (m), 2878 (w), 1775 (vs), 1697 (s), 1487 (w), 1453 (w), 1375 (s), 1300 (m), 1203 (vs), 1143 (w), 1120 (m), 1101 (m), 1054 (w), 1015 (w), 989 (m), 954 (m), 904 (w), 818 (w), 776 (w), 709 (w), 640 (w), 537 (w), 441 (w).

(4*S*)-3-((*E*)-3-hydroxy-2,5,9-trimethyldeca-4,8-dienoyl)-4-isopropyloxazolidin-2-one (4.16c)



$C_{19}H_{31}NO_4$
337,45 g/mol

Prepared according to the general procedure from **4.4c,d** and **4.13a** after flash chromatography (61 mg, 18 %) as a colourless oil. $[\alpha]^{20}_D$ = +38.3 (c 1.2, CH_2Cl_2).

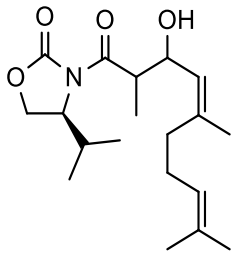
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.16 (d, *J* = 9.1 Hz, 1H), 5.09-4.99 (m, 1H), 4.58-4.44 (m, 2H), 4.30-4.13 (m, 2H), 3.82 (dq, *J* = 14.1, 7.0 Hz, 1H), 2.38-2.29 (m, 1H), 2.15-1.97 (m, 4H) 1.68 (s, 3H), 1.65 (s, 3H), 1.58 (s, 3H), 1.10 (d, *J* = 7.0 Hz, 3H), 0.91 (d, *J* = 7.0 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.6, 154.0, 140.9, 131.9, 125.2, 123.9, 76.1.8, 63.6, 58.6, 44.5, 39.7, 28.7, 26.4, 25.5, 18.0, 17.8, 16.9, 14.9, 14.7 ppm.

MS (ESI): *m/z* = calcd for$[C_{18}H_{29}NO_4^+]$: 360.2151, found 360.2145.

FT-IR (ATR): ν = 3445 (w), 2645 (m), 2928 (m), 2878 (w), 1778 (vs), 1696 (s), 1485 (w), 1455 (m), 1382 (vs), 1300 (m), 1256 (s), 1225 (s), 1202 (vs), 1142 (m), 1120 (m), 1104 (m), 1076 (m), 1055 (m), 988 (s), 954 (m), 901 (w), 846 (w), 818 (w), 775 (w), 760 (w), 733,6 (w), 707 (m), 638 (w), 527 (w), 461 (w).

(4*S*)-3-((*Z*)-3-hydroxy-2,5,9-trimethyldeca-4,8-dienoyl)-4-isopropyloxazolidin-2-one (4.17a)



$C_{19}H_{31}NO_4$
337,45 g/mol

Prepared according to the general procedure from **4.4c,d** and **4.13b** after flash chromatography (25 mg, 7 %) as a colourless oil. $[\alpha]^{20}_D$ = +30.2 (c 1.2, CH_2Cl_2).

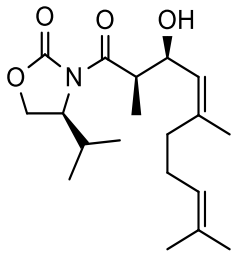
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.18 (d, *J* = 9.2 Hz, 1H), 5.14-5.00 (m, 1H), 4.51-4.36 (m, 2H), 4.34-4.15 (m, 3H), 3.95-3.82 (m, 1H), 2.46-2.30 (m, 1H), 2.19-2.03 (m, 4H) 1.73 (s, 3H), 1.66 (s, 3H), 1.58 (s, 3H), 1.06 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 7.1 Hz, 3H), 0.88 (d, *J* = 7.0 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.7, 154.3, 141.2, 132.3, 126.6, 123.9, 71.3, 63.3, 59.9, 44.9, 38.6, 28.0, 26.6, 25.7, 23.4, 18.2, 17.9, 14.6, 14.4 ppm.

MS (ESI): *m/z* = calcd for[$C_{18}H_{29}NO_4^+$]: 360.2151, found 360.2168.

FT-IR (ATR): ν = 3517 (w), 2965 (m), 2931 (m), 2877 (w), 1776 (vs), 1700 (s), 1487 (w), 1450 (w), 1386 (s), 1373 (s), 1301 (w), 1248 (m), 1205 (s), 1143 (w), 1121 (m), 1056 (m), 1015 (m), 990 (m), 953 (w), 900 (w), 816 (w), 773 (w), 757 (w), 698 (w), 634 (w), 533 (w).

(*S*)-3-((2*R*,3*S*,*Z*)-3-hydroxy-2,5,9-trimethyldeca-4,8-dienoyl)-4-isopropyloxazolidin-2-one (4.17b)



$C_{19}H_{31}NO_4$
337,45 g/mol

Prepared according to the general procedure from **4.4c,d** and **4.13b** after flash chromatography (201 mg, 59 %) as a colourless oil. $[\alpha]^{20}_D$ = +26.7 (c 1.2, CH_2Cl_2).

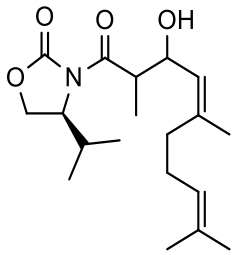
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.18 (d, *J* = 9.3 Hz, 1H), 5.15-5.05 (m, 1H), 4.56-4.44 (m, 2H), 4.31-4.15 (m, 2H), 3.88-3.77 (m, 1H), 2.42-2.26 (m, 1H), 2.22-1.98 (m, 4H) 1.74 (s, 3H), 1.67 (s, 3H), 1.60 (s, 3H), 1.12 (d, *J* = 7.0 Hz, 3H), 0.91 (d, *J* = 7.0 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.6 (C-1), 154.0 (C-3), 141.4 (C-5'), 132.4 (C-9'), 126.1 (C-4'), 123.9 (C-8'), 70.3 (C-4), 63.6 (C-5), 58.6 (C-3'), 44.3 (C-2'), 32.5 (C-6'), 28.7 ($\underline{C}H(CH_3)_2$), 26.7 (C-7'), 25.7 (C-10'), 23,.5 (CH_3), 18.0 (CH_3), 17.8 (CH_3), 15.0 (2 CH_3) ppm.

MS (ESI): *m/z* = calcd for[$C_{18}H_{29}NO_4^+$]: 360.2151, found 360.2164.

FT-IR (ATR): ν = 3497 (w), 2965 (m), 2926 (m), 2877 (m), 1774 (vs), 1696 (s), 1487 (w), 1454 (m), 1374 (vs), 1300 (m), 1201 (vs), 1142 (m), 1119 (m), 1100 (m), 1054 (m), 1014 (m), 989 (s), 965 (m), 954 (m), 904 (w), 845 (w), 819 (w), 775 (w), 761 (w), 709 (m), 640 (w), 555 (w), 533 (w), 451 (w).

(4*S*)-3-((*Z*)-3-hydroxy-2,5,9-trimethyldeca-4,8-dienoyl)-4-isopropyloxazolidin-2-one (4.17c)



$C_{19}H_{31}NO_4$
337,45 g/mol

Prepared according to the general procedure from **4.4c,d** and **4.13b** after flash chromatography (56 mg, 17 %) as a colourless oil. $[\alpha]^{20}_D$ = +24.6 (c 1.1, CH_2Cl_2).

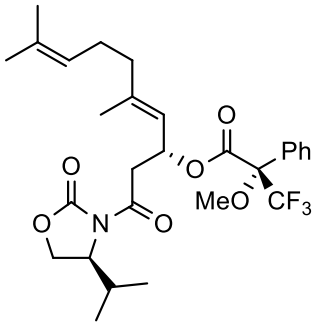
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.18 (d, *J* = 9.3 Hz, 1H), 5.15-5.05 (m, 1H), 4.56-4.44 (m, 2H), 4.31-4.15 (m, 2H), 3.88-3.77 (m, 1H), 2.42-2.26 (m, 1H), 2.22-1.98 (m, 4H) 1.74 (s, 3H), 1.67 (s, 3H), 1.60 (s, 3H), 1.12 (d, *J* = 7.0 Hz, 3H), 0.91 (d, *J* = 7.0 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.6, 154.0, 141.4, 132.4, 126.1, 123.9, 70.3, 63.6, 58.6, 44.3, 32.5, 28.7, 26.7, 25.7, 23,.5, 18.0, 17.8, 15.0 ppm.

MS (ESI): *m/z* = calcd for[$C_{18}H_{29}NO_4^+$]: 360.2151, found 360.2158.

FT-IR (ATR): ν = 3469 (w), 2964 (m), 2931 (m), 2977 (w), 1777 (s), 1696 (s), 1455 (m), 1382 (s), 1300 (m), 1255 (m), 1225 (s), 1202 (s), 1143 (w), 1120 (m), 1101 (m), 1055 (m), 988 (s), 954 (m), 901 (w), 848 (w), 819 (w), 775 (w), 707 (m), 638 (w), 595 (w), 528 (w), 449 (w).

(*R*,*E*)-1-((*S*)-4-isopropyl-2-oxooxazolidin-3-yl)-5,9-dimethyl-1-oxodeca-4,8-dien-3-yl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate ((*S*)-4.20)



$C_{28}H_{36}F_3NO_6$
539,58 g/mol

To a solution of **4.14b** (19.9 mg) in anhydrous CH_2Cl_2 (2 ml), anhydrous pyridine (10.5 µL), (*R*)-MTPACl (20.0 mg) dissolved in anhydrous CH_2Cl_2 (1 ml) and DMAP (3.70 mg) were added in this order at 0 °C. The reaction mixture was slowly warmed to room temperature and stirred overnight. The solvent was removed under vacuum and the residue was purified by flash chromatography (SiO_2, 6:1 petroleum ether/ethyl acetate) to give **(*S*)-4.20** (28 mg, 85 %) as a colourless oil.

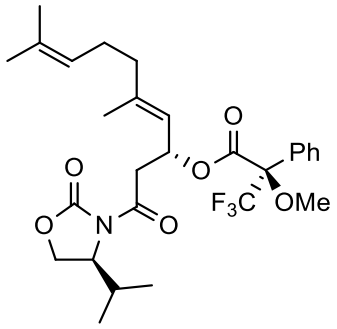
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49-7.46 (m, 2H), 7.41-7.34 (m, 4H), 6.21 (td, *J* = 9.5, 3.7 Hz, 1H), 5.09-5.03 (m, 2H), 4.39-4.33 (m, 1H), 4.26-4.16 (m, 2H), 3.52 (s, 3H), 3.44 (dd, *J* = 17.1, 9.4 Hz, 1H), 3.16 (dd, *J* = 17.1, 3.7 Hz, 1H), 2.41-2.30 (m, 1H), 2.11-1.96 (m, 4H), 1.86 (s, 3H), 1.66 (s, 3H), 1.59 (s, 3H), 0.91 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 7.0 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.3, 165.6, 154.1, 143.5, 132.6, 132.1, 128.3, 127.7, 124.2, 123.6, 122.6, 120.5, 70.5, 63.6, 58.7, 55.7, 40.4, 39.6, 29.8, 26.4, 25.8, 18.1, 17.8, 17.1, 14.7 ppm.

MS (ESI): *m/z* = calcd for[$C_{28}H_{36}F_3NO_6^+$]: 562.2393, found 562.2387.

FT-IR (ATR): ν = 2960 (m), 2924 (m), 2854 (w), 1782 (vs), 1746 (s), 1703 (s), 1488 (w), 1452 (w), 1452 (m), 1387 (s), 1301 (m), 1269 (s), 1238 (s), 1169 (vs), 1121 (s), 1080 (m), 1018 (m), 991 (m), 920 (w), 831 (w), 766 (w), 719 (m), 641 (w).

(*R*,*E*)-1-((S)-4-isopropyl-2-oxooxazolidin-3-yl)-5,9-dimethyl-1-oxodeca-4,8-dien-3-yl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate ((*S*)-4.20)



$C_{28}H_{36}F_3NO_6$
539,58 g/mol

Prepared as described for **(*S*)-4.20** from **4.14b** (19.9 mg) in anhydrous CH_2Cl_2 (2 ml), anhydrous pyridine (10.5 µL), (*S*)-MTPACl (20.0 mg) in anhydrous CH_2Cl_2 (1 ml), and DMAP (3.70 mg) to give **(*R*)-4.20** (27 mg, 84 %) after flash chromatography (SiO_2, 6:1 petroleum ether/ethyl acetate) as a colourless oil.

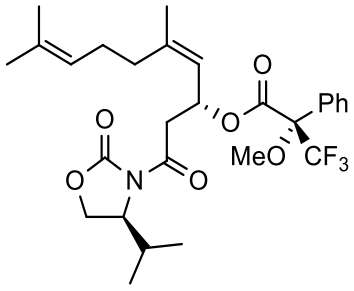
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.54-7.49 (m, 2H), 7.39-7.33 (m, 4H), 6.26 (td, *J* = 9.5, 3.8 Hz, 1H), 5.23 (d, *J* = 9.6 Hz, 1H), 5.09-5.03 (m, 1H), 4.22-4.07 (m, 3H), 3.54 (s, 3H), 3.54-3.49 (m, 1H), 3.03 (dd, *J* = 16.9, 3.8 Hz, 1H), 2.36-2.27 (m, 1H), 2.12-1.99 (m, 4H), 1.87 (s, 3H), 1.66 (s, 3H), 1.60 (s, 3H), 0.88 (t, *J* = 7.9 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.0, 165.4, 154.1, 143.9, 132.8, 132.2, 129.5, 128.3, 127.6, 124.3, 123.5, 122.6, 120.6, 70.4, 63.6, 58.6, 55.6, 40.1, 39.6, 28.5, 26.4, 25.8, 18.1, 17.8, 17.2, 14.7 ppm.

MS (ESI): *m/z* = calcd for[$C_{28}H_{36}F_3NO_6^+$]: 562.2393, found 562.2387.

FT-IR (ATR): ν = 2964 (w), 2925 (w), 2854 (w), 1780 (s), 1747 (s), 1703 (s), 1488 (w), 1451 (w), 1386 (s), 1258 (s), 1237 (s), 1204 (s), 1167 (vs), 1120 (s), 1106 (s), 1080 (m), 1017 (s), 989 (s), 971 (s), 914 (m), 824 (w), 765 (m), 731 (s), 717 (s), 697 (s), 642 (m), 579 (w), 553 (w), 510 (w), 442 (w).

(*R*,*Z*)-1-((S)-4-isopropyl-2-oxooxazolidin-3-yl)-5,9-dimethyl-1-oxodeca-4,8-dien-3-yl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate ((*S*)-4.21)



$C_{28}H_{36}F_3NO_6$
539,58 g/mol

Prepared as described for **(*S*)-4.20** from **4.15b** (19.9 mg) in anhydrous CH_2Cl_2 (2 ml), anhydrous pyridine (10.5 µL), (*R*)-MTPACl (20.0 mg) in anhydrous CH_2Cl_2 (1 ml), and DMAP (3.70 mg) to give **(*S*)-4.21** (23 mg, 70 %) after flash chromatography (SiO_2, 5:1 petroleum ether/ethyl acetate) as a colourless oil.

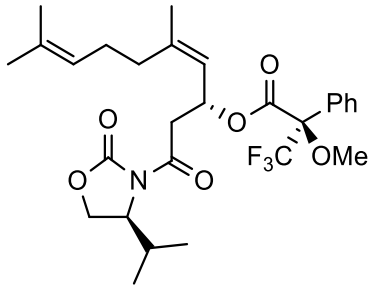
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50-7.45 (m, 2H), 7.41-7.34 (m, 4H), 6.24-6.16 (m, 1H), 5.16-5.11 (m, 1H), 5.11-5.04 (m, 1H), 4.38-4.32 (m, 1H), 4.24-4.18 (m, 2H), 3.51 (s, 3H), 3.47 (dd, *J* = 17.4, 9.7 Hz, 1H), 3.07 (dd, *J* = 17.4, 3.3 Hz, 1H), 2.42-2.32 (m, 1H), 2.21-2.12 (m, 2H), 2.12-2.04 (m, 1H), 1.73 (s, 3H), 1.66 (s, 3H), 1.61 (s, 3H), 0.92 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 7.0 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.3, 165.5, 154.1, 143.3, 132.8, 132.6, 129.6, 128.3, 127.7, 124.0, 121.4, 70.0, 63.6, 58.7, 55.7, 40.5, 32.6, 28.8, 26.6, 25.8, 18.1, 17.8, 14.7, 14.3 ppm.

MS (ESI): *m/z* = calcd for[$C_{28}H_{36}F_3NO_6^+$]: 562.2393, found 562.2401.

FT-IR (ATR): ν = 2962 (m), 2924 (m), 2854 (w), 1782 (vs), 1747 8s), 1702 (s), 1591 (w), 1487 (w), 1451 (w), 1387 (s), 1270 (s), 1238 (s), 1206 (s), 1183 8vs), 1169 (vs), 1121 (s), 1107 (m), 1080 (m), 1019 (m), 991 (m), 972 (m), 919 (w), 823 (w), 765 (w), 719 (m), 641 (w), 600 (w), 559 (w), 502 (w), 450 (w).

(*R*,*Z*)-1-((S)-4-isopropyl-2-oxooxazolidin-3-yl)-5,9-dimethyl-1-oxodeca-4,8-dien-3-yl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate ((*R*)-4.21)



$C_{28}H_{36}F_3NO_6$
539,58 g/mol

Prepared as described for **(*S*)-4.20** from **4.15b** (19.9 mg) in anhydrous CH_2Cl_2 (2 ml), anhydrous pyridine (10.5 µL), (*S*)-MTPACl (20.0 mg) in anhydrous CH_2Cl_2 (1 ml), and DMAP (3.70 mg,) to give **(*R*)-4.21** (26 mg 80 %) after flash chromatography (SiO_2, 6:1 petroleum ether/ethyl acetate) as a colourless oil.

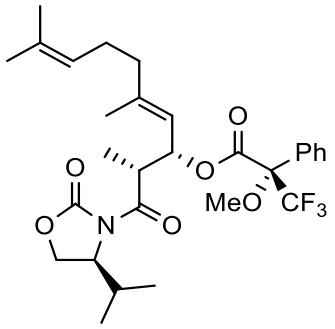
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53-7.49 (m, 2H), 7.38-7.35 (m, 4H), 6.27 (td, *J* = 9.8, 3.4 Hz), 5.24 (d, *J* = 9.8 Hz, 1H), 5.16-5.10 (m, 1H), 4.20-4.05 (m, 3H), 3.59-3.50(m, 1H), 3.54 (s, 3H), 2.94 (dd, *J* = 17.2, 3.4 Hz, 1H), 2.38-2.27 (m, 1H), 2.22-2.02 (m, 4H), 1.76 (s, 3H), 1.65 (s, 3H), 1.61 (s, 3H), 0.89 (d, *J* = 7.0 Hz, 3H), 0.83 (d, *J* = 7.0 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.9, 165.3, 154.1, 143.6, 132.9, 132.4, 129.4, 128.3, 127.7, 123.9, 121.4, 70.0, 63.6, 58.6, 55.6, 40.2, 32.6, 28.5, 26.6, 25.8, 23.5, 18.1, 17.8, 14.7 ppm.

MS (ESI): *m/z* = calcd for[$C_{28}H_{36}F_3NO_6^+$]: 562.2393, found 562.2387.

FT-IR (ATR): ν = 2965 (w), 2925 (w), 2854 (w)1780 (vs), 1748 (s), 1703 (s), 1487 (w), 1451 (w), 1386 (s), 1237 (s), 1168 (vs), 1120 (s), 1106 (s), 1080 (m), 1018 (s), 989 (s), 971 8s), 917 (w), 825 (w), 765 (m), 718 (s), 697 (m), 642 (w), 581 (w), 553 8w), 507 (w), 452 (w).

(2*R*,2*S*,*E*)-1-((*S*)-4-isopropyl-2-oxooxazolidin-3-yl)-2,5,9-trimethyl-1-oxodeca-4,8-dien-3-yl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate ((*S*)-4.22)



$C_{29}H_{38}F_3NO_6$
553,61 g/mol

Prepared as described for **(*S*)-4.20** from **4.16b** (20.7 mg) in anhydrous CH_2Cl_2 (2 ml), anhydrous pyridine (10.5 µL), (*R*)-MTPACl (20.0 mg) in anhydrous CH_2Cl_2 (1 ml), and DMAP (3.70 mg) to give **(*S*)-4.22** (29 mg, 88 %) after flash chromatography (SiO_2, 6:1 petroleum ether/ethyl acetate) as a colourless oil.

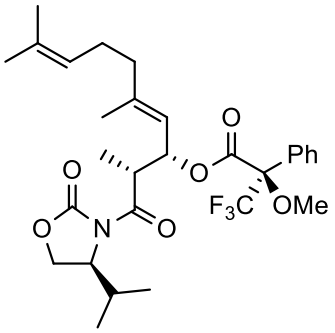
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.51-7.45 (m, 2H), 7.40-7.34 (m, 4H), 5.99 (dd, *J* = 9.6, 7.1 Hz, 1H), 5.09 (dd, *J* = 9.6, 0.8 Hz, 1H), 5.06-5.02 (m, 1H), 4.44-4.38 (m, 1H), 4.28-4.14 (m, 3H), 3.55 (s, 3H), 2.23-2.15 (m, 1H), 2.23-2.15 (m, 1H), 2.09-1.94 (m, 5H), 1.84 (s, 3H), 1.65 (s, 3H), 1.59 (s, 3H), 1.23 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 7.1 Hz, 3H), 0.79 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.1, 165.9, 153.8, 144.5, 132.4, 132.1, 129.6, 128.4, 127.6, 124.2, 123.7, 122.6, 119.4, 75.3, 63.2, 58.6, 55.7, 41.9, 39.8, 28.4, 26.4, 25.8, 18.0, 17.8, 17.2, 14.5, 14.3, 13.2 ppm.

MS (ESI): *m/z* = calcd for[$C_{28}H_{36}F_3NO_6^+$]: 576.2549, found 576.2558.

FT-IR (ATR): ν = 2965 (w), 2925 (w), 2855 (w), 1778 (s), 1744 (s), 1700 (s), 1488 (w), 1452 (m), 1385 (s), 1299 (m), 1260 (s), 1229 (s), 1184 (vs), 1120 (s), 1104 (s), 1081 (m), 1056 (m), 1014 (vs), 990 (s), 965 (m), 910 (s), 803 (m), 765 (m), 731 (vs), 719 (vs), 697 (s), 648 (m), 550 (w), 521 (w), 445 (w), 411 (w).

(2*R*,3*S*,*E*)-1-((*S*)-4-isopropyl-2-oxooxazolidin-3-yl)-2,5,9-trimethyl-1-oxodeca-4,8-dien-3-yl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate ((*R*)-4.22)



$C_{29}H_{38}F_3NO_6$
553,61 g/mol

Prepared as described for **(*S*)-4.20** from **4.16b** (20.7 mg) in anhydrous CH_2Cl_2 (2 ml), anhydrous pyridine (10.5 µL), (*S*)-MTPACl (20.0 mg) in anhydrous CH_2Cl_2 (1 ml), and DMAP (3.70 mg) to give **(*R*)-4.22** (29 mg, 87 %) after chromatography (SiO_2, 6:1 petroleum ether/ethyl acetate) as a colourless oil.

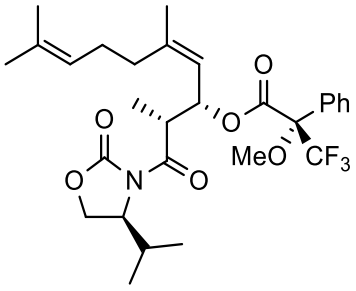
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.55-7.49 (m, 2H), 7.41-7.33 (m, 4H), 5.96 (dd, *J* = 9.5, 8.3 Hz, 1H), 5.24 (d, *J* = 9.7 Hz, 1H), 5.07-4.98 (m, 1H), 4.45-4.34 (m, 1H), 4.26-4.15 (m, 3H), 3.54 (s, 3H), 2.25-2.15 (m, 1H,), 2.11-1.96 (m, 5H), 1.83 (s, 3H), 1.64 (s, 3H), 1.58 (s, 3H), 1.08 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 7.1 Hz, 3H), 0.81 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.5, 165.8, 153.8, 144.9, 132.7, 132.2, 129.6, 128.4, 127.4, 124.3, 123.6, 119.7, 75.2, 63.2, 58.6, 55.6, 41.9, 39.8, 28.4, 26.4, 25.8, 18.0, 17.8, 17.2, 14.5, 14.3, 13.4 ppm.

MS (ESI): *m/z* = calcd for[$C_{28}H_{36}F_3NO_6^+$]: 576.2549, found 562.2560.

FT-IR (ATR): ν = 2964 (w), 2924 (w), 2853 (w), 1778 (s), 1746 (s), 1699 (s), 1488 (w), 1452 (m), 1384 (s), 1299 (m), 1268 (s), 1229 (s), 1168 (vs), 1120 (s), 1104 (s), 1081 (m), 1055 (m), 1014 (vs), 989 (s), 964 (m), 932 (m), 910 (s), 805 (m), 765 (m), 731 (vs), 719 (vs), 697 (s), 648 (m), 577 (w), 553 (w), 520 (w), 444 (w), 414 (w).

(2*R*,3*S*,*Z*)-1-((*S*)-4-isopropyl-2-oxooxazolidin-3-yl)-2,5,9-trimethyl-1-oxodeca-4,8-dien-3-yl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate ((*S*)-4.23)



$C_{29}H_{38}F_3NO_6$
553,61

Prepared as described for **(*S*)-4.20** from **4.17b** (20.7 mg) in anhydrous CH_2Cl_2 (2 ml), anhydrous pyridine (10.5 µL), (*R*)-MTPACl (20.0 mg) in anhydrous CH_2Cl_2 (1 ml), and DMAP (3.70 mg) to give **(*S*)-4.23** (28 mg, 85 %) after chromatography (SiO_2,5:1 petroleum ether/ethyl acetate) as a colourless oil.

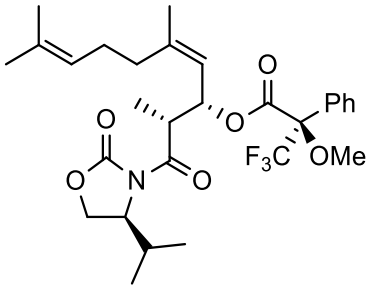
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50-7.46 (m, 2H), 7.41-7.34 (m, 4H), 6.03 (dd, *J* = 9.7, 7.4 Hz, 1H), 5.22-5.13 (m, 1H), 5.10 (dd, *J* = 23.9, 7.9 Hz, 1H), 4.45-4.37 (m, 1H), 4.29-4.13 (m, 3H), 3.55 (s, 3H), 2.33-2.02 (m, 5H), 1.69 (s, 6H), 1.64 (s, 3H), 1.23 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 7.4 Hz, 3H), 0.80 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.6, 165.8, 153.8, 144.6, 132.4, 132.0, 129.6, 128.3, 127.7, 124.2, 120.2, 74.7, 63.1, 58.6, 55.7, 41.9, 29.7, 28.3, 26.5, 25.8, 18.1, 17.7, 14.3, 13.5 ppm.

MS (ESI): *m/z* = calcd for[$C_{28}H_{36}F_3NO_6^+$]: 576.2549, found 562.2563.

FT-IR (ATR): ν = 2965 (m), 2925 (m), 2876 (w), 2854 (w), 1779 (vs), 1745 (s), 1700 (s), 1488 (w), 1452 (m), 1384 (s), 1300 (m), 1261 (s), 1232 (s), 1184 (vs), 1121 (s), 1105 (s), 1081 (m), 1056 (m), 1014 (s), 990 (s), 965 (m), 909 (m), 803 (m), 765 (m), 731 (s), 718 (s), 697 (m), 648 (w), 517 (w), 449 (w), 412 (w).

(2*R*,3*S*,*Z*)-1-((*S*)-4-isopropyl-2-oxooxazolidin-3-yl)-2,5,9-trimethyl-1-oxodeca-4,8-dien-3-yl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate ((*R*)-4.23)



$C_{29}H_{38}F_3NO_6$
553,61 g/mol

Prepared as described for **(*S*)-4.20** from **4.17b** (20.7 mg) in anhydrous CH_2Cl_2 (2 ml), anhydrous pyridine (10.5 µL), (*S*)-MTPACl (20.0 mg) in anhydrous CH_2Cl_2 (1 ml), and DMAP (3.70 mg) to give **(*R*)-4.23** (29 mg, 88 %) after chromatography (SiO_2, 5:1 petroleum ether/ethyl acetate) as a colourless oil.

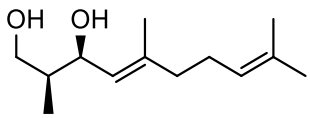
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.56-7.49 (m, 2H), 7.41-7.32 (m, 4H), 6.02 (dd, *J* = 9.8, 8.4 Hz, 1H), 5.27 (d, *J* = 9.9 Hz), 5.18-5.12 (m, 1H), 4.42-4.37 (m, 1H), 4.26-4.15 (m, 3H), 3.54 (s, 3H), 2.33-2.19 (m, 3H), 2.11-2.03 (m, 2H), 1.72 (s, 3H), 1.66 (s, 3H), 1.62 (s, 3H), 1.07 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 7.1 Hz, 3H), 0.81 (t, *J* = 6.8 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.6, 165.7, 153.8, 145.0, 132.8, 132.1, 129.6, 128.4, 127.5, 124.1, 120.5, 74.6, 63.1, 58.6, 55.6, 42.0, 32.7, 28.2, 26.5, 25.8, 23.6, 18.1, 17.7, 14.4, 13.6 ppm.

MS (ESI): *m/z* = calcd for[$C_{28}H_{36}F_3NO_6^+$]: 576.2549, found 562.2562.

FT-IR (ATR): ν = 2965 (m), 2923 (m), 2877 (w), 2854 (w), 1778 (vs), 1746 (s), 1699 (s), 1488 (w), 1452 (m), 1383 (s), 1299 (m), 1232 (vs), 1167 (vs), 1120 (s), 1103 (s), 1080 (s), 1055 (s), 1014 (vs), 989 (vs), 964 (s), 931 (s), 909 (s), 803 (s), 765 (m), 730 (s), 718 (vs), 697 (s), 648 (m), 595 (w), 554 (w), 509 (w), 450 (w), 408 (w).

(2*S*,3*S*,4*E*)-2,5,9-trimethyldeca-4,8-diene-1,3-diol (4.24)



$C_{13}H_{24}O_2$
212,33 g/mol

To a solution of **4.16b** (100 mg) in abs. THF/abs. MeOH (5:0.05 mL), $LiBH_4$ (4 M in THF, 222 µL) was added dropwise at 0 °C and the reaction mixture was stirred for 3 h. Then, a saturated solution of $NaHCO_3$ (10 mL) was added, the layers were separated, and the aqueous layer was extracted with ethyl acetate (3×15 mL). The combined organic layers were dried with $MgSO_4$ and the solvent was removed under vacuo. The residue was purified by flash chromatography (SiO_2, 2:1 petroleum ether/ethyl acetate) to give the product **4.24** (40 mg, 64 %) as a colourless oil. $[\alpha]^{20}_D$ = +65.2 (c 1.0, CH_2Cl_2).

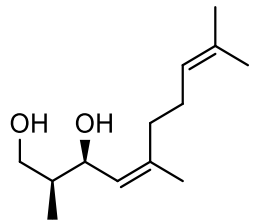
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.29 (dd, *J* = 9.0, 1.1 Hz, 1H), 5.11-4.99 (m, 1H), 4.50 (dd, *J* = 9.0, 4.1 Hz, 1H), 3.68 (dd, *J* = 10.7, 7.6 Hz, 1H), 3.57 (dd, *J* = 10.8, 4.6 Hz, 1H), 2.52 (s, 2H), 2.16-1.98 (m, 4H), 1.99-1.85 (m, 1H), 1.66 (s, 6H), 1.59 (s, 3H), 0.86 (d, *J* = 7.0 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 139.4, 131.9, 124.9, 124.0, 71.9, 66.3, 40.6, 39.9, 26.5, 25.8, 17.8, 16.8, 11.9 ppm.

MS (EI): *m/z* = calcd for[$C_{13}H_{24}O_2^+$]: 212.1776, found 212.1776.

FT-IR (ATR): ν = 3338 (m), 2965 (m), 2921 (m), 2064 (w), 1731 (w), 1669 (w), 1669 (w), 1449 (m), 1376 (m), 1261 (w), 1106 (m), 1082 (m), 1030 (s), 970 (s), 816 (w), 562 (w), 447 (w), 419 (w).

(2*S*,3*S*,4*Z*)-2,5,9-trimethyldeca-4,8-diene-1,3-diol (4.25)



$C_{13}H_{24}O_2$
212,33 g/mol

Prepared as described for **4.24**, from **4.17b** (100 mg) in abs. THF/abs. MeOH (5:0.05 mL), $LiBH_4$ (4 M in THF, 222 µL), saturated solution of $NaHCO_3$ (10 mL), extraction with ethyl acetate (3×15 mL) and flash chromatography (SiO_2, 2:1 petroleum ether/ethyl acetate) gave **4.25** (38 mg, 60 %) as a colourless oil. $[\alpha]^{20}_D$ = +68.6 (c 1.2, CH_2Cl_2).

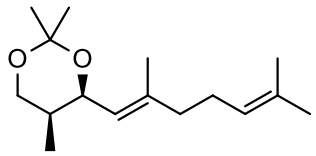
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.34 (d, *J* = 9.2 Hz, 1H), 5.15-5.02 (m, 1H), 4.47 (dd, *J* = 9.3, 4.3 Hz, 1H), 3.68 (dd, *J* = 10.8, 7.2 Hz, 1H), 3.64-3.52 (m, 1H), 2.39 (s, 2H), 2.20-1.98 (m, 4H), 1.96-1.81 (m, 1H), 1.75 (s, 3H), 1.67 (s, 3H) 1.59 (s, 3H), 0.88 (d, *J* = 7.0 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 139.9, 132.4, 125.5, 123.9, 71.2, 66.3, 40.2, 32.2, 26.5, 25.6, 23.5, 17.6, 11.7 ppm.

MS (EI): *m/z* = calcd for[$C_{13}H_{24}O_2^+$]: 212.1776, found 212.1768.

FT-IR (ATR): ν = 3346 (m), 2965 (m), 2916 (m), 2876 (m), 1994 (w), 1972 (w), 1726 (w), 1665 (w), 1449 (m), 1376 (m), 1262 (w), 1104 (m), 1080 (m), 1031 (s), 968 (s), 857 (w), 820 (w), 731 (w), 596 (m), 453 (w).

(4*S*,5*S*)-4-[(1*E*)-2,6-dimethylhepta-1,5-dien-1-yl]-2,2,5-trimethyl-1,3-dioxane (4.26)



$C_{16}H_{28}O_2$
252,39 g/mol

To a solution of **4.24** (20 mg) in anhydrous acetone (1 mL) dimethoxypropane (98.4 µL) and PPTS (1 mg) were added at room temperature. The reaction mixture was stirred for 3 h and then the solvent was removed under vacuum. The residue was purified by flash chromatography (SiO_2, 10:1 petroleum ether/ethyl acetate and 1 vol.-% Et_3N) to give the product **4.26** (21 mg, 90 %) as a colourless oil. $[\alpha]^{20}_D$ = +52.8 (c 1.1, CH_2Cl_2).

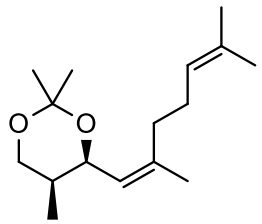
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.21 (dd, *J* = 7.7, 1.2 Hz, 1H), 5.13-5.02 (m, 1H), 4.75 (dd, *J* = 7.7, 2.8 Hz, 1H), 4.15 (dd, *J* = 11.5, 2.9 Hz, 1H), 3.59 (dd, *J* = 11.5, 1.7 Hz, 1H), 2.18-1.96 (m, 4H), 1.67 (s, 3H) 1.66 (s, 3H), 1.50 (s, 3H), 1.49-1.44 (m, 1H), 1.42 (s, 3H), 1.11 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.3, 131.8, 124.2, 124.0, 98.8, 69.3, 66.7, 39.8, 32.9, 29.9, 26.5, 25.8, 19.3, 17.8, 17.0, 11.3 ppm.

MS (EI): *m/z* = calcd for[$C_{16}H_{28}O_2^+$]: 252.2089, found 252.2097.

FT-IR (ATR): ν = 3411 (w), 2965 (m), 2925 (m), 2858 (m), 2125 (w), 1671 (w), 1450 (m), 1378 (m), 1270 (m), 1239 (m), 1195 (m), 1166 (m), 1137 (m), 1102 (m), 1087 (m), 1008 (s), 983 (m), 963 (m), 914 (m), 862 (m), 843 (m), 817 (m), 753 (w), 540 (w), 517 (m), 433 (w).

(4*S*,5*S*)-4-[(1*Z*)-2,6-dimethylhepta-1,5-dien-1-yl]-2,2,5-trimethyl-1,3-dioxane (4.27)



$C_{16}H_{28}O_2$
252,39 g/mol

Prepared as described for **4.26**, from **4.25** (20 mg) in anhydrous acetone (1 mL), dimethoxypropane (98.4 µL), PPTS (1 mg), after flash chromatography (SiO_2, 10:1 petroleum ether/ethyl acetate and 1 vol.-% Et_3N) gave the product **4.27** (20 mg, 88 %) as a colourless oil. $[\alpha]^{20}_D$ = +57.7 (c 1.1, CH_2Cl_2).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.24 (dd, *J* = 8.2, 1.1 Hz, 1H), 5.17-5.04 (m, 1H), 4.74 (dd, *J* = 8.2, 2.7 Hz, 1H), 4.13 (dd, *J* = 11.5, 2.9 Hz, 1H), 3.59 (dd, *J* = 11.5, 1.7 Hz, 1H), 2.22-1.95 (m, 4H), 1.75 (s, 3H) 1.68 (s, 3H), 1.61 (s, 3H), 1.49 (s, 3H), 1.47-1.42 (m, 1H), 1.41 (s, 3H), 1.13 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.3, 131.8, 124.2, 124.0, 98.8, 69.3, 66.7, 39.8, 32.9, 29.9, 26.5, 25.8, 19.3, 17.8, 17.0, 11.3 ppm.

MS (EI): *m/z* = calcd for[$C_{16}H_{28}O_2^+$]: 252.2089, found 252.2080.

FT-IR (ATR): ν = 3359 (w), 2962 (m), 2925 (m), 2856 (m), 2168 (w), 2071 (w), 2037 (w), 2026 (w), 1734 (w), 1455 (m), 1377 (m), 1261 (w), 1104 (w), 1034 (m), 967 (m), 512 (w), 451 (w), 422 (w), 407 (w).

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5 APPROACH TO THE SYNTHESIS OF NATURAL HERBICIDE AUSCALITOXIN AGLYCONE

5.1 Introduction

The globally widespread diffusion of resistance in weed populations has led to the loss of effectiveness of entire classes of commercial herbicides. Furthermore, no herbicides with a novel mode of action have been launched on the market in the last twenty years.[1,2] Thus, the discovery of herbicides with novel mechanism of actions is crucial to avoid the loss of crops productivity caused by weeds competition, and the related increase of the costs of weeds management.

As discussed in **Chapter 1**, natural compounds represent an important source of lead compounds for the discovery and development of new agrochemicals. Despite of this, natural and natural-derived herbicides are underrepresented if compared to synthetic agents.[3]

It is often challenging to achieve natural compounds in adequate amounts , thus the development of efficient synthetic strategies able to overcome the chemical complexity of these compounds is pivotal to perform the required research and development studies.

The development of a strategy for the total synthesis of molecules with such a chemical complexity requires a sequence of reactions with high degree of selectivity. The development of new reagents, methodologies, and the application of existing reagents underpins the advance of new synthetic strategies as it enriches the arsenal of chemical tools available to chemists to face the chemical complexity of natural compounds.

In this Chapter the results of our efforts towards the total synthesis of the fungal toxin with herbicidal activity auscalitoxin aglycone **5.1** are discussed.

5.1.1 Auscalitoxin and auscalitoxin aglycone

Ascochyta caulina (P. Karst.) v.d. Aa & v Kest. is a plant pathogenic fungus, that has been proposed as biocontrol agent for the globally widespread weed *Chenopodium album*. This fungus causes the formation of necrotic areas on leaves and stem and can lead to retard in the growth or death of the weed.[4]

As several species of the genus *Ascochyta* are known to produce toxic metabolites,[5] the filtrate of *in vitro* culture of *A. caulina* were tested to assess the production of phytotoxic compounds. From the filtrate, three compounds with herbicidal activity were isolated and characterized. The three phytotoxins were *trans*-4-amino-*D*-proline[6] **5.3**, auscalitoxin[4] **5.2**, and its aglycone[7] **5.1**. The chemical structures are highlighted in **Figure 5.1**.

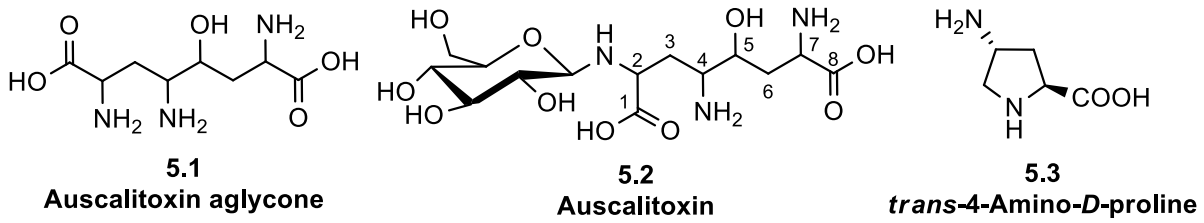


Figure 5.1 Structure of toxins isolated from *A. caulina*

The main compound auscalitoxin **5.2**, is a N^2-glucoside of the non-proteinogenic *bis*-aminoacid 2,4,7-triamino-5-hydroxyoctandioic acid. The absolute configuration of the four stereogenic centres of the naturally occurring compound is still undetermined, but the relative configuration was determined by Bassarello et al. in 2001 through a *J*-based NMR configurational analysis. The authors suggest that the configuration at carbons C-4 and C-5 is opposite to that at carbons C-2 and C-7.[8]

In preliminary studies, the aglycone of auscalitoxin **5.1** has proven to show a more potent phytotoxic activity than auscalitoxin. The mechanism of action was investigated by Duke et al. in 2011. It was demonstrated that the aglycone **5.1** shows a strong herbicidal activity against *Lemna paucicostata* with IC_{50} below 1 μM, and most of proteinogenic aminoacids, non-proteinogenic aminoacids (ornithine and citrulline), *D*-aminoacids and oxaloacetate were able to reverse the phytotoxicity. Furthermore, the aglycone of auscalitoxin **5.1** has proven to induce alterations on metabolites levels with changes in aminoacids, enhanced levels of intermediates of citrate cycle and reduction in concentration of antioxidants. The authors suggest that the aglycone **5.1** could act as a protoxin or its phytotoxic activity could be mediated by inhibition of one or more aminoacid transporters.[9]

Because of the high herbicidal activity and the possible novel mechanism of action, the aglycone of auscalitoxin **5.1** represents a valuable lead compound for the development of new natural compound-based herbicides.

Besides of the herbicidal activity, our interest in the aglycone of the auscalitoxin **5.1** is related to its chemical structure that represents a challenging task to the development of a strategy for its total synthesis. The presence of different stereogenic centres requires the development of a series of reactions characterized by high degrees of stereoselectivity. Furthermore, the presence of multiple amino groups and one hydroxy entail reactions with high degree of chemoselectivity and an efficient strategy of protection. In our approach to the total synthesis of **5.1** we decided to apply the chemistry of samarium and in particular a samarium(II) iodide-

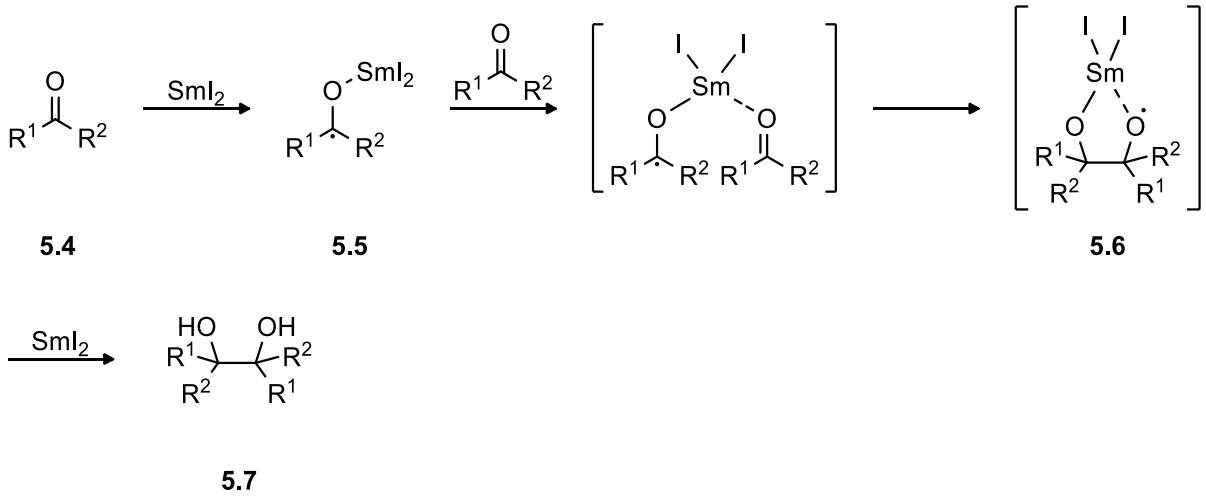
mediated pinacol coupling as the key reaction for the synthesis of the central amino alcohol moiety.

5.1.2 Samarium(II) iodide-mediated pinacol coupling

As discussed in **Chapter 4** Samarium(II) iodide has become a valuable tool in organic synthesis with hundreds of different applications because of the unique combination of chemical reactivity and versatility combined with selectivity and a great functional-group tolerance. The possibility to mediate the formation of new carbon-carbon bonds combined with selectivity and its mild nature, has made samarium(II) iodide a powerful tool in total synthesis of natural compounds.[10]

A valuable application of samarium(II) iodide's ability in the formation of new carbon-carbon bond is represented by SmI_2-pinacol coupling. Samarium(II) iodide-mediated pinacol coupling was first described by Kagan et al. in 1983,[11] while the application of the intramolecular version of this reaction for the formation of rings was first reported by Molander and Kenny in 1988.[12]

Pinacol coupling is the formation of a diol by reductive coupling of two carbonyl groups. The general accepted mechanism for the SmI_2-mediated coupling is highlighted in **Figure 5.2**. The SmI_2-mediated reduction of the carbonyl compound **5.4** leads to the formation of the ketyl radical **5.5**, which can attack a second carbonyl group generating the oxygen radical **5.6**. Then, a second rapid SmI_2-mediated reduction and the subsequent quench of the alkoxide provides the product of pinacol coupling **5.7**.



5.4 **5.5** **5.6** **5.7**

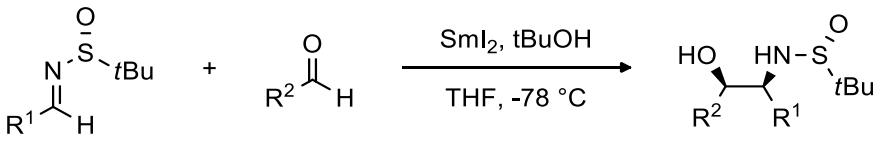
Figure 5.2 Mechanism SmI_2-mediated pinacol coupling

Intermolecular SmI_2-mediated pinacol coupling is generally characterized by poor stereoselectivity. Conversely, the intramolecular version of the reaction exhibits a high degree of stereoselectivity in favour of *cis*-diol, because of the chelation of the ketyl radical. The presence of an alkoxy group close to one of the carbonyls leads to the formation of the *anti*- rather than the *cis*-diol.[13]

SmI_2-mediated pinacol-type reactions can also be performed to couple a ketone or an aldehyde with oximes,[14] nitriles,[15] or hydrazones.[16]

A samarium diiodide-mediated asymmetric pinacol-type cross-coupling between an aldehyde and a chiral *N*-sulfinyl imine was described by Zhong et al. in 2005. In this procedure, different chiral *N*-*tert*-butanesulfinyl imines were coupled with aldehydes affording the corresponding amino alcohol with *anti*-stereochemistry with high yield and high degree of diastereo- and enantioselectivity.[17] The results of that research are reported in **Table 5.1**.

Table 5.1 SmI_2-mediated cross-coupling of aldehydes with *N*-*tert*-butanesulfinyl imine[17]



Entry	R^1	R^2	Yield (%)	dr	ee
1	4-$CH_3C_6H_4$	*i*Pr	92	>99:1	98
2	4-$CH_3C_6H_4$	C_6H_{11}	90	99:1	>99
3	4-$CH_3C_6H_4$	$(Et)_2CH$	73	>99:1	99
4	4-$CH_3C_6H_4$	*n*-C_5H_{11}	90	91:9	95
5	4-$CH_3C_6H_4$	PhC_2H_4	95	88:12	95
6	Ph	*i*Pr	86	99:1	97
7	4-FC_6H_4	*i*Pr	89	98:2	>99
8	4-ClC_6H_4	*i*Pr	71	99:1	98

9	4-BrC_6H_4	*i*Pr	70	>99:1	>99
10	4-$AcOC_6H_4$	*i*Pr	82	>99:1	>99
11	4-CH_3OC6H4	*i*Pr	84	>99:1	>99
12	3,4-$(MeO)_2C_6H_3$	*i*Pr	90	>99:1	>99
13	2,4-$(MeO)_2C_6H_3$	*i*Pr	73	>99:1	>99
14	*i*Pr	*i*Pr	88	>99:1	98
15	PhC_2H_4	*i*Pr	87	96:4	>99
16	*n*-C_5H_{11}	*i*Pr	95	98:2	97
17	$BnOCH_2$	*i*Pr	82	>99:1	97

Diasteromeric ration was determined by HPLC-MS and ^{1}H NMR of the crude materials. Enantiomeric excess was determined for the free β-amino alcohols after acidic hydrolysis.

The authors do not discuss the possible mechanism of this reaction. It can be speculated that the reaction occurs following the accepted mechanism of the SmI_2-mediated pinacol coupling discussed above. The observed high diastereo- and enantioselectivity arise from the chiral sulfinyl imine that could be involved in the chelated transition state.

5.2 Results and discussion

5.2.1 Retrosynthetic analysis

Our retro-synthetic approach for the synthesis of aglycone of auscalitoxin **5.1** started with the disconnection between the carbons C-4 and C-5, to obtain the two key fragments **5.10** and **5.11**, as highlighted in **Figure 5.3**. The assembly of the aldehyde **5.10** and the chiral *N*-sulfinyl imine **5.11** could be performed using the samarium diiodide-mediated asymmetric pinacol-type cross-coupling developed by Zhong et al. in 2005 discussed above.[17]

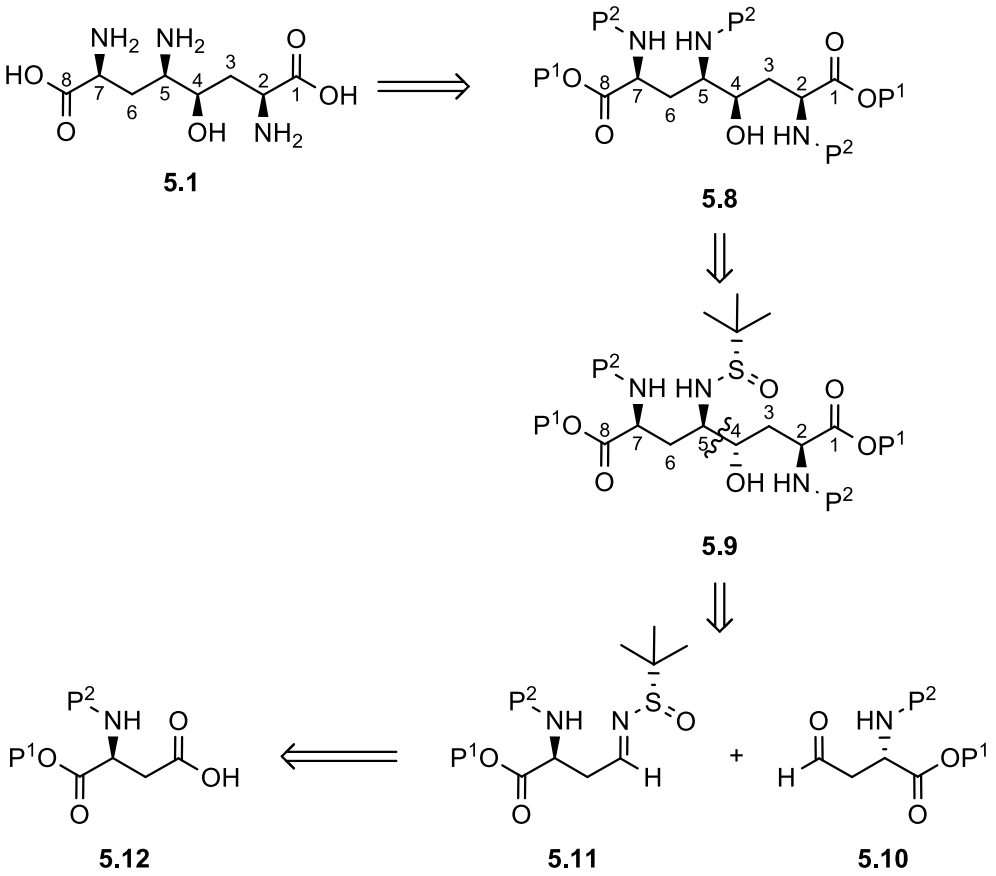


Figure 5.3 Retrosynthetic analysis

The main advantage of this strategy is the possibility to build the carbon backbone and to insert all the functional group at the same time. The choice of the chiral sulfinyl imine allows to introduce the amino group at the carbon C-5 with the desired configuration. Since the main product of the reaction is the *anti*-1,2-amino alcohol, the configuration at the carbon C-4 must be inverted, after the condensation at the level of intermediate **5.9**, to obtain the desired compound.

The inversion of the stereochemistry at the carbon C-4 could be performed in a multistep process involving the deprotection and the subsequent protection with *tert*-butyloxycarbonyl group (Boc) of the amino group at the carbon C-5, conversion of the hydroxy group at the carbon C-4 in a suitable leaving group, and, finally, inversion via S_N2 cyclization to oxazolidinone. The strategy is highlighted in **Figure 5.4**.

Selective cleavage of the *N*- *tert*-butylsulfinyl group could be obtained in presence of iodine in neutral condition following the procedure reported by Chen et al. in 2014.[18] The inversion via S_N2 cyclization to oxazolidinone could be performed by treatment with methanesulfonyl chloride as described by Benedetti and Norbedo in 2000 or mediated by a Ph_3P-CCl_4-Et_3N system as reported by Madhusudhan et al. in 2003. [19,20] Finally, the formed oxazolidinone ring

could be opened in mild condition by hydrolysis with Cs_2CO_3 after Boc protection of the nitrogen, following the procedure described by Ishizuka and Kunieda in 1987.[21]

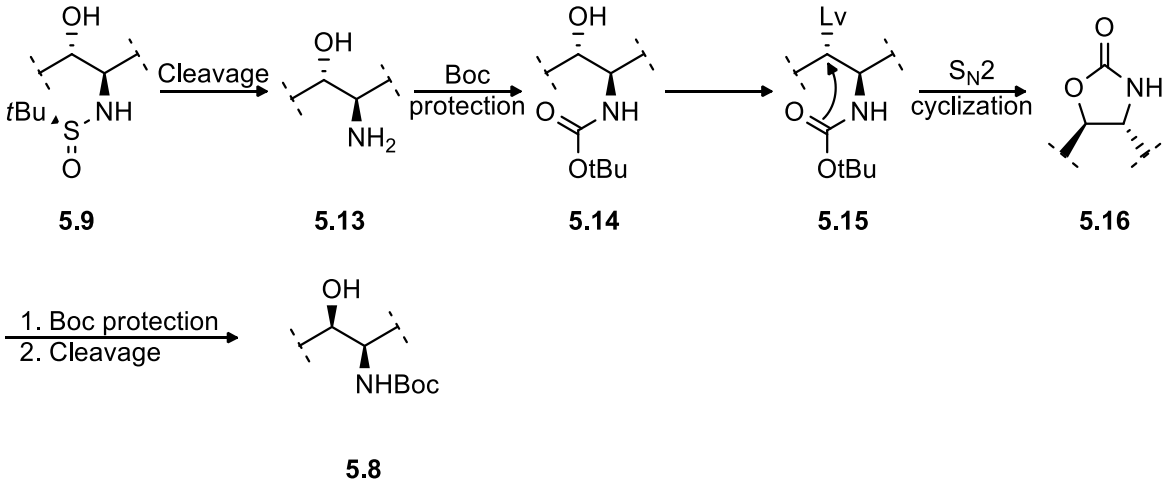


Figure 5.4 Strategy for the inversion of the alcohol group at C-4

Both the fragment **5.10** and **5.11** can be prepared starting from a commercially available protected aspartic acid **5.12**.

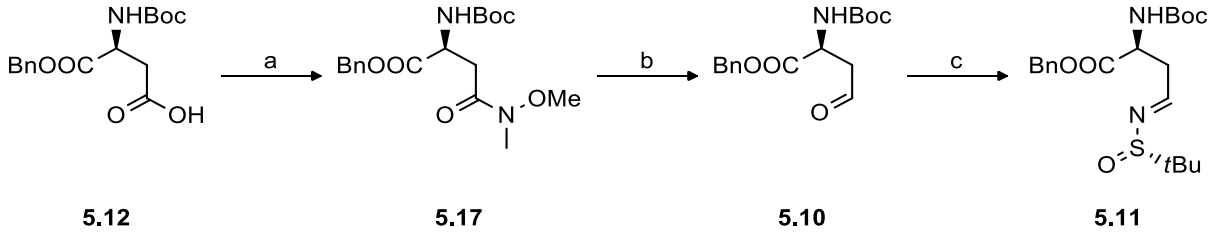
Considering the obvious high polarity of the target molecule, the protecting group strategy was designed to reduce the number of deprotection steps in the end of the synthesis and to simplify the handling and the purification of the last intermediates. Thus, *tert*-butyloxycarbonyl was selected for the protection of the amino groups at the carbon C-2 and C-7 because it can be removed in mild acid condition. To protect the carboxylic acid moieties, the conversion in benzyl esters was selected, considering that the benzyl esters can be removed in mild condition by catalytic hydrogenation, orthogonally to the nitrogen protecting groups.

As the absolute configuration of the natural occurring aglycone of auscalitoxin **5.1** in still undetermined, and due to the necessity of testing the viability of this synthetic approach, we decided to start from a less expensive protected Boc-*L*-aspartic acid 1-benzyl ester **5.12**. In the case this approach was successful, the synthetic could be repeated with the *D*-isomer.

*5.2.2 Synthesis of precursors **5.10** and **5.11***

The aspartic acid derivative **5.12** was converted with high yields in the Weireb's amide **5.17** by activation of the free carboxylic group with 1,1'-carbonyldiimidazole, followed by reaction of the *in situ* generated mixed anhydrides with *N*,*O*-dimethylhydroxylamine hydrochloride.[22] Aldehyde **5.10**, was obtained in quantitative yield by reduction of the Weireb's amide **5.17** by

diisobutylaluminium hydride at -78 °C.[23] Finally, compound **5.11** was obtained from the aldehyde **5.10** by reaction with the commercially available Ellman's chiral sulfinamide in presence of anhydrous $CuSO_4$ as dehydrating agent.[24]



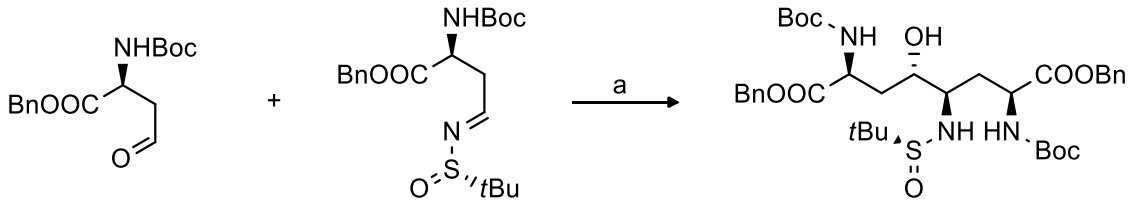
5.12 **5.17** **5.10** **5.11**

Figure 5.5 Synthesis of precursors **5.10** and **5.11**. (a) 1,1'-carbonyldiimidazole, HNMeOMe·HCl, CH_2Cl_2, rt 80 %; (b) DIBAL, THF, -78 °C, quantitative yield; (c) Ellman's sulfinamide, $CuSO_4$, CH_2Cl_2, rt, 87 %

5.2.3 SmI_2-mediated asymmetric pinacol-type cross-coupling

To build the carbon backbone of the target molecule inclusive of the amino alcoholic function at carbons C-4 and C-5, fragments **5.10** and **5.11** were coupled in a samarium(II) iodide-mediated pinacol-type cross-coupling.[17] The coupling of the compound **5.11** with an excess (1.5 eq.) of **5.10** was performed at -78 °C, with two equivalents of samarium(II) iodide in solution of absolute tetrahydrofuran, in presence of *tert*-butanol as proton donor. The reaction led to the formation of two diastereomers in ratio 84:16. The ratio was determined by ^{1}H-NMR. The major diastereomer was obtained in 54 % yield after flash chromatography. The minor diastereomer was recovered in 1:1 mixture with the major one.

The major diastereomer was identified as the coupling products **5.9** by ^{1}H-and ^{13}C-NMR. The structure of compound **5.9** is highlighted in **Figure 5.5**; the reported configuration at C-4 and C-5 is the expected one according to the results reported by Zhong et al. in 2005.[17] The reaction did not go to completion, but total consumption of samarium(II) iodide was observed by colour change from dark blue to yellow. In the end of the reaction, 25% of **5.11** could still be recovered.



5.10 **5.11** **5.9**

Figure 5.6 SmI2-mediated cross-coupling. (a) SmI_2 0.1 M in abs. THF, -78 °C, 54 %

With this promising result, a screening of different conditions was started to optimize the reaction. Firstly, the amount samarium(II) iodide added at the beginning of the reaction was progressively increased up to six equivalents. No effects on the consumption of the starting material and improvements in the yield were observed. Furthermore, samarium(III) hydroxides formed during the work up of the reaction are poorly soluble in organic solvents and in neutral or slightly acid aqueous solution. This favoured the formation of emulsions during liquid-liquid extraction and made the work up of the reaction particularly tedious. Similarly, addition of samarium(II) iodide during the progression of the reaction had no effect on the outcome of the coupling.

The reaction was repeated with freshly prepared samarium(II) iodide solutions in order to exclude problems related to the title of the solutions of the commercially available reagent. The solution of samarium(II) iodide in anhydrous THF was prepared from commercially available samarium metallic powder and iodide, following the procedure described by Concellón et al.[25] The obtained dark blue solution was used immediately after the preparation, unfortunately, in this case too, no improvement in the reaction was observed.

With these results in hands, we concluded that the problems of the reactions were not due to samarium(II) iodide. Thus, we decided to change the order of addition of the reactants to the reaction, however the addition of the chiral *N*-sulfinyl imine **5.11** to the samarium(II) iodide solution, followed by the aldehyde **5.10** and *tert*-butanol had no effect on the outcome of the reaction. Conversely, addition first of the aldehyde **5.10** led to a formation of small amounts of unidentified by-products, probably derived by aldehyde pinacol coupling, with a reduction in the yield. Also, *tert*-butanol was found to be essential for the reaction, as variation in the order of addition led to reduction of the yields and formation of many by-products.

5.2.4 Absolute configuration at C-4 and C-5

Once the coupling product **5.9** was obtained, the attention was focused to the investigation of the absolute configuration at the carbons C-4 and C-5. Despite the stereochemistry of the amino alcohol derived from the samarium(II) iodide-mediated pinacol cross-coupling is known[17], the presence of other groups able to coordinate samarium(III) ions in the two products **5.10** and **5.11** could interfere with the mechanism of the reaction, leading to a different stereochemistry.

Since the carbon at C-4 of **5.9** bears a free hydroxyl group, we decided to apply the Mosher's method.[26] Compound **5.9** was treated with both the enantiomers of the Mosher's acid under Steglich's esterification condition,[27] but formation of the desired chiral esters was not observed. Thus, the same reaction was performed with the more reactive Mosher's acyl chlorides in presence of a catalytic amount of DMAP and triethylamine as base.[28] The formation of the same product was observed from the reactions with both the enantiomers of the Mosher's acyl chloride and it was identified as a lactonization product by ^{1}H-NMR analysis.

As highlighted in **Figure 5.7**, involvement of the carboxylic group at C-1 or at C-8 can lead to the formation of two different cyclic compound, one with six atoms and the other with five atoms. Because only one compound was observed, we were interested to determine the structure of the lactone. Furthermore, since the formation of the cycle involves the stereogenic centres at the carbons C-4 and C-5, the determination of the structure of the lactone could be useful to assign the stereochemistry. Thus, compound **5.9** was treated with a catalytic amount of DMAP and triethylamine, in the same condition used for the acylation with the Mosher's acyl chloride, in order to prepare an amount of lactone suitable to the NMR studies. We found that this lactonization reaction occurs very easily under these conditions and in general in presence of a base. The reaction occurs in neutral and slightly acid solution as well.

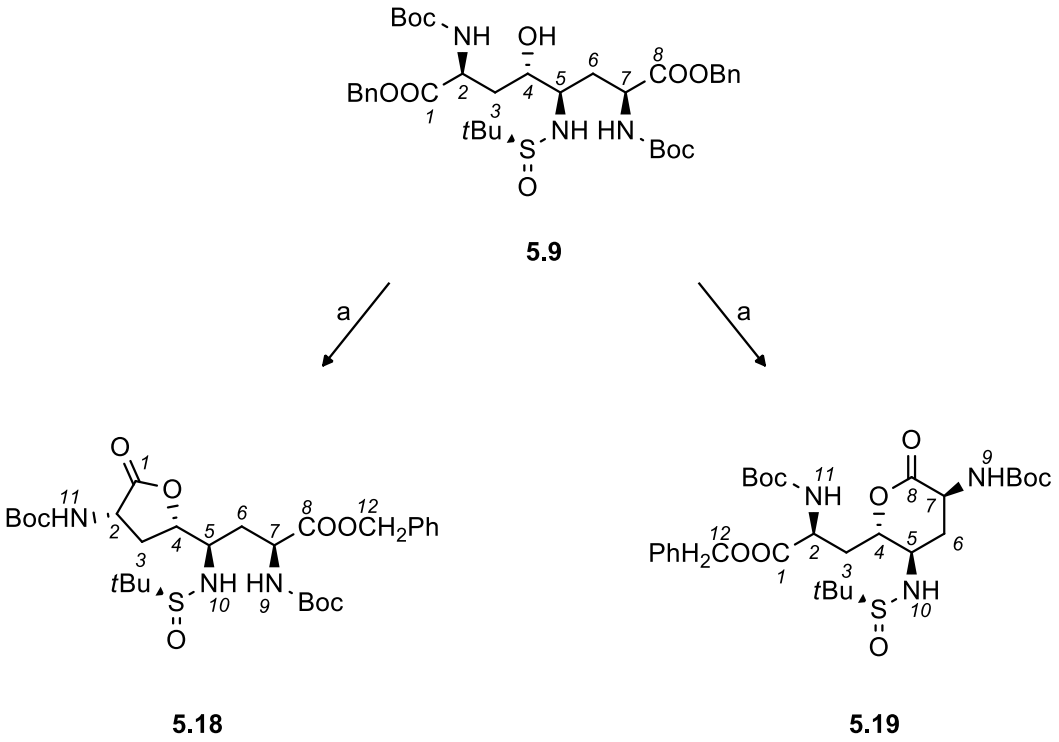


Figure 5.7 Possible pathways for lactonization reaction. (a) Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 63 %

To determine which of the two possible proposed structures was obtained from the cyclization a series of NMR experiments were performed. Correlation spectroscopy (COSY), heteronuclear single-quantum correlation spectroscopy (HSQC), and Long-Range HETCOR experiments were used to obtain the complete assignment.

The starting point of our investigation was the identification of the signal belonging to the proton and relative carbon at the position C-4. Proton directly linked to the heteroatom were identified in a Heteronuclear Single-Quantum Correlation (HSQC) experiment as the signal which does not correlate with any carbon signal. In the same experiment each proton correlates with the directly bound carbon. Thus, the signals belonging to C-2, C-4, C-5, and C-7 were identified. Out of these selected signals one resulted to be very different from the others in term of chemical shift and was identified as the C-4.

The structure of the molecule was determined by a Long-Range HETCOR experiment performed at low temperature (7 °C). Long range correlations were observed between the proton at the position C-4 and the carbons C-3, C-2, and C-1. These correlations, and in particular the one with C-2, suggest the involvement of a five atoms cycle. The involvement of the six atoms cycle was excluded. In fact, C-2 in the structure **5.18** is equivalent to C-7 in structure **5.19**, but in the case of this second structure no correlation between proton on C-4 should be observed with C-7.

Long-Range HETCOR experiments permitted to assign all the other signals. The most relevant signal in the assignment of the structure are listed in **Table 5.2**.

Table 5.2 Assignment of the most relevant protons and carbons

Position	^{1}H δ (ppm)	^{13}C δ (ppm)
1	—	174.00
2	4.39	50.97
3	2.70/1.90	32.01
4	4.28	78.92
5	3.72	55.03
6	2.16/1.94	32.69
7	4.59	49.52
8	—	172.57
9-NH	5.87	—
10-NH	4.77	—
11-NH	5.02	—
$PhCH_2$	5.26/5.11	67.64
CO (Boc)	—	155.73/155.38
C-ipso	—	135.12
CH arom.	7.45-7.26	128.63/128.43/128.16

Correlations were observed between the benzylic proton C-12 and the carbonyl carbon C-8 and the carbon ipso of the aromatic ring. Proton at C-2 and C-7 showed a coupling with the carbonyl carbons of the Boc groups. For proton at C-7 a correlation was observed with carbons C-5 and C-6. Proton at C-2 coupled with C-3, but no correlation with C-4 was observed. Interestingly, only one proton at C-3 exhibited a correlation with C-4. Protons at C-6 correlated with C-5, C-7 and C-8.

For NH-9 and NH-11 correlations with C-2 and C-7 respectively were observed. The quaternary carbon of the *tert*-butyl of the sulfinyl amide group was identified by a correlation with NH-10.

A nuclear Overhauser effect spectroscopy (NOESY) experiment was performed to assign the absolute configuration at the carbon C-4 and C-5. Observed NOE effects are listed in **Table 5.3**.

Table 5.3 Observed NOE

Proton	^{1}H δ (ppm)	Observed NOE
2	4.39	H-3a; H-3b (vw); NH-11
3a	2.70	H-2; H-4
3b	1.90	H-2 (vw); H-4 (w); H-5; NH-10; NH-11
4	4.28	H-3a; H-3b (w); H-5; H-6a; H-6b
5	3.72	H-3b; H-4; H-6a; H-6b; H-7; NH-9; NH-10
6a	2.16	H-4; H-5; H-7; NH-9
6b	1.94	H-4; H-5; H-7; NH-9
7	4.59	H-4 (w); H-5; H-6a; H-6b; NH-9; NH-10
9-NH	5.87	H-5; H-6a (w); H-6b (w); H-7
10-NH	4.77	H-3b (w); H-4 (w); H-5; H-6b; H-7
11-NH	5.02	H-2; H-3b

Weak (w); very weak (vw)

A strong NOE was observed for the proton C-2 with one of the protons at C-3 (proton **3a**), while a very weak NOE was observed with the other (proton **3b**) at the same position. This suggests that protons **2** and **3a** are oriented on the same plane of the ring, while **3b** is oriented on the opposite side. Proton **3b** showed a weak NOE with proton at C-4 and two strong NOE with NH-10 and NH-11. Conversely, for proton **3a** a very strong NOE was observed with proton at C-4. This suggests that protons **4**, **3a** and **2** are oriented on the same side of the ring. (**Figure 5.8**)

The absolute configuration at C-2 is known since it is derived by the protected aspartic acid **5.12** used as starting material. This permits to assign the absolute configuration at C-4. Since protons C-2 and C-4 are oriented on the upper side of the ring, the absolute configuration at the C-4 is *S*. This is in agreement with the expected absolute configuration deriving by the Sm(II) iodide-mediated cross-coupling.

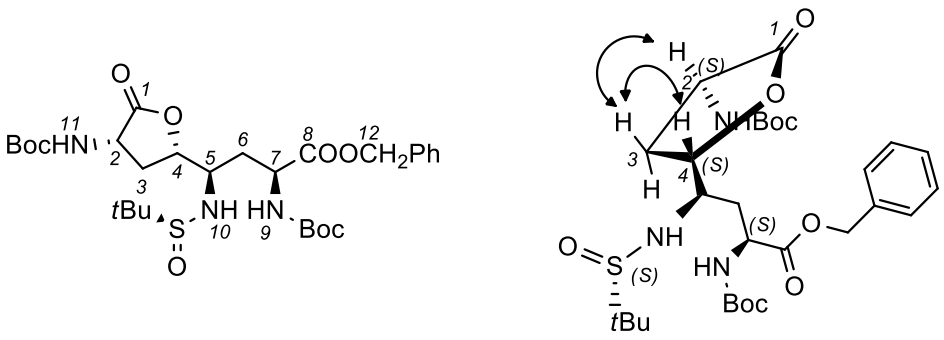


Figure 5.8 Observed NOE for **5.18**

The absolute configuration at the position C-5 can not be determined by NOESY experiment. Since rotation around the bond connecting C-4 and C-5 is allowed it was not possible to identify NOE correlations appropriate to determine the orientation in the space of the proton at C-5. Further investigations are required assign the absolute configuration at C-5 and confirm the stereoselectivity of the samarium(II) iodide-mediated pinacol coupling.

5.3 Summary

In this chapter the results of our efforts in developing a strategy for the synthesis of auscalitoxin aglycone **5.1** are described.

The retrosynthetic analysis of the molecule was performed in order to define the most appropriate synthetic pathway. Considering the structure of the target molecule, in our approach we decided to disconnect the molecule at the level of the central amino alcoholic group. This approach has the advantage that all the stereogenic centres are present in the two moieties used in the condensation step. Indeed, the terminal aminoacidic functions can be obtained by the use the appropriate commercially available aminoacids as starting materials. To synthetize the key C-4-C-5 α-amino alcoholic group a samarium(II) iodide mediated pinacol coupling was selected. This procedure provides an evidence of the power of samarium(II) iodide as reagent in the synthesis of natural compounds since it permits to build the carbon backbone of the molecule and to install the functional group with high degree of stereoselectivity.

The synthesis of the precursors **5.10** and **5.11** was successfully performed starting from the Weireb's amide **5.17** that was easily prepared starting from commercially available protected aspartic acid **5.12**.

Samarium(II) iodide-mediated pinacol coupling of compounds **5.10** and **5.11** led to the formation of compound **5.9** in 55 % together with a minor diastereomer. Diastereomeric ratio was determined by ^{1}H-NMR and resulted to be 84:16. Complete consumption of the starting materials was not observed, indeed 25 % of unreacted compound **5.11** was recovered at the end of the reaction.

Any attempts to optimize the reaction failed. Changing in the amount of SmI_2, the use of freshly prepared SmI_2 solution, and modification of the order of addition of the reagents led to no improvements and in some cases revealed to be detrimental for the reaction.

Product **5.9** turned out to be prone to lactonization. The first evidence of this behaviour was observed during the esterification reaction to prepare the Mosher's derivatives.

The product of lactonization was exploited to confirm the stereochemistry of the coupling product **5.9**. A series of NMR experiments was used to determine the structure of the lactone that was identified to be the compound **5.18**.

The absolute configuration at the C-4 was determined by a NOESY experiment. It was assigned the absolute configuration at the C-4 as *S* , in agreement with the SmI_2-pinacol coupling mechanism. It was not possible to determine the absolute configuration at the C-5 because of the conformational mobility of the chain. Further investigations are needed to

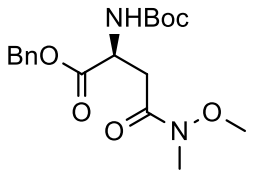
assign the absolute configuration at the C-5. Selective cleavage of the sulfinyl chiral auxiliary could be performed in order to induce different cyclization pattern. Thus, the obtained compounds could be exploited for further NMR studies.

Overall, the samarium(II) iodide-mediated pinacol coupling demonstrated to be a reliable approach for the construction of the amino alcohol moiety of the aglycone of auscalitoxin **5.1**. Further investigation must be performed to identify the detrimental factors and to improve the outcome of the reaction. In particular, modification of the samarium(II) iodide reactivity by use of additives and cosolvent is a strategy that must be evaluate.

The observed lactonization of compound **5.9** is a limiting factor in the progress of the total synthesis. Variation of the protecting group of the terminal carboxyl groups could be useful to limit this problem.

5.4 Experimental part

Benzyl (*S*)-2-(*tert*-butoxycarbonylamino)-4-(methoxy(methyl)amino)-4-oxobutanoate (5.17)



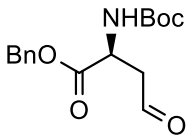
$C_{18}H_{26}N_2O_6$
366,41 g/mol

To a solution of Boc-aspartic acid-*O*-benzyl ester **5.12** (1.0 g 1.0 eq.) in abs. CH_2Cl_2 (10 mL) was added at room temperature 1,1'-carbonyldiimidazole (551.5 mg, 1.1 eq.) and the resulting solution was stirred for 1 h at the same temperature. After that, solid *N*,*O*-dimethylhydroxylamine hydrochloride (331.5 mg, 1.1 eq.) was added, and the solution was stirred at room temperature for 12 h. The reaction mixture was diluted with ethyl acetate (15 mL) and washed with aqueous 1 M HCl (1×15 mL), saturated aqueous solution of $NaHCO_3$ (1×15 mL), and a saturated solution of NaCl (1×15 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed at reduced pressure. The crude was purified by flash chromatography (SiO_2, 3:1 → 2:1 petroleum ether/ethyl acetate) to afford **5.17** (906 mg, 80 %) as colourless oil. $[\alpha]_D^{20}$ +19.8 (c 1.0 in $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ =7.40-7.27 (m, 5H,), 5.76 (d, 1H, *J* = 8.9 Hz), 5.25-5.08 (m, 2H), 4.68-4.57 (m, 1H), 3.63 (s, 3H), 3.28-2.64 (m, 1H), 3.13 (s, 3H), 1.42 (s, 9H) ppm.

Spectroscopic data were in accordance with the literature.[29]

Benzyl (*S*)-2-tert-butoxycarbonylamino-4-oxobutanoate (5.10)



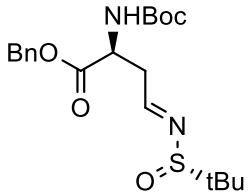
$C_{16}H_{21}NO_5$
307,34 g/mol

To a solution of **5.17** (900 mg, 1.0 eq.) in abs THF (19 mL) solution of DIBAL 1 M in CH_2Cl_2 (3.7 mL, 1.5 eq.) was added dropwise at -78 °C a. The resulting solution was stirred for 2 h at the same temperature. After that, 0.35 M aqueous solution of $NaHSO_4$ (33 mL) was carefully added dropwise at -78 °C. The reaction mixture was slowly warmed to room temperature and layers were separated. The aqueous layer was extracted with Et_2O (3×25 mL). The combined organic extracts were washed with 1 M aqueous HCl (3×20 mL), saturated aqueous solution of $NaHCO_3$ (3×20 mL), and a saturated solution of NaCl (3×20 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed at reduced pressure obtaining **5.10** (756 mg, quantitative yield) as colourless oil. $[\alpha]_D^{20}$ +16.5 (c 1.0 in $CHCl_3$). The crude product was used for the next steps without further purification.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.71 (s, 1H), 7.41-7.28 (m, 5H), 5.40 (d, *J* = 7.1 Hz, 1H), 5.17 (s, 2H), 3.11 (dd, *J* = 18.3, 5.2 Hz, 1H), 3.01 (dd, *J* = 18.3, 4.6 Hz), 1.42 (s, 9H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ = 199.3, 171.0, 155.2, 135.4, 128.6, 128.4, 128.3, 128.2, 80.3, 67.8, 49.1, 45.4, 27.6 ppm.

Benzyl [2*S*, N(*E*), S(*S*)]-2-*tert*-butyloxycarbonyl-4-(2-methyl-propane-2-sulfynilimino)-butanoate (5.11)



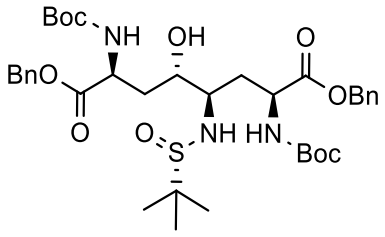
$C_{20}H_{30}N_2O_5S$
410,53 g/mol

In a round bottom flask a solution of **5.10** (375 mg, 1.0 eq.) in abs. CH_2Cl_2 (7 mL) and solid (*S*)-2-methyl-2-propanesulfinamide were added to anhydrous $CuSO_4$ (778.8 mg, 4.0 eq.) and the resulting suspension was stirred for 10 h at room temperature. The solid was removed by filtration over a pad of celite and the solvent was removed at reduced pressure. The crude was purified by flash chromatography (SiO_2, 4:1 → 2:1 hexane/ethyl acetate) to afford **5.11** (436 mg, 87 %) as white solid: mp 106-108 °C. $[\alpha]_D^{20}$ +24.1 (c 1.0 in $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.02 (dd, *J* = 3.5, 3.5 Hz, 1H), 7.41-7.27 (m, 5H,), 5.33 (d, *J* = 7.6 Hz, 1H), 5.16 (s, 2H), 4.71-4.57 (m, 1H), 3.22-2.97 (m, 2H), 1.41 (s, 9H), 1.16 (s, 9H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.0, 165.4, 155.1, 135.2, 129.0, 128.6, 128.0, 80.2, 67.6, 50.7, 38.1, 28.4, 22.4 ppm.

1,8-Dibenzyl [2*S*, 4*R*, 7*S*, S(*S*)]-2,7-bis-(*tert*-butyloxycarbonylamino)-4-hydroxy-5-(2-methyl-propane-2-sulfinylamino)-octadioate (5.9)



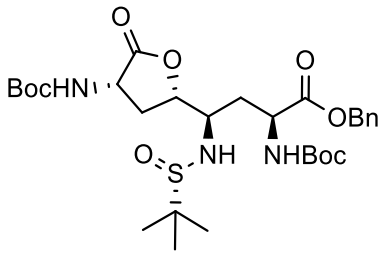
$C_{36}H_{53}N_3O_{10}S$
719,89 g/mol

To a solution of SmI_2 0.1 M in abs. THF (13 mL, 2.0 eq.) at -78 °C was added dropwise a solution of **5.11** (267 mg, 1.0 eq.), **5.10** (300 mg 1.5 eq.), and *tert*-butanol (96.5 mg, 2.0 eq.) in abs. THF (7 mL). The resulting solution was stirred for 5 h at the same temperature. The reaction was quenched by dropwise addition of a saturated solution of $Na_2S_2O_3$ (20 mL) at -78 °C. The solution was slowly warmed to room temperature and then the layers were separated. The aqueous layer was extracted with ethyl acetate (3×15 mL). The unite organic layers were dried with Na_2SO_4 and the solvent was removed at reduced pressure. The residue was purified by flash chromatography (SiO_2, 3:1:1 hexane/ethyl acetate/acetone) to afford **5.5** (260 mg, 55 %) as white waxy solid. $[\alpha]_D^{20}$ +28.7 (c 1.0 in $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.44-7.28 (m, 10H,), 5.78 (d, *J* = 5.2 Hz, 1H), 5.51 (d, *J* = 8.0 Hz, 1H), 5.28-5.05 (m, 4H), 4.57-4.43 (m, 2H), 4.37 (d, *J* = 4.9 Hz, 1H), 3.94 (bs, 1H), 3.59-3.46 (m, 1H), 3.36-3.22 (m, 1H), 2.22-2.09 (m, 1H), 2.08-1.93 (m, 1H), 1.88-1.71 (m, 1H), 1.70-1.53 (m, 1H), 1.42 8 (s, 9H), 1.41 (s, 9H), 1.18 (s, 9H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.5, 172.8, 157.7, 157.0, 142.0, 141.8, 130.0, 129.7, 129.6, 129.4, 129.3, 129.2, 73.4, 72.8, 71.0, 70.6, 68.0, 64.1, 54.9, 54.2, 53.9, 34.8, 32.0, 26.5, 26.1, 24.3 ppm.

Benzyl [2*S*, S(*S*), 2′*S*, 4′*S*]-2-tert-butoxycarbonylamino-4-(4′-tert-butoxycarbonylamino-5′-oxo-tetrahydro-furan-2′-yl)-4-(2-methyl-propane-2-sulfinylamino)-butanoate (5.18)



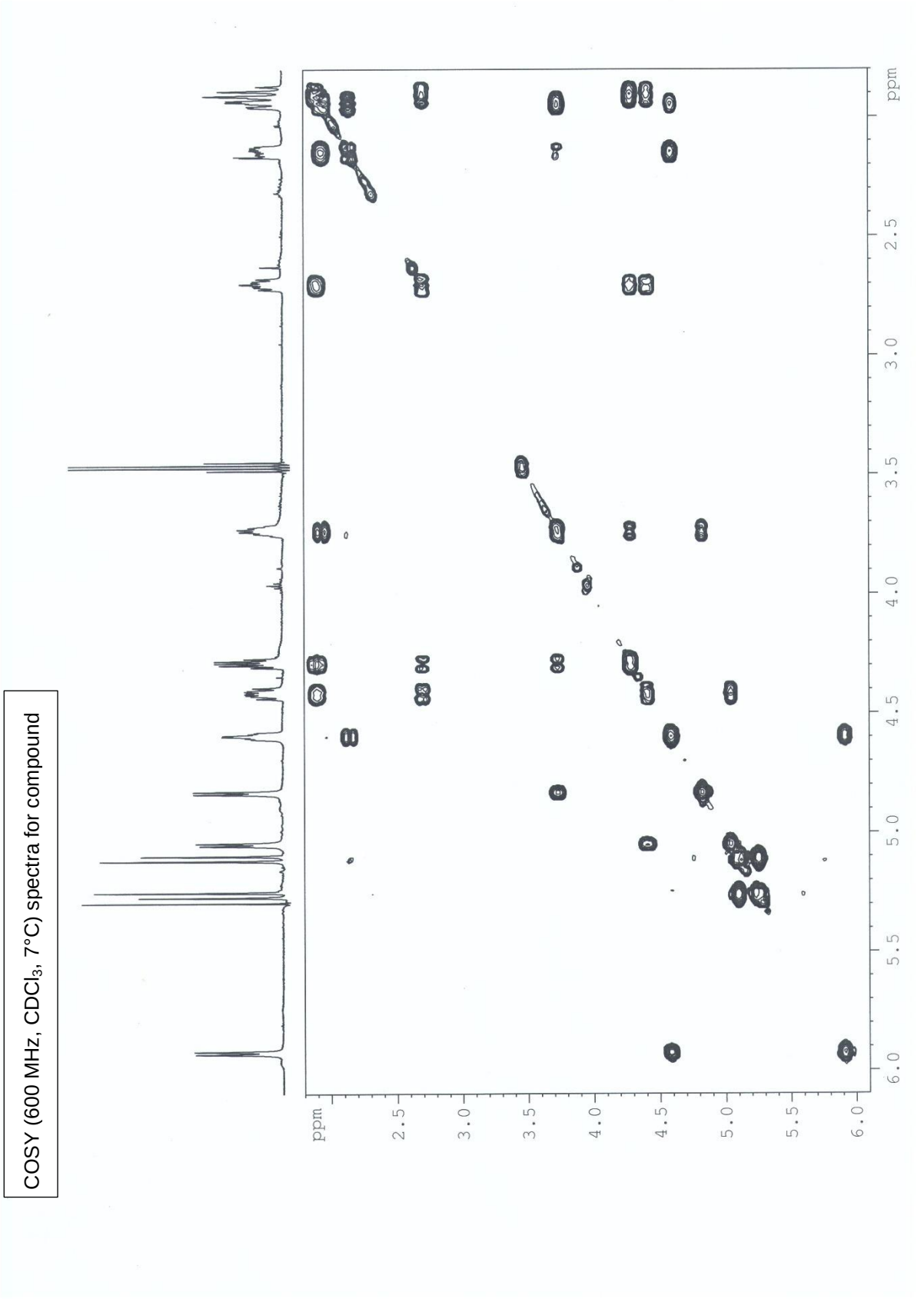
$C_{29}H_{45}N_3O_9S$
611,75 g/mol

To a solution of **5.9** (200 mg, 1.0 eq.) in anhydrous CH_2Cl_2 (2 mL) at 0 °C were added DMAP (3.4 mg, 0.1 eq.) and dropwise triethylamine (116 μL, 3.0 eq.). The resulting solution was stirred for 8 h at room temperature. The solution was diluted with ethyl acetate (10 mL) and washed with saturated aqueous solution of NH_4Cl (3×10 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed at reduced pressure. The crude was purified by flash chromatography (SiO_2, 3:1:1 hexane/ethyl acetate/acetone) to afford **5.18** (107 mg, 63 %) as white waxy solid. $[\alpha]_D^{20}$ +21.3 (c 1.0 in $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46-7.23 (m, 5H), 5.88 (bs, 1H), 5.23-5.10 (m, 2H), 5.05 (bs, 1H), 4.76 (bs, 1H), 4.60-4.52 (m, 1H), 4.44-4.37 (m, 1H), 4.33-4.24 (m, 1H), 3.36-3.24 (m, 1H), 2.70-2.64 (m, 1H), 2.25-2.17 (m, 1H), 1.98-1.94 (m, 1H), 1.92-1.82 (m, 1H), 1.47 (s, 9H), 1.42 (s, 9H), 1.20 (s, 9H) ppm.

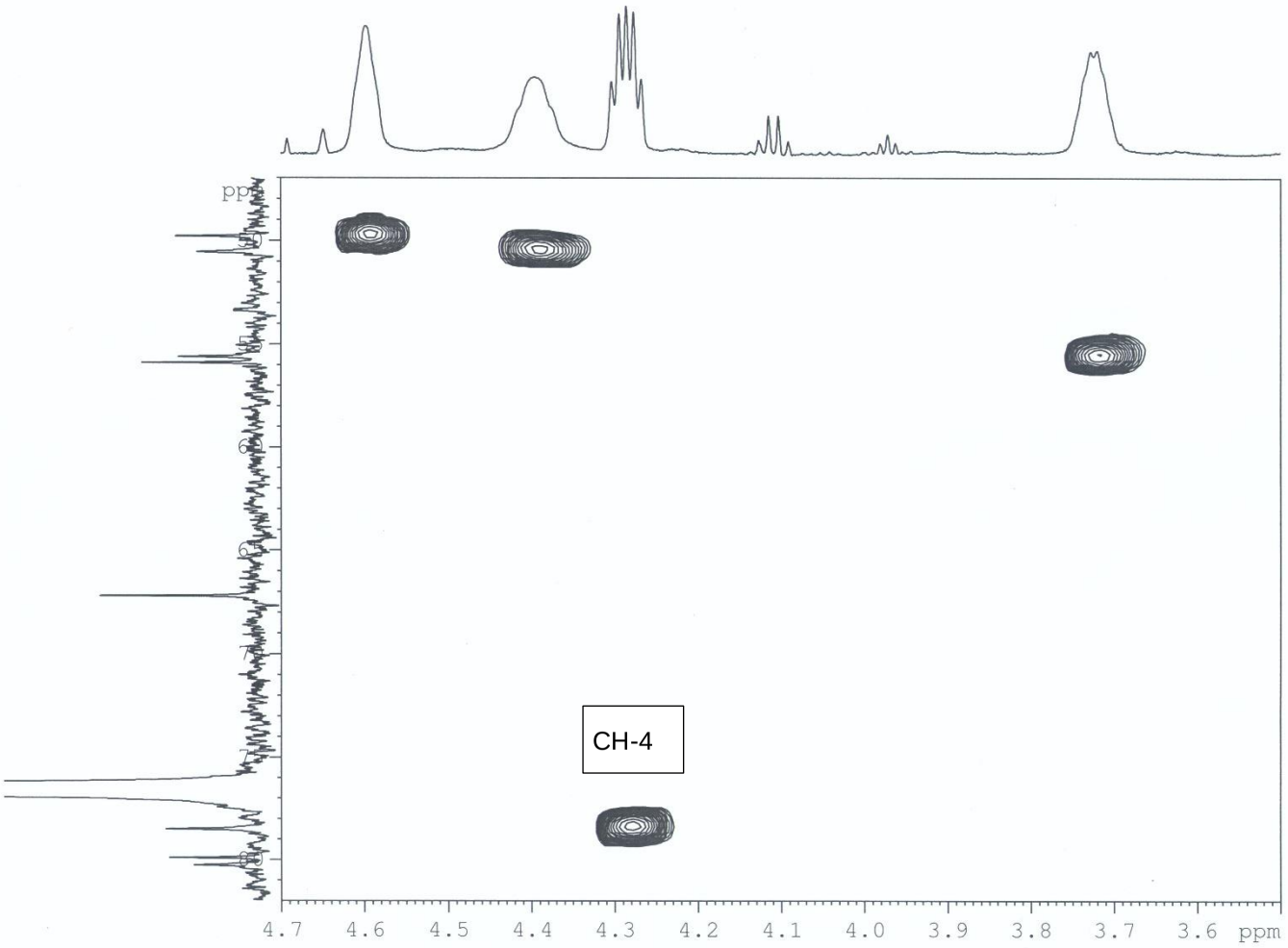
^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.0, 172.6, 155.7, 155.4, 135.12, 128.6, 128.3, 128.2, 80.7, 80.3, 65.8, 29.7, 28.3, 28. ppm.

5.5 NMR spectra

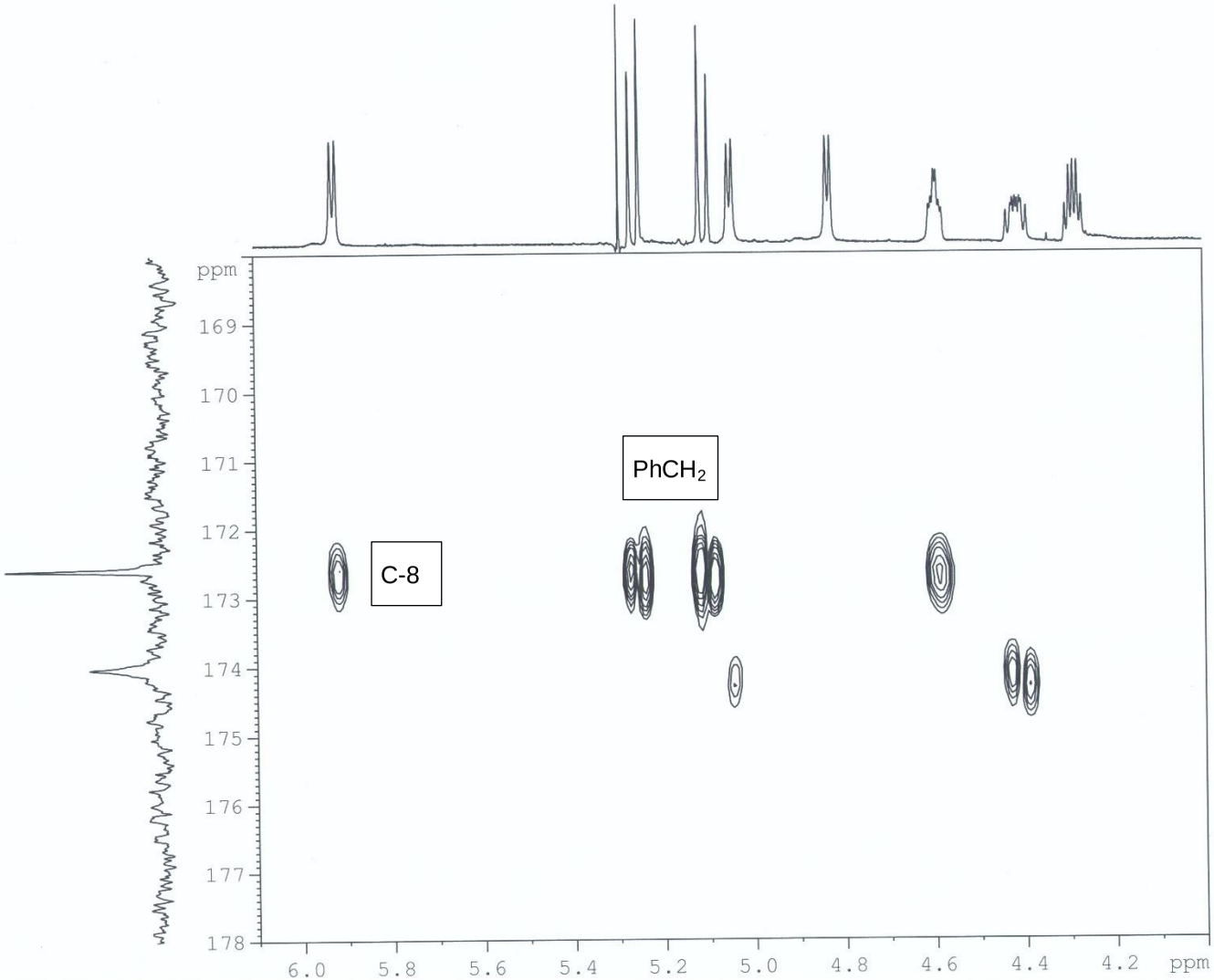


COSY (600 MHz, $CDCl_3$, 7°C) spectra for compound

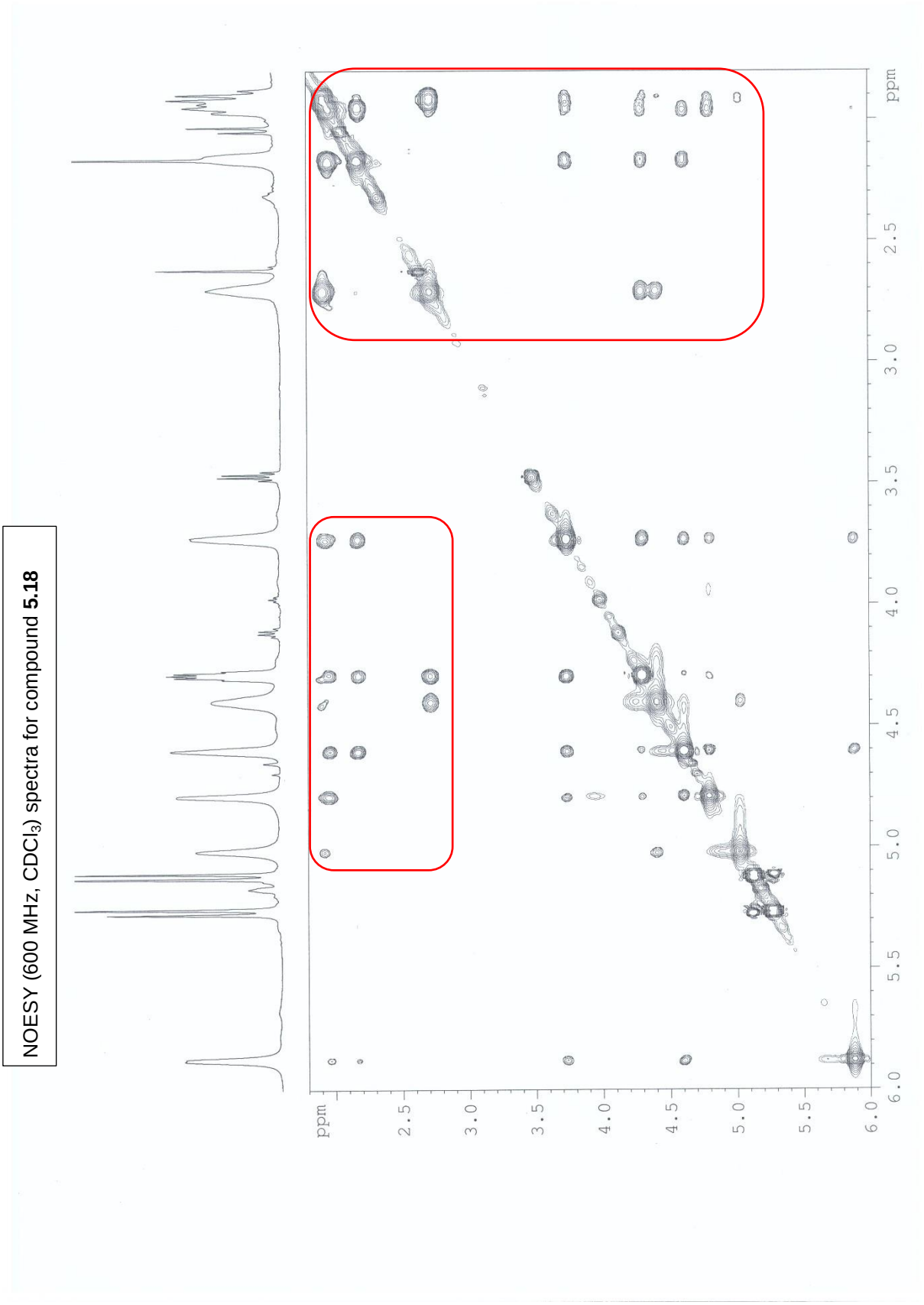
HSQC (600 MHz, $CDCl_3$) spectra for compound **5.18**



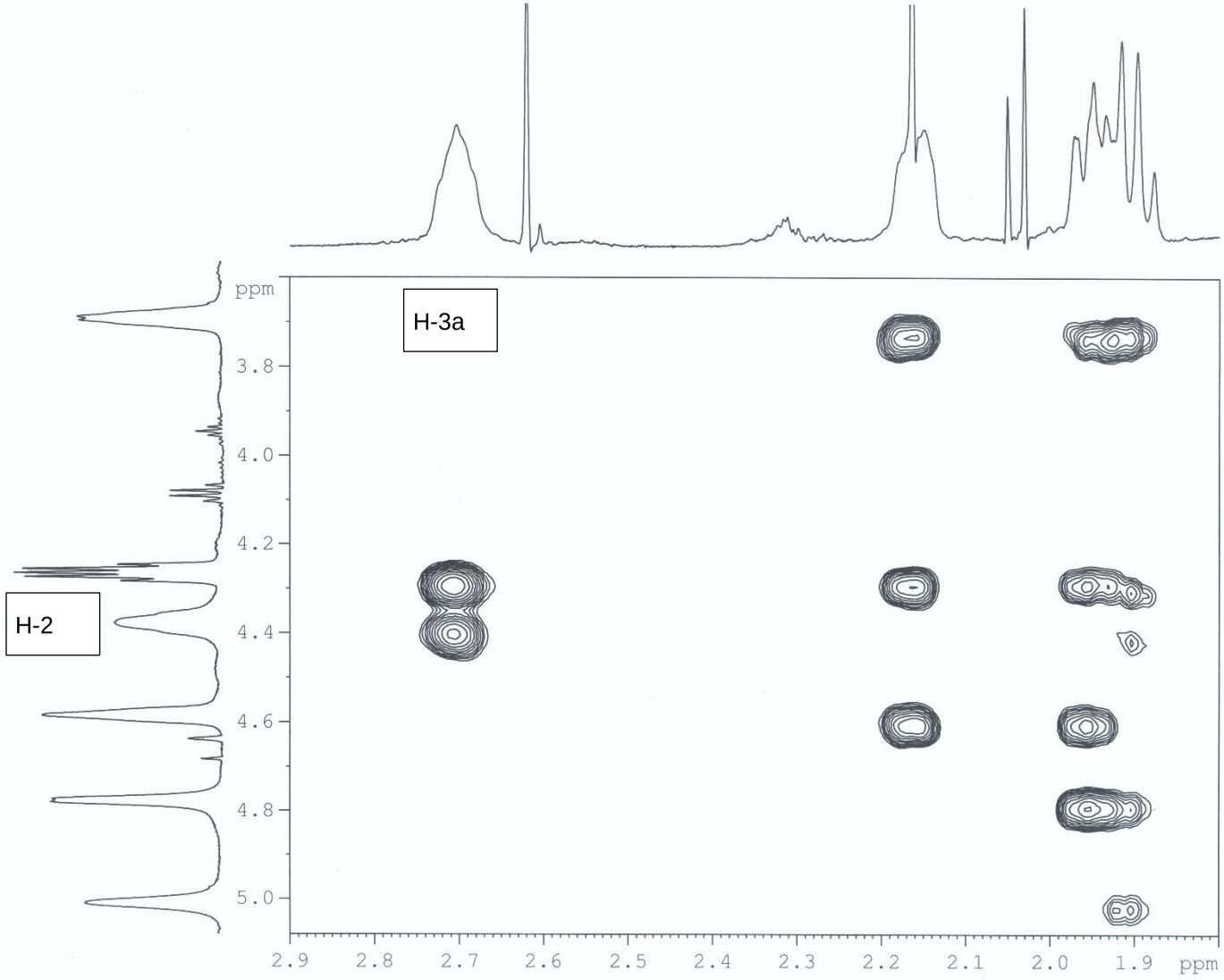
HMBC (600 MHz, $CDCl_3$) spectra for compound **5.18**



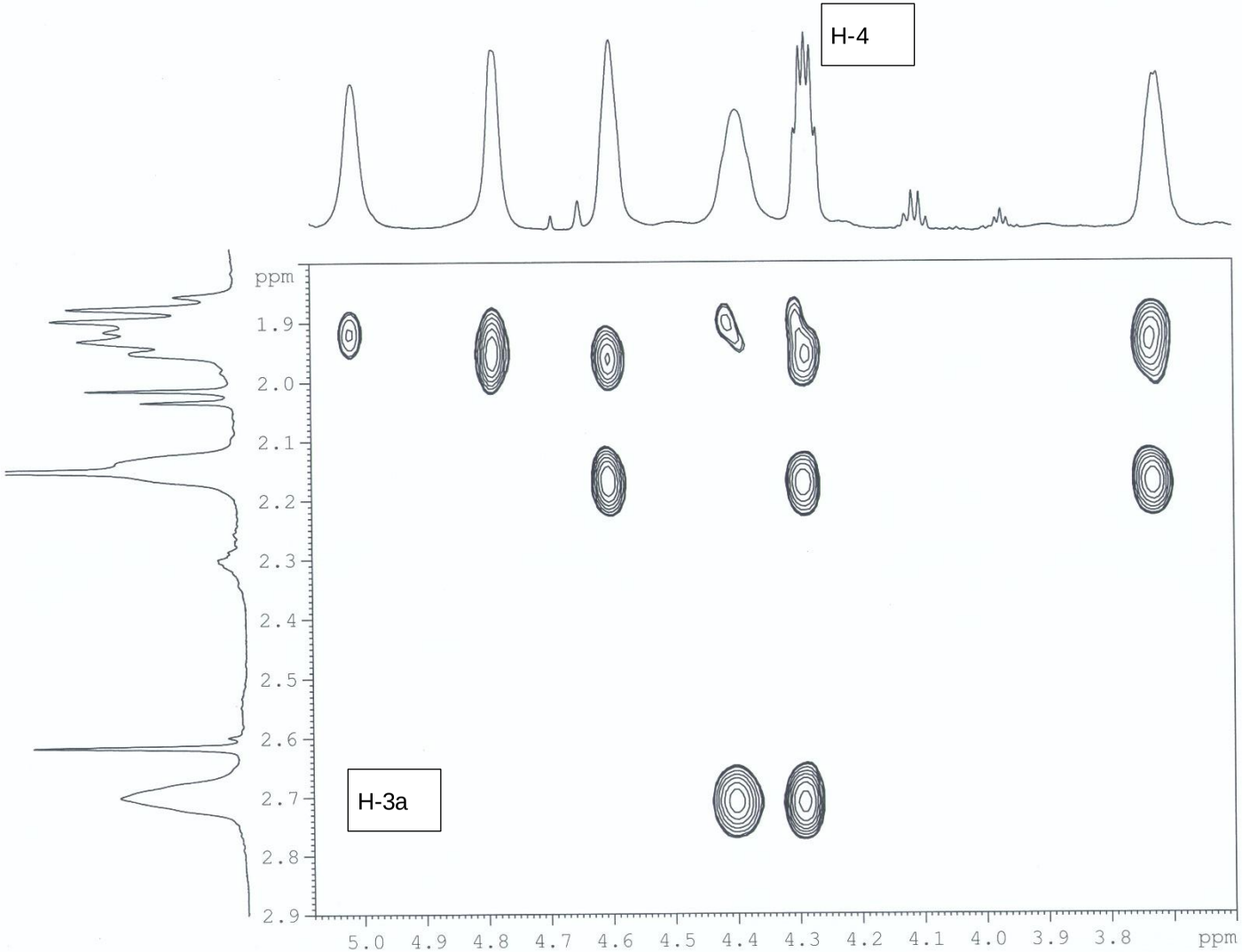
NOESY (600 MHz, $CDCl_3$) spectra for compound **5.18**



NOESY (600 MHz, $CDCl_3$) spectra for compound **5.18**



NOESY (600 MHz, $CDCl_3$) spectra for compound **5.18**



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6 CONCLUSIONS

In conclusion, through this Ph.D. thesis it was possible to develop novel approaches for the discovery and development of new agrochemicals. This is particularly important considering the crucial role of this agents in maximizing crop production and the continuous need of new products to face the changes of the agronomic systems.

In the first part of the thesis we demonstrated the value of the design of dual-target active compounds as a novel approach to the discovery and development of agrochemicals. In particular, we first confirmed that is possible to combine strobilurin fungicides with succinate dehydrogenase inhibitors obtaining compounds able to act on both target enzymes. Three compounds **3.23a-c** expressing both strobilurin that SDH inhibitory activity have been identified. Out of these three compounds, **3.23a** exhibits a remarkable antifungal activity against the fungus *Pyricularia oryzae*, which is the causal agent of one of the most serious diseases of cultivated rice, and responsible for 10-30 % yield loss of rice at harvest every year. Compound **3.23a** was selected as a candidate for *in vivo* studies. Because of the promising antifungal activity, compounds **3.23a-c** represent a valuable starting point for further optimization focused on the improvement of the activity and the spectrum of action.

In the second part of the work, two different applications of the chemistry of samarium(II) iodide were investigated as versatile approaches in the synthesis of different bioactive compounds. Overall, we confirmed the synthetic utility of this reagent in developing alternative synthetic strategy.

A SmI_2-mediated Reformatsky reaction of Evans' *N*-acyl-oxazolidinones with terpene aldehydes was studied as key step for the synthesis of cembranoids. The reaction was successfully performed and the role of a series of different factors (e.g. halide, double bond configuration, temperature etc.) on the outcome of the reaction was determined. The absolute configuration of the products of the reaction was successfully determined. Overall, we demonstrated that this reaction represents a complementary approach to Evans aldol reaction for the synthesis of cembranoids precursors, providing mainly products with *syn*-configuration

A SmI_2-mediated pinacol coupling was studied as key step for the total synthesis of the natural herbicide auscalitoxin aglycone **5.1**. We demonstrated the reliability of this approach, but further studies are needed to improve the reaction and to identify the factors that can influence the outcome of the reaction. We found that the coupling product **5.9** is unstable

since it easily undergoes to a cyclization reaction with the formation of a lactone. The instability is a limiting factor for further development of the synthesis. Variation of the protecting groups could be a strategy to overcome this problem.

We determined the absolute configuration at C-4 of compounds **5.9** exploiting the lactonization product in a series of NMR experiments. Lactonization compound was successfully identified as compound **5.18**. It was demonstrated a *S* configuration at the C-4. It was not possible to determine the absolute configuration at the C-5 because of the conformational mobility of the molecule. Further investigations are needed to confirm the configuration at this position. A possible approach could be the selective removal of the chiral auxiliary on the nitrogen at C-5. In this way, alternative cyclization pathways will be available leading to conformationally blocked compounds that could be used in further NMR studies.

7 MATERIALS AND METHODS

7.1 Solvents and general experimental procedures

All the chemicals were provided by supplier and used without further purification unless otherwise stated. The solvents were purified by distillation and dried using various desiccants as show in **Table 7.1**.

Table 7.1 Absolute solvents and their respective dessicants

Solvent	Dessicant
Tetrahydrofuran	Sodium/Benzophenone (Ch. 3,5) or Potassium/Benzophenone (Ch. 4)
Dichlorometane	Phosphorous pentoxide (Ch. 3,5) or Calcium hydride (Ch. 4)
Acetone	4 Åmolecular sieves
Methanol	3 Å molecular sieves

Samarium (II) iodide solution was prepared as described by Concellón et al. in 2003 by sonication of metallic samarium powder and iodine in dry degassed THF.[1]

When necessary solvents were degassed by bubbling nitrogen through the solvent for 30 minutes. All reactions requiring anhydrous conditions were performed under a positive nitrogen flow with with Schlenk-type oven-dried and/or flame-dried glassware.

Low temperature reactions were carried out using *Kryostat TC100E* (Huber).

7.2 Analytics and Apparatus

The following equipment was used for the characterization of compounds

7.2.1 NMR Spectroscopy

NMR spectra were recorded on *Varian Mercury 300* (^{1}H, 300 MHz; ^{13}C 75 MHz) and *Bruker AV600* (^{1}H, 600 MHz; ^{13}C 150 MHz) spectrometers **(Chapter 3,5)**, or on *Bruker Avance 700* (^{1}H, 700 MHz; ^{13}C 175 MHz), *Bruker Avance 500* (^{1}H, 500 MHz; ^{13}C 126 MHz), *Bruker Avance 400* (^{1}H, 400 MHz; ^{13}C 100 MHz), *Bruker Avance 300* (^{1}H, 300 MHz; ^{13}C 75 MHz). **(Chapter 4)**

TMS was used as an internal standard. $CDCl_3$ was used as solvent for the spectroscopy. All the spectra were recorded at room temperature.

^1H signals were referred to by the following abbreviations: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), m (multiplet).

7.2.2 IR Spectroscopy

FT-IR were recorded on *Bruker FT-IR-Spectrometer Vector 22* with *MKII Golden Gate Single Reflection Diamant ATR system*. The absorption bands have been rounded to the nearest wavenumber and the intensity of each band was abbreviated to: vs (very strong), s (strong), m (medium), w (weak). (**Chapter 4**)

7.2.3 Mass spectrometry

Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded with a *Bruker Daltonics MicroOTOF-Q* spectrometer for measurements with electrospray ionisation (ESI) or a *Finnigan MAT MAT 95* spectrometers for electron impact ionisation (EI). (**Chapter 4**)

7.2.4 Polarimetry

Optical rotations were measured with P-2000 polarimeter (*Jasco*) (**Chapter 3,5**) or with Perkin-Elmer 241 polarimeter (**Chapter 4**) at 20 °C

7.2.5 Thin layer chromatography

Analytical TLC was conducted on TLC plates (silica gel 60 F254, aluminum foil). Substances were detected with a UV-light source (λ = 254 or 365 nm) or stained with by spraying with 10% phosphomolybdic acid solution [10.0 g phosphomolybdic acid in 100 mL of abs. ethanol] or with ninhydrin solution [1.5 g ninhydrin, 3.0 mL acetic acid in 100 mL of abs. ethanol]. **(Chapter 3,5)**

ALUGRAM® Xtra SIL G/UV$_{254}$ Aluminium sheets pre-coated with 0.2 mm of silica gel 60 F^{254} (*Macherey-Nagel*) were used for thin layer chromatography (TLC). Substances were detected with a UV-light source (λ = 254 nm) or stained by spraying with anisaldehyde solution [6.0 g anisaldehyde, 1.5 mL conc. H_2SO_4 in 250 mL ethanol]. (**Chapter 4**)

7.2.6 Column chromatography

Isolation and purification of the compounds were performed by flash column chromatography on silica gel 60 with grain size within 40-63 μm (*Sigma*), **(Chapter 3,5)**, or on silica gel with grain size within 40-63 μm (*Fluka*). **(Chapter 4)**

7.3 Antifungal activity evaluation

7.3.1 Fungal strains

The following fungal and oomycete strains, belonging to a collection maintained in the laboratory of Plant Pathology at DeFENS, University of Milan, Italy, were used in the study: *Botrytis cinerea* BC4A and BC-2F-2016, *Curvularia* sp. CURV3, *Fusarium fujikuroi* FFM2 and FFM4, *Pyricularia oryzae* A2.5.2, TA102, A.4.31.1-0 and A4.31.1-1, *Pythium ultimum* FW407 and *Sclerotinia sclerotiorum* SW361. *Rhizoctonia solani* FW408 was obtained from Dr. Andrea Minuto (Centro di Sperimentazione e Assistenza Agricola, Albenga, Italy). The strains were maintained as single-spore isolates in malt-agar medium slants (MA: 20 g/L malt extract, Difco Laboratories, USA; 15 g/L agar, Applichem, Germany) at 4 °C.

7.3.2 Fungicides

The following commercially available fungicides were used in the study: azoxystrobin (AZX, Amistar SC – suspension concentrate, 22.9 % active ingredient., Syngenta Crop Protection), kresoxim-methyl (KM, Sovran WG – wettable granules, 50 % active ingredient., BASF Italia, S.p.A.), fluopyram (FLP, Luna Privilege SC, 50 % active ingredient., Bayer CropScience, S.r.l) and fluxapyroxad (FXP, Sercadis EC – emulsifiable concentrate, 30 % active ingredient., BASF Italia, S.p.A.).

7.3.3 Inhibition of mycelium growth on media supplemented with fungicides

A mycelium plug (0.5 cm in diameter) obtained from actively growing fungal colonies of *B. cinerea* BC4A and BC-2F-2016, *Curvularia* sp. CURV3, *F. fujikuroi* FFM2 and FFM4, *P. oryzae* A2.5.2 and TA102, *P. ultimum* FW407, *R. solani* FW408 and *S. sclerotiorum* SW361 was transferred to MA medium plates supplemented with fungicides at the concentration 25 mg/L ai. Because the tested molecules had low solubility in water, they were dissolved in acetone. Therefore, two controls were included: MA medium not supplemented with fungicides (NTC), and MA medium supplemented with acetone at the final concentration 1% v/v (ACT). The plates were incubated at 24 °C in the dark. The mycelium growth was

measured 2-7 days after inoculation (dai) and the inhibition of mycelium growth (%) was calculated by comparing the mycelium growth on control and fungicide-supplemented plates.
The mycelium growth of four strains of *P. oryzae* (A2.5.2, TA102, A.4.31.1-0 and A4.31.1-1) was evaluated on MA plates supplemented with increasing concentrations of selected compounds and. commercial fungicides. Again, NTC and ACT were included as controls. The mycelium growth was measured after 3-12 dai and the inhibition (%) was calculated as described above.

7.4 Cell-free enzymatic assay

7.4.1 Preparation of P. oryzae submitochondrial fraction

The submitochondrial fraction was prepared according to Ye et al. with some modifications.[2] Mycelium liquid culture (200 mL, 20 g/L malt extract medium, ML, in 1 L flask) was inoculated with 10 MA plugs of actively-grow *P. oryzae* (A2.5.2) and incubated at 25 °C in an orbital shaker (124 rpm) in the dark for 5 days. Mycelium was cooled on ice and harvested by vacuum filtration through Miracloth (Calbiochem®, La Jolla, CA, U.S.A.). During vacuum filtration residual culture medium was removed by washing 3-times with 30 mL of ice pre-cooled water. The collected well-drained mycelium was chopped in small pieces by scalpel and thoroughly powdered in mortar and pestle in the presence of liquid nitrogen. The mycelium powder (~ 6 g) was suspended in 10 volumes of ice-cooled mitochondrial buffer (0.5 M sucrose, 10 mM KH_2PO_4, 10 mM KCl, 10 mM $MgCl_2$, 0.2 mM EDTA, pH 7.2), and mildly mixed 60 min by magnetic stirrer at 4 °C. The suspension was centrifuged two-times 10 min at 4 °C, 5500 rpm (rotor JA20, Beckman). The cleared supernatant was furtherly centrifuged 20 min at 4 °C, 11250 rpm (rotor JA20, Beckman). The collected pellet (~ 33 mg) was suspended in mitochondrial buffer (1 mL / centrifuge tube), fractionated in 2 Eppendorf tubes and centrifuged again 20 min at 4 °C, 10000 rpm (rotor F45-24-11, Eppendorf). Each pellet was suspended in 0.2 mL of the same buffer and stored at - 80 °C till use.

7.4.2 Cell-free NADH-consumption assay

The mitochondrial NADH-driven respiration was evaluated monitoring the oxidation rate of NADH in the presence of the submitochondrial fraction according to Steinfeld et al. with some modifications.[3] The assay was carried out using a spectrophotometer (Lambda2S; Perkin Elmer, UK) equipped with a thermostated (30°C) cell holder (Peltier Temperature Programmer, PTP6; Perkin Elmer, UK) in a cuvette containing the assay buffer (82 mM HEPES, 8 mM $MgSO_4$, pH 8), 0.2 mM NADH (added from a 4 mM stock in 0.05 M

Tris/HCl, 1 mM EDTA, pH 7.7) in the presence of azoxystrobin (Pestanal®) and selected compounds (20-200 µM; from stock in DMSO), or DMSO (negative inhibition control). The reaction was started with the addition of the submitochondrial fraction suspension (10 µL). The time-drive absorbance (λ = 340 nm) decrease in the first 60 sec time-window was used for the measurement of the initial NADH-oxidation rate. Each rate was subtracted from the NADH autoxidation rate (recorded soon before the addition of submitochondrial fraction). The rate of the negative inhibition control was used to calculate the percent inhibition value.

7.4.3 Cell-free DBH_2-Cyt c oxidoreductase activity assay

The inhibition of DBH_2-Cyt *c* oxidoreductase activity was evaluated monitoring the reduction rate of Cyt *c* in the presence of the submitochondrial fraction according to Zhu et al. with some modifications.[4] The assay was carried out using a spectrophotometer (Lambda2S; Perkin Elmer, UK) equipped with a thermostated (30°C) cell holder (Peltier Temperature Programmer, PTP6; Perkin Elmer, UK) in a quartz cuvette containing the assay buffer (KH_2PO_4 50 mM previous degassed by stirring under vacuo, 3 mM NaN_3, 0,01 % lauryl maltoside, pH 7.4), Cyt *c* (added from a stock 2 mg/mL in stock in 0.05 M Tris/HCl, 1 mM EDTA, pH 7.7), and after 30 seconds, reduced decilubichinone (added from a stock reduced decylubichinone in 245 µL HCl 1 M in ethanol degassed with a stream of nitrogen), in the presence of azoxystrobin (Pestanal®), fluxapyroxad (Pestanal®) and selected compounds (20-200 µM; from stock in DMSO), or DMSO (negative inhibition control). The reaction was started with the addition of the submitochondrial fraction suspension (10 µL). The time-drive absorbance (λ = 550 nm) decrease in the first 6 sec. Each rate was subtracted from the Cyt *c* autoreduction rate (recorded soon before the addition of submitochondrial fraction). The rate of the negative inhibition control was used to calculate the percent inhibition value.

Reduced decylubiquinone was prepared from 2,3-dimethoxy-5-methyl *n*-decyl-1,4-benzoquinone (*Sigma*) following the procedure described by Gudz et al.[5] After the reaction the solvent was removed by flushing with a stream of nitrogen and the product was liofilized (Savant Speedvad Concentrator). The product was dissolved in degassed ethanol acidified with 10mM HCl and stored at -80 °C.

7.4.4 Cell-free SDH activity assay

The inhibition of SDH activity was evaluated monitoring by comolorimetric assay in the presence of the submitochondrial fraction according to Ye et al. with some modifications.[2] The assay was carried out using a spectrophotometer (Lambda2S; Perkin Elmer, UK)

equipped with a thermostated (30°C) cell holder (Peltier Temperature Programmer, PTP6; Perkin Elmer, UK) in a microtitration plate containing assay buffer (sodium succinate 50 mM, saccharose 250 mM , 3 mM NaN_3, pH 7.2), the submitochondrial fraction suspension (preactivated with sodium succinate 10 mM at 30 °C for 30 min.; 5-20 μL according to activity). 0.2mM 2,3-dimethoxy-5-methyl-1,4-benzoquinone, 140 μM DCPIP, in the presence of fluxapyroxad (Pestanal®), azoxystrobin (Pestanal®) and selected compounds (20-200 μM; from stock in DMSO), or DMSO (negative inhibition control). The reaction was started with the addition of 10 mM sodium succinate. The time-drive absorbance (λ = 595 nm) was measured after 2, 5, 10, 20, 30, 40, 50, and 60 min. The rate of the negative inhibition control was used to calculate the percent inhibition value.

7.5 Molecular modelling

The CytB homology model was based on the primary sequence of the CytB protein from *P. grisea* (Uniprot code: Q85KP9). The structure of CytB from *P. grisea* was modelled by using the resolved CytB structure from *S. cerevisiae* in complex with the inhibitor Stigmatellin A (PDB Id: 3CX5) as the template.

The SDH structure is composed of four subunits which have to be modelled together since the binding site is located at the interface between them. In detail, the homology model was based on three primary sequences from *P. oryzae* (i.e. Subunit A: G4NE44; Subunit B: L7JQS7; Subunit C: G4N2P5) while the sequence from *P. grisea* was used for subunit D (Q5G5B2) since the corresponding sequence from *P. oryzae* is unavailable. Then the SDH structure was generated based on the resolved structure of the porcine enzyme in complex with N-[(4-tert-butylphenyl)methyl]-2-(trifluoromethyl)benzamide (E23).

In both cases, the sequence alignment was performed by NIH Cobalt[6] while the homology models were generated by Modeller9.16.[7] Among the proposed models, the best ones were selected based on DOPE and GA341 scores and optimized by keeping fixed the backbone atoms to preserve the predicted folding. The Stigmatellin A and E23 ligands were then accommodated within the binding sites of the selected models by superimposing the key residues with those of the utilized templates. The so obtained complexes were finally minimized by keeping fixed all atoms outside a 10 Å radius sphere around the bound ligands. The so optimized complexes revealed a set of stabilizing interactions very similar to those seen in the resolved structures, a finding which emphasizes the reliability of the generated homology models.

The conformational profile of the considered ligands was explored by Monte Carlo procedures as described elsewhere.[8]

The docking simulations were performed by PLANTS focusing the search on a 8.0 radius sphere around the bound ligands.[9] For each ligand, 10 poses were generated and scored by the ChemPLP score with a speed equal to 1. Along with the computed primary score, the best complexes were also selected based on the interaction similarity with the complexes with Stigmatellin A and E23. The so obtained complexes were finally minimized by keeping fixed all atoms outside a 10 Å radius sphere around the bound ligand.

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8 PRODUCTS

8.1 Abstract of papers

Marco Zuccolo, Sabrina Dallavalle, Raffaella Cincinelli, Luce Mattio, Stefania Mazzini, Michelandrea De Cesare, Loana Musso. 2-Acryloyl-4,5-methylenedioxyphenol: A Small Molecule Endowed with Antidermatophytic Activity. *Letters in Drug Design & Discovery*, 2018.
DOI: 10.2174/1570180815666180803115347

Marco Zuccolo, Andrea Kunova, Loana Musso, Fabio Forlani, Giulio Vistoli, Silvia Gervasoni, Paolo Cortesi, Sabrina Dallavalle. Exploring dual-action antifungal compounds: merging the strobilurin- and succinate dehydrogenase inhibitor-pharmacophores (SUBMITTED)

2-Acryloyl-4,5-methylenedioxyphenol: A Small Molecule Endowed with Antidermatophytic Activity

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ABSTRACT

BACKGROUND: Superficial fungal infections are the most common fungal diseases in humans, affecting more than 25% of the population worldwide. Methods: In the present study, we have investigated the activity of kakuol, a natural compound isolated from the rhizomes of *Asarum sieboldii*, and some analogues, against various dermatophytes and pharmacologically relevant yeasts.

RESULTS: One of the tested compounds, 2-acryloyl-4,5-methylenedioxyphenol, showed a broadspectrum activity against most of the fungal species assayed, resulting particularly effective against dermatophyte strains (MIC values in the range of 0.25-0.5 μg/mL, two/four-fold lower than the positive control miconazole).

CONCLUSION: The results suggest that this molecule can be considered a promising starting point for the development of new antifungal compounds.

Keywords: kakuol, *Asarum sieboldii*, antifungal agents, *Candida albicans*, dermatophytes, 3,4-methylenedioxyphenol.

Exploring dual-action antifungal compounds: merging the strobilurin- and succinate dehydrogenase inhibitor-pharmacophores (SUBMITTED)

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ABSTRACT

BACKGROUND: Crop disease management often implies repeated application of fungicides. However, hazardous effects of fungicides to human health and environment and the increasing emergence of fungicide-resistant pathogens are the major drawbacks to their uses. Tank-mix combination treatments using fungicides with different modes of action is in general hard to manage either by farmers or chemical companies. An alternative and unexploited strategy is based on the use of bifunctional fungicides, i.e. compounds resulting from conjugation of the pharmacophores of fungicides with different mechanisms of action.

RESULTS: In this paper we report our efforts to develop a new approach to antifungal treatment based on the synthesis of dual agents. The hybrids were obtained by merging into a new entity the pharmacophores of strobilurins and SDH inhibitors. The selected compound inhibits the growth of P. oryzae with activity comparable with kresoxim-methyl, and even better than that of fluopyram. Inhibition of the NADH-driven respiration in cell-free submitochondrial preparations of P. oryzae confirmed the hypothesis that the mitochondrial respiratory electron-flow is targeted by this molecule.

Molecular modelling studies showed that the selected compound is capable of forming stable complexes with both CytB subunit and succinate dehydrogenase enzyme, providing a computational validation to the design of strobilurin-SDHI-dual molecules.

CONCLUSION: Due to the call for additional molecules active against P. oryzae, one of the most detrimental pathogens worldwide, this work can be considered an important first step towards the development of novel dual-action agents with optimized structure and improved interaction with the targets.

Keywords: dual-action inhibitors, antifungal compounds, succinate dehydrogenase inhibitors (SDHI), strobilurins, mitochondrial respiration.

8.2 Abstract of oral communications and poster

Marco Zuccolo, Andrea Kunova, Ilaria Teresa Di Gennaro, Fabio Forlani, Paolo Cortesi, Sabrina Dallavalle. Design and synthesis of hybrid bifunctional fungicides with dual mechanism of action. Avanced School: "Food Proteins" SIB-Gruppo Proteine, Bergamo, Italy, 2-4 May 2018. *Oral Communication*

Marco Zuccolo, Sabrina Dallavalle. Development of SDHI-strobilurin hybrids as novel fungicides for crop protection. XIII Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology, Oristano, Italy, 19-21 September 2018. *Oral Communication*

Marco Zuccolo, Sabrina Dallavalle, Laura Polito, Andrea Kunova, Paolo Cortesi. Design and preliminary evaluations of gold nanoparticles as potential nanocarriers for agrochemicals. Ischia Advanced School of Organic Chemistry (IASOC 2016), Ischia, Italy, 25-29 September 2016. *Poster presentation*

Avanced School: "Food Proteins" SIB-Gruppo Proteine, Bergamo, Italy, 2-4 May 2018

Design and synthesis of hybrid bifunctional fungicides with dual mechanism of action

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Plant pathogenic fungi are among the major causes of crop losses worldwide. Although these losses may be reduced using suitable agronomical practices, fungicides remain a critical element to increase crop production. Fungicides play also an important role in the control of mycotoxin-producing fungi ensuring the quality of food.

Due to a substantial reduction of available fungicides after the EU revision of agrochemicals and because of the risk of fungicide resistance emergence in pathogen populations, new fungicides must be continuously developed. Resistance to fungicides is one of the most serious problems in disease management and generally the use of a single molecule is not able to provide a durable control of fungi.

The efficacy of fungicides could be improved using hybrid bifunctional molecules, i.e. compounds containing the pharmocophores of different fungicides designed to inhibit simultaneously multiple targets relevant to pathogen growth/survival.

Here we report the results of our efforts to develop new hybrid fungicides. In particular, we combined the pharmacophoric features of two classes of commercially validated antifungal compounds: strobilurines and succinate dehydrogenase inhibitors.

The dual inhibitors were designed based on reported SAR studies on both classes of fungicides, which allowed to define the key moieties of the new hybrid molecules. A simple divergent synthetic strategy was developed for the preparation of the hybrids and the antifungal activity was assessed in vitro against different pathogenic fungi of agronomical interest. Moreover, the inhibitory activity on the enzymatic target was assessed in vitro on mitochondrial fraction to elucidate the mechanism of action of the new synthesized molecules.

XIII Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology, Oristano, Italy, 19-21 September 2018

Development of SDHI-strobilurin hybrids as novel fungicides for crop protection

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This Ph.D. thesis dealt with the exploration of a new approach for antifungal agents discovery based on the synthesis of hybrid bifunctional compounds. The molecules were obtained by merging the pharmacophoric features of existing fungicides belonging to the class of succinate dehydrogenase inhibitors (SDHI) and of strobilurins.

Ischia Advanced School of Organic Chemistry (IASOC 2016), Ischia, Italy, 25-29 September 2016

Design and preliminary evaluations of gold nanoparticles as potential nanocarriers for agrochemicals

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Nanomaterials possess physico-chemical properties that are different from the same material on macroscale. For this reason, they are very interesting for applications in many disciplines such as medicine, food and environment sciences. Several studies show that nanomaterials could be successfully applied also to agricultural production and crop protection. In fact, there are evidences that some nanomaterials could be used to enhance seeds germination and plants growth, as nanosensors for detection of pesticide residue and pathogens and for improvement of existing arochemicals.

As regards agrochemicals, the use of nanomaterials and more generally of the nanotechnologies is emerging in recent years and it is expected to be in rapid development in the near future. In particular, the efforts are oriented to the development of nanoformulations, i. e., formulations that consist in or contain elements with dimensions on nanoscale. The aims of nanoformulations are basically the improvement of the solubility and therefore of the bioavailabilty of the active ingredients and the realization of controlled/targeted release.

More interestingly, nanomaterials could be used as nanocarriers for pesticides. Indeed, they could provide a better penetration and distribution of the active ingredients in the plants, resulting in an increased efficacy and a possible reduction of the required doses. Despite the potential advantages of the use of pesticide nanocarriers this topic is still underexplored and very little is reported in the scientific literature. For these reasons we planned to explore the possibility of using gold nanoparticles as nanocarriers for fungicides and to evaluate their activity against well known pathogens of agronomical interest. Here we describe the synthesis

and the functionalization of gold nanoparticles with a triazole fungicide. The active moiety of tetraconazole was derivatized with a spacer chain in order to provide an uncleavable linker, and then it was linked to gold nanoparticle by a PEG chain. The functionalized nanoparticles were fully caracterized and then both their preventive and curative antifungal activity was assessed against experimental infection with powdery mildew (*Podosphaera fusca*) of cucumber plants (*Cucumis sativus*) under greenhouse conditions.