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SYNTHETIC APPROACHES TO NOVEL DERIVATIVES OF NATURAL HEMIASTERLINS AND EPOTHILONE B AS POTENTIAL ANTICANCER DRUGS INTERFERING WITH MICROTUBULE DYNAMICS

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Chapter 1

General introduction

1. General introduction

By definition, the word *natural* refers to something that is present in or produced by nature and not artificial or man-made. Natural products may be extracted from tissue of plants, marine organisms or microorganism fermentation broths.

Structurally, natural products include different classes of compounds as terpenoids, polyketides, aminoacids, peptides, proteins, carbohydrates, lipids, nucleic acid bases, ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and so forth, usually provided with biological and pharmacological activity.

They are considered the most productive source of leads for the development of drugs.

Indeed from 2005 to 2007 thirteen natural product-related drugs were approved, particularly as anti-infectives and anticancer agents.[1,2]

1.1. Tumor: what it is?

Cancer is a disease characterized by uncontrolled and diffuse multiplication of abnormal shape cells in the organism.[3]

Biologically, cancer is composed of more than one distinct disease, each with its own etiology and pathology, which is most closely related to the tissue of origin.

The treatment of these diseases has involved the development of many strategies, together with the discovery of mechanisms that allow disorderly cell growth, and many naturally-derived molecules have been found and employed to improve therapies. Moreover, natural compounds have often

been the starting point of a drug discovery process that was used to better understand targets and pathways of tumor onset.

For this reason, improvements in the area of cancer therapy are essential, considering that is forecasted to be major cause of death in the 21st century, in particular in industrialized countries.[4]

1.2. Defects of the mechanisms of cell replication and death.

During eukaryotic cell division, the mother cell must replicate its chromosomes exactly once in the synthetic phase (S phase of cell cycle), and then must separate the replicated chromosomes at the end of the mitotic phase to the two daughter cells provided with the same genetic heritage of the mother cell.

Defects in the coordination of chromosomes replication and segregation can have severe consequences leading to genetic instability and aneuploidy, and eventually fostering tumor malignancy.

To ensure correct transmission of genetic material during cell division, cells have evolved cellular regulatory mechanisms termed “cell cycle checkpoints”.

This control system prevents or delays cell cycle progression if certain cellular processes or proteins are disrupted, to gain time to repair the damage before cell division occurs.

When the damage is irreparable, the cell undergoes apoptosis through the triggering of specific biochemical pathways.

However, cancer cells often elude the control systems allowing uncontrolled cell proliferation, even when cell division does not occur properly.

About the 50% of human malignant tumor are induced by mutation of p53 gene, that codifies for the namesake protein involved in the reparation mechanism of DNA.[3]

1.3. Cancer chemotherapy

The aim of conventional antitumor therapy to date is to slow and hopefully halt the growth and spread of a cancer cells. There are three goals associated with the use of the most common anticancer agents:

- To damage the DNA of cancer cells.
- To stop cells replication by inhibition of the synthesis of new DNA strands.
- Block of mitosis, or the actual splitting of the original cell into two new daughter cells during replication.

In general, chemotherapy agents can be divided into three main categories based on their mechanism of action.

- *Stop of the synthesis of pre-DNA molecule building blocks*. DNA building blocks are folic acid, heterocyclic bases, and nucleotides, which are made naturally in the cells.

  All of these agents work to block some steps in the formation of nucleotides or deoxyribonucleotides (necessary for making DNA) and without these the cells can't replicate.

  Examples of drugs in this class include methotrexate (Abitrexate®) and fluorouracil (Adrucil®).
- *Direct damage to the DNA in the cell nucleus*. These agents chemically damage DNA and RNA, either blocking totally replication or causing the formation of nonsense DNA or RNA (i.e. the new DNA or RNA does not code for anything useful).

Examples of drugs in this class include cisplatin (Platinol®) and antibiotics such as doxorubicin (Adriamycin®).

- *Effect the synthesis or breakdown of the mitotic spindles.* Mitotic spindles serve as molecular railroads during cell replication, since they help to split the new copied DNA so that every copy goes to each of the two new cells during cell division.
  These drugs paclitaxel (Taxol®) and vincristine (Oncovin®) disrupt the formation of the spindles and therefore interrupt cell division.
  Mitotic spindle structure is made up of dynamic substructures named microtubules.[5]

1.4. Microtubules

Microtubules are major dynamic structural components in cells since they are involved in very important cellular functions, such as the development and maintenance of cell shape, cell reproduction and division, cell signalling and movement.

For this reason, microtubules are the target of structurally different groups of anticancer drugs, most of which derive from natural products.

The mitotic inhibitors represent the single best cancer target identified to date.[6]

Microtubules are highly dynamic polymers of heterodimers of two closely related 55 KDa proteins termed α and β tubulin, arranged parallel to a cylindrical axis to form tubes of 25 nm diameter that may be many μm long. The proteins are encoded by separate genes or small gene families, whose sequences are highly conserved throughout the eukaryotic kingdom.

Another member of the tubulin family, γ-tubulin, is involved in the nucleation and polar orientation of microtubules.

It is found primarily in centrosomes and spindle bodies, since there are areas of most abundant microtubules nucleation.

Polymerization of microtubules occurs by a nucleation-elongation mechanism in which the formation of a short microtubules "nucleus" is followed by elongation phase characterized by reversible, noncovalent addition of tubulin dimers to the end of microtubule itself.

They exhibit complex polymerization dynamics that use energy provided by the hydrolysis of GTP.

Tubulin binds GTP in solution with high affinity, and little by little tubulin-GTP is added to the end of a growing microtubule, the GTP is gradually hydrolyzed to GDP and P_i.

Ultimately, the P_i dissociates from the microtubules, leaving a microtubule core consisting of tubulin with stoichiometrically-bound GDP. The nucleotide remains non-dissociable and non-exchangeable until the tubulin subunit dissociates from the microtubule.

Microtubules dynamic is regulated by two phenomena, from one side "treadmilling" is the net growth at one microtubule end, on the other side "dynamic instability" is a process in which the individual microtubules ends switch between phases of relatively slow sustained growth and rapid shortening.

The transition between growth and shortening appears to be regulated by the presence or absence of the region of GTP-containing-tubulin at the microtubule end.

A microtubule can grow as long as maintains a stabilizing cap of tubulin-GTP or tubulin-GDP-P_i at its end. The loss of the cap induces depolymerization of microtubules.

Hydrolysis of tubulin bound GTP and the subsequent release of P_i establish conformational changes in the tubulin molecule, that destabilize the microtubule polymer, resulting in "catastrophe" and shortening of the microtubules.

The two ends of microtubules are not equivalent; one end, termed "plus end" is kinetically more dynamic than the "minus end" and although both ends can either grow or shorten, the changes in length at the first one end are much larger than the changes in length at the other one.[7,8]

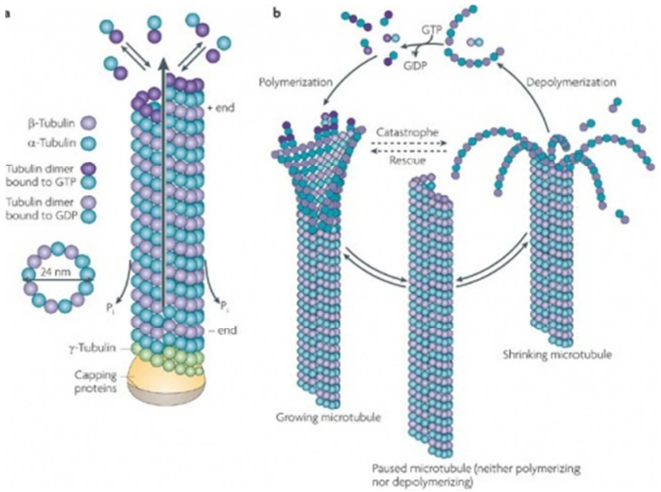


Figure I-1. (a) Schematization of a microtubule made up of dimers of α and β tubulin. (b) Representation of main microtubule dynamics.

1.4.1. How microtubules work throughout the cell division cycle

In a typical cell during the interphase of the cell cycle, the microtubules radiate from a central site near the nucleus, called "microtubules-organizing

centre" (MTOC). In animal cells, the MTOC consists of a centrosome made up of a lattice of MAPs, γ-tubulin and a pair of centrioles.

While the minus end of the microtubules lies in or near the centrosome, the plus end extends out toward the cell periphery. Before of the cell division, the cytosol is permeated by a fixed net of microtubules organized in a spindle-shape "mitotic spindle" that is the real director of the chromosomes segregation at anaphase.[9]

Mitotic spindle consists of three kinds of microtubules:

- Astral microtubules are shorter and stabler because they link the two poles of mitotic spindle to the cell ends.
- Polar microtubules are longer, rather stable and they look overlaid each other because of their structural role during mitosis.
- Kinetochore microtubules are unstable and directly linked to the chromosomes on the equatorial plate. They are the object of dynamic instability phenomenon given that the rapid removal of tubulin subunit determine the separation of the chromosomes.[10]

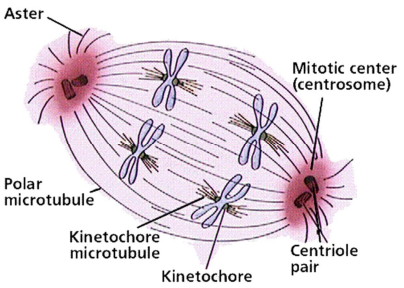


Figure I-2. Representation of microtubules network during the separation of chromosomes.

The interphase microtubules network disassembles at the onset of mitosis and is replaced by a new population of spindle microtubules that are 4 to 100 times more dynamic than microtubules in the interphase cytoskeleton.
Mitotic spindle exchange its tubulin with that in the soluble pool with half-times in the order of 10-30 seconds. Recent studies have indicated that several antimitotic anticancer drugs appear to inhibit mitosis at the methaphase/anaphase transition by suppressing spindle tubules dynamics.
In the presence of low, but effective drugs concentrations, spindle forms and mitosis can progress as far as the methaphase/anaphase transition.
However, the spindles are completely unable to pass the mitotic cell cycle checkpoint and to initiate anaphase movements, or do so only after a long period of mitotic blockage, apparently due to their suppressed dynamics. Mitotically-blocked cells eventually die by apoptosis.[6]

1.4.2. Tubulin binding site

Tubulin was identified for the first time as the "colchicines-binding protein" by Borisy and Taylor in 1967, and the ability of colchicine to block cells in prometaphase/metaphase step of mitosis played a key role in the development of antimitotic drugs.
Antitubulin agents are divided into two categories based on their ability to bind to tubulin and change the ratio between assembled microtubules and dimeric tubulin.
In vitro, the equilibrium between the dimeric and polymeric forms of tubulin can be altered by different effecters, such as DMSO, cofactors [Mg^{2+}, guanosine-5'-triphosphate (GTP), guanosine-5'-diphospahte (GDP)], or small molecule, which alter the stability of tubulin dimers or the polymerization process.[6]

Three major classes of tubulin-binding agents have been identified: the colchicine-site binding agents, the vinca domain inhibitors, which block microtubules growth, taxanes and epothilones binding site inhibitors which stabilize microtubules.

Both the vinca alkaloids and the taxane drugs bind to the tubulin, but at different location on the protein: the first group binds to β-tubulin between amino acids 175 and 213, while paclitaxel (Taxol®) binds both to N-terminal unit on β-tubulin and to the region bounded by amino acids 217-231.

Colchicine that is not a clinically used drug for cancer binds to the β-subunit at the interface with α-monomer of the some tubulin molecule.[9]

1.4.2.1. Colchicine binding site

Colchicine (**1**), was isolated from the autumn crocus *Colchicum autumnale.*

Although colchicine has significant *in vitro* antitumor effects, its medical uses, as well as use of its derivatives, has been limited because of its high toxicity, low bioavailability and poor water solubility (fig. I-3).[11]

The effect of colchicine on microtubule dynamics depends on drugs concentration. Thus, relatively high colchicine concentrations inhibit microtubule polymerization and depolymerize preformed microtubules.

Colchicine binds to soluble tubulin and forms a poorly reversible tubulin-colchicine (TC) complex, which then is incorporate at the microtubule ends.[6]

A group of tubulin inhibitors that bind at this site include CA-4 (**2**) and ZD6126, both of which are currently in clinical development.

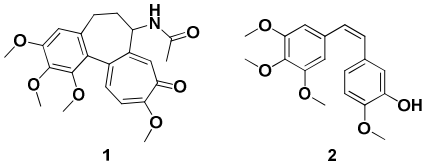


Figure I-3. Structures of colchicine and CA-4.

1.4.2.2. The vinca domain

The antimitotic vinca alkaloids vinblastine (**3**), vincristine (**4**), vindesine and vinorelbine are widely used both as single agent and in combination with other antitumor drugs in cancer chemotherapy, particularly in a variety of hematologic and solid tumor (fig. I-4).

The two complex indole alkaloids, vinblastine and vincristine were originally isolated from the plant *Catharantus Rosea*, while vindensine and vinorelbine are semisinthetic compounds.

The binding site of vinblastine on tubulin also binds other compounds (hemiasterlins, crytophycins and halicondrins) and this kind of binding induces a conformationally change in tubulin that becomes more akin to vinblastine with the formation of vinblastine-tubulin spiral oligomers.[9]

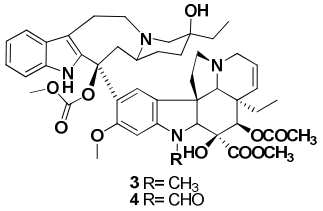


Figure I.4. Structures of vincristine and vinblastine.

1.4.2.3. Taxol binding site

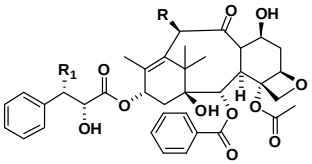
Taxol® (paclitaxel, **5**) was isolated in 1971 by Well and Wani from the pacific yew *Taxus Brevifolia* and is effective used in the treatment of the breast, ovarian, and lung carcinomas.

Taxol appears to arrest cell in mitosis by stabilizing spindle microtubules, in particular this alkaloid induces the formation of morfologically-altered tubulin polymers, that organize themselves in 12 protofilaments rather than 13, both *in vitro* with pure tubulin and *in vivo*.[6]

Like vinblastine and colchicine, taxol slows and blocks mitosis at the metaphase/anaphase transition in a number of cell types, inducing accumulation in a metaphase-like state and ultimately apoptosis.[12]

The success of paclitaxel has spurred enormous interest in finding better pharmacokinetic profile analogs.

To date the only one approved for clinical use in the U.S. is docetaxel (**6**) that has a semi-synthetic origin.



5 R= $OCOCH_3$, R_1= NHCOPh
6 R= OH; R_1= O^tBu

Figure I-5. Structure of paclitaxel and docetaxel.

Both paclitaxel and docetaxel occupy the same binding site in the subunit of tubulin with a 1:1 stoichiometry.

In the following tab (Table I-1) are reported the class, at the moment, more promising of tubulin inhibitors, most of which are in clinic trials.

However, there is an unmet need of new drugs since current therapies suffer from limited extension of survival time due to inherent or acquired resistance which is often associated with expression of the P-glycoprotein drug transporter.

Compound class	Approved agents	Compounds in develop	Effect(s) on microtubules
Taxanes	paclitaxel docetaxel	DJ-927	Polymerization and/or depolymerization
Epothilones	Ixabepilone	KOS-1584 Epothilone B	
Vinca alkaloids	Vincristine Vinblastine Vinorelbine		Depolymerization and/or destabilization
Halicondrin b		Erubiline mesylate	

Table I-1. Classes of MTIs

1.4.3. Multi drug resistance

Multidrug resistance, the principal mechanism by which many cancers develop resistance to chemotherapy drugs, is a major factor in the failure of many forms of chemotherapy.

It affects patients with a variety of blood cancers and solid tumors, including breast, ovarian, lung, and lower gastrointestinal tract cancers.

Tumors usually consist of mixed populations of malignant cells, some of which are drug-sensitive while others are drug-resistant. Chemotherapy kills drug-sensitive cells, but leaves behind a higher proportion of drug-resistant cells.

As the tumor begins to grow again, chemotherapy may fail because the remaining tumor cells are now resistant.

Resistance to therapy has been correlated to the presence of at least two molecular "pumps" in tumor-cell membranes that actively expel chemotherapy drugs from the interior. This allows tumor cells to avoid the toxic effects of the drug or molecular processes within the nucleus or the cytoplasm.

The two pumps commonly found to confer chemoresistance in cancer are P-glycoprotein and the so-called multidrug resistance–associated protein (MRP).

Drug resistance is a multifactor phenomenon in which different mechanisms are involved as failure of physiologic apoptosis, modified drug activation or degradation and modified transport of the drugs through the membrane because of a change its permeability.

Pgp (Fig. I-6) is a trans-membrane protein, product of gene *mdr-1*, over-expressed in tumor cells, in particular those targets of chemotherapy.

The primary structure of this protein is made up of 1280 amino acids, organized into two repeated units, everyone of 610 amino acids, linked by a bridge. Its trans-membrane domain (TMD) is directly involved in drugs binding, but is independent from ATP activity.[13]

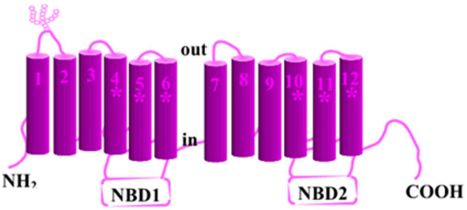


Figure I-6. Scheme of P-glycoprotein and its functional domain. Domains directly involved in this bond are starred.

In this Ph.D. thesis I focused on two modified analogs of natural tubulin inhibitors, with the same target but different action mechanism that are respectively hemiasterlin and epothilone B.

These compounds are the result of the drug discovery process starting from natural template, aimed at finding new drugs that elude multi-drug resistance phenomenon.

Chapter 2

Hemiasterlins

2. Microtubules depolymerization inducing agents

2.1. Hemiasterlins

Hemiasterlin (**7**), hemiasterlins A (**8**), B (**9**), and C (**10**) are members of a small family of cytotoxic tri-peptides that have been isolated from a South Africa sea sponge *Hemiasterella minor* (fig. II-1).

Structurally, hemiasterlins are characterized by the presence of tri- or tetramethylated tryptophan, *tert*-leucine, and N-methylvinylogous valine residues.

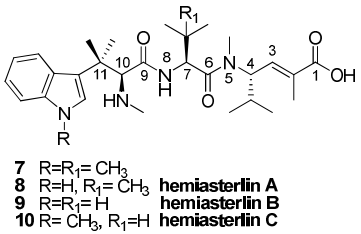


Figure II-1. Structures of hemiasterlins and relative numeration.

These natural substances show potent *in vitro* cytotoxicity against murine leukemia P388 and human breast, ovarian, colon, and lung cancer cell lines. Against human breast cancer MCF7 cells, compounds (**7**) and (**8**) are more cytotoxic and more potent mitotic blockers than vincristine, paclitaxel and nocodazole, while hemiasterlin C is the least potent derivative.[14]

The potency of hemiasterlin was also confirmed by the lowest IC_{50} values (about 2 pM) obtained in the human tumor cell lines OVCAR-3 and NCI-H460.

It was found that hemiasterlin strongly inhibited tubulin assembly, with activity comparable to dolastatin 10 (**11**) and cryptophycin 1 (**12**) (IC_{50}

values are 0.59, 0.98, and 1.1 μM for dolastatin 10, hemiasterlin, and cryptophycin 1, respectively).

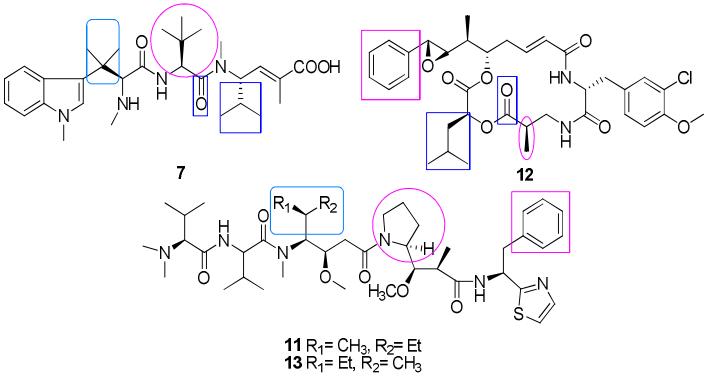


Figure II-2. Structure of hemiasterlin, cryptophicin 1, dolastatin. Boxed region represents areas of common overlap identified through docking studies.

Several experimental studies probing the binding of this class of mitotic inhibitors to tubulin have appeared in literature.[15]

However, the complexity and diversity of tubulin-ligand binding compounded with the lack of corroborating structural evidence have served as limitation to an understanding of the detailed molecular interaction present in the system under study.

Rai and Wolff have localized the binding region of the microtubule destabilizing agent vinblastine on β-subunit, while the three peptides, probably, bind in a site distinct from that at which the vinca alkaloids bind, since they all noncompetitively inhibit the binding of radiolabeled vinca alkaloids to tubulin.

In contrast, hemiasterlin as well as cryptophycin 1 and chiral isomer of dolastatin 10 (**13**) competitively inhibit each other for binding of [^{3}H]dolastatin 10 to tubulin (Table II-1).

Table II-1. Inhibition by hemiasterlin and other vinca domain drugs of the binding of [^{3}H]vinblastine and [^{3}H]dolastatin 10 to Tubulin[a]

Drug added	% inhibition of [^{3}H]vinblastine binding	% inhibition of [^{3}H]dolastatin 10 binding
Hemiasterlin	27	43
Dolastatin 10	44	
Cryptophicin 1	36	42
Vinblastine		2

[a] The 0.4 ml reaction mixtures contained 10 µM tubulin, 0.5% DMSO, the indicated potential inhibitor at 0.5 µM. Incubation was fro 30 min, and centrifugal gel filtrations of duplicated 0.19 ml aliquots were at room temperature. Avarages from two independent experiments are presented in the table. Stoichiometry of binding in the control reaction mixtures: 0.57 mol vinblastine and 0.59 mol of dolastatine 10 per mole of tubulin.

Dolastatin 10 is the most active, but phase I/II clinical trials revealed bone marrow toxicity, neuropathy together with a poor therapeutic index.

Despite their structurally diversity, these antimitotic agents bind at the same active site, that is adjacent to the exchangeable GTP site on β-tubulin and is composed primarily of residues Ser171, Lys174, Val175, Asp177, Asn204, Glu205, Tyr208, Asp209, Phe212, Pro220, and Tyr222.[16]

Each of these antimitotic agents destabilizes and depolymerizes microtubules, resulting in the formation of aberrant non-microtubules rings and oligomers, in this case hemiasterlin-tubulin rings are 45 nm in diameter, and both contain 14 tubulin dimers.

Thanks to molecular dynamics simulations and molecular docking studies it was possible to understand which functional groups of the hemiasterlin

were important for the interaction with the binding site, comparing the three hydrophobic peptides.

Starting from the N-terminus of the hemiasterlin, the Trp residue provided with the two methyl group could be overlaid with the aliphatic side chain, while the Val residue probably overlaps the pyrrolidine ring of the dolastatin 10 (fig.II-2).

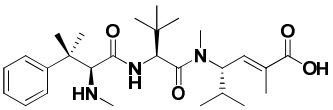
An overlap can be exist between the carbonyl of Val moieties and the aliphatic side chain of the Val, moreover Dil/Leu residues may show structural overlap. These results indicate that the Val and Ile/Leu residues may be the common elements forming the pharmacophore.[15]

2.2. From Hemiasterlin to HTI-286

Nieman and co-workers synthetized a number of analogues in order to define what portions of the structure were required for cytotoxicity and antimitotic activity and to prepare more potent analogs easier to synthesize.[17]

A great result of this study was the identification of a Synthetic Peptide Analogue 110 (SPA 110 or HTI-286, **14**), obtained changing indole of hemiasterlin with phenyl ring.

At the moment, HTI-286 is in clinical trial after demonstration of biological activity in preclinical cancer models.



14

Figure II-3. Structure of HTI-286.

The library of products was prepared considering that the geminal methyl couldn't be removed because they were thought to protect the tripeptide from proteolysis and/or to establish a preferred conformation that may be critical to biological activity.

Hemiasterlin has been the object of a series of single-point changes and corresponding new compounds have been tested for both antimitotic activity in MCF-7 cells expressing a dominant-negative mutant p-53 tumor suppressor gene and *in vitro* cytotoxicity.

It was found that there was a linear relation between antimitotic and cytotoxicity activity over a wide range of structural variations; this correlation suggest that, in these cells, cytotoxicity is solely due to inhibition of tubulin function at mitosis, and that hemiasterlins are pure antimitotic agents.

2.2.1. SAR study on natural hemiasterlin

C-10 trimethylammonium ion and the substitution of the isopropylic chain with a hydrogen atom caused a loss of activity, proving that the two functionality are crucial to metabolic stability and/or tubulin binding.

The same outcome had the hydrogenation of the double bond to give a mixture of epimers that reduce the potency by roughly an order of magnitude.

The aromatic function is essential at N-terminus as demonstrated by the loss of potency due to the replacement of the N-methylindole ring with a methyl or hydrogen atom to give respectively *tert*-LEU and Val-hemiasterlins.

Other modifications are well tolerated resulting in no significant loss in potency; these include the conversion of the B residue *tert*-Leu to Val,

formation of the methyl ester at and the replacement of the N-5 methyl substituent with a proton.

Only one change, the replacement of N-methylindole by aromatic ring made HTI-286 3-fold more potent than the natural product.

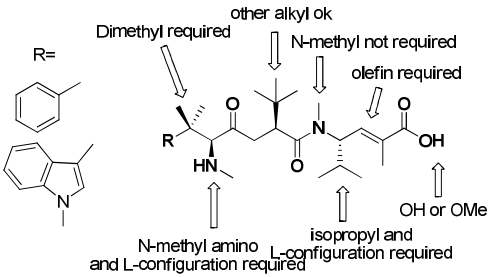


Figure II-4. Structural requirement for optimal cytotoxicity (IC_{50}< 1 nM) nd antimitotic activity (IC_{50} < 1 NM)

The result of this study has highlighted four regions of hemiasterlins where single structural changes are possible without seriously compromising antimitotic activity:

- replacing N-methylindole with phenyl and methyl groups
- replacing the *tert*-Leu residue with Valine
- removing the N-5 methyl substituent
- making the C-terminal residue methyl ester

C-11 methyl substituents, the C-10 methylamino group, and the C-4 isopropyl groups are extremely important structural elements for potent antimitotic activity.[18]

With HTI-286 in hand, Rush and co-workers from Wyeth have undertaken a docking study to better understand the interaction of HTI-286 with the

tubulin and according their predictions, there are several important factors contributing to the HTI-286/tubulin interaction.

These include different hydrophobic interactions, hydrogen bonding, and electrostatic complementarity.

It is hypothesized the U-shaped curvature of the backbone of HTI-286, in particular the tertiary butyl group is found to occupy a spacious cavity in β-tubulin, proximal to the location of the guanosine 5'-diphosphate (GDP) (fig II.5).

The binding model finds the C-1 position of HTI-286 to be oriented in a region of β-tubulin that is flanked by two ASN residues (residues 186 and 101 of the β-tubulin subunit) providing hydrogen bonding opportunities with the carbonyl or hydroxyl group of the ligand.

The heteroatoms of the amide backbone of the HTI-286 are seen forming β-sheet-like interactions with the backbone atoms of nearby amino acids residues.

The interaction between the basic NH group of HTI-286 and the Asp179 residue of the β-tubulin subunit are quite significant in fact deleting them, the enthalpic and desolvatation contributions to binding might be perturbated.

The binding model indicates an association between Ser 174 and Ser 178 of the β-tubulin and the C-9, C-6 and N-8 position of the HTI-286 scaffold (Fig. II-4).[19]

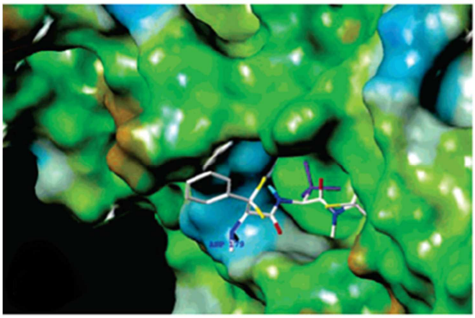


Figure II-5. Surface view of HTI-286 docked to the *β*-tubulin portion of the interdimer interface, emphasizing the binding pocket. The α-tubulin subunit is not shown for clarity. The tubulin surface is colored by lipophilic potential (brown, hydrophobic; green, neutral; and blue, hydrophilic). Hydrogens are removed for simplicity. The yellow-colored carbon atoms are poised for intramolecular interactions, and the *tert*-butyl carbons are colored purple.

Chapter 3

Scopes and aims

3. Scopes and aims

3.1. Development of synthetic routes for the synthesis of A-fragment

In literature there are different total synthesis of hemisterlin, but the first one was completed by Andersen and co-workers in 1997 and it is the result of a sequence of coupling reactions between three modified amino acids A, B and C. Every amino acid apart the commercially available N-Boc-*tert*-leucine is synthesized singularly.

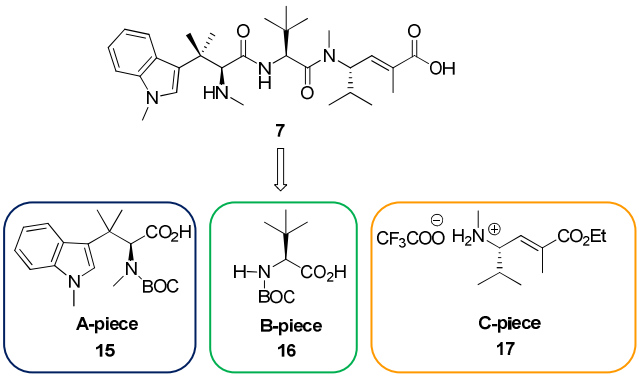
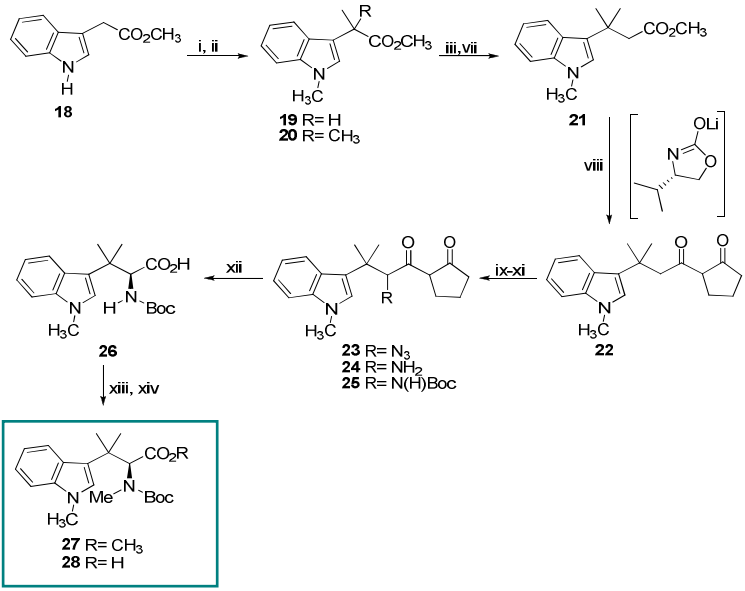


Figure III-1. Retrosynthetic analysis of hemiasterlin.

Structural assignment based on NMR and degradation studies are supported by X-ray diffraction analysis of hemiasterlin methyl ester, and it was found that the members of this family are known to have L-configuration (Fig. III-1).

The hardest step is the synthesis of A-piece (**15**) because of the enantiocontrolled installment of a chiral amine group.

The first approach was based on Evans' oxazolidinone chemistry employing the electrophylic nitrogen source triisopropylphenylsulfonyl azide. The reduction of azide (**23**), followed by Boc-protection of amine (**24**) gave the desired functionalized tryptophan (**28**) in a good enantiomeric excess (> 98%).[14]



Scheme III-2. Reagents and conditions: (**i**): $KN(SiCH_3)_2$, THF, -78°C to 0°C, 3 h; CH_3I, -78°C to 0°C, 2 h; (**ii**): $KN(SiCH_3)_2$ (1.5 eq), then as in (**i**); (**iii**): i-Bu_2AlH, Et_2O; (**iv**): TPAP, NMO, CH_2Cl_2, 4Å sieves; (**v**): Ph_3P=CHOMe, THF, r.t.; (**vi**): p-TsOH, H_2O, dioxane, 60° C, 16 h; (**vii**): $NaClO_2$, NaH_2PO_4, 2-methylbut-2-ene, *t*-BuOH, H_2O, 0°C; (**viii**):CH_3CCOCl, TEA, THF, -78°C; (**ix**): $KN(SiMe_3)_2$, THF, -78°C; 2,4,6-triisopropylbenzenesulfonyl azide, THF, -78°C, 1 min; AcOH, 30-40°C, 1h; (**x**): $SnCl_2$, dioxane, H_2O, r.t., 36 h; (**xi**): $(CH_3CO_2C)_2O$, dioxane, H_2O, r.t., 16 h; (**xii**): LiOOH, THF, H_2O, r.t., 16 h; citric acid, H_2O; (**xiii**): NaH, DMF, CH_3I, r.t., 16 h; (**xiv**): LiOH, MeOH, H_2O, 60°C, 24 h; citric acid, H_2O.

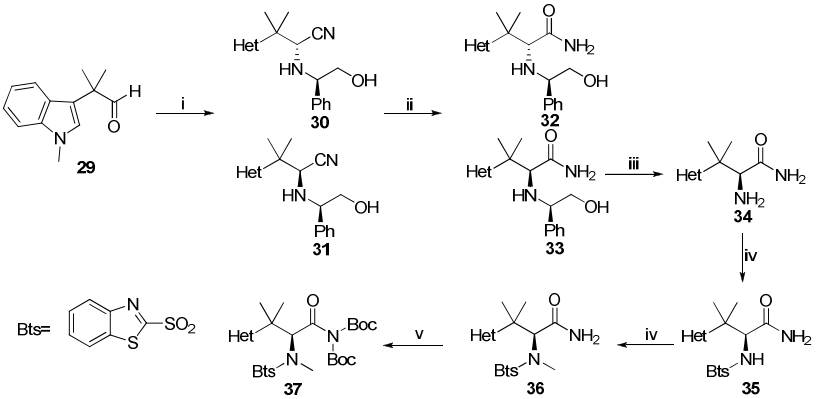
The reported synthesis of the tryptophan moiety starting from methyl ester of 3-indoylacetic acid (**18**) was 16 steps long and suffered from low yields of coupling reactions.

Several research groups tried to find a successful convergent approach that could give analogues more readily.

This would open the possibility of generating not only significant quantities of hemiasterlin itself but also analogues for screening and further biological assays.

3.1.1. Vedej's synthetic approach to fragment A.

An improved enantiocontrolled route to the tetramethyltryptophan subunit (**36**) was developed by Vedejs' group using an asymmetric Strecker synthesis (5 steps, 50% yield from **29**).



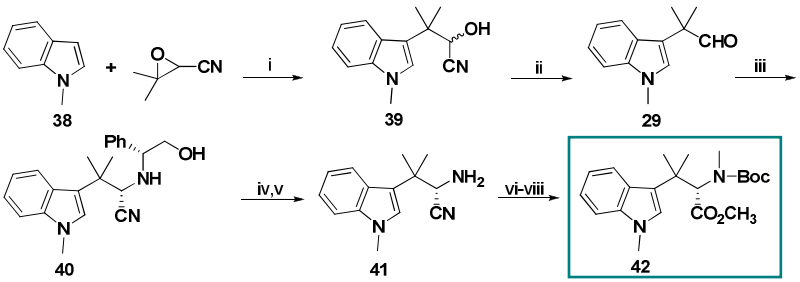
Scheme III-3. Reagents and conditions: (**i**) $Sc(OTf)_3$, (*R*)-2-phenylglycinol, CH_2Cl_2, r.t.; Bu_3SnCN, 0°C to r.t.; (**ii**) H_2O_2, K_2CO_3, DMSO, MeOH, 45°C; (**iii**) H_2, $Pd(OH)_2/C$, MeOH, r.t.; (**iv**) BtsCl, Na_2CO_3, CH_2Cl_2-H_2O, 0°C to r.t.; (**v**) CH_3I, DMF, 35°C; (**vi**) BOC_2, DMAP,CH_3CN, r.t.

This method exploits the high reactivity of a Bts-protected amino acid chloride in the difficult peptide coupling of sterically hindered amino acids residue like BC dipeptide.[20]

3.1.2. Durst's synthetic approach to fragment A.

The same principle was followed by Durst's group to get A-piece starting from N-methylindole (**38**), that was reacted with tin tetrachloride mediated ring opening cyanoepoxide.

The treating of the cyanohydrin (**39**) with NaOH gave the corresponding aldehyde (**29**) that was used in asymmetric Strecker methodology. This sequence led to the formation of an 85:15 diastereomeric mixture of α-cyano amines (**41**) direct precursor of Andersen intermediate (**42**).[21]



Scheme III-4. Reagents and conditions: (**i**) $SnCl_4$, CH_2Cl_2, -78 °C, 70%; (**ii**) NaOH, EtOH 95%; (**iii**) (*R*)-phenylglycinol, CH_2Cl_2, TMSCN; (**iv**) $Pb(OAc)_4$, $MeOH/CH_2Cl_2$; (**v**) 3 N HCl, Et_2O, 55% over two steps; (**vi**) concentrated HCl reflux; (**vii**) $(Boc)_2O$, Na_2CO_3, THF/H_2O; (**viii**) NaH, MeI, DMF, 65% over three steps.

A-piece, whatever its origin, was coupled with the segment BC though amide bond formation.

To date, the first proposed synthetic route was extensively followed for SAR studies, but it is resulted poorly versatile, because every structurally different A amino acid has to prepared on purpose.

Moreover, this strategy is hardly scalable to obtain considerable amount of product.

3.2. Toward the synthesis new analogues of HTI-286

The aim of my Ph.D. project is the possibility to easily access to a series of synthetic analogues of HTI-286, opportunely modified at N-terminus.

Molecular modeling studies, not reported in this context, have highlighted the ability of the binding site on the tubulin to host bulky derivatives at N-terminus.

For this reason, we considered the use of a common precursor from which a series of derivatives with modified A-portion could be obtained with one or very few steps.

Following previous works in the field, we have exploited the synthetic potentiality of 2-bromoacyl-peptides as versatile synthetic intermediates in reactions in which the pool of natural amino acids is used as starting material.

The modifications were directed mainly N-terminus of hemiastelin structure, but also the C-terminus, was involved in SAR studies.

At first we thought to invert the α,α-dimethylbenzyl group with the α-N-methyl group in order to get derivatives of the alanine (**43**) that was incorporated in the backbone of the hemiasterlin thanks to two following coupling reactions.[22]

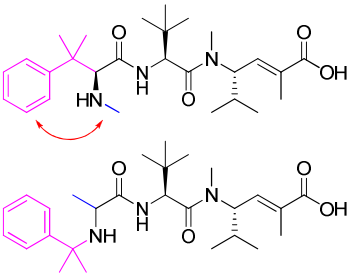


Figure III-2. Inversion of methyl group with dimethylbenzyl radical at N-terminus.

From one side, a key role was played by silver oxide as promoter of substitution reactions with controlled chemistry, employed to introduce particular functional groups at N-terminus.[23]

Then, in order to further explore the size of the binding site, bulkier groups were introduced at N-terminus, employing bromoacyl peptides (**44**) derived from phenylalanine instead of alanine as starting materials.

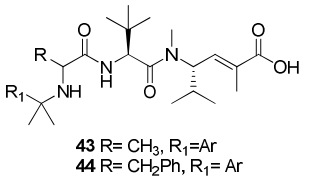


Figure III-3. General structure of the synthesized products related to HTI-286.

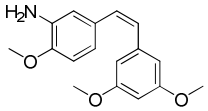
Besides that, we also tried to obtain the direct analogue of natural hemiasterlin, bearing 2-(1-methyl-1*H*-indol-3-yl)propan-2-amine at C-10 of the tripeptidic backbone as derivative of alanine, but so far we haven't obtained it.

New anticancer therapies are based on synergic interaction between drugs that have the same action mechanism and pharmacologic effect.

Thus, we have thought to take advantage of this principle, planning to prepare a conjugate between the most active candidate and stilbene 5c, known to inhibit tubulin assembly (IC_{50} values from 2 to 8 nM) by binding to a site different from hemiasterlin, thanks to a linkage easily hydrolysable *in situ*. The idea of a pro-drug came up also from the possible improvement of the water-solubility of stilbene 5c.

By definition two or more agents or substances act in synergy if they produce an effect greater than the sum of their individual effects.[24,25]

In this context, this CA-4 analog was termed exactly as in a paper published by prof. Simoni and al.[24,25]



stilbene 5c

Chapter 4

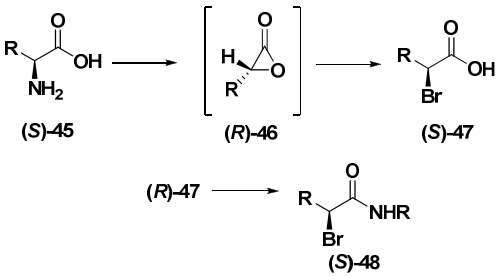
Results and discussion

4. Results and discussion

4.1. Potentiality of silver oxide in nucleophile substitution reaction

The research group, in which I carried out this Ph.D. project, has experience in the knowledge of the silver oxide chemistry as promoter of nucleophile substitution on substrates as peptides, depsi-peptides and pseudo-peptides.[26] These applications are indicative of the great versatility of 2-bromoamides, and of the potential involvement of natural aminoacids.

Thus, the novelty of our synthetic approach is based on the consideration that *(S)*-*α*-amino-acid (**45**) can be diazotization and halogenated to give the corresponding *(S)*-*α*-bromo-acid (**47**) in turn derivatized to *(S)*-α-bromoacylpeptide or *(S)*-α-bromoacylamide (**48**) depending on the nature of R, maintaining the same configuration of the starting material.[27]



Scheme IV-1. Stereospecific synthesis of *(S)*-α-bromoacylpeptide from *(S)*-α-aminoacid

This is the result of a double inversion of configuration in the sequence *(S)*-**45** → *(R)*-**46** → *(S)*-**48** in which the unstable lactone is involved.

In literature there are some examples that highlight the bromoacylamide (**48**) reactivity towards the substitution of the bromine with a nucleophile, according to different factors:

- nature of the nucleophile
- solvent characteristics
- presence of a silver promoter
- nature of alkylic group in α position

Thus, the reaction of *(S)*-**48** with an amine gives substitution compounds, whose N-terminus bears the side chain of *(S)*-**45**.

In absence or in presence of a soft Lewis acid Ag^+, starting from *(S)*-bromoacylpeptide **49** the product with inverted configuration is obtained *(R)*-**51**.

This reaction is quite slow, it takes hours to go to an end, and is performed with 1°, 2° amines and enolate that are good nucleophiles.

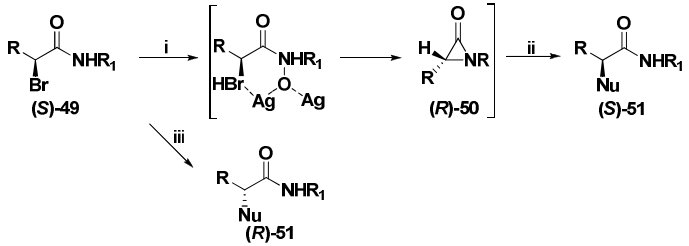
The combination of Ag^+/hindered amine induced the amine to behave both as base and as nucleophile, thanks to a shift from a mechanism of electrophilic assistance to an alternative one, where Ag^+ is responsible of an acidity-enhancement mechanism.

The addition of covalent silver oxide increases the rate of the reaction, in fact it was observed that with whatever amine, the product has the same configuration *(S)*-**51** of the parent 2-bromoamide **49** in high *ee* was obtained.

Stereoselectivity depends on a favorable ratio between the rates of two competitive mechanisms that can operate.

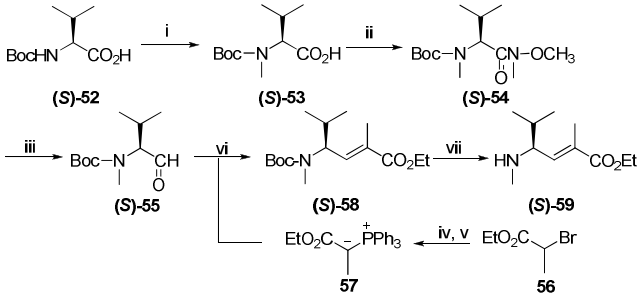
By using an insufficient amount of Ag_2O, in fact, the optical activity of the produced aminoamide decreas, because the rate of promoted and unpromoted reaction approach to each other.

Interaction between Ag_2O and the solution-species would make the mechanism a complex one characterized by a labile aziridinone (α-lactam, *(R)*-**50**).



Scheme IV-2. Reagents and conditions: (**i**) Ag_2O; (**ii**) Nu:; (**iii**) Nu:, Ag^+ $CF_3SO_3^-$

4.2. Synthesis of the fragment BC



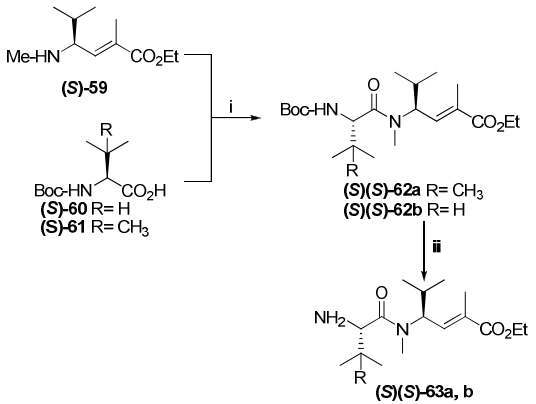
Scheme IV-3. Reagents and conditions: (**i**) CH_3I, NaH 60% min. oil, THF; (**ii**) $CH_3NHOCH_3 \cdot HCl$, 1-HOBT, WSC, NMM; (**iii**) $LiAlH_4$, THF; (**iv**) PPh_3, toluene; (**v**) NaOH; (**vi**) **57**, CH_2Cl_2; (**vii**) TFA, $CHCl_3$

The schemes IV-3 and IV-4, show the synthesis of the intermediates *(S)(S)*-**63a**, **b** as exactly reported in literature for HTI-286.[28]

Boc-N-methyl-(*S*)-valinale **52** obtained from reduction with $LiAlH_4$ of the Weinreb amide *(S)*-**54**, was reacted with a stabilized Wittig reagent (**57**), previously prepared from 2-bromoester (**56**) to get γ-aminoester α,β-unsaturated (scheme 6).

The olefination was performed in CH_2Cl_2 affording stereoselectively the *E*-2-alkenoate (**59**) (scheme IV-3).

After deprotection under acidic conditions, the intermediate *(S)*-**59** was coupled with *(S)*-Boc-Valine **60** or *(S)*-Boc-*Tert*-Leucine **61** employing TMAC to activate the amino acid (scheme IV-4).

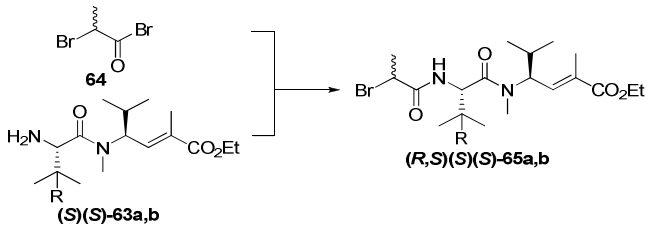


Scheme IV-4. Reagents and conditions: (**i**) TMAC, DIPEA, THF, -78°C, 2h; (**ii**) TFA, $CHCl_3$, r.t.

4.3. Synthesis of racemic mixtures of bromoderivatives

From this known intermediates, we started our own project by reacting *(S)(S)*-**63a,b** with bromide of *(R, S)*-2-bromo-propionic acid **64** obtaining 2-

bromoacyl-peptides *(R,S)(S)(S)*-**65a,b** as diasteromeric mixtures (scheme IV-5).



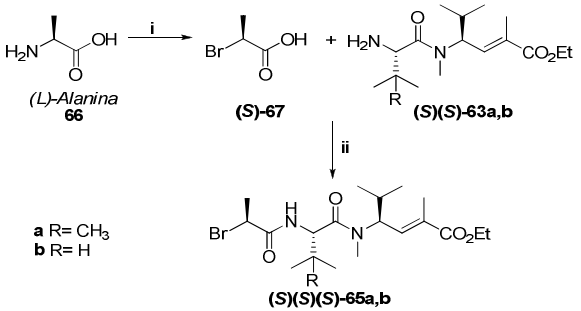
Scheme IV-5. Reagents and conditions: (**i**) TEA, DCM, 0°C, to r.t. overnight.

At first, the products were synthesized as mixture of diastereoisomers, in order to achieve easily both compounds, whose activity might be quickly tested and compared to each other. The most promising compounds were then prepared as single diasteroisomers in order to establish the identity of which one was responsible of the activity.

In this perspective, silver oxide was employed as coupling agent granting good yields and the correct stereochemistry of the product.

In this case, bromo-derivatives *(S)(S)(S)*-**65a**,**b** are the result of the reaction between *(S)(S)*-**63a**,**b** with *(S)*-2-propanoic acid **67**, in turn obtained by diazotization-bromination of amino acid (L)-alanine **66**.

Similarly, the *(R)(S)(S)*-**65a**,**b** series display both valine and *tert*-leucine were prepared using (D)-alanine as starting material (scheme IV-6).[27]



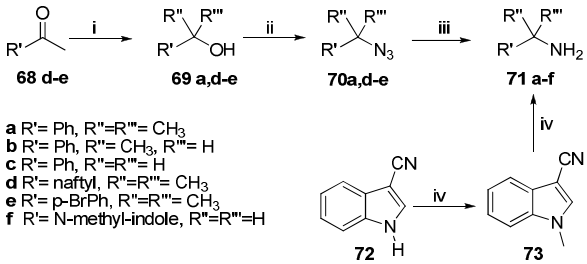
Scheme IV-6. Reagents and conditions: (**i**) $NaNO_2/H^+$, KBr; (**ii**) TMAC, DIPEA, THF, -78°C

4.4. Nucleophilic substitution reaction catalyzed by silver oxide

The prepared bromoacyl intermediates **65a**,**b** were subsequently reacted with different nucleophyles, mainly amines. Some amine were not commercial and were synthesized.

Indeed, most of amines **71a,d-e** were prepared treating tertiary alcohol with sodium azide and TFA at 0°C, followed by catalytic hydrogenation with palladium on charcoal, while non commercially available alcohols **69d-e** were obtained adding the correspondent ketone **68d-e** on to freshly prepared Grignard's reagent.[29]

With the aim to understand the importance of the two methyl groups R" and R'", also amines as **71b**,**c**, and N-methyl-indol-3-yl-methanamine **71f**, yielded by reduction of the corresponding nitrile **73**, were employed (scheme IV-7).



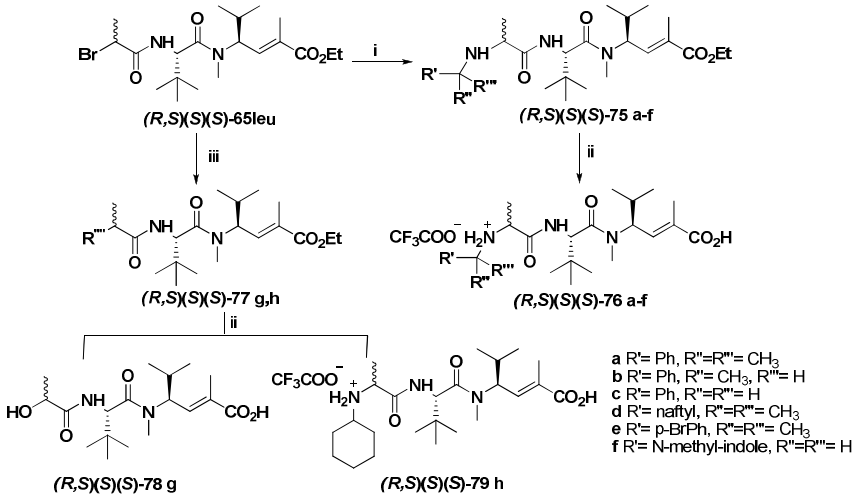
Scheme IV-7. Reagents and conditions: (**i**) CH_3I, Mg, Et_2O; (**ii**) NaN_3, TFA, $CHCl_3$; (**iii**) H_2, Pd/C; (**iv**) H_2, Pd/C, EtOAc

In the same perspective, cyclohexylamine was used even though its structure is different from what is represented in the general scheme IV-7.

4.4.1. Synthesis of *tert*-Leucine derivatives

As regarding the reactivity, only the reaction with benzylamine **71c** could run without Ag_2O, while the other amines have required this promoter as they were quite bulky.

Also water was used as nucleophile even though hydroxy derivatives *(R,S)(S)(S)*-**78g** and *(R,S)(S)(S)*-**83g** were reaction by-products more or less present if the environment of the reaction was not strictly dry (scheme IV-8).[22]



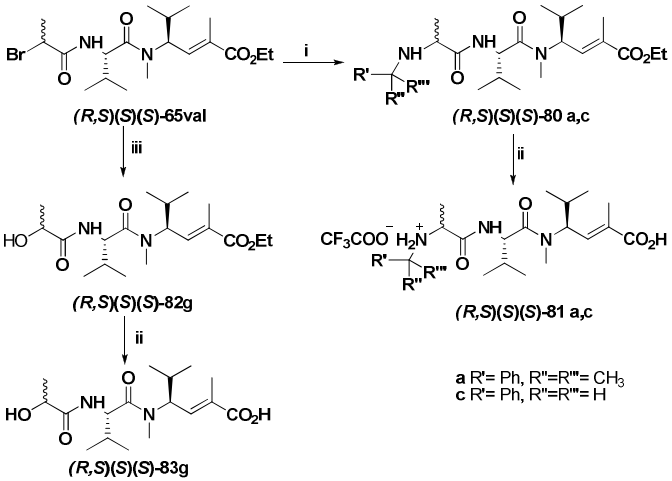
Scheme IV-8. Reagents and conditions: (**i**) Ag_2O, 70a-f, toluene, reflux; (**iii**) Ag_2O, Nu: (**g**: H_2O or **h**:Cyclohexylamine), toluene; (**ii**) LiOH/MeOH/water, than TFA.

4.4.2. Synthesis of Valine derivatives

The following scheme shows the synthesis of some valine derivatives *(R,S)(S)(S)*-**81a**,**c** in which only the amine **71a**,**c** were used (scheme IV-9).

The mixtures *(R,S)(S)(S)*-**75a**,**c**, *(R,S)(S)(S)*-**76a**,**c** and *(R,S)(S)(S)*-**80a**,**c**, *(R,S)(S)(S)*-**81a**,**c** weren't separable either by flash chromatography or HPLC, therefore the single diasteroisomers were stereoselectively synthetized starting from both *(R)* and *(S)(S)(S)*-**65a**,**b** thanks to the potentiality of Ag_2O.

The *de* of the products is 95% and was measured by HPLC, ^{1}H NMR and comparing the results with those of diasteromeric mixtures previously obtained.

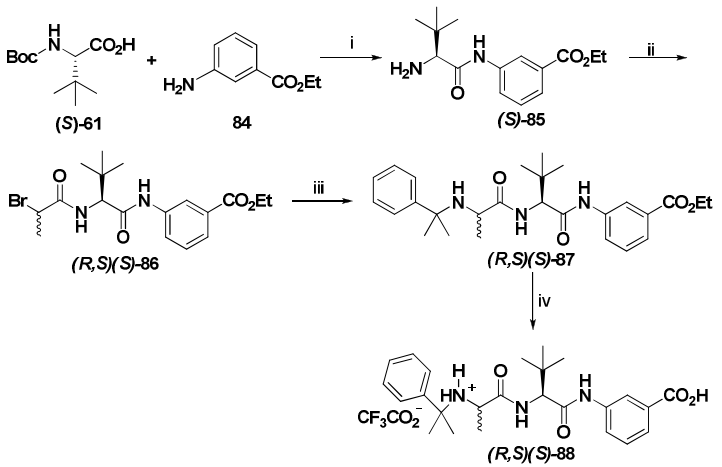


Scheme IV-9. Reagents and conditions: (**i**) Ag_2O, 71a,c, toluene, reflux; (**iii**) Ag_2O, H_2O, toluene; (**ii**) LiOH/MeOH/water, than TFA.

Apart the hydroxy-derivatives *(R,S)(S)(S)*-**78g**, **83g**, the final products *(R,S)(S)(S)*-**76a-f**, *(R,S)(S)(S)*-**79h**, *(R,S)(S)(S)*-**81a,c** were all tested as trifluoroacetate salts, after basic hydrolysis of ethyl ester and HPLC purification.

Moreover, we wanted expand our investigation on the versatility of synthetic procedure, together with the need of further SAR studies on a series of products modified at carboxyl-end position.

It was, therefore, easily synthetized a compound *(R,S)(S)*-**88** whose fragment C was a rigid aromatic derivative of γ-aminoester α,β-unsaturated *(S)(S)*-**63b**, condensing Boc-*(L)-tert*-leucine *(S)*-**61** and 3-aminobenzoic ethyl ester **84**, in order to investigate if a modification at C-terminus might afford a series of products modified at this position (scheme IV-10).



Scheme IV-10. Reagents and conditions: (**i**) (1). DIPEA, TMAC, THF, -78°C, (2) TFA; (**ii**) DIPEA, TMAC, (*R*,*S*)-2-bromo-propionic acid (**iii**) Ag_2O, toluene, reflux; (**iv**) LiOH, MeOH, H_2O, then TFA.

4.4.3. Synthesis of Phenylalanine derivatives at N-terminus

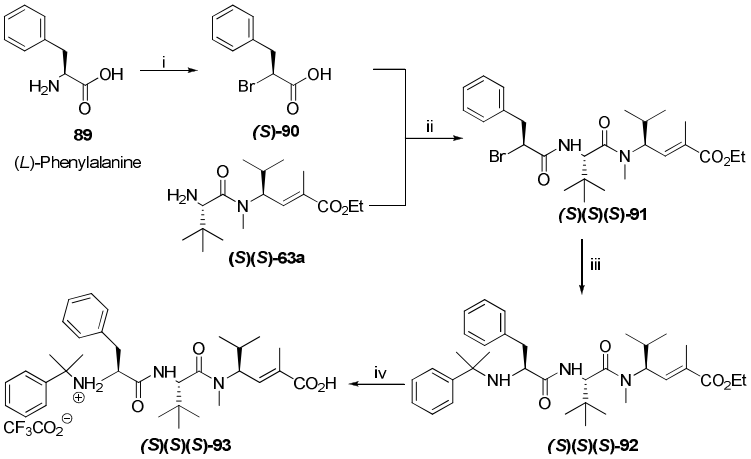
Computational studies suggested that the portion of the binding site that host the aromatic ring of HTI-286 was enough large to accommodate bulkier groups.

Moreover the most interesting derivatives were those bearing the dimethylbenzyl-amino group.

Thus, a bivalent ligand was prepared, including a further benzylic group at N-terminus.

The two diasteroisomers were synthetized separately following the same synthetic route shown in the scheme 12, but in place of alanine, (D) and (L)-phenylalanine **89** were used, as starting material for the corresponding bromo-acids *(S)*-**90** and *(R)*-**90** (scheme IV-11).

In the scheme is represented only the synthesis of diasteroisomer *(S)(S)(S)*-**93**, but also the other *(R)(S)(S)*-**93** were obtained.



Scheme IV-11. Reagents and conditions: (**i**) $NaNO_2/H^+$, KBr; (**ii**) TMAC, DIPEA, THF; (**iii**) Ag_2O, 71a, toluene, reflux; (**iv**) LiOH, H_2O, MeOH, then TFA.

Chapter 5

Biological evaluation

5. Biological evaluation

Small groups of compounds structurally different to each other were synthetized, tested and the biologic results have led us in our SAR study.[22]

The first synthetized compounds as racemic mixtures, bearing phenyl group at N-terminus apart series **g** that are hydroxyl-derivative, *(R,S)(S)(S)*-**76a,c**, *(R,S)(S)(S)*-**81a,c**, *(R,S)(S)(S)*-**78g**, **83g** were tested on lung cancer cells A549.

While *(R,S)(S)(S)*-**81c**, *(R,S)(S)(S)*-**78g** and *(R,S)(S)(S)*-**83g** were inactive at the highest concentration used (3 μM), *(R,S)(S)(S)*-**81a** at the same concentration has shown weak activity.

On the other hand, *tert*-leucine derivatives *(R,S)(S)(S)*-**76a** and *(R,S)(S)(S)*-**81a** have a very interesting biologic profile, as they inhibit tumor cell growth at nanomolar level.

It was also demonstrate that such concentrations are necessary to completely disrupt microtubules network and to arrest cell cycle at G_2/M phase.

On purpose, immunofluorescent staining of A549 cells treated with *(R,S)(S)(S)*-**76a** and *(R,S)(S)(S)*-**81a** was performed and it was found that when these cells were treated with 100 nM of *(R,S)(S)(S)*-**83g** (inactive compound), there are no difference from the control cells with fully intactive microtubule network and normal mitotic spindles.

Cells treated with *(R,S)(S)(S)*-**76a** were blocked at mitotic phase as evidence of condensed chromosomes in DAPI (4'-6'-diammidine-2-phenylindole) staining. The same effect was due to cells treatment with *(R,S)(S)(S)*-**81a** that exhibited a different pattern, in fact the microtubule network is not fully disrupted in the non-mitotic cells that are present occasionally.

In the mitotic cells identified by DAPI staining, the mitotic spindle shows a radial pattern characteristic for monastral spindle.

Because the structures of *(R,S)(S)(S)*-**76a** and *(R,S)(S)(S)*-**81a** are very similar except an additional methyl group in the B-piece, we thought the mechanism of these two compounds should be similar, and the different pattern seen in the immunofluorescent staining might be due to the potency.

The two most active compounds were tested on several cellular panels after 48 hours incubation and it was observed IC_{50} values around 10 nM for both of them, but the ability of tumor cells growth inhibition of *(R,S)(S)(S)*-**76a** is higher than *(R,S)(S)(S)*-**81a**.

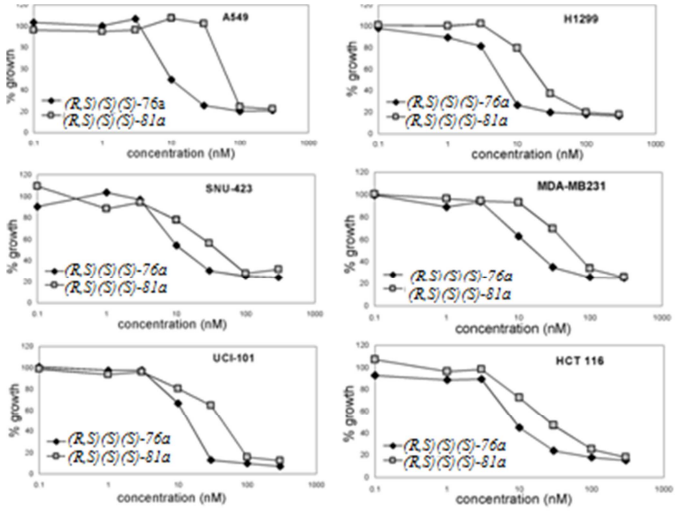


Figure V-1. Effect on cell growth of A-549 and H-1299 (cancer lung cells), SNU-423 (hepatocellular cancer cells), MDA-MB (breast cancer cells), UCI-101 (ovarian cancer cells), HCT-116 (colon cancer cells) of compounds *(R,S)(S)(S)*-**76a** and *(R,S)(S)(S)*-**81a** after 48 hours incubation.

As *(R,S)(S)(S)*-**76a** and *(R,S)(S)(S)*-**81a** have proved to have the best pharmacologic profile, they were synthetized as single diastereoisomers and the cytotoxicty was examined in UCI-101 human ovarian cancer cells.

Their activity is represented in figure V-2 together with racemic mixtures of new derivatives *(R,S)(S)*-**88**, *(R,S)(S)(S)*-**76d** and *(R,S)(S)(S)*-**79h**.

The two most active compounds are *(R)(S)(S)*-**76a**, **81a** with IC_{50} of 20 nM, whereas *(R,S)(S)(S)*-**79h** and *(R,S)(S)*-**88** have no cytotoxic activity even at 1 µM. Other two compounds *(S)(S)(S)*-**76a** and *(R,S)(S)(S)*-**76d** have IC_{50} at 200 nM. The fact that *(R)(S)(S)*-**76a** and **81a** are more potent than corresponding stereoisomers *(S)(S)(S)*-**76a** and **81a** highlights the essential role of the *(R)* configuration at N-terminus. This is not in line with taltobulin derivatives stereochemistry, in which *(S)(S)(S)* configurations are reported to have potent activity.

However, the bulky dimethyl benzyl group in the most active diastereomers occupies the same place in both series. It seems, therefore, that the correct placement of dimethyl benzyl group is more important than the secondary amine to give functional interaction with binding site.

The poor activity found with the aromatic 2-naphthyl-2-propyl group of **76d**, suggests the presence of a large pocket that could be occupied by an aromatic group, similarly to the indole ring in natural parent compound hemiasterlin.

Lack of the aryl portion also seems to be incompatible with growth inhibition, which is consistent with the results described in taltobulin series.

Both other non aromatic substituents at nitrogen of fragment A as cyclohexyl and the rigid modified at fragment C analogue *(R,S)(S)*-**88** led to loss of activity.

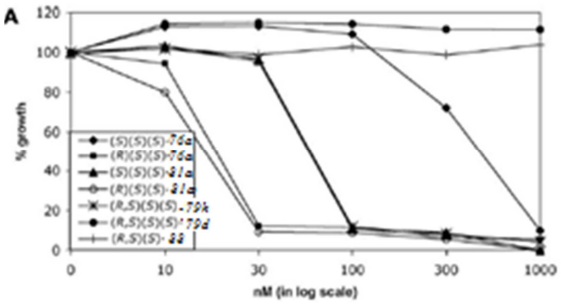


Figure V-2. (A) UCI-101 cells were treated with various concentrations of compounds for two days. The tumor growth suppression was determined by Alamar blue staining and plotted against concentrations of the treated drugs. Each concentrations was repeated in triplicates.

The lack of activity of *(R,S)(S)(S)*-**79 h** was unexpected as molecular modeling studies suggested that the pocket was enough large to host bulky groups. This aspect led us to synthetized other compounds aromatic like indole *(R,S)(S)(S)*-**76f** derivative just to confirm the data previously obtained.

It was found to be completely inactive in E2S cell viability test after 48 hours incubation.

Two diastereoisomers bearing p-bromine on the aromatic ring of dimethylbenzylamine were tested as they added and modified steric bulkiness at N-terminus.

They were found inactive with IC_{50} of 600 nM for *(R)(S)(S)*-**76e** and 100 nM for *(S)(S)(S)*-**76e** in a MTT cell vitality assay on MCF-7 breast cancer cells, and in this case there is a discrepancy referring the data collected so far, since diasteroisomer *(R)(S)(S)* was expected to be more active than the other one.

The same biologic assay was applied to test compounds with only one of the geminal methyl groups. With *(S)*-1-phenylethamine, a further

asymmetry centre was introduced at the most investigated region of the tripeptide that was tested as racemic mixture *(R)(R,S)(S)(S)*-**76b,** but it was found to be inactive showing IC_{50} >100 nM.

Phenylalanine was employed as source of bromoacyl intermediates for other derivatives were examined on MCF-7 with the aim to understand if the addition of a further bulky benzylic moiety in place of the simple methyl group of the alanine at N-terminus might increase the activity.

Thus, a couple of diastereoisomers *(S)(S)(S)*-**93** and *(R)(S)(S)*-**93** was synthetized and their respective activity compared with *(R,S)(S)(S)*-**76a**; unfortunately, the preliminary biologic data were not encouraging showing IC_{50} of 367 nM for *(R)(S)(S)*-**93** and 240 nM for the other one in E2S cell viability test (figure V-3). Interestingly, in this case the activity of diasteroisomers showed that *(S)* is more active than *(R)*, as for the couple bearing bromine, but with less difference.

In light of the results data, it seems that the presence at N-terminus, of bulkier aromatic groups decreases the activity, in fact *(R,S)(S)(S)*-**76f** and *(R,S)(S)(S)*-**76d** are inactive.

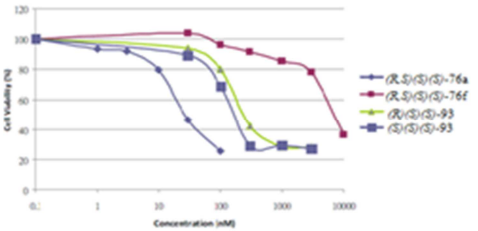


Figure V-3. Cytotoxicity in ES-2 cells (duplicates, IC_{50} was calculated using Sigma plot).

Chapter 6

Conclusions and perspectives

6. Conclusions and perspectives

This Ph.D. thesis reports the synthesis of a small numbers of compounds inhibitors of tubulin polymerization, involved in cells replication.

These product are isosters of patented tripeptide, HTI-286, and have been obtained with a modified synthetic route developed in our research group regarding mainly the N-terminus end.

Generally, the modification that we have done, are known in literature, but some of them have put forward again, with the aim to obtain compounds possibly more active than the parental, considering our modification at N-terminus.

Our synthetic approach has been studied to be more versatile thanks to the possibility to access to a variety of final products starting from a common building block, the bromoacylderivative characterized by a desidered stereochemistry.

The substitution of the bromine with a nucleophile, generally an amine, catalyzed by silver oxide, is the key step as it allowed us to obtain the derivative both as racemic mixture, both as single diasteroisomers.

Several compounds were examined in different biologic tests and IC_{50} values compared to establish some structural-activity relationships among them.

Our biological result are in line with the SAR data both of Nieman' s group and Zask's laboratory.[17,18]

- Compounds in which the B-piece is a valine aminoacid are less active than the corresponding with the *tert*-leucine.

- Both in Valine and in *tert*-leucine series, the presence of the geminal dimethyl group at A-fragment is essential for the activity, as demonstrate literature information.
- Among derivatives with the aromatic ring at N-terminus, the single diasteroisomers *(R)(S)(S)* are more active than the other series with *(S)(S)(S)*stereochemistry, that is also the natural stereochemistry.

  Inverting the groups bound on nitrogen and at C-α, only the first series might probably expose the radical in a correct spatial conformation, similar to that of HTI-286.

  In the parental compounds, Zask's group has demonstrate the stereochemistry *(S)(S)(S)* is critical for the activity of HTI-286 derivatives, in fact *(S)(R)(S)* and *(S)(S)(R)* isomers were completely inactive in inhibition polymerization tubulin assay.
- Bulkier groups were introduce on the nitrogen at N-terminus as suggested by molecular modeling informations.

  We expected more potent compounds, but unfortunately they revealed a weak activity.

  We have supposed, on the basis of the results, that the activity could be better related to the two methyl radicals near the bulky groups.
- Modifications at C-terminus that conferred rigidity to the backbone of the tripeptide were not tolerated.
- The most active compound is the *(R)(S)(S)*-**76a** with IC_{50} 20 nM, value of the same order of magnitude of the referential compound, HTI-286.

6.1. Toward the pro-drug with stilbene 5c

On the basis of the reported results, a further progress in this project regarding antitubulin compounds in our research group, has been envisaged in the possible development of a pro-drug linking *(R)(S)(S)*-**76a** and stilbene 5c, whose activity is 10 nM in some solid tumor.

This compound suffers from low water solubility, and in the past our aim was to increase its *in vivo* biologic profile, by a linkage with a water soluble carrier.

As first information, we needed to know of any possible synergic activity between stilbene 5c and the most active hemiasterlin derivative.[29]

6.1.1. Synergistic effect between *(R,S)(S)(S)*-76a, *(R,S)(S)(S)*-81a and stilbene 5c.

When the structure model of tubulin bound with colchicine and vinblastine was solved, the mechanism of interaction and how colchicine and vinblastine affect tubulin polymerization were unveiled. The tubulin-colchicine complex was kept in a curve conformation and unable to change into a straight conformation for polymerization, so that tubulin cannot be assembled.

The formation of a curve conformation in tubulin with colchicine site inhibitors also enhances the binding of vincristine by stabilization of the vincristine binding site in tubulin.

This structure information thus suggests a synergistic effect between colchicine site inhibitors and vincristine site inhibitors.

Since stilbene 5c is a colchicine-site tubulin inhibitor and *(R,S)(S)(S)*-**76a** and *(R,S)(S)(S)*-**81a** are inhibitors with non-colchicine related mechanism, synergistic effect between the two compounds and stilbene 5c was tested.

At first, it was carried out *in vitro* tubulin polymerization study by incubation of 1 μM stilbene 5c and *(R,S)(S)(S)*-**76a** individually and in combination. This concentration is barely effective in suppressing *in vitro* tubulin polymerization as shown in Figure VI-1.

However, the combination of both stilbene 5c and *(R,S)(S)(S)*-**76a** shows a much more robust inhibition of in vitro tubulin polymerization, suggesting a synergistic effect between them.

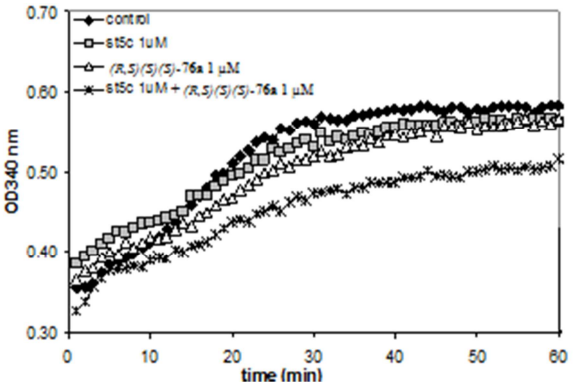


Figure VI-1. In vitro tubulin polymerization study. Purified tubulin was incubated in a 96-well plate with polymerization buffer and 1 μM stilbene 5c, *(R,S)(S)(S)*-**76a** individually or in combination. OD340 was measured every min for 30 min in a plate reader at 37°C.

6.1.2. Project for a reciprocal prodrug between stilbene 5c and *(S)(S)(S)* 76a

In order to further exploit the synergic effects of stilbene and hemiasterlin derivatives, as above reported, the synthesis of a mutual prodrug could be thought in order to possibly improve farmacokinetic of both components. The most important requirement is to find a linkage that is an *in vivo* hydrolysable function.

We would exploit the amine group of stilbene 5c and, on the other side, we have planned to react the bromo-acylpeptide with an amine conveniently bearing a group that is useful to be reacted with a linker (OH, NH_2) and, in the same time maintaning a high biologic activity.

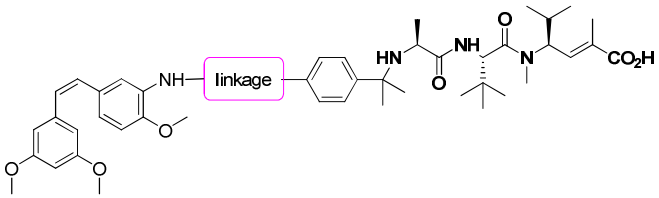


Figure VI-2. Model of the pro-drug between stilbene 5c and the compound with the best biologic profile.

Chapter 7

Experimental section

7. Experimental section

7.1. General methods

^{1}H and ^{13}C NMR spectra were determined with a Mercury Place Varian spectroscope at 400 MHz. Chemical shifts (*d*) are reported in parts per million relative to residual chloroform (7.26 ppm) or dimethyl sulfoxide (2.49 ppm), TMS (0 ppm) as an internal reference. Coupling constants (*J*) are reported in Hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet, br, broad. Electrospray (ES) mass spectra were recorded on a Micromass ZMD 2000 and MALDI mass on a Bruker Omniflex. IR spectra were recorded with an FT-IR Perkin Elmer Paragon 1000 using cm^{-1} as units. Optical rotation were determined in a polarimeter with a 10 cm cell, operating at 589 nm (sodium D line) at 20°C. The concentration was 1-2% in $CHCl_3$. Chromatographic purifications were performed by flash chromatography using Merck 0.040-0.063 mm silica gel. Thin-layer chromatography (TLC) was performed on Merck silica gel precoated plates (Merck F254) using the indicated solvent systems. Analytical HPLC was performed with a Beckman System Gold with a 166 detector (visualization at 254 nm) and was run on a TSK Gel Super ODS (C18) column (4.6 mm x 10 cm, 2 mm) using a gradient solvent system (solvent A: 0.1% TFA/H_2O, solvent B: 0.1% TFA/CH_3CN). Unless otherwise noted, the gradient was: t = 0 min: 0% B, t = 25 min: 100% B. Retention times (t_R) are reported. Preparative HPLC was performed with a Waters Delta Prep 4000 and was run on a XTerra (C18) column (30 x 50 mm, 5 mm) using a gradient solvent system (solvent A: 0:1% TFA/H_2O, solvent B: 0:1% TFA/40% H_2O/60% CH_3CN). Unless otherwise noted, the gradient was: t = 0 min: 0% B, t = 25 min: 50%

B, t = 35 min: 100% B. All reactions mixtures were analyzed by HPLC and ^{1}H NMR to check the diastereomeric distribution before and after column chromatography, to avoid wrong conclusions, due to diastereomeric enrichment. Reagents and promoters are purchased from Alfa Aesar.

7.2. Experimental procedures and analytical data.

7.2.1. Synthesis of fragment BC

(*S*)-2-(*tert*-buthoxycarbonyl(methyl)amino)-3-methylbutanoic acid, [(*S*)-53]

To a solution of Boc-L-Valine (14.7 g, 67.7 mmol) in dry THF (350 ml), methyl iodide (42.2 ml, 667 mol) was added dropwise.

At 0°C, NaH 60% min. oil (32.5 g, 667 mol) was added portion wise. The mixture was left stirring overnight at r.t.

After quench with water, the reaction solvent was concentrated *in vacuo*. The crude was suspended 150 ml of water and washed with 50 ml of EtOAc. The water phase was acidified with citric acid until pH 3.5, extracted with AcOEt (3x100 ml).

The organic phase, was washed with brine (3x50 ml) and dried over Na_2SO_4 to afford (15 g, 60 mmol, 96%) of yellow oil, that was used without any purification for the next reaction.

^{1}H NMR ($CDCl_3$, 200 MHz) : δ 0.90 (d, 3H, *J* = 6.6 Hz), 1.01 (d, 3H, *J* = 6.6 Hz), 1.44 (s, 9H), 2.14 (m, 1H), 2.86 (s, 3H), 4.16 (m, 1H)

^{13}C NMR ($CDCl_3$, 400 MHz) : δ 19.0, 19.9, 27.7, 28.4, 31.9, 65.6, 80.9, 157.1, 175.7

(*S*)-tert-butyl 1-(methoxy(methyl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate [*(S)*-54]

To a cold (0° C.) solution of N-Boc-N-methylvaline *(S)*-**53** (15 g, 65 mmol) in 150 ml of DMF, 1-HOBT (9.64 g, 71.4 mmol), EDCI (13.7 g, 71.4 mmol) and N,O-dimethylhydroxylamine hydrochloride (9.1 g, 91 mmol), were added. Then, at 0°C 4-methylmorpholine (17, 86 ml, 162 mmol) was added and after 10' the reaction was left stirring overnight at r.t.

The reaction solvent was remover under vacuo, and the crude was suspended in AcOEt (150 ml). After washing with citric acid (2x50 ml) to pH 5, the organic layer was washed with $NaHCO_3$ 5% (2x50 ml). The organic layer was washed with brine, dried with sodium sulfate and the solvent was evaporated to get (11 g, 40.14 mmol, 60%) of a yellow oil, that was used without any purification for next step.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.85 (d, 3H, *J* = 4 Hz), 0.89 (d, 3H, *J*=4 Hz), 1.44 (s, 9H), 2.25 (m, 1H), 2.81 (s, 3H), 3.19 (s, 3H), 3.70 (s, 3H), 4.66 (m, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz) : δ 18.6, 19.4, 27.5, 28.3, 31.7, 37.0, 60.7, 61.6, 19.7, 156.1, 170.2

R_f (Hexane/EtOAc 6/1): 0.25

(*S*)-tert-butyl methyl(3-methyl-1-oxobutan-2-yl)carbamate [*(S)*-55]

Lithium aluminum hydride (342 mg, 9 mmol) was added at 0°C to a solution of (**54**) (2.0 g, 7.2 mmol) in dry THF (20 mL) and the reaction mixture was stirred for 2 h at r.t. The mixture was carefully quenched with water at 0°C, diluted with diethyl ether (75 mL) and treated sequentially with 3 N hydrochloric acid (3×20 mL), aqueous sodium hydrogen carbonate (3×20 mL), and saturated with brine (3×20 mL). The organic layer was dried over sodium sulfate and the solvent was evaporated to yield the crude

aldehyde (1.2 g, 5.58 mmol, 92% yield), that was used without any purification.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.86 (d, 3H, J = 7 Hz), 0.95 (d, 3H, J = 6.8 Hz), 1.44 (s, 9H), 2.25 (m, 1H), 2.74 (s, 3H), 4.65 (m, 1H), 9.63 (s, 1H).

R_f (Hexane/EtOAc 6/1): 0.4

(*S*, *E*)-ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-2,5-dimethylhex-2-enoate [(*S*)-58].

To a solution of aldehyde **55** (840 mg, 3.9 mmol) in dry CH_2Cl_2 (10 mL) under an argon atmosphere at room temperature was added (carbethoxyethylidene)triphenylphosphorane (2.8 g, 7.8 mmol) and it was refluxed 5 h. The reaction mixture was concentrated in vacuo and the crude was triturated with Hexane several times (20 ml x5). The combined organic extracts were concentrated *in vacuo* to afford the required E-2-alkenoate as a yellow oil (0.940 g, 3.14 mmol, 81%).

^{1} H-NMR (200 MHz, $CDCl_3$) 0.74 (d, J=6 Hz, 3H), 0.79 (d, J=6 Hz, 3H), 1.17 (t, J=0.7 Hz, 3H), 1.34 (s, 9H), 1.72 (m, 1H), 1.78 (s, 3H), 2.60 (bs, 3H), 4.08 (q, J=7 Hz, 2H), 4.15-4.20 (m, 0.5H), 4.21-4.32 (m, 0.5H), 6.54 (d, J=8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 13.2, 14.2, 19.5, 59.6, 60.7, 79.7, 132.0, 138.8, 155.7, 168.0

R_f (Hexane /EtOAc 6/1): 0.5

Hylide of ethylic ester of 2-(triphenylphosponium)propionic acid (57).

To a solution of triphenylphosphine (60.5 g, 0.23 mol) in toluene (100 ml), ethyl 2-bromopropanoate **56** (30 ml, 0.23 mol) was added.

The suspension was stirred for 48 h at r.t. After that the mixture was filtered on Gooch and a white solid was obtained (71.3 g, 0.16 mol, 70%). The solid

(19.5 g, 44 mmol) was solved in 75 ml of water and to this solution, a saturated solution of NaOH was added until pH 9.

The mixture was left stirring until the formation of a precipitate, that was filtered and the solid was triturated with water (10 ml x3). The yellow solid was concentrated *in vacuo*, solved in AcOEt and crystallized from petroleum ether to afford a yellow solid (10.4 g, 28.7 mmol, 65%)

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.44 (t, 3H, J = 7.0 Hz), 1.6 (d, 3H, J = 7.0 Hz), 3.7 (q, 2H, J = 7.2 Hz), 7.53 (m, 15H). ^{13}C NMR ($CDCl_3$. 400 MHz): δ 12.8, 14.2, 57.4, 76.5, 128.5, 132.0, 133.6, 168.0.

(*S*, *E*)-6-ethoxy-*N*, 2,5-trimethyl-6-oxohex-4-en-3-aminium 2,2,2-trifluroacetate [*(S)*-59]

[*(S)*-58] (641 mg, 2.1 mmol) solved in 2.2. ml of CH_2Cl_2 was treated with TFA/CH_2Cl_2 (0.1 mmol/1 mL) at room temperature for 2 h. Removal of the solvent *in vacuo*, followed by repeated rinsing of the residual material with methanol (3×5 mL) and evaporation of the remaining traces of solvent afforded the TFA salt of the amino acid ester in quantitative yield, that was used in next step without any purification.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.98 (d, 3H, J = 6.4 Hz), 1.01 (d, 3H, J = 6.2 Hz), 1.31 (t, 3H, J = 7 Hz), 1.93 (d, 3H, J = 1.2 Hz), 2.2 (m, 1H), 2.59 (t, 3H, J = 5.2 Hz), 3.8 (m, 1H), 4.23 (q, 2H, J = 7 Hz), 6.65 (dd, 1H, J = 1.2 Hz, J = 9.2 Hz), 8.9 (s, 1H); 9.1 (s,1H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ 13.4, 14.0, 17.1, 19.0, 31.4, 31.6, 61.6, 63.1, 130.9, 136.7, 166.6. IR (KBr): ν = 2974, 1715, 1669, 1173, 1130.

General procedure 1: pivaloyl chloride-mediated amide bond formation [*(S)(S)*-62a,b]

To a cold (-78°C) solution of carboxylic acid (1.1 eq) in dry THF (1 ml/mmol) of amino acid ester) under an argon atmosphere was added DIPEA (1.5 eq) and pivaloyl chloride (1.2 eq). The resulting mixture was warmed to 0°C for 1 h and then re-cooled to -78°C.

DIPEA (2.2 eq) was added to the reaction flask followed by the addition, *via syringes*, of the TFA salt of the amino acid ester (1 eq) in dry THF (0.5 ml/mmol of amino acid ester) at -78°C.

Stirring was continued for 1 h and then quenched by the addition of H_2O. The mixture was allowed to warm to r.t. and extracted three times with diethyl ether. The combined organic layer was dried over sodium sulphate and concentrated in vacuo. The crude oil was purified by silica gel column chromatography using a EtOAc/petroleum ether mixture as the mobile phase.

(*S*,*E*)-ethyl 4-((*S*)-2-(*tert*-butoxycarbonylamino)-N, 3,3-trimethylbutanaido)-2,5-dimethylhex-2-enoate 62a

It was obtained following the general procedure 1: (70%, colorless oil)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (d, 6H, *J* = 2.4 Hz), 0.9 (d, 6H, *J* = 1.6 Hz), 1.29 (t, 3H, *J* = 7.2 Hz), 1.41 (s, 9H), 1.87 (d, 3H, *J* = 1.2 Hz), 2.85 (m, 1H), 2.94 (s, 3H), 3.1 (m, 1H), 4.19 (q, 2H, *J* = 7.2 Hz), 4.35 (dd, 1H, *J* = 6.8 Hz, *J* = 9.2 Hz), 5.04 (dd, 1H, *J* = 9.2 Hz, *J* = 10.8 Hz), 6.63 (dd, 1H, *J* = 1.2 Hz, *J* = 9.2 Hz), 7.5 (m, 1H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ 13.7, 14.3, 17.6, 18.8, 19.5, 28.3, 30.0, 30.4, 31.0, 55.5, 56.4, 60.9, 79.5, 133.9, 138.4, 156.1, 167.8, 172.6. R_f (Hexane /EtOAc 2/1): 0.85

(*S*,*E*)-ethyl 4-((S)-2-(*tert*-butoxycarbonylamino)-N, 3-dimethylbutanaido)-2,5-dimethylhex-2-enoate 62b

It was obtained following the general procedure 1: (62%, colorless oil)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (d, 6H, *J* = 2.4 Hz), 0.9 (d, 6H, *J* = 1.6 Hz), 1.29 (t, 3H, *J* = 7.2 Hz), 1.41 (s, 9H), 1.87 (d, 3H, *J* = 1.2 Hz), 2.85 (m, 1H), 2.94 (s, 3H), 3.1 (m, 1H), 4.19 (q, 2H, *J* = 7.2 Hz), 4.35 (dd, 1H, *J* = 6.8 Hz, *J* = 9.2 Hz), 5.04 (dd, 1H, *J* = 9.2 Hz, *J* = 10.8 Hz), 6.63 (dd, 1H, *J* = 1.2 Hz, *J* = 9.2 Hz), 7.5 (m, 1H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ 13.7, 14.3, 17.6, 18.8, 19.5, 28.3, 30.0, 30.4, 31.0, 55.5, 56.4, 60.9, 79.5, 133.9, 138.4, 156.1, 167.8, 172.6. R_f (Hexane/EtOAc 2/1): 0.75

General procedure 2: Trifluoacetic acid mediated cleavage of N-Boc group of dipeptides [*(S)(S)*-63a,b]

N-Boc-dipeptide (1.0 equiv.) was treated with TFA/CH_2Cl_2 (0.1 mmol/1 mL) at room temperature for 2 h. Removal of the solvent in vacuo, followed by repeated rinsing of the residual material with CH_2Cl_2 (3×5 mL) and evaporation of the remaining traces of solvent afforded the TFA salt of the amino acid ester in quantitative yield. TFA salts were used without further purification.

Dipeptide [*(S)(S)*-63a]

It was obtained following the general procedure 2: (85%, colorless oil)

^{1}H NMR (DMSO-$_{d6}$, 400 MHz): δ 0.95 (d, 3H, *J* = 7.6 Hz), 0.98 (s, 9H), 1.00 (d, 3H, *J* = 6.0 Hz), 1.21 (t, 3H, *J* = 7.2 Hz), 1.82 (d, 3H, *J* = 1.6 Hz), 2.05 (m, 1H), 2.93 (s, 3H), 4.14 (q, 2H, *J* = 7.2 Hz), 4.21 (m, 1H), 4.93 (t, 1H, *J* = 10.0 Hz), 6.67 (dd, 1H, *J* = 1.6 Hz, *J* = 10.8 Hz), 7.5 (m, 3H). ^{13}C

NMR (DMSO-$_{d6}$, 400 MHz): δ 13.9, 16.5, 18.8, 19.2, 25.9, 32.9, 34.1, 52.3, 56.5, 60.9, 127.0, 137.9, 167.9, 169.0

Dipeptide [*(S)(S)*-63b]

It was obtained following the general procedure 2: (85%, colorless oil)

^{1}H NMR (DMSO-$_{d6}$, 400 MHz): δ 0.82 (d, 6H, *J* = 6.8 Hz), 0.85 (d, 6H, *J* = 6.8 Hz), 1.22 (t, 3H, *J* = 7.2 Hz), 1.80 (d, 3H, *J* = 1.6 Hz), 2.05 (m, 1H), 2.10 (m, 1H), 2.89 (s, 3H), 4.13 (q, 2H, *J* = 7.2 Hz), 4.23 (m, 1H), 4.83 (dd, 1H, *J* = 10.2 Hz, *J* = 10.0 Hz), 6.67 (dd, 1H, *J* = 1.4 Hz, *J* = 9.6 Hz), 8.08 (m, 3H). ^{13}C NMR (DMSO-$_{d6}$, 400 MHz): δ 13.3, 13.9, 16.4, 17.7, 18.8, 28.5, 30.2, 31.1, 54.2, 56.9, 60.8, 133.1, 137.9, 166.7, 168.3

***(R,S)(S)(S)* 2-bromopropanoyl-dipeptide ethyl ester**

[*(R,S)(S)(S)*-65a]: To a solution of dipeptide ethyl ester **63a** (0,96 mmol, 273 mg) and Et_3N (0.96 mmol, 0.13 mL) in anhydrous CH_2Cl_2 (5 mL) was dropped at 0° C, (*R,S*)-2-bromopropanoyl bromide **64** (0.96 mmol, 0.1 mL) dissolved in CH_2Cl_2 (3 mL). The mixture was allowed to warm at r.t. and stirred overnight. The resulting solution was diluted with CH_2Cl_2 (30 mL) and washed 3 times x 5 mL, in the order, with citric acid (10%), $NaHCO_3$ (5%), brine and dried over Na_2SO_4. Evaporation of organic phase to constant weight gave the bromo acyl-peptide **65a** as a diastereomeric mixture of an oil (267 mg, 69%). R_f (Hexane/EtOAc 4/1): 0.35 and 0.25. Column chromatography of *(R,S)(S)(S)*-6**5a** (Hexane/EtOAc 3/1) allowed us to obtain the single diastereoisomers *(S)(S)(S)*-**65a** and *(R)(S)(S)*-**65a**. Absolute configurations were assigned by indipendent syntheses of the two single diastereoisomers by reaction of *(S)*- or *(R)*-2-bromopropanoic acid (**67**) with *(S)(S)*-**63a** (see in next context).

For *(S)(S)(S)*-**65a**: R_f (Hexane/AcOEt 4/1) 0.25.

^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.78 (d, 3H, *J* = 6.4), 0.85 (d, 3H, *J* = 6.4 Hz), 0.96 (s, 9H), 1.27 (t, 3H, *J* = 7.2 Hz), 1.82 (d, 3H, *J* = 6.8 Hz), 1.81-1.88 (m, 1H), 1.89 (d, 3H, *J* = 1.6 Hz), 2.96 (s, 3H); 4.17 (q, 2H, *J* = 7.2 Hz), 4.37 (q, 1H, *J* = 6.8 Hz), 4.77 (d, 1H, *J* = 9.6 Hz), 5.07 (dd, 1H, *J* = 9.6 and 9.8 Hz), 6.61 (dd, 1H, *J* = 1.6 and 9.8 Hz), 6.89 (d, 1H, *J* = 9.6 Hz).

^{13}C NMR ($CDCl_3$, 400 MHz): *d* 13.8, 14.2, 18.7, 19.4, 22.8, 26.4, 30.0, 31.1, 36.1, 44.2, 55.2, 56.2, 61.0, 132.8, 138.2, 167.8, 169.1, 171.0.

For *(R)(S)(S)*-**65a**: R_f (Hexane/EtOAc 4/1) 0.35.

^{1}H NMR: ($CDCl_3$, 400 MHz): *d* 0.80 (d, 3H, *J* = 6.8 Hz), 0.87 (d, 3H, *J* = 6.8 Hz), 0.97 (s, 9H), 1.29 (t, 3H, *J* = 7.2 Hz), 1.82 (d, 3H, *J* = 7.2 Hz), 1.82-1.90 (m, 1H), 1.90 (d, 3H, *J* = 1.6 Hz), 2.97 (s, 3H), 4.19 (q, 2H, *J* = 7.2 Hz), 4.40 (q, 1H, *J* = 7.2 Hz), 4.76 (d, 1H, *J* = 9.4 Hz), 5.10 (dd, 1H, *J* = 7.3 and 7.1 Hz), 6.63 (dd, 1H, *J* = 1.6 and 7.3 Hz), 6.93 (d, 1H, *J* = 9.4 Hz).

^{13}C NMR ($CDCl_3$, 400 MHz): *d* 13.3, 14.3, 18.7, 19.5, 23.0, 26.5, 30.1, 31.1, 35.6, 44.6, 55.5, 56.1, 60.9, 132.9, 138.3, 167.8, 169.3, 171.0

_(S)_-2-bromopropionic acid [_(S)_-67].

To a solution of (L)-alanine **66** (3 g, 34 mmol) in H_2SO_4 2.5 N (140 ml), KBr (14, 119 mmol) was added. At 0°C, $NaNO_2$ (3.7 g, 54 mmol) was added portionwise. The mixture was stirred at r.t. for 3 h.

The yellow solution was extracted with ethylic ether (3x15 ml)and the organic phase was washed with $Na_2S_2O_3$ (3x75 ml), brine (3x75 ml). The organic phase was dried over Na_2SO_4 to afford a colorless oil (2.9 g, 18.95 mmol, 56%).

^{1}H NMR (CD_3OD, 200 MHz): δ 1.92 (d, 3H), 4.48 (q, 1H). ^{13}C NMR (CD_3OD, 200 MHz): δ 21.1, 42.3, 171.0

$[\alpha]^{20}_D$ = -30.4

The same procedure was followed to get the other enantiomer ***(R)*-2-bromopionic acid [*(R)*-67]**, starting from (D)-alanine **66** (3 g, 34 mmol). The product is a colorless oil (2.6 g, 17 mmol, 50%).

^{1}H NMR (CD3OD, 200 MHz): δ 1.92 (d, 3H), 4.48 (q, 1H). ^{13}C NMR (CD_3OD, 200 MHz): δ 21.3, 42.5, 171.2

$[\alpha]^{20}_D$ = +30.4

General procedure 3: diastereoselective synthesis of Bromopropanoyl-dipeptide ethyl ester.

To a cold (-78°C) stirred solution of (*S*)-2-bromopionic acid (1.1 equiv.) in dry THF (1 mL/mmol) under argon atmosphere was added DIEA (1.5 equiv.) and trimethylacetyl chloride (1.2 equiv.). The resulting mixture was warmed to 0°C for 1 h and then re-cooled to -78° C. DIEA (1.5 equiv.) and dipeptide ester ***(S)(S)*-63a** or **b** (1 equiv.) dissolved in dry THF (1 mL/mol) were added *via syringes*. Stirring was continued for 1 h and H_2O (30 mL). The mixture was allowed to warm to room temperature, and extracted with diethyl ether (3 x 30 mL) was added. The combined organic extracts were washed with brine (30mL), dried and concentrated.

[*(S)(S)(S)*-65a] Oil (62%). R_f (hexane/AcOEt 4/1) 0.25. See above for spectroscopy data.

Following the same procedure, [***(R)(S)(S)*-65a]** was obtained as an oil (62%). R_f (hexane/AcOEt 4/1) 0.25. See above for spectroscopy data.

2-Bromopropanoyl-dipeptide ethyl ester [*(R,S)(S)(S)*-65b]

It was obtained from dipeptide ethyl ester [***(S)(S)*-63b]** and *(R,S)*-2-bomopropanoyl bromide **64**, following the general procedure 3. The product

was obtained as colorless oil (85%). R_f (Hexane/AcOEt 2:1): 0.5 and 0.53. Due to the difficulty to separate the diastereomeric mixture by chromatography, ***(S)(S)(S)*-65b** and ***(R)(S)(S)*-65b** were obtained by independent syntheses following the general procedure 4.

2-Bromopropanoyl-dipeptide ethyl ester [*(S)(S)(S)*-65b].

According to general procedure 4, it was obtained from dipeptide ethyl ester ***(S)(S)*-63b** and ***(S)*-**2-bromopropanoic acid **[*(S)*-67**]

^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.83 (d, 3H, *J* = 6.4 Hz), 0.84 (d, 3H, *J* = 6.4 Hz), 0.86 (d, 3H, *J* = 6.8 Hz), 0.93 (d, 3H, *J* = 6.8 Hz), 1.31 (t, 3H, *J* = 7.2 Hz), 1.82-1.88 (m, 1H), 1.86 (d, 3H, *J* = 7.0 Hz), 1.89 (d, 3H, *J* = 1.6 Hz), 1.96-2.06 (m, 1H), 2.94 (s, 3H), 4.2 (q, 2H, *J* = 7.2 Hz), 4.40 (q, 1H, *J* = 7.0 Hz), 4.74 (dd, 1H, J = 8.6 and 9.0 Hz), 5.04 (dd, 1H, *J* = 9.2 and 10.5 Hz), 6.64 (dd, 1H, *J* = 1.6 and 9.2 Hz), 6.98 (d, 1H, *J* = 8.6 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): *d* 13.7, 14.2, 17.1, 18.8, 19.4, 19.6, 22.7, 26.5, 29.8, 30.3, 31.5, 44.1, 54.2, 56.6, 60.9, 132.9, 137.9, 167.7, 169.3, 171.3.

R_f (Hexane/AcOEt 2:1): 0.5

2-Bromopropanoyl-dipeptide ethyl ester [*(R)(S)(S)*-65b].

It was obtained from dipeptide ethyl ester [***(S)(S)*-63b]** and *(R)*-2-bromopropanoic acid [***(R)*-67**], according to general procedure 3.

^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.85 (d, 3H, *J* = 6.4 Hz), 0.87-0.93 (m, 9H), 1.28 (t, 3H, *J* = 7.6 Hz), 1.84 (d, 3H, *J* = 6.8 Hz), 1.89 (d, 3H, *J* = 1.6 Hz), 2.95 (s, 3H), 4.20 (q, 2H, *J* = 7.6 Hz), 4.19 (q, 1H, *J* = 6.8 Hz), 4.68 (dd, 1H, *J* = 6.4 and 8.8 Hz), 5.05 (dd, 1H, *J* = 9.6 and 10.8 Hz), 6.63 (dd, 1H, *J* = 1.6 and 9.6 Hz), 6.99 (br d, 1H).

^{13}C NMR ($CDCl_3$, 400 MHz): *d* 13.7, 14.3, 17.6, 18.9, 19.5, 19.6, 22.9, 29.9, 30.4, 31.5, 44.44, 54.7, 56.7, 61.0, 132.0, 138.1, 167.8, 169.4, 171.4.
R_f (Hexane/EtOAc 2/1) 0.53

General Procedure 4: reduction of tertiary alcohol

To a suspension of Mg (2 eq) in 10 ml of dry ethylic ether, methyl iodide (2.2 eq) was added dropwise and stirred for several minutes while carefully observing the mixture for signs of reaction. When the reaction has started the solvent began to reflux vigorously. Once the addition is complete, a solution of ketone (1 eq) in 15 ml of ethylic ether was added dropwise and the mixture was stirred at r.t. for 2 h.

The mixture was quenched with NH_4Cl (3 ml) until pH 6-7 and extracted with ethylic ether (3x 15 ml). The organic phase was dried over sodium sulfate, concentrated in vacuo to give the desired product.

2-(naphthalen-2-yl)propan-2-ol 69d

The product is a yellow solid, 93%.

^{1}H NMR ($CDCl_3$, 400 MHz): *d* 1.68 (s, 6H), 7.38 (m, 2H), 7.10 (dd, 1H), 7.80 (m, 3H), 7.88 (dd, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): *d* 31.7, 72.8, 122.44, 123.6, 125.8, 126.1, 127.5, 128.0, 128.2, 132.3, 133.2, 146.5.

R_f (Hexane/AcOEt 4:1) 0.3

2-(4-bromophenyl)propan-2-ol 69e

The product is an oil, 91%.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.56 (s, 6H), 1.79 (s, 1H), 7.35 (d, 2H, J = 8.8 Hz), 7.45 (d, 2H, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 31.8, 72.4, 120.6, 126.4, 131.3, 148.2.

R_f (petroleum ether/diethyl ether 1:1) 0.5

General procedure 5: synthesis of the azide 70a, 70d, 70e from the corresponding alcohols 69a, 69d, 69e.

A solution of alcohol (1 eq), and NaN_3 (2 eq) in $CHCl_3$ (1 ml/1 mmol) was cooled at -5°C. At this temperature, a mixture of $CHCl_3$ and trifluroacetic acid (5 eq) was added portionwise, paying attention to the temperature increase. The mixture was left stirring at r.t. for 24 h, after that it was quenched with NH_4OH until pH 9.

The organic phase was washed with water, dried over Na_2SO_4 and concentrated in vacuo to give the product as an oil.

(2-azidopropan-2-yl)benzene 70a

The product is a colourless oil, 60%.

^1H NMR ($CDCl_3$, 200 MHz): δ 1.64 (s, 6H), 7.37 (m, 5H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 30.4, 63.0, 125.9, 126.1, 128.4, 138.3.

R_f (Hexane/ AcOEt 1:1): 0.85

2-(2-azidopropan-2-yl)naphthalene 70d

The product is a yellow oil, 86%.

^1H NMR ($CDCl_3$, 200 MHz): δ 1.74 (s, 6H), 7.54 (m, 2H), 7.85 (m, 5H). ^{13}C NMR ($CDCl_3$, 400 MHz): *d* 31.7, 74.0, 122.44, 123.6, 125.8, 126.1, 127.5, 128.0, 128.2, 132.3, 133.2, 146.5. R_f (Hexane/ AcOEt 4:1) 0.4

1-(2-azidopropan-2-yl)-4-bromobenzene 70e

The product is an oil, 85%.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.56 (s, 1H), 1.61 (s, 6H), 7.31 (d, 2H, J = 8.8 Hz), 7.48 (d, 2H, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 28.4, 63.4, 121.5, 127.1, 131.7. R_f (Hexane/AcOEt 4:1) 0.8

General procedure 6: reduction of azide 70a, 70d, 70e, to the corresponding amine 71a, 71d, 71e.

To a solution of amine in EtOAc, it was added portionwise C/Pd previously suspended in EtOAc. The suspension hydrogenated for 6 h, at r.t., at 50 psi. The reaction mixture was filtered on celite and rinsed with EtOAc. The solvent was concentrated in vacuo to afford the desired product.

2-phenylpropan-2-amine (71a)

The product is an oil , 85% .

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.51 (s, 6H), 2.09 (s, 2H), 7.37 (m, 5H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 34.6, 55.5, 125.9, 126.1, 128.4, 144.6

R_f (Hexane/EOAc 1:1): 0.15

2-(naphathalen-2-yl)propan-2-amine (71d)

The crude is a colourless oil, 14%, after distillation from the yellow crude.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.60 (s, 6H), 7.37 (m, 2H), 7.67 (dd, 1H), 7.83 (m, 3 H), ^{13}C NMR ($CDCl_3$, 400 MHz): d 34.6, 55.9, 125.1, 126.0, 127.0, 127.3, 127.5, 127.6, 128.2, 131.8, 133.7, 135.2.

R_f (Hexane/EtOAc 1:1) 0.18

2-(4-bromophenyl)propan-2-amine (71e)

The product is an oil , 85%

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.45 (s, 6H), 7.41 (m, 4H). ^{13}C NMR ($CDCl_3$, 400 MHz) : δ 34.6, 55.5, 120.3, 124.9, 125.5, 143.6

1-methyl-1H-indole-3-carbonitrile (73)

To a suspension of KOH (158 mg, 2.8 mmol) in DMSO (5 ml), indole **72** (200 mg, 1.4 mmol) and methyl iodide (104.6 µl, 1.68 mmol) were added. The reaction mixture was left stirring at r.t. for 2 h.

After addition of water, the mixture was extracted with EtOAc (3x 20 ml), washed with brine (5 ml), dried over Na_2SO_4 and concentrated to get a red oil that was purified by flash chromatography (EtOAc: petroleum ether 1:2). It was obtained (174 mg, 1.1 mmol, 80%) of a pale red oil.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.85 (s, 3H), 7.33 (m, 3H), 7.57 (s, 1H), 7.76 (dd, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 29.8, 33.7, 110.4, 120.0, 122.23, 123.9, 135.6. R_f (Hexane/EtOAc 1:2) 0.7

MS, m/z (ES+): 157 [M+H]

(1-methyl-1H-indol-3-yl)methanamine 71f

The product was obtained according the general procedure 6 starting from **73**.

The product was a yellow oil 85%.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.69 (s, 3H), 4.24 (s, 2H), 6.35 (s, 1H), 7.02 (m, 1H), 7.37 (m, 1H), 7.51-7.56 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 42.5, 34.0, 109.6, 112.5, 118.8, 119.8, 121.7, 126.5, 127.7, 137.5. Check

General procedure 7: nucleophilic substitution reaction Ag_2O catalyzed.

To a suspension of Ag_2O (2eq) in dry toluene (2.5 mL/eq), [*(S)(S)(S)*-**65a**] (1eq) and amine 71a-f (3eq) were added. The mixture was refluxed for 2½ h under argon atmosphere filtered over celite. Evaporation of the solvent gave the crude product as a yellow oil that was purified by column chromatography to afford the desired product.

- **Amine 71a**

Tripeptide ethyl ester [*(S)(S)(S)*-75a]

It was obtained following the general procedure 6, after purification by flash chromatography (toluene/AcOEt 3.5:3) to afford the product as solid, 90%, R_f (toluene/EtOAc 3.5: 3) 0.3.

By comparison with TLC and NMR of the diastereomeric mixture obtained from [*(R,S)(S)(S)*-**65a**], we confirmed the optical purity.

MS (m/z) MALDI: 502 ($M+H^+$), 524 ($M+ Na^+$), 540 ($M+K^+$).

^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.79 (d, 3H, *J* = 6.8 Hz), 0.87 (d, 3H, *J* = 6.8 Hz), 0.97 (s, 9H), 1.21 (s, 3H), 1.32 (t, 3H, *J* = 7.2 Hz), 1.40 (d, 3H, *J* = 6.8 Hz), 1.52 (s, 3H), 1.87-1.91 (m, 1H), 1.90 (d, 3H, *J* = 0.8 Hz), 2.90-3.01 (br, 2H), 3.01 (s, 3H), 4.17-4.22 (m, 3H), 4.79 (d, 1H, *J* = 9.6 Hz), 5.08 (dd, 1H, *J* = 10 and 10.2 Hz), 6.63 (dd, 1H, *J* = 9.6 and 0.8 Hz), 7.19-7.49 (m, 5H).

^{13}C NMR *d* 13.9, 14.3, 18.8, 19.5, 21.3, 26.5, 27.3, 30.0, 31.2, 32.2, 35.4, 54.7, 56.3, 61.0, 68.2, 124.6, 126.6, 128.4, 132.8, 138.2, 150.1, 167.8, 171.8, 174.6.

Tripeptide ethyl ester [*(R)(S)(S)*-75a]

It was obtained following the general procedure 7, starting from bromoacyl-dipeptide [*(R)(S)(S)*-65a]leu**.**

R_f (Toluene/AcOEt 3.5:3) 0.25.

MS, (m/z) MALDI: 502 ($M+H^+$), 524 ($M+ Na^+$), 540 ($M+K^+$)

^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.77 (d, 3H, *J* = 6.4 Hz), 0.86 (d, 3H, *J* = 6.4 Hz), 1.00 (s, 9H), 1.06 (d, 3H, *J* = 6.8 Hz), 1.30 (t, 3H, *J* = 7.2 Hz), 1.42 (s, 3H), 1.47 (s, 3H), 1.8 (br, 1H), 1.82-1.89 (m, 1H), 1.91 (d, 3H, *J* = 1.6 Hz), 2.95 (q, 1H, *J* = 6.8 Hz), 2.98 (s, 3H), 4.19 (q, 2H, *J* = 7.2 Hz), 4.75 (d, 1H, *J* = 10.0 Hz), 5.11 (dd, 1H, *J* =9.6 and 9.8 Hz), 6.62 (dd, 1H, *J* = 10 and 1.6 Hz), 7.20-7.42 (m, 5H), 8.14 (d, 1H, *J* = 10.0 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): d 13.9, 14.3, 18.7, 19.5, 21.4, 26.6, 27.0, 30.1, 31.0, 31.1, 31.7, 35.2, 52.9, 54.6, 55.9, 56.6, 60.9, 125.6, 126.7, 128.3, 132.8, 138.6, 147.2, 167.8, 171.8, 176.2.

- **Reaction with amine 71b**

Tripeptide ethyl ester [*(R)(R,S)(S)(S)*-75b]

It was obtained as colourless oil (55%), following the general procedure 7.

R_f (AcOEt/petroleum ether 1:2) 0.26.

It is a diastereomeric mixture, thus spectroscopic data are not reported, as they are all multiplets.

MS, *m/z* (ES+): 488 [M+H]

- **Reaction with amine 71c**

Tripeptide ethyl ester [*(S)(S)(S)*-75c]

It was obtained as a solid (79%) from *(S)(S)(S)*-65leu, following the general procedure 7**.**

R_f (Hexane/AcOEt 1:8) 0.3. MS, *m/z* (ES+): 474 [M+H], 496 [M+Na].
^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.83 (d, 3H, *J* = 6.5 Hz), 0.85-0.90 (m, 9H), 1.11 (d, 3H, *J* = 7.2 Hz), 1.35 (t, 3H, *J* = 7.1 Hz), 1.49 (s, 3H), 1.55 (s, 3H), 1.80 (br s, 1H), 1.92 (d, 3H, *J* = 1.4 Hz), 1.99-2.18 (m, 2H), 4.21 (q, 2H, *J* = 7.1 Hz), 4.28 (q, 1H, *J* = 7.2 Hz), 4.76 (dd, 1H, *J* = 7.2 and 9.8 Hz), 5.17 (dd, 1H, *J* = 9.7 and 10.5 Hz), 6.33 (dd, 1H, *J* = 1.4 and 9.2 Hz), 7.22-7.40 (m, 5H), 8.14 (d, 1H, *J* = 9.6 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): *d* 14.9, 15,4, 16.6, 17.7, 18.4, 19.5, 19.7, 21.0, 21.4, 31.3, 31.9, 32.3, 53.1, 54.9, 56.9, 57.9, 61.3, 127.6, 127.9, 129.0, 132.3, 138.5, 138.7, 167.6, 172.7, 175.9.

Tripeptide ethyl ester [*(R)(S)(S)*-75c]

It was obtained as a solid (76%) from *(R)(S)(S)*-65leu according to general procedure 7.

R_f (Hexane/EtOAc 1:8) 0.3; MS, *m/z* (ES+): 474 [M+H], 496 [M+Na].
^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.80 (d, 3H, *J* = 6.4 Hz), 0.87-0.93 (m, 9H), 1.09 (d, 3H, *J* = 7.2 Hz), 1.31 (t, 3H, *J* = 7.2 Hz), 1.46 (s, 3H), 1.50 (s, 3H), 1.80 (br s, 1H), 1.91 (d, 3H, *J* = 1.6 Hz), 1.93-2.11 (m, 2H), 4.20 (q, 2H, *J* = 7.2 Hz), 4.25 (q, 1H, *J* = 7.2 Hz), 4.66 (dd, 1H, *J* = 7.2 and 9.6 Hz), 5.07 (dd, 1H, *J* = 9.6 and 10.8 Hz), 6.34 (dd, 1H, *J* = 1.6 and 9.2 Hz), 7.21-7.42 (m, 5H), 8.01 (d, 1H, *J* = 9.6 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): *d* 14.5, 15,1, 16.9, 17.9, 18.6, 19.8, 19.9, 20.6, 20.4, 31.1, 31.4, 31.7, 52.9, 54.7, 56.0, 57.8, 61.2, 127.4, 128.8, 128.9, 132.1, 138.2, 138.5, 167.9, 172.9, 175.5.

- **Reaction with amine 71d**

Tripeptide ethyl ester [*(R,S)(S)(S)*-75d]

It was obtained from *(R,S)(S)(S)*-65leu, as yellow oil (80%) according to general procedure 7.

R_f (AcOEt/Hexane 1:1) 0.4

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.78 (d, 3H, J = 6.8 Hz), 0.82 (d, 3H, J = 6.8 Hz), 0.96 (s, 9H), 1.20 (m, 3H), 1.48 (m, 3H) 1.57 (s, 3H), 1.90-2.04 (m, 4H), 2.8-3.00 (m, 4H), 4.16 (q, 2H, J = 6.8 Hz), 4.75 (dd, 1H, J = 3.2-7.2 Hz),5.07-5.14 (m, 1H), 6.65 (d, 1H, J = 9.6 Hz), 7.08-7.82 (m, 7H), 8.23 (d, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.0, 14.3, 18.9, 19.5, 21.7, 21.8, 25.5, 25.8, 26.5, 27.1, 29.8, 29.9, 31.2, 31.6, 32.1, 35.7, 53.3, 53.9, 55.9, 56.5, 56.7, 60.9, 122.5, 125.5, 126.6, 127.5, 128.3, 131.3, 132.7, 138.7, 138.8, 146.7, 147.6, 167.8, 171.2, 173.09, 175.9, 176.2, 186.80.

- **Reaction with amine 71e**

Tripeptide ethyl ester [*(S)(S)(S)*-75e]

It was obtained from [*(R,S)(S)(S)*-65a] as yellow oil (41 %) according to general procedure 7.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.77 (d, 3H, J = 6.8 Hz), 0.87 (d, 3H, J = 6.8 Hz), 0.96 (s, 9H), 1.18 (d, 3H, J = 6.8 Hz), 1.29 (t, 3H, J = 7.2 Hz) 1.34 (s, 3H), 1.35 (s, 3H), 1.85-1.94 (m, 1H), 1.86-1.95 (m, 1H), 3.00 (s, 3H), 4.18 (q, 1H, J = 6.8 Hz), 4.75 (dd, 1H, J = 3.2-7.2 Hz),5.07-5.14 (m, 1H), 6.65 (d, 1H, J = 9.6 Hz), 7.25-7.43 (m, 4H), 8.23 (d, 1H, J = 14 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.0, 14.3, 18.9, 19.5, 21.7, 21.8, 25.5, 25.8, 26.5, 27.1, 29.8, 29.9, 31.2, 31.6, 32.1, 35.7, 53.3, 53.9, 55.9, 56.5, 56.7, 60.9, 12.5, 125.5, 126.6, 127.5, 128.3, 131.3, 132.7, 138.7, 138.8, 146.7, 147.6, 167.8, 171.2, 175.9, 176.2 .

Tripeptide ethyl ester [*(R)(S)(S)*-75e]

It was obtained from [*(R,S)(S)(S)*-65a] as white solid (80%), according to general procedure 7.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.77 (d, 3H, J = 6.4 Hz), 0.87 (d, 3H, J = 6.4 Hz), 0.99 (s, 9H), 1.09 (d, 3H, J = 6.8 Hz), 1.31 (t, 3H, J = 7.2 Hz), 1.41 (s, 3H), 1.45 (s, 3H), 1.85-1.94 (m, 1H), 1.92 (s, 3H), 2.89-2.96 (m, 1H), 2.99 (s, 3H), 4.20 (q, 2H, J = 7.2 Hz), 4.76 (d, 1H, J = 10 Hz), 5.11 (t, 1H, J = 10 Hz), 6.63 (d, 1H, J = 9.2 Hz), 7.30 (d, 2H, J = 8.4 Hz), 7.44 (d, 2H, J = 8.8 Hz), 7.99 (d, 1H, J = 10 Hz).

^{13}C NMR ($CDCl_3$, 400 MHz): δ 13.9, 14.3, 18.7, 19.5, 21.6, 26.6, 26.8, 27.1, 29.8, 30.1, 31.1, 31.5, 35.3, 53.0, 54.7, 55.9, 56.5, 60.9, 120.7, 127.6, 131.4, 138.6, 167.8, 171.8, 175.9

MS, *m/z* (ES+): 580 (M+H), 582 (M+2)

Tripeptide ethyl ester [*(R,S)(S)(S)*-75f]

It was obtained from [*(R,S)(S)(S)*-**65a**] as an oil (80%) according to general procedure 7.

^{1}H NMR($CDCl_3$, 400 MHz) : δ 0.5-1 (m, 6H), 0.97 (s, 9H), 1.00 (s, 9H), 1.18 (d, 3H), 1.22 (d, 2H), 1.20-1.35 (m, 3H), 1.50-1.70 (m 1H), 1.78-2.00 (m, 1H), 1.91 (s, 3H), 1.93 (s, 1H), 3.01 (s, 3H), 3.03 (s, 3H), 3.25-3.35 (m, 1H), 3.76 (s, 3H), 3.85-4.02 (m, 1H), 6.60-6.70 (m, 1H), 7.03 (s, 1H), 7.10-7.30 (m, 3H), 7.60-7.70 (m, 1H), 7.98 (d, 1H), 8.00 (d, 1H).

^{13}C NMR ($CDCl_3$, 400 MHz): δ 13.8, 13.9, 14.3, 17.6, 18.7, 18.8, 19.5, 19.8, 20.3, 20.8, 23.9, 26.6, 29.8, 30.2, 31.2, 32.7, 35.1, 35.4, 43.1, 43.8, 54.2, 54.3, 55.9, 57.9, 60.9, 109.3, 112.8, 112.9, 118.9, 119.0, 119.2, 121.7, 121.9, 127.4, 127.9, 132.8, 138.8, 167.9, 171.7, 175.0, 175.1.

2-Hydroxypropanoyl-dipeptide ethyl ester [*(S)(S)(S)*-77g]

It was obtained as solid (98%) according general procedure 7.

R_f (Hexane/AcOEt 1:1) 0.28. MS, *m/z* (ES+): 384 (M+H).

^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.79 (d, 3H, *J* = 6.8 Hz), 0.87 (d, 3H, *J* = 6.8 Hz), 0.97 (s, 9H), 1.32 (t, 3H, *J* = 7.2 Hz), 1.40 (d, 3H, *J* = 6.8 Hz), 1.83-1.94 (m, 1H), 1.90 (d, 3H, *J* = 0.8 Hz), 2.88 (br s, 1H), 3.01 (s, 1H), 4.18-4.22 (m, 3H), 4.79 (d, 1H, *J* = 9.6 Hz), 5.08 (dd, 1H, *J* = 10.0 and 10.2 Hz), 6.63 (dd, 1h, *J* = 10.0 and 0.8 Hz), 7.21 (d, 1H, *J* = 9.6 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): *d* 13.9, 14.3, 18.8, 19.5, 21.3, 26.5, 30.0, 31.2, 35.4, 54.7, 56.3, 61.0, 68.3, 132.8, 138.2, 167.8, 171.8, 174.6.

2-Hydroxypropanoyl-dipeptide ethyl ester [*(R)(S)(S)*-77g]

It was obtained as for [*(S)(S)(S)*-**77g**] but starting from bromoacyl-dipeptide [*(R)(S)(S)*-65]**.**

R_f (Hexane/AcOEt 1:1) 0.5, (toluene/EtOAc) 0.4. MS, *m/z* (ES+): 384 (M+H).

^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.78 (d, 3H, *J* = 6.4 Hz), 0.86 (d, 3H, *J* = 6.4 Hz), 0.97 (s, 9H), 1.30 (t, 3H, *J* = 7.2 Hz), 1.38 (d, 3H, *J* = 6.4 Hz), 1.87-1.92 (m, 1H), 1.89 (d, 3H, *J* = 1.2 Hz), 3:00 (s, 3H), 3.70 (br, 1H), 4.19 (q, 2H, *J* = 7.2 Hz), 4.26 (q, 1H, *J* = 6.4 Hz), 4.81 (d, 1H, *J* = 9.6 Hz), 5.07 (dd, 1H, *J* = 10.8 and 9.6 Hz), 6.62 (dd, 1H, *J* = 1.2 and 9.6 Hz), 7.20 (d, 1H, *J* = 9.6 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): *d* 13.9, 14.2, 18.7, 19.5, 21.3, 26.5, 30.1, 31.2, 35.4, 54.6, 56.2, 61.0, 68.2, 132.9, 138.3, 167.8, 171.8, 174.4.

Tripeptide ethyl ester [*(R,S)(S)(S)*-77h]

It was obtained as oil (98%), following the general procedure 7, purified by flash chromatography (Hexan/AcOEt 1:2) starting from [*(R,S)(S)(S)*-**65a**] e cyclohexylamine.

R_f (Hexan/AcOEt 1:2) 0.2; MS, *m/z* (MALDI): 488 (M+ Na^+)

^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.78 (d, 3H, *J* = 6.4 Hz), 0.86 (d, 3H, *J* = 6.4), 0.97 (s, 9H), 1.11-2.11 (m, 4H), 1.30 (t, 3H, *J* = 7.2 Hz), 1.38 (d, 3H, *J* = 6.4 Hz), 1.3-1.6 (m, 4H), 1.5-1.6 (m, 2H), 1.6 (m, 1H), 1.86 (s, 3H), 1.90 (m, 1H), 2.10 (m, 1H), 2.90 (s, 3H), 3.21 (m, 1H), 4.18 (q, 2H), 4.8 (m, 1H), 5.10 (m, 1H), 6.60 (m, 1H), 8.10 (m, 1H).^{13}C NMR ($CDCl_3$, 400 MHz): *d* 13.1, 14.2, 20.0, 23.7, 25.7, 26.0, 29.7, 33.2, 33.9, 35.5, 56.6, 61.7, 64.9, 126.8, 137.3, 162.3, 171.0, 171.9

General procedure 8: hydrolysis of ethylic ester at C-terminus.

To a solution of tripeptide ethyl ester (60 mg, 0.12 mmol) in MeOH (2 mL) and H_2O (1 mL), LiOH monohydrate (50 mg, 1.2 mmol) was added and the reaction mixture stirred for 3 h at r. t. The mixture was acidified with TFA and then extracted with AcOEt (3 x 5 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the product as trifluoroacetate salt. The crude product was then purified by trituration with Et_2O (3 x 3mL).

Tripeptide trifluoroacetate salt [*(S)(S)(S)*-76a]

The crude product was purified by trituration with Et_2O (3 x 3mL), to afford a solid (86%). R_t*:* 19.81 MS, *m/z* (ES+): 474 (M+H)

^{1}H NMR (CD_3OD, 400 MHz): 0.77 (d, 3H, J = 6.4 Hz), 0.86 (d, 3H, J = 6.4 Hz), 0.9 (s, 9H), (d, 3H, *J* = 7.2 Hz), 1.75 (s, 3H), 1.80 (s, 3H), 1.84 (d, 3H,

J = 0.6 Hz), 1.90-2.03 (m, 1H), 2.62 (d, 2H, *J* = 15.6 Hz), 3.03 (s, 3H), 3.80 (q, 1H, *J* = 7.2 Hz), 4.58 (s, 1H), 4.95 (dd, 1H, *J* = 10 and 9.6 Hz), 6.65 (dd, 1H, *J* = 9.6 and 0.6 Hz), 7.42-7.56 (m, 5H). ^{13}C NMR (CD_3OD, 400 MHz) *d* 13.4, 15.1, 18.6, 19.3, 19.5, 19.6, 26.0, 26.2, 28.7, 30.8, 34.7, 42.8, 52.1, 54.4, 55.8, 64.8, 72.1, 125.8, 127.6, 128.5, 131.2, 131.8, 138.3, 168.5, 171.2, 174.8.

Tripeptide trifluoroacetate salt [*(R)(S)(S)*-76a]

It was obtained following the general procedure 8.

R_t: 21.02. MS, *m/z* (ES+): 475 (M+H)

^{1}H NMR (CD_3OD, 400 MHz) d 0.89 (d, 3H, *J* = 6.8 Hz), 0.92 (d, 3H, *J* = 6.4 Hz), 0.96 (s, 9H), 1.39 (d, 3H, *J* = 6.8 Hz), 1.63 (s, 3H), 1.75 (s, 3H), 1.89 (d, 3H, *J* = 0.8 Hz), 2.01-2.10 (m, 1H), 3.09 (s, 3H), 3.78 (q, 1h, *J* = 6.8 Hz), 4.73 (s, 1H), 5.01 (dd, 1H, *J* = 9.8 and 9.4 Hz), 6.90 (dd ,1H, *J* = 0.8 and 9.0 Hz), 7.42-7.57 (m, 5H).

^{13}C NMR(CD_3OD, 400 MHz): *d* 14.2, 18.9, 19.4, 19.9, 26.2, 26.8, 27.1, 31.1, 31.9, 35.6, 53.9, 57.9, 58.2, 64.4, 127.6, 130.6, 130.7, 134.0, 139.6, 139.9, 168.6, 171.0, 172.9.

Tripeptide trifluoroacetate salt [*(R)(R,S)(S)(S)*-76b]

It was obtained following the general procedure 8.

R_t: 11.66. MS, *m/z* (ES+): 460 (M+H)

^{1}H NMR (CD_3OD, 400 MHz) d 0.89 (d, 3H, *J* = 6.8 Hz), 0.92 (d, 3H, *J* = 6.4 Hz), 0.96 (s, 9H), 1.39 (d, 3H, *J* = 6.8 Hz), 1.63 (m, 3H), 1.77 (s, 3H), 1.94 (d, 1H, *J* = 0.8 Hz), 2.01-2.10 (m, 1H), 3.09 (s, 3H), 3.78 (q, 1H), 4.73 (m, 1H), 5.01 (d, 1H, *J* = 9.8), 6.90 (dd ,1H, *J* = 0.8 and 9.0 Hz), 7.42-7.57 (m, 5H).

^{13}C NMR(CD_3OD, 400 MHz): *d* 14.2, 18.9, 19.4, 19.9, 26.2, 26.8, 27.1, 31.1, 31.9, 35.6, 53.9, 57.9, 58.2, 64.4, 127.6, 130.6, 130.7, 134.0, 139.6, 139.9,161.44 168.6, 171.0, 172.9.

Tripeptide trifluoroacetate salt [*(S)(S)(S)*-76c]

It was obtained according general procedure 8 after purification by preparative HPLC to get a solid (84%).

R_t: 12.26. MS, *m/z* (ES+): 446 [M+H], 468 [M+Na].

^{1}H NMR (CD_3OD, 400 MHz): *d* 0.81 (d, 3H, *J* = 6.4 Hz), 0.89 (d, 3H, *J* = 6.5 Hz), 1.01 (s, 9H), 1.44 (d, 2H, *J* = 6.8 Hz), 1.89 (dd, 3H, *J* = 1.4 and 6.9 Hz), 1.95-2.10 (m, 1H), 3.13 (s, 3H), 3.95-4.20 (m, 3H), 4.76 (s, 1H), 5.04 (dd, 1H, *J* = 10 and 9.8 Hz), 6.74 (dd, 1H, *J* = 9.6 and 6.9 Hz), 7.46 (s, 5H). ^{13}C NMR (CD_3OD, 400 MHz): *d* 14.1, 16.8, 19.8, 26.7, 27.0, 30.9, 31.8, 35.6, 51.0, 56.2, 57.8, 58.0, 130.4, 131.1, 131.2, 132.2, 133.9, 170.1, 170.9, 172.7.

Tripeptide trifluoroacetate salt [*(R)(S)(S)*-76c]

It was obtained following the general procedure 8 after purification by preparative HPLC as solid (84%).

R_t: 12.12. MS, *m/z* (ES+): 446 [M+H], 468 [M+Na].

^{1}H NMR(CD3OD, 400 MHz): *d* 0.81 (d, 3H, *J* = 6.4 Hz), 0.90 (d, 3H, *J* = 6.5 Hz), 1.00 (s, 9H), 1.44 (d, 2H, *J* = 6.8 Hz), 1.88 (dd, 3H, *J* = 1.6 and 6.8 Hz), 1.95-2.10 (m, 1H), 3.09 (s, 3H), 4.83 (s, 1H), 5.09 (dd, 1H, *J* = 10 and 9.8 Hz), 6.72 (dd, 1H, *J* = 9.5 and 7.0 Hz), 7.46 (s, 5H). ^{13}C NMR (CD_3OD, 400 MHz): *d* 14.1, 17.3, 19.2, 26.7, 26.9, 31.0, 31.7, 35.9, 50.8, 56.6, 57.4, 58.2, 130.5, 130.8, 131.0, 132.1, 140.0, 170.2, 170.9, 172.8.

Tripeptide TFA salt [*(R,S)(S)(S)*-76d]

It was obtained according to the general procedure 8 as white solid (70%).

^{1}H NMR (CD_3OD, 400 MHz): δ 0.78 (d, 3H, J = 6.8 Hz), 0.82 (d, 3H, J = 6.8 Hz), 0.96 (s, 9H), 1.20 (m, 3H), 1.57 (s, 3H), 1.90-2.04 (m, 4H), 2.8-3.00 (m, 4H), 4.75 (dd, 1H, J = 3.2-7.2 Hz), 5.07-5.14 (m, 1H), 6.65 (d, 1H, J = 9.6 Hz), 7.08-7.82 (m, 7H), 8.23 (d, 1H). ^{13}C NMR (CD_3OD, 400 MHz): δ 14.0, 14.3, 18.9, 19.5, 21.7, 21.8, 25.5, 25.8, 26.5, 27.1, 29.8, 29.9, 31.2, 31.6, 32.1, 35.7, 53.3, 53.9, 55.9, 56.5, 56.7, 60.9, 122.5, 125.5, 126.6, 127.5, 128.3, 131.3, 132.7, 138.7, 138.8, 146.7, 147.6, 167.8, 171.2, 173.09, 175.9, 176.2, 186.80.

Tripeptide TFA salt [*(S)(S)(S)*-76e]

It was obtained following the general procedure 8.(95%)

^{1}H NMR (CD_3OD, 400 MHz): δ 0.77 (d, 3H, J = 6.8 Hz), 0.86 (d, 3H, J = 6.8 Hz), 0.90 (s, 9H), 1.32 (d, 3H, J = 7.2 Hz), 1.73 (s, 3H), 1.77 (s, 3H), 1.85 (s, 3H), 1.91-2.01 (m, 1H), 3.02 (s, 3H), 3.79 (q, 1H, J = 7.2 Hz), 4.50 (s, 1H), 4.98 (t, 1H, J = 10 Hz), 6.71 (d, 1H, J = 9.6 Hz), 7.47 (d, 2H, J = 8.8 Hz), 7.61 (d, 2H, J = 8.8 Hz). ^{13}C NMR (CD_3OD, 400 MHz): δ 14.1, 18.9, 19.2, 19.8, 25.9, 26.6, 26.9, 30.9, 31.7, 35.4, 53.6, 57.6, 58.1, 63.4, 124.6, 129.8, 133.3, 133.8, 139.8, 170.8, 171.1, 172.7.

Tripeptide TFA salt [*(R)(S)(S)*-76e]

It was obtained according to procedure 8 (95%)

^{1}H NMR (CD_3OD, 400 MHz): δ 0.88-0.97 (m, 6H,), 1.01 (s, 9H), 1.38 (d, 3H, J = 6.8 Hz), 1.51 (s, 3H), 1.67 (s, 3H), 1.95 (s, 3H), 1.98-2.01 (m, 1H), 3.13 (s, 3H), 3.70 (q, 1H, J = 7.2 Hz), 4.96 (t, 1H, J = 4.4 Hz), 6.73 (dd, 1H, J = 1.3-4.1 Hz), 7.41 (d, 2H, J = 6.8 Hz), 7.61 (d, 2H, J = 6.8 Hz). ^{13}C NMR

(CD_3OD, 400 MHz): δ 14.1, 18.8, 18.9, 19.9, 20.1, 24.3, 26.9, 28.3, 30.7, 31.8, 35.8, 53.9, 57.6, 59.6, 63.8, 124.5, 129.2, 132.9, 133.6, 138.9, 139.3, 170.8, 171.1, 172.9.

Tripeptide TFA salt [*(R,S)(S)(S)*-76f]

It was obtained following general procedure 8 (95%)

^{1}H NMR (CD3OD, 400 MHz): δ 0.82 (d, 6H, J = 6.6 Hz), 0.89 (d, 6H, J = 6.5 Hz), 1.01 (s, 9H), 1.04 (s, 9H), 1.43 (d, 3H, J = 7 Hz), 1.51 (d, 3H, J = 7 Hz), 1.87 (d, 3H, J = 1.5 Hz), 1.91 (d, 3H, 1,5), 1.95-2.08 (m, 1H), 3.09 (s, 3H), 3.16 (s, 1H), 3.85 (s, 3H), 3.98-4.05 (m, 1H), 4.09 (q, 1H, J = 7 Hz), 4.18 (d, 1H, J = 13 Hz), 4.31 (d, 1H, J = 13 Hz), 4.79 (s, 1H), 4.82 (s, 1H), 5.04 (t, 1H, J = 10 Hz), 5.10 (t, 1H, J = 10 Hz), 6.72-6.79 (m, 1H), 7.15-7.20 (m, 1H), 7.24-7.29 (m, 1H), 7.37 (s, 1H), 7.40 (s, 1H), 7.43 (d, 1H, J = 8 Hz), 7.45 (d, 1H, J = 8 Hz), 7.69-7.74 (m, 1H)

^{13}C NMR (CD_3OD, 400 MHz): δ 14.3, 14.7, 17.2, 17.6, 19.5, 20.0, 22.4, 24.4, 27.1, 27.2, 28.2, 31.1, 31.2, 32.0, 32.1, 33.3, 35.8, 36.1, 42.2, 55.5, 55.6, 57.7, 58.1, 58.2, 58.5, 61.7, 104.5, 104.6, 111.1, 111.2, 119.6, 119.7, 121.5, 123.8, 128.7, 132.4, 132.7, 133.9, 134.1, 138.8, 140.0, 170.5, 171.0, 173.0, 173.2.

2-Hydroxypropanoyl-dipeptide [*(S)(S)(S)*-78g]

It was obtained following the general procedure 8 (94%).

R_t: 15.3; MS, *m/z* (ES+): 357 (M+H).

^{1}H NMR (DMSO-D_6): *d* 0.70 (d, 3H, *J* = 6.8 Hz), 0.79 (d, 3H, *J* = 6.8 Hz), 0.89 (s, 9H), 1.21 (d, 3H, *J* = 6.8 Hz), 1.77 (d, 3H, *J* = 0.8 Hz), 1.82-1.98 (m, 1H), 2.93 (s, 3H), 3.98 (q, 1H, *J* = 6.8 Hz), 4.69 (d, 1H, *J* = 10.0 Hz), 4.92 (dd, 1H, *J* = 10.0 and 10.3 Hz), 5.40 (br s, 1H), 6.62 (dd, 1h, *J* = 10.0

and 0.8 Hz), 7.44 (d, 1H, *J* = 9.6 Hz). ^{13}C NMR (DMSO-D_6): *d* 13.9, 18.5, 19.3, 21.6, 26.0, 28.8, 42.6, 53.1, 55.6, 67.0, 72.3, 131.8, 138.2, 168.5, 171.2, 174.5.

2-Hydroxypropanoyl-dipeptide [*(R)(S)(S)*-78g]

It was obtained according to procedure 8 (90%).

R_f: 14.7; MS, *m/z* (ES+): 357 (M+H).

^{1}H NMR (DMSO-D_6): *d* 0.73 (d, 3H, *J* = 6.7 Hz), 0.81 (d, 3H, *J* = 6.8 Hz), 0.93 (s, 9H), 1.18 (d, 3H, *J* = 6.9 Hz), 1.71 (d, 3H, *J* = 0.7 Hz), 1.86-2.01 (m, 1H), 2.95 (s, 3H), 4.04 (q, 1H, *J* = 6.8 Hz), 4.62 (d, 1H, *J* = 10.1 Hz), 4.99 (dd, 1H, *J* = 10.1 and 10.4 Hz), 5.42 (br s, 1H), 6.66 (dd, 1h, *J* = 10.4 and 0.7 Hz), 7.51 (d, 1H, *J* = 10.1 Hz). ^{13}C NMR (DMSO-D_6): *d* 13.7, 19.1, 19.2, 21.9, 26.3, 28.0, 42.4, 53.5, 55.9, 66.8, 72.5, 131.9, 138.2, 168.4, 171.5, 174.7.

Tripeptide TFA salt [*(R,S)(S)(S)*-79h]

It was obtained according to general procedure 8.(white solid, 60%)

^{1}H NMR (CD_3OD, 400 MHz): *d* 0.78 (d, 3H, *J* = 6.4 Hz), 0.86 (d, 3H, *J* = 6.4), 0.97 (s, 9H), 1.11-2.11 (m, 4H), 1.38 (d, 3H, *J* = 6.4 Hz), 1.3-1.6 (m, 4H), 1.5-1.6 (m, 2H), 1.6 (m, 1H), 1.86 (s, 3H), 1.90 (m, 1H), 2.10 (m, 1H), 2.90 (s, 3H), 3.21 (m, 1H), 4.8 (m, 1H), 5.10 (m, 1H), 6.60 (m, 1H), 8.10 (m, 1H).^{13}C NMR (CD_3OD, 400 MHz): *d*, 20.0, 23.7, 25.7, 26.0, 29.7, 33.2, 33.9, 35.5, 56.6, 61.7, 64.9, 126.8, 137.3, 162.3, 171.0, 171.9

Tripeptide ethyl ester valine derivative [*(S)(S)(S)*-80a]

It was obtained according to general procedure 8 (white solid, 64%).

MS, *m/z* (ES+): 439 [M+H], 460 [M+Na].

^{1}H NMR ($CDCl_3$, 400 MHz) 0.81 (d, 3H, *J* = 6.4 Hz), 0.89 (d, 3H, *J* = 6.5 Hz), 1.01 (s, 9H), 1.44 (d, 2H, *J* = 6.8 Hz), 1.89 (dd, 3H, *J* = 1.4 and 6.9 Hz), 1.95-2.10 (m, 1H), 3.13 (s, 3H), 3.95-4.20 (m, 3H), 4.76 (s, 1H), 5.04 (dd, 1H, *J* = 10 and 9.8 Hz), 6.74 (dd, 1H, *J* = 9.6 and 6.9 Hz), 7.46 (s, 5H). ^{13}C NMR ($CDCl_3$, 400 MHz) 14.1, 16.8, 19.8, 26.7, 27.0, 30.9, 31.8, 35.6, 51.0, 56.2, 57.8, 58.0, 130.4, 131.1, 131.2, 132.2, 133.9, 170.1, 170.9, 172.7.

Tripeptide ethyl ester [*(R)(S)(S)*-80a]

It was obtained following the general procedure 7 after purification by column chromatography (Hexane/AcOEt 1/1) to get a solid (92%).

R_f (Hexane/AcOEt 1/3) 0.36

^{1}NMR ($CDCl_3$, 400 MHz): d 0.82 (d, 3H), 0.89 (d, 9H, J = 6.4 Hz), 1.17 (d, 3H, *J* = 7.2 Hz), 1.31 (t, 3H, *J* = 6.8), 1.38 (s, 3H), 1.40 (s, 3H), 1.63 (br, 1H), 1.88-1.95 (m, 1H), 1.91 (d, 3H, *J* = 0.8 Hz), 1.97-2.08 (m, 1H), 2.90 (q, 1H, *J* = 7.2 Hz), 2.97 (s, 3H), 4.20 (q, 2H, *J* = 6.8 Hz), 4.68 (dd, 1H, *J* = 7.2 and 9.2 Hz), 5.08 (dd, 1H, *J* = 10.0 and 6.0 Hz), 6.65 (dd, 1H, *J* = 0.8 and 9.2 Hz), 7.13-7.38 (m, 5H), 8.18 (d, 1H, *J* = 9.2 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): *d* 13.8, 14.3, 17.7, 18.9, 19.5, 19.6, 21.9, 25.6, 30.0, 30.6, 31.7, 32.0, 53.2, 53.5, 56.3, 56.8, 60.9, 103.7, 125.6, 126.6, 128.3, 138.6, 147.5, 167.9, 171.8, 176.6.

Tripeptide ethyl ester [*(S)(S)(S)*-80c]

It was obtained following the general procedure 7 as a solid (89%).

R_f (CH_2Cl_2/MeOH/Toluene 17/2/2.5) 0.3. MS, *m/z* (ES+): 460 [M+H], 482 [M+Na].

^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.80 (d, 3H, *J* = 6.8 Hz), 0.86-0.95 (m, 9H), 1.28 (t, 3H, *J* = 7.1 Hz), 1.43 (d, 3H, *J* = 6.9 Hz), 1.80 (br s, 1H), 1.90-1.96 (m, 4H), 1.99-2.11 (m, 1H), 2.98 (s, 3H), 3.24 (q, 1H, *J* = 6.9 Hz), 3.59-3.69 (m, 2H), 4.22 (q, 2H, *J* = 7.0 Hz), 4.78 (dd, 1H, *J* = 9.6 and 10.3 Hz), 5.02 (dd, 1H, *J* = 9.6 and 7.0 Hz), 6.62 (dd, 1H, *J* = 1.2 and 7.0 Hz), 7.31-7.33 (m, 5H), 7.75 (d, 1H, *J* = 9.6 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): *d* 14.3, 17.5, 18.8, 19.0, 19.5, 19.6, 20.2, 29.9, 30.4, 31.3, 52.6, 53.7, 56.6, 57.7, 61.0, 127.3, 128.6, 128.8, 132.9, 138.1, 138.3, 167.8, 172.1, 175.1.

Tripeptide ethyl ester [*(R)(S)(S)*-80c]

It was obtained following the general procedures 8 as white solid (81%).

R_f (CH_2Cl_2/MeOH/Toluene 17/2/2.5) 0.3. MS, *m/z* (ES+): 460 [M+H], 482 [M+Na].

^{1}H NMR ($CDCl_3$, 400 MHz): *d* 0.82 (d, 3H, *J* = 6.8 Hz), 0.85-0.95 (m, 9H), 1.27 (t, 3H, *J* = 7.0 Hz), 1.41 (d, 3H, *J* = 6.9 Hz), 1.84 (br s, 1H), 1.92-1.98 (m, 4H), 2.00-2.15 (m, 1H), 2.97 (s, 3H), 3.22 (q, 1H, *J* = 6.9 Hz), 3.48-3.59 (m, 2H), 4.20 (q, 2H, *J* = 7.0 Hz), 4.80 (dd, 1H, *J* = 9.7 and 10.4 Hz), 5.05 (dd, 1H, *J* = 9.6 and 7.0 Hz), 6.62 (dd, 1H, *J* = 1.2 and 7.1), 7.30-7.33 (m, 5H), 7.75 (d, 1H, *J* = 9.7 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): *d* 13.7, 14.2, 17.5, 17.8, 18.8, 19.4, 19.6, 20.2, 21.4, 29.9, 30.4, 31.1, 31.3, 52.6, 53.4, 53.7, 56.3, 56.6, 57.7, 57.9, 61.0, 68.3, 127.3, 128.1, 128.4, 128.6, 132.9, 138.1, 138.4, 138.5, 167.8, 172.0.

Tripeptide trifluroacetate salt [(*S)(S)(S)*-81a]

It was obtained following the general procedure 8 as white solid (92%).

R_t: 16.28. MS, *m/z* (ES+): 488 [M+H].

^{1}H NMR (CD_3OD, 400 MHz): *d* 0.79 (d, 3H, *J* = 6.0 Hz), 0.83-0.88 (m, 6H), 1.33 (d, 3H, *J* = 7.2 Hz), 1.76 (s, 3H), 1.80 (s, 3H), 1.84 (d, 3H, *J* = 1.2Hz), 1.83-1.99 (m, 2H), 3.00 (s, 3H), 3.68 (q, 1H, *J* = 7.2Hz), 4.35 (dd, 1H, *J* = 9.3 and 9.9 Hz), 4.96 (dd, 1H, *J* = 10.1 and 9.6 Hz), 6.71 (dd, 1H, *J* = 9.9 and 1.2 Hz), 7.43-7.58 (m, 5H). ^{13}C NMR (CD_3OD): *d* 13.8, 15.6, 18.8, 18.9, 19.0, 19.7, 25.0, 29.4, 30.1, 30.6, 43.1, 52.2, 54.9, 56.5, 65.4, 72.9, 126.8, 129.2, 132.4, 138.6, 169.0, 171.3, 171.7, 175.0.

Tripeptide trifluoroacetate salt [*(R)(S)(S)*-81a]

It was obtained following the general procedure 8 as white solid (95%).

R_t: 21.0, MS, *m/z* (ES+): 342, 459 [M+H].

^{1}H NMR (CD_3OD, 400 MHz) *d* 0.88-0.93 (m, 12H), 1.39 (d, 3H, *J* = 7.2 Hz), 1.65 (s, 3H), 1.76 (s, 3H), 1.88 (d, 3H, *J* = 1.6 Hz), 1.92-2.10 (m, 2H), 3.07 (s, 3H), 3.66 (q, 1H, *J* = 7.2 Hz), 4.53 (d, 1H, *J* = 7.6 Hz), 4.99 (dd, 1H, *J* = 10.4 and 10.0 Hz), 6.76 (dd, 1H, *J* = 1.6 and 9.2 Hz), 7.45-7.59 (m, 5H). ^{13}C NMR (CD_3OD, 400 MHz): *d* 12.5, 17.0, 17.7, 17.9, 18.3, 23.0, 27.0, 29.4, 29.8, 30.2, 52.7, 55.3, 57.4, 63.0, 110.0, 125.8, 129.0, 129.1, 132.3, 138.0, 138.0, 161.0, 169.3, 171.8.

Tripeptide trifluoroacetate salt [*(S)(S)(S)*-81c]

It was obtained following the general procedure 8 after purification by preparative HPLC as solid (87%).

R_t: 16.06, MS, *m/z* (ES+): 432 [M+H]

^{1}H NMR (CD_3OD, 400 MHz) *d* 0.83 (d, 3H, *J* = 6.4 Hz), 0.89-1.00 (m, 9H), 1.46 (d, 3H, *J* = 6.8 Hz), 1.86 (d, 3H, *J* = 1.6 Hz), 1.90-1.98 (m, 2H), 3.12 (s, 3H), 3.95-4.15 (m, 3H), 4.60 (d, 1H, *J* = 8.1Hz), 5.01 (dd, 1h, *J* = 10.4 and 9.6 Hz), 6.75 (dd, 1H, *J* = 1.6 and 9.6 Hz), 7.46 (s, 5H). ^{13}C NMR

(CD_3OD, 400 MHz) *d* 14.0, 17.8, 18.9, 19.4, 19.5, 20.5, 21.4, 30.0, 30.5, 31.0, 52.6, 53.5, 56.3, 57.9, 68.4, 128.1, 128.4, 128.8, 132.9, 138.0, 138.4, 167.8, 172.0, 174.5.

Tripeptide trifluoroacetate salt [*(R)(S)(S)*-81c]

It was obtained by hydrolysis of the relative ester after purification by preparative HPLC to afford a solid (85%).

R_t: 16.45. MS, *m/z* (ES+): 432 [M+H]

^{1}H NMR (CD_3OD, 400 MHz): *d* 0.89 (d, 3H, *J* = 6.5 Hz), 0.91-1.02 (m, 9H), 1.53 (d, 3H, *J* = 6.8 Hz), 1.88 (d, 3H, *J* = 1.6 Hz), 1.98-2.05 (m, 2H), 3.09 (s, 3H), 3.91-4.10 (m, 3H), 4.56 (d, 1H, *J* = 8 Hz), 5.04 (dd, 1h, *J* = 10.4 and 9.5 Hz), 6.75 (dd, 1H, *J* = 1.6 and 9.5 Hz), 7.45 (s, 5H).^{13}C NMR (CD_3OD, 400 MHz) *d* 14.1, 16.7, 18.9, 19.3, 19.5, 19.8, 30.9, 31.2, 31.4, 50.8, 56.4, 56.9, 58.6, 130.3, 130.8, 131.1, 132.1, 133.8, 139.5, 169.9, 170.8, 173.8.

2-Hydroxypropanoyl-dipeptide ethyl ester [*(S)(S)(S)*-82g]

It was obtained following the general procedure 7 as solid (97%).

R_f (hexane/AcOEt 1/3) 0.61.

^{1}H NMR (DMSO-$_{d6}$, 400 MHz): *d* 0.79 (d, 3H, *J* = 6.8 Hz), 0.86-0.92 (m, 6H), 1.29 (t, 3H, *J* = 76.8 Hz), 1.46 (d, 3H, *J* = 7.2 Hz), 1.83-1.88 (m, 1H), 1.86 (d, 1H, *J* = 0.7 Hz), 1.88-2.07 (m, 1H), 2.91 (s, 3H), 2.95 (br s, 1H), 4.19 /q, 2H, *J* = 7.2 Hz), 4.78 (dd, 1H, *J* = 9.6 and 7.3 Hz), 5.02 (dd, 1H, *J* = 10.1 and 6.7 Hz), 5.18 (q, 1H, *J* = 7.2 Hz), 6.62 (dd, 1H, *J* = 0.7 and 9.6 Hz), 6.68 (d, 1H, *J* = 10.1 Hz). ^{13}C NMR (DMSO-$_{d6}$, 400 MHz): *d* 13.8, 14.3, 17.3, 17.6, 18.0, 19.4, 19.6, 27.1, 30.0, 31.6, 53.3, 56.4, 60.1, 70.4, 133.0, 138.3, 167.7, 171.4, 177.0.

2-Hydroxypropanoyl-dipeptide ethyl ester [*(R)(S)(S)*-82g]

It was obtained following the general procedure 7 as white solid (88%).

R_f (Hexane/AcOEt 1/3) 0.42; (hexane/AcOEt 1/2) 0.2.

^{1}H NMR: (CD_3OD, 400 Mhz) *d* 0.81 (d, 3H, *J* = 6.8 Hz), 0.88-0.92 (m, 9H), 1.30 (t, 3H, *J* = 7.6 Hz), 1.40 (d, 3H, *J* = 7.2 Hz), 1.72 (br s, 1H), 1.85-1.98 (m, 1H), 1.88 (d, 3H, *J* = 1.6 Hz), 1.98-2.01 (m, 1H), 2.97 (s, 3H), 4.20 (q, 2H, *J* = 7.6 Hz), 4.26 (q, 1H, *J* = 7.2 Hz), 4.73 (dd, 1H, *J* = 6.8 and 8.8 Hz), 5.02 (dd, 1H, *J* = 10 and 9.9 Hz), 6.63 (dd, 1H, *J* = 1.6 and 8.8Hz), 7.08 (d, 1H, *J* = 9.2Hz). ^{13}C NMR (CD_3OD, 400 MHz) *d* 13.7, 14.3, 17.7, 18.8, 19.4, 19.6, 21.4, 30.0, 31.1, 31.2, 53.9, 56.6, 61.0, 68.2, 133.0, 138.1, 167.8, 172.1, 174.5.

2-Hydroxypropanoyl-dipeptide [*(S)(S)(S)*-83g]

It was obtained following the general procedure 8 as white solid (90%).

^{1}H NMR (CD_3OD, 400 MHz): d 0.71 (d, 3H, *J* = 6.4 Hz), 0.77-0.86 (m, 9H), 1.15 (d, 3H, *J* = 6.4 Hz), 1.76 (d, 3H, *J* = 1.2 Hz), 1.90-2.03 (m, 2H), 2.92 (s, 3H), 3.97 (q, 1H, *J* = 6.4 Hz), 4.54 (dd, 1H, *J* = 6.8 and 9.0 Hz), 4.85 (dd, 1H, *J* = 9.8 and 9.5 Hz), 5.2 (br, 2H), 6.61 (dd, 1H, *J* = 1.2 and 9.3 Hz), 7.45 (d, 1H, *J* = 8.3 Hz). ^{13}C NMR d 13.2, 17.6, 18.4, 18.6, 18.9, 19.0, 21.2, 28.8, 30.2, 52.7, 67.0, 113.8, 138.3, 168.4, 171.0, 174.0.

2-Hydroxypropanoyl-dipeptide [*(R)(S)(S)*-83g]

It was obtained following the general procedure 8 as solid (87%).

^{1}H NMR (CD_3OD, 400 MHz) *d* 0.72 (d, 3H, *J* = 6.4 Hz), 0.79-0.82 (m, 9H), 1.20 (d, 3H, *J* = 6.4 Hz), 1.75 (d, 3H, *J* = 1.2 Hz), 1.91-2.06 (m, 2H), 2.91 (s, 3H), 4.02 (q, 1H, *J* = 6.4 Hz), 4.52 (dd, 1H, *J* = 6.9 and 9.0 Hz), 4.87 (dd, 1H, *J* = 9.9 and 9.6 Hz), 5.8 (br, 2H), 6.62 (dd, 1H, *J* = 1.2 and 9.3 Hz),

7.58 (d, 1H, *J* = 8.2 Hz). ^{13}C NMR (CD_3OD, 400 MHz): *d* 13.2, 17.4, 18.5, 18.7, 18.9, 19.2, 20.9, 28.9, 30.1, 52.9, 56.0, 56.1, 66.9, 113.9, 138.1, 168.4, 171.1, 174.0.

_(S)_-2-bromo-3-phenylpropanoic acid [_(S)_-90]

It was prepared following the same procedure for [*(S)*-67] as solid 60%.

^{1}H NMR (CD_3OD, 200 MHz): δ 3.18-3.30 (dd, 1H, *J* = 8, *J* = 10 Hz), 3.42-3.54 (dd, 1H, *J* = 7.2, *J* = 10 Hz), 4.43 (t, 1H, *J* = 7.4 Hz), 7.18-7.38 (m, 5H)

^{13}C NMR(CD_3OD, 400 MHz): δ 39.1, 53.1, 125.9, 127.7, 128.6, 139.4, 171.0

_(R)_-2-bromo-3-phenylpropanoic acid [_(R)_-89]

It was obtained following the same procedure for [***(S)***-67] as solid 60%.

^{1}H NMR (CD_3OD, 200 MHz): δ 3.19-3.30 (dd, 1H, *J* = 8, *J* = 10 Hz), 3.42-3.53 (dd, 1H, *J* = 7.2, *J* = 10 Hz), 4.43 (t, 1H, *J* = 7.4 Hz), 7.19-7.39 (m, 5H)

^{13}C NMR (CD_3OD, 200 MHz): δ 39.2, 53.2, 127.8, 126.0, 128.7, 139.5, 171.1

Bromoacyl-dipeptide [*(S)(S)(S)*-91]

It was obtained following the general procedure 3, after chromatography purification. as an oil, 60% (Hexan/AcOEt 5/1): R_f = 0.2

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.71 (d, 3H, J = 6.4 Hz), 0.86 (d, 3H, J = 6.4 Hz), 0.94 (s, 9H), 1.30 (t, 3H, J = 7.2 Hz), 1.81-1.92 (m, 1H), 1.89 (s, 3H), 2.94 (s,3H), 3.19-3.57 (dd, 2H, J = 8, 6.4 Hz), 4.19 (q, 2H, J = 7.2 Hz), 4.44 (t, 1H, J = 6.4 Hz), 4.78 (d, 1H, J = 9.6 Hz), 5.06 (t, 1H, J = 9.6 Hz), 6.62

(d, 1H, 5.2 Hz), 6.84 (d, 1H, J = 9.2 Hz), 7.19-7.31 (m, 5H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 13.9, 14.3, 18.8, 19.5, 26.5, 30.0, 31.1, 36.2, 41.3, 50.2, 55.3, 56.2, 60.9, 127.2, 128.6, 129.3, 132.9, 137.1, 138.2, 167.8, 167.9, 171.0.

Bromoacyl-dipeptide [*(R)(S)(S)*-91]

It was obtained following the general procedure 3, affording the product as oil (60%)

^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 0.82 (d, 3H, *J* = 6.5 Hz), 0.86 (s, 9H), 0.88 (d, 3H, *J* = 6.5 Hz), 1.30 (t, 3H, *J* = 7.2 Hz), 1.89 (s, 3H), 1.85-1.95 (m, 1H), 2.97 (s, 3H), 3.13-3.54 (dd, 2H, *J* = 7.2-7.6 Hz), 4.19 (q, 2H, *J* = 7.2 Hz), 4.39 (t, 1H, *J* = 7.2 Hz, 4.77 (d, 1H, *J* = 9.6 Hz), 5.09 (t, 1H, *J* = 5.6 Hz), 6.63 (d, 1H, *J* = 9.2 Hz), 6.80 (d, 1H, *J* = 9.2 Hz), 7.19-7.32 (m, 5H). ^{13}C NMR ($CDCl_3$, 400 MHz): *δ* 13.8, 14.3, 18.9, 19.5, 26.4, 30.0, 31.1, 35.6, 41.7, 50.0, 55.4, 56.2, 60.9, 127.3, 128.6, 129.3, 132.9, 137.1, 138.3, 167.8, 168.1, 171.1

Tripeptide ethyl ester[*(S)(S)(S)*-92]

It was obtained following the general procedure 7, R_f (Hexan/AcOEt 3/1) 0.20 as solid (85%)

^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 0.81 (d, 3H, *J* = 6.8 Hz), 0.88 (d, 3H, *J* = 6.4 Hz), 0.98 (s, 9H), 1.19 (t, 3H), 1.29 (s, 6H), 1.93 (s, 3H), 1.85-1.95 (m, 1H), 2.55 (dd, 1H, *J* = 4, *J* = 9.2 Hz), 2.99 (dd, 1H, *J* = 4, *J* = 10 Hz), 3.05 (s, 3H), 3.13 (dd, 1H, *J* = 4, *J* = 5.6 Hz), 4.21 (q, 2H, *J* = 7.2 Hz), 4.80 (d, 1H, *J* = 9.6 Hz), 5.13 (t, 1H, *J* = 10 Hz), 6.67 (d, 1H, *J* = 9.6 Hz), 7.01 (m, 4H), 7.11 (m, 3H), 7.21 (m, 3H), 8.31 (d, 1H, *J* = 9.6 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): *δ* 14.1, 14.3, 19.0, 19.5, 25.2, 26.6, 29.8, 29.9, 31.3, 35.6, 40.6, 54.2,

56.0, 56.2, 59.0, 60.9, 125.4, 126.3, 126.9, 128.2, 128.8, 129.4, 132.6, 137.2, 138.8, 146.9, 167.9, 171.1, 175.4

Tripeptide ethyl ester [*(R)(S)(S)*-92]

It was obtained following the general procedure 8, as solid 85%.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.80 (d, 3H, J = 6.4 Hz), 0.87 (d, 3H, J = 6.4 Hz), 0.99 (s, 9H), 1.26 (t, 3H), 1.30 (s, 6H), 1.82-1.95 (m, 1H), 1.93 (s, 3H), 2.49 (q, 1H, J = 4, J = 9.6 Hz), 2.89 (dd, 1H, J = 4, J = 9.6 Hz), 3.02 (s, 3H), 3.20 (dd, 1H, J = 4.4, J = 5.2 Hz), 4.20 (q, 2H, J = 7.2 Hz), 4.79 (d, 1H, J = 10 Hz), 5.13 (t, 1H, J = 10 Hz), 6.64 (d, 1H, J = 8 Hz), 6.94-7.26 (m, 10H), 7.95 (d, 1H, J = 9.6 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 13.9, 14.3, 18.9, 19.5, 26.5, 26.7, 30.0, 31.1, 31.2, 35.4, 40.354.9, 55.9, 56.1, 58.8, 60.9, 125.4, 126.4, 126.8, 128.2, 128.8, 129.4, 132.7, 137.2, 138.5, 146.9, 167.9, 171.7, 175.6

Tripeptide TFA salt [*(S)(S)(S)*-93]

It was obtained following general procedure 8, as a solid (95%).

^{1}H NMR (CD_3OD, 400 MHz): δ 0.83 (s, 9H), 0.88 (d, 3H, J = 6.4 Hz), 0.93 (d, 3H, J = 6.4 Hz), 0.94-1.01 (m, 1H), 1.68 (s, 3H), 1.77 (s, 3H), 1.87 (s, 3H), 1.85-2.00 (m, 1H), 3.00 (s, 3H), 2.91-3.01 (m, 1H), 3.45-3.52 (m, 1H), 4.72 (d, 1H, J = 8.4 Hz), 5.00 (t, 1H, J = 10 Hz), 6.48 (d, 1H, J = 10 Hz), 6.55 (d, 1H, J = 9.2 Hz 6.67 (d, 1H, J = 6.8Hz), 6.74 (d, 1H, J = 8.8 Hz), 6.83 (m, 2H). 7.15-7.25 (m, 3H), 7.33-7.37 (m, 3H), 7.41-7.46 (m, 2H). ^{13}C NMR (CD_3OD, 400 MHz): δ 13.7, 18.9, 19.3, 19.6, 20.4, 24.4, 26.4, 26.6, 27.8, 29.8, 31.6, 35.3, 37.8, 56.2, 57.1, 58.9, 62.3, 63.1, 126.2, 126.4, 127.8, 128.8, 128.9, 129.1, 129.2, 129.5, 131.9, 134.5, 138.8, 139.9, 162.0, 169.2, 170.9.

Tripeptide TFA salt [*(R)(S)(S)*-93]

It was obtained following the general procedure 8, as solid (95%)

^{1}H NMR (CD_3OD, 400 MHz): δ 0.61 (s, 9H), 0.85 (d, 3H, J = 6.4 Hz), 0.90 (d, 3H, J = 6.4 Hz), 0.95-1.02 (m, 1H Hz), 1.58 (s, 3H), 1.84 (s, 3H), 1.87 (s, 3H), 1.88-2.01 (m, 1H), 3.01 (s, 3H), 2.94-3.03 (m, 1H), 3.11-3.19 (m, 1H), 3.55-3.61 (dd, 1H, J = 4.8 Hz), 4.61 (d, 1H, J = 8.4 Hz), 5.05 (t, 1H, J = 10 Hz), 6.43 (m, 1H), 6.74 (d, 1H, J = 10 Hz), 6.78-6.81 (m, 2H), 7.13-7.21 (m, 3H), 7.37-7.47 (m, 3H), 7.57-7.61 (m, 2H). ^{13}C NMR (CD_3OD, 400 MHz): δ 13.7, 19.0, 19.4, 26.1, 26.5, 28.6, 29.7, 31.3, 34.8, 37.8, 56.7, 57.2, 58.7, 63.3, 126.4, 127.9, 129.0, 129.3, 129.5, 129.6, 132.0, 134.3, 138.1, 139.4, 161.7, 170.9, 171.3

(S)-ethyl (3-(2-(*tert*-butoxycarbonylamino)-3,3-dimethylbutanamido)benzoate [*(S)*-85]

It was obtained following the general procedure 1 as oil (73%) after purification (Hexan/AcOEt 2:1).

R_f (Hexan/AcOEt 2:1) 0.5, *m/z* (ES+): 432 [M+H]

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.79 (s, 9 H), 1.32-1.43 (m, 9H), 4.36 (q, 2H, J = 7.2 Hz), 5.2 (d, 1H), 7.23 (s, 1H), 7.7 (m, 2H), 7.9 (m, 1H), 8.03 (s, 1H), 8.13 (d, J = 2 Hz, 1 H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ 13.8, 25.8, 28.4, 45.1, 60.8, 66.8, 79.5, 119.5, 124.1, 127.4, 133.7, 137.1, 156.0, 164.1, 172.0, 185.6.

(S)-ethyl 3-(2-amino-3,3-dimethylbutanamido)benzoate [*(S)*-85a]

It was obtained following the general procedure 2 as oil (90%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.98 (s, 9 H), 1.29 (t, 3H, J = 7.2 Hz), 4.36 (q, 2H, J = 7.2 Hz), 5.2 (s, 1H), 7.23 (s, 1H), 7.7 (dd, 1H), 7.9 (dd, 1H), 8.03 (d, 1H), 8.13 (d, J = 2 Hz, 1 H), 9.02 (bs, 2H).

^{13}C NMR ($CDCl_3$, 400 MHz): δ 13.8, 25.8, 28.4, 45.1, 60.8, 73.2, 119.5, 124.1, 127.4, 133.7, 138.1, 144.7, 164.1, 172.0, 185.6.

[*(R,S)(S)*-86] bromo-acyl-peptide.

It was obtained following the general procedure 3 as oil (80%) after purification (Hexan/AcOEt 2:1) 0.48

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.98 (s, 9 H), 1.29 (t, 3H, J = 7.2 Hz), 1.97 (d, 3H), 4.36 (q, 2H, J = 7.2 Hz), 5.2 (s, 1H), 7.23 (s, 1H), 7.54 (dd, 1H), 7.8-8.3 (m, 2H), 8.03 (s, 1H), 8.3 (d, J = 2 Hz, 1 H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.1, 22.0, 25.8, 35.2, 45.6, 60.9, 64.6, 120.6, 125.5, 128.8, 130.3, 141.4, 165.9, 172.0, 175.4.

[*(R,S)(S)*-87] tripeptide

It was obtained following the general procedure 7 as oil (78%)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.94 (s, 9H), 1.27-1.29 (m, 9H), 2.0 (s, 1H), 3.74 (m, 1H), 4.30 (q, 2H), 4.51 (s, 1H), 7.23-7.37 (m, 6H), 7.54 (d, 1H), 7.82 (m, 1H), 8.03 (s, 1H), 8.29 (d, 1H).

^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.1, 24.0, 25.8, 32.0, 35.2, 56.8, 60.9, 65.3, 120.6, 125.9, 126.1, 128.4, 130.3, 141.4, 144.6, 165.9, 171.7

[*(R,S)(S)*-88] Tripeptide salt.

It was obtained following the general procedure 8 as white solid (55%).

^{1}H NMR (CD_3OD, 400 MHz): δ 0.97 (s, 9H), 1.42 (d, 1H), 1.73 (s, 3H), 1.73 (s, 3H), 3.9 (m, 1H), 7.2 (t, 1H), 7.2 (t, 2H), 7.48-7.52 (m, 3H), 7.82 (m, 2H), 8.31 (d, 1H).

^{13}C NMR (CD_3OD, 400 MHz): δ 18.97, 25.8, 35.2, 54.5, 56.9, 65.3, 120.6, 125.9, 126.1, 128.4, 130.3, 130.6, 133, 138.8, 140.0, 165.9, 170.3, 170.7

m/z (ES+): 440 [M+H]

Chapter 8

Hemiasterlin: references

8. Hemiasterlin: references

1. Glad, S.C. *Drug Discovery Handbook* **2005**, John Wiley & Sons, Inc.
2. Harvey, A.L. *Drug Discovery Today* **2008**, *13*, 894-90.
3. Rang, H.P., Dale, M.M., Ritter, J.M., *Farmacologia*, II ed.
4. Ravelo, A. G.; Estevez-Braun, A.;Chavez-Orellana, H.; Perez-Sacau, E.; Mesa-Siviero, D. J. *Curr.Top.Med.Chem.* **2004**, *4*, 241-265.
5. http://www.elmhurst.edu/~chm/vchembook/655cancer.html
6. Jordan, M. A. *Curr.Med.Chem.-Anti-Cancer Agents* **2002**, *2*, 1-17.
7. (a) Xu, F.L., Saunders, W.S. *Cancer Cells* **2008**, 14, 197-199.
8. Valiron, O., Caudron, N., Tob, D. *Cell. Mol. Life Sci.* **2001**, *58*, 2069-2084.
9. Zhou, J., Giannakakou, P. *Curr. Med. Chem.- Anti Agents* **2005**, *5*, 65-71.
10. Solomon, P., Berg, L.R., D.W. Martin Villee, *Biologia*, Edises.
11. Kozawa, T., Nakagawa-Goto, K., Shi, Q., Lay, C.Y, Hamel, E., Bastow, K.F., Brossi, A., Lee, K.H. *Bioorg.Med.Chem.Lett*. **2010**, *20* (14), 4091-4.
12. Lobert, S., Vulevic, B., Correira J.J. *Biochemistry* **1996**, 35, 6806-6814.
13. (a) Loganzo, F., Hari, M., Annable, T. et al. *Mol. Cancer Ther.* **2004**, 3(*10*), 1319-1327. (b) Loganzo F., Discafani C. M., Annable, Beyer T. C., S., M Musto., Hari X. Tan, C. Hardy, R. Hernandez, M. Baxter, T. Singanallore, Khafizova G., Poruchynsky M. S., T Fojo., Nieman J. A., S. Ayral-Kaloustian, A. Zask, R. J. Anderson, L. M. Greenberg *Cancer Research* **2003**, *63*, 1838-1845.
14. Andersen, R.J.; Coleman, J.E.; Piers, E; Wallace, D.J. *Tetrahedron Letters* **1997**, *38*(3), 317-320.
15. Mitra, A., Sept, D. *Biochemistry* **2004**, *43*, 13955-13962.
16. Bai, R.; Durso, N. A.; Sackett, D. L.; Hamel, E. *Biochemistry* **1999**, *38*(43), 14302-14310.
17. Nieman, J.A.; Coleman, J. E.; Wallace, D. J.; Piers, E.; Lim, L.Y.; Roberge, M.; Andersen, R. J. *Journal of Natural Products* **2003**,*66*(2),183-199.
18. (a) Zask, A.; Birnberg, G.; Cheung, K.; Kaplan, J.; Niu, C.; Norton, E.; Suayan, R.; Yamashita, A.; et al. *Journal of Medicinal Chemistry* **2004**, *47*(19),4774-4786. (b) Zask, A.; Kaplan, J.; Musto, S.; Loganzo, F. *Journal of the American Chemical Society* **2005**, 127(50), 17667-17671.
19. Ravi, M.; Zask, A.; Rush, T.S., III. *Biochemistry* **2005**, *44*(48),15871-15879.
20. Vedejes, E., Kongkittingam, C. *J.Org.Chem.* **2001**, *66*, 7355-7364.
21. Reddy, R.; Jaquith, J.B., Neelagiri, V.R., Saleh-Hanna, S., Durst, T. *Org. Lett.* **2002**, *4*(5), 695-697.
22. D. Simoni, Ray M. Lee, David E. Durrant, Nai-When Chi, Riccardo Baruchello, Riccardo Rondanin, Cinzia Rullo, Paolo Marchetti *Bioorganic & Medicinal Chemistry Letters*, **2010**, *20*, 3431-3435.
23. D'Angeli F., Marchetti P., Cavicchioni G., Bertolasi, V., Maran, F. *Tetrahedron: Asimmetry*, **1991**, *2* (11), 1111-1121.
24. Cao TM, Durrant D, Tripathi A, Liu J, Tsai S, Kellogg GE, Simoni D, Lee RM. *Am J Hematol*. **2008** ;*83*(5), 390-7.

25. Durrant D, Richards JE, Walker WT, Baker KA, Simoni D, Lee RM. *Gynecol Oncol*. **2008**; *110*(1):110-7.
26. (a) D'Angeli F., Marchetti, P. Bertolasi V. *J. Org. Chem*., **1995**, *60*, 4013-4016. (b) Marchetti P. *Tetrahedron Letters* **2003**, *44*, 4121-4123. (c) F. Maran *J. Am. Chem. Soc*. **1993**, *115*, 6557.
27. D'Angeli F., Marchetti P., Salvadori S., Balboni G. *J. Chem. Soc. Chem. Commun*. **1993**, *3*, 304-305.
28. R. Andersen, E. Piers, J. Nieman, J. Coleman, M. Roberge Hemiasterlin Analogs, 1999, WO99/32509
29. Durrant DE, Richards J, Tripathi A, Kellogg GE, Marchetti P, Eleopra M, Grisolia G, Simoni D, Lee RM. Invest New Drugs. 2009 Feb;27(1):41-52.

Chapter 9

Synthesis of a benzothiazole analog of Epothilone B

(January-September 2010)

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9. Microtubules depolymerization inducing agents

9.1. Epothilones

Epothilones are a family of macrocyclic poliketides, isolated from the culture broth of the myxobacterial strain *Sorangium cellulosum* SoCe90, which was found in soil samples collected along the Zambezi River in Africa.[1]

This class is represented by the natural epothilones A and B, discovered as antifungal agents by Höfle and Reichenbach.

They became interesting when Bollag et al. in 1995 reported that they had the same mechanism of action of paclitaxel, stabilizing the tubulin polymers and causing apoptotic cell death.[2]

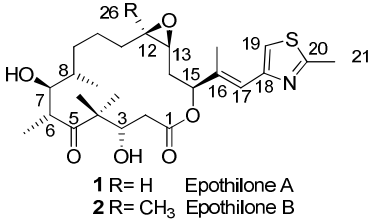


Figure IX-1. Structure of Epothilones A and B.

Despite the structural difference between taxol and epothilones, it was observed a competitive pattern of inhibition by epothilone A and B on the binding of paclitaxel to polymer formed with purified tubulin.

Epothilone B was the most effective compound in inducing assembly of tubulin polymers, while the potency of Epo A was similar to that of paclitaxel.[3]

The epothilones seem to have different potential advantage in comparison to Taxol®.

First of all, epothilones have a simpler structure, that is adapt to be easily manipulated both in total synthesis and in the synthesis of analogues for structure-activity relationship (SAR) studies. This wasn't possible for taxol because of its complex structure.[4]

Even more interestingly, epothilones A and B retain their activity also in PgP-overexpressing taxol-resistant cancer cells-lines.[5]

Taxol was scarcely soluble, it was administered together with vehicles such as Cremophor EL (polythoxylated castor oil), which is responsible of cardiac problems and cause severe hypersensitivity responses.[3]

Whereas, epothilones have a higher water solubility, which suggest the possibility of an easier formulation.

Several research groups have started a synthetic exploration, which led quickly to the total synthesis of epothilones A and B.[6-9]

Resolution of the trickiest key step of the synthetic route allowed extensively SAR studies, in order to find analogs with a better pharmacological profile.

Table IX-1. Inhibition of human carcinomas cell lines by taxol, EPO A, Epo B.

Cell lines	**IC_{50} [nM]**		
	Taxol	**Epo A**	**Epo B**
HCT-116 (colon)	2.79	2.51	0.32
PC-3M (prostate)	4.77	4.27	0.52
A549 (lung)	3.19	2.67	0.23
MCF-7 (breast)	1.8	1.49	0.18
NCI-ADR[a,b]	9105	27.5	2.92
KB-31 (cervix)	2.31	2.1	0.19
KB-8511[b,c]	533	1.9	0.19

[a]Multi-drug resistant cell-lines. [B] Multiple resistance mechanisms/MDR. [C] P-gp overexpression/MDR.

In this last years, a huge number of different epothilones have been synthesized, and tested in several cancer cell lines.[3]

The previous table (Table IX-1) shows human carcinoma cell growth inhibition data of the Epo A and B in comparison with taxol.

9.2. Structure and SAR of epothilones

The epothilones chemical structure is characterized by a poliketide core featuring a 16-membered lactone, a ketone at position 5, and an aromatic side chain attached at C15 of the core ring. The compounds were termed "epothilones" by Reichenbach and Höfle to reflect their structural features, which includes an *epo*xide moiety, a *thia*zole-containing side chain, and a single keto*(ne)* function.[3]

So far, six natural epothilones have been discovered (Epo A-F) that have been considered as starting point for further structural modifications.

Both the importance of the macrolactone ring substitution pattern and stereochemistry for biological activity have been extensively investigated in the context of SAR studies, as well as many modifications of the side chain.[3]

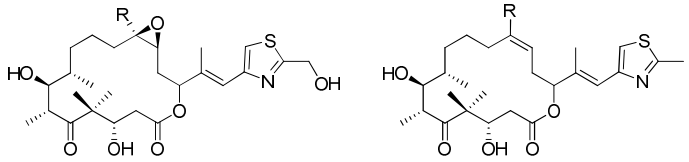


Figure IX-2. Structures of Epothilones C, D, E, F.

9.2.1. C12-C13 modifications

The epoxide moiety at C12-C13 of the macrolactone ring was object of wide interest for SAR study and analyzing deoxyepothilones (Epo C and D), it was found that their biological activity is similar to that of the parent compounds.

Efforts have been made to explore the effect of modifications at position (C26)[10]; some of the smaller substituents (ethyl, halomethyl) produced activities comparable with epothilones B, while large or polar groups proved to be detrimental.[11]

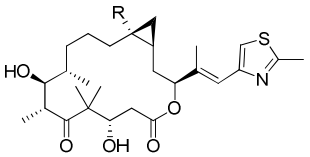
Moreover, Epo C and D microtubules stabilization efficiency and the potent human cancer cell growth inhibition don't depend on the presence of an epoxide moiety, conversely to previous beliefs about its important role as hydrogen-bond acceptor in the binding to tubulin.[12]

Since the most active epoxide derivatives have a *trans* geometry, several analogs were synthesized bearing small rings such as a cyclopropane fused at the C12-C13 bond with the macrocyclic core.

The new compounds are equipotent with the epoxide-derived natural product (for example, IC_{50} values against cell line HCT-116 are 1.4 nM for **7** and 0.7 nM for **8**, compared with 4.4 nM and 0.8 nM for Epo A and Epo B, respectively).[3]

This result suggests that the contribution of the epoxide moiety is mainly conformational-type and serve to stabilize the proper bioactive conformation of the macrolactone ring.[13]

The activity of the trans- cyclopropane derivatives of Epo A and its analogs makes them promising compounds.



7 R= H
8 R= CH_3

Figure IX-3. Cyclopropane analogs of Epothilone A and B.

9.2.2. Side chain modifications

The aromatic side chain is the most obvious site for structural modifications of epothilones, because it doesn't involve the macrocycle believed crucial for the activity.

The thiazole ring in Epo A-D and in cyclopropanated Epo A and B has been replaced by other heterocycles or simple phenyl groups, and the synthesis of C16-desmethyl Epo B.[3]

Modifications have been also involve the methyl group on the thiazole, while small substituents such as hydroxymethyl[14], aminomethyl, fluoromethyl, methylthio[15], or ethyl are well tolerated, bulkier substituents cause a significant loss in potency.[16] Moreover, the natural stereochemistry at C-15 is preferred.[17]

The atom of sulphur was found not necessary for biological activity as well as the presence of a five-membered heterocycle at C-16, while six-rings members are well tolerated such as pyridine.[17]

Based on this SAR data, Epo D analogs provided with a benzoheterocycle (**9**, **10**) were prepared, whose cellular activity is higher in those with the nitrogen in natural position.[18]

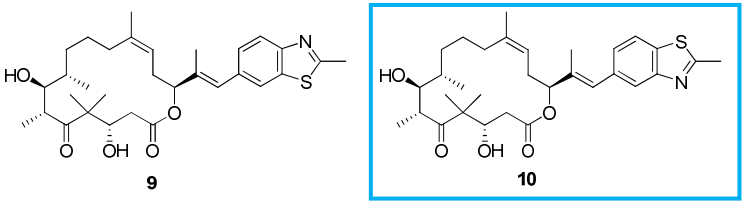


Figure IX-4. Analogs of Epo D bearing a benzoheterocyclic side chain, and pyridine Epo B analogs.

The benzoheterocyclic analogs derives from the observations that in the bioactive conformation of epothilones, the torsion angle about C16-C17-C18-N portion of the molecule is 180° and the consequent inclusion of this part in a rigid structure, as the bicyclic side chain should be tolerated.[19]

9.2.3. C1-C5 and C6-C11 fragment modifications.

This part of the macrocycle has been investigated in order to understand the importance of the hydroxy group at C-3 (**11**) and its stereochemistry, that can' t be inverted, because of the lack of activity.[20]

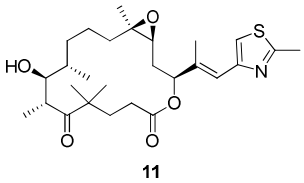
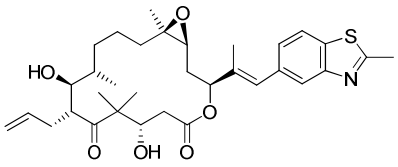


Figure IX-5. C3-deoxy-Epo B.

SAR investigations established that changes of stereochemistry or substitution pattern at C8 or simultaneous inversion of the stereochemistry at both C6 and C7 lead to a lack of biological activity.[17]

Generally, C6-C11 fragment is unchanged, apart an interesting derivative (**12**) that is in phase III of clinical trial (ZK-EPO).[22]



12, ZK-EPO

Figure IX-6. Structure of C-6 allylated Epo B derivative, ZK-EPO.

This C-6 allylated compound has been considered as a model for our project on benzothiazole analog synthesis.

9.2.4. Epothilones in clinical trials

Among all epothilones analogs, only a small numbers of derivative are in clinical trials.

At the moment the most advanced of these compounds is the lactam analog of Epo B, Ixabepilone, which received FDA approval for the treatment of metastatic breast cancer in 2007 and marked under the trade name Ixempra ®, by Bristol Mayer Squibb.[3]

Clinical evaluation of this drug is going for a large number of prostate, pancreatic and ovarian cancer.

Epothilone B (patupilone), developed by Novartis is at an advanced stage of clinical trials against ovarian cancer cells (phase III).

Epothilone D has entered clinical trials supported by Kosan –Roche (Kos-862) but when it was at II phase, the company chose to interest to an unsaturated epothilones D analog (Kos-1584), which is in phase I of investigation for breast cancer.[3]

Sagopilone, (ZK-EPO) is a fully synthetic epothilones analog developed by Bayer-Schering pharma, and is currently undergoing in phase II clinical trials for a number of malignancies, including prostate, ovarian, and lung cancer and melanoma.[22]

Chapter 10

Scopes and aims

10. Project aims

10.1. Synthetic approaches to an epothilone B analogue provided with tumor targeting moiety.

Conventional cytotoxic therapies of cancer often suffer from a lack of specificity, as they present low therapeutic index and considerable toxicity to healthy organs.

A new approach to overcome this disadvantage is the selective delivery of drugs to the tumor site by their conjugation to an antibody (carrier) molecule specific for a tumor-associated molecular marker, as the tumor vasculature expresses markers, attractive targets for a site-specific pharmacodelivery through blood flow.[23]

Recent research in this field, has demonstrated that the construction of *in vivo* active conjugates between tumor specific antibodies and cytotoxic agents requires the latter to have *in vitro* potency in the sub-nM range.

Thanks to SAR information we know that not all modifications are compatible with the retention of cytotoxic activity of epothilones .[3, 10-21]

Moreover, aware of the clinical trials progresses of ZK-EPO (**12**), the group of Prof. Altmann, in which I spent a period of 9 months as visiting Ph. D. student, has designed a benzothiazole analog of Epo B.

It is provided with a functional "handle" (Fig. X-1, red chain)for antibody conjugation, in which the natural 12,13-epoxide moiety has been replaced by a cyclopropane ring.

This modification eliminates potential problems of chemical and/or metabolic stability linked to the epoxide functionality; indeed, the

cyclopropane doesn't lead to any loss of antiproliferative activity.[3] The replacement of thiazole with benzothiazole leads to enhanced *in vitro* activity, the most important requirement for the construction of antibody-drug conjugates (ADCs).

In fact, ZK-EPO shows a mean IC_{50} value below 1 nM in antiproliferative test on different human tumor cell lines.[22]

It is reported to be more active than parental compound Epo B against a variety of drug sensitive cancer cells and to overcome the problem linked to resistance P-gp-mediated in some kind of tumor.[22]

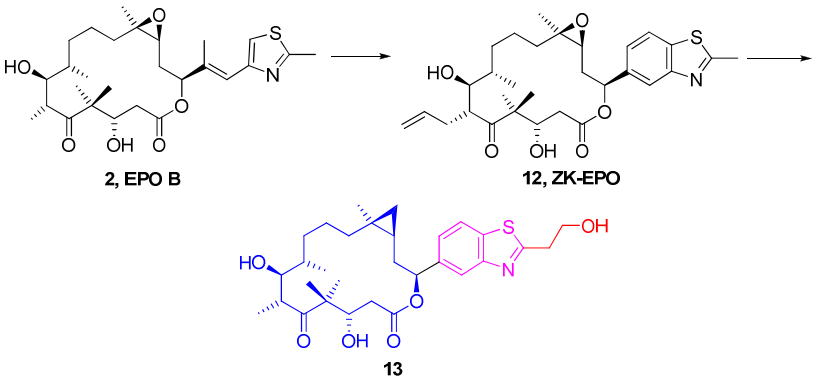


Figure X-1. From Epo B, through ZK-EPO to target compound **13**.

It was thought to introduce a hydroxyethyl side chain at position 2 of the heterocyclic core, that is expected to be tolerated. Indeed, benzothiazole-base epothilones analogs with substituents different from methyl group in this position have never been investigated.

It is important to notice that the handle is not bulky, since we don't know anything about the effect of additional volume.

Although the final compound **13**, shows 3 hydroxyl groups, only the primary alcohol will be used for the linkage, given that the other two are quite hindered.

In the following scheme is represented the structure of the conjugate between the carrier and the epothilone linked together by means of an opportune linker; in this context it hasn't been explained the nature of this linker, as the scope of my project is strictly limited to the synthesis of the intermediate **15**.

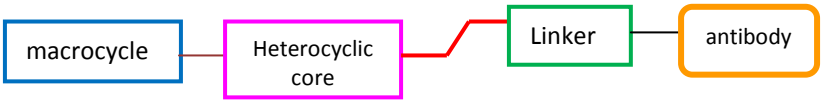


Figure X-2. Scheme of antibody tumor targeting as therapeutic approach.

10.2. Retrosynthetic analysis

The inclusion of C16-C18 olefin double bond in a six-membered aromatic ring which also incorporated C17, C19 and C22 of the original epothilones structure, led to a coplanar arrangement between these two moieties reducing the conformationally entropy of the heterocycle side chain.

A highly convergent strategy was followed that allowed us to introduce structural modification at every position of the 16-membered ring macrocycle. [7, 10, 24,]

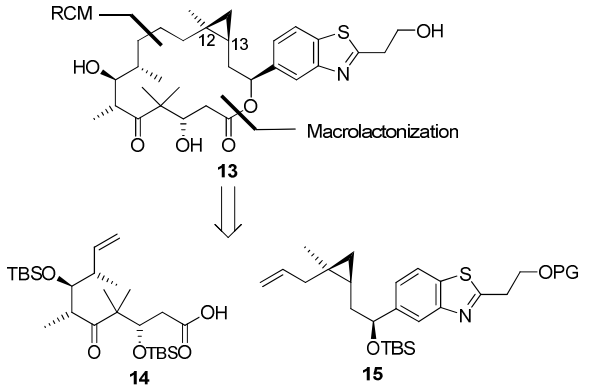
The introduction of the hydroxyethyl side chain at position 2 of the benzothiazole mandated the synthesis of the heterocycle to occur at the very beginning of the synthetic route.

Moreover, Charette cyclopropanation[25] of the C12-C13 double bond, requiring the presence of an allylic alcohol **22** to occur, took place before

ring closure metathesis reaction (RCM) in the west-northern part of the molecule.[26]

It was observed, that the cyclopropanation on C-12 desmethyl analog is highly stereoselective, providing the desired cyclopropane as single isomer, but the presence of an additional methyl group C2 at the double bond, caused the decrease of the selectivity.[26]

A ratio of both possible products in the range of 15:1 is considered acceptable to go toward the target molecule **13**.



Scheme X-1 Retrosynthetic analysis of **13**: disconnections into two building blocks.

Worst situation would lead us to give up with the synthesis of **13**; in this context is the meaning of my project.

Two crucial disconnections of the macrocyclic core allowed **14** and **15** to be traced back to two primary building blocks (scheme X-1).

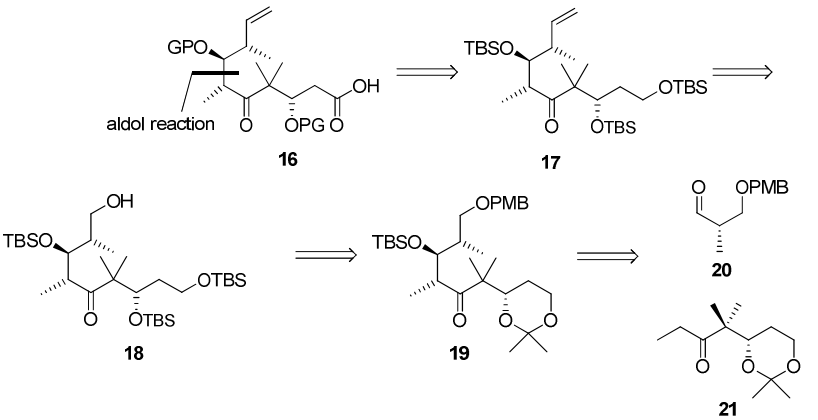
Building block **16**, which contains the heterocycle provided with the side chain, will be coupled with **15** through a Yamaguchi esterification after

selective deprotection of the secondary alcohol at C15 and hydrolysis of methyl ester at C1.[20]

The synthesis is accomplished with the cleavage of the two silyl ethers on O3 and O7 and of the side chain protecting group.[26]

As we need the primary alcohol at the very end of the synthetic route, for the linkage with antibodies, we decided to employ a different protecting group from the others at C3 and C7.

Building block **16** with its three stereocenters at C6, C7 and C8 might be accessed by a stereoselective aldol reaction of the known Schinzer ketone[24, 27] **21** and a chiral aldehyde[28] **20** as the key step: this was followed by a series of functional group manipulation to provide carboxylic acid in 57% overall yield from aldol product (scheme X-2). The synthesis of building block 16, was performed in prof. Altmann research group. [29]



Scheme X-2. Retrosynthetic analysis of building block **16**.

The allylic alcohol **22** is the result of Still-Gennari olefination on the aldehyde **23**.

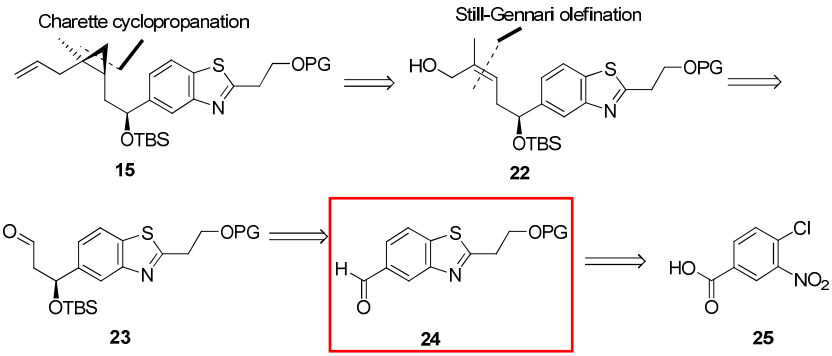
Apart the cyclopropanation, other key steps have characterized the achievement of **15**.

First of all, the synthesis of the heterocyclic core **22** bearing the hydroxyethyl side chain, was required to be high yielding and possibly adapt to be scaled-up (g-quantities).

All attempts to get the benzoheterocycle functionalized [22, 30] have been made starting from only one precursor, 4-chloro-3-nitro benzoic acid **24**.

The introduction of the chiral centre at C15 of **22**, was a challenge and it was planned as an enantioselective reaction of the achiral aldehyde; on purpose several methods were available.

At first, we tried with asymmetric allylation reactions,[31, 32] but because of the lack of selectivity, Evans chiral auxiliaries were employed in a stereoselective aldol reaction (scheme X-3).



Scheme X-3. Retrosynthetic analysis of building block **15**.

Chapter 11

Results and discussion

11. Results and discussion

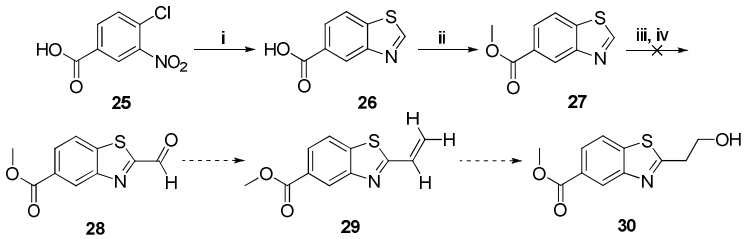
11.1 Synthesis of heterocyclic core

11.1.1 First synthetic approach

The synthesis of the benzothiazole core provided with hydroxyethyl side chain at position 2 started from commercially available 4-chloro-3-nitro-benzoic acid **25**.[22] The heterocycle **26** was obtained heating together **25** and sodium sulfide nonahydrate, followed by quick addition of mixed acetic formic anhydride in a nucleophilic aromatic substitution reaction of chlorine ion.

This reaction was performed to 60% yield (1 gram scale) working under argon atmosphere, because *in situ* prepared mixed anhydride was found easily hydrolysable.

After protection of the acid moiety as methyl ester **27**, none of the formylation attempts at position 2, afforded the aldehyde **28** that would have afforded monosubstituted olefin **29,** by hydroboration.[33]

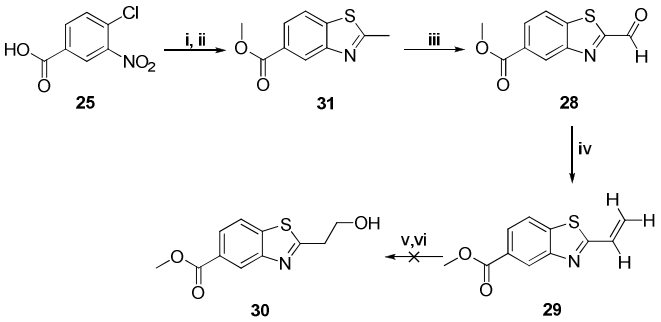


Scheme XI-1. Reagents and conditions: (**i**) Na_2S nonahydrate, acetic formic anhydride, CH_3COOH, 120°C, 60%; (**ii**) H_2SO_4, MeOH, 65°C, 98%; (**iii**) THF, n-BuLi 1.6 M in hexan, -78°C, 1h, DMF, -78°C to r.t. or (**iv**) THF, n-BuLi 1.6 M in hexane, -78°C to -50°C, 27, -78°C.

Direct formylation of **27** was tried following two general procedures [34a,b] that differ each other in both addition order and number of equivalents of reagents, but was unsuccessfully (Scheme XI-1).

11.1.2. Second synthetic approach

This approach was based on the direct oxidation of the methyl group of 2-methylbenzo[*d*]thiazole-5-carboxylate **31**.[34, 35]



Scheme XI-2. Reagents and conditions: (**i**) Na_2S nonahydrate, CH_3COOH, acetic anhydride, 120°C, 2h, 60%; (**ii**) H_2SO_4, MeOH, reflux, overnight, 98% ; (**iii**) SeO_2, CH_3COOH, reflux, 4 h, 50%; (**iv**) base, CH_3PPh_3Br, THF; (**v**) (1) 9-BBN 0.5 M THF, 0°C to r.t. overnight, (2) H_2O, NaOH 4N, 30% H_2O_2; (**vi**) (1) $BH_3 \cdot SCH_3$, THF, -10°C to 0°C, 2h; (2) 2M NaOH, H_2O_2 30%

Intermediate **31** has been obtained following the procedure described in the scheme XI-1, employing acetic anhydride in place of mixed acetic formic anhydride.

It is worth noting that on large scale (from 5 to 15 grams) the yield decreases detrimentally, probably because of the formation of a slurry mixture in which the product is trapped, during the work up.

Indeed, a by-product due to nitro group reduction, 3-amino-4-chlorobenzoic acid represented 50% of the crude product, difficult to separate in the chromatography purification.

The aldehyde **28** was reacted with non-stabilized ylide, prepared treating the methyl triphenylphosphonium salt with a strong base under inert conditions. We noticed that even changing kind of base (LiHMDS, NaH, and n-BuLi)[36,28] the yield of Wittig reaction was always around 20-50%, despite the complete consumption of starting material.

The best outcome (yield 50 %) was related to a quick filtration of the crude on a small pad of silica gel, in order to avoid possible degradation of the product.

The evidence of its instability was confirmed by literature data, that described the low yield (36%) of the of 2-vinylbenzo[*d*]thiazole.[37]

However, the negative outcome of the next hydroboration step, using both $BH_3 \cdot SMe_2$ and 9-BBN, have made us to give up further olefination reaction trials (scheme XI-2).[36, 38]

11.1.3. Third synthetic approach

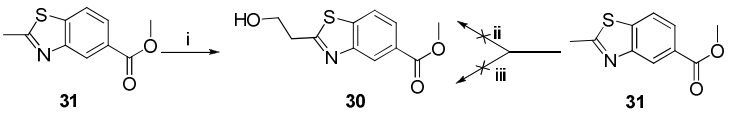
The third attempt was based on the direct elongation of the methyl group, heating together, at 165°C in an oil bath, **31** and paraformaldehyde in a sealed microwave vial.[39]

Although the desired product was achieved in low yield (18%), the bright side of this reaction was the complete recovery of unreacted starting material by chromatography.

Thus, at this stage of the synthetic route, substantial amount of compound **30** derived from as series of parallel reactions on 1 gram scale of **31**.

It was observed that higher amount of starting material **31** was detrimental also in term of isolation of the product, because of a "glue" formation difficult to solve with common chromatographic solvent and consequently to purify.

Whereas a base-(LDA) mediated reaction of **31** with paraformaldehyde failed completely in affording the hydroxyethyl derivative (scheme XI-3).[40]

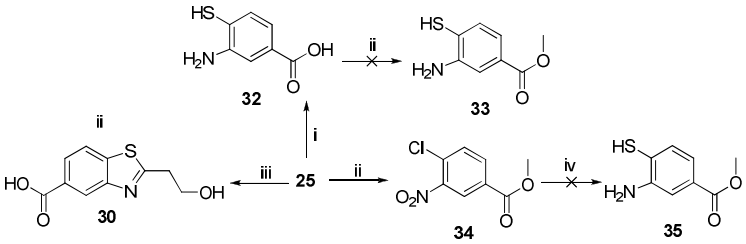


Scheme XI-3. Reagents and conditions: (**i**) $(HCHO)_n$, 165°C, 3h, 18% (**ii**) (1) LDA, THF; (2) DMF, (3) MeOH, acetic acid, $NaBH_4$; (**iii**) (1) LDA 2M THF, (2) $(HCHO)_n$, THF.

11.1.4. Fourth synthetic approach

The fourth synthetic approach was devised on the condensation of benzoic acid 3-amino-4-mercapto methyl ester **32** with *in situ* prepared propionaldehyde by oxidation with PCC of the correspondent 1,3-propanediol **36**. [41]

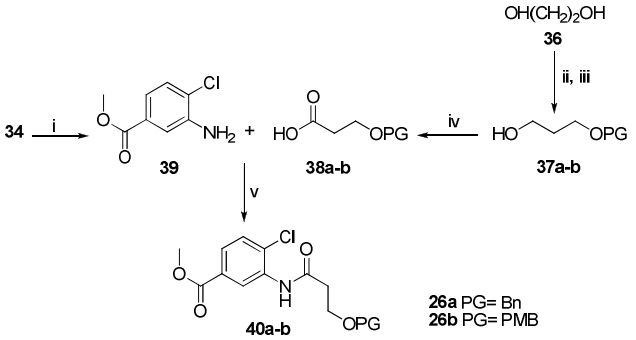
As, the isolation of the intermediate **32** from the melted mixture between **25** and sodium sulfide was hardous, it was employed in the next reaction without any characterization (scheme XI-4). [42]



Scheme XI-4. Reagents and conditions: (**i**) Na_2S nonahydrate, 120°C, 2h; (**ii**) H_2SO_4, MeOH, reflux, overnight, 98%; (**iii**) Na_2S nonahydrate, 120°C, 2h, slow addition of MeOH, solution A: 1,3-propandiol **36**, PCC, DDQ

11.1.5. Fifth synthetic approach

The 1,3-propanediol **36**, was also the starting material of the fifth synthetic approach, in which opportunely monoprotected 3-hydroxypropanoic acid **37a-b** was coupled with methyl 3-amino-4-chlorobenzoate **39** to afford the correspondent amide **40a-b**.



Scheme XI-5. Reagents and conditions: (**i**) $SnCl_2$, MeOH, reflux, 1h, 90%; (**ii**) NaH 60% min. oil, THF, 0° C, BnBr, TBAI, r.t., 2h, 90% or (**iii**) NaH 60% min. oil, THF, 0°C, 1h, p-CH_3OBnCl, TBAI, 0°C to r.t. 2h, 25% (**iv**) Jones reagent 1.26 M, acetone, 0°C; (**a**) 76%, (**b**) 36%; (**v**) $SOCl_2$, DMA, 0°C, r.t. overnight, (**a**)79%, (**b**) 44%

11.1.6. Choice of the best protecting group

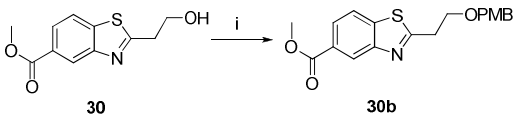
Since we need hydroxyethyl moiety at the very end of the synthetic route, for the linkage with antibodies, we decided to distinguish alcohols in the molecule, choosing PMB for primary alcohol, and TBS for secondary alcohol, at the future C15 position of the epothilones macrocycle. [22,26]

We have also chosen TBS, with the aim not to change the reactivity of this molecule portion, before the key step of Charette cyclopropanation, which is known to work with TBS protected alcohol, as reported in literaure.[26]

Unfortunately, in the new synthetic route, PMB monoprotected propandiol **37b** was found incompatible with Jones oxidation conditions to get **38b**, since it is removed in oxidative environment by DDQ; moreover, the yield of thiation reaction was very low (18%).[43]

Otherwise, before trying this new approach (schemes XI-3 and XI-6), alcohol **30b** obtained as shown in the scheme XI-3, was protected with PMB via imidate in moderate yield (56-60 %).[44]

Intermediate **30b**, was used to explore the whole synthetic route until the cyclopropanation step.



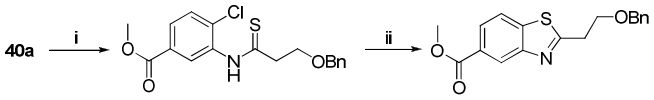
Scheme XI-6. Reagents and conditions: (**i**) PMB imidate, PPTS, DCM, r.t., 24 h, 60%

11.1.6.1. Benzyl side chain protection

A good alternative was the benzyl protecting group **30a**, that could be removed at the end of the synthetic route.

We will have to verify if its cleavage conditions keep intact the cyclopropane from the ring opening.

The amide **40a** was changed into the thioamide **41a** with Lawesson's reagent that is a mild and convenient thionating agent for ketones.



Scheme XI-7. Reagents and conditions: (**i**) Lawesson's reagent , dioxane, reflux, 2 h, 81%, (**ii**) NaH 60%, NMP,160°C, 1h, 72%

Thus, only the benzyl protected derivatives afforded the desired heterocycle **30a** in good yield 72% and in large amount (scheme XI-7).[45]

Reaction time longer than one's due, gave the unsubstituted olefin at the side chain, main product against our expectations, instead of **30a**.[42]

An excess of cheap 1,3-propandiol has been required to afford the monoprotected alcohol **37a,** as the main product over dibenzylated derivative, obtained following other procedures, in which equimolar amounts of diol and benzyl bromide were used.[46]

The acid **38a** was coupled with the correspondent aromatic amine **39** in presence of condensing agents like CDI (entry 2, table XI-1) or EDCI (entry 1, table XI-1).[47, 48]

High yields were obtained when the acid was activated as acyl- derivative and dimethylacetamide was employed instead of dichloromethane (entry 5, table XI-1).[42,49]

Entry	Acid (eq)	Amine (eq)	Conditions
1	1	1	EDCI, 1-HOBT, DMAP, DMF
2	1	1	CDI, THF
3	1	1	Oxalyl chloride, DCM
4	1.1	1	Oxalyl chloride, TEA, DMF (drops) DCM
5	1	1	$SOCl_2$, DMA

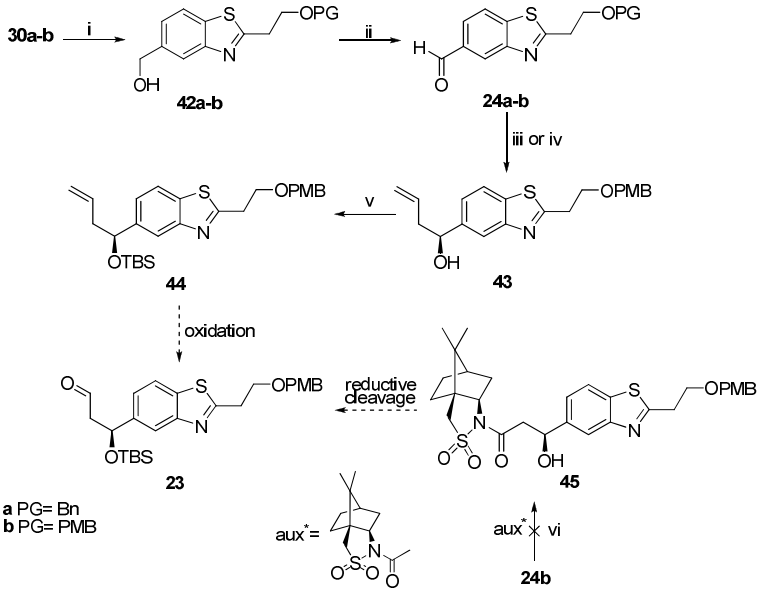
Table XI-1. Conditions of coupling reaction.

11.2. Synthesis of the homoallylic alcohol.

Intermediates **30a-b** have been reduced and the correspondent primary alcohols **42a-b** were oxidized to aldehydes **24a,b** by Swern oxidation.[50]

Our main goal was the enantioselective allylation of the aldehyde **30b** followed by ozonolysis, that would have furnished the precursor for Charette cyclopropanation, after a sequence of Still-Gennari olefination/reduction of the α,β-unsaturated ester. [26]

The aldehyde **24b** was allylated following Keck conditions, that might have achieved high level of enantioselectivity, employing catalytic amount of a chiral Lewis acid, prepared by addition of trifluoromethanesulfonic acid to a mixture of *(S)*-binaphtol and titanium tetraisopropoxide.[31]



Scheme XI-8. Reagents and conditions: (**i**) DIBAL-H 1M DMC,DCM, -78°C; 1½ h, 72% (**ii**) DMSO, oxalyl chloride, TEA, -78°C to r.t., 87%; (**iii**) allyltri-n-butylstannane, *(S)*-BINOL, Ti(*Oi*Pr)$_4$, CF_3SO_3H, DCM, -78°C, 60 h, 52%; (**iv**) (1) solution A: C_3H_5MgBr, (-)-IPc_2BCl, Et_2O, -78°C to r.t., slow addition of solution A, -100°C, 54%; (2) Ethanolamine, MeOH; (**v**) Triethylborane, 1M hexane, CF_3SO_3H, DIPEA, DCM, 2h,-78°C

Unfortunately, the enantioselectivity of this reaction was limited, giving the product **43** as a racemic mixture.

For this reason, Brown (-)-β-allyldiisopinocamphenylborane has been employed as chiral ligand to install the asymmetric centre at C15 (scheme X-8).[32]

Better results, only in terms of selectivity (*ee* 75%, in favour of the desired enantiomer), were obtained from Brown allylation, because the low yield was always variable from trial to trial.

However, the moderate value of enantiomeric excess, measured by Mosher ester analysis, wasn't satisfactory in the perspective to arrive at cyclopropanation, possibly with only one enantiomer, given that the selectivity of this key step is unpredictable on this substrate.[51]

The *ee* of **43** decreased from 75% to 50% when dichloromethane was used as reaction solvent under alternative Brown's conditions (other solvent were allowed, such as dichloromethane, THF, chloroform, in place of the diethyl ether).[32]

The low solubility of **30b** in ethylic ether was thought to be related to the lipophilic nature of protecting group (PMB). Thus, a more polar one was chosen for the hydroxyethyl moiety (**30**).[52]

TBS-protecting group was easier to install than PMB, but it wasn't resistant to Swern oxidation condition, because of HCl produced during the reaction, that cleaved the protection of this primary alcohol.

11.2.1. Diastereoselective aldol reaction

We tried to obtain the product as mixture of diastereoisomers easily separable, through a stereoselective aldol reaction.

Our first approach made use of the boron enolate of the acetylated Oppolzer's camphor sultam, a chiral auxiliary.

Its reductive cleavage (**45**), after silylation of the alcohol, would have afford the aldehyde **23** ready to be used for the following Still- Gennari olefination (scheme XI-8).[53]

After several unsuccessful attempts in term of yield, we decided to exploit Evans oxazolidinone chemistry.

They are a class of oxazolidinones substituted at 4 and 5 position and thanks to their steric hindrance, they direct any aldol reaction to the α position of the carbonyl of the substrate (scheme XI-10).[54]

Structurally different oxazolidinones were acetylated with the aim to find the best combination *Lewis acid/chiral auxiliary* that could ensure high level of diastereoselectivity or lead to a mixture of separable distereoisomers, (table XI-2).[55]

11.2.1.1. Evans aldol reaction

The potentiality of the aldol reaction is the highly stereoselective C-C bond formation.

This reaction proceeds via enolate, using a Lewis acid and a weak base as di-n-butylborontriflate/ DIPEA [36] or DIPEA /$TiCl_4$.[37]

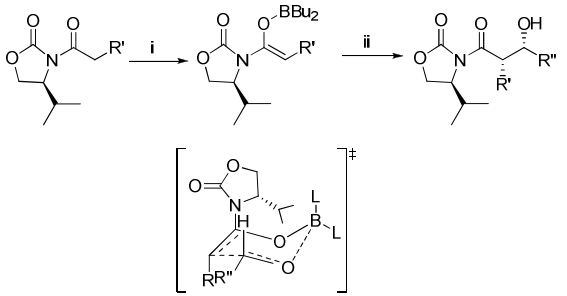
For example, the reaction of an achiral aldehyde with (Z)-boron enolate of N-acyloxazolidinone gives the *syn* aldol product, while (E)-enolate afford the *anti* product, as confirmed by Zimmermann-Traxler six-membered transition state characterized by a chair-conformation (scheme XI-9).

Unfortunately, the selectivity was about 1:1 and the two possible diastereoisomers were neither separable by flash chromatography, nor as C15-OTBS ethers (table XI-2, entry 1-4).

The negative result was expected, as boron enolate doesn't give high level of selectivity with simple N-acetyloxazolidinones.

In the perspective of the synthetic route exploration, our main aim was to collect enough desired distereoisomer to go straight to cyclopropanation step. The stereochemistry of aldol products was confirmed comparing the spectroscopic and analytic data of the product obtained from two different

reaction conditions, employing the two enantiomers of the same oxazolidinone (table XI-2, entry 1, 2).



Scheme XI-9. Reagents and conditions: (**i**) DIPEA, Bu_2BOtf, DCM, 0°C; (**ii**) (1) R"CHO, DCM, -78°C to r.t., (2) oxidative work-up.

A strong base like LDA in which lithium cation is replaced with zinc, furnished by $ZnCl_2$, provided control of stereochemistry.[22]

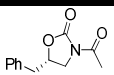
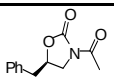
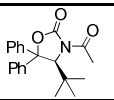
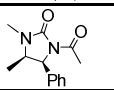
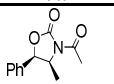
Evans auxiliary	Conditions	Outcome
	di-n-BuOTf, DIPEA, DCM **A**	53% (3:2 mixture), separable as TBS protected
	$TiCl_4$, DIPEA, DCM **B**	54% (1:1 mixture)
	di-n-BuOTf, DIPEA, DCM **C**	54% (3:7 mixture, 85% brsm) not separable neither as TBS protected
	di-n-BuOTf, DIPEA, DCM	No product
	LDA, $ZnCl_2$ 0.5 M in THF, THF	64% (5:1 mixture, 85% brsm) separable as TBS protected

Table XI-2. Aldol reaction of **24b** with Evans chiral auxiliaries.

Thus, zinc enolate of a N-acetylated (1*R*, 2*S*)-(-)-norephedrine derivative **46** allowed us to obtain a 5:1 diastereomeric mixture (**47**) in 64% yield.

The two diastereoisomers have been separated by flash chromatography as TBS protected **48** (scheme XI-10).

Every mixture of diastereoisomers and their silylated derivatives has been analyzed by HPLC just to determine the ratio of two components, instead of Mosher ester analysis.

11.3. Toward the synthesis of the cyclopropane key intermediate 15.

Among Evans auxiliary removal methods, the most convenient was the reductive cleavage with $LiBH_4$[59] because the corresponding primary alcohol might be easily oxidized to aldehyde **23**, but migration of TBS protecting group from new chiral centre to primary alcohol was observed.

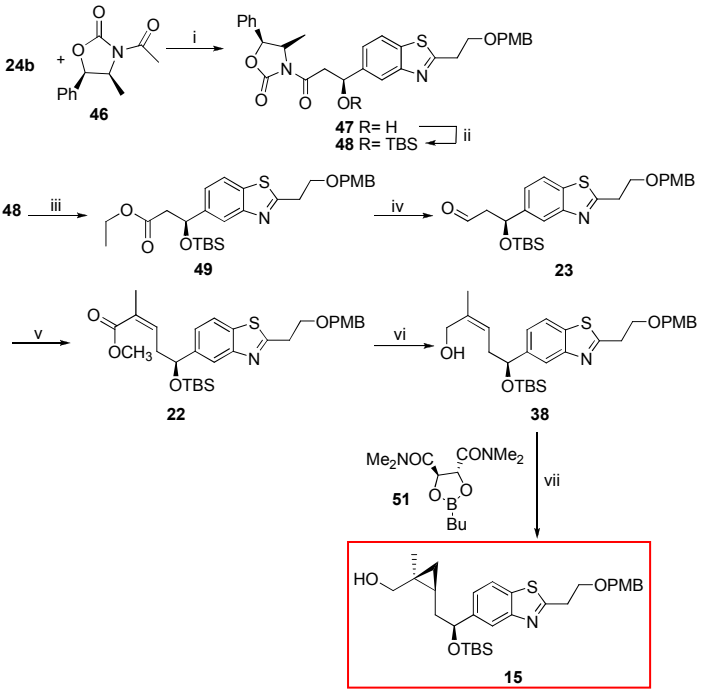
Thus, **48** was esterified in refluxing EtOH with freshly distilled $Ti(OEt)_4$ to give **49**, subsequently reduced to aldehyde **23**.

The storage of the aldehyde **23** led to formation of a completely conjugated π-electron system, the more stable α,β-unsaturated aldehyde via TBS-OH elimination.[22]

After a filtration on a small plug of silica, the aldehyde **23** was used for Still-Gennari olefination which is Z-selective, as confirmed by NOESY experiment on intermediate 50.[60]

The reduction of **50** with DIBAL-H, gave the allylic alcohol **15** which was reacted with an excess of Et_2Zn/CH_2I_2 in presence of Charette ligand **51**, to give the desired product as a mixture of diasteroisomers in good yield.[25]

The *dr* was in the range of 15:1 in favour of the right diastereoismer as confirmed by ^1H NMR.[22, 25]



Scheme XI-10. Reagents and conditions: (**i**) LDA, $ZnCl_2$ 0.5 M THF, THF, -70°C, 2½ h, 64%; (**ii**) TBS-Cl, imidazole, DMF, r.t.,overnight, 90%; (**iii**) $Ti(OEt)_4$, EtOH, 78°C, 98%; (**iv**) DIBAL-H 1.2 M toluene, DCM, -78°C, 30';(**v**) KHMDS, 18-crown-6, $(CF_3CH_2O)P(O)C(CH_3)CO_2Et$, -78°C, 30', 54%; (**vi**) DIBAL-H 1M DCM, DCM, -78°C, 1 ½ h; (**vii**) Et_2Zn, CH_2I_2, DCM, Charette ligand, 0°C to r.t.,1½ h

Charette cyclopropanation is a stereoselective method to facilitate the access to enantioenriched cyclopropanes, and is based on haloalkylzinc, widely used in Simmons-Smith reaction, that gave very low selectivity.

The cyclopropanation might be explosive because of the exotermicity of the formation of $Zn(CH_2I)_2$, for this reason it is carried out on scale lower than 8 mmol. This methodology is specific for allylic alcohol, through a

transition state, in which it is thought that the bulkier butyl substituent on the dioxaborolane adopts the less congested pseudoequtorial position and the allylic alkoxide the more stable pseudoaxial position. This allows the complex to act as a bidentate ligand.

The zinc reagent should then be complexed simultaneously by both the highly basic carbonyl amide of the dioxaborolane ligand and the oxygen atom of the allylic alkoxide.

The most suitable conformation for the methylene delivery is that in which the allylic chain is in its most stable conformation. This model correctly predicts the absolute configuration for the cyclopropanation of all the allylic alcohols.

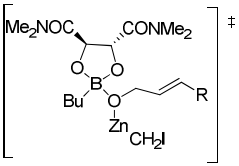


Figure XI-1. Transition state of Charette cyclopropanation reaction.

11.4 Conclusions

This experience of Ph.D. visiting student has been accomplished with the achievement of the result, that was to arrive to the key step 15.

The synthetic route to obtain the heterocyclic core in high yield was designed that allowed to go straight to cyclopropanated intermediate.

The positive outcome both in term of yield, both in term of selectivity are promising in the perspective to arrive at the very end of the synthetic route.

Chapter 12

Experimental section

12 Experimental procedures

12.1. General Informations

All solvents used for reactions were purchased as anhydrous grade from Fluka (puriss.; dried over molecular sieves; H_2O < 0.005%) and used without further purification. Solvents for extractions, flash column chromatography (FC) and thin layer chromatography (TLC) were purchased as commercial grade and distilled prior to use. All non-aqueous reactions were performed under an argon atmosphere using flame-dried glassware and standard syringe/septa techniques. All other commercially available reagents were used without further purification, unless otherwise noted. In general, reactions were magnetically stirred and monitored by TLC performed on Merck TLC aluminum sheets (silica gel 60 F254). Spots were visualized with UV light (λ = 254 nm) or through staining with $Ce_2(SO_4)_3$/phosphomolybdic acid/H_2SO_4 or $KMnO_4$/K_2CO_3.

Melting points were obtained in open capillary tubes using a Büchi melting point apparatus B-540 and are uncorrected. 1H- and 13C-NMR spectra were recorded in $CDCl_3$ (unless otherwise noted) on a Bruker AV-400 400 MHz at room temperature. Chemical shifts (δ) are reported in ppm and are referenced to added tetramethyl silane as an internal standard (otherwise to chloroform; δ 7.26 ppm for 1H, δ 77.16 ppm for 13C). All 13C-NMR spectra were measured with complete proton decoupling. Data for NMR spectra are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal, J = coupling constant in Hz.

Infrared spectra (IR) were recorded on a Jasco FT/IR-6200 instrument. Resonance frequencies are given as wavenumbers in cm-1. Data for IR spectra are reported as follows: w = weak, m = medium, s = strong, br =

broad signal. Optical rotations were measured on a Jasco P-1020 polarimeter. Mass spectra were recorded by the ETH Zürich MS service; HRMS (ESI) spectra were obtained on a Varian IonSpec spectrometer.

Synthesis of benzo[*d*]thiazole-5-carboxylic acid (26).

An equimolar mixture of formic acid (1 g, 21.7 mmol) and acetic anhydride (2.22 g, 21.7 mmol) were heated up to 65 °C for 15 minutes. After cooling down to 0 °C, this solution was used in the following reaction without any purification.

A mixture of 4-chloro-3-nitrobenzoic acid (548 mg, 2.72 mmol) and sodium sulphide nonahydrate (1.96 g, 8.15 mmol) was heated at 120°C for 30 min, and then cooled to 0°C in an ice bath. Mixed anhydride (718 mg, 8.15 mmol) was added rapidly dropwise, followed by dropwise addition of acetic acid (256.4 µl, 4.07 mmol). The resulting mixture was refluxed for 4 h, then allowed to cool to room temperature and stirred overnight.

The mixture was distributed in water (5 ml) and AcOEt (20 ml), and the precipitated sulphur was removed by filtration on celite, the organic phase was washed with brine (5 ml), dried over magnesium sulphate, and concentrated in vacuo to give a yellow solid in 60 % of yield (292 mg, 1.63 mmol).

For spectroscopic data of compound **26** and the corresponding methyl benzo[d]thiazole-5-carboxylate **13** see:

Walker, D. P.; Bioorg. Med. Chem. **2006**, *14*(24), 8219-8248

Vel'tman, R. P.; Zhurnal Obshchei Khimii **1996**, *26*, 3388-91.

Synthesis of methyl 2-methylbenzo[*d*]thiazole-5-carboxylate (31).

3-nitro-4-chloro-benzoic acid (25g, 0.124 mol) and sodium sulphide nonahydrate (89.37 g, 0.372 mol) were heated until melting (bath temperature 120°C, caution: reaction is highly exothermic).

After 30 min, the heating bath was removed, cooled to 0°C in an ice bath and acetic anhydride (35.15 ml, 0.37 mol) (caution: after adding 50% of acetic anhydride, a highly exothermic reaction started) followed by acetic acid (11.7 ml, 0.186 mol) were added rapidly, dropwise.

The resulting mixture was refluxed for 2 h, then allowed to cool to room temperature and stirred overnight.

The mixture was distributed in water (50 ml) and AcOEt (200 ml), and stirring was continued for 30 minutes. The suspension was filtered through celite to remove sulfur, the aqueous layer was washed with ethyl acetate and the combined organic layers were washed with brine (10 ml), dried over $MgSO_4$ and concentrated in vacuo to give 10 g of yellow powder.

The filter was washed several times with EtOH, and concentrated to give a further batch that was combined with the first one and purified by flash chromatography (AcOEt and 1% of acetic acid) to afford 5 g of 2-methylbenzo[*d*]thiazole-5-carboxylic acid (21%).

A solution of 2-methylbenzo[*d*]thiazole-5-carboxylic acid (5 g, 0.026 mol) in 30 ml of MeOH was cooled to 0° C, then $SOCl_2$ was carefully added (4.75 ml, 0.065 mol) dropwise.

The mixture was heated to 70° C for two hours, and concentrated under reduced pressure. To the mixture were added 5 ml of water and 15 ml of ethylic ether (3 x 15 ml). The organic phase was washed with Na_2CO_3 sat. and with brine. Finally it was dried over Mg_2SO_4 and evaporated. The crude

was purified by fc (Hexane/AcOEt 12:1) to get 3 g of **31** as yellow solid (55%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (s, 1H), 8.03 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.87 (d, *J* = 8.5 Hz, 1H), 3.97 (s, 3H), 2.87 (s, 3H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 168.5, 167.1, 153.5, 140.8, 128.5, 125.6, 124.1, 121.4, 52.6, 20.4; **IR** (film, $CHCl_3$): 2954 (w), 2902 (w), 1715 (s), 1431 (m), 1300 (s), 1240 (m), 1090 (s), 904 (m), 758 (m); **R_f** 0.4 (Hexane/Ethylic ether: 1:1); **M.p.**: 96-98°C; **MS (ESI)**: 208.0423 $[M + H]^+$

Synthesis of methyl 2-formylbenzo[*d*]thiazole-5-carboxylate (28).

A solution of methyl-benzothiazole (500 mg, 2.41 mmol) and SeO_2 (1.07 mg, 9.65 mmol) in 12 ml of AcOH was refluxed for 4 h.

The mixture was filtered through celite to remove the dark precipitate and the filtrate was concentrated. The residue was neutralized with aq. saturated $NaHCO_3$ solution (4 ml) and extracted with AcOEt (12 ml x 3). The combined organic extracts were dried on Mg_2SO_4 and evaporated to give after purification by fc (AcOEt/Hexane 1:11) 303 mg of white solid (57%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 10. 17 (s, 1H), 8.94 (dd, *J* = 1.7, 0.5 Hz, 1H), 8.22 (dd, *J* = 8.5, 1.8 Hz, 1H), 8.05 (dd, *J* =8.5 Hz, 0.5 Hz, 1H), 4.00 (s, 1H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 185.4, 166.8, 166.3, 153.5, 140.8, 129.97, 128.8, 127.6, 122.8; **IR** (film, $CHCl_3$): 2957 (w), 2921 (w), 1720 (s), 1685 (s), 1488 (w), 1314 (m), 1206 (m), 754 (m); **R_f** 0.59 (Hexane/AcOEt 5:1); **M.p.**: 137°C (decomposition); **MS (ESI)**: m/z = 220.0425

Synthesis of methyl 2-vinylbenzo[*d*]thiazole-5-carboxylate (29)

To a methyltriphenylphosponium bromide (30 mg, 0.085 mmol) in 1.5 ml of THF was added LiHMDS 1M in THF (85 µl, 0.085 mmol) at 0°C and it was left stirring for 30 min at that temperature. Then the aldehyde **14** (15 mg, 0.068 mmol) dissolved in 0.5 ml of THF was added and the reaction mixture was left stirring for 1 h at 0°C. The reaction was cautiously quenched by addition of NH_4Cl, water and afterwards diluted with ethylic ether. The phases were separated and the aqueous phase was extracted with ethylic ether, the combined ethereal layers were dried over $MgSO_4$, concentrated under reduced pressure and purified by fc to afford (8 mg, 0.036 mmol) of desired product (54%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1H), 8.06 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.05 (dd, *J* = 17.9, 11 Hz, 1H), 6.22 (d, *J* = 17.7 Hz, 1H), 5.82 (d, *J* = 10.2 Hz, 1H), 3.98 (s, 3H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 168. 5, 166.9, 153.72, 139.3, 131.4, 128.8, 126.4, 124.90, 124.3, 121.6, 52.6; **IR** (film $CHCl_3$): 2949, 1741, 1454, 1433, 1304, 1208, 1091, 756; **R_f**: (Hexane/AcOEt 4:1) 0.48; **M.p.**: 81.4°C; **MS (ESI)**: m/z = 220.0417 $[M+H]^+$

Synthesis of Methyl 2-(2-hydroxyethyl)benzo[*d*]thiazole-5-carboxylate (30).

Methyl 2-methylbenzo[*d*]thiazole-5-carboxylate (1.00 g, 0.05 mol) and paraformaldehyde (291.36 mg, 0.010 mol) were heated together in a sealed microwave vial at 165°C in an oil bath for 6 hours. After cooling to room temperature, the reaction mixture was carefully transferred in a chromatography column and purified by fc (AcOEt/Hexane 1:1) to give 196 mg (0.83 mmol, 17%) of yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (d, J= 1.4 Hz, 1H), 8.05 (dd, J= 8.4, 1.6 Hz, 1H), 7.90 (d, J= 8.4 Hz, 1H), 4.15 (q, J= 5.8 Hz, 2H), 3.97 (s, 3H), 3.35 (t, J= 5.7, 2H), 3.20 (t, J= 6.0 Hz, 1H)
^{13}C NMR (400 MHz, $CDCl_3$): δ 171.1, 167.0, 153.0, 139.8, 128.6, 125.9, 124.3, 121.6, 61.1, 52.6, 36.9; **IR** ($CHCl_3$, film): 3382 (br, 3600-3000), 1718 (s), 1432 (m), 1418 (w), 1301 (s), 1287 (m), 1219 (m), 1094 (m), 888 (m), 702 (m); **R_f** 0.5 (AcOEt/Hexane 5:1); **M.p.**: 101-103°C; **MS (ESI)**: m/z = 238.0528 $[M + H]^+$

Synthesis of Methyl 2-(2-(4-methoxybenzyloxy)ethyl)benzo[d]thiazole-5-carboxylate (30b)

To a solution of 4-methoxybenzyl alcohol (1.5 g, 10.86 mmol) in 10 ml of ethylic ether was added at r.t. NaH 60% (40.4 mg). The resulting suspension was allowed to stir until the solid has dissolved and the gas evolution had ceased (approximately 1h).

Then the mixture was cooled to 0 °C and trichloroacetonitrile (1.09 ml, 10.86 mmol) was added over 15 min dropwise. The mixture was stirred at 0°C for another 5 min and at r.t. for 20 min.

It was then transferred to a separatory funnel, washed with saturated $NaHCO_3$-solution (3 ml) and brine, dried over $MgSO_4$, and concentrated in vacuo to yield the crude 4-methoxybenzyl trichloroacetimidate as yellow oil (2.92 g, 95%). To 2.19 g of imidate in 8 ml of DCM were added methyl 2-(2-hydroxyethyl)benzo[*d*]thiazole-5-carboxylate (832 mg, 3.5 mmol) and the pyridinium p-toluenesulphonate (35 mg, 0.14 mmol)

The mixture was stirred at room temperature overnight, then washed with saturated $NaHCO_3$-solution (4 ml), brine (4 ml), dried $MgSO_4$, and concentrated in vacuo.

The crude was purified by flash chromatography (AcOEt/Hexane 1:1) to give 640 mg of pale yellow solid (51%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (d, J= 1.5 Hz, 1H), 8.04 (dd, J= 8.4, 1.5 Hz, 1H); 7.89 (dd, J= 8.4, 1.5 Hz, 1H), 7.26 (d, J= 8.0 Hz, 2H), 6.86 (d, J= 8.0 Hz, 2H), 4.52 (s, 2H), 3.97 (s, 3H), 3.92 (t, J= 6.2 Hz, 2H), 3.8 (s, 3H), 3.42 (t, J= 6.3 Hz, 2H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 170. 7, 167.2, 159.4, 153.0, 140.6, 130.1, 129.5, 128.4, 125.6, 124.3, 121.5, 114, 73.1, 68.1, 55.4, 52.4, 35.2; **IR**: 1718 (s), 1609 (w), 1512 (m), 1433 (w), 1298 (s), 1245 (s), 1093 (m), 1033 (w), 909 (m), 757(m)

R_f 0.2 (AcOEt/Hexane 1:3); **M.p.:** 58 °C; **MS (ESI)**: m/z = 358.1108 $[M + H]^+$

Synthesis of (2-(2-(4-methoxybenzyloxy)ethyl)benzo[d]thiazol-5-yl)methanol (42b).

To a solution of methyl 2-(2-(4-methoxybenzyloxy)ethyl)benzo[d]thiazole-5-carboxylate (621.6 mg, 1.74 mmol) in 7 ml of DCM was added DIBAL-H 1M in DCM (4.35 ml, 4.35 mmol) at -78°C under argon. After 1h, Et_2O (3 ml) was added and the mixture was allowed to warm to r.t. After the addition of further ethylic ether (3 ml) and Rochelle salt, the mixture was stirred until the solution become clear. The mixture was concentrated and the crude was suspended in DCM (15 ml) and washed with water (3x 3ml). The organic phase was dried over $MgSO_4$, and concentrated in vacuo. The crude was purified by fc (AcOEt/Hexane 1:1) to give (335.6 mg, 1.02 mmol) of yellow solid 59%.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (dd, J= 1.9, 0.8 Hz, 1H), 7.75 (d, J= 8.3 Hz, 1H), 7.31 (dd, J= 8.3, 1.6 Hz, 1H), 7.18 (d, J= 8.8 Hz, 2H), 6.78 (d, J= 8.8 Hz, 2H), 6.78 (d, J= 8.8 Hz, 2H), 4.75 (d, J= 5.2 Hz, 2H), 4.44 (s,

2H), 3.82 (t, J= 6.44 Hz, 2H), 3.72 (s, 3H), 3.32 (t, J= 6.4 Hz, 2H), 1.96 (t, J= 6.4 Hz, 1H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 169.9, 159.5, 153.4, 139.42, 134.82, 130.13, 129.5, 124.2, 121.7, 121.0, 113.98, 73.1, 68.4, 65.4, 55.5, 35.2; **IR**: 3314 (br, 3500-3100), 2920 (m), 2858 (m), 1611 (m), 1508 (s), 1366 (m), 1304 (w), 1175 (m), 1154 (m), 1245 (s), 1090 (s), 1033 (s), 813 (s), 584 (m); **R_f** 0.14 (AcOEt/Hexane 1:1); **M.p.**: 84-85°C; **MS (ESI)**: m/z = 330 .1160

Synthesis of (2-(2-(4-methoxybenzyloxy)ethyl)benzo[d]thiazole-5-carbaldehyde (24b).

DMSO (289.4 ml, 4.075 mmol) was added to a solution of oxalyl chloride(131.3 ml, 1.528 mmol) in DCM (3.5 ml) at -78°C. After 5 min alcohol (335.6 mg, 1.02 mmol) in DCM (2.5 ml) was added. Stirring was continued at -78°C for an additional 15 min, whereupon TEA (495.6 µl, 3.56 mmol) was added. The ice bath was removed and the reaction was stirred at r.t. for additional 2.5 h. The reaction was quenched by addition of NH_4Cl (3 ml). The aqueous phase was extracted with DCM (10 ml x 3), the combined organic phases were washed with brine and dried over $MgSO_4$. The yellow crude was purified by chromatography on silica gel (AcOEt/Hexane 1:3→ AcOEt/Hexane **1:1)** to give mg 280 (84%) as yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.13 (s, 1H), 8.42 (dd, J= 1.6, 0.6 Hz, 1H), 7.99 (d, J= 8.3, 1H), 7.90 (dd, J= 8.3, 1.6 Hz, 1H), 7.26 (d, J= 8.8, Hz, 2H), 6.87 (d, J= 8.8 Hz, 2H), 4.53 (s, 1H), 3.92 (t, J= 6.2 Hz, 2H), 3.8 (s, 3H), 3.43 (t, J= 6.2 Hz, 2H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 191.8, 171.3, 159.52, 153.22, 142.22, 135.06, 130.0, 129.55, 125.6, 124.3, 122.3, 114.0, 73.12, 68.01, 55.43, 35.29; **IR** (film, $CHCl_3$): 1691 (s), 1602 (m), 1511 (s),

1282 (m), 1244 (s), 1093 (m), 1062 (w), 1033 (m), 813 8m); **M.p.**: 83.6-84.6°C; $\mathbf{R_f}$ 0.67 (AcOEt/Hexane 3:1); **MS (ESI)**: m/z = 328.0987 $[M+ H]^+$

Synthesis of methyl 4-chloro-3-nitrobenzoate (34).

Concentrated sulphuric acid (200 µl) was cautiously added to a chilled (0°C) solution of 4-chloro-3-nitrobenzoic acid (1 g, 5 mmol) in methanol (10 ml) and the resulting mixture heated under reflux for 16 h. The reaction mixture was evaporated do dryness, solved in AcOEt (9 ml) and washed with sat. $NaHCO_3$ (3 ml), dried over $MgSO_4$ and concentrated under reduced pressure to afford the title compound as white solid 950 mg (88%).

$\mathbf{R_f}$ 0.7 (AcOEt/Hexane 1:1); **IR** (film, $CHCl_3$): 1729 (w), 904, 45 (s), 723. 66 (s).

^{1}H, **^{13}C NMR**, **M.p.** are described in: Joensson, D., Warrington, B.H., & Ladlow, M. (**2004**) Journal of Combinatorial Chemistry, *6*, 584-595.

Synthesis of Methyl 3-amino-4-chlorobenzoate (39).

To a solution of methyl 4-chloro-3-nitrobenzoate (1.66 g, 8 mmol) in MeOH 65 ml was added anhydrous $SnCl_2$ (14.6 g, 77 mmol) and the resulting reaction mixture was refluxed for 1 h. The reaction mixture was cooled to 0°C followed by dropwise addition of a saturated solution of $NaHCO_3$ to pH = 8.

The reaction mixture was filtered and the filtrate was extracted with AcOEt (3x 30 ml). The combined organic extracted were dried over $MgSO_4$, filtered and concentrated under reduced pressure to obtain a orange crude that was purified (AcOEt/Hexane 1:2) to get 1.34 g (90%) of pale yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.4 (d, J= 1.89 Hz, 1H), 7.3 (dd, J= 8.4, 1.89 Hz, 1H), 7.23 (d, J= 8.4 Hz, 1H), 4.12 (bs, 1H), 3.85 (s, 3H); **^{13}C NMR**

(400 MHz, $CDCl_3$): δ 166.6, 142.8, 129.63, 129.42, 119.87, 116.56, 53.1; **IR** (film, $CHCl_3$): 1701 (m), 1253 (s), 1097 (m), 755 (s); $\mathbf{R_f}$ 0.7 (AcOEt/Hexane 1:1); **M.p.**: 82-83°C; **MS (ESI)**: m/z = 186.0318 $[M+ H]^+$

Synthesis of methyl-3-(3-(benzyloxy)propanamido)-4-chlorobenzoate (40a).

3-(benzyloxy)propanoic acid (615 mg, 3.41 mmol) in 20 ml DMA was treated with thionyl chloride (250 µl, 3.41 mmol) at 0°C. The mixture was stirred for 30 min, and then methyl 3-amino-4-chlorobenzoate (633.5 mg, 3.41 mmol) was added. The resulting suspension was stirred overnight and then quenched with 5 ml of $NaHCO_3$. The reaction mixture was extracted with AcOEt (10 ml x 3), washed with brine, dried over MgSO4 and concentrated under reduced pressure. The crude was purified by fc (Et_2O/Hexane 1:2) to get 1.09 gr (90 %) of white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.93 (d, *J*= 1.9 Hz, 1H), 8.86 (s, 1H), 7.60 (dd, *J*= 8.3, 2 Hz, 1H), 7.30 (d, *J*= 8.3 Hz, 1H), 7.29-7.19 (m, 5H), 4.56 (s, 1H), 3.80 (s, 3H), 3.75 (t, *J*= 5.4 Hz, 2H), 2.66 (t, J= 5.4 Hz, 2H); **^{13}C-NMR** (400 MHz, $CDCl_3$) δ 137.1, 135.2, 129.7, 129.0, 128.5, 128.1, 128, 125.5, 122.7, 73.9, 66.0, 52.4, 38.6; **IR** (film, $CHCl_3$): 3306 (w), 2950, 2867 (w), 1723 (s), 1697 8M9, 1584 (m), 1531 (m), 1416 (m), 1293 (s), 1293 (s), 1106 (s), 760 (m); $\mathbf{R_f}$ 0.16 (Hexane/AcOEt 5:1); **M.p**.: 78°C; **MS (ESI)**: m/z = 348 .0991 $[M + H]^+$

Synthesis of methyl-3-(3-(benzyloxy)propanethioamido)-4-chlorobenzoate (41).

To a solution of methyl-3-(3-(benzyloxy)propanamido)-4-chlorobenzoate (1g, 3 mmol) in dioxane (18 ml) was added Lawesson's reagent (1.28 g, 3

mmol) and the reaction mixture was refluxed for 3 h. The solvent was evaporated and the crude was directly purified by fc (two columns, AcOEt/Hexane 1:6) to obtain 881.8 mg (81%) of yellow oil.

^{1}H NMR (400 MHz; $CDCl_3$): δ10.23 (s, 1H), 9.13 (d, *J*= 2.6 Hz, 1H), 7.8 (dd, *J*= 8.4, 2.0 Hz, 1H), 7.49 (d, *J*= 8.4 Hz, 1H), 7.38-7.27 (m, 5H), 4.64 (s, 2H), 3.92 (s, 3H), 3.9 (t, *J*= 5.5 Hz, 2H), 3.26 (t, J= 5.5 Hz, 2H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 203.7, 165.8, 137.0, 136.0, 132.1, 129.6, 129.1, 128.6, 128.2, 128.1, 128.0, 126.9, 73.8, 68.2, 52.5, 48.7; **R_f** 0.5 (Hexane/AcOEt 2:1); **IR** (film, $CHCl_3$): 3260(w), 3030, 2950 (w), 1723 (s), 1436 (w), 1376 (s), 1302 (s), 1248 (s), 1108 (s), 760 (m), 747 (m); **MS (ESI)**: m/z = 364.0761 $[M + H]^+$

Synthesis of methyl-2-(2-(benzyloxy)ethyl)benzo[*d*]thiazole-5-carboxylate (30a).

To a solution of **26a** in NMP (20 ml) was added portionwise NaH 60 % min oil (185 mg). The reaction was refluxed for 1h at 160°C. The reaction mixture was poured into a flask with water and crushed ice.

The organic phase was extracted with AcOEt (3x 50 ml) and washed with brine. The combined organic phases were dried over $MgSO_4$, concentrated to get an orange oil as crude that was directly purified by fc (AcOEt/Hexane 1:5) to get 1 g (63%) of pale yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (d, *J*= 1.3 Hz, 1H), 8.04 (dd, *J*= 8.3, 1.7 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 1H), 7.36-7.26 (m, 5H), 4.6 (s, 2H), 3.96 (s, 3H), 3.94 (t, *J*= 6.3 Hz, 2H), 3.44 (t, *J*= 6.3 Hz, 2H), **^{13}C NMR** (400 MHz, $CDCl_3$): δ 170.5, 167.1, 153.8, 140.5, 138.0, 128.5, 127.8, 127.8, 125.5, 124.3, 121.4, 73.5, 68.6, 52.4, 35.5; **IR** (film, $CDCl_3$): 2949.6 (w),

1716 (s), 1299 (s), 1284 (s), 1209 (s), 1091 (s), 758 (s), 698 (s); **R_f** 0.74 (Hexane/AcOEt: 2:1); **M.p.**: 45-46°C; **MS (ESI)**: m/z = 328.0999 $[M + H]^+$

Synthesis of (2-(2-benzyloxy)ethyl)benzo[*d*]thiazol-5-yl)methanol (28a).

To a solution of **42a** (1g, 4 mmol) in 16 ml of DCM was added slowly 7.5 ml of DIBAL-H 1.2 M in toluene at -78°C. The mixture was stirred for 1 ¼ h and after the addition of 10 ml of ethylic ether, the reaction was allowed to reach r.t.

360 µl of water, 360 µl of NaOH 15%, 900 µl of water were added following this order, after stirring for 15 min at r.t., a small amount of $MgSO_4$ was added and it was left stirring for 15 min at r.t. The mixture was filtrated, the filtrate was transferred in a funnel and the organic phase was extracted with Et_2O, dried over MgSO4, concentrated to get a yellow oil as crude.

The crude was purified by fc (AcOEt/Hexane 1: 2.5→1:2→1:1→2:1) to get 773.5 mg (72%) of yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.9 (dd, *J*= 2.2, 0.9 Hz, 1H), 7.82 (d, *J*= 8.4 Hz, 1H), 7.38 (dd, *J*= 8.2, 1.6 Hz, 1H), 7.36-7.26 (m, 5H), 4.8 (s, 2H), 4.6 (s,. 2H), 3.93 (t, *J*= 6.2 Hz, 2H), 3.42 (t, *J*= 6.2 Hz, 2H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 169.7, 153.5, 139.4, 138.2, 135, 129, 128, 124.2, 121.7, 121.0, 73.4, 68.7, 65.4, 35.1; **IR** (film, $CHCl_3$): 3344 (bs, w), 2856 (w), 1099 (m), 884 (m), (747 (m), 697 (s); **R_f** 0.1 (Hexane/AcOEt 2:1); **MS (ESI)**: m/z = 300.1055 $[M + H]^+$

Synthesis of the 2-(2-(benzyloxy)ethyl)benzo[*d*]thiazole-5-carbaldehyde (24a).

To a cooled solution (-78°C) of oxalyl chloride (332.8 µl, 3.875 mmol) in 9 ml of DCM was added DMSO (734 µl, 9.7 mmol) as a solution in DCM (2.5 ml), over a period of 5 min.

After 10 min of stirring at -78°C the alcohol (725 mg, 2.42 mmol) solved in 10 ml of DCM was added over 5 min and stirring was continued for 15 min. 1.18 ml of TEA was added to the mixture, the cold bath was removed and it was allowed to stir at r.t. for 2.5 h. After the addition of 4 ml of NH_4Cl (3 ml), the aqueous phase was extracted with DCM (10 ml x 3), the combined organic phases were washed with brine and dried over $MgSO_4$. The yellow crude was purified by fc (AcOEt/Hexane 1:3) to give mg 280 (84%) to get the product as yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.15 (s, 1H), 8.42, (dd, J= 1.7, 0.5 Hz, 1H), 7.99 (d, J=8.5 Hz, 1H), 7.90 (dd, 8.3, 1.5 Hz, 2H), 7.34- 7.26 (m, 5H), 4.6 (s, 2H), 3.9 (t, J= 6.2 Hz, 2H), 3.46 (t, J= 6.2 Hz, 2H); **^{13}C NMR** (400 MHZ, $CDCl_3$): δ 191.94, 171.12, 153.33, 142.27, 137.95, 134.93, 128.53, 127.94, 127.87, 125.5, 124.27, 122.33, 73, 48, 68.37, 35.28; **IR** (film, $CHCl_3$): 2860 (W); 1695 (s), 1598.7 (w), 1103.08 (w).

Aldol reaction of 24b with 3-acetyl-4-benzyloxazolidin-2-one

Procedure with di-n-butylborontriflate/DIPEA(table X-2, entry 1)

To (*S*)-3-acetyl-4-benzyloxazolidin-2-one[43] (0.151 mmol, 33.1 mg) in 1.5 ml of DCM, di-n-butylboron triflate 1M in DCM (0.151 ml, 0.15 mmol) and DIPEA (35 µl, 0.202 mmol) were added at 0°C. The pale yellow solution was cooled to -78°C and the aldehyde **29b** (33 mg, 0.101 mmol) in

1 ml of DCM was added dropwise. The solution was stirred for 30 min at -78°C and then slowly warmed to 0°C (in 25 min). It was then stirred for 2 hours at 0°C. Phospate buffer solution (1 ml) and (1ml) MeOH/ 30% H_2O_2 solution (2:1) were added and the resulting two layers were stirred for 1 h at 0°C.

Sat. aq. NH_4Cl solution (3 ml) and AcOEt were added. The layers were separated and the aq. layer was extracted with AcOEt (3x 10 ml). The combined organic layers were washed with brine (2 ml), dried over $MgSO_4$ and concentrated under reduced pressure. Purification by fc (AcOEt/Hexane 1:1) to separate the starting material from the product, afforded as white solid (29.5 mg, 53%, dr = 3:2).

^{1}H NMR (400 MHz, $CDCl_3$): data for major isomer: **(*S*)-4-benzyl-3-((*S*)-3-hydroxy-3-(2-(2-(4-methoxybenzyloxy)benzo[*d*]thiazol-5-yl)propanoyl)oxazolidin-2-one**: δ 8.03 (t, *J*= 3.1, 1.7 Hz, 1H), 7.85 (d, *J*= 8.3 HZ, 1H), 7.47 (dt, *J*= 8.3, 3.1, 1.5 Hz, 1H), 7.38-7.16 (m, 9H), 6.87 (d, 2H), 5.4 (m, 1H), 4.72 (m, 1H), 4.52 (s, 2H), 4.26 (m, 2H), 3.9 (t, *J*= 6.4 Hz, 2H), 3.8 (s, 3H), 3.53-3.26 (m, 5H); **IR**: 3385 (bs), 2915 (w), 1777 (s), 1698 (m), 1512 (m), 1388 (m), 1245 (s); $\mathbf{R_f}$ 0.35, 0.30 both diastereoisomers (Hexane/AcOEt 1:1); **M.p.**: 99-100°C; **R_t:** 8.77 (38.79%), 9.24 (61.21%) Method 50-60%; **MS (ESI):** m/z = 547.1893 $[M + H]^+$

Procedure with $TiCl_4$, DIPEA (table X, entry 2)

$TiCl_4$ 1M in DCM (84 µl, 0.084 mmol) was added dropwise to a solution 0.2 M of (*R*)-3-acetyl-4-benzyloxazolidin-2-one[43] (16.7 mg, 0.076 mmol) in 0.5 ml of DCM at -78°C under argon giving a yellow slurry. After 2 min, DIPEA (16 µl, 0.092 mmol) was added dropwise, and the resulting deep red solution was stirred at -78°C under argon for 1.5 h. After the dropwise

addition of aldehyde (30 mg, 0.092 mmol) in 0.8 ml of DCM, stirring was continued at -78°C for 2h and for 1 h at -20°C.

The reaction was quenched with 1.2 ml of NH_4Cl and extracted with Et_2O (3x 5 ml). The combined organic layers were washed with brine, dried over MgSO4 and concentrate. The crude was purified by fc (Hexane/AcOEt 1:1) to afford the product as white solid (27 mg, 54%).

The dr in this case hasn't been calculated as the mixture was not separable.

Synthesis of corresponding OTBS-derivative.

In a mass vial to (*S*)-4-benzyl-3-((*S*),(R)-3-hydroxy-3-(2-(2-(4-methoxybenzyloxy)benzo[*d*]thiazol-5-yl)propanoyl)oxazolidin-2-one (8.8 mg, 0.016 mmol) (dr = 1:1), in 15 µl of DMF, imidazole (2.41 mg, 0.035 mmol) and TBS-Cl (2.7 mg, 0.018 mmol) were added and the mixture was stirred at r.t. overnight

^{1}H NMR (400 MHz, $CDCl_3$): data for major isomer (*S*)-4-benzyl-3-((*S*)-3-(*tert*-butyldimethylsilyloxy)-3-(2-(methoxybenzyloxy)ethyl)benzo[*d*]thiazol-5-ylpropanoyl)oxazolidin-2-one): δ 7.98 (s, 1H), 7.81 (d, *J*= 8.3 Hz, 1H), 7.43 (m, 1H), 7.37-7.13 (m, 7H), 6.87 (d, *J*= 8.5 Hz, 2H), 5.44 (m, 1H), 4.70-4.60 (m, 1H), 4.52 (s, 2H), 4.14 (m, 2H), 3.90 (t, *J*= 6.9 Hz, 2H), 3.80 (s, 3H), 3.79-3.70 (m, 0.5H), 3.58-3.50 (m, 0.5 H), 3.40 (t, J= 6.9, 2H), 3.34- 3.20 (m, 1.5 H), 3.10-3.06 (m, 0.5 H), 2.94- 2.65 (m, 1H), 0.87, 0.85 (2s, 9H), 0.08, 0.06 (s, 3H), -0.13, -0.16 (2s, 3H); **IR** (film, $CHCl_3$): 2932 (m), 2858 (m), 1781 (s), 1702 (m), 1515 (m), 1248 (s), 1090 (s), 833 (s); $\mathbf{R_f}$ 0.57, 0.46 of both isomers (Hexane/AcOEt 1:1).

Synthesis of (*S*)-3-acetyl-4-*tert*-butyl-5,5-diphenyloxazolidin-2-one (C)

To a suspension of *(S)*-4-*tert*-butyl-5,5-diphenyloxazolidin-2-one in 3 ml THF, (0.45 ml, 0.71 mmol) of n-BuLi 1.6 M in hexane was slowly added at 0°C in an ice bath. To the resulting clear solution, acetyl chloride (58 µl, 1.2 mmol) was added in one portion. The mixture was allowed to warm slowly to r.t. overnight, treated with aq. sat. NH_4Cl (3 ml), and diluted with Et_2O (10 ml). The organic phase was washed with 1M HCl (3 ml), 1M NaOH (3 ml), brine (3 ml), dried over MgSO4, and evaporated.

Purification by fc EtOAc/Hexane 1:12, afforded the product as white solid (190 mg, 83%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52-7.47 (m, 3H), 7.36-7.23 (m, 7H), 5.33 (s, 1H), 2.33 (s, 3H), 0.82 (s, 9H), **^{13}C NMR** (400 MHz, $CDCl_3$): δ 169.8, 143.8, 137.8, 129.0, 128.6, 128.2, 128.1, 125.6, 90.5, 67.2, 37.2, 27.8, 23.4; **R_f** 0.37 (Hexane/EtOAc 10:1); **M.p.**: 166-167°C; **$[\alpha]_D^{19}$**: -260. 59 °(c = 0.67, $CHCl_3$); **MS (ESI)**: m/z = 338.1752 $[M + H]^+$

Aldol reaction of 24b with (*S*)-3-acetyl-4-*tert*-butyl-5,5-diphenyloxazolidin-2-one (table X, entry 3).

To (*S*)-3-acetyl-4-*tert*-butyl-5,5-diphenyloxazolidin-2-one (0.14 mmol, 46.4 mg) in DCM (1.5 ml) was added di-n-butylboron triflate 1M in DCM (0.140 ml, 0.14 mmol) and DIPEA (32 µl, 0.183 mmol) at 0°C. The pale yellow solution was cooled to -78°C and the aldehyde (30 mg, 0.092 mmol) in 1 ml of DCM was added dropwise. The solution was stirred for 5 ½ h at -78°C and as it was not moving anymore it was quenched at -78°C with phosphate buffer solution (1 ml) and (1ml) MeOH/ 30% H_2O_2 solution (2:1) and the resulting two layers were stirred for 20 min at 0°C.

Sat. aq. NH_4Cl solution (3 ml) and Et_2O (9 ml) were added. The two layers were separated and the aq. layer was extracted with Et_2O (3x 5 ml). The combined organic layers were washed with brine (2 ml), dried over $MgSO_4$ and concentrated under reduced pressure. Purification of 120 mg of crude by fc (AcOEt/Hexane 1:3 → 1:2) to mg 32.7 (54%, 85% brsm) of product as white foam (dr = 3:7, the two diasteroisomers are not separable).

^{1}H NMR (400 MHz, $CDCl_3$), data for major isomer (*S*)-4-*tert*-butyl-3-((*S*)-3-hydroxy-3-(2-(2-4.methoxybenzyloxy)ethyl)benzo[*d*]-thiazol-5-yl)propanoyl)-5,5-diphenyloxazolidin-2-one) δ 7.92 (d, *J*= 1.8 Hz, 1H), 7.75 (d, *J*= 8.5 Hz, 1H), 7.52-7.45 (m, 3H), 7.36-7.23 (m, 10 H), 6.87 (d, *J*= 9.15 Hz, 2H), 5.37 (s, 1H), 5.26 (m, 1H), 4.50(s, 2H), 3.90 (, *J*= 6.62 Hz, 2H), 3.79 (s, 3H), 3.39 (t, *J*=6.62 Hz, 2H), 3.36 -3.3 (m, 1H), 3.0 (m, 1H), 0.83 (s, 9H).

IR (film, $CHCl_3$): 2959 (w), 1780 (s), 1703 (m), 1512 (m), 1338 (m), 1246.8 (s), 1182 (m), 1164 (m), 730 (s), 702 (s); $\mathbf{R_f}$ 0.42 (Hexane/AcOEt 1:1); $\mathbf{R_t}$: 17.14 (28.7%), 17.41 (71.3%) (50-60% method); **MS (ESI)**: m/z = 665.2672.

Synthesis of the corresponding O-TBS derivative.

To a solution of (*S*)-4-*tert*-butyl-3-((*S*),(*R*)-3-hydroxy-3-(2-(2-4.methoxybenzyloxy)ethyl)benzo[*d*]-thiazol-5-yl)propanoyl)-5,5-diphenyloxazolidin-2-one (9 mg, 0.013 mmol) in 10 µl of DMF, was added imidazole (2.04 mg, 0.03 mmol), TBS-Cl (2.15 mg, 0.014 mmol), and the reaction mixture was stirred at r.t. overnight. The crude was directly purified by fc (AcOEt/Hexane 1:2) to obtain an inseparable mixture of two diastereoisomers (mg 7.1, 57% yield) as colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86, 7.83 (d, *J*= 1.6 Hz, 1H), 7.73, 7.69 (d, *J*= 8.5 Hz, 1H), 7.52, 7.33 (m, 3H), 7.36-7.23 (m, 10 H), 6.87 (d, *J*= 8.7Hz, 2H), 5.31, 5.30 (s, 1H), 5.27 (m, 1H), 4.53,4.52 (s, 2H), 3.90 (t, *J*= 6.62 Hz, 2H), 3.80, 3.79 (s, 2H), 3.39 (t, *J*= 6.62 Hz, 2H), 3.25 -3.18 (m, 1H), 0.83 (s, 9H), 0.76 (s, 9H), -0.017 (3H), -0.23 (3H); **IR** (film, $CHCl_3$): 2955.4 (m), 2931 (m), 1785 (s), 1710 (w), 1513 (w), 1249 (s), 1090 (s); **R_f** 0.66, 0.62 (Hexane/AcOEt 1:1) and R_f 0.84 (AcOEt/Hexane 1:2); **MS (ESI)**: m/z =779.3538

Synthesis of (4*S*, 5*S*)-3-((*S*)-3-hydroxy-3-(2-(2-4-methoxybenzyloxy)ethyl)benzo[d]thiazol-5-yl)propanoyl-4-methyl-5-phenyloxazolidin-2-one (47).

To a solution of diisopropylamine (16.2 µl, 0.116 mmol) in 0.380 ml of THF was added n-BuLi 2.5 M in hexan (50 µl) at -30°C. After 15 min the mixture was cooled to -70°C and oxazolidinone[39] (23.8 mg, 0.109 mmol) solved in 0.2 ml of THF was added slowly. After stirring for 15 min, 0.220 ml of $ZnCl_2$ 0.5 M in THF was added and stirring continued for additional 15 min,

Afterwards, the aldehyde (22 mg, 0.067 mmol) in 0.25 ml of THF was added. The mixture was stirred for 30 min and poured into concentrated aq. NH_4Cl (2 ml), extracted with AcOEt (8 ml), dried over $MgSO_4$ and concentrated under reduced pressure.

The crude was purified by fc (two colums, AcOEt/Hexane 1:2→ 1:1) to get 23 mg (64%) of product as white foam (dr = 4:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02, (d, *J* = 1.6 Hz, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.50 - 7.34 (m, 4H), 7.34 -7.23 (m, 4H), 6.87 (d, *J* = 8.6 Hz, 2H), 5.68 (d, *J*= 7.4 Hz, 1H), 5.37 (m, 1H), 4.8 (m, 1H), 4.52 (s, 2H), 3.9 (t, *J*=

6.2 Hz, 2H), 3.6- 3.3 (m, 5H) 3.1 (s, 3 H), 0.94 (s, 3H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 159.5, 141.0, 133.2, 130.1, 129.5, 129.0, 128.9, 125.8, 122.9, 121.8, 120.0, 114.0, 79.4, 73.1, 70.4, 68.4, 55.5, 55.1, 44.9, 35.3, 14.6; **IR** (film, $CHCl_3$): 1778 (s), 1700 (m), 1512 (w), 1366 (m), 1348 (m), 1245 (w), 1195 (m); **R_f** 0.28 (AcOEt/Hexane 1:2); **MS (ESI)**: m/z = 547.1878 $[M+ H]^+$

Synthesis of (4S, 5S)-3-((S)-3-(tert-butyldimethylsilyloxy)-3-(2-(2-4-methoxybenzyloxy)ethyl)benzo[d]thiazol-5-yl)propanoyl-4-methyl-5-phenyloxazolidin-2-one (48).

To a solution of alcohol **34** (8 mg, 0.015 mmol) in 10 µl of DMF, was added imidazole (2.19 mg, 0.032 mmol), TBS-Cl (2.4 mg, 0.016 mmol), and the reaction mixture was stirred at r.t. overnight. The crude was directly purified by fc with AcOEt to get 6.1 mg of colourless oil (67% referred to the main diasteroisomer).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, *J*= 1.7 Hz, 1H), 7.81 (d, *J*= 8.3 Hz, 1H), 7.47 (dd, J= 8.3, 1.6 Hz, 2H), 7.3-7.26 (m, 5H), 6.87 (d, *J*= 8.7 Hz, 2H), 5.54 (d, *J*= 7.1 Hz , 1H), 5.42 (dd, *J*= 8.4, 3.1 Hz, 1H), 4.7 (m, 1H), 4.5 (s, 2H), 3.9 (t, *J*= 6.5 Hz,), 3.8 (s, 3H); 3.55 (m, 1H), 3.4 (t, *J*= 6.5 Hz, 2H), 3.26 (m, 1H), 0.86 (s, 9H), 0.06 (s, 3H), -0.01 (s, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): 170.2, 169.5, 159.4, 153.1, 142.7, 142.6, 134.7, 134.4, 130.2, 129.5, 128.9, 128.86, 125.8, 123.3, 121.5, 120.2, 114, 79.1, 73.0, 71.6, 63.4, 55.4, 35.0, 47.2, 35.2, 25.9, 18.3, 14.8, -4.3, -4.94.

IR (film, $CHCl_3$): 1780 (m), 1701 (m), 1346 (m), 907 (m), 728 (s); **R_f** 0.23 (Hexane/AcOEt 3:1), 0.5 (Hexane/AcOEt 2:1); **MS (ESI)**: m/z = 661.2746; **R_t**: 10.13 (19.19%), 10.18 (80.81%) 50-90%

$[\alpha]^{19}_D$ = -36.62° (c = 0.475 M, $CHCl_3$)

Synthesis of (S)-ethyl 3-(tert-butyldimethylsilyloxy)-3-(2-(2-(4-methoxybenzyloxy)ethyl)benzo[d]thiazole-5-yl)propanoate (49)

In a microwave vial to **35** (180 mg, 0.272 mmol) in 1 ml of EtOH was added titanium (IV) ethoxide (26 µl, 0.124 mmol) and the mixture was refluxed for 6 h at 78°C.

3 ml of AcOEt and 1 ml of water were added and after 30 minutes of stirring the organic layer was removed from the precipitate by filtration and concentrated under reduced pressure. The residue was purified by fc (hexane /AcOEt 1:3) to yield 134. 7 mg (94%) of pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (d, *J*= 1.6 Hz, 1H), 7.79 (d, *J*= 8.3 Hz, 1H), 7.40 (dd, J= 8.3, 1.6 Hz, 2H), 7.27 (d, *J*= 8.84 Hz, 2H), 6.87 (d, *J*= 8.84 Hz, 2H), 5.29 (dd, *J*=13.4, 4.4 HZ, 1H), 4.52 (s, 2H), 4.14 (m, 2H), 3.9 (t, *J*= 6.5 Hz, 2H), 3.8 (s, 3H); 3.4 (t, *J*= 6.5 Hz, 2H), 2.78 (m, 2H), 2.60 (m, 1H), 1.25 (t, *J*= 6.3 Hz, 3H) 0.86 (s, 9H), 0.06 (s, 3H), -0.01 (s, 3H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 171.75, 169.70, 159.5, 153.3, 142.8, 134.8, 130.2, 129.5, 123, 121.5, 120, 114, 73.05, 72.3, 68.4, 60.7, 55.42, 46.84, 35.15, 25.9, 18.2, 14.4, -4.4, -5.13; **IR** (film, $CHCl_3$): 1735 (m), 1513 (m), 1249 (s), 1092 (s), 1172 (m), 1039 (m), 833 (m), 780 (m); **$[\alpha]_D^{19}$**= - 40.92° (c= 0.48 M, CHCl3); **R_f** 0.71 (Hexane/AcOEt 1:1); **MS (ESI)**: m/z = 530.2385 $[M+H]^+$

Synthesis of (S)-ethyl 3-(tert-butyldimethylsilyloxy)-3-(2-(2-(4-methoxybenzyloxy)ethyl)benzo[d]thiazole-5-yl)propanale (23)

To a stirred solution of **36** (39.60 mg, 0.075 mmol) in 0.65 ml of DCM at -78°C was added 1.2 M DIBAL-H (90 µl, 0.187 mmol). The mixture was stirred for 3 h at -78°C, then it was quenched with MeOH (30 µl) and it was left to reach r.t.

2ml of DCM and 1 ml of water were added and the mixture was stirred for ½ h to get a precipitate that was filtered on celite. The two phases were separated and the organic phase was washed with water (1 ml), brine (1 ml), dried over $MgSO_4$ and concentrated to get a yellow oil as crude. Purification by fc (AcOEt/Hexane 1:3) to get 18 mg (50%) of pale yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.8 (t, 1H, *J*= 2.5 Hz), 7.44 (d, J= 8.84 Hz,1H), 7.82 (d, *J*= 8.4 Hz, 1H), 7.37 (dd, *J*= 8.37, 1.86 Hz, 1H), 7.27 (d, *J*= 8.8 Hz, 2H), 6.87 (d, *J*= 8.8 Hz, 2H), 5.35 (dd, *J*= 8.0, 4.0 Hz, 1H), 4.53 (s, 1H), 3.9 (t, *J*= 6.34 Hz, 2H), 3.8 (s, 1H), 3.4 (t, *J*= 6.34 Hz, 2H), 2.9 (m, 1H), 2.68 (m, 1H), 0.88 (s, 9H), 0.06 (s, 3H), -0.13 (s, 3H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 201.2, 170.0, 159.5, 153.3, 142.3, 135.0, 130.2, 129.54, 122.80, 121.72, 119.80, 114.01, 73.05, 70.84, 68.32, 55.46, 54.43, 35.14, 28.85, 25.97, 18.25, -4.35, -4.96; **IR** (film, $CHCl_3$): 1725 (m), 1513 (m), 1248 (s), 1092 (s), 1034 (m), 836 (s), 778 (m), 2931 (w); **R_f** 0.70 (Hexane/AcOEt 1:1).

$[\alpha]_D^{19}$= -56.5 ° (c = 0.50 M, $CHCl_3$); **MS (ESI)**: m/z = 518.2569 (this aldehyde isn't so stable).

Synthesis of (S, Z)-methyl 5-(tert-butyldimethylsilyloxy)-5-(2-(2-(4-(methoxybenzyloxy)ethyl)benzo[d]thiazol-5-yl)-2-methylpent-2-enoate (50).

A solution of phosphonate (16 mg, 0.48 mmol), 18-crown-6 (63.7 mg, 0.24 mmol) in 1 ml of dry THF is cooled at -78°C under nitrogen and treated with KHMDS (9.6 mg, 0.048 mmol). After ½ h, the aldehyde (23.4 mg, 0.048 mmol) in 0.5 ml was added and the resulting mixture was stirred for 3 h at-78°C.

The reaction was cautiously quenched by addition of 1 ml sat. NH_4Cl, the aq. layer was extracted with Et_2O (5 ml x 3) and the combined ethereal layers were dried over $MgSO_4$. Purification by fc (AcOEt/Hexane 1:8 → 1:7) afforded 9.3 mg (35%) of product as a colourless oil.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.92 (s, 1H), 7.78 (d, *J*= 8.2 Hz, 1H), Hz, 1H), 7.36 (dd, *J*= 8.27, 1.70 Hz, 1H), 7.27 (d, *J*= 8.8 Hz, 2H), 6.87 (d, *J*= 8.7 Hz, 2H), 6.04 (m, 1H), 4.92 (m, 1H), 4.53 (s, 2H), 3.91 (t, *J*= 6.6 Hz, 2H), 3.80 (s, 3H), 3.70 (s, 3H), 3.41 (t, *J*= 6.6 Hz, 2H), 2.9 (m, 2H),1.88 (s, 3H), 0.90 (s, 9H), 0.04 (s, 3H), -0.11 (s, 3H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 190.0, 169.4, 168.3, 159.5, 153.2, 143.7, 139.3, 134.2, 130.2, 129.5, 128.7, 123.1, 121.3, 119.9, 114.0, 74.7, 73.1, 68.4, 55.4, 51.4, 41.06, 35.2, 26.0, 20.9, 18.4, - 4.40, - 4.8; **R_f** 0.49 (Hexane/AcOEt 2:1).

$[\alpha]_D^{19}$= -140.90° (c = 0.59 M, $CHCl_3$); **MS (ESI)**: m/z = 556.2538 $[M + H]^+$

Synthesis of (*S*, Z)-methyl 5-(tert-butyldimethylsilyloxy)-5-(2-(2-(4-(methoxybenzyloxy)ethyl)benzo[d]thiazol-5-yl)-2-methylpent-2-en-1-ol (22).

To a solution of **32** (9 mg, 0.016 mmol) in 120 µl of DCM, was added slowly (40 µl, 0.039 mmol) of DIBAL-H 1 M in DCM at -78°C. The reaction mixture was left to stir at this temperature for 1 h ½ and then warmed at 0°C and kept at that temperature for 30 min.

1 ml of ethylic ether was added, cooled to 0°C and after the addition of 1.56 µl of water, 1.56 µl of NaOH15%, 3.9 µl of water, the mixture was warmed to r.t. and stirred for 15'.

$MgSO_4$ was added, the mixture stirred for further 15 min and at the end it was filtered on a very small plug of celite. The crude was directly purified by fc (AcOEt/Hexane 1:2) to get mg 5.7 (67%) of product as colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (d, *J*= 1.7 Hz, 1H), 7.92 (d, *J*= 8.1 Hz, 1H), 7.35 (dd, *J*= 8.37, 1.56 Hz, 1H), 7.28 (d, *J*= 8.6 Hz, 2H), 6.87 (d, *J*= 8.6 Hz, 2H), 5.33 (m, 1H), 4.82 (m, 1H), 4.53 (s, 2H), 4.06 (d, *J*= 11.8 Hz, 1H), 3.96-3.88 (m, 3H), 3.8 (s, 3H), 3.41 (t, *J*= 6.9 Hz, 2H), 2.65- 2.54 (m, 1H), 2.43-2.35 (m, 1H), 1.8 (s, 3H), 0.88 (s, 9H), 0.01 (s, 3H), -0.08 (s, 3H); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 159.4, 143.5, 138.0, 134.2, 130.1, 129.5.124.2, 123, 121.2, 119.8, 113.9, 74.9, 73.0, 68.3, 61.9, 55.4, 39.8, 35.2, 29.8, 26.0, 22.1, 18.5, -4.5, -4.8; **IR** (film, $CHCl_3$): 3369 (w), 2929 (m), 2857 (m), 1512 (m), 1461 (w), 1248 (s), 1087 (s), 1030 (m), 831 (s), 780 (m); $\mathbf{R_f}$ 0.31 (Hexane/AcOEt 1:2); $\mathbf{[\alpha]_D^{19}}$= -55.97 ° (c = 0.25M, $CHCl_3$)

Synthesis of ((1S, 2S)-2-((S)-2-(tert-butyldimethylsilyloxy)-2-(2-(2-(4-methoxybenzyloxy)ethyl)benzo[d]thiazol-5-yl)ethyl-1-methylcyclopropyl)methanol (15).

To a stirred solution of Et_2Zn 1M in hexane (17 µl, 0.106 mmol) in 0.5 ml of dry DCM at 0°C was added CH_2I_2 (12.7 µl, 0.158 mmol). The mixture was stirred at 0°C for 10 min and a preformed solution of Charette ligand (3 mg, 0.012 mmol) and **38** in 0.2 ml of dry DCM was rapidly added via syringe. The resulting mixture was allowed to reach r.t and stirred for 1½ h. Aq. Sat. NH_4Cl (1 ml) was added and the mixture extracted with (3 ml x 3) of DCM. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give after purification by fc (AcOEt/Hexane 1:3 →1:2) 4 mg of product as white foam.

^{1}H NMR (400 MHz, $CDCl_3$): 7.87 (d, *J*= 1.66 Hz, 1H), 7.8 (d, *J*= 8.3 Hz, 1H), 7.37 (dd, *J*= 8.2, 1.5 Hz, 1H), 7.2 (d, *J*= 8.4 HZ, 2H), 6.87 (d, *J*= 8.4 Hz, 2H), 4.90 (m, 1H), 4.53 (s, 1H), 3.9 (t, *J*= 6.2 Hz, 2H), 3.8 (s, 3H), 3.67 (m, 1H), 3.4 (t, *J*= 6.2 Hz, 2 H), 3.3 (d, *J*= 12.3 , 1H), 1.9 (m, 1H), 1.7- 1.57

(m, 2H), 1.2 (s, 3H), 0.89 (s, 9 H); 0.45 (m, 1H), 0.04 (s, 3H), -0.07 (s, 3H); **^{13}C NMR** (400 MHZ, $CDCl_3$): 169.6, 159.5, 153.1, 144.2, 134.4, 130.2, 129,5, 123.0, 121.5, 119.8, 114, 73.07, 68.37, 67.02, 55.4, 41.5, 35.2, 29.9, 26.2, 23.2, 22.8, 22.0, 18.53, 16.54, 14.3, -4.32; **IR** (film, $CHCl_3$): 1761 (s), 1716 8s), 1390, 1355, (m), 1259, 1199 (m); $\mathbf{R_f}$ 0.35 (Hexan/AcOEt 2:1); **MS (ESI)**: m/z = 542.2754 $[M+H]^+$; $\mathbf{[\alpha]_D^{19}}$= -72. 02 ° (c = 0.19M; $CHCl_3$).

Chapter 13

Epothilones references

13 References

1) Gerth, K.; Bedorf, N.; Hofle, G.; Irschik, H.; Reichenbach, H. *The journal of Antibiotics* **1996**, *49*, 560.
2) Hofle, G.; Bedorf, N.; Steinmetz, H; Schomburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem. Int. Ed*. **1996**, *35*, 1567.
3) Altmann, K.-H., Pfeiffer, B., Arseniyadis, S., Pratt, B.A., Nicolau, K.C. *ChemMedChem.* **2007**, *2*, 396-423.
4) Bollag, D.M.; McQueney, P.A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.;Woods, C.M. *Cancer Research* **1995**, *55*, 2325.
5) Kowalski, R.J.; Giannakakou, P.; Hamel, E. *Journal of Biological Chemistry* **1997**, *272*, 2534.
6) Balog, A.; Meng, D.; Kamenecka, T.; Bertinato, P.; Su, D.-S.; Sorensen, E. J.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **1996**, *35*, 280.
7) Yang, Z.; He, Y.; Vourloumis, D.; Volberg, H.; Nicolau, K. C. *Angew. Chem. Int. Ed*. **1997**, *36*, 166
8) Meng, D.; Bertinato, P.; Balog, A.; Su, D.-S.; Kamenecka, T.; Sorensen, E. J.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **1997**, *119*, 10073.
9) Su, D.-S.; Meng, D.; Bertinato, P.; Balog, A.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S.B. *Angew. Chem. Int. Ed.* **1997**, 36, 757.
10) Nicolau, K.C.; Ninkovic, S.; Finlay, M. R. V.; Sarabia, F.; Li, T. *Chem. Comm*. **1997**, 2343.
11) Newman, R.; Yang, J.; Finlay, R.; Cabral, F.; Vourloumis, D.; Stephens, C.; Troncoso, P.; Wu, X.; Logothetis, C.; Nicolau, K. C.; Navone, N. *Cancer Chemotheraphy and Pharmacology*, **2001**, *48*, 319.
12) Nicolau, K. C.; Winssinger, N.; Pastor, J.; Ninkovic, S.; Sarabia, F.; He, Y.; Vourloumis, D.; Yang, Z.; Li, T.; Giannakakou, P.; Hemel, E. *Nature*, **1997**, *387*, 286
13) Nicolau, K. C.; Namoto, K.; Ritzen, A.; Ulven, T.; Shoji, M.;Li, T.; D'Amico, G.; Liotta, D.; French, C.T.; Wartmann, M.; Altmann, K.-H.; Giannakakou, P.; *JACS*, **2001**, *123*, 939.
14) Höfle, G.; Glaser, N.; Kiffe, M.; Hecht, H.-J.; Sasse, F.; Reichenbach, H. *Angew. Chem. Int. Ed.* **1999**, *38*, 1971.
15) Nicolau K.C.; Ritzén, A.; Namoto, K.; Buey, R. M.; Díaz, J.F.; Andreu, J.M.; Wartmann, M.; Altmann, K.-H.; O'Brate, A.; Giannakakou, P. *Tetrahedron*, **2002**, *58*, 6413.
16) Nicolau, K.C.; Sasmal, P.K.; Rassias, G.; Reddy, M.V.; Altmann, K.-H.; Wartmann, M.; O'Briate, A.; Giannakakou, P. *Angew. Chem. Int Ed.* **2003**, *42*, 3515.
17) Nicolau, K. C.; Vourloumis, D.; Li, T.; Pastor, J.; Winssinger, N.; He, J.; Ninkovic, S.; Sarabia, F.; Vallberg, H.; Roschangar, F.; King, N.P.; Finlay,

M.V.R.; Giannakakou, P.; Verdier-Pinard, P.; Hamel, E. *Angew. Chem. Int, Ed*. **1997**, *36*, 2097.
18) Nicolau, K.C.; Scarpelli, R.; Bollbuck, B.; Werschunk, B.; Pereira, M.M.A.; Wartmann, M.; Altmann, K.-H.; Zaharevitz, D.; Gussio, R.; Giannakakou, P.; *Chem Biol.* **2000**, *7*, 593-599
19) Altmann, K.-H.; Bold, G.; Caravatti, G.; Flörsheimer, A.; Guagnano, V.; Wartmann, M. *Bioorg. Med. Chem. Lett*, **2000**, *10*, 2765.
20) Cachoux, F.; Isarno, T.; Wartmann, M.; Altmann, K.-H. *Angew, Chem, Int, Ed*, **2005**, *44*, 7469.
21) Borzilleri, R.M.; Zheng, X.; Schmidt, R. J.; Johnson, J.A.; Kim, S.-H.; Di Marco, J.D.; Fairchild, C.R.; Gougoutas, J.Z.; Lee, F.Y.F.; Long, B.H.; Vite, G.D. *JACS*, **2000**, *122*, 8890.
22) Klar, U.; Buchmann, B.; Schwede, W.; Skuballa, W.; Hoffmann, J.; Lichtener, R. B. *Angew. Chem. Int. Ed*. **2006**, *45*, 7942.
23) Schliemann, C.; Neri, D. *Biochimica et Biophysica Acta* 1776, **2007**, 175-192
24) Schinzer, D.; Limberg A.; Bauer, A.; Böhm, M. O.; Cordes, M. *Angew. Chem. Int. Ed. Engl*. **1997**, *36* (5), 523-524.
25) Charette, A.B. et al. *J. Am. Chem. Soc*. **1998**, *120*, 11943-11952.
26) Kuzniewski N.C.; Getsch, J.; Wartmann, M.; Altmann K.-H. *Org. Lett.* **2008**, *10* (6), 1183-1186.
27) Altmann, K.-H.; Bold, G.; Caravatti, G.; Denni, D.; Flörsheimer, A.; Schmidt, A.; Rihs, G.; Wartmann, M. *Helv. Chim. Acta* **2002**, *85*, 4086-4110.
28) White, J.D.; Carter, R.G.; Sundermann, K.F.; Wartmann, M. *JACS*, **2001**, *123*, 5407-541.
29) (a)Feyen, F.; Jiantsch, A.; Hauenstain, K.; Pfeiffer, B.; Altmann, K.-H. Tetrahedron **2008**, *65*, 7456; (b) *Angew. Chem. Int. Ed*. **2006**, *45*, 5580-5885.
30) Bold, G.; Wojeik, S.; Caravatti, G.; Lindauer, R.; Stierlin, C.; Gertsch, J.; Wartmann, M.; Altmann, K.-H. *ChemMedChem* **2006**, *1*, 37, reference [12].
31) Keck, G.E., Tarbet, K.H., Geraci, L.S. *J. Am. Chem. Soc*. **1993**, *115*, 8467.
32) Brown H.C., Racherla, U.S., Pellecchia, P.J. *J. Org. Chem*. **1990**, *55*, 1868-1874.
33) Crepin A., Wattier N. *Org. Biomol. Chem*. **2009**, *7*, 128-134.
34) For formylation procedures see: (a) Chiurato M., Routier S., Troin Y., Guillaumet, G. *Europ. J. Org. Chem*. **2009**, 3011-3021. (b) Hrobárik P., Sigmundová I. *Synthesis* **2005**, *4*, 600-604.
35) Aulakh V. S., Ciufolini M. *J. Org. Chem.* **2009**, *74* (15), 5750-5753.
36) (a) Cho S.J. et al. *J. Med. Chem.* **2009**, *52* (7), 1885-1902. (b) Onado T., Takikawa, Y., Fujimoto, T., Yashi, Y., Suzuki, K., Matsumoto, T. *Synlett*, **2009**, *7*, 1041-1046.
37) Zajac, M., Hrobárik, P., Magdolen, P., Foltínová, P., Zahradník, P. *Tetrahedron*, **2008**, *64* (46), 10605-10618.
38) Das S.K., Panda, G. *Tetrahedron*, **2008**, *64* (19), 4162-4173.
39) Young R.C., Ganellin C. R., Griffith R., Mitchell R. C., Parsons M. E., Saunders D., Sore N.E. *Europ. J. Med. Chem.* **1993**, *28*, 201-211.

40) Langendoen, A., Koomen, G-J., Pandit, U.K. *Tetrahedron*, **1988**, *44* (12), 3627-3631.
41) (a) Park, C-H., Yous, S., Nativelle-Serpentini, C., Seralini, G-E., Chang, S-J., Lesieur, D., Patent WO/2005/033104. (c) Richter U., Patent WO200580406.
42) Kaneko, T., Clark R.S.J., Ohi., Kawahara T., Akamutsu H., Ozaki F., Kamada A., Okano K., Yokohama H., Muramoto K., Okhuro M., Takenaka O., Kobayashi S. *Chem. Pharm. Bull*, **2002**, *50* (7) 922-929.
43) (a) Yadav J.S. et alt. *Tetrahedron: Asimmetry* **2001**, *12*, 2129-2135. (b) Shibahara S., Fujino M., Tashiro Y., Okamoto N., Esumi T., Takahashi K., Ishihara J., Hatakeyama S. *Synthesis*, **2009**, *17*, 2935-2953.
44) White J.D., Tiller T., Ohba Y., Porter W. J., Jackson R. W., Wang S., Hanselmann R. *Chem. Comm.* **1998**, 79.
45) Haydan D., et al., *J. Med. Chem.* **2010**, *53* (10), 3927-3036.
46) Heumann L.V., Keck G.E. *Organic Letters*, **2007**, *9* (10), 1951-1954.
47) Albrecht M. et al. *Tetrahedron*, **2002**, *58*, 561-567.
48) Todd M. H. *J. Org. Chem*. **2002**, *67* (12), 3985-3988.
49) Zhang X. *J. Med. Chem*. **2007**, *50*, 3857-3869.
50) Rodriguez , J.B., *Tetrahedron* **1999**, 55, 2157-2170.
51) Hoye T. R., Jeffrey C. S., Shao F. *Natural Protocols*, **2007**, *2* (10), 2451-2458.
52) Li P., Li Y., Arikau F., Ahlbrecht W., Dieckmann M., Menche D. *J. Org. Chem*. **2010**, *75*, 2429-2444.
53) Dietrich S. A., Lindauer R., Stierlin C., Gertsch J., Matesanz R., Notararigo S., Díaz J. F., Altmann K.-H. *Chem. Eur. J*. **2009**, *15*, 10144 – 10157.
54) Hintermann T., Seebach D., *Helvetica Chimica Acta* **1998**, *81*, 2093-2125.
55) Ho G-J., Mathre D. J. *J. Org. Chem.* **1995**, *60*, 2271.
56) Blakemore, P.R. et al. *J. Org. Chem*. **2005**, *70*, 5449-5460
57) Evans D. A., Rieger D.L., Bilodeau M. T., Urpi F. *J. Am. Chem. Soc.* **1991**, *113* (3) 1047-1049.
58) House H.O., Crumrine D.S., Teranishi A.Y., Olmstead H.D. *J. Am. Chem. Soc.* 1973, *95* (10), 3310–3324.
59) Wullschleger, C., Altmann K-H., *Organic Letters* **2010**, *12*(5), 1120-112.
60) Still, W.C., Gennari, C., *Tetrahedron letters* **1983,** *24*, 4405-4408.

List of abbreviation.

9-BBN-H	9-Borabicyclo[3.3.1]nonane
(S)-BINOL	S-(-)-1,1'-Bi-2-naphthol
Bn	Benzyl
Boc	*t*-Butyloxycarbonyl
BTAF	Benzyltrimethylammonium Fluoride
CA-4	Combretastatine A-4
Cbz	Carbobenzyloxy
CDI	Carbnyldiimidazole
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL (DIBAH)	Diisobutylaluminum Hydride
DIPEA	N,N-diisopropylethylamine
DMA	Dimethylacetamide
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
de	Diastereomeric excess
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	Enantiomeric Excess
ESI	Electrospray ionization
GTP	Guanosine-5'-triphosphate
FDA	Food and Drug Administration
HBT	Hydroxybenzotriazole
Ipc_2BH	Bisisopinocampheylborane
KHMDS	Potassium bis-trimethylsilylamide
Lawesson' s reagent	2,4-bis(4-methoxyphenyl)-1,3,2,4-

	dithiadiphosphetane-2,4-disulfide
LDA	Lithium Diisopropylamide
Leu	Leucine
LHMDS	Lithium Hexamethyldisilazide
Lys	Lysine
MS	Mass spectrometry
NMP	N-methyl-2-pyrrolidone
NMR	Nuclear Magnetic Resonance
PCC	Pyridinium chlorochromate
PG	Protecting group
PMB	p-Methoxybenzyl
PPTS	Pyridinium p-toluensulfonate
R_f	Retention factor
RCM	Ring closure methatesis
r.t.	Room temperature (23°C)
SAR	Structure activity relationship
TBAF	Tetra-*n*-butylammonium fluoride
TBS	t-Butyldimethylsilyl (also TBDMS)
TEA	Triethylamine
TFA	Trifluoroacetic(yl)
THF	tetrahydofurane
TMAC	Tymethylacethyl chloride
TPAP	Tetra-n-propylammonium Perruthenate
Val	Valine
Z	Cis