Università degli Studi di Ferrara

DOTTORATO DI RICERCA IN
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CICLO XXV

COORDINATORE Prof. Carlo Alberto Bignozzi

Synthesis and biological applications of non natural α-amino acids

Settore Scientifico Disciplinare CHIM/06

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All of old. Nothing else ever. Ever tried. Ever failed.
No matter. Try again. Fail again. Fail better.
– Samuel Beckett

There is excitement, adventure, and challenge,
and there can be great art in organic synthesis.
— R.B. Woodward

Alla mia famiglia.
E a chi ha sempre creduto in me.

SOMMARIO

In questo lavoro di Tesi è stata affrontata la sintesi e l'applicazione in campo biomedico di α-amminoacidi non naturali in tre differenti progetti.

È stata messa a punto la sintesi "one-pot" di *N*-acetamido acrilato di mentile otticamente attivo e puro a partire da reattivi commerciali a basso costo, ottenendolo in buone rese ed identificando il maggiore sottoprodotto di reazione. Tale acrilato è stato utilizzato come accettore in reazioni di Michael per la formazione di nuovi legami C-C e C-S, valutando l'induzione asimmetrica apportata dal $(-)$-mentolo. Diversi nucleofili sono stati utilizzati e molteplici basi testate per cercare di ottenere una conversione completa. Si è dunque indagata la possibilità di reazione della molecola con opportuni nucleofili a dare cicli a cinque termini tetraidrotiofenici ed altri intermedi a struttura di biciclo[3.1.0]esano e biciclo[4.1.0]eptano. Tutto ciò è stato fatto nell'ottica di ottenere metodi alternativi per la sintesi di analoghi a struttura costretta del glutammato, in precedenza identificati da Ely Lilly come potenti, selettivi e attivi agonisti dei recettori mGluR.

Il secondo progetto, svolto presso il laboratorio del Professor Matteo Zanda all'Università di Aberdeen, riguarda la sintesi di piccoli peptidi contenenti arginina ed analoghi dello stesso amminoacido. I piccoli peptidi sono costituiti dal residuo argininico accoppiato con differenti basi a struttura picolinica, mentre gli analoghi contengono un sostituente alfa-metilico ed un triplo legame, od un gruppo trifluorometilico ed il triplo legame – purtroppo questa parte del progetto non è stata conclusa per motivi di tempo. L'interesse per questo tipo di analoghi e peptidi risale al fatto che l'arginina è il substrato per le Nitric Oxide Synthase, classe di enzimi coinvolti nella regolazione del rilassamento muscolare e della pressione sanguigna attraverso la sintesi di ossido nitrico a partire dal summenzionato amminoacido. I substrati progettati e sintetizzati, grazie alla loro struttura, risultano polarizzabili per mezzo di spettroscopia NMR e tecniche particolari con molecole di paraidrogeno. Così, sarebbe possibile preparare specifici "tracers" per Risonanza Magnetica, permettendo una facile e sicura rivelazione di malattie attraverso una semplice e veloce scansione. Infatti, alcuni dei peptidi sintetizzati e polarizzati sono stati testati in vitro e risultano compatibili con l'enzima. Infine, è stato messo a punto un sistema per la determinazione del contenuto di controione trifluoroacetato all'interno delle molecole.

Il terzo progetto ha riguardato l'individuazione e la messa a punto di una via alternativa alla sintesi a poco costo dell'amminoacido 2,6-dimetil-L-tirosina, un potente analogo della tirosina usato in peptidi attivi sui recettori degli oppiacei. Come reagente di partenza è stato utilizzato un derivato analogo dell'estere di Hagemann. Tale reagente è stato utilizzato come punto di partenza per due vie sintetiche: una stereoselettiva grazie all'impiego di un sintone chirale a struttura 2,5-dichetopiperazinica, ed una seconda che ha visto come reazione-chiave una reazione di Michael per l'introduzione del residuo amminoacidico.

ABSTRACT

In this PhD Thesis work, the synthesis and the biomedical application of non natural α-amino acids was accomplished in three different projects.

A "one-pot" procedure for the synthesis of optically active and pure methyl *N*-acetamido acrylate starting from cheap and commercial sources was developed and fine-tuned, obtaining the target compound in good yields and identifying the major reaction by product. This acrylate was used as acceptor in Michael reaction conditions for the formation of new C-C and C-S bonds, evaluating the asymmetry induced by the (−)-menthol. Different nucleophiles were used and different bases were tested in order to obtain a full conversion. After that, reaction of the aforementioned molecule with opportune nucleophiles to synthesise five-membered tetrahydrothiophene rings and other intermediates with a bicyclo[3.1.0]hexane and bicyclo[4.1.0]heptane structure was investigated. The project was undertaken in order to find new synthetic ways obtain constrained glutamate analogues, which have been identified by Eli Lilly as potent, selective and active agonists of mGluR receptors.

The second project, developed in the laboratory of Professor Matteo Zanda at the Univesity of Aberdeen, is focused on the synthesis of small arginine-containing peptides and arginine analogues. The small peptides are obtained by simple coupling of the amino acid residue with different picolyl amines, while analogues of the parent amino acid will contain a triple bond and a methyl or trifluoromethyl substituent on the α-carbon of the amino acid skeleton. Interest in these two type of compounds was risen because arginine is a substrate for Nitric Oxide Synthase, a class of enzymes involved in the regulation of muscular relax and blood pressure through nitric oxide synthesis starting from the aforementioned amino acid. The designed and synthesised substrates result being able to polarise their signal under specific NMR conditions in the presence of parahydrogen. In this way, a possibility towards the synthesis of of specific and target-focused tracers for Magnetic Resonance Imaging would be achieved, allowing for a simple and secure detection of illness via a simple and quick MRI scan. Indeed, some of the synthesised compounds polarise and are substrates for the Enzyme. On the last note, a method for the determination of the amount of trifluoroacetic counter-ion was developed.

The third project is focused on the development of an alternative synthetic route for the cheap synthesis of 2,6-dimethyl-L-tyrosine, a potent amino acid analogue to tyrosine and employed in peptides which target opioid receptors. An analogue of Hagemann's ester 's was used as starting point for the entire process and it was used in two synthetic pathways. The first one is stereoselective in the fact that involves alkylation of the chiral synthon 2,5-diketopiperazine, while in the second one the key-step involves a Michael addition for the introduction of the amino acidic residue.

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Acronyms

Ac Acetyl.

ACN Acetonitryle.

aq aqueous.

ADMET Absorbition, Distribution, Metabolism, Excretion, Toxicity.

Acronym used in pharmacokynetics and pharmacodynamics to indicate the processes sustained by the drug in the organism from its assumption until its elimination, and its potential toxicity.

Arg Arginine.

Asn Asparagine.

Asp Aspartic Acid.

Boc *tert*-butoxycarbonyl.

conc. concentrated.

CADD Computer-Aided Drug Design

Cy cyclohexane.

Cbz Carboxybenzyl

DBU 1,8-Diazabicyclo[5.4.0]undec-7-ene.

DCC dicyclohexylcarbodiimide.

DCM dichloromethane.

de Diastereomeric excess.

DMAP 4-Dimethylaminopyridine.

DMF *N,N*-dimethylformamide.

DMP Dess-Martin Periodinane.

DMSO Dimethylsulfoxide.

Dmt 2′,6′-dimethyl-L-tyrosine

EA Elemental Analysis.

ee enantiomeric excess.

EDC 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide.

Et ethyl.

EtOAc Ethyl acetate.

EWG Electro-Withdrawing Group.

FDA Food and Drug Administration.

Fmoc 9-Fluorenylmethyloxycarbonyl.

Glu Glutammic Acid.

Gly Gyicine.

HTS High Throughput Screening.
Highly efficient and quick pharmacological screening technique.

i-Pr iso-Propyl.

LDA Lithium diisopropylamide.

LG Leaving Group.

LHMDS or LiHMDS Lithium hexamethyldisilazide.

MCPBA *m*-Chloroperbenzoic acid.

Me Methyl.

MeOH Methanol.

MRI Magnetic Resonance Imaging.

Ms Mesyl.

MW Molecular Weight.

n-Bu normal-butyl.

NMR Nuclear Magnetic Resonance.

NOS Nitric Oxide Synthases

o.n. Over night.

p-H_2 *para*-Hydrogen

Pbf 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl

PHIP ParaHydrogen Induced Polarisation

Ph Phenyl.

Phe Phenylalanine.

PTC Phase Transfer Catalysis.

PTSA or p-TsOH p-Toluenesulfonic acid.

PTSCl p-Toluenesulfonyl chloride.

Py Pyridine.

QSAR Quantitative Structure-Activity Relationship.

r.t. room temperature.

rflx Refluxing temperature.

SAR Structure-Activity Relationship.

sat. sol. Saturated solution.

SABRE Signal Amplification By Reversible Exchange

SPPS Soli-phase peptide synthesis.

SNR Signal to Noise Ratio

STABASE 1,1,4,4-tetramethyldisilylazacyclopentane

t-Bu *tert*-butyl.

TEA Triethylamine.

TFA Trifluoroacetic acid.

THF Tetrahydrofuran.

TMG 1,1,3,3-Tetramethylguanidine.

Ts Tosyl (p-Toluenesulfonyl)

TsCl Tosyl Chloride (p-Toluenesulfonyl chloride)

Val Valine.

1 Introduction

Chemistry is said to be one of the most complicated sciences. Yet almost everything which is around us today is related to or has been made by chemists, from the shining rubber of the car's dashboard and the catalysts in the car's exhaust, to the new drug targeting malaria or the new analytic technique to investigate and isolate metals and other pollutants in water or country soil.

As was beautifully stated in a recent paper by K. C. Nicolau, "chemistry is the scientific bridge that connects the macrocosm with the microcosm, our perceived visible world with the invisible world of atoms and molecules. [...] The power of chemistry is primarily derived from its ability to understand molecular structure, synthesize it, and build function within it through molecular design and synthesis." [1]

In fact, the chemists are applying old and new techniques to produce, improve, analyse and rationalise all the goods and substances which are now available in the world. They are also working on envisaging new ones, progressing past the constant challenges and eventually finding new answers to old problems.

Still in the words of KCN, "one of the most vital and valued subdisciplines of chemistry is the science of organic synthesis, without which much of science and industry would have remained paralyzed and sterile." [1]

Organic chemistry is fairly young, having started to develop in the 19th century, with the serendipitous synthesis of urea by Friedrich Wohler [2], who treated silver isocyanate with ammonium chloride, as shown in the following reaction.

$$AgNCO + NH_4Cl \longrightarrow (NH_2)_2CO + AgCl$$

This simple yet surprising reaction for the era was just the beginning of a series of reactions which enabled chemists to reach more and more complex structures. It also allowed them to start addressing the problem of solving the structure of natural products, and gave input to begin new investigations, which ended in providing some serendipitously discovered material and goods, answers to human needs at the time. An outstanding example is the discovery of synthetic dyes; mauveine is shown in Figure 1.0.1 on the following page, found by a young William H. Perkin on his quest for quinine. In the mid 19th century, when he discovered his non-fading dye and developed the processes for the mass-production and use of his new dye,

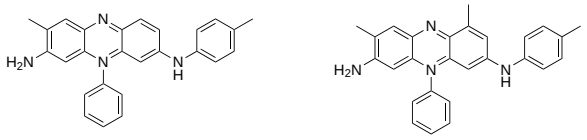
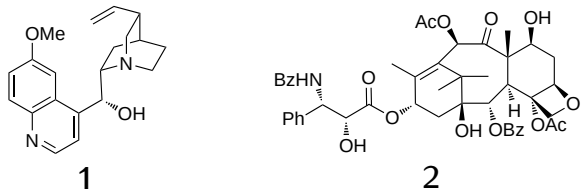


FIGURE 1.0.1: The two major components of mauveine.

coloured substances were highly valued and much sought after as raw materials. [3] Dye research also led to the introduction of sulfonamides in 1936, and therefore to the development of the original sulpha drugs against bacterial infections.

As it has been said, synthesis, with complementary analysis, was often a matter of utilitarian necessity rather than the creative, elegant art form revealed by the work of many of the great synthetic chemists who characterised the second half of the 20th century. And "chemistry blossomed between the two World Wars, at an ever-accelerating pace of discovery. Work done in chemical physics and physical chemistry did much to transform notions of how molecules are held together, how bonds are formed and broken, and how reactions occur." [4]

Organic synthesis made interesting progress too; for example, scientist R. B. Woodward, in April 1944 finally accomplished a first total synthesis of the elusive molecule of quinine **1**, [5] whose supplies during the Second World War were scarce for the Allies Army.



SCHEME 1.1: Quinine **1** and Taxol **2**, two of the most sought after natural products.

In the words of R. B. Woodward, which are reported at the beginning of this work, an organic synthesis can be considered mostly an adventure: the chemist is tackling one simple compound but facing a series of problems and challenges which can be depressing at points, but also enlightening and exciting and lead to the final achievement of the synthesised final product. Many are in fact routes which could be undertaken for just one step, but the outcomes can be so different, just because of the reactivity of the molecule. There can be also failure along the way, but trial and error are part of the deal a chemist signs. Moreover, even in the most detailed and best planned strategy there could be unexpected failure because of the unusual reactivity of an intermediate. It is therefore necessary for a chemist to be open minded and not surprised by the constant trials on the way.

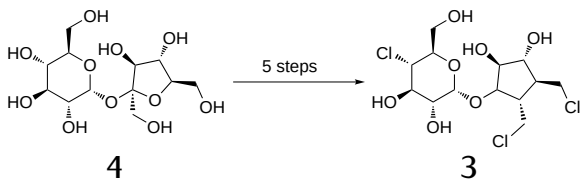
But this outstanding giant of organic chemistry started an era where organic chemistry grew up, and new reagents, reactions, concepts and strategies have been devised and can be employed. This "new era was brought about by an array of technological advances in instrumentation that brought structural representations closer to real materials and their properties. This reification was realised through

physical data collected by infrared (IR), ultraviolet (UV), and NMR spectroscopies, mass spectrometry (MS), and X-ray crystallography instruments. The plethora of data on large numbers of compounds allowed for generalisations and rules that facilitated structural elucidations of natural and synthetic compounds and, to some extent, made obsolete the methods of analysis of the past, namely elemental analysis, degradation, and reactivity. " [1]

The Woodward era was followed by an even more impressive period of expansion led by Elias J. Corey: new principles and theories like retrosynthetic analysis and rational strategy design, new reagents and catalysts, but also the demonstration of the strong connection between natural products synthesis to biological investigations and medical applications were brought to the world-wide attention of chemists. [1]

Nowadays, new principles like atom economy, stereo control, simplicity and scalability, and environmental impact are orienting the development of the discipline. And there is therefore evolution over the years from synthesis to synthesis, based on the previous findings but always learning from the master of synthesis which is nature, with its high yielding processes and stereocontrol.
There are in fact themes or trends of compounds whose synthesis continue to be explored and tuned, according to the state of the art of the moment and the capacity of the research group. Two examples are the aforementioned quinine **1**, whose story almost spans the history of Organic Chemistry [4], and the taxol **2** with its seven total syntheses in over two decades. [6–15]

Corey's fundamental work "also demonstrated and inspired the design and synthesis of natural product analogues for the purpose of biological investigations as well as process development for the large-scale production of pharmaceuticals." [1] In fact, the advent of new techniques and computer-aided drug-design, and the possibility given by high-throughput screening techniques for biological essays opened the way for the organic chemists and their collaborating biologists to study not only what effect a natural compound could have, but also the biological impact a modification of a natural scaffold could have in the organism.
An example is sucralose **3**, the main ingredient in commercially available sweeteners, shown in Scheme 1.2. This molecule is quite similar to the parent compound, sucrose **4**, the only difference being three hydroxyl groups replaced by three chlorine atoms. This makes the sweetener rather indigestible but also about 600 times sweeter than the natural, starting material. Moreover, as it contains fewer than 5 calories per serving the US FDA allows it to be sold as "zero calorie".



4 3

SCHEME 1.2: Synthetic scheme for transforming sucrose into sucralose.

As stated earlier, lots of compounds have an important role nowadays because of their useful biological activity: in fact they exert their action by interacting with a

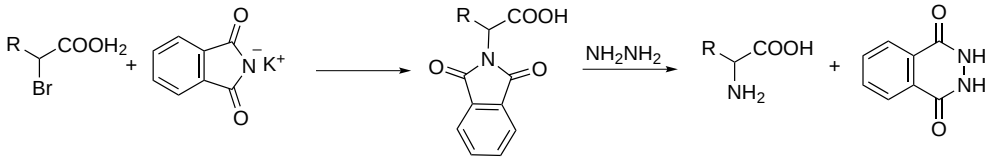
specific site within a biomolecule, *i.e.* an enzyme or a receptor. This site is made by chiral building blocks, normally amino acids and carbohydrates, which have a specific structure and spatial configuration and are present as a singular enantiomer.
It is therefore important to notice that the stereochemistry of a molecule has important and profound effects on the interactions and the consequent biological response.

In most cases, in fact, two enantiomers can have very different activity levels and can be considered two different compounds biologically speaking. This is due to the fact that these two molecules can be metabolised with different speed or by different paths because of their different interactions with enzymes and receptors, which are chiral molecules. An outstanding case is thalidomide.

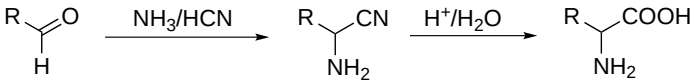
A racemic mixture needs then to be resolved in order to get the two compounds and test them separately, while employing the methods of Asymmetric Synthesis can clearly lead to obtaining a preponderance or even just one of the two enantiomers. This can be achieved by employing chiral reagents, solvents, or catalysts because the transition states of the reactions would then be diastereomeric and at different energy levels, leading to the preferred formation of just one of the enantiomers or diastereomers.
It is then possible to start from the so-called *chiral pool*, a collection of cheap, readily available enantiomerically pure natural products, usually amino acids or sugars, from which pieces containing the required chiral centres can be taken and incorporated into the product.
There could be employment of a *chiral catalyst* on a prochiral substrate with an achiral reagent: the new stereogenic centre is formed when the chiral catalyst will put in contact substrate and reagent.
A final way to accomplish an asymmetric synthesis is the employment of *chiral auxiliaries* that can be attached to the prochiral substrate just for the reaction, in order to obtain a diastereomeric mixture. Like reagents from the chiral pool, a chiral auxiliary needs to be enantiomerically pure, economical and easy to install and to remove.

1.1 Amino acids

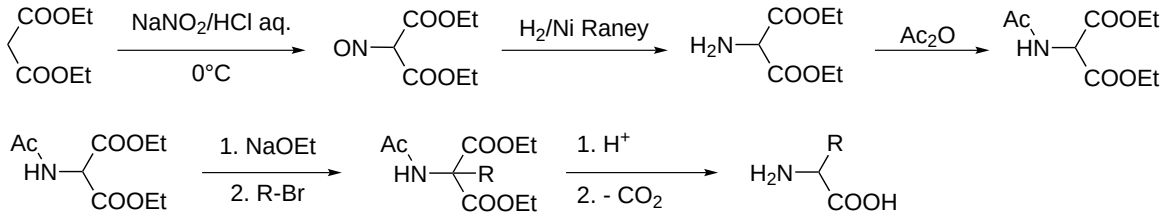
In this thesis work the attention was focused on obtaining amino acids by laboratory synthesis. A few methods have been developed by chemists during the 20th century, and are reported in the following Scheme 1.3 on the facing page. Notable and classic routes are Gabriel and Strecker synthesis, which are simple and low cost.
In Gabriel synthesis the initial reaction of the α-bromoacid with the potassium salt of phtalimide is followed by hydrazinolysis to yield the amino acid anche the cyclic hydrazine.
Strecker synthesis is characterised by a multicomponent reaction, in which the aldehyde, the ammonia and the cyanide concur to the formation of the α-aminonitrile which, upon hydrolysis, gives the expected α-amino acid.
In the final method, the crucial step is the attack of the primary or allylic/benzylic bromide by a carbanion; moreover, it is interesting to note that the acetamidoma-



(A) Gabriel Synthesis.



(B) Strecker Synthesis.

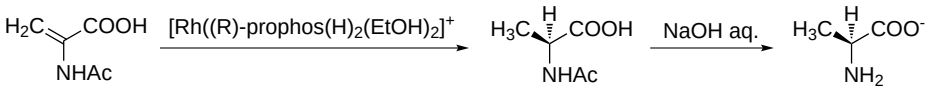


(C) Alkylation of the acetamidomalonate.

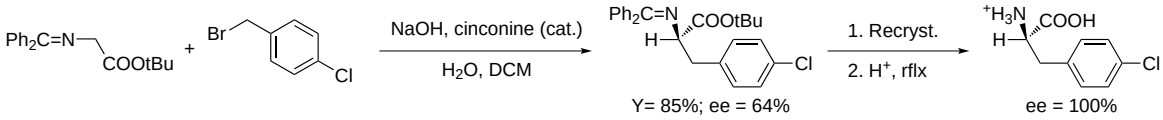
SCHEME 1.3: Classic synthetic strategies for synthesising amino acids.

lonate intermediate is very versatile and the C−H bond can be easily activated by a base.
These routes can be easily tuned in order to obtain all the different natural amino acids and, as it has been said already, they are simple and low cost. The only big draw-back is that the final product is a racemic mixture that has to be resolved in order to obtain the pure enantiomers.

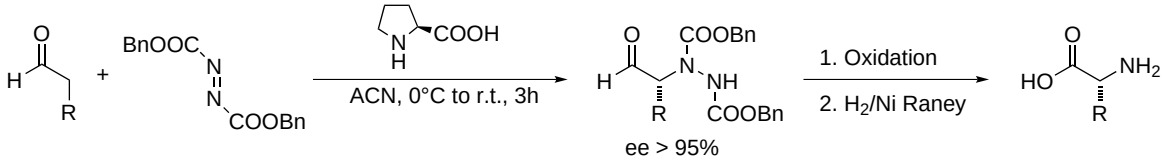
To overcome this problem, stereoselective synthesis of amino acids has been developed over the years, as it is shown in the following Scheme 1.4. In the first case,



(A) Enantioselective reduction to *(S)*-alanine.



(B) Enantioselective alkylation to *(R)*-(4-chlorophenyl)alanine.



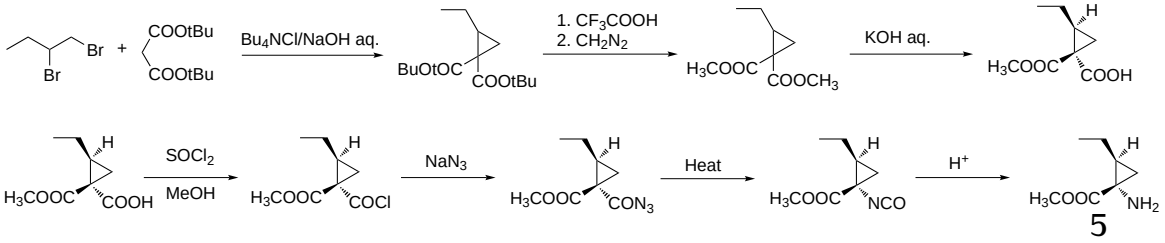
(C) Proline-catalized enantionselective amino acid synthesis.

SCHEME 1.4: Principal anantioselective strategies for synthesising amino acids.

a chiral catalyst is used to hydrogenate the double bond of the α-amino alkene. It

is obvious that this route is very expensive, although highly efficient and the enantiomeric excess is such that it is possible to obtain the pure *(S)* enantiomer.
In the second case, the acid and basic moiety of the α-amino acid are protected as ester and imine functions respectively. The alkylation is stereocontrolled by the organocatalyst (+)-cinconine, a phase transfer catalyst which exerts its deprotonating action in the organic medium; in its protonated form, it returns back to the aqueous phase where the inorganic base regenerates it to come back to the organic phase.
The last example is a proline-catalised synthesis of amino acid: the proline, an amino acid used in catalytic quantities, or an organocatalyst, is used to promote and catalyse the condensation of the aldehyde with the azadicarboxilate in a stereoselective fashion, with high ee. Then, after oxidation of the aldehyde to carboxylic acid and hydrogenation to get rid of the benzoyl moieties, the final amino acid is obtained with high enantioselectivity. [16]

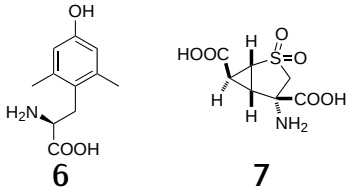
Some unusual amino acids can be found naturally, in particular cyclic amino acids like aminocyclopropanecarboxylic acid or coronamic acid **5**, the biogenetic precursor of ethene, which is in turn involved in the ripening of the fruit. The



SCHEME 1.5: Synthesis of coronamic acid.

carbanion from the malonate ester forms a three-membered cycle in the first step by nucleophilic substitution. After conversion of the ester and hydrolysis of the least hindered one, the carboxylic acid is transformed into acyl chloride and then isocyanate via Curtius rearrangement. The product is then finally hydrolysed to give the amino acid. [17]

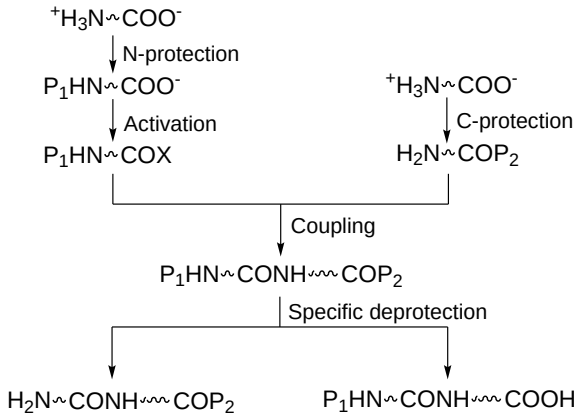
More complicated manipulations of the scaffold could also be undertaken and explored, like the addition of substituents in order to gain steric effects or developing the amino acid around a cyclic structure in order to impose the molecule a single conformation. Two examples, 2',6'-dimethyl-L-tyrosine **6** and (−)-*(1R,4S,5S,6S)*-4-Amino-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic Acid 2,2-Dioxide **7**, are shown in the following Scheme 1.6. The first compound **6** is a tyrosine analogue bearing



SCHEME 1.6: Constrained amino acids.

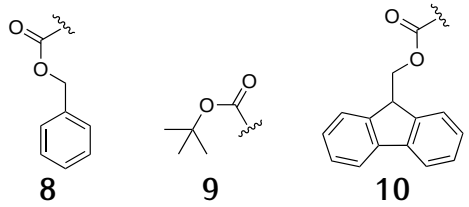
two methyl groups on the aromatic ring. This allows it to be used in place of normal tyrosine in small peptides active on the opioid receptors with higher affinity for the receptor than the normal amino acid. The second compound **7**, known also as LY404039, [18] is a potential and nonclassical antipsychotic agent now in clinical trials based on glutamic acid structure. Its bicyclo[3.1.0]hexane structure allows it to be in a very constrained form and in just one conformation: for this reason, there is a higher affinity of this substrate than the normal glutamic acid to the target receptor, rendering it highly interesting and more potent than others.
The conformational rigidity on arginine backbone and its effect on the affinity of the obtained analogues on the biological target has also been studied [19], and insights on the mechanism and the preferred conformation for the substrate were gained in this manner.

Beside all of this, chemists have developed means of coupling amino acids with each other in order to synthesise polypeptides and proteins. Over the years, the technique evolved and became state of the art, but it is based on a few important steps: protection, activation, coupling and specific deprotection, as it is possible to notice from the Scheme 1.7. Amino acids are in fact characterised by two different



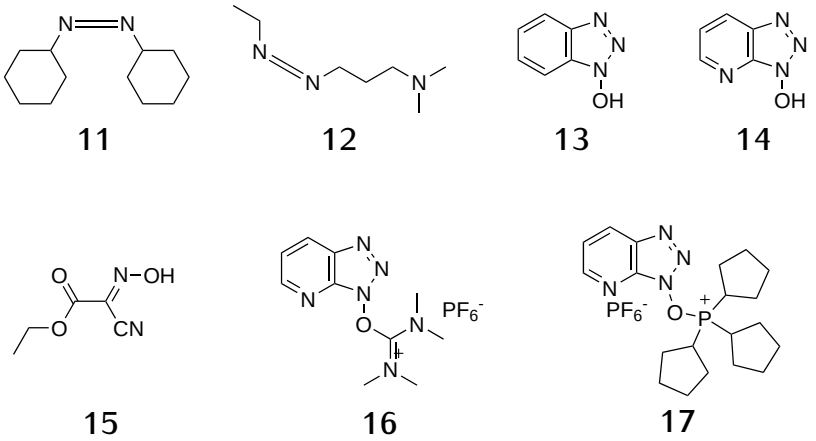
SCHEME 1.7: Steps for the synthesis of a simple dipeptide.

reactive moieties – they are bifunctional compounds – and have at least one active stereocentre, hence the special care in their handling to avoid racemisation.
Both amine and acid moiety require protecting groups to be applied and removed selectively and under mild conditions. For the amino group, the protecting group is placed in order to decrease the nucleophilicity of the amine and hence avoid its participation in the coupling reaction; nonetheless, it needs to be removed under mild conditions in order not to cleave the peptidic bond just formed. The most used are the benzyloxycarbonyl **8** (Z or Cbz) and the *tert*-butoxycarbonyl **9** (Boc) groups alongside the Fluorenylmethyloxycarbonyl **10** (FMOC), shown in the Scheme 1.8 on the following page. Protection of the carboxyl group is needed in order to prevent it from participating during the coupling reaction. The most used and usual method is achieved by acid esterification with methanol, ethanol or *tert*-butanol.
It is obvious that the coupling reaction is carried out between a free amino acid carboxylic group and the free amino group of another amino acid, as shown in Scheme 1.7. There is although need to enhance the electrophilicity of the carboxylate group by



SCHEME 1.8: Amine protecting groups.

introducing a good leaving group on the acid moiety: the most used at the moment is the "active ester" method, and the most known and commonly used reagent is the DCC **11**, although it has been superseded by EDC **12** and some new reagents. The carboxyl oxygen atom acts as a nucleophile, attacking the central carbon in DCC and thus forming the so-called *O*-acylurea. DCC is temporarily attached to the former carboxylate group, which is now an ester group, making nucleophilic attack by an amino group by the attaching amino acid to the former carbonyl group more efficient. With this method, although, some racemisation might occur: to solve this



SCHEME 1.9: A few examples of activating agents for amino acids coupling reactions.

problem, triazoles derivatives have been introduced. The most important ones are 1-hydroxy-benzotriazole **13** (HOBt) and 1-hydroxy-7-aza-benzotriazole (HOAt) **14**. These substances can react with the O-acylurea to form an active ester which is less reactive and less in danger of racemization. HOAt is especially favourable because of a neighbouring group effect. Alternatives to HOBt and HOAt have also been introduced because of the dangerous effects these reagents can exert. One of the most promising and inexpensive alternatives is ethyl 2-cyano-2-(hydroxyimino)acetate known as Oxyma Pure **15**, which is not explosive and has a reactivity between HOBt and HOAt. Newer developments omit the carbodiimides totally, and the active ester is introduced as a uronium or phosphonium salt of a non-nucleophilic anion: two examples of the two categories of compounds are, respectively, HATU **16** and PyBOP **17**. [20]

After the coupling takes place and the peptide bond has been formed, the following step is the deprotection of the unwanted protecting group on the newly formed

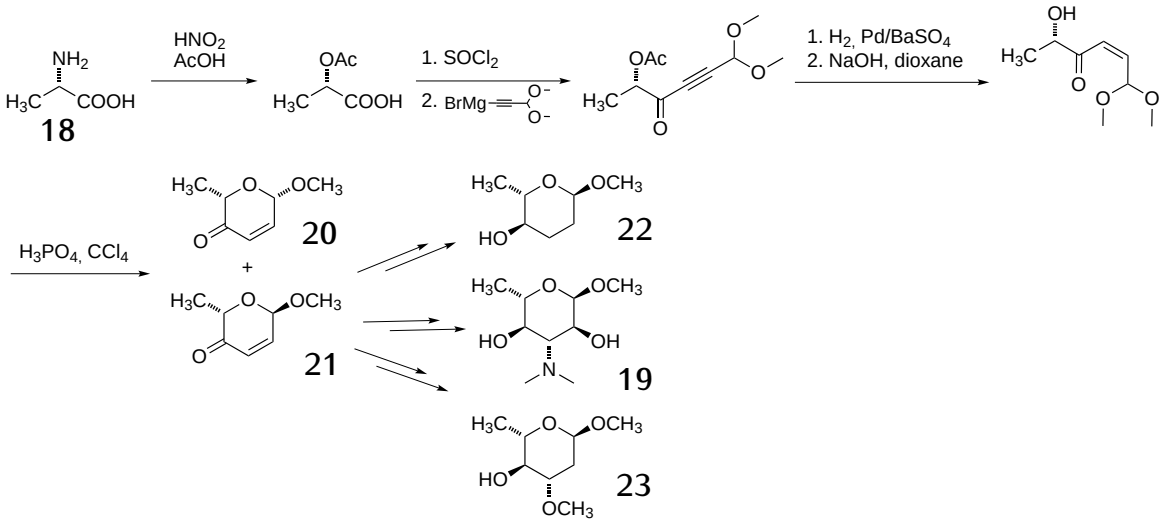
peptidic chain in order to continue with the synthesis of the peptide, or a final deprotection step to obtain the pure compound.

The technique describers so far involves the classical approach of liquid-phase peptide synthesis. This method has although been replaced in most labs by solid-phase synthesis, but the underlying principles of the two techniques remain the same. A few words on this latter technique are deserved.
Solid-phase peptide synthesis (SPPS), pioneered by Robert Bruce Merrifield, requires the peptide to be "immobilised" on the solid phase and retained during a filtration process, whereas liquid-phase reagents and by-products of synthesis are flushed away. The general principle of SPPS is one of repeated cycles of coupling-wash-deprotection-wash. [21]

Introducing a new moiety or functionality or replacing a carbon atom with a halogen atom are just a few examples of the variety of means the chemist can exploit in order to achieve higher diversity and different effects.

It needs to be mentioned, finally, that amino acids are useful synthons in organic chemistry: they constitute a big part of the so-called *chiral pool* from which asymmetric synthesis can start from. In fact, compounds can incorporate the chiral carbons of the amino acid into their carbon skeleton or the amino acid's chirality can be used to induce asymmetry in reaction which would not usually be asymmetric.

An interesting example of the first case of employment of amino acids in asymmetric synthesis is reported in Scheme 1.10: L-alanine **18** is transformed into α-L-mycaminoside **19**. The asymmetric carbon atom is incorporated into the ring sys-

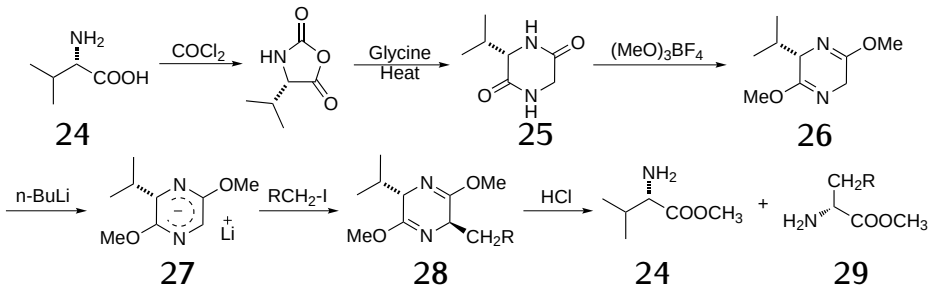


SCHEME 1.10: Elaboration of alanine into different sugars.

tem and its chirality is able to induce and control the chirality of the contiguous asymmetric centres.
L-alanine **18** is deaminated with nitrous acid and elaborated to the anomeric mixture of compounds **20** and **21**. This last compound is the common intermediate of a series of interesting sugars: methyl α-L-amicetoside **22**, α-L-mycaminoside **19** and α-L-oleandroside **23**.

An example of the second case is the so-called Schöllkopf Bis-Lactim Amino

Acid Synthesis: amino acids can be converted to diketopiperazines or mixed diketopiperazines and undergo a variety of reactions resulting in high asymmetric induction. [22–24]



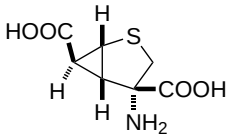
SCHEME 1.11: Formation and alkylation of Schöllkopf Bis-Lactim ether for the asymmetric synthesis of amino acids.

Glycine and *(R)*-valine **24** are easily converted to a diketopiperazine **25** with high yields. A twofold methylation with methyloxonium tetrafluoroborate forms the bis-lactim ether **26**. Lithiation with η-BuLi of the prochiral position on C-3 occurs regiospecifically to form the charged species **27**, which can react with various electrophiles.
This is the step that decides the stereoselectivity of the method: one face of the carbanionic centre is shielded by steric hindrance from the isopropyl residue on valinine. The reaction of the anion with an alkyl halide will form the alkylated product **28** indicating that the alkylating agent approaches C-3 *trans* to the isopropyl group at C-6, with a diastereoselectivity of up to 95%.
In the final step the heterocyclic ring is cleaved by careful acidic hydrolysis to produce the regenerated chiral auxiliary methyl L-valinate and the *(R)*-amino acid ester **29** which can be separated from each other.

The scope of the faction can be further extended to include preparation of unsaturated amino acids, or even α-methyl amino acids, as reported in [25]. Nevertheless, the method is limited to the laboratory for the synthesis of exotic amino acids, as industrial applications are not known. A major disadvantage of this method is the limited atom economy.

1.2 The synthetic target – Project 1

Compounds with a bicyclo[3.1.0]hexane structure are quite well-known in the pharmaceutical industry because of their constrained and rigid scaffold. An example is the well-known patented amino acid LY354740 **30** by Eli Lilly. The cyclopentane ring is condensed with a cyclopropane ring, mimicking a cyclohexane structure but not giving way to the famous boat equilibrium typical of these last structures. In this way, molecules based on such a scaffold can show high affinity with the receptorial site of interest as they are not submitted to usual conformational movements that can lower the lingand-receptor interaction.



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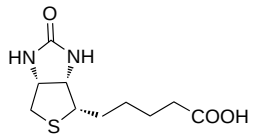
It was for these reasons and the possibility to study the affinity and understand the conformational configuration of the glutamate in the CNS that the group of James A. Monn reported the synthesis of the constrained amino acid in 1997. [26] It is in fact well known that L-Glutamic acid is the principal excitatory amino acid neurotransmitter in the mammalian central nervous system. It exerts its effects via activation of two types of receptors: those which form ligand-gated cation channels (ionotropic glutamate receptors, iGluRs) and those which are coupled via G-proteins to intracellular enzyme systems which influence the production of second messengers (metabotropic glutamate receptors, mGluRs). There are currently eight distinct mGluR proteins (mGluR1-8), and this bicyclic amino acid has been designed specifically to target mGluR 2 receptors, and – as it has been reported in the aforementioned work – the conformational restrictions imposed on the glutamic acid structure render it highly potent and selective toward the biological target.

Further developments on this molecule has led the group to explore different decorations and substitution on the cyclopentane framework. [27, 28] Eventually the group addressed the problem of studying the effects of the presence of an heteroatom on the ring. In particular, it was finally synthesised, modelled and tested compound LY404039 **7**. [18] They found out that the oxidised sulphur atom can add interactions similar to those of the natural, parent compound. In fact, the oxygen atoms of the oxidised sulphur stabilise furthermore the structure of the molecule inside the target receptor, rendering this molecule a useful tool for exploring the effects of mGlu2/3 receptor activation in mammals.

There is in fact widespread occurrence of thia-ring systems in natural compounds and drugs. In particular, dihydro- and especially tetra-hydrothiophenes have attracted attention because these are recurring ring system motifs in natural and non-natural products displaying a broad spectrum of biological activities. [29] A few examples are biotin **31**, an essential coenzyme, unnatural L-nucleoside **32** which displays potent anti-HIV activity without significant toxicity and Sulopenem **33**. However, despite the importance of these scaffolds and the high benefits in



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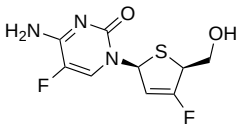

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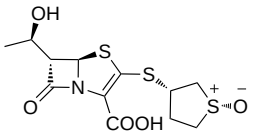

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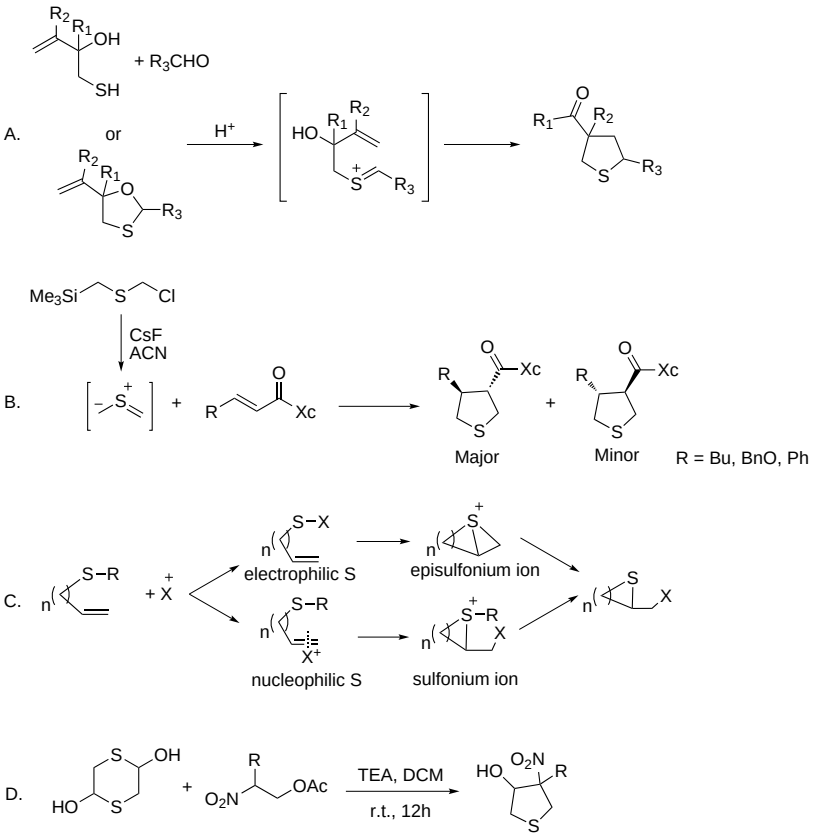
SCHEME 1.12: Three tetrahydrothiphene-containing molecules: biotin **31**, the unnatural L-nucleoside **32** and Sulopenem **33**.

a variety of applications, few methods for their asymmetric synthesis have been reported, and only over the past decade has this topic emerged as an important research area.

A few methods have been developed to synthesise substituted tetrahydrothiophenes, either involving carbon-carbon or carbon-sulfur bond formation reactions. In 1952 Grob and von Sprecher described a nitroaldolization approach to the thio-

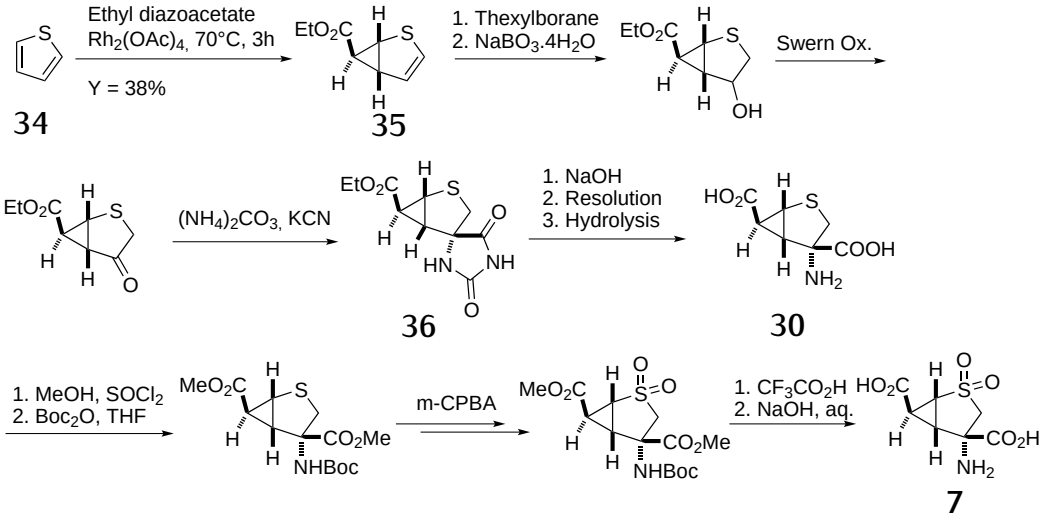


SCHEME 1.13: Approaches to thiophene synthesis. A. Overman approach. B. Karlsson approach. C. Castillon approach. D. Benetti *et al*. approach.

phane ring system in their efforts to synthesise biotin. [30]
More recently, Ponce Molina and Overman reported a new method for the stereocontrolled synthesis of a variety of acyltetrahydrothiophenes by acid-promoted condensation of mercapto allylic alcohols and carbonyl compounds or by rearrangement of suitable oxathiolanes, the ring-formation step being based upon a carbon-carbon bond formation. [31]
In another approach that has been recently developed by Castillon *et al*., a thioetherification reaction was the key step for synthesising homochiral substituted tetrahydrothiophenes by carbon-sulfur bond formation. [32]
In the same period, Karlsson *et al*. exploited 1,3-dipolar cycloadditions – a C-C bond forming strategy – of sulfur-containing 1,3-dipole to α,β-unsaturated acyl derivatives dipolarophiles, which resulted in the formation of *trans*-3,4-disubstituted tetrahydrothiophenes. [33]
A final approach was studied in the group where part of this thesis work was developed: it consisted in the use of the commercially available 1,4-dithiane-2,5-diol in a domino Michael-Henry reaction with nitroalkenes *in situ* generated [34] – more on the domino reaction in 1.2.2 on page 17.
The strategy entailed on the use of as a suitable bifunctional reagent possess- ing a sulfur nucleophile able to undergo Michael intermolecular addition to in situ generated nitroalkenes producing an intermediate nitroalkane adduct, which bears a

suitably placed aldehyde group required for the subsequent intramolecular Henry nitroaldol reaction

The synthetic target for the first project is represented by compound **7**. Although the tested compound failed clinical trials for phase III, [35] it remains an interesting synthetic target. It is always challenging to introduce and fuse two carbon rings together, not to mention the interesting feature of the presence of an heteroatom on the main cycle. The original synthesis envisaged by Monn *et al*. starts from



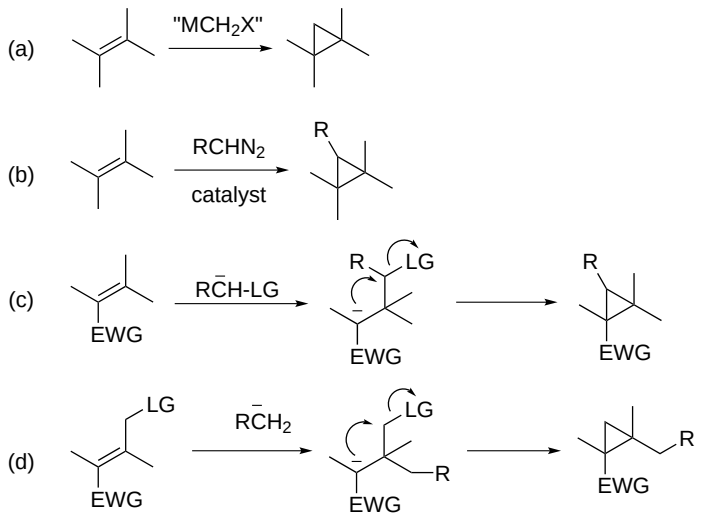
SCHEME 1.14: Original synthesis for constrained amino acid LY404039 as reported by Monn *et al*. [18, 27]

optically active and pure compound **30**, which is protected as ester on the acid moiety and as Boc-anhydride on the amine moiety, and oxidized on the sulfur atom to sulfone. After removal of the protecting groups, final compound **7** is obtained. Compound **30** is obtained from thiophene **34**, which is cyclopropanated to **35**. Upon hydroboration and oxidation of the introduced hydroxyl moiety, hydantoin cycle is constructed **36** to give upon hydrolysis the amino acid moiety. Resolution and separation of the two optical antipodes would result in optically active and pure **30**. [18, 27]

1.2.1 Cyclopropanation Reaction

There are three types of stereoselective cyclopropanation reactions from olefins, which are shown in Scheme 1.15 on the following page: the halomethyl-metal-mediated cyclopropanation reactions (a), the transition metal-catalyzed decomposition of diazo compounds (b), and the nucleophilic addition-ring closure sequence, also called Michael-Initiated Ring Closure (c) and (d). [36]

The chemistry for the first type of reaction shown above has been developed by Simmons and Smith during the 1960s with the discovery and use of reagents of the type $IZnCH_2I$ for stereospecific conversion of alkenes to cyclopropanes. [37] A major advantage of this approach is its excellent compatibility and chemoselectivity as it has a wide scope, being applicable to a variety of olefins and compatible with

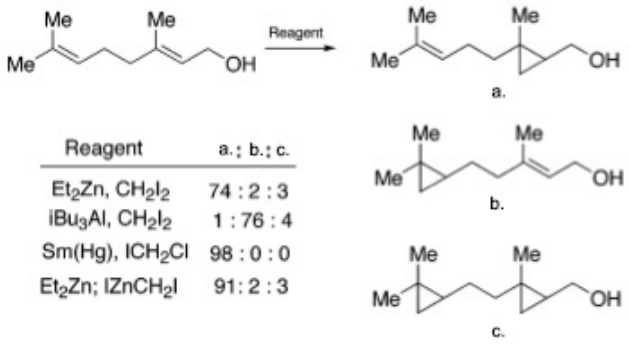


SCHEME 1.15: Main type of reactions for the cyclopropane formation.

several functional group like enamines, enol ethers, esters, ketones.
Further developments led to the substitution of the original Zn/Cu couple with the more practical and easy to handle $ZnEt_2$ by Furukawa and co-workers in 1966. [38] [39]

Other cyclopropanating reagents of the proposed general structure MCH_2X have also been prepared. For example, samarium carbenoids were generated by the use of a samarium/mercury amalgam in conjunction with CH_2I_2 by Molander [40], while Yamamoto discovered the analogous R_2AlCH_2I reagent, which displays a unique reactivity that complements that of the zinc- and samarium-mediated cyclopropanation reaction. [41]

The synthetic utility and scope of these reagents is clearly illustrated by the chemoselectivity observed in the cyclopropanation of geraniol Scheme 1.16. [42] The allylic alcohol group can be cyclopropanated in the presence of an isolated



Reagent	a. : b. : c.
Et_2Zn, CH_2I_2	74 : 2 : 3
iBu_3Al, CH_2I_2	1 : 76 : 4
Sm(Hg), ICH_2Cl	98 : 0 : 0
Et_2Zn; $IZnCH_2I$	91 : 2 : 3

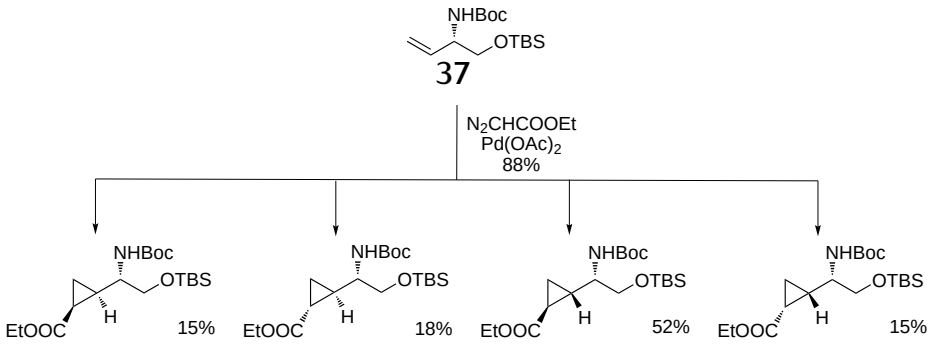
SCHEME 1.16: Cyclopropanation of the geraniol. [42]

olefin with zinc- or samarium-derived reagents. Conversely, the aluminium reagent can convert the isolated olefinic group into the corresponding cyclopropane with outstanding chemoselectivity. Optimization of the reaction conditions has shown that zinc-based reagents could also be effectively used to achieve good chemoselec-

tivities in that reaction. The reason behind this selectivity is thought to involve a prior coordination of the zinc or samarium reagent with the hydroxy group or the corresponding metal alkoxide to direct the addition of methylene to the neighboring alkene, in fact enhancing the rate of the reaction. Along with steric effects, it has been the main controlling element for the high stereocontrol in these reactions.

On the other hand, the cyclopropanation of olefins using the transition metal-catalyzed decomposition of diazoalkanes – case (b) in Scheme 1.15 on the facing page – is one of the most extensively studied reactions of the organic chemist's arsenal, with inter- and intra-molecular version being developed and studied over the years.

The most exhaustively studied and employed diazo reagents for intermolecular cyclopropanation reactions are the α-diazoesters used in presence of a metal catalyst. Usually, Rh, Ru, Co, and Cu metal carbenes react faster with electron-rich alkenes, whereas Pd metal carbenes are optimal for electron-deficient alkenes. An example of this methodology is reported in Scheme 1.17 where compound **37** is cyclopropanated with the aid of ethyl diazoacetate in presence of a palladium catalyst. [43]

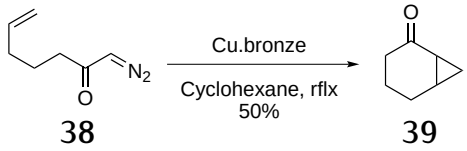


SCHEME 1.17: Example of a cyclopropanation reaction of a double bond by decomposition of diazo-compound in presence of a metal catalyst.

When both functionalities of the diazo unit and the alkenes are in the same molecule, an intramolecular cyclopropanation is possible with the appropriate catalyst, thus producing bicyclic products. In contrast to the intermolecular reaction, only one diastereoisomer is obtained when forming five- or six-membered rings. However, it is important to consider the chemoselectivity, as, in some cases, the C-H insertion may become the major pathway.

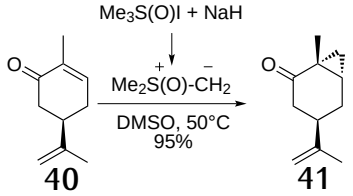
Most of the successful systems involve cyclization of either γ,δ-unsaturated diazocarbonyl or δ,ϵ-unsaturated diazocarbonyl systems, leading to fused [3.1.0] or [4.1.0] bicyclic systems, as reported in Scheme 1.18 on the following page, where the diazoketone **38** rearranges to give the aforementioned [4.1.0] structure **39**. [44]

The last methods for the generation of the cyclopropane ring are based on the ring closure initiated by a Michael reaction, exemplified by situations (c) and (d) in Scheme 1.15 on the preceding page: a conjugate addition to an electrophilic alkene produces an enolate ion which subsequently undergoes an intramolecular ring closure – Michael-initiated ring closure (MIRC) reactions. [36] Two eventualities fall under this category: in case (c) the leaving group is present on the nucle-



SCHEME 1.18: Intramolecular cyclopropanation reaction.

ophile molecule, while in case (d) the formation of cyclopropanes is obtained by nucleophilic addition to electrophilic substrates containing a leaving group. The first method was previously shown in Scheme 1.5 on page 6 for the synthesis of coronamic acid **5**, whilst the second method is exemplified particularly by the use of sulfur ylide reagents.
Usually this type of reactions are nonstereospecific, and both *(E)*- and *(Z)*-olefins give the *trans*-cyclopropanes. The most outstanding example was reported by Co-

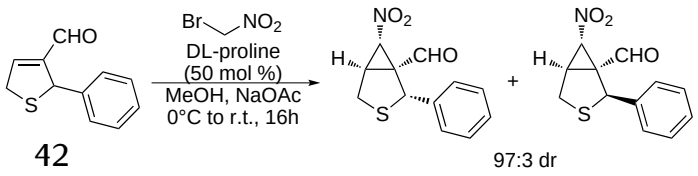


SCHEME 1.19: MIRC reaction.

rey [45] on the cyclopropanation reaction of *(R)*-(−)-carvone **40** with methylen dimethylsulfoxonium *in situ* generated to provide the desired cyclopropylcarvone **41** in 95% yield, as a single diastereoisomer, which results from the attack of the ylide on the less hindered face. Its homologue ethyl (dimethyl sulfuranylidene) acetate (EDSA) has been described by Payne. [46], and reacts with α,β-unsaturated esters, ketones, aldehydes and nitriles to afford cyclopropanated adducts as mixtures of diastereomers depending on the substrate. This compound and its applications with MIRC reaction has been studied by several groups in the world, particularly by Monn *et al*. at the Eli Lilly research centres. In fact, this compound, in presence of DBU or of catalytic amounts of TMG reacts with cyclic or acyclic substrates in cyclopropanation reactions studied for the preparation of bicyclo[3.1.0]hexane-2-one-6- carboxylic acid ethyl ester. [47, 48] This procedure was further developed and scaled-up, as reported in [49].

Nitrocyclopropanes are an emerging area of interest as they have been recognised widely as a useful and riveting synthetic target in recent years. Several groups approached the synthetic problem, developing also an enantioselective version of this cascade reaction. Bromonitromethane was the chosen reagent: it rapidly undergoes conjugate addition reaction to α,β-unsaturated enone system; subsequent elimination of the bromine atom would yield the cyclopropylated adduct. [50] An example is the recently reported nitrocyclopropanation of suitable 2,5-dihydrothiophene-3-carbaldehydes **42** by De Risi *et al*. and shown in Scheme 1.20 on the next page. [51] These compounds are expected to act as Michael acceptors toward the anion of bromonitromethane, through an Iminium ion activation with DL-proline in the presence of basic co-catalysts such as TEA or NaOAc forming 6-nitro-3-thiabi-

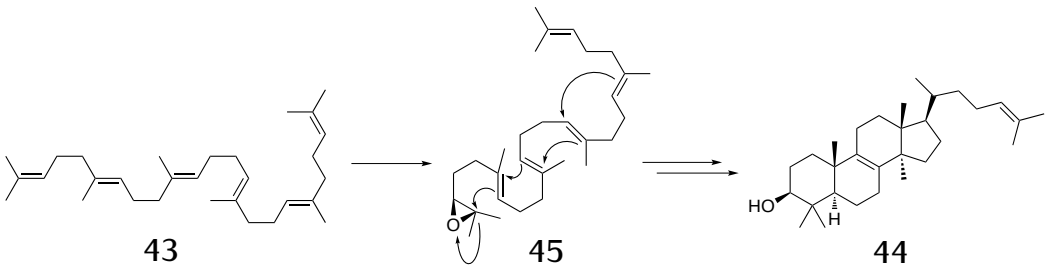
cyclo[3.1.0]hexane-1-carbaldehyde derivatives, possibly through a subsequent intramolecular alkylation cyclization, with diastereoselectivities depending strongly on the substrate.



SCHEME 1.20: Approach to the synthesis of 6-nitro-3-thiabicyclo[3.1.0]hexane-1-carbaldehyde.

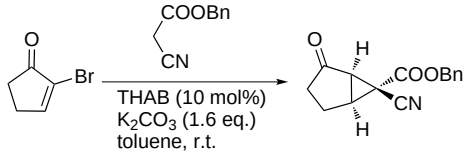
1.2.2 Tandem reactions

A "domino" or "tandem" reaction consists of series of a consecutive intramolecular reaction which allows for the synthesis of usually cyclic compounds in high a fast and clean way, with high atom economy; finally, a lot of molecular complexity is generated effectively at once.
The inspiration for this type of reactions is of course taken from Nature: squalene **43** is oxidised and then enzymatically cyclized to give lanosterol **44**, a steroid which can be further elaborated by the organism into other steroidal compounds.



SCHEME 1.21: Nature's tandem reaction for the synthesis of Lanosterol: squalene **43** is enzymatically oxidised to squalene epoxide **45** by squalene monooxygenase, and then the enzyme lanosterol synthase complete the cascade reaction to yield lanosterol **44**.

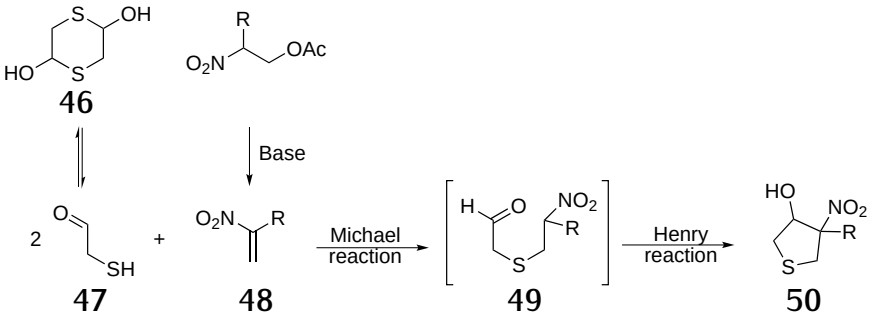
The aforementioned MIRC reaction mentioned in the previous section as a strategy for synthesising cyclopropyl compounds can be considered an example to such a class of reaction employed in the chemical lab routine. Arai *et al*. have in fact studied in the late 1990s the stereoselective cyclopropanation reaction under phase-transfer-catalyzed conditions of cyclic and acyclic α-haloenones. These compounds are indeed quite reactive: the conjugated double bond works as suitable Michael acceptor and the halogen atom as a good leaving group for a subsequent attack on the α-carbon. There is then an intermolecular Michael addition followed by an intramolecular cyclisation process. All of the above processes are indeed conducted under phase transfer conditions, using K_2CO_3 and some quaternary ammonium salts, or quinidine-derived quaternary ammonium salts for the asymmetric version



SCHEME 1.22: Arai *et al*. PTC approach for the synthesis of the cyclopropane ring via domino reaction.

of the approach.
Malonates, soft carbon nucleophiles, nitromethane with highly acid protons and cyanoacetates bearing an active methylene and all their derivatives were screened in the two studies. [52, 53]
Carlson *et al*. studied and exploited the same reaction in presence of ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate ($[BMIM]PF_6$) and were able to direct the synthesis to dihydrofuran rings as well as bicyclo[3.1.0]hexanones. [54]

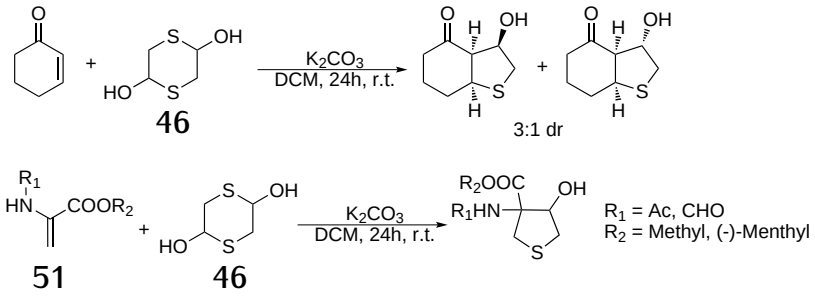
On the other side, tandem reactions have long been studied in the group where part of this thesis work was researched in order to synthesise variously substituted tetrahydrothiophenes, as it has been mentioned in 1.2 on page 12 and reported in [34], where a tandem Michael-Henry reaction has been optimised to yield 3,4-substituted tetrahydrothiphenes.
Starting from the dimer **46** of mercapto-acetaldehyde **47**, the nucleophilic sulphur atom was found able to undergo a Michael addition to the *in situ* generated nitroalkenes **48**; these led to formation of intermediate compound **49** bearing an aldehyde group which in turn would undergo intramolecular Henry nitroaldol reaction to finally build the thiophene ring **50**, as reported in 1.2 on page 12 and shown more in detail in Scheme 1.23. In a recent publication from the same group, com-



SCHEME 1.23: Sequence of Michael-Henry tandem reaction for the synthesis of variously substituted thiophenes.

pound **46** was reacted with α,β-unsaturated ketones in the tandem Michael-aldol reaction process, resulting in the efficient preparation of monocyclic and bicyclic 3-hydroxythiophanes in good yields and high diastereoselectivity.
Dehydroalanine esters **51** were also considered as counterparts of compound **46** in the tandem sulfa-Michael/aldol reaction process. These compounds have been used as Michael acceptors for conjugate addition reactions even though they are typically considered poor electrophiles because of the electron-donating effects of the lone pair of the nitrogen atom. Nonetheless, amidoacrilates were reacted with

mercaptoacetaldehyde dimer in presence of potassium carbonate at room temperature in the same domino process to give the tetrahydrothiophane ring system. [55]



SCHEME 1.24: Synthesis of variously substituted thiophenes.

1.3 The synthetic target – Project 2

1.3.1 MRI contrasting agents

Magnetic Resonance Imaging is a powerful technique which allows for the visualisation of internal structures of the body in detail by imaging nuclei atoms inside the body. This technique exploits the magnetic properties of atomic nuclei, in particular the most abundant one in our bodies: ^{1}H from the water molecule. Different tissues will have different water concentration, hence the different response to the applied magnetic field and the different grey gradient in the final picture. More in detail, an MRI scanner is a device in which the patient lies within a large, powerful magnet where the magnetic field is used to align the magnetization of some atomic nuclei in the body, and radio frequency magnetic fields are applied to systematically alter the alignment of this magnetization. [57] This causes the nuclei to produce a rotating magnetic field detectable by the scanner and this information is recorded to construct an image of the scanned area of the body. [58] Magnetic field gradients cause nuclei at different locations to precess at different speeds, which allows spatial information to be recovered using Fourier analysis of the measured signal. By using gradients in different directions 2D images or 3D volumes can be obtained in any arbitrary orientation.

MRI provides good contrast between the different soft tissues of the body, which makes it especially useful in imaging the brain, muscles, the heart, and cancers compared with other medical imaging techniques such as computed tomography (CT) or X-rays, but it requires quite long scan times to acquire a proper image.

To enhance the visibility of internal body structures in MRI, different contrast media can be used, and the most common compounds are gadolinium-based. These type of agents works by altering the relaxation times of atoms within body tissues after oral or intravenous administration.

In fact, MRI and NMR – which is the respective technique used routinely in chemistry – is quite an insensitive technique: for example, it is quite astounding

the difference in concentration in the preparation of the sample to be submitted to a normal $^{1}H-NMR$ experiment against the compound quantity submitted to an MS analysis. Another example is the time and concentration requested to run a normal $^{13}C-NMR$ experiment, because, as it is well known, the ^{13}C is the least abundant of all the isotopes of Carbon.
Several scans are also needed in order to achieve a good to optimal Signal-to-Noise Ratio – at least 16 for normal ^{1}H-NMR: δ, while thousands for ^{13}C-NMR: δ. Finally, reaction intermediates are not possible to detect.

The reason behind all of this is that the signal intensity is related to relative populations of the magnetic levels probed during the experiment, a mechanism which is called *polarization*. At thermal equilibrium the energy levels are close, or in other words there is little energy needed to promote the transition from one level to the other one. Moreover, the Boltzman distribution predicts that almost an equal number of nuclei will be in the two different energy levels, even in highest magnetic fields.
An altered distribution of the nuclei population in the two levels would give raise to an increase in NMR signal because more nuclei are subjected to transition. This mechanism is called *hyperpolarization*.

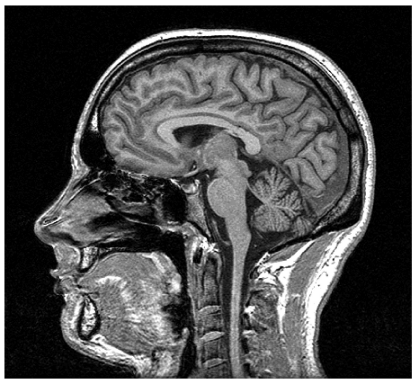


FIGURE 1.3.1: Sagittal MRI scan. [56]

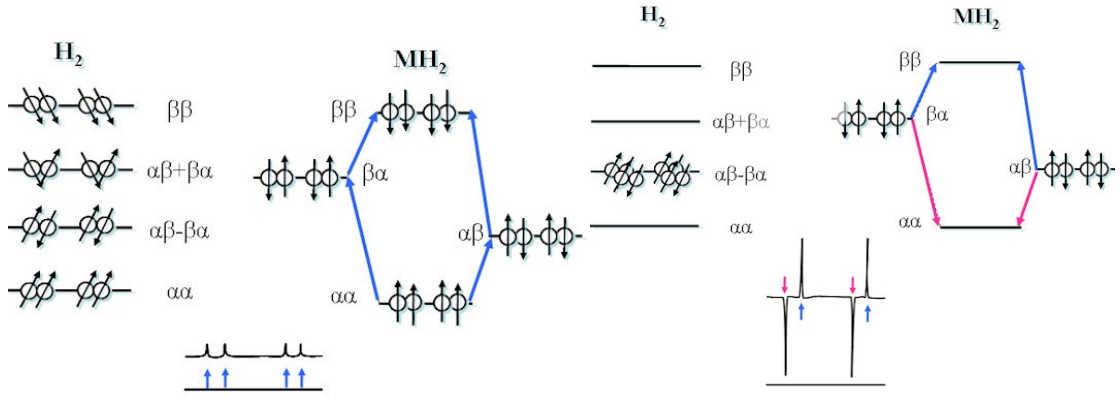
1.3.2 para-Hydrogen Induced Polarisation

ParaHydrogen Induced Polarisation is a technique used extensively today to induce polarisation into molecules, and this phenomenon has been exploited for the generation of PHIP enhanced contrast agents for use in Magnetic Resonance Imaging. [59]

In the H_2 molecule the spins of the two hydrogen nuclei posses a spin $= \frac{1}{2}$ and is therefore NMR active, showing a singlet at around 4.55 ppm. In addition, this two spins can couple to form a triply degenerate and symmetric with respect to the exchange of nuclei state (orthohydrogen), while the remaining configuration is antisymmetric with respect to exchange of nuclei and results in a singlet state, the parahydrogen.
These spin configurations are extremely close together in energy, and therefore all four are populated essentially equally at room temperature. But p-H_2 is the most stable isomer by quantum mechanics and it dominates at low temperatures. The change from one isomer to the other is although symmetry forbidden: the spins cannot simply flip from one state to another even if the thermodynamics are favourable and suitable to drive the process forward. There is therefore need for a paramagnetic catalyst like charcoal, Fe_2O_3 or $Fe(OH)_3$.

When hydrogen reacts and its symmetry is broken in an oxidative addition reaction to a metal centre, novel NMR effects can be seen provided the reaction proceeds

in a spin correlated manner. Because hydrogen naturally contains approximately equal numbers of molecules in each of its four spin configurations, the four possible configurations in the product are also populated approximately equally. Since the intensity of an NMR signal is proportional to the population difference between the energy levels, *i.e.*, the number of nuclei capable of undergoing the transition normally is very low, low intensity signals are detected, as depicted in figure Figure 1.3.2. On the other hand, if it is possible to use only one of the spin configura-



(A) Standard Boltzmann-derived distribution of population and resulting transitions and NMR signals.

(B) p-H_2 -derived distribution of population and resulting NMR signal.

FIGURE 1.3.2: Population of the energy levels of ortho- and para-hydrogen.

tions of the molecular hydrogen, the $\alpha\beta - \beta\alpha$ state, after the oxidative addition to the metal centre only one of the spin states in the product would be selectively populated, with a resulting hyperpolarisation. In this case, as it has already been said, the populations of each energy level are greatly different to the usual Boltzmann distribution and therefore the NMR signals of the product are greatly increased. It is easy to see if the enhancement has worked since the resultant signals are now antiphase, one set in absorption and the other in emission – Figure 1.3.2. Once the enhancement has been observed by NMR, the sample relaxes back to thermal equilibrium. As a result, it is often necessary to have the chemical system "refresh" itself by reaction with more p-H_2 throughout the experiment.

There are a few ways to exploit the p-H_2 -induced polarisation: the two oldest techniques are called ALTADENA, Adiabatic Longitudinal Transport After Dissociation Engenders Nuclear Alignment, and PASADENA, Parahydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment, and both requires that the subjected molecules contain a suitable proton acceptor, be it a double or a triple bond.

A newer approach to the generation of PHIP sensitised materials is SABRE - signal amplification by reversible exchange - and is achieved without any chemical modification of the substrate. [60] [61] In this new approach, developed at the University of York, UK, in the group of Professor Simon Duckett, there is no chemical modification of the hyperpolarized material; it generate long lived spin states,

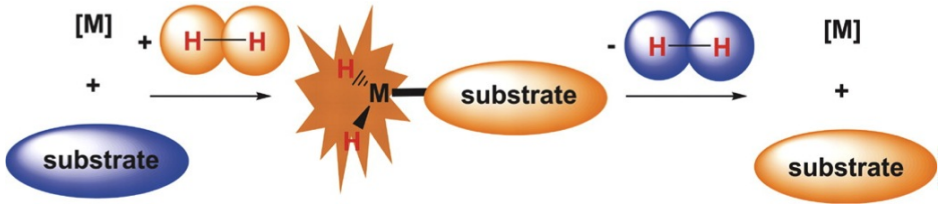


FIGURE 1.3.3: SABRE mechanism for transfer of polarisation from p-H_2 to the substrate. [61]

i.e. longer lifetimes which render this approach suitable to develop an MRI application. Finally, the polarization can be transferred to the heteronuclei through a suitable NMR sequence. The only requirement is that the substrate needs to incorporate a pyridyl moiety that can obviously coordinate with the metal centre in the catalyst. Hence, it seems that the metal centre serves as a template for allowing the p-H_2 and the substrate molecule to get close and participate in the magnetisation exchange. Most unfortunately, the real mechanism by which the exchange takes place is still unknown.

1.3.3 Arginine and NOS

Arginine, or 2-Amino-5-guanidinopentanoic acid, is a natural amino acid and one of the 20 proteinogenic L-amino acids. Thanks to the guanidine end, it is one of the most basic residues, with a pKa of 12.48.

Arginine is also the substrate for a class of enzymes called Nitric Oxide Synthases (NOS). This class of enzymes accounts for three different isozyme forms that catalyse the conversion of arginine to citrulline and nitric oxide: the Neuronal NOS, the Endothelial NOS and the Inducible NOS. NO is produced in neuronal tissues to be used as an intracellular second messenger for neurotransmission; NO is involved in the regulation of smooth muscle relaxation and blood pressure in the endothelial tissue; finally, in macrophage cells, NO is an answer to inflammatory stimuli. The uncontrolled production of NO has been implicated in numerous disease states, and it is outstanding the number of inhibitors that have been developed. The ac-

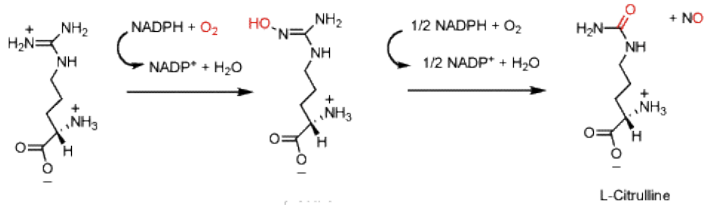
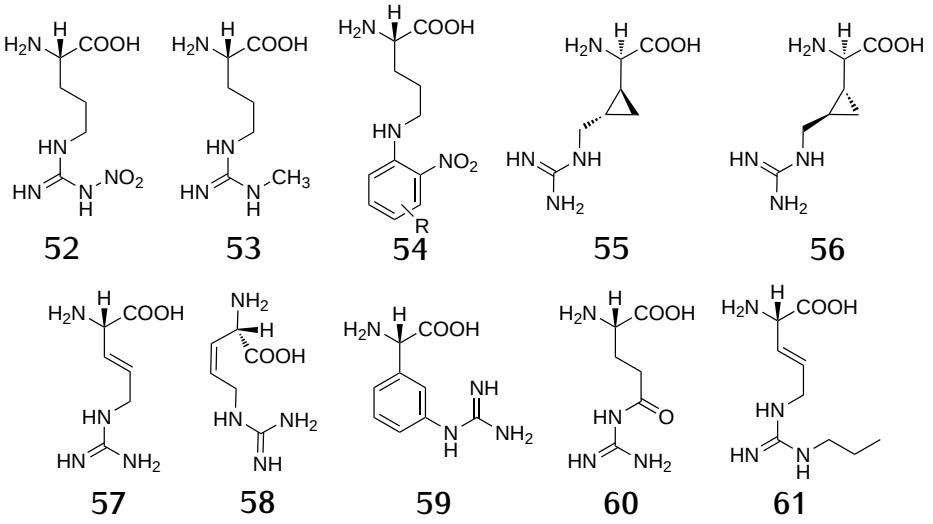


FIGURE 1.3.4: NOS catalytic cycle.

tion mechanism is shown in Figure 1.3.4: the guanidine moiety of the arginine is oxidized into urea, the arginine becoming cytrulline upon nitric oxide release.

A lot of inhibitors structures are based on L-arginine, and changing motifs and adding simple modification to the scaffold or capping an amine functionality could target only one of the different isoforms of the NOS enzyme. [19]



SCHEME 1.25: Some representative NOS inhibitors.

The classical NOS inhibitors, most significantly N^G-nitroarginine (L-NNA, **52**) and N^G-methylarginine (L-NMA, **53**), are analogues of the NOS substrate, L-arginine, but display low selectivity for inhibition of nNOS versus eNOS; therefore, Cowart *et al*. described the synthesis and activity of a series of N^ω-2-nitroaryl amino acid analogues **54**, where the R group is methyl or more methyl groups on the aromatic ring. A rationale for the preparation of this type of analogues was to create a molecule combining features of the substrate amino acid arginine with an aromatic moiety having the possibility of an interaction with one of the known NOS enzyme cofactors such as flavin adenine dinucleotide (FAD), tetrahydrobiopterin, flavin adenine mononucleotide (FMN), NADPH, or heme. Nitroarylated amino acids are readily synthesized by N-alkylation of NR-Boc-protected amino acids with 2-fluoro nitroaromatics, followed by removal of the protecting group. [62]
Lajoie *et al*. instead analysed the effect of structural constraints on the arginine structure. The aim of their study was in fact to probe the active site of the three NOS isoforms and retrieve informations about the enzyme binding and mechanism, determining the conformational preference of each isoform and evaluating the impact of the cyclopropyl moiety on isoform selectivity. They then developed a simple procedure for the preparation of syn- and anti-trans-cyclopropyl arginine **55** and **56**. [63, 64]
Finally, the conformational space was further probed by Silverman *et al*. by designing and synthesising other conformationally-restricted arginine analogues like **57**, **58**, **59**, **60**, **61**. [19]

1.4 The synthetic target – Project 3

2',6'-Dimethyl-L-tyrosine **6** is an interesting target from a synthetic and pharmacological point of view. It is an analogue of the natural amino acid L-tyrosine with two methyl groups on the phenyl ring in *m* position with respect to the oxydryl group

of the parent compound. The presence of these two moieties renders the analogue's side chain more constrained as fewer conformations can be populated because of the steric hindrance deriving from the two methyl groups.

It is well-known that secondary and tertiary structures of a peptide are crucial for the biological activity, along with its sequence, *i.e.* the primary structure; and it is also well established the relationship between the torsional angles ϕ, ψ and ω of the amino acid residues and the secondary structure of peptides – helices, sheets, turns. [65, 66] At the end of the 1990s an interest developed in studying the so-

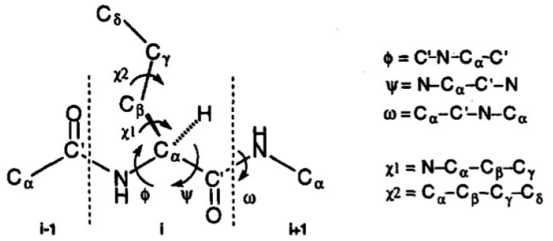


FIGURE 1.4.1: Definition of the dihedral angles ϕ, ψ, ω and χ of a peptide. [67]

called χ-space, or the exploration of the possible conformers of the side chain of the amino acids and how they influence the three-dimensional structure of peptides. The χ angle, in fact, in conjunction with the backbone angles define the position of side-chain functional groups in space: it is therefore a piece of great importance to understand the mode of action of peptides – Figure 1.4.1. [67]

As reported by the Hruby group [68] and shown in Figure 1.4.2, a single residue

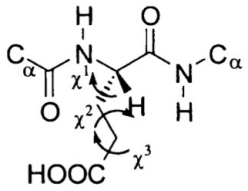
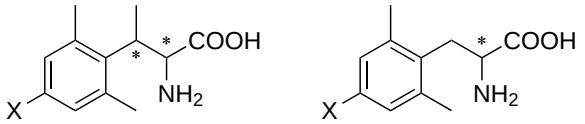


FIGURE 1.4.2: Up to 27 (3*x*3*x*3) different bioactive conformations are possible for a single glutamic acid residue, based on the χ angle. [68]

of glutamic acid in a peptide could give rise to up to 27 possible biologically active side-chain conformations as a result of three χ-angles determining the amino acid 3D structure.

On these bases, χ-constrained α-amino acids were designed alongside Dmt: β,2',6'-trimethyl-phenylalanine (β-TMP) **62**, β,2',6'-trimethyl-tyrosine (β-TMT) **63**, 2',6'-dimethylphenylalanine **64**. [67, 68] Their incorporation in strategic positions of peptides, giving raise to local side-chain constraints, has allowed for a reduction of the corresponding side-chain conformers and for increased selectivity and/or potency of several target peptides.

The focus of this project is on the development of an alternative synthetic route to Dmt **6**. In the following paragraph a short overview of all the synthetic routes entertained so far, while in the following section there will space for presenting



62 X = H (β-TMP)
63 X = OH (β-TMT)

64 X = H (DMP)
6 X = OH (DMT)

SCHEME 1.26: Some χ-constrained amino acids.

the pharmacological activity of this residue in some important target compounds. Finally, there will be focus over the parent compound from which the target compound is derived in the new devised plan.

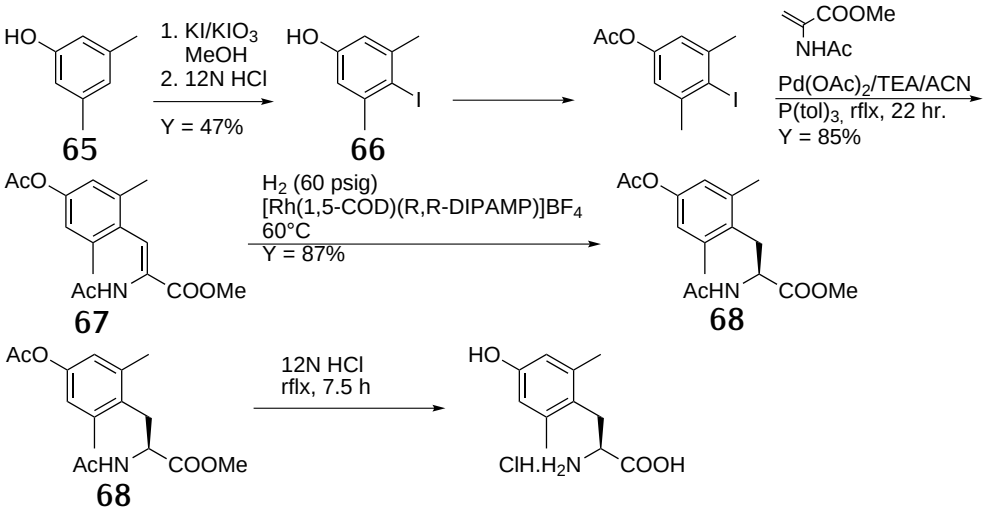
1.4.1 Preceding enantioselective synthesis

Only three enantioselective synthesis of Dmt have been reported so far in the literature. Beside these, a chemoenzymatic approach have also been reported: the enzymatic hydrolysis of *α-N*-acetyl-DL-2′,6′-dimethyltyrosine methyl ester in presence of *α*-chimotrypsin by Abrash *et al*. [69] allowed for the recovery of the single L-isomer.

The first asymmetric synthesis is reported by Beck *et al*. [70] in 1992. Starting from 3,5-dimethylphenol **65**, iodophenol **66** is obtained and acetylated to provide a suitable substrate for the Heck coupling with 2-acetamidoacrylate. This last step was repeated on a kilogram scale with an isolated yield of 85%. The desired dehydroamino acid **67** was hydrogenated at 60°C under 60 psig of hydrogen in ethyl acetate as solvent in the presence of 1.0 mole percent [Rh(1,5-COD)-*(R,R)*-DIPAMP]BF_4 in 12 to 24 hours. Protected-Dmt **68** was obtained under these conditions in 87% yield with an enantiomer ratio of 96:4 (L:D). The reaction rate for the hydrogenation reaction was expected to be slow because of the sterical congestion of the olefinic bond in the dehydroamino acid. A final hydrolysis with 12 N hydrochloric acid provided the expected compound in 97% yield with no loss of optical purity in this final step. The drawback of this synthesis is the quite lengthy hydrogenation step to be run under high pressure, which renders this route not approachable from a scale up point of view. Moreover, the the final ee is 92%. In fact, this procedure was revised and scaled up by Praquin *et al*. recently, addressing the aforementioned issues. [71]

A few years later, Soloshonok and Hruby [68, 72] reported the asymmetric synthesis of Dmt via alkylation of a chiral equivalent of a nucleophilic glycine with a suitable benzyl bromide. The chiral equivalent is based on a Ni(II) complex of the chiral non-racemic Schiff base of glycine with *(S)-o*-[*N*-(*N*-benzylprolyl)amino]benzophenone ([*(S)*-BPB]), introduced by Belokon' *et al*. [73], compound **69** in Scheme 1.28 on page 27.

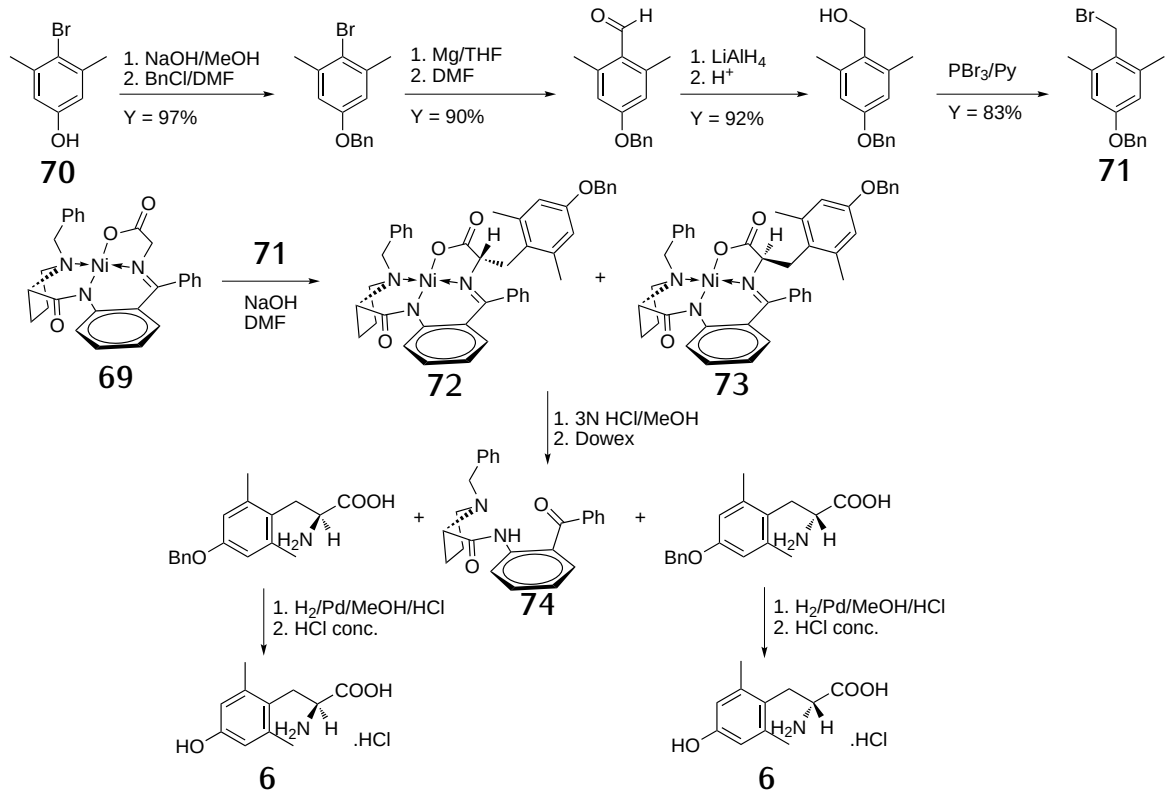
The synthesis started from commercially available bromodimethyl phenol **70** whose phenol moiety was protected as benzyl ether. The bromine atom was then converted to aldehyde and then to benzyl alcohol, which was finally reacted with PBr_3 to give the benzyl bromide **71** – this final reaction is described by the authors



SCHEME 1.27: Enantioselective synthesis reported by Beck. [70]

as rather difficult, most probably due to the steric hindrance on the benzyl position. In general, benzyl bromides are more reactive than chlorides, and therefore more suitable to sustain allylation from complex **69** at room temperature in presence of KOH or NaOH.
The reaction between Ni(II)-complex **69** and benzyl bromide **71** was conducted at room temperature in DMF using powdered NaOH as a base. The benzylation occurred with an unexpectedly high reaction rate (5 min), furnishing two products **72** and **73** in a ratio of 8:1 and in 95% chemical yield. These two diastereomers were separated via column chromatography and decampsed to afford the free amino acids, with recovery of the chiral ligand **74**.
Hydrogenation and hydrolysis of the obtained compounds lead to the obtainment of both enantiomers of Dmt **6**.
This short and rapid synthesis has a broader application [68] and is based on the simple alkylation reaction of a chiral metal complex which can be easily recycled, with high enantioselectivities from inexpensive and readily available reagents and solvents.

Some years later, another short but enantiomerically pure synthesis was published by Balducci *et al*. [74]. The key feature involves a kinetically controlled reaction between lithium-derived enolates of a diketopiperazine – an asymmetric glycine-moiety equivalent – and alkyl halides under very mild conditions at -78°C. Once again the synthesis started from commercially available **65** which was protected with ethyl chloroformate to be chloroformylated to give benzyl chloride **75**, as already reported in [69]. This last compound was then converted into benzyl iodide **76** via Finkelstein reaction over 50 hours.
This iodide compound would undergo alkylation from the lithium enolate formed by diketopiperazine **77** and the lithium base LHMDS to yield compound **78**. Upon reflux with 57% iodidric acid and exchange resin, final Dmt **6** was obtained.
The chiral synthon **77** is easily obtained in two steps as reported in [75]. A prominent feature of the method resides mostly in the stereocontrolled alkylation of the



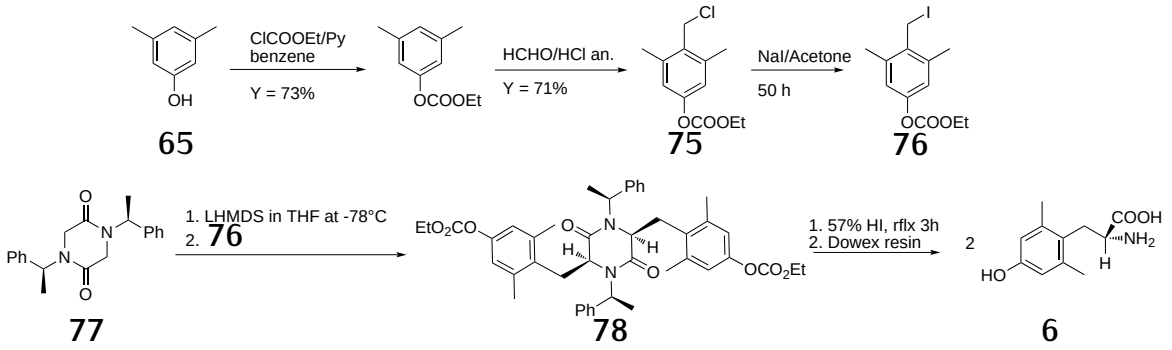
SCHEME 1.28: Enantioselective synthesis reported by Soloshonok *et al*. [72]

aforementioned synthon, which occurs with practically total diastereoselectivity to furnish the cis-derivative *(3S,6S)*-**78** A further interesting feature of this procedure is the total conversion of *(S)*-phenylethylamine to *(S)*-Dmt: two units of the target α-amino acid are obtained from one unit of the chiral synthon **77**. [74]

1.4.2 Pharmacological activity

2',6-Dimethyl-L-tyrosine is a widely used amino acids in the synthesis of opioid peptides and pseudopeptides [76] and it was first introduced in this type of compounds by Hansen *et al*. [77] The incorporation of this amino acid into simple peptide sequence has lead to important alterations in activities through the elevation of affinity, modification of receptor selectivity, and change in the spectrum of their bioactivity profile. Although new analogues are always been sought by chemists and pharmacologist, the use of Dmt in opioids still remains unsurpassed. [78]

Opioid receptors are a group of G protein-coupled receptors found widely in the brain, spinal cord and digestive tract. The major types are called δ, κ and μ. With their ligands they are involved in the path and mechanism of controlling pain with exceptional efficacy. Efforts to understand and then avoid the causes of opioid-related side effects such as physical dependence, tolerance, and respiratory depression, have generated a number of classes of opioid ligands ranging from derivatives of the endogenous ligands (enkephalins, endorphins, endomorphins, dynorphins) to frog skin peptides (deltorphins and dermorphins) and the alkaloids (morphine



SCHEME 1.29: Enantioselective synthesis reported by Balducci *et al*. [74]

and naloxone). In addition, it has been found that opioid antagonists, originally designed to reverse the adverse effects of opioid analgesics, are being useful in treatments of a number of significant human illnesses like addictions from cocaine and alcohol and autism. [79] Starting from investigating opioid analogues derived form frog skin secretions, Salvadori *et al*. developed synthetic opioid agonists and antagonists containing Dmt; in particular, they were able to identify the H-2',6'-dimethyl-L-tyrosine-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (H-Dmt-Tic-OH) **79**, as a δ-opioid antagonist pharmacophore for the Dmt-Tic series of peptides. In fact, incorporation of the Dmt amino acid into H-Tyr-Tic-OH peptide increased the δ-receptor affinity 8500-fold. Moreover, it has been found that enclosure of Dmt in place of Tyr at the N-terminus of diverse groups of opioid peptides produced a broad range of activities, turning the respective peptide into an agonist or antagonist at time. [79]
This outstanding characteristics arises from the presence of the hydroxyl group on the aromatic ring; the steric properties – on which we spent a few words on in the introductory paragraph of this section – induced by the presence of the methyl moiety in position 2' and 6' of the aromatic ring; aromatic ring which gives alignment and stabilisation through ring stacking or $\pi - \pi$ interactions between receptor and ligand; finally, the presence of a *N*-terminal amine.

An example of potency of action is found in the dermorphin-derived tetrapeptide [Dmt^1]DALDA (H-Dmt-D-Arg-Phe-Lys-NH_2), where Dmt is incorporated in the first position of the sequence. This peptide is a highly potent μ opioid agonist, characterised by a 7-fold higher binding affinity with respect to morphine and a high μ receptor selectivity. Moreover, pharmacokinetics studies found that the half-life of this peptide is four times longer than that of morphine, and recent studies indicates that it is also capable of crossing the blood-brain barrier. Its analgesic mode of action is thought to be triple: activation of the target opioid receptor, inhibition of norepinephrine uptake and release of endogenous opioid peptides. [76]

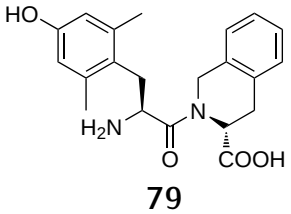


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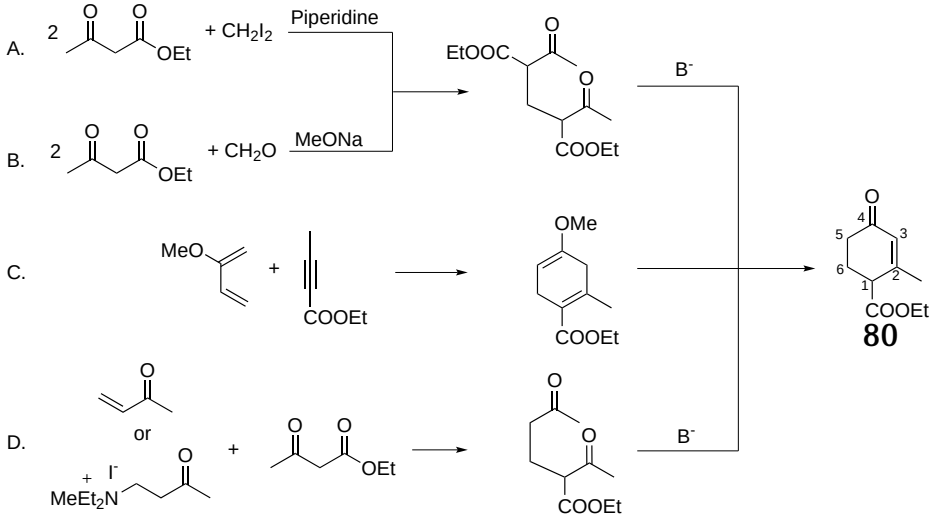
FIGURE 1.4.3: Dmt-Tic pharmacophore.

More recently, Tourwé *et al*. reported the synthesis and pharmacological studies of a novel dermorphin tetrapeptide in which Tyr^1 has been replaced by Dmt^1 because of its enhancement of the μ and δ receptor interactions and significant increase in opi-

oid agonist potency. Other modifications at other residues has been made, and this lead to identification of the ligand H-Dmt-NMe-D-Ala-Aba-Gly-NH_2 with mixed μ/δ opioid agonist properties. This is part of an emerging approach because various studies proved the existence of physical and functional interactions between the μ and δ receptors. [80]

1.4.3 Hagemann's Ester as building block

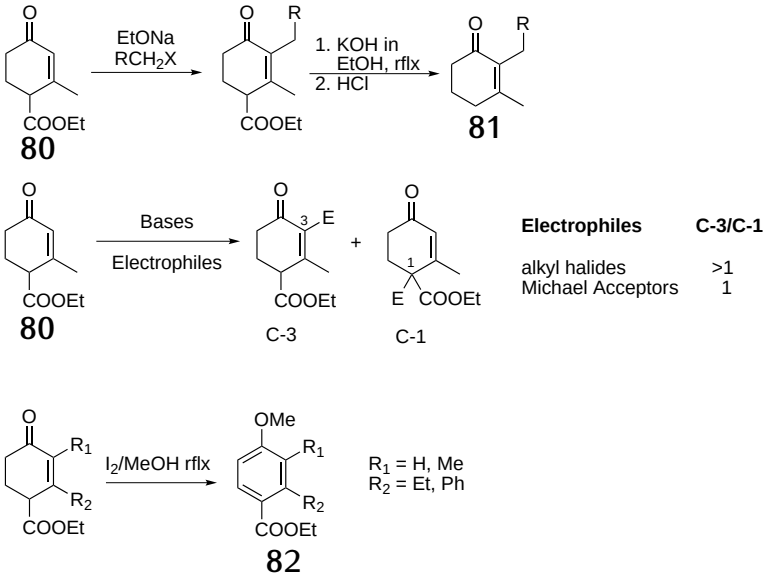
Hagemann's ester, or ethyl 2-methyl-4-oxocyclohex-2-enecarboxylate **80**, its derivatives and C-1 and C-6 alkylated analogues have long been used as a starting point for modification and manipulation at their functional moieties in order to easily deliver interesting compounds
Its original synthesis has been improved over the years, but there are mainly four different approaches, shown in Scheme 1.30: the original approach involves the condensation of two equivalents of ethyl acetoacetate and methylene iodide in the presence of sodium methoxide and subsequent cyclisation of the resulting diethyl ester; Knoevenagel used a domino Knoevenagel-Michael-intramolecular aldol reaction starting from two equivalents of acetoacetate and formaldehyde in the presence of basis; Newman and Lloyd exploited a Diels-Alder reaction and subsequent hydrolysis to obtain the final compound; finally, Mannich and fourneau prepared the ester by the action of sodium ethoxide on acetoacetic ester and a precursor of methyl vinyl ketone. Hagemann's ester finds useful application in the synthesis of



SCHEME 1.30: A. Hagemann's original approach. B. Knoevenagel's approach. C. Newman and Lloyd's approach. D. Mannich and Fourneau approach. [81]

2-alkyl-3methyl-2-cyclohexen-1-ones **81**, resulting from C-3 alkylation with suitable halide followed by one-pot hydrolysis and decarboxylation. At the same time, it is a convenient starting material to obtain differently decorated benzoates **82** by aromatisation with iodine in methanol at reflux.
On the former theme, an interesting study was published a few years ago on the selectivity of C-3 versus C-1 alkylation products, and the ratio of the products was

found to be more dependent on the electrophilic agent rather than on the basic conditions of the reaction. At the same time, reaction of the title compound with suitable Michael acceptor were screened and it was found that these conditions are not selective, giving C-1 and C-3 adducts in almost comparable yields. [82] As

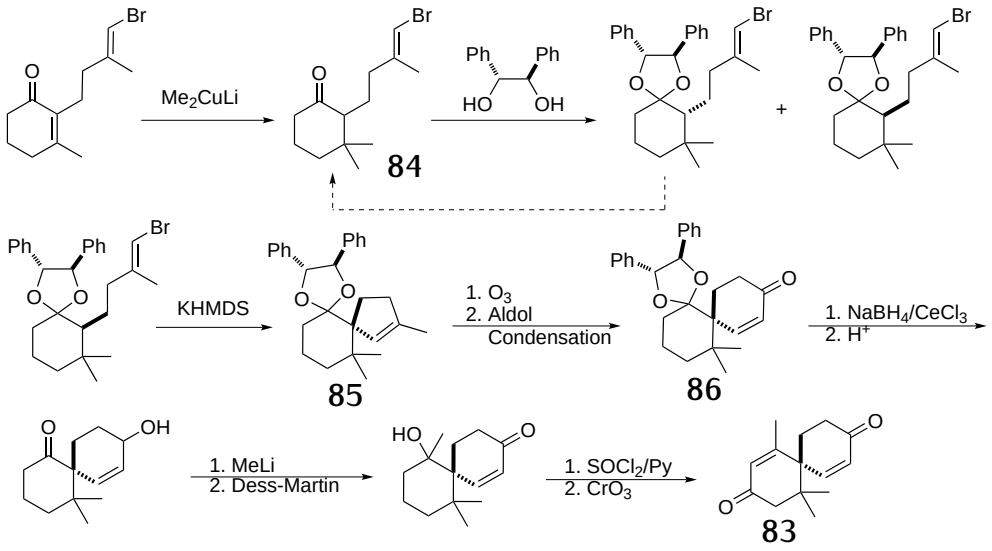


SCHEME 1.31: Alkylation and aromatisation reaction of Hagemann's ester . [81, 82]

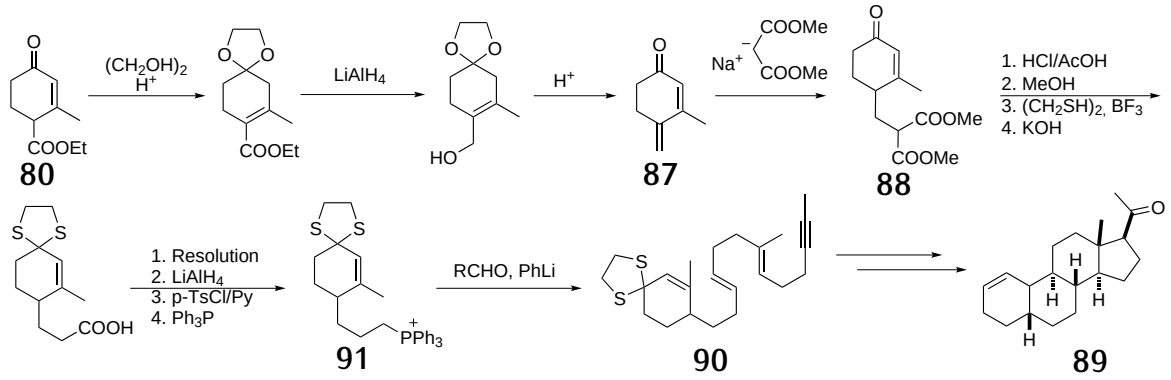
already mentioned, Hagemann's ester can be alkylated at different carbon atoms, mainly C-3 and C-1, but also at C-5; its ester moiety can be removed, preserved or elaborated; finally, its ring can be opened in order to deliver a more useful carbon scaffold. [81] In the following paragraphs two selected examples of synthetic utility of Hagemann's ester are presented and discussed.

An example of the utility of Hagemann's ester for the synthesis of natural products can be found in a recent synthesis of (+)-majuscolone **83** which features a spiro-fused six-membered ring at the C-3 position of original Hagemann's ester . Starting from trisubstituted **84** derived from previous alkylation with suitable derivative and decarboxylation of Hagemann's ester , ketalization with useful chiral auxiliary allowed for the separation of the two epimers at C-3 – the undesired one is easily recycled. **85** was easily obtained by intramolecular stereocontrolled insertion reaction. After ozonolysis and aldol reaction, ring expansion was achieved to give **86**. Chemoselective reduction and subsequent removal of the ketal group gave the keto-alcohol. On this substrate, tertiary alcohol was generated alongside a new ketone moiety. Final dehydration and allylic oxidation gave the spiro-compound **83**. [83]

Hagemann's ester **80** has been used in the past as a starting point for synthesising a steroid analog. [84] After ketalisation subsequent reduction and deprotection of starting **80**, derivative **87** is submitted to Michael reaction with dimethylmalonate to yield **88**. This latter is further manipulated to arrive phosphonium salt **89**, which is reacted with the appropriate aldehyde in a Wittig-Schlosser reaction to give the



SCHEME 1.32: Synthesis of (+)-majuscolone. [83]



SCHEME 1.33: Synthesis of steroid scaffold. [84]

trans,*trans*-trienyne thioketal **90**. After two more steps the final tettracycle **91** is obtained from a stereocontrolled acid-promoted polyene cyclisation.

Part I

First Project: Synthesis of Bicyclo[3.1.0]hexanes Amino Acids

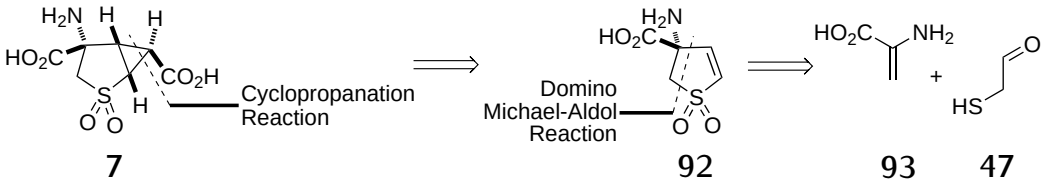
2 Aim

The aim of this project is to develop an alternative and stereoselective route for the synthesis of compound **7** and to find new ways of synthesising bicyclo[3.1.0]hexanes.

2.1 Studies Towards the Synthesis of Compound LY404039

The original synthesis of compound LY404039 **7** has already been shown in Scheme 1.14 on page 13 and originally reported in [18, 27]. This synthesis is not stereoselective: there is in fact need for a resolution step in order to differentiate and isolate the two different enantiomers of the compound. Moreover, it is quite lengthy and the first step is not at all efficient, as the best yield in target compound is 38%.

An alternative retrosynthetic analysis for synthesising the compound can be found in Scheme 2.1. First disconnection on the cyclopropyl moiety could lead to dihydrothiophene **92**: by the experience matured in the group on synthesis of this type of compounds, we disconnected further this ring and opened it into dehydroalanine **93** and mercaptoacetaldehyde **47**. In fact, as shown in 1.2.2 on page 17



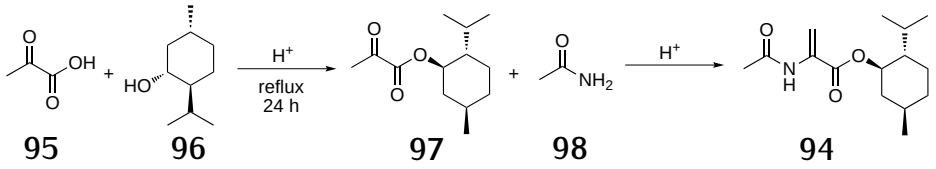
SCHEME 2.1: Retrosynthetic analysis for the synthesis of compound **7**.

and reported in [55], it is possible to construct the tetrahydrothiophene ring by a tandem Michael-Aldol reaction of dithiane **46** – the dimer of **47** – onto a suitable acceptor like an amino acrylate – structurally related to dehydroalanine **93**.

To control the stereoselection and induce asymmetry in the tetrahydrothiophene ring synthesis, it was thought to use a chiral auxiliary. (−)-Menthol esters had already found application in controlling the stereochemistry of in the literature in the copper-promoted 1,4-conjugate addition of phenylmagnesium bromide to chiral 2-acetamidoacrylates to produce N-acetylphenylalanine esters in high chemical yields and good diastereoselectivity. [85]

2.1.1 Studies toward the Synthesis of Appropriate Amino Acrylate

The first step of the synthesis was then to investigate and tune the synthesis of (−)-menthyl 2-acetamidoacrylate **94**, which could be conveniently obtained through a known two-step procedure. [86]
This preparation has been tested and standardised for the first time in our labs. The starting materials are cheap and the obtained acrylate is optically active and pure, making it a perfect substrate for reaction such as 1,4-conjugate addition. The overall synthetic process is easy and do not require complex work-up procedures; most important, it is scalable up to 10 grams of starting materials, with good yields (41%) and easy work-up.
Esterification of pyruvic acid **95** with (−)-menthol **96** in refluxing benzene would give ester **97**. This intermediate is treated in a one-pot-reaction with acetamide **98** in refluxing benzene to finally yield the target chiral acetamidoacrylate **94**.



SCHEME 2.2: Preparation of the (−)-menthyl N-acetamido acrylate **94**.

A series of experiments to study the best conditions for the two reactions, which have been combineed together in a one-pot procedure, have been conducted.
For what is concerning the first step, several esterification reactions had been conducted: the results are shown in Table 2.1.1. A few acid catalysts have been screened, and the best ones are sulphuric acid and p-toluensulfonic acid. Acid catalysis by pyridinium p-toluensulfonate and acidic Dowex resin were not successful attempts in gaining more reaction yield or an easier work-up.

TABLE 2.1.1: Screening for best conditions to obtain ester **97**.

Pyruvic Acid		Menthol		Other conditions and results					
g	mmol	g	mmol	eq	catalyst	solvent ml	T (°C)	time (h)	Y (%)
1	11.36	1.42	9.088	0.8	pTosOH	20	110	o.n.	62
1.5	17	2.12	13.6	0.8	PPTS	30	112	48	22
1.5	17	2.12	13.6	0.8	H_2SO_4	30	110	16	60
1.5	17	2.12	13.6	0.8	resin	30	110	48	NR

In this step, the formation of the by-product is observed as the reactive ketoester reacts with itself to form the (−)-menthyl ester of the isotetronic acid; in the second step, it is supposed to react with the acetamide to form the amidoester of the isotetronic acid. Both compounds have been isolated and characterised.

For what is concerning the second step, changing the equivalents of acetamide or using sulphuric acid as a catalyst is not suitable for the second reaction step, as it can be seen in Table 2.1.2 on the facing page.

TABLE 2.1.2: Screening for the best conditions to obtain (−)-menthyl N-acetamido acrylate **94**.

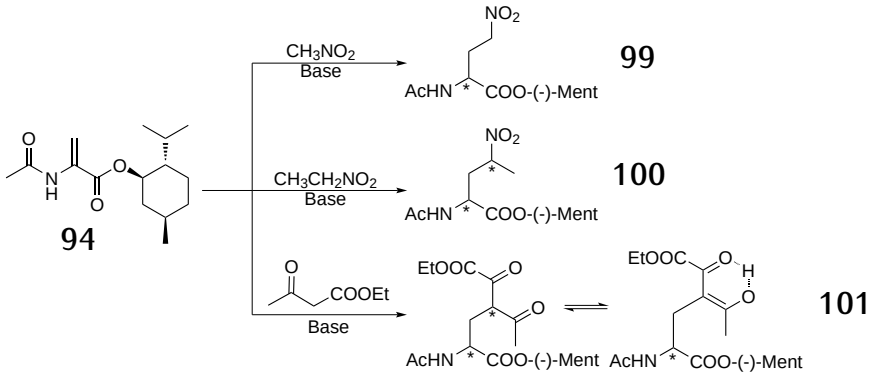
Ester		Acetamide			Other conditions and results				
g	mmol	g	mmol	eq	catalyst	solvent ml	T (°C)	time (h)	Y (%)
1.28	5.66	0.67	11.3	2	pTosOH	30	110	48	24
6.23	27.55	1.63	27.55	1	PPTS	60	112	24	22
3.12	13.8	1.63	27.6	2	H_2SO_4	60	110	48	42

In Table 2.1.3 on the next page all the different experiments for the one-pot reaction are reported, alongside with the results and yields. In addition, scale-up experiments for this synthesis are included, from 1,5 g up to 10 grams.

As it can be seen, catalysis by sulphuric acid was not used for the one-pot reaction because it interferes in second step. Moreover, lower yields are obtained when toluene is used as solvent or when longer reaction times are employed: this is probably due to high temperatures causing the reagents and the product to react and give some other byproducts.

2.1.2 Reaction of the N-Acetamido acrylate of (−)-Menthol with Nucleophiles as Michael Acceptor

The double bond of the acrylate is conjugate and thus can undergo addition from a nucleophile. We studied the reactivity of the double bond and the stereoselectivity of the addition reaction led by the (-)-Menthol inserted in the molecule in presence of different types of nucleophiles before turning the attention to the synthesis of the tetrahydrothiophene ring.



SCHEME 2.3: Reaction of (−)-menthyl N-acetamido acrylate **94** with Michael acceptors.

Given the easy outcome of the reaction, reaction of compound **94** with nitromethane has been taken as a model reaction and therefore thoroughly investigated: different types and kinds of bases have been used.
Only one product **99** is always formed. In Table 2.1.4 on page 39 all the carried out experiments are reported. As it is also possible to notice, no reaction is reported for

TABLE 2.1.3: Experiments for the one-pot-reaction shown in Scheme 2.2 on page 36

Pyruvic Acid		Menthol			Conditions and results for Ester				Acetamide			Conditions and results		
g	mmol	g	mmol	eq	cat.	benz. (ml)	T (°C)	time (h)	g	mmol	eq	benz. (ml)	time (h)	Y (%)
1.5	17	2.12	13.6	0.8	pTosOH	30	110	24	1.61	27.2	2	130	72	37.5
1.5	17	2.12	13.6	0.8	pTosOH	30	114	24	1.61	27.2	2	130	48	57
1.5	17	2.12	13.6	0.8	pTosOH	30	114	24	1.61	27.2	2	130	24	57
5	56.8	7.1	45.44	0.8	pTosOH	tol. 100	130	24	6.7	113.6	2	tol. 125	24	32
5	56.8	7.1	45.44	0.8	pTosOH	100	110	20	6.7	113.6	2	125	20	36
1.5	17	2.12	13.6	0.8	pTosOH	30	110	24	1.61	27.2	2	130	24	38
1.5	17	2.12	13.6	0.8	H_2SO_4 /pTosOH	30	110	6	1.61	27.2	2	130	24	27
10	113	14.2	0.091	0.8	pTosOH	200	105	3	10.75	182	2	200	48	41

bases like L-proline or 2-hydroxy pyridine, probably due to the limited basicity of these compounds and the scarce possible interactions.

TABLE 2.1.4

Base	Eq.	Y (%)	Time
K_2CO_3/TEA	2 /0,1	86	6 days
TEA	0.1	NR	24 hrs
K_2CO_3	2	37	6 days
Cs_2CO_3	2	18	4 days
K_3PO_4	6	28	6 days
L-Proline	0.1	NR	6 days
2-Pyridone	0.1	NR	6 days
KF	1	11	12 days

Most unfortunately, the chiral HPLC analysis on the resulting compounds showed that the only formed product is a racemic mixture, the (−)-menthol too far from the reactive centre and hence not able to control the stereochemistry of the final step of the Michael addition, where the negative charge is quenched with a proton.

Reaction with nitroethane to give **100** was then investigated: in this reaction two new stereocenters are formed. As a result, two compounds which are diastereomeric are expected and in fact obtained, with different diastereomeric excess as it is reported in Table 2.1.5. In this case, too, a few bases were assessed on the reaction.

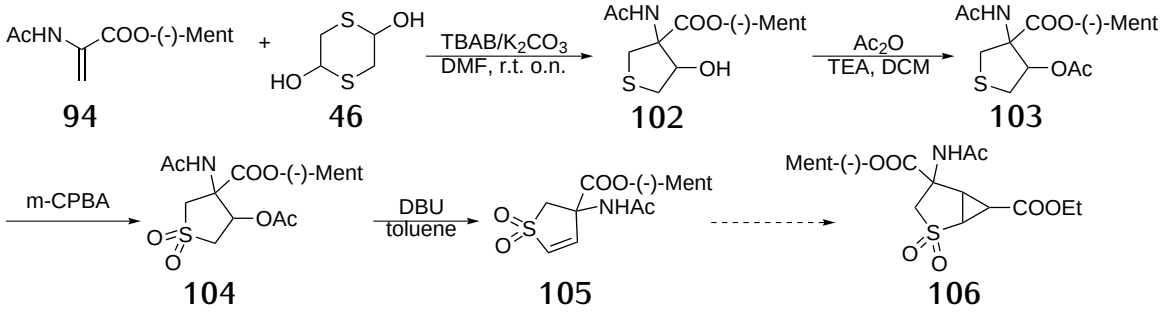
TABLE 2.1.5

Base	Eq.	Y (%)	d.e. (%)	Time
K_2CO_3/TEA	2 /0,1	86	18	6 days
TMG	0.1	NR	-	24 hrs
Cs_2CO_3	2	20.4	13	4 days

The previous studies and experiments conducted on the reactivity of the double bond of the acrylate lead us to use the K_2CO_3/TEA base couple in the ratio 2:0,1 equivalents with respect to the acrylate protocol to generate the nucleophile in Michael-type reaction.
This was in fact tested in the reaction with Ethyl Acetoacetate to give **101**: two products are expected, but only one diastereomer is formed because of the keto-enolic equilibrium, shown in Scheme 2.3 on page 37.

2.1.3 Synthesis of the Tetrahydrothiophene-compound

In the following Scheme 2.4 on the next page is reported the synthetic pathway for the synthesis of the target bicyclo[3.1.0]hexane amino acid **7**.



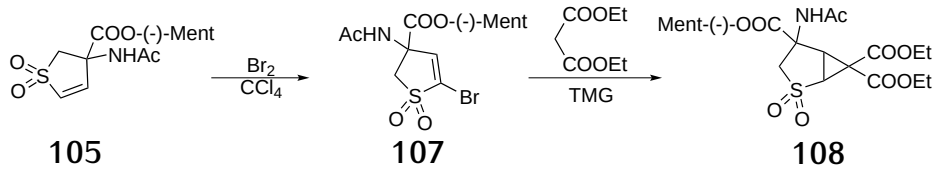
SCHEME 2.4: Envisaged synthetic pathway to achieve formation of compound **102**.

The first reaction is the Michael-Aldol tandem reaction previously studied in our laboratories to form **103**. It is the result of further improvements on the protocol developed and reported in the previous paragraph 2.1.2 on page 37.
Conditions were although altered: in particular, change of solvent was of great help in improving the reaction yield. In fact, shift from DCM to DMF greatly improved solubility of the dimer **46** of mercapto-acetaldehyde **47**. Potassium carbonate was still used as base, but a better phase transfer agent was chosen: tetrabutyl ammonium bromide was indeed more efficient with respect to TEA to transfer the base between the two phases. These two fact greatly improved reaction yield and time.
Moreover, some diastereoselectivity was observed in this reaction, as we were able to obtain two different diastereomers in a 3:1 ratio. Most unfortunately, due to the nature of the compounds, it has not been possible to perform a more in depth chiral analysis via HPLC in order to discern and separate the diastereomeric mixture.

Compound **103** was then acetylated: in this way the alcohol functionality is protected from oxidation in the following step, but is also transformed in a good leaving group for the subsequent elimination reaction. The sulphur atom of the acetylated compound **104** was oxidised with μ-chloroperbenzoicacid to sulphone **105**. The obtained compound was submitted to elimination reaction to achieve synthesis of the double-bonded adduct **106**. This is the final adduct before the construction of the bicyclo[3.1.0]hexane scaffold and achieve synthesis of compound **102**, to be obtained via a suitable cyclopropanation reaction.

A few trials to obtain the cyclopropanated compound **102** were made with ethyl diazoacetate and catalysts, but most unfortunately they did not result in isolable quantities of the target compound.

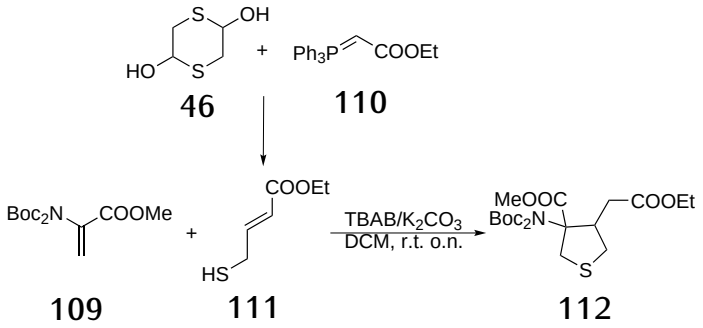
Another strategy was then probed on the basis of MIRC reaction studies conducted in the same lab and whose results are shown in 2.2 on the next page. A



SCHEME 2.5: Alternative synthetic pathway to achieve formation of compound **102**.

bromine atom was successfully inserted on the α carbon of compound **106** by ordinary bromination with molecular bromine to obtain bromodihydrothiophene **107**. This latter compound would then react in MIRC reaction with TMG-produced anion of diethyl malonate to give the dicarboxylate compound **108**.

Further studies have been conducted on a multicomponent Michael-Aldol-Wittig tandem reaction, in order to add and generate more diversity in one place at one time with the reaction of acrylate **109**, mercaptoacetaldehyde dimer **46** and phosphorane **110**. They were all added to the reaction vessel in a one-pot fashion. DCM was chosen as a solvent even if dithiane has difficulties in being solved in it: in this way, it reacts under basic conditions to give a Wittig adduct **111** that reacts with acrylate upon formation in the tandem Michael-Aldol reaction to afford **112**.



SCHEME 2.6: Alternative synthetic pathway to achieve formation of compound **102**.

Compound **111** was not isolated because of its high reactivity: it dimerises rapidly. Screening of addition time for the reaction components lead to consider a one-pot one-step reaction, or, in other words, the three components were added altogether in pone place at one time. The choice of DCM as solvent rendered the dissolution of dithiane very slow, making it possible to react primarily with phosphorane and subsequently undergoing the Michael-Aldol reaction in order to achieve the desired product **112**.
This methodology was applied also on acrylate **94**, but it proved less satisfactory.

2.2 Cyclic ketones and MIRC Reactions

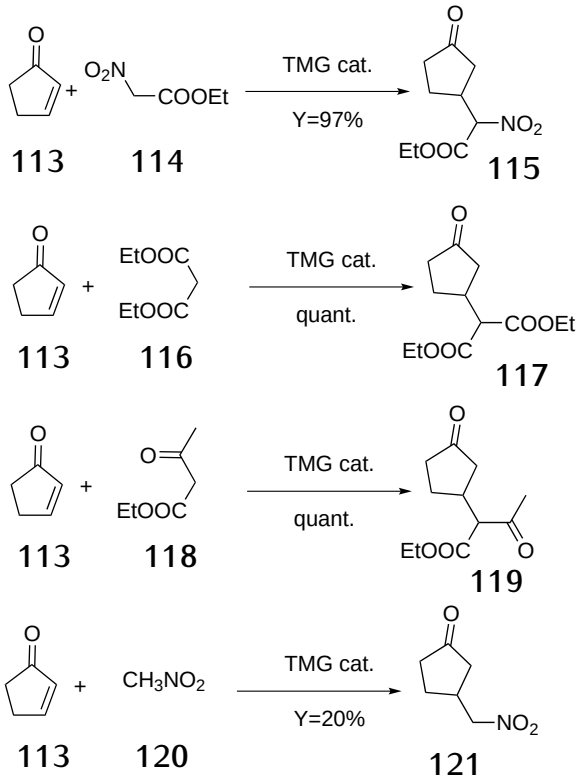
2.2.1 Cyclopentenone

In the group where part of this thesis work was done, Michael reaction has been throughly investigated as a mean for forming new C-C bonds. Efforts have therefore been turned towards using 1,4-conjugate addition reaction for synthesising bicyclo[3.1.0]compounds, obviously exploiting and trying to develop a MIRC reaction – see 1.2.1 on page 15.

The investigation started by exploring simple TMG-catalysed Michael addition to cyclopentenone **113** of some nucleophiles. TEA was not found suitable for the transformation because of the scarce conversion – isolation of unreacted starting

material. In addition, the reaction was conducted in neat conditions. The conversion is total with excellent yields in less than an hour's time. The scope of the reaction is shown in Scheme 2.7.

A particular mention is the last case, where nitromethane **119** was employed: the



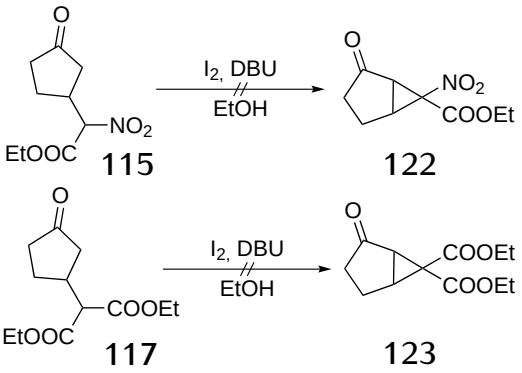
SCHEME 2.7: Michael-addition reaction of cyclopent-2-enone with different enolates.

yields were scarce, possibly because of the high reactivity of the compound.
A MIRC reaction with molecular iodine was assessed on compounds **120** and **121**, under the conditions reported by [87]. This iodo-induced cyclization would allow to achieve the bicyclo[3.1.0]hexanes structures **122** and **123** reported in Scheme 2.8 on the facing page. Most unfortunately this procedure proved unsatisfactory because of the very low yields. Moreover, decomposition of the starting material **120** was also observed, as it was possible to isolate the dimethyl malonate **115** which was used in the preparation of the starting material.

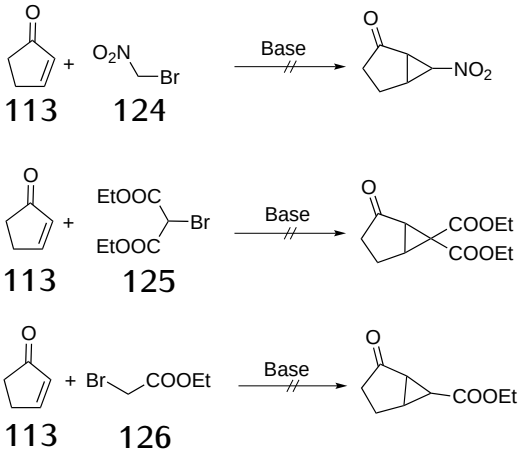
Nonetheless, this failure prompted us to explore two different possibilities for the MIRC reaction: 1. installation of the halogen atom onto the nucleophile and 2. the installation of an halogen atom onto the cyclopent-2-enone ring.

In the first case, reaction with bromonitromethane **124**, bromo diethyl malonate **125** or bromoacetato **126** were unsuccessful. Both catalytic and equimolar ratios of TMG and TEA were used in the reaction, but only the starting material were recovered after appropriate work-up – Scheme 2.9 on the next page.

After this series of unsatisfying results, attention was then turned toward the second option, *i.e.* insertion of the halogen atom onto the cyclopent-2-enone ring.



SCHEME 2.8: Iodo-induced cyclization reaction.

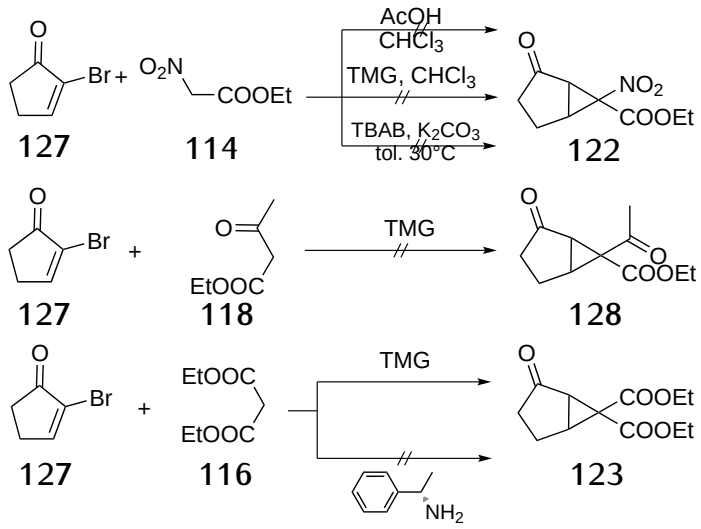


SCHEME 2.9: cyclopent-2-enone reaction with halogen-containing nucleophiles.

Synthesis of 2-bromocyclopent-2-enone **127** was achieved following literature procedure with satisfying yields [88]. It was then reacted in different ways with nucleophiles, *i.e.* the enolates of nitroacetate **114**, of ethyl acetoacetate **116** and of diethyl malonate **115** – see Scheme 2.10 on the following page. The method developed and which lead to the synthesis of ethyl bicyclo[3.1.0]hexanonedicarboxylate **123** sees the employment of equimolar ratios of the starting materials **127** and **115** and of the base, TMG, in solvent-free conditions; the reaction time is less than an hour.
Most unfortunately, these conditions were successful only for the reaction of **127** with **115**. No reaction took place, in fact, when the method was applied with other different enolates. Outstanding is the reaction of **127** with **114**: the expected compound **122** was not produced in all the screened conditions. Neither ethyl acetoacetate **116** gave the expected bicyclo[3.1.0]hexanes structure **128**. In these last two cases, the starting materials were recovered.

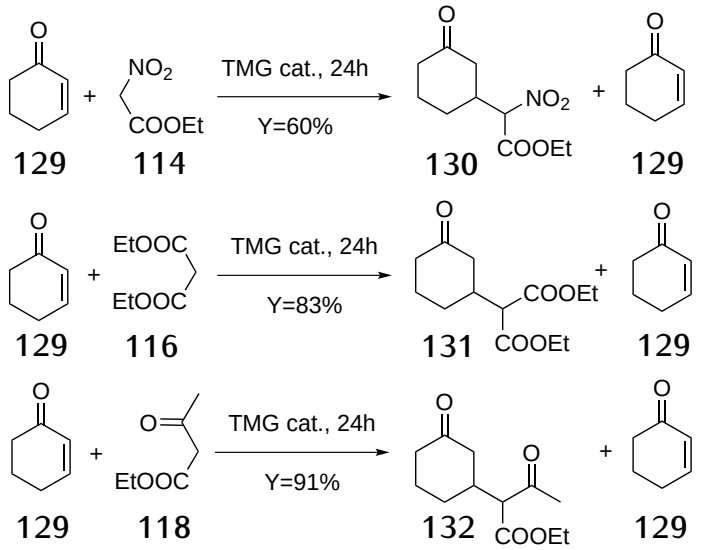
2.2.2 Cyclohexenone

The Michael addition of suitable nucleophiles methodology which was studied on the cyclopent-2-enone was then applied to it superior homologue, cyclohexen-1-



SCHEME 2.10: 2-bromocyclopent-2-enone reaction with nucleophiles.

one **129**, as reported in Scheme 2.11. It is possible to notice a few things from the



SCHEME 2.11: Cyclohexen-1-one reaction with nucleophiles.

reported scheme: a. the reaction conversion is lower with respect to the previous case, where cyclopentenone was used, as the starting ketone was isolated from reaction medium; b. reaction time is also higher with respect to the previous case.

In this case too bromocyclopent-2-enone was synthesised and reacted with diethyl malonate in order to synthesise a bicyclo[4.1.0]hexane dicarboxylate structure in the conditions found suitable from the previous section. Most unfortunately, this only lead to recovery of the starting materials.

3 Conclusions and Perspectives

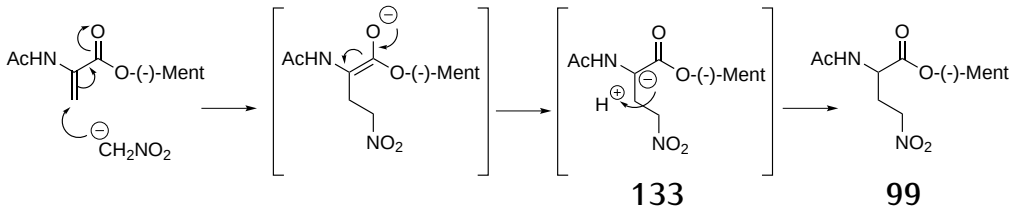
3.1 On the synthesis of LY404039

A new synthetic pathway for the synthesis of compound LY404039 **7** was devised.

The focal point of the synthesis is the use of a tandem Michael-Aldol reaction to synthesise the tetrahydrothiophene ring. This is also the key stereochemical passage, because in just one step two new stereogenic centres are formed. Controlling the stereochemistry of this passage is therefore central in planning an asymmetric synthesis.

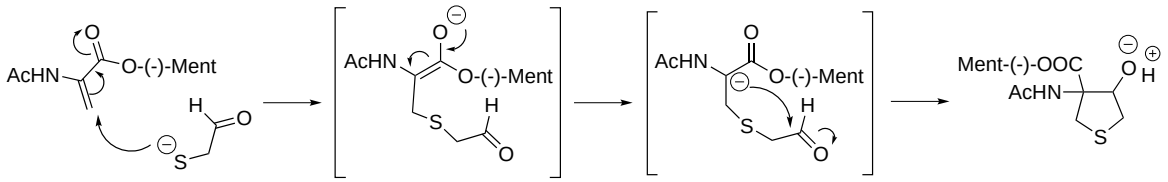
The envisioned method of using (−)-menthol as a chiral auxiliary inserted as ester moiety most unfortunately resulted in very poor enantioselectivity, as demonstrated by the chiral HPLC analysis performed on nitromethane and nitroethane derivatives **99** and **100**.

The reason behind this comes from the reaction mechanism, which can be seen in Scheme 3.1. In fact, the esterificated menthol is too far away from the reactive centre to control the stereoselectivity of the reaction: the key step that defines the stereochemistry is the quenching of the negative charge by a proton – intermediate **130** in Scheme 3.1. Here the negative charge is in no way conditioned to attack the the proton in a stereoselective way, because the far away methol cannot influence this attack.



SCHEME 3.1: Intramolecular cyclopropanation reaction.

Different is the case for the tandem Michael-Aldol reaction for the synthesis of the tetrahydrothiophene ring, shown in Scheme 3.2 on the following page. For instance, the quenching of the negative charge formed by Michael reaction and which found itself "resting" at the branching of the amino acid moieties does not



SCHEME 3.2: Intramolecular cyclopropanation reaction.

take place. After the first Michael attack by the mercaptoacetaòdehyde, which we could argue occurs preferably at one side of the molecule because of the steric incumbrance of menthol, the negative charge attacks the electrophilic centre on the carbonyl moiety of the mercaptoacetaldehyde. In this way the subsequent aldol reaction takes place, finally forming the tetrahydrothiophene ring. It is therefore possible that in this case the esterificated (−)-menthol and its bulky presence could in some way influence the stereochemistry of this second reaction. In fact, it is possible to separate by chromatographic column two compounds, with the same m/z ratio and similar ^{1}H-NMR signals, in a 3:1 ratio. Most unfortunately it was not possible to analyse and separate these compounds on a chiral HPLC column.
Some would argue that determination of the products configuration is futile at this stage because in the following steps the alcohol is eliminated to yield a double bond. Anyway, determining the configuration is vital to understand if the chosen chiral auxiliary can or can not guide and control stereoselection in the reaction. Moreover, since the double bond is to be cyclopropanated, the same stereoselection would be expected.

It would be interesting to calculate the conformations and the energies for the transition states, in order to see if and how the (−)-menthol influences the reaction space.

A way to improve stereoselectivity in this step could be the employment of chiral quaternary ammonium salts, derived from cinchona alkaloids, as phase transfer catalysts. The Chiral Phase-Transfer Catalysis has in fact proven reliable and viable for synthetic applications also on a large and even industrial scale, even in Michael Reaction cases. [89]
A tandem Michael-Aldol reaction with simple quinine and K_2CO_3 was indeed tried, but the resulting yields were not encouraging. This is possibly due to the fact that the employed compound is not a real transfer agent – *i.e.* a quaternary ammonium salt – and therefore its behaviour is like the one shown by the TEA/K_2CO_3 couple at the beginning of the investigations on the Michael reaction on the selected compounds, reported in 2.1.2 on page 37.

The other key step in the synthetic pathway is the final cyclopropanation reaction. Decomposition of ethyl diazoacetate with catalysts to give suitable carbene to react with double bond did not produced the expected cyclopropyl compounds. Instead, decomposition of the starting materials was observed. $Rh_2(OAc)_4$ and Cu(I) trifluoromethane sulfonate were found inactive as catalysts, according to literature, but also active Cu(II) acetylacetonate catalyst was found not reactive towards the compound's double bond.

There is also to consider that, while metallic catalysts are at their best with active double bonds, the considered double bond is in reality not so active because of the conjugation with the sulphone moiety. It is possible that Pd-containing catalysts are more efficient towards these type of bonds.
A trace of target compound was eventually isolated from the MIRC reaction between the brominated double bonded compound **107** and the diethyl malonate ester. This is an application of the developed method for the synthesis of bicyclo[3.1.0]hexanes compounds with TMG and diethyl malonate, reported in 2.2.1 on page 41. Most unfortunately, it was not possible to isolate it in reasonable yields to get a full characterisation.

Finally, the multicomponent reaction resulted quite well by developing a one-pot one-step Wittig-Michael-Aldol reaction. The slow dissolution of dithiane **46** in DCM allowed it to react primarily with phosphorane **111**. The so formed, very reactive adduct was allowed to react with acrylate**109** in order to give the desired tetrahydrothiophene. In this way, in just one step more diversity was generated.

3.2 Cycloenones and Michael Reactions

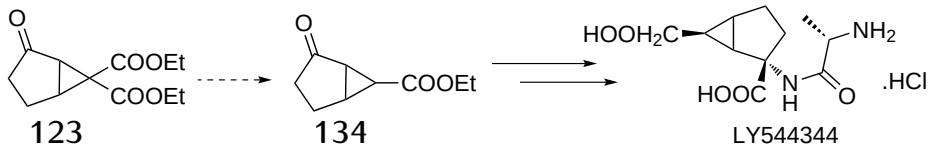
Two cycloenones were chosen as the target for a simple Michael reaction and then subsequent MIRC reaction was studied.

It is quite outstanding the difference in reaction times between cyclopenten-1-one and cyclohexen-1-one in the studied Michael reaction conditions – a catalytic amount of TMG and different enolates. This difference in reactivity is thought to reside in the ring strain: the five-membered ring is more strained than the six-membered, also because of the conjugated double bond. After the addition reaction the double bond becomes a single bond and the strain is reduced.
Cyclohexen-1-one has more freedom of rotation in its ring and therefore is less reactive.

Asymmetric addition was tried with α-phenylethylamine, but no reaction was observed. The same result was found when APTC conditions were used, although an asymmetric version of this MIRC reaction with PTC has been developed and throughly studied and applied. [52, 53]

It is also quite outstanding that the methodology developed so-far was effective only on the malonic ester derivative. It is although possible that the exothermicity of the reaction and the higher reactivity of the other compounds used as nucleophiles required different conditions which was not possible to study given the short time this methodology has been studied.

The interest in researching how to construct this scaffolds arises from the fact that these are intermediate in the synthesis of LY544344, as reported in [49] and shown in Scheme 3.3 on the next page. The dicarboxylate compound **123** can in fact be decarboxylated by basic hydrolysis followed by acidic treatment to yield compound **131**. This latter can be further manipulated to finally yield the target compound.



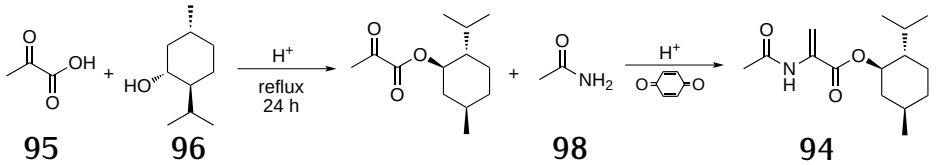
SCHEME 3.3: Manipulation of the dicarboxylic compound.

4

Experiments

The TLC layers are Macherey-Nagel Poligram SIL G/UV_{254} 0.20 mm; a 1% solution of $KMnO_4$, 2,4-dinitrophenylhydrazine in acidic solution were used to visualise spots.
Chromatographic purifications were run over silica gel Macherey-Nagel 60M 230-400 mesh.
^{1}H-NMR and ^{13}C-NMR were registered with Varian spectrometers at 300 MHz and 400 MHz at room temperature. Chemical shifts (δ) are reported with respect to trimethylsilane in the following manner: chemical shift (multiplicity, coupling constants, integer value). Signal multiplicity are shortened in the following manner: *s* for singlet; *d* for doublet; *t* for triplet; *q* for quartet; *br* for broad signal; *m* for multiplet; *dd* for double doublet.
Mass spectra were recorded on Mass spectra were recorded on Waters Micromass ZMD 2000, ESI-Q-TOF 6520 Agilent Technologies, Agilent 6520 Q-TOF LC/MS System and LCQ Duo Finningan.

4.1 One pot preparation of *(1S,2R,5S)*-2-isopropyl-5-methylcyclohexyl 2-acetamido-2-propenoate

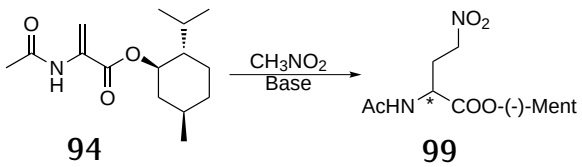


95 **96** **98** **94**

In a round-bottomed-flask, 1,5 g (17 mmol) of pyruvic acid **95** were dissolved in 30 ml of benzene under vigorous stirring. Next, 0.8 eq. of (−)-Menthol **96** and 0.1 eq of p-toluensulfonic acid mono hydrate as catalyst were added.
The reaction was heated at reflux at about 106°C for at least 24 hours with a Dean-Stark apparatus. The reaction was followed via TLC EtOAc:Cy 1/4. After depletion of menthol, the reaction is considered finished and treated as following.

In the same reaction vessel from the previous reaction, 2 eq (with respect to menthol) of acetamide **98** were added, together with 0.05 eq (with respect to menthol) of dihydroquinone. The reaction is stirred at reflux at around 105°C for about 24 hours. The reaction was monitored via TLC EtOAc:Cy 1/4. The final product has an Rf=0.4.
After the reaction is complete, it is washed with a saturated solution of $NaHCO_3$ 3x100 ml, and the two layers are separated. The aqueous layer is extracted with 3x100 ml EtOAc (it is possible that the organic layers combined form a slightly blurred solution). The combined organic layers are dried over anhydrous Na_2SO_4 and concentrated under vacuum, obtaining an oily and yellow compound which is then purified via flash chromatography EtOAc/Cy 1:9 then 2:8.
Compound **94** is finally isolated as a yellow and dense oil. The overall yield for the whole process is circa 50%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.76 (s, 1H), 6.56 (s, 1H), 5.85 (s, 1H), 4,79 (td, J = 10.9, 4.4, 1H), 2.12 (s, 3H), 2.02-1.97 (m, 1H), 1.83 (td, J = 7.0, 2.68, 1H), 1.73-1.42 (m, 5H), 1.13-1.02 (m, 2H), 0.93-0.85 (t, J =7.0, 8H), 0.77 (d, J = 7.0, 3H).
^{13}C-NMR (100 MHz, $CDCl_3$): δ 168.8, 163.3, 131.2, 108, 47.04, 40.57, 34.09, 31.39, 26.45, 23.55, 21.95, 20.61, 16.45.

4.2 Synthesis of (−)-Menthyl 2-acetamido-4-nitrobutanoate

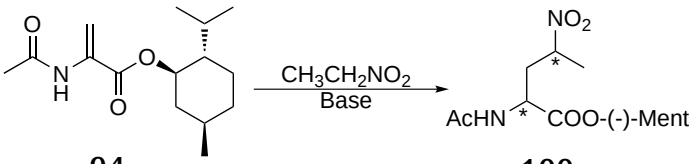


94 **99**

Typical reaction conditions are as following.
The base or the base mixture is added to a round-bottomed-flask filled with 5 ml

of nitromethane; then the acrylate **94** is included and the reaction is stirred at 25°C. The reaction is followed by TLC EtOAc/Cy 1:1; upon formation of the product, a white spot is seen after revelation of the TLC with $KMnO_4$.
The starting material **94** does not disappear entirely during the reaction; mixing the nitromethane with other solvents such as THF for improving the solubilisation of the solid base does not result in change of the reaction rate, which is actually quite slow.
When the reaction is finished, the organic layer is washed with 5 ml HCl 1N, and the two phases are separated. The organic layer is dried over anhydrous sodium sulphate and concentrated at reduced pressure. The product **99** is then purified via flash chromatography EtOAc/Cy 7:3, then 1:1. In table Table 2.1.4 on page 39 all the screened conditions and yields are reported.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.27 (br s, 1H), 4.78-4.70 (m, 1H), 4.65 (ddd, J = 12.5, 8.0, 4.67, 1H), 4.51-4.44 (m, 1H), 4.44-4.37 (m, 1H), 2.67-2.59 (m, 1H), 2.31 (tq, J = 6.95, 6.55, 1H), 2.01 (s, 3H), 2.00-1.92 (m, 1H), 1.79 (ddd, J =10.4, 6.97, 3.45, 1H), 1.72-1.67 (m, 3H), 1.51-1.37 (m, 2H), 1.09-0.96 (m, 2H), 0.92-0.84 (m, 6H), 0,73 (d, 3H).

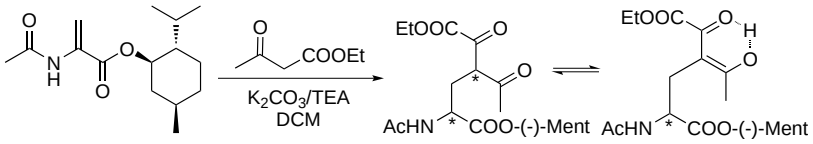
4.3 Synthesis of (−)-Menthyl 2-acetamido-4-nitropentanoate



94 **100**

Typical reaction conditions are as following. The base or the base mixture is added to a round-bottomed-flask filled with 5 ml of nitromethane; then the acrylate is included and the reaction is stirred at 25°C. The reaction is followed by TLC EtOAc/Cy 1:1; upon formation of the products, two white spots can be seen after revelation of the TLC with $KMnO_4$. The starting material does not disappear entirely during the reaction; mixing the nitromethane with other solvents such as THF for improving the solubilisation of the solid base does not result in change of the reaction rate, which is actually quite slow.
When the reaction is finished, the organic layer is washed with 5 ml HCl 1N, and the two phases are separated. The organic layer is dried over anhydrous sodium sulphate and concentrated at reduced pressure. The product **100** is then purified via flash chromatography EtOAc/Cy 7:3, then 1:1. In table Table 2.1.4 on page 39 all the screened conditions and yields are reported.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.38 (br dd, 1H), 4.72 (m, 1H), 4.61 (m, 1H), 4.58 (m, 1H), 4.24 (m, 1H), 2.39 (m. 1H), 1.99 (m, 1H), 1.82 (s, 4H), 1.68-1.58 (m, 2H), 1.52 (d, J=7 3H), 1.50-1.32 (m, 2H), 1.12-0.98 (m, 2H), 0.90 (m, 7H), 0.78-0.75 (d, J = 7, 3H).

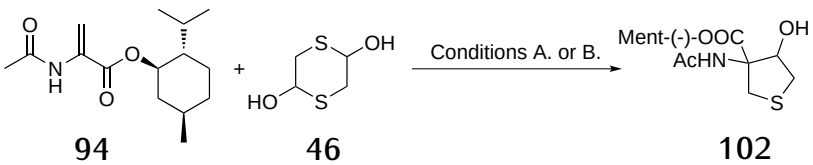
4.4 Synthesis of 1-ethyl 6-(−)-Menthyl 5-acetamido-3-acetyl-2-oxohexanedioate



94 **101**

0,9 mmol of acrylate **94** are reacted with one equivalent of ethyl acetoacetate in DCM in presence of two equivalents of K_2CO_3 and 0,1 equivalents of TEA in a round-bottomed flask for several days.
The reaction is followed via TLC EtOAc/Cy 1:1. The product's spot – Rf=0,27 – is white upon revelation with $KMnO_4$, then it becomes yellow.
The reaction is washed with HCl 2N until acid pH; emulsion is formed, and the solution is filtered over a celite pad. The two phases are then separated and th organic layer is dried over anhydrous Na_2SO_4 and concentrated under reduced pressure, obtaining 0,290 g of crude product. The crude product is purified via flash chromatography EtOAc/Cy 1:2, then 1:1. The two starting materials, the acrylate and the ethyl acetoacetate which did not react are recovered; at last, only 40 mg of purified product **101** are recovered, with an overall yield of 13%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.28 (br s, 1H), 4.55 (m, 1H), 4.11 (br q, 3H), 3.68 (m, 1H), 2.23 (s, 1H), 2.19 (s, 1H), 2.09 (s, 6H), 1.86-1.79 (m, 3H), 1.68-1.49 (m, 2H), 1.48-1.2.29 (m, 2H), 1.25-1.16 (m, 3H), 0.91-0.81 (m, 10H), 0.69 (d, J = 7, 3H).

4.5 Synthesis of (−)-Menthyl 3-acetamido-4-hydroxythiophene-3-carboxylate



94 **46** **102**

Condition A.

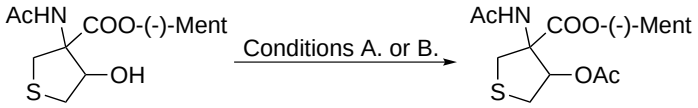
Acrylate **94** [1,38 g; 5,16mmol] is dissolved in DCM. To this solution, 393,1 mg of 1,4-dithiane-2,5-diol **46** [0,5 eq; 2,58 mmol], 1,4 g of K_2CO_3 [2 eq; 10,32 mmol] and 1 eq TEA are added. After a few minutes, the yellow solutions turns red. The reaction is left under stirring for four days at r.t. TLC AcOEt/Cy 1:1 monitor shows that a big part of acrylate did not react. The reaction is so worked-up to recover the starting materials by washing the organic phase with HCL 1N and then $NaHCO_3$ sat.

sol. The crude product is dried over sodium sulphate anhydrous and concentrated under vacuum. A red solid is finally obtain.

Condition B.

Acrylate **94** [926,4 mg; 3,47mmol] is dissolved in DMF. Then, 263,9 mg of 1,4-dithiane-2,5-diol **46** [0,5 eq; 1,73 mmol], 959mg of K_2CO_3 [2 eq; 6,94 mmol] and 1,119g of tetrabutylammonium bromide (TBAB) [1 eq; 3,47mmol] are added, and the solution is stirred at room temperature for 24 hours. After a few minutes, the yellow solutions turns red. The reaction is monitored via TLC AcOEt/Cy 1:1.
After reaction is complete, *i.e.* starting material **94** has been consumed, the solution is filtered to remove the undissolved K_2CO_3 and TBAB. Diethyl ether is added and the organic phase is washed with distilled water and then with HCl 1N and a saturated solution of $NaHCO_3$. The organic solution is finally dried over anhydrous sodium sulphate. The crude reaction product is concentrated *in vacuo* to give a brown solid which is run over a chromatographic column eluted with EtOAc/Cy 1:1 to afford 600 mg of **103**, with a final yield of 62%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6,4 (s, 1H); 4,75 (m, 1H); 4,50 (dt; 1H), 3,6 (dd, 1H); 3,1 (dd, 1H), 3,0 (d, 1H); 2,85 (m, 1H), 2,05 (s, 3H), da 1,95 a 0,95 (m, 9H), 0,9 (m, 6H), 0,75 (d, 3H).
M^+= 343,9 m/z

4.6 Synthesis of (−)-Menthyl 3-acetamido-4-acetoxythiophene-3-carboxylate



102 **103**

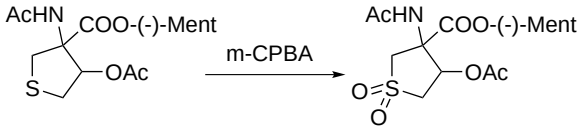
Condition A.

217 mg of compound **103** [0.63 mmol] are dissolved in circa 5 ml of DCM. At 0°C, 179 μl of acetyl chloride [4 eq; 2,52mmol] and 430 μl of DIPEA [4 eq; 2,52mmol] are slowly added through a syringe. The reaction is then stirred at r.t. for 24 h and monitored via TLC EtOAc/Cy 1:1.
Once the reaction is over, DCM is evaporated under reduced pressure and the crude is redissolved in EtOAc. The organic phase is washed with HCl 1N and a saturated solution of $NaHCO_3$, dried over anhydrous sodium sulphate and concentrated *in vacuo*. The crude product is purified over chromatographic column eluted with EtOAc/Cy 1,5:1 to afford 37 mg of products, and a final yield of 15%.

Condition B.

376 mg of compound **103** [1,098 mmol] are dissolved in circa 4 ml of pyridine. At 0°C circa 3 ml of acetic anhydride are added very carefully and very slowly. The reaction is stirred for 24 h at room temperature and monitored via TLC EtOAc/Cy 2:1. AcOEt is then added, and the organic phase is washed with HCl 1N and a saturated solution of $NaHCO_3$, dried over anhydrous sodium sulphate and concentrated *in vacuo*.
The crude reaction product **104** is then used in the following step without further purification.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6 (d, 1H); 5,6 (m, 1H); 4,75 (m, 1H); 3,6 (dd, 1H); 3,45 (dd, 1H); 3,25 (dd, 1H); 2,9 (m, 1H); 2,1 (s, 3H); 2,05 (s, 3H); 1,99 (m, 1H); 1,90-1,2 (m, 6H); 0,95 (m, 9H).
M^+: 385,8 m/z

4.7 Synthesis of (−)-Menthyl -3-acetamido-4-acetoxysulpholan-3-carboxylate

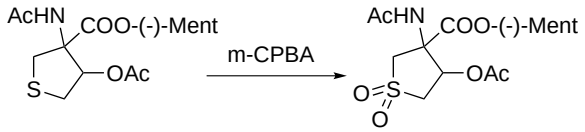


103 **104**

384 mg of compound **104** [0.998 mmol] are dissolved in circa 10 ml of DCM. 559 mg of *m*-chloro-perbenzoic acid 70% are added. The slightly yellowish solution becomes blurry with the reaction proceeding. The reaction is stirred for 24 h at room temperature and monitored via TLC EtOAc/Cy 1:1. The organic phase is then washed with a saturated solution of $NaHCO_3$ and brine. A white emulsion is formed and removed with filtration over celite. The organic layer is then dried over anhydrous sodium sulphate and concentrated under reduced pressure.
258 mg of crude product **105** are obtained. There was no need to further purify the crude.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6,4 (d, 1H); 5,7 (m, 1H); 4,8 (m, 1H); 4 (m, 1H); 3,7 (m, 1H); 2,1- 1,8 (m, 7H); 1,2 (m, 9H).
M^+ = 417,8 m/z

4.8 Synthesis of (−)-Menthyl -3-acetamido-2,3-disulpholan-3-carboxylate

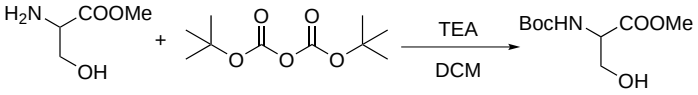
220 mg of compound **105** [0,53 mmol] are dissolved in circa 10 ml of toluene, upon which 158 μl of DBU [2 eq; 1,06 mmol] are added. The solution reaction is then stirred at r.t. for 24 h and monitored via TLC AcOEt/Cy 2:1.



TMP4 **104**

Once the reaction is deemed completed, toluene is evaporated under reduced pressure, and the crude product is redissolved in EtOAc. The organic phase is washed with HCl 1N and then with a saturated solution of $NaHCO_3$, dried over anhydrous sodium sulphate and concentrated *in vacuo*.
After purification over chromatographic column eluted with EtOAc/Cy 1:1, 58 mg of compound **106** are obtained with a 31% yield.
^{1}H-NMR (300 MHz, CDl_3): δ 6,85 (d, 1H); 6,67 (d, 1H); 6,55 (d, 1H); 4,75 (m, 1H); 3,95 (dd, 1H); 3,5 (dd, 1H); 2,05 (s, 3H); 2,0- 1,2 (m, 7H); 0,95 (m, 9H).

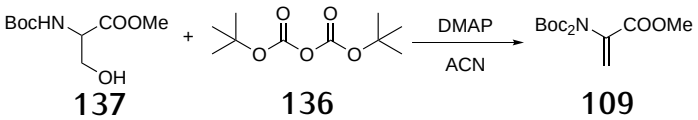
4.9 Synthesis of *N*-tert-butoxycarbonyl serine methyl ester



135 **136** **137**

2 g [12,9 mmol] of serine methyl ester chlorohydrate **132** are treated with 4,224 g of Boc_2O **133** [1,5 eq; 19,35 mmol] and 1,788 ml of trietilammina [1 eq; 12,9 mmol] in DCM for 24 h at room temperature.
The reaction is monitored via TLC EtOAc/Cy 2:1. Upon completion, DCM is removed under vacuum and the crude is redissolved in EtOAc, washed with HCl 2N and then with a saturated solution of $NaHCO_3$. The water phase are extracted with EtOAc 1x5 ml. The organic phases are combined, dried over anhydrous sodium sulphate and concentrated *in vacuo*.
The product is used as it is in following step, without further purification.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 5,5 (s; 1H); 3,97 (dd, 1H); 3,86 (dd, 1H); 3,78 (s, 3H); 1.58 (s; 9H); 1,43 (s, 9H).

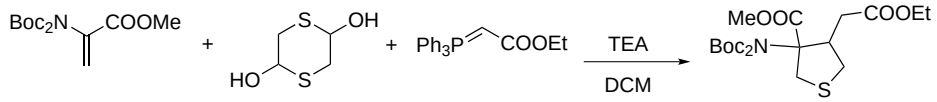
4.10 Synthesis of methyl 2-(bis(*tert*-butoxycarbonyl)amino)acrylate



137 **136** **109**

To a solution of compound **134** [2,67 g; 12.19mmol] in dry ACN, 147,8 mg of DMAP [0,1 eq; 1,21 mmol] and 7g of Boc_2O **133** [2,5 eq; 30,5 mmol] are added. The reaction is then stirred at r. t. for 24 h and monitored via TLC Et_2O/Cy 1:1. At the end the reaction solution turns yellowish. The reaction solvent is evaporate under reduced pressure and the crude is redissolved in Et_2O.
The organic phase is washed with 100 ml of $KHSO_4$ 1M, then with a saturated solution of $NaHCO_3$ and 3 x 50 ml of brine. The solution is finally dried over anhydrous sodium sulphate and concentrated *in vacuo*.
The product **109** is used as it is, without further purification.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 6,3 (s, 1H); 5,6 (s, 1H); 3,8 (s, 3H); 1,23 (s, 18H).

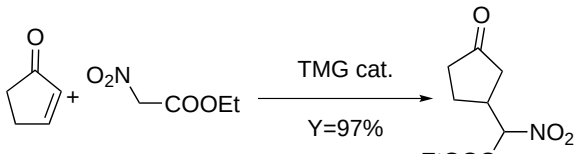
4.11 Synthesis of methyl 3-((di-Boc)amino)-4-(2-ethoxy-2-oxoethyl)tetrahydrothiophene-3-carboxylate



109 **46** **110** **112**

In a round-bottomed flask 200 mg [0,66 mmol] of compound **109** are treated with 51 mg [0,5 eq; 0,33 mmol] of compound **46** and 290 mg [1eq; 0,66 mmol] of **110** in DCM with a few drops of TEA, these last added when the reaction solution turns clear. The eraction id stirred at r.t. for 24 h and monitored via TLC EtOAc/Cy 1:3.
Once the reaction is over, the organic phase is washed with HCl 1N and then with a saturated solution of $NaHCO_3$, dried over anhydrous sodium sulphate and concentrated under reduced pressure.
The crude compound is purified by chromatographic column eluted with EtOAc/Cy 1:5, then 1:3, to afford 98 mg of product **112** with a 32% yield.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 4,2 (q, 2H); 3,4 (d, 1H); 3,3 (d; 1H); 3,1 (dd, 1H); 2,9 (dd, 1H); 2,7-2,2 (m, 3H); 2,2 (s, 3H); 1,4 (s, 18H); 1,1 (t; 3H).
$[M - 202] = 247m/z$, where 202 is the weight of the two *tert*-butoxycarbonyl moieties lost in the mass analysis.

4.12 Synthesis of ethyl 2-nitro-2-(3-oxocyclopentyl)acetate

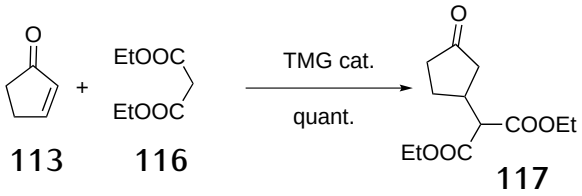


113 **114** **115**

0.2 g [0,244 mmol] of compound **113** and 1 eq [0,27 ml] of ethyl nitroacetate **114** were reacted together in the same reaction vessel without any solvent in the pres-

ence of 10 μl of TMG. The reaction was stirred for five hours and followed via TLC EtOAc/Cy 1:1.
Upon completion of the reaction, diethyl ether is added; the organic phase is washed with a few drops of HCl 2N and then with a few drops of a saturated solution of $NaHCO_3$. the aqueous phase was extracted with 4 x 5ml Et_2O and then 3 x 5 ml EtOAc. The crude is dried over anhydrous sodium sulphate and concentrated under reduced pressure, affording 0,510 g of compound **120** which is not further purified. The yield is 97%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.07 (1 H, dd, J 9.3 and 4.0), 4.25-4.34 (2 H, m), 3.09-3.19 (1 H, m), 2.50-2.60 (1 H, m), 2.04-2.45 (5 H, m), 1.69-1.89 (2 H, m), 1.26-1.35 (3 H, m).
^{13}C-NMR (100 MHz, CDl_3): δ 90.60 (CH), 62.71 (CH_2), 41.47 (CH_2), 37.49 (CH_2), 36.82 (CH), 26.20 (CH_2), 13.58 (CH_3).

4.13 Synthesis of diethyl 2-(3-oxocyclopentyl)malonate

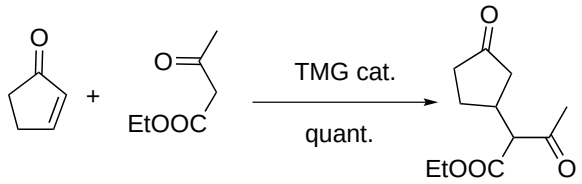


113 116 117

0.2 g [0,244 mmol] of compound **113** and 1 eq [0,37 ml] of ethyl nitroacetate **115** were reacted together in the same reaction vessel without any solvent in the presence of 10 μl of TMG. The reaction was stirred for five hours and followed via TLC EtOAc/Cy 1:1.
Upon completion of the reaction, diethyl ether is added; the organic phase is washed with a few drops of HCl 2N and then with a few drops of a saturated solution of $NaHCO_3$. the aqueous phase was extracted with 4 x 5ml Et_2O and then 3 x 5 ml EtOAc. The crude is dried over anhydrous sodium sulphate and concentrated under reduced pressure, affording 0,598 g of compound **121** which is not further purified. The yield is quantitative.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.24-4.16 (m, 4H), 3.32 (d, J = 9.5 Hz, 1H), 2.83 (s, 1H), 2.50 (dd, J = 18.4, 7.5 Hz, 1H), 2.33-2.19 (m, 3H), 2.01 (ddd, J = 18.4, 11.1, 1.4 Hz, 1H), 1.68-1.63 (m, 1H), 1.29-1.24 (m, 6H).

4.14 Synthesis of ethyl 3-oxo-2-(3-oxocyclopentyl)butanoate

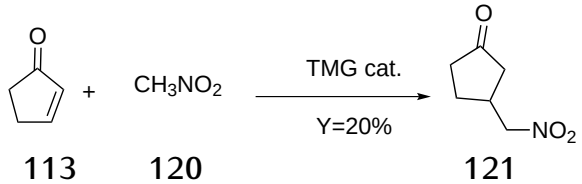
0.2 g [0,244 mmol] of compound **113** and 1 eq [0,37 ml] of ethyl acetoacetate **116** were reacted together in the same reaction vessel without any solvent in the presence of 10 μl of TMG. The reaction was stirred for three hours and followed via TLC EtOAc/Cy 1:2.
Upon completion of the reaction, diethyl ether is added; the organic phase is washed



113 **118** **119**

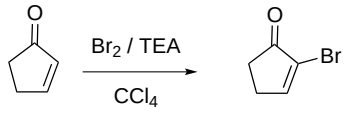
with a few drops of HCl 2N and then with a few drops of a saturated solution of $NaHCO_3$. the aqueous phase was extracted with 3 x 5ml Et_2O and then 3 x 5 ml EtOAc. The crude is dried over anhydrous sodium sulphate and concentrated under reduced pressure, affording 0,544 g of crude that is purified over chromatographic column eluted with EtOAc/Cy 1:2 to afford 0.433 g of compound **117** as a mixture of diastereomers. The yield is 84%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.25-4.17 (m, 2H), 3.42 (dd, J = 9.8, 6.4 Hz, 1H), 2.91-2.82 (m, 1H), 2.50-2.43 (m, 1H), 2.35-2.13 (m, 6H), 1.95 (ddd, J = 18.4, 11.1, 1.4 Hz, 1H), 1.81 (ddd, J = 18.3, 11.0, 1.4 Hz, 1H), 1.65-1.47 (m, 1H), 1.31-1.24 (m, 3H).
^{13}C-NMR (100 MHz, $CDCl_3$): δ 217.077, 217.019, 201.563, 168.295, 64.823, 64.606, 61.697, 42.955, 42.700, 38.163, 38.016, 35.836, 35.744, 29.458, 29.220, 27.615, 27.331, 14.105, 14.069.

4.15 Synthesis of 3-(nitromethyl)cyclopentanone



113 **120** **121**

0.2 g [0,244 mmol] of compound **113** and 1 eq [132 μl] of nitromethane **119** were reacted together in the same reaction vessel without any solvent in the presence of 10 μl of TMG. The reaction was stirred o.n. hours and followed via TLC EtOAc/Cy 1:2.
Upon completion of the reaction, which is turned orange, 2 ml EtOAc is added; the organic phase is washed with a few drops of HCl 2N and then with a few drops of a saturated solution of $NaHCO_3$. The aqueous phase was extracted with 2 x 5 ml EtOAc. The crude is dried over anhydrous sodium sulphate and concentrated under reduced pressure, affording 0,384 g of crude yellow oil that is purified over chromatographic column eluted with EtOAc/Cy 1:1 to afford 0.072 g of compound **118**. The yield is 20%.
^{1}H-NMR (400 MHz, $CDCL_3$): δ missing!!!

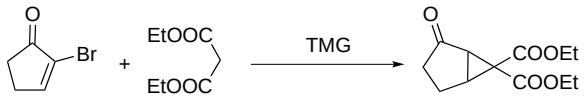


113 127

4.16 Synthesis of 2-bromocyclopent-2-enone

In a round-bottomed flask 1 g of cyclopenten-1-one **113** [12.2 mmol] is dissolved in 100 ml CCl_4. After cooling at 0°C the reaction vessel, molecular bromine [1.1 eq, 13.4 mmol, 0,70 ml] in 10 ml of solvent is added dropwise in an hour.
After this, and under much vigorous stirring, TEA [1.5 eq, 2,34 ml] is added dropwise in an hour at 0°C. Upon addition, the clear yellowish solution becomes blurry and then brown. The reaction is left stirring at room temperature for another 2 hours.
The slurry is then filtered over celite, washed twice with 25 ml HCl 2N, once with 25 ml saturated solution of sodium carbonate and finally with 25 ml of brine. The crude is dried over anhydrous sodium sulphate and concentrated under reduced pressure, affording 1,727 g of oily compound **127** which solidifies upon standing at r.t. The final yield is 86%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.78 (t, J = 3.0 Hz, 1H), 2.72-2.68 (m, 2H), 2.55-2.52 (m, 2H).

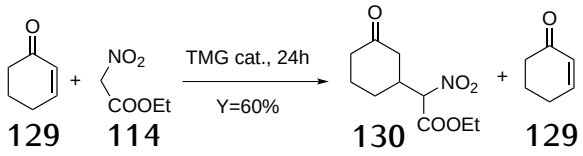
4.17 Synthesis of diethyl 2-oxobicyclo[3.1.0]hexane-6,6-dicarboxylate



127 116 123

In a round-bottomed flask 0.2 g of 2-bromocyclopent-2-enone are reacted with 1 eq [0.186 ml] of diethyl malonate **115** with 1 eq [0.15 ml] of TMG in 2 ml of chloroform. The reaction is left stirring at 30°C and monitored via TLC EtOAc/Cy 1:2.
Upon completion after a few hours, the reaction is washed with HCl 2N and a saturated solution of $NaHCO_3$, dried over anhydrous sodium sulphate and concentrated under reduced pressure, affording 0,232 g of crude compound which is purified via chromatographic column eluted with EtOAc/Cy 1:4 to afford 0,206 g of **123** as a yellow oil. The final Yield is 78%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.23-4.17 (m, 4H), 2.74 (td, J = 5.7, 0.6 Hz, 1H), 2.52 (d, J = 5.8 Hz, 1H), 2.36-2.17 (m, 3H), 1.87-1.78 (m, 1H), 1.30-1.24 (m, 9H).
^{13}C-NMR (100 MHz, $CDCl_3$): δ 209.77, 166.595, 165.603, 62.469, 61.479, 61.464, 40.744, 39.738, 35.014, 33.970, 20.655, 14.017, 13.812.

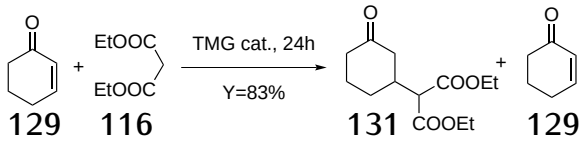
4.18 Synthesis of ethyl 2-nitro-2-(3-oxocyclohexyl)acetate



129 114 130 129

0.23 g [0,244 mmol] of compound **129** and 1 eq [0.27 ml] of ethyl nitroacetate **135** were reacted together in the same reaction vessel without any solvent in the presence of 10 μl of TMG. The reaction was stirred o.n. hours and followed via TLC EtOAc/Cy 1:2.
Upon completion of the reaction, which is turned orange, 2 ml EtOAc are added; the organic phase is washed with a few drops of HCl 2N and then with a few drops of a saturated solution of $NaHCO_3$. The aqueous phase was extracted with 2 x 5 ml EtOAc. The crude is dried over anhydrous sodium sulphate and concentrated under reduced pressure, affording 0,66 g of crude yellow oil that is purified over chromatographic column eluted with EtOAc/Cy 1:1 to afford 0.561 g of compound **136** as a diastereomeric mixture. The yield is 57%.
^{1}H-NMR (400 MHz, $CDCL_3$): δ5.03 (dd, J = 18.3, 7.3 Hz, 1H), 4.36-4.26 (m, 2H), 2.86-2.72 (m, 1H), 2.50-2.40 (m, 3H), 2.37-2.24 (m, 2H), 2.13 (dtt, J = 13.3, 6.6, 3.3 Hz, 1H), 2.06-1.93 (m, 2H), 1.78-1.65 (m, 2H), 1.65-1.50 (m, 1H), 1.34-1.28 (m, 3H).

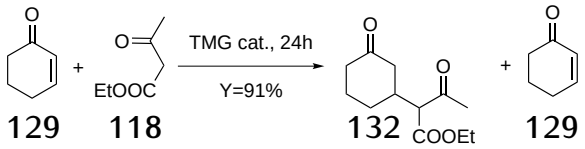
4.19 Synthesis of diethyl 2-(3-oxocyclohexyl)malonate



129 116 131 129

0.23 g [0,244 mmol] of compound **113** and 1 eq [0,37 ml] of diethyl malonate **115** were reacted together in the same reaction vessel without any solvent in the presence of 10 μl of TMG. The reaction was stirred o.n. hours and followed via TLC EtOAc/Cy 1:2.
Upon completion of the reaction, which becomes brown, 2 ml EtOAc is added; the organic phase is washed with a few drops of HCl 2N and then with a few drops of a saturated solution of $NaHCO_3$ and brine. The aqueous phase was extracted with 2 x 5 ml EtOAc. The crude is dried over anhydrous sodium sulphate and concentrated under reduced pressure, affording 0,65 g of crude yellow oil that is purified over chromatographic column eluted with EtOAc/Cy 1:3 to afford 0.522 g of compound **137**. The yield is 83%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.23-4.16 (m, 4H), 3.28 (d, J = 7.9 Hz, 1H), 2.57-2.48 (m, 1H), 2.46-2.32 (m, 3H), 2.30-2.20 (m, 2H), 2.10-2.00 (m, 2H), 1.95 (dddt, J = 13.0, 5.3, 3.6, 1.8 Hz, 1H), 1.73-1.62 (m, 1H), 1.55-1.45 (m, 1H), 1.29-1.24 (m, 6H).

4.20 Synthesis of ethyl 3-oxo-2-(3-oxocyclohexyl)butanoate



129 118 132 129

0.23 g [0,244 mmol] of compound **113** and 1 eq [0.31 μl] of ethyl acetoacetate **138** were reacted together in the same reaction vessel without any solvent in the presence of 10 μl of TMG. The reaction was stirred o.n. hours and followed via TLC EtOAc/Cy 1:2.
Upon completion of the reaction, which becomes brown, 2 ml EtOAc is added; the organic phase is washed with a few drops of HCl 2N and then with a few drops of a saturated solution of $NaHCO_3$ and finally brine. The aqueous phase was extracted with 2 x 5 ml EtOAc. The crude is dried over anhydrous sodium sulphate and concentrated under reduced pressure, affording 0,612 g of crude yellow oil that is purified over chromatographic column eluted with EtOAc/Cy 1:3 and then 1:1 to afford 0.504 g of compound **139**. The yield is 91%.
^{1}H-NMR (400 MHz, $CDCL_3$): δ 4.23-4.15 (m, 2H), 3.37 (dd, J = 8.7, 7.7 Hz, 1H), 2.62-2.54 (m, 1H), 2.42-2.32 (m, 2H), 2.30-2.19 (m, 4H), 2.14 (dt, J = 13.7, 1.6 Hz, 1H), 2.07-2.00 (m, 1H), 1.92-1.82 (m, 1H), 1.73-1.63 (m, 1H), 1.50-1.37 (m, 1H), 1.29-1.24 (m, 3H).

Part II

Second Project: Synthesis of L-arginine analogues as potential MRI contrast agents

5 Aim

The second project is focused on the synthesis of arginine analogues that could be potential substrate for eNOS enzyme and also could be easily polarised by either the PHIP or the SABRE techniques reported in the introduction.

The structural modifications devised are based on two different scaffolds: a. an arginine residue coupled with different picolyl bases and b. an α-methyl arginine or α-trifluoromethyl arginine structure incorporating a double or triple bond. This project has been developed in collaboration with the group of Professor Matteo Zanda at the University of Aberdeen, UK. This project was developed between the group of Professor Zanda, the group of Professor Michael Frenneaux and doctor Dana Dawson at the University of Ferrara and the group of Professor Simon B. Duckett at the University of York.

Part of the project was funded under the program "Bando Rivolto a Giovani Ricercatori Non Strutturati dell'Università degli Studi di Ferrara per il Finanziamento di Progetti di Ricerca e Mobilità Internazionale – Fondi 5x1000 Anno 2009".

The practical application of this project resides on the fact that peculiar analogues and peptides involved in the catalytic process of the synthesis of the Nitric Oxide could be detected through Magnetic Resonance Imaging if these compounds could be source of their NMR signal. In other words, there is interest in developing arginine analogues that could work as MRI contrasting agents which are target-specific because they would be located only into the NOS enzymes. This could result in the MRI technique being turned into a more sensitive and faster technique.

5.1 Arginine coupled with picolyl moieties

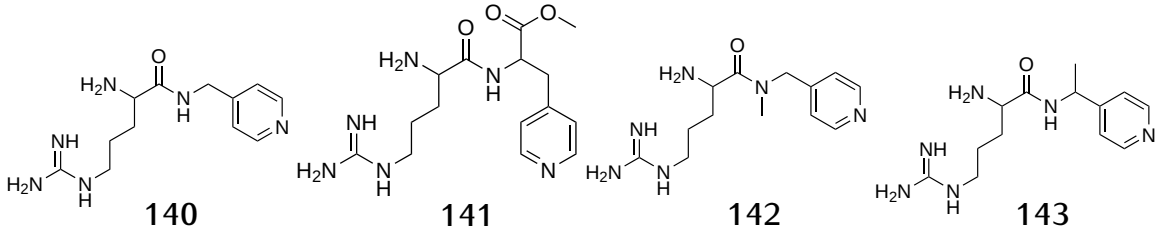
The aim of this work is to synthesise arginine analogues which can be hyperpolarised through the SABRE technique. A series of analogues will therefore incorporate a pyridyl/pycolyl moiety. A biological assay will be performed to test the affinity with the eNOS enzyme.

This type of compounds are in fact suitable substrates for the transfer of polarization by p-H_2 molecules through the SABRE technique.[61] A synthetic route for these analogues and small dipeptides coupled with an arginine residue has been

devised: the question for these compounds is whether they could be substrates for these enzymes and could be polarised with p-H_2 .

5.1.1 First Generation

Four analogues **140**, **141**, **142**, **143**, were synthesised in good yield exploiting the Boc-Cbz chemistry. They are shown in scheme Scheme 5.1; they had been submitted for biological test and their correspondent protected precursors had been polarised with good results by the group of professor Simon B. Duckett at the Univesrity of York, UK. As it is possible to notice, these little dipeptides contains a picolyl moiety,



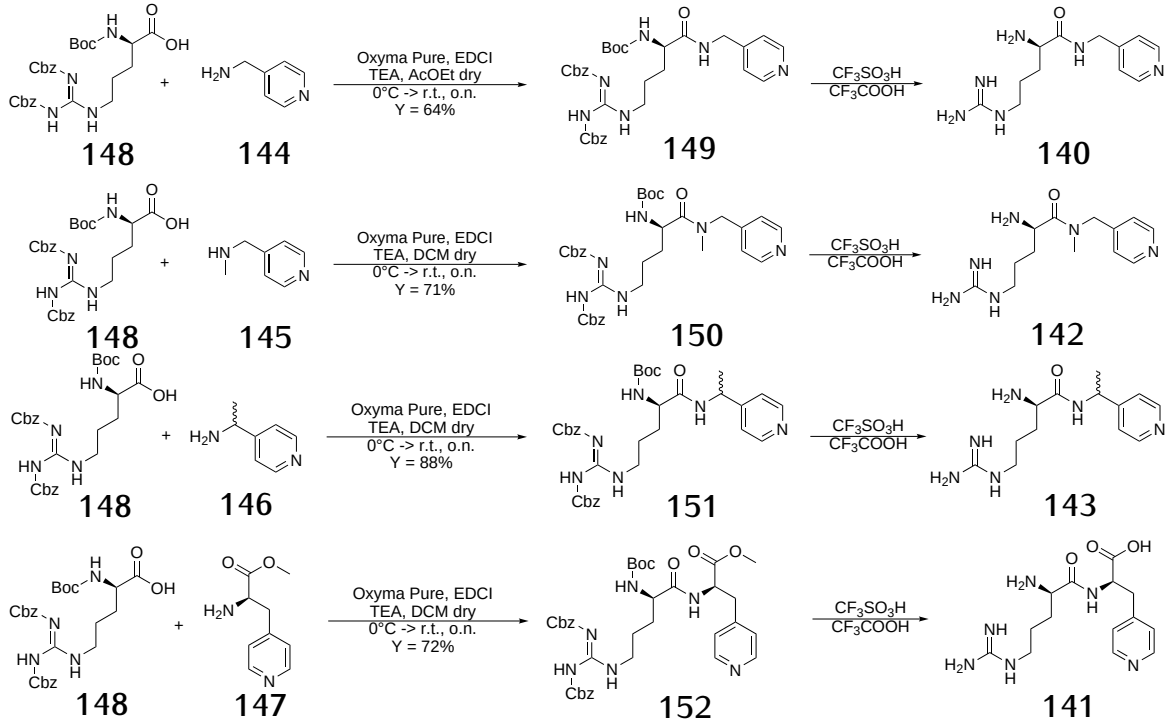
SCHEME 5.1: Analogues synthesised in 2011.

each one bearing a substituent place in a different position of the moiety, in order to change their : **144**, **145**, **146** and **147**.

These four analogues were synthesised starting from Boc-Arg(Cbz)-OH **148** using a standard peptide coupling method with EDC.HCl and Oxyma Pure in DCM with TEA.

The four deprotected amino acids **140**, **141**, **142**, **143**, have been obtained by treating the protected precursors with a solution of trifluoroacetic acid and trifluoromethanesulfonic acid.
The usual deprotection chemistry, involving treatment with TFA to release the Boc-protected amine and then subsequent hydrogenation with hydrogen on Pd/C in order to remove the Cbz group, lead to hydrogenation at the pyridine ring and was therefore discarded. It has also been found that the use of the scavengers thioanisole and m-cresol was not necessary for the triflic and trifluoroacetic deprotection protocol, but instead counterproductive as it was almost impossible to fully remove them from the resulting mixture..
Another deprotection method was tried with trimethylsilyl iodide in acetonitrile: a total of 7.2 equivalents of deblocking agent were needed in order to achieve complete deprotection of the peptide, and this method was therefore discarded.

After deprotection reaction, most unfortunately, it was not possible to the complete remove TFA and triflic acid from the reaction mixture; nonetheless, the resulting oily and difficult to handle residue was submitted for biological tests and polarisation studies.
It has been thought of using a cationic exchange resin to exchange the counterions with the more common chloride anion, but most unfortunately all the deprotected compounds were lost once put on the resin.



SCHEME 5.2: Synthetic route to the analogues.

Despite all, the biological tests gave good results, as it is shown in Table 5.1.1 on the following page, but the polarisation did not work on the submitted compounds.

5.1.2 Polarisation Tests

The samples that were tested using the SABRE technique were compounds **149**, **150**, **151**, **152**. The preparation of the NMR tube for the first compound **144** is reported as following: 19 mg of sample were dissolved in 0.6 ml CD_3OD with catalyst [Ir(IMes)(COD)Cl]. The Acquisition parameters were modified to single scan acquisition with receiver gain= 1.
Factors like shaking time, temperature and magnetic field at which the magnetic exchange would take place were changed in order to find the best condition to obtain the best enhancement for each compound. In the end, a shaking time of 8 seconds at Earth's magnetic field and 40°C were found to be the best conditions for the exchange to occur.
All the tables summarising the performed experiments and the proton NMRs of the enhanced molecules are reported in the appendix A on page 113. In the following Figure 5.1.1 on the following page is reported a superimposition of the aromatic portion of the ^{1}H-NMR spectra for the most enhanced experiments of all the tested compounds, where it is possible to make comparisons between the different enhanced molecules.

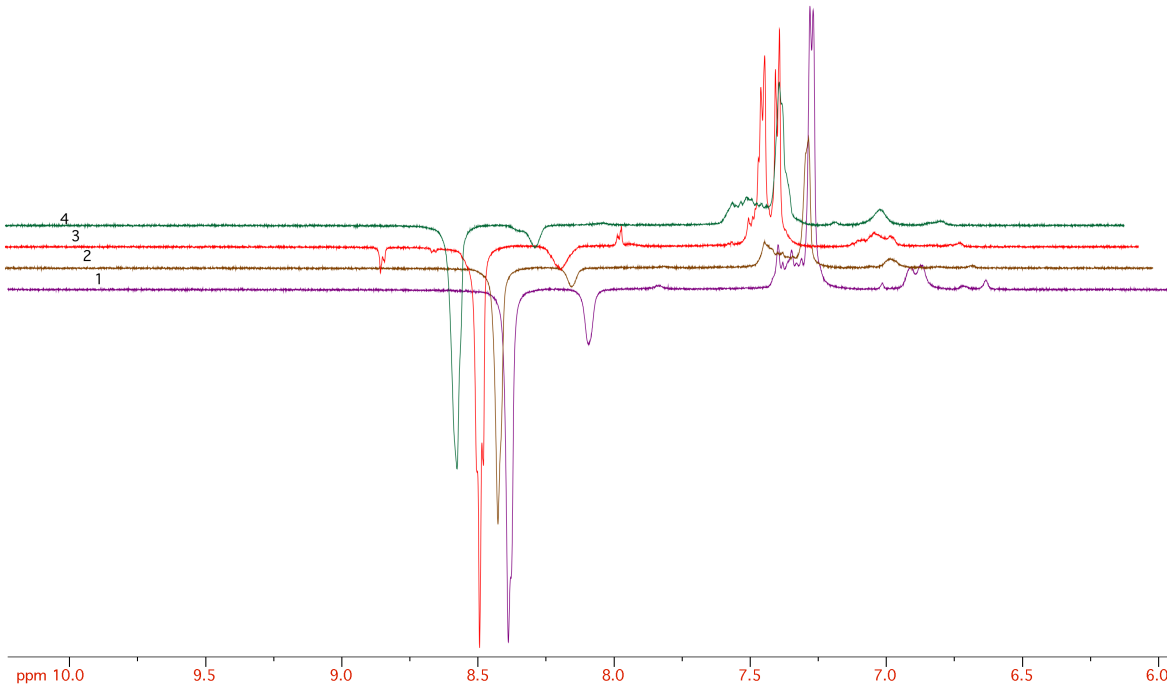


FIGURE 5.1.1: Expansion of the aromatic region of the 1 H-NMR for the compounds.

5.1.3 Biological Tests

The deprotected compounds **141**, **143**, **142** and **140** were finally tested with the eNOS enzyme according to a protocol based on a standard Nitrate-Nitrite colorimetric assay protocol; the full protocol is reported in the appendix B.1 on page 121.

In the following Table 5.1.1 the results of the tested compounds are reported in comparison with L-arginine, the substrate of the enzyme. As it is possible to notice, the synthesised compounds can be considered analogues to the enzyme substrate.

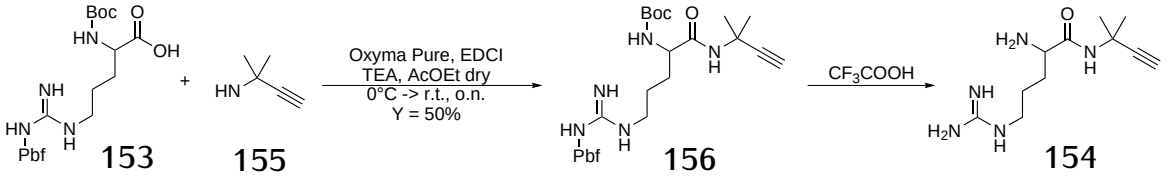
TABLE 5.1.1: Biological tests.

Sample	eNOS unit	Average O.D.	Final Nitrate Concentration (μM)
L-arginine	1	0.192	12.93
141	1	0.186	12.43
143	1	0.219	14.95
142	1	0.199	13.42
140	1	0.170	11.28

5.1.4 Second Generation

A different approach to the synthesis was planned, involving the use of a Boc- and Pbf-protected arginine **153** as a starting material, using the conventional coupling method with EDC and Oxyma Pure with TEA in DCM.

In this way, a first synthesis on a mg-scale was attempted to obtain compound **154**, as shown in Scheme 5.3.
Compound **154** is interesting in the fact that it is formed by an arginine, substrate for the eNOS enzyme, coupled with amine **155** bearing a triple bond, which can be easily hydrogenated with para-hydrogen and a suitable catalyst. The carbon adjacent to the triple bond is instead bonded to two methyl groups, making it a quaternary carbon: for this reason, its T_1 is higher and the the half-life of the enhanced, polarised 1 H-NMR signal should be extended.



SCHEME 5.3: Synthetic route to the analogue.

Upon synthesising compound **156** in moderate yield, it was successfully deprotected with TFA – proving the new strategy works – to give **154**. The compound was precipitated in diethyl ether and recovered as a white powder. The product was then freeze-dried and the excess TFA removed at the same time.

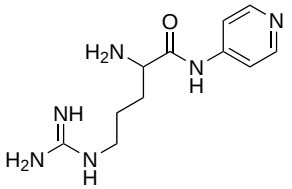
Studying more carefully the ^{1}H-NMR spectrum for compound **154**, it was found that other protons signals were present alongside the expected signals for the product. Moreover, the MS spectra for the aforementioned deprotected compound didn't show any remaining peak for the starting material **156**.
It was therefore thought that these signals could be coming from a remaining byproduct encaged in some manner in the mixture deriving from the deprotection with TFA of **156**. Most unfortunately, a complete removal of the remaining byproduct was not possible with the usual mean of washing and stripping the precipitate with Et_2O.

This byproduct was finally removed using C-18 cartridges.

Finally, a sixth analogue was thought to be of spectroscopic interest: compound **157**, shown in Scheme 5.4 on the following page, bearing the pyridyl moiety directly attached to the amide bond – coupling with **158**. There is hope that in this way the *para*-hydrogen induced polarisation could spread over the entire dipeptide skeleton, and eventually a polarised ^{13}C-NMR for the molecule could be recorded.

In the end, then, the five analogues were re-synthesised starting from Boc-Arg(Pbf)-OH **153** initially using the standard peptide coupling method with EDC.HCl and Oxyma Pure in DCM with TEA. Most unfortunately, this protocol was found not suitable for the new starting material, as moderate yields of protected peptides were recovered.

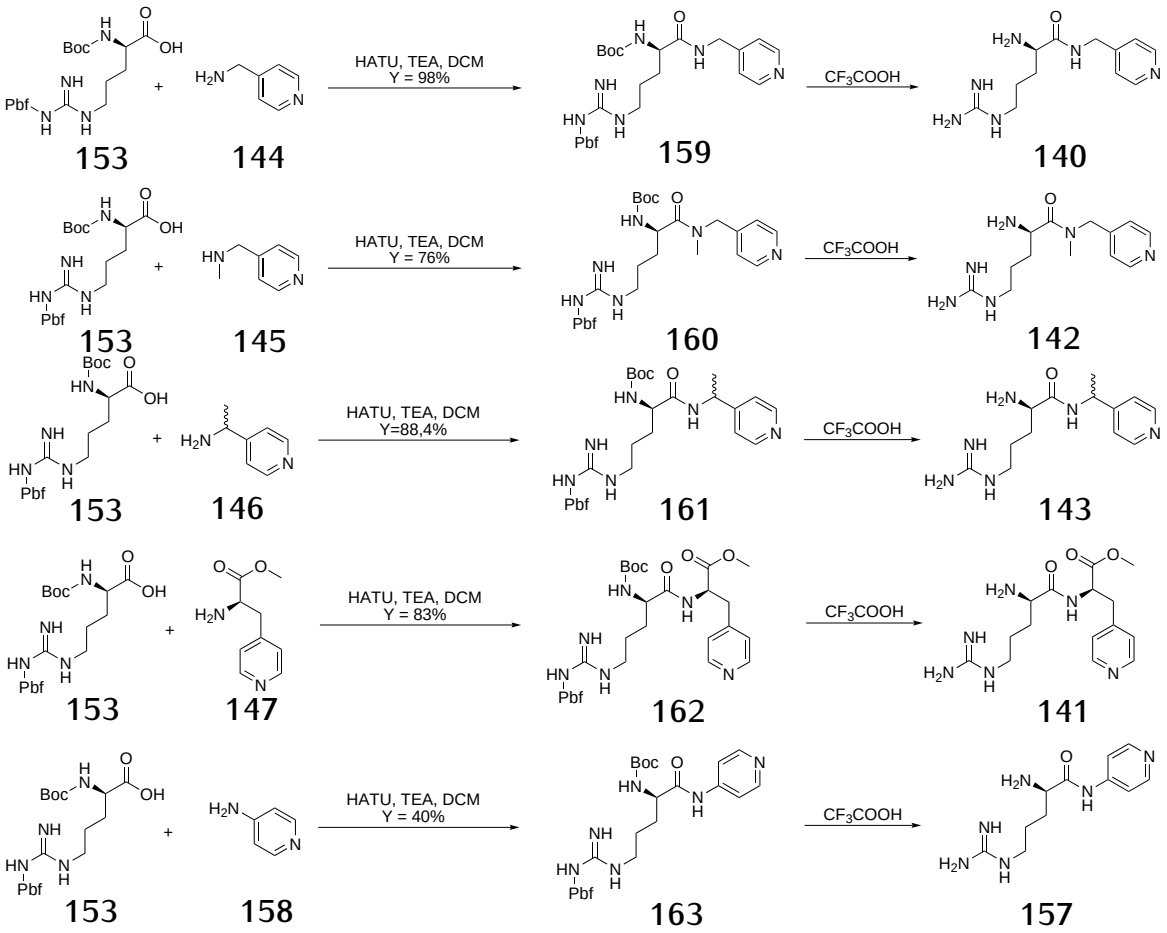
The synthetic procedure was therefore changed to the coupling method with



157

SCHEME 5.4: The sixth analogue.

HATU and TEA in DCM and is reported in the following Scheme 5.5.



SCHEME 5.5: Synthetic route to the analogues.

All the final compounds **140, 142, 143, 141** and **157** were analysed via HPLC-MS in order to assess their purity.

5.1.5 Determination of the Molecular Weight: accounting for the amount of TFA counterion

The synthesised molecules contain more than one possible protonation site: it is therefore our aim to determine the concentration of the TFA in the samples by

TABLE 5.1.2: Correlation Compounds-Yields.

Compound	Yield	Deprotected compound
159	97.7%	**140**
160	76.5%	**142**
161	88.4%	**143**
162	82,8%	**141**
163	40%	**157**

means of ERETIC-2 experiments in MeOH$-d_6$ or D_2O and Elemental Analysis. In this way, we would be able to finally assess the molecular weight of our compounds, and offer a more reliable estimation of the millimoles in the samples we are going to submit to biological tests and the NMR polarisation experiments.

It is known that the deprotected compounds are precipitated from diethyl ether as trifluoroacetic salts. This fact and their structure, altogether with the impossibility of eluting the free compounds on an ion-exchange resin has lead to think that these compounds are strongly basic.

The question which arises is then how many protonatoin sites there are on the synthesised molecules, and therefore how many counter-ions – the trifluoroacetic anions – there are. This simple and trivial question is nonetheless a very important one, because only accounting for the actual number of counter-ions is possible to state the exact molecular weight of the molecule, given the molecular weight of TFA of 114 g/mol.
The amount of TFA trapped as a counterion may be predicted based on the pKa(s) of the considered molecule; however, this is not always straightforward with complex structures containing multiple ionizable functional groups. Additionally, the method of compound isolation and purification plays a role in the final amount of counterion present.[90]

Two techniques were taken into account to determine the amount of counterions: the Elemental Analysis and the ^{19}F-NMR.
The first technique is widely used and renown and it allows the determination of the composition of any submitted compound. Also NMR is a primary method for Quantitation, for the NMR-signal (integral) is directly proportional to the number of atoms (nuclei) and molecules present in the sample. It is therefore an analytical technique with which it is possible to analyse mixtures of compounds directly. It is possible to achieve qNMR in at least two ways: by setting up an ERETIC-2 experiment or by interpolation on a calibration curve.
This last method consists of a calibration curve developed using least square linear regression, and the NMR integral area is obtained from serial dilutions of stock solution of the reference compound. Analyte test samples are then recorded using the same experimental parameters and the integral area is compared with the calibration curve to calculate the concentration. [91]
The ERETIC-2 type of experiment implemented in Bruker's TOPSpin is basically quantification via an external reference sample of the same nucleus. If the spectra

are acquired in a properly quantitative way, it is possible to really determine absolute concentrations by reference to an external standard, to quite high accuracy. This method is based on the PULCON sequence.

Most unfortunately, EA was conducted only on sample from compounds **157** since it was the only ones which, upon freeze-drying, exhibited a powdery structure. Moreover, the detector used in the analyser from the Department of Chemistry at the University of Ferrara is not able to detect and reveal fluorine atoms: therefore, the analyses were carried out anyway and the data collected only for C, N, H and S atoms.
In the following paragraph the determination of the amount of TFA and therefore the final weight for compound **157** is accounted for.

Determination of the weight of compound *N*-(pyridin-4-yl)arginine amide

In the following table Table 5.1.3 it is possible to view the data from the Elemental Analysis of compound **157**. The sample was analysed twice.

TABLE 5.1.3: Elemental Analysis results for the elected compound.

Sample name	Element %			
	Nitrogen	Carbon	Hydrogen	Sulfur
157-1	17,65	35,54	6,18	5,45
157-2	16,04	32,98	5,97	4,93

As it is possible to notice, elemental sulphur has been unexpectedly found in the sample. Moreover, it is not in small quantity, nor it was found in the blank: therefore, the detection of the element is not a mistake and it belongs in some manner to the analysed sample. This led to think that the deprotection process was incomplete, but, revising the data from the HPLC-MS analyses run on the final, purified reaction product, this possibility was discarded: only one peak for the deprotected compound can be observed.
A few simulations of possible structures of the final product were then envisaged and their elemental analysis calculated with ChemDraw, accounting for a remaining SO_2 from the protecting group. It came out that a deprotected **157** with two trifluoroacetic counterions and an SO_2 group attached in some manner to the structure has a calculated Elemental analysis accounting for the following quantities: Elemental Analysis: C, 33.28; H, 3.54; F, 21.05; N, 15.52; O, 20.69; S, 5.92. As it is possible to notice, the calculated formula is quite close to what was found experimentally. The final Chemical Formula of compound would then be $C_{15}H_{19}F_6N_6O_7S$, with a molecular weight of 541,40 g/mol.
It was then possible to "infuse" a 10 ppm sample in ACN aliquot of the sample into an ESI-MS system equipped with linear ion trap MS analyser. The base-peak was at 126.08 *m/z* – $[M + 2H]^{2+}$ – , and the molecular peak is at 251.12 *m/z*, ie. the $[M + H]^+$. The $[M + H]^+$ was then isolated and fragmented: the two major transitions were 251.12 m/z $\longrightarrow$ 157.00 m/z and 251.12 m/z $\longrightarrow$ 94.92 m/z. Then, *m/z*

540.00, the expected molecular weight for the compound under study, was identified as a tiny peak among the noise and fragmented, giving the following main *m/z*: 251.08, 256.17, 257.25, 283.25, 284.25, 311.25, 312.25 and 415.17. It is not possible to see it in great abundance because of the ion pairing effect from TFA. [92] The first *m/z* fragment was therefore isolated and fragmented once more, showing the same fragmentation pattern of 251.12.

This was the final proof we needed to state that the molecular weight of **157** is really 541.40 g/mol.

With this knowledge, 4.9 mg of the freeze-dried compound were dissolved in 1 ml D_2O and a ^{19}F-ERETIC-2-NMR experiment was run. The automatic calculation gave a concentration of TFA 24.1 mM. Assuming a MW = 541.40 g/mol, then 4.9 mg = 0,009 mmol. This quantity dissolved in 1 ml deuterated water accounts for a concentration 0,009 M, *i.e.* 9 mM. Doubling this, that is acknowledging the presence of two TFA counterions, we obtain 18 mM, which is roughly in the same order of quantitation.

5.2 α-Methyl- and α-trifluoromethyl arginine

The aim of this part of the second project is to synthesise arginine analogues which can be hyperpolarised through the PHIP technique, *i.e.* containing a double or triple bond to be hydrogenated in the presence of p-H_2 . A biological assay will be performed to test the affinity with the eNOS enzyme.

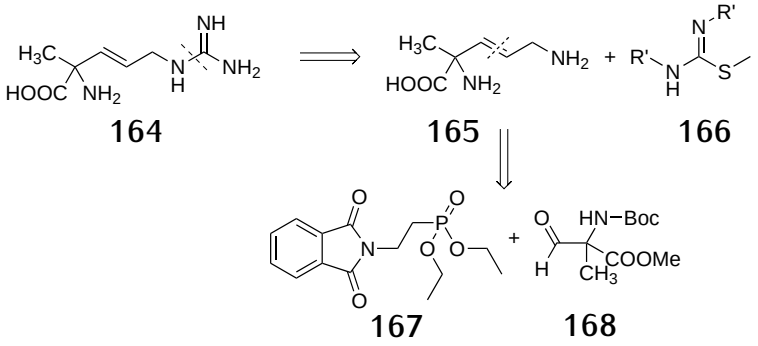
The presence of a methyl or a trifluoromethyl group on the α carbon of the amino acid is relevant because in this way the T_1 of the adjacent protons is raised. Moreover, the presence of a *alpha*-trifluoromethylic moiety on the α carbon would to only result in higher T_1 time for the adjacent hydrogens, but also the fluorine atom could be used as a spectroscopic probe in MRI: it is in fact known that ^{19}F is magnetically active and not abundant in our body, hence its incorporation in our studies.

In addition, analogues of the Arginine with a double bond are known and work effectively on the eNOS enzymes: not so much is known of α-methyl arginine derivatives. [19] It is therefore one aim of this second project to devise a synthesis for these analogues.

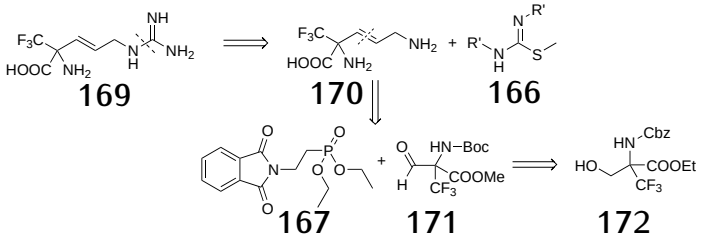
Two double-bonded α-substituted arginine analogues synthetic pathways were devised, and three triple-bonded α-substituted arginine analogues synthetic pathways were envisaged.

The retrosynthetic pathway for a double-bonded α-methyl arginine, or *(E)*-2-amino-5-guanidino-2-methylpent-3-enoic acid **164** can be seen in Scheme 5.6 on the next page. Guanidine moiety can be introduced on the double-bonded α-mehyl ornithine **165** via coupling reaction with protected isothiourea **166**. Adduct **165** is obtained by a Wittig-Horner-Emmons reaction between phosphonate **167** and aldehyde **168**. This latter is obtained by oxidation of opportunely protected α-methyl serine, easily obtainable from commercial sources.

The α-trifluoromethyl homologue **169** of the previously described compound **164** can be obtained in a similar way, as shown in Scheme 5.7 on the following page. Guanidine moiety can be in fact introduced on the double-bonded α-trifluoromehyl



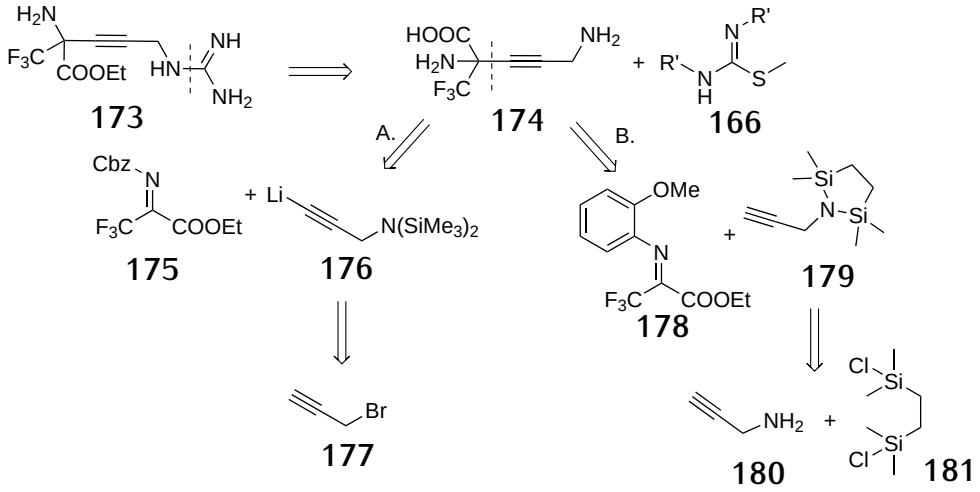
SCHEME 5.6: Synthetic route to the analogues.



SCHEME 5.7: Synthetic route to the analogues.

ornithine **170** via coupling reaction with protected isothiourea **166**. Adduct **170** is obtained by a Wittig-Horner-Emmons reaction between phosphonate **167** and aldehyde **171**. This latter is obtained by oxidation of opportunely protected α-methyl serine **172**, which can be synthesised as reported in [93].

Two different routes were devised for the triple-bonded α-trifluoromethyl arginine **173**: route A. is inspired from [94], while route B. has been inspired form a recent work by Zhang *et al*. [95]. Compound **174** can be in fact obtained by coupling



SCHEME 5.8: Synthetic route to the analogues.

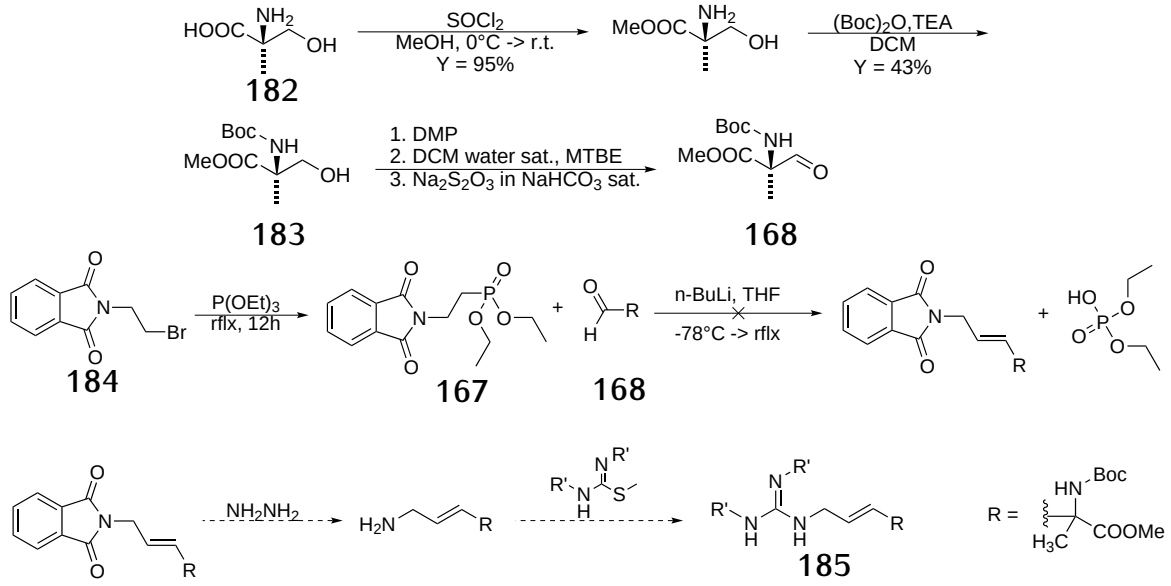
of imine **175** with adduct **176** formed from propargyl bromide **177** and LHMDS. For what is concerning the second route, **174** is obtained by dimethyl zinc-mediated

coupling between **178** and STABASE adduct **179**, which is in turn obtained from its precursors **180** and **181**.

In the following paragraphs the synthesis of the aforementioned analogues is explained.

5.2.1 α-Methyl doublebonded arginine

Commercially available α-methyl serine **182** was protected as methyl ester and Boc-anhydride to give compound **183**. This adduct was then oxidised with Dess-Martin periodinane to give *(R)*-methyl 2-((*tert*-butoxycarbonyl)amino)-2-methyl-3-oxopropanoate **168**. This was reacted under Wittig-Horner-Emmons conditions with phosphonate **167**. This latter compound was obtained by refluxing bromoethyl phtalimide **184** in triethylphosphite. Most unfortunately, in the used conditions the Wittig reaction did not work.
The Wittig adduct would have then undergone deprotection on the phtalimide moiety and subsequent coupling with an opportunely protected thioisourea in order to put in place the guanidine moiety. Final deprotection of compound **185** would have lead to compound **164**.

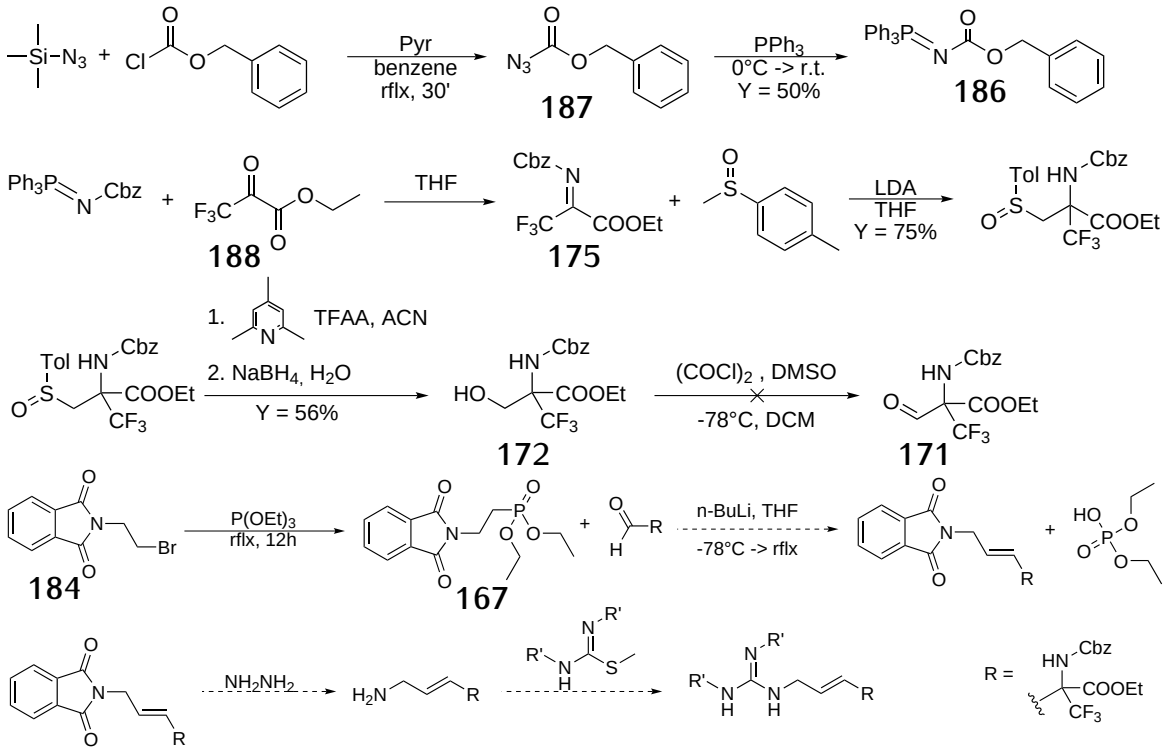


SCHEME 5.9: Synthetic route to the analogues.

5.2.2 α-trifluoromethyl arginine

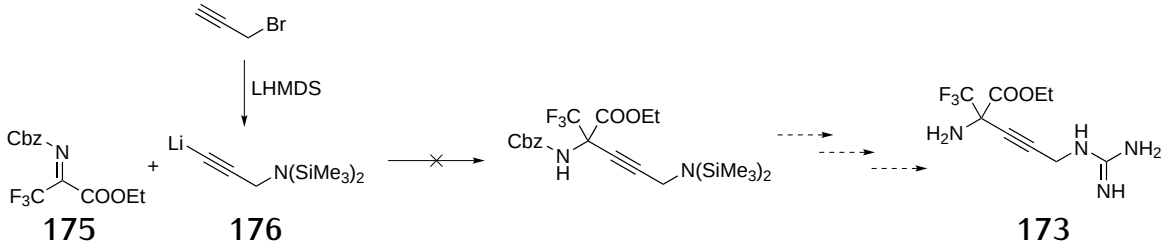
A first attempt to synthesise **169** started from aza-phosphorane **186**, easily obtained through a Staudinger reaction between azide **187** and triphenyl phosphine. It is then reacted with ethyl 3,3,3-trifluoro-pyruvate **188** to yield imine **175**, which is treated with methyl-tolylsulfoxide and LDA to give an intermediate which is readily treated under so-called Non-oxidative Pummerer Reaction conditions [93] to give protected α-trifluoromethyl serine **172**. Most unfortunately, Swern oxidation of

this compound to aldehyde **171** was unsuccessful, and therefore the devised route to the final compound **169**, similar if not equal to the one just described for the α-methyl aldehyde, was not undertaken, but it is nonetheless shown in Scheme 5.10.



SCHEME 5.10: Synthetic route to the analogues.

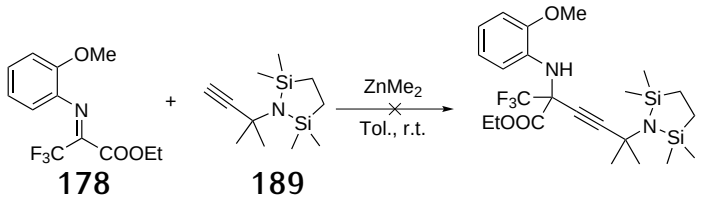
It seemed easier incorporation of a triple bond into the arginine skeleton: attention was therefore turned to method A. reported in a previous publication [94], where the adduct **176** between propargyl bromide and LHMDS is reacted *in situ* with **175** – synthesised as previously shown in Scheme 5.10 – to give an intermediate that, upon further manipulations, would finally result in **173**. This route, shown in Scheme 5.11, most unfortunately failed to react at the beginning as it was not possible to isolate a product from the coupling reaction.



SCHEME 5.11: Synthetic route to the analogues.

Therefore, route B. was undertaken: dimethyl propargyl amine was reacted with 1,2-bis(chlorodimethylsilyl)ethane to give the STABASE adduct **189** as reported in

the literature. [96] Imine **178** was then prepared according to literature procedure and coupling in the presence of dimethyl zinc in toluene was tried, but most unfortunately it was not possible to isolate the expected product in reasonable yield. The plan did not proceed further and was therefore abandoned.



SCHEME 5.12: Synthetic route to the analogues.

6

Conclusions and Perspectives

It can be said that from the positive results obtained fro the first generation of picolyl-coupled arginine analogues on the spectroscopic and biological side, the results presented here take us a step further toward the goal of responsive high-sensitivity MR-based methods for the diagnosis of disease.

Most unfortunately, there is still a lot of work to be done in order to achieve a full understanding and comprehension of the mechanism for the transfer of polarisation, but the results are very promising.
Moreover, this is one of the first deployment of such a method on organic molecules other than normal SABRE substrates, *i.e.* pyridine. It was also demonstrated that these compounds are substrates for the target enzyme.

In the following paragraphs the different generations of analogues, the problems encountered, the possible solutions and perspectives will be analysed.

6.1 On the picolyl-coupled arginines

For what is concerning the first generation of the picolyl-coupled arginine analogues, the protected analogues polarised quite well and it was demonstrated that they can be considered arginine analogues.

Most unfortunately the complete removal from the reaction mixture of TFA and triflic acid was not possible for these compounds, although the resulting oily and difficult to handle residue was submitted anyway for biological tests and polarisation studies.

Despite all, the biological tests gave good results, as it is shown in Table 5.1.1 on page 68, but the polarisation did not work on the submitted compounds.
A possible explanation for this might be that the guanidine moiety is interacting with the catalyst's metal centre instead of the pyridyl moiety, hence impeding or altering the transfer of polarisation – which could transferred, in this case, onto the nitrogen atoms.

A second synthetic generation was then developed: it was interesting to notice how the change of just one protecting group to another one affected the whole synthetic pathway. Indeed, the previous developed coupling method needed to be

changed: the standard EDC.HCl and Oxyma Pure in DCM with TEA was changed to HATU and TEA in DCM. Deprotection and isolation of compound were finally accomplished in an easy way.

6.2 On the double- and triple bond-containing α-methyl- and α-trifluoromethyl arginines

Synthesis of double- and triple bonded arginine with an α-methyl or α-trifluoromethyl moiety was not easy if not impossible to achieve.

The Wittig methodology failed, most probably because of elimination reactions taking place at the phthalimide moiety instead of the proper Wittig reaction for the construction of the double bond.

Not so much can be said about the reason for the failure of the installation of the triple bond on the skeleton of the arginine. While some methods need to be more thoroughly investigated, other gave inexplicable results.

6.3 Perspectives

A common trait for all of the synthesised compounds is the determination of the molecular weight: in fact, it has been said that it is a central and focal point the determination of such an entity in order to offer a sure amount of the millimoles in the samples that are going to be submitted to biological tests and the NMR polarisation experiments.

While for the first analogues' generation an estimation based on the protonation sites was made – accounting for four possible protonation site –, for what is concerning the second generation a more precise and accurate method was devised. In fact, EA and qNMR were employed with good accordance on compound **157**.
Elemental Analysis on the remaining compounds and respective ERETIC-2 NMR experiments are on going for the determination of the MW of the remaining samples.

Finally, it can be said that with this type of compounds it would be possible to address problems like sensitivity and functionality in the MRI spectroscopy.
Indeed, with PHIP the sensitivity of the NMR technique can be raised. In addition, target-oriented development of organic tracers could and should open the door, in the future, to a possible easy detection, in this case, of heart-stroke and related diseases by a simple MRI scan with great improvement in the patients' health. In other words, another step will be made in the direction of turning the MRI from an insensitive technique into a functional and rather fast one.

7 Experiments

Commercially available reagents were purchased from Sigma-Aldrich, Fisher, Acros Organic, Merck and VWR Prolabo. All of these chemicals were the highest grade and used without further purification.
TLCs were performed on Merck silica gel glass plates (60 F_{254}). Visualisation was accomplished by irradiation with a UV lamp and/or staining with a ceric ammonium molybdate or $KMnO_4$ solution. Flash chromatography was performed on Silica gel Si 60 (40-63 μm).
NMR data were recorded on Bruker ADVANCE III for 1H at 400 MHz, for 13C at 100 MHz and for 19F at 376 MHz, or on Varian VNMRS-400 spectrometer. All chemical shifts (δ) are expressed in ppm and coupling constant (J) are given in Hertz. The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet-doublet, dt = doublet-triplet, m = multiplet, br = broad.
LC-MS experiments were perfomed on an Agilent Technologies 1200 Series HPLC system equipped with a DAD and a 6120 MS detector composed by a ESI ionization source and a Single Quadrupole mass selective detector using a Analytical C18 RP column (Phenomenex Luna, C18(2) 250x4.60 mm, 5 μ, 100). HPLC purifications were performed on the Agilent 1200 system using a Semipreparative C18 RP column (Phenomenex Luna, 250x10.00 mm, 5μ, 100).

7.1 First generation picolyl-coupled arginine analogues

7.1.1 Synthesis

The four analogues were synthesised starting from Boc-Arg(Cbz)-OH **148** using a standard peptide coupling method with EDC.HCl and Oxyma Pure in DCM with TEA.
A typical procedure can be found in the following lines: The protected amino acid is dissolved in DCM and then 1.2 equivalents of coupling agent are added followed by 2 equivalents of TEA, and finally by 1.2 equivalents of Oxyma Pure. The mixture is lightly yellow and, upon completion of the coupling reaction, becomes orange, if not red. The reaction is then worked up by washing it with 3x10 ml $KHSO_4$ 1M – this gives raise to an emulsion –, then the organic phase is washed with 2x20 ml $NaHCO_3$ saturated solution and finally with 2x10 ml of brine. After drying over sodium sulphate anhydrous, the crude material is purified with a chromatographic column.

Compound Boc-Arg(Cbz)-*N*-(pyridin-4-ylmethyl)amide

Compound **149** was obtained in 54% yield.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.36-9.27 (m, 2H), 8.36 (d, J = 5.2 Hz, 2H), 7.35-7.13 (m, 10H), 7.10-7.01 (m, 1H), 6.87 (d, J = 5.7 Hz, 2H), 5.82 (d, J = 8.3 Hz, 1H), 5.22-5.15 (m, 3H), 4.98 (d, J = 12.4 Hz, 1H), 4.88-4.85 (m, 1H), 4.29-4.22 (m, 1H), 4.01-3.90 (m, 2H), 3.78-3.70 (m, 1H), 1.75-1.48 (m, 5H), 1.43-1.32 (m, 9H).

Compound Boc-Arg(Cbz)-*N*-methyl-*N*-(pyridin-4-ylmethyl)amide

Compound **150** was obtained in 71% yield.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.45 (dd, J = 4.4, 1.5 Hz, 2H), 7.35-7.20 (m, 9H), 6.98-6.97 (m, 1H), 6.94 (dd, J = 5.4, 2.4 Hz,), 5.23-5.22 (m, 2H), 5.16 (q, J = 5.4 Hz, 2H), 5.04 (t, J = 3.9 Hz, 2H), 4.59-4.48 (m, 2H), 4.25 (d, J = 15.6 Hz, 1H), 3.96-3.90 (m, 1H), 2.85-2.83 (m, 2H), 2.76 (s, 1H), 1.68-1.44 (m, 6H), 1.35-1.33 (m, 6H).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 160.5, 150.0, 136.7, 128.8, 128.38, 128.31, 128.1, 127.9, 122.3, 68.1, 67.0, 50.5, 50.1, 49.2, 44.3, 35.3, 29.9, 28.3, 24.7.

Compound Boc-Arg(Cbz)-*N*-(1-(pyridin-4-yl)ethyl)amide

Compound **151** was obtained in 88% yield.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.35 (dd, J = 18.1, 5.5 Hz, 2H), 7.35-7.14 (m, 9H), 6.96 (dd, J = 7.9, 5.9 Hz, 3H), 5.80 (dd, J = 8.7, 3.0 Hz, 1H), 5.18-5.16 (m, 1H), 5.14-5.11 (m, 2H), 5.03 (dq, J = 23.1, 11.2 Hz, 2H), 4.91-4.81 (m, 2H), 4.20 (s, 1H), 3.95-3.84 (m, 1H), 3.80-3.69 (m, 1H), 1.71-1.47 (m, 4H), 1.33-1.28 (m, 8H), 1.14-1.05 (m, 3H).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 160.5, 149.5, 136.7, 128.8, 128.38, 128.3, 128.1, 127.8, 120.6, 67.08, 53.80, 47.55, 43.65, 28.03, 24.51, 20.60

Compound *(R)*-methyl 2-Boc-Arg(Cbz)-2-(pyridin-4-yl)acetate

Compound **152** was obtained in 72% yield.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.47-9.25 (m, 3H), 8.46-8.38 (m, 2H), 7.46-7.26 (m, 11H), 6.95-6.92 (m, 2H), 5.29-5.04 (m, 5H), 4.78-4.73 (m, 1H), 4.25-4.18 (m, 1H), 3.91-3.73 (m, 3H), 3.67-3.63 (m, 3H), 3.00-2.95 (m, 1H), 2.68 (dt, J = 13.2, 8.1 Hz, 1H), 1.77-1.49 (m, 6H), 1.44-1.38 (m, 9H).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 160.5, 150.0, 136.7, 128.8, 128.38, 128.31, 128.1, 127.9, 124.17, 68.31, 67.92, 66.75, 53.86, 51.91, 43.71, 36.68, 29.9, 28.3, 24.7.

7.1.2 Deprotection reaction

A typical procedure is reported as follows.
To a stirring solution of protected compound in 3 ml of trifluoroacetic acid, 6 eq. of trifluoromethanesulfonic acid are added. the reaction is followed via NMR and MS. Upon completion, the acidic mixture is evaporated under a nitrogen flux. A few millilitres of HCl 1 N are added and the aqueous phase is extracted with EtOAc. The aqueous phase is then concentrated under high-vacuum to yield the final deprotected amino acid as a clear oil.

Compound *(R)*-2-Arginine-3-(pyridin-4-yl)propanoic acid

Compound **141** was isolated as a clear oil in the amount of 0.280 g.
^{1}H-NMR (400 MHz, CD_3OD): δ 8.61 (d, J = 6.8 Hz, 2H), 7.92 (t, J = 5.7 Hz, 2H), 4.94 (dt, J = 9.1, 4.4 Hz, 1H), 3.83 (dt, J = 5.7, 3.1 Hz, 1H), 3.55-3.50 (m, 1H), 3.30-3.23 (m, 3H), 3.16-3.10 (m, 2H), 1.90-1.54 (m, 6H).
^{13}C-NMR (101 MHz, D_2O): δ 172.7, 169.3, 158.7, 156.6, 140.7, 127.7, 117.9, 52.5, 52.2, 40.2, 36.4, 27.9, 23.2.

Compound *N*-(1-(pyridin-4-yl)ethyl)arginine amide

Compound **143** was isolated as a clear oil in the amount of 0.198 g.
^{1}H-NMR (400 MHz, CD_3OD): δ 8.73-8.70 (m, 1H), 8.00-7.97 (m, 1H), 5.14-5.09 (m, 1H), 3.93-3.87 (m, 1H), 3.22 (d, J = 1.6 Hz, 11H), 3.20-3.14 (m, 1H), 1.93-1.55 (m, 5H), 1.51-1.48 (m, 2H).
^{13}C-NMR (101 MHz, D_2O): δ 169.14, 163.86, 156.63, 141.20, 124.46, 52.56, 49.7, 40.15, 27.92, 23.70, 19.75.

Compound *N*-methyl-*N*-(pyridin-4-ylmethyl)argininamide

Compound **142** was isolated as a clear oil in the amount of 0.114 g.
^{1}H-NMR (400 MHz, CD_3OD): δ 8.72-8.70 (m, 2H), 7.89 (d, J = 6.8 Hz, 2H), 4.50 (dd, J = 7.9, 4.4 Hz, 1H), 3.13 (d, J = 1.7 Hz, 3H), 2.00-1.91 (m, 2H), 1.88-1.79 (m, 2H), 1.71-1.62 (m, 3H).

Compound *N*-(pyridin-4-ylmethyl)argininamide

Compound **140** was isolated as a clear oil in the amount of 0.222 g.
^{1}H-NMR (400 MHz, D_2O): δ 8.57 (d, J = 6.4 Hz, 2H), 7.81 (d, J = 6.3 Hz, 2H), 4.64-4.54 (m, 2H), 4.01 (t, J = 6.6 Hz, 1H), 3.10 (t, J = 6.9 Hz, 2H), 1.90-1.79 (m, 2H), 1.54 (quintet, J = 7.7 Hz, 2H).
^{13}C-NMR (101 MHz, D_2O): δ 170.1, 159.3, 141.0, 125.1, 52.8, 42.5, 40.2, 27.9, 23.6.

7.2 Second generation picolyl-coupled arginine analogues

7.2.1 Synthesis of the protected derivatives

The protected amino acid **153** is dissolved in DCM and then 1.4 equivalents of coupling agent HATU are added, followed by 2.9 equivalents of TEA, and finally by 1.1 equivalents of the desired pycolyl-amine. The resulting mixture is lightly yellow and, upon completion of the coupling reaction, becomes dark orange. The reaction is then worked up by washing with HCl 1N and a saturated solution of sodium bicarbonate. After drying over sodium sulphate anhydrous, the mixture is concentrated in vacuo and the crude material is purified with a chromatographic column (DCM/MeOH 95:5).
All the reactions were first tried on a 50 mg scale and then scaled up to 0.5 g of starting material **153**.

Compound Boc-Arg(Pbf)-*N*-(pyridin-4-ylmethyl)amide

Compound **159** was isolated as a yellowish transparent solid in the amount of 0.572 g with a final yield of 97%.
^{1}H-NMR (400 MHz, D_2O): δ 8.52-8.50 (m, 2H), 7.24-7.22 (m, 2H), 5.64-5.61 (m, 1H), 4.50-4.36 (m, 2H), 4.36-4.28 (m, 1H), 3.34-3.27 (m, 2H), 2.96 (d, J = 5.1 Hz, 2H), 2.56-2.55 (m, 3H), 2.48-2.46 (m, 3H), 2.14-2.08 (m, 4H), 1.89-1.83 (m, 1H), 1.71-1.58 (m, 3H), 1.48-1.38 (m, 12H).

Compound Boc-Arg(Pbf)-*N*-(pyridin-4-yl)amide

Compound **157** was isolated as white solid in the amount of 0.602 g. with a final yield of 40%.
^{1}H-NMR (400 MHz, D_2O): δ 8.43 (d, J = 6.3 Hz, 2H), 7.62 (td, J = 1.7, 0.3 Hz, 2H), 6.30 (d, J = 0.2 Hz, 2H), 5.72-5.70 (m, 1H), 3.32-3.28 (m, 2H), 2.97 (s, 2H), 2.61 (s, 3H), 2.53 (s, 3H), 2.11 (s, 3H), 1.95-1.82 (m, 3H), 1.71-1.64 (m, 3H), 1.47-1.40 (m, 15H).

Compound Boc-Arg(Pbf)-*N*-(1-(pyridin-4-yl)ethyl)amide

Compound **160** was isolated as a whitish solid, with a final yield of 76.5%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.61-8.57 (m, 2H), 7.15-7.12 (m, 2H), 5.71-5.68 (m,

1H), 4.70-4.53 (m, 3H), 3.43-3.41 (m, 1H), 3.23-3.19 (m, 1H), 3.02-2.90 (m, 6H), 2.59 (d, J = 7.3 Hz, 3H), 2.53 (s, 3H), 2.10 (s, 3H), 1.75-1.60 (m, 5H), 1.43-1.42 (m, 13H).

Compound *(R)*-methyl 2-Boc-Arg(Pbf)-2-(pyridin-4-yl)acetate

Compound **162** was isolated as a solid in the amount of 0.552 g and a yield of 83%. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.53 (s, 2H), 7.20 (s, 2H), 4.88 (s, 1H), 4.15 (s, 1H), 3.77-3.74 (m, 3H), 3.36 (m, 1H), 3.23 (m, 2H), 3.15 (m, 1H), 2.98 (s, 2H), 2.62 (s, 3H), 2.55 (s, 3H), 2.13 (s, 3H), 1.81 (m, 1H), 1.64 (m, 9H), 1.46 (d, J = 18.1 Hz, 13H).

Compound Boc-Arg(Pbf)-*N*-(1-(pyridin-4-yl)ethyl)amide

Compound **151** was obtained in 88% yield.
Compound **161** was isolated as a transparent solid in the amount of 0.530 g with a yield of 88.4%. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.49 (dd, J = 11.5, 5.2 Hz, 2H), 7.28 (d, J = 7.8 Hz, 2H), 5.02-4.96 (m, 1H), 4.26-4.23 (m, 1H), 2.96 (s, 2H), 2.58 (d, J = 12.1 Hz, 3H), 2.51 (s, 3H), 2.10 (s, 3H), 1.81-1.75 (m, 1H), 1.57-1.50 (m, 2H), 1.46-1.44 (m, 8H), 1.42 (d, J = 14.7 Hz, 10H).

Compound Boc-Arg(Pbf)-*N*-(2-methylbut-3-yn-2-yl)amide

Compound **156** was synthesised according to the procedure reported in section 7.1.1 on page 82. It was isolated as a yellowish solid in the amount of 0.344 g with a yield of 50%. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.10-4.05 (m, 2H), 3.49 (s, 1H), 3.22-3.21 (m, 2H), 2.95 (s, 2H), 2.59 (d, J = 5.0 Hz, 3H), 2.53 (s, 3H), 2.33-2.32 (m, 1H), 2.09 (s, 3H), 1.85-1.80 (m, 2H), 1.64-1.61 (m, 8H), 1.48-1.44 (m, 6H), 1.43 (d, J = 9.1 Hz, 9H).

7.2.2 Deprotection Reaction

The protected compounds were then deprotected with neat TFA (2-5 ml). After the evaporation of the acid under a nitrogen stream, the product was precipitated by help of a few millilitres of diethyl ether. After stripping several times with diethyl ether and centrifuging the recovered solid, it was dissolved in water and eluted through the C-18 cartridge; the water fractions were collected. The collected water fractions were united and freeze-dried to give the pure product of interest. Under the freeze-drying conditions the excess of TFA was removed as well.
All the reactions were first tried on a 50 mg scale and then scaled up to 0.5 g of starting material **153**.
All the final compounds **140**, **142**, **143**, **141** and **157** were analysed via HPLC-MS in order to assess their purity.

Compound *N*-(pyridin-4-ylmethyl)argininamide

Compound **140** was isolated as a solid in the amount of 0.24 g.
^{1}H-NMR (400 MHz, D_2O): δ 8.67 (d, J = 6.6 Hz, 2H), 7.91-7.89 (m, 2H), 4.13-4.10 (m,

1H), 3.49 (q, J = 7.1 Hz, 1H), 3.20-3.16 (m, 2H), 1.97-1.90 (m, 2H), 1.66-1.58 (m, 2H), 1.10 (dd, J = 8.4, 5.8 Hz, 2H).

Compound *N*-(pyridin-4-yl)arginineamide

Compound **157** was isolated as a solid in the amount of 0.119 g.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.58 (m, 2H), 8.0 (m, 2H), 4.3 (m, 1H), 3.17 (m, 1H), 2.00 (m, 2H), 1.67 (m, 2H).

Compound *N*-(pyridin-4-ylmethyl)argininamide

Compound **142** was isolated as a clear solid in the amount of 0.289 g.
^{1}H-NMR (400 MHz, D_2O): δ 8.77-8.63 (m, 2H), 7.92-7.75 (m, 2H), 5.01-4.88 (m, 1H), 3.52-3.47 (m, 1H), 3.24-3.18 (m, 4H), 3.00 (d, J = 5.0 Hz, 1H), 1.98-1.78 (m, 2H), 1.72-1.55 (m, 2H).

Compound *(R)*-2-Arginine-3-(pyridin-4-yl)propanoic acid

Compound **152** was isolated as a solid in the amount of 0.240 g.
^{1}H-NMR (400 MHz, D_2O): δ 8.65-8.62 (m, 2H), 7.93-7.89 (m, 2H), 5.01-4.97 (m, 1H), 3.96-3.92 (m, 1H), 3.59-3.47 (m, 2H), 3.35-3.28 (m, 1H), 3.19-3.14 (m, 2H), 1.89-1.83 (m, 2H), 1.66-1.54 (m, 2H), 1.11 (tt, J = 7.1, 0.8 Hz, 1H).

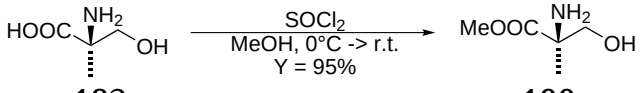
Compound *N*-(1-(pyridin-4-yl)ethyl)arginine amide

Compound **143** was isolated as a clear oil in the amount of 0.336 g.
^{1}H-NMR (400 MHz, D_2O): δ 8.70-8.68 (m, 1H), 7.90-7.85 (m, 1H), 5.10-5.06 (m, 1H), 3.93-3.87 (m, 1H), 3.16 (d, J = 1.6 Hz, 11H), 3.20-3.14 (m, 1H), 1.93-1.55 (m, 5H), 1.51-1.48 (m, 2H).

Compound *N*-(2-methylbut-3-yn-2-yl)arginineamide

Compound **154** was isolated as a white solid in the amount of 71.2 mg.
^{1}H-NMR (400 MHz, CD_3OD): δ 3.88-3.84 (m, 1H), 3.28-3.22 (m, 2H), 2.74-2.72 (m, 1H), 1.99-1.90 (m, 2H), 1.76-1.67 (m, 2H), 1.66-1.63 (m, 3H), 1.63 (dd, J = 8.6, 0.4 Hz, 2H), 1.46-1.44 (m, 2H).

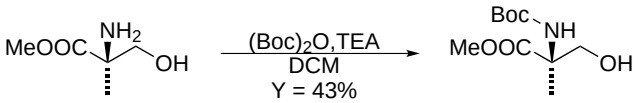
7.3 Synthesis of *(R)*-methyl 2-amino-3-hydroxy-2-methyl-propanoate



182 **190**

In a round-bottomed flask, 0.64 ml [2.1 eq.] of thionyl chloride were added to a cooled solution of 15 ml of MeOH under stirring. After this, 0.5 g of starting material **182** were added. The readtioin is allowed to stir and monitored via TLC DCM/MeOH 4:1 for two days, and additional 2.1 eq. of thionyl chloride were added.
After evaporation of the solvent under reduced pressure, 0.734 g of crude compound **190** were obtained and used as is in the following reaction.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.45 (d, J = 10.0 Hz, 2H), 4.00 (d, J = 12.2 Hz, 1H), 3.93 (d, J = 12.1 Hz, 1H), 3.80 (s, 2H), 3.42-3.42 (m, 3H), 1.61 (d, J = 6.0 Hz, 2H).

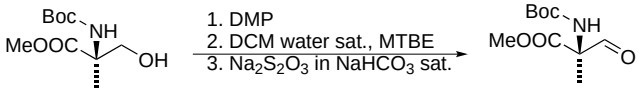
7.4 Synthesis of *(R)*-methyl 2-((*tert*-butoxycarbonyl)amino)-3-hydroxy-2-methylpropanoate



190 **183**

In a round-bottomed flask, crude **190** was dissolved in DCM; 0.76 ml [1 eq] of TEA were then added, followed by 1.19 g [1 eq.] of Boc anhydride. The reaction was left stirring at r.t. for 24 h, then additional 0.5 eq. of TEA and 0.5 eq. of Boc anhydride were added. The reaction was followed via TLC DCM/meOH 4:1. The reaction is then refused o.n.
It was then worked-up by washing with HCl until acidic pH, then washed with a few portions of a saturated solution of sodium bicarbonate. The aqueous phase was extracted with EtOAc, dried over anhydrous sodium sulfate and concentrated *in vacuo*, obtaining 0.95 g of crude product. This latter was purified with a chromatographic column eluted with DCM/MeOH 4:1, affording 0.544 g of product with a yield of 43%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.70 (s, 1H), 4.89 (t, J = 6.2 Hz, 1H), 3.55 (d, J = 8.3 Hz, 3H), 3.48 (tt, J = 10.5, 5.2 Hz, 2H), 1.30 (d, J = 39.1 Hz, 11H).

7.5 Synthesis of *(R)*-methyl 2-((*tert*-butoxycarbonyl)amino)-2-methyl-3-oxopropanoate

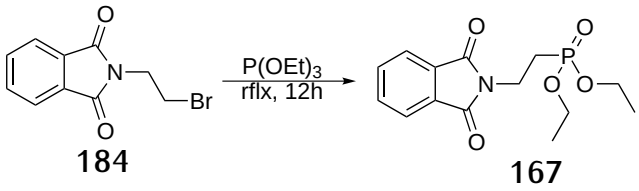


183 **168**

In a round-bottomed flask 0.291 starting material **183** was dissolved in DCM wet and 1.06 g [2 eq.] of DMP were added under stirring at r.t. The reaction becomes

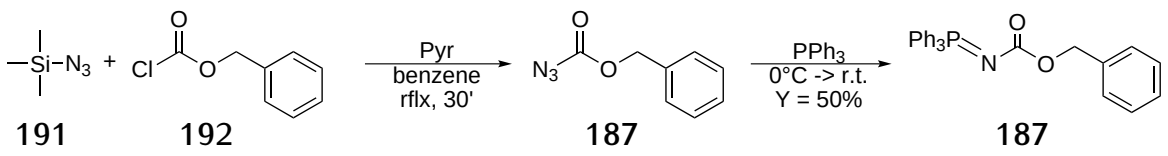
opalescent and is followed via TLC EtOAc/Cy 1:1. After six hours from an additional 1 eq. of DMP were added, MTBE and a saturated solution of $NaHCO_3$ and $Na_2S_2O_3$ were added under stirring.
The two phases are separated. The aqueous phase is extracted with 2 x 20 ml DCM. The collected organic phases are washed with 50 ml of $NaHCO_3$ saturated solution; 2 x 50 ml of distilled water and 2 x 50 ml of brine – inversion of phase can be noticed at this point.
After drying over anhydrous sodium sulfate and concentration *in vacuo*, obtaining 0.225 g of crude product purification over chromatographic column eluted with EtOAc/Hex 3:7 is accomplished, affording 0.158 g of product with a yield of 60%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.56 (s, 1H), 3.80-3.79 (m, 3H), 1.63-1.60 (m, 3H), 1.41 (s, 9H).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 193.8, 169.3, 53.3, 28.2, 19.2.

7.6 Synthesis of diethyl (2-(1,3-dioxoisoindolin-2-yl)ethyl)-phosphonate



In a round-bottomed flask, to a solution of 6.74 ml [5 eq.] of triethyl phosphite phthalimide **184** was added. The resulting mixture id refluxed for 12 h. The excess triethylphosphite was removed under vacuum distillation.
After addition of EtOH and further stripping, 2.85 g of oily **167** were recovered and used as is.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.80-7.77 (m, 2H), 7.67-7.64 (m, 2H), 4.10-3.99 (m, 5H), 3.93-3.86 (m, 2H), 2.19-2.11 (m, 2H), 1.27-1.21 (m, 7H).

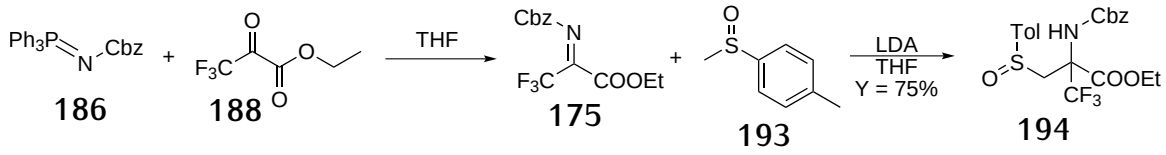
7.7 Synthesis of (Benzyloxycarbonyl)amino triphenylphosphorane



To a solution of 1.0g trimethylsilylazide **191** [8.7 mmol] and 0.48 mL benzyl chloroformate **192** [6.2 mmol] in 10 ml of dry benzene under nitrogen atmosphere, were added three drops of pyridine, and the solution was heated for 30 min. at reflux

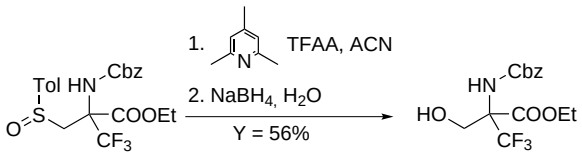
temperature, then diluted with further 10 ml of benzene and cooled to 0°C. This solution, containing intermediate **187**, was slowly added to a solution of 1.6 g of triphenyl phosphine [1 eq.] in 15 ml of benzene at 0°C. When nitrogen evolution ceased, the mixture was stirred for 30 min. at rt., then the solvent was removed under reduced pressure. Crystallisation of the residue from AcOEt afforded the required iminophosphorane **186** as white crystals (2.5g, 98%).
^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.05 (s, 2H).

7.8 Synthesis of Sulfinylamine



Under an inert atmosphere, to a solution of 0.5 g of **186** in dry THF, 0.17 ml [1.05 eq.] of trifluoroethyl pyruvate **188** were added. The reaction was stirred at r.t. and monitored via TLC EtOAc/Hex 1:1.
After this reaction is complete, it is added via cannula to a cooled (-78°C) solution of 0.19 g [1 eq.] of methyl tolyl sulfoxide **193** in the presence of 0.66 ml [1.1 eq.] of LDA.
After 20 minutes, reaction is then quenched with a saturated solution of NH_4Cl. The crude amount of 1.05 g is purified over a chromatographic column, affording 0.420 g of a diastreomeric mixture of the desired product **194**, and a yield of 65%
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.44 (d, J = 8.1 Hz, 2H), 7.34-7.25 (m, 7H), 6.53 (s, 1H), 5.09 (dt, J = 18.4, 9.0 Hz, 2H), 4.35 (qd, J = 7.2, 4.3 Hz, 2H), 4.12 (d, J = 13.0 Hz, 1H), 3.50 (d, J = 13.0 Hz, 1H), 2.35 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H).

7.9 Synthesis of α-trifluoromethyl serine



To a cooled (0 °C) and stirred solution of 0.187 g of **194** [0.435 mmol] in 4 ml of acetonitrile, under nitrogen, was added 0.170 ml of sym-collidine [3 eq.], followed by 0.30 ml of trifluoroacetic acid [5 eq.]. After 2 min at room temperature, TLC analysis EtOAc/Hex 3:7 showed the disappearance of the starting material. To the reaction mixture, cooled longer at 0 °C, was added one drop of water, and portionwise one drop of water was added at 0 °C, followed by an excess of $NaBH_4$ [about 3 eq.]. After 2 min, the reaction was warmed at r.t. and stirred for 1.5 h. The mixture

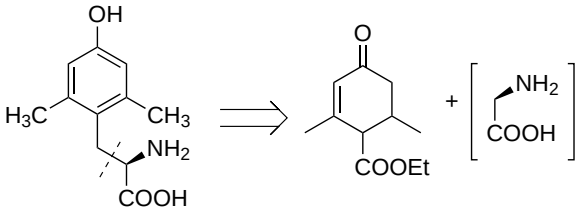
was quenched, at 0 °C with a saturated aqueous solution of NH_4Cl and extracted with 3 x 5 ml EtOAc. The collected organic layers were washed twice with a 1 N HCl solution to remove sym- collidine and then with aqueous $NaHCO_3$. After routine workup, the crude was purified by FC EtOAc/Hex 3:7 affording 93 mg of **172** with a 60% yield.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.32-7.25 (m, 4H), 5.89 (s, 1H), 5.08-5.04 (m, 2H), 4.55-4.52 (m, 1H), 4.26 (dq, J = 13.7, 6.6 Hz, 2H), 4.14 (d, J = 12.1 Hz, 1H), 4.04 (qd, J = 7.2, 1.0 Hz, 1H), 1.26-1.22 (m, 3H).

Part III

Third Project: Studies toward the Synthesis of 2',6'-dimethyl-L-tyrosine

8 Aim

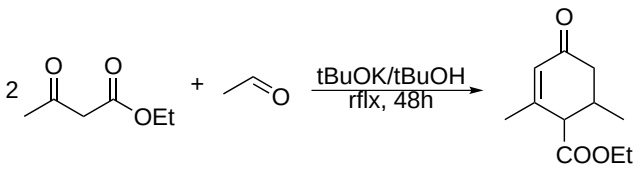
The aim of this third and last project is to find a new synthetic pathway to 2′,6′-dimethyl-L-tyrosine, a potent and well-known substitute for L-tyrosine in opioid peptides.



6 **195** **196**

SCHEME 8.1: Disconnection for the synthetic plan for Dmt.

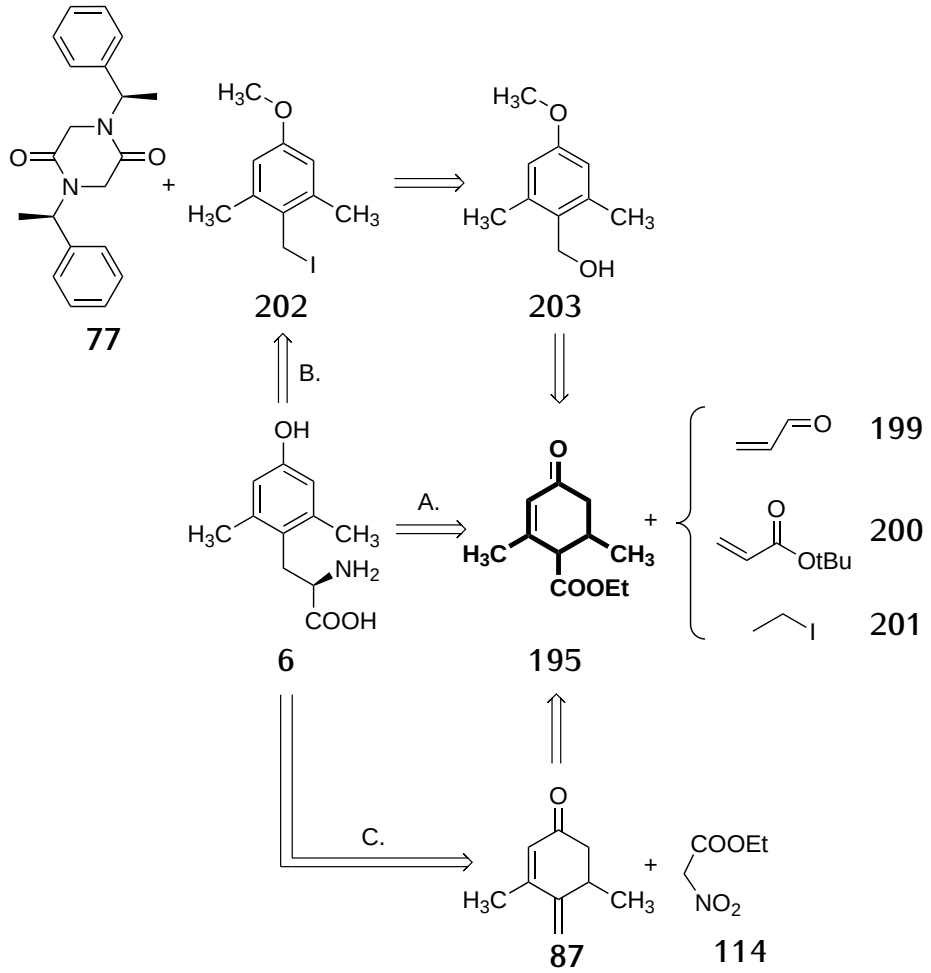
As it is shown in Scheme 8.1, disconnection of the amino acidic residue on C1 of the phenyl ring of Dmt **6** leads to a 4-hydroxy-2,6-dimethylphenyl residue whose skeleton resembles the Hagemann's ester 's derivative **196**. This residue would then be coupled with a surrogate of the amino acid moiety **195**.
In this way, three different routes were designed to achieve the final product **6**, all of them starting from compound **196**, which is easily obtained by tandem Michael addition-aldol condensation reaction of two equivalents of acetoacetate **197** with acetaldehyde **198** in good yield. [97] This method is an improvement of the original Knoevenagel approach, and Chong *et al*. report that the annulation reaction proceeds smoothly using potassium *tert*-butoxide in *tert*-butanol at refluxing temperature, as shown in Scheme 8.2.



118 **198** **195**

SCHEME 8.2: Synthesis of Hagemann's ester 's derivative **196**. [97]

The three different investigated routes for the synthesis of **6** are shown in the following Scheme 8.3. First of all, suitable Michael addition of acrolein **199**, *tert*-butyl

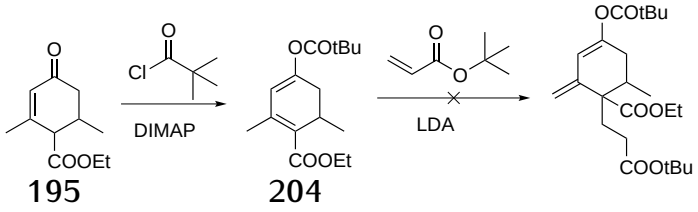


SCHEME 8.3: Synthetic routes for Dmt.

acrylate **200** and iodoethane **201** on Hagemann's ester 's C1 position was envisaged – route A. This C1 alkylation would have led to compounds whose alkyl chain could have been manipulated and turned into the amino acid moiety. After alkylation, aromatisation reaction of the obtained product would have been required in order to build the Dmt skeleton.
On the other hand, Dmt can be obtained by alkylation of benzyl iodide **202** with diketopiperazine **77**; the former can be afforded by opportune transformation of benzyl alcohol **203**, which in turn is derived from Hagemann's ester as a starting material – route B. This route can be considered an evolution or improvement of what has been previously reported in [74].
Finally, aromatisation of the Michael adduct between the Hagemann's ester -derived dienone **87** and nitroacetate **114** can yield racemic Dmt – route C. As it is possible to notice, Hagemann's ester is very versatile and quite a useful compound!

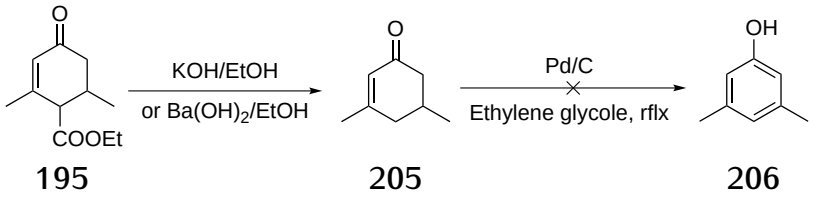
8.1 Route A.

For what is concerning investigations on the first route, Hagemann's ester **196** was reacted in the presence of TMG or $Yb(OTf)_3$ in different solvents with the aforementioned electrophiles **199**, **200** and **201**: as previously reported in the literature, these approach was not satisfying because of the presence of compounds alkylated in other positions.
An alternative was then alkylation with *tert*-butyl acrylate **200** of compound **204** in the presence of LDA, where the former was obtained from *O*-pivaloyl protected Hagemann's ester : unfortunately also this last reaction did not result in the expected alkylated product, as it is possible to see in the following Scheme 8.4.



SCHEME 8.4: Synthesis of Hagemann's ester 's derivatives.

In the mean time, attempts at aromatisation of the ester was attempted. The whole process started with elimination of the ester moiety by basic hydrolysis followed by decarboxylation in acidic medium. Promising results were achieved in the presence of $Ba(OH)_2$, while also KOH in ethanol gave good results; decomposition of the starting material was observed under the Krapko conditions.
Unfortunately, aromatisation of compound **205** with Pd/C in ethylene glycol in order to study the feasibility of the procedure to arrive to the 3,5-dimethylphenol **206** was unsuccessful.



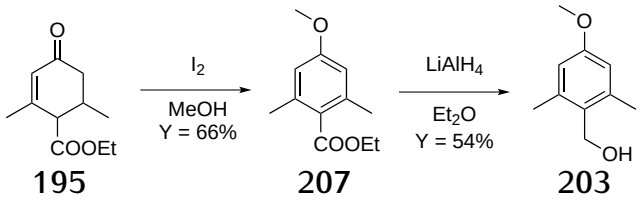
SCHEME 8.5: Studies towards aromatisation.

The failure of all the experiments for the envisaged route A., the synthetic approach was changed in favour of route B.

8.2 Route B.

Route B. sees the inversion of the synthetic "logic" shown in path A.: in fact, aromatisation reaction in this case occurs as the first step. Following Kotnis findings, Hagemann's ester was aromatised in refluxing methanol in the presence of molecular iodine. [98] A mechanism is not certain for this transformation, nor it has been

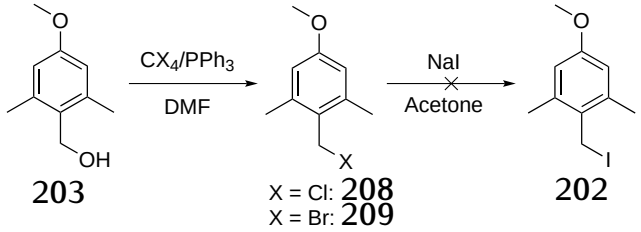
proposed, but most likely oxidation of the ring occurs by mean of one molecule of iodine. At the same time, oxidation produces hydrogen iodide *in situ* which protonates the methanol: this latter can act as electrophile or form iodomethane, finally leading to *p*-methoxybenzoate **207**. Finally, the benzoate was reduced to benzylic alcohol **203** easily in the presence of $LiAlH_4$ – Scheme 8.6.



SCHEME 8.6: Studies towards aromatisation.

At this point, investigations began on how it could be possible to transform the benzylic alcohol into a better leaving group. This might seem a trivial problem, but the two methyl groups in the *o* position to the benzylic alcohol hinder greatly this position, actually blocking and impeding the reactivity. It is therefore important to transform this moiety in a more reactive centre, for example into an halide. In fact, attempts to synthesise a mesyl- or tosyl derivative of **203** failed.
A few halogen derivatives were then synthesised with different methodologies.

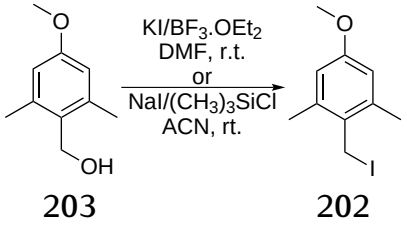
Firstly, benzyl chloride **208**: reaction with thionyl chloride was unsuccessful, but simple Appel reaction with CCl_4 and triphenyl phosphine in DCM or DMF gave the expected product in moderate yield. This approach required although removal of unwanted triphenyl phosphoxide by means of a chromatographic column, leading to loss of product during the purification process. The obtained chloride was then subjected to Finkelstein retain with NaI in acetone, in order to exchange the chloride with the iodide atom. It is in fact well-known that generally iodide-containing compounds are better substrates and reactive towards alkylating agents. Unfortunately, this exchange reaction did not work.
In the meantime, synthesis of the benzylic bromide was accomplished, always under Appel reaction conditions with CBr_4 and triphenyl phosphine in DMF. The C-Br bond is more reactive, and even though the crude was purified, compound **209** was obtained in better yields than its chloride homologue.



SCHEME 8.7: Studies towards formation of benzylic halide – part 1.

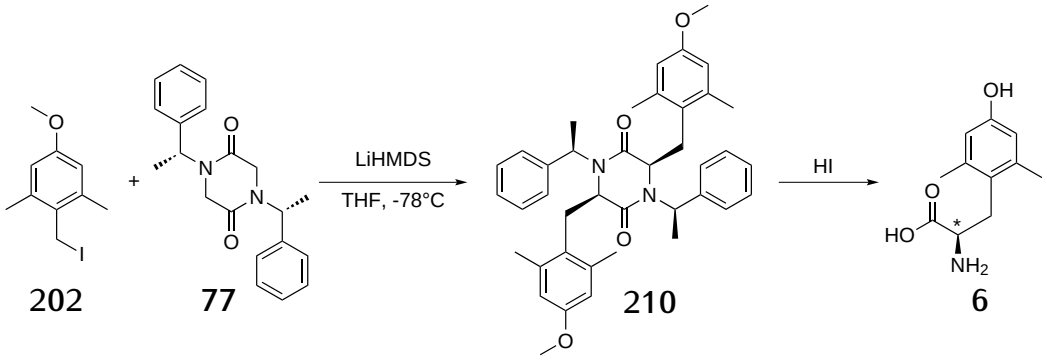
Nonetheless, the real goal was obtainment of iodide **202** in good yields and quantities. Under specific Appel conditions for the synthesis of iodides, *i.e.* with triphenyl phosphine and imidazole, in the presence of molecular iodide in DMF at

r.t., the expected product was not formed. [99]
It turned out that a modification of a literature procedure was found working on the substrate: KI and $BF_3 \cdot OEt_2$ were employed in anhydrous conditions in diethyl ether and a few drops of DMF. [100] The reaction in this case was successful, but unreacted starting material was found from the crude ^{1}H-NMR spectrum. Unfortunately, purification of compound did not lead to isolation of significant amount of desired product, nor its ^{1}H-NMR purity was deemed sufficient for using it in the following step. This is a confirmation of the fact that the iodide is the sought for, reactive compound for the alkylation reaction.
An improvement in the synthesis of **202** was achieved by reacting benzylic alcohol **203** with NaI in the presence of trimethyl chlorosilane in dry ACN: in this case the yield was finally quantitative and the product could be used in the following step without any further purification.



SCHEME 8.8: Studies towards formation of benzylic halide – part 2: synthesis of the benzylic iodide.

The finally obtained compound **202** was alkylated by 2,5-diketopiperazine **77** in the presence of LHMDS in anhydrous THF, as reported in [74]. The alkylated adduct **210** was then hydrolysed in refluxing hydrogen iodide to finally afford the amino acid L-Dmt with good stereoselectivity, thanks to the chiral synthon **77**.

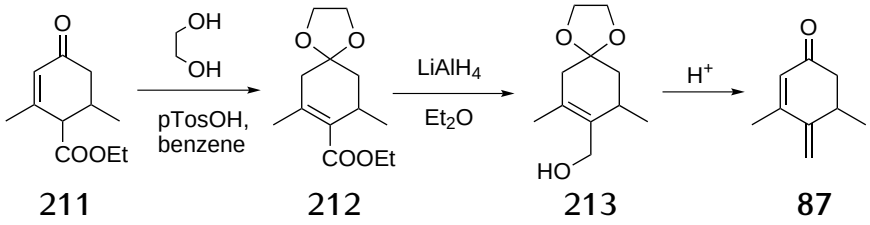


SCHEME 8.9: Alkylation reaction and release of the amino acid.

8.3 Route C.

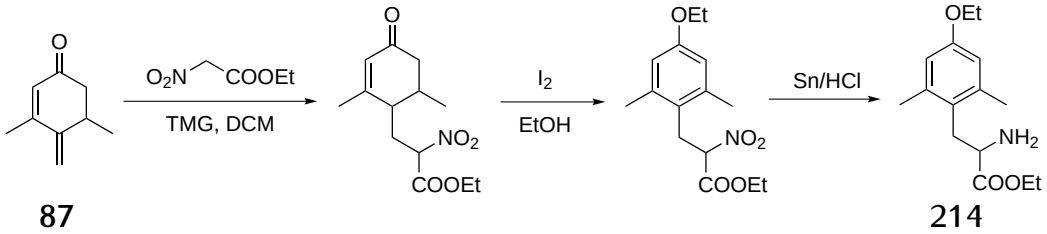
Racemic Dmt can be also obtained by means of Michael addition of ethyl nitroacetate on dienone **87**. This latter is obtained starting from Hagemann's ester **211**:

protection of the ketone as dioxolane **212** is followed by selective reduction of the ester moiety in the presence of $LiAlH_4$. Stirring the obtained alcohol **213** in acidic medium for a few hours easily led to stable compound **87**. [101]



SCHEME 8.10: Synthesis of the dienone.

Construction of the Dmt skeleton was then accomplished by simple Michael addition of ethyl nitroacetate on dienone **87** followed by iodine-mediated aromatisation, as shown in the following Scheme 8.11. The nitrate group was eventually reduced to amine by Sn/HCl, leading to the racemic ethyl ester of Dmt **214**.



SCHEME 8.11: Alkylation reaction and release of the amino acid.

9

Conclusions and Perspectives

Hagemann's ester is a very useful and versatile building block: it has been shown before in the introduction and from th recent publication [81], but also in this last project.

It is in fact very easy to synthesise and purify and can be manipulated in quite a few different manners to achieve the most varied structures. We chose Hagemann's ester because of its substituents position resemblance to the Dmt aromatic skeleton. Moreover, it is well known that the ester moiety can be easily transformed.

A stereoselective synthesis, based on previous work [74], was accomplished. The crucial step is the alkylation on the part of the chiral synthon 2,5-diketopiperazine on the benzylic iodide: all of the compounds can be easily synthesised and do not require chiral catalysts. Moreover, the aforementioned diketopiperazine and its employment fall under the auspices of the *atom economy*: in fact, all of its atoms concur and are in the end part of the amino acid.

It was also possible to develop an easy route to benzyl iodide **202**.

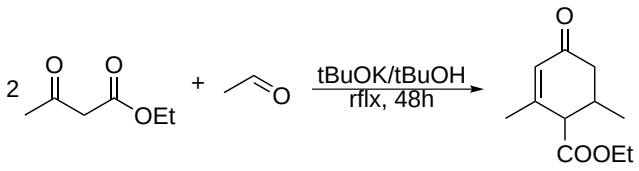
One aim of the following work is to study the feasibility of the employment of the 2,5-diketopiperazine chiral synthon in the Michael addition to dienone **87**.

10

Experiments

The TLC layers are Macherey-Nagel Poligram SIL G/UV_{254} 0.20 mm; a 1% solution of $KMnO_4$, 2,4-dinitrophenylhydrazine in acidic solution were used to visualise spots.
Chromatographic purifications were run over silica gel Macherey-Nagel 60M 230-400 mesh.
^{1}H-NMR and ^{13}C-NMR were registered with Varian spectrometers at 300 MHz and 400 MHz at room temperature. Chemical shifts (δ) are reported with respect to trimethylsilane in the following manner: chemical shift (multiplicity, coupling constants, integer value). Signal multiplicity are shortened in the following manner: *s* for singlet; *d* for doublet; *t* for triplet; *q* for quartet; *br* for broad signal; *m* for multiplet; *dd* for double doublet.
Mass spectra were recorded on Waters Micromass ZMD 2000, ESI-Q-TOF 6520 Agilent Technologies, Agilent 6520 Q-TOF LC/MS System and LCQ Duo Finningan.

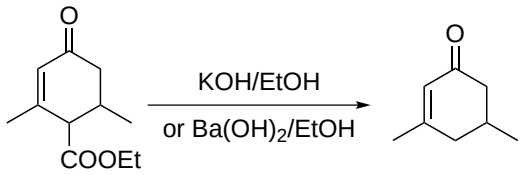
10.1 Synthesis of ethyl 2,6-dimethyl-4-oxocyclohexen-2-encarboxylate



118 **198** **195**

In a round-bottomed flask, 5.2 g of ethyl acetate **116** [39,96 mmol, 2eq], 1,11 ml of acetaldehyde **215** and 40 ml of *tert*-butanol were stirred together. At 0°C, 0,16 g of *tert*-butanol [0,1 eq] were added, and the mixture was stirred at the same temperature for about one hour. After this, additional 0.4 g [0.25 eq] of *tert*-butanol were added, and the reaction was heated under reflux for about 48 hours and followed via TLC EtOAc/Cy 1:2.
After this time, the reaction was cooled to room temperature, washed with HCl 1N followed by a saturated solution of sodium bicarbonate, NaOH 1N, distilled water and brine. The aqueous phases were extracted with EtOAc. The combined organic layers are dried over anhydrous Na_2SO_4 and concentrated under vacuum, obtaining an oily and yellow compound which is then purified via flash chromatography EtOAc/Cy 1:4, affording 2.57 g of product **196** with a final yield of 65%.
MS [ESI] = 197.1 m/z [MH^+]
^{1}H-NMR (400 MHz, $CDCl_3$): δ 5,9 (s, 1H), 4,15 (m, 2H), 3,10 (m, 1H), 3 (m, 1H), 2,85 (m, 1H), 2,6 (m, 1H), 1,95 (s, 3H), 1,3 (m, 3H), 1,1 (m, 3H).

10.2 Synthesis of 3,5-dimethylcyclohexen-2-one



195 **205**

10.2.1 Procedure A.

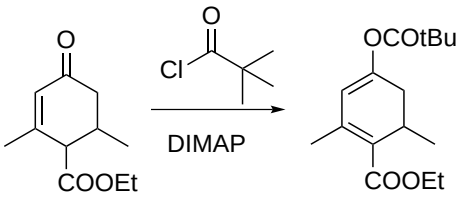
500 mg of **196** [2.55 mmol] were dissolved in 13 ml of ethanol and added to a EtOH/water mixture containing 1 g of KOH [7 eq.]. The mixture was heated under refluxing conditions over night.
The solution was dissolved in 10 ml of water and the ethanol was evaporated under reduced pressure. HCl 6N was added and the mixture refluxed for additional 2 hours.

The aqueous phase was then cooled and extracted thrice with diethyl ether and washed with brine. The combined organic layers are dried over anhydrous Na_2SO_4 and concentrated under vacuum, obtaining 118.6 mg of crude compound **205** which is then filtered over a silica pad, affording 52 mg of product with a final yield of 16%.

10.2.2 Procedure B.

396 mg of **196** [2.02 mmol] were dissolved in 8 ml of ethanol; 2.83 g of $Ba(OH)_2$ [8.86 mmol] were added to the mixture, which was then was heated at reflux temperature over night.
The solution was the cooled and acidified and extracted thrice with diethyl ether. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum, obtaining 281 mg of crude compound **205** which is then purified via chromatographic column EtOAc/Cy 1:3, affording 109 mg of product with a final yield of 44%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 5,9 (s, 1H); 2,4 (m, 2H); 2,2 (m, 1H); 2 (m, 2H) 1,9 (s, 3H); 1,05 (d, 3H, J= 12 Hz).

10.3 Synthesis of 2,6-dimethyl-4-(pivaloxy)cyclohexen-2-encarboxylate



195 **204**

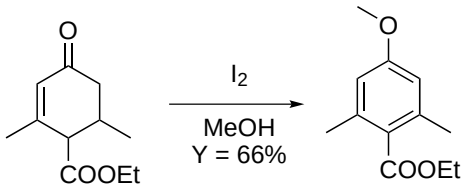
10.3.1 Procedure A.

In a round-bottomed flask, 204 mg [1.04 mmol] of compound **196** were dissolved in THF. Then, 2 eq. [0.25 ml] of pivaloyl chloride and 2.5 eq. [0.39 ml] of TMEDA were added, and the reaction was stirred at room temperature o.n.; a white precipitate started to form.
After TLC analysis the following day, starting material was still present: additional 2.5 eq. of base were added. Following further monitoring of the reaction, additional pivaloyl chloride was added too, but the reaction was not proceeding anymore.
The reaction was then worked-up: the organic phase was washed with HCl 1N, and the aqueous phase was extracted with diethyl ether. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*, obtaining 318 mg of crude compound **204** which is then purified via chromatographic column EtOAc/Cy 1:4, affording 74 mg of product and 107 mg of starting material **196**, with a final yield of 44%.

10.3.2 Procedure B.

In a round-bottomed flask, 507 mg of compound **196** were dissolved in THF. Then, 2 eq. of pivaloyl chloride and 2.5 eq. [0.39 ml] of DMAP were added, and the reaction was stirred at room temperature o.n.; a white precipitate started to form.
The reaction was then worked-up: the organic phase was washed with HCl 1N and the aqueous phase was extracted with diethyl ether. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*, obtaining 700 mg of crude compound **204** which is then purified via chromatographic column EtOAc/Cy 1:4, finally affording 349 mg of product and 272 mg of starting material **196**, with a final yield of 44%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 5,6 (s, 1H); 4,20 (m, 2H); 3 (m, 1H); 2,8 (m, 1H); 2,2 (s, 3H), 2 (m, 2H); 1,3 (t, 2H, J=7 Hz) 1,2 (m, 9H); 1,1(d, 3H J= 6,9 Hz).
^{13}C-NMR (100 MHz, $CDCl_3$): δ 176,489; 167,838; 152,492; 141,496; 124,424; 116,056; 59,919; 39,008; 33,332; 29,850; 26,980; 21,076; 18,348; 14,330.

10.4 Synthesis of 4-methoxy-2,6-dimethylbenzene

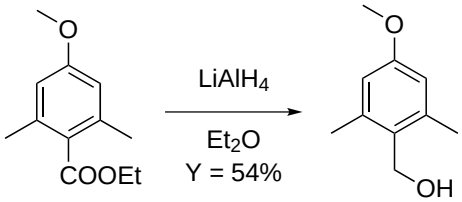


195 **207**

3 g of ester **196** [67.6 mmol] and 7.7 g [2.2 eq] of molecular iodine were dissolved in 20 ml of methanol and refluxed for a few hours.
After reaction completion, methanol was evaporated under reduced pressure and the crude was taken up in EtOAc. The organic phase was washed with a saturated solution of sodium bicarbonate and with a saturated solution of sodium thiosulfate, followed by water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*, obtaining 2.7 g of crude compound **207** which is then purified via chromatographic column EtOAc/Cy 5:95, finally affording 2.11 g of final product **216**, with a final yield of 66%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6,559 (s, 2H); 4,369 (q, 2H, J= 7 Hz); 3,779 (s, 3H); 2,320(s, 6H), 1,372 (t, 3H, J= 7 Hz).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 169.8, 159.9, 137.4, 126.4, 113.1, 60.7, 55.1, 20.3, 14.3.

10.5 Synthesis of (4-methoxy-2,6-dimethyl)methanol

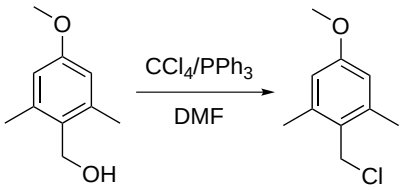
Under nitrogen atmosphere, 1 g of starting material **216** was added drowse to a suspension of 68 mg [0.75 eq] of $LiAlH_4$ in 10 ml of diethyl ether.
After two hours of reaction, total conversion to product **203** was accomplished.
Reaction was then treated with NaOH 2N to destroy the excess of reducing agent



207 **203**

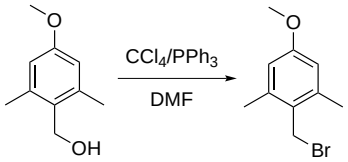
and filtered over a celite pad. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*, obtaining 0.24 g of crude compound **203** which is then purified via chromatographic column EtOAc/Cy 1:2, finally affording 0.21 g of final product **203**, with a final yield of 54%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6,6 (s, 2H), 4,7 (s, 2H), 3,8 (s, 3H), 2,4 (s, 6H).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 158.9, 139.0, 113.6, 66.1, 59.0, 55.1, 41.5, 19.7.

10.6 Synthesis of 2-(chloromethyl)-5-methoxy-1,3-dimethylbenzene



203 **208**

To a solution of 201 mg of **203** in 5 ml of DMF, 0.12 ml [1 eq.] of CCl_4 and 317 mg [1 eq.] of triphenyl phosphine were added. The reaction mixture was allowed to stir at r.t.
After 30 minutes, TLC-monitoring did not show anymore the presence of the starting material. Water was added and the solution was extracted in η-hexane. The organic phase was dried over anhydrous sodium sulphate and concentrated under reduce pressure to give 174 mg of crude, which was purified on silica gel eluted with EtOAc/Cy 1:8, affording 34 mg of desired product **208** as a white solid, with a final yield of 15%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6,554 (s, 2H); 4,513 (s, 2H); 3,752 (s, 3H); 2,358 (s, 6H).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 158,779; 139,482; 126,925; 113,365; 66,102; 55,074; 19,830.

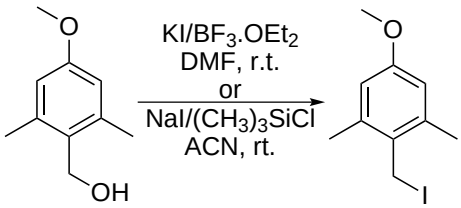


203 **209**

10.7 Synthesis of 2-(bromomethyl)-5-methoxy-1,3-dimethylbenzene

To a solution of 200 mg of **203** in 3 ml of DMF, 0.4 g [1 eq.] of CBr_4 and 315 mg [1 eq.] of triphenyl phosphine were added. The reaction mixture was allowed to stir at r.t. for three days.
After completion of the reaction, water was added and the solution was extracted in η-hexane. The organic phase was dried over anhydrous sodium sulphate and concentrated under reduce pressure to give 762 mg of crude product, which was purified on silica gel eluted with EtOAc/Cy 1:6, affording 136 mg of desired product **209** as a white solid, with a final yield of 49%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6,55 (s, 2H); 4,5 (s, 2H), 3,78 (s, 3H), 2,4 (s, 6H).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 158.8, 139.5, 126.9, 113.4, 66.1, 55.1, 19.8.

10.8 Synthesis of 2-(iodomethyl)-5-methoxy-1,3-dimethylbenzene



203 **202**

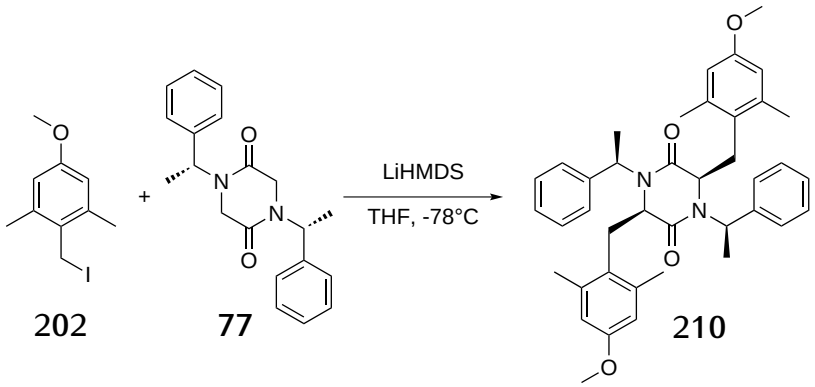
10.8.1 Procedure A.

Under an inert atmosphere, 200 mg [1.2 mmol] of compound **203** was dissolved in 5 ml of dry diethyl ether. To this, 200 mg [1 eq.] of KI dissolved in anhydrous DMF and 0.15 ml [1 eq.] of $BF_3 \cdot OEt_2$ were added under stirring, and the reaction was left at r.t. for one day.
Upon reacting, a change of colour can be observed: complete reaction is in fact dark orange.
The reaction solution was washed with cooled water, extracted thrice with diethyl ether, dried over anhydrous sodium sulphate and concentrated under reduce pressure to give 257 mg of crude product **202**. Purification with Isolera automatic column yielded not quantifiable and extremely impure final product.

10.8.2 Procedure B.

Under an inert atmosphere, 1,5 g [9.04 mmol] of compound **203**, 1.35 g [1 eq.] of NaI, 1.35 ml [1 eq.] of chloro trimethylsilane were dissolved in 10 ml ACN. A white precipitate is formed and reaction colour becomes dark red. After 30 minutes and TLC monitoring, reaction is complete.
The reaction solution was washed with water, and a saturated solution of sodium thiosulfate, extracted thrice with diethyl ether, dried over anhydrous sodium sulphate and concentrated *in vacuo* to give 2,36 g of crude product **202** which was not further purified.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6,56 (s, 2H); 4,47 (s, 2H); 3,765 (s, 3H); 2,33 (s, 6H).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 159,108; 138,694; 127,001; 113,931; 55,100; 19,710; 5,101.

10.9 Synthesis of *(3S,6S)*-3,6-bis(4-methoxy-2,6-dimethylbenzyl)-1,4-bis(*(S)*-1-phenylethyl)piperazyn-2,5-dione

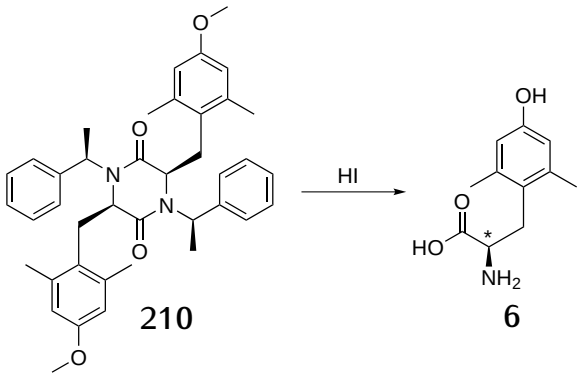


202 **77** **210**

Under an inert atmosphere in a round-bottomed flask, 875 mg [0.5 eq] of **77** are dissolved in 5 ml of freshly distilled THF. At -10°C, 3 ml of LHMDS 1M in THF [0.5 eq.] are added. After circa 30-40 minutes, 750 mg [0.5 eq] of benzyl iodide **202** are added at -78°C. The reaction is stirred at -78°C for an hour and followed via TLC. after depletion of starting material **202**, another 3 ml of LHMDS 1M in THF [0.5 eq.] are added at -10°C, and after 40 minutes 750 mg [0.5 eq] of benzyl iodide **202** are added at -78°C. the reaction is then allowed to reach r.t. and is stirred for circa 5 hours.
The reaction was then worked-up by washing with HCl 1N; the aqueous phase is extracted with EtOAc and dried over anhydrous sodium sulphate and concentrated *in vacuo* to afford 2.5 g of crude product **210**.
The crude compound was purified over chromatographic column eluted with EtOAc/Cy 1:3, obtaining 0.8 g of pure compound **217**, with a yield of 47 %.
MS (ESI) = [MH^+]: 619,7 *m/z*
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7,289 (m, 6H); 7,004 (m, 4H); 6,608 (s, 4H); 5,718 (q, 2H, J=7); 4,046 (t, 2H, J=8); 3,782 (s, 6H); 3,43 (m, 4H), 2,322 (s, 12H); 1,33 (d, 2H,

J=7).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 167,89; 158,02; 138,28; 128,69; 128,01; 127,25; 113,95; 58,1; 55,04; 35,56; 26,90; 21,61.

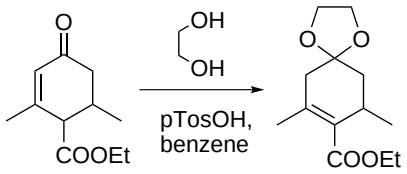
10.10 Synthesis of 2,6-dimethyl-L-tyrosine



210 6

0.8 g of alkylated diketopiperazine **210** are reacted with 10 ml of HI 57% concentrated at refluxing temperature for about three hours. After this time, the acidic solution was concentrated *in vacuo*, diluted with water and extracted with EtOAc. It was then purified over ion-exchenge resin Dowex 50 WX 8 (50-100 mesh), eluted with a solution of ammonium hydroxide 5M. After evaporation of the ammonia solution, 100 mg of final S-Dmt is isolated aswhite powder, with an overall yield of 74%.
^{1}H-NMR (400 MHz, D_2O): δ 6,43 (s, 2H); 3,6 (t, 1H, JAX =8); 3,06 (dd, 1H, JBX =8, JAB = 14); 2,8 (dd, 1H, JAX = 8, JAB = 14), 2,1 (s, 6H).

10.11 Synthesis of ethyl 7,9-dimethyl-1,4-dioxaspiro[4.5]-dec-7-ene-8-carboxylate

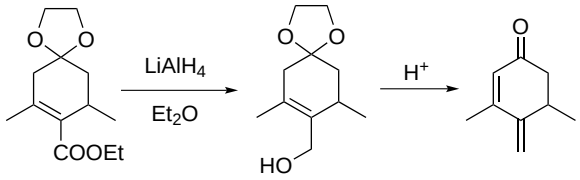


211 212

2.87 g [14.62 mmol] of Hagemann's ester **211** were refluxed under Dean-Stark apparatus in 100 ml of benzene in the presence of 2.86 ml [51.17 mmol 3.5 eq.] of ethylene glycol and a catalytic amount of p-TosOH over night.
After complete conversion in product **212**, the reaction mixture is cooled at r.t., washed with a saturated solution of $NAHCO_3$ and brine, dried over anhydrous sodium sulphate and concentrated *in vacuo* to afford 3.22 g of crude product **212**.

This latter was purified over chromatographic column eluted with EtOAc/Cy 1:3, obtaining 2.21 g of pure compound and a finale yield of 63%.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 4,2 (m, 6H), 2,9 (m, 1H), 2,4 (m, 2H), 2 (m, 2H), 1,9 (s, 3H), 1,3 (t, 3H, J=7 Hz), 1,1 (d, 3H, J= 6,5 Hz).

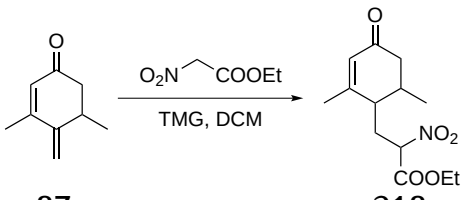
10.12 Synthesis of 3,5-dimethyl-4-methylenecyclohex-2-enone



212 213 87

Compound **212** [5,42 g] was added in a single portion to a suspension of 1 g of $LiAlH_4$ in 20 ml of diethyl ether and 20 ml of freshly distilled THF at 0°C. After stirring at this temperature for additional 20 minutes, the reaction vessel was allowed to reach room temperature.
After elimination of excess of reducing agent by carefully adding water and EtOAc to the reaction flask, the organic phase is decanted and concentrated under reduced pressure. It is then taken up with chloroform and stirred for 5 hours in the presence of 20 ml of HCl 2N.
The organic phase is the separated and dried over anhydrous sodium sulphate; finally, the organic solvent was evaporated, affording 2.9 g of target compound **87**.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 5,9 (s, 1H), 5,4 (d, 1H, J= 4 Hz), 5,3 (d, 1H, J= 4 Hz), 2,9 (m, 1H), 2,6 (m, 1H), 2,2 (m, 1H), 2 (s, 3H), 1,2 (d, 3H, J= 6,5). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 199.4, 154.1, 147.6, 127.2, 114.8, 45.4, 35.9, 20.5, 19.9.

10.13 Synthesis of ethyl 3-(2,6-dimethyl-4-oxocyclohex-2-en-1-yl)-2-nitropropanoate

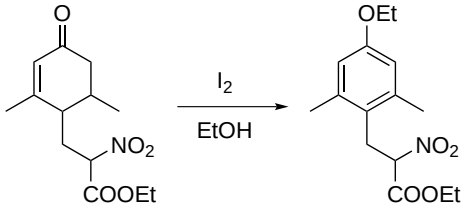


87 218

In a round bottomed-flask, 1,4 g of compound **87** is dissolved in 5 ml DCM; to this solution, 1.15 ml [1 eq.] of ethyl nitroacetate is added in the presence of 4-5 drops of TMG. The reaction is stirred at r.t. until completion over one day.

The reaction mixture is washed with HCl 1N and extracted with diethyl ether. After drying over anhydrous sodium sulphate, the organic solvent was evaporated, affording 0.9 g of target compound **218**.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 5,9 (s, 1H), 5,3 (m, 3H), 4,3 (m, 3H), 2,4 (m, 6H), 2 (s, 3H), 1,3 (m, 6H), 1 (m, 3H).

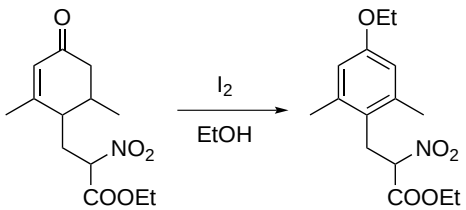
10.14 Synthesis of ethyl 3-(4-ethoxy-2,6-dimethylphenyl)--2-nitropropanoate



218 **219**

In a round-bottomed flask, 0.9 g [3.34 mmol] of compound **218** are dissolved in 10 ml of ethanol in the presence of 1.7 g [2 eq.] of molecular iodine. The reaction is then heated at refluxing conditions for 24 hours and is monitored via TLC.
After completion of the reaction, the organic phase is washed with a saturated solution of sodium thiosulfate, then a saturated solution of sodium bicarbonate and finally with brine. After drying over anhydrous sodium sulphate, the organic solvent was evaporated and the crude purified over chromatographic column eluted with EtOAc/Cy 1:3, affording the target compound **219** with a 70% yield.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6,6 (s, 2H), 4,2 (q, 2H, J= 7 Hz), 4 (q, 2H, J=6,9 Hz), 3,5 (m, 3H), 2,3 (s, 6H), 1,3 (t, 3H, J= 6,9 Hz), 1,2 (t, 3H, J= 7 Hz).

10.15 Synthesis of ethyl 2-amino-3-(4-ethoxy-2,6-dimethylphenyl)propanoate



219 **214**

Previously isolated 0.27g [2.34 mmol] of compound **219** were dissolved in ethanol; then, 0.3g of powdered tin and 1 ml of concentrated HCl were added. The reaction was stirred at room temperature for a few hours and monitored via TLC.
Upon completion, the organic phase is washed with NaOH 1M and the aqueous

phase is extracted with DCM, dried over anhydrous sodium sulphate and concentrated *in vacuo* to afford 0.2 g of **214** as a pure product.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6,6 (s, 2H), 4,2 (q, 2H, J= 7 Hz), 4,1 (m, 1H), 4 (q, 2H, J=6,9 Hz), 3 (m, 2H), 2,3 (s, 6H), 1,3 (t, 3H, J= 6,9 Hz), 1,2 (t, 3H, J= 7 Hz).

A

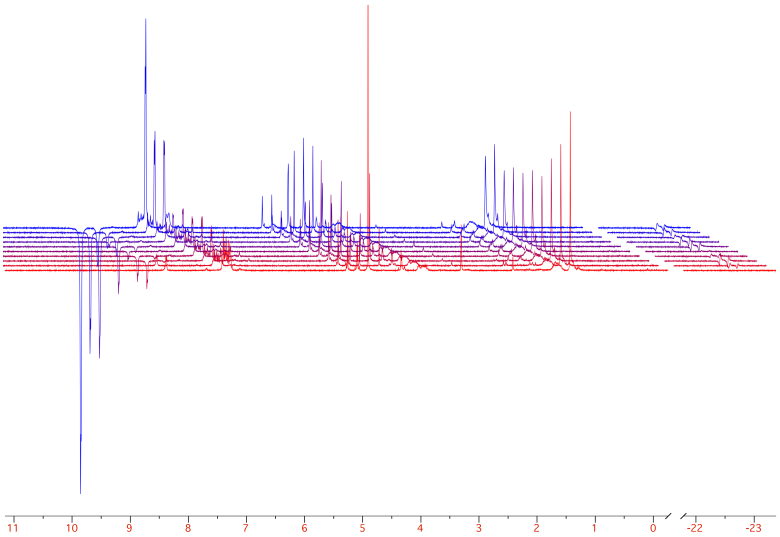
Polarization experiments

A.1 Compound Boc-Arg(Cbz)-*N*-(pyridin-4-ylmethyl)amide

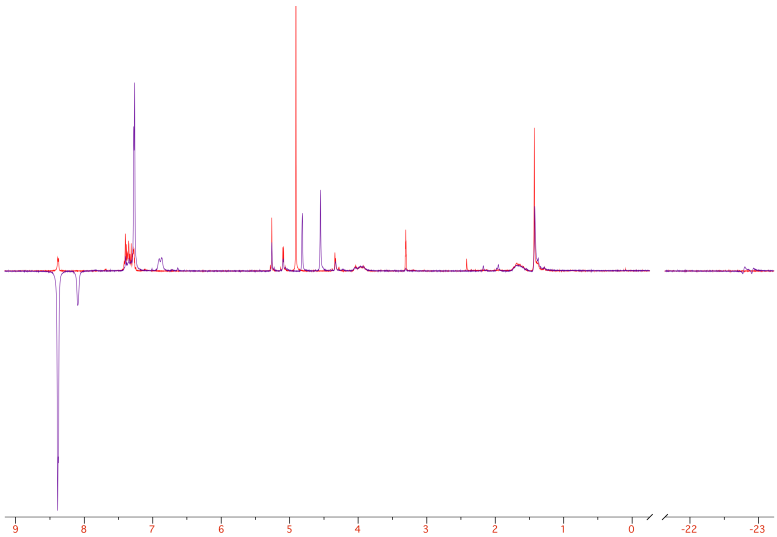
Adduct **149** was polarised as previously described.
The reference peak for calculating the enhancements was set at 8.38 ppm.

TABLE A.1.1: Polarisation test for compound Boc-Arg(Cbz)-*N*-(pyridin-4-ylmethyl)amide.

	1	3	4	6	10	11	13	14	23	24
Enhancement	1.00	0.72	−1.72	−1.58	−0.58	−3.23	−0.33	−8.77	−7.75	−17.59
T (°C)	r.t.	r.t.	r.t.	r.t.	r.t.	r.t.	r.t.	40	r.t.	60
B (G)	0	0	0	0	0	68	0	0	0	0
Shake time (sec)	8	8	8	8	8	8	8	8	20	8



(A) Superimposition of all the protonic NMR spectra for the compound.



(B) Thermal proton NMR superimposed to the one with the higher enhancement.

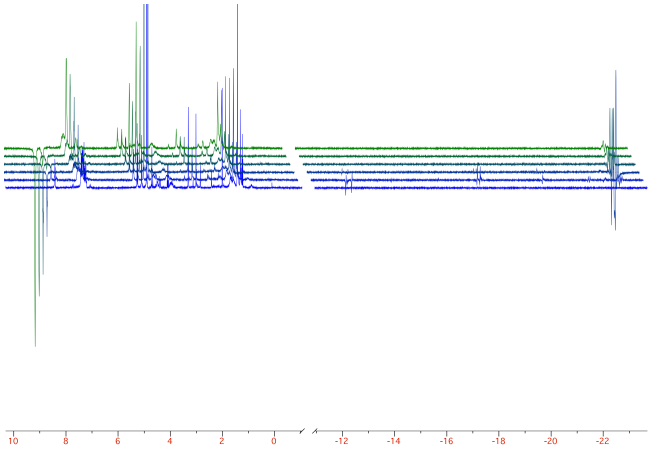
FIGURE A.1.1

A.2 Compound Boc-Arg(Cbz)-*N*-methyl-*N*-(pyridin-4-ylmethyl)amid

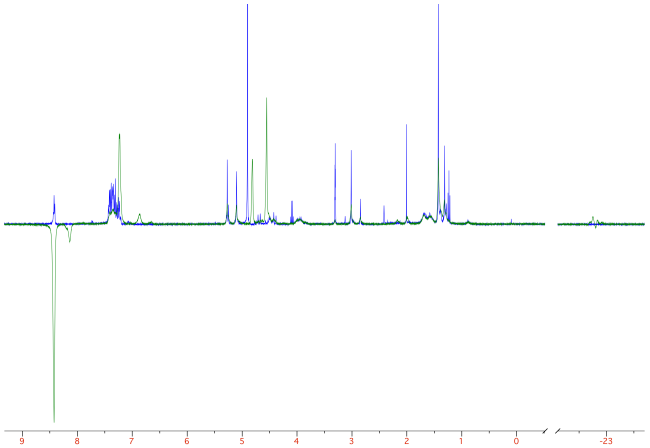
Adduct **150** was polarised as previously described.
The reference peak for calculating the enhancements was set at 8.43 ppm. The shake time was set being 8 sec.

TABLE A.2.1: Polarisation test for Boc-Arg(Cbz)-*N*-methyl-*N*-(pyridin-4-ylmethyl)amide.

	27	29	30	31	35	39
Enhancement	1.00	0.587	−2.306	−3.933	−8.013	−11.568
T (°C)	r.t.	r.t.	r.t.	r.t..	40	60



(A) Superimposition of all the protonic NMR spectra for the compound.



(B) Thermal proton NMR superimposed to the one with the higher enhancement.

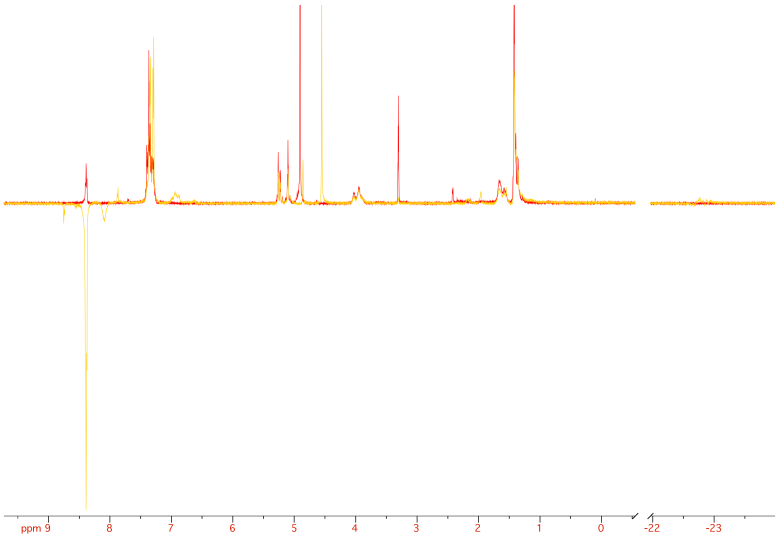
FIGURE A.2.1

A.3 Compound Boc-Arg(Cbz)-*N*-(1-(pyridin-4-yl)ethyl)amide

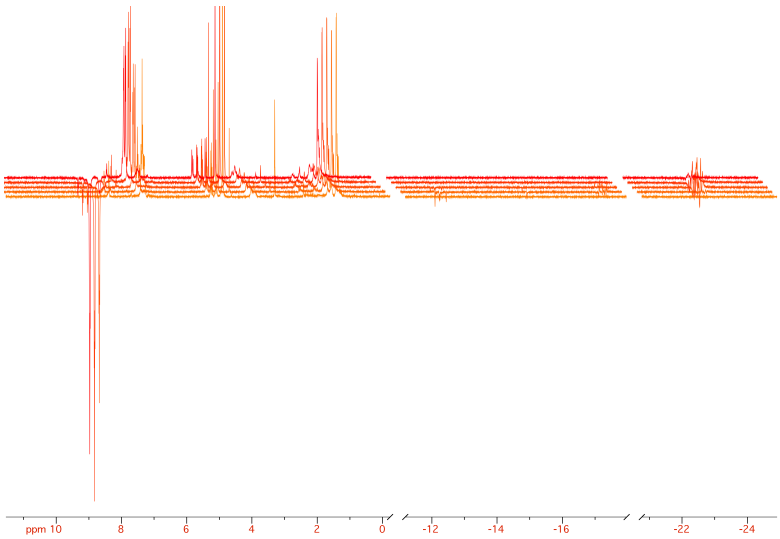
Adduct **151** was polarised as previously described.
The reference peak for calculating the enhancements was set at 8.41 ppm. The shake time was set being 8 sec.

TABLE A.3.1: Polarisation test for compound Boc-Arg(Cbz)-*N*-(1-(pyridin-4-yl)ethyl)amide.

	43	45	46	47	51
Enhancement	1.00	0.629	−5.981	−8.992	−8.358
T (°C)	r.t.	r.t.	r.t.	r.t.	40



(A) Thermal proton NMR superimposed to the one with the higher enhancement.



(B) Superimposition of all the protonic NMR spectra for the compound.

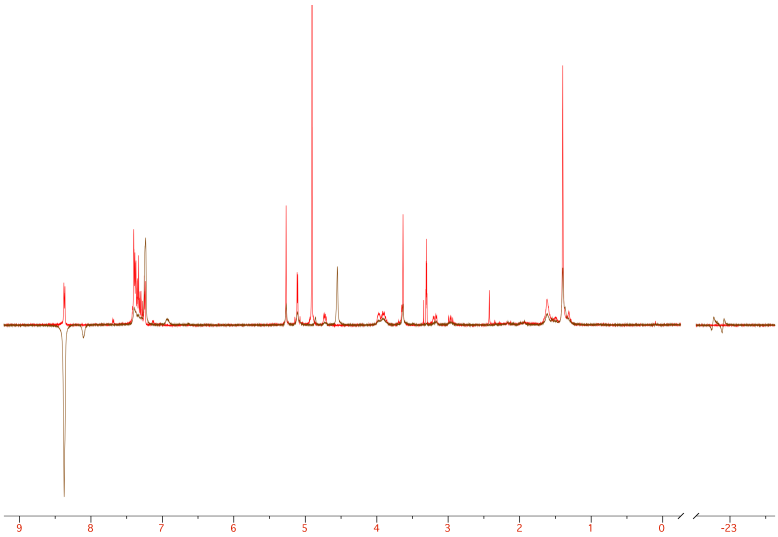
FIGURE A.3.1

A.4 Compound *(R)*-methyl 2-Boc-Arg(Cbz)-2-(pyridin-4-yl)-acetate

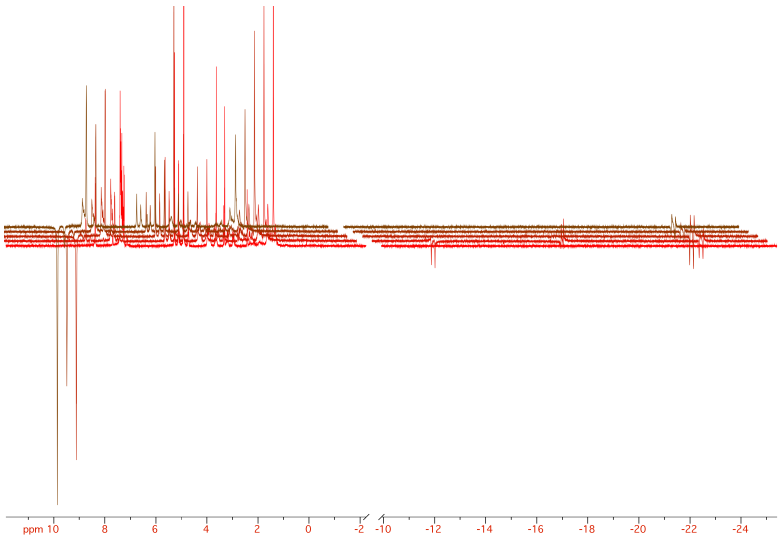
Adduct **152** was polarised as previously described.
The reference peak for calculating the enhancements was set at 8.38 ppm. The shake time was set being 8 sec.

TABLE A.4.1: Polarisation test for compound *(R)*-methyl 2-Boc-Arg(Cbz)-2-(pyridin-4-yl)acetate.

	54	56	57	58	61	
Enhancement	1.00	0.494	−4.480	−1.58	−3.375	−6.049
T (°C)	r.t.	r.t.	r.t.	r.t.	r.t.	40



(A) Thermal proton NMR superimposed to the one with the higher enhancement.



(B) Superimposition of all the protonic NMR spectra for the compound.

FIGURE A.4.1

B

Biological Assay

B.1 Protocol for testing L-arginine analogues

B.1.1 Ingredients for the NOS reaction

The ingredients for the NOS reaction were found to be from the Cayman eNOS product info sheet, cat no 60880:

1 unit eNOS (eNOS supplied in 50 mM HEPES, pH 7.4, with 10% glycerol, 5mM CHAPS and 100μM DTT);

50 mM HEPES, pH 7.4;

1mM CaCl2;

20 μg/ml calmodulin;

0.1 mM NADPH;

50 μM L-arginine or L-arginine analogue;

12 μM BH4 (tetrahydrobiopterin).

Prepare the above for a 200μl amount for each time point below (5 time points).
One Unit of eNOS produces 1 nM NO/minute at 37°C. Follow all instructions regarding storage and lab use from the eNOS data sheet, especially with regards to temperature. The assay should be performed first with L-arginine in order to draw a response curve (incubation times of 5 min, 10 min, 20 min, 40 min and 1 hour). The assay should be performed afterwards with each L-arginine analogue, also with all the incubation times above.
After incubation for x min at 37°C, the reaction is terminated by the addition of 3 ml of ice-cold stop buffer (20 mM HEPES pH: 5.5, 2 mM EDTA, 2 mM EGTA as Ca^{2+} chelators).

For all not mentioned in the following lines please refers to the Nitrate-Nitrite colorimetric assay protocol. Follow the instructions of the standard wells (A-F). Follow all the instructions of the rest of the reaction for each analogue and for each analogue concentration to test.

B.1.2 Stock solutions preparation

The stock solutions are prepared 10 times of above concentrations, so that 10 μl of each component below is used for a 100 μl reaction:

HEPES (Sigma, H3375, 25g, £22.9), Mol weight=238.3. Make stock solution 500 mM (0.5M): 11.9 g HEPES in 100 mls water Make it in 90 mls water first and pH with NaOH to 7.4, then dilute to a total of 100 mls.

$CaCl_2$ (VWR, 250g, £35), Mol weight=147.01. Make stock solution10 mM: 147 mg $CaCl_2$ dihydrate in 100 mls water.

Calmodulin (Millipore, 14-368A, 100μg, £76) Make stock solution of 200μg/ml, so 100μg into 0.5 ml water. Check the size of the bottle in which it comes as 100 μg powder, dilute with 500 μl water and aliquote to freeze at -20°C. Keep on ice at all times, whilst preparation in the lab, waiting times. From the amount calculated there will be 200 μl left over to freeze.

L-arginine (Sigma, 11009, 25g, £18), Mol weight = 174.2. Make stock solution 500 μM (0.5 mM). 1M=174.2 g/L, so 0.5M=87.1g/L, so 0.5mM=0.0871 g/L = 87.1mg in 1000 mls, therefore equivalent to adding 8.71 mg to 100 mls water. Make fresh every time it is used.

NADPH (Sigma, N1630, 25 mg, £45.8) Mol weight = 833.35. Make stock solution 1mM. Add 8.33 mg to 10 mls water. Use fresh every time, make new if more needed.

BH4 (Sigma, T4425, 5 mg, £32.2), Mol weight=314.2. Make 120 mM stock solution (this is 1000x of the reaction concentration, due to small amount of BH4 provided, so will need further dilution, see below).
Add 5 mg BH4 to 132 μl water; aliquot and freeze in 5 μl aliquots. On the day of the experiment dilute the 5 μl aliquot in 5000 μl water to get the 120 μM stock. From this, use the 10 μl for the 100 μl reaction.

For each 100 μl reaction use the following dose from the 10x concentrated stocks above:

10 μl (0.5M) HEPES
10 μl (10 mM) $CaCl_2$
10 μl (200 μg/ml) CaM
10 μl (500 μM) L-arginine
10 μl (1 mM) NADPH
10 μl (120 μM) BH4

Add them in the reaction in exact the above order. BH4 needs to be added after NADPH to prevent its oxidation.

Then add the following amounts of water/eNOS, to vary the concentration of eNOS in the reaction:

The eNOS needs to be added last and the timing of the reaction is calculated from this moment onwards.

So in total, for each reaction to happen in duplicate, at 5 time points and with 5 different concentrations of enzyme, 50 wells are needed.

At this point stop the reaction and then go onto point 2 from the first page and follow the instructions from the colorimetric assay.

eNOS quantity (U)	eNOS μl	Water (μl)
0	0	40
0.25	1.375	38.625
0.5	2.75	37.25
1	5.5	34.5
2	11	29

B.1.3 Preparation of the Analogues solutions

L-Arginine analogues: assigned molecular weights are the following.

Compound 141 : 921.19 mol;
Compound 143 : 878.19 mol;
Compound 142 : 878.19 mol;
Compound 140 : 864.17 mol.

We need 0.5 mM concentration for all of these, as with L-arginine, which are calculated as following:

Compound 141: MW = 921.19 g/mol.
Furnished 22.8 mg. Dilute in 49.4 mls water to make stock solution 0.5 mM.

$$\text{mol} = \frac{g}{MW} = \frac{0.0228}{921.19} = 0,0000247 \text{ mol}$$
$$M = \frac{\text{mol}}{\text{Vol (l)}} = \frac{0,0000247 \text{ mol}}{0,0494 \text{ l}} = 0,0005 \text{ mol/l} = 0.5 \text{ mM} = 500\mu M$$

Compound 143: MW = 878.19 g/mol.
Furnished 23.1 mg. Dilute in 52.6 mls water to make stock solution 0.5 mM.

$$\text{mol} = \frac{g}{MW} = \frac{0.0231}{878,19} = 0,0000263 \text{ mol}$$
$$M = \frac{\text{mol}}{\text{Vol (l)}} = \frac{0,0000263 \text{ mol}}{0,0526 \text{ l}} = 0,00050 \text{ mol/l} = 0.50 \text{ mM} = 500\mu M$$

Compound 142: MW = 878.19 g/mol.
Furnished 23 mg. Dilute in 52.2 mls water to make stock solution 0.5 mM.

$$\text{mol} = \frac{g}{MW} = \frac{0.023}{878,19} = 0,0000261 \text{ mol}$$
$$M = \frac{\text{mol}}{\text{Vol (l)}} = \frac{0,0000261 \text{ mol}}{0,0522 \text{ l}} = 0,00050 \text{ mol/l} = 0.50 \text{ mM} = 500\mu M$$

Compound 140: MW = 864.17 g/mol.
Furnished 21.9 mg. Dilute in 50.7 mls water to make stock solution 0.5 mM.

$$\text{mol} = \frac{g}{MW} = \frac{0.0219}{864,17} = 0,0000253 \text{ mol}$$
$$M = \frac{\text{mol}}{\text{Vol (l)}} = \frac{0,0000253 \text{ mol}}{0,0507 \text{ l}} = 0,0005 \text{ mol/l} = 0.50 \text{ mM} = 500\mu M$$

B.1.4 Preparation of the Stop solution

Quantity to prepare: 100 mls.

20 mM HEPES pH = 5.5, 2 mM EDTA, 2 mM EGTA as Ca^{2+} chelators. Make stock solutions 10x more concentrated so that 10 mls of each stock solution below can be used for a total of 100 mls stop solution:

1. HEPES (Sigma, H3375, 25g, £22.9), Mol weight=238.3. Make stock 200 mM: 1M concentration is 238.3 g/L, so 200 mM=47.66 g/L or 4.76 g in 100 mls. Put 4.76 g in 90 mls water, pH with HCl to 5.5 and then add water to 100 mls.

2. EDTA, mol mass = 372.24. Make stock 20 mM: 1M concentration is 372.24 g/L, so 20 mM = 740 mg in 100 mls.

3. EGTA, mol mass=380.35. Make stock 20 mM: 1M concentration =380.35 g/L, so 20 mM = 760 mg in 100 mls.

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1,4-Dithiane-2,5-diol as an efficient synthon for a straightforward synthesis of functionalized tetrahydrothiophenes via sulfa-Michael/aldol-type reactions with electrophilic alkenes

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ABSTRACT

'One-pot' tandem reactions of commercially available 1,4-dithiane-2,5-diol (the dimer of mercaptoacetaldehyde) with electrophilic alkenes resulted in the facile formation of substituted tetrahydrothiophene derivatives. Thus, sulfa-Michael/Henry and sulfa-Michael/aldol sequences provided polysubstituted tetrahydrothiophenes using in situ generated nitroalkenes and α,β-unsaturated carbonyl compounds as the electrophilic partners of mercaptoacetaldehyde dimer, respectively.

1. Introduction

The tetrahydrothiophene moiety is the core structural component of many natural products, bioactive compounds, and synthetic intermediates. Tetrahydrothiophene-based compounds include the essential coenzyme biotin **1**, a water-soluble vitamin involved in important biological functions,[1] the cholecystokinin type-B receptor antagonist tetronothiodin **2**,[2] the nucleoside **3** showing potent activity against human cytomegalovirus,[3] and glucosidase inhibitors, such as kotalanol **4**[4] and salacinol **5**[5] (Fig. 1).

Furthermore, tetrahydrothiophene derivatives have been used in a range of chemical transformations, including asymmetric hydrogenation,[6] catalytic asymmetric epoxidation,[7] and catalytic intramolecular cyclopropanation.[8] The synthetic usefulness and the wide range of biological activities give tetrahydrothiophenes a privileged role in organic chemistry. Accordingly, various approaches to these interesting scaffolds have been developed, the earliest and present most common ones being listed in our recent paper dealing with the synthesis of nitrohydroxylated tetrahydrothiophenes by 'one-pot' tandem sulfa-Michael/Henry reactions.[9]

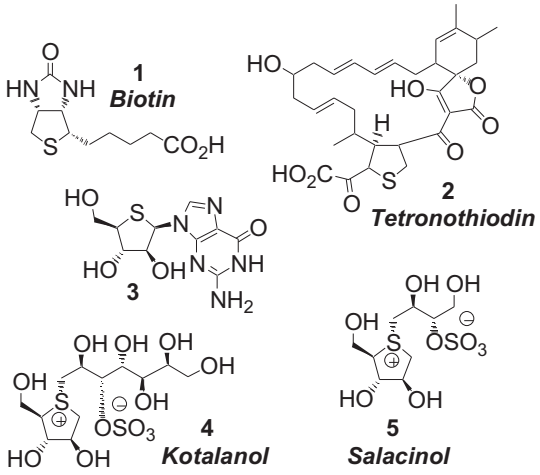

Fig. 1. Structure of bioactive tetrahydrothiophenes **1**–**5**.

2. Results and discussion

Our studies entailed the use of 1,4-dithiane-2,5-diol **6**, the mercaptoacetaldehyde dimer, as a convenient and efficient synthon incorporating a thiol group able to add to in situ generated

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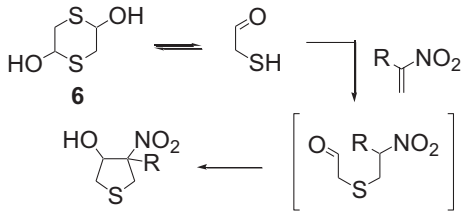
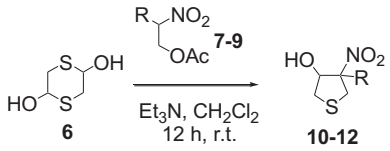
nitroalkenes. The derived nitroalkane adducts provided 4-nitro-tetrahydrothiophen-3-ol scaffolds through a subsequent intramolecular nitroaldol reaction (Scheme 1).



Scheme 1. General approach to the synthesis of 4-nitro-tetrahydrothiophen-3-ol derivatives via tandem reactions.

Thus, tandem sulfa-Michael/Henry sequences smoothly took place by reaction of 2-nitroethylacetates **7**–**9**, used as stable precursors for the corresponding nitroalkenes, with dimer **6** in dichloromethane containing triethylamine providing good yields of the expected 4-nitro-tetrahydrothiophen-3-ols **10**–**12** as 1.5:1 mixtures of diastereomers (Table 1).[9,10] The ratio was determined by integration of characteristic signals in their ^{1}H NMR spectra.

Table 1
Synthesis of tetrahydrothiophene compounds **10**–**12**

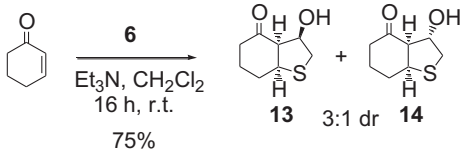


Nitroacetate	R	Product (dr)	Yield[a] (%)
7	H	**10** (1.5:1)	65
8	CH_2OH	**11** (1.5:1)	70
9	$(CH_2)_2OCH_2OMe$	**12** (1.5:1)	80

[a] Isolated yield after purification by column chromatography.

Quite surprisingly, the simple two-carbon atom unit incorporating a thiol group and an additional electrophilic functionality, such as the aldehydic group, has been only occasionally used in domino reactions with α,β-unsaturated carbonyl compounds.[11]

We envisioned the reaction of 1,4-dithiane-2,5-diol **6** with cyclohexenone as a straightforward route to the hexahydro-benzothiophen-4-one nucleus, the scaffold of a diterpenoid isolated from the seeds of Japanese Morning Glory (*Ipomoea violacea*),[12] which seems to regulate the activity of gibberellin A_3. Indeed, the reaction, performed in dichloromethane containing catalytic triethylamine (5 mol %), proceeded smoothly via a sulfa-Michael/aldol reaction sequence leading to the formation in good yield (75%) of a 3:1 mixture of the diastereomeric hexahydro-benzothiophen-4-ones **13** and **14**, easily separated by column chromatography (Scheme 2).



Scheme 2. Synthesis of tetrahydrothiophenes **13** and **14**.

The structure of the prevalent compound **13** has been unequivocally assigned through single-crystal X-ray analysis (Fig. 2).[13]

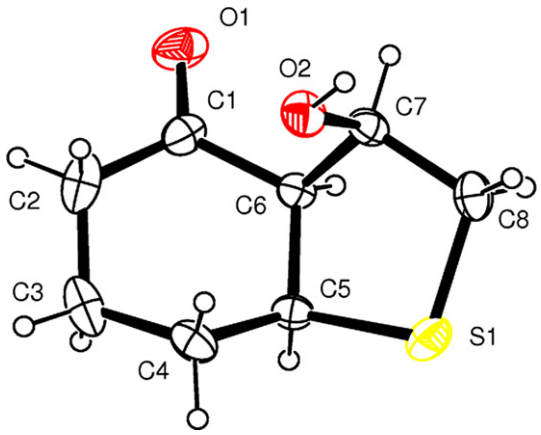
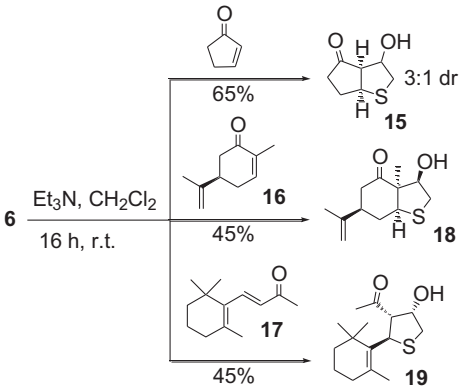


Fig. 2. ORTEP view of compound **13** displaying the thermal ellipsoids at 30% probability.

Analogously, use of different α,β-unsaturated ketones as partners of mercaptoacetaldehyde dimer in the tandem Michael-aldol reaction process resulted in the efficient preparation of monocyclic and bicyclic 3-hydroxythiophanes in good yields and high diastereoselectivity.

As shown in Scheme 3, treatment of **6** with cyclopentenone under the same conditions as above gave rise to an inseparable diastereomeric mixture of bicyclic derivatives **15** (3:1 ratio from ^{1}H NMR, 65% yield), while single stereoisomers **18** and **19** could be obtained in 45% yield through reaction of **6** with (*S*)-carvone **16** and β-ionone **17**, respectively.



Scheme 3. Synthesis of tetrahydrothiophenes **15**, **18**, and **19**.

The stereochemistry of compound **18** was tentatively assigned by NOE experiments, while X-ray crystallographic analysis allowed us to assign the structure of tetrahydrothiophene **19** (Fig. 3).[13]

In the context of our studies, we considered also dehydroalanine esters as counterparts of mercaptoacetaldehyde dimer in the tandem sulfa-Michael/aldol reaction process. These compounds have been largely utilized as Michael acceptors for conjugate addition reactions[14] even though typically considered poor electrophiles due to the electron-donating effects of the nitrogen lone pair. However, to the best of our knowledge, dehydroalanine esters have not been hitherto employed in tandem reaction processes. Therefore, we were intrigued to test the reactivity of differently *N*-

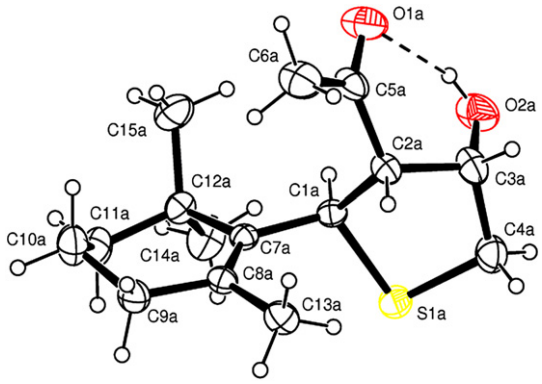
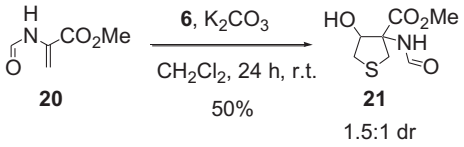


Fig. 3. ORTEP view of compound **19** displaying the thermal ellipsoids at 30% probability.

protected dehydroalanine esters in cascade reactions with a simple bifunctional reagent, such as mercaptoacetaldehyde dimer.

Thus, the reaction between **6** and methyl 2-formamidoacrylate **20**, in turn easily obtained from serine methyl ester hydrochloride and methyl formate as described in the literature,[15] performed in dichloromethane at room temperature in the presence of potassium carbonate gave the interesting 3,4-trisubstituted tetrahydrothiophene **21** in satisfactory yield (50%) as an inseparable 1.5:1 mixture of diastereomers (Scheme 4). The ratio was determined by integration of characteristic signals in their ^{1}H NMR spectra.



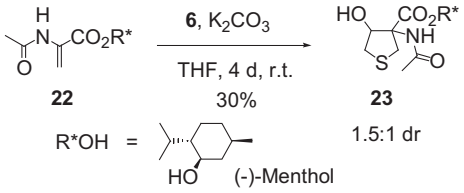
Scheme 4. Synthesis of tetrahydrothiophene **21**.

Unsuccessful attempts to induce asymmetry in this domino process using conventional organocatalysts (e.g., quinine and some derived thioureas, proline and (*S*)-diphenylprolinol TMS ether) led us to turn our attention to a chiral auxiliary-assisted approach using (−)-menthyl 2-acetamidoacrylate **22**, which could be conveniently obtained through a known two-step procedure.[16]

Menthyl esters have been conveniently applied in the copper-promoted 1,4-conjugate addition of phenylmagnesium bromide to chiral 2-acetamidoacrylates to produce *N*-acetylphenylalanine esters in high chemical yields and good diastereoselectivity.[17]

Based on these findings, we were confident that the domino reaction between mercaptoacetaldehyde dimer and (−)-menthyl 2-acetamidoacrylate **22** could be stereocontrolled by the bulky chiral auxiliary group.

Treatment of **22** with **6** in THF at room temperature in the presence of potassium carbonate provided tetrahydrothiophene **23** in disappointing low yield (30%) and diastereoselectivity (dr 1.5:1 from ^{1}H NMR) (Scheme 5). All attempts to improve the reaction outcome proved unsuccessful.



Scheme 5. Synthesis of tetrahydrothiophene **23**.

Notwithstanding, compound **23** could be considered a very interesting intermediate to accomplish a new synthetic approach to (−)-4-amino-2-thiabicyclo-[3.1.0]hexane-4,6-dicarboxylate **24**, or its *S*-oxidized variants **25** and **26** (Fig. 4), which have been shown to be highly potent and selective agonists of metabotropic glutamate receptors 2 (mGlu2) and 3 (mGlu3).[18]

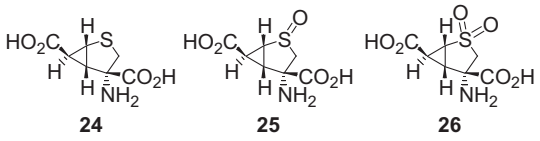
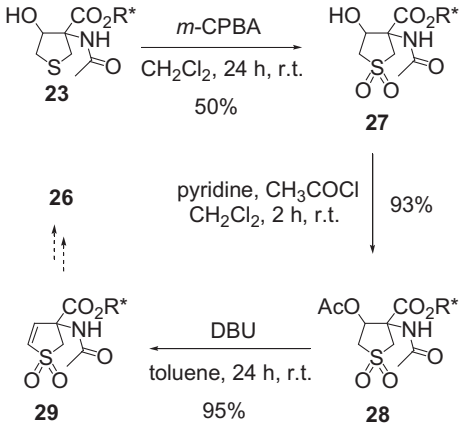


Fig. 4. Agonists of metabotropic glutamate receptors.

As shown in Scheme 6, tetrahydrothiophene **23** has been readily converted into the dihydrothiophene compound **29**, which represents an advanced intermediate toward **26**.



Scheme 6. Synthesis of compound **29**.

Thus, *m*-chloroperbenzoic acid (*m*-CPBA) oxidation of **23** produced the intermediate sulfone **27** (50% yield), which took part in a subsequent acylation step providing the acetoxy derivative **28** in 93% yield. The latter has been eventually taken to the target compound **29** through a quantitative DBU-promoted elimination reaction.

3. Conclusion

In summary, we have developed highly efficient tandem reactions to form tetrahydrothiophene ring systems using the mercaptoacetaldehyde dimer as a common and convenient starting material. The diverse functional groups in the products obtained will permit further manipulation for synthesizing bioactive compounds.

4. Experimental

4.1. General methods

Melting points were determined on a Büchi-Tottoli apparatus.

IR spectra were recorded using a Perkin–Elmer FT-IR SPECTRUM 100 spectrophotometer equipped with ATR (diamond/ZnSe serial No. 14031), and only the more representative frequencies (cm^{-1}) are reported.

^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer. Chemical shifts (δ) are given in parts per million and coupling constants (J) in Hertz. Data are reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

Solvents were distilled prior to use and reactions were performed under nitrogen or argon atmosphere. Organic solutions were dried over anhydrous magnesium sulfate and evaporated

with a rotary evaporator. Chromatographic purifications were carried out using 70–230 mesh silica gel.

Nitroacetates **7** and **8** were prepared from commercially available 2-nitroethanol and acetyl chloride by adopting the same procedure used for the synthesis of the corresponding nitrobenzoates,[19] while nitroacetate **12** was obtained through already reported directions.[20]

4.2. General procedure for the preparation of compounds 10–12

A solution of nitroacetate (1.5 mmol) in CH_2Cl_2 (2 mL) was added to a stirred suspension of 1,4-dithiane-2,5-diol **6** (0.75 mmol) in CH_2Cl_2 (2 mL) containing triethylamine (1.65 mmol). The reaction mixture was stirred at room temperature for 12 h, then the solvent was evaporated. The residual oil was purified by flash chromatography (silica gel, EtOAc/cyclohexane 1:4).

*4.2.1. 4-Nitro-tetrahydrothiophen-3-ol (**10**).* Oil (0.14 g, 65%). Data for the major isomer: IR (neat) 3410, 1545 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.81 (dd, J=7.0, 14.0 Hz, 1H), 3.07 (dd, J=7.0, 14.0 Hz, 1H), 3.34 (dd, J=7.0, 14.0 Hz, 1H), 3.38 (dd, J=7.0, 14.0 Hz, 1H), 4.80 (q, J=7.0 Hz, 1H), 5.06 (q, J=7.0 Hz, 1H), 5.90 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 31.9, 37.3, 72.7, 93.9; $C_4H_7NO_3S$ (149.17): calcd C 32.21, H 4.73, N 9.39; found C 32.23, H 4.70, N 9.35. Selected data for the minor isomer: ^1H NMR (300 MHz, DMSO-d_6) δ 2.78 (dd, J=7.0, 14.0 Hz, 1H), 3.03 (dd, J=6.8, 13.6 Hz, 1H), 3.30 (dd, J=7.0, 13.0 Hz, 1H), 3.35 (dd, J=7.0, 13.0 Hz, 1H), 4.71–4.82 (m, 2H), 5.60 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 31.9, 37.2, 73.0, 94.0.

*4.2.2. 4-Hydroxymethyl-4-nitro-tetrahydrothiophen-3-ol (**11**).* Oil (0.19 g, 70%). Data for the major isomer: IR (neat) 3400, 1540 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.87 (dd, J=7.2, 14.6 Hz, 1H), 3.10 (d, J=8.2 Hz, 1H), 3.35 (dd, J=7.2, 14.6 Hz, 1H), 3.57 (d, J=8.2 Hz, 1H), 3.90 (d, J=14.0 Hz, 1H), 4.30 (d, J=14.0 Hz, 1H), 4.72 (br s, 1H), 5.60 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 36.3, 37.6, 60.5, 71.8, 94.7; $C_5H_9NO_4S$ (179.20): calcd C 33.51, H 5.06, N 7.82; found C 33.55, H 5.08, N 7.84. Selected data for the minor isomer: ^1H NMR (300 MHz, $CDCl_3$) δ 3.10 (dd, J=4.0, 13.5 Hz, 1H), 3.15 (d, J=8.0 Hz, 1H), 3.20 (dd, J=6.8, 13.5 Hz, 1H), 3.61 (d, J=8.0 Hz, 1H), 3.80 (dd, J=15.0, 5.0 Hz, 1H), 3.95 (dd, J=4.5, 15.0 Hz, 1H), 4.18 (s, 2H), 5.10 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.8, 38.6, 72.0, 96.0.

*4.2.3. 4-(2-Methoxymethoxy-ethyl)-4-nitro-tetrahydrothiophen-3-ol (**12**).* Oil (0.28 g, 80%). Data for the major isomer: IR (neat) 3460, 1545 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6, 160 °C) δ 2.25 (t, J=6.0 Hz, 1H), 2.46 (t, J=6.0 Hz, 1H), 2.85 (dd, J=6.0, 15.0 Hz, 1H), 3.20 (d, J=14.0 Hz, 1H), 3.30 (dd, J=6.0, 15.0 Hz, 1H), 3.38 (s, 3H), 3.60 (m, 3H), 3.65 (d, J=14.0 Hz, 1H), 4.58 (s, 2H), 5.20 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.3, 36.4, 37.6, 55.5, 64.5, 71.3, 94.0, 97.3; $C_8H_{15}NO_5S$ (237.27): calcd C 40.50, H 6.37, N 5.90; found C 40.53, H 6.33, N 5.88. Selected data for the minor isomer: ^1H NMR (300 MHz, DMSO-d_6, 160 °C) δ 2.35 (t, J=6.0 Hz, 1H), 2.39 (t, J=6.0 Hz, 1H), 3.05 (dd, J=3.0, 15.0 Hz, 1H), 3.30 (dd, J=5.0, 15.0 Hz, 1H), 3.35 (s, 3H), 3.65 (m, 3H), 4.62 (s, 2H), 5.30 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.2, 36.0, 55.7, 63.4, 76.0, 98.5.

4.3. General procedure for the preparation of compounds 13, 14, 15, 18, and 19

A suspension of dithiane **6** (1.30 mmol), enone (2.60 mmol), and triethylamine (0.13 mmol) in CH_2Cl_2 (8 mL) was stirred at room temperature for 16 h. After this time, the reaction mixture was concentrated under reduced pressure and the residue obtained was purified by column chromatography (silica gel, EtOAc/cyclohexane 1:4).

*4.3.1. 3-Hydroxy-hexahydro-benzo[b]thiophen-4-one (**13**).* White solid (0.25 g, 57%); mp 68–70 °C. IR (neat) 3500, 1715 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.80–1.90 (m, 2H), 2.20–2.55 (m, 6H), 3.10 (m, 1H), 3.80 (m, 1H), 4.35 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.8, 30.3, 38.1, 39.6, 47.0, 59.9, 76.9, 207.9; $C_8H_{12}O_2S$ (172.25): calcd C 55.78, H 7.02; found C 55.71, H 7.10.

*4.3.2. 3-Hydroxy-hexahydro-benzo[b]thiophen-4-one (**14**).* White solid (0.08 g, 18%); mp 78–79 °C. IR (neat) 3500, 1715 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.80–1.90 (m, 2H), 1.95–2.60 (m, 6H), 2.87 (m, 1H), 3.20 (m, 1H), 4.20 (m, 1H), 5.00 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.6, 28.2, 35.3, 42.1, 44.5, 56.0, 77.7, 214.5; $C_8H_{12}O_2S$ (172.25): calcd C 55.78, H 7.02; found C 55.71, H 7.10.

*4.3.3. 3-Hydroxy-hexahydro-cyclopenta[b]thiophen-4-one (**15**).* White solid (0.27 g, 65%). Data for the major isomer: IR (neat) 3600, 1745 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.10–2.40 (m, 4H), 2.70 (m, 1H), 2.85 (m, 1H), 3.15 (m, 1H), 3.90–4.10 (br s, 1H), 4.02 (m, 1H), 4.60 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.8, 36.8, 38.1, 58.2, 58.3, 75.3, 218.1; $C_7H_{10}O_2S$ (158.22): calcd C 53.14, H 6.37; found C 53.20, H 6.28. Selected data for the minor isomer: ^1H NMR (300 MHz, $CDCl_3$) δ 2.09–2.38 (m, 2H), 2.60 (m, 1H), 2.80 (m, 1H), 3.21 (m, 1H), 3.80–4.00 (br s, 1H), 4.30 (m, 1H), 4.80 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.5, 36.2, 39.7, 58.2, 58.4, 55.8, 75.4, 220.5.

*4.3.4. (3R,3aR,6S,7aS)-3-Hydroxy-6-isopropenyl-3a-methyl-hexahydro-benzo[b]thiophen-4-one (**18**).* Oil (0.26 g, 45%). IR (neat) 3300, 1715 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.95 (s, 3H), 1.63 (m, 1H), 1.66 (s, 3H), 2.00–2.40 (m, 4H), 2.82 (dd, J=12.0, 8.0 Hz, 1H), 3.09 (t, J=7.5 Hz, 1H), 3.23 (dd, J=12.0, 7.5 Hz, 1H), 4.61 (s, 1H), 4.72 (s, 1H), 4.84 (t, J=7.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.5, 20.4, 36.3, 37.2, 42.7, 44.5, 49.7, 65.4, 74.5, 109.9, 149.3, 200.0; $C_{12}H_{18}O_2S$ (226.34): calcd C 63.68, H 8.02; found C 63.75, H 7.90.

*4.3.5. 1-[4-Hydroxy-2-(2,6,6-trimethyl-cyclohex-1-enyl)-tetrahydrothiophen-3-yl]-ethanone (**19**).* White solid (0.31 g, 45%). IR (neat) 3400, 1725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 0.85 (s, 3H), 1.05 (s, 3H), 1.40–1.60 (m, 5H), 1.98 (s, 3H), 2.00 (m, 1H), 2.40 (s, 3H), 3.00 (d, J=9.0 Hz, 1H); 3.30 (m, 1H), 3.60 (dd, J=9.0, 5.0 Hz, 1H), 4.20 (s, 1H), 4.50 (d, J=10.0 Hz, 1H), 4.80 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.3, 20.4, 29.0, 30.8, 34.4, 37.3, 38.3, 39.2, 51.2, 63.4, 79.7, 133.7, 135.0, 211.3; $C_{15}H_{24}O_2S$ (268.42): calcd C 67.12, H 9.01; found C 67.20; H 8.90.

4.4. Synthesis of compounds 21, and 27–29

*4.4.1. 3-Formylamino-4-hydroxy-tetrahydrothiophene-3-carboxylic acid, methyl ester (**21**).* Dithiane **6** (0.94 g, 6.20 mmol) and K_2CO_3 (1.71 g, 12.4 mmol) were added to a solution of acrylate **20** (1.60 g, 12.4 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred at room temperature for 24 h, then filtered and evaporated. The residue obtained was purified by column chromatography (silica gel, EtOAc/cyclohexane 3:1) to furnish tetrahydrothiophene **21** (1.27 g, 50%) as an oil. Data for the major isomer: IR (neat) 3450, 1740, 1670 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.82–3.17 (m, 2H), 3.18 (d, J=10.7 Hz, 1H), 3.60 (d, J=10.7 Hz, 1H), 3.81 (s, 3H), 4.62 (t, J=7.1 Hz, 1H), 6.70 (br s, 1H), 8.23 (s, 1H); the ^{13}C NMR data have not been recorded as the compound slowly decomposes under the long accumulation times required to obtain the spectrum; $C_7H_{11}NO_4S$ (205.23): calcd C 40.97, H 5.40, N 6.82; found C 41.06, H 5.33, N 6.75. Selected data for the minor isomer: ^1H NMR (300 MHz, $CDCl_3$)

δ 2.75–3.01 (m, 3H), 3.50 (d, *J*=10.0 Hz, 1H), 3.75 (s, 3H), 4.80 (t, *J*=7.0 Hz, 1H), 6.40 (br s, 1H), 8.15 (s, 1H).

*4.4.2. 3-Acetylamino-4-hydroxy-tetrahydrothiophene-3-carboxylic acid, (−)-menthyl ester (**23**).* K_2CO_3 (0.22 g, 1.58 mmol) and a few drops of triethylamine were added to a stirred suspension of dithiane **6** (0.24 g, 1.58 mmol) and acrylate **22** (0.42 g, 1.58 mmol) in THF (4 mL). The reaction mixture was stirred at room temperature for 4 days, then filtered, and evaporated. The residue obtained was purified by column chromatography (silica gel, EtOAc/cyclohexane 1:1) to furnish tetrahydrothiophene **23** (0.16 g, 30%) as an oil. Data for the major isomer: IR (neat) 3500, 1660, 1750 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.75 (d, *J*=7.0 Hz, 3H), 0.90 (d, *J*=7.0 Hz, 6H), 0.95–2.20 (m, 9H), 2.05 (s, 3H), 2,85 (dd, *J*=7.0, 14.0 Hz, 1H), 3.05 (d, *J*=14.2 Hz, 1H), 3.15 (dd, *J*=6.8, 14.0 Hz, 1H), 3.55 (d, *J*=14.2 Hz, 1H), 4.58 (m, 1H), 4.75 (m, 1H), 6.50 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.5, 20.6, 22.3, 23.1, 23.6, 28.1, 30.9, 34.1, 37.6, 38.4, 41.2, 47.1, 74.9, 76.4, 78.5, 169.9, 171.8; $C_{17}H_{29}NO_4S$ (343.48): calcd C 59.44, H 8.51, N 4.08; found C 59.50, H 8.47, N 4.00. Selected data for the minor isomer: ^1H NMR (300 MHz, $CDCl_3$) δ 0.70 (d, *J*=7.0 Hz, 3H), 0.89 (d, *J*=7.0 Hz, 6H), 0.95–1.95 (m, 9H), 2.04 (s, 3H), 2.80 (dd, *J*=14.0, 7.0 Hz, 1H), 3.10 (d, *J*=14.0 Hz, 1H), 3.20 (dd, *J*=7.0, 14.0 Hz, 1H), 3.60 (d, *J*=14.0 Hz, 1H), 4.80 (m, 1H), 6.40 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.5, 20.0, 23.1, 24.2, 24.8, 27.9, 32.0, 35.0, 38.5, 39.6, 40.3, 46.1, 75.2, 77.1, 79.1, 170.0, 172.0.

*4.4.3. 3-Acetylamino-4-hydroxy-1,1-dioxo-tetrahydro-1λ⁶-thiophene-3-carboxylic acid, (−)-menthyl ester (**27**).* A solution of **23** (0.27 g, 0.80 mmol) in CH_2Cl_2 (10 mL) was treated with *m*-chloroperbenzoic acid (0.49 g, 2.00 mmol, 70%) and stirred at room temperature for 24 h. Aqueous saturated $NaHCO_3$ solution was added under stirring and the mixture was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were washed with brine, dried, and evaporated under reduced pressure, yielding **27** (0.15 g, 50%) as an oil, which was sufficiently pure to be used in the next step without further purification. Data for the major isomer: IR (neat) 3450, 1660, 1750 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.78 (d, *J*=7.0 Hz, 3H), 0.90 (d, *J*=7.0 Hz, 6H), 0.95–2.20 (m, 9H), 2.05 (s, 3H), 3.35 (dd, *J*=6.5, 14.0 Hz, 1H), 3.45 (dd, *J*=6.5, 14.0 Hz, 1H), 3.65 (d, *J*=14.4 Hz, 1H), 4.15 (d, *J*=14.4 Hz, 1H), 4.58 (m, 1H), 4.75 (m, 1H), 6.90 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.6, 20.7, 22.3, 23.1, 23.6, 28.1, 30.9, 34.1, 38.4, 47.1, 59.0, 59.8, 70.6, 73.5, 78.5, 169.7, 171.8; $C_{17}H_{29}NO_6S$ (375.48): calcd C 54.38, H 7.78, N 3.73; found C 54.35, H, 7.82, N 3.78. Selected data for the minor isomer: ^1H NMR (300 MHz, $CDCl_3$) δ 0.72 (d, *J*=7.0 Hz, 3H), 0.89 (d, *J*=7.0 Hz, 6H), 0.95–1.95 (m, 9H), 2.04 (s, 3H), 3.30 (dd, *J*=7.0, 14.0 Hz, 1H), 3.40 (dd *J*=7.0, 14.0 Hz, 1H), 3.70 (d, *J*=14.0 Hz, 1H), 4.20 (d, *J*=14.0 Hz, 1H), 4.70 (m, 1H), 4.80 (m, 1H), 6.50 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.5, 20.6, 22.1, 23.2, 24.1, 28.9, 31.0, 35.2, 38.7, 47.6, 59.3, 60.1, 71.2, 77.6, 79.5, 170.1, 172.3.

*4.4.4. Synthesis of 4-acetoxy-3-acetylamino-1,1-dioxo-tetrahydro-1λ⁶-thiophene-3-carboxylic acid, (−)-menthyl ester (**28**).* Pyridine (0.16 mL, 2.00 mmol) and acetyl chloride (0.14 mL, 2.00 mmol) were added to a solution of compound **27** (0.15 g, 0.40 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred for 2 h at room temperature, then water was added, the organic layer separated and sequentially washed with HCl 1 N and aqueous saturated $NaHCO_3$ solution. The organic extracts were dried and evaporated to give crude **28** (0.15 g, 93%) as an oil, which was used in the next step without further purification. Data for the major isomer: IR (neat) 1660, 1735, 1750 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.78 (d, *J*=7.0 Hz, 3H), 0.90 (d, *J*=7.0 Hz, 6H), 0.95–2.20 (m, 9H), 2.10 (s, 3H), 2.18 (s, 3H), 3.41 (dd, *J*=6.2, 14.0 Hz, 1H), 3.65 (dd, *J*=6.2, 14.0 Hz, 1H), 4.05 (d, *J*=16.0 Hz, 1H), 4.10 (d, *J*=16.0 Hz, 1H), 4.58 (m, 1H), 5.65 (m, 1H), 6.35 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.5 (×2), 21.3, 22.3, 23.1, 23.6, 28.1, 30.9, 34.1, 38.4, 47.1, 56.9, 59.4, 72.8, 73.9, 78.5, 168.6, 171.2, 171.8; $C_{19}H_{31}NO_7S$ (417.52): calcd C 54.66, H 7.48, N 3.35; found: C 54.60, H 7.55, N 3.40. Selected data for the minor isomer: ^1H NMR (300 MHz, $CDCl_3$) δ 0.75 (d, *J*=7.0 Hz, 3H), 0.87 (d, *J*=7.0 Hz, 6H), 0.95–2.00 (m, 9H), 2.15 (s, 3H), 2.50 (s, 3H), 3.40 (dd, *J*=7.0, 14.0 Hz, 1H), 3.75 (dd *J*=7.0, 14.0 Hz, 1H), 4.00 (d, *J*=14.0 Hz, 1H), 4.15 (d, *J*=14.0 Hz, 1H), 4.80 (m, 1H), 5.60 (m, 1H), 6.50 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.5 (×2), 21.8, 23.2, 23.8, 29.0, 31.0, 38.7, 38.9, 47.6, 59.5, 60.1, 71.2, 76.6, 79.5, 170.2, 173.0.

*4.4.5. Synthesis of 3-acetylamino-1,1-dioxo-2,3-dihydro-1H-1λ⁶-thiophene-3-carboxylic acid, (−)-menthyl ester (**29**).* Compound **28** (0.22 g, 0.53 mmol) was dissolved in toluene (8 mL) and treated with DBU (0.16 mL, 1.06 mmol). The reaction mixture was stirred at room temperature for 24 h and evaporated. The oily residue was dissolved in CH_2Cl_2 (10 mL) and sequentially washed with HCl 1 N and aqueous saturated $NaHCO_3$ solution. The organic extracts were dried and evaporated, and the crude product was purified by column chromatography (silica gel, EtOAc/cyclohexane 1:2) to give **29** (0.18 g, 95%) as an oil. IR (neat) 1660, 1735 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.78 (d, *J*=7.0 Hz, 3H), 0.90 (d, *J*=7.0 Hz, 6H), 0.95–2.02 (m, 9H), 2.03 (s, 3H), 3.50 (d, *J*=16.0 Hz, 1H), 3.95 (d, *J*=16.0 Hz, 1H), 4.75 (m, 1H), 6.80 (d, *J*=7.0 Hz, 1H), 7.45 (d, *J*=7.0 Hz, 1H), 9.20 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.7, 20.8, 21.9, 22.7, 23.0, 26.0, 31.4, 33.9, 40.0, 46.7, 56.8, 63.5, 78.3, 135.0, 137.0, 167.1, 170.0; $C_{17}H_{27}NO_5S$ (357.47): calcd C 57.12, H 7.61, N 3.92; found C 57.20, H 7.55, N 3.85.

4.5. X-ray structure determinations of compounds 13 and 19

X-ray diffraction data for compounds **13** and **19** were collected at room temperature, 295 K, on a Nonius Kappa CCD diffractometer with graphite monochromated Mo Kα radiation (λ=0.7107 Å). The structures were solved by direct methods (SIR97)[21] and refined (SHELXL-97)[22] by full matrix least squares with anisotropic non-hydrogen atoms. For compound **13** the hydrogen atoms were refined isotropically while for compound **19** the hydrogens were included on calculated positions, riding on their carrier atoms, except the O–H ones, which were refined isotropically.

Crystal data: **13**, $C_8H_{12}O_2S$; monoclinic, space group $P2_1/a$, *a*=11.5646(3), *b*=6.3291(2), *c*=12.5747(4) Å, β=114.702(1)°, *V*=836.16(4) $Å^3$, *Z*=4, D_c=1.368 g cm^{-3}. Intensity data collected with θ≤30°; 2393 independent reflections measured; 2070 observed [$I>2\sigma(I)$]. Final *R* index=0.0433 (observed reflections), *wR*=0.1138 (all reflections), *S*=1.023. CCDC N. 827486.

ORTEP[23] view of compound **13** is shown in Fig. 2. The molecules in the crystal are linked in chains by means of intermolecular O2–H···O1(1/2+*x*, −1/2−*y*, *z*) hydrogen bond with O2···O1 distance of 2.830(2) Å.

Compound **19**, $C_{15}H_{24}O_2S$; monoclinic, space group $P2_1/a$, *a*=15.0396(3), *b*=7.2266(1), *c*=28.0275(7) Å, β=104.504(1)°, *V*=2949.1(1) $Å^3$, *Z*=8, D_c=1.209 g cm^{-3}. Intensity data collected with θ≤26°; 5767 independent reflections measured; 3570 observed [$I>2\sigma(I)$]. Final *R* index=0.0483 (observed reflections), *wR*=0.1247 (all reflections), *S*=1.009. CCDC N. 827487.

The asymmetric unit contains two independent molecules. ORTEP[23] view of molecule A is shown in Fig. 3. Both molecules display an intramolecular O2–H···O1 hydrogen bond having O2···O1 distances of 2.737(3) and 2.750(3) Å, for molecules A and B, respectively.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2011.10.064.

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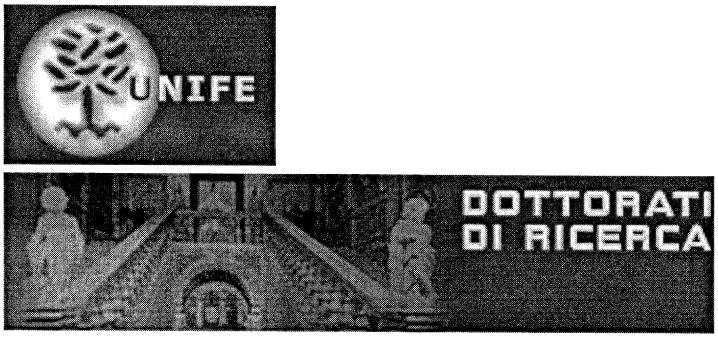

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NOTA: Il lavoro è composto con il font *Palatino*, di Hermann Zapf. Le strutture chimiche sono state disegnate tramite *CS ChemDraw*.

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