Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Clinical and Molecular Medicine

Eivind Halvorsen Brenna

Synthesis of Scopularide A and B

Master's thesis in Pharmacy
Supervisor: Eirik Johansson Solum
September 2020

NTNU
Norwegian University of Science and Technology

OSLOMET
OSLO METROPOLITAN UNIVERSITY
STORBYUNIVERSITETET

Eivind Halvorsen Brenna

Synthesis of Scopularide A and B

Master's thesis in Pharmacy
Supervisor: Eirik Johansson Solum
September 2020

Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Clinical and Molecular Medicine

NTNU
Norwegian University of
Science and Technology

Synthesis of Scopulariside A and B

Master's thesis

Eivind Halvorsen Brenna

List of abbreviations and symbols (alphabetical)

Ac	Acetyl
Ala	Alanine
Ar	Aryl
Boc	*tert*-butoxycarbonyl
Bz	Benzyl
°C	Degrees Celsius
$CDCl_3$	Deuterated chloroform
DBBT	Dibutylboron trifluoromethanesulfonate
DCM	Dichloromethane
d	Doublet (NMR)
dd	Doublet of doublet (NMR)
ddd	Doublet of doublet of doublet (NMR)
dddd	Doublet of doublet of doublet of doublet (NMR)
δ	chemical shift (NMR)
DIPEA	*N, N*-diisopropylethylamine
DMAP	4-*N, N*-dimethylaminopyridine
DMF	*N, N*-dimethylformamide
Dq	double quartet (NMR)
dsept	double septet (NMR)
E	Entgenen (enolate)
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
Eq	Equivalent(s)
FDPP	Pentafluorophenyl diphenylphosphinate
G	Gram
Gly	Glycine
^1H	Proton

HOBt	Hydroxybenzotriazole
Hz	Hertz
IPr	isopropyl
Leu	Leucine
LiBr	Lithium bromide
m	Multiplet (NMR)
MeOH	Methanol
MHz	Megahertz
mL	Millilitre
mmol	Millimol
min	Minutes
NaHMDS	Sodium bis(trimethylsilyl)amide
n-BuLi	n-butyllithium
NHC	*N*-heterocyclic carbene
NMP	*N*-Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
No.	Number
NOESY	Nuclear overhauser effect spectroscopy
oct	Octet (NMR)
OH	Hydroxyl
OMe	Methyl ether/ester
Phe	Phenylalanine
ppm	Parts per million
quint	quintet (NMR)
s	Singlet (NMR)
sext	sextet (NMR)
tert	tertiary
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography

Val	Valine
Z	zusammen (enolate)

Abstract

Scopularide A and B is a part of a family of cyclohexadepsipeptides who has shown to have significant effect against tumor growth in certain tumor cell lines. To study this effect in more detail, a synthetic pathway to produce these molecules were attempted in this project.
The synthesis strategy was to synthesize two major fragments, a peptide fragment and a non-peptide fragment, and then to cyclize the molecules.
The synthesis of the peptide fragment was completed using standard liquid phase coupling reagents and conditions. The synthesis of the non-peptide fragment was planned to be completed in three steps, in either of two ways. The two paths differed in the first step, either including a Negishi cross-coupling or an asymmetric alkylation, then both paths required a reduction, before an asymmetric aldol addition completed the non-peptide fragment. To finalize the molecules a lactamization was planned to cyclize the two fragments into Scopularide A and B.

Acknowledgements

This master's thesis was carried out at OsloMet in the period between August 2019 and September 2020. This was a cooperation project between the Department of Clinical and Molecular Medicine, NTNU and Department of Life Sciences and Health, OsloMet.

I would like to thank my supervisor, Dr. Eirik Johansson Solum, for presenting me to a very exciting and interesting project, and for always being available with his insight and theoretical knowledge.

I would also like to express gratitude towards my other supervisor, Dr. Bjørg Siw Møller Tannæs, who spent countless hours discussing and providing motivation through the inevitable dark periods of this kind of project.

My family and friends deserve an apology for my retraction from normal social interaction these last few months and should be applauded for their patience.

I want to give great thanks to professor Frode Rise and Dirk Petersen for all the practical and theoretical help and valuable advice related to NMR experiments.

I am also grateful towards Karina Ervik, who has faced my never-ending supply of dry humour bravely and generally made the lab-work more enjoyable.

Table of content:

Chapter 1: Introduction

1.1 Introduction

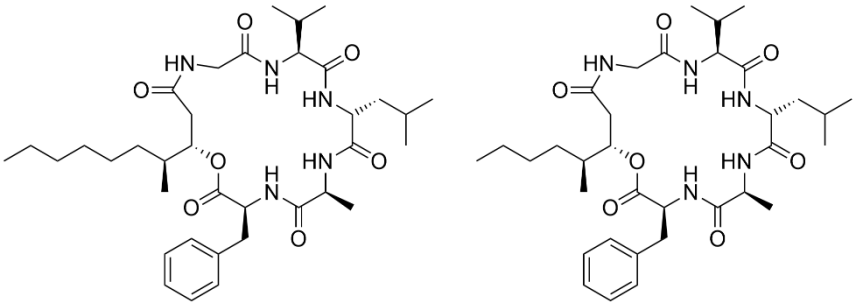
When penicillin and natural antibiotics were discovered nearly 100 years ago, it was thought that problems with infectious diseases would disappear.
History would show us otherwise, as antibiotic resistance among microorganisms developed quickly and now it is currently listed by WHO as one of the biggest threats to global health.[1, 2]
As bacterial resistance to antibiotics became apparent, the search for new compounds with antibiotic effect intensified.

As the most available eco-systems to humans are the terrestrial ones, most of the exploration into new compounds have been done on terrestrial life.[3]
However, as the terrestrial world is relatively well mapped, the marine world is relatively unexplored. Although the search for novel drugs from life forms in maritime environments is still in an early phase, there has been many successes, and it is generally viewed to be a vast source of information.[4]

In the search of natural products with antibiotic effect, it has also been discovered compounds with other beneficial effects.
The discovery and study of Actinomycin D was the first natural compound that revealed other significant uses than antibiotics, as it showed anti-cancerous activity in its ability to inhibit transcription in cells.[5]
The aftermath of this discovery has been an increase in the focus on the anti-cancer effect of natural compounds, and many similar molecules with similar or other anti-cancer effects have been found.[3]
However, like the ongoing search for new antibiotics, there's a similar search to find new compounds for new cancer treatments, and to optimize the current treatments.[6]

Among the discoveries both in terrestrial and maritime environment are a group of cyclic depsipeptides (CDP) derived from fungi. These CDP's have been reported to exhibit various biological activities, such as cytotoxic, antimicrobial and antitumoral activities.[7]

Among these CDP's a family of cyclohexadepsipeptides known as *Scopularides* has been discovered.[8]



Scopularide A (**1a**) Scopularide B (**1b**)

Figure 1.1.1: *Scopularide A (**1a**) and B (**1b**).*

The focus of this project are the Scopularides A (**1a**) and B (**1b**), which has been identified and isolated from the marine sponge-derived fungus *Scopulariopsis brevicaulis* as well as from the coral-derived fungus *Penicillium chrysogenum*.[8, 9]
These scopularides have shown weak antibiotic activity against bacteria, but significant cytotoxic activity against colon- and pancreatic tumour cell lines.[8]

Nevertheless, due to the small amounts isolated from the fungi, a synthetic route to acquire these compounds could prove significant. Synthesis on large scale would open for more elaborate testing of the molecules, as well as serve as a base for production if they should be applied in medicine.

1.2 Retrospective analysis

The synthetic strategy for assembling Scopularide A (**1a**) and B (**1b**) were envisioned by completing the following main steps:

1. Synthesizing the peptide fragment (either as tetra- or pentapeptide).
2. Synthesizing the non-peptide fragment with different alkyl chain lengths.

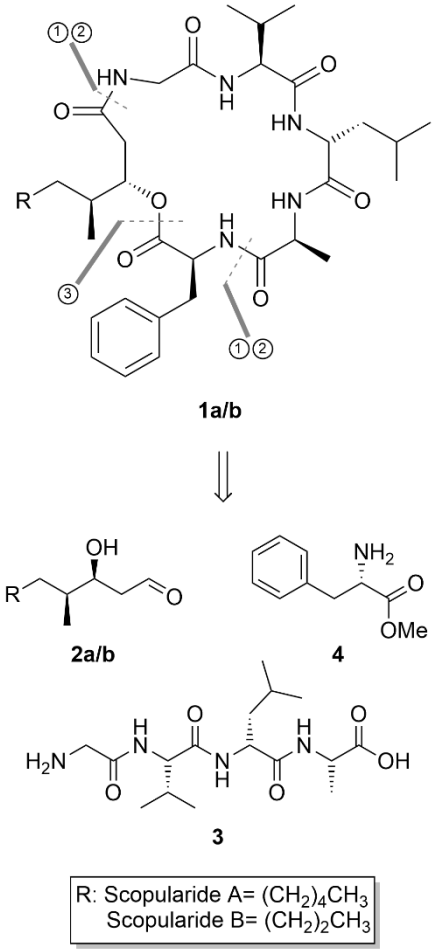


Figure 1.2.1: *Retrosynthetic analysis of the Scopularides (**1a/b**).*

These steps were broken down further into the following reactions:

① Peptide couplings, to couple together the amino acids and peptides.

② A macrolactamization, as one alternative for cyclization of the molecule or

③ a macrolactonization, as the other alternative of cyclization of the molecule.

④ A Negishi cross-coupling, to get the different length alkyl chains on the non-peptide fragment.

⑤ An aldol addition, to create the necessary aldol molecule with hydroxyl-group for completing the macrolactonization/esterification.

⑥ Alkylation, as an alternative path to the Negishi-reaction, as to add different lengths of alkyl chains to the non-peptide fragment.

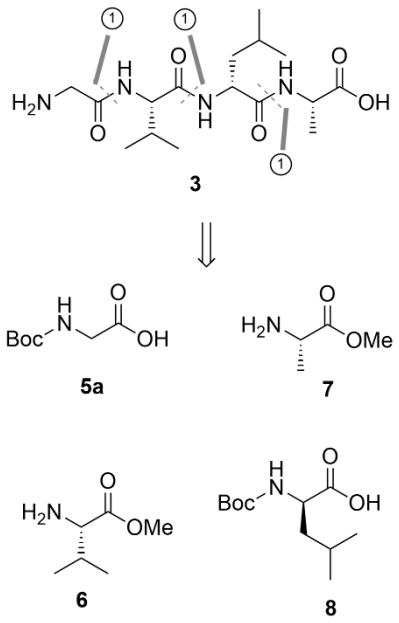


Figure 1.2.2: *Retrosynthetic analysis of tetrapeptide (**3**).*

The tetrapeptide (**3**) consists of Gly (glycine), L-Val (valine), D-Leu (leucine) and L-Ala (alanine) in this respective order (figure 1.2.2). The commercially available amino acids used in the project were the salts of Boc-protected Gly (**5a**), Leu (**8**) and the esters of Val (**6**) and Ala (**7**) (and Phe (**4**)).

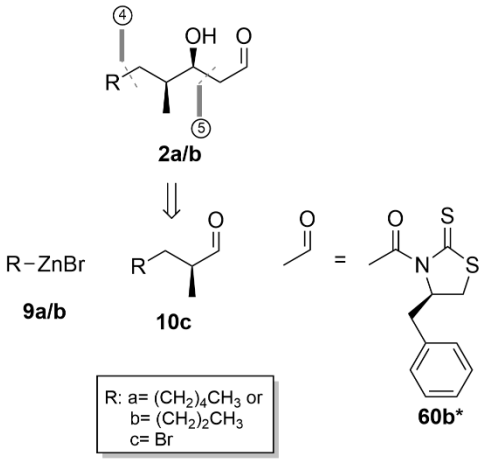


Figure 1.2.3: *Retrosynthetic analysis of non-peptide fragment (**2a/b**).*
**This synthon was replaced with a chiral auxiliary*

The retrospective analysis (figure 1.2.3) of the non-peptide fragment (**2a/b**) yields 3 synthons when analysed. Of these synthons, compound **10c** were commercially available for a reasonable price, and was decided to be the starting point of the synthesis of the non-peptide fragment (**2a/b**). The two other synthons were decided based on this starting molecule, and to add the alkyl chain, it was decided to use a Negishi cross-coupling reaction, making the ZnBr-compound (**9a/b**) necessary. To produce the chiral centre connected to the hydroxyl group of **2a/b**, a stereoselectivity-inducing compound would have to be applied, and the decision to use a chiral auxiliary (**60b**) was made.

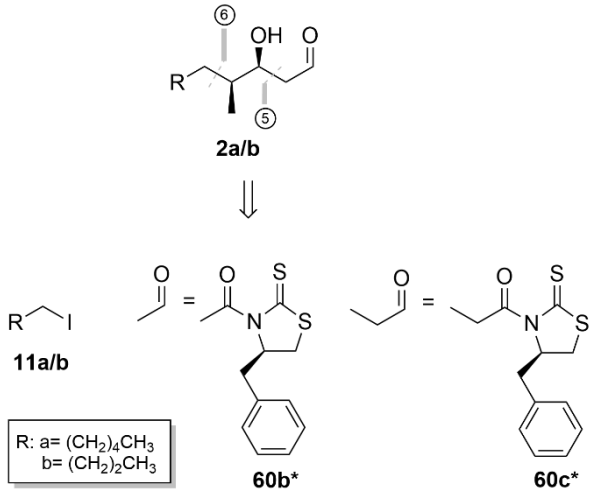


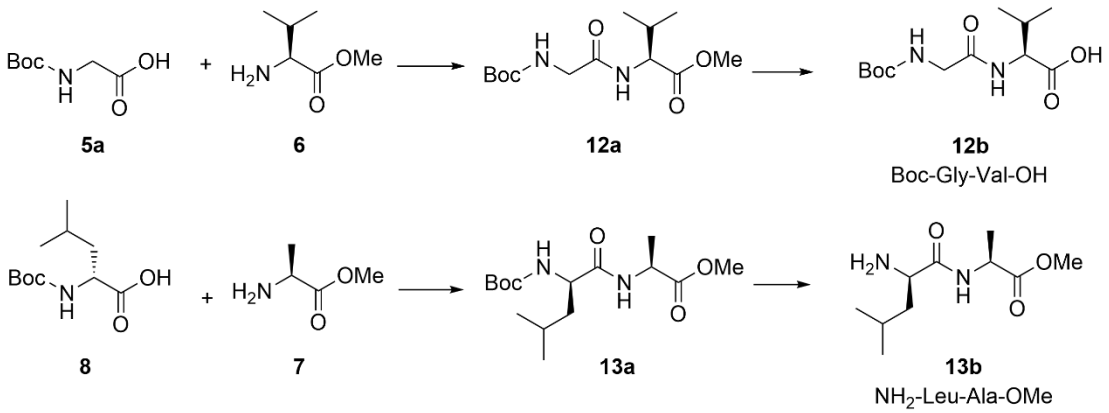
Figure 1.2.4: *Alternative retrosynthetic analysis of non-peptide fragment (**2a/b**).*
**This synthon was replaced with a chiral auxiliary*

A contingency plan was also made (figure 1.2.4), as an alternative way of synthesising the non-peptide fragment (**2a/b**). The same plan for achieving the chiral centre connected to the hydroxyl group of **2a/b**, but an additional chiral auxiliary would have to be used to make the other chiral centre of the molecule. An asymmetric alkylation of

a chiral auxiliary was the reaction that was decided upon to produce this second chiral centre.

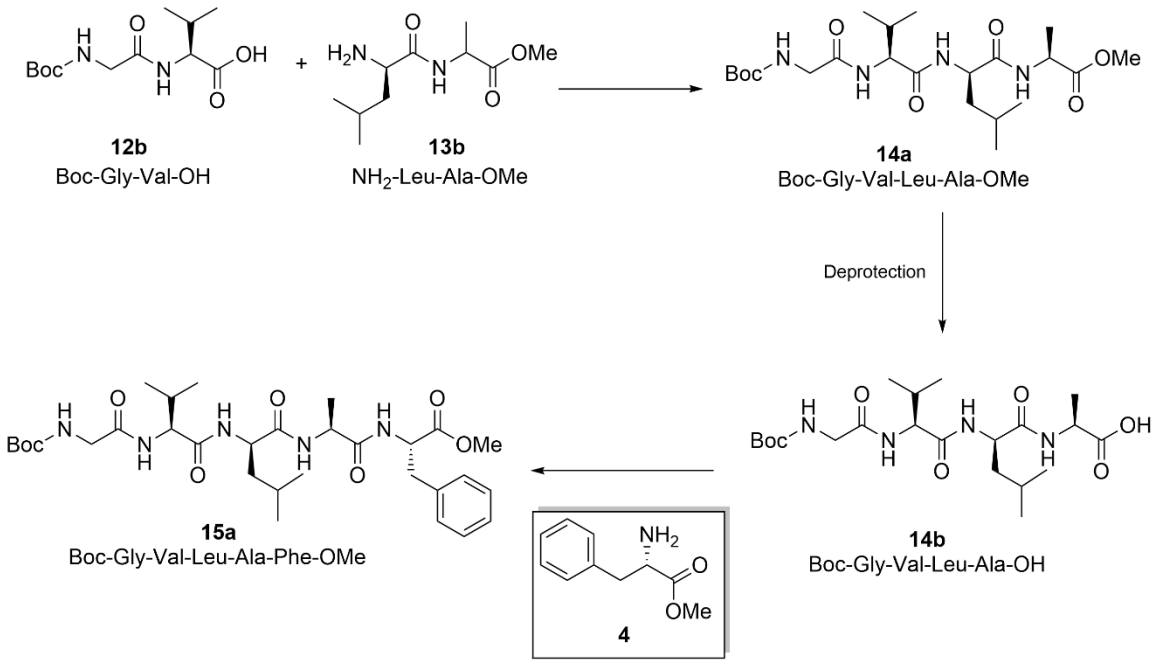
1.3 General plan

This aim of the project was to synthesise Scopulariide A and B, following the steps described in the retrospective analysis. The rough idea was to synthesise the peptide fragment (scheme 1.3.1 and 1.3.2), then synthesise the non-peptide fragment separately (scheme 1.3.3) before connecting the two fragments and finally cyclizing the molecule (scheme 1.3.4). As a general plan, it seemed reasonable to arrange the project chronologically along this line of though.



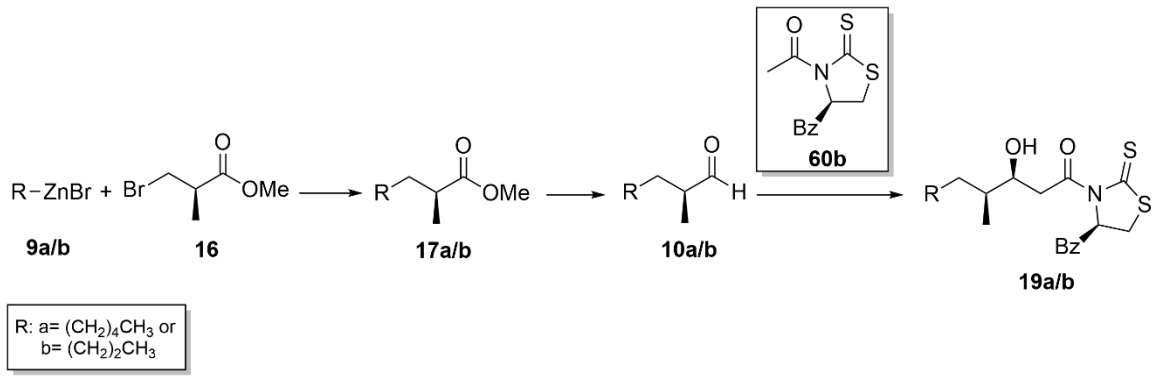
Scheme 1.3.1: *Plan for the synthesis of dipeptides.*

The strategy was to synthesise two dipeptides, using standard peptide coupling techniques and protecting groups (scheme 1.3.1). Then to use these two dipeptides to synthesise the tetrapeptide (scheme 1.3.2).



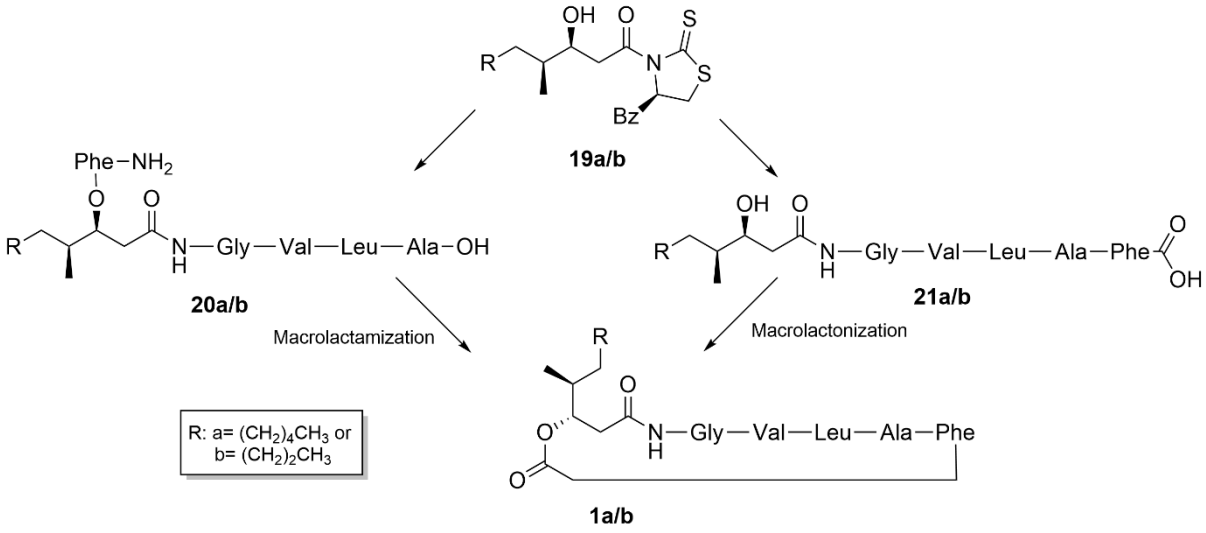
Scheme 1.3.2: *Plan for the synthesis of tetra- and pentapeptide.*

The synthesis of the peptide fragment were left somewhat open at the last stages, as it was thought that the cyclization could either be tried with a lactonization technique or a lactamization technique.
However, the same techniques would be applied for completing the tetra- and pentapeptides as were used to complete the dipeptides.



Scheme 1.3.3: *Plan for non-peptide fragment synthesis.*

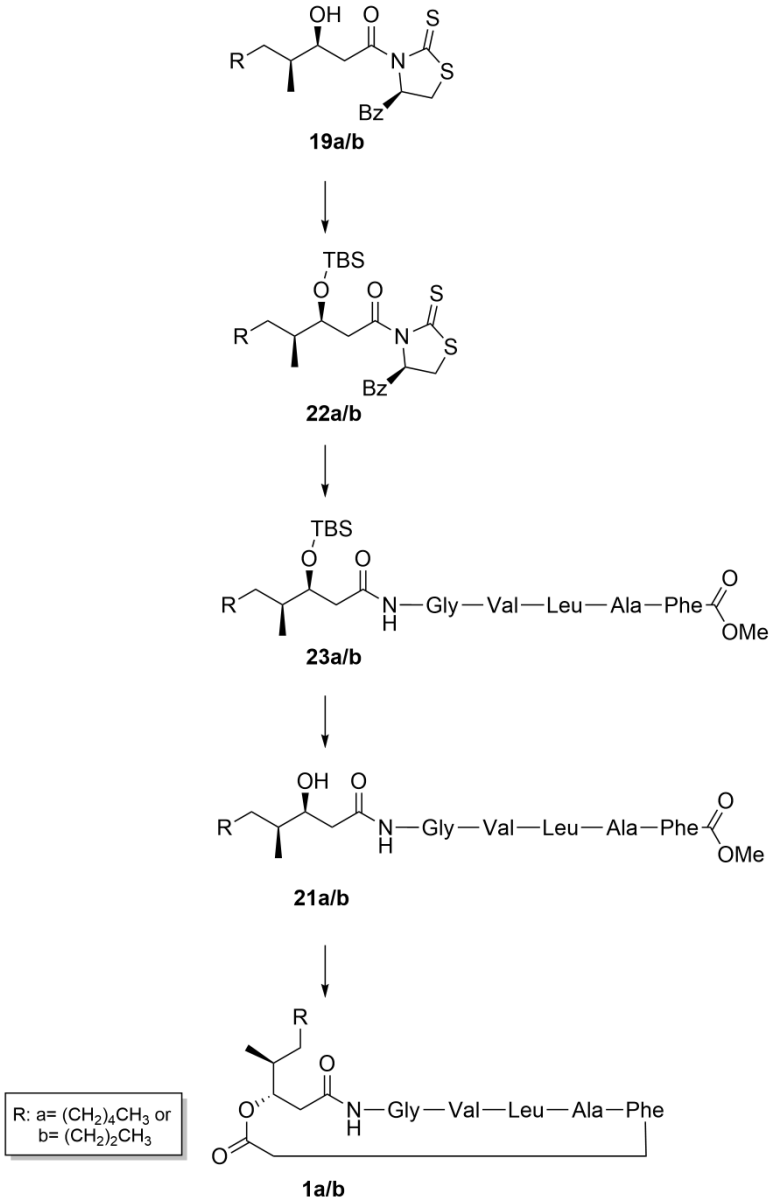
The synthesis strategy for the non-peptide fragment of the scopularides was based on a sp^3-sp^3 Negishi cross-coupling reaction followed by a reduction of the ester, before finally using an aldol addition to complete the non-peptide fragment (scheme 1.3.3). The aldol addition is necessary to create the necessary alcohol and to inducing the right stereochemistry needed to perform the cyclization.



Scheme 1.3.4: *Coupling the fragments and cyclization.*

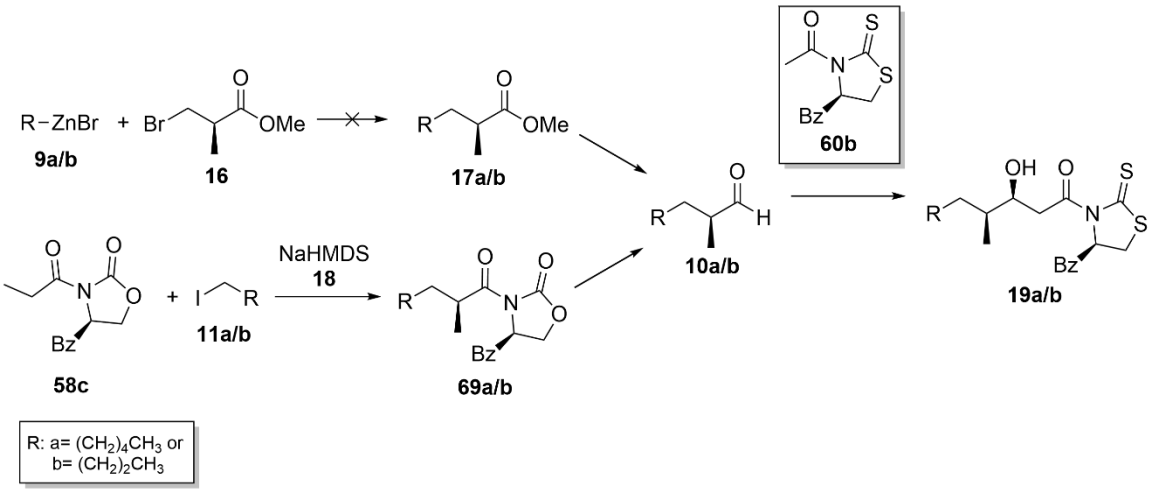
The last part of the plan was to couple the non-peptide fragment (**19**) together with either the pentapeptide (**15**), followed by a lactonization, or coupled to the tetrapeptide (**14**) and the Phe (**4**), followed by a lactamization. (scheme 1.3.4).

The layout of the general plan for the synthesis of Scopularides A and B are shown and described in these schemes, but a multitude of methods of completing each step were discussed. As some of the methods were tested and deemed unsuccessful, there were several additions and adjustments to the plan as the project moved forward.



Scheme 1.3.5: *Protection/deprotecting of hydroxyl group.*

An example of the necessity of adjusting the project was as the protection of the hydroxyl groups in the molecule became apparent, so an addition of a hydroxyl-protection step was added (**scheme 1.3.5**). And as this hydroxyl group became protected to hinder the occurrence of unwanted side reactions to this group, and later to deprotect the same hydroxyl group as its deprotection was essential for the cyclization of the molecule in the final steps.



Scheme 1.3.6: *Alternative path to the non-peptide fragment.*

Another big change in the plan was when the Negishi cross-coupling had to be replaced by another reaction designed to ultimately get the same non-peptide fragment.

Chapter 2: Background information and theory

2.1 Peptide coupling

There is predominantly two ways of synthesising peptides, which is either a solid phase or liquid phase peptide synthesis. Each of these techniques have their own advantages, but the focus of this project was the liquid phase synthesis.

The plan was to synthesise two dipeptides, Gly-L-Val (**12a**) and D-Leu-L-Ala (**13a**) before coupling them together to from a tetrapeptide (**14a**). The final step of the peptide synthesis would be to either add the last amino acid, L-Phe (**4**) to form the complete pentapeptide fragment (**15a**) to use in a lactonization or to use the tetrapeptide (**14a**) and L-Phe (**4**) to complete a lactamization (scheme 1.3.4). The dipeptides and the tetra- and pentapeptide is depicted in figure 2.1.1.

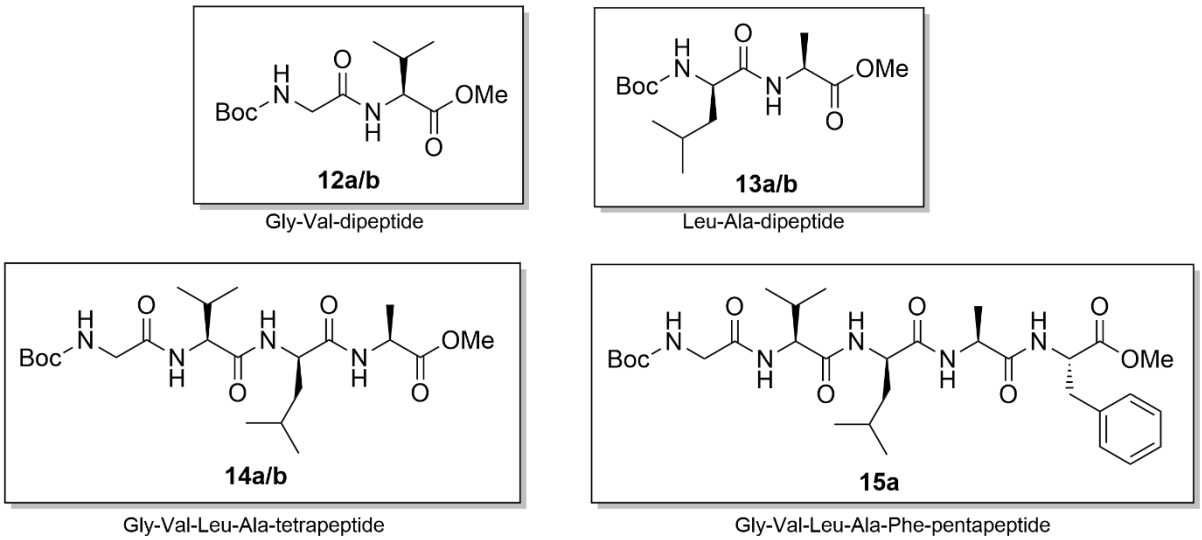


Figure 2.1.1: *The peptides needed to complete the scopularides.*

The procedure was based on the work done by T.K Chakraborty, and the synthesis of dipeptides were carried out using commercially available amino acids, where one amino acid is protected on the *N*-terminus with a Boc-group and the other amino acid is an ester hydrochloride (figure 2.1.2**).**[10]

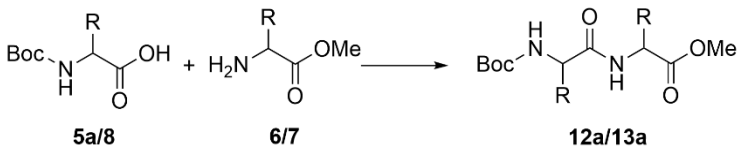
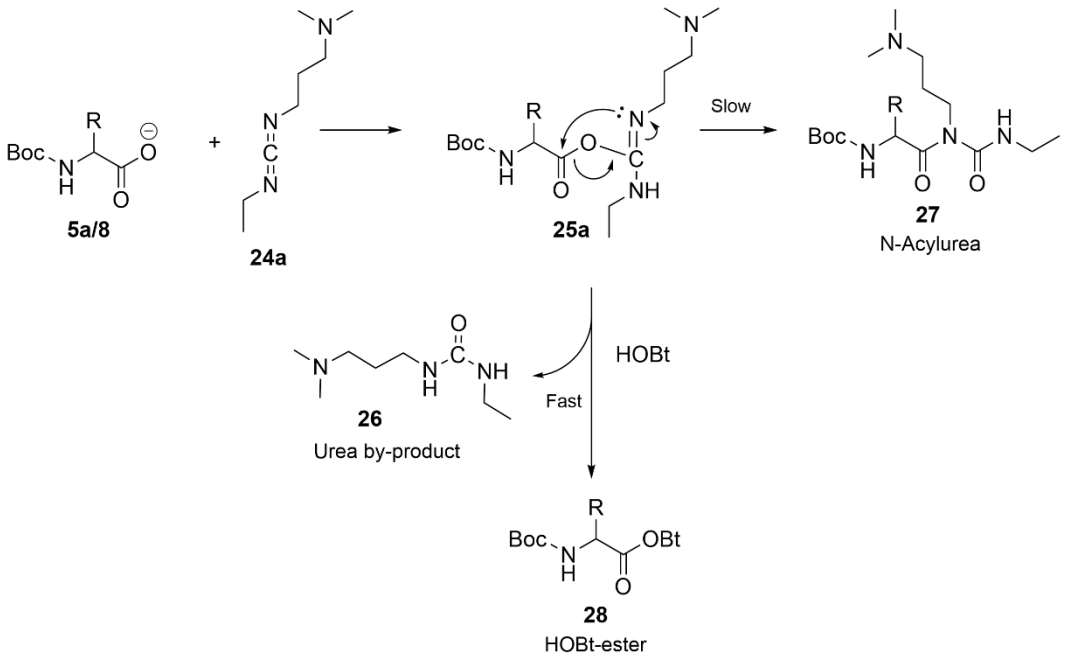


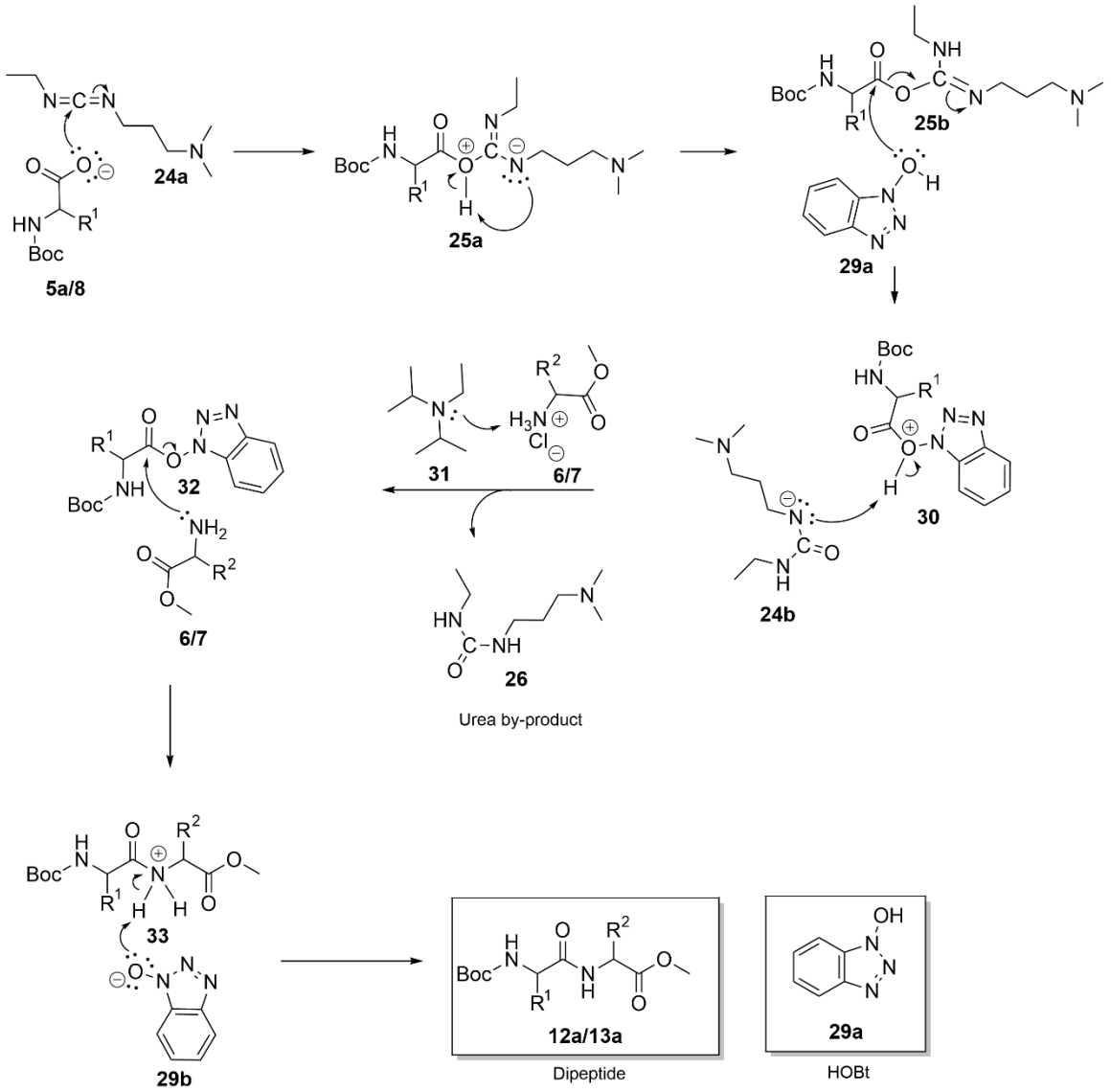
Figure 2.1.2: *General peptide coupling.*

This reaction includes several intermediary products and one of these products, an amino acid-carbodiimide-ester, can rearrange itself and result in the formation of an unreactive *N*-acylurea (**27,** scheme 2.1.1).[11, 12]



Scheme 2.1.1: *Use of HOBt to avoid formation of N-acylurea (27).*

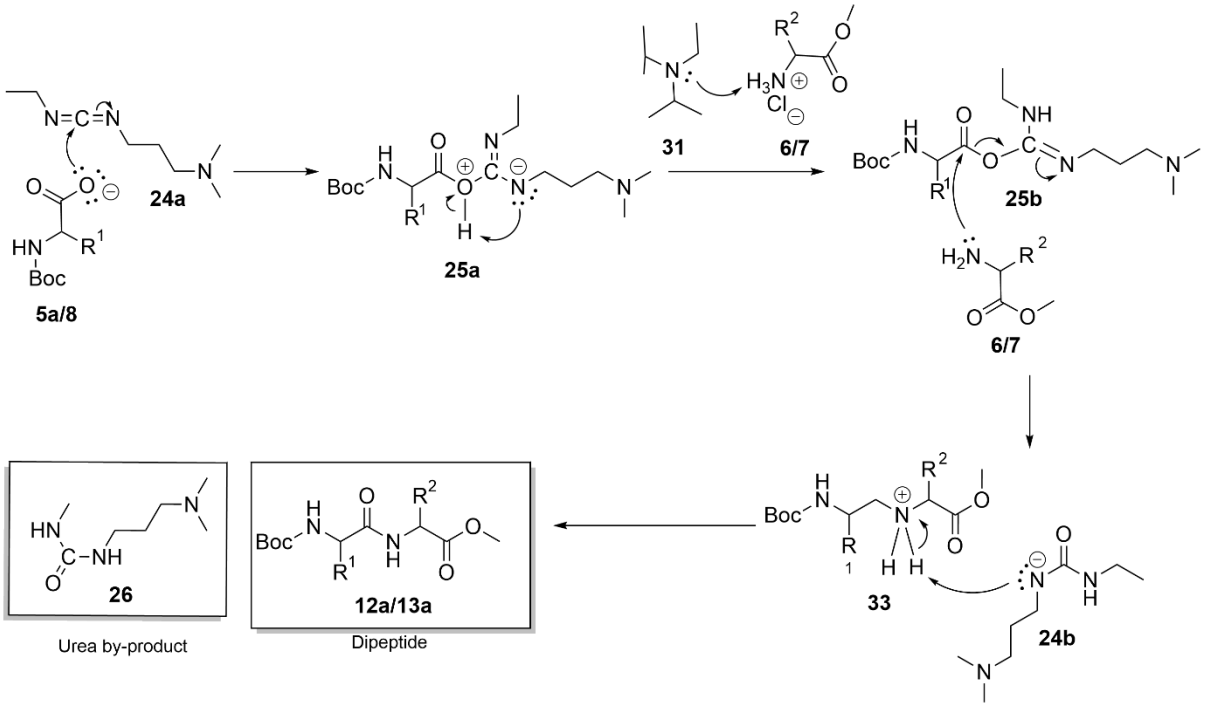
To avoid the formation of the *N*-acylurea by-product, the reaction included HOBt. When added, a secondary intermediary product with HOBt is made. This reaction is much faster reaction than the reaction that produces *N*-acylurea, and it results in an amino acid-HOBt-ester (scheme 2.1.1) which does not form unwanted by-products.[12, 13]



Scheme 2.1.2: *Mechanism for forming peptides, using HOBt.*[13]

The mechanism of the peptide coupling (scheme 2.1.2) starts with a nucleophilic attack by the first amino acid (**5a** or **8**), which has a free C-terminus, on the carbodiimide (**24a**, EDC) forming an ester with it (**25a**). This is followed by another nucleophilic attack by the hydroxyl group of HOBt (**29a**) on the carbonyl of newly formed amino acid-EDC-ester (**25b**). HOBt has now substituted EDC in the ester, and EDC results in a urea by-product (**26**). The second amino acid (**6** or **7**), which has a free *N*-terminus, initiates a nucleophilic attack on the same carbonyl of the ester as HOBt attacked, expelling HOBt and the result is a dipeptide (**12a/13a**).

During the project, the reaction was also attempted without the use of HOBt (scheme 2.1.3), reacting the amino acid-EDC-ester directly with the second amino acid to produce the dipeptide.
This method was based on the work done by K. Luthman and co-workers. [14]
The thought behind it was that as one of the intermediary products were removed from the reaction, the workup of the product would be simplified.



Scheme 2.1.3: *Mechanism for forming peptide, without the use of HOBt.*[12]

The mechanism for this reaction (scheme 2.1.3) was essentially same as with HOBt (scheme 2.1.2), but without the HOBt-intermediary ester, and where the reaction time was shortened to avoid the formation of *N*-acylurea.

The amino acids used to synthesise the dipeptides were commercially available amino acids which were protected on one of their termini and therefor ready to be used in coupling reactions. However, the dipeptides and the tetrapeptide synthesized in the project were protected on both termini, and as such were not in any condition to react. (figure 2.1.3).

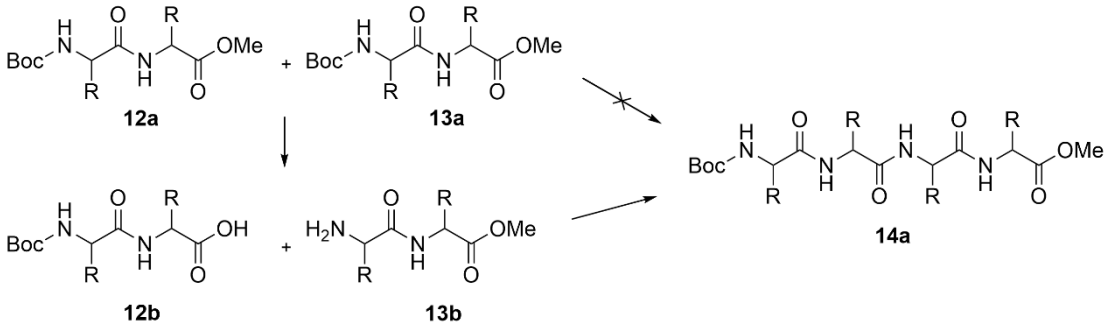


Figure 2.1.3: *The necessity of deprotecting peptides to use them in further synthesis.*

The obvious solution to this problem was to deprotect one the termini of the peptide that was needed to react with another amino acid or peptide (figure 2.1.3). These reactions are quite elementary, where the peptide was either treated with acid or base depending on which terminus that should be deprotected (figure 2.1.4).

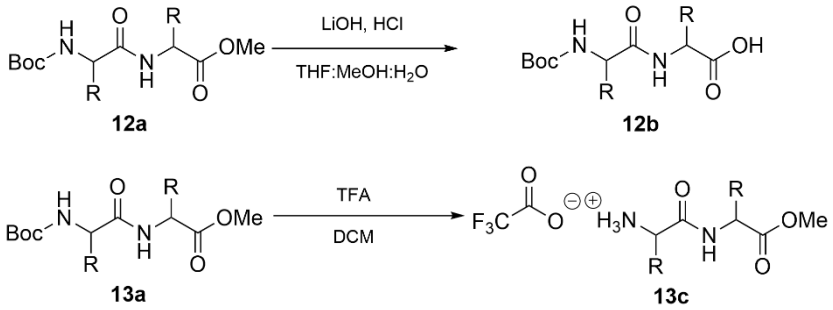


Figure 2.1.4**: Deprotection of the dipeptides**.

By combining the same deprotection methods and the peptide coupling methods described in this part, it is in theory possible to synthesize peptide chains of greater lengths.

2.2 Negishi cross-coupling

The Negishi cross-coupling technique is frequently used in organic synthesis, along with several other techniques such as the Heck- and the Suzuki cross-coupling techniques.[15] These techniques all typically use palladium as a metal catalyst, but they are different in what conditions they can be performed and what kind of coupling reagents that are used.[16]
The reactants used in a Negishi cross-coupling, which separates these reactions from each other, are organozinc compounds coupled with organohalides or pseudohalides, along with triflates, tosylates and mesylates.[17]
While the mentioned cross-coupling reactions are similar in the way that they can couple together sp^2-carbons, the Negishi technique also can couple sp- and sp^3-hybridized carbons (figure 2.2.1), which made the technique ideal for this project.

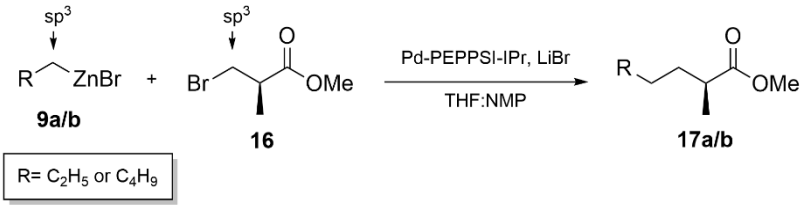
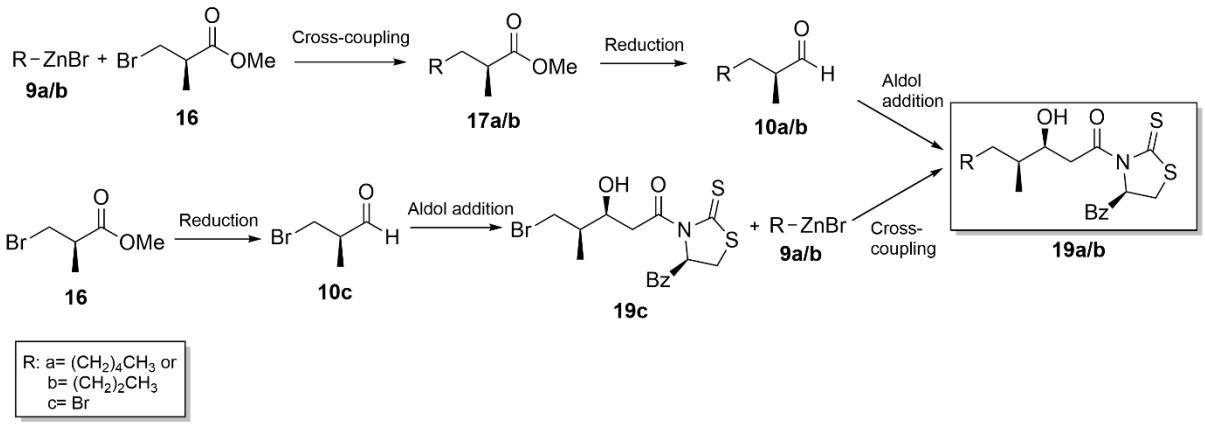


Figure 2.2.1: *The Negishi cross-coupling reagents used for connecting the sp^3-hybridized carbons.*

Fortunately, as cross-coupling reactions are quite popular, they have also been developed quite a bit since when they were discovered more than a century ago. These developments include the gradual movement from nickel- to palladium catalysts, due to environmental and cost concerns, which is why palladium was the chosen catalyst used for this project.[18]

The original plan for the cross-coupling was to synthesise compound **17**, from **9** and **16**, while later the plan changed somewhat to the synthesis of **25a** and **25b** from **25c** and **9** (Scheme 2.2.1).



Scheme 2.2.1: *The different stages of the plan which included the Negishi cross-coupling technique.*

Additionally, the synthesis of compound **35b** was tried attempted as a test reaction (figure 2.2.2), but the method remained the same for all these reactions.

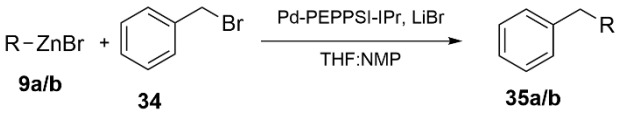


Figure 2.2.2: *Test reaction with benzyl bromide.*

The mechanistic approach to these reactions is quite similar, and they revolve around the catalytic cycle of a select few metals, of which metals such as palladium or nickel are typically used.[19, 20]
The cycle involves three main steps: an oxidative addition of the catalyst followed by a transmetalation, and lastly a reductive elimination to restore the catalyst.[21]
This cycle is illustrated in the following figure (figure 2.2.3).

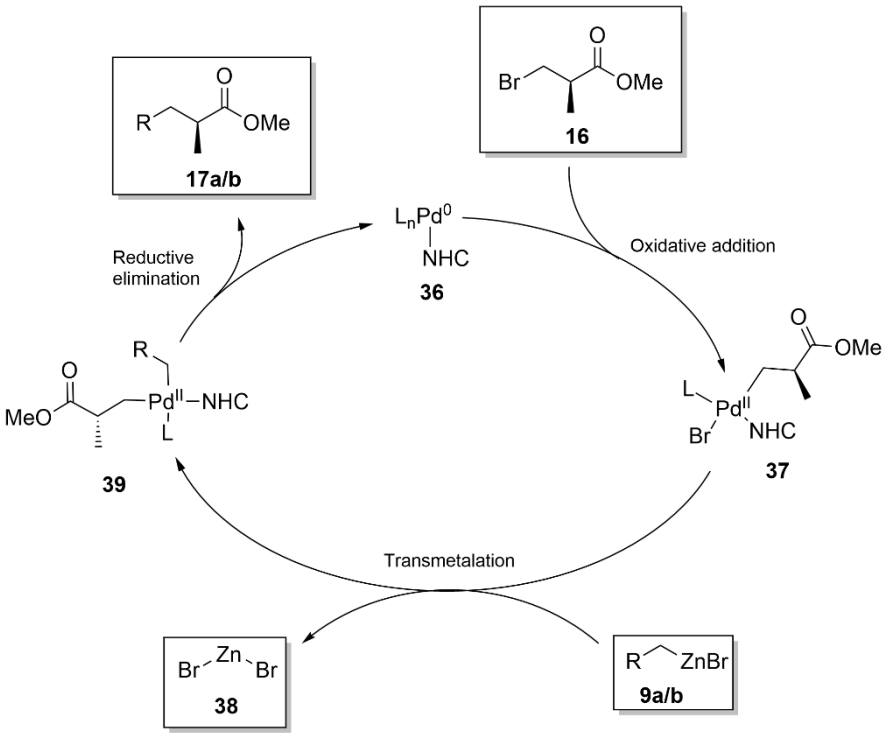


Figure 2.2.3: *Palladium catalysation cycle.*

The initiation of the reaction is done by an electrophilic addition to the electron-dense Pd-atom (figure 2.2.3). This produces a mono-organopalladium complex which then undergoes a transmetallation to produce a di-organopalladium complex. The final step is the C-C bond formation which results from a reductive elimination.[22]
Nevertheless, there are several side reactions that may occur during a cross-coupling reaction, such as homo-couplings, β-hydride elimination, reduction of halides or loss of ligands (figure 2.2.4).[23, 24]

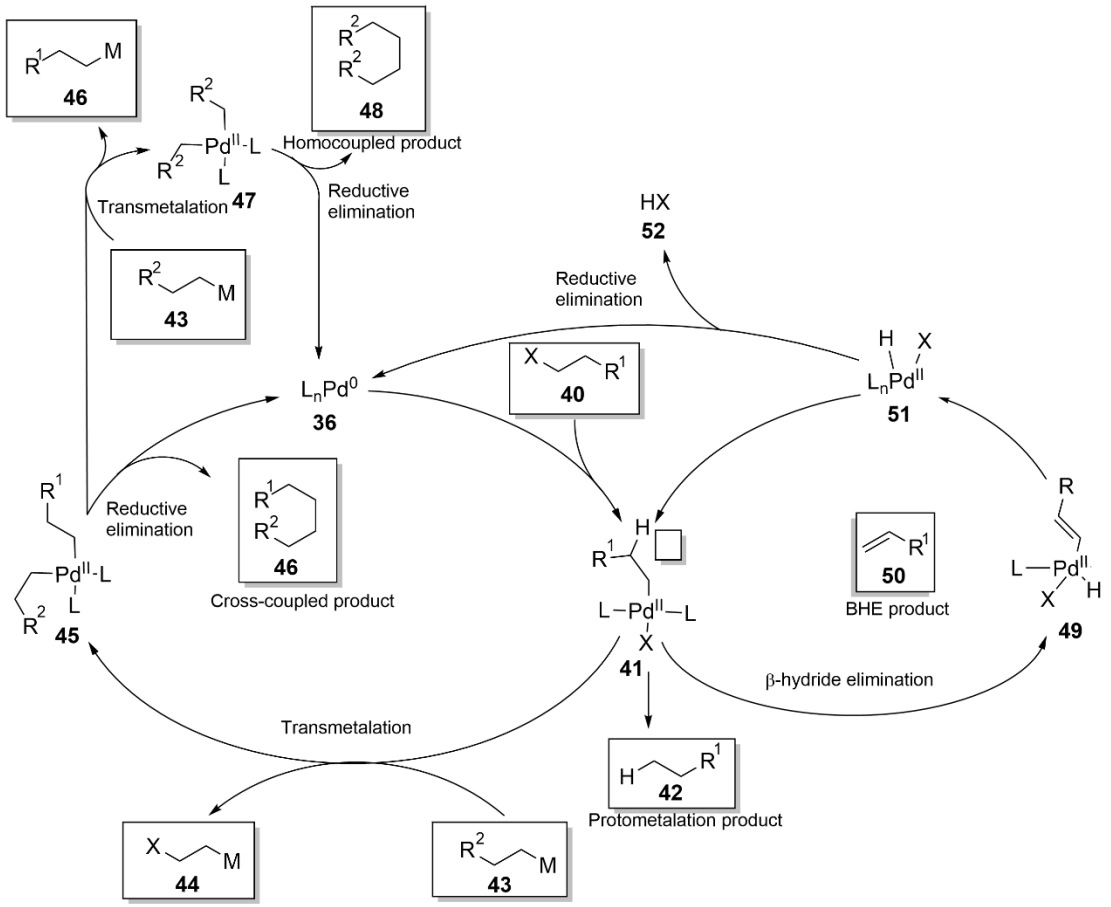
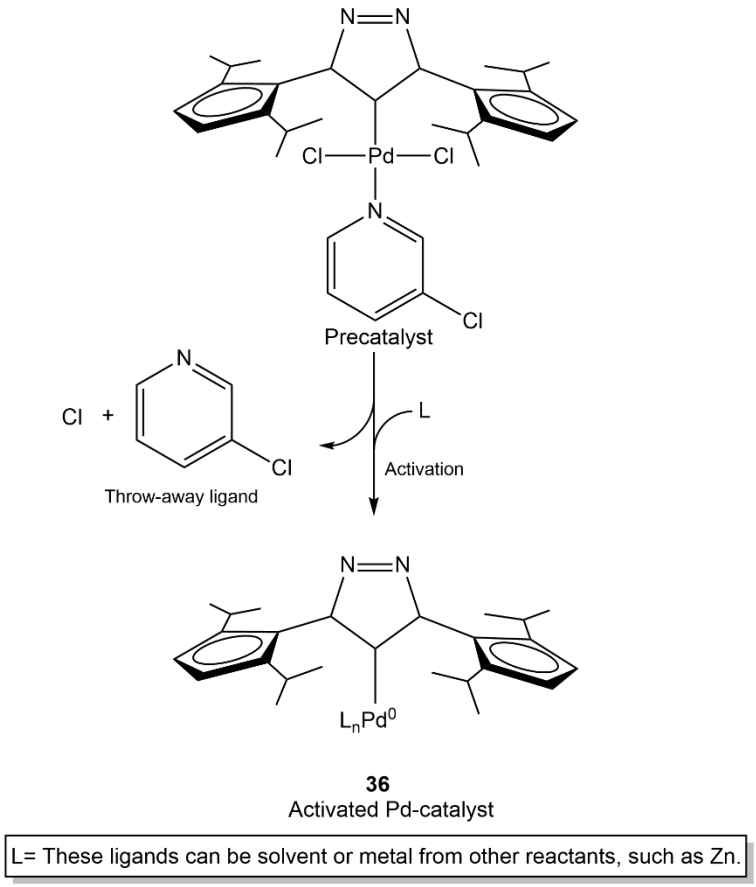


Figure 2.2.4: *Map of the different reactions and side reactions that may occur during the catalytic cycle.*[25]

This will almost inevitably consume a part of the reagents, but steps can be made to decrease the portion of reagents that will vanish in these unwanted reactions. Examples of such steps would be to conduct the experiments in inert atmosphere, exercise temperature control or use specialized ligands.

The specific palladium catalyst used in the project (figure 2.2.5) is a part of a family of palladium pre-catalysts developed by Organ and co-workers called Pd-PEPPSI (Pyridine-Enhanced Pre-Catalyst Preparation Stabilization and Initiation).
The benefit of using this family of pre-catalysts is firstly that there's no need to attach ligands to the palladium-containing molecule while they are air stable in addition to maintaining a high reactivity to organozinc compounds.[17]
It also has shown to resist the formation of unwanted side reactions such as the β-hydride elimination.[26]

The PEPPSI pre-catalyst needs to be activated to function as a catalyst, and as a part of the design of this pre-catalyst both the pyridinium chloride and the chloride atoms coupled to Pd dissociates readily when solved.[27]
This activated Pd-catalysator will however still be connected to the NHC (*N*-heterocyclic carbene) as shown in figure 2.2.5, as this NHC-part is what gives this catalyst its beneficial properties during the coupling reaction.



36
Activated Pd-catalyst

L= These ligands can be solvent or metal from other reactants, such as Zn.

Figure 2.2.5: *Activation of Pd-PEPPSI-IPr.*

Another essential part of the cross coupling is the addition of the LiBr salt, but the reasoning behind this is still discussed. However, what's observed in this type of reaction is that there are none of the desired cross-coupled products in reactions absent of LiBr, while the desired cross-coupled products are present in the reactions which include LiBr.[25]

The procedures for the coupling-reactions done during the project was derived from the work done by Organ and co-workers, who as a part of developing the PEPPSI-family, has done a great deal of work dealing with Pd-catalysts.[17]

2.3 Reduction of ester to aldehyde

The original plan for making the non-peptide fragment includes the following reaction (**figure 2.3.1**), which is a reduction of an ester to an aldehyde. This was in order to couple the aldehyde to a chiral auxiliary, in which the latter is necessary for the stereoselectivity of the non-peptide fragment.

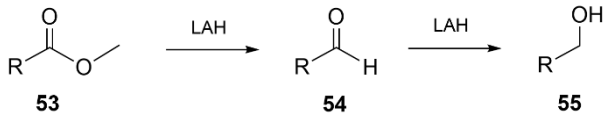


Figure 2.3.1: *General reduction of ester with LAH as reducing agent.*

The procedure used in this project is based on work done by H.F Olivo and co-workers, and the reaction is based upon DIBAL-H functioning as a reducing agent.[28]

The reduction of esters and aldehydes is a well-known and used technique, and the reaction is described in many textbooks.
The reaction is quite simple, and several reducing agents can be used to complete the reaction. The completion of the reaction however, does not necessary stop when the aldehyde is produced, but can continue to produce an alcohol.[29]

However, the purpose of this step in the plan was to produce an aldehyde, which in most accounts are more reactive than esters, and this makes the reaction a bit more complicated. To stop the reduction at the aldehyde step and not continue towards the alcohol, DIBAL was used as a reducing agent at low temperature (figure 2.3.2), as it has been reported to be a method to control and stop the reduction at the wanted aldehyde stage, as opposed to other reagents such as lithium aluminium hydride.[30, 31]

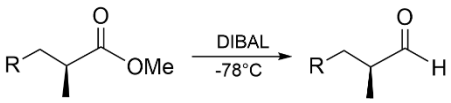


Figure 2.3.2: *Reduction of ester with DIBAL as reducing agent in low temperature.*

2.4 Aldol addition

The aldol addition (figure 2.4.1) was another essential part of the synthesis of the non-peptide fragment of the product molecule. The general aldol reaction has been continuously developed since its discovery in the 19th century, but there have been specific developments of this technique that has been important for this project.[32] D. Evans and co-workers developed a way of performing an stereoselective aldol reaction in the 1970's and it has since then often been referred to as "Evans aldol reaction".[33]

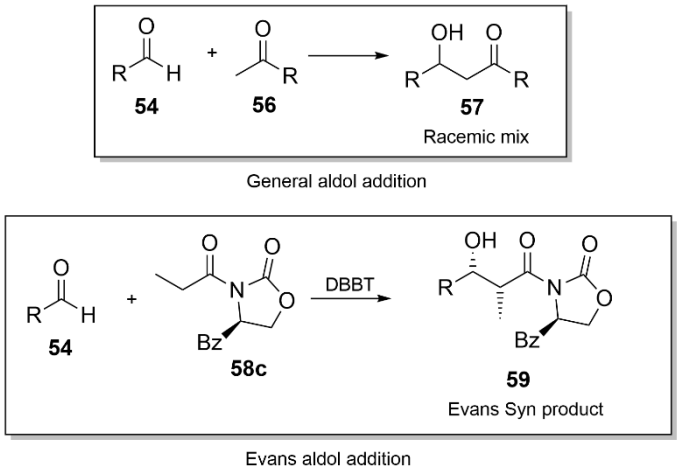


Figure 2.4.1: *General- and Evans aldol addition.*

The significance of his development to the aldol reactions for this project was his focus on the stereochemistry of the aldol-products. His research group used chiral auxiliaries of the oxazolidinone family to provide steric hinderance and DBBT as a chelation agent to direct the reaction towards stereoselectivity (figure 2.4.1).[33]

About 20 years later, M. Crimmins in his work with asymmetric aldol additions used oxazolidinones along with thiazolidinethiones as chiral auxiliaries, and he additionally replaced DBBT with $TiCl_4$ as a chelating agent (figure 2.4.2).[34]

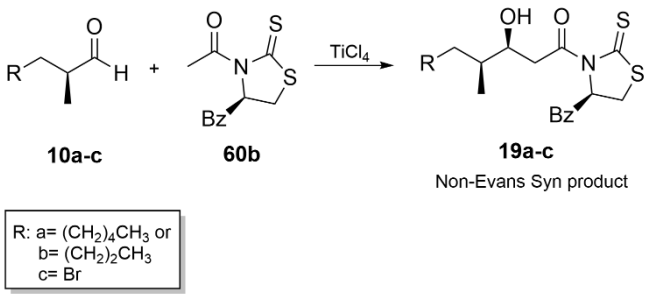


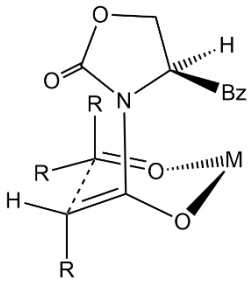
Figure 2.4.2: *The aldol addition with $TiCl_4$ and a thiazolidinethione (60b) as chiral auxiliary.*

The fundamental procedure in this project was developed by M. Crimmins and co-workers and the procedures used in the project were based on the refined ones developed by Olivio and co-workers and A. Phillips/N. Guz.[28, 35, 36]
One of the results of Crimmins' work with these reagents was the inverse products from the "Evans aldol products", and they were aptly named "non-Evans aldol products".

With the reagents used by Crimmins, he reported that adjusting the equivalents of different reagents, one could also direct the stereoselectivity of the resulting products, which is a practical aspect when conducting this type of synthesis.[34]
The chiral auxiliaries that were in focus during this project were of the thiazolidinethiones family used by Crimmins, and the resulting products were the non-Evans products.

The original plan for this project was to synthesise compound **60b** from commercially available (R)-4-Benzylthiazolidine-2-thione (**60a**) and acetyl chloride (**73a**) to create a chiral auxiliary (**60b**) (figure 2.6.1, page 38).
Next was to combine it with the aldehyde (**18a/b**) from the reduction of compound **17a/b** (figure 2.3.2) to create compound **19a/b** (figure 2.4.2).

One of the prominent theories of how this reaction takes place is through a chair-like intermediary state theorized by H. Zimmerman and M.D Traxler in 1957 called the Zimmerman-Traxler model (figure 2.4.3).[37]
This model shows how a metal ion coordinates with the aldehyde and/or ketone reactants into a six membered, chair-like complex.[38]



61

Figure 2.4.3: *Zimmerman-Traxler chair-like complex.*

According to the Zimmerman-Traxler model the aldol addition typically yields anti products with E-enolates, whereas Z-enolates yield syn products (figure 2.4.4).[33, 39, 40]
This is due to the stereochemistry in the six membered chair-like transition state, where the favoured state(s) with both isomers is a state where the R-groups of the aldehydes or ketones are found equatorial to their respective axial counterpart. This is most likely to avoid the steric hinderance of syn-pentane interactions.[41]

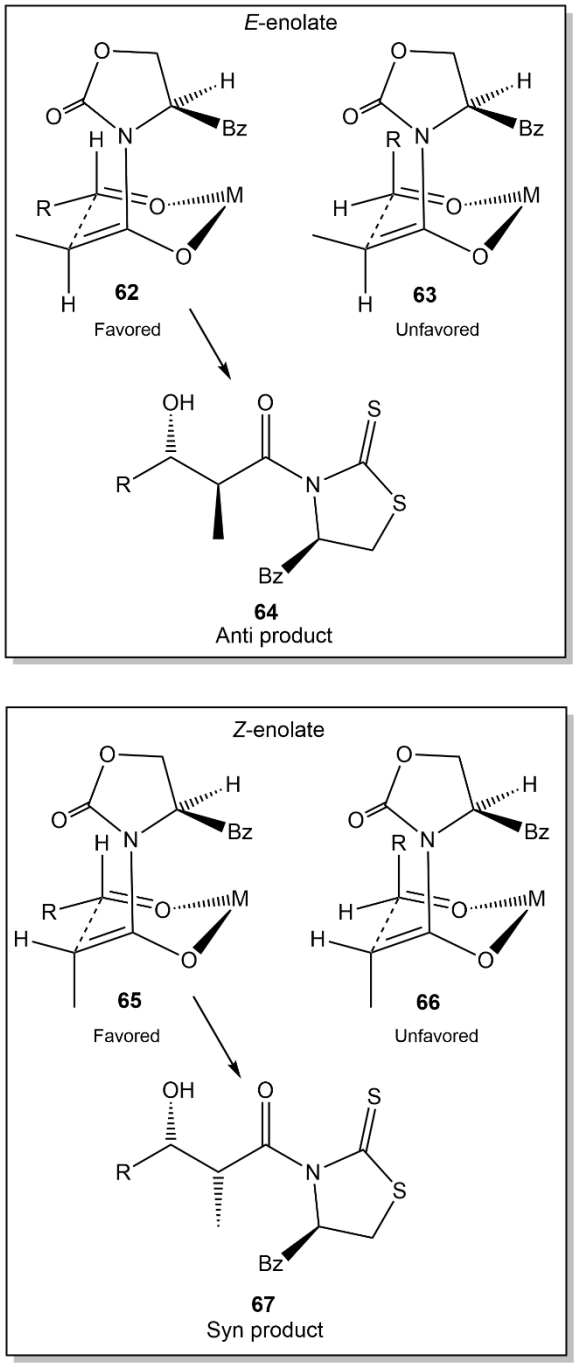


Figure 2.4.4: *E-enolates yield anti products and Z-enolates yield syn products.*

The carbonyl oxygens will chelate to the metal ion and open up for rearrangement in the six-member-like ring, and when the metal ion is removed and the chair-like structure opened up, the product will be a racemic mixture of the aldol. However, the earlier mentioned steric hinderance provided by the chiral auxiliaries guides this reaction to towards stereoselectivity.[33]

However, an alternative yet similar model for the transition state has been suggested by W. Oppolzer, based on a chelation model made by Y. Nagao during his work with aldol-type reactions (figure 2.4.5).[42, 43]
This chelation complex has a tin atom in the centre, but the same model is applied by Crimmins to his titanium chelation complex, where the titanium and it's bonded atoms makes and octahedral shape (figure 2.4.5).

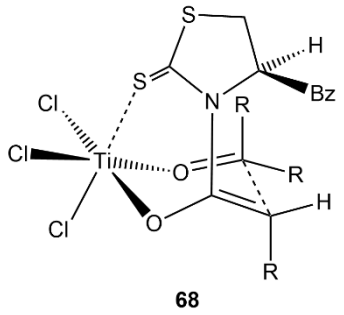


Figure 2.4.5: *The alternative six membered chair-like complex, as proposed by Oppolzer and Crimmins.*[34]

This Crimmins-developed $TiCl_4$-chelated aldol addition which was applied in this project uses this alternative chelation model to explain the resulting inverse non-Evans products. This is due to the chelation and coordination to the $TiCl_4$-molecule by the reactants, which results in the mirrored six-membered chair-like conformation (figure 2.4.6). Nevertheless, the axial/equatorial positions remain as they are in the traditional Zimmerman-Traxler model.

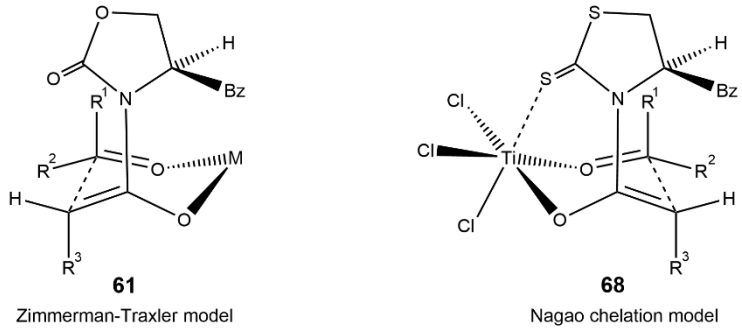


Figure 2.4.6: *The axial and equatorial positions are the same in both the Zimmerman-Traxler model and the Nagao chelation model.*

As the chiral auxiliary used in this project also contains a chiral centre, of which the appended benzyl-group contributes steric hinderance (figure 2.4.7). The *R*-configuration of the chiral auxiliary is furthering the selectivity towards the non-Evans syn products.[35]

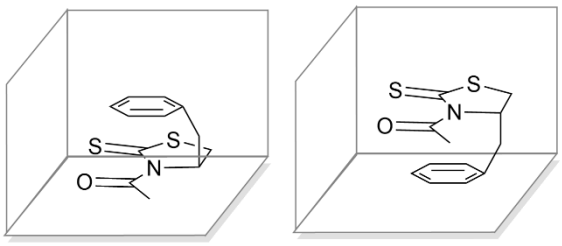


Figure 2.4.7: *The steric hinderance of the chiral auxiliary depends on the stereochemistry of the substituent on the heterocyclic ring.*

With the use of oxazolidinethiones, the carbonyl is nucleophilic enough to displace the chloride ion of the $TiCl_4$, but the addition of a second equivalent of $TiCl_4$ to the mixture, a chloride ion is abstracted from the titanium, opening up another coordination site.[38] This results in Evans syn products with the use of 1 equivalent of $TiCl_4$ and non-Evans syn products with the use of 2 equivalents of $TiCl_4$, due to the mirrored transition states depicted in figure 2.4.6.
However, when using thiozolidinethiones, the increased nucleophilicity of the results in an ordered chelated transition state where only 1 equivalent of $TiCl_4$ is needed to produce the non-Evans syn products. In this system, the addition of a second equivalent of chiral ligands results in the reversal to Evans syn products. This is possibly because of the binding of these ligands to the titanium disfavours its coordination with the thiocarbonyl.[34]

2.5 Asymmetric alkylation

The alkylation of carbonyl groups can be done with a multitude of reagents and in different conditions.[44]
In this project the alkylation of the α-carbon illustrated in the following figure were suggested as an alternate route to the original plan, as to produce compound **69a/b** (figure 2.5.1).

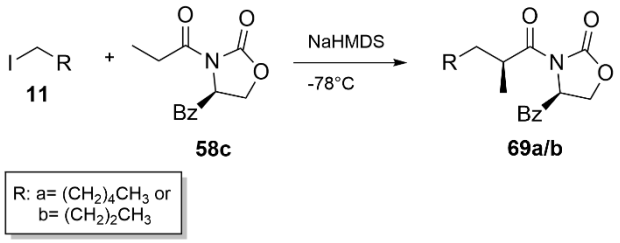


Figure 2.5.1: *Asymmetric alkylation using chiral auxiliary.*

The method used was developed based on work done M. Kohler, who has done work with asymmetric α-alkylation of carbonyls.[45]
The starting molecule of this reaction was a prochiral molecule, which made the use of chiral auxiliaries essential, as to induce the right stereoseletivity.[45, 46]

This reaction is similar to the aldol addition, though there are some slight differences which makes these reactions different.
The metal chelation centre is the Na atom of the NaHMDS molecule, and the two carbonyls (or carbonyl and thiocarbonyl) of the chiral auxiliary will form bonds to it (figure 2.5.2).[47, 48]

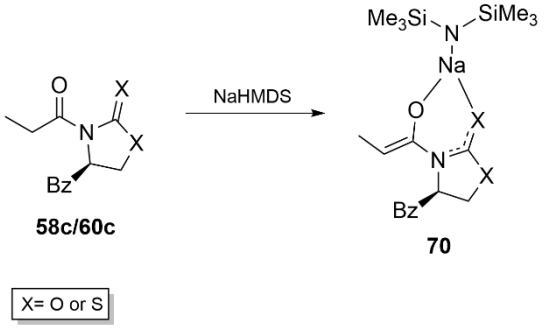


FIGURE 2.5.2: *The chelated NaHMDS-complex.*

This enolate makes it possible for the nucleophilic attack on the iodine compound to occur (figure 2.5.3).

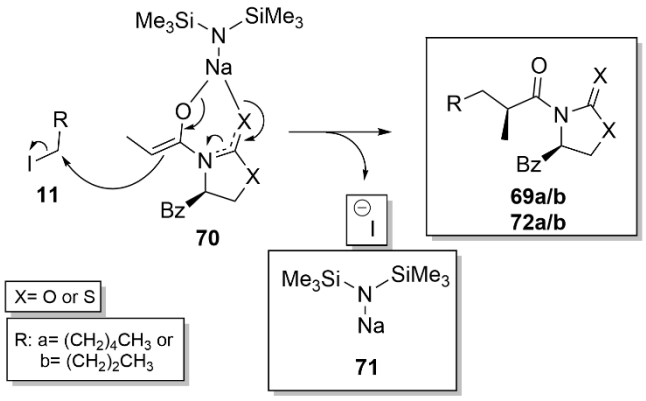


FIGURE 2.5.3: *Proposed mechanism of the alkylation.*

Based on several articles and the work done with the aldol addition, it was theorized that the chiral auxiliaries with R-configuration would result in the second chiral centre would be S-configured (figure 2.5.3).[45-48]

This due to the steric hinderance which would make the nucleophilic attack happen from the Re-face of the prochiral molecule (figure 2.5.4). The steric hinderance would be applied by the benzyl group of the chiral auxiliary, which is connected to the 5-membered ring with a rotatable single bond.

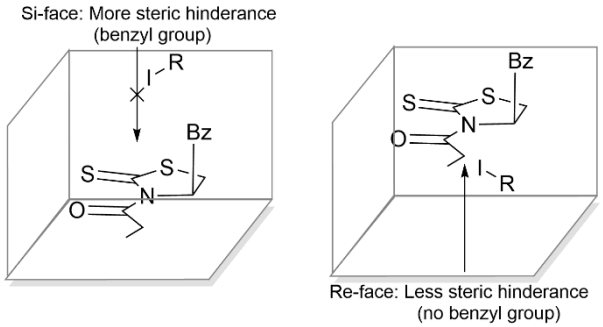
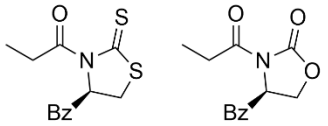


Figure 2.5.4: *Due to steric hinderance, the alkylation takes place on the Re-face of the prochiral molecule.*

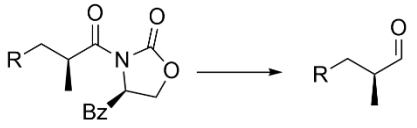
The chiral auxiliaries used for this alkylation was both an oxazolidine (**58c**) and a thiazolidinethione (**60c**) (figure 2.5.5), of which very similar chiral auxiliaries were used for the aldol reaction (**60b**). This reflects the similarity of these reactions and the importance of the chiral auxiliary used, as to achieve products with the desired stereochemistry.



60c **58c**

Figure 2.5.5: *The chiral auxiliaries used.*

After the ended alkylation, the chiral auxiliary used would need to be removed by a reduction reaction, as to produce aldehyde needed to complete the aldol addition (figure 2.5.6).



69a/b **10a/b**

Figure 2.5.6: *Reduction of the molecule into an aldehyde to be used in the aldol addition.*

2.6 Chiral auxiliaries

As indicated in the previous sections, the chiral auxiliaries were of great importance for this project. The asymmetric alkylation and the aldol addition and were attempted to different molecules, and the stereoselectivity of these reactions was guided by the chiral auxiliaries. The function of these chiral auxiliaries is to induce a certain stereochemistry to a product then be removed, preferably under mild conditions.[49]

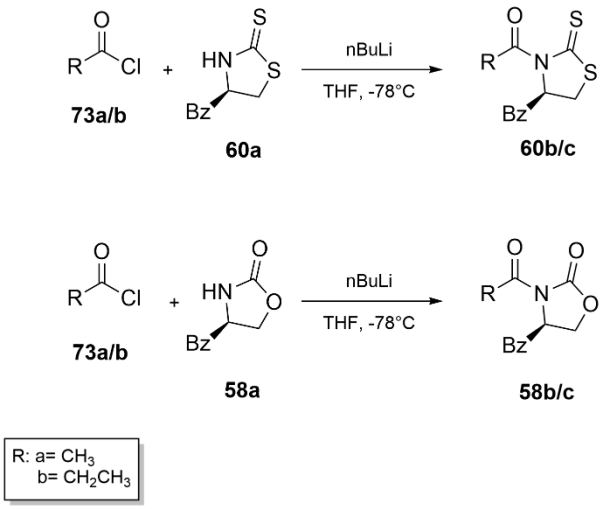


Figure 2.6.1: *Acylation of the chiral auxiliaries with different acylating agents.*

The different lengths of the R-group on the acid chlorides are due to the different applications of the auxiliaries, either to use for aldol additions or the iodine-asymmetric alkylation (figure 2.6.1).

The mechanism of the reaction is an elementary acylation, where the amine of the chiral auxiliary works as a nucleophile which will attack the carbonyl of the acid chloride, expelling the chloride ion, which of course is an excellent leaving group. [29]

The procedures for the synthesis of both oxazolidinethiones and thiazolidinethiones were based on work by P. Romea and his research group, whose work includes preparation of thiozolidinethiones.[50]

The stereoselective-inducing properties of the chiral auxiliary can could be attributed to the steric hinderance of the ring structure, but mainly due to the substituents on the ring, such as isopropyl or benzyl groups (**figure 2.6.2**).[49]
This is due to the steric hinderance provided by these substituents, depicted in figure 2.4.7 (page 34).

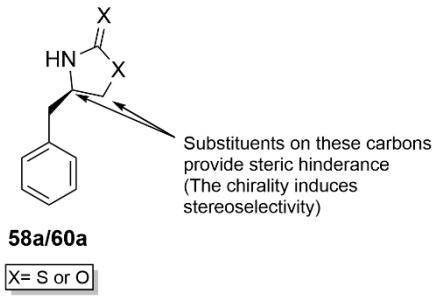


Figure 2.6.2: *The placement of the substituents that induce selectivity in connected molecules.*

There are many chiral auxiliaries to choose from, and most published papers claim that their chiral auxiliaries are the best. However, it was decided to apply the procedures developed by M. Crimmins and H. Olivo and their respective research groups, as they applied reasonably similar reagents in their articles as the ones that were used in the aldol addition of this project (figure 2.6.3).[28, 34]

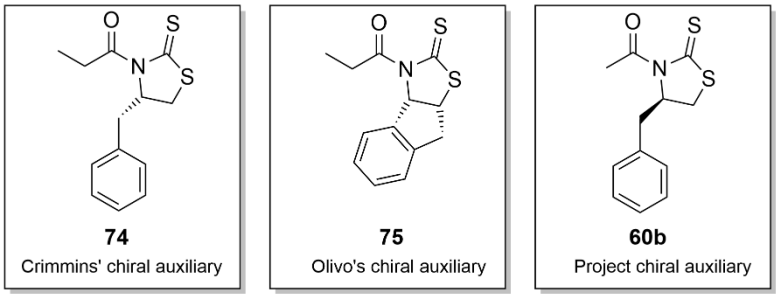


Figure 2.6.3: *Similarity of the chiral auxiliaries.*

As an alternative path to complete the non-peptide fragment was implemented to the project, it was decided to synthesise variants of the thiazolidinethiones and oxazolidinones, to test the success of the different auxiliaries in different reactions. The general reaction of synthesizing both thiazolidinethiones and oxazolidinones was the same, where the only difference was what acid chlorides were used to acylate the heterocyclic compounds (figure 2.6.1).

2.7 TBS-protection

The protection of hydroxyl groups is a rather known method of ensuring that this functional group will not undergo any unwanted reactions, as it is quite reactive. The thought behind this reaction is to reduce the nucleophilic properties of the OH-group by substituting the hydrogen of the hydroxyl with a silyl-group, making a silyl ether (figure 2.7.1).

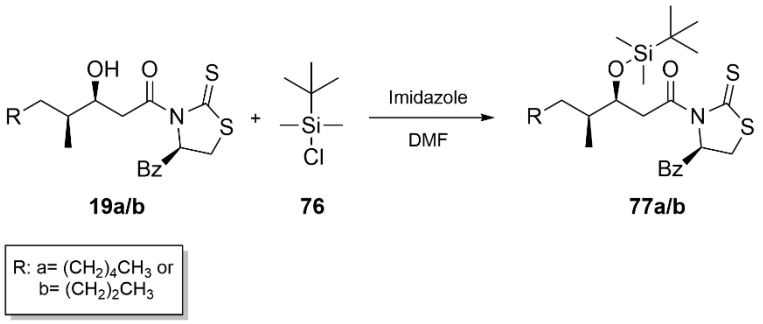
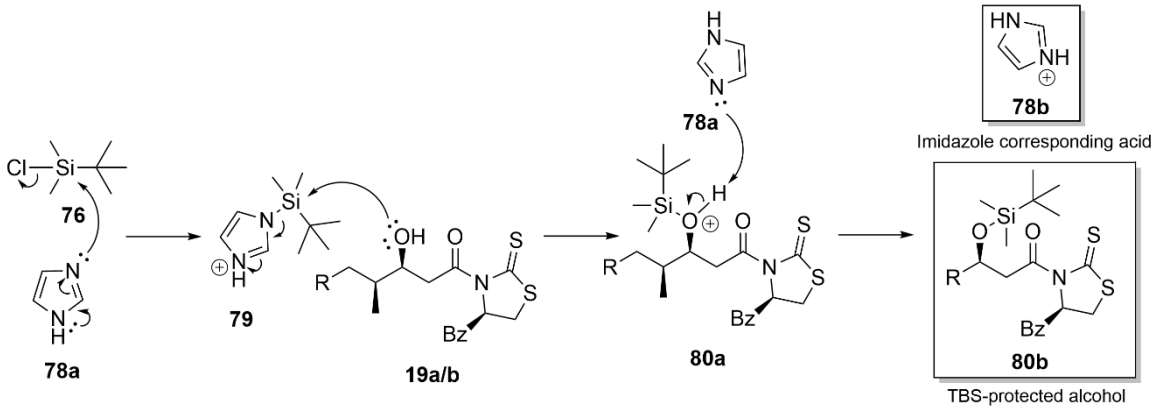


Figure 2.7.1: *The TBS-protection reaction.*

The reagents used for the reaction is traditionally an amine base along with the hydroxyl-containing compound and the silyl chloride. As a solvent, DMF is used as it acts as a Lewis-basic solvent. This traditional approach was used in the project, where imidazole was used as a base and TBSCl as the silyl chloride (scheme 2.7.1). Different hydroxyl-containing compounds were also used, as several of the compounds needed protecting.

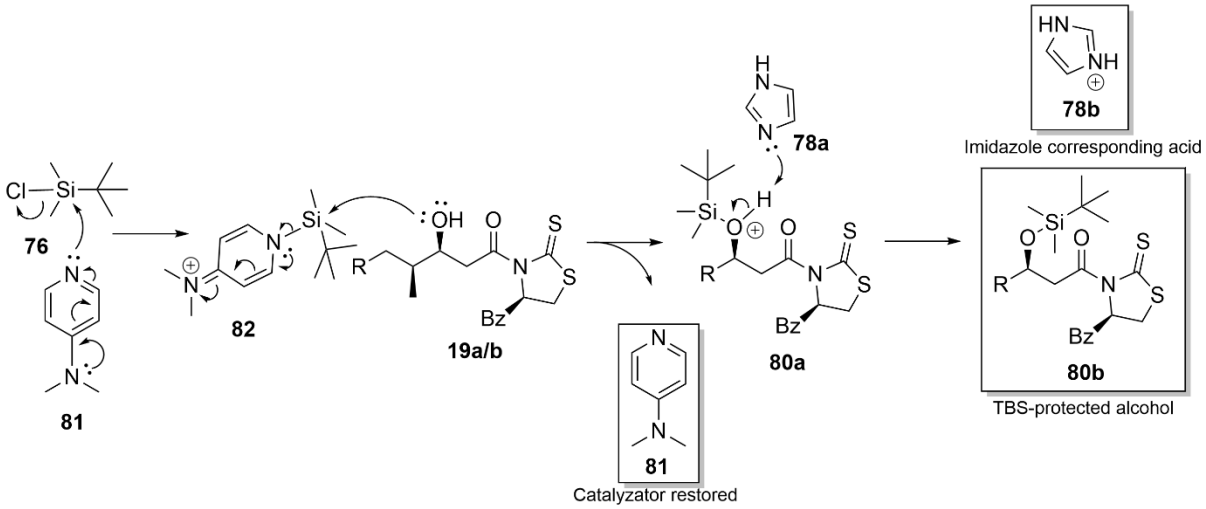


Scheme 2.7.1: *Proposed mechanism for TBS protection with imidazole as base.*

The procedure for this approach was developed were based on work done by E. Solum.

Additionally, a protection method utilizing a Lewis base-catalyst system were used, based on work done by P. Patschinki and co-workers (scheme 2.7.2).[51]
The advantage of using this method is that one is able to speed up the reaction time while using less polar solvents than DMF, such as DCM or chloroform. This would be

advantageous for the workup of the product, as DMF can be quite troublesome to remove from reaction mixtures.



Scheme 2.7.2: *Proposed mechanism of TBS protection, using DMAP as catalysator.*

2.8 Nagao aminolysis

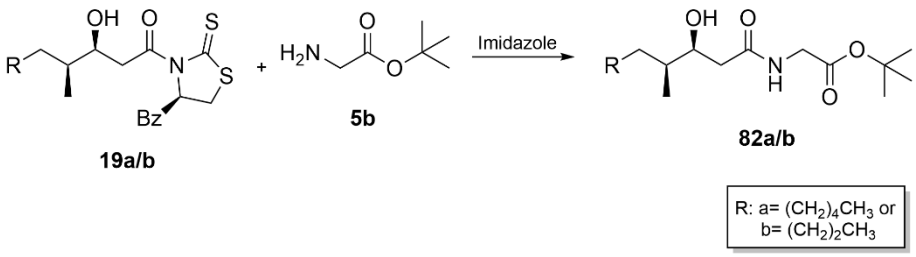


Figure 2.8.1: *Nagao aminolysis.*

Following the use of chiral auxiliaries, the removal of the chiral auxiliary was an inevitable step for finishing the non-peptide fragment of the project. This reaction is an aminolysis of the chiral auxiliary, and it should be carried out in mild conditions, utilizing the good leaving qualities of the heterocyclic thiocarbonyl group.[52]

The reaction used in this project (figure 2.8.1) was developed by Y. Nagao and what made this reaction ideal for this project was that it is a method specifically developed to displace chiral auxiliaries. Also, because of said auxiliaries, the reaction mixtures often have a yellow colour which disappears after ended reaction. This makes the reaction easily monitorable.[52]

This reaction requires a nucleophile to instigate a nucleophilic attack where it substitutes the heterocyclic ring of the chiral auxiliary part of the molecule (figure 2.8.2).

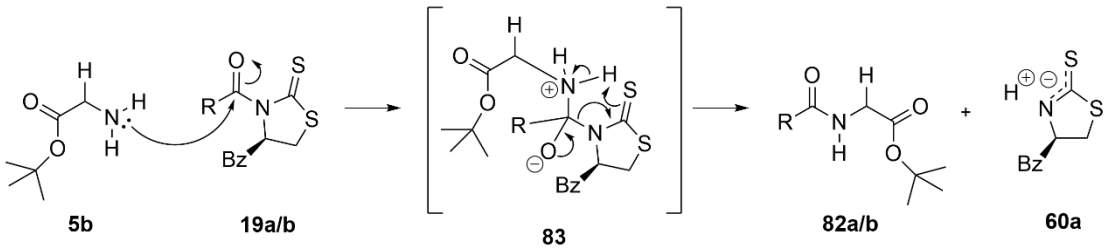


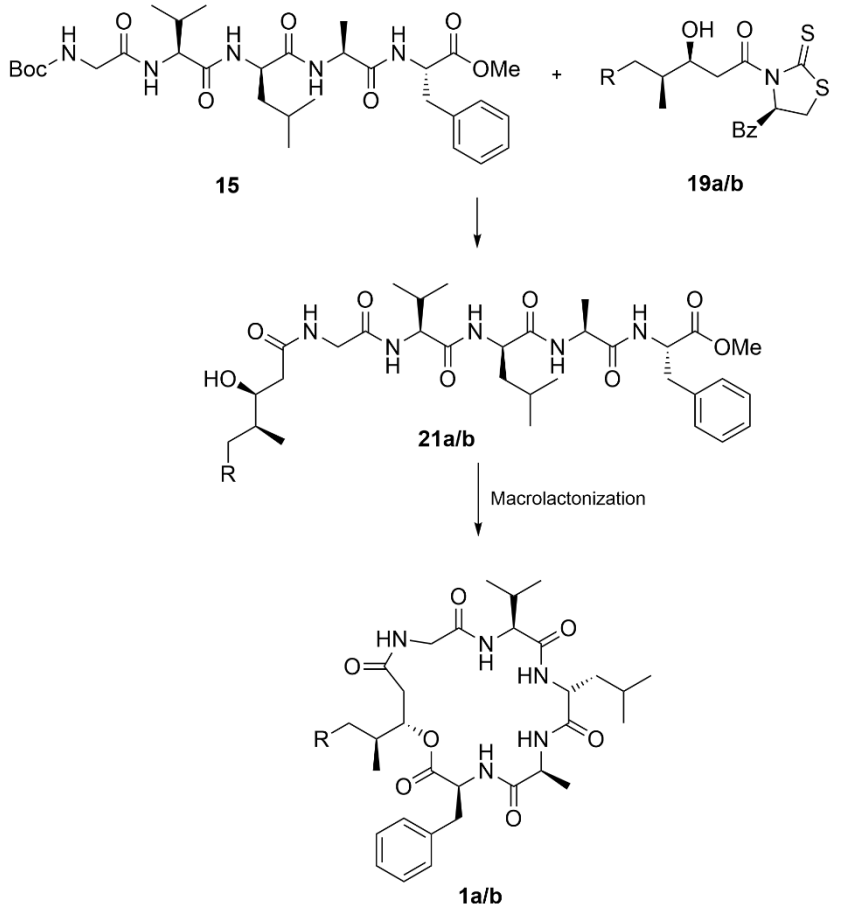
Figure 2.8.2: *Nagao's proposed mechanism.*[52]

Since the base used for the reaction substitutes the chiral auxiliary, it is important that it either is a base that is easily displaceable while still nucleophilic enough to displace the chiral auxiliary. However, the more logical approach would be to use an amine that is not needed to be replaced, which for this project meant to use the *C*-terminus protected amino acid glycine (**5b**) or either the tetra-(**14**) or pentapeptide (**15**) of which both contains glycine on one end.

2.9 Cyclization

The cyclization of the molecule is a step that can be achieved by using several different methods, but the principles are somewhat similar.

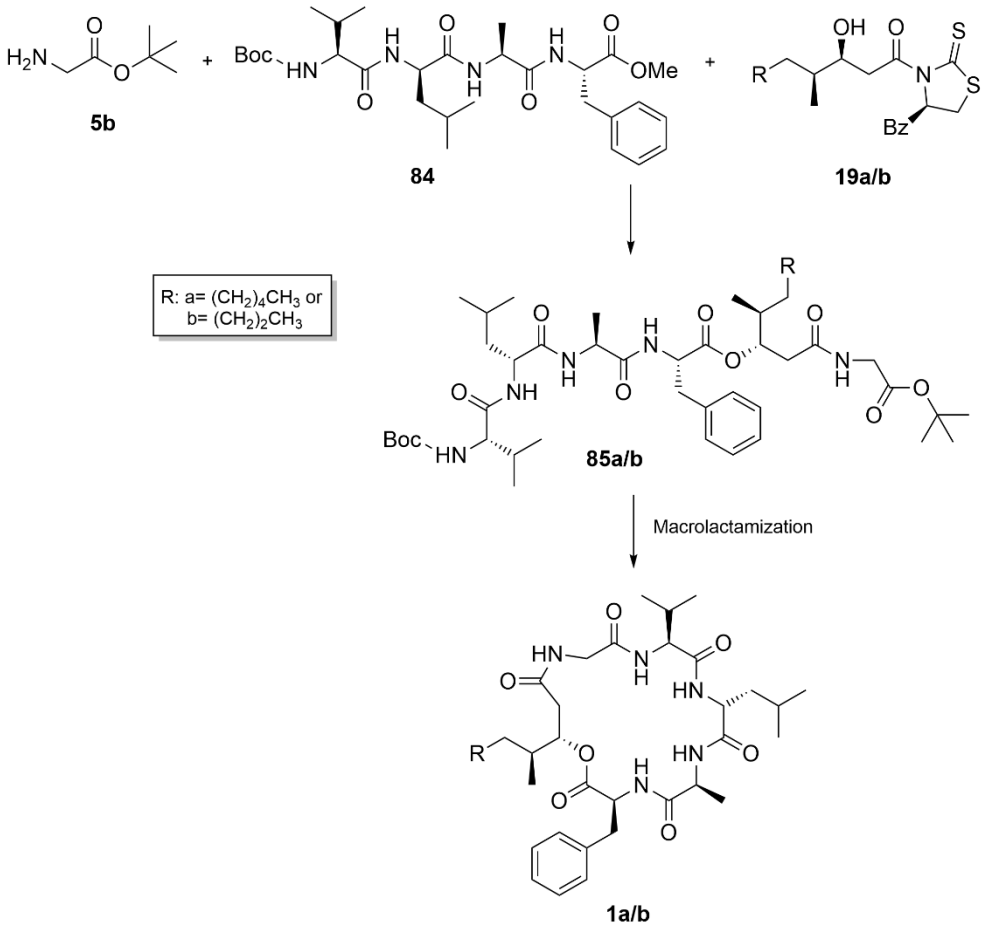
The original plan for cyclizing the molecules were a macrolactonization (scheme 1.3.4, page 16). It would start by connecting the pentapeptide to the non-peptide fragment with an aminolysis, then using a macrolactonization technique to cyclize the molecule (scheme 2.9.1).
As macrolactonization of cyclic peptides is well documented in literature, there were several reports that were considered as basis for the procedure. [53, 54]



Scheme 2.9.1: *The proposed macrolactonization.*

The alternative way considered for cyclizing the molecules was a lactamization (scheme 1.3.4, page 16).
This would require the peptide fragments to be split up, where one part of the peptide would be connected to the hydroxyl group of the non-peptide fragment, while the other part of the peptide would be connected to the carbonyl.
The last step would then be to form a bond between the *N*- and *C* termini of the peptides and thus, cyclizing the molecule. (scheme 2.9.2)

This could be completed in two ways. Either by coupling the *C*-terminus of Phe (**4**) and the *N*-terminus of the tetrapeptide (**14**) to the non-peptide fragment (**19a/b**) then connecting the peptide with the amino acid through a lactamization (scheme 2.9.2). The other way would be to couple the *N*-terminus of Gly (**5b**) and the *C*-terminus of the alternative tetrapeptide (**84**) to the non-peptide, and then connecting the peptide to the amino acid through lactamization (scheme 2.9.2).
The macrolactamization of cyclic peptides is just as well documented as the macrolactonization, and input from several articles were used to form this procedure as well.[55-57]



Scheme 2.9.2: *The proposed macrolactamization.*

In the end, it was decided to go for the macrolactamization path, as there was reported problems completing a macrolactonization of a similar molecule (figure 2.9.1) to the scopularides in one of the articles.[56]

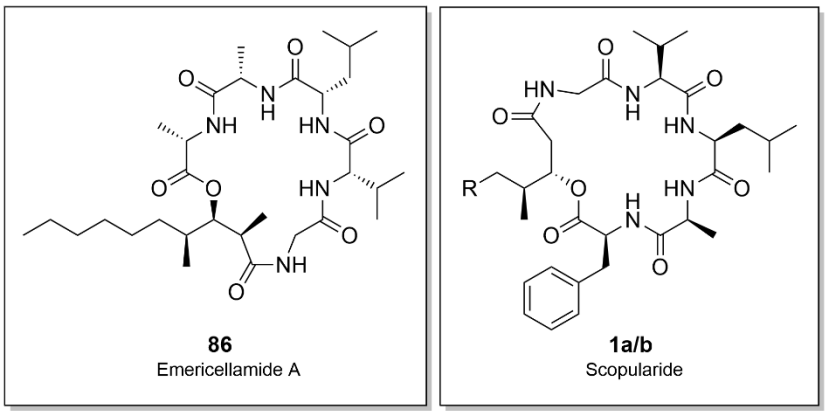


FIGURE 2.9.1: *Side-by-side comparison of Scopularide and the similar molecule from the article.*

The fundamental thought for this pathway was to perform a Nagao-aminolysis of the heterocyclic ring of the chiral auxiliary where a *t*-Butyl protected glycine is replacing the auxiliary, before introducing the tetrapeptide on the hydroxyl group. The reason behind the aminolysis happening first was to reduce the chance of a reaction to occur on a different than intended part of the molecule, as the introduction of a multi-amide containing peptide chain on the molecule could provide additional sites for the reaction to happen (figure 2.9.2). These new points of attack are sterically hindered by the R-groups of the peptide, but it was thought to minimize the risk of these side-reactions to happening in any way possible.

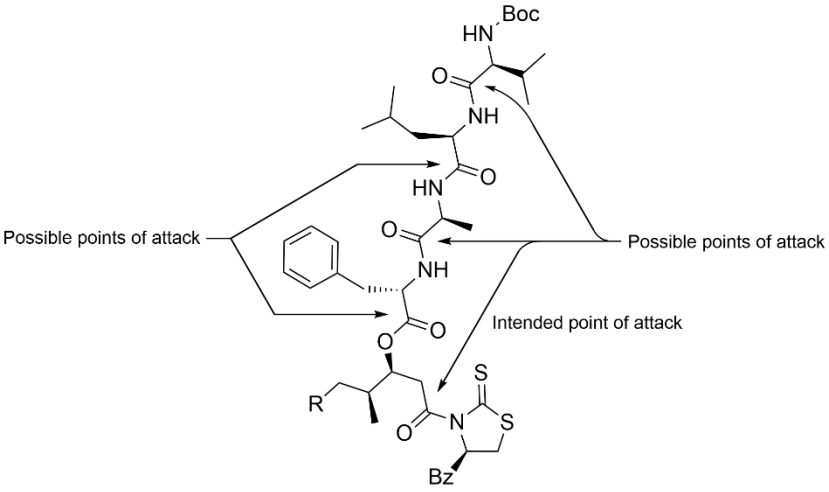
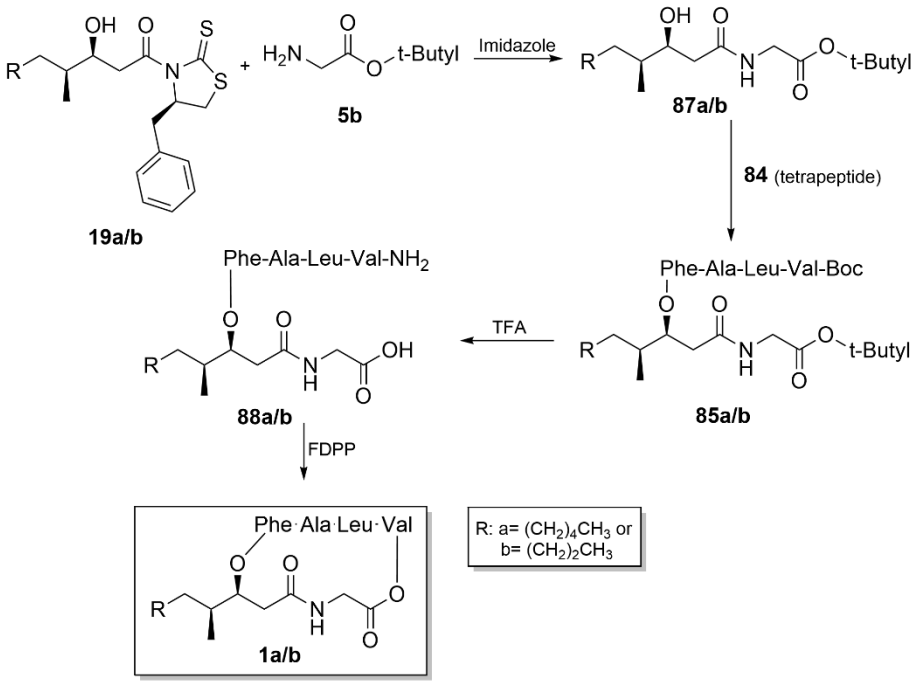


Figure 2.9.2: *Possible attack points during the aminolysis.*

Next is the deprotection of the Boc-group of the tetrapeptide, which conveniently also is the method for deprotecting *t*-butyl protected glycine, before finally connecting the termini of the opposing amino acids to a cyclic molecule, using FDPP as a coupling agent (**scheme 2.9.3**).



Scheme 2.9.3: *The reagents and the sequence of reactions of the lactamization of the Scopularides (**1a/b**).*

Chapter 3: Results and discussion

3.1 Synthesis of peptides

The peptide synthesis was one of the reactions that were successful from the start, but which were developed to improve the yields during the project from the range of 42 % to 86 %.

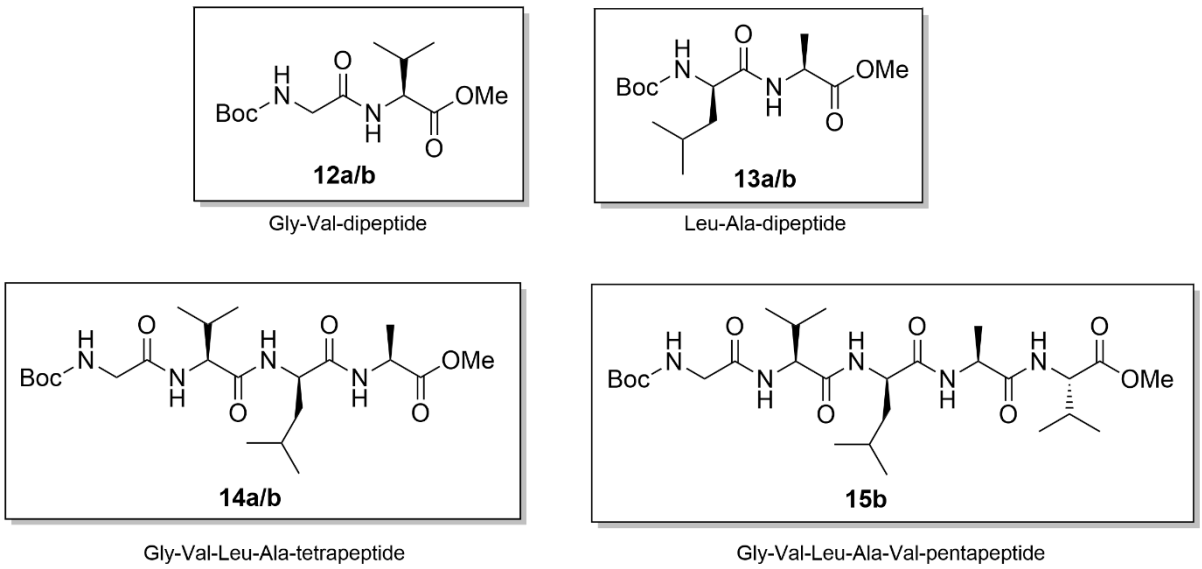
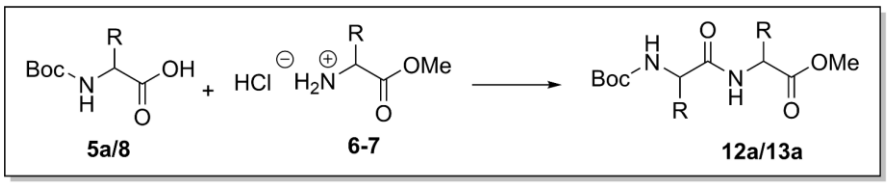


Figure 3.1.1: *The synthesised peptides.*

Four different peptides were synthesised during this project (figure 3.1.1).
A distinction has to be made between the reactions carried out with the amino acids which were acquired commercially, and the reactions done with dipeptides that were synthesised during the project. The reactions carried out with the commercially acquired reagents to produce dipeptides were generally the reactions with the best yields (figure 3.1.2, table 3.1.1-3.1.3).



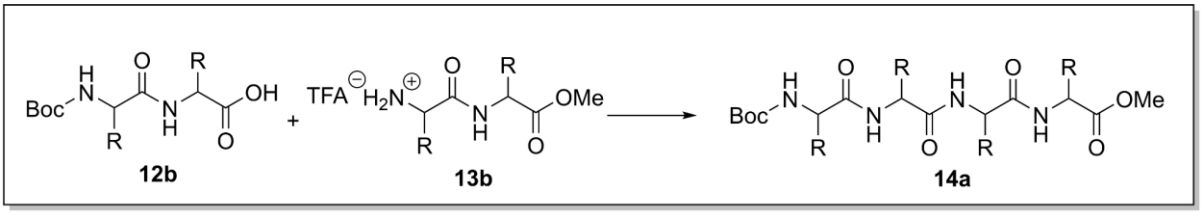


Figure 3.1.2: *The commercially acquired amino acids generally gave better yields than when the synthesised peptides were used as reagents.*

A representative amount of NMR-analyses was made for each peptide and confirmed through NMR (spectras 1-4, page 100-103), and later the products were identified using TLC.

The synthesis of dipeptides started with procedures based on the work done by T.K. Chakraborty and his research group, using standard conditions of peptide synthesis with EDC and HOBt as coupling reagents. This procedure eventually resulted good yields (**table 3.1.1** and **3.1.2**), and the products were which were confirmed with NMR.

The yield of the dipeptide reactions started quite low, with around 40-50 % of theoretical output, but as the general lab-technique improved, so did the yield. By mainly improving technique, the yield increased to above 65 %, and several reactions yielded over 90 %. It was also shown that the lengthier experiments gave similar yields to the shorter ones, and if the right eluents were used, a short silica-filled column could be used for purifying the product instead of the longer columns used in flash chromatography. This means that the synthesis could be done with smaller amounts of time used, and less materials used for the work-up and purification. The scale of the reactions did not seem to affect the yields much either, so the synthesis could potentially be run at a large scale. The method for synthesising the dipeptide without the use of HOBt was also deemed a success, as the yield were high. However, the time needed for this experiment was relatively lengthy, and therefore the time aspect should be considered when using this method.

The need for HOBt was considered, as this coupling reagents function was to inhibit the formation of *N*-acylurea (see chapter 2.1). It was theorized that if the reaction time could be reduced it could be conducted without the use of HOBt. A test reaction was then completed without the use of HOBt, based on work done by K. Luthman and her research group.[14]
This test was a success, where the same dot (Rf-value and colour) appeared on the TLC-plate as the earlier NMR-confirmed product, which served as a fundamental confirmation

of the product. As there were no observable by-products, it was decided that HOBt could be removed as a coupling reagent.
As a result of this the workup became simpler which in turn increased the yield of the reactions and reduced the total time spent on the reaction. The yields were equal to yields provided by the reactions including HOBt, as shown in table 3.1.1 and 3.1.2.

Figure 3.1.3: *Synthesis of Gly-Val dipeptide*

Entry. no.	Reagents	Eq and mmol	Solvents	Duration (hours)	Workup	Yield (%)
1	**5a**, **6**, EDC, HOBt, DIPEA	1, 0.47 1, 0.47 3, 1.4 3, 1.4 3, 1.4	DCM	18	Wash, flash	46
2	**5a**, **6**, EDC, HOBt, DIPEA	1, 1.88 1, 1.88 1, 1.88 1, 1.88 2, 3.76	DCM	18	Wash, flash	52
3	**5a**, **6**, EDC, HOBt, DIPEA	1, 0.47 1, 0.47 1, 0.47 1, 0.47 2, 0.94	DCM	18	Wash, flash	89
4	**5a**, **6**, EDC, HOBt, DIPEA	1, 0.94 1, 0.94 1, 0.94 1, 0.94 2, 1.88	DCM	2	Wash, short column	86
5	**5a**, **6**, EDC, HOBt, DIPEA	1, 0.43 1, 0.43 1, 0.43 1, 0.43 2, 0.86	DCM	2	Wash, short column	88
6	**5a**, **6**, EDC,	1, 1.88 1, 1.88 1, 1.88	DCM	2	Wash, short column	97

	HOBt, DIPEA	1, 1.88 2, 3.76				
7	**5a**, **6**, EDC, DIPEA	1, 0.5 1.27, 0.635 1.27, 0.635 1.27, 0.635	DCM	18	Washed	89

Table 3.1.1: *Results of Gly-Val dipeptide syntheses.*

Comparing the experiments conducted making the Gly-Val dipeptide (figure 3.1.3), there were several factors that could be considered for optimizing the synthesis.
The equivalents used for the synthesis were reduced through the project. Table 3.1.1 and 3.1.2 shows that the yields were just as good, or better when using the lower equivalents compared to the higher equivalents in the same conditions.

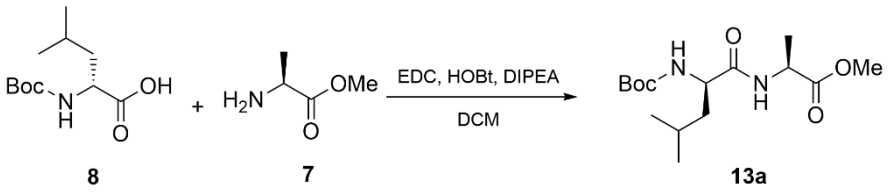


Figure 3.1.4: Synthesis of Leu-Ala dipeptide.

Entry. no.	Reagents	Eq and mmol	Solvents	Duration (hours)	Workup	Yield (%)
1	**8**, **7**, EDC, HOBt, DIPEA	1, 0.43 1, 0.43 3, 1.29 3, 1.29 3, 1.29	DCM	18	Wash, flash	43
2	**8**, **7**, EDC, HOBt, DIPEA	1, 0.43 1, 0.43 1, 0.43 1, 0.43 2, 0.86	DCM and DMF	18	Wash, flash	87
3	**8**, **7**, EDC, HOBt, DIPEA	1, 1.72 1, 1.72 1, 1.72 1, 1.72 2, 3.44	DCM and DMF	18	Wash, short column	55
4	**8**, **7**, EDC, HOBt,	1, 0.43 1, 0.43 1, 0.43 1, 0.43	DCM and DMF	18	Wash, short column	67

	DIPEA	2, 0.86				
5	**8**, **7**, EDC, HOBt, DIPEA	1, 0.43 1, 0.43 1, 0.43 1, 0.43 2, 0.86	DCM and DMF	2	Wash, short column	96
6	**8**, **7**, EDC, HOBt, DIPEA	1, 1.88 1, 1.88 1, 1.88 1, 1.88 2, 3.76	DCM and DMF	18	Wash, short column	74
7	**8**, **7**, EDC, HOBt, DIPEA	1, 0.43 1, 0.43 1, 0.43 1, 0.43 2, 0.86	DCM	2	Wash, short column	58
8	**8**, **7**, EDC, HOBt, DIPEA	1, 1.88 1, 1.88 1, 1.88 1, 1.88 2, 3.76	DCM	2	Wash, short column	77
9	**8**, **7**, EDC, DIPEA	1, 3 1.3, 3.9 1.3, 3.9 1.3, 3.9	DCM	2	Wash, short column	68

Table 3.1.2: *Results of Leu-Ala dipeptide syntheses.*

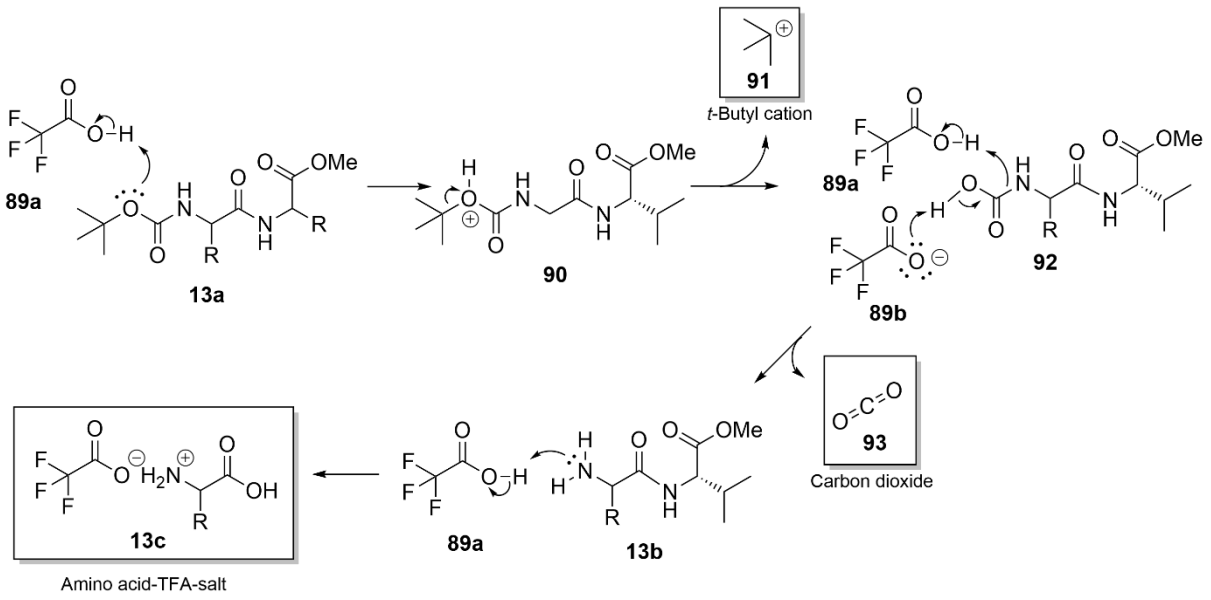
The conclusions drawn for the synthesis of the Gly-Val-dipeptide (figure 3.1.4) could be applied to the synthesis of Leu-Ala-dipeptide, but there were some additional points that had to be addressed. The Gly-Val synthesis were completed using only DCM as solvent, but as the AlaOMe compound (**7**) was poorly soluble in DCM, some slight adjustments to the procedure had to be made. The first adjustment made was to add DMF as solvent to the reaction, and this gave an increased yield, though the work-up and purification of the product became slightly more difficult. As DMF has a high boiling point, it does not evaporate easily and had to be removed by several rounds of washing and in some experiments a couple of rounds through silica-gel filled columns.

It was observed that some of the compounds that were not easily solved during the peptide synthesis seemed to dissolve after the addition of DIPEA to the reaction mixture. It was theorized that DIPEA as a proton scavenger would deprotonate the amino acid salts used in the synthesis, and that it could help dissolve compound **7**. This turned out somewhat successful, as compound **7** (in its salt form: HCl-Ala-OMe) dissolved after it was added directly to the reaction in its dry form, followed by the addition of DIPEA. Despite this, the yield seemed to slightly decline in the experiments where this was done.
The scale of the reactions conducted during the Leu-Ala-syntheses, as with the Gly-Val syntheses, seemed to be have no apparent effect on the yield, as the small scale experiments yielded about the same results as the large scale experiments.

When the dipeptides were coupled further, either when two dipeptides were coupled together to form tetrapeptides or the tetrapeptide with a single amino acid to form pentapeptide, the yields fell dramatically. (Table 3.1.3)

The reason for this could be in the method used for removing the amine-protecting Boc-group of the amino acid.

This method includes TFA as the deprotecting agent, and in theory what is left after an ended reaction is a mixture of compounds: a trifluoracetate salt of the amino acid and by-products such as trifluoroacetate, excess trifluoracetic acid, carbon dioxide and possible either a free *t*-butyl cation or a *t*-butyl bound to trifluoroacetate as a salt (scheme 3.1.1).



Scheme 3.1.1: *Proposed mechanism for deprotection of the amine.*

The removal of the impurities from the reaction mixture turned out to be quite difficult, and the important impurity was perhaps the excess TFA as it made the mixture acidic. Even after 24 hours on a high-vacuum pump, pH-strips revealed that the mixture was acidic which indicated TFA in the mixture. As a result, the products were put for even longer periods of time on the vacuum pump, but it did not seem to have any significant effect. Basic extraction was also tried, but the amino acid was too soluble in water compared to organic solvents, and water could not be used as solvent in further reactions. However, a method was tried in the next step of the plan, when coupling the slightly acidic dipeptide with another amino acid/dipeptide, where it was tried to add higher equivalents of DIPEA.

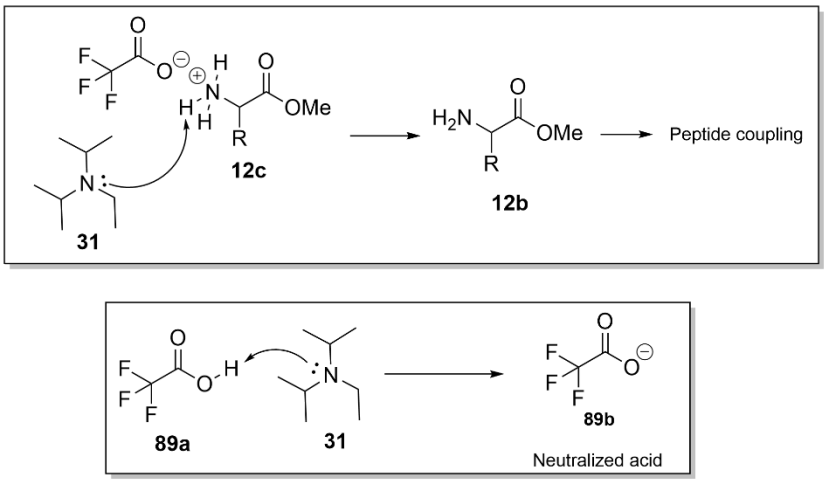


Figure 3.1.5: *DIPEA used to neutralize acid in addition to deprotonating amino acids.*

DIPEA was included in the reaction as a proton scavenger, but the amounts specified in the procedure was not measured to neutralize acid, but rather to deprotonate the amino acids. However, the basic property of DIPEA was used more extensively, as the equivalent was increased to neutralize the trace amounts of acid in the deprotected dipeptide mixtures (figure 3.1.5). The mixture was monitored with pH-strips as DIPEA was added to slightly basic conditions, and the observed yields increased to equal the dipeptide synthesis yields. However, this method was tried but once before the COVID-19 shutdown and NMR showed that the coupling of the dipeptide (Gly-Phe) did not couple with the additional glycine.

When the reaction mixture was analysed with TLC, a new spot appeared which indicates a new compound in the mixture. However, the NMR-spectrum of this mixture indicated that the Boc-protecting group no longer appeared in the molecules, so this new compound was not the wanted product.

The pentapeptide (**15a**) synthesised in this project (table 3.1.3) was made with Val (**6**) as the final amino acid instead of with Phe (**4**), which is the last amino acid in the Scopularides. This was due to the availability of amino acids at the time, but the reaction should work just as well with Phe as with Val.
However, generally the workup of the multi-peptides proved more difficult than the dipeptides, and the purification through flash chromatography did not yield an isolated product, but contained some impurities.

The NMR-spectrum of the tetrapeptide (**14a**) showed that the sample did not contain the isolated tetrapeptide, however it was possible to identify the peaks corresponding to the R-groups of the amino acids. Both the patterns of the peaks, and if the integral values of these peaks are compared to each other, they match the different amino acids that should be found in the tetrapeptide. (spectrum 3, page 102)

The NMR-spectrum of the pentapeptide (**15a**) was done of the crude product and like the tetrapeptide, it had contaminants which made the analysis of the spectrum more difficult. However, one can determine the corresponding peak patterns and the integral values to the different R-groups of the amino acids as to identify the pentapeptide (spectrum 4).

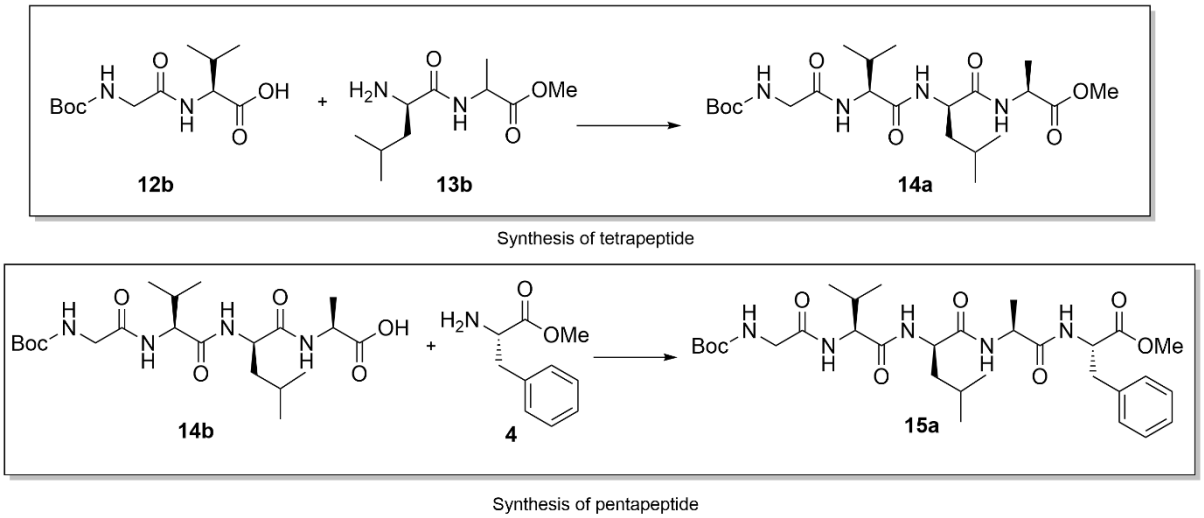


Figure 3.1.6: *The synthesis of tetra- and pentapeptide.*

Entry. no.	Reagents	Eq and mmol	Solvents	Duration (hours)	Workup	Product	Yield (%)
1	**12b**, **13b**, EDC, HOBt, DIPEA	1, 0.42 1, 0.42 1, 0.42 1, 0.42 2, 0.84	DCM	8	Wash, flash	**14a**	52
2	**14b**, **4**, EDC, HOBt, DIPEA	1, 0.22 1, 0.22 1, 0.22 1, 0.22 2, 0.44	DCM	8	Wash, flash	**15a**	15

Table 3.1.3: *Results of tetra- and pentapeptide syntheses.*

3.2 Negishi cross-coupling

The Negishi cross-coupling was a technique that seemed promising based on the success of several research groups, of which there were several articles to develop a procedure from.[17, 25]

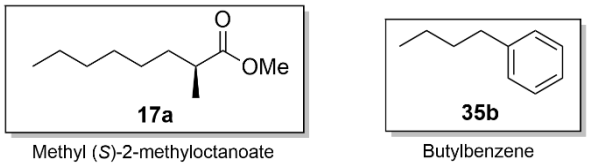


Figure 3.2.1: *The desired products of the cross-coupling reactions.*

However, there was some trouble reproducing the successful results reported in the published work which the procedures were based on.
Initially there was no easy way to monitor the reaction, as there were no visible changes in the reaction mixture and there was not found any TLC-stain that could show either reactants or products on the TLC-plate.

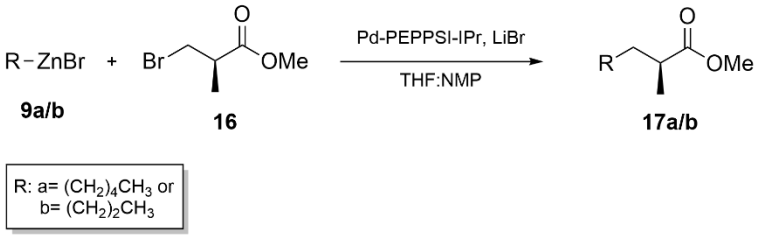


Figure 3.2.2: *Reactants and desired products for the initial cross-coupling.*

Due to the difficulties monitoring the reaction, the way used to decide if the reaction had been successful was to use instrumental analyses of the reaction mixture after the reaction had ended, such as GC-FID, GC-MS and NMR.

GC-MS analysis of the mixtures produced by the first attempts at the cross-coupling showed that there were no fragments that matched the expected product, but instead a β-hydride elimination product.
This alternative reaction path is summarized by McCann in his work on Negishi cross-couplings (figure 2.2.4, page 27), but he also reports that the use of the catalyst family of PEPPSI that were used in this project shows a tendency to resist β-hydride elimination (BHE).[25]

A likely explanation to this is that the conjugated system that appears in the molecule after the BHE is so preferable that it precedes the BHE-resistant property of the catalyst.

To work around this problem an alternative plan was made to remove the possibility for this conjugated system, by performing the cross-coupling at a later stage in the total synthesis. At the stage suggested, the molecule's carbonyl would not be in a position to form a conjugated system with the coupled alkyl (figure 3.2.3).

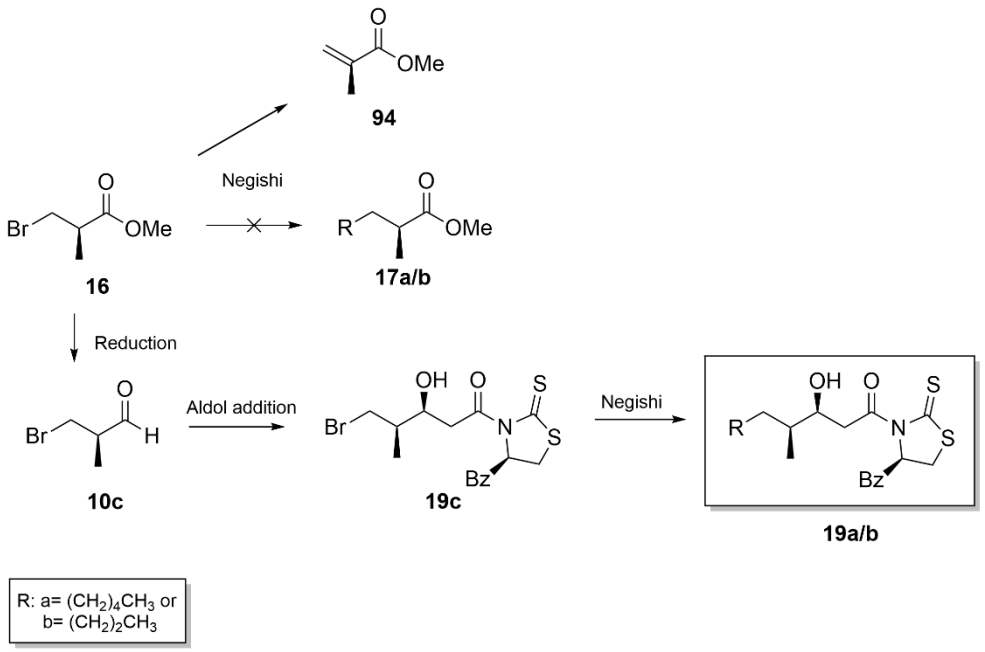


Figure 3.2.3*: Cross coupling at an alternate stage in the project, from compound* ***19c*** *instead of* ***16****.*

However, as there were difficulties producing successful results of the cross-coupling reaction, a series experiments were set up where the bromo-ester (**16**) were replaced by another, cheaper bromo-reagent (**34**) to check the general reproducibility of the reaction in the conditions proposed by Organ and McCann (figure 3.2.4).[17, 25]

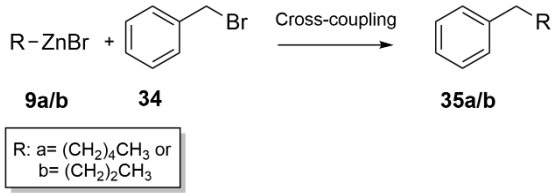


Figure 3.2.4: *Test reaction with benzyl bromide.*

This reaction was easier to monitor, as it was possible to use TLC due to the phenyl ring absorbing UV-light. The results from these tests did not show any products, but for one

reaction carried out in a pressure sealed test tube at 80 °C, where there were observed a new dot on the TLC-plate. This product was analysed with H-NMR, but it was not the desired product.
Due to the Covid-19 shutdown, it was not possible to run the necessary detailed analyses of the reaction with the new compound to determine the identity of it. The ^{1}H-NMR-spectrum did however give enough information as to exclude the desired product as a possibility.

In theory, the probability of success with the benzyl bromide (**34**) should be higher than with compound **16**. This due to the increased reactivity of benzyl bromide (**34**), as the phenyl ring's added stability to the molecule if the bromide were to act as a leaving group.

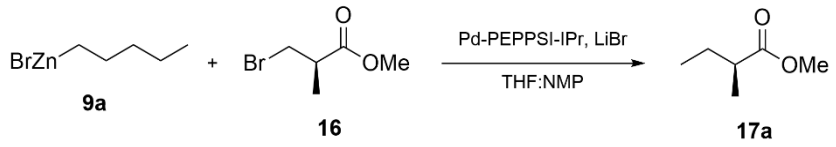


Figure: 3.2.5: *The cross-coupling reactions attempted with the bromo-ester (**16**).*

Entry. no.	Reagents	Eq	Solvents	Temperature (°C)	Time (hours)
1	**16**, **9a**, LiBr, Pd-PEPPSI-IPr	1 1.6 3.2 0.001	NMP	RT	4
2	**16**, **9a**, LiBr, Pd-PEPPSI-IPr	1 1.6 3.2 0.001	NMP	0 → RT	3
3*	**16**, **9a**, LiBr, Pd-PEPPSI-IPr	1 1.3 3 0.04	THF/NMP	0 → RT	4
4	**16**, **9a**, LiBr, Pd-PEPPSI-IPr	1 1.3 3 0.04	THF/NMP	0 → RT → 40	3
5	**16**, **9a**, LiBr, Pd-PEPPSI-IPr	1 1.3 3 0.04	THF/NMP	0 → RT → 55	3

Table 3.2.1: *Reagents and conditions used for the Negishi cross-coupling with compound **16** as reactant. None of the attempts yielded any desired products.*

**Procedure changed after two experiments, from Organ's conditions to Lucas' conditions.*[17, 25]

As shown in the table, one of the main variables in the experiments conducted was the temperature. The experiments that ran for more than 3-4 hours did so after it had been determined that there were no new products and was then allowed to continue through the night.

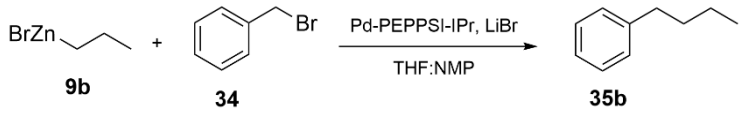


Figure 3.2.6: *The cross-coupling reactions attempted with benzyl bromide (**34**).*

Entry. no.	Reagents	Eq	Solvents	Temperature (°C)	Time (hours)
1	**34**, **9b**, LiBr, Pd-PEPPSI-IPr	1 1.3 3 0.04	THF/NMP	0 → RT	12
2	**34**, **9b**, LiBr, Pd-PEPPSI-IPr	1 1.3 3 0.04	THF/NMP	0 → RT → 60	20
3	**34**, **9b**, LiBr, Pd-PEPPSI-IPr	1 1.3 3 0.1	THF/NMP	0 → RT → 90	3
4	**34**, **9b**, LiBr, Pd-PEPPSI-IPr	1 1.3 3 0.1	THF/NMP	0 → RT → 50	3
5	**34**, **9b**, LiBr, Pd-PEPPSI-IPr	1 1.3 3 0.1	THF/NMP	0 to RT	12

Table 3.2.2: *Reagents and conditions used for the Negishi cross-coupling with compound **16** as reactant. None of the attempts yielded any desired products.*

The other big variable was the testing of compound benzyl bromide (**34**) instead of the bromo-ester (**16**), which underwent a similar temperature-variation sequence of experiments (table 3.2.2).

One of the possible reasons for the absence of success with this reaction, could be that the catalysator was more susceptible to air/moisture than has been reported.
However, the reactions were carried out in inert atmosphere, and there were done a multitude of tests under various conditions, where the only constant was the catalysator, which makes this theory unlikely.
Also, as illustrated in figure 2.2.4 (page 27), there is also several alternative by-products that could have been made.

As none of the reactions either with the bromo-ester (**16**) or with benzyl bromide (**34**) showed any sign of success, the cross-coupling reaction was discontinued, and an alternative plan for producing the non-peptide fragment was developed (chapter 2.5 and 3.5).

3.3 Reduction of ester to aldehyde

The reduction of the theorized and actual bromo-esters to their corresponding aldehydes (figure 3.3.1) was a reaction that was included in the original plan to complete the non-peptide fragment. However, as the Negishi cross-coupling technique was shelved, so was also the ester, which made this reaction unnecessary.

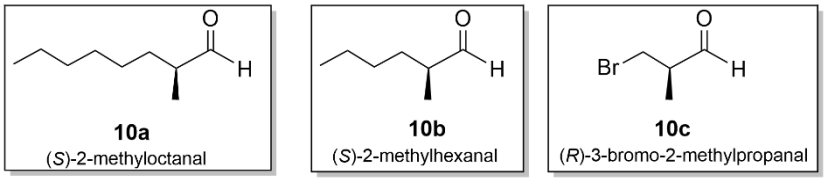


Figure 3.3.1: *The desired products of the reduction of different esters.*

As the Negishi cross-coupling (chapter 3.2) did not yield results, compound **10a/b** could not be made, but the reduction of the bromo-ester (**16**) to the corresponding aldehyde was completed (figure 3.3.2).

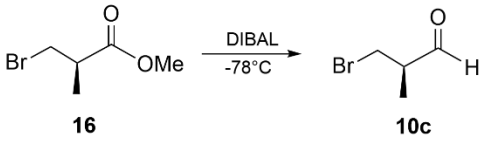


Figure 3.3.2: *The reduction of bromo-ester (**16**) to its corresponding aldehyde (**10c**).*

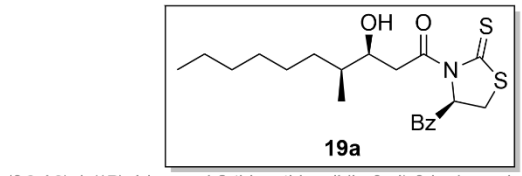
This reaction was possible to monitor with TLC, but it became apparent that it was difficulties during the workup and when determining the yield. The NMR-spectrum of the **10c**-aldehyde (spectrum 5, page 104) was run on the crude product, as the aldehyde was unstable and could not undergo further workup without degradation. Nevertheless, one can identify the aldehyde quite easily by its singlet in the ^{1}H-NMR-spectrum, which has a higher chemical shift-value than most protons, and which is clearly present in the spectrum.

When another NMR-analysis were run on the same sample later, several singlets in the aldehyde area had appeared, indicating a possible decomposition of the product.

Due to the apparent instability of the aldehyde, the remaining reactions involving the DIBAL-reduction were made into one-pot syntheses, as to improve the yield of the aldehyde and its following products.

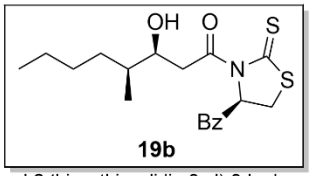
3.4 Aldol addition

The aldol addition would produce compounds **19a/b** (figure 3.4.1), which for this project made it equivalent to the non-peptide fragment (**2a/b**, figure 1.2.1, page 11). In addition, compound **95** was synthesised as a test of the reaction's conditions (figure 3.4.1).



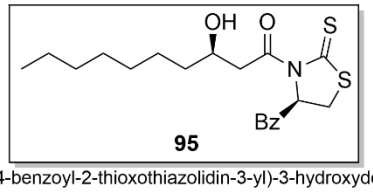
19a

(3*S*,4*S*)-1-((*R*)-4-benzoyl-2-thioxothiazolidin-3-yl)-3-hydroxy-4-methyldecan-1-one



19b

(3*S*,4*S*)-1-((*R*)-4-benzoyl-2-thioxothiazolidin-3-yl)-3-hydroxy-4-methyloctan-1-one



95

(*R*)-1-((*R*)-4-benzoyl-2-thioxothiazolidin-3-yl)-3-hydroxydecan-1-one

Figure 3.4.1: *The desired products of the aldol addition.*

As the Negishi cross-coupling reaction had been discontinued (chapter 3.2) when the aldol reaction really got into focus during the project. This meant that compound **16** was no longer to be used to make the non-peptide fragment (**19a/b**, figure 3.4.2), but rather compounds **69** and **72** which were produced by the asymmetric alkylation reaction discussed in the next chapter (chapter 3.5).

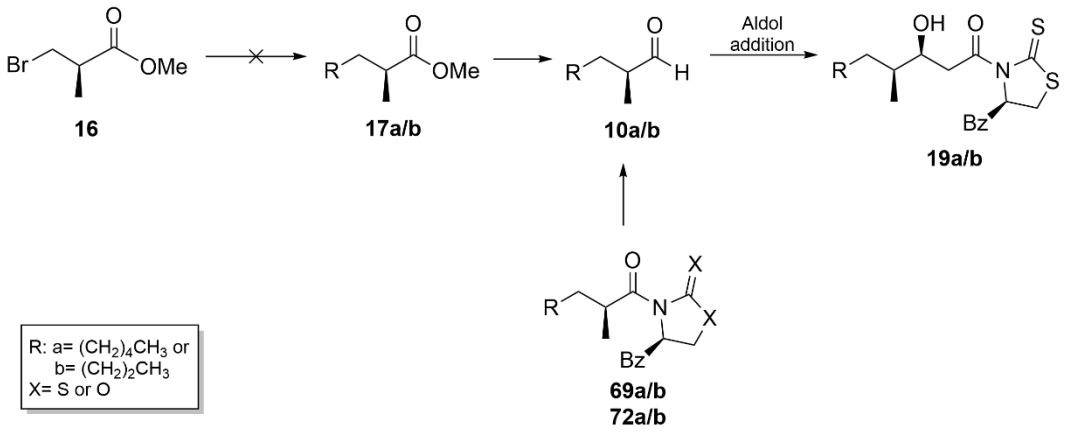


***Figure 3.4.2**: The alternative path to synthesise the **10a/b** reagent needed for the aldol addition.*

While the reagents needed to attempt this alternative synthesis of compounds **19a/b** was ordered, octanal (**96**) was used along with the chiral auxiliary (**60b**) to test the reaction (figure 3.4.3). This due to it being available at the time and having an alkyl chain of about the same length as **19a**, in addition to octanals' lack of chiral centre which made it less expensive.

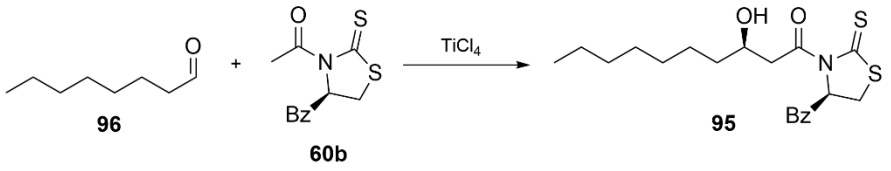


Figure 3.4.3: The aldol reaction with octanal and chiral auxiliary.

Thus, most of reactions conducted, and all the product-yielding reactions, were the ones using octanal (**96**) as a reagent.

The test reactions done with octanal were in part done to test different conditions for the reaction, as to optimize it for the actual products. The conditions are listed in the following table (**table 3.4.1**).

Entry. no.	Reagents	Eq and mmol	Temperature (°C)	Time (hours)	Yield (%)
1	**96**, **60b**, DIPEA, $TiCl_4$	0.86, 0.86. 1, 1. 0.44, 0.44. 0.95, 0.95.	-78	12	30
2	**96**, **60b**, DIPEA, $TiCl_4$	1, 0.5. 1, 0.5. 1, 0.5. 1.3, 0.65.	0 → -78	2-3	46
3	**96**, **60b**, DIPEA, $TiCl_4$	2, 1. 1, 0.5. 1, 0.5. 1.1, 0.65.	0 →-78	2-3	43
4	**96**, **60b**, DIPEA, $TiCl_4$	1, 0.55. 1, 0.55. 1, 0.55. 2, 1.	0	2-3	19
5	**96**, **60b**, DIPEA, $TiCl_4$	1, 1.7. 1, 1.7. 1, 1.7. 1.3, 2.2.	0 → -78	2-3	?

Table 3.4.1: *Results and conditions from/for the aldol addition.*

Of the experiments completed towards the aldol addition in this project, entry no. 2 was the most promising, where the equivalents of the reagents were about the same with a slight excess of $TiCl_4$. This reaction started at 0 °C and was kept at this temperature while the thiazolidinethione (**60b**), $TiCl_4$ and DIPEA was mixed and giving the mixture a deep red colour, indicating the chelation of **60b**-$TiCl_4$. Following this, the temperature was lowered to -78 °C to counteract the heat being made when adding the last reagent, octanal (**96**).

The results from reaction entry no. 3 were quite similar, and as the only difference between this and no. 2 was the equivalents used of **96** and $TiCl_4$, it implies that the complex formation of these two reagents may be the limiting factor to the reaction.

Entry 1 was conducted based on the conditions set by H. Olivo, and the remaining entries were conducted based on the conditions set by A. Phillips in an attempt to increase yield.[28, 36]
However, the conditions of entry no. 3 and 4 were modified, based on reports by a multitude of reports by M. Crimmins concerning equivalents and temperatures used.[34, 35, 38, 58]

Entry no. 4, in which the temperature was kept at a constant 0 °C the yield was reduced considerably, indicating that the low, sub -70 °C temperature indeed is an important factor for the formation rate the wanted product. However, this reaction mixture was not analysed with any chromatographic or spectrographic method of detail, and it proved difficult to determine what by-products were formed during this experiment.

Entry no. 5 was done with the same equivalents and conditions as entry no. 2, as it was the experiment that had given the highest yield. However, the amounts used were increased, to check if this system would give the same yields when upscaled. Alas, due to the sudden Covid-19-pandemic shutdown, the product, which underwent the fundamental workup, was left for over a month before further analyses could be done. At that point, the product had decomposed, and only trace amounts of the desired product could be found in the NMR-spectrum.

This one pot synthesis introduced impurities to the aldol reaction as the aldehyde product mixture was not purified. However, there should be most of the intended aldehyde product compared to the impurities.
These impurities were trace amounts of compounds used in the DIBAL-reduction and perhaps some degenerated form of the aldehyde, as this was quite unstable.
Nonetheless, even if the reaction mixture contained some impurities, the probability of the impurities destroying all the necessary reactants of the reaction was quite low, which in turn makes the probability of achieving the reaction with the intended reactants high.

As for confirmation of this product, the peaks in the COSY spectrum of **95** (spectrum 13, page 112) confirms the coupling between the chiral hydrogen connecting the benzyl group and the sulphur containing heterocyclic ring. As this is a characteristic identifier of this chiral auxiliary, the spectrum could be used to identify the chiral auxiliary in other compounds containing the same chiral auxiliary.

The CH_2-group that connects the sulphur atom and the chiral centre of the heterocyclic ring does not behave entirely as expected, as only one of the protons are coupling with the chiral centre while the other only shows a geminal coupling. This is shown in the coupling patterns of the 1D ^{1}H-NMR spectra, and in the COSY spectra of the molecules containing this chiral auxiliary molecule. It is possible that the CH_2-group in question could be the other CH_2-group connected to the chiral centre, in the benzyl-group. However, it is more likely that a neighbouring sulphur atom is inducing this coupling pattern than that a neighbouring benzene-group is inducing it.

It was hoped that NOESY spectrum of compound **95** could confirm that the wanted R-isomer of the molecule is produced, but the distance between the rotating benzyl group was too far away from anything in the molecule to register but its connecting CH-CH_2-chain.
However, comparing the spectral data to the data of a similar molecule (**97**, figure 3.4.3) found in an article from 2008, the chemical shift values are virtually identical, heavily implying that it is the R-isomer which is present in the mixture.[59]

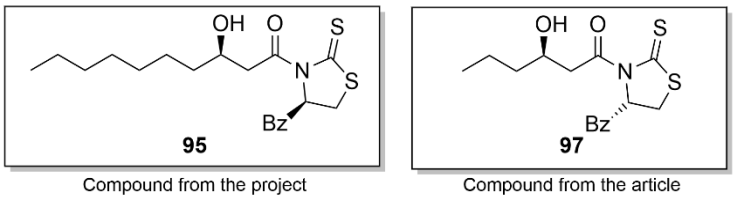


Figure 3.4.3: *Comparison of the molecule from the project and the one from article.*

Crimmins reports in his article from 2001 that this chiral centre's (figure 3.4.4) different stereoisomers (**98** and **99**) have different shift values, which also serves to strengthen the claim that the product of the reaction was the R-isomer.[38]

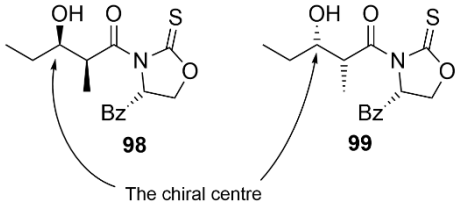
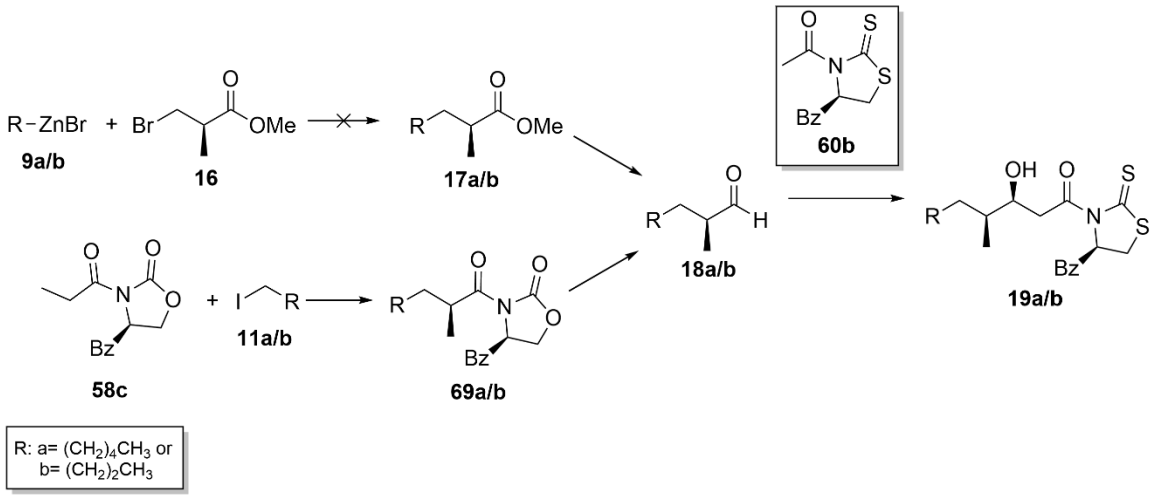


Figure 3.4.4: *The two molecules in Crimmins' article with reported different chemical shift values for the chiral centres.*

3.5 Asymmetric alkylation

This alkylation method (figure 3.5.2) was attempted as an alternate path to complete the non-peptide fragment of the molecule (scheme 3.5.1), as the Negishi cross-coupling had not turned out any successful/wanted products.



Scheme 3.5.1: *Alternative path to* ***19a/b****, as presented in chapter 1.3.*

Following the original plan with the Negishi cross-coupling, the starting molecule (**16**) when had the correct stereochemistry. Following the alternative plan, the stereochemistry in the compounds (**69** and **72)** had to be obtained using chiral auxiliary compounds (figure 3.5.2).

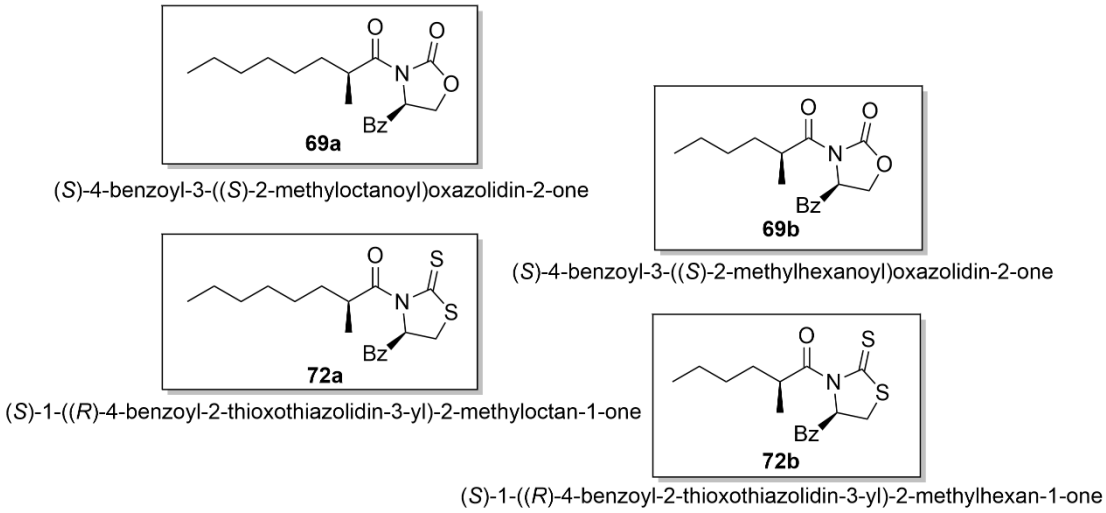


Figure 3.5.1: *The desired products of the asymmetric alkylation.*

The planned products of this alkylation are listed in figure 3.5.1, but only **69b** and **72b** were attempted to be synthesised.
This due to that the alkylation was attempted using butyl iodide (**11b**) in all the attempts, but it was used two different chiral auxiliaries (**58c** and **60c**) and the chelation agent also differed between the attempts (NaHMDS and $TiCl_4$).

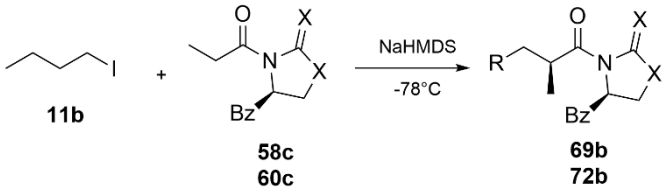


Figure 3.5.2: *The asymmetric alkylation.*

Entry. no.	Reagents	Eq	Solvent	Time (hours)
1	**58c**, NaHMDS, **11b**	1 1.15 5	THF	2
2	**58c**, NaHMDS, **11b**	1 1.15 5	THF	2

Table 3.5.1: *Conditions and reagents for the attempted alkyl addition to produce* ***69b.***

Entry. no.	Reagents	Eq	Solvent	Time (hours)
1	**60c**, NaHMDS, **11b**	1 1.15 5	THF	**2**
2	**60c**, $TiCl_4$, DIPEA, **11b**	1 1.3 1 1	DCM	**12***

Table 3.5.2: *Conditions and reagents for the attempted alkyl addition to produce* ***72b.***

**1 hour at -78 °C, then 12 hours at RT*

All the reactions were monitored using TLC, and the entry no.1 with thiazolidine auxiliary (**60c**) revealed by that the propionylated chiral auxiliary molecule lost its propionyl chain and reverted to the fundamental molecule (**60a**) after the addition of NaHMDS.

As shown in table 3.5.1 and 3.5.2, the only repeated reaction was done with the oxazolidine chiral auxiliary (**58c**), as it did not degenerate during the reaction. There difference between entry no. 1 and 2 (table 3.5.1) was that the latter reaction the inert atmosphere was kept with extreme care, where all the glass equipment was flame-dried, and the nitrogen gas were supplied/flushed quite generously.

The reactions where the oxazolidine chiral auxiliary (**58c**) were used seemed more promising based on TLC, where the decomposition of the chiral auxiliary did not seem to take place. However, when analysed with GC-FID and NMR, it was revealed that it was not the desired product.

As for the thiazolidine auxiliary (**60c**), it was theorized that the chelating agent (NaHMDS) possibly could remove the propionyl chain from the thiazolidine chiral auxiliary. As an alternative, it was tried to substitute NaHMDS with $TiCl_4$ as a chelating agent, as the two chelating agents had somewhat similar function.

The first day during the experiment, a colour change to deep red while the reaction was cooled down to -78 °C indicated a complex formation with $TiCl_4$. TLC-analysis did however show that there was no new product, so the temperature was gradually increased to room temperature and was continued through the night.
The next day the deep red colour had been replaced by a brown colour, and TLC-analysis showed no change from the previous day. The reaction was then quenched, purified, and then analysed with NMR. The NMR-spectrum showed that there was nothing of the wanted product in the reaction mixture.

Post-experiment research revealed articles that reported success with similar molecules, but where methyl iodide was used instead of iodides with longer alkyl chains, as were used in this project.[60]
This could indicate that the iodides with longer alkyl chains exercises too much steric hinderance for the reaction to take place.

3.6 Chiral auxiliaries

The method for synthesising the chiral auxiliaries (figure 3.6.1) were based on work by P. Romea and his research group.[50]

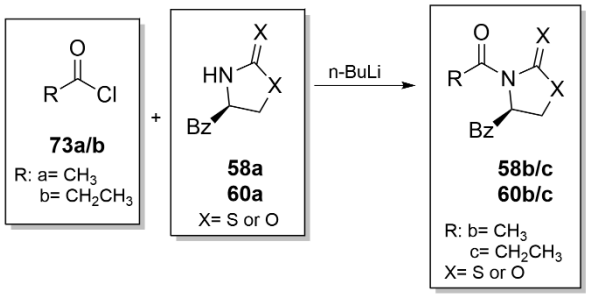


Figure 3.6.1: *The synthesis of the chiral auxiliaries.*

Although the reagents used in the project were not identical to the ones used in this publication, the differences were of such low significance that they did not matter for the reproducibility of the procedure.
By most accounts, the synthesis of the chiral auxiliaries was the most successful reactions in the entire project with yields ranging from 89 %-97 %. (**Table 3.6.1**)

When monitoring with TLC, the plates showed that the starting compounds disappeared either entirely or close to entirely during the reaction. The small percentages that were unaccounted for were probably lost during workup or due to transfer loss when transferring between reaction vessels etc.

The thiazolidine products (**60b/c**) and **58c** were confirmed using NMR-spectroscopy (spectra 8, 9 and 10), and its confirmation is discussed in **chapter 3.4**, as there was run a bigger array of spectrums on the aldol-products which contained the same heterocyclic chiral auxiliaries. As there were only reactant and wanted product spots on these TLC-plates, it was thought of as a temporary confirmation of **58b** as well when they showed the equivalent spots. The confirmation of **58b** through NMR-analysis could unfortunately not be done, due to the COVID-19 pandemic-shutdown.

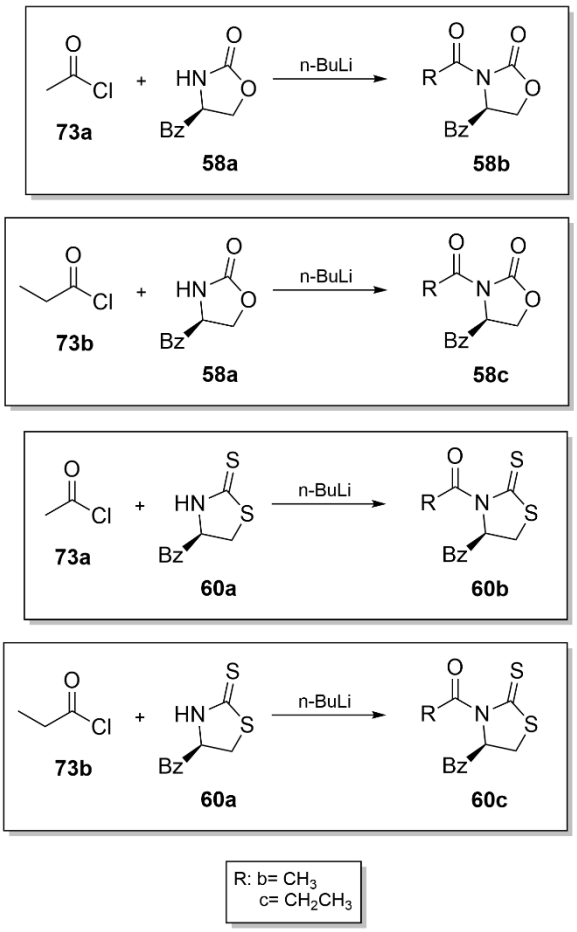


Figure 3.6.2: *The individual reactions for the synthesis of the different chiral auxiliaries.*

Entry. no.	Reagents	Eq and mmol	Workup	Product	Yield (%)
1	**60a**, n-BuLi, **73a**	1, 0.5 1.1, 0.55 1.3, 0.65	Extracted, flashed	**60b**	91
2	**60a**, n-BuLi, **73a**	1, 1.5 1.1, 1.65 1.3, 1.95	Extracted, flashed	**60b**	90
3	**60a**, n-BuLi, **73a**	1, 3 1.1, 3.3 1.3, 3.9	Extracted, flashed	**60b**	96
4	**58a**, n-BuLi, **73b**	1, 3 1.1, 3.3 1.3, 3.9	Extracted, flashed	**58c**	96
5	**60a**, n-BuLi, **73b**	1, 3 1.1, 3.3 1.3, 3.9	Extracted, flashed	**60c**	97

Table 3.6.1: *Results of chiral auxiliary syntheses.*

As shown in the table (table 3.6.2), the same equivalents and procedures were used for all the experiments, and the only variables were the reactants and the amounts used. Neither of these variables seemed to have any impact on the yield.

3.7 TBS-protection

The TBS-protection experiments were carried out by using 2-octanol as a substitute compound, in preparation of using the procedures on the molecules that eventually could become the end products.

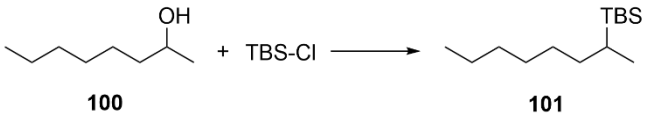


Figure 3.7.1: *The general TBS-protection reaction.*

The reason for this choice was because of 2-octanol's properties as a secondary alcohol, which was somewhat similar to the secondary alcohol in alkyl chain length and being a secondary alcohol compared to the molecules that were synthesised in the towards the goal of the project (figure 3.7.2).

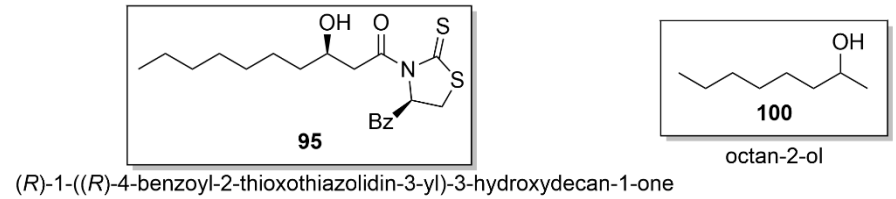


Figure 3.7.2: *Comparison of the secondary alcohol produced during the project and 2-octanol, used as a placeholder molecule.*

As these were brief test reactions, which were meant to give an inkling to as if the reactions could be successfully conducted in the reported conditions, not much time were spent on the purification of the product. NMR spectra were produced on crude product or on product that had a brief workup.

However, all the reactions were monitorable by TLC, and the NMR-spectra of the different TBS-protected molecules showed that the protective reactions were successful.

There were two procedures used in the project, as one method used a DMAP-catalysation system and the other did not. The reaction without DMAP, did include DMF as solvent, as opposed to the DMAP catalysed which used DCM as solvent.
The procedure developed featuring a DMAP-catalysation system with an auxiliary base was based on work by P. Patschinski.[51]

The test reactions on 2-octanol (**100**) were successful, but TBS-protection of the hydroxyl group in compound **95** was not, and it was theorized that DMAP could react with the thiocarbonyl heterocyclic ring of the chiral auxiliary or the carbonyl group of the aldol, as illustrated in figure 3.7.3.

Also, during post-experimental research, an article written by Y. Wu in 2004 was found which substantiates this theory, as it is about the removal of thiazolidinethione auxiliaries mediated by DMAP.[61]

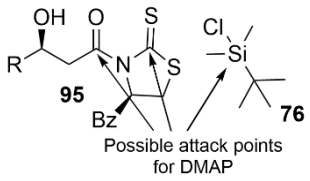


Figure 3.7.3: *DMAP has different possible attack points.*

Because of this unfortunate mechanism, the return to the traditional way of TBS-protecting hydroxyl groups seemed logical, as it did not include DMAP.

The test reactions for the traditional, DMAP-absent variation of the method were carried out on 2-octanol, as this reagent was plentiful while compound **95** was not abundant.

3.8 Nagao aminolysis

The purpose of this reaction was to remove the heterocyclic part of the chiral auxiliary and substituting it with an amino acid, so it could be further used to cyclize the molecule.

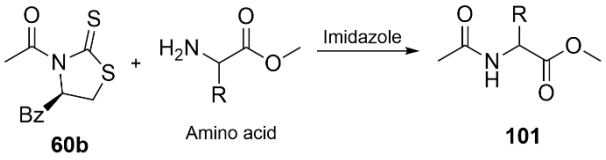


Figure 3.8.1: *General aminolysis.*

This reaction was quite easily monitorable as the mixtures containing the chiral auxiliary compounds were yellow-coloured, and which lost its colour as the chiral auxiliary was replaced with the aminolysed product. This was an easily observable indicator, but the reactions were also monitored with TLC, which corresponded with the colour change in all the experiments.

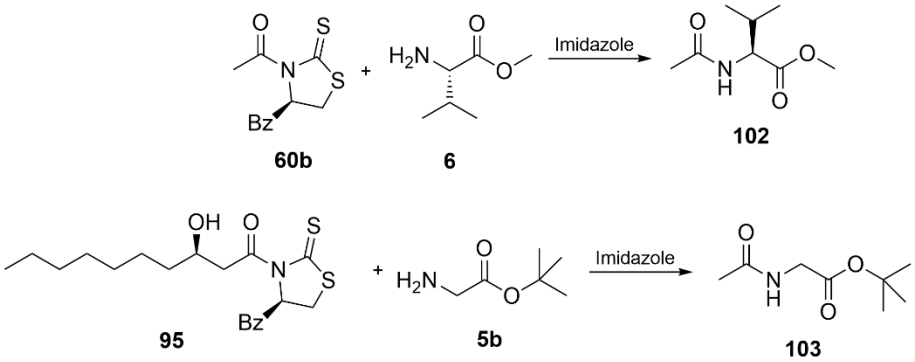


Figure 3.8.2: *The aminolysis of the chiral auxiliaries.*

Entry. no.	Reagents	Eq	Colour change	Desired product	Yield (%)
1	**60b**, **6**	1 1.1	No	**102**	-
2	**60b**, **6**, imidazole	1 1.1 3	Yes	**102**	78
3	**95**, **5b**, imidazole	1 1.1 2	No	**103**	-
4	**95**, **5b**, DIPEA	1 1.1 1.1	No	**103**	-

Table 3.8.1: *Results from the aminolysis reaction*.

A factor that played a role in the successful experiment of this reaction was the addition of imidazole. Its role is not entirely clear, but a theory is that it works as an auxiliary base which can deprotonate the nucleophilic base, making the latter base more reactive towards the carbonyl-carbon (figure 3.8.3).

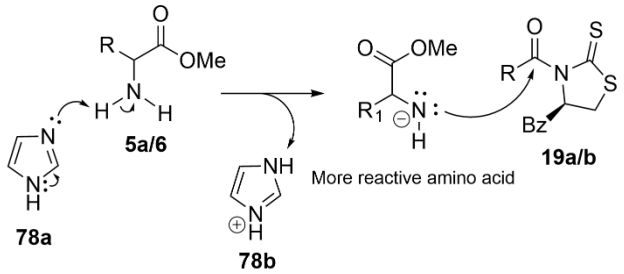


Figure 3.8.3: *Proposed function of imidazole.*

The reactions that were unsuccessful could be due to differences in electronegativity of the amines of the amino acids used, as the successful reaction was done with Val (**6**), while the unsuccessful were carried out with Gly (**5b**). The obvious difference between these amino acids are the R-group, but there is also a difference in the protecting group of the *C*-terminus of the amino acids. In Val (**6**) it is ester-protected, while the Gly (**5b**) is protected by a *t*-butyl group (figure 3.8.2). This could possibly affect the reaction, but it could also be a result of the addition of the long alkyl chain of the chiral auxiliary molecule (**95**), which could apply steric hinderance.

The NMR-spectrum of compound **102** (spectrum 11, page 110) contained traces of solvent, and the CH_3-peak of the molecule is hidden within the singlet of ethyl acetate, which was used as a solvent in the reaction. However, except for this hidden peak the rest of the molecule is clearly visible with easily identifiable peaks in the spectrum.

3.9 Macrolactamization

The macrolactamization of the molecule was one of the projects final obstacles to overcome, as to complete the final Scopularide molecules (figure 3.9.1).

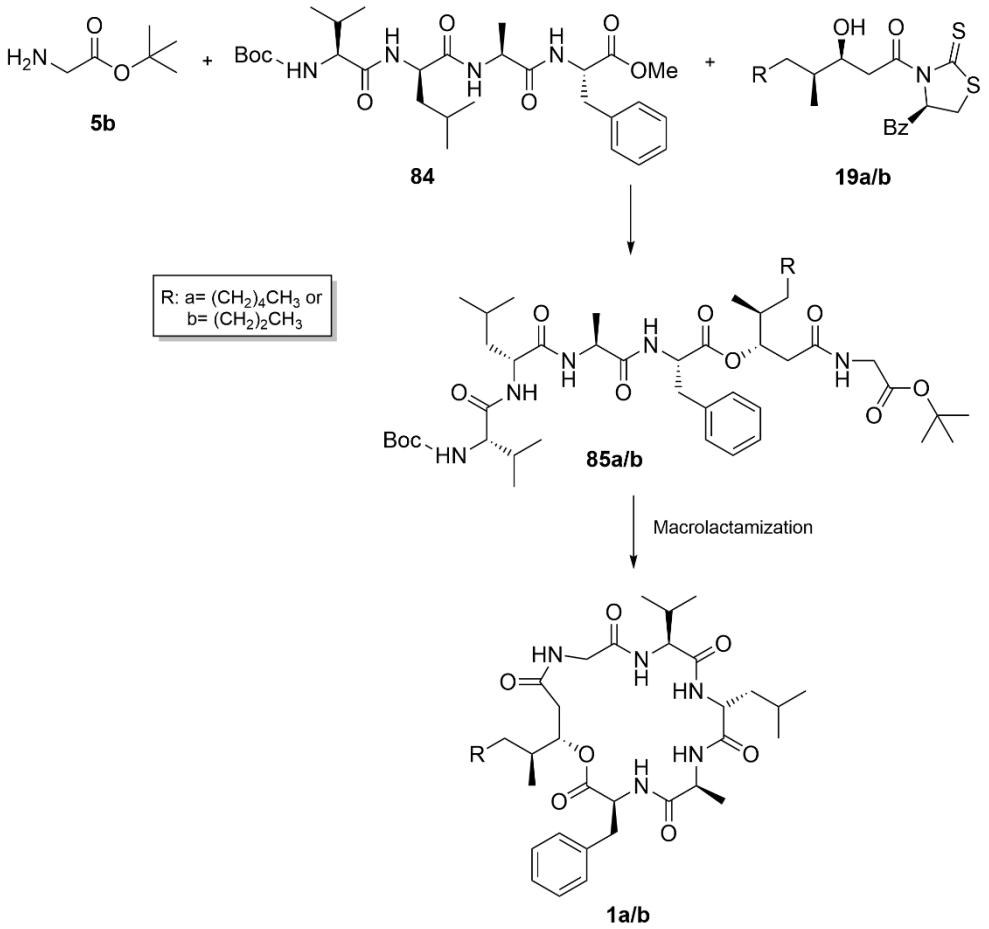


Figure 3.9.1: *The macrolactamization, as presented in chapter 2.9.*

This plan was initiated with a test reaction, where an amino acid was appended to a hydroxyl group of octan-2-ol (**100**) with an acylation reaction (figure 3.9.2). The hydroxyl containing molecule used for the first experiments was 2-octanol (**100**) which acted as a placeholder molecule for compound **19a/b** as both of these molecules contain an alkyl chain of similar length in addition to the hydroxyl (figure 3.7.2, page 72).

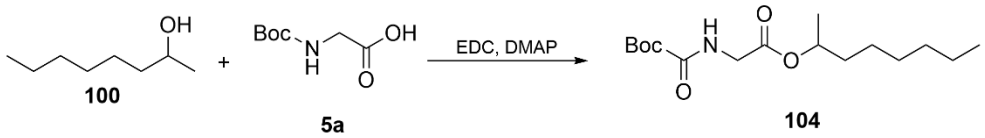


Figure 3.9.2: *The acylation test reaction.*

The experiment using octan-2-ol (**100**) was carried out as a test to see if the acylation reaction could be completed, and NMR-analysis of the product solution showed that it was a successful reaction (spectrum 12, page 111).
The other essential reaction was to connect the other end of the peptide chain to the non-peptide fragment through an aminolysis, as was discussed in the previous chapter (chapter 3.8).

This reaction did not go as planned, and TLC-analysis showed a multitude of new products. This could be due to the same problem that was encountered during the TBS-protection of the same molecule (chapter 3.7), and the suspected culprit is DMAP. Although unconfirmed, the several points of attack for the DMAP-base would account for the multitude of products.

Unfortunately, before we could isolate and analyse any of these products the covid-19 pandemic hit, and the lab was shut down.

Chapter 4: Conclusions and future outlook

4.1 Conclusion

The synthesis of the target molecules was not achieved during this project. The synthetic pathway must be altered to synthesise Scopularide. Nevertheless, several of the reactions/methods used were successful and could be used in future projects.

The peptide fragment of four to five amino acids in length is very much obtainable, although the procedure for deprotection and purification needs to be optimized. Even so, several factors were optimized during the project, such as equivalents, solvents and reagents used along with the optimal time used for synthesising dipeptides.

None of the plans for synthesising the complete non-peptide fragment were successful, but alterations or changes from reactions to similar reactions may well result in successful syntheses.

The Negishi cross-coupling was unsuccessful, but using a cross-coupling technique should still be considered, as they in general are powerful tools of forming carbon-carbon bonds. The reactants and catalysator-system should be changed, as the absence of desired products using them indicates strongly that they are not ideal for this specific bond-formation.

The aldol addition was successful, but it should be determined if the complex formation between chiral auxiliary and $TiCl_4$ is the limiting factor of the aldol reaction, as to improve the yield of the reaction. This could be done by varying the equivalents used while the time- and temperature conditions are kept static, then systematically change single conditions.
The formation of the aldehydes needed for the aldol addition were also successful, but the technique should be optimized, and the products should ideally be purified.

The synthesis of the chiral auxiliaries during this project was easily reproduced from articles, and they are a powerful and necessary tool to achieve asymmetric syntheses such as in the aldol addition and the alkylation of a chiral auxiliary.

The few attempts of cyclization of the scopularides during this project showed limited success. However, this could still turn out to be a viable path, and it could be attempted either through lactonization or lactamization, and possibly both. The focus should however be at finding a stable way of appending the *N*-terminus of the peptide-fragment to the non-peptide fragment, perhaps alternatives to the Nagao-aminolysis.

Unfortunately, as the molecules were not finished, neither was there any possibility of any elaborate evaluation of the potential biological effect of the compounds.

4.2 Future Outlook

In general, the different Scopularides still needs to be assessed, along with the A and B versions which was attempted to synthesise in this project. The development of a viable synthesis route is therefore essential for the large-scale synthesis, which is needed for such assessments as opposed to the tiny amounts isolated from natural sources.

Several/most of the structures should be confirmed by more than ^{1}H-NMR, using for instance either with more detailed NMR techniques (COSY, NOESY, HMBC/HSQC etc) or with HR-MS.

The use of solid phase peptide synthesis should be considered, as it is generally viewed as a superior way of synthesising peptides.

Isolation of the aldehyde of the DIBAL-reduction could be beneficial, as of to identify the molecule and to determine the yields of this reaction.

Alternative routes of producing the non-peptide fragment should be explored. The alkylation method attempted in this project could potentially be tried with methyl iodide instead of hexyl-/butyl iodide, as articles indicates successful reactions using methyl iodide.

The chiral auxiliaries were produced with success, and both synthesis and use of these compounds were confirmed during the project. In further work, chiral auxiliaries with different R-groups on the ring structure could be tested.

The Nagao-aminolysis should be explored further, and what reactants that can be used for successful reactions should be mapped. Also, the function of imidazole should be elaborated.

Chapter 5: Experimental section

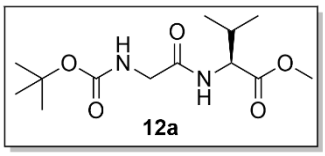
5.1.1 Peptide couplings

With HOBt,

General procedure:

A dry round-bottomed flask with magnetic stirring bar was flushed with nitrogen gas. The BOC-protected amino acid (1 eq) was solved in DCM, then the solution was cooled down to 0 °C°C before HOBt (1 eq) and EDC (1 eq) was added to the mixture and stirred for 15 minutes. The methyl ester amino acid (1 eq) was dissolved in a separate, pear-shaped flask and sealed with a septum before this mixture was transferred dropwise to the reaction mixture through a teflon tube. DIPEA (2 eq) was added to the mixture along with the second amino acid. The reaction then continued in room temperature.
The reaction was monitored with TLC, treating the plates with ninhydrin to develop the spots made by peptides.
After the reaction ended the mixture was diluted with EtOAc and then washed with aqueous, saturated NH_4Cl-solution and brine before the organic phase was dried over $MgSO_4$. The $MgSO_4$ was then filtered away and the product was purified through various silica-gel filled columns.

5.1.2 Synthesis of compound **12a**, methyl (*tert*-butoxycarbonyl)glycyl-L-valinate.

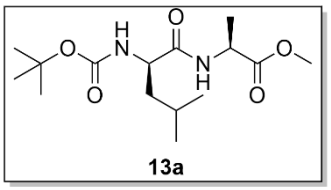


0,335 g Boc-Glycine-OH (1 eq) was added to a flask and solved in 6 mL dry DCM. The solution was cooled down to about 0 °C before 0,259 g HOBt (1 eq) and 0,367 g EDC (1 eq) was added to the mixture. 0,325 g L-valine methyl ester hydrochloride (1 eq) was added to a dry pear-shaped flask and dissolved in 6 mL dry DCM. After 15 minutes the Valine/DCM-solution and 0,72 mL DIPEA (2 eq) was added to the reaction mixture. The reaction then continued through the night before it underwent workup. The product was concentrated in vacuo and purified through a short silica-gel filled column under reduced pressure, using EtOAc as eluent.

Yield: 97 %

Spectral data for compound **12a**:
^{1}H NMR (400MHz, $CDCl_3$): δ 0.81 (dd, *J* 7.5 Hz and ^{2}*J* 6.8 Hz, 6H, CH(*CH*$_3$*)2*), 1.34 (s, 9H, OC(*CH*$_3$*)3*), 2.05 (dsept, *J* 6.8 Hz and *J* 6.6 Hz and *J* 6 Hz, 1H, NC*CH*), 3.62 (s, 3H, O*CH*$_3$), 3.72 (m, 2H, N*CH2*), 4.42 (dd, *J* 6.6 Hz and *J* 6.0 Hz, 1H, N*CH*), 5.54 (s, 1H, *NH*), 6.88 (s, 1H, *NH*).

5.1.3 Synthesis of compound **13a**, methyl (*tert*-butoxycarbonyl)-D-leucyl-L-alaninate.

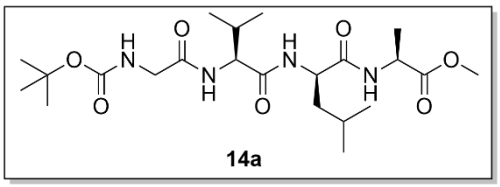


0,100 g Boc-D-Leucine-OH (1 eq) was added to the flask and 1,8 mL dry DCM was used as solvent. The solution was cooled down to about 0 °C before 0,056 g HOBt (1 eq) and 0,082 g EDC (1 eq) was added to the mixture. 0,060 g L-alanine methyl ester hydrochloride (1 eq) was added to a 5 mL dry pear-shaped flask and dissolved in 1,2 mL DMF. After 15 minutes the Valine/DMF-solution and 0,13 mL DIPEA (2 eq) was added to the reaction mixture.
The reaction then continued through the night before it underwent workup. After the general workup, the product was concentrated in vacuo and purified with flash chromatography, using hexane:EtOAc (2:1) as eluent.

Yield: 96 %.

Spectral data for compound **13a**:
^{1}H NMR (400MHz, $CDCl_3$): δ 0.15 (dd, *J* 3.5 Hz and *J* 2.9 Hz and *J* 3.4 Hz (^{2}J 5.0 or 2.2 Hz 6H, CH(CH_3)$_2$), 0.61 (d, *J* 7.3 Hz, 3H, CHCH_3), 0.66 (s, 9H, OC(CH_3)$_3$, 0.70-0.89 (m, 3H, CH-CH_2-CH-(CH_3)$_2$ and CH-CH_2-CH-(CH_3)$_2$, 2.94 (s, 3H, OCH_3), 3.42 (s, 1H, $CH$$CH_3$), 3.77 (quint, *J* 7.3 Hz, 1H, N-CH-CH_2), 4.69 (s, 1H, NH), 6.50 (s, 1H, *NH*).

5.1.4 Synthesis of compound **14a**, methyl (*tert*-butoxycarbonyl)glycyl-L-valyl-D-leucyl-L-alaninate.



0,091 g Boc-Leu-Ala-OMe (1 eq) was added to a flask and was solved in DCM, before the solution was put on a cooling bath and cooled down to 0 °C. An excess of TFA (1 mL) was added to the mixture via syringe, and the reaction was stirred for 30 minutes. The product, TFA-Leu-Ala-OMe, was then concentrated in vacuo.

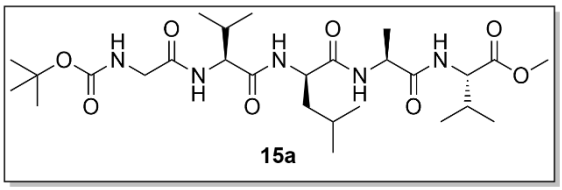
0,116 g Boc-Gly-Val-OMe (1 eq) was added to a flask and solved in a solvent-combination of THF, MeOH and water (3:1:1).
The reaction was put on a cooling bath and cooled down to 0 °C, before 0,032 g LiOH (3 eq) was added. The reaction stood for 60 minutes before the pH of the reaction was adjusted to 2, using 1M HCl solution. The reaction was diluted with EtOAc and washed with brine before the organic phase was dried over $MgSO_4$.
The reaction was filtered, and the filtrate was concentrated in vacuo in which the resulting product was Boc-Gly-Val-OH.

Approximately 0,422 mmol of the Boc-Gly-Val-OH was dissolved in DCM. The solution was cooled down to about 0 °C before 0,057 g HOBt (1 eq) and 0,081 g EDC (1 eq) was added to the mixture. Approximately 0,421 mmol Leucine-Alanine methyl ester trifluoracetate (1 eq) was dissolved in DCM, and after 15 minutes of stirring the reaction, this second peptide was added dropwise to the reaction mixture along with 0,16 mL DIPEA (2 eq). After the reaction ended reaction and general workup, the product was concentrated in vacuo and purified through a short silica-gel filled column under reduced pressure, using EtOAc as eluent.
The NMR-spectrum for this product contains contaminants.

Yield: 52 %.

Spectral data for compound **14a**:
^{1}H NMR (400MHz, $CDCl_3$): δ 0.84-0.93 (m, 12H, CH-CH(*CH₃*)₂ and $CH_2CH(CH_3)_2$), 1.34 (d, *J* 7.2 Hz, 3H, NCH*CH₃*), 1.40 (s, 9H, OC(*CH₃*)₃), 1.51-1-68 (m, 4H, CH-*CH₂CH*-$(CH_3)_2$ and CH-*CH*-$(CH_3)_2$), 3.67 (s, 3H, O*CH₃*).

5.1.5 Synthesis of compound **15a**, methyl (*tert*-butoxycarbonyl)glycyl-L-valyl-D-leucyl-L-alanyl-L-valinate.



0,103 g Boc-Gly-Val-Leu-Ala-OMe (1 eq) was added to a flask and solved in a solvent-combination of THF, MeOH and water (3:1:1).
The reaction was put on a cooling bath and cooled down to 0 °C, before 0,032 g LiOH (3 eq) was added. The reaction stood for 60 minutes before the pH of the reaction was adjusted to 2, using 1M HCl solution. The reaction was diluted with EtOAc and washed with brine before the organic phase was dried over $MgSO_4$.
The reaction was filtered, and the filtrate was concentrated in vacuo in which the result was the deprotected peptide.

0,101 g Boc-Gly-Val-Leu-Ala-OH was then dissolved in 1,5 mL DCM. The solution was cooled down to about 0 °C before 0,0300 g HOBt (1 eq) and 0,042 g EDC (1 eq) was added to the mixture. 0,037 g L-valine methyl ester hydrochloride (1 eq) was added to a 5 mL dry pear-shaped flask and dissolved in 1,5 mL dry DCM. After 15 minutes the Valine/DCM-solution and 0,084 mL DIPEA (2 eq) was added to the reaction mixture. After the reaction ended reaction the product underwent general workup and no further purification.
The NMR-spectrum for this product contains contaminants.

Yield: 15 %.

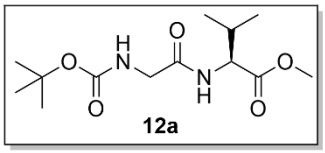
Spectral data for compound **15a**:
^{1}H NMR (400MHz, $CDCl_3$): δ 0.84-0.94 (m, 18H, 2x CH(CH_3)$_2$ and $CH_2CH(CH_3)_2$), 1.37 (d, *J* 6.9 Hz, 3H, $CHCH_3$), 1.42 (s, 9H, $OCCH_3$), 3.69 (s, 3H, OCH_3).

Without HOBt

General procedure:

A dry round bottomed flask with magnetic stirring bar was flushed with nitrogen gas. The esterified amino acid (1 eq) was solved in DCM and cooled down to 0 °C, before DIPEA (1,27 eq) was added.
The reaction was stirred at 0 °C for 40minutes before EDC and the Boc-protected amino acid were added. The reaction was stirred for another 3 hours at 0 °C, before the cooling bath was removed, and the reaction kept going through the night.
The following day the reaction mixture was diluted with DCM and washed with 10% aqueous citric acid solution, before the organic phase was dried over $MgSO_4$.
$MgSO_4$ was filtered away and the filtrate was concentrated in vacuo.

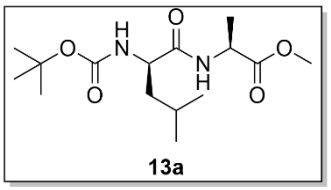
5.1.6 Alternative synthesis of compound **12a**, methyl (*tert*-butoxycarbonyl)glycyl-L-valinate.



0,106 g HCl-Gly-OMe(1,27 eq) was dissolved in 5 mL dry DCM. The solution was cooled down to about 0 °C before 0,12 mL DIPEA (1,27 eq) was added. The mixture was stirred for 40 min at 0 °C before 0,088 g Boc-Glycine-OH (1 eq) and 0,122 g EDC (1,27 eq) was added to the mixture. The general procedure was used to complete the reaction, and after the workup, TLC showed that the product was isolated, and no further purification was needed.

Yield: 89 %.

5.1.7 Alternative synthesis of compound **13a**, methyl (*tert*-butoxycarbonyl)-D-leucyl-L-alaninate.



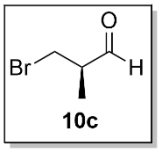
13a

0,544 g HCl-Ala-OMe (1,27 eq) was dissolved in 9 mL dry DCM. The solution was cooled down to about 0 °C before 0,75 mL DIPEA (1,27 eq) was added. The amino acid did not completely dissolve until the addition of DIPEA.
The mixture was stirred for 40 min at 0 °C before 0,694 g Boc-Leucine-OH (1 eq) and 0,748 g EDC (1,27 eq) was added to the mixture. The general procedure was used to complete the reaction, and after the workup, TLC showed that the product was isolated, and no further purification was needed.

Yield: 68 %.

5.2.1 Reduction

5.2.2 Synthesis of compound **10c**, *(R)*-3-bromo-2-methylpropanal



10c

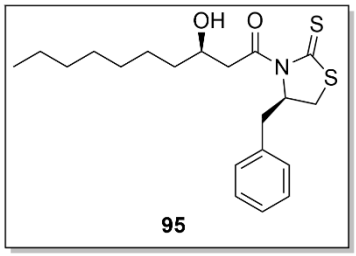
A dry round bottomed flask was prepared with a magnetic stirring rod, flushed with nitrogen gas, and fitted with a septum. 0,7mL methyl (R)-(+)-3-bromo-2-methyl propionate (1 eq) was added to the flask, followed by 30 mL dry DCM. The solution was put on a cooling bath (acetone/dry ice), and cooled down to -78 °C, before 0,58 mL DIBAL (1,05 eq) was added dropwise. The reaction was stirred at -78 °C for 15 minutes before it was quenched with 0,4 mL methanol and stirred for another 5 minutes. 15 mL of aqueous solution of saturated Rochelle's salt was added to the mixture before it was warmed to room temperature and stirred for another 30 minutes.
The mixture was then extracted with DCM and the organic layer was dried with $MgSO_4$ before it was filtered away.
The then attempted to purify the product through a short silica-gel filled column, using DCM as eluent.

Yield: 41 %.

Spectral data for compound **10c**:
^{1}H NMR (400MHz, $CDCl_3$): δ 1.24 (d, *J* 7.1 Hz, 3H, CH*CH*$_3$), 9.6 (s, 1H, C*H*O).

5.3.1 Aldol

5.3.2 Synthesis of compound **95**, 1-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxynonan-1-one.



95

A dry round-bottomed flask was prepared with a magnetic stirring rod and flushed with nitrogen gas. 0,128 g R-1-(4-phenyl-2-thioxothiazolidine-3-yl-)ethan-1-one (1 eq) was added to the flask and solved with 2 mL dry DCM, then fitted with a septum before the solution was put on a cooling bath (water/ice) and cooled down to 0 °C.
0,65 mL $TiCl_4$ (1,3 eq) was added dropwise to the solution and a colour change to orange was observed. The reaction was stirred for 10 minutes before 0,1 mL DIPEA (1 eq) was mixed with 1 mL dry DCM and added dropwise to the reaction mixture. A colour change from orange to deep red was observed at this point. The reaction mixture was stirred for 20 minutes before it was put on another cooling bath (acetone/dry ice) and cooled down to -78 °C. A solution of 0,064 g octanal (1 eq) in 2 mL dry DCM was then added dropwise to the reaction mixture, while the temperature was kept below -70 °C.
The reaction mixture was then stirred for 1 hour before the cooling bath was removed and the reaction mixture allowed to reach room temperature. It was then quenched with aqueous, half-saturated NH_4Cl-solution.
The phases of the reaction mixture were separated, and the water phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, before it was filtered and concentrated in vacuo.
The product was then purified through a short silica-gel filled column under reduced pressure, using hexane:EtOAc (1:1) as eluent.

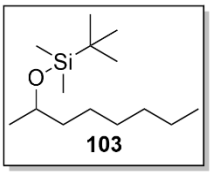
Yield: 46 %.

Spectral data for compound **95**:
^{1}H NMR (400MHz, $CDCl_3$): δ 0.85 (t, *J* 7.1 Hz, 3H, CH_3), 1.22-1.59 (m, 10H, $CH_3(CH_2)_5$), 2.86 (d, *J* 11.6 Hz, 1H, SCH_2), 3.01 (dd, *J* 13.2 Hz and ^2J 10.5 Hz, 1H, $ArCH_2$), 3.08 (dd, *J* 17.8 Hz and ^2J 9.5 Hz, 1H, $OCCH_2$), 3.19 (dd, *J* 13.2 Hz and ^2J 3.9 Hz, 1H, $ArCH_2$), 3.36 (dd, *J* 11.6 Hz and ^2J 7.2 Hz, 1H, SCH_2), 3.61 (dd, *J* 17.8 Hz and ^2J 2.4 Hz, 1H, $OCCH_2$), 4.11 (m, 1H, HOCH), 5.36 (ddd, *J* 4.0 Hz and *J* 3.7 Hz and *J* 3.0 Hz, 1H, SCH_2-CH), 7.22-7.33 (m, 5H, ArH).

5.4.1 TBS-protection

5.4.2 Synthesis of compound **101**, *tert*-butyldimethyl(octan-2-yloxy)silane.

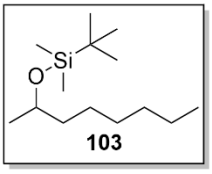
With DMAP



A round bottomed flask was prepared with a magnetic stirring rod. 0,16mL octan-2-ol (1 eq) and 0,166 g TBSCl (1,1 eq) was added to the flask and solved in 2 mL DCM. 0,22 mL DIPEA (1,2 eq) was added before 0,037g DMAP (0,3 eq) was added to the mixture and the reaction was stirred through the night.
The products were then concentrated in vacuo and purified through a short silica-gel filled column under reduced pressure, using hexane:etOAc (1:1) as eluent.
The NMR-spectrum for this product was from the crude product and contained contaminants.

Spectral data for compound **101**:
^{1}H NMR (600MHz, $CDCl_3$): δ 0.03 (s, 6H, Si(CH_3)$_2$), 0.87 (s, 9H, CH_3C-Si), 1.10 (d, *J* 6.1 Hz, 3H, CH_3CH), 1.25-1.43 (m, 10H, 5x CH_2), 3.75 (sext, *J* 6.1 Hz and *J* 6.0 Hz and *J* 5.7 Hz, 1H, *CH*).

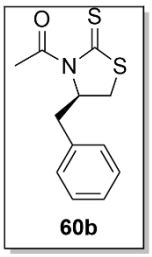
Without DMAP



A dry round bottomed flask was prepared with a magnetic stirring rod. Octa-2-nol (1 mmol, 1 eq) was added to the flask and solved in dimethylformamide (5 mL). TBSCl (1,05 mmol, 1,05 eq) and imidazole (1,2 mmol, 1,2 eq) was added. The reaction was stirred in room temperature for 48 hours under nitrogen atmosphere.
Brine was added, and the mixture was extracted with ethyl acetate. The combined organic extract was washed with brine, aqueous AcOH (10 %) and aqueous $NaHCO_3$ before it was dried over $MgSO_4$ and evaporated *in vacuo*.
The product mixture was compared to the one conducted with DMAP with TLC-analysis, and they resulted in the same product spots.

5.5.1 Chiral auxiliaries

5.5.2 Synthesis of compound **60b**, (*R*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)ethan-1-one.
(General procedure)



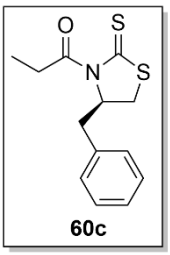
60b

A dry two-necked round bottomed flask was prepared with a magnetic stirring rod and a thermometer. 0,626 g 4-benzyl thiazolidine (1 eq) was added to the flask and solved in 10 mL dry THF and the flask was fitted with a septum. The solution was put on a cooling bath (acetone/dry ice) and cooled to -78 °C before 2,05 mL n-BuLi (1,1 eq) was added dropwise to the mixture, while the temperature was kept below -70 °C. The reaction was stirred for 15 minutes before 0,28 mL acetyl chloride (1,3 eq) was added dropwise, while the temperature still was being monitored.
The mixture was stirred for 5 minutes before the cooling bath was removed, and the reaction was stirred for another 1,5 hours in room temperature. The reaction was then put on another cooling bath (ice) and quenched with aqueous saturated NH_4Cl solution before it was extracted with DCM and the organic layer dried over $MgSO_4$.
The mixture was filtered, concentrated in vacuo and purified through flash chromatography, using DCM as eluent.

Yield: 96 %.

Spectral data for compound **60b**:
^{1}H NMR (400MHz, $CDCl_3$): δ 2.77 (s, 3H, *CH*$_3$), 2,86 (d, *J* 11.5 Hz, 1H, Ar*CH*$_2$), 3.01 (dd, *J* 13.1 Hz and ^{2}*J* 10.4 Hz, 1H, S*CH*$_2$), 3.19 (dd, *J* 13.1 Hz and ^{2}*J* 3.8 Hz, 1H, S*CH*$_2$), 3.36 (dd, *J* 11.5 Hz and ^{2}*J* 7.2 Hz, 1H, Ar*CH*$_2$), 5.35 (ddd, *J* 3.8 Hz and *J* 7.2Hz and *J* 10.8 Hz,1H, *CH*), 7.24-7.34 (m, 5H, Ar*H*).

5.5.3 Synthesis of compound **60c**, (*R*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)propan-1-one.



60c

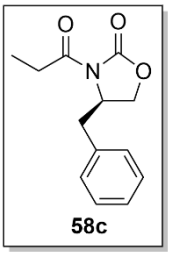
This reaction was performed following the general procedure with same equivalents.

0,627 g 4-benzyl thiazolidine was used along with 2 mL n-BuLi and 0,34 mL propionyl chloride, which replaced acetyl chloride.

Yield: 97 %

Spectral data for compound **60c**:
^{1}H NMR (400MHz, $CDCl_3$): δ 1.17 (t, *J* 7.2 Hz, 3H, CH_2CH_3), 2.86 (d, *J* 11.5Hz, 1H, SCH_2), 3.03 (dd, *J* 13.1 Hz and ^{2}J 10.5 Hz, 1H, ArCH_2), 3.11 (dq, *J* 7.2 Hz and ^{2}J 18 Hz, 1H, CH_3CH_2), 3.19 (dd, *J* 13.1 Hz and ^{2}J 3.7 Hz, 1H, ArCH_2), 3.37 (dd, *J* 11.7 Hz and ^{2}J 7.3 Hz, 1H, SCH_2), 3.40 (dq, *J* 7.3 Hz, 1H, CH_3CH_2), 5.37 (ddd, *J* 3.8 Hz and *J* 6.8 Hz and *J* 10.6 Hz, 1H, CH), 7.25-7.34 (m, 5H, Ar*H*)

5.5.4 Synthesis of compound **58c**, (*R*)-4-benzyl-3-propionyloxazolidin-2-one.



This reaction was performed following the general procedure with same equivalents.

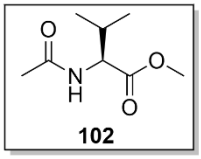
0,532 g (R)-4-benzyl-2-oxazolidinone was used instead of 4-benzyl thiazolidine along with 2 mL n-BuLi and 0,34 mL propionyl chloride, which replaced acetyl chloride.

Yield: 96 %.

Spectral data for compound **58c**:
^{1}H NMR (400MHz, $CDCl_3$): δ 1.24 (t, *J* 7.3 Hz, 3H, *CH*$_3$), 2.81 (dd, *J* 13.4 Hz and ^{2}J 9.6 Hz, 1H,), 2.91-3.08 (m, 1H,), 3.00 (dd, *J* 9.7 Hz and ^{2}J 7.4 Hz, 1H, CH_3*CH*$_2$), 3.34 (dd, *J* 13.4 and ^{2}J 3.3, 1H,), 4.19-4.26 (m, 2H,), 4.71 (dddd, *J* 3.3 Hz and *J* 3.6 Hz *J* 3.7 Hz and *J* 2.3 Hz, 1H, *CH*), 7.24-7.40 (m, 5H, *ArH*).

5.6.1 Aminolysis

5.6.2 Synthesis of compound **102**, methyl acetyl-L-valinate.



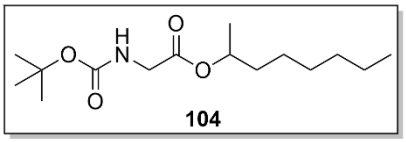
A dry round bottomed flask was prepared with a magnetic stirring rod. 0,050 g (R)-1-(4-benzyl-2-thioxothiazolidin-3-yl)ethan-1-one (1 eq) was added to the flask and solved in 1 mL dry DCM. A dry pear-shaped flask was prepared and 0,395 g Val-OMe (1,1 eq) was added and solved in 1 mL dry DCM. The content of the pear-shaped flask was transferred to the round bottomed flask, before 0,042 g imidazole (3 eq) was added. The reaction mixture was stirred in room temperature through the night, and a colour change from yellow to clear was observed.
The mixture was then diluted with DCM and washed with saturated aqueous NH_4Cl-solution before the organic phase was dried over $MgSO_4$.
The solution was then filtered and concentrated in vacuo, before it was purified through a short silica-gel filled column under reduced pressure, using a gradient eluent system. This gradient system comprised of hexane:EtOAc (1:1) and DCM:MeOH (10:1).

Yield: 78 %.

Spectral data for compound **102**:
^{1}H NMR (600MHz, $CDCl_3$): δ 0.87 (dd, *J* 6.9 Hz and ^2J 18.9 Hz, 6H, CH(CH_3)$_2$), 1.99 (s, 3H, OCCH_3), (oct, *J* 6.5 Hz, 1H, $CH(CH_3)_2$), 3.69 (s, 3H, OCH_3), 4.51 (m, 1H, N*CH*), 6.08 (s, 1H, NH).

5.7.1 Cyclization

5.7.2 Synthesis of **104**, octan-2-yl (*tert*-butoxycarbonyl)glycinate.



104

A dry round bottomed flask was prepared with a magnetic stirring rod. Boc-protected amino acid (1,5 eq) was added to the flask and solved in dry DCM. The secondary alcohol (1 eq) was added to the solution and the mixture was cooled down to 0 °C on a cooling bath (ice). *
EDC was added (3 eq) before DMAP (0,135 eq) was added to the reaction mixture and the cooling bath was then removed and the reaction was stirred for 4 hours in room temperature.
The products were filtered through 0,5cm celite in a short column before it was washed with aqueous saturated NH_4Cl-solution and brine and dried over $MgSO_4$.
The mixture was then filtered and concentrated in vacuo.
The NMR-spectrum for this product was from the crude product and contains contaminants.

Spectral data for compound **104**:
^1H NMR (600MHz, $CDCl_3$): δ 0.75 (t, *J* 7 Hz, 3H, CH_2CH_3), 1.10 (d, *J* 6.3 Hz, 3 Hz, $CHCH_3$), 1.11-1.22 (m, 5x CH_2), 1.32 (s, 9H, $OC(CH_3)_3$), 3.74 (d, *J* 5.5 Hz, 2H, NCH_2), 4.83 (sext, *J* 6.3 Hz, 1H, *CH*), 5.12(s, 1H, *NH*).

References

1. WHO/Rada Akbar QM. Ten threats to global health in 2019. https://www.who.int/news-room/feature-stories/ten-threats-to-global-health-in-2019: WHO, 2019.
2. WHO. Urgent health challenges for the next decade. https://www.who.int/news-room/photo-story/photo-story-detail/urgent-health-challenges-for-the-next-decade: WHO, 2020.
3. Demain AL and Vaishnav P. Natural products for cancer chemotherapy. *Microb Biotechnol*. 2011; 4: 687-99.
4. Nalini S, Sandy Richard D, Mohammed Riyaz SU, Kavitha G and Inbakandan D. Antibacterial macro molecules from marine organisms. *Int J Biol Macromol*. 2018; 115: 696-710.
5. Hollstein U. Actinomycin - Chemistry and Mechanism of Action. *Chem Rev*. 1974; 74: 625-52.
6. Seca AML and Pinto D. Plant Secondary Metabolites as Anticancer Agents: Successes in Clinical Trials and Therapeutic Application. *Int J Mol Sci*. 2018; 19.
7. Wang X, Gong X, Li P, Lai D and Zhou L. Structural Diversity and Biological Activities of Cyclic Depsipeptides from Fungi. *Molecules*. 2018; 23.
8. Yu Z, Lang G, Kajahn I, Schmaljohann R and Imhoff JF. Scopularides A and B, cyclodepsipeptides from a marine sponge-derived fungus, Scopulariopsis brevicaulis. *J Nat Prod*. 2008; 71: 1052-4.
9. Hou XM, Li YY, Shi YW, et al. Integrating Molecular Networking and (1)H NMR To Target the Isolation of Chrysogeamides from a Library of Marine-Derived Penicillium Fungi. *J Org Chem*. 2019; 84: 1228-37.
10. Chakraborty TK, Ghosh S, Jayaprakash S, et al. Synthesis and conformational studies of peptidomimetics containing furanoid sugar amino acids and a sugar diacid. *J Org Chem*. 2000; 65: 6441-57.
11. Al-Warhi TI, Al-Hazimi HMA and El-Faham A. Recent development in peptide coupling reagents. *J Saudi Chem Soc*. 2012; 16: 97-116.
12. Montalbetti CAGN and Falque V. Amide bond formation and peptide coupling. *Tetrahedron*. 2005; 61: 10827-52.
13. Joullie MM and Lassen KM. Evolution of amide bond formation. *Arkivoc*. 2010: 189-250.
14. Tullberg M, Grotli M and Luthman K. Efficient synthesis of 2,5-diketopiperazines using microwave assisted heating. *Tetrahedron*. 2006; 62: 7484-91.
15. Campeau LC and Hazari N. Cross-Coupling and Related Reactions: Connecting Past Success to the Development of New Reactions for the Future. *Organometallics*. 2019; 38: 3-35.
16. Biffis A, Centomo P, Del Zotto A and Zeccal M. Pd Metal Catalysts for Cross-Couplings and Related Reactions in the 21st Century: A Critical Review. *Chem Rev*. 2018; 118: 2249-95.
17. Organ MG, Avola S, Dubovyk I, et al. A user-friendly, all-purpose Pd-NHC (NHC=N-heterocyclic carbene) precatalyst for the negishi reaction: a step towards a universal cross-coupling catalyst. *Chemistry*. 2006; 12: 4749-55.
18. Haas D, Hammann JM, Greiner R and Knochel P. Recent Developments in Negishi Cross-Coupling Reactions. *Acs Catal*. 2016; 6: 1540-52.
19. Phapale VB and Cardenas DJ. Nickel-catalysed Negishi cross-coupling reactions: scope and mechanisms. *Chem Soc Rev*. 2009; 38: 1598-607.

20. Bricout H, Carpentier JF and Mortreux A. Nickel vs. palladium catalysts for coupling reactions of allyl alcohol with soft nucleophiles: activities and deactivation processes. *J Mol Catal a-Chem*. 1998; 136: 243-51.
21. Chen X, Engle KM, Wang DH and Yu JQ. Palladium(II)-catalyzed C-H activation/C-C cross-coupling reactions: versatility and practicality. *Angew Chem Int Ed Engl*. 2009; 48: 5094-115.
22. Knappke CEI and Jacobi von Wangelin A. 35 years of palladium-catalyzed cross-coupling with Grignard reagents: how far have we come? *Chem Soc Rev*. 2011; 40: 4948-62.
23. Liu Q, Lan Y, Liu J, Li G, Wu YD and Lei AW. Revealing a Second Transmetalation Step in the Negishi Coupling and Its Competition with Reductive Elimination: Improvement in the Interpretation of the Mechanism of Biaryl Syntheses. *J Am Chem Soc*. 2009; 131: 10201-10.
24. Valente C, Belowich ME, Hadei N and Organ MG. Pd-PEPPSI Complexes and the Negishi Reaction. *Eur J Org Chem*. 2010; 2010: 4343-54.
25. McCann LC. Mechanistic insight in alkyl-alkyl and aryl-aryl Negishi cross-coupling. *Graduate program in chemistry*. York University Toronto, Ontario., 2015, p. 1-168.
26. Hadei N, Kantchev EAB, O'Brien CJ and Organ MG. Room-temperature Negishi cross-coupling of unactivated alkyl bromides with alkyl organozinc reagents utilizing a Pd/N-heterocyclic carbene catalyst. *Journal of Organic Chemistry*. 2005; 70: 8503-7.
27. O'Brien CJ, Kantchev EA, Valente C, et al. Easily prepared air- and moisture-stable Pd-NHC (NHC=N-heterocyclic carbene) complexes: a reliable, user-friendly, highly active palladium precatalyst for the Suzuki-Miyaura reaction. *Chemistry*. 2006; 12: 4743-8.
28. Osorio-Lozada A and Olivo HF. Indene-based thiazolidinethione chiral auxiliary for propionate and acetate aldol additions. *Org Lett*. 2008; 10: 617-20.
29. Klein D. *Organic Chemistry*. 2nd ed.: Wiley, 2013, p.1344p.
30. Zakharkin LI and Khorlina IM. Reduction of Esters of Carboxylic Acids into Aldehydes with Diisobutylaluminium Hydride. *Tetrahedron Lett*. 1962: 619-20.
31. Yoon NM and Gyoung YS. Reaction of Diisobutylaluminum Hydride with Selected Organic-Compounds Containing Representative Functional-Groups. *Journal of Organic Chemistry*. 1985; 50: 2443-50.
32. Podlech J. "Try and fall sick .."-the composer, chemist, and surgeon Aleksandr Borodin. *Angew Chem Int Ed Engl*. 2010; 49: 6490-5.
33. Evans DA, Vogel E and Nelson JV. Stereoselective Aldol Condensations Via Boron Enolates. *J Am Chem Soc*. 1979; 101: 6120-3.
34. Crimmins MT and Chaudhary K. Titanium enolates of thiazolidinethione chiral auxiliaries: versatile tools for asymmetric aldol additions. *Org Lett*. 2000; 2: 775-7.
35. Crimmins MT and Shamszad M. Highly selective acetate aldol additions using mesityl-substituted chiral auxiliaries. *Organic Letters*. 2007; 9: 149-52.
36. Guz NR and Phillips AJ. Practical and highly selective oxazolidinethione-based asymmetric acetate aldol reactions with aliphatic aldehydes. *Organic Letters*. 2002; 4: 2253-6.
37. Zimmerman HE and Traxler MD. The Stereochemistry of the Ivanov and Reformatsky Reactions .1. *J Am Chem Soc*. 1957; 79: 1920-3.
38. Crimmins MT, King BW, Tabet EA and Chaudhary K. Asymmetric aldol additions: Use of titanium tetrachloride and (-)-sparteine for the soft enolization of N-acyl oxazolidinones, oxazolidinethiones, and thiazolidinethiones. *Journal of Organic Chemistry*. 2001; 66: 894-902.

39. Vanhorn DE and Masamune S. Stereoselective Synthesis of Beta-Hydroxy-Alpha-Methylketones Via Z-Vinyloxyboranes and E-Vinyloxyboranes Generated Directly from Cyclohexyl Ethyl Ketone. *Tetrahedron Lett*. 1979: 2229-32.
40. Brown HC, Dhar RK, Bakshi RK, Pandiarajan PK and Singaram B. Major Effect of the Leaving Group in Dialkylboron Chlorides and Triflates in Controlling the Stereospecific Conversion of Ketones into Either (E)-Enol or (Z)-Enol Borinates. *J Am Chem Soc*. 1989; 111: 3441-2.
41. Heathcock CH, Buse CT, Kleschick WA, Pirrung MC, Sohn JE and Lampe J. Acyclic Stereoselection .7. Stereoselective Synthesis of 2-Alkyl-3-Hydroxy Carbonyl-Compounds by Aldol Condensation. *Journal of Organic Chemistry*. 1980; 45: 1066-81.
42. Oppolzer W, Blagg J, Rodriguez I and Walther E. Bornanesultam-Directed Asymmetric-Synthesis of Crystalline, Enantiomerically Pure Syn Aldols. *J Am Chem Soc*. 1990; 112: 2767-72.
43. Nagao Y, Hagiwara Y, Kumagai T, et al. New C4-Chiral 1,3-Thiazolidine-2-Thiones - Excellent Chiral Auxiliaries for Highly Diastereocontrolled Aldol-Type Reactions of Acetic-Acid and Alpha,Beta-Unsaturated Aldehydes. *Journal of Organic Chemistry*. 1986; 51: 2391-3.
44. Noyori R, Suga S, Kawai K, Okada S and Kitamura M. Enantioselective Alkylation of Carbonyl-Compounds - from Stoichiometric to Catalytic Asymmetric Induction. *Pure Appl Chem*. 1988; 60: 1597-606.
45. Kohler MC, Wengryniuk, S.E. and Coltart, D.M. Asymmetric α-Alkylation of Aldehydes, Ketones, and Carboxylic Acids. *Stereoselective Synthesis of Drugs and Natural Products*. 2013, p. 1-31.
46. Evans DA, Ennis MD and Mathre DJ. Asymmetric Alkylation Reactions of Chiral Imide Enolates - a Practical Approach to the Enantioselective Synthesis of Alpha-Substituted Carboxylic-Acid Derivatives. *J Am Chem Soc*. 1982; 104: 1737-9.
47. Ojeda-Amador AI, Martinez-Martinez AJ, Kennedy AR, Armstrong DR and O'Hara CT. Monodentate coordination of the normally chelating chiral diamine (R,R)-TMCDA. *Chem Commun*. 2017; 53: 324-7.
48. Zhang ZR and Collum DB. Structures and Reactivities of Sodiated Evans Enolates: Role of Solvation and Mixed Aggregation on the Stereochemistry and Mechanism of Alkylations. *J Am Chem Soc*. 2019; 141: 388-401.
49. Heravi MM, Zadsirjan V and Farajpour B. Applications of oxazolidinones as chiral auxiliaries in the asymmetric alkylation reaction applied to total synthesis. *Rsc Adv*. 2016; 6: 30498-551.
50. P. Romea FU, E. Gálvez, J. Jewett. PREPARATION OF (S)-4-ISOPROPYL-N-PROPANOYL-1,3-THIAZOLIDINE-2-THIONE. *Organic Syntheses*. 2009; 86: p70-80.
51. Patschinski P, Zhang C and Zipse H. The Lewis Base-Catalyzed Silylation of Alcohols-A Mechanistic Analysis. *Journal of Organic Chemistry*. 2014; 79: 8348-57.
52. Nagao Y, Seno K, Kawabata K, Miyasaka T, Takao S and Fujita E. Monitored Aminolysis of 3-Acylthiazolidine-2-Thione - New Convenient Synthesis of Amide. *Tetrahedron Lett*. 1980; 21: 841-4.
53. Cochrane JR, Yoon DH, McErlean CSP and Jolliffe KA. A macrolactonization approach to the total synthesis of the antimicrobial cyclic depsipeptide LI-F04a and diastereoisomeric analogues. *Beilstein J Org Chem*. 2012; 8: 1344-51.
54. Narayanaswamy VK, Albericio F, Coovadia YM, et al. Total synthesis of a depsidomycin analogue by convergent solid-phase peptide synthesis and macrolactonization strategy for antitubercular activity. *J Pept Sci*. 2011; 17: 683-9.

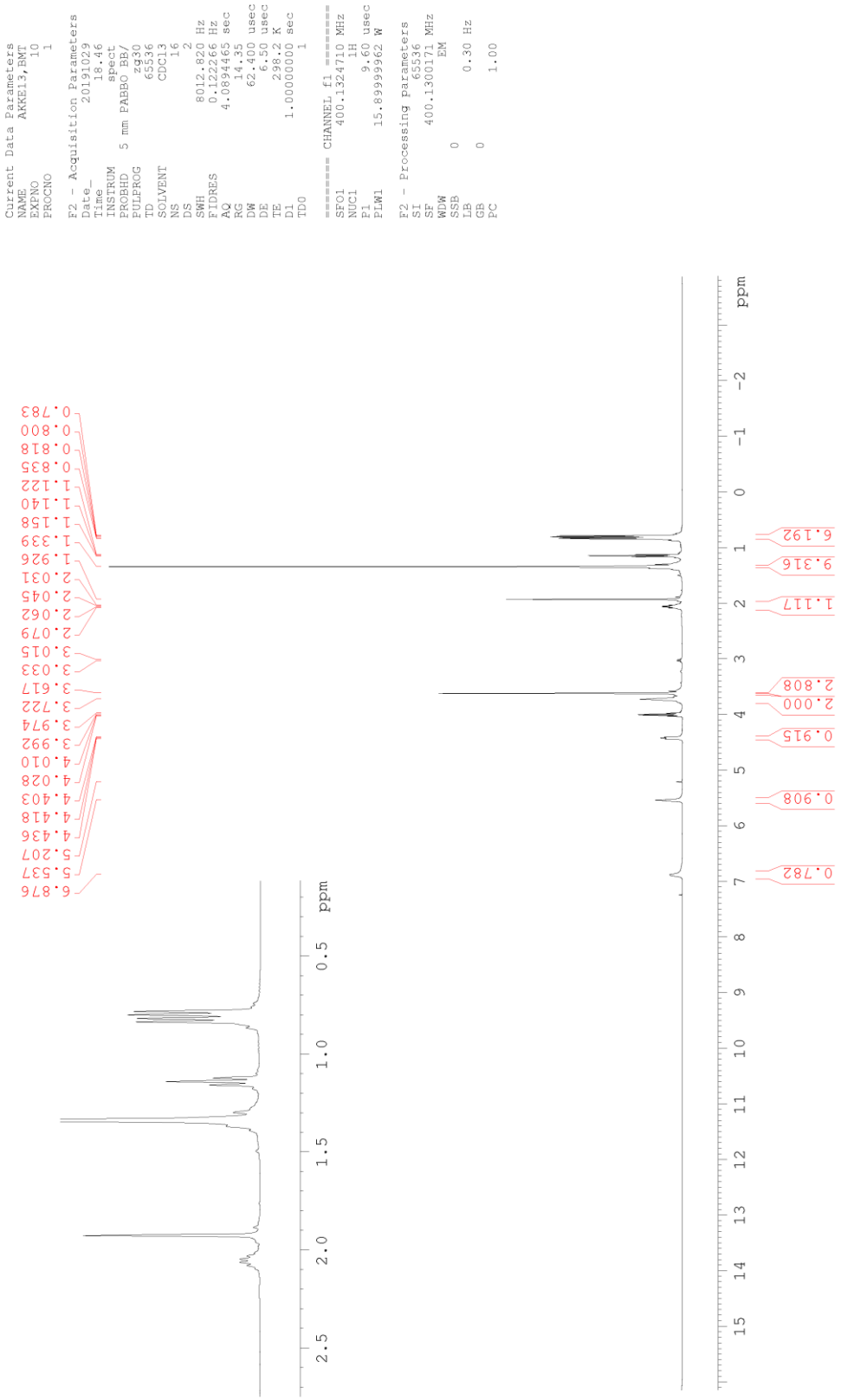
55. Ghosh S and Pradhan TK. The first total synthesis of emericellamide A. *Tetrahedron Lett*. 2008; 49: 3697-700.
56. Pradhan TK, Reddy KM and Ghosh S. Total synthesis of emericellamides A and B. *Tetrahedron-Asymmetr*. 2013; 24: 1042-51.
57. Chandrasekhar S, Sathish K, Reddy GPK and Mainkar PS. Total syntheses of arenamides A, B and C. *Tetrahedron-Asymmetr*. 2014; 25: 348-55.
58. Crimmins MT, King BW and Tabet EA. Asymmetric aldol additions with titanium enolates of acyloxazolidinethiones: Dependence of selectivity on amine base and Lewis acid stoichiometry. *J Am Chem Soc*. 1997; 119: 7883-4.
59. Yadav JS, Kumar VN, Rao RS and Srihari P. A concise stereoselective total synthesis of herbarumin III. *Synthesis-Stuttgart*. 2008: 1938-42.
60. Schinzer D, Limberg A, Bauer A, Bohm OM and Cordes M. Total synthesis of (-)-epothilone A. *Angew Chem Int Edit*. 1997; 36: 523-4.
61. Wu YK, Sun YP, Yang YQ, Hu Q and Zhang Q. Removal of thiazolidinethione auxiliaries with benzyl alcohol mediated by DMAP. *Journal of Organic Chemistry*. 2004; 69: 6141-4.

Appendices

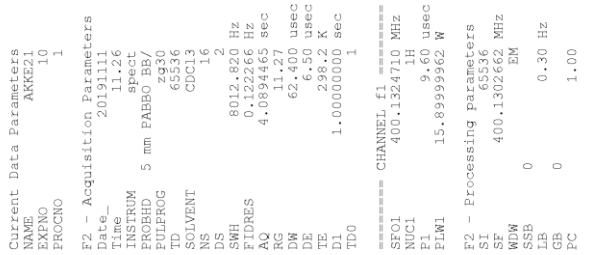
NMR-Spectra

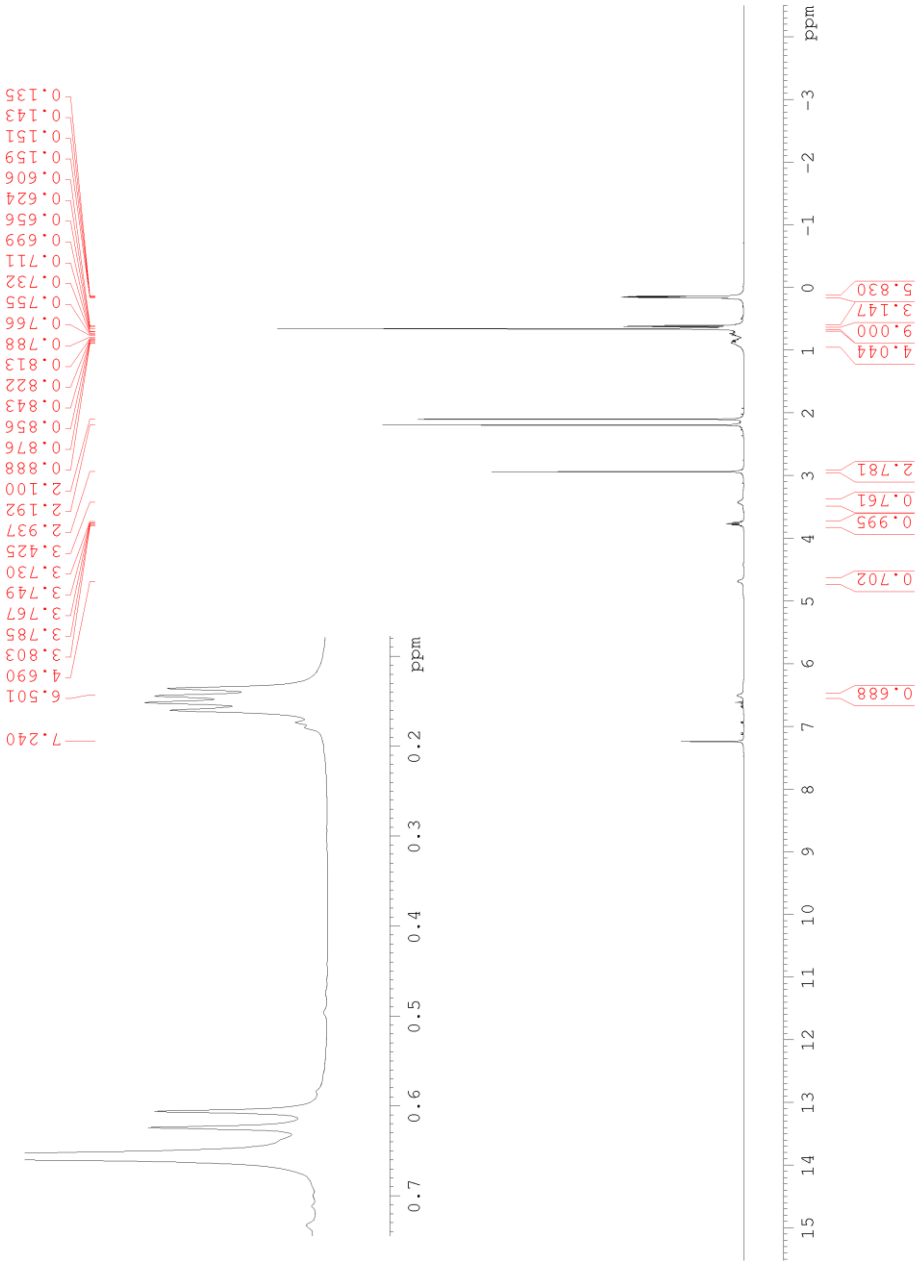
^{1}H-NMR:

Spectrum 1, of compound **12a**: Methyl (*tert*-butoxycarbonyl)glycyl-L-valinate.



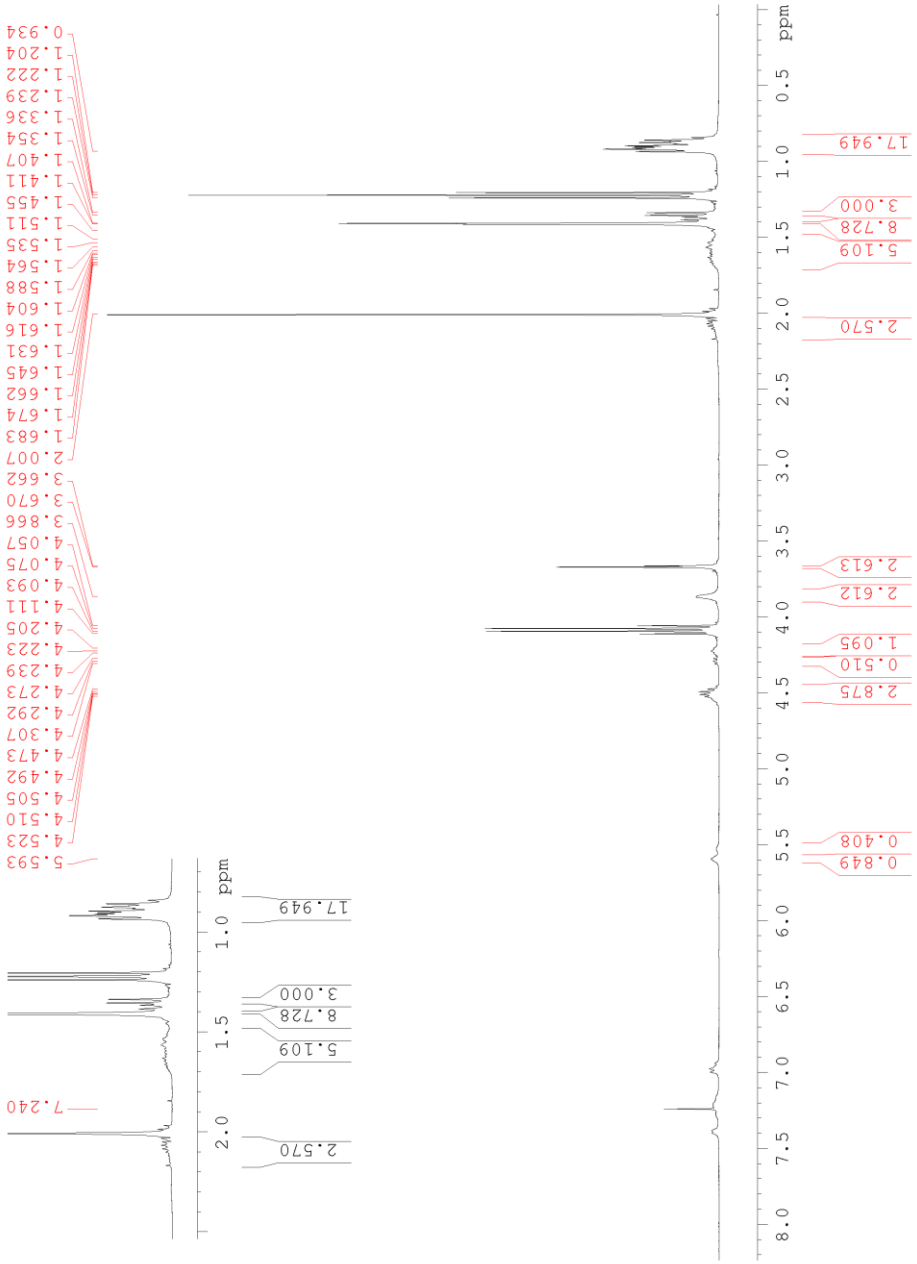
Spectrum 2, of compound **13a**: Methyl (*tert*-butoxycarbonyl)-D-leucyl-L-alaninate.



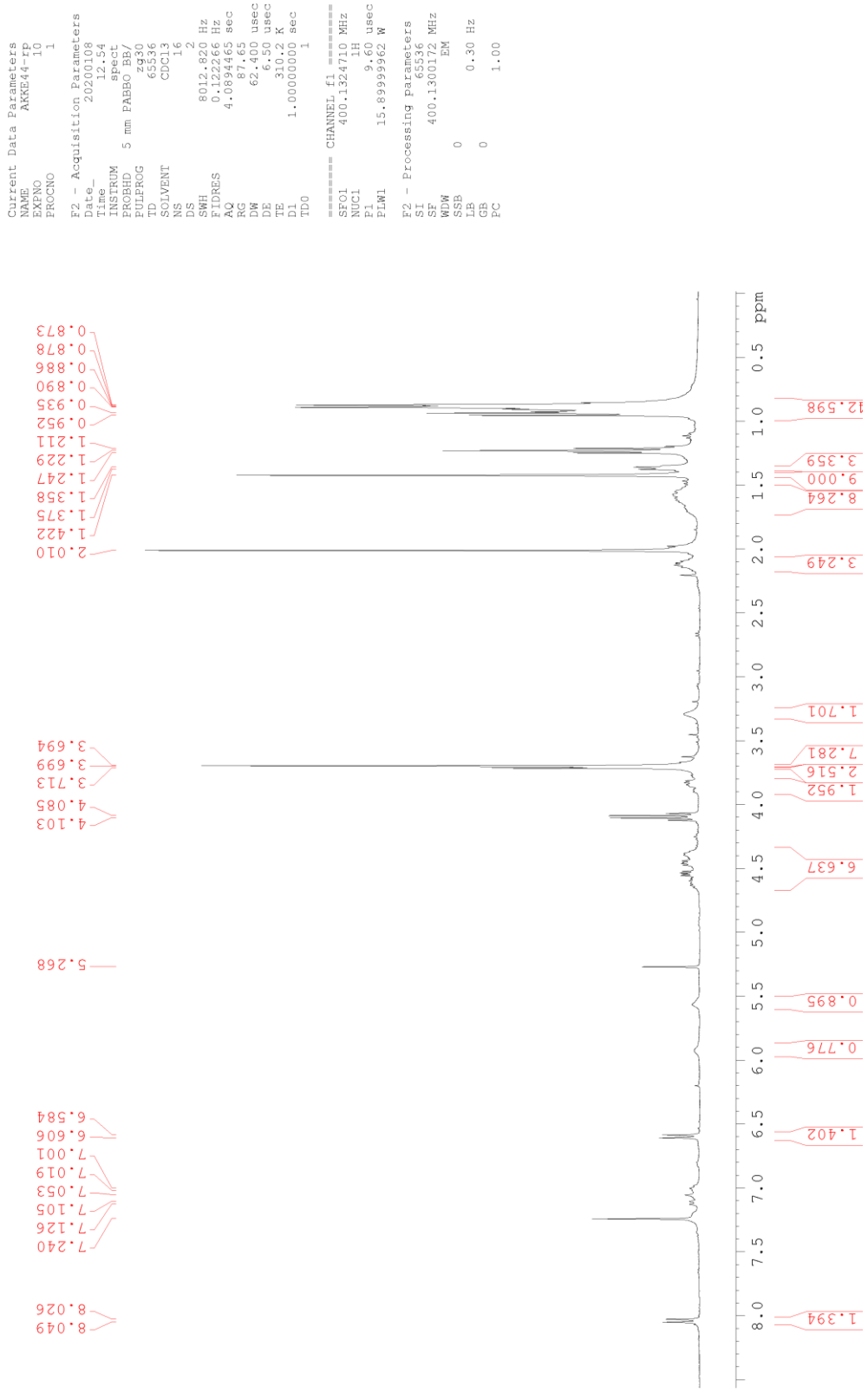


Spectrum 3, of compound **14a**: Methyl (*tert*-butoxycarbonyl)glycyl-L-valyl-D-leucyl-L-alaninate.

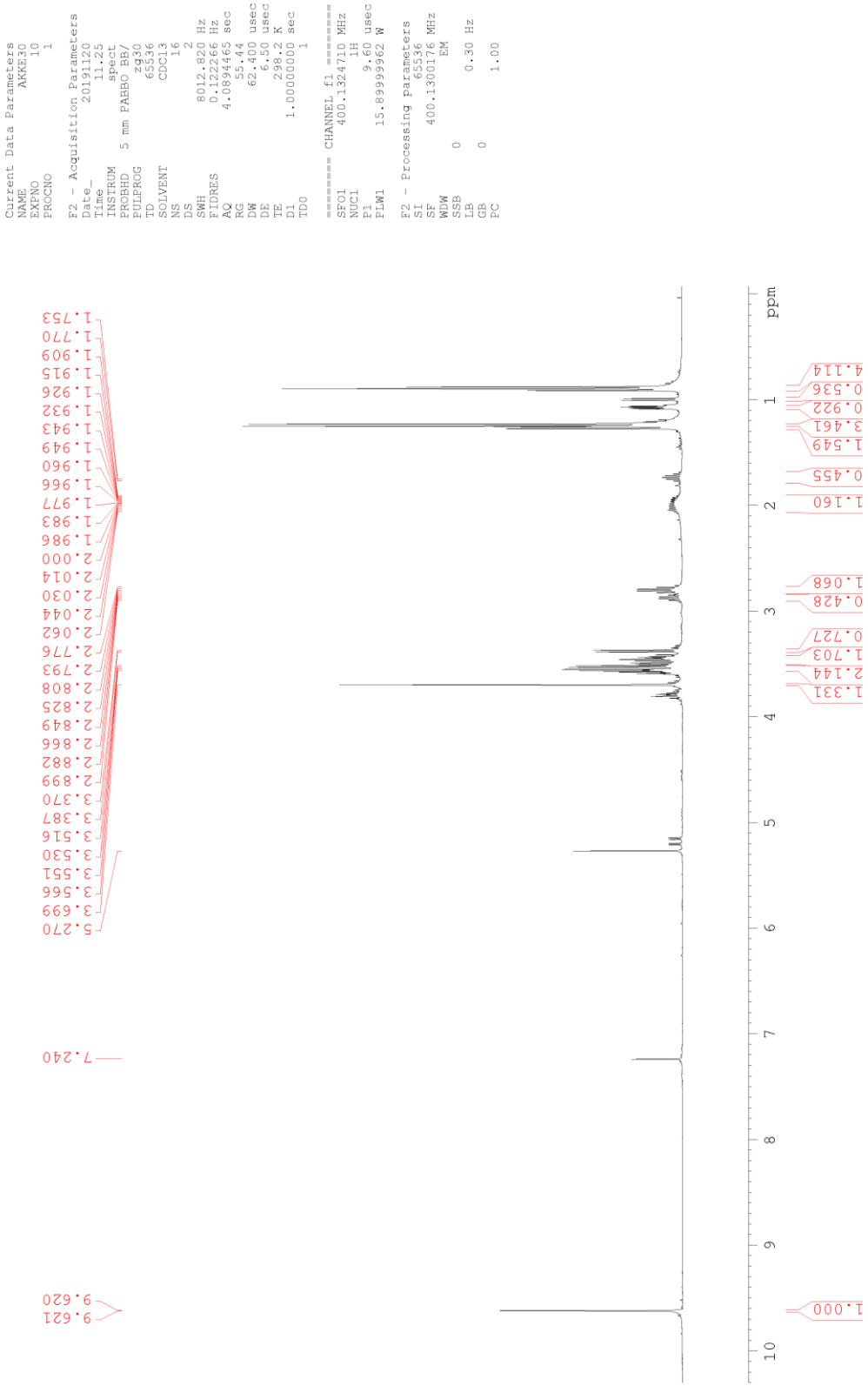
```
Current Data Parameters
NAME          AKE26
EXPNO            10
PROCNO            1

F2 - Acquisition Parameters
Date_        20191120
Time_            11.18
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG           zg30
TD               65536
SOLVENT          CDCl3
NS                  16
DS                   2
SWH           8012.820 Hz
FIDRES        0.122266 Hz
AQ            4.0894465 sec
RG                55.44
DW               62.400 usec
DE                6.50 usec
TE              298.2 K
D1              1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1         400.1324710 MHz
NUC1               1H
P1                9.60 usec
PLW1        15.89999962 W

F2 - Processing parameters
SI               65536
SF          400.1300174 MHz
WDW                EM
SSB                 0
LB               0.30 Hz
GB                  0
PC                1.00
```



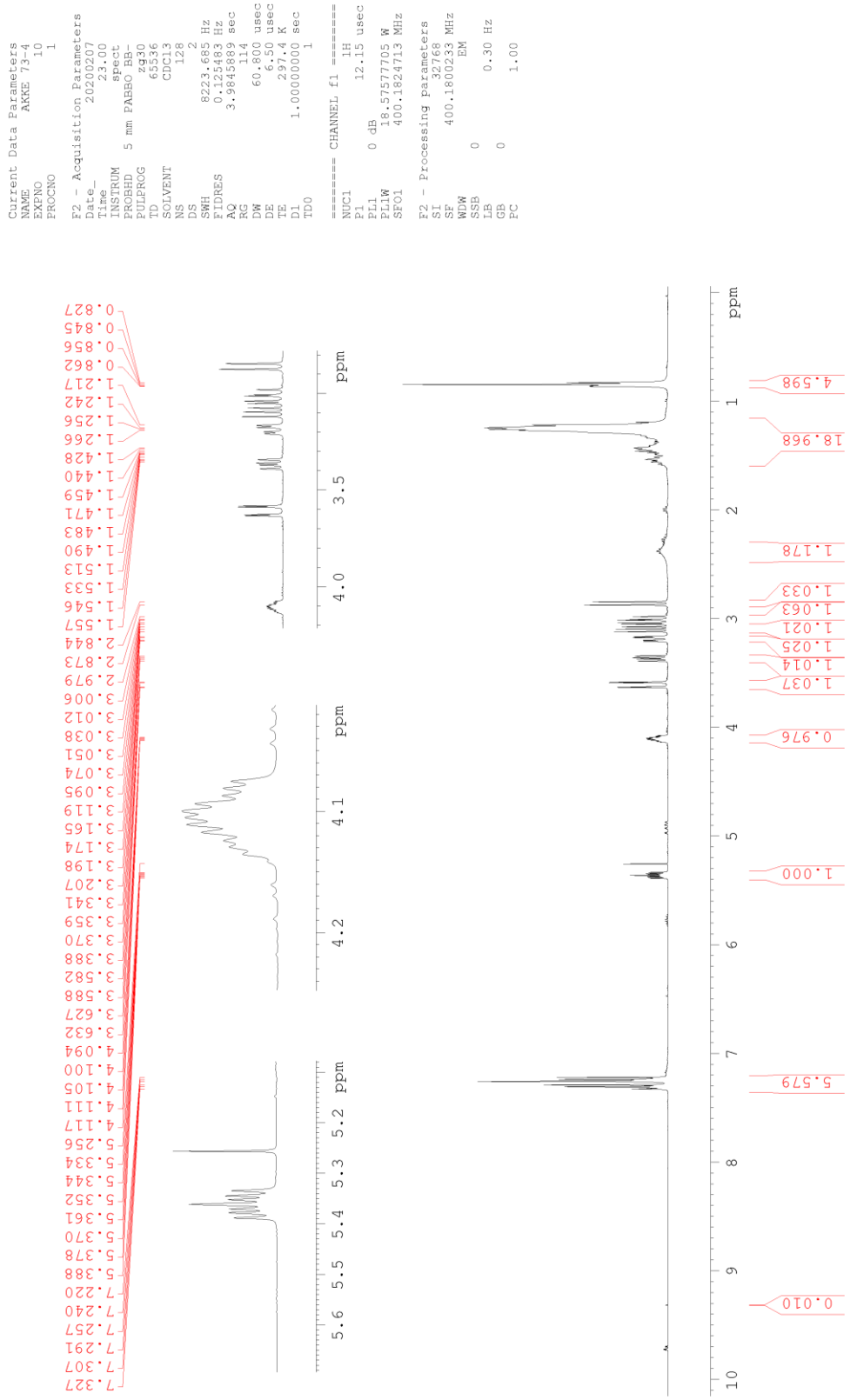
Spectrum 4, of compound **15a**: Methyl (*tert*-butoxycarbonyl)glycyl-L-valyl-D-leucyl-L-alanyl-L-valinate.



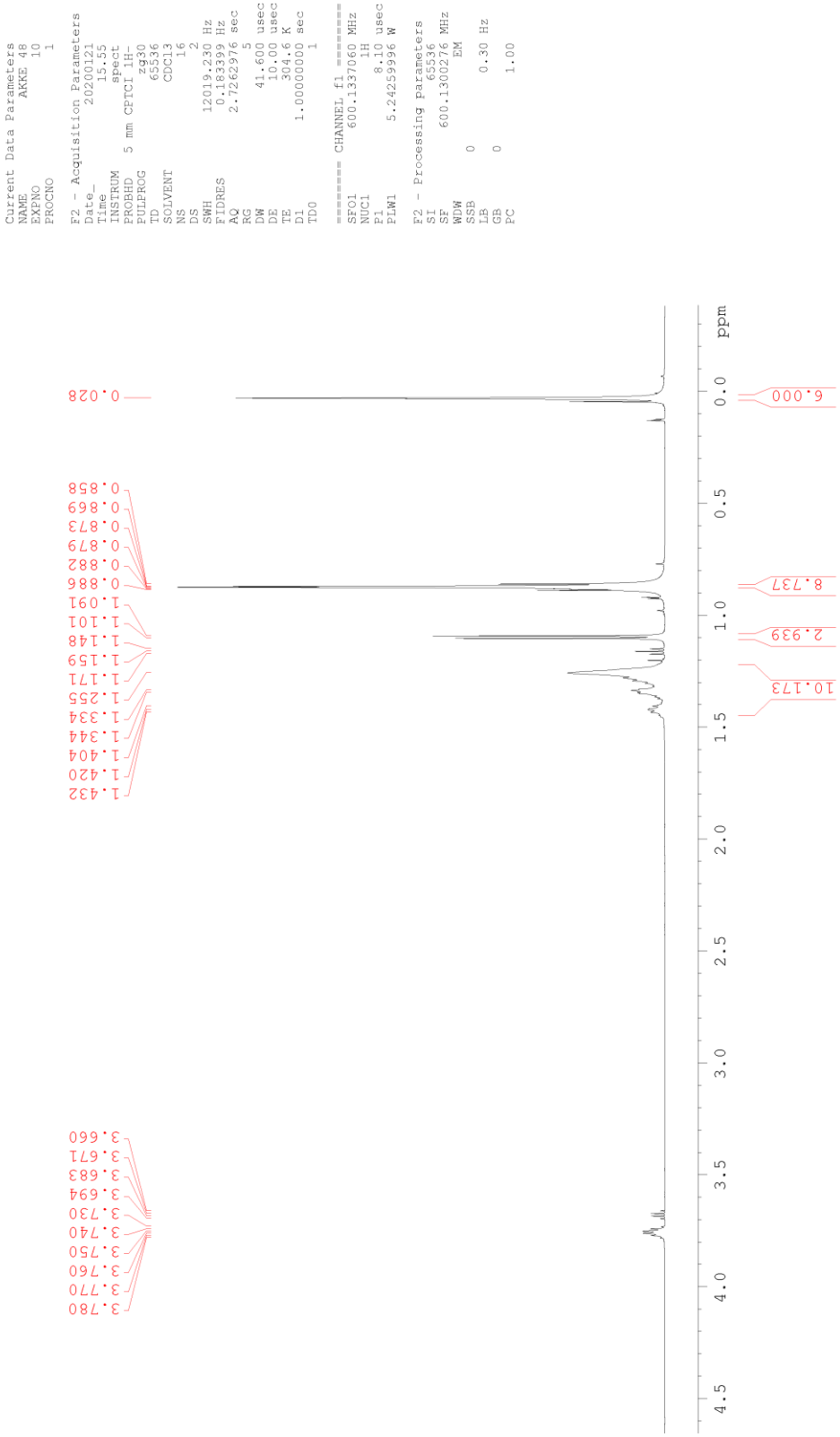
Spectrum 5, of compound **10c**: (*R*)-3-bromo-2-methylpropanal.



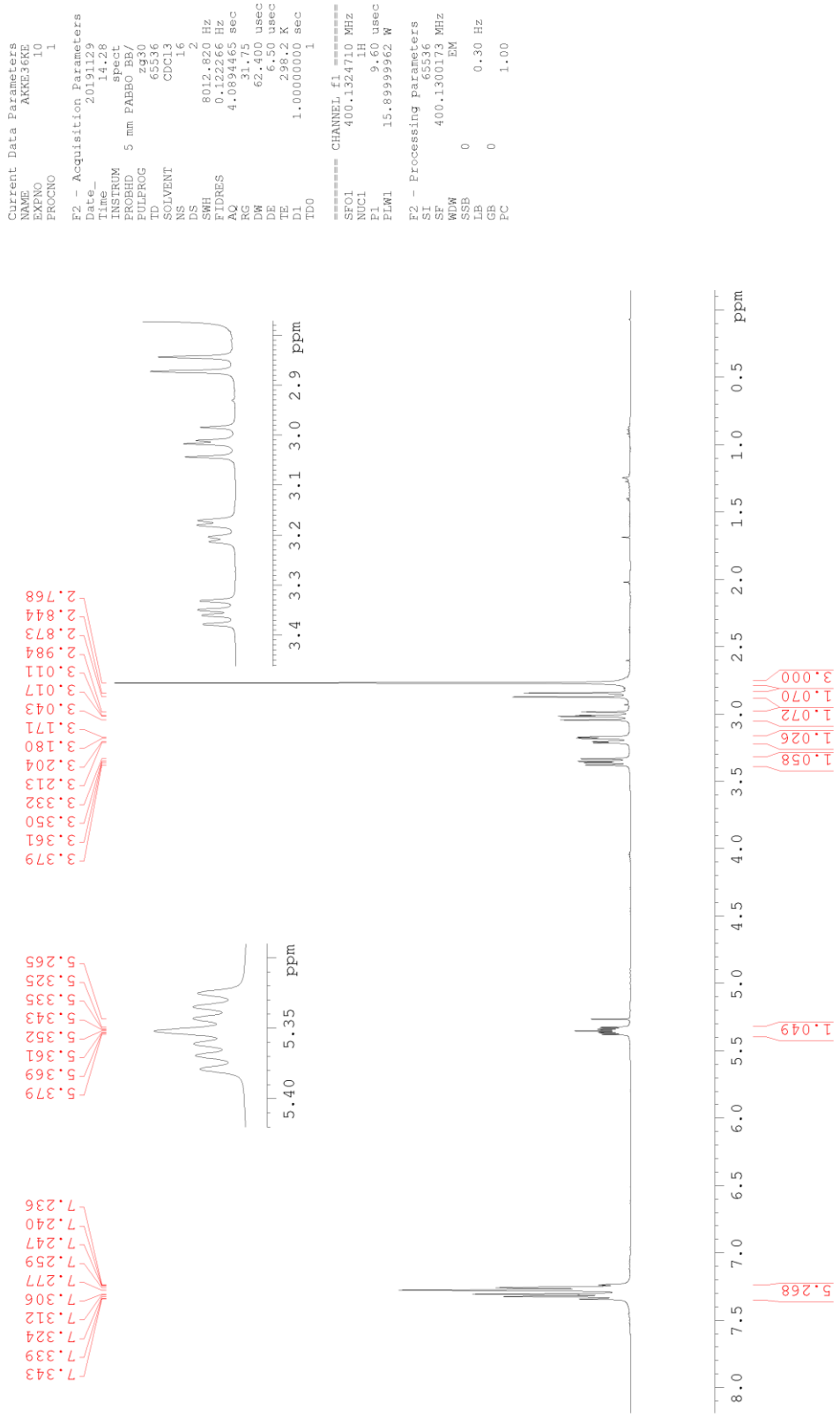
Spectrum 6, of compound 95: 1-((R)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxynonan-1-one.



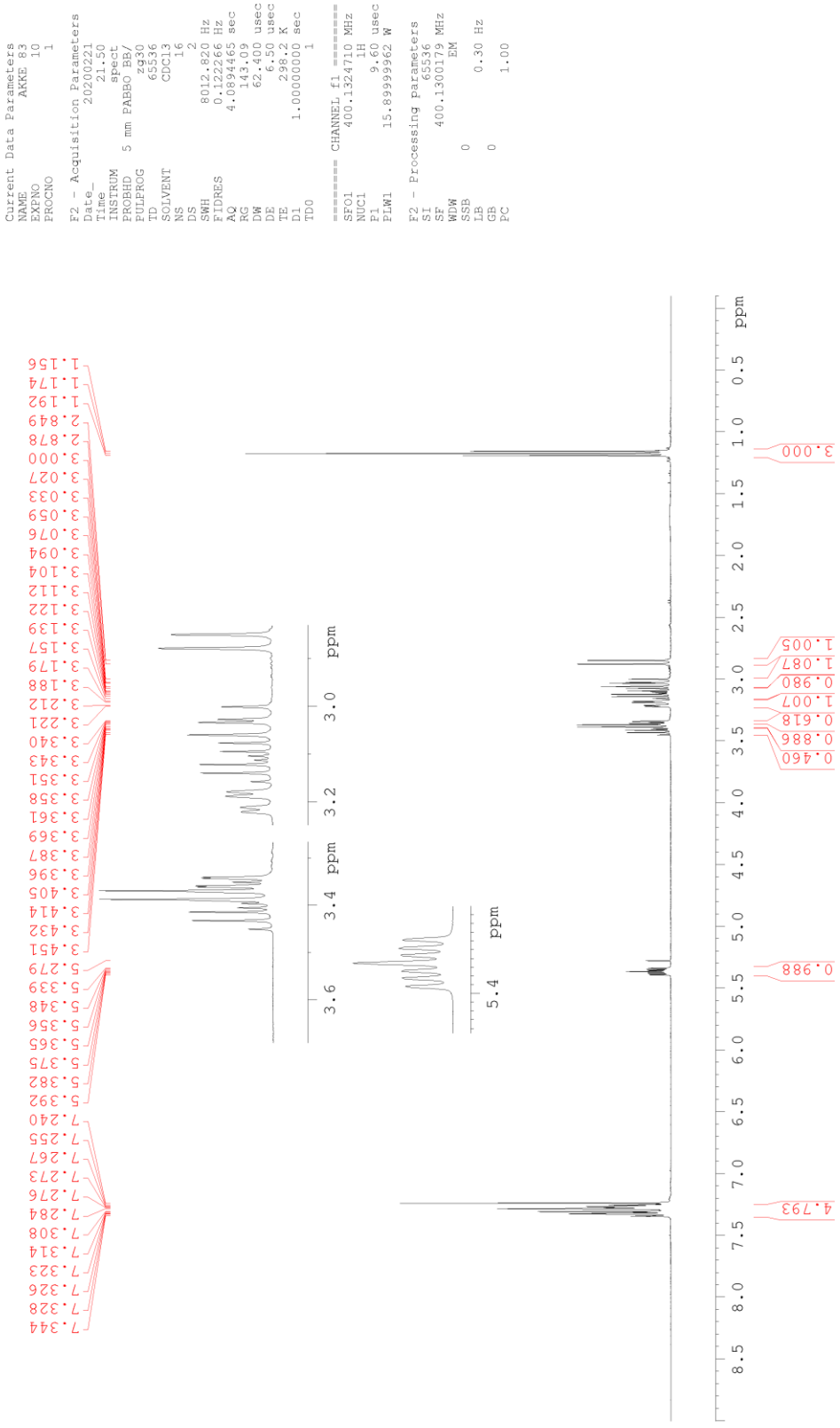
Spectrum 7, of compound **101**: *Tert*-butyldimethyl(octan-2-yloxy)silane.



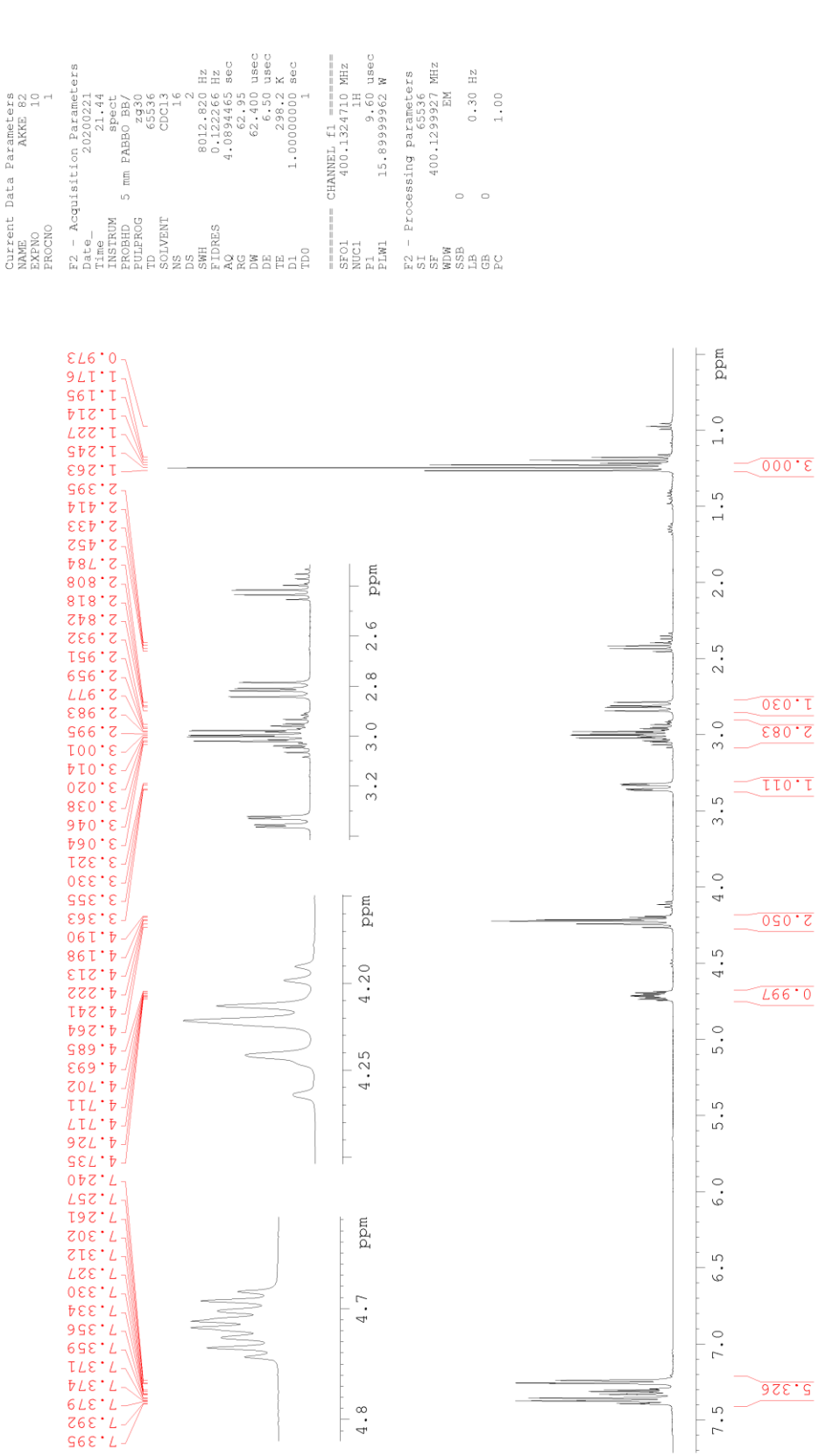
Spectrum 8, of compound **60b**: (*R*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)ethan-1-one.



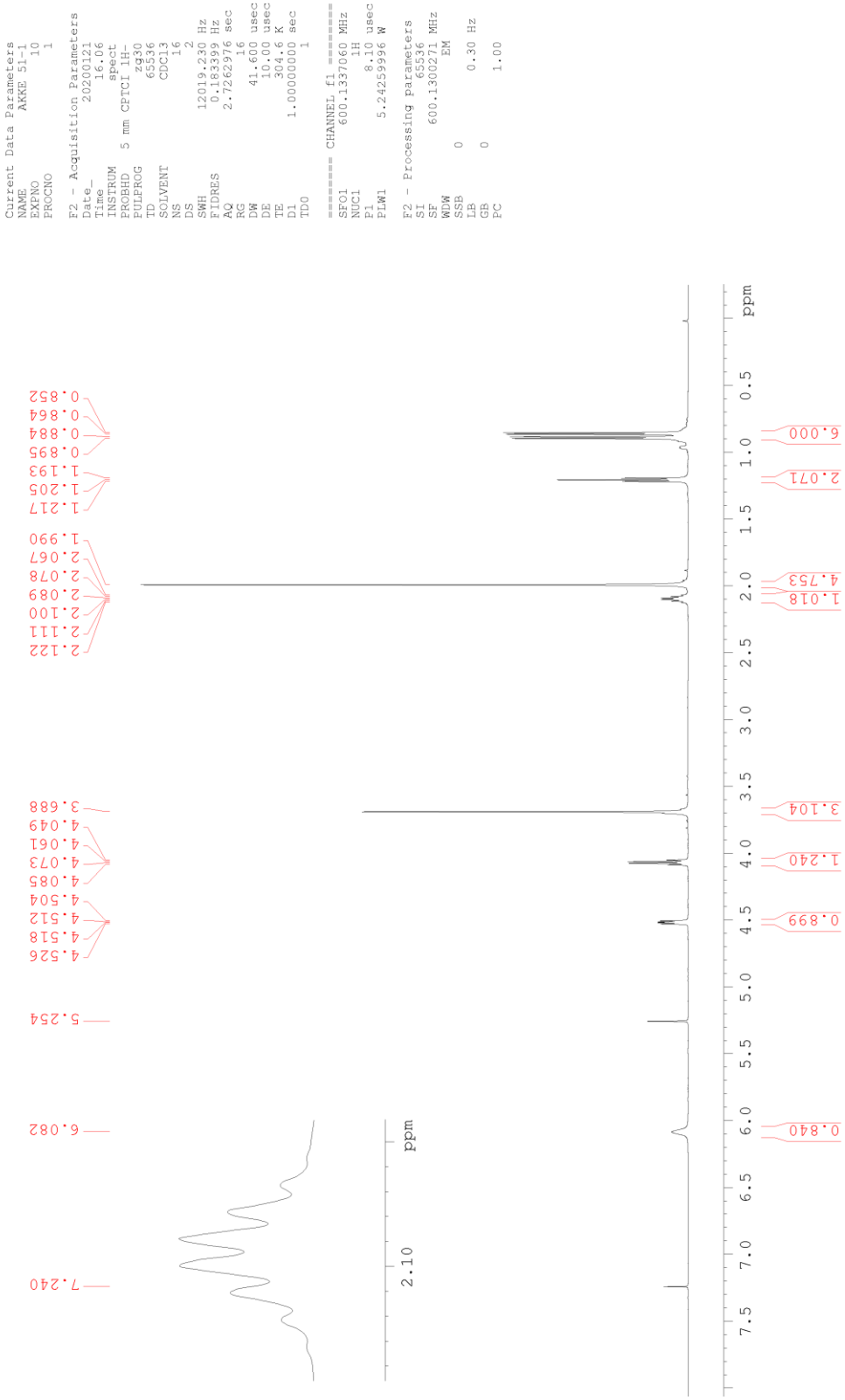
Spectrum 9, of compound **60c**: (*R*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)propan-1-one.



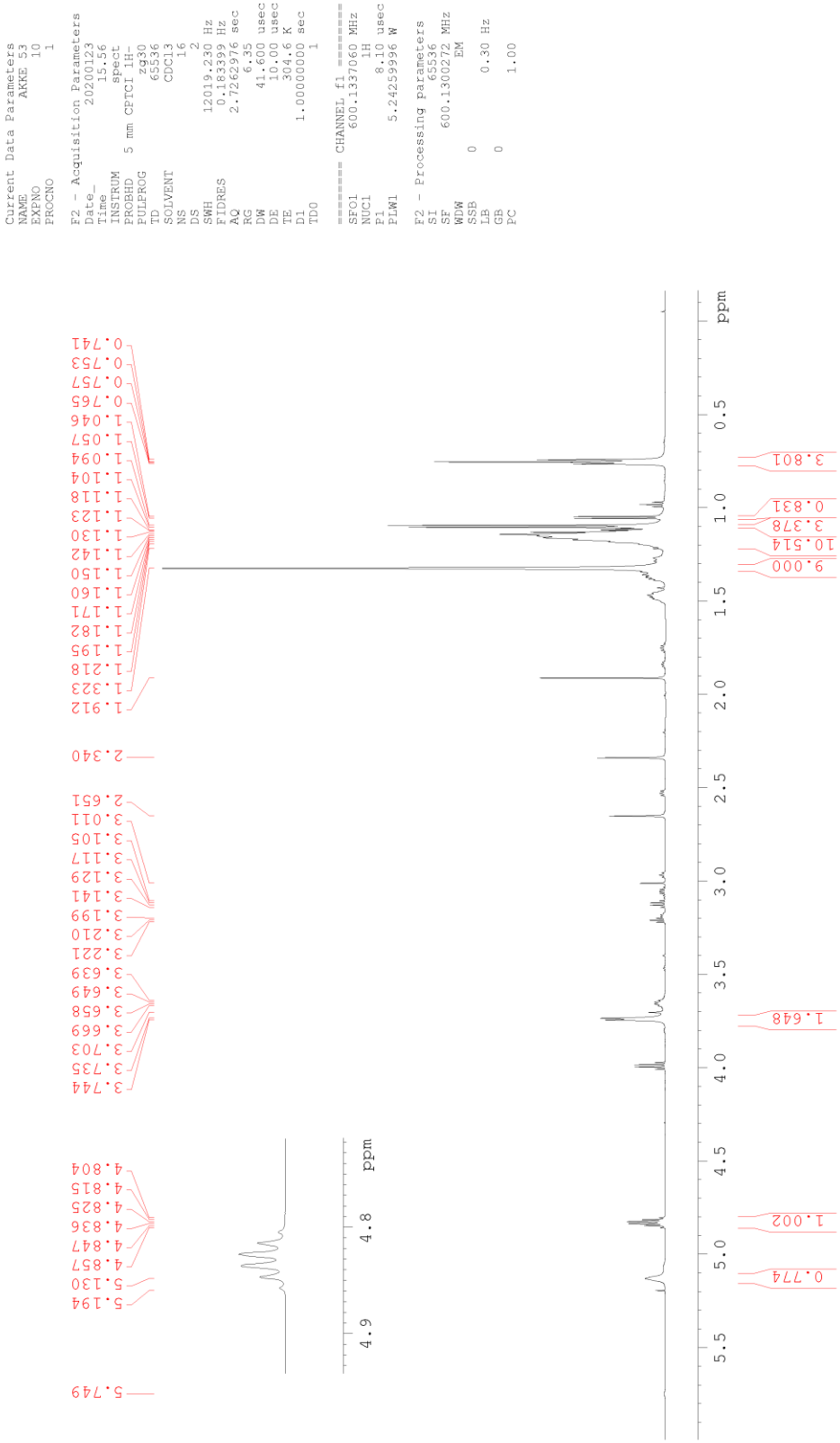
Spectrum 10, of compound **58c**: (*R*)-4-benzyl-3-propionyloxazolidin-2-one.



Spectrum 11, of compound **102**: Methyl acetyl-L-valinate.

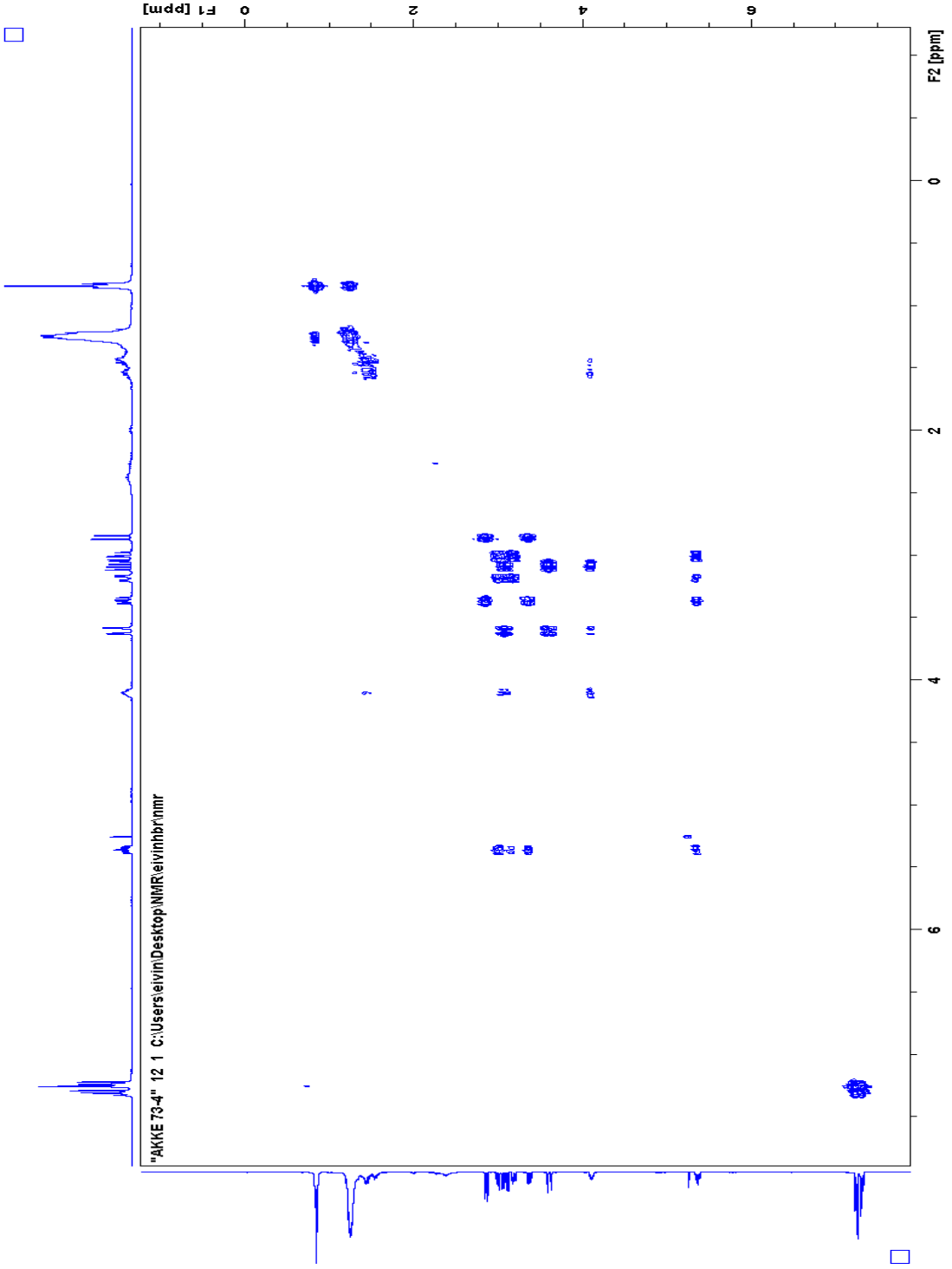


Spectrum 12, of compound **104**: Octan-2-yl (*tert*-butoxycarbonyl)glycinate.



COSY

Spectrum 13, COSY-spectrum of compound **95**: 1-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxynonan-1-one.



Eivind Halvorsen Brenna

Synthesis of Scopulariside A and B

NTNU
Norwegian University of
Science and Technology

OSLOMET
OSLO METROPOLITAN UNIVERSITY
STORBYUNIVERSITETET