Synthesis of 15-aza-salicylihalamide A analogue

Dan Balan  B Sci (Hons)

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

November 2012

School of Applied Sciences (Applied Chemistry)
RMIT University
Statement of Authenticity

I certify that except where due acknowledgement has been made the thesis comprises only my original work. This thesis has not previously been submitted, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work that has been carried out during the approved research program.

Dan Balan
26/11/2012
WORK IN THIS THESIS HAS APPEARED IN THE FOLLOWING PUBLICATION

This thesis is dedicated to the memory of my father.
1931-2010
ACKNOWLEDGEMENTS

While it is difficult to acknowledge everyone as I appreciated the support and guidance of many people, I will start acknowledging the greatest contribution to this work that comes from my supervisors, Associate professor Helmut Hügel from RMIT University and Professor Mark Rizzacasa from the School of Chemistry, the Bio21 Institute, the University of Melbourne. They appropriately criticized, praised, cajoled and restrained me.

Professor Helmut Hügel’s help and guidance as well as his valuable input and support throughout my research turned out to be very exciting as well as challenging. It has been a pleasure working with Professor Hügel on my project as he always offered exceptional advice and encouragement when I needed most. I also owe him thanks for important suggestions and for providing guidance throughout for my work that led to a scientific improvement of this research.

Professor Mark Rizzacasa whose years of experience with salicylihalamide analogues and whose appreciation of the complexity of this research constantly challenged my thinking. I am very grateful to Professor Rizzacasa for many fruitful discussions and invaluable suggestions that have contributed to the 15-aza-salicylihalamide analogue A synthesis. Without his patience, guidance, inspiration and help, everything what I have achieved would not have been possible. It has been a privilege to work with him on an exciting target as salicylihalamide analogues.
I would also like to thank all the staff at the RMIT University School of Applied Science and the Bio21 Institute at the University of Melbourne that provided the much needed administrative, technical and financial resources throughout my candidature years.

Finally I am fortunate to have a large network of family and friends. It is the unquantifiable moral support that I have received that made this research possible.

Thank you!
ABSTRACT

Salicylihalamide A, a potent cytotoxic natural product, was isolated by Boyd and co-workers in 1997 from a marine sponge *Haliclona* sp. collected from waters around Rottnest Island which is situated 18 km off the coast of southern Western Australia. This compound is characterized by a unique structure as well as by different biological activities with interesting mechanisms of action, displaying cytotoxicity at nanomolar levels against several human tumor cell lines.

The majority of SAR studies have been conducted on salicylihalamide derivatives with modified enamide side chains as well as on the 12-membered ring and it has been shown that some small modification of the enamide moiety or of the macrolactone ring are tolerated without substantial loss of cytotoxic activity. For example, the semisynthetic 15-aza-epothilone B was found to exhibit a better therapeutic range than epothilone B because of the stability of the macrolactam.

*This led us to our hypothesis that the modification of the 12-membered macrolactone in salicylihalamide A to a macrolactam would provide a more stable macrocyclic system and thus would be an interesting target for synthesis and biological evaluation.*

A key step in the synthesis of the new lactam analogue of salicylihalamide A was a photochemical acylation reaction. In order to prepare the desired *E*-lactam we have investigated the formation of the macrocyle using a RCM procedure. Conversion of macrolactam into the vinyl iodide followed by Cu catalysed cross coupling with the 2,4-(Z,Z)-heptadienoic amide gave the 15-aza-salicylihalamide A analogue in good yield.

Due to its promising bioactivity profile, the first total synthesis of the novel salicylihalamide A lactam analogue was accomplished in 17 linear steps from 2,6-
dihydroxybenzoic acid with an overall yield of 0.70%.

The new analogue was tested for cell growth inhibition against several leukemia cell lines. The results showed that the 15-aza-salicylihalamide A analogue exhibited antiproliferative effects at sub-micromolar concentrations.
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<tr>
<td>[α]</td>
<td>specific rotation</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BINAP</td>
<td>2,2'-bis(diphenylphosphino)-1,1'-binaphthyl</td>
</tr>
<tr>
<td>bp</td>
<td>boiling point</td>
</tr>
<tr>
<td>br</td>
<td>broad</td>
</tr>
<tr>
<td>bu</td>
<td>butyl</td>
</tr>
<tr>
<td>conc</td>
<td>concentrated</td>
</tr>
<tr>
<td>CuTc</td>
<td>copper(l) thiophenecarboxylate</td>
</tr>
<tr>
<td>d</td>
<td>day(s) or doublet</td>
</tr>
<tr>
<td>DBU</td>
<td>diazobicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DDQ</td>
<td>2,3-dichloro-5,6-dicyanobenzoquinone</td>
</tr>
<tr>
<td>DIAD</td>
<td>diisopropyl azodicarboxylate</td>
</tr>
<tr>
<td>DIBAL-H</td>
<td>diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DIPA</td>
<td>diisopropylamine</td>
</tr>
<tr>
<td>DMA</td>
<td>dimethylacetamide</td>
</tr>
<tr>
<td>DMAP</td>
<td>N,N-dimethyl-4-aminopyridine</td>
</tr>
<tr>
<td>DME</td>
<td>dimethoxyethane</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N'-dimethylformamide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
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<td>DPPA</td>
<td>diphenylphosphorazide</td>
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<td>equiv</td>
<td>equivalent(s)</td>
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<td>Et</td>
<td>ethyl</td>
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<tr>
<td>g</td>
<td>gram(s)</td>
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<tr>
<td>GI&lt;sub&gt;50&lt;/sub&gt;</td>
<td>concentration at which 50% growth inhibition is observed</td>
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<tr>
<td>h</td>
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<tr>
<td>HMDS</td>
<td>hexamethyldisilazide</td>
</tr>
<tr>
<td>HMPA</td>
<td>hexamethylphosphoramide</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrum or spectrometry</td>
</tr>
<tr>
<td>i</td>
<td>iso</td>
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<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>inhibitory concentration (for 50% of a biological sample)</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant in Hertz</td>
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<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>mCPBA</td>
<td>meta-chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
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<td>mg</td>
<td>milligram(s)</td>
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MOM…………………………………  methoxymethyl
mp…………………………………  melting point
n………………………………………  normal
NBS…………………………………  N-bromosuccinimide
NCI…………………………………  National Cancer Institute
nM………………………………………  nanomolar
NMP…………………………………  1-methyl-2-pyrrolidinone
NMR…………………………………  nuclear magnetic resonance
°C………………………………………  degrees Celsius
PMB…………………………………  p-methoxybenzyl
pyr…………………………………  pyridine
q…………………………………………  quartet
R…………………………………………  rectus (configuration)
RCM…………………………………  ring-closing metathesis
Rf………………………………………  retention factor
rt………………………………………  room temperature
s………………………………………  singlet
S…………………………………………  sinister (configuration)
SAR…………………………………  structure-activity relationship
SN2…………………………………  bimolecular nucleophilic substitution reaction
t…………………………………………  triplet
t…………………………………………  tertiary
TBAF…………………………………  tetrabutylammonium fluoride
TBS…………………………………  tert-butylidimethylsilyl
TEA…………………………………  triethylamine
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<tr>
<td>Tf</td>
<td>trifluoromethanesulfonyl</td>
</tr>
<tr>
<td>TFP</td>
<td>tris(2-furyl)phosphine</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TIPS</td>
<td>triisopropylsilyl</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer chromatography</td>
</tr>
<tr>
<td>TMEDA</td>
<td>1,1,4,4-tetramethylethylene diamine</td>
</tr>
<tr>
<td>TPAP</td>
<td>tetrapropylammonium perruthenate</td>
</tr>
<tr>
<td>uv</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>z</td>
<td>benzene</td>
</tr>
<tr>
<td>Z</td>
<td>Zusammen (configuration)</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift in parts per million from tetramethylsilane</td>
</tr>
<tr>
<td>µM</td>
<td>micromolar</td>
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Chapter One:

Introduction-Salicylihalamides:

Isolation, Biological Activity And Structure Elucidation
1.1 Introduction

The marine environment has proven to be a rich source of novel bioactive natural compounds with cytotoxic effects or specific effects on cellular targets. These natural products have demonstrated the invaluable role played in the drug discovery process related to all disease types and, in particular, in the areas of cancer and infectious diseases. They can be extremely potent in culture, with inhibitory concentrations generally in the nanomolar concentrations.\(^1\)

Salicylihalamides A (1a) and B (1c) (Figure 1) are marine metabolites which are characterised by a unique structure as well as by different biological activities with interesting mechanisms of action. Both salicylihalamide A (1a) and B (1c) are members of a new family of cytotoxic natural products isolated from the sponge *Haliclona* sp. by Boyd and co-workers in 1997 and display cytotoxicity at nanomolar levels against several human tumor cell lines.\(^2\)

![Salicylihalamide A and B](image)

**Figure 1. Salicylihalamide A and B**

Structurally, these compounds feature a 12-membered macrolide ring that includes a salicylic acid residue with the macrolactone carrying a multiunsaturated side chain consisting of an unusual acyl enamine moiety and a (2Z,4Z)-heptadienamide system.

Testing of salicylihalamide A (1a) in the NCI-60 cell line human tumour screen gave a
striking pattern of differential toxicity, which did not correlate with the profiles of any known antitumor compounds in the standard database. These results suggest that compound 1a has a novel mechanism-of-action and further biological evaluation revealed that compound 1a was the first of a new class of mammalian specific vacuolar-type (H⁺)-ATPase inhibitor.

1.2 Biological significance of Vacuolar (H⁺) – ATPases

Eukaryotic cells contain single membrane-bounded organelles, such as Golgi apparatus, coated vesicles, vacuoles of plants and fungi, endosomes, lysosomes, endoplasmic reticulum, secretory granules and synaptic vesicles. Their lumina are acidic, with pH from 4.5 to 6.5. Environmental pH regulation is essential to the function of many biological systems and each organelle maintains a characteristic internal pH close to the physiological range, which is crucial to normal cell function due to the narrow pH optima of many cellular processes. A defect in organellar acidification in mammalian cells is correlated with an increasing number of diseases such as male infertility, renal acidosis, tumor metastasis and osteopetrosis (thickening of the bones and skull and skeletal defects due to the inability of osteoclasts to degrade bone). The acidic environment in organelles is necessary to promote the activity of hydrolases responsible for degradation of proteins and other macromolecules and the release of ligands from receptors. It is well established that the acidic pH in organelles is generated by the action of a vacuolar-type H⁺-ATPase (V-ATPase), a primary proton pump that plays a key role in the proper functioning of internal organelles such as vacuoles, endosomes, lysosomes, synaptic vesicles, secretory granules, and the Golgi apparatus as well as on plasma membranes of diverse organelles. The core function of protein complex V-ATPase is to pump protons out of the cytoplasm into the
organelle or the extracellular space using the chemical energy released by adenosine triphosphate (ATP) hydrolysis. This multi-subunit electrogenic proton pump (V-ATPase) plays crucial roles in sperm storage and maturation (maintaining sperm in an immotile state in the epididymis and vas deferens), body acid-base regulation (renal proximal tubule and collecting duct intercalated cells), inhibition of bone remodeling (osteoclasts), pH homeostasis, coupled transport and tumor metastasis.

Loss of V-ATPase activity due to protein mutations in particular cell types has been implicated in diverse pathophysiological states such as kidney and bone disease (inadequate bone resorption caused by osteoclast dysfunction), cutis laxa syndrome (wrinkly skin syndrome - loose and redundant skin folds and decreased elasticity of the skin) and sensorineural deafness. Thus V-ATPase functions in a very broad array of physiological processes, performing diverse biological activities within cells.

1.3 Structure of the V-ATPases and relationship to F-ATPases

V-ATPases are large (900-kDa) multisubunit complex enzymes containing at least 13 distinct subunits, some of which occur in mammalian tissues as multiple isoforms. They are comprised of two domains, a membrane-embedded $V_0$ domain and an extramembranal soluble $V_1$ domain. The energy gained from ATP-hydrolysis by the $V_1$ domain is used by the $V_0$ domain to pump protons from the cytosol to the non-cytosolic space. The soluble $V_1$ domain is a complex of 640 kDa including at least eight different subunits (A–H) and is responsible for ATP hydrolysis. The A and B subunits serve as the ATP binding sites, with the catalytic site located on the A subunit. The membrane-embedded $V_0$ a 260 kDa subcomplex, includes subunits $a$, $c$, $c'$, $c''$, $d$, and $e$ and is responsible for proton translocation across the membrane in which it is anchored. The $a$ subunit of the proton
pump is considered essential for coupling of ATP hydrolysis ($V_1$) and proton transport ($V_0$). Subunit $c$ seems to significantly influence the proliferation and metastasis of tumor cells and inhibition of this subunit resulted in the suppression of growth and metastasis which is overexpressed in most human cancers, including liver carcinoma.

![Comparison of ATPases](image)

**Figure 2. Comparison of ATPases**

There are three known types of ATPases – P-ATPases, F-ATPases, and V-ATPases (Figure 2). The P-type ATPases are membrane proteins responsible for the transport of a variety of cations across cell membranes using ATP hydrolysis for energy. There are many different classes of P-ATPases, each of which transports a specific type of ion: $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Ag^+$ and $Ag^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Pb^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Cu^{3+}$, $Cu^{2+}$. V-ATPases have similar structure and mechanism of action as F-ATPase. In contrast to F-ATPases, whose primary function is to synthesize ATP from ADP, V-ATPases uses the energy derived from ATP hydrolysis to transport protons across the lysosomal membrane.
1.4 Role of V-ATPases inhibitors in cancer

Tumor cells in vivo often exist in a poor oxygenated region with a lower extracellular pH than that of surrounding normal tissues (pH 6.9-7.1), the acidic environment contributing to tissue damage, metastatic progression as well as activation of destructive enzymes. \(^{25}\)

The majority of tumor cells demonstrate substantially increased glucose uptake compared with normal tissue, converting glucose to ATP even in the presence of abundant oxygen, resulting in tumor cell acid production, which contributes, to the proliferation and invasion of cancer cells during the process of tumorigenesis and metastasis. \(^{26}\) In solid tumors, both intracellular and extracellular pH differ, thus tumor cells express various pH regulators to avoid apoptosis. However, cancer cells usually have neutral to alkaline cytosolic pH in the acidic extracellular microenvironment. \(^{27}\)

Recently, much attention has been paid to the roles that the highly active V-ATPase plays in the pH regulation in tumor cells. \(^{28}\) V-ATPases located in the membranes of vacuolar systems of animal cells act as important proton transporters that regulate the cytosolic pH maintaining an alkaline intracellular environment favorable for growth, whereas the V-ATPases present in the plasma membrane of tumor and endothelial cells are involved in extracellular pH regulation. \(^{29}\)

1.5 V-ATPase inhibitors

In general, intracellular pH is similar in both solid tumor and normal tissues while extracellular pH is higher in normal tissue and lower in solid tumors, scientific evidence suggesting that the acidic tumor microenvironment is key to managing cancer development
and metastasis. As a consequence, these findings offer a possibility to develop novel compounds as agents that specifically target the mechanism(s) responsible for the acidic pH of tumors. Among the key regulators of the tumor acidic microenvironment, V-ATPases play an important role because they can be inhibited by proton pump inhibitors.\textsuperscript{30} Attempts to develop V-ATPase-targeted drugs have focused on the naturally occurring macrolide antibiotics concanamycins and bafilomycins (Figure 3).

Figure 3. Bafilomycin A\textsubscript{1} and Concanamycin A

This class of antibiotics has no effect on F-ATPases (which are structurally homologous to the V-ATPase) whereas some P-type ATPases are inhibited in the micromolar and V-ATPases in the nanomolar concentration range.\textsuperscript{31} Concanamycins (an 18-membered plecomacrolides) are better and more specific inhibitors of V-ATPases than the bafilomycins with bafilomycin A\textsubscript{1}, (a 16-membered macrolactone) inhibiting bone resorption both \textit{in vitro}\textsuperscript{32} and \textit{in vivo}\textsuperscript{33}. However, bafilomycin A\textsubscript{1} cannot be administrated even in animal experiments, because it inhibits all the essential V-ATPases leading to
systemic alteration of cellular physiology and severe toxic effects.\textsuperscript{34}

Another class of natural products that selectively inhibit V-ATPase activity \textit{in vitro} is chondropsins that display potent cytotoxicity and produce a characteristic pattern of differential activity in the NCI 60-cell line antitumor screen.\textsuperscript{35} This class of macrolides has no inhibitory activity on F- and P-type ATPases enzyme and shows a high COMPARE correlation coefficient with the bafilomycin and concanamycin.\textsuperscript{36}

Since the discovery of the salicylihalamides, the isolation of other natural products with the common characteristics of this new benzolactone enamide class has been reported. This family includes the apicularens with an identical enamide side chain\textsuperscript{37} and the lobatamides, oximidines I and II, CJ-12,950, and CJ-13,357 each with the highly unsaturated enamide side chain terminating in an \textit{O-methyl} oxime as illustrated in Figure 4.\textsuperscript{38} These natural compounds possess common structural features – a 12 or 15 – membered ring macrolactone and an $\alpha,\beta$-unsaturated enamide side chain - and are the only known potent inhibitors that show a clear preference for inhibition of mammalian vacuolar ATPase activity and is ineffective toward V-ATPase from yeast and fungi in contrast to bafilomycins or concanamycins. They were shown to inhibit human kidney (IC\textsubscript{50} 0.58 nM),\textsuperscript{39} liver (IC\textsubscript{50} 0.62 nM)\textsuperscript{40,41} and human osteoclast (IC\textsubscript{50} 0.40 nM)\textsuperscript{42,43} V-ATPase in membrane preparations at low nanomolar concentrations, but were inactive against V-ATPases of fungi (IC\textsubscript{50} > 10,000 nM).\textsuperscript{44} In biological testing, these benzolactone enamides showed potent cytotoxic activity when tested in vitro for their ability to inhibit growth of 60 human cancer cell lines from different organs of origin. Studies of structure-activity relationships (SAR) of the salicylihalamide A and chemical analogues have helped to identify the minimal structural requirements for inhibition of V-APases.

Since V-ATPases play crucial roles for many cellular processes, salicylihalamide is expected to be a promising anticancer drug candidate.
Figure 4. Salicylhalamides and structurally related benzolactone acylenamine natural products.
1.6 Total synthesis of salicylihalamides

Immediately after their isolation and structural elucidation, the salicylihalamides have received considerable attention from various chemical synthesis research groups, due to their novel structural features and outstanding biological activity. The novel class of natural products known as the benzolactone enamide has a salicylic acid moiety, a macrolactone and an unusual highly unsaturated enamide side chain. Given the sensitive nature of the enamide side chain of this macrolide, the installation of this chain moiety has been done at a late stage in the synthesis. The 12-membered macrolactone can either be secured by ring closing metathesis or by intramolecular cross coupling reactions. The general route for the Mitsunobu esterification/ring closing metathesis strategy followed by the enamide side-chain installation\(^{45}\) is shown in Scheme 1.
Scheme 1. General route for the salicylhalamide syntheses (Mitsunobu esterification/ring closing metathesis/enamide side-chain installation)

1.6.1. Synthetic strategies for the salicylate precursors

De Brabander and co-workers commenced the construction of the macrolactone core of the salicylhalamides 1 (Scheme 2) from commercially available 2,6-
dihydroxybenzoic acid 2. Treatment of the corresponding acid with acetone produced an isopropylidene intermediate, which was converted to acetonide triflate 3. The allyl substituent introduced by a Stille coupling of 3 with allylstannane, was followed by a Grignard addition to give allyl ester 4. Subsequent methylation and deprotection of the allyl group provided the allyl-anisic acid 5.

Labrecque et al. used the most direct route to prepare the same 6-allyl-2-methoxybenzoic acid 5 by allylation of lithium o-anisate starting from the known o-anisic acid 6. Although the yields in this reaction are poor (18-48%), this method gives a quick access to the salicylate precursor.

According to Fürstner’s procedure, triflate 3 was converted to salicylic acid derivative 8 via allylation in high yield by a modified Suzuki-type reaction, followed by the cleavage of the isopropylidene group of 7.

Snider and Song introduced the allyl substituent by a Stille coupling reaction. Reaction of 3 with allyltributyltin using Pd\textsubscript{2}(dba)\textsubscript{3} and TFP followed by hydrolysis of the acetonide under basic conditions afforded 8 in a very good yield.

Smith and Zheng adopted a different strategy to obtain the same salicylate precursor, starting from the known amide 9. Using a conventional procedure involving the use of iodine in aqueous tetrahydrofuran mixture, the amide 9 was converted to an iodolactone 10, which was then treated with zinc in acetic acid to provide the allyl-anisic acid 5.

Maier et al. employed 5 as the starting material, which was converted to vinyl iodide 11 by formation of the aldehyde intermediate followed by Takai iodo-olefination.

According to Rizzacasa’s procedure, 6-methyl salicylic acid 12 was converted to bromoacetonide 13 via acetonide formation followed by radical bromination, in order to construct the macrolacton core through a Stille coupling reaction.
Scheme 2. Synthetic strategies for the salicylate precursors
1.6.2. Synthetic strategies for the alcohol precursors

De Brabander and co-workers\textsuperscript{46} initiated their synthesis of the Mitsunobu coupling precursor with the enantioselective allylation of the aldehyde 14 followed by the silylation and oxidative double-bond cleavage of 15 to afford aldehyde 16 (Scheme 3). To set the absolute stereochemistry at C15, this aldehyde was treated with (Z)-O-titanium enolate derived from 17 producing one diastereomeric aldol product 18. Alcohol protection of 18, followed by tosylation formation, reduction and desilylation afforded the alcohol 19.

\begin{center}
\textbf{Scheme 3. Synthesis of the diastereomERICALLY pure alcohol precursor 19 (De Brabander et al.)}\textsuperscript{46}
\end{center}
Rizzacasa et al.\textsuperscript{52} developed a stereocontrolled construction of the vinyl stannane 26 starting with the known optically pure acetal 20, which was then subjected to ozonolysis/reductive workup to afford the alcohol 21. Subsequent tosylation, lithium acetylide displacement, and DIBAL reduction of the acetal functionality, furnished the alcohol 23 (Scheme 4). Oxidation of the resultant alcohol with Dess-Martin periodinane and addition of allylmagnesium bromide afforded alcohol 25, which was separated from its diastereomeric contaminant via flash chromatography. The alcohol 24 could be converted to 25 by Mitsunobu inversion. The synthesis of 26 was completed by regioselective palladium-catalyzed hydrostannylation of terminal alkyne.

Scheme 4. Synthesis of the diastereomerically pure alcohol precursor 26 (Rizzacasa et al.)\textsuperscript{52}
Fürstner and co-workers synthesized the alcohol precursor 35 as outlined in Scheme 5. After prenylation of Oppolzer’s bornanesultam 27 the desired compound 28 was prepared with excellent diastereoselectivity and useful yield (79%). Saponification of 28 with aqueous LiOH in THF, converting the resulting acid 29 to acid chloride 30 followed by chain extension provided β-exo ester 31. Chemo- and stereoselective hydrogenation of this ester gave the corresponding secondary alcohol 32, which was protected by a MOM group preparing the conditions for the construction of the chiral center at C15.

Scheme 5. Synthesis of the diastereomerically pure alcohol precursor 35 (Fürstner et al.)
Smith and Zheng\textsuperscript{50} prepared the requisite alcohol precursor 41 by the route depicted in Scheme 6. Roush crotylboration coupling of aldehyde 36 and allyl boronate 37 proceeded with excellent diastereoselectivity to give alcohol 38 in 88% yield and 90% diastereoselectivity. Protection of the secondary alcohol 38 as the \( t \)-butyldimethylsilyl ether, followed by hydroboration, Swern oxidation and Wittig methylenation led to alkene 39 which was deprotected with aqueous trifluoroacetic acid to give the intermediate diol that was converted to the epoxide 40. Finally, treatment of the epoxide with vinyl Grignard in catalytic conditions gave the targeted intermediate 41 in 76% yield.

Scheme 6. Synthesis of the diastereomerically pure alcohol precursor 41 (Smith \textit{et al.})\textsuperscript{50}
Labrecque et al.\textsuperscript{47} described the use of the known epoxide 42 for the preparation of Mitsunobu coupling precursor by the route presented in Scheme 7. The known epoxide was converted to the protected 1,3-diol 43 via sequential reduction and \textit{in situ} protection with \textit{p}-anisaldehyde. Cleavage of the \textit{O}-protecting groups in the adduct by DIBAL-H followed by Dess–Martin oxidation and Roush crotylation afforded 44. This secondary alcohol was then submitted to silylation and hydroboration, which gave alcohol 45 in good overall yield. Dess–Martin oxidation followed by Wittig homologation and PMB deprotection with DDQ gave the desired alcohol 46.

**Scheme 7. Synthesis of the diastereomERICally pure alcohol precursor 46** (Labrecque et al.)\textsuperscript{47}
The Snider and Song\textsuperscript{49} stereocontrolled synthesis of pure alcohol 53 is outlined in Scheme 8. The synthesis of building block 53 commenced with the LiBH\textsubscript{4} reduction of the amide 47 followed by the Swern oxidation to provide aldehyde 48. This aldehyde was then subjected to asymmetric Carreira aldol condensation with 49, which gave an inseparable 4.2:1 mixture of 50. Treatment of the alcohol 50 gave \(\beta\)-keto ester 51, which was reduced to the syn alcohol and cyclized to the hydroxylactone 52.

\(\text{NaBH}_4\) reduction to the lactol followed by the methyl acetal protection afforded cyclic ester 53 in an overall yield of 83%.

Scheme 8. Synthesis of the diastereomerically pure alcohol precursor 52 (Snider and Song)\textsuperscript{49}
Maier et al. have used as starting material aldehyde 55 and obtained the enantiomerically pure alcohol 61 in good yield (Scheme 9). The aldehyde 55 was subjected to Duthaler aldol reaction with tert-butyl acetate to give 3-hydroxy ester 56 in 83% ee. Protection of the resulting secondary hydroxyl group as the TIPS ether, followed by DIBALH reduction gave aldehyde 57, which was subjected to an Evans aldol reaction with 58 to afford 59 in quite good yield. Completion of alcohol 61 entailed formation of the MOM-ether, reductive removal of the chiral auxiliary with NaBH₄, elimination in the presence of NaI and DBU, silyl deprotection and inversion of the alcohol 60.

Scheme 9. Synthesis of the diastereomerically pure alcohol precursor 61 (Maier et al.)⁵¹
Georg et al.\textsuperscript{53} reported the synthesis of 66 in a nine step procedure, beginning with the known alcohol 63, which was prepared in five straightforward steps from diacetone-D-glucose 62 as a source for the three chiral centres of the molecule (chiral pool approach) (Scheme 10). Conversion of the alcohol 63 to the corresponding triflate followed by displacement with a higher order allylcyanocuprate provided alcohol 65. The acetonide function was cleaved with acetic acid and the resulting anomeric hydroxy group selectively oxidized to generate lactone 66.

\begin{scheme}
\begin{center}
\includegraphics[scale=0.5]{scheme10}
\end{center}
\end{scheme}

\textbf{Scheme 10. Synthesis of the diastereomerically pure alcohol precursor 66 (Georg et al.)\textsuperscript{53}}

Georg and co-workers on the basis of a similar strategy employed for compound 66, performed the synthesis of the other diastereomer 71 adopting the chiral pool strategy toward the synthesis of the (-)-salicylihalamides (Scheme 11).\textsuperscript{54} Conversion of the hydroxyl group of 67 to the corresponding bromide 68 followed by the allylation set the
absolute stereochemistry at C12. Acidic hydrolysis of the acetonide moiety in 69 gave 70, and subsequently the lactol was oxidized selectively to lactone 71.

Scheme 11. Synthesis of the diastereomerically pure alcohol precursor 71 (Georg et al.)

Hanson and co-workers\(^{55,56}\) have utilized temporary tethers in a recent tactic to join complex synthetic building blocks in moderate to good yield preparing the Mitsunobu coupling precursor 81 by the route outlined in Scheme 12.

Construction of alcohol 81 began with the union of the 1,3-anti-Diol 72 and phosphoryl trichloride using the protocol of Rychnovsky and co-workers,\(^{57}\) to give the coupling product phosphoryl monochloride 73 in 90% yield. Formation of phosphate triester triene 74 was next readily achieved, in one step, by addition of lithium allyloxide into 73, followed by ring-closing metathesis (RCM) to provide phosphate 75 in good yield.

Alcohol 76, secured via a chemoselective hydroboration followed by oxidation, was elaborated to homologated phosphate ester 78 by PMB protection, diastereoselective cuprate addition and methylation. Completion of 81, the alcohol coupling partner, was
achieved in six steps. First, phosphate 78 was converted via a three-step sequence to the corresponding diol 79, and then selectively protected as a TIPS-ether followed by MOM protection to furnish the fully protected triol 80. Desilylation of the resultant triol then furnished the alcohol 81 in 95% yield.

A comparison of the overall yields for the synthesis of the alcohol precursors discussed above is shown in Table 6 (Appendix C, page 167).
1.6.3 Methodology related to macrolactone ring formation

The first total stereoselective synthesis of salicylihalamides A was reported by De Brabander and co-workers (Scheme 13). The synthesis commenced with allylation of aldehyde 82 that provided the absolute stereochemistry at C15 for the known homoallyl alcohol 83. The other two chiral C12 and C13 centers were set by a diastereoselective aldol reaction on aldehyde 84. Condensation of allysalicylic acid 85 with diastereomERICALLY alchhol 86 under Mitsunobu conditions followed by RCM produced macrolactone 87. To complete the total synthesis of 1a, the next step was the introduction of the acylated side-chain via an acyl azide formation and Curtius rearrangement followed by the addition of a nucleophile.

Scheme 13. De Brabander and co-workers synthesis of the ring-closing metathesis precursor of (-) salicylihalamide A

The route developed by Fürstner and co-workers to the total synthesis of (-) Salicylihalamidade A began with the construction of the stereocenter at C12 by prenylation of Oppolzer’s boranesultam 88 (Scheme 14). Stereoselective hydrogenation of ester 89 creates the C13 chiral center while the remaining stereocenter at C15 is introduced by the
The reduction of $\beta$-oxo ester $90$, using the same catalyst but under slightly modified conditions. The macrocyclic core was built by ring-closing metathesis of the diene derived from compound $91$ and alcohol $92$ by Mitsunobu macrolactonization. A Cu-cross coupling process installed the amide $93$.

Scheme 14. Fürstner and co-workers synthesis of the ring-closing metathesis precursor of $(-)$-Salicylihalamide A

Smith and co-workers also completed the total synthesis of $(-)$-salicylihalamide A (Scheme 15). The formation of the required 12-membered lactone was achieved by means of Mitsunobu esterification followed by ring-closing metathesis of diene $94$, which was assembled by coupling the two advanced fragments $5$ and $41$. Fragment $5$ has been synthesized from the amide $9$ (Scheme 2), while fragment $41$ from the known aldehyde $36$. The labile enamide bond was installed via $N$-acylation of the enecarbamate $96$ with acid chloride $95$. 
Scheme 15. Smith and co-workers synthesis of the ring-closing metathesis precursor of (-) salicylihalamide A

Snider and Song\(^49\) prepared the diene 97 via Mitsunobu coupling protocol of acid 8 and cyclic ether 53 (Scheme 16). What is important to note is that the RCM of 96 did not proceed, “presumably because the diequatorial substituents on the tetrahydropyran ring kept the alkenes too far apart.”\(^49\) Hydrolysis of 96 in aqueous acetic acid and alkene homologation of the resulting lactol followed by TBS protection gave diene precursor 98, which was subjected to ring-closing metathesis to afford the macrolactone core. Completion of the (-)-salicylihalamide A was then achieved in eight steps.

Scheme 16. Snider and co-workers synthesis of the ring-closing metathesis precursor of (-) salicylihalamide A
In the Labrecque et al. synthesis of (+)-salicylihalamides A (1a) and B (1b) outlined in Scheme 17, the diene 99 was constructed from Mitsunobu coupling of acid 5 and alcohol 46, which upon ring-closing metathesis gave the macrolactone core 100. This benzolactone was converted to an aldehyde that was condensed with amide 101 to afford \( N,N' \)-bis-acylated aminal 102. By adopting the same synthetic sequence Labrecque’s group synthesized (-) salicylihalamides A and B using the enantiomer of 46.

Scheme 17. Labrecque and co-workers synthesis of the ring-closing metathesis precursor of (-) salicylihalamide A

Georg et al. used the RCM reaction technique to synthesize the macrocyclic core structure of Salicylihalamides A and B with potential of adding the enamide side chain at a later stage to achieve total synthesis (Scheme 18). Esterification under Mitsunobu conditions of the hydroxyl function at C15 of lactone 71 with aromatic fragment 8 provided diene 103, but this lactone proved unreactive upon treatment with first generation
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Grubbs catalyst. Consequently the lactone was reduced to lactol 104 using DIBAL-H followed by a Wittig reaction with stabilized ylide 105 to afford acryl nitrile 106. The right chirality at C13 was achieved by a Mitsunobu esterification with p-nitrobenzoic acid, and the resulting acryl nitrile was subjected to a RCM reaction that provided macrocycle 107. Cleavage of the phenolic methyl ether followed by removal of Z-isomer and hydrolysis of the PNB group furnished the macrocyclic core structure 108.

Scheme 18. Georg and co-workers synthesis of the ring-closing metathesis precursor of (-) salicylihalamide A

Rizzacasa and co-workers have used a totally different strategy for the creation of the unsaturated benzolactone core of the salicylihalamides A and B (Scheme 19). Their approach relied on a Stille cross-coupling reaction between vinyl stannane 26 and bromoacetonide 13 to afford alkene 109 in good yield. Macrolactonization of the latter compound (NaH, THF) led to the intermediate macrolactone, from which the PMB group was removed (DDQ) followed by the TBS reprotection and cross metathesis (Grubbs’
second generation catalyst) with acrylic acid to afford $\alpha,\beta$-unsaturated acid 110. The latter intermediate had been used for De Brabander’s and Smith’s syntheses thereby completing the formal total synthesis of salicylihalamides A and B.

**Scheme 19.** Rizzacasa and co-workers synthesis of the ring-closing metathesis precursor of (-) salicylihalamide A

Maier and co-workers\textsuperscript{58} achieved the total synthesis of salicylihalamides A and B, by preparing benzolactone 114 either by Suzuki cross-coupling/intramolecular Mitsunobu strategy or by the intermolecular Mitsunobu/ring closing metathesis strategy. While the ring-closing metathesis approach requires eight steps (22.8\% overall yield), the Suzuki coupling/macrolactonization strategy requires nine steps but the overall yield (26.6\%) is slightly higher. As illustrated in Scheme 20, the synthesis of 114 commenced with the hydroboration of alkene 111 followed by a Suzuki cross-coupling reaction with vinyl iodide 11 which gave the trans-alkene 112. Removal of the silyl ether group followed by the cleavage of the methyl ester moiety afforded the seco acid 113, which was converted to macrolactone 114 by a Mitsunobu reaction. After conversion of the latter compound to
aldehyde 115 over four steps, the amide 101 was introduced through hemiaminal formation and formal elimination of water.

Scheme 20. Maier and co-workers synthesis of the ring-closing metathesis precursor of (-) salicylihalamide A

The convergent strategy Hanson et al.\textsuperscript{55} implemented toward the synthesis of the salicylihalamides A and B is based on the construction of the macrolactone core (Scheme 21). The synthesis of the latter stared with alcohol 81, which was obtained in 11 steps from the bicycle (\textit{R,R,Rp})-75. Addition of benzodioxinone 7 to alcohol 81 in the presence of NaH provided the functionalized aromatic alcohol 116, which was subsequently methylated to provide the RCM precursor 117 in 90\% yield. Ring closing metathesis of diene 117 with second-generation Grubbs catalyst provided the salicylihalamides macrolide 118 in an \textit{E/Z} ratio of 10:1.
Scheme 21. Hanson and co-workers synthesis of the ring-closing metathesis precursor of salicylihalamide A and B

1.6.4. Methodology related to installation of enamide side chain

In the total synthesis of (-) salicylihalamides A (1a), completed by Snider and co-workers, the dienyl enamides were incorporated through the addition of hexadienyllithium cuprate 112 (prepared in situ from ethyllithium, copper bromide dimethyl sulfide complex and acetylene) to a solution of isocyanate 113 (derived from the corresponding (E)- α,β-unsaturated carboxylic acid by acyl azide formation followed by Curtius rearrangement). The desired compound 114 was obtained in 43% yield together with two other byproducts (Scheme 22).
Scheme 22. Snider’s synthesis of model enamide side chain \(114^{49}\)

Smith and co-workers\(^{50}\) developed a similar but more efficient system for the incorporation of the salicylihalamide side chain using the electrophile \(115\) instead of the nucleophilic hexadienyl intermediate \(112\) by trapping the latter with carbon dioxide to furnish 2,4-(Z,Z)-hexadienyl acid \(115\) (Scheme 23). \(N\)-acylation of sodium salt of \(117\) (obtained by Curtius rearrangement and trapping of the resulting isocyanate with trimethylsilylethanol) with dienyl acyl chloride \(116\) provided protected salicylihalamides \(118\) in 81% yield and as a single isomer.

Scheme 23. Smith’s \(N\)-acylation of enecarbamate \(117^{50}\)
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De Brabander and co-workers\textsuperscript{59} reported an alternative pathway to the dienyl enamide via a lithium precursor instead of a cuprate species (Scheme 24). The first total synthesis of (\(+\))-salicylihalamides A features a late-stage vinyllithium addition to an isocyanate for the installation of the enamide side chain. The hexadienyllithium \textbf{124} prepared \textit{in situ} by metal-halogen exchange from \textbf{123} was added to a solution of isocyanate \textbf{125} to afford compound \textbf{126} as an inseparable mixture of (22Z)- and (22E)-isomers in 20\% yield together with a dimer resulting from the addition of the intermediate lithamid to the isocyanate \textbf{125}.

\begin{equation}
\text{CH}_2\text{Cl}_2 \quad \text{Br}_2 \quad \text{COOH} \quad \text{Br} \quad \text{COOH} \quad \text{Br} \quad \text{Br} \quad \text{NaHCO}_3, \text{DMF} \quad \text{NaHCO}_3, \text{DMF} \quad \text{TMS acetylene, Pd(PPh}_3)_4, \text{CuI, n-Pr}_3\text{N} \quad \text{SiMe}_3 \quad \text{NBS, AgNO}_3, \text{acetone} \quad \text{Br} \quad \text{BH}_3\text{-SMe}_2, \text{Et}_2\text{O} \quad \text{cyclohexene} \quad \text{Br} \quad \text{t-ButLi} \quad \text{Et}_2\text{O} \quad \text{TBSO} \quad \text{O} \quad \text{N}=\text{C}=\text{O} \quad \text{Me} \quad \text{OTBS} \quad \text{125} \quad \text{124} \quad \text{TBSO} \quad \text{O} \quad \text{Me} \quad \text{OTBS} \quad \text{126}
\end{equation}

Scheme 24. \textit{De Brabander’s hexadienyllithium addition to the isocyanate} \textbf{125}\textsuperscript{59}

Unlike the salicylihalamides that have an amide side chain that terminates in an \(\alpha,\beta\)-unsaturated alkyl chain, oximidines have an amide side chain that terminate in \(\text{O}\)-methyloxime. Kitahara’s group\textsuperscript{60} described a strategy for an efficient stereocontrolled introduction of the (\(Z\))-enamide side chains of oximidines (Scheme 25). Exposure of the
known alcohol 127 to n-butyllithium followed by addition of iodine and manganese dioxide gave the aldehyde, which was subsequently transformed into the corresponding O-methyloxime 128 by treatment with O-methylhydroxylamine hydrochloride. Completion of 130, the vinyl lithium coupling partner of (Z)-acyl azide 133, was achieved in two steps. Treatment of a solution of isocyanate 133 in toluene with reagent 130 allowed for the assembly of the two fragments providing the desired (Z)-enamide 134.

Scheme 25. Kitahara’s synthesis of the model oximidine side chain 134

One year later the same group reported a facile method for stereoselective synthesis of (Z)-enamides, particularly, indolic enamide compounds, when they accomplished the total syntheses of coscinamides, chondriamides and igzamide. The synthesis of the key intermediate 138 of both Chondriamide A and C began with the protection of the aldehyde 135 as the Teoc-carbamate by using Roush’s method to afford 136 (Scheme 26). After condensation of the latter with malonic acid the (E)-N-Teoc-3-indole-acrylic acid 137 was converted to N-Teoc-alkenylcarbamate 138 in 85% yield over three steps. Treatment of
138 with NaHMDS in THF, followed by addition of acid chloride 139 in THF gave the desired enamide 141 in 82% yield with retention of the configuration. Deprotection of the Teoc group with TBAF in THF provided chondriamide A (142).

Chondriamide C (145) was also synthesized from the key intermediate 138 (Scheme 26). Irradiation of 138 with a high-pressure mercury arc lamp produced a mixture of the (Z)- and (E)-alkenylcarbamates from which the (Z)-enamide 143 was isolated and subjected to NaHMDS, followed by addition of 139 to afford 145 in 79% yield with retention of the (Z)-configuration.

Scheme 26. Kitahara’s stereoselective synthesis of (Z)-enamide 145
Rizzacasa and co-workers\textsuperscript{62} applied a similar approach to the synthesis of the (+) Crocacin D by using NaH and phosphoryl azide to provide the N-acylazide 148 with minimal isomerization of Z-alkene; this is then followed by a Curtius rearrangement to give vinylisocyanate 149 (Scheme 27).\textsuperscript{63} Treatment of the latter compound with trimethylsilylethanol 150 provided the enecarbamate 151. Reaction of the anion derived from 151 with acid chloride 152 led to the protected enamide 153 in 32\% yield.

Scheme 27.  \textit{Rizzacasa’s stereoselective synthesis of enamide 153}\textsuperscript{62}

A convenient route to macrolides containing unsaturated enamide side chains is reported by Taylor\textsuperscript{64} and co-workers in an effort to synthesise the naturally occurring enamides Lansamide-I and Lansiumamides A and B. The synthesis of Lansamide-I (159) is outlined in Scheme 28. This makes use of the cinnamic acid 154, which is converted to adduct 158 by addition of styryl Grignard 157 to the isocyanate 156.
Scheme 28. Taylor’s synthesis\textsuperscript{64} of lansamide I

Under similar conditions as before, \((Z)\)-cinnamic acid did not afford the desired \((Z)\)-vinyl enamides and the only product that was observed was the isomerised \(E\)-adduct 158. To prevent the unwanted vinyl isocyanate isomerization, Taylor’s group has explored the installation of a protecting group at the \(\alpha\)-position of \((Z)\)-cinnamic acid in an effort to maintain the stereochemistry as in 160 (Scheme 29). Reaction of the acid 160 with DPPA provided the acyl azide, which was converted into vinyl isocyanate. Addition of the styryl Grignard reagent 157 afforded a 1:1 mixture of \((E)\)- and \((Z)\)-vinyl enamides, of which the latter was used for the preparation of Lansiumamides A (163) and B (164).

Scheme 29. Taylor’s stereoselective synthesis\textsuperscript{64} of lansiumamide A and B
1.6.4.1 Synthesis of unsaturated 2,4-(Z,Z)-heptadienoic amide

Scheme 30. Synthesis of the (Z,Z)-heptadienamide from pentenoic acid

Scheme 31. Synthesis of the (Z,Z)-heptadienamide from 1,2-dibromobutane

Scheme 32. Synthesis of the (Z,Z)-heptadienamide from 1-bromobut-1-yne

Scheme 33. Synthesis of the (Z,Z)-heptadienamide from propionaldehyde
Several examples of the synthesis of the highly unsaturated (Z,Z)-amide 101 (Schemes 30 to 32) were reported in 2004 by Maier and Bayer.\textsuperscript{65}

Their first approach to amide 101 started with bromination of trans-α,β-unsaturated carboxylic acid 167 which was converted to 2,3-dibromopentanoic acid followed by microwave irradiation to afford the corresponding (Z)-vinyl bromide 168 in excellent yield and high (Z)-selectivities.\textsuperscript{66} The metallation of 168 with lithium to the vinyl lithium intermediate, followed by transmetallation with ZnCl\textsubscript{2} afforded the precursors for the construction of the (Z,Z)-configurated ester 169. Aminolysis of the ester group provided the amide 101 in low yield (36%).

The second method is a variation of the Fürstner strategy (Scheme 31).\textsuperscript{67} First, the Z-iodoacrylate 166 was prepared by the reaction of lithium iodide with 2-propynoate (165) in acetic acid with both the stereoselectivity and yield depending on the reaction temperature.\textsuperscript{68} Cross-coupling reaction of butynylzinc (prepared from 171) with the (Z)-methyl 3-iodoacrylate 166 followed by Lindlar reduction of the enyne 172 gave Z,Z-diene ester 169 in high yield. Finally, aminolysis of 169 converted it to amide 101 in low yield (36%) but changing the order of events (aminolysis of the ester 172 followed by Lindlar hydrogenation) dramatically improved the yield of the desired amide 101 to 80%.

The last approach to amide 101 by Maier and Bayer began with a methanol solution of the propiolamide 175 (prepared from methyl propiolate 174)\textsuperscript{69} that was coupled in the presence of CuCl with 1-bromobut-1-yn-1 providing the diyne 171 in 86% yield (Scheme 32). Lindlar hydrogenation of the latter provided (Z,Z) amide 101 in 80% yield.

Fürstner’s synthesis makes use of the Z-iodoacrylate 166 which was converted into the enyne 172 using a Negishi coupling reaction with butynylzinc chloride in catalytic condition (Scheme 34). Lindlar reduction followed by aminolysis of the resulting ester 169 yielded the required amide 101 (62%).
Scheme 34. Fürstner’s synthesis of 2,4-(Z,Z)-heptadienoic amide

Labrecque et al. reported an alternative pathway to the amide 101 by using alcohol 180 as the starting material (Scheme 35). Oxidation of the primary alcohol 180 with TPAP in the presence of NMO provided the unsaturated aldehyde 181 that was subjected to Horner–Emmons coupling reaction to afford the (Z,Z)-configured ester 169 in 73% yield. The resulting ester was then hydrolyzed using barium hydroxide followed by amidation via mixed anhydride to afford amide 101 in 63% yield.

Scheme 35. Labrecque’s synthesis of 2,4-(Z,Z)-heptadienoic amide
1.7 Installation of the enamide side chain

1.7.1 Condensation of aldehydes and amides

The strategy used by Maier’s group\(^5\) in their total synthesis of salicylihalamides involved the elimination of water from a N-acylhemiaminal to give the enamide functionality, a reaction that usually gives mixture of isomers. Treating aldehyde 183 with aluminum carboximidoate, derived from amide 101 and DIBAL, gave the hemiaminal 184 which underwent elimination of water upon treatment with acetic anhydride and pyridine to afford the desired \((E)\)-enamide 185 in 45% yield along with 14% of the \(Z\)-isomer (Scheme 36).

![Scheme 36. Preparation of enamide via Maier’s approach\(^5\)](image)

Starting with the same precursor but using different reaction conditions, Labrecque and co-workers\(^4\) installed the enamide side chain using the condensation of an excess of amide 101 with aldehyde 186 which provided the key intermediate \(N,N'\)-bis-acylated aminal (incorporating two side chain residues). The corresponding intermediate 187 underwent elimination upon treatment with sodium hydride in trifluorotoluene at 60 °C to give a mixture of enamides 188a and 188b in 18% yield (Scheme 37).
1.7.2 Copper-mediated coupling reactions

Enamides are key structural features in a number of natural products. In addition to conventional approaches for their preparation, which has been mentioned previously in sections 1.5.4.1 and 1.5.4.3, a number of transition metal-catalyzed methods for the syntheses of enamides are known. However, these protocols often suffer from either low yields or are unable to control double bond stereochemistry. For these reasons, the copper-catalyzed coupling of amides with vinyl halides appears to be widely applicable procedures in the total synthesis of natural products. This section is not intended to be exhaustive as it focuses on only a number of general and widely applicable procedures.

The basis of today’s developments of copper-mediated C-N, C-O, and C-C bond formation reactions was first reported more than a hundred years ago in the pioneering work of Fritz Ullmann and Irma Goldberg. In 1901 Ullmann reported the use of o-
Bromonitrobenzene for the synthesis of the corresponding biaryl 190 by direct coupling of two molecules of 189 in the presence of metallic copper (Scheme 38).\textsuperscript{70}

\[
\begin{array}{ccc}
\text{Br} & \text{O}_2\text{N} & \text{Cu} \\
\text{O}_2\text{N} & \text{Br} & \text{Cu} \\
\end{array}
\xrightarrow{210-220 ^\circ\text{C}}
\begin{array}{ccc}
\text{O}_2\text{N} & \text{Br} & \text{Cu} \\
\text{O}_2\text{N} & \text{Br} & \text{Cu} \\
\end{array}
\]

Scheme 38. Ullmann reaction

The first copper iodide-catalyzed coupling reaction between vinyl bromides and potassium amides was successfully reported by Ogawa and co-workers\textsuperscript{71} (Scheme 39) in which enamides products were obtained in low to moderate yields by using stoichiometric amounts of copper(I) in HMPA at 130 °C.

\[
\begin{array}{ccc}
\text{Br} & \text{C} & \text{NH}_{3} \\
\text{R} & \text{NH} & \text{R} \\
\end{array}
\xrightarrow{\text{CuI (1.0 equiv)}}
\begin{array}{ccc}
\text{Br} & \text{C} & \text{NH} \\
\text{R} & \text{NH} & \text{R} \\
\end{array}
\]

Scheme 39. Cu(I)-catalyzed C-N bond construction by Ogawa \textit{et al.}\textsuperscript{71}

Based on this precedent, Porco and co-workers\textsuperscript{72,73} developed a copper-mediated amide vinylation method that occurred at mild temperatures and was suitable for the installation of potentially labile enamides. After screening a variety of catalyst systems, bases and solvents, they found that using the Liebeskind catalyst, copper(I) thiophene carboxylate (CuTC \textsuperscript{193}), cesium carbonate as base, and terminal (E)-vinyl iodides in polar aprotic solvents (NMP or DMSO) at 90 °C, a series of (E)-enamides could be prepared as shown in Scheme 40. Higher conversion (>95\%) was obtained when the catalyst loading was increased to 30 mol % and the reaction mixture was rigorously degassed.

As an extension of this methodology, the copper-catalyzed cross coupling process provided a guide for the design of another catalytic system based on ligand \textsuperscript{194}, Cu(CH\textsubscript{3}CN)\textsubscript{4}PF\textsubscript{6}
and Rb$_2$CO$_3$ in DMA (Scheme 40)\textsuperscript{74} starting from amides 195 and (E)-allyl 3-idoacrylate 196 or (E)-N-benzyl-3-idoacrylamide 197.

\begin{align*}
\text{195} & \quad + \quad \text{I} \quad \text{193} \\
\begin{array}{c}
\text{CuI, Cs$_2$CO$_3$, NMP, 90 °C} \\
10 \text{ examples, 36-75% yield}
\end{array}
\end{align*}

\begin{align*}
\text{195} & \quad + \quad \text{I} \quad \text{196} \\
\begin{array}{c}
\text{Cu(\text{CN})$_2$PF$_6$, Rb$_2$CO$_3$} \\
\text{DMA, 45 °C} \\
11 \text{ examples, 7-92% yield}
\end{array}
\end{align*}

Scheme 40. CuTC-Catalyzed coupling of vinyl iodides and amides\textsuperscript{74}

Buchwald and co-workers\textsuperscript{75} have also reported the formation of enamides under the mild conditions and developed an experimentally simple and inexpensive catalytic system based on the use of 1,2-diamine ligand 198 (Scheme 41), K$_2$CO$_3$ or Cs$_2$CO$_3$ as base and substituted vinyl iodides 199 and bromides 200. This operationally simple protocol uses Cs$_2$CO$_3$, CuI as the catalyst and 198 as ligand in THF at temperatures ranging from 50 to 70 °C when vinyl iodides 199 are used as substrates, whereas vinyl bromides 200 require the use of potassium carbonate in toluene at 110 °C.

\begin{align*}
\text{198} & \quad \text{MeHN} \\
\begin{array}{c}
\text{Cul, THF or Cs$_2$CO$_3$} \\
\text{50-110 °C}
\end{array}
\end{align*}

Scheme 41. Formation of enamides under Buchwald’s conditions\textsuperscript{75}
In 2004, Coleman and Liu\textsuperscript{76} reported a unified strategy for the divergent and stereocontrolled introduction of the \((E)\)- and \((Z)\)-enamide side chains of oximidines I-III, salicylihalamides A and B, CJ-12,950, lobatamides A and D, all starting from vinyl iodides \((E)\)-202 and \((Z)\)-202 that can be prepared from isovaleraldehyde by Takai or Stork-Zhao olefination, respectively. Their strategy involved the use of a common intermediate (the cyclic hemiaminal 203) to prepare diverse members of a family of natural products (Scheme 42).

A stereospecific copper-promoted C-N coupling of \((E)\)-202 and \((Z)\)-202 vinyl iodides with a protected maleimide hemiaminal 201 followed by deprotection gives the key intermediate 203. Reaction of the resulting \((E)\)- or \((Z)\)-enelactam hemiaminals with \(O\)-methylhydroxylamine afforded the corresponding ring-opened \(O\)-methylloxime ether \((Z)\)-

\textbf{Scheme 42.} Strategy for the divergent and stereocontrolled introduction of the \((E)\)- and \((Z)\)-enamide side chains of family of natural products\textsuperscript{76}
205 (78%), characteristic of oximidine I and II, and (E)-205 (71%), characteristic of oximidine III, lobatamides, and CJ-12,950 while cis-selective Wittig olefination of (E)-203 and (Z)-203 using propylidenetriphosphorane afforded (E)-206, characteristic of salicylihalamide A, and (Z)-206, characteristic of salicylihalamide B in high yields (Scheme 43).

Scheme 43.  Ring opening of the hemiaminals with O-methylhydroxylamine or propylidenetriphenylphosphorane.

With the development of new and efficient catalytic system that enable reactions to be conducted in mild conditions, the copper(I) thiophenecarboxylate mediated substitution of vinyl iodides with amides, has became a valuable strategy for the preparation of enamides. The utility of this synthetic strategy was later demonstrated in a
total synthesis of Lobatamide C \textbf{210} (Scheme 44).\textsuperscript{77} The key step featured a Copper(I) thiophenecarboxylate (CuTC, \textbf{193})-mediated vinylic substitution of \textbf{208} with (\textit{E})-O-methyloxime amide \textbf{207} with base and additives and led to a 52\% yield of the C1-C10 enamide fragment \textbf{209}, along with 10\% of (\textit{Z})-oxime stereoisomer.

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\includegraphics[width=\textwidth]{example.png}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 44. Porco’s synthesis of lobatamide C}\textsuperscript{77}

It has been reported by Porco \textit{et al.}\textsuperscript{78} in two separate publications the installation of the side chain of oximidines II \textbf{213} and III \textbf{215} by using a modified, ligand-assisted system (Scheme 45). Amidation of (\textit{Z})-vinyl iodide \textbf{211} with oxime amide \textbf{212} using standard conditions led to low yield due to competitive elimination under basic conditions, whereas the use of an additional ligand (\textit{N,N}'- dimethylethlenediamine \textbf{198}) allowed for a smooth coupling reaction. Oximidine II \textbf{213} was secured after desilylation in 44\% yield and with high stereospecificity. Treatment of a 1/1 mixture of vinyl iodide isomers \textbf{214} with oxime amide \textbf{212} under the same conditions, gave after 1h a mixture (\textit{E}:\textit{Z}=7:1) of the required oximidine III in 45\% yield. Particularly in the latter case, extended reaction times resulted in decomposition of \textbf{215} and a higher yield of \textit{Z} enamide.
The intermolecular cross-coupling reaction using Liebeskind’s promoter CuTC 193 was next extended by the Nicolaou\textsuperscript{79} and Panek\textsuperscript{80} groups to the preparation of apicularen A (218). Su and Panek installed the highly sensitive enamide side chain at a late stage, using Porco’s protocol for the CuTC catalyzed substitution of vinyl iodide 211 with amide 101, which proceeded in 40% yield at 58 °C (Scheme 46). It was revealed that the reaction temperature and ligand-base combination were, as it is in a lot of cases, an important factor for the success of this coupling reaction.

A similar catalytic system was used in Nicolaou’s synthesis when ligandless conditions proved to be more efficient for vinylation of amide 101 with (E)-vinyl iodide 219, which provided the desired apicularen precursor 220, with 90% yield at 50% conversion (Scheme 46).
Scheme 46. Synthesis of apicularen A by Panek and Nicolaou groups.\textsuperscript{79,80}
1.8 Structure-activity relationships of benzolactone enamide family

1.8.1 Salicylihalamide enamide analogues and biological activity

Salicylihalamide A, an interesting cytotoxic natural product, is a potent inhibitor of both vacuolar ATPase and proton pumps (IC$_{50}$ below 1 nM)\textsuperscript{81} which are highly expressed in metastatic cancer cells\textsuperscript{82} where they modulate pH. De Brabander and co-workers\textsuperscript{83} have shown that the inhibition site(s) of salicylihalamides is within the membrane-bound V$_0$ sector of the V-ATPase where the inhibition is caused by blocking of the V$_0$ proton channel through a mechanism distinct from concanamycin or bafilomycin. Evidence suggests\textsuperscript{84} that inhibition of these proton pumps eventually leads to cell deaths via apoptosis. In the course of their research to study the binding site of salicylihalamides and its mechanism, De Brabander’s group\textsuperscript{85} synthesized various analogues to perform some structure-activity relationship studies (SAR), identifying some structural characteristics that influence cytotoxic activity. The mechanism for the irreversible inhibition of the V-ATPase proposed by De Brabander’s group is based on the N-acyliminium ion \textsuperscript{222} generated from N-acyl enamine \textsuperscript{221} under acidic conditions (Figure 5). The iminium ion \textsuperscript{222} could then react with a nucleophilic amino acid side chain within the V-ATPase. The complex formed is then fragmented to a covalent protein \textsuperscript{226} with loss of the side chain \textsuperscript{227}. 
Some representative examples of the salicylihalamide and apicularen side chain modified analogues developed by De Brabander, Nicolaou\cite{86} and Maier are shown in Figure 6.

Based on extensive screening studies of systematically modified derivatives of salicylihalamide, it was revealed that side-chain-modified analogues 228-232 (Figure 6) all retain the ability to inhibit V-ATPase activity at concentrations similar to those of the parent compound with irreversible inhibition of the enzyme. The saturated hexane side chain analog 228 is almost equally as potent as salicylihalamide A but is ten-fold less active against the SK-MEL-5 melanoma cancer cell line. These studies also provided evidence that the hexadienoyl moiety did not affect the activity dramatically. When replacing the Z,Z-heptadienylamide with either 229 or 230 the analogues retained most of their activity. Additionally, it could be seen that the IC$_{50}$ values increased with increasing the size and the bulkiness of the side chain, shown by the analogues 235-238.
The structure-activity study of salicylihalamide analogues showed that the enamide subunit is responsible for potent inhibitory activity. Changing the \(N\)-acyl enamine functionality resulted in a significant loss of cytotoxicity as shown by analogs 233 and 235, the latter being more than 1400 times less active than salicylihalamide A. It should be noted, however, that the enamide is important, but that modifications on the hexadienoyl fragment are tolerated up to 6-7 carbon atoms.

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**Table 1.** Cytotoxicity values (IC\(_{50}\), nM) of several benzolactone enamides.

A structure-activity study showed that the apicularen A, a highly cytostatic 12-membered macrolide, shares an identical, acid labile, \(N\)-acyl enamine side chain with salicylihalamide A. With respect to the important role of the enamide subunit, several analogues of apicularen A have been prepared and then tested against various cell lines, and the corresponding IC\(_{50}\) values are listed in Table 1 and Figure 6.
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**IC<sub>50</sub>**

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**IC<sub>50</sub>**

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<tr>
<td>5</td>
<td>500&lt;sup&gt;84&lt;/sup&gt;</td>
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<td>6</td>
<td>60&lt;sup&gt;84&lt;/sup&gt;</td>
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<td>7</td>
<td>450&lt;sup&gt;84&lt;/sup&gt;</td>
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<td>8</td>
<td>900&lt;sup&gt;84&lt;/sup&gt;</td>
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<td>9</td>
<td>&gt;20x10&lt;sup&gt;3&lt;/sup&gt;&lt;sup&gt;84&lt;/sup&gt;</td>
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<tr>
<td>10</td>
<td>1.7&lt;sup&gt;83&lt;/sup&gt;</td>
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<td>11</td>
<td>24&lt;sup&gt;83&lt;/sup&gt;</td>
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<tr>
<td>12</td>
<td>250&lt;sup&gt;84&lt;/sup&gt;</td>
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<tr>
<td>13</td>
<td>805.5&lt;sup&gt;84&lt;/sup&gt;</td>
<td></td>
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</tbody>
</table>

**Figure 6.** Salicylihalamide and apicularen enamide analogues and biological activity
Furthermore the crucial role of the enamide moiety was confirmed by the loss of inhibitory activity of melanoma cell line SK-MEL-5 with analogues 240 and 246, while inverting the stereochemistry in the enamide olefin (247) resulted in a 100-fold loss of potency, respectively (Figure 6). Similarly as with salicylihalamide A analogues, substitution of the hexadienyl moiety with short aromatic chains (250 and 251) showed weak cytostatic affects relative to their congeners, whereas those analogues (248 and 249) with comparable side chain as that of apicularen A showed significant potency for cell inhibitions.

A careful analysis of a series of simplified analogs (252 and 261) reveals the importance of the macrolactone core for the biological function of salicylihalamide A (Figure 7). Blocking of either the phenolic hydroxy group (256) or the hydroxyl group on the macrolactone core (257) led to a decrease of activity by 60-fold and respectively by 100-fold compared to that of the parent compound, suggesting the important role of the hydrogen-bonding in the enzymatic interaction between salicylihalamide and V-ATPase.

Smith and co-workers synthesized two modified macrolactone analogues (252 and 253) and their results showed that while the removal of OH at C13 as well as the methyl group at C12 (252) had a moderate effect on the GI50 values (10-fold decrease from prostate cell-line DU-145), removal of the double bond reduced the activity slightly on several cell lines. They suggested that the removal of substituents from the lactone ring and the C-9 double bond might be important but not critical for the biological activity.

Following this work, a number of other simplified salicylihalamide analogues were synthesized in an attempt to evaluate whether the V-ATPase is a molecular target for cancer therapy. The results showed that fluorinated analog of salicylihalamide A (258) acted as a potent inhibitor of V-ATPase (IC50=2 nM), the truncated analogue 259 had only modest activity (1000-fold less active than the parent natural product), while the
macrolactone ether analogue 261 retained inhibition similar to the natural product but was weakly cytotoxic.

Figure 7. Salicylhalamide core analogues and biological activity
It can be concluded that none of the new analogues identified were more potent than the parent compound, however the minimum structural requirements for cytotoxic activity have been identified. The key elements necessary for a biological activity include:

- changing the N-acyl enamine functionality attenuates the biological activity, but without the enamide subunit there is no inhibition of the V-ATPase protein;
- the macrolactone ring is important and minor modifications in the aliphatic area cause only a small loss of activity;
- the removal of the hydroxyl group on the macrolactone at C13 as well as the methyl group at C12 attenuates the biological activity;
- the macrocyclic double bond is important but is not crucial for cytotoxicity;
- the free phenolic hydroxyl group and the chiral center at C15 are essential for the cytotoxicity.
Chapter Two:

Results and Discussion-

Synthesis of a 15-aza-Salicylihalamide A

Analogue
2.1 Initial considerations

In the course of ongoing investigations aiming at new drug candidates, we hypothesized that a new modification of salicylihalamide (1a) would be to change the 12-membered macrolactone to a more stable macrolactam as in the 15-aza-salicylihalamide A analogue 301, that would be an interesting target for synthesis and biological evaluation.

Is the lactam linkage less susceptible to cleavage than the lactone of the natural product? Would it impart stability to the macrolide system, possibly increase \textit{in vivo} activity as was observed for the aza-epothilone?

The majority of SAR studies have been conducted on salicylihalamide derivatives with modified enamide side chains and it has been shown that the presence of some form of enamide functionality is critical for activity.

What changes in the macrolactone are tolerated without substantial loss of cytotoxic activity?

All these questions attracted our interest and we have launched into the synthesis and biological studies of the lactam analogue 301 of salicylihalamide A.
2.2 Retrosynthetic analysis

A concise examination of the structure of compound 301 reveals a 12-membered salicylate macrolactam ring, an unusual N-acylated enamide side chain at C15 and an E-endocyclic olefin at C9 as interesting structural motifs that need special attention from a synthetic point of view due to their stereochemical features.

Given these considerations, and some interesting structural issues which we hoped to address, we planned to utilize late-stage copper(I)-mediated cross-coupling of (E)-vinyl iodide derived from intermediate 265 with the known amide 101 to attach the enamide side chain (Figure 8). The macrolactam could be synthesised via a ring-closing metathesis reaction (RCM) conducted on diene 290. The critical amide bond could then be secured by a photochemical acylation between known dioxinone 266 and amine 285. This reaction involves the photolysis of 266, which produces an intermediate reactive quinoketene that is trapped by amine 285 to afford the amide 290. Amine 285 would be constructed from optically pure L-aspartic acid 278.

![Figure 8. Retrosynthetic analysis of 15-aza-salicylihalamide A](image-url)
Our proposed retrosynthetic approach for analogue 301 ultimately led to the alkene 266. The first part of the project therefore involves the synthesis of this intermediate for coupling to the amine 285.

2.3 Synthesis of terminal alkene fragment 266

Following the synthetic pathway illustrated in Scheme 47 we began the synthesis of the alkene fragment from commercially available and inexpensive 2,6-dihydroxybenzoic acid 262. Initially we considered two protecting groups (acetone and benzophenone), which concomitantly mask both the carboxylic acid and one of the free hydroxyl moieties. It was anticipated that at this stage, the two benzodioxinones (265 and 266) would possess sufficient variation in reactivity to allow them to be photolysed in the presence of amine 285. As shown in the following section (section 2.5) using benzophenone proved to be a good choice.

```
[a] benzophenone, SOCl₂, DMAP, DME, 44%; [b] Tf₂O, CH₂Cl₂, pyridine, 96%; [c] LiCl, Pd(PPh₃)₄, allyltributyltin, DMF, 74%
```

Scheme 47. A linear synthesis of alkene 266
Following a modified Hadfield’s experimental procedure,\(^{87}\) the synthesis of the 2,2-diphenyl 1,3-dioxin-4-one (263) was attempted. In our hands, initial attempts at this reaction gave the benzodioxinone 263 in very low yield (less than 20\%). Allowing the reaction to stir for one hour upon completion of the addition of the reagents, as described in the reported procedure, had little effect on the conversion of symmetrical acid 262 to 263 and purification of the latter by sublimation was found to be very difficult. A superior procedure, however, involved maintaining the anhydrous condition more efficiently and performing two recrystallizations from ethanol to provide a highly pure product not requiring chromatographic purification. A satisfactory yield of 44\% was achieved. This benzodioxinone was then allowed to react with triflic anhydride and pyridine in dichloromethane, whereby the remaining hydroxyl group was converted to the corresponding triflate 264 in 96\% yield.

Next, we directed our attention towards the substitution of an allyl group for the triflate group using a Stille-type reaction.

The Stille reaction\(^{89}\) involves the palladium-catalysed coupling of organotin reagents with a large variety of electrophiles and is now in widespread use in organic synthesis due to the stability to air and moisture of organostannanes and their compatibility with virtually any functional group. Various organic electrophiles can be used but halides and triflates are the most common due to their excellent leaving group properties. Typical palladium sources which have been used in such coupling reactions include Pd(PPh\(_3\))\(_4\), Pd\(_2\)(dba)\(_3\), Pd(OAc)\(_2\), PdCl\(_2\)(PPh\(_3\))\(_2\), PdCl\(_2\)(PhCN)\(_2\), PdCl\(_2\)(MeCN)\(_2\) with the most common being tetrakis(triphenylphosphine)palladium, which has shown excellent activity and functional group tolerance.\(^{90}\) The Stille reaction is complex and can follow different pathways depending on the solvent, additives (e.g. LiCl) and the nature of the substrates involved.
At this point it would be useful to gain some insight into the mechanism of the Stille reaction before attempting an intermolecular coupling reaction between the precursor 264 and allyltributyltin.

The generally accepted catalytic active complex involved in the Stille reaction is believed to be the 14-electron Pd(0) species L₂Pd⁰ (Scheme 48). The initial step is the oxidative addition of the catalyst to the triflate 264 in which the organopalladium intermediate 264 a is formed, followed by transmetalation that has been established as the rate-determining step. To increase the reaction rate at which this process occurs, LiCl or Cu(I) salts are added. Finally, a reductive elimination reaction gives the coupled product 266 and regenerates palladium(0) species to complete the catalytic cycle.

Scheme 48. General mechanism of the Stille cross coupling reaction

Catalyst: Palladium Source: Pd(PPh₃)₄ or Pd₂(dba)₃
L= Ligand
S= L or solvent
Taking advantage of the significant Stille methodology, we also wanted to take the opportunity to move away from THF, and run the reaction between the triflate 264 and allyltributyltin in DMF, a polar solvent that has been shown to promote reactions where moderately-polar THF had failed.\textsuperscript{92} Thus, the triflate 264 was allowed to react in a Stille fashion with allylstannane in the presence of catalytic Pd(PPh\textsubscript{3})\textsubscript{4} and LiCl in DMF at 80 °C to afford terminal olefin 266 in 74% yield.

Despite the versatility and synthetic utility of organotin reagents, a significant drawback of the Stille cross-coupling reaction is the difficulty in removing the tin by-products from product mixture. In an effort to circumvent the problem associated with removing organotin impurities, we identified KF as a potential alternative to access the pure product.\textsuperscript{93} The incorporation of finely ground potassium fluoride into slurry silica gel used as a stationary phase for chromatographic purification of product mixture containing tin residues and a second chromatographic purification led to the isolation of the product in a high state of purity (Figure 9).

Some product may have been lost during each purification step but it is of particular interest to note that under these conditions the desired product was easily separated from the organotin residue, as verified by the \textsuperscript{1}H NMR spectra.

![Figure 9. 1H NMR spectra of alkene 266: a) after KF-silica column and b) before silica column](image-url)
2.4 Synthesis of amine fragment 285

2.4.1 Model study towards the synthesis of primary amine

Initially, the most pressing research issue to be addressed was whether or not our key photochemical reaction would be feasible for an acylation coupling between chiral amine 285 and dioxinone 266 to form the amide 290 using a reported procedure.\textsuperscript{98}

Before attempting the photoacylation of our authentic substrates, we decided to carry out a simple model study on a simpler amine. Although, later on, this amine had to be abandoned because it lacked the important chirality, it did help to trial the reaction conditions and to illustrate a few synthetic steps that were later incorporated into the synthesis of the genuine amine.

For the preparation of a model amine 277, the first key step involved protection of the commercial 3-butene-1-ol (267) as its p-methoxybenzyl (PMB) ether 270 as shown in Scheme 49.
Synthesis of model amine substrate 277

The mono-PMB-protected olefin 270 was then smoothly and efficiently epoxidized with mCPBA to secure the epoxide 271 as a mixture of diastereoisomers (89% yield), that were inseparable on silica gel. Ring opening of 271 with the Grignard reagent 273 provided the alcohol 274 in 80% yield.\textsuperscript{50} Having obtained the desired precursor for the synthesis of

\textbf{Reagents and conditions:}\,[a] HBr 48%, rt, 1h, 96%; [b] NaH, THF, 90%; [c] CH\textsubscript{2}Cl\textsubscript{2}, mCPBA, rt, 89%; [d] Et\textsubscript{2}O, I\textsubscript{2}; [e] Li\textsubscript{2}CuCl\textsubscript{4}, THF, 80%; [f] MsCl, NE\textsubscript{t}\textsubscript{3}, Et\textsubscript{2}O; [g] NaN\textsubscript{3}, DMF; [h] PPh\textsubscript{3}, DIAD, DPPA, THF, 0\textdegree C; [i] PPh\textsubscript{3}, H\textsubscript{2}O; [j] PMe\textsubscript{3}, THF, H\textsubscript{2}O.
amine fragment 277, efforts were focused towards the final product at this stage. The initial, motivating retrosynthetic thinking regarding the construction of the amine centered on the use of an azidation reaction. Two options were generally considered. Provided that an azide species could be generated from an alcohol in the presence of DPPA (an alternative to hydrazoic acid), DIAD and PPh$_3$ using Mitsunobu conditions, a relatively easy reduction process of the azide group could then be used to form the amine 277 (Scheme 49, method b).

In the event that this reaction was not feasible, an alternative was to take advantage of the known ability of alcohols to be activated, followed by nucleophilic displacement of an oxy anion by azide and subsequent reduction would constitute a route for the synthesis of the target amine (Scheme 49, method a).

Both methods allow for efficient preparations of amines: whereas the mesylation reaction seems to be a little bit more efficient because the crude mesylate can be used directly for the next step without further purification, the Mitsunobu approach was shown to have technical difficulties in the separation and purification of 276 before the azide group reduction to the amine.

The first approach (method a) is one step longer, but is both more efficient and reproducible with excellent yields for each step. Importantly, both strategies come together to a common amine precursor 276, adding flexibility to the synthesis.

The alcohol 274 was then converted to azide 276 via a mesylation and azide displacement sequence. Mesylation of the alcohol using MsCl and triethylamine in the presence of diethyl ether, afforded the mesylate in 94% yield and its presence confirmed by the singlet at 2.95 ppm in the $^1$H NMR (Appendix B, page 146), corresponding to the methyl protons of the sulfonate group. Displacement of the mesylate using sodium azide in DMF afforded azide 276 in 90% yield. In the $^1$H NMR spectrum the multiplet at 3.5 ppm
moved upfield from its position in the mesylate at 4.9 (Appendix B, page 147). The observation of strong IR absorptions at 2096 and 1245 cm\(^{-1}\), corresponding to the azide group asymmetrical and symmetrical stretches respectively, confirmed the identity of the azide as 276 (Appendix A, page 135).

Next in the synthesis was the conversion of the latter azide to the target amine. Reduction of the azide to the amine is a standard process and is of considerable importance for the introduction of primary amino group in organic synthesis, a process that could be done by using a variety of reducing agents. Initially, we tried to reduce the azide group to an amine group using trimethylphosphine as an alternative reagent of the Staudinger reaction, but we could not isolate the pure compound. After intensive trials of this reaction with no success this approach was abandoned. At that stage, a variety of azide reduction methods were surveyed with essentially no success. Consequently, alternatives were sought, and we decided to revisit the Staudinger reaction, the classical pathway to the iminophosphorane, a useful intermediate for the transformation of azides to amines when the reaction is carried out in an aqueous solvent.

Gratifyingly, changing the reagent from trimethylphosphine to triphenylphosphine enabled amine 277 to be isolated in 65% yield after 3h at 70 °C.

The disadvantage of this method was the formation of the very stable triphenylphosphine oxide byproduct that was generated in the reaction, which was difficult to remove, involving multiple chromatographic purifications. In an effort to circumvent this problem associated with this undesired byproduct, an extraction of the amine into aqueous HCl was sought. Two extractions with aqueous hydrochloric acid were necessary to recover more than 93% of amine 277 before neutralization and extraction into the organic layer. Concentration under reduced pressure gave amine product as an oil in a high
state of purity (65% yield). $^1$H NMR, $^{13}$C NMR, HRMS and IR spectroscopy analysis supported the structure of amine 277.

### 2.4.2 Synthesis of chiral amine 285

The favorable outcome of our model study was encouraging. Having established an effective route to the requisite amine 277 and based on the insights gained from this model, we were prepared to test our model on the genuine substrate 278. To that end, we decided to use $L$-aspartic acid as a template for our synthesis that would allow us not only to reduce the number of steps, but also as a means of introducing the chirality with greater efficiency into 285.

The synthesis of the chiral amine required for the production of amide 286 began with commercially available $L$-aspartic acid 278, which was converted to bromosuccinic acid 279 using sodium nitrite in the presence of potassium bromide and sulfuric acid (Scheme 50).

![Scheme 50. Synthesis of diacid fragment 279](image)

Reduction of the diacid with borane dimethylsulfide complex afforded bromodiol 280 in 88% yield. One-pot intramolecular cyclisation of the diol using NaH in THF afforded the corresponding epoxide, followed by the protection of the hydroxyl group as a PMB ether (Scheme 51).
Applying the previously model study conditions, exposure of the enantiopure epoxide 281 to the Grignard reagent (derived from 5-bromo-pent-1-ene) and Kochi’s catalyst (Li$_2$CuCl$_4$) in THF led to an efficient ring opening of the epoxide providing alcohol product 282 in good yield (Scheme 52).

Scheme 51. Synthesis of epoxide fragment 281

Scheme 52. Synthesis of alcohol 282

A drawback of this synthesis is the use of the 5-bromo-pent-1-ene for the Grignard reagent, which is expensive to perform on a large scale. However, we were pleased to find that this reaction proceeded smoothly in 78% yield via attack of an in situ generated Grignard reagent on the epoxide.
To complete the synthesis of the azide, the free secondary alcohol in 282 was activated by mesylate formation (MsCl, NEt₃, Et₂O), and then converted to azide 284 (NaN₃, DMF) in 84% yield over two steps. The reaction was stereospecific and resulted in an inversion of the stereochemistry at C7 (Scheme 53) in accordance with a bimolecular nucleophilic substitution (SN₂) reaction. The strategy has led to an efficient and stereospecific synthesis of the chiral azide 284.

Scheme 53. Preparation of azide 284

Following the success of the model reduction procedure reported in Scheme 49, the Staudinger reaction proceeded smoothly furnishing the desired amine product 285 as an oil, in good yield (65%) and in a high state of purity (Scheme 54).

Scheme 54. Preparation of amine product 285

Initial analysis by TLC indicated complete consumption of the starting material, and no IR absorbance in the azide range (2096 cm⁻¹) had been observed for the product.
The IR spectrum displayed an absorbance at 3369 cm\(^{-1}\), validating the formation of the amine product 285 (Appendix A, page 136). In the \(^1\)H NMR spectrum the multiplet at 2.8 ppm moved upfield from its position in the azide at 3.5 (Appendix B, page 149).

2.5 Synthesis of amide fragment 286

The initial goal of the synthetic plan was to construct amide 286 (Scheme 56) in a stereocontrolled manner using the shortest and most efficient route possible. Before attempting the acylation of our authentic substrates, we decided to carry out a simple model study on 2,6-dihydroxybenzoic acid and different amines. To this end, we decided to investigate the possibility of protecting the phenolic hydroxyl groups or activating the acid as an acyl chloride followed by synthesis of the amide product (Figure 10).

\[
\begin{array}{c}
\text{OR} \\
\text{R} = \text{H, -OCOCH}_3 \\
\text{R}_1 = \text{-OCH}_3, \text{Cl} \\
\text{R}_2 = \text{-CH}_2(\text{CH}_3)_4\text{CH}_3, \text{-CH}_2\text{CH}_3, \\
\text{-(CH(CH}_3)_2)_2 \\
\end{array}
\]

Figure 10. Various approaches to the amide product

Initial attempts to construct the amide bond were explored by starting from 2,6-dihydroxybenzoic acid and using different conditions with model substrates (Table 2). It should be noted that this acid is relatively unreactive due to steric hindrance exerted by two groups in the ortho position.
Table 2. Attempted salicylate amide formation

The larger part of these traditional amine-acid coupling approaches relies upon activation of the acyl carbon allowing for greater access to the electrophile by the amine nucleophile under the reaction conditions.

Our attempts to install the amide bond using traditional amine-acid coupling strategies, proved unsuccessful. These attempts to link carbonyl group and amine in the acylation event were unsuccessful most likely due to steric hindrance of the ortho ester/hydroxyl groups which retarding the approach of the amine to the carbonyl group.

Figure 11. Steric hindrance impeding the amine nucleophilic attack
These disappointing results led us to broaden our search of amidation methods to include less conventional approaches. As a result, we began to explore alternate strategies that would install the amide bond. Consequently, we elected to investigate the elegant photochemical method reported by De Brabander’s group, utilizing ketenes as useful intermediates in the synthesis of hindered ortho-substituted salicylate amides under essentially neutral conditions. In view of this finding and previous work by our group\textsuperscript{88} we decided to utilize the highly reactive quinoketene intermediate for the photochemical acylation reaction to acetonide 265 leading to the formation of salicylate amide 265 A in 12\% yield (Scheme 55).

![](image)

**Scheme 55. Photochemical acylation of the diene precursor 265\textsuperscript{88}**

The low yield of this reaction could be attributed to the poor radical stabilization by the dimethyl group in acetonide 265. To improve the radical stability and therefore the yield of this photochemical reaction, the methyl groups were replaced by the phenyl groups, generating benzodioxinone 266 (Scheme 56).
Scheme 56.  Photochemical acylation of the diene precursors

When dioxinone 266 was photolysed, the *in-situ* generated quinoketene 287 was captured by the amine 285 and the yield of 286 improved to 20% supporting the claim that the ortho-quinoketene intermediate generated from 266 was more efficient leading to the formation of the desired 286 amide product (Scheme 57).

These results add support to the original proposal\(^9\) that the quinoketene 287 is most likely formed by homolytic C–O bond fission, since an intermediate diphenylmethyl radical would be more stable than the dialkyl analogue. The proposed reaction mechanism is detailed in Scheme 57, whereby formation of the biradical 266 a and elimination of the benzophenone molecule is followed by formation of hydrogen bonded complex 287. Rearrangement of the 287 a and 287 b provided the amide product 286.
Several attempts were made to optimize the reaction conditions for this transformation, such as varying the benzodioxinone to amine ratio from 1.5:1 to 2.5:1 or changing the concentration of the reagents.

After much optimization studies it was found that the most favorable conditions for this reaction were using a reaction time of 6 h, 1.5 equivalent of benzodioxinone, a concentration of 0.04 M, in a non-polar solvent and using a quartz reaction vessel (which allows more of the light to pass through) instead of a borosilicate test tube. Employing these conditions, the conversion of the required salicylate amide 286 was raised to 55% yield. To date, this reaction remains challenging but this approach to the required salicylate amide is notably short, efficient, and scalable.

Scheme 57. Proposed reaction mechanism of photolysis via quinoketene intermediate
The IR spectrum showed a broad absorbance at 3240 cm\(^{-1}\), indicating presence of the hydroxyl and also an absorbance at 1632 cm\(^{-1}\), providing confirmation of the amide bond (Appendix A, page 137). The \(^1\)H NMR spectrum of 286 displayed all the expected resonances, indicated a one proton singlet at 10.65 ppm (corresponding to phenolic hydroxyl) as well as a six proton in the alkenyl range of 6.05-4.90 ppm. Non-protonated \(^13\)C NMR signal at \(\delta\) 169.2 ppm indicated that a carbonyl group was present in the product (Appendix B, page 151).

2.6 Ring closing metathesis reaction

With diene 286 in hand, the stage was set for the exploration of the key step in forming the macrolactam 293. To construct the unsaturated 12-member macrolactam core with an isolated (E)-double bond configuration, we relied on a ring closing metathesis reaction (RCM) of diene substrate 286. While RCM has been demonstrated to be a versatile technique in carbon-carbon double bond formation it is important to note that the prediction and control of stereoselectivity of the reaction is often challenging and usually provides a mixture of the (E)- and (Z)- isomers.

Several studies have been published on this RCM reaction,\(^{48,77,86,100}\) all of which revealed the influence that functionality remote from the double bond in the RCM can affect the \(E/Z\)-ratio of the configured cyclic olefins. It is worthy to note that Fürstner speculated that the hydrogen bond between the phenolic hydroxyl and the carbonyl group most likely was responsible for influencing the stereochemical outcome of the cyclization.

Other studies directed toward the synthesis of salicylialamid A\(^{81,86}\) have shown that using the appropriate ruthenium catalyst could exert control of the double bond geometry.
Based on these observations, we decided to introduce an appropriate protecting group to mask the aromatic free hydroxyl group in 286 to favor the formation of the desired $E$-isomer.

The exploration of the stereoselective formation of macrocyclic $E$ alkenes involved a different subset of protecting groups at the phenol position to enhance the $E:Z$ selectivity of the ring-closing metathesis reaction. The list of potential protecting groups was rather long, but limited to groups that could be easily removed at a later stage and were tolerant of basic and oxidative conditions. We considered that the three best candidates were TBS ether, methyl and acetyl protecting groups, although the exact nature of the protecting group had little effect on the RCM cyclization (Scheme 58).

Scheme 58. Protection of the phenol group in 286

Treatment of 286 with TBSCI in imidazole and DCM afforded the desired TBS protected product 288 in 68% yield while exchanging TBS silyl ether for a methyl group (using MeI and K$_2$CO$_3$ in acetone) provided 289 in a better yield (88 %). In contrast to the previous results, the use of an acetyl-protecting group proved to be exceptionally efficient (90% yield) and could be used for the next step without purification. Consequently, we chose to use an acetyl-protecting group for our ring-closing metathesis.
Recrystallisation of 290 from CH₂Cl₂/pentane gave small crystals suitable for X-ray diffraction. Single crystal X-ray analysis confirmed the absolute configuration as R and allowed an unambiguous assignment of the molecular structure of 290 to be made (Figure 12).

Figure 12. Absolute stereochemistry of acetate 290

The structure corroborates the data from ¹H NMR, ¹³C NMR, IR spectroscopy and HRMS analysis. The appearance of IR absorption at 1764 cm⁻¹ for the ester carbonyl functional group of 290, along with the disappearance of absorption at 3240 cm⁻¹ for the alcohol functional group of 286, was observed to confirm that acylation was successful (Appendix A, page 138). The methyl singlet at δ 2.27 in ¹H NMR and the carbonyl signal at δ 165.5 ppm in ¹³C NMR positively confirmed the presence of acetyl group in 290 (Appendix B, page 153).

Having constructed the key element of the RCM precursor 290, the stage was set to explore the reactivity of this compound for macrocyclisation. In regard to the construction of the macrocyclic ring, the RCM reaction was conducted on 290 using the highly active ruthenium-based catalyst A and B (Scheme 59).
Scheme 59. Synthesis of the macrolactam core using RCM

There was no evidence for the formation of RCM product when diene 290 was treated with Grubbs 1st generation catalyst (A) in toluene at room temperature; when increased to the reflux temperature of toluene, decomposition occurred. All other attempts using Grubbs 2nd generation catalyst (B) in refluxing toluene also did not produce any desired product.

In order to address this lack of reactivity we decided to attempt the cyclization in a different solvent using same catalysts and substrate.

As previously mentioned, we expected that the stereochemical outcome to be determined during the cyclisation in which the protection of the phenolic hydroxyl group
would have a major role. Thus, when the phenolic hydroxyl group in 286 was protected as the tert-butyldimethylsilyl (TBS) ether, using CH₂Cl₂ and Grubbs 1st generation catalyst (A), we were able to form the macrolactam 291, with $E$-isomer predominating (Table 3).

**Table 3.** RCM studies of 287 to 295. All reaction were carried out using Grubbs 1st generation catalyst A (1 mol%) in CH₂Cl₂

<table>
<thead>
<tr>
<th>entry</th>
<th>substrate</th>
<th>t(h)</th>
<th>yield(%)</th>
<th>$E:Z$</th>
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<tr>
<td>1</td>
<td>288</td>
<td>24</td>
<td>34</td>
<td>64:35</td>
</tr>
<tr>
<td>2</td>
<td>289</td>
<td>24</td>
<td>65</td>
<td>72:28</td>
</tr>
<tr>
<td>3</td>
<td>290</td>
<td>24</td>
<td>77</td>
<td>87:13</td>
</tr>
</tbody>
</table>

Ratio $E:Z$ determined by $^1$H NMR analysis

A methyl or acetyl phenol protecting group produced similar outcomes. Treatment of 290 with ruthenium catalyst A in refluxing CH₂Cl₂ under rigorously anhydrous and air-free conditions afforded the desired macrolactam ($E$)-293 as the major product, and was isolated in 66% yield while ($Z$)-296 could be separated from the $E$-isomer by flash chromatography in 10% yield.

It should be noted here that when the reaction was performed using the second-generation Grubbs’ catalyst B (1 mol %) in CH₂Cl₂, the results were comparable, contrary to what has been reported in the literature (Grubbs 2nd generation catalyst had been shown to favor production of the $Z$-configured product).¹⁴⁸

The well-established mechanism of the ring-closing metathesis of diene 290 is an intramolecular process and consists of two successive metathesis reactions (Scheme 60).
Scheme 60.  The mechanism of ring-closing olefin ruthenium-catalyzed metathesis

Initially, the synthesis of 293 was not very satisfactory as yields were in the 45% range and the macrolcyclisation reaction performed poorly on scale-up above 300 mg. In an effort to optimize the reaction conditions, we found that by adding a solution of the ruthenium catalyst A (1 mol%) (via cannula over a 5 min period) to the RCM substrate in CH₂Cl₂ at 45 °C, the yield of the reaction increased (the addition sequence was important for this reaction). Finally it was found that performing the reaction in a more diluted solution (substrate concentration of 0.005 g/mL CH₂Cl₂ versus 0.05 g/mL CH₂Cl₂) improved the yield to 77%.
The assignment of the protons in $E$-endocyclic alkene system of 293 is based on the analysis of the signals in the $^1$H NMR spectrum where the H(10) proton appeared at $\delta$ 5.26 (ddddd) and exhibited a $trans$ coupling of 14.9 Hz with H(9) at $\delta$ 5.50. The $^1$H NMR spectrum of $Z$-olefin system possessed the expected signal from the H(9) proton which appeared at $\delta$ 5.46 and exhibited a $cis$ coupling of 10.3 Hz with H(10) at $\delta$ 5.18 (Appendix B, page 157). Mass spectrometry data also confirmed the formation of the E (293) and Z (296) product, with a peak at 474.22504 m/z, which is the calculated mass plus a sodium ion.

Ruthenium catalysts A and B despite of their importance in the preparation of macrocyclic rings and of their remarkable tolerance of different organic functional groups, share some disadvantages. The colored and toxic ruthenium byproducts are difficult to remove completely from the reaction products even after purification by column chromatography.

Several interesting protocols to remove ruthenium-related byproducts have been reported. Use of DMSO, oxidation of the ruthenium products with lead tetraacetate or the use of a combination of silica gel and activated charcoal were reported to reduce the ruthenium content in the crude product.

In view of reported methods we applied the last procedure to our crude product and found that after stirring with activated charcoal for 12 h at room temperature followed by silica gel column chromatography, the level of ruthenium byproduct was reduced dramatically.
2.7 Synthesis of the vinyliodide fragment 299

Having secured an effective sequence to the macrolactam 293, we set out to prepare vinyl iodide 299, for a copper (I) mediated cross-coupling reaction with 101 to complete the synthesis. Therefore, benzolactam 293 was deprotected by oxidative cleavage of the p-methoxybenzyl ether using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) under pH 7.0 buffered conditions (6:1, CH$_2$Cl$_2$/pH 7.0 buffer) to give the primary alcohol at C17 in 84% yield (Scheme 61).

Scheme 61. Synthesis of the vinyl iodide 299

Monitoring the reaction by TLC showed that the cleavage of the protecting group went to completion and alcohol 297 was formed after 2 hours and the crude product was
purified by flash chromatography to afford the primary alcohol as a white solid in 84% yield.

The resulting primary alcohol 297 was then oxidized using the Dess-Martin reagent to yield the corresponding aldehyde 298 required to carry out the necessary Takai olefination to provide the E-vinyl iodide 299 for the final coupling step (Scheme 61). We chose to use Dess-Martin periodinane as it has been reported to be non-acidic and also the most reliable reagent for oxidation of primary alcohols to aldehydes under mild conditions.

The addition of four equivalents of Dess-Martin periodinane in one portion to alcohol 297 in CH₂Cl₂ at 0 °C gave aldehyde 298 in 90% yield. Colorless crystals of 298 of suitable quality and size for X-ray structural analysis were obtained from recrystallization from EtOAc at room temperature, giving, once again, the opportunity to confirm the absolute stereochemical configuration at C15 (Figure 13).

The ¹H NMR spectrum showed the aldehyde proton as a triplet at δ 9.81 (J 2.1 Hz) whereas the aldehyde carbon atom appeared at δ 200.4 in the ¹³C NMR spectrum (Appendix B, page 161). The IR spectrum of 298 showed the aldehyde carbonyl absorption at 1724 cm⁻¹ (Appendix A, page 142). The X-ray crystal structures of 298 also showed that the endocyclic alkene in the 12-membered ring possesses the E geometry.

Figure 13. Absolute stereochemistry of aldehyde 298
At that point, there was sufficient material to continue with the synthesis of the vinyliodide compound.

A literature search revealed that a stereoselective method for the conversion of aldehyde to the corresponding (E)-vinyl halides have been published in 1986 by Takai et al.99 The reaction involves the use of CHX₃ (X=I, Br or Cl) and the chromium salt, CrCl₂, usually in large excess.

Since an E-iodolakene was required, we chose the Takai reaction as it is known to be highly E stereoselective. Using the chromium-mediated approach, aldehyde 298 was subjected to a Takai iodo-olefination by treatment with CHI₃ and CrCl₂ in THF to furnish the desired trans-iodo-olefin 299 as an inseparable mixture of isomers (E:Z in a ratio of 8:1) as determined by ¹H NMR analysis (only one single spot was visible on the TLC plate for both isomers). Regarding the result of this one-carbon homologation reaction, one should keep in mind a practical and important parameter: the outcome was highly dependent on the quality of the CrCl₂ knowing that this reagent is very hygroscopic and air-sensitive.

A possible mechanism has been suggested for this reaction, involving a geminal dichromium reagent (D) formation as the key step, followed by addition of the aldehyde to afford the organometallic compound 298 A which then eliminates to give olefin 299 with mainly E geometry in 72% yield (Figure 14).

The ¹H NMR spectrum of the vinyliodide 299 showed the H(17) signal resonance at δ 6.37 (dtd) and exhibited a trans coupling of 14.3 Hz with H(18) at δ 6.09. Mass spectrometry data also confirmed the formation of the olefin product 299, with a peak at 476.06950 m/z, which is the calculated mass plus a sodium ion.
2.8 Completion of the total synthesis of 15-aza-salicylihalamide A analogue

Having completed the efficient synthesis of 299 and heptadienyl amide 101, attention was focused on the coupling of both fragments.

According with our proposed retroynthetic approach (Figure 8, Section 2.2), the assembly of iodo-olefin 299 and amide fragment 101 was planned to be done at a late stage in the synthesis with the latter fragment synthesized according to the protocol developed by Fürstner et al, Maier et al, and Erickson et al (Chapter 1, Scheme 33).

To assess the utilization of copper-mediated coupling reaction, we commenced with a model study to determine the most practical method to convert an iodo-olefin into
an enamide. Heptamide 300, a readily available starting material prepared from heptanoic acid and urea, served as a coupling partner of iodo alkene 299. Originally we had proposed that the coupling of these two fragments would be accomplished via a modern variant of the Goldberg reaction developed by Porco and co-workers. This group used an efficient approach to the assembly of enamides using Liebeskind catalyst (copper(I) thiophene carboxylate) as a source of Cu(I), Cs₂CO₃ or K₂CO₃ as base and NMP, DMA or DMSO as a polar aprotic solvent (Chapter 1, Section 1.7.2.1). Consequently, we applied the reported reaction conditions to our system (entries 1-6) but our attempts to assemble the enamide moiety failed (Table 4).

As a result, we turned our attention to alternative strategies to install the enamide side chain. To that end, we explored the Buchwald protocol that used the ligand N,N'-ethylene diamine and Cs₂CO₃ in THF at temperatures ranging from 50 to 70 °C. However, in the few trials run under these conditions (entries 7-9, Table 4), no reaction occurred as monitored by TLC and confirmed by ¹H NMR analysis.

Various attempts were made to optimize the reaction, such as freshly distilled solvent, varying the heating times and changing the equivalents of the reagents, but unfortunately, under this variety of conditions we were unable to achieve the task.
The inability to advance our model system to the enamide formation, prompted us to speculate that the absence of conjugated dienes in the amide model 300, prevented the copper-mediated C-N bond formation from occurring. As a result we decided to use the real amide 101 (prepared from propionaldehyde 176 in five steps, Scheme 33) in the synthesis of the new analogue.

Cross coupling of iodide 299 with dienamide 101 was conducted under the conditions developed by Buchwald and utilised in a recent total synthesis of the related compound apicularen A. This reaction formed the desired enamide and resulted in the concomitant cleavage of the phenolic acetate to provide the analogue (−)-301 in 64% yield (Scheme 62).
Scheme 62. Completion of the total synthesis of 301

The mechanism of copper-catalyzed C-N bond-forming reaction between \( N \)-nucleophile 101 and vinyl iodide 299 is shown in (Scheme 63).\(^{109}\)

Scheme 63. Proposed mechanism for the Cu(I)-catalyzed \( N \)-Alkylation of dienamide 101\(^{109}\)
The $^1$H NMR spectrum of the analogue 301 showed the signal of H(17) at $\delta$ 5.41 (m) and the proton H(18) appeared as a doublet at $\delta$ 6.80 and exhibited a trans coupling of $J = 14.4$ Hz with H(17) (Appendix A, page 165). The corresponding signal in the $^{13}$C NMR spectrum for C(17) was observed at $\delta$ 112.1 and that of C(18) at $\delta$ 126.3 whereas C(20) signal appeared at $\delta$ 120.6 (Appendix B, page 166). In the IR spectrum the absorption of the OH group appeared at 3348 cm$^{-1}$ along with the disappearance of absorption at 1760 cm$^{-1}$ for the ester carbonyl functional group of 299 (Appendix A, page 143). Mass spectrometry data also confirmed the formation of the analogue 301, with a peak at 431.23053 $m/z$, which is the calculated mass plus a sodium ion.
Scheme 64. Complete synthetic route of 15-aza-salicylihalamide A analogue
2.9 Biological assays

The analogue 301 was assayed against the NCI-60 leukaemia cell lines [i.e. CCRF-CEM ( acute lymphoblastic leukaemia), HL-60 ( acute promyelocytic leukaemia), K562 ( chronic myeloid leukaemia), MOLT-4 ( acute lymphoblastic leukaemia) and RPMI 8266 ( myeloma)] and exhibited antiproliferative effects at sub-micromolar concentrations (Table 5). For comparison, bafilomycin A1 (Figure 15) was also assayed against the same cell lines since both this compound and salicylihalamide A (1) target vacuolar-type (H\(^+\))-ATPase in the lysosome.\(^{38,106,107}\) Compound 301 was very active against MOLT-4 (IC\(_{50}\) = 277 nM) and CCRF-CEM (IC\(_{50}\) = 371 nM) but was most active against RPMI 8226 and HL-60 (IC\(_{50}\) = 117 and 116 nM for each respectively).

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Compound 301 IC(_{50}) (nM)</th>
<th>Bafilomycin A1 IC(_{50}) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCRF-CEM</td>
<td>371 ± 39</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>HL-60</td>
<td>116 ± 14</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>K562</td>
<td>506 ± 47</td>
<td>43 ± 15</td>
</tr>
<tr>
<td>MOLT-4</td>
<td>277 ± 9</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>RPMI-8226</td>
<td>117 ± 4</td>
<td>13 ± 1</td>
</tr>
</tbody>
</table>

Table 5 Growth inhibitory activities of compound 301 and bafilomycin A1

Bafilomycin A1 was approximately 10 fold higher in activity than the aza-salicylihalamide analogue 301 across all the cell lines. In addition, the sensitivity profiles of bafilomycin A1 and analogue 301 were strongly correlated (Figure16) supporting a similar mode of action against vacuolar-type (H\(^+\))-ATPase.\(^1\)

\[\text{Figure 15. Bafilomycin A1}\]

\(^1\) We thank Professor David C. S. Huang (Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria) for the biological assays of the analogs.
Fig 16. Sensitivity of NCI-60 leukemia cells to baflomycin A₁ versus the aza-salicylihalamide analogue 301
2.10 Conclusions

The aza-salicylihalamide analogue 301 was synthesised for the first time in a short sequence that is amenable to large scale. Key steps include a photochemical acylation to form the amide linkage, a $E$-selective ring closing metathesis to secure the macrolactam and a Cu mediated cross coupling to introduce the enamide and remove the phenolic acetate. Analogue 301 showed potent activity against several leukemia cell lines correlating strongly with bafilomycin A1 and shows promise for further development as an anti-cancer compound. The preliminary biological results demonstrate that the introduction of a stable lactam linkage in the salicylihalamide structure affords a potent simplified analogue 301 of this family of compounds which compares well to other reported analogues and the natural product itself. Further tests to determine the $in$ $vivo$ activity and stability of this compound and derivatives are underway.
Chapter Three:

Experimental
Experimental Procedures

I. General Techniques

Unless noted otherwise, commercially available materials were used without further purification. Solvents used for moisture sensitive operations were distilled from drying agents under a nitrogen atmosphere: Et$_2$O and THF from sodium benzophenone ketyl; benzene and toluene from sodium; CH$_2$Cl$_2$, NEt$_3$ and pyridine from CaH$_2$. All other commercial reagents were used as received.

Optical rotations were recorded in a 10 cm microcell. High resolution mass spectra (HRMS) were run using electrospray ionisation (ESI). Chemical shifts (δ) are given in ppm relative to residual solvent (usually chloroform; δ 7.27 for $^1$H NMR or δ 77.5 for proton decoupled $^{13}$C NMR) and coupling constants (J) in Hz. Multiplicity is tabulated as s for singlet, d for doublet, t for triplet, q for quadruplet, and m for multiplet, whereby the prefix app is applied in cases where the true multiplicity is unresolved, and br when the signal in question is broadened. Infrared spectra were recorded on a Perkin-Elmer 1000 series FTIR with wavenumbers expressed in cm$^{-1}$ using samples prepared as thin films between salt plates.

Analytical thin layer chromatography (TLC) was conducted on aluminium backed 2 mm thick silica gel GF254. Compounds were visualized with solutions of 20% w/w phosphomolybdic acid in ethanol or under UV (365 nm). All air and moisture sensitive reactions were performed in glassware that was either flame dried under an atmosphere of dry argon or oven dried at 150 °C.
II. Experimentals

1. Synthesis of terminal alkene fragment 8

**Benzodioxinone 263**

To an oven dried flask containing 2,6-dihydroxybenzoic acid 262 (18 g, 116.8 mmol, 1 equiv) and DMAP (2.13 g, 17.5 mmol, 0.15 equiv), ethyleneglycol dimethyl ether (100 mL) and benzophenone (31.91 g, 175.2 mmol, 1.5 equiv) were added under a nitrogen atmosphere. This solution was cooled to 0 °C and SOCl₂ (14.42 mL, 198.5 mmol, 1.7 equiv) was added dropwise with stirring over a period of 30 minutes. The mixture was allowed to reach room temperature slowly and stirred for 18 h and then quenched with saturated NaHCO₃ solution. The solution was extracted with EtOAc (3 x 50 mL) and the separated organic layers were washed with water and brine, dried over anhydrous MgSO₄ and concentrated in vacuo. The residue was passed through a plug of silica gel using CH₂Cl₂/hexane 1:1 as the eluent. After removal of the solvent the solid was recrystallized from ethanol to give 263 (11g, 44%) as a white powder.

The **¹H NMR** and **¹³C NMR** spectra of 263 were identical with those reported in the literature.⁸⁷,⁸⁸

**TLC:** \( R_f \) (50% CH₂Cl₂/hexane) 0.60;

**IR** \( \nu_{max} \): 3257, 3063, 1696, 1629, 1585, 1470, 1334, 1200, 1157, 1106, 1056, 810, 754, 693 cm⁻¹;
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$^{1}\text{H NMR}$ (CDCl$_3$, 300 MHz): $\delta = 10.14$ (1H, br. s, Ar-OH), 7.59 (4H, m, Ar-H, 2’, 6’, 2”, 6”), 7.40 (7H, m, 7H, Ar-H 3’, 4’, 5’, 3”, 4”, 5””), 6.66 (1H, dd, $J = 8.1$, 0.95 Hz, 6H), 6.57 (1H, d, $J = 8.5$, 0.95 Hz, 8H);

$^{13}\text{C NMR}$ (CDCl$_3$, 75.5 MHz): = 165.6, 161.5, 156.0, 139.1, 138.1, 129.5, 128.7, 126.5, 111.2, 107.6, 107.5, 100.9.

**Triflate 264**$^{110}$

In a 250 mL round bottom flask bezodioxinone 263 (8.8 g, 27.64 mmol, 1 equiv) was dissolved in CH$_2$Cl$_2$ (130 mL) at room temperature and pyridine (11.23 mL, 138.3 mmol, 5 equiv) was added to the reaction mixture via syringe. The flask was cooled to -30 °C under a nitrogen atmosphere, and trifluoromethanesulfonic anhydride (6.05 mL, 35.97 mmol, 1.3 equiv) was added dropwise to the reaction mixture. The mixture was stirred in the cold for 10 minutes and for 2 h at room temperature, then quenched with saturated NaHCO$_3$ and extracted into EtOAc. The organic extracts were successively washed with water, saturated CuSO$_4$ solution, water and brine, dried with MgSO$_4$ and the solvent was evaporated to give the crude triflate 264 (11.94 g, 96%) as a yellow powder. Generally, the product is very clean and does not require any further purification.

The $^{1}\text{H NMR}$ and $^{13}\text{C NMR}$ spectra of 264 were identical with those reported in the literature.$^{110}$

**TLC:** $R_f$ (50% CH$_2$Cl$_2$/hexane) 0.62;

**IR** $\nu_{\text{max}}$: 3075, 1750, 1620, 1579, 1472, 1428, 1288, 1206, 837, 749, 699 cm$^{-1}$;
**Experimental**

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\(^1\text{H NMR}\) (CDCl\(_3\), 300 MHz): \(\delta = 7.57\) (5H, m, \(7\text{H}, \text{Ar-}H\ 2', 6', 2'', 6''\)), 7.37 (6H, m, \(\text{Ar-H}\ 3', 4', 5', 3'', 4'', 5''\)), 7.24 (1H, dd, \(J = 8.5, 0.95\) Hz, \(6\text{H}\)), 6.95 (1H, d, \(J = 8.3\) Hz, \(8\text{H}\));

\(^{13}\text{C NMR}\) (CDCl\(_3\), 75.5 MHz): \(\delta = 157.8, 157.1, 148.7, 138.6, 136.6, 129.6, 128.6, 126.6, 118.2, 117.1, 116.4, 109.4, 107.5\).

**Alkene 266\(^89\)**

![Diagram of reaction](image)

To an oven dried, 250 mL Schlenk flask with Teflon stir bar, containing a solution of LiCl (2.24 g, 52.88 mmol, 3.5 equiv) and Pd(PPh\(_3\))\(_4\) (0.34 g, 0.3 mmol, 0.02 equiv) in dry DMF (100 mL) was added, under the nitrogen, a solution of triflate 264 (6.8 g, 15.11 mmol, 1 equiv) in DMF (20 mL) via cannula, and the reaction mixture was stirred at room temperature for 1.5 h. Allyltributyltin (6.95 mL, 22.6 mmol, 1.5 equiv) was added and the reaction mixture was heated at 80 °C with stirring for 20 h. The flask was cooled to 0 °C followed by the addition of 1.0 M KF solution and the reaction mixture stirred for 30 minutes at the same temperature and then extracted with Et\(_2\)O (3 x 50 mL). The organic layers were combined, washed with water and brine, dried over MgSO\(_4\) and concentrated in vacuo. Purification on silica gel (30% EtOAc/hexane) impregnated with 10% w/w of finely grounded KF afforded alkene 266 (3.82 g, 74%) as a white solid in a high state of purity.

The \(^1\text{H NMR}\) and \(^{13}\text{C NMR}\) spectra of 266 were identical with those reported in the literature.\(^89\)
TLC: $R_f$ (30 % EtOAc/hexane) 0.81;

IR $\nu_{\max}$: 3072, 3031, 1738, 1605, 1581, 1445, 1304, 1288, 1267, 1203, 1100, 1064, 988 cm$^{-1}$;

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta = 7.56$ (4H, m, Ar-H 2’, 6’, 2”, 6”), 7.35 (1H, t, $J = 8.01$ Hz, 7H), 7.26 (6H, m, Ar-H 3’, 4’, 5’, 3”, 4”, 5”), 6.91 (1H, dd, $J = 8.2$, 1.1 Hz, 8H), 6.81 (1H, br. d, $J = 7.6$ Hz, 6H), 5.87 (1H, ddt, $J = 17.1$, 10.1, 6.3 Hz, -CH=CH$_2$), 4.88 (1H, ddt, $J = 10.2$, 1.5, 1.5 Hz, cis CH=CH$_2$), 4.67 (1H, ddt, $J = 17.1$, 1.7 Hz, trans CH=CH$_2$), 3.77 (2H, br. d, $J = 6.3$ Hz, Ar-CH$_2$-CH=CH$_2$);

$^{13}$C NMR (CDCl$_3$, 75.5 MHz): $\delta = 160.2, 157.5, 145.2, 139.7, 136.3, 135.5, 129.1, 128.5, 126.6, 125.3, 115.9, 115.8, 113.6, 106.0, 38.1$.

2. Synthesis of amine fragment 285

(S)-2-Bromosuccinic acid 279$^{111}$

A 100 mL round bottomed flask fitted with a mechanical stirrer was charged with L-aspartic acid 278 (12 g, 89.82 mmol, 1 equiv) and KBr (37.41 g, 314.3 mol, 3.5 equiv). Then, H$_2$SO$_4$ (4N, 200 mL) was added in one portion and the resulting solution was cooled to -10 °C (with an ice/methanol cooling bath). To this mixture a solution of sodium nitrite (9.24 g, 134.73 mmol, 1.5 equiv) in H$_2$O (20 mL) was continuously added over a period of 40 minutes, under a nitrogen atmosphere. The reaction mixture was stirred for an additional 2 h at -10 °C and then extracted with Et$_2$O (3 x 50 mL). The combined ether
layers were washed with brine and dried (MgSO$_4$), filtered and concentrated under reduced pressure to yield the crude product 279 as a white solid (17.73 g, 84%).

The $^1$H NMR and $^{13}$C NMR spectra of 279 were identical with those reported in the literature.$^{111}$

$^1$H NMR ((CD$_3$)$_2$CO, 300 MHz): δ = 11.40 (1H, br, s, 1C-OH), 4.64 (1H, dd, $J = 8.6$, 6.2 Hz, 2H), 3.78 (1H, br, s, 4C-OH), 3.25 (1H, dd, $J = 17.2$, 8.6, 3H), 3.02 (1H, dd, $J = 17.2$, 6.1, 3H);

$^{13}$C NMR ((CD$_3$)$_2$CO, 75.5 MHz): δ = 171.1, 170.4, 40.1, 39.8

Bromodiol 280$^{111}$

Employing flame-dried glassware under a nitrogen atmosphere, (S)-2-bromosuccinic acid 279 (16 g, 81.2 mmol, 1 equiv) was dissolved in freshly distilled THF (100 mL) and the mixture cooled to -20 °C. Borane dimethylsulfide complex (21.6 mL, 243.6 mmol, 2.0 M solution in THF, 3 equiv) was added via cannula and the reaction mixture was allowed to warm slowly to room temperature when a slightly exothermic reaction commenced. The mixture was stirred and maintained under a sweep of nitrogen gas for 16 h then quenched by dropwise addition of THF/H$_2$O (20 mL, 1:1). K$_2$CO$_3$ (40 g) was added and a white solid appeared within several minutes, which was removed by decantation and washed with Et$_2$O (2 x 50 mL). The organic phases were concentrated to an oily residue, which was then triturated with Et$_2$O (3 x 50 mL) and borate salts removed by filtration. The ether extracts were combined, dried over MgSO$_4$, filtered and then
concentrated. The residue (yellow oil) was purified by flash column chromatography to afford the desired product as an oil (12.07 g, 86%).

The $^1$H NMR and $^{13}$C NMR spectra of 280 were identical with those reported in the literature.\textsuperscript{111}

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 4.32 (1H, m), 3.85 (4H, m), 2.88 (2H, br, s), 2.12 (2H, m);

$^{13}$C NMR (CDCl$_3$, 75.5 MHz): $\delta$ = 67.1, 60.0, 55.0, 37.7

**Methoxybenzyl bromide (B)**

To a flask containing 4-methoxybenzyl alcohol A (14 g, 173 mmol, 1 equiv) was added a solution of HBr (15 mL 48%), and the mixture was vigorously stirred at room temperature for 1 hour. The resulting mixture was diluted with Et$_2$O (40 mL) and the organic layer was successively washed with saturated NHCO$_3$, water, saturated aqueous NaCl, dried over K$_2$CO$_3$, filtered and concentrated to give unstable 4-methoxybenzyl bromide B (19.46 g, 96%) as a colorless oil. The crude product was found to be of sufficient purity for use in the next step without need for further purification.

The $^1$H NMR and $^{13}$C NMR spectra of 263 were identical with those reported in the literature.\textsuperscript{111}

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 7.35 (2H, d, $J$ = 8.7 Hz, \textit{o-Ph-H}), 6.89 (2H, $J$ = 8.7, Hz, \textit{m-Ph-H}), 4.53 (2H, s, Br-CH$_2$-Ph-), 3.82 (3H, s, Ph-O-CH$_3$);

$^{13}$C NMR (CDCl$_3$, 75.5 MHz): $\delta$ = 159.7, 130.4, 129.9, 114.2, 55.3, 34.0.
Under an atmosphere of nitrogen, sodium hydride (60% in mineral oil, 6.66 g, 166.67 mmol, 3.5 equiv) was suspended in dry THF (75 mL) after being washed with hexane. The suspension was cooled to –10 °C and (S)-2-bromo-1,4-butanediol 280 (8 g, 47.62 mmol, 1 equiv) in dry THF (20 mL) was added via cannula. After 1.5 h of stirring at –10 °C a solution of 4-methoxybenzyl bromide B (14.28 g, 71.43 mmol, 1.5 equiv) in THF (50 mL) was added followed by a solution of TBAI (1.75 g, 4.76 mmol, 0.1 equiv) in THF (10 mL). The white suspension was stirred for further 15 minutes at the same temperature and then allowed to warm to room temperature over 5 h (cooling bath slowly warmed to rt). The reaction was quenched by addition of saturated NH₄Cl solution (50 mL), stirring was continued for 15 min, and the phases were separated. The aqueous layer was extracted with EtOAc (3 x 40 mL) and the combined organic extracts were washed with H₂O, brine and dried with Na₂SO₄. After filtration and evaporation of the solvent, the crude product was purified by flash chromatography (50 % EtOAc /hexane) to provide the pure product 281 (6.93 g, 64 %) as a colorless oil.

The ¹H NMR and ¹³C NMR spectra of 263 were identical with those reported in the literature.¹¹¹

**TLC:** \( R_f = 0.58 \);  
**IR**: \( v_{max} : 2997, 2926, 2859, 2838, 1736, 1612, 1585, 1512, 1464, 1361, 1301, 1244, 1173, 1088, 1032, 907, 819 \text{ cm}^{-1} ;\)
Experimental Chapter 3

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta = 7.27$ (2H, d, $J = 8.6$ Hz, $o$-Ph-H), 6.89 (2H, d, $J = 8.6$ Hz, $m$-Ph-H), 4.47 (2H, s, -O-CH$_2$-Ph-), 3.81 (3H, s, -Ph-O-CH$_3$), 3.61 (2H, m, 5H), 3.07 (1H, m, 3H), 2.78 (1H, app. dd, $J = 4.8$, 0.7 Hz, 2H), 2.53 (1H, dd, $J = 5.0$, 2.7 Hz, 2H), 1.84 (2H, m, 4H);

$^{13}$C NMR (CDCl$_3$, 75.5 MHz): $\delta = 159.2$, 130.3, 129.2, 113.8, 72.7, 66.7, 55.2, 50.0, 47.1, 32.9.

**Alcohol 282**

To a flame-dried two necked round bottom flask equipped with a magnetic stirring bar, a condenser and a rubber septum cap was added magnesium turnings (1.08 g, 45 mmol, 1.5 equiv) and Et$_2$O (20 mL). The suspension was stirred at room temperature for 10 min, the solvent removed via syringe and Mg dried under a flame. Freshly distilled THF (23 mL, 2M with respect to Mg) was added to the flask and after 10 min a small crystal of I$_2$ was added and stirred for another 10 min at room temperature when the mixture turned from yellow to turbid gray. 5-bromo-1-pentene (4.5 g, 30 mmol, 1 equiv) was added dropwise and the resulting solution was stirred at reflux temperature for 30 min. THF (30 mL) was added to this solution of the Grignard reagent, and after cooling to -78°C a solution of Li$_2$CuCl$_4$ (0.1M in THF, 9.4 mL, 0.9 mmol, 0.03 equiv) was added dropwise via syringe. After the mixture was stirred for 25 min at the same temperature, a solution of 281 (3.12 g, 15 mmol, 0.5 equiv) in THF (20 mL) was added dropwise. After the resulting mixture was stirred at -78 °C for 15 min, it was allowed to warm to room temperature over
a period of 2 h. A saturated aqueous solution of NH₄Cl (30 mL) was added and the reaction mixture was extracted with Et₂O (3 x 50 mL). The organic layers were washed with water and brine, dried (Na₂SO₄) and the solvent removed by evaporation at reduced pressure. The residue was purified by flash chromatography (40 % EtOAc/hexane) to afford the desired product 282 (3.41 g, 78%) as a colorless oil.

The ¹H NMR and ¹³C NMR spectra of 263 were identical with those reported in the literature.⁵⁰

TLC: Rₜ = (40% EtOAc/hexane) 0.61;

[α]²⁶³: -9.13 (c 1.390, CH₂Cl₂);

IR νmax: 3462, 2932, 2857, 1612, 1512, 1245, 1173, 1087, 1033, 910, 819 cm⁻¹;

¹H NMR (CDCl₃, 300 MHz): δ = 7.26 (2H, d, J = 8.5 Hz, o-Ph-H), 6.88 (2H, d, J = 8.6 Hz, m-Ph-H), 5.82 (1H, ddt, J = 17.1, 10.2, 6.6 Hz, CH₂=CH-), 5.01 (1H, ddt, J = 17.1, 2.07, 1.5 Hz, trans CH=CH₂), 4.94 (1H, ddt, J = 10.2, 1.1, 1.1 Hz, cis CH=CH₂), 4.46 (2H, s, -O-CH₂-Ph-), 3.81 (3H, s, -Ph-O-CH₃), 3.66 (2H, m, -CH₂-OPMB), 2.93 (1H, d, J = 3.0 Hz, -OH), 2.06 (2H, q, J = 6.3 Hz, CH₂=CH-CH₂-), 1.73 (2H, m, -CH₂-CH₂-OPMB), 1.4 (6H, m, CH₂=CH-CH₂-(CH₂)-CH-OH);

¹³C NMR (CDCl₃, 75.5 MHz): δ = 159.2, 138.9, 130.0, 129.3, 114.3, 113.85, 72.9, 71.4, 68.9, 55.2, 37.2, 36.3, 33.7, 28.9, 25.0.

HRMS (ESI) calculated for C₁₇H₂₆O₃ [M+H]⁺ 301.17742 found 301.17737.
Mesylate 283

To a cooled (0 °C) solution of alcohol 282 (2.4 g, 8.62 mmol, 1 equiv) in Et₂O (60 mL) was added triethylamine (4.23 mL, 30.17 mmol, 3.5 equiv) and methanesulfonyl chloride (2.33 mL, 30.17 mmol, 3.5 equiv). After 15 min of stirring at 0 °C the reaction mixture was warmed to room temperature and the reaction was left to proceed for further 2h. Then the resulting mixture was quenched with water, the organic layer was separated and the aqueous solution was extracted with Et₂O (3 × 50 mL). The combined organic extracts were washed successively with 50 mL H₂O and 50 mL saturated aqueous NaCl, dried over Na₂SO₄ and concentrated affording the crude mesylate (2.88 g, 94%) directly used for the next step without further purification.

TLC: \( R_f = (40\% \text{ EtOAc/hexane}) 0.56; \)

\(^1\text{H NMR}\) (CDCl₃, 300 MHz): \( \delta = 7.26 \) (2H, d, \( J = 8.6 \) Hz, \( o\text{-Ph-H} \)), 6.88 (2H, d, \( J = 8.6 \) Hz, \( m\text{-Ph-H} \)), 5.81 (1H, ddt, \( J = 17.1, 10.2, 6.6 \) Hz, \( -\text{CH=CH}_2 \)), 5.01 (1H, ddt, \( J = 17.1, 1.9, 1.6 \) Hz, trans \( \text{CH=CH}_2 \)), 4.95 (1H, ddt, \( J = 10.3, 1.3, 1.1 \) Hz, cis \( \text{CH=CH}_2 \)), 4.90 (1H, q, \( J = 6.16 \) Hz, \( -\text{SO}_2(\text{CH}_3)\text{-O-CH} \)), 4.46 (2H, ABq, \( J = 11.3 \) Hz, \( -\text{O-CH}_2\text{-Ph} \)), 3.80 (3H, s, \( -\text{Ph-O-CH}_3 \)), 3.56 (2H, m, \( -\text{CH}_2\text{-OPMB} \)), 2.95 (3H, s, \( -\text{SO}_2\text{-CH}_3 \)), 2.06 (2H, m, \( \text{CH}_2\text{=CH-CH}_2 \)), 1.98 (2H, q, \( J = 6.1 \) Hz, \( \text{MsO-CH-CH}_2\text{-OPMB} \)), 1.74 (2H, m, \( \text{CH}_2\text{=CH-(CH}_2)_3\text{-CH}_2 \)), 1.41 (4H, m, \( \text{CH}_2\text{=CH-CH}_2\text{-}(\text{CH}_2)_2 \));

\(^{13}\text{C NMR}\) (CDCl₃, 75.5 MHz): \( \delta = 159.2, 138.5, 130.1, 129.4, 114.6, 113.8, 81.2, 72.7, 65.5, 55.2, 38.3, 34.8, 34.5, 33.4, 26.5, 24.1. \)

\( \text{HRMS (ESI) calculated for C}_{18}\text{H}_{28}\text{O}_2\text{SNa [M+Na]}^+ 379.15497 \) found 379.15494.
Azide 284\textsuperscript{113}

\begin{align*}
\text{PMBO} \quad \text{OMs} \quad 283 \quad \text{NaN}_3, \text{DMF} \quad 90\% \quad \text{PMBO} \quad \text{N}_3 \quad 284
\end{align*}

To a solution of mesylate 283 (2.80 g, 7.86 mmol, 1 equiv) in DMF (30 mL) was added \text{NaN}_3 (1.12 g, 17.29 mmol, 2.2 equiv) and the mixture was stirred at reflux temperature (oil bath 72 °C) for 20 h. After cooling to room temperature the reaction mixture was quenched with water and extracted with diethyl ether (3 x 50 mL). The combined organic extracts were washed with water and brine and the organic layer was dried over MgSO\textsubscript{4}, filtered and concentrated in vacuo to give the crude product, which was purified by silica gel column chromatography (10% Et\textsubscript{2}O/hexane) to give 284 (2.14 g, 90%) as a colorless oil.

[\alpha]_D^{26}: -27.78 (c 1.115, CH\textsubscript{2}Cl\textsubscript{2});

TLC: \(R_f\) (10% Et\textsubscript{2}O/hexane) 0.58;

IR \(\nu_{\text{max}}\): 2933, 2858, 2096, 1612, 1512, 1245, 1089, 1034, 910, 819 cm\textsuperscript{-1};

\(^1\text{H} \text{NMR} \) (CDCl\textsubscript{3}, 300 MHz): \(\delta = 7.30 \) (2H, d, \(J = 8.5\) Hz, \(\text{o-Ph-H}\), 6.92 (2H, d, \(J = 8.6\) Hz, \(\text{m-Ph-H}\)), 5.84 (1H, ddt, \(J = 17.1, 10.2, 6.6\) Hz, \(-\text{CH=}\text{CH}_2\)), 5.03 (2H, m, \(\text{CH=}\text{CH}_2\)), 4.48 (2H, ABq, \(J = 11.6\) Hz, \(-\text{O-CH}_2\text{-Ph}\)), 3.83 (3H, s, \(-\text{Ph-O-CH}_3\)), 3.59 (2H, m, \(-\text{CH}_2\text{-OPMB}\)), 3.54 (1H, m, \(\text{N}_3\text{-CH-}\)) 2.10 (2H, m, \(\text{CH}_2\text{=CH-CH}_2\)), 1.79 (2H, m, \(\text{N}_3\text{-CH-CH}_2\text{-CH}_2\text{-OPMB}\)) 1.50 (6H, m, \(\text{CH}_2\text{=CH-CH}_2\text{-CH}_3\));

\(^{13}\text{C} \text{NMR} \) (CDCl\textsubscript{3}, 75.5 MHz): \(\delta = 159.2, 138.6, 130.3, 129.3, 114.6, 113.8, 72.8, 66.5, 60.1, 55.2, 34.7, 34.5, 33.6, 28.6, 25.5\);

HRMS (ESI) calculated for C\textsubscript{17}H\textsubscript{25}N\textsubscript{3}O\textsubscript{2}Na [M+Na]\textsuperscript{+} 326.18390, found 326.18396.
To a solution of azide 284 (4.8 g, 15.83 mmol, 1 equiv) and triphenylphosphine (16.86 g, 63.32 mmol, 4 equiv) in THF (100 mL) was added water (3.13 mL, 174.13 mmol, 11 equiv) and the mixture was stirred for 3 h at 70 °C. The reaction mixture was cooled to room temperature and extracted with 0.5% solution of HCl. The aqueous phase was successively washed with Et₂O (3 x 40 mL) and 5% aq. NaOH and then extracted with CH₂Cl₂ (3 x 40 mL). The combined organic extracts were dried over MgSO₄, filtered and then concentrated under reduced pressure to give amine 285 (4.08 g, 65%) as an oil in a high state of purity.

\([\alpha]^{26}_D + 3.13 \ (c \ 1.280, \text{CH}_2\text{Cl}_2)\);

**TLC**: \(R_f\) (10% MeOH/CH₂Cl₂) 0.55;

**IR** \(\nu_{\text{max}}\): 3369, 2927, 2854, 1612, 1586, 1512, 1245, 1092, 1034, 908, 819, 721 cm⁻¹;

**¹H NMR** (CDCl₃, 300 MHz): \(\delta = 7.25\) (2H, d, \(J = 8.6\) Hz, -Ph-H), 6.86 (2H, d, 8.6 Hz, -Ph-H), 5.79 (1H, ddt, \(J = 17.1, 10.2, 6.6\) Hz, -CH=CH₂), 4.99 (1H, ddt, \(J = 17.1, 1.9, 1.6\) Hz, trans CH=CH₂), 4.92 (1H, ddt, \(J = 10.2, 1.1, 1.1\) Hz, cis CH=CH₂), 4.42 (2H, br, s, -O-CH₂-Ph-), 3.78 (3H, s, -Ph-O-CH₂), 3.54(2H, m, -CH₂-OPMB), 2.86(1H, m, NH₂-CH-), 2.04 (2H, m, CH₂=CH-CH₂-), 1.79 (2H, m, NH₂-CH₂-CH₂-OPMB), 1.50 (2H, m, CH₂=CH-(CH₂)₂-CH₂-), 1.38 (4H, m, CH₂=CH-CH₂-(CH₂)₂-);

**¹³C NMR** (CDCl₃, 75.5 MHz): \(\delta = 159.1, 138.9, 130.5, 129.2, 114.3, 113.7, 72.6, 67.8, 55.2, 49.1, 38.2, 37.7, 33.7, 28.9, 25.5;\)

**HRMS** (ESI) calculated for C₁₇H₂ₘNO₂ [M+H]⁺ 278.21146, found 278.21155.
3. Synthesis of core of salicylhalamide 301

Preparation of amide 286

To an oven-dried quartz test tube was added amine 285 (116.4 mg, 0.42 mmol, 1 equiv.) and benzodioxinone 266 (215.5 mg, 0.63 mmol, 1.5 equiv.). The tube was then sealed with a rubber septum and after three vacuum/N$_2$ cycles to remove air from the reaction tube, dry CH$_2$Cl$_2$ (10 mL) was added via syringe. After stirring for 5 min at room temperature, the reaction mixture was placed in a Rayonet photochemical reactor equipped with 8 x 300 nm lamps and the degassed solution was irradiated for 6 h. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography affording 286 (151.4 mg, 55%) as a colorless oil.

$[\alpha]_D^{23}$: -1.6 (c 0.790, CH$_2$Cl$_2$);

TLC: $R_f$ (30% EtOAc/hexane) 0.55;

IR $\nu_{\text{max}}$: 3280, 3240, 2932, 2857, 1631, 1517, 1462, 1245, 1091, 909, 817, 761 cm$^{-1}$;

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 10.65 (1H, s, Ph-OH), 7.25 (1H, t, $J$ = 7.9 Hz, H5), 7.19 (2H, d, $J$ = 8.6 Hz, o-Ph-H), 6.87 (1H, dd, $J$ = 1.1, 8.2 Hz, H6), 6.81 (2H, d, $J$ = 8.6 Hz, m-Ph-H), 6.66 (1H, dd, $J$ = 7.5, 1.1 Hz, H4), 6.61 (1H, d, $J$ = 8.5 Hz, -NH), 6.05 (1H, ddt $J$ = 17.2, 10.2, 5.1 Hz, H9), 5.79 (1H, ddt, $J$ = 17.1, 10.2, 6.6 Hz, H12), 5.20 (1H, ddt, $J$ = 10.2, 1.7, 1.7 Hz, cis H10), 5.02 (1H, ddt, $J$ = 17.2, 1.9, 1.6 Hz trans H11), 4.94 (1H, ddt,
$J = 10.1, 1.2, 0.9 \text{ Hz, cis H}11$), 4.90 (1H, ddt, $J = 17.3, 1.8, 1.5 \text{ Hz trans H}10$), 4.40 (2H, s, H20), 4.28 (1H, m, H17), 3.78 (3H, s, H23), 3.55 (2H, m, H19), 3.43 (2H, m, H8), 2.05 (2H, dt, $J = 6.9, 6.9 \text{ Hz, H}13$), 1.93 (1H, m, H18), 1.72 (1H, m, H18), 1.54 (2H, m, H16), 1.39 (4H, m, H14, H15);

$^{13}$C NMR (CDCl$_3$, 75.5 MHz): $\delta = 169.2, 159.3, 159.2, 138.6, 138.1, 136.4, 131.8, 130.0, 129.4, 122.6, 118.8, 117.6, 115.8, 114.5, 113.7, 72.9, 67.0, 55.2, 48.3, 38.4, 34.4, 34.2, 33.5, 28.6, 25.4;

HRMS (ESI) calculated for C$_{27}$H$_{35}$NO$_4$Na [M+Na]$^+$ 460.24583, found 460.24586.

**Acetyl protected phenol 290$^{115}$**

To a solution of 286 (0.909 g, 2.07 mmol, 1.00 equiv) and 4-(dimethylamino) pyridine (DMAP, 378 mg, 1.5 equiv) in CH$_2$Cl$_2$ (10 mL) was added pyridine (0.84 mL, 10.3 mmol, 5 equiv) and freshly distilled acetic anhydride (0.29 mL, 3 mmol, 1.5 equiv). After stirring for 12 h at room temperature, the mixture was diluted with Et$_2$O (20 mL), quenched with aqueous NaHCO$_3$ (40 mL) and stirred at ambient temperature for 10 min. The organic layer was separated and the aqueous layer was extracted with Et$_2$O (3x40 mL). The combined organic layers were washed successively with CuSO$_4$ solution, water, NaHCO$_3$ solution and saturated aqueous NaCl, dried over Na$_2$SO$_4$ and evaporated. The residue was purified by flash chromatography (3%, EtOAc/ CH$_2$Cl$_2$) to give 290 as a white solid (915 mg, 90%) suitable for X-ray crystallographic analysis.
Experimental

Chapter 3

mp 55 °C;

**TLC:** $R_t$ (3%, EtOAc/ CH$_2$Cl$_2$) 0.91;

$R_t$ (40%, EtOAc/ CH$_2$Cl$_2$) 0.52;

$[\alpha]_D^{23}$: +2.4 ($c$ 0.910, CH$_2$Cl$_2$);

**IR** $\nu_{\text{max}}$: 3296, 3074, 2933, 2858, 1764, 1641, 1610, 1512, 1456, 1246, 1198, 1092, 1018, 911, 820, 733 cm$^{-1}$;

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 7.33 (1H, t, $J = 7.9$ Hz, H$_5$), 7.19 (2H, d, $J = 8.6$ Hz, o-Ph-H), 7.12 (1H, d, $J = 7.6$ Hz, H$_6$), 6.95 (1H, d, $J = 8.1$ Hz, H$_4$), 6.82 (2H, d, $J = 8.6$ Hz, m-Ph-H), 6.07 (1H, d, $J = 8.8$ Hz, -NH), 5.93 (1H, ddt, $J = 17.0$, 10.2, 6.5 Hz, H$_9$), 5.80 (1H, ddt, $J = 17.1$, 10.2, 6.6, Hz, H$_{12}$), 5.07 (1H, ddt, $J = 10.2$, 1.5, 1.3 Hz, cis H$_{10}$), 4.95 (1H, ddt, $J = 17.0$, 1.7, 1.5 Hz trans H$_{10}$), 4.94 (1H, ddt, $J = 10.0$, 1.9, 1.1 Hz, cis H$_{11}$), 4.41 (2H, ABq, $J = 11.5$ Hz, H$_{20}$), 4.23 (1H, m, H$_{17}$), 3.79 (3H, s, H$_{23}$), 3.56 (2H, m, H$_{19}$), 3.33 (2H, d, $J = 6.4$ Hz, H$_8$), 2.27 (3H, s, -O-CO-CH$_3$), 2.05 (2H, m, H$_{13}$), 1.82 (1H, m, H$_{18}$), 1.64 (1H, m, H$_{18}$), 1.43 (2H, m, H$_{16}$), 1.32 (4H, m, H$_{14}$, H$_{15}$);

$^{13}$C NMR (CDCl$_3$, 75.5 MHz): $\delta$ = 170.0, 165.5, 159.1, 147.0, 138.8, 138.6, 136.6, 131.0, 130.1, 129.6, 129.3, 127.3, 120.5, 116.5, 114.5, 113.7, 72.8, 66.8, 55.2, 47.7, 37.0, 34.2, 34.0, 33.6, 28.8, 25.4, 21.0;

**HRMS** (ESI) calculated for C$_{29}$H$_{37}$NO$_5$Na [M+Na]$^+$ 502.25639, found 502.25646.
Experimental Chapter 3

**Macrolactam 293 and 296**

A 100 mL, flame-dried, round bottomed flask equipped with a reflux condenser and a magnetic stirring bar, was charged with 290 (390 mg, 0.814 mmol, 10 equiv) before being sealed with a rubber septum and evacuated and backfilled with N₂ three times. Freshly distilled CH₂Cl₂ was introduced and the resulting solution was stirred at room temperature for 2 min. A solution of Grubbs 1st-generation catalyst (Cy₃P)₂Cl₂Ru=CHPh (66.9 mg, 0.081 mmol, 1 equiv) in degassed CH₂Cl₂ (8 mL) was added dropwise and the reaction mixture was heated to reflux for 24 h. The cooled (room temperature) solution was evaporated until ca. 30 mL remained and then filtered through a short plug of silica gel eluting with EtOAc. After the filtered solution was stirred with activated charcoal (20g, 50 equiv wt of crude product) for 12 h at room temperature, the carbon was removed by filtration. The removal of solvent in vacuo and purification on a silica gel chromatographic column (EtOAc/hexane 1:20 → 1:4 → 1:1) afforded the (E)-macrolactam 293 (257.76 mg, 66%) and (Z)-macrolactam 296 (41.2 mg, 10%) as white solids.

**(E)-macrolactam 293**

mp 117 °C;

TLC: Rₜ (50% EtOAc/hexane) 0.44;

[α]D²³: -98.3 (c 0.720, CH₂Cl₂);
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**Experimental**

**IR** \( \nu_{\text{max}} \): 3293, 2926, 2855, 1765, 1644, 1455, 1246, 1200, 1089, 1020, 804, 733 cm\(^{-1}\);

**\(^{1}\text{H NMR}** (CDCl\(_3\), 300 MHz): \( \delta = 7.30 \) (1H, t, \( J = 7.8 \) Hz, H\(_5\)), 7.26 (2H, d, \( J = 8.6 \) Hz, o-Ph-H), 7.11 (1H, d, \( J = 7.5 \) Hz, H\(_6\)), 6.92 (1H, d, \( J = 8.1 \) Hz, H\(_4\)), 6.88 (2H, d, \( J = 8.6 \) Hz, m-Ph-H), 5.51 (1H, d, \( J = 8.6 \) Hz, -NH), 5.50 (1H, m, trans H\(_9\)), 5.26 (1H, dddd, \( J = 14.9, 10.3, 3.3, 1.3 \) Hz, trans H\(_{10}\)), 4.46 (2H, ABq, \( J = 11.6 \) Hz, H\(_{18}\)), 4.11 (1H, m, H\(_{15}\)), 3.92 (1H, dd, 14.2, 3.8 Hz, H\(_8\)), 3.73 (3H, s, H\(_{23}\)), 3.58 (2H, app. t, \( J = 7.3 \) Hz, H\(_{17}\)), 3.21 (1H, dd, \( J = 14.2, 2.5 \) Hz, H\(_8\)), 2.16 (3H, s, 3C-O-CO-CH\(_3\)), 1.72 (4H, m, H\(_{16}\), H\(_{14}\)), 1.41 (4H, m, H\(_{11}\), H\(_{13}\)), 1.11 (2H, m, H\(_{12}\));

**\(^{13}\text{C NMR}** (CDCl\(_3\), 75.5 MHz): \( \delta = 170.7, 165.3, 159.1, 147.3, 140.6, 133.1, 130.7, 130.4, 129.9, 129.4, 128.5, 128.0, 120.8, 113.8, 72.8, 67.3, 55.2, 45.0, 37.9, 36.7, 32.7, 32.6, 24.7, 20.9, 20.7;

**HRMS** (ESI) calculated for C\(_{27}\)H\(_{33}\)NO\(_5\)Na [M+Na]\(^+\) 474.22509, found 474.22504.

**\((Z)\)-macrolactam 296**

**mp** 119-120 °C

**TLC:** \( R_f \) (50% EtOAc/hexane) 0.43;

\([\alpha]_{D}^{27} \): - 8.82 (c 0.720, CH\(_2\)Cl\(_2\));

**IR** \( \nu_{\text{max}} \): 3289, 2928, 2855, 1764, 1642, 1512, 1455, 1245, 1200, 1094, 1023, 818,733cm\(^{-1}\);

**\(^{1}\text{H NMR}** (CDCl\(_3\), 300 MHz): \( \delta = 7.35 \) (1H, t, \( J = 7.8 \) Hz, H\(_5\)), 7.24 (2H, d, \( J = 8.6 \) Hz, o-Ph-H), 7.20 (1H, d, \( J = 7.8 \) Hz, H\(_6\)), 6.93 (1H, d, \( J = 8.1 \) Hz, H\(_4\)), 6.87 (2H, d, \( J = 8.5 \) Hz, m-Ph-H), 5.80 (1H, d, \( J = 8.1 \) Hz, -NH), 5.46 (1H, app. t, \( J = 10.3 \) Hz, cis H\(_9\)), 5.18 (1H, app. t, \( J = 10.3 \) Hz, cis H\(_{10}\)), 4.44 (2H, ABq, \( J = 11.9 \) Hz, H\(_{18}\)), 4.28 (1H, m, H\(_{15}\)), 3.81 (3H, s, H\(_{23}\)), 3.70 (1H, dd, \( J = 14.9, 4.8 \) Hz, H\(_8\)), 3.52 (2H, m, H\(_{17}\)), 3.22 (1H, br d, \( J = 14.9 \) Hz, H\(_8\)), 2.18 (3H, s, 3C-O-CO-CH\(_3\)), 1.94 (2H, m, H\(_{16}\)), 1.73 (2H, m, H\(_{14}\)), 1.42 (2H, m, H\(_{11}\), H\(_{13}\)), 1.02 (2H, m, H\(_{12}\));

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To a solution of PMB ether 293 (0.120 mg, 0.266 mmol, 1 equiv) in CH₂Cl₂ (40 mL) at 0 °C was added pH 7 buffer (6 mL) followed by DDQ (301.91 mg, 1.33 mmol, 5 equiv). After being stirred at 0 °C for 5 min and at room temperature for 2 h, the reaction was quenched with saturate aqueous NaHCO₃ and saturated aqueous Na₂S₂O₃, and extracted with CH₂Cl₂ (3x20 mL). The combined organic extracts were washed with brine, dried over MgSO₄, filtered and then concentrated under reduced pressure. The residue was purified by flash chromatography to afford 297 (81 mg, 84%) as a white solid.

**mp** 156-157 °C;

**TLC:** *R*ᵣ (40% EtOAc/hexane) 0.47;

[*α*]ᵣ²⁺ : -169.5 (c 0.710, CH₂Cl₂);

**IR** νₘₚₓ : 3369, 3264, 2944, 2926, 2862, 1759, 1548, 1456, 1215, 966, 750 cm⁻¹;

**¹H NMR** (CDCl₃, 300 MHz): δ = 7.33 (1H, t, J = 7.9 Hz, H5), 7.13 (1H, d, J = 7.5 Hz, H6), 6.94 (1H, d, J = 8.2, H4), 5.47 (1H, d, J = 8.7 Hz, NH), 5.48 (1H, m, H10), 5.27 (1H, m, H9), 4.15 (1H, m, H15), 3.89 (1H, dd, J = 14.0, 3.3 Hz, H8), 3.72 (2H, m, H17), 3.20
(1H, app. dd, J = 14.0, 2.3 Hz, H8), 2.26 (3H, s, 3C-O-CO-CH3), 1.81 (2H, m, H16), 1.61 (2H, m, H14), 1.48 (2H, m, H11), 1.33 (2H, m, H13), 1.13 (2H, m, H12);

13C NMR (CDCl3, 75.5 MHz): δ = 170.9, 167.6, 147.2, 140.4, 133.3, 130.3, 130.1, 128.6, 128.1, 120.9, 58.3, 43.6, 40.0, 37.9, 33.8, 32.5, 24.4, 20.9, 20.8;


Synthesis of Aldehyde 298

Dess-Martin periodinane (1.53 g, 3.625 mmol, 4 equiv) was added in one portion to a cooled (0 °C) solution of alcohol 297 (300 mg, 0.906 mmol, 1.00 equiv) in CH2Cl2 (50 mL) and the resultant mixture was stirred for 45 min at 25 °C. The white suspension was then cooled to 0 °C and aqueous solution of Na2S2O3 (20%, 100 mL) and saturated aqueous solution of NaHCO3 (100 mL) were added and the resulting mixture stirred for 30 minutes until it became clear. The mixture was diluted with CH2Cl2 (50 mL), the organic layer was separated and the aqueous layer was extracted with CH2Cl2 (3 x 50 mL). The combined organic extracts were washed with a 40% aqueous solution of sodium thiosulfate (200mL) and brine, dried over MgSO4 and concentrated. Flash chromatography provided 298 (250.47 mg, 90%) as a white solid, which was recrystallized from EtOAc affording colorless crystals suitable for X-ray crystallographic analysis.

mp 168 °C;

TLC: Rf (80% EtOAc/hexane) 0.78;
Experimental

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[α]_D^{23}: -112.5 (c 1.030, CH₂Cl₂);

**IR** ν<sub>max</sub>: 3254, 2930, 2852, 2737, 1763, 1724, 1634, 1546, 1458, 1202, 1019, 973, 756 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ = 9.81 (1H, t, J = 2.1 Hz, H₁₇), 7.28 (1H, t, J = 7.8 Hz, H₅), 7.08 (1H d, J = 7.4 Hz, H₆), 6.90 (1H, d, J = 8.1 Hz, H₄), 5.74 (1H, d, J = 7.8 Hz, NH), 5.47 (1H, dddd, J = 14.9, 10.1, 4.5, 1.6 Hz, H₁₀), 5.26 (1H, dddd, J = 14.9, 10.1, 3.4, 1.3 Hz, H₉), 4.44 (1H, m, H₁₅), 3.82 (1H, dd, J = 14.1, 3.9 Hz, H₈), 3.21 (1H, dq, J = 14.1, 2.7 Hz, H₈), 2.57, (2H, m, H₁₆), 2.29 (3H, s, 3C-O-CO-CH₃), 2.18 (1H, m, H₁₄), 1.69 (1H, m, H₁₄), 1.56 (2H, m, H₁₁), 1.43 (2H, m H₁₃), 1.11 (2H, m, H₁₂);

¹³C NMR (CDCl₃, 75.5 MHz): δ = 200.4, 171.0, 165.7, 147.3, 140.4, 132.7, 130.2, 130.1, 128.49, 128.44, 120.9, 50.2, 43.0, 37.9, 32.9, 32.7, 24.6, 21.0, 20.8;

HRMS (ESI) calculated for C₁₉H₂₃NO₄Na [M+Na]⁺ 352.15193, found 352.15199.

**Vinyl iodide 299**

![Chemical structure of vinyl iodide 299](image)

Chromium chloride (II) (112.95 mg, 0.919 mmol, 7 equiv) was added to a dry 50 mL round bottomed flask and was flame dried at reduced pressure for 5 min. After cooling to room temperature under a nitrogen atmosphere, dry THF (5 mL) was added and the slurry solution was stirred for 30 min at the same temperature followed by 5 min at 0 °C. A nitrogen purged solution of aldehyde 298 (43.20 mg, 0.131 mmol, 1.00 equiv) and iodoform (155.09 mg, 0.394 mmol, 3 equiv) in dry THF (4 mL) was then added via cannula and the initial grey suspension turned almost immediately reddish-brown. After
being stirred at room temperature for 8 h, the reaction mixture was diluted with water and extracted in Et₂O. The aqueous phase was extracted with Et₂O (3 x 20 mL) and the combined organic layers were washed with aqueous solution of Na₂S₂O₃, brine, dried over Na₂SO₄, filtered and concentrated in vacuo. The product was purified by chromatography (30% EtOAc/hexane) affording the (E)-iodoalkene 299 (38.06 mg, 72%) as a white solid and a small amount of Z-isomer 299 A.

**(E)-iodoalkene 299**

mp 154-156 °C;  
TLC: \( R_f \) (80% Et₂O/hexane) 0.72;  
\([\alpha]_{D}^{23}\) : -96.4° (c 0.805, CH₂Cl₂);  
IR \( \nu_{\text{max}} \): 3255, 2924, 1760, 1456, 1207, 1019, 966, 873, 708 cm⁻¹;  
\(^1\)H NMR (CDCl₃, 300 MHz): \( \delta = 7.32 \) (1H, t, \( J = 7.9 \) Hz, H₅), 7.16 (1H, d, \( J = 7.6 \) Hz, H₆), 6.91 (1H, dd, \( J = 8.08, 0.93 \) Hz, H₄), 6.37 (1H, dtd, \( J = 14.3, 7.3, 7.1 \) Hz, H₁₇), 6.09 (1H, app. dt, \( J = 14.3, 1.2 \) Hz, H₁₈), 5.92 (1H, d, \( J = 8.1 \) Hz, NH), 5.42 (1H, app. t, \( J = 10.0 \) Hz, H₉), 5.13 (1H, app. t, \( J = 10.5 \) Hz, H₁₀), 4.10 (1H, m, H₁₅), 3.61 (1H, dd, 15.1, 4.7 Hz, H₈), 3.17 (1H, br dd, \( J = 14.9, 2.5 \) Hz, H₈), 2.38 (1H, m, H₁₁), 2.29 (3H, m, 3C-O-CO-CH₃), 2.17 (2H, m, H₁₆), 1.94 (1H, m, H₁₁), 1.83 (1H, m, H₁₃), 1.48 (1H, m, H₁₃), 1.37 (2H, m, H₁₄), 1.16 (2H, m, H₁₂);  
\(^{13}\)C NMR (CDCl₃, 75.5 MHz): \( \delta = 170.14, 166.0, 146.8, 142.3, 140.1, 130.7, 130.5, 130.0, 129.9, 127.6, 120.2, 77.5, 49.0, 39.2, 31.4, 30.1, 26.2, 24.2, 21.4, 18.5;  
HRMS (ESI) calculated for C₂₀H₂₄INO₃Na \([M+Na]^+\) 476.06931, found 476.06950.
Experimental

Chapter 3

15 – aza - salicylamide analogue 301\textsuperscript{105}

\[
\begin{align*}
\text{OAc} & \quad \text{CuI; Cs}_2\text{CO}_3 \\
\text{N, N'-ethylene diamine} & \quad \text{DMF, rt} \\
\text{64}\% & \quad \rightarrow
\end{align*}
\]

A 10 mL, flame-dried, round bottomed flask equipped with a magnetic stirring bar, was charged with iodoalkene 299 (34.2 mg, 0.075 mmol, 1.00 equiv), Cs\textsubscript{2}CO\textsubscript{3} (132.3 mg, 0.375 mmol, 5 equiv), CuI (28.75 mg, 0.151 mmol, 2 equiv), N, N'-ethylenediamine (40.96 µL, 0.375 mmol, 5 equiv) and dienamide 101 (49 mg, 0.375 mmol, 5 equiv) before being sealed with a rubber septum and evacuated and backfilled with N\textsubscript{2} three times. To a separate dried flask was added dry DMF (2 mL), degassed rigorously (3 x) by the freeze-pump-thaw technique and transferred via cannula to the first flask. The mixture was stirred under nitrogen at room temperature and monitored by TLC. After 2 h, the reaction mixture was diluted with water, the organic layer was separated and the aqueous layer was extracted with EtOAc (3 x 15 mL). The combined organic extracts were washed with water and brine, dried over MgSO\textsubscript{4} and concentrated. Flash chromatography provided 301 (19.7 mg, 64\%) as a white solid.

\textbf{mp} 151-152 °C;

\textbf{TLC}: \textit{R}_f (55\% EtOAc/hexane) 0.41;

[\alpha]_{\text{D}}^{23} = -13.4^\circ (c 0.210, \text{CH}_2\text{Cl}_2);

\textbf{IR} \quad \nu_{\text{max}}: 3348, 2931, 2471, 2243, 2141, 2071, 1633, 1595, 1461, 1121, 1090, 972, 821 cm\textsuperscript{-1}

\textbf{\textsuperscript{1}H NMR} (CD\textsubscript{3}OD, 300 MHz): \delta = 7.32 (1H, dt, \textit{J} = 10.8, 1.2 Hz, \textbf{H22}), 7.08 (1H, t, \textit{J} = 7.9 Hz, \textbf{H5}), 6.87 (1H, dd, \textit{J} = 11.6, 1.1 Hz, \textbf{H21}), 6.80 (1H, d, \textit{J} = 14.4 Hz, \textbf{H18})
(1H, d, J = 7.9 Hz, H4), 6.6 (1H, d, J = 7.7 Hz, H6), 5.83 (1H, dddd, J = 10.8, 7.7, 7.5, 1.3, 1.1 Hz, H23), 5.70 (1H, d, J = 11.6 Hz, H20), 5.41 (2H, m, H17, H10), 5.26 (1H, m, H9), 3.97 (1H, m, H15), 3.76 (1H, dd, J = 14.3, 4.1 Hz, H8), 3.13 (1H, br dd, J = 14.3, 2.3 Hz, H8) 2.31 (2H, dquin, J = 7.5, 7.5, 7.5, 7.5, 1.5 Hz, H24), 2.25 (2H, m, H16), 2.16 (1H, m, H11), 1.69 (1H, m, H11), 1.56 (2H, m, H12), 1.42 (2H, m, H14), 1.20 (2H, m, H13), 1.03 (3H, t, J = 7.5 Hz, H25);

13C NMR (CD3OD, 75.5 MHz): δ = 170.0, 165.9, 155.9, 142.6, 141.0, 137.7, 133.4, 131.0, 130.0, 126.3, 125.7, 125.5, 122.5, 120.6, 115.2, 112.1, 49.6, 39.3, 37.0, 33.9, 32.9, 26.3, 22.2, 21.6, 14.5;


4. Synthesis of amide fragment 10165,67,94

Synthesis of gem-dibromide 17767

\[
\begin{align*}
\text{176} & \xrightarrow{\text{CBr}_4, \text{Ph}_3\text{P}} \xrightarrow{\text{CH}_2\text{Cl}_2, -5\text{C to 25\text{C}}} \xrightarrow{68\%} \text{177}
\end{align*}
\]

An oven dried 100 mL two-necked round-bottomed flask equipped with a stirrer and a calcium chloride drying tube is charged with 40 mL of CH2Cl2 and cooled at -5 °C. Carbon tetrabromide (2 g, 6.03 mmol, 1 equiv) was added in one portion and the solution was stirred in the cold for 25 minutes. To this solution was added freshly distilled propionaldehyde 176 (0.35 g, 6.03 mmol, 1 equiv) and PPh3 (3.95 g, 15.07 mmol, 2.5 equiv) over 30 minutes. The mixture was stirred at the same temperature for 20 minutes, allowed to warm to room temperature and stirred for additional 2 hours. Upon
consumption of the starting material, 20 mL n-pentane was added, under stirring, when the reaction mixture thickened noticeably. The slurry was filtered and the solid was washed with n-pentane; the filtrates were combined and the solvent was removed under vacuum. The resulting liquid was distilled under reduced pressure to yield 177 (0.87 g, 68%) as a colorless liquid.

The \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra of 177 were identical with those reported in the literature.

\textbf{IR} $\nu_{\text{max}}$: 2970, 2923, 1713, 1139, 805, 674 cm$^{-1}$;  

\textbf{\textsuperscript{1}H NMR} (CDCl$_3$, 300 MHz): $\delta = 6.40$ (1H, t, $J = 7.19$ Hz), 2.13 (1H, d, $J = 7.4$ Hz), 1.05 (1H, t, $J = 7.5$ Hz);  

\textbf{\textsuperscript{13}C NMR} (CDCl$_3$, 75.5 MHz): $\delta = 140.1$, 88.2, 26.5, 12.3.

\textbf{Z – iodoacrylate 166$^{67}$}

\begin{center}
\begin{tikzpicture}
\node [draw] (a) at (0,0) {179};
\node [draw] (b) at (1.5,0) {166};
\node [draw] (c) at (0.75,0.5) {Li, CH$_3$COOH};
\node [draw] (d) at (1.25,0.5) {CH$_3$CN, 70$^\circ$C};
\node [draw] (e) at (0.75,-0.5) {79\%};
\end{tikzpicture}
\end{center}

To a stirred solution of LiI (10.12 g, 75.61 mmol, 1.5 equiv) in acetonitrile (74 mL) was added methyl propiolate 179 (4.23 g, 50.35 mmol, 1 equiv) and acetic acid (6 mL). After heating at 70 $^\circ$C for 19 h, the reaction mixture was diluted with 200 mL of H$_2$O and the mixture was extracted with diethyl ether (3 x 100 mL). The combined organic extracts were sequentially washed with a saturated aqueous solution of sodium thiosulfate and brine. The organic layer was dried over MgSO$_4$, filtered and concentrated in vacuo to give the crude product, which was purified by silica gel column chromatography (20% Et$_2$O/hexane) to give 166 (8.42 g, 79%) as a colorless oil.
The $^1$H NMR and $^{13}$C NMR spectra of 166 were identical with those reported in the literature.

**TLC:** $R_f$ (20% Et$_2$O/hexane) 0.67;

**IR** $\nu_{\text{max}}$: 3031, 2950, 2840, 1724, 1596, 1324, 1200, 1161, 806 cm$^{-1}$.

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 7.48 (1H, d, 8.9 Hz), 6.91 (1H, d, 8.9 Hz), 3.78 (3H, s);

$^{13}$C NMR (CDCl$_3$, 75.5 MHz): $\delta$ = 164.9, 129.5, 95.3, 51.6.

**Preparation of enyne 172**

Under a nitrogen atmosphere, to a solution of 1,1-dibromobutene 177 (2.78 g, 13 mmol, 1 equiv) in dry THF (40 mL) at -78 °C was added dropwise n-BuLi (11.44 mL, 2.5 M in hexane, 2.2 equiv), and the mixture was stirred at the same temperature for 1.5h and for 1h at room temperature. The reaction was then brought to -50°C and a solution of ZnCl$_2$ (2.04 g, 15 mmol, 1.15 equiv) in THF (20 mL) was added via syringe. The reaction mixture was allowed to warm to ambient temperature. A solution of ester 305 (2.09 g, 9.86 mmol, 0.76 equiv) in THF (20 mL) and Pd(PPh$_3$)$_4$ (232 mg, 0.20 mmol, 2 mol%) in THF (20 mL) were subsequently introduced, and stirring of the mixture was continued for 5h. The reaction was quenched with water followed by extraction of the aqueous layer with diethyl ether. The combined organic layers were washed with water and brine, dried with MgSO$_4$, and filtered. The solvent was removed in vacuo to give crude product that was chromatographed on silica gel to provide 172 (1.19g, 88 %) as a colorless liquid.

The $^1$H NMR and $^{13}$C NMR spectra of 172 were identical with those reported in the
Experimental Chapter 3

literature.

**TLC**: $R_f = 0.55$ (5% EtOAc/hexane).

**IR**: $\nu_{\text{max}}$: 3012, 2979, 2950, 2880, 2210, 1726, 1609, 1436, 1404, 1290, 1232, 1192, 1170, 814 cm$^{-1}$.

**$^1$H NMR** (CDCl$_3$, 300 MHz): $\delta = 6.14$ (1H, dt, $J = 11.3$, 2. 3 Hz), $6.03$ (1H, $J =11.3$ Hz), 3.74 (3H, s), 2.45 (2H, qd, $J = 7.5$, 2.3 Hz), 1.21 (3H, t, $J = 7.5$);

**$^{13}$C NMR** (CDCl$_3$, 75.5 MHz): $\delta = 165.3, 126.9, 124.2, 105.4, 77.0, 51.3, 13.7, 13.4$.

**Synthesis of dienoate 169$^{67}$**

In a two-neck flask was placed a solution of 172 (74.9 mg, 0.57 mmol, 1 equiv) in CH$_2$Cl$_2$ (36 mL, dried over calcium hydride). After three vacuum/H$_2$ cycles to remove air from the reaction flask, Lindlar’s catalyst (3.176 mg, 4 wt %) and freshly distilled quinoline (4µL, 6 mol%) were added under positive H$_2$ pressure. The resulting suspension was stirred and hydrogenated at balloon pressure and room temperature for 3h. The resulting mixture was filtered through Celite to remove the catalyst, washed with CH$_2$Cl$_2$ (3x40mL) and the filtrated was concentrated in vacuo. The resulting crude product was purified by flash chromatography (5% Et$_2$O/hexane). The diester 169 was obtained as a colorless liquid (51.67g, 68%).

The **$^1$H NMR** and **$^{13}$C NMR** spectra of 169 were identical with those reported in the literature.
Experimental Chapter 3

**TLC:** \( R_f \) (5% Et₂O/hexane) 0.48;

**\(^1\)H NMR** (CDCl₃, 300 MHz): \( \delta = 7.16 \) (1 H, dd, \( J = 11.1, 1.1 \) Hz), 6.87 (1 H, \( J = 11.8, 1.0 \) Hz), 5.89-5.78 (1H, m), 5.60 (1 H, d, \( J = 11.4 \) Hz), 3.65 (3H, s), 2.20 (2 H, dq, \( J = 7.5, 1.5 \) Hz), 0.96 (3H, t, \( J = 7.5 \) Hz);

**\(^{13}\)C NMR** (CDCl₃, 75.5 MHz): \( \delta = 166.9, 143.2, 139.0, 123.7, 116.9, 77.4, 77.0, 76.6, 51.1, 20.8, 13.9 \).

**ZZ** - heptadienamide 101

\[
\begin{array}{c}
\text{O} \\
\text{OCH₃}
\end{array} \xrightarrow{\text{NH₄OH, CH₃OH} 46\%} \begin{array}{c}
\text{O} \\
\text{NH₂}
\end{array}
\]

A mixture of diene 172 (183 mg, 1.30 mmol, 1 equiv) and 28% solution of NH₄OH (35 mL/mmol) at room temperature was stirred for 4 days. Cold water was added and after stirring for 15 minutes the mixture was extracted with ethyl acetate (3 x 40 mL). The combined organic extracts were washed successively with water and brine. Following drying over anhydrous MgSO₄, the suspension was filtered, volatiles were removed under reduced pressure, and the residue was subjected to silica gel column chromatography (eluting with ethyl acetate) to afford 101 as a white solid (51.52 mg, 46%).

The **\(^1\)H NMR and \(^{13}\)C NMR** spectra of 101 were identical with those reported in the literature.

**TLC:** \( R_f \) (EtOAc) 0.64;

**IR** \( \nu_{max} \): 3395, 3187, 3010, 2968, 2934, 1650, 1604, 1590, 1453, 1324, 806 cm⁻¹;
$^1$H NMR (CDCl$_3$, 300 MHz): $\delta = 7.13$ (1H, m), 6.73 (dd, $J = 11.6$, 1.1 Hz), 6.11 – 5.88 (br s, 2H), 5.82 – 5.71 (1H, m), 5.59 (1H, d, $J = 11.5$ Hz), 2.19 (2H, dq, $J = 7.5$, 1.5 Hz), 0.9 (3H, t, $J = 7.5$ Hz).

$^{13}$C NMR (CDCl$_3$, 75.5 MHz): $\delta = 168.8$, 141.8, 136.1, 123.6, 119.4, 20.7, 13.9.
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References

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References


88. White, C. *Honours Thesis*, University of Melbourne, **2006**.


APPENDICES
IR - Alcohol 282
IR - Amine 285
Appendix A

[Graph with data points and wavelengths in cm⁻¹]

IR - RCM cis (296)
Appendix A

IR - Primary alcohol 297
Appendix A

IR - Aldehyde 298
Appendix B

ppm

PMBO

N

284
PMBO\textsubscript{2}NH

284

285

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm H N O OH OPMB 286
293

H

N

OAc

OPMB
$^1$H NMR (CDCl$_3$, 300 MHz)
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Diagram: [Chemical Structure Image]
Table 6

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<th>Total steps</th>
<th>Overall yield</th>
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<td>43.8%</td>
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