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Synthesis, Design, and Biological Evaluation of Inhibitors and Activators of Src Homology 2 Domain-Containing Inositol Phosphatase (SHIP) and Synthetic Studies of Apicularen A and Maoecrystal V

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ABSTRACT OF THE DISSERTATION

This dissertation involves work both on the syntheses of small molecules with potential therapeutic applications and synthetic studies on natural products with unusual core skeletons.

The first part of this work involves the synthesis, design, and biological evaluation of aminosteroids as inhibitors for the SH2–containing inositol phosphatase 1 (SHIP1). SHIP1, a 145 kDa protein, is involved in regulating the formation of new blood cells. High–throughput screening identified several SHIP inhibitors including NSC23922, a mixture of 3α– and 3β–amincholestan. Both 3α– and 3β–amincholestan were synthesized from dihydrocholesterol and the 3α form was verified as SHIP1 inhibitor. Comparison of the 3α– and 3β–amincholestan showed that the former was the more active form. Further biological studies on 3α–amincholestan (3AC) showed similar biological activities observed with NSC23922 such as rapid and increased recovery of blood components in myelosuppressed hosts, increased myeloid immunoregulatory (MIR) cells, and reduced survival of cancer cells that express SHIP1. Synthetic studies were undertaken to determine which portions of the aminosteroid SHIP1 inhibitors are important for its biological activity and to improve the water solubility of the aminosteroid. This led to the synthesis of androsterone based derivatives with greater potency and solubility. Syntheses of other derivatives with additional polar functional group on ring A were also explored. While these molecules showed better SHIP1 inhibitory activity compared with 3AC, many showed significant inhibition of SHIP2, suggesting that not all aminosteroids are SHIP1 selective inhibitors. Some known
SHIP1 activators were also pursued, as these molecules would be useful for validation of screening techniques and biological assays. The reported structure containing a sulfone moiety was problematic to test due to its low solubility in water or dimethyl sulfoxide (DMSO).

The second part of this work involves the synthesis of thiophene based SHIP2 inhibitors AS1949490 and AS1938909 and some analogs of these molecules. SHIP2 inhibitors have potential therapeutic application to breast cancer treatment since overexpression of SHIP2 in breast cancer supports cell proliferation while its suspension retards cancer growth. In addition, the inhibitory activities against SHIP1 of these molecules were evaluated. Although both molecules did not show any inhibitory potency against SHIP1, both molecules showed comparable SHIP2 inhibitory activity. The analogs of AS1949490, particularly the 4–bromobenzyl and 4–methoxybenzyl analogs showed almost a 3–fold increase in potency against SHIP2 compared to the parent molecule.

The third part of this work involved studies towards the synthesis of apicularen A. Apicularen A is a macrosalicylic natural product isolated from the myxobacterial genus *Chondromyces* and showed potent cytotoxic activity against several cancer cell lines. A synthetic route towards apicularen A was investigated featuring an intramolecular Diels–Alder reaction using an aldehyde as a dienophile as the key step.

The fourth part of this work involves the synthetic studies on maocystal V. Maocystal V is a diterpenoid containing a unique kaurane skeleton. This compound was isolated from the leaves of *Isodon eriocalyx* and shows selective cytotoxic activity
towards HeLa cells having an IC$_{50}$ of 0.02 µg/mL. The syntheses of the tricylic core of this molecule were explored using a Diels–Alder reaction followed by a subsequent annulation reaction.
SYNTHESIS, DESIGN, AND BIOLOGICAL EVALUATION OF INHIBITORS AND ACTIVATORS OF Src HOMOLOGY 2 DOMAIN–CONTAINING INOSITOL PHOSPHATASE (SHIP) AND SYNTHETIC STUDIES ON APICULAREN A AND MAOECRYSTAL V

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DISSERTATION

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By

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Graduate school was difficult and stressful, not to mention doing all of this away from home. Nevertheless, I have enjoyed everything. The whole journey was amazing and I am truly humbled by all of these experiences. There are a lot of people for whom I am most thankful. These people inspired me to be better and helped me in my journey of finding my own eureka moment. Also, they made this journey less difficult than it could have been. I would not have accomplished anything without them.

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<th>Description</th>
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<tbody>
<tr>
<td>[α]</td>
<td>Specific rotation</td>
</tr>
<tr>
<td>3AC</td>
<td>3α–aminocholestane</td>
</tr>
<tr>
<td>Akt</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>Akt1</td>
<td>Protein kinase B 1</td>
</tr>
<tr>
<td>Akt2</td>
<td>Protein kinase B 2</td>
</tr>
<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
<tr>
<td>AML</td>
<td>Acute myelogenous leukemia</td>
</tr>
<tr>
<td>Anal.</td>
<td>Combustion elemental analysis</td>
</tr>
<tr>
<td>anhyd</td>
<td>Anhydrous</td>
</tr>
<tr>
<td>ATG</td>
<td>Autophagy–related</td>
</tr>
<tr>
<td>BAECs</td>
<td>Bovine aortic endothelial cells</td>
</tr>
<tr>
<td>bFGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated hydroxytoluene</td>
</tr>
<tr>
<td>BM</td>
<td>Bone marrow</td>
</tr>
<tr>
<td>BMMC</td>
<td>Bone marrow mast cell</td>
</tr>
<tr>
<td>bs</td>
<td>Broad singlet</td>
</tr>
<tr>
<td>Btk</td>
<td>Bruton’s tyrosine kinase</td>
</tr>
<tr>
<td>calc</td>
<td>Calculated</td>
</tr>
<tr>
<td>CD</td>
<td>Crohn’s Disease</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>CI</td>
<td>Chemical ionization</td>
</tr>
<tr>
<td>CLogP</td>
<td>Calculated partition coefficient</td>
</tr>
<tr>
<td>cod</td>
<td>1,5–Cyclooctadiene</td>
</tr>
<tr>
<td>compd</td>
<td>Compound</td>
</tr>
<tr>
<td>concd</td>
<td>Concentrated</td>
</tr>
<tr>
<td>COSMIC</td>
<td>College of Science Major Instrumentation</td>
</tr>
<tr>
<td>CSA</td>
<td>Camphorsulfonic acid</td>
</tr>
<tr>
<td>Cy</td>
<td>Cyclohexyl</td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift in part per million</td>
</tr>
<tr>
<td>DCE</td>
<td>1,2–Dichloroethane</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
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<tr>
<td>DBU</td>
<td>1,8–Diazabicyclo[5.4.0]undec–7–ene</td>
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<tr>
<td>DEPT</td>
<td>Distortionless enhancement by polarization transfer</td>
</tr>
<tr>
<td>DIAD</td>
<td>Diisopropyl azodicarboxylate</td>
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<td>DIBAL</td>
<td>Diisobutylaluminium hydride</td>
</tr>
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<td>DMAP</td>
<td>4–Dimethyl aminopyridine</td>
</tr>
<tr>
<td>dba</td>
<td>Dibenzylideneacetone</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
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<tr>
<td>DMP</td>
<td>Dess–Martin periodinane</td>
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<td>DMPU</td>
<td>1,3–dimethyl–3,4,5,6–tetrahydro–2(1H)–pyrimidinone</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>ERK</td>
<td>Extracellular regulated kinase</td>
</tr>
<tr>
<td>ES</td>
<td>Embryonic stem</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>FP</td>
<td>Fluorescence polarization</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform</td>
</tr>
<tr>
<td>Gab</td>
<td>Grb2–associated binding</td>
</tr>
<tr>
<td>Glut4</td>
<td>Glucose transporter type 4</td>
</tr>
<tr>
<td>Grp1</td>
<td>General receptor for phosphoinositides 1</td>
</tr>
<tr>
<td>GSK3β</td>
<td>Glycogen synthase kinase 3β</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>GvHD</td>
<td>Graft vs. Host disease</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>HGF</td>
<td>Hepatocyte growth factor</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear multiple bond correlation</td>
</tr>
<tr>
<td>HRMS</td>
<td>High–resolution mass spectroscopy</td>
</tr>
<tr>
<td>HSC</td>
<td>Hematopoietic stem cells</td>
</tr>
<tr>
<td>HTS</td>
<td>High–throughput screening</td>
</tr>
<tr>
<td>HWE</td>
<td>Horner–Wadsworth–Emmons</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
<td>I–1,3,4,5–P&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Inositol–1,3,4,5–tetrakisphosphate</td>
</tr>
<tr>
<td>IL–1β</td>
<td>Interleukin–1β</td>
</tr>
<tr>
<td>IMDA</td>
<td>Intramolecular Diels–Alder</td>
</tr>
<tr>
<td>IMHDA</td>
<td>Intramolecular hetero Diels–Alder reaction</td>
</tr>
<tr>
<td>IP</td>
<td>Inositol phospholipid</td>
</tr>
<tr>
<td>IP&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Inositol–1,2,4,5–tetrakisphosphate</td>
</tr>
<tr>
<td>JNK</td>
<td>c–Jun N–terminal kinases</td>
</tr>
<tr>
<td>K&lt;sub&gt;d&lt;/sub&gt;</td>
<td>Equilibrium dissociation constant</td>
</tr>
<tr>
<td>LAH</td>
<td>Lithium aluminum hydride</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropylamine</td>
</tr>
<tr>
<td>lit.</td>
<td>Literature value</td>
</tr>
<tr>
<td>LN</td>
<td>Lymph node</td>
</tr>
<tr>
<td>MAP</td>
<td>Mitogen–activated protein</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen–activated protein kinases</td>
</tr>
<tr>
<td>m–CPBA</td>
<td>meta–Chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>MEF</td>
<td>Mouse embryonic fibroblasts</td>
</tr>
<tr>
<td>Mes</td>
<td>2,4,6–Trimethylphenyl (mesityl)</td>
</tr>
<tr>
<td>MDCK</td>
<td>Madin–Darby canine kidney</td>
</tr>
<tr>
<td>MG&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Malachite Green</td>
</tr>
<tr>
<td>MIR</td>
<td>Myeloid immunoregulatory</td>
</tr>
<tr>
<td>MM</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>MOM</td>
<td>Methoxymethyl</td>
</tr>
<tr>
<td>Ms</td>
<td>Methylsulfonyl (mesyl)</td>
</tr>
<tr>
<td>MS</td>
<td>Molecular sieves</td>
</tr>
<tr>
<td>MySCs</td>
<td>Myeloid suppressor cells</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
</tr>
<tr>
<td>NHK</td>
<td>Nozaki–Hiyama–Kishi</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>NMO</td>
<td>N–Methylmorpholine N–oxide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NO</td>
<td>Nitrite</td>
</tr>
<tr>
<td>NOESY</td>
<td>Nuclear Overhausser effect spectroscopy</td>
</tr>
<tr>
<td>PCC</td>
<td>Pyridinium chlorochromate</td>
</tr>
<tr>
<td>PDC</td>
<td>Pyridinium dichlorochromate</td>
</tr>
<tr>
<td>PDK1</td>
<td>Phosphatidylinositol kinase 1</td>
</tr>
<tr>
<td>PH</td>
<td>Pleckstrin homology</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphatidylinositol–3–kinase</td>
</tr>
<tr>
<td>PI–3,4–P₂</td>
<td>Phosphatidylinositol–3,4–bisphosphate</td>
</tr>
<tr>
<td>PI–3,4,5–P₃</td>
<td>Phosphatidylinositol–3,4,5–trisphosphate</td>
</tr>
<tr>
<td>IPn</td>
<td>Phosphoinositides</td>
</tr>
<tr>
<td>PPTS</td>
<td>Pyridinium para–toluenesulfonate</td>
</tr>
<tr>
<td>Piv</td>
<td>Pivalate</td>
</tr>
<tr>
<td>PKB</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>PLC–γ</td>
<td>Phospholipase C–γ</td>
</tr>
<tr>
<td>PMP</td>
<td>para–Methoxyphenyl</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and tensin homolog</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>p–TsCl</td>
<td>para–Toluenesulfonyl chloride</td>
</tr>
<tr>
<td>Ras</td>
<td>Receptor tyrosine kinases</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cell</td>
</tr>
<tr>
<td>RCM</td>
<td>Ring closing metathesis</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure–activity relationship</td>
</tr>
<tr>
<td>Shc</td>
<td>Src homology 2–containing</td>
</tr>
<tr>
<td>SH2</td>
<td>Src homology 2 containing</td>
</tr>
<tr>
<td>SHIP</td>
<td>Src homology 2 domain–containing inositol 5’–phosphatase</td>
</tr>
<tr>
<td>SHIP1</td>
<td>Src homology 2 domain–containing inositol 5’–phosphatase 1</td>
</tr>
<tr>
<td>SNPs</td>
<td>Single–nucleotide polymorphisms</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetrabutylammonium fluoride</td>
</tr>
<tr>
<td>TBS</td>
<td>tert–Butyldimethylsilyl</td>
</tr>
<tr>
<td>TBDDS</td>
<td>tert–Butyldiphenylsilyl</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6–Tetramethylpiperidin–1–oxyl</td>
</tr>
<tr>
<td>TES</td>
<td>Triethylsilyl</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TFAA</td>
<td>Trifluoroacetic anhydride</td>
</tr>
<tr>
<td>TFP</td>
<td>Tri–2–furylphosphine</td>
</tr>
<tr>
<td>THP</td>
<td>Tetrahydropyran–2–yl</td>
</tr>
<tr>
<td>TMEDA</td>
<td>N,N,N,N–Tetramethylethylenediamine</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethyldisilane</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TIPS</td>
<td>Triisopropyl</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>Tf</td>
<td>Trifluoromethanesulfonyl (triflyl)</td>
</tr>
<tr>
<td>Ts</td>
<td>para–Toluenesulfonyl (tosyl)</td>
</tr>
<tr>
<td>V–ATPases</td>
<td>Vacuolar (H⁺)–ATPases</td>
</tr>
<tr>
<td>Yphos</td>
<td>Tyrosine phosphorylated</td>
</tr>
</tbody>
</table>
DEDICATION

For Papa, Mama, Cherrymaine, Lola Lita, and Lola Etang

“I think if you do something and it turns out pretty good, then you should go do something else wonderful, not dwell on it for too long. Just figure out what’s next.”

Steven Paul Jobs
CHAPTER 1

THERAPEUTIC POTENTIAL OF INOSITOL PHOSPHATASE MODULATORS

Introduction

In order to react to changes in their environment, eukaryotic cells must be able to transfer information about the extracellular environment through the plasma membrane inside the cell to the nucleus. Passages of these signals through the membrane are often mediated by receptors, which are activated by a variety of extracellular stimuli. These receptors then initiate a signaling cascade through a complex network of enzymes and second messengers inside the cell, resulting in a number of intracellular events. In many of these signaling pathways phosphatidyl inositols have become recognized as key participants. Phosphoinositides (PIPs) are minor phospholipid components of the cell membranes in eukaryotic cells. The pattern of phosphorylation present on the inositol ring acts as a key recognition element for several protein kinases contributing to the communication between cells and their external environments. The plethora of complex kinases and phosphatases known to metabolize PIPs demonstrates their significance in cellular physiology and the need for their tight regulation. Phosphatidylinositols play an important role in a vast array of cellular functions including mitogenesis, vesicle trafficking, secretion, motility, adherence, and death.¹ These inositol–containing phospholipids also manifest cellular effects by acting as important secondary messengers in major signaling networks controlled by various proteins, kinases, phosphatases, and phospholipases.²
The phosphatidylinositol–3–kinase (PI3K) pathway is one of the most well known phosphatidylinositol containing signaling pathways, and has been shown to have widespread effects cellular physiology. The PI3K pathway cannot perform its diverse effects on cellular function without its key secondary messenger, phosphatidylinositol–3,4,5–trisphosphate **1.2** (PI–3,4,5–P₃, **Figure 1–1**), which the cell normally maintains at low concentration. However, upon stimulation by extracellular stimuli, PI3K can quickly synthesize PI–3,4,5–P₃ from phosphatidylinositol–4,5–bisphosphate **1.1** (PI–4,5–P₂) and rapidly increase the intracellular concentration of this secondary messenger. PI–3,4,5–P₃, located on the plasma membrane, directly binds and thereby drafts various signaling proteins with pleckstrin homology (PH) domains to the membrane. The PH domains, consists of approximately 120 amino acids, occurs in proteins that are involved in intracellular signaling. Some of these proteins are protein serine–threonine kinases, such as protein kinase B (Akt) and phosphoinositide kinase 1 (PDK1); protein tyrosine kinases, such as the Tec family; exchange factors for guanosine triphosphate (GTP) – binding proteins, such as general receptor for phosphoinositide 1 (Grp1) and the Rac family (subfamily of Rho GTP–binding proteins); and adaptor proteins. Upon activation, these proteins then help to initiate even more signaling cascades, which affect numerous cellular functions. Catimel has shown that PI–3,4,5–P₃ **1.2** ultimately influences molecular trafficking, protein trafficking, vesicle mediated transport, regulation of the actin cytoskeleton and GTPase function, cellular signaling, development, movement, organization, growth, and proliferation. In addition, Prestwich has also shown that PI–3,4,5–P₃ **1.2** influences neutrophil migration and insulin action. Due to the pervasive influences of PI–3,4,5–P₃ **1.2** and its associated proteins, the over or under activation of
the PI3K pathway has been implicated in many altered human metabolic states such as
cancer, diabetes, cardiovascular disease, Alzheimer’s disease, bacterial infections,
allergies, and autoimmune disorders.\textsuperscript{2} As a result, studies of the regulators of this
significant pathway may lead to the discovery of major pharmacological targets for
therapeutic intervention.\textsuperscript{3}

Figure 1–1. Modification of inositols mediated by PI3K, PTEN, SHIP and INPP4.

PI–3,4,5–P\textsubscript{3} levels are also governed by its degradation by the inositol
phosphatases PTEN (phosphatase and tensin homolog protein) and SHIP (src homology 2
(SH2) – containing inositol 5′–phosphatase).\textsuperscript{6–7} Although PTEN and the SHIP both
negatively regulate the PI3K pathway, they do so in different ways. PTEN converts PI–
3,4,5–P\textsubscript{3} \textbf{1.2} to PI–4,5–P\textsubscript{2} \textbf{1.1} while the SHIP isoforms convert PI–3,4,5–P\textsubscript{3} \textbf{1.2} to
phosphatidylinositol–3,4–bisphosphate \textbf{1.3} (PI–3,4–P\textsubscript{2}).\textbf{(Figure 1–1)}\textsuperscript{6} By decreasing the
cellular concentration of PI–3,4,5–P\textsubscript{3} \textbf{1.2}, PTEN and SHIP can control many of the
pathway’s downstream effector cascades.

PTEN is a 403 amino acid protein located on the human chromosome 10q23, a
region that is often deleted in many malignant tumors.\textsuperscript{6–7} The enzyme’s N–terminus
contains a 3–phosphatase catalytic domain that is directly adjacent to a C2 domain.\textsuperscript{6} PTEN is primarily known as a tumor suppressor gene as PTEN mutations or deletions are found in a high percentage of a wide variety of both hereditary and non–hereditary human cancers.\textsuperscript{8} PTEN heterozygous mutations have been associated with Cowden disease,\textsuperscript{6} endometrial cancer, malignant melanomas,\textsuperscript{7} glioblastomas, prostate cancer, breast cancer, and T–cell and B–cell lymphomas.\textsuperscript{8} PTEN is actually now known as one of the most commonly mutated genes in human cancer.\textsuperscript{7} PTEN functions as an inositol lipid phosphatase; however, it also functions as a specific protein phosphatase. Despite this dual functionality, its role as an inositol lipid phosphatase is crucial for its tumor suppressing activity. By hydrolyzing PI–3,4,5–P\textsubscript{3} \textbf{1.2} to PI–4,5–P\textsubscript{2} \textbf{1.1}, PTEN acts as a gatekeeper, keeping Akt from becoming hyperactivated.\textsuperscript{9} Hyperactivation of Akt prevents cells from responding to their normal apoptotic stimuli. As a result, cells do not undergo apoptosis when they normally should and survive much longer than normal, leading to abnormal growth in tissues.\textsuperscript{8}

While PTEN converts PI–3,4,5–P\textsubscript{3} \textbf{1.2} to PI–4,5–P\textsubscript{2} \textbf{1.1}, SHIP converts PI–3,4,5–P\textsubscript{3} \textbf{1.2} to PI–3,4–P\textsubscript{2} \textbf{1.3} and is therefore classified as inositol phospholipid 5’–phosphatase. Two major forms of SHIP are associated with the PI3K pathway: SHIP1 and SHIP2. SHIP1 consists of a SH2 domain at the N–terminus, a central phosphatase catalytic region, and two PTB domains at the proline–rich C–terminal.\textsuperscript{10} The SH2 domain gives rise to an antiparallel β–sheet that lies between two α–helices.\textsuperscript{11} The two PTB domains are each NPXY motifs – one contains asparagine (NPNY) and the other contains leucine (NPLY). The C–terminus also contains a glycine–rich P–loop, a conserved motif in many adenosine triphosphate (ATP) and GTP–binding proteins.\textsuperscript{12} Expression of SHIP1
is primarily confined to cells of the hematopoietic lineage, primarily blood and bone marrow cells.

Several forms of SHIP1 have been detected, including isoforms weighing 145kDa (SHIP1α), 135KDa (SHIP1β) and 110KDa (SHIP1δ). Additionally several truncated SHIP1 proteins have also been discovered. Typically these truncated forms have different protein binding properties owing to the deletion or altered expression of protein binding domains. One example of a truncated SHIP is sSHIP, which is missing the SH2 domain but retains the C2 domain, catalytic domain and proline rich domain. This version of SHIP (and its human homologue, SIP–110) are typically localized at the plasma membrane rather than the cytoplasm. While expression of sSHIP was first thought to be restricted to embryonic STEM cells, the enzyme has also been recently reported in adult hematopoietic cells.

A paralog of SHIP1, SHIP2, is expressed in all cells in the human body. SHIP2 consists of a N–terminal SH2 domain, a central lipid 5–phosphatase domain, and a proline rich C–terminal region containing a phosphotyrosine binding (PTB) consensus sequence. The PTB consensus sequence contains a NPYX motif, a site for tyrosine phosphorylation, in which N is asparagine, P is proline, X is any amino acid, and Y is tyrosine. In humans, especially high levels of SHIP2 are found in the heart, skeletal muscle, and the placenta.

SHIP1 and SHIP2 share a high level of amino acid conservation. The overall amino acid sequence identity between the two proteins is 42.7%. Their SH2 domains have 54% identity in primary structure while their catalytic domains have 64% identity. Despite this homology in amino acid sequence, SHIP1 and SHIP2 vastly differ in their
expression and binding kinetics. As mentioned above, SHIP2 is ubiquitously expressed across various cell types and is especially prevalent in skeletal muscle, the heart, and the brain.\textsuperscript{16} SHIP1’s expression, however, is limited mostly to hematopoietic cells, including hematopoietic stem cells (HSCs), and some endothelial cells.\textsuperscript{17} SHIP1 is preferentially recruited to the plasma membrane over SHIP2 in hematopoietic cells, whereas SHIP2 is recruited to all other cells in the body. Furthermore, the binding kinetics of their SH2 domains also varies. The SH2 domain of SHIP2 associates and disassociates from its ligand proteins very slowly. As a result, determining the equilibrium dissociation constant ($K_D$) with certain peptides is very difficult. Meanwhile, the SH2 domain of SHIP1 quickly associates and disassociates with its ligand proteins. In fact, SHIP1 can reach its binding equilibrium in less than 30 seconds with certain peptides.\textsuperscript{16}

These differences in cellular expression and binding kinetics may explain the varying roles these two homologous proteins play in cellular pathology. As briefly discussed above, SHIP2 acts as an important negative regulator of the insulin–signaling pathway. SHIP1, on the other hand, functions as an important negative controller in immunoreceptor signaling\textsuperscript{8} and hematopoietic progenitor cell proliferation/survival,\textsuperscript{15} and as an inducer of cellular apoptosis.\textsuperscript{13} Interestingly, SHIP1 has also been implicated both as a hematopoietic tumor suppressor and activator.\textsuperscript{18} SHIP1 knockout mice demonstrate the significance of the overall role SHIP1 plays in the cell. While these mice are viable and fertile, they display several abnormal pathologies, such as progressive splenomegaly\textsuperscript{15} (enlargement of the spleen), massive infiltration and consolidation of the lungs by macrophages\textsuperscript{13} (a type of white blood cell involved in phagocytosis),\textsuperscript{19} and a shortened life span. By the time these mice are only 14 weeks old, their chance of
survival is only 40%. This combined data confirms the importance of SHIP1 in the proper functioning of the cell.

While the exact role SHIP1 plays in cellular processes is still under investigation, a general understanding seems to be emerging. Under normal cellular conditions, SHIP1 resides in the cytoplasm. However, its primary enzymatic substrate, PI–3,4,5–P₃ 1,2, is found on the plasma membrane. To perform its catalytic function, SHIP1 must somehow translocate to the plasma membrane. This translocation is accomplished through the association of SHIP1 with a wide range of major receptor complexes. SHIP1 may be recruited to these receptor complexes with either adaptor and scaffold proteins and/or direct binding via the SH2 domain. Important adaptor proteins that bind to SHIP1 are Shc, Grb2, and downstream of tyrosine kinase (Dok) proteins. Major scaffold proteins that bind to SHIP1 and help facilitate its recruitment to receptor complexes are Grb2–associated binding (Gab) proteins – Gab1 and Gab2. In addition, SHIP1 can directly bind to certain receptor complexes through its SH2 domain. Once SHIP1 becomes associated with a receptor complex, its location at the plasma membrane now allows it to hydrolyze PI–3,4,5–P₃ 1,2. Hydrolysis of PI–3,4,5–P₃ 1,2 blocks PI3K effector pathways and thereby blocks the further recruitment of many proteins – including important kinases, such as Akt, Bruton’s tyrosine kinase (Btk), and phospholipase C–gamma (PLC–g). As a result, SHIP1 can potentially influence many aspects of cellular pathology. (Figure 1–2)
Figure 1–2. The PI3K/Akt/mTor Pathway.

Inositol Phosphatase Inhibition in the Treatment of Disease

Modulation of PI(3,4,5)P₃ levels has become a hotly pursued goal as this molecule plays a critical role in signal transduction. Controlling the synthesis of PI(3,4,5)P₃ by inhibiting PI3K has been the most heavily pursued strategy and while several excellent inhibitors have been developed, efforts have been complicated by the requirement of selectively targeting numerous PI3K isoforms to disrupt PI3K signaling. An alternative approach to lowering PI(3,4,5)P₃ levels in cells is to upregulate the phosphatase enzymes that degrade PI(3,4,5)P₃, specifically PTEN or SHIP. Modulation of SHIP activity also raises the possibility of tissue specific treatments, as SHIP1 is expressed primarily in hematopoietic cells while SHIP2 is utilized in other cell types. A number of specific diseases related to the modulation of inositol phosphatases are under investigation. In many of these cases genetic studies have indicated that
modulation of inositol phosphatases may play a role in the development and progression of the disease state. Recently small molecule modulators of these key regulatory enzymes have been developed to further explore these molecular targets for the treatment of cancer, anaphylaxis, diabetes and other diseases. A more detailed discussion of several of these diseases is presented below.

Cancer

Inositol phospholipids play a crucial role in all aspects of cell biology; from cell survival, differentiation and migration, to immune function, organ development and tumor growth. Their production is carefully regulated by a wide range of lipid kinases and phosphatases.\(^{18,20}\) The most studied of these is PI3K, which produces the inositol phospholipids \(\text{Pl}(3)\text{P}_1\) \(^{1.4}\), \(\text{Pl}(3,4)\text{P}_2\) \(^{1.3}\) and \(\text{Pl}(3,4,5)\text{P}_3\) \(^{1.2}\). Many tumors, including breast cancer\(^{21–22}\) and hematological malignancies such as the plasma cell disorder multiple myeloma (MM), present with constitutive activation of the PI3K–AKT–mTOR pathway.\(^{23}\) Activating mutations in the PI3K gene (PIK3CA) have been described, but constitutive PI3K signaling may also occur as a secondary effect.\(^{24}\) Activating mutations in receptor tyrosine kinases (Ras), oncogenic translocation products, and autocrine cytokine signaling loops have been described as contributing factors.\(^{25–26}\) Whatever the activating mechanism, targeting the PI3K–AKT–mTOR pathway with specific inhibitors is believed to be a promising approach in cancer treatment.\(^{27–28}\)

Due to its essential role in cell survival, the PI3K–AKT–mTOR pathway has received much attention in cancer treatment. AKT is recruited to the membrane by both the PI3K product \(\text{Pl}(3,4,5)\text{P}_3\) \(^{1.2}\) and the SHIP phosphatase product \(\text{Pl}(3,4)\text{P}_2\) \(^{1.3}\), where it is subsequently activated by kinases such as PDK1.\(^{29–30}\) Next, AKT activates mTOR by
phosphorylating TSC2, thus lifting the inhibitory effect of the TSC1/TSC2 complex on Reb and mTOR.\textsuperscript{31} The PI3K survival signal stems in part from AKT–mediated phosphorylation and inactivation of pro–apoptotic proteins such as Bim and Bad.\textsuperscript{32–35} Furthermore, AKT may activate transcription factors like AP–1 and NFkB, which results in transcription of anti–apoptotic genes.\textsuperscript{36–37} In addition, mTOR activation has been shown extensively to inhibit the cellular process of autophagy.\textsuperscript{38} Autophagy, or autophagocytosis, is a reversible process in which intracellular components are sequestered and degraded in double membrane autophagosomes.\textsuperscript{39} Upon cellular stresses such as nutrient deficiency, mTOR is deactivated and autophagy is initiated. Although this may temporarily protect cells from cytotoxic stimuli, progression to apoptosis under prolonged cellular stress may also occur.

The role of autophagy in tumorigenesis remains elusive.\textsuperscript{38,40–41} Both increased and decreased expression of Beclin–1 in tumor cells has been correlated to poor prognosis. Autophagy may serve to protect cells from apoptosis–inducing agents as evidence shows that autophagy can be used by cancer cells to escape therapy and hence contributes to drug resistance.\textsuperscript{42–43} On the other hand, autophagy leads to a block in proliferation and can result in cell death. Indeed, induction of autophagic cell death is a key strategy of several therapeutic agents being investigated for the treatment of breast cancer, including the mTOR inhibitors curcumin, everolimus, niclosamide, and temsirolimus.\textsuperscript{38}

Generally PI3K signaling is thought to be counteracted by lipid phosphatases. One of these is the phosphatase and tensin homolog PTEN, which hydrolyzes PI(3,4,5)P\textsubscript{3} \textbf{1.2} to PI(4,5)P\textsubscript{2} \textbf{1.1}, thereby decreasing the PI3K–induced survival signal and acting as a tumor suppressor.\textsuperscript{44} Others are the SH2–domain containing inositol phosphatases SHIP1
and SHIP2, which dephosphorylate inositol lipids at the 5’ position of the inositol ring, thereby generating PI(3,4)P₂. SHIP1 and SHIP2 share 38% sequence homology, but may have different cellular functions, as SHIP2 is ubiquitously expressed, whereas SHIP1 is predominantly found in cells from the hematopoietic lineage and bone forming osteolineage cells.⁴⁵–⁴⁶

SHIP1, SHIP2 and PTEN are typically viewed as opposing the activity of the PI3K/AKT signaling axis that promotes survival of cancer cells and tumors. However, there is emerging evidence that SHIP1 and SHIP2 may actually facilitate, rather than suppress, tumor cell survival in contrast to PTEN.⁴⁷–⁵¹ The enzymatic activities of SHIP1 and SHIP2 are quite distinct from that of PTEN, as PTEN reverses the PI3K reaction to generate PI(4,5)P₃ 1.1 from PI(3,4,5)P₃ 1.2, while the 5’ poly–phosphatase activity of SHIP1/2 converts PI(3,4,5)P₃ 1.2 to PI(3,4)P₂ 1.3. Accordingly, PTEN has a very different structure when compared to SHIP, sharing less than 20% homology with SHIP, and PTEN does not possess an SH2 domain. Also noteworthy is the product of the SHIP hydrolysis, PI(3,4)P₂ 1.3 has a greater affinity for the PH domain of AKT than PI(3,4,5)P₃ 1.2, and thus PI(3,4)P₂ 1.3 more potently stimulates AKT activity.²⁹ PTEN and SHIP also show significant differences in in vivo settings, as PTEN mutant knockout mice develop leukemia quite rapidly,⁵² while no malignancy of any type has been reported in either SHIP1⁻/⁻ or SHIP2⁻/⁻ mice.⁵³–⁵⁸ These distinctions are crucial as they demonstrate that SHIP1/2 and PTEN exhibit distinctly different effects on AKT signaling. Thus, via generation of PI(3,4)P₂ 1.3, SHIP1/2 could in some contexts amplify survival or proliferative signals in neoplastic cells by providing additional docking sites at the plasma membrane for recruitment and activation of PH–domain containing kinases.
such as AKT. In support of this hypothesis, PI(3,4)P₂ 1,3 levels are increased in leukemic cells⁵⁹ and increased levels of PI(3,4)P₂ 1,3 due to mutations in INPP4A/B genes promotes the transformation and tumorigenicity of mouse embryonic fibroblasts (MEF) and breast tumor formation.⁶⁰-⁶² While both AKT and mTOR can be targeted directly with small molecule inhibitors, SHIP inhibition provides a new molecular mechanism to influence this pathway. This differentiation could be advantageous for treating tumors that are resistant to AKT and/or mTOR inhibitors, as they may still be susceptible to SHIP modulation.

Recently we found that a SHIP1 selective inhibitor, 3α–aminocholestane 1,5 (3AC), reduces AKT activation and promotes apoptosis of human blood cell cancers that express SHIP1.⁴⁷,⁶³ We further confirmed a role for PI(3,4)P₂ 1,3 in cancer signaling by showing that introduction of exogenous PI(3,4)P₂ 1,3 into leukemia cells or breast cancer cells protects them from SHIP1 or SHIP2 inhibition in a dose–responsive fashion.⁴⁷,⁶³ Thus, SHIP1 inhibitors can now be considered as potential therapeutics to decrease the growth and survival of certain hematologic malignancies like MM. There may also be applications for SHIP2 inhibitors in non–hematologic cancers as SHIP2 expression is increased in breast cancer and promotes survival signals from EGF–R in these tumors.⁴⁹,⁵¹ We have begun to explore this aspect of SHIP biology, with pan–SHIP1/2 inhibitors showing excellent activity against breast cancer cell lines which are dependent on SHIP2.⁶³
Figure 1–3. 3α–Aminocholestane 1.5 (3AC).

Through the generation of PI(3,4)P₂ 1.3, SHIP1/2 could enhance survival or proliferative signals in neoplastic cells by providing additional docking sites at the plasma membrane for recruitment and activation of PH–domain containing kinases such as AKT or PDK1. We refer to this as the “Two PIP Hypothesis” where a certain amount of both PI(3,4,5)P₃ 1.2 and PI(3,4)P₂ 1.3 are required to promote a malignant state (Figure 1–4). Consistent with the ‘Two PIP Hypothesis’, a SHIP agonistic compound also reduces the growth of MM cells in vitro.⁶⁴ That both antagonistic and agonistic SHIP modulators can kill MM cells highlights the delicate balance of both PI(3,4,5)P₃ 1.2 and PI(3,4)P₂ 1.3 that a cancer cell must maintain in order to achieve and sustain the malignant state. Multiple configurations of PI3K, PTEN, SHIP1/2 and/or INPP4 mutations (or alterations in their expression), are likely permissible under the “Two PIP Hypothesis” in order to increase levels of both PIP species in order for a cell to achieve a “malignant state”. Thus, different perturbations in the PI3K–PTEN–SHIP1/2–INPP4 signaling cassette are possible in order for the cancer cell to satisfy the ‘Two PIP Hypothesis’.
Figure 1–4. The ‘Two PIP Hypothesis’ in Cancer. Signals emanating from both PI(3,4,5)P$_3$ 1.2 and PI(3,4)P$_2$ 1.3 are necessary for the cancer cell to achieve and sustain the malignant state.

Another way of evaluating SHIP1’s role in cellular physiology has evolved from this line of reasoning. Rather than accepting SHIP1 as an inhibitor of the PI3K pathway, some scientists propose that SHIP1 is actually a “redirector” of signaling from PI–3,4,5–P$_3$ 1.2 to PI–3,4–P$_2$ 1.3, thereby recruiting a completely different set of PH–domain containing proteins to the plasma membrane. This new view on SHIP1 also leads to further conclusions. For cellular functions in which SHIP1 has a negative effect, PI–3,4,5–P$_3$ 1.2 must be the main phospholipid secondary messenger that initiates the requisite pathways for the manifestation of that particular function. Conversely, for cellular functions in which SHIP1 has a positive effect, PI–3,4–P$_2$ 1.2 must be the major secondary messenger responsible for initiating the necessary pathways to effect that specific function.$^{18}$

These theories imply that SHIP1 antagonists as well as agonists can potentially serve as new drug targets for many maladies, no matter the severity. Since PI–3,4–P$_2$ 1.3 actually binds Akt more strongly than PI–3,4,5–P$_3$ 1.2 and consequently induces more potent Akt activity, it can even be hypothesized that SHIP1 antagonists may display more potent activity than agonists. Some major pathologies that SHIP1 antagonists may
alleviate or treat are autoimmune deficiency syndrome (AIDS), cancers, especially blood cancers, Graft vs. Host disease (GvHD), solid organ transplantation, and bone marrow transplantation.

SHIP1 antagonists can also be therapeutic for cancers due to its effects on hepatocyte growth factor (HGF). Many types of cancer patients exhibit an overproduction of HGF, which mediates cell junction degradation, the distribution of epithelial cell colonies, and tubulogenesis. Given its major functions, an abnormally high level of HGF often leads to tumorigenesis. SHIP1 has been shown to enhance HGF–induced tubulogenesis. Tamura and co–workers reveal that Madin–Darby canine kidney (MDCK) cells induced to overexpress SHIP1 experience a change in morphology that resembles an epithelial–mesenchymal like transition. These cells lose cortical actin, develop actin stress fibers, and spontaneously mobilize – all these morphological changes occurred without HGF treatment. However, SHIP1−/− MDCK cells did not display any of these changes. These results signify that SHIP1 plays an important role in the regulation of cellular adhesion and dispersion. Therefore, SHIP1 antagonists can potentially prevent or delay tumorigenesis.

SHIP1 antagonists clearly present possible new therapies for blood cancers. They manifest this potential remedial effect by influencing multiple pathways. The initial studies described above strengthen the potential role these antagonists can have in cancer therapies. The use of SHIP1 antagonists has a major advantage over current therapies such as chemotherapy and other radiation–based methods because of their tissue selective effects. The fact that SHIP1 is expressed in only hematopoietic cells makes the use of its antagonists even more attractive because these potential drugs will only target blood cells
and may not affect any cells that do not express SHIP1. Therefore, SHIP1 antagonists and
their effects should be further studied so that their applicability as drugs may be explored.

SHIP2 inhibition may also be a viable route for the treatment of breast cancer. SHIP2 has an atypical effect on EGF–induced signaling in various breast cancer cell
lines. Instead of repressing cellular responses to EGF, SHIP2 actually causes an increase
in EGF–induced signaling by elevating the level of the EGF receptor (EGFR). In fact,
multiple breast cancer cell lines, including MDA–231, SKBR–3, MDA–468, MDA–436,
MCF–7, and ZR–75, all overexpress SHIP2 and display enhanced levels of EGFR. Due
to their increase in EGF–induced signaling, these breast cancer cell lines exhibit an
increased rate of cellular proliferation. However, if SHIP2 is silenced in the MDA–231
breast cancer cell line, cell proliferation is dramatically decreased. Furthermore, SHIP2
silencing in MDA–231 also produces a 60% increase in apoptosis in response to EGFR
inhibitors. These results imply that SHIP2 may play a significant role in oncogenesis,
especially in breast cancer.

To further explore the effect of SHIP2 on tumorigenesis, orthotopic implantation
was conducted on nude mice. Tumors that originated from SHIP2–/– cells display delayed
growth by almost three weeks as compared to tumors that came from SHIP2+/+ cells. In
addition, mice with tumors from SHIP2–silenced cells exhibit profoundly decreased
spontaneous lung metastases. These mice show either no detectable masses or just merely
micromasses in their lungs while mice with tumors from control cells display extensive
lung metastases that cover more than 25% of their lungs. These findings confirm the
positive role SHIP2 has upon cancer growth and metastasis. These results also
demonstrate that SHIP2 plays an especially significant role in breast cancer development
and proliferation given that it is highly expressed in multiple breast cancer cell lines. As a result, SHIP2 is also emerging as an important clinical anticancer target.

**Inflammation (Asthma)**

SHIP1 knockout mice have been shown to display systemic mast cell hyperplasia, increased serum levels of IL–6, TNF, and IL–5, and heightened anaphylactic response. These effects were verified to be attributed to the loss of function of the SHIP1 enzyme. Given this phenotype, it seems clear that the use of SHIP1 agonists could be used to treat anaphylactic events with allergy sufferers. These results have led to the development of AQX–1125, which is under development for the treatment of pulmonary inflammation and asthma.

**Diabetes**

In response to extracellular stimuli, including growth factors and cytokines, SHIP2 becomes tyrosine phosphorylated (Yphos) and associates with the src homology 2–containing (Shc) adaptor protein, a substrate of various tyrosine kinases. This association brings SHIP2 to the cellular membrane and thereby brings it close to its enzymatic substrate, PI–3,4,5–P₃. Sasaoka and co–workers cloned SHIP2 from rat skeletal muscle and overexpressed the enzyme in Rat1 fibroblasts with human insulin receptors. This abnormally increased activation of SHIP2 inhibited the association of Shc with growth factor receptor–bound protein 2 (Grb2), a normal occurrence after the insulin receptor is Yphos. In addition, overexpression of SHIP2 decreased the activation of insulin–stimulated mitogen activated protein (MAP) kinase, inhibited insulin–induced Akt activation, and decreased insulin–stimulated thymidine incorporation. These
combined results indicate that SHIP2 may play a critical role in the negative regulation of insulin signaling and therefore involved in diabetes.\textsuperscript{67}

Schurmans and co–workers demonstrated further evidence of SHIP2’s role in insulin signaling by generating SHIP2 knockout mice and adult mice heterozygous for the SHIP2 mutation. SHIP2 deficient mice portrayed a metabolic state with an abnormally increased sensitivity to insulin. These mice exhibited severe neonatal hypoglycemia, decreased expression of the genes involved in gluconeogenesis, and perinatal death. The heterozygous mutated SHIP2 adult mice also showed an increased sensitivity to insulin as well as a higher tolerance for glucose. These mice showed an overexpression of glucose transporter type 4 (GLUT4) and an increase in glycogen synthesis in skeletal muscles.\textsuperscript{56} These results clearly demonstrate that SHIP2 acts as a negative regulator of insulin signaling.

Dysfunctional insulin signaling often leads to diabetes mellitus. Type 2 diabetes continues to be a growing global health issue. As a result, components of the insulin signaling pathway, including SHIP2, are becoming important targets for the discovery of new therapeutic agents.\textsuperscript{68} However, diabetes is not the only reason for exploring SHIP2 as a pharmacological target. In addition to insulin regulation, SHIP2 also inhibits cellular responses to epidermal growth factor (EGF) and platelet–derived growth factors.\textsuperscript{69} These additional roles increase SHIP2’s attraction for the pharmacological industry.

**Bone Marrow Transplantation**

Due to an increased expression of Ly49A and Ly49C receptors, SHIP1 knockout mice are able to accept allogeneic bone marrow grafts because they do not develop GvHD. In contrast, less than half the wild type mice given allogeneic bone marrow
transplants survive. The major cause of death in these mice was GvHD. The increase in Ly49A and Ly49C receptors is postulated to diminish the capacity of the NK pool as a whole to secrete inflammatory cytokines such as INF-γ and TNFα in response to MHC class I ligands exhibited by the allogeneic BM cells. As a result, this greatly reduces the ability of the donor T cells to manifest a GvHD reaction. Intriguingly, SHIP1–deficient mice do reject MHC class I deficient BM, however this implies that the NK component of the immune system is not completely compromised with SHIP1–deficiency.\textsuperscript{54} Therefore, SHIP1 antagonists can be utilized to selectively impair the NK pool function in order to limit the initiation and severity of GvHD. This may help ensure that allogeneic bone marrow transplants succeed.

SHIP1 deficiency also results in NK cells that overexpress not only Ly49A and Ly49C but also the MHC–independent inhibitory receptor, 2B4. NKG2D has been established as a key player in the ability of mice to acutely reject mismatched BM grafts. Exposure of a NKG2D receptor to a MHC class I ligand on allogeneic bone marrow cells results in the activation of the NK cell, which then proceeds to kill the donor BM cell. This manifestation of GvHD ultimately leads to the rejection of the allogeneic BM graft and in many cases, death. However, SHIP1\textsuperscript{−−} mice do not exhibit GvHD and consequently do not reject multiple kinds of mismatched bone marrow grafts. The acceptance of mismatched MHC bone marrow grafts in SHIP1 knockout mice results from the reduced cytotoxic function of the NK compartment. Since SHIP1 deficiency leads to reduced NKG2D–mediated killing, SHIP1 deficiency also leads to reduced NKG2D cytolysis of allogeneic BM cells. SHIP1 clearly acts to negatively control 2B4 expression so that it does not handicap the ability of activating receptors to effect normal
NK cytotoxicity.\textsuperscript{70} Therefore, by inducing NK cell hyporesponsiveness, especially to mismatched BM grafts, SHIP1 antagonists may play a crucial role in the alleviation of GvHD and the ensured success of allogeneic bone marrow transplantations.

Furthermore, a SHIP1 antagonist may also help abrogate clinical GvHD by increasing the population of myeloid suppressor cells. Myeloid suppressor cells (MySCs) gather in lymphoid organs where an intense immune response is activated. Once there, MySCs then inhibit the functions of both B and T cells. SHIP1–deficient mice display a MySC pool in the lymph node and spleen that is 10–20 times greater than that of wild type. In addition, SHIP1\textsuperscript{−/−} splenocytes and lymph node (LN) cells exhibit a profoundly reduced ability to prime allogeneic T cell responses, such as proliferation and cytokine secretion. This reduced ability to initiate allogeneic T cell responses is not due to decreased APC function nor decreased APC number because SHIP1\textsuperscript{−/−} mice do not display other defects in adaptive immune responses. For example, SHIP1–deficient APCs can still prime antigen–specific proliferation and differentiation of naive T cells into helper and cytotoxic T cells. This finding implicates an external source for this reduced priming of allogeneic T cell responses.\textsuperscript{69}

The dramatically weakened allogeneic T cell response in SHIP1–deficient mice greatly contributes to the ability of these mice to abrogate GvHD, which heavily depends on the activity of donor T–lymphocytes. Normally, donor T cells are activated upon exposure to host APCs expressing MHC antigens.\textsuperscript{71} Once activated, the donor T cells then initiate a series of events which leads to the death of host cells, which cumulatively then gives rise to GvHD. SHIP1 deficiency, however, prevents this chain of events from occurring. In fact, induction of SHIP1 deficiency in adult mice prevents acute and lethal
GvHD after a T cell–replete, MHC–mismatched BM transplant. As a result, these mice accept instead of rejecting allogeneic BM grafts. After MHC–mismatched BM transplantation, SHIP1 knockout mice exhibit a 94% survival rate while their wild type counterparts exhibit only a 57% survival rate. These findings suggest that SHIP1 antagonists, by reducing allogeneic T cell responses, can play a powerful role in the prevention of GvHD in organ transplant procedures.

SHIP1 antagonists display extensive therapeutic potential in averting GvHD. This ameliorative effect has a profound significance in organ transplants, especially that of the bone marrow. Bone marrow transplantation constitutes a major treatment option for various cancers and genetic disorders. Unfortunately, serious complications exist with bone marrow transplants due to the high risk of lethal GvHD. Many allogeneic bone marrow transplants are often rejected by the host, which can ultimately lead to death depending on the severity of GvHD. Moreover, solid organ transplants are also an important curative option for many types of cancer. But, just like bone marrow transplantation, GvHD poses a major obstacle to the success of organ transplant treatments. Therefore, by inhibiting the occurrence of GvHD, SHIP1 antagonists serve as extremely titillating therapeutic targets because they open up new possibilities in the continuously developing field of organ transplants and engraftments.

**Crohn's disease**

SHIP1 knockout mice develop a severe inflammatory disease in their small intestine. This disease closely resembles human Crohn’s Disease (CD). Recent studies indicate that this inflammatory disease does not result from an autoimmune attack, but rather from a lack of a specific immune cell type in the small intestine called T cells. A
profound deficit in both CD4 and CD8 T cells in the small intestine where disease occurs was also observed, suggesting a positive role for SHIP1 in promoting trafficking and/or persistence of T cells at mucosal sites. Thus, SHIP1 could potentially also be a genetic determinant of susceptibility to CD in humans. Consistent with this hypothesis there are single–nucleotide polymorphisms (SNPs) found at 2q37 in the human genome where the human SHIP1/INPP5D gene is located that are highly enriched in CD and ulcerative colitis patients. Studies are continuing, but these results may have important implications for patients with all forms of inflammatory bowel disease (IBD). With the development of small molecule SHIP modulators, studies to determine the role of SHIP in CD seem to be a prime area of research.

**Cystic Fibrosis**

Recently work by Biwas showed that SHIP1 may play an important role in cystic fibrosis (CF). SHIP1 knockout mice develop a significant neutrophil infiltration in the lungs, similar to the massive proinflammatory phenotype in the lung observed in cystic fibrosis patients. This lung pathology has been traced to the overexpression of proinflammatory genes like IL–8, which are regulated by posttranscriptional mechanisms. Expression of this gene was linked to the microRNA miR–155, which may play an important role in the regulation of inflammation in CF lung epithelial cells. The specific mechanism of miR–155 action in CF cells appeared to be an inhibition of the translation of SHIP1, leading to a loss of SHIP1 function. This loss in SHIP1 function lead to the downstream overexpression of IL–8, which was hypothesized to contribute to the lung infiltration seen in CF patients. This study suggests a role for SHIP1 agonists in the treatment of CF.
**Blood Cell Production Enhancement**

Inhibition of SHIP1 in healthy cells should lead to an increase in PI(3,4,5)P3 1.2, resulting in an increase in the amount of cell division specific to blood and other hematopoietic cells. Treatments to increase blood cell production currently rely on the recombinant endogenous growth factors epogen and neupogen, which are protein–based and therefore must be administered by injection. A small molecule SHIP1 inhibitor could be taken orally, which may be advantageous in some situations. Studies with a selective SHIP1 inhibitor recently showed that increases in blood cell production could be detected *in vivo* in mice.⁴⁷ Typically neutropenia and anemia are problems associated as side effects with cancer chemotherapy, so SHIP1 inhibitors could be used to help alleviate these conditions. Radiation poisoning also leads to similar blood cell nadirs, so SHIP1 antagonists may be useful for the treatment of accidental radiation exposure.⁷⁵–⁷⁶

**STEM Cells Mobilization**

SHIP1 knockout mice show a characteristic proliferation of hematopoietic STEM cells (HSCs) in circulating plasma. This is counterintuitive as SHIP1 knockout mice also show expansion of the hematopoietic stem cell niche in the bone marrow. While the larger niche should accommodate the greater number of STEM cells, the SHIP1 deficient HSCs home poorly in the bone marrow. The bone marrow in SHIP1 deficient mice also shows a reduced capacity for long–term repopulation of HSCs. Use of a SHIP1 antagonist may therefore be used to mobilize hematopoietic STEM cells from the bone marrow into the bloodstream, where they can be harvested before reintroduction to the host. This method would avoid removing the STEM cell from the bone marrow through a more invasive procedure.
AIDS

SHIP1 antagonists can theoretically treat a wide spectrum of diseases. One increasingly prevalent disease that SHIP1 antagonists can potentially influence is AIDS. Patients with chronic human immunodeficiency virus (HIV) – 1 infection exhibit impaired natural killer (NK) cell function. In these patients, natural killer cells cannot secrete cytokines, lyse MHC–deficient target cells, and undergo antibody–dependent cell–mediated cytotoxicity. This decrease in NK cell function has been partially attributed to elevated levels of SHIP1.77 Thus, the application of SHIP1 antagonists, such as 3AC, may help alleviate the physical symptoms of HIV–1 infection, treat AIDS patients, or lead to the discovery of new cures.

PTEN Modulation

An alternative phosphatase whose modulation would significantly affect the PI3K pathway is PTEN, the specific phosphatase and tensin homolog deleted on chromosome 10. PTEN is a well–known tumor suppressor,78–79 with studies showing that it may be the second most common loss of function mutant in tumor cells. Given its role, it seems a prime target for the development of agonists (currently, the only known phosphatase agonists are the pelorol analogs in development by Aquinox Pharmaceuticals as SHIP1 agonists). Unfortunately, no PTEN agonists are known even though there has been some call for their development.

Given its role as a tumor suppressor, there would seem to be little call for PTEN inhibitors. Recent work has shown otherwise, however, as PTEN plays an important role in limiting the regrowth of nerve cells.80–86 An important role for PTEN inhibitors in this
process would be to facilitate nerve regrowth, but obviously the use of these compounds would have to be monitored closely as the side effects could be severe.

**Small Molecule Phosphatase Modulators**

Given the significant effects changes in the expression and activity of inositol phosphatases has on numerous biological pathways, new ways of modulating these enzymes are of great interest. Efforts have become focused on the development small molecule agonists and antagonists for SHIP1, SHIP2 and PTEN. A summary of these molecules are shown in below. *(Figure 1–5–Figure 1–9)*

**SHIP1 Agonists**

The inositol phosphatase signaling pathways are especially appealing targets for pharmaceutical intervention, as these events are near the origin of the signal, which can be stopped or started with a smaller number of molecular interactions. As SHIP1 has been shown to participate in these signaling events, and has been shown to influence a multitude of physiological pathways, it has become an increasingly active target for workers in academia and the pharmaceutical industry. In addition, SHIP1’s restriction to mostly hematopoietic cells augments its attraction, as it can selectively modify the cellular processes of blood cells without affecting other cell types. The ability to control SHIP1 activity may lead to novel therapies for many pathological disorders of varying severity. These disorders range from allergies to autoimmune disorders to cancer. SHIP1 activity can be activated using small–molecule agonists or inhibited using small molecule antagonists. Because SHIP1 opposes PI3K signaling, and overactive PI3K activity has
been shown to facilitate tumor growth, the analysis of SHIP1 agonists as potential new drugs became an appealing area of medicinal research.

![Chemical Structures]

**Figure 1–5.** SHIP1 Agonists and Analogs.

Krystal and Mui working in collaboration with Andersen at the University of British Columbia screened crude extracts of marine invertebrates in an effort to discover potential SHIP1 activators. In this initial screening, the naturally occurring sesquiterpene, pelorol 1.6, was found to enhance SHIP1 activity.\(^{87}\) Pelorol was isolated from a marine sponge, *Dactylospongia elegans*, in Papua New Guinea. Since only small amounts of pelorol could be obtained from *D. elegans*, a synthetic route to this molecule was also developed. This route allowed for the synthesis of the natural product in 9 steps with an over – all yield of 6% from the commercially available terpenoid, (+)–sclareolide.\(^{87}\)
Pelorol had been isolated independently by König\textsuperscript{88} and Schmitz\textsuperscript{89} in 2000 from \textit{D. elegans} and the Micronesian sponge \textit{Petrosaspongia metachromia}, respectively, but they were unable to determine the molecule’s enzymatic target.

Several analogues of pelorol were also synthesized and tested for SHIP1 activity.\textsuperscript{87} This study led to the discovery of analogue \textbf{1.7}, which exhibited improved biological activity.\textsuperscript{87} In fact, analogue \textbf{1.7} was three times more potent than pelorol at the same molar concentration.\textsuperscript{90} Anderson and co–workers synthesized methyl agonist \textbf{1.7} starting from (+)–sclareolide through a series of 6 steps with an overall yield of 29\%.\textsuperscript{87} The methyl analogue \textbf{1.7} was shown to inhibit degranulation and TNF\textsubscript{α} production in SHIP1\textsuperscript{+/+} murine mast cells stimulated with IgE. However, it exhibited no activity in SHIP1\textsuperscript{−/−} murine mast cells. These results demonstrate that agonist \textbf{1.7} selectively targets SHIP1, which made it a very promising lead molecule. Additional testing showed that agonist \textbf{1.7} shows similar anti–inflammatory effects to that of dexamethasone, the reference standard, in murine models of ear edema and sepsis syndrome.\textsuperscript{87}

Agonist \textbf{1.7} was designated AQX–016A and it was discerned that it preferentially activates SHIP1 over SHIP2.\textsuperscript{90} The appeal of \textbf{1.7} was diminished by the presence of its catechol moiety, which may cause problems in real–life applications. Catechols are typically undesireable in potential medicinal compounds because they can produce unwanted “side–effects” that are independent of their specific protein pocket binding interactions. Some examples of these “side–effects” include metal–binding and oxidation to an orthoquinone. Orthoquinones are especially undesired because they can covalently modify proteins and DNA through Michael reactions, which will lead to even more unexpected results.\textsuperscript{90} This issue was addressed by removing the hydroxyl functionality at
C–17 to form a modified version designated as AQX–MN100 1.8. Analysis of AQX–MN100 1.8 biological activity show that its potency is equivalent to that of the diol AQX–016A 1.7. Furthermore, similarly to diol 1.7, phenol AQX–MN100 1.8 also preferentially activates SHIP1 over SHIP2. Initial toxicology studies provided evidence that AQX–MN100 is well tolerated and does not have a large impact on peripheral blood cell counts, bone marrow progenitor numbers, liver function, and kidney function.

The discovery of these small molecule agonists of SHIP1 also contributed to the identification of SHIP1 as an allosterically activated enzyme. Classical enzyme kinetic analysis of SHIP1 phosphatase activity with inositol–1,2,4,5– tetrakisphosphate (IP4) substrate verifies its sigmoidal reaction kinetics, a characteristic of allosteric enzymes, with its phosphatase activity. Furthermore, both AQX–MN100 1.8 and SHIP1’s end product, PI–3,4–P₂ 1.3, both enhance SHIP1’s activity and bind to the C2 domain of SHIP1, as confirmed by scintillation proximity assays. These combined findings strongly implicate SHIP1 as an allosteric enzyme. As an allosteric activator, AQX–MN100 1.8 increases its appeal for medicinal purposes because allosteric sites tend to be better targets than active sites. Another advantage of allosteric regulators is that they also tend to have higher selectivity than active site regulators.⁹⁰

In 2009, Mui and co–workers showed that AQX–MN100 1.8 was active in several assays against multiple myeloma (MM). Multiple myeloma is a plasma cell neoplasia that exhibits an increased number of both malignant B cells and associated monoclonal immunoglobulin proteins in the bone marrow. The current treatment options consist of high–dose chemotherapy and stem cell transplantation. However, most patients cannot be cured and the disease often recurs and is then much more difficult to treat due to drug
resistance. AQX–MN100 1.8 inhibits proliferation and induces of apoptosis of MM cell lines by activating SHIP1. Consistent with the molecules selective SHIP1 activity, phenol 1.8 displays no significant effects on SHIP1–deficient non–hematopoietic cells. Furthermore, AQX–MN100 1.8 activation of SHIP1 elevates the therapeutic effects of dexamethasone and bortezomib, both of which are common cytotoxic drugs used to fight MM.64

These results reveal that AQX–MN100 1.8 may be used to treat MM. This corroborates the theorized potential of small molecule SHIP1 agonists to be used as treatments for cancer, especially blood cancers. However, other evidence also suggests that SHIP1 agonists may also be used to treat common gastrointestinal problems such as diarrhea caused by certain bacteria.91 In addition, other studies demonstrate that SHIP1 agonists may potentially alleviate more serious medical pathologies, such as Crohn’s disease, a chronic inflammatory bowel disease,72 and leukemia.92 By enhancing SHIP1’s ability to inhibit the PI3K pathway and its downstream effector proteins, agonists may provide new therapies for several disorders. Therefore, the identification of more SHIP1 agonists besides AQX–MN100 1.8 is an important newly developing area of pharmacological research.

Additional analog studies by Aquinox pharmaceuticals have resulted in the development of even more pelerol analogs as SHIP1 agonists. These studies appear to have culminated in the development of sulfone containing derivatives such as 1.9 and 1.10, which were disclosed in the patent literature.93 These molecules are readily synthesized from (+)–sclareolide and appear to be less lipophilic than the previous pelerol based analogs, which likely improves their pharmacodynamic properties and oral
availability. Sulfone 1.9 showed the highest activity in SHIP1 agonist assays, while sulfone 1.10 showed the best activity in the OPM2 multiple myeloma cell assay. Unfortunately the data that is presented is incomplete, with information for enhancement of SHIP1 activity being given for only a subset of compounds, and the data is given in a qualitative format, so little conclusion can be made about the relative activities of these compounds. Aquinox has begun Phase II clinical trials in the Netherlands of a SHIP1 agonist which is being used to treat airway inflammation. While the structure of the compound being evaluated in clinical trials (AQX–1125) has not been disclosed, it most likely is one of the compounds described in this patent (approximately 125 compounds are covered).

A second class of terpenoid SHIP1 agonist was also found from screening natural product isolates that were donated from Pfizer when they shut down their natural product screening efforts.94 The active constituent of an isolate from the soft coral Cladiella sp. collected in Pohnpei was shown to activate SHIP1. The isolate was then fractionated and the structure of the active constituent determined by x–ray crystallography. This led to the determination that australin E 1.11 is the SHIP1 agonist present in the isolate. Australin E 1.11 shows little resemblance to the pelorol based agonists, with the structure belonging to the eunicellin diterpenoids. While the eunicellin diterpenoids have shown in vitro cytotoxicity against cancer cell lines, this is the first example of a SHIP1 inhibitor with this structural type. Three other australin analogs were also identified from the isolate, but showed no activity against SHIP1. These included australin F 1.12, which only differs in alkene regiochemistry, which implicates the endocyclic alkene as important for biological activity.
Recently a cyclic depsipeptide was also shown to have the ability to activate SHIP1, providing a third structural class of SHIP1 agonists.\textsuperscript{95} Isolated from a strain of \textit{Bacillus sp.} that was cultured from a sediment sample collected from the sea floor at a depth of 100m near Turnagain Island in Howe Sound, British Columbia, these compounds differed only in the stereochemistry of the ester linkage. The structures of these molecules were verified by synthesis of the linear seco acids followed by macrocyclization with a carbodiimide reagent. Turnagainolide B \textbf{1.13} was found to have similar potency as a SHIP1 activator to AQX–MN100. Interestingly, turnagainolide A \textbf{1.14}, which only differs in the stereochemistry of the ester, was found to have no activity as a SHIP1 agonist.

**SHIP1 Antagonists**

Using a high–throughput screening (HTS) approach, 3AC \textbf{1.5} was identified as a potent SHIP1 inhibitor.\textsuperscript{47} 3AC \textbf{1.5} displays a detectable level of inhibition at 2 mM and 50% inhibition at 10 mM in the Malachite green phosphate assay. 3AC \textbf{1.5} also selectively inhibits SHIP1 over both SHIP2 and PTEN. In fact, \textbf{1.5} shows not even a detectable level of inhibition for either SHIP2 and PTEN at 1 mM. Further analysis of \textbf{1.5}’s biological activity demonstrated that it expands the myeloid immunoregulatory cell pool and increases myeloid immunoregulatory cell function in peripheral lymphoid tissues, increases granulocyte production, significantly elevates red blood cell number in myelosuppressed hosts, and reduces the growth and survival of hematopoietic cancer cells. These results all mirror the effects observed in SHIP knockout mice.\textsuperscript{53} These observations make \textbf{1.5} an attractive new lead for new agents that stimulate blood cell production and facilitate bone marrow transplantation. Applications of \textbf{1.5} in mice did not
result in the typical myeloid lung infiltration, splenomegaly, and shortened life span observed in SHIP1 knockout mice. In fact, mice treated with 1.5 are generally healthy and do not seem to have any detrimental physiologies. Surprisingly, given SHIP1’s negative regulation of the PI3K pathway, treatment of leukemia and multiple myeloma cell lines with 1.5 lead to a significant decrease in cell viability. Control experiments verified that this decrease in cell viability was due to the inhibition of SHIP1, which can be explained by a redirection of the signaling using the “Two PIP Hypothesis”.

![Diagram of SHIP1 Inhibitors](image)

**Figure 1–6.** SHIP1 Inhibitors.

As cancer is a heterogenous disease, the effects of 3AC were evaluated on three common MM cell lines, U266, RPMI8226 and OPM2, to determine if each would be equally effected by the SHIP1 inhibitor. While treatment of OPM2 cells with 1.5 lead to apoptosis, the less proliferative RPMI8226 and U266 cells appeared to manifest their lack of viability through autophagy instead. Responding to 1.5 treatment with autophagy may protect cells in the short term from 1.5 treatment, but can progress to apoptosis under
conditions of prolonged cellular stress. In RPMI8226 and U266 cells prolonged exposure to 1.5 also lead to degradation of the SHIP1 protein, but not SHIP2 or PTEN.

Treatment of MM with 1.5 in vivo, employing a tumor xenograft mouse model and OPM2 cells, was also explored. Treatment with the aminosteroid resulted in reduced tumor growth and reduced numbers of circulating OPM2 cells. More significantly, 1.5 treatment resulted in a significantly enhanced survival of mice after the tumor challenge. Evaluation of the tumor cells from mice that resisted treatment showed a significant upregulation of SHIP2, suggesting that SHIP1 inhibition may select for tumor cells with increased SHIP2 expression. These results suggest that complementing SHIP1 inhibitors with SHIP2 inhibitors may be an even more effective treatment regime for MM.

In addition to 1.5, other SHIP1 antagonists have been identified. Prestwich designed several metabolically stabilized analogues of PI–(3,4,5)–P3 1.2 in an attempt to develop probes to better understand the role of this lipid in cell physiology. These molecules are very similar to PI–(3,4,5)–P3 1.2 but contain a phosphorthioate or a methyleneosphphonate instead of a phosphate group at the 5’ position of the inositol. These molecules were evaluated for their ability to inhibit SHIP1 and SHIP2 in the hydrolysis of radiolabelled Ins(1,3,4,5)P4, with compounds 1.15, 1.16, and 1.17 showing significant inhibition of SHIP1 at 100μM concentration. PI–3,4,5–P3 1.2 analogues 1.15–1.17 also inhibit SHIP2 to some extent. This difference in inhibition highlights the difference in the inositol binding pockets of the two enzymes. No inhibition of PTEN was observed. Interestingly, similar inositols with a phosphorthiolate or a
methylenephosphonate at the C3 position also showed modest activity as inhibitors of SHIP1.

**SHIP2 Inhibitors**

Phosphorylated polyphenols like 1.18, 1.19 and 1.20 have been shown to be effective inhibitors of SHIP2. These compounds were investigated when three-dimensional modeling revealed a high homology between the phosphorylated phenols and phosphorylated inositol substrates. The number of phosphates and the position of the phosphates on the benzene ring greatly influenced the observed SHIP2 inhibition. For example, Bz(1,2,4,5)P₄ 1.19 showed an IC₅₀ of 11.2 μM, while Bz(1,2,3,4)P₄ 1.20 showed an IC₅₀ of 19.6 μM. None of the phosphorylated phenol inhibitors appeared to be acting as a substrate for the phosphatase, instead the results appeared to be consistent with a competitive inhibitor, with the phosphorylated phenols displacing [³H]Ins(1,4,5) in Sf9 cells overexpressing IP₃R1. The most active phosphorylated polyphenol SHIP2 inhibitor was biphenyl(2,3’,4,5’,6)P₅ 1.18, which showed an IC₅₀ of 1.8 μM. While no data for inhibition of SHIP1 or PTEN was given, these inhibitors did inhibit Type-I 5-phosphatase, which is another common 5-phosphatase. Given the highly polar structure of the phosphorylated phenols it is unlikely that they can enter cells in an unmodified form, and while the authors do mention protecting the phosphate groups to create cell permeable analogs no results to this effect have yet been disclosed.
Recently the crystal structure of the active site of SHIP2 was solved bound to biphenyl(2,3’,4,5’,6)P$_5$ \textbf{1.18}.\textsuperscript{97} The enzyme crystallized with two protein monomers in the unit cell, but only one of the enzymes had the inhibitor bound in the binding pocket. The inhibitor also had additional interactions with residues of a second SHIP2 molecule that was present in the crystal lattice. As SHIP2 is normally not present in high concentrations, these interactions with a second molecule of SHIP2 are likely not important in understanding the binding of the inhibitor to the active site. In order to gain a greater understanding of the interaction between SHIP2 and the inhibitor, molecular dynamics calculations were performed after removing the second SHIP2 molecule. The simulations revealed a loop of the protein that acts as a P4–interacting motif (P4IM). This
portion of the protein was disordered and located above the binding site in the crystal structure but during the dynamics calculation this flexible region moved to interact with the inhibitor and become part of the binding pocket. Evaluation of the sequence alignment of 11 human inositol 5’-phosphatases showed that only SHIP1 and SHIP2 possessed this flexible loop P41M motif. Additional modeling was then performed to evaluate the binding of the synthetic inositol di-C8–PtdIns(3,4,5)P3 and the SHIP2 inhibitor AS1949490 1.21. This work provides a very important advance, as now that the enzyme binding pocket has been described drug discovery can move forward in a more rational manner. Given the high homology between SHIP1 and SHIP2 this will also likely allow researchers to use this information in the design and \textit{in silico} evaluation of new SHIP1 inhibitors.

Other SHIP2 inhibitors are almost certainly known behind the closed doors of pharmaceutical companies. For example, a high–throughput assay utilizing SHIP2 was conducted using a 91,060 compound library, with over 700 inhibitors being identified as having \( \geq 70\% \) inhibition of SHIP2 at 25\( \mu \)M.\textsuperscript{98} One compound was claimed to have an IC\(_{50}\) of 0.37 \( \mu \)M against SHIP2, and also showed significant activity against SHIP1 and PTEN (IC\(_{50}\) = 0.90 \( \mu \)M against SHIP1 and 2.2 \( \mu \)M against PTEN). Unfortunately no structures have been reported, and no other follow–up studies have been published on these inhibitors. Over time the expectation is that these compounds will become known when either the clinical trials begin or when the research program is closed. In either case, it may be several years before these structures and the results of the studies on SHIP2 inhibitors becomes known.
A thiophene–based small molecule inhibitor of SHIP2, AS1949490 1.21 was reported by workers at Astellas pharmaceuticals. This molecule was reported to have an IC$_{50}$ value of 0.62 µM against SHIP2 and was selective for SHIP2 compared to SHIP1 (IC50 against SHIP1 of 13 µM). Treatment of L6 myoblasts with 1.21 led to the increased phosphorylation and subsequent activation of Akt. Interestingly, this inhibitor affects only protein kinase B 2 (Akt2), an isoform of Akt that plays an especially predominant role in insulin signaling, while it has very minimal, if any, inhibitory effects on protein kinase B 1 (Akt1). In addition, 1.21 was shown to increase glucose metabolism, resulting in an increase in both glucose uptake and consumption. A rat hematoma cell line derived from H35 cells (FAO) treated with 1.21 displayed reduced insulin–dependent gluconeogenesis and underexpression of related genes. Similarly, normal adult mice acutely treated with 1.21 also showed reduced gluconeogenesis in the liver, demonstrating that 1.21 can control gluconeogenesis both in vitro and in vivo. Diabetic db/db mice chronically treated with 1.21 displayed increased stimulation of insulin signaling through the increased phosphorylation of glycogen synthase kinase 3b (GSK3b). These mice showed a 37% reduction in fasting blood glucose as compared to vehicles. The physiological effects of 1.21 clearly demonstrate the importance of SHIP2 in the regulation of insulin signaling.

Later efforts by the Astellas group revealed a similar thiophene based inhibitor, AS1938909 1.19. This compound was reported to have a higher affinity for SHIP2 than 1.19 (IC$_{50}$ of 0.57 µM), as well as a greater selectivity for SHIP2 over SHIP1 (IC$_{50}$ for SHIP1 of 21 µM). Administration of 1.19 was shown to increase Akt phosphorylation, glucose consumption and glucose uptake in L6 myotubes. Longer term
administration of \textbf{1.19} was also found to alter gene expression patterns, specifically upregulating the GLUT1 gene but not GLUT4. GLUT1 is a glucose transporter, typically localized on near the cell surface that has a significant role in basal glucose uptake and has been linked to the PI3K/Akt signaling pathway. These studies were all performed in cell culture, but still demonstrate the potential of SHIP2 inhibition for the treatment of diabetes. For these lead molecules to proceed further analogs will need to be prepared with better solubility properties so that dosing can be performed orally, as the thiophenes show poor pharmacokinetic properties and have limited cell permeability.\textsuperscript{101}

Three other types of heterocyclic SHIP2 inhibitors were found by a novel type of HTS involving mass spectroscopy implemented by Neogenesis pharmaceuticals.\textsuperscript{102} The publication presents a number of these inhibitors, with the most active molecules being shown in \textbf{Figure 1–7}. One of these compounds (NGD–61185 \textbf{1.23}) bears a strong resemblance to the compounds investigated by Astellas, as its phenol–based core is similar to the thiophene inhibitors of the Astellas compounds. The other two inhibitors possess highly functionalized pyrazole core structures. The most active compound mentioned in the paper is NGD–78700 ($K_d = 0.82 \pm 0.14 \ \mu M$), but the structure of this material is not revealed, and most other experiments are performed with NGD–61338 \textbf{1.24}. NGD–61338 \textbf{1.24} is quite active, with a reported IC\textsubscript{50} of 1.1 \ \mu M against SHIP2. Kinetic analysis of \textbf{1.24} binding to SHIP2 showed that binding of the inhibitor was mutually exclusive with PIP(3,4,5)P\textsubscript{3} \textbf{1.2} analogs, which suggests that \textbf{1.23} is binding at the active site of the enzyme. No data is given on the selectivity of these inhibitors with regard to other phosphatases. The effects of these compounds in cells and their
bioavailability also remain to be investigated, as well as in vivo studies to determine toxicity.

**Pan SHIP1/SHIP2 Inhibitors**

HTS efforts in the Kerr group also lead to the discovery of several structures which inhibit both SHIP1 and SHIP2. These inhibitors included the two quinoline aminoalcohols, 1.26 and 1.27 and tryptamine 1.28. These compounds showed inhibition of SHIP1 and SHIP2, but did not show inhibition of OCRL, another human inositol 5’–phosphatase, suggesting that the molecules are not general phosphatase inhibitors but have some specificity towards the two SHIP paralogs. All three of these molecules showed activity against the MM cell lines RPMI8226, U266 and OPM2. Cell cycle studies showed significant arrest at the G2/M phase in all cell lines along with a significant increase in sub–G0–G1 phase cells. Typically these results are an indication of apoptotic cell death. Breast cancer cell lines were also evaluated, as SHIP2 is overexpressed in a significant number of breast carcinomas. Both MDA–MB–231 and MCF–7 breast cancer cells were treated with the pan–SHIP1/2 inhibitors. These breast cancer cell lines do not express SHIP1, and therefore selective SHIP1 inhibitor 1.5 had no effect on these cells. In contrast, cell viability was severely reduced in both breast cancer cell lines with increasing concentrations of the pan SHIP1/2 inhibitors. To further prove that the SHIP2 inhibition was responsible for the observed cell death, exogenous PI(3,4)P2 1.3 was added to rescue cells from the effects of the pan–SHIP1/2 inhibitors. Cell viability improved in the case of each inhibitor, but no change was seen when PI(3,5)P2 was used, providing further evidence that the SHIP inhibition is responsible for the observed effects.
PTEN Inhibitors

SHIP is not the only phosphatase that is a negative regulator of the PI3K pathway, as PTEN also degrades PI(3,4,5)P₃ **1.2** to another inositol product, in this case PI(4,5)P₂ **1.1**. At first, PTEN would not seem to hold any promise as a therapeutic target, however, as it has been shown that loss of PTEN function leads to the development of leukemia, and PTEN is therefore widely known as a tumor suppressor.¹⁰³ Indeed, loss of functioning PTEN is a very common mutation in a number of cancers.¹⁰⁴ This has not stopped the development of PTEN inhibitors as biological probes, however, with several molecules being found from HTS methods, including SF1670 **1.29** and quinone **1.30**.¹⁰⁵ Additional inhibitors based on vanadium complexes (like **1.31**) have also been reported.¹⁰⁶ Prestwich has also prepared metabolically stabilized inositol analogs as which function as PTEN inhibitors.¹⁰⁷ Both the 3–phosphothiolate **1.32** and the 3–methylenephosphonate **1.33** gave significant inhibition of PTEN, with the phosphorthioate providing a higher degree of inhibition (>90% inhibition at 0.4 µM) than the methylenephosphonate (>90% inhibition at 40 µM).
Figure 1–9. PTEN Inhibitors.

While it may seem counterintuitive to inhibit a tumor suppressor in order to treat disease, this has not stopped some researchers in evaluating the effects of PTEN inhibition on neutropenia–associated pneumonia. As increased levels of PI(3,4,5)P3 1.2 have been shown to enhance neutrophil function, inhibition of PTEN has been shown to slow neutrophil death and provide neutrophils with enhanced sensitivity to chemoattractant stimulation.\textsuperscript{108} PTEN inhibitors were therefore evaluated with regard to neutropenia–associated pneumonia and found to decrease mortality rates in mice.\textsuperscript{109} The most effective PTEN inhibitor was shown to be 1.30, which appears to be an analog of SF1670 1.29. In addition to their work with pneumonia, the same group has recently demonstrated that PTEN inhibition with 1.29 can increase the efficacy of granulocyte transfusion, which is often used to treat patients suffering from neutropenia due to chemotherapy.\textsuperscript{110} The increased effectiveness of the transfusion with the PTEN inhibitor is attributed to elevating the sensitivity of the neutrophils, as was demonstrated in the pneumonia treatment. This transfusion method also limits the exposure of the patient to the possibly problematic PTEN inhibitor, as it may be used only transiently and the cells
are treated while they are outside the transplant recipient. The authors also note that in myeloid specific PTEN knockout mice no tumors were detected until 3 months after birth.\textsuperscript{111} This shows that while elevated signaling due to high PI(3,4,5)P\textsubscript{3} levels does lead to cancer, it is likely a progressive process that takes months or perhaps even years in humans.

PTEN inhibition has also been implicated in nerve cell regeneration.\textsuperscript{84–86} This finding is based on PTENs role as a negative regulator of the PI3K/Akt/mTOR signaling pathway, which is known to be a common survival pathway downstream of neuronal growth factors. PTEN deletion was shown to enable adult neurons to affect a significant regenerative response that is typically not observed in similar cases of spinal or optic nerve injury in mice. While currently all studies have appeared have used genetic approaches for exploring the effects of PTEN on neuroregeneration, it appears to be just a matter of time before the use of small molecule PTEN inhibitors will be explored in this area.\textsuperscript{84}

**Conclusion**

Given the significant biological relevance that the PI3K pathway has on human health and the promise PI3K inhibitors show for the treatment of disease, attention has turned to the development of small molecule modulators of the inositol phosphatases as alternative method to attenuate PI3K signaling pathways. Both agonists and antagonists of the phosphatase SHIP1 have been developed, with inhibitors of SHIP2 and PTEN also being discovered in the last few years. While the modulation of the phosphatases of this pathway remains in their infancy when compared to the kinase, initial results show great promise in the treatment of many disease states.
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CHAPTER 2

SYNTHESIS AND BIOLOGICAL EVALUATION OF AMINOSTEROID INHIBITORS OF THE INOSITOL PHOSPHATASE SHIP1

Introduction

Inositol phospholipid (IP) signaling pathways play a critical role in cell proliferation, survival, differentiation and effector function.\(^1\)\(^-\)\(^3\) Phospholipids are ubiquitous minor constituents of cell membranes that are composed of a fatty acid chain which anchors an inositol ring decorated with phosphate groups. The position and number of these phosphates on the inositol ring act as mediators of cellular signaling. The pattern of phosphorylation is controlled by intracellular enzymes known as phosphatases and kinases.

SH2–containing inositol phosphatase–1 (SHIP1), a 145 kDa enzyme,\(^4\) is predominantly found in hematopoietic cells\(^5\)-\(^7\) but can also be found in endothelial cells\(^8\) and embryonic stem (ES) cells.\(^9\) This enzyme selectively hydrolyzes the 5’–phosphate from inositol polyphosphates, such as phosphatidylinositol–3,4,5–triphosphate (PI–3,4,5–P\(_3\)) \(2.1\) and inositol–1,3,4,5–tetrakisphosphate (I–1,3,4,5–P\(_4\)) \(2.3\). These inositol polyphosphates play important roles in growth factor mediated signaling.
Figure 2–1. SHIP1 Mediated Hydrolysis of Inositol Phosphates 2.1 and 2.3.

SHIP1 has also been shown to regulate Natural Killer (NK) cells$^{10-12}$ and T cells$^{13}$ that mediate bone marrow graft rejection and Graft–versus–Host–Disease (GvHD). Inhibition of SHIP increases the efficacy and utility of allogeneic bone marrow (BM) transplantation, a well utilized therapy for other diseases such as leukemia.$^{14-15}$ SHIP1 also prevents Steel factor (SF) from triggering degranulation of normal bone marrow–derived mast cells (BMMCs)$^{16}$ and regulates osteoclast formation and function.$^{17}$ In addition, SHIP1 plays an important role in the development of marginal zone macrophages$^{17}$ and B lymphocytes as well as antibody reproduction.$^{18-19}$

Specific chemical inhibitors of prominent signaling enzymes such as phosphoinositide 3–kinases (PI3K),$^{20}$ mitogen–activated protein kinases (MAPK),$^{21}$ extracellular–regulated kinases (ERK),$^{22}$ c–Jun N–terminal kinases (JNK),$^{23}$ and protein kinase B (PKB)$^{24}$ enable instantaneous and reversible inhibition and have greatly facilitated the exploration of the role these enzymes play with regard to other signaling
components. These inhibitors also helped determine the purpose of these signaling components in stem cell biology, cancer, immunity, and as well as other tissue function. Similar studies with SHIP phosphatases have been limited due to the unavailability of chemical inhibitors which can cross cell membranes.\textsuperscript{25–26}

In order to explore similar biochemical studies on SHIP1, a high–throughput screening (HTS) was performed using a fluorescence polarization (FP) assay on a library of compounds obtained from the National Cancer Institute. This screen identified NSC23922 \textbf{2.5} as an inhibitor of SHIP1 enzymatic activity.\textsuperscript{27}

![Chemical structures of NSC23922 2.5 and the International Union of Pure and Applied Chemistry (IUPAC) Steroid Numbering System.](image)

**Figure 2–2.** Structure of NSC23922 \textbf{2.5} and the International Union of Pure and Applied Chemistry (IUPAC) Steroid Numbering System.

**Background and Significance**

The NSC23922 \textbf{2.5} obtained from the National Cancer Institute (NCI), exhibited a detectable inhibitory activity against 0.1 \(\mu\)g of recombinant SHIP at 2 \(\mu\)M and showed 50\% inhibition at 10 \(\mu\)M. The activity of \textbf{2.5} with SHIP was also compared to its activity towards SHIP2 and PTEN (\textbf{Figure 2–3}), other IP modifying enzymes that hydrolyze the phosphates on PI(3,4,5)–P\(_3\). These studies showed that \textbf{2.5} is a selective inhibitor of SHIP1. The observed selectivity is important since the loss of PTEN function promotes malignancy in parenchymal and hematopoietic tissues.\textsuperscript{28–30} Also, the NCI obtained NSC23922 \textbf{2.5} showed biological activities \textit{in vivo} comparable to genetic phenotypes observed in SHIP\(^{-/-}\) mice.\textsuperscript{31–32}
**Figure 2–3.** The NSC23922 2.5 Inhibitory Activity Against SHIP1, SHIP2 and PTEN at 1 mM as Measured by Malachite Green Assay.

The ability of NSC23922 to expand the Mac1\(^+\)Gr1\(^+\) myeloid immunoregulatory (MIR) cell compartment in peripheral lymphoid tissues was investigated since the expansion of these cells usually suppresses GvHD.\(^{13–14,33}\) Treatment with 2.5 increased the number of MIR cells significantly in both the spleen and lymph node of treated mice, shown in **Figure 2–4**, while no significant change was observed for the vehicle compared controls.\(^{27}\) Thus, inhibiting SHIP in adult mice expands the MIR cell compartment which is consistent with SHIP deficient mice.\(^{14}\) In addition to the MIR cell compartment expansion observed by inhibiting SHIP with NSC23922, it also was found to reduce the priming of human and rodent allogeneic T cell responses.
Figure 2–4. Fluorescence Activated Cell Sorting (FACS) Quantification of Mac1⁺Gr1⁺ Myeloid Immunoregulatory (MIR) Cells in Spleen (A) and Lymph Node (B) of C57BL/6 Mice Treated with NSC23922 (Compound), Vehicle (Ethanol) and Normal (Unmanipulated) Controls.

Furthermore, NSC23922 (2.5) inhibition of SHIP resulted in an increased production of myeloid cells like granulocytes and neutrophils in the absence of the pneumonia and lung consolidation that is lethal for adult SHIP⁻/⁻ mice (Figure 2–5).¹⁶,³² Treatment with NSC23922 led to a 5– to 6-fold increase in circulating granulocytes in peripheral blood relative to the vehicle and unmanipulated controls shown in Figure 2–6.

Figure 2–5. Representative Hematoxylin and Eosin (H&E) Stained Lung Sections From NSC23922–Treated (A), Vehicle Treated (B) and Unmanipulated (C) C57BL6 Mouse.²⁷
**Figure 2–6.** Comparison of Circulating Granulocyte/Neutrophil Numbers of NSC23922–Treated, Vehicle Treated and Unmanipulated (Normal) Mouse.

Moreover, SHIP inhibition with NSC23922 2.5 reduced the survival of several human blood cancer cells *in vitro* such as the AML cell line KG–1. Comparable growth inhibitory effects were also observed for multiple myeloma (MM) cells. However, toxicity was not observed for cell lines that lack SHIP expression such as the osteoblast cell line MG63 (**Figure 2–7**).

**Figure 2–7.** MTT Proliferation Assay of KG1 and MG63 Cells Treated With Increasing NSC23922 Concentration.

**Objectives**

The primary objective of this work was to synthetically prepare both diastereomers of NSC23922 2.5 separately using optimized procedures to allow large scale synthesis. This would involve the verification of which diastereomer of NSC23922
2.5 has the same biological properties as the material obtained from NCI and determine which diastereomer is more biologically active. Also, analogs and derivatives of NSC23922 2.5 with improved solubility were also pursued, because of the limited solubility of 2.5 in water and DMSO (typically ethanol was used to dose 2.5). Ultimately better inhibitory activity would also be desirable, so determining and structure–activity relationships from these initial synthetic studies would also become an important goal.

The NSC23922 analogs and derivatives will be evaluated for their SHIP inhibition potency using the malachite green assay and the FP assay for inositol phosphatase activity. The assays allowed for the determination of role of several functional groups in SHIP inhibition. Also, when significant SHIP inhibitory activity was confirmed for any of the synthetic molecules, biological testing in vitro with human cells and in vivo in mice may be conducted. The synthesized SHIP inhibitors may also be used to facilitate biological exploration of SHIP function and the role SHIP plays with regard to other cellular signaling components.

Results and Discussion

The 1H NMR of NSC23922 2.5 showed that the compound tested is a mixture of α– and β–amine hydrochloride salts 2.9 and 2.13 (71:29). To prove this hypothesis, both 2.9 and 2.13 were synthesized. The starting point for both syntheses of 2.9 and 2.13 was the commercially available 5α–cholestan–3β–ol 2.6.

The β–alcohol 2.6 was converted into the corresponding α–azide 2.7 using a Mitsunobu reaction,34 which resulted in an inversion of the C–3 stereochemistry. Reduction of the α–azide 2.7 to the α–amine 2.8 was accomplished using lithium aluminum hydride (LAH).35 The α–amine 2.8 was then converted to the hydrochloride
salt 2.9 in a high yield using a solution of hydrogen chloride in diethyl ether. Methanolic hydrogen chloride while it resulted in the formation of hydrochloride salt, led to the isolation of product that was contaminated with unidentifiable impurities. (Scheme 2–1)

**Scheme 2–1** Synthesis of 3α–Amine Hydrochloride 2.9

![Diagram](image)

αReagents and conditions: (a) PPh₃, DIAD, (PhO)₂PON₃, THF, rt, 24 h 79% (b) LAH, THF, 4 h, reflux, 99% (c) HCl, Et₂O, 10 min, rt, 99%.

Synthesis of β–diastereomer 2.13 began with converting the β–alcohol 2.6 into the α–iodide 2.10. Use of a nucleophilic substitution reaction with NaN₃ in dimethyl sulfoxide (DMSO) followed by reduction using LAH provided β–amine 2.12. The β–amine 2.12 was then converted to the β–hydrochloride salt 2.13 using dry HCl gas generated from the reaction of calcium chloride with concentrated sulfuric acid. (Scheme 2–2)
Scheme 2–2a  Synthesis of β–Amine Hydrochloride 2.13

Reagents and conditions: (a) PPh₃, DIAD, CH₂I₂, PhH, 24 h, rt, 86%  (b) NaN₃, DMSO, 5 h, 90 °C, 91%  (c) LAH, THF, 4 h, 76 °C, 61%  (d) HCl, Et₂O, 10 min, rt, 63%

Comparison of the ¹H NMR of both synthetic α– and β–amine hydrochlorides with the NSC23922 obtained from NCI confirmed that NSC23922 was a mixture of 2.9 and 2.13 (Figure 2–8). In addition, the ¹³C NMR together with the mass spectral data of NCI obtained NSC23922 matched well with the synthesized hydrochloride salt 2.9 and 2.13 and further confirmed its structure.
Both 2.9 and 2.13 together with their synthetic intermediates were evaluated for their SHIP inhibition using the malachite green assay to determine the amount of inorganic free phosphate.\(^{39}\) This assay is a widely used procedure to quantitate protein phosphatase activity.\(^{40-42}\) The assay is based on the ion association of phosphomolybdic acid with the cationic malachite green (MG\(^+\)) dye at low pH.\(^{43-44}\) This coloration reaction is multi–step which is shown in Figure 2–9. The formation of the green molybdophosphoric acid complex measured directly at 620–640 nm and is directly related to the free phosphate concentration.\(^{44}\)
Figure 2–9. Coloration Reaction of Phophomolybdate with Malachite Green Under Acidic Conditions.\(^{45}\)

Initially the following synthetic products were tested and evaluated for their ability to inhibit SHIP1: alcohol 2.6, azide 2.7, amine 2.8, and hydrochloride salts 2.9 and 2.13. Synthetic products such as iodide 2.10, azide 2.11, and amine 2.12 were not tested because of their insolubility with the vehicle (ethanol). The results of the malachite green assay (Figure 2–10) revealed that amine 2.8 and hydrochloride salts 2.5 and 2.12 showed potent inhibitory activity against SHIP1. Careful inspection appears to indicate that the more active constituent of the NSC23922 obtained from NCI was amine 2.9 but both synthetically prepared 2.9 and 2.13 are both SHIP inhibitors.
Figure 2–10. Malachite Green Phosphatase Assay of 2.9, 2.13, and Its Synthetic Intermediates.\textsuperscript{46}

\textsuperscript{46} The malachite green assay was performed by Robert Brooks from SUNY Upstate Medical University, Syracuse, NY.
The SHIP inhibitory activity of NSC23922 promotes a rapid and effective recovery of neutrophils and platelets in myelosuppressed hosts. Although both 2.9 and 2.13 showed inhibition of SHIP1, only the α–amine hydrochloride 2.9 was used for in vivo studies because of the lower solubility of 2.13 in the vehicle. To test whether the synthetically prepared 2.9 will show the same activity as NSC23922, daily treatment of 2.9 (3AC) for 7 days to sublethally irradiated mice followed by analysis of their key blood components such as red blood cells (RBC), neutrophils and platelets were conducted and was compared to the vehicle–treated cohorts of mice. This assay simulates what happens from accidental radiation poisoning.

The treatment of 2.9 promoted a significant increase in RBC count 8 days after irradiation as shown in Figure 2–11A. In addition, the RBC level did not drop below normal after 15 days post irradiation which is expected for an irradiated host. Both 2.9 and vehicle–treated group’s neutrophils and platelet nadirs are significant which is shown in Figure 2–11B and Figure 2–11C, respectively. However, hosts that are treated with 2.9 showed faster and more robust recovery of neutrophils and platelets.
Figure 2–11. Comparison and Analysis of Red Blood Cells (RBC) (A), Neutrophils (B), and Platelets (C) of 2.9– and Vehicle–Sublethally Irradiated Mice.\textsuperscript{46}\textdagger

Additionally, treatment of SHIP1 inhibitor 3AC showed rapid and robust recovery of white blood cells (WBC) and lymphocytes. After day 22, all the 2.9–treated mice recovered normal WBC (Figure 2–12A) and lymphocyte count (Figure 2–12B) compared to the vehicle–treated mice where the WBC and lymphocyte count are near the lower limit and below the normal range, respectively.

\textsuperscript{46}\textdagger The blood component of 2.9– and Vehicle–Sublethally Irradiated Mice were analyzed by Robert Brooks and Sonia Iyer from SUNY Upstate Medical University.
Figure 2–12. White Blood Cells (A) and Lymphocytes (B) Count of 9 and Vehicle–Sublethally Irradiated Mice in 15, 22, and 32 days After Treatment.46†

Western blotting was performed on SHIP1–expressing OPM2 cell lysates to determine the mechanism of cell death in hematopoietic cancer cells after treatment of 3AC 2.9. Significant increase were observed after treatment of 3AC in both PARP and Caspase 3 cleavage that indicates 3AC–induced cell death proceeds via apoptosis in these cells. In addition, assays showed an increased frequency of Annexin V positive cells compared to the vehicle–treated controlled, indicating apoptosis.26–27

† The blood component of 2.9– and Vehicle–Sublethally Irradiated Mice were analyzed by Robert Brooks and Sonia Iyer from SUNY Upstate Medical University.
**Figure 2–13.** Staining of Annexin V Analyzed by Flow Cytometry and Western Blotting of OPM2 cells treated with 3AC.\textsuperscript{26}

SHIP1 inhibition also reduces basal and IGF–1 induced activation levels of Akt.\textsuperscript{27} This effect is consistent on Akt in MM cells, with 3AC showing cytotoxicity in MM cells.\textsuperscript{26} A tumor xenograft model of MM was employed to determine if previous \textit{in vitro} effects on cancer cell survival were sufficiently sustained and robust to enable abrogation of MM growth \textit{in vivo}. Immunodeficient NOD/SCID/IL2RγC (NSG) triple mutant mice were challenged with 1X10\textsuperscript{7} OPM2 cells which provided an \textit{in vivo} model for MM. OPM2 cells are considered a high-risk of MM\textsuperscript{47} because it harbors an internal deletion of the PTEN gene that ablates its expression that increases PI3K/Akt signaling and thus enhancing survival capacity.\textsuperscript{48–49} Mice received an initial injection of either 3AC or vehicle 6 hours after injection of MM cells and then daily for one week (\textit{induction phase}) followed by twice weekly injections of 3AC (\textbf{2.9}) or vehicle for the subsequent 15 weeks of the study (\textit{maintenance phase}). Treatment with 3AC reduced MM growth \textit{in vivo} which was determined by the reduction of free human Ig κ light chain levels in the plasma. In addition, 3AC treatment reduced the numbers of circulating OPM2 MM cells in PBMC and showed increased survival after OPM2 challenge. (\textbf{Figure 2–14}) Thus,
treatment with the SHIP1 selective inhibitor 3AC also significantly abrogates MM tumor growth *in vivo*.

![Graph A](image1)

**Figure 2–14.** SHIP1 Inhibition Triggers MM Cell Death, Reduces MM Growth *In Vivo* and Enhances Host Survival. (A) ELISA Quantitation of Human Ig λ Light Chain in Serum of OPM2 Challenged Mice (3AC or Vehicle Treated) or Unchallenged NSG Mice (B) Circulating OPM2 MM Cells of Challenged Mice (3AC or Vehicle Treated) or Unchallenged NSG Mice and (C) Survival of 3AC and Vehicle Treated OPM2 Challenged Mice.26

In a separate experiment, NSG mice were challenged with 10⁷ OPM2 cells stably expressing a luciferase transgene (12 mice/group). Five weeks after challenge and initiation of 3AC therapy or vehicle treatment, mice were injected with luciferin substrate and imaged. (**Figure 2–15**) Tumors were readily detected in all vehicle control mice. On the other hand, 5 out of 12 3AC–treated mice had undetectable MM growth after 5 weeks of treatment. (One of the 12 vehicle treatment mice had already succumbed to MM prior to these image in **Figure 2–15**.)
Figure 2–15. Luciferase Based Assay of the *In Vivo* Anti-MM Effect of 3AC Imaged with the IVIS50.\(^{50f}\)

Furthermore, no significant morbidity, mortality or weight loss was observed in 3AC–treated mice and there was no evidence of pathology in major organs of mice treated with the SHIP1 inhibitor.\(^{27}\) These results contrast significantly with germline SHIP knockout mice that develop a severe lung pathology related to a myeloid consolidation of the alveolar spaces\(^{51}\) and a Crohn’s like ileitis,\(^{52}\) routinely observed in MxCreSHIP\(^{\text{flox/flox}}\) mice after induced genomic ablation of SHIP expression, but not in polyI/C–treated SHIP\(^{\text{flox/flox}}\) controls.\(^{52}\) In addition, SHIP1 inhibition by 3AC treatment for 7 days does not cause pulmonary myeloid infiltration and lung consolidation\(^{27}\) that is uniformly lethal for adult SHIP1\(^{-/-}\) mice.\(^{16,49}\) This lack of significant toxicity or effects on host survival following small molecule inhibition of SHIP1 suggests that 3AC and SHIP1 inhibition with small molecules could be a safe and effective treatment for MM.

\(^{\dagger}\) The luciferase based assay experiments were conducted by Mi–young Park from SUNY Upstate Medical University.
Figure 2–16. SHIP1 Inhibition of 3AC Does Not Cause Morbidity or Pathology. (A) Comparison of Body Weight of 3AC and Vehicle Treated Mice (B) Examined Tissues of 3AC Treated Mice that Shows No Evidence of Organ Pathology Determined by a Veterinary Pathologist.\textsuperscript{27}

The citrate salt of amine 2.8 (Scheme 2–3) was also prepared and was evaluated for its activity and solubility. Compared to 2.9, which is insoluble in DMSO and only soluble in ethanol, amine citrate 2.16 was soluble in DMSO. In addition, 2.16 showed a comparable biological activity.
**Scheme 2–3** Synthesis of Amine Citrate Salt 2.16.

\[ \text{Reagents and conditions: (a) 2.15, THF, rt, 19 h, 87\%.} \]

Furthermore, functionalization of the amine moiety, such as acylation and alkylation as shown in **Scheme 2–4** was investigated to study the effect of these changes on the structure with the biological activity. Acylation of the \( \alpha \)-amine 2.8 using acetyl chloride afforded amide 2.17. Reduction of the acetamide to the ethylamine (2.18) was achieved through reaction with LAH. Both amide 2.17 and amine 2.18 were subjected to the malachite green assay. While the ethylamine showed no activity, amide 2.17 initially showed promising SHIP1 inhibition, but this was later shown to be an artifact as later evaluations showed minimal SHIP1 inhibition.

**Scheme 2–4** Acylation and Alkylation of 3\( \alpha \)–Amine 2.18

\[ \text{Reagents and conditions: (a) acetyl chloride, Et}_3\text{N, THF, 0 °C to rt, 30 min, 65\% (b) LAH, THF, 16 h, 76 °C, 61\%.} \]

Several other amides (2.19–2.30) were also synthesized (summarized in **Table 2–1**). These molecules were to be used to evaluate whether the alkyl group on the amide can improve its inhibitory activity towards SHIP, as the acetamide was considered highly
active. However, all these amides showed no inhibitory activity. This led to reevaluation of the acetamide 2.17 which then showed minimal SHIP1 activity. This study indicated that a free amine or amine salt is necessary for the exhibited SHIP1 inhibitory activity.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
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<td>13</td>
</tr>
<tr>
<td>2</td>
<td>2.20</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>2.21</td>
<td>81</td>
</tr>
<tr>
<td>4</td>
<td>2.22</td>
<td>72</td>
</tr>
<tr>
<td>5</td>
<td>2.23</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>2.24</td>
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<tr>
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<td>2.25</td>
<td>52</td>
</tr>
<tr>
<td>8</td>
<td>2.26</td>
<td>80</td>
</tr>
<tr>
<td>9</td>
<td>2.27</td>
<td>63</td>
</tr>
</tbody>
</table>
Table 2–1. Synthesis of Amides 2.19–2.30.

In addition, the effect of different functional groups at the C–3 position of the steroid on the biological activity was investigated. The β–alcohol 2.6 was converted to the α–thioacetate 2.31 through Mitsunobu reaction which was reduced to the α–thiol 2.32. (Scheme 2–5) The SHIP inhibitory activity of both 2.31 and 2.32 were determined by malachite green assay however, these molecules do not show any inhibitory activity.

Scheme 2–5a Synthesis of 3α–Thiol 2.32

Scheme 2–6 aReagents and conditions: (a) PPh3, DIAD, CH3COSH, THF, 24 h, 0 °C to rt, 100% (b) LAH, Et2O, 24 h, 0 °C to rt, 66%.

The effect of moving the amine further outside the A ring of the steroid nucleus was also explored as shown in Scheme 2–6. First, the β–alcohol 2.6 was converted to the tosylate 2.33.34 Nucleophilic substitution reaction of 2.33 with NaCN in DMSO afforded 3α–cyanide 2.34 which was reduced using LAH to give amine 2.41. However, isolation
of amine 2.41 was not observed. Consumption of the starting material were observed however no identifiable product were recovered.

**Scheme 2–6**

First Generation Synthesis of Methanamium Chloride 2.36

---

Scheme 2–6

First Generation Synthesis of Methanamium Chloride 2.36

---

$^a$Reagents and conditions: (a) $p$–TsCl, pyridine, rt, 24 h, 93% (b) NaCN, DMSO, reflux, 18 h, 69% (6:94) (c) LAH, THF, 4 h, 76 °C (g) HCl, Et$_2$O, 10 min, rt

Alternatively, the desired methylamine 2.35 may be accessed by reduction of the nitrile to the alcohol followed by a Mitsonobu reaction and reduction, which is a similar synthetic strategy for the synthesis of 2.9. Diisobutylaluminium hydride (DIBAL) reduction of the $\alpha$–cyanide 2.34 afforded an inseparable mixture of $\alpha$– and $\beta$–carbaldehydes 2.37 and 2.38 in 80% (72:28) that epimerized on standing. Since the $\alpha$–carbaldehyde 2.37 was much desired, both diastereomers were converted to the alcohol through a NaBH$_4$ reduction to determine if the $\alpha$–methanol 2.39 could be separated from the $\beta$–methanol 2.40. The crude $^1$H NMR of the reaction mixture revealed the conversion to methanols 2.39 and 2.40. Unfortunately, these compounds were inseparable and the route was discontinued.
Scheme 2–7\textsuperscript{a} Second Generation Synthesis of Methanamium Chloride 2.36

\textsuperscript{a}Reagents and conditions: (a) DIBAL, DCM, \(-78\,^\circ\text{C}\), 1 h, 80\% (2.6:1) (b) NaBH\(_4\), MeOH, 0 °C, 1 h (c) PPh\(_3\), DIAD, (PhO)\(_2\)PON\(_3\), THF, rt, 24 h (d) LAH, THF, 4 h, 76 °C (e) HCl, Et\(_2\)O, 10 min, rt.

Because of the solubility problems with the amides 2.17, 2.19–2.30 as well as amines 2.9 and 2.13, synthesis of analogs with improved solubility and greater druglikeness were designed keeping in mind Lipinski’s rules.\textsuperscript{55} Lipinski’s rules, also known as the rule of five, evaluates molecule’s druglikeness. These concepts describe the importance of molecular properties for druglike pharmacokinetics, however, the rules do not predict if a compound will be pharmacologically active, only if it will have the correct solubility properties to function in a cellular environment. The two possible ways to increase the solubility of these steroids are incorporating additional polar functional groups (\textit{e.g.} 2.42 and 2.43), removing alkyl groups (\textit{e.g.} 2.44) or both (\textit{e.g.} 2.45, 2.46, and 2.47). These modifications also can be quantified using the calculated partition coefficient (CLogP) which is based on the differential solubility of the compound between 1–octanol and water. Some CLogP values of proposed inhibitors are shown in
Figure 2–17. Most drug like molecules have LogP measurements of between 1 and 5, so the molecules with similar CLogP numbers were targeted first (2.44–2.47). Also, it is important to remember is that the CLogP calculations were performed on the free amine; the amine salts should be more water soluble.

Figure 2–17. Comparison of Calculated Partition Coefficient (cLogP) of 2.8 and Its Analogs.

Pursuant to the synthesis of amines 2.44 and 2.45, trans–androsterone 2.48 was chosen as a starting material. The synthesis of amine 2.44 commenced from reduction of the ketone 2.48 to alkane 2.49 using a Wolff–Kishner reaction.\(^6\) Unexpectedly, besides the conversion of the ketone to the alkane, formation of a small amount of the 3α–alcohol 2.50 was also observed (Scheme 2–8). Several side reactions of Wolff–Kishner reaction has been reported which includes double bond migration,\(^6\) hydrazone cyclizations,\(^5\)–\(^8\) \(\alpha\)–substituted ketone cleavage,\(^6\) olefin reduction,\(^6\)\(^1\) and aldehyde derivative base catalyzed decomposition,\(^6\)\(^2\) however, none have reported inversion of secondary alcohols.
**Scheme 2–8**a Reduction of the Ketone 2.50 Using Wolff–Kishner Reaction

```
\begin{align*}
\text{2.48} & \xrightarrow{a} \text{2.49} + \text{2.50} \\
\end{align*}
```

*aReagents and conditions: (a) N\textsubscript{2}H\textsubscript{4}, KOH, HOCH\textsubscript{2}CH\textsubscript{2}OH, 206 °C, 23 h, 85% (24:1).

The β–alcohol 2.49 was converted to the α–azide 2.51 as shown in **Scheme 2–9**.

LAH reduction of 2.51 yielded α–amine 2.44 which was then reacted with a solution of hydrogen chloride in diethyl ether which resulted in the precipitation of amine hydrochloride 2.52. In addition to the hydrochloride salt 2.52, acetamide 2.53 and citrate 2.54 were also prepared using the similar methodology for the synthesis of 2.17 and 2.16, respectively.

**Scheme 2–9**a Synthesis of Hydrochloride Salt 2.52, Acetamide 2.53, and Citrate 2.54

```
\begin{align*}
\text{2.49} & \xrightarrow{a} \text{2.51} (R = \text{N}_3) \xrightarrow{b} \text{2.44} (R = \text{NH}_2) \xrightarrow{c} \text{2.52} \\
\text{2.53} & \xrightarrow{d} \text{2.54} \\
\end{align*}
```

*aReagents and conditions: (a) PPh\textsubscript{3}, DIAD, (PhO)\textsubscript{2}PON\textsubscript{3}, THF, rt, 24 h, 74% (b) LAH, THF, 4 h, 76 °C, 72%, (c) HCl, Et\textsubscript{2}O, 10 min, rt, 65%, (d) acetyl chloride, Et\textsubscript{3}N, THF, 0 °C to rt, 30 min, 22% (e) 2.15, THF, rt, 78%.
Amine hydrochloride 2.57 was also synthesized by converting the alcohol 2.48 to the azide 2.56 through a substitution reaction of the β-tosylate 2.55 with sodium azide in DMSO. Azide reduction using a Staudinger reaction followed by reaction with hydrogen chloride in diethyl ether gave the desired amine hydrochloride 2.57. (Scheme 2–10)

**Scheme 2–10**

Synthesis of Hydrochloride Salt 2.57

![Scheme Diagram]

Reagents and conditions: (a) TsCl, pyridine, rt, 24 h, 87% (b) NaN₃, DMSO, 5 h, 100 °C, 67% (c) (i) PPh₃, THF, rt, 18 h (ii) H₂O, 80 °C, 1 h (iii) HCl, Et₂O, 10 min, rt, 37% (over two steps).

Both amine hydrochloride salts 2.52 and 2.57, acetamide 2.53, and citrate 2.54 showed improved solubility compared to the original hydrochloride salt 2.9 which are summarized in Table 2–2. Furthermore, 2.54 exhibited solubility in mixture of dimethylsulfoxide (DMSO):water (~1:3).
<table>
<thead>
<tr>
<th>Compound</th>
<th>Vehicle</th>
<th>SHIP1 %Inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Compound 2.52" /></td>
<td>DMSO</td>
<td>67.0%</td>
</tr>
<tr>
<td><img src="image2.png" alt="Compound 2.57" /></td>
<td>DMSO</td>
<td>2.6%</td>
</tr>
<tr>
<td><img src="image3.png" alt="Compound 2.53" /></td>
<td>DMSO</td>
<td>Not Determined</td>
</tr>
<tr>
<td><img src="image4.png" alt="Compound 2.54" /></td>
<td>DMSO</td>
<td>Not Determined</td>
</tr>
<tr>
<td><img src="image5.png" alt="Compound 2.9" /></td>
<td>Ethanol</td>
<td>50.9%</td>
</tr>
</tbody>
</table>

*Determinated at 1 mM Concentration Using Malachite Green Assay

**Table 2–2.** Solubility of Amine Hydrochloride Salts 2.53 and 2.57, Acetamide 2.54, and Citrate 2.54 Compared to 2.9.

The recombinant SHIP activity of these compounds were measured by malachite green assay and revealed that hydrochloride salt 2.57 showed no inhibitory activity against SHIP. On the other hand, 2.52 and 2.54 showed a comparable or improved activity with 2.9. This suggests that incorporation of polar functional groups (e.g. C=O) in ring D, particularly at the C–17 position, may result in a decrease or no inhibition
towards SHIP. This leads to the hypothesis that the steroid A ring is in a polar portion of the binding pocket and the D ring is in a nonpolar portion. (Figure 2–18)

![Polar binding pocket](image)

**Figure 2–18.** Structure–Activity Relationship (SAR) of Aminosteroid.

Also, an MTT assay for cell viability was conducted using C1498 leukemia cells, with the results as shown in **Figure 2–19**. This assay revealed that 2.52 (3A5AS) is more potent in the killing of blood cancer cells (C1498 leukemia cells) compared to 2.9 (3AC). Amine hydrochloride 2.52 also showed greater potency in inducing the formation of MIR cells *in vivo* as it can induce a comparable MIR cell compartment growth at 15 µM compared to the 60 µM showed by 2.9. At this stage, it’s difficult to determine if the greater activity is due to increased solubility or increased potency.

![MTT Assay](image)

**Figure 2–19.** MTT Assay for Cell Viability C1498 Leukemia Cells Result of 2.9 (3AC) and 2.52 (3A5AS).\(^\dagger\)

\(^\dagger\) The MTT assay for cell viability was conducted by Robert Brooks from SUNY Upstate Medical University.
Synthesis of the **2.52** diastereomer hydrochloride salt, **2.62** and its corresponding citrate salt **2.62**, were also conducted. These molecules were synthesized to establish whether the difference between the SHIP inhibitory activity exhibited by **2.9** and **2.13** was due to the difference in stereochemistry or just simply a difference in solubility. The strategy for the synthesis of these molecules is homologous to the synthesis of amine salt **2.13.** (Scheme 2–11) Conversion of the β-alcohol **2.49** to α-iodo **2.58** followed by substitution and reduction yielded amine **2.60**. Reaction of amine **2.61** with hydrogen chloride in diethyl ether and citric acid in THF provided **2.61** and **2.62**, respectively.

**Scheme 2–11** Synthesis of Amine salts **2.61** and **2.62**

\[
\begin{align*}
\text{2.49} & \xrightarrow{\text{a}} \text{2.58} & \xrightarrow{\text{b}} \text{2.59} \\
\text{2.61} & \xrightarrow{\text{d}} \text{2.60} & \xrightarrow{\text{e}} \text{2.62}
\end{align*}
\]

^aReagents and conditions: (a) PPh₃, DIAD, CH₃I, PhH, 24 h, rt, 75% (b) NaN₃, DMSO, 5 h, 90 °C, 32% (c) LAH, THF, 4 h, 76 °C (d) HCl, Et₂O, 10 min, rt, 74% (over two steps) (e) citric acid, THF, rt, 12 h, 76% (over 2 steps).

Hydrochloride salt **2.61** is one of the most soluble analogs yet synthesized being soluble in water when heated compared to **2.52** and **2.62** that are only soluble in DMSO. The compound **2.61** was still soluble even after it was cooled to room temperature. Comparison of the SHIP1 %inhibition of **2.61** and **2.52** showed that their activity is
comparable thus establishing that the difference in inhibitory activity of the parent compounds 2.9 and 2.13 is more likely due to their difference in solubility than the stereochemistry.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solubility</th>
<th>Vehicle</th>
<th>SHIP1 %Inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>DMSO</td>
<td>DMSO</td>
<td>61.7%</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>Hot H₂O</td>
<td>H₂O</td>
<td>58.5%</td>
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<tr>
<td><img src="image" alt="Structure" /></td>
<td>Hot DMSO</td>
<td>DMSO</td>
<td>72.3%</td>
</tr>
</tbody>
</table>

* Determined at 1 mM Concentration Using Malachite Green Assay

Table 2–3. Solubility and %SHIP1 Inhibition of Amine Hydrochloride Salts 2.53 and 2.61 and Citrate 2.62.†

Incorporation of the amine functionality into the ring A (2.63 and 2.64) was also pursued. These compounds were deemed interesting because they oriented the amine in the same area as the other aminosteroids but were more conformationally restricted than other molecules.

† The %SHIP1 inhibition of the molecules was determined through malachite green assay conducted by Amanda Balch from SUNY Upstate Medical University.
The synthesis of these derivatives commenced by oxidizing 2.49 to ketone 2.65 which is shown in Scheme 2–12. The ketone 2.65 was converted to oxime 2.66 in good yields with hydroxylamine. The oxime was subjected to a SOCl₂–catalyzed Beckmann rearrangement which gave an equal mixture of lactams 2.67 and 2.68 which were inseparable. Alternatively, unsaturated oxime 2.70 and its O–tosylate oxime 2.71 were synthesized since it was reported that α–unsaturated oximes undergo Beckmann rearrangement in which the alkyl migration does not occur on the sp²–α–carbon which should lead to the formation of 2.72. The expected lactam to be formed would then be reduced to form the cyclic amine 2.64. Enone 2.69, synthesized from an elimination of bromoketone using lithium bromide and potassium carbonate, was converted to the O–methyl oxime and was subjected to the SOCl₂–catalyzed Beckmann rearrangement to give unsaturated lactams 2.72 as the major product. However, in some cases the ratio between unsaturated lactam 2.72 and 2.73 are not consistent. In addition, these lactams like 2.67 and 2.68 were inseparable. With the problems associated with the synthesis of these compounds attention was refocused on other derivatives.
Scheme 2–12a Syntheses of Lactams 2.67 and 2.68

\[ \text{Scheme 2–12} \]

\[
\begin{align*}
\text{2.49} & \xrightarrow{a} \text{2.65} \xrightarrow{b} \text{2.66} \xrightarrow{c} \text{2.67} \quad \text{2.68} \\
\text{2.69} & \xrightarrow{e} \text{2.70} \xrightarrow{f} \text{2.71} \xrightarrow{g} \text{2.72} \quad \text{2.73}
\end{align*}
\]

aReagents and conditions: (a) PCC, silica gel, DCM, rt, 2 h, 93% (b) NH₂OH · HCl, NaOAc, MeOH, H₂O, 110 °C, 4 h. (c) i. SOCl₂, –78 °C ii. NaOH (d) i. PCC, silica gel, DCM, rt, 2 h, 93% ii. C₅H₅N · HBr₃, CH₃COOH, 50 °C, rt, 65% iii. LiBr, Li₂CO₃, DMF, 175 °C, 3 h. 76% (e) NH₂OH · HCl, NaOAc, MeOH, H₂O, 110 °C, 4 h., 100% (f) p–TsCl, pyridine, rt, 3 h., quantitative (g) i. SOCl₂, –78 °C ii. NaOH, 38%.

Given that polar substituents are not tolerated on the D ring, the incorporation of multiple polar groups on A ring was investigated. This may lead to multiple polar contacts with the enzyme and therefore greater potency. In support to this hypothesis, another aminosteroid identified as a SHIP inhibitor from HTS is NSC54340 2.74. This molecule leads to the design for the synthesis of aminoalcohols 2.75 and 2.76. (Figure 2–21)

\[ \text{Figure 2–21. NSC54340 2.74 and Aminoalcohols 2.75 and 2.76.} \]
The syntheses of these aminoalcohols commenced from known ketone 2.65 (Scheme 2–13). The ketone 2.65 was subjected to a regioselective and diastereoselective α–bromination using pyridinium tribromide to give α–bromide 2.77. Treatment of the bromide 2.77 with sodium azide in dimethylformamide (DMF) afforded 2α–azide 2.78. The resulting stereochemistry of the azide is likely thermodynamically controlled with epimerization being facile under the reaction conditions.

Scheme 2–13† Synthesis of Azidoketone 2.78

\[ \text{Ketone} \ 2.78 \rightarrow \text{Alcohol} \]

†Reagents and conditions: (a) PCC, silica gel, DCM, rt, 2 h, 93% (b) C₅H₅N · HBr₃, CH₃COOH, 50 °C, rt, 65% (c) NaN₃, DMF, 2 h, 28 °C, 80%.

Ketone (2.78) was reduced to the alcohol utilizing several reducing protocols as shown in Table 2–4. Most of the protocols gave moderate yields and mixtures of azidoalcohols 2.79 and 2.80. As expected when borohydride reducing agents were used 2.80 was formed preferentially, and when L–selectride was used as the hydride source 2.79 was the predominant product. Hydride sources such as NaBH₄ and LiBH₄ prefer to deliver the hydride through an axial attack to form an equatorial alcohol like 2.80. The transition state for axial attack minimizes the torsional strain in the transition state, favoring formation of the equatorial alcohol. On the other hand, hydride sources such as L–selectride prefer to deliver the hydride through an equatorial attack forming axial alcohol 2.79. This avoids steric interactions between the bulky hydride reagent and the axial hydrogen on C–2 and methyl substituent in C–10.
Table 2–4. Reduction of Ketone (2.79).

In order to verify our initial assignments of the axial and equatorial alcohols 2.79 and 2.80 we turned to analysis of the $^1$H NMR coupling constants. Unfortunately, analysis of the coupling constants of the aminoalcohols was complicated by the coupling of the alcohol protons and overlapping peaks. In order to shift the hydrogen next to the alcohol downfield and minimize the undesired couplings, the acetate of alcohols 2.79 and 2.80 were synthesized (Scheme 2–14).

Scheme 2–14$^a$ Acylation of Alcohol (2.81)

$^a$Reagents and conditions: (a) 2.82, pyridine, rt, 24 h.

Analysis of the observed coupling constants for the acetate derivative of azidoalcohol 2.79 showed that methine proton adjacent to the azide functionality (H$_1$) is in an axial position due to the one large ($J = 12.8$ Hz) and two small coupling constants ($J = 4.3, 3.3$ Hz) observed in the $^1$H NMR spectra. However, determination of the coupling
constants for the methine proton adjacent to the acetate group (H₂) was difficult because of the observed broad multiplicity. This is consistent with an equatorial orientation of this proton as it would have three coupling constants of a similar but different magnitude which may lead to a complex signal in the ¹H NMR. Both methine protons H₁ and H₂ of the acetate derivative of azidoalcohol 2.80 showed two large coupling constants, with J = 12.6, 10.2 Hz and J = 11.1 and 10.2 Hz, respectively. This implies that both methine protons are in an axial orientation. These analyses confirm the stereochemical outcome of the reduction reaction as well as confirmation that epimerization at the C–2 position did not occur.

![Diagram](image)

**Figure 2–22.** Determination of C–2 and C–3 Stereochemistry of Azidoalcohol 2.79 and 2.80 Using Coupling Constant.

Azidoalcohols 2.79 and 2.80 were reduced using LAH and the resulted amines were treated with hydrogen chloride in diethyl ether to form amine salts 2.86 and 2.87, respectively, as shown in **Scheme 2–15**.
Scheme 2–15

Synthesis of Aminoalcohols 2.86 and 2.87

\[\text{Reagents and conditions: (a) NaBH}_4, \text{ CH}_3\text{OH, DCM, } -78^\circ\text{C, 2 h, 68\% (35:65) (b) LAH, THF, 80^\circ\text{C, 4 h (c) HCl, Et}_2\text{O, rt, 10 min. (50 \% over 2 steps) (d) LAH, THF, 80^\circ\text{C, 4 h (e) HCl, Et}_2\text{O, rt, 10 min. (62 \% over 2 steps).}\]

Biological evaluation of the amine salts (2.86 and 2.87) using the malachite green assay showed that both of these compounds have improved SHIP1 inhibitory activity compared to 2.9 (Table 2–5). Both hydrochloride salts 2.86 and 2.87 showed better inhibitory activity towards SHIP1 compared to 2.9. In addition, SHIP2 inhibition of these molecules was also evaluated using the same assay. Surprisingly, 2.86 and 2.87 showed better potency against SHIP2 compared to 2.9 with 83.8\% and 53.4\% SHIP2 %inhibition, respectively. These data show that the aminosteroid class of inhibitors will not all be SHIP1 selective inhibitors, but depending on the pattern of the functional group on the A ring they may be pan SHIP1/SHIP2 inhibitors. In addition, these molecules showed better solubility compared to 2.9. Aminehydrochloride salts 2.86 and 2.87 were soluble in mixture of DMSO and H\(_2\)O (80:20) and hot H\(_2\)O, respectively.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Solubility</th>
<th>%Inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.86</td>
<td>80:20 DMSO:H₂O</td>
<td>68.7% 83.8%</td>
</tr>
<tr>
<td>2.87</td>
<td>Hot H₂O</td>
<td>61.4% 53.4%</td>
</tr>
<tr>
<td>2.9</td>
<td>EtOH</td>
<td>50.9% 31.8%</td>
</tr>
</tbody>
</table>

* Determined at 1 mM Concentration Using Malachite Green Assay

Table 2–5. Comparison of Solubility and SHIP1 and SHIP2 %Inhibition of Amine Hydrochloride Salts 2.86, 2.87 and 2.9.†

Attempts were also made to synthesize the corresponding diamines like 2.47. The synthesis of these molecules were first attempted using azidoketone 2.78 (Scheme 2–16).

The azidoketone 2.78 was converted to the azido oxime 2.88 using hydroxylamine. The azide could then be reduced using a Staudinger reaction followed by a borane reduction of the oxime which would afford the desired diamine 2.47. The reduction of the azide (2.88) using triphenylphosphine and water afforded 2.90. However, the removal of the triphenylphosphine oxide, a byproduct of the Staudinger reaction, was very difficult. In addition, full conversion of the azide to the amine was not observed which indicates that the Staudinger reaction does not go to completion. This was supported by the conversion

† The %SHIP1 and %SHIP2 inhibition of the molecules were determined through malachite green assay conducted by Amanda Balch from SUNY Upstate Medical University.
of the aminooxime to the dihydrochloride salt 2.91 that gave only 17% yield over two steps.

**Scheme 2–16**  
First Generation Synthesis of cis–Diamine 2.47

\[ \text{N}_3 \rightarrow \text{H} \quad \text{H}_3\text{CO} \rightarrow \text{N} \]

\[ 2.78 \quad \rightarrow \quad 2.88 \]

\[ \quad \rightarrow \quad 2.90 \quad \text{R}_1 = \text{NH}_3, \text{R}_2 = \text{NOCH}_3 \]

\[ \quad \rightarrow \quad 2.91 \quad \text{R}_1 = \text{NH}_3^+\text{Cl}^- \]

Reagents and conditions: (a) CH\textsubscript{3}ONH\textsubscript{2} · HCl, pyridine, rt, 24 h, 78% (b) i. PPh\textsubscript{3}, H\textsubscript{2}O, THF, rt, 48 h ii. BH\textsubscript{3}–THF, rt → 78 °C, 1.5 h (c) HCl, Et\textsubscript{2}O, rt, 10 min (d) PPh\textsubscript{3}, H\textsubscript{2}O, THF, rt, 48 h, 41% (e) HCl, Et\textsubscript{2}O, rt, 10 min, 17% over 2 steps from 2.88.

A second route to the diamine 2.47 could be envisioned from 2.80 (Scheme 2–16). The azidoalcohol 2.80 can be transformed to the cis–diazide using a Misononbu reaction to convert the alcohol to the azide, however this methodology may not be compatible with the substrate since the triphenylphosphine reacts with the azide moiety. In addition, the use of a direct conversion of alcohols to azides using diphenyl phosphorazidate and DBU\textsuperscript{69} was explored. Although starting material was consumed, no desired and identifiable product could be isolated.

The alcohol (2.80) was then converted to the mesylate 2.92. Attempts to convert the alcohol to its tosylate were also attempted, however the reaction did not proceed to full conversion, possibly due to steric factors with the relatively hindered alcohol. The
mesylate 2.92 was then displaced with azide using S_N2 conditions. Several protocols were explored for the conversion of mesylate 2.92 to the cis--diazide 2.93 which are shown in Table 2–6. Isolation and purification of 2.93 using silica gel chromatography seemed to decompose during silica gel chromatography. Thus, conditions that would provide relatively pure 2.93 after work–up with no further purification were necessary. Reaction of 2.92 with 4 equivalents of sodium azide in DMF at 90 °C for 20 h gave a good yield of 2.93. This procedure (Entry 5) uses milder reaction conditions to the first attempt (Entry 1). Lastly, LAH reduction followed by reaction with hydrogen chloride in diethyl ether afforded the desired cis--diamine salt 2.89.

Scheme 2–17a  Second Generation Synthesis of Diamine 2.47

Reagents and conditions: (a) MsCl, pyridine, rt, 24 h, 92% (b) NaN₃, DMF, 100 °C, 20 h, 90% (c) (PhO)₂PON₃, DBU, 0 °C to rt, 18 h (d) LAH, THF, reflux, 4 h (d) HCl, Et₂O, rt, 10 min, 77% over 2 steps.
Table 2–6. Synthesis of cis–Diazide 2.93.

A similar strategy was used to synthesize the trans–diamine salt 2.96. Mesylation of alcohol 2.79 using methanesulfonyl chloride in pyridine provided 2.94 in good yields (Scheme 2–18). The mesylate 2.94 was then converted to the trans–diazide 2.95. Like 2.93, several conditions, as shown in Table 2–7 were evaluated to optimize this transformation. Reaction of 2.94 with 2 equivalents of sodium azide in DMSO at 90 °C provided pure 2.95 with no chromatography needed. LAH reduction followed by a reaction of the resulting diamine with a solution of hydrogen chloride in diethyl ether provided the desired trans–diamine salt 2.97.
Scheme 2–18\textsuperscript{a} Synthesis of trans–Diamine 2.96

\[ \text{Scheme 2–18 (trans–Diamine 2.96)} \]

\[ \text{Reagents and conditions: (a) MsCl, pyridine, rt, 24 h, 100\%} \text{ (b) } \text{NaN}_3, \text{ DMSO, 90 }^\circ\text{C, 5 h, 92\%} \text{ (c) LAH, THF, 80 }^\circ\text{C, 4 h} \text{ (d) HCl, Et}_2\text{O, rt, 10 min, 100\% over 2 steps.} \]

\[ \text{Table 2–7. Synthesis of trans–diamine salt 2.95.} \]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sodium Azide (equiv)</th>
<th>Solvent</th>
<th>( t ) Time</th>
<th>% conversion</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>DMF</td>
<td>reflux</td>
<td>5 h</td>
<td>100%</td>
</tr>
<tr>
<td>2*</td>
<td>2</td>
<td>DMSO</td>
<td>90 ( ^\circ\text{C} )</td>
<td>5 h</td>
<td>100%</td>
</tr>
</tbody>
</table>

\*In some case, formation of 2.95 and 2.92 were observed. [2.95:2.92 (94:6)]

SHIP1 and SHIP2 inhibitory activity of diaminohydrochloride salts 2.89 and 2.97 were evaluated and was compared to 2.9. Both of these compounds showed improved SHIP1 inhibition compared to 2.9. Comparison of diaminohydrochloride salt 2.89’s inhibitory activity against SHIP1 and SHIP2 showed that it is more selective SHIP1 inhibitor. On the other hand, 2.97 has a better SHIP2 inhibitory activity that shows that 2.97 is a pan SHIP1/SHIP2 inhibitor.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Solubility</th>
<th>%Inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SHIP1</td>
</tr>
<tr>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>H₂O at rt</td>
<td>68.9%</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>60:40 DMSO:H₂O</td>
<td>56.2%</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure 3" /></td>
<td>EtOH</td>
<td>58.6%</td>
</tr>
</tbody>
</table>

*Determined at 1 mM Concentration Using Malachite Green Assay

**Table 2–8.** SHIP1 and SHIP2 %Inhibition of Amine Hydrochloride Salts 2.89, 2.97 and 2.9.⁶⁴†

Attention was also focused on the evaluation of SHIP1 agonists. Interestingly, SHIP acts as a negative regulator of mast cell and macrophage activation,⁷⁰ osteoclast formation, resorptive function,⁷¹ and has been reported to be tumor suppressor in acute myelogenous leukemia (AML)⁷² and chronic myelogenous leukemia (CML).⁷³ Selective activators of SHIP would be useful experimental tools and potential drug candidates to provide proof of principle validation for a new approach to the treatment of inflammation, osteoporosis, and leukemia. Although there is interest in exploring therapeutic potential of phosphatase inhibitors,⁷⁴ there has been few endeavors for phosphatase activators.⁷⁵ Recently, sulfones 2.98 and 2.99 have been identified as SHIP

---

† The %SHIP1 and %SHIP2 inhibition of the molecules were determined through malachite green assay conducted by Amanda Balch from SUNY Upstate Medical University.
activators. Sulfones 2.98 and 2.99 would be quite useful to validate screening techniques and biological assays.

Figure 2–23. SHIP Agonists: Sulfones 2.98 and 2.99

The synthetic strategy for sulfone 2.98 is shown in Figure 2–24. Sulfone 2.98 can arise from oxidation of the Friedel–Crafts alkylation product of sulfide 2.100. The sulfide 2.100 can come from S–alkylation of benzenethiol 2.102 with mesylate 2.101. Mesylate 2.101 may be synthesized from commercially available sclareolide 2.103. The thiophenol 2.102 can be obtained from the hydrolysis of the intramolecular aryl migration product of O–thiocarbamate derivative of 2.104.

Figure 2–24. Retrosynthetic Analysis of Sulfone 2.98.

The synthesis of mesylate 2.101 began with the addition of methyllithium to lactone 2.103, which has been reported to afford the hydroxyl ketone 2.105 (Scheme 2–
19). Initially, Baeyer–Villiger oxidation of ketone (2.105) to the ester 2.106 was explored using perfluoroacetic acid prepared in situ from trifluoroacetic anhydride and hydrogen peroxide. This reaction has been reported to provide the product in good yields on this substrate when buffered with sodium bicarbonate.\textsuperscript{78} However, these conditions only resulted in poor yields of 2.106, possibly because this method is strongly affected by minor deviations in the ratio of sodium bicarbonate to trifluoroacetic anhydride used. If the ratio is either greater than, or less than 1:1, the yield of 2.106 drops dramatically because of the formed dehydration products of 2.105 as well as the formation of 12–hydroperoxy–8α,12–epoxy–ll–homodrimane 2.108.\textsuperscript{77–79} Alternatively, oxidation using permaleic acid consistently afforded improved amounts of 2.106.\textsuperscript{80} Deacetylation followed by a selective methanesulfonylation afforded the desired mesylate 2.101.

**Scheme 2–19\textsuperscript{a}** Synthesis of Mesylate 2.101

\textsuperscript{a}Reagents and conditions: (a) MeLi, Et\textsubscript{2}O, 0 °C, 5 min, 61\% (b) acetic anhydride, H\textsubscript{2}O\textsubscript{2}, maleic anhydride, DCM, 0 °C to rt, 28 h, 62\% (c) KOH, MeOH, rt, 1 h, 92\% (d) MsCl, Et\textsubscript{3}N, DMF, 0 °C to rt, 3 h, 89\%.
Entry | Reaction Conditions | Yield |
--- | --- | --- |
1 | a. (CF$_3$CO)$_2$O, H$_2$O$_2$, DCM, 0 °C, 10 min  
   b.) NaHCO$_3$, rt, 38 min | 15% |
2 | a. (CF$_3$CO)$_2$O, H$_2$O$_2$, DCM, 0 °C, 10 min  
   b.) NaHCO$_3$, rt, 24 h | 0% |
3 | a. (CF$_3$CO)$_2$O, H$_2$O$_2$, DCM, 0 °C, 10 min  
   b.) NaHCO$_3$, 0 °C, 2.5 h | 17% |
4 | m-CPBA, DCM, 0 °C to rt, 48 h | – |
5 | (CH$_3$CO)$_2$O, H$_2$O$_2$, maleic anhydride, DCM, 0 °C to rt, 28 h | 62% |

Table 2–9. Baeyer–Villiger Oxidation of Ketone 2.105.

Figure 2–25. 12-Hydroperoxy–8α,12-epoxy–ll–homodrimane 2.108.

Benzenethiol 2.102 was prepared from commercially available 3,5–dimethoxyphenol 2.104. O–Alkylation of 2.104 with N,N–dimethylthiocarbamyl chloride using sodium hydride in DMF afforded the O–thiocarbamate 2.109. Conversion of 2.109 into the S–thiocarbamate 2.110 was performed using Newman–Kwart rearrangement (NKR), an intramolecular aryl migration. The NKR was performed neat at high temperature (>300 °C), and therefore several methods were explored to find more mild reaction conditions. Palladium catalysis$^{81}$ of the NKR has been reported, however this
only gave small conversion to the product with this substrate. Another possible alternative is the use of microwave synthesis,\(^{82-83}\) however, it has not yet been attempted because of the unavailability of microwave reactor. Hydrolysis of 2.110 gave the desired thiophenol 2.102.

**Scheme 2–20**  
Synthesis of Benzenethiol 2.102

\[ \begin{align*}
\text{2.104} & \xrightarrow{a} \text{2.109} & \text{2.110} & \xrightarrow{s} \text{2.102} \\
\end{align*} \]

\(^{\text{a}}\text{Reagents and conditions: (a) } N,N\text{–dimethylthiocarbonyl chloride, NaH, DMF, 0 °C to 70 °C, 1 h, 66% (b) } >300 \text{ °C, 4 h, 74% (c) } \text{KOH, MeOH, reflux, 3 h, 58%.} \)

The mesylate 2.101 was coupled with benzenethiol 2.102 using cesium carbonate in acetonitrile to afford sulfide 2.100. Tin (IV) chloride (SnCl$_4$) catalyzed intramolecular Friedel–Crafts alkylation followed by oxidation using \(m\text{–chloroperoxybenzoic acid (}m\text{–CPBA)}\) afforded the desired sulfone 2.98.\(^{76}\) (**Scheme 2–21**)
Scheme 2–21a  Synthesis of Sulfone 2.98

\[
\begin{align*}
\text{2.101} + \text{2.102} & \xrightarrow{a} \text{2.100} \\
\text{2.98} & \xrightarrow{b} \text{2.111}
\end{align*}
\]

*aReagents and conditions: (a) Cs₂CO₃, CH₃CN, rt to reflux, 25 h, 37% (b) SnCl₄, DCM, –78 °C to 0 °C to rt, 4 h, 70% (c) m–CPBA, DCM, rt, 2 h, quantitative.

However, subjecting sulfone 2.98 in malachite green assay was problematic because of the observed precipitation of the molecule from DMSO. Because of this, attempts were made to synthesize sulfone 2.99 through deprotection of the methyl ethers. The formation of the desired 2.99 was observed; however purification of the reaction mixture either through column chromatography or recrystallization was not successful.

Scheme 2–22a  Synthesis of Sulfone 2.99

\[
\begin{align*}
\text{2.98} & \xrightarrow{a} \text{2.99}
\end{align*}
\]

*aReagents and conditions: (a) BBr₃, DCM, 0 °C to rt, 48 h.
Conclusion

Aminosteroid NSC23922 2.5, a mixture of α– and β–aminocholestan hydrochloride obtained from NCI, was identified as a selective SHIP1 inhibitor. SHIP1 inhibition of 2.5 showed an increase in the number of MIR cells, reduced priming of human and rodent allogeneic T cell responses, increased production of myeloid cells in the absence of pneumonia and lung consolidation, and reduced survival of several human blood cancer cells.

Both the α– and β–aminocholestan hydrochlorides (2.9 and 2.13) were synthesized using dihydrocholesterol and were evaluated for their SHIP1 inhibition using the Malachite Green assay. Both 2.9 and 2.13 showed potent inhibitory activity against SHIP1. However, only 2.9 was used for in vivo studies because of the lower solubility of 2.13 in ethanol. Treatment of sublethally irradiated mice with 2.9 showed an increase in RBC as well as faster and robust recovery of neutrophils, platelets, WBC, and lymphocytes. In addition, treatment of 2.9 also showed reduction of human blood cancer cells through apoptosis, which was determined by Western blotting. Tumor xenograft models of MM revealed treatment of 2.9 significantly abrogates MM tumor growth in vivo. Lastly, treatment of 2.9 in mice did not show any significant morbidity, mortality or weight loss and no evidence of pathology in major organs which 3AC and SHIP1 inhibition with small molecules could be a safe and effective treatment for MM.

Several derivatives of 2.9 such as functionalized amine and different functional groups at the C–3 position of the steroid nucleus were synthesized to study the effect of these changes on the structure with the biological activity. However, none of these molecules showed improved or any SHIP1 inhibitory activity, which indicated that a free
amine or amine salt is necessary for the exhibited inhibitory activity. In addition, these molecules also showed lower solubility in ethanol which led to the design of molecules with improved solubility and greater druglikeness keeping in mind Lipinski’s rules.

More soluble derivatives of 2.9 such as 2.52 and 2.57 were synthesized using trans–androsterone 2.48 as starting material. Aminehydrochloride 2.57 showed no inhibitory activity against SHIP and on the other hand, 2.52 showed an improved activity against SHIP compared to 2.9 which was determined with malachite green assay. Thus suggesting that incorporation of polar functional groups in ring D results to decrease in inhibition towards SHIP which leads to the hypothesis that the steroid A ring is in a polar portion of the binding pocket and the D ring is in a nonpolar portion. In addition, 2.52 showed greater potency in killing blood cancer cells as well as inducing the formation of MIR cells in vivo.

The diastereomer of 2.52 hydrochloride salt, 2.61 and its corresponding citrate salt 2.62 were also synthesized. These compounds showed better solubility such as 2.61 was soluble in hot H₂O. Comparison of the SHIP1 inhibition of 2.52 and 2.61 showed that their activity is comparable thus establishing that the difference in inhibitory activity of the parent compounds 2.9 and 2.13 is most likely due to their difference in solubility than the stereochemistry which is supported by the exhibited SHIP inhibitory activity of the more soluble citrate 2.62. In addition,

Syntheses of molecules with multiple polar groups in ring A such as hydroxyaminehydrochlorides 2.86 and 2.87 and diaminehydrochlorides 2.89 and 2.97 were also conducted. As expected, these molecules were more soluble than 2.9. Surprisingly, 2.87 and 2.89 were soluble in H₂O. Evaluation of their SHIP inhibitory
activity using malachite green assay showed better potency against SHIP1 in comparison to 2.9. However in contrast to the SHIP1 selectivity showed by 2.9, hydroxyaminehydrochloride 2.86 and diaminehydrochloride 2.89 were pan SHIP1/SHIP2 inhibitor which are also exhibited by 2.61.

Lastly, the synthesis of sulfone 2.98, which was identified as SHIP activators, was also conducted. Selective SHIP activators would be useful experimental tools and potential drug candidates for treatment of inflammation, osteoporosis, and leukemia. Sulfone 2.98 will be used to validate screening techniques and biological assays for identification of potential SHIP1 agonists. However, biological evaluation of this molecule using malachite green assay became challenging because of the low solubility in water, DMSO, and ethanol.

**Future Work**

Future work will involve *in vitro* studies with human cancer cells on the identified SHIP1 inhibitors as well as molecules that are pan SHIP1/SHIP2 inhibitors. In addition, *in vivo* studies in mice will be conducted particularly for compounds that show the best potency in the cell line assays.

In addition, design and syntheses of aminosteroid derivatives with potential SHIP inhibitory activity will continue. Possible compounds that can be synthesized are compounds with polar functional groups incorporated in ring B and ring C of the steroid nucleus as well as compounds containing polar functional groups on C–1 and C–5 positions. These molecules may be able to provide information on how extent the polar binding pocket of SHIP1.
Lastly, synthesis of the other identified SHIP agonist sulfone 2.99 will be pursued. DE protection of the methyl ether (2.111) using boron tribromide followed by $m$–CPBA oxidation may afford the desired sulfone 2.99. (Scheme 2–23)

**Scheme 2–23** Synthesis of Sulfone 2.99

$^a$Reagents and conditions: (a) BBr$_3$, DCM, 0 °C to rt, 48 h. (b) $m$–CPBA, DCM, rt, 2 h.

**Experimental Section**

**General Experimental Procedure**

**Reactions:** Except as otherwise noted, reactions were carried out under an argon atmosphere in an oven– or flame–dried glassware. Flasks were flame dried under a stream of nitrogen or argon or under vacuum. Pyridine, toluene, and triethylamine were distilled from calcium hydride. Reaction solvents were dried by a solvent purification apparatus (benzene, dichloromethane (DCM), tetrahydrofuran (THF), diethyl ether) using alumina as the drying agent. All other reagents were reagent grade and were purified as necessary.

**Methods:** Analytical thin layer chromatography (TLC) was performed using 250 μm commercial silica gel plates (EMD Chemicals, silica gel 60 F$_{254}$) and visualized by UV illumination or by exposure to potassium permanganate or $p$–anisaldehyde solution followed by heating using a heat gun. Flash chromatography was carried using commercially available 40–60 μm silica gel (Silicycle).

**Identity:** Melting points were recorded using an Electrothermal® Melting Point Apparatus and EZ–Melt Automated Melting Point Apparatus in open capillaries and are uncorrected. Infrared spectra were obtained using Thermo Nicolet IR100 and IR200 spectrometers. Proton and carbon nuclear magnetic resonance ($^1$H and $^{13}$C NMR) were
recorded using Bruker DPX–300 spectrometer. Chemical shifts are reported in delta (δ) units, downfield from tetramethylsilane (TMS) with reference to residual solvents CDCl₃ (¹H: 7.26; ¹³C: 77.23), CD₂OD (¹H: 4.78; ¹³C: 49.15) and (CD₃)₂SO (¹H: 2.50; ¹³C: 39.51). Coupling constants are reported in Hertz (Hz). Electron impact and high-resolution mass spectra was obtained from a Bruker 12 APEX –Qe FTICR–MS instrument with an Apollo II ion source in the College of Science Major Instrumentation (COSMIC) at Old Dominion University at Northfolk, VA 23529. Elemental Analysis data was obtained from a Carlo Erba Elemental Analyzer 1108 in Atlantic Microlab Inc., in Norcross, GA 30071.

Experimental

![Chemical Structures]

3α–Azido–5α–cholestane (2.7)


In a round bottom flask, 5α–cholestan–3β–ol 2.6 (3.00 g, 7.72 mmol) was dissolved in THF (77 mL). Triphenylphosphine (2.02 g, 7.72 mmol) was added into the clear solution followed by diisopropyl azodicarboxylate (DIAD) (1.50 mL, 7.72 mmol). Diphenylphosphoryl azide (1.67 mmol, 7.72 mmol) was then added dropwise over about 20 min. The resulting light yellow solution was stirred continuously at rt for 24 h. The reaction mixture was concd under reduced pressure and the residue was purified using flash column chromatography with 50% benzene in hexane to afford α–azide 2.7 as white solid (2.51 g, 79%).

**2.7.** m.p. = 46.7–49.9 °C (DCM) (Lit:*⁸⁴ 50–52 °C); TLC Rf = 0.92 (hexane:benzene, 1:1); IR (KBr) 2932, 2866, 2087, 1458, 1381, 1265 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 3.88 (t, J = 2.7 Hz, 1H), 1.96 (td, J = 6.3, 3.3 Hz, 1H), 1.75–1.88 (m, 1H), 1.66–1.71 (m, 2H), 1.61–1.65 (m, 1H), 1.53–1.58 (m, 1H), 1.45–1.51 (m, 4H), 1.40–1.41 (m, 1H), 1.30–1.37
(m, 5H), 1.25–1.28 (m, 2H), 1.17–1.22 (m, 4H), 1.09–1.15 (m, 4H), 1.06 (s, 1H), 0.98–1.03 (m, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.87 (d, J = 1.4 Hz, 3H), 0.85 (d, J = 1.4 Hz, 3H), 0.78 (s, 3H), 0.68–0.76 (m, 1H), 0.64 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 58.4, 56.7, 56.4, 54.3, 42.8, 40.2, 40.2, 39.7, 36.4, 36.1, 36.0, 35.6, 33.1, 32.8, 32.1, 28.5, 28.4, 28.2, 25.8, 24.4, 24.1, 23.0, 22.8, 21.0, 18.9, 12.3, 11.8.

![Diagram](image)

3α–Amino–5α–cholestane (2.8)


In round bottom flask, lithium aluminum hydride (LAH) (0.54 g, 15.0 mmol, 95%) was suspended in THF (15 mL). The suspension was cooled at 0 °C using ice/water cold bath before adding a solution of α–azide 2.7 (1.88 g, 4.54 mmol) in THF (4 mL). The solution was warmed to rt and was then refluxed for 4 h. The reaction mixture was allowed to cool to rt before diluting the solution with THF (19 mL). The diluted reaction mixture was cooled at 0 °C and quenched using the Fieser method$^{85}$ by adding cold water (1.62 mL), sodium hydroxide solution (1.62 mL, 15% in water) and cold water (0.54 mL), successively. The reaction mixture was stirred continuously until it turned into a milky white suspension. The solution was then filtered through celite and washed with THF. The filtrate was dried over sodium sulfate and concd under reduced pressure to afford α–amine 2.8 (1.75 g, 99%) as white solid. No further purification was necessary.

2.8. m.p. = 72.4–78.0 °C (DCM); TLC $R_f$ = 0.40 (DCM:methanol:ammonium hydroxide, 90:9:1); IR (KBr) 3449, 2932, 2867, 2850, 1467, 1382 cm$^{-1}$; 1H NMR (300 MHz, CDCl$_3$) δ 3.15 (bs, 1H), 1.94 (td, J = 6.1, 3.2 Hz, 1H), 1.69–1.85 (m, 2H), 1.63–1.67 (m, 1H), 1.59–1.62 (m, 1H), 1.29–1.58 (m, 12H), 1.21–1.26 (m, 2H), 1.07–1.20 (m, 8H), 1.03 (s, 1H), 0.95–1.00 (m, 2H), 0.87 (d, J = 6.6 Hz, 3H), 0.85 (s, 3H), 0.83 (s, 3H), 0.75 (s, 3H), 0.66–0.71 (m, 1H), 0.62 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 56.8, 56.4, 54.7,
46.0, 42.8, 40.2, 39.7, 39.4, 36.5, 36.4, 36.0, 35.7, 32.3, 29.3, 28.9, 28.4, 28.2, 24.4, 24.0, 23.0, 22.8, 21.0, 18.9, 12.3, 11.5.

3α–Amino–5α–cholestane hydrochloride (2.9)

A solution of hydrogen chloride in diethyl ether (5.16 mL, 10.32 mmol, 2.0 M in diethyl ether) was added into the clear solution of α–amine 2.8 (2.00 g, 5.16 mmol) in diethyl ether (103 mL) which resulted into the formation of a white suspension. The white precipitate was filtered through vacuum and washed with diethyl ether. Vacuum drying afforded hydrochloride 2.9 (2.15 g, 99%).

2.9. m.p. = 262.9 (dec.) (diethyl ether); IR (KBr) 3346, 2930, 2866, 1608, 1498, 1382 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 5.06 (bs, 1H), 3.37 (bs, 1H), 1.94 (d, J = 10.8 Hz, 1H), 1.70–1.86 (m, 2H), 1.61–1.66 (m, 2H), 1.47–1.56 (m, 6H), 1.30–1.40 (m, 7H), 1.25–1.27 (m, 1H), 1.17–1.21 (m, 4H), 1.10–1.13 (m, 4H), 0.93–1.04 (m, 5H), 0.88 (d, J = 6.5 Hz, 3H), 0.86 (d, J = 1.3 Hz, 3H), 0.84 (d, J = 1.3 Hz, 3H), 0.77 (s, 3H), 0.63 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 56.5, 56.2, 53.0, 48.0, 42.8, 39.9, 39.7, 38.9, 38.9, 36.4, 36.2, 36.2, 35.7, 32.0, 31.6, 31.5, 28.5, 28.2, 24.9, 24.4, 24.3, 23.0, 22.8, 21.0, 19.0, 12.2, 11.6. HRMS (ESI) m/z calculated for C₂₇H₅₀N⁺ (M⁺): 388.3938. Found: 388.3932. Anal. calcd for C₂₇H₅₀ClN: C, 76.46; H, 11.88; N, 3.30. Found: C, 76.25; H, 11.98; N, 3.33.
3α–Iodo–5α–cholestane (2.10)


A solution of DIAD (0.55 mL, 2.83 mmol) in benzene (6 mL) was added dropwise into a solution of 5α–cholestan–3β–ol 2.6 (1.00 g, 2.57 mmol) and triphenylphosphine (0.74 g, 2.83 mmol) in benzene (14 mL). The resulting yellow solution was stirred continuously at rt. After approximately 15 min, a solution of iodomethane (0.18 mL, 2.83 mmol) in benzene (6 mL) was added in a dropwise fashion after which the solution was continuously stirred at rt for 24 h. The reaction mixture was concd under reduced pressure and the concentrate was purified through flash column chromatography using 50% benzene in hexane as eluent to afford α–iodo 2.10 (1.10 g, 86%) as an off white solid.

2.10. m.p. = 108.2–110.0 °C (DCM); TLC Rf = 0.95 (hexane:benzene, 1:1); IR (KBr) 2936, 2864, 1445, 1242, 964, 474 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 4.95 (s, 1H), 1.94–2.00 (m, 1H), 1.87–1.89 (m, 1H), 1.76–1.85 (m, 1H), 1.65–1.72 (m, 3H), 1.61–1.64 (m, 1H), 1.48–1.58 (m, 5H), 1.45 (d, J = 3.0 Hz, 1H), 1.31–1.40 (m, 5H), 1.09–1.14 (m, 4H), 1.18–1.19 (m, 1H), 1.07 (s, 1H), 1.05–1.00 (m, 3H), 0.94–0.98 (m, 1H), 0.90 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 1.3 Hz, 3H), 0.85 (d, J = 1.3 Hz, 3H), 0.79 (s, 3H), 0.65 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 56.6, 56.5, 54.0, 42.8, 42.2, 40.2, 39.7, 39.0, 38.5, 36.7, 36.4, 36.0, 35.6, 34.6, 32.9, 32.0, 28.5, 28.2, 28.0, 24.4, 24.1, 23.1, 22.8, 21.0, 18.9, 13.6, 12.3.
3β–Azido–5α–cholestan (2.11)

In a round bottom flask, α–iodide 2.10 (1.10 g, 2.21 mmol) and sodium azide (1.44 g, 22.1 mmol) were suspended in DMSO (55 mL). The suspension was then warmed to 90 °C. After approximately 5 h, the reaction mixture was cooled to rt before adding water (55 mL). The solution was extracted with diethyl ether (3 x 50 mL) and all organic layers were combined, collected, washed over brine solution (3 x 50 mL), dried over sodium sulfate, and concd under reduced pressure. Purification of the residue using flash column chromatography with hexane as eluent afforded β–azide 2.11 (0.83 g, 91%) as a white solid.

2.11. m.p. = 55.7–59.9 °C (DCM); TLC Rf = 0.90 (hexane); IR (KBr) 2931, 2850, 2091, 1468, 1256, 1172 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 3.20–3.31 (m, 1H), 1.96 (td, J = 5.9, 3.4 Hz, 1H), 1.78–1.85 (m, 2H), 1.73–1.75 (m, 1H), 1.63–1.70 (m, 1H), 1.53–1.61 (m, 3H), 1.49–1.51 (m, 1H), 1.42–1.47 (m, 2H), 1.24–1.37 (m, 9H), 1.17–1.22 (m, 1H), 1.16–1.18 (m, 6H), 1.04–1.07 (m, 1H), 0.96–1.02 (m, 3H), 0.89 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 1.4 Hz, 3H), 0.85 (d, J = 1.4 Hz, 3H), 0.80 (s, 3H), 0.66 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 60.8, 56.6, 56.4, 54.4, 45.4, 42.7, 40.1, 39.7, 37.3, 36.3, 35.9, 35.6, 35.6, 34.2, 32.1, 28.7, 28.4, 28.2, 27.8, 24.3, 24.0, 23.0, 22.7, 21.3, 18.8, 12.4, 12.2.
3β–Amino–5α–cholestanate (2.12)

A suspension of LAH (0.25 g, 6.40 mmol, 95%) in THF (6 mL) was cooled at 0 °C using an ice/water bath. β–Azide 2.11 (0.79 g, 1.91 mmol) in THF (2 mL) was added into the cooled suspension. The solution was stirred continuously as it warmed up at rt. A refluxing unit was added and the reaction was heated to reflux at 76 °C for 4 h. The reaction mixture was cooled to rt, diluted with THF (8 mL), cooled to 0 °C and quenched using the Fieser method. The resulting milky white solution was filtered through celite and washed with THF. The filtrate was dried over sodium sulfate and concd under reduced pressure to provide a solid residue. Purification using silica gel chromatography with 10–20% methanol in DCM as eluent followed by 1% triethylamine in methanol as eluent afforded β–amine 2.12 (0.45 g, 61%) as white solid.

2.12. TLC Rf = 0.32 (DCM:methanol:ammonium hydroxide, 90:9:1); 1H NMR (300 MHz, CDCl3) δ 2.59–2.67 (m, 1H), 1.95 (td, J = 6.2, 3.2 Hz, 1H), 1.74–1.86 (m, 1H), 1.60–1.71 (m, 5H), 1.55–1.57 (m, 1H), 1.49–1.55 (m, 2H), 1.44–1.47 (m, 1H), 1.38–1.42 (m, 1H), 1.28–1.34 (m, 4H), 1.20–1.26 (m, 4H), 1.04–1.17 (m, 9H), 0.95–1.01 (m, 3H), 0.89 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 1.4 Hz, 3H), 0.84 (d, J = 1.3 Hz, 3H), 0.77 (s, 3H), 0.66 (s, 3H); 13C NMR (75 MHz, CDCl3) δ 60.8, 56.6, 56.4, 54.4, 45.4, 42.7, 40.1, 39.7, 37.3, 36.3, 35.9, 35.6, 35.6, 34.2, 32.1, 28.7, 28.4, 28.2, 27.8, 24.3, 24.0, 23.0, 22.7, 21.3, 18.8, 12.4, 12.2.
3β–Amino–5α–cholestane hydrochloride (2.13)

The β–amine 2.12 (0.28 g, 0.72 mmol) was dissolved in CHCl₃ (15 mL). Dry hydrogen chloride gas, produced from reacting calcium chloride with concd hydrochloric acid, was purged into the solution resulting in the formation of a precipitate. The solution was filtered and the precipitate was collected, washed with diethyl ether, and dried under vacuum to afford amine salt 2.13 (0.19 g, 63%) as an off–white solid.

2.13. m.p. = 230.4 °C (dec.) (CHCl₃); IR (KBr) 3423, 2933, 2866, 1608, 1502, 1383 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.29 (bs, 3H), 3.12 (bs, 1H), 1.79–2.01 (m, 2H), 1.71–1.79 (m, 4H), 1.66–1.68 (m, 1H), 1.62–1.64 (m, 1H), 1.58 (s, 2H), 1.53 (s, 1H), 1.47–1.51 (m, 2H), 1.42–1.45 (m, 1H), 1.28–1.34 (m, 6H), 1.21–1.25 (m, 2H), 1.05–1.11 (m, 6H), 1.00–1.01 (m, 2H), 0.96–0.97 (m, 1H), 0.89 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 1.3 Hz, 3H), 0.85 (d, J = 1.2 Hz, 3H), 0.82 (s, 3H), 0.64 (s, 3H); ¹³C NMR (75 MHz, CD₃OD) δ 57.9, 57.8, 55.6, 51.9, 46.2, 43.9, 41.5, 40.9, 37.9, 37.5, 37.3, 36.9, 36.6, 34.1, 33.2, 29.7, 29.5, 29.3, 27.7, 25.4, 25.1, 23.4, 23.1, 22.4, 19.4, 12.7.

3β–Amino–5α–cholestane citrate (2.16)

In a flame dried 50 mL round bottom flask, amine 2.8 (0.20 g, 0.52 mmol) was dissolved in THF (1 mL). A solution of citric acid 2.10 (0.10 g, 0.52 mmol) in THF (1 mL) was added to the amine solution and the resulting suspension was continuously stirred at rt.
After 19 h, the resulting solid is filtered and washed over THF to afford citrate **2.16** (0.26 g, 87%).

**2.16.** IR (KBr) 3440, 2935, 2867, 1719, 1618, 1234 cm⁻¹; ¹H NMR (300 MHz, DMSO) δ 3.58 (q, 1H, J = 0.8 Hz), 3.38 (bs, 1H), 1.92 (d, J = 11.4 Hz, 1H), 1.67–1.78 (m, 3H), 1.59–1.63 (m, 2H), 1.39–1.54 (m, 6H), 1.24–1.36 (m, 6H), 1.03–1.21 (m, 1H), 0.91–0.99 (m, 3H), 0.86 (d, J = 6.2 Hz, 3H), 0.83 (s, 3H), 0.81 (s, 3H), 0.73 (s, 3H), 0.61 (s, 3H).

![Chemical structure](image)

**3α–Acetamido–5α–cholestanate (2.17)**

The α–amine **2.8** (0.29 g, 0.75 mmol) was dissolved in THF (2.21 mL) in a round bottom flask. Triethylamine (0.12 mL, 0.90 mmol) was added dropwise and the resulting solution was cooled to 0 °C. Acetyl chloride **2.112** (0.06 mL, 0.83 mmol) was added dropwise into the cooled solution which resulted on the formation of white precipitate. The milky white suspension was stirred continuously for 15 min at 0 °C before allowing the reaction mixture to warm up to rt. DCM (5 mL) was added and the diluted solution was washed with aqueous hydrochloric acid solution (10 mL, 1 M), brine (10 mL), and water (10 mL). The organic layer was collected, dried over sodium sulfate, and concd under reduced pressure. Recrystallization of the solid residue using ethyl alcohol afforded amide **2.17** (0.22 g, 65 %) as off white solid.

**2.17.** m.p. = 203.6–212.8 °C (ethyl alcohol); IR (KBr) 3263, 3069, 2927, 2864, 2846, 1635 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 5.71 (bs, 1H), 4.13 (bs, 1H), 1.99 (s, 3H), 1.96 (t, J = 3.0 Hz, 1H), 1.79 (m 1H), 1.60–1.65 (m, 2H), 1.45–1.60 (m, 7H), 1.31–1.36 (m, 6H), 1.27–1.28 (m, 1H), 1.03–1.04 (m, 2H), 0.96–1.00 (m, 5H), 0.94–0.96 (m, 1H), 0.87 (s, J = 1.2 Hz, 3H), 0.85 (d, J = 1.2 Hz, 3H), 0.80 (s, 3H), 0.68–0.73 (m, 1H), 0.65 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 169.4, 56.7, 56.4, 54.7, 44.9, 42.7, 41.0, 40.2, 39.6, 36.3, 36.1, 35.9, 35.5, 33.3, 33.0, 32.1, 28.6, 28.4, 28.1, 26.1, 24.3, 24.0, 23.8, 23.0, 22.7, 20.9, 18.8, 12.2, 11.6.
General Procedure for 2.19 – 2.30

The α-amine 2.8 (1 equiv) was dissolved in THF (3 mL per 1 mmol of 2.8) in a round bottom flask. Triethylamine (1.2 equiv) was added dropwise and the resulting solution was cooled to 0 °C. Acyl chloride (1.1 equiv) was added dropwise into the cooled solution. The solution was stirred continuously for 15 min at 0 °C before allowing the reaction mixture to warm up to rt. DCM was added and the diluted solution was washed with aqueous hydrochloric acid solution (1 M), brine, and water. The organic layer was collected, dried over sodium sulfate, and concd under reduced pressure. The crude product was purified by recrystallization from ethyl alcohol.

\[ \text{N–(3α,5α–cholestan–2–methyl–2–propenamide (2.19)} \]

2.19. m.p. = 183.0–185.0 °C (ethyl alcohol); IR (KBr) 3296, 2929, 2866, 1652, 1613, 912 cm\(^{-1}\); \([\alpha]_{D}^{20.3} = +37.8\ (c\ 0.90,\ DCM)\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta 6.04\) (bs, 1H), 5.67 (t, \(J = 1.0\) Hz, 3H), 5.32 (quint, \(J = 1.4\) Hz, 1 H), 4.17 (bs, 1H), 1.98 (q, \(J = 0.9\) Hz, 3H), 1.73–1.86 (m, 2H), 1.60–1.71 (m, 4H), 1.45–1.59 (m, 1H), 1.41–1.45 (m, 1H), 1.27–1.39 (m, 6H), 1.16–1.26 (m, 5H), 1.06–1.16 (m, 7H), 1.05 (bs, 1H), 0.98–1.03 (m, 3H), 0.93–0.97 (m, 1H), 0.89 (d, \(J = 6.5\) Hz, 3H), 0.87 (d, \(J = 1.5\) Hz, 3H), 0.82 (s, 3H), 0.66–0.72 (m, 1H), 0.65 (s, 3H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta 167.8, 140.9, 119.2, 56.8, 56.5, 54.8, 45.0, 42.8, 41.5, 40.2, 39.7, 36.4, 36.3, 36.0, 35.6, 33.6, 32.9, 32.2, 28.7, 28.4, 28.2, 26.2, 24.4, 24.1, 23.0, 22.8, 21.0, 19.0, 18.9, 12.3, 11.7.
\(N-(3\alpha,5\alpha)-\text{cholestan-3-yl-3-phenyl-2-propenamide}\) \((2.20)\)

2.20. m.p. = 188.6–192.8 °C (ethyl alcohol); IR (KBr) 3285, 3061, 2931, 1615, 1220, 767 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.64 (d, \(J = 15.6\) Hz, 1H), 7.50 (d, \(J = 5.6\) Hz, 2H), 7.33 (d, \(J = 4.1\) Hz, 2H), 6.57 (d, \(J = 15.6\) Hz, 1H), 6.37 (d, \(J = 7.4\) Hz, 1H), 4.29 (bs, 1H), 1.95 (d, \(J = 12.0\) Hz, 1H), 1.67–1.75 (m, 4H), 1.47–1.62 (m, 9H), 1.32–1.35 (m, 5H), 1.26 (bs, 2H), 1.12 (bs, 4H), 0.96–1.03 (m, 5H), 0.88 (s, 3H), 0.86 (s, 3H), 0.80 (s, 3H), 0.63 (s, 3H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) d 165.3, 140.8, 135.2, 129.6, 128.9, 127.9, 121.5, 56.7, 56.4, 54.6, 45.2, 42.7, 41.1, 40.2, 39.7, 36.3, 26.2, 35.9, 35.5, 33.4, 33.1, 32.1, 28.7, 28.4, 28.2, 26.3, 24.3, 24.0, 23.0, 22.7, 20.9, 18.8, 12.2, 11.6.

\(N-(3\alpha,5\alpha)-\text{cholestanbenzamide}\) \((2.21)\)

2.21. m.p. = 207.0–209.0 °C (ethyl alcohol); IR (KBr) 3267, 3056, 2928, 1627, 1330, 722 cm\(^{-1}\); \([\alpha]_D^{20.8}=+27.6\) (c 6.46, DCM); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.77 (dt, \(J = 6.5, 1.4\) Hz, 2H), 7.40–7.52 (m, 3H), 6.40 (d, \(J = 7.1\) Hz, 1H), 4.32 (bs, 1H), 1.98 (dt, \(J = 12.2, 3.1\) Hz, 1H), 1.64–1.70 (m, 2H), 1.57–1.61 (m, 2H), 1.51–1.53 (m, 2H), 1.47–1.49 (m, 2H), 1.29–1.35 (m, 5H), 1.21–1.25 (m, 5H), 1.07–1.13 (m, 6H), 1.05 (bs, 1H), 0.96–1.03 (m, 4H), 0.90 (d, \(J = 6.5\) Hz, 3H), 0.87 (d, \(J = 1.4\) Hz, 3H), 0.85 (d, \(J = 1.3\) Hz, 3H), 0.84 (s, 3H), 0.70–0.77 (m, 1H), 0.65 (s, 3H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 166.9, 135.4,

\[ \text{N–}(3\alpha,5\alpha)\text{-cholestan–2,2–dimethylpropenamide (2.22)} \]

**2.22.** m.p. = 167.0–170.0 °C (ethyl alcohol); IR (KBr) 3351, 2928, 1633, 1467, 1203, 742 cm\(^{-1}\); [\( \alpha \)]\(_D^{21,7}\) = +30.8 (c 6.02, DCM); \(^1\)H NMR (300 MHz, CDCl\(_3\) \( \delta \) 5.89 (d, \( J = 8.2 \) Hz, 1H), 4.07 (bs, 1H), 1.95 (dt, \( J = 12.1, 3.0 \) Hz, 1H), 1.71–1.85 (m, 2H), 1.67–1.69 (m, 1H), 1.62–1.65 (m, 1H), 1.56–1.59 (m, 2H), 1.49–1.54 (m, 3H), 1.45–1.47 (m, 1H), 1.30–1.35 (m, 5H), 1.21–1.26 (m, 3H), 1.18 (s, 9H), 1.03–1.11 (m, 6H), 0.95–1.00 (m, 3H), 0.87 (d, \( J = 6.6 \) Hz, 3H), 0.85 (d, \( J = 1.4 \) Hz, 3H), 0.82 (d, \( J = 1.1 \) Hz, 3H), 0.78 (s, 3H), 0.63 (s, 3H); \(^1^3\)C NMR (75 MHz, CDCl\(_3\) \( \delta \) 177.5, 56.7, 56.5, 54.9, 44.4, 42.8, 41.6, 40.1, 39.7, 38.9, 36.3, 36.2, 36.0, 35.6, 33.6, 32.9, 32.2, 28.7, 28.4, 28.2, 27.8, 26.1, 24.3, 24.0, 23.0, 22.7, 21.0, 18.8, 12.3, 11.6.

\[ \text{N–}(3\alpha,5\alpha)\text{-cholestan–2E–propenamide (2.23)} \]

**2.23.** m.p. = 195.0–198.0 °C (ethyl alcohol); IR (KBr) 3289, 2931, 2869, 1625, 1543, 975 cm\(^{-1}\); [\( \alpha \)]\(_D^{22,2}\) = +39.5 (c 0.62, DCM); \(^1\)H NMR (300 MHz, CDCl\(_3\) \( \delta \) 6.79–6.89 (m, 1H), 5.82 (dd, \( J = 13.5, 1.6 \) Hz, 1H), 5.69 (d, \( J = 7.3 \) Hz, 1H), 4.19 (bs, 1H), 1.97 (dt, \( J = 12.2, 2.9 \) Hz, 3H), 1.85 (dd, \( J = 5.4, 1.5 \) Hz, 3H), 1.72–1.81 (m, 1H), 1.64–1.69 (m, 3H), 1.45–1.60 (m, 6H), 1.32–1.41 (m, 5H), 1.16–1.25 (m, 5H), 1.13–1.08 (m, 4H), 1.05 (bs, 1H), 117.
1.02–0.96 (m, 4H), 0.90 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 1.2 Hz, 3H), 0.85 (d, J = 1.1 Hz, 3H), 0.80 (s, 3H), 0.67–0.72 (m, 1H), 0.65 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 165.3, 139.7, 125.7, 56.8, 56.5, 54.8, 44.8, 42.8, 41.3, 40.2, 39.7, 36.4, 36.2, 36.0, 35.6, 33.5, 33.0, 32.2, 28.7, 28.4, 28.2, 26.2, 24.3, 24.0, 23.0, 22.8, 21.0, 18.9, 17.9, 12.3, 11.6.

![Chemical structure](image1)

$N$–(3α,5α)–cholestancyclopropanecarboxamide (2.24)

2.24. m.p. = 223.0–225.0 °C (ethyl alcohol); IR (KBr) 3302, 2932, 1633, 1547, 1457, 1238 cm$^{-1}$; $[\alpha]_{D}^{23.4} = +34.9$ (c 3.68, DCM); $^1$H NMR (300 MHz, CDCl$_3$) δ 6.07 (d, J = 7.6 Hz, 1H), 4.13 (bs, 1H), 1.96 (dt, J = 12.2, 2.8 Hz, 1H), 1.71–1.85 (m, 2H), 1.67–1.69 (m, 1H), 1.62–1.63 (m, 2H), 1.56–1.57 (m, 1H), 1.50–1.53 (m, 3H), 1.45–1.47 (m, 1H), 1.30–1.40 (m, 6H), 1.17–1.23 (m, 5H), 1.03–1.11 (m, 6H), 0.97–1.01 (m, 2H), 0.91–0.95 (m, 3H), 0.88 (d, J = 6.5 Hz, 3H), 0.85 (d, J = 0.7 Hz, 3H), 0.83 (d, J = 0.1 Hz), 0.78 (s, 3H), 0.69 (d, J = 3.1 Hz, 1H), 0.66 (d, J = 2.9 Hz, 1H), 0.63 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 172.7, 56.8, 56.5, 54.7, 44.9, 42.7, 41.1, 40.2, 39.7, 36.3, 36.2, 36.0, 35.6, 33.4, 33.1, 32.2, 28.7, 28.4, 28.2, 26.3, 24.3, 24.0, 23.0, 22.7, 21.0, 18.8, 15.1, 12.2, 11.6, 7.2, 7.2.

![Chemical structure](image2)

$N$–[(3α,5α)–cholestan–3–yl]–2–trifluoroacetamide (2.25)

2.25. m.p. = 213.0–215.0 °C (ethyl alcohol); IR (KBr) 3268, 2929, 1652, 1548, 1244, 1171 cm$^{-1}$; $[\alpha]_{D}^{22.8} = +41.3$ (c 3.74, DCM); $^1$H NMR (300 MHz, CDCl$_3$) δ 6.28 (dd, J =
15.2, 1.7 Hz, 1H), 6.12 (dd, \( J = 10.1, 6.9 \text{ Hz}, 1H \)), 5.91 (d, \( J = 7.1 \text{ Hz}, 1H \)), 5.62 (dd, \( J = 8.3, 1.7 \text{ Hz}, 1H \)), 4.21 (bs, 1H), 1.97 (dt, \( J = 6.1, 3.2 \text{ Hz}, 1H \)), 1.72–1.84 (m, 2H), 1.63–1.70 (m, 3H), 1.60 (d, \( J = 3.3 \text{ Hz}, 1H \)), 1.48–1.56 (m, 4H), 1.42–1.46 (m, 1H), 1.36–1.37 (m, 1H), 1.31–1.34 (m, 3H), 1.26–1.28 (m, 1H), 1.24–1.25 (m, 1H), 1.18–1.20 (m, 3H), 1.09–1.12 (m, 3H), 1.07 (bs, 1H), 1.04–1.06 (m, 1H), 0.89 (d, \( J = 6.5 \text{ Hz}, 3H \)), 0.87 (d, \( J = 1.4 \text{ Hz}, 3H \)), 0.84 (d, \( J = 1.4 \text{ Hz}, 3H \)), 0.80 (s, 3H), 0.67–0.74 (m, 1H), 0.64 (s, 3H); \(^{13}\text{C} \text{ NMR (75 MHz, CDCl}_3 \)) \( \delta \) 164.9, 131.5, 126.3, 56.8, 56.5, 54.7, 45.0, 42.8, 41.2, 40.2, 39.7, 36.2, 36.0, 33.4, 32.9, 32.2, 28.7, 28.4, 28.2, 26.1, 24.3, 24.0, 23.0, 22.8, 21.0, 18.9, 12.2, 11.6.

![Chemical structure](image)

**N-[(3α,5α)-cholestan-3-yl]-2,2,2-trifluoroacetamide (2.26)**

**2.26.** m.p. = 164.9–169.0 °C (DCM); IR (KBr) 3284, 2935, 1720, 1315, 1211, 1190 cm\(^{-1}\); \(^1\text{H} \text{ NMR (300 MHz, CDCl}_3 \)) \( \delta \) 6.55 (bs, 1H), 4.22 (bs, 1H), 2.02 (dt, \( J = 12.2, 3.2 \text{ Hz}, 1H \)), 1.80–1.89 (m, 2H), 1.73–1.78 (m, 2H), 1.70–1.71 (m, 1H), 1.63–1.66 (m, 1H), 1.60–1.62 (m, 1H), 1.54–1.58 (m, 2H), 1.48–1.51 (m, 1H), 1.37–1.44 (m, 4H), 1.33 (d, \( J = 3.6 \text{ Hz}, 1H \)), 1.26–1.29 (m, 2H), 1.23–1.24 (m, 1H), 1.13–1.20 (m, 5H), 1.10–1.11 (m, 1H), 1.02–1.07 (m, 3H), 0.98–1.00 (m, 1H), 0.94 (d, \( J = 6.5 \text{ Hz}, 3H \)), 0.92 (d, \( J = 1.4 \text{ Hz}, 3H \)), 0.89 (d, \( J = 1.3 \text{ Hz}, 3H \)), 0.86 (s, 3H), 0.74–0.79 (m, 1H), 0.70 (s, 3H); \(^{13}\text{C} \text{ NMR (75 MHz, CDCl}_3 \)) \( \delta \) 156.2, 156.3, 118.0, 114.2, 56.7, 56.5, 54.5, 46.2, 42.8, 41.2, 40.1, 39.7, 36.4, 36.2, 36.0, 35.6, 33.2, 32.4, 32.0, 28.5, 28.4, 28.2, 25.6, 24.3, 24.0, 23.0, 22.8, 21.0, 18.9, 12.3, 11.6; \(^{19}\text{F} \text{ NMR (282 MHz, CDCl}_3 \)): −76.2 (s).
\[ N-(3\alpha,5\alpha)-\text{cholestan-4-bromobenzamide (2.27)} \]

**2.27.** m.p. = 164.0–168.0 °C (DCM); IR (KBr) 3297, 2931, 2868, 1630, 1265, 759 cm\(^{-1}\); \([\alpha]_D^{23.1} = +21.3 (c 5.41, DCM); \]^1H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.63 (d, \(J = 8.7\) Hz, 2H), 7.56 (d, \(J = 8.6\) Hz, 2H), 6.36 (d, \(J = 7.2\) Hz, 1H), 4.29 (bs, 1H), 1.98 (dt, \(J = 12.2, 3.5\) Hz, 1H), 1.73–1.83 (m, 3H), 1.59–1.65 (m, 4H), 1.45–1.53 (m, 4H), 1.32–1.34 (m, 4H), 1.19–1.23 (m, 4H), 1.04–1.13 (m, 7H), 0.96–1.02 (m, 3H), 0.90 (s, \(J = 6.5\) Hz, 3H), 0.87 (d, \(J = 1.2\) Hz, 3H), 0.85 (s, \(J = 1.2\) Hz, 3H), 0.83 (s, 3H), 0.69–0.74 (m, 1H), 0.65 (s, 3H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 165.9, 134.2, 131.9, 128.7, 126.1, 56.8, 56.5, 54.8, 45.6, 42.8, 41.5, 40.2, 39.7, 36.4, 36.3, 36.0, 35.6, 33.6, 33.0, 32.1, 28.7, 28.4, 28.2, 26.2, 24.3, 24.1, 23.0, 22.8, 21.0, 18.9, 12.3, 11.7.

\[ N-(3\alpha,5\alpha)-\text{cholestan-4-nitrobenzamide (2.28)} \]

**2.28.** m.p. = 213.0–216.0 °C (ethyl alcohol); IR (KBr) 3278, 2929, 2867, 1631, 1599, 1349 cm\(^{-1}\); \([\alpha]_D^{23.8} = +18.3 (c 3.56, DCM); \]^1H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.22 (td, \(J = 8.9, 2.0\) Hz, 2H), 7.90 (td, \(J = 8.9, 2.0\) Hz, 2H), 6.62 (d, \(J = 7.3\) Hz, 1H), 4.29 (bs, 1H), 1.95 (dt, \(J = 12.3, 3.1\) Hz, 1H), 1.78–1.81 (m, 1H), 1.74–1.76 (m, 2H), 1.62–1.67 (m, 2H), 1.57–1.60 (m, 1H), 1.43–1.53 (m, 4H), 1.26–1.37 (m, 6H), 1.19–1.23 (m, 4H), 1.08–1.11 (m, 4H), 1.00–1.05 (m, 3H), 0.93–0.98 (m, 2H), 0.87 (d, \(J = 6.5\) Hz, 3H), 0.85 (d, \(J = 1.3\) Hz, 3H), 0.85 (d, \(J = 1.3\) Hz, 3H), 0.82 (s, 3H), 0.67–0.72 (m, 1H), 0.63 (s, 3H); \(^{13}\)C
NMR (75 MHz, CDCl₃) δ 164.9, 149.6, 141.0, 128.3, 124.0, 56.7, 56.5, 54.7, 46.0, 42.7, 41.5, 40.5, 39.7, 36.3, 36.0, 35.6, 32.9, 32.1, 28.6, 28.4, 28.2, 26.1, 24.3, 24.0, 23.0, 22.7, 21.0, 18.8, 12.2, 11.6.

\[ \text{N–}(3\alpha,5\alpha)\text{–cholestan–4–methylbenzenesulfonamide (2.29)} \]

29. IR (KBr): 3282, 2932, 2868, 1383, 1157, 707 cm⁻¹; \(^1\)H NMR (300 MHz, CDCl₃) δ 7.77 (dt, \(J = 8.3, 1.7\) Hz, 2H), 7.29 (d, \(J = 8.0\) Hz, 2H), 3.48 (bs, 1H), 4.93 (d, \(J = 7.0\) Hz, 1H), 2.42 (s, 3H), 1.94 (dt, \(J = 11.2, 3.1\) Hz, 1H), 1.75–1.8 (m 1H), 1.50–1.64 (m, 4H), 1.38–1.37 (m, 5H), 1.35–1.30 (m, 3H), 1.16–1.27 (m, 6H), 1.11–1.13 (m, 3H), 1.08–1.09 (m, 2H), 1.03–1.06 (m, 3H) 0.96–1.00 (m, 2H), 0.89 (d, \(J = 6.8\) Hz, 3H), 0.88 (d, \(J = 1.5\) Hz, 3H), 0.85 (d, \(J = 1.4\) Hz, 3H), 0.78–0.83 (m, 1H), 0.71 (s, 3H), 0.62 (s, 3H); \(^13\)C NMR (75 MHz, CDCl₃) δ 143.2, 138.2, 129.8, 127.2, 56.6, 56.4, 54.3, 49.6, 42.7, 40.1, 40.0, 39.7, 36.3, 36.0, 35.9, 35.5, 33.8, 32.7, 32.0, 28.5, 28.4, 28.2, 27.0, 24.3, 24.0, 23.0, 22.8, 21.7, 20.9, 18.8, 12.2, 11.7.

\[ \text{N–}(3\alpha,5\alpha)\text{–cholestanmethanesulfonamide (2.30)} \]

2.30. m.p. = 152.9–157.1 °C (DCM); IR (KBr) 3257, 2931, 2868, 1445, 1315, 1135 cm⁻¹; \(^1\)H NMR (300 MHz, CDCl₃) δ 4.83 (d, \(J = 7.3\) Hz, 1H), 3.70 (quint, \(J = 3.3\) Hz, 1H), 2.95 (s, 3H), 1.96 (dt, \(J = 12.0, 3.1\) Hz, 1H), 1.73–1.85 (m, 2H), 1.62–1.71 (m, 3H), 1.56–1.58 (m, 2H), 1.51–1.58 (m, 2H), 1.41–1.48 (m, 2H), 1.36–1.37 (m, 1H), 1.29–1.34 (m,
3H), 1.24–1.27 (m, 2H), 1.18–1.22 (m, 3H), 1.14 (d, J = 3.9 Hz, 1H), 1.09–1.12 (m, 4H), 1.06 (bs, 1H), 0.96–1.03 (m, 3H), 0.89 (d, J = 6.5 Hz, 3H), 0.86 (d, J = 1.2 Hz, 3H), 0.84 (d, J = 1.4 Hz, 3H), 0.78 (s, 3H), 0.66–0.74 (m, 1H), 0.63 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 56.6, 56.4, 54.5, 50.0, 42.7, 41.5, 40.3, 40.1, 40.0, 36.3, 36.0, 36.0, 35.6, 34.4, 32.8, 32.1, 28.6, 28.4, 28.2, 27.6, 24.3, 24.0, 23.0, 22.8, 20.9, 18.8, 12.3, 11.7.

3β–N–ethylamine–5α–cholestane (2.18)

In a 50 mL round bottom flask, LAH (0.28 g, 7.05 mmol, 95%) was suspended in THF (9 mL). α–Amide 2.17 (1.00 g, 2.35 mmol) was added slowly into the suspension and the solution was heated to reflux. After approximately 22 h, the solution was cooled at rt before diluting it with THF (9 mL). The diluted suspension was cooled to 0 °C and the reaction was quenched by adding cold water (0.84 mL), sodium hydroxide solution (0.84 mL, 15% in water), and cold water (0.28 mL), successively. The resulting milky white suspension was filtered through celite and the filtrate was coned under reduced pressure which afforded ethylamine 2.18 (0.59 g, 61%) as a light yellow oil.

2.18. $^1$H NMR (300 MHz, CDCl$_3$) δ 2.82 (t, J = 2.4 Hz, 1H), 2.59 (q, J = 7.4 Hz, 2H), 1.94 (td, J = 6.1, 3.1 Hz, 1H), 1.73–1.85 (m, 2H), 1.69–1.64 (m, 1H), 1.55–1.60 (m, 3H), 1.50 (t, J = 6.5 Hz, 2H), 1.44 (t, J = 4.6 Hz, 1H), 1.38–1.39 (m, 1H), 1.28–1.35 (m, 6H), 1.23–1.24 (m, 1H), 1.19 (s, 1H), 1.16 (d, J = 2.9 Hz, 2H), 1.07–1.12 (m, 6H), 0.93–1.04 (m, 4H), 0.88 (d, J = 6.5 Hz, 3H), 0.86 (d, J = 1.2 Hz, 3H), 0.84 (d, J = 1.2 Hz, 3H), 0.77 (s, 3H), 0.63 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 56.0, 55.6, 53.7, 51.5, 42.0, 40.9, 39.4, 39.1, 38.9, 35.6, 35.2, 34.9, 32.5, 32.0, 31.4, 28.1, 27.7, 27.4, 25.1, 23.6, 23.2, 22.2, 22.0, 20.2, 18.1, 14.8, 11.5, 10.9.
3β-Thiolacetate–5α-cholestanate (2.31)

In a round bottom flask, triphenylphosphine (0.68 g, 2.58 mmol) was dissolved in THF (5.16 mL). The clear solution was cooled to 0 °C before adding DIAD (0.50 mL, 2.58 mmol). After 30 min of stirring at 0 °C, a solution of 5α–cholestan–β–ol 2.6 (0.50 g, 1.29 mmol) in THF (3.79 mL) was added followed by thioacetic acid 2.125 (0.19 mL, 2.58 mmol, 95%). The resulting light yellow orange solution was stirred continuously at 0 °C. After 1 h, the solution was allowed to warm up to rt and stirred continuously for 24 h. The reaction mixture was concd under reduced pressure and purification of the residue using flash column chromatography with 0–5% ethyl acetate in hexane as eluent afforded α–thioacetate 2.31 (0.58 g, 100%) as white solid.

2.31. m.p. = 119.3–121.1 °C (ethyl acetate:hexane) (Lit.86 120–121 °C); TLC Rf = 0.88 (ethyl acetate:hexane, 3:7); 1H NMR (300 MHz, CDCl3) δ 3.96 (bs, 1H), 2.27 (s, 3H), 1.94 (td, J = 6.5, 3.1 Hz, 2H), 1.69–1.89 (m, 3H), 1.58–1.65 (m, 3H), 1.43–1.55 (m, 3H), 1.23–1.36 (m, 7H), 1.16–1.19 (m, 3H), 1.07–1.13 (m, 5H), 1.04 (s, 1H), 0.93–1.00 (s, 3H), 0.88 (d, J = 6.5 Hz, 3H), 0.85 (d, J = 1.3 Hz, 3H), 0.83 (d, J = 1.3 Hz, 3H), 0.77 (s, 3H), 0.63 (s, 3H).

3α–Thiol–5α–cholestanate (2.32)

In a 25 mL flame dried round bottom flask, LAH (0.03 g, 0.76 mmol, 95%) was suspended in diethyl ether (3.80 mL). The suspension was cooled to 0 °C using ice/water cold bath. A solution of thioacetate 2.31 (0.11 g, 0.25 mmol) in diethyl ether (1 mL) was
added to the cooled suspension in a dropwise manner after which the solution was stirred continuously as it warmed up to rt. After 24 h, the reaction mixture was diluted with diethyl ether and cooled at 0 °C. The reaction was quenched using the Fieser method. The resulting milky white suspension was filtered through celite, dried over sodium sulfate, and concd under reduced pressure to give α–thiol 2.32 (0.07 g, 66%) as white solid.

**2.32.** IR (KBr) 2928, 2865, 2849, 1466, 1382, 1260 cm⁻¹; \(^1\)H NMR (300 MHz, CDCl₃) δ 3.10 (bs, 1H), 1.88 (d, \(J = 11.9\) Hz, 1H), 1.63–1.76 (m, 3H), 1.55–1.61 (m, 2H), 1.34–1.52 (m, 8H), 1.19–1.27 (m, 4H), 1.06–1.16 (m, 7H), 1.01–1.05 (m, 5H), 0.90–0.94 (m, 2H), 0.84 (d, \(J = 7.4\) Hz, 3H), 0.79 (s, 3H), 0.77 (s, 3H), 0.69 (s, 3H), 0.56 (s, 3H); \(^1^3\)C NMR (75 MHz, CDCl₃): δ 56.7, 56.5, 54.4, 42.8, 40.2, 39.7, 39.7, 37.0, 36.7, 36.6, 35.7, 32.5, 32.1, 30.1, 28.6, 28.5, 28.2, 24.4, 24.1, 23.1, 22.8, 21.0, 18.9, 12.3, 12.0.

3β–Tosyloxy–5α–cholestan-2.33
e (2.33)


In 10 mL round bottom flask, 5α–cholestan–3β–ol 2.6 (0.50 g, 1.29 mmol) was dissolved in pyridine (1.61 mL). \(p\)-Toluenesulfonyl chloride (0.57 g, 2.97 mmol) was added into the clear solution and the reaction mixture was stirred continuously at rt. After 24 h, the reaction mixture was quenched by adding water (10 mL) and it was extracted with DCM (3 x 20 mL). All organic layers were collected, combined together, washed with hydrochloric acid solution (3 x 20 mL, 2 M), brine solution (3 x 20 mL), and water (3 x 20 mL), dried over sodium sulfate, and concd under reduced pressure which afforded tosylate 2.33 (0.65 g, 93%) as white solid. No further purification was needed.

**2.33.** \(^1\)H NMR (300 MHz, CDCl₃) δ 7.76 (dt, \(J = 8.3, 1.8\) Hz, 2H), 7.30 (dd, \(J = 8.2, 0.5\) Hz, 2H), 4.39 (septet, \(J = 5.6\) Hz, 1H), 2.42 (s, 3H), 1.92 (td, \(J = 6.7, 2.3\) Hz, 1H), 1.67–
1.81 (m, 3H), 1.49–1.64 (m, 7H), 1.46–1.47 (d, $J = 1.7$ Hz, 1H), 1.37–1.42 (m, 1H), 1.30–1.34 (m, 3H), 1.25 (t, $J = 4.1$ Hz, 1H), 1.05–1.11 (m, 5H), 1.00–1.02 (m, 2H), 0.94–0.97 (m, 2H), 0.86 (d, $J = 6.7$ Hz, 3H), 0.85 (d, $J = 1.4$ Hz, 3H), 0.83 (d, $J = 1.3$ Hz, 3H), 0.75 (s, 3H), 0.61 (s, 3H), 0.53 (dd, $J = 7.7$, 3.0 Hz, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 144.4, 134.9, 129.9, 127.7, 82.7, 56.5, 56.4, 54.2, 44.9, 42.7, 40.0, 39.6, 36.9, 36.3, 35.9, 35.5, 35.0, 32.0, 28.6, 28.5, 28.4, 28.1, 24.3, 24.0, 23.0, 22.7, 21.8, 21.3, 18.8, 12.3, 12.2.

![Image](233)  
NaCN, DMSO  
reflux, 18 h  
65%

![Image](234)  
DRV-III-65b

3α–Cyano–5α–cholestan (2.34)

A suspension of tosylate 2.33 (0.65 g, 1.20 mmol) and sodium cyanide (0.59 g, 12.0 mmol) in DMSO (30 mL) was heated to 90 °C. After approximately 18 h, the reaction mixture was cooled to rt before adding water (10 mL). The diluted solution was extracted with diethyl ether (3 x 20 mL). All organic layers were collected, washed with brine solution (3 x 20 mL), dried over magnesium sulfate, and concd under reduced pressure. The crude mixture was purified using flash column chromatography with 0–5–10 % ethyl acetate in hexane as eluent to afford cyanide 2.34 (0.31 g, 65 %) as a white solid.

2.34. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 2.94 (bs, 1H), 1.95 (td, $J = 11.9$, 3.1 Hz, 1H), 1.73–1.87 (m, 3H), 1.63–1.71 (m, 3H), 1.46–1.58 (m, 6H), 1.29–1.35 (m, 5H), 1.24–1.26 (m, 2H), 1.20 (bs, 1H), 1.16 (t, $J = 1.5$ Hz, 1H), 1.09–1.12 (m, 4H), 1.06 (bs, 1H), 0.95–1.01 (m, 4H), 0.89 (d, $J = 6.5$ Hz, 3H), 0.86 (d, $J = 1.3$ Hz, 3H), 0.84 (d, $J = 1.3$ Hz, 3H), 0.77 (s, 3H), 0.63 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 123.0, 56.5, 56.3, 54.0, 42.7, 42.5, 40.0, 39.7, 36.3, 36.1, 35.9, 35.5, 34.5, 31.8, 30.6, 28.4, 28.3, 28.2, 27.7, 24.4, 24.3, 24.0, 23.0, 22.7, 20.9, 18.8, 12.2, 12.0.
5β–Cholestane–3–carboxaldehyde (2.37) and 5β–Cholestane–3–carboxaldehyde (2.38)

In a round bottom flask, cyanide 2.34 (0.25 mmol, 0.10 g) was dissolved in DCM (5 mL). The solution was cooled at –78 °C before adding a solution of diisobutylaluminum hydride (0.63 mL, 1.0 M in hexane) dropwise. The solution was stirred continuously at –78 °C. After an hour, the reaction mixture was quenched by adding ethyl acetate. The quenched reaction mixture was washed with Rochelle’s salt solution followed by brine and dried over sodium sulfate before concentrating under reduced pressure. The residue was purified using flash column chromatography with 10% ethyl acetate in hexane as eluent to afford an inseparable mixture of aldehydes 2.37 and 2.38 (0.08 g, 80% (2.6:1)).

2.37 and 2.38. 1H NMR (300 MHz, CDCl₃) δ 9.70 (bs, 1H), 9.60 (d, J = 1.7 Hz, 1H), 2.40 (t, J = 5.7 Hz, 1H), 2.20–2.31 (m, 1H), 2.02–2.10 (m, 1H), 1.91–2.00 (m, 2H), 1.72–1.83 (m, 4H), 1.63–1.71 (m, 2H), 1.46–1.61 (m, 13H), 1.39–1.43 (m, 2H), 1.22–1.36 (m, 16H), 1.06–1.19 (m, 14), 0.95–1.03 (m, 7H), 0.88–0.90 (m, 6H), 0.86–0.87 (m, 6H), 0.84–0.85 (m, 6H), 0.79 (s, 3H), 0.77 (s, 3H), 0.77 (s, 3H), 0.65 (s, 3H), 0.63 (s, 3H), 0.55–0.60 (m, 1H).

5α–Androstan–3β–ol (2.49)

In a flame–dried flask, potassium hydroxide (1.58 g, 28.2 mmol) was dissolved in ethylene glycol (10 mL) by heating. The solution was cooled to rt before adding trans–androsterone 2.50 (2.00 g, 6.89 mmol) and hydrazine hydrate (0.98 mL, 20.2 mmol). The
solution was heated to reflux. After 23 h, the solution was cooled to rt and the reaction mixture was quenched by adding hydrochloric acid solution (14.1 mL, 2M). The mixture was extracted with DCM (4 x 30 mL). The organic layers were collected, combined, dried over sodium sulfate, and concd under reduced pressure. The resulting solid residue was recrystallized in methanol to afford alcohol 2.49 (1.56 g, 82%).

2.49. m.p. = 149.3–150.7 °C (DCM) (Lit.87 151–152 °C); TLC Rf = 0.33 (ethyl acetate:hexane, 1:4); IR (KBr) 3350, 2930, 2845, 1447, 1377, 1133 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 3.58 (hept, J = 4.9 Hz, 1H), 1.76–1.82 (m, 1H), 1.70–1.75 (m, 2H), 1.65–1.69 (m, 2H), 1.61–1.63 (m, 1H), 1.57–1.60 (m, 1H), 1.52–1.57 (m, 2H), 1.47–1.50 (m, 1H), 1.40–1.45 (m, 1H), 1.33–1.39 (m, 1H), 1.29–1.30 (m, 1H), 1.22–1.28 (m, 4H), 1.04–1.17 (m, 4H), 0.9–1.02 (m, 1H), 0.85–0.93 (m, 2H), 0.80 (s, 3H), 0.68 (s, 3H) 0.60–0.65 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 71.6, 54.8, 54.7, 45.1, 41.0, 40.6, 39.1, 38.4, 37.3, 36.1, 35.8, 32.7, 31.7, 29.0, 25.7, 21.5, 20.7, 17.7, 12.6.

![Diagram](image-url)

(3R,5S,8S,9S,10S,13S,14S)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-ol (2.50)

In a round bottom flask, trans-androsterone 2.48 (5.00 g, 17.2 mmol) and hydrazine hydrate (13.39 mL, 275.2 mmol) was added to a solution of KOH (13.51 g, 240.8 mmol) dissolved in ethylene glycol (215 mL). The suspension was heated to reflux. After approximately 4.5 h, the solution was cooled to rt after which potassium hydroxide (13.51 g, 240.8 mmol) was added. The suspension was heated to reflux for 24 h. The reaction mixture was cooled and quenched by adding hydrochloric acid solution. The quenched reaction mixture was extracted with DCM. All organic layers were collected, combined, dried over magnesium sulfate, and concd under reduced pressure. The residue was purified using flash column chromatography with 10–15% ethyl acetate in hexane afforded 2.50 as white solid (0.13 g, 3%).

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2.50. m.p. = 131.0–140.9 °C (DCM); TLC Rf = 0.55 (hexane/ethyl acetate, 9:1); IR (KBr) 3292, 2930, 2867, 1449, 1163, 1035 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 4.04 (quint, J = 2.3 Hz, 1H), 1.71–1.73 (m, 1H), 1.67–1.69 (m, 2H), 1.62–1.66 (m, 2H), 1.56–1.61 (m, 3H), 1.49–1.55 (m, 3H), 1.44–1.47 (m, 1H), 1.38–1.42 (m, 1H), 1.33–1.36 (m, 1H), 1.26–1.31 (m, 2H), 1.22–1.24 (m, 1H), 1.17–1.21 (m, 2H), 1.13–1.15 (m, 1H), 1.05–1.11 (m, 1H), 0.94–1.02 (m, 1H), 0.85–0.93 (m, 1H), 0.72–0.82 (m, 1H), 0.78 (s, 3H), 0.69 (s, 3H), ¹³C NMR (75 MHz, CDCl₃) δ 66.8, 54.7, 41.0, 40.6, 39.3, 39.1, 36.4, 36.0, 32.6, 32.4, 29.2, 28.8, 25.7, 21.0, 20.7, 17.7, 11.4.

![Reaction Scheme](image)

3α–Azido–5α–androstan (2.51)

In a 50 mL round bottom flask, 5α–androstan–3β–ol 2.49 (1.12 g, 4.05 mmol) was dissolved in THF (20 mL). Triphenylphosphine (1.06 g, 4.04 mmol) was added into the solution followed by diisopropyl azodicarboxylate (DIAD) (0.83 mL, 4.05 mmol). The resulting yellow solution was stirred continuously at rt for 10 min before adding diphenylphosphoryl azide (0.88 mL, 4.05 mmol). The solution was stirred continuously at rt. After 24 h, the reaction mixture was concd and the residue was recrystallized in EtOH to afford α–azide 2.51 as a white solid (0.90 g, 74%).

2.51. ¹H NMR (300 MHz, CDCl₃) δ 3.88 (quint, J = 2.8 Hz, 1H), 1.71–1.72 (m, 1H), 1.67–1.70 (m, 3H), 1.59–1.64 (m, 2H), 1.57–1.53 (m, 3H), 1.45–1.52 (m, 3H), 1.36–1.42 (m, 2H), 1.26–1.31 (m, 1H), 1.18–1.24 (m, 3H), 1.14–1.17 (m, 2H), 1.13–1.10 (m, 1H), 0.85–1.03 (m, 2H), 0.79 (s, 3H), 0.72–0.77 (m, 1H), 0.69 (s, 3H).
3α–Amino–5α–androstane (2.44)

In round bottom flask, LAH (0.39 g, 9.83 mmol, 95%) was suspended in THF (10 mL). The suspension was cooled at 0 °C using ice/water cold bath before adding the solution of α–azide 2.52 (0.90 g, 2.98 mmol) in THF (5 mL). The solution was warmed to rt and then refluxed for 4 h. The reaction was then cooled to rt before diluting the solution with THF (15 mL). The diluted reaction mixture was cooled at 0 °C and quenched using a Fieser method. The reaction mixture was stirred continuously until it turned into a milky white suspension. The suspension was then filtered through celite and washed with THF. The filtrate was dried over sodium sulfate and concd under reduced pressure to afford α–amine 2.44 (0.59 g, 72%).

2.44. IR (KBr) 2926, 2855, 1472, 1378, 1124, 753 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 3.18 (bs, 1H), 1.71–1.73 (m, 2H), 1.65–1.69 (m, 3H), 1.61–1.63 (m, 1H), 1.59–1.60 (m, 1H), 1.55–1.57 (m, 2H), 1.50–1.53 (m, 1H), 1.40–1.45 (m, 3H), 1.30–1.32 (m, 1H), 1.23–1.29 (m, 3H), 1.18–1.21 (m, 3H), 1.14–1.18 (m, 2H), 1.07–1.10 (m, 2H), 0.89–1.99 (m, 2H), 0.78 (s, 3H), 0.69 (s, 3H).

3α–Amino–5α–androstane hydrochloride (2.52)

The α–amine 2.44 (0.20 g, 0.73 mmol) was dissolved in diethyl ether (5 mL). A solution of hydrogen chloride in diethyl ether (0.73 mL, 2 M) was added dropwise which resulted in precipitate formation. The suspension was filtered and the precipitate was collected, washed with diethyl ether, and dried under vacuum to afford amine salt 2.52 (0.15 g, 65%) as a white solid.
2.52. m.p. = 252.2 °C (diethyl ether) (dec.); IR (KBr) 3320, 2945, 1619, 1495, 1443, 1379 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.45 (bs, 3H), 3.60 (bs, 1H), 1.84 (bs, 2H), 1.62–1.69 (m, 8H), 1.51–1.58 (m, 4H), 1.37–1.44 (m, 1H), 1.23–1.29 (m, 2H), 1.09–1.20 (m, 4H), 0.92–1.07 (m, 3H), 0.79 (s, 3H), 0.69 (s, 3H); ¹³C NMR (75 MHz, CD₃OD) δ 54.2, 53.3, 48.0, 41.0, 40.6, 38.9, 38.8, 36.3, 36.0, 32.3, 31.6, 28.6, 25.6, 25.0, 20.9, 20.7, 17.8, 11.6.

3α–Acetamido–5α–androstan-25 (2.53)

The α–amine 2.44 (0.20 g, 0.73 mmol) was dissolved in THF (3 mL) in a round bottom flask. Triethylamine (0.12 mL, 0.88 mmol) was added dropwise and the resulting solution was cooled to 0 °C. Acetyl chloride 2.112 (0.05 mL, 0.80 mmol) was added dropwise into the cooled solution which resulted on the formation of white precipitate. The milky white suspension was stirred continuously for 15 min at 0 °C before allowing the reaction mixture to warm up to rt. DCM (5 mL) was added and the diluted solution was washed with hydrochloric acid (10 mL, 1 M), brine (10 mL), and water (10 mL). The organic layer was collected, dried over sodium sulfate, and coned under reduced pressure. Recrystallization of the solid residue using ethyl alcohol afforded amide 2.53 (0.05 g, 22 %) as white solid.

2.53. m.p. = 248.5–251.2 °C (ethyl alcohol); IR (KBr) 3264, 3077, 2933, 2834, 1637, 1558 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 5.70 (bs, 1H), 4.12 (m, 1H), 1.99 (s, 3H), 1.72–1.76 (m, 1H), 1.68–1.71 (m, 2H), 1.62–1.66 (m, 2H), 1.60–1.62 (m, 2H), 1.56–1.58 (m, 1H), 1.52–1.55 (m, 1H), 1.48–1.51 (m, 1H), 1.42–1.46 (m, 1H), 1.36–1.39 (m, 1H), 1.29–1.34 (m, 2H), 1.23–1.27 (m, 1H), 1.21 (d, J = 3.0 Hz, 1H), 1.18–1.19 (m, 1H), 1.12–1.17 (m, 2H), 1.08–1.11 (m, 1H), 1.00–1.06 (m, 1H), 0.92–0.97 (m, 1H), 0.84–0.90 (m, 1H), 0.81 (s, 3H), 0.71–0.77 (m, 1H), 0.69 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ
169.4, 55.0, 54.9, 45.0, 41.3, 41.0, 40.6, 39.1, 36.4, 36.0, 33.5, 33.0, 32.6, 28.7, 26.2, 25.6, 24.0, 21.0, 20.7, 17.7, 11.7.

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\text{(3R,5S,8S,9S,10S,13S,14S)}-10,13-\text{dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-aminium 3,4-dicarboxy-3-hydroxybutanoate (2.54)}
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In a round bottom flask, α-amine 2.44 (0.21 g, 0.76 mmol) was dissolved in THF (0.75 mL). A citric acid 2.15 (0.32 g, 1.67 mmol) solution in THF (0.75 mL) was added dropwise to the α-amine solution. The resulting solution was stirred continuously at rt. After stirring overnight, diethyl ether was added and the suspension was stirred continuously at rt. The precipitate that was formed after several minutes was filtered, collected, and dried to afforded citrate 2.54 (0.28 g, 78%).

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\text{2.54. } ^1\text{H NMR (300 MHz, CD}_3\text{OD) } \delta 3.47 \text{ (sextet, } J = 1.6 \text{ Hz, 1H), 2.73 (q, } J = 16.4 \text{ Hz, 4H), 1.88 (tt, } J = 15.2, 4.6 \text{ Hz, 1H), 1.65-1.75 (m, 4H), 1.49-1.63 (m, 5H), 1.44 (q, } J = 2.3 \text{ Hz, 1H), 1.39 (d, } J = 2.1 \text{ Hz, 1H), 1.34-1.37 (m, 1H), 1.31 (t, } J = 3.8 \text{ Hz, 1H), 1.25-1.28 (m, 1H), 1.21-1.24 (m, 2H), 1.16-1.19 (m, 1H), 1.09-1.14 (m, 2H), 0.88-1.06 (m, 2H), 0.76-0.85 (m, 1H), 0.82 (s, 3H), 0.69 (s, 3H).}
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\text{3β-Tosyloxy-5α-androstan-17-one (2.55)}
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In a 25 mL round bottom flask, trans-androsterone 2.48 (1.00 g, 3.44 mmol) and p-toluenesulfonyl chloride (1.51 g, 7.91 mmol) were dissolved in pyridine (4.30 mL). The reaction mixture was stirred continuously at rt. After 24 h, the reaction mixture was
quenched by adding water (10 mL) and it was extracted with DCM (3 x 20 mL). All organic layers were collected, combined, and washed with hydrochloric acid (3 x 20 mL, 2 M), brine solution (3 x 20 mL), and water (3 x 20 mL), dried over sodium sulfate, and concd under reduced pressure which afforded tosylate 2.55 (1.33 g, 87%) as white solid.

2.55. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.79 (dt, $J = 8.3, 1.9$ Hz, 2H), 7.33 (dd, $J = 8.0, 0.5$ Hz, 2H), 4.42 (heptet $J = 5.9$ Hz, 1H), 2.44 (s, 3H), 2.38–2.47 (m, 1H), 1.99–2.11 (m, 1H), 1.86–1.95 (m, 1H), 1.78–1.80 (m, 1H), 1.71–1.77 (m, 2H), 1.65–1.69 (m, 1H), 1.56–1.64 (m, 3H), 1.44–1.55 (m, 3H), 1.30–1.31 (m, 1H), 1.28–1.29 (m, 2H), 1.22–1.24 (m, 1H), 1.18–1.20 (m, 1H), 1.04–1.16 (m, 1H), 0.85–1.00 (m, 2H), 0.84 (s, 3H), 0.80 (s, 1H), 0.60–0.69 (m, 1H).

![Diagram](image)

3α–Azido–5α–androstan–17–one (2.56)

A suspension of tosylate 2.55 (1.33 g, 2.99 mmol) and sodium azide (1.94 g, 29.9 mmol) in DMSO (75 mL) was heated to 90 °C. After approximately 5 h, the reaction mixture was cooled at rt before adding water (10 mL). The diluted solution was extracted with diethyl ether (3 x 20 mL). All organic layers were collected, dried over magnesium sulfate, and concd under reduced pressure. The solid residue was recrystallized from ethyl alcohol to afford azide 2.56 (0.28 g, 30%).

2.56. $^1$H NMR (300 MHz, CDCl$_3$) δ 3.88 (quint, $J = 2.6$ Hz, 1H), 2.43 (dd, $J = 10.3, 9.6$ Hz, 1H), 2.00–2.12 (m, 1H), 1.88–1.97 (m, 1H), 1.81–1.83 (m, 1H), 1.76–1.78 (m, 1H), 1.66–1.72 (m, 2H), 1.62–1.65 (m, 1H), 1.51–1.56 (m, 2H), 1.39–1.49 (m, 4H), 1.17–1.34 (m, 7H), 0.94–1.08 (m, 1H), 0.85 (s, 3H), 0.81 (s, 3H).
3α–Amino–5α–androstan–17–one hydrochloride (2.57)

In a flame dried flask, azide 2.56 (0.28 g, 0.89 mmol) and triphenylphosphine (0.36 g, 1.37 mmol) was dissolved in THF (15 mL). The solution was stirred continuously at rt. After approximately 18 h, water (3 mL) was added and the solution was heated to reflux. After 1 h, the solution was cooled at rt. The organic layer was collected, dried over sodium sulfate, and concd under reduced pressure. The residue was dissolved in diethyl ether (7 mL) and a solution of hydrochloric acid (0.89 mL, 2 M) was added which resulted to formation of precipitate. The precipitate was filtered, washed with diethyl ether, and dried to afford hydrochloride 2.57 as white solid (0.10 g, 37%).

2.57. IR (KBr) 3326, 2923, 1737, 1496, 1455, 731 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.42 (bs, 3H), 3.61 (bs, 1H), 2.42 (dd, J = 11.1, 8.7 Hz, 1 H), 2.00–2.13 (m, 1H), 1.86–1.94 (m, 2H), 1.76–1.83 (m, 3H), 1.44–1.64 (m, 7H), 1.19–1.38 (m, 6H), 0.95–1.13 (m, 2H), 0.84 (s, 3H), 0.81 (s, 3H).

(3R,5S,8S,9S,10S,13S,14S)–3–iodo–10,13–dimethylhexadecahydro–1H–cyclopenta[a]phenanthrene (2.58)

In a flame dried round bottom flask, β–alcohol 2.49 (1.00 g, 3.62 mmol) and triphenylphosphine (1.04 g, 3.98 mmol) were dissolved in benzene (18 mL). A solution of DIAD (0.77 mL, 3.98 mmol) in benzene (7.50 mL) was added dropwise over several minutes followed by a solution of iodomethane (0.25 mL, 3.08 mmol) in benzene (7.50 mL). The resulting milky yellow solution was stirred continuously at rt. After
approximately 24 h, the reaction mixture was concd and the residue was purified through
flash column chromatography eluting with hexane which afforded 2.58 (1.05 g, 75%).

2.58. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 4.94 (quint, \(J = 2.6\) Hz, 1H), 1.91 (pt, \(J = 15.4, 3.3\)
Hz, 1H), 1.70–1.76 (m, 1H), 1.66–1.69 (m, 2H), 1.59–1.64 (m, 3H), 1.56–1.58 (m, 1H),
1.52–1.54 (m, 1H), 1.49 (t, \(J = 3.3\) Hz, 1H), 1.45 (t, \(J = 2.2\) Hz m 1H), 1.39–1.43 (m, 1H),
1.30–1.36 (m, 1H), 1.28 (d, \(J = 4.0\) Hz, 1H), 1.22–1.26 (m, 2H), 1.18–1.21 (m, 1H),
1.13–1.17 (m, 2H), 1.07–1.11 (m, 1H), 0.97–1.04 (m, 1H), 0.89–0.95 (m, 1H), 0.83–0.87
(m, 1H), 0.79 (s, 3H), 0.69 (s, 3H).

(3S,5S,8S,9S,10S,13S,14S)–3–azido–10,13–dimethylhexadecahydro–1H–
cyclopenta[a]phenanthrene (2.59)

In a flame dried–round bottom flask, iodide 2.58 (1.05 g, 2.72 mmol) and sodium azide
(1.77 g, 27.2 mmol) were suspended in DMSO (68 mL). The suspension was heated to 90
\(^\circ\)C. After 5 h, the solution was cooled at rt before quenching the reaction by adding water
(50 mL). The quenched reaction mixture was extracted with diethyl ether (3 \(\times\) 30 mL).
The organic layers were collected, combined, dried over magnesium sulfate, and concd
under reduced pressure. Purification using flash column chromatography with hexane as
eluent followed by recrystallization using ethanol as solvent afforded azide 2.59 (0.26 g,
32%) as white solid.

2.59. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 3.25 (dt, \(J = 12.9, 4.5\) Hz, 1H), 1.78–1.86 (m, 1H),
1.72–1.76 (m, 1H), 1.64–1.71 (m, 2H), 1.58–1.63 (m, 2H), 1.52–1.57 (m, 2H), 1.48–1.51
(m, 1H), 1.44–1.47 (m, 1H), 1.40–1.43 (m, 1H), 1.33–1.39 (m, 1H), 1.20–1.31 (m, 4H),
1.02–1.19 (m, 4H), 0.83–0.99 (m, 3H), 0.80 (s, 3H), 0.68 (s, 3H), 0.61–0.70 (m, 1H).
(3S,5S,8S,9S,10S,13S,14S)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-aminium chloride (2.61)

A solution of β-azide 2.59 (0.26 g, 0.86 mmol) in THF (2.50 mL) was added to an ice cold suspension of LAH (0.11 g, 2.84 mmol) in THF (3 mL). The solution was stirred continuously at 0 °C for 10 min before allowing it to warm up to rt after which it is heated to reflux. After approximately 4 h, the reaction mixture was allowed to cool at rt and was diluted with THF (5.50 mL). The diluted reaction mixture was cooled at 0 °C using an ice bath and was quenched using the Fieser method. The resulting milky white suspension was filtered through celite and washed with THF. The filtrate was dried over magnesium sulfate and concd under reduced pressure. The residue was then redissolved in CHCl₃. The solution was filtered to remove any undissolved solids. A solution of hydrochloric acid (0.86 mL, 2.0 M in diethyl ether) was added to the filtrate which resulted to the formation of precipitate. The precipitate was filtered, collected and dried to afford β-amine salt 2.61 (0.20 g, 74%) as white powdery solid.

2.61. m.p. = 276.2 °C (diethyl ether) (dec.); IR (KBr) 3449, 2928, 2361, 1984, 1451, 1377 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.29 (bs, 3H), 3.13 (bs, 1H), 1.99 (app d, 1H), 1.55–1.580 (m, 10H), 1.38–1.48 (m, 2H), 1.23–1.35 (m, 4H), 1.06–1.19 (m, 4H), 0.89–1.02 (m, 3H), 0.84 (s, 3H), 0.68 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 54.7, 54.5, 51.5, 45.3, 41.0, 40.6, 39.0, 36.9, 35.9, 35.7, 33.3, 32.4, 28.5, 27.1, 25.7, 21.3, 20.7, 17.8, 12.5.
(3S,5S,8S,9S,10S,13S,14S)–10,13–dimethylhexadecahydro–1H–

Amine 2.60 (0.21 g, 0.76 mmol) was dissolved in THF (50 mL). The solution was filtered
to remove all undissolved solids. A solution of citric acid 2.15 (0.15 g, 0.76 mmol) in
THF (3 mL) was added to the solution. The resulting cloudy solution was stirred
continuously at room temperature. After 12 h, the precipitate was filtered, collected,
washed over diethyl ether, and dried to afford amine citrate 2.62 (0.27 g, 76%) as white
solid.

2.62. m.p. = 201.3 °C (THF) (dec.); ¹H NMR (300 MHz, CD₂OD) δ 3.04–3.14 (m, 1H),
2.79 (q, J = 15.5 Hz, 4H), 1.81–1.88 (m, 2H), 1.71–1.78 (m, 3H), 1.63–1.68 (m, 2H),
1.51–1.62 (m, 4H), 1.41–1.47 (m, 2H), 1.34–1.39 (m, 3H), 1.29–1.32 (m, 1H), 1.25–1.28
(m, 1H), 1.20–1.23 (m, 1H), 1.14–1.18 (m, 3H), 1.08–1.11 (m, 1H), 1.02–1.06 (m, 1H),
0.91–1.00 (m, 2H), 0.88 (s, 3H), 0.71 (s, 3H).

(5S,8S,9S,10S,13S,14S)–10,13–dimethyltetradecahydro–1H–
cyclopenta[a]phenanthren–3(2H)–one (2.65)

A solution of alcohol 2.49 (1.00 g, 3.62 mmol) in DCM (20 mL) was added to a
suspension of pyridinium chlorochromate (1.56 g, 7.24 mmol) and silica gel (1.56 g) in
DCM (5 mL). The resulting black orange solution was stirred continuously at rt for 2 h.
The reaction mixture was filtered through a plug of silica gel eluting with DCM. The
filtrate was coned under reduced pressure which afforded ketone 2.65 (0.92 g, 93%).
2.65. $^1$H NMR (300 MHz, CDCl$_3$) δ 2.18 – 2.40 (m, 3H), 1.94–2.06 (m, 2H), 1.64–1.71 (m, 2H), 1.57–1.63 (m, 2H), 1.44–1.55 (m, 3H), 1.33–1.43 (m, 2H), 1.24–1.33 (m, 4H), 1.04–1.21 (m, 3H), 0.97 (s, 3H), 0.82–0.95 (m, 2H), 0.73 (dd, $J = 10.7, 4.2$ Hz, 1H), 0.67 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 210.7, 54.0, 53.8, 46.4, 44.3, 40.5, 40.1, 38.5, 38.3, 37.8, 35.4, 35.4, 31.8, 28.7, 25.2, 21.2, 20.2, 17.2.

\[ \text{N}^\text{N} - \text{H} \text{Br}_3 \]

(2R,5S,8S,9S,10S,13S,14S)–2–bromo–10,13–dimethyltetradecahydro–1H–cyclopenta[a]phenanthren–3(2H)–one (2.77)

A solution of ketone 2.65 (0.18 g, 0.66 mmol) in acetic acid (6.60 mL) was warmed to 50 °C. Pyridinium tribromide 2.126 (0.23 g, 0.66 mmol) was added in one portion and the solution was stirred continuously. After several seconds, a precipitate was formed. The precipitate was filtered, collected, and dried to afford bromide 2.77 (0.15 g, 65%) as white powdery solid.

2.77. IR (KBr) 2924, 2865, 2846, 1716, 1656, 1311 cm$^{-1}$; $[\alpha]_D^{19.1} = +29.2$ (c 1.13, DCM); $^1$H NMR (300 MHz, CDCl$_3$) δ 4.75 (dd, $J = 13.4, 6.3$ Hz, 1H), 2.64 (dd, $J = 12.6, 6.3$ Hz, 1H), 2.37–2.48 (m, 2H), 1.84 (d, $J = 13.4$ Hz, 1H), 1.74–1.77 (m, 1H), 1.69–1.73 (m, 1H), 1.52–1.67 (m, 6H), 1.40–1.47 (m, 2H), 1.32–1.38 (m, 2H), 1.21–1.31 (m, 1H), 1.12–1.19 (m, 2H), 1.08 (s, 3H), 0.88–1.01 (m, 2H), 0.76–0.82 (m, 1H), 0.71 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 201.5, 54.9, 54.4, 54.1, 52.1, 47.7, 44.2, 41.1, 40.5, 39.4, 38.8, 35.5, 32.1, 28.7, 25.7, 21.8, 20.7, 178, 12.4.
(2R,5S,8S,9S,10S,13S,14S)-10,13-dimethyl-4,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3H-cyclopenta[a]phenanthren-3-one oxime (2.70)

A suspension of bromoketone 2.77 (0.22 g, 0.62 mmol), lithium bromide (0.32 g, 3.72 mmol), and lithium carbonate (0.27 g, 3.72 mmol) in DMF (62 mL) was heated to reflux. After approximately 3 h, the reaction mixture was cooled to rt before adding it to crushed ice. The quenched reaction mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were collected, combined, washed with cold water and brine, dried over sodium sulfate, and concd under reduced pressure. The concd reaction mixture was purified with flash column chromatography using 20% ethyl acetate in hexane as the eluent which afforded 2.69 (0.13 g, 76%) as an off-white solid.

2.69. TLC Rf = 0.52 (hexane:ethyl acetate, 4:1); 1H NMR (300 MHz, CDCl3) δ 7.14 (d, J = 10.2 Hz, 1H), 5.83 (d, J = 10.2 Hz, 1H), 2.35 (dd, J = 17.6, 14.0 Hz, 1H), 2.19 (dd, J = 17.7, 3.8 Hz, 1H), 1.83–1.95 (m, 1H), 1.68–1.80 (m, 3H), 1.51–1.67 (m, 3H), 1.32–1.50 (m, 5H), 1.09–1.23 (m, 3H), 0.90–1.05 (m, 3H), 0.99 (s, 3H), 0.71 (s, 3H); 13C NMR (75 MHz, CDCl3) δ 200.4, 158.8, 127.5, 54.6, 50.4, 44.5, 41.2, 41.1, 40.4, 39.2, 38.8, 36.2, 31.8, 27.8, 25.5, 21.4, 20.7, 13.2.

(5S,8S,9S,10S,13S,14S)-2–azido–10,13–dimethyltetradecahydro–1H–cyclopenta[a]phenanthren–3(2H)–one oxime (2.70)

In a round bottom flask, enone 2.69 (0.12 g, 0.44 mmol) was suspended in methanol:water (5 mL, 1:1). Hydroxylamine hydrochloride (0.12 g, 1.74 mmol) and sodium acetate (0.17 g, 2.07 mmol) were added to the suspension and the reaction mixture was heated to reflux. After 4 h, the reaction mixture was cooled and water (2.50
mL) was added and the reaction mixture was refluxed for another 5 mins. The reaction mixture was cooled at rt and was extracted with DCM (3 x 10 mL). The organic layers were collected, combined, washed with brine, dried over sodium sulfate, and concd under reduced pressure to afford oxime 2.70 (0.13 g, 100%) as a beige solid.

2.70. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.42 (d, $J = 10.1$ Hz, 1H), 6.00 (d, $J = 10.1$ Hz, 1H), 2.81 (dd, $J = 18.0$, 4.2 Hz, 1H), 2.11 (dd, $J = 18.1$, 13.5 Hz, 1H), 1.69–1.78 (m, 3H), 1.57–1.66 (m, 3H), 1.49–1.55 (m, 1H), 1.25–1.47 (m, 5H), 1.05–1.19 (m, 3H), 0.89–1.02 (m, 3H), 0.89 (s, 3H), 0.72 (s, 3H).


In a round bottom flask, oxime 2.70 (0.11 g, 0.38 mmol) was dissolved in pyridine (3.45 mL). $p$–Toluenesulfonyl chloride 2.123 (0.15 g, 0.79 mmol) was added and the reaction mixture was stirred continously at rt for 3 h. The reaction was quenched by adding water (5 mL). The quenched reaction mixture was extracted with DCM (3 x 10 mL). The organic layers were collected, combined, washed with hydrochloric acid (20 mL, 1 M), dried over sodium sulfate, and concd under reduced pressure to afford O–tosyl oxime 2.71 (0.22 g, quantitative) as a white solid.

2.71. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.79 (d, $J = 8.3$ Hz, 2H), 7.25 (d, $J = 8.1$ Hz, 2H), 6.57 (d, $J = 10.3$ Hz, 1H), 5.87 (d, $J = 10.2$ Hz, 1H), 2.71 (dd, $J = 18.3$, 4.2 Hz, 1H), 2.36 (s, 3H), 2.10 (dd, $J = 18.5$, 13.4 Hz, 1H), 1.65–1.71 (m, 2H), 1.56–1.63 (m, 2H), 1.50–1.55 (m, 2H), 1.41–1.48 (m, 1H), 1.34–1.38 (m, 2H), 1.25–1.32 (m, 2H), 1.18–1.24 (m, 1H), 1.01–1.12 (m, 3H), 0.84–0.94 (m, 2H), 0.80–0.94 (m, 1H), 0.76 (s, 3H), 0.63 (s, 3H).
(5aR,5bS,7aS,10aS,10bR,12aR)–5a,7a–dimethyl–1,2,5b,6,7,7a,8,9,10,10a,10b,11,12,12a–tetradecahydrocyclopenta[5,6]naphtho[2,1–c]azepin–3(5aH)–one (2.72)

Oxime 2.70 (0.13 g, 0.45 mmol) was cooled at −78 °C using a dry ice/acetone bath. Thionyl chloride (1.29 mL, 17.69 mmol) was added and the solution was stirred continuously at −78 °C. After 20 min, the reaction mixture was added to a solution of sodium hydroxide which resulted to formation of a precipitate. The precipitate was collected, washed over water, dried, and was purified using flash column chromatography with 20% ethyl acetate in hexane as eluent to afford unsaturated lactam 2.72 (0.05 g, 38%) as a off–white solid.

2.72. 1H NMR (300 MHz, CDCl3) δ 9.00 (bs, 1H), 6.43 (d, J = 10.1 Hz), 5.98 (d, J = 10.1 Hz, 1H), 2.81 (dd, J = 18.1, 4.2 Hz, 1H), 2.10 (dd, J = 18.0, 13.5 Hz, 1H), 1.69–1.77 (m, 3H), 1.57–1.66 (m, 3H), 1.50–1.54 (m, 1H), 1.42–1.48 (m, 2H), 1.24–1.40 (m, 3H), 1.10–1.21 (m, 3H), 0.91–1.01 (m, 3H), 0.87 (s, 3H), 0.71 (s, 3H).

(2R,5S,8S,9S,10S,13S,14S)–2–azido–10,13–dimethyltetradecahydro–1H–cyclopenta[a]phenanthren–3(2H)–one (2.78)

In a flamed dried round bottom flask, bromide 2.77 (1.89 g, 5.35 mmol) was suspended in DMF (90 mL). Sodium azide (0.42 g, 6.42 mmol) was added to the suspension and the reaction mixture was stirred continuously at 28 °C. After approximately 2 h, the reaction mixture was poured onto crushed ice. After the ice melted it was extracted with ethyl acetate (3 x 50 mL). The organic layers were collected, combined, washed with cold water (3 x 50 mL) and brine, dried over magnesium sulfate, and concd under reduced
pressure. The residue was recrystallized from ethyl alcohol to afford azide 2.78 (1.35 g, 80%) as light brown solid.

2.78. m.p. = 131.5–132.6 °C (ethyl alcohol); TLC Rf = 0.63 (hexane:ethyl acetate, 4:1); IR (KBr) 2928, 2833, 2105, 1714, 1282, 1156 cm⁻¹; [α]D⁻¹⁴ = +54.8 (c 1.00, DCM); ¹H NMR (300 MHz, CDCl₃) δ 3.98 (dd, J = 13.1, 6.4 Hz, 1H), 2.21–2.42 (m, 3H), 1.69–1.77 (m, 2H), 1.52–1.67 (m, 6H), 1.21–1.48 (m, 6H), 1.13–1.19 (m, 2H), 1.09 (s, 3H), 0.88–1.01 (m, 2H), 0.76–0.86 (m, 1H), 0.71 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 205.5, 64.2, 54.4, 54.2, 47.9, 45.8, 44.0, 41.1, 40.5, 38.8, 37.3, 35.4, 32.1, 28.7, 25.7, 21.8, 20.7, 17.7, 12.8.

![Chemical structure of 2.78, 2.79, and 2.80](image)


A pre–cooled solution of azidoketone 2.78 (1.38 g, 4.37 mmol) in DCM (31 mL) was added to a cooled suspension of sodium borohydride (0.44 g, 11.5 mmol) in DCM:methanol (146 mL, 1:1) at −78 °C using an dry ice/acetone bath with a canula. The reaction mixture was stirred continuously at −78 °C. After approximately 2 h, the reaction mixture was quenched by adding sodium hydroxide solution (40 mL, 2 M). The organic layer was collected, dried over sodium sulfate, and concd under reduced pressure. The concentrate was purified through a gravity column chromatography using 10–15–20% ethyl acetate in hexane to afford 2.79 (0.19 g, 24%) as white solid and 2.80 (0.61 g, 44%) as a clear colorless needle crystals.

2.79. m.p. = 76.4–81.3 °C (DCM); TLC Rf = 0.69 (hexane:ethyl acetate, 4:1); IR (film) 3435, 2927, 2099, 1451, 1248, 1038 cm⁻¹; [α]D⁻⁰⁸ = −60.7 (c 10.94, DCM); ¹H NMR (300 MHz, CDCl₃) δ 3.96 (bs, 3H), 3.52 (dq, J = 12.5, 4.6, 3.0, Hz, 1H), 2.07 (s, 1H), 1.65–1.80 (m, 3H), 1.53–1.62 (m, 5H), 1.35–1.50 (m, 3H), 1.21–1.32 (m, 3H), 1.08–1.18 (m,
4H), 0.87–1.01 (m, 3H), 0.82 (s, 3H), 0.68 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 68.2, 61.2, 54.6, 54.6, 41.0, 40.6, 38.9, 38.3, 37.2, 37.1, 35.6, 34.5, 32.4, 27.9, 25.7, 21.2, 20.7, 17.8, 12.5.

2.80. m.p. = 127.7–130.0 °C (DCM); TLC $R_f = 0.67$ (hexane:ethyl acetate, 4:1); IR (film) 3352, 2932, 2862, 2103, 1452, 1262 cm$^{-1}$; $[\alpha]^{9.9}_{D} = -31.4$ (c 9.23, DCM); $^1$H NMR (300 MHz, CDCl$_3$) δ 3.32–3.48 (m, 2H), 2.14 (d, J = 2.9, Hz, 1H), 2.04 (dd, J = 12.8, 4.4 Hz, 1H), 1.67–1.76 (m, 3H), 1.59–1.65 (m, 2H), 1.50–1.58 (m, 2H), 1.38–1.46 (m, 2H), 1.26–1.36 (m, 3H), 1.19–1.23 (m, 1H), 1.11–1.19 (m, 3H), 0.97–1.09 (m, 2H), 0.83–0.93 (m, 2H), 0.87 (s, 3H), 0.73–0.78 (m, 1H), 0.69 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 74.3, 64.8, 54.6, 54.6, 44.6, 42.3, 41.0, 40.6, 38.9, 37.4, 35.9, 35.5, 32.4, 28.2, 25.7, 21.6, 20.7, 17.8, 13.4.

![Chemical Structure](image)


In a round bottom flask, a solution of 2.78 (0.20 g, 0.63 mmol) was dissolved in THF (12.6 mL). The solution was cooled to −78 °C using ice/acetone bath. A solution of L–selectride in THF (0.97 mL, 0.97 mmol, 1M in THF) was added dropwise to the solution. The reaction mixture was stirred continuously at −78 °C. After approximately 2 h, the reaction mixture was transferred to an ice/water bath and was quenched by adding sat. aqueous ammonium chloride solution. The reaction mixture was extracted with ethyl acetate (3 x 10 mL) and the organic layers were collected, combined, washed over sodium sulfate, and concd under reduced pressure. The residue was purified with flash column chromatography using 10% ethyl acetate in hexane as eluent to afford 2.79 (0.12 g, 60%).

2.79. m.p. = 76.4–81.3 °C (DCM); TLC $R_f = 0.69$ (hexane:ethyl acetate, 4:1); IR (KBr): 3435, 2927, 2099, 1451, 1248, 1038 cm$^{-1}$; $[\alpha]^{20.8}_{D} = -60.7$ (c 10.94, DCM); $^1$H NMR
(300 MHz, CDCl$_3$) $\delta$ 3.96 (broad, 3H), 3.52 (dq, $J$ = 12.5, 4.6, 3.0, Hz, 1H), 2.07 (s, 1H), 1.65–1.80 (m, 3H), 1.53–1.62 (m, 5H), 1.35–1.50 (m, 3H), 1.21–1.32 (m, 3H), 1.08–1.18 (m, 4H), 0.87–1.01 (m, 3H), 0.82 (s, 3H), 0.68 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 68.2, 61.2, 54.6, 54.6, 41.0, 40.6, 38.9, 38.3, 37.2, 37.1, 35.6, 34.5, 32.4, 27.9, 25.7, 21.2, 20.7, 17.8, 12.5.

(2R,3S,5S,8S,9S,10S,13S,14S)$\text{–}2$–azido–10,13–dimethylhexadecahydro–1H–cyclopenta[a]phenanthren–3–yl acetate (2.84)

In a round bottom flask, azidoalcohol 2.79 (0.01 g, 0.03 mmol) was dissolved in pyridine (2 mL). Acetic anhydride 2.82 (1.00 mL, 10.6 mmol) was added dropwise and the reaction mixture was stirred continuously at rt. After approximately 24 h, water was added to quench the reaction. The quenched reaction mixture was extracted with DCM (3 x 10 mL). The organic layers were collected, combined, washed with hydrochloric acid (3 x 10 mL, 1M), dried over sodium sulfate and concd under reduced pressure. The concentrate was purified using flash column chromatography with 10% ethyl acetate in hexane to afford 2.84 (0.01 g, 100%) as an off–white solid.

2.84. TLC $R_f$ = 0.63 (hexane:ethyl acetate, 4:1); IR (film) 2931, 2868, 2098, 1745, 1368, 1243 cm$^{-1}$; $[\alpha]_{D}^{22.8} = +22.5$ (c 1.00, DCM); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.19 (bs, 1H), 3.30 (ddd, $J$ = 12.8, 4.3, 3.3, Hz, 1H), 2.12 (s, 3H), 1.87 (dd, $J$ = 12.0, 4.1 Hz, 1H), 1.73–1.78 (m, 1H), 1.66–1.71 (m, 1H), 1.60–1.65 (m, 2H), 1.53–1.59 (m, 2H), 1.47–1.50 (m, 1H), 1.40–1.45 (m, 2H), 1.33–1.38 (m, 1H), 1.28–1.31 (m, 1H), 1.25 (bs, 2H), 1.16–1.21 (m, 2H), 1.09–1.14 (m, 2H), 0.92–1.01 (m, 2H), 0.87–0.91 (m, 2H), 0.84 (s, 3H), 0.70 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 170.7, 71.4, 57.6, 54.7, 54.6, 41.1, 40.6, 39.5, 39.0, 37.9, 37.2, 35.6, 32.9, 32.4, 27.8, 25.7, 21.6, 21.2, 20.8, 17.8.
(2R,3R,5S,8S,9S,10S,13S,14S)-2-azido-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate (2.85)

In a round bottom flask, azidoalcohol 2.80 (0.02 g, 0.06 mmol) was dissolved in pyridine (4 mL). Acetic anhydride 2.82 (2.00 mL, 21.2 mmol) was added dropwise and the reaction mixture was stirred continuously at rt. After approximately 24 h, water was added to quench the reaction. The quenched reaction mixture was extracted with DCM (3 x 10 mL). The organic layers were collected, combined, washed with hydrochloric acid (3 x 10 mL, 1M), dried over sodium sulfate and concd under reduced pressure. The concentrate was purified with flash column chromatography using 10% ethyl acetate in hexane to afford 2.85 (0.02 g, 100%) as a white solid.

2.85. TLC Rf = 0.65 (hexane:ethyl acetate, 4:1); IR (film) 2939, 2853, 2105, 1736, 1234, 1032 cm⁻¹; [α]D²₂.₈ = –20.8 (c 2.00, DCM); ¹H NMR (300 MHz, CDCl₃) δ 4.68 (dt, J = 11.1, 10.2, 5.1 Hz, 1H), 3.56 (ddd, J = 15.0, 9.0, 4.8 Hz, 1H), 2.08 (s, 3H), 2.03 (dd, J = 13.0, 4.8 Hz, 1H), 1.70–1.80 (m, 2H), 1.65–1.69 (m, 1H), 1.52–1.63 (m, 3H), 1.37–1.50 (m, 3H), 1.28–1.34 (m, 3H), 1.18–1.25 (m, 3H), 1.11–1.17 (m, 2H), 0.98–1.08 (m, 2H), 0.90–0.94 (m, 1H), 0.87 (s, 3H), 0.75 (dd, J = 12.1, 4.1 Hz, 1H), 0.69 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 170.7, 76.6, 60.9, 54.5, 54.5, 44.3, 43.0, 41.0, 40.6, 38.9, 37.0, 35.5, 33.3, 32.3, 28.0, 25.7, 21.5, 21.5, 20.7, 17.8, 13.2.

A suspension of LAH (0.08 g, 1.98 mmol) in THF (4 mL) was cooled at 0 °C using an ice/water bath. A solution of 2.79 (0.19 g, 0.60 mmol) in THF (4 mL) was added dropwise to the cooled suspension. After approximately 10 min, the reaction mixture was allowed to warm to rt after which it was heated to reflux. After 4 h, the reaction mixture was cooled to rt and diluted with THF (8 mL). The reaction mixture was then cooled to 0 °C and quenched using the Fieser method. The quenched reaction mixture was filtered through celite, dried over sodium sulfate, and concd under reduced pressure. The residue was next dissolved in diethyl ether:chloroform (20 mL, 1:1) and was filtered to remove any undissolved solids. A solution of hydrochloric acid in diethyl ether (0.60 mL, 1.20 mmol, 2M) was added dropwise to the solution which resulted in the formation of white precipitate. The precipitate was filtered, washed over diethyl ether, and dried to afford 2.86 (0.10 g, 50% over 2 steps).

2.86 m.p. = 235.3 °C (diethyl ether)(dec.); IR (KBr) 3399, 3043, 2926, 1970, 1600, 1085 cm⁻¹; [α]D²².⁸ = +17.4 (c 0.33, methanol); ¹H NMR (300 MHz, CD₃OD) δ 3.99 (bs, 1H), 3.26–3.32 (m, 1H), 1.71–1.78 (m, 4H), 1.57–1.70 (m, 5H), 1.51–1.56 (m, 2H), 1.40–1.47 (m, 2H), 1.33–1.38 (m, 2H), 1.25–1.29 (m, 2H), 1.15–1.23 (m, 3H), 0.92–1.12 (m, 2H), 0.88 (s, 3H), 0.83 (dd, J = 10.5, 2.6 Hz, 1H), 0.73 (s, 3H); ¹³C NMR (75 MHz, CD₃OD) δ 66.3, 51.6, 42.1, 41.6, 40.1, 39.4, 38.2, 38.0, 36.9, 36.4, 33.6, 29.0, 26.6, 22.2, 21.5, 18.1, 12.5.
(2R,3R,5S,8S,9S,10S,13S,14S)-3-hydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-2-aminium chloride (2.87)

A solution of 2.80 (0.20 g, 0.63 mmol) in THF (4 mL) was added dropwise to a cooled suspension of LAH (0.08 g, 2.08 mmol) in THF (4 mL). The reaction mixture was warmed to rt and then reflux. After approximately 4 h, the reaction mixture was cooled to rt and diluted with THF (8 mL). The diluted reaction mixture was cooled to 0 °C and was quenched using the Fieser method. The quenched reaction mixture was filtered through celite, dried over sodium sulfate, and concd under reduced pressure. The residue was dissolved in diethyl ether (10 mL) and chlorform (10 mL). A solution of hydrogen chloride in diethyl ether (0.63 mL, 1.26 mmol, 2M) was added dropwise to the solution which resulted to the formation of precipitate. The precipate was filtered, collected and dried to afford 2.87 (0.13 g, 62%) as white solid.

2.87 IR (KBr) 3387, 2931, 2860, 1624, 1376, 1047 cm⁻¹; [α]D²¹ = +13.6 (c 1.00, methanol); ¹H NMR (300 MHz, CD₃OD) δ 3.45 (dt, J = 10.4, 5.0 Hz, 1H), 3.00–3.08 (m, 1H), 2.03 (dd, J = 12.5, 4.4 Hz, 1H), 1.64–1.78 (m, 6H), 1.52–1.62 (m, 2H), 1.42–1.50 (m, 2H), 1.33–1.39 (m, 3H), 1.24–1.30 (m, 2H), 1.09–1.21 (m, 4H), 0.95–1.05 (m, 2H), 0.92 (s, 3H), 0.80 (dd, J = 12.0, 4.2 Hz, 1H), 0.74 (s, 3H); ¹³C NMR (75 MHz, CD₃OD) δ 72.9, 55.8, 55.2, 46.0, 42.5, 42.1, 41.6, 40.1, 38.3, 37.8, 36.8, 33.5, 29.2, 26.6, 22.7, 21.5, 18.0, 13.4.
(2R,5S,8S,9S,10S,13S,14S)-2-azido-10-methyltetradecahydro-1H-cyclopenta[a]phenanthrene-3(2H)-one O-methyl oxime (2.88)

In a flame dried round bottom flask, azidoketone 2.78 (0.66 g, 2.09 mmol) and methoxylamine hydrochloride (0.52 g, 6.27 mmol) were dissolved in pyridine (41.8 mL). After stirring the reaction mixture for approximately 24 h, ethyl acetate (10 mL) was added and the mixture was filtered through celite. The filtrate was concd under reduced pressure. The concentrate was dissolved in DCM (10 mL) and it was washed with hydrochloric acid (3 x 10 mL, 2M). The organic layer was collected, dried over sodium sulfate, and concd under reduced pressure to afford O-methyloxime 2.88 (0.56 g, 78%) as an off-white solid.

2.88. m.p. = 121.1–126.6 °C (DCM); ¹H NMR (300 MHz, CDCl₃) δ 3.97 (dd, J = 12.2, 5.7 Hz, 1H), 3.90 (s, 3H), 3.01 (dd, J = 15.1, 3.4 Hz, 1H), 2.19 (dd, J = 12.5, 5.5 Hz, 1H), 1.68–1.76 (m, 3H), 1.60–1.65 (m, 3H), 1.50–1.56 (m, 2H), 1.39–1.43 (m, 2H), 1.23–1.36 (m, 5H), 1.070–1.21 (m, 4H), 0.84–0.98 (m, 2H), 0.91 (s, 3H), 0.71–0.80 (m, 1H), 0.69 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 155.9, 62.1, 58.0, 54.5, 54.3, 45.2, 45.2, 41.0, 40.6, 40.5, 39.0, 38.8, 36.9, 35.4, 32.1, 28.4, 27.7, 25.7, 21.5, 20.7, 17.7, 12.7.

(2R,5S,8S,9S,10S,13S,14S)-2-amino-10,13-dimethyltetradecahydro-1H-cyclopenta[a]phenanthrene-3(2H)-one O-methyl oxime (2.90)

Azidooxime 2.88 (0.23 g, 0.67 mmol) was dissolved in THF (8.35 mL). Triphenylphosphine (0.53 g, 2.01 mmol) was added followed by water (0.26 mL, 14.3 mmol). The resulting homogenous solution was stirred continuously at rt for 24 h. The reaction mixture was concd under reduced pressure and water was removed.
azeotropically using toluene. The residue was purified using flash column chromatography with a gradien
e of 0–10–20–30% methanol in ethyl acetate as eluent to afford amino oxime 2.90 (0.09 g, 41%) as an off–white solid.

2.90. TLC Rf = 0.46 (DCM:methanol:ammonium hydroxide, 90:9:1); 1H NMR (300 MHz, CDCl3): δ 4.39 (bs, 2H), 3.84 (s, 3H), 3.63 (dd, J = 12.3, 5.0 Hz, 1H), 2.96 (dd, J = 15.0, 2.7 Hz, 1H), 2.29 (dd, J = 12.4, 5.1 Hz, 1H), 1.52–1.72 (m, 7H), 1.33–1.44 (m, 3H), 1.20–1.30 (m, 3H), 1.01–1.18 (m, 4H), 0.82–0.97 (m, 1H), 0.92 (s, 3H), 0.70–0.76 (m, 1H), 0.68 (s, 3H).

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\text{(2R,5S,8R,9S,10S,13S,14S,Z)}-3\text{–(methyleneiminio–10,13–dimethylhexadecahy}
\text{dro–1}H\text{–cyclopenta[}a\text{]phenanthren–2–aminium chloride (2.91)}
\]

In a round bottom flask, azido N–methyl oxime 2.88 (0.61 g, 1.77 mmol) and triphenylphosphine (1.39 g, 5.31 mmol) was dissolved in THF (45 mL). Water (37.9 mmol, 0.68 mL) was added and the reaction mixture was stirred continously at rt. After 48 h, the reaction mixture was concd under reduced pressure and water was removed azeotropically using toluene. The residue was dissolved in diethyl ether (20 mL). A solution of hydrogen chloride in diethyl ether (7.08 mmol, 3.54 mL, 2.0 M in HCl) was added dropwise which resulted in the formation of a white precipitate. The precipitate was filtered and then suspended in diethyl ether and stirred continously overnight. The precipitate was filtered, washed with diethyl ether, dried, and collected to give 2.91 (0.10 g, 17% over 2 steps) as a white solid.

2.91. IR (KBr) 3425, 2934, 2869, 1655, 1047, 957 cm\(^{-1}\); 1H NMR (300 MHz, CD\(_3\)OD) 3.99 (dd, J = 12.8, 5.0 Hz, 1H), 3.89 (s, 3H), 3.06 (dd, J = 15.0, 3.6 Hz, 1H), 2.30 (dd, J = 12.1, 5.2 Hz, 1H), 1.73–1.83 (m, 3H), 1.64–1.71 (m, 2H), 1.54–1.63 (m, 2H), 1.41–1.51 (m, 3H), 1.27–1.38 (m, 3H), 1.12–1.24 (m, 4H), 1.02 (s, 3H), 0.90–1.00 (m, 2H), 0.83 (dt, J = 12.1, 2.9 Hz, 1H), 0.75 (s, 3H); 13C NMR (75 MHz, CD\(_3\)OD): δ 155.6, 62.5, 55.7, 55.5, 47.1, 45.1, 42.0, 41.5, 40.0, 38.0, 36.8, 33.3, 29.4, 28.1, 26.6, 22.6, 21.5, 18.0, 12.6.
cyclopenta[a]phenanthren–3–yl methanesulfonate (2.92)

In a round bottom flask, azidoalcohol 2.80 (0.10 g, 0.31 mmol) was dissolved in pyridine
(1.00 mL). Methanesulfonyl chloride (0.04 mL, 0.53 mmol) was added dropwise and the
reaction mixture was stirred continuously for 24 h. Water (5 mL) was then added to the
reaction mixture to quench the reaction. The quenched reaction mixture was extracted
with DCM (3 x 20 mL). The organic layers were collected, washed with hydrochloric
acid (2 x 10 mL, 6 M) followed by sat. sodium bicarbonate (2 x 10 mL) and water (2 x 10
mL). The solution was dried over sodium sulfate and concd under reduced pressure to
afford mesylate 2.92 as an off–white solid (0.11 g, 92%).

2.92. m.p. = 115.0–118.2 °C (DCM); IR (KBr) 2933, 2867, 2105, 1362, 1173, 947 cm⁻¹;

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\left[\alpha\right]_{D}^{21.3} = -19.8 \, (c \, 1.67, \, DCM) \; ; \; \text{\textsuperscript{1}H NMR (300 MHz, CDCl}_3) \, \delta \, 4.35 \, (dt, \, J = 7.8, 5.6 \, Hz, \, 1H), \, 3.57–3.66 \, (m, \, 1H), \, 3.09 \, (s, \, 3H), \, 2.10 \, (dd, \, J = 13.0, 4.9 \, 1H), \, 1.94 \, (ddd, \, J = 13.1, 5.4, \, 2.3 \, Hz, \, 1H), \, 1.65–1.74 \, (m, \, 3H), \, 1.55–1.64 \, (m, \, 3H), \, 1.41–1.53 \, (m, \, 2H), \, 1.32–1.38 \, (m, \, 2H), \, 1.18–1.30 \, (m, \, 4H), \, 1.05–1.15 \, (m, \, 4H), \, 0.89–1.01 \, (m, \, 1H), \, 0.87 \, (s, \, 3H), \, 0.75 \, (dd, \, J = 12.0, 4.1 \, Hz, \, 1H), \, 0.68 \, (s, \, 3H); \; \text{\textsuperscript{13}C NMR (75 MHz, CDCl}_3) \, \delta \, 84.1, \, 60.7, \, 54.2, \, 54.1, \, 44.2, \, 42.8, \, 40.3, \, 38.6, \, 35.2, \, 34.7, \, 32.0, \, 27.4, \, 25.5, \, 21.3, \, 20.5, \, 17.5, \, 12.9.
\]

(2R,3S,5S,8R,9S,10S,13S,14S)–2,3–diazi–10,13–dimethylhexadecahydro–1H–
cyclopenta[a]phenanthrene (2.93)

A suspension of azidomesylate 2.91 (0.32 g, 0.30 mmol) and sodium azide (0.04 g, 0.60
mmol) in DMF (3 mL) was heated to 100 °C. After 20 h, the reaction mixture was cooled
and was added to crushed ice. The quenched reaction mixture was extracted with ethyl acetate (3 x 10 mL) and the organic layers were collected, combined, washed with water (5 x 10 mL), dried over sodium sulfate and concd under reduced pressure to give diazidocholestanate \textbf{2.93} (0.09 g, 90\%) as an off–white solid.

\textbf{2.93}. m.p. = 247.5 °C (ethyl acetate) (dec.); IR (KBr) 2930, 2861, 2100, 1452, 1363, 1263 cm\(^{-1}\); [\(\alpha\)]\textsubscript{D}\textsuperscript{213} = −12.1 (c 0.66, DCM); \(^1\)H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta\) 3.91 (q, \(J\) = 2.7 Hz, 1H), 3.49 (td, \(J\) = 12.4, 3.9 Hz, 1H), 1.80 (dd, \(J\) = 12.5, 3.9 Hz, 1H), 1.67–1.76 (m, 2H), 1.62–1.66 (m, 1H), 1.57–1.61 (m, 2H), 1.50–1.56 (m, 2H), 1.45–1.47 (m, 1H), 1.39–1.43 (m, 2H), 1.29–1.37 (m, 2H), 1.21–1.27 (m, 2H), 1.14–1.18(m, 2H), 1.07–1.12 (m, 2H), 0.96–1.03 (m, 1H), 0.86–0.94 (m, 2H), 0.82 (s, 3H), 0.69 (s, 3H); \(^{13}\)C NMR (75 MHz, CDCl\textsubscript{3}) \(\delta\) 61.9, 58.9, 54.5, 54.4, 41.0, 40.5, 39.3, 38.8, 37.6, 37.1, 35.4, 32.7, 32.2, 27.7, 25.6, 21.1, 20.6, 17.7, 12.8.

![Diagram](image)

\((2R,3S,5S,8R,9S,10S,13S,14S)-10,13–dimethylhexadecahydro–1H–cyclopenta[a]phenanthrene–2,3–diaminium chloride (2.89)\)

In a round bottom flask, LAH (0.16 g, 4.03 mmol) was suspended in THF (2 mL). The suspension was cooled at 0 °C using an ice/water bath. A solution of \textit{cis}–diazidocholestanate \textbf{2.93} (0.21 g, 0.61 mmol) in THF (3 mL) was added. The reaction mixture was warmed to rt and then heated to reflux for 4 h. It was then cooled to rt and diluted with THF (5 mL). The diluted reaction mixture was cooled at 0 °C using ice/water bath and was quenched using the Fieser method.\textsuperscript{85} The quenched reaction mixture was filtered through celite, dried over sodium sulfate, and concd under reduced pressure. The residue was dissolved in diethyl ether:CHCl\textsubscript{3} (20 mL, 1:1). The solution was heated and filtered to remove any undissolved amine. A solution of HCl in diethyl ether (1.04 mmol, 0.52 mL, 2.0 M in diethyl ether) was then added dropwise which resulted to formation of white precipitate. The precipitate was filtered, washed with diethyl ether, and dried to afford \textit{cis}–diamine salt \textbf{2.89} (0.17 g, 77\% over 2 steps).
2.89. m.p. = 261.4 °C (diethyl ether) (dec.); IR (KBr) 3430, 2931, 2861, 1602, 1094, 1030 cm⁻¹; ¹H NMR (300 MHz, CD₃OD) δ 3.85 (app d, J = 2.9 Hz, 1H), 3.70 (td, J = 13.4, 3.7 Hz, 1H), 1.87–1.99 (m, 2H), 1.70–1.80 (m, 3H), 1.65–1.68 (m, 2H), 1.55–1.62 (m, 3H), 1.40–1.50 (m, 3H), 1.30–1.37 (m, 3H), 1.11–1.27 (m, 4H), 0.98–1.09 (m, 2H), 0.95 (s, 3H), 0.74 (s, 3H); ¹³C NMR (75 MHz, CD₃OD) δ 55.7, 54.9, 50.8, 49.7, 42.1, 41.5, 39.9, 39.6, 38.5, 37.6, 36.7, 33.1, 32.1, 28.5, 26.6, 22.2, 21.5, 18.0, 12.8.

![Chemical structure](image)


Methanesulfonyl chloride (0.04 mL, 0.53 mmol) was added dropwise to a solution of azidoalcohol 2.79 (0.10 g, 0.31 mmol) dissolved in pyridine (1.00 mL). The reaction mixture was stirred continuously at rt. After approximately 24 h, water (5 mL) was added to quench the reaction. The quenched reaction mixture was extracted with DCM (20 mL). The organic layer was collected, washed with hydrochloric acid solution (20 mL, 3 M) followed by sat. sodium bicarbonate solution (20 mL) and water (20 mL). The organic layer was dried over sodium sulfate and concd under reduced pressure to obtain azidomesylate 2.94 (0.12 g, 100%).

2.94. m.p. = 150.3 °C (DCM) (dec.); IR (KBr): 2960, 2926, 2872, 2101, 1328, 1181 cm⁻¹; [α]D²⁰ = −39.3 (c 8.00, DCM); ¹H NMR (300 MHz, CDCl₃) δ 4.85 (bs, 1H), 3.09 (s, 3H), 3.43 (ddd, J = 12.8, 4.4, 3.0 Hz, 1H), 1.85 (dt, J = 12.5, 3.5 Hz, 2H), 1.63–1.78 (m, 3H), 1.55–1.62 (m, 4H), 1.37–1.52 (m, 3H), 1.21–1.36 (m, 3H), 1.07–1.17 (m, 4H), 0.85–1.02 (m, 3H), 0.83 (m, 3H), 0.66 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 80.1, 57.6, 54.5, 54.2, 40.9, 40.4, 38.8, 38.6, 38.5, 37.3, 36.9, 35.4, 34.0, 32.0, 27.4, 25.6, 21.1, 20.6, 17.7, 12.7.
(2R,3R,5S,8R,9S,10S,13S,14S)–2,3–diazido–10,13–dimethylhexadecahydro–1H–cyclopenta[a]phenanthrene (2.95)

In a round bottom flask, mesylate 2.94 (0.14 g, 0.35 mmol) and sodium azide (0.05 g, 0.70 mmol) were suspended in DMSO (2.50 mL). The suspension was stirred continuously and heated to 90 °C. After approximately 5 h, the reaction mixture was cooled to rt and was quenched by adding water (5 mL). The quenched reaction mixture was then extracted with diethyl ether (3 x 10 mL). The organic layers were collected, combined, washed with water (5 x 10 mL), dried over magnesium sulfate and coned under reduced pressure to afford trans–1,2–diazide 2.95 (0.11 g, 92%) as a light brown solid.

2.95. IR (KBr) 2927, 2864, 2845, 2102, 1451, 1257 cm⁻¹; [α]D²⁰.⁵ = −25.4 (c 11.50, DCM); ¹H NMR (300 MHz, CDCl₃) δ 3.35 (dt, J = 11.2, 4.7 Hz, 1H), 3.21 (dt, J = 11.5, 5.0 Hz, 1H), 2.06 (dd, J = 12.9, 4.6 Hz, 1H), 1.68–1.75 (m, 3H), 1.60–1.66 (m, 2H) 1.48–1.58 (m, 2H), 1.33–1.46 (m, 4H), 1.27 (dd, J = 12.4, 3.7 Hz, 2H), 1.19–1.22 (m, 1H), 1.13–1.18 (m, 2H), 1.07–1.11 (m, 1H), 0.97–1.06 (m, 1H), 0.86–0.94 (m, 2H), 0.84 (s, 3H), 0.72–0.77 (m, 1H), 0.68 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 65.2, 62.2, 54.5, 54.4, 44.7, 43.2, 40.9, 40.9, 40.5, 38.8, 37.0, 35.4, 32.2, 27.9, 25.6, 21.4, 20.6, 17.7, 13.1.

(2R,3R,5S,8R,9S,10S,13S,14S)–10,13–dimethylhexadecahydro–1H–cyclopenta[a]phenanthrene–2,3–diaminium chloride (2.97)

A solution of trans–diazidcholestan 2.94 (0.12 g, 0.35 mmol) in THF (3 mL) was added dropwise to a pre–cooled suspension of LAH (0.09 g, 2.31 mmol) in THF (3 mL). The reaction mixture was stirred continuously at 0 °C for 10 min and then was allowed to warm up to rt and heated to reflux. After approximately 4 h, the reaction mixture was
cooled to rt and diluted with THF (6 mL). The diluted reaction mixture was cooled at 0 °C using an ice/water bath and was quenched using the Fieser method. The quenched reaction mixture was filtered through celite, dried over sodium sulfate, and concd under reduced pressure. The residue was then dissolved in diethyl ether:CHCl₃ (20 mL, 1:1). The solution was heated to make sure all residue was dissolved and was filtered to remove any undissolved solids. A solution of hydrogen chloride in diethyl ether (0.70 mmol, 0.35 mL, 2.0 M in diethyl ether) was then added dropwise which resulted to formation of white precipitate. The precipitate was filtered, washed with diethyl ether, and dried to afford trans–diamine salt 2.97 (0.13 g, 100% over 2 steps).

2.97. IR (KBr) 3422, 2929, 1604, 1501, 1059, 1020 cm⁻¹; ¹H NMR (300 MHz, D₂O) δ 3.57 (dt, J = 11.2, 4.6 Hz, 1H), 3.41 (dt, J = 10.9, 5.3 Hz, 1H), 2.17 (dd, J = 12.6, 4.3 Hz, 1H), 1.81–1.86 (m, 1H), 1.55–1.72 (m, 6H), 1.43–1.51 (m, 2H), 1.23–1.39 (m, 7H), 1.06–1.18 (m, 3H), 0.93–1.01 (m, 2H), 0.88 (s, 3H), 0.78–0.82 (m, 1H), 0.68 (s, 3H); ¹³C NMR (75 MHz, D₂O) δ 54.0, 53.4, 52.6, 50.0, 43.6, 41.5, 40.4, 40.1, 38.4, 36.3, 35.0, 31.7, 31.6, 27.1, 25.2, 21.0, 20.2, 16.9, 11.9.

![Chemical structure](image)

1–((1R,2R,4aS,8aS)–2–hydroxy–2,5,5,8a–tetramethyldecahydronaphthalen–1–yl)propan–2–one (2.105)


In a round bottom flask, sclareolide 2.103 (1.00 g, 3.99 mmol) was dissolved in diethyl ether (30 mL). The solution was cooled to 0 °C. Methyl lithium solution in diethyl ether (4.39 mmol, 2.74 mL, 1.6 M in diethyl ether) was added dropwise. The reaction mixture was stirred continuously at 0 °C. After 5 min, a solution of sulfuric acid (8 mL, 10% aq.) was added dropwise to quench the reaction. The organic layer was collected and the aqueous layer was extracted with diethyl ether (2 x 10 mL). The organic layers were collected, combined, washed over sodium hydroxide solution (2 x 20 mL, 1% aq.)
followed by water (2 x 20 mL), dried over magnesium sulfate, and concd under reduced pressure. The residue was purified by flash column chromatography using 40% diethyl ether in hexane as eluent to afford ketone 2.105 (0.65 g, 61%) as colorless oil.

2.105. TLC Rf = 0.12 (hexane:diethyl ether, 3:2); IR (film) 3433, 2924, 1706, 1461, 1386, 1239 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 2.49 (dq, J = 17.6, 5.7 Hz, 2H), 2.20 (s, 3H), 1.90–1.96 (m, 2H), 1.65–1.71 (m, 1H), 1.53–1.60 (m, 2H), 1.46–1.51 (m, 1H), 1.42–1.44 (m, 1H), 1.38–1.39 (m, 1H), 1.33–1.35 (m, 1H), 1.24–1.29 (m, 1H), 1.13–1.21 (m, 1H), 1.11 (s, 3H), 1.02 (dd, J = 12.2, 2.4 Hz, 1H), 0.92 (dd, J = 13.1, 3.6 Hz, 1H), 0.87 (s, 3H), 0.79 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 210.0, 72.8, 55.7, 55.7, 44.3, 41.6, 39.4, 39.2, 38.1, 33.2, 33.0, 30.1, 23.0, 21.2, 20.4, 18.2, 15.5.

![Chemical Structure Image]

((1S,2R,4aS,8aS)–2–hydroxy–2,5,5,8a–tetramethyldecahydroacenaphthalen–1–yl)methyl acetate (2.106)


A hydrogen peroxide solution (11.4 mmol, 1.16 mL, 30% aq.) was added dropwise to a pre–cooled solution of acetic anhydride (15.5 mmol, 1.46 mL) in DCM (1.86 mL) in an ice bath. The solution was stirred continuously at 0 °C using an ice/water bath. After 1 h, maleic anhydride 2.128 (0.86 g, 8.75 mmol) was added in portions after which the solution was allowed to stir continuously at 0 °C for 1 h and then allowed to warm to rt. The reaction mixture was stirred continuously at rt for 1.5 h before adding a solution of 2.105 (0.29 g, 1.21 mmol) in DCM (7.11 mL) dropwise. The solution was stirred continuously for 23 h. The reaction mixture was then diluted with DCM (20 mL) and washed with water (2 x 10 mL), sat. sodium bicarbonate solution (2 x 20 mL), and sat. sodium chloride solution (2 x 20 mL), dried over sodium sulfate, and concd under
reduced pressure. The residue was purified by flash column chromatography using 25% ethyl acetate in hexane as eluent to afford 2.106 (0.21 g, 62%) as off–white solid.

2.106. m.p. = 76.1–79.2 °C (DCM) (Lit.:88 77–79 °C); TLC Rf = 0.23(hexane:ethyl acetate, 3:1); IR (film) 3494, 2965, 2852, 1706, 1263, 1047 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 4.23 (dd, J = 11.6, 4.2 Hz, 1H), 4.11 (dd, J = 12.1, 5.7 Hz, 1H), 2.39 (bs, 1H), 1.92 (s, 3H), 1.76 (dt, J = 12.5, 3.1 Hz, 1H), 1.44–1.58 (m, 3H), 1.31–1.38 (m, 3H), 1.20–1.29 (m, 2H), 1.07–1.16 (m, 1H), 1.05 (s, 3H), 0.91–0.99 (m, 1H), 0.81–0.88 (m, 1H), 0.76 (s, 3H), 0.74 (s, 3H), 0.68 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 171.5, 72.7, 62.7, 60.1, 55.8, 44.1, 41.8, 39.8, 38.2, 33.6, 33.3, 24.7, 21.7, 21.4, 20.4, 18.5, 15.9.

(1S,2R,4aS,8aS)–1–(hydroxymethyl)–2,5,5,8a–tetramethyldecahydronaphthalen–2–ol (2.107)


In a round bottom flask, acetate 2.106 (0.86 g, 3.05 mmol), and potassium hydroxide (1.69 g, 30.2 mmol) were dissolved in methanol (16.94 mL). The reaction mixture was stirred continuously at rt. After 1 h, water (20 mL) was added and the quenched reaction mixture was extracted with diethyl ether (3 x 20 mL). The organic layers were collected, combined, washed with sat. sodium chloride solution (3 x 20 mL), dried over sodium sulfate, and concd under reduced pressure to afford diol 2.107 (0.67 g, 92%) as white solid.

2.107. m.p. = 122.7–123.5 °C (diethyl ether) (Lit.88 119–120°C); TLC Rf = 0.30 (hexane:ethyl acetate, 3:2); IR (film) 3326, 3003, 2924, 2864, 1456, 1021 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 4.21 (bs, 2H), 3.87 (d, J = 8.0 Hz, 2H), 1.83 (dt, J = 12.3, 2.9 Hz, 1H), 1.56–1.71 (m, 2H), 1.48–1.53 (m, 3H), 1.32–1.45 (m, 2H), 1.29 (s, 3H), 1.22–1.23 (m, 1H), 1.01–1.14 (m, 2H), 0.93 (d, J = 11.9 Hz, 1H), 0.84 (s, 3H), 0.75 (s, 3H); ¹³C
NMR (75 MHz, CDCl₃) δ 75.0, 61.0, 60.3, 56.1, 44.2, 41.9, 40.2, 37.6, 33.7, 33.4, 24.4, 21.8, 20.3, 18.8, 16.2.

((1S,2R,4aS,8aS)–2–hydroxy–2,5,5,8a–tetramethyldecahydro-naphthalen–1–yl)methyl methanesulfonate (2.101)


In a round bottom flask, alcohol 2.107 (0.14 g, 0.58 mmol) was dissolved in DMF (1.18 mL). Triethylamine (0.21 mL, 1.51 mmol) was added dropwise and the reaction mixture was stirred continuously and cooled to 0 °C using ice/water bath. Methanesulfonyl chloride (0.05 mL, 0.70 mmol) was added dropwise to the cooled reaction mixture and the solution was stirred continuously as it warmed up to rt. After approximately 3 h, the reaction mixture was diluted with diethyl ether (20 mL) and was washed over hydrochloric acid solution (2 x 20 mL, 3 M in water) followed by sat. sodium bicarbonate (2 x 20 mL) and brine solution (2 x 20 mL), dried over magnesium sulfate, and concd under reduced pressure to afford mesylate 2.101 (0.16 g, 89%) as a light yellow oil.

2.101. IR (film) 3527, 2927, 2861, 1388, 11173, 943 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 4.56 (dd, J = 10.5, 2.7 Hz, 1H), 4.31 (dd, J = 10.5, 5.7 Hz, 1H), 3.01 (s, 3H), 1.85–1.92 (m, 2H), 1.65–1.73 (m, 2H), 1.57–1.62 (m, 1H), 1.46–1.53 (m, 2H), 1.32–1.42 (m, 2H), 1.16–1.29 (m, 2H), 1.14 (s, 3H), 1.08 (dd, J = 12.6, 4.0 Hz, 1H), 0.95 (dd, J = 12.0, 2.2 Hz, 1H), 0.87 (s, 3H), 0.86 (s, 3H), 0.79 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 72.6, 68.2, 60.8, 55.7, 44.5, 41.8, 39.7, 38.3, 37.4, 33.5, 33.3, 24.6, 21.6, 20.5, 18.4, 16.1.
**O–3,5–dimethoxyphenyl dimethylcarbamothioate (2.109)**


NaH (0.78 g, 19.4 mmol, 60% in mineral oil) was added in several portions to a solution of 3,5–dimethoxyphenol 2.104 in DMF (6.50 mL). The reaction mixture was cooled to 0 °C using ice/water bath before adding a solution of *N*,*N*–dimethylthiocarbomyl chloride 2.129 (2.20 g, 17.8 mmol) in DMF (3.27 mL). The solution was stirred continuously as it warmed up to rt followed by heating at 70 °C for 1 h. The reaction mixture was cooled to rt and was poured into potassium hydroxide solution (16 mL, 5% in water). The resulting precipitate was filtered, washed with water, dried, and dissolved in diethyl ether. The solution was washed over water (3 x 20 mL), dried over magnesium sulfate, and concd under reduced pressure. Purification of the residue with silica gel chromatography using 20% ethyl acetate in hexane afforded 2.109 (2.59 g, 66%) as an off–white/white solid.

2.109. m.p. = 76.7–79.0 °C (DCM) (Lit.89 76–77°C); TLC Rf = 0.42 (hexane:ethyl acetate, 4:1); IR (KBr) 3004, 2946, 1613, 1185, 1304, 1046 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 6.35 (t, *J* = 2.2 Hz, 1H), 6.24 (d, *J* =2.3 Hz, 2H), 3.76 (s, 6H), 3.44 (s, 3H), 3.31 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 187.5, 161.0, 155.6, 101.6, 98.5, 55.6, 43.4, 38.9.
\( \text{S–3,5–dimethoxyphenyl dimethylcarbamothioate (2.109)} \)


*O–Carbamate 2.109* (0.62 g, 2.56 mmol) was placed in a round bottom flask. The flask was then purged with argon and heated to \( \sim 300 \, ^\circ\text{C} \) neat. After 4 h, the resulting molten product was cooled to rt and purified using flash column chromatography with 20% ethyl acetate in hexane as eluent to afford 2.110 (0.46 g, 74%) as light yellow oil.

2.110. TLC \( R_f = 0.29 \) (hexane:ethyl acetate, 4:1); IR (film) 2937, 2835, 1668, 1592, 1204, 1155 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 6.64 (d, \( J = 2.3 \, \text{Hz}, 2\text{H} \)), 6.45 (t, \( J = 2.3 \, \text{Hz}, 1\text{H} \)), 3.74 (s, 6H), 3.00 (bs, 6H); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 166.6, 160.5, 130.0, 113.4, 102.0, 55.4, 36.8.

\( \text{3,5–dimethoxybenzenethiol (2.102)} \)


In a round bottom flask, \( \text{S–carbamothioate 2.110} \) (0.46 g, 1.91 mmol) and potassium hydroxide (0.63 g, 11.2 mmol) were dissolved in methanol (21.13 mL). The resulting light yellow solution was stirred continuously and heated to reflux. After 3 h, the reaction mixture was cooled to rt and concd under reduced pressure. The residue was dissolved in ethyl acetate (20 mL), washed with hydrochloric acid solution (2 x 10 mL, 3 M in water), dried over sodium sulfate, and concd under reduced pressure. The residue was purified
through flash column chromatography using 10% ethyl acetate in hexane as eluent to afford benzenethiol 2.102 (0.19 g, 58%) as colorless oil.

2.102. TLC \( R_f = 0.57 \) (hexane:ethyl acetate, 4:1); IR (film) 3001, 2938, 2564, 1587, 1299, 1156 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta 6.42 \) (d, \( J = 2.3 \) Hz, 2H), 6.26 (t, \( J = 2.2 \) Hz, 1H), 3.74 (s, 6H), 3.51 (bs, 1H); \(^1^3\)C NMR (75 MHz, CDCl\(_3\)) \( \delta 160.9, 132.7, 107.1, 98.1, 55.2.

![Chemical structure of 2.101 and 2.102](image)

\((1R,2R,4aS,8aS)\)-1-((3,5-dimethoxyphenylthio)methyl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (2.100)


In a round bottom flask, mesylate 2.101 (0.62 g, 1.95 mmol), benzenethiol 2.102 (0.33 g, 1.95 mmol), and cesium carbonate (1.90 g, 5.83 mmol) were suspended in acetonitrile (27.86 mL). The reaction mixture was degassed with argon for about 10 min and was stirred continuously at rt. After approximately 1 h, the reaction mixture was heated at reflux for about 24 h. The reaction mixture was cooled to rt and was concd under reduced pressure. The residue was purified through flash column chromatography using 10–15–20% ethyl acetate in hexane as eluent to afford sulfide 2.100 (0.28 g, 37%) as yellow oil.

2.100. TLC \( R_f = 0.31 \) (hexane:ethyl acetate, 4:1); IR (film) 3430, 2935, 1587, 1455, 1204, 1155 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta 6.50 \) (d, \( J = 2.2 \) Hz, 2H), 6.26 (t, \( J = 2.2 \) Hz, 1H), 3.76 (s, 3H), 3.09 (dd, \( J = 12.4, 5.5 \) Hz, 1H), 2.95 (dd, \( J = 12.3, 4.5 \) Hz, 1H), 2.07 (bs, 1H), 1.88 (dt, \( J = 12.4, 3.2 \) Hz, 1H), 1.74 (app d, \( J = 12.7 \) Hz, 1H), 1.54–1.67 (m, 2H), 1.42–1.52 (m, 3H), 1.22–1.41 (m, 2H), 1.20 (s, 3H), 1.11 (dd, \( J = 12.9, 4.2 \) Hz, 1H), 1.03 (dd, \( J = 12.7, 3.8 \) Hz, 1H), 0.93 (dd, \( J = 12.1, 2.1 \) Hz, 1H), 0.86 (s, 3H), 0.84 (s, 3H), 0.61 (s, 18H).

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0.78 (s, 3H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) \(\delta\) 161.0, 140.0, 106.5, 98.3, 73.9, 61.0, 56.0, 55.5, 44.2, 41.8, 40.2, 39.2, 33.5, 33.4, 29.4, 24.2, 21.7, 20.4, 18.6, 15.6.

\[
\begin{align*}
\text{H}_3\text{CO} & \quad \text{OCH}_3 \\
\text{S} & \quad \text{OH} \\
\text{H} & \quad \text{SnCl}_4 \text{CH}_2\text{CH}_2 \\
\text{2.100} & \quad \text{-78 °C to 0 °C to rt, 4 h} \\
\text{H} & \quad \text{70%} \\
\text{2.111} & \quad \text{H} \quad \text{OCH}_3
\end{align*}
\]

\(\text{(4aS,4bR,10bR,12aS)}\text{–8,10–dimethoxy–1,1,4a,10b–tetramethyl–2,3,4a,4b,5,10b,11,12,12a–decahydro–1}\text{H–naphtho[1,2–c]}\text{thiochromene (2.111)}\)


In a round bottom flask, sulfide \textbf{2.100} (0.28 g, 0.72 mmol) was dissolved in DCM (14.4 mL). The solution was cooled at −78 °C using dry ice/acetone bath. Tin (IV) chloride (2.88 mmol, 0.34 mL) was added dropwise to the reaction mixture and the solution was stirred continuously at −78 °C. After approximately 1 h, the reaction mixture was transferred to an ice/water bath and was stirred for another 1 h before being allowed to warm up to rt. After 2 h, water (5 mL) was added to quench the reaction. The organic layer was collected, dried over sodium sulfate, and concd under reduced pressure. Purification of the residue with flash column chromatography using 10% ethyl acetate in hexane as eluent afforded \textbf{2.111} (0.19 g, 70 %) as light yellow foam.

\textbf{2.111}. TLC \textit{R}y= 0.77 (hexane:ethyl acetate, 4:1); IR (film) 2930, 2861, 1596, 1457, 1288, 1155 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta\) 6.20 (d, \(J = 2.6\) Hz, 1H), 6.17 (d, \(J = 2.6\) Hz, 1H), 3.75 (s, 3H), 3.73 (s, 3H), 3.28 (dt, \(J = 13.2, 3.4\) Hz, 1H), 2.89 (dd, \(J = 12.6, 10.9\) Hz, 1H), 2.74 (app d, \(J = 11.6\) Hz, 1H), 1.83 (dt, \(J = 12.3, 3.5\) Hz, 1H), 1.71 (app d, \(J = 11.0, 1.3\) Hz, 1H), 1.56–1.66 (m, 2H), 1.46–1.52 (m, 1H), 1.35–1.44 (m, 2H), 1.33 (s, 3H), 1.18–1.27 (m, 1H), 1.07–1.15 (m, 1H), 0.97–1.04 (m, 1H), 0.92 (s, 3H), 0.89 (d, \(J = 2.6\) Hz, 1H), 0.86 (s, 3H), 0.84 (s, 3H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) \(\delta\) 160.6, 156.0, 134.2, 127.6, 102.0, 97.1, 58.7, 56.1, 55.4, 55.2, 41.9, 40.2, 39.5, 39.0, 38.4, 33.4, 21.6, 21.5, 21.2, 19.3, 18.8, 17.1.
(4aR,4bR,10bR,12aS)--8,10--dimethoxy--1,1,4a,10b,12a--pentamethyl--2,3,4,4a,4b,5,10b,11,12,12a--decahydro--1H--naphtho[1,2--c]thiochromene 6,6--dioxide (2.98)


Thiochroman 2.111 (0.19 g, 0.51 mmol) was dissolved in DCM (10.2 mL). m--Chloroperoxybenzoic acid (0.25 g, 1.02 mmol, 70--75%) was added to the solution and the reaction mixture was stirred continuously at rt. After 2 h, the solution was diluted with DCM (20 mL), washed with sat. sodium bicarbonate solution (3 x 20 mL), dried over sodium sulfate, and concd under reduced pressure. The residue was recrystallized using methanol/DCM (1:1) to afford 2.98 (0.23 g, quantitative) as white long needles.

2.98. TLC Rf = 0.32 (hexane:ethyl acetate, 4:1); IR (film) 2992, 2937, 2838, 1292, 1186, 1049 cm^-1; ^1H NMR (300 MHz, CDCl3) δ 6.93 (d, J = 2.5 Hz, 1H), 6.53 (d, J = 2.9 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 3H), 3.13--3.33 (m, 3H), 2.25 (d, J = 12.0 Hz, 1H), 1.57--1.70 (m, 3H), 1.39--1.52 (m, 3H), 1.35 (s, 3H), 1.23--1.29 (m, 1H), 0.96--1.04 (m, 1H), 0.92 (s, 3H), 0.83 (s, 3H), 0.80 (s, 3H); ^13C NMR (75 MHz, CDCl3) δ 159.4, 158.9, 139.6, 128.2, 104.5, 98.1, 55.8, 55.8, 55.7, 53.0, 45.7, 41.7, 41.5, 39.3, 38.5, 37.6, 33.3, 33.0, 21.2, 18.7, 18.6, 18.4, 17.3.

References


66.) Djerassi, C.; Scholz, C. R. “Brominations with pyridine hydrobromide perbromide, $\text{C}_3\text{H}_5\text{N}.\text{HBr}.\text{Br}_2$” J. Am. Chem. Soc. 1948, 70, 417–418.


CHAPTER 3

SYNTHESIS OF SHIP2 INHIBITORS AS1949490, AS1938909 AND SOME STRUCTURAL ANALOGS

Introduction

Phosphatidylinositol 3’–kinase (PI3K) is a critical component of signaling for many growth factors and hormones.¹ Several components of this pathway have been targeted for drug discovery since deregulation of this pathway results in the development of cancer and diabetes.²,³

PI3K generates phosphoinositides specifically phosphotidylinositol–3,4,5–trisphosphate (PIP3), which are second messengers that are involved in regulating cell functions.⁴ SH2–containing inositol phosphatase (SHIP) helps regulate these phosphoinositide pools⁵ by hydrolyzing phosphatidylinositol (3,4,5)–trisphosphate (PI–(3,4,5)–P3) into phosphatidylinositol (3,4)–bisphosphate (PI–(3,4)–P2). There are three isoforms of SHIP: SHIP1, sSHIP, and SHIP2. SHIP1 is expressed only in blood and bone marrow cells while sSHIP is primarily expressed in stem cells. SHIP2, on the other hand, is expressed ubiquitously throughout the body.

A number of studies showed that SHIP2 has a possible role in a breast cancer–relevant pathway. SHIP2 has an atypical effect on epidermal growth factor (EGF) induced signaling in various breast cancer cell lines. Overexpression of this enzyme causes an increase in EGF–induced signaling by elevating the level of the EGF receptor (EGFR). Multiple breast cancer cell lines displayed significantly higher levels of EGFR. In addition, SHIP2 was shown to also be overexpressed in these multiple cancer cell lines. Clinical specimens of breast cancer showed a similar overexpression of both EGFR
and SHIP2. Additionally, gene array and RNA interference studies have suggested SHIP2 is required for growth and survival of breast cancer cells.\textsuperscript{6–9} Furthermore, suppression of SHIP2 with EGFR inhibitors showed 60\% increase in apoptosis.\textsuperscript{6}

In 2009, AS1949490 \textbf{3.1} was identified as a potent, small molecule SHIP2 inhibitor through a high-throughput screening (HTS). This molecule showed selective potency against human (IC\textsubscript{50} = 0.62 \(\mu\)M) and mouse (IC\textsubscript{50} = 0.34 \(\mu\)M) SHIP2. In addition, kinetic inhibition studies showed that \textbf{3.1} was a competitive inhibitor (K\textsubscript{i} \approx 0.44 mM for human SHIP2), meaning it shared a binding site with the inositol substrate.\textsuperscript{10}

![Chemical structure of AS1949490 3.1](image)

**Figure 3–1.** AS1949490 \textbf{3.1}.

<table>
<thead>
<tr>
<th>Polyphosphatases</th>
<th>IC\textsubscript{50} ((\mu)M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human SHIP2</td>
<td>0.62 ± 0.02</td>
</tr>
<tr>
<td>Human SHIP1</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>Mouse SHIP2</td>
<td>0.34 ± 0.10</td>
</tr>
<tr>
<td>Human PTEN</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Human synaptojanin</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Human myotubularin</td>
<td>&gt; 50</td>
</tr>
</tbody>
</table>

**Table 3–1.** \textit{In–vitro} affinity of \textbf{3.1} Towards Several Polyphosphatases.\textsuperscript{10}

Recently, AS1938909 \textbf{3.2}, an analog of \textbf{3.1}, was also identified as SHIP2 inhibitor. This molecule exhibited inhibitory activity against human and mouse SHIP2 with IC\textsubscript{50} values of 0.57 and 0.18 \(\mu\)M, respectively. The inhibitory activity of \textbf{3.2} against human SHIP1 was weaker than AS1949490. In addition, it showed no inhibitory activity
against PTEN, synaptojanin, or myotublarin (Table 3–2) indicating that 3.2 is a selective and potent SHIP2 inhibitor.\textsuperscript{11}

![Figure 3–2. AS1938909 3.2.](image)

<table>
<thead>
<tr>
<th>Polyphosphatases</th>
<th>IC(_{50}) ((\mu)M)</th>
<th>(K_i) ((\mu)M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human SHIP2</td>
<td>0.57 ± 0.11</td>
<td>0.44 ± 0.07</td>
</tr>
<tr>
<td>Human SHIP1</td>
<td>21 ± 2</td>
<td>12 ± 4.4</td>
</tr>
<tr>
<td>Mouse SHIP2</td>
<td>0.18 ± 0.02</td>
<td>Not Determined</td>
</tr>
<tr>
<td>Human PTEN</td>
<td>&gt; 50</td>
<td>Not Determined</td>
</tr>
<tr>
<td>Human synaptojanin</td>
<td>&gt; 50</td>
<td>Not Determined</td>
</tr>
<tr>
<td>Human myotubularin</td>
<td>&gt; 50</td>
<td>Not Determined</td>
</tr>
</tbody>
</table>

Table 3–2. \textit{In Vitro} Affinity of 3.2 Towards SHIP2, SHIP1, PTEN, synaptojanin, and myotubularin.\textsuperscript{11}

Objectives

The primary objective of this work was to synthetically prepare AS1949490 3.1 and AS1938909 3.2 and evaluate their biological activity. We were interested on the SHIP1 activity of these molecules thus these molecules were tested for its SHIP1 activity. In addition, the SHIP inhibitory activity of these compounds were compared to the pan SHIP1/SHIP2 inhibitors that we discovered.\textsuperscript{12} Also, these molecules will be tested for their cytotoxicity on breast cancer cell lines to verify the role of SHIP2 inhibition on the growth of breast cancer cells. Lastly, analogs of 3.1 and 3.2 were synthesized and was
tested for their SHIP inhibitory activity to determine some preliminary structure activity relationships.

Results and Discussion

The literature synthetic route for the synthesis of AS1949490 3.1 is shown in Scheme 3–1. The synthesis commenced from the commercially available methyl thioglycolate 3.3 and methyl 2,3–dichloropropionate 3.4. Cyclization of 3.3 and 3.4 using sodium bicarbonate in methanol afforded thiophene 3.5. Methyl ester (3.5) has been reported to be hydrolyzed to the acid 3.6 using lithium hydroxide in water. However, attempts to reproduce this reaction were not successful, and only resulted in the recovery or decomposition of the starting material.

Scheme 3–1 First Generation Synthesis of AS1949490 3.1

Reagents and conditions: (a) sodium bicarbonate, MeOH, overnight, reflux, 39% (b) i. LiOH, THF, MeOH, water, rt, 22 h ii. 55 °C, 5 h (c) (S)–(−)–α–methylbenzylamine, EDC, DCM, rt, overnight (d) 4–chlorobenzylechloride, K₂CO₃, DMF, 80 °C, 18 h.

To circumvent issues with the ester hydrolysis, the alcohol (3.5) was protected using benzyl bromide (BnBr). Hydrolysis of methyl ester (3.8) gave acid 3.9 in good yield. The acid was then coupled with (S)–(−)–α–methylbenzylamine using 1–ethyl–3–
(3–dimethylaminopropyl) carbodiimide (EDC). Deprotection of the benzyl ether (3.10) followed alkylation provided the desired AS1949490 3.1. (Scheme 3–2)

**SCHEME 3–2** Second Generation Synthesis of AS1949490 3.2

\[
\text{3.5} \xrightarrow{a} \text{3.8} \xrightarrow{b} \text{3.9} \xrightarrow{c} \text{3.1} \xrightarrow{d} \text{3.10} \xrightarrow{e} \text{3.11}
\]

\( ^a \text{Reagents and conditions: } (a) \text{BnBr, } K_2CO_3, \text{ DMF, } 80 \, ^\circ\text{C, } 26 \, h, \text{92\% } (b) \text{NaOH, THF, MeOH, water, } 50 \, ^\circ\text{C, } 20 \, h, \text{81\% } (c) \text{(S)-(--)–}\alpha–\text{methylbenzylamine, EDCI, DCM, rt, overnight, 83\% } (d) \text{BBr}_3, \text{ DCM, } 0 \, ^\circ\text{C, } 3 \, h, \text{76\% } (e) \text{4–chlorobenzyl chloride, } K_2CO_3, \text{ DMF, } 80 \, ^\circ\text{C, } 18 \, h, \text{78\%.}
\]

In addition, halo– and methoxybenzyl halides were used to alkylate the thiophen–3–ol (3.11) as shown in **Table 3–3** below. These molecules were tested for their inhibitory activity against SHIP2 and were compared with AS1949490. These molecules will help determine the effect of the changes in the substitution as well as the position of these substituents in the benzyl ether moiety towards SHIP2 inhibitory activity.
<table>
<thead>
<tr>
<th>Entry</th>
<th>G</th>
<th>X</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
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<tr>
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<td>2–Br</td>
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<td><img src="image" alt="3.14" /></td>
<td>88%</td>
</tr>
<tr>
<td>2</td>
<td>4–Br</td>
<td>Br</td>
<td><img src="image" alt="3.15" /></td>
<td>88%</td>
</tr>
<tr>
<td>3</td>
<td>2–F</td>
<td>Br</td>
<td><img src="image" alt="3.16" /></td>
<td>64%</td>
</tr>
<tr>
<td>4</td>
<td>4–F</td>
<td>Br</td>
<td><img src="image" alt="3.17" /></td>
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<tr>
<td>5</td>
<td>2–Cl</td>
<td>Cl</td>
<td><img src="image" alt="3.18" /></td>
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</tr>
<tr>
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<tr>
<td>#</td>
<td>Substituent</td>
<td>Functional Group</td>
<td>Yield</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-------------</td>
<td>-----------------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2,4–Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Cl</td>
<td>80%</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4–OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Cl</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>H</td>
<td>Br</td>
<td>92%</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3–3.** Synthesized AS1949490 3.1 Analogs.

The synthesis of 3.2 was performed in an analogous manner to AS1949490 3.1 as shown in **Scheme 3–3**. Coupling of acid 3.9 with 2,6–difluorobenzyl amine using EDC followed by deprotection of the benzyl ether using BBr<sub>3</sub> then alkylation of thiophen–3–ol (3.23) with 2,4–dichlorobenzyl chloride afforded the desired thiophene AS1938909 3.2.
**SCHEME 3–3** Synthesis of AS1938909 3.2

\[
\begin{align*}
3.5 & \xrightarrow{a} 3.8 \xrightarrow{b} 3.9 \xrightarrow{c} 3.2 \xrightarrow{e} 3.23 \xrightarrow{d} 3.22
\end{align*}
\]

\[\text{a} \text{Reagents and conditions: (a) BnBr, K}_2\text{CO}_3, \text{DMF, 80 °C, 26 h, 98% (b) NaOH, THF, MeOH, water, 50 °C, 20 h, 8% (c) 2,6--difluorobenzylamine, EDC, DCM, rt, overnight, 83% (d) BBr}_3, \text{DCM, 0 °C, 3 h, 63% (e) 4--chlorobenzyl chloride, K}_2\text{CO}_3, \text{DMF, 80 °C, 18 h, 80%}}
\]

In addition to the synthesis of AS1938909, analogs were also synthesized and included 3.25–3.22.

<table>
<thead>
<tr>
<th>Entry</th>
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<th>Yield</th>
</tr>
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<tbody>
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<td>-------</td>
<td>----------------</td>
<td>----------------</td>
<td>-------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4-Br</td>
<td>Br</td>
<td>65%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2-F</td>
<td>Br</td>
<td>76%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4-F</td>
<td>Br</td>
<td>79%</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
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<td>6</td>
<td>3-Cl</td>
<td>Cl</td>
<td>83%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4-Cl</td>
<td>Cl</td>
<td>83%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4-OCH₃</td>
<td>Cl</td>
<td>83%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3–4.** Synthesized AS1938909 3.22 Analogs.
The SHIP inhibitory activity of these molecules is summarized in Table 3–5. Compounds 3.1, 3.2, and its analogs, however, did not show any inhibitory activity towards SHIP1 which was determined using malachite green assay. In the malachite green assay, comparison of the SHIP2 inhibitory activity of the parent molecules 3.1 and 3.2 showed both molecules have comparable activity, but this activity was significantly lower than was reported in the literature.\textsuperscript{10–11} Analogs of 3.1 showed increased inhibitory activity particularly analogs with bromide substituents (3.14 and 3.15) as well as methoxy analog (3.21). These molecules showed more than twice inhibition exhibited by 3.1. Alternatively, analogs of 3.2 did not show any improved (3.25 and 3.29) or any inhibition (3.22, 3.27–3.28, 3.29) towards SHIP2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>%Inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textbf{SHIP1}</td>
</tr>
<tr>
<td><img src="image" alt="Compound 3.1" /></td>
<td>No Inhibition</td>
</tr>
<tr>
<td><img src="image" alt="Compound 3.14" /></td>
<td>No Inhibition</td>
</tr>
<tr>
<td><img src="image" alt="Compound 3.15" /></td>
<td>No Inhibition</td>
</tr>
</tbody>
</table>
No Inhibition  44.2%
No Inhibition  No Inhibition
No Inhibition  29.6%
No Inhibition  25.2%
No Inhibition  27.9%
No Inhibition  50.7%
No Inhibition  31.0%
No Inhibition 20.7%

No Inhibition  No Inhibition

No Inhibition 10.9%

Not Determined**  Not Determined**

No Inhibition  No Inhibition

No Inhibition  No Inhibition

No Inhibition 16.7%
Table 3–5. SHIP1 and SHIP2 %Inhibition of AS1949490 **3.1**, AS1938909 **3.2**, and Analogs.\(^\dagger\)

**Conclusion and Future Work**

SHIP2 has been shown to have possible role in a breast cancer–relevant cell signaling pathway. Recently it was demonstrated that SHIP2 is overexpressed in many cancer cell lines as well as clinical specimens of breast cancer. The overexpression of SHIP2 in breast cancer appears to supports cell proliferation while suspension of SHIP2 retards cancer growth. The molecules AS1949490 **3.1** and AS1938909 **3.2**, identified as SHIP2 inhibitors, were synthesized. In addition, syntheses of analogs of these molecules with varying substituents on the O–benzyl substituent were pursued. Evaluation of these molecules using malachite green assay showed that parent molecules and their analogs

\(^\dagger\) The %SHIP1 and %SHIP2 inhibition of the molecules were determined through malachite green assay conducted by Amanda Balch from SUNY Upstate Medical University.
did not exhibit any SHIP1 inhibitory activity. On the other hand, evaluation of SHIP2 inhibitory activity of the parent molecules showed comparable activity of the two molecules, however this potency was lower than was reported in the literature. Only the analogs of 3.1 showed better potency against SHIP2 some of which exhibited more than double the potency of 3.1. The analogs of 3.2 showed less potency or no inhibitory activity against SHIP2. In the future, biological testing against breast cancer cells \textit{in vitro} will be conducted with the most active to molecules to determine if they will be effective in slowing the growth of these tumor cells.

**Experimental Section**

**General Experimental Procedure**

**Reactions:** Except as otherwise noted, reactions were carried out under argon atmosphere in an oven– or flame–dried glassware. Flasks were flame dried under a stream of nitrogen or argon or under vacuum. Dimethylformamide (DMF) was distilled from molecular sieves. Reaction solvents were dried by a solvent purification system (benzene, dichloromethane (DCM), tetrahydrofuran (THF)) using alumina as the drying agent. All other reagents were reagent grade and purified as necessary.

**Methods:** Analytical thin layer chromatography (TLC) was performed using 250 μm commercial silica gel plates (EMD Chemicals, silica gel 60 F_{254}) and visualized by UV illumination or by exposure to p–anisaldehyde solution followed by heating using a heat gun. Flash chromatography was carried using commercially available 40–60 μm silica gel (Silicycle).

**Identity:** Melting points were recorded using an EZ–Melt Automated Melting Point Apparatus in open capillaries and are uncorrected. Infrared spectra were obtained using Thermo Nicolet IR200 and IR100 spectrometers. The specific rotations were determined at the sodium line (589 nm) and recorded on a Rudolph Autopol III using DCM as solvent. Proton, carbon, and fluorine nuclear magnetic resonance (^{1}H, ^{13}C, and ^{19}F NMR)
were recorded using Bruker DPX–300 spectrometer. Chemical shifts are reported in delta (δ) units, downfield from tetramethylsilane (TMS) with reference to residual solvents CDCl$_3$ ($^1$H: 7.26; $^{13}$C: 77.23) and CD$_3$OD ($^1$H: 4.78; $^{13}$C: 49.15). Coupling constants are reported in Hertz (Hz).

**Experimental**

![Chemical Reaction](image)

**Methyl 3–(benzyloxy)thiophene–2–carboxylate (3.8)**

In a round bottom flask, thiophen–3–ol 3.5 (1.87 g, 11.8 mmol), benzyl bromide 3.33 (1.69 mL, 14.2 mmol), and potassium carbonate (2.45 g, 17.7 mmol) were suspended in DMF (59 mL). The reaction mixture was stirred continuously and heated to 80 °C. After approximately 22 h, the reaction mixture was cooled at room temperature before quenching the reaction by adding water (100 mL). The quenched reaction mixture was extracted with ethyl acetate (3 x 100 mL). The organic layers were collected, combined, washed over sat. sodium chloride solution (3 x 100 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified through flash column chromatography using 10–15% ethyl acetate in hexane as eluent to afford benzyl ether 3.8 (2.86 g, 98%) as an off white solid.

**3.8.** m.p. = 65.0–67.3 °C (DCM); TLC R$_f$ = 0.56 (hexane:ethyl acetate, 4:1); IR (KBr) 3404, 3107, 3032, 1645, 1233, 1067 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.45 (d, J = 7.4 Hz, 2H), 7.26–7.38 (m, 3H), 6.79 (d, J = 5.5 Hz, 1H), 5.21 (s, 2H), 3.83 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 162.1, 160.9, 136.5, 130.6, 128.6, 128.0, 117.6, 110.4, 73.2, 51.6.
3–(benzyloxy)thiophene–2–carboxylic acid (3.9)

A solution of sodium hydroxide (5.75 mL, 34.5 mmol, 6.0 M in water) was added to the solution of methyl ester 3.8 (2.86 g, 11.5 mmol) in THF (14.40 mL) and methanol (28.75 mL). The reaction mixture was stirred continuously and heated to 50 °C. After approximately 22 h, the reaction mixture was cooled to room temperature and diluted by adding ethyl acetate (100 mL). The reaction was quenched by adding hydrochloric acid solution (15 mL, 1 M). The organic layer was washed with brine solution (20 mL), dried over sodium sulfate, and concentrated under reduced pressure. Purification with flash column chromatography using 50–60% ethyl acetate in hexane as eluent afforded acid 3.9 (2.39 g, 89%) as an off–white solid.

3.9. m.p. = 115.3–121.8 °C (DCM); TLC Rf = 0.30 (hexane:ethyl acetate, 3:2); IR (KBr) 3387, 3032, 2948, 2881, 1668, 1067 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.50 (d, J = 5.5 Hz, 1H), 7.40–7.46 (m, 4H), 7.24–7.39 (m, 2H), 6.88 (d, J = 5.5 Hz, 1H) 5.29 (s, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 160.2, 135.4, 132.5, 129.0, 128.7, 128.7, 127.4, 116.9, 111.5, 74.1.

(S)–3–(4–chlorobenzyloxy)–N–(1–phenylethyl)thiophene–2–carboxamide (3.1)

In a round bottom flask, thiophen–3–ol 3.11 (0.10 g, 0.40 mmol), 4–chlorobenzyl chloride 3.34 (0.07 g, 0.48 mmol), and potassium carbonate (0.08 g, 0.60 mmol) were suspended in DMF (2 mL). The reaction mixture was stirred continuously and heated to 80 °C. After approximately 18 h, water (10 mL) was added. The quenched reaction
mixture was extracted with ethyl acetate (3 x 10 mL). The organic layers were collected, combined, washed with sat. aq. sodium chloride solution (3 x 10 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified using flash column chromatography with 20% ethyl acetate in hexane as eluent to afford 3.1 (0.14 g, 78%) as off–white solid.

3.1. m.p. = 92.4–93.8 °C (DCM); TLC Rf = 0.22 (hexane:ethyl acetate, 4:1); IR (KBr) 3410, 3104, 2965, 1637, 1230, 1071 cm⁻¹; [α]D²³⁵ = +72.7 (c 2.33, DCM); ¹H NMR (300 MHz, CDCl₃) δ 7.37 (d, J = 7.6 Hz, 1H), 7.22–7.28 (m, 3H), 7.15–7.18 (m, 4H), 7.07–7.12 (m, 2H), 6.79 (d, J = 5.5 Hz, 1H), 5.13 (quint, J = 7.2 Hz, 1H), 5.01 (dd, J = 13.8, 11.0 Hz, 2H), 1.37 (d, J = 6.8 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 160.9, 155.0, 143.4, 134.8, 134.0, 129.4, 129.2, 129.1, 128.7, 127.3, 126.1, 118.0, 116.2, 73.2, 48.9, 22.5.

**General Procedure for 3.10 and 3.14–3.21**

In a round bottom flask, thiophenol 3.11 (1 equiv), benzyl halide 3.12 (1.2 equiv), and potassium carbonate (1.5 equiv) were suspended in DMF (5 mL per 1 mmol). The reaction mixture was stirred continuously and heated to 80 °C. After approximately 18 h, water was added. The quenched reaction mixture was extracted with ethyl acetate. The organic layers were collected, combined, washed over sat. sodium chloride solution (3 x 10 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified using flash column chromatography with 20% ethyl acetate in hexane as eluent.
(S)-3-(benzyloxy)-N-(1-phenylethyl)thiophene–2–carboxamide (3.10)

3.10. TLC Rf = 0.45 (hexane:ethyl acetate, 4:1); IR (film) 3390, 3030, 2970, 1642, 1231, 1059 cm⁻¹; [α]D²⁰.⁹ = +76.3 (c 2.00, DCM); ¹H NMR (300 MHz, CDCl₃) δ 7.47 (d, J = 7.7 Hz, 1H), 7.24–7.32 (m, 5H), 7.11–7.18 (m, 3H), 7.06–7.10 (m, 2H), 6.81 (d, J = 5.5 Hz, 1H), 5.14 (quint, J = 7.4 Hz, 1H), 5.06 (dd, J = 11.4, 9.2 Hz, 2H), 1.33 (d, J = 6.9 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 161.0, 155.4, 143.7, 135.6, 129.0, 129.0, 128.1, 128.8, 127.2, 126.2, 118.0, 116.4, 74.1, 48.9, 22.7.

(S)-3-(2-bromobenzylxyloxy)-N-(1-phenylethyl)thiophene–2–carboxamide (3.14)

3.14. TLC Rf = 0.28 (hexane:ethyl acetate, 4:1); IR (film) 3390, 3107, 2970, 1640, 1060, 762 cm⁻¹; [α]D²⁰.⁶ = +58.9 (c 2.66, DCM); ¹H NMR (300 MHz, CDCl₃) δ 7.53 (dd, J = 10.1, 7.5 Hz, 1H), 7.43 (d, J = 7.6 Hz, 1H), 7.27–7.30 (m, 2H), 7.17–7.24 (m, 2H), 7.12–7.15 (m, 4H), 6.83 (d, J = 5.5 Hz, 1H), 5.20–5.29 (m, 3H), 1.39 (d, J = 6.9 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 160.9, 154.9, 143.6, 134.9, 133.3, 130.6, 130.1, 129.1, 128.7, 128.0, 127.3, 126.2, 123.6, 118.2, 116.3, 73.5, 49.0, 22.6.
**(S)-3-(4-bromobenzyloxy)-N-(1-phenylethyl)thiophene-2-carboxamide (3.15)**

3.15. m.p. = 84.9–86.4 °C (DCM); TLC R_f = 0.38 (hexane:ethyl acetate, 4:1); IR (KBr) 3409, 3105, 2868, 1634, 1071, 650 cm⁻¹; [α]_D¹⁰⁰ = +66.5 (c 2.33, DCM); ^1^H NMR (300 MHz, CDCl₃) δ 7.38 (d, J = 8.4 Hz, 2H), 7.34 (bs, 1H), 7.14–7.18 (m, 3H), 7.07–7.11 (m, 4H), 7.27 (d, J = 5.5 Hz, 1H), 6.78 (d, J = 5.5 Hz, 1H), 5.14 (quint, J = 7.2 Hz, 1H), 4.99 (dd, J = 13.8, 11.1 Hz, 2H), 1.37 (d, J = 6.8 Hz, 3H); ^1^C NMR (75 MHz, CDCl₃) δ 160.9, 155.0, 143.4, 134.5, 132.2, 129.7, 129.1, 128.8, 127.4, 126.2, 123.0, 118.1, 116.2, 73.3, 49.0, 22.5.

**(S)-3-(2-fluorobenzyloxy)-N-(1-phenylethyl)thiophene-2-carboxamide (3.16)**

3.16. TLC R_f = 0.28 (hexane:ethyl acetate, 4:1); IR (film) 3410, 3103, 2929, 1639, 1230, 1071 cm⁻¹; [α]_D²¹⁴ = +62.3 (c 1.33, DCM); ^1^H NMR (300 MHz, CDCl₃) δ 7.39 (d, J = 7.5 Hz, 1H), 7.28 (d, J = 5.5 Hz, 1H), 7.20–7.25 (m, 2H), 7.13–7.17 (m, 2H), 7.07–7.12 (m, 2H), 6.81 (d, J = 5.5 Hz, 1H), 5.13 (quint, J = 6.9 Hz, 1H), 5.02 (dd, J = 13.1, 10.8 Hz, 2H), 1.35 (d, J = 6.8 Hz, 3H); ^1^C NMR (75 MHz, CDCl₃) δ 160.9 (d, J = 247.1 Hz), 160.9, 154.9, 143.7, 131.0 (d, J = 8.2 Hz), 130.4 (d, J = 3.5 Hz), 129.0, 128.7, 127.2, 126.1, 124.6 (d, J = 3.6 Hz), 122.8 (d, J = 14.3 Hz), 118.3, 116.3, 115.9 (d, J = 20.9 Hz), 67.9 (d, J = 3.8 Hz), 48.8, 22.6; ^1^F NMR (282 MHz, CDCl₃) –118.1–(–118.2) (m).
(S)-3-(4-fluorobenzzyloxy)-N-(1-phenylethyl)thiophene-2-carboxamide (3.17)

3.17. TLC R<sub>f</sub> = 0.38 (hexane:ethyl acetate, 4:1); IR (KBr) 3392, 3107, 2923, 1638, 1232, 1058 cm<sup>-1</sup>; [α]<sub>D</sub><sup>22.0</sup> = +74.4 (c 2.00, DCM); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.45 (d, <i>J</i> = 7.6 Hz, 1H), 7.23–7.33 (m, 2H), 7.09–7.20 (m, 4H), 6.99–7.08 (m, 2H), 6.86 (d, <i>J</i> = 5.5 Hz, 1H), 5.10–5.20 (m, 3H), 1.37 (d, <i>J</i> = 6.9 Hz, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 163.0 (d, <i>J</i> = 246.3 Hz), 160.9, 155.1, 143.5, 131.4 (d, <i>J</i> = 3.2 Hz), 130.1 (d, <i>J</i> = 8.3 Hz), 129.1, 128.8, 127.3, 126.2, 118.1, 116.3, 116.0 (d, <i>J</i> = 21.5 Hz), 73.4, 48.9, 22.5; <sup>19</sup>F NMR (282 MHz, CDCl<sub>3</sub>) -112.9 (sept, <i>J</i> = 5.0 Hz).

(S)-3-(2-chlorobenzzyloxy)-N-(1-phenylethyl)thiophene-2-carboxamide (3.18)

3.18. TLC R<sub>f</sub> = 0.30 (hexane:ethyl acetate, 4:1); IR (film) 3391, 3062, 2970, 1641, 1063, 762 cm<sup>-1</sup>; [α]<sub>D</sub><sup>22.7</sup> = +58.4 (c 2.66, DCM); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.44 (d, <i>J</i> = 7.7 Hz, 1H), 7.34 (dd, <i>J</i> = 8.0, 1.0 Hz, 1H), 7.27–7.30 (m, 2H), 7.22–7.25 (m, 1H), 7.12–7.19 (m, 5H), 6.83 (d, <i>J</i> = 5.5 Hz, 1H), 5.11–5.23 (m, 3H), 1.38 (d, <i>J</i> = 6.9 Hz, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 160.9, 155.0, 143.6, 133.8, 133.3, 130.4, 130.0, 129.1, 128.7, 127.4, 127.2, 126.2, 118.2, 116.3, 71.3, 48.9, 22.6.
(S)-3-(3-chlorobenzyloxy)-N-(1-phenylethyl)thiophene-2-carboxamide (3.19)

3.19. m.p. = 75.5–79.1 °C (DCM); TLC R_f = 0.22 (hexane:ethyl acetate, 4:1); IR (KBr) 3417, 3103, 2922, 1640, 1282, 766 cm^{-1}; [\alpha]_D^{23.1} = +71.1 (c 2.33, DCM); ^1H NMR (300 MHz, CDCl_3) δ 7.39 (d, J = 7.6 Hz, 1H), 7.27–7.32 (m, 3H), 7.23 (d, J = 7.6 Hz, 1H), 7.10–7.21 (m, 6H), 6.79 (d, J = 5.5 Hz, 1H), 5.15 (quint, J = 7.2 Hz, 1H), 5.03 (dd, J = 13.3, 11.3 Hz, 2H), 1.38 (d, J = 6.9 Hz, 3H); ^13C NMR (75 MHz, CDCl_3) δ 160.9, 154.9, 143.6, 137.6, 134.9, 130.4, 129.2, 129.1, 128.8, 128.0, 127.3, 126.1, 126.0, 118.2, 116.2, 73.2, 48.9, 22.6.

(S)-3-(2,4-dichlorobenzyloxy)-N-(1-phenylethyl)thiophene-2-carboxamide (3.20)

3.20. TLC R_f = 0.27 (hexane:ethyl acetate, 4:1); IR (KBr) 3421, 3107, 2961, 1656, 1227, 1080 cm^{-1}; [\alpha]_D^{23.8} = +56.4 (c 3.00, DCM); ^1H NMR (300 MHz, CDCl_3) δ 7.31–7.34 (m, 2H), 7.27 (d, J = 5.5 Hz, 1H), 7.09–7.19 (m, 7H), 6.79 (d, J = 5.5 Hz, 1H), 5.06–5.18 (m, 3H), 1.40 (d, J = 6.9 Hz, 3H); ^13C NMR (75 MHz, CDCl_3) δ 160.8, 154.6, 143.4, 135.5, 134.5, 131.8, 130.8, 129.9, 129.1, 18.7, 127.6, 127.3, 126.2, 118.3, 116.2, 116.2, 70.6, 49.0, 22.3.
(S)-3-(4-methoxybenzyl)-N-(1-phenylethyl)thiophene-2-carboxamide (3.21)

3.21. TLC $R_f = 0.17$ (hexane:ethyl acetate, 4:1); IR (film) 3383, 3101, 2968, 1638, 1250, 1054 cm$^{-1}$; $[\alpha]_{D}^{20.6} = +65.5$ (c 5.33, DCM); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.49 (d, $J = 7.8$ Hz, 1H), 7.25 (d, $J = 5.5$ Hz, 1H), 7.11–7.20 (m, 5H), 7.04–7.09 (m, 2H), 6.77–6.82 (m, 3H), 5.12 (quint, $J = 7.2$ Hz, 1H), 4.97 (dd, $J = 12.6$, 10.4 Hz, 2H), 3.73 (s, 3H), 1.33 (d, $J = 6.8$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 161.0, 160.1, 155.4, 143.6, 129.8, 128.9, 128.7, 127.6, 127.2, 126.1, 117.8, 116.4, 114.3, 73.9, 55.4, 48.9, 22.8.

3-(benzyl)-N-(2,6-difluorobenzyl)thiophene-2-carboxamide (3.22)

In a round bottom flask, acid 3.9 (0.93 g, 3.97 mmol), EDC (0.76 g, 3.97 mmol), and 2,6-difluorobenzylamine 3.45 (0.47 mL, 3.97 mmol) was suspended in DCM (12 mL). The reaction mixture was stirred continuously at room temperature for 22 h. A hydrochloric acid solution (20 mL, 1 M) was added to quench the reaction. The organic layer was collected, washed over sat. sodium chloride (3 x 20 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified with flash column chromatography using 30% ethyl acetate in hexane as eluent to afford 3.22 (1.18 g, 83%) as an off-white solid.

3.22. m.p. = 89.3–90.1 °C (DCM); TLC (hexane:ethyl acetate, 7:3) $R_f = 0.38$; IR (KBr) 3410, 3088, 2996, 1638, 1266, 1067 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.50 (t, $J = 5.0$ Hz, 1H), 7.19–7.26 (m, 6H), 7.06–7.17 (m, 1H), 6.69–6.78 (m, 3H), 5.04 (s, 2H), 4.56 (d,
\[ J = 6.0 \text{ Hz, 2H}; \] 13C NMR (75 MHz, CDCl3) \( \delta \) 161.5 (dd, \( J = 247.7, 8.0 \text{ Hz} \)), 161.5, 155.4, 135.5, 129.4 (t, \( J = 10.2 \text{ Hz} \)), 129.2, 128.8, 128.6, 127.6, 117.2, 116.4, 114.1 (t, \( J = 19.4 \text{ Hz} \)), 111.4 (dd, \( J = 17.4, 7.6 \text{ Hz} \)), 73.9, 31.1 (t, \( J = 3.8 \text{ Hz} \)); 19F NMR (282 MHz, CDCl3) \( \delta \) –115.2 (t, \( J = 6.3 \text{ Hz} \)).

![Chemical Structure](image)

**\( N\)-(2,6-difluorobenzyl)-3-hydroxythiophene-2-carboxamide (3.23)\)**

A solution of boron tribromide in DCM (3.18 mmol, 3.18 mL, 1.0 M in DCM) was added dropwise to a cooled solution of benzyl ether 3.22 (1.04 g, 2.89 mmol) in DCM (19 mL). The reaction mixture was stirred continuously at 0 °C using an ice/water bath. After 3 h, the reaction was quenched by adding sat. sodium bicarbonate solution (50 mL). The organic layer was collected and the aqueous layer was extracted with DCM (3 x 30 mL). The organic layers were collected, combined, washed over sat. sodium bicarbonate solution (3 x 30 mL) followed by water (3 x 30 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified through flash column chromatography using 20–30% ethyl acetate in hexane as eluent to afford thiophenol 3.23 (0.49 g, 63%) as white solid.

3.23. m.p. = 192.0–193.4 °C (DCM); TLC \( R_f = 0.35 \) (hexane:ethyl acetate, 4:1); IR (KBr) 3366, 2956, 2768, 1549, 1232, 1064 cm\(^{-1}\); 1H NMR (300 MHz, CD3OD) \( \delta \) 7.44 (d, \( J = 5.4 \text{ Hz} \), 1H), 7.29–7.39 (m, 1H), 6.94–7.04 (m, 2H), 6.69 (d, \( J = 5.4 \text{ Hz} \), 1H), 4.66 (s, 2H); 13C NMR (75 MHz, CD3OD) \( \delta \) 164.1, 161.8 (dd, \( J = 246.7, 8.0 \text{ Hz} \)), 158.0, 129.6 (t, \( J = 10.4 \text{ Hz} \)), 128.7, 119.2, 114.1 (t, \( J = 19.7 \text{ Hz} \)), 111.1 (dd, \( J = 17.6, 8.2 \text{ Hz} \)), 110.7, 30.7 (t, \( J = 4.2 \text{ Hz} \)); 19F NMR (282 MHz, CDCl3) \( \delta \) –117.6 (quint, \( J = 7.2 \text{ Hz} \)).
3–(2,4–dichlorobenzylxoy)–N–(2,6–difluorobenzyl)thiophene–2–carboxamide (3.2)

In a round bottom flask, thiophen–3–ol 3.23 (0.15 g, 0.58 mmol), 2,4–dichlorobenzyl chloride 3.42 (0.10 mL, 0.70 mmol), and potassium carbonate (0.12 g, 0.87 mmol) were suspended in DMF (2.90 mL). The reaction mixture was stirred continuously and was heated at 80 °C. After 19 h, water (10 ml) was added to the reaction mixture. The quenched reaction mixture was extracted with ethyl acetate (3 x 10 mL). The organic layers were collected, combined, washed over sat. sodium chloride solution (3 x 10 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified with flash column chromatography using 10–15% ethyl acetate in hexane as eluent to afford 3.2 (0.20 g, 80%) as an off white solid.

3.2. m.p. = 113.3–115.9 °C (DCM); TLC Rf = 0.29 (hexane:ethyl acetate, 7:3); IR (KBr) 3408, 3087, 2948, 1627, 1255, 1066 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.43 (t, J = 5.4 Hz, 1H), 7.35–7.38 (m, 2H), 7.24–7.30 (m, 1H), 7.18–7.23 (m, 2H), 6.79–6.88 (m, 3H), 5.20 (s, 2H), 4.65 (d, J = 5.6 Hz, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 161.6 (dd, J = 247.6, 7.9 Hz), 161.4, 154.8, 135.4, 134.3, 131.9, 131.4, 130.6, 129.7 (t, J = 10.3 Hz), 129.4, 127.6, 118.0, 116.2, 114.1 (t, J = 19.4 Hz), 111.5 (dd, J = 17.5, 7.7 Hz), 70.6, 31.3 (t, J = 3.6 Hz); ¹⁹F NMR (282 MHz, CDCl₃) δ –115.2 (t, J = 6.6 Hz).

General Procedure for 3.25–3.32

In a round bottom flask, thiophen–3–ol 3.23 (1 equiv), benzyl halide (1.2 equiv), and potassium carbonate (1.5 equiv) were suspended in DMF (5 mL per 1 mmol). The
reaction mixture was stirred continuously and heated to 80 °C. After approximately 19 h, water was added. The quenched reaction mixture was extracted with ethyl acetate. The organic layers were collected, combined, washed over sat. sodium chloride solution (3 x 10 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified using flash column chromatography with 10–15% ethyl acetate in hexane as eluent.

3–(2-bromobenzzyloxy)–N–(2,6-difluorobenzyl)thiophene–2–carboxamide (3.25)

3.25. TLC R_f = 0.17 (hexane:ethyl acetate, 4:1); IR (film) 3399, 3067, 2860, 1647, 1233, 1068 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.55 (dd, J = 7.7, 1.3 Hz, 2H), 7.33–7.36 (m, 2H), 7.25–7.30 (s, 1H), 7.16–7.24 (m, 2H), 6.78–6.87 (m, 3H), 5.22 (s, 2H), 4.67 (d, J = 5.7 Hz, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 161.6 (dd, J = 247.7, 8.0 Hz), 161.4, 155.0, 134.8, 133.1, 130.3, 129.6, 129.5 (t, J = 10.3 Hz), 129.3, 127.9, 123.2, 117.7, 116.3, 114.1 (t, J = 19.3 Hz), 111.5 (dd, J = 17.6, 7.8 Hz), 73.3, 31.2 (t, J = 3.7 Hz); ¹⁹F NMR (282 MHz, CDCl₃) δ –117.6 (t, J = 6.7 Hz).

3–(4-bromobenzzyloxy)–N–(2,6-difluorobenzyl)thiophene–2–carboxamide (3.26)

3.26. m.p. = 156.3–158.5 °C (DCM); TLC R_f = 0.18 (hexane:ethyl acetate, 4:1); IR (film) 3394, 3086, 2936, 1636, 1235, 1067 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.45 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 5.2 Hz, 1H), 7.24–7.29 (m, 1H), 7.18–7.22 (m, 2H), 6.80–6.88 (m, 3H), 5.08 (s, 2H), 4.63 (d, J = 5.5 Hz, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 161.6 (dd, J =
247.6, 8.0 Hz), 161.5, 155.2, 134.5, 132.2, 129.6 (t, J = 10.1 Hz), 129.5, 129.4, 122.8, 117.6, 116.2, 114.0 (t, J = 19.3 Hz), 111.5 (dd, J = 17.6, 7.7 Hz), 73.2, 31.3 (t, J = 3.7 Hz); $^{19}$F NMR (282 MHz, CDCl$_3$) δ −115.2 (t, J = 6.7 Hz).

$\text{N–(2,6–difluorobenzyl)–3–(2–fluorobenzyloxy)thiophene–2–carboxamide (3.27)}$

3.27. m.p. = 103.9–106.1 °C (DCM); TLC $R_f$ = 0.19 (hexane:ethyl acetate, 4:1); IR (KBr) 3401, 3096, 2944, 1647, 1233, 1067 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.55 (t, J = 5.0 Hz, 1H), 7.31–7.39 (m, 3H), 7.16–7.26 (m, 1H), 7.03–7.14 (m, 2H), 6.90 (d, J = 5.9 Hz, 1H), 6.79–6.87 (m, 2H), 5.22 (s, 2H), 4.66 (d, J = 6.0 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 161.7 (dd, J = 247.8, 8.0 Hz), 160.8 (d, J = 246.7 Hz), 161.5, 155.1, 130.8 (d, J = 8.3 Hz), 130.1 (d, J = 3.5 Hz), 129.5 (t, J = 10.4 Hz), 129.3, 124.6 (d, J = 3.8 Hz), 122.7 (d, J = 14.3 Hz), 117.9, 116.3, 115.8 (d, J = 21.1 Hz), 114.2 (t, J = 19.4 Hz), 111.5 (dd, J = 17.5, 7.6 Hz), 67.8 (d, J = 4.1 Hz), 31.2 (t, J = 3.6 Hz); $^{19}$F NMR (282 MHz, CDCl$_3$) δ −115.2 (t, J = 6.5 Hz), −118.6 (q, J = 7.8 Hz).

$\text{N–(2,6–difluorobenzyl)–3–(4–fluorobenzyloxy)thiophene–2–carboxamide (3.28)}$

3.28. m.p. = 104.4–105.2 °C (DCM); TLC (hexane:ethyl acetate, 4:1) $R_f$ = 0.20; IR (KBr) 3404, 3107, 3032, 1645, 1233, 1067 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.55 (t, J = 5.0 Hz, 1H), 7.31–7.39 (m, 3H), 7.16–7.26 (m, 1H), 7.03–7.14 (m, 2H), 6.90 (d, J = 5.9 Hz, 1H), 6.79–6.87 (m, 2H), 5.22 (s, 2H), 4.66 (d, J = 6.0 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 162.9 (d, J = 245.9 Hz), 161.6 (dd, J = 247.7, 8.0 Hz), 161.5, 155.3, 131.4 (d, J
= 3.2 Hz), 129.9 (d, \( J = 8.3 \) Hz), 129.5 (d, \( J = 10.2 \) Hz), 129.3, 117.6, 116.3, 115.9 (d, \( J = 21.5 \) Hz), 114.1 (t, \( J = 19.4 \) Hz), 111.5 (dd, \( J = 17.6, 7.8 \) Hz), 73.3, 31.3 (t, \( J = 3.6 \) Hz); \(^{19}\)F NMR (282 MHz, CDCl\(_3\)) \( \delta \) –111.3–(–113.4) (m), –115.3 (t, \( J = 6.5 \) Hz).

3–(2–chlorobenzyloxy)–N–(2,6–difluorobenzyl)thiophene–2–carboxamide (3.29)

3.29. TLC \( R_f = 0.30 \) (hexane:ethyl acetate, 7:3); IR (film) 3400, 3080, 2950, 1645, 1233, 1068 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.56 (t, \( J = 5.3 \) Hz, 1H), 7.32–7.40 (m, 3H), 7.20–7.30 (m, 3H), 6.78–6.88 (m, 3H), 5.25 (s, 2H), 4.67 (d, \( J = 5.7 \) Hz, 2H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 161.6 (dd, \( J = 247.8, 8.0 \) Hz), 161.5, 155.1, 133.5, 133.2, 130.1, 129.9, 129.6, 129.5 (t, \( J = 10.3 \) Hz), 129.3, 127.3, 117.7, 116.3, 114.2 (t, \( J = 19.3 \) Hz), 111.5 (dd, \( J = 17.5, 7.6 \) Hz), 71.2, 31.2 (t, \( J = 3.7 \) Hz); \(^{19}\)F NMR (282 MHz, CDCl\(_3\)) \( \delta \) –115.1 (t, \( J = 6.7 \) Hz).

3–(3–chlorobenzyloxy)–N–(2,6–difluorobenzyl)thiophene–2–carboxamide (3.30)

3.30. m.p. = 90.5–92.7 \( ^\circ \)C (DCM); TLC \( R_f = 0.23 \) (hexane:ethyl acetate, 7:3); IR (KBr) 3404, 3087, 2883, 1642, 1232, 1070 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.52 (t, \( J = 5.1 \) Hz, 1H), 7.30–7.35 (m, 3H), 7.25–7.28 (m, 1H), 7.15–7.22 (m, 2H), 6.79–6.88 (m, 3H), 5.11 (s, 2H), 4.65 (d, \( J = 5.7 \) Hz, 2H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 161.5 (dd, \( J = 249.2, 8.1 \) Hz), 161.4, 155.1, 137.5, 134.8, 130.3, 129.7, 129.5 (t, \( J = 10.3 \) Hz), 129.0,
127.9, 125.9, 117.7, 116.2, 114.2 (t, J = 19.4 Hz), 111.5 (dd, J = 25.3, 10.0 Hz), 73.1, 31.3 (t, J = 3.7 Hz); $^{19}$F NMR (282 MHz, CDCl$_3$) δ –115.3 (t, J = 6.7 Hz).

![Chemical Structure](image)

$^3$–(4–chlorobenzyloxy)–N–(2,6–difluorobenzyl)thiophene–2–carboxamide 3.31

3.31. m.p. = 142.9–144.3 °C; TLC R$_f$ = 0.29 (hexane:ethyl acetate, 7:3); IR (KBr) 3393, 3086, 2851, 1636, 1235, 1068 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.46 (t, J = 5.1 Hz, 1H), 7.34 (d, J = 6.2 Hz, 1H), 7.18–7.31 (m, 5H), 6.79–6.87 (m, 3H), 5.09 (s, 2H), 4.63 (d, J = 5.7 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 161.5 (dd, J = 247.7, 8.0 Hz), 161.4, 155.2, 134.6, 134.0, 129.6 (t, J = 10.2 Hz), 129.3, 129.2, 129.1, 117.5, 116.2, 114.0 (t, J = 19.4 Hz), 111.5 (dd, J = 17.6, 7.7 Hz), 73.1, 31.2 (t, J = 3.6 Hz); $^{19}$F NMR (282 MHz, CDCl$_3$) δ –115.2 (t, J = 6.7 Hz).

![Chemical Structure](image)

$^N$–(2,6–difluorobenzyl)–$^3$–(4–methoxybenzyloxy)thiophene–2–carboxamide (3.33)

3.33. TLC R$_f$ = 0.18 (hexane:ethyl acetate, 4:1); IR (film) 3401, 3110, 2837, 1631, 1246, 1067 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.58 (t, J = 6.5 Hz, 1H), 7.35 (d, J = 4.9 Hz, 1H), 7.16–7.22 (m, 3H), 6.79–6.88 (m, 5H), 5.07 (s, 2H), 4.63 (d, J = 5.2 Hz, 2H), 3.83 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 161.6 (dd, J = 247.9, 8.0 Hz), 161.6, 160.0, 155.6, 129.5, 129.4 (t, J = 10.2 Hz), 129.2, 127.6, 117.5, 116.5, 114.3 (t, J = 18.2 Hz), 111.5 (dd, J = 17.6, 7.7 Hz), 73.8, 55.4, 31.2 (t, J = 3.8 Hz); $^{19}$F NMR (282 MHz, CDCl$_3$) δ –115.1 (quintet, J = 5.0 Hz).

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References


CHAPTER 4

INTRAMOLECULAR DIELS–ALDER REACTIONS USING AN ALDEHYDE DIENOPHILE: A NEW SYNTHETIC APPROACH TO THE SYNTHESIS OF THE CYTOTOXIC MACROLIDE APICULAREN A

Introduction

Multiple macrosalicylic antitumor natural products have been isolated from several sources such as marine organisms, terrestrial plants and even microbial and fungal broths. These compounds include salicylihalimide,1 the lobatamides,2 CJ–12,950,3 and the oximidines.4 Another interesting molecule belonging from these class of natural products is apicularen A (Figure 4–1).

![Chemical structures of macrosalicylic antitumor natural products](image_url)

**Figure 4–1.** Macrosalicylic Antitumor Natural Products.
Apicularen A 4.9 is a salicylate structurally related to salicylhalimide 4.10. This molecule was isolated and identified during a screening of various strains of the myxobacterial genus *Chondromyces* (i.e. *C. apiculatus*, *C. lanuginosus*, *C. pediculatus*, and *C. robustus*) for biologically active metabolites in 1998 by Jansen and co–workers. Some unusual structural features of this molecule include a macrocyclic salicylate ester core skeleton, an embedded *trans*–tetrahydropyran ring, and a doubly unsaturated acylenamine moiety. Biosynthetic studies showed that apicularen A is acetate–derived except for C–17 which comes from glycine and C–18 and C–25 which comes from methionine.6

![Structures of Apicularen A 4.9 and Salicylhalimide 4.10.](image)

**Figure 4–2.** Structures of Apicularen A 4.9 and Salicylhalimide 4.10.

Apicularen A showed no antimicrobial activity however it exhibits potent cytotoxic activity against several human cancer cell lines including the multi–drug resistant line KB–VI. The IC₅₀ values of 4.9 range between 0.3 to 3.0 ng/mL and are summarized in Table 4–1.5
<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Origin</th>
<th>IC$_{50}$ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB–3–1</td>
<td>cervix carcinoma</td>
<td>1.0</td>
</tr>
<tr>
<td>KB–V1</td>
<td>cervix carcinoma</td>
<td>0.4</td>
</tr>
<tr>
<td>K–562</td>
<td>chronic myelogenous leukemia</td>
<td>2.0</td>
</tr>
<tr>
<td>HL–60</td>
<td>acute myeloid leukemia</td>
<td>3.0</td>
</tr>
<tr>
<td>U–937</td>
<td>histocytic carcinoma</td>
<td>1.5</td>
</tr>
<tr>
<td>A–498</td>
<td>kidney carcinoma</td>
<td>0.3</td>
</tr>
<tr>
<td>A–549</td>
<td>lung carcinoma</td>
<td>0.1</td>
</tr>
<tr>
<td>PC–3</td>
<td>prostate carcinoma</td>
<td>0.5</td>
</tr>
<tr>
<td>SK–OV–3</td>
<td>ovarian carcinoma</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**Table 4–1. Cytostatic Effects of Apicularen A on Human Cell Lines.**

Exploration of the mode of action of this compound that apicularen A induced nitrite (NO) production which induced apoptosis$^7$ in mouse leukaemic monocyte cell line RAW 264.7$^8$. Apicularen A induces apoptosis in human promyelocytic leukemia cell line HL–60 as well.$^9$ The natural product also induced cell death in HM7 cells through regulating the Fas ligand and disrupting microtubules with down–regulation of tubulin level. Such a mechanism indicates that it can also be a promising microtubule targeting compound.$^{10}$

Recent studies also show that 4.9 is also an inhibitor of mammalian vacuolar (H$^+$)–ATPases (V–ATPases).$^{11–12}$ Such impressive activity against V–ATPases makes apicularen A analogues candidate for the treatment of several diseases such as diabetes, Alzheimer’s disease, cardiovascular disorders, osteoporosis, and cancer.$^{13–15}$ The biological activity of 4.9 has already been evaluated for osteoporosis$^{16}$ and cancer.$^{17}$ Apicularen A inhibited bafilomycin A1–sensitive ATP–dependent proton transport into microsome vesicles and also showed better inhibition of bone resorption in cultures of
mouse calvariae either induced by human parathyroid hormone (PTH) or interleukin–1β (IL–1β) probably due to the inhibition of V–ATPase.$^{16}$

In addition, 4.9 potently inhibited the proliferation of bovine aortic endothelial cells (BAECs) in a dose–dependent manner. Apicularen A showed inhibition of basic fibroblast growth factor (bFGF)–induced invasion and capillary tube formation of BAECs which demonstrates 4.9 as a novel antiangiogenic agent and may suppress the growth of tumors through inhibition of neovascularization.$^{17}$ Because of such amazing biological activity, several synthetic apicularen A analogues have also been subjected to biological evaluation.$^{18–21}$

**Background and Significance**

**Synthetic Strategy for the Formation of Apicularen A’s Functionalized Pyran Ring**

Because of its potent biological activity, unique structural features, and limited availability (210 mg / 90–L fermentation),$^{5}$ apicularen A has been an attractive synthetic target. Many research groups have completed the total synthesis of apicularen A.$^{18–20,22–28}$ Also, several formal syntheses$^{29–36}$ and many synthetic studies$^{37–47}$ have been reported. In these total and formal syntheses of the apicularen A, many different methodologies were employed for the construction of the functionalized tetrahydropyran moiety of 4.9 which includes allylation,$^{19,21}$ annulation,$^{22}$ Diels–Alder reaction,$^{18,38}$ trans–annular etherification,$^{20,24,34–35}$ cyclization,$^{27–28,34–35}$ coupling reaction,$^{31,36}$ and metathesis.$^{35}$ The key disconnections and their strategies for the formation of tetrahydropyran moiety are summarized in Figure 4–3.
Figure 4–3. Key Disconnections and Strategies for the Formation of the Functionalized Tetrahydropyran of Apicularen A 4.9.

The first completed total synthesis of apicularen A was reported by De Brabander and co–workers18,38 shown in Scheme 4–1. De Brabander utilized a Diels–Alder reaction for the construction of the tetrahydropyran moiety. A chiral chromium–catalyzed hetero–Diels–Alder reaction between aldehyde 4.11 and a Danishefsky diene 4.12 followed by the conjugate addition of vinyl cuprate afforded the 1,6–trans–tetrahydropyranone 4.14. NaBH₄ reduction followed by the protection of the resulting alcohol with TBSCI furnished the functionalized pyran ring of apicularen A.
Scheme 4–1

De Brabander’s Synthetic Route to (−)–Apicularen A

Reagents and conditions: (a) i. cat 4.13, 4 Å MS, (CH₃)₂CO, rt, 24 h, ii. CF₃CO₂H, DCM, 0 °C, 1 h (b) CH₂=CHMgBr, Cul, DMPU, −78 °C for 3 h to −40 °C for 1 h, 49% over 2 steps (c) NaBH₄, MeOH (d) TBSCl, imidazole, cat. DMAP, DMF, 98% over 2 steps.

Nicolaou¹⁹,²¹ also synthesized apicularen A employing an alkylation–ozonolysis reiterations to form the THP ring. Allylation of the aldehyde 4.11 with (−)-Ipc₂Ballyl produced an alcohol which was protected as its triethylsilyl ether 4.16. Ozonolysis of the vinyl group (4.16) followed by Brown allylation and mild acidic treatment furnished the 1,3–anti diol 4.17. Ozonolysis of the terminal alkene followed by acetylation then resulted to the formation of 4.18. Diacetate 4.18 was subjected to an anomeric allylation with allylsilane 4.19 in the presence of BF₃·OEt₂ which gave 4.20. Tetrahydropyran 4.20 was then used to complete the synthesis of apicularen A.
Scheme 4–2\textsuperscript{a} Nicolaou’s Total Synthesis of Apicularen A

\textsuperscript{a}Reagents and conditions: (a) (–)–Ipc\textsubscript{2}Ballyl, Et\textsubscript{2}O, –100 °C, 2 h (b) TESOTf, 2,6–lutidine, DCM, 25 °C, 3 h, 58% over 2 steps (c) i. O\textsubscript{3}, DCM, –78 °C, 1 h ii. Me\textsubscript{2}S, 25 °C (d) PPh\textsubscript{3}, 1 h (e) (Ipc)\textsubscript{2}B’allyl, Et\textsubscript{2}O, –100 °C, 2 h (f) HCl, 4 h, 59% over 3 steps (g) i. O\textsubscript{3}, DCM, –78 °C, 1 h ii. PPh\textsubscript{3}, 1 h (g) Ac\textsubscript{2}O, pyridine, 25 °C, 1 h, 83% over 2 steps (h) 4.19, BF\textsubscript{3}.Et\textsubscript{2}O, CH\textsubscript{3}CN, 0 °C, 1 h, 97%.

Several other research groups have employed \textit{trans}–annular etherification as a synthetic strategy for the formation of the pyran ring including reports from Maier,\textsuperscript{20,24} O’Doherty,\textsuperscript{34} and Tae.\textsuperscript{35} Maier’s approach used a cross–coupling reaction of the triflate 4.22 with stannane 4.23 that furnished salicylate 4.24. Hydrolysis of the acetal and ester functionalities gave the dihydroxy acid 4.25. Macrolactonization of 4.25 was achieved by converting the acid to an ethoxyvinyl ester followed by the addition of camphorsulfonic acid (CSA). Treatment of the lactone 4.26 with mercuric trifluoroacetate followed by reductive demercuration resulted to the formation of the \textit{trans}–pyran 4.27 which was used to complete the synthesis of 4.9.\textsuperscript{20,24}
**Scheme 4–3**

Maier’s Total Synthesis of Apicularen A

\[ \text{Scheme 4–3} \]

\[
\begin{array}{c}
\text{OMe} \quad \text{OMe} \\
\text{OMe} \quad \text{OMe} \\
\text{O} \\
\text{Bu}_3\text{Sn} \\
4.23
\end{array}
\]

\[
\text{+}
\]

\[
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{Bn} \\
\text{Bn} \\
\text{Bn} \\
\text{Bn} \\
4.24
\end{array}
\]

\[
\text{\textit{a}}
\]

\[
\begin{array}{c}
\text{OMe} \quad \text{O} \\
\text{O} \\
\text{O} \\
\text{Bn} \\
\text{Bn} \\
\text{Bn} \\
\text{Bn} \\
\text{Bn} \\
\text{OH} \\
\text{OH} \\
\text{OH} \\
\text{Bn} \\
\text{4.25}
\end{array}
\]

\[
\text{\textit{b,c}}
\]

\[
\begin{array}{c}
\text{OMe} \quad \text{O} \\
\text{O} \\
\text{O} \\
\text{Bn} \\
\text{Bn} \\
\text{Bn} \\
\text{Bn} \\
\text{Bn} \\
\text{OH} \\
\text{OH} \\
\text{OH} \\
\text{Bn} \\
\text{4.26}
\end{array}
\]

\[
\text{\textit{d,e}}
\]

\[
\begin{array}{c}
\text{OMe} \quad \text{O} \\
\text{O} \\
\text{O} \\
\text{Bn} \\
\text{Bn} \\
\text{Bn} \\
\text{Bn} \\
\text{Bn} \\
\text{OH} \\
\text{OH} \\
\text{OH} \\
\text{Bn} \\
\text{4.27}
\end{array}
\]

\[
\rightarrow \text{APICULAREN A}
\]

---

\text{Reagents and conditions: (a) P(furyl)$_3$, Pd$_2$(dba)$_3$, LiCl, NMP, 60 °C, 48 h (b) AcOH, 23 °C, 80 min (c) LiOH, MeOH/H$_2$O, 60 °C, 72 h, 98% over 2 steps (d) \{[RuCl$_2$(p–cymene)$_2]\}, ethoxyacetylene (e) CSA, toluene, 80 °C, 63% over 2 steps (f) \textit{i.} Hg(O$_2$CCF$_3$)$_2$, DCM, 23 °C, 30 min \textit{ii.} NaCl, 23 °C (g) Et$_3$B, LiBH$_4$, THF, −78 °C, 1 h, 89% over 2 steps.}

O’Doherty\textsuperscript{34} used a similar transannular cyclization in his synthesis of apicularen A. An olefin cross metathesis between alkenes 4.28 and 4.29 followed by a weakly acidic deprotection of the benzylidene provided diol 4.31. Hydrolysis of the methyl ester using LiOH resulted to the formation of the \textit{seco} acid which was subjected to a modified Yamaguchi lactonization procedure to selectively produced the 12–membered macrolactone 4.26. Formation of the tetrahydropyran with base (\textit{t–BuOK, 1 equiv})
yielded the desired macrolide 4.27 that is identical to the material previously reported by Maier.\textsuperscript{20,24}

\textbf{Scheme 4–4} \textsuperscript{a} O’Doherty’s Formal Synthesis of Apicularen A

\textsuperscript{a}Reagents and conditions: (a) 4.30, DCM, reflux, 24 h (b) AcOH, 60 °C, 12 h, 71% over 2 steps (c) LiOH, CH\textsubscript{3}OH, 65 °C, 72 h (d) 4.32, 4–DMAP, Et\textsubscript{3}N, 66% over 2 steps (e) t–BuOK, THF, 0 °C, 3 h, 83%.

Tae and co–workers also reported a formal synthesis of apicularen A using a transannular cyclization approach.\textsuperscript{35} Cycloaddition of alkyne (4.34) and diene 4.33 resulted into the salicylate 4.35, which was followed by macrolactonization using a ring–closing metathesis (RCM) utilizing a second generation Grubbs’ catalyst 4.30. Deprotection of TBS protecting group and \textit{trans}–annular etherification resulted in the tetrahydropyran moiety of apicularen A. Deselenization followed by deprotection
afforded alcohol 4.37 that can be converted to the vinyl iodide 4.38, a known intermediate to Nicolaou’s synthetic approach\textsuperscript{19,21} to 4.9.

Scheme 4–5\textsuperscript{a} Tae’s Formal Synthesis of Apicularen A

\[\text{OCH}_3 + \text{O} \rightarrow \text{a} \rightarrow \text{b} \rightarrow \text{c} \]

\[4.33 + 4.34 \rightarrow 4.35 \]

\[4.36 \leftrightarrow 4.37 \rightarrow 4.38 \]

\textsuperscript{a}Reagents and conditions: (a) 200 °C, neat, 36 h, 60% (b) 4.30, DCM, reflux, 8 h (c) HF, THF, 65% over 2 steps (d) \textit{i}. PhSeCl, pyr. DCM \textit{ii}. \textit{n}–Bu\textsubscript{3}SnH, AIBN, PhCH\textsubscript{3}, 80 °C (e) H\textsubscript{2}, Pd/C, EtOH, 72% over 2 steps.

Similarly, Rizzacasa employed \textit{trans}–annular conjugate addition as synthetic strategy for the formation of the pyran ring\textsuperscript{31,36}. Stille coupling of bromide 4.39 and stannane 4.40 using tri–2–furylphosphine (TFP) as ligand produced the cyclization precursor 4.41. Treatment of 4.41 with NaH resulted in the lactone formation after which global desilylation followed by an allylic alcohol oxidation with MnO\textsubscript{2} produced \textit{\alpha},\textit{\beta}–unsaturated ketone 4.42. Heating of 4.42 in CDCl\textsubscript{3} in the presence of amberlyst–15 gave the desired \textit{trans}–pyranone 4.43 (>10:1) which can be converted to 4.44, a common intermediate to Taylor’s\textsuperscript{20–30} and Rychnovsky’s\textsuperscript{32} synthesis of 4.9.
Scheme 4–6a  Tae’s Formal Synthesis of Apicularen A

\[ \text{Scheme 4–6a} \]

Tae’s Formal Synthesis of Apicularen A

\[ \begin{align*}
4.39 & \quad \text{Bu}_3\text{Sn} \quad \text{R}^1 = \text{TIPS, R}^2 = \text{TBS} \\
4.40 + & \quad \text{R}^1 = \text{TIPS, R}^2 = \text{TBS} \\
4.41 & \quad \text{OH} \\
4.42 & \quad \text{OMe} \\
4.43 & \quad \text{OH} \\
4.44 & \quad \text{OMe} \\
4.45 & \quad \text{OH} \\
4.46 & \quad \text{OMe}
\end{align*} \]

\[ a \]

Reagents and conditions: (a) \( \text{Pd}_2(\text{dba})_3 \), TFP, NMP, 60 °C, 85% (b) \( \text{NaH} \), THF then MeI (c) TBAF (d) \( \text{MnO}_2 \), 49% over 3 steps (e) Amberlyst–15, CDCl3, reflux, 18 h, 90% (dr = >10:1).

Annulation was Panek’s strategy for the construction of the dihyropyran of apicularen A focused on the reaction of aldehyde 4.45 and chiral organosilane 4.46 in the presence of TMSOTf. This quickly afforded dihydropyran 4.47 (Figure 4–10). Methanolysis followed by protection of the free alcohol afforded silyl ether 4.48. Treatment of alkene (4.48) with \( m \)-CPBA then yielded epoxide 4.49 (\( \alpha: \beta = 7.5:1 \)). The epoxide was subjected to a regioselective opening with DIBAL–H and the resulting alcohol stereochemistry was inverted using a Mitsonobu reaction to install the correct stereochemistry for the functionalized pyran ring.\(^{22}\)
Scheme 4–7a  Panek’s Total Synthesis of Apicularen A

![Scheme 4–7](image)

*aReagents and conditions: (a) TMSOTf, −50 °C, 90% (dr = >30:1) (b) K₂CO₃, MeOH
(c) TBDPSCl, imidazole, 85% over 2 steps (d) m–CPBA, CCl₄, 0 °C, 85% (7.5:1, α:β)
(e) DIBAL–H, DCM, −78 °C (f) PPh₃, DIAD, CICH₂COOH, PhH, 0 to 25 °C, 52% over
2 steps.

Rychnovsky and co–workers reported a formal synthesis of apicularen A³²–³³ employing
the formation of an acetal and its reaction with 2,4–pentadienyltrimethylsilane 4.55. Coupling between nitrile 4.51 and iodide 4.52 followed by diastereoselective lithium ammonia reduction yielded 4.53. Oxidation of the primary alcohol with Dess–Martin periodinane (DMP) followed by deprotection of TES and acetamide groups and protection of the secondary alcohols afforded THP acetal 4.54. Treatment of acetal 4.54 with 4.55 and BF₃·OEt₂ formed the trans–tetrahydropyran 4.56.
exclusively. Diene (4.56) was used for the formation of the salicylate moiety of 4.9 through an intramolecular Diels–Alder reaction to synthesize 4.57, a known intermediate in De Brabander’s total synthesis of 4.9.\textsuperscript{18,38} (Scheme 4–8)

\textbf{Scheme 4–8}\textsuperscript{a}  
Rychnovsky’s Formal Synthesis of Apicularen A

\textsuperscript{a}Reagents and conditions: (a) LDA, DMPU, THF, \textdegree{}78 \textdegree{}C to \textdegree{}40 \textdegree{}C (b) i. Li, NH\textsubscript{3} ii. isoprene quench, 81\% over 2 steps (c) Dess–Martin periodinane, DCM (d) i. DOWEX, MeOH ii. TFAA, DMAP, pyridine, DCM, 89\% over 2 steps (e) 4.55, BF\textsubscript{3}O·Et\textsubscript{2}, CH\textsubscript{3}CN, 0 \textdegree{}C (f) K\textsubscript{2}CO\textsubscript{3}, MeOH, H\textsubscript{2}O, 98\% over 2 steps.

Recently, Uenishi’s synthetic strategy included another unusual cyclization reaction.\textsuperscript{27–28} Nozaki–Hiyama–Kishi (NHK) reaction of vinyl iodide 4.58 and aldehyde 4.59 afforded allylic alcohol 4.60. Deprotection of the benzylidene acetal yielded triol 4.61 which was subjected to a Pd(II)–catalyzed cyclization gave the 2,6–\textit{trans}–dihydropyran 4.62. Ester hydrolysis followed by a Yamaguchi lactonization afforded the core macrolactone 4.63. Installation of the C–11 \(\alpha\)–hydroxy isomer was accomplished through an oxymercuration and successive reductive demercuration providing the \(\beta\)–hydroxy isomer 4.64 in a 3:1 ratio with the undesired \(\alpha\)–product.
**Scheme 4–9**

Uenishi’s Total Synthesis of Apicularen A

\[
\begin{align*}
\text{Scheme 4–9}^a & \quad \text{Uenishi’s Total Synthesis of Apicularen A} \\
\text{4.58} & \quad + \\
\text{b} & \quad \rightarrow \\
\text{4.60} & \quad \text{a}
\end{align*}
\]

\[
\begin{align*}
\text{4.59} & \quad \rightarrow \\
\text{4.61} & \quad \text{4.62} \\
\text{4.63} & \quad \rightarrow \\
\text{4.64} & \quad \text{c}
\end{align*}
\]

\[\text{Reagents and conditions: (a) NiCl}_2 (0.1\%), \text{Cr}_2\text{Cl}_2, \text{DMSO, rt, 20 h, 75\% (dr = 1.3:1)} \]
(b) AcOH, 60 °C, 8 h, 77\% (c) PdCl}_2(\text{CH}_3\text{CN})_2 (20 \text{ mol\%}), \text{THF, rt, 8 h, 72\% (d) LiOH, Dioxane/H}_2\text{O, 80 °C, 48 h (e) 4.32, 4-DMAP, Et}_3\text{N, THF, 76\% over 2 steps (f) Hg(OCOCF}_3)_2, THF/H}_2\text{O, rt, 1 h (g) NaOH, NaBH}_4, \text{rt, 1 min, 80\% (dr = 3:1).}
\]

**Retrosynthetic Analysis**

Apicularen A 4.9 provides an excellent opportunity to evaluate an intramolecular Diels–Alder reaction using an aldehyde dienophile,\textsuperscript{48–59} an underutilized transformation in the synthesis of natural products.

The primary objective of this work is explore the use of a new type of Diels–Alder reaction in the synthesis of apicularen A 4.9. Our synthetic strategy for apicularen A is shown in the retrosynthetic analysis in **Figure 4–4**. The natural product 4.9 was
envisioned to arise from dihydropyran 4.65, accessible from a cycloaddition of diene 4.66. Also formation of intermediate 4.65 may be achieved by stepwise addition of the enol to the aldehyde followed by a conjugate addition. Such methodology would be explored for the assembly of the functionalized pyran ring. Finally, diene 4.66 would be achieved by coupling of alcohol 4.67 and carboxylic acid 4.68.

![Diagram](image)

**Figure 4–4.** Retrosynthetic Analysis of Apicularen A.

**Intramolecular Diels–Alder Reaction using Aldehyde Dienophile**

The intramolecular Diels–Alder reaction is powerful tool in synthesis. The variations on the conditions of this reaction led to the discovery of numerous hetero Diels–Alder reactions. The first intramolecular Diels–Alder reaction was documented in 1953, however it was not until 1970s that the reactions use became common in the literature. The intramolecular Diels–Alder reaction has several advantages compared to its intermolecular counterpart. The reaction involves the formation of two rings simultaneously where the size of one of the rings can be varied. In addition, since the
reactive moieties are part of the same molecule, the reaction is more ordered than the intermolecular reaction and often has lower activation energies leading to milder reaction conditions. Also, because of the constraints on the reaction imposed by the tether, the reaction often gives excellent stereoselectivity.\textsuperscript{63}

![Chemical structure](image)

**Figure 4–5.** Intramolecular Diels–Alder Reaction.

Similarly, hetero Diels–Alder reaction using an aldehyde as dienophile (**Figure 4–6**) only proved to be useful years after its discovery. Gersham and Steadman reported the use of formaldehyde as dienophile under thermal conditions in 1951. Later studies showed activation of the aldehyde with a strong electron withdrawing group was necessary for good yields. Danishefsky evaluated various dienes, aldehydes, and catalysts that allowed the control of the stereochemical outcome of the reaction. These also led the use of milder conditions of the reaction\textsuperscript{61} which were valuable to the syntheses of natural products.

![Chemical structure](image)

**Figure 4–6.** Hetero–Diels Alder Reaction Using Aldehyde as Dienophile.

Thus far, the combination of these two types of Diels–Alder reactions, the intramolecular Diels–Alder reaction and hetero Diels–Alder reaction using an aldehyde as the dienophile (**Figure 4–7**), are rare in literature.
Figure 4–7. The Intramolecular Hetero–Diels–Alder Reaction Using Aldehyde as Dienophile.

Oppolzer reported the first intramolecular Diels–Alder reaction with an aldehyde dienophile in 1972. The cyclobutane (4.76) was heated in bromobenzene for 23 h, where the cyclobutane ring opens up to create highly reactive diene 4.77. The spontaneous Diels–Alder reaction formed a mixture of 4.78 and 4.79.

Scheme 4–10a The Intramolecular Cycloadditions of Aldehyde to o-Quinodimethanes

\[ \text{Scheme 4–10a} \]

Reagents and conditions: (a) PhBr, 155 °C, 25%.

Rigby utilized the intramolecular Diels–Alder reaction of aldehyde 4.84 for the formation of tricyclic system 4.85. This system was then used for the assembly of guaianolides like (±)-dehydrocostus lactone 4.86 and the somewhat more highly functionalized (±)-estafiatin 4.87. The syntheses of these natural products commenced from the addition of Grignard reagent 4.81 to tropone 4.80. Reduction of the ketone (4.82) followed by methylation of the resulting alcohol provided methyl ether 4.83. Removal of the acetal formed the aldehyde 4.84 which was subjected to Lewis acids leading to Diels–Alder product in excellent yields with the cyclopentane ring having an exclusively cis–conformation. The Diels–Alder reaction established stereocenters on two additional carbons atom, setting a total of 4 stereocenters in one
step. Several Lewis acids catalyzed this reaction; however, BF$_3$OEt$_2$ was the most efficient promoter. Surprisingly, treatment of acetal 4.83 with 50% aqueous trifluoroacetic acid (TFA) at ambient temperature gave 4.85 in yields comparable to the original two-step sequence. The cyclic ether 4.85 was then converted to the 4.86 over several steps. Treatment of 4.86 with BF$_3$OEt$_2$ in benzene followed by regio- and stereoselective epoxidation to provide 4.87.

**Scheme 4–11**

Rigby’s Total Synthesis of Guaianolides: (±)–Dehydrocostus Lactone 4.86 and (±)–Estafiatin 4.87

![](image)

$^a$Reagents and conditions: (a) THF, 0 °C, 96% (b) NaBH$_4$, EtOH, 0 °C, (c) CH$_3$I, NaH, THF, 0 °C, 82% over 2 steps (d) 5% aq TFA, acetone (e) BF$_3$OEt$_2$, DCM, 0 °C, 92% (f) 50% aq TFA, DCM, 77% (g) BF$_3$OEt$_2$, DCM, 0 °C, 3 h (h) m-CPBA, DCM, 0 °C, 2.5 h, 35% over 2 steps.
More recently, Snider and co–workers used this methodology for the synthesis of (±)–deoxyxypenostatin A 4.88 which was used as a model system for the synthesis of penostatins A 4.89 and B 4.90.57–58

![Figure 4–8. (±)–Deoxyxypenostatin A 4.88, Penostatin A 4.89 and B 4.90.](image)

Treatment of the epoxycyclopentane 4.91 with Ph₃P=CH₂ followed by reaction with dienal 4.92 provided trienol 4.93. Trienol 4.93 was converted to partially hydrated glyoxylate 4.94 through several functional group interconversions. Yb(OTf)₃–catalyzed Diels–Alder reaction in the presence of 2,6–di–tert–butylpyridine, which was added to prevent isomerization, provided adduct 4.95. Reaction of lactone 4.95 with LiCH₂PO(OMe)₂ followed by Dess–Martin periodinane and treatment with NaH in THF afforded cyclohexenone 4.96. Isomerization of 4.96 with K₂CO₃ in MeOH afforded 4.88 in 28%.
Scheme 4–12* Snider’s Total Synthesis of (±)-Deoxyxynostatin A 4.88

\[ \text{Reagents and conditions: (a) Ph}_3\text{P} \equiv \text{CH}_2, \text{THF, LiBr, 25 °C, 16 h (b) sec--BuLi then 4.92 (c) BrCH}_2\text{COBr, pyr. (d) NaI, acetone (e) AgNO}_3, \text{CH}_3\text{CN (f) NaOAc, DMSO (g) Yb(OTf)}_3, \text{2,6-di--t--butylpyridine, CH}_3\text{CN, DCM, 25 °C, 18 h 21% from 4.93 (h) MePO(O\text{Me})}_2, \text{n--BuLi, THF, --78 °C (i) Dess--Martin periodinane, DCM (j) NaH, THF, --30 °C, 12 h, 55% over 3 steps (k) K}_2\text{CO}_3, \text{MeOH, 12 h, 25 °C, 28%} \]

This reaction can be applied to numerous other structures in literature. In addition, no systematic study has been conducted on some of these molecules. Therefore, we chose to further explore this reaction in the context of natural product synthesis.

Results and Discussion

The starting point for our macrolide synthesis was the commercially available 3--methoxyphenol 4.97 (Scheme 4–13). This molecule was converted to its corresponding benzoic acid derivative 4.99 by subjecting THP ether 4.98 to a metellation reaction with \text{n--butyllithium (n--BuLi)} in dry diethyl ether. Deprotonation of 4.98 is selective for the 2--position, the common ortho--site.\(^6^4\)
Scheme 4–13† Synthesis of Benzoic Acid 4.99

\[
\text{4.97} \xrightarrow{a} \text{4.98} \xrightarrow{b} \text{4.99}
\]

*Reagents and conditions: (a) 3,4-dihydro–2H–pyran, pyridinium p–toluenesulfonate, DCM, rt, 19 h, 72% (b) *i.* n–BuLi (2 eq), Et₂O, 0 °C to rt, 24 h *ii.* CO₂ *iii.* H₂O⁺, rt, 66%.

Kuhnert and co–workers³⁹ reported that using one equivalent of ether protected 4.98 to n–BuLi would be able to lithiate 4.98 which then can be used to synthesize 4.99. However when these conditions were employed, 4.99 was not formed Several solutions were explored to solve this problem. Attempts were made to isolate 4.100, the corresponding ester derivative of 4.99, by extracting the reaction mixture of the aryl lithiation reacted with solid CO₂ and subjecting it with methylation; however, formation of 4.100 was not observed. This prompted an investigation into whether aryllithium 4.101 was produced by quenching the reaction with D₂O to form the deuterated THP ether 4.102. Results showed that, in our hands, more than an equivalent of n–butyllithium was needed to form 4.101.

Scheme 4–14† *o–*Lithiation Reaction of THP Ether Protected 4.98

\[
\text{4.98} \xrightarrow{a} \text{4.100} \xrightarrow{b} \text{4.101} \xrightarrow{d} \text{4.102}
\]

*Reagents and conditions: (a) *i.* n–BuLi (2 eq), Et₂O, 0 °C to rt, 24 h *ii.* CO₂ *iii.* H₂O⁺, rt *iv.* Mel, DBU, DCM, rt, 24 h (b) n–BuLi (1 eq), Et₂O, 0 °C to rt, 24 h (c) n–BuLi (2 eq), Et₂O, 0 °C to rt, 24 h (d) D₂O.
Table 4–2. Ortholithiation of 4.98.

Chemoselective methylation of 4.99 was accomplished in high yield using 1,8–
diazabicyclo[5.4.0]undec–7–ene (DBU) and iodomethane.\textsuperscript{65} The phenol 4.100 was then
converted to its triflate 4.103, also in a good yield. (Scheme 4–15)

\textbf{Scheme 4–15}\textsuperscript{a} Synthesis of Triflate 4.103

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scheme415.png}
\caption{Synthesis of Triflate 4.103}
\end{figure}

\textsuperscript{a}Reagents and conditions: (a) MeI, DBU, DCM, rt, 24 h , 83% (b) Tf\textsubscript{2}O, pyridine, DCM, 24 h, rt, 86 %.

The triflate 4.103 was converted to allylbenzene 4.104 through a palladium
catalyzed coupling with allylttributylstannane.\textsuperscript{66} However, although the reaction was
successful, it required two days and provided 4.104 in a low yield (14%). Since the initial
synthesis of 4.104 gave 5% yield over–all in five steps, a second route was explored.
ortho–Metalation of methoxybenzoic acids\textsuperscript{67–68} can also be used to give 4.106, the carboxylic acid analogue of 4.104. Exploring this route, 2–methoxybenzoic acid 4.105 was subjected to ortho–metallation using sec–butyllithium at low temperature. The resulting bislithium anion was quenched with allylbromide to yield 4.106. (Scheme 4–16) This route, while more direct, also resulted in low yields (13\%) compared to the literature (40–50\%). In addition, the reaction proved challenging in that using this methodology required use of a mechanical stirrer at very low temperature under inert atmosphere with the solution freezes at −90 °C which interfered with stirring.

**Scheme 4–16\textsuperscript{a}** Synthesis of Allyl Ester 4.104 and Ally Acid 4.106

\textsuperscript{a}Reagents and conditions: (a) allyltributyltin, LiCl, Pd(PPh\textsubscript{3})\textsubscript{4}, 2,6–di–tert–butyl–4–methylphenol, 1,4–dioxane, reflux, 48 h, 14\% (b) sec–BuLi, TMEDA, allylbromide, THF, −90 °C to rt, 13\%.

Because of these issues, the effective director property for ortho–lithiation of tertiary amide\textsuperscript{69} was explored to synthesize 4.108, a tertiary amide derivative of 4.106. The acid 4.105 was converted to its corresponding diethylamide 4.107 using thionyl chloride and diethylamine.\textsuperscript{70} The amide was then subjected to ortho–lithiation using sec–BuLi and TMEDA in the presence of CuCN–LiCl complex followed by quenching with allyl bromide to form allyl amide 4.108 in 49\% over 2 steps.
Scheme 4–17\textsuperscript{a}  Synthesis of Allyl Diethylamide 4.108

\begin{align*}
\begin{array}{c}
\text{OMe} \\
\text{O Me} \quad \text{OH} \\
4.105
\end{array} & \xrightarrow{a} \\
\begin{array}{c}
\text{OMe} \\
\text{O Me} \\
4.107
\end{array} & \xrightarrow{b} \\
\begin{array}{c}
\text{OMe} \\
\text{O Me} \\
\text{NEt}_2 \\
4.108
\end{array}
\end{align*}

\textsuperscript{a}Reagents and conditions: (a) SOCl\textsubscript{2}, CHCl\textsubscript{3}, reflux, 24 h \textit{ii}. Et\textsubscript{2}NH, 24 h, 93\% (b) \textit{i}. sec–BuLi, TMEDA, THF, –78 °C, 30 min \textit{ii}. CuCN–LiCl, THF, –78 °C, 45 min \textit{iii}. allyl bromide, THF, –78 °C to rt, 4 h, 53\%.

Several oxidative cleavage procedures\textsuperscript{71–73} (Table 4–3) were explored to convert allyl acid 4.106 and allyl diethylamide 4.108 to their corresponding aldehydes 4.68 and 4.109, respectively. However, none of the cleavage reactions were successful and only resulted in formation of complex mixture.

\begin{align*}
\begin{array}{c}
\text{OMe} \\
\text{O Me} \\
4.106 \quad R = \text{OH} \\
4.108 \quad R = \text{NEt}_2
\end{array} & \xrightarrow{\text{conditions}} \\
\begin{array}{c}
\text{OMe} \\
\text{O Me} \\
4.68 \quad R = \text{OH} \\
4.109 \quad R = \text{NEt}_2
\end{array}
\end{align*}

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Reaction Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OH</td>
<td>OsO\textsubscript{4}, NaIO\textsubscript{4}, 2,6–lutidine, dioxane/H\textsubscript{2}O</td>
</tr>
</tbody>
</table>
| 2     | OH | a.) OsO\textsubscript{4}, Et\textsubscript{2}O/H\textsubscript{2}O  \\
|       |    | b.) NaIO\textsubscript{4}                   |
| 3     | OH | a.) NMO, OsO\textsubscript{4}, acetone  \\
|       |    | b.) NaIO\textsubscript{4}, H\textsubscript{2}O |
| 4     | NEt\textsubscript{2} | a.) NMO, OsO\textsubscript{4}, acetone  \\
|       |    | b.) NaIO\textsubscript{4}, H\textsubscript{2}O |
| 5     | NEt\textsubscript{2} | a.) NMO, OsO\textsubscript{4}, acetone  \\
|       |    | b.) Pb(OAc)\textsubscript{4}, H\textsubscript{2}O |

Table 4–3. Oxidative Cleavage of Allyl Acid 4.106 and Allyl Diethylamide 4.108.

This prompted us to investigate whether the formation of the intermediate diol 4.110 or its cleavage was the problematic step. Allyl diethylamide 4.108 was subjected to a dihydroxylation reaction using OsO\textsubscript{4}; however, an unidentifiable mixture was isolated.
(Scheme 4–18) Because of these problems, the o–metallated dianion of t–butylbenzamide 4.111 and added to a functionalized epoxide in an attempt to produce key intermediate 4.68. However, decomposition of the starting material was observed.

Scheme 4–18† Oxidative Cleavage of Allyl Ethylamidine 4.108 and o–Metalated Dianions of t–Butylbenzamides and Functionalized Epoxides for the Formation of Intermediate 4.68

†Reagents and conditions: (a) NMO, OsO₄, acetone (b) NaIO₄, H₂O (c) i. SOCl₂, CHCl₃, reflux, 24 h ii. t-BuNH₂, 16 h, 77% (d) i. n-BuLi, TMEDA ii. tert–butyldimethylsilyl glycidyl ether (e) TBAF (f) i. p–TsOH ii. NaOH, NaIO₄, H₂O.

Erickson74 has recently shown that aldehyde 4.68 readily forms orthoformates and acetals as shown in Figure 4–9. Revisitation of the NMR spectra of crude mixture from the oxidative cleavage OsO₄–NaIO₄ experiments for the synthesis of intermediate aldehyde 4.68 did show peaks at δ 5.80 and 3.22 which are evidence that the formation of cyclic pseudoacid 4.116 was observed.75
Figure 4–9. Pseudoacid Forms of 4.68.

Progress was also made toward the key intermediate 4.67 which was synthesized starting from 1,4–butanediol 4.117 as shown in Scheme 4–19. Monoprotection of 4.117 was carried out with the use of sodium hydride and BnBr to give rise to the monoprotected alcohol 4.118.\textsuperscript{76} Several oxidation protocols were explored, as shown in Table 4–4, to convert 4.118 to aldehyde 4.119; however, oxidation using pyridinium chlorochromate\textsuperscript{77} (PCC) generated the aldehyde 4.119 in the best yield and shortest reaction time.

Scheme 4–19a  
Synthesis of Aldehyde 4.119

\[
\begin{align*}
\text{HO–CH} & \overset{a}{\text{CH}} \quad \text{HO–CH} \\
4.117 & \quad \text{BnO–CH} \\
4.118 & \quad \text{BnO–CH} \\
4.119 & \\
\end{align*}
\]

\textsuperscript{a}Reagents and conditions: (a) \textit{i}. NaH, THF, 0 °C to rt, 2 h \textit{ii}. BnBr, THF, 0 °C to rt, 12 h, 84% (b) PCC, silica gel, DCM, rt, 2 h, 81%.
Entry | Oxidizing Agent / Additive | Solvents | Time | $t$ | Yield
---|---|---|---|---|---
1 | 2,2,6,6–<sub>T</sub>Tetramethylpiperidin–1–oxyl (TEMPO), KBr, NaOCl, NaHCO<sub>3</sub> | DCM | 20 min | rt | 15%
2 | oxalyl chloride, DMSO, Et<sub>3</sub>N | DCM | 2 h | −78 °C | 2%
3 | 2–Iodoxybenzoic acid (IBX) | EtOAc | 16 h | 80 °C | 0%*<br>4 | DMP, NaHCO<sub>3</sub> | DCM | 16 h | rt | 9%
5 | Pyridinium dichlorochromate (PDC) | DCM | 5 h | rt | —
6 | PCC, silica gel | DCM | 2 h | rt | 81%

*No conversion was observed.

**Table 4–4. Oxidation of Alcohol 4.118 to Aldehyde 4.119.**

Initially, we planned on using enantioselective allylation of aldehyde 4.119 using (+)–β–chlorodiisopinocampheylborane with allylmagnesium bromide<sup>78</sup> however the protocol proved difficult to reproduce. Alternatively, Grignard reaction of 4.119 using an allylmagnesium bromide provided racemic homoallylic alcohol 4.121 which was useful for model studies. (Scheme 4–20)

**Scheme 4–20**

**Allylation of Aldehyde 4.119**

<sup>a</sup>Reagents and conditions: (a) <i>i</i>. (−)–Ipc<sub>2</sub>BAcclyl, Et<sub>2</sub>O, 4 h, −78 °C to rt <i>ii</i>. MeOH, NaOH, H<sub>2</sub>O<sub>2</sub>, THF, 10 h, −90 °C to rt (b) allylmagnesium bromide, Et<sub>2</sub>O, −78 °C to rt, 77%.
Homoallylic alcohol 4.121 was protected by trimethylacetyl chloride to form pivalate 4.122.\textsuperscript{77} The pivalate was converted to aldehyde 4.123 by olefinic cleavage using OsO\textsubscript{4}–NaIO\textsubscript{4}.\textsuperscript{73} Wittig olefination of the aldehyde 4.123 using acetonylphosphonium chloride and LiHMDS did not result in the formation of the desired unsaturated ketone 4.124. The pivalate 4.123 was consumed; however, no identifiable product could be isolated. Alternatively, reaction of 4.123 to 1–(triphenylphosphoranylidene)acetone using sodium carbonate gave unsaturated ketone 4.124 and diene 4.125 in a 2:1 ratio. (Scheme 4–21)

Scheme 4–21\textsuperscript{a}  
Wittig Olefination for the Synthesis of 4.124

\textsuperscript{a}Reagents and conditions: (a) trimethylacetyl chloride, 4–DMAP, pyridine, rt, 24 h, 91% (b) i. NMO, OsO\textsubscript{4}, acetone, rt, 24 h ii. NaIO\textsubscript{4}, H\textsubscript{2}O, rt, 12 h, 64% (c) i. acetonylphosphonium chloride, LiHMDS, 0 °C to rt ii. reflux, 14 h (d) 1–(triphenylphosphoranylidene)acetone, THF, reflux, 33% (2:1).
In order to avoid this elimination reaction, an alternative route was explored. The homoallylic alcohol 4.121 was subjected to an oxidative cleavage in attempt to synthesize aldehyde 4.127. Aldehyde 4.127, which has a hydroxyl moiety that is a poorer leaving group than the pivalate moiety present in 4.123, would be subjected to an olefination reaction. However, formation of the aldehyde 4.127 was not observed. Vinylogous Aldol addition\textsuperscript{79} reaction of 4.130 and 4.119 were also explored to synthesize 4.131, however complex mixture was observed. (Scheme 4–22)

**Scheme 4–22** Vinylogous Aldol Addition of 4.130 and 4.119

\[\text{Scheme 4–22}\]

- Reagents and conditions: (a) NMO, OsO\textsubscript{4}, acetone (b) NaIO\textsubscript{4}, H\textsubscript{2}O (c) Et\textsubscript{3}N, morpholine, benzene, 0 °C, 1 h, 72% (d) KHMDS, THF, TBSCI, DCM, –78 °C to rt, 45 min, 15% (e) BF\textsubscript{3}·OEt\textsubscript{2}, DCM, –78 °C to rt.

Alternatively, construction of 4.124 and its corresponding free alcohol 4.132 could be formed using a Grubbs catalyzed olefin metathesis with methyl vinyl ketone.\textsuperscript{80}

While 4.132 was formed in only 25% yield no elimination product was observed, so further optimization of this reaction seemed warranted. (Scheme 4–23)
Scheme 4–23<sup>a</sup> Olefin Metathesis for the Synthesis of α,β–Unsaturated Ketones 4.124 and 4.132

4.122

4.121

<sup>a</sup>Reagents and conditions: (a) methyl vinyl ketone, Grubbs Catalyst (1<sup>st</sup> Generation), DCM, rt, 18 h (b) methyl vinyl ketone, Grubbs Catalyst (3<sup>rd</sup> Generation), DCM, rt, 17 h (c) methyl vinyl ketone, Grubbs Catalyst (1<sup>st</sup> Generation), DCM, rt, 53 h (d) methyl vinyl ketone, Grubbs Catalyst (2<sup>nd</sup> Generation), DCM, rt, 25%.

At this juncture, Panek published an improved synthesis of 4.9 that called for a revision on the synthetic route.<sup>22</sup> Apicularen A can also be synthesized through an advanced intermediate 4.133 which is similar but contains fewer less carbon than our target advanced intermediate 4.65. Panek showed that through Takai iodoolefination of the aldehyde 4.134 would result to the formation of the vinyl ketone 4.135. Deprotection followed by copper(I) thiophene–2–carboxylate (CuTC) catalyzed substitution of 4.136 with (2Z,4Z)–hepta–2,4–dienamide 4.137 gave rise to 4.9. (Scheme 4–24)
Scheme 4–24a  Panek’s Synthetic Sequence for the Completion of the Synthesis of Apicularen A

\[ \text{Scheme 4–24} \]

a. Reagents and conditions: (a) CrCl$_2$, HCl, THF/dioxane, 0 °C to rt, 24 h, 85% (E:Z = 6:1) (b) BCl$_3$, DCM, –78 °C, 8 h, 80% (c) 4.136, 4.137, CuTC, Rb$_2$CO$_3$, DMA, 58 °C, 5 h, 40% (E:Z = 8:1).

Taking this method for installation of the side chain into account, the synthetic approach to 4.9 was revised. The synthetic approach to the aldehyde 4.141, shown in Figure 4–10, is through coupling of alcohol 4.138 and carboxylic acid 4.68. Intramolecular hetero Diels–Alder reaction followed by selective deprotection and oxidation was expected to result to 4.141.
Homoallylic alcohol 4.145 was constructed by monoprotection of diol 4.142 with BnBr followed by oxidation using PCC and Grignard reaction with allylmagnesium chloride. (Scheme 4–25)

Scheme 4–25* Synthesis of Homoallylic Alcohol 4.145

*aReagents and conditions: (a) i. NaH, THF, rt, 1 h ii. BnBr, THF, reflux, 12 h, 72% (b) PCC, silica gel, DCM, rt, 3 h, 62% (c) allylmagnesium chloride, THF, −78 °C, 2 h, 63%.

Homoallylic alcohol 4.145 was protected with a pivalate group. Construction of the α,β–unsaturated ketone 4.148 could be achieved by subjecting the pivalate 4.146 to
an oxidative cleavage followed by a Horner–Wadsworth–Emmons (HWE) reaction.\textsuperscript{81} However, a 2\textsuperscript{nd} generation Grubbs–Hoveyda catalyzed olefin metathesis of 4.146 with methyl vinyl ketone afforded 4.148 in a high yield. (Scheme 4–26)

\textbf{Scheme 4–26\textsuperscript{a}} Synthesis of \(\alpha,\beta\)–Unsaturated Ketone 4.148

\begin{center}
\begin{tikzcd}
\text{BnO} \quad \text{4.145} & \quad \begin{array}{c}
\text{OH} \\
\text{OPiv}
\end{array} \\
\text{4.146} & \quad \begin{array}{c}
\text{BnO} \\
\text{OPiv}
\end{array} \\
\quad \text{4.147} \\
\quad \text{4.148}
\end{tikzcd}
\end{center}

\textsuperscript{a}Reagents and conditions: (a) trimethylacetyl chloride, 4–DMAP, pyridine, rt, 24 h, 36\% (b) \textit{i}. NMO, OsO\textsubscript{4}, acetone, rt, 24 h \textit{ii}. NaIO\textsubscript{4}, H\textsubscript{2}O, rt, 12 h, 64\% (c) dimethyl 2–oxopropyl phosphonate, NaH, THF (d) methyl vinyl ketone, Grubbs–Hoveyda Catalyst, 2\textsuperscript{nd} Generation, DCM, rt, 24 h, 92\%.

\textbf{Conclusion and Future Work}

Apicularen A is a macrosalicylic antitumor natural product that has been isolated from various strains of the myxobacterial genus \textit{Chondromyces}. This molecule showed potent cytotoxicity against several cancer cell lines. Because of its biological activity as well as its interesting structural features, several research groups have reported the syntheses of 4.9.

A new type of Diels–Alder reaction for the synthesis of 4.9 was explored. The macrosalicylic salicylate ester core and the embedded \textit{trans}–tetrahydropyrany ring of 4.9 were envisioned to come from coupling of alcohol 4.67 and acid 4.68 followed by an intramolecular cycloaddition. Several routes were explored for the synthesis of 4.68 which includes Stille coupling or \(o\)–metallation of \(o\)–methoxybenzoic acids and tertiary amides followed by an oxidative cleavage. Although in some cases the pseudoacid 4.116

\section*{233}
were observed, the synthetic precursor for 4.68 such as 4.106 and 4.108 as well as the oxidative cleavage of this precursor proved to be difficult to synthesize.

The synthesis of the key intermediate 4.67 was also explored starting from diol 4.117. Monoprotection of 4.117 followed by an oxidation gave aldehyde 4.119. Enantioselective allylation of 4.119 were attempted however this protocol was difficult to reproduce. Alternatively, Grignard reaction of 4.119 with allylmagnesium bromide provided racemic 4.121. The racemate 4.121 were converted into pivalate followed by an oxidative cleavage which formed aldehyde 4.123. Wittig olefination of 4.123, however, gave a mixture of inseparable 4.124 and 4.125. Because of the unwanted elimination reaction, alternative route were explored such vinylogous Aldol addition as well as olefin metathesis. Although the olefin metathesis only gave a 25% of the desired product, this reaction did not provide any elimination product.

Alternatively, Panek published an improved synthesis for 4.9 which lead for the revision on our synthetic route. The molecule 4.9 can be synthesized on an advanced intermediate 4.133 which is similar but contains one less carbon than the original target advanced intermediate 4.65. Monoprotection of diol 4.142 followed by oxidation and Grignard reaction gave alcohol 4.145. The alcohol was protected with a pivalate group followed by a Grubbs–Hoveyda catalyzed olefin metathesis provided the desired 4.148.

The future work involves the conversion of the α,β–unsaturated ketone 4.148 to the tert–butylsilyl enolate 4.149 followed by deprotection to afford 4.150. Alcohol 4.150 will be coupled to the benzoic acid 4.68 followed by intramolecular Diels–Alder reaction to afford the advanced intermediate 4.152. (Figure 4–11)
Experimental Section

General Experimental Procedure

Reactions: Except as otherwise noted, reactions were carried out under argon atmosphere in an oven– or flame–dried glasswares. Flasks were flame dried under a stream of nitrogen or argon or under vacuum. Pyridine and triethylamine were distilled from calcium hydride while 1,4– dioxane was distilled from sodium. Reaction solvents were dried by a solvent purification apparatus (benzene, dichloromethane (DCM), tetrahydrofuran (THF), and diethylether using alumina as the drying agent. $n$–BuLi (2.5 M in cyclohexane) and sec–BuLi (1.4 M in cyclohexane) were titrated prior to use according to the procedure reported in literature.$^{82}$ All other reagents were reagent grade and were purified as necessary.

Methods: Analytical thin layer chromatography (TLC) was performed using 250 μm commercial silica gel plates (EMD Chemicals, silica gel 60 F$_{254}$) and visualized by UV illumination or by exposure to potassium permanganate or $p$–anisaldehyde solution followed by heating using a heat gun. Flash chromatography was carried using commercially available 40–60 μm silica gel (Silicycle).
Identity: Melting points were recorded using an Electrothermal® Melting Point Apparatus in open capillaries and are uncorrected. Infrared spectra were obtained using Thermo Nicolet IR100 and IR200 spectrometers. Proton and carbon nuclear magnetic resonance (\(^1\)H and \(^13\)C NMR) were recorded using Bruker DPX–300 spectrometer. Chemical shifts are reported in delta (δ) units, downfield from tetramethylsilane (TMS) with reference to the CDCl\(_3\) solvent signal (\(^1\)H, 7.26; \(^13\)C, 77.23) Coupling constants are reported in Hertz (Hz). Electron impact and high–resolution mass spectra were obtained from a Bruker 12 APEX–Qe FTICR–MS instrument with an Apollo II ion source in the College of Science Major Instrumentation (COSMIC) at Old Dominion University at Northfolk, VA 23529.

Experimental

![Chemical Reaction](image)


To a solution of 3–methoxyphenol 4.97 (15.00 g, 120.8 mmol) and pyridinium p–toluenesulfonate (9.18 g, 48.3 mmol) in DCM (130 mL), 3,4–dihydro–2\(H\)–pyran 4.97 (11.17 g, 132.8 mmol) was added dropwise. The reaction mixture was stirred continuously at room temperature overnight. The solvent was removed under vacuo and the residue was partitioned between ethyl acetate (210 mL) and 5% sodium bicarbonate solution (180 mL). The organic layer was collected, dried over sodium sulfate, and concd under reduced pressure. Purification using flash column chromatography (5–15% ethyl acetate in hexane) yielded 4.98 as a clear colorless oil (18.13 g, 72%).

4.98. TLC \(R_f\) = 0.56 (hexane:ethyl acetate, 4:1); IR (film): 2944, 2874, 2851, 1263, 1198, 1152 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) δ 7.18 (t, \(J = 8.1\) Hz, 1H), 6.68 (dd, \(J = 1.5, 0.9\) Hz , 1H), 6.63–6.67 (m, 1H), 6.55 (ddd, \(J = 8.3, 3.3, 0.7\) Hz, 1H), 5.41 (t, 1H, \(J = 3.2\) Hz, 1H), 2.36 (q, 1H, \(J = 7.2\) Hz, 1H), 2.27 (t, \(J = 7.2\) Hz, 1H), 2.18 (q, \(J = 7.2\) Hz, 1H), 2.09 (t, \(J = 7.2\) Hz, 1H).
Hz.), 3.92 (dt, $J = 11.3, 3.2$ Hz, 1H), 3.79 (s, 3H), 3.57–3.64 (m, 1H), 1.55–2.04 (m, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 160.9, 158.5, 129.4, 108.8, 107.4, 102.9, 96.6, 62.2, 55.4, 30.5, 25.4, 19.0.

2–Hydroxy–6–methoxybenzoic acid (4.99).


$n$–Butyllithium (2.08 M in hexane, 55.53 mL) was added dropwise to a cooled solution of 4.98 in diethyl ether (22 mL) at 0 °C. The reaction mixture was stirred continuously for 24 h and then allowed to warm up to room temperature. The resulting solution was added to crushed dry ice (31 g) using a syringe and was diluted with diethyl ether (12 mL). The excess dry ice was allowed to sublime. The solution was extracted with H$_2$O (3 x 10 mL) and the aqueous layer was collected and acidified using coned hydrochloric acid until the solution’s pH was equal to 1. The resulting white solid was collected and washed with cold H$_2$O. The solid was air dried to afford 4.99 as an off–white solid (4.29 g, 66%).

4.99. TLC $R_f$ = 0.24 (petroleum ether:ethyl acetate, 1:1); IR (KBr) 3421, 3154, 1689, 1580, 1508, 1241 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 12.17 (s, 1H), 11.33 (bs, 1H), 7.42 (t, $J = 8.4$ Hz, 1H), 6.73 (dt, $J = 8.5, 0.8$ Hz, 1H), 6.50 (d, $J = 8.4$ Hz, 1H), 4.07 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 171.1, 164.3, 158.6, 135.8, 112.4, 101.6, 101.5, 57.3.

Methyl 2–hydroxy–6–methoxybenzoate (4.100).


2–Hydroxy–6–methoxybenzoic acid 4.99 (1.07 g, 6.36 mmol) was dissolved in THF (34 mL). 1,8–Diazabicyclo[5.4.0]undec–7–ene (0.95 mL, 6.40 mmol) was added dropwise
followed by iodomethane (0.39 mL, 6.36 mmol). The reaction mixture was stirred continuously at room temperature for 24 h. The resulting iodide amine salt was filtered off and the filtrate was concd under reduced pressure. Purification by flash column chromatography using 20% ethyl acetate in hexane afforded **4.100** as a light yellow solid (0.96 g, 83%).

**4.100.** TLC *R*<sub>f</sub> = 0.74 (petroleum ether/ethyl acetate, 1:1); IR (KBr) 3415, 3011, 2956, 1654, 1258, 1234 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 11.47 (s, 1H), 7.28 (t, *J* = 8.4 Hz, 1H), 6.56 (dd, *J* = 7.5, 0.9 Hz, 1H), 6.37 (d, *J* = 8.4 Hz, 1H), 3.91 (s, 3H), 3.81 (s, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 171.7, 163.7, 161.0, 135.1, 109.9, 103.1, 102.6, 55.9, 52.2.

Trifluoromethanesulfonic anhydride (5.00 g, 17.7 mmol) was added dropwise to a solution of methyl 2–hydroxy–6–methoxybenzoate **4.102** (3.22 g, 17.7 mmol) in pyridine (55 mL). The reaction mixture was stirred continuously at room temperature. After approximately 22 h, it was quenched with H<sub>2</sub>O (161 mL). A solution of HCl (184 mL, 2 N) was added followed by acidification using conc. HCl until the solution’s pH reached 1. The reaction mixture was extracted with ethyl acetate (3 x 161 mL). The organic layers were collected, combined, washed with sat. NaHCO<sub>3</sub>, and dried over magnesium sulfate. The dried organic layers were concd under reduced pressure which afforded **4.103** as red orange oil (4.78 g, 86%).

**4.103.** TLC *R*<sub>f</sub> = 0.29 (hexane:ethyl acetate, 4:1); IR (film) 3013, 2957, 2846, 1613, 1425, 1075 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.45 (t, *J* = 8.4 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 3.94 (s, 3H), 3.89 (s, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 163.7, 158.5, 147.0, 132.2, 120.8, 117.5, 113.5, 111.5, 56.6, 52.9.
Methyl 2–Allyl–6–methoxybenzoate (4.104).


To a solution of 4.103 dissolved in a freshly distilled 1,4–dioxane (2.41 mL), allyltributyltin 4.154 (0.17 mL, 0.55 mmole), LiCl (0.07 g, 1.59 mmole), Pd(PPh₃)₄ (0.01 g, 0.01 mmole), and a few crystals of 2,6–di–tert–butyl–4–methylphenol 4.155 were added. The solution was heated to reflux for 48 h. A solution of KF·2H₂O (0.54 mL, 0.76 mmole) was added and the resulting solution was stirred at room temperature for 17 h. The reaction mixture was diluted with diethyl ether (20 mL). The organic layer was separated from the aqueous layer and washed with H₂O, HCl (1 N), and sat. aq. NaCl. The organic layer was dried using magnesium sulfate and the solvent was removed under reduced pressure. Purification by flash column chromatography using 5–10–20–50% ethyl acetate in hexane afforded 4.104 (0.014 g, 14%) as a light yellow orange oil. This compound has been synthesized previously.⁸³

4.104. TLC Rₜ = 0.79 (petroleum ether:ethyl acetate, 1:1); IR (film) 3072, 3003, 2950, 2840, 1733, 1265 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.30 (d, J = 7.8 Hz, 1H), 6.78–6.84 (m, 2H), 5.83–5.97 (m, 1H), 5.02–5.09 (m, 2H), 3.89 (s, 3H), 3.82 (s, 3H), 3.35 (dt, J = 4.0, 1.3 Hz, 2H).
2–Allyl–6methoxybenzoic acid (4.107).


In a dried 100 mL three-neck round bottom flask equipped with a thermometer, mechanical stirrer, and a dropping funnel, N,N,N,N–tetramethylethylenediamine (4.35 mL, 28.8 mmol) was dissolved in THF (5.04 mL). The flask was flushed with argon and cooled to −90 °C using hexane/liquid N₂ cold bath. sec–Butyllithium (sec–BuLi) (20.6 mL, 1.4 M in cyclohexane) was added dropwise maintaining an internal temperature of −90 °C. The resulting yellow solution was stirred further for 30 min before adding a solution of 2–methoxybenzoic acid 4.105 (2.00 g, 13.1 mmol) in THF (6 mL) dropwise for over 30 min. The reaction mixture was stirred further as it warmed up to −78 °C until the color of the solution turned orange. Allyl bromide 4.156 (4.68 mL, 53.7 mmol) was added and stirred for an hour at −78 °C. The reaction was stirred continuously for another 30 min and then quenched with H₂O (25 mL). The aqueous layer was separated from the organic layer and it was extracted with diethyl ether (2 x 11 mL). The aqueous layer was collected and acidified with HCl (6 N, 17 mL) followed by extraction using DCM (3 x 10 mL). The organic layer was collected, combined, and dried over magnesium sulfate. The solvent was removed under vacuo and was purified through flash column chromatography using 30–50% ethyl acetate in hexane afforded 4.106 (0.34 g, 13%) as a clear light yellow crystal.

4.106. TLC RF = 0.30 (hexane:ethyl acetate, 4:1); IR (film) 3420, 2960, 2656, 1699, 1293, 1265 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 10.54 (broad, 1H), 7.33 (t, J = 8.4 Hz, 1H), 6.88 (d, J = 7.5 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 5.97 (m, 1H), 5.12 (m, 2H), 3.88 (s, 3H), 3.53 (d, J = 6.6 Hz, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 173.7, 156.9, 139.4, 136.5, 131.3, 122.4, 122.3, 116.6, 109.4, 56.2, 38.0.
N,N-diethyl–2–methoxybenzamide (4.107).

To a solution of o–anisic acid 4.105 (3.00 g, 19.7 mmol) in CHCl₃ (150 mL), thionyl chloride (3.60 mL, 49.3 mmol) was added dropwise. The solution was heated to reflux for 24 h. The excess SOCl₂ was removed by distillation. Diethylamine (5.12 mL, 49.3 mmol) was added and the resulting solution was stirred at room temperature overnight. The reaction was quenched with aq. HCl (25 mL, 2 M) and extracted with ethyl acetate (3 x 50 mL). The organic layers were combined, washed with NaOH (3 x 50 mL, 2 M), dried over sodium sulfate, and concd under reduced pressure. Purification with flash column chromatography afforded 4.107 (3.79 g, 93%) as a light yellow oil.

4.107. TLC R₇ = 0.33 (hexane:ethyl acetate, 1:1); ¹H NMR (300 MHz, CDCl₃) δ 7.32 (dq, J = 7.0, 1.8 Hz, 1H), 7.18 (dd, J = 7.4, 1.8 Hz, 1H), 6.96 (dt, J = 7.5, 0.9 Hz, 1H), 6.90 (d, J = 8.3 Hz, 1H), 3.81 (s, 3H), 3.57 (t, J = 5.2 Hz, 2H), 3.14 (q, J = 7.1 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H), 1.03 (t, J = 7.1 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 168.9, 155.3, 130.0, 127.6, 120.8, 111.1, 55.6, 42.9, 38.9, 14.1, 13.0.

N,N-diethyl–2–allyl–6–methoxybenzamide (4.108)

To a solution of TMEDA (0.40 mL, 2.65 mmol) in THF (14 mL) at −78 °C, sec–BuLi (0.31 M in hexane, 8.55 mL) was added. The solution was stirred continuously for 10 min before adding a solution of 4.107 (0.50 g, 2.41 mmol) in THF (4.1 mL). The resulting dark orange solution was continuously stirred. After 30 min, CuCN (0.24 g, 2.65 mmol) and LiCl (0.11 g, 2.65 mmol) in THF (4.1 mL) were added and the solution was stirred for another 45 min. Allyl bromide 4.156 was then added dropwise and the reaction mixture was cooled and stirred at −78 °C for the next 2 h. After 2 h, the reaction
mixture was allowed to warm up to room temperature as it was stirred continuously for another 2 h. The reaction mixture was then quenched by adding H₂O (20 mL) and was diluted with diethyl ether (30 mL). The layers were separated and the organic layer was washed with sat. aq. NH₄Cl (3 x 30 mL) and sat. aq. NaCl (3 x 30 mL), dried over magnesium sulfate and concd under reduced pressure. Flash column chromatography using 40–50–60% ethyl acetate in hexane afforded **4.108** (0.32 g, 53%) as a light yellow oil. This compound has been synthesized previously.³⁴

**4.108.** TLC *R*ₖ = 0.31 (hexane:ethyl acetate, 1:1); ¹H NMR (300 MHz, CDCl₃) δ 7.22 (d, *J* = 8.0 Hz, 1H), 6.85 (d, *J* = 7.7 Hz, 1H), 6.75 (d, *J* = 8.3 Hz, 1H), 5.86–5.99 (m, 1H). 4.97–5.11 (m, 2H), 3.79 (s, 3H), 3.28–3.44 (m, 2H), 3.00–3.17 (m, 2H), 1.25 (dt, *J* = 7.2, 0.4 Hz, 3H), 1.03 (dt, *J* = 7.3, 0.4 Hz, 3H).

![Chemical Structure](image)

**N–(1,1–dimethylethyl)–2–methoxybenzamide (4.111).**

To a solution of o–anisic acid **4.105** (1.00 g, 6.57 mmol) in CHCl₃ (150 mL), thionyl chloride (1.20 mL, 16.4 mmol) was added. The solution was heated to reflux for 24 h. The excess SOCl₂ was removed through distillation. t–Butylamine (1.73 mL, 16.4 mmol) was added and the resulting solution was stirred at room temperature overnight. The reaction was quenched with aq. HCl (10 mL, 2 M) and the mixture was extracted with ethyl acetate (3 x 50 mL). The organic layers were combined, washed with NaOH (3 x 50 mL, 2 M) and dried over sodium sulfate. The dried solution was concd under reduced pressure and purified with flash column chromatography to afford **4.111** (1.05 g, 77%) as a light yellow oil.

**4.111.** TLC (hexane:ethyl acetate, 1:1) *R*ₖ = 0.77; ¹H NMR (300 MHz, CDCl₃) δ 8.18 (dd, *J* = 5.9, 1.9 Hz, 1H), 7.83 (bs, 1H), 7.41 (qt, *J* = 8.2, 0.9 Hz, 1H), 7.07 (dt, *J* = 7.3, 1.0, 0.6 Hz, 1H), 6.95 (d, *J* = 8.3 Hz, 1H), 3.95 (s, 3H), 1.46 (s, 9H).
**tert–Butyldimethylsilyl glycidyl ether (4.157).**

TBSCl (0.52 g, 3.42 mmol) and imidazole (0.26 g, 3.76 mmol) were dissolved in DCM (15 mL). The resulting cloudy solution was cooled at 0 °C using an ice/H2O bath. Glycidol 4.157 (0.25 mL, 3.76 mmol) was added and the solution was stirred continuously for 12 h. H2O (20 mL) was added and the aqueous layer was separated from the organic layer. The aqueous layer was extracted with DCM (3 x 20 mL). The organic layers were combined, dried over NaSO4, and concd under reduced pressure. Purification using flash column chromatography afforded 4.158 (0.43 g, 67%) as a colorless oil. This compound has been synthesized previously.77

4.158 TLC Rf = 0.67 (hexane:ethyl acetate, 4:1); 1H NMR (300 MHz, CDCl3) δ 3.85 (dd, J = 8.7, 3.2 Hz, 1H), 3.66 (dd, J = 7.1, 4.7 Hz, 1H), 3.06–3.11 (m, 1H), 2.76 (dd, J = 4.1, 1.1 Hz, 2H), 2.63 (q, J = 2.7 Hz, 1H) 0.90 (s, 9H), 0.08 (s, 6H).

**4–Benzyloxy–butan–1–ol (4.118).**


To a cooled suspension of NaH (60%, 2.27 g, 56.7 mmol) in THF (200 mL) at 0 °C, a solution of 1,4–butanediol 4.117 (24.98 g, 277.1 mmol) in THF (94 mL) was added. The reaction mixture was stirred at 0 °C for 10 min and continuously stirred as it warmed up at room temperature. After 2 h, the resulting solution was cooled at 0 °C using an ice/H2O bath before adding a solution of benzyl bromide (9.48 g, 55.4 mmol) in THF (74 mL). The reaction was stirred at 0 °C for 10 min and continuously stirred as it warmed up at room temperature. After approximately 12 h, the solution was quenched with sat. NH4Cl solution (60 mL). The organic layer was collected and the aqueous layer was extracted with ethyl acetate (3 x 150 mL). The organic layers were combined, dried over sodium
sulfate, and concd under vacuo. Purification by flash column chromatography using 40–60% ethyl acetate in hexane as eluent afforded 4.118 (8.43 g, 84%) as a clear colorless oil.

4.118. TLC \( R_f = 0.10 \) (hexane:ethyl acetate, 4:1); IR (film) 3294, 3088, 3063, 2941, 1257, 1205 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.28–7.38 (m, 5H), 4.52 (s, 2H), 3.66 (q, \( J = 5.4 \) Hz, 2H), 3.53 (t, \( J = 6.0 \) Hz, 2H), 2.17 (bs, 1H), 1.61–1.77 (m, 2H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 138.4, 128.6, 127.9, 127.8, 73.2, 70.5, 62.6, 30.1, 26.7.

![Diagram 4.118](image)

4–Benzyloxy–butyraldehyde (4.119).


To a mixture of pyridinium chlorochromate (23.93, 111.0 mmol) and silica gel (23.93 g) in DCM (191 mL), a solution of 4.118 (10.00 g, 55.5 mmol) dissolved in DCM (68 mL) was added. The reaction mixture was stirred at room temperature for about 2.5 h. The reaction mixture was then filtered through a plug of silica eluting with DCM to afford 4.119 (8.01 g, 81%) as clear colorless oil.

4.119. TLC \( R_f = 0.42 \) (hexane:ethyl acetate, 1:1); IR (film) 3088, 3064, 2932, 2852, 1728, 1205 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 9.79 (t, \( J = 1.6 \) Hz, 1H), 7.28 (m, 5H), 4.49 (s, 2H), 3.51 (t, \( J = 6.1 \) Hz, 2H), 2.55 (dt, \( J = 7.1, 1.6 \) Hz, 2H), 1.55 (quint, \( J = 6.0 \) Hz, 2H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 202.6, 138.4, 128.6, 73.1, 69.3, 41.1, 22.7.

![Diagram 4.119](image)


Allylmagnesium bromide 4.159 (1.0 M in diethyl ether, 0.89 mL) was added dropwise to a solution of 4.119 (0.11 g, 0.59 mmole) in dry diethyl ether (2 mL) that was pre–cooled at −78 °C in an acetone/dry ice cold bath. The reaction was allowed to stir at −78 °C and
was continuously stirred as it warmed up to room temperature. After 2 h, the solution was cooled again at −78 °C before adding an additional amount of allylmagnesium bromide (0.59 mL, 0.59 mmole). The reaction was stirred at −78 °C and stirred continuously as it warmed up at room temperature. After approximately 1 h, the reaction mixture was quenched with sat. aq. NH₄Cl solution (10 mL). The organic layer was separated from the aqueous layer and the aqueous layer was extracted with diethyl ether (3 x 10 mL). The organic layers were collected, combined, dried over magnesium sulfate, and were concd under reduced pressure which afforded 4.121 (0.10 g, 77%) as a light orange oil. No further purification was needed.

4.121. TLC R₇ = 0.39 (hexane:ethyl acetate, 4:1); IR (film) 3431, 3069, 3030, 2927, 2858, 1205 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.25–7.35 (m, 5H), 5.76–5.90 (m, 1H), 5.07–5.14 (m, 2H), 4.50 (s, 2H), 3.65 (sept, J = 4.2 Hz, 1H). 3.50 (t, 2H, J = 6.0 Hz), 2.79 (bs, 1H), 2.13–2.28 (m, 1H), 1.57–1.80 (m, H), 1.41–1.54 (m, 1H), 1.18–1.27 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 138.4, 135.3, 128.5, 127.8, 127.8, 117.7, 73.1, 70.7, 70.6, 42.1, 34.0, 26.3.

![Chemical structure of 4.121](image)


Homoallylic alcohol 4.121 (0.06 g, 0.27 mmol) was dissolved in pyridine (0.14 mL). Into the resulting clear solution, 4–dimethylaminopyridine (0.007 g, 0.05 mmole) was added followed by trimethylacetylchloride 4.160 (0.04 mL, 0.30 mmol). The resulting solution was stirred continuously and the reaction was monitored by TLC. After approximately 24 h, the reaction mixture was quenched by the addition of H₂O (15 mL). The quenched reaction mixture was extracted with diethyl ether (3 x 10 mL). The organic layers were collected, combined, washed with H₂O (3 x 10 mL) and sat. sodium chloride (3 x 10 mL), dried over magnesium sulfate and concd under reduced pressure. The residue was purified by flash column chromatography using 15–20% ethyl acetate in hexane as eluent which afforded 4.122 (0.08 g, 91%) as an oil.
2,2–Dimethyl–propionic acid 4–benzyloxy–1–(2–oxo–ethyl)–butyl ester (4.123)

N–Methylmorpholine–N–oxide (0.81 g, 3.19 mmol) was added into a solution of 4.122 (0.90 g, 2.95 mmol) in acetone (10.5 mL). Osmium tetroxide (2.5 % solution in t–BuOOH, 0.5 mL, 0.04 mmol) was added and the resulting solution was stirred continuously at room temperature. The reaction was monitored by TLC and after 2 h, additional osmium tetroxide (0.2 mL, 0.02 mmol) was added. After 24 h, the solution was diluted by adding ethyl acetate (10 mL). The solution was washed with HCl (1 N, 3 x 15 mL), dried over sodium sulfate, and concd under reduced pressure. The residue was dissolved in THF (22.7 mL) and a solution of sodium periodate (1.16 g, 5.43 mmol) in H₂O (25 mL) was added. The solution was continuously stirred at room temperature. The solvent was removed under reduced pressure after 12 h and the residue was poured into H₂O (50 mL). The water layer was then extracted with DCM (3 x 25 mL) and the organic layers were collected, combined, dried over sodium sulfate, and concd under reduced pressure. Purification using flash column chromatography using 40–60% ethyl acetate in hexane as eluent to afford 4.123 (0.58 g, 64%) as an oil.

4.123. TLC Rₐ = 0.81 (hexane:ethyl acetate, 1:1); ¹H NMR (300 MHz, CDCl₃) δ 9.71 (dt, J = 2.4, 0.6 Hz, 1H), 7.26–7.37 (m, 5H), 5.31 (quint, 1H, J = 6.0 Hz), 4.94 (s, 2H), 3.48 (quint, J = 6.0 Hz, 2H), 2.62–2.65 (m, 2H), 1.56–1.78 (m, 4H), 1.19 (s, 9H).
1–(triphenylphosphoranylidene)acetone (4.162)


Acetonilorphosphonium chloride 4.161 (3.13 mmol, 1.11 g) was added to a solution of Na$_2$CO$_3$ (16.7 mmol, 1.40 g) in H$_2$O (14 mL). The solution was stirred continuously at room temperature. After 26 h, the resulting solid was vacuum filtered, washed with H$_2$O and dried under vacuum to afford 4.162 as white solid (0.96 g, 96%).

4.162. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.62–7.69 (m, 5H), 7.51–7.58 (m, 5H), 7.42–7.49 (m, 5H), 3.70 (d, $J$ = 27.1 Hz, 1H), 2.09 (d, $J$ = 1.8 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 190.7, 190.6, 133.0, 132.9, 131.9, 131.9, 128.8, 128.6, 52.3, 50.9, 28.6, 28.4.

(E)–4–(1–Oxo–2–butenyl)morpholine (4.129)

To a cooled solution of morpholine 4.163 (0.76 mL, 8.70 mmol) in benzene (40 mL), Et$_3$N (1.21 mL, 8.70 mmol) was added followed by a solution of trans–crotonyl chloride 4.128 (0.92 mL, 9.60 mmol) in benzene (8.70 mL). The resulting cloudy solution was stirred continuously at 0 °C. After 1 h, the solvent was removed under reduced pressure and the resulting residue was dissolved in ethyl acetate before the solution was filtered. The filtrate was concd under reduced pressure and purification with flash column chromatography using 70–80–90% ethyl acetate in hexane to yield 4.129 (1.05 g, 79%) as a light yellow oil. This compound has been synthesized previously.

4.129. TLC $R_f$ = 0.11 (hexane:ethyl acetate, 1:1); $^1$H NMR (300 MHz, CDCl$_3$) δ 6.84–6.96 (m, 1H), 6.22 (qd, 1H, $J$ = 15.0, 1.7 Hz), 3.68 (bs, 8H), 1.88 (dd, $J$ = 6.9, 1.7 Hz, 3H).
(Z)–1–[1–(tert–Butyldimethylsilanyloxy)–1,3–butadienyl]morpholine (4.130)


A solution of **4.129** (0.50 g, 2.95 mmol) in THF (15 mL) was cooled at −78 °C using a dry ice/acetone cold bath. KHMDS (0.5 M in toluene, 6.50 mL) was added in a dropwise fashion and the resulting yellow solution was continuously stirred at −78 °C for 40 min. A solution of TBSCI (0.49 g, 3.25 mmol) in THF (4 mL) was added and the solution was continuously stirred as it warmed up to room temperature. The solvent was removed under reduced pressure and pentane was added to the residue. The solution was filtered through celite and the filtrate was concd under reduced pressure. The crude product was purified by Kugelrohr distillation which afforded **4.130** as a colorless oil (0.12 g, 15%).

**4.130.** ^1^H NMR (300 MHz, CDCl_3) δ 6.47–6.60 (m, 1H), 4.87 (dd, *J* = 14.9, 2.2 Hz, 1H), 4.65 (dt, *J* = 8.9, 2.2 Hz, 1H), 3.68 (t, *J* = 4.7 Hz, 4H), 2.88 (t, *J* = 4.8 Hz, 4H), 0.99 (s, 9H), 0.17 (s, 6H).


1,3–Propanediol **4.142** was added in a suspension of NaH (1.66 g, 41.5 mmol) in THF (415 mL). The resulting cloudy solution was stirred continuously. After 1 h, BnBr (4.94 mL, 41.5 mmol) was added and the solution was heated to reflux. After 12 h, the reaction was quenched with H_2O (200 mL). The quenched reaction mixture was extracted with diethyl ether (3 x 100 mL). The organic layers were collected, combined, washed with sat. aq. NaCl solution, dried over magnesium sulfate, and concd under reduced pressure. The residue was purified using flash column chromatography with 50–60–70% ethyl
acetate in hexane to afford 4.143 (4.96 g, 72%) as colorless oil.

4.143. TLC $R_f = 0.26$ (hexane:ethyl acetate, 4:1); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.27–7.38 (m, 5H), 4.53 (s, 2H), 3.79 (q, $J = 5.5$ Hz, 2H), 3.67 (t, $J = 5.8$ Hz, 2H), 2.25 (t, $J = 5.5$ Hz, 1H), 1.88 (quint, $J = 5.7$ Hz, 2H).

3–Benzyloxy–propionaldehyde (4.144)


A solution of 4.143 (1.00 g, 6.02 mmol) in DCM (30 mL) was added in a suspension of pyridinium chlorochromate (2.60 g, 12.04 mmol) and silica gel (2.60 g) in DCM (20 mL). The black orange solution was stirred continuously at room temperature for 3 h. The solution was filtered through a plug of silica gel eluting with DCM. The filtrate was concd under reduced pressure and purification by Kugelrohr distillation to afford 4.144 (0.61 g, 62%) as colorless oil.

4.144. TLC $R_f = 0.46$ (hexane:ethyl acetate, 4:1); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 9.80 (t, $J = 1.9$ Hz, 1H), 7.28–7.38 (m, 5H), 4.54 (s, 2H), 3.82 (t, $J = 6.1$ Hz, 2H), 2.70 (dt, $J = 6.1$, 1.9 Hz, 3H).


A solution of 4.144 (0.53 g, 3.23 mmol) in THF (9 mL) was cooled to −78 °C using a dry ice/acetone cold bath. Allylmagnesium chloride (1.7 M in THF, 4.30 mL) was added dropwise and the solution was stirred continuously as it warmed up to room temperature. The reaction was monitored by TLC. After approximately 1.5 h, the reaction was quenched with sat. aq. NH$_4$Cl solution (10 mL) and was extracted with diethyl ether. The organic layers were collected, combined, dried over magnesium sulfate, and concd under
reduced pressure to give the crude product. Purification of the crude product using flash column chromatography with 15–20% ethyl acetate gave 4.145 (0.42 g, 63%) as light yellow oil. This compound has been synthesized previously.  

4.145. TLC $R_f = 0.22$ (hexane:ethyl acetate, 4:1); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.28–7.43 (m, 5H), 5.77–5.91 (m, 1H), 5.08–5.15 (m, 2H), 4.53 (s, 2H), 3.88 (quint, $J = 6.2$ Hz, 1H), 3.61–3.76 (m, 2H), 2.25 (dt, $J = 6.9$, 1.1 Hz, 2H), 1.74–1.84 (m, 2H).

![Reaction diagram]


In a dried round bottom flask, 4.145 (0.10 g, 0.48 mmol) was dissolved in DCM (2 mL). DMAP (0.002 g, 0.02 mmol) was added followed by Et$_3$N (0.13 mL, 0.96 mmol). The solution was stirred for some minutes before adding trimethylacetyl chloride 4.160 (0.09 mL, 0.72 mmol). The resulting solution was stirred continuously at room temperature. After approximately 24 h, the reaction was quenched by adding H$_2$O (10 mL). The organic layer was separated from the aqueous layer and the aqueous layer was extracted with diethyl ether (3 x 10 mL). All organic layers were collected, combined, dried over magnesium sulfate, and concd under vacuo. Purification with flash column chromatography using 5% ethyl acetate in hexane as eluent afforded 4.146 (0.05 g, 36%) as orange oil.

4.146. TLC $R_f = 0.74$ (hexane:ethyl acetate, 4:1); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.26–7.34 (m, 5H), 5.67–5.81 (m, 1H), 5.03–5.11 (m, 3H), 4.48 (s, 2H), 3.41–3.51 (m, 2H), 2.27–2.40 (m, 2H), 1.84–1.92 (m, 2H), 1.15 (s, 9H).
(E)-1-(benzyloxy)-7-oxooct-5-en-3-yl pivalate (4.148).

To a solution of 4.146 in DCM (1 mL), methyl vinyl ketone 4.162 (0.06 mL, 0.68 mmol) was added dropwise. The solution was degassed for about 10 min before adding Grubbs–Hoveyda catalyst (0.0006 g, 0.01 mmol) 4.166. The resulting green solution was stirred continuously at room temperature for 24 h. Purification using flash column chromatography with a dry packed column eluting with 20% ethyl acetate in hexane gave 4.148 as brown oil (0.05 g, 92%).

4.148. TLC $R_f = 0.86$ (hexane:ethyl acetate, 1:1); IR (film) 2969, 2926, 1725, 1677, 1479, 1159 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.26–7.33 (m, 5H), 6.64–6.75 (m, 1H), 6.03–6.08 (d, $J = 16.0$ Hz, 1 H), 5.12–5.20 (m, 1H), 4.47 (s, 2H), 3.42–3.54 (m, 2H), 2.41–2.61 (m, 2H), 2.21 (s, 3H), 1.89 (q, $J = 6.3$ Hz, 2H), 1.13 (s, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 198.4, 178.0, 143.1, 138.3, 134.0, 128.6, 127.9, 127.9, 73.4, 69.5, 66.3, 39.0, 37.9, 34.4, 29.9, 27.3, 26.9. HRMS (ESI): $m/z$ calcd for C$_{20}$H$_{28}$O$_4$Na$^+$ (M+Na$^+$): 355.1880. Found: 355.1873.

References


CHAPTER 5

SYNTHETIC STUDIES ON MAOECRYSTAL V

Introduction

Since 1976, more than 50 plants of the genus *Isodon* have been examined for bioactive constituents. Some of the new secondary metabolites isolated and identified from these sources consist of highly diversified oxygenated structures\(^1\) such as gerardianin A,\(^2\) the alboatisins,\(^3\) and the sculponins.\(^4\) A number of these natural products showed potent anti–tumor activities which make them good lead molecules for the development of anticancer agents. One of the most complex molecules isolated from genus *Isodon* is maoecrystal V 5.1. (Figure 5–1)\(^5\)

![Figure 5–1. Maoecrystal V 5.1.](image)

Maoecrystal V 5.1 is a C\(_{19}\) diterpenoid containing a unique 6,7–seco–6–nor–15(8→9)–abeo–5,8–epoxy–*ent*–kaurane skeleton which has similar structural features to epi–eriocalyxin A 5.2, a known *ent*–kaurane.\(^6\) (Figure 5–2) Maoecrystal V was isolated from the leaves of *Isodon eriocalyx* (Dunn.) Hara, a perennial herb widely distributed in China. This herb has been long used as folk medicine for treatment of sore throat, inflammation, influenza, hypertension, and dermatophytosis.\(^7\) The skeleton of 5.1, 5.9, is

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believed to have a similar biosynthetic pathway as maocrystral Z 5.6 which is shown in
Figure 5–3. Xu has proposed the formation of 5.1 from the cleavage of the C–6/C–7
bond of commonly occurring 7,20–epoxy–ent–kaurane 5.3 followed by oxidation and a
consequent decarboxylation. \(^8\)

![Figure 5–2. Epi–eriocalyxin A 5.2.](image)

![Figure 5–3. The Proposed Biosynthetic Pathway for the Skeleton of Maocrystral V 5.1 and Maocrystral Z 5.6.](image)

The biological activity of 5.1 was partially evaluated and this revealed a
remarkable selective cytotoxicity against HeLa cells with an IC\(_{50}\) of 0.02 µg/mL.
Cytotoxic activities of 5.1 were compared to cis–platin against several cancer cell lines
and are is summarized in Table 5–1. \(^5\)

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<th>Cell Line</th>
<th>Origin</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µg/mL)</th>
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<th>cis–Platin</th>
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<td>HeLa</td>
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Table 5–1.  Cytostatic Effects of Maoecrystal V and cis–Platin on Human Cancer Cell Lines.

Because of the structural complexity of 5.1 coupled with its biological activity, several research groups were prompted to undertake studies for its synthesis. The core structure for this diterpenoid has been synthesized employing metal–mediated arylation and oxidative dearomatization and an intramolecular Diels–Alder (IMDA) reaction as key steps. Also, several other synthetic routes are being explored for the synthesis of this interesting natural product including intramolecular hetero Diels–Alder reaction (IMHDA), IMDA reactions, carbonyl–based cyclizations an intramolecular 1,5–hydride shift, an Aldol reaction, and rhodium–catalyzed C–H functionalization. The key disconnections and their strategies for the synthesis of 5.1 are summarized in Figure 5–4.
**Figure 5–4.** The Key Disconnections and Strategies for the Synthesis of the Core Skeleton of Maoecrystal V 5.1.

The first total synthesis of (±)-maoecrystal V was reported by Yang and co-workers in 2010.²⁶ Carbomethoxylation on enone 5.10 followed by an oxidative arylation using plumbane 5.11 afforded β–ketoester 5.12. The diol 5.13, formed from the reduction of 5.12, was coupled with acid 5.14. Treatment of 5.14 with TsN₂ followed by a Rh₂(OAc)₄–catalyzed O–H bond insertion lead to lactone 5.15. Horner–Wadsworth–Emmons using phosphonate (5.15) and paraformaldehyde followed by a deprotection of the MOM ether leads to the formation of phenol 5.16. Wessely oxidative acetoxylation of 5.16 afforded stable o–quinol acetates which without purification undergo an IMDA to give mixture of adducts 5.17, 5.18, and 5.19. The adduct 5.19 was brominated and subsequently treated with Bu₃SnH to generate an allylic radical that was trapped with TEMPO. Regioselective reductive cleavage of the adduct afforded product 5.20. Lastly, regioselective hydrogenation using the Lindlar catalyst followed by oxidation using Dess–Martin periodinane and treatment with DBU provided the target natural product. (Scheme 5–1)
Scheme 5–1a Yang’s Total Synthesis of Maoecrystal V 5.1

Reagents and conditions: (a) CH$_3$CO$_2$CH$_3$, NaH, THF (b) 5.11, pyr., CHCl$_3$, 81% over 2 steps (c) 5.14, EDCI, DMAP, DCM (d) TsN$_3$, DBU (e) Rh$_2$(OAc)$_4$, PhH, 40% over 3 steps (f) t-BuOK, (HCHO)$_n$, THF (g) TFA, DCM, rt, 86% over 2 steps (h) Pb(OAc)$_4$, AcOH, then PhMe, 145 °C 76% (2.33:1:3) (i) Lindlar cat., MeOH, THF (j) Dess–Martin periodinane, DCM, rt (k) DBU, toluene, 39% over 3 steps.
Similarly, Baran’s synthesis of the carbon skeleton of 5.1 is parallel to Yang’s synthesis. Barton arylation of 5.21 with 5.22 led to the formation of 5.23. Reduction of aldehyde (5.23) followed by acetylation and MOM deprotection afforded the hemiketal 5.24. Wessely oxidation of 5.24 afforded mixture of diastereomeric acetate 5.25 and 5.26 which was heated to 165 °C to form endo–cycloadducts 5.27 and 5.28, respectively. Hydrogenation of the bicyclic olefins 5.27 and 5.28 followed by acetate group removal using SmI₂ gave α–methyl ketone 5.29 as a mixture of diastereomers.
Scheme 5–2a  Baran’s Synthesis of Maocryystal V’s Carbon Skeleton

\[ \text{Scheme 5–2a  Baran’s Synthesis of Maocryystal V’s Carbon Skeleton} \]

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Michael addition as key steps. 1,4–Addition, enol ether trapping, and ozonolysis would give rise to the advanced intermediate 5.34. Lastly, lactone 5.34 would then be converted to 5.1 using several reactions such as intramolecular ring closing reaction and oxidation, reduction, and deprotection reactions.

![Diagram](image)

**Figure 5–5.** Synthetic Approach for Maoecrystal V 5.1.

**Results and Discussion**

Synthesis of tricyclic ketone 5.33 requires diene 5.30 for use in a Diels–Alder reaction. Synthesis of diene 5.30 begins with the conversion of trans–crotonaldehyde 5.35 to the diethylamino butadiene 5.37 using the method of Hunig.\(^\text{28}\) Diels–Alder reaction of 5.37 with ethyl acrylate followed by a Hoffman elimination provided diene ester 5.38.\(^\text{29}\) The diene ester 5.38 was subjected to several reduction protocols; however, lithium aluminum hydride (LAH) reduction afforded alcohol 5.30 in the best yield (74%).\(^\text{30}\) The alcohol was then protected using DMAP and TBSCl to synthesize silyl ether 5.30. (Scheme 5–3)
Scheme 5–3a Synthesis of Diene 5.30

\[
\begin{align*}
\text{5.35} & \xrightarrow{a} \left[ \begin{array}{c}
\text{Et}_2\text{N} \\
\text{5.36}
\end{array} \right] \xrightarrow{b} \text{5.37} \\
\text{5.30} & \xrightarrow{e} \text{5.39} \xrightarrow{d} \text{5.38}
\end{align*}
\]

Reagents and conditions: (a) K₂CO₃, HNEt₂, Et₂O, 0 °C to rt, 21 h (b) Δ, 61% (c) i. ethyl acrylate, 40 °C, 48 h, ii. Mel, DCM, rt, 18 h iii. DBU, DCM, 0 °C to rt, 23 h, 57%, (d) LAH, AlCl₃ THF, 0 °C to rt, 23 h, 74%, (e) TBSCl, DMAP, DCM, rt, 19 h, 66%.

The tert–butyldimethylsilyl protected alcohol 5.30 and nitrobenzoate dienophile 5.40, synthesized from enolate trapping of 2,3–butadiene with p–nitrobenzoyl chloride, was subjected to a thermal Diels–Alder reaction. Several conditions were varied for optimization (Table 5–2) and the reaction conducted in dichloroethane (DCE) at reflux showed a 38% yield of the Diels–Alder products 5.41 and 5.42 (1:2). These products could be separated by silica gel chromatography. The relative stereochemistry of both alkenes 5.41 and 5.42 were established based on detailed analysis of Nuclear Overhausser Effect Spectroscopy (NOESY). The key NOESY correlations of alkenes 5.41 and 5.42 are shown in Figure 5–6.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time</th>
<th>( t )</th>
<th>Additive</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>toluene</td>
<td>24 h</td>
<td>110 °C</td>
<td>hydroquinone</td>
<td>23% (1:15)*</td>
</tr>
<tr>
<td>2</td>
<td>toluene</td>
<td>24 h</td>
<td>150 °C</td>
<td>hydroquinone</td>
<td>27% (1:3.5)**</td>
</tr>
<tr>
<td>3</td>
<td>DCE</td>
<td>24 h</td>
<td>reflux</td>
<td>none</td>
<td>38% (1:2)**</td>
</tr>
</tbody>
</table>

The ratios were determined either through *NMR or **isolated ratio.

**Table 5–2.** Diels–Alder Reaction of the tert–Butyldimethylsilyl Protected Alcohol 5.30 and Nitrobenzoate Dienophile 5.40.

**Figure 5–6.** The Key NOESY Correlation of 5.41 and 5.42.

Resubjecting the Diels–Alder products 5.41 and 5.42 separately with the same conditions in **Entry 3** of **Table 5–2** suggests that the cycloaddition is not reversible, as no equilibration was observed. Carberry also explored similar Lewis acid catalyzed Diels–Alder reactions however with Lewis acids the reaction either led to the decomposition of the starting materials or no reaction at all.\(^{31}\)

Also, the free and TBS–protected diene alcohol 5.39 and 5.30, respectively, were subjected to a Diels–Alder addition with a TMS–protected dienophile however none of the desired Diels–Alder adducts 5.44, 5.45, 5.46, and 5.47 were observed. (**Scheme 5–4**) Diels–Alder reaction between 5.39 and 5.43 resulted to the decomposition of the starting
materials. On the other hand, TBS–protected diene alcohol 5.30 was recovered from the purification of the reaction mixture of the cycloaddition reaction between 5.30 and 5.43. However, no dienophile 5.43 was recovered which possibly was hydrolyzed back to the volatile 2,3–butadione.

**Scheme 5–4**

Thermal Diels–Alder Reaction of Free and TBS–Protected Diene Alcohol with Dienophile 5.43

Reagents and conditions: (a) toluene, 110 °C, 24 h (b) toluene, reflux, 20 h.

The nitrobenzoate group of the alkene 5.41 was hydrolyzed with K$_2$CO$_3$ in MeOH to afford alcohol 5.48. (Scheme 5–5) The alcohol 5.48 was subjected to an epoxidation reaction using $m$–CPBA which resulted in a mixture of inseparable epoxides 5.49 and 5.50 in 17% yield with a ratio of 1:9:1. Alternatively, alcohol–directed epoxidation using VO(acac)$_2$ with $t$–BuOOH afforded only epoxide 5.50. The relative stereochemistry was also established using a 2D NOESY experiment and the key correlations of epoxide 5.44 is shown in Figure 5–7.
Scheme 5–5a  Stereoselective Synthesis of Epoxide 5.50

\[
\begin{align*}
5.41 & \xrightarrow{\text{a}} 5.48 \\
\text{a} & \text{Reagents and conditions: (a) K}_2\text{CO}_3, \text{MeOH, rt, 50 h, 44% (b) m–CPBA, NaHCO}_3, \\
 & \text{DCM, 0 °C to rt, 24 h, 17% (1.9:1) (c) VO(acac)}_2, \text{t–BuOOH, DCM, rt, 15 h, 50%}}.
\end{align*}
\]

Figure 5–7. The Key NOESY Correlation of 5.50.

Epoxide opening of 5.44 was attempted using several nucleophiles which are summarized in Table 5–3. However, in no case no desired products (5.48–5.51) were observed. The reaction of the epoxide 5.44 afforded a complex of mixture. On the other hand, starting material was recovered from the reaction of 5.44 with NaCN.
Table 5–3. Epoxide Opening of 5.50.

Because of the low reactivity of the epoxide 5.50 to several nucleophiles, a model system was designed to explore the possibility of synthesizing the tricyclic skeleton of maoecrystal V 5.1. The retrosynthetic analysis in Figure 5–8 shows the synthetic strategy for model system 5.57. The model system 5.57 can be synthesized from β–hydroxy ketone 5.58. This ketone can come from lactone 5.59 through an intramolecular Aldol reaction. A 1,4–addition of the unsaturated ketone 5.60 and dithiane 5.61 followed by trapping of enol ether and ozonolysis would afford the lactone 5.59.
Figure 5–8. Retrosynthetic Analysis for the Model System for Maoecrystal V 5.1.

The synthesis of the unsaturated ketone 5.60 commenced using the Diels–Alder addition of the diene 5.39 and the dieneophile 5.62. (Figure 5–11) The protecting group of the dieneophile was changed from nitrobenzoate group (5.40) to triisopropylsilyl group (5.62) to evaluate its reactivity with 5.39 towards Diels–Alder reaction. The dieneophile was synthesized using TIPSOTf and Et3N. Several attempts to synthesize 5.62 from the less expensive TIPSCI and various bases were also explored however none of which yielded desired dieneophile. (Table 5–4)
Table 5–4. Synthesis of the Dienophile 5.69.

The cycloaddition of 5.39 and 5.62, however, afforded an inseparable mixture of alkenes 5.63 and 5.64 with a low yield of 15% with the endo product 5.63 as the major product.

Scheme 5–6a Diels–Alder Reaction Between Diene 5.39 and Dienophile 5.62

\[
\begin{align*}
\text{5.39} + \text{5.62} &\xrightarrow{a} \text{5.63} + \text{5.64} \\
&\text{aReagents and conditions: (a) toluene, 180 °C, 17 h, 15% (4.1:1).}
\end{align*}
\]

Oxidation of alcohols 5.63 and 5.64 using PCC followed by palladium–catalyzed hydrogenation afforded alkane aldehyde 5.68. (Scheme 5–7) Aldol reaction of 5.68 with KOH was attempted however formation of the unsaturated ketone 5.55 was not observed. No starting material was recovered from this reaction; however, \(^1\)H NMR of the crude mixture suggests the formation of the \(\alpha\)–hydroxy ketone, the intermediate of the Aldol reaction.
Scheme 5–7 Synthesis of Unsaturated Ketone 5.60.

\[ \text{Scheme 5–7 Synthesis of Unsaturated Ketone 5.60.} \]

\[ \text{HO} \text{OTIPS} + \text{HO} \text{OTIPS} \rightarrow ^{a} \text{HO} \text{OTIPS} + \text{HO} \text{OTIPS} \]

\[ \text{5.63} \stackrel{a}{\rightarrow} \text{5.66, 5.67} \]

\[ \text{HO} \text{OTIPS} \rightarrow \text{HO} \text{OTIPS} \]

\[ \text{5.69} \rightarrow \text{5.68} \]

\[ ^{a}\text{Reagents and conditions: (a) PCC, silica gel, DCM, rt, 4 h, 81\% (4.1:1) (b) Pd/C, H}_2, \text{EtOH, rt, 2 h, 23\% (c) KOH, THF:H}_2\text{O, 80 }^\circ\text{C, 22 h.} \]

Several other conditions were explored to attempt to synthesize the tricyclic enone 5.60 however, none of the attempts were successful. These conditions are summarized in Table 5–5. Reaction conditions (Entry 1 and 3) resulted to decomposition of the starting material or formation of complex mixtures. On the other hand, reaction condition using potassium carbonate in \( t \)-butyl alcohol (Entry 2) may have resulted to the formation of 5.69 similar to the reaction using potassium hydroxide in THF:H\(_2\)O.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Solvent</th>
<th>Time</th>
<th>$t$</th>
<th>Yield</th>
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<tbody>
<tr>
<td>1</td>
<td>KOH</td>
<td>THF:H$_2$O</td>
<td>22 h</td>
<td>80 °C</td>
<td>0 %</td>
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<tr>
<td>2</td>
<td>DBU</td>
<td>DMF</td>
<td>42 h</td>
<td>50 °C</td>
<td>0 %</td>
</tr>
<tr>
<td>3</td>
<td>K$_2$CO$_3$</td>
<td>$t$–BuOH</td>
<td>16 h</td>
<td>reflux</td>
<td>0 %</td>
</tr>
<tr>
<td>4</td>
<td>LDA</td>
<td>THF</td>
<td>2 h</td>
<td>$-78$ °C to rt</td>
<td>0 %</td>
</tr>
</tbody>
</table>

**Table 5–5.** Synthesis of the Tricyclic Enone 5.60.

**Conclusion and Future Work**

Maoecrystal V 5.1 is a diterpenoid isolated from the leaves of Chinese herb *Isodon eriocalyx*. The natural product 5.1 contains 6,7–seco–6–nor–15(8→9)–abeo–5,8–epoxy–*ent*–kaurane skeleton. In addition, 5.1 showed selective cytotoxicity against HeLa cells. Because of the interesting structural feature of 5.1 as well as its biological activity, several research groups were prompted to undertake studies for its synthesis. The first reported total synthesis of 5.1 was reported in 2010 and employed Wessely oxidative dearomatization, intramolecular Diels–Alder reaction and rhodium–catalyzed O–H bond insertion as key steps.

The synthesis of the core of the natural product 5.1 was explored. Attempts were made to synthesize the desired advanced intermediate tricyclic ketone 5.33 using a Diels–Alder reaction with diene 5.30 and the nitrobenzoate dienophile 5.40. The Diels–Alder reaction provided a mixture of separable adducts 5.41 and 5.42 in which the less desired adduct 5.42 is the major product. Other diene such as 5.39 and dienophile such as 5.43 were also explored however these resulted in the recovery or hydrolysis of the starting...
material. The adduct 5.41 was hydrolyzed to alcohol 5.48 and was subjected to an alcohol
directed epoxidation afforded 5.50. Epoxide opening of 5.50 using different nucleophiles
were attempted however, in no case epoxide opening were observed.

Because of the low reactivity of the epoxide 5.50, a model system was designed
to explore the possibility of synthesizing the tricyclic skeleton of maoecrystal V 5.1. The
cycloaddition between diene alcohol 5.39 and dienophile 5.62 provided adducts 5.63 and
5.64. PCC oxidation followed by hydrogenation afforded 5.68. The ketoaldehyde 5.68
was subjected to several conditions for Aldol reaction. However, no desired tricyclic
eneone 5.60 were formed but in some cases the possible formation of the α–hydroxy
ketone 5.69 were observed.

The future work involves the synthesis of the desired tricyclic 5.60 and its use in
the synthesis of model system 5.57. (Figure 5–9) In addition, these compounds would
then be tested for toxicity against cancer cell lines.

Figure 5–9. Synthesis of Tricyclic 5.60.

Experimental Section

General Experimental Procedure

Reactions: Except as otherwise noted, reactions were carried out under an argon
atmosphere either in an oven– or flame–dried glassware. Flasks were flame dried under
vacuum. Pyridine, toluene, and triethylamine were distilled from calcium hydride.
Reaction solvents were dried by a solvent purification apparatus (benzene,
dichloromethane (DCM), tetrahydrofuran (THF), diethylether) using alumina as the
drying agent.
**Methods:** Analytical thin layer chromatography (TLC) was performed using 250 μm commercial silica gel plates (EMD Chemicals, silica gel 60 F254) and visualized by UV illumination or by exposure to potassium permanganate or p-anisaldehyde solution followed by heating using a heat gun. Flash chromatography was carried using commercially available 40–60 μm silica gel (Silicycle).

**Identity:** Melting points were recorded using an Electrothermal® Melting Point Apparatus in open capillaries and are uncorrected. Infrared spectra were obtained using Thermo Nicolet IR100 and IR200 spectrometers. Proton and carbon nuclear magnetic resonance (1H and 13C NMR) were recorded using Bruker DPX–300 spectrometer. The NOESY spectra were recorded using Bruker DRX–500 spectrometer. Chemical shifts are reported in delta (δ) units, downfield from tetramethylsilane (TMS) with reference to CDCl3 solvent signal (1H, 7.26; 13C, 77.23) Coupling constants are reported in Hertz (Hz). Electron impact and high–resolution mass spectra were obtained from a Bruker 12 APEX –Qe FTICR–MS instrument with an Apollo II ion source in the College of Science Major Instrumentation (COSMIC) at Old Dominion University at Northfolk, VA 23529.

**Experimental**

\[
\begin{align*}
\text{\textbullet} & \quad \text{K}_2\text{CO}_3, \text{NHEt}_2 \\
\text{Et}_2\text{O}, 0^\circ \text{C} \to \text{rt}, 21 \text{h} & \quad \begin{array}{c}
\text{5.35} \\
\text{5.36}
\end{array} \quad \Delta \quad \text{61%} \\
& \quad \begin{array}{c}
\text{5.37} \\
\text{DRV-II-132}
\end{array}
\end{align*}
\]

*(E*–N,N–diethylbuta–1,3–dien–1–amine (5.37))


Potassium carbonate (3.34 g, 24.1 mmol) was placed in a flame–dried round bottom flask. The flask was cooled to 0 ºC using an ice/H₂O bath. Diethylamine (19.89 mL, 193.1 mmol) was added to potassium carbonate followed by a solution of trans–crotonaldehyde (8.00 mL, 96.6 mmol) in diethyl ether (10 mL). The resulting reaction mixture was allowed to warm to rt. After approximately 21 h, the reaction mixture was filtered and concd under reduced pressure. Purification using vacuum distillation (fractions from 35–40 ºC) afforded 5.37 (7.36 g, 61%) as an oil.
5.37. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.21 – 6.34 (m, 2H), 5.03 (dd, $J$ = 10.7, 2.7 Hz, 1H), 4.71 (dd, $J$ = 14.7, 2.0 Hz, 1H), 4.45 (dd, $J$ = 8.0, 2.1 Hz, 1H), 0.99–1.15 (m, 12H).

![Diagram](image)

**Ethyl cyclohexa-1,3-dienecarboxylate (5.38)**


Ethyl acrylate 5.70 (7.68 mL, 70.6 mmol) was added to diene 5.37 (7.36 g, 58.8 mmol) in an oven-dried round bottom flask. The neat solution was warmed to 40 °C. After approximately 48 h the reaction was cooled to rt and taken up in DCM (25 mL). MeI (18.30 mL, 294.0 mmol) was then added. After 18 h, the reaction mixture was concd under reduced pressure, the concd residue was dissolved in DCM (90 mL) and cooled to 0 °C. DBU (17.59 mL, 117.6 mmol) was added in a dropwise fashion and the solution was allowed to warm to rt. After 23 h, the reaction was quenched by adding HCl solution (10 mL, 1 M) and was extracted thrice with DCM. All organic layers were collected, combined, dried over sodium sulfate and concd under reduced pressure. Distillation under high vacuum provided diene 5.38 (5.11 g, 57%) as an oil.

5.38. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.99–7.00 (m, 1H), 6.11–6.17 (m, 1H), 6.03–6.09 (m, 1H), 4.21 (dq, $J$ = 7.0, 0.7 Hz, 2H), 2.42–2.49 (m, 2H), 2.22–2.30 (m, 2H), 1.30 (ddd, $J$ = 6.4, 4.4, 0.7 Hz, 4H).
**Cyclohexa-1,3-dienylmethanol (5.39)**


THF (63 mL) was placed in a flame dried round bottom flask. Lithium aluminum hydride (4.47 g, 111.9 mmol, 95%) was then added in several portions. The suspension was cooled to 0 °C using ice/H₂O, and aluminum chloride (5.15 g, 38.6 mmol) was added in several portions. After 30 min., ester **5.38** (5.11 g, 33.6 mmol) was added dropwise. The resulting solution was allowed to warm to rt. After approximately 24 h, the reaction mixture was cooled to 0 °C. The reaction was quenched by adding cooled H₂O (15 mL), NaOH (15 mL, 15%) and H₂O (5 mL) sequentially in a dropwise fashion. The reaction mixture was then filtered through celite and the filtrate was dried over magnesium sulfate and concd under reduced pressure to afford alcohol **5.39** (2.75 g, 74%) as a light yellow oil.

**5.39.**¹H NMR (300 MHz, CDCl₃) δ 5.87–5.96 (m, 2H), 5.72–5.80 (m, 1H), 4.11 (d, J = 4.9 Hz, 2H), 2.12–2.26 (m, 4H).

**tert-Butyl-(cyclohexa-1,3-dienylmethoxy)-dimethyl-silane (5.30)**

In a flame dried round bottom flask, alcohol **5.39** was dissolved in DCM (4.54 mL). Diisoproylethylamine (1.11 mL, 6.36 mmol) was added to the solution dropwise followed by 4–dimethylaminopyridine (0.03 g, 0.23 mmol). After several minutes of stirring, tert–butyldimethylsilyl chloride (0.82 g, 5.45 mmol) was added in several portions. After approximately 19 h, the reaction mixture was concd under reduced pressure. H₂O (10 mL) was added to the residue and the mixture was extracted with DCM (3 × 10 mL). All
organic layers were collected, combined, dried over sodium sulfate, and concd under reduced pressure. Purification using flash column chromatography with 5% ethyl acetate in hexane afforded 5.30 (0.67 g, 66%) as a clear oil.

5.30. TLC Rf = 0.71 (hexane:ethyl acetate, 97:3); IR (neat) 3039, 2955, 2854, 1252, 1094, 837 cm−1; 1H NMR (300 MHz, CDCl3) δ 5.85–5.94 (m, 2H, 5.68–5.74 (m, 1H), 4.12 (s, 2H), 2.16–2.23 (m, 2H), 2.05–2.11 (m, 2H), 0.92 (s, 9H), 0.08 (s, 6H); 13C NMR (75 MHz, CDCl3) δ 138.5, 125.1, 124.5, 118.1, 66.3, 26.1, 23.5, 22.8, 18.6, –5.1.

![Chemical structure](image)


In a flame–dried round bottom flask, 5.30 (0.49 g, 2.18 mmol) and 5.40 (0.77 g, 3.27 mmol) were dissolved in anhydrous DCE (2.20 mL). The solution was heated to reflux for 48 h. The reaction mixture was allowed to cool to rt and was concd under reduced pressure. Purification using flash column chromatography with 7.5–10% ethyl acetate in hexane as eluent gave 5.41 (0.13 g, 13%) and 5.42 (0.25 g, 25%) both as light yellow crystals.

5.41. m.p. = 142.5–145.0 °C (CHCl3); TLC Rf = 0.57 (hexane:ethyl acetate, 4:1); IR (film) 3155 2948, 2858, 1709, 1526, 1303, 1100 cm−1; 1H NMR (300 MHz, CDCl3) δ 8.28–8.32 (m, 2H), 8.14–8.18 (m, 2H), 6.43 (dd, J = 8.1, 6.5 Hz, 1H), 6.07–6.10 (m, 1H), 4.07 (d, J = 10.4 Hz, 1H), 3.81 (d, J = 10.4 Hz, 1H), 2.65–2.70 (m, 2H), 2.17 (s, 3H), 1.75–1.77 (m, 1H), 1.60 (dt, J = 14.9, 3.7 Hz, 1H), 1.21–1.39 (m, 2H), 1.04–1.14 (m, 1H), 0.88 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H); 13C NMR (75 MHz, CDCl3) δ 205.9, 164.3, 150.9, 135.9, 135.7, 132.2, 131.0, 123.9, 92.9, 64.9, 47.2, 41.9, 31.0, 29.9, 27.8, 26.1, 26.1, 24.4, 23.4, 18.7, –5.3. HRMS (ESI): m/z calcd for C24H33O6SiNa⁺ (M+Na⁺): 482.1969. Found: 482.1958.
5.42. m.p. 106.0–108.5 °C (CHCl₃); TLC Rₜ = 0.54 (hexane:ethyl acetate, 4:1); IR (film) 2953, 2857, 1722, 1530, 1286, 1102 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.30–8.33 (m, 2H), 8.18–8.22 (m, 2H), 6.51 (dd, J = 8.2, 6.6 Hz, 1H), 6.18–6.21 (m, 1H), 3.88 (d, J = 10.4 Hz, 1H), 3.65 (d, J = 10.4 Hz, 1H), 2.81–2.19 (m, 1H), 2.48 (dt, J = 14.7, 2.9 Hz, 1H), 2.15 (s, 3H), 2.06–2.15 (m, 1H), 1.93 (dd, J = 14.8, 2.6 Hz, 1H), 1.59–1.69 (m, 1H), 1.42–1.53 (m, 1H), 1.14–1.25 (m, 1H), 0.88 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 203.7, 164.3, 150.8, 136.4, 135.8, 131.9, 130.9, 123.8, 90.5, 64.1, 47.1, 39.4, 30.8, 28.2, 26.1, 24.9, 24.8, 18.7, –5.5, –5.4. HRMS (ESI): m/z calcd for C₂₄H₃₃O₆SiNa⁺ (M+Na⁺): 482.1969. Found: 482.1959.

![Chemical Structure](image)

3–(trimethylsilyloxy)but–3–en–2–one (5.43)


In a flame–dried round bottom flask, 2,3–butadione 5.65 (4.30 g, 49.9 mmol) was dissolved in THF (70 mL). Triethylamine (16.70 mL, 119.8 mmol) was added dropwise followed by freshly distilled trimethylsilyl chloride (7.60 mL, 59.9 mmol). The solution was heated to reflux. After 35 h, it was filtered and concd under reduced pressure. The concentrate was dissolved in diethyl ether (35 mL) and washed with HCl (3 x 15 mL, 1 M), sat. aq. NaHCO₃ (2 x 10 mL) and brine (2 x 20 mL). The resulting solution was dried over magnesium sulfate and concd under *vacuo*. The residue was purified by distillation to afford 5.43 as an oil (0.63 g, 8%).

5.63. ¹H NMR (300 MHz, CDCl₃) δ 5.35 (d, J = 1.7 Hz, 1H), 4.84 (d, J = 1.7 Hz, 1H), 2.29 (s, 3H), 0.24 (s, 9H).

In a round bottom flask, nitrobenzoate 5.41 (0.13 g, 0.28 mmol) was dissolved in CH$_3$OH (5.60 mL). Potassium carbonate (0.08 g, 0.56 mmol) was added and the solution was stirred at rt for 50 h. The reaction mixture was then taken up in DCM (10 mL) and washed with HCl (1 M). The organic layer was then dried the organic layer over sodium sulfate. The dried extracts were concd under reduced pressure and purified through flash column chromatography using 5–10% ethyl acetate in hexane to afford 5.48 (0.04 g, 44%) as a yellow oil.

5.48. TLC $R_f$ = 0.52 (hexane:ethyl acetate, 4:1); IR (neat) 3489, 2934, 2860, 1707, 1471, 1255, 837 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.49 (dd, $J$ = 8.0, 6.7 Hz, 1H), 6.10 (d, $J$ = 8.2 Hz, 1H), 4.66 (br, s, 1H), 3.79 (d, $J$ = 10.4 Hz, 1H), 3.70 (d, $J$ = 10.4 Hz, 1H), 2.71–2.65 (m, 1H), 2.36 (s, 3H), 2.00 (dd, $J$ = 13.1, 1.9 Hz, 1H), 1.72–1.57 (m, 2H), 1.36–1.24 (m, 2H), 0.92 (s, 9H), 0.81–0.70 (m, 1H), 0.12 (s, 3H), 0.10 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 215.0, 135.1, 133.2, 85.0, 67.6, 46.0, 43.1, 31.0, 28.4, 26.0, 25.2, 23.2, 18.3, –5.5, –5.5. HRMS (ESI): $m/z$ calcd for C$_{17}$H$_30$O$_3$SiNa$^+$ (M$+$Na$^+$): 333.1856. Found: 333.1851.

1–[5–(tert–Butyl–dimethyl–silanyloxymethyl)–6–hydroxy–3–oxa–tricyclo[3.2.2.0$^{2,4}$]non–6–yl]–ethanone (5.49 and 5.50)

In a round bottom flask, alcohol 5.48 (0.02 g, 0.06 mmol) was dissolved in DCM (6 mL). The solution was cooled to 0 °C using an ice/H$_2$O bath. Sodium bicarbonate (0.03 g, 0.30 mmol) was added to the solution followed by $m$–CPBA (0.03 g, 0.12 mmol). The
resulting suspension was stirred continuously as it warmed up to rt. After approximately
25 h, the reaction mixture was quenched by adding sat. aq. sodium bicarbonate solution
(10 mL). The solution was extracted with ethyl acetate, dried over sodium sulfate, and
concd under reduced pressure. Purification of the residue using flash column
chromatography with 10% ethyl acetate in hexane as eluent afforded inseparable epoxides 5.49 and 5.50 as an oil.

5.49 and 5.50. TLC Rf = 0.31 (hexane:ethyl acetate, 9:1); 1H NMR (300 MHz, CDCl₃) δ
4.29 (t, J = 6.8 Hz, 1H), 3.42 (t, J = 4.7 Hz, 1H), 3.19 (d, J = 5.0 Hz, 1H), 2.36–2.43 (m,
1H), 2.21 (s, 3H), 1.92 (dd, J = 11.5, 2.6 Hz, 1H), 1.65–1.76 (m, 1H), 1.51–1.60 (m, 2H),
1.23–1.29 (m, 2H), 0.87 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H).

![Chemical structure of 5.48 and 5.50](image)

1–[5–(tert–Butyl–dimethyl–silanyloxymethyl)–6–hydroxy–3–oxa–tricyclo[3.2.2.0₂,₄]non–6–yl]–ethanone (5.50)

In a round bottom flask, alcohol 5.48 (0.06 g, 0.19 mmol) was dissolved in DCM (3 mL).
VO(acac)₂ (0.0005 g, 0.02 mmol) was added followed by t–BuOOH (0.19 mL, 0.95
mmol, 5.0–6.0 M). The resulting bright red solution was stirred at rt for 15 h. Ethyl
acetate (10 mL) was added and the reaction was quenched by adding sat. aq. sodium
thiosulfate solution. The organic layer was collected and the aqueous layer was extracted
thrice with ethyl acetate. All organic layers were collected, combined, dried over sodium
sulfate, and concd under reduced pressure. The residue was purified with silica column
chromatography using 10% ethyl acetate in hexane to afford 5.50 (0.03 g, 50%) as an oil.

5.50. TLC Rf = 0.31 (hexane:ethyl acetate, 9:1); IR (film) 3528, 2934, 2859, 1713, 1466,
1348 cm⁻¹; 1H NMR (300 MHz, CDCl₃) δ 3.42 (t, J = 4.7 Hz), 3.19 (d, J = 5.0 Hz), 2.36–
2.43 (m, 1H), 2.21 (s, 3H), 1.92 (dd, J = 11.5, 2.6 Hz, 1H), 1.65–1.76 (m, 1H), 1.51–1.60
(m, 2H), 1.23–1.29 (m, 2H), 0.87 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H); 13C NMR (75 MHz,
CDCl₃) δ 212.9, 80.4, 64.8, 54.7, 53.8, 46.0, 40.2, 29.7, 28.8, 26.1, 22.7, 21.6, 18.9, −5.4,
1-(1-Hydroxymethyl-2-triisopropylsilanyloxy-bicyclo[2.2.2]oct-5-en-2-yl)-ethanone (5.63 and 5.64)

Alcohol 5.63 (0.74 g, 6.71 mmol) and dienophile 5.62 (0.74 g, 3.05 mmol) were dissolved in toluene (4.00 mL) in a pressure tube. The reaction mixture was purged with argon and heated to 180 °C. After approximately 17 h, the reaction mixture was cooled at rt and it was purified through flash column chromatography using 15–20–30% ethyl acetate in hexane as eluent to afford 5.63 and 5.64 (0.16 g, 15%) as a yellow oil.

5.63 and 5.64. TLC Rf = 0.43 (hexane:ethyl acetate, 9:1); IR (film) 3435, 2944, 2866, 1709, 1465 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 6.37–6.44 (m, 1H), 6.26–6.28 (m, 1H)exo, 6.22 (dd, J = 8.1, 0.8 Hz, 1H)endo, 3.68–3.84 (m, 2H), 3.55 (dd, J = 11.0, 3.5 Hz, 1H)endo, 3.37 (dd, J = 8.1, 2.0 Hz, 1H)exo, 2.73–2.80 (m, 1H)endo, 2.63–2.68 (m, 1H)exo, 2.48 (dd, J = 13.3, 2.4 Hz, 1H)exo, 2.42 (s, 3H)exo, 2.19 (ddd, J = 12.6, 9.7, 3.4 Hz, 1H)endo, 2.15 (s, 3H)endo, 2.04 (dt, J = 13.5, 2.9 Hz, 1H)endo, 1.91 (dd, J = 10.8, 6.7 Hz, 1H)endo, 1.74–1.84 (m, 1H), 1.58 (dd, J = 13.5, 2.6 Hz, 1H)endo, 1.34–1.45 (m, 2H), 1.11–1.29 (m, 6H), 1.02–1.06 (m, 32H), 0.76–0.91 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 211.1, 135.3, 134.8, 134.1, 133.7, 86.3, 67.0, 65.0, 48.4, 46.7, 43.3, 41.8, 31.2, 31.0, 29.0, 28.8, 25.1, 24.5, 24.4, 23.9, 19.1, 19.1, 18.7, 14.6. HRMS (ESI): m/z calcd for C₂₀H₃₆O₃SiNa⁺ (M+Na⁺): 375.2326. Found: 375.2322.

3–Triisopropylsilanyloxy–but–3–en–2–one (5.62)


In a flame–dried round bottom flask, 2,3–butadione 5.65 (1.15 g, 13.4 mmol) was dissolved in THF (27 mL). The resulting yellow solution was cooled at 0 °C.
Triethylamine (2.98 mL, 21.4 mmol) was added in a dropwise manner and after approximately 10 min., triisopropylsilyl trifluoromethanesulfonate (2.41 mL, 8.93 mmol) was added. The reaction was allowed to warm to rt and stirred for 20 h. The reaction mixture was then concd under vacuo. The residue was dissolved in diethyl ether and was washed with HCl (1 M). The aqueous layer was extracted thrice with diethyl ether and all organic layers were collected, combined, dried over magnesium sulfate, and concd under reduced pressure. Purification of the residue through flash column chromatography with 5–10–20 % ethyl acetate in hexane as eluent afforded 5.62 a colorless oil (1.03 g, 47%).

5.62. TLC Rf = 0.51 (hexane:ethyl acetate, 92:8); IR (neat) 2991, 2846, 1697, 1609, 1150, 1017 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 5.31 (d, \(J = 1.6\) Hz, 1H), 4.67 (d, \(J = 1.6\) Hz, 1H), 2.32 (s, 3H), 1.18–1.30 (m, 3H), 1.10 (d, \(J = 6.8\) Hz, 18H); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 197.3, 154.6, 99.3, 26.4, 18.1, 12.8. HRMS (ESI): m/z calcd for C\(_{13}\)H\(_{28}\)O\(_2\)SiNa\(^+\) (M+Na\(^+\)): 265.1594. Found: 265.1594.

![Reaction Scheme](attachment:image.png)

2,3–Bis–triisopropylsilyloxy–buta–1,3–diene (5.71)


In a flame–dried round bottom flask, 2,3–butadiene 5.65 (0.25 mL, 2.9 mmol) was dissolved in THF (9.6 mL). Et\(_3\)N (0.65 mL, 4.63 mL) was added to resulting yellow solution and the reaction mixture was cooled to 0 °C. Triisopropylsilyl trifluoromethanesulfonate (0.59 g, 1.93 mmol) was added in a dropwise fashion. The reaction mixture was allowed to warm to rt and stirred for 25 h. The reaction was quenched by adding sat. aq. ammonium chloride solution. The quenched reaction mixture was extracted with DCM (3 x 10 mL). The organic layers were collected, combined, dried over sodium sulfate, and concd under reduced pressure. Purification by flash column chromatography using 0–10% ethyl acetate in hexane as eluent provided diene 5.71 (0.10 g, 17%).
5.71. m.p. = 45.9–47.2 °C (CHCl₃); TLC Rₛ = 0.90 (hexane:ethyl acetate, 95:5); IR (film) 2946, 2869, 2894, 1590, 1465 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 4.90 (s, 2H), 4.31 (s, 2H), 1.18–1.33 (m, 6H), 1.11 (d, J = 6.7 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 153.5, 91.1, 18.3, 13.0. HRMS (ESI): m/z calcld for C₂₂H₄₆O₂Si₂Na⁺ (M+Na⁺): 421.2929. Found: 421.2923.

![Chemical structure](image)

6–Acetyl–6–triisopropylsilyloxy–bicyclo[2.2.2]oct–2–ene–1–carbaldehyde (5.66 and 5.67)

PCC (0.20 g, 0.91 mmol) and silica gel (0.20 g) were combined and placed in a flame dried 50 mL round bottom flask. A solution of alcohol 5.58 and 5.59 (0.16 g, 0.45 mmol) in DCM (4.50 mL) was added dropwise to the suspension. The resulting suspension was continuously stirred at rt for 4 h. The reaction was filtered through silica gel using DCM as eluent and purified through silica gel chromatography using 6% ethyl acetate in hexane to afford aldehydes 5.60 and 5.61 (0.13 g, 81% (4:1:1)) as a clear oil.

5.60. TLC Rₛ = 0.46 (hexane:ethyl acetate, 9:1); IR (film) 3050, 2967, 2868, 2718, 1716, 1464 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 10.01 (d, J = 0.8 Hz, 1H), 6.32–6.40 (m, 2H), 2.75–2.82 (m, 1H), 2.60 (ddd, J = 12.3, 9.7, 4.5 Hz, 1H), 2.41 (ddd, J = 13.4, 3.9, 3.0 Hz, 1H), 2.08 (s, 3H), 1.80–1.90 (m, 1H), 1.51 (dd, J = 13.1, 2.1 Hz, 1H), 1.35–1.45 (m, 1H), 0.99–1.13 (m, 22H); ¹³C NMR (75 MHz, CDCl₃) δ 207.1, 203.5, 136.3, 127.5, 86.5, 55.8, 38.3, 31.1, 27.6, 26.0, 22.5, 18.7, 13.7. HRMS (ESI): m/z calcld for C₂₀H₃₄O₃SiNa⁺ (M+Na⁺): 373.2169. Found: 373.2167.

5.61. TLC (hexane:ethyl acetate, 9:1) Rₛ = 0.53. IR (film): 2944, 2866, 2718, 1722, 1463, 1176 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ 10.25 (s, 1H), 6.54 (dd, J = 8.2, 6.6 Hz, 1H), 6.38–6.35 (m, 1H), 2.80–2.73 (m, 1H), 2.65 (dd, J = 13.0, 2.7 Hz, 1H), 2.22 (s, 3H), 1.63–1.54 (m, 1H), 1.52 (m, 1H), 1.34 (dt, J = 13.0, 2.9 Hz, 1H), 1.26–1.16 (m, 1H), 1.13–1.02 (m, 22H). ¹³C NMR (75 MHz, CDCl₃): δ 210.1, 205.1, 136.1, 128.7, 89.2,
56.9, 41.4, 31.5, 27.4, 22.4, 22.2, 18.6, 13.7. (ESI): m/z calcld for C_{20}H_{34}O_{3}SiNa^+ (M+Na^+): 373.2169. Found: 373.2167.

2–Acetyl–2–triisopropylsilyloxy–bicyclo[2.2.2]octane–1–carbaldehyde (5.68)

Palladium on carbon (0.01 g, 0.07 mmol, 10% on C) was added to a solution of alkenes 5.66 and 5.67 (0.13 g, 0.37 mmol) in absolute EtOH (4.5 mL). The reaction mixture was stirred continuously at rt under H₂ atmosphere. After 2 h, the reaction mixture was filtered through celite. The filtrate was concd under reduced pressure to afford alkane 5.68 (0.03 g, 23%) as an oil.

5.68. TLC Rᶠ = 0.38 (hexane:ethyl acetate, 9:1); IR (neat) 2969, 2866, 2725, 1714, 1153, 1024 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 10.06 (s, 1H), 2.76 (ddd, J = 13.5, 4.0, 2.6 Hz, 1H), 2.35–2.47 (m, 1H), 2.10 (s, 3H), 1.85 (m, 1H), 1.69–1.77 (m, 1H), 1.39–1.55 (m, 5H), 1.17–1.29 (m, 2H), 1.03–1.10 (m, 21H); ¹³C NMR (75 MHz, CDCl₃) δ 208.8, 206.9, 85.5, 49.6, 37.2, 26.5, 25.5, 25.0, 23.1, 22.2, 21.5, 18.6, 18.5, 13.7. HRMS (ESI): m/z calcld for C_{20}H_{36}O_{3}SiNa^+ (M+Na^+): 375.2326. Found: 375.2319.

References


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APPENDIX A

$^1$H AND $^{13}$C NMR SPECTRA SUPPLEMENT TO CHAPTER 2
APPENDIX B

$^1$H AND $^{13}$C NMR SPECTRA SUPPLEMENT TO CHAPTER 3
APPENDIX C

$^1$H AND $^{13}$C NMR SPECTRA SUPPLEMENT TO CHAPTER 4
APPENDIX D

$^1$H AND $^{13}$C NMR SPECTRA SUPPLEMENT TO CHAPTER 5
NOESY Spectra of 5.41
NOESY Spectra of 5.42

TBSO

5.42

NO₂

ppm
NOESY Spectra of 5.50
NOESY Spectra of 5.50 (Expansion)
Education

- **Syracuse University, Doctor of Philosophy**
  - August 2006 – September 2012
    - Major field of study: Medicinal and Organic Chemistry
    - Research advisor: John D. Chisholm
    - Dissertation: “Syntheses, Design, and Biological Evaluation of Inhibitors and Activators of Src Homology 2 Domain–Containing Inositol Phosphatase (SHIP) and Synthetic Studies on Apicularen A and Maocrystat V”

- **Syracuse University, Master of Philosophy**
  - August 2006 – April 2008

- **De La Salle University – Manila, Master of Science** May 2005 (Candidate)
  - September 2003 – May 2006
    - Major field of study: Chemistry

- **De La Salle University – Manila, Bachelor of Science** October 2003
  - May 2000 – August 2003
    - Major field of study: Chemistry
    - Thesis: "In Search of a New Potential Liquid Crystal Compound: Reaction of Chloroaceto-p-hexyphenone with 2,3,6,7-Tetrakis(2’cyanoethylthio)tetrathiafulvalene”

Work Experience

- **Part–Time Instructor**, Department of Chemistry, Syracuse University, Syracuse, NY
  - May 2012 – present
    - Teach undergraduate organic chemistry laboratory
    - Conducts lectures relevant to the experiments to be performed
    - Develop students’ knowledge in chemistry including laboratory techniques essential in handling glasswares, reagents, and equipments
    - Hold weekly office hours for students to provide extra help

- **Teaching Associate**, Department of Chemistry, Syracuse University, Syracuse, NY
  - August 2006 – May 2011
    - Teach undergraduate physical and general chemistry laboratory
    - Conducts lectures relevant to the experiments to be performed
    - Develop students’ knowledge in chemistry including laboratory techniques essential in handling glasswares, reagents, and equipments
    - Designed worksheets, practice exams, and their corresponding answer keys for students
    - Facilitated discussion relevant to organic chemistry topics discussed in the lecture
    - Assist in proctoring and grading exams
    - Hold weekly office hours for students to provide extra help
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- Lecturer, Chemistry Department, De La Salle University – Manila, Philippines
  - August 2003 – July 2006
    - Teach and supervise undergraduate laboratory courses which includes general, organic, physical and analytical chemistry; served as coordinators
    - Conducts lectures relevant to the experiments to be performed
    - Develop students’ knowledge in chemistry including laboratory techniques essential in handling glasswares, reagents, and equipments
    - Make worksheets so students can have extra practice
    - Assist in writing and grading exams
    - Holds office hours for students’ consultation
    - Handles Science Summer Camp

Educational Experience

- Graduate Research Assistant, Syracuse University, Advisor: John D. Chisholm
  - January 2007 – Present
    - Organic Synthesis
    - Exploration of various conditions for Hetero–Intramolecular Diels–Alder Cycloadditions
    - Synthetic Studies Toward Apicularen A and Maoecrystal V
    - Synthesis and Structure Activity Relationship Studies of SHIP Inhibitors
    - Characterization of Novel Organic Compounds, including $^1$H, $^{13}$C, $^{19}$F DEPT–135 NMR spectra, IR, & HRMS
    - Assist undergraduate students and Summer Research Experience for Undergraduate (REU) participants

- Graduate Research Assistant, Department of Microbiology and Immunology, State University of New York Upstate Medical University, Syracuse, NY, Advisor: William G. Kerr
  - May 2009 – August 2009
    - Synthesis and Structure Activity Relationship Studies of SHIP Inhibitors
    - Conducted Biological Activity Test Using Malachite Green Assay and Fluorescence Polarization Assay (FP) Test

- Undergraduate Research Assistant, De La Salle University – Manila, Philippines
  - May 2002 – August 2003
    - Organic Synthesis
    - Exploration of Potential Tetrathiafulvalene Based Liquid Crystal

Honors and Distinctions

- Phi Beta Delta Honor Society (2008)
- Nominee, Student Leadership Award and Community Development Award (2003)
Publications


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