Synthetic Investigation of Small-Molecule Probes

by

Caleb Bromba
Bachelor of Science with Honours, University of Victoria, 2007

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Abstract

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A series of small molecules was synthesized to probe three protein targets in order to elucidate the key small molecule-protein interactions required for potency. Triclosan is an antibacterial compound that has surfaced as a potential environmental hazard and is hypothesized to cause perturbations in the thyroid hormone response of frogs. Using a C-fin assay and a GH3 cell line, our work suggests that triclosan itself may not in fact be the cause of the observed endocrine disruptions. Instead, methyl triclosan (a result of biological methylation during waste water treatment) was shown to disrupt the thyroid hormone response in tadpoles. Secondly, a set of probes was designed based on a cyclopentane scaffold derived from the known neuraminidase inhibitor peramivir. Kinetic assays using both a recombinant neuraminidase protein and an inactivated sample of influenza virus showed that the guanidine group contributes a 10 fold increase in potency while the α-hydroxyl group was observed to have little to no effect. This result suggests that future neuraminidase drug design based on a cyclopentane scaffold may forgo the use of both the guanidinium group and the hydroxyl group to potentially increase the oral availability of these drugs while sacrificing little in the way of potency. Finally, a series of truncated analogues related to the western half of the natural product didemnaketal A was synthesized. These compounds will be used as probes to better understand the mechanism of didemnaketal-mediated protease inhibition. It is hypothesized that a more rigid structure (due to molecular gearing enforced by the presence of additional methyl groups, relative to previously examined analogues) will increase the potency of these molecules toward HIV-1 protease and may lead to new information for designing next-generation dissociative inhibitors. Work was also begun toward the total synthesis of the natural product itself.
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<td>Proton Nuclear Magnetic Resonance</td>
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<td>cRNA</td>
<td>complimentary ribonucleic acid</td>
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CSA          camphorsulfonic acid
D            dimeric enzyme
d            doublet
DCC          dicyclohexylcarbodiimide
dd           doublet of doublet
ddd          doublet of doublet of doublets
DDQ          2,3-dichloro-5,6-dicyano-1,4-benzoquinone
ddq          doublet of doublet of quartets
DEAD         di-tert-butylazodicarboxylate
DEPT         distortionless enhancement by polarization transfer
DI           dimer-inhibitor complex
DIBAL-H      diisobutylaluminum hydride
DMAP         N,N-dimethylaminopyridine
DMF          dimethylformamide
DMP          Dess-Martin periodinane
DMSO         dimethyl sulfoxide
DNA          deoxyribonucleic acid
DOS          diversity-oriented synthesis
dq           doublet of quartets
dr           diastereomeric ratio
DS           dimer-substrate complex
DSI          dimer-substrate-inhibitor complex
dt           doublet of triplets
DTS          diverted total synthesis
E            enzyme
e.g.        for example
EAATs        excitatory amino-acid transporters
EDC•HCl      1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
ee           enantiomeric excess
EFC          ethanol-free chloroform
EI           enzyme-inhibitor complex
ENR enoyl-acyl carrier protein reductase
ent enantiomer
epi epimer
equiv. equivalents
ES enzyme-substrate complex
ESI enzyme-substrate-inhibitor complex
Et ethyl
Et$_3$N triethylamine
FAS fatty acid synthesis
FKBP FK506 binding protein
FOS functional-oriented synthesis
FRB FKBP-rapamycin binding domain
FT-IR Fourier Transform Infrared
g grams
GCMS gas chromatography/mass spectrometry
GH growth hormone
GISA glycopeptide-intermediate S. aureus
GLAST glutamate aspartate transporter
GTP guanosine triphosphate
HA hemagglutinin
HATs (KATs) histone acetyltransferases
HDACs (KDACs) histone deacetylases
HIV human immunodeficiency virus
HSP30 heat shock protein 30
HSP70 heat shock protein 70
Hz hertz, s$^{-1}$
i iso
i.e. that is
IC$_{50}$ maximal inhibitory concentration
IL-2 interlukin-2
InhA inhibin $\alpha$
IR  infrared spectroscopy
J  coupling constant
K'  uncompetitive rate constant
Kc  classical competitive rate constant
k_{cat}  catalytic rate constant
Kd  dissociation rate constant
kDa  kilodalton
k_f  forward rate constant
KHMDS  potassium hexamethyldisilazide
K_i  inhibition rate constant
K_m  Michaelis-Menten rate constant
k_r  reverse rate constant
L  liter
LC-MS  liquid chromatography-mass spectrometry
LD_{50}  lethal dose, 50%
LDA  lithium diisopropylamide
LiHMDS  lithium hexamethyldisilazide
lit.  literature
M  molar
M  monomer
m  multiplet
M^+  molecular ion
m-CPBA  \textit{m}-chloroperbenzoic acid
mg  milligrams
MHz  megahertz
MI  monomer-inhibitor complex
MICs  minimal inhibitory concentrations
mM  millimolar
mmol  millimoles
mol  moles
mp  melting point
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<td>MRSA</td>
<td>methicillin-resistant <em>S. aureus</em></td>
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<td>mammalian target of rapamycin</td>
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<td>neuraminidase</td>
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<td>nuclear factor of activated T-cells</td>
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<td><em>N</em>-methylmorpholine oxide</td>
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<td>nuclear Overhauser enhancement</td>
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<td>proliferating cell nuclear antigen</td>
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<td>protecting group</td>
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<td>picomolar</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>PMB</td>
<td>p-methoxybenzyl</td>
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<tr>
<td>q</td>
<td>quartet</td>
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<td>QPCR</td>
<td>quantitative real-time polymerase chain reaction</td>
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<td>R</td>
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<td>Rana larval keratin type I</td>
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<td>reduced potassium dependency 3</td>
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<td>room temperature</td>
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<td>tobacco etch virus</td>
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<td>trimethylsilyl</td>
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<td>TOS</td>
<td>target-oriented synthesis</td>
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<tr>
<td>TRH</td>
<td>thyrotropin-releasing hormone</td>
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</table>
TRα  thyroid hormone receptor α
TRβ  thyroid hormone receptor β
Ts  p-toluenesulfonyl
TSH  thyroid-stimulating hormone
UGTs  uridine 5’-diphospho-glucuronosyltransferases
US EPA  United States Environmental Protection Agency
US FDA  United States Food and Drug Administration
UV  ultraviolet
vRNA  viral ribonucleic acid
w/w  weight percentage
WHO  World Health Organization
WT  wild type
X  generalized functional group
Acknowledgments

I would like to start by thanking the “bossman” Dr. Jeremy Wulff. You have been a great teacher over the past five years. If it wasn’t for you critiquing this work and giving me such prompt feedback, I doubt this thesis would ever have been done in time. Thanks to the UVic lab staff (Nichole Taylor, Peter Marrs, Jane Browning, Dave Berry and Kelli Fawkes) who taught me not only how to run a reaction, but how to teach others the same thing while bettering myself as a person. Jane, I fear the demo room will never be as organized once you and I leave UVic. As for Kelli, I do not think I will ever attend another meeting in my career where I will be fed so well.

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Finally, I’d like to thank all my friends and family who kept me sane during the arduous years of my graduate life. Here’s to never going “bananas”.
Chapter 1 – Introduction

1.1.0 Chemical Biology and Chemical Genetics

1.1.1 Merging of Chemistry and Biology

Through the use of scientific methodology, natural science seeks to elucidate the rules that govern the natural world. Tremendous advances in knowledge and technology over the past few centuries can be attributed to the subfields astronomy, biology, chemistry, earth science and physics. However, in order to develop new ideas and push the boundaries of science, a coalition between these disciplines is required. An important example is biology, whose adaptation from a descriptive (phenomenological) science to a molecular one has progressed through collaborative efforts with chemists. This invaluable partnership has generated new disciplines under the umbrella of natural science.\textsuperscript{1,2}

Chemical biology became a new discipline to bridge the gap between synthetic organic chemistry and molecular, structural and cellular biology. It includes proteomics (the study of the proteome, enrichment techniques, and design of enzyme probes), glycobiology (the study of sugars), combinatorial chemistry (automated synthesis of diverse compounds) and molecular sensing (the study of biological processes through molecular imaging).

Genetics is a subclass of biology and specifically deals with the molecular structure and function of genes, as well as heredity and gene distribution. This subclass can be further divided into classical forward genetics and classical reverse genetics. In classical forward genetics, analysis starts with an outward physical characteristic called a phenotype and ends with the identification of the gene or gene product that is responsible for it. In classical reverse genetics, scientists start by irreversibly deleting or mutating specific sequences in a gene and then analyze the phenotype when the gene is mutated. While providing a wealth of information, these techniques are not without limitations such as global side effects and difficulty moving to higher mammalian systems.\textsuperscript{3} Therefore, to investigate living systems and their biochemical processes, a modification to the classical approach was required.

Just as genetics underpins biology so too does chemical genetics support chemical biology. However, the focus is now on modulation at the protein level rather than the genetic level. The principal goals of chemical genetics are to explain the function and responses associated with interactions between a small molecule and a biological macromolecule. Several
advantages over classical genetics include reversible, temporal and dose-dependent control of gene products and greater versatility when moving to higher order systems. Furthermore, the fine-tuning ability available for small molecules can be used to increase specificity toward a target protein. This specificity can then lead to a perturbation of a single protein function. In contrast, modulation of a gene, as is the case for classical genetics, can lead to global side effects for the gene product.

As with classical genetics, chemical genetics can be further subdivided into forward and reverse chemical genetics. Forward chemical genetics starts with the identification of a specific phenotype of interest (Figure 1). Assays are used to monitor the phenotype and commonly include the use of cells or cell lysates in well-plates; alternatively one could utilize genetically tractable model organisms such as *Drosophila melanogaster* (fruit fly), *Caenorhabditis elegans* (nematode worm), *Saccharomyces cerevisiae* (budding yeast), *Arabidopsis thalina* (plant), *Danio rerio* (zebrafish) or *Mus musculus* (mice). Next, a series of small molecules are added to the biological system in order to produce a phenotypic response. A “hit” occurs when a small molecule generates such an outcome. It is generally assumed that the phenotype response is due to the small molecule interacting with a single biomolecule (or at least a subset of biomolecules) associated with this phenotype, although this cannot be stated with certainty. The last and most challenging part is the determination of which protein(s) the small molecule is interacting with. To solve this, the small molecule is generally re-synthesized with a radioactive isotope, biotin or fluorophore and re-subjected to the phenotypic assay in hopes of selecting a specific target(s) associated with the phenotype. The use of these tags and labels will be discussed later on in this introduction.

Reverse chemical genetics is the more common approach and starts with the identification of a specific biomolecule (Figure 1). Once a specific protein is selected, a series of small molecules can then be assayed against this target until a perturbation for the protein is found. These candidates are fine-tuned and re-tested under several conditions and only those that pass these subsequent tests will be used to alter the protein’s function in cells or animals. Analysis of the phenotypic response at this stage can provide information about the binding or modulation of the protein as well as information on the genetic pathway(s). With the completion of human genome sequencing, information for the primary (sequence) structure has provided a defined set of potential protein targets. Furthermore, understanding of primary
structure allows predictions for the secondary (domain folds) and tertiary (overall threedimensional) structures. Insights into tertiary structures coupled with information about known proteins with similar sequences, can help to predict functional sites. The roles of uninvestigated proteins can then be used to define new chemical challenges, e.g. development of small molecules to probe protein structures and to help explain key biological interactions needed to cause inhibition of the target protein.

**Figure 1.** Forward and reverse chemical genetics

### 1.1.2 Chemical Space and Biological Space

“Small” molecules are low molecular weight organic compounds, usually less than 500 to 700 Daltons. This low molecular weight allows potential rapid diffusion across cell membranes enabling the small molecule to reach intracellular sites of action. Whether synthetic or natural, these molecules find applications as pharmacological prototypes, precursors to more diverse chemical entities or as instrumentation in the study of biological processes. However, with a vast number of “small” molecules available, how does one narrow down the possibilities to lead to an effective chemical probe?

Molecules are characterized by shape, physical properties (molecular mass, nucleophilicity, lipophilicity, and dipolar moment), topology, etc. Therefore, the term “chemical space” encompasses all these characterizations for all compounds that could be theoretically conceived. Similarities among their properties allow further subcategorizing to compound
families. The virtual chemical space as well as the natural product space is immense. However, the number of available synthetic targets can be reduced since it is calculated that approximately $10^{60}$ chemical compounds could potentially be bioactive molecules and would show pharmacological properties.\textsuperscript{6,7} While theoretically there are a variety of amino acid sequences able to lead to a vast number of potential proteins, the human genome only synthesizes a defined number of proteins. Thus the biologically relevant chemical space, encompassing synthetic or natural small molecules able to interact with biomolecules, is a mere fraction of the overall possible compounds that could be synthesized (Figure 2).

\textbf{Figure 2.} Chemical and biological space relationship

\textbf{1.1.3 Chemical Probes}

Understanding biological processes often comes from directly perturbing a protein and observing the effects. The ability to alter these functions with chemical probes on short timescales and to vary enzymatic response through dose dependency has led to valuable insight for many dynamic biological pathways.\textsuperscript{8} Living systems have evolved over billions of years to generate chemical compounds under specific conditions, typically aqueous media and temperatures of 0 °C to 40 °C.\textsuperscript{1} Enzymes, in conjunction with other proteins and nucleic acids, are responsible for the synthesis, transport and degradation of all small molecules within a living organism. The small molecule-protein interaction requires the two structures to complement each other in shape. Emil Fischer invented the concept of a “Lock-and-Key” interaction between a small molecule and its biological target. However, he was unaware at the time of the dynamic
and flexible nature of protein structures.\textsuperscript{9} To take into account this flexibility, Fischer’s original concept was refined to a “Hand-and-Glove” notion which includes the adaptive motion of the binding partners.\textsuperscript{10} In order to develop a quality chemical probe, a reasonably high specificity between the protein and the small molecule is required. Creating vast libraries of small molecules lacking a natural product scaffold would rely on serendipity to discover a successful chemical probe, whereas the co-evolution of natural products and their macromolecule counterparts offers superior diversity, specificity, binding efficiency and affinity that would otherwise be unavailable or too time consuming to obtain from the best synthetic moieties.\textsuperscript{11}

Two major impacts in biology resulting from the use of chemical probes were the synthesis and study of trapoxin and of rapamycin.\textsuperscript{12} Trapoxin causes arrest in mammalian cells by inhibiting histone deacetylation whereas rapamycin interferes with a mammalian gene product (subsequently given the name mammalian target of rapamycin or mTOR) that plays an important role in immune system response.

In 1996, Schreiber used trapoxin to achieve the first molecular characterization of a histone deacetylase.\textsuperscript{13} Prior to this work, isolation of the protein had proved challenging for many groups. Posttranslational modification of histones regulates gene expression. Modifications include acetylation, phosphorylation, methylation and ubiquitination.\textsuperscript{12} These modifications are also reversible allowing the molecule to be deacetylated, dephosphorylated and demethylated. Gene transcription is activated by acetylation of ε-lysine residues of histone tails by histone acetyltransferases (HATs; now called KATs). Conversely, transcriptional silencing and chromatin condensation are achieved by deacetylation using histone deacetylases (HDACs; now KDACs). Schreiber and co-workers\textsuperscript{13} used trapoxin, a cyclotetrapeptide isolated from the fungus \textit{Helicoma ambiens}, to probe the HDAC molecular target and found that it induced morphological reversion of \textit{v-sis}-transformed NIH/3T3 fibroblasts causing an accumulation of acetylated histone cores.

Previous work by several groups showed that hydrolysis or reduction of the epoxide in trapoxin led to abolishment of the inhibitory activity toward HDAC in mammalian cell lines.\textsuperscript{13,14} This suggested that trapoxin may act as an irreversible inhibitor through covalent bond formation (Figure 3). The development of a trapoxin-based affinity resin, known as K-trap, utilized the electrophilic epoxy-ketone to facilitate enrichment and eventual identification of the HDAC protein target, subsequently named HD1.\textsuperscript{13} The protein possessing histone deacetylase activity
was also 60% identical to reduced potassium dependency 3 (Rpd3), a transcriptional repressor in yeast. As such, these studies showed correlations between histone deacetylases and transcriptional regulation/cell cycle progression. This research ultimately led to the development of HDAC inhibitors for cancer treatment.\textsuperscript{15,16}

![Chemical structures of Trapoxin and Acetylated Lysine](image1)

**Figure 3.** Trapoxin and its affinity resin K-Trap

Another important natural product is rapamycin (Rap), a macrolide produced by *Streptomyces hygroscopicus*. Although structurally similar to FK506 (an immunosuppressive drug) (Figure 4), the two compounds exhibit different activities, prompting the use of rapamycin and its derivatives for the examination of several cellular processes including cell growth, proliferation, transcription, survival and protein synthesis.\textsuperscript{17,18}

![Chemical structures of Rapamycin and FK506](image2)

**Figure 4.** Rapamycin and FK506 have similar structures but influence T-cell signalling by different mechanisms
Figure 5. The FKBP-rapamycin complex targets interleukin-2 response whereas the FKBP-FK506 complex inhibits calcineurin. RAP: rapamycin; FKBP: FK506 binding protein; mTOR: mammalian target of rapamycin; NFATc: nuclear factor of activated T-cells; Calc: calcineurin; P: phosphorus; FRB: FKBP-rapamycin binding domain; IL-2: interleukin-2

Rapamycin and FK506 both interact with a peptidyl-prolyl cis/trans isomerase (subsequently given the name FK506 binding protein or FKBP). The FKBP-FK506 complex directly inhibits calcineurin, whereas the FKBP-rapamycin complex does not (Figure 5). Calcineurin (Calc) is a protein phosphatase that activates T-cells of the immune system by
dephosphorylating a nuclear factor of activated T-cells (NFATc). The activated NFATc is translocated into the nucleus where it upregulates interleukin-2 (IL-2), a cytokine signalling molecule used to attract lymphocytes. Therefore, direct inhibition of calcineurin by the FKBP-FK506 complex reduces interleukin-2 production leading to an overall downregulation in T-cell production.12

Conversely, the FKBP-rapamycin complex does not target calcineurin but rather forms a complex with mTOR through a binding domain subsequently called the FKBP-rapamycin binding domain or FRB. This complex blocks the response to interleukin-2. Using this research, Kapoor was able to identify the lipid kinases mTOR1 and mTOR2 and prove that these proteins possessed homology to the mammalian phosphatidyl inositol-3-kinases involved in cell cycle progression.12 Furthermore, the low toxicity of rapamycin versus common calcineurin inhibitors, especially toward kidneys, has allowed it to find applications in transplant therapy. By suppressing the host’s immune system, rejection to the new organ is minimized. A particular advantage is seen for patients with kidney transplants for haemolytic-uremic syndrome, as this disease is likely to reoccur when using calcineurin inhibitors but less likely to reoccur when using rapamycin.19

1.1.4 Activity-Based Probes

Enzyme activity (in vivo) is dependent on regulation by substrate co-localization, post-translational modification, allosteric interactions and/or co-regulation by endogenous inhibitors.20 Due to the complex behaviour of enzymes in vivo, cell-free experiments with recombinant enzymes may only offer limited knowledge and have the potential to be misleading. This is due to the temporal difference between catalysis and gene expression as well as the time required for dynamic messenger ribonucleic acid (mRNA) processing. Due to these variations a poorly-resolved picture of protein abundance and therefore active enzyme concentrations is obtained. Activity-based protein profiling is a proteomic technology that uses specially designed chemical probes to react mechanistically with distinct enzymes, providing insight into their activity. Knowledge of the enzyme’s catalytic mechanism ensures that detection occurs for catalytically-active species only.20 Unlike substrate mimics and reversible inhibitors, once in the active site activity-based probes form covalent bonds. Since the probe acts as a mimic of the endogenous substrate, the catalytic conformation of the enzyme can be trapped through the formation of this covalent bond. Isolation then provides information on the active site
transformations required for catalysis of the endogenous substrate. Another advantage of activity-based probes are their cross-species portability which provides biological information on pathological species. Finally, activity-based probes have the ability to pick up specific activities in a homologous group of enzymes, potentially leading to the understanding of parallel biochemical pathways.

Figure 6. Activity-based probes (ABP) vs. Affinity-based probes (AfBP). Activity-based probes label the enzyme through direct binding to the active site. Affinity-based probes label based on affinity (not necessarily in the active site), followed by stabilization via a non-specific cross-linking
Before continuing, an important distinction between activity-based probes (ABP) and affinity-based probes (AfBP) must be made. An affinity-based probe binds at a specific site on a protein (not necessarily the active site) followed by a non-specific covalent bond-forming event, usually triggered by photochemical cross-linking or by spontaneous trapping of a nearby functional group on the protein (Figure 6). For example, if a catalytic site residue required for catalysis but not for substrate binding is inactivated then an activity-based probe will no longer function, whereas an affinity-based probe may still be able to function normally.

Initial studies of activity-based probes used solid-phase affinity chromatography, where the probe is directly bound to a solid matrix, incubated in cell lysates and then purified. Unfortunately, binding experiments are therefore limited to tissue and cell lysates since the solid-phase is definitely not cell permeable. To fully utilize the activity-based probe technology, advances have been made toward developing new soluble and cell permeable probes.

Figure 7. Bioorthogonal ligation introduces a substrate modified with a bioorthogonal functional group to a cell. A reporter molecule containing the complementary functional group is introduced to react and label the substrate.

Bioorthogonal ligation (or “click” chemistry) can be used to link a fluorophore to the probe after the warhead has been administered and activated by the enzyme. This offers the advantage of no longer having a bulky fluorophore or solid matrix attached to the probe prior to binding. Since the warhead can be designed to be cell permeable, the assay is no longer restricted
to tissue and cell lysates. Furthermore, use of bioorthogonal functional groups avoids non-specific labelling and is known to be highly efficient. In the most common strategy, the fluorophore contains an azide whereas the probe contains an alkyne, both being inert to biological environments (Figure 7). Using a [3+2] copper (I) catalyzed Huisgen cyclisation as described by Sharpless, a triazole is formed in situ, followed by analysis using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Other common bioorthogonal functional groups include phosphines which undergo a Staudinger reaction with the azide or the use of cyclic alkynes to undergo a copper-free [3+2] cyclisation.

Fine tuning of activity-based probes allows for either a specific interaction with a distinct protein or a broader targeting of protein families. The probe is often based on an electrophilic irreversible inhibitor of an enzyme class and incorporates a “warhead”, a specificity domain (sometimes), a linker region and a tag or label. As would be expected, the design of these activity-based probes follows the same design progression of an effective enzyme inhibitor and thus many of these probes have been molecularly engineered from the discovery of both irreversible and reversible inhibitors. The “warhead” is designed as an electrophilic trap, such as an epoxide or Michael acceptor, which reacts irreversibly to form a new covalent bond and trap the enzyme in its active form (Figure 8). Care must be taken to design an electrophilic warhead that will interact with a nucleophilic amino acid residue in the active site but will not react non-specifically with other potential nucleophiles such as thiols, amines and alcohols. Moreover, the quality of the warhead is dependent on both its affinity and chemical reactivity toward the enzyme’s active site. Thus, a highly reactive warhead may be able to target a variety of enzymes while a less reactive warhead can be used to target a single enzyme.

While there are a variety of potential functional groups available for the warhead, the use of stable leaving groups adjacent to ketones is common when targeting cysteine and serine proteases. Furthermore, the ability of a hard electrophile to react selectively with serine and a soft electrophile to react selectively with cysteine allows the development of warheads with orthogonal reactivity. Other electrophiles such as acyl and aryloxymethyl ketones, fluorophosphonates, and vinyl sulfones have been used in the same manner. Metalloproteases encompass another large and diverse class of enzymes. Activity-based probes can selectively chelate the zinc metal found in the metalloprotease active site with hydroxamates, providing
information on the enzyme’s function which would otherwise be challenging to obtain when using conventional genomic and proteomic methods.

Figure 8. Key features of activity-based probes

The key feature that sets probes apart from a standard irreversible inhibitor or suicide inhibitor is the presence of a tag or label. Three of the most common tags/labels used are fluorophores, radioactive isotopes, and biotin. The size and properties of the tag can have significant influences on the behaviour of the probe since if directly linked to the warhead they have the possibility of interfering with the coordination activity due to their bulk. Therefore, in order to avoid this, fluorophores and biotin are usually attached to the warhead using a linker. Linkers fall into two categories: non-cleavable and cleavable. Non-cleavable linkers include polyethylene glycol (PEG) units that can be fine tuned to enhance solubility or membrane permeability. With high affinity tags (such as biotin), cleavage generally requires harsh conditions that would denature the enzyme thereby often requiring further purification by gel
electrophoresis prior to analysis by mass spectrometry (MS). Cleavable linkers avoid this by facilitating cleavage between the probe-bound protein and the affinity tag bound to the resin. Two examples of cleavable linkers are disulfide bonds and linkers containing a functional group able to react with tobacco etch virus proteases (TEV). Disulfide bonds are the simpler of the two but suffer drawbacks due to the nucleophilic nature of proteases and non-specific disulfide formation. Cravatt\(^\text{25}\) has highlighted the use of tobacco etch virus proteases that cleave at a specific site on the linker. Therefore, the probe bound to the active enzyme can be isolated through solid-phase binding experiments (e.g. biotin bound to streptavidin) and then cleaved under less harsh conditions to isolate the desired enzyme for analysis.\(^\text{24}\)

Fluorophores are one of the most common tags used in the identification of enzyme-bound probes. While having similar sensitivity to that of radiolabels, the diversity, commercial availability, absorbance and emission spectra, safety and cost provide a significant advantage.\(^\text{21}\) Analysis of the fluorescence by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) enables a direct read-out of probe labelled proteins using laser scanners. Since this can be done directly while still in the gel cassette, fluorophores offer a greater means to gain quantitative measurements over both radiolabels and biotin which require processing of the gel prior to analysis.\(^\text{21}\) One of the downfalls of fluorophores is their size. This bulk can have effects on affinity of the small molecule for the enzyme active site as well as membrane permeability. The affinity dilemma has already been addressed in the previous paragraph with the use of linkers, whereas the use of “click” chemistry to avoid cell permeability issues was discussed earlier.

Radioactive isotopes (\(^{125}\text{I}, ^{3}\text{H}, \text{etc}\)) are widely used in the detection of biomolecules due to their high sensitivity. Since incorporation of the radioactive isotope does little to affect cell permeability or affinity, the labelled probes have found use in diverse samples such as intact cells, cell or tissue lysates and whole organisms.\(^\text{21}\) The bound proteins are isolated by chromatography, followed by SDS-PAGE and then the proteins are analyzed using liquid chromatography-mass spectrometry (LC-MS) or detected with Western-blot antibody candidates (Figure 9).

Biotin (vitamin H or B\(_7\)) is a common tag for activity-based probes because it facilitates isolation and enrichment of the target proteins through binding with avidin (or streptavidin) on a solid-phase network.\(^\text{21}\) Biotin has a high affinity for avidin with a dissociation constant (K\(_d\)) of
$10^{15}$ M making it one of the strongest non-covalent protein-ligand interactions. A common experiment uses a biotinylated probe pre-complexed to streptavidin bound to a solid-support resin and incubated with live cells. The probe and its bound protein are removed, and eluted from the avidin resin by 8 M guanidine hydrochloride, pH 1.5 or by boiling in an SDS-PAGE denaturing loading dye. Subsequent analysis is performed using LC-MS or detection with candidate antibodies.

**Figure 9.** General strategy for target identification. Activity-based probes are added to proteomes (live cells or lysates) and reacted with their target protein. Probe-bound proteins are enriched by affinity chromatography and analyzed by SDS-PAGE. Target identification proceeds via LC-MS or detection with candidate antibodies.
candidate antibodies (Figure 9). A disadvantage of this route is the non-specific binding of endogenous biotin-containing proteins to the streptavidin resin. However, this problem is avoided by using control and competition experiments to exclude this data. Finally, similar to fluorophores, biotin suffers the potential drawback of reduced cell permeability due to its size. As described earlier, use of bioorthogonal ligation allows binding of a probe to the enzyme target followed by coupling of the biotin label.

1.1.5 Thesis Goals

A chemical probe is a small molecule synthetically designed to interact with a specific protein in order to explore its role in a broader biological context. The design of a chemical probe is often based on a natural product since this approach offers more diversified structures, greater specificity and better affinity due to the co-evolution of natural products with their biomolecule counterpart. Conversely, a chemical probe can be designed around a simplified core which serves to project functional groups toward key interactions with the target protein. Understanding the structure and function of the biological target and/or knowing information about the biological phenomenon being studied will have a large impact on the strategy taken toward designing these chemical probes. Ultimately, any small molecule, whether natural or synthetic, that is able to selectively perturb the function of a biomolecule can be used as a chemical probe.

This thesis describes the synthesis of small molecules to probe three protein targets. Triclosan, an aromatic ether, is an antibacterial compound that has surfaced as a potential environmental hazard by perturbing the thyroid hormone response of frogs. Since the receptor being targeted is unknown, a series of compounds will be synthesized that contain the aromatic scaffold of triclosan but differ in functionality around the ring. Testing will be done with tadpoles and those compounds showing an interaction with the frog’s thyroid receptors will be resynthesized with a biotin tag in order to analyze the specific receptor(s) being targeted. The second target is the H1N1 neuraminidase protein of the influenza virus. Design of the chemical probe will involve the use of a cyclopentane scaffold, similar to peramivir (a known neuraminidase inhibitor), to understand the contribution of both the guanidine and the hydroxyl functional groups toward the inhibition of H1N1 neuraminidase. Finally, didemnaketal A is a natural product containing an acyclic esterified polyol western half and a spiroketal eastern moiety with the acyclic portion of the molecule showing potency toward HIV-1 protease. A
series of analogues based on the acyclic western half of the molecule will be used to better understand the mechanistic origins of HIV-1 inhibition by didemnaketal A. Furthermore, stereochemical and functional modifications of these analogues will aid in determining which key interactions are required for optimal inhibition of the target protein. Utilizing the methodology designed for the acyclic didemnaketal A analogues, a similar synthetic strategy will be used to design the western fragment of didemnaketal A and couple it to the spiroketal portion, thus generating the natural product.

1.2.0 Triclosan

1.2.1 Applications and Specific Targets

Triclosan has been used for nearly forty years as an antibacterial and antifungal agent in many commercial products such as toothpaste, deodorant, bodywash, cosmetics, antiseptic soaps, carpets, plastic kitchenware and toys. Triclosan at low doses is bacteriostatic and acts by inhibiting the enzyme enoyl-acyl carrier protein reductase (ENR) in bacteria in a similar fashion to diazaborine (another antibacterial agent), effectively blocking lipid biosynthesis. The enzyme ENR (FabI) catalyses the final regulatory step in the fatty acid synthase cycle by reducing a carbon-carbon double bond in an enoyl moiety (Figure 10). The mechanism is thought to proceed via hydride transfer from the nicotinamide C-4 position of NADH or NADPH to the C-3 position of the enoyl moiety. This forms an enolate intermediate which receives further donation of a proton resulting in the reduced product. An overlay of the phenol in triclosan and the enolate intermediate indicates good mimicry in that the C-3 of the enoyl and the C-5 of triclosan bind at the same site (Figure 11c). Since the ENR enzyme is a key component in type II fatty acid synthesis (FAS) it provides an attractive target because of its significant role in metabolism as well as its sequence conservation across many bacterial species. Furthermore, the structure of ENR in bacteria is distinct from that of the mammalian ENR required for fatty acid synthesis which allows for higher specificity.
Figure 10. Enoyl-acyl carrier protein reductase (ENR), coenzymes $\text{NAD}^+$/NADH and $\text{NADP}^+$/NADPH and inhibitors. A) Inhibitors triclosan and diazaborine. B) NAD$^+$/NADH and $\text{NADP}^+$/NADPH are cofactors that aid in the reduction of double bonds via ENR. C) The reduction of an enoyl moiety by NADH in the presence of the enzyme ENR. ACP: acetyl-carrier protein.

Using *Escherichia coli* ENR (FabI) complexed with NAD$^+$ and triclosan, Larson$^{26}$ showed that the phenol ring of triclosan forms a $\pi$-$\pi$ stacking interaction with the nicotinamide ring of NAD$^+$ (Figure 11a). Furthermore, van der Waals interactions between the active site residues and triclosan contribute good substrate binding in the enzyme pocket. Finally, favourable hydrogen bonding exists between the hydroxyl group on triclosan, the 2’ OH of the nicotinamide ribose and the phenolic oxygen of tyrosine 156, believed to be the proton donor during the catalytic cycle of ENR.$^{26}$ Mutations G93V, M159T, and F203L are known in triclosan- and diazaborine-resistant strains of *E. coli* and *S. aureus* (Figure 11b). The G93V mutation is of most significance as the valine causes a 100-fold inhibitory loss due to steric
hindrance of the 2,4-dichlorophenoxy ring of triclosan. Triclosan is bactericidal at higher doses and inhibits bacterial growth through multiple cytoplasmic and membrane site interactions including the reduction in membrane potential during oxidative phosphorylation. A reduction in membrane potential depletes ATP, a key molecule required in many biological processes, thus causing a decrease in bacterial growth through the inhibition of multiple targets. Access to multiple targets is believed to be the cause for low triclosan resistance amongst many bacteria.

**Figure 11.** Triclosan bound to *E. coli* ENR. A) A key hydrogen bond between tyrosine 156, the phenol of triclosan and the 2’ OH of NAD⁺. Measurements are shown in Å. B) Interactions of NAD⁺ and triclosan with residues commonly mutated to produce triclosan and diazaborine resistance. C) Overlay of triclosan and the enoyl-acyl carrier protein fragment. Enoyl: aqua; Triclosan: orange; NAD⁺: brown. Acetyl-carrier protein (ACP).
One of the first applications of triclosan was its widespread use in controlling plaque formation on teeth.\textsuperscript{27,28} Since it is non-ionic, it can be easily formulated into common dentifrices and oral mouthwashes. Two common formulations have been triclosan bound to a polyvinylmethyl ether maleic acid copolymer and triclosan mixed with zinc citrate. Both approaches have been applied to decrease gingivitis in adolescents and adults as well as decreasing further root degradation during periodontitis.\textsuperscript{28} Triclosan has also been known to reduce skin and mucosal inflammation in the gingiva. However, widespread use of triclosan in other materials where no known contribution is validated has led researchers to worry about possible triclosan resistance.

\textbf{1.2.2 Triclosan Resistance}

Since entering the market, triclosan has become a controversial molecule based on research into triclosan-resistant bacteria as well as its impact on the environment. While some believe in the removal of this drug, others believe that there remains insufficient evidence to validate any claims. Triclosan is a primary agent in oral hygiene although its proven benefit in other applications is often criticized. It has been hypothesized that since triclosan mechanistically impacts bacteria in a similar fashion to antibiotics, continued use/overuse of triclosan will eventually lead to resistance. Antibiotics have a specific cellular target to which they interact with in order to cause bacterial destruction. If a mutation occurs at this specific target then the antibiotic will be rendered ineffective and the organism will be considered resistant. In contrast, antiseptics rely on multiple, non-specific modes and are thus less likely to be affected by a specific cellular mutation. Known mechanisms of resistance toward triclosan include: i) the overproduction of the enoyl-ACP reductase enzyme, ii) production of a triclosan nonsusceptible enzyme (i.e. a triclosan-resistant enoyl-ACP reductase with a point mutation impeding the formation of the FabI-NAD$^+$-triclosan tertiary complex), iii) the production of triclosan-degrading enzymes, iv) moderation of outer membrane permeability barriers and v) upregulation or adaption of pre-existing efflux pumps.

Laboratory research has demonstrated triclosan resistance, although the use of enteric bacteria and pseudomonads in these studies is problematic since triclosan is not known to be active against either.\textsuperscript{29,30} In fact pseudomonads have a natural resistance to triclosan with strong evidence showing an inability of triclosan to pass through a cell barrier augmented by the expression of efflux pumps capable of removing hostile molecules from the cell. While triclosan
does not directly influence the efflux pumps, it is believed that sub-bactericidal exposure to triclosan may result in selection for spontaneous hyper-expressing efflux mutants. These efflux mutants put a heavy energy-cost on the bacteria thus making these mutations less likely to occur in nature. However, several studies have shown efflux pumps to be the predominant mechanism in triclosan resistance.

Clinical isolates of *Staphylococcus epidermidis* and *Staphylococcus aureus* including methicillin-resistant *S. aureus* (MRSA) showed reduced susceptibility to triclosan while glycopeptide-intermediate *S. aureus* (GISA) and heterogeneous GISA strains were less susceptible to triclosan when compared to MRSA. In contrast, triclosan susceptibility measurements using fresh natural isolates (mouth, nose, etc.) versus cultured *E. coli* showed minimal decreases in triclosan activity leading to the hypothesis that such susceptibilities may only be applicable to *E. coli* and are not universal. Furthermore, exposure of *E. coli* with long-term, sub-lethal concentrations of triclosan has generated mutations in either the enoyl-reductase enzyme (FabI) (G93V, M159T, and F203L) or has caused overexpression of multidrug efflux pumps in laboratory settings. Similar effects have been observed using *Salmonella*. Overexpression of efflux pumps led to an increase in minimal inhibitory concentrations (MICs) for trimethoprim, tetracycline, ciprofloxacin and erythromycin in *Ps. aeruginosa*. Furthermore, work by McMurray demonstrated that mutations of InhA (homologous to FabI) residues like those of triclosan-resistant *E. coli* led to resistance of triclosan in *Mycobacterium smegmatis*. This suggests that like isoniazid and diazaborine, triclosan binds to the InhA target in these particular bacteria and thus mutations against triclosan would result in cross-resistance for isoniazid and diazaborine. Finally, the four key residue mutations that influence triclosan resistance in *M. smegmatis* (S93, M103, A124 and M161) are conserved in *M. tuberculosis*. However, it is unclear whether *M. tuberculosis* resistance is due to a natural triclosan resistant acyl-carrier protein (ACP) or whether there exists other mechanisms such as endogenous efflux pumps. While some studies are inconclusive in demonstrating triclosan resistance as a result of prolonged exposure, other studies have verified such resistance. Many of the resistances shown clinically cannot be verified as having come from triclosan since other antibiotics targeting the same enzyme could have led to the original mutation. Thus, while triclosan resistance cannot be ruled out there remains a lack of confirmation of triclosan-induced resistant bacterial populations or an overgrowth of opportunistic bacteria in environments outside the laboratory.
1.2.3 Human Exposure and Environmental Impact

Millions of pounds of triclosan are produced annually each year for use in clinics and personal care products. Exposure of the general population through ingestion or dermal contact with consumer products containing triclosan or through the consumption of food and drinking water contaminated with triclosan is of great concern. Triclosan reaches the circulatory system via absorption through the mucous membranes and gastrointestinal tract when taken orally, through the skin with dermal exposure, and through mucosal tissues from intra-vaginal administration. During the manufacturing process, workers are exposed by dermal contact or inhalation. A National Occupational Exposure Survey estimated that nearly 190,000 workers in 16 different industries were potentially exposed to triclosan from 1981 to 1983.

A study monitoring triclosan levels in human breast milk from Australia found that while detectable, the results were variable and not associated with demographics.\(^{35}\) In contrast to the previous study, research from Sweden has shown direct correlations between towns with contaminated water or heavy use of triclosan-containing products and the detected concentrations of triclosan in urine. It was concluded that the levels of exposure to triclosan in Australia must be low, possibly because of individual influence (e.g. not using commercial products containing triclosan). Sixty percent of randomly selected Swedish human milk samples contained triclosan at <20 to 300 µg/kg lipid,\(^{36}\) whereas concentrations of 81 to 345 µg/kg lipid were found in 50% of milk samples isolated from American lactating women with no known exposure to triclosan.\(^{37}\) Additionally, 62 single samples of breast milk donated to the Mother Milk Bank in San Jose, CA and Austin, TX yielded a range of triclosan from undetectable (two samples) and barely detectable (nine samples) to readily detectable at levels of 100 to 2100 µg/kg lipid (51 samples).\(^{38}\) Triclosan has also been detected in human plasma samples from 0.1 to 8.1 ng/mL in ten individuals of whom five were exposed to triclosan and five were not.\(^{37}\)

Finally, regardless of the usage of personal care products containing triclosan, nursing mothers had detectable levels in both their breast milk and their plasma. However, the levels of triclosan were higher in mothers who used personal care products containing triclosan (0.4 to 38.0 ng/g in plasma and 0.022 to 0.95 ng/g in milk) than in those who did not (0.01 to 19 ng/g in plasma and <0.018 to 0.35 ng/g in milk).\(^{39,40}\) While triclosan can be found in both breast milk and plasma, there exists no evidence to suggest that these levels are harmful to humans.
Triclosan is metabolized to glucuronide and sulfate conjugates as demonstrated by in vitro experiments using skin\textsuperscript{41} and liver microsomes or cytosol\textsuperscript{42} (Figure 12). Dermal metabolism of triclosan in diffusion cells with human skin showed that only the sulfate conjugate metabolites were present in the first 4 hours, while both the sulfate conjugates and glucuronides were present at 8 and 24 hours. At all times, there was more unchanged triclosan than either of the conjugates.

Triclosan is excreted in both feces and urine with urine being the major route of elimination. The excretion of triclosan in urine has been reported at rates of 0.1 to 743 µg/day from a random selection of ten Swedish men\textsuperscript{40} and at concentrations of 2.4 to 3790 µg/L urine from a random selection of 2517 participants 6 years of age or older from the United States.\textsuperscript{43} Urinary excretion in humans increases after exposure with the major fraction being eliminated in the first 24 hours. Between 24% and 83% of a 4 mg dose was excreted mainly as its metabolites in the first 4 days with the remainder being excreted within 8 days of exposure.\textsuperscript{36}

**Figure 12.** Triclosan metabolism. P450: cytochrome P450; UGTs: UDP-glucuronosyltransferases; SULTs: sulfotransferases.
Extensive research has addressed the acute toxicity, subchronic toxicity, skin sensitization, reproductive/developmental toxicity and genotoxicity/mutagenicity of triclosan. The LD$_{50}$ for oral administration of triclosan is 3750 to >5000 mg/kg by weight in rats,$^{44}$ 4350 mg/kg by weight in mice$^{45}$ and >5000 mg/kg by weight in dogs.$^{46}$ The route of administration has a significant influence on the toxicity of triclosan. Intravenous administration showed a LD$_{50}$ of 19 mg/kg by weight in mice$^{45}$ and 29 mg/kg by weight in rats$^{46}$ while intraperitoneal injections led to LD$_{50}$ values of 184 to 1090 mg/kg by weight in mice. Furthermore, dermal application to rabbit skin as a slurry in propylene glycol gave a LD$_{50}$ of >9300 mg/kg by weight.$^{46}$ Finally, subcutaneous administration in rats had a LD$_{50}$ of 14,700 mg/kg by weight.$^{46}$

Subchronic toxicity experiments using dental products$^{46}$ (toothpaste, mouthwash and aqueous slurries) and skin sensitization using triclosan in a water-isopropanol mixture$^{47}$ showed no adverse effects. While a two-generation reproduction study on rats and a developmental study on mice, rabbits and rats sponsored by triclosan manufacturers showed only minor neonatal toxicity at 3000 ppm of triclosan, oral administration of triclosan to pregnant mice resulted in both maternal and fetal toxicity represented by death of dams, a reduction in litter size and a decrease in pup weight.$^{45}$ Finally, triclosan generated no mutagenic or genotoxic properties but did cause significant hepatocellular adenomas and carcinomas in mice although not in rats or hamsters. Thus, more research needs to be conducted on the chronic use of triclosan.

Triclosan and triclocarban are among the highest detected chemicals in waste water treatment plant effluent due to variability in their elimination (dependent on the processing) and are found to bioaccumulate along with methyl triclosan (Figure 13). In fact, methyl triclosan has been reported as high as 2100 ng/g in fish lipids.$^{48}$ Triclosan is readily eliminated under aerobic conditions but not under anaerobic conditions. Along with waste water treatment plant effluent, this antimicrobial has also been detected in network streams across the United States (85 of 139 samples),$^{49}$ drinking water in California, surface water and environmental sediments. Although bioaccumulation in plants and algae has been shown for both methyl triclosan and triclosan, there exists contradicting evidence as to which molecule accumulates to the higher degree. The amount of bioaccumulation in plant and algae is hypothesized to be dependant on the pH of the aquatic environment, although this has yet to be investigated. The sensitivity of invertebrates, algae, fish, amphibians and plants to triclosan and methyl triclosan are very dependant on exposure times, pH and concentration. While animal studies demonstrate that triclosan is not an
oral toxicant, a carcinogen or a mutant and while it was found to be safe in reproductive studies, exposure can potentially lead to adverse effects in metabolic processes and hormone homeostasis.\textsuperscript{50}

\[ \text{Triclosan} \quad \text{Methyl Triclosan} \quad \text{Triclocarban} \]

**Figure 13.** Triclosan, methyl triclosan and triclocarban

While there is no evidence to suggest that triclosan is harmful to humans, degradation products produced during waste water treatment could potentially create molecules with far greater toxic effects. During waste water treatment triclosan is converted to methyl triclosan via biological methylation.\textsuperscript{51} Since methyl triclosan is more lipophilic and is more resistant to biodegradation and photolysis than its parent compound it tends to persist in the environment for far greater periods of time. Triclosan is also readily chlorinated by sodium hypochlorite or hypochlorous acid during waste water treatment (Figure 14). The generation of these toxic chlorophenols can also occur at low levels of chlorine or chloramines and arises from reactions with the ionized phenolate form of triclosan. These by-products have been flagged by US EPA as priority pollutants and are found to be relatively stable over time. Furthermore, triclosan can be converted to di- and trichlorodibenzo-\textit{p}-dioxin by photodegradation or alternatively upon heating to temperatures above 400 °C, as occurs during the production of fabrics. Photodegradation of triclosan to produce dioxins is of great concern since this constitutes a principal route of elimination in aquatic environments. This degradation has been shown to take place in pure water and waste water at low light intensity under UV light (254, 313 and 365 nm), simulated solar light or artificial white light (Figure 14).\textsuperscript{52-57} From these studies, it is apparent that the decomposition of triclosan to dioxin is reliant on pH and the irradiation wavelength, while the degree of photolytic conversion is dependent on the pH and the organic matter content of the sample. Finally the photolysis of triclosan occurs 44 to 586 times faster for the phenolate than for the phenol, suggesting that the active species in photodegradation is the ionized form of
triclosan. While it is known that triclosan converts to dioxins, the kinetics and degree of environmental impact are still currently being investigated.

![Diagram of triclosan photodecomposition products]

**Figure 14.** Triclosan photodecomposition products

Due to the ever growing occurrence of triclosan in aquatic environments, its effect on marine animals is currently under investigation. Of particular interest is the endocrine disruption effect of triclosan, especially with such strong evidence for its presence in human breast milk (vide supra). The thyroid hormones thyroxine (T\(_4\)) and triiodothyronine (T\(_3\)) are tyrosine-based hormones that control cardiac output, heart rate, ventilation rate, basal metabolic rate, brain development, metabolism of proteins and carbohydrates and much more (Figure 15). The major form of thyroid hormones in the blood stream is T\(_4\) which can be converted to the more active form T\(_3\) via deiodinases in the cell. There is very little unbound or free T\(_4\) circulating in the blood as most is bound to transport proteins.
Thyrotropin-releasing hormone (TRH) produced by the hypothalamus travels along the hypothalamo-hypophyseal portal system to stimulate the release of thyroid-stimulating hormone (TSH) from the anterior pituitary gland (Figure 16). Somatostatin (SS) is also produced by the hypothalamus and acts to inhibit the production of TSH. The interaction of TSH with TSH receptors located on thyroid follicular cells stimulates the production of the thyroid hormones (T$_4$ and T$_3$). Thyroglobulin is produced by the rough endoplasmic reticulum while a sodium-iodide symporter pumps iodide actively into the cell. The iodide is oxidized to iodine by an enzyme called thyroid peroxidase and then subsequently reacted with the tyrosine residues of thyroglobulin to produce monoiodotyrosine and diiodotyrosine. Linking of these moieties

![Image of Thyroxine (T$_4$) and Triiodothyronine (T$_3$)](image-url)

**Figure 15.** Thyroid hormones thyroxine (T$_4$) and triiodothyronine (T$_3$)

![Diagram of Thyroid Hormone Regulation](image-url)

**Figure 16.** Regulation of thyroid hormone levels. TRH: Thyrotropin-releasing hormone; SS: Somatostatin; TSH: Thyroid-stimulating hormone; T3: Triiodothyronine; T4: Thyroxine.
produces either T₄ or T₃. The concentrations of T₄ and T₃ in the blood help to regulate the production of TSH by the pituitary gland via a negative feedback loop. Therefore, if the concentrations of T₄ and T₃ are low, more TSH is produced whereas if the levels of T₄ and T₃ are high, somatostatin is produced, effectively inhibiting TSH output.

The structural similarity of triclosan to known estrogenic and androgenic endocrine disrupting chemicals such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and bisphenol A as well as its similarity to thyroid hormones would suggest that triclosan could exhibit endocrine disruption (Figure 17). In fact, several studies have shown that this antimicrobial agent has the ability to influence endocrine function in a variety of species, although human studies published on its thyroidal effects are still limited. Still, these studies are of great significance since there exists regularly a large concentration of triclosan detected in human plasma, urine, breast milk and aquatic environments.

**Figure 17.** Estrogenic and androgenic endocrine disruptors

Triclosan was first investigated for its effects on thyroid hormone metabolism by Schuur in 1998. In vitro studies using rat liver cytosol showed triclosan inhibited diiodothyronine (T₂) sulfotransferase with an IC₅₀ of 3.1 ± 0.7 µM. Sulfotransferases sulfonate free alcohols and amines making them more soluble as well as increasing the compound’s half-life, thus allowing prolonged storage. This is particularly useful as a means to store estrogen during the development of the fetus, where nearly all the estrogen circulating in the fetal blood stream comes from sulfoconjugated estradiol and sulfoconjugated estrone. The sulfoconjugated estradiol and estrone are secreted from the placenta to the fetus where sulfatases remove the sulfate to yield estrogen. Schuur postulated that inhibition of thyroid hormone sulfotransferases from triclosan and triclosan metabolite exposure would decrease the estrogen available to the fetus, thus potentially leading to developmental consequences including pregnancy loss. However, further analysis by this group using in vivo disruption as well as the determination of effects on
embryos and developing fetuses is still underway. In 2010, James tested the vulnerability of sheep placenta and found that triclosan inhibited estradiol sulfonation with an IC$_{50}$ of 0.6 nM.$^{59}$ This further augmented the argument on the dangers of triclosan exposure. Finally, triclosan has been shown to decrease serum T$_4$ levels without effecting thyroid stimulating hormone levels in female rats with high dosage (>30 mg/kg).$^{60}$ In a similar study, male rats also showed a significant decrease in serum T$_4$ levels without affecting the thyroid-stimulating hormone (>30 mg/kg triclosan).$^{61}$

To further assess the endocrine potential of triclosan, Veldhoen and co-workers studied its ability to alter thyroid-mediated developmental processes in premetamorphic North American bullfrog tadpoles (Rana catesbeiana).$^{50}$ Pretreatment of triclosan at concentrations as low as 0.15 µg/L accelerated metamorphological changes when followed by subsequent administration of T$_3$. Within 48 hours of T$_3$ administration, the T$_3$ mediated thyroid hormone receptor β (TRβ) mRNA expression in the tadpole tails decreased and the levels of proliferating nuclear cell antigen in the brain increased. In the absence of T$_3$, triclosan alone affected the thyroid hormone receptor α (TRα) transcripts in the brain resulting in transitory weight loss. Thus, it was concluded that environmentally relevant levels of triclosan are capable of disrupting developmental processes that are reliant on thyroid hormones in the bullfrog. In contrast, Fort exposed South African clawed frog larvae (Xenopus laevis) to 0.6 to 32.3 µg/L of triclosan over a 21-day trial and concluded that it had no effect on larvae development based on thyroid histology, plasma thyroxine levels and/or survivorship.$^{62}$ However, the reported data did suggest an effect on postembryonic development. Moreover, there was a significant difference between exposed and control groups in terms of the expression of TRβ as well as a reduction in larval growth. Thus, these studies have shown that amphibians are sensitive to triclosan, but more research is required.

To date, a study by Allmyr is the only examination of the effects of triclosan on human thyroid homeostasis.$^{63}$ Participants brushing with Colgate Total containing 0.3% (w/w) triclosan for a 14-day period showed elevated concentrations of triclosan in their plasma at the end of the exposure period. Despite this significant difference, triclosan had no effect on circulating levels of 4β-hydroxycholesterol (a cholesterol metabolite used as an indicator of CYP3A4 activity), thyroid-stimulating hormone, free T$_4$ or free T$_3$. Thus, it was concluded that toothpaste containing triclosan does not induce CYP3A4 activity or disrupt thyroid homeostasis. However,
this study was conducted over a small period of time and therefore limits the validity of these results. Future work should include multiple exposure pathways for prolonged periods of time.

Finally, the structural similarity between triclosan and anthropogenic estrogens, diethylstilbestrol and bisphenol A led to the investigation of its effect on sex hormones. In one study, a weak androgenic effect was identified to cause fin length and sex ratio changes in Japanese Medaka fish\textsuperscript{64} while another study showed triclosan to be toxic and showed a weak estrogen effect with a potential to produce vitellogenin (an egg yolk precursor protein) in male Medaka.\textsuperscript{65} Intraperitoneal injection of triclosan at 4, 40 and 400 µg/g by weight in male frogs showed lower plasma vitellogenin and testosterone levels but at environmentally relevant concentrations no estrogenic effects were detected.\textsuperscript{66} Furthermore, Gee demonstrated intrinsic estrogenic and androgenic activity for triclosan in human breast cancer cells and with rat androgen receptors.\textsuperscript{67} While there is good evidence for endocrine disrupting effects of triclosan, it remains unclear whether it acts to cause (anti)estrogenic affects, (anti)androgenic effects, or both.

1.2.4 Genetic Investigation of Triclosan

With the environmentally relevant levels of triclosan shown to induce endocrine disruption, a collaboration was proposed with Dr. Caren Helbing of the University of Victoria Biochemistry and Microbiology Department in order to better understand the means by which triclosan and its metabolites affect the thyroid hormone response. Previous research by Helbing in collaboration with Dr. Veldhoen\textsuperscript{50} demonstrated an increase in hindlimb development and a decrease in body weight following T\textsubscript{3} administration to tadpoles subjected to environmentally-relevant levels of triclosan. Furthermore, triclosan exposure was shown to induce T\textsubscript{3}-mediated TRβ mRNA expression in tadpole tail fins and caused an increase in proliferating cell nuclear antigen (PCNA) transcript levels in the brains of premetamorphic tadpoles. Finally, exposure of XTC-2 cells to T\textsubscript{3} plus nominal levels of triclosan altered thyroid hormone receptor mRNA expression and disrupted thyroid hormone-associated gene expression and the overall rate of postembryonic anuran development.

While the previous studies by Veldhoen have established triclosan-induced endocrine perturbations, the precise biomolecular target responsible for these effects is unknown. In fact, triclosan may be able to modulate activities of a number of different regulatory receptors leading to the possibility of receptor crosstalk in biological systems. Furthermore, the disruptive action
observed is not known to be caused by triclosan itself. In fact, this parent compound could be further metabolized by the animal physiology to generate more potent bioactive compounds such as methyl triclosan. Both of these problems will be addressed using a forward chemical genetics approach. The overall goals of this project will be to first synthesize a series of compounds (Figure 18) keeping the overall diphenyl ether scaffold. Variations in the substituents surrounding the aromatic rings will help to unravel the key interactions needed to sustain the already observed endocrine disruption. Secondly, a subset of biotin-containing compounds

![Figure 18. Triclosan analogues](image-url)
Figure 19. Free amine and biotin containing triclosan analogues

(Figure 19) will be used to identify the exact binding protein responsible for the observed thyroid hormone disruption. Once the protein is known, insight into the metabolic pathway and the ability of these disruptions to occur in other species such as humans will be addressed. Finally, the precise mechanism for the methylation of triclosan to form methyl triclosan is unknown. (Figure 19) will be used to identify the exact binding protein responsible for the observed thyroid hormone disruption. Once the protein is known, insight into the metabolic pathway and the ability of these disruptions to occur in other species such as humans will be addressed. Finally,
the precise mechanism for the methylation of triclosan to form methyl triclosan is unknown. Therefore, the use of biotin-labelled triclosan may help to unlock the key process required to generate this molecule and help to identify the potential bacteria responsible. To achieve these goals, the synthesis of compounds 1 through 20 will be done by this author and then passed along to our collaborators for further analysis.

1.3.0 Neuraminidase

1.3.1 Influenza Virus Background

Influenza is an infectious disease caused by RNA viruses. These viruses target birds and mammals and infect approximately 600,000,000 people globally each year. Although the World Health Organization (WHO) has implemented a monitored vaccine program, it still remains a significant health concern. This is due in part to rapid mutations leading to new strains of the influenza virus as well as the long lead-time required to produce the trivalent influenza vaccine (TIV) each year resulting in vaccinated individuals remaining susceptible to these new strains.

The influenza virus can be subdivided into viral subtypes A, B and C. Commonly, influenza A targets wild aquatic birds, influenza B targets predominantly humans and influenza C targets humans, dogs and pigs. However, influenza B and C are less common subtypes, whereas influenza A constitutes most of the worldwide influenza virus (the “common” flu) seen each year. Influenza A can be transmitted from wild avians to humans resulting in infection and in turn possible influenza pandemics. In contrast, pandemics are less prevalent in influenza B and C, since influenza C is an infrequent subtype and influenza B has a low rate of antigenic change. All three viral subtypes possess a structure called a viral envelope containing two main types of glycoproteins wrapped around a central core housing negative sense, single stranded, segmented RNA genomes. This RNA codes for 10 proteins, including three polymerases, hemagglutinin (HA), neuraminidase (NA) and the M2 protein. Hemagglutinin and neuraminidase (also known as sialidase) are the two large glycoproteins on the outside of the virus. Hemagglutinin is a lectin (sugar-binding protein) that mediates the binding of the virus to the target cell allowing entry of the viral genome into the host cell. Neuraminidase is a glycoside hydrolase enzyme that catalyzes the hydrolysis of a terminal sialic acid residue from the newly formed virion and the host cell receptor. Influenza A is further classified as several groups with the type of hemagglutinin designated by HX and the type of neuraminidase designated by NX,
where $X$ is the number of a specific antigen. There are currently 16 different H antigens (H1 to H16) and 9 different N antigens split into group 1 (N1, N4, N5, N8) and group 2 (N2, N3, N6, N7, N9). Each group is susceptible to mutations, resulting in a variety of daughter strains with differing pathogenic profiles.

Viruses cannot replicate outside of a living cell. Instead, the influenza virus initiates the maturation cycle by binding the glycoprotein hemagglutinin onto sialic acid sugars on the surface of epithelial cells (Figure 20). Next, receptor-induced endocytosis produces an endosome containing the virion. Once inside the cell, the cell triggers digestion of the endosome contents by acidifying its interior resulting in two events. First, the hemagglutinin protein fuses the viral envelope to the vacuole membrane, then an M2 ion channel pumps protons into the viral envelope where the acidity of the core (pH < 6.0) causes it to disassemble and release the viral RNA (vRNA) and core proteins. These subsequently form a complex (ribonucleoprotein; RNP) that is transported into the nucleus. Once inside, the negative-sense vRNA is transcribed into positive-sense messenger RNA (mRNA) by a RNA polymerase attached to the RNP. Due to the absence of RNA proofreading enzymes, errors in copying causes antigenic drift leading to rapid mutations of the virus. Furthermore, the assortment of genetic material between animal and human strains can introduce new antigens which the immune system cannot recognize. The mRNA is either exported into the cytoplasm or retained in the nucleus. mRNA retained in the nucleus can use the cell’s complementary RNA (cRNA) synthesis mechanism to replicate new vRNA. Since the mRNA generated by the virus is a mirror image of the mRNA used by the cell, mRNA exported into the cytoplasm can also use the cell’s ribosomes to translate the mRNA and generate new viral proteins. These proteins are either secreted through the Golgi apparatus onto the cell surface (in the case of neuraminidase and hemagglutinin) or transported back into the nucleus to aid in vRNA replication. Future viral genomes located in the nucleus can then assemble into a virion and exit into the cytoplasm. The virion travels toward a cell membrane protrusion, triggering a viral budding event to generate a spherical host phospholipid membrane surrounded by hemagglutinin and neuraminidase. Once again, the newly formed bud is adhered to the cell by a hemagglutinin-sialic acid interaction. Cleavage of the sialic acid residue by neuraminidase allows release of the newly formed virus. Eventually, the viral replication takes over so much of the host’s machinery that the cell dies.
Figure 20. Influenza A virus replication © Qiagen, all rights reserved. Reproduced with permission.
1.3.2 Types of Antiviral Influenza Drugs

There are currently two classes of influenza inhibitors approved by the US Food and Drug Administration (FDA). The first drugs designed were amantadine and rimantadine which target the N-terminal hydrophobic pore of the M2 channel, a homo-tetrameric proton-selective channel involved in the uncoating of the endosome following fusion with the cell. This interaction prevents the uptake of protons through an electrostatic repulsion with the positively charged amino group (Figure 21). Disruption of this proton uptake inhibits the uncoating of the viral envelope, thus preventing the vRNA from entering the cytoplasm. Unfortunately, as of the 2005–2006 influenza season, it was estimated that >90% of all influenza strains were resistant to amantadine and rimantadine due to a single point mutation S31N (Ser31Asn), prompting the Centers for Disease Control (CDC) to remove their recommendation for treatment of influenza by these drugs.

Figure 21. M2 ion channel inhibitors

The second class of antiviral drugs are the neuraminidase inhibitors consisting of zanamivir (relenza®), oseltamivir (tamiflu®) and peramivir (rapiacta®). Neuraminidase is a 240 kDa glycoprotein composed of four identical subunits anchored by a hydrophobic stem embedded into the lipid bilayer envelope of the virion. It is estimated that each virion consists of approximately 100 copies of neuraminidases and 300 copies of hemagglutinin. Terminal sialic acid residues present in glycoproteins and glycolipids act as surface cell receptors for the binding of hemagglutinin, leading to penetration of the host cell. Following viral replication, the newly formed virion remains bound to the cell surface through a hemagglutinin-sialic acid interaction. Viral neuraminidases specifically cleave terminal α-2,3- and α-2,6-sialic acid-galactose linkages allowing the virion to detach and propagate throughout the body. Administration of neuraminidase inhibitors prevents the cleavage of the viral bud, thus causing the particles to aggregate. It is currently unknown whether these aggregates are a result of the
virion unable to detach from the host cell or from the clustering of virions to other virions or both. Regardless, these aggregates can then be successfully removed through respiratory secretions.

The enzymatic mechanism for the influenza viral sialidase was analyzed by Bennet and co-workers using kinetic isotopes. Based on the proximity of adjacent isotopic nuclei (either spin active or non-spin active) the shifts observed from $^{13}$C NMR spectroscopy were monitored and used to determine the second-order rate constant, $k_{cat}/K_m$. If the change in the rate constant is large compared to the unlabelled experiments, then a chemical bond is being broken or formed during the rate limiting step. Conversely, if there is very little change in the rate compared to the unlabelled substrate then the substitution (i.e. the position isotopically labelled) is not involved in bond breaking or forming. Based on this research, Bennet proposed that upon binding of the substrate to the enzyme, the complex distorts the $\alpha$-sialoside leading to a $^{4}S_2$ skew-boat conformation (Scheme 1). Previous studies suggested the formation of an oxonium transition state prior to glycosylation; however, this proposed structure was inconsistent with the kinetic isotope effects observed by Bennet. Instead, the glycosidic bond cleavage, through the controlled addition of Tyr409, occurs as the anomic carbon motion generates a $^{2}C_5$ chair conformation of the intermediate by passing through a $^{4}H_5$ half-chair transition state. An Asp151 controlled addition of water then yields the initial $\alpha$-isomer intermediate which quickly mutarotates to the more thermodynamically stable $\beta$-isomer.
The viral neuraminidase active site is highly conserved amongst group 1 and group 2 neuraminidases even with amino acid sequence variations of 30 to 60% between neuraminidase strains and up to 70% variation between influenza A and influenza B subtypes. Furthermore, site-directed mutagenesis has determined that substitution of Asp151, Trp178, Glu276, Glu277, Tyr409 or Arg371 residues results in a decrease in neuraminidase activity when compared to the wild-type enzyme. Therefore, these residues must play a pivotal role in catalysis. Design of new neuraminidase inhibitors would do well to target these specific residues in order to increase potency.

Zanamivir (relenza®) was developed by GlaxoSmithKline using computer modeling of the known sialidase inhibitor DANA (a dehydrated sialic acid analog; Figure 22) bound to the neuraminidase active site. At the time of development, it was hypothesized that the hydrolysis of the endogenous substrate by viral neuraminidase proceeded through an oxonium-based transition state. Therefore, both zanamivir and DANA were developed to mimic this boat-shaped oxonium transition state of sialic acid. Recent work by Bennet has shown that zanamivir,
DANA and even oseltamivir do not mimic the transition state for sialic acid. However, they still remain potent inhibitors due to key ground state interactions with the hydrophilic and hydrophobic pockets of the enzyme. Replacement of the hydroxyl group in DANA with a guanidine resulted in a >1000 fold increase in potency (Figure 22). However, due to poor membrane solubility, zanamivir must be administered by inhalation twice a day with a bioavailability of 10% (intranasally) and 25% (inhaled dose). The pro-drug lanamivir is currently in phase I/II trials as a longer acting neuraminidase inhibitor and is predicted to improve upon the pharmacokinetics of zanamivir.

Gilead Sciences designed oseltamivir (tamiflu®) to similarly mimic the hypothesized boat-shaped sialic acid hydrolysis transition state. However, instead of employing the pyran scaffold a more chemically versatile cyclohexane isostere was used while still allowing the correct orientation of the functional groups to bind to the enzyme active site. The key structural differences between zanamivir and oseltamivir are the replacement of the glycerol side
chain with a 3-pentyl group and the replacement of the guanidine with an amine (Figure 23). This substantial increase in lipophilicity allows the active form GS-4071 (orally inactive; 5% bioavailability) to be administered as the ethyl ester pro-drug oseltamivir (orally active; 80% bioavailability).\textsuperscript{89,90} The ethyl ester of GS-4109 was not orally active as guanidines tend to have low intestinal permeability.\textsuperscript{91,92}

![Chemical structures](image1.png)

**Figure 23.** Cyclohexane isostere neuraminidase inhibitors

Peramivir (rapiacta\textsuperscript{®}) was developed by BioCryst Pharmaceuticals and makes use of a cyclopentane ring which orients the carboxylate and the guanidine toward similar pockets of the neuraminidase enzyme when compared to zanamivir and oseltamivir.\textsuperscript{93,94} Although currently in phase II/III trials as an injectible due to poor oral bioavailability, peramivir was used on an emergency basis during the recent 2009 H1N1 pandemic in the US and is currently approved for use in Japan.

![Chemical structure](image2.png)

**Figure 24.** Cyclopentane derived neuraminidase inhibitor peramivir
1.3.3 Pharmacokinetics and Pharmacodynamics

Pharmacokinetics dictates the fate of a substance administered externally to a living organism. This generally includes the routes of absorption and distribution of the drug, the rate and duration of effects, and the excretion of metabolites. On the other hand, pharmacodynamics is the study of the biochemical and physiological effects of drugs on the body. This involves the mechanism of drug action and the relationship between the concentration of the drug and its overall effect including toxicology. Simply put, pharmacokinetics is what the body does to the drug, whereas pharmacodynamics is what the drug does to the body.

Development of potential therapeutics usually starts with the identification and validation of a target protein. Identification of a pharmacophore provides a lead compound which can be further optimized through computational chemistry and high-throughput screening. Analysis of these compounds is continued to generate sub-libraries that can be used to further investigate the stability, solubility, permeability and toxicity of each molecule. From here, one or two compounds will be chosen as candidate drugs and further developed.95

Lipinski’s rules of five are based on empirical observations and while they do not necessarily predict a compound’s pharmacology, they are nonetheless used by many researchers as a general guideline when developing lead structures.96 To dictate these rules, Lipinski analyzed known compounds which had previously been shown to be orally active. Observed trends comparing specific parameters (molecular weight, lipophilicity, and hydrogen bond donors/acceptors) led Lipinski to define a set of rules in which 90% of these compounds followed these trends. Therefore, in general, an orally active drug should not violate more than one of the following statements:

- No more than five hydrogen bond donors
- No more than ten hydrogen bond acceptors
- A molecular weight of less than 500 g mol\(^{-1}\)
- A partition coefficient (logP) between −1 and 5

These rules have been further updated by various researchers in order to fine-tune profiling techniques for use in making orally active drugs.97,98
Chemical probes possessing drug-like properties may not have all the attributes required to be a drug, but have the advantage that should this probe modulate a target of therapeutic interest, the small molecule can then act as a suitable starting point for future drug development. Small molecules without drug-like properties can still be used for biological probing but lack the ability to be quickly turned into a lead compound. Another issue associated with these molecules is toxicity. If the toxicity of the chemical probe does not present itself during the timescale of the experiment or if the toxicity targets a biomolecule(s) outside the intended system being analyzed, then the chemical probe can be used to analyze a target biomolecule(s). For example, a small molecule could be highly toxic against liver cells, but if the probe is being used to analyze a specific protein without ever coming into contact with liver cells then its toxicity is of no concern. Nevertheless, small molecule probes still need to be selective since promiscuous probes will complicate analyses by binding to multiple targets. Finally, if the aim is to probe in vivo rather than in vitro, pharmacokinetics may play a more pivotal role.

1.3.4 Neuraminidase Inhibitor Structure–Activity Relationship

The viral neuraminidase active site is highly conserved amongst group 1 and group 2 neuraminidases and can be further subdivided into five distinct subsites (Figure 25). Common amongst zanamivir, oseltamivir and peramivir, the central scaffold does not interact directly with the enzyme and serves only to orientate the substituents for optimum interaction within each binding pocket. In order to maximize potency and produce low nanomolar inhibition, all five subsites need to be occupied. Although there are several cyclic scaffold templates, only the core structures pertaining to zanamivir, oseltamivir and peramivir will be discussed.
Figure 25. Important interacting residues present in the sialic acid binding domain of neuraminidase.

The S1 subsite is a positively charged pocket composed of an Arg118-Arg371-Arg292 triad, Tyr409 and Tyr347 (Figure 26). The arginine triad is highly conserved across the neuraminidase strains as it binds the carboxylate of sialic acid through a salt bridge, thereby making it essential for orienting the substrate during catalysis. Furthermore, Tyr409 is structurally important as it stabilizes the $^{4}H_{2}$ half-chair transition state during hydrolysis. While nearly all potent neuraminidase inhibitors contain an anionic carboxylate bound to this pocket, work by Wong has shown that a significant increase in activity is achieved by replacement of the
carboxylate with a phosphonate in both zanamivir and oseltamivir (Table 1).\textsuperscript{100,101} The phosphonate is generally used as a bioisostere in drug design and exhibits stronger electrostatic interactions with the guanidinium ion. A similar structural replacement for peramivir has yet to be done, but would be hypothesized to show a comparable outcome as the binding of the carboxylate to the arginines is highly conserved for all three molecules.

\textbf{Figure 26.} Peramivir bound to the S1 subsite of a N8 neuraminidase. Measurements (Å) represent the distance between hydrogen bonded heteroatoms.
Table 1. Structure–activity relationship studies for the S1 binding pocket.\textsuperscript{100,101} A) Phosphonate analogues for zanamivir. B) Phosphonate analogues for oseltamivir.

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<td>22b</td>
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The S2 subsite is composed of three negatively charged residues (Glu119, Glu227 and Asp151), along with Leu135 and the backbone carbonyl of Trp178 (Figure 27).\textsuperscript{78} The amino group of oseltamivir carboxylate (22a) interacts with the residues Asp151 and Glu119.\textsuperscript{89} Alternatively, the guanidines in peramivir (23a) and zanamivir (21a) stack parallel to Glu119 forming a strong electrostatic interaction and allow additional interactions with Glu227 and the backbone carbonyl of Trp178.\textsuperscript{93}

The guanidinylated zanamivir (21a) showed a 40-fold increase in potency compared to the amine derivative 21c (Table 2a), whereas only a 2-fold increase in potency was observed for oseltamivir carboxylate (Table 2b; 22a versus 22c). The use of guanidinium groups is known to be associated with poor intestinal permeability; therefore, in the case of oseltamivir, an increase in bioavailability was achieved by using the amine without decreasing the potency of the overall drug by a substantial margin. The epimer of zanamivir at the guanidinium position (epi-21a) no longer interacts with the key Glu119 residue in the S2 binding pocket, thereby reducing the charge-charge interaction. Furthermore, mono- and di-methylation of amine 21c increases the K\textsubscript{i} due to similar removal of the glutamic acid charge contacts at this enzyme pocket required for potency.
During the design of peramivir, an inseparable mixture of four diastereomers was generated with each product comprising of a racemate mixture (8 total products). This mixture of products was subjected to neuraminidase crystal soaking experiments resulting in the isolation of the most active isomer. Surprisingly, the most active compound $23a$ contained the opposite stereochemistry at the guanidine position when compared to zanamivir $21a$ (Table 2). Irrespective of their orientation, zanamivir and peramivir equally probe the S2 pocket and stack parallel to one another and parallel to Glu119 in most neuraminidase subtypes. An exception is the S2 pocket of the N8 subtype where peramivir’s guanidine points further into the pocket than does zanamivir’s guanidine. This subtle difference in stereochemistry may play an important role for neuraminidase drug-resistant influenza strains. Since zanamivir contains a key interaction with Glu119, mutation of this residue results in partial resistance for zanamivir and complete resistance in oseltamivir. In contrast, peramivir remains relatively potent toward Glu119 mutants during in vitro studies of some neuraminidase subtypes (Table 7).

Figure 27. Peramivir bound to the S2 subsite of a N8 neuraminidase. Measurements (Å) represent the distance between hydrogen bonded heteroatoms.
While synthesizing peramivir, a β-hydroxyl group was installed as part of a palladium-catalyzed [3+2] cycloaddition rather than to target any key residue. This alcohol does show a hydrogen bond to Asp151, however, comparison of compounds 23a and 24a reveals similar activity (Table 2c). Therefore, this interaction between the β-hydroxyl group and Asp151 is superfluous for potent inhibition.94

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Table 2. Structure–activity relationship studies for the S2 binding pocket<sup>80,94,103,104</sup>

The subsite S3 is a small hydrophobic pocket containing Trp178 and Ile222 which interact with the methyl group of the NHAc, a critical interaction for substrate recognition (Figure 28). This site also contains Arg152 which hydrogen bonds to the carbonyl of the NHAc group. Inhibition of Arg152 is important as both this residue and Asp151 are required for the orientation of a water molecule during the hydrolysis of sialic acid. While there is little room for increasing bulk toward the hydrophobic pocket (Table 3), it was proposed that a tertiary amine (21m) could displace this water molecule from the enzyme. However, this was not the case (Table 3a). Therefore, other than a small increase in activity for the trifluoromethyl 21i, the S3 site remains extremely sensitive to any minor changes.

![Figure 28. Peramivir bound to the S3 subsite of a N8 neuraminidase. Measurements (Å) represent the distance between hydrogen bonded heteroatoms. Blue lines represent potential hydrophobic interactions and red lines represent hydrogen bonding.](image)
Table 3. Structure–activity relationship studies for the S3 binding pocket.\textsuperscript{105,106} A) Analogues for zanamivir. B) Analogues for oseltamivir.

The S4 binding pocket is comprised of an extended hydrophobic surface consisting of Ala246, Ile222 and the side chain methylenes of Arg224 (Figure 29).\textsuperscript{78} The S5 subsite consists of Glu276 which can orient in two distinct conformations. Both sialic acid and zanamivir bind to
Glu276 through a bidentate hydrogen bond using the C-8 and C-9 hydroxyl groups of their glycerol side chain.\textsuperscript{78} However branched aliphatic groups, like those of peramivir and oseltamivir, can force Glu276 to rotate outside the active site where it forms a salt bridge with Arg224, exposing the hydrophobic surface of the side chain methylenes of Glu276. Reorientation of Glu276 has an increased energetic cost for most influenza B strains due to bulkier residues surrounding the S4/S5 pocket.\textsuperscript{89} This often causes a two to three order of magnitude loss in inhibition relative to influenza A strains (\textsuperscript{21x, 26a, and 26b}) which tend to have smaller residues surrounding the S4/S5 pocket.\textsuperscript{80} However, zanamivir, oseltamivir and peramivir are all tight binders and thus are equally active against both strains.

\textbf{Figure 29.} Peramivir bound to the S4 and S5 subsites of a N8 neuraminidase.
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<tr>
<td>21w</td>
<td>O-N</td>
<td>4</td>
</tr>
<tr>
<td>21x</td>
<td>O-N</td>
<td>2 (influenza A) 500 (influenza B)</td>
</tr>
</tbody>
</table>

Table 4. Zanamivir analogue structure–activity relationship studies for the S4/S5 binding pocket$^{107-110}$
Zanamivir contains a glycerol side chain whereby the C-8 and C-9 hydroxyl groups interact with Glu276 to form a bidentate hydrogen bond. X-ray crystallography studies by von Itzstein\(^7\) showed that the C-7 hydroxyl group had no direct contact with the enzyme. This was later confirmed by Honda through the addition of small lipophilic substituents or the complete removal of the C-7 hydroxyl group resulting in little variation in potency (21r, 21s; Table 4).\(^{107}\) Increasing the bulk of tertiary amide derivatives coordinated to the scaffold through the carbonyl carbon (21v, 21w, 21x) resulted in an increase in potency toward influenza A due to the conformational change of Glu276, while influenza B showed a decrease in potency (Table 4).\(^{109}\)

The balance of lipophilicity and water solubility is important in drug design in order to maximize absorption by the intestinal tract. Kim conducted structure–activity relationship studies using the oseltamivir scaffold and determined the maximum extension of linear and branched alkyl chains able to bind in the S4 and S5 enzyme pocket.\(^{89,111}\) While poor neuraminidase inhibitory potency was observed with hydrocarbons extending beyond n-nonyl, there was very little increase in activity for alkyl chains longer than n-propyl (Table 5). Branching at the β-position showed no increase in activity (22n), however, branching in the α-position provided an increase in inhibition up to derivative 22a. Further extension of these alkyl branches resulted in steric clashes with the enzyme residues. Chirality of the branching isomers had no effect suggesting that bond rotation allows an interaction of one branch with the S4 pocket and one branch with the S5 pocket. Compounds containing cyclopentane and cyclohexane rings showed similar results to the branched alkyl groups (good hydrophobic contacts), while the allylic and aromatic side-chains proved less successful.
Similarly to oseltamivir, the alkyl chains of peramivir showed good hydrophobic contact with the S4 and S5 enzyme pocket (Table 6). Interestingly, the n-butyl side chain of derivatives 24c and 25b (Table 6a) adopted different binding modes for influenza A and influenza B. In influenza B, the hydrocarbon oriented alongside a hydrophobic surface consisting of Ala246, Ile222 and the methylenes of Arg224 (S4 pocket). On the other hand, in influenza A, the n-butyl side chain occupied a region whereupon the Glu276 residue had reoriented. This result was used to confirm previous observations of conformational changes seen during neuraminidase inhibition by oseltamivir.

**Table 5.** Oseltamivir analogue structure–activity relationship studies for the S4 and S5 binding pocket.\(^\text{106,111}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>IC(_{50}) (nM)</th>
<th>Compound</th>
<th>R</th>
<th>IC(_{50}) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22i</td>
<td>H</td>
<td>6300</td>
<td>22o</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>22j</td>
<td>Me</td>
<td>3700</td>
<td>22a (Oseltamivir Carboxylate)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>22k</td>
<td></td>
<td>2000</td>
<td>22p</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>22l</td>
<td></td>
<td>180</td>
<td>22q</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>22m</td>
<td></td>
<td>300</td>
<td>22r</td>
<td></td>
<td>2200</td>
</tr>
<tr>
<td>22n</td>
<td></td>
<td>200</td>
<td>22s</td>
<td></td>
<td>620</td>
</tr>
</tbody>
</table>

Table 5. Oseltamivir analogue structure–activity relationship studies for the S4 and S5 binding pocket.\(^\text{106,111}\)
Table 6. Peramivir analogue structure–activity relationship studies for the S4 and S5 binding pocket.\textsuperscript{93,94}

1.3.5 Mutation-Induced Resistance

Neuraminidase inhibitors were once thought to be non-susceptible to mutation-induced resistance as less than 1% of isolates showed resistance prior to 2007.\textsuperscript{112} In fact before the 2007–2008 flu season, detection of oseltamivir-resistant viruses in humans had only been reported for those individuals taking oseltamivir, with no known human to human transfer of neuraminidase inhibitor-resistant viruses. However, in the 2007–2008 influenza season, a His274Tyr (H274Y)
point mutation was isolated from 12% of H1N1 viruses tested in the United States. Furthermore, during the 2008–2009 flu season the number of H274Y oseltamivir-resistant isolates for the H1N1 subtype increased to 98.5%. Fortuitously, the 2009–2010 H1N1 swine flu pandemic did not contain the H274Y mutant and was therefore susceptible to treatment by oseltamivir.

The point mutations Asn294Ser (N294S) or H274Y prevent Glu276 from rotating out of the active site which is required to effectively bind one of the arms of the branched alkyl side chain of oseltamivir and peramivir into the S5 pocket. A crystal structure of the H274Y mutant shows the bulkier tyrosine displaces Glu276 further into the pocket thereby limiting the size of the hydrophobic surface. This results in a 754-fold decrease in potency for oseltamivir. Since zanamivir forms a bidentate hydrogen bond with Glu276 requiring no conformational change, its potency toward the H274Y mutant is unaffected (Table 7). Finally, N2 neuraminidase subtypes have a less bulky residue at the 252-position and can therefore accommodate the increase in bulk caused by the H274Y mutation. Thus, oseltamivir shows no loss of potency toward N2 H274Y mutants.

The mutants Glu119Val (E119V), Asn294Ser (N294S), and Arg292Lys (R292K) have only been isolated from patients treated with oseltamivir and are not known to significantly transfer from human to human. Furthermore, while multiple Glu119 mutants have been identified in vitro in the presence of zanamivir, only the mutant R292K has been isolated in patients treated with zanamivir. The neuraminidase enzyme contains a series of water molecules that aid in the hydrogen bond networking of the active site. Glu119 mutations create subtle changes in the solvent structure thereby disrupting the surrounding hydrogen bond network in the S2 pocket. Oseltamivir resistance also occurs for the E119V mutation due to a complete loss of binding for the amino group. In the case of Glu119Asp (E119D), loss of the electrostatic interaction for zanamivir’s guanidine results in an appreciable loss of activity. However, since peramivir’s guanidine occupies a slightly different conformation, it remains less susceptible to this particular mutation.
Table 7. Activity of neuraminidase inhibitors against known neuraminidase mutants

<table>
<thead>
<tr>
<th>Mutant NA Protein</th>
<th>NA Activity versus WT</th>
<th>Fold Decrease in IC₅₀ versus WT (Oseltamivir)</th>
<th>Fold Decrease in IC₅₀ versus WT (Zanamivir)</th>
<th>Fold Decrease in IC₅₀ versus WT (Peramivir)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1 H274Y</td>
<td>80%</td>
<td>754x</td>
<td>1x</td>
<td>260x</td>
</tr>
<tr>
<td>N2 H274Y</td>
<td>34%</td>
<td>7x</td>
<td>5x</td>
<td>1x</td>
</tr>
<tr>
<td>N1 N294S</td>
<td>115%</td>
<td>197x</td>
<td>5x</td>
<td>12x</td>
</tr>
<tr>
<td>N2 N294S</td>
<td>-</td>
<td>1879x</td>
<td>8x</td>
<td>0.8x</td>
</tr>
<tr>
<td>N2 E119V</td>
<td>81%</td>
<td>1028x</td>
<td>7x</td>
<td>3x</td>
</tr>
<tr>
<td>N2 E119D</td>
<td>19%</td>
<td>4.5x</td>
<td>323x</td>
<td>33x</td>
</tr>
<tr>
<td>N2 R292K</td>
<td>29%</td>
<td>1500x</td>
<td>8x</td>
<td>43x</td>
</tr>
</tbody>
</table>

Table 7. Activity of neuraminidase inhibitors against known neuraminidase mutants

WT: wild type; NA: neuraminidase

1.3.6 Structure–Activity Relationship Studies for Peramivir

While there has been extensive structure–activity relationship studies done for oseltamivir and zanamivir, less information is known about peramivir. This investigation is of far greater importance with the emergence of oseltamivir resistance through point mutations and the lack of bioavailability for both zanamivir and peramivir. Guanidinylation of oseltamivir (22a versus 22c) has been shown to have little effect on its potency, whereas the removal of the guanidine from zanamivir (21a versus 21c) results in a 40-fold increase in Kᵢ. However, peramivir’s guanidine is positioned in a slightly different conformation than zanamivir’s guanidine due to their opposing stereochemistry. Therefore, the removal of the guanidine from peramivir may not have as significant an impact as seen in zanamivir’s case and may in fact increase peramivir’s intestinal permeability while still allowing it to remain a relatively potent drug.

55
neuraminidase inhibitor. Furthermore, zanamivir is less susceptible to the H274Y mutation compared to oseltamivir because it does not require a conformational change in Glu276 to bind, but rather hydrogen bonds using the alcohols of the glycerol side chain. Using the ideal properties of oseltamivir, zanamivir and peramivir, a hybrid neuraminidase inhibitor can be envisioned. Removal of the guanidine from peramivir would decrease the number of hydrogen bond acceptors/donors and potentially increase its bioavailability while showing minimal loss in potency. Moreover, decreasing the number of hydrogen bond acceptors/donors could also allow for the replacement of the branched alkyl side chain with a glycerol side chain that more closely mimics zanamivir’s functional group. This could potentially lead to an analogue which would be less susceptible to the H274Y mutation, as is the case with zanamivir.

![Peramivir (BCX-1812)](image)

**Figure 30. Proposed peramivir analogue**

The β-hydroxyl group of peramivir has been shown to hydrogen bond to Asp151. However, a comparison of peramivir’s potency with a de-hydroxylated peramivir analogue revealed similar activity (23a versus 24a). Since the hydroxyl group was not installed to generate a key interaction with the active site residues but rather was a product of the synthesis, understanding its full impact could shed some light on its usefulness when designing future cyclopentane-like scaffolds. Based on the guidelines set by Lipinski, if the hydroxyl group proves to be superfluous then removal of this substituent would not only remove a hydrogen bond donor but would also increase the lipophilicity of the compound. Both outcomes could lead to an increase in bioavailability and allow the potential to install functionality at other positions around the scaffold. To test the usefulness of the hydroxyl group, a simplified cyclopentane scaffold mimicking peramivir’s scaffold (Figure 31) will be used, which will possess a carboxylic acid and a guanidine. The dehydroxylated analogue (29), the α-hydroxy carboxylic
acid (30) and the β-hydroxy carboxylic acid (31) will be synthesized and their inhibitions analyzed against the neuraminidase enzyme.

![Figure 31. Proposed hydroxy analogues of peramivir](image)

**1.4.0 Didemnaketal A**

**1.4.1 Human Immunodeficiency Virus (HIV)**

As of 2010, the World Health Organization (WHO) estimated that nearly 34 million people were living with human immunodeficiency virus (HIV), with 2.7 million people being newly infected in 2010. Acquired immunodeficiency syndrome (AIDS) has begun to decrease since 2005 partly due to advances in antiretroviral therapy (ART) in which three or more antiretroviral drugs are used in combination to limit resistance through viral mutations. Using a mathematical model Ho established the life span of HIV (from viral entry of the cell to the release of additional virions) to be as short as 1.5 days. HIV also lacks any proofreading enzymes to correct errors when converting genomic RNA to proviral DNA using reverse transcriptase. Both the short life span and the high error rate associated with the proviral DNA can cause the virus to mutate rapidly. Most of these mutations fail to offer any advantages, however some can become superior compared to the parent virus and lead to drug resistance. Improper use of antiretroviral drugs, especially reverse transcriptase inhibitors, can also lead to the development of multi-drug resistant mutations. Thus, antiretroviral combination therapy aims to suppress HIV replication through the inhibition of multiple targets, thereby reducing the chance of generating a superior mutation.

HIV can only replicate inside a human cell. Once inside the body, HIV binds to dendrites which carry the virus to CD4 T-helper cells. Entry to these host cells occurs through binding of the viral envelope protein gp120 to a CD4 receptor protein on the surface of the cell as well as
binding to other co-receptors (Figure 32). Once fused to the cell, the virus empties its three major viral enzymes (HIV reverse transcriptase, integrase and protease). Each of these is required for the maturation of HIV. HIV reverse transcriptase opens the RNA into single strands and generates a DNA copy which is transported into the cell’s nucleus along with HIV integrase. HIV integrase then integrates the viral DNA into human DNA. Using the host’s own cellular machinery, mRNA (for viral proteins) and genomic RNA (for new viral particles) are produced and exit the nucleus. The viral mRNA is used to generate a polypeptide precursor which is then acted on by a third viral enzyme, HIV protease, to yield the three major viral enzymes HIV reverse transcriptase, integrase and protease. The viral proteins and genomic RNA then move toward the cell membrane to form a new viral bud. Common HIV treatment uses drug combinations comprised of two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor or protease inhibitor. Next-generation therapeutics are now focussing on inhibiting the entry/fusion of the virus, with enfuvirtide (2007) and maraviroc (2003) being the only known drugs of this kind to be approved by the FDA.

**Figure 32.** HIV maturation cycle and key targets for antiretroviral drugs
HIV-1 protease is a homodimer made up of twin 99 amino acid polypeptides. The dimer is generated from the N- and C-termini of one monomer self-assembling with the N- and C-termini of another monomer to form a β-sheet (Figure 33a). The active site of the dimer contains an identical Asp25 from each monomer. These two aspartic acids are essential in controlling the nucleophilic attack of water during the proteolysis of the substrate (Figure 33b). Due to its importance during the maturation of HIV, HIV-1 protease is a prime target for drug therapy. Current drugs inhibit the enzyme by mimicking the tetrahedral intermediate that occurs during the hydrolysis of the substrate. The structural similarity among protease inhibiting drugs (Figure 34) and the high mutation rates of the retrovirus could possibly lead to multi-drug resistance. Therefore, new means of inhibiting HIV-1 protease have been sought. Of particular interest is dissociative inhibition which would inhibit the enzyme at the β-sheets of the N- and C-termini rather than the aspartyl active site. Since the active site of HIV-1 protease is present only in the dimeric form, a dissociative inhibitor could be used to block the formation of the dimer resulting in an absence of the active site. Drugs that function through dissociative inhibition could be combined with drugs that function through active-site inhibition and would reduce the likelihood of drug-resistance through structural mutations.

Figure 33. HIV-1 protease. A) The N- and C-termini of both monomers link to form a β-sheet and generate the HIV-1 protease dimer. B) Catalytic diad active site containing Asp25 controls the nucleophilic attack of water during proteolysis.
Figure 34. Common HIV protease inhibitors
1.4.2 Background and Isolation of Didemnaketals

A sample from the magenta ascidian *Didemnum sp.* was isolated by Faulkner from the coast of Auluptagel, Palau and stored at –20 ºC in methanol for several years. With increasing interest in potential HIV/AIDS chemotherapeutics, natural product screening was used in 1991 to help identify new candidates for development. The isolated products didemnaketal A (32) and B (33) (Figure 35) were shown to inhibit HIV-1 protease with an IC$_{50}$ of 2 µM and 10 µM, respectively. Using a combination of 1-D NMR spectroscopy and 2-D correlation spectroscopy, the regiochemistry of didemnaketal A was determined. Didemnaketal A contained a pentaester fragment and a spiroketal fragment. Comparison of the data for didemnaketal A to didemnaketal B revealed that the compounds were identical in their C-1 to C-20 region. The differences were the additional carbons constituting an extension of the polyisoprenoid chain such that an oxidative cleavage of the C-22/C-23 olefin would result in didemnaketal A. The stereochemistry of the two compounds could not be determined at the time due to the small amount of natural product isolated. Didemnaketal B is a linear heptaprenoid, a rare class of terpenoids. The isolation of terpenoids from ascidians is unusual since most ascidians produce metabolites that are amino acid derived. However, new research into symbiotic bacterial relationships with ascidians suggests the possibility of these terpenoids coming from bacterial symbiotes rather than the tunicates themselves.

![Figure 35](image-url)

**Figure 35.** Didemnaketal A, B and C isolated from the ascidian *Didemnum sp.*

<table>
<thead>
<tr>
<th></th>
<th>R =</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><img src="image-url" alt="A" /></td>
</tr>
<tr>
<td>B</td>
<td><img src="image-url" alt="B" /></td>
</tr>
<tr>
<td>C</td>
<td><img src="image-url" alt="C" /></td>
</tr>
</tbody>
</table>
A fresh collection of the ascidian organism was collected from Topkukau, Palau by Faulkner in 1993. However, the new sample did not contain compounds 32 or 33 but rather the major secondary metabolite didemnaketal C (34), which upon hydrolysis in methanol produced didemnaketal B (33). Therefore it was assumed that didemnaketal A and B were degradation products of didemnaketal C produced from the prolonged storage in methanol. Interestingly, didemnaketal C showed no inhibition toward HIV-1 protease. Using a series of degradation and derivatization experiments in conjunction with chiral shift methods and comparison of fragments to known compounds, the absolute stereochemistry of didemnaketal C was determined in 2002 to be 5S, 6S, 7R, 8R, 10S, 11S, 12S, 14S, 16S, 18R, 20S, 21S, and 26S.

In 1998, the Rich group synthesized a series of simplified didemnaketal A analogues. Most reported HIV-1 protease inhibitors contain a free hydroxyl group (Figure 34) that can interact with the catalytic aspartic acids in the active site. Therefore, Rich first examined analogue 38 encompassing the C-8 to C-23 portion of didemnaketal A and containing an α-hydroxy ketone function. At the time, the stereochemistry at the methyl substituents was unknown. Therefore, the ambiguous methyl groups (C-10, C-14, and C-18) were deleted, leaving four possible diastereomers (at the C-11 and C-21 carbon centers) as targets. These analogues were synthesized as pairs of diastereomers (C-11/epi-C-11 and C-21/epi-C-21) from the known diol 37 in 14 steps (Scheme 2) and subsequently tested. However, none of the spiroketal analogues inhibited HIV-1 protease. Therefore, the C-1 to C-11 portion was synthesized in 16 steps from 1,5-diene-3-ol (35), again removing the ambiguous methyl groups (C-6 and C-10) to generate compound 36 as eight possible diastereomers (Scheme 2). Of the eight compounds synthesized, the most potent of these (39) showed a K_i value of 2.1 µM which was comparable to the natural product didemnaketal A. Interestingly, this particular molecule is an epimer of the natural product at C-8. In fact the analogue matching the absolute stereochemistry, determined by Faulkner in 2002, showed a K_i value of 10 µM which is comparable to that of didemnaketal B. Additional analogues containing hydroxyl groups in place of the esters were not active suggesting that full activity does not require a free hydroxyl group. The mechanism of HIV-1 protease inhibition for analogue 39 was then studied kinetically.
1.4.3 Enzyme Kinetics

Reversible inhibitors bind to an enzyme with non-covalent interactions such as hydrogen bonding, hydrophobic interactions and ionic bonds. Unlike irreversible inhibitors or substrates, reversible inhibitors do not undergo a chemical reaction once complexed with the enzyme. Seminal work by Michaelis and Menten provides a simplified model of enzyme kinetics. In this
model, the initial bimolecular reaction between the enzyme (E) and the substrate (S) provides an
enzyme-substrate complex (ES). This complex can then undergo a unimolecular reaction to

\[
E + S \xrightarrow{k_f} ES \xrightarrow{k_{cat}} E + P
\]

\[
I \quad I
\]

\[
K_i \parallel K_i'
\]

\[
EI \quad ESI
\]

**Scheme 3.** Michaelis-Menten enzyme kinetics for reversible inhibitors

produce a product (P) (Scheme 3). To gain information about the enzyme, the initial rate (\(v\)) of
the reaction can be measured over a series of substrate concentrations. The rate of the reaction

\[
v = V_{max}[S] / (K_m + [S])
\]  

(1)

where \(V_{max} = k_{cat}[E]\)

\[K_m = (k_{cat} + k_r) / k_f \approx K_d\]

will increase with increasing substrate concentrations until all the individual enzyme copies
become occupied and a maximum rate, \(V_{max}\), is obtained. The Michaelis constant, \(K_m\), is the
substrate concentration at which the reaction rate reaches half-maximum and is inversely
proportional to the affinity of the substrate for the enzyme, i.e. a small \(K_m\) indicates a high
affinity. Put simply, if the substrate has a high affinity for the enzyme then the rate will approach
\(V_{max}\) at a lower concentration of substrate. The Michaelis-Menten equation (equation 1) contains
two crucial assumptions other than the consideration that there is no intermediate and no product
inhibition. The first assumption is called the quasi-steady-state hypothesis and requires that the
enzyme-substrate complex does not change significantly over time when compared to the
product and substrate concentrations. The second assumption is that the total enzyme
concentration is also constant. If the rate determining step is slow compared to dissociation (\(k_{cat}
<< k_r\)) then \(K_m\) is roughly equal to the dissociation constant \(K_d\). Furthermore, if the substrate
concentration is small compared to \(K_m\) and the concentration of the ES complex is also small, the
Michaelis-Menten equation can be simplified to equation 2, where \( \frac{k_{cat}}{K_m} \) is a pseudo-second order rate constant.

\[
v_0 = \left( \frac{k_{cat}}{K_m} \right) [E][S] \quad \text{if } [S] << K_m
\]  

(2)

A plot of rate versus [S] shows a logarithmic function that asymptotically approaches \( V_{\max} \). In order to calculate accurate values for \( V_{\max} \) and \( K_m \), a nonlinear regression analysis is performed. Alternatively, one could use a variety of graphical methods including the Eadie-Hofstee diagram, the Hanes-Woolf plot and the Lineweaver-Burk plot. Depending on where the errors lie in the data, certain analyses may offer more accurate results. Therefore, it is advisable to interpret the data using a few different models and compare the outcomes. Since this thesis will focus on both nonlinear regression and the Lineweaver-Burk plot for analysis of its data, the other models will not be discussed further. The Lineweaver-Burk is a double inverse plot comparing \( \frac{1}{\text{rate}} \) versus \( \frac{1}{[S]} \) for varying substrate concentrations at a series of inhibitor concentrations. That is, each line is associated with a different concentration of inhibitor which is tested over a range of substrate concentrations. This generates a linear graph whose y-intercept for each line will provide a \( V_{\max} \) value and the x-intercept for each line will provide a \( K_m \) value. Since each line is associated with a different concentration of inhibitor, the point at which all the lines cross can help to elucidate the type of inhibition observed (Figure 36).

In competitive inhibition the substrate and the inhibitor have a high affinity for the active site but both cannot be bound to the enzyme at the same time (Scheme 3). This type of inhibition can be overcome by using sufficient concentrations of substrate to bias the probability of forming the ES versus EI complex. Once the substrate binds to the enzyme to form the ES complex, catalysis can occur resulting in the formation of product. Since the inhibitor cannot bind to the ES complex under competitive inhibition, it will not have any effect on the catalytic turnover and the maximum rate (\( V_{\max} \)) will remain constant (Figure 36b). In the presence of increasing concentrations of inhibitor, the substrate’s ability to form the ES complex will be reduced. Therefore, a higher concentration of substrate is required to reach half-\( V_{\max} \), i.e. \( K_m \) increases in the presence of an inhibitor.
In uncompetitive inhibition, the inhibitor only binds to the ES complex. As a result, $V_{\text{max}}$ decreases relative to the inhibitor-free system since binding of the inhibitor to the ES complex slows down the rate of catalysis (Figure 36e). Moreover, $K_m$ will also decrease since the ES complex is reacting with the inhibitor to form the ESI complex and shifting the equilibrium toward the formation of more ES complex.

In mixed inhibition the inhibitor can bind to the enzyme at an allosteric site at the same time as the substrate (i.e. a combination of competitive and uncompetitive inhibition). Binding of the inhibitor to this allosteric site changes the tertiary conformation of the enzyme active site thereby reducing the substrate’s affinity. Increasing the amount of substrate will reduce this inhibition, but won’t completely overcome it. This in turn will affect the catalysis rate, increasing $K_m$ and decreasing $V_{\text{max}}$ as the concentration of inhibitor increases. This is represented by a Lineweaver-Burk plot with a crossing of lines at any point other than the x- or y-axes. Non-competitive inhibition is a special case of mixed inhibition where binding of the inhibitor reduces the enzyme’s activity but does not affect the substrate’s affinity. Since the affinity remains...
constant, so too does $K_m$ (Figure 36d). However, disruption of the catalysis by reducing enzyme activity will result in a decrease in $V_{max}$.

<table>
<thead>
<tr>
<th>Type of Inhibition</th>
<th>$V_{max}$</th>
<th>$K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competitive</td>
<td>Constant</td>
<td>Increases</td>
</tr>
<tr>
<td>Uncompetitive</td>
<td>Decreases</td>
<td>Decreases</td>
</tr>
<tr>
<td>Mixed</td>
<td>Decreases</td>
<td>Increases</td>
</tr>
<tr>
<td>Non-competitive</td>
<td>Decreases</td>
<td>Constant</td>
</tr>
</tbody>
</table>

Table 8. Summary of the types of inhibition and their effects on $V_{max}$ and $K_m$ with increasing inhibitor concentrations

Inhibition of obligatory dimeric enzymes by interfering with dimerization (known as dissociative inhibition) occurs via a second-order rate constant and precludes the possibility of displaying the kinetic characteristics of any simple, classical type of inhibition. This is of particular interest for HIV-1 protease whose enzymatic catalysis was proven to be dependent on its dimerization. If a dimeric enzyme (D) is in rapid equilibrium with its monomer (M) and can react with a substrate (S), then an inhibitor (I) can bind not only to the dimer (competitive inhibition) but also to the monomer (dissociative inhibition), to the dimer-substrate complex (uncompetitive inhibition) or to both the dimer and the dimer-substrate complex (non-competitive inhibition).

If the equilibria are faster than the catalytic steps, then employing the equations defining the dissociation constants and the mass balance equations generates an expression for enzyme concentration ($E_0$) as a function of rate ($v$), substrate concentration ($S$) and inhibitor concentration ($I$). The first term in equation 3 corresponds to the classical competitive ($K_c$) and
uncompetitive ($K'_c$) processes which obey Michaelis-Menten type kinetics, while the second term reflects the dissociation of the dimeric enzyme into its inactive monomers ($K_d$) and subsequent dissociative inhibition ($K_i$).

\[
E_0 = \frac{v}{S} \frac{K_m(1 + \frac{I}{K_c}) + S(1 + \frac{I}{K'_c})}{k_{cat} + k'_{cat} \frac{I}{K'_c}} + \left(\frac{v}{S}\right)^{1/2} \times \frac{\left(\frac{K_d K_m}{4}\right)^{1/2} \left(1 + \frac{I}{K_i}\right)}{\left(k_{cat} + \frac{k'_{cat} I}{K'_i}\right)^{1/2}}
\]

(3)

The substrate used to probe the active enzyme should not perturb the equilibria between the enzyme and the inhibitor. To monitor this interaction, a fluorescent-labelled substrate is used and should ideally have a relatively high $K_m$ (low affinity) and a large fluorescence upon hydrolysis. Therefore, if $S_0 \ll K_m$, the reaction will follow first-order kinetics and $k_{exp}$ can be determined to routinely good precision and reproducibility. Using these first-order conditions, DS and DSI become negligible with respect to all other species and so the term $S(1 + I/K'_c)$ can be removed. Inserting the term $v/S = k_{exp}$, equation 3 can then be expressed as:

\[
\frac{E_0}{(k_{exp})^{1/2}} = \left(k_{exp}\right)^{1/2} \frac{K_m(1 + \frac{I}{K_c})}{k_{cat} + k'_{cat} \frac{I}{K'_c}} + \left(\frac{K_d K_m}{4}\right)^{1/2} \left(1 + \frac{I}{K_i}\right) \left(k_{cat} + \frac{k'_{cat} I}{K'_i}\right)^{1/2}
\]

(4)

In the absence of an inhibitor, equation 4 is further simplified to:

\[
\frac{E_0}{(k_{exp})^{1/2}} = \left(k_{exp}\right)^{1/2} \frac{K_m}{k_{cat}} + \left(\frac{K_d K_m}{4k_{cat}}\right)^{1/2}
\]

(5)
The $k_{\text{exp}}$ must first be determined for a variety of enzyme concentrations based on the linear plot $v = k_{\text{exp}}[S]$. Once known, plotting $E_0/(k_{\text{exp}}^{1/2})$ as a function of $(k_{\text{exp}}^{1/2})$ will generate a line with a positive slope and a positive intercept. If the intercept is significantly non-zero then the enzyme will show significant dissociation even at low enzyme concentrations. Using this data, both $k_{\text{cat}}/K_m$ and $K_d$ can be determined.

Carrying out a series of experiments where the initial substrate concentration is kept constant ($S_0 << K_m$) and then subsequently varying the enzyme concentration and maintaining a constant inhibitor concentration, one can again plot $E_0/(k_{\text{exp}}^{1/2})$ as a function of $(k_{\text{exp}}^{1/2})$ to generate a line (Figure 36). If the graph generates a similar slope to the case without an inhibitor then the $K_c$ term is very large in relation to $K_m$ and $k’_{\text{cat}}/K’_c$ is negligible (i.e. neither competitive nor non-competitive inhibition occurs). If the intercept is higher than in the case with no inhibitor added, then the contribution is due to the $K_d$ term, thus the inhibitor acts as a dissociative inhibitor. Knowing the values for the enzyme concentration ($E_0$), $k_{\text{exp}}$, the slope $K_m/k_{\text{cat}}$ and the dissociation constant $K_d$, the value for $K_i$ can be calculated using equation 4.

The most potent analogue (39) synthesized by Rich was subjected to this Zhang model to determine if the inhibition of HIV-1 protease was caused by a competitive, a non-competitive or a dissociative inhibition mechanism. Based on the data obtained, the inhibition of HIV-1 protease by the simplified acyclic side chain was said to proceed by a dissociative pathway. This particular molecule would fail to provide a useful drug candidate due to the lability of the esters. However, it provides a useful model to gain a better understanding of how HIV-1 protease can be inhibited by a dissociative pathway. This knowledge can in turn lead to the development of future drug candidates that can be administered in conjunction with the already available competitive HIV protease inhibitors in order to avoid multi-drug resistance through mutations.

1.4.4 Previous Synthetic Attempts

The total synthesis of the didemnaketals has not yet been accomplished. However, several synthetic attempts or partial syntheses have been published. These will be briefly discussed in this section with emphasis on the key steps involved. The first attempt at the synthesis of the didemnaketals was by Tu in 2001 (Scheme 5). Although the relative stereochemistry of the target had not yet been established, an approach toward two possible epimers (at C-11) of the spiroketal 50 was envisioned to proceed through the coupling of sulfone
and aldehyde 48. Both the sulfone and the aldehyde share the “1-oxygen-3-methyl” moiety common to (R)-(+-)pualexone (40) which is readily abundant. Furthermore, a key methyl stereocenter can be established from this starting material. Methylation and oxidative cleavage of the olefin in starting material 40 generated an α-hydroxy ketone 41 which was then converted to an allylic alcohol. The allylic alcohol underwent an oxidative rearrangement using pyridinium chlorochromate followed by reduction and epoxidation to give compound 42 as a single diastereomer. Selective opening of the epoxide under basic conditions yielded an ene-diol that was further cleaved by ozone to generate compound 43. Trivial protection and functional-group manipulation of this intermediate then led to the desired sulfone coupling partner 44.

Access to aldehyde 48 was envisioned to proceed from the same starting material 40. The known allylic alcohol 45, accessed using a previous synthesis by Crabbé,131 underwent epoxidation to give a 3:1 mixture of 46 and its syn-epoxide isomer which were separated using a pyridinium chlorochromate method developed by Tu. A Lewis acid-mediated rearrangement provided an allylic alcohol which was cleaved by ozone to give hemiacetal 47. Opening of the hemiacetal and further protection yielded the second coupling partner 48 in a 5:1 inseparable diastereomeric ratio. Coupling of the two fragments followed by global deprotection and acid-mediated spiroketalization generated the desired compound 50 in 18 linear steps and an overall yield of 0.0063%.
Scheme 5. Tu’s retrosynthesis of the spiroketal moiety using a sulfone-aldehyde coupling

A year later the same group published a similar synthesis of the spiroketal, starting again from \((R)-(+)-pulegone 40\), but instead aiming to couple the two fragments using a \(S_N2\) dithiane addition to increase the flexibility of the reaction (Scheme 6).\(^{132}\) A model coupling of a common terpenoid iodide with dithiane 51 and a separate model coupling of a common dithiane derived from benzaldehyde with compound 52 showed promising results. Unfortunately, the intended
coupling of compounds 51 and 52 yielded no reaction. Believed to be caused by steric factors, the synthesis was repeated with exchange of the acetonides for *tert*-butyldimethylsilyl (TBS) ethers, however, the coupling reaction still failed to proceed. Exchanging the iodide for a sulfone and removal of the dithiane to give the aldehyde (as seen in their previous paper) allowed for the generation of compound 49 followed by global deprotection and acid-mediated spiroketalization to afford compound 50 in 20 linear steps and an overall yield of 0.0019%.

Scheme 6. Tu’s retrosynthesis of the spiroketal moiety using a key S_N2 dithiane coupling

In the same year, Tu published another paper showing the synthesis of a spiroketal variant of didemnaketal (α-hydroxy ketone exchanged for an isopropyl). Starting from commercially available (−)-menthone (53), a similar approach to the previous two syntheses generated compounds 54 and 55. Of particular interest is the now syn-diol in compound 56 (Scheme 7) which was previously synthesized as the anti-diol. Therefore, compound 57 was generated as the epimer at C-16 and C-20, with respect to Tu’s previous syntheses of compound 50, in 12 linear steps and an overall yield of 0.096%.
With the determination of the absolute stereochemistry of didemnaketal by Faulkner in 2002, the acyclic polyol fragment also became a target for synthesis. Again at the forefront was Tu who in 2004 proposed a synthesis starting from (S)-carvone (58) and employed a key Gröb fragmentation to generate the C-7 OH, C-6 methyl and the terminal C-1 to C-3 olefin framework (Scheme 8).\(^\text{134}\) Epoxidation of 58 followed by an organoselenium-mediated catalytic reductive ring opening of the resulting oxirane afforded β-hydroxy ketone 59. The ketone was protected, followed by protection of the β-hydroxyl group and then subsequent deprotection of the ketone. This round-about route was employed due to elimination issues associated with directly protecting the β-hydroxyl group of 59 in the presence of a free ketone. A Shapiro reaction\(^\text{135}\) using tosyl hydrazine then generated diene 60. In the case where \(R^1\) is a tert-butyl-diphenylsilyl (TBDPS) ether, the steric effects biased the epoxidation of compound 60 to the external olefin producing a 1:1 diastereomeric ratio. Dihydroxylation of the remaining olefin and protection of the resultant alcohols gave intermediate 61 which then underwent a Gröb fragmentation to furnish the Z-allylic alcohol 62 as a single isomer. Interestingly, the configuration of the epoxide

**Scheme 7.** Tu’s retrosynthesis of the spiroketal moiety starting from (–)-menthone
had no effect on the Gröb fragmentation reaction. Further oxidation provided compound 63 in 15 linear steps and an overall yield of 0.033%.

Scheme 8. Tu's retrosynthesis of the acyclic ester moiety

In 2005, Tu revised the synthesis of the spiroketal fragment to employ a more stereoselective approach, once again starting from \((R)\)-pulegone and utilizing the key aldehyde-sulfone coupling reaction. The spiroketal was generated in 21 linear steps and an overall yield of 0.016%. In 2007, their group published the C-17 to C-28 fragment of didemnaketal B using previously established methodologies. However, with all these key intermediates, there still remains no concise way to couple the two halves of the target together.

In 2008, Ito devised a convergent synthesis of the C-9 to C-28 spiroketal subunit of didemnaketal B. Starting from commercially available 64, protection of the alcohol followed by reduction of the ester gave an aldehyde which was then reacted with the Ohira-Bestmann reagent to generate a terminal alkyne 65. Hydrozirconation of 65 gave the desired vinyl iodide 66 which underwent a Suzuki-Miyaura cross-coupling with alkyl bromide 67 to afford a
cross-coupled alkene intermediate 68. Compound 68 was then subjected to a Sharpless dihydroxylation followed by acetonide protection and conversion of the alcohol to an alkyl bromide, generating the key intermediate 69.

Starting from L-glutamic acid (70), the known lactone 71 was synthesized in three steps. Methylation alpha to the carbonyl followed by opening of the lactone gave compound 72 in a 10:1 mixture of diastereomers. Allyl protection and cleavage of the trityl ether followed by oxidation of the subsequent alcohol provided the aldehyde intermediate 73. Another coupling fragment 76 was synthesized from a desymmetrizing anhydride opening of 74 with lipase (92% ee) to provide an intermediate possessing an n-propyl ester at one end and an acid at the other end. Both the ester and the acid were further reduced and the subsequent alcohols were monoprotected to give compound 75. Installation of a propyne functional group followed by hydrozirconation with the Schwartz reagent\textsuperscript{141} produced compound 76. Lithiation of 76 and addition to 73 generated a 1:1 inseparable mixture of diastereomers. Cleavage of the allyl ethers with zirconocene and acetonide protection of the 1,2-diol permitted separation of the two diastereomers. Oxidation of the remaining primary alcohol produced the aldehyde intermediate 77 which was then coupled to the alkyl bromide 69 again using a lithiation/addition protocol. Oxidation of the coupled intermediate and global deprotection of the acetonides with acid-induced spiroketalization provided the desired product 78 in 16 linear steps and an overall yield of 0.028\% (Scheme 9).
Scheme 9. Ito's retrosynthesis for the C-9 to C-28 subunit of didemnaketal B.
The most recent synthesis of the C-9 to C-28 subunit of didemnaketal B was reported by Fuwa in 2010 employing a Suzuki-Miyaura coupling and a Nozaki-Hiyama-Kishi (NHK) coupling as the key fragment assembly processes (Scheme 10). The synthesis of the C-9 to C-15 iodide 83 proceeded through a Julia-Kozcienski olefination between aldehyde 80 and sulfone 81 to yield 82 as an inseparable mixture of E/Z isomers (16:1). A Sharpless dihydroxylation, subsequent protections and PMB removal followed by a Finkelstein reaction to convert a free alcohol to an alkyl iodide generated compound 83. The approach toward the C-16 to C-21 enol phosphate 89 started from a known epoxide 85 which was regioselectively opened with a vinyl Grignard to give the homoallylic alcohol 86 which in turn was esterified with acryloyl chloride to provide 87. A ring-closing methathesis using Grubbs second generation catalyst followed by a Michael addition with lithium dimethylcuprate afforded lactone 88. Finally, installation of the phosphate gave compound 89. Conversion of iodide 83 to alkylborate 84 followed by a β-alkyl Suzuki coupling with 89, in a single pot, yielded intermediate 90. After removal of the TIPS protecting groups and addition of an acid catalyst, spiroketalization occurred with the correct stereochemistry as confirmed by NOE experiments. Finally, iodide 92 was synthesized from a known alkyne employing a key silylcupration step with a 7:1 regioselectivity and an E/Z ratio of 6:1 when installing the vinyl iodide. Coupling of iodide 92 with aldehyde 91 using a Nozaki-Hiyama-Kishi coupling afforded a separable 1.4:1 mixture of 93 and the corresponding C-21 epimer. The epimer could be converted to 93 by a two-step oxidation and reduction sequence giving a synthesis with 17 linear steps and an overall yield of 0.21%.
Scheme 10. Fuwa's retrosynthesis for the C-9 to C-28 subunit of didemnaketal B
1.4.5 Proposed Synthesis of Didemnaketal A

While various fragments of didemnaketal A and B have been targets for other research groups, the total synthesis has yet to be completed; although both Fuwa and Ito are potentially close to completing their routes. In our group, the total synthesis of didemnaketal A has been pursued through a collaborative effort with my colleague Jason Davy. To this end, we envisioned a late stage bond formation between C-10 and C-11 employing a Nozaki-Hiyama-Kishi coupling (Scheme 11). This key retrosynthetic step roughly divides the molecule in half, providing a convergent approach toward the target compound. Jason Davy is working to synthesize the spiroketal fragment 96 while I undertake the synthesis of the alkyl sidechain 102. The spiroketal is doubly anomeric and should be accessible from spiroketalization of tetraol 95 using an acid catalyst. The initially planned route to compound 95 called for a ring opening/cross metathesis of the meso-nononenone 94 followed by a desymmetrizing Sharpless dihydroxylation.

Accessing compound 102 is one of the principal goals for this thesis. Not only does it provide a key intermediate required for the total synthesis, but it also offers a route toward potential analogues. The acyclic fragment 102 was envisioned to start from 97 employing an Evans aldol reaction to install the syn C-6 and C-7 geometry. Cleavage of the auxiliary would generate a 1,3-diol which could be subsequently protected as the p-methoxybenzylidene acetal 98. The steric bias of this acetal could be used to control the allylation of 98, generating the desired syn C-8 stereochemistry. Oxidation of the terminal olefin to a ketone using a Wacker oxidation would afford compound 99 followed by functional group interconversion to generate the aldehyde intermediate 100. An asymmetric Brown allylation would provide the final stereocenter C-5 and a Grubbs cross metathesis with methyl methacrylate would generate compound 101. Finally, formation of either a vinyl halide or a vinyl triflate and coupling to the spiroketal fragment 96 would complete the total synthesis of didemnaketal A.
Scheme 11. Retrosynthesis of didemnaketal A
As discussed previously, Rich reported the synthesis of simplified analogues of the western half of didemnaketal A and showed that they were dissociative inhibitors of HIV-1 protease. However, these molecules were lacking the methyl groups at C-6 and C-10, and his most potent analogue 39 was the C-8 epimer of the natural product. That being stated, both 39 and didemnaketal A showed similar potency toward HIV-1 protease. Since ascidians lack co-evolution with HIV, it is not surprising that the natural product could be modified to increase its potency toward its target. If indeed these fragments do target the β-sheets of HIV-1 protease, then rigidity of these molecules may play a pivotal role in inhibition. Therefore, it would prove interesting to see what effects enhancing the rigidity of the acyclic molecule will have on the overall potency. Gaining an understanding of the contributions from both the C-6 and C-10 methyl groups toward the inhibition of HIV-1 protease could help to develop future therapeutics which function through a dissociative pathway. To meet these goals, a series of analogues based on the natural product scaffold will be generated which bear either the C-6 methyl group or the C-6 and C-10 methyl groups and will be tested against HIV-1 protease. Using this data, the claims made by Rich in regards to whether these molecules act through a dissociative pathway will be confirmed or refuted. Furthermore, the notion of whether these molecules act by disrupting the intercolating β-sheets of the dimer or whether they bind to the monomer and inhibit the formation of the dimer will also be addressed.

Analogues 105 and 106 were envisioned to utilize methodology previously designed in the synthesis of the natural product. Therefore, access to both route A and route B would start from the key intermediate 103 (Scheme 12). Route A would follow previous methodology to afford 102 which would then be coupled to an alkyl aldehyde using a Nozaki-Hiyama-Kishi coupling followed by stereoselective hydrogenation and esterification with isovaleric anhydride of the subsequent diols to provide analogue 106. Route B would involve hydroboration of 103 followed by esterification with isovaleric anhydride to afforded compound 104. Using an asymmetric Brown allylation and a Grubbs cross metathesis, analogue 105 could be generated containing only the C-6 methyl group.
Scheme 12. Retrosynthesis of didemnaketal A analogues
Chapter 2 – Triclosan

2.1.0 Introduction

Triclosan (107) is an antibacterial and antifungal agent that has previously been shown to induce endocrine disruption in amphibians.\textsuperscript{50,143} While triclosan’s off-target activity has been evaluated for multiple organisms, the precise protein-binding partner remains unknown as well as the biological mechanism of inhibition. In fact, diaryl ethers have been shown to target a range of biomolecules. For example, diphenyl ethers structurally similar to triclosan act as potent non-nucleoside HIV reverse transcriptase inhibitors.\textsuperscript{144} Using computationally-guided optimization involving free-energy perturbations, compound 108 was synthesized and showed a 55 pM inhibition toward HIV reverse transcriptase (Figure 37). Interestingly, ethyl triclosan (2) was used computationally as a model inhibitor in this study to determine the energetic gain associated with the addition of two chlorines to the aromatic rings. Merck and Roche have also recently developed compounds 109 and 110, respectively, as diaryl ether non-nucleoside reverse transcriptase inhibitors possessing a diphenyl ether scaffold.
transcriptase inhibitors.\textsuperscript{145-147} Finally, similar diphenyl ether scaffolds have been used to develop second generation inhibitors which are currently effective against Lys103Asn (K103N) and Tyr181Cys (Y181C) mutations commonly seen for HIV reverse transcriptase. Both the K103N and the Y181C mutations have resulted in resistance for efavirenz and nevirapine, two approved non-nucleoside reverse transcriptase inhibitors (Figure 38).\textsuperscript{148}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{efavirenz_nevirapine.png}
\caption{Approved non-nucleoside reverse transcriptase inhibitors}
\end{figure}

In 2001, Merck research laboratories designed a series of farnesyltransferase inhibitors based around a diaryl ether scaffold (Figure 39).\textsuperscript{149} Farnesyltransferase adds a 15-carbon isoprenoid unit to the thiol of a cysteine residue of a protein possessing a terminal CaaX motif (C: cysteine; a: aliphatic amino acid; X: a variable). Specific targets include members of the Ras superfamily, which are guanosine triphosphate (GTP)-binding proteins critical in cell cycle progression. Farnesylated Ras can loosely insert into the membrane of the endoplasmic reticulum or other cellular membranes eventually leading to its transport. In cancer, Ras signalling becomes overactive. Development of farnesyltransferase inhibitors as anti-cancer agents results in a downregulation of Ras signalling and subsequently a decrease in cancer cell growth.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{farnesyltransferase_inhibitor.png}
\caption{General farnesyltransferase inhibitor}
\end{figure}
Yet another example of diaryl ether-biomolecule interactions is in the targeting of excitatory amino-acid transporters (EAATs). Regulation of glutamate is important in order to control many physiological and neurological actions.\textsuperscript{150} Tight monitoring of glutamate levels is accomplished by a family of high-affinity Na\textsuperscript{+}-dependent glutamate transporters of which five have been identified by molecular cloning and designated EAAT1/GLAST, EAAT2/GLT-1, EAAT3/EAAC1, EAAT4, and EAAT5. Amongst these, EAAT2 accounts for the bulk of transport activity measured. Compound WAY-213613 (Figure 40) was shown to be a highly potent inhibitor of EAAT2 with an IC\textsubscript{50} of 85 nM and a selectivity toward EAAT2 versus EAAT1 (59-fold) and EAAT3 (45-fold).

![WAY-213613](image)

**Figure 40.** A highly potent and selective EAAT2 inhibitor

Finally, aryl ethers have found applications as anti-inflammatory and analgesic medicines including selective inhibition of cyclooxygenase-2 (COX-2).\textsuperscript{151} In fact, triclosan has been shown to reduce inflammation caused by gingivitis when applied as a triclosan/zinc citrate solution.\textsuperscript{152}

These few examples presented above, along with the antibacterial/antifungal properties of triclosan discussed in chapter 1, are but a mere subset of the available roles aryl ethers can play in biology and medicinal chemistry. With such a variety of potential protein targets, the endocrine disruption seen in amphibians and other organisms by triclosan may not be due to a direct interaction with thyroid receptors. In fact, it remains possible that these observations are caused by the disruption of other proteins, including the inhibition of transporter proteins, which can lead to a downstream effect on the thyroid signal. To fully elucidate triclosan’s ability to cause endocrine disruption and to probe the mechanism of action, a forward chemical genetics approach was undertaken whereby a family of triclosan-based probes were synthesized and then passed along to our collaborators for evaluation (Figure 41). Using structure-activity relationship studies, various functional groups were proposed in order to probe putative triclosan-binding partners and provide information about the tolerability of these functional groups at specific sites.
around the scaffold. With this information, a second series of compounds could then be synthesized possessing a tethered biotin label. In the event of a slow turnaround rate for the evaluation of our compounds, a broader selection of biotin labeled triclosan derivatives could still be synthesized by this author and evaluated at a later date by our collaborators. Using affinity chromatography, the specific triclosan-binding protein(s) could then be isolated. Control experiments can be done using structurally similar compounds lacking the biotin label which will be synthesized en route to the biotin labelled derivatives. Understanding the specific protein(s) involved in endocrine disruption will allow future analogue design to probe more specific areas of the target biomolecule(s) and provide further information on its biological role. Thirdly, the mechanism for the methylation of triclosan’s phenol is unknown. While already possessing the biotin-containing compounds, future work will involve a select subset of these compounds that will be used to ultimately elucidate the protein(s) or organism responsible for the generation of environmentally-relevant methyl triclosan.

![Figure 41. Triclosan scaffold](image)

### 2.2.0 Synthesis of Triclosan Analogues

Before continuing with the synthesis of the triclosan-based probes, a set of nomenclature used by this author should be addressed. When performing reactions, regardless of the target (i.e. triclosan, neuraminidase or didemnaketal), four plausible outcomes were expected. The first involves no reaction occurring and the result is denoted by “starting material”. The second is when the product has decomposed so that no discernible patterns in the NMR can be used to evaluate the outcome and is denoted by “decomposed”. Thirdly, if a side product was isolated via purification and can be accurately assigned using standard spectroscopy, then the side product will be shown and designated a number written in bold. However, if a compound is isolated but can neither be confirmed as the desired product nor can it be accurately assigned by conventional spectroscopy, then the result will be indicated by “no desired product”.

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The first set of analogues was focused on functionality at the phenol of triclosan. In particular, comparison of the assay data between methyl triclosan (1) and triclosan (107) would dictate whether a key interaction (hydrogen bond, etc.) was required between the phenol and the protein target(s) for binding. As such, the biologically relevant methyl triclosan (1) was synthesized in near quantitative yield (Scheme 13). Compounds 2 and 3 were also generated containing an extra carbon and a branched aliphatic group, respectively. While methyl triclosan (1) and ethyl triclosan (2) were produced in excellent yields, the isopropyl variant (3) was isolated in a reduced yield likely due to the need for the $S_{N}2$ reaction to occur at a secondary center instead of a primary center. Next, information was required as to the placement of the tether and biotin about the aromatic ether. Two possible considerations based on synthetic design were to install the tether at the phenol or to install the tether at the C-2 or C-4 position on the opposing ring. Considering first the phenol, the tolerability of the space surrounding this functional group would need to be probed.

There are two common types of tethers: ethylene glycol chains for hydrophilic regions and alkyl chains for hydrophobic regions. Using a Mitsunobu reaction, analogues containing a hydrophilic tetraethylene glycol chain (4) and a lipophilic decyl chain (5) were synthesized in good yields, although the by-product triphenylphosphine oxide proved challenging to fully remove. Comparison of the assay data for these two molecules would elucidate the precise tether tolerated by this system. Coupled with the steric information from compounds 2 and 3, a series of biotinylated compounds could then be generated. Unfortunately, since neither compounds 2, 3, 4 or 5 were analyzed by our collaborators, a series of triclosan analogues were synthesized containing a biotin label at both the phenol position and the C-4 position on the opposing ring. The tetraethylene glycol tether was chosen as it was deemed the most common choice among literature searches involving biotin-containing affinity chromatography. Synthesis of these biotin-containing molecules will be discussed later on.
In pursuit of additional structural diversity that could be used for the generation of molecular probes, a set of analogues was generated concentrating on the functionality at the carbon para to the aromatic ether linkage (C-4 position) (Scheme 14). Commercially available 111 and 112 were coupled together under basic conditions to produce compound 6. Once again, the tolerance for different functional groups was probed through the synthesis of acid 8 and amide 10. The decyl amide 10 could also be used to probe the tolerance for aliphatic chains at this position of the aromatic ring. This would once again provide insight as to whether an alkyl tether could be used to attach the biotin to the aromatic scaffold. At the time of synthesizing these molecules, it was still unknown whether the phenol contributed any key interactions with its biological target(s), thus compounds 6, 8 and 10 were demethylated to afford compounds 7, 9 and 11 for testing. Following similar methodology, analogues 12, 13, 14 and 15 were generated to install functionality ortho to the aromatic ether linkage (C-2 position) (Scheme 15).
Scheme 14. Synthesis of triclosan analogues with functionality at the carbon center para to the aromatic ether.
Scheme 15. Synthesis of triclosan analogues with functionality at the carbon center ortho to the aromatic ether

With only a single compound tested (methyl triclosan; see section 2.3.0 for results), attention was turned to generate the biotin-containing analogues required for future affinity chromatography experiments. As stated previously, the most common tetraethylene glycol tether was used to connect the biotin to the aromatic scaffold. Starting from tetraethylene glycol 115, two routes were taken to generate the known tethers 118 and 121 (Scheme 16). The first route involved monotosylation of 115 with tosyl chloride to produce compound 116 which then underwent an $S_{N}2$ reaction to form azide 117 followed by hydrogenation to afford amino alcohol 118. Likewise, bistosylation of 115 afforded the fully tosylated 119 which then underwent an $S_{N}2$ reaction to generate bisazide 120 followed by hydrogenation to yield diamine 121.
Surprisingly, amide formation to couple the tether to biotin proved far more challenging than would have been expected (Scheme 17). Attempts to generate the acid chloride of biotin were unsuccessful (entries 1 and 5) while coupling conditions involving $N,N'$-dicyclohexylcarbodiimide (DCC) failed to produce any desired product (entries 2, 3 and 6). Switching to 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC•HCl) yielded a small amount of 124 (entry 4), but the reaction was not reproducible. Due to the polarity of compound 124, purification also proved difficult.

Scheme 16. Synthesis of the amino alcohol tether and the diamine tether
Since directly coupling biotin to the tether was unsuccessful and the purification of 124 was challenging, a new strategy was undertaken. Coupling the tether to the aromatic scaffold could be beneficial as it would decrease the polarity of the subsequent tether and provide a useful chromophore during purification. As such, both amino alcohol 118 and diamine 121 were monoprotected with di-tert-butyl dicarbonate to produce compounds 125 and 126, respectively (Scheme 18). Using previously established Mitsunobu conditions, triclosan 107 was coupled with the Boc-protected tether 125 to afford compound 127. Cleavage of the protecting group with trifluoroacetic acid provided the useful analogue 16 which could be used as a control during the affinity chromatography experiments. Attempts to couple the amine with biotin were however unsuccessful. Although the use of PyBOP yielded a small amount of the desired compound 17, the reproducibility of this reaction was low. Attempts were made to adjust both solvent and temperature but failed to increase the yield. Attempts to try other coupling reagents also proved futile. It was believed that the reaction might proceed more effectively if the biotin tag possessed a better leaving group such as an N-hydroxysuccinimide. The succinimidyl ester
**Scheme 18. Synthesis of biotinylated triclosan 17**

128 was produced in modest yield from biotin 122 and subsequently reacted with amine 16 to produce the biotin labelled triclosan derivative 17 (Scheme 19), thus confirming our hypothesis.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>N</em>-methylmorpholine, 122, PyBOP, CH$_2$Cl$_2$</td>
<td>17</td>
<td>5%</td>
</tr>
<tr>
<td>2</td>
<td>122, EDC•HCl, HOBT, Et$_3$N, DMF</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>3</td>
<td>122, Cl-HOBt, iPr$_2$NEt, DMF</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>4</td>
<td>122, HBTU, iPr$_2$NEt, DMF</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Scheme 19. Synthesis of the biotin-containing triclosan derivative 17 using a biotin succinimidyl ester.

With the success of the biotin succinimidyl ester, it was thought that the amide coupling between acid 8 and amine 126 might also proceed more effectively with a similar installation of an $N$-hydroxysuccinimide leaving group (Scheme 20). Three methodologies were attempted for

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
<th>Yield</th>
</tr>
</thead>
</table>
| 1     | 1. SOCl₂, benzene, 90 °C  
       | 2. $N$-hydroxysuccinimide, Et₂N, EtOAc | 129 | 85% |
| 2     | $N$-hydroxysuccinimide, DCC, DMAP, DMF | 129 | 75% |
| 3     | $N$-hydroxysuccinimide, triphosgene, Et₂N, CH₂Cl₂ | 129 | 49% |

the synthesis of ester 129. While the direct coupling conditions using DCC resulted in a decent yield, conversion to the acid chloride followed by addition of N-hydroxysuccinimide provided 129 in the highest yield. Unfortunately, attempts to couple amine 126 to the succinimidyl ester 129 proved rather challenging. Therefore, a series of standard amide coupling reactions were screened in the hope of synthesizing compound 130 from compounds 8 and 126.

![Scheme 21](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>126, iPr₂NEt, THF</td>
<td>Starting Material (129)</td>
</tr>
<tr>
<td>2</td>
<td>126, NaHCO₃, H₂O/acetone (1:1)</td>
<td>Starting Material (129)</td>
</tr>
</tbody>
</table>

Scheme 21. Coupling conditions to form amide 130 from succinimidyl ester 129 and amine 123

While there are a variety of coupling reagents used in the literature to form amides, only a handful of conditions were attempted (Scheme 22). Conversion of acid 8 to the acid chloride using thionyl chloride followed by the addition of amine 126 only yielded a small amount of product when using toluene and resulted in starting material when using benzene (entries 1 and 2, respectively). Carbodiimide based reagents (entries 4, 5 and 6) failed to produce any desired product even when varying the nucleophilic catalyst. Turning our attention to aminium based reagents (entries 3 and 7), the reactions still failed to provide any desired product. Finally, phosphonium based reagents (entries 8 through 11) were used both at room temperature and at 40 °C in a microwave reactor while varying the time of the reaction. While PyBOP at room temperature (entry 8) did generate the desired product 130, the reaction could not be reproduced on larger scales. As a final attempt, the reaction conditions established in entry 4 were once again used but instead the reaction was left for 24 hours based on ideas adopted from various synthetic chemistry blogs (entry 12). Fortunately, compound 130 was isolated in an 85% yield.
Cleavage of the Boc-protected amine in intermediate 130 with trifluoroacetic acid afforded analogue 18 in quantitative yield (Scheme 23). Similarly to amine 16, this analogue can be used as a control for the affinity chromatography experiments. As with compound 16, issues arose when trying to couple the tethered amine 18 to biotin. Instead, monoprotected diamine 126 was coupled to biotin using the phosphonium coupling catalyst HBTU followed by the addition of compound 8 using the previously established EDC•HCl amide coupling conditions to afford the biotin labelled methyl triclosan 19. To further probe the biological target(s) associated with

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1. SOCl₂, toluene, 90 °C 2. 126, THF</td>
<td>minor 130</td>
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<tr>
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<td>starting material (8)</td>
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<tr>
<td>3</td>
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<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>4</td>
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<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
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<td>n/a</td>
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<td>7</td>
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</tr>
<tr>
<td>8</td>
<td>N-methylmorpholine, PyBOP, 126, CH₂Cl₂</td>
<td>130 36%</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>N-methylmorpholine, PyBOP, 126, CH₂Cl₂, 40 °C, 20 hrs, microwave</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>10</td>
<td>N-methylmorpholine, BOP, 126, CH₂Cl₂</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>11</td>
<td>N-methylmorpholine, BOP, 126, CH₂Cl₂, 40 °C, 8 hrs, microwave</td>
<td>no desired product</td>
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<tr>
<td>12</td>
<td>EDC•HCl, Et₃N, HOBT, 126, DMF, 24 hrs</td>
<td>130 85%</td>
<td></td>
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</table>

Scheme 22. Coupling conditions between analogue 8 and amine 126
Scheme 23. Synthesis of the biotinylated methyl triclosan and the biotinylated triclosan
the methylation of triclosan, demethylation of compound 19 using boron tribromide was attempted. Unfortunately this reaction failed to proceed, possibly because of the age of the boron tribromide being used. Instead, biotin labelled tether 131 was coupled directly with compound 9, albeit in very low yield.

2.3.0 Preliminary Biological Results for Methyl Triclosan, Triclosan and Triclocarban

Preliminary testing for endocrine disruption by triclosan (107), methyl triclosan (1) and triclocarban in Rana catesbeiana tadpoles was conducted by Helbing and co-workers of the University of Victoria Biochemistry and Microbiology Department. Using a cultured frog tadpole tail fin biopsy assay (C-fin) and a thyroid hormone rat pituitary GH3 cell line, all three compounds were analyzed by quantitative real-time polymerase chain reaction (QPCR) for responses associated with thyroid hormone signalling and cellular stress. In particular, the C-fin assay monitored thyroid hormone response using mRNA abundance in transcript levels for both the thyroid hormone receptor β and the Rana larval keratin type I (RLKI) gene. Thyroid hormone receptor β transcript levels are known to upregulate in the presence of triiodothyronine, whereas RLKI transcript levels are known to decrease in the presence of triiodothyronine. Cellular stress was monitored by both heat shock protein 30 (HSP30) and catalase, which are upregulated and downregulated, respectively, upon exposure to thyroid hormones. All data sets for the C-fin assay were analyzed using a non-parametric statistical hypothesis test (Wilcoxon signed-rank test). The GH3 cell line was used to examine the gene transcripts encoding growth hormone, deiodinase I and prolactin as a means to monitor thyroid hormone response. Both the growth hormone and deiodinase I transcripts are upregulated in the presence of triiodothyronine, whereas prolactin is downregulated in the presence of triiodothyronine. Cellular stress for this particular assay was analyzed with heat shock protein 70 (HSP70). All data sets for the GH3 cell line were analyzed first globally using a non-parametric one-way analysis of variance (Kruskal-Wallis) followed by a non-parametric statistical hypothesis test (Mann-Whitney U). The results and interpretation of these analyses will be summarized below.

In the C-fin assay, four of the eight tail biopsies from each individual tadpole were subjected to solvent control and three concentrations of either triclosan (1, 10 and 100 nM), methyl triclosan (1, 10 and 100 nM) or triclocarban (10, 100 and 1000 nM). The remaining four tail biopsies were subjected to 10 nM triiodothyronine and 10 nM triiodothyronine with three
concentrations of either triclosan (1, 10 and 100 nM), methyl triclosan (1, 10 and 100 nM) or triclocarban (10, 100 and 1000 nM). Triclosan treatment with and without triiodothyronine was found to have no affect on both thyroid hormone receptor β and RLKI transcript levels. Likewise, triclosan alone had no affect on the mammalian GH3 cell line hormone transcripts. These results were surprising since previous research suggested a thyroid hormone disruptive effect. It is possible that this effect could be dependent on species or may require the intact specimen rather than a biopsy. A small amount of hormone disruption was observed in the presence of triiodothyronine resulting in prolactin transcripts upregulating by 2-fold at a concentration of 1000 nM triclosan.

Somewhat surprisingly, methyl triclosan treatment exhibited a much greater effect. The transcript levels of thyroid hormone receptor β were increased by 1.4-fold with 10 nM methyl triclosan, whereas RLKI transcript levels were increased by 1.3-fold with 1 nM methyl triclosan. In the GH3 cell line, methyl triclosan at 1000 nM concentration reduced growth hormone transcripts by 2.1-fold but had no affect on deiodinase I transcripts. In the presence of triiodothyronine, application of 1000 nM methyl triclosan was observed to have a significant downregulation of growth hormone and deiodinase I transcript levels with a response of 7.9-fold and 35-fold, respectively. Moreover, methyl triclosan reduced prolactin transcript levels at 100 and 1000 nM concentrations by 1.6-fold and 2.8-fold, respectively, and also reduced prolactin by 18-fold with triiodothyronine and treatment with 1000 nM methyl triclosan. In conjunction with the data for triclosan, evidence from the biological metabolite, methyl triclosan, suggests an alternative mechanism for hormone disruption. It is plausible that the active metabolite may act to disrupt the thyroid hormone rather than triclosan itself.

Triclocarban did not affect thyroid hormone receptor β transcript levels but did trigger a 1.5-fold decrease in RLKI transcript levels at a 1000 nM concentration. In the GH3 cell line, a 1000 nM concentration of triclocarban resulted in a downregulation of growth hormone, deiodinase I and prolactin transcripts by 1.6-fold, 2.6-fold and 2.2-fold, respectively. In the presence of triiodothyronine, all hormone transcripts showed significant change with emphasis on 1000 nM triclocarban which had a response of 3.1-fold, 1.8-fold and 11-fold for growth hormone, deiodinase I and prolactin, respectively. This data was consistent with previous research.\textsuperscript{157}
All three chemical treatments showed some alteration to at least one indicator of cellular stress. In the C-fin assay, HSP30 transcript levels were shown to increase by 4.5-fold and 3.7-fold at triclosan concentrations of 1 nM and 10 nM, respectively. Catalase transcript levels were also shown to increase by 2.4-fold with 10 nM triclosan. Methyl triclosan decreased HSP30 transcript levels at a concentration of 10 nM by 1.6-fold but had no significant affect on catalase. In the presence of triiodothyronine, neither triclosan nor methyl triclosan had any effect on HSP30 and catalase transcription levels. Based on these results, cellular stress could possibly influence metamorphosis in frogs. The difference between triclosan and methyl triclosan could be attributed to differences in the induction kinetics. In the GH3 cell line, triclosan at 1 nM and 10 nM increased HSP70 transcript levels by 2.6-fold and 1.5-fold, respectively. At 1000 nM, triclosan showed a slight decrease in HSP70 transcripts by 1.2-fold. However, in the presence of triiodothyronine, triclosan had no effect on HSP70. Methyl triclosan, at 1 and 10 nM concentrations, significantly increased HSP70 transcript levels by 2.8-fold and 3.3-fold, respectively. Furthermore, HSP70 transcript levels were increased with 10 nM methyl triclosan in the presence of triiodothyronine by 3.6-fold. Comparison of these results for triclosan and methyl triclosan in the GH3 cell line further supports the idea of a common mechanism between the two metabolites when inducing cellular stress.

Finally, 1000 nM triclocarban upregulated HSP30 transcript levels significantly, but only upregulated catalase by 1.29-fold. Addition of triiodothyronine to the biopsies upregulated HSP30 transcript levels by 10-fold and decreased catalase transcript levels by 1.4-fold. In contrast, a 7.5-fold and 6.2-fold increase in HSP30 transcript levels were observed with triiodothyronine and triclocarban at 100 and 1000 nM, respectively. However, no effect was seen with catalase. In the GH3 cell line, HSP70 transcript levels were also altered at all concentrations tested. These results revealed downregulating by 3.9-fold at 10 nM triclocarban, by 1.5-fold at 100 nM triclocarban and upregulating by 2.5-fold at 1000 nM triclocarban. Furthermore, all concentrations of triclocarban (10, 100, 1000 nM) in the presence of triiodothyronine downregulated HSP70 with a response of 2.2-fold, 6.3-fold and 2.9-fold, respectively. It is possible that a negative feedback loop may exist involving HSP70. If this were true, then at the time point taken for these analyses the abundance of mRNA could have already been on its way down.
2.4.0 Future Work

The results from the C-fin assay and the GH3 cell line suggests that endocrine disruption may be present for methyl triclosan but is absent for triclosan. It is therefore plausible that triclosan is converted to methyl triclosan either by bacteria in the environment or by the organism itself and then subsequently used to induce endocrine disruption. As the assay used to determine this data was quite difficult, only a single analogue synthesized (methyl triclosan) was tested. The remaining analogues were originally generated to probe the tolerance of the biological target(s) to provide information on where to position the biotin tag. Since a set of biotin-labelled molecules has already been produced, it would seem more efficient to direct our efforts towards the elucidation of the precise biological target(s) binding to triclosan and methyl triclosan through affinity chromatography experiments. If these results fail, then the previously generated analogues can still be used to obtain information on the biological tolerance and a new set of biotin-containing analogues can be synthesized, where the position of the biotin has changed (i.e. to the C-2 position instead of the C-4 position). Secondly, using a similar assay the route for the conversion of triclosan to its metabolite methyl triclosan can be determined by subjecting the biotin-labelled analogues to various methylating bacteria. Finally, it would be interesting to assay the ethyl and isopropyl triclosan to determine the effect of extending the aliphatic chain and of branching the chain. With this mind, stock solutions (100 mM in dimethylsulfoxide) of analogues 2 through 20 have been prepared. Results from our collaborator Dr. Helbing are still pending.
3.1.0 Introduction

The influenza virus remains a significant health concern even with the implementation of a vaccine program monitored by the World Health Organization (WHO). These health risks are due in part to rapid mutations leading to new strains of the influenza virus as well as the long lead-time required to produce new vaccines each year. This results in already vaccinated patients remaining susceptible to newer strains of the virus. M2 proton channel inhibitors and neuraminidase inhibitors are two distinct classes of antivirals. These drugs were once thought to protect against future influenza pandemics. However, mutation-induced resistance has led to the M2 proton channel inhibitors, rimantadine and amantadine, no longer being recommended for use. Genetic variation in the hemagglutinin protein can also result in influenza strains evading yearly vaccination treatments.

There are currently three clinically approved neuraminidase inhibitors: oseltamivir, zanamivir and peramivir. Oseltamivir (orally active, IC$_{50}$ = 1 nM) and zanamivir (orally inactive, IC$_{50}$ = 5 nM) were once thought to be non-susceptible to resistance, since less than 1% of resistant isolates were detected prior to 2007. In the 2007–2008 flu season, an H274Y point mutation triggered oseltamivir resistance in 12% of the H1N1 viruses tested in the United States. In the 2008–2009 flu season, this number skyrocketed to 98.5%. Fortunately, both the 2009–2010 H1N1 “swine flu” and the H5N1 “avian flu” pandemics lacked this particular mutation and were treatable with oseltamivir. Zanamivir is less susceptible to H274Y mutation-induced resistance but suffers from poor oral availability. The third neuraminidase inhibitor, peramivir (IC$_{50}$ = 0.1 nM), is currently in phase II/III clinical trials in the United States but suffers from poor oral activity and is also ineffective against the H274Y mutant.

The cyclic cores used in the construction of neuraminidase inhibitors have included aromatic rings, dihydropyrans (e.g. zanamivir), cyclohexenes (e.g. oseltamivir), cyclopentanes (e.g. peramivir), and tetrahydropyrroles. The scaffold makes no direct contact with the neuraminidase protein, but rather functions to point the substituents around the ring toward five subsites (S1 to S5) of the neuraminidase active site (Figure 42). All potent neuraminidase inhibitors require a carboxylic acid (or phosphonic acid) to bind to the
positively charged Arg118-Arg371-Arg292 triad. An acetamide is also required and serves both to bind the positively charged guanidine in Arg152 (through the carbonyl group) and also to allow the methyl group of the acetamide to fill the $S_3$ lipophilic pocket. Of these two roles, the latter function is the most important. The acetamide is also conserved amongst the neuraminidase inhibitors as it is present in the natural substrate, sialic acid, and acts as an important recognition element.

Most inhibitors also incorporate either an amine or guanidine to bind to the acidic $S_2$ site, though there is some indication that lipophilic substituents can also be accommodated here.\textsuperscript{165}
The majority of the inhibitor potency comes from filling the $S4/S5$ subsite of the neuraminidase active site. Unfortunately, this region is also highly susceptible to mutations leading to drug-resistance. For example, the widespread H274Y mutation changes the histidine-274 to a larger tyrosine residue. As a result, glutamic acid-276 can no longer rotate out of the $S4/S5$ binding site. This leads to the H274Y mutant retaining a more polar active site which can no longer effectively bind the 3-pentyl group of oseltamivir and peramivir. Zanamivir’s triol sidechain, however, can still hydrogen-bond to Glu276.$^{166}$

With both oseltamivir and peramivir showing susceptibility to the H274Y mutant and both zanamivir and peramivir being orally inactive, second generation neuraminidase inhibitors are needed. The design of these next-generation antivirals would do well to focus on targeting the $S4/S5$ subsite with moderately polar functional groups (as for zanamivir), while maintaining oral activity (as for oseltamivir) and high potency (as for peramivir). To design an optimal second-generation inhibitor, the contributions of each functional group upon binding to its subsequent subsite should be known in order to understand which variations in the substituents will be tolerated. Peramivir is the most potent of the three neuraminidase inhibitors, but has been the least studied from a structure–activity perspective.

While the carboxylic acid and the acetamide in peramivir are known to be required for potency, it is unknown what the consequences of varying the remaining substituents of peramivir will be. To this end, the relative contributions of two particular functional groups present in peramivir, the guanidine and the $\beta$-hydroxy acid, will be analyzed. The published structure for neuraminidase-bound peramivir$^{167}$ reveals a distorted cyclopentane ring, which upon binding places both the carboxylate and the guanidinium functional groups into pseudo-equatorial positions, thus directing them toward the $S1$ and $S2$ pocket, respectively. Furthermore, the X-ray structure suggests that the $\beta$-hydroxyl group may be hydrogen bonding to the Asp151 residue. Understanding these two functional groups’ contributions toward the inhibition of neuraminidase may have implications when designing second-generation inhibitors based on a cyclopentane scaffold. This information will advance ideas aimed at increasing oral availability as well as the design of inhibitors resistant to the H274Y mutant.

One of the ways to increase oral availability might be to reduce the number of hydrogen bond donors and acceptors in accordance with Lipinski’s rules of five.$^{96,168}$ A guanidine group contains three nitrogen atoms which together can contribute multiple hydrogen bond donors and
acceptors. As a result, compounds containing guanidine functions are more likely to have difficulty in being absorbed by the intestinal lining. Although many guanidinylated drugs can still be transported using other means, relying on these transport processes is non-ideal. A perfect example of the impact a guanidine group can have on oral availability is the comparison of oseltamivir and guanidinylated oseltamivir. Oseltamivir carboxylate (22a) possesses a primary amine and has an IC$_{50}$ of 1 nM whereas guanidinylated oseltamivir carboxylate 22c has an IC$_{50}$ of 0.5 nM (Figure 43). Both compounds are highly potent, however neither are orally available in their carboxylate form, possibly due to their high polarity. Interestingly, the pro-drug oseltamivir (ethyl ester of compound 22a) is orally active whereas the ethyl ester of compound 22c is not orally available, presumably due to the presence of the guanidine. Thus, the two-fold increase in activity for compound 22c (compared to 22a) is not enough to offset the benefits of having an orally available antiviral. When designing new drugs, oral availability is highly sought after since patients can administer the drugs themselves resulting in a greater chance of patient compliance. Zanamivir’s and peramivir’s guanidines bind to the S2 pocket of the neuraminidase enzyme by stacking parallel to the Glu119 residue. Zanamivir (21a) has a K$_i$ of 1 nM, whereas the de-guanidinylated zanamivir analogue 21c has a 40-fold decrease in activity with a K$_i$ of 40 nM. It should be noted that neither of these compounds are orally active but regardless, a 40-fold decrease in potency is substantial. Peramivir’s guanidine functions similarly to zanamivir’s guanidine but the two are epimeric at the guanidine-bearing carbon center. Therefore, peramivir may not exhibit the same 40-fold decrease in potency observed for zanamivir when comparing it with de-guanidinylated peramivir 28. De-guanidinylated peramivir 28 was previously synthesized by Chand but activity data for this compound were unavailable. If comparison of the activity data for peramivir (23a) and de-guanidinylated peramivir 28 follows that of zanamivir’s case, then retaining the guanidine in structurally similar second-generation inhibitors will be of utmost importance. However, if the observations follow more closely to that of oseltamivir’s case, then removal of the guanidine when designing next-generation inhibitors may result in an increase in oral availability. Furthermore, if the guanidine is superfluous, then removal of this polar functional group will allow the installation of a subsequent polar group aimed at binding in the S4/S5 pocket while still maintaining a desirable log P value for the overall molecule. Thus, when combating the H274Y mutant possessing a more polar S4/S5
subsite, newly designed inhibitors should be able to effectively bind to this region much like zanamivir’s triol.

**Figure 43.** Comparison of guanidine and de-guanidinylated compounds

The synthesis of peramivir and its analogues is synthetically challenging and requires a significant number of steps. Therefore, a structurally similar but simplified cyclopentane scaffold will be used to further evaluate other potential functional groups and their contributions. To confirm that our simplified cyclopentane scaffold is an acceptable model in which to probe the neuraminidase enzyme, the activity data between peramivir (23a) and de-guanidinylated peramivir 28 will be compared to the activity data between compound 30 and compound 132 (Figure 43). Since the simplified scaffold is missing some key functional groups, the activity of these molecules is expected to be low. However, trends observed for these simplified structures should nonetheless provide meaningful information when designing more elaborate second-generation inhibitors based on the same cyclopentane scaffold.
During the synthesis of peramivir, a [3+2] cycloaddition involving a neopentyl-derived aldoxime was used to install the nitrogen of the acetamide as well as the neopentyl group. The β-hydroxyl group was a consequence of this synthetic methodology. Although this hydroxyl group does seem to be able to hydrogen bond with an Asp151 residue (as seen in the x-ray structure), direct comparison of peramivir (23a) and deoxy peramivir 24a revealed no change in activity (i.e. both were 0.1 nM inhibitors; Figure 44). This suggests that the β-hydroxyl group lacks any significant contribution toward the potency of peramivir. Peramivir (23a) can be synthesized in seven steps from readily available intermediates, whereas deoxy peramivir 24a requires additional manipulations. Therefore, since both compounds showed similar potency but compound 23a was easier to synthesize, it was selected for further clinical trials and eventually emerged as the drug candidate.

![Peramivir and Deoxy Peramivir](image)

**Figure 44.** Comparison of peramivir and deoxy peramivir

While the β-hydroxyl group in peramivir was installed for synthetic ease rather than to invoke a key interaction in the neuraminidase active-site, it was unknown whether this observation would be consistent for future, structurally-related neuraminidase inhibitors. Therefore, to evaluate the potential contributions to the activity for the hydroxyl group in a more generalized system, our simplified scaffold was used to target deoxy compound 29, α-hydroxy acid 30 and β-hydroxy acid 31 (Figure 45). Similarly to the guanidine analysis above, if the hydroxyl group offers no significant interaction with the neuraminidase active site, then its removal will not only simplify future synthetic designs but may increase oral availability through the removal of a hydrogen bond donor and acceptor, and/or allow the installation of a polar group elsewhere on the molecule in order to bind to the S4/S5 pocket.
Information from these studies will be useful for two related projects focused on designing novel second-generation neuraminidase inhibitors currently being carried out by Michael Brant and Jeremy Mason of our group. A one-pot tandem vinylogous 1,2-addition/anionic oxy-Cope reaction followed by a second vinylogous ketone addition developed by Michael Brant yielded a rigid bicyclic sulfone.\textsuperscript{169} Computational docking experiments conducted in MOLOC\textsuperscript{170} showed the bicyclic scaffold overlaid nicely with the cyclopentane scaffold of peramivir. The neuraminidase-bound peramivir\textsuperscript{167} reveals significant cyclopentane ring distortion upon binding. It was therefore hypothesized that the bicyclic sulfone scaffold would fix the ring geometry in place, thereby reducing the entropy of binding and enhancing the target selectivity. Furthermore, functionalization of this scaffold would allow targeting of the same residues located in the neuraminidase active-site that are targeted by peramivir (Figure 46). This fortuitous outcome triggered the design of a new novel neuraminidase inhibitor 133, where four of the six stereocenters were installed during the one-pot two-step bicycle formation. β-amino sulfones are of particular interest in medicinal chemistry due to the greatly attenuated basicity of the amine nitrogen, compared to other secondary amines.\textsuperscript{171} The synthesis of a second neuraminidase inhibitor 134 has been undertaken by Jeremy Mason of our group, wherein the sulfone in compound 133 has been removed. As stated above, the carboxylic acid and the acetamide are required for the potent inhibition of the neuraminidase enzyme, but it is unclear if and to what extent other functionality about the bicyclic scaffold can be altered. Since these structures overlay with the cyclopentane scaffold of peramivir, the trends observed for the analysis of the guanidine and the β-hydroxyl group in the simplified probes may shed some light on future designs of these bicyclic structures.
3.2.0 Synthesis of De-guanidinylated Peramivir

To directly compare de-guanidinylated peramivir 28 with peramivir (23a), the synthesis of both molecules was attempted following existing literature protocols. Freshly generated methanolic hydrochloride was bubbled through a solution containing commercially available lactam 135 to produce an amino ester intermediate, followed by protection of the amine with di-tert-butyl dicarbonate to yield 136 (Scheme 24). Unfortunately, the literature conditions for the [3+2] cycloaddition of compound 136 with the aldoxime 139 failed to generate any product (Scheme 24; entry 1).

Heating the mixture in a pressure vial for 24 hours provided a small amount of desired material 137 (entry 2), while heating for 3 days gave a range of yields from 17% to 74% but the overall reaction was not reproducible (entry 3). The variation in yields was unexpected since no changes were made to the synthetic procedure. Therefore, a variety of conditions were scanned in order to optimize this protocol. Due to the time required for the reaction to proceed at refluxing temperatures, a microwave reactor was used for testing so that temperatures above the boiling point of the solvent could be reached and thus push this sluggish reaction. Entry 4 is a summation of several attempts to generate compound 137. At lower temperatures in the microwave the reaction was slow, while at higher temperatures both the production of side products and the decomposition of starting material 136 were observed. Varying the reaction
time from an hour to three hours showed only a minor difference in the conversion. Using optimal conditions (microwave 100 °C, 3 hours), a 1:1 mixture of starting material 136 and desired product 137 was achieved although separation of the two compounds by chromatography was rather difficult. Changing from 1,2-dichloroethane to a more common yet polar solvent such as methanol (entry 5) resulted in the isolation of clean starting material 136, while the use of a trifluoroacetic acid catalyst gave no desired product (entry 6).

Hypervalent iodine species have been reported in literature to provide a more efficient oxidation of hydroxylamines to the nitrone (required for the [3+2] cycloaddition) than sodium hypochlorite.\textsuperscript{173} Therefore, reactions involving both IBX (entry 7 and 8) and \textbf{Dess-Martin periodinane} (DMP) (entry 9) were attempted with only DMP showing any progression toward the generation of 137. Since the reaction failed to proceed further than a 50% conversion rate, it was theorized that the reactants may have been used up in some side reaction. Increasing the equivalents of triethylamine and 5% sodium hypochlorite aqueous solution failed to provide any product at all, suggesting that this reaction was sensitive to the molar ratios of each material. Instead, triethylamine and 5% sodium hypochlorite aqueous solution were added portionwise over 3 hours and heated to 100 °C in the microwave (entry 11). Initially this showed promising results, so the conditions were adapted to a syringe pump with the slow addition of these reagents over a 2 day period while heating to reflux (entry 12). While the reproducibility was slightly better than in entry 3, the reaction still failed to proceed past a 50% conversion rate and the separation of the product from the starting material proved difficult due to the inability to see the product using standard stains and UV.
Scheme 24. Synthesis of compound 136 and subsequent [3+2] cycloaddition conditions

<table>
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<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>139, Et₃N, CH₂Cl₂, 5% NaOCl, Δ, 20 hrs</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>2</td>
<td>139, Et₃N, CH₂Cl₂, 5% NaOCl, 40 °C, 23 hrs, pressure vial</td>
<td>137</td>
<td>29%</td>
</tr>
<tr>
<td>3</td>
<td>139, Et₃N, CH₂Cl₂, 5% NaOCl, 50 °C, 3 days, pressure vial</td>
<td>137</td>
<td>17% to 74%</td>
</tr>
<tr>
<td>4</td>
<td>139, Et₃N, ClCH₂CH₂Cl, 5% NaOCl, microwave 100 °C to 180 °C, 1 to 3 hrs</td>
<td>136:137 (1:1)</td>
<td>n/a</td>
</tr>
<tr>
<td>5</td>
<td>139, Et₃N, CH₃OH, 5% NaOCl, microwave 100 °C, 1 hr</td>
<td>136</td>
<td>n/a</td>
</tr>
<tr>
<td>6</td>
<td>139, Et₃N, CH₃OH, 5% NaOCl, microwave 100 °C, CF₃CO₂H, 1 hr</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>7</td>
<td>139, ClCH₂CH₂Cl, IBX, microwave 100 °C, 3 hrs</td>
<td>136</td>
<td>n/a</td>
</tr>
<tr>
<td>8</td>
<td>139, CH₃OH, IBX, microwave 100 °C, 3 hrs</td>
<td>136</td>
<td>n/a</td>
</tr>
<tr>
<td>9</td>
<td>139, CH₃OH, Dess-Martin periodinane, microwave 100 °C, 20 hrs</td>
<td>136:137 (2:1)</td>
<td>n/a</td>
</tr>
<tr>
<td>10</td>
<td>139, ClCH₂CH₂Cl, 5% NaOCl, microwave 100 °C, 3 hrs</td>
<td>136:137</td>
<td>n/a</td>
</tr>
<tr>
<td>11</td>
<td>139, ClCH₂CH₂Cl, 5% NaOCl, Et₃N, microwave 100 °C, 3 hrs, portionwise addition of reagents</td>
<td>137</td>
<td>20%</td>
</tr>
<tr>
<td>12</td>
<td>139, CH₂Cl₂, 5% NaOCl, Et₃N, Δ, 3 days, slow addition of reagents via syringe pump</td>
<td>136:137 (1:1)</td>
<td>11% to 54%</td>
</tr>
</tbody>
</table>
With a small amount of material in hand, cleavage of the nitrogen-oxygen bond of isoxazole 137 via hydrogenation followed by subsequent acetylation of the amine gave compound 140 (Scheme 25). Controlling the amount of concentrated hydrochloric acid proved pivotal for this reaction (Scheme 25; entry 1), as too much acid quenched the catalyst and resulted in decomposition. The difference in yield from hydrogenating for 24 hours to 3 days (entry 2 versus entry 3) was minimal, with the shorter reaction time providing slightly more pure material. Due to the polar nature of the amino alcohol intermediate, purification proved rather difficult and care had to be taken when removing the platinum catalyst by filtration.

**Scheme 25.** Opening of the isoxazole ring by hydrogenation and acetylation of the amine intermediate

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1. H₂ (100 psi), PtO₂, CH₃OH, excess HCl conc., 24 hrs 2. Et₃N, Ac₂O, CH₂Cl₂</td>
<td>decomposed</td>
<td>n/a</td>
</tr>
<tr>
<td>2</td>
<td>1. H₂ (100 psi), PtO₂, CH₃OH, HCl conc., 3 days 2. Et₃N, Ac₂O, CH₂Cl₂</td>
<td>140</td>
<td>72% over two steps</td>
</tr>
<tr>
<td>3</td>
<td>1. H₂ (100 psi), PtO₂, CH₃OH, HCl conc., 24 hrs 2. Et₃N, Ac₂O, CH₂Cl₂</td>
<td>140</td>
<td>64% over two steps</td>
</tr>
</tbody>
</table>

Following literature protocol, compound 140 was treated with 1 M hydrochloric acid in ether to remove the tert-butyloxycarbonyl (Boc) protecting group and generate amine hydrochloride salt 141 (Scheme 26). Not surprisingly, under acidic conditions the trace amount of water present in distilled ether was enough to hydrolyse the methyl ester. Fortuitously, this did provide access to the desired de-guanidinylated peramivir 28. Improvement on this literature
procedure was done using 1 M hydrochloric acid in methanol so that the excess methanol would re-esterify compound 140 in case of hydrolysis. This new methodology worked very well and produced amine hydrochloride salt 141 as a white solid, which was triturated with ether and taken crude onto the next step. Guanidinylation of compound 141 was performed using a Boc-protected guanidine to afford compound 143. However, removal of the protecting group under standard acidic conditions failed to yield any product. Therefore, attention was turned to the use of the carboxybenzoyl (Cbz)-protected guanidine. Unfortunately, attempts to remove the protecting groups by hydrogenation also failed to produce peramivir.

Scheme 26. Synthesis of de-guanidinylated peramivir

At the time of undertaking this synthesis, peramivir was rather expensive to purchase at $350 / 1 mg from Euroasia. However, after over a year of attempting this synthesis the price
dramatically dropped to $295 / 1 g from DLChiral. Thus, it seemed more economical to purchase peramivir and compare the commercially available product to the synthesized de-guanidinylated peramivir 28.

3.3.0 Contribution of Peramivir's Guanidine toward the Inhibition of Neuraminidase

Peramivir (23a) and de-guanidinylated peramivir 28 were analyzed for their ability to inhibit viral neuraminidase using a soluble recombinant neuraminidase enzyme adapted from the influenza A/Brevig Mission/1/1918 (H1N1) and a native neuraminidase protein present in NP40-inactivated influenza A/Brisbane/59/2007 (H1N1).\textsuperscript{174,175} When tested against the recombinant virus using a ten minute pre-incubation period, compound 28 remained a relatively potent inhibitor of neuraminidase with an IC\textsubscript{50} of 18.39 ± 2.46 nM compared to peramivir which showed an IC\textsubscript{50} of 3.60 ± 0.35 nM (Figure 47). Using the inactivated virus and the same ten minute pre-incubation period, a lower IC\textsubscript{50} was observed for both compounds with peramivir and compound 28 showing an IC\textsubscript{50} of 0.82 ± 0.18 nM and 7.30 ± 1.32 nM, respectively. Zanamivir,\textsuperscript{176} oseltamivir,\textsuperscript{177} peramivir\textsuperscript{178} and other potent anti-neuraminidase compounds\textsuperscript{165} have been reported as slow-binding inhibitors. Therefore, comparison of peramivir and compound 28 was repeated utilizing a 2 hour pre-incubation period with the inactivated virus. This analysis could not be performed using the recombinant virus, as this particular synthesized protein was susceptible to decomposition over this time frame. A significant difference in inhibition was observed for the 2 hour pre-incubation period with peramivir and compound 28 showing an IC\textsubscript{50} of 0.13 ± 0.009 nM and 1.80 ± 0.83 nM, respectively. The observed decrease in IC\textsubscript{50} values from the longer incubation period suggests that both compounds act as slow-binders. Errors associated with the IC\textsubscript{50} values were determined using a 95% confidence interval for the fit of the curve (XLfit; sigmoidal model).
Figure 47. Inhibition data for peramivir (blue) and de-guanidinylated peramivir 28 (red). A) Dose-response curve for both compounds against recombinant viral neuraminidase. B) Dose-response curve for both compounds against inactivated influenza virus (10 min. pre-incubation). C) Dose-response curve for both compounds against inactivated influenza virus (2 hour pre-incubation). Error bars are equal to the standard deviation for each measurement.
The mechanism of inhibition for both peramivir and compound 28 were analyzed with the recombinant viral neuraminidase using a Michaelis-Menten plot (Figure 48a). Although the inactivated influenza virus should provide results more consistent with the literature, access to this protein was unavailable at the time of this particular analysis. Upon obtaining the inactivated virus, the synthesis of compound 28 was redone but unfortunately the [3+2] cycloaddition proved challenging (vide supra). Since the kinetic analysis requires a large amount of material, only the IC\textsubscript{50} values were completed with the inactivated influenza virus.

The Michaelis-Menten plot for both compounds shows a constant V\textsubscript{max} and an increasing apparent Michaelis-Menten constant (K\textsubscript{m,App}) over increasing inhibitor concentrations, thereby confirming a competitive mechanism of inhibition. The inhibition constant (K\textsubscript{i}) was determined following equation 6, where K\textsubscript{m} is the Michaelis-Menten constant in the absence of inhibitor, K\textsubscript{m,App} is the apparent Michaelis-Menten constant in the presence of inhibitor, [I] is the inhibitor concentration and K\textsubscript{i} is the inhibition constant.

\[
K_{m,App} = K_m \cdot \left\{1 + \left(\frac{[I]}{K_i}\right)\right\}
\]  

Plotting K\textsubscript{m,App} versus [inhibitor] affords K\textsubscript{i} from the x-intercept. K\textsubscript{m,App} was determined using non-linear regression analysis of the Michaelis data and the standard error was taken from a 95% confidence interval for the fit of the curve associated with the Michaelis-Menten plot. Since peramivir and compound 28 are highly potent, small errors associated with plotting the Michaelis data resulted in larger errors when determining the K\textsubscript{i} using a graphical representation of equation 6. Instead, K\textsubscript{m,App}, K\textsubscript{m} and [I] were directly used in equation 6 for different inhibitor concentrations and the resulting K\textsubscript{i} values were averaged. K\textsubscript{i} for peramivir was determined to be 1.68 ± 0.23 nM while the K\textsubscript{i} for compound 28 was determined to be 19.79 ± 4.21 nM. Errors associated with these values were obtained from the standard deviation of the measurement. To further confirm the competitive nature of these two molecules, a double-inverse Lineweaver-Burk plot was done where the lines associated with varying inhibitor concentrations should cross at a single point along the y-axis under competitive inhibition (Figure 48b).
Figure 48. Kinetic data for peramivir (blue) and de-guanidinylated peramivir 28 (red). A) Michaelis-Menten representation of kinetic data at various inhibitor concentrations. B) Lineweaver-Burk representation of kinetic data at various inhibitor concentrations. Inhibitor concentrations: circles – 0 nM inhibitor; triangles – 0.75 nM peramivir or 3.75 nM 28; squares – 1.5 nM peramivir or 7.5 nM 28; diamonds – 3 nM peramivir or 15 nM 28. Error bars not included.
An approximate 10-fold loss in potency was observed upon the de-guanidinylation of peramivir to produce compound 28 (Table 9). This result was surprising, since it was theorized that the contribution of the guanidine would more closely follow the observations for zanamivir (40-fold loss in potency). Since peramivir is epimeric at the guanidine-containing carbon center compared to zanamivir, it is possible that peramivir’s guanidine is binding to a slightly different position in the neuraminidase active site than is zanamivir’s. Even with a 10-fold decrease, compound 28 remains highly potent against the influenza virus. Considering the implications of this result for the design of next-generation neuraminidase inhibitors allows for some interesting analyses to be performed. First, a large number of hydrogen bond donors and acceptors present in the guanidine makes these particular functional groups a potential liability when designing orally available drugs, since they often exhibit difficulty in diffusing across the intestinal membrane. If the data for peramivir and compound 28 hold true for structurally-related inhibitors based on the cyclopentane scaffold, then removal of the guanidine group may be able to increase the oral availability of these inhibitors while maintaining high potency. At the very least, removal of the guanidine will free up hydrogen bond donors and acceptors from the S2 subsite interaction, so that the S4/S5 subsite interactions can be optimized to combat drug-resistance caused by the H274Y mutation. This will hopefully result in an increase in the inhibitor’s activity while still maintaining a desirable log P value for the overall molecule.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Peramivir</th>
<th>Compound 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$ versus recombinant soluble neuraminidase</td>
<td>3.60 ± 0.35 nM</td>
<td>18.39 ± 2.46 nM</td>
</tr>
<tr>
<td>IC$_{50}$ versus inactivated virus (10 min pre-incubation)</td>
<td>0.82 ± 0.18 nM</td>
<td>7.30 ± 1.32 nM</td>
</tr>
<tr>
<td>IC$_{50}$ versus inactivated virus (2 hours pre-incubation)</td>
<td>0.13 ± 0.009 nM</td>
<td>1.80 ± 0.83 nM</td>
</tr>
<tr>
<td>K$_{i}$ versus recombinant soluble neuraminidase</td>
<td>1.68 ± 0.23 nM</td>
<td>19.79 ± 4.21 nM</td>
</tr>
<tr>
<td>Calculated LogP</td>
<td>−1.37 ± 0.45</td>
<td>+0.33 ± 0.40</td>
</tr>
<tr>
<td>Hydrogen-bond acceptors</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Hydrogen-bond donors</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table 9. Summary of kinetic data for peramivir and compound 28**

As proof of concept, Michael Brant has synthesized compounds 144 and 145, whose K$_{i}$’s are 5 μM and 20 μM, respectively. Although these molecules lack the alkyl substituents (R) to
make them highly potent, these early results are even better than that predicted by peramivir’s case with only a 4-fold decrease in potency upon de-guanidinylation (Figure 49). Since the bicyclic scaffold overlays nicely with the cyclopentane scaffold of peramivir, these results give hope that the contribution of the guanidine will be relatively conserved across a variety of structurally-related neuraminidase inhibitors possessing either the cyclopentane or bicyclic scaffold.

![Figure 49. Comparison of guanidinylation of the bicyclic scaffold as synthesized by Michael Brant](image)

3.4.0 Synthesis of Deoxy Analogue

The deoxy analogue 29 was envisioned to come from the cleavage of the amide bond of lactam 135 followed by guanidinylation of the amine to give compound 146. Hydrogenation to both cleave the Cbz protecting groups and reduce the olefin, followed by hydrolysis should afford the desired analogue 29 (Scheme 27).

![Scheme 27. Retrosynthesis of the deoxy cyclopentane analogue](image)

Similarly to the peramivir synthesis, lactam 135 was heated to reflux for 24 hours in freshly prepared methanolic hydrogen chloride (Scheme 28). The resulting amino ester
intermediate was treated with Cbz-protected guanidine to afford compound 146 in near quantitative yield over two steps. Hydrogenation of compound 146 reduced the olefin and cleaved the Cbz protecting groups. Hydrolysis of compound 147 followed by neutralization afforded the desired deoxy analogue 29 which was used in the enzymatic assays without further purification.

![Scheme 28. Synthesis of the deoxy cyclopentane analogue](image)

### 3.5.0 Synthesis of α-Hydroxy Carboxylic Acid Analogue

The α-hydroxy carboxylic acid analogue 30 was envisioned to come from a conjugate addition of a protected (PG) amine onto 2-cyclopenten-1-one (148) followed by cyanation of the ketone to give compound 149. Deprotection to generate the free amine and subsequent guanidinylation was expected to provide compound 150. Finally, hydrolysis of the cyano group and hydrogenation to cleave the Cbz-protecting groups of compound 150 should afford α-hydroxy carboxylic acid 30 (Scheme 29).
Scheme 29. Retrosynthesis of the α-hydroxy carboxylic acid analogue

Starting from 2-cyclopenten-1-one (148), the installation of the amine was first attempted using a conjugate addition with potassium phthalimide (Scheme 30; entry 1) but this failed to yield any desired product, even upon heating to 50 ºC in dimethylformamide (entry 2). Next, the installation of an azide which could later be reduced to the amine was tried (entry 3), however, no desired product was observed. Attention was then turned to a palladium-catalyzed hydroamination with p-methoxybenzamide. However, due to solubility issues this reaction failed to generate any desired product (entry 4). Using the same conditions (entry 5), benzamide and compound 148 were heated neat to 60 ºC to provide compound 154. Although this reaction worked best on large scale, 2-cyclopenten-1-one is an expensive reagent. To decrease the volume of compound 148 needed, solvents such as tetrahydrofuran were used (entry 6) but this resulted in a decrease in yield. While a variety of other solvents were attempted along with variations in reaction time and temperature, most of these conditions failed to produce any desired product.
Cyanation of compound 154 with potassium cyanide under acidic conditions generated compound 149a in low yield and as a 1:1 mixture of diastereomers (Scheme 31). Efforts to increase the yield by heating to 40 °C proved futile and provided no desired product. With a small amount of compound 149a on hand, removal of the protecting group on the amine was attempted. While the reaction did provide compound 155, the molecule could not be adequately purified.

Scheme 30. Installation of the amine functionality for the α-hydroxy carboxylic acid analogue

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>potassium phthalimide, CH₃OH, 25 hrs</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>2</td>
<td>potassium phthalimide, DMF, 50 ºC, 25 hrs</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>3</td>
<td>NaN₃, conc. HCl, Et₃N, CH₂Cl₂, 24 hrs</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>4</td>
<td>p-methoxybenzamide, PdCl₂(PhCN)₂, 60 ºC, 24 hrs</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>5</td>
<td>benzamide, PdCl₂(PhCN)₂, 60 ºC, 24 hrs</td>
<td>154 62%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>benzamide, PdCl₂(PhCN)₂, THF, 60 ºC, 24 hrs</td>
<td>154 49%</td>
<td></td>
</tr>
</tbody>
</table>
Scheme 31. Cyanation and deprotection of the α-hydroxy carboxylic acid analogue

With such poor diastereoselectivity, a new and more elegant route was envisioned to start from the guanidinylated amine 146. Selenium dioxide can oxidize alpha to a carbonyl center, known as a Riley oxidation, as well as oxidize at the allylic position of an olefin. Compound 146 possesses two allylic positions with one also being alpha to a carbonyl. Therefore, it was theorized that a selenium oxidation would not only provide the desired regioselectivity but that the steric influence of the guanidinium group would also provide the correct stereoselectivity. Under standard conditions (Scheme 32; entry 1) only starting material was isolated. Next, attempts were made to switch to a more polar solvent, 1,4-dioxane, (entry 2) and was heated to reflux for several days (entry 3). Unfortunately, the reaction still failed to generate any desired product and only starting material was observed. It was hypothesized that the oxidation of

Scheme 32. Selenium oxidation conditions
compound 146 was too sterically encumbered for the reaction to take place due to the presence of the guanidinium group. Attempts to push the reaction with high temperatures in the microwave resulted in only a minor amount of desired product 156 (entry 4).

Although this reaction was unsuccessful for compound 146 due to steric factors, using a smaller Cbz protecting group on the amine (157) provided the α-hydroxy methyl ester 158 in good overall yield (Scheme 33). Hydrogenation of compound 158 reduced the olefin and also cleaved the carboxybenzoyl protecting group to generate compound 159. Attempts to

\[
\text{Scheme 33. Synthesis of analogue 30}
\]
directly guanidinylate compound 159 to give compound 161 were unsuccessful. Instead, the carboxybenzoyl-protected guanidine analogue 160 was generated followed by hydrogenation and hydrolysis to yield the desired α-hydroxy carboxylic acid analogue 30.

A 10-fold decrease in potency was observed upon de-guanidinylation of peramivir (23a) to provide compound 28. However, both of these molecules proved challenging to synthesize. It would prove interesting if the same trend was observed when using the simplified cyclopentane scaffold. If this were the case, then not only would this further validate the contribution of the guanidine (as shown previously), but it would also validate the use of this simplified model. The consequence of this would be to use this simplified structure to determine the contribution of other functionality about the ring and then apply this knowledge to the design of next-generation neuraminidase inhibitors based on either the cyclopentane or the bicyclic scaffolds. To confirm the observations seen for the contribution of the guanidine functional group in peramivir, amino ester 159 was hydrolysed to the α-hydroxy amino acid 132 and was compared to analogue 30.

3.6.0 Synthesis of β-Hydroxy Carboxylic Acid Analogue

Similarly to the synthesis of the α-hydroxy carboxylic acid 30, both the regiochemistry and the stereochemistry for analogue 31 were envisioned to be controlled by the steric influence of lactam 135. Hydroboration of lactam 135 was expected to provide alcohol 162, which should subsequently undergo methanolysis and guanidinylation to afford compound 163. Finally,

Scheme 34. Retrosynthesis of β-hydroxy carboxylic acid analogue
hydrogenation should cleave the carboxybenzoyl protecting groups, followed by hydrolysis to yield the desired analogue 31 (Scheme 34).

Hydroboration of compound 135 with borane-tetrahydrofuran complex resulted in no desired product, possibly due to the reduction of the amide followed by some undetermined side reaction (Scheme 35; entry 1). Borane is known to reduce an amide to an amine but this unwanted reaction was expected to occur at a slower rate compared to the oxidation of the olefin. A bulkier borane, 9-BBN, was used to control the regioselectivity of the reaction and to avoid any reduction of the amide, but it still failed to produce any product (entry 2). Increasing the duration of the reaction as well as the temperature also resulted in no desired product being isolated (entry 3 and entry 4). Next, a series of hydrosilations\textsuperscript{183} were attempted while varying both temperature and catalyst, but all failed to convert the starting material to the desired product. It was believed that the steric influence of the rigid bicycle 135 was the cause for these reactions failing to progress.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1. BH\textsubscript{3} • THF, THF 2. 10% NaOH, 30% H\textsubscript{2}O\textsubscript{2}</td>
<td>no desired product</td>
</tr>
<tr>
<td>2</td>
<td>1. 9-BBN, THF, 2 hrs 2. 10% NaOH, 30% H\textsubscript{2}O\textsubscript{2}</td>
<td>135</td>
</tr>
<tr>
<td>3</td>
<td>1. 9-BBN, THF, 24 hrs 2. 10% NaOH, 30% H\textsubscript{2}O\textsubscript{2}</td>
<td>135</td>
</tr>
<tr>
<td>4</td>
<td>1. 9-BBN, THF, \Delta, 24 hrs 2. 10% NaOH, 30% H\textsubscript{2}O\textsubscript{2}</td>
<td>135</td>
</tr>
<tr>
<td>5</td>
<td>Et\textsubscript{3}SiH, CH\textsubscript{2}Cl\textsubscript{2}, Karstedt's cat., 24 hrs</td>
<td>135</td>
</tr>
<tr>
<td>6</td>
<td>Et\textsubscript{3}SiH, CH\textsubscript{2}Cl\textsubscript{2}, Karstedt's cat., \Delta, 24 hrs</td>
<td>135</td>
</tr>
<tr>
<td>7</td>
<td>Et\textsubscript{3}SiH, CH\textsubscript{2}Cl\textsubscript{2}, B(C\textsubscript{6}F\textsubscript{5})\textsubscript{3}, 24 hrs</td>
<td>135</td>
</tr>
<tr>
<td>8</td>
<td>Et\textsubscript{3}SiH, Karstedt's cat., 24 hrs</td>
<td>135</td>
</tr>
</tbody>
</table>

Scheme 35. Attempts to install the β-hydroxy functional group using the steric influence of bicycle 135
The same series of conditions (Scheme 35; entries 1 through 8) were attempted using the previously synthesized compound 146 (Scheme 36; entries 1 through 6). The added advantages of this route were fewer steps to the target analogue 31 and the use of the protected-guanidine to help control the regioselectivity. Unfortunately, all attempts to install the β-hydroxy functional group resulted in either the recovery of starting material (entries 2 through 6) or no desired product (entry 1). A final effort was made trying both a green microwave-assisted\(^{184}\) addition of water (entry 7) and a more traditional hydrolysis (entry 8) but both provided no useful product. Thus, a new synthetic pathway toward analogue 31 was devised.

Scheme 36. Attempts to install the β-hydroxy functional group using a protected guanidine functional group

[Table]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1. BH(_3) • THF, THF 2.10% NaOH, 30% H(_2)O(_2)</td>
<td>no desired product</td>
</tr>
<tr>
<td>2</td>
<td>1. 9-BBN, THF, Δ, 24 hrs 2.10% NaOH, 30% H(_2)O(_2)</td>
<td>146</td>
</tr>
<tr>
<td>3</td>
<td>Et(_3)SiH, CH(_2)Cl(_2), Karstedt's cat., 24 hrs</td>
<td>146</td>
</tr>
<tr>
<td>4</td>
<td>Et(_3)SiH, CH(_2)Cl(_2), Karstedt's cat., Δ, 24 hrs</td>
<td>146</td>
</tr>
<tr>
<td>5</td>
<td>Et(_3)SiH, CH(_2)Cl(_2), B(C(_6)F(_5))(_3), 24 hrs</td>
<td>146</td>
</tr>
<tr>
<td>6</td>
<td>Et(_3)SiH, Karstedt's cat., 24 hrs</td>
<td>146</td>
</tr>
<tr>
<td>7</td>
<td>microwave 200 °C, 2 to 8 hrs, H(_2)O</td>
<td>no desired product</td>
</tr>
<tr>
<td>8</td>
<td>H(_2)SO(_4), H(_2)O, 3 hrs</td>
<td>no desired product</td>
</tr>
</tbody>
</table>

Starting once again from commercially available lactam 135, protection of the amide and stereoselective epoxidation of the olefin was envisioned to generate epoxide 164. Cleavage of the amide bond and protection of the primary alcohol followed by a regioselective opening of the epoxide could then provide access to compound 166. Selective oxidation of the primary alcohol
to the carboxylic acid and deprotection of the amine should afford amino acid 167. Finally, compound 167 will undergo guanidinylation with a protected guanidine source followed by hydrogenation to cleave the Cbz protecting groups and yield the desired analogue 31 (Scheme 37).

Scheme 37. Revised retrosynthesis of β-hydroxy carboxylic acid analogue

To reduce the number of synthetic steps, direct epoxidation of the bicyclic amide 135 was first attempted using oxone to produce compound 168 in modest yield and a 6:1 diastereomeric ratio. However, selective opening of the epoxide ring proved unsuccessful despite several attempts with various reducing agents (Scheme 38).
Alternatively, compound 135 was protected with di-tert-butyl dicarbonate, followed by epoxidation to yield compound 164 as a single diastereomer (Scheme 39). Ethanol-free chloroform (EFC) was used for both reactions, as trace amounts of ethanol have previously been
shown to decrease the yield. Next, selective cleavage of the amide bond with sodium borohydride gave alcohol 169, which was then protected to give silyl ether 165. It is noteworthy that opening of the lactam under these conditions does not affect the epoxide present in the molecule. A sterically bulky silyl ether was required so as to bias the regioselectivity during the lithium aluminum hydride-induced epoxide opening to generate diol 166. Fortunately, the silyl ether was also cleaved during this reaction. Unfortunately, efforts to selectively oxidize the primary alcohol to the aldehyde (Scheme 40; entries 1, 4 and 5) failed. Attempts to proceed directly to the carboxylic acid (entries 2 and 3) unexpectedly provided minor amounts of the desired aldehyde, although purification was a major issue due to competing decomposition. Any attempt to further optimize these procedures was unsuccessful.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SO₃•pyridine, Et₃N, CH₂Cl₂:(CH₃)₂SO (2:1)</td>
<td>no desired product</td>
</tr>
<tr>
<td>2</td>
<td>PDC, DMF, 24 hrs</td>
<td>minor 171</td>
</tr>
<tr>
<td>3</td>
<td>PtO₂, H₂O:EtOAc, (1:5), 40 °C</td>
<td>minor 171</td>
</tr>
<tr>
<td>4</td>
<td>oxalyl chloride, (CH₃)₂SO, Et₃N, CH₂Cl₂</td>
<td>no desired product</td>
</tr>
<tr>
<td>5</td>
<td>Dess-Martin periodinane, CH₂Cl₂</td>
<td>no desired product</td>
</tr>
</tbody>
</table>

Scheme 40. Selective oxidation of diol 166

With a lack of success from directly oxidizing the primary alcohol in compound 166, the diol was instead re-protected as a silyl ether to afford compound 172 (Scheme 41). Protection of the secondary alcohol with benzyl bromide generated no desired product (Scheme 41; entry 3); however, upon the in situ generation of benzyl iodide (entry 4) the unexpected bis-protected silyl ether 173 was obtained. Efforts to remove the Boc-protecting group under acidic conditions also resulted in the cleavage of the silyl ethers. Based on these results, bis-protection of diol 166 as
the \( p \)-methoxybenzyl ether (entry 1) or the acetal (entry 2) were tried, but both failed to produce any desired product.

\[
\begin{array}{ccc}
\text{BocHN}_2\text{OH} & \text{TBSCl, imidazole, DMF} & \text{BocHN}_2\text{OTBS} \\
166 & (93\%) & 172 \\
\end{array}
\]

**Conditions**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LiHMDS, THF, then PMBBr</td>
<td>no desired product</td>
</tr>
<tr>
<td>2</td>
<td>PMPCH(OMe)(_2), CSA, CH(_2)Cl(_2)</td>
<td>no desired product</td>
</tr>
</tbody>
</table>

**Scheme 41. Protection of diol 166**

Attention was turned to the cleavage of the Boc protecting group and guanidinylation of the subsequent amine prior to oxidation in the hope that this new molecule might yield better results and lead to the desired analogue 31 (Scheme 42). Cleavage of the Boc protecting group was accomplished using 1 M hydrochloric acid in ether to afford the amino hydrochloride salt which was taken crude and guanidinylated to afford 174. Using standard Swern oxidation conditions to generate the aldehyde at the primary alcohol (Scheme 42; entry 1), compound 174 failed to oxidize. Changing the order of addition had no effect on the reaction, while heating to reflux caused decomposition (entry 2). Using hypervalent iodine oxidizing reagents such as DMP (entry 3) and IBX (entry 4) to produce the aldehyde resulted in decomposition via a side reaction pathway and recovered starting material, respectively. Next, chromium-based reagents were used with PDC (entry 5) and DCC (entry 7) failing to convert to product and the harsh conditions of chromium (VI) oxide (entry 8) resulting in decomposition. PCC oxidation (entry 6)
**Scheme 42.** Oxidation of the Cbz-protected guanidine diol
provided a minor amount of keto-acid but the product was difficult to isolate and purify from the chromium by-product species. A Parikh-Doering oxidation (entry 9) also failed to produce any product. Efforts to use radical-based oxidations such as TEMPO (entries 10 to 13) all resulted in the recovery of starting material. Likewise, N-methylmorpholine oxide (NMO) (entry 14) and platinum (IV) oxide (entry 15) oxidations provided only recovered starting material, as did the attempted oxidation using oxone (entry 16).

As has been the case with previous analogue syntheses involving the Cbz-protected guanidine, the issue of a steric influence from the bulky protecting groups was proposed as the problem with these oxidations. To validate this claim, compound 174 was hydrogenated to provide guanidinylated diol 175, which was subsequently subjected to a select set of the most promising oxidizing conditions (Scheme 43). Unfortunately, none of these conditions provided any desired product.

![Scheme 43. Oxidation of the unprotected guanidine diol](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TEMPO, N-chlorosuccinimide, Bu₄NCl, pH 8.6, CH₂Cl₂</td>
<td>175</td>
</tr>
<tr>
<td>2</td>
<td>IBX, CH₃CN</td>
<td>175</td>
</tr>
<tr>
<td>3</td>
<td>oxalyl chloride, (CH₃)₂SO, Et₃N, CH₂Cl₂</td>
<td>no desired product</td>
</tr>
</tbody>
</table>

Finally, the order of reactions was altered so that the oxidation of the primary alcohol in compound 170 was oxidized prior to the opening of the epoxide ring (Scheme 44). As was the case with the other oxidation attempts (vide supra), these too failed to provide any desired product, while the minor amount of aldehyde isolated from the Swern oxidation (Scheme 44; entry 3) could not be reproduced. It was hoped that the aldehyde could be converted to the acid
and esterified prior to the epoxide opening and then de-esterified afterwards. Unfortunately the β-hydroxy carboxylic acid could not be synthesized and remained elusive, albeit not from a lack of effort.

![Scheme 44](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TEMPO, N-chlorosuccinimide, Bu₄NCl, pH 8.6, CH₂Cl₂</td>
<td>no desired product</td>
</tr>
<tr>
<td>2</td>
<td>IBX, CH₃CN</td>
<td>170</td>
</tr>
<tr>
<td>3</td>
<td>oxaly chloride, (CH₃)₂SO, Et₃N, CH₂Cl₂</td>
<td>minor 176</td>
</tr>
<tr>
<td>4</td>
<td>CrO₃, H₂SO₄ (aq), acetone</td>
<td>no desired product</td>
</tr>
<tr>
<td>5</td>
<td>PDC, DMF, 24 hrs</td>
<td>no desired product</td>
</tr>
</tbody>
</table>

**Scheme 44.** Oxidation attempts for compound 170

### 3.7.0 Contribution of Peramivir's Hydroxyl Group toward the Inhibition of Neuraminidase

Compounds 29 (deoxy), 30 (α-hydroxy acid), 132 (de-guanidinylated α-hydroxy acid) and 175 (guanidine diol) were analyzed against a recombinant soluble neuraminidase protein (Figure 50). Compound 29 had an IC₅₀ of 2.06 ± 0.33 mM while compound 30 had an IC₅₀ of 4.05 ± 0.60 mM. This data suggests that no major contribution was gained upon adding the hydroxyl group to the alpha position. Unfortunately, the β-hydroxy carboxylic acid analogue 31 could not be synthesized, but using the data by Chand⁹⁴ for peramivir (23a) and deoxy peramivir (24a) the overall contribution of the alcohol would seem to be minor at best. Therefore, when designing new neuraminidase inhibitors based on the cyclopentane scaffold, deletion of the hydroxyl group should allow easier synthetic access to more complicated inhibitors while sacrificing little to no potency. Furthermore, removal of the hydroxyl group will provide the ability to install more polar functional groups that can interact with the S₄/S₅ subsite. This will
hopefully lead to a reduction in H274Y mutation-induced drug resistance, while maintaining a desirable log P and possibly increasing oral availability.

Similarly to peramivir and compound 28, compounds 30 and 132 were compared to further validate the effect of the guanidine functional group. Compound 132 gave an IC$_{50}$ of 18.74 ± 5.78 mM, a roughly 5-fold decrease in potency compared to 30 (Table 10). The guanidine contribution has thus been analyzed using peramivir, a simplified cyclopentane

![Figure 50](image).

**Figure 50.** Inhibition data analyzed against a recombinant soluble neuraminidase protein. A) Dose-response curve for compound 29 (brown). B) Dose-response curve for compound 30 (green). C) Dose-response curve for compound 132 (purple). D) Dose-response curve for compound 175 (light blue). Error bars are equal to the standard deviation for each measurement.
scaffold and the bicyclic scaffold, showing an increase in potency toward neuraminidase by ≤ 1 order of magnitude. These results are encouraging as our simplified structure has been validated and can now be used to determine the contributions of other potential functional groups.

Finally, since the β-hydroxy analogue 31 proved difficult to synthesize, the intermediate 175 was tested but provided an uninspiring IC\textsubscript{50} of 35.40 ± 16.34 mM. Errors associated with the IC\textsubscript{50} values were determined using a 95% confidence interval for the fit of the curve (XLfit; sigmoidal model).

<table>
<thead>
<tr>
<th>Properties</th>
<th>Compound 29</th>
<th>Compound 30</th>
<th>Compound 132</th>
<th>Compound 175</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC\textsubscript{50} versus recombinant soluble neuraminidase (mM)</td>
<td>2.06 ± 0.33</td>
<td>4.05 ± 0.60</td>
<td>18.74 ± 5.78</td>
<td>35.40 ± 16.34</td>
</tr>
</tbody>
</table>

*Table 10. Summary of kinetic data for compounds 29, 30, 132 and 175*

### 3.8.0 Future Work

Comparison of peramivir and compound 28 as well as the comparison of compound 30 and compound 132 have shown that the guanidine functional group provides approximately 5 to 10 fold increase in potency. These results were further emphasized by compounds 144 and 145 (synthesized by Michael Brant) which showed a 4-fold increase in potency upon guanidinyating the amine in the bicyclic scaffold. Drug molecules containing a large number of hydrogen bond donors and acceptors are less likely to be orally active as they suffer from poor permeability across the intestinal membrane. If next-generation neuraminidase inhibitors based on either the cyclopentane or the bicyclic scaffolds are observed to be highly active, then sacrificing 5 to 10 fold in potency may be an acceptable loss if oral availability is gained. Moreover, removal of the guanidine group may be offset by a gain in activity from the installation of hydrogen bonding groups elsewhere in the molecule to target the S4/S5 subsite of the H274Y mutant. Knowing the contribution of the guanidine now allows future designs to become more open and not as limited in scope to what key functional groups have to be present in order to obtain subnanomolar potency.

Preliminary data comparing compounds 29 and 30 as well as previous studies by Chand\textsuperscript{94} have shown that the hydroxyl group offers very little toward the activity of either peramivir or the simplified scaffold against neuraminidase. While further work is needed in synthesizing the
β-hydroxy analogue to utterly confirm whether the complete removal of the hydroxyl group will have any significant effect on inhibitor activity, it would seem that for the time being future designs involving the structurally-related scaffolds would do well to avoid intentionally installing the hydroxyl group.

With information gathered for the contributions associated with the guanidine, carboxylic acid, hydroxyl group and the $N$-acetyl group, attention should now be turned to the alkyl functional group. Based on the results obtained from the simplified scaffold, this cyclopentane should continue to be used to determine the overall effect of various alkyl substituents toward the inhibition of the neuraminidase enzyme. Work toward the alkyl group contributions is currently underway in our group, which should ultimately lead to new and exciting designs for novel next-generation neuraminidase inhibitors.
Chapter 4 – Synthesis of the C-6 Methyl Didemnaketal A Analogue

4.1.0 Introduction

Didemnaketal A (32) and B (33) are degradation products of didemnaketal C (34) and have been shown to inhibit HIV-1 protease with an IC\textsubscript{50} of 2 μM and 10 μM, respectively (Figure 51).\textsuperscript{121} Interestingly, the natural product didemnaketal C has no activity against HIV-1 protease. In 1998, the Rich group synthesized a series of simplified analogues corresponding to either the C-8 to C-23 eastern half or the C-1 to C-11 western half of didemnaketal A.\textsuperscript{124} Since the relative stereochemistry of didemnaketal A was unknown at the time, the ambiguous C-6, C-10, C-14 and C-18 methyl groups were deleted from Rich’s structures for simplicity. Rich’s initial hypothesis was that since most potent competitive HIV protease inhibitors contain a free hydroxyl group to interact with the aspartyl active site, the spiroketal eastern fragment would likely be the cause of the inhibition seen for the natural product. However, none of the C-8 to C-23 analogues synthesized by Rich were active against HIV-1 protease. Instead, attention was

![Figure 51. A) Structures of didemnaketal A, B and C. B) Rich's most potent analogue](image-url)
turned to the pentaester western fragment. Rich’s most potent analogue (39) showed a $K_i$ of 2.1 μM, which was comparable to the activity reported for didemnaketal A. Interestingly, this particular molecule is epimeric at the C-8 carbon compared to the natural product (Figure 51). In fact, the analogue possessing the same stereochemistry as the natural product showed a $K_i$ of 10 μM, which was comparable to the activity reported for didemnaketal B. To confirm that the activity of these analogues did not require a free hydroxyl group, Rich synthesized the de-esterified C-1 to C-11 analogues and observed that none were active. Using activity assays developed by Zhang,129 Rich showed that his most potent analogue 39 inhibited HIV-1 protease via a dissociative pathway.

Current HIV protease drugs function through competitive inhibition by mimicking the tetrahedral intermediate that occurs upon hydrolysis of the natural substrate. Initially, these inhibitors have a dramatic effect, lowering the virus load to less than 1% of pre-treatment levels.187-189 Unfortunately, the therapeutic benefit of these drugs is short-lived due to rapid viral resistance caused by mutations.190,191 The primary route by which these retroviruses develop resistance is thought to occur during transcription of the single-stranded RNA by reverse transcriptase.190 DNA polymerase does not possess a proofreading function. Therefore, mismatches in base pairing leads to errors in the viral DNA which becomes integrated to form the proviral DNA, serving as a template for all new viral transcripts and passing along any mutations. In a short period of time, a large set of genetic mutations can accumulate, which is further made possible by large pools of infected T-cells with an extremely rapid turnover. Coffin calculated that approximately $10^9$ new cells were infected each day in a typical HIV patient during the latent or steady-state stages of infection.192 If each point mutation that could occur along the viral genome did occur, then the frequency of these mutations would be between $10^4$ and $10^5$ times a day.192 These arbitrary mutations can be further compounded by serial monotherapy. In the presence of a drug selecting for a certain type of mutation present in a protein, random mutations can lead to a higher probability of encoding new mutants which develop a reduced affinity for the inhibitor while retaining enough enzymatic activity to still interact with the substrate.193-196 The use of antiviral cocktails has been shown to reduce these effects, although recent studies have reported the isolation of latent infective reservoirs of HIV in some patients currently using multi-drug therapy.197 This problem generally arises from the inadequate suppression of virus replication using suboptimal treatments.198 Furthermore, high
mutation rates can also arise from the lack of adherence to complex and toxic regimes as well as treatment of HIV in later stages.  

Another pathway leading to drug resistance involves person-to-person transmission of a mutant HIV protease that is already resistant to common HIV drugs. While this pathway is a cause of great concern, passive mutations of the retrovirus, especially during drug administration, remain the target of many researchers. Even more alarming, multi-drug resistance has been detected whereby distal mutations act in a cooperating manner. Drug resistance was shown by Freire to be minimal for either active site, flap, or dimer interface submutants, however, a combination of all three mutations lowered the activity of common HIV protease drugs by 2 to 3 orders of magnitude. Furthermore, investigation of HIV protease resistant to ritonavir showed an ordered accumulation of mutations, suggesting that early detection and therapy might help to prolong viral suppression. With significant structural similarity among protease inhibiting drugs and the high mutation rates of the retrovirus leading to multi-drug resistance, it would be prudent to search for new inhibitors of HIV protease that are less sensitive to mutations.

HIV-1 protease is comprised of twin monomers that form the active dimer through interdigitation of β-strands from the N- and C-termini for each monomer, thus forming an antiparallel β-sheet. Structure-based thermodynamic modeling suggests that the terminal amino residues Pro1, Ile3 and Leu5 along with the carboxyl end residues Cys95, Thr96, Leu97, Asn98, and Phe99 constitute 75% of the total binding energy for dimerization. While most drug resistance occurs from a series of point mutations in either the active site or the structural loops (also known as the flaps), the dimer interface region is generally mutation free and is highly conserved among the mutant variants when compared to the wild-type enzyme. Therefore, the design of new HIV protease inhibitors would do well to target the disruption of the dimer and/or bind to the individual monomers. Early work into dissociative inhibitors focused on using truncated peptides mimicking either the N- or C-terminal residues. These ideas were further developed to bind two peptides with either flexible or rigid linkers to form “molecular tongs”. The activity of these inhibitors was shown to increase when applying a more rigid conformation resulting from a minimum change in entropy upon binding to the protein.
The stability of the HIV protease dimer is strongly temperature and pH dependent. The dissociation constant, $K_d$, has been reported as ranging from 9 nM to 130 nM at pH 6.0 and 37 °C depending on the type of analyses used.\textsuperscript{126,127,129,218,224,225} Higher temperatures increase the dissociation of the dimer into its subsequent monomers while low pH helps to retain the dimeric form. In calculating the dissociation constant, Zhang assumed a rapid equilibrium between the monomer and the dimer.\textsuperscript{129} However, work by Darke suggests that the equilibrium is much slower.\textsuperscript{224} To overcome the slow dissociation of the dimer to its subsequent monomers as proposed by Darke, a longer incubation time (> 1 hour) at 37 °C can be utilized when performing the kinetic analysis. Although the monomers are generally perceived as the main targets due to the low nanomolar dissociation constant (i.e. dimer is tightly bound), work by Louis using size exclusion chromatography coupled with multiangle light scattering and refractive index measurements (SMR) showed ~15% of their antibodies were targeting the dimeric form of HIV-2 protease.\textsuperscript{217}

Rich’s analysis has already shown that the pentaester analogues of didemnaketal A act to inhibit HIV-1 protease via a dissociative pathway.\textsuperscript{124} However, these western fragment analogues of didemnaketal A are not ideal drug candidates to be used in an antiviral cocktail due to the lability of the esters. Instead, they should provide an excellent probe with which to understand the key interactions required for dissociative inhibition of HIV-1 protease by non-peptidic molecules. This in turn will provide new insights into designing novel classes of HIV protease inhibitors that act through a dissociative pathway. Ultimately, since the active site of HIV-1 protease is present only in the dimeric form, a dissociative inhibitor could be used to block the formation of the dimer resulting in an absence of the active site. Drugs that function through dissociative inhibition could then be combined with drugs that function through active-site inhibition and would reduce the likelihood of drug resistance through structural mutations.

To better understand how these molecules function, a series of analogues based on the western half of the natural product, didemnaketal A, will be synthesized and their activities monitored by Zhang plots (Figure 52).\textsuperscript{129} In particular, three questions regarding rigidity, stereochemistry and ester functionality will be addressed.
If these molecules are indeed acting as β-strand mimics during the inhibition of HIV-1 protease, then the rigidity of these compounds might play an important role in their potency. Rich’s analogues lacked the C-6 and C-10 methyl groups present in the natural product. It is our proposal that installation of either the C-6 methyl group (105) or the C-6 and C-10 methyl groups (106) will increase the potency of these molecules toward the target enzyme by decreasing the number of conformational rotomers available. By synthesizing both compounds 105 and 106, the contribution of each methyl group can be evaluated.

Rich’s most potent analogue 39 is epimeric at the C-8 position compared to the natural product. Since neither the ascidian nor the bacteria living amongst it have had direct encounters with HIV, it is not surprising that the didemnaketal structures could be optimized to increase their potency against HIV-1 protease. An interesting question would be how the stereochemistry of the molecule would affect the overall potency. To address this question, both enantiomers of the above compounds (Figure 52) will be synthesized and their activities compared to the analogues possessing the natural stereochemistry. Furthermore, during the design of these molecules, each stereocenter will be constructed so that its epimer can easily be accessed through similar chemistry. This will allow future analogues to encompass a wide range of potential diastereomers, providing a better outlook on what is required for future dissociative HIV protease inhibitors.

Figure 52. Proposed didemnaketal A analogues
Finally, as stated above, the didemnaketal analogue is an unoptimized structure. Therefore, while esterification of the polyol is required, it is unknown what particular types of esters are needed at the C-5, C-7, C-8 and C-11 positions. To probe this, compounds 177 and 178 will be synthesized and their activities compared to structures 105 and 106, respectively, possessing the natural product esters.

The synthesis of the western half of the natural product, didemnaketal A, and the C6/C10 methyl analogue (106) is derived from a common intermediate. Therefore, the methodology toward both products will be discussed later on in chapter 5. In the meantime, the route to the C-6 methyl analogue (105) was envisioned to proceed with an Evans aldol reaction\textsuperscript{226-228} to install the desired \textit{syn} stereochemistry at the C-6 and C-7 positions. Reduction of the aldol product to cleave the chiral auxiliary followed by a 1,3-diol protection and subsequent oxidation should provide aldehyde 182 (Scheme 45). A substrate-controlled allylation of aldehyde 182 should generate compound 103 with the desired \textit{syn} C-8 stereochemistry. Hydroboration of the olefin and di-esterification with isovaleric anhydride followed by functional group

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{scheme45}
\caption{Retrosynthesis of the C-6 methyl didemnaketal A analogue}
\end{scheme}
interconversion should lead to intermediate 104. Finally, an asymmetric allylation\textsuperscript{229} to set the C-5 stereochemistry, esterification with propionic anhydride and a stereoselective Grubbs cross metathesis\textsuperscript{230} with methyl methacrylate should yield the desired C-6 methyl analogue 105.

4.2.0 Control of the C-6 and C-7 Stereochemistry

4.2.1 Determination of Stereochemistry for the Stereoselective Evans Aldol

The aldol reaction is a powerful method for generating carbon-carbon bonds and typically involves the nucleophilic addition of a ketone enolate to an aldehyde. The Zimmerman-Traxler model was originally developed to predict the outcome of a Reformatsky reaction but has been adopted to predict the outcome for aldol reactions using a six-membered transition state formed by a chelating metal such as lithium or boron (Scheme 46).\textsuperscript{231} During deprotonation there are two possible enolates, $E$ or $Z$. The $E$-enolate will lead to 1,2-\textit{anti} stereochemistry in the product while the $Z$-enolate will give \textit{syn} stereochemistry.\textsuperscript{231} The stereochemical control comes from placing substituents in the equatorial position and reducing the number of 1,3-diaxial interactions in the concerted transition state. Although this model is useful, only some metals such as lithium and boron reliably follow this pathway.\textsuperscript{231} Dialkylboron triflates typically afford the $Z$-enolate with little sensitivity toward the amine used or the sterics of the alkyl groups on the boron reagent. In the case of dialkylboron chlorides, the geometry of the product enolate is much more sensitive to variations in the amine and to the alkyl groups on the boron. However, the use of (c-hex)$_2$BCl and triethylamine (Et$_3$N) provides preferentially the $E$-enolate.\textsuperscript{231}
Scheme 46. Zimmerman-Traxler model to predict the relative stereochemical outcome of an aldol reaction
To better understand the specificity of the protein for the didemnakineta A analogues, an enantioselective approach is required. Since a standard aldol creates two stereocenters, there are four possible stereoisomers. Therefore, to control both relative stereochemistry (syn versus anti) and absolute stereochemistry (R versus S) the widely used method involving Evans’ acyl oxazolidinone was used. Boron-mediated soft enolization reliably forms the Z-enolate (>100:1 Z:E). Boron can coordinate with both carbonyls of the imide which positions the acyl methyl group gauche to the carbonyl to avoid steric interactions with both the chiral carbon center possessing the benzyl group on the oxazolidinone and the alkyl group on boron (Scheme 47). This will therefore generate the Z-enolate.

Scheme 47. Evans chiral auxiliary and generation of the Z-enolate
An open coordination site is required for the concerted aldol reaction (Scheme 48). Nucleophilic attack of the enolate on the aldehyde can occur from the *Re* or the *Si* face. Attack on the *Re* face places the benzyl group back and away from the six-membered transition state.

**Scheme 48.** Evans aldol transition state generating a single enantiomer
making it highly favourable. In contrast, attack from the Si face will position the benzyl group above the cyclic transition state causing steric interactions and thus makes it unfavourable. Use of this chiral auxiliary will therefore generate a single enantiomer.

4.2.2 Preparation of Compound 190 using an Evans Isopropyl Oxazolidinone

When designing the methodology for the synthesis of the didemnaketal A analogues, the cheaper L-valine was used. Although this will generate the enantiomer of the natural product scaffold, a repetition of the known reaction conditions starting with D-valine will provide the correct scaffold and allow the testing of both enantiomers against HIV-1 protease.

The chiral auxiliary ent-179 required for the Evans’ aldol reaction is commercially available but rather expensive. Since a large quantity was required for this synthesis, the cheaper commercially available L-valine (ent-183) was reduced to form the known amino alcohol ent-184 in quantitative yield (Scheme 49). Formation of the Evans auxiliary using di-tert-butyl dicarbonate (Boc₂O) proved unsuccessful; however the use of diethyl carbonate along with in situ generation of sodium ethoxide gave compound ent-185. Reaction of the oxazolidinone ent-185 with propionyl chloride reliably yielded the desired chiral imide ent-179 on multi-gram scale.

Scheme 49. Synthesis of Evans chiral imide ent-179
The Evans aldol reaction to install the 1,2-syn C-6 and C-7 stereochemistry was originally envisioned to proceed using aldehyde 180. The generation of aldehyde 180 began with mono-protecting glycerol (186) with tert-butylidemethylsilyl chloride (TBSCl) followed by oxidation using Swern conditions (Scheme 50).\textsuperscript{233} Unfortunately, the aldehyde was unstable to purification and only a well optimized copper sulphate work-up allowed the crude aldehyde 180 to be used in the subsequent aldol reaction. Somewhat later in the project, further testing of other known oxidation conditions revealed Dess-Martin periodinane (DMP) as a successful replacement for generating the desired aldehyde 180. Not only did it increase the yield, but it also improved the purity of the aldehyde.

\begin{center}
\textbf{Scheme 50. Synthesis of aldehyde 180}
\end{center}

The outcome for the Evans aldol reaction is predicted to be the desired C-6/C-7 syn stereochemistry when using dibutylboron triflate. However, unforeseen circumstances arose when trying to isolate the final aldol product ent-192. While the crude organoborane intermediate ent-188 could be isolated, standard work-up conditions involving hydrogen peroxide and base to remove the borane resulted in a retro-aldol and regeneration of the starting material ent-179 (Scheme 51). Moreover, direct purification of intermediate ent-188 also resulted in a retro-aldol. Serendipitously, the subsequent step to cleave the chiral auxiliary using lithium borohydride provided an organoborane species ent-189 nicely cyclized into a six-membered ring. Using this structure, the stereochemical outcome for the aldol reaction was confirmed using NOESY spectroscopy and coupling constants (as will be discussed later). The cleavage of the borane to generate the diol intermediate ent-190 could now be accomplished, although the resulting product was unstable during purification. In an attempt to avoid protecting groups, the primary alcohol of diol ent-190 was selectively oxidized in the presence of the secondary alcohol using

149
DMP followed by esterification with acetic anhydride to produce compound ent-191. While proving successful, compound ent-191 was unfortunately also unstable during purification. Due to the high sensitivity of these molecules and the inability to carry the crude products through numerous steps, a revised synthetic design was investigated.

![Scheme 51. Synthesis of the intermediate diol ent-190](image)

Problems associated with building the C-5 through C-8 carbon skeleton arose mainly from the sensitivity of aldehyde 180. While scanning the literature, acrolein (181) was found to be a suitable aldehyde replacement for use in the Evans aldol reaction. First, since the aldol product ent-193 is a known compound, comparison of the spectroscopic data with the literature would provide confirmation for the correct syn stereochemistry. Secondly, acrolein contains an alkene which can be oxidized at a later stage, thus only minor revisions were needed when redesigning the synthesis of the C-6 methyl analogue. Using the same chiral auxiliary ent-179, the Evans aldol reaction with acrolein proceeded in quantitative yield (Scheme 52). However,
alcohol, which was then reduced using lithium borohydride to give the desired diol. Attempts to use this method to prepare the title compound resulted in a mixture of diastereomers and other by-products (to be discussed further on). Since the starting material was contaminated with auxiliary, it was not known whether these issues arose from the reactions themselves or from the contaminant auxiliary.

Scheme 52. Synthesis of diol 194 using Evans isopropyl auxiliary and acrolein

4.2.3 New Approach toward the Preparation of the Carbon Skeleton C-5 to C-8

While the Evans aldol reaction provided the desired stereochemistry at the C-6 and C-7 positions, purification issues made this synthetic route far more challenging than was originally hypothesized. Therefore, two completely new routes for the synthesis of the C-5 to C-8 fragment were investigated. The first route involved the use of maleic acid (195) which has the alcohols for the C-5 to C-8 fragment already installed. For the sake of methodology, the racemate was used with the notion that the correct enantiomer could be purchased later. Esterification of acid 195 followed by α-methylation yielded compound 197 as a single diastereomer, albeit with the
1,2-anti stereochemistry (Scheme 53). This was not a concern, since a Mitsunobu reaction\textsuperscript{153} can be used to invert the stereochemistry at the C-8 position when installing the ester functionality, thus providing the required 1,2-syn product. Reduction of the bis-ethyl ester 197 to triol 198 was met with isolation issues as unsurprisingly the molecule proved to be rather polar. With no successful way to cleanly isolate the desired product 198, this methodology was abandoned.

Scheme 53. Synthesis of the C-5 to C-8 fragment of the C-6 methyl analogue using DL-maleic acid

The second proposal to establish the C-5 to C-8 stereochemistry for the C-6 methyl analogue was envisioned to proceed with the epoxidation of known cis-2-butene-1,4-diol (199; or protected form 202 or 205) using meta-chloroperoxybenzoic acid (mCPBA) to generate epoxide 200 (or 203 or 206). Opening of the epoxide ring with methyl magnesium bromide only succeeded with the protected diol to give compound 204 (or 207) with the desired 1,2-syn stereochemistry (Scheme 54). When the diol was unprotected (compound 200), isolation of the resulting triol 201 proved to be as much of a challenge as it was for the maleic acid synthetic route (Scheme 53). Protection of diol 199 with either a benzyl (Bn) or a TBS group, prior to epoxidation and methylation, simplified purification. However, the subsequent deprotection step gave either starting material (as in the case of 204) or the resulting triol was too polar to isolate during an aqueous workup (as in the case of 207).

While there was hope that these methods would be an improvement compared to the Evans aldol reaction, the use of the Evans auxiliary seemed to be the better choice. Therefore, a
new investigation was undertaken to try and improve upon the original issues associated with purification when using the valine-derived auxiliary.

**Scheme 54.** Synthesis of the C-5 to C-8 fragment of the C-6 methyl analogue using *cis*-2-butene-1,4-diol

4.2.4 Preparation of Compound 194 using Evans' Benzyl Oxazolidinone

When choosing a chiral auxiliary, the functional group at the chiral center of the oxazolidinone is used to bias the diastereoselectivity during the aldol reaction. Since such selectivity had not been an issue when using the valine-derived oxazolidinone 185, no other chiral auxiliaries were tried. However, in light of the purification issues discussed above, a search of the literature revealed phenylalanine-derived oxazolidinone 210 as a popular choice when doing an Evans aldol reaction. It was hypothesized that the addition of the aromatic substituent would create enough of a difference in polarity between auxiliary 210 and diol 194 to allow an adequate separation during purification. Similarly to before, chiral auxiliary 97 is commercially available but can be made on multigram scale cheaply. Therefore, compound 97 was generated from the cheaper commercially available amino acid 208 in near quantitative yield (Scheme 55). Since access to both enantiomers of the C-6 methyl analogue was required, both *D-*
and L-phenylalanine were used in developing this methodology. The reaction schemes shown below will depict the natural product stereochemistry starting from D-phenylalanine.

Following a similar protocol used to access auxiliary 179, compound 97 was generated in near quantitative yield over three steps (Scheme 55). A procedural change was required when going from amino alcohol 209 to auxiliary 210, as the in situ generation of sodium ethoxide (as used before) resulted in low product yields due to inadequate stirring from the gelling of the reaction mixture. Changing the base from sodium ethoxide to potassium carbonate proved very successful. As before, the aldol product 211 generated using the chiral auxiliary 97 and acrolein was not purified due to problems with a retro-aldol reaction. Again this was believed to be caused by the type of silica gel being used during purification. Fortunately, the desired molecule 211 was obtained without any need for purification using the same work-up procedure optimized during the synthesis of the valine-derived aldol product 193. Finally, cleavage of the chiral auxiliary from aldol product 211 under reductive conditions yielded diol 194. As predicted, separation of diol 194 from auxiliary 210 (while far from trivial) was attainable. Thus, the C-6 and C-7 stereochemistry was installed and access to diol 194 could now be performed on large scale (starting with 50 grams of phenylalanine 208).

Scheme 55. Synthesis of diol 194 using a chiral auxiliary derived from phenylalanine

4.2.5 Confirmation of the C-6 and C-7 Stereochemistry

To better understand the specificity of HIV-1 protease toward our dissociative inhibitors, an enantioselective synthesis of our analogues is required. To confirm the stereochemistry at the
C-6 and C-7 position, three compounds were used. First, diol 194 is a known compound, and comparison of its data to both the literature spectroscopic data (to confirm diastereoselectivity) and the optical rotation (to confirm enantioselectivity) verified that the correct product had been obtained.234

Secondly, since organoborane ent-189 had been synthesized during the initial aldol attempts (Scheme 51), its structure was used to further elucidate the diastereoselectivity of the Evans aldol reaction. Since the Evans aldol reaction is known to be diastereoselective, there are two plausible diastereomers for compound ent-189 (R,S and R,R; Figure 53). The R,S configuration corresponds to the expected 1,2-syn product, while the R,R configuration corresponds to the 1,2-anti product. The R,R configuration can exist as one of two chair conformations in solution. The first conformation would be expected to show two axial proton interactions. However, since there was no axial-axial proton coupling interactions observed in the ¹H NMR, the methyl group at the C-6 position had to be axial instead of the proton. For the R,S configuration and the other R,R chair conformation, axial-equatorial and equatorial-equatorial proton interactions were expected and were observed with coupling constants of 4.2 Hz and 6.2 Hz. To differentiate between these two structures, an observable NOE interaction

![Diastereoselectivity confirmation using organoborane ent-189. NOE interactions are shown in red. Coupling constants are shown in blue.](image)

**Figure 53.** Diastereoselectivity confirmation using organoborane ent-189. NOE interactions are shown in red. Coupling constants are shown in blue.
between the methyl group and the –CH₂OTBS protons would be expected for the \textit{R,S} configuration but not for the \textit{R,R} configuration. This key NOE interaction further confirmed that the correct 1,2-syn product \textit{(R,S)} had been obtained.

Finally, the \textit{p}-methoxybenzylidene acetal 98 was generated as a useful precursor toward the synthesis of analogue 105 (see section 4.3.1) and was used to confirm the stereochemistry at the C-6 and C-7 positions (Figure 54). While a dimethyl ketal might have been advantageous by avoiding the introduction of a third chiral center, it was unfortunately too volatile to isolate. Compound 98 is a known compound, and therefore its data was compared to both the literature spectroscopic data and optical rotation to again verify the 1,2-syn stereochemistry. Further evaluation involved the observation of NOE interactions between the three axial protons, helping to fully authenticate the structure. Thus, with two known literature compounds and two fully characterized molecules using 2-D NMR spectroscopy, it is confidently claimed that the stereocenters at the C-6 and C-7 positions are correct.

\textbf{Figure 54.} Confirmation of diastereoselectivity using compound 98. NOE interactions are shown in red.

\textbf{4.3.0 Control of the C-8 Stereochemistry}

\textbf{4.3.1 Installation of the C-8 Stereochemistry using a Substrate-controlled Allylation}

The first approach toward controlling the stereochemistry at the C-8 position involved the selective protection of diol ent-194 (derived from aldol product ent-193). The primary alcohol of diol ent-194 was selectively protected with \textit{tert}-butyldimethylsilyl chloride to provide compound ent-212, but subsequent esterification or oxidation led to decomposition of compounds ent-213
and ent-214, respectively (Scheme 56). To avoid these issues, compound ent-194 was fully protected to afford compound ent-215 and then subsequently oxidized to give aldehyde ent-216.

Scheme 56. Monoprotection of diol ent-194

Scheme 57. Allylation of bis-TBS protected diol ent-216

Quenching of the ozonide was done with a variety of reagents in order to avoid purification since aldehyde ent-216 was unstable. Of the reagents, both triethylamine and trimethylphosphine provided the highest yields and purity. Due to the decomposition of compound ent-216 during
purification, the aldehyde was taken on in crude form for the subsequent substrate-controlled allylation. It was hypothesized that the bulky protecting group alpha to the aldehyde would help to control the diastereoselectivity during the allylation reaction. However, this was not the case as allylmagnesium bromide and allylboronic acid pinacol ester both generated an inseparable mixture of diastereomers (1:1 $dr$). The use of an asymmetric allylation reaction involving (−)-$B$-allyldiisopinocampheylborane increased the selectivity slightly and improved the purification, but again the diastereomeric products could not be separated (Scheme 57).

Steric bulk at the $\alpha$-carbon was unsuccessful in controlling the diastereoselectivity since the molecule has the ability to freely rotate. Alternatively, it was envisioned that cyclizing the 1,3-diol into an acetal might provide a more effective means to control the stereochemical outcome during a substrate-controlled allylation. While the aldehyde can still freely rotate, the dipole of the carbon-oxygen bond alpha to the aldehyde will be set relative to the aldehyde’s dipole. Depending on whether the allylation reaction follows a Conforth model or a polar Felkin-Ahn model, predictions for the stereochemical outcome may be plausible. The cyclic acetal group also has the advantage of functioning as a useful protecting group thus avoiding issues highlighted in Scheme 56 and Scheme 57.

![Scheme 58. Acetal protection of diol ent-194 contaminated with isopropyl auxiliary ent-185](image)

Initial protection of the 1,3-diol was done using ent-194 which was contaminated with the inseparable auxiliary ent-185. Protection of compound ent-194 as either the benzilidene acetal
(ent-218) or the p-methoxybenzilidene acetal (ent-219) gave a mixture of products with a 1:3 dr
(anti : syn). While the diastereomers were separable, repetition of this purification proved
difficult (Scheme 58). Furthermore, co-elution of either benzaldehyde or p-anisaldehyde during
purification proved problematic as the later allylation step would have three possible aldehydes
to react with (two diastereomers and the by-product aldehyde). Since this early work on
generating the acetal was done using an inseparable mixture of diol ent-194 and Evans auxiliary
ent-185, it was unknown whether the reaction or the impurity ent-185 was the cause of the
reduced diastereoselectivity.

As the epimerisation of the acetal CH made the above route synthetically difficult, efforts
were made to synthesize the dimethyl ketal, thereby negating this stereocenter. Although the
dimethyl ketal was produced efficiently, this molecule unfortunately proved to be too volatile
and distillation of the product was unsuccessful. Therefore, the focus became on the modification
of the original acetal protection conditions to isolate a single diastereomer. While both the
benzylidene acetal and the p-methoxybenzylidene acetal were equally promising and the
benzylidene acetal was more robust, the p-methoxybenzylidene acetal was ultimately used as it
provided an easier NMR spectrum to interpret.

Starting with non-contaminated diol 194 (derived from auxiliary 210) and avoiding heat
in hopes to steer clear of epimerisation, the p-methoxybenzylidene acetal 98 was isolated as a
single diastereomer over two steps using camphorsulfonic acid (CSA) as a catalyst (Scheme
59). As before, the generation of p-methoxybenzaldehyde made purification difficult.
However, the addition of sodium borohydride reduced the aldehyde to p-methoxybenzyl alcohol
without reacting with acetal 98. This result was rather fortuitous since acetal migration occurs
under reductive conditions (discussed later), which could lead to the epimerisation of the acetal
CH proton. However, the use of a mild reducing agent such as sodium borohydride enables the
reduction of the aldehyde to occur faster than the reduction of the acetal. In order to avoid de-
acetalization and regeneration of p-methoxybenzaldehyde caused by the acidic nature of the
silica during purification, a 1% triethylamine treatment of the silica gel was used. This treatment
was further utilized on all compounds containing this acetal.

Oxidation of alkene 98 using a one-pot osmium (IV) tetroxide dihydroxylation followed
by a sodium periodate oxidation yielded crude aldehyde 182. As seen for other aldehydes during
this synthesis, purification was unsuccessful due to the decomposition of the molecule. Since
aldehyde 182 could not be adequately purified, it was unknown whether trace amounts of the osmium catalyst would interfere by catalysing side reactions during the allylation step. While the yields were initially high for the osmium tetroxide/sodium periodate oxidation, scalability was a major issue. Reactions above 100 to 200 mg suffered from drastically reduced yields and since this was a key intermediate, more adaptable conditions were sought out.

Ozonolysis of compound 98 originally resulted in its decomposition. The problem was initially perceived to be the reduction of the ozonide rather than its generation. Therefore, various workup conditions were tried including dimethyl sulfide, amines and phosphines as well as changing both the quenching time and the temperature. All of these efforts proved ineffective. Further research into this matter revealed ozone as a means to de-acetalize molecules, suggesting that the acetal was being cleaved and the resulting compound was unstable in solution. Thus, even though the osmium tetroxide/sodium periodate oxidation suffered drawbacks, it seemed the most reliable method for generating the desired aldehyde 182.

Scheme 59. Substrate-controlled allylation to install the C-8 stereochemistry
However, more than three years after initial efforts to generate aldehyde 182 using ozonolysis, recent work on similar molecules suggested that it was the generation of acid during the ozonide degradation that was cleaving the acetal rather than the ozone itself. Therefore, addition of a mild base, 2,6-lutidine, prior to quenching with dimethyl sulfide allowed the successful synthesis of aldehyde 182 in high yield. This new methodology proved easily scalable.

In order to avoid expensive asymmetric reagents, the substrate-controlled allylation of aldehyde 182 was first attempted using allylborane pinacol ester. Serendipitously, a single diastereomer (>20:1 dr) resulted. On large scale reactions (> 1 gram), the minor diastereomer can be observed but is easily separable. It was not immediately obvious whether compound 103 or its epimer had been produced as the major product. However, either diastereomer would be useful from both an analogue and a natural product methodology perspective. If the epimer at the C-8 position was the major product, the C-6 and C-6/C-10 methyl group compounds would be similar to Rich’s most potent analogue (39; epimeric at the C-8 position compared to the natural product) and a direct comparison for the analogue’s inhibition of HIV-1 protease could be made. Similarly, the methodology toward the western coupling fragment for the natural product synthesis could be accomplished knowing that either an inversion of this stereocenter at a later stage or an asymmetric allylation of aldehyde 182 could be performed after all the subsequent steps had been established.

4.3.2 Determination of the C-8 Stereochemistry

In order to determine whether compound 103 or its epimer had been produced in the substrate-controlled allylation, the less expensive acetal ent-103 (or epimer) was deprotected using hydrochloric acid to yield compound ent-221 (Scheme 60). The primary alcohol of triol ent-221 was selectively protected using tert-butyldimethylsilyl chloride to furnish compound ent-222. The yield for the selective protection of triol 221 was low as the molecule was highly polar and difficult to isolate following the aqueous work-up. Furthermore, selectively protecting the primary alcohol was difficult, resulting in the generation of the secondary protected alcohol products as well. Finally, formation of dioxalan-2-one ent-223 by protecting the 1,2-diol with triphosgene was expected to help establish the stereochemistry at the C-8 position through the use of coupling constants and NOE interactions.
Scheme 60. Synthesis of the dioxalan-2-one scaffold for C-8 stereochemistry determination

The Karplus equation describes the correlation between the vicinal $\^{3}$J-coupling constant and the dihedral torsion angle in NMR. The magnitude of the coupling constant is based on the cosine function which has maxima at 0º and 180º and a minimum at 90º.

$$J(\phi) = A \cos^2 \phi + B \cos \phi + C$$  \hspace{1cm} (7)  

where J is the $\^{3}$J-coupling constant, $\phi$ is the dihedral torsion angle and A, B, and C are empirically-derived constants whose values depend on the specific molecule involved.$^{236}$

The stereochemistry at the C-6 and C-7 positions has been previously established. For the purposes of this analysis, it is assumed that the dioxalan-2-one ring is relatively flat since it is unknown how much the ring will distort in order to relieve sterically unfavourable gauche interactions. Depending on the outcome of the allylation reaction, the proton (H) at the C-8 position can either be syn or anti to the C-7 proton (H) (Figure 55). If it were syn, the dihedral angle would be approximately 0º and a maximum coupling constant would be observed ($^{3}$J$\_{\text{HH}} = 7$ to 9 Hz). In contrast, if the two protons were anti, then the dihedral angle would be approximately 120º and the coupling constant ($^{3}$J$\_{\text{HH}}$) would be 4 to 6 Hz. Since these predicted dihedral angles, and thus the coupling constants, are based on the assumption that the ring is flat, a search of the literature was performed to confirm that our estimation of the structure would be suitable. Molecules with a similar anti dioxalan-2-one structure have been shown to have an
average dihedral angle between proton H and proton H of 128º which is close to our approximated value of 120º. Literature data for similar dioxalan-2-one compounds predicted the anti case to have a coupling constant of 3.6 to 4.2 Hz, whereas the syn case was predicted to have a coupling constant of 7.7 to 8.1 Hz. It should be noted that these literature compounds all contained an aromatic ketone at the C-8 position, possibly making them more sterically crowded than compound ent-223 (or ent-224). Attempts to find a broader range of dioxalan-2-one compounds with which to compare were unsuccessful. Either the molecules were too complex and the desired coupling constants were difficult to obtain due to overlapping signals or the simpler compounds were not analyzed by the authors. Comparison of acetonide compounds similar to compound ent-223 (or ent-224) (of which there was a larger variety) showed coupling constants for the anti case to be 6.7 to 7.1 Hz, whereas the syn case showed coupling constants of 7.4 Hz. With such similarity in the coupling constants amongst these structures, these were unfortunately not helpful.

Figure 55. Structure and dihedral angles for the two possible diastereomers resulting from the substrate controlled allylation of aldehyde ent-182

The measured coupling constant ($^3J_{HH}$) for the dioxalan-2-one compound obtained from the NMR data was 6.9 Hz. Unfortunately, since this coupling constant is close to that predicted for both the syn and the anti confirmation, the stereochemistry at the C-8 position remained ambiguous (Figure 55). Therefore, NOESY spectroscopy was used instead and showed an interaction between the allyl CH$_2$ protons at C-9 and the proton at C-6 (Figure 56). Moreover,
there were no NOE interactions observed between either the methyl or the C-6 proton and the C-8 proton nor between the allyl CH₂ protons at C-9 and the C-7 proton. This further suggests that compound ent-224 had been synthesized.

![ent-224](image)

**Figure 56.** Confirmation of ent-224. NOE interactions shown in red.

To justify the outcome observed during the allylation reaction, two common models for α-heteroatom-substituted aldehydes were compared (Figure 57). During the transition state, the polar Felkin-Ahn model positions the α-heteroatom perpendicular to the carbonyl group. This allows hyperconjugative interactions from the newly formed bond (HOMO) to the best vicinal acceptor, the C-X bond (LUMO). In contrast, the Cornforth model aims to minimize the dipole interactions between the C-X bond and the carbonyl bond. Using both these models, the C-8 epimer of the natural product scaffold is predicted. To further confirm these results, the chemical shifts and coupling constants for the C-7 and C-8 protons of the natural product were compared to the C-7 and C-8 protons of a truncated intermediate (to be discussed in detail later on). While the chemical shifts were similar to that of the natural product, the coupling constants for the C-7 proton differed. Based on this information, conclude that the undesired C-8 epimer was the molecule synthesized.

While the undesired stereochemistry was obtained during the allylation reaction of aldehyde 182, the fact that a single diastereomer was isolated cleanly proved rather fortunate. Rich’s group showed that their most potent analogue (39) was epimeric at the C-8 position compared to the natural product and has been proven to be a dissociative inhibitor of HIV-1 protease. Therefore, in synthesizing the C-8 epimer of the natural product, our analogues possessing a C-6 methyl group can now be directly compared to analogue 39, synthesized by
Rich, in order to account for the potency obtained/lossed from increasing the rigidity of the molecule.

![Figure 57. Comparison of the polar Felkin-Ahn model and the Cornforth model in predicting the stereochemical outcome for the allylation of aldehyde 182](image)

### 4.4.0 Methodology Toward Compound 234

#### 4.4.1 Preparation of Compound 234 using a Direct Esterification Route

The design of the C-8 epimer of analogue 105 was envisioned to proceed with the direct esterification of the alcohols as they were formed. This would remove any cumbersome protection/deprotection steps and thus constitute a convenient synthesis toward the didemnaketal A analogues.

Hydroboration of intermediate ent-225 was first attempted using 9-borabicyclo(3.3.1)nonane. While the reaction proceeded quantitatively based on integrated signals, separation of the product from the borane by-product proved challenging as both co-eluted during purification. Therefore, regioselective hydroboration was accomplished using a borane-tetrahydrofuran complex to give compound ent-226 followed by subsequent esterification of the diol with isovaleric anhydride to provide diester ent-227 (Scheme 61). Since it was previously known that the acetal group was sensitive to acidic conditions such as silica gel purification, a dilute solution of acid was used to cleave the acetal. The concentration of
hydrochloric acid had to remain low since higher concentrations of acid have the ability to cleave the ester functional groups as well. While this was originally successful, future efforts resulted in difficulties separating the desired compound from the co-product \( p \)-anisaldehyde, especially when working on scales greater than 50 milligrams.

Scheme 61. Synthesis of diol ent-228

Selective oxidation of the primary alcohol in the presence of the secondary alcohol in diol ent-228 was attempted under a variety of conditions (Scheme 62). Unfortunately, most conditions provided either no desired product (Scheme 62; entries 2 to 4) or starting material (entries 5 to 7, 10). A TEMPO oxidation (entry 8) did generate a minor amount of the desired product ent-229, however, even upon heating the solution to 40 °C for 3 days, only a 20% yield was obtained. Using Dess-Martin periodinane (entry 1), a similar yield was achieved although this material possessed a higher purity. However, in light of these low yields and an inability to optimize the reactions further, a slight adaptation to the synthesis was required.
Since the direct oxidation and esterification of diol ent-228 was unsuccessful, a selective protection of the primary alcohol with tert-butyldimethylsilyl chloride was used to generate compound ent-230. Subsequent esterification of compound ent-230 with acetic anhydride afforded compound ent-231 in excellent yield (Scheme 63). Unfortunately, standard conditions for cleaving the tert-butyldimethylsilyl group using tetrabutylammonium fluoride resulted in no desired product, possibly due to decomposition of the molecule from the excess fluoride (Scheme 64; entry 1). Therefore, the reaction was repeated using one equivalent of tetrabutylammonium fluoride and a reduced reaction time (entry 2), which gave the desired

### Scheme 62. Synthesis of compound ent-229

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMP, CH₂Cl₂, 2.5 hrs</td>
<td>ent-229</td>
<td>21%</td>
</tr>
<tr>
<td>2</td>
<td>oxalyl chloride, (CH₃)₂SO, Et₃N, CH₂Cl₂, 2.5 hrs</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>3</td>
<td>SO₃•C₅H₅N, Et₃N, CH₂Cl₂/(CH₃)₂SO (2:1), 1 hr</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>4</td>
<td>PCC, CH₂Cl₂, celite, 2 hrs</td>
<td>no desired product</td>
<td>n/a</td>
</tr>
<tr>
<td>5</td>
<td>MnO₂, CH₂Cl₂, 24 hrs</td>
<td>ent-228</td>
<td>n/a</td>
</tr>
<tr>
<td>6</td>
<td>DCC, (CH₃)₂SO, Et₂O, 2hrs</td>
<td>ent-228</td>
<td>n/a</td>
</tr>
<tr>
<td>7</td>
<td>TEMPO, CH₃CN, 2 hrs</td>
<td>ent-228</td>
<td>n/a</td>
</tr>
<tr>
<td>8</td>
<td>TEMPO, KBr, sat. NaHCO₃, NaOCl, CH₂Cl₂</td>
<td>ent-228 + minor ent-229</td>
<td>n/a</td>
</tr>
<tr>
<td>9</td>
<td>TEMPO, KBr, sat. NaHCO₃, NaOCl, CH₂Cl₂, 40 °C, 3d</td>
<td>ent-228 + 20% ent-229</td>
<td>n/a</td>
</tr>
<tr>
<td>10</td>
<td>NMO, TPAP, CH₃CN, 3Å MS, 1.5 hrs</td>
<td>ent-228</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Scheme 63. Synthesis of compound ent-231

Scheme 64. Silyl cleavage of TBS protected ent-231

product but also migrated the acetate to yield a 1:1 mixture of the desired ent-232 and undesired ent-233. In order to avoid this migration, believed to be caused by the basicity of the fluoride...
solution, a buffered solution containing acetic acid was used (entry 3). However, only starting material was isolated. Finally, a slightly acidic environment involving hydrogen fluoride resulted in the desired product ent-232 with no acetate migration. Regrettably, repetition of these results proved to be rather challenging. To solve this problem, variations in both the temperature and the equivalents of hydrogen fluoride added were attempted but these only generated small amounts of the desired product ent-232.

With difficulty in removing the tert-butyldimethylsilyl group, alternative silyl protecting groups were attempted. First, a one-pot protection using trimethylsilyl chloride followed by esterification/deprotection was attempted but found little success (Scheme 65). Secondly, triisopropylsilyl chloride and p-methoxybenzyl bromide protections were undertaken since either one of these groups can be cleaved using fluoride-free conditions. However, at the time of these reactions diol ent-228 could not be adequately purified and separated from p-anisaldehyde. Therefore, in the presence of the nucleophilic catalyst N,N-dimethylaminopyridine (DMAP), p-anisaldehyde present in the crude mixture reacted with diol ent-228 to reform acetal ent-227.

\[
\text{Scheme 65. Alternative silyl protections of diol ent-228}
\]
After multiple reaction attempts to generate compound ent-232, a small amount of this material was able to be isolated. With this molecule in hand, alcohol ent-232 was oxidized to aldehyde ent-234 using Dess-Martin periodinane conditions. As with most aldehydes isolated throughout this synthesis, the sensitivity of compound ent-234 during purification was taken into account and thus taken on crude for the allylation reaction. Hoping that the substrate would once again control the stereochemical outcome of the allylation, allylboronic acid pinacol ester was used as the allylating agent. Unfortunately, no desired product was observed during this reaction. Due to the scalability and the reproducibility of various reactions along this methodology, producing enough pure ent-232 to lead to an adequate number of analogues became a significant challenge. Thus, regrettably, the sensitivity of these molecules and problems associated with the cleavage of the acetal protecting group meant a new synthetic strategy was needed.

Scheme 66. Synthesis of compound ent-234

4.5.0 Methodology Toward a Fully Protected Carbon Skeleton

4.5.1 Preparation of the Fully Protected Intermediate 237

With a growing number of issues associated with the direct esterification route, an alternate orthogonally protected route toward the C-8 epimer of compound 105 was proposed. While orthogonal protection of the alcohols will add additional steps to the synthesis, it can be used to create a single compound that could later be selectively deprotected and esterified. The
advantage of this would be the ability to scramble the esters along the backbone of the didemnaketal framework, thus generating a variety of analogues in a short period of time from a central molecule. Furthermore, in completing the methodology toward the fully protected carbon skeleton of the C-8 epimer of analogue 105, a more precise understanding of what reactions are tolerated and the sensitivity of the overall molecule will be gained. Using this information, a more direct route to the final target can be achieved later on.

Starting from intermediate 225, benzyl protection of the alcohol provided compound 235. Close monitoring of the benzyl protection was required as prolonged heating began to produce elimination products. Regioselective hydroboration and subsequent tert-butylidimethylsilyl protection of the alcohol yielded compound 237 in near quantitative yield (Scheme 67). Surprisingly, efforts to protect the alcohol of compound 236 with tert-butyldimethylsilyl chloride resulted in the isolation of clean starting material. It was hypothesized that chloride was not a good enough leaving group to facilitate the reaction for this particular molecule. Therefore, a leaving group exchange with triflic acid was performed to generate tert-butyldimethylsilyl triflate, resulting in a substantial increase in yield.

Scheme 67. Synthesis of the fully protected compound 237

4.5.2 Model Studies Used to Determine the Acetal Migration, Allylation and Cross Metathesis Conditions

Before continuing on to install the final pieces of the carbon skeleton, a set of simplified test substrates were used to work out the acetal migration, allylation and cross metathesis
conditions. Compound ent-225 possessed an ideal carbon framework along with a sensitive acetal group and could be synthesized in gram-scale quantities, making it an ideal substrate for analyzing cross-metathesis conditions. Likewise, compound ent-98 could be synthesized in similarly large quantities and was used to identify conditions for both the acetal migration and the allylation reaction.

Following literature protocols, the regioselective acetal migration of ent-98 with diisobutylaluminum hydride gave compound ent-238 in an 88% yield (Scheme 68). Only a minor amount of the other regioisomer was detected and was easily separated during purification. Oxidation of the primary alcohol to the aldehyde failed when using IBX (Scheme 68; entry 1) and when using Swern conditions (entry 2). On the other hand, oxidation using TEMPO (entry 3) provided fairly clean product that was then subjected to various allylating conditions.

![Diagram of chemical reaction]

**Scheme 68.** Acetal migration conditions using ent-98 as a test substrate

Ideally, allylation of compound ent-239 to generate compound ent-240 would proceed using a non-asymmetric reagent with control of the newly formed C-5 stereocenter arising from the electronic influence of the molecule (Scheme 69). However, as predicted from the Felkin-
Ahn model, the diastereomeric ratio (dr) for the product was consistently 2:1 in favour of the S-alcohol (epimer of the natural product). This result was good since the electronic influence during the substrate controlled allylation was low and could potentially be overcome using an asymmetric allylating reagent. B-allyldiisopinocampheylborane can be used to overcome the stereoselectivity imposed by the molecule, but this reaction was performed on the actual substrate rather than this model system (to be discussed later on). To monitor the sensitivity of this reaction, efforts were made to bias the selectivity via solvent and temperature changes. Changes to the temperature had no effect (Scheme 69; entries 2 through 5), while attempts to bias the stereocontrol using solvents such as acetonitrile or tetrahydrofuran did little to sway the selectivity (entries 6 and 7).

![Scheme 69](image)

**Scheme 69.** Allylation conditions starting from ent-98 as a test substrate

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
<th>Yield (over two steps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>allylboronic acid pinacol ester, CH₂Cl₂, 25 °C, 17 hrs</td>
<td>ent-240 + epi-ent-240</td>
<td>32% (2:1 dr)</td>
</tr>
<tr>
<td>2</td>
<td>allylboronic acid pinacol ester, CH₂Cl₂, 0 °C, 17 hrs</td>
<td>ent-240 + epi-ent-240</td>
<td>n/a (2:1 dr)</td>
</tr>
<tr>
<td>3</td>
<td>allylboronic acid pinacol ester, CH₂Cl₂, 4 °C, 17 hrs</td>
<td>ent-240 + epi-ent-240</td>
<td>n/a (2:1 dr)</td>
</tr>
<tr>
<td>4</td>
<td>allylboronic acid pinacol ester, CH₂Cl₂, 25 °C, 17 hrs</td>
<td>ent-240 + epi-ent-240</td>
<td>n/a (2:1 dr)</td>
</tr>
<tr>
<td>5</td>
<td>allylboronic acid pinacol ester, CH₂Cl₂, 40 °C, 17 hrs</td>
<td>ent-240 + epi-ent-240</td>
<td>n/a (2:1 dr)</td>
</tr>
<tr>
<td>6</td>
<td>allylboronic acid pinacol ester, CH₃CN, 25 °C, 17 hrs</td>
<td>ent-240 + epi-ent-240</td>
<td>21% (2:1 dr)</td>
</tr>
<tr>
<td>7</td>
<td>allylboronic acid pinacol ester, THF, 25 °C, 17 hrs</td>
<td>ent-240 + epi-ent-240</td>
<td>28% (2:1 dr)</td>
</tr>
</tbody>
</table>
Conditions for the Grubbs cross metathesis were determined using compound ent-225. The first generation Grubbs catalyst has a lower activity than the second generation Grubbs catalyst, but has been shown to be extremely robust in the presence of water, air, and polar functional groups. Unfortunately, no desired product was observed when using standard conditions (Scheme 70; entry 1). Therefore, the more active second generation Grubbs catalyst was tried (entry 2). Regrettably, no reaction occurred possibly because of quenching of the catalyst by the free alcohol present in compound ent-225.

Scheme 70. Grubbs cross metathesis with methyl methacrylate using the test substrate ent-225
Scheme 71. Hoveyda-Grubbs cross metathesis with methyl methacrylate using the protected compound ent-235

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting Material</th>
<th>Conditions</th>
<th>Results</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ent-235</td>
<td>Grubbs II (5 mol %), CH₂Cl₂ 5 eq. methyl methacrylate, Δ</td>
<td>ent-242</td>
<td>79%</td>
</tr>
<tr>
<td>2</td>
<td>ent-235</td>
<td>Hoveyda-Grubbs (10 mol %), CH₂Cl₂ 30 eq. methyl methacrylate</td>
<td>ent-235</td>
<td>n/a</td>
</tr>
<tr>
<td>3</td>
<td>ent-235</td>
<td>Hoveyda-Grubbs (5 mol %), CH₂Cl₂ 5 eq. methyl methacrylate, Δ</td>
<td>ent-242</td>
<td>100%</td>
</tr>
<tr>
<td>4</td>
<td>ent-235</td>
<td>Hoveyda-Grubbs (5 mol %), toluene 5 eq. methyl methacrylate, Δ</td>
<td>ent-241</td>
<td>92%</td>
</tr>
<tr>
<td>5</td>
<td>ent-242</td>
<td>Hoveyda-Grubbs (5 mol %), toluene 5 eq. methyl methacrylate, Δ</td>
<td>ent-241</td>
<td>92%</td>
</tr>
</tbody>
</table>
Since the free alcohol in compound ent-225 seemed to be an issue, benzyl protected ent-235 was used instead as a model substrate. Cross metathesis using the second generation Grubbs catalyst (Scheme 71; entry 1) occurred to produce dimer ent-242. It was originally proposed that the second generation Grubbs catalyst was still not reactive enough to push the reaction to completion. Therefore, further cross metathesis attempts were done using an even more active second generation Hoveyda-Grubbs catalyst. At room temperature with Hoveyda-Grubbs catalyst (entry 2) the reaction failed to proceed; however, upon heating to reflux in dichloromethane the dimer ent-242 was once again formed (entry 3). Manipulation of the order and rate of addition of the reagents had no effect on this result. Similarly, increasing the equivalents of methyl methacrylate as well as adding it portionwise had no effect and the same dimer ent-242 was observed. With these results, an alternative theory was proposed. It was hypothesized that the formation of the dimer was a result of the methyl methacrylate possibly being too hindered to react at lower temperatures. Therefore, using a higher boiling solvent might provide a significant boost in energy to force the reaction toward the product. To test this idea, both dimer ent-242 and compound ent-235 were set up simultaneously (entries 4 and 5) using the second generation Hoveyda-Grubbs catalyst but instead heated to reflux in toluene. This increase in temperature was enough to force the reaction in both cases past the dimerization stage and generate the desired product in 92% yield with a >20:1 isomeric ratio (Scheme 71). With this result, the acetal migration and cross metathesis conditions were established using the model systems and could now be used with compound 237 to generate the C-6 methyl didemnaketal analogue 105 epimeric at the C-8 position. As for the allylation reaction, an asymmetric allylating reagent would be used on the actual substrate to install the C-5 stereocenter.

4.6.0 Control of the C-5 Stereocenter

4.6.1 Synthetic Strategies toward Compound 243

With conditions for the acetal migration step having been identified from the model study, compound 237 was subjected to diisobutylaluminum hydride in dichloromethane (Scheme 72; entry 1) but unfortunately the reaction failed to generate any desired product. Attention was then turned to stronger reducing agents such as vitride and lithium aluminum hydride but all
<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 eq. DIBAL-H, CH₂Cl₂, 25 °C, 18 hrs</td>
<td>237</td>
<td>10</td>
<td>Bu₂BOTf, BH₃•THF, THF, 0 °C, 20 min.</td>
<td>no desired product</td>
</tr>
<tr>
<td>2</td>
<td>10 eq. DIBAL-H, THF, 40 °C, 22 hrs</td>
<td>237</td>
<td>11</td>
<td>BH₃•THF, THF, Δ, 23 hrs</td>
<td>no desired product</td>
</tr>
<tr>
<td>3</td>
<td>5 eq. DIBAL-H, toluene, 25 °C, 23 hrs</td>
<td>243 (48%)</td>
<td>12</td>
<td>(CH₃)₃SiOTf, BH₃•THF, CH₂Cl₂, 25 °C, 22 hrs</td>
<td>no desired product</td>
</tr>
<tr>
<td>4</td>
<td>5 eq. vitride, CH₂Cl₂, 0 °C, 2 hrs</td>
<td>237</td>
<td>13</td>
<td>Kardstedt's cat., Et₃SiH</td>
<td>237</td>
</tr>
<tr>
<td>5</td>
<td>5 eq. vitride, CH₂Cl₂, 40 °C, 21 hrs</td>
<td>237</td>
<td>14</td>
<td>PhBCl₂, Et₃SiH, Et₂O, 25 °C, 24 hrs</td>
<td>no desired product</td>
</tr>
<tr>
<td>6</td>
<td>5 eq. LiAlH₄, CH₂Cl₂, 25 °C, 2 hrs</td>
<td>237</td>
<td>15</td>
<td>TiCl₄, Et₃SiH, Et₂O, 25 °C, 23 hrs</td>
<td>243 (unclean) (27%)</td>
</tr>
<tr>
<td>7</td>
<td>5 eq. LiAlH₄, AlCl₃, THF, 25 °C, 4 hrs</td>
<td>237</td>
<td>16</td>
<td>1. O₃, – 78 °C, CH₂Cl₂ 2. Me₂S, 25 °C</td>
<td>244 (11%)</td>
</tr>
<tr>
<td>8</td>
<td>10 eq. LiAlH₄, AlCl₃, Et₂O, D, 25 hrs</td>
<td>237</td>
<td>17</td>
<td>N-bromosuccinimide, BaCO₃, CH₂Cl₂/H₂O (1:1), 25 °C, 23 hrs</td>
<td>no desired product</td>
</tr>
<tr>
<td>9</td>
<td>20 eq. LiAlH₄, Et₂O, silica gel, 25 °C, 3d</td>
<td>237</td>
<td>18</td>
<td>DDQ, CuBr₂, Bu₄NBr, CH₂Cl₂, 25 °C, 24 hrs</td>
<td>no desired product</td>
</tr>
</tbody>
</table>

**Scheme 72.** Acetal migration conditions for compound 237
were equally unsuccessful (entries 4, 5, and 6). It was thought that the oxonium formation during cleavage might be slow for compound 237, so addition of a Lewis acid (aluminum trichloride) was attempted as well as efforts to address any solvent effects (entries 7 and 8). Unfortunately, both of these reactions yielded only starting material. Since the acetal had proven rather sensitive to silica gel during purification, silica gel was used as an acid source alongside lithium aluminum hydride. Surprisingly this too was unsuccessful even after stirring for three days! Instead, focus was directed on finding an adequate alternative for the acetal migration. The use of borane (entries 10, 11, 12 and 14) at least showed a disappearance of the starting material 237, but no desired product was ever observed. Efforts to migrate the acetal and form a primary silyl protected alcohol using Kardstedt’s catalyst (entry 13) also resulted in the isolation of starting material. Titanium (IV) chloride as a Lewis acid (entry 15) proved somewhat successful, although the product proved difficult to purify due to titanium contaminants. Alternative methods were attempted to purify this reaction further but were unsuccessful. Finally, attention was turned to an oxidative cleavage of the acetal to form a benzoyl protected alcohol (entries 16 through 18). Ozone showed the most promise but again the purification proved challenging. It was thought that since the acetal was sensitive to ozonolysis during previous reactions that at least the acetal group might be fully cleaved, but this too failed to occur. As a last ditch effort, diisobutylaluminum hydride in toluene (entry 3). Serendipitously, a 48% yield of desired product with the correct regiochemistry was obtained. Based on other products isolated from purification, it was observed that the silyl protecting group was being cleaved as well under these specific set of conditions. As this result showed the most promise, further investigation into optimizing this reaction was done.

The side reaction involving the cleavage of the silyl protecting group was first theorized to occur at higher temperatures (Scheme 73; entry 1). Therefore, the acetal migration was repeated at –78 ºC (entry 2) but suffered from lower yields as the migration began to slow down as well. While the reaction at 0 ºC proceeded rapidly, cleavage of the silyl group was still observed after one hour (entry 3). With the temperature optimized, the number of equivalents of diisobutylaluminum hydride was reduced. However, upon attempting this, the reaction failed to go to completion. Interestingly, the addition of the remaining three equivalents of diisobutylaluminum hydride (entry 4) after one hour resulted in complete conversion of compound 237 to form alcohol 243 in less than twenty minutes. Thus, the optimized conditions
were found to be five equivalents of diisobutylaluminum hydride at 0 °C for fifteen minutes. Close monitoring by thin layer chromatography aided in reducing the amount of silyl cleavage product observed.

![Scheme 73](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Equivalents of DIBAL-H</th>
<th>Temperature</th>
<th>Reaction Time</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>25 °C</td>
<td>23 hrs</td>
<td>48%</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>−78 °C</td>
<td>7 hr</td>
<td>14%</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0 °C</td>
<td>1 hr</td>
<td>32%</td>
</tr>
<tr>
<td>4</td>
<td>2 then 3 more after 1 hr</td>
<td>0 °C</td>
<td>1 hr 20 min</td>
<td>49%</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0 °C</td>
<td>15 min</td>
<td>69%</td>
</tr>
</tbody>
</table>

**Scheme 73.** Optimization of acetal migration conditions for compound 237

### 4.6.2 Synthetic Strategies Toward the C-5 Stereochemistry

With the alcohol in hand, control of the C-5 stereocenter was envisioned to proceed via a Brown allylation.\(^{229}\) The stereochemistry of the Brown allylation is controlled by a chair-like transition state involving two sterically cumbersome pinene groups. The R-group of the aldehyde positions itself equatorially to avoid 1,3-diaxial interactions and the aldehyde facial selectivity derives from minimization of steric interactions between the isopinocampheyl ligand and the allyl group (Scheme 74). In the case shown below, addition of the allyl group will occur from the Si face, producing a newly formed alcohol with the desired R-geometry.

For chiral α-substituted aldehydes a matched or mismatched stereochemical outcome can be observed. The matched case occurs when both the facial selectivity of the chiral borane and the facial preference of the aldehyde are the same. In contrast, the mismatched case arises when
the facial preference of the aldehyde and the facial selectivity of the chiral borane oppose one another. Diastereofacial selectivity of the $B$-allyldiisopinocampherylborane reagent often overrides any facial preference of the aldehyde during nucleophilic attack. However, there are cases where a mixture of diastereomers are generated due to the inability of the borane to sufficiently bias the reaction. In order to generate the desired stereocenter at the C-5 position (mismatched) when allylating compound ent-245, the (+)-$B$-allyldiisopinocampherylborane was required. To yield the desired mismatched stereochemistry at the C-5 position when allylating the analogue containing the natural product stereochemistry (i.e. compound 245), the (−)-$B$-allyldiisopinocampherylborane was required.

Scheme 74. Transition state for the Brown allylation

With only a mere four steps from the final analogue, the enantiomer of compound 243 (ent-243 derived from the cheaper amino acid L-phenylalanine) was used to derive the necessary conditions needed for both the Brown allylation and the Grubbs cross metathesis. Oxidation of
compound ent-243 to aldehyde ent-245 was first attempted using the TEMPO conditions adopted from the test substrate reaction (Scheme 68; entry 3). Similarly to before, while these ideal conditions worked well for the model substrate they provided lower yields when used to oxidize alcohol ent-243. Investigation into alternate oxidative conditions revealed no aldehyde production when using Swern conditions and Parikh-Doering conditions. Fortunately, Dess-Martin periodinane provided the desired aldehyde cleanly in a quantitative yield (Scheme 75). Next, the Brown allylation was attempted using both (+)- and (−)-B-allyldiisopinocampheylborane at low temperatures (−78 °C) to afford the near instantaneous allylboration of aldehyde ent-245. (−)-B-allyldiisopinocampheylborane was used to generate the matched stereochemistry (ent-247). This granted a point of reference in which to compare the reaction for aldehyde ent-245 with (+)-B-allyldiisopinocampheylborane. If a single diastereomer epimeric at the C-5 position was observed (compound ent-246), then this would provide confirmation that the facial selectivity of aldehyde ent-245 was being overridden (Scheme 75). Unfortunately, allylation of aldehyde ent-245 using (+)-B-allyldiisopinocampheylborane to generate the desired C-5 stereocenter gave two inseparable diastereomers in a 2:1 dr

![Scheme 75. Brown allylation to generate the C-5 stereocenter](image)

$$ \text{OH} \quad \text{OPMB} \quad \text{TBS} $$

$$ \text{OBn} $$

$$ \text{ent-243} $$

$$ \text{DMP, CH}_2\text{Cl}_2 $$

$$ (100\%) $$

$$ \text{OH} \quad \text{OPMB} \quad \text{TBS} $$

$$ \text{OBn} $$

$$ \text{ent-245} $$

$$ (+)-(\text{ipc})_2\text{B(allyl),} $$

$$ \text{Et}_2\text{O} $$

$$ (57\% \text{ over two steps}) $$

$$ \text{OH} \quad \text{OPMB} \quad \text{TBS} $$

$$ \text{OBn} $$

$$ \text{ent-247} $$

$$ (+)-(\text{ipc})_2\text{B(allyl),} $$

$$ \text{Et}_2\text{O} $$

$$ (52\% \text{ over two steps}) $$

$$ \text{OH} \quad \text{OPMB} \quad \text{TBS} $$

$$ \text{OBn} $$

$$ \text{ent-245} $$

$$ (--)\text{B(allyl),} $$

$$ \text{Et}_2\text{O} $$

$$ (52\% \text{ over two steps}) $$

$$ \text{OH} \quad \text{OPMB} \quad \text{TBS} $$

$$ \text{OBn} $$

$$ \text{ent-246} $$

$$ + $$

$$ \text{ent-247} $$

$$ \text{Scheme 75. Brown allylation to generate the C-5 stereocenter} $$
(mismatched:matched). While ill-fated, this result did improve upon the ratio of desired product observed when using the model compound ent-239 and utilizing the electronic influence of the substrate. It may be plausible that with this substrate, there exists a secondary effect (in conjunction with the electronic effect) whereby the three chiral groups in the α, β, and γ positions may be influencing the reaction and making it more difficult for the chiral borane to control the reaction. If this were the case, it was theorized that the β-position containing a p-methoxybenzyl protected alcohol might be causing the largest bias of the three positions based on work done using similar compounds (to be discussed further on).

Although the C-5 stereocenter proved challenging, the methodology to establish the full carbon skeleton of the didemnaketal A analogue was close at hand. Using compound ent-247, esterification with propionic anhydride followed by a Grubbs cross metathesis with methyl methacrylate, using previously established conditions, afforded compound ent-249 in excellent yield (Scheme 76). Although this was the wrong diastereomer, this proved that the carbon skeleton of the didemnaketal A analogue could be synthesized using this route as well as generating another possible analogue for testing.

![Scheme 76](image.png)

**Scheme 76.** Synthesis of the C-5 epimer carbon skeleton

### 4.6.3 Confirmation of the C-5 Stereochemistry

Both epimers of the C-5 position were synthesized from the Brown allylation using either (−)-B-allyldiisopinocampheylborane to provide the matched case (ent-247) or (+)-B-
allyldiisopinocampheylborane to provide the mismatched case (ent-246). Originally, the determination of the stereochemistry at C-5 was envisioned to proceed via cleavage of the p-methoxybenzyl protecting group and then subsequent re-protection using an acetonide. Instead, addition of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) regenerated the p-methoxybenzylidene acetal (Scheme 77). Not only did this grant a cyclic structure upon which to analyze the C-5 stereocenter, it also provided insight into future deprotection reactions, i.e. deprotection of the p-methoxybenzyl group can not proceed in the presence of an unprotected alcohol in the β-position. The rigidity of the cyclic structure also allowed for the separation of compound ent-250 from ent-251, which explains the low yield of 17%.

![Scheme 77. Acetal formation for determining the C-5 stereochemistry](image)

When using (−)-B-allyldiisopinocampheylborane to generate the matched case (compound ent-251), the C-5 stereocenter is S (when starting from L-phenylalanine). This was confirmed by strong 1-D NOE interactions between the two axial protons at the C-5 and C-7 positions and the acetal proton (Figure 58). In contrast, only the proton at the C-7 position and the acetal proton show an NOE interaction for compound ent-250, generated using (+)-B-allyldiisopinocampheylborane. Furthermore, an interaction between the C-7 proton, the acetal
proton and the allylic CH$_2$ protons are seen when C-5 is the $R$ diastereomer (allyl group axial) but not when C-5 is the $S$ diastereomer.

![Chemical structures](image)

**Figure 58.** 1-D NOE interactions for compound ent-250 and compound ent-251. NOE interactions are shown in pink.

### 4.6.4 Synthetic Strategies toward the C-5 Stereochemistry using Alternate Routes

The methodology toward the synthesis of the fully protected analogue 249 has endured some minor struggles but overall possesses scalable, high-yielding steps. From aldehyde ent-245, it is only a mere three steps to obtain the final carbon skeleton for the desired C-8 epimer analogue. Therefore, a way to utilize the generation of the unexpected matched allylation product ent-247 was sought. Compound ent-247 can be isolated cleanly as a single diastereomer and in adequate yield from the Brown allylation of aldehyde ent-245 (Scheme 75). Using a Mitsunobu reaction, one could in principle epimerize the C-5 stereocenter while simultaneously esterifying the alcohol (Scheme 78).
Scheme 78. Mitsunobu mechanism

Using standard Mitsunobu conditions (Scheme 79; entry 1), the desired product ent-252 was not isolated. This result reinforced the idea that the C-7 protected alcohol might be too bulky and was most likely interfering with the reaction. Although compound ent-247 looks to be able to freely rotate, it may be possible that the presence of so many bulkier protecting groups actually creates a partial rigidity to the molecule. When designing probes for the triclosan project, higher temperatures and longer reaction times were often needed to push the Mitsunobu reaction. Therefore, the reaction was attempted again in refluxing tetrahydrofuran for three days (entry 2). Unfortunately, only starting material was re-isolated. The use of a smaller phosphine reagent (trimethylphosphine) to reduce steric interference also failed to lead to any desired product (entry 3). Finally, Mitsunobu reactions have been known to be sensitive to the order of addition of the reagents. Thus, the pre-formation of the betaine was accomplished prior to the addition of alcohol ent-247 and the propionic acid but this made no difference in the outcome.
With the inability to generate the desired stereochemistry either by using a Mitsunobu reaction or by overcoming the stereochemical bias of compound ent-245 using a Brown allylation, it seemed imperative to develop a modified strategy. Two proposals were considered with the first utilizing a Corey-Bakshi-Shibata (CBS) reduction to install the C-5 stereocenter.

The first step in the CBS reduction involves the coordination of borane to the nitrogen of the oxazaborolidine catalyst (Scheme 80; I). This serves to activate the borane as a hydride donor as well as enhancing the Lewis acidity of the catalyst’s boron atom (II). Coordination of the ketone (III) positions the smaller R-group near the butyl group of the catalyst in order to reduce steric interactions and aligns the carbonyl for nucleophilic attack. The hydride is donated from the front when using the R-enantiomer of the catalyst (IV). Coordination of the borane (V) to the newly formed alcohol gives alcohol VI and regenerates the catalyst (I). Alternatively, a second molecule of borane can coordinate to V and upon cleavage of alcohol VI, the intermediate II is formed.
The CBS reaction involves the stereoselective reduction of a ketone to an alcohol. Using a non-asymmetric allylation, the alcohol at the C-5 position could be installed as a mixture of diastereomers and then subsequently oxidized to a ketone and then reduced back to an alcohol albeit as a single diastereomer. Since an asymmetric allylating reagent would no longer be required, allylation of aldehyde ent-245 was first attempted with allylborane pinacol ester but was unsuccessful as it yielded only starting material. It is possible that the steric influence of the pinacol group with the p-methoxybenzyl protected alcohol at the C-7 position was the cause of this failed reaction. Instead, allylmagnesium bromide was used and provided a 1:1 dr of the desired allyl alcohol ent-246/ent-247 albeit in poor yield (Scheme 81). Once again, the steric influence of the p-methoxybenzyl protected alcohol was perceived to be the culprit. Oxidation of the secondary alcohol with Dess-Martin periodinane afforded ketone ent-253 followed by cleavage of the p-methoxybenzyl group to give alcohol ent-254. Esterification of alcohol ent-254 with acetic anhydride then yielded compound ent-255 whereby the external olefin had migrated under these conditions to become conjugated with the ketone. Although this was an undesired product, the stereoselective CBS reduction of ketone ent-255 was attempted anyways, but failed to yield any desired product. Based on the NMR data, it appeared as though the borane used in the CBS reduction preferentially reduced the olefin. While this was not surprising, it was the hope that the hydroboration reaction would proceed slower than the reduction of the ketone at
−78 °C. However, at this time parallel progress toward the C-8 epimer of compound 105 was being pursued by our secondary strategy and offered a more accessible route. Thus, no further work into optimizing the CBS conditions was done.

Scheme 81. Installation of the C-5 stereocenter using a Corey-Bakshi-Shibata reduction

The second proposed route toward the installation of the C-5 stereochemistry was to remove the p-methoxybenzyl protecting group prior to the asymmetric allylation. This alternative and rather elegant route would not add any new synthetic steps to the pre-existing methodology (Scheme 82). Due to the instability of other aldehydes previously synthesized, this route was initially predicted to be quite challenging and its success was doubtful. Fortunately, however the aldehyde was far more robust than would have been predicted. Deprotection of the C-7 alcohol provided compound ent-257 which was subsequently esterified with acetic anhydride to give compound ent-258 in a fairly decent overall yield. While the allylation of aldehyde ent-258 proceeded in low yield, the correct diastereomer at the C-5 position was formed with no traces of the corresponding epimer. This solidified the notion that the C-7 p-methoxybenzyl group rather
than the C-6 methyl group was too bulky to allow either the Mitsunobu or the original Brown allylation to install the desired stereochemistry. Since both the esterification using propionic anhydride and the Grubbs cross metathesis had been previously shown to be successful (compound ent-247 to ent-249), access to the final analogue was not expected to be a major issue.

Scheme 82. Installation of the C-5 stereocenter using the key intermediate ent-245

Although the methodology toward the C-8 epimer of analogue 105 was close at hand, it was apparent that the use of protecting groups added a number of additional steps to an already growing synthesis and that the bottle neck of this methodology now lay in the allylation. While there are a variety of allylating reagents, the Brown allylation was initially chosen based on literature precedent stating that this particular reagent was the most suitable for overcoming the diastereofacial selectivity of the aldehyde when encountering a mismatched case. To try and increase the yield while maintaining the mismatched stereochemistry, a titanium based reagent[250-254] was synthesized and reacted with aldehyde ent-245 (Scheme 83). As was the case with the Brown allylation, compound 259 was touted for having a strong ability to overcome the diastereofacial selectivity of the aldehyde. Unfortunately, the reaction failed to produce any desired product. It is possible that the titanium species 259 is too bulky to be able to coordinate with aldehyde ent-245. This further suggested that aldehyde ent-245 may in fact be a lot more rigid than originally thought.
4.6.5 Revisiting the Direct Esterification Route: New Ways to Install the C-5 Stereocenter

While the fully protected route was designed to synthesize a single protected polyol which could later be selectively deprotected and esterified in any order, recent challenges associated with the steric influence of the protected C-7 position suggests that this may not be ideal. Furthermore, low yields for both the Brown allylation step and the acetal migration step have resulted in a bottleneck, thereby reducing the ability to make synthetic analogues quickly and efficiently. Although this work was useful in gaining a better understanding of the sensitivity and the reactivity of the different intermediates, a re-investigation of the earlier synthetic methodology was done.

With a clearer understanding of the challenges ahead, compound 225 was subjected to hydroboration and esterification to yield the di-ester 227 in quantitative yield (Scheme 84). Previously, cleavage of the acetal under acidic conditions proved difficult to reproduce on large scale. Therefore, a new strategy was employed involving hydrogenation at 300 psi in a high pressure PARR reactor. Initial attempts to cleave the acetal under these conditions resulted in the observation of the isovaleric ester at the C-8 position migrating between the C-7 and C-8 positions to form an inseparable mixture of the two compounds. The overall reaction was slightly basic which was believed to be the cause of this migration. By adding a few drops of acetic acid to the reaction mixture, the newly formed alcohols were readily protonated, negating this migration and generating diol 228 in quantitative yield. Direct oxidation of the primary alcohol in the presence of the secondary alcohol had already been shown to be ineffective. Furthermore, the protection of the primary alcohol with a silyl group was problematic due to ester migration issues during its subsequent deprotection. Since the isovaleric esters were already proven to be stable during hydrogenation, the bulky trityl bromide reagent was used to selectively protect the
primary alcohol affording compound 260. Installation of the third ester to give compound 261 followed by cleavage of the trityl protecting group using hydrogen yielded compound 232.

Scheme 84. Revisiting the methodology for the synthesis of compound 232

As described above, our initial efforts to generate diol 228 suffered from unwanted migration of the isovaleric ester. Before discovering that this migration could be avoided with the addition of acetic acid, an idea was proposed to generate a useful analogue possessing four isovaleric esters. This would thereby negate the dilemma of the ester migration. Toward this goal, compound 260 was esterified with isovaleric anhydride to yield triester 262 in an 80% yield
(Scheme 85). Hydrogenation was then done to cleave the trityl protecting group and provide compound 263.

Scheme 85. Synthesis of triisovaleric ester 263

With compound 263 in hand, the Brown allylation was attempted in order to gauge how large the substituent on the C-7 position (β to the aldehyde) can be before shutting down the reaction. An acetate had previously been shown to work, but it was unknown whether the isovalerate would make the molecule too sterically encumbered. Oxidation of the primary alcohol in compound 263 with Dess-Martin periodinane gave aldehyde 264 (Scheme 86). A Brown allylation of crude aldehyde 264 failed to produce any product, with only decomposition being observed. It was theorized that the initial allylation was not proceeding. However, upon using standard workup conditions (10% sodium hydroxide and 30% hydrogen peroxide), it was possible that the esters were being cleaved and the resulting tetraol was being lost to the aqueous layer. To test this theory, the Brown allylation was once again tried with aldehyde 264, but instead the reaction was quenched with brine. Isolation of clean aldehyde 264 proved that not only was the reaction failing to proceed (possibly because the isovalerates were too big), but that the sodium hydroxide/hydrogen peroxide workup was detrimental to retaining the ester functionality.
4.7.0 C-6 Methyl Analogue Synthesis

4.7.1 Synthesis of Truncated Didemnaketal A Analogues

In 2003, Reboud-Ravaux showed that the length of their lipopeptides was essential for dissociative inhibition of HIV-1 protease. While the tripeptides acted strictly as dissociative inhibitors, the tetramer was observed to be a mixed inhibitor, interacting with both the active site and the dimer site. The hexamer acted solely as a competitive inhibitor. This does not imply that all tetrapeptides are mixed inhibitors, as Schramm has synthesized a number of tetramers that act strictly as dissociative inhibitors. With so many problems associated with the Brown allylation, access to the final carbon skeleton of the didemnaketal A analogues was becoming increasingly challenging. Therefore, the synthesis of a series of truncated analogues was proposed whereby the final carbon fragment (C-1 to C-4) will be deleted (Figure 59). Regardless of the outcome for the enzymatic assays, these molecules will provide a pivotal step toward understanding the key interactions needed to cause dissociative inhibition of HIV-1 protease. If these analogues prove to be more potent than Rich’s analogue, then the synthetic ease to accessing compounds similar to these analogues and diversifying them will be a great advantage for future designs. If, however, these molecules are less potent, then it can be hypothesized that the C-1 to C-4 carbon fragment is required for optimal potency. Finally, comparison of compound 265 to its enantiomer as well as compound 266 with its enantiomer should also help to shed some light on which enantiomeric form is preferred for the inhibition of HIV-1 protease.
or if there is any requirement at all. This information will help to understand the specificity of the enzyme toward our dissociative inhibitors.

![Figure 59. Truncated analogues](image)

Access to the truncated analogue possessing the natural product esters (compound 265) was achieved through the esterification of alcohol 232 with propionic anhydride (Scheme 87). Following the same methodology (except starting from L-phenylalanine) enantiomer ent-265 was also synthesized.

![Scheme 87. Synthesis of analogue 265](image)
While synthesizing compound 232, the acetate ester analogue 269 was being synthesized in parallel (Figure 60). Therefore, following a similar methodology to compound 232, compound 274 was generated in five steps from diol intermediate 226 (Scheme 88). Even in the presence of

![Figure 60. Compound 269](image_url)

Scheme 88. Synthesis of intermediate 274
acetic acid, a small amount of ester migration was observed during the hydrogenation of acetal 270. The truncated analogue 266 was obtained from esterification of alcohol 274. Alternatively, compound 266 was synthesized using a more direct route from diol 271 (Scheme 89). The low yield for both reactions was attributed to tetraacetate 266 being difficult to isolate during purification as many standard tlc stains failed to reveal the product. This issue can be solved in the future with the use of the department’s new liquid-chromatography mass spectrometer (LC-MS). The enantiomer ent-266 was generated using the same methodology but starting from L-phenylalanine.

![Scheme 89. Synthesis of analogue 266](image)

The propionic ester analogue 267 was synthesized from the intermediate diol 226 using the more direct and efficient route (Scheme 90). Finally, the tetraisovalerate compound 268 was synthesized from compound 263 in a 90% yield (Scheme 91). Alternatively, the more direct approach could again be used to esterify diol 228 with isovaleric anhydride to give tetraester 268 in a similar yield. Both analogues 267 and 268 were synthesized with the natural product stereochemistry for the C-6 and C-7 positions.
4.7.2 Re-investigation of the C-8 Stereochemistry

As stated previously (section 4.3.2) the stereochemistry at the C-8 position was determined to be the epimer of the natural product based on the polar Felkin-Ahn model, the Conforth model and the spectroscopic information available. The full carbon skeleton of the C-6 methyl analogue 105 could not be synthesized at the present time, but the truncated analogue 265...
still offered an excellent scaffold in which to compare the proton shifts and coupling constants with the natural product data as described by Faulkner (Table 11).\textsuperscript{121,123} Compound 265 is missing the methyl group at the C-10 position, which may cause the chemical shifts for the surrounding carbon centers to shift upfield slightly. Nevertheless, the protons at the C-7 and C-8 positions were used for comparison since they both should be far enough away from the truncated ends thus minimizing the effect on their chemical shifts. The natural product, didemnaketal A (32), shows the C-7 and C-8 proton shifts to be at 5.16 (dd) and 5.22 (t), respectively. The C-7 and C-8 proton shifts for compound 265 were observed at 5.11 (dd) and 5.12 (m), respectively. These chemical shifts for both the \textsuperscript{1}H NMR and \textsuperscript{13}C NMR correspond closely to that of the natural product. However, the coupling constant between the C-7 proton and the C-8 proton is 2 Hz in the natural product and 5.6 Hz in analogue 265. This evidence further supports the earlier notion that the epimer at the C-8 position was synthesized during the allylation reaction.

<table>
<thead>
<tr>
<th></th>
<th>Didemnaketal A (32) \textsuperscript{1}H NMR</th>
<th>Analogue 265 \textsuperscript{1}H NMR</th>
<th>Didemnaketal A (32) \textsuperscript{13}C NMR</th>
<th>Analogue 265 \textsuperscript{13}C NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-7</td>
<td>5.16 (dd, 9, 2)</td>
<td>5.11 (dd, 9.6, 5.6)</td>
<td>72.9</td>
<td>73.1</td>
</tr>
<tr>
<td>C-8</td>
<td>5.22 (t, 10)</td>
<td>5.12 (m)</td>
<td>70.9</td>
<td>71.9</td>
</tr>
</tbody>
</table>

Table 11. Comparison of didemnaketal A (32) and truncated analogue 265

To fully confirm the stereochemistry at the C-8 position, two plausible methods should be attempted in the future and compared with the data already obtained. The first idea would be to use Mosher esterification.\textsuperscript{255} In 1969, Mosher established a method to determine the enantiopurity of any given compound using a Mosher acid (\(\alpha\)-methoxy-\(\alpha\)- (trifluoromethyl)phenylacetic acid, MTPA). Advantages of using this acid include: 1) the
generally excellent separation of both proton and fluorine NMR signals of the diastereomers; 2) the presence of the trifluoromethyl group permitting the use of fluorine NMR, which occurs in an uncongested region of the spectrum; 3) its stability toward racemization even under severe conditions such as acidity, basicity and temperature; 4) its relative ease of preparation and resolution; 5) its inherent volatility which allows lower molecular weight derivatives to be purified and 6) its versatility, i.e. it may be used for determination of enantiomeric composition of primary and secondary amines as well as carbinols. The method works by first obtaining an NMR of the original compound. Then both the R- and the S-Mosher ester are generated and the change in chemical shifts analyzed. The Mosher ester will preferentially position itself to reduce the steric interactions between the bulky R¹ and R² groups and the carbonyl (Figure 61). Furthermore, the dipole of the electronegative CF₃ group will align in order to minimize its interaction with the carbonyl’s LUMO axis. When using the S-Mosher ester, the phenyl group and the R¹ group will be on the same side and R¹ will be shielded and shift upfield with respect to the original spectrum. The R² group will feel a through space electron withdrawing effect from the methoxy group and subsequently be deshielded and shifted downfield relative to the original spectrum. In contrast, using the R-Mosher ester will deshield the R¹ group (shifts downfield) and shield the R² group (shifts upfield).

![Figure 61. Mosher esterification method for determining absolute and relative stereochemistry](image)

The second method, Murata’s J-based approach, uses coupling constants to evaluate the relative stereochemistry of acyclic structures. In particular, vicinal ¹H-¹H, geminal
$^{13}$C-$^1$H and vicinal $^{13}$C-$^1$H signals are used for structural analysis and help to determine the rotomers of the compound (Figure 62). In order to provide accurate results, the proton signals must be adequately separated from the carbon of interest. As a general rule, the number of bonds separating asymmetric centers should be less than three. Murata’s method assumes that there are three staggered rotomers that the molecule can adopt for each rotatable carbon-carbon bond plus the possibility of either an erythro or a threo conformation (total of six rotomers). This holds true for acyclic structures possessing hydroxyl and methyl substituents. Murata analyzed over 30 diastereorelationships for over 10 natural products and synthetic compounds and found that the angle of these staggered rotomers varied by less than 10° compared to his predictions. The $^3J_{H,H}$ coupling constants can be accurately obtained using decoupling difference spectra or COSY-type experiments. However, the $^3J_{C,H}$ and the $^2J_{C,H}$ coupling constants are harder to obtain accurately using HMBC or other spectroscopic techniques (only accurate to a single digit) due to low signal-to-noise ratios and limited digital resolutions for 2-D methods. The four conformers (A-1, A-2, B-1 and B-2) each have a distinct pattern for their coupling constants (Figure 62). The conformers A-3 and B-3 possessing an H/H anti orientation can not be distinguished using the $^3J_{H,H}$, $^3J_{C,H}$ and $^2J_{C,H}$ coupling constants. However, acyclic organic compounds with methyl and hydroxyl groups generally organize in a C/C anti orientation to which no NOE interaction should be seen between H$^1$ and H$^4$ (B-3 conformer). If the orientation is H/H anti C/C gauche (conformer A-3), then the protons H$^1$ and H$^4$ should be close enough to show a NOE interaction. Thus, all six conformers can be discriminated and their relative stereochemistry (threo and erythro) assigned. Once assigned, the combination of two asymmetric centers should also now possess a distinct pattern. These assignments can continue through the acyclic chain until all stereocenters have been identified.
Figure 62. Murata’s J-based approach toward determining the relative stereochemistry of acyclic compounds
4.8.0 HIV-1 Protease Inhibition Assays

Kinetic assays of the truncated analogues with HIV-1 protease were attempted. Regrettably, the protein purchased from Sigma-Aldrich failed to cleave the fluorescent substrate and was thus perceived to be inactive. Future work toward these assays is still in progress.

4.9.0 Future Work

During the synthesis toward analogue 105, numerous issues pertaining to the various functional groups arose. In dealing with these problems, a great deal of information was gathered and has led to a better overall understanding of the sensitivity of different functional groups and their reactivities with respect to these particular molecules. Unfortunately, the final allylation step to install the C-5 stereocenter remains a significant challenge to overcome. The focus of future work should be concentrated on solving this particular issue. With the identification of an adequate asymmetric allylation, the remaining two steps will be trivial and have already been developed by this author. Progress toward novel allylations is ongoing in the lab, and some of these routes will be discussed in more detail later on in chapter 5. Having access to the allylation reaction will also allow for a direct comparison between compound 105 and compound 265, thus revealing whether the western fragment of didemnaketal A can be truncated for the purposes of probe design.

While the allylation to install the C-8 stereochemistry generated the epimer of the natural product, future work should be able to overcome this using either an asymmetric allylation or a Mitsunobu reaction. Combined with the work toward the C-5 allylation reaction, the natural product possessing the C-6 methyl group (compound 105) should be obtained rather quickly.

Once the allylation reaction is known, it would be interesting to re-visit the stereochemistry of these analogues to truly understand their structure based affinity toward the inhibition of HIV-1 protease. Rich’s most potent analogue (39) is epimeric at the C-8 position and was shown to be more potent than the natural product scaffold when inhibiting HIV-1 protease. It would therefore be worthwhile to generate new analogues whereby the natural framework of didemnaketal A is conserved while epimerising other stereocenters since the methodology developed by this author lends itself to the synthesis of either diastereomer for each stereocenter-generating step.

Finally, with the unfortunate inactivity of the HIV-1 protease purchased from Sigma-Aldrich, testing of the truncated analogues 265, ent-265, 266, ent-266, 267 and 268 still needs to
be completed. These results will have a significant impact on this group’s design of next-generation dissociative inhibitors of HIV-1 protease.
Chapter 5 – Synthesis of Didemnaketal A

5.1.0 Introduction

5.1.1 Origins of Didemnaketal

Didemnaketal A (32) and B (33) are degradation products of didemnaketal C (34) which was isolated from the magenta ascidian *Didemnum sp.* off the coast of Auluptagel, Palau (Figure 63).\(^{121}\) The family didemnidae consists of 578 species (21% of the total species), while the remaining 25 families of the ascidian class contain 2237 species.\(^{257}\) The didemnids can be found in a variety of habitats such as soft sediments, coral reefs and rocky substrates. It is not uncommon to find a diverse set of species (both from didemnidae and from other ascidian families) in a single location since these sea squirts tend to be colonial in nature, especially in tropical settings.

![Diagram of Didemnaketal A, B, and C](image)

**Figure 63.** Didemnaketals A, B and C

Over the past 40 years, marine natural products chemists have isolated nearly 1100 compounds from ascidians of which many have been used as clinical drugs, preclinical leads and potent bioactive molecules.\(^{258,259}\) As such, the ascidians are an excellent source of active compounds with novel structural motifs. The didemnids have played a prominent role, being responsible for at least 375 (~35%) known ascidian compounds. Some of the earliest didemnid chemicals isolated were the cyclic peptides ulicyclamide and ulithiacyclamide, reported by
Ireland and Scheuer in 1980, which were shown to have cancerostatic and anti-tumour properties, respectively (Figure 64).\textsuperscript{260} Despite the rich source of molecules for pharmaceutical discovery, many natural products chemists shy away from collecting representatives of the didemnidae family. This is mainly due to the colonial nature of these ascidians which makes separating out the different species a challenge. Therefore, access to sufficient material is difficult and sometimes unreliable.\textsuperscript{122} However, despite these limitations, the ability of the ascidians to combine with other species, and in many cases still maintain individual genomic signatures, offers a great advantage by increasing the potential number of available natural products. Simply put, nature has found a way to develop its own combinatorial library and is just waiting for it to be harnessed.

\textbf{Figure 64.} Cyclic peptides ulicyclamide and ulithiacyclamide isolated from didemnidae

The didemnids are able to synthesize a variety of natural products and metabolites, but also play host to symbiotic microorganisms that possess their own individual biosynthetic pathways.\textsuperscript{261} The biosynthetic origin of the ascidian metabolites is therefore an interesting one. Symbiotic bacteria that live among the ascidians have been identified and are responsible for producing many complex “ascidian” molecules.\textsuperscript{261-265} Evidence to support the claim that marine animal compounds originate from bacteria is often based on the structural similarity between these marine molecules and those produced naturally by similar bacteria.\textsuperscript{122} One of the most common and well-studied cyanobacteria, \textit{Prochloron didemni}, found in the ascidians exhibits
unique properties such as an unusual photosynthetic apparatus.\textsuperscript{266} The \textit{Prochloron} can provide nearly all the nitrogen and carbon required for the ascidian’s survival via photosynthesis and nitrogen recycling.\textsuperscript{122} This allows the ascidians to thrive in nutrient-low environments. There are other symbiotic bacteria that provide chemicals to the tunicates (other cyanobacteria and proteobacteria), but these bacteria have yet to be studied in full. Some of the more interesting compounds synthesized by the \textit{Prochloron} bacteria are lipids \textbf{278} and \textbf{279} whose applications may include biofuels and mycosporine \textbf{277} which shields the ascidian from ultraviolet radiation (Figure 65).

\textbf{Figure 65.} Compounds originating from \textit{P. didemni}

Didemnaketal C is a polyketide and closely resembles molecules made by bacteria rather than the polypeptides produced by the ascidians themselves. Therefore, it is most likely that didemnaketal C is produced by a symbiotic bacteria rather than the ascidian. Unfortunately, there remains no evidence to suggest the true application of this molecule with respect to the ascidian and its survival. Nevertheless, the degradation product of didemnaketal C (didemnaketal A) remains an interesting target from a synthetic perspective and while many groups have attempted its total synthesis\textsuperscript{130,132-134,136-138,142} none have succeeded at the present time.

\textbf{5.1.2 Potential Coupling Reactions in the Design of Didemnaketal A}

Didemnaketal A possesses an acyclic polyol western fragment and a spiroketal eastern fragment. In designing a convergent synthesis, the natural product can be divided into two roughly equal coupling partners whereby there are three potential points along the carbon skeleton in which to couple the two halves (Scheme 92). The first potential coupling occurs between C-8 and C-9 and would involve an aldol reaction. The advantage of this route is that aldehyde \textbf{182} has already been synthesized during the design of the C-6 methyl analogues.
Moreover, the spiroketal coupling partner 280 is partially symmetrical and should require fewer steps to access. Ideally, modifications to the molecule post-coupling should be minimized in order to achieve an optimal convergent synthesis (i.e. decrease the total number of linear steps). The disadvantages of the aldol coupling route are the need to install the C-1 to C-4 carbon fragment (potentially 3 steps) and install the ester functionality after coupling the two halves. Furthermore, it may not prove trivial to install the C-10 methyl group stereoselectively.

Scheme 92. Potential cleavage points leading to a convergent synthesis of didemnaketal A

The second possibility is to couple the molecule between C-9 and C-10, most likely via a β-alkyl Suzuki cross coupling reaction (Scheme 92). One of the advantages of this route is the
variety of potential alkyl coupling partners 281 that can be used. Previous literature has shown excellent results when using alkyl halides, alkyl triflates and alkylborates.\textsuperscript{267,268} In fact, Fuwa employed a Suzuki-Miyaura cross coupling reaction involving an alkylborate during the synthesis of the spiroketal eastern half of didemnaketal B, albeit not to produce the C-9/C-10 bond.\textsuperscript{142,269} Compound 280, meanwhile could potentially be converted to a vinyl triflate, vinyl halide or vinyl phosphonate en route to a β-alkyl Suzuki reaction. Thus, there exists a large variety of compounds to mix-and-match in order to achieve the desired product. Similarly to the aldol coupling, the use of a partially symmetrical spiroketal fragment 280 should also decrease the total number of steps during the synthesis. Finally, standard Suzuki conditions should be tolerant of the ester functionality and will reduce the number of post-coupling steps. However, if these standard conditions fail, then the alkylborate can be used as described by Fuwa.\textsuperscript{142} Unfortunately, installation of the alkylborate using Fuwa’s conditions will involve a strong base and could possibly lead to β-hydride elimination in compound 281. Furthermore, using Fuwa’s protocols, it is unknown whether the esters on compound 281 will survive. If not, then an orthogonally protected route will be required which will add a number of post-coupling reactions to install the various ester functionality. While there are other ways to design alkylborates\textsuperscript{267} involving mild conditions which may be tolerant of the ester functionality, the precedent by Fuwa in designing a similar molecule will be attempted first. A third disadvantage of the β-alkyl Suzuki cross coupling reaction is the need to selectively enolize one of the ketones in compound 280 which will likely be quite challenging.

The third route involves the formation of the C-10 and C-11 bond using a Nozaki-Hiyama-Kishi (NHK) coupling (Scheme 92).\textsuperscript{270} The advantages of this reaction are the tolerance of the NHK conditions toward the labile esters on the western fragment 282 and the selectivity toward reaction with the aldehyde instead of the ketone in compound 283. Other advantages are fewer post-coupling reactions to access the natural product and less guess work on which compounds will be required to achieve an optimal coupling. The latter point is particularly useful since designing a variety of molecules in a longer synthesis will prove taxing. One of the disadvantages of this route is the need to produce an asymmetric spiroketal 283. Compound 280 is a likely precursor to compound 283, in which case the stereocenter at the C-11 position would first be destroyed in order to truncate the spiroketal half only to then install it again using a NHK
coupling. Furthermore, it is unknown whether the diastereoselectivity of the NHK coupling will in fact regenerate the desired stereoisomer at the C-11 position.

All three potential coupling routes have their advantages and disadvantages. Since it is unknown which route will be optimal, methodology toward all three approaches will be developed using common intermediates (Figure 66). The western coupling partner will be constructed from compound 98, while the spiroketal eastern fragment will be synthesized from nonenone 94. Acetal 98 has already been synthesized during the design of the C-6 methyl analogues. A colleague, Jason Davy, is working on the spiroketal eastern half while I simultaneously synthesize the acyclic western fragment. Since the design of the natural product utilizes a convergent synthesis, an enantioselective approach was required. This would avoid the generation of four possible stereoisomers when coupling the two fragments together.

![Figure 66](https://via.placeholder.com/150)

**Figure 66.** Key intermediates toward the pentaester western coupling partner and the spiroketal eastern coupling partner

### 5.1.3 Retrosynthesis of Didemnaketal A Possessing a Key NHK Coupling

Of the three potential coupling reactions, the NHK showed the most promise and was therefore chosen as the first attempt toward the natural product. Since the ester groups were expected to be tolerant of the NHK conditions, the simultaneous synthesis of both the western half of didemnaketal A and the C-6/C-10 methyl analogues was also employed. The spiroketal eastern fragment was envisioned to proceed through a ring opening/cross metathesis of nonenone 94 with methyl vinyl ketone followed by an asymmetric Sharpless dihydroxylation which should afford tetraol 95 (Scheme 93). An acid-mediated spiroketalization followed by monoprotection of one of the hydroxyl groups and subsequent selective oxidation of the other α-ketone should then afford the desired aldehyde 96. For the western acyclic fragment, the previously synthesized
aldehyde derived from acetal 98 was expected to undergo an asymmetric alkynylation followed by protection of the newly formed alcohol with a benzyl group. Hydrozirconation of alkyne 284 with a halogenation workup should provide compound 285 which should then undergo an acetal migration and oxidation to yield 286. An asymmetric allylation of aldehyde 286 followed by a stereoselective Grubbs cross metathesis and alcohol protection should afford the coupling partner.
Protection of the alcohol at the C-5 position may be altered to an esterification if the coupling between compounds 287 and 96 shows tolerance to ester functionality. If not, orthogonal deprotection and esterification can be accomplished after the NHK coupling. Following the coupling reaction, a stereoselective hydrogenation should set the desired C-10 stereocenter and cleave the benzyl protecting group in a single operation, after which esterification with isovaleric anhydride should provide didemnaketal A. Once the key steps have been determined, this methodology can be adapted to install the ester groups as the alcohols are formed, thus minimizing the total number of steps.

Scheme 94. Retrosynthesis of the C-6/C-10 methyl analogue

Previously (chapter 4), the importance of generating both the C-6 and C-6/C-10 methyl analogues was described. Access to the C-6/C-10 methyl analogue 106 could be achieved using a nearly identical route to that for the natural product (Scheme 94). Therefore, once compound 287 is in hand, orthogonal deprotection of the C-5 and C-7 alcohols and subsequent esterification can be accomplished. The desired analogue 106 should be obtained using the same NHK coupling conditions optimized for the natural product followed by a stereoselective hydrogenation to install the C-10 methyl group and esterification with isovaleric anhydride. Similarly to the natural product synthesis, this methodology can be adapted to include prior esterification of the C-5 and C-7 alcohols to reduce the overall number of steps.
5.2.0 Total Synthesis of the Didemnaketal A Western Fragment for use in a Nozaki-Hiyama-Kishi Coupling

5.2.1 Preparation of Compound 280 using an Asymmetric Alkynylation

Acetal 98 was previously synthesized en route to developing the truncated C-6 methyl analogues (chapter 4). Unfortunately, the C-8 epimer of the natural product was isolated when allylating the subsequent aldehyde 182. However, it was unknown whether the same outcome would occur during an alkynylation of aldehyde 182. As was the case with the analogue design, the methodology for the natural product was initially developed using the less expensive L-phenylalanine enantiomer.

To understand the stereochemical bias of substrate ent-182, no chiral catalyst was initially used when generating alkyne ent-289. Trimethylsilyl acetylene (Scheme 95; entry 1) was used as a test substrate to develop the ideal conditions for the alkynylation of aldehyde ent-182 and resulted in the generation of alkyne ent-288 as a 1:1 mixture of diastereomers. This result suggested that there was little stereochemical control by the substrate. Regardless, the reaction was repeated using propargyl trimethylsilane but failed to generate any product (entries 2-4).

![Scheme 95. Substrate-controlled alkynylation of aldehyde ent-182](image.png)
2 and 3). It was hypothesized that a terminal alkyne was required in order for the reaction to proceed. Addition of a nucleophile to aldehyde ent-\textbf{182} using a chelating reagent was predicted to follow the chelating Felkin-Ahn model and would be expected to generate the propargyl alcohol with the desired stereochemistry. Therefore, propargyl magnesium bromide was used (entry 4) in the hope that chelation of the substrate would bias the stereochemical outcome. Unfortunately, this too resulted in the isolation of clean starting material ent-\textbf{182}.

Although the substrate-controlled alkynylation was unsuccessful, an asymmetric alkynylation was attempted.\textsuperscript{271,272} Access to propyne was rather limited, so 1-hexyne was used as a model substrate. Using (S)-BINOL as a chiral catalyst, both the indium (III)/BINOL complex and the titanium dinuclear zinc/BINOL complex (Scheme 96; entries 1 and 2 respectively) failed to provide any desired product.

![Scheme 96. Asymmetric alkynylation of aldehyde ent-\textbf{182}](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(\text{Bu} = \text{H}) InBr(_3), (S)-BINOL, Cy(_2)NMe, CH(_2)Cl(_2), 40 °C, 2d</td>
<td>ent-\textbf{182}</td>
</tr>
<tr>
<td>2</td>
<td>(\text{Bu} = \text{H}) Ti(iPrO)(_4), (S)-BINOL, Et(_2)Zn, toluene, Et(_2)O, 25 °C, 4 hrs</td>
<td>ent-\textbf{182}</td>
</tr>
</tbody>
</table>

Since the allylation of aldehyde \textbf{182} provided a single diastereomer, it seemed a shame not to further utilize this reaction in the development of the natural product methodology. Although compound \textbf{225} is epimeric at the C-8 position compared to the natural product based on the evidence provided thus far, this issue was expected to be fixed after developing a useful route to the full carbon skeleton. Either an asymmetric allylation of aldehyde \textbf{182} or a Mitsunobu reaction to epimerize the C-8 stereocenter during esterification should allow access to the desired
stereochemistry for the natural product. With this in mind, a modified synthetic pathway was employed making use of the previous analogue methodology.

5.2.2 Revised Retrosynthesis of Didemnaketal A

Compound 102 was envisioned to begin with the oxidation and asymmetric allylation of acetal 98 which should provide compound 103 (Scheme 97). Benzyl protection of the alcohol and a Wacker oxidation to convert the terminal olefin to a ketone should afford compound 99. Using some functional group interconversion, compound 100 should be obtained and then undergo subsequent asymmetric allylation, esterification and cross metathesis to yield compound 101. Installation of a vinyl halide (or triflate) terminus should then provide compound 102. Finally, NHK coupling with Jason’s eastern half (96) followed by a stereoselective hydrogenation and then esterification should give the desired natural product.
Scheme 97. Revised retrosynthesis of didemnaketal A utilizing a key NHK coupling
5.2.3 Preparation of a Vinyl Triflate Involving a Cyclic \( \rho \)-Methoxybenzylidene Acetal

As stated previously, the C-8 epimer 225 will be used to develop the methodology leading to the carbon skeleton of the western fragment of the natural product. Furthermore, its less costly enantiomer, derived from \( L \)-phenylalanine, will be used.

A Wacker oxidation\(^{273} \) of olefin ent-235 (derived from compound ent-225) provided ketone ent-291 in good yield (Scheme 98). Unfortunately, attempts to install either a vinyl bromide, iodide or triflate were met with significant challenges (Scheme 99). Attempted formation of the vinyl bromide ent-292 using triphenylphosphite and bromine (Scheme 99; entry 2) led to decomposition of starting material ent-291. At first it was thought that the phenyl groups might be sterically clashing with the cyclized acetal and thus a longer reaction time would be needed to push the reaction to completion. If a smaller phosphite was used, then the reaction time could be minimized and decomposition of the starting material may not be as significant. Therefore, the smaller triethylphosphite (entry 1) was tried. Unfortunately, this too failed to produce any desired product in the early stages of the reaction and led to decomposition at longer reaction times. An attempt to generate a vinyl iodide using chromium (II) chloride also led to decomposition. The inefficiency of these three reactions was believed to be due to the sensitivity of the acetal. Therefore, formation of a vinyl triflate was attempted using a bulky base and Comins reagent (entries 4 and 5). Unfortunately, when using lithium bis(trimethylsilyl) amide the conjugated ketone ent-293 was observed and when using potassium bis(trimethylsilyl) amide the conjugated vinyl triflate ent-294 was observed. It would seem that under these conditions, the benzyl protecting group is most likely being cleaved followed by the deprotonation of the acidic \( \alpha \)-proton to generate the trans olefin. To avoid this, a milder base

![Scheme 98. Wacker oxidation of olefin ent-235](image)

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(2,6-lutidine) was tried with triflic anhydride (entry 6) but resulted only in the isolation of clean ketone ent-291.

\[ \text{Scheme 99. Synthesis of a vinyl halide (or triflate) using a cyclic acetal scaffold} \]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>X</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(EtO)₃P, Br₂, Et₃N, CH₂Cl₂, 25 °C, 18 hrs</td>
<td>Br</td>
<td>decomposition</td>
</tr>
<tr>
<td>2</td>
<td>(PhO)₃P, Br₂, Et₃N, CH₂Cl₂, 25 °C, 18 hrs</td>
<td>Br</td>
<td>decomposition</td>
</tr>
<tr>
<td>3</td>
<td>CrCl₂, CHI₃, THF, 40 °C, 28 hrs</td>
<td>I</td>
<td>decomposition</td>
</tr>
<tr>
<td>4</td>
<td>1. LiHMDS, THF, –78 °C, 30 min. 2. Comins Reagent, –78 °C, 15 min</td>
<td>OTf</td>
<td>ent-293</td>
</tr>
<tr>
<td>5</td>
<td>1. KHMDS, THF, –78 °C, 30 min. 2. Comins Reagent, –78 °C, 15 min</td>
<td>OTf</td>
<td>ent-294</td>
</tr>
<tr>
<td>6</td>
<td>2,6-lutidine, CH₂Cl₂, (CF₃SO₂)₂O, 25 °C, 19 hrs</td>
<td>OTf</td>
<td>ent-291</td>
</tr>
</tbody>
</table>

While the sensitivity of the acetal can easily be addressed by removing it prior to the installation of the vinyl halide (or triflate), it was uncertain whether the cleavage of the benzyl group might be solved at the same time. Since the vinyl triflate seemed more promising than the vinyl halides it was used for further development of this methodology.

### 5.2.4 Preparation of the Vinyl Triflate From a De-acetalated Synthon

When designing this revised approach, a previously established acetal migration reaction (chapter 4) was to be utilized to cleave the acetal protecting group. This acetal migration reaction requires the use of diisobutylaluminum hydride which would also cleave any ester functional groups present in the molecule. Therefore, the route to the vinyl triflate will be designed using
orthogonally protected alcohols. The revised synthetic plan began with the previously established acetal migration conditions to convert acetal ent-235 to alcohol ent-295 with an 81% yield (Scheme 100). Subsequent protection of the C-5 hydroxyl group with tert-butyldimethylsilyl triflate provided compound ent-296 followed by a Wacker oxidation to yield the intermediate ketone ent-297. Attempted formation of the vinyl triflate using triflic anhydride resulted in decomposition. However, the use of Comins reagent provided the desired vinyl triflate in a 32% yield as the terminal olefin only.

**Scheme 100. Synthesis of vinyl triflate ent-298**

While developing this synthetic route, progress toward the spiroketal fragment generated an interesting result. At the outset to this research, it was unknown whether the predicted stereochemistry for the eastern fragment could be achieved. However, following ring opening/cross metathesis of nonenone 94 with ethyl vinyl ketone, Jason Davy produced an intermediate which upon dihydroxylation and acid-induced spiroketalization yielded the spiroketal fragment with the desired natural product stereochemistry. More importantly, this reaction generated the correct stereochemistry at the C-11 center. With this result, it would seem foolhardy to obliterate this center and then re-install it later on with the Nozaki-Hiyama-Kishi
reaction. Since it seemed less problematic to truncate the acyclic coupling partner, a new synthetic route was considered where the western fragment would be shortened by two carbon atoms. In doing so, the coupling of both halves of the natural product would now use the β-alkyl Suzuki coupling described earlier. In light of this new evidence the above synthesis of the vinyl triflate was left unoptimized.

5.3.0 Total Synthesis of the Didemnaketal A Western Fragment for use in a β-alkyl Suzuki Cross Coupling

5.3.1 Second-Generation Retrosynthesis of Didemnaketal A

The eastern coupling partner 302 currently being developed by Jason Davy was still envisioned to come from nonenone 94 but now possessed an additional two carbons as well as the desired stereochemistry at the C-11 position. Truncation of the western fragment was envisioned to proceed via a poisoned Grubbs I catalyzed isomerization of the terminal olefin in benzyl protected 103 to an internal alkene (Scheme 101). Oxidation of this newly formed double bond followed by reduction of the aldehyde should generate a primary alcohol. This alcohol can then be converted to either a halide or a mesylate (compound 299). Acetal migration of compound 299 and oxidation of the subsequent alcohol should give compound 300. Finally, an asymmetric allylation and Grubbs cross metathesis should furnish the desired compound 301. Prior to the β-alkyl Suzuki cross coupling reaction, the alkyl halide can be converted to either an alkylborane, an alkylborate or an alkylboronate.269 Previously, Fuwa utilized an alkylborate during the synthesis of the spiroketal fragment of didemnaketal B.142 Therefore, initial attempts will focus on synthesizing a similar boron adduct for use in our cross coupling reaction. As has been the case previously, the p-methoxybenzyl protecting group at the C-7 alcohol may prove problematic when undergoing the asymmetric allylation of compound 300. Therefore, this protecting group may need to be cleaved prior to the allylation. With the two coupling partners in hand, a Suzuki cross coupling reaction and orthogonal deprotection and esterification should yield the desired natural product.
Scheme 101. Second-generation retrosynthesis of didemnaketal A

5.3.2 Synthesis of Compound 301

Starting from the previously synthesized compound ent-235, isomerization of the terminal olefin to the internal olefin proceeded to give compound ent-303 in quantitative yield.
and a 5:1 ratio of geometric isomers (Scheme 102). Oxidation of alkene ent-303 with osmium tetroxide provided the crude aldehyde which was unstable to column chromatography. Therefore, it was taken directly on to the reduction step to provide alcohol ent-304 in a 63% yield over two steps.

Scheme 102. Synthesis of alcohol ent-304

Generation of the alkyl bromide under Appel conditions was unsuccessful at room temperature. It was hypothesized that the triphenylphosphine might be too sterically encumbered and was causing unfavourable steric interactions with the substituents around the six-membered ring of compound ent-304. Upon heating to reflux, evidence suggests that alkyl bromide ent-305 may have been generated but immediately reacted with alcohol ent-304 to produce alkyl ether ent-308 (Scheme 103; entry 1). Alternatively, since the reaction was in acidic media, the generation of the ether may have occurred without the intermediate alkyl bromide ent-305. Attempts to buffer the solution with the addition of triethylamine (entry 2) resulted in the isolation of clean alcohol ent-304. Efforts were made using other brominating agents such as N-bromosuccinimide but were equally ineffective (entry 3) and led to neither desired product nor starting material being observed upon heating to reflux in dichloromethane (entry 4). By contrast, generation of the iodide through tosylation of the alcohol proved successful but only gave a 12% yield (entry 6). Again steric interactions of the tosylate group interacting with the substituents on the ring were believed to be the cause of this low yield. Therefore, the reaction was repeated using the smaller mesylate to generate alkyl bromide ent-305 (entry 5) but an
increase in yield was not observed. However, this issue was believed to be caused by the ineffective generation of the intermediate sulfoxyl species required for adequate mesylation using the conditions in entry 5. Modification of these conditions (entry 7) generated the mesylate effectively but upon subjection of compound ent-307 to the acetal migration step, the reaction failed to go to completion. Therefore, challenges associated with the steric effects of the acetal ring meant a slight revision was required in the route.

Scheme 103. Synthesis of alkyl halides and alkyl mesylates using an acetal scaffold
5.3.3 Revised Synthesis of Compound 301

Issues involving the sterically encumbered six-membered ring meant that the acetal needed to be cleaved prior to the formation of the alkyl halide. Access to compound ent-310 was envisioned to proceed via two possible routes. The advantages of both routes were the use of previously established chemistry and high yields (Scheme 104). Previous work into synthesizing vinyl triflate ent-298 had provided compound ent-296. This substrate was isomerized using a poisoned Grubbs I catalyst to provide the desired compound ent-310 (Route A). Alternatively, compound ent-235 was isomerized first followed by subsequent acetal migration and tert-butylidydimethylsilyl protection of the primary alcohol to yield compound ent-310 (Route B). While both routes are high yielding, route A has a slight advantage by avoiding geometric isomers until the final step. This allows for easier interpretation of the proton NMR.

Scheme 104. Two possible routes for the synthesis of compound ent-310
Without the \( p \)-methoxybenzylidene acetal group, the more robust and scalable ozonolysis of olefin ent-310 could now be done rather than the unreliable oxidation using osmium (IV) tetroxide. In the absence of base, oxidation of alkene ent-310 to the aldehyde proceeded cleanly. However, the acidity of the solution from quenching the ozonide with dimethyl sulfide removed the tert-butyltrimethylsilyl protecting group thereby yielding hemiacetal ent-312. This was a significant result since it led to the realization that the generation of acid during the ozonide quenching step was the problem associated with the production of compound 182 early on in the synthesis. Thus, addition of a mild base 2,6-lutidine during the oxidation of compound ent-310 negated the formation of the hemiacetal. The oxidation of compound ent-310 was first attempted as a stepwise process involving isolation of the aldehyde, which could be purified by column chromatography, followed by subsequent reduction of the aldehyde to alcohol ent-311 with sodium borohydride. This reaction took approximately a day and a half, required two columns and gave a yield of 68%. Alternatively a one-pot reaction involving oxidation of olefin ent-310 followed by directly quenching the ozonide with sodium borohydride, rather than dimethyl sulfide, produced the desired alcohol ent-311 with the same yield, no chromatography and only required three hours to complete (Scheme 105). This optimization also no longer required the use of the mild base.

**Scheme 105. Ozonolysis of compound ent-310**

Mesylation of the primary alcohol in compound ent-311 proceeded in quantitative yield. However, addition of tetrabutylammonium bromide (Scheme 106; entry 1) failed to generate compound ent-313. While there are a variety of reactions that could be preformed to make the alkyl halide, the apparent robustness of the mesylate resulted in an intriguing idea to use it as a “protecting group”. Thus, the alkyl mesylate could potentially be used in a more generic coupling of the two halves en route to didemnaketal A. Alternatively, it could be carried through
the synthesis of the western fragment and converted to either an alkyl halide (and then an alkylborate) or an alkylborate prior to the β-alkyl Suzuki cross coupling. This idea was further enhanced by the notion that the C-7 p-methoxybenzyl protected alcohol was shown to be somewhat sterically crowding when attempting the Brown allylation and thus might serve to protect the mesylate from any S_N_2 reactions.

![Diagram](image)

**Scheme 106.** Mesylation of compound ent-311

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>X</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1. MsCl, pyridine, CH_2Cl_2, 25 °C, 24 hrs 2. Bu_4NBr, THF, 40 °C, 17 hrs</td>
<td>Br</td>
<td>ent-314</td>
</tr>
<tr>
<td>2</td>
<td>MsCl, pyridine, CH_2Cl_2, 25 °C, 24 hrs</td>
<td>OMs</td>
<td>ent-314 (100%)</td>
</tr>
</tbody>
</table>

With mesylate ent-314 in hand, the next step toward the methodology for the synthesis of didemnaketal A was to selectively remove the tert-butyldimethylsilyl protecting group without displacing the mesylate (Scheme 107). It was recognized that the reaction conditions chosen would need to be slightly acidic in order to rapidly quench the alkoxide intermediate before it could intramolecularly attack the C-9 mesylate and form pyran ent-316. Hydrogen fluoride pyridinium complex (Scheme 107; entry 1) was unsuccessful in removing the protecting group whereas an acetic acid buffered tetrabutylammonium fluoride solution (entry 2) only generated a small amount of compound ent-315. Since the fluoride-mediated deprotections proved unsuccessful, acid catalyzed cleavage was attempted using a DOWEX resin (entry 3) in the hope that purification might be easier since the resin could be filtered away. Unfortunately, only a small amount of compound ent-315 was isolated. The use of p-toluenesulfonic acid showed the most promise (entry 4), while pyridinium p-toluenesulfonate (entry 5) only generated a tiny amount of desired material. Finally, attention was turned to the oxidative cleavage of the silyl group which formed pyran ent-316 when using oxone (entry 6) and was ineffective when using sodium periodate (entry 7).

With the synthesis of compounds 314 and 315 in hand, access to two useful cross coupling intermediates was achieved. As well, access to the useful aldol coupling intermediate 182 has also been established. While undertaking the synthetic route toward the Suzuki cross coupling intermediate 301, new advances toward the methodology for the didemnaketal A analogues (chapter 4) led to some interesting chemistry. Not only did these new ideas help to circumvent earlier issues associated with generating the NHK coupling partner 102, but the total number of steps could be significantly reduced when compared to the Suzuki cross coupling route since an orthogonally protected scaffold was no longer required. As such, a reinvestigation into the NHK coupling partner 102 was done, knowing that the chemistry completed thus far in the Suzuki coupling route could still be used if required.
5.4.0 Revisiting the Total Synthesis of the Didemnaketal A Western Fragment for use in a Nozaki-Hiyama-Kishi Coupling

5.4.1 Third-Generation Retrosynthesis of Didemnaketal A

From the chemistry completed thus far, the major issues pertaining to the isolation of compound 102 were addressed in this third-generation retrosynthesis of didemnaketal A (Scheme 108). It is known that the stereoselective allylation at the C-5 position cannot occur in the presence of a p-methoxybenzyl protected β-alcohol. Furthermore, formation of the vinyl triflate from the subsequent ketone cannot be accomplished in the presence of the cyclic acetal due to steric interactions. With this in mind, compound 102 was envisioned to proceed from ketone 99 following two possible pathways. Following route A, the acetal in compound 99 should be fully cleaved to yield a diol. Selective oxidation of the primary alcohol followed by esterification using acetic anhydride should provide compound 100. Alternatively (route B), compound 99 could undergo an acetal migration with diisobutylaluminum hydride. It is expected that under these conditions, both the acetal migration and reduction of the ketone in compound 99 should occur to provide compound 317. However, oxidation of this diol followed by cleavage of the p-methoxybenzyl protecting group and subsequent esterification using acetic anhydride should yield compound 100, albeit in one extra step. Compound 100 (if it is stable) could then undergo an asymmetric allylation, esterification and cross metathesis to afford compound 101. Finally, installation of the vinyl halide (or most likely a vinyl triflate) should afford coupling partner 102. Coupling of compound 102 with compound 96 under NHK conditions followed by a stereoselective hydrogenation and esterification of the alcohols with isovaleric anhydride should yield the desired natural product.
Scheme 108. Third-generation retrosynthesis of didemnaketal A
5.4.2 Synthesis of Keto-aldehyde 100

As was the case with the previous attempts toward both the NHK coupling partner 102 and the Suzuki coupling partner intermediate 301, the C-8 epimer of compound 99 (compound 291) was used to develop the methodology.

Using previously established protocols, cleavage of the acetal in compound 291 was attempted using dilute acid (Scheme 109). Unfortunately, only starting material was isolated. Upon increasing the concentration of hydrochloric acid, compound 291 eventually decomposed.

![Scheme 109: Deacetalization of compound 291 following route A](image)

Using an alternative route (route B), compound 291 underwent an acetal migration, and concomitant ketone reduction, to afford diol 318 as a 2:1 mixture of diastereomers (Scheme 110). Oxidation of diol 318 with Dess-Martin periodinane generated keto-aldehyde 319.

![Scheme 110: Synthesis of keto-aldehyde 319 following route B](image)

Standard conditions to remove the p-methoxybenzyl protecting group (Scheme 111; entry 1) failed to afford the desired compound. It was proposed that alcohol 320 was produced, but the sensitivity of the molecule led to immediate decomposition. To try and trap the alcohol before it
could decompose, acetic anhydride was added to the solution (entry 2) but alas only compound 319 was isolated. Likewise, use of a mild base (entry 3) failed to yield any desired product. With these results, a variety of oxidizing conditions were screened in the hopes of achieving an adequate cleavage of the protecting group. While both ceric ammonium nitrate (CAN)\textsuperscript{276} and zirconium (IV) chloride resulted in the re-isolation of compound 319 (entries 5 and 7), the use of tin (IV) chloride,\textsuperscript{277} tin (II) chloride as well as iodine resulted in decomposition (entries 6, 9, and 10). Cerium (III) chloride\textsuperscript{278} gave back compound 319 but had epimerised a stereocenter (most likely the C-6 position which is alpha to the aldehyde) to generate a 1:1 mixture of diastereomers (entry 8). An attempt to selectively deprotect the p-methoxybenzyl group in the presence of a benzyl protecting group with hydrogen also led to decomposition (entry 4). Finally, Oriyama had previously shown that a p-methoxybenzyl group could be exchanged for a tert-butyldimethylsilyl

![Scheme 111](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DDQ, CH(_2)Cl(_2)</td>
<td>decomposition</td>
</tr>
<tr>
<td>2</td>
<td>DDQ, Ac(_2)O, DMAP, Et(_3)N, CH(_2)Cl(_2)</td>
<td>319</td>
</tr>
<tr>
<td>3</td>
<td>DDQ, 2,6-lutidine, CH(_2)Cl(_2), 24 hrs</td>
<td>319</td>
</tr>
<tr>
<td>4</td>
<td>H(_2) (1 atm), 10% Pd/C, CH(_3)OH, 24 hrs</td>
<td>decomposition</td>
</tr>
<tr>
<td>5</td>
<td>CAN, CH(_2)Cl(_2)/H(_2)O (9:1)</td>
<td>319</td>
</tr>
<tr>
<td>6</td>
<td>SnCl(_4), PhSH, CH(_2)Cl(_2)</td>
<td>decomposition</td>
</tr>
<tr>
<td>7</td>
<td>ZrCl(_4), CH(_3)CN</td>
<td>319</td>
</tr>
<tr>
<td>8</td>
<td>CeCl(_3)(\cdot)7 H(_2)O, NaI, CH(_3)CN, (\Delta)</td>
<td>319/epi-319</td>
</tr>
<tr>
<td>9</td>
<td>I(_2), MeOH, (\Delta)</td>
<td>decomposition</td>
</tr>
<tr>
<td>10</td>
<td>SnCl(_2)(\cdot)2 H(_2)O, AcCl, CH(_2)Cl(_2)</td>
<td>decomposition</td>
</tr>
<tr>
<td>11</td>
<td>TBSOTf, Et(_3)N, CH(_2)Cl(_2)</td>
<td>319/epi-319</td>
</tr>
</tbody>
</table>

**Scheme 111.** Conditions for cleaving the p-methoxybenzyl group in compound 319
protecting group.\textsuperscript{279} Although this would gain an extra step, it was believed that the silyl protecting group might prove easier to cleave. Unfortunately, only epimerized starting material was isolated (entry 11).

With such an unexpected road block toward the natural product, yet another route was required. Hall’s group has developed some elegant and interesting organoborane chemistry for over ten years. Of particular interest is their design of novel chiral catalysts for use in asymmetric allylations and crotylations.\textsuperscript{280-288} Interestingly, Hall’s group was able to install a vinyl bromide in a single step using optimized allylating conditions with excellent diastereoselective control.\textsuperscript{282,285,287,288} With this new information in hand, a revised methodology toward the synthesis of didemnaketal A was proposed.

\section*{5.4.3 Fourth-Generation Retrosynthesis of Didemnaketal A}

Based on precedent from Hall’s group, the natural product didemnaketal A was envisioned to proceed through an allylation of the known aldehyde \textsuperscript{182} followed by subsequent esterification of the newly generated alcohol which should afford compound \textsuperscript{321} (Scheme 112). Since the alkyl boronate species involved in the allylation is bulkier than the allylboronic acid pinacol ester used previously, it may be possible to bias the stereocenter at the C-8 position using substrate control to afford the desired diastereomer. Acetal migration of compound \textsuperscript{321} using a different reducing reagent than previously employed should selectively place the protecting group on the primary alcohol. Esterification with acetic acid should then afford compound \textsuperscript{322}. Cleavage of the \textit{p}-methoxybenzyl protecting group and subsequent oxidation of the primary alcohol should generate aldehyde \textsuperscript{323}. Finally, an asymmetric allylation, esterification, and cross metathesis should provide the desired coupling partner \textsuperscript{324}. It is unknown whether the vinyl bromide will participate in the cross metathesis reaction or whether it might act to poison the catalyst similar to the effects of methyl vinyl ether. If the vinyl bromide does interfere with the cross metathesis reaction, then coupling of compound \textsuperscript{322} with aldehyde \textsuperscript{96} could be done prior to the allylation and cross metathesis step. If the vinyl bromide is unreactive during the cross metathesis step, then coupling of compounds \textsuperscript{324} and \textsuperscript{96} using a NHK reaction followed by a stereoselective hydrogenation and esterification should yield the desired natural product.
Scheme 112. Fourth-generation retrosynthesis of didemnaketal A

1. NHK Coupling, 96
2. Stereoselective Hydrogenation
3. Esterification

1. Asymmetric Allylation
2. Esterification
3. Cross Metathesis

1. Deprotection
2. [O]

1. Acetal Migration
2. Esterification

1. Allylation
2. Esterification

R = \text{phenol}

\[ R = \text{phenol} \]
5.4.4 Determination of Allylating Conditions Using Compound 225 as a Model Substrate

Before undertaking the synthesis of compound 324, the reaction conditions were determined using compound 225 as a model substrate. The acetal migration of compound 225 has been done previously (vide supra) using diisobutylaluminum hydride to place the $p$-methoxybenzyl protecting group on the secondary alcohol. To migrate the acetal and protect the primary alcohol, sodium cyanoborohydride is used in the presence of an acid. Using a 10% aqueous solution of hydrochloric acid and sodium cyanoborohydride (Scheme 113; entry 1), the acetal was completely cleaved to yield diol 336. A more common acid for these reactions was trifluoroacetic acid. Using 1.2 equivalents and warming from 0 °C to room temperature (entry 2), a modest 31% yield of the desired compound 335 was obtained. The loss of yield was mainly attributed to a low conversion rate of only 48% but also suffered from side reactions such as migration to protect the secondary alcohol and formation of diol 336 occurring at room temperature. Therefore, to deal with the formation of side products the reaction was repeated but kept at 4 °C (entry 3). The yield for this reaction increased to 50% as did the conversion rate (71%) and there were less side reactions observed. To try and push the reaction to completion, 2 equivalents of sodium cyanoborohydride were used at room temperature (entry 4). While both the yield and conversion rate increased, the amount of diol 336 and secondary protected alcohol product increased as well. Finally, using 2.2 equivalents of sodium cyanoborohydride and allowing a gradual increase in temperature over 24 hours (entry 5), an optimal 68% yield for compound 335 was achieved with a 75% conversion rate. On going from a 20 milligram scale reaction to a 250 milligram scale reaction, the conversion rate decreased. Fortunately, starting material 225 can be re-isolated and the reaction redone.
Scheme 113. Acetal migration conditions using compound 225 as a test substrate

With compound 335 in hand, esterification with acetic anhydride generated compound 337 (Scheme 114). Cleavage of the p-methoxybenzyl protecting group using DDQ followed by oxidation with Dess-Martin periodinane gave aldehyde 339 in excellent yield. Using the same methodology, ent-339 was also synthesized and used in determining optimal allylation conditions.

Scheme 114. Synthesis of aldehyde 339
A number of substrate-controlled and asymmetric allylations have been performed previously in chapter 4. Since none were particularly successful, attempts were made using allyl silanes and allyl stannanes as well as the usual allyl boronates. \((-\text{ipc})_2\text{B(allyl)}\) was reacted with aldehyde \textbf{339} but only starting material was isolated (Scheme 115; entry 1). Based on previous reactions with this allylating reagent, a brine workup was used since cleavage of the borane with the standard 30\% hydrogen peroxide and 10\% sodium hydroxide was predicted to lead to decomposition of aldehyde \textbf{339}. Attention was then turned to allyltrimethylsilane (entry 2) which had no control over the stereoselectivity of the newly formed alcohol, but did provide the desired compound \textbf{340} and epi-\textbf{340} in a 59\% yield. Unfortunately, the two diastereomers could not be separated. Furthermore, a minor amount of compound \textbf{341} and epi-\textbf{341} were detected whereby the acetate functional group is observed to be migrating. The lack of stereochemical control in the absence of a chiral catalyst was encouraging, since with no substrate control for the reaction, a chiral catalyst should be more effective in controlling the stereochemical bias. Therefore, the reaction was repeated in the presence of (S)-\text{BINOL} (entry 3) but generated four inseparable compounds (\textbf{340}:epi-\textbf{340}:\textbf{341}:epi-\textbf{341}) in a 1:1:1:1 mixture. The titanium reagent \textbf{259} had been tried previously to allylate stereoselectively with little success and ultimately led to the same result here with no desired product able to be isolated. Hall’s group has had success with allylations and crotylations using bulky diol catalysts such as Vivol-F.\textsuperscript{282,285,287,288} However, one of the issues associated with these reactions is the competing non-catalyzed allylation which is especially prominent at higher temperatures. To see if this would be of concern, aldehyde \textbf{339} was reacted with allylboronic acid pinacol borane without a chiral catalyst and warmed to room temperature (entry 5). The recovery of aldehyde \textbf{339} meant that at least the non-catalyzed reaction was not expected to interfere even upon warming to higher temperatures. Using the optimized conditions developed by Hall but in the presence of a simplified diol catalyst (S)-\text{BINOL}, aldehyde \textbf{339} still failed to react.
While these results were disheartening, a final attempt was made using aldehyde 342 to test two allyl stannane reactions (Scheme 116). Unfortunately, both failed to provide any desired allyl product.
5.4.5 Synthesis of Compound 324

Previous work with model substrates has shown that although useful, sometimes slight differences in the structure or electronics of the compound can have drastic affects on the reaction. Therefore, it was envisioned that the allylation of the desired intermediate 323 might yield a better outcome.

The known compound 346 was produced from allene in decent yield over three steps on a 25 gram scale (Scheme 117). Allylation of aldehyde 182 with compound 346 was initially performed utilizing substrate-control rather than a chiral catalyst as per the original reaction of aldehyde 182 with allylboronic acid pinacol ester (Scheme 118; entry 1). While there was some improvement from the substrate for the stereochemical bias toward the desired compound 348 (5:1 \textit{dr} compared to >20:1 \textit{dr} for the original allylation), the overall yield was lower and the two diastereomers could not be separated as they could in the original allylation. To improve upon this diastereomeric ratio, the allylation was repeated but this time using compound 345 (entry 2).
When using compound 345, the ratio of diastereomers remained the same, but a drop in yield was noticed.

![Scheme 118. Allylation of aldehyde 182 with a 2-bromoallyl derivative](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
<th>Yield over two steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>346, CH₂Cl₂, −78 °C to 25 °C, 22 hrs</td>
<td>347:348 (5:1)</td>
<td>54%</td>
</tr>
<tr>
<td>2</td>
<td>345, CH₂Cl₂, −78 °C to 25 °C, 22 hrs</td>
<td>347:348 (5:1)</td>
<td>47%</td>
</tr>
<tr>
<td>3</td>
<td>346, BF₃•OEt₂, CH₂Cl₂, −78 °C</td>
<td>decomposition</td>
<td>n/a</td>
</tr>
<tr>
<td>4</td>
<td>346, CF₃CO₂H, CH₂Cl₂, −78 °C</td>
<td>decomposition</td>
<td>n/a</td>
</tr>
</tbody>
</table>

There are two types of models for predicting the stereochemical outcome during an allylation reaction involving metal reagents. Type I involves a closed cyclic, six-membered chair-like transition state and generally involves metals such as boron, lithium, aluminum and trichlorosilanes. The boron (or other metal) internally activates the carbonyl of the aldehyde and the resulting transition state becomes highly diastereoselective, diastereospecific and predictable. In contrast, a type II class uses an open transition state and involves metals such as trialkylsilanes and trialkylstannanes. An external Lewis acid is required to activate the carbonyl of the aldehyde. The diastereoselectivity is generally lower in these cases and is predicted by a Felkin-Ahn model. Using the Felkin-Ahn model, the desired compound 348 is predicted as the major product. Since the allylation of aldehyde 182 with compound 346 mainly afforded the undesired C-8 epimer 347, the use of either a chiral catalyst or an open transition state might help to improve the allylation reaction toward the desired product. Work by Hall’s group using Vivol-F has shown great results when overriding the stereochemical bias of the substrate. In these reactions tin (IV) chloride is used as a Lewis acid. If using an open transition state with a
trialkylsilane or trialkylstannane, then a Lewis acid would also be required. The acetal in compound 182 has previously been shown to be sensitive toward acidic media. Therefore, to establish whether compound 182 could survive either the reactions done by Hall or a Lewis acid directed allylation, it was reacted with compound 346 in the presence of boron trifluoride etherate (entry 3) and trifluoroacetic acid (entry 4). In both cases, the acetal group was cleaved, resulting in the decomposition of compound 182.

As was the case when performing the original allylation, the C-8 epimer 347 was taken on to develop the methodology toward the synthesis of didemnaketal A. Similarly, it can be used to design the C-6/C-10 methyl analogues whereby the C-8 stereocenter matches the C-8 stereocenter in Rich’s most potent molecule 39. Upon completion of this methodology, fixing the allylation step to generate compound 348 will then allow access to the natural product and another set of analogues using well established chemistry. The 5:1 mixture of compounds 347 and 348 were esterified using isovaleric anhydride (Scheme 119). Fortunately, the diastereomers could now be separated to give compound 349 cleanly. It was unknown whether the use of sodium cyanoborohydride to migrate the acetal group would also reduce the newly installed ester. Happily, the mild reducing agent generated compound 350 without any detectable cleavage of the ester, secondary protected alcohol or fully cleaved diol. The issue with the reaction going to completion was still problematic, especially on larger scale. Esterification with acetic anhydride followed by removal of the p-methoxybenzyl protecting group generated compound 352 in near quantitative yield. Finally, alcohol 352 was oxidized with Dess-Martin periodinane to provide aldehyde 353 which was filtered to remove excess DMP and taken on crude to the allylation step.

Using compound 352, the stereochemistry at the C-8 position was confirmed through comparison of the coupling constant ($^{3}$J$_{8,7}$) with the natural product. Comparison of the chemical shift was not as useful this time around since the vinyl bromide shifted the C-8 proton significantly as one would expect. The coupling constant between the C-7 and the C-8 protons in the natural product is 2 Hz. The coupling constant observed for compound 352 was 6.4 Hz which is similar to the coupling constant obtained for the C-8 epimer when using the original allylation reaction (6.9 Hz). Therefore, the C-8 center is confirmed as the epimer of the natural product based on the evidence thus far.
The remaining few reactions offered two potential problems. The first was whether the allylation would preferentially react with the aldehyde or whether the vinyl bromide might undergo some subsequent side reaction. The second was how the vinyl bromide would fare during the Grubbs cross metathesis. It was predicted that the vinyl bromide might act to poison the Grubbs catalyst similar to the case seen when using ethyl vinyl ether. In the event of either one of these issues arising, the vinyl bromide could always be coupled to the eastern half of didemnaketal A prior to either the allylation reaction or Grubbs cross metathesis. While slightly less elegant, this still remains a viable option.

The allylation conditions to install the C-5 stereocenter were attempted using ent-353. Using allyltrimethylsilane, aldehyde ent-353 reacted to form a small amount of alcohol ent-354 as a single diastereomer (Scheme 120; entry 1). The lower yield was attributed to side reactions.
occurring at the vinyl bromide end as confirmed by the isolation of these products during purification. While trying this reaction for a second time, only the side products were isolated and in a very low yield. Using the conditions developed by Hall’s group\textsuperscript{282,285,287,288} involving Vivol-F,\textsuperscript{290} no desired product was isolated as the same side reactions were observed (entry 2). It would seem that the vinyl bromide portion of the molecule reacts faster than the aldehyde even at \(-78 \, ^\circ\text{C}\). Therefore, it may be prudent then to couple the two halves of the molecule using the NHK reaction prior to the allylation. In light of these results, no further work was done toward developing new allylation conditions.

\begin{center}
\begin{tabular}{|c|c|c|}
\hline
Entry & Conditions & Result \\
\hline
1 & \(-\text{SiMe}_3, \text{SnCl}_4, \text{CH}_2\text{Cl}_2, -78 \, ^\circ\text{C}\) & \text{ent-354 (10\%)} \\
\hline
2 & allylboronic acid pinacol ester, \textit{R,R}-Vivol, \text{SnCl}_4, \text{Na}_2\text{CO}_3, 4 \, \text{Å MS}, \text{toluene}, -78 \, ^\circ\text{C} \text{ to } 4 \, ^\circ\text{C} & \text{no desired product} \\
\hline
\end{tabular}
\end{center}

\textbf{Scheme 120}. Allylation conditions to install the C-5 stereochemistry

\textbf{5.5.0 Future Work}

Work done toward the synthesis of didemnaketal A and the subsequent C-6/C-10 methyl analogue has endured some significant trials and tribulations. Nevertheless, a great deal of information is now known about the sensitivity of these molecules. With respect to the C-6/C-10 methyl analogue, there remain two pathways toward either the full carbon skeleton or the truncated fragment that still need to be accomplished. In light of the evidence provided during the synthesis of the truncated C-6 methyl analogues described in chapter 4, a C-6/C-10 methyl truncated analogue was envisioned to proceed from the esterification of alcohol \textbf{352} with propionic acid followed by a NHK coupling with acetaldehyde which should provide compound
Finally, esterification with isovaleric anhydride followed by subsequent stereoselective hydrogenation should provide the truncated analogue 356. This would be a useful substrate to have so as to directly compare it with the truncated C-6 methyl analogue 265. This will help to elucidate the contribution of the second methyl group in relation to the potency of these analogues toward the dissociative inhibition of HIV-1 protease.

Scheme 121. Proposed synthesis toward the C-6/C-10 methyl truncated analogue 356

Similarly, future work should focus on generating the full carbon skeleton to compare with the truncated analogue 356. This would be envisioned to proceed through the NHK coupling of compound 351 with acetaldehyde followed by esterification with isovaleric anhydride of the newly generated alcohol (Scheme 122). A stereoselective hydrogenation of compound 357 should set both the methyl group at the C-10 position and cleave the p-methoxybenzyl protecting group. Oxidation should then lead to compound 358. Finally, an asymmetric allylation, esterification and stereoselective cross metathesis should yield the full carbon skeleton of the C-6/C-10 methyl analogue 359.
Scheme 122. Proposed synthesis toward the full carbon skeleton of the C-6/C-10 methyl analogue 359

In regards to didemnaketal A, problems associated with the allylation reaction to set either the C-5 or the C-8 stereocenter have created the constant need for new or modified routes to the natural product. Therefore, it would be of the utmost importance to devote a significant amount of time toward designing an adequate means to allylate these molecules. This is important since both the total synthesis of didemnaketal A and the analogue design have been bottlenecked at these steps. Once optimized allylating conditions are in hand, access to the natural product and both analogues can be achieved since the remaining reactions have all been established by this author.
Chapter 6 – Experimental

6.0.0 General Remarks

All reactions were carried out in flame-dried glassware fitted with rubber septa under a positive pressure of argon. Dichloromethane, diethyl ether, methanol, and acetonitrile were purified by a solvent purification system, unless otherwise stated. Tetrahydrofuran and 1,4-dioxane were distilled over sodium and benzophenone. Dimethylformamide, benzene, triethylamine and toluene were distilled over calcium hydride. Liquid reagents were transferred via glass microsyringe. Organic solutions were concentrated at 35 °C by rotary evaporation under vacuum, unless otherwise stated. The vacuum line pressure for distillation was determined using a mercury manometer.

Optical activity was analyzed by a Rudolph Research Autopol III Automatic Polarimeter using a Rudolph Research Analytical 1.5 mL polarimeter cell. NMR was analyzed using a Bruker 500 MHz instrument and infrared spectroscopy (IR) was analyzed using a Perkin-Elmer FT-IR Spectrum Spectrometer 1000. High-resolution mass spectra were done at the UVic Proteomics Center on an Orbitrap Velos system by Thermo scientific. Samples of 1mg/ml diluted 1:100 in methanol or acetonitrile were directly infused at a 5-10ul/min flow rate through an ESI source. Spectra obtained at about 1Hz scanning rate 100–2000 range with 60,000 resolution and less than 1 ppm accuracy in most cases. Analytical thin-layer chromatography (TLC) was performed using aluminum plates pre-coated with silica gel (0.20 mm, 60 Å pore size, 230-400 mesh, Macherey-Nagel) impregnated with a fluorescent indicator (254 nm). Flash chromatography was performed as described by Still with either silica (60 Å, 63-200 µM, Caledon) or alumina (basic, acidic or neutral). Unless otherwise noted, all compounds isolated by chromatography were sufficiently pure (>95 % by NMR) for use in subsequent preparative reactions.

Assignment of proton and carbon NMR was done with the aid of 2-D NMR spectroscopy (COSY, NOESY, 1-D NOE, HSQC and HBMC). When 2-D NMR was unavailable, assignments were performed using shift prediction tables and coupling constants.
6.1.0 Triclosan Analogues

6.1.1 2,4,4′-trichloro-2′-methoxydiphenyl ether (1)

To a solution of irgasan (107; 184 mg, 0.636 mmol) and potassium carbonate (178 mg, 1.29 mmol) in 20 mL anhydrous tetrahydrofuran was added methyl iodide (0.16 mL, 2.6 mmol) dropwise via syringe. The resulting mixture was heated to reflux for 20.5 hours, after which saturated ammonium chloride (20 mL) was added. The aqueous layer was extracted with three portions of dichloromethane (20 mL) and the combined organic layers were washed with 10% aqueous sodium hydroxide (2 x 30 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated in vacuo to yield 1 as a white solid (187 mg, 0.616 mmol, 96.8%).

Melting Point: 25 ºC

IR (cm⁻¹, neat) 3081, 3014, 1597, 1496, 1267, 1113, 853, 819, 802, 791, 749, 702, 651

¹H NMR (300 MHz, CDCl₃) δ 7.41 (d, J = 2.5 Hz, 1H, H-11), 7.09 (dd, J = 8.8, 2.5 Hz, 1H, H-9), 6.96 (d, J = 2.2 Hz, 1H, H-2), 6.88 (dd, J = 8.5, 2.2 Hz, 1H, H-4), 6.82 (d, J = 8.5 Hz, 1H, H-5), 6.66 (d, J = 8.8 Hz, 1H, H-8), 3.79 (s, 3H, H-13)

¹³C NMR (75 MHz, CDCl₃) δ 152.1 (C-7), 151.6 (C-1), 143.2 (C-6), 130.5 (C-10), 130.4 (C-11), 128.4 (C-3), 127.9 (C-9), 125.0 (C-12), 121.3 (C-8), 121.1 (C-4), 118.6 (C-5), 113.8 (C-2), 56.4 (C-13)

6.1.2 2,4,4′-trichloro-2′-ethoxydiphenyl ether (2)

To a solution of irgasan (107; 159 mg, 0.549 mmol) and potassium carbonate (155 mg, 1.12 mmol) in 20 mL anhydrous tetrahydrofuran was added ethyl iodide (0.18 mL, 2.2 mmol) dropwise via syringe. The resulting mixture was heated to reflux for 20.5 hours, after which saturated ammonium chloride (20 mL) was added. The aqueous layer was extracted with three portions of dichloromethane (20 mL) and the combined organic layers were washed with 10% aqueous sodium hydroxide (2 x 30 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated in vacuo to yield 2 as a white solid (153 mg, 0.482 mmol, 87.7%).

Melting Point: 41 – 43 ºC

IR (cm⁻¹, film) 3053, 2980, 1594, 1468, 1264, 1116, 942, 869, 847, 825, 802, 735, 699

¹H NMR (300 MHz, CDCl₃) δ 7.40 (d, J = 2.5 Hz, 1H, H-11), 7.07 (dd, J = 8.8, 2.6 Hz, 1H, H-9), 6.94 (dd, J = 1.3, 1.3 (4J) Hz, 1H, H-2), 6.89 (s, 1H, H-4), 6.88 (s, 1H, H-5), 6.65 (d, J = 8.8 Hz, 1H, H-8), 3.99 (q, J = 7.1 Hz, 2H, H-13), 1.26 (t, J = 7.1 Hz, 3H, H-14)

¹³C NMR (75 MHz, CDCl₃) δ 152.6 (C-7), 151.0 (C-1), 143.5 (C-6), 130.6 (C-10), 130.3 (C-11), 128.2 (C-3), 127.8 (C-9), 124.9 (C-12), 121.9 (C-8), 121.1 (C-4), 118.5 (C-5), 115.1 (C-2), 65.1 (C-13), 14.7 (C-14)

6.1.3 2,4,4′-trichloro-2′-isopropoxydiphenyl ether (3)

To a solution of irgasan (107; 159 mg, 0.550 mmol) and potassium carbonate (154 mg, 1.11 mmol) in 20 mL anhydrous tetrahydrofuran was added 2-iodopropane (0.25 mL, 2.2 mmol) dropwise via syringe. The resulting mixture was heated to reflux for 20.5 hours, after which saturated ammonium chloride (20 mL) was added. The aqueous layer was extracted with three portions of dichloromethane (20 mL) and the combined organic layers were washed with 10 % aqueous sodium hydroxide (2 x 30 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated in vacuo to yield 3 as a colourless oil (101 mg, 0.303 mmol, 55.2%).

IR (cm⁻¹, neat) 3086, 1597, 1490, 1264, 962, 864, 808, 794, 749, 707, 696, 662, 648

¹H NMR (300 MHz, CDCl₃) δ 7.41 (d, J = 2.4 Hz, 1H, H-11), 7.06 (dd, J = 8.7, 2.5 Hz, 1H, H-9), 6.96 (d, J = 2.2 Hz, 1H, H-2), 6.92 (d, J = 8.2 Hz, 1H, H-4), 6.89 (dd, J = 8.6, 2.2 Hz, 1H, H-5), 6.63 (d, J = 8.8 Hz, 1H, H-8), 4.45 (septet, J = 6.1 Hz, 1H, H-13), 1.19 (d, J = 6.1 Hz, 6H, H-14)

¹³C NMR (75 MHz, CDCl₃) δ 152.7 (C-7), 150.1 (C-1), 144.5 (C-6), 130.6 (C-10), 130.3 (C-11), 128.0 (C-3), 127.7 (C-9), 124.7 (C-12), 122.4 (C-8), 121.4 (C-4), 118.4 (C-5), 117.4 (C-2), 72.4 (C-13), 22.0 (C-14)

6.1.4 2,4,4′-trichloro-2′-(2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy)di phenyl ether (4)

To a solution of irgasan (107; 152 mg, 0.523 mmol), 2-[2-(2-(2-Methoxy-ethoxy)-eth oxy)-ethoxy]-ethanol (131 mg, 0.629 mmol) and triphenylphosphine (159 mg, 0.606 mmol) in 20 mL anhydrous tetrahydrofuran was added diethyl azodicarboxylate (40% by weight in toluene, 280 mg, 0.642 mmol) dropwise via syringe. The resulting mixture was heated to reflux for 89 hours. The solvent was removed in vacuo followed by the addition of hexanes:ether (1:1). The white solid (triphenylphosphine oxide) was removed and the filtrate was concentrated in vacuo. Flash-chromatography of the crude material (silica, 10% methanol in chloroform, Rf 0.34) yielded compound 4 as a colourless oil with some triphenylphosphine oxide (216 mg, 0.449 mmol, 85.9 %).\textsuperscript{153}

IR (cm\textsuperscript{-1}, neat) 3064, 1594, 1493, 1262, 1116, 951, 850, 794, 749, 718, 696, 662, 648

\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 7.37 (d, J = 2.5 Hz, 1H, H-11), 7.05 (dd, J = 8.8, 2.4 Hz, 1H, H-9), 6.97 (dd, J = 1.2, 1.2 Hz (\textsuperscript{4}J), 1H, H-2), 6.88-6.90 (m, 2H, H-4, H-5), 6.61 (d, J = 8.8 Hz, 1H, H-8), 4.05 (t, J = 4.9 Hz, 2H, H-13), 3.45-3.70 (m, 14H, H-14 to H-20), 3.33 (s, 3H, H-21)

\textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) δ 152.6 (C-7), 151.0 (C-1), 143.2 (C-6), 130.7 (C-10), 130.2 (C-11), 127.9 (C-3), 127.8 (C-9), 124.5 (C-12), 122.2 (C-8), 121.5 (C-4), 118.1 (C-5), 115.5 (C-2), 72.1 (C-13), 71.0 (C-14), 70.6 (C-15, C-16, C-17), 70.5 (C-18), 69.5 (C-19), 69.4 (C-20), 59.2 (C-21)

HR-ESIMS calculated for [M+Na]\textsuperscript{+} C\textsubscript{21}H\textsubscript{25}Cl\textsubscript{3}O\textsubscript{6}Na: 501.0614. Found 501.0625.
6.1.5 2,4,4′-trichloro-2′-decoxydiphenyl ether (5)

To a solution of irgasan (107; 149 mg, 0.513 mmol), decyl alcohol (0.11 mL, 0.56 mmol) and triphenylphosphine (148 mg, 0.564 mmol) in 20 mL anhydrous tetrahydrofuran was added diethyl azodicarboxylate (40% by weight in toluene, 246 mg, 0.564 mmol) dropwise via syringe. The resulting mixture was heated to reflux for 65 hours. The solvent was removed in vacuo followed by the addition of hexanes:ether (1:1). The white solid (triphenylphosphine oxide) was removed and the filtrate was concentrated in vacuo. Flash-chromatography of the crude material (silica, hexanes:ether 9:1, Rf 0.86) yielded compound 5 as a white solid (171 mg, 0.399 mmol, 77.8%).

Melting Point: 29 °C

IR (cm⁻¹, film) 3047, 1597, 1496, 1264, 1110, 892, 864, 839, 808, 797, 738, 704, 662, 651

¹H NMR (300 MHz, CDCl₃) δ 7.39 (d, J = 2.5 Hz, 1H, H-11), 7.05 (dd, J = 8.8, 2.5 Hz, 1H, H-9), 6.96 (d, J = 8.5 Hz, 1H, H-5), 6.92 (d, J = 2.1 Hz, 1H, H-2), 6.89 (dd, J = 8.4, 2.3 Hz, 1H, H-4), 6.59 (d, J = 8.8 Hz, 1H, H-8), 3.87 (t, J = 6.3 Hz, 2H, H-13), 1.58 (quintet, J = 6.8 Hz, 2H, H-14), 1.18-1.35 (m, 14H, H-15 to H-21), 0.87 (t, J = 7.0 Hz, 3H, H-22)

¹³C NMR (75 MHz, CDCl₃) δ 152.9 (C-7), 151.3 (C-1), 143.0 (C-6), 130.9 (C-10), 130.2 (C-11), 127.8 (C-3), 127.6 (C-9), 124.4 (C-12), 122.5 (C-8), 120.9 (C-4), 117.7 (C-5), 114.8 (C-2), 69.2 (C-13), 32.1 (C-14), 29.7 (C-15), 29.5 (C-16), 29.4 (C-17, C-18), 29.1 (C-19), 25.9 (C-20), 22.9 (C-21), 14.3 (C-22)

6.1.6 3-Chloro-4-(4-chloro-2-methoxy-phenoxy)-benzonitrile (6)

4-chloro-2-methoxy-phenol (111; 610 µL, 5.00 mmol) was added to a solution of 3-chloro-4-fluoro-benzonitrile (112; 780 mg, 5.01 mmol) and potassium carbonate (1.38 g, 9.98 mmol) in 5 mL anhydrous dimethylsulfoxide and heated to 100 ºC for 90 minutes. The reaction mixture was quenched with 10% aqueous sodium hydroxide (20 mL) and extracted with three portions of ethyl acetate (20 mL). The combined organic layers were dried over anhydrous magnesium sulphate and concentrated in vacuo to yield compound 6 as a white solid with a small amount of dimethylsulfoxide (1.47 g, 5.00 mmol, quantitative).

Melting Point: 101 – 104 ºC

IR (cm\(^{-1}\) film) 3053, 2229, 1594, 1490, 1261, 1110, 889, 875, 841, 816, 735, 702, 662

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.70 (d, \(J = 2.0\) Hz, 1H, H-11), 7.38 (dd, \(J = 8.6, 2.0\) Hz, 1H, H-9), 7.01 (d, \(J = 8.5\) Hz, 1H, H-5), 6.99 (s, 1H, H-2), 6.96 (dd, \(J = 8.3, 2.2\) Hz, 1H, H-4), 6.62 (d, \(J = 8.6\) Hz, 1H, H-8), 3.75 (s, 3H, H-13)

\(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 157.6 (C-7), 152.0 (C-1), 141.2 (C-6), 134.3 (C-11), 132.3 (C-3), 132.1 (C-9), 124.0 (C-12), 123.3 (C-8), 121.5 (C-4), 117.8 (C-14), 116.1 (C-5), 114.0 (C-2), 106.7 (C-10), 56.4 (C-13)

HR-ESIMS calculated for [M+Na]\(^+\) \(\text{C}_{14}\text{H}_9\text{Cl}_2\text{NO}_2\text{Na}: 315.9908\). Found 315.9910.

6.1.7 3-Chloro-4-(4-chloro-2-hydroxy-phenoxy)-benzonitrile (7)

Boron tribromide (1.0 M in dichloromethane, 5.0 mL, 5.0 mmol) was added slowly via syringe to a solution containing compound 6 (368 mg, 1.25 mmol) in 5 mL anhydrous dichloromethane at –78 ºC. The red-orange mixture was warmed gradually to room temperature
over 16 hours. The reaction was quenched with distilled water (20 mL) at 0 ºC resulting in the formation of a yellow precipitate. The aqueous layer was extracted with three portions of dichloromethane (20 mL) and added to a flask containing a small amount of methanol. The combined yellow organic phases were dried over anhydrous magnesium sulphate and concentrated in vacuo. Flash-chromatography of the crude material (silica, hexanes:ethyl acetate 9:1 to 4:1, Rf 0.35) yielded compound 7 as a white solid (269 mg, 0.961 mmol, 76.9%).

Melting Point: 129 ºC

IR (cm⁻¹, film) 3338, 2229, 1485, 1415, 1278, 1104, 914, 886, 850, 813

¹H NMR (300 MHz, CDCl₃) δ 7.74 (d, J = 2.0 Hz, 1H, H-11), 7.47 (dd, J = 8.5, 2.0 Hz, 1H, H-9), 7.08 (d, J = 2.3 Hz, 1H, H-2), 6.90 (d, J = 8.6 Hz, 1H, H-5), 6.89 (dd, J = 8.7, 2.3 Hz, 1H, H-4), 6.83 (d, J = 8.7 Hz, 1H, H-8), 5.61 (s br, 1H, OH)

¹³C NMR (75 MHz, CDCl₃) δ 156.5 (C-7), 148.3 (C-1), 140.4 (C-6), 134.7 (C-11), 132.5 (C-9), 131.9 (C-3), 125.4 (C-12), 121.5 (C-4, C-8), 121.0 (C-5), 118.0 (C-2), 117.3 (C-13), 108.2 (C-10)

HR-ESIMS calculated for [M+Na]⁺ C₁₃H₇Cl₂NO₂Na: 301.9752. Found 301.9752.

6.1.8 3-Chloro-4-(4-chloro-2-methoxy-phenoxy)-benzoic acid (8)

5 mL 10% aqueous potassium hydroxide was added to a solution of compound 6 (540 mg, 1.84 mmol) in 10 mL methanol and heated to 100 ºC for 20 hours. The methanol was removed in vacuo and the reaction mixture was partitioned between 10% aqueous sodium hydroxide and ether, then cooled to 0 ºC and acidified with concentrated hydrochloric acid to yield a white precipitate. The aqueous layer was extracted with three portions of dichloromethane (30 mL), and the combined organic layers (with a small amount of methanol added) were dried over anhydrous sodium sulphate and concentrated in vacuo to yield compound 8 as a white solid (522 mg, 1.67 mmol, 90.6%).

Melting Point: 224 ºC
IR (cm$^{-1}$, film) 3372, 1667, 1465, 1272, 1180, 1107, 1026

$^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 7.99 (d, $J = 2.0$ Hz, 1H, H-11), 7.78 (dd, $J = 8.6$, 2.0 Hz, 1H, H-9), 7.31 (d, $J = 1.9$ Hz, 1H, H-2), 7.19 (d, $J = 8.6$ Hz, 1H, H-5), 7.07 (dd, $J = 8.5$, 2.2 Hz, 1H, H-4), 6.72 (d, $J = 8.6$ Hz, 1H, H-8), 3.74 (s, 3H, H-13)

$^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$ 165.7 (C-14), 156.4 (C-7), 151.7 (C-1), 141.1 (C-6), 131.3 (C-11), 130.6 (C-3), 129.9 (C-9), 126.0 (C-10), 123.2 (C-8), 121.5 (C-12), 121.0 (C-4), 115.5 (C-5), 114.1 (C-2), 56.3 (C-13)

HR-ESIMS calculated for [M+Na]$^+$ C$_{14}$H$_{10}$Cl$_2$O$_4$Na: 334.9854. Found 334.9850.

6.1.9 3-Chloro-4-(4-chloro-2-hydroxy-phenoxo)-benzoic acid (9)

5 mL 10% aqueous potassium hydroxide was added to a solution of compound 7 (235 mg, 0.838 mmol) in 10 mL methanol and heated to 100 ºC for 20 hours. The methanol was removed in vacuo and the reaction mixture was partitioned between 10% aqueous sodium hydroxide and ether, then cooled to 0 ºC and acidified with concentrated hydrochloric acid to yield a white precipitate. The aqueous layer was extracted with three portions of dichloromethane (30 mL), and the combined organic layers (with a small amount of methanol added) were dried over anhydrous sodium sulphate and concentrated in vacuo to yield compound 9 as a white solid (237 mg, 0.791 mmol, 94.4%).

Melting Point: 209 – 210 ºC

IR (cm$^{-1}$, film) 3361, 1664, 1448, 1269, 1183, 1107, 1023

$^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 8.07 (d, $J = 2.0$ Hz, 1H, H-11), 7.83 (dd, $J = 8.6$, 2.0 Hz, 1H, H-9), 6.98 (d, $J = 2.2$ Hz, 1H, H-2), 6.96 (d, $J = 8.6$ Hz, 1H, H-5), 6.86 (dd, $J = 8.6$, 2.4 Hz, 1H, H-4), 6.75 (d, $J = 8.7$ Hz, 1H, H-8)

$^{13}$C NMR (75 MHz, CD$_3$OD) $\delta$ 168.2 (C-13), 158.6 (C-7), 151.3 (C-1), 142.4 (C-6), 133.0 (C-11), 132.1 (C-3), 130.8 (C-9), 127.1 (C-10), 124.2 (C-12), 123.8 (C-8), 121.1 (C-4), 118.5 (C-5), 117.0 (C-2)

HR-ESIMS calculated for [M-H]$^-$ C$_{13}$H$_7$Cl$_2$O$_4$: 296.9721. Found 296.9724.
6.1.10 3-Chloro-4-(4-chloro-2-methoxy-phenoxy)-N-decyl-benzamide (10)

Thionyl chloride (57 µL, 0.78 mmol) was added to a solution of compound 8 (123 mg, 0.392 mmol) in 3 mL anhydrous benzene. The white suspension was heated to 90 ºC for 2 hours at which time the solution began to go clear. The solvent was removed in vacuo and the residue was washed with three aliquots of anhydrous benzene and concentrated in vacuo again.

Decyl amine (0.31 mL, 1.6 mmol) was added to a solution of crude acid chloride in 3 mL anhydrous tetrahydrofuran and stirred at room temperature for 20 hours. The reaction mixture was quenched with saturated ammonium chloride (15 mL) and extracted with three portions of ether (30 mL). The combined organic layers were dried over anhydrous sodium sulphate and concentrated in vacuo. Flash chromatography of the crude material (silica, hexanes:ethyl acetate 4:1, Rf 0.5) yielded compound 10 as a white solid (141 mg, 0.311 mmol, 79.4%).

Melting Point: 97 ºC

IR (cm\(^{-1}\), film) 3422, 3058, 1655, 1594, 1493, 1261, 1172, 1118, 895, 841, 830, 813, 735

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta 7.82\) (d, \(J = 2.2\) Hz, 1H, H-11), 7.51 (dd, \(J = 8.6, 2.2\) Hz, 1H, H-9), 6.98 (t, \(J = 1.3, 1.3\) (\(^4\)J) Hz, 1H, H-2), 6.92 (d, \(J = 1.3\) Hz, 2H, H-4, H-5), 6.66 (d, \(J = 8.6\) Hz, 1H, H-8), 5.97 (t br, \(J = 5.3\) Hz, 1H, NH), 3.77 (s, 3H, H-24), 3.40 (td, \(J = 7.2, 5.4\) Hz, 2H, H-14), 1.58 (quintet, \(J = 6.8\) Hz, 2H, H-15), 1.18-1.40 (m, 14H, H-16 to H-22), 0.86 (t, \(J = 6.5\) Hz, 3H, H-23)

\(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta 165.9\) (C-13), 156.0 (C-7), 151.9 (C-1), 142.5 (C-6), 131.2 (C-3), 130.3 (C-10), 129.6 (C-11), 126.8 (C-9), 123.8 (C-12), 122.4 (C-8), 121.3 (C-4), 116.5 (C-5), 113.9 (C-2), 56.4 (C-24), 40.4 (C-14), 32.1 (C-15), 29.8 (C-16 to C-19), 29.5 (C-20), 27.2 (C-21), 22.9 (C-22), 14.3 (C-23)

HR-ESIMS calculated for [M+H]^+ C\(_{24}\)H\(_{31}\)Cl\(_2\)NO\(_3\)H: 452.1759. Found 452.1753.
6.1.11 3-Chloro-4-(4-chloro-2-hydroxy-phenoxy)-N-decyl-benzamide (11)

Boron tribromide (1.0 M in dichloromethane, 0.97 mL, 0.97 mmol) was added slowly via syringe to a solution containing compound 10 (110 mg, 2.42 mmol) in 5 mL anhydrous dichloromethane at −78 °C. The red-orange mixture was warmed gradually to room temperature over 4 hours, and then quenched with distilled water (20 mL) at 0 °C resulting in the formation of a yellow precipitate. The aqueous layer was extracted with three portions of dichloromethane (20 mL) and added to a flask containing a small amount of methanol. The combined yellow organic phases were dried over anhydrous sodium sulphate and concentrated in vacuo. Flash-chromatography of the crude material (silica, hexanes:ethyl acetate 4:1, Rf 0.60) yielded compound 11 as an off-white solid (93 mg, 0.21 mmol, 88%).

Melting Point: 118 °C

IR (cm⁻¹, film) 3310, 3092, 1633, 1549, 1485, 1272, 1177, 1113, 914, 858, 813

¹H NMR (300 MHz, CDCl₃) δ 7.65 (d, J = 2.2 Hz, 1H, H-11), 7.45 (dd, J = 8.7, 2.2 Hz, 1H, H-9), 7.08 (d, J = 2.3 Hz, 1H, H-2), 6.80 (dd, J = 8.7, 2.3 Hz, 1H, H-4), 6.77 (d, J = 9.4 Hz, 1H, H-5), 6.74 (d, J = 8.7 Hz, 1H, H-8), 6.08 (t br, J = 5.8 Hz, 1H, NH), 3.41 (td, J = 7.0, 6.0 Hz, 2H, H-14), 1.59 (quintet, J = 6.6 Hz, 2H, H-15), 1.15-1.37 (m, 14H, H-16 to H-22), 0.85 (t, J = 6.4 Hz, 3H, H-23)

¹³C NMR (75 MHz, CDCl₃) δ 166.0 (C-13), 155.3 (C-7), 148.9 (C-1), 141.4 (C-6), 131.1 (C-3), 130.6 (C-10), 129.8 (C-11), 126.7 (C-9), 124.5 (C-12), 120.8 (C-8, C-4), 117.9 (C-5), 117.7 (C-2), 40.7 (C-14), 32.1 (C-15), 29.6 (C-16 to C-20), 27.2 (C-21), 22.9 (C-22), 14.3 (C-23)

6.1.12 5-Chloro-2-(4-chloro-2-methoxy-phenoxy)-benzonitrile (12)

4-chloro-2-methoxy-phenol (113; 244 µL, 2.00 mmol) was added to a solution of 5-chloro-2-fluoro-benzonitrile (114; 312 mg, 2.01 mmol) and potassium carbonate (552 mg, 3.99 mmol) in 3 mL anhydrous dimethylsulfoxide and heated to 100 ºC for 90 minutes. The reaction mixture was quenched with 10% aqueous sodium hydroxide (20 mL) and extracted with three portions of ethyl acetate (20 mL). The combined organic layers were dried over anhydrous magnesium sulphate and concentrated in vacuo to yield compound 12 as a white solid with a small amount of dimethylsulfoxide (588 mg, 2.00 mmol, quantitative).

Melting Point: 70 ºC

IR (cm⁻¹, film) 3070, 2229, 1597, 1499, 1479, 1398, 1272, 1177, 1118, 872, 813, 735, 716, 668

¹H NMR (300 MHz, CDCl₃) δ 7.56 (d, J = 2.6 Hz, 1H, H-11), 7.33 (dd, J = 9.0, 2.6 Hz, 1H, H-9), 7.03 (d, J = 8.4 Hz, 1H, H-5), 6.97 (d, J = 2.1 Hz, 1H, H-2), 6.94 (dd, J = 8.3, 2.2 Hz, 1H, H-4), 6.57 (d, J = 9.0 Hz, 1H, H-8), 3.74 (s, 3H, H-13)

¹³C NMR (75 MHz, CDCl₃) δ 158.9 (C-7), 152.2 (C-1), 141.8 (C-6), 134.3 (C-11), 133.1 (C-9), 132.2 (C-3), 127.5 (C-10), 123.5 (C-8), 121.4 (C-4), 116.4 (C-5), 115.0 (C-14), 114.1 (C-2), 103.9 (C-12), 56.4 (C-13)


6.1.13 5-Chloro-2-(4-chloro-2-methoxy-phenoxy)-benzoic acid (13)

5 mL 10% aqueous potassium hydroxide was added to a solution of compound 12 (510 mg, 1.73 mmol) in 10 mL methanol and heated to 100 ºC for 12 hours. The methanol was removed in vacuo and the reaction mixture was partitioned between 10% aqueous sodium
hydroxide and ether, then cooled to 0 ºC and acidified with concentrated hydrochloric acid to yield a white precipitate. The aqueous layer was extracted with three portions of dichloromethane (30 mL), and the combined organic layers (with a small amount of methanol added) were dried over anhydrous sodium sulphate and concentrated in vacuo to yield compound 13 as a white solid (371 mg, 1.18 mmol, 68.5%).

Melting Point: 152 – 153 ºC

IR (cm
\(^{-1}\), film) 3333, 1697, 1594, 1493, 1476, 1267, 1177, 1116, 892, 867, 816

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.06 (d, \(J = 2.7\) Hz, 1H, H-11), 7.33 (dd, \(J = 8.9, 2.7\) Hz, 1H, H-9), 7.03 (d, \(J = 8.3\) Hz, 1H, H-5), 6.98 (d, \(J = 2.0\) Hz, 1H, H-2), 6.95 (dd, \(J = 8.4, 2.2\) Hz, 1H, H-4), 6.66 (d, \(J = 8.8\) Hz, 1H, H-8), 3.75 (s, 3H, H-13)

\(^1\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 166.3 (C-14), 156.2 (C-7), 151.9 (C-1), 141.6 (C-6), 134.5 (C-11), 132.9 (C-9), 132.2 (C-3), 128.6 (C-10), 123.2 (C-8), 121.3 (C-12, C-4), 117.7 (C-5), 114.0 (C-2), 56.4 (C-13)

HR-ESIMS calculated for [M+Na]\(^+\) C\(_{14}\)H\(_{10}\)Cl\(_2\)O\(_4\)Na: 334.9854. Found 334.9850.

6.1.14 5-Chloro-2-(4-chloro-2-methoxy-phenoxy)-N-decyl-benzamide (14)

Thionyl chloride (46 µL, 0.64 mmol) was added to a solution of compound 13 (100 mg, 0.319 mmol) in 4 mL anhydrous benzene. The white suspension was heated to 90 ºC for 2 hours at which time the solution began to go clear. The solvent was removed in vacuo and the residue was washed with three aliquots of anhydrous benzene and concentrated in vacuo again.

Decyl amine (128 µL, 0.64 mmol) and triethylamine (89 µL, 0.64 mmol) were added sequentially to a solution of crude acid chloride in 3 mL anhydrous tetrahydrofuran and stirred at room temperature for 20 hours. The reaction mixture was quenched with saturated ammonium chloride (15 mL) and extracted with three portions of ether (30 mL). The combined organic layers were washed with saturated sodium bicarbonate (20 mL), dried over anhydrous sodium sulphate and concentrated in vacuo. Flash chromatography of the crude material (silica,
hexanes:ethyl acetate 9:1 to 4:1, R_f 0.63) yielded compound 14 as a white solid (129 mg, 0.284 mmol, 89.0%).

Melting Point: 48 ºC

IR (cm⁻¹, film) 3422, 3058, 1655, 1594, 1493, 1261, 1172, 1118, 895, 841, 830, 813, 735

¹H NMR (300 MHz, CDCl₃) δ 8.13 (d, J = 2.7 Hz, 1H, H-11), 7.66 (t br, J = 5.3 Hz, 1H, NH), 7.23 (dd, J = 8.8, 2.7 Hz, 1H, H-9), 7.00 (d, J = 2.7 Hz, 1H, H-2), 6.97 (d, J = 8.7 Hz, 1H, H-5), 6.97 (d, J = 2.7 Hz, 1H, H-4), 6.62 (d, J = 8.8 Hz, 1H, H-8), 3.77 (s, 3H, H-24), 3.44 (td, J = 6.7, 5.3 Hz, 2H, H-14), 1.54 (quintet, J = 6.8 Hz, 2H, H-15), 1.18-1.35 (m, 14H, H-16 to H-22), 0.86 (t, J = 6.8 Hz, 3H, H-23)

¹³C NMR (75 MHz, CDCl₃) δ 163.7 (C-13), 154.3 (C-7), 151.8 (C-1), 141.9 (C-6), 132.0 (C-9), 131.6 (C-3), 128.8 (C-10), 125.1 (C-12), 122.8 (C-11), 121.3 (C-8, C-4), 117.4 (C-5), 113.8 (C-2), 56.2 (C-24), 40.1 (C-14), 32.0 (C-15), 29.6 (C-16 to C-20), 27.1 (C-21), 22.8 (C-22), 14.3 (C-23)


6.1.15 5-Chloro-2-(4-chloro-2-hydroxy-phenoxy)-N-decyl-benzamide (15)

Boron tribromide (1.0 M in dichloromethane, 0.60 mL, 0.60 mmol) was added slowly via syringe to a solution containing compound 14 (68 mg, 0.15 mmol) in 3 mL anhydrous dichloromethane at −78 ºC. The red-orange mixture was warmed gradually to room temperature over 4 hours. The reaction was quenched with distilled water (20 mL) at 0 ºC resulting in the formation of a yellow precipitate. The aqueous layer was extracted with three portions of dichloromethane (20 mL) and added to a flask containing a small amount of methanol. The combined yellow organic phases were dried over anhydrous sodium sulphate and concentrated in vacuo. Flash-chromatography of the crude material (silica, hexanes:ethyl acetate 9:1 to 4:1, R_f 0.60) yielded compound 15 as a white solid (66 mg, 0.15 mmol, quantitative).

Melting Point: 25 ºC
IR (cm\textsuperscript{-1}, film) 3305, 3103, 1636, 1479, 1275, 1174, 1107, 911, 855, 813, 758
\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta 7.52\) (d, \(J = 2.7\) Hz, 1H, H-11), 7.25 (dd, \(J = 8.9, 2.7\) Hz, 1H, H-9), 7.03 (d, \(J = 2.5\) Hz, 1H, H-2), 7.00 (d, \(J = 8.5\) Hz, 1H, H-5), 6.82 (dd, \(J = 8.6, 2.5\) Hz, 1H, H-4), 6.81 (d, \(J = 8.9\) Hz, 1H, H-8), 6.74 (t br, \(J = 5.1\) Hz, 1H, NH), 3.34 (td, \(J = 7.1, 5.2\) Hz, 2H, H-14), 1.55 (quintet, \(J = 7.1\) Hz, 2H, H-15), 1.18-1.35 (m, 14H, H-16 to H-22), 0.86 (t, \(J = 7.0\) Hz, 3H, H-23)
\textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) \(\delta 166.4\) (C-13), 154.6 (C-7), 150.8 (C-1), 141.5 (C-6), 132.4 (C-9), 132.1 (C-3), 129.3 (C-11), 128.9 (C-10), 126.2 (C-12), 123.4 (C-8), 120.1 (C-4), 119.0 (C-5), 118.8 (C-2), 40.6 (C-14), 32.1 (C-15), 29.6 (C-16 to C-20), 27.2 (C-21), 22.9 (C-22), 14.3 (C-23)
HR-ESIMS calculated for [M+Na]\textsuperscript{+} \(C_{23}H_{29}Cl_2NO_3Na\): 460.1422. Found 460.1422.

6.1.16 \{2-[2-[2-[5-Chloro-2-(2,4-dichloro-phenoxy)-phenoxy]-ethoxy]-ethoxy]-ethoxy]-ethyl\}-carbamic acid tert-butyl ester (127)

To a solution of irgasan (107; 102 mg, 0.352 mmol), (2-[2-[2-(2-Hydroxy-ethoxy)-ethoxy]-ethoxy]-ethyl)-carbamic acid tert-butyl ester\textsuperscript{154} (125; 115 mg, 0.389 mmol) and triphenylphosphine (104 mg, 0.395 mmol) in 10 mL anhydrous tetrahydrofuran was added diethyl azodicarboxylate (40% by weight in toluene, 172 mg, 0.395 mmol) dropwise via syringe. The resulting mixture was heated to reflux for 89 hours. The solvent was removed in vacuo and then taken up in 40 mL diethyl ether and washed with 3 x 20 mL aliquots of 10% aqueous sodium hydroxide. The organic layer was dried over anhydrous sodium sulphate and concentrated in vacuo. Flash-chromatography of the crude material (silica, hexanes:ether 1:2, \(R_f\) 0.19) yielded compound 127 as a yellow oil (139 mg, 0.246 mmol, 70.0%).\textsuperscript{153}
IR (cm\textsuperscript{-1}, neat) 1711, 1597, 1493, 1269, 1188, 951, 858, 794
\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta 7.39\) (d, \(J = 2.5\) Hz, 1H, H-11), 7.06 (dd, \(J = 8.8, 2.5\) Hz, 1H, H-9), 6.98 (dd, \(J = 1.4, 1.4\) Hz \(^4\)J, 1H, H-2), 6.89-6.91 (m, 2H, H-4, H-5), 6.62 (d, \(J = 8.8\) Hz,
1H, H-8), 4.98 (s, br, 1H, NH), 4.07 (dd, J = 4.9, 4.9 Hz, 2H, H-13), 3.68 (dd, J = 4.9, 4.9 Hz, 2H, H-14), 3.46-3.60 (m, 10H, H-15 to H-19), 3.23-3.32 (m, 2H, H-20), 1.41 (s, 9H, H-23)

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 156.2 (C-21), 152.6 (C-7), 151.0 (C-1), 143.3 (C-6), 130.8 (C-10), 130.3 (C-11), 128.0 (C-3), 127.8 (C-9), 124.6 (C-12), 122.3 (C-8), 121.6 (C-4), 118.2 (C-5), 115.6 (C-2), 79.4 (C-22), 71.1 (C-13), 69.5-70.8 (C-14 to C-19), 40.6 (C-20), 28.6 (C-23)


6.1.17 2-[2-(2-[5-Chloro-2-(2,4-dichloro-phenoxy)-phenoxy]-ethoxy)-ethoxy]-ethoxy]-ethylamine (16)

Compound 127 (115 mg, 0.204 mmol) was dissolved in neat trifluoroacetic acid (3 mL) containing 0.03 mL anisole and stirred for 1 hr at room temperature. The trifluoroacetic acid was removed in vacuo and the subsequent yellow oil was washed with 3 x 3 mL aliquots of toluene (concentrated in vacuo between each aliquot). The crude material was purified by flash chromatography (silica, chloroform:methanol 9:1, R$_f$ 0.20) to yield compound 16 as a yellow oil (95 mg, 0.163 mmol, 80.1%).

IR (cm$^{-1}$, neat) 3422, 3075, 1597, 1493, 1267, 1197, 948, 850, 797, 730, 662, 648

$^1$H NMR (300 MHz, CDCl$_3$) δ 7.41 (d, J = 2.5 Hz, 1H, H-11), 7.13 (dd, J = 8.8, 2.5 Hz, 1H, H-9), 6.99 (d, J = 2.3 Hz, 1H, H-2), 6.89 (dd, J = 8.6, 2.3 Hz, 1H, H-4), 6.78 (d, J = 8.6 Hz, 1H, H-5), 6.77 (d, J = 8.8 Hz, 1H, H-8), 4.14 (dd, J = 4.4, 4.4 Hz, 2H, H-13), 3.76 (dd, J = 4.4, 4.4 Hz, 2H, H-14), 3.46-3.71 (m, 10H, H-15 to H-19), 2.91-3.04 (m, 2H, H-20)

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 156.2 (C-7), 150.6 (C-1), 143.4 (C-6), 130.8 (C-10), 130.3 (C-11), 128.4 (C-3), 128.1 (C-9), 124.8 (C-12), 121.8 (C-8), 121.7 (C-4), 118.7 (C-5), 115.5 (C-2), 70.8 (C-13), 69.4-70.4 (C-14 to C-20)

HR-ESIMS calculated for [M+Na]$^+$ C$_{20}$H$_{24}$Cl$_3$NO$_7$: 486.0618. Found 486.0610.
6.1.18 5-(2-Oxo-hexahydro-thieno[3,4-d]imidazol-6-yl)-pentanoic acid {2-[2-[2-[5-chloro-2-[2,4-dichloro-phenoxy]-phenoxy]-ethoxy]-ethoxy]ethoxy}-ethyl]-amide (17)

To a solution of compound 16 (20 mg, 0.034 mmol) and triethylamine (19 µL, 0.14 mmol) in 3 mL anhydrous dimethylformamide was added D-biotin succinimidyl ester (128; 9 mg, 0.03 mmol) and the resulting mixture was stirred for 24 hours. The dimethylformamide was removed in vacuo and the crude material was purified by flash-chromatography (silica, chloroform:methanol 97:3, R<sub>f</sub> 0.03) to yield compound 17 as a colourless oil (19 mg, 0.027 mmol, quantitative).

IR (cm<sup>-1</sup>, neat) 1686, 1644, 1493, 1468, 1264, 1188, 1113, 1096, 945

<sup>1</sup>H NMR (500 MHz, MeOD) δ 7.51 (d, J = 2.5 Hz, 1H, H-11), 7.21 (dd, J = 8.8, 2.5 Hz, 1H, H-9), 7.21 (d, J = 2.4 Hz, 1H, H-2), 7.04 (d, J = 8.6 Hz, 1H, H-5), 7.00 (dd, J = 8.6, 2.0 Hz, 1H, H-4), 6.71 (d, J = 8.8 Hz, 1H, H-8), 4.48 (dd, J = 8.0, 4.9 Hz, 1H, H-29), 4.29 (dd, J = 8.0, 4.5 Hz, 1H, H-27), 4.12 (dd, J = 4.2, 4.2 Hz, 2H, H-13), 3.68 (dd, J = 4.3, 4.3 Hz, 2H, H-14), 3.56-3.61 (m, 4H, H-15, H-19), 3.50-3.55 (m, 6H, H-16, H-17, H-18), 3.34 (d, J = 5.0 Hz, 2H, H-20), 3.16-3.21 (m, 1H, H-26), 2.91 (dd, J = 12.8, 5.2 Hz, 1H, H-30), 2.70 (d, J = 12.8 Hz, 1H, H-30), 2.20 (t, J = 7.2 Hz, 2H, H-22), 1.65-1.75 (m, 2H, H-25), 1.56-1.65 (m, 2H, H-23), 1.42 (quintet, J = 7.5 Hz, 2H, H-24)

<sup>1</sup>C NMR (125 MHz, MeOD) δ 176.2 (C-21), 166.2 (C-28), 154.21 (C-7), 152.6 (C-1), 144.3 (C-6), 132.1 (C-10), 131.2 (C-11), 129.2 (C-9), 128.9 (C-3), 125.4 (C-12), 123.8 (C-8), 122.6 (C-4), 119.2 (C-5), 116.4 (C-2), 70.5-71.9 (C-13 to C-19), 63.5 (C-27), 61.76 (C-29), 57.1 (C-26), 41.2 (C-20), 40.5 (C-22), 36.9 (C-30), 29.9 (C-25), 29.6 (C-24), 27.0 (C-23)

HR-ESIMS calculated for [M+Na]<sup>+</sup> C<sub>30</sub>H<sub>38</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>7</sub>SNa: 714.1370. Found 714.1367.
6.1.19 {3-[2-{3-[3-Chloro-4-(4-chloro-2-methoxy-phenoxy)-benzoylamino]-propoxy}-ethoxy]-ethoxy]-propyl]-carbamic acid tert-butyl ester (130)

A solution of compound 8 (39 mg, 0.12 mmol), hydroxybenzotriazole (22 mg, 0.16 mmol), 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (32 mg, 0.17 mmol) and triethylamine (5 µL, 0.4 mmol) in 3 mL anhydrous dimethylformamide were stirred for 30 min before being transferred dropwise via cannula to a solution of (3-[2-{2-(3-amino-propoxy)-ethoxy]-ethoxy]-propyl)-carbamic acid tert-butyl ester^{293} (126; 72 mg, 0.23 mmol) in 2 mL anhydrous dimethylformamide. The resulting mixture was stirred for 17 hours followed by removal of the solvent in vacuo. The crude material was purified by flash-chromatography (silica, chloroform:methanol 97:3, R_f 0.02) to yield compound 130 as a yellow oil (65 mg, 0.11 mmol, 85%).

IR (neat, cm^{-1}) 3347, 1704, 1699, 1652, 1604, 1495, 1272, 1178, 839, 780, 760, 699

^{1}H NMR (300 MHz, MeOD) δ 7.94 (d, J = 2.2 Hz, 1H, H-11), 7.64 (dd, J = 8.6, 2.2 Hz, 1H, H-9), 7.18 (d, J = 2.2 Hz, 1H, H-2), 7.06 (d, J = 8.3 Hz, 1H, H-5), 7.01 (dd, J = 8.6, 2.2 Hz, 1H, H-4), 6.67 (d, J = 8.6 Hz, 1H, H-8), 3.78 (s, 3H, H-13), 3.42-3.68 (m, 14H, H-15, H-17 to H-22), 3.10 (td, J = 6.6, 2.3 Hz, 2H, H-24), 1.87 (quintet, J = 6.2 Hz, 2H, H-16), 1.69 (quintet, J = 6.4 Hz, 2H, H-23), 1.42 (s, 9H, H-27)

^{13}C NMR (75 MHz, MeOD) δ 168.0 (C-14), 158.4 (C-25), 157.5 (C-7), 153.5 (C-1), 143.3 (C-6), 132.5 (C-3), 130.72 (C-10, C-11), 128.2 (C-9), 124.0 (C-8), 123.9 (C-12), 122.2 (C-4), 116.6 (C-5), 115.0 (C-2), 79.6 (C-26), 69.85-71.5 (C-17 to C-22), 56.7 (C-13), 38.8 (C-24), 38.7 (C-15), 30.9 (C-16), 30.3 (C-23), 28.8 (C-27)

HR-ESIMS calculated for [M+Na]^+ C_{29}H_{40}Cl_{2}N_{2}O_{8}Na: 637.2059. Found 637.2059.
6.1.20 *N*-(3-{2-[2-(3-Amino-propoxy)-ethoxy]-ethoxy}-propyl)-3-chloro-4-(4-chloro-2-methoxy-phenoxy)-benzamide (18)

Compound 130 (53 mg, 0.085 mmol) was dissolved in neat trifluoroacetic acid (3 mL) containing 0.03 mL anisole and stirred for 1 hr at room temperature. The trifluoroacetic acid was removed in vacuo and the subsequent yellow oil was purified by flash chromatography (silica, chloroform:methanol 9:1, *R*$_f$ 0.09) to yield compound 18 as a yellow oil (54 mg, 0.085 mmol, quantitative).

IR (neat, cm$^{-1}$) 3389, 1704, 1694, 1683, 1495, 1273, 1204, 1133, 1028, 840, 801, 723

$^1$H NMR (300 MHz, MeOD) $\delta$ 7.95 (d, $J = 2.2$ Hz, 1H, H-11), 7.64 (dd, $J = 8.7$, 2.2 Hz, 1H, H-9), 7.20 (d, $J = 2.2$ Hz, 1H, H-2), 7.07 (d, $J = 8.4$ Hz, 1H, H-5), 7.02 (dd, $J = 8.7$, 2.2 Hz, 1H, H-4), 6.68 (d, $J = 8.7$ Hz, 1H, H-8), 3.78 (s, 3H, H-13), 3.54-3.68 (m, 12H, H-17 to H-22), 3.45 (t, $J = 6.8$ Hz, 2H, H-15), 3.09 (t, $J = 6.5$ Hz, 2H, H-24), 1.89 (septet, $J = 6.5$ Hz, 4H, H-16, H-23)

$^{13}$C NMR (75 MHz, MeOD) $\delta$ 168.1 (C-14), 162.9 (TFA), 157.5 (C-7), 153.4 (C-1), 143.2 (C-6), 132.5 (C-3), 130.8 (C-11), 130.5 (C-10), 128.2 (C-9), 123.9 (C-8), 123.9 (C-12), 122.2 (C-4), 116.6 (C-5), 115.0 (C-2), 70.0-71.3 (C-17 to C-22), 56.7 (C-13), 40.0 (C-24), 38.6 (C-15), 30.4 (C-16), 28.2 (C-23)

HR-ESIMS calculated for [M+H]$^+$ C$_{24}$H$_{32}$Cl$_2$N$_2$O$_6$H: 515.1716. Found 515.1716.
6.1.21 3-Chloro-4-(4-chloro-2-methoxy-phenoxy)-N-{3-[2-(2-[3-[5-(2-oxo-hexahydro-thieno[3,4-d]imidazol-4-yl)-pentanoylamino]-propoxy]-ethoxy)-ethoxy]-propyl}-benzamide (19)

{3-[2-(2-[3-[5-(2-Oxo-hexahydro-thieno[3,4-d]imidazol-4-yl)-pentanoylamino]-propoxy]-ethoxy)-ethoxy]-propyl}-carbamic acid tert-butyl ester^{293} (131; 63 mg, 0.12 mmol) was dissolved in neat trifluoroacetic acid (3 mL) containing 0.03 mL anisole and stirred for 1 hr at room temperature. The trifluoroacetic acid was removed in vacuo and the crude salt was taken directly onto the next step.

A solution of compound 8 (29 mg, 0.092 mmol), hydroxybenzotriazole (15 mg, 0.11 mmol), 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (26 mg, 0.14 mmol) and triethylamine (5.1 µL, 0.37 mmol) in 3 mL anhydrous dimethylformamide were stirred for 30 min before being transferred dropwise via cannula to a solution of crude 5-(2-Oxo-hexahydro-thieno[3,4-d]imidazol-4-yl)-pentanoic acid (3-[2-(3-amino-propoxy)-ethoxy]-propyl)-amide in 2 mL anhydrous dimethylformamide. The resulting mixture was stirred for 17 hours followed by removal of the solvent in vacuo. The crude material was purified by flash-chromatography (silica, chloroform to 10% methanol in chloroform, R_f 0.05 for chloroform:methanol 97:3) to yield compound 19 as a yellow oil (17 mg, 0.023 mmol, 25% over two steps).

IR (neat, cm\(^{-1}\)) 3314, 1697, 1648, 1497, 1271, 1216, 1117, 1057, 867, 842, 754, 666

\(^1\)H NMR (500 MHz, MeOD) δ 7.95 (d, J = 2.1 Hz, 1H, H-11), 7.64 (dd, J = 8.7, 2.1 Hz, 1H, H-9), 7.20 (d, J = 2.1 Hz, 1H, H-2), 7.07 (d, J = 8.4 Hz, 1H, H-5), 7.02 (dd, J = 8.4, 2.1 Hz, 1H, H-4), 6.68 (d, J = 8.7 Hz, 1H, H-8), 4.48 (dd, J = 7.7, 5.1 Hz, 1H, H-32), 4.29 (dd, J = 7.7, 4.5 Hz, 1H, H-33), 3.78 (s, 3H, H-13), 3.43-3.67 (m, 14H, H-17 to H-22, H-24), 3.24 (t, J = 6.6 Hz, 2H, H-15), 3.19 (quintet, J = 4.4 Hz, 1H, H-30), 2.91 (dd, J = 12.8, 5.0 Hz, 1H, H-31), 2.70 (d, J = 12.8 Hz, 1H, H-31), 2.18 (t, J = 7.4 Hz, 2H, H-26), 1.87 (quintet, J = 6.2 Hz, 2H, H-29), 1.68-1.77 (m, 4H, H-16, H-23), 1.54 – 1.68 (m, 2H, H-27), 1.42 (quintet, J = 7.6 Hz, 2H, H-28)
$^{13}$C NMR (125 MHz, MeOD) $\delta$ 176.2 (C-25), 168.3 (C-34), 166.4 (C-14), 157.7 (C-7), 153.7 (C-1), 143.5 (C-6), 132.9 (C-3), 130.9 (C-10, C-11), 128.4 (C-9), 124.2 (C-8, C-12), 122.6 (C-4), 116.8 (C-5), 115.1 (C-2), 70.1-71.7 (C-17 to C-22), 63.6 (C-33), 61.8 (C-32), 57.2 (C-30), 56.9 (C-13), 41.2 (C-24), 39.0 (C-15), 38.0 (C-26), 37.0 (C-31), 30.6 (C-16), 30.5 (C-23), 30.0 (C-29), 29.7 (C-28), 27.1 (C-27)

HR-ESIMS calculated for [M+Na]$^+$ C$_{34}$H$_{46}$Cl$_2$N$_4$O$_8$SNa: 763.2311. Found 763.2313.

6.1.22 3-Chloro-4-(4-chloro-2-hydroxy-phenoxy)-N-(3-[2-(2-[3-([2-oxo-hexahydro-thieno[3,4-d]imidazol-4-yl]-pentanoylamino]propanoylpropoxy)-ethoxy]-ethoxy]-propyl]-benzamide (20)

{3-[2-(2-[3-([2-Oxo-hexahydro-thieno[3,4-d]imidazol-4-yl]-pentanoylamino]propanoylpropoxy]-ethoxy]-ethoxy]-propyl]-carbamic acid tert-butyl ester$^{293}$ (131; 157 mg, 0.286 mmol) was dissolved in neat trifluoroacetic acid (6 mL) containing 0.06 mL anisole and stirred for 1 hr at room temperature. The trifluoroacetic acid was removed in vacuo and the crude salt was taken directly onto the next step.

A solution of compound 9 (50 mg, 0.17 mmol), hydroxybenzotriazole (35 mg, 0.255 mmol), 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (52 mg, 0.270 mmol) and triethylamine (16 µL, 1.1 mmol) in 5 mL anhydrous dimethylformamide were stirred for 30 min before being transferred dropwise via cannula to a solution of crude 5-(2-Oxo-hexahydro-thieno[3,4-d]imidazol-4-yl)-pentanoic acid (3-[2-(3-amino-propanoyl)-ethoxy]-ethoxy]-propyl)-amide in 2 mL anhydrous dimethylformamide. The resulting mixture was stirred for 23 hours followed by removal of the solvent in vacuo. The crude material was purified by flash-chromatography (silica, 9:1 chloroform:methanol, $R_f$ 0.32) to yield compound 20 as a yellow oil (18 mg, 0.025 mmol, 8.7% over two steps).

IR (neat, cm$^{-1}$) 3405, 1682, 1486, 1398, 1251, 1202, 1132, 831, 800, 720

$^1$H NMR (500 MHz, MeOD) $\delta$ 7.95 (d, $J = 2.2$ Hz, 1H, H-11), 7.67 (dd, $J = 8.6, 2.2$ Hz, 1H, H-9), 6.99 (d, $J = 2.2$ Hz, 1H, H-2), 6.95 (d, $J = 8.6$ Hz, 1H, H-5), 6.87 (dd, $J = 8.6, 2.4$ Hz, 1H,
H-4), 6.76 (d, J = 8.6 Hz, 1H, H-8), 4.48 (dd, J = 7.9, 5.1 Hz, 1H, H-31), 4.28 (dd, J = 7.9, 4.6 Hz, 1H, H-32), 3.43-3.66 (m, 14H, H-16 to H-21, H-23), 3.24 (t, J = 6.9 Hz, 2H, H-14), 3.18 (quintet, J = 4.5 Hz, 1H, H-29), 2.91 (dd, J = 12.6, 5.0 Hz, 1H, H-30), 2.70 (d, J = 12.9 Hz, 1H, H-30), 2.18 (t, J = 7.2 Hz, 2H, H-25), 1.87 (quintet, J = 6.2 Hz, 2H, H-28), 1.54 – 1.77 (m, 6H, H-15, H-22, H-26), 1.42 (quintet, J = 7.7 Hz, 2H, H-27)

$^{13}$C NMR (125 MHz, MeOD) δ 176.0 (C-24), 168.1 (C-33), 166.1 (C-13), 157.4 (C-7), 151.3 (C-1), 142.6 (C-6), 131.9 (C-3), 130.8 (C-10, C-11), 128.2 (C-9), 124.4 (C-12), 123.7 (C-8), 121.1 (C-4), 118.5 (C-5), 117.4 (C-2), 69.971.5 (C-16 to C-21), 63.4 (C-32), 61.6 (C-31), 57.0 (C-29), 41.1 (C-23), 38.9 (C-14), 37.8 (C-25), 36.9 (C-30), 30.4 (C-15), 30.4 (C-22), 29.8 (C-28), 29.5 (C-27), 26.9 (C-26)

HR-ESIMS calculated for [M+Na]$^+$ $C_{33}H_{44}Cl_2N_4O_8$Na: 749.2155. Found 749.2155.

### 6.2.0 De-Guanidinylated Peramivir Analogue

![Diagram of De-Guanidinylated Peramivir Analogue](image)

#### 6.2.1 (1S,4R)-(-)-Methyl-4-[(tert-Butoxycarbonyl)amino]cyclopent-2-ene-1-carboxylate (136)

(1R,4S)-(--)2-azabicyclo[2.2.1]hept-5-en-3-one (135; 432 mg, 3.96 mmol) was heated to reflux for 18 hours in methanolic hydrochloride (10 mL). The solvent was removed in vacuo to yield (1S,4R)-(--)-methyl-4-aminocyclopent-2-en-1-carboxylate hydrochloride as a white solid.

To a mixture of the crude hydrochloride salt and di-tert-butyl dicarbonate (963 mg, 4.41 mmol) in 30 mL anhydrous dichloromethane at 0 °C was added triethylamine (610 µL, 4.36 mmol) dropwise via syringe. After stirring for 1 hour the solvent was removed in vacuo to yield a crude white solid, which was further purified by washing with 4:1 hexanes:ethyl acetate. The filtrate was collected and concentrated under reduced pressure to provide compound 136 as a white solid (956 mg, 3.96 mmol, quantitative over two steps). Spectral data were consistent with that reported in the literature.\(^{94}\)
\( ^1 \)H NMR (300 MHz, CDCl\textsubscript{3}) \( \delta \) 5.81-5.88 (m, 2H, H-4, H-5), 4.87 (br s, 1H, NH), 4.71-4.82 (m, 1H, H-1), 3.69 (s, 3H, H-7), 3.46 (ddd, \( J = 8.5, 4.4, 0.8 \) Hz, 1H, H-3), 2.49 (dt, \( J = 13.8, 8.5 \) Hz, 1H, H-2), 1.84 (dt, \( J = 13.8, 4.1 \) Hz, 1H, H-2), 1.42 (s, 9H, H-10)

\[ \text{6.2.2 (3aR,4R,6S,6aS)-(+) -Methyl-4-[[(tert-Butoxycarbonyl)amino]-3-(1-ethylpropyl)-4,5,6,6a-tetrahydro-3aH-cyclopenta[d]isoxazole-6-carboxylate (137)} \]

A solution of sodium hypochlorite (5% solution in water, 12.7 mL, 8.55 mmol) and a solution of 2-ethyl-butyraldehyde oxime (139; 499 mg, 4.33 mmol) in 12 mL dichloromethane were added dropwise via a syringe pump over two days to a mixture of compound 136 (229 mg, 0.950 mmol) and triethylamine (40 \( \mu \)L, 0.29 mmol) in 20 mL dichloromethane and heated to reflux. The solution was stirred at reflux for an additional 24 hours before being quenched with 40 mL saturated brine. The aqueous layer was extracted with 3 x 40 mL dichloromethane, and the combined organic phases were dried over anhydrous sodium sulphate and concentrated in vacuo to yield a crude yellow oil. Flash-column chromatography (silica, hexanes:ethyl acetate 4:1, \( R_f \) 0.20) afforded compound 137 as a colourless oil (180 mg, 0.508 mmol, 53.5%). Spectral data were consistent with that reported in literature.\textsuperscript{172}

\( ^1 \)H NMR (300 MHz, CDCl\textsubscript{3}) \( \delta \) 5.57 (d, \( J = 7.5 \) Hz, 1H, NH), 5.18 (dd, \( J = 9.1, 0.9 \) Hz, 1H, H-4), 4.15-4.24 (m, 1H, H-1), 3.74 (s, 3H, H-13), 3.56 (d, \( J = 9.2 \) Hz, 1H, H-3), 3.18 (d, \( J = 6.4 \) Hz, 1H, H-5), 2.42-2.55 (m, 1H, H-7), 2.10 (ddd, \( J = 14.4, 7.5, 1.0 \) Hz, 1H, H-2), 1.99 (dt, \( J = 14.1, 2.8 \) Hz, 1H, H-2), 1.53-1.78 (m, 4H, H-8, H-9), 1.42 (s, 9H, H-16), 0.90 (t, \( J = 7.0 \) Hz, 3H, H-10 or H-11), 0.86 (t, \( J = 7.0 \) Hz, 3H, H-10 or H-11)
6.2.3 (1S,2S,3R,4R)-(-)-Methyl-3-[(1S)-1-(Acetylamino)-2-ethylbutyl]-4-[(tert-butoxycarbonyl)amino]-2-hydroxy-cyclopentanecarboxylate (140)

To 137 (17 mg, 0.048 mmol) in 2 mL anhydrous methanol were added concentrated hydrochloric acid (4 µL, 0.048 mmol) and platinum (VI) oxide. The mixture was stirred vigorously at 100 psi hydrogen pressure (PARR reactor) for 24 hours. The catalyst was removed by filtration through a minimum amount of celite and the filtrate was concentrated in vacuo to give (1S,2S,3R,4R)-(-)-methyl-[(1S)-1-(amino)-2-ethylbutyl]-4-[(tert-butoxycarbonyl)amino]-2-hydroxycyclopentanecarboxylate hydrochloride, which was used crude for acetylation.

To the amine hydrochloride in 2 mL anhydrous dichloromethane were added triethylamine (7 µL, 0.05 mmol) and acetic anhydride (5 µL, 0.05 mmol) at room temperature. The mixture was stirred for 2 hours and then washed with 10 mL distilled water. The aqueous layer was extracted with 3 x 15 mL dichloromethane, and the combined organic layer was dried over anhydrous sodium sulphate and concentrated in vacuo to yield a colourless oil. Flash-column chromatography (silica, hexanes:ethyl acetate 1:1, Rf 0.11) afforded compound 140 as a colourless oil (12 mg, 0.031 mmol, 64% over two steps). Spectral data were consistent with that reported in the literature.94

1H NMR (300 MHz, CDCl₃) δ 7.59 (d, J = 9.5 Hz, 1H, NHAc), 4.72 (d, J = 9.4 Hz, 1H, NHBoc), 4.21 (dd, J = 4.6, 1.2 Hz, 1H, H-4), 4.07-4.15 (m, 1H, H-1), 3.96-4.05 (m, 1H, H-6), 3.68 (s, 3H, H-13), 2.80 (ddd, J = 9.0, 8.9, 1.7 Hz, 1H, H-5), 2.47 (dt, J = 13.7, 7.9 Hz, 1H, H-3), 2.07 (s, 3H, H-18), 1.96 (dd, J = 10.8, 4.3 Hz, 1H, H-2), 1.60-1.73 (m, 1H, H-2), 1.42 (s, 9H, H-16), 1.18-1.30 (m, 6H, H-7, H-8, H-9, OH), 0.83 (t, J = 7.0 Hz, 3H, H-10 or H-11), 0.77 (t, J = 7.0 Hz, 3H, H-10 or H-11)
6.2.4 (1S,2S,3R,4R)-(−)-3-[(1S)-1-(Acetylamino)-2-ethylbutyl]-4-amino-2-hydroxy-cyclopentanecarboxylic acid (28)

To a solution of 140 (12 mg, 0.031 mmol) in 3 mL diethyl ether was added hydrochloric acid (1.0 M aq., 110 µL, 0.11 mmol) at room temperature. The mixture was stirred for 24 hours and the solvent was removed in vacuo to yield compound 28 as a viscous, colourless oil (9 mg, 0.031 mmol, quantitative).

1H NMR (300 MHz, D2O) δ 4.52 (dd, J = 5.1, 1.5 Hz, 1H, H-4), 4.38 (dd, J = 10.5, 2.3 Hz, 1H, H-1), 3.61-3.72 (m, 1H, H-6), 3.02-3.08 (m, 1H, H-3), 2.54-2.77 (m, 1H, H-5), 2.36-2.47 (m, 1H, H-2), 2.05 (s, 3H, H-14), 1.36-1.57 (m, 4H, H-2, H-7, H-8), 0.82-0.98 (m, 8H, H-9, H-10, H-11)

6.3.0 Neuraminidase Analogues

6.3.1 (1S,4R)-4-[1,3-Bis(benzyloxycarbonyl)guanidino]-cyclopent-2-enecarboxylic acid methyl ester (146)

(1R,4S)-(−)-2-azabicyclo[2.2.1]hept-5-en-3-one (135; 109 mg, 1.00 mmol) was heated to reflux for 18 hours in methanolic hydrochloride (10 mL). The solvent was removed in vacuo to yield (1S,4R)-(−)-methyl-4-aminocyclopent-2-en-1-carboxylate hydrochloride as a white solid.

To a mixture of the crude hydrochloride salt and triethylamine (630 µL, 4.50 mmol) in 5 mL anhydrous dimethylformamide was added 1,3-Bis(benzyloxycarbonyl)-2-methyl-2-thiopseudourea (359 mg, 1.00 mmol) and mercury (II) chloride (272 mg, 1.00 mmol). After stirring for 20 hours, ethyl acetate was added and the solution was filtered through celite. The
solvent was removed in vacuo to yield a brown oil. Flash-column chromatography (silica, dichloromethane, Rf 0.24) afforded compound 146 as a white, viscous oil (424 mg, 0.940 mmol, 94.0%).

IR (cm⁻¹, film) 3325, 3064, 1730, 1691, 1638, 1617

¹H NMR (500 MHz, CDCl₃) δ 11.71 (s, 1H, NH), 8.58 (d, J = 8.5 Hz, 1H, NH), 7.25-7.40 (m, 10H, H-14 to H-16, H-18 to H-20), 5.88-5.94 (m, 2H, H-4, H-5), 5.28-5.34 (m, 1H, H-1), 5.16 (s, 2H, H-12), 5.11 (s, 2H, H-11), 3.72 (s, 3H, H-7), 3.47-3.52 (m, 1H, H-3), 2.52 (dt, J = 15.0, 8.2 Hz, 1H, H-2), 1.98 (dt, J = 14.3, 4.3 Hz, 1H, H-2)

¹³C NMR (125 MHz, CDCl₃) δ 174.4 (C-6), 163.9 (C-8), 155.3 (C-10), 153.7 (C-9), 136.9 (C-13), 134.8 (C-17), 133.7 (C-5), 132.6 (C-4), 128.1-128.9 (C-14 to C-16, C-18 to C-20), 68.3 (C-12), 67.3 (C-11), 56.1 (C-7), 52.5 (C-1), 49.7 (C-3), 34.2 (C-2)

6.3.2 (1S,3S)-3-Guanidino-cyclopentanecarboxylic acid methyl ester (147)

To 146 (72 mg, 0.16 mmol) in 10 mL anhydrous methanol was added 10% palladium on carbon. The mixture was stirred vigorously under a balloon of hydrogen for 21 hours. The catalyst was removed by filtration through cotton and the filtrate was concentrated in vacuo to afford compound 147 as a colourless oil (30 mg, 0.16 mmol, quantitative).

IR (cm⁻¹, neat) 3325, 1740, 1638, 1617

¹H NMR (500 MHz, D₂O) δ 3.88-3.95 (m, 1H, H-1), 3.71 (s, 3H, H-7), 2.95-3.02 (m, 1H, H-3), 2.30-2.39 (m, 1H, H-2), 1.98-2.07 (m, 2H, H-4, H-5), 1.89-1.98 (m, 1H, H-4), 1.81-1.88 (m, 1H, H-2), 1.67-1.75 (m, 1H, H-5)

¹³C NMR (125 MHz, D₂O) δ 179.2 (C-6), 156.4 (C-8), 52.8 (C-7), 52.7 (C-1), 41.9 (C-3), 35.3 (C-2), 31.8 (C-5), 27.3 (C-4)
6.3.3 (1S,3S)-3-Guanidino-cyclopentanecarboxylic acid (29)

To 147 (30 mg, 0.16 mmol) was added 5% aqueous sodium hydroxide (160 µL) and the solution was stirred at room temperature for 4 hours. The mixture was neutralized with concentrated hydrochloric acid (20 µL) and used directly for kinetic assays.

6.3.4 (1S,4R)-4-(Benzyloxycarbonyl)amino-cyclopent-2-enecarboxylic acid methyl ester (157)

(1R,4S)-(−)-2-azabicyclo[2.2.1]hept-5-ene-3-one (135; 70 mg, 0.640 mmol) was heated to reflux for 18 hours in methanolic hydrochloride (10 mL). The solvent was removed in vacuo to yield (1S,4R)-(−)-methyl-4-aminocyclopent-2-en-1-carboxylate hydrochloride as a white solid.

To a mixture of the crude hydrochloride salt and sodium carbonate (207 mg, 1.95 mmol) in 10 mL distilled water at 0 °C was added benzyl chloroformate dropwise via a syringe (100 µL, 0.70 mmol). The solution was stirred for 30 minutes at 0 °C and 30 minutes at room temperature. The aqueous layer was extracted with 3 x 20 mL dichloromethane and the organic layers were combined and dried over anhydrous sodium sulphate. The solvent was removed in vacuo to yield a colourless oil. Flash-column chromatography (silica, hexanes:ether 2:1, Rf 0.22) afforded compound 157 as a white solid (105 mg, 0.382 mmol, 59.7%).

Melting Point: 155 °C

IR (cm⁻¹, film) 3428, 1731, 1716, 1506

1H NMR (500 MHz, CDCl₃) δ 7.27-7.36 (m, 5H, H-11 to H-13), 5.84-5.90 (m, 2H, H-4, H-5), 5.16 (d, J = 9.7 Hz, 1H, NH), 5.08 (s, 2H, H-9), 4.80-4.87 (m, 1H, H-1), 3.68 (s, 3H, H-7), 3.43-3.49 (m, 1H, H-3), 2.48 (dt, J = 14.2, 8.4 Hz, 1H, H-2), 1.88 (dt, J = 14.2, 3.8 Hz, 1H, H-2)
\[ ^{13}\text{C NMR} (125 \text{ MHz}, \text{CDCl}_3) \delta 175.4 (\text{C-6}), 155.8 (\text{C-8}), 136.8 (\text{C-10}), 134.7 (\text{C-5}), 131.8 (\text{C-4}), 128.7 (\text{C-11}), 128.3 (\text{C-12}), 128.3 (\text{C-13}), 66.8 (\text{C-9}), 56.5 (\text{C-7}), 52.4 (\text{C-1}), 49.4 (\text{C-3}), 34.7 (\text{C-2}) \]

![Diagram of compound 157](image)

### 6.3.5 (1S,4R)-4-(Benzyloxy carbonyl) amino-1-hydroxy-cyclopent-2-enecarboxylic acid methyl ester (158)

Compound 157 (151 mg, 0.547 mmol) and selenium oxide (74 mg, 0.67 mmol) in 15 mL anhydrous dichloromethane were heated to 100 °C in a microwave for 8 hours. The solvent was removed in vacuo to yield a brown oil. Flash-column chromatography (silica, hexanes:ether 1:2, \( R_f \) 0.10) afforded compound 158 as a colourless oil (99 mg, 0.34 mmol, 62%).

\[ ^1\text{H NMR} (500 \text{ MHz}, \text{CDCl}_3) \delta 7.26-7.39 (m, 5\text{H}, \text{H-11 to H-13}), 6.04 (d, J = 5.4 \text{ Hz}, \text{1H}, \text{NH}), 5.74 (d, J = 5.4 \text{ Hz}, \text{1H}, \text{H-1}), 5.05-5.10 (m, 4\text{H}, \text{H-4, H-5, H-9}), 3.80 (s, 3\text{H}, \text{H-7}), 3.30 (s \text{ br}, \text{1H}, \text{OH}), 2.43 (dd, J = 14.3, 7.6 \text{ Hz}, \text{1H}, \text{H-2}), 2.15 (dd, J = 14.3, 2.5 \text{ Hz}, \text{1H}, \text{H-2}) \]

\[ ^{13}\text{C NMR} (125 \text{ MHz}, \text{CDCl}_3) \delta 175.8 (\text{C-6}), 156.1 (\text{C-8}), 137.3 (\text{C-5}), 136.6 (\text{C-10}), 135.6 (\text{C-4}), 128.8 (\text{C-11}), 128.4 (\text{C-12}), 128.4 (\text{C-13}), 84.8 (\text{C-3}), 67.0 (\text{C-9}), 56.4 (\text{C-7}), 53.7 (\text{C-1}), 44.7 (\text{C-2}) \]

![Diagram of compound 159](image)

### 6.3.6 (1S,3S)-3-Amino-1-hydroxy-cyclopentanecarboxylic acid methyl ester (159)

To 158 (82 mg, 0.28 mmol) in 15 mL anhydrous methanol was added 10% palladium on carbon. The mixture was stirred vigorously at 300 psi hydrogen pressure (PARR reactor) for 21 hours. The catalyst was removed by filtration through cotton and the filtrate was concentrated in vacuo to afford compound 159 as a white solid (43 mg, 0.27 mmol, 97%).
Melting Point: 111 °C
IR (cm\(^{-1}\), film) 3365, 1719

\(^1\)H NMR (500 MHz, D\(_2\)O) \(\delta\) 3.90-3.97 (m, 1H, H-1), 3.80 (s, 3H, H-7), 2.31-2.41 (m, 3H, H-2, H-4), 2.26 (dd, \(J = 14.1, 7.8\) Hz, 1H, H-2), 1.86-2.00 (m, 2H, H-5)

\(^{13}\)C NMR (125 MHz, D\(_2\)O) \(\delta\) 176.7 (C-6), 81.2 (C-3), 53.4 (C-7), 50.8 (C-1), 42.8 (C-2), 36.8 (C-4), 28.9 (C-5)

6.3.7 \((1S,3S)-3\)-Amino-1-hydroxy-cyclopentanecarboxylic acid (132)

To 159 (21 mg, 0.13 mmol) was added 5% aqueous sodium hydroxide (80 \(\mu\)L) and the solution was stirred at room temperature for 4 hours. The mixture was neutralized with concentrated hydrochloric acid (10 \(\mu\)L) and used directly for assays.

6.3.8 \((1S,3S)-3\)-[1,3-Bis(benzyloxycarbonyl)guanidino]-1-hydroxy-cyclopentanecarboxylic acid methyl ester (160)

To 159 (22 mg, 0.14 mmol) and triethylamine (85 \(\mu\)L, 0.61 mmol) in 2 mL anhydrous dimethylformamide was added 1,3-Bis(benzyloxycarbonyl)-2-methyl-2-thiopseudourea (54 mg, 0.15 mmol) and mercury (II) chloride (41 mg, 0.15 mmol). After stirring for 18 hours, ethyl acetate was added and the solution was filtered through cotton. The solvent was removed in vacuo to yield a brown oil. Flash-column chromatography (silica, hexanes:ether 1:2, \(R_f\) 0.19) afforded compound 160 as a colourless oil (30 mg, 0.064 mmol, 46%).
IR (cm\(^{-1}\), neat) 3325, 3064, 1730, 1691, 1638, 1617

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 11.80 (s, 1H, NH), 8.56 (d, \(J = 8.04\) Hz, 1H, NH), 7.25-7.39 (m, 10H, H-14 to H-16, H-18 to H-20), 5.16 (s, 2H, H-11), 5.11 (s, 2H, H-12), 4.74-4.82 (m, 1H, H-1), 3.82 (s, 3H, H-7), 3.18 (s br, 1H, OH), 2.18-2.33 (m, 3H, H-2, H-4), 2.08 (dd, \(J = 14.5, 5.6\) Hz, 1H, H-2), 1.82-1.88 (m, 1H, H-5), 1.72-1.80 (m, 1H, H-5)

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 177.2 (C-6), 163.9 (C-8), 155.5 (C-9), 153.9 (C-10), 137.0 (C-13), 134.8 (C-17), 128.1-129.0 (C-14 to C-16, C-18 to C-20), 80.6 (C-3), 68.5 (C-11), 68.3 (C-12), 53.3 (C-7), 52.1 (C-1), 45.9 (C-2), 38.5 (C-4), 32.5 (C-5)

6.3.9 (1S,3S)-3-guanidino-1-hydroxy-cyclopentanecarboxylic acid methyl ester (161)

To 160 (30 mg, 0.064 mmol) in 10 mL anhydrous methanol was added 10% palladium on carbon. The mixture was stirred vigorously at 300 psi hydrogen pressure (PARR reactor) for 24 hours. The catalyst was removed by filtration through cotton and the filtrate was concentrated in vacuo to afford compound 161 as a yellow oil (11 mg, 0.053 mmol, 83%).

\(^1\)H NMR (300 MHz, D\(_2\)O) \(\delta\) 4.10-4.25 (m, 1H, H-1), 3.80 (s, 3H, H-7), 2.25-2.41 (m, 3H, H-2, H-4), 2.20 (dd, \(J = 14.3, 7.3\) Hz, 1H, H-2), 1.75-1.94 (m, 2H, H-5)

6.3.10 (1S,3S)-3-guanidino-1-hydroxy-cyclopentanecarboxylic acid (30)

To 161 (11 mg, 0.053 mmol) was added 5% aqueous sodium hydroxide (80 µL) and the solution was stirred at room temperature for 4 hours. The mixture was neutralized with concentrated hydrochloric acid (10 µL) and used directly for assays.
6.3.11 tert-Butyl (1S,4R)-3-oxo-2-azabicyclo[2.2.1]hept-5-en-2-carboxylate (169)

To a solution of (1S)-(+)2-azabicyclo[2.2.1]hept-5-en-3-one (135; 653 mg, 5.98 mmol), di-tert-butyl dicarbonate (2.124 g, 9.732 mmol) and 4-dimethylaminopyridine (734 mg, 6.01 mmol) in 10 mL anhydrous ethanol-free chloroform (EFC) was added triethylamine (830 µL, 5.98 mmol). The mixture was stirred at room temperature for 26 hours after which the solvent was concentrated in vacuo. The residue was purified by flash-column chromatography (silica, petroleum ether:ether 1:3, Rf 0.71) to yield compound 169 as a white solid (1.251 g, 5.980 mmol, quantitative). The spectral data were consistent with that reported in literature.\(^{186,294}\)

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 6.84 (ddd, \(J = 5.3, 2.1, 0.7\) Hz, 1H, H-5), 6.60 (ddd, \(J = 5.4, 3.3, 1.5\) Hz, 1H, H-4), 4.89-4.91 (m, 1H, H-1), 3.32-3.34 (m, 1H, H-3), 2.29 (dt, \(J = 8.3, 1.9\) Hz, 1H, H-2), 2.10 (dt, \(J = 8.4, 1.4\) Hz, 1H, H-2), 1.45 (s, 9H, H-9)

\(^13\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 176.4 (C-6), 150.5 (C-7), 140.1 (C-5), 138.3 (C-4), 82.7 (C-8), 62.5 (C-1), 55.0 (C-2), 54.5 (C-3), 28.2 (C-9)

6.3.12 tert-Butyl (1R,2S,4R,5S)-7-oxo-3-oxa-6-azatricyclo[3.2.1.0]octan-6-carboxylate (164)

Compound 169 (1.99 g, 9.3 mmol) and 3-chloroperbenzoic acid (8.41 g, 38 mmol) were dissolved in 80 mL anhydrous ethanol-free chloroform (EFC). The mixture was stirred at room temperature for three days, after which the reaction was diluted with 300 mL chloroform and
washed with 3 x 100 mL saturated sodium carbonate aqueous. The organic phases were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The white solid was purified by flash-column chromatography (silica, petroleum ether:ether 3:1, Rf 0.17) to yield compound 164 as a white solid (1.84 g, 8.2 mmol, 88%). Spectral data were consistent with that reported in the literature.\textsuperscript{186,294}

\textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ 4.59-4.61 (m, 1H, H-1), 3.75 (dd, J = 3.4, 1.1 Hz, 1H, H-5), 3.59 (dd, J = 3.5, 1.3 Hz, 1H, H-4), 3.05 (quintet, J = 1.5 Hz, 1H, H-3), 1.79 (dt, J = 10.5, 1.7 Hz, 1H, H-2), 1.62 (dt, J = 10.2, 1.7 Hz, 1H, H-2), 1.50 (s, 9H, H-9)

\textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}) δ 173.7 (C-6), 150.0 (C-7), 83.6 (C-8), 59.2 (C-1), 53.3 (C-5), 50.2 (C-4), 48.6 (C-3), 28.3 (C-9), 27.3 (C-2)

\textbf{6.3.13 tert-Butyl N-[(1S,2R,4R,5S)-4-(hydroxymethyl)-6-oxabicyclo[3.1.0]hex-2-yl]-carbamate (170)}

Epoxide 164 (106 mg, 0.470 mmol) was dissolved in 15 mL anhydrous methanol and sodium borohydride (87 mg, 2.3 mmol) was added over 5 minutes at 0 °C. The reaction was stirred at 0 °C for 30 min, and then neutralized with a solution of 10% acetic acid in methanol. The neutralization process was carefully monitored by spot testing on wet pH paper. The solvent was concentrated in vacuo and 20 mL saturated ammonium chloride solution was added to the residue. The mixture was extracted with 4 x 15 mL chloroform, and the combined organic phases were dried over anhydrous sodium sulphate. The solvent was removed in vacuo to afford compound 170 as a colourless oil (108 mg, 0.470 mmol, quantitative), without any further purification required. Alternatively, an analytical sample could be purified by flash-column chromatography (silica, toluene:ethyl acetate 1:1, R\textsubscript{f} 0.29). Spectral data were consistent with that reported in the literature.\textsuperscript{186}
\[^1\]H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 5.74 (s br, 1H, NH), 4.18-4.33 (m, 1H, H-1), 3.91 (dt, \(J = 10.1, 2.9\) Hz, 1H, H-5), 3.67 (dt, \(J = 10.1, 3.6\) Hz, 1H, H-4), 3.39-3.43 (m, 2H, H-6), 2.36-2.43 (m, 1H, H-3), 2.04-2.17 (m, 1H, H-2), 1.67 (s br, 1H, OH), 1.38-1.47 (m, 10H, H-2, H-9)

\[^{13}\]C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 156.1 (C-7), 79.7 (C-8), 63.0 (C-5), 60.0 (C-4), 59.6 (C-6), 49.9 (C-1), 40.5 (C-3), 33.0 (C-2), 28.6 (C-9)


Alcohol 170 (837 mg, 3.65 mmol), imidazole (549.4 mg, 8.07 mmol) and \textit{tert}-butyldimethylsilyl chloride (1.216 g, 8.069 mmol) were dissolved in 5 mL anhydrous dimethylformamide and stirred for 3 hours at room temperature. The reaction was diluted with 40 mL ethyl acetate and washed with 2 x 40 mL saturated aqueous sodium bicarbonate solution and 4 x 40 mL brine. The organic phase was dried over anhydrous sodium sulphate, and concentrated under reduced pressure to yield a yellow oil. The crude product was purified by flash-column chromatography (silica, toluene:ethyl acetate 6:1, \(R_f\) 0.53) to afford compound 165 as yellow oil (1.229 g, 3.577 mmol, 98\%). Spectral data were consistent with that reported in the literature.\(^{186}\)

\[^1\]H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 5.67 (d, \(J = 9.9\) Hz, 1H, NH), 4.22 (t, \(J = 8.9\) Hz, 1H, H-1), 3.84 (dd, \(J = 10.6, 2.4\) Hz, 1H, H-5), 3.61 (dd, \(J = 10.4, 2.8\) Hz, 1H, H-4), 3.35 (d, \(J = 2.1\) Hz, 1H, H-6), 3.31 (d, \(J = 2.1\) Hz, 1H, H-6), 2.30-2.35 (m, 1H, H-3), 2.03-2.11 (m, 1H, H-2), 1.40 (s, 9H, H-9), 1.35 (d, \(J = 13.9\) Hz, 1H, H-2), 0.90 (s, 9H, H-12), 0.11 (s, 6H, H-10)

\[^{13}\]C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 155.4 (C-7), 79.2 (C-8), 64.9 (C-5), 60.0 (C-4), 59.6 (C-6), 49.8 (C-1), 40.9 (C-3), 33.6 (C-2), 28.6 (C-9), 26.1 (C-12), 18.9 (C-11), -5.2 (C-10)
6.3.15 (3S-Hydroxy-4R-hydroxymethyl-cyclopentyl)-R-carbamic acid tert-butyl ester (166)

Lithium aluminum hydride (109 mg, 2.87 mmol) was added slowly to compound 165 (440 mg, 1.28 mmol) in 10 mL anhydrous diethyl ether at room temperature and stirred for 3 hours. The solution was then quenched with Rochelle’s salt and stirred for 20 hours. The aqueous layer was extracted with 3 x 30 mL chloroform, and the combined organic layers were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, 10% methanol in chloroform, Rf 0.10) to afford compound 166 as a white solid (192 mg, 0.832 mmol, 65.0%). Spectral data were consistent with that reported in the literature.\(^{186}\)

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.62 (s br, 1H, NH), 4.16 (q, \(J = 6.9\) Hz, 1H, H-1), 4.08-4.13 (m, 1H, H-4), 3.79 (dd, \(J = 10.2, 4.9\) Hz, 1H, H-6), 3.58 (dd, \(J = 10.5, 7.9\) Hz, H-6), 2.22-2.32 (m, 2H, H-2, OH), 1.93-2.05 (m, 3H, H-3, H-5, OH), 1.82 (dt, \(J = 13.5, 6.9\) Hz, 1H, H-5), 1.41 (s, 9H, H-9), 1.10 (ddd, \(J = 13.2, 9.5, 7.8\) Hz, 1H, H-2)

\(^13\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 155.7 (C-7), 79.7 (C-8), 75.6 (C-4), 65.6 (C-6), 51.4 (C-1), 49.1 (C-3), 42.4 (C-5), 34.8 (C-2), 28.8 (C-9)
6.3.16 (1R,3S,4R)-1-[1,3-Bis(benzyloxy carbonyl)guanidino]-3-hydroxy-4-hydroxymethyl-cyclopentane (174)

Compound 166 (60 mg, 0.26 mmol) was stirred in 1.0 M hydrochloric acid in anhydrous ether at room temperature for 22 hours. The solvent was removed in vacuo to afford the crude amino hydrochloride salt as a white solid.

To the crude salt and triethylamine (160 µL, 1.17 mmol) in 5 mL anhydrous dimethylformamide was added 1,3-Bis(benzyloxy carbonyl)-2-methyl-2-thiopseudourea (93 mg, 0.26 mmol) and mercury (II) chloride (71 mg, 0.26 mmol). After stirring for 18 hours, ethyl acetate was added and the solution was filtered through cotton. The solvent was removed in vacuo to yield a yellow oil. Flash-column chromatography (silica, ether, Rf 0.06) afforded compound 174 as a white solid (88 mg, 0.20 mmol, 76% over two steps).

IR (cm⁻¹, film) 3328, 3064, 1732, 1621, 1617

¹H NMR (500 MHz, CDCl₃) δ 11.71 (s br, 1H, NH), 8.35 (d, J = 7.0 Hz, 1H, NH), 7.24-7.39 (m, 10H, H-13 to H-15), 5.14 (s, 2H, H-10), 5.09 (s, 2H, H-11), 4.54 (sextet, J = 7.8 Hz, 1H, H-1), 4.07 (dt, J = 7.0, 5.3 Hz, H-4), 3.62 (dd, J = 10.4, 4.6 Hz, 1H, H-6), 3.44 (dd, J = 10.3, 7.6 Hz, 2H, H-6, OH), 3.09 (s br, 1H, OH), 2.27 (dt, J = 12.9, 5.4 Hz, 1H, H-5), 2.02-2.09 (m, 1H, H-2), 1.94-2.09 (m, 1H, H-3), 1.71 (dt, J = 13.9, 7.5 Hz, 1H, H-2), 1.04 (dt, J = 13.1, 9.2 Hz, 1H, H-5)

¹³C NMR (125 MHz, CDCl₃) δ 163.7 (C-7), 155.6 (C-8), 153.9 (C-9), 134.7 (C-16), 136.7 (C-12), 128.2-128.9 (C-13 to C-15, C-17 to C-19), 74.6 (C-4), 68.3 (C-10), 67.4 (C-11), 64.9 (C-6), 49.6 (C-1), 48.9 (C-3), 41.4 (C-5), 34.3 (C-2)
6.3.17 \((1R,3S,4R)-1\text{-guanidino-3-hydroxy-4-hydroxymethyl-cyclopentane}\) (175)

To 174 (44 mg, 0.099 mmol) in 10 mL anhydrous methanol was added 10% palladium on carbon. The mixture was stirred vigorously at 300 psi hydrogen pressure (PARR reactor) for 24 hours. The catalyst was removed by filtration through cotton and the filtrate was concentrated in vacuo to afford compound 175 as a colourless oil (17 mg, 0.099 mmol, quantitative).

IR (cm\(^{-1}\), neat) 3328, 1623

\(^1\)H NMR (500 MHz, MeOD) \(\delta\) 4.04 (dt, \(J = 6.7, 4.8\) Hz, 1H, H-4), 3.98 (quintet, \(J = 7.6\) Hz, 1H, H-1), 3.57 (dd, \(J = 10.8, 5.7\) Hz, 1H, H-6), 3.51 (dd, \(J = 10.8, 6.2\) Hz, 1H, H-6), 3.27 (t, \(J = 3.3\) Hz, 1H, OH), 2.33 (dddd, \(J = 13.0, 7.8, 7.8, 1.1\) Hz, 1H, H-2), 1.94-2.00 (m, 2H, H-3, H-5), 1.77 (ddd, \(J = 13.5, 7.6, 6.9\) Hz, 1H, H-5), 1.26 (dt, \(J = 13.1, 8.4\) Hz, 1H, H-2)

\(^13\)C NMR (125 MHz, MeOD) \(\delta\) 158.3 (C-7), 73.9 (C-4), 64.4 (C-6), 51.8 (C-1), 50.4 (C-3), 42.2 (C-5), 35.4 (C-2)

6.4.0 Neuraminidase Biology

6.4.1 Recombinant A/Brevig Mission/1/1918 flu virus Expression

A synthetic gene codon-optimized for expression in insect cells was designed (Genescript) encoding a hexahistidine tag, a tetramerization domain from the human vasodilator-stimulated phosphoprotein and a thrombin cleavage site, followed by amino acid residues 82–467 of the ectodomain of the neuraminidase from the A/Brevig Mission/1/1918 flu virus strain. The synthetic gene was cloned in a modified pAcGP67B vector (BD Biosciences) and co-transfected into Sf9 cells with Sapphire baculovirus (Orbigen) in the presence of Cellfectin (Invitrogen) according to the manufacturer’s instructions. After two rounds of amplification in SF9 cells, the recombinant virus was used to infect Hi5 cells. A small-scale pilot experiment was performed to determine the optimal amount of virus to use. In a typical preparation, 4-5 L of Hi5 cells at a density of 1.8 x 10^6 cells/mL were used. After 3 days of incubation at 28 °C, the cells were removed by centrifugation and 10 µL of protease inhibitor cocktail (Roche) was added per
liter of culture. The supernatant was then filtered through 3 Millipore filters (5, 1 and 0.45 µm pores) and concentrated by tangential flow/diafiltration (10 kDa cut-off). The sample was buffer-exchanged to 20 mM HEPES, 1 M NaCl and 30 mM imidazole, pH 8. The soluble neuraminidase was recovered by metal affinity chromatography using Ni-charged Chelating Sepharose Fast Flow Beads (Amersham Pharmacia). Following elution with imidazole, the samples were concentrated and buffer-exchanged with a spin column to the Neuraminidase Assay Buffer (50 mM Tris–HCl pH 7.5, 200 mM NaCl, 5 mM CaCl₂). Aliquots were flash-frozen on liquid nitrogen and stored at 70 °C.

6.4.2 Influenza A/Brisbane/59/2007 (H1N1) Virus Propagation

Influenza A/Brisbane/59/2007 (H1N1) virus was propagated in Madin-Darby Canine Kidney (MDCK) cells. Seed virus was inoculated into 10 confluent 75 cm² monolayers which were incubated at 37 °C for 2-3 days and harvested when full cytopathic effect was observed.

6.4.3 Influenza A/Brisbane/59/2007 (H1N1) Virus Purification

The pooled cell lysates were frozen and thawed and clarified by centrifugation at 3000g for 20 minutes. The supernatant was subjected to ultracentrifugation at 25,000 rpm for 90 minutes and the virus containing pellet was resuspended in 2 mL of MegaVir medium (Hyclone). Virus concentration was assessed by hemagglutination (HA) at 40,960 HA units.

6.4.4 Influenza A/Brisbane/59/2007 (H1N1) Virus Inactivation

NP40 (Fluka) was added to the purified influenza virus at a final concentration of 0.2% and the mixture was incubated at room temperature for a total of 3 hours and at 4 °C for 6 hours. The inactivation of the virus was confirmed by the Tissue Culture Infectious Dose assay. This virus preparation had a titre of 10⁷ TCID₅₀ per 100 µL before inactivation and <10² TCID₅₀ per 100 µL after NP40 treatment.

6.4.5 Enzyme Assay

The following solutions were prepared for the enzyme assays: (1) Assay buffer: 50 mM Tris, 5 mM CaCl₂, 200 mM NaCl, pH 7.5; (2) Protein stock solution: recombinant neuraminidase
was diluted in assay buffer to 1000 ng/mL, or inactivated virus suspension was diluted to obtain a similar activity; (3) Substrate stock solution: 2-\((4\text{-methylumbelliferyl})\)-\(\alpha\)-D-\(N\)acetylneuraminic acid (Aldrich) was dissolved in dimethylsulfoxide (DMSO) to a concentration of 10 mM; (4) Substrate working solution: 40 μL of the substrate stock solution was diluted to 1000 μL with assay buffer, for a final concentration of 400 μM (4% DMSO); (5) Inhibitor solutions: Inhibitors were diluted in assay buffer to provide a range of working concentrations. For IC\text{50} measurements with recombinant neuraminidase, sample wells of a black 96-well plate (Nunc, optical bottom) were charged with 40 μL of protein stock solution (1000 ng/mL), followed by 10 μL of inhibitor solution and 50 μL of substrate working solution (400 μM substrate, 4% DMSO). The samples (each containing 100 μL total volume, 400 ng/mL enzyme, 200 μM substrate, and 1.5% total DMSO, in 100 μL total sample volume) were mixed briefly by pipetting. Fluorescence was monitored over 5 minutes \((k_{\text{exc}} = 365 \text{ nm}; k_{\text{em}} = 445 \text{ nm})\). Experiments with inactivated virus were conducted similarly, except that the inhibitor solutions were added to wells containing inactivated virus, and these mixtures were allowed to incubate for either 10 minutes or 2 hours at room temperature prior to the addition of working solution. For kinetic data, the working solution was subjected to serial dilution in assay buffer containing 4% DMSO. Progress of the reaction was measured over 10 minutes at various concentrations of substrate and inhibitor. Control experiments (substrate buffer only) showed no significant background reaction. Data was plotted using XLfit (IDBS software).

\textbf{6.5.0 Didemnaketal A Analogue Possessing the C-6 Methyl Group}

![Chemical structure of the compound](image)

\textbf{6.5.1 2-Butyl-4S-(tert-butyl-dimethyl-silyloxy)methyl-5R-methyl-[1,3,2]dioxaborinane (189)}

Dibutylboron triflate \((1.0 \text{ M in dichloromethane, 3.00 mL, 3.00 mmol})\) and diisopropylethylamine \((570 \mu\text{L, 3.26 mmol})\) were added sequentially to a solution of compound
ent-179 (504 mg, 2.72 mmol) in anhydrous dichloromethane (20 mL) at 0 °C. The yellow solution was stirred at 0 °C for 45 minutes then cooled down to −78 °C before compound 181 (905 mg, 5.19 mmol) was added. The yellow mixture was stirred at −78 °C for 30 minutes then gradually warmed to room temperature for 3 hours. The dark yellow solution was quenched with saturated ammonium chloride (40 mL) and extracted with dichloromethane (3 x 50 mL). The combined organic fractions were dried over anhydrous sodium sulphate and the solvent was removed in vacuo to yield compound 188 as a crude viscous orange.

A 2 M solution of lithium borohydride (143 mg, 6.53 mmol) in anhydrous ether was added dropwise via a cannula to a solution of crude compound 188 in anhydrous ether (37.3 mL) and anhydrous methanol (0.7 mL) at 0 °C. The mixture was stirred at 0 °C for 2 hours then quenched with Rochelle’s salt (40 mL) and warmed to room temperature over 24 hours. The following day, the solvent was removed in vacuo and the aqueous layer was extracted with ether (3 x 30 mL). The combined organic fractions were dried over anhydrous sodium sulphate and the solvent removed in vacuo to yield compound 189 as a yellow oil. Flash-column chromatography of the crude material (silica, hexanes:ether 1:1, Rf 0.71) yielded compound 189 as a yellow oil (331 mg, 1.23 mmol, 45.0% over two steps).

$^1$H NMR (500 MHz, CDCl$_3$) δ 3.94 (dt, J = 6.2, 4.2 Hz, 1H, H-3), 3.92 (dd, J = 10.9, 4.2 Hz, 1H, H-1), 3.83 (dd, J = 10.9, 6.4 Hz, 1H, H-1), 3.71 (dd, J = 10.6, 4.3 Hz, 1H, H-4), 3.58 (dd, J = 10.5, 5.9 Hz, 1H, H-4), 2.12-2.20 (m, 1H, H-2), 1.21-1.35 (m, 4H, H-10, H-11), 0.93 (d, J = 7.0 Hz, 3H, H-5), 0.87 (s, 9H, H-8), 0.85 (d, J = 7.3 Hz, 3H, H-12), 0.67 (d, J = 7.3 Hz, 2H, H-9), 0.04 (s, 3H, H-6), 0.04 (s, 3H, H-6)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 73.8 (C-3), 67.4 (C-1), 63.7 (C-4), 31.9 (C-2), 26.6 (C-10), 26.0 (C-8), 25.7 (C-11), 18.4 (C-7), 14.2 (C-12), 10.9 (C-5), −5.2 (C-6), −5.3 (C-6)
6.5.2 *D*-phenylalaninol (209)

To a suspension of sodium borohydride (5.765 g, 152.4 mmol) in 150 mL anhydrous tetrahydrofuran at 0 °C was added *D*-phenylalanine (208; 10.004 g, 60.56 mmol) in one portion. Iodine (20.058 g, 79.03 mmol) in 100 mL anhydrous tetrahydrofuran was added via a dropping funnel over thirty minutes and the resulting solution was heated to reflux for 24 hours. The reaction mixture was cooled and methanol was added until the gas evolution ceased and the solution went clear. The solvents were removed in vacuo to afford a white paste. A solution of 20% (w/w) aqueous potassium hydroxide (100 mL) was added and the resulting solution was stirred overnight. The aqueous phase was extracted with dichloromethane (3 x 200 mL), and the combined organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. Recrystallization from toluene afforded compound 209 as a white crystalline solid after two crops (9.112 g, 60.26 mmol, 99.5 %). Spectral data were consistent with that reported in the literature.\(^{232}\)

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.26-7.32 (m, 2H, H-5), 7.15-7.23 (m, 3H, H-6, H-7), 3.61 (dd, J = 10.8, 3.8 Hz, 1H, H-1), 3.37 (dd, J = 10.7, 7.3 Hz, 1H, H-1), 3.09 (dddd, J = 8.9, 7.3, 5.2, 3.7 Hz, 1H, H-2), 2.77 (dd, J = 13.6, 5.2 Hz, 1H, H-3), 2.50 (dd, J = 13.6, 8.7 Hz, 1H, H-3), 2.16 (br s, 1H, OH)

\(^1^3\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 138.8 (C-4), 129.4 (C-5), 128.7 (C-6), 126.6 (C-7), 66.4 (C-1), 54.4 (C-2), 41.0 (C-3)

\([\alpha]_D^{20} = +22.2^\circ\) (c = 0.0100 g/mL; CHCl\(_3\)); lit.\(^{295}\) \([\alpha]_D^{20} = +21.7^\circ\) (c = 1.0 g / 100 mL; CHCl\(_3\))

**Ent-209** \([\alpha]_D^{20} = \ -22.0^\circ\) (c = 0.0100 g/mL; CHCl\(_3\))
6.5.3 (S)-(−)-4-benzyl-2-oxazolidinone (210)

Compound 209 (5.007 g, 33.11 mmol), potassium carbonate (462 mg, 3.340 mmol) and diethyl carbonate (8.02 mL, 66.2 mmol) were added to a flask equipped with a vigreux column and a short path distillation apparatus and heated to 135 °C. Ethanol was removed as it formed over a 2 hour period. The solution was cooled down and dichloromethane (100 mL) was added. The organic phase was washed with 10% aqueous hydrochloric acid (3 x 30 mL), 10% aqueous sodium bicarbonate (30 mL) and brine (30 mL). The organic phase was then dried over anhydrous sodium sulphate and the solvent removed in vacuo to yield compound 210 as a white solid (5.52 g, 31.1 mmol, 94.0%) without further purification required. Alternatively, an analytically pure sample could be obtained through recrystallization from hexanes/ethyl acetate. Spectral data were consistent with that reported in the literature.$^{226}$

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.29-7.33 (m, 2H, H-6), 7.23-7.27 (m, 1H, H-7), 7.14-7.17 (m, 2H, H-5), 5.91 (br s, 1H, NH), 4.41 (dd, J = 8.6, 7.9 Hz, 1H, H-1), 4.12 (dd, J = 8.4, 5.5 Hz, 1H, H-1), 4.07 (m, 1H, H-2), 2.90 (dd, J = 13.4, 7.2 Hz, 1H, H-3), 2.83 (dd, J = 13.4, 6.7 Hz, 1H, H-3)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 159.6 (C-8), 136.1 (C-4), 129.2 (C-5), 129.2 (C-6), 127.4 (C-7), 69.8 (C-1), 53.9 (C-2), 41.6 (C-3)

$[\alpha]_D^{20} = + 61.6 ^\circ$ (c = 0.0100 g/mL; CHCl$_3$); lit.$^{227}$ $[\alpha]_D^{20} = + 62 ^\circ$ (c = 1.0 g / 100 mL; CHCl$_3$)

Ent-210 $[\alpha]_D^{20} = - 61.8 ^\circ$ (c = 0.0100 g/mL; CHCl$_3$)
6.5.4 (S)-(–)-4-benzyl-3-propionyl-2-oxazolidinone (97)

*n*-butyllithium (14.0 mL, 35.9 mmol) was added dropwise via syringe to a solution of compound 210 (5.62 g, 31.7 mmol) in anhydrous tetrahydrofuran (100 mL) at –78 ºC. The solution was warmed gradually to 0 ºC for 30 minutes and then cooled back down to –78 ºC before freshly distilled propionyl chloride (3.20 mL, 34.9 mmol) was added. The yellow reaction mixture was gradually warmed to 0 ºC for 1 hour, then to room temperature for 1 hour followed by the addition of saturated ammonium chloride and extraction with dichloromethane (3 x 150 mL). The combined organic fractions were dried over anhydrous sodium sulphate and the solvent removed in vacuo to yield crude compound 97 as a yellow oil. Flash-column chromatography of the crude material (silica, hexanes:ether 2:1, Rf 0.25) afforded compound 97 as a white solid (7.394 g, 31.70 mmol, quantitative). Spectral data were consistent with that reported in the literature.  

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.29-7.33 (m, 2H, H-6), 7.23-7.27 (m, 1H, H-7), 7.17-7.20 (m, 2H, H-5), 4.65 (dddd, J = 9.5, 7.4, 3.3, 3.3 Hz, 1H, H-2), 4.17 (dd, J = 7.4, 0.7 Hz, 1H, H-1), 4.15 (dd, J = 9.1, 3.2 Hz, 1H, H-1), 3.28 (dd, J = 13.4, 3.3 Hz, 1H, H-3), 2.94 (dq, J = 17.8, 7.2 Hz, 2H, H-10), 2.75 (dd, J = 13.3, 9.5 Hz, 1H, H-3), 1.19 (t, J = 7.3 Hz, 3H, H-11)  

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 174.3 (C-9), 153.8 (C-8), 135.5 (C-4), 129.6 (C-5), 129.2 (C-6), 127.5 (C-7), 66.4 (C-1), 55.4 (C-2), 38.1 (C-3), 29.4 (C-10), 8.5 (C-11)  

$[^{[\alpha]}]_{D}^{20} = -55.0^\circ$ (c = 0.0100 g/mL; CHCl$_3$); lit.$^{296}$ $[^{[\alpha]}]_{D}^{20} = -55.6^\circ$ (c = 1.27 g / 100 mL; CHCl$_3$)  

Ent-97 $[^{[\alpha]}]_{D}^{20} = +55.0^\circ$ (c = 0.0100 g/mL; CHCl$_3$)
6.5.5 (2S,3R)-2-Methyl-pent-4-ene-1,3-diol (194)

Dibutylboron triflate (1.0 M in dichloromethane, 41.4 mL, 41.4 mmol) and diisopropylethylamine (7.20 mL, 41.4 mmol) were added sequentially to a solution of compound 97 (8.045 g, 34.49 mmol) in anhydrous dichloromethane (150 mL) at 0 ºC. The yellow solution was stirred at 0 ºC for 45 minutes then cooled down to −78 ºC before fresh acrolein (6.90 mL, 104 mmol; passed through a neutral alumina plug) was added. The yellow mixture was stirred at −78 ºC for 30 minutes then gradually warmed to room temperature for 3 hours. The dark yellow solution was quenched with Rochelle’s salt (100 mL) and extracted with ether (3 x 150 mL). The solvent was removed in vacuo followed by addition of methanol (140 mL) and then cooled to 0 ºC. 30% aqueous hydrogen peroxide (70 mL) was added dropwise via a pressure-equalising dropping funnel and the resulting orange solution was stirred at 0 ºC for one hour. Distilled water was added and the methanol was removed in vacuo. The resulting aqueous phase was extracted with ether (3 x 150 mL) and the combined organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo to yield compound 211 as a viscous orange oil (9.979 g, 34.49 mmol, quantitative). Alternatively, an analytically pure sample could be obtained through flash-column chromatography of the crude material (silica, hexanes:ether 1:2, R<sub>f</sub> 0.23) to provide a colourless oil. Spectral data were consistent with that reported in the literature.234

A 2 M solution of lithium borohydride (1.991 g, 91.41 mmol) in anhydrous tetrahydrofuran was added dropwise via a cannula to a solution of crude compound 211 (9.979 g, 34.49 mmol) in anhydrous tetrahydrofuran (400 mL) and anhydrous methanol (3.74 mL, 86.6 mmol) at 0 ºC. The mixture was stirred at 0 ºC for 2 hours then quenched with 10% aqueous sodium hydroxide (200 mL) and warmed to room temperature over 24 hours. The following day, the solvent was removed in vacuo and the aqueous layer was extracted with ether (4 x 200 mL). The combined organic fractions were dried over anhydrous sodium sulphate and the solvent removed in vacuo to yield compound 194 as a colourless oil and compound 210 as a white solid as a 1:1 mixture. Flash-column chromatography of the crude material (silica,
chloroform:methanol 97:3, R_f 0.07) yielded compound 194 as a colourless oil (4.006 g, 34.49 mmol, quantitative over two steps) and compound 210 as a white solid (4.20 g, 23.7 mmol, 60.2% recovery). Spectral data were consistent with that reported in the literature.\textsuperscript{234,297}

\begin{align*}
^1\text{H NMR} (500 \text{ MHz, CDCl}_3) &\delta 5.85 (\text{ddd, } J = 17.3, 10.6, 5.9 \text{ Hz, } 1\text{H, H-4}), 5.23 (\text{dt, } J = 17.2, 1.4, 1.4 (4\text{J} \text{ Hz, } 1\text{H, H-5}), 5.14 (\text{dt, } J = 10.4, 1.3, 1.3 (4\text{J} \text{ Hz, } 1\text{H, H-5}), 4.26 (\text{m, } 1\text{H, H-3}), 3.61 (\text{dd, } J = 10.6, 7.8 \text{ Hz, } 1\text{H, H-1}), 3.55 (\text{dd, } J = 10.8, 4.5 \text{ Hz, } 1\text{H, H-1}), 1.84-1.93 (\text{m, } 1\text{H, H-2}), 1.36 (\text{br s, } 1\text{H, OH}), 1.24 (\text{br s, } 1\text{H, OH}), 0.80 (\text{d, } J = 7.0 \text{ Hz, } 3\text{H, H-6}) \\
^{13}\text{C NMR} (125 \text{ MHz, CDCl}_3) &\delta 138.5 (\text{C-4}), 115.7 (\text{C-5}), 75.4 (\text{C-3}), 65.9 (\text{C-1}), 39.8 (\text{C-2}), 11.2 (\text{C-6})
\end{align*}

\[ \left[\alpha\right]_D^{20} = -17.0^\circ \text{ (c = 3.00 g/mL; CH}_2\text{Cl}_2); \text{lit.}^{234} \left[\alpha\right]_D^{20} = -17.6^\circ \text{ (c = 3.00 g/mL; CH}_2\text{Cl}_2) \]

\textbf{Ent-194} \[ \left[\alpha\right]_D^{20} = +17.4^\circ \text{ (c = 3.00 g/mL; CH}_2\text{Cl}_2) \]

6.5.6 (2\textbf{R},4\textbf{R},5\textbf{S})-2-(4-Methoxy-phenyl)-5-methyl-4-vinyl-[1,3]dioxane (98)

A solution of compound 194 (144 mg, 1.24 mmol), camphorsulfonic acid (37 mg, 0.16 mmol) and \( p \)-anisaldehyde dimethyl acetal (0.52 mL, 3.1 mmol) in anhydrous dichloromethane (5 mL) was stirred at room temperature for 24 hours. The mixture was quenched with saturated sodium bicarbonate (5 mL) and the aqueous phase was extracted with dichloromethane (3 x 20 mL). The combined organic fractions were dried over anhydrous sodium sulphate and the solvent removed in vacuo.

The crude material was dissolved in anhydrous tetrahydrofuran (10 mL) and sodium borohydride (1.5 eq) was added. The solution was stirred at room temperature for 1 hour then quenched with methanol till the evolution of gas ceased and the solution went clear. The solvents were removed in vacuo to yield a white paste which was taken directly on to flash-column
chromatography (silica, hexanes:ether 97:3, Rf 0.36) to yield compound 98 as a white solid (285 mg, 1.22 mmol, 98.0% over two steps). Spectral data were consistent with that reported in the literature.\textsuperscript{234}

\begin{align*}
\text{H NMR (500 MHz, CDCl}_3\text{)} \delta & \ 7.44 (d, J = 8.9 Hz, 2H, H-9), 6.88 (d, J = 8.8 Hz, 2H, H-10), 5.83 (ddd, J = 17.2, 10.6, 4.8 Hz, 1H, H-4), 5.51 (s, 1H, H-7), 5.31 (dt, J = 17.2, 1.7, 1.7 (\textsuperscript{4}J) Hz, 1H, H-5), 5.18 (dt, J = 10.7, 1.9, 1.9 (\textsuperscript{4}J) Hz, 1H, H-5), 4.48-4.50 (m, 1H, H-3), 4.11 (dd, J = 11.2, 2.3 Hz, 1H, H-1), 3.78 (s, 3H, H-12), 1.62-1.68 (m, 1H, H-2), 1.16 (d, J = 7.0 Hz, 3H, H-6)
\end{align*}

\begin{align*}
\text{C NMR (125 MHz, CDCl}_3\text{)} \delta & \ 160.1 (C-11), 136.9 (C-4), 131.6 (C-8), 127.7 (C-9), 115.5 (C-5), 113.8 (C-10), 101.7 (C-7), 80.3 (C-3), 73.5 (C-1), 55.5 (C-12), 32.9 (C-2), 11.6 (C-6)
\end{align*}

\begin{align*}
[a]_{D\textsuperscript{20}} & = -20.2^\circ \text{ (c = 1.00 g/mL; CHCl}_3\text{); lit.} \text{[a]_{D\textsuperscript{20}}} = -20.3^\circ \text{ (c = 1.00 g/mL; CHCl}_3\text{)}
\end{align*}

Ent-98 $[\alpha]_D^{20} = +20.1^\circ \text{ (c = 1.00 g/mL; CHCl}_3\text{)}$

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{structure.png}
\caption{Structural diagram of compound 98.}
\end{figure}

6.5.7 \textit{(2R,4R,5S)-2-(4-Methoxy-phenyl)-5-methyl-[1,3]dioxane-4-carbaldehyde (182)}

Ozone was bubbled through a solution of compound 98 (578 mg, 2.47 mmol) and 2,6-lutidine (0.34 mL, 3.0 mmol) in 20 mL anhydrous dichloromethane at –78 °C till pale blue. The ozone was stopped and argon was bubbled through till the blue colour dissipated, followed by the addition of dimethyl sulfide (0.22 mL, 3.0 mmol). The solution was warmed to room temperature and stirred for 22 hours. The solvent was removed in vacuo and the resulting aldehyde 182 was taken on crude to the next step. Alternatively, an analytical sample could be purified via flash-column chromatography (florisil, hexanes:ethyl acetate 9:1, Rf 0.03). Spectral data were consistent with that in the literature.\textsuperscript{234}
$^1$H NMR (500 MHz, CDCl$_3$) δ 9.67 (s, 1H, H-4), 7.46 (d, J = 8.8 Hz, 2H, H-8), 6.91 (d, J = 8.8 Hz, 2H, H-9), 5.54 (s, 1H, H-6), 4.36 (d, J = 2.9 Hz, 1H, H-3), 4.10 (ddd, J = 11.4, 2.4, 0.8 (J) Hz, 1H, H-1), 4.02 (dd, J = 11.4, 1.3 Hz, 1H, H-1), 3.80 (s, 3H, H-11), 2.07-2.13 (m, 1H, H-2), 1.20 (d, J = 6.7 Hz, 3H, H-5)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 202.5 (C-4), 160.5 (C-10), 130.5 (C-7), 127.7 (C-8), 114.0 (C-9), 102.0 (C-6), 83.7 (C-3), 73.3 (C-1), 55.5 (C-11), 30.6 (C-2), 12.1 (C-5)

6.5.8 1-[(2R,4R,5S)-2-(4-Methoxy-phenyl)-5-methyl-[1,3]dioxan-4-yl]-1(1S)-but-3-en-1-ol (225)

To crude compound 182 in anhydrous dichloromethane (10 mL) at −78 ºC was added allylboronic acid pinacol ester (507 mg, 3.02 mmol). The solution was gradually warmed to room temperature over 23 hours then quenched with saturated ammonium chloride (10 mL). The aqueous phase was extracted with dichloromethane (3 x 20 mL) and the combined organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude yellow oil was purified using flash-column chromatography (1% triethylamine treated silica, hexanes:ether 1:1, R$_f$ 0.09) to yield compound 225 as a white solid (>20:1 dr, 551 mg, 1.98 mmol, 80.2 % over two steps).

Melting Point: 74-75 ºC

IR (cm$^{-1}$, film) 3383, 990, 910

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.40 (d, J = 8.8 Hz, 2H, H-8), 6.88 (d, J = 9.0 Hz, 2H, H-9), 5.84 (ddddd, J = 17.9, 9.6, 8.4, 6.2 Hz, 1H, H-13), 5.45 (s, 1H, H-6), 5.18-5.20 (m, 1H, H-14), 5.15-5.17 (m, 1H, H-14), 4.06 (dd, J = 11.4, 2.3 Hz, 1H, H-1), 4.03 (dd, J = 11.4, 1.5 Hz, 1H, H-1),
3.79 (s, 3H, H-11), 3.65-3.71 (m, 2H, H-3, H-4), 2.59-2.64 (m, 1H, H-12), 2.11-2.19 (m, 1H, H-12), 1.86-1.92 (m, 1H, H-2), 1.68 (d, J = 3.7 Hz, 1H, OH), 1.24 (d, J = 6.9 Hz, 3H, H-5)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 160.1 (C-10), 134.6 (C-13), 131.5 (C-7), 127.5 (C-8), 119.2 (C-14), 113.8 (C-9), 101.8 (C-6), 81.6 (C-3), 74.1 (C-1), 69.2 (C-4), 55.5 (C-11), 38.6 (C-12), 29.4 (C-2), 11.7 (C-5)

$\left[\alpha\right]_{D}^{20} = -26.0^\circ$ (c = 0.00510 g/mL; CHCl$_3$)

Ent-225 $\left[\alpha\right]_{D}^{20} = +25.9^\circ$ (c = 0.00510 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{16}$H$_{22}$O$_4$Na: 301.1416. Found 301.1416.

6.5.9 2R-Methyl-hept-6-ene-1,3S,4R-triol (ent-221)

A solution of compound ent-225 (171 mg, 0.588 mmol) in 10 mL 0.01 M hydrochloric acid and 10 mL tetrahydrofuran was stirred at room temperature for 24 hours. Tetrahydrofuran was removed in vacuo and the aqueous layer was extracted with ethyl acetate (5 x 40 mL). The organic layers were combined, dried over anhydrous sodium sulphate and concentrated under reduced pressure to give crude ent-221 as a white solid. Flash-column chromatography (silica, 10% methanol in dichloromethane, R$_f$ 0.19) of the crude material yielded compound ent-221 (87 mg, 0.54 mmol, 92%) as a white solid.

$^1$H NMR (300 MHz, D$_2$O) $\delta$ 5.84 (ddt, J = 17.3, 10.1, 7.1 Hz, 1H, H-7), 4.90-5.05 (m, 2H, H-8), 3.47 (dd, J = 10.6, 6.9 Hz, 1H, H-3), 3.34-3.43 (m, 3H, H-1, H-4), 3.21 (quintet, 3 x OH), 2.40-2.50 (m, 1H, H-2), 1.89-2.11 (m, 2H, H-6), 0.80 (d, J = 6.8 Hz, 3H, H-5)
6.5.10 1-(tert-Butyl-dimethyl-silanyloxy)-2R-methyl-hept-6-ene-3S,4R-diol (ent-222)

*tert*-Butyldimethylsilyl chloride (19 mg, 0.13 mmol) was added to a solution of ent-221 (22 mg, 0.13 mmol) and imidazole (22 mg, 0.32 mmol) in 2 mL anhydrous dimethylformamide. The mixture was heated to reflux for 17 hours, then quenched with saturated ammonium chloride (10 mL) and extracted with dichloromethane (3 x 20 mL). The organic fractions were combined, dried over sodium sulphate and concentrated in vacuo. Flash-column chromatography (silica, hexanes:ether 1:2, R_f 0.54) of the crude material yielded compound ent-222 (14 mg, 0.050 mmol, 38%) as a colourless oil.

1H NMR (300 MHz, CDCl_3) δ 5.80-5.95 (m, 1H, H-7), 5.13-5.21 (m, 2H, H-8), 3.83 (dd, J = 10.0, 3.2 Hz, 1H, H-1), 3.70 (dd, J = 10.0, 4.0 Hz, 1H, H-1), 3.66 (dt, J = 9.4, 2.0 Hz, 1H, H-3), 3.55-3.63 (m, 1H, H-4), 3.37 (d, J = 1.8 Hz, 1H, OH), 2.53-2.63 (m, 1H, H-6), 2.17 (dtt, J = 14.1, 8.4, 1.1 Hz, 1H, H-6), 1.94-2.05 (m, 1H, H-2), 1.84 (d, J = 4.0 Hz, 1H, OH), 1.02 (d, J = 7.3 Hz, 3H, H-5), 0.88 (s, 9H, H-11), 0.06 (s, 6H, H-9)

6.5.11 4R-Allyl-5S-[2-(tert-butyl-dimethyl-silanyloxy)-1R-methyl-ethyl]-[1,3]dioxolan-2-one (ent-224)

Triphosgene (19 mg, 0.064 mmol) and pyridine (12 µL, 0.15 mmol) were added to a solution of ent-222 (19 mg, 0.068 mmol) in 5 mL anhydrous dichloromethane at 0 ºC and stirred for 40 minutes. The mixture was diluted with 15 mL ether and washed with saturated copper (II) sulfate (5 mL) and brine (5 mL). The organic layer was dried over sodium sulphate and concentrated in vacuo to yield compound ent-224 (13 mg, 0.042 mmol, 61%) without any further purification.
\[^1\text{H} \text{NMR} (500 \text{ MHz, CDCl}_3) \ \delta \ 5.80 \ (\text{ddt, } J = 17.1, 10.2, 6.8 \text{ Hz, 1H, H-7}), \ 5.15-5.21 \ (\text{m, 2H, H-8}), \ 4.71 \ (\text{ddd, } J = 8.9, 6.8, 4.2 \text{ Hz, 1H, H-4}), \ 4.62 \ (\text{t, } J = 6.9 \text{ Hz, 1H, H-3}), \ 3.57 \ (\text{dd, } J = 10.3, 4.8 \text{ Hz, 1 H, H-1}), \ 3.39 \ (\text{dd, } J = 9.9, 9.2 \text{ Hz, 1H, H-4}), \ 2.45-2.58 \ (\text{m, 2H, H-6}), \ 2.13-2.20 \ (\text{m, 1H, H-2}), \ 0.98 \ (\text{d, } J = 6.8 \text{ Hz, 3H, H-5}), \ 0.87 \ (\text{s, 9H, H-11}), \ 0.04 \ (\text{s, 6H, H-9})

6.5.12 (2R, 4R, 5S)-4-(1S-Benzyloxy-but-3-enyl)-2-(4-methoxy-phenyl)-5-methyl-[1,3]dioxane (235)

To a solution of 225 (109 mg, 0.392 mmol) and benzyl bromide (93 μL, 0.78 mmol) in 10 mL anhydrous tetrahydrofuran was added 60% wt. sodium hydride (94 mg, 3.92 mmol) in a single portion and heated to reflux for 2 hours. The mixture was quenched with 15 mL brine and extracted with 3 x 20 mL aliquots of dichloromethane. The organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo to yield a crude yellow oil. The crude material was purified by flash chromatography (1% triethylamine treated silica, hexanes:ether 4:1, R\text{f} 0.32) to yield compound 235 as a colourless oil (144 mg, 0.392 mmol, quantitative).

IR (cm\(^{-1}\), film) 3014, 1212, 759

\[^1\text{H} \text{NMR} (500 \text{ MHz, CDCl}_3) \ \delta \ 7.40 \ (\text{d, } J = 8.7 \text{ Hz, 2H, H-8}), \ 7.26-7.35 \ (\text{m, 5H, H-17 to H-19}), \ 6.88 \ (\text{d, } J = 8.7 \text{ Hz, 2H, H-9}), \ 5.95 \ (\text{dddd, } J = 16.4, 10.1, 8.2, 6.2, 1H, H-13), \ 5.42 \ (\text{s, 2H, H-6}), \ 5.11-5.18 \ (\text{m, 2H, H-14}), \ 4.73 \ (\text{d, } J = 11.7 \text{ Hz, 1H, H-15}), \ 4.44 \ (\text{d, } J = 11.7 \text{ Hz, 1H, H-15}), \ 4.05 \ (\text{dd, } J = 11.6, 2.7 \text{ Hz, 1H, H-1}), \ 4.01 \ (\text{dd, } J = 11.1, 1.6 \text{ Hz, 1H, H-1}), \ 3.89 \ (\text{dd, } J = 9.3, 2.3 \text{ Hz,}}
1H, H-3), 3.79 (s, 3H, H-11), 3.61 (dt, J = 9.4, 4.1 Hz, 1H, H-4), 2.62-2.68 (m, 1H, H-12), 2.39-2.46 (m, 1H, H-12), 1.89-1.95 (m, 1H, H-2), 1.14 (d, J = 6.8 Hz, 3H, H-15)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 160.1 (C-10), 138.5 (C-16), 134.3 (C-13), 131.6 (C-7), 127.5-128.6 (C-8, C-17, C-18, C-19), 117.9 (C-14), 113.8 (C-9), 101.8 (C-6), 79.6 (C-3), 76.7 (C-4), 74.2 (C-15), 71.7 (C-1), 55.5 (C-11), 33.9 (C-12), 29.3 (C-2), 11.7 (C-5)

[α]$_{D}^{20}$ = + 30.5º (c = 0.00586 g/mL; CHCl$_3$)

Ent-235 [α]$_{D}^{20}$ = − 30.5º (c = 0.00586 g/mL; CHCl$_3$)


6.5.13 4S-Benzyloxy-4R-[2R-(4-methoxy-phenyl)-5S-methyl-[1,3]dioxan-4-yl]-butan-1-ol (236)

Borane tetrahydrofuran complex (1.0 M in tetrahydrofuran, 1.68 mL, 1.68 mmol) was added dropwise via syringe to a solution of 235 (310 mg, 0.841 mmol) in 20 mL anhydrous tetrahydrofuran and stirred at room temperature for 2 hours. The solution was cooled to 0 ºC and 12 mL 10% sodium hydroxide aqueous and 6 mL 30% hydrogen peroxide aqueous were added dropwise and sequentially. The resulting mixture was stirred at 0 ºC for 30 minutes, followed by removal of the tetrahydrofuran in vacuo. Saturated sodium bicarbonate was added and the aqueous layer was extracted with 3 x 50 mL dichloromethane. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo to yield compound 236 as a colourless oil requiring no further purification (325 mg, 0.841 mmol, quantitative).

IR (cm$^{-1}$, film) 3439, 1613, 1516, 1244, 1107, 903, 827, 730, 648
$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.38 (d, $J = 8.7$ Hz, 2H, H-8), 7.27-7.36 (m, 5H, H-17 to H-19), 6.87 (d, $J = 8.7$ Hz, 2H, H-9), 5.44 (s, 1H, H-6), 4.66 (d, $J = 11.2$ Hz, 1H, H-15), 4.47 (d, $J = 11.2$ Hz, 1H, H-15), 4.03 (dd, $J = 11.4$, 2.5 Hz, 1H, H-1), 4.01 (dd, $J = 11.4$, 1.5 Hz, 1H, H-1), 3.91 (dd, $J = 9.2$, 2.3 Hz, 1H, H-3), 3.78 (s, 3H, H-11), 3.58-3.66 (m, 3H, H-4, H-14), 1.88-1.84 (m, 1H, H-2), 1.81-1.88 (m, 1H, H-12), 1.75-1.81 (m, 1H, H-12), 1.69-1.74 (m, 2H, H-13), 1.15 (d, $J = 7.0$ Hz, 3H, H-5)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 160.1 (C-10), 138.3 (C-16), 131.5 (C-7), 127.5-128.7 (C-8, C-17 to C-19), 113.8 (C-9), 102.0 (C-6), 79.9 (C-3), 77.0 (C-4), 74.2 (C-15), 71.8 (C-1), 63.4 (C-14), 55.5 (C-11), 29.4 (C-2), 27.4 (C-13), 26.1 (C-12), 11.8 (C-5)

$[\alpha]_D^{20} = +13.9^\circ$ (c = 0.240 g/mL; CHCl$_3$)

Ent-236 $[\alpha]_D^{20} = -13.8^\circ$ (c = 0.242 g/mL; CHCl$_3$)


6.5.14 {4S-Benzylxy-4R-[2R-(4-methoxy-phenyl)-5S-methyl-[1,3]dioxan-4-yl]-butoxy}-tert-butyldimethylsilane (237)

A solution of 236 (200 mg, 0.518 mmol), dimethylaminopyridine (8 mg, 0.06 mmol), and triethylamine (0.27 mL, 1.9 mmol) in 10 mL anhydrous dichloromethane was added dropwise via syringe to a solution of tert-butyldimethylsilyl trifluoromethane sulfonate (354 mg, 1.59 mmol) in 15 mL anhydrous dichloromethane and heated to reflux for an hour. The mixture was quenched with 15 mL saturated ammonium chloride and extracted with 3 x 50 mL dichloromethane. The organic layers were combined, dried over anhydrous sodium sulphate and
concentrated in vacuo. The crude material was purified by flash chromatography (1% triethylamine treated silica, hexanes:ether 4:1, $R_f$ 0.76 for hexanes:ether 1:1) to yield compound 237 as a colourless oil (249 mg, 0.497 mmol, 96.0%).

IR (cm$^{-1}$, film) 1617, 1518, 1249, 1113, 776, 734, 697, 665

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.39 (d, $J = 8.7$ Hz, 2H, H-8), 7.26-7.34 (m, 5H, H-17 to H-19), 6.87 (d, $J = 8.7$ Hz, 2H, H-9), 5.44 (s, 1H, H-6), 4.65 (d, $J = 11.2$ Hz, 1H, H-15), 4.45 (d, $J = 11.2$ Hz, 1H, H-15), 4.06 (dd, $J = 11.3$, 2.6 Hz, 1H, H-1), 4.01 (dd, $J = 11.3$, 1.5 Hz, 1H, H-1), 3.88 (dd, $J = 9.3$, 2.2 Hz, 1H, H-3), 3.79 (s, 3H, H-11), 3.60-3.65 (m, 3H, H-4, H-14), 1.89-1.95 (m, 1H, H-2), 1.82-1.89 (m, 1H, H-12), 1.65-1.75 (m, 3H, H-12, H-13), 1.16 (d, $J = 7.0$ Hz, 3H, H-5), 0.88 (s, 9H, H-22), 0.03 (s, 6H, H-20)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 160.0 (C-10), 138.7 (C-16), 131.6 (C-7), 127.5-128.6 (C-8, C-17 to C-19), 113.8 (C-9), 101.9 (C-6), 80.1 (C-3), 77.0 (C-4), 74.3 (C-15), 71.6 (C-1), 63.6 (C-14), 55.5 (C-11), 29.5 (C-2), 27.3 (C-13), 26.2 (C-22), 25.8 (C-12), 18.6 (C-21), 11.8 (C-5), −5.1 (C-20)

$[\alpha]_D^{20} = +4.4^\circ$ (c = 0.0191 g/mL; CHCl$_3$)

**Ent-237** $[\alpha]_D^{20} = −4.7^\circ$ (c = 0.0191 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{29}$H$_{44}$O$_5$SiNa: 523.2856. Found 523.2855.
6.5.15 4S-Benzylxy-7-(tert-butyl-dimethyl-silanyloxy)-3R-(4-methoxy-benzyloxy)-2S-methyl-heptan-1-ol (243)

DIBAL-H (1.0 M in hexanes, 1.27 mL, 1.27 mmol) was added dropwise via syringe to a solution of 237 (128 mg, 0.255 mmol) in 10 mL anhydrous toluene at 0 °C. After 15 minutes, the mixture was quenched with 10 mL Rochelle’s salt and warmed to room temperature. The aqueous layer was extracted with 3 x 20 mL dichloromethane and the organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash chromatography (1% triethylamine treated silica, hexanes:ether 1:1, Rf 0.27) to yield compound 243 as a colourless oil (88 mg, 0.175 mmol, 68.6%).

IR (cm⁻¹, film) 3439, 1611, 1513, 1462, 1247, 1172, 1090, 1037, 774, 696, 662

¹H NMR (500 MHz, CDCl₃) δ 7.27-7.35 (m, 5H, H-17 to H-19), 7.25 (d, J = 8.7 Hz, 2H, H-8), 6.85 (d, J = 8.7 Hz, 2H, H-9), 4.65 (d, J = 11.2 Hz, 1H, H-15), 4.59 (d, J = 11.2 Hz, 1H, H-6), 4.53 (d, 1H, H-6), 4.48 (d, J = 11.2 Hz, 1H, H-15), 3.78 (s, 3H, H-11), 3.57-3.65 (m, 4H, H-3, H-4, H-14), 3.50-3.57 (m, 2H, H-1), 1.98-2.06 (m, 1H, H-2), 1.80 (t, J = 4.3 Hz, 1H, OH), 1.68-1.76 (m, 3H, H-12, H-13), 1.57-1.63 (m, 1H, H-12), 0.96 (d, J = 6.9 Hz, 3H, H-5), 0.88 (s, 9H, H-22), 0.03 (s, 6H, H-20)

¹³C NMR (125 MHz, CDCl₃) δ 159.4 (C-10), 138.8 (C-16), 131.0 (C-7), 127.8-129.7 (C-8, C-17 to C-19), 114.0 (C-9), 80.9 (C-3), 79.9 (C-4), 73.4 (C-15), 72.1 (C-6), 66.4 (C-14), 63.4 (C-1), 55.5 (C-11), 37.7 (C-2), 29.1 (C-13), 27.2 (C-12), 26.2 (C-22), 18.6 (C-21), 12.3 (C-5), -5.0 (C-20)

[α]D²⁰ = + 10.3º (c = 0.00785 g/mL: CHCl₃)
Ent-243 $[\alpha]_{D}^{20} = -10.3^\circ$ (c = 0.00787 g/mL: CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{29}$H$_{46}$O$_5$SiNa: 525.3007. Found 525.3007.

6.5.16 4R-Benzylxy-7-(tert-butyl-dimethyl-silanyloxy)-3S-(4-methoxy-benzylxy)-2S-methylheptanal (ent-245)

Dess-Martin periodinane (68 mg, 0.16 mmol) was added to a solution of compound ent-243 (39 mg, 0.077 mmol) in 5 mL anhydrous dichloromethane and stirred for 2 hour. The mixture was quenched with 10 mL brine and extracted with 3 x 10 mL dichloromethane. The organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was subjected to a short plug (silica, hexanes:ether 1:1, R$_f$ 0.72) to yield compound ent-245 as a colourless oil (39 mg, 0.077 mmol, quantitative).

$^1$H NMR (300 MHz, CDCl$_3$) δ 9.69 (d, J = 1.4 Hz, 1H, H-1), 7.25-7.36 (m, 5H, H-17 to H-19), 7.19 (d, J = 8.4 Hz, 2H, H-8), 6.83 (d, J = 8.4 Hz, 2H, H-9), 4.56 (d, J = 11.7 Hz, 1H, H-6), 4.47 (d, J = 11.7 Hz, 1H, H-15), 4.44 (d, J = 11.2 Hz, 1H, H-15), 4.38 (d, J = 11.0 Hz, 1H, H-6), 3.94 (dd, J = 6.8, 3.8 Hz, 1H, H-3), 3.78 (s, 3H, H-11), 3.60 (t, J = 6.0 Hz, 2H, H-14), 3.49-3.55 (m, 1H, H-4), 2.70-2.81 (m, 1H, H-2), 1.58-1.80 (m, 4H, H-12, H-13), 1.13 (d, J = 7.2 Hz, 3H, H-5), 0.88 (s, 9H, H-22), 0.03 (s, 6H, H-20)
6.5.17 7\textit{R}-Benzyloxy-10-(\textit{tert}-butyl-dimethyl-silanyloxy)-6\textit{S}-(4-methoxy-benzyloxy)-5\textit{R}-methyl-dec-1-en-4\textit{R}-ol (ent-246)

Compound ent-245 (10 mg, 0.020 mmol) was dissolved in 2 mL anhydrous ether and added dropwise via syringe to a solution of (+)-\textit{B}-allyldiisopinocampheylborane solution (1.0 M in dioxane, 8 μL, 0.76 mmol) in 3 mL anhydrous ether at -78 °C. The solution was stirred for 2 hours before warming to 0 °C and quenching (slowly) with 3 mL 10% sodium hydroxide aqueous and 30% hydrogen peroxide aqueous. The mixture was then stirred at 0 °C for 30 minutes, followed by extraction of the aqueous layer with 3 x 20 mL ether. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash chromatography (silica, hexanes:ether 4:1, \( R_f \) 0.19) to yield an inseparable mixture (2:1 \( dr \)) of compounds ent-246 and ent-247 as a colourless oil (20 mg, 0.036 mmol, 57%).

IR (cm\(^{-1}\), film) 3439, 1611, 1513, 1460, 1244, 1172, 1093, 1034, 911, 830, 774, 735, 696, 662

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.26-7.35 (m, 5H, H-17 to H-19), 7.25 (d, \( J = 8.7 \) Hz, 2H, H-8), 6.84 (d, \( J = 8.7 \) Hz, 2H, H-9), 5.71-5.83 (m, 1H, H-24), 5.12 (m, 2H, H-25), 4.72 (d, \( J = 11.0 \) Hz, 1H, H-15), 4.63 (d, \( J = 11.2 \) Hz, 1H, H-6), 4.52 (d, \( J = 11.2 \) Hz, 1H, H-6), 4.46 (d, \( J = 11.0 \) Hz, 1H, H-15), 3.78 (s, 3H, H-11), 3.73-3.77 (m, 1H, H-3), 3.57-3.68 (m, 4H, H-4, H-14), 2.65 (s, 1H, OH), 2.21-2.29 (m, 1H, H-23), 2.12-2.29 (m, 1H, H-23), 1.82-1.89 (m, 1H, H-2), 1.66-1.80 (m, 3H, H-12, H-13), 1.54-1.60 (m, 1H, H-12), 0.99 (d, \( J = 6.9 \) Hz, 3H, H-5), 0.87 (s, 9H, H-22), 0.03 (s, 6H, H-20)

HR-ESIMS calculated for [M+Na]\(^+\) \( C_{32}H_{50}O_5SiNa \): 565.3320. Found 565.3321.
6.5.18 7R-Benzylxy-10-(tert-butyl-dimethyl-silanyloxy)-6S-(4-methoxy-benzylxyloxy)-5R-methyl-dec-1-en-4S-ol (ent-247)

Compound ent-245 (14 mg, 0.028 mmol) was dissolved in 2 mL anhydrous ether and added dropwise via syringe to a solution of (–)-B-allyldiisopinocampheylborane solution (1.0 M in dioxane, 0.12 mL, 0.12 mmol) in 3 mL anhydrous ether at –78 °C. The solution was stirred for 2 hours before warming to 0 °C and quenching (slowly) with 3 mL 10% sodium hydroxide aqueous and 30% hydrogen peroxide aqueous. The mixture was then stirred at 0 °C for 30 minutes, followed by extraction of the aqueous layer with 3 x 20 mL ether. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash chromatography (silica, hexanes:ether 4:1, R_f 0.19) to yield compound ent-247 as a colourless oil (29 mg, 0.054 mmol, 52%).

IR (cm⁻¹, film) 3439, 1611, 1513, 1460, 1244, 1172, 1093, 1034, 911, 830, 774, 735, 696, 662

¹H NMR (500 MHz, CDCl₃) δ 7.26-7.35 (m, 5H, H-17 to H-19), 7.25 (d, J = 8.7 Hz, 2H, H-8), 6.84 (d, J = 8.7 Hz, 2H, H-9), 5.71-5.83 (m, 1H, H-24), 5.12 (m, 2H, H-25), 4.72 (d, J = 11.0 Hz, 1H, H-15), 4.63 (d, J = 11.2 Hz, 1H, H-6), 4.52 (d, J = 11.2 Hz, 1H, H-6), 4.46 (d, J = 11.0 Hz, 1H, H-15), 3.78 (s, 3H, H-11), 3.73-3.77 (m, 1H, H-4), 3.57-3.68 (m, 4H, H-1, H-3, H-14), 2.65 (s, 1H, OH), 2.21-2.29 (m, 1H, H-23), 2.12-2.29 (m, 1H, H-23), 1.82-1.89 (m, 1H, H-2), 1.66-1.80 (m, 3H, H-12, H-13), 1.54-1.60 (m, 1H, H-12), 0.99 (d, J = 6.9 Hz, 3H, H-5), 0.87 (s, 9H, H-22), 0.03 (s, 6H, H-20)

¹³C NMR (125 MHz, CDCl₃) δ 159.4 (C-10), 138.8 (C-16), 135.6 (C-24), 130.6 (C-7), 127.8-129.7 (C-8, C-17 to C-19), 117.0 (C-25), 114.1 (C-9), 84.1 (C-3), 80.5 (C-4), 73.8 (C-1), 73.1
(C-15), 72.4 (C-6), 63.3 (C-14), 55.5 (C-23), 38.9 (C-2), 29.4 (C-13), 27.3 (C-12), 26.2 (C-22), 18.6 (C-21), 8.1 (C-5), -5.1 (C-20)

$[\alpha]_D^{20} = -11.5^\circ$ (c = 0.00650 g/mL: CHCl$_3$

HR-ESIMS calculated for [M+Na]$^+$ C$_{32}$H$_{50}$O$_5$SiNa: 565.3320. Found 565.3321.

6.5.19 Propionic acid 1S-allyl-4R-benzyloxy-7-(tert-butyl-dimethyl-silanyloxy)-3S-(4-methoxy-benzyloxy)-2R-methyl-heptyl ester (ent-248)

Propionic anhydride (5 µL, 0.04 mmol) was added dropwise via syringe to a solution of ent-247 (19 mg, 0.035 mmol), triethylamine (6 µL, 0.04 mmol) and 4-dimethylaminopyridine (0.4 mg, 0.003 mmol) in 10 mL anhydrous dichloromethane. The solution was stirred for 19.5 hours then quenched with 5 mL saturated ammonium chloride. The aqueous layer was extracted with 3 x 10 mL aliquots of dichloromethane then the organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash chromatography (silica, hexanes:ether 4:1, R$_f$ 0.47) to yield compound ent-248 as a colourless oil (17 mg, 0.028 mmol, 81%).

IR (cm$^{-1}$, film) 1733, 1613, 1514, 1463, 1249, 1187, 1096, 1038, 917, 835, 776, 735, 698, 664

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.26-7.33 (m, 5H, H-17 to H-19), 7.25 (d, J = 8.7 Hz, 2H, H-8), 6.84 (d, J = 8.7 Hz, 2H, H-9), 5.61-5.71 (m, 1H, H-24), 4.95-5.06 (m, 3H, H-1, H-25), 4.68 (d, J = 10.6 Hz, 1H, H-15), 4.58 (d, J = 11.0 Hz, 1H, H-6), 4.47 (d, J = 11.0 Hz, 1H, H-6), 4.43 (d, J = 10.6 Hz, 1H, H-15), 3.78 (s, 3H, H-11), 3.55-3.63 (m, 3H, H-4, H-14), 3.50 (dd, J = 5.2, 4.1 Hz, 1H, H-3), 2.22-2.34 (m, 4H, H-23, H-27), 1.95-2.03 (m, 1H, H-2), 1.69-1.79 (m, 1H, H-12),
1.51-1.69 (m, 3H, H-12, H-13), 1.09 (t, J = 7.1 Hz, 3H, H-28), 1.03 (d, J = 6.9 Hz, 3H, H-5), 0.87 (s, 9H, H-22), 0.02 (s, 6H, H-20)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 174.5 (C-26), 159.3 (C-10), 139.0 (C-16), 134.2 (C-24), 131.2 (C-7), 127.7-129.5 (C-8, C-17 to C-19), 117.8 (C-25), 113.9 (C-9), 80.7 (C-3), 80.5 (C-4), 74.7 (C-1), 73.3 (C-15), 72.3 (C-6), 63.4 (C-14), 55.5 (C-11), 38.0 (C-2), 36.8 (C-23), 29.5 (C-13), 28.1 (C-12), 26.8 (C-27), 26.2 (C-22), 18.6 (C-21), 10.5 (C-28), 9.5 (C-5), −5.1 (C-20)

$\alpha^\text{H}\text{D}_{20}^\text{m} = −2.8^\circ$ (c = 0.00430 g/mL: CHCl$_3$

HR-ESIMS calculated for [M+Na]$^+$ C$_{35}$H$_{54}$O$_6$SiNa: 621.3582. Found 621.3583.

6.5.20 8R-Benzylxoy-11-(tert-butyl-dimethyl-silanyloxy)-7S-(4-methoxy-benzyloxy)-2,6R-dimethyl-5S-propionyloxy-undec-2-enoic acid methyl ester (ent-249)

A solution of compound ent-248 (17 mg, 0.028 mmol), distilled methyl methacrylate (15 μL, 0.14 mmol) and Hoveyda-Grubbs II catalyst (5 mol %, 0.9 mg, 0.0014 mmol) in 10 mL anhydrous toluene was refluxed for 20 hours. The solvent was removed under reduced pressure and the crude material was purified by flash-column chromatography (silica, hexanes:ether 4:1, R$_f$ 0.23) to yield compound ent-249 as a colourless oil (15 mg, 0.023 mmol, 82%).

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.19-7.31 (m, 5H, H-8, H-17 to H-19), 6.82 (d, J = 8.5 Hz, 2H, H-9), 6.61 (dq, J = 7.4, 1.6 Hz, 1H, H-24), 4.96-5.04 (m, 1H, H-1), 4.66 (d, J = 11.2 Hz, 1H, H-6), 4.58 (d, J = 11.2 Hz, 1H, H-15), 4.47 (d, J = 11.2 Hz, 1H, H-15), 4.40 (d, J = 11.2 Hz, 1H, H-6), 3.76 (s, 3H, H-11), 3.74-3.76 (m, 1H, H-3), 3.67 (s, 3H, H-31), 3.53-3.60 (m, 3H, H-4, H-14), 2.33-2.42 (m, 2H, H-23), 2.24 (qd, J = 7.8, 1.1 Hz, 2H, H-27), 1.95-2.05 (m, 1H, H-2),
6.5.21 \{4\text{-}[6R\text{-}\text{Allyl}\text{-}2S\text{-}(4\text{-methoxy\text{-}phenyl)}\text{-}5R\text{-}methyl\text{-}[1,3]\text{dioxan\text{-}4S\text{-}yl]}\text{-}4R\text{-}benzyloxy\text{-}butoxy\}\text{-}\text{tert\text{-}butyl\text{-}dimethyl\text{-}silane (ent-250)}

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (5.4 mg, 0.024 mmol) was added to a solution of compound ent-246 and ent-247 (11 mg, 0.020 mmol) in 5 mL anhydrous dichloromethane and stirred for 26 hours. The mixture was quenched with 5 mL brine and extracted with 3 x 10 mL dichloromethane. The organic layers were dried over anhydrous sodium sulphate and concentrated in vacuo. Flash-column chromatography (silica, hexanes:ether 4:1, \text{R}_f \ 0.55) of the crude material yielded compound ent-250 (2 mg, 0.0033 mmol, 17\%) as a colourless oil.

\(^1\text{H NMR (500 MHz, CDCl}_3 \) \(\delta \ 7.36 \ (d, \ J = 8.4 \ Hz, \ 2H, \ H-8), \ 7.25\text{-}7.35 \ (m, \ 5H, \ H-17 \ to \ H-19), \ 6.85 \ (d, \ J = 6.7 \ Hz, \ 2H, \ H-9), \ 5.77\text{-}5.89 \ (m, \ 1H, \ H-24), \ 5.70 \ (s, \ 1H, \ H-6), \ 5.12 \ (d, \ J = 17.1 \ Hz, \ 1H, \ H-25), \ 5.08 \ (d, \ J = 10.3 \ Hz, \ 1H, \ H-25), \ 4.62 \ (d, \ J = 11.4 \ Hz, \ 1H, \ H-15), \ 4.44 \ (d, \ J = 11.4 \ Hz, \ 1H, \ H-15), \ 4.05 \ (dd, \ J = 9.2, 1.9 \ Hz, \ 1H, \ H-3), \ 3.94 \ (dd, \ J = 8.2, 7.2 \ Hz, \ 1H, \ H-1), \ 3.78 \ (s, \ 3H, \ H-11), \ 3.59\text{-}3.63 \ (m, \ 3H, \ H-4, \ H-14), \ 2.86 \ (\text{quintet}, \ J = 7.5 \ Hz, \ 1H, \ H-23), \ 2.43 \ (\text{quintet}, \ J = 7.5 \ Hz, \ 1H, \ H-23), \ 1.81\text{-}1.89 \ (m, \ 2H, \ H-2, \ H-12), \ 1.63\text{-}1.75 \ (m, \ 3H, \ H-12, \ H-13), \ 1.19 \ (d, \ J = 6.9 \ Hz, \ 3H, \ H-5), \ 0.87 \ (s, \ 9H, \ H-22), \ 0.02 \ (s, \ 6H, \ H-20)
6.5.22 \{4\{6S-\text{Allyl}-2S\{-4\text{-methoxy-phenyl}\}-5R\text{-methyl}-[1,3]dioxan-4S-yl\}-4R\text{-benzyloxy-butoxy}\}-\text{tert-butyl-dimethyl-silane (ent-251)}

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (5 mg, 0.022 mmol) was added to a solution of compound ent-247 (10 mg, 0.019 mmol) in 5 mL anhydrous dichloromethane and stirred for 26 hours. The mixture was quenched with 5 mL brine and extracted with 3 x 10 mL dichloromethane. The organic layers were dried over anhydrous sodium sulphate and concentrated in vacuo. Flash-column chromatography (silica, hexanes:ether 4:1, \(R_f\) 0.59) of the crude material yielded compound ent-251 (6 mg, 0.010 mmol, 55\%) as a colourless oil.

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.38 (d, \(J = 8.4\) Hz, 2H, H-8), 7.25-7.36 (m, 5H, H-17 to H-19), 6.85 (d, \(J = 6.7\) Hz, 2H, H-9), 5.77-5.87 (m, 1H, H-24), 5.46 (s, 1H, H-6), 5.12 (d, \(J = 17.1\) Hz, 1H, H-25), 5.05 (d, \(J = 10.3\) Hz, 1H, H-25), 4.63 (d, \(J = 11.4\) Hz, 1H, H-15), 4.44 (d, \(J = 11.4\) Hz, 1H, H-15), 3.85 (ddd, \(J = 7.2, 7.0, 1.9\) Hz, 1H, H-1), 3.78 (dd, \(J = 8.9, 2.1\) Hz, 1H, H-3), 3.78 (s, 3H, H-11), 3.65 (dd, \(J = 9.3, 4.4\) Hz, 1H, H-4), 3.61 (t, \(J = 6.2\) Hz, 2H, H-14), 2.45 (quintet, \(J = 7.5\) Hz, 1H, H-23), 2.23 (quintet, \(J = 7.5\) Hz, 1H, H-23), 1.81-1.91 (m, 2H, H-2, H-12), 1.62-1.76 (m, 3H, H-12, H-13), 0.94 (d, \(J = 6.9\) Hz, 3H, H-5), 0.87 (s, 9H, H-22), 0.02 (s, 6H, H-20)
6.5.23 7R-Benzylxy-10-(tert-butyl-dimethyl-silanyloxy)-6S-(4-methoxy-benzylxy)-5S-methyl-dec-1-en-4-one (ent-253)

Dess-Martin periodinane (47 mg, 0.112 mmol) was added to a solution of ent-246 and ent-247 (1:1 dr, 29.3 mg, 0.054 mmol) in 5 mL anhydrous dichloromethane and stirred for 2 hours. The mixture was quenched with 5 mL brine and extracted with 3 x 10 mL dichloromethane. The organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. Flash-chromatography (silica, hexanes:ether 4:1, Rf 0.44) of the crude material yielded compound ent-253 (29 mg, 0.054 mmol, quantitative) as a colourless oil.

\(^{1}\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.23-7.32 (m, 5H, H-17 to H-19), 7.21 (d, J = 8.9 Hz, 2H, H-8), 6.82 (d, J = 8.9 Hz, 2H, H-9), 5.83 (ddt, J = 16.8, 10.2, 6.6 Hz, 1H, H-24), 5.11 (dq, J = 10.2, 1.5 Hz, 1H, H-25), 5.02 (dq, J = 17.1, 1.5 Hz, 1H, H-25), 4.56 (d, J = 10.6 Hz, 1H, H-15), 4.55 (d, J = 11.2 Hz, 1H, H-6), 4.46 (d, J = 11.2 Hz, 1H, H-6), 4.44 (d, J = 10.6 Hz, 1H, H-15), 3.88 (dd, J = 6.8, 4.7 Hz, 1H, H-3), 3.76 (s, H-11), 3.54 (t, J = 5.8 Hz, 2H, H-14), 3.33 (dd, J = 10.0, 4.7 Hz, 1H, H-23), 3.16 (dq, J = 7.0, 1.4 Hz, 1H, H-23), 2.85 (quintet, J = 6.8 Hz, 1H, H-2), 1.45-1.69 (m, 4H, H-12, H-13), 1.14 (d, J = 7.3 Hz, 3H, H-5), 0.86 (s, 9H, H-22), 0.00 (s, 6H, H-20)
6.5.24 7R-Benzyloxy-10-(tert-butyl-dimethyl-silanyloxy)-6S-hydroxy-5S-methyl-dec-1-en-4-one (ent-254)

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (3 mg, 0.013 mmol) was added to compound ent-253 (6 mg, 0.012 mmol) in 5 mL anhydrous dichloromethane and stirred for 18.5 hours. The mixture was quenched with 10 mL brine and extracted with 3 x 10 mL dichloromethane. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. Flash-chromatography of the crude material (silica, hexanes:ether 4:1, R<sub>f</sub> 0.10) gave compound ent-254 as a colourless oil (2 mg, 0.0057 mmol, 50%).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.26-7.37 (m, 5H, H-14 to H-16), 5.86 (ddt, J = 16.8, 10.1, 6.7 Hz, 1H, H-2), 5.16 (dq, J = 10.1, 1.5 Hz, 1H, H-1), 5.10 (dq, J = 17.1, 1.5 Hz, 1H, H-1), 4.57 (d, J = 11.7 Hz, 1H, H-12), 4.44 (d, J = 11.7 Hz, 1H, H-12), 3.95-4.01 (m, 1H, H-7), 3.54 (d, J = 6.2, 4.1 Hz, 2H, H-10), 3.36-3.43 (m, 1H, H-6), 3.22 (tt, J = 6.4, 1.2 Hz, 1H, H-3), 2.86-2.96 (m, 1H, H-5), 2.71 (d, J = 3.0 Hz, 1H, OH), 1.56-1.76 (m, 4H, H-8, H-9), 1.11 (d, J = 7.7 Hz, 3H, H-11), 0.87 (s, 9H, H-19), 0.03 (s, 6H, H-17)
6.5.25 Acetic acid 1S-[1R-benzyloxy-4-(tert-butyl-dimethyl-silanyloxy)-butyl]-2S-methyl-3-oxo-hex-4-enyl ester (ent-255)

Acetic anhydride (0.6 µL, 0.0063 mmol) was added to a solution containing compound ent-254 (2 mg, 0.0057 mmol), 4-dimethylaminopyridine (0.1 mg, 0.0008 mmol) and triethylamine (1 µL, 0.0068 mmol) in 5 mL anhydrous dichloromethane and stirred for 23 hours. The mixture was quenched with 5 mL saturated ammonium chloride and extracted with 3 x 10 mL dichloromethane. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. Flash-chromatography of the crude material (silica, hexanes:ether 4:1, Rf 0.25) yielded compound ent-255 as a colourless oil (2 mg, 0.0032 mmol, 57%).

1H NMR (300 MHz, CDCl3) δ 7.25-7.33 (m, 5H, H-14 to H-16), 6.85 (dq, J = 15.7, 6.8 Hz, 1H, H-2), 6.13 (dq, J = 15.7, 1.5 Hz, 1H, H-3), 5.51 (dd, J = 7.9, 5.3 Hz, 1H, H-6), 4.60 (d, J = 11.2 Hz, 1H, H-12), 4.45 (d, J = 11.2 Hz, 1H, H-12), 3.48-3.60 (m, 2H, H-10), 3.39-3.46 (m, 1H, H-7), 3.10 (quintet, J = 6.8 Hz, 1H, H-5), 2.04 (s, 3H, H-21), 1.86 (dd, J = 6.8, 1.6 Hz, 3H, H-1), 1.57-1.67 (m, 2H, H-8), 1.41-1.51 (m, 2H, H-9), 1.07 (d, J = 7.0 Hz, 3H, H-11), 0.86 (s, 9H, H-19), 0.00 (s, 6H, H-17)
6.5.26 4R-Benzylxy-7-(tert-butyl-dimethyl-silanyloxy)-3S-hydroxy-2S-methyl-heptanal (ent-257)

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (12 mg, 0.054 mmol) was added to compound ent-245 (21 mg, 0.041 mmol) in 5 mL anhydrous dichloromethane and stirred for 18.5 hours. The mixture was quenched with 10 mL brine and extracted with 3 x 10 mL dichloromethane. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. Flash-chromatography of the crude material (silica, hexanes:ether 1:1, R_f 0.41) gave compound ent-257 as a colourless oil (11 mg, 0.028 mmol, 68%).

^{1}H NMR (300 MHz, CDCl_3) δ 9.64 (d, J = 1.4 Hz, 1H, H-1), 7.27-7.34 (m, 5H, H-11 to H-13), 4.58 (d, J = 11.4 Hz, 1H, H-9), 4.41 (d, J = 11.4 Hz, 1H, H-9), 4.10 (dt, J = 7.5, 4.4 Hz, 1H, H-4), 3.63 (t, J = 7.0 Hz, 2H, H-8), 3.48 (m, 1H, H-3), 2.69 (qdd, J = 7.4, 4.5, 1.5 Hz, 1H, H-2), 2.50 (d, J = 4.9 Hz, 1H, OH), 1.60-1.81 (m, 4H, H-6, H-7), 1.11 (d, J = 7.4 Hz, 3H, H-5), 0.88 (s, 9H, H-16), 0.04 (s, 6H, H-14)
6.5.27 Acetic acid 2R-benzyloxy-5-(tert-butyl-dimethyl-silyloxy)-1S-(1S-methyl-2-oxo-ethyl)-pentyl ester (ent-258)

Acetic anhydride (3 µL, 0.030 mmol) was added to a solution containing compound ent-257 (11 mg, 0.028 mmol), 4-dimethylaminopyridine (0.3 mg, 0.003 mmol) and triethylamine (5 µL, 0.033 mmol) in 5 mL anhydrous dichloromethane and stirred for 23 hours. The mixture was quenched with 5 mL saturated ammonium chloride and extracted with 3 x 10 mL dichloromethane. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. Flash-chromatography of the crude material (silica, hexanes:ether 1:1, Rf 0.67) yielded compound ent-258 as a colourless oil (10 mg, 0.024 mmol, 87%).

$^1$H NMR (300 MHz, CDCl$_3$) δ 9.62 (d, J = 1.3 Hz, 1H, H-1), 7.27-7.33 (m, 5H, H-11 to H-13), 5.37 (dd, J = 7.1, 4.4 Hz, 1H, H-3), 4.56 (d, J = 11.2 Hz, 1H, H-9), 4.48 (d, J = 11.2 Hz, 1H, H-9), 3.55-3.63 (m, 3H, H-4, H-8), 2.79 (qdd, J = 7.1, 4.5, 1.3 Hz, 1H, H-2), 2.02 (s, 3H, H-18), 1.57-1.77 (m, 4H, H-6, H-7), 1.04 (d, J = 7.1 Hz, 3H, H-5), 0.87 (s, 9H, H-16), 0.02 (s, 6H, H-14)
6.5.28 Acetic acid 1S-[1R-benzylloxy-4-(tert-butyl-dimethyl-silanyloxy)-butyl]-3R-hydroxy-2R-methyl-hex-5-enyl ester (ent-256)

Compound ent-258 (10 mg, 0.024 mmol) was dissolved in 2 mL anhydrous ether and added dropwise via syringe to a solution of (+)-B-allyldiisopinocampheylborane solution (1.0 M in dioxane, 3 µL, 0.03 mmol) in 3 mL anhydrous ether at –78 ºC. The solution was stirred for 2 hours before warming to 0 ºC and quenching (slowly) with 3 mL 10% sodium hydroxide aqueous and 30% hydrogen peroxide aqueous. The mixture was then stirred at 0 ºC for 30 minutes, followed by extraction of the aqueous layer with 3 x 20 mL ether. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash chromatography (silica, hexanes:ether 1:1, Rf 0.38) to yield compound ent-256 as a colourless oil (3 mg, 0.0073 mmol, 31% over two steps).

\[ ^{1}H \text{ NMR (300 MHz, CDCl}_{3} \] \( \delta \) 7.25-7.36 (m, 5H, H-14 to H-16), 5.67-5.95 (m, 1H, H-2), 5.05-5.30 (m, 3H, H-1, H-6), 4.59 (d, J = 11.4 Hz, 1H, H-12), 4.48 (d, J = 11.4 Hz, 1H, H-12), 3.50-3.72 (m, 4H, H-4, H-7, H-10), 2.18-2.24 (m, 1H, H-3), 2.05 (s, 3H, H-21), 1.94-1.99 (m, 1H, H-3), 1.46-1.73 (m, 5H, H-5, H-9, H-10), 0.92 (d, J = 7.4 Hz, 3H, H-11), 0.87 (s, 9H, H-19), 0.01 (s, 6H, H-17)
Borane-tetrahydrofuran complex (1.0 M in tetrahydrofuran, 0.36 mL, 0.36 mmol) was added to a solution of compound 225 (50 mg, 0.18 mmol) in anhydrous tetrahydrofuran (10 mL) at room temperature. The reaction mixture was stirred for 2 hours, where upon 10% aqueous sodium hydroxide (5 mL) was added followed by the careful addition of 30% aqueous hydrogen peroxide (2 mL). The mixture was stirred for an additional 30 minutes before the tetrahydrofuran was removed in vacuo. The resulting aqueous suspension was diluted with 10 mL 10% aqueous sodium bicarbonate then extracted with ethyl acetate (3 x 20 mL). The combined organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo to yield compound 226 as a white solid (53 mg, 0.18 mmol, quantitative) without further purification required. Alternatively, an analytical sample could be purified by flash-column chromatography (1% triethylamine treated silica, chloroform:methanol 97:3, R_f 0.14).

Melting Point: 103-104 °C

IR (cm\(^{-1}\),film) 3394, 1300, 1247, 1163, 1102, 1029, 827

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.38 (d, J = 9.1 Hz, 2H, H-8), 6.87 (d, J = 8.9 Hz, 2H, H-9), 5.44 (s, 1H, H-6), 4.04 (dd, J = 11.3, 2.6 Hz, 1H, H-1), 4.01 (dd, J = 11.2, 1.5 Hz, 1H, H-1), 3.77 (s, 3H, H-11), 3.55-3.69 (m, 4H, H-3, H-4, H-14), 2.96 (br s, 1H, OH), 2.04 (br s, 1H, OH), 1.92-2.00 (m, 1H, H-12), 1.85-1.90 (m, 1H, H-2), 1.62-1.74 (m, 2H, H-12, H-13), 1.37-1.47 (m, 1H, H-13), 1.22 (d, J = 7.0 Hz, 3H, H-5)

\(^13\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 160.1 (C-10), 131.5 (C-7), 127.5 (C-8), 113.8 (C-9), 101.8 (C-6), 82.2 (C-3), 74.2 (C-1), 70.6 (C-4), 63.1 (C-14), 55.5 (C-11), 31.4 (C-12), 29.3 (C-2), 28.9 (C-13), 11.8 (C-5)

\([\alpha]_D^{20} = -18.4^\circ\) (c = 0.00850 g/mL; CHCl\(_3\))
\textbf{Ent-226} \([\alpha]_D^{20} = +18.2^\circ\) (c = 0.00854 g/mL; CHCl\(_3\))

HR-ESIMS calculated for [M+Na]^+ \(C_{16}H_{24}O_5Na\): 319.1521. Found 319.1537.

\[ \begin{array}{c}
\text{6.5.30 3-Methyl-butyric acid 1-[2R-(4-methoxy-phenyl)-5S-methyl-[1,3]dioxan-4R-yl]-4S-(3-methyl-butyryloxy)-butyl ester (227)}
\end{array} \]

Isovaleric anhydride (0.15 mL, 0.75 mmol) and 4-dimethylaminopyridine (7 mg, 0.06 mmol) were added to compound \textbf{226} (100.2 mg, 0.338 mmol) and anhydrous triethylamine (0.14 mL, 1.0 mmol) in anhydrous dichloromethane (10 mL) at 0 °C. The reaction mixture was gradually warmed to room temperature over 24 hours. The solution was quenched with saturated ammonium chloride (10 mL) and extracted with dichloromethane (3 x 20 mL). The combined organic fractions were washed with 10% aqueous sodium bicarbonate (2 x 20 mL) then dried over anhydrous sodium sulphate. The solvent was removed in vacuo to yield compound \textbf{227} as a colourless oil (157 mg, 0.338 mmol, quantitative) without further purification required. Alternatively, an analytical sample could be purified by flash-column chromatography (1% triethylamine treated silica, hexanes:ether 4:1, R\(_f\) 0.19).

IR (cm\(^{-1}\), neat) 1732, 1615, 1519, 1466, 1370, 1293, 1249, 1185, 1115, 1033, 1004, 828, 788, 738

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.37 (d, \(J = 8.9\) Hz, 2H, H-8), 6.86 (d, \(J = 8.9\) Hz, 2H, H-9), 5.44 (s, 1H, H-6), 4.99-5.04 (m, 1H, H-4), 3.97-4.06 (m, 4H, H-1, H-14), 3.89 (dd, \(J = 9.3, 2.3\) Hz, 1H, H-3), 3.77 (s, 3H, H-11), 2.18 (dd, \(J = 7.0, 1.3\) Hz, 2H, H-10 or H-15), 2.11 (dd, \(J = 7.4, 0.7\) Hz, 2H, H-10 or H-16), 2.00-2.10 (m, 2H, H-17, H-18), 1.85-1.91 (m, 1H, H-12), 1.56-1.69 (m, 4H, H-2, H-12, H-13), 1.14 (d, \(J = 7.0\) Hz, 3H, H-5), 0.94 (d, \(J = 6.7\) Hz, 3H, H-19 or H-20, or
H-21 or H-22), 0.93 (d, J = 6.6 Hz, 3H, H-19 or H-20, or H-21 or H-22), 0.98 (d, J = 6.6 Hz, 6H, H-19 or H-20, or H-21 or H-22)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.3 (C-23 or C-24), 172.3 (C-23 or C-24), 160.2 (C-10), 131.2 (C-7), 127.5 (C-8), 113.8 (C-9), 102.0 (C-6), 80.0 (C-3), 73.8 (C-1), 71.4 (C-4), 64.1 (C-14), 55.5 (C-11), 43.7 (C-15 or C-16), 43.6 (C-15 or C-16), 29.4 (C-2), 28.0 (C-12), 25.9 (C-17 or C-18), 25.8 (C-17 or C-18), 24.3 (C-13), 22.6 (C-19, C-20, C-21, C-22), 11.5 (C-5)

[α]$_D^{20}$ = $-$ 8.01º (c = 0.0397 g/mL; CHCl$_3$)

Ent-227 [α]$_D^{20}$ = + 8.00º (c = 0.0400 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{26}$H$_{40}$O$_7$Na: 487.2672. Found 487.2672.

6.5.31 3-Methyl-butryic acid 2R,4-dihydroxy-3S-methyl-1R-[3-(3-methyl-butryloxy)-propyl]-butyl ester (228)

Compound 227 (32 mg, 0.068 mmol) was added to a catalytic amount of 10% palladium on carbon in anhydrous methanol (10 mL) with a few drops of acetic acid and pressurized with hydrogen gas (300 psi) for 18 hours. The solution was filtered through a cotton plug with a minimum amount of celite and the filtrate was concentrated in vacuo. Purification of the crude material by flash-column chromatography (silica, 10% methanol in chloroform, R$_f$ 0.50) yielded compound 228 (24 mg, 0.068 mmol, quantitative) as a colourless oil.

IR (cm$^{-1}$, neat) 3443, 1737, 1732, 1466, 1369, 1295, 1253, 1190, 1120, 1096, 1032, 995, 918, 733

$^1$H NMR (500MHz, CDCl$_3$) δ 4.96 (td, J = 7.1, 2.8 Hz, 1H, H-4), 4.00-4.09 (m, 2H, H-8), 3.82 (dd, J = 7.4, 2.8 Hz, 1H, H-3), 3.72 (dd, J = 10.6, 4.3 Hz, H-1), 3.66 (dd, J = 10.8, 5.2 Hz, 1H, H-1), 2.75 (s, br, 1H, OH), 2.17 (d, J = 6.9 Hz, 2H, H-9 or H-10), 2.14 (d, J = 6.9 Hz, 2H, H-9 or
H-10), 2.01-2.10 (m, 3H, H-11, H-12, OH), 1.78-1.85 (m, 1H, H-6), 1.72-1.78 (m, 1H, H-2), 1.58-1.71 (m, 3H, H-6, H-7), 0.94 (d, J = 7.0 Hz, 3H, H-5), 0.91-0.94 (m, 12H, H-13 to H-16)

$^{13}$C NMR (125MHz, CDCl$_3$) $\delta$ 173.5 (C-17 or C-18), 173.0 (C-17 or C-18), 74.6 (C-4), 73.8 (C-3), 67.5 (C-8), 64.1 (C-1), 43.8 (C-9 or C-10), 43.7 (C-9 or C-10), 35.9 (C-2), 27.3 (C-6), 25.9 (C-11, C-12), 24.7 (C-7), 22.6 (C-13 to C-16), 10.2 (C-5)

$[\alpha]_{D}^{20} = +0.35^\circ$ (c = 0.0369 g/mL; CHCl$_3$)

Ent-228 $[\alpha]_{D}^{20} = -0.35^\circ$ (c = 0.0375 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{18}$H$_{34}$O$_6$Na: 369.2247. Found 369.2254.

6.5.32 3-Methyl-butyric acid 4-(tert-butyl-dimethyl-silyloxy)-2S-hydroxy-3R-methyl-1R-[3-(3-methyl-butyryloxy)-propyl]-butyl ester (ent-230)

tert-butyldimethylysilyl chloride (219 mg, 1.45 mmol) was added portion-wise to a solution of ent-228 (229 mg, 0.661 mmol), dimethylaminopyridine (22 mg, 0.18 mmol) and triethylamine (220 µL, 1.58 mmol) in 15 mL anhydrous dichloromethane and heated to reflux for 24 hours. The reaction was quenched with saturated aqueous ammonium chloride and the aqueous layer extracted with 3 x 20 mL dichloromethane. The organic layers were dried over sodium sulphate and concentrated in vacuo to yield a colourless oil and a white solid. The crude material was purified by flash-column chromatography (florisil, hexanes:ether 4:1, R$_f$ 0.61) to yield compound ent-230 as a colourless oil (216 mg, 0.469 mmol, 70.9%).

IR (cm$^{-1}$, neat) 3503, 1735, 1465, 1369, 1294, 1254, 1187, 1095, 837, 776, 665

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.92-4.96 (m, 1H, H-4), 4.02-4.06 (m, 2H, H-8), 3.81 (dt, J = 8.3, 2.2 Hz, 1H, H-3), 3.78 (dd, J = 9.7, 3.4 Hz, 1H, H-1), 3.66 (dd, J = 9.9, 4.4 Hz, 1H, H-1), 3.28 (d, J = 2.2 Hz, 1H, OH), 2.16 (d, J = 6.9 Hz, 2H, H-9 or H-10), 2.14 (d, J = 6.9 Hz, 2H, H-9 or H-10), 2.01-2.13 (m, 2H, H-11, H-12), 1.82-1.90 (m, 1H, H-6), 1.59-1.74 (m, 4H, H-2, H-6,
6.5.33 3-Methyl-butyric acid 2S-acetoxy-4-(tert-butyl-dimethyl-silyl-oxy)-3R-methyl-1R-[3-(3-methyl-butyryloxy)-propyl]-butyl ester (ent-231)

To a solution of ent-230 (43 mg, 0.094 mmol), dimethylaminopyridine (2 mg, 0.02 mmol), and triethylamine (14 µL, 0.10 mmol) in 10 mL anhydrous dichloromethane at 0 ºC was added acetic anhydride (9 µL, 0.09 mmol) and then warmed to room temperature for 24 hours. The mixture was diluted with saturated aqueous ammonium chloride (10 mL) and the aqueous layer was extracted with 3 x 20 mL dichloromethane. The organic layers were combined, washed with saturated sodium bicarbonate (10 mL) then dried over sodium sulphate and concentrated in vacuo to afford compound ent-231 (45 mg, 0.090 mmol, 95%) as a white film without any further purification.

IR (cm⁻¹, film) 1738, 1470, 1369, 1293, 1233, 1185, 1094, 1026, 837, 776, 665

¹H NMR (500 MHz, CDCl₃) δ 5.12-5.16 (m, 1H, H-4), 5.06 (t, J = 4.8 Hz, 1H, H-3), 4.01 (t, J = 5.6 Hz, 2H, H-8), 3.41 (dd, J = 10.0, 7.4 Hz, 1H, H-1), 3.37 (dd, J = 9.9, 5.8 Hz, 1H, H-1), 2.14 (d, J = 6.6 Hz, 4H, H-9, H-10), 1.97-2.11 (m, 2H, H-11, H-12), 2.00 (s, 3H, H-23), 1.85-1.92 (m, 1H, H-6), 1.53-1.67 (m, 4H, H-2, H-6, H-7), 0.92 (d, J = 6.6 Hz, 6H, H-13, H-14, H-15

H-7), 0.94 (d, J = 7.0 Hz, 3H, H-5), 0.91-0.94 (m, 12H, H-13 to H-16), 0.87 (s, 9H, H-21), 0.05 (s, 3H, H-19), 0.04 (s, 3H, H-19)

¹³C NMR (125 MHz, CDCl₃) δ 173.4 (C-17 or C-18), 172.7 (C-17 or C-18), 75.6 (C-4), 73.3 (C-3), 69.2 (C-1), 64.3 (C-8), 43.8 (C-9 or C-10), 43.7 (C-9 or C-10), 35.3 (C-2), 27.7 (C-6), 26.0 (C-21), 25.9 (C-11, C-12), 24.6 (C-7), 22.6 (C-13 to C-16), 18.4 (C-20), 10.1 (C-5), -5.4 (C-19), -5.5 (C-19)

[α]D²⁰ = + 3.34° (c = 0.0222 g/mL; CHCl₃)
or H-16), 0.91 (d, J = 6.6 Hz, 6H, H-13, H-14, H-15 or H-16), 0.86 (d, J = 7.0 Hz, 3H, H-5), 0.85 (s, 9H, H-21), -0.01 (s, 6H, H-19)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.3 (C-17 or C-18), 172.7 (C-17 or C-18), 170.4 (C-22), 74.0 (C-3), 72.6 (C-4), 65.4 (C-1), 63.9 (C-8), 43.7 (C-9 or C-10), 43.6 (C-9 or C-10), 36.6 (C-2), 26.6 (C-6), 26.0 (C-21), 25.9 (C-11 or C-12), 25.7 (C-11 or C-12), 22.6 (C-13 to C-16), 18.4 (C-20), 8.8 (C-5), -5.4 (C-19)

$[\alpha]_D^{20} = -4.53^\circ$ (c = 0.0225 g/mL; CHCl$_3$)

6.5.34 3-Methyl-butyric acid 2R-hydroxy-3S-methyl-1S-[3-(3-methyl-butyryloxy)-propyl]-4-trityloxy-butyl ester (260)

Trityl bromide (37 mg, 0.12 mmol) was added to a solution of compound 228 (31 mg, 0.090 mmol), dimethylaminopyridine (1 mg, 0.009 mmol) and triethylamine (18 μL, 0.13 mmol) in 10 mL anhydrous dichloromethane and refluxed for 24 hours. The reaction was quenched with 10 mL saturated aqueous ammonium chloride and extracted with 3 x 20 mL dichloromethane. The organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 2:1, R$_f$ 0.07) to yield compound 260 as a colourless oil (39 mg, 0.066 mmol, 73%).

IR (cm$^{-1}$, neat) 3501, 1731, 1488, 1446, 1367, 1292, 1253, 1183, 1088, 1068, 1029, 909, 730, 704, 626

$^1$H NMR (500MHz, CDCl$_3$) δ 7.20-7.43 (m, 15H, H-20 to H-22), 4.93 (dt, J = 7.9, 3.1 Hz, 1H, H-4), 4.03 (t, J = 6.1 Hz, 2H, H-8), 3.70 (dt, J = 7.9, 3.2 Hz, 1H, H-3), 3.24 (dd, J = 9.2, 5.6 Hz, H-1), 3.14 (dd, J = 9.5, 4.2 Hz, 1H, H-1), 2.72 (d, J = 2.8 Hz, 1H, OH), 2.12-2.17 (m, 4H, H-9, H-10), 2.02-2.10 (m, 2H, H-11, H-12), 1.75-1.84 (m, 2H, H-2, H-6), 1.56-1.66 (m, 1H, H-6,
H-7), 1.03 (d, J = 7.1 Hz, 3H, H-5), 0.92 (d, J = 6.6 Hz, 6H, H-13, H-14 or H-15, H-16), 0.92 (d, J = 6.6 Hz, 6H, H-13, H-14 or H-15, H-16)

$^{13}$C NMR (125MHz, CDCl$_3$) $\delta$ 173.4 (C-17 or C-18), 172.6 (C-17 or C-18), 143.9 (C-19), 127.3-128.8 (C-20 to C-22), 87.3 (C-23), 74.8 (C-4), 73.3 (C-3), 68.1 (C-1), 64.2 (C-8), 43.8 (C-9 or C-10), 43.6 (C-9 or C-10), 34.8 (C-2), 27.6 (C-6), 25.9 (C-11, C-12), 24.6 (C-7), 22.6 (C-13 to C-16), 10.8 (C-5)

$\lbrack \alpha \rbrack_D^{20} = -18.0^\circ$ (c = 0.00325 g/mL; CHCl$_3$)

Ent-260 $\lbrack \alpha \rbrack_D^{20} = +18.2^\circ$ (c = 0.00325 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{37}$H$_{48}$O$_6$Na: 611.3343. Found 611.3343.

6.5.35 3-Methyl-butyric acid 2$R$-acetoxy-3$S$-methyl-1$S$-[3-(3-methyl-butyryloxy)-propyl]-4-trityloxy-butyl ester (261)

Acetic anhydride (20 $\mu$L, 0.21 mmol) was added to a solution of compound 260 (105 mg, 0.178 mmol), dimethylaminopyridine (2 mg, 0.014 mmol) and triethylamine (32 $\mu$L, 0.23 mmol) in 10 mL anhydrous dichloromethane and stirred for 20 hours. The reaction was quenched with 10 mL saturated aqueous ammonium chloride and extracted with 3 x 20 mL dichloromethane. The organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 4:1, R$_f$ 0.28) to yield compound 261 as a colourless oil (112 mg, 0.178 mmol, quantitative).

IR (cm$^{-1}$, neat) 1742, 1734, 1728, 1485, 1446, 1367, 1292, 1230, 1183, 1088, 1068, 1023, 760, 744, 702, 632
\[
\begin{align*}
\text{\textsuperscript{1}H NMR (500MHz, CDCl}\textsubscript{3}) & \delta 7.18-7.43 (m, 15H, H-20 to H-22), 5.17 (t, J = 5.1 Hz, 1H, H-3), 5.08-5.13 (m, 1H, H-4), 4.02 (t, J = 6.0 Hz, 2H, H-8), 2.97 (dd, J = 9.5, 5.8 Hz, 1H, H-1), 2.93 (J = 9.1, 7.3 Hz, 1H, H-1), 2.13-2.17 (m, 4H, H-9, H-10), 2.07 (septet, J = 6.6 Hz, 2H, H-11, H-12), 1.98-2.03 (m, 1H, H-2), 1.90 (s, 3H, H-25), 1.53-1.66 (m, 4H, H-6, H-7), 0.92-0.95 (m, 12H, H-13, H-14, H-15, H-16), 0.88 (d, J = 6.9 Hz, 3H, H-5) \\
\text{\textsuperscript{13}C NMR (125MHz, CDCl}\textsubscript{3}) & \delta 173.3 (C-17 or C-18), 172.6 (C-17 or C-18), 170.3 (C-24), 144.2 (C-19), 127.2-128.9 (C-20 to C-22), 86.9 (C-23), 74.0 (C-3), 72.5 (C-3), 65.4 (C-8), 63.9 (C-1), 43.7 (C-9 or C-10), 43.6 (C-9 or C-10), 34.7 (C-2), 26.6 (C-6), 25.9 (C-11 or C-12), 25.8 (C-11 or C-12), 22.7 (C-13 to C-16), 22.6 (C-7), 21.0 (C-25), 12.7 (C-5) \\
[\alpha]_D^{20} & = +13.00^\circ (c = 0.00545 \text{ g/mL}; \text{CHCl}_3) \\
\text{Ent-261} & [\alpha]_D^{20} = -12.95^\circ (c = 0.00542 \text{ g/mL}; \text{CHCl}_3) \\
\text{HR-ESIMS calculated for [M+Na]} & ^+ \text{C}_{39}\text{H}_{50}\text{O}_7\text{Na: 653.3449. Found 653.3450.}
\end{align*}
\]

6.5.36 3-Methyl-butyric acid 2R-acetoxy-4-hydroxy-3S-methyl-1S-[3-(3-methyl-butyryloxy)-propyl]-butyl ester (232)

Compound 261 (108 mg, 0.172 mmol) was added to a catalytic amount of 10% palladium on carbon in anhydrous methanol (10 mL) with a few drops of acetic acid and pressurized with hydrogen gas (300 psi) for 18 hours. The solution was filtered through a cotton plug with a minimum amount of celite and the filtrate was concentrated in vacuo. Purification of the crude material by flash-column chromatography (silica, hexanes:ether 1:2, R\textsubscript{f} 0.17) yielded compound 232 (67 mg, 0.172 mmol, quantitative) as a colourless oil.

IR (cm\textsuperscript{-1}, neat) 3482, 1733, 1464, 1363, 1291, 1237, 1187, 1165, 1119, 1093, 1025

\[
\begin{align*}
\text{\textsuperscript{1}H NMR (500MHz, CDCl}\textsubscript{3}) & \delta 5.12-5.17 (m, 1H, H-4), 5.10 (dd, J = 6.5, 3.5 Hz, 1H, H-3), 4.03 (t, J = 5.3 Hz, 2H, H-8), 3.47 (ddd, J = 11.4, 7.9, 5.2 Hz, 1H, H-1), 3.25 (ddd, J = 11.5, 9.1, 5.2
\end{align*}
\]
Hz, 1H, H-1), 2.48 (dd, J = 7.8, 5.3 Hz, 1H, OH), 2.18 (d, J = 7.2 Hz, 2H, H-9 or H-10), 2.15 (d, J = 7.0 Hz, 2H, H-9 or H-10), 2.06-2.11 (m, 1H, H-11 or H-12), 2.08 (s, 3H, H-20), 2.03-2.06 (m, 1H, H-11 or H-12), 1.90-1.98 (m, 1H, H-2), 1.62-1.68 (m, 2H, H-6), 1.55-1.62 (m, 2H, H-7), 0.94 (d, J = 5.3 Hz, 6H, H-13 or H-14 or H-15 or H-16), 0.92 (d, J = 5.3 Hz, 6H, H-13 or H-14 or H-15 or H-16), 0.82 (d, J = 7.1 Hz, 3H, H-5)

$^{13}$C NMR (125MHz, CDCl$_3$) δ 173.3 (C-17 or C-18), 172.8 (C-17 or C-18), 171.6 (C-19), 73.4 (C-3), 71.5 (C-4), 64.6 (C-1), 63.8 (C-8), 43.7 (C-9 or C-10), 43.6 (C-9 or C-10), 36.2 (C-2), 27.3 (C-6), 25.9 (C-11 or C-12), 25.8 (C-11 or C-12), 24.7 (C-7), 22.6 (C-13 to C-16), 21.0 (C-20), 10.8 (C-5)

$[^{\alpha}]_D^{20} = -1.59^\circ$ (c = 0.00755 g/mL; CHCl$_3$)

Ent-232 $[^{\alpha}]_D^{20} = +1.65^\circ$ (c = 0.00760 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{20}$H$_{36}$O$_7$Na: 411.2353. Found 411.2352.

6.5.37 3-Methyl-butyric acid 2R-acetoxy-3S-methyl-1S-[3-(3-methyl-butyryloxy)-propyl]-4-propionyloxy-butyl ester (265)

Propionic anhydride (5 µL, 0.039 mmol) was added to a solution of compound 232 (13 mg, 0.033 mmol), dimethylaminopyridine (0.4 mg, 0.003 mmol) and triethylamine (6 µL, 0.042 mmol) in 5 mL anhydrous dichloromethane and stirred for 20 hours. The reaction was quenched with 5 mL saturated aqueous ammonium chloride and extracted with 3 x 20 mL dichloromethane. The organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 2:1, R$_f$ 0.26) to yield compound 265 as a colourless oil (15 mg, 0.033 mmol, quantitative).

IR (cm$^{-1}$, neat) 1738, 1465, 1370, 1295, 1234, 1186, 1116, 1094, 1022, 756, 666
$^1$H NMR (500MHz, CDCl$_3$) δ 5.11 (dd, J = 9.6, 5.6 Hz, 1H, H-3), 5.05-5.12 (m, 1H, H-4), 4.03 (t, J = 5.4 Hz, 2H, H-8), 3.90 (d, J = 6.5 Hz, 2H, H-1), 2.32 (q, J = 7.7 Hz, 2H, H-22), 2.16 (d, J = 7.2 Hz, 2H, H-9 or H-10), 2.15 (d, J = 7.2 Hz, 2H, H-9 or H-10), 2.01-2.13 (m, 3H, H-2, H-11, H-12), 2.04 (s, 3H, H-20), 1.56-1.68 (m, 4H, H-6, H-7), 1.12 (t, J= 7.6 Hz, 3H, H-23), 0.90-0.97 (m, 15H, H-5, H-13, H-14, H-15, H-16)

$^{13}$C NMR (125MHz, CDCl$_3$) δ 174.5 (C-21), 173.3 (C-17 or C-18), 172.6 (C-17 or C-18), 170.4 (C-19), 73.1 (C-3), 71.9 (C-4), 65.9 (C-1), 63.8 (C-8), 43.7 (C-9 or C-10), 43.6 (C-9 or C-10), 33.4 (C-2), 27.7 (C-6), 26.8 (C-22), 25.9 (C-11 or C-12), 25.8 (C-11 or C-12), 24.9 (C-7), 22.6 (C-13 to C-16), 21.0 (C-20), 11.8 (C-5), 9.3 (C-23)

$[^\alpha]_{D}^{20} = + 0.82^o$ (c = 0.0122 g/mL; CHCl$_3$)

Ent-265 $[^\alpha]_{D}^{20} = - 0.83^o$ (c = 0.0125 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{23}$H$_{40}$O$_8$Na: 467.2615. Found 467.2612.

6.5.38 Acetic acid 4S-acetoxy-1R-[2R-(4-methoxy-phenyl)-5S-methyl-[1,3]dioxan-4-yl]-butyl ester (270)

Acetic anhydride (0.12 mL, 1.2 mmol) and 4-dimethylaminopyridine (7 mg, 0.06 mmol) were added to compound 226 (166.2 mg, 0.561 mmol) and anhydrous triethylamine (0.18 mL, 1.3 mmol) in anhydrous dichloromethane (10 mL) at 0 ºC. The reaction mixture was gradually warmed to room temperature for 24 hours. The solution was quenched with saturated ammonium chloride (10 mL) and extracted with dichloromethane (3 x 20 mL). The combined organic fractions were dried over anhydrous sodium sulphate and the solvent was removed in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether

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2:1, \( R_f \) 0.13) to yield compound 270 as a white solid with a minor impurity (201 mg, 0.527 mmol, 94.0%).

Melting Point: 74 °C

IR (cm\(^{-1}\), neat) 1733, 1615, 1514, 1367, 1298, 1241, 1165, 1108, 1036, 827, 784, 734

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.38 (d, \( J = 8.7 \) Hz, 2H, H-8), 6.87 (d, \( J = 8.7 \) Hz, 2H, H-9), 5.44 (s, 1H, H-6), 4.97-5.03 (m, 1H, H-4), 3.97-4.06 (m, 4H, H-1, H-14), 3.89 (dd, \( J = 9.2, 2.5 \) Hz, 1H, H-3), 3.78 (s, 3H, H-11), 2.06 (s, 3H, H-16 or H-18), 1.99 (s, 3H, H-16 or H-18), 1.84-1.92 (m, 1H, H-2), 1.60-1.69 (m, 4H, H-13, H-14), 1.14 (d, \( J = 6.8 \) Hz, 3H, H-5)

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \( \delta \) 171.3 (C-15 or C-17), 170.3 (C-15 or C-17), 160.2 (C-10), 131.2 (C-7), 127.5 (C-8), 113.8 (C-9), 102.0 (C-6), 80.1 (C-3), 73.8 (C-1), 71.8 (C-4), 64.4 (C-14), 55.5 (C-11), 29.4 (C-2), 27.9 (C-12), 24.2 (C-13), 21.2 (C-16 or C-18), 21.1 (C-16 or C-18), 11.6 (C-5)

\([\alpha]_D^{20} = -4.44^\circ \) (c = 0.00765 g/mL; CHCl\(_3\))

**Ent-270** \([\alpha]_D^{20} = +4.46^\circ \) (c = 0.00767 g/mL; CHCl\(_3\))

HR-ESIMS calculated for [M+Na]\(^+\) \( C_{20}H_{28}O_{7}Na \): 403.1727. Found 403.1730.

### 6.5.39 Acetic acid 1S-(3-acetoxy-propyl)-2R,4-dihydroxy-3S-methyl-butyl ester (271)

Compound 270 (52 mg, 0.14 mmol) was added to a catalytic amount of 10% palladium on carbon in anhydrous methanol (10 mL) with a few drops of acetic acid and pressurized with hydrogen gas (300 psi) for 18 hours. The solution was filtered through a cotton plug with a minimum amount of celite and the filtrate was concentrated in vacuo. Purification of the crude material by flash-column chromatography (silica, 10% methanol in chloroform, \( R_f \) 0.38) yielded compound 271 (36 mg, 0.14 mmol, quantitative) as a colourless oil.

IR (cm\(^{-1}\), neat) 3431, 1730, 1370, 1241, 1140, 1029, 910, 730
$^1$H NMR (500MHz, CDCl$_3$) δ 4.96 (td, J = 7.8, 2.7 Hz, 1H, H-4), 4.00-4.09 (m, 2H, H-7), 3.81 (dd, J = 7.2, 2.9 Hz, 1H, H-3), 3.71 (dd, J = 10.5, 4.1 Hz, H-1), 3.65 (dd, J = 10.7, 5.9 Hz, 1H, H-1), 2.87 (s, br, 1H, OH), 2.32 (s, br, 1H, OH), 2.04 (s, 3H, H-11), 2.01 (s, 3H, H-9), 1.78-1.84 (m, 1H, H-5), 1.72-1.78 (m, 1H, H-2), 1.59-1.71 (m, 3H, H-5, H-6), 0.94 (d, J = 7.1 Hz, 3H, H-14)

$^{13}$C NMR (125MHz, CDCl$_3$) δ 171.5 (C-8 or C-10), 171.0 (C-8 or C-10), 74.6 (C-4), 74.1 (C-3), 67.4 (C-7), 64.1 (C-1), 36.0 (C-2), 27.2 (C-5), 24.6 (C-6), 21.3 (C-9 or C-11), 21.2 (C-9 or C-11), 10.3 (C-14)

[$\alpha$]$_D^{20}$ = + 3.17º (c = 0.01765 g/mL; CHCl$_3$)

Ent-271 [$\alpha$]$_D^{20}$ = − 3.20º (c = 0.01765 g/mL; CHCl$_3$)


6.5.40 Acetic acid 1S-(3-acetoxy-propyl)-2R-hydroxy-3S-methyl-4-trityloxy-butyl ester (272)

Trityl bromide (93 mg, 0.29 mmol) was added to a solution of compound 271 (63 mg, 0.24 mmol), dimethylaminopyridine (4 mg, 0.034 mmol) and triethylamine (43 μL, 0.31 mmol) in 10 mL anhydrous dichloromethane and refluxed for 24 hours. The reaction was quenched with 10 mL saturated aqueous ammonium chloride and extracted with 3 x 20 mL dichloromethane. The organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 1:1, $R_f$ 0.23) to yield compound 272 as a colourless oil (67 mg, 0.13 mmol, 55%).

IR (cm$^{-1}$, neat) 3496, 1741, 1723 1485, 1446, 1370, 1241, 1151, 1065, 1029, 903, 763, 741, 727, 698
$^1$H NMR (500MHz, CDCl$_3$) δ 7.19-7.45 (m, 15H, H-15 to H-17), 4.93 (dt, J = 7.5, 2.5 Hz, 1H, H-4), 4.03 (t, J = 5.7 Hz, 2H, H-8), 3.70 (dt, J = 7.6, 2.9 Hz, 1H, H-3), 3.24 (dd, J = 9.3, 5.8 Hz, H-1), 3.15 (dd, J = 9.3, 3.9 Hz, 1H, H-1), 2.72 (d, J = 3.1 Hz, 1H, OH), 2.01 (s, 6H, H-10, H-12), 1.76-1.86 (m, 2H, H-2, H-6), 1.62-1.66 (m, 1H, H-6), 1.57-1.62 (m, 2H, H-7), 1.04 (d, J = 7.1 Hz, 3H, H-5)

$^{13}$C NMR (125MHz, CDCl$_3$) δ 171.3 (C-9 or C-11), 170.5 (C-9 or C-11), 143.9 (C-14), 127.3-128.8 (C-15 to C-17), 87.3 (C-13), 74.9 (C-4), 73.7 (C-3), 68.1 (C-), 64.5 (C-8), 34.8 (C-2), 27.5 (C-6), 24.6 (C-7), 21.3 (C-10 or C-12), 21.2 (C-10 or C-12), 10.9 (C-5)

$[\alpha]_{D}^{20} = -14.19^\circ$ (c = 0.03150 g/mL; CHCl$_3$)

Ent-272 $[\alpha]_{D}^{20} = +14.25^\circ$ (c = 0.03175 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{31}$H$_{36}$O$_6$Na: 527.2404. Found 527.2408.

6.5.41 Acetic acid 2R-acetoxy-1S-(3-acetoxy-propyl)-3S-methyl-4-trityloxy-butyl ester (273)

Acetic anhydride (15 µL, 0.16 mmol) was added via syringe to a solution of compound 272 (67 mg, 0.13 mmol), triethylamine (24 µL, 0.17 mmol) and dimethylaminopyridine (2 mg, 0.016 mmol) in 10 mL anhydrous dichloromethane and stirred for 24 hours. The reaction was quenched with 10 mL saturated ammonium chloride and the aqueous layer was extracted with three aliquots of 20 mL dichloromethane. The organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 1:1, R$_f$ 0.30) to yield compound 273 as a colourless oil (71 mg, 0.13 mmol, quantitative).

IR (cm$^{-1}$, neat) 1737, 1489, 1446, 1367, 1226, 1065, 1029, 971, 763, 745, 702, 630
**1H NMR (500MHz, CDCl\textsubscript{3}) δ 7.18-7.43 (m, 15H, H-15 to H-17), 5.12 (t, J = 4.9 Hz, 1H, H-3), 5.07 (q, J = 5.8 Hz, 1H, H-4), 4.00 (t, J = 6.2 Hz, 2H, H-8), 3.24 (dd, J = 9.3, 5.8 Hz, 1H, H-1), 2.98 (dd, J = 9.5, 5.7 Hz, 1H, H-1), 2.95 (dd, J = 9.5, 7.2 Hz, 1H, H-1), 2.02 (s, 3H, H-12 or H-19), 1.93 (s, 3H, H-10), 1.47-1.67 (m, 5H, H-2, H-6, H-7), 0.88 (d, J = 7.1 Hz, 3H, H-5)**

**13C NMR (125MHz, CDCl\textsubscript{3}) δ 171.3 (C-9 or C-11 or C-18), 170.6 (C-9 or C-11 or C-18), 170.4 (C-9 or C-11 or C-18), 144.2 (C-14), 127.2-128.9 (C-15 to C-17), 87.0 (C-13), 74.2 (C-3), 72.9 (C-4), 65.5 (C-1), 64.3 (C-8), 34.7 (C-2), 26.3 (C-6), 25.0 (C-7), 21.2 (C-10 or C-12 or C-19), 21.2 (C-10 or C-12 or C-19), 21.0 (C-10 or C-12 or C-19), 12.9 (C-5)**

$\left[\alpha\right]_D^{20} = + 19.53^\circ$ (c = 0.02125 g/mL; CHCl\textsubscript{3})

Ent-273 $\left[\alpha\right]_D^{20} = - 19.50^\circ$ (c = 0.02130 g/mL; CHCl\textsubscript{3})

HR-ESIMS calculated for [M+Na]\textsuperscript{+} C\textsubscript{33}H\textsubscript{38}O\textsubscript{7}Na: 569.2510. Found 569.2511.

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**6.5.42 Acetic acid 2R-acetoxy-1S-(3-acetoxy-propyl)-4-hydroxy-3S-methyl-butyl ester (274)**

Compound 273 (56 mg, 0.10 mmol) was added to a catalytic amount of 10% palladium on carbon in anhydrous methanol (10 mL) with a few drops of acetic acid and pressurized with hydrogen gas (300 psi) for 18 hours. The solution was filtered through a cotton plug with a minimum amount of celite and the filtrate was concentrated in vacuo. Purification of the crude material by flash-column chromatography (silica, hexanes:ether 1:2, R\textsubscript{f} 0.11) yielded compound 274 as a colourless oil (30 mg, 0.10 mmol, quantitative).

IR (cm\textsuperscript{-1}, neat) 3490, 1731, 1446, 1370, 1236, 1104, 1029, 911, 730, 646, 604

$\text{1H NMR (500MHz, CDCl}_3\text{) δ 5.08-5.13 (m, 1H, H-4), 5.07 (dd, J = 6.3, 4.0 Hz, 1H, H-3), 4.00 (t, J = 5.5 Hz, 2H, H-8), 3.42-3.49 (m, 1H, H-1), 3.26 (dd, J = 9.0, 8.8 Hz, 1H, H-1), 2.49 (s br, 1H, OH), 2.07 (s, 3H, H-12 or H-14), 2.03 (s, 3H, H-12 or H-14), 2.00 (s, 3H, H-10), 1.88-1.95 (m, 1H, H-2), 1.54-1.67 (m, 4H, H-6, H-7), 0.81 (d, J = 7.1 Hz, 3H, H-5)$
$^{13}$C NMR (125MHz, CDCl$_3$) δ 171.6 (C-9 or C-11 or C-13), 171.3 (C-9 or C-11 or C-13), 170.7 (C-9 or C-11 or C-13), 73.5 (C-3), 71.9 (C-4), 64.6 (C-8), 64.2 (C-1), 36.2 (C-2), 27.0 (C-6), 24.6 (C-7), 21.2 (C-10 or C-12 or C-14), 21.1 (C-10 or C-12 or C-14), 21.0 (C-10 or C-12 or C-14), 10.9 (C-5)

$\alpha$$_D^{20} = +1.26^\circ$ (c = 0.00950 g/mL; CHCl$_3$)

**Ent-274** $\alpha$$_D^{20} = -1.30^\circ$ (c = 0.00950 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{14}$H$_{24}$O$_7$Na: 327.1414. Found 327.1412.

**6.5.43 Acetic acid 2R,4-diacetoxy-1S-(3-acetoxy-propyl)-3S-methyl-butyl ester (266)**

Acetic anhydride (6 µL, 0.065 mmol) was added via syringe to a solution of compound 274 (17 mg, 0.054 mmol), triethylamine (10 µL, 0.070 mmol) and dimethylaminopyridine (0.7 mg, 0.006 mmol) in 5 mL anhydrous dichloromethane and stirred for 24 hours. The reaction was quenched with 5 mL saturated ammonium chloride and the aqueous layer was extracted with three aliquots of 10 mL dichloromethane. The organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 2:1, R$_f$ 0.19) to yield compound 266 as a colourless oil (10 mg, 0.030 mmol, 55%).

Alternatively, acetic anhydride (13 µL, 0.13 mmol) was added via syringe to a solution of compound 271 (19 mg, 0.061 mmol), triethylamine (20 µL, 0.14 mmol) and dimethylaminopyridine (0.7 mg, 0.006 mmol) in 5 mL anhydrous dichloromethane and stirred for 24 hours. The reaction was quenched with 5 mL saturated ammonium chloride and the aqueous layer was extracted with three aliquots of 10 mL dichloromethane. The organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 2:1, R$_f$ 0.19) to yield compound 266 as a colourless oil (11 mg, 0.031 mmol, 51%).
IR (cm\(^{-1}\), neat) 1738, 1370, 1224, 1027
\(^1\)H NMR (500MHz, CDCl\(_3\)) \(\delta\) 5.01-5.09 (m, 2H, H-3, H-4), 4.03 (t, J = 5.5 Hz, 2H, H-8), 3.90 (d, J = 6.1 Hz, 2H, H-1), 2.07-2.12 (m, 1H, H-2), 2.05 (s, 3H, H-10 or H-12 or H-14 or H-16), 2.04 (s, 3H, H-10 or H-12 or H-14 or H-16), 2.03 (s, 3H, H-10 or H-12 or H-14 or H-16), 2.02 (s, 3H, H-10 or H-12 or H-14 or H-16), 1.58-1.70 (m, 4H, H-6, H-7), 0.94 (d, J = 7.3 Hz, 3H, H-5)
\(^{13}\)C NMR (125MHz, CDCl\(_3\)) \(\delta\) 171.3 (C-9 or C-11 or C-13 or C-15), 171.1 (C-9 or C-11 or C-13 or C-15), 170.6 (C-9 or C-11 or C-13 or C-15), 170.5 (C-9 or C-11 or C-13 or C-15), 73.2 (C-3), 72.2 (C-4), 66.0 (C-1), 64.2 (C-8), 33.4 (C-2), 26.5 (C-6), 24.8 (C-7), 21.2 (C-10 or C-12 or C-14 or C-16), 21.1 (C-10 or C-12 or C-14 or C-16), 21.1 (C-10 or C-12 or C-14 or C-16), 21.0 (C-10 or C-12 or C-14 or C-16), 12.0 (C-5)
\([\alpha]_D^{20}\) = + 5.83º (c = 0.0048 g/mL; CHCl\(_3\))
Ent-266 \([\alpha]_D^{20}\) = - 5.86º (c = 0.0050 g/mL; CHCl\(_3\))
HR-ESIMS calculated for [M+Na]\(^+\) C\(_{16}\)H\(_{26}\)O\(_8\)Na: 369.1520. Found 369.1518.

6.5.44 Propionic acid 1\(R\)-2\(R\)-(4-methoxy-phenyl)-5\(S\)-methyl-[1,3]dioxan-4-yl]-4\(S\)-propionyloxy-butyl ester (275)

Propionic anhydride (0.13 mL, 0.99 mmol) was added via syringe to a solution of compound 226 (133 mg, 0.449 mmol), triethylamine (0.14 mL, 1.0 mmol) and dimethylaminopyridine (7 mg, 0.057 mmol) in 10 mL anhydrous dichloromethane and stirred for 24 hours. The reaction was quenched with 10 mL saturated ammonium chloride and the aqueous layer was extracted with three aliquots of 20 mL dichloromethane. The organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was
purified by flash-column chromatography (silica, hexanes:ether 1:1, R<sub>f</sub> 0.4) to yield compound 275 as a colourless oil (167 mg, 0.408 mmol, 90.8%).

IR (cm<sup>-1</sup>, neat) 1738, 1616, 1519, 1464, 1423, 1369, 1295, 1249, 1184, 1114, 1084, 1033, 1012, 829, 809, 755, 666

<sup>1</sup>H NMR (500MHz, CDCl<sub>3</sub>) δ 7.37 (d, J = 8.5 Hz, 2H, H-8), 6.87 (d, J = 8.5 Hz, 2H, H-9), 5.44 (s, 1H, H-6), 4.98-5.04 (m, 1H, H-4), 3.98-4.06 (m, 4H, H-1, H-14), 3.90 (dd, J = 9.1, 2.3 Hz, 1H, H-3), 3.79 (s, 3H, H-11), 2.33 (q, J = 7.6 Hz, 2H, H-16 or H-19), 2.26 (q, J = 7.6 Hz, 2H, H-16 or H-19), 1.85-1.93 (m, 1H, H-2), 1.61-1.68 (m, 4H, H-12, H-13), 1.14 (t, J = 7.6 Hz, 3H, H-17 or H-20), 1.14 (d, J = 6.9 Hz, 3H, H-5), 1.08 (t, J = 7.6 Hz, 3H, H-17 or H-20)

<sup>1</sup>3C NMR (125MHz, CDCl<sub>3</sub>) δ 174.7 (C-15 or C-18), 173.8 (C-15 or C-18), 160.2 (C-10), 131.2 (C-7), 127.5 (C-8), 113.8 (C-9), 102.0 (C-6), 80.1 (C-3), 73.9 (C-1), 71.6 (C-4), 64.3 (C-14), 55.5 (C-11), 29.5 (C-2), 28.0 (C-12), 27.8 (C-16, C-19), 24.3 (C-13), 11.6 (C-5), 9.5 (C-17 or C-20), 9.3 (C-17 or C-20)

[α]<sub>D</sub><sup>20</sup> = -5.77° (c = 0.0026 g/mL; CHCl<sub>3</sub>)

**Ent-275** [α]<sub>D</sub><sup>20</sup> = +5.80° (c = 0.0024 g/mL; CHCl<sub>3</sub>)

HR-ESIMS calculated for [M+Na]<sup>+</sup> C<sub>22</sub>H<sub>32</sub>O<sub>7</sub>Na: 431.2040. Found 431.2037.

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6.5.45 Propionic acid 2R,4-dihydroxy-3S-methyl-1S-(3-propionyloxy-propyl)-butyl ester (276)

Compound 275 (140 mg, 0.343 mmol) was added to a catalytic amount of 10% palladium on carbon in anhydrous methanol (10 mL) with a few drops of acetic acid and pressurized with hydrogen gas (300 psi) for 18 hours. The solution was filtered through a cotton plug with a minimum amount of celite and the filtrate was concentrated in vacuo. Purification of the crude material by flash-column chromatography (silica, 10% methanol in chloroform, R<sub>f</sub> 0.09) yielded compound 276 as a colourless oil (93 mg, 0.320 mmol, 93.2%).

IR (cm<sup>-1</sup>, neat) 3422, 1735, 1464, 1424, 1352, 1277, 1193, 1084, 1028, 938, 886, 808, 665
$^1$H NMR (500MHz, CDCl$_3$) δ 4.96 (dt, J = 7.2, 2.8 Hz, 1H, H-4), 4.00-4.13 (m, 2H, H-7), 3.80-3.93 (m, 1H, H-3), 3.71 (dd, J = 10.4, 3.6 Hz, 1H, H-1), 3.65 (dd, J = 10.4, 5.6 Hz, 1H, H-1), 2.80 (d, J = 3.7 Hz, 1H, OH), 2.24-2.40 (m, 5H, H-2, H-9, H-12), 1.55-1.83 (m, 5H, H-5, H-6, OH), 1.11 (t, J = 7.5 Hz, 3H, H-10 or H-13), 1.10 (t, J = 7.5 Hz, 3H, H-10 or H-13), 0.94 (d, J = 7.1 Hz, 3H, H-14)

$^{13}$C NMR (125MHz, CDCl$_3$) δ 174.9 (C-8 or C-11), 174.4 (C-8 or C-11), 74.6 (C-4), 73.9 (C-3), 67.4 (C-7), 64.3 (C-1), 37.0 (C-2), 28.0 (C-5), 27.8 (C-9, C-12), 24.7 (C-6), 10.3 (C-14), 9.4 (C-10 or C-13), 9.3 (C-10 or C-13)

$[\alpha]_D^{20} =$ –1.52° (c = 0.00725 g/mL; CHCl$_3$)

Ent-276 $[\alpha]_D^{20} =$ +1.55° (c = 0.00730 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{14}$H$_{26}$O$_6$Na: 313.1622. Found 313.1625.

6.5.46 Propionic acid 3S-methyl-2R,4-bis-propionyloxy-1S-(3-propionyloxy-propyl)-butyl ester (267)

Propionic anhydride (67 µL, 0.52 mmol) was added via syringe to a solution of compound 276 (69 mg, 0.24 mmol), triethylamine (76 µL, 0.54 mmol) and dimethylaminopyridine (3 mg, 0.028 mmol) in 10 mL anhydrous dichloromethane and stirred for 24 hours. The reaction was quenched with 10 mL saturated ammonium chloride and the aqueous layer was extracted with three aliquots of 20 mL dichloromethane. The organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 2:1, R$_f$ 0.24) to yield compound 267 as a colourless oil (87 mg, 0.22 mmol, 91%).

IR (cm$^{-1}$, neat) 1735, 1464, 1424, 1351, 1274, 1181, 1083, 1020, 807, 666

$^1$H NMR (500MHz, CDCl$_3$) δ 5.02-5.16 (m, 2H, H-3, H-4), 4.01 (t, J = 5.5 Hz, 2H, H-8), 3.88 (d, J = 6.8 Hz, 2H, H-1), 2.23-2.24 (m, 8H, H-10, H-13, H-16, H-19), 2.03-2.12 (m, 1H, H-2), 1.50-
1.70 (m, 4H, H-6, H-7), 1.06-1.14 (m, 12H, H-11, H-14, H-17, H-20), 0.91 (d, J = 7.0 Hz, 3H, H-5)

$^{13}$C NMR (125MHz, CDCl$_3$) δ 174.6 (C-9 or C-12 or C-15 or C-18), 174.4 (C-9 or C-12 or C-15 or C-18), 173.9 (C-9 or C-12 or C-15 or C-18), 173.7 (C-9 or C-12 or C-15 or C-18), 72.8 (C-3), 72.0 (C-4), 65.8 (C-1), 64.0 (C-8), 33.4 (C-2), 27.8 (C-10 or C-13 or C-16 or C-19), 27.7 (C-10 or C-13 or C-16 or C-19), 27.7 (C-10 or C-13 or C-16 or C-19), 27.6 (C-10 or C-13 or C-16 or C-19), 26.6 (C-6), 24.8 (C-7), 11.9 (C-5), 9.4 (C-11 or C-14 or C-17 or C-20), 9.3 (C-11 or C-14 or C-17 or C-20), 9.3 (C-11 or C-14 or C-17 or C-20), 9.2 (C-11 or C-14 or C-17 or C-20)

$[\alpha]_D^{20} = +6.52^\circ$ (c = 0.03315 g/mL; CHCl$_3$)

Ent-267 $[\alpha]_D^{20} = -6.50^\circ$ (c = 0.03325 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{20}$H$_{34}$O$_8$Na: 425.2146. Found 425.2142.

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6.5.47 3-Methyl-butyric acid 3S-methyl-2R,4-bis-(3-methyl-butyryloxy)-1S-[3-(3-methyl-butyryloxy)-propyl]-butyl ester (268)

Isovaleric anhydride (10 µL, 0.051 mmol) was added via syringe to a solution of compound 263 (20 mg, 0.046 mmol), triethylamine (8 µL, 0.055 mmol) and dimethylaminopyridine (0.6 mg, 0.005 mmol) in 5 mL anhydrous dichloromethane and stirred for 24 hours. The reaction was quenched with 5 mL saturated ammonium chloride and the aqueous layer was extracted with three aliquots of 10 mL dichloromethane. The organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 2:1, R$_f$ 0.24) to yield compound 268 as a colourless oil (24 mg, 0.046 mmol, 90%).
Alternatively, isovaleric anhydride (81 µL, 0.41 mmol) was added via syringe to a solution of compound 228 (64 mg, 0.19 mmol), triethylamine (60 µL, 0.43 mmol) and dimethylaminopyridine (3 mg, 0.02 mmol) in 15 mL anhydrous dichloromethane and stirred for 24 hours. The reaction was quenched with 15 mL saturated ammonium chloride and the aqueous layer was extracted with three aliquots of 30 mL dichloromethane. The organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 2:1, Rf 0.24) to yield compound 268 as a colourless oil (88 mg, 0.17 mmol, 92%).

IR (cm\(^{-1}\), neat) 1738, 1467, 1370, 1294, 1250, 1186, 1167, 1119, 1096, 1013, 916, 734, 666, 648

\(^1\)H NMR (500MHz, CDCl\(_3\) ) δ 5.03-5.16 (m, 2H, H-3, H-4), 4.01 (t, J = 5.7 Hz, 2H, H-8), 3.89 (dd, J = 11.1, 6.1 Hz, 1H, H-1), 3.85 (dd, J = 11.1, 7.3 Hz, 1H, H-1), 2.11-2.21 (m, 8H, H-10, H-14, H-18, H-22), 1.99-2.11 (m, 5H, H-2, H-11, H-15, H-19, H-23), 1.52-1.78 (m, 4H, H-6, H-7), 0.89-0.94 (m, 27H, H-5, H-12, H-16, H-20, H-24)

\(^{13}\)C NMR (125MHz, CDCl\(_3\) ) δ 173.2 (C-9 or C-13 or C-17 or C-21), 173.0 (C-9 or C-13 or C-17 or C-21), 172.5 (C-9 or C-13 or C-17 or C-21), 172.3 (C-9 or C-13 or C-17 or C-21), 72.7 (C-3), 71.9 (C-4), 65.7 (C-1), 63.8 (C-8), 43.6 (C-10 or C-14 or C-18 or C-22), 43.5 (C-10 or C-14 or C-18 or C-22), 43.5 (C-10 or C-14 or C-18 or C-22), 43.4 (C-10 or C-14 or C-18 or C-22), 33.4 (C-2), 26.7 (C-6), 25.8 (C-11 or C-15 or C-19 or C-23), 25.8 (C-11 or C-15 or C-19 or C-23), 25.7 (C-11 or C-15 or C-19 or C-23), 25.7 (C-11 or C-15 or C-19 or C-23), 24.8 (C-7), 22.6 (C-12 or C-16 or C-20 or C-24), 22.5 (C-12 or C-16 or C-20 or C-24), 22.5 (C-12 or C-16 or C-20 or C-24), 22.4 (C-12 or C-16 or C-20 or C-24), 11.9 (C-5)

\([\alpha]_D^{20} = +1.45^\circ (c = 0.0393 \text{ g/mL; CHCl}_3)\)

**Ent-268** \([\alpha]_D^{20} = −1.50^\circ (c = 0.0390 \text{ g/mL; CHCl}_3)\)

HR-ESIMS calculated for [M+Na]^+ C\(_{28}\)H\(_{50}\)O\(_8\)Na: 537.3398. Found 537.3397.
6.6.0 Didemnaketal A Natural Product and C-6/C-10 Methyl Analogues

6.6.1 4S-Benzylxy-4R-[2R-(4-methoxy-phenyl)-5S-methyl-[1,3]dioxan-4-yl]-butan-2-one (291)

Oxygen gas (1 atm) was bubbled through a solution of 235 (60 mg, 0.16 mmol), palladium (II) chloride (29 mg, 0.16 mmol) and copper (II) acetate monohydrate (6.2 mg, 0.03 mmol) in 8 mL dimethylformamide/water (7:1) at room temperature for 24 hours. The mixture was quenched with 10 mL brine and extracted with 3 x 20 mL aliquots of diethyl ether. The organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo to yield a crude yellow oil. The crude material was purified by flash chromatography (1% triethylamine treated silica, hexanes:ether 1:1, Rf 0.23) to yield compound 291 as a colourless oil (54 mg, 0.14 mmol, 86%).

IR (cm\(^{-1}\), film) 1711, 1613, 1518, 1244, 1110, 827, 735, 696

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.27-7.37 (m, 7H, H-8, H-17 to H-19), 6.87 (d, J = 8.9 Hz, 2H, H-9), 5.41 (s, 1H, H-6), 4.65 (d, J = 11.5 Hz, 1H, H-15), 4.53 (d, J = 11.5 Hz, 1H, H-15), 4.08 (ddd, J = 9.2, 6.2, 4.9 Hz, 1H, H-4), 4.03 (dd, J = 11.5, 2.7 Hz, 1H, H-1), 4.00 (dd, J = 11.5, 1.7 Hz, 1H, H-1), 3.82 (dd, J = 9.1, 2.2 Hz, 1H, H-3), 3.78 (s, 3H, H-11), 2.85 (dd, J = 16.2, 5.1 Hz, 1H, H-12), 2.70 (dd, J = 16.2, 6.2 Hz, 1H, H-12), 2.07 (s, 3H, H-14), 1.89-1.96 (m, 1H, H-2), 1.17 (d, J = 6.9 Hz, 3H, H-5)
$^{13}$C NMR (125 MHz, CDCl$_3$) δ 207.4 (C-13), 160.2 (C-10), 138.3 (C-16), 131.2 (C-7), 127.6-128.6 (C-8, C-17 to C-19), 113.7 (C-9), 102.2 (C-6), 81.5 (C-3), 74.7 (C-4), 74.0 (C-15), 72.7 (C-1), 55.5 (C-11), 46.4 (C-12), 31.4 (C-2), 29.5 (C-14), 11.8 (C-5)

[α]$_D^{20}$ = + 6.3° (c = 0.0214 g/mL; CHCl$_3$)

**Ent-291** [α]$_D^{20}$ = − 6.1° (c = 0.0210 g/mL; CHCl$_3$)


6.6.2 4S-Benzzyloxy-3R-(4-methoxy-benzyloxy)-2S-methyl-hept-6-en-1-ol (295)

DIBAL-H (1.0 M in hexanes, 0.52 mL, 0.52 mmol) was added dropwise via syringe to a solution of 235 (38 mg, 0.10 mmol) in 5 mL anhydrous toluene at 0 °C. After 15 minutes, the mixture was quenched with 10 mL Rochelle’s salt and warmed to room temperature. The aqueous layer was extracted with 3 x 20 mL dichloromethane and the organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash chromatography (1% triethylamine treated silica, hexanes:ether 1:1, R$_f$ 0.14) to yield compound 295 as a colourless oil (31 mg, 0.083 mmol, 81%).

IR (cm$^{-1}$, film) 3428, 1611, 1510, 1247, 911, 819, 752, 693

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.27-7.34 (m, 5H, H-17 to H-19), 7.25 (d, J = 8.7 Hz, 2H, H-8), 6.86 (d, J = 8.7 Hz, 2H, H-9), 5.90-5.99 (m, 1H, H-13), 5.15 (dd, J = 17.3, 2.0 Hz, 1H, H-14), 5.09 (dd, J = 10.5, 2.0 Hz, 1H, H-14), 4.62 (d, J = 11.2 Hz, 2H, H-6, H-15), 4.54 (d, J = 11.2 Hz, 1H, H-15), 4.50 (d, J = 11.2 Hz, 1H, H-6), 3.78 (s, 3H, H-11), 3.62-3.67 (m, 2H, H-3, H-4), 3.52-
3.58 (m, 2H, H-1), 2.43-2.58 (m, 2H, H-12), 2.03-2.12 (m, 1H, H-2), 1.82 (t, J = 3.8 Hz, 1H, OH), 0.94 (d, J = 6.9 Hz, 3H, H-5)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 159.4 (C-10), 138.6 (C-16), 135.5 (C-13), 130.9 (C-7), 127.8-129.6 (C-8, C-17 to C-19), 117.3 (C-14), 114.0 (C-9), 80.5 (C-3), 79.5 (C-4), 73.5 (C-15), 72.1 (C-6), 66.3 (C-1), 55.4 (C-11), 37.4 (C-2), 35.4 (C-12), 12.0 (C-5)

$[^\alpha]_D^{20} = + 20.0^\circ$ (c = 0.0156 g/mL; CHCl$_3$)

Ent-295 $[^\alpha]_D^{20} = - 19.7^\circ$ (c = 0.0154 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{23}$H$_{30}$O$_4$Na: 393.2036. Found 393.2036.

6.6.3 [4S-Benzylxy-3R-(4-methoxy-benzyloxy)-2S-methyl-hept-6-enyloxy]-tert-butyl-dimethyl-silane (296)

A solution of 295 (74 mg, 0.20 mmol), dimethylaminopyridine (2 mg, 0.02 mmol), and triethylamine (6.6 µL, 0.47 mmol) in 5 mL anhydrous dichloromethane was added dropwise via syringe to a solution of tert-butyldimethylsilyl trifluoromethane sulfonate (87 mg, 0.39 mmol) in 5 mL anhydrous dichloromethane and heated to reflux for an hour. The mixture was quenched with 10 mL brine and extracted with 3 x 20 mL dichloromethane. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash chromatography (silica, hexanes:ether 4:1, R$_f$ 0.69) to yield compound 296 as a colourless oil (85 mg, 0.18 mmol, 89%).

IR (cm$^{-1}$, neat) 1641, 1608, 1583 1513, 1244, 1088, 911, 833, 772, 732 696, 665
\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.29-7.37 (m, 5H, H-17 to H-19), 7.27 (d, J = 8.7 Hz, 2H, H-8), 6.86 (d, J = 8.7 Hz, 2H, H-9), 5.90-6.00 (m, 1H, H-13), 5.13 (d, J = 17.2 Hz, 1H, H-14), 5.07 (d, J = 10.2 Hz, 1H, H-14), 4.64 (d, J = 10.7 Hz, 1H, H-6), 4.59 (s, 2H, H-15), 4.51 (d, J = 10.7 Hz, 1H, H-6), 3.79 (s, 3H, H-11), 3.71 (t, J = 4.5 Hz, 1H, H-3), 3.64 (dd, J = 10.6, 5.7 Hz, 1H, H-13), 3.47-3.56 (m, 2H, H-1), 2.46 (t, J = 6.2 Hz, 2H, H-12), 1.96-2.06 (m, 1H, H-2), 0.93 (d, J = 6.9 Hz, 3H, H-5), 0.90 (s, 9H, H-22), 0.04 (2 x s, 3H, H-20)

\(^1\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 159.3 (C-10), 139.0 (C-16), 136.0 (C-13), 131.5 (C-7), 127.6-129.5 (C-8, C-17 to C-19), 116.9 (C-14), 113.9 (C-9), 80.0 (C-3), 79.8 (C-4), 73.9 (C-15), 72.0 (C-6), 66.0 (C-1), 55.5 (C-11), 37.6 (C-2), 35.4 (C-12), 26.2 (C-22), 18.5 (C-21), 12.0 (C-5), -5.1 (C-21), -5.2 (C-21)

\([\alpha]_D^{20}\) = + 8.6° (c = 0.0258 g/mL; CHCl\(_3\))

**Ent-296** \([\alpha]_D^{20}\) = − 8.8° (c = 0.0261 g/mL; CHCl\(_3\))

HR-ESIMS calculated for [M+Na]^+ \(C_{29}H_{44}O_4SiNa\): 507.2901. Found 507.2900.

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6.6.4 4R-Benzylxoy-7-(tert-butyl-dimethyl-silanyloxy)-5S-(4-methoxy-benzylxoy)-6R-methyl-heptan-2-one (ent-297)

Oxygen gas (1 atm) was bubbled through a solution of ent-296 (85 mg, 0.18 mmol), palladium (II) chloride (32 mg, 0.18 mmol) and copper (II) acetate monohydrate (8 mg, 0.04 mmol) in 8 mL dimethylformamide/water (7:1) at room temperature for 24 hours. The mixture was quenched with 10 mL brine and extracted with 3 x 20 mL aliquots of diethyl ether. The organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in
vacuo to yield a crude yellow oil. The crude material was purified by flash chromatography (silica, hexanes:ether 4:1, R_f 0.21) to yield compound ent-297 as a colourless oil (65 mg, 0.13 mmol, 73%).

IR (cm⁻¹, film) 1717, 1611, 1513, 1247, 1082, 934, 909, 833, 774, 735, 696, 665

¹H NMR (500 MHz, CDCl₃) δ 7.26-7.35 (m, 5H, H-17 to H-19), 7.26 (d, J = 8.7 Hz, 2H, H-8), 6.85 (d, J = 8.7 Hz, 2H, H-9), 4.69 (d, J = 11.1 Hz, 1H, H-6), 4.62 (d, J = 11.1 Hz, 1H, H-15), 4.53 (d, J = 11.1 Hz, 1H, H-15), 4.50 (d, J = 11.1 Hz, 1H, H-6), 4.20 (dt, J = 8.2, 3.9 Hz, 1H, H-4), 3.79 (s, 3H, H-11), 3.71, (dd, J = 4.3, 4.3 Hz, 1H, H-3), 3.52 (dd, J = 9.9, 5.1 Hz, 1H, H-1), 3.48 (dd, J = 9.9, 7.3 Hz, 1H, H-1), 2.85 (dd, J = 16.7, 8.7 Hz, 1H, H-12), 2.62 (dd, J = 16.7, 3.1 Hz, 1H, H-12), 2.12 (s, 3H, H-14), 1.78-1.85 (m, 1H, H-2), 0.95 (d, J = 6.9 Hz, 3H, H-5), 0.90 (s, 9H, H-22), 0.05 (s, 3H, H-20), 0.03 (s, 3H, H-20)

¹³C NMR (125 MHz, CDCl₃) δ 207.7 (C-13), 159.3 (C-10), 138.8 (C-16), 131.3 (C-7), 127.7-129.6 (C-8, C-17 to C-19), 113.9 (C-9), 80.2 (C-3), 77.5 (C-4), 74.0 (C-15), 72.5 (C-6), 65.7 (C-1), 55.4 (C-11), 45.5 (C-12), 38.1 (C-2), 31.2 (C-14), 26.1 (C-22), 18.5 (C-21), 12.8 (C-5), –5.2 (C-21), –5.2 (C-21)

[α]D₂⁰ = + 4.1º (c = 0.0293 g/mL; CHCl₃)

6.6.5 Trifluoro-methanesulfonic acid 3R-benzyloxy-6-(tert-butyl-dimethyl-silyloxy)-4S-(4-methoxy-benzyloxy)-5R-methyl-1-methylene-hexyl ester (ent-298)

Lithium bis(trimethylsilyl)amide (55 µL, 0.055 mmol) was added to a solution of ent-297 (25 mg, 0.050 mmol) in 5 mL anhydrous tetrahydrofuran at –78 ºC. The mixture was stirred for 30 minutes, followed by addition of N-(5-chloro-2-pyridyl)bis(trifluromethanesulfonylimide) (Comins’ reagent, 28 mg, 0.70 mmol). The solution was warmed to room temperature and stirred for 18.5 hours. The reaction was quenched with 10 mL saturated sodium chloride and extracted with 3 x 15 mL dichloromethane. The organic layers were combined, dried over sodium sulphate and concentrated in vacuo. Purification of the crude material by flash-chromatography (silica, hexanes:ether 9:1, Rf 0.16) yielded compound ent-298 as a colourless oil (10 mg, 0.016 mmol, 32%; not optimized).

IR (cm⁻¹, film) 1639, 1611, 1510, 1465, 1415, 1300, 1247, 1211, 1138, 1085, 1037, 942, 895, 833, 777, 746, 732, 696, 665, 606

¹H NMR (500 MHz, CDCl₃) δ 7.26-7.33 (m, 5H, H-17 to H-19), 7.23 (d, J = 8.5 Hz, 2H, H-8), 6.85 (d, J = 8.5 Hz, 2H, H-9), 5.14 (d, J = 3.2 Hz, 1H, H-14), 5.04 (d, J = 3.2 Hz, 1H, H-14), 4.66 (d, J = 11.3 Hz, 1H, H-6), 4.63 (d, J = 11.3 Hz, 1H, H-15), 4.51 (d, J = 11.3 Hz, 1H, H-15), 4.50 (d, J = 11.3 Hz, 1H, H-6), 3.86 (dt, J = 8.7, 3.3 Hz, 1H, H-4), 3.78-3.80 (m, 1H, H-3), 3.78 (s, 3H, H-11), 3.52 (dd, J = 10.1, 5.4 Hz, 1H, H-1), 3.48 (dd, J = 9.9, 6.7 Hz, 1H, H-1), 2.67 (dd, J = 15.6, 8.4 Hz, 1H, H-12), 2.61 (dd, J = 15.6, 3.4 Hz, 1H, H-12), 1.77-1.86 (m, 1H, H-2), 0.95 (d, J = 6.9 Hz, 3H, H-5), 0.89 (s, 9H, H-22), 0.03 (s, 3H, H-20), 0.03 (s, 3H, H-20)
$^{13}$C NMR (125 MHz, CDCl$_3$) δ 168 (C-13, not seen), 159.5 (C-10), 154.9 (C-23), 138.4 (C-16), 131.2 (C-7), 127.9-129.6 (C-8, C-17 to C-19), 113.9 (C-9), 106.9 (C-14), 79.3 (C-3), 77.5 (C-4), 74.2 (C-15), 72.3 (C-6), 65.5 (C-1), 55.5 (C-11), 37.9 (C-2), 36.0 (C-12), 26.1 (C-22), 18.5 (C-21), 12.9 (C-5), –5.2 (C-21), –5.3 (C-21)

[$\alpha$]$_D$$_{20}^0$ = –6.0° (c = 0.00500 g/mL; CHCl$_3$)


6.6.6 4R-(1S-Benzylolxy-but-2-enyl)-2R-(4-methoxy-phenyl)-5S-methyl-[1,3]dioxane (303)

A solution of 235 (111 mg, 0.300 mmol), Grubbs’ I catalyst (14 mg, 0.017 mmol) and ethyl vinyl ether (0.29 mL, 3.0 mmol) in 10 mL anhydrous toluene was heated to reflux for 24 hours. The solvent was removed in vacuo and the crude material was purified by flash chromatography (1% triethylamine treated silica, hexanes:ether 4:1, R$_f$ 0.36) to yield compound 303 as a colourless oil (111 mg, 0.300 mmol, quantitative; 5:1 geometric isomers).

IR (cm$^{-1}$, film) 1613, 1518, 1247, 1110, 909, 825, 732, 696

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.37 (d, J = 8.7 Hz, 2H, H-8), 7.26-7.35 (m, 5H, H-17 to H-19), 6.86 (d, J = 8.7 Hz, 2H, H-9), 5.86 (ddq, J = 15.5, 6.6, 0.7 (J Hz, 0.2H, H-13; minor isomer), 5.77 (ddq, J = 15.5, 6.6, 0.7 (J Hz, 0.8H, H-13; major isomer), 5.34-5.49 (m, 2H, H-6, H-12), 4.61 (d, J = 11.4 Hz, 1H, H-15), 4.30 (d, J = 11.4 Hz, 1H, H-15), 4.05 (dd, J = 11.3, 2.3 Hz, 1H, H-1), 3.99 (dd, J = 11.3, 1.6 Hz, 1H, H-1), 3.84 (dd, J = 9.0, 2.3 Hz, 1H, H-3), 3.78 (s, 3H, H-11), 3.74 (dd, J = 7.7, 7.5 Hz, 1H, H-4), 1.91-1.98 (m, 1H, H-2), 1.77 (dd, J = 6.6, 1.9 Hz,
2.5H, H-14; major isomer), 1.68 (dd, J = 6.6, 1.9 Hz, 0.5H, H-14; minor isomer), 1.14 (d, J = 7.0 Hz, 0.5H, H-5; minor isomer), 1.09 (d, J = 7.0 Hz, 2.5H, H-5; major isomer)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 160.0 (C-10), 138.6 (C-16), 131.7 (C-7), 127.4-131.1 (C-8, C-12, C-13, C-17 to C-19), 113.7 (C-9), 101.6 (C-6), 81.5 (C-3), 78.7 (C-4), 74.0 (C-15), 70.3 (C-1), 55.5 (C-11), 34.1 (C-2), 29.6 (C-14), 18.2 (C-5)


6.6.7 2S-Benzylxy-2R-[2R-(4-methoxy-phenyl)-5S-methyl-[1,3]dioxan-4-yl]-ethanol (304)

To 8 mL dioxane/water (3:1) at 0ºC was added 303 (93 mg, 0.252 mmol), osmium tetroxide (4% solution in water, 16 µL, 0.0025 mmol), sodium metaperiodate (222 mg, 1.04 mmol) and 2,6-lutidine (58 µL, 0.50 mmol). The mixture was warmed to 4 ºC for 23 hours, then quenched with 15 mL brine and extracted with 3 x 20 mL dichloromethane. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo.

Sodium borohydride (11 mg, 0.30 mmol) was then added to the crude aldehyde in 10 mL anhydrous tetrahydrofuran and stirred for an hour at room temperature. The mixture was quenched with methanol and the solvent removed in vacuo. The crude material was purified by flash chromatography (1% triethylamine treated silica, hexanes:ether 1:1, R$_f$ 0.15) to yield compound 304 as a white solid (57 mg, 0.159 mmol, 63.0 % over two steps).

Melting Point: 70 ºC

IR (cm$^{-1}$, film) 3469, 1615, 1518, 1249, 1107, 1028, 911, 830, 732, 699
\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.28-7.39 (m, 7H, H-8, H-17 to H-19), 6.86 (d, \(J = 8.7\) Hz, 2H, H-9), 5.47 (s, 1H, H-6), 4.71 (d, \(J = 11.4\) Hz, 1H, H-15), 4.53 (d, \(J = 11.4\) Hz, 1H, H-15), 4.09 (dd, \(J = 9.7, 2.5\) Hz, 1H, H-3), 4.07 (dd, \(J = 12.1, 2.2\) Hz, 1H, H-1), 4.01 (dd, \(J = 11.2, 1.1\) Hz, 1H, H-3), 3.86-3.91 (m, 1H, H-12), 3.79-3.83 (m, 1H, H-12), 3.78 (s, 3H, H-11), 3.54 (ddd, \(J = 9.3, 4.4, 2.6\) Hz, 1H, H-4), 2.00 (dd, \(J = 9.2, 2.4\) Hz, 1H, OH), 1.87-1.94 (m, 1H, H-2), 1.14 (d, \(J = 6.9\) Hz, 3H, H-5)

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 160.2 (C-10), 138.0 (C-16), 131.2 (C-7), 127.6-128.8 (C-8, C-17 to C-19), 113.9 (C-9), 102.0 (C-6), 79.1 (C-3), 77.5 (C-4), 74.1 (C-15), 72.2 (C-1), 61.0 (C-12), 55.5 (C-11), 29.3 (C-2), 11.7 (C-5)

\([\alpha]_{D}^{20}\) = +10.5° (c = 0.01150 g/mL; CHCl\(_3\))

\(\text{Ent-304} [\alpha]_{D}^{20} = -10.6\)° (c = 0.01155 g/mL; CHCl\(_3\))

HR-ESIMS calculated for [M+Na]\(^+\) C\(_{21}\)H\(_{26}\)O\(_5\)Na: 381.1678. Found 381.1672.

6.6.6 4S-Benzyløxy-3R-(4-methoxy-benzyløxy)-2S-methyl-hept-5-en-1-ol (309)

DIBAL-H (1.0 M in hexanes, 1.29 mL, 1.29 mmol) was added dropwise via syringe to a solution of 303 (95 mg, 0.26 mmol) in 5 mL anhydrous toluene at 0 °C. After 15 minutes, the mixture was quenched with 10 mL Rochelle’s salt and warmed to room temperature. The aqueous layer was extracted with 3 x 20 mL dichloromethane and the organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash chromatography (1% triethylamine treated silica, hexanes:ether 1:1, \(R_f\) 0.14) to yield compound 309 as a colourless oil (83 mg, 0.22 mmol, 86.3%).
IR (cm⁻¹, film) 3428, 1611, 1513, 1244, 970, 909, 822, 730, 696, 665

¹H NMR (500 MHz, CDCl₃) δ 7.24-7.34 (m, 5H, H-17 to H-19), 7.22 (d, J = 8.7 Hz, 2H, H-8), 6.84 (d, J = 8.7 Hz, 2H, H-9), 5.87 (dq, J = 15.5, 6.3 Hz, 0.2H, H-13; minor isomer), 5.76 (dq, J = 15.5, 6.3 Hz, 0.8H, H-13; major isomer), 5.52 (ddq, J = 15.5, 8.3, 1.2 Hz, 1H, H-12), 4.61 (d, J = 11.0 Hz, 1H, H-6), 4.59 (d, J = 11.0 Hz, 1H, H-15), 4.43 (d, J = 11.0 Hz, 1H, H-15), 4.30 (d, J = 11.0 Hz, 1H, H-6), 3.82 (dd, J = 8.2, 6.7 Hz, 1H, H-3), 3.78 (s, 3H, H-11), 3.63 (dd, J = 6.4, 3.4 Hz, 0.2H, H-4; minor isomer), 3.59 (dd, J = 6.4, 3.4 Hz, 0.8H, H-4; major isomer), 3.51-3.56 (m, 2H, H-1), 2.03-2.13 (m, 1H, H-2), 1.78 (dd, J = 6.6, 1.6 Hz, 3.4H, OH, H-14; major isomer), 1.67 (dd, J = 6.6, 1.6 Hz, 0.6H, H-14; minor isomer), 0.89 (d, J = 6.9 Hz, 0.5H, H-5; minor isomer), 0.86 (d, J = 6.9 Hz, 2.5H, H-5; major isomer)

¹³C NMR (125 MHz, CDCl₃) δ 159.4 (C-10), 138.8 (C-16), 131.2 (C-7), 127.7-130.0 (C-8, C-12, C-13, C-17 to C-19), 113.9 (C-9), 82.0 (C-3), 81.1 (C-4), 73.8 (C-15), 70.0 (C-6), 66.3 (C-1), 55.5 (C-11), 37.5 (C-2), 18.2 (C-14), 11.6 (C-5)


6.6.9 [4S-Benzyloxy-3R-(4-methoxy-benzyloxy)-2S-methyl-hept-5-enyloxy]-tert-butyl-dimethyl-silane (310)

A solution of 309 (70 mg, 0.19 mmol), dimethylaminopyridine (2 mg, 0.02 mmol), and triethylamine (6.7 µL, 0.48 mmol) in 5 mL anhydrous dichloromethane was added dropwise via syringe to a solution of tert-butyldimethylsilyl trifluoromethane sulfonate (89 mg, 0.40 mmol) in 5 mL anhydrous dichloromethane and heated to reflux for an hour. The mixture was quenched
with 10 mL brine and extracted with 3 x 20 mL dichloromethane. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash chromatography (silica, hexanes:ether 4:1, Rf 0.69) to yield compound 310 as a colourless oil (88 mg, 0.18 mmol, 95%).

Alternatively, a solution of 296 (30 mg, 0.062 mmol), Grubbs’ I catalyst (3 mg, 0.0031 mmol) and ethyl vinyl ether (59 µL, 0.62 mmol) in 10 mL anhydrous toluene was heated to reflux for 24 hours. The solvent was removed in vacuo and the crude material was purified by flash chromatography (silica, hexanes:ether 4:1, Rf 0.69) to yield compound 310 as a colourless oil (30 mg, 0.062 mmol, quantitative; 5:1 geometric isomers).

IR (cm\(^{-1}\), neat) 1611, 1586, 1513, 1247, 1169, 1088, 970, 934, 836, 774, 732, 693, 665
\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.24-7.34 (m, 5H, H-17 to H-19), 7.23 (d, J = 8.7 Hz, 2H, H-8), 6.83 (d, J = 8.7 Hz, 2H, H-9), 5.81 (dq, J = 15.4, 6.6 Hz, 0.2H, H-13; minor isomer), 5.72 (dq, J = 15.5, 6.6 Hz, 0.8H, H-13; major isomer), 5.48-5.57 (m, 1H, H-12), 4.62 (d, J = 10.4 Hz, 1H, H-6), 4.59 (d, J = 10.4 Hz, 1H, H-15), 4.45 (d, J = 10.4 Hz, 1H, H-15), 4.32 (d, J = 10.4 Hz, 1H, H-6), 3.81 (dd, J = 7.4, 5.9 Hz, 1H, H-3), 3.78 (s, 3H, H-11), 3.67 (dd, J = 6.0, 3.9 Hz, 0.2H, H-4; minor isomer), 3.62 (dd, J = 6.0, 3.9 Hz, 0.8H, H-4; major isomer), 3.50 (dd, J = 9.7, 7.6 Hz, 1H, H-1), 3.44 (dd, J = 9.6, 6.2 Hz, 1H, H-1), 1.96-2.06 (m, 1H, H-2), 1.76 (d, J = 6.4 Hz, 2.5H, H-14; major isomer), 1.64 (d, J = 6.4 Hz, 0.5H, H-14; minor isomer), 0.88 (s, 9H, H-22), 0.84 (d, J = 6.9 Hz, 0.5H, H-5; minor isomer), 0.82 (d, J = 6.9 Hz, 2.5H, H-5; major isomer), 0.02 (2 x s, 6H, H-20)

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 159.2 (C-10), 139.2 (C-16), 131.6 (C-7), 127.5-130.8 (C-8, C-12, C-13, C-17 to C-19), 113.8 (C-9), 81.3 (C-3), 81.0 (C-4), 74.2 (C-15), 69.9 (C-6), 65.8 (C-1), 55.5 (C-11), 37.5 (C-2), 26.2 (C-22), 18.5 (C-21), 18.2 (C-14), 11.5 (C-5), -5.1 (C-20), -5.2 (C-20)

HR-ESIMS calculated for [M+Na]\(^+\) \(\text{C}_{29}\text{H}_{44}\text{O}_{4}\text{SiNa}: 507.2901\). Found 507.2898.
6.6.10 2S-Benzyloxy-5-(tert-butyl-dimethyl-silanyloxy)-3R-(4-methoxy-benzyloxy)-4S-methyl-pentan-1-ol (311)

Ozone was bubbled through a solution of compound 310 (58 mg, 0.12 mmol) and 2,6-lutidine (15 μL, 0.13 mmol) in 20 mL anhydrous dichloromethane at –78 ºC till pale blue. The ozone was stopped and argon was bubbled through till the blue colour dissipated. Sodium borohydride (10 mg, 0.26 mmol) was then added and the solution was warmed to room temperature over 3 hours. The solvent was removed in vacuo and the crude material was purified by flash chromatography (silica, hexanes:ether 1:1, Rf 0.31) to yield compound 311 as a colourless oil (39 mg, 0.081 mmol, 67% over two steps).

IR (cm⁻¹, film) 3445, 1608, 1513, 1460, 1297, 1250, 1169, 1093, 1034, 937, 836, 774, 735

¹H NMR (500 MHz, CDCl₃) δ 7.27-7.35 (m, 5H, H-15 to H-17), 7.25 (d, J = 8.6 Hz, 2H, H-8), 6.85 (d, J = 8.6 Hz, 2H, H-9), 4.64 (d, J = 10.9 Hz, 1H, H-6), 4.61 (d, J = 10.9 Hz, 1H, H-13), 4.59 (d, J = 10.9 Hz, 1H, H-13), 4.53 (d, J = 10.9 Hz, 1H, H-6), 3.80-3.86 (m, 2H, H-3, H-12), 3.79-3.81 (m, 1H, H-12), 3.78 (s, 3H, H-11), 3.58 (dd, J = 10.5, 4.0 Hz, 1H, H-4), 3.51 (d, J = 6.9 Hz, 2H, H-1), 2.28 (t, 5.3 Hz, 1H, OH), 1.94-2.03 (m, 1H, H-2), 0.89 (s, 9H, H-20), 0.87 (d, J = 6.9 Hz, 3H, H-5), 0.03 (s, 3H, H-18), 0.02 (s, 3H, H-18)

¹³C NMR (125 MHz, CDCl₃) δ 159.2 (C-10), 138.4 (C-14), 131.0 (C-7), 128.0-129.7 (C-8, C-15 to C-17), 114.0 (C-9), 79.8 (C-3), 78.9 (C-4), 74.5 (C-13), 72.0 (C-6), 65.8 (C-1), 61.5 (C-12), 55.4 (C-11), 37.8 (C-2), 26.1 (C-20), 18.5 (C-19), 11.8 (C-5), −5.1 (C-18), −5.2 (C-18)

[α]D²⁰ = + 7.24º (c = 0.00899 g/mL; CHCl₃)

Ent-311 [α]D²⁰ = − 7.26º (c = 0.00895 g/mL; CHCl₃)
HR-ESIMS calculated for [M+Na]⁺ C_{27}H_{42}O_5SiNa: 497.2694. Found 497.2694.

6.6.11 Methanesulfonic acid 2S-benzyloxy-5-(tert-butyl-dimethyl-silanyloxy)-3R-(4-methoxy-benzyloxy)-4S-methyl-pentyl ester (314)

Mesyl chloride (4 μL, 0.04 mmol) and pyridine (5 μL, 0.06 mmol) were added sequentially to compound 311 (14 mg, 0.029 mmol) in 5 mL anhydrous dichloromethane and stirred for 19 hours. The solution was quenched with brine and the aqueous layer extracted with dichloromethane (3 x 10 mL). The combined organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 1:1, Rf 0.40) to yield compound 314 as a colourless oil (16 mg, 0.029 mmol, quantitative).

IR (cm⁻¹, neat) 1611, 1513, 1462, 1356, 1300, 1247, 1174, 1096, 1032, 833, 774, 744

¹H NMR (500 MHz, CDCl₃) δ 7.27-7.37 (m, 5H, H-15 to H-17), 7.25 (d, J = 8.6 Hz, 2H, H-8), 6.86 (d, J = 8.6 Hz, 2H, H-9), 4.69 (d, J = 11.3 Hz, 1H, H-6), 4.61 (d, J = 11.0 Hz, 1H, H-13), 4.58 (d, J = 11.0 Hz, 1H, H-13), 4.57 (m, 1H, H-12), 4.55 (d, J = 11.3 Hz, 1H, H-13), 4.36 (dd, J = 11.1, 5.2 Hz, 1H, H-12), 3.83 (dd, J = 11.0, 6.8 Hz, 1H, H-3), 3.78 (s, 3H, H-11), 3.76 (dt, J = 7.8, 5.8, Hz, 1H, H-4), 3.54 (dd, J = 6.2, 2.9 Hz, 2H, H-1), 2.94 (s, 3H, H-21), 1.95-2.04 (m, 1H, H-2), 0.90 (s, 9H, H-20), 0.85 (d, J = 6.9 Hz, 3H, H-5), 0.05 (s, 3H, H-18), 0.05 (s, 3H, H-18)

¹³C NMR (125 MHz, CDCl₃) δ 159.4 (C-10), 137.9 (C-14), 130.8 (C-7), 128.0-129.7 (C-8, C-15 to C-17), 114.0 (C-9), 78.2 (C-3), 77.6 (C-4), 74.4 (C-13), 72.6 (C-6), 69.8 (C-12), 65.4 (C-1), 54.6, 18.6, 18.4, 18.2, 18.1, 18.0, 17.9, 17.8, 17.7, 17.6, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 16.9, 16.8, 16.7, 16.6, 16.5, 16.4, 16.3, 16.2, 16.1, 16.0, 15.9, 15.8, 15.7, 15.6, 15.5, 15.4, 15.3, 15.2, 15.1, 15.0, 14.9, 14.8, 14.7, 14.6, 14.5, 14.4, 14.3, 14.2, 14.1, 14.0, 13.9, 13.8, 13.7, 13.6, 13.5, 13.4, 13.3, 13.2, 13.1, 13.0, 12.9, 12.8, 12.7, 12.6, 12.5, 12.4, 12.3, 12.2, 12.1, 12.0, 11.9, 11.8, 11.7, 11.6, 11.5, 11.4, 11.3, 11.2, 11.1, 11.0, 10.9, 10.8, 10.7, 10.6, 10.5, 10.4, 10.3, 10.2, 10.1, 10.0, 9.9, 9.8, 9.7, 9.6, 9.5, 9.4, 9.3, 9.2, 9.1, 9.0, 8.9, 8.8, 8.7, 8.6, 8.5, 8.4, 8.3, 8.2, 8.1, 8.0, 7.9, 7.8, 7.7, 7.6, 7.5, 7.4, 7.3, 7.2, 7.1, 7.0, 6.9, 6.8, 6.7, 6.6, 6.5, 6.4, 6.3, 6.2, 6.1, 6.0, 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, 5.1, 5.0, 4.9, 4.8, 4.7, 4.6, 4.5, 4.4, 4.3, 4.2, 4.1, 4.0, 3.9, 3.8, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2, 3.1, 3.0, 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3, 2.2, 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.0
55.4 (C-11), 37.7 (C-21), 37.5 (C-2), 26.1 (C-20), 18.4 (C-19), 11.8 (C-5), –5.2 (C-18), –5.2 (C-18)

$[\alpha]_D^{20} = -2.52^\circ$ (c = 0.0578 g/mL; CHCl$_3$)

**Ent-314** $[\alpha]_D^{20} = +2.54^\circ$ (c = 0.0575 g/mL; CHCl$_3$)

HR-ESIMS calculated for [M+Na]$^+$ C$_{28}$H$_{44}$O$_7$SSiNa: 575.2469. Found 575.2463.

6.6.12 Methanesulfonic acid 2S-benzyloxy-5-hydroxy-3R(4-methoxy-benzyloxy)-4S-methyl-pentyl ester (315)

Compound 314 (60 mg, 0.11 mmol) and p-toluenesulfonic acid (2 mg, 0.012 mmol) in 10 mL tetrahydrofuran:water (20:1) were heated to 40 °C for 24 hours. The solution was quenched with 10 mL brine and extracted with 3 x 10 mL dichloromethane. The organic fractions were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 1:2, R$_f$ 0.09) to yield compound 315 as a colourless oil (21 mg, 0.047 mmol, 49%).

IR (cm$^{-1}$, neat) 3439, 1611, 1510, 1457, 1351, 1297, 1244, 1172, 1090, 1029, 967, 822, 752, 696, 662

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.27-7.37 (m, 5H, H-15 to H-17), 7.25 (d, J = 8.6 Hz, 2H, H-8), 6.86 (d, J = 8.6 Hz, 2H, H-9), 4.70 (d, J = 11.0 Hz, 1H, H-6), 4.60 (dd, J = 11.1, 1.9 Hz, 1H, H-12), 4.59 (d, J = 10.6 Hz, 1H, H-13), 4.55 (d, J = 11.0 Hz, 1H, H-6), 4.53 (d, J = 10.6 Hz, 1H, H-13), 4.35 (dd, J = 11.1, 4.6 Hz, 1H, H-12), 3.78 (s, 3H, H-11), 3.73-3.78 (m, 2H, H-3, H-4),...
3.58 (dd, J = 10.8, 5.4 Hz, 1H, H-1), 3.53 (dd, J = 10.8, 7.8 Hz, 1H, H-1), 2.95 (s, 3H, H-18),
1.99-2.08 (m, 2H, H-2, OH), 0.86 (d, J = 6.9 Hz, 3H, H-5)
$^{13}$C NMR (125 MHz, CDCl$_3$) δ 159.6 (C-10), 137.6 (C-14), 130.4 (C-7), 128.3-129.9 (C-8, C-15
to C-17), 114.1 (C-9), 78.0 (C-3), 78.0 (C-4), 74.2 (C-13), 72.6 (C-6), 69.3 (C-12), 65.6 (C-1),
55.5 (C-11), 37.8 (C-18), 37.4 (C-2), 11.5 (C-5)
$[^{[a]}]_{D}^{20} = + 9.43^\circ$ (c = 0.00935 g/mL; CHCl$_3$)
Ent-315 $[^{[a]}]_{D}^{20} = - 9.47^\circ$ (c = 0.00940 g/mL; CHCl$_3$)
HR-ESIMS calculated for [M+Na]$^+$ C$_{22}$H$_{30}$O$_7$SNa: 461.1604. Found 461.1606.

6.6.13 4S-Benzylxy-3R-(4-methoxy-benzylxy)-2S-methyl-heptane-1,6-diol (318)

Diisobutylaluminum hydride (1.0 M in hexanes, 2.6 mL, 2.6 mmol) was added to
compound 291 (202 mg, 0.525 mmol) in 10 mL anhydrous toluene at 0 ºC. The solution was
stirred for 30 minutes and then quenched with 30 mL Rochelle’s salt and warmed to room
temperature. The mixture was stirred vigorously until the two phases became clear, followed by
extraction of the aqueous phase with 3 x 50 mL dichloromethane. The organic fractions were
combined, dried over anhydrous sodium sulphate and concentrated in vacuo. Purification by
flash-column chromatography (silica, ether, R$_f$ 0.35) yielded compound 318 (2:1 dr) as a
colourless oil (184 mg, 0.473 mmol, 90.2%).
IR (cm$^{-1}$, neat) 3389, 1611, 1513, 1454, 1370, 1300, 1247, 1169, 1090, 1034, 909, 822, 730, 693
$^1$H NMR (500 MHz, CDCl$_3$) δ 7.27-7.37 (m, 5H, H-17 to H-19), 7.26 (d, J = 7.5 Hz, 2H, H-8),
6.86 (d, J = 7.5 Hz, 2H, H-9), 4.76 (d, J = 10.9 Hz, 0.33H, H-6 minor), 4.74 (d,
J = 11.2 Hz, 0.33H, H-15 minor), 4.69 (d, J = 10.9 Hz, 0.66H, H-6 major), 4.65 (d, J = 11.2 Hz, 0.66H, H-15 major), 4.56 (d, J = 11.2 Hz, 0.66H, H-15 major), 4.51 (d, J = 11.2 Hz, 0.33H, H-15 minor), 4.50 (d, J = 10.9 Hz, 0.66H, H-6 major), 4.49 (d, J = 10.9 Hz, 0.33H, H-6 minor), 3.95-4.08 (m, 1H, H-3), 3.88-3.93 (m, 1H, H-4), 3.78 (s, 3H, H-11), 3.67-3.71 (m, 1H, H-13), 3.48-3.57 (m, 2H, H-1), 1.86-1.98 (m, 1H, H-12), 1.79-1.86 (m, 1H, H-2), 1.61-1.71 (m, 1H, H-12), 1.17 (d, J = 6.3 Hz, 2H, H-14 major), 1.15 (d, J = 6.3 Hz, 1H, H-14 minor), 1.01 (d, J = 6.8 Hz, 1H, H-5 minor), 0.97 (d, J = 6.8 Hz, 2H, H-5 major)

13C NMR (125 MHz, CDCl3) δ 159.4 (C-10), 138.4 (C-16 major), 138.0 (C-16 minor), 130.9 (C-7 major), 130.7 (C-7 minor), 128.1-129.8 (C-8, C-17 to C-19), 114.0 (C-9), 81.3 (C-3 minor), 80.9 (C-3 major), 80.7 (C-4 minor), 78.0 (C-4 major), 73.7 (C-15 major), 73.6 (C-15 minor), 72.3 (C-6 major), 72.1 (C-6 minor), 67.2 (C-13 minor), 65.9 (C-1), 65.3 (C-13 major), 55.5 (C-11), 39.4 (C-12 major), 39.3 (C-12 minor), 37.9 (C-2), 24.3 (C-14 major), 23.8 (C-14 minor), 13.2 (C-5 major), 13.0 (C-5 minor)


6.6.14 4S-Benzzyloxy-3R-(4-methoxy-benzylxy)-2R-methyl-6-oxo-heptanal (319)

Compound 318 (92 mg, 0.237 mmol) and Dess-Martin periodinane (304 mg, 0.716 mmol) were stirred in 10 mL anhydrous dichloromethane for 2 hours. The solvent was removed in vacuo to yield a white solid to which hexanes:ether 4:1 was added. The solid was filtered through a cotton plug with a small amount of celite and the resulting filtrate was
concentrated in vacuo to yield compound 319 as a yellow oil (88 mg, 0.229 mmol, 96.6%) with no further purification required.

IR (cm\(^{-1}\), neat) 1714, 1608, 1583, 1510, 1457, 1359, 1300, 1244, 1174, 1090, 1032, 738, 696

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 9.69 (d, \(J = 0.9\) Hz, 1H, H-1), 7.25-7.34 (m, 5H, H-17 to H-19), 7.18 (d, \(J = 8.7\) Hz, 2H, H-8), 6.84 (d, \(J = 8.7\) Hz, 2H, H-9), 4.58 (d, \(J = 10.9\) Hz, 1H, H-6), 4.52 (d, \(J = 11.1\) Hz, 1H, H-15), 4.42 (d, \(J = 10.9\) Hz, 1H, H-15), 4.38 (d, \(J = 11.1\) Hz, 1H, H-6), 4.04 (dd, \(J = 7.4, 6.1\) Hz, 1H, H-3), 3.94 (dd, \(J = 6.1, 3.7\) Hz, 1H, H-4), 3.78 (s, 3H, H-11), 2.73 (d, \(J = 6.4\) Hz, 1H, H-12), 2.72 (d, \(J = 4.9\) Hz, 1H, H-12), 1.65-1.71 (m, 1H, H-2), 2.09 (s, 3H, H-14), 1.11 (d, \(J = 7.4\) Hz, 3H, H-5)

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 207.1 (C-14), 203.8 (C-1), 159.5 (C-10), 138.0 (C-16), 130.0 (C-7), 127.7-129.8 (C-8, C-17 to C-19), 114.0 (C-9), 79.0 (C-3), 76.2 (C-4), 73.1 (C-15), 72.8 (C-6), 55.5 (C-11), 48.7 (C-2), 45.9 (C-12), 31.2 (C-14), 8.7 (C-5)

\([\alpha]_D^{20} = + 0.61^\circ\) (c = 0.003740 g/mL; CHCl\(_3\))

Ent-319 \([\alpha]_D^{20} = - 0.61^\circ\) (c = 0.03745 g/mL; CHCl\(_3\))

HR-ESIMS calculated for [M+Na]\(^+\) \(C_{23}H_{28}O_5Na\): 407.1829. Found 407.1833.

6.6.15 4S-Benzylxoy-1-(4-methoxy-benzyloxy)-2S-methyl-hept-6-en-3R-ol (335)

To a solution of 225 (122 mg, 0.331 mmol) and sodium cyanoborohydride (1.0 M in tetrahydrofuran, 0.40 mL, 0.40 mmol) in 3 mL anhydrous dimethylformamide at 0 °C was added dropwise via syringe a solution of trifluoroacetic acid (31 μL, 0.40 mmol) in 2 mL anhydrous
dimethylformamide. The resulting mixture was warmed to room temperature and continued to stir for 23 hours. The solution was quenched with 20 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 40 mL). The combined organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 1:1, Rf 0.48) to afford compound 335 as a colourless oil (83 mg, 0.23 mmol, 68%).

IR (cm⁻¹, film) 3478, 2920, 2863, 1636, 1608, 1582, 1514, 1457, 1360, 1302, 1244, 1173, 1090, 1032, 910, 817, 737, 694

¹H NMR (500 MHz, CDCl₃) δ 7.23-7.35 (m, 5H, H-17 to H-19), 7.22 (d, J = 8.1 Hz, 2H, H-8), 6.86 (d, J = 8.1 Hz, 2H, H-9), 5.90-5.99 (m, 1H, H-13), 5.15 (dd, J = 17.1, 1.4 Hz, 1H, H-14), 5.09 (dd, J = 10.5, 1.2 Hz, 1H, H-14), 4.63 (d, J = 11.3 Hz, 1H, H-6), 4.45 (d, J = 11.3 Hz, 1H, H-15), 4.44 (d, J = 11.3 Hz, 1H, H-15), 4.38 (d, J = 11.3 Hz, 1H, H-6), 3.79-3.81 (m, 1H, H-4), 3.79 (s, 3H, H-11), 3.52 (dd, J = 8.8, 3.8 Hz, 1H, H-1), 3.45-3.49 (m, 1H, H-3), 3.46 (dd, J = 8.8, 4.9 Hz, 1H, H-1), 2.80 (s, br, 1H, OH), 2.50-2.56 (m, 1H, H-12), 2.39-2.46 (m, 1H, H-12), 2.08-2.15 (m, 1H, H-2), 0.97 (d, J = 7.0 Hz, 3H, H-5)

¹³C NMR (125 MHz, CDCl₃) δ 159.4 (C-10), 138.6 (C-16), 135.2 (C-13), 130.3 (C-7), 127.8-129.4 (C-8, C-17 to C-19), 117.3 (C-14), 114.0 (C-9), 79.1 (C-4), 75.3 (C-1), 74.6 (C-3), 73.2 (C-15), 71.8 (C-6), 55.4 (C-11), 34.4 (C-12), 34.2 (C-2), 11.2 (C-5)

6.6.16 Acetic acid 2S-benzyloxy-1\text{R}-[2-(4-methoxy-benzyloxy)-1\text{S}-methyl-ethyl]-pent-4-enyl ester (337)

Acetic anhydride (11 µL, 0.12 mmol) was added via syringe to a solution of compound 335 (36 mg, 0.096 mmol), triethylamine (17 µL, 0.13 mmol) and dimethylaminopyridine (1 mg, 0.0098 mmol) in 10 mL anhydrous dichloromethane and stirred for 24 hours. The reaction was quenched with 10 mL saturated ammonium chloride and the aqueous layer was extracted with three aliquots of 20 mL dichloromethane. The organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 4:1, Rf 0.23) to yield compound 337 as a colourless oil (36 mg, 0.086 mmol, 89%).

IR (cm\(^{-1}\), film) 2920, 2863, 1733, 1636, 1611, 1579, 1511, 1453, 1367, 1298, 1241, 1173, 1090, 1029, 906, 820, 730, 698, 644

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.24-7.32 (m, 5H, H-17 to H-19), 7.21 (d, \(J = 8.7\) Hz, 2H, H-8), 6.84 (d, \(J = 8.7\) Hz, 2H, H-9), 5.83 (ddt, \(J = 17.2, 10.3, 7.0\) Hz, 1H, H-13), 5.21 (t, \(J = 5.3\) Hz, 1H, H-3), 5.04 (dd, \(J = 17.2, 1.7\) Hz, 1H, H-14), 5.02 (dd, \(J = 10.5, 1.7\) Hz, 1H, H-14), 4.56 (d, \(J = 11.3\) Hz, 1H, H-15), 4.49 (d, \(J = 11.3\) Hz, 1H, H-15), 4.36 (s, 2H, H-6), 3.78 (s, 3H, H-11), 3.61 (dt, \(J = 6.8, 5.1\) Hz, 1H, H-4), 3.29 (dd, \(J = 9.3, 6.4\) Hz, 1H, H-1), 3.25 (dd, \(J = 9.3, 5.7\) Hz, 1H, H-1), 2.29-2.34 (m, 2H, H-12), 2.19 (septet, \(J = 5.8\) Hz, 1H, H-2), 2.01 (s, 3H, H-21), 0.94 (d, \(J = 7.1\) Hz, 3H, H-5)
$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.6 (C-20), 159.3 (C-10), 138.5 (C-16), 135.1 (C-13), 130.6 (C-7), 127.8-129.5 (C-8, C-17 to C-19), 117.1 (C-14), 113.9 (C-9), 78.2 (C-3), 74.4 (C-4), 73.0 (C-1), 72.9 (C-15), 71.7 (C-6), 55.5 (C-11), 34.8 (C-12), 34.4 (C-2), 21.2 (C-21), 12.7 (C-5)


6.6.17 Acetic acid 2S-benzyloxy-1R-(2-hydroxy-1S-methyl-ethyl)-pent-4-enyl ester (338)

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (32 mg, 0.14 mmol) was added to a solution of compound 337 (48 mg, 0.12 mmol) in 10 mL anhydrous dichloromethane and stirred for 24 hours. The solution was quenched with 10 mL brine and extracted with 3 x 15 mL dichloromethane. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 1:1, R$_f$ 0.19) to yield compound 338 as a colourless oil (32 mg, 0.11 mmol, 91%).

IR (cm$^{-1}$, film) 3456, 3064, 3025, 2963, 2924, 2873, 1734, 1639, 1454, 1434, 1370, 1239, 1074, 1023, 911, 741, 696

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.24-7.35 (m, 5H, H-11 to H-13), 5.85 (ddt, J = 17.3, 9.8, 7.2 Hz, 1H, H-7), 5.04-5.13 (m, 3H, H-3, H-8), 4.63 (d, J = 11.3 Hz, 1H, H-9), 4.50 (d, J = 11.3 Hz, 1H, H-9), 3.66 (dt, J = 7.8, 5.1 Hz, 1H, H-4), 3.41-3.48 (m, 1H, H-1), 3.2 (td, J = 10.0, 2.5 Hz, 1H, H-1), 2.58-2.64 (m, 1H, OH), 2.41-2.49 (m, 1H, H-6), 2.26-2.34 (m, 1H, H-6), 2.17-2.26 (m, 1H, H-2), 2.07 (s, 3H, H-15), 0.77 (d, J = 6.8 Hz, 3H, H-5)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 171.9 (C-14), 138.2 (C-10), 134.1 (C-7), 128.0-128.6 (C-11 to C-13), 117.8 (C-8), 77.0 (C-3), 73.6 (C-4), 71.8 (C-9), 64.7 (C-1), 36.0 (C-2), 35.2 (C-6), 21.2 (C-15), 10.5 (C-5)

6.6.18 3-Bromo-1-[2R-(4-methoxy-phenyl)-5S-methyl-[1,3]dioxan-4R-yl]-but-3-en-1S-ol (347)

Compound 346 (44 mg, 0.18 mmol) was added to a solution of crude compound 182 (derived from compound 98) in 10 mL anhydrous dichloromethane at –78 ºC. The mixture was warmed gradually to room temperature over 23 hours then quenched with 10 mL brine. The aqueous layer was extracted with 3 x 15 mL dichloromethane. The organic fractions were combined, dried over sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (1% triethylamine treated silica, hexanes:ether 1:1, Rf 0.2) to yield compound 347 and 348 as a colourless oil (5:1 dr, 29 mg, 0.080 mmol, 54% over two steps).

IR (cm⁻¹, film) 3445, 3045, 2358, 2341, 1630, 1615, 1519, 1464, 1303, 1299, 1164, 1106, 1078, 1031, 996, 891, 830, 666

¹H NMR (500 MHz, CDCl₃) δ 7.39 (d, J = 8.7 Hz, 2H, H-8), 6.88 (d, J = 8.7 Hz, 2H, H-9), 5.71 (t, J = 1.5 Hz, 1H, H-14), 5.57 (d, J = 1.5 Hz, 1H, H-14), 5.43 (s, 1H, H-6), 4.07 (dd, J = 11.1, 2.3 Hz, 1H, H-1), 4.03 (dd, J = 11.1, 1.3 Hz, 1H, H-1), 3.95 (td, J = 8.8, 2.0 Hz, 1H, H-4), 3.79 (s, 3H, H-11), 3.75 (dd, J = 8.8, 2.3 Hz, 1H, H-3), 2.96 (dt, J = 14.5, 1.5 Hz, 1H, H-12), 2.50 (dd, J = 14.5, 9.2 Hz, 1H, H-12), 1.90-1.95 (m, 1H, H-2), 1.82-1.90 (m, 1H, OH), 1.25 (d, J = 6.9 Hz, 3H, H-5)

¹³C NMR (125 MHz, CDCl₃) δ 160.2 (C-10), 131.3 (C-7), 130.4 (C-13), 127.5 (C-8), 120.5 (C-14), 113.8 (C-9), 101.8 (C-6), 81.4 (C-3), 74.0 (C-1), 68.4 (C-4), 55.5 (C-11), 46.1 (C-12), 29.4 (C-2), 11.7 (C-5)

[α]D²⁰ = − 0.86º (c = 0.00700 g/mL; CHCl₃)

Ent-347 [α]D²⁰ = + 0.90º (c = 0.00725 g/mL; CHCl₃)

6.6.19 3-Methyl-butyric acid 3-bromo-1S-[2R-(4-methoxy-phenyl)-5S-methyl-[1,3]dioxan-4R-yl]-but-3-enyl ester (349)

Isovaleric anhydride (51 µL, 0.26 mmol) was added via syringe to a solution of compound 347/348 (77 mg, 0.22 mmol), triethylamine (39 µL, 0.28 mmol) and dimethylaminopyridine (4 mg, 0.03 mmol) in 10 mL anhydrous dichloromethane and stirred for 24 hours. The reaction was quenched with 10 mL saturated ammonium chloride and the aqueous layer was extracted with three aliquots of 20 mL dichloromethane. The organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 4:1, Rf 0.42) to yield compound 349 as a colourless oil (68 mg, 0.15 mmol, 71%).

IR (cm⁻¹, film) 3054, 1735, 1616, 1589, 1518, 1466, 1392, 1249, 1165, 1115, 1058, 1034, 895, 830, 738, 704, 666

¹H NMR (500 MHz, CDCl₃) δ 7.38 (d, J = 8.8 Hz, 2H, H-8), 6.88 (d, J = 8.8 Hz, 2H, H-9), 5.60 (t, J = 1.4 Hz, 1H, H-14), 5.50 (d, J = 1.4 Hz, 1H, H-14), 5.43 (s, 1H, H-6), 5.19 (ddd, J = 9.7, 6.7, 3.2 Hz, 1H, H-4), 4.06 (dd, J = 11.5, 2.4 Hz, 1H, H-1), 4.02 (dd, J = 9.0, 2.2 Hz, 1H, H-3), 4.00 (dd, J = 11.5, 1.4 Hz, 1H, H-1), 3.79 (s, 3H, H-11), 2.95 (ddd, J = 15.4, 3.0, 0.9 Hz, 1H, H-12), 2.76 (dd, J = 15.5, 7.5 Hz, 1H, H-12), 2.19 (d, J = 7.3 Hz, 2H, H-16), 2.10 (septet, J = 6.7 Hz, 1H, H-17), 1.66-1.73 (m, 1H, H-2), 1.19 (d, J = 7.0 Hz, 3H, H-5), 0.94 (d, J = 6.7 Hz, 6H, H-18)
$^{13}$C NMR (125 MHz, CDCl$_3$) δ 172.1 (C-15), 160.2 (C-10), 131.1 (C-7), 128.8 (C-13), 127.5 (C-8), 120.4 (C-14), 113.8 (C-9), 102.0 (C-6), 79.2 (C-3), 73.8 (C-1), 70.0 (C-4), 55.5 (C-11), 43.7 (C-16), 42.6 (C-12), 29.5 (C-2), 25.8 (C-17), 22.6 (C-18), 11.6 (C-5)

$[\alpha]_D^{20} = + 5.86^\circ$ (c = 0.00990 g/mL; CHCl$_3$)

Ent-349 $[\alpha]_D^{20} = -5.80^\circ$ (c = 0.0100 g/mL; CHCl$_3$)


Trifluoroacetic acid (0.21 mL, 2.7 mmol) was added dropwise via syringe to a solution of compound 349 (399 mg, 0.904 mmol) and sodium cyanoborohydride (1.0 M in tetrahydrofuran; 2.71 mL, 2.71 mmol) in 10 mL anhydrous dimethylformamide at 0 ºC. The mixture was warmed gradually to room temperature over 21 hours and then quenched with 20 mL saturated sodium bicarbonate. The aqueous layer was extracted with 3 x 50 mL dichloromethane and the combined organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 1:1, R$_f$ 0.48) to yield compound 350 as a colourless oil (292 mg, 0.657 mmol, 72.7%).

IR (cm$^{-1}$, film) 3482, 1733, 1615, 1511, 1460, 1360, 1295, 1244, 1180, 1169, 1093, 1032, 1000, 892, 820

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.22 (d, J = 8.6 Hz, 2H, H-8), 6.86 (d, J = 8.6 Hz, 2H, H-9), 5.60 (s, 1H, H-14), 5.44 (d, J = 1.2 Hz, 1H, H-14), 5.19 (ddd, J = 9.0, 7.2, 2.6 Hz, 1H, H-4), 4.44 (d, J = 11.9 Hz, 1H, H-6), 4.41 (d, J = 11.9 Hz, 1H, H-6), 3.79-3.83 (m, 1H, H-3), 3.78 (s, 3H,
H-11), 3.52 (dd, J = 9.3, 4.0 Hz, 1H, H-1), 3.47 (dd, J = 9.1, 5.5 Hz, 1H, H-1), 2.90 (dd, J = 14.9, 2.0, Hz, 1H, H-12), 2.85 (d, J = 3.1 Hz, OH), 2.71 (dd, J = 15.0, 9.2 Hz, 1H, H-12), 2.15 (d, J = 7.5 Hz, 1H, H-16), 2.15 (d, J = 6.8 Hz, 1H, H-16), 2.07 (octet, J = 6.7 Hz, 1H, H-17), 1.81-1.88 (m, 1H, H-2), 0.99 (d, J = 7.1 Hz, 3H, H-5), 0.92 (d, J = 6.7 Hz, 6H, H-18)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 172.3 (C-15), 159.5 (C-10), 130.1 (C-7), 129.9 (C-8), 129.5 (C-13), 119.7 (C-14), 114.1 (C-9), 74.9 (C-4), 74.8 (C-1), 73.4 (C-6), 71.8 (C-3), 55.5 (C-11), 43.7 (C-16), 43.0 (C-12), 34.9 (C-2), 25.8 (C-17), 22.6 (C-18), 11.1 (C-5)

$[^{[a]}]_D^{20} = -3.33^\circ$ (c = 0.00420 g/mL; CHCl$_3$)  

Ent-350 $[^{[a]}]_D^{20} = +3.35^\circ$ (c = 0.00415 g/mL; CHCl$_3$)  


6.6.21 3-Methyl-butyric acid 1S-[1R-acetoxy-3-(4-methoxy-benzyl)-2S-methyl-propyl]-3-bromo-but-3-enyl ester (351)

Acetic anhydride (1.5 µL, 0.016 mmol) was added via syringe to a solution of compound 350 (7 mg, 0.015 mmol), triethylamine (2.4 µL, 0.018 mmol) and dimethylaminopyridine (0.2 mg, 0.001 mmol) in 5 mL anhydrous dichloromethane and stirred for 24 hours. The reaction was quenched with 5 mL saturated ammonium chloride and the aqueous layer was extracted with three aliquots of 10 mL dichloromethane. The organic fractions were dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 4:1, R$_f$ 0.22) to yield compound 351 as a colourless oil (7 mg, 0.015 mmol, quantitative).
IR (cm⁻¹, film) 1739, 1627, 1611, 1510, 1462, 1365, 1292, 1241, 1233, 1177, 1113, 1090, 1034, 970, 889, 819, 755

¹H NMR (500 MHz, CDCl₃) δ 7.25 (d, J = 8.5 Hz, 2H, H-8), 6.85 (d, J = 8.5 Hz, 2H, H-9), 5.57 (s, 1H, H-14), 5.45 (ddd, J = 9.3, 5.1, 3.6 Hz, 1H, H-4), 5.41 (d, J = 1.5 Hz, 1H, H-14), 5.15 (t, J = 5.4 Hz, 1H, H-3), 4.42 (d, J = 11.7 Hz, 1H, H-6), 4.39 (d, J = 11.7 Hz, 1H, H-6), 3.79 (s, 3H, H-11), 3.30 (dd, J = 9.4, 7.1 Hz, 1H, H-1), 3.28 (dd, J = 9.2, 5.7 Hz, 1H, H-1), 2.62-2.74 (m, 2H, H-12), 2.13 (d, J = 7.0 Hz, 2H, H-16), 1.96-2.10 (m, 2H, H-2, H-17), 2.03 (s, 3H, H-20), 0.92 (d, J = 6.4 Hz, 3H, H-5), 0.92 (d, J = 6.7 Hz, 6H, H-18)

¹³C NMR (125 MHz, CDCl₃) δ 172.3 (C-15), 170.5 (C-19), 159.4 (C-10), 130.4 (C-7), 129.7 (C-8), 129.4 (C-13), 119.6 (C-14), 113.9 (C-9), 74.6 (C-3), 73.2 (C-6), 72.2 (C-1), 70.4 (C-4), 55.5 (C-11), 43.6 (C-16), 42.0 (C-12), 34.9 (C-2), 25.7 (C-17), 22.6 (C-18), 21.1 (C-20), 12.8 (C-5)

[α]D²⁰ = + 7.03° (c = 0.00370 g/mL; CHCl₃)

Ent-351 [α]D²⁰ = − 6.99° (c = 0.00370 g/mL; CHCl₃)


6.6.22 3-Methyl-butyric acid 1S-(1R-acetoxy-3-hydroxy-2S-methyl-propyl)-3-bromo-but-3-enyl ester (352)

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (195 mg, 0.860 mmol) was added to a solution of compound 351 (344 mg, 0.709 mmol) in 200 mL anhydrous dichloromethane and stirred for 24 hours. The solution was quenched with 100 mL brine and extracted with 3 x 150 mL dichloromethane. The organic layers were combined, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude material was purified by flash-column chromatography (silica, hexanes:ether 1:1, Rf 0.22) to yield compound 352 as a colourless oil (239 mg, 0.653 mmol, 92.1%).
IR (cm\(^{-1}\), film) 3464, 1742, 1632, 1467, 1370, 1294, 1237, 1186, 1167, 1118, 1094, 1030, 972, 894, 757, 666

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 5.62 (s, 1H, H-8), 5.46 (d, \(J = 1.6\) Hz, 1H, H-8), 5.42 (ddd, \(J = 8.0, 6.5, 4.5\) Hz, 1H, H-4), 5.16 (dd, \(J = 6.4, 4.3\) Hz, 1H, H-3), 3.50-3.57 (m, 1H, H-1), 3.30 (ddd, \(J = 12.6, 7.7, 4.2\) Hz, 1H, H-1), 2.71 (dd, \(J = 14.8, 7.8\) Hz, 1H, H-6), 2.67 (dd, \(J = 14.8, 4.5\) Hz, 1H, H-6), 2.45 (t, \(J = 6.1\) Hz, 1H, OH), 2.17 (d, \(J = 7.3\) Hz, 2H, H-10), 2.10 (s, 3H, H-14), 2.06 (septet, \(J = 6.8\) Hz, 1H, H-11), 1.89-1.98 (m, 1H, H-2), 0.93 (d, \(J = 6.7\) Hz, 3H, H-12), 0.92 (d, \(J = 6.7\) Hz, 3H, H-12), 0.86 (d, \(J = 6.9\) Hz, 3H, H-5)

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 172.4 (C-9), 171.5 (C-13), 128.9 (C-7), 120.1 (C-8), 73.6 (C-3), 69.7 (C-4), 64.5 (C-1), 43.5 (C-10), 43.0 (C-6), 36.5 (C-2), 25.8 (C-11), 22.6 (C-12), 21.1 (C-14), 11.1 (C-5)

\([\alpha]_D^{20} = + 2.55^\circ (c = 0.00510 \text{ g/mL; CHCl}_3)\)

\textbf{Ent-352} \([\alpha]_D^{20} = - 2.63^\circ (c = 0.00530 \text{ g/mL; CHCl}_3)\)

HR-ESIMS calculated for [M+Na]\(^{+}\) \(C_{15}H_{25}BrO_{5}Na\): 387.0777. Found 387.0779.
References


Wang, L.-Q.; Falany, C. N.; James, M. O. *Drug Metab. Dispos.* **2004**, *32*, 1162-1169.


(59) James, M. O.; Li, W.; Summerlot, D. P.; Rowland-Faux, L.; Wood, C. E. Environ Int 2010, 36, 942-949.


(68) Influenza. WHO Fact Sheet No. 211.


(73) Bouvier, N. M.; Palese, P. Vaccine 2008, 26, Supplement 4, D49-D53.

(74) Cros, J. F.; Palese, P. Virus Res. 2003, 95, 3-12.


(167) Protein Data Bank structure 2HTU can be accessed at www.pdb.org.


(250) Nicholls-Allison, E., Thanks Emma for helping me get in touch with my inner inorganic chemist and for making the TiCp*Cl3.
(290) Thanks to the Hall group for supplying me with 40 mg of Vivol-F. Much appreciated.
Appendix A. $^1$H and $^{13}$C NMR for Triclosan Compounds

1. $^1$H NMR for Compound 1 in CDCl$_3$

2. $^{13}$C NMR for Compound 2 in CDCl$_3$
3. $^1$H NMR for Compound 2 in CDCl$_3$

![H NMR Image]

4. $^{13}$C NMR for Compound 2 in CDCl$_3$

![C NMR Image]
5. $^1$H NMR for Compound 3 in CDCl$_3$

![H NMR spectrum of Compound 3 in CDCl$_3$]

6. $^{13}$C NMR for Compound 3 in CDCl$_3$

![C NMR spectrum of Compound 3 in CDCl$_3$]
7. $^1$H NMR for Compound 4 in CDCl$_3$

8. $^{13}$C NMR for Compound 4 in CDCl$_3$
9. $^1$H NMR for Compound 5 in CDCl$_3$

10. $^{13}$C NMR for Compound 5 in CDCl$_3$
11. $^1$H NMR for Compound 6 in CDCl$_3$

![H NMR spectrum for Compound 6 in CDCl$_3$]

12. $^{13}$C NMR for Compound 6 in CDCl$_3$

![C NMR spectrum for Compound 6 in CDCl$_3$]
13. $^1$H NMR for Compound 7 in CDCl$_3$

14. $^{13}$C NMR for Compound 7 in CDCl$_3$
15. $^1$H NMR for Compound 8 in (CD$_3$)$_2$SO

16. $^{13}$C NMR for Compound 8 in (CD$_3$)$_2$SO
17. $^1$H NMR for Compound 9 in CD$_3$OD

18. $^{13}$C NMR for Compound 9 in CD$_3$OD
19. $^1$H NMR for Compound 10 in CDCl$_3$

![H NMR spectrum of Compound 10 in CDCl$_3$]

20. $^{13}$C NMR for Compound 10 in CDCl$_3$

![C NMR spectrum of Compound 10 in CDCl$_3$]
21. $^1$H NMR for Compound 11 in CDCl$_3$

![NMR spectrum](image1)

22. $^1$H NMR for Compound 11 in CDCl$_3$

![NMR spectrum](image2)
23. \textsuperscript{1}H NMR for Compound 12 in CDCl\textsubscript{3}

![1H NMR spectrum of Compound 12 in CDCl3]

24. \textsuperscript{13}C NMR for Compound 12 in CDCl\textsubscript{3}

![13C NMR spectrum of Compound 12 in CDCl3]
25. $^1$H NMR for Compound 13 in CDCl$_3$

![1H NMR spectrum for Compound 13 in CDCl$_3$](image)

26. $^{13}$C for Compound 13 in CDCl$_3$

![13C NMR spectrum for Compound 13 in CDCl$_3$](image)
27. $^1$H NMR for Compound 14 in CDCl$_3$

28. $^{13}$C NMR for Compound 14 in CDCl$_3$
29. $^1$H NMR for Compound 15 in CDCl$_3$

30. $^{13}$C NMR for Compound 15 in CDCl$_3$
31. $^1$H NMR for Compound 127 in CDCl$_3$

32. $^{13}$C NMR for Compound 127 in CDCl$_3$
33. $^1$H NMR for Compound 16 in CDCl$_3$

34. $^{13}$C NMR for Compound 16 in CDCl$_3$
35. \(^1\)H NMR for Compound 17 in CDCl\(_3\)

36. \(^{13}\)C NMR for Compound 17 in CDCl\(_3\)
37. $^1$H NMR for Compound 130 in CD$_3$OD

38. $^{13}$C NMR for Compound 130 in CD$_3$OD
39. $^1$H NMR for Compound 18 in CD$_3$OD

40. $^{13}$C NMR for Compound 18 in CD$_3$OD
41. $^1$H NMR for Compound 19 in CD$_3$OD

42. $^{13}$C NMR for Compound 19 in CD$_3$OD
43. $^1$H NMR for Compound 20 in CD$_3$OD

![H NMR spectrum](image1)

44. $^{13}$C NMR for Compound 20 in CD$_3$OD

![C NMR spectrum](image2)
Appendix B. $^1$H and $^{13}$C NMR for De-Guanidinylated Peramivir Analogue

1. $^1$H NMR for Compound 28 in D$_2$O
Appendix C. $^1$H and $^{13}$C NMR for Neuraminidase Analogue Compounds

1. $^1$H NMR for Compound 146 in CDCl$_3$

2. $^{13}$C NMR for Compound 146 in CDCl$_3$
3. $^1$H NMR for Compound 147 in D$_2$O

4. $^{13}$C NMR for Compound 147 in D$_2$O
5. $^1H$ NMR for Compound 157 in CDCl$_3$

6. $^{13}C$ NMR for Compound 157 in CDCl$_3$
7. $^1$H NMR for Compound 158 in CDCl$_3$

8. $^{13}$C NMR for Compound 158 in CDCl$_3$
9. $^1$H NMR for Compound 159 in D$_2$O

10. $^{13}$C NMR for Compound 159 in D$_2$O
11. $^1$H NMR for Compound 160 in CDCl$_3$

![1H NMR Spectrum](image1)

12. $^{13}$C NMR for Compound 160 in CDCl$_3$

![13C NMR Spectrum](image2)
13. $^1$H NMR for Compound 161 in D$_2$O
14. $^1$H NMR for Compound 169 in CDCl$_3$

15. $^{13}$C NMR for Compound 169 in CDCl$_3$
16. $^1$H NMR for Compound 164 in CDCl$_3$

17. $^{13}$C NMR for Compound 164 in CDCl$_3$
18. $^1$H NMR for Compound 170 in CDCl$_3$

19. $^{13}$C NMR for Compound 170 in CDCl$_3$
20. $^1$H NMR for Compound 165 in CDCl$_3$

21. $^{13}$C NMR for Compound 165 in CDCl$_3$
22. $^1$H NMR for Compound 166 in CDCl$_3$

![H NMR spectrum](image1.png)

23. $^{13}$C NMR for Compound 166 in CDCl$_3$

![C NMR spectrum](image2.png)
24. $^1$H NMR for Compound 174 in CDCl$_3$

25. $^{13}$C NMR for Compound 174 in CDCl$_3$
26. $^1$H NMR for Compound 175 in CD$_3$OD

![H NMR spectrum for Compound 175 in CD$_3$OD](image)

27. $^{13}$C NMR for Compound 175 in CD$_3$OD

![C NMR spectrum for Compound 175 in CD$_3$OD](image)
Appendix D. $^1$H and $^{13}$C NMR for Didemnaketal A C-6 Methyl Analogue Compounds

1. $^1$H NMR for Compound ent-189 in CDCl$_3$

2. $^{13}$C NMR for Compound ent-189 in CDCl$_3$
3. COSY for Compound ent-189 in CDCl₃

4. NOESY for Compound ent-189 in CDCl₃
5. $^1$H NMR for Compound 209 in CDCl$_3$

6. $^{13}$C NMR for Compound 209 in CDCl$_3$
7. $^1$H NMR for Compound 210 in CDCl$_3$

8. $^{13}$C NMR for Compound 210 in CDCl$_3$
9. $^1H$ NMR for Compound 97 in CDCl$_3$

![H NMR spectrum for Compound 97 in CDCl$_3$]

10. $^{13}C$ NMR for Compound 97 in CDCl$_3$

![C NMR spectrum for Compound 97 in CDCl$_3$]
11. $^1$H NMR for Compound 194 in CDCl$_3$

12. $^{13}$C NMR for Compound 194 in CDCl$_3$
13. $^1$H NMR for Compound 98 in CDCl$_3$

14. $^{13}$C NMR for Compound 98 in CDCl$_3$
15. COSY for Compound 98 in CDCl₃

16. NOESY for Compound 98 in CDCl₃
17. $^1$H NMR for Compound 182 in CDCl$_3$

18. $^{13}$C NMR for Compound 182 in CDCl$_3$
19. $^1$H NMR for Compound 225 in CDCl$_3$

20. $^{13}$C NMR for Compound 225 in CDCl$_3$
21. $^1$H NMR for Compound ent-221 in D$_2$O

22. $^1$H NMR for Compound ent-222 in CDCl$_3$
23. $^1$H NMR for Compound ent-224 in CDCl$_3$

24. NOESY for Compound ent-224 in CDCl$_3$
25. $^1$H NMR for Compound 235 in CDCl$_3$ 

![H NMR spectrum for Compound 235](image)

26. $^{13}$C NMR for Compound 235 in CDCl$_3$ 

![C NMR spectrum for Compound 235](image)
27. $^1$H NMR for Compound 236 in CDCl$_3$

28. $^{13}$C NMR for Compound 236 in CDCl$_3$
29. $^1$H NMR for Compound 237 in CDCl$_3$

30. $^{13}$C NMR for Compound 237 in CDCl$_3$
31. $^1$H NMR for Compound 243 in CDCl$_3$

32. $^{13}$C NMR for Compound 243 in CDCl$_3$
33. $^1$H NMR for Compound ent-245 in CDCl$_3$

34. $^1$H NMR for Compound ent-246 in CDCl$_3$
35. $^1$H NMR for Compound ent-247 in CDCl$_3$

36. $^{13}$C NMR for Compound ent-247 in CDCl$_3$
37. $^1$H NMR for Compound ent-248 in CDCl$_3$

![H NMR spectrum](image)

38. $^{13}$C NMR for Compound ent-248 in CDCl$_3$

![C NMR spectrum](image)
39. \(^1\)H NMR for Compound ent-249 in CDCl\(_3\)
40. $^1$H NMR for Compound ent-250 in CDCl$_3$

42. $^1$H NMR for Compound ent-251 in CDCl$_3$

44. $^1$H NMR for Compound ent-253 in CDCl$_3$

45. $^1$H NMR for Compound ent-254 in CDCl$_3$
46. $^1H$ NMR for Compound ent-255 in CDCl$_3$

![H NMR spectrum of Compound ent-255 in CDCl$_3$](image)

47. $^1H$ NMR for Compound ent-257 in CDCl$_3$

![H NMR spectrum of Compound ent-257 in CDCl$_3$](image)
48. $^1$H NMR for Compound ent-258 in CDCl$_3$

49. $^1$H for Compound ent-256 in CDCl$_3$
50. $^1$H NMR for Compound 226 in CDCl$_3$

51. $^{13}$C NMR for Compound 226 in CDCl$_3$
52. $^{1}\text{H} \text{NMR for Compound 227 in CDCl}_3$

53. $^{13}\text{C} \text{NMR for Compound 227 in CDCl}_3$
54. $^1$H NMR for Compound 228 in CDCl$_3$

55. $^{13}$C NMR for Compound 228 in CDCl$_3$
56. $^1$H NMR for Compound ent-230 in CDCl$_3$

57. $^{13}$C NMR for Compound ent-230 in CDCl$_3$
58. $^1$H NMR for Compound ent-231 in CDCl$_3$

![H NMR spectrum](image1)

69. $^{13}$C NMR for Compound ent-231 in CDCl$_3$

![C NMR spectrum](image2)
70. $^1$H NMR for Compound 260 in CDCl$_3$

71. $^{13}$C NMR for Compound 260 in CDCl$_3$
72. $^1$H NMR for Compound 261 in CDCl$_3$

73. $^{13}$C NMR for compound 261 in CDCl$_3$
74. $^1$H NMR for Compound 232 in CDCl$_3$

75. $^{13}$C NMR for Compound 232 in CDCl$_3$
76. $^1$H NMR for Compound **265** in CDCl$_3$

![H NMR spectrum for Compound 265 in CDCl$_3$](image)

77. $^{13}$C NMR for Compound **265** in CDCl$_3$

![C NMR spectrum for Compound 265 in CDCl$_3$](image)
78. $^1$H NMR for Compound 270 in CDCl$_3$

79. $^{13}$C NMR for Compound 270 in CDCl$_3$
80. $^1$H NMR for Compound 271 in CDCl$_3$

81. $^{13}$C NMR for Compound 271 in CDCl$_3$
82. $^1$H NMR for Compound 272 in CDCl$_3$

83. $^{13}$C NMR for Compound 272 in CDCl$_3$
84. $^1$H NMR for Compound 273 in CDCl$_3$

85. $^{13}$C NMR for Compound 273 in CDCl$_3$
86. $^1$H NMR for Compound 274 in CDCl$_3$

87. $^{13}$C NMR for Compound 274 in CDCl$_3$
88. $^1$H NMR for Compound 266 in CDCl$_3$

89. $^{13}$C NMR for Compound 266 in CDCl$_3$
90. $^1$H NMR for Compound 275 in CDCl$_3$

![H NMR spectrum](image)

91. $^{13}$C NMR for Compound 275 in CDCl$_3$

![C NMR spectrum](image)
92. $^1$H NMR for Compound 276 in CDCl$_3$

93. $^{13}$C NMR for Compound 276 in CDCl$_3$
94. $^1$H NMR for Compound 267 in CDCl$_3$

95. $^{13}$C NMR for Compound 267 in CDCl$_3$
96. $^1$H NMR for Compound 268 in CDCl$_3$

97. $^{13}$C NMR for Compound 268 in CDCl$_3$
Appendix E. $^1$H and $^{13}$C NMR for Didemnaketal A C-6/C-10 Methyl Analogue and Natural Product Compounds

1. $^1$H NMR for Compound 291 in CDCl$_3$

[Chemical structure image]

2. $^{13}$C NMR for Compound 291 in CDCl$_3$

[Chemical structure image]
3. $^1$H NMR for Compound 295 in CDCl$_3$

4. $^{13}$C NMR for Compound 295 in CDCl$_3$
5. $^1$H NMR for Compound 296 in CDCl$_3$

6. $^{13}$C NMR for Compound 296 in CDCl$_3$
7. $^1$H NMR for Compound ent-297 in CDCl$_3$

8. $^{13}$C NMR for Compound ent-297 in CDCl$_3$
9. $^1$H NMR for Compound ent-298 in CDCl₃

10. $^{13}$C NMR for Compound ent-298 in CDCl₃
11. $^1$H NMR for Compound 303 in CDCl$_3$

12. $^{13}$C NMR for Compound 303 in CDCl$_3$
13. $^1$H NMR for Compound 304 in CDCl$_3$

14. $^{13}$C NMR for Compound 304 in CDCl$_3$
15. $^1$H NMR for Compound 309 in CDCl$_3$

16. $^{13}$C NMR for Compound 309 in CDCl$_3$
17. $^1$H NMR for Compound 310 in CDCl$_3$

![H NMR spectrum of Compound 310 in CDCl$_3$](image)

18. $^{13}$C NMR for Compound 310 in CDCl$_3$

![C NMR spectrum of Compound 310 in CDCl$_3$](image)
19. $^1$H NMR for Compound 311 in CDCl$_3$

20. $^{13}$C NMR for Compound 311 in CDCl$_3$
21. $^1\text{H}$ NMR for Compound 314 in CDCl$_3$

![H NMR Spectrum](image1)

22. $^{13}\text{C}$ NMR for Compound 314 in CDCl$_3$

![C NMR Spectrum](image2)
23. $^1$H NMR for Compound 315 in CDCl$_3$

24. $^{13}$C NMR for Compound 315 in CDCl$_3$
25. $^1$H NMR for Compound 318 in CDCl$_3$

26. $^{13}$C NMR for Compound 318 in CDCl$_3$
27. $^1$H NMR for Compound 319 in CDCl$_3$

![H NMR spectrum of Compound 319 in CDCl$_3$]

28. $^{13}$C NMR for Compound 319 in CDCl$_3$

![C NMR spectrum of Compound 319 in CDCl$_3$]
29. $^1$H NMR for Compound 335 in CDCl$_3$

30. $^{13}$C NMR for Compound 335 in CDCl$_3$
31. $^1$H NMR for Compound 337 in CDCl$_3$

32. $^{13}$C NMR for Compound 337 in CDCl$_3$
33. $^1$H NMR for Compound 338 in CDCl$_3$

34. $^{13}$C NMR for Compound 338 in CDCl$_3$
35. $^1$H NMR for Compound 347 in CDCl$_3$

![H NMR spectrum for Compound 347 in CDCl$_3$](image)

36. $^{13}$C NMR for Compound 347 in CDCl$_3$

![C NMR spectrum for Compound 347 in CDCl$_3$](image)
37. $^1$H NMR for Compound 349 in CDCl$_3$

38. $^{13}$C NMR for Compound 349 in CDCl$_3$
39. $^1$H NMR for Compound 350 in CDCl$_3$

40. $^{13}$C NMR for Compound 350 in CDCl$_3$
41. $^1$H NMR for Compound 351 in CDCl$_3$

42. $^{13}$C NMR for Compound 351 in CDCl$_3$
43. $^1$H NMR for Compound 352 in CDCl$_3$

![H NMR spectrum for Compound 352 in CDCl$_3$](image)

44. $^{13}$C NMR for Compound 352 in CDCl$_3$

![C NMR spectrum for Compound 352 in CDCl$_3$](image)