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Sugars, Thiosugars and Thiophenes

A thesis submitted to the University of Dublin for the degree of Doctor of Philosophy

By

Cornelius John O’ Connor B.A. (Mod.)

Under the supervision of

Dr. Mike Southern

School of Chemistry
Trinity College Dublin
Dublin

March 2009
Declaration

I declare that this work has not been submitted as an exercise for degree at this or any other university, and that it is entirely my own work. Due acknowledgements and references are given to the work of others. The library is granted permission to lend or copy this thesis upon request.
Summary

This project was concerned with expanding and exploiting the proven medical potential of 4'-thionucleosides analogues (4'-TNAs), with the long term aim of providing improved treatments for viral, bacterial and cancer based diseases. To date, no general synthesis of this class of compounds has been reported restricting the range of new compounds that can be accessed. A versatile synthesis of 4'-TNAs is essential if their true potential as medicinal therapeutics is to be fully realised. The aim of this project was to develop a key intermediate compound which could be modified readily affording a plethora of thiosugars with a variety of substitution patterns and configurations with either natural or unnatural stereochemistry. Preliminary investigations focused on a de novo synthesis of Erythrulose, (a target molecule possessing the desired α-hydroxy ketone motif of the proposed key intermediate compound). Several novel highly functionalised small molecules were synthesised. In particular, several vinyl halides and vinyl ethers were synthesised as single isomers using novel methodology. Sharpless asymmetric dihydroxylolation was performed on several of the trisubstituted alkenes but with limited success. This reaction was concisely reviewed.

A route was developed for the synthesis of the key intermediate compound by desymmetrisation of a C2-symmetric tartaric acid derivative. 3,4-Dihydroxy thiolane was synthesised and subsequently desymmetrised. The resulting monoprotected 1,2-diol was oxidised forming a ketone. The thiolane derivative was then oxidised forming a diastereomerically enriched sulfoxide. This, the key intermediate was unstable but it is believed that the use of alternative protecting groups may both increase stability of the compound and improve the diastereomeric ratio obtained.

Thiophenes are a diverse class of compound possessing a wide range of properties with a long and distinguished chemical history. Thiophenes have significant biological applications; exempli gratia, the thiophene derivative Plavix is a blockbuster drug and PaTrin-2 is a thiophene containing MGMT inhibitor currently in clinical trials. Thiophenes have also generated considerable interest, in the field of material science, as organic semiconductors; with potential applications as OLEDs, OFETs and organic solar cells.
A synthesis of 3-nitro-2-substitued thiophenes was developed using solution based thermal chemistry, solution based microwave chemistry and solid phase microwave chemistry. The methodology developed possesses significant advantages over traditional protocols as it obviates the need for a blocking group at the 5-position and removes the necessity for chromatography in most cases. The nitro group is a particularly useful functional group; it will ensure exclusive (rather than preferential) electrophilic aromatic substitution reactivity at the 5-position and a wide range of functionality can be derived from it.
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Abstract

This project was concerned with expanding and exploiting the proven medical potential of 4'-thionucleosides analogues (4'-TNAs), with the long term aim of providing improved treatments for viral, bacterial and cancer based diseases. To date, no general synthesis of this class of compounds has been reported restricting the range of new compounds that can be accessed. A versatile synthesis of 4'-TNAs is essential if their true potential as medicinal therapeutics is to be fully realised. The aim of this project was to develop a key intermediate compound which could be modified readily affording a plethora of thiosugars with a variety of substitution patterns and configurations with either natural or unnatural stereochemistry. Preliminary investigations focused on a de novo synthesis of Erythrulose, (a target molecule possessing the desired $\alpha$-hydroxy ketone motif of the proposed key intermediate compound), with limited success. Subsequently, a route was developed for the synthesis of the key intermediate compound by desymmetrisation of a C2-symmetric tartaric acid derivative.

Thiophenes are a diverse class of compound possessing a wide range of properties with a long and distinguished chemical history. Thiophenes have significant biological applications; exempli gratia, the thiophene derivative Plavix is a blockbuster drug and PaTrin-2 is a thiophene containing MGMT inhibitor currently in clinical trials. Thiophenes have also generated considerable interest, in the field of material science, as organic semiconductors; with potential applications as OLEDs, OFETs and organic solar cells.

A synthesis of 3-nitro-2-substituted thiophenes was developed using solution based thermal chemistry, solution based microwave chemistry and solid phase microwave chemistry. The methodology developed possesses significant advantages over traditional protocols as it obviates the need for a blocking group at the 5-position and removes the necessity for chromatography in most cases. The nitro group is a particularly useful functional group; it will ensure exclusive (rather than preferential) electrophilic aromatic substitution reactivity at the 5-position and a wide range of functionality can be derived from it.
Abbreviations

3TC  lamivudine  
4'-seleno-d4Ns  2',3'-didehydro-2',3'-dideoxy-4'-selenonucleosides  
5'-NT  5'-nucleotidase  
ACV  acyclovir  
ADA  adenosine deaminase  
ADP  adenosine diphosphate  
AICAR  5'-phosphoribosyl-5-aminoimidazole-4-carboxamide  
AIDS  acquired Immune deficiency syndrome  
ALL  acute lymphoblastic leukemia  
AML  acute myeloid leukemia  
AMP  adenosine monophosphate  
ANP  acyclic nucleotide phosphonates  
ANPpp  diphosphorylated acyclic nucleotide phosphonate  
APAF-1  apoptotic protease activating factor-1  
API  active product ingredient  
ara-A  arabinosyladenosine  
ara-C  cytarabine  
ATP  adenosine triphosphate  
AZT  zidovudine  
BAEC  bovine aorta endothelial cell  
BCH-189  2,3-Dideoxy-3'-thiacytidine  
BHV-1  bovid herpesvims type 1  
BIFC  benzimidazolium fluorochromate  
BVDU  brivudine  
BVU  E-5-(2-bromovinyl)-uracil  
CAFdA  clofarabine  
calcd.  calculated  
CB  cannabinoid
CCN  Criegee-Corey-Noe
CdA  cladribine
CLL  chronic lymphocytic leukemia
CML  chronic myeloid leukemia
CMV  cytomegalovirus
CTP  cytidine triphosphate
d4T  stavudine
dA  deoxyadenosine
DABCO  1,4-diazabicyclo[2.2.2]octane
DBU  1,8-Diazabicycloundec-7-ene
dCK  deoxycytidine kinase
DDC  zalcitibine
DDI  didanosine
DDNs  2',3'-dideoxynucleosides
DDNTPs  dideoxynucleoside triphosphates
DDQ  2,3-dichloro-5,6-dicyano-p-benzoquinone
dFdC  gemcitabine
dG  deoxyguanosine
dGK  deoxyguanosine kinase
DHQ  dihydroquinine
DHQD  dihydroquinidine
DHT  dihydrothiophene
dI  deoxyinosine
DIBAL  diisobutylaluminium hydride
DIPA  diisopropylamine
DMAP  dimethylaminopyridine
DMF  dimethylformamide
DMP  Dess-Martin periodinane
DMSO  dimethyl sulfoxide
<table>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNA</td>
<td>deoxynucleoside analogues</td>
</tr>
<tr>
<td>dNTP</td>
<td>deoxynucleotide triphosphates</td>
</tr>
<tr>
<td>DP</td>
<td>diphosphate</td>
</tr>
<tr>
<td>DPD</td>
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</tr>
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</tr>
<tr>
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</tr>
<tr>
<td>GSK</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td>HAART</td>
<td>highly active antiretroviral therapy</td>
</tr>
<tr>
<td>HBV</td>
<td>hepatitis B virus</td>
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<tr>
<td>HCV</td>
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<td>HHV</td>
<td>human herpes virus</td>
</tr>
<tr>
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<td>human immunodeficiency virus</td>
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<td>HMBC</td>
<td>Heteronuclear multiple bond connectivity</td>
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<tr>
<td>HSQC</td>
<td>heteronuclear single quantum coherance</td>
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<tr>
<td>HSV</td>
<td>herpes simplix virus</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
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<td>-------------</td>
</tr>
<tr>
<td>HSVtk</td>
<td>HSV thymidine kinase</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>50% inhibitory concentration</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IMOMM</td>
<td>integrated molecular orbital molecular mechanics</td>
</tr>
<tr>
<td>IMP</td>
<td>inosine 5'-monophosphate</td>
</tr>
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<td>IR</td>
<td>infra red</td>
</tr>
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<td>ligand acceleration effect</td>
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<tr>
<td>m</td>
<td>meta</td>
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<tr>
<td>mCPBA</td>
<td>meta-chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>MGMT</td>
<td>O&lt;sup&gt;6&lt;/sup&gt;-methyl-guanine DNA methyl transferase</td>
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<tr>
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<td>molecular mechanics</td>
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<tr>
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<td>monophosphate</td>
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<td>NA</td>
<td>nucleoside analogue</td>
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<tr>
<td>NAD</td>
<td>nicotinamide adenine dinucleotide</td>
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<tr>
<td>NBS</td>
<td>N-bromosuccinimide</td>
</tr>
<tr>
<td>NIS</td>
<td>N-iodosuccinimide</td>
</tr>
<tr>
<td>NMO</td>
<td>N-methyl-morpholine-N-oxide</td>
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<td>NMR</td>
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<td>non-nucleoside reverse transcriptase inhibitors</td>
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<tr>
<td>NO</td>
<td>nitroolefin</td>
</tr>
<tr>
<td>nOe</td>
<td>nuclear Overhauser effect</td>
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<td>nucleotide reverse transcriptase inhibitors</td>
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<td>o</td>
<td>ortho</td>
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<td>OFET</td>
<td>organic field effect transistors</td>
</tr>
<tr>
<td>OLED</td>
<td>organic light emitting diode</td>
</tr>
<tr>
<td>p</td>
<td>para</td>
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</table>
PARP: poly (ADP-ribose) polymerase
PBM: peripheral blood mononuclear
PCV: penciclovir
PNAs: purine nucleoside analogues
PT: phenylthiophene
PTSA: para-toluene sulfonic acid
PyNAs: pyrimidine nucleoside analogues
PYDZ: pyridazine
QFC: quinolium fluorochromate
QM: quantum mechanical
RNA: ribonucleic acid
RR: ribonucleotide reductase
RSV: respiratory syncytial virus
RT: room temperature
SAD: Sharpless asymmetric dihydroxylation
SAH: S-adenosylhomocysteine
S-BVDU: E-5-(2-bromovinyl)-4'-thio-2'-deoxyuridine
SHV-1: suid herpesvirus type 1
SR141716A: rimonabrant
ssDNA: single stranded deoxyribonucleic acid
ssRNA: single stranded ribonucleic acid
SVV: simian varicella virus
T-ara-C: 4'-thio-β-D-arabinofuranosylcytosine
T-ara-U: 4'-thio-β-D-arabinofuranosyluracil
TBAF: tetrabutylammonium fluoride
TBDPS: tert-butylidiphenylsilyl
TBS: tert-butylidimethylsilyl
TEA: triethylamine
TFAA: trifluoroacetic anhydride
THF: tetrahydrofuran

xvi
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>THT</td>
<td>tetrahydrothiophene</td>
</tr>
<tr>
<td>TIPS</td>
<td>triisopropylsilyl</td>
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<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
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<td>trimethylsilyl</td>
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<td>trimethylsilyl chloride</td>
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<td>TMSOTf</td>
<td>trimethylsilyl triflate</td>
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<td>thionucleoside analogues</td>
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<td>TOCSY</td>
<td>total correlation spectroscopy</td>
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<td>TP</td>
<td>triphosphate</td>
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<td>TPS</td>
<td>2,4,6-triisopropylbenzenesulfonyl</td>
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<tr>
<td>TPSCI</td>
<td>2,4,6-triisopropylbenzenesulfonyl chloride</td>
</tr>
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<td>valaciclovir</td>
</tr>
<tr>
<td>VGCV</td>
<td>valganciclovir</td>
</tr>
<tr>
<td>VZV</td>
<td>Varicella Zoster virus</td>
</tr>
<tr>
<td>VZVtk</td>
<td>VZV thymidine kinase</td>
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Chapter 1

Introduction to nucleoside analogues
1 Introduction to nucleoside analogues

1.1 Viruses and viral diseases

Viruses are non-cellular infectious agents which take over a host cell in order to survive and multiply. They are parasitic in nature and multiply at the expense of the host cell’s metabolic system. Viruses may start reproducing immediately upon infection of a suitable host but they also have the ability to remain dormant within a host cell for extended periods of time until an etiological agent instigates commencement of the reproductive cycle. Viral infections may be transmitted by a variety of routes. The viruses responsible for diseases such as influenza, chicken pox, measles, mumps and rubella can be transmitted through the air by an infected host coughing or sneezing. Other viruses can be transmitted through insects. For example, ticks act as vectors for the virus responsible for yellow fever and Colorado tick fever. Viruses can also be transmitted through physical contact: through respiratory, gastrointestinal and genital tracts, urine, skin, blood and placenta. Finally, food-borne and water-borne viruses can lead to hepatitis A and E, polymyelitis and viral gastroenteritis.

Historically, viral infections have had catastrophic effects on human populations. Small pox was responsible for the decimation of the Americas’ indigenous tribes during European colonisation. Various flu epidemics and pandemics have also proved devastating. For example, the post world-war-one flu pandemic is estimated to have killed over 20 million people, a figure far greater than the number killed through military action in the war. In the past viral diseases such as Ebola were contained within certain population groups. However, with the advent of cheap and readily available air-travel tourists are able to visit remote areas and thus the threat of spreading rare diseases around the globe is far greater.

Given the increasing numbers of patients infected with potentially deadly viruses, the number of new virus strains, which are constantly emerging, and the considerable threat posed by bioterrorism, it is perhaps axiomatic to point out that notwithstanding the considerable advancements that have been made in the treatment of viral infections in the
last few decades, far too many viral diseases still remain untreatable. As the following statistics clearly disclose, research into effective antiviral drugs is a major priority.

In 2000, 27.6 million people were infected with human immunodeficiency virus (HIV). In 2007, the world health organisation estimated this number to have increased to 33.2 million people worldwide. Sub-Saharan Africa continues to be the region most affected by HIV/AIDS. In 2007, approximately two in every three people worldwide living with HIV lived in sub-Saharan Africa, a total of 22.5 million people.

![Number of people (in millions) living with HIV globally, 1990-2007](image)

**Figure 1.1:** Chart illustrating the number of people in millions living with HIV globally.

The percentage prevalence has stabilised in the last few years but continuing new infections contribute to the estimated number of persons infected with HIV being greater than ever before (Figure 1.1). The numbers are rising and the message is clear: more and better antiviral products are urgently required.

In 2007, 170 million people were thought to be chronically infected with hepatitis C virus (HCV) (Figure 1.2) and currently an estimated 3 to 4 million people are newly infected with HCV each year. Post-transfusion hepatitis C, which used to be a major cause of transmission, has almost disappeared in Western countries and intravenous drug abuse is
the main route of transmission. However, because the infected can remain asymptomatic for several years, many people are unaware of their infection. Infected persons serve as a source of transmission to others and are themselves at risk for chronic liver disease or other HCV-related chronic diseases. More than 80% of people infected with HCV progress to a chronic form of the disease. Approximately 20% of people with chronic hepatitis C develop cirrhosis in 10-20 years and may die of complications of cirrhosis in the absence of liver transplantation. Of those with cirrhosis, up to 4% develop hepatocellular carcinoma.

Thirty years ago, just 3 drugs were licensed for the treatment of viral infections. Since then significant progress in developing new antiviral agents has been made and there are now more than 40 licensed antiviral drugs, most of which are for the treatment of HIV. In the 1980s, genome sequencing and structure based drug design were developed which facilitated a new approach to targeting viral enzymes, guided by rational design. Greater understanding of viral life cycles has resulted in the identification and validation of several targets for therapeutic intervention, but there are still a lack of potent therapies for several viral infections, and established treatments are not always efficacious or well tolerated. For example, a combination of three or more drugs is usually used in the treatment of HIV to create an effective antiviral therapy and combat the development of drug resistance. However, combination therapy for those patients with advanced acquired immune
deficiency syndrome (AIDS) has only increased life expectancy from 1.53 years to 2.91 years. Clearly, new therapies with novel mechanisms of action or unique resistance profiles are seriously needed.

Several potentially fatal viral pathogens, such as smallpox, polio, measles, mumps and rubella have been, or may be eradicated due to the development of effective vaccines. However, other viral diseases such as HIV and HCV have so far proved intractable to the vaccine approach; this is mainly due to the notoriously high mutation rate of the viruses. The lack of vaccines for most respiratory-tract virus infections (adenovirus, rhinovirus, parainfluenza virus and respiratory syncytial virus (RSV)), the highly prevalent herpesviruses (herpes simplex virus types 1 and 2 (HSV-1,-2), varicella zoster virus (VZV), Epstein Barr virus (EBV), cytomegalovirus (CMV) and the human herpes viruses types 6, 7 and 8 (HHV-6,-7,-8) and the vast array of haemorhagic fever viruses (such as Ebola) highlights the need for new and effective antiviral drugs.

Dmg discovery and development efforts for HIV, based on advances in the understanding of the viral life cycle, in particular the genome sequencing of the virus in the 1990s, have transformed HIV infection from a death sentence to a chronically manageable disease. HIV can be controlled for many years through combination therapies with different classes of antiviral dmgs - known as highly active antiretroviral therapy (HAART) provided early diagnosis has been made.

Considerable advancements have also been made in the treatment of HCV in the past decade. At present, a combination of pegylated interferon (IFN) and ribavirin are used to treat chronically infected HCV sufferers. Increased knowledge of the viral life cycle is providing new opportunities for therapeutic intervention and the first drugs developed specifically to target HCV enzymes are showing promise in clinical trials.

1.1.1 Virus Structure and Classification

Viruses are microorganisms that can be viewed as protein packages transmitting foreign nucleic acid between host cells. They are the smallest of all self-replicating organisms and vary in size between 0.02-0.04 μm; they can be viewed with the aid of an
Viruses consist of a nucleic acid core that contains either DNA or RNA but not both. They are therefore classified as being either DNA or RNA viruses. Most RNA viruses contain single stranded RNA (ssRNA) but double stranded RNA (dsRNA) viruses also exist. If the base sequence of the RNA is identical to that of the viral mRNA, it is described as the positive (+) strand. If however the base sequence is complementary to that of the viral mRNA, it is called the negative (-) strand. Most DNA viruses contain double stranded DNA (dsDNA) but some single stranded DNA (ssDNA) viruses have also been identified. A protein coat known as the capsid surrounds the nucleic acid core. The capsid is formed during a process called self assembly and is made up of protein sub-units called protomers. The capsid containing the nucleic acid core is called the nucleocapsid. The nucleocapsid often contains certain enzymes which are critical to the replication of the virus. Most viruses can be classified into two groups on the basis of the structure of their nucleocapsid. One group of viruses may show helical symmetry and the other icosahedral symmetry (there are however other types of nucleocapsid morphology). The nucleocapsid may or may not be coated by a protein coat called an envelope. The envelope is composed of glycoproteins that can be recognised by the immune system as antigens. The arrangement of coat proteins defines the overall shape of the viruses. A mature infectious virus particle is called a virion.

Figure 1.3: Diagrammatic representation of a flu virus
1.1.2 Life Cycle of Viruses:

When a virus attacks a host cell it must initially adsorb onto the surface of the host cell. It does so by binding to a specific receptor on the surface of the cell. The virus then penetrates the surface of the cell and removes its protective coat, introducing its nucleic acid material into the host cell. The virus then replicates its genetic material using viral enzymes which also catalyse the synthesis of mRNA molecules for the formation of viral structural and non-structural proteins. The assembled virus must then leave the cell by either inducing host cell lysis, which results in the destruction of the host cell or by a budding process, leaving the host cell intact.

![Diagram of the life cycle of a DNA virus such as herpes simplex]

Figure 1.4: Life cycle of a DNA virus such as herpes simplex

In DNA viruses, viral DNA is integrated into the host cell's DNA by an enzyme called DNA integrase. The viral DNA therefore becomes a permanent part of the host's genomic make up. The virus may remain latent for years or begin the replication process once more, producing progeny viral DNA during cell division. Viral DNA transcribes into early and late mRNA, which is then translated into viral proteins by both viral and host enzymes. Viral proteins synthesised by an infectious DNA virus may cause the host cell to
alter its normal functions or morphological characteristics. The lifecycle of a DNA virus such as herpes simplex is outlined in Figure 1.4.

RNA viruses use an RNA directed RNA polymerase to replicate their RNA which can then be employed to prepare their required proteins. Retroviruses like HIV, on the other hand, must convert their RNA to DNA in a process called reverse transcription which is catalysed by an enzyme called reverse transcriptase. Before the viral DNA can be integrated into the host cell chromosomes a double stranded helix (RNA/DNA duplex) is formed in which one strand is the original viral RNA and the other strand is the new complementary DNA chain. An enzyme called RNase H then catalyses the removal of the viral RNA strand, leaving a single stranded DNA molecule. The virus then harnesses host cell enzymes to replicate the viral DNA creating a DNA duplex which is then integrated into the host cell genome by DNA integrase. This integrated copy of the viral RNA into the host DNA is called a "provirus," this will be transmitted to new daughter cells during cell replication similar to any other host cell genes. The provirus is later transcribed into mRNA, which is translated into viral proteins by cellular enzymes. As was the cases for the DNA viruses, the viral proteins can change the cell’s functions and morphological characteristics.
1.2 DNA structure and DNA Replication

There are two major forms of nucleic acid, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) both of which are long linear polymers. DNA stores and carries the genetic information of a cell from one generation to the next whereas RNA is intricately involved in the conversion of this information into the protein domain. They are macromolecules consisting of a large number of linked nucleotides, each composed of a sugar, a phosphate and a nucleobase.

![DNA Nucleosides](image1)

![RNA Nucleosides](image2)

**Figure 1.5: Structures of DNA and RNA nucleosides**

The absence of a phosphate on the structure, *id est* a unit consisting of a base bonded to a sugar is referred to as a nucleoside, a term proposed by Levene and Jacobs in 1909. The
four nucleoside units in DNA are deoxyadenosine, deoxyguanosine, deoxycytidine and deoxythymidine whereas those in RNA are adenosine, guanosine, cytidine and uridine. These nucleosides can be isolated by either enzymatic or chemical hydrolysis of the appropriate nucleic acid.

Figure 1.6: Outline of structure of DNA

In 1953, Watson and Crick correctly proposed the double helical structure of DNA based on the analysis of x-ray diffraction patterns and careful model building. The two sugar-phosphate back-bones are on the exterior of the double helix and the bases project into the interior. The adjoining bases in each strand stack on top of one another in parallel planes. The two strands are orientated anti-parallel to each other id est their 5'→3' directions are opposite. The bases are paired with adenine always binding with thymine through two hydrogen bonds and similarly cytidine always binds with guanine through three hydrogen bonds. The Watson-Crick base pairs are visible in the segment of DNA shown in Figure 1.6.
Before a cell can divide, it must initially replicate its DNA. DNA replication is a semi-conservative process and occurs in the S phase of the cell cycle. In order to replicate, DNA must first be unwound by specific enzymes called DNA gyrase and DNA helicase. This process is initiated at specific points within DNA called origins. These are specific DNA sequences recognised by replication initiator proteins which promote the unwinding action of helicase and gyrase (topoisomerase) resulting in the formation of a replication bubble containing two replication forks (most DNA replication in both prokaryotes and eukaryotes is bidirectional). A specialised RNA primase then forms short RNA primers complementary to the exposed template. Special proteins called single strand binding are bound to the separated strands and prevent re-annealing of the exposed chains. Each primer is then elongated by the action of DNA polymerase which catalyses the addition of nucleotide units complementary to the template chain onto the growing chain. DNA polymerase can only synthesise DNA in the 5’→3’ direction; because the strands of the parental duplex are anti-parallel, different mechanisms are required for the synthesis of both complementary strands.
Figure 1.8: DNA replication fork. Reproduced with permission from Nature Publishing Group, *Nature* 2003

Figure 1.9: DNA polymerase catalysed addition of nucleotides to growing chain

At each growing fork, one strand called the leading strand is synthesised continuously by DNA polymerase (Pol δ) from a single RNA primer on the leading strand.
template and grows in the 5'→3' direction. This is the same direction as the movement of the growing fork. However, growth of the other daughter strand, called the lagging strand, must also occur in the 5'→3' direction, which is the opposite direction to the movement of the replication fork. This problem is overcome by the production of short RNA primers by RNA primase along the length of the lagging strand template. Each of these primers, base paired to their template strand, is elongated in the 5'→3' direction by DNA polymerase (Pol α), forming discontinuous segments called Okazaki fragments (after their discoverer Reiji Okazaki). The RNA primers of each Okazaki fragment are then removed and replaced by DNA chain extended from an adjacent Okazaki fragment. Finally an enzyme called DNA ligase joins the adjacent fragments completing the replication process.16
1.3 Structure of nucleosides:

A nucleoside is a unit consisting of a nucleobase bonded to a sugar. The carbohydrate moiety in RNA is ribose (β-D-ribofuranose), whereas in DNA the sugar component is 2-deoxyribose (2-deoxy-β-D-ribofuranose). The sugar is linked to a purine or pyrimidine base through a β-N-glycosidic bond, through \( N^\beta \) of the purine and \( N^1 \) of the pyrimidine. In addition to the nucleosides of RNA and DNA, several naturally occurring nucleosides have also been isolated and the configuration at the anomeric centre (C1') is invariably β.\(^{17}\)

1.3.1 Nucleoside Conformation

Three parameters are used to describe the conformation of nucleosides: the geometry of the glycosyl link between the nucleobase and the sugar ring, the rotation about the exocyclic C4'-C5' bond, and the puckering of the sugar ring.

1.3.1.1 Glycosyl Link Geometry

The rotation of the nucleobase about the glycosidic bond is restricted due to steric hindrance exerted by the endo-proton on C2'. This forces the nucleoside to adopt one of two conformations: when the O2 of pyrimidine nucleosides; or the N3 of purine nucleosides lies above the sugar ring the conformation is \( \text{syn} \) and when they point away from the sugar ring the conformation is \( \text{anti} \).\(^{18}\) The conformation can also be determined by measuring the torsional angle \( \chi(C2-N1-C1'-O4') \) where N1 and C2 belong to the nucleobase and C1' and O4' belong to the ribose ring. This angle is illustrated below, (Figure 1.10) the view point is along the N1-C1' bond axis and the torsional angle \( \chi \) is the angle between the C2-N1 bond and the C1'-O4' bond.
The value of $\chi$ indicates whether the conformation is anti (-120 ± 60°) or syn (70 ± 20°). Pyrimidine nucleosides tend to have an anti conformation with $-180 < \chi < -115^\circ$, in purine bases a high anti value of -60° is allowed. Anti conformations are preferable over syn conformations as there is reduced steric hindrance between the nucleobase and the sugar ring.\(^{17}\)

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{glycosidic_link.png}
\caption{Glycosidic Link Geometry\(^{17}\)}
\end{figure}

\section*{1.3.1.2 $C4'$-$C5'$ Bond Geometry}

A torsional angle $\gamma$(C3'-C4'-C5'-O5') is used to describe the geometry of the exocyclic bond relative to the ribose ring. There are three possible orientations of the O5'-hydroxyl group, ap (trans, t), +sc (gauche, g+), and -sc (gauche, g-), the corresponding $\gamma$ angles are approximately 180°, 60°, and -60° respectively, see \textbf{Figure 1. 12} and \textbf{Figure 1. 13}.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{c4_c5_bond.png}
\caption{C4'-C5' Bond Geometry\(^{17}\)}
\end{figure}
Pyrimidine nucleosides tend to have a $+sc$ conformation whereas purine nucleosides adopt $ap$ and $+sc$ with equal preference.

**Figure 1.12:** C4'-C5' Conformation

**Figure 1.13:** Illustration of possible conformations about the C4'-C5' bond. A=C3', B=C4', C=C5', D=O5'
1.3.1.3 **Ring Puckering**

A planar five-membered sugar ring is sterically and energetically very unfavorable; the ring is therefore puckered with one or two atoms positioned above or below the plane releasing the steric strain, lowering the energy and producing a stable conformation. The puckering of the ring gives rise to two main structural conformations, envelope (E) and twist (T). The conformation with C3' out of the plane of C4'-O4'-C1'-C2' and on the same side as the nucleobase is called C3'-endo (C3'-exo has the C3' on the opposite side to the nucleobase). The conformation with C2' out of the plane, and on the same side as the base, is C2'-endo. These two conformations approximate those found in DNA and RNA. ¹⁹

![Figure 1.14: Sugar conformations – ring puckering](image)

C3'-endo/C2'-exo  
C2'-endo/C3'-exo
1.4 Targets in the viral life cycle

Understanding the life cycle of a virus enables the identification of specific drug targets. There are several viral functions that can be clearly distinguished from the cellular functions, thus allowing the development of newer antiviral agents based on the difference in viral-host metabolism. Antiviral drug design could, in principle, be targeted at either viral proteins or cellular proteins. Targeting specific viral proteins affords more selective specialised compounds that are likely to be less toxic, with a lower chance of side effects. There is however, increased likelihood of drug resistance development and a narrower spectrum of activity. Targeting cellular proteins might produce antiviral compounds with a broader spectrum of activity and with less chance of resistance development but it is likely that these compounds will be more toxic. The desirable features that novel antiviral drugs should display include: high antiviral activity against both wild-type and mutant variants, high oral bioavailability and long elimination half-life, allowing once-daily oral treatment at low doses; minimal adverse effects and ease of synthesis and formulation.

HIV is a retrovirus that replicates through a proviral double stranded DNA intermediate, whereas HCV is a (+) RNA virus that replicates through a (-) RNA intermediate. Figure 1.15 outlines the life cycles of both HIV and HCV complete with established and potential points for therapeutic intervention. There are a number of analogous steps in both life cycles which include viral entry, replication of the viral genome by polymerase enzymes and proteolytic processing of virus polyproteins. HIV integrase provides a virus specific target. Once the retrovirus has completed reverse transcription, the resulting proviral DNA becomes integrated into the cellular genome by HIV integrase. Specific aspects of viral entry and subsequent assembly also differ for the two viruses. Current and future drug-based strategies for combating viral infections include targeting: virus adsorption, virus-cell fusion, viral genome replication inhibitors, viral protease and neuraminidase inhibitors and two host cellular enzymes - inosine 5'-monophosphate (IMP) dehydrogenase (IMP dehydrogenase) and S-adenosylhomocysteine (SAH) hydrolase could also provide targets for certain classes of antiviral agents.
Figure 1.15: Simplified flow charts of the life cycles of HIV and HCV.
Established and potential targets for therapeutic intervention are highlighted, together with the class of antiviral drugs. CLDN-1, claudin-1; GAG, glycosaminoglycans; LDLR, low density lipoprotein receptor; NNRRI, non-nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NtRTI, nucleotide reverse transcriptase inhibitor; SR-BI, scavenger receptor class B type.
For the purpose of this thesis only certain viral genome replication inhibitors shall be considered; namely nucleoside and nucleotide analogues.
1.5 Nucleoside mimetics as viral genome replication inhibitors

The potential of nucleosides as therapeutic agents emerged in the 1950s and the 1960s with the discovery of agents such as arabinosyladenosine (ara-A), which exhibited antiviral activity, and toyocamycin and the synthetic nucleosides 5-fluoro-2'-deoxyuridine (FUDR) and arabinosylcytidine (ara-C), which exhibited anticancer activity.17

Figure 1.16: Diagram of ara-A, toyocamycin, FUDR & ara-C

1.5.1 Inhibitors targeted at reverse transcriptase

With the emergence of HIV in the 1980s the potential of nucleoside analogues as antiviral compounds was further investigated. The first anti-HIV drugs targeted the enzyme reverse transcriptase. There were two types of inhibitor: those that target the catalytic substrate (3'-deoxynucleoside triphosphate (dNTP)) binding site, which are known as nucleoside reverse transcriptase inhibitors, (NRTIs), and those that target an allosteric site, which is distinct from the substrate site, known as non-nucleoside reverse transcriptase inhibitors (NNRTIs). NRTIs are prodrugs that require three phosphorylation steps by cellular kinases, to be converted into their active metabolites.10 The first class of drugs licensed for the treatment of HIV were 2',3'-dideoxynucleosides (DDNs). Zidovudine (AZT) which was the first drug approved by the US Food and Drug
The active metabolite for AZT is its triphosphate form AZT-TP, which acts as a competitive inhibitor or alternative substrate with respect to the normal substrate deoxythymidine triphosphate (dTTP), during the DNA replication process. Without the 3'-hydroxy group the DNA chain cannot extend, therefore dideoxynucleoside triphosphates (DDNTPs) inhibit the action of reverse transcriptase, by premature termination of chain elongation. The ability of a DDN to inhibit reverse transcriptase and suppress HIV is directly related to its intracellular metabolism to the 5'-triphosphate. In the case of AZT, the rate determining step is the formation of the diphosphate AZT-DP from the
monophosphate AZT-MP. This is due to the ability of AZT-MP to inhibit dTMP kinase, which catalyses the conversion of AZT-MP to AZT-DP. So far, seven NRTIs have been approved by the US FDA for the treatment of HIV, these include zidovudine, didanosine, zalcitibine, stavudine, lamivudine, abacavir and emtricitabine, see Figure 1. (vide supra).

Figure 1. 18: (a) AZT must be phosphorylated to form AZT-TP, the active metabolite. (b) AZT is incorporated into the growing DNA chain by reverse transcriptase.

Some of the underlying problems associated with phosphorylation of the 5'-hydroxy group can be overcome by introducing a phosphonate group. The phosphonate group is sufficiently similar to a phosphate group to be recognised as a substrate by reverse
transcriptase and only two phosphorylation steps are then required to generate the active metabolite. These phosphonate derivatives are referred to as nucleotide reverse transcriptase inhibitors (NtRTIs).

Unlike phosphate groups, phosphonate groups cannot be cleaved by esterases that would normally convert nucleoside monophosphates back to their nucleoside form. Presently there is only one NtRTI, tenofovir disoproxil fumarate, approved by the US FDA.

1.5.2 Inhibitors targeted at RNA polymerase

In HCV, RNA replicase is an RNA dependent RNA polymerase which replicates the HCV genome before viral assembly. Nucleoside analogues designed to inhibit RNA replicase are referred to as nucleoside RNA replicase inhibitors (NRRIs), these too have to be phosphorylated to their triphosphate form to become active metabolites. They behave as competitive inhibitors with the natural substrate for RNA replicase cytidine triphosphate (CTP).

![Structures of some NRRIs](Figure 1.19: Structures of some NRRIs)

Unlike NRTIs, NRRIs do possess a 3' hydroxy group and therefore chain termination is not accomplished in the same manner as is for NRTIs. The presence of a methyl (or fluorine) group in the 2' position, or an azido group in the 4' position, is vital
to the anti-HCV activity of the current repertoire of NRRIs. Their anti-HCV activity is achieved by interfering with the subsequent elongation step through steric hindrance of the aforementioned azide or methyl groups, resulting in chain termination.

### 1.5.3 Inhibitors Targeted at DNA polymerase

Analogous to the mode of action of NRTIs and NRRIs, inhibitors of DNA viruses such as HBV, HSV, EBV, VZV and CMV, inhibit DNA polymerase resulting in chain termination and/or DNA strand breakage to effect their antiviral activity. The acyclic nucleosides acyclovir (ACV), ganciclovir (GCV), penciclovir (PCV) and their oral prodrug forms valaciclovir (VCV), valganciclovir (VGCV) and famciclovir (FCV) are recognised as substrates by HSV and VZV encoded thymidine kinase (ensuring activity only in viral infected cells). The viral thymidine kinase phosphorylates these substrates to their monophosphate form and then cellular kinases complete the formation of the triphosphate active metabolites. The triphosphates act as substrates for DNA polymerase and once incorporated into the growing viral DNA, chain termination occurs. Cidofovir (HPMPC) on the other hand, is active in its diphosphate form which acts as a competitive inhibitor of DNA polymerase resulting in chain termination or incorporation into the growing DNA chain resulting in the formation of aberrant DNA.
Chain termination is not the only possible way for nucleoside analogues to exhibit antiviral activity. Some nucleosides disrupt the stability and functioning of DNA during the replication and transcription processes. Brivudin (BVDU), which was originally synthesised in 1976, as a potential radiation sensitizing agent by Walker and co-workers at the University of Birmingham, was found to exhibit excellent anti-HSV-1 and anti-VZV properties. Later it was discovered that the compound had a much broader spectrum of activity and was effective against several other herpes viruses such as suid herpesvims type 1 (SHV-1), bovid herpesvirus type 1 (BHV-1), simian varicella virus (SVV), herpesvirus saimiri, and herpesvirus platyrrhinae. EBV is also rather sensitive to BVDU. The mechanism of action of BVDU against HSV-1 and VZV depends on a specific phosphorylation by the virus-encoded thymidine kinase to its 5'-mono- and 5'-di-phosphate forms. Cellular kinases complete the formation of the triphosphate, BVDU-TP, which
then acts as a competitive inhibitor with respect to the natural substrate (dTTP) or as an alternative substrate, allowing the incorporation of BVDU-TP into the growing chain resulting in the formation of defective DNA fragments. Results from *in vitro* experiments carried out by Mancini *et al.* indicate a close correlation between the incorporation of BVDU into HSV-1 DNA, DNA integrity, and viral infectivity.²⁶

![Brivudine (BVDU)](image)

**Figure 1.20:** Structure of anti-herpetic agent Brivudine

1.5.5 Nucleoside analogues as selective antiviral therapeutics

Target specificity and selectivity is a crucial factor in the development of modern antiviral therapeutics. The more selective a drug is for its target, the less chance it has of causing unwanted side effects. When one considers some of the aforementioned virus targets – DNA polymerase & RNA polymerase - it is vital that the therapeutic agent in question is selective for virally infected cells and does not inhibit normal cellular genomic replication.

The mode of action of ACV involves: (i) phosphorylation forming ACV-MP by viral thymidine kinase. ACV is a poor substrate for normal thymidine kinase and therefore is only activated in virally infected cells; (ii) viral DNA polymerase is competitively inhibited by ACV-TP at lower concentrations than is cellular DNA polymerase; (iii) ACV is preferentially taken up by cells infected with HSV than by healthy cells.²⁷ The combined result is a highly beneficial therapy with a negligible level of unwanted toxic side effects.
BVDU has also proved to be a potent yet selective antiviral agent. De Clercq et al. examined the inhibitory effects of BVDU and several other 5-substituted-2'-deoxyuridine compounds against HSV-1 and vaccinia virus. They discovered that BVDU had to be administered in a 10,000-fold higher concentration than that required to inhibit HSV-1 before it had an adverse effect on normal cell metabolism (BVDU did not markedly affect vaccinia virus multiplication, which further points to its selectivity as an anti-herpes agent).

Cheng et al. measured the affinity of various 5-(2-halovinyl)-2'-deoxyuridines for deoxythymidine kinases (dTKs) of various origins and discovered that the substrates were effectively phosphorylated by viral thymidine kinases but none served as an alternative substrate for cytosol dTK. Consequently, the phosphorylation of BVDU (as well as some other 5-(2-halovinyl)-2'-deoxyuridines) to its triphosphate form BVDU-TP is restricted to virus infected cells. BVDU-TP is a considerably stronger inhibitor of HSV-1 DNA polymerase than of cellular DNA polymerases.

In addition, while the initial phosphorylation of BVDU to BVDU-MP is selective for viral thymidine kinase, the second phosphorylation step forming BVDU-DP is even more selective and is also dependent on HSV-1 thymidine kinase. This further highlights the specificity and selectivity of this drug.
1.6 Nucleoside analogues as cancer therapeutics

Nucleoside analogues exert their antiviral activity by disrupting the metabolism of virus infected cells. They can however, also behave as cytotoxic antimetabolites in non-infected cells and because of this cytotoxic nucleoside analogues were among the first chemotherapeutic agents to be introduced for the medical treatment of cancer. They exert their cytotoxic activity by being incorporated into and altering DNA and RNA, by interfering with various enzymes involved in the synthesis of nucleic acids, or by modifying the metabolism of physiological nucleosides.

Nucleoside analogues act against targets that are present in both normal and cancerous cells. Because the pharmacologic targets of nucleoside analogues are intracellular, permeation through the plasma membrane is an obligatory first step in the manifestation of cytotoxicity. However, as nucleoside analogues are generally hydrophilic, diffusion through the cell membrane is slow, and transporter-mediated uptake is the major route of drug influx. In general, cancer cells are rapidly dividing and present a higher level of expression of nucleoside transporters than their normal counterparts. This can lead to an accumulation of nutrients, synthetic building blocks and drugs more quickly than in normal cells, and gives some selectivity to cytotoxic nucleoside analogues.

1.6.1 Purine Nucleoside Analogues (PNA)

In the late 1980s a novel group of cytotoxic purine nucleoside analogues with a high level of antineoplastic activity were developed. Two of most successful reagents from this class of compound are adenosine derivatives - Fludarabine (FA) and Cladribine (CdA), (see Figure 1.22). Both were approved by the US FDA for the treatment of neoplastic diseases, they are especially active in lymphoid and myeloid malignancies. FA is administered for the treatment of chronic lymphocytic leukemia (CLL) and CdA is used for treating hairy-cell leukemias. These drugs share activity against other indolent lymphoid malignant disorders including low grade non-Hodgkin lymphomas, Waldeström's
macroglobulinaemia, and cutaneous T-cell lymphomas, but lack activity against multiple myeloma and most solid tumours. FA and CdA share several similar characteristics including transport into cells, phosphorylation by deoxycytidine kinase (dCK) and dephosphorylation by 5'-nucleotidase. They also have similar mechanisms of toxicity both in proliferating and quiescent cells, such as inhibition of DNA synthesis, inhibition of DNA repair and accumulation of DNA strand breaks. Induction of apoptosis through the mitochondrial pathway, direct binding to apoptosome or modulation of p53 expression lead to programmed cell death, which is the main end point of PNA action.

![Fludarabine (FA) and Cladrabine (CdA)](image)

**Figure 1.22:** Cytotoxic purine nucleoside analogues

Since the development of FA and CdA, considerable progress has been made in the field of PNAs and their potential as effective antineoplastic therapeutics, when administered both in combination or as single treatments, continues to improve the lives of leukemia sufferers. Although CLL is currently considered an incurable disease, improved treatment regimes aim to alleviate symptoms and prevent life threatening complications. The field has expanded considerably and several novel PNAs have been discovered, for example pentostatin (DCF) and Clofarabine (CAFdA), which were recently approved by the US FDA as antineoplastic agents, see **Figure 1.23**.
The first step in the cellular activity of FA, CAFdA and CdA is their uptake into cells. Limited solubility of FA and consequent difficulties in formulation led to the synthesis of the prodrug, fludarabine monophosphate FA-MP,\textsuperscript{36} which is dephosphorylated by membrane \textit{ecto-5'}-nucleotidase (5'-NT) prior to cell entry. The PNAs are transported across the cell membrane by nucleoside-specific membrane transporters. After their uptake into cells they must be converted to their metabolically active triphosphate forms. All cells maintain a pool of deoxynucleotide triphosphates (dNTPs) for DNA synthesis; these dNTPs are in an equilibrative state with their dephosphorylated deoxynucleotide precursors. For example, deoxyadenosine (dA) is continually being phosphorylated to dATP \textit{via} dAMP and dADP. The rate limiting step in this sequence is the formation of dAMP from dA. Conversely, dATP is continually being dephosphorylated by 5'-NT back to dA, and the rate limiting step is formation of dA from dAMP. In lymphoid cells, the relative activities of dCK and 5'-NT strongly favor the formation of dATP. However, dA may also be converted to deoxyinosine (dI) by adenosine deaminase (ADA); which subsequently leads to the formation of uric acid. ADA plays a crucial role in limiting the intracellular accumulation of dATP, which is cytotoxic at high concentrations.\textsuperscript{37}

Two enzymes are involved in PNA phosphorylation: dCK and deoxyguanosine kinase, (dGK) (localised in the mitochondria). CAFdA, CdA and FA are intracellularly converted (mainly by dCK) to their 5'-monophosphate metabolites and then by mono- and diphosphokinases to their active 5'-triphosphate forms.\textsuperscript{36} The efficacy of conversion of CAFdA and CdA to their phosphorylated forms by dGK is comparable to that of 2'-
deoxyguanosine (dG), the preferred substrate of the enzyme, and approximately 10-fold higher than that of FA. In lymphoid cells, a high ratio of dCK/5'-NT activity favors the accumulation of phosphorylated metabolites, which inhibit various processes involved in DNA and RNA synthesis, modulate apoptosis, influence cell cycle control and signal transduction pathways.  

1.1 Mechanism of action

An important part in the mechanism of PNA action is their incorporation into DNA and inhibition of either DNA synthesis or DNA repair which leads to an accumulation of DNA breaks and mainly results in apoptosis. DNA polymerases incorporate dNTPs into a new DNA strand during replication. Some nucleoside analogues exert their cytotoxicity by inhibiting DNA polymerase. The triphosphates of FA, CdA and CAFdA compete with dATP for utilisation by DNA polymerases. Thus, the likelihood of drug incorporation is strongly determined by the ratio of the cellular concentrations of dNTP to deoxynucleoside analogue triphosphate (dNTP).

Most nucleotide analogues act as chain terminators, reducing the ability of DNA polymerase to use the 3'-terminal nucleotide analogue as a substrate for addition of a subsequent nucleotide. Huang et al evaluated the action of FA on DNA synthesis and discovered that more than 94% of the analogue incorporated in DNA was at the 3'-termini, indicating a very strong chain terminating action. However, in the case of CdA which DNA polymerases can insert and extend quite efficiently as a single nucleotide, DNA polymerase is weakly inhibited and only on insertion of an increasing number of tandemly incorporated nucleotide analogue residues, does the polymerase activity slow and ultimately stop.

Several DNA polymerases possess exonuclease activities which are activated when errors occur in DNA synthesis. They act as proof-readers removing mismatched nucleotides. The switch from polymerase to excision function is probably triggered by a conformational change in the DNA template due to lack of hydrogen bonding in the mismatched bases. 3'-Terminal FA is recognised by exonucleases with a 38-fold greater
affinity than an identical sequence terminated with mismatched dAMP. Nevertheless, the rate of excision is 37-fold less than that of the natural nucleotide. Furthermore once excision has been completed it appears that the reaction products remain in association with the polymerase and this has been associated with loss of function of the enzyme and therefore inhibition of DNA polymerase.40

Ribonucleotide reductase (RR) is a key enzyme involved in DNA replication. It reduces ribonucleosides forming deoxribonucleosides which are subsequently phosphorylated to their active triphosphate form. FA, CdA and CAFdA are potent RR inhibitors. A decrease in dNTPs levels, caused by RR inhibition causes an increase in competition between analogue and natural substrate for incorporation into DNA. In addition, a decrease in dCTP causes an increase in dCK activity, (because the enzyme has a feed-back inhibition system in place and its activity is regulated by the concentration of dCTP), leading to increased phosphorylation of the nucleoside analogues to their triphosphate form. Therefore, the cytotoxicity of the RR inhibiting nucleoside analogues is enhanced by self-potentiation.32, 40, 41

Although there are multiple target sites for the action of nucleoside analogues, and the spectrum of activity at different sites differs for different analogues, the incorporation of analogues into cellular DNA and the subsequent termination of DNA synthesis appear to be the most prominent modes of action for all analogues. Experiments carried out on a variety of cell lines in vitro incubated with different nucleoside analogues indicate that the incorporation of the nucleoside analogues into DNA correlates with cytotoxicity.38, 42 These observations led to the hypothesis that incorporation of nucleoside analogues into DNA may be the key event in the initiation of apoptosis.38, 40

Unlike other antineoplastic drugs which only exert cytotoxicity in proliferating cells, PNAs act cytotoxically in both mitotic and quiescent phases of the cell cycle. Because quiescent cells are not actively replicating, incorporation of nucleoside analogues into DNA through DNA replication is obviously not a key event in drug induced apoptosis. However, as the length of time it takes for analogues to induce apoptosis in dividing cells is rapid (4-5 hours) and the drug induced cell death of quiescent cells takes considerably
longer (24-48 hours) this suggests that there are different drug induced apoptotic pathways in the two populations.\textsuperscript{40}

Although quiescent cells are not actively replicating their genome, they do possess single stranded DNA breaks; therefore, nucleoside analogues can be incorporated into the DNA during the repair synthesis. However, because the rate of incorporation of analogue into DNA is much slower than by replication synthesis in proliferating cells, a longer time is required for the quiescent cells to accumulate sufficient analogue to trigger apoptosis.

When DNA breaks occur, \textit{id est} when DNA is damaged, an enzyme called poly(ADP-ribose) polymerase (PARP) is activated. If the DNA is mildly damaged, PARP orchestrates the reparations leading to cell survival, however, if DNA is severely damaged PARP mediated nicotinamide adenine dinucleoside (NAD\textsuperscript{+}) depletion occurs leading to subsequent ATP depletion. The result is \textit{in vitro} cell death by a necrotic pathway but \textit{in vivo} the apoptotic cell is rapidly phagocytosed.\textsuperscript{37}

Disruption in DNA integrity whether it is in proliferating or quiescent cells, ultimately leads to the production of p53. p53 is a tumour suppressor protein that protects the genome from mutation by either eliminating damaged cells through the induction of apoptosis or by facilitating DNA repair through cell cycle arrest. Purine analogues have been shown to be potent activators of p53 in both thymocytes\textsuperscript{43} and CLL cells.\textsuperscript{37} If extensive DNA damage is evident the concentration of p53 increases leading to stimulation of pro-apoptotic proteins, which cause changes in the mitochondria of the cell leading to production of cytochrome c or apoptosis inducing factor. Cytochrome c released into the cytosol binds to apoptotic protease activating factor-1 (APAF-1) and procaspase 9 forming a complex known as an apoptosome. Formation of the apoptosome in turn leads to activation of the caspase cascade and subsequently apoptosis, (caspases are protease enzymes which destroy the cells protein).

In 2000, Gennini \textit{et al.} demonstrated that CdA and CAFdA (but not FA) can directly disrupt the integrity of mitochondria from primary chronic lymphophatic leukemia (B-CLL) cells, leading to the release of pro-apoptotic mitochondrial proteins cytochrome c and apoptosis inducing factor.\textsuperscript{44} The mitochondrial effects of CdA and CAFdA may
explain why these drugs are toxic to CLL cells at concentrations 5- to 10-fold lower than FA.  

There is a final extrinsic route by which PNAs can induce cell death. Here, PNAs stimulate death activator proteins which can bind to cell membrane proteins called tumour necrosis factor receptors. This triggers a signaling process initiating apoptosis. A diagrammatic illustration of the different mechanisms of action of PNAs is illustrated in Figure 1.24.

![Diagram of mechanisms of action of PNAs](image)

**Figure 1.24:** Mechanism of action of CdA, FA and CAFdA.

1.6.2 **Pyrimidine Nucleoside Analogues (PyNA)**

Pyrimidine nucleoside analogues are also clinically important anticancer drugs. PyNAs are not only essential components in the arsenal against hematological
malignancies; they are active against solid tumours as well. These agents act as antimitabolites, competing with physiological pyrimidine nucleosides and behave similarly to PNAs in their mode of action – exerting cytotoxicity by disrupting DNA synthesis and inducing apoptosis. Two PyNAs currently approved by the US FDA are Cytarabine (ara-C) and Gemcitabine (dFdC), see Figure 1. 25.

Cytarabine is used in the treatment of acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myloid leukemia (CML) and meningeal leukemia. Gemcitabine is used as a therapeutic in non-small cell lung cancer, pancreatic cancer, bladder cancer and breast cancer. As was the case for the PNAs the PyNAs must be phosphorylated once inside the cell by dCK to their active triphosphate forms. Unlike the PNAs and dFdC, ara-C is a good substrate for deaminase enzymes and is readily metabolised to an inactive form arabinofuranosyluracil. Although ara-C and dFdC are structurally similar, there are major differences in the antitumour activity of these compounds. ara-C is one of the reagents of choice for the treatment of adult acute myelogenous leukemia but it has limited activity against most solid tumours because of its inactivation by deaminase enzymes. In contrast, dFdC has a broad spectrum of activity against a variety of solid tumours. ara-C exerts its antineoplastic activity through inhibition of DNA polymerase resulting in disruption of DNA formation. dFdC on the other hand is a fair inhibitor of DNA polymerase but its metabolites are excellent inhibitors.
of RR and so it can enhance its own cytotoxicity by self-potentiation, and the result is the induction of apoptosis.
1.7 Strategies for improving nucleoside analogues

Deeper understanding of the metabolism and mechanism of action of nucleoside analogues has created opportunities for improving their antitumour efficacy. By understanding what reduces the cytotoxic capacity of a drug, steps can be taken to reduce its inactivation and enhance its potency. For example, different combinations of NAs or other cytotoxic drugs potentiate the activity of NAs by increasing drug triphosphate formation. One example of such a combination therapy is the administration of FA and ara-C. FA-TP exerts its cytotoxicity by inhibiting several critical enzymes involved in DNA synthesis and repair, including DNA polymerase, RR and DNA ligase (amongst others). FA-TP potentiates dCK activity and because of this it is considered a good candidate for combination with other NAs that require dCK for their activation. Because ara-C is a good substrate for cytidine deaminase and is readily converted to inactive arabinofuranosyluracil it is essential that ara-C is converted to its active metabolite and exerts its cytotoxic effect at a greater rate than that of its deactivation. Prior administration of FA before ara-C enhances ara-CTP accumulation in leukemic blast cells. This combination regimen has proved particularly successful in the treatment of AML. Several other combination therapies are also under investigation, for example the use of NAs in combination with DNA-damaging agents, such as cis-platin. Inhibition of DNA repair by NAs may also increase accumulation of DNA lesions induced by DNA-damaging agents, and slow their removal, thereby potentiating cytotoxic effects.

Since the approval of cytarabine in 1969, considerable progress has been made in the field of cytotoxic NAs as cancer therapeutics. Several novel therapies have been created with different modes of action, which act cytotoxically in both mitotic and quiescent phases of the cell cycle. However, the use of NAs in the clinic is limited by primary and acquired resistance. The area of resistance to cytotoxic NAs has been an active area of study during the last four decades and has been extensively reviewed. The advent of combination therapies has led and may lead, to the creation of highly effective combination regimens, which helps offset the development of resistance.
However, because of the adverse side effects and limited specificity of NAs, novel drugs with enhanced selectivity and new routes of administration are required.

In 1996 the highly active antiretroviral therapy (HAART) programme for HIV, based on a combination of different antiviral drugs, was developed. Over the past decade or so, HAART has gradually evolved from drug regimens with more than 20 pills daily (including stavudine, lamivudine and indinavir) in 1996, to 3 pills daily (zidovudine/lamivudine (Combivir) twice daily and efavirenz once daily) in 2003, to 2 pills daily (emtricitabine/tenofovir disoproxil fumarate (Truvada) and efavirenz) in 2004, and finally one pill daily in 2006 (tenofovir disoproxil fumarate/emtrictabine/efavirenz (Atripla)). These combination regimens are proving highly effective in delaying the evolution of nucleoside analogue resistant strains of HIV. Even the co-administration of two different drugs belonging to the same class requires more than one mutation to develop to infer resistance to the treatment. Treating with one particular nucleoside analogue leads to the evolution of mutations in reverse transcriptase. In resistant strains RT must retain the ability to incorporate normal dNTPs reasonably well, yet have an enhanced ability to discriminate against the DNA-TPs. This enhanced discrimination can either occur in the incorporation step or, alternatively, the enzyme can selectively excise the analogue after it has been incorporated. Resistance to the analogue 3TC, for example, involves an enhanced discrimination at the incorporation step and usually arises by either an M184I or M184V mutation in HIV-1; both of these mutations selectively interfere with the incorporation of 3TCTP. The underlying mechanism is steric hindrance; a β-branched amino acid at position 184 clashes with the oxathiolane ring of 3TCTP. Mutagenesis is not a stagnant phenomenon; therefore antiviral treatments must likewise evolve to combat the development of “super-viruses”. As previously mentioned, with the advent of cheap travel there is an underlying risk that new and rare viruses which have up until now been contained due to population size, could emerge and like the plagues of the middle ages have a devastating effect on humanity. Clearly, new therapies with novel mechanisms of action or unique resistance profiles are seriously needed and the administration of both current and future drugs from the antiviral repertoire should be carefully monitored to avoid the development of resistant variants.
1.8 Modified Nucleoside Analogues

Modification of conventional nucleosides can produce nucleoside classes with very specific enzyme targets. In the quest for effective antiviral and antitumour agents, a variety of strategies have been devised to synthesise nucleoside analogues. These strategies have involved several modifications from the naturally occurring nucleosides, but can be divided into three categories: (i) phosphate modified (ii) base modified and (iii) sugar modified. The phosphate modified nucleosides are a more recent development and most of the more commonly known active nucleosides are either base or sugar modified. The main focus of this thesis is sugar modified nucleosides but a brief overview of the other classes is presented first.

1.8.1 Phosphate Modified Nucleosides

The intracellular level of deoxynucleotide triphosphates is maintained through the action of kinase and nucleotidase enzymes. In general, NAs must be phosphorylated to their triphosphate form via three phosphorylation steps by cytosolic enzymes in order to exert their therapeutic effect. The initial phosphorylation step is often the rate limiting step in this sequence of events. To circumvent this issue, various phosphorus modified nucleotide analogues that do not require the first phosphorylation step were investigated. One such group exhibiting broad spectrum antiviral activity is the acyclic nucleotide phosphonates (ANP). The US FDA has approved three acyclic nucleoside phosphonates so far (see Figure 1.26).

ANPs behave in a similar manner to 2',3'-dideoxynucleotide analogues but unlike the original ddNAs (such as ACV, AZT and 3TC) they do not require the initial phosphorylation needed for activation. They are phosphorylated by diphosphate and triphosphate kinases to their active metabolites - diphosphorylated ANPs (ANPpp), and are potent DNA polymerase inhibitors. The antiviral activity of ANPs is the result of the higher affinity of the ANPpp metabolite for viral DNA polymerases than for cellular DNA.
polymerases. Because the initial phosphorylation step is not dependent on viral thymidine kinase, (as is the case for ACV, BVDU etc.), they exhibit a more expansive spectrum of activity against DNA viruses, retroviruses and some RNA viruses.

![Chemical structures: Cidofovir, Adefovir dipivoxil, Tenofovir disoproxil fumarate](image)

**Cidofovir**
(S)-HPMPC

**Adefovir dipivoxil**
Bis(PO4)-PMEA

**Tenofovir disoproxil fumarate**
Bis(PO4)-PMPA fumarate

**Figure 1. 26:** US FDA approved acyclic nucleoside phosphonates

The phosphonate group is a bioisostere for the phosphate group and is sufficiently similar so as to be metabolised by diphosphate and triphosphate kinase enzymes. Because of the flexibility of the acyclic carbohydrate structure the ANPpps remain good substrates for DNA polymerase enzymes and the extra methylene group between the nucleobase and the carbohydrate moiety (see **Figure 1. 26**) gives added stability to the intermediate metabolites which are resistant to enzymatic degradation. The increased stability of ANPs lends them an enhanced antiviral response as compared with their acyclic nucleoside counterparts. They have considerably longer half-lives and therefore can be administered less frequently. For example, cidofovir only has to be administered once a week if given systemically or once a day if given topically.

The ANPs have a broad spectrum of antiviral activity. Cidofovir is effective against herpesviridae, adenoviridae and papovaviridae. Adefovir is unique in that it is active against both retro- and hepadna- viruses as well as herpesviruses. Tenofovir is particularly effective against hepadna- and retroviruses. De Clercq has reviewed the clinical potential of ANPs as therapeutics for DNA and retrovirus infections. Although, ANPs are the only
phosphate modified nucleoside analogues discussed here (in brief), there are others, for example, some phosphoramidate derivatives have exhibited potent anti-HIV activity.\textsuperscript{54}

**1.8.2 Base Modified Nucleosides**

Modification of the heterocyclic base moiety of nucleosides has been extensively studied and has proved to be an extremely effective method of potentiating biological activity. As mentioned previously, purine nucleosides such as deoxyadenosine are subject to deamination by adenine deaminase (ADA). This reduces their antitumour effect considerably. Fludarabine (FA), Cladribine (CdA) and Clofarabine (CAFdA) are all examples of purine analogues with halogen substitution in the 2 position. The 2-halogenated analogues of deoxyadenosine are potent antineoplastic agents but no longer serve as substrates for ADA.\textsuperscript{55}

Pyrimidine modified nucleoside analogues have also been developed. Brivudin \((E)-5-(2\text{-bromovinyl})\text{-uracil (BVDU)) which is a potent antiherpetic drug has a \((E)-2\text{-bromovinyl} substiutuent in the 5 position of the uracil base that is essential to its biological activity. The substituent has to have the “Entgegen” or \textit{trans} configuration; the “Zusammen” or \textit{cis} isomer is considerably less active. The potency and selectivity of BVDU in addition to its mode of action and activity spectrum have been extensively reviewed.\textsuperscript{23, 24, 56} In a comparative study of several antiherpetic drugs against VZV, BVDU proved to be greater than one thousand fold more potent \textit{in vivo} than ACV.\textsuperscript{24}

The use of other heterocyclic bases has also been studied. For example, nucleoside analogues with a 5-membered heterocyclic base in place of the nucleobase, once phosphorylated to their monophosphate form mimic 5’-phosphoribosyl-5-aminimidazole-4-carboxamide (AICAR) which is involved in the \textit{de novo} biosynthesis of purine nucleotides (see Figure 1.\textsuperscript{27}) and inhibit some of the key enzymes involved in purine biosynthesis. Inhibitors of IMP dehydrogenase have exhibited a broad spectrum of biological activity including antiviral and anticancer activities.\textsuperscript{57} One of the most notable members of this group is ribavirin which has broad spectrum antiviral activity against a variety of RNA viruses. Ribavirin is used in the treatment of respiratory syncytial virus
and Lassa virus infections and is used in combination with pegylated interferon for the treatment of hepatitis C.\textsuperscript{58,59} The structure of Ribavirin is illustrated in \textbf{Figure 1. 28}

\centering
\includegraphics[width=0.5\textwidth]{Ribavirin_structure.png}

\textbf{Figure 1. 28}: The structure of the anti-HCV agent, Ribavirin
Figure 1.27: Outline of de novo synthesis of GMP and AMP
1.8.3 \textbf{Sugar Modified Nucleosides}

1.8.3.1 \textit{2',3'-Dideoxynucleotides}

Modification of the carbohydrate moiety of nucleosides has been extensively studied and reviewed.\textsuperscript{50, 60-62} When the potential of NAs as important medicinal therapeutics was realised, the quest to improve their biological response began. The development of NAs with low toxicity that selectively inhibit kinase and polymerase enzymes was required in order to increase the therapeutic arsenal to combat viral and neoplastic diseases. Modification of the 2'- and 3'- positions of the sugar ring has resulted in compounds with a broad range of biological activity. Zidovudine (AZT), the first compound approved for the treatment of AIDS is a 2',3'-dideoxynucleoside (ddN). AZT (as mentioned previously) is a HIV-RT inhibitor. The azide group in the 3'- position of AZT prevents chain elongation once the active form AZT-TP is incorporated by HIV reverse transcriptase into a growing chain. There are certain side effects associated with the prolonged use of AZT including anemia and leucopenia however it is considered that these complications are outweighed by the clinical benefits achieved.\textsuperscript{63} Soon after AZT was reported in clinical use two other ddNs, zalcitabine (DDC) and didanosine (DDI) were discovered to act as inhibitors of HIV. Both were approved for use by the US FDA for the treatment of HIV infections in the early 1990s.

1.8.3.2 \textit{Acyclic NAs}

Acyclic nucleoside analogues are modified ddNs. As their name implies they do not possess a complete sugar ring. There are several acyclic guanosine analogues \textit{id est}: acyclovir, penciclovir, ganciclovir and their oral pro-drug forms valaciclovir, famciclovir and valganciclovir, and all of these compounds exert their antiviral activity by inhibiting DNA polymerase once phosphorylated to their active triphosphate form.
I.8.3.3 Configurationally Modified NAs

Cytarabine is also a sugar modified NA. The sugar moiety of naturally occurring nucleosides is generally ribose or 2’-deoxyribose, cytarabine has an arabinose sugar structure and inhibits DNA polymerase very efficiently once its triphosphate form ara-CTP is incorporated into a growing DNA chain. The cytarabine analogue gemcitabine has the same sugar configuration however it has two geminal fluorine atoms in the 2’-position. These atoms change the mode of action of the drug, and affect its antineoplastic activity against a variety of solid tumours. The modifications render gemcitabine resistant to degradation by deaminase enzymes and in addition a potent inhibitor of ribonucleotide reductase (RR) enabling it to increase its own cytotoxicity by self-potentiation, unlike cytarabine.

I.8.3.4 Carbocyclic NAs

Greater understanding of metabolism has guided structural based design of new NAs. The issue of degradation of the N-glycosyl bond by phosphorylase and hydrolase enzymes was addressed by modifying the carbohydrate moiety. Replacing the oxygen of the furanose ring with a methylene unit furnishes carbocyclic NAs which are no longer subject to in vivo degradation by phosphorylases and hydrolases. When the natural carbocyclic nucleosides aristeromycin and neplanocin A (see Figure 1. 29) were discovered to exhibit both antitumour and antibacterial activity the hunt for novel carbocyclic nucleosides with enhanced biological activity began.62
In 1998, Abacavir became the first member of the carbocyclic NAs to be approved by the US FDA as an anti-HIV drug. Abacavir (a prodrug form of carbovir) is initially phosphorylated by adenosine phosphotransferase to form abacavir-5'-monophosphate; the next step is deamination by a cytosolic enzyme to form carbovir-5'-monophosphate and subsequent phosphorylation reactions by cellular kinases afford the active metabolite carbovir-5'-triphosphate. Carbovir-TP is a competitive inhibitor of HIV-RT, competing with the natural substrate dGTP and once incorporated into a developing DNA chain acts as a chain terminator. Although carbovir-TP is a potent inhibitor of HIV-RT it is a poor substrate for cellular DNA polymerase, making it a highly selective drug. The initial phosphorylation step forming abacavir-MP is different to that of any other NRTIs; this suggests that competition for phosphorylation with other NRTIs is unlikely and highlights the benefits of utilizing combination regimens to combat viruses. Several combination regimens containing Abacavir have been developed including Trizivir (abacavir sulfate/lamivudine/zidovudine) and Epzicom (abacavir sulfate/lamivudine) which were approved by the US FDA in 2000 and 2004 respectively.

### 1.8.3.5 Azanucleoside Analogues

When the oxygen of the sugar ring is replaced by nitrogen the resulting NAs are referred to as azanucleosides. Azanucleosides are an important class of NAs and the synthesis and biological activities of several azanucleosides was reviewed by Yokoyama et al. The discovery of Nojirimycin - a potent glycosidase inhibitor, in which the oxygen
of β-D-glucosylpyranose is replaced by nitrogen, has led to increasing interest in similar alkaloidal sugar mimics.⁶⁵

In 1994, Altman et al. reported the synthesis and incorporation of N-acetyl azathymidine (see Figure 1. 30) into oligonucleotides and commented on the potential of azanucleotides as antisense therapeutics for protein inhibition. The concept of using antisense oligonucleotides for the inhibition of protein synthesis aims to exploit the selective hybridisation of an antisense oligonucleotide to a complementary base sequence on an appropriate messenger RNA in order to block the expression of a disease related protein. Since natural oligonucleotides are rapidly degraded under physiological conditions, more robust oligonucleotides are required to determine the therapeutic potential of the antisense approach.

![N-acetyl azathymidine](image)

**Figure 1. 30:** Structure of N-acetyl azathymidine

Altman et al. discovered that oligonucleotides containing N-acetyl azathymidine hybridise to complementary RNA with similar specificity as the corresponding wild type oligodeoxynucleotides. They also investigated the resistance of the azathymidine containing oligonucleotides to degradation by 3'-exonucleases in 10% heat-inactivated fetal calf serum and found that the modified oligonucleotide displayed a >20 fold increase in nuclease resistance than the corresponding wild type oligonucleotide.

In the last few years, several azanucleosides have been synthesised, where the carbohydrate moiety is linked to the nucleobase via the heteroatom. Oxetanocin A is isolated from *Bacillus megaterium* NK84-0218, and is a broad spectrum nucleoside
antibiotic which also inhibits HIV which contains an oxetane ring in place of the usual furanose ring system.\textsuperscript{66} Its mechanism of action is thought to be disruption of DNA synthesis by inhibition of DNA polymerase. Nishiyama \textit{et al.} synthesised two oxetanocin-A analogues which exhibited potent anti-HIV activity at approximately the same level as ddi\textsuperscript{67} (see \textbf{Figure 1.31}). Further research may yield other potentially useful therapeutics.

\begin{center}
\includegraphics[width=\textwidth]{figure1_31.png}
\end{center}

\textbf{Figure 1.31:} \textit{[2',3'-(S,S)-(bishydroxymethyl)-azetidin-1-yl] purine nucleosides: Analogues of Oxetanocin-A}

\textit{4'-Selenonucleoside Analogues}

In 2008, 4'-selenonucleosides were synthesised for the first time, by Jeong \textit{et al.}\textsuperscript{68} Selenium, like the methylene group of carbocyclic NAs is a bioisostere of oxygen. 4'-Selenouridine, unlike its oxygen based counterpart, where the dominant driving force leading to the \textit{N} (North) conformation in the purine base is the anomeric effect, has a \textit{S} (South) conformation. This is due to steric hindrance caused by the considerably larger selenium atom.\textsuperscript{68} This unusual conformation may have potential therapeutic value and requires further investigation. Jeong\textsuperscript{69} and co-workers synthesised several 2',3'-didehydro-2',3'-dideoxy-4'-selenonucleosides (4'-seleno-d4Ns) analogues (\textit{vide infra}), of the potent HIV-RT inhibitor stavudine (d4T), and evaluated their anti-HIV activity in MT-4 cells; unfortunately they showed neither anti-HIV activity nor cytotoxicity up to 100 \(\mu\)M. The reason(s) for their loss in anti-HIV activity as compared with their d4T counterparts is unclear. It may be that they are poor substrates for kinase enzymes and are not easily

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converted to their triphosphate form or that the triphosphate form does not inhibit HIV-RT.\textsuperscript{69} This is currently under investigation in the Jeong group.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{selenium_analogues.png}
\caption{Selenium analogues of stavudine prepared by Jeong and co-workers\textsuperscript{69}}
\end{figure}

\subsection{4'-Thionucleoside Analogues}

Sulfur also has a bioisosteric relationship with oxygen and selenium. NAs where the oxygen of the sugar ring is replaced by a sulfur atom are referred to as 4'-thionucleosides (4'-TNAs). These compounds have exhibited a broad spectrum of biological activities including antibiotic,\textsuperscript{70} antiviral\textsuperscript{71} and antitumour\textsuperscript{72} and show considerable potential as therapeutics for numerous diseases. Resistance to degradation by phosphorylase and hydrolase enzymes enhances their intracellular half-lives and makes them a potentially attractive alternative to their oxygen based counterparts.
1.9 4'-Thionucleoside Analogues (4'-TNAs)

In 1972 Bobek et al. synthesised a number of 4'-thio analogues of the antibiotic toyocamycin in an attempt at decreasing its toxicity. They made structural modifications to the compounds they tested replacing the oxygen of the sugar ring with a sulfur atom and substituting the 6 position of the heterocycle ring with bromine or amino groups. They tested the effect of the 4'-thionucleosides on the growth of *Streptococcus faecium* and leukemia L-1210 cells.

![Figure 1.33: 4'-Thiotoyocamycin analogues](image)

In the bacterial test systems 4'-thiotoyocamycin proved to be approximately 10 times more potent while the 4'-thio-6-amino derivative was 3 times more effective than toyocamycin. The 6-bromine derivative however was inactive at >10^{-3} M. The active 4'-thio analogues were also equally effective against toyocamycin and tubercidin resistant strains of *S. faecium*. However all of the 4'-thionucleosides proved less active than toyocamycin against the leukemia L-1210 cells although the 6-bromine derivative which was inactive in the bacterial system, was markedly inhibitory of the tumour cell growth.
In 1991, Walker et al. synthesised E-5-(2-bromovinyl)-4'-thio-2'-deoxyuridine (S-BVDU) the 4'-TNA of the anti-herpetic agent BVDU and tested its antiviral activity. BVDU is a potent and selective inhibitor of HSV-1 and also of VZV replication and its antiviral activity also encompasses several other herpesviruses (see section 1.5.4). In a comparative study of various anti-herpes drugs against VZV, BVDU proved to be approximately one thousand fold more effective compared to acyclovir - the EC$_{50}$ (50% effective concentration) of BVDU was 0.0024 µg/mL, as compared to 4.64 µg/mL for acyclovir. BVDU has a high therapeutic index of ca. 10000 due to a combination of its efficacy and low toxicity as a result of its selective phosphorylation to BVDU-MP and BVDU-DP by viral thymidine kinases. However, BVDU is rapidly degraded by pyrimidine phosphorylase in vivo into E-5-(2-bromovinyl)-uracil (BVU) and 2-deoxyribose-1-phosphate. S-BVDU on the other hand is a poor substrate for pyrimidine phosphorylase so it is not rapidly degraded and it retains the potent antiviral activity of its oxygen counterpart. In vitro, the anti-herpetic activity of S-BVDU was shown to be essentially the same as that for BVDU, however, in vivo, because it is more robust and less subject to degradation, very high serum levels of S-BVDU were achieved and maintained for several hours. Therefore a lower dose of S-BVDU is required to effect the same results. It is noteworthy that BVU itself is a potent inhibitor of dihydrothymidine dehydrogenase (DPD) - an enzyme involved in the de novo biosynthesis of pyrimidines. This intracellular degradation of BVDU to form BVU has been exploited to potentiate the activity of 5-fluorouracil in anticancer therapy.
In 1991 Secrist et al. synthesised several 2'-deoxy-4'-thiopyrimidine nucleosides and tested their biological activity. The compounds were tested for in vitro activity against three neoplastic cell lines: murine leukemia L-1210, human epidermoid carcinoma #2 and human T-cell leukemia CCRF-CEM. The most cytotoxic compound was 4'-thiothymidine but 2'-deoxy-4'-thiocytidine and 2'-deoxy-4'-thiouridine also exhibited some cytotoxicity (see Figure 1.35). 4'-Thiothymidine had IC$_{50}$ values of 0.12 µM against L-1210 cells, 0.14 µM against H-Ep-2 and 0.67 µM against CCRF-CEM cells.

The compounds were also tested for antiviral activity and 4'-thiothymidine exhibited activity against human cytomegalovirus in MRC5 cells that was comparable to ganciclovir however because this NA is cytotoxic to rapidly dividing cells it is unlikely that this compound would exhibit in vivo activity at tolerated doses.

In 2000, Secrist et al. published a concise synthesis of 4'-thio-β-D-arabinofuranosylcytosine (T-ara-C) and did a comparative study of its anticancer activity with that of ara-C. Athymic nude mice were implanted subcutaneously with eight different human tumour cell lines. T-ara-C proved to be cytotoxic to most human tumour cell lines at micromolar concentrations but ara-C was in general more cytotoxic. However, when T-ara-C was administered intraperitoneally for 9 consecutive days either as a single daily injection or three injections per day - one every 4 hours, it exhibited potent activity against HCT-116 colon and CAK-1 renal tumours. T-ara-C treatment proved curative against HCT-116 and caused complete regression of CAK-1 renal tumours. In contrast ara-C proved to be much less active at the maximum tolerated dose.
Parker et al. later that year elaborated on the metabolism of T-ara-C in CEM cells. They carried out a comparative study with ara-C, an agent that is currently used in the treatment of AML and analyzed differences in their modes of action. Although the mechanism of action of ara-C and T-ara-C were largely similar, there were several dissimilarities: (i) T-ara-C was phosphorylated to the active metabolite T-ara-CTP at 1% the rate of ara-C; (ii) T-ara-CTP was 10- to 20- fold more potent an inhibitor of DNA synthesis than ara-CTP; (iii) the half life of T-ara-CTP was twice that of ara-CTP; (iv) T-ara-C was a poorer substrate for cytidine deaminase than ara-C, and the monophosphate analogues also differed in their catalytic efficiency with deoxycytidine 5'-monophosphate deaminase, the enzyme having a greater affinity for ara-CMP than T-ara-CMP.

The active metabolite ara-CTP causes the induction of apoptosis by inhibiting DNA synthesis which occurs mainly in the S phase of the cell cycle. Solid tumours have a very low percentage of cells in the S phase of the cell cycle and as ara-C and its phosphates are quickly metabolised by deaminase and phosphorylase enzymes, the accumulation of active metabolite in the S phase of the cell cycle is minimal ergo ara-C is not as potent an antineoplastic agent in solid tumours. This is where 4'-thionucleosides offer a huge advantage over their oxygen based counterparts by virtue of their vastly improved metabolic stability. T-ara-C has a longer half life and so sufficient quantities accumulate by the S phase of the cell cycle to exert a cytotoxic effect in solid tumours. These results indicate how relatively minor structural changes can have a considerable effect on drug metabolism and biological activity.

![Figure 1.36: Structures of lamivudine stereoisomers](image-url)
In 1992, Coates et al. discovered that the unnatural L-isomer of (±)-2,3-Dideoxy-3'-thiacytidine ((±)-BCH-189) was more potent and less toxic than its D-isomer.79 (±)-BCH-189 is an anti-HIV agent which was originally synthesised as a racemate by Belleau and co-workers in 1989.80 The L-isomer of (±)-BCH-189, (also known as lamivudine or 3TC), has been approved by the US FDA; for the treatment of AIDS in 1995 and HBV in 1998.

3TC was found to be more potent in peripheral blood mononuclear (PBM) cells \( (EC_{50}=1.8 \text{ nM}) \) than either the racemate \((±)-BCH-189 \ (EC_{50}=0.02-0.06 \ \mu M) \) or the D-isomer alone \((+)-BCH-189 \ (EC_{50}=0.21 \ \mu M) \) against HIV-1.81 3TC also was a superior anti-HBV agent \( (EC_{50}=0.01 \ \mu M) \) than its racemate \( (EC_{50}=0.05 \ \mu M) \) and was markedly less toxic.82 Since then several L-nucleoside analogues have been synthesised and biologically evaluated and in 2006 the US FDA approved telbivudine \( (vide \ infra) \) for the treatment of hepatitis B virus (HBV). Telbivudine triphosphate inhibits HBV DNA polymerase by competing with its natural substrate thymidine 5'-triphosphate, resulting in the inhibition of HBV replication.83 Importantly, telbivudine 5'-triphosphate does not inhibit human DNA polymerases, which has been associated with the mitochondrial toxicity of some other nucleoside analogues used in antiviral therapy83 - mitochondrial toxicity including cases of pancreatitis, lactic acidosis with liver failure and hepatic steatosis was experienced by a subset of patients co-infected with both HIV and HCV which were treated with ribivarin in combination with didanosine.84

![Figure 1.37: Structure of Telbivudine](image)

The benefits of biologically active unnatural L-isomer nucleosides include favourable toxicological profiles and greater metabolic stability (3TC is a poorer substrate...
than the D-isomer counterpart for deaminase enzymes; therefore its half life is increased). Consequently, Satoh *et al.* synthesised and evaluated the biological activity of L-4'-thioarabinonucleosides however they were considerably less potent than their D-counterparts. These results should however be taken into context, there were a considerable number of D-nucleoside analogues synthesised since the discovery of the potential of nucleosides as therapeutics which exhibited little or no biological activity, yet the more successful analogues are key components of the antiviral and anticancer repertoires. Therefore, further investigation and structural based design could produce several L-4'-TNAs with potent biological activities and possibly lower toxicities.

In the early 1990s, Culver and co-workers demonstrated the feasibility of using ‘suicide genes’ to alter the metabolic pathways of tumour cells in order to make them susceptible to chemotherapeutic agents. This technique involved using a retroviral-mediated gene transfer of the HSV thymidine kinase (HSVtk) gene into tumour cells. Retroviral-mediated gene transfer is limited in its usefulness because murine retroviruses stably integrate their genes only in actively dividing cells. Culver *et al.* exploited this inability to transfer genes into non-dividing cells as a delivery system to target proliferating tumour cells in an organ in which normal cells are not dividing. Working with rats carrying 9L glioma implanted in their brains Culver *et al.*, injected intratumourally HSV-tk retroviral vector producing murine fibroblasts by stereotaxic guidance. Once the tumour cells were transduced with the murine fibroblasts subsequent treatment with the antitherpetic agent ganciclovir (GCV), (which is phosphorylated to its monophosphate form by HSVtk and then to its di- and triphosphate forms by cellular kinases), caused tumour regression in the animals by inhibition of DNA polymerase *ipso facto* apoptosis.

Degreve *et al.* employed VZV thymidine kinase (VZVtk) as a pro-drug activating enzyme because of the fact that BVDU and various BVDU analogues including S-BVDU were up to 80- fold more potent *in vitro* inhibitors of VZV replication than of HSV-1 replication. S-BVDU has an EC<sub>50</sub> value of ~1 ng/mL against VZV. S-BVDU proved highly efficacious in this combined gene/chemotherapy approach, being highly cytotoxic to osteosarcoma cells containing the VZVtk gene, with an IC<sub>50</sub> value of 44 nM. The
enhanced stability of the glycosidic bond in S-BVDU prolongs the length of duration of the active metabolite and increases the cytotoxic effect of the drug as compared with BVDU.
1.10 Synthesis of 4'-Thionucleoside Analogues

1.10.1 Whistler et al. synthesis of T-ara-C

The synthesis of 4'-TNAs is quite well established and has been reviewed extensively, however the protocols employed to date often involve complex carbohydrate chemistry with numerous protection and deprotection steps resulting in laborious linear synthesis and removing the possibility of readily creating a library of compounds. Whistler et al. were the first group to report the synthesis of T-ara-C. The synthetic route is illustrated in Figure 1. 38 and Figure 1. 39. Starting with 1, which can be synthesised from D-glucose, (and is now commercially available), the 3'-hydroxy group was benzylated by treatment with sodium in dry diethyl ether to form the alkoxide followed by the addition of benzyl bromide. The 5,6-isopropylidene group of 2 was then selectively hydrolysed using aqueous acetic acid and the resulting diol was then acetylated affording 3 in 78% yield over two steps. Deacetylation of 3 was achieved using a catalytic quantity of sodium methoxide and the primary hydroxyl group was selectively benzoylated by treatment with benzoyl chloride in pyridine at -15 °C. The resulting compound 5 was tosylated using tosyl chloride in pyridine and compound 6 was isolated in 92% yield. The benzoyl group was then removed by treatment with methanolic sodium methoxide, affording a primary alkoxide anion which subsequently cyclised to form the epoxide 7. Thiourea was then introduced and reacted with the epoxide to form the episulfide 8 with consequent inversion of stereochemistry. The thirane was then ring opened with acetate at the least hindered position and isolated as the thioacetate 9 in 79% yield from compound 6.
The 1,2-isopropylidene group of compound 9, (an intermediate prepared by Whistler and co-workers for the synthesis of 5-thio-D-glucopyranose), was selectively hydrolysed using 50% aqueous acetic acid at 70 °C producing 10 as a mixture of anomers in 78% yield and the undesired 6-membered ring side product 11 in approximately 16% yield. The compounds were separated by column chromatography and 10 was treated with sodium periodate to furnish acyclic 12 by oxidative cleavage. Subsequent treatment with 0.5% methanolic hydrogen chloride at reflux gave anomeric 2,5-di-O-benzyl-4-thio-D-arabinofuranoside. The α- and β- anomers were formed in a ratio of 1:1.2 and were easily
separated by column chromatography. Debenzylation of the desired β anomer – 13 was achieved employing sodium in liquid ammonia affording 15 quantitatively.

![Chemical Structure]

(i) BzCl, Py; (ii) HBr, AcOH; (iii) trimethylsilyl-N-acetyl cytosine; (iv) 2,4-Bis trimethylsilyl uracil; (v) P2S5, Py; (vi) MeOH, NH3

**Figure 1.39:** Whistler et al. synthesis of T-ara-C

Benzoylation of the thiosugar 15 was executed by treatment with benzoyl chloride and the anomeric methoxy group was substituted for a bromine using hydrogen bromide in glacial acetic acid (using a procedure previously described by Ness and Fletcher) yielding 17 and a small quantity of the α anomer. Whistler et al. completed the synthesis of the desired T-ara-C via two routes as illustrated in **Figure 1.39.** The first route involved direct condensation of 17 with trimethylsilyl-N-acetylcytosine forming an anomeric mixture from which the β-D anomer 18 crystallises. Subsequent treatment with a saturated solution of ammonia in methanol afforded T-ara-C (21). The second route involved the formation of an anomeric mixture of T-ara-U. Incorporation of the nucleobase was achieved in a similar
manner, this time employing \textit{bis} trimethylsilyl-uracil giving 19. The \(\beta\)-D anomer predominated and was isolated by crystallisation; subsequent treatment with phosphorus pentasulfide formed the corresponding thio derivative 20. Exposure of compound 20 to a saturated solution of ammonia in methanol gave T-ara-C (21) in 23\% overall yield (from compound 15).

\subsection{1.10.2 Tiwari et al. synthesis of T-ara-C}

The convoluted nature of the synthetic route described by Whistler \textit{et al.} to T-ara-C (18 steps) only afforded a sufficient quantity to evaluate its cytotoxicity in a single cell line (KB\textsuperscript{1}, \(EC_{50} = 0.42\, \mu\text{M}\)).\textsuperscript{76} No further biological testing was performed until the end of the 1990s when Tiwari \textit{et al.} developed a more concise synthetic procedure that afforded gram quantities of T-ara-C. This enabled Tiwari \textit{et al.} to evaluate the \textit{in vitro} and \textit{in vivo} activities of T-ara-C against a range of human cancers. A comparative study of the biochemical pharmacology of T-ara-C and ara-C was performed to explain the differences in antitumour activity of the two compounds.\textsuperscript{76}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Tiwari synthesis of 4-Thio-D-arabinofuranose}
\end{figure}

(i) MeOH, HCl; (ii) BnBr, NaH, Bu\textsubscript{4}NT; (iii) BnSH, SnCl\textsubscript{4}; (iv) Ph\textsubscript{3}P, Imidazole, I\textsubscript{2}; (v) Hg(OAc)\textsubscript{2}, AcOH.

\textsuperscript{1} KB cells are derived from a human carcinoma of the nasopharynx.
Tiwari's synthesis exploited the work of Secrist et al. who described the synthesis of intermediate compounds 25 and 26 (see Figure 1. 40) in a synthetic protocol for the synthesis of 4'-Thio-D-arabinofuranosylpurine nucleosides.\(^9\) Compound 26 is a readily accessible intermediate that can be coupled with a variety of nucleobases affording a library of 4'-thioarabinino NAs with both α and β anomers available for biological testing.\(^9\) Treatment of L-xylose with methanolic hydrogen chloride followed by tris benzylolation of the resulting triol afforded furanoside 23. Conversion to the acyclic dibenzyl thioacetal, was accomplished by exposure to benzyl mercaptan and stannic chloride and stirring at room temperature overnight. The next step involved cyclisation at C-4 with a single inversion generating the D-arabino configuration. This was achieved by treatment with triphenylphosphine, iodine and imidazole resulting in spontaneous cyclisation with inversion of the C4 centre forming thioacetal 25 with the correct D-thioarabinose stereochemistry. The synthesis of intermediate 26 was completed by treatment of 25 with mercuric acetate in acetic acid. The protected thioarabinose derivative was isolated in 30% overall yield.\(^9\)
The intermediate 26 was obtained as a ca. 1:1 mixture of α and β anomers, which is considerably superior to a previously reported synthesis by Yoshimura et al. which required 13 steps beginning with 1,2:5,6 di-O-isopropylidene-α-D-glucofuranose and had overall yield of <10%.^2^ The intermediate 26 was obtained as a ca. 1:1 mixture of α and β anomers, which is considerably superior to a previously reported synthesis by Yoshimura et al. which required 13 steps beginning with 1,2:5,6 di-O-isopropylidene-α-D-glucofuranose and had overall yield of <10%.^2^

Tiwari and co-workers devised two alternative protocols for the synthesis of T-ara-C from 26. The first involved generating silylated cytosine in situ which was accomplished by treating cytosine with hexamethyldisilazane (HMDS) and then introducing trimethylsilyl chloride (TMSCl). The silylated cytosine was coupled with 26 using trimethylsilyl triflate (TMSOTf) as a catalyst producing a 2:1 mixture of α and β anomers of tri-O-benzyl cytosine nucleoside 27 in 77.5% yield. Deprotection was necessary in order to separate the anomers. Boron trichloride was employed to debenzylate 27 and compound 28 was isolated in 85% yield as a mixture of anomers; subsequent
treatment with 4,4'-dimethoxytrityl chloride in pyridine furnished the mono-protected 5'-O-dimethoxytrityl derivative 29 which enabled separation by column chromatography of the two anomers. β-29 was recovered in 90% yield based on the 2:1 anomeromic mixture of compound 28. Deprotection of β-29 was achieved using trifluoroacetic acid to give the desired T-ara-C (21) in 85% yield. The overall yield of T-ara-C was 5% from L-xylose.

In order to improve the anomic ratio (β:α), thiosugar 25 was coupled with silylated uracil using N-bromosuccinimide and molecular sieves in acetonitrile. This gave an enhanced ratio of α:β (1:1.15), however coupling of sugar 25 with cytosine as base using the same procedure gave a considerably lower yield of cytosine nucleoside. In addition separation of the uracil compound proved more facile than the corresponding cytosine compound. β-30 was converted to its 2,4,6-triisopropylbenzenesulfonate derivative β-31 using 2,4,6-triisopropylbenzenesulfonyl chloride, dimethylaminopyridine (DMAP) and triethylamine (TEA) in acetonitrile and conversion of the sulfonate to the cytosine analogue was accomplished by treatment of β-31 with ammonium hydroxide. The yield for this reaction was a disappointing 50% and so offset the improved anomic ratio. β-27 was deprotected using the previously employed boron trichloride procedure affording the desired T-ara-C (21) in a similar yield, ca. 5% overall.

The successful development of a synthetic route whereby gram quantities of T-ara-C were generated enabled biological evaluation of the compound and led to the discovery of its highly potent cytotoxic effects and its broad spectrum of activity in various tumour cell lines. The only structural difference between ara-C and T-ara-C is replacement of the oxygen of the sugar ring with a sulfur atom, yet they have profoundly different antitumour activities. The excellent broad spectrum in vivo activity exhibited by T-ara-C led OSI Pharmaceuticals Inc. (Melville, NY) to evaluate the effectiveness of T-ara-C in a number of patients with a variety of advanced refractory solid tumours, which they marketed as OSI-7836, in clinical trials. However in 2004 OSI Pharmaceuticals decided to halt further development of OSI-7836 because they were unable to overcome certain toxicity issues – fatigue was found to be the main dose limiting toxicity in one particular phase I clinical trial.
Nucleoside analogues have in general been synthesised using carbohydrate chemistry. However, the carbohydrate moiety has also been synthesised from achiral starting materials in de novo syntheses. Using non-carbohydrate, achiral starting materials, Uenishi et al. employed asymmetric synthesis to install the required stereochemistry in their synthesis of several 4'-thionucleoside analogues, see (Figure 1. 42). They employed Sharpless asymmetric epoxidation to form epoxide 38 and subsequently formed thiirane 40 with inversion of stereochemistry. Sharpless asymmetric epoxidation is a very powerful technique for generating chiral epoxides from allyllic alcohols and has been reviewed extensively. Uenishi et al. generated 37 in five steps starting with 3-benzylxoypropenal (32). Aldehyde 32 was initially protected as an acetal by treatment with triethyl orthoformate and zinc chloride. Debenzylation of 33 was achieved by exposure to lithium in liquid ammonia and a subsequent Swem oxidation of the resulting hydroxyl group afforded compound 34. Aldehyde 34 was then reacted with a β-stabilised phosphorus ylide in a Horner-Wadsworth-Emmons reaction to generate alkene 36 (any Z-alkene was removed at this point by column chromatography). Reduction of the methyl ester to the corresponding allylic alcohol was accomplished by treatment of 36 with diisobutylaluminium hydride and 37 was isolated in 63% overall yield (5 steps).

Sharpless asymmetric epoxidation of alkene 37 afforded the desired epoxide 38 in high enantiomeric excess (>95%) and in 69% yield. Treatment of 38 with potassium hydride and carbon disulfide generated xanthate 39 in 86% yield as a single stereoisomer. This stereospecific reaction involved initial formation of a potassium xanthate anion followed by ring opening of the epoxide in a 5-exo-tetragonal fashion. Silylation of the resulting secondary alcohol with tert-butyldisilylsilyl triflate followed by treatment with potassium carbonate in methanol provided episulfide 40 in 90% yield. The episulfide was then converted to the desired thiosugar 41 by treatment with sodium acetate in acetic acid. Compound 41 was isolated in 87% yield as approximately a 1:1 mixture of anomers, which were separated by column chromatography.
Figure 1. 42: Uenishi de novo synthesis of 4'-TNA

The nucleobase was subsequently introduced using silylated uracil and trimethylsilyl triflate in the usual manner and α-42 and β-42 were isolated in 23% and 35% yields respectively. Removal of the protecting group in β-42 was achieved in two steps, desilylation by treatment with tetrabutylammonium fluoride (TBAF) followed by basic hydrolysis of the acetyl group using potassium carbonate in methanol. The desired 4'-TNA β-43 was isolated in 75% yield.96
1.11 General synthesis of 4’-TNAs

The broad spectrum of disease, their potency, good specificity and increased metabolic stability (compared with their oxygen based counterparts) make 4’-TNAs attractive as drug candidates. However, to date their development has been hampered because no general convergent synthesis of such compounds has been published. In addition, most previous syntheses require carbohydrate based starting materials meaning that the production of the unnatural L-isomer can be prohibitively expensive. For example, D-ribose costs approximately €1000/kg whereas L-ribose costs €138000/kg (Sigma-Aldrich). This is of particular importance because some L-nucleosides have proved to be extremely potent anti-parasitic agents (cf. 3TC) with reduced side effects owed to the poor affinity of mammalian enzymes for the unnatural isomer substrates. In conclusion, 4’-TNAs are often more potent and selective than their oxygen based analogues and they are significantly more metabolically stable. Such a profile makes them ideal for further investigation and excellent candidates for drug development. Heretofore, no general synthetic route to 4’-thionucleosides has been developed that can readily supply both DNA and RNA analogues as either natural or unnatural stereoisomers. The focus of this project was to develop a key intermediate 4’-thiosugar, which could be readily manipulated, affording either L- or D- stereochemistry and a vast array of substitution patterns around the sugar ring. Minor alterations to the proposed methodology used in this project would permit access to a range of alternative heterosugars. It should be mentioned that the incorporation of nitrogen, selenium and other elements in place of the oxygen of the sugar ring is possible. However this was beyond the scope of this project. A versatile route to a common precursor to 4’-thiofuranoses is described in the following chapters.
1.12 Overview of Retrosynthetic Pathway

The aim of this project was to develop new stereoselective routes to a thiosugar intermediate which could be regio- and stereoselectively functionalised generating 4'-TNAs with a vast array of substitution patterns. In order to maximise the versatility of the key intermediate, both the natural and unnatural isomers should be obtainable without significant modification of the synthetic route, for this reason, stereochemical information was introduced, using chiral catalysis which could be modified accordingly, to furnish either stereoisomer. Several different synthetic strategies have been devised to prepare 44 but they fall into three general categories. The first disconnection involved installation of stereochemical information prior to ring formation (Disconnection 1). In the second route the desired α-hydroxy ketone motif was generated via desymmetrisation of a C2 symmetric tartaric acid derivative (Disconnection 2). The third route involved formation of the sulfur heterocycle followed by the introduction of stereochemistry (Disconnection 3).

![Figure 1.43: Retrosynthetic analysis for generation of key intermediate 44](image)

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Chapter 2

*De Novo* synthesis of Erythrulose
2 De novo synthesis of Erythrulose

2.1 Introduction

Traditionally carbohydrate chemistry has been employed to synthesise purine and pyrimidine nucleosides. Condensation of the imidazolo sodium salt with a halogenated sugar derivative found wide applications in the stereo- and regioselective synthesis of imidazole nucleosides.\(^{100}\) The Vorbruggen glycosylation, the reaction of silylated heterocyclic bases with peracylated sugars in the presence of Lewis acids to yield natural \(\beta\)-nucleosides,\(^{101}\) is also popular as is the Hilbert–Johnson reaction to yield pyrimidine nucleosides.\(^{102}\) The biological properties of 4'-thionucleoside analogues (4'-TNAs) were discovered long after those of nucleoside analogues (NAs) and as a result their synthesis was developed using methodologies previously employed to synthesise their oxygen based counterparts.

4'-TNAs are often more potent and selective than their oxygen based counterparts and they are significantly more metabolically stable. However, they are also quite difficult to synthesise, and several long and laborious syntheses of 4'-TNAs have been published.\(^{76,88}\) In order to maximise the medicinal potential of this class of compound the synthesis of libraries of compounds is essential, enabling high-throughput screening against a large number of diseases. To date, syntheses usually involve generating a thiosugar with fixed stereochemistry and coupling with a variety of nucleobases. The aim of this project was to develop a key intermediate which could be modified readily, affording thiosugars with a variety of substitution patterns and configurations with either natural or unnatural stereochemistry. The vast majority of syntheses reported thus far employ carbohydrate starting materials with existing stereochemistry and appropriate manipulation of the compounds generates the desired stereochemistry. This methodology has proved very successful and a large number of analogues have been synthesised in this manner, however, it may prove limited because of the reliance on the chiral pool to furnish compounds with appropriate
stereochemistry. For this reason, the decision to synthesise the proposed key intermediate compound 44 using chiral catalysis was made, thereby enabling synthesis of either stereoisomer.

![Scheme 2.1: Retrosynthetic analysis showing key intermediate 44](image)

This project describes the synthesis of key intermediate compound 44 which can be elaborated to generate libraries of nucleoside analogues (Scheme 2.1). Although the oxygen of the sugar ring was replaced with sulfur, the methodology could also easily be extended to synthesise azanucleosides, selenonucleosides and telluronucleosides, this is however beyond the scope of this particular project.

### 2.1.1 Elaboration of key intermediate 44

The intermediate can be elaborated by regio- and stereoselective functionalisation at any of the vacant sites. In this regard, a suitably protected alcohol can direct the stereochemistry of the system by blocking one face of the molecule or directing the attack to the same face through oxygen coordination to the incoming moiety. The synthesis of RNA and DNA monomer analogues with controlled regio- and stereochemistry is thus possible.

Scheme 2.3 outlines a general synthesis of a 4'-TNA from compound 44. Incorporation of a 5'-hydroxyl group is achieved by treating the intermediate with a suitable base followed by the addition of formaldehyde (or equivalent compound). Perhaps more importantly, different electrophiles may be employed to synthesise an expanded array of analogues for example those with an elongated chain (a class of
compounds yet to be explored in depth). Reduction of the ketone can be performed to provide either epimer of the differentially protected thiofuranose derivative. Selective protection of the initial hydroxyl groups can be used to direct the reduction, whether by coordination or by blocking access with steric bulk.

![Diagram](image)

**Scheme 2. 3: Elaboration of key intermediate**

The final step in the preparation of the 4'-TNA is to form the glycosidic link between the nucleobase and the sugar moiety. This could be achieved by employing Pummerer/Polonovski chemistry as was used by Naka *et al.* in their synthesis of a variety of purine 4'-TNAs. The introduction of the nucleobase (or an alternative nucleophile) in the final step is preferential given the possible sensitivity of the nucleobase and their inability to survive severe conditions; another advantage is that it permits a combinatorial approach to base/nucleophile incorporation on a specific framework.

Three general synthetic routes to intermediate 44 were proposed: **Disconnection 1**, where stereochemistry was introduced prior to ring formation;
Disconnection 2, which relied on desymmetrisation of a C2-symmetric tartaric acid derivative to generate the α-hydroxy ketone motif, and Disconnection 3, whereby cyclisation preceded the introduction of stereochemistry. Disconnection 1 is discussed in this chapter.

Scheme 2.4: Retrosynthesis overview
2.2 Retrosynthetic Analysis of Disconnection 1:

A detailed retrosynthetic analysis of Disconnection 1 is outlined in Scheme 2. It was envisaged that appropriate functionalisation of cis-2-butene-1,4-diol (49), with either molecular bromine or N-bromosuccinimide (NBS) and methanol, would produce dibromide 50 or bromoether 51 respectively. Subsequent dehydrohalogenation provides trisubstituted vinyl bromide 52 or vinyl ether 53, as single stereoisomers. Sharpless asymmetric dihydroxylation (SAD) of the trisubstituted olefin affords an enantioenriched α-hydroxy ketone 54, which when deprotected gives the natural sugar erythritol. Activation of the primary hydroxyl groups and treatment with sodium sulfide produces a thiolane derivative. The thiolane derivative can then be stereoselectively oxidised from the sulfide to the sulfoxide using meta-chloroperoxybenzoic acid (mCPBA), with the secondary hydroxyl in its unprotected or protected forms providing a chiral handle within the molecule.

Scheme 2. 5: Retrosynthetic Analysis of Disconnection 1

The key reaction in the synthetic sequence is a Sharpless asymmetric dihydroxylation which merits detailed discussion (vide infra).
2.3 Catalytic Asymmetric Dihydroxylation

The cis-dihydroxylation of olefins by osmium tetroxide was discovered early in the 20th century. However, osmium tetroxide is a very expensive reagent (Sigma-Aldrich; €289.5 per gram), making the stoichiometric reaction uneconomical for small scale reactions and prohibitively expensive for larger scale reactions. In 1912, Hoffmann was the first to report the possibility of doing the reaction catalytically using chlorate solutions as re-oxidants. A few years later, Criegee discovered that the rate of the stoichiometric reaction was enhanced considerably upon the addition of pyridine to the reaction mixture. Considerable advancements have been made since the pioneering work of Criegee and Hoffmann to make the reaction catalytic, using inexpensive, relatively inert re-oxidants to regenerate Os(VIII) from the Os(VI) glycolate ester and these have greatly enhanced the synthetic utility of the reaction. Metal chlorates were the first co-oxidants to be used in the reaction and hydrogen peroxide has also been employed. However, these reagents often over oxidised the desired products leading to diminished yields. A breakthrough came in 1976 when Sharpless et al. reported the efficacy of alkaline tert-butyl hydroperoxide as a co-oxidant for the reaction. In the same year, VanRheenen et al. at the Upjohn Company, reported the use of tertiary amine oxides – in particular N-methylmorpholine-N-oxide (NMO), as a co-oxidant for the reaction which provided much better results. In 1990, Minato et al. demonstrated the efficacy of potassium ferricyanide (K₃Fe(CN)₆) in the presence of potassium carbonate as a co-oxidant system for the osmium tetroxide-catalysed dihydroxylation of olefins.

In addition to their work on co-oxidants, Sharpless and co-workers were also interested in developing a stereoselective variant of the dihydroxylation reaction. They sought to induce enantioselectivity in the osmylation reaction by exploiting the ligand acceleration effect (LAE) discovered by Criegee using chiral pyridine derivatives. The initial enantioselectivities obtained were low (3-18% ee) and this was attributed to the poor binding affinity of the pyridine ligands for OsO₄. It was found
that the binding constant of a ligand is extremely sensitive to the steric hindrance near the reacting centre. However when modified quinuclidine derivatives were used (Figure 2.1), a considerable improvement in binding affinity and consequently enantioselectivity was observed - the birth of the Sharpless Asymmetric Dihydroxylation (SAD) had taken place.

Figure 2.1: Cinchona alkaloid ligands for AD under catalytic conditions

2.3.1.1 Development of the Asymmetric Dihydroxylation:

Initially the SAD was carried out using stoichiometric OsO₄ but in 1988 Jacobsen et al. discovered that the reaction proceeded catalytically using NMO as a co-oxidant although a decrease in enantioselectivity was observed. The cause of the decreased enantioselectivity was later attributed to the existence of a second catalytic cycle shown in Scheme 2.6 (vide infra). In the first catalytic cycle, (primary cycle, Scheme 2.6) an osmium glycolate product 57 is formed when the ligand-osmium tetroxide complex reacts with the olefin. This complex is oxidised by the co-oxidant from Os(VI) to Os(VIII) which is catalytically active and subsequent hydrolysis of the osmium ester produces the enantioenriched diol and OsO₄.
If however, the hydrolysis step is slow, the active Os(VIII) ester can react with another molecule of olefin in the absence of the chiral alkaloid ligand and this reaction therefore proceeds with low enantioselectivity. This problem was partially remedied by the slow addition of olefin to the reaction system, ensuring a low concentration of olefin in the reaction medium and enabling the hydrolysis of 58 prior to reaction with a second molecule of olefin and entry into the second catalytic cycle (vide infra).

Scheme 2. 6: The two catalytic cycles for the AD using NMO as co-oxidant
The problem of lower enantioselectivities was alleviated when Kwong et al. discovered that participation of the second catalytic cycle can be precluded by performing the reaction under bi-phasic conditions with potassium ferricyanide as the co-oxidant and a tert-butanol/water biphasic solvent system. Under these conditions the only oxidant present in the organic layer is OsO$_4$ and the osmium-glycolate ester is hydrolysed at the interface between the two layers, see Scheme 2.7.

Scheme 2.7: Catalytic cycle of AD reaction with potassium ferricyanide as co-oxidant

In the early 1990s Hartung$^{117}$ and Crispino$^{118}$ reported that ligands containing two cinchona alkaloid derivatives joined by a heterocyclic spacer induced high levels of enantioselectivity across a broad range of substrates in SAD reactions. The heterocyclic spacers employed are shown in Figure 2.3.

Amberg$^{117}$ and Xu$^{118}$ also discovered that methane sulfonamide considerably accelerates the rate of hydrolysis of the osmium glycolate ester, enabling asymmetric dihydroxylation of olefins which normally require elevated temperatures to be performed at 0 °C. Taken together, these advancements increased the scope of the
reaction significantly and led to one general procedure that is applicable to a wide range of olefinic substrates.

![Phthalazine (PHAL) and Diphenylpyrimidine (PYR)](image)

**Figure 2.3:** Second generation ligands

These combined discoveries and the use of potassium osmate dihydrate as a non-volatile source of OsO₄ (potassium osmate dihydrate is oxidised *in situ* to OsO₄) also enabled the formation of the pre-mix formulation containing potassium osmate dihydrate, potassium ferricyanide, potassium carbonate and ligand, commercially marketed as AD-mix, which has greatly enhanced the simplicity of the reaction. In addition, safety issues concerned with handling the volatile and toxic OsO₄ were reduced. There are two AD-mix formulations available, α and β. The ligand in AD-mix α is a dihydroquinine (DHQ) derivative and the ligand in AD-mix β is a dihydroquinidine (DHQD) derivative (*vide infra*).

![Cinchona Alkaloid Ligands in AD-mix reagents](image)

**Figure 2.4:** Cinchona Alkaloid Ligands in AD-mix reagents

AD-mix reagents are now used extensively in modern day organic synthesis. Some particularly relevant examples exhibiting the versatility of the reagents and synthetic utility of the reaction include: Harris *et al.*'s synthesis of four D- and L-
hexoses\textsuperscript{119} and also their synthesis of D- and L- mannose, gulose and talose,\textsuperscript{120} Somfai et al. \textquoteleft s total synthesis of (+)-1-deoxynojirimycin;\textsuperscript{121} and Boger et al. \textquoteleft s total syntheses of vancomycin aglycon\textsuperscript{122} and camptothecin.\textsuperscript{123} In this project, the AD-mix pre-formulations were used to dihydroxylate trisubstituted olefins and form \(\alpha\)-hydroxy ketones.

\textbf{2.3.1.2 Mechanistic Considerations:}

The mechanistic discussion concerning the mode of interaction between OsO\textsubscript{4} and the olefin leading to the intermediate glycolate was hotly debated for many years. In the early 1920s, Böskken was the first to comment and he suggested that osmylations might proceed in a similar fashion to permanganate oxidations of alkenes to diols.\textsuperscript{124} Böskken, and later Criegee, proposed that dihydroxylation proceeded \textit{via} a concerted [3+2] cycloaddition reaction of the olefin with OsO\textsubscript{4} forming glycolate 65 directly.\textsuperscript{105, 106, 124}

\begin{center}
\textbf{Scheme 2. 8: Stepwise [2+2] and Concerted [3+2] Mechanisms}
\end{center}
In 1977, Sharpless et al. proposed an alternative stepwise mechanism\textsuperscript{125} (both mechanisms are outlined in Scheme 2. 8). Sharpless proposed a [2+2] mechanism for the osmylation of olefins analogous to the oxidation of olefins by $d^2$ metals such as chromium (\textit{exempli gratia} $\text{CrO}_2\text{Cl}_2$ oxidation of alkenes). They suggested a [2+2] addition of the olefin across an osmium oxygen double bond forming a metallaoxetane intermediate 62 which would subsequently rearrange to form the glycolate intermediate product 65.\textsuperscript{125}

In 1986, Hoffmann and co-workers\textsuperscript{126} carried out a frontier orbital study of the formation of intermediates in the \textit{cis} dihydroxylation of olefins by OsO$_4$ and concluded that “the frontier orbitals in osmium tetroxide are set up for a [3+2] cycloaddition reaction, whereas a geometric distortion of osmium tetroxide would have to take place if the reaction were a [2+2] cycloaddition reaction, followed by a second deformation back to the symmetric osmium ester complex”.

Despite these calculations, one of the most convincing pieces of evidence in favour of the [2+2] mechanism was provided by the Sharpless group who carried out a series of experiments on the effect of temperature on the enantioselectivities of stoichiometric AD reactions using a variety of catalysts.\textsuperscript{127} They analyzed the temperature dependence of enantioselectivities using Eyring plots and discovered two linear regions and one characteristic inversion point for each olefin/catalyst combination. Non-linear Eyring behavior is observed if a change in the rate-determining step occurs in the temperature window under investigation.\textsuperscript{128} These results were attributed to the reversible formation of two diastereomeric osmaoxetanes (major and minor) and subsequent rearrangement to the glycolate.\textsuperscript{127} More support for the [2+2] mechanism stemmed from \textit{ab initio} calculations performed by Veldkamp\textsuperscript{129} \textit{et al.} in 1994.

The hotly debated topic as to whether the mechanism was indeed the [3+2] concerted mechanism or the stepwise [2+2] mechanism was eventually solved in 1996-1997 when three independent groups presented quantum mechanical results on the reaction of OsO$_4$ with ethylene.\textsuperscript{130} Frenking,\textsuperscript{131} Morokuma,\textsuperscript{132} Ziegler,\textsuperscript{133} and co-workers calculated the activation energy of the [2+2] addition to be prohibitively high
(>39 kcal/mol), whereas the activation energy of the [3+2] addition is much smaller (<10 kcal/mol) indicating that the reaction does indeed follow the concerted [3+2] mechanism.\textsuperscript{130} In addition the Houk, Sharpless and Singleton laboratories compared computed and measured kinetic isotope effects and their results supported the formation of a highly symmetrical transition state thus corroborating a rate limiting [3+2] cycloaddition mechanism.\textsuperscript{134}

2.3.1.3 \textit{Rationalisation of the face selectivity:}

The enantioselectivity of the SAD reaction does not alone stem from a concerted [3+2] cyclisation. Several models based on both the concerted and stepwise mechanisms have been advanced and they converged on the principle that the face selectivity was thought to arise from a reaction between the olefin and OsO\textsubscript{4} within a chiral binding pocket created by the aromatic groups of the ligand.\textsuperscript{113}

Corey and Noe carried out extensive studies in order to explain the stereoselectivity in the dihydroxylation reaction.\textsuperscript{135-138} They supported Criegee’s theory of a concerted [3+2] mechanism and pioneered the Criegee-Corey-Noe (CCN) [3+2] model to explain the high level of enantiofacial selectivity in SAD reactions. They proposed that the enantioselectivity arose from binding interactions between the substrate and the ligand bound OsO\textsubscript{4} (L•OsO\textsubscript{4}) in an enzyme-like binding pocket (\textbf{Scheme 2. 9}). They suggested that the methoxyquinoline units of the ligand act as “walls” and the phthalazine spacer acts as the “floor” of the “active site” giving a U-shape to the binding pocket. OsO\textsubscript{4} is bound to a quinuclidine moiety in a staggered conformation and the substrate is pre-complexed to the L•OsO\textsubscript{4} complex by two binding interactions:\textsuperscript{139}

(i) Aryl-aryl interactions between the aromatic substituent of the substrate and the methoxy quinoline “walls,” and

(ii) A \pi-orbital interaction between the HOMO of the olefin and the low lying empty \textit{d-}orbitals of the osmium.
The optimum $\pi$-$d$ interaction is attained when the osmium oxygen bonds of the $\text{OsO}_4$ are eclipsed with the C-N bonds of the quinuclidine ring. Subsequent rotation of the $\text{OsO}_4$ unit about the Os-N bond occurs to relieve the eclipsing strain and results in placing one equatorial and one axial oxygen atom in close proximity to the double bond. This facilitates the [3+2] cycloaddition and enantioselective formation of the osmate ester. Dihydroxylation of the opposite face is disfavoured due to the lack of a suitable 3-dimensional arrangement for simultaneous interaction of the substrate with $\text{OsO}_4$ and the catalyst binding pocket.\textsuperscript{139, 140}

Scheme 2. 9: Proposed CCN Pathway for the enantioselective formation of Os(VI) ester with styrene

However, Sharpless \textit{et al.} maintained that the Criegee-Corey-Noe [3+2] model involved the unfavourable formation of a 20 electron species ($67, \text{L}^*\text{OsO}_4$) and that the rotation about the Os-N bond brought about by the eclipsing interaction would be minimal due to the long Os-N bond length in the complex ($2.48 \text{ Å}$). The U-shaped
binding pocket in the CCN model also cannot explain the enantiofacial selectivity of the first generation catalyst, which only possess one methoxyquinoline unit.

The Sharpless model involved a stepwise \([2+2]\) reaction mechanism with an L-shaped binding pocket. It was based on molecular mechanics (MM) calculations parameterised to fit the experimental selectivities and proposed the flat aromatic linker between the two alkaloid moieties to form the “floor” with one of the methoxyquinoline units acting as the perpendicular “wall” of the binding pocket. This structure is one of the most stable conformations of the ligand. A substrate entering the binding cleft will adopt an orientation stabilised by aromatic face-to-face interactions with the aromatic linker and edge-to-face interactions with the methoxyquinoline “wall” (see Figure 2. 5).

![Figure 2. 5: Sharpless’ proposed L-shaped binding cleft](image)

The stepwise \([2+2]\) mechanism, with an osmaoxetane intermediate was consistent with the observation that the AD reaction proceeds \(via\) at least two different pairs of diastereomeric transition states, as was deduced from Eyring plots correlating the temperature dependence of enantioselectivities \(\text{vide supra}\). Norrby \textit{et al.} invoked the Hammond postulate, which suggests that an intermediate that is energetically above the ground state should be similar in structure to both of the transition states on the reaction path. With this assumption, the relative stabilities of both diastereotopic transition states can be estimated by comparing the
relative energies of both diastereomeric metallaoxetane/ligand intermediates 72 and 73 (vide infra).

Figure 2.6: Rationalisation for enantiofacial selectivity in AD reaction based on the interplay of attractive and repulsive interactions

The MM model suggests the enantiofacial selectivity is governed by two main factors:

(i) Stabilising interactions between R'' on the oxetane and the OR substituent on C9 of the ligand (see Figure 2.6)

(ii) Destabilising/repulsive interactions between another oxetane substituent (H₈) and H₉ of the ligand (see Figure 2.6)

Compounds 72 and 73 are diastereomers leading to the major and minor enantiomeric products respectively.

In 72, there is an attractive interaction between R'' and OR of C9 leading to rate enhancement, there is also minimum repulsion between H₉ and the oxetane oxygen. Similarly, in 73, there is a favourable stacking interaction between R'' and OR of C9 but in this instance H₉ encounters severe steric repulsion from H₈ on the oxetane ring, rendering this the less favoured intermediate. This model suggests that the AD reaction is primarily dependent on non-covalent attractive interactions for its high enantiofacial selectivity and ligand acceleration.²⁰⁷,¹⁴⁰,¹⁴¹ The interplay of the
non-covalent interactions favour one diastereomeric transition state arrangement with minimised repulsive steric effects (72) over the other (73). A second level of selectivity may result from the impeded rearrangement of oxetane 73, to form the osmate ester, due to increased H$_2$-H9 interactions in the course of rearrangement. The Sharpless model unlike the CCN model can readily be extended to the first generation ligands.

More recent theoretical calculations on the origin of enantioselectivity in SAD reactions using quantum mechanical/molecular mechanical (QM/MM) methods revealed that the models originally proposed by Sharpless and Corey were indeed the lowest energy paths found for the olefin approaching the catalyst. Maseras et al. studied the SAD reaction of styrene with (DHQD)$_2$PYDZ·OsO$_4$ (67, PYDZ=pyridazine, Scheme 2. 9) using the integrated molecular orbital molecular mechanics (IMOMM) method (a computational hybrid method mixing quantum mechanics and molecular mechanics descriptions for different parts of the system). They identified the factors governing the enantiofacial selectivity to be face-to-face interactions between the phenyl ring of the olefin and the two methoxyquinoline rings, as well as edge to face interactions between the substrate and the pyridazine ring. Norrby and co-workers also rationalised the stereoselectivity in SAD reactions using quantum mechanics-guided molecular mechanics and their results supported those of Maseras. Furthermore, they suggested that rather than a definite U-shaped or L-shaped binding pocket as was indicated by Corey et al. and Sharpless et al., respectively, the position of the bystander methoxyquinoline was quite flexible. Depending on the distance between the alkene and its aromatic substituent the bystander quinoline unit could rotate so as to maximise stabilization. This implied that in olefinic substrates where the aromatic moiety is allylic or homoallylic, the methoxyquinoline unit could rotate to form a proper sandwich structure as was suggested by Corey in his investigations of allyl benzoates.
2.3.1.4  SAD summary:

The SAD is a powerful tool for asymmetric synthesis. The reaction forms cis-1,2-diols from alkenes with a high degree of stereoselectivity. The reaction typically proceeds with high chemoselectivity and enantioselectivity and the face selectivity of the reaction is reliably predicted using a simple “mnemonic device” (see Kob review). The reaction has a broad scope tolerating numerous organic functional groups and usually exhibits a high catalytic turnover number allowing low catalyst loading and good yields. The advent of the AD-mix pre-formulations has enhanced the ease at which these reactions can be performed and their use is perhaps the most common and practical method for small scale asymmetric dihydroxylations.
2.4 Results

The total synthesis of Erythrulose (74) has not yet been reported and as this tetrose sugar contains the desired α-hydroxy ketone motif of intermediate 44 it seemed an obvious initial target molecule. Erythrulose (74) which was not commercially available at the outset of this project can now be purchased from Sigma-Aldrich as the S isomer but not as the R isomer; it is currently prepared enzymatically by the aerobic fermentation of gluconobacter and multistep purification processes but it can also be isolated from plants.

![Scheme 2.10: Retrosynthetic analysis of Erythrulose](image)

The method employed involved the synthesis of a suitably substituted olefin which was to furnish a handle within the molecule, allowing the in situ generation of an enantioenriched α-hydroxy ketone. This was to be achieved by performing Sharpless asymmetric dihydroxylation (SAD) on the vinyl bromide (52) or vinyl ether (53) delivering initially a 1,1-bromohydrin or hemiacetal that would collapse to generate the required ketone. Subsequent removal of any protecting groups employed would yield the desired tetrose. Although SAD reactions of trisubstituted vinyl ethers had been reported, similar reactions employing vinyl bromides have not been reported. It was expected that the synthesis of the vinyl halide would be more facile than the corresponding vinyl ether, the focus of preliminary investigations was therefore concentrated in this direction.

Following the disconnection shown in Scheme 2.10, the first reaction in the sequence was the dibromination of cis-2-butene-1,4-diol (49). This was executed by the slow addition of a solution of bromine in dry dichloromethane to a solution of the
diol in dichloromethane at 0 °C. Once all the bromine had been added, the reaction system was allowed to warm to room temperature and stirring was continued overnight. Unsurprisingly, the highly polar product is partially soluble in water, and so, the work up required some refinement. The best results were obtained when the reaction was quenched with 10% sodium thiosulfate solution and the resulting mixture was extracted several times with ethyl acetate. A single recrystallisation gave the desired dibromide 75 as a white solid in 83% yield.

\[
\begin{align*}
&\text{HO} - \text{CH}_2\text{OH} \\
&\text{Br}^+\text{CH}_2\text{Cl}_2 \\
&\text{AcjO, TEA, DMAP, THF} \\
&\text{Br}^-\text{Ac} \\
&\text{K}_2\text{CO}_3, \text{DMSO}
\end{align*}
\]

\[
\begin{align*}
&\text{HO} - \text{CH}_2\text{OH} \\
&\text{Br}^+\text{CH}_2\text{Cl}_2 \\
&\text{AcjO, TEA, DMAP, THF} \\
&\text{Br}^-\text{Ac} \\
&\text{K}_2\text{CO}_3, \text{DMSO}
\end{align*}
\]

Scheme 2. 11: Synthesis of erythrulose via vinyl halide

Protection of the alcohol groups was necessary to prevent epoxide formation upon treatment with base in a subsequent step. The initial approach was to protect cis-2-butene-1,4-diol (49) as a diacetate prior to bromination; however, upon treatment with bromine a mixture of products resulted. Fortunately, protection of the dibromo-diol 75 as a diacetate 76 was facile, thus precluding possible epoxide formation in the next step. The acetylation reagent of choice (the use of acetyl chloride or acetic anhydride made little difference) was added to a solution of diol 75 in tetrahydrofuran at 0 °C and 2.2 equivalents of triethylamine were added. After column chromatography, the desired dibromo-diacetate 76 was isolated, as a yellow oil, in 94% yield.

The next step in the reaction series was to synthesise vinyl halide 77, from dibromide 76 in an elimination reaction. Vinyl halides are important reagents in organic chemistry and several methodologies to their synthesis have been
developed. The elimination reaction was accomplished by adding 1.1 equivalents of potassium carbonate to a solution of 76 in dry dimethyl sulfoxide and stirring overnight at 45 °C. TLC analysis indicated that all the starting material had been consumed (after approximately 16 hours). After column chromatography the desired vinyl halide 77 was obtained as a yellow oil in 76% yield as a single isomer. It was assumed that the reaction would proceed via an E2 elimination mechanism. An E1 mechanism was unlikely because departure of the bromide ion would leave an unstable secondary carbocation of relatively high energy. As there is no anion stabilising group α to the proton being removed, E1cB could be ruled out as a possible mechanism. E2 eliminations require an anti-periplanar transition state. The best overlap occurs when the C-H bond and the C-Br bond are fully parallel, as shown in Scheme 2. Although the (Z) alkene was formed from the (Z) butene diol, it is important to note that the CH₂OAc groups have switched from being cis to each other to being trans to each other (the bromine dominates the numbering sequence). (Note: Bromination of cis-2-butene-1,4-diol led to the formation of the anti-periplanar vicinal dibromo species 75). Polar aprotic solvents such as dimethyl sulfoxide are used in E2 eliminations because they increase base strength by coordination with the cation. Pearson et al. found that in non-polar solvents, clustering of base is at a maximum and the ability to react with a proton is at a minimum. Conversely, in dimethyl sulfoxide clustering of base is minimised and the ability to react with a proton is maximised. The large potassium cation can complex with solvent leaving the carbonate anion free for proton abstraction. The greater the complexation between the potassium cation and the solvent, the more "naked" the anion, the stronger is the base and the faster the rate of reaction.
Scheme 2.12: E2 elimination forming vinyl halide

With the vinyl bromide in hand, the next step in the synthesis was a Sharpless asymmetric dihydroxylation. Dihydroxylation of the vinyl bromide should initially generate an α-hydroxy bromohydrin 79 which should then collapse, delivering the desired enantioenriched α-hydroxyketone 78, as shown in Scheme 2.13.

Scheme 2.13: SAD on vinyl halide

This reaction was attempted several times employing standard AD conditions but unfortunately vinyl bromide 77 did not react even with extended reaction times (up to 72 hours) or at elevated temperature (25 °C). No significant difference was observed when the number of equivalents of AD-mix reagent were doubled and tripled; with the starting material being recovered on each occasion. Osmium tetroxide is an electrophilic reagent and consequently, osmium mediated dihydroxylations proceed more slowly with electron deficient olefins. The inductively withdrawing halide and
acetate groups render the alkene of the vinyl halide quite electron deficient. The nucleophilicity of the double bond is increased by electron donating groups and decreased by electron withdrawing groups. The relative reactivity of some alkenes towards bromine, for example, is indicated in Table 2.1.

Table 2.1: Relative reactivity of some alkenes toward bromine in acetic acid at 24 °C

<table>
<thead>
<tr>
<th>Alkene</th>
<th>Relative Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhCH=CH₂</td>
<td>Very Fast</td>
</tr>
<tr>
<td>PhCH=CHPh</td>
<td>18</td>
</tr>
<tr>
<td>CH₂=CHCH₂Cl</td>
<td>1.6</td>
</tr>
<tr>
<td>CH₂=CHCH₂Br</td>
<td>1.0</td>
</tr>
<tr>
<td>PhCH=CHBr</td>
<td>0.11</td>
</tr>
<tr>
<td>CH₂=CHBr</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

However, SAD has been performed on numerous electron deficient substrates; *exempli gratia* Evans *et al.* performed SAD on vinyl sulfones and nitroolefins generating enantioenriched α-hydroxy aldehydes.

Scheme 2.14: Evans *et al.* SAD of electron deficient olefins
The asymmetric dihydroxylation of vinyl ethers furnishing enantioenriched α-hydroxy ketones is well documented,\textsuperscript{134} therefore the decision to synthesise a more electron rich vinyl ether 85 and attempt the dihydroxylation reaction once more, was made.

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) [draw, fill=white] {49};
\node (b) at (1,0) [draw, fill=white] {NBS, MeOH};
\node (c) at (2,0) [draw, fill=white] {Br};
\node (d) at (3,0) [draw, fill=white] {83};
\node (e) at (4,0) [draw, fill=white] {AcO\textsubscript{2}O, H\textsubscript{2}SO\textsubscript{4}};
\node (f) at (5,0) [draw, fill=white] {AcO\textsubscript{2}O};
\node (g) at (0,-1) [draw, fill=white] {K\textsubscript{2}CO\textsubscript{3}, DMSO};
\node (h) at (1,-1) [draw, fill=white] {MeO\textsubscript{3}, OAc};
\node (i) at (2,-1) [draw, fill=white] {85};
\node (j) at (3,-1) [draw, fill=white] {SAD};
\node (k) at (4,-1) [draw, fill=white] {78};
\node (l) at (5,-1) [draw, fill=white] {84};
\node (m) at (6,-1) [draw, fill=white] {Steps};
\draw [-] (a) -- (b) -- (c) -- (d) -- (e) -- (f) -- (g) -- (h) -- (i) -- (j) -- (k) -- (l) -- (m);
\end{tikzpicture}
\end{center}

\textbf{Scheme 2.22: Vinyl ether route to erythrulose}

The first step in the synthesis was the formation of bromoether 83. This was achieved by adding \textit{cis}-2-butene-1,4-diol (49) to a mixture of \textit{N}-bromosuccinimide and methanol. This reaction is similar in mechanism to the bromination of an alkene, exposure of 49 to \textit{N}-bromosuccinimide results in the formation of a bromonium ion intermediate which is subsequently attacked by methanol generating the \textit{anti}-bromoether 83. Following the removal of volatiles, the reaction was placed in a freezer overnight to facilitate crystallisation of the by-products. The solid succinimide by-product was then removed by filtration and the desired product 83 was recovered as a yellow oil in 87% yield without the need for further purification.

Once again the hydroxy groups of the haloether had to be protected, to avoid epoxide formation in the subsequent elimination reaction. Acetate groups were once again employed; however, on this occasion, their electron withdrawing effect was not a cause of concern, due to the strong electron donating nature of the methoxy substituent. Initially, similar conditions to those used for the formation of dibromide 76 were used, (acetyl chloride, triethylamine, tetrahydrofuran, 0 °C), which gave the desired diacetate 84 as a yellow oil in 50% yield after column chromatography. Later, a more efficient acetylation procedure was discovered. Acetic anhydride was used as
solvent, the bromoether was introduced and a drop of sulfuric acid was used as the catalyst for the reaction. When this protocol was followed considerably better yields were obtained (>90%).

\[
\begin{align*}
\text{Br} & \quad \text{OMe} \\
\text{HO} & \quad \text{OH}
\end{align*}
\]

83

\[
\begin{align*}
\text{Ac}_2\text{O}, \text{cat. } \text{H}_2\text{SO}_4 \\
\text{Br} & \quad \text{OMe} \\
\text{AcO} & \quad \text{OAc}
\end{align*}
\]

84

Scheme 2.23: Alternative acetylation procedure

The conditions previously in force to effect the elimination reaction (dimethyl sulfoxide, potassium carbonate at 45 °C), had to be modified slightly, elevating the temperature to 70 °C to afford the desired vinyl ether. Column chromatography on silica gel gave the vinyl ether, 85, as a yellow oil in good yield (69%).

With the vinyl ether in hand, SAD was attempted a number of times using various concentrations of the AD-mixes. Unfortunately, in all cases, the reaction failed with none of the desired 78 being recovered. A control reaction was then carried out on the parent alkene itself (cis-2-butene-1,4-diacetate), to ascertain whether it would undergo SAD. It did not. Fortunately, Prof. Sharpless was amenable to consultation and he suggested the use of alternative protecting groups, commenting also that larger molecules perform better under SAD conditions than smaller molecules.

\[
\begin{align*}
\text{TPSO} & \quad \text{86} \\
\text{Ph} & \quad \text{Ph} \\
\text{OTPS} & \quad \text{OAc} \quad \text{OAc} \quad \text{OAc}
\end{align*}
\]

Scheme 2.24: Evans et al. dihydroxylation of a silyl protected 1,4-diol

In their synthesis of D-Altritol, Evans et al. successfully dihydroxylated an olefin with a 1,4-diol system, similar to that in the instant case, using a large silicon protecting group (see Scheme 2.24); and so, the possibility of synthesising and
dihydroxylating silyl protected vinyl halide 88 and silyl protected vinyl ether 89 was investigated.

Before synthesising compounds 88 and 89 a control reaction was performed. cis-2-Butene-1,4-diol (49) was protected as the bis silyl ether 92 and dihydroxylation was performed under standard conditions. The reaction was a success and diol 93 was isolated in 73% yield as a colourless oil. The syntheses of both trisubstituted olefins 88 and 89 were then undertaken.

The initial step in the synthesis of 88 was to protect the hydroxyl groups of the previously synthesised dibromo-diol 75, as the bis silyl ether, 90. This was accomplished by adding diol 75 to a solution of tert-butyldimethylsilyl chloride (2.0 equivalents) and imidazole (4.5 equivalents) in dimethylformamide and stirring at room temperature overnight. After column chromatography, the desired bis silyl ether 90, was obtained in 98% yield as a colourless liquid.
The next step in the reaction series was the preparation of vinyl halide 88, by elimination of HBr from dibromide 90. The first attempt was executed under the same conditions used to effect the elimination of HBr from the bis acetate 76 (dimethyl sulfoxide/potassium carbonate/RT); however on this occasion potassium carbonate proved unsuccessful. The elimination reaction was successfully realised by adding 1.05 equivalents of DBU to a solution of 90 in dry dimethyl sulfoxide and stirring overnight (ca. 16 hours) at 45 °C. After purification by column chromatography the desired vinyl halide was obtained as a colourless oil in 92% yield.

Initial attempts at the dihydroxylation of 88 employed the standard SAD conditions. A solution of 88 in tert-butanol was added to a mixture of AD-mix β (1.0 equivalent) and methane sulfonamide (1.0 equivalent) in a tert-butanol-water (1:1) solvent system at 0 °C. Unfortunately, after 8 hours stirring no starting material had been consumed. The temperature was therefore elevated to room temperature and the reaction was stirred for a further 16 hours, however once again no starting material was consumed. The standard response to stubborn substrates is to increase the number of equivalents of AD-mix and also if necessary to increase the catalytic loading of osmium by adding extra potassium osmate dihydrate (K₂OsO₄(OH)₄). After some optimisation, the dihydroxylation of 88 was achieved by treatment with 3.0 equivalents of AD-mix reagents; 1.0 equivalent of methane sulfonamide; and, an additional 2.0
mole percent potassium osmate dihydrate. Isolation of the hydroxy ketone 94 proved to be very difficult due to the ability of the silicon groups to “walk” from the primary oxygen to the secondary oxygen. To minimise this problem the crude reaction mixture was treated with tert-butylidimethylsilyl chloride (1.1 equivalents) and imidazole (1.1 equivalents) in dry dimethylformamide to form the tris protected silyl ether 95 which proved less difficult to isolate. The overall yield for the reaction was disappointingly low (14% over 2 steps).

The synthesis of vinyl ether 89 was undertaken in an attempt to circumvent the issues of poor yield and sluggish reactivity of vinyl halide 88 under SAD conditions (see Scheme 2.28). The initial step in the reaction sequence was to protect the bromoether 83 as the bis silyl ether 91. This was accomplished by adding 2.15 equivalents of tert-butylidimethylsilyl chloride and 3.5 equivalents of imidazole to a solution of 83 in dry dimethylformamide. The product was purified by column chromatography using silica gel and 91 was isolated in 79% yield as a colourless oil.

\[
\text{TBSCI, Imidazole, DMF} \rightarrow \text{Br} \quad \text{OTBS} \quad \text{MeO} \quad \text{OTBS} \quad 91
\]

\[
\text{TBSCI, Imidazole, DMF} \rightarrow \text{TBSO} \quad \text{OH} \quad \text{OH} \quad 94
\]

\[
\text{AD-mix β, MeSO₂NH₂, K₂OsO₄(OH)₄, t-BuOH-H₂O (1:1), RT} \rightarrow \text{TBSO} \quad \text{OH} \quad \text{OH} \quad 94
\]

\[
\text{TBSCI, Imidazole, DMF} \rightarrow \text{TBSO} \quad \text{OH} \quad \text{OH} \quad 74
\]

Scheme 2.28: Bis tert-butylidimethylsilyl protected vinyl ether route to erythrulose
Table 2.2: pKₐ values for bases tested to effect elimination of 91

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>pKₐ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Imidazole</td>
<td>6.95</td>
</tr>
<tr>
<td>2</td>
<td>TEA</td>
<td>10.6</td>
</tr>
<tr>
<td>3</td>
<td>DMAP</td>
<td>9.2</td>
</tr>
<tr>
<td>4</td>
<td>DABCO</td>
<td>8.82</td>
</tr>
<tr>
<td>5</td>
<td>Hünigs</td>
<td>10.5</td>
</tr>
<tr>
<td>6</td>
<td>K₂CO₃</td>
<td>6.37</td>
</tr>
<tr>
<td>7</td>
<td>DBU</td>
<td>(12)</td>
</tr>
<tr>
<td>8</td>
<td>Sodium Succinimide</td>
<td>(14.7)</td>
</tr>
<tr>
<td>9</td>
<td>Sodium hydride (60% dispersion in oil)</td>
<td>38</td>
</tr>
</tbody>
</table>

Compounds in brackets have pKₐ values shown in DMSO

The subsequent conversion from bromoether 91 to vinyl ether 89 proved to be extremely troublesome. Potassium carbonate, which successfully furnished the bis acetate vinyl ether 85, was tested at various temperatures, and concentrations and using different numbers of equivalents of base but none of the conditions tested proved successful.

Consequently, a number of bases were tested to ascertain which conditions would effect the elimination. The reactions were performed employing 2.0 equivalents of base in dimethyl sulfoxide with a reaction concentration of 0.16 M. Starting material was recovered in all cases except for when sodium hydride (60% dispersion in mineral oil) was used (Entry 8, Table 2.2), where a complex mixture of products was obtained and DBU (Entry 7, Table 2.2), which proved somewhat more successful.
Table 2.3: DBU optimisation experiments for formation of bis silyl vinyl ether

<table>
<thead>
<tr>
<th>Entry</th>
<th>No. Equiv.</th>
<th>Time (hours)</th>
<th>Temp. (°C)</th>
<th>91</th>
<th>89</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>16</td>
<td>25</td>
<td>MP</td>
<td>-</td>
<td>No Rxn</td>
</tr>
<tr>
<td>2</td>
<td>1.1</td>
<td>24</td>
<td>25</td>
<td>MP</td>
<td>-</td>
<td>No Rxn</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>16</td>
<td>25</td>
<td>MP</td>
<td>-</td>
<td>No Rxn</td>
</tr>
<tr>
<td>4</td>
<td>1.1</td>
<td>16</td>
<td>50</td>
<td>MP</td>
<td>-</td>
<td>No Rxn</td>
</tr>
<tr>
<td>5</td>
<td>1.1</td>
<td>24</td>
<td>50</td>
<td>MP</td>
<td>-</td>
<td>No Rxn</td>
</tr>
<tr>
<td>6</td>
<td>2.0</td>
<td>16</td>
<td>50</td>
<td>MP</td>
<td>-</td>
<td>No Rxn</td>
</tr>
<tr>
<td>7</td>
<td>1.1</td>
<td>16</td>
<td>75</td>
<td>1</td>
<td>1.7</td>
<td>RR</td>
</tr>
<tr>
<td>8</td>
<td>2.0</td>
<td>16</td>
<td>75</td>
<td>1</td>
<td>2.2</td>
<td>RR</td>
</tr>
<tr>
<td>9</td>
<td>2.0</td>
<td>25</td>
<td>75</td>
<td>1</td>
<td>3.3</td>
<td>RR(^a)</td>
</tr>
<tr>
<td>10</td>
<td>2.0</td>
<td>72</td>
<td>75</td>
<td>0.1</td>
<td>2.24</td>
<td>RR(^a)</td>
</tr>
</tbody>
</table>

MP=major product; \(a=\)Additional unsaturated products formed; RR=relative ratios

The reaction with DBU was carried out several times varying the temperature, the length of reaction and the number of equivalents of base (see Table 2.3). At low temperature, none of the starting material was consumed even with extended reaction times and increased number of equivalents of base. The temperature was therefore elevated further and at 75 °C, some of the desired vinyl ether 89 was observed. When the reaction was performed at temperatures above 75 °C complex mixtures containing unwanted unsaturated side-products were formed. These unwanted side-products were also observed, with extended reaction periods, when the reaction was performed at 75 °C. Different solvents were also tested for the reaction (tetrahydrofuran, dimethylformamide and dimethyl sulfoxide) but dimethyl sulfoxide proved far superior to the others. The best results were obtained, when a solution of bromoether 91, in dimethyl sulfoxide was treated with 2.0 equivalents of DBU and the reaction was stirred at 75 °C for 16 hours.
The desired vinyl ether 89 was unstable on both silica and alumina and, for that reason could not be purified by column chromatography. The reaction did not go to completion at this temperature, but the quantities of undesirable, unsaturated compounds were minimised. The unwanted, unsaturated side-products would react under SAD conditions and, therefore, it was essential that the quantities of these compounds were minimised. However, the results for this experiment were not reproducible. To provide confirmation of this, the reaction was carried out several times using the exact same conditions and on each occasion the ratio of products to starting material differed. Using an internal standard (2,5-diphenyl furan) an NMR yield of 37% was calculated from the crude reaction mixture.

Despite these considerable difficulties it was deemed prudent to examine how the vinyl ether behaved in a SAD reaction. The quantity of vinyl ether 89 present in the crude reaction mixture was calculated by $^1$H NMR using the internal standard. This crude reaction mixture was then subjected to the modified SAD conditions, previously employed to effect dihydroxylation of the vinyl halide (3.0 equivalents AD-mix $\beta$ /1.0 equivalents methanesulfonamide/2.0 mole percent potassium osmate dihydrate/RT). The reaction went to completion in 8 hours. Once again the yield was estimated by NMR analysis (employing 2,5-diphenyl furan as the internal standard) and proved to be greatly enhanced (ca. 45%) when compared to the SAD reaction of vinyl halide 88 (ca. 10%). Again, silyl migration was an issue so the crude product was then treated with tert-butyldimethylsilyl chloride and imidazole to form the tris silylated product. Although the vinyl ether 89 behaved much better under SAD conditions, the instability of the resulting hydroxy ketone makes isolation very difficult and indicates that tert-butyldimethylsilyl protecting groups are not the protecting group of choice for this particular transformation.

While optimization experiments to synthesise 89 were ongoing the preparation of an alternative vinyl ether, with tert-butylidiphenylsilyl (TBDPS) protecting groups, was attempted. It was hoped that the use of the larger protecting group would minimise subsequent silyl migration once SAD had been performed. Due to the difficulties encountered generating vinyl ether 89, from its precursor bromoether 91,
the synthesis of an analogous iodoether 97, was attempted; in the hope that, the subsequent elimination would prove less troublesome. The synthesis of the bis tert-butyldiphenylsilyl protected vinyl ether 98 is shown in Scheme 2. 29.

![Scheme 2. 29: Bis tert-butyldiphenylsilyl vinyl ether route to erythrulose](image)

The decision to use tert-butyldiphenylsilyl protecting groups instead of tert-butyldimethylsilyl groups was taken to preclude O,O-migration after SAD had been performed on 98. Usually elevated temperatures and a suitable base are required to facilitate tert-butyldiphenylsilyl protection of a secondary alcohol. Although migration might still be possible it was hoped that any migration would be reversible and that the high steric demand of tert-butyldiphenylsilyl would favour the primary silyl ether.

The synthetic strategy employed was analogous to that used for the formation of vinyl ether 89. The first step in the reaction sequence was the formation of iodoether 96 which was achieved by adding cis-2-butene-1,4-diol (49) to a suspension of N-iodosuccinimide (NIS) in methanol at 0 °C. After column chromatography iodoether 96 was isolated as a yellow oil in moderate yield (52%). Diol 96 was then protected as the bis tert-butyldiphenylsilyl ether 97 by treatment of a solution of 96 in dimethylformamide with 2.7 equivalents of tert-butyldiphenylsilyl chloride, 3.8 equivalents of imidazole and a catalytic quantity of dimethylaminopyridine (0.1
equivalents) at 0 °C. The desired bis tert-butyldiphenylsilyl iodoether 97 was recovered as a white solid in excellent yield (86%) after column chromatography.

The elimination of 97 was attempted several times using a variety of bases, (DBU, sodium hydroxide, sodium hydride), at different temperatures, and in different solvents (dimethylformamide, dimethyl sulfoxide, benzene). However, none of the experiments produced the desired vinyl ether 98. When an account by Corey et al., describing the poor enantiofacial selectivities obtained, when SAD was performed on substrates with bulky allylic tert-butyldiphenylsilyl ethers, was discovered; it was deemed prudent to abandon this particular route and focus instead on the deprotection of the tris tert-butyldimethylsilyl ether 95 (see Scheme 2.30).

\[
\begin{array}{c}
\text{TBSO} \quad \text{OTBS} \\
\text{95} \\
\text{OTBS} \quad \text{OTBS} \\
\text{HO} \quad \text{OH} \\
\text{74}
\end{array}
\]

**Scheme 2.30: Deprotection of 95**

Silyl ethers are generally stable to basic conditions but can be cleaved in an acidic environment. Initial attempts at deprotection of tris silyl ether 95 were made using p-toluene sulfonic acid in a tetrahydrofuran-methanol (1:10) solvent system. It was anticipated that the reaction would either deliver the desired erythulose 74, or, that it would remove the two tert-butyldimethylsilyl groups protecting the primary hydroxyl groups. The reaction was partially successful; the disappearance of starting material was observed by TLC analysis and NMR analysis indicated that the silyl groups had been removed. Isolation of the deprotected product 74 proved problematic. Aqueous work ups were futile as the product itself is very soluble in water and column chromatography was also redundant because of the extreme polarity of the sugar. Demonstrably, an alternative method of deprotection was required.

The silicon fluorine bond is one of the strongest bonds known (Si-F, \( \Delta H^\circ = 135 \) kcal mol\(^{-1} \)); making fluoride containing compounds such as quaternary amine salts very popular for cleaving silyl ethers. The driving force for such reactions is provided by the formation of the strong Si-F bond. These reagents are not acidic (in fact they
can be quite basic) and so if other acid labile groups are present in a molecule, the use of fluoride enables the selective removal of silyl groups. Tetrabutylammonium fluoride (TBAF) is one of the most common reagents for removing silicon protecting groups, for this reason, an attempt was made to deprotect 95 with TBAF. Unfortunately, this was also unsuccessful due to the necessity for a consequent aqueous work up. Endeavouring to eliminate the requirement of such a work up, deprotection of 95 was also attempted with a silicon bound version of TBAF; disappointingly, this proved unsuccessful and none of the desired 74 was isolated.

Keeping with fluoride as a means of deprotecting 95, it was decided to treat the tris silylether with various concentrations of (extremely dangerous) hydrogen fluoride pyridine. Hydrogen fluoride pyridine was added to a solution of tris tert-butyldimethylsilyl erythrulose in tetrahydrofuran and the reaction was stirred at room temperature overnight. TLC analysis indicated consumption of the starting material and the formation of a highly polar product which did not move on the TLC plate; however, once again isolation proved troublesome. The isolation problems for all of the protocols described thus far, stemmed from the inability to separate the water soluble erythrulose from unwanted side-products. Deprotection of 95 was therefore attempted with a solution of HCl in methanol thereby enabling separation of the silyl products in organic solvent, and in the absence of salts, the erythrulose could be isolated from the methanol/water layer by removing the volatiles at reduced pressure. Unfortunately, this resulted in the isolation of a complex mixture of products and any further attempts to deprotect tris silyl ether 95 were abandoned.

Thus far, assessment of stereoselectivity in the SAD reactions could not be made. In their investigation of the stereoselective coupling of thymine with meso-thiolane-3,4-diol-1-oxide via the Pummerer reaction, Naka et al.\textsuperscript{158} used benzoyl protecting groups to protect cis-2-butene-1,4-diol and subsequently dihydroxylated the product in good yield. Benzoyl protecting groups are similar electronically to acetate protecting groups and, as the synthesis of the vinyl ether 77 was facile it was decided to prepare 101 to see whether it would react well under SAD conditions and if the
resulting product would have enhanced stability, compared to its silicon protected counterpart.

![Chemical structure](image)

Scheme 2.31: Benzoyl protected vinyl ether route to erythrulose

The first step in the reaction sequence (Scheme 2.31) was the formation of the bis benzoate 100. Benzoylation of the bromoether was accomplished by the treatment of a solution of 83 with 3.5 equivalents benzoyl chloride and 3.5 equivalents triethylamine in dichloromethane at 0 °C. After work up the crude reaction mixture was purified by column chromatography and the desired compound 100, was isolated in 88% yield as a white solid.

The next task was to prepare the vinyl ether 101 by elimination of HBr. This was achieved using DBU in dry dimethyl sulfoxide and heating to 60 °C overnight (ca. 16 hours). Vinyl ether 101 was isolated in 49% yield as a white solid after column chromatography.

With the bis benzoyl vinyl ether in hand the SAD reaction was attempted a number of times. The first two trials were performed under the same conditions as those used to dihydroxylate the bis tert-butylidimethylsilyl vinyl ether 89 (3.0 equivalents AD-mix β /1.0 equivalents methanesulfonamide/2.0 mole percent potassium osmate dihydrate/RT). In both instances, the starting material was consumed, albeit slowly. The product 102 was, however, unstable and rapidly decomposed on both silica and alumina, although this was not apparent by 2 dimensional-TLC analysis, _id est_ the product was lost when column chromatography was performed. Scrambling of the benzoyl groups was a potential route of
decomposition *id est* once the α-hydroxy ketone motif was in place the benzoyl groups could potentially "walk" from one hydroxyl group to another. This behaviour however, was not observed by Naka and co-workers,158 but has been reported by Shibuya and co-workers159 in a series of AD kinetic resolution experiments they carried out on compounds containing an allylic acetate group. In addition, the reasonable leaving group ability of the benzoate is a potential route of decomposition.

Another attempt at SAD was performed on a larger scale using 6 mole percent potassium osmate dihydrate with the intention of derivatising the crude product. Surprisingly, the reaction proceeded extremely slowly (*ca.* 48 hours) even with the increased osmium loading and upon examination of the solubility of the starting material it was discovered that the vinyl ether was crashing out of solution in the reaction vessel, prolonging reaction times. A final attempt at dihydroxylation of 101 was made and on this occasion the vinyl ether was administered as a solution made up in acetone. The dihydroxylation proceeded in *ca.* 16 hours employing 1.0 equivalent of AD-mix β, 2 mole percent potassium osmate dihydrate and 1.0 equivalent of methane sulfonamide in a tert-butanol-water solvent system (1:1). After work up the α-hydroxyl ketone 102 was isolated in 71% crude yield. Benzoylation of the crude reaction mixture (see Scheme 2.32) to generate the *tris* benzoyl ether 103 was attempted; however a mixture of products formed, due to decomposition of 102 with the elimination of benzoic acid.

\[
\text{BzO} - \text{OBz} \quad \text{BzO} - \text{OBz}
\]

**Scheme 2.32:** Benzoylation of *bis* benzoyl erythrulose

In addition, an effort was made to estimate the enantiofacial selectivity of the SAD reaction on 102 using europium (III) *tris* [3-(heptafluoropropylhydroxymethylene)-*d*-camphorate] (Eu(hfc)₃) – see Figure 2.7) as a chiral shift reagent. Chiral shift reagents function by forming a complex with chiral
compounds, converting a mixture of enantiomers into a mixture of diastereomers. This causes chemical shift inequivalence and the integration gives a direct measure of the diastereoisomeric composition which can be related to the enantiomeric composition of the original mixture.

Figure 2. 7: Chiral shift reagent Eu(hfc)$_3$

The $\alpha$-hydroxyl ketone 102 complexed with the chiral lanthanide shift reagent and a downfield shift was observed in the peaks of the $^1$H NMR spectrum. Splitting of the peaks representing major and minor diastereomers was not, however, observed. Moreover, after several additions of shift reagent were made, the downfield shift gradually returned upfield and cloudiness was observed in the NMR tube indicating that the complex was crashing out of solution. Disappointingly the enantiomeric excess could not be determined. A further review of the literature revealed that $p$-methoxybenzoyl groups are less prone to scrambling and induce very high levels of enantiofacial selectivity in SAD reactions because of their ability to form a non-covalent sandwich pre-complex in the binding cleft of the catalyst. The $p$-methoxybenzoyl group has been used extensively by Corey et al. in their comprehensive studies of the origin of enantioselectivity in the SAD reaction$^{135-137, 144}$ and they also employed the protecting group in a total synthesis of (-)-ovalicin, a potent inhibitor of angiogenesis. Furthermore, they compared the enantioselectivities obtained with a $p$-methoxybenzoate derivative, to those obtained with allylic alcohols, benzyl and triisopropyl silyl ethers and pivalate esters, each of which were considerably poorer substrates inducing fair to very poor levels of enantiofacial
selectivity. However, should \( p \)-methoxybenzoyl protecting groups be utilised in this \textit{de novo} synthesis of erythulose, their removal could prove difficult, with a potentially detrimental effect on selectivities.
2.5 Conclusion

Several highly functionalised, novel, small molecules were successfully synthesised but despite exhaustive efforts the target molecule, erythrulose 74, has not been isolated in its non-protected form. The key reaction in all synthetic sequences to form the desired sugar was a Sharpless asymmetric dihydroxylation reaction, which has been concisely reviewed in the introduction to this chapter. The SAD of several novel vinyl halides, and vinyl ethers (surprisingly not previously reported as single isomers), was attempted and considerable difficulties were encountered in two main areas: (i) reactivity of the substrates under standard SAD conditions and (ii) the isolation of the products, a major inconvenience being the water solubility of erythrulose, and the inability to perform column chromatography on several compounds due to protection group scrambling and decomposition of products.
Chapter 3

Synthesis of Key Intermediate from Tartaric Acid Derivatives
3 Synthesis of key intermediate from tartaric acid derivatives

3.1 Introduction

In order to truly discover the medicinal potential of 4'-TNAs the synthesis of libraries of compounds is essential. As discussed in Chapter 2 the development of a synthetic route to a key intermediate compound which could be further manipulated to furnish a variety of NAs with various substitution patterns of either natural or unnatural stereochemistry was the basis for this project.

Three general synthetic routes to intermediate 44 were investigated (see Scheme 3.1). This chapter focuses on Disconnection 2 which relied on desymmetrisation of a C2-symmetric tartaric acid derivative to generate the required α-hydroxy ketone motif of intermediate 44.

Scheme 3.1: Retrosynthesis overview

An important consideration in the choice of starting material was the availability of both isomers; it was therefore decided to employ tartrate derivatives which are commercially available in both D- and L- forms and are relatively inexpensive.
3.2 Retrosynthetic Analysis

A detailed retrosynthetic analysis is outlined in Scheme 3.2 (vide infra). It was envisaged that readily available D- or L-dimethyl tartrate, could be suitably protected, enabling reduction of the ester groups to form a tetrose sugar 109. Suitable activation of the primary hydroxyl groups, as tosylates for example, followed by deprotection of the dioxolane group and subsequent treatment with sodium sulfide furnishes the cyclic thiolane 106. Monoprotection of the thiolane moiety would desymmetrise the molecule providing an enantiopure protected thiolane. Oxidation of the remaining free hydroxy group provides the desired α-hydroxy ketone motif. Treatment of the cyclic ketone with meta-chloroperoxybenzoic acid (mCPBA) affords the sulfoxide intermediate with a degree of stereocontrol governed either by the influence of the hydroxyl protecting group or by hydrogen bonding if the free alcohol were employed.

Scheme 3.2: Retrosynthetic analysis for tartrate route to key intermediate
3.3 Results:

The first step in the reaction sequence was to protect the 1,2-diol of L-dimethyl tartrate (8) as an isopropylidene group (see Scheme 3.3). This was accomplished using a modified version of the method reported by Hemperley and co-workers. L-Dimethyl tartrate (111), 2,2'-dimethyoxypropane and a catalytic quantity of p-toluene sulfonic acid (PTSA) were heated at reflux in cyclohexane for ca. 8 hours and a Dean-Stark apparatus was employed to remove the methanol by-product thus driving the reaction to completion. Following the removal of the solvent; purification by column chromatography; and Kugelrohr distillation; the desired product 110 was isolated in 93% yield as a yellow oil.

Scheme 3.3: Formation of 2,3-isopropylidene-L-threitol

The next step in the reaction was to reduce the ester groups and form 2,3-isopropylidene-L-threitol (109). This was executed using lithium aluminium hydride as the reducing agent in a diethyl ether solvent system. Unfortunately the procedure necessitated a lengthy and protracted work up technique. Firstly, the aluminium salts were extracted using a Soxhlet apparatus; the resulting extracts were purified by filtration through a pad of silica and finally Kugelrohr distillation was performed, generating the product in 47% yield as a colourless oil.

The next task was to activate the primary hydroxyl groups as tosylates to increase their leaving group ability. This was achieved following the method employed by Kielbasiński et al. Compound 109 was treated with purified tosyl chloride in pyridine at 0 °C. The reaction was allowed to warm to room temperature and stirred overnight (ca. 16 hours). After work up and purification by recrystallisation the desired compound 108, was
recovered in excellent yield (92%) as a white solid. Kielbasiński et al.\textsuperscript{161} synthesised chiral hydroxy thiolanes as potential catalysts for organozinc additions to carbonyl groups; 3,4-dihydroxythiolane was synthesised accordingly (shown in Scheme 3.4).

\[ HO \rightarrow OX \xrightarrow{[X]} HO \quad \text{Scheme 3.5: Attempted monofunctionalisation of 3,4-dihydroxythiolane} \]

Following the tosylation reaction, the isopropylidene group had to be removed. This was accomplished by acidic hydrolysis in methanol. After recrystallisation from hot ethanol and chloroform, the desired diol 107, was isolated quantitatively as a white solid. The next reaction involved the cyclisation of the \textit{bis} tosylate to form the 3,4-dihydroxy thiolane 106. The \textit{bis} tosylate was treated with sodium sulfide nonohydrate in ethanol and the reaction was heated at reflux overnight (\textit{ca.} 16 hours). The crude reaction mixture was purified by column chromatography and thiolane 106 was isolated in 57% yield as a white solid. Some optimisation experiments were performed in an attempt to improve the yield of the cyclisation. Sodium sulfide nonahydrate was replaced with anhydrous sodium sulfide and experiments were carried out at a number of different temperatures, in both dimethyl sulfoxide and dimethylformamide (solvents we had previously found to be successful in such reactions). Unfortunately none of the alterations improved the yield; consequently, trials were abandoned to proceed to the next step.
Diol \textbf{106} is an enantiomerically pure C2-symmetric molecule. Mono protection of one of the hydroxyl groups desymmetrises the molecule leaving the remaining hydroxyl group free for further functionalisation of the thiolane ring. Kielbasiński \textit{et al.}\textsuperscript{161} performed several mono protection reactions on \textbf{106}, however the ratios of mono to \textit{bis} protected diol were not particularly encouraging (Table 3.1).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents</th>
<th>X</th>
<th>Monofunctionalised Yield (%)</th>
<th>Difunctionalised Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BuLi/Me$_3$SiCl</td>
<td>SiMe$_3$</td>
<td>28</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>Ac$_2$O/Yb(OTf)$_3$</td>
<td>Ac</td>
<td>54</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>NaH/BnBr</td>
<td>Bn</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>TsCl/Et$_3$N</td>
<td>Ts</td>
<td>46</td>
<td>36</td>
</tr>
</tbody>
</table>

In Entries 1 and 3 (Table 3.1) the monoanion was formed prior to the addition of the appropriate electrophile, however despite this enhancement of nucleophilicity, significant quantities of difunctionalised compounds were recovered. The particularly large quantity of difunctionalised product observed in Entry 1 was not discussed in detail by Kielbasiński \textit{et al.}\textsuperscript{161} but perhaps can be explained by the vacant $d$- orbitals available on the silicon atom (Scheme 3.6). If compound \textbf{112} is deprotonated, the resulting alkoxide anion can attack the neighbouring silyl group, leading to the formation of a reactive trigonal bipyramidal ate- complex \textbf{112a}; prior to reacting with a second molecule of trimethylsilyl chloride (Scheme 3.6).
tert-Butyldimethylsilyl groups were chosen to monoprotect 106 and imidazole was employed as the base for the reaction. The decision to use imidazole was influenced by the limited success of Kiefbsinski et al. with stronger bases. The reaction was performed by treating a solution of 106 in dimethylformamide with 1.0 equivalent tert-butyldimethylsilyl chloride and 2.0 equivalents imidazole and heating the reaction mixture at 75 °C overnight (Scheme 3.7). The yields obtained were comparable to those in Entry 2 of Table 3.1; the desired monofunctionalised material 114 was recovered in 52% yield and the unwanted difunctionalised material 115 was isolated in 17% yield.

Scheme 3.7: tert-butyldimethylsilyl protection of 106
Monoprotection of 106 was also attempted using a tert-butylidiphenylsilyl group in the hope that its considerable steric bulk would further hinder bis protection. However the yield for the reaction was poor and the bis silyl compound was formed as before (Table 3. 2, Entry 2).

In their synthesis of Taxol, Mukaiyama and co-workers employed a tert-butyldimethylsilyl group as a hydroxyl protecting group for a secondary alcohol. tert-Butyldimethylsilyl triflate served as the silylating reagent and 2,6-lutidine was the base used for the reaction.

\[
\text{Scheme 3. 8: Attempted protection of 3,4-dihydroxy thiolane}
\]

**Table 3. 2: Attempted monosilylation of diol 106**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents</th>
<th>X</th>
<th>Monosilylation Yield (%)</th>
<th>Disilylation Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TBSCl/Imidazole</td>
<td>TBS</td>
<td>52</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>TPSCI/Imidazole</td>
<td>TPS</td>
<td>41</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>TBSOTf/2,6-lutidine</td>
<td>TBS</td>
<td>45</td>
<td>53</td>
</tr>
<tr>
<td>4</td>
<td>TBSOTf/BuLi</td>
<td>TBS</td>
<td>69</td>
<td>-</td>
</tr>
</tbody>
</table>

tert-Butyldimethylsilyl triflate is a more electrophilic silylating reagent than its chloro counterpart; the question arose as to whether it was possible to exploit its greater reactivity by reacting it with the monoanion of 106. This was investigated by treatment of a solution of thiolane 106 in dichloromethane with 1.05 equivalents n-butyl lithium at -60 °C; after 10 minutes a solution of tert-butyldimethylsilyl triflate in dichloromethane was introduced and the reaction was allowed to warm to room temperature. After work up and
column chromatography the monoprotected thiolane 114 was isolated in 69% yield as a white solid without contamination by the bis substituted thiolane 115 (Table 3. 2, Entry 3).

The moderate yields for the monoprotection and cyclisation steps proved very costly in the context of a linear synthesis. Modification of the synthetic route was deemed necessary. To improve its efficiency, the use of a benzylidene group, rather than the isopropylidene group, to protect the diol of L-dimethyl tartrate (111) was investigated. It was expected that the benzylidene group would be preferable to the isopropylidene group because it can be reductively cleaved providing the monoprotected diol directly; thus enabling deprotection of the diol and desymmetrisation, in a single step.

The reductive cleavage of benzylidene acetals has been performed with a mixture of lithium aluminium hydride and aluminium chloride in the syntheses of some biologically important products such as mitosenes and platelet activating factors. However, due to the fact that lithium aluminium hydride is such a powerful reducing agent (and can be used to reduce tosylates to alkanes), this method of benzylidene cleavage was deemed inappropriate for the reductive cleavage of 117 (Scheme 3. 9). A milder method developed by Takano et al. involving the use of diisobutyl aluminium hydride (DIBAL) to cleave benzylidene acetals, emerged as more conducive to the instant case. Fortunately, 2,3-O-benzylidene-D-threitol (116) is commercially available; in consideration of that fact the synthetic route outlined in Scheme 3. 9 using diisobutyl aluminium hydride as the reducing agent was developed.

Scheme 3. 9: Outline of benzylidene approach to key intermediate
Using 2.5 equivalents of diisobutyl aluminium hydride in toluene at 0 °C the reaction was performed under the conditions described by Takano et al.\textsuperscript{165} Unfortunately no reaction occurred and the starting material was recovered untouched. A review of the literature indicated that Holmes et al.\textsuperscript{166} had cleaved a 1,3-benzylidene acetal using diisobutyl aluminium hydride but the reaction took 96 hours, at room temperature, to reach completion. The reaction was thus repeated and stirring was continued for two days at room temperature. Disappointingly, even after this extended period, none of the starting material had been consumed and the procedure was abandoned to pursue an alternative protocol.

In 1996, Saito et al.\textsuperscript{167} reported a novel reducing system for the reduction of acetals, employing a combination of borane dimethyl sulfide complex and boron trifluoride diethyletherate. When the discovery was originally made, it was conducted using acetals with neighbouring hydroxyl groups; but it was later realised, that the procedure could be extended to simple acetal cleavage reactions. For example, the reduction of the benzylidene acetal 120 led to 1-(O-benzyl)propane-1,3-diol (121) in 84\% yield, within 10 minutes at room temperature, upon treatment with the BH\textsubscript{3}·BF\textsubscript{3} reagent in dichloromethane (Scheme 3. 10). The cleavage of 117, was therefore attempted using the method described by Saito et al.\textsuperscript{167} However, once again none of the desired product was isolated.

\begin{center}
\includegraphics[width=0.5\textwidth]{scheme3_10.png}
\end{center}

\textbf{Scheme 3. 10:} Saito et al.\textsuperscript{167} reduction of benzylidene acetics

Cyclisation of 117 was also attempted, using the same conditions employed previously (2.5 equivalents sodium sulfide nonohydrate in ethanol and heating at reflux) but this was also unsuccessful (Scheme 3. 11).
Scheme 3. 11: Attempted cyclisation of 117

The possibility of desymmetrising a C2-symmetric carbonate tartrate derivative 123 was also examined (see Scheme 3. 12). A solution of compound 107 in tetrahydrofuran was treated with 0.43 equivalents triphosgene and 3.0 equivalents triethylamine at 0 °C; the reaction was allowed to warm to room temperature and stirred overnight. The desired carbonate 123 was purified by column chromatography and isolated in 97% yield. The crystal structure of 123 is shown in Figure 3. 1.

Scheme 3. 12: Carbonate route to key intermediate
Cyclisation of carbonate 123 was attempted a number of times; treatment with sodium sulfide nonohydrate in ethanol and heating at reflux overnight, yielded a mixture of compounds, the major product being 3,4-dihydroxy thiolane (106). A possible explanation for this is outlined in Scheme 3.13. Ring opening could occur due to attack on the cyclic carbonate by ethanol forming an acyclic ethyl carbonate derivative 125. This would relieve the ring strain, and facilitate the cyclisation of bis tosylate 125, with sodium sulfide forming 126. Removal of the carbonate functionality in 126 could be accomplished by attack of ethanol, generating thiolane 11 and diethyl carbonate. The observation of monoprotected thiolane 126 in the crude $^1$H NMR spectrum lends support to this proposed mechanism. Unfortunately when column chromatography was attempted this product decomposed forming thiolane 106.
The reaction was repeated at 50 °C, but no reaction occurred and the starting material was recovered. The use of methanol as solvent for the reaction was also investigated, but none of the desired product was formed. In addition, cyclisation of 123 to form 124 was investigated, employing dimethylformamide as solvent but unfortunately this too proved unsuccessful.

Due to time constraints, it was determined that the reductive cleavage deprotection strategy, ought to be abandoned and also attempts at desymmetrising the C2-symmetric carbonate moiety. The original route (Scheme 3.14), despite the poor yields obtained for the cyclisation and monoprotection reactions, was resumed.

With the monoprotected thiolane in hand, the next task was oxidation of the primary hydroxyl group, forming ketone 127. The first attempt employed the mild oxidising agent
Dess-Martin periodinane (DMP). While a small quantity of the desired product was formed, several unidentified side products were also generated; therefore, alternative oxidative procedures were investigated. One possible side reaction was the removal of the silyl protecting group due to liberation of acetic acid during the reaction.

\[
\begin{align*}
\text{AcO} & \quad \text{OAc} \\
& \quad \text{OAc} \\
\text{R} & \quad \text{R'} \\
\text{AcOH} & \quad \text{AcOH}
\end{align*}
\]

\text{Scheme 3. 15: Dess-Martin oxidation of a secondary alcohol to a ketone}

A possible manner in which to circumvent this problem involved the monooxidation of diol 106, forming α-hydroxy ketone 128 (Scheme 3. 16). The possibility of a second oxidation of 128 was unlikely, due to the unfavourable generation of a vicinal diketone.

\[
\begin{align*}
\text{HO}_2 & \quad \text{OH} \\
\text{106} & \quad \text{DMP, MeCN, RT} \\
\text{COH} & \quad \text{128}
\end{align*}
\]

\text{Scheme 3. 16: DMP oxidation of 3,4-dihydroxythiolane 106}

To this end, diol 106 was exposed to DMP in acetonitrile at room temperature and left stirring overnight. Unfortunately, in this instance C-C bond cleavage occurred, a reaction that has been previously observed when using DMP in the presence of 1,2-diols, an alternative protocol was clearly required.

In their synthesis of an L-thioarabinose derivative, Jeong \textit{et al.}, oxidised a hydroxy substituted tetrahydrothiophene, using acetic anhydride in dimethyl sulfoxide. Following from this, a similar oxidation procedure on compound 114 was attempted. A solution of 114 in dimethyl sulfoxide was treated with acetic anhydride at room temperature and the reaction was stirred overnight, however, none of the desired ketone
was recovered. Once again removal of the silicon protecting group was observed affording compound 106. Undeterred, a traditional Swern oxidation reaction was attempted and this proved more fruitful. The reaction proceeded cleanly, but it was difficult to remove the triethylamine hydrochloride side product, washing with base was not an option because of the potential epimerisation of the stereocentre. Unfortunately, ketone 127 is unstable on both silica and alumina, thus preventing further purification by column chromatography. The instability of ketone 127 forced advancement with the crude product in the subsequent oxidation step.

Scheme 3.17: Formation of key intermediate

The protected hydroxyl group in compound 127, should serve as a chiral handle and direct the oxidation of the sulfide to the sulfoxide, by blocking one face of the molecule; resulting in the formation of the anti sulfoxide 44a (Scheme 3. 18). Similarly, if the protecting group was removed, the free hydroxyl group may direct sulfoxide formation through co-ordination to the oxidising agent (meta-chloroperoxybenzoic acid); thereby forming the syn sulfoxide 44b (Scheme 3. 18).

Scheme 3. 18: Formation of sulfoxide intermediate

Oxidation of sulfide 127 to sulfoxide 44a was accomplished, by treating a solution of unpurified 127 in dichloromethane with meta-chloroperoxybenzoic acid and potassium
carbonate at -80 °C, and allowing the reaction to warm to room temperature. Unfortunately, the key intermediate product 44a was unstable on silica and alumina, thus precluding column chromatography once more.

Although confident that ketosulfoxide 44a had been successfully prepared, failure to purify the compound was a matter of concern. The possibility of generating, the potentially more stable syn monoprotected diol 48, by reduction of ketone 44a was considered. The steric influence of the tert-butyldimethylsilyl group would ensure that hydride delivery came from the opposite face to the bulky protecting group. Ideally, this would allow for generation of a compound amenable to purification and also prove that the transformations, previously described, had been executed. It was elected upon to repeat the experiment and to do a reductive work up, isolating alcohol 48 as shown in Scheme 3.19.

\[
\begin{align*}
\text{OTBS} & \quad \text{OTBS} \\
\text{HO} & \quad \text{O} \\
\text{(COCl)}_2 & \quad \text{DMSO, TEA, CH}_2\text{Cl}_2, -80 ^\circ\text{C} \\
114 & \quad \text{127} \\
\text{m-CPBA, K}_2\text{CO}_3, \text{CH}_2\text{Cl}_2, -80 ^\circ\text{C} & \quad \text{OTBS}
\end{align*}
\]

Scheme 3.19: Reductive work up on 44a

Alcohol 48 was isolated as a 1:3.6 mixture of diastereomers. The tert-butyldimethylsilyl group had sufficient steric influence to direct the hydride delivery from the opposite face and furnish the monoprotected syn diol. As alcohol 48 was isolated as a mixture of diastereomers this suggested that the formation of sulfoxide 44a was not completely stereocontrolled and that both syn and anti sulfoxides were formed, albeit in favour of the anti sulfoxide.

The general stability and stereoselectivity, of the intermediate compounds might be improved upon, by using different larger protecting groups, which would have a greater influence on the stereochemical outcome of the oxidation reaction, to the sulfoxide. In
addition, the generation of the syn sulfoxide by oxidation of 128 merits further investigation in any future considerations of this project.
3.4 Conclusion and Future Work

The key intermediate compound 44a was synthesised but unfortunately, it was unstable as the tert-butyldimethyl silyl ether, therefore the ketone was reduced to the secondary alcohol 48, which has enhanced stability and purification was possible. Compound 48 was isolated as a mixture of diastereomers; this indicates that the tert-butyldimethyl silyl group did not have a substantial stereoimpact on the formation of the chiral sulfoxide.

The instability of 44a precluded purification of the compound and also implies that this particular compound is not suitable as a key intermediate for the generation of libraries of compounds of thionucleosides. However, if a larger more robust protecting group is employed the stability of the compound may be enhanced and the protecting group can be used as a handle for stereocontrolled elaboration of the molecule.

In their syntheses of various asymmetric cyclopentanones Bertus et al.\textsuperscript{172} employed \textit{p}-methoxybenzylidene as a protecting group for a 1,2-diol tartrate derivative very similar to 117; they employed diisobutyl aluminium hydride in toluene (as was attempted for cleavage of benzylidene 117) to cleave the acetal and the reaction proceeded in excellent yield (89\%). Their synthetic route is outlined in Scheme 3. 20.

![Scheme 3. 20: Outline of Bertus desymmetrisation of tartrate derivative](image-url)

\textbf{Scheme 3. 20}: Outline of Bertus desymmetrisation of tartrate derivative
Their successful use of the \( p \)-methoxybenzylidene group should be considered in the future development of the synthetic route to the key intermediate compound. The \textit{bis} tosyl benzyl ether was obtained in excellent yield. This would remove a single step in the originally proposed synthetic sequence – the monoprotection of the dihydroxythiolane which was problematic due to \textit{bis} protection. After cyclisation and oxidation to the ketone it is possible that the resulting thiophenone would have enhanced stability when compared to 44\textit{a} enabling purification and storage, thus making it a more suitable candidate for the key intermediate compound.

Despite the inability to effect cyclisation of 123 with sodium sulfide, a C-Se bond should be sufficiently long to enable cyclisation, forming the analogous selenium compound with a view to synthesising a key intermediate compound for the combinatorial synthesis of 4'-selenonucleoside analogues, which are becoming increasingly popular as potential drug candidates.\textsuperscript{173}
Chapter 4

A novel route to

3-Nitro-2-Substituted-Thiophenes
The Synthesis of 2,3,4-Substituted Tetrahydrothiophenes and 2,3-Substituted Thiophenes

4.1 Introduction

A versatile synthesis of 4'-thionucleoside analogues (4'-TNAs) is essential if their true potential as medicinal therapeutics is to be fully realised. The synthesis of a vast array of 4'-TNAs and subsequent screening of these compounds against a large number of diseases will determine their utility as potential drug targets. The aim of this project was to develop a key intermediate compound 44 which could be modified readily affording a plethora of thiosugars with a variety of substitution patterns and configurations with either natural or unnatural stereochemistry. As outlined in Chapter 1 there are three general synthetic routes to intermediate 44. This chapter focuses on Disconnection 3 where cyclisation precedes the introduction of stereochemistry.

Scheme 4.1: Retrosynthesis overview

Although intermediate 44 could be accessed via Disconnection 1 or Disconnection 3 using Sharpless Asymmetric Dihydroxylation (SAD) as a means of generating the desired α-hydroxy ketone motif, it quickly became apparent that Disconnection 3 also afforded the opportunity to synthesise a variety of highly substituted heterocycles. Therefore Disconnection 3 was developed as a means of generating 2,3,4-substituted tetrahydrothiophenes (THTs) and 2,3-substituted thiophenes.
4.1.1 Some Biologically Significant Tetrahydrothiophenes:

The tetrahydrothiophene (THT) motif, has widespread occurrence in biologically significant natural products, exempli gratia, the essential coenzyme biotin. Biotin is one of the B vitamins and plays an essential role in carboxylation reactions relating to biochemical processes, such as gluconeogenesis and fatty acid biosynthesis. Because humans and other mammals cannot synthesise biotin, it must be derived from dietary sources. Good sources of biotin include liver, kidney, pancreas, yeast, milk and egg yolk.

Biotinidase is the enzyme responsible for cleaving and recycling biotin from biocytin (which is lysine bound biotin) and from dietary protein-bound sources. Mutations in the genes coding for this enzyme, result in biotinidase deficiency and individuals (children) suffering from this disorder (if untreated), may exhibit seizures, hypotonia, skin rash, alopecia, vision problems, hearing loss, and developmental delay, with accompanying ketolactic acidosis and organic aciduria. Biotin supplementation can markedly improve the neurological and cutaneous symptoms of affected children and many countries screen for biotinidase deficiency in their newborn screening programmes.

![Figure 4.1: Structure of biotin](image)

In 2005, studies carried out in the Mock laboratory, investigating biotin status during pregnancy, indicated that a marginal degree of biotin deficiency develops in a substantial proportion of women during normal pregnancy. The degree of deficiency was not substantial enough, to produce the classical cutaneous and behavioural manifestations of biotin deficiency and therefore goes unrecognised. However, in animal
models marginal biotin deficiency has proved teratogenic, leading to the formation of malformed fetuses.\textsuperscript{177}

Biotin deficiency also has a serious effect on poultry leading to reduced growth and the formation of epidermal and bone lesions. The first signs of biotin deficiency in growing chickens and turkeys occur in epidermal tissues: impaired feathering, periocular dermatitis, encrustation and fissures in the angles of the beak and eyelids, and on the foot pads and toes. These symptoms worsen with the severity of the deficiency and also with increased time.\textsuperscript{178}

A second biotin related affliction of young broilers is fatty liver and kidney syndrome (FLKS). This is a metabolic disorder which results in the death of the young birds. It is usually associated with diets that have marginal levels of biotin but there may be none of the previously described signs and symptoms. FLKS is caused by a failure of hepatic gluconeogenesis and results in extensive fatty infiltration of body tissues, leading to enlarged liver, kidneys and heart. A reduction in hepatic gluconeogenesis, caused by decreased pyruvate carboxylase activity, leads to severe hypoglycaemia, which results in damage to the nervous system and ultimately death.\textsuperscript{178} These nutritional deficiencies can be avoided by using biotin as a food additive, hence its commercial importance.\textsuperscript{175} Since the elucidation of the structure of biotin, many racemic and subsequently enantioselective syntheses, have been reported. The total syntheses of biotin have been exhaustively reviewed by De Clercq.\textsuperscript{175}

Despite the significant importance of the THT motif in nature and the considerable medicinal potential of thiolane derivatives, such as 4'-thionucleoside analogues (discussed in Chapters 1, 2 and 3), salacinol\textsuperscript{179} (a glucosidase inhibitor used in the traditional treatment of diabetes) and biotin; the main focus of this chapter is the synthesis of 2,3-substituted thiophenes.
4.1.2 Biologically Significant Thiophenes:

Thiophenes are a diverse class of compound possessing a wide range of properties with a long and distinguished chemical history. They are found in abundance in nature, some recent examples include xanthopapins A, B and C (see Scheme 4.4); these were isolated from *Xanthopapus subacaulis*, an endemic plant in mainland China. These compounds have exhibited potent photoactivated insecticidal activity against larvae of the Asian tiger mosquito *Aedes albopictus*.

![Figure 4.2: Photoactivated Insecticidal Thiophene Derivatives from *Xanthopappus subacaulis*](image)

Another example is byranthrathiophene (see Figure 4.3), a thiophene containing compound isolated from the bryozoan *Watersipora subtorquata*, which exhibited antiangiogenic behavior on bovine aorta endothelial cell (BAEC) proliferation.

![Figure 4.3: Structure of bryoanthrathiophene](image)

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A vast quantity of thiophenes have been isolated from *Compositae* plants and an exhaustive list of the species from which these thiophenes were derived was reported by Bohlmann and Zdero.\textsuperscript{182}

![Structure of Plavix](image)

**Figure 4.4: Structure of Plavix**

Plavix (clopidogrel) is another biologically significant thiophene containing molecule. Plavix is a potent antiplatelet agent used in the treatment of coronary artery disease, peripheral vascular disease and cerebrovascular disease. Plavix is one of the top selling drugs in the world marketed by Bristol Myers Squibb and in 2007 net sales reached almost USD 7 billion.\textsuperscript{183}

Thiophene can act as a bioisostere for benzene and therefore the replacement of phenyl groups with thiophene ring systems in biologically active compounds is a common exercise in medicinal chemistry. Bioisosteres, as defined by Friedman, are "functional groups or molecules that have chemical and physical similarities producing broadly similar biological properties."\textsuperscript{184} Recently, Burger expanded this definition to take into account biochemical views of biological activity, "Bioisosteres are compounds or groups that possess near equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties such as hydrophobicity. Bioisosteric compounds affect the same biochemically associated systems as agonist or antagonists and thereby produce biological properties that are related to each other."\textsuperscript{185,186}
**Scheme 4.2: Bioisosteric strategy in drug design**

Recently Tai *et al.* designed, synthesised and evaluated the biological activity of novel alkenylthiophenes as potent and selective CB₁ cannabinoid receptor inverse agonists. Selective receptor antagonists of CB₁ receptors have exhibited considerable potential as appetite suppressants and thus, are being investigated to determine their efficacy as anti-obesity drugs. Obesity has become a global epidemic and according to the World Health Organisation, in 2005 there were approximately 400 million people suffering from obesity; this figure is expected to grow to 700 million people by 2015. Obesity is a major cause of cardiovascular disease, diabetes and some cancers. Rimonabant (SR141716A) is a potent CB₁ receptor inverse agonist which was launched in Europe in 2006. Tai *et al.* designed and synthesised several Rimonabant mimetics using a strategy of bioisosterism in their modifications (see Scheme 4.2). They chose to investigate the pharmacological effects exerted by C5 substitution and also the effect of 1,3-transposition of the carbonyl at the pyrazole C3 atom. Tai *et al.* discovered several compounds with potent biological activity towards CB₁ receptors and good CB₁/CB₂ selectivity; the most promising mimetic is shown in Scheme 4.2.

There is a plethora of pharmacologically active thiophene containing molecules; one particularly important compound is the local anaesthetic Articaine (see Figure 4.5)
Local anaesthetics block nerve conduction by reversibly binding with proteins of the voltage gated sodium channels in the nerve membrane. This action is intracellular, requiring diffusion of the anaesthetics across the lipophilic nerve membrane. The pH inside the cell is lower than that outside and once inside the cell, an ionised form of the drug is generated. The ionised drug blocks receptors in sodium channels of the cell and if a sufficient number of sodium channels are blocked, the threshold potential will not be reached and impulse conduction stops.

The unionised local anaesthetic can also disrupt the intra-membrane portion of the ion channels, resulting in a blockade of potassium channels and calcium channels, all of which are involved in the neurological response.

There is a broad similarity in the structures of local anaesthetics; they consist of a lipophilic aromatic ring connected to a hydrophilic amine group (see Figure 4.5). The thiophene ring in Articaine gives it enhanced lipophilicity as compared to other amide local anaesthetics. Lipid solubility is important in determining the ability of local anaesthetics to diffuse across the lipid rich nerve membrane and access target receptors. The higher the lipid solubility the greater the potency of the local anaesthetic. Articaine, like lidocaine, has a low pKa (~7.8 and ~7.7 respectively); this ensures a rapid onset of action. Articaine is the most commonly used dental anaesthetic in Europe, where it has been available since 1976 and it was first approved for use by the US FDA in 2000.

Dolman et al. recently reported thiophene substituted willardiine derivatives as excellent selective antagonists of the GLU$_{K5}$ receptor. The GLU$_{K5}$ receptor is a kainate receptor, which are a class of transmembrane ligand gated ion channels. They are located at synapses and convert the chemical signal of a presynaptically released neurotransmitter.
into a postsynaptic electrical signal. Kainate receptors are activated by the neurotransmitter (S)-glutamate.\textsuperscript{193, 194}

To date, only a few selective kainate receptor antagonists have been reported. Compounds LY293-558 and LY382-884 (see Figure 4.6) are compounds based around a decahydroisoquinoline nucleus and have been reported to be competitive GLU\textsubscript{K5} receptor antagonists. These antagonists have been used to provide evidence that GLU\textsubscript{K5} receptors feature in a range of central nervous system disorders such as epilepsy, chronic pain, ischemia and migraine.\textsuperscript{194}

\textbf{Figure 4.6: Some GLU\textsubscript{K5} antagonists}

The most potent derivatives synthesised by Dolman et al. are shown in Figure 4.7. These are among the most potent and selective GLU\textsubscript{K5} containing kainate receptor antagonists reported to date.\textsuperscript{193}

\textbf{Figure 4.7: Thiophene substituted willardiine derivatives with potent GLU\textsubscript{K5} kainate receptor antagonist activity}

Substituted thiophenes with electron withdrawing substituents have displayed a wide range of biocidal activity; for example 2,4-dinitrothiophene (131) and related
derivatives 132-134 are fungicides and 2-acetyl-3,5-dinitrothiophene 132 is a potent antibiotic (see Figure 4.8).\cite{195}

![Figure 4.8: Some biologically active nitrothiophenes](image)

The precise mode of action of these compounds is not known but Morley et al.\cite{195,196} have suggested that the mechanism of action of compounds such as 133 and 134, bearing good leaving groups (Cl and Br) may be associated with nucleophilic attack by intracellular thiols at the 2-position of the ring leading to displacement of the halogen atom.

![Scheme 4.3: Possible mode of action of nitrothiophenes, resulting in disruption of protein function in anti-oxidant processes](image)

On the other hand other derivatives such as 131 and 132 are thought to act by forming Meisenheimer complexes as shown in Scheme 4.4. The overall biological effect is thought to occur by disruption of anti-oxidant processes.\cite{195}

These Meisenheimer complexes are formed by attack of a nucleophile at the 2- or 5-position of the thiophene ring and the negative charge is thought to be delocalised by both the nitro group(s) and the vacant $d$- orbitals on the sulfur atom (some similar complexes were found to be remarkably stable in solution).\cite{195}
Scheme 4.4: Formation of Mesienheimer complexes between 2-nitrothiophenes and nucleophiles

Another important biologically active thiophene containing molecule is PaTrin-2 (see Figure 4.9).

Figure 4.9: Structure of PaTrin-2

PaTrin-2 was developed by McElhinney et al. of Trinity College Dublin in collaboration with Margison et al. of the Paterson Institute for Cancer Research in Manchester. PaTrin-2 is an inhibitor of the DNA repair enzyme O\(^6\)-methylguanine-DNA methyl transferase (MGMT).\(^{197}\) MGMT is the first line of defence against alkylating agents; its possible mode of action is outlined in Scheme 4.5.
Scheme 4.5: Possible mode of action of MGMT in removing $O^6$-methyl groups from alkylated guanine residues in DNA

Alkylating agents are key chemotherapeutics in the arsenal against neoplastic diseases. However their cytotoxic effects are impeded by the DNA repair enzyme (MGMT). Therefore by administering an MGMT inhibitor in a combination regimen with an alkylating agent the chemotherapeutic effect of the alkylating agent is potentiated by the enzyme inhibitor. The in vitro MGMT inactivating activity of PaTrin-2 was $IC_{50}=3.4$ nM and in clinical trials PaTrin-2 has been administered with the alkylating agent temozolomide (see Figure 4.10).

Figure 4.10: Structure of the alkylating agent temozolomide

4.1.3 Thiophenes with Applications in Materials Science

In addition to the extensive biological activity exhibited by thiophenes, oligomeric and polymeric thiophenes have generated significant interest as organic semiconductors and
are extensively studied by materials chemists. Recently, organic semiconductors have found applications in optoelectronic devices such as organic light emitting diodes (OLEDs), organic solar cells and organic field effect transistors (OFETs). Some advantages of using organic semiconductors include ease of fabrication, mechanical flexibility and low cost.

Unlike inorganic semiconductors, organic semiconductors are composed of individual molecules and are only weakly bound together by van der Waals, hydrogen bonding and π-π stacking interactions. Charge delocalisation can only occur along the conjugated backbone of a single molecule, or between the π-orbitals of adjacent molecules. Charge transport is thought to rely on charge hopping from these localised states, with electron transfer between a charged oligomer/polymer and a neutral oligomer/polymer.

Organic semiconductors are commonly classified as either p-type (hole conducting) or n-type (electron conducting), depending on which type of charge carrier is more efficiently transported through the material. P-type organic semiconductors are much more common than n-type semiconductors; this is partly due to the methods of synthesis. N-type semiconductors are chemically synthesised, by treatment with reducing agents, such as alkali metals, resulting in the formation of electron rich polarons, which are unstable in the presence of oxygen. P-type organic semiconductors can be generated by chemical doping, using oxidants such as iodine, bromine or trifluoroacetic acid.

![Scheme 4.6: Formation of a polaron in polythiophene](image)

In recent years, the synthesis of n-type organic semiconductors, for the development of OFETs, has received considerable attention and the discovery that π-conjugated systems
with electron withdrawing groups have n-type semiconducting properties, has led to the synthesis of several electronegative oligomeric thiophenes. For example, le et al. synthesised a number of perfluorinated thiophene derivatives which exhibited n-type semiconductor conductivity (see Figure 4.11).

Despite the historical interest in the chemistry of aromatic heterocycles, the production of 2,3-disubstituted thiophenes is not always trivial due to the preferential reaction at the 2- and 5-positions, necessitating the use and removal of blocking groups. The route discussed in this chapter, which involves the synthesis of 2-substituted-3-nitro thiophenes, possesses significant advantages over much current methodology as it obviates the need for a blocking group at the 5-position and removes the necessity for chromatography in most cases. Also the nitro group is a particularly useful functional group, its powerful directing effect will ensure exclusive (rather than preferential) electrophilic aromatic substitution reactivity at the 5 position and it has been described as a "chemical chameleon" due to the range of functionality that can be derived from it.
4.2 Retrosynthetic Analysis:

A detailed retrosynthetic analysis for the synthesis of 2,3-substituted thiophenes is outlined in Scheme 4.7. The approach involved a tandem Michael-Henry reaction. It was envisaged that Michael-addition of a thiolate anion (generated in situ from the dithiane 135) to a nitroolefin 136 followed by an intramolecular attack of the resultant α-nitroanion onto the nearby aldehyde would furnish a diastereomeric mixture of the desired tetrahydrothiophene (THT). Subsequent dehydration and oxidation affords the 2-substituted-3-nitrothiophene (NT) 6.

Scheme 4.7: Retrosynthetic analysis for generation of 2-substituted-3-nitrothiophenes
4.3 Results:

4.3.1 Synthesis of Tetrahydrothiophenes:

The first step in the reaction sequence was a tandem Michael-Henry reaction between a thiolate anion and a nitroolefin leading to the formation of a 2,3,4-substituted tetrahydrothiophene (THT). The commercially available trans-β-nitrostyrene (136) was initially chosen to develop the methodology. The reaction proceeded by treating dithiane 135 with catalytic triethylamine, generating the thiolate anion in situ followed by a Michael reaction with nitroolefin 136 leading to the formation of a stabilised α-nitroanion 140. A subsequent intramolecular Henry reaction provided the desired THT 137 as a 3:2 mixture of diastereomers in excellent yield (99%) after purification by column chromatography.

\[
\text{HO}^\text{S} \xrightarrow{\text{TEA, CH}_2\text{Cl}_2} \text{Et}_3\text{NH}^+ \quad \text{R} \xrightarrow{\text{NO}_2} \quad \text{O}_2\text{N}^+ \quad \text{R} \xrightarrow{\text{OH}} \quad \text{O}_2\text{N}^+ \quad \text{OH}
\]

**Scheme 4.8**: Formation of THT via tandem Michael-Henry reaction

Despite the inevitable loss of the chiral centres upon aromatisation to 3-nitro-2-phenyl thiophene (138), to ease the analysis of the THT, the diastereomers of the test compound 137 were separated using column chromatography, thereby enabling more facile NMR assignment for subsequent diastereomeric mixtures. The assigned \(^1\text{H}\) NMR spectra (in deuterated dimethyl sulfoxide) for the THT 137 are shown in **Figure 4.12**.
4.3.1.1 *Synthesis of Nitroolefins:*

Having successfully developed methodology for the synthesis of the THT motif, the scope of the reaction had to be determined, using a variety of nitroolefins. Nitroolefins have been extensively used in organic chemistry due to their ease of conversion into a variety of functionalities; they are powerful dienophiles in Diels-Alder reactions and undergo addition reactions with many different nucleophiles. In recent years they have also become of particular importance in organocatalysis. Some nitroolefins were commercially available but the others were synthesised from their aldehyde precursors. The nitroolefins were prepared *via* two methodologies (Scheme 4. 9 and Scheme 4. 10) and the results are summarised in Table 4. 1.
The first approach was a two step process whereby an aldehyde was initially converted into a nitroalcohol, which was subsequently dehydrated, furnishing the desired nitroalkene (Scheme 4.9). The first step involved the addition of nitromethane to a solution of potassium tert-butoxide (0.1 equivalents) in tetrahydrofuran/tert-butanol (1:1) at 0 °C. After 1 hour a solution of aldehyde was added and the reaction was allowed to warm to room temperature and stirred overnight (ca. 16 hours). After work up and column chromatography the nitroalcohols were isolated in varying yields (46-89%). This considerable variance is due to product stability, with some products being prone to decomposition on silica and alumina. Dehydration of the nitroalcohols was accomplished by treatment of a solution of nitroalcohol at -5 °C with 1.1 equivalents of trifluoroacetic anhydride (TFAA), and after 5 minutes 2.2 equivalents of triethylamine (TEA) were added dropwise over a 30 minute period. After a further 30 minutes stirring and aqueous work up the nitroolefins were isolated in good yield after column chromatography (67-92%).

The second procedure for generation of nitroolefins was a one pot protocol whereby the appropriate aldehyde was heated at reflux in nitromethane with ammonium acetate as a catalyst (see Scheme 4.10). After work up and column chromatography the nitroolefins were isolated once again in varying yields (25-80%). Once again product stability was an issue, in some cases resulting in a reduction in yields.

Scheme 4.9: Two step formation of nitroolefins

Scheme 4.10: One pot formation of nitroolefins
4.3.1.2 Nitroalcohols, Nitroolefins and Tetrahydrothiophenes Prepared:

The nitroalcohols and nitroolefins prepared via both procedures (Scheme 4.9 and Scheme 4.10) and the corresponding THTs prepared are shown in Table 4.1

Table 4.1: Nitroalcohols (NA), Nitroolefins (NO) and Tetrahydrothiophenes (THT) prepared

<table>
<thead>
<tr>
<th>R=</th>
<th>% Yield</th>
<th>Product No.</th>
<th>% Yield</th>
<th>Product No.</th>
<th>% Yield</th>
<th>Product No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NA</td>
<td></td>
<td>NO</td>
<td></td>
<td>THT</td>
<td></td>
</tr>
<tr>
<td>CH$_3$</td>
<td>-</td>
<td>-</td>
<td>a</td>
<td>136</td>
<td>99</td>
<td>137</td>
</tr>
<tr>
<td>O$_2$N</td>
<td>64</td>
<td>144</td>
<td>77</td>
<td>145</td>
<td>74</td>
<td>146</td>
</tr>
<tr>
<td>Br$_2$</td>
<td>-</td>
<td>-</td>
<td>a</td>
<td>147</td>
<td>87</td>
<td>148</td>
</tr>
<tr>
<td>NO$_2$</td>
<td>56</td>
<td>149</td>
<td>76</td>
<td>150</td>
<td>80</td>
<td>151</td>
</tr>
<tr>
<td>Br$_2$</td>
<td>-</td>
<td>-</td>
<td>a</td>
<td>152</td>
<td>84</td>
<td>153</td>
</tr>
</tbody>
</table>
Table 4.1: Nitroalcohols (NA), Nitroolefins (NO) and Tetrahydrothiophenes (THT) prepared

<table>
<thead>
<tr>
<th>R</th>
<th>% Yield NA</th>
<th>Product No.</th>
<th>% Yield NO</th>
<th>Product No.</th>
<th>% Yield THT</th>
<th>Product No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMe</td>
<td>81</td>
<td>156</td>
<td>76</td>
<td>154</td>
<td>76</td>
<td>155</td>
</tr>
<tr>
<td>CF₃</td>
<td>75</td>
<td>159</td>
<td>82</td>
<td>160</td>
<td>71</td>
<td>161</td>
</tr>
<tr>
<td>O</td>
<td>54</td>
<td>164</td>
<td>34</td>
<td>165</td>
<td>59</td>
<td>166</td>
</tr>
<tr>
<td>Br</td>
<td>46</td>
<td>167</td>
<td>25</td>
<td>168</td>
<td>34</td>
<td>169</td>
</tr>
<tr>
<td>N</td>
<td>-</td>
<td>-</td>
<td>47</td>
<td>170</td>
<td>73</td>
<td>171</td>
</tr>
<tr>
<td>N</td>
<td>-</td>
<td>-</td>
<td>80</td>
<td>172</td>
<td>67</td>
<td>173</td>
</tr>
</tbody>
</table>
Table 4.1: Nitroalcohols (NA), Nitroolefins (NO) and Tetrahydrothiophenes (THT) prepared

<table>
<thead>
<tr>
<th>R=</th>
<th>% Yield NA</th>
<th>Product No.</th>
<th>% Yield NO</th>
<th>Product No.</th>
<th>% Yield THT</th>
<th>Product No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Pyridine" /></td>
<td>79</td>
<td>174</td>
<td>69</td>
<td>175</td>
<td>33</td>
<td>176</td>
</tr>
<tr>
<td><img src="image2.png" alt="Cyclohexane" /></td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>177</td>
<td>77</td>
<td>178</td>
</tr>
<tr>
<td><img src="image3.png" alt="Tetrahydrofuran" /></td>
<td>83</td>
<td>179</td>
<td>92</td>
<td>180</td>
<td>94</td>
<td>181</td>
</tr>
<tr>
<td><img src="image4.png" alt="Tetrahydrothiophene" /></td>
<td>89</td>
<td>182a</td>
<td>-</td>
<td>183</td>
<td>60</td>
<td>184</td>
</tr>
<tr>
<td><img src="image5.png" alt="Tetrahydrofurane" /></td>
<td>68</td>
<td>185a</td>
<td>67</td>
<td>186</td>
<td>71</td>
<td>187</td>
</tr>
<tr>
<td><img src="image6.png" alt="Tetrahydrofuran" /></td>
<td>89</td>
<td>188</td>
<td>70</td>
<td>189</td>
<td>89</td>
<td>190</td>
</tr>
</tbody>
</table>

*a=commercially available; b=synthesised by Dr. Mark Roydhouse*
All of the THTs synthesised in **Table 4.1** are novel compounds. In general the diastereomeric mixtures of THT were not separated, however, in the case of the furanyl substituted THT 163, separation of the diastereomers was particularly facile and crystals suitable for x-ray analysis were grown of the major diastereomer (see **Figure 4.13**).

![Crystal structure of furanyl THT](image)

**Figure 4.13**: Crystal structure of furanyl THT

Analysis of the $^1$H NMR spectra of 163 revealed large coupling constants between H5 and H4 in both the major ($J=10.0$ Hz) and minor ($J=10.0$ Hz) diastereomers, thus indicating an *anti* arrangement (**Figure 4.14**). The coupling constant between H4 and H3 in the major isomer, was also large ($J=9.5$ Hz), therefore H4 maj and H3 maj have an *anti* relationship. However in the minor isomer the coupling constant between H4 and H3 was significantly smaller ($J=3.5$ Hz). This implies that H4 min and H3 min are *syn* to each other.

The crystal structure of the major diastereomer in **Figure 4.13**, clearly depicts the *anti* relationship between the nitro group and the furanyl group, and also the *anti* stereochemistry of H4 maj and H3 maj. Similarly, the crystal structure of the minor *iso*-butyl substituted THT 181 in **Figure 4.15**, clearly shows the *anti* relationship between the nitro group and the *iso*-butyl group, as well as the *syn* stereochemistry of H4 min and H3 min.
Figure 4.14: Furanyl-THT 163 \(^1\)H NMR spectra

- Complete separation of the minor isomer was not achieved but the spectrum containing it as the major component is shown for comparison. (The lower spectrum is of a column fraction which contained both major and minor diastereomers)

Figure 4.15: Crystal structure of minor diastereomer of iso-butyl-THT 181
4.3.2 Dehydration of THT:

\[
\text{O}_2\text{N} - \text{OH} \quad \rightarrow \quad \text{O}_2\text{N} - \text{S} - \text{Ph} \\
\text{Ph} \quad \text{Dehydration} \quad \text{S} - \text{Ph} \\
\text{137} \quad \text{191} \quad \text{138}
\]

Scheme 4.11: Dehydration/aromatisation of THT 137

In order to prepare the thiophenes from the THTs dehydration and aromatisation had to be performed. Initial attempts at dehydration of THT 137 were performed in a similar manner to the synthesis of the nitroolefins. A solution of 137 in dichloromethane at -5 °C was treated with 1.1 equivalents of trifluoroacetic anhydride for 5 minutes before the addition of 2.2 equivalents of triethylamine dropwise over 30 minutes. The reaction afforded the dihydrothiophene (DHT) 191 in moderate yield (60%) and so various optimisation experiments were subsequently carried out. By reducing the temperature to -15 °C and freshly distilling the reagents immediately prior to use a marked improvement in yield was observed and DHT 191 was isolated in 85% yield.

Alternative procedures involving tosylation and mesylation of 137 followed by in situ elimination were also attempted but both proved less successful than when the trifluoroacetic anhydride/triethylamine protocol was used.

A two pot procedure was also investigated to ascertain whether yields for the dehydration could be improved further. In this instance 137 was initially acetylated forming 192 which was subsequently treated with base to effect the elimination (Scheme 4.12). Acetylation forming 192 was accomplished, by stirring a solution of the 137 in acetic anhydride at 0 °C and introducing a catalytic quantity of sulfuric acid. After work up and column chromatography the desired acetate 192 was isolated as a white solid in 92% yield. A series of experiments were carried out whereby 192 was treated with a variety of bases to ascertain which if any effected dehydration/aromatisation.
Scheme 4. 12: Dehydration/aromatisation of THT

Treatment of solutions of 192 in dichloromethane with a variety of bases gave complex mixtures of products after stirring for ca. 16 hours at room temperature. There were 4 main products observed in the $^1$H NMR spectra: DHT 191, nitrothiophene (NT) 138, phenylthiophene (PT) 193, and an unknown aromatic compound (which could not be isolated). Undesired PT 193 was thought to arise from loss of HNO$_2$, with aromatisation being the driving force for the reaction. The end product 138 was thought to arise from oxidation of an intermediate compound to form the desired aromatic compound. The ratios of products obtained using various bases are outlined in Table 4. 2.

Table 4. 2: Elimination of acetic acid from THT 192

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Solvent</th>
<th>192</th>
<th>191</th>
<th>138</th>
<th>193</th>
<th>Unknown</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DBU</td>
<td>CH$_2$Cl$_2$</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>2</td>
<td>0.15</td>
<td>RR</td>
</tr>
<tr>
<td>2</td>
<td>TEA</td>
<td>CH$_2$Cl$_2$</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>1.8</td>
<td>0.4</td>
<td>RR</td>
</tr>
<tr>
<td>3</td>
<td>DMAP</td>
<td>CH$_2$Cl$_2$</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>0.52</td>
<td>0.48</td>
<td>RR</td>
</tr>
<tr>
<td>4</td>
<td>DABCO</td>
<td>CH$_2$Cl$_2$</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>0.7</td>
<td>RR</td>
</tr>
<tr>
<td>5</td>
<td>DIPA</td>
<td>CH$_2$Cl$_2$</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>1.52</td>
<td>0.18</td>
<td>RR</td>
</tr>
</tbody>
</table>

RR=Relative Ratios, which were determined from the crude $^1$H NMR spectra; MP=major product
Table 4.2: Elimination of acetic acid from THT 192

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Solvent</th>
<th>192</th>
<th>191</th>
<th>138</th>
<th>193</th>
<th>Unknown</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>N-methyl morpholine</td>
<td>CH₂Cl₂</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>1.9</td>
<td>0.2</td>
<td>RR</td>
</tr>
<tr>
<td>7</td>
<td>Hünigs Base</td>
<td>CH₂Cl₂</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>0.87</td>
<td>0.29</td>
<td>RR</td>
</tr>
<tr>
<td>8</td>
<td>N-methyl imidazole</td>
<td>CH₂Cl₂</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>MP</td>
<td>MP</td>
</tr>
<tr>
<td>9</td>
<td>N-methyl pyrrolidine</td>
<td>CH₂Cl₂</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>0.22</td>
<td>0.61</td>
<td>RR</td>
</tr>
<tr>
<td>10</td>
<td>NaOAc</td>
<td>Et₂O</td>
<td>0.4</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td>RR</td>
</tr>
</tbody>
</table>

RR=Relative Ratios, which were determined from the crude ¹H NMR spectra; MP=major product.

The ¹H NMR spectra of crude reaction mixtures derived from the basic eliminations of acetic acid from 192 were very complex. TLC analysis also indicated a number of products had formed in most cases, with Entries 8 and 10 (Table 4.2) being the only exceptions. Therefore only relative ratios were calculated from integrals in the ¹H NMR spectra. Of the reactions in Table 4.2, none of the conditions described afforded either 191 or 138 in appreciable yield; consequently this route was abandoned in favour of the trifluoroacetic anhydride/triethylamine method (Scheme 4.13)

Scheme 4.13: Dehydration of THT 137
4.3.3 Aromatisation of THT/DHT:

Initially, the oxidations leading to formation of 138, observed in Table 4.2 were thought to arise from oxidation of intermediary compounds present in the reaction, by trace quantities of oxygen. However, when a solution of DHT 191 in dichloromethane was stirred in air, formation of 138 did not occur and the denitro compound 193 was not observed after 24 hours (Scheme 4.14). Aromatisation of 191 to 138 was therefore attempted using 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) as an oxidizing agent. DDQ is a hydride acceptor molecule which is used extensively as an oxidizing agent, and has proved to be particularly useful for aromatisation reactions. Fortunately, when DDQ was used to oxidise DHT 191 nitrothiophene 138 was formed and none of the denitro compound 193 was observed.

Scheme 4.14: Aromatisation of DHT 191

A series of optimisation experiments varying quantities of DDQ, temperature and reaction time were performed, the results of which are shown in Table 4.3.
Table 4. 3: Aromatisation of DHT to THT with DDQ experimental results

<table>
<thead>
<tr>
<th>Entry</th>
<th>Oxidant</th>
<th>No. Equiv.</th>
<th>Time (h)</th>
<th>Temp. (°C)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DDQ</td>
<td>1.0</td>
<td>24</td>
<td>RT</td>
<td>A large quantity of starting material remained</td>
</tr>
<tr>
<td>2</td>
<td>DDQ</td>
<td>1.0</td>
<td>24</td>
<td>40</td>
<td>All starting material consumed, 47% isolated yield</td>
</tr>
<tr>
<td>3</td>
<td>DDQ</td>
<td>2.0</td>
<td>36</td>
<td>RT</td>
<td>All starting material consumed, 44% isolated yield</td>
</tr>
<tr>
<td>4</td>
<td>DDQ</td>
<td>2.0</td>
<td>18</td>
<td>40</td>
<td>All starting material consumed, 63% isolated yield</td>
</tr>
<tr>
<td>5</td>
<td>DDQ</td>
<td>4.0</td>
<td>24</td>
<td>40</td>
<td>Work up very difficult, no improvement in yield</td>
</tr>
</tbody>
</table>

When 1.0 equivalent of DDQ in dichloromethane was used to effect aromatisation of DHT 191, after 24 hours at room temperature (Entry 1), a large quantity of starting material remained. The reaction was therefore repeated; on this occasion heating at reflux and after 24 hours, all of the starting material had been consumed and the product was isolated in 47% yield (Entry 2). The effect of altering the number of equivalents of oxidant was also tested. At room temperature 2.0 equivalents of DDQ completed oxidation of DHT 191 within 36 hours (Entry 3) in 44% yield. By elevating the temperature of the reaction to 40 °C, the reaction was complete within 18 hours and an increase in yield to 63% was observed. However, when the quantity of DDQ was elevated further to 4.0 equivalents, the work up for the reaction became very difficult and isolation of product was complex.

Alternative solvents were also assessed, including dioxane (anhydrous and wet) and toluene. When dioxane was employed, the majority of the starting material remained after 48 hours with only a minor quantity of product (the relative ratio of 191 to 138 was 10:1). When toluene was used, the product was observed but the reaction also produced a number
of by-products and work up was once again difficult. Neither solvent proved as successful as dichloromethane.

Table 4. 4: Formation of Nitrothiophenes (NTs) from tetrahydrothiophenes (THTs)

<table>
<thead>
<tr>
<th>R=</th>
<th>% Yield THT</th>
<th>Product No.</th>
<th>% Yield NT</th>
<th>Product No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>99</td>
<td>137</td>
<td>60</td>
<td>138</td>
</tr>
<tr>
<td>BnO</td>
<td>87</td>
<td>148</td>
<td>44</td>
<td>194</td>
</tr>
<tr>
<td>Br</td>
<td>84</td>
<td>153</td>
<td>30</td>
<td>195</td>
</tr>
<tr>
<td>O Bn</td>
<td>76</td>
<td>155</td>
<td>30</td>
<td>196</td>
</tr>
<tr>
<td>O</td>
<td>44</td>
<td>163</td>
<td>33</td>
<td>197</td>
</tr>
<tr>
<td>94</td>
<td>181</td>
<td>29</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>187</td>
<td>42</td>
<td>199</td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>190</td>
<td>-</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

*a=Reaction time was extended to 48 h*
Aromatisation of 137 to the desired 138 was also attempted in a one pot procedure employing the trifluoroacetic anhydride/triethylamine route for generation of 191, then treating with DDQ and heating at reflux. Yields varied between 30-40% when attempted on several occasions and therefore the 2 pot procedure which has an overall yield of approximately 60% is preferable.

The scope of the two step process was then tested using some of the THTs previously prepared. The results are outlined in Table 4.4. The isolated thiophene yields were disappointing, especially for the aliphatic compounds, even when the reaction time was extended to 48 hours. In the case of THT 190 (R='Bu) only starting material was recovered. However these initial results led to collaboration with GlaxoSmithKline (GSK) who funded a post-doctoral researcher, Dr. Mark Roydhouse. Optimisation of the dehydration/aromatisation step was continued by O'Connor and Roydhouse.

4.3.4 Optimisation of Aromatisation/Dehydration using aromatic substituted THT 138

The use of surface mediated solid phase reactions involving inorganic solids is becoming increasingly popular in synthetic chemistry. In 1992, Ballini et al. described the synthesis of nitroalkenes, by dehydration of nitroalcohols using basic alumina in refluxing dichloromethane (Scheme 4.15); this procedure was attempted on 138 but after 41 hours the reaction had not reached completion and once again a mixture of products was observed.

Scheme 4.15: Dehydration of nitroalcohols using basic alumina
As the dehydration could potentially be performed under basic or acidic catalysis, acidic and neutral alumina were also investigated as dehydrating agents (Table 4.5). The neutral alumina gave similar results to the basic alumina reactions with a ratio of 138:193 of 1:1. Acidic alumina gave a more encouraging ratio of 138:193 of approximately 2:1 after 41 hours. It was hoped that the addition of DDQ would prevent the formation of the denitro product 193 by *in situ* oxidation of 191 to NT 138. The reaction proceeded with clean conversion of 137 to 138 but after 62 hours some of the starting material remained. Unfortunately difficulties with the work up prevented accurate determination of the yield for the reaction but nonetheless the combination of acidic alumina and DDQ to effect aromatisation proved encouraging.

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{OH} \\
\text{Ph} & \quad \text{S} \\
\text{137} & \quad \text{193}
\end{align*}
\]

**Scheme 4.16: Dehydration/aromatisation of THT 137 using solid support**

To try and improve the rate of conversion, the reaction was repeated employing chloroform as solvent, to exploit its higher boiling point. After heating for 40 hours at reflux a ratio of 137:138 of 30:70 was observed. As no 191 was observed it was concluded that the dehydration step was rate limiting and possibly reversible so, to try to combat this the reaction was repeated with molecular sieves (200% w/w) and after 64 hours at reflux an isolated yield of 88% 138 was obtained.

Solvents with higher boiling points were also investigated in an attempt to further reduce the reaction time but none proved as successful as chloroform. Interestingly, when silica gel (400% w/w) was used in place of acidic alumina a similar yield (85%) of 138 was formed with a reduced reaction time of 24 hours.
Table 4.5: Optimisation experiments for dehydration/aromatisation using solid support

<table>
<thead>
<tr>
<th>Entry</th>
<th>Support % w/w</th>
<th>Solvent</th>
<th>Temp. (°C)</th>
<th>Oxidant</th>
<th>Mol. Sieves % w/w</th>
<th>Time (hours)</th>
<th>137</th>
<th>191</th>
<th>138</th>
<th>193</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BA (200)</td>
<td>CH₂Cl₂</td>
<td>40</td>
<td>Air</td>
<td>-</td>
<td>41</td>
<td>44</td>
<td>41</td>
<td>6</td>
<td>9</td>
<td>RR</td>
</tr>
<tr>
<td>2</td>
<td>NA (200)</td>
<td>CH₂Cl₂</td>
<td>40</td>
<td>Air</td>
<td>-</td>
<td>41</td>
<td>40</td>
<td>35</td>
<td>12.5</td>
<td>12.5</td>
<td>RR</td>
</tr>
<tr>
<td>3</td>
<td>AA (200)</td>
<td>CH₂Cl₂</td>
<td>40</td>
<td>Air</td>
<td>-</td>
<td>41</td>
<td>36</td>
<td>30</td>
<td>21</td>
<td>12</td>
<td>RR</td>
</tr>
<tr>
<td>4</td>
<td>AA (400)</td>
<td>CH₂Cl₂</td>
<td>40</td>
<td>DDQ (2.0 eq.)</td>
<td>-</td>
<td>62</td>
<td>No yield data available</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>AA (400)</td>
<td>CHCl₃</td>
<td>61</td>
<td>DDQ (2.0 eq.)</td>
<td>-</td>
<td>41</td>
<td>29</td>
<td>-</td>
<td>-</td>
<td>71</td>
<td>RR</td>
</tr>
<tr>
<td>6</td>
<td>AA (400)</td>
<td>CHCl₃</td>
<td>61</td>
<td>DDQ (2.0 eq.)</td>
<td>200</td>
<td>61</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>88</td>
<td>Isolated Yield</td>
</tr>
<tr>
<td>7</td>
<td>Silica gel (400)</td>
<td>CHCl₃</td>
<td>61</td>
<td>DDQ (2.0 eq.)</td>
<td>200</td>
<td>61</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>85</td>
<td>Isolated Yield</td>
</tr>
</tbody>
</table>

BA=basic alumina; NA=neutral alumina; AA=acidic alumina; RR=relative ratios calculated from ¹H NMR integrals
4.3.5 Optimisation of Aromatisation/Dehydration using aliphatic substituted THT:

Dehydration/aromatisation of the alkyl THTs once again proved more difficult than for the aromatic substituted THTs. When 181 was subjected to the standard dehydration/oxidation conditions (silica gel (400% w/w), 2.0 equivalents DDQ, molecular sieves (200% w/w), chloroform, 61 °C) after 80 hours the desired product 198 was formed in a 1:1 ratio with the starting material. In an attempt to increase the rate of reaction and maximise yields of the desired product 198, a series of optimisation experiments were performed. Doubling the reagents had little effect and so a number of alternative solvents were tested (toluene, chlorobenzene, hexane, ethyl acetate). Of these ethyl acetate (B.p. 77 °C) gave a very clean conversion of 181 to 198 was observed albeit very slowly. To further increase the rate of the reaction, alternative acetate solvents (e.g. n-propyl, n-butyl) with higher boiling points were investigated.

When THT 181 was subjected to the standard dehydration/oxidation conditions (silica gel (400% w/w), 2.0 equivalents DDQ, molecular sieves (200% w/w), in n-propyl acetate (B.p. 102 °C), the desired product 198 was formed in a ratio of 1:5.4 (198:181) with the starting material after 16 hours. When the number of equivalents of silica and DDQ were increased to 4.0, a further improvement was observed, with the desired NT 198 isolated in 39% after 16 hours.
When THT 181 was reacted under standard conditions (silica gel (400% w/w), 2.0 equivalents DDQ, molecular sieves (200% w/w), in n-butyl acetate (B.p. 126 °C) a very encouraging ratio of 0.3:1 181:198 was observed with an isolated yield of 39% after 16 hours at reflux. By replacing the oxidant DDQ with chloranil (a similar, but more robust and less toxic oxidant than DDQ) a comparable yield was observed, albeit after 40 hours. The highest yield (55%) for the reaction was observed when 181 was treated with acidic alumina (800% w/w), molecular sieves (400% w/w), 4.0 equivalents of chloranil and the reaction was heated at reflux for 40 hours (see Scheme 4.18).

Scheme 4.18: Aromatisation of 181

4.3.6 Microwave-assisted synthesis of thiophenes:

Given the disappointing yields and lengthy reaction times in forming alkynitrothiophenes via conventional thermal heating, microwave heating was investigated. Microwave assisted organic synthesis (MAOS) is a relatively new and rapidly expanding area of synthetic organic chemistry. This synthetic technique is based on the empirical observation that some organic reactions proceed much faster and with higher yields under microwave irradiation compared to conventional thermal heating. Recently Prediger et al. reported the oxidation of 2-arylindolines into 2-arylindoles using DDQ in toluene under microwave irradiation for 4 hours with yields up to 69%. The corresponding reaction using conventional heating took 5 days in toluene at reflux to achieve comparable yields (Scheme 4.19).
Initial attempts at microwave assisted oxidation of THT 181 were performed in a conventional domestic microwave. THT 181 was treated with silica (800% w/w), 4.0 equivalents of DDQ and molecular sieves (400% w/w) in ethyl acetate and heated in a domestic microwave for 30 minutes (Scheme 4. 20). Disappointingly only a ratio of 1:9 of product to starting material was observed.

Since the appearance of the first article on the application of microwaves for chemical synthesis in polar solvents,213 the approach has developed considerably and is now considered a general and useful technique for a variety of applications in organic synthesis and functional group transformations.210, 214-216 In recent years the focus has shifted to solvent-free methods wherein neat reactants, often in the presence of mineral oxides or supported catalysts undergo facile reactions to provide high yields of pure products thus eliminating or minimizing the use of organic solvents.210, 214-216 Solvent-free organic syntheses using supported reagents and microwave irradiation has been concisely reviewed by Varma.217

In their synthesis of fluorenone, Loupy and co-workers performed oxidation reactions on arene substrates using potassium permanganate adsorbed onto alumina in the
absence of solvent. Loupy commented that solid supports are rather poor thermal conductors, (with a consequent gradient in temperature inside the materials), whereas, they behave as strong microwave adsorbants, (with consequent homogeneity in temperature under microwave conditions). Loupy and co-workers successfully oxidised a number of arenes quantitatively, with vastly reduced reaction times when compared with analogous reactions performed with conventional heating.

\[ 
\text{Scheme 4. 21: Loupy and co-workers oxidation of arenes in dry media under focused microwaves} \]

Sivamurugan et al. have also reported the aromatisation of 1,4-dihydropyridines using benzimidazolium fluorochromate (BIFC) and quinolium fluorochromate (QFC) adsorbed onto alumina as oxidants under solvent-free microwave irradiation.

Given the disappointing yields and slow rates observed for the oxidation of THT when using solution based chemistry with both conventional and microwave heating, the use of microwave heating in the absence of solvent was considered.

To this end, alkyl substituted THT 181 was adsorbed onto acidic alumina (10 equivalents w/w) and the resulting solid was ground to homogeneity in a mortar with chloranil (2 equivalents). The resulting pale yellow solids were heated in an open vessel on full power (800 W) for 4 minutes. The solids were then stirred in dichloromethane and removed by filtration. The filtrate was concentrated at reduced pressure and an NMR yield of 41% of the desired thiophene 198 was observed with a small quantity of unreacted starting material still present. When the quantities of alumina and chloranil were doubled and the reaction time was increased to 8 minutes the NMR yield rose to 55% with full consumption of starting material.
Scheme 4.22: Oxidation of 26 under MW irradiation

Initially the chloranil/phenol by-products of the reaction were removed by column chromatography, however it was later discovered that these impurities could be removed by stirring the reaction solids in dichloromethane for 1 hour followed by the introduction of charcoal (2.5 equivalents w/w) and powdered potassium hydroxide (2.5 equivalents w/w), with continued stirring for a further 1 hour. Subsequent filtration through a pad of silica and removal of solvent at reduced pressure furnished the desired NT 198. The improved work up resulted in a considerable enhancement in yield with NT 198 isolated in 85% yield.

A CEM discovery chemical microwave was purchased and the dehydration/aromatisation reaction was optimised further, under the more controlled conditions possible available with the more sophisticated chemical microwave. Using the aromatic THT 137 once more as a test substrate, optimisation experiments were carried out varying the concentration of reagents and reaction time. The best yield was obtained when 137 was treated with acidic alumina (20 equivalents w/w) and chloranil (1.5 equivalents) at 125 °C for 4 minutes. After the optimised work up described above (dichloromethane/charcoal/potassium hydroxide) was performed thiophene 138 was isolated in 91% yield, without the need for further purification.
Figure 4.16: Crude $^1$H NMR spectrum of 5'-Bromo-3-nitro-[2,2']bithiophenyl
The newly optimised conditions were then tested on a range of THT substrates with both aromatic and aliphatic side chains. The list of the nitrothiophenes prepared is shown in Table 4.6. The yields were generally good to excellent with a few notable exceptions. The yields obtained for the aliphatic substituted thiophenes were lower than for those with aromatic substituents. When the reaction was performed on several nitrogen containing heterocycles (R=\text{N-methylpyrrole}, \text{pyridine}, \text{N-methylindole}) only one furnished the desired product (R=\text{N-methylindole}) albeit in poor yield (Table 4.6, compound 210). The poor yield obtained for bis nitrothiophene 211 was attributed to the low solubility of the product. Overall, the use of the solid phase microwave approach produced a marked improvement in yields over the solution based thermal chemistry. For example, using the thermal based solution chemistry the best yield obtained for the synthesis of compound 195 was 30%. However when the solid phase microwave protocol was employed an excellent yield of 82% was obtained. The x-ray crystal structure for compound 195 is shown in Figure 4.17.
Table 4. 6: List of NTs prepared employing microwave procedure

<table>
<thead>
<tr>
<th>R=</th>
<th>% Yield</th>
<th>Product No.</th>
<th>% Yield</th>
<th>Product No.</th>
</tr>
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<tr>
<td><img src="image1" alt="Chemical Structure" /></td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td><img src="image4" alt="Chemical Structure" /></td>
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<td></td>
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<td>146</td>
</tr>
<tr>
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<td><img src="image12" alt="Chemical Structure" /></td>
<td><img src="image13" alt="Chemical Structure" /></td>
<td><img src="image14" alt="Chemical Structure" /></td>
<td><img src="image15" alt="Chemical Structure" /></td>
</tr>
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<td>153</td>
</tr>
<tr>
<td><img src="image16" alt="Chemical Structure" /></td>
<td><img src="image17" alt="Chemical Structure" /></td>
<td><img src="image18" alt="Chemical Structure" /></td>
<td><img src="image19" alt="Chemical Structure" /></td>
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<td>161</td>
</tr>
<tr>
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<td><img src="image22" alt="Chemical Structure" /></td>
<td><img src="image23" alt="Chemical Structure" /></td>
<td><img src="image24" alt="Chemical Structure" /></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>73</td>
<td>171</td>
</tr>
</tbody>
</table>
Table 4.6: List of NTs prepared employing microwave procedure

<table>
<thead>
<tr>
<th>R=</th>
<th>% Yield THT</th>
<th>Product No.</th>
<th>% Yield NT</th>
<th>Product No.</th>
</tr>
</thead>
<tbody>
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<td><img src="image1" alt="R1" /></td>
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<td>178</td>
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<td>211</td>
</tr>
<tr>
<td><img src="image2" alt="R2" /></td>
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<td>184</td>
<td>57</td>
<td>212</td>
</tr>
<tr>
<td><img src="image3" alt="R3" /></td>
<td>71</td>
<td>187</td>
<td>52</td>
<td>213</td>
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<tr>
<td><img src="image4" alt="R4" /></td>
<td>59</td>
<td>166</td>
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<td>214</td>
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<td><img src="image5" alt="R5" /></td>
<td>67</td>
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<td>-</td>
<td>215</td>
</tr>
<tr>
<td><img src="image6" alt="R6" /></td>
<td>33</td>
<td>176</td>
<td>-</td>
<td>216</td>
</tr>
</tbody>
</table>

Figure 4.17: X-ray crystal structure of 195
A considerable enhancement in yields was also observed for several other compounds when synthesised using the solid phase microwave procedure, a few representative examples are shown in Table 4.7. Of particular note is THT 190 (R='Bu) which did not aromatise under solution based thermal conditions but the aromatic product 217 was recovered in 64% yield when the solid phase microwave procedure was employed.

<table>
<thead>
<tr>
<th>R=</th>
<th>THT Product No.</th>
<th>% Yield NT Thermal/Solution</th>
<th>% Yield NT Microwave/Solid Phase</th>
<th>NT Product No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>137</td>
<td>60</td>
<td>91</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>153</td>
<td>30</td>
<td>82</td>
<td>195</td>
</tr>
<tr>
<td></td>
<td>190</td>
<td>0</td>
<td>64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>217</td>
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<tr>
<td></td>
<td>181</td>
<td>29</td>
<td>85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>198</td>
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<td>163</td>
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<td>56&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td></td>
<td>187</td>
<td>42</td>
<td>52</td>
<td>199</td>
</tr>
</tbody>
</table>

<sup>a</sup>=Prepared by Dr. Mark Roydhouse
Analysis of the 3-nitro-2-substituted thiophenes proved difficult. The $^{13}$C spectra had to be run with longer recycle times (2.5 seconds rather than the normal 1.0 seconds) otherwise the quaternary carbon bearing the nitro group was not observed. Several different types of mass spectrometry techniques have been performed on all of the compounds employing numerous solvents but with limited success. Electrospray mass spectrometry in both positive and negative ionisation modes was attempted using a Waters micromass LCT machine; however this proved unsuccessful in a number of alternative solvents (methanol, acetonitrile, dichloromethane). Electron impact and chemical ionisation mass spectrometry were carried out using a Waters GCT Premier machine but once again neither nominal nor accurate mass were obtained (even when different ionisation gases (methane/ammonia) were employed). A test NT sample, 3-nitro-2-(3-nitrophenyl)-thiophene (206) Table 4. 6, was therefore submitted to the EPSRC National Mass Spectrometry Service Centre in Swansea and using a Quattro-II mass spectrometer equipped with a direct injection sampling accessory in El mode both a nominal and an accurate mass were determined for the compound. The alkyl substituted THTs also proved difficult to analyse using mass spectrometry but it is hoped that employing El mass spectrometry with a direct injection sampling accessory (as was done for the 3-nitro-2-(3-nitrophenyl)-thiophene (206)) should prove more fruitful. This facility is currently only commercially available; any outstanding mass spectra will be obtained in the near future.

4.3.7 Further Development of Thiophenes:

The presence of the bromo substituent in compound 195 makes it a particularly useful synthetic building block enabling functionalisation using palladium chemistry, for example. In collaboration with the Draper group at Trinity College Dublin, compound 195 was coupled with a ferrocenyl moiety 218, furnishing a fully conjugated organometallic compound 219. This compound was prepared to investigate its potential non-linear optical properties arising from the electronic push-pull system between the electron withdrawing nitro group and the electron donating ferrocene.
Scheme 4. 24: Sonogashira cross coupling reaction

Draper et al. have formed a collaboration with Professor Isabelle Ledoux at Laboratoire de Photonique Quantique et Moleculaire at Cachan, France and intend to determine the NLO properties of 219 in the near future. Depending on the outcome of this result several other analogous thiophenes will be synthesised and have their NLO properties determined.
4.4 Conclusion

A new synthetic route for the synthesis of 2,3,4-substituted tetrahydrothiophenes from nitroolefins has been developed. Conditions for the aromatisation of the tetrahydrothiophenes to their thiophene counterparts were subsequently optimised using solution based thermal chemistry, solution based microwave chemistry and solid phase microwave assisted chemistry. The synthesis of a large number of novel tetrahydrothiophenes and thiophenes has been accomplished.

In summary, a new, rapid and general route to synthetically useful 3-nitro-2-substituted-thiophenes, without the need for blocking groups, has been developed. The rapid work up/isolation procedure precludes the requirement for chromatography for the majority of post-synthetic applications. The nitro group can serve two further purposes, its powerful directing effect will ensure exclusive (rather than preferential) electrophilic aromatic substitution reactivity at the 5-position (note that under standard nitration conditions a ratio of 85:15 of 2-nitro to 3-nitro substitution is observed). The nitro group also provides an excellent functional handle to install a variety of other substituents by reduction, diazotisation and substitution with a variety of nucleophiles.
4.5 Future Work

Although the focus of this thesis has been on the synthesis of 3-nitro-2-substituted-thiophenes, the methodology developed could potentially be extended to the synthesis of other aromatic heterocycles *exempli gratia* selenophenes, pyrroles and furans from the corresponding unsaturated heterocycles. This work is currently underway in the Southern research group and thus far, the first pyrrole has been prepared by analogous methodology.

Depending on the outcome of the NLO measurements made by Draper and co-workers, several other halo-substituted thiophenes will be synthesised and coupled with ferrocenyl derivatives with a view to developing a new class of compound with potential applications in NLO devices.
Chapter 5

Experimental
5 Experimental

5.1 General

Melting points were determined using a standard melting point apparatus and are uncorrected. Infrared spectra were obtained on a Perkin Elmer Spectrum 100 FT-IR spectrometer equipped with a universal ATR sampling accessory. The Microanalytical Laboratory, University College Dublin, performed elemental analyses. Proton nuclear magnetic resonance (NMR) spectra were recorded on: Bruker Avance III 400 MHz, Bruker DPX400 400 MHz and Bruker Avance II 600 MHz spectrometers (¹H NMR spectra were recorded at 400.23 MHz, 400.13 MHz and 600.13 MHz respectively). Chemical shifts are reported in ppm relative to tetramethylsilane and coupling constants (J) are quoted in Hertz. Carbon NMR spectra were recorded on the previously mentioned instruments (100.64 MHz, 100.61 MHz & 150.9 MHz, respectively) with total proton decoupling. Fluorine NMR spectra were recorded on the Bruker DPX400 machine at 376.5 MHz). HSQC, HMBC, TOCSY and nOe NMR experiments were used to aid assignment of NMR peaks when required. A Waters micromass LCT-tof mass spectrometer was used in ES positive and ES negative modes for electrospray mass spectrometry. Electron impact mass spectra were determined on a Quatro-II mass spectrometer in the EI mode. Mass spectra were recorded in CSCB Trinity College Dublin, CSCB University College Dublin and ESPRC National Mass Spectrometry Service Centre, Swansea. Flash chromatography was performed using Merk Kiesegel 60 (art. 9385) and aluminium oxide 90, standardized (activity II-III). Merk precoated Kiesegel 60F₂₅₄ and alumina (neutral, type E) were used for thin-layer chromatography and slides were visualised by UV irradiation, KMnO₄, or anisaldehyde staining. Specific rotation measurements were made on a Rudolph research analytical Autopol IV instrument and are quoted in units of 10⁻¹degcm²g⁻¹. Tetrahydrofuran and diethyl ether were distilled over sodium-benzophenone ketyl radical before use. Dichloromethane, toluene and triethylamine were distilled from calcium hydride. Toluene-p-sulfonyl chloride was purified as described by Vogel.²²⁰ All reactions were carried out under a protective nitrogen or argon atmosphere unless otherwise stated.
5.2 De Novo Synthesis of Erythrulose

5.2.1 Acetate Protected Vinyl Halide Route

5.2.1.1 (Z)-2-Butene-1,4-diacetate:

To a stirred solution of cis-2-butene-1,4-diol (49a) (1.00 mL, 12.20 mmol) in anhydrous THF (20 mL) at 0 °C were added acetic anhydride (2.53 mL, 26.80 mmol), triethylamine (3.73 mL, 26.7 mmol) and dimethylaminopyridine (100 mg, 0.82 mmol) under an argon atmosphere. The reaction was allowed to warm to room temperature and stirred overnight (ca 16 h). The reaction mixture was partitioned between 15% HCl solution (20 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL) and the organic solution was washed with water (2 x 50 mL) and brine (50 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo. The resulting residue was then purified by flash chromatography on silica, eluting with 15% EtOAc in hexane to give 49b (1.95 g, 11.33 mmol, 93% yield) as a pale yellow oil. Rf (30% EtOAc/Hexane) 0.39.

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 3304, 2814, 2499, 1735, 1476, 1397, 1370, 1227, 1155, 1060, 844.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 2.09 (s, 6H, H1'), 4.70 (d, \( J=4.5 \) Hz, 4H, H1), 5.77 (t, \( J=4.5 \) Hz, 2H, H2).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 20.4 (C1'), 59.5 (C1), 127.5 (C2), 170.3 (q, C2').
5.2.1.2 2,3-syn-Dibromobutane-1,4-diol:

To a stirred solution of cis-2-butene-1,4-diol (49a) (8.00 mL, 97.40 mmol) in CH₂Cl₂ (100 mL) was added drop-wise a solution of liquid bromine (5.20 mL, 101.50 mmol) in CH₂Cl₂ (100 mL) at 0 °C. The reaction system was allowed to warm to room temperature and left stirring overnight. Excess bromine was removed by adding 10% sodium thiosulfate solution until the brown bromine colour disappeared. The reaction system was stirred for a further 30 minutes and then the aqueous layer was extracted with EtOAc (2 x 50 mL). The CH₂Cl₂ layer was concentrated at reduced pressure and the resulting residue was dissolved in EtOAc (200 mL), which was subsequently washed with water (2 x 50 mL) and brine (100 mL). The combined organic layers were dried over magnesium sulfate and concentrated in vacuo. The crude product was purified by recrystallisation from CH₂Cl₂ and EtOAc to yield compound 75 (19.90 g, 80.27 mmol, 82% yield) as a white solid. M.p. 88-91 °C lit. 21 89.5-90.5 °C. Rₚ (100% EtOAc) 0.74.

IR ʋₘₐₓ (cm⁻¹): 3220, 2944, 2867, 1449, 1224, 1079, 1027, 1006, 940.

¹H NMR (CDCl₃, 400 MHz): δ (ppm) 4.49 (m, 1H, H2), 4.14 (m, 1H, H1), 3.95 (m, 1H, H1).

¹³C NMR (CDCl₃, 100 MHz): δ (ppm) 63.55 (C2), 55.15 (C1).

HRMS: (m/z-ES) calcd. for C₄H₇⁷⁹Br₂O₂ (M-H)⁺ 244.8813, found 244.8820.
5.2.1.3 2,3-syn-Dibromobutane-1,4-diacetate:

To a stirred solution of 75 (2.02 g, 8.15 mmol) in anhydrous THF (40 mL) at 0 °C were added acetyl chloride (1.40 mL, 19.6 mmol), triethylamine (2.72 mL, 19.6 mmol) and dimethylaminopyridine (100 mg, 0.8 mmol) under an argon atmosphere. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was partitioned between 15% HCl solution (20 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL) and the organic solution was washed with water (2 x 50 mL) and brine (50 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The resulting residue was then purified by flash chromatography on silica, eluting with 20% EtOAc in hexane to give 76 (2.30 g, 94% yield) as a yellow oil. \( R_f \) (30% EtOAc/Hexane) 0.33.

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 2961, 1740, 1366, 1213, 1031, 836.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 2.13 (s, 6H, H1'), 4.33-4.45 (m, 4H, H1 & H2), 4.52 (dd, \( J=4.5 \), 10.0 Hz, 2H, H1).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 20.3 (C1'), 48.8 (C1), 65.3 (C2), 169.6 (q, C2').

HRMS: \( m/z - ES \) calcd. for C\(_8\)H\(_{13}\)O\(_4\)Na\(^{79}\)Br\(_2\) (M+Na)\(^+\) 352.9000, found 352.9009.
5.2.1.4 (Z)-2-Bromobut-2-ene-1,4-diacetate:

To a stirred solution of compound 76 (1.99 g, 6.00 mmol) in dry DMSO (40 mL) was added potassium carbonate (0.91 g, 6.60 mmol) under an argon atmosphere. The reaction mixture was heated to 45 °C and stirred overnight. The reaction mixture was partitioned between 15% HCl solution (20 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL) and the organic solution was washed with water (2 x 50 mL) and brine (50 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The resulting residue was then purified by flash chromatography on silica, eluting with 30% EtOAc in hexane to give 77 (1.15 g, 4.58 mmol, 76% yield) as a pale yellow oil. $R_f$ (20% EtOAc/Hexane) 0.2.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 2940, 1737, 1379, 1364, 1212, 1025, 967;

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 2.10 (s, 3H, H1'), 2.14 (s, 3H, H4'), 4.74 (d, $J$=6.0 Hz, 2H, H4), 4.76 (s, 2H, H1), 6.25 (t, $J$=6.0 Hz, 1H, H3).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 20.3 (C1' & C4'), 62.8 (C4), 67.4 (C1), 122.8 (q, C2), 126.6 (C3), 169.6 (q, C3'), 170.2 (q, C2').

HRMS: (m/z – EI) calcd. for $C_9H_{12}O_4^{79}$Br (M+H)$^+$ 250.9919, found 250.9913.
5.2.2 Acetate Protected Vinyl Ether Route

5.2.2.1 *syn*-2-Bromo-3-methoxy-butane-1,4-diol:

\[
\begin{array}{c}
\text{Br} \\
\text{O} \\
\text{HO} \\
\text{2} \\
\text{3} \\
\text{5} \\
\text{4} \\
\text{HO} \\
\text{1} \\
\end{array}
\]

To a stirred suspension of *N*-Bromosuccinimide (8.66 g, 48.70 mmol) in methanol (40 mL) was added a solution of *cis*-2-butene-1,4-diol (49a) (4.00 mL, 48.60 mmol) in methanol (20 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The volatiles were removed *in vacuo* and the resulting suspension was placed in a freezer for 1 hour. The solid succinimide was then removed by filtration through a sintered frit affording 83 (8.39 g, 42.15 mmol, 87% yield) as a colourless oil. 

\[ R_f \ (100\% \ \text{EtOAc}) \ 0.2. \]

IR \( \nu_{\max} \ \text{cm}^{-1} \): 3150, 3077, 2955, 2827, 1770, 1688, 1370, 1355, 1292, 1239, 1182, 1055, 1003, 935, 842, 818.

\[ ^1H \text{ NMR (CDCl}_3, 400 \text{ MHz)} \delta \ (ppm) \]

3.54 (s, 3H, H5), 3.59 (dd, \( J=4.5 \), 9.3 Hz, 1H, H3), 3.89 (d, \( J=4.5 \) Hz, 2H, H4), 3.92 (dd, \( J=5.0 \), 12.5 Hz, 1H, H1), 4.00 (dd, \( J=5.0 \), 12.5, 1H, H1) 4.32 (dd, \( J=5.0 \), 9.3 Hz, 1H, H2).

\[ ^{13}C \text{ NMR (CDCl}_3, 100 \text{ MHz)} \delta \ (ppm) \]

54.8 (C2), 58.3 (C5), 60.3 (C4), 62.9 (C1), 81.5 (C3).
5.2.2.2 *syn*-3-Bromo-2-methoxy-butane-1,4-diacetate:

To a stirred solution of 83 (3.25 g, 16.30 mmol) in anhydrous THF (90 mL) were added acetic anhydride (6.20 mL, 65.30 mmol), triethylamine (9.10 mL, 65.30 mmol) and 4'-dimethylaminopyridine (1.00 g, 8.17 mmol) at 0 °C under an argon atmosphere. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was partitioned between 15% HCl solution (100 mL) and EtOAc (100 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL) and the organic solution was washed with water (2 x 50 mL) and brine (50 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The resulting residue was purified by flash chromatography on silica, eluting with 30% EtOAc in hexane to give compound 84 (4.199 g, 91% yield) as a colourless oil. $R_f$ (30% EtOAc/Hexane) 0.28.

IR $\nu_{max}$ (cm$^{-1}$): 2939, 1738, 1445, 1368, 1218, 1033, 897, 837.

$^1$H NMR (CDCl$_3$, 400 MHz)$\delta$ (ppm) 2.12 (s, 3H, H4'), 2.13 (s, 3H, H1'), 3.54 (s, 3H, H5), 3.58-3.64 (m, 1H, H2), 4.23-4.30 (m, 2H, H3 & H4), 4.35 (dd, $J$=6.3, 12.0 Hz, 1H, H4), 4.45 (d, $J$=6.3 Hz, 2H, H1).

$^{13}$C NMR (CDCl$_3$, 100 MHz)$\delta$ (ppm) 20.3 (C4’), 20.4 (C1’), 49.1 (C5), 58.8 (C3), 63.6 (C4), 64.0 (C1), 77.1 (C2), 169.8 (q, C3’), 170.1 (q, C2’).

HRMS: ($m/z$ – ES) calcd. for C$_9$H$_{16}$BrO$_5$ (M+H)$^+$ 283.0181, found 283.0178.
5.2.2.3  (Z)-2-methoxy-but-2-ene-1,4-diacetate:

To a solution of 84 (2.02 g, 7.12 mmol) in anhydrous DMSO (40 mL) was added potassium carbonate (1.97 g, 14.24 mmol) under an argon atmosphere. The reaction was heated to 80 °C and stirred overnight. Water (100 mL) was added and the reaction mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with water (2 x 100 mL) and brine (100 mL) and dried over magnesium sulfate. The volatiles were removed in vacuo, and the resulting oil was purified by flash chromatography on silica, using a gradient eluant of 2 – 10% EtOAc in hexane to give 85 (0.84 g, 4.16 mmol, 58% yield) as a yellow oil. \( R_f \) (30% EtOAc/Hexane) 0.30.

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 3462, 2957, 1733, 1371, 1221, 1043, 842.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 2.07 (s, 3H, H4'), 2.13, (s, 3H, H1'), 3.69 (s, 3H, H5), 4.63 (s, 2H, H1), 4.70 (d, J=7.0 Hz, 2H, H4), 5.04 (t, J=7.0 Hz, 1H, H3).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 20.9 (C1'), 21.0 (C4'), 56.9 (C5), 58.5 (C4), 61.9 (C1), 107.1 (C3), 153.1 (q, C2), 170.3 (q, C3'), 171.0 (q, C2').

HRMS: \( (m/z - \text{ES}) \) calcd. for C\(_9\)H\(_{15}\)O\(_5\) (M+H)\(^+\) 203.0919, found 203.0929.
5.2.3 Silyl Protected Vinyl Halide Route

5.2.3.1 syn-2,3-Dibromo-1,4-bis-(tert-butyl-dimethyl-silanyloxy)-butane:

To a stirred solution of tert-butyldimethylsilyl chloride (5.20 g, 34.5 mmol) and imidazole (4.92 g, 72.29 mmol) in DMF (12 mL) at 0 °C was added compound 75 (4.00 g, 16.14 mmol) under an argon atmosphere. The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was partitioned between water (30 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (2 x 30 mL) and the organic solution was washed with water (2 x 30 mL) and brine (2 x 30 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The resulting oil was purified by flash chromatography on silica, eluting with 5% EtOAc in hexane to give 90 (7.51 g, 15.75 mmol, 98% yield) as a colourless oil. \( R_f \) (5% EtOAc/Hexane): 0.77.

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 2955, 2930, 2858, 1472, 1276, 1260, 1091, 837, 765, 750.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 0.11 & 0.12 (\( \{s, \text{6H}\} & \{s, \text{6H}\}, \text{H3'} & \text{H4'} \)), 0.92 (s, 18H, H1’), 3.86-3.98 (m, 4H, H1), 4.39-4.47 (m, 2H, H2).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) -5.81 (C3’), -5.75 (C4’), 17.8 (q, C2’), 25.3 (C1’), 53.1 (C1), 64.8 (C2).

HRMS: \( m/z \) – ES calcd. for \( C_{16}H_{36}O_2NaSi_2^{79}\)Br\(_2\) (M+Na\(^+\)) 497.0518, found 497.0538.
5.2.3.2 (Z)-2-Bromo-1,4-bis-(tert-butyl-dimethyl-silanyloxy)-but-2-ene:

To a solution of 90 (2.0 g, 4.20 mmol) in anhydrous DMSO (30 mL), was added DBU (0.67 mL, 4.40 mmol) under an argon atmosphere. The reaction was heated to 45 °C and stirred overnight. The reaction mixture was partitioned between water (50 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL) and the organic solution was washed with water (2 x 50 mL) and brine (2 x 50 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The resulting oil was then purified by flash chromatography on silica, using a gradient eluant of 2–5% EtOAc in hexane to give 88 (1.52 g, 3.84 mmol, 92% yield) as a yellow oil. \( R_f \) (2% EtOAc/Hexane): 0.66.

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 3006, 2990, 1463, 1276, 1260, 837, 764, 750.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 0.107 (s, 6H, H3' or H4'), 0.119 (s, 6H, H3' or H4'), 0.93 (s, 9H, H6'), 0.94 (s, 9H, H6'), 4.246-4.27 (m, 2H, H1), 4.35-4.39 (m, 2H, H4), 6.204-6.27 (m, 1H, H3).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) -5.78 & -5.61 (C3' & C4'), 17.83 & 17.89 (q, C2' & C5'), 25.36 & 25.45 (C1' & C6'), 62.42 (C4), 67.2 (C1), 124.2 (q, C2), 127.57 (C3).

HRMS: \( m/z - \text{ES} \) calcd. for C\(_{16}\)H\(_{35}\)O\(_2\)NaSi\(_2\)\(^{79}\)Br (M+Na)\(^+\) 417.1257, found 417.1275.
5.2.3.3 (S)-1,4-Bis-(tert-butyl-dimethyl-silanyloxy)-3-hydroxy-butan-2-one:

To a stirred solution of AD-mix α (0.67 g), methane sulfonamide (100 mg, 0.51 mmol) and potassium osmate dihydrate (4 mg, 9.6 x 10⁻³ mmol) in tert-butanol (1.4 mL) and water (2.4 mL) was added a solution of 88 (0.20 g, 0.51 mmol) in tert-butanol (1.0 mL). The reaction was stirred at room temperature for ca. 72 hours. The reaction was quenched by adding a solution of sodium sulfite (0.72 g, 5.71 mmol) in water (10 mL) and stirring for a further hour. The aqueous layers were extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with water (20 mL) and brine (20 mL), and then dried over magnesium sulfate. The volatiles were removed in vacuo, and the crude reaction mixture purified by flash chromatography on silica, using a gradient eluant of 5-7.5% EtOAc in hexane to give 94 (15 mg, 0.04 mmol, 8% yield) as a colourless oil. \( R_f \) (5% EtOAc/Hexane): 0.77.

IR \( \nu_{\text{max}} \) (cm⁻¹): 3493, 2954, 2929, 2857, 2383, 2358, 2300, 1728, 1472, 1463, 1362, 1255, 1110, 834, 776.

\(^1\)H NMR (CDCl₃, 400 MHz) \( \delta \) (ppm) 0.05-0.13 (m, 12H, H3', H4', H5' & H6'), 0.88 (s, 9H, H1'), 0.94 (s, 9H, H8'), 3.48 (d, \( J=6.5 \) Hz, 1H, -OH), 3.85 (dd, \( J=3.8, 10.5 \) Hz, 1H, H1), 4.12 (dd, \( J=3.8, 10.5 \) Hz, 1H, H1), 4.36-4.53 (m, 3H, H3 & H4).

\(^13\)C NMR (CDCl₃, 100 MHz) \( \delta \) (ppm) -6.06, -6.04, -5.98 & -5.89 (C3', C4', C5' & C6'), 17.8 & 17.9 (q, C2' & C7'), 25.3 (C1' & C8'), 64.0 (C1), 67.4 (C4), 75.3 (C3), 209.2 (q, C2).
5.2.3.4 (S)-1,3,4-Tris-(tert-butyl-dimethyl-silyloxy)-butan-2-one:

To a stirred solution of crude 94 (1.10 g)* in anhydrous DMF (5 mL) at 0 °C were added tert-butyldimethylsilyl chloride (0.52 g, 3.46 mmol) and imidazole (0.24 g, 3.46 mmol) under an argon atmosphere. The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was partitioned between water (10 mL) and EtOAc (10 mL). The aqueous layer was extracted with EtOAc (2 x 10 mL) and the organic solution was washed with water (2 x 10 mL) and brine (2 x 10 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The resulting oil was then purified by flash chromatography on alumina eluting with 1% EtOAc in hexane affording 95 (0.21 g, 0.45 mmol, 14% yield) as a colourless oil. Rf (2% EtOAc/Hexane): 0.34. [α]_D^{20} = 12.4 (CHCl3, c 1.0).

IR ν_max (cm⁻¹): 2953, 2929, 2857, 1736, 1472, 1463, 1253, 1096, 830, 774.

^1H NMR (CDCl₃, 600 MHz) δ (ppm): 0.05, 0.06, 0.09, 0.09 & 0.10 (s, 18H, H3′, H4′, H5′, H6′, H9′ & H10′), 0.89 & 0.93-0.94 (s, 9H) & {m, 18H}, H1′, H8′ & H12′), 3.75 (dd, J=3.8, 10.4 Hz, 1H, H4), 3.84 (dd, J=4.5, 10.4 Hz, 1H, H4), 4.25 (app t, J=3.8 Hz, 1H, H3), 4.56 (d, J=18.0 Hz, 1H, H1).
$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) -5.52, -5.45, -5.40, -5.34, -5.19, -4.65 (C3', C4', C5', C6', C9' & C10'), 18.11, 18.34, 18.52 (q, C2', C7' & C11'), 25.73, 25.86, 25.87 (C1', C8' & C12'), 66.2 (C4), 68.6 (C1), 78.9 (C3), 209.5 (q, C2).

HRMS: (m/z – ES) calcd. for C$_{22}$H$_{50}$NaO$_4$Si$_3$ (M+Na)$^+$ 485.2915, found 485.2915.

*Crude 94 was prepared from 88 (1.68 g, 4.25 mmol) as previously described.

5.2.4 Silyl Protected Vinyl Ether Route

5.2.4.1 syn-2-Bromo-1,4-bis-(tert-butyl-dimethyl-silanyloxy)-3-methoxy-butane:

To a solution of 83 (2.80 g, 14.07 mmol) in anhydrous DMF (11 mL) at 0 °C were added tert-butyldimethylsilyl chloride (4.56 g, 30.25 mmol) and imidazole (3.35 g, 49.25 mmol). The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was partitioned between water (30 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (2 x 30 mL) and the organic solution was washed with water (2 x 30 mL) and brine (2 x 30 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo. The resulting oil was then purified by flash chromatography on silica, using a gradient eluant of 2–5% EtOAc in hexane to give 91 (4.82 g, 11.27 mmol, 80% yield) as a colourless liquid. $R_f$ (5% EtOAc/Hexane): 0.78.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 2955, 2930, 2858, 1472, 1276, 1260, 1092, 837, 765, 750.
\(^1\)H NMR (CDCl\(_3\), 400 MHz)\(\delta\) (ppm): 0.10 (s, 12H, H3', H4', H5' & H6'), 0.92 & 0.93
({s, 9H} & {s, 9H}, H1' & H8'), 3.53 (s, 3H, H5),
3.55-3.60 (m, 1H, H3), 3.70 (dd, \(J=7.0\), 10.0 Hz,
1H, H1), 3.81-3.90 (m, 2H, H1 & H4), 3.97 (t, \(J=9.2\) Hz, 1H, H4), 4.21 (ddd, \(J=2.0\), 5.0, 9.2 Hz,
1H, H2).

\(^1\)C NMR (CDCl\(_3\), 100 MHz)\(\delta\) (ppm): -5.87, -5.82 & -5.76 (C3', C4', C5' & C6'), 17.7
(q, C2' & C7'), 25.3 & 25.4 (C1' & C8'), 54.4
(C2), 58.9 (C5), 62.4 (C1), 63.1 (C4), 78.1 (C3).

HRMS: \((m/z - ES)\) calcd. for C\(_{17}\)H\(_{40}\)\(^{79}\)BrO\(_3\)Si\(_2\) (M+H)\(^+\) 427.1699, found 427.1711.

5.2.4.2 (Z)-1,4-Bis-(tert-butyldimethylsilyloxy)-2-methoxy-but-2-ene:

\(\text{89}\)

To a stirred solution of 91 (4.50 g, 10.52 mmol) in anhydrous DMSO (22.5 mL) was added
DBU (3.15 mL, 21.04 mmol) under an argon atmosphere. The reaction was heated to 75
°C and stirred overnight. The reaction mixture was partitioned between water (150 mL)
and EtOAc (100 mL). The aqueous layer was extracted with EtOAc (3 x 100 mL) and the
organic solution was washed with water (2 x 100 mL) and brine (2 x 100 mL). The organic
layer was then dried over magnesium sulfate and concentrated in vacuo. The yield of
desired compound 89 (1.36 g, 3.92 mmol, 37% yield) was determined by proton NMR of
the crude reaction mixture using a known quantity of diphenyl furan as an internal standard.
The product decomposed on both silica and alumina so purification by column
chromatography was not possible.
IR $\nu_{\text{max}}$ (cm$^{-1}$): 2954, 2929, 2857, 1733, 1472, 1463, 1253, 1093, 832, 774.

$^1$H NMR (CDCl$_3$, 600 MHz) $\delta$ (ppm) 0.06-0.14 (m, 12H, H3' & H4'), 0.88-0.97 (m, 18H, H1' & H6'), 3.70 (s, 3H, H5), 4.16 (s, 2H, H1), 4.30 (d, $J$=6.6 Hz, 2H, H4), 4.89 (t, $J$=6.6 Hz, 1H, H3).

$^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ (ppm) -5.4 (C4'), -5.3 (C3'), 18.1 & 18.2 (q, C2'& C5'), 25.7 & 25.9 (C1' & C6'), 56.7 (C5), 57.4 (C4), 61.9 (C1), 109.3 (C3), 153.9 (q, C2).

5.2.4.3 (S)-1,4-Bis-(tert-butyl-dimethyl-silyloxy)-3-hydroxy-butan-2-one:

To a stirred solution of AD-mix $\alpha$ (5.33 g), methane sulfonamide (0.24 g, 2.54 mmol) and potassium osmate dihydrate (9 mg, 2.5 x 10$^{-4}$ mmol) in tert-butanol (6 mL) and water (6 mL) at 0 °C was added a solution of 89 (0.37 g, 1.07 mmol) in tert-butanol (2 mL). The reaction was stirred at 0 °C for 1 hour and then allowed to warm to room temperature and stirred for a further 8 hours. The reaction was quenched by adding a solution of sodium sulfate (4.7 g, 37.45 mmol) in water (30 mL) and stirring for a further hour. The aqueous layers were extracted with EtOAc (2 x 100 mL) and the combined organic layers were then washed with water (2 x 100 mL) and brine (2 x 100 mL), and then dried over magnesium sulfate. The volatiles were removed in vacuo, and the yield of 94 (0.11 g, 0.33 mmol, 30% yield) was determined by proton NMR of the crude reaction mixture using a known quantity of diphenyl furan as an internal standard.
To a stirred suspension of N-iodosuccinimide (4.106 g, 18.25 mmol) in methanol (25 mL) was added *cis*-2-butene-1,4-diol (49a) (1.5 mL, 18.25 mmol) at 0 °C. The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was partitioned between saturated thiosulfate solution (50 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (3 x 50 mL) and the organic solution was washed with water (50 mL) saturated bicarbonate solution (2 x 40 mL) and brine (2 x 50 mL). The organic layer was then dried over magnesium sulfate and concentrated *in vacuo*. The resulting oil was then purified by flash chromatography on silica, using a gradient eluant of 40–60% EtOAc in hexane affording 96 (2.356 g, 9.58 mmol, 52% yield) as a yellowish oil. *R*<sub>f</sub> (50% EtOAc/Hexane): 0.21.

IR *ν*<sub>max</sub> (cm<sup>-1</sup>): 3351, 2934, 2830, 1703, 1351, 1184, 1049, 1025, 816.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz): δ (ppm) 3.41 (dd, *J*=4.6, 11.5 Hz, 1H, H2), 3.54 (s, 3H, H5), 3.84-3.88 (m, 2H, H1 & H4), 3.91-3.96 (m, 2H, H1 & H4), 4.45 (dd, *J*=4.6, 10.2 Hz, 1H, H3).

<sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ (ppm) 36.6 (C3), 58.3 (C5), 61.9 (C1), 64.7 (C4), 82.5 (C2).
5.2.4.5 syn-1,4-bis-tert-butyl-diphenyl-silyloxy-2-iodo-3-methoxy-butane:

To a stirred solution of 96 (0.33 g, 1.32 mmol) in anhydrous DMF (6 mL) at 0 °C were added tert-butyl diphenyl silyl chloride (0.91 mL, 3.50 mmol), imidazole (0.34 g, 4.95 mmol) and dimethylaminopyridine (16 mg, 0.13 mmol) under an argon atmosphere. The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was partitioned between saturated ammonium chloride solution (30 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL) and the organic solution was washed with water (2 x 30 mL) and brine (50 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The resulting residue was then purified by flash chromatography on silica, eluting with 5% EtOAc in hexane affording compound 97 (0.82 g, 1.14 mmol, 86% yield) as a white solid. $R_f$ (5% EtOAc/Hexane): 0.59. M.p. 89–91 °C.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3071, 2952, 2926, 2854, 2826, 1470, 1426, 1104, 1070, 1005, 823, 786, 699;

$^1$H NMR (CDCl$_3$, 600 MHz) $\delta$ (ppm) 1.10 (s, 9H, H1'), 1.12 (s, 9H, H12'), 3.21-3.25 (m, 1H, H3), 3.41 (s, 3H, H5), 3.67 (dd, $J=7.3$, 10.3 Hz, 1H, H4), 3.86 (dd, $J=5.1$, 10.3, 1H, H4),
3.98 (dd, J = 5.1, 10.3 Hz, 1H, H1), 4.08 (app t, J = 10.3 Hz, 1H, H1), 4.52 (ddd, J = 2.2, 5.1, 10.3 Hz, 1H, H2), 7.38-7.41 (m, 8H, H4', H4'', H8' & H8''), 7.44-7.48 (m, 4H, H6', H6'', H10' & H10''), 7.67-7.74 (m, 8H, H5', H5'', H9' & H9'').

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 19.2 & 19.3 (q, C2', & C11'), 26.8 (C1', & C12'), 37.2 (C2), 58.8 (C5), 65.5 (C4), 65.9 (C1), 78.3 (C3), 127.78 & 127.83 (C4', C4'', C8' & C8''), 129.79, 129.85 & 129.89 (C6', C6'', C10' & C10''), 133.1, 133.2 & 133.3 (q, C3', C3'', C7' & C7''), 135.51, 135.54 & 135.65 (C5', C5'', C9' & C9'').

HRMS: ($m/z$ – ES) calcd. for C$_{37}$H$_{48}$IO$_3$Si$_2$ (M+H)$^+$ 723.2187, found 723.2215.

5.2.5 Benzoyl Protected Vinyl Ether Route

5.2.5.1 syn-2-Bromo-3-methoxy-1,4-benzoyloxy-bntane:

To a stirred solution of 83 (3.038 g, 15.26 mmol) in CH$_2$Cl$_2$ (60 mL) at 0 °C were added benzoyl chloride (6.15 mL, 52.8 mmol) and triethylamine (7.35 mL, 52.8 mmol) under an argon atmosphere. The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was partitioned between water (50 mL) and CH$_2$Cl$_2$ (50 mL). The aqueous layer was extracted with CH$_2$Cl$_2$ (2 x 50 mL) and the organic solution
was washed with water (2 x 50 mL) and brine (2 x 50 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The resulting residue was then purified by flash chromatography on silica, eluting with 10% EtOAc in hexane affording compound 100 (5.453 g, 13.39 mmol, 88% yield) as a white solid. 

Rf (10% EtOAc/Hexane): 0.38. M.p. 69–71 °C.

IR ν<sub>max</sub> (cm<sup>-1</sup>): 3062, 2985, 2932, 2887, 2834, 1713, 1600, 1449, 1262, 1095, 1058, 1026, 706.

1H NMR (CDCl<sub>3</sub>, 600 MHz): δ (ppm) 3.61 (s, 3H, H5), 3.86-3.89 (m, 1H, H3), 4.50-4.53 (m, 1H, H2), 4.59 (dd, J=5.9, 11.7 Hz, 1H, H4), 4.66 (dd, J=5.9, 11.7 Hz, 1H, H4), 4.75 (d, J=2.2 Hz, 1H, H1), 7.45-7.51 (m, 4H, H4' & H9'), 7.58-7.63 (m, 2H, H5' & H10').

13C NMR (CDCl<sub>3</sub>, 100 MHz): δ (ppm) 49.8 (C2), 59.5 (C5), 64.8 (C4), 64.9 (C1), 77.9 (C3), 128.49 & 128.52 (C4' & C9'), 129.5 & 129.6 (q, C2' & C7'), 129.66 & 129.71 (C3' & C8'), 133.29 & 133.36 (C5' & C10'), 165.8 & 166.1 (q, C1' & C6').

5.2.5.2 (Z)-1,4-Bisbenzoyloxy-2-methoxy-but-2-ene:

To a stirred solution of 100 (1.20 g, 2.95 mmol) in anhydrous DMSO (7.40 mL) was added DBU (0.88 mL, 5.90 mmol) under an argon atmosphere. The reaction was heated to 60 °C.
and stirred overnight. The reaction mixture was partitioned between water (50 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL) and the organic solution was washed with water (2 x 30 mL) and brine (2 x 30 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The resulting oil was purified by flash chromatography on silica, eluting with 5% EtOAc in hexane affording compound 101 (0.48 g, 1.46 mmol, 49% yield) as a yellow oil. 

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3063, 3033, 2945, 2852, 1713, 1678, 1450, 1259, 1174, 1094, 1067, 1024, 937, 705;

$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 3.81 (s, 3H, H5), 4.93 (s, 2H, H1), 5.01 (d, $J=7.0$ Hz, 2H, H4), 5.28 (t, $J=7.0$ Hz, 1H, H3), 7.46 (t, $J=7.7$ Hz, 2H, H9'), 7.49 (t, $J=7.7$ Hz, 2H, H4'), 7.58 (t, $J=7.3$ Hz, 1H, H10'), 7.61 (t, $J=7.3$ Hz, 1H, H5'), 8.07-8.12 (m, 4H, H8' & H3').

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 57.2 (C5), 59.1 (C4), 62.5 (C1), 107.5 (C3), 128.3 (C9'), 128.5 (C4'), 129.6 (C8'), 129.68 (q, C2'), 129.74 (C3'), 130.4 (q, C7'), 132.9 (C10'), 133.3 (C5'), 153.3 (q, C2), 165.9 (q, C1'), 166.6 (q, C6').

HRMS: (m/z – ES) calcd. for C$_{19}$H$_{18}$O$_3$Na (M+Na)$^+$ 349.1052, found 349.1050.
A stirred solution of dimethyl L-tartrate (111) (35.00 g, 0.20 mol), 2, 2′-dimethoxypropane (60 mL, 0.48 mol) and catalytic p-toluene sulfonic acid (0.373 g, 1.96 mmol) in cyclohexane (300 mL) was heated at reflux using a Dean-Stark apparatus and the cyclohexane-MeOH azeotrope (20 mL) was removed from the reaction mixture, repeatedly. After 8 hours, volatiles were removed at reduced pressure and the residue was dissolved in EtOAc (100 mL) and then washed with saturated bicarbonate solution (2 x 50 mL). The organic layer was then dried over magnesium sulfate and volatiles were removed in vacuo. The crude product was passed through a short-pad column (silica gel, 200 mL, 10:1 hexane/EtOAc), followed by Kugelrohr distillation (B.p. 122–128 °C, 3.0 mm Hg) to give compound 110 (39.87 g, 0.18 mol, 93% yield) as a pale yellow oil. \([\alpha]_D^{20} = -44.4 \text{ (CHCl}_3, c 10.0), \text{lit.}^{160} [\alpha]_D^{24} = -42.6 \text{ (CHCl}_3, c 5.1), \text{lit.}^{222} [\alpha]_D^{20} = -49.4 \text{ (neat).}

IR \(\nu_{\text{max}}\) (cm\(^{-1}\)):

- 2993, 2957, 1739, 1438, 1384, 1374, 1205, 1105, 1011, 855.

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) (ppm):

- 1.52 (s, 6H, H1'), 3.85 (s, 6H, H3'), 4.83 (s, 2H, H2).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) (ppm):

- 28.8 (C1'), 52.4 (C3'), 76.5 (C2), 113.4 (q, C2'), 169.6 (C1).

HRMS: \(m/z - ES\) calcd. for C\(_9\)H\(_{15}\)O\(_6\) (M+H)\(^+\) 219.0689, found 219.0878.
A stirred suspension of lithium aluminium hydride (13.66 g, 0.36 mol) in anhydrous diethyl ether (200 mL) was heated at reflux for 1 hour under an argon atmosphere. The suspension was cooled to 0 °C and a solution of compound III (39.31 g, 0.18 mol) in diethyl ether (100 mL) was added over a 2 hour period. The ice bath was then removed and the reaction was heated at reflux for 3 hours. The heat was then removed and the reaction was stirred at room temperature overnight. The reaction was cooled to 0 °C and after successive cautious additions of water (12 mL), sodium hydroxide solution (4N, 12 mL) and more water (30 mL) were made, the inorganic precipitate which had formed was removed by filtration and extracted thoroughly with THF (Soxhlet). The extracts were combined with the ethereal filtrate, dried over magnesium sulfate and concentrated in vacuo. The resulting residue was taken up in a small quantity of EtOAc and then passed through a plug of silica eluting with EtOAc. Volatiles were removed at reduced pressure and the crude product was further purified by Kugelrohr distillation (B.p. 128-135 °C, 3.0 mm Hg), affording compound 109 (13.69 g, 0.08 mol, 47% yield) as a colourless oil. \( [\alpha]_D^{20} + 3.9 \) (CHCl₃, c 5.0), lit.* \( [\alpha]_D^{24} +2.78 \) (CHCl₃, c 4.67); lit.** \( [\alpha]_D^{20} +4.1 \) (CHCl₃, c 5).

IR \( \nu_{\text{max}} \) (cm⁻¹): 3376, 2987, 2935, 2880, 1456, 1372, 1214, 1164, 1046, 842.

\(^1\)H NMR (\( \delta^\text{DMSO}, 600 \text{MHz} \)) \( \delta \) (ppm) 1.31 (s, 6H, H1'), 3.46-3.56 (m, 4H, H1), 3.74-3.78 (m, 2H, H2), 4.79 (s, broad, 2H, -OH).

\(^{13}\)C NMR (\( \delta^\text{DMSO}, 150 \text{MHz} \)) \( \delta \) (ppm) 27.4 (C1'), 62.3 (C1), 79.1 (C2), 108.3 (q, C2').
5.3.1.3 1,4-Ditosyl-2,3-O-isopropylidene-L-threitol

To a stirred solution of compound 109 (0.50 g, 3.08 mmol) in anhydrous pyridine (4.50 mL) at -10 °C was added purified tosyl chloride (1.81 g, 9.50 mmol) under an argon atmosphere. The reaction was allowed to warm to room temperature and stirred overnight. Water (20 mL) was introduced and the reaction was refrigerated for 1.5 hours. The white precipitate which formed was removed by filtration and washed with water (2 x 20 mL), petrol ether (20 mL) and cold EtOH (20 mL). The product was recrystallised from hot EtOH and compound 108 (1.43 g, 3.03 mmol, 98% yield) was isolated as a white solid. \( R_f \) (30% EtOAc/Hexane) 0.52. M.p. 91-93 °C; lit.\(^{224} \) 92 °C. \([\alpha]_D^{20} = 11.2\) (CHCl\(_3\), c 1.05), lit.\(^{161} \) [\(\alpha\)]\(_D\) -12.75 (CHCl\(_3\), c 1.09).

IR \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3037, 2984, 2934, 1596, 1355, 1171, 1096, 969, 810, 784, 662.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) (ppm) 1.32 (s, 6H, H1'), 2.49 (s, 6H, H7'), 4.00-4.07 (m, 2H, H2), 4.09-4.14 (m, 4H, H1), 7.39 (d, \(J=8.5\) Hz, 4H, H5'), 7.81 (d, \(J=8.5\) Hz, 4H, H4').

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) (ppm) 21.3 (C7'), 26.3 (C1'), 67.9 (C2), 74.6 (C1), 110.4 (q, C2'), 127.6 (C4'), 129.6 (C5'), 132.0 (q, C3'), 144.8 (q, C6').

HRMS: \(m/z - ES\) calcd. for C\(_{21}\)H\(_{25}\)O\(_8\)S\(_2\) (M+H)\(^+\) 471.1147, found 471.1146.
5.3.1.4 1,4-Ditosyl-L-threitol:

To a stirred solution of compound 108 (14.0 g, 29.75 mmol) in MeOH (230 mL) was added HCl solution (1.0 M, 23 mL). The reaction was heated at reflux overnight. Volatiles were removed at reduced pressure and the resulting residue was dissolved in CHCl₃, dried over magnesium sulfate and concentrated in vacuo. This afforded an off-white solid which was purified by recrystallisation from hot EtOH and CHCl₃. The desired compound 107 (12.512 g, 29.1 mmol, 98% yield) was recovered as a white solid. Rf (10% MeOH/CHCl₃) 0.71. M.p. 75-77 °C; lit. 224 76-77 °C. [α]¹⁰ +1.1 (CHCl₃, c 5.0), lit.¹⁰¹ [α]¹⁰ +1.05 (CHCl₃, c 1.33).

IR νmax (cm⁻¹): 3503, 3416, 3023, 2994, 2958, 1348, 1171, 1095, 1067, 959, 810, 750, 661.

¹H NMR (d⁶ DMSO, 600 MHz): δ (ppm) 2.44 (s, 6H, H5'), 3.63-3.67 (m, 2H, H2), 3.84 (dd, J=7.2, 9.8 Hz, 2H, H1), 3.99 (dd, J=3.8, 9.8 Hz, 2H, H1), 7.49 (d, J=8.3 Hz, 4H, H3'), 7.77 (d, J=8.3 Hz, 4H, H2').

¹³C NMR (d⁶ DMSO, 150 MHz): δ (ppm) 21.4 (C5'), 68.5 (C1), 71.4 (C2), 127.9 (C3'), 130.5 (C2'), 132.6 (q, C4'), 145.2 (q, C1').

HRMS: (m/z - ES) calcd. for C₁₈H₂₃O₈S₂ (M+H)^+ 431.0834, found 431.0848.
A mixture of compound 107 (12.00 g, 27.88 mmol) and sodium sulfide nonohydrate (16.74 g, 69.69 mmol) in EtOH (230 mL) was heated at reflux overnight. After filtration through Celite and evaporation the residue was purified by flash chromatography on silica using a gradient eluant of 10% hexane in CHCl₃ increasing the polarity gradually to 10% MeOH in CHCl₃, to give compound 106 (1.93 g, 16.10 mmol, 57% yield) as a white solid. Rf (10% MeOH/CHCl₃) 0.33. M.p. 56-58 °C; lit.²²⁵ 57-60 °C. [α]D²⁰ + 79.3 (MeOH, c 1.3), lit.¹⁶¹ [α]D +76.9 (MeOH, c 1.22).

IR νₘₐₓ (cm⁻¹): 3357, 2922, 2852, 1645, 1426, 1146, 1020.

¹H NMR (CDCl₃, 400 MHz): δ (ppm) 1.99 (d, J=5.5 Hz, 2H, -OH), 2.81 (dd, J=3.3, 11.8 Hz, 2H, H2), 3.19 (dd, J=4.3, 11.8 Hz, 2H, H2), 4.31-4.36 (m, 2H, H3).

¹³C NMR (CDCl₃, 100 MHz): δ (ppm) 35.5 (C2), 78.1 (C3).
5.3.2 C2-Desymmetrisation of 3,4-dihydroxy thiolane and manipulation of derivatives

5.3.2.1 (R,R)-4-(tert-Butyl-dimethyl-silanyloxy)-3-hydroxythiolane

To a stirred solution of compound 106 (1.33 g, 11.07 mmol) in anhydrous DMF (65 mL) were added tert-butyldimethylsilyl chloride (1.93 g, 12.78 mmol) and imidazole (1.58 g, 23.26 mmol) under an argon atmosphere. The reaction was heated to 75 °C and stirred overnight. The reaction mixture was partitioned between water (100 mL) and EtOAc (100 mL). The aqueous layer was extracted with EtOAc (3 x 50 mL) and the organic solution was washed with water (50 mL) and brine (2 x 50 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The resulting residue was then purified by flash chromatography on silica, eluting in gradient with hexane to 20% EtOAc in hexane to give compound 114 (1.36 g, 5.80 mmol, 52% yield) as a white solid. Rf (20% EtOAc/hexane) 0.5. M.p. 56-58 °C. [α]D° + 71.8 (CHCl3, c 1.3).

IR νmax (cm⁻¹): 3319, 2928, 2894, 2855, 1470, 1462, 1248, 1143, 1052, 1016, 826, 775, 701.

1H NMR (CDCl3, 400 MHz) δ (ppm) 0.12 (s, 6H, H1' & H2'), 0.91 (s, 9H, H4'), 1.95 (s, broad, 1H, -OH), 2.67-2.83 (m, 2H, H2 & H5), 3.11-3.29 (m, 2H, H2 & H5), 4.14-4.22 (m, 1H, H3), 4.25-4.31 (m, 1H, H4).

13C NMR (CDCl3, 100 MHz) δ (ppm) -4.74 & -4.70 (C1' & C2'), 18.0 (q, C3'), 25.7 (C4'), 35.5 (C2 & C5), 78.47 (C3), 78.50 (C4).
Elemental Analysis: Anal. calcd. for C_{16}H_{22}O_{2}SSi: C, 51.23; H, 9.46; S, 13.68. Found: C, 51.34; H, 9.54; S, 13.64.

The bis-silylated compound 115 (0.65 g, 1.86 mmol, 17% yield) was also recovered.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 0.01 (s, 6H, H1'), 0.02 (s, 6H, H2'), 0.816 (s, 18H, H4'), 2.54-2.62 (m, 2H, H2), 2.94-3.02 (m, 2H, H2), 4.05-4.10 (m, 2H, H3).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) -5.18 & -5.10 (C1' & C2'), 17.5 (q, C3'), 25.3 (C4'), 35.7 (C2), 78.5 (C3).

5.3.2.2 4-(S)-(tert-Butyl-dimethyl-silanyloxy)-3-(R)-hydroxy-1-oxo-thiolane

To a stirred solution of oxalyl chloride (180 µL, 2.04 mmol) in CH$_2$Cl$_2$ (8 mL) at -80 °C was added dropwise anhydrous DMSO (290 µL, 4.08 mmol) over ca. 5 min under an argon atmosphere. Stirring was continued for 10 min followed by addition of a solution of compound 106 (0.40 g, 1.70 mmol) in CH$_2$Cl$_2$ (6 mL) over ca. 5 min. The reaction was stirred for a further 15 minutes whereupon triethylamine (120 µL, 8.50 mmol) was added over ca. 5 min with stirring at -80 °C. The cooling bath was then removed and the reaction was allowed to warm to room temperature. The cooling bath was then removed and the reaction was allowed to warm to room temperature. The reaction was diluted with CH$_2$Cl$_2$ (30 mL) and washed with water (30 mL). The aqueous phase was extracted with CH$_2$Cl$_2$ (2 x 30 mL) and the combined organic layers were washed with water (30 mL) and brine (2 x 30 mL) and dried over magnesium sulfate. Volatiles were removed at reduced pressure. The
resulting residue was dissolved in anhydrous CH$_2$Cl$_2$ (28 mL) and cooled to -80 °C. Purified *meta*-chloroperoxy benzoic acid (0.31 mg, 1.79 mmol) and potassium carbonate (0.28 mg, 2.04 mmol) were added and the reaction was stirred for a further 15 min whereupon the cooling bath was removed and the reaction was allowed to warm to room temperature over *ca.* 1.5 h. The solid precipitate which formed was removed by filtration and volatiles were removed *in vacuo*. The resulting residue was dissolved in MeOH (11 mL) and cooled to -40 °C. Sodium borohydride (0.32 g, 8.50 mmol) was introduced in small portions over *ca.* 15 min. The reaction was allowed to warm to 0 °C and stirred for 1 h. The reaction was diluted with EtOAc (40 mL) and washed with water (30 mL). The aqueous layer was extracted with EtOAc (2 x 40 mL). The combined organic layers were then washed with water (30 mL) and brine (2 x 30 mL) and dried over magnesium sulfate. Volatiles were removed *in vacuo* and the resulting residue was purified by flash chromatography on silica eluting with 2% MeOH in EtOAc to give compound 48 (0.06 g, 0.24 mmol, 14% yield) as approximately a 1:3.6 mixture of diastereomers: $R_f$ (2% MeOH/EtOAc) 0.58.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3293, 2952, 2929, 2856, 1471, 1462, 1391, 1253, 1065, 1004, 837, 778.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 0.12 (s, 6H, H1' maj & H2' maj), 0.18 (s, 6H, H1' min & H2' min), 0.88 (s, 9H, H4' maj), 0.95 (s, 9H, H4' min), 2.57 (s, broad, 1H, -OH min), 2.90-2.96 (m, 1H, H2 min), 2.97-3.03 (m, 1H, H2 maj), 3.03-3.08 (m, 2H, H5 min), 3.25 (d, $J=14.3$ Hz, 1H, H2 maj), 3.33-3.43 (m, 2H, H5 maj), 3.48-3.54 (m, 1H, H2 min), 4.15 (s, broad, 1H, -OH maj), 4.55-4.60 (m, 1H, H3 maj), 4.60-4.64 (m, 1H, H3 min), 4.92-5.00 (m, 2H, H4 both isomers).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) -4.99 (C1' maj), -4.97 (C1' min), -4.9 (C2' maj), -4.7 (C2' min), 17.8 (q, C3' maj), 18.0 (q, C3' min), 25.6 (C4' maj), 25.7 (C4' min), 55.4 (C2 maj), 55.8 (C5 min), 58.9 (C2 min), 63.9 (C5 min).
5.3.2.3 *1,4-Ditosyl-2,3-O-carbonate-L-threitol*

To a solution of compound 107 (1.00 g, 2.32 mmol) in THF (50 mL) at 0 °C was added triphosgene (0.30 g, 1.00 mmol) under an argon atmosphere. The reaction was stirred for ca. 5 min whereupon freshly distilled triethylamine (0.97 mL, 6.96 mmol) was added. The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was partitioned between water (100 mL) and EtOAc (100 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL) and the organic solution was washed with water (2 x 50 mL) and brine (2 x 50 mL). The organic layer was then dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was then purified by flash chromatography on silica, using a gradient eluant of 40-60% EtOAc in hexane to give compound 123 (0.82 g, 1.80 mmol, 77% yield) as a white solid. $R_f$ (40% hexane/EtOAc) 0.73. M.p. 99-101 °C. $[\alpha]_D^{20} + 1.9$ (CHCl₃, c 1.1).

**IR $\nu_{\text{max}}$ (cm⁻¹):** 1808, 1729, 1597, 1351, 1173, 1095, 972, 814, 663.

$^1$H NMR (CDCl₃, 400 MHz): $\delta$ (ppm) 2.488 (s, 6H, H5'), 4.22-4.27 (m, 4H, H1), 4.73-4.78 (m, 2H, H2), 7.39-7.42 (m, 4H, H3'), 7.78-7.82 (m, 4H, H2').

$^{13}$C NMR (CDCl₃, 100 MHz): $\delta$ (ppm) 21.7 (C5'), 66.8 (C1), 74.3 (C2), 128.0 (C2'), 130.2 (C3'), 131.6 (q, C1'), 145.9 (q, C4'), 152.4 (q, C3).
Elemental Analysis:  *Anal. calcd.* for C_{19}H_{20}O_{9}S_{2}: C, 49.99; H, 4.42.  *Found*: C, 50.02; H, 4.58.

5.4 A Novel Route to Highly Substituted Aliphatic and Aromatic Heterocycles

5.4.1 General Procedure for synthesis of Nitroalcohols, Procedure A:

To a stirred solution of potassium tert-butoxide (0.17 g, 1.50 mmol) in tert-butanol (12 mL) and THF (6 mL) at -10 °C was added nitromethane (1.22 mL, 22.50 mmol). Stirring was continued for ca. 1 h whereupon a solution of aldehyde (15.00 mmol) in THF (6 mL) was added. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was diluted with EtOAc (50 mL) and saturated ammonium chloride solution (30 mL) was introduced. The aqueous phase was extracted with EtOAc (2 x 30 mL) and the combined organic layers were washed with water (50 mL) and brine (50 mL) before drying over magnesium sulfate. Volatiles were removed under reduced pressure and the resulting residue was purified by column chromatography.

5.4.1.1 2-Nitro-1-(3-nitro-phenyl)-ethanol:

![Image](image-url)

Prepared as per procedure A using potassium tert-butoxide (0.17 g, 1.50 mmol), nitromethane (1.22 mL, 22.50 mmol) and 3-nitrobenzaldehyde (2.27 g, 15.00 mmol). Flash chromatography using gradient elution from 1:1 CH₂Cl₂-hexane to 9:1 CH₂Cl₂-EtOAc and recrystallisation from CH₂Cl₂-hexane afforded compound 149 (1.79 g, 8.43...
mmol, 56% yield) as a white solid. M.p. 71-74 °C; lit.\textsuperscript{226} 70-71 °C. \(R_f\) (30\% EtOAc/hexane) 0.38.

IR \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3487, 3097, 3037, 2902, 1548, 1518, 1346, 1314, 1094, 1069, 900, 893, 832, 808, 735, 682.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) (ppm) 4.58-4.72 (m, 2H, H2'), 5.62-5.68 (m, 1H, H1'), 7.65 (t, \(J=8.3\) Hz, 1H, H5), 7.77-7.84 (m, 1H, H6), 8.26 (ddd, \(J=1.0, 2.2, 8.3\) Hz, 1H, H4), 8.34-8.38 (m, 1H, H2).

\(^13\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) (ppm) 69.9 (C1'), 80.6 (C2'), 121.2 (C2), 123.9 (C4), 130.2 (C5), 132.0 (C6), 140.1 (q, C1), 148.6 (q, C3).

5.4.1.2 2-Nitro-1-(4-nitro-phenyl)-ethanol:

\[
\begin{array}{c}
\text{O}_2\text{N} \\
\text{OH} \\
\text{O}_2\text{N} \\
\text{NO}_2 \\
\text{O}_2\text{N} \\
\text{OH} \\
\text{O}_2\text{N} \\
\text{NO}_2 \\
\end{array}
\]

Prepared as per procedure A using potassium tert-butoxide (0.17 g, 1.50 mmol), nitromethane (1.22 mL, 22.50 mmol) and 4-nitrobenzaldehyde (2.27 g, 15.00 mmol).

Flash chromatography, flushing initially with 1:1 CH\(_2\)Cl\(_2\)-hexane and then eluting with 30\% EtOAc in hexane followed by recrystallisation from CH\(_2\)Cl\(_2\)-hexane gave compound \textbf{144} (2.05 g, 9.66 mmol, 64\% yield) as an orange solid. M.p. 84-86 °C; lit.\textsuperscript{226} 84 °C. \(R_f\) (20\% EtOAc/Hexane) 0.24.

IR \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3446, 3112, 3081, 3052, 1603, 1540, 1517, 1338, 1321, 1215, 1085, 873, 858, 825, 744, 693.
$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 3.23 (broad s, 1H, -OH), 4.56-4.69 (m, 2H, H2'), 5.59-5.69 (m, 1H, H1'), 7.64-7.68 (m, 2H, H2), 8.27-8.31 (m, 2H, H3).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 70.0 (C1'), 80.6 (C2'), 124.2 (C2), 127.0 (C3), 145.0 (q, C1), 148.1 (q, C4).

5.4.1.3 2-Nitro-1-(2-trifluoromethyl-phenyl)-ethanol:

[Chemical structure image]

Prepared as per procedure A using potassium tert-butoxide (0.17 g, 1.50 mmol), nitromethane (1.22 mL, 22.50 mmol) and 2-trifluoromethyl benzaldehyde (1.94 mL, 15.00 mmol).

Flash chromatography, eluting with 10% EtOAc in hexane furnished compound 159 (2.63 g, 11.20 mmol, 75% yield) as a colourless oil. $R_f$ (20% EtOAc/Hexane) 0.54.

IR $\nu_{max}$ (cm$^{-1}$): 3507, 1554, 1379, 1310, 1161, 1108, 1033, 769.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 2.95 (s, 1H, -OH), 4.50-4.54 (m, 2H, H2), 5.91 (app t, $J$=5.9 Hz, 1H, H1), 7.46-7.52 (m, 1H, H4'), 7.63-7.72 (m, 2H, H5' & H3'), 7.86 (dd, $J$=0.5, 7.8 Hz, 1H, H6').

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 66.6 (C1), 80.6 (C2), 124.1 (quartet, $J_{C,F}$=273.5 Hz, C7'), 126.1 (quartet, $J_{C,F}$=5.4 Hz, C3'), 127.0 (quartet, $J_{C,F}$=30.5 Hz, q, C2'), 128.0 (C6'), 129.0 (C4'), 132.7 (C5'), 136.8 (q, C1')

207
5.4.1.4 1-(2-Methoxy-phenyl)-2-nitro-ethanol:

Prepared as per procedure A using potassium tert-butoxide (0.17 g, 1.50 mmol), nitromethane (1.22 mL, 22.50 mmol) and o-anisaldehyde (2.04 g, 15.00 mmol). Flash chromatography, eluting with 10% EtOAc in hexane furnished compound 156 (2.401 g, 12.18 mmol, 81% yield) as a yellow oil. $R_f$ (20% EtOAc/Hexane) 0.41.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3525, 3011, 2942, 2841, 1602, 1547, 1490, 1377, 1240, 1070, 1022, 754.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 3.15 (d, $J$=6.0 Hz, -OH), 3.91 (s, 3H, H7), 4.61 (dd, $J$=9.5, 13.0 Hz, 1H, H2'), 4.68 (dd, $J$=3.5, 13.0 Hz, 1H, H2'), 5.64-5.70 (m, 1H, H1'), 6.94 (d, $J$=8.5 Hz, 1H, H6), 7.05 (app t, $J$=7.5 Hz, 1H, H4), 7.34-7.39 (m, 1H, H5), 7.48 (dd, $J$=1.5, 7.5 Hz, 1H, H3).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 55.4 (C7), 67.9 (C1'), 79.9 (C2'), 110.5 (C6), 121.2 (C4), 125.9 (q, C2), 127.3 (C3), 129.9 (C5), 156.0 (q, C1).

HRMS: (m/z-ES) calcd. for C$_9$H$_{10}$NO$_4$ (M) 196.0610, found 196.0614.
5.4.1.5 2-Nitro-1-pyridin-3-yl-ethanol:

![Image of 174]

Prepared as per procedure A using potassium tert-butoxide (0.17 g, 1.50 mmol), nitromethane (1.22 mL, 22.50 mmol) and pyridine-3-carboxaldehyde (1.42 mL, 15.00 mmol).

Compound 174 (1.99 g, 11.85 mmol, 79% yield) was recovered as a yellow oil. $R_f$ (20% hexane/EtOAc) 0.42.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3094, 2918, 2851, 1547, 1424, 1376, 1027, 807, 707.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 4.57 (dd, $J$=3.0, 13.3 Hz, 1H, H2'), 4.67 (dd, $J$=9.5, 13.3 Hz, 1H, H2'), 5.57 (dd, $J$=3.0, 9.5 Hz, 1H, H1'), 7.40 (dd, $J$=5.0, 8.0 Hz, 1H, H5), 7.81-7.87 (m, 1H, H4), 8.58 (d, $J$=5.0 Hz, 1H, H6), 8.62 (s, 1H, H2).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 68.7 (C2'), 80.9 (C1'), 124.0 (C5), 134.1 (C4), 134.3 (q, C3), 147.5 (C2), 149.9 (C6).

HRMS: (m/z-ES) calcd. for C$_7$H$_7$N$_2$O$_3$ (M) 167.0457, found 167.0455.

5.4.1.6 1-(5-Bromo-thiophen-2-yl)-2-nitro-ethanol:

![Image of 167]

209
Prepared as per procedure A using potassium tert-butoxide (0.17 g, 1.50 mmol), nitromethane (1.22 mL, 22.50 mmol) and 5-bromothiophen-2-carboxaldehyde (1.78 mL, 15.00 mmol).

Flash chromatography, flushing initially with 1:1 CH₂Cl₂-hexane and then eluting with 25% EtOAc gave compound 167 (1.73 g, 6.88 mmol, 46% yield) as a brown oil. *R*ₗ (100% CH₂Cl₂) 0.32.

IR *ν*ₘₐₓ (cm⁻¹): 3395, 3100, 2922, 1654, 1549, 1414, 1376, 1074, 1054, 968, 797, 669.

¹H NMR (CDCl₃, 400 MHz): δ (ppm) 3.18 (d, *J*=4.0 Hz, 1H, -OH), 4.58 (dd, *J*=3.5, 13.6 Hz, 1H, H₂'), 4.67 (dd, *J*=9.0, 13.6 Hz, 1H, H₂'), 5.61-5.66 (m, 1H, H₁'), 6.81 (d, *J*=0.5 Hz, 1H, H₃), 6.96 (d, *J*=0.5 Hz, 1H, H₄).

¹³C NMR (CDCl₃, 100 MHz): δ (ppm) 67.2 (C₁'), 80.4 (C₂'), 113.3 (q, C₅), 125.3 (C₃), 130.0 (C₄), 142.8 (q, C₂).

HRMS: *m/z*-ES calcd. for C₉H₇NO₂Br (M⁺) 249.9174, found 249.9170.

5.4.1.7 1-(5-Iodo-furan-2-yl)-2-nitro-ethanol:

![164]

Prepared as per procedure A using potassium tert-butoxide (24 mg, 0.20 mmol), nitromethane (162 µL, 3.00 mmol) and 5-iodo-2-furaldehyde (0.44 g, 2.00 mmol).

Flash chromatography eluting with CH₂Cl₂ gave compound 164 (0.31 g, 1.08 mmol, 54% yield) as a dark brown paste. *R*ₗ (100% CH₂Cl₂) 0.30.
IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 3438, 3110, 2923, 1752, 1628, 1550, 1376, 1315, 1095, 1017, 911, 820, 794.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 2.88 (d, \( J=5.0 \) Hz, 1H, -OH), 4.67 (dd, \( J=3.5, 13.5 \) Hz, 1H, H2), 4.77 (dd, \( J=9.0, 13.5 \) Hz, 1H, H2), 5.44-5.51 (m, 1H, H1), 6.34 (d, \( J=3.3 \) Hz, 1H, H3'), 6.54 (d, \( J=3.3 \) Hz, 1H, H4').

\(^13\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 64.7 (C1), 78.2 (C2), 89.0 (q, C5'), 111.3 (C4'), 121.3 (C3'), 156.0 (q, C2').

HRMS: (m/z-ES) calcd. for C\(_6\)H\(_5\)NO\(_3\)I (M) 281.9263, found 281.9276.

5.4.1.8 1-Nitro-nonan-2-ol: \( 185a \)

Prepared as per procedure A using potassium tert-butoxide (0.17 g, 1.50 mmol), nitromethane (1.22 mL, 22.50 mmol) and octanal (2.34 mL, 15.00 mmol).

Flash chromatography eluting with 10% EtOAc in hexane afforded \( 185a \) (1.94 g, 10.23 mmol, 68% yield) as a colourless oil. \( R_f \) (20% hexane/EtOAc) 0.58.

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 3427, 2925, 2856, 1550, 1378, 1091, 881, 722.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 0.890 (m, 3H, H9), 1.255-1.668 (m, 12H, H3-H8), 2.752 (broad s, 1H, -OH), 4.319-4.531 (m, 3H, H1 & H2).

\(^13\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 14.1 (C9), 22.7 (C8), 25.2 (C7), 29.2 (C6), 29.3 (C5), 31.8 (C4), 33.8 (C3), 68.8 (C2), 80.7 (C1).
5.4.1.9 3-Methyl-1-nitro-butan-2-ol:

Prepared as per procedure A using potassium tert-butoxide (0.17 g, 1.50 mmol), nitromethane (1.22 mL, 22.50 mmol) and isobutyraldehyde (1.37 mL, 15.00 mmol). Compound 182a (1.80 g, 13.49 mmol, 89% yield) was recovered as a colourless oil. $R_f$ (20% hexane/EtOAc) 0.53.

IR $\nu_{max}$ (cm$^{-1}$): 3393, 2967, 2935, 1550, 1382, 1203, 1069, 886, 713.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 1.02 (m, 6H, H4 & H4'), 1.76-1.90 (m, 1H, H3), 2.44 (s, 1H, -OH), 4.08-4.18 (m, 1H, H2), 4.43 (dd, $J$=9.0, 13.1 Hz, 1H, H1), 4.50 (dd, $J$=3.0, 13.1 Hz, 1H, H1).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 17.0 & 18.0 (C4 & C4'), 31.3 (C3), 72.9 (C2), 78.8 (C1).

5.4.1.10 4-Methyl-1-nitro-pentan-2-ol:

Prepared as per procedure A using potassium tert-butoxide (0.69 g, 6.15 mmol), nitromethane (5.0 mL, 92.30 mmol) and isovaleraldehyde (6.6 mL, 61.50 mmol).
Compound 179 (7.53 g, 51.17 mmol, 83% yield) was recovered as a pale yellow oil. $R_f$ (30% hexane/EtOAc) 0.61.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3405, 2958, 2934, 2872, 1548, 1383, 1368, 1088, 890, 734.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 0.97 (d, $J=6.0$ Hz, 3H, H5), 0.99 (d, $J=6.0$ Hz, 3H, H5), 1.21-1.31 (m, 1H, H3), 1.49-1.58 (m, 1H, H3), 1.80-1.92 (m, 1H, H4), 2.52 (s broad, 1H, -OH), 4.35-4.47 (m, 3H, H1 & H2).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 21.3 (C5), 22.7 (C5), 23.8 (C4), 41.9 (C3), 66.5 (C2), 80.5 (C1).

HRMS: ($m/\text{z-ES}$) calcd. for C$_6$H$_{12}$NO$_3$ (M-H)$^-$ 146.0817, found 146.0809.

5.4.1.11 3,3-Dimethyl-1-nitro-butan-2-ol:

Prepared as per procedure A using potassium tert-butoxide (0.41 g, 3.68 mmol), nitromethane (3.00 mL, 55.24 mmol) and pivaldehyde (4.00 mL, 36.83 mmol).

Compound 188 (4.80 g, 32.63 mmol, 89% yield) was recovered as a colourless oil. $R_f$ (30% hexane/EtOAc) 0.61.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3332, 2965, 1548, 1384, 1191, 1080, 1068, 1009, 882, 701.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 1.00 (s, 9H, H4), 2.44 (d, $J=4.4$ Hz, 1H, -OH), 4.06 (dd, $J=1.5$, 10.0 Hz, 1H, H2), 4.39 (dd, $J=10.0$, 12.9 Hz, 1H, H1), 4.55 (dd, $J=1.5$, 12.9 Hz, 1H, H1).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 25.6 (C4), 34.3 (q, C3), 76.2 (C2), 78.4 (C1).
5.4.2 General procedure for acetate protection of nitroalcohols, Procedure B:

To a stirred solution of the appropriate nitroalcohol (2.58 mmol) in acetic anhydride (5.00 mL, 45.26 mmol) at 0 °C was added concentrated sulfuric acid (ca. 2 drops). The reaction mixture was stirred for ca. 1 h and then partitioned between water (50 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL) and the organic solution was washed with water (2 x 50 mL) and brine (2 x 50 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo furnishing the desired nitroacetate.

5.4.2.1 2-Acetoxy-1-nitrononane:

Prepared as per procedure B using 1-nitro-nonan-2-ol (1.00 g, 5.30 mmol). Compound 185b (1.14 g, 4.93 mmol, 93% yield) was isolated as a colourless oil. \( R_f \) (30% EtOAc/hexane) 0.91.

\[ \text{1H NMR (CDCl}_3, 400 MHz): \delta \text{ (ppm)} \]

\[ 0.91 \text{ (t, } J=6.5 \text{ Hz, 3H, H9), 1.21-1.80 (m, 12 H, H3-H8), 2.10 (s, 3H, H2' ), 4.53-4.57 (m, 2H, H1), 5.47 (quintet, } J=6.2 \text{ Hz, 1H, H2). } \]
5.4.2.2 2-Acetoxy-3-methyl-1-nitrobutane:

![Chemical Structure](image)

Prepared as per procedure B using 1-nitro-3-methyl-butan-2-ol (1.00 g, 7.51 mmol). Compound **182b** (1.20 g, 6.83 mmol, 91% yield) was isolated as a pale yellow oil: \( R_f \) (30% EtOAc/hexane) 0.84.

\[ ^1H \text{ NMR (CDCl}_3, 400 MHz): \delta \text{ (ppm) } 1.00 \text{ (d, } J=1.8 \text{ Hz, 3H, H4), 1.02 \text{ (d, } J=1.8 \text{ Hz, 3H, H4'}, 1.99-2.09 \text{ (m, 1H, H3), 2.11 \text{ (s, 3H, H2'), 4.53-4.58 \text{ (m, 2H, H1), 5.36-5.42 \text{ (m, 1H, H2).}} \]

5.4.3 Synthesis of Nitroolefins, Procedure C

To a stirred solution of the appropriate nitroalcohol (5.98 mmol) in CH\(_2\)Cl\(_2\) (30 mL) at -15 °C was added freshly distilled trifluoroacetic anhydride (0.95 mL, 6.58 mmol). Stirring was continued for ca. 5 min whereupon anhydrous triethylamine (1.90 mL, 13.16 mmol) was added over 30 min maintaining the temperature at -15 °C; stirring was continued for a further 30 minutes. Saturated ammonium chloride solution (30 mL) was then introduced and the aqueous layer was extracted with CH\(_2\)Cl\(_2\) (2 x 30 mL). The combined organic layers were then washed with water (30 mL) and brine (30 mL) before drying over magnesium sulfate and concentrating \textit{in vacuo}. The resulting residue was purified by column chromatography.
5.4.3.1 1-Nitro-3-(2-nitro-vinyl)-benzene:

\[
\begin{array}{c}
\text{HO}_2 \text{C} \\
\text{HO}_2 \\
\text{C}\text{H} \\
\text{C}\text{H} \\
\text{C}\text{H} \\
\text{C}\text{H} \\
\text{C}\text{H} \\
\end{array}
\]

Prepared as per procedure C using compound 149 (1.27 g, 5.98 mmol), trifluoroacetic anhydride (0.95 mL, 6.58 mmol) and triethylamine (1.90 mL, 13.16 mmol). Flash chromatography eluting with 100% CH\(_2\)Cl\(_2\) afforded compound 150 (0.88 g, 4.52 mmol, 76% yield) as a white solid. M.p. 126-128 °C; lit.\(^{227}\) 126 °C. \(R_f\) (30% EtOAc/hexane) 0.68.

IR \(\nu_{\text{max}}\) (\(\text{cm}^{-1}\)): 3103, 3056, 1683, 1524, 1505, 1475, 1344, 1263, 1201, 1099, 970, 809, 738, 809, 738, 708, 659.

\(^1\)H NMR (CDCl\(_3\), 600 MHz): \(\delta\) (ppm) 7.66-7.73 (m, 2H, H\(_2\)' & H\(_5\)'), 7.90 (d, \(J=7.9\) Hz, 1H, H\(_4\)), 8.08 (d, \(J=13.9\) Hz, 1H, H\(_1\)'), 8.38 (d, \(J=8.3\) Hz, 1H, H\(_6\)), 8.45 (s, 1H, H\(_2\)).

\(^13\)C NMR (CDCl\(_3\), 150 MHz): \(\delta\) (ppm) 123.4 (C\(_2\)), 126.2 (C\(_6\)), 130.6 (C\(_5\)), 131.8 (q, C\(_3\)), 134.4 (C\(_4\)), 136.2 (C\(_1\)'), 139.3 (C\(_2\)'), 148.9 (q, C\(_1\)).

HRMS: (\(m/z\)-El) calcd. for C\(_8\)H\(_6\)N\(_2\)O\(_4\) (M) 194.0328, found 194.0334.
5.4.3.2 1-Methoxy-2-(2-nitro-vinyl)-benzene:

Prepared as per procedure C using compound 156 (1.18 g, 5.98 mmol), trifluoroacetic anhydride (0.95 mL, 6.58 mmol) and triethylamine (1.90 mL, 13.16 mmol).

Flash chromatography eluting with 100% CH$_2$Cl$_2$ afforded compound 157 (0.81 g, 4.54 mmol, 76% yield) as a yellow solid. M.p. 50-53 °C; lit.$^{228}$ 50-51 °C. $R_f$ (30% EtOAc/hexane) 0.7.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3107, 1624, 1596, 1499, 1484, 1468, 1429, 1335, 1238, 1162, 1106, 1017, 965, 843, 747.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 3.99 (s, 3H, H7), 6.99-7.08 (m, 2H, H6 & H4), 7.46-7.53 (m, 2H, H5 & H3), 7.92 (d, $J=13.6$ Hz, 1H, H2'), 8.18 (d, $J=13.6$ Hz, 1H, H1').

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 55.7 (C7), 111.4 (C6), 119.2 (q, C1), 112.1 (C4), 132.6 (C3), 133.5 (C5), 135.6 (C1'), 138.3 (C2'), 159.5 (q, C2).

HRMS: (m/z-El) calcd. for C$_9$H$_8$NO$_3$ (M) 179.0582, found 179.0576.
5.4.3.3 1-(2-Nitro-vinyl)-2-trifluoromethyl-benzene:

Prepared as per procedure C using compound 159 (1.18 g, 5.98 mmol), trifluoroacetic anhydride (0.95 mL, 6.58 mmol) and triethylamine (1.90 mL, 13.16 mmol). Flash chromatography eluting with 100% CH₂Cl₂ afforded compound 160 (1.06 g, 4.89 mmol, 82% yield) as a yellow solid. M.p. 64-66 °C. \( R_f \) (30% EtOAc/hexane) 0.55.

IR \( \nu_{\text{max}} \) (cm⁻¹): 3107, 1643, 1531, 1514, 1488, 1346, 1311, 1156, 1103, 1034, 955, 767, 729.

\(^1\)H NMR (CDCl₃, 400 MHz): \( \delta \) (ppm) 7.54 (d, \( J=13.5 \) Hz, 1H, H2'), 7.61-7.74 (m, 3H, H4, H5 & H6), 7.82 (d, \( J=6.8 \) Hz, 1H, H3), 8.41 (dd, \( J=1.5, 13.5 \) Hz, 1H, H1').

\(^13\)C NMR (CDCl₃, 100 MHz): \( \delta \) (ppm) 123.6 (quartet, \( J_{C,F}=274.2 \) Hz, C7), 126.8 (quartet, \( J_{C,F}=5.4 \) Hz, C3), 128.5 (C6), 128.6 (q, C1), 129.9 (quartet, \( J_{C,F}=30.7 \) Hz, q, C2), 131.4 (C4), 132.5 (C5), 134.77 (quartet, \( J_{C,F}=2.0 \) Hz, C1'), 139.8 (C2').

HRMS: \( m/z-\text{El} \) calcd. for C₉H₆NO₂F₃ (M) 194.0328, found 194.0334.
5.4.3.4 \textit{1-Nitro-4-(2-nitro-vinyl)-benzene}:

\begin{center}
\begin{tikzpicture}
  \node (a1) at (0,0) {O2N};
  \node (a2) at (1,0) {};  \node (a3) at (1,-0.5) {1};
  \node (a4) at (1.5,-0.5) {2};  \node (a5) at (2,-0.5) {3};
  \node (a6) at (2.5,-0.5) {4};
  \node (a7) at (3,-0.5) {5};
  \node (a8) at (3.5,-0.5) {1'}

  \draw[thick] (a1) -- (a2) -- (a3) -- (a4) -- (a5) -- (a6) -- (a7) -- (a8);
  \draw[thick] (a3) -- (a4) -- (a5);
\end{tikzpicture}
\end{center}

Prepared as per procedure C using compound 144 (1.27 g, 5.98 mmol), trifluoroacetic anhydride (0.95 mL, 6.58 mmol) and triethylamine (1.90 mL, 13.16 mmol). Flash chromatography eluting with 100\% CH$_2$Cl$_2$, followed by recrystallisation from acetone-hexane afforded compound \textbf{145} (0.88 g, 4.54 mmol, 77\% yield) as a yellow solid. M.p. 203-205 °C; lit.$^{229}$ 203 °C. \(R_f\) (20\% EtOAc/hexane) 0.58.

IR \(v_{\text{max}}\) (cm$^{-1}$): 3110, 1638, 1601, 1524, 1332, 1310, 1261, 1196, 1111, 964, 836, 741, 707, 670.

$^1$H NMR (CDCl$_3$, 400 MHz): \(\delta\) (ppm) 7.66 (d, \(J=13.9\) Hz, 1H, H2'), 7.76 (d, \(J=7.2\) Hz, 2H, H3), 8.06 (d, \(J=13.9\) Hz, 1H, H1'), 8.35 (d, \(J=7.2\) Hz, 2H, H2).

$^{13}$C NMR (CDCl$_3$, 100 MHz): \(\delta\) (ppm) 124.5 (C2), 129.7 (C3), 136.0 (C1'), 136.1 (q, C4), 139.7 (C2'), 149.5 (q, C1).

5.4.3.5 \textit{4-Methyl-1-nitro-pent-1-ene}:

\begin{center}
\begin{tikzpicture}
  \node (a1) at (0,0) {NO$_2$};
  \node (a2) at (1,0) {};  \node (a3) at (1,-0.5) {1};
  \node (a4) at (1.5,-0.5) {2};  \node (a5) at (2,-0.5) {3};
  \node (a6) at (2.5,-0.5) {4};
  \node (a7) at (3,-0.5) {5};

  \draw[thick] (a1) -- (a2) -- (a3) -- (a4) -- (a5) -- (a6) -- (a7);
\end{tikzpicture}
\end{center}
Prepared as per procedure C using compound 179 (7.23 g, 49.10 mmol), trifluoroacetic anhydride (7.51 mL, 54.01 mmol) and triethylamine (15.05 mL, 108.02 mmol). Compound 180 (5.86 g, 45.4 mmol, 92% yield) was isolated as a yellow oil. \( R_f \) (30% EtOAc/hexane) 0.61.

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 2959, 2931, 2872, 1649, 1520, 1347, 966, 875, 733.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 0.99 (d, \( J=7.0 \) Hz, 6H, H5), 1.86 (heptet, \( J=7.0 \) Hz, 1H, H4), 2.14-2.21 (m, 2H, H3), 6.96-7.03 (m, 1H, H1), 7.23-7.33 (m, 1H, H2).

\(^1^3\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 21.8 (C5), 27.3 (C4), 36.8 (C3), 139.7 (C1), 141.2 (C2).

5.4.3.6 3,3-Dimethyl-1-nitro-but-1-ene:

\[
\begin{array}{c}
\text{4} \\
\text{3} \\
\text{2} \\
\text{1} \\
\text{NO}_2
\end{array}
\]

189

Prepared as per procedure C using compound 188 (3.83 g, 26.40 mmol), trifluoroacetic anhydride (4.04 mL, 29.04 mmol) and triethylamine (8.10 mL, 58.08 mmol). Compound 189 (2.36 g, 18.58 mmol, 70% yield) was isolated as a yellow oil. \( R_f \) (2% EtOAc/hexane) 0.25.

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 2966, 2870, 1643, 1524, 1347, 968, 925, 842, 785, 721.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 1.19 (s, 9H, H4), 6.93 (d, \( J=13.2 \) Hz, 1H, H1), 7.29 (d, \( J=13.2 \) Hz, 1H, H2).

\(^1^3\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 28.3 (C4), 32.6 (q, C3), 137.0 (C1), 151.8 (C2).
5.4.4 Synthesis of Nitroolefins, Procedure D

A reaction vessel containing a stirring bar was charged with the appropriate aldehyde (4.5 mmol) and ammonium acetate (0.19 g, 2.5 mmol). Nitromethane (4 mL) was introduced and the reaction was heated at reflux overnight. The reaction was diluted with EtOAc (30 mL) and washed with saturated bicarbonate solution (2 x 30 mL). The combined aqueous layers were extracted with EtOAc (2 x 30 mL) and the combined organic layers were then washed with water (30 mL) and brine (2 x 30 mL). The organic solution was then dried over magnesium sulfate and concentrated in vacuo. The resulting residue was purified by column chromatography.

5.4.4.1 2-Bromo-5-(2-nitro-vinyl)-thiophene:

![Image of 2-Bromo-5-(2-nitro-vinyl)-thiophene]

Prepared as per procedure D using 5-bromothiophene-2-carboxaldehyde (1.0 mL, 8.41 mmol) and ammonium acetate (0.357 g, 4.62 mmol). Flash chromatography eluting with 1:1 CH₂Cl₂-hexane gave compound 168 (0.49 g, 2.09 mmol, 25% yield) as a brown solid. M.p. 89-91 °C; lit.²¹ 90-91 °C. Rf (10% EtOAc/hexane) 0.56.

IR \( \nu_{\text{max}} \) (cm⁻¹): 3096, 1617, 1522, 1494, 1485, 1419, 1314, 1233, 1192, 1057, 954, 798.

\(^1\)H NMR (CDCl₃, 400 MHz): δ (ppm) 7.12 (d, \( J=3.9 \) Hz, 1H, H3), 7.21 (d, \( J=3.9 \) Hz, 1H, H4), 7.38 (d, \( J=13.3 \) Hz, 1H, H2''), 8.03 (d, \( J=13.3 \) Hz, 1H, H1 '').

\(^13\)C NMR (CDCl₃, 100 MHz): δ (ppm) 119.7 (q, C2), 131.1 (C1''), 131.9 (C3), 135.1 (C4), 135.2 (q, C5), 135.6 (C2 '').

221
5.4.4.2 *N*-Methyl-3-(2-nitro-vinyl)-indole:

Prepared as per procedure D using *N*-methyl-3-formyl-indole (2.00 g, 12.56 mmol) and ammonium acetate (0.53 g, 6.91 mmol).

Flash chromatography using gradient elution from 1:1 CH$_2$Cl$_2$-hexane to 100% CH$_2$Cl$_2$ gave compound 170 (1.18 g, 5.85 mmol, 47% yield) as light brown needles. M.p. 169-172 °C; lit.$^{231}$ 166-169 °C; lit.$^{232}$ 171-172 °C. *R*$_f$ (30% EtOAc/hexane) 0.45.

IR *ν*$_{max}$ (cm$^{-1}$): 3110, 3058, 1614, 1531, 1491, 1478, 1458, 1294 1247, 948, 787, 740.

$^1$H NMR (CDCl$_3$, 400 MHz): δ (ppm) 3.91 (s, 3H, H10), 7.35-7.47 (m, 3H, H6, H7 & H8), 7.56 (s, 1H, H2), 7.79-7.84 (m, 1H, H5), 7.80 (d, *J*=13.5 Hz, 1H, H2'), 8.29 (d, *J*=13.5 Hz, 1H, H1').

$^{13}$C NMR (CDCl$_3$, 100 MHz): δ (ppm) 33.6 (C10), 108.2 (q, C4), 110.6 (C8), 120.7 (C5), 122.6 (C6), 124.0 (C7), 125.5 (q, C3), 132.1 (C2'), 133.4 (C1'), 136.7 (C2), 138.5 (q, C9).
5.4.4.3 1-Methyl-2-(2-nitro-vinyl)-1H-pyrrole:

\[
\begin{align*}
\text{5.4.4.3 } & 1-	ext{Methyl-2-(2-nitro-vinyl)-1H-pyrrole:} \\
\end{align*}
\]

Prepared as per procedure D using N-methyl-2-formyl-pyrrole (2.00 g, 18.3 mmol) and ammonium acetate (0.71 g, 9.15 mmol). Flash chromatography eluting with 100% CH$_2$Cl$_2$ followed by recrystallisation from CH$_2$Cl$_2$-petroleum ether gave compound 172 (2.22 g, 14.62 mmol, 80% yield) as light brown needles. M.p. 103-107 °C; lit. 98-103 °C. $R_f$ (40% EtOAc/hexane) 0.74.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3116, 3091, 1607, 1527, 1464, 1407, 1302, 1241, 1069, 957, 814, 728, 715, 656.

$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 3.80 (s, 3H, H6), 6.30 (ddd, $J$=0.7, 2.5, 3.4 Hz, 1H, H4), 6.83 (dd, $J$=1.7, 3.7 Hz, 1H, H3), 6.96 (dd, $J$=1.7, 2.5 Hz, 1H, H5), 7.48 (d, $J$=12.8 Hz, H2'), 8.01 (d, $J$=12.8 Hz, 1H, H1').

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 34.8 (C6), 111.2 (C4), 116.3 (C3), 125.0 (q, C2), 127.4 (C1'), 130.7 (C5), 131.9 (C2').

5.4.4.4 2-Iodo-5-(2-nitro-vinyl)-furan:

\[
\begin{align*}
\end{align*}
\]
Prepared as per procedure D using 5-iodo-furaldehyde (1.00 g, 4.50 mmol) and ammonium acetate (0.19 g, 2.50 mmol).

Flash chromatography eluting with 1:1 CH₂Cl₂-hexane gave compound 165 (0.41 g, 1.55 mmol, 34% yield) as a dark red solid. M.p. 113-115 °C; lit. 113-114 °C. $R_f$ (40% CH₂Cl₂/hexane) 0.45.

IR $\nu_{\text{max}}$ (cm⁻¹): 3111, 3056, 1628, 1496, 1483, 1311, 1258, 1020, 957, 949, 818, 795, 696.

$^1$H NMR (CDCl₃, 400 MHz): $\delta$ (ppm) 6.76 (d, $J=3.5$ Hz, 1H, H4), 6.78 (d, $J=3.5$ Hz, 1H, H3), 7.54 (d, $J=13.1$ Hz, 1H, H2'), 7.70 (d, $J=13.1$ Hz, 1H, H1').

$^{13}$C NMR (CDCl₃, 100 MHz): $\delta$ (ppm) 95.9 (q, C2), 121.9 (C3), 123.9 (C1'), 124.1 (C4), 135.4 (C2'), 151.7 (q, C5).

HRMS: (m/z-ES) calcd. for C₆H₅NO₃I (M-H)- 263.9158, found 263.9168.

5.4.4.5 3-(2-Nitro-vinyl)-pyridine:

To a stirred solution of compound 174 (0.90 g, 5.35 mmol) in CH₂Cl₂ (27 mL) were added acetic anhydride (0.60 mL, 6.34 mmol) and dimethylaminopyridine (33 mg, 0.27 mmol) at room temperature. Stirring was continued for 2 h whereupon the reaction was diluted with CH₂Cl₂ (50 mL) and washed with saturated bicarbonate solution (20 mL). The aqueous layer was extracted with CH₂Cl₂ (2 x 30 mL) and the combined organic layers were washed with water (50 mL) and brine (50 mL) before drying over magnesium sulfate. Volatiles were removed under reduced pressure and the resulting residue was recrystallised from
EtOAc-petroleum ether to give compound 175 (0.55 g, 3.63 mmol, 69% yield) as light brown needles. M.p. 141-144 °C; lit.211 141 °C. Rf (10% EtOAc/CH2Cl2) 0.28.

IR ν max (cm⁻¹): 3077, 1633, 1515, 1488, 1417, 1342, 1215, 1027, 974, 968, 957, 857, 803, 744, 692.

¹H NMR (CDCl3, 400 MHz): δ (ppm) 7.45 (dd, J=4.5, 8.0 Hz, 1H, H5), 7.66 (d, J=13.9 Hz, 1H, H2'), 7.86 (m, 1H, H4), 8.04 (d, J=13.9 Hz, 1H, H1'), 8.75 (dd, J=1.7, 4.5 Hz, 1H, H6), 8.83 (d, J=1.7 Hz, 1H, H2).

¹³C NMR (CDCl3, 100 MHz): δ (ppm) 124.1 (C5), 126.2 (q, C3), 135.2 (C4), 135.5 (C1'), 138.4 (C2'), 150.4 (C2), 152.7 (C6).

HRMS: (m/z-EI) calcd. for C7H6N2O2 (M) 150.0429, found 150.0426.

5.4.5 General Procedure for the Synthesis of Tetrahydrothiophenes, Procedure E

To a stirred solution of the appropriate nitroolefin (2.00 mmol) in CH2Cl2 (5.6 mL) were added 2,5-dihydroxy-1,4-dithiane (0.23 g, 1.50 mmol) and triethylamine (56 µL, 0.40 mmol) under an argon atmosphere. The reaction was stirred at room temperature overnight. The reaction was diluted with CH2Cl2 (20 mL) and the suspended solid was removed by filtration. A solution of saturated ammonium chloride (20 mL) was added to the filtrate and the aqueous layer was extracted with CH2Cl2 (2 x 20 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL) and then dried over magnesium sulfate. Volatiles were removed under reduced pressure and the resulting residue was purified by column chromatography.
5.4.5.1 4-Nitro-5-phenyl-tetrahydro-thiophen-3-ol:

![Chemical Structure](image)

Prepared as per procedure E using trans-β-nitrostyrene (0.80 g, 5.36 mmol), 2,5-dihydroxy-1,4-dithiane (0.61 g, 4.02 mmol) and triethylamine (150 μL, 1.08 mmol).

Flash chromatography using a gradient eluant of 10-25% EtOAc in hexane gave compound 137 (1.20 g, 5.31 mmol, 99% yield) in approximately a 3:2 mixture of diastereomers. \( R_f \) maj (20% EtOAc/Hexane) 0.24; \( R_f \) min (20% EtOAc/Hexane) 0.16.

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 3383, 2940, 1547, 1371, 1075, 729.

\(^1\)H NMR (\(d^6\) DMSO, 400 MHz): \( \delta \) (ppm) 3.10 (dd, \( J=9.2, 10.1 \) Hz, 1H, H2 maj), 3.20 (dd, \( J=7.6, 10.1 \) Hz, 1H, H2 maj), 4.74-4.83 (m, 1H, H3 maj), 4.89 (d, \( J=10.3 \) Hz, 1H, H5 maj), 5.18 (dd, \( J=9.3, 10.3 \) Hz, 1H, H4 maj), 6.30 (d, \( J=6.3 \), 1H, -OH maj), 7.30-7.39 (m, 3H, H2’ maj & H4’ maj), 7.46-7.51 (m, 2H, H3’ maj).

\(^13\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 34.4 (C2 maj), 50.0 (C5 maj), 77.4 (C3 maj), 98.4 (C4 maj), 128.3 (C3’ maj), 129.4 (C4’ maj), 129.6 (C2’ maj), 137.1 (q, C1’ maj).

\(^1\)H NMR (\(d^6\) DMSO, 400 MHz): \( \delta \) (ppm) 2.84 (dd, \( J=1.5, 11.3 \) Hz, 1H, H2 min), 3.54 (dd, \( J=4.0, 11.3 \) Hz, 1H, H2 min), 4.91-4.97 (m, 1H, H3 min), 5.14 (d, \( J=10.5 \) Hz, 1H, H5 min), 5.612 (dd, \( J=4.0, 10.5 \) Hz, 1H, H4 min), 6.23 (d, \( J=4.0 \)
1H NMR (CDCl₃, 600 MHz); δ (ppm) 3.06 (dd, J=1.9, 12.0 Hz, 1H, H2 min), 3.17 (dd, J=8.3, 10.9 Hz, 1H, H2 maj), 3.30 (dd, J=7.2, 10.9 Hz, 1H, H2 maj), 3.52 (dd, J=4.5, 12.0, 1H, H2 min), 4.87 (d, J=8.5 Hz, 1H, H5 maj), 4.92 (app. t, J=8.5 Hz, 1H, H4 maj), 4.94 (m, 2H, H3 maj & H4 min), 5.06-5.11 (m, 1H, H3 min), 5.22 (d, J=9.8 Hz, 1H, H5 min), 7.31 (d, J=8.7 Hz, 5.4.5.2 5-(4-Bromo-phenyl)-4-nitro-tetrahydro-thiophen-3-ol

Prepared as per procedure E using p-bromo-trans-β-nitrostyrene (150 mg, 0.66 mmol), 2,5-dihydroxy-1,4-dithiane (75 mg, 0.49 mmol) and triethylamine (27 μL, 0.19 mmol).

Flash chromatography using a gradient eluant of 10-20% EtOAc in hexane afforded compound 153 (169 mg, 0.55 mmol, 84%) in approximately a 1.8:1 mixture of diastereomers. Rf maj (30% EtOAc/Hexane) 0.45; Rf min (30% EtOAc/Hexane) 0.35.

IR νmax (cm⁻¹): 3393, 1547, 1487, 1370, 1072, 1009, 832, 750.

13C NMR (CDCl₃, 100 MHz); δ (ppm) 35.7 (C2 min), 47.9 (C5 min), 75.0 (C3 min), 95.1 (C4 min), 127.7 (C3' min), 128.2 (C4' min), 128.5 (C2' min), 136.1 (q, C1' min).

HRMS: (m/z - El) calcd. for C₁₀H₁₁NO₃S (M) 225.0453, found 225.0460.
2H, H2' maj), 7.38 (d, J=8.3 Hz, 2H, H2' min), 7.48-7.52 (m, 4H, H3' both isomers).

13C NMR (CDCl3, 150 MHz): δ (ppm)
33.6 (C2 maj), 36.0 (C2 min), 47.4 (C5 min), 48.7 (C5 maj), 75.3 (C3 min), 76.6 (C3 maj), 95.3 (C4 min), 97.6 (C4 maj), 122.5 (q, C4' min), 122.8 (q, C4' maj), 129.4 (C2' maj), 130.0 (C2' min), 132.0 (C3' min), 132.1 (C3' maj), 135.6 (q, C1' min), 135.7 (q, C1' maj).

HRMS: (m/z-ES) calcd. for C10H79BrNO3S (M-H)– 301.9487, found 301.9476.

5.4.5.3 5-Furan-2-yl-4-nitro-tetrahydro-thiophen-3-ol:

[Chemical structure image]

Prepared as per procedure E using 2-(2-nitrovinyl)-furan (2.00 g, 14.40 mmol), 2,5-dihydroxy-1,4-dithiane (1.64 g, 10.80 mmol) and triethylamine (0.30 mL, 2.2 mmol).
Flash chromatography using a gradient eluant of 10–20% EtOAc in hexane afforded compound 163 (1.36 g, 6.32 mmol, 44% yield) in approximately a 1:1.4 ratio of diastereomers. Rf maj (40% EtOAc/Hexane) 0.39; Rf min (40% EtOAc/Hexane) 0.45.

IR νmax (cm⁻¹): 3535, 1545, 1372, 1147, 1069, 1011, 928, 753.

1H NMR (d6 DMSO, 400 MHz): δ (ppm) 2.85 (dd, J=1.0, 11.3 Hz, 1H, H2 min), 3.01 (dd, J=10.0, 10.5 Hz, 1H, H2 maj), 3.18 (dd, J=7.0, 10.5 Hz, 1H, H2 maj), 3.46 (dd, J=4.0, 11.3 Hz, 1H, H2 min), 4.72-4.82 (m, 1H, H5 maj), 4.72-4.82 (m, 1H, H3 maj), 4.90-4.96 (m, 1H, H3 min), 228
5.27 (d, \( J=9.8 \) Hz, 1H, H5 min), 5.56 (dd, \( J=3.5, 9.8 \) Hz, 1H, H4 min), 5.72 (app. t, \( J=9.5 \) Hz, 1H, H4 maj), 6.27 (d, \( J=4.5 \) Hz, 1H, -OH min), 6.34 (d, \( J=6.5 \) Hz, 1H, -OH maj), 6.40-6.44 (m, 2H, H4' both isomers), 6.46-6.50 (m, 2H, H3' both isomers), 7.61-7.64 (m, 1H, H5' min), 7.67-7.70 (m, 1H, H5' maj).

\[^{13}\text{C} \text{NMR (d}^6\text{DMSO, 100 MHz):} \delta (\text{ppm})32.5 \text{ (C2 maj), 35.8 (C2 min), 40.2 (C5 min), 40.5 (C5 maj), 74.4 (C3 min), 76.4 (C3 maj), 91.5 (C4 min), 94.5 (C4 maj), 107.8 (C4' min), 108.6 (C4' maj), 110.83 (C3' maj), 110.87 (C3' min), 143.0 (C5' min), 143.8 (C5' maj), 149.9 (q, C2' maj), 150.5 (q, C2' min).}

HRMS: \( m/z-\text{ES} \) calcd. for \( \text{C}_8\text{H}_8\text{N}_0\text{O}_4\text{S} \) (M-H)' 214.0174, found 214.0175.

5.4.5.4 5-Isobutyl-4-nitro-tetrahydrothiophen-3-ol:

Prepared as per procedure E using compound 180 (0.31 g, 2.40 mmol), 2,5-dihydroxy-1,4-dithiane (0.28 g, 1.80 mmol) and triethylamine (50 \( \mu \text{L}, 0.36 \) mmol).

Flash chromatography eluting with 10% EtOAc in hexane afforded compound 181 (0.46 g, 2.26 mmol, 94% yield) in approximately a 2:1 ratio of diastereomers. \( R_f \) maj (30% EtOAc/Hexane) 0.72; \( R_f \) min (30% EtOAc/Hexane) 0.61.

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 3409, 2958, 1547, 1368, 1083, 773.
$^1$H NMR (400 MHz, CDCl$_3$) δ (ppm)

0.90 (d, $J$=6.4 Hz, 3H, H3' min), 0.95 (d, $J$=6.4 Hz, 3H, H3' min), 0.96 (d, $J$=4.8 Hz, 3H, H3' maj), 0.98 (d, $J$=4.8 Hz, 3H, H3' maj), 1.51-1.79 (m, 6H, H1' & H2' both isomers), 2.72 (d, $J$=6.0 Hz, 1H, -OH min), 2.80 (d, $J$=6.0 Hz, 1H, -OH maj), 2.94 (dd, $J$=8.0 & 11.0 Hz, 1H, H2 min), 2.98 (dd, $J$=2.5, 12.1 Hz, 1H, H2 maj), 3.18 (dd, $J$=6.5, 11.0 Hz, 1H, H2 min), 3.24 (dd, $J$=4.5, 12.1 Hz, 1H, H2 maj), 3.79-3.89 (m, 1H, H5 min), 4.09-4.19 (m, 1H, H5 maj), 4.57 (dd, $J$=7.5, 8.0 Hz, 1H, H4 min), 4.61 (dd, $J$=4.0, 8.6 Hz, 1H, H4 maj) & 4.86-5.04 (m, 2H, H3 both isomers).

$^{13}$C NMR (CDCl$_3$, 100 MHz): δ (ppm)

21.12 (C3' maj), 21.14 (C3' min), 23.3 (C3' min), 23.5 (C3' maj), 26.8 (C2' min), 27.7 (C2' maj), 33.4 (C2 min), 35.0 (C2 maj), 43.4 (C5 maj), 44.1 (C1' maj), 44.5 (C1' min), 44.7 (C5 min), 75.2 (C3 maj), 76.9 (C3 min), 94.4 (C4 min), 97.0 (C4 maj).

HRMS: (m/z – El) calcd. for C$_8$H$_{15}$NO$_3$S (M) 205.0773, found 205.0768.
5.4.5.5 5-(4-Benzyloxy-phenyl)-4-nitro-tetrahydro-thiophen-3-ol:

Prepared as per procedure E using 4-benzoyloxy-trans-ß-nitrostyrene (0.50 g, 1.96 mmol), 2,5-dihydroxy-1,4-dithiane (0.22 g, 1.47 mmol) and triethylamine (55 µL, 0.39 mmol).
Flash chromatography eluting with 10% EtOAc in hexane afforded compound 148 (0.57 g, 1.71 mmol, 87% yield) as a 1.6:1 mixture of diastereomers. \( R_f \) maj (30% EtOAc/Hexane) 0.33; \( R_f \) min (30% EtOAc/Hexane) 0.23.

IR \( \nu_{max} \) (cm\(^{-1}\)): 3504, 3032, 2996, 2933, 2907, 1609, 1549, 1509, 1383, 1371, 1242, 1174, 1078, 1013, 837, 749, 701.

\(^1\)H NMR (600 MHz, CDCl\(_3\)) \( \delta \) (ppm) 2.66 (d, \( J=5.1 \) Hz, 1H, -OH maj), 2.76 (d, \( J=5.1 \) Hz, 1H, -OH min), 3.09 (dd, \( J=2.2 \), 11.7 Hz, 1H, H2 min), 3.171 (dd, \( J=8.1 \), 11.0 Hz, 1H, H2 maj), 3.33 (dd, \( J=7.4 \), 11.0 Hz, 1H, H2 maj), 3.52 (dd, \( J=4.4 \), 11.7 Hz, 1H, H2 min), 4.89-5.03 (m, 4H, H5 maj, H4 both isomers & H3 maj), 5.05-5.10 (m, 1H, H3 min), 5.08 (s, 4H, H1’’ both isomers), 5.23 (d, \( J=9.5 \) Hz, 1H, H5 min), 6.96-6.99 (m, 4H, H3’ both isomers), 7.338-7.379 (m, 4H, H4’’ maj & H5’’ both isomers), 7.40-7.46 (m, 6H, H4’’ min, H2’ & H3’’ both isomers).
$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 33.9 (C2 maj), 35.9 (C2 min), 47.9 (C5 min), 49.2 (C5 maj), 70.0 (C1’’ both isomers), 75.1 (C3 min), 76.7 (C3 maj), 95.4 (C4 min), 97.9 (C4 maj), 115.1 (C3’ min), 115.2 (C3’ maj), 127.29 (C3’’ min), 127.31 (C3’’ maj), 127.4 (q, C1’ min), 127.91 (C5’’ min), 127.92 (C5’’ maj), 128.3 (q, C1’ maj), 128.5 (C2’ both isomers), 128.8 (C4’’ maj), 129.2 (C4’’ min), 136.5 (q, C2’’ maj), 136.6 (q, C2’’ min), 159.0 (q, C4’ maj).

HRMS: ($m/z$-ES) calcd. for C$_{17}$H$_{17}$NO$_4$NaS (M+Na)$^+$ 354.0776, found 354.0774.

5.4.5.6 5-tert-Butyl-4-nitro-tetrahydro-thiophen-3-ol:

Prepared as per procedure E using compound 189 (0.57 g, 4.54 mmol), 2,5-dihydroxy-1,4-dithiane (0.52 g, 3.4 mmol) and triethylamine (127 µL, 0.91 mmol).

Flash chromatography using a gradient eluant of 5-10% EtOAc in hexane afforded compound 190 (0.83 g, 4.04 mmol, 89% yield) as approximately a 1:3.3 mixture of diastereomers. $R_f$-maj (20% EtOAc/Hexane) 0.35; $R_f$-min (20% EtOAc/Hexane) 0.24.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3312, 2964, 2869, 1553, 1469, 1368, 1347, 1126, 1087, 1047, 755, 743.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (ppm) 1.01 (s, 9H, H2’ min), 1.02 (s, 9H, H2’ maj), 2.86 (dd, $J$=8.0, 11.3 Hz, 1H, H2 maj), 3.03 (dd, $J$=4.5, 11.5 Hz, 1H, H2 min), 3.09 (dd, $J$=4.5, 232
11.5 Hz, 1H, H2 min), 3.11 (dd, J=6.0, 11.3 Hz, 1H, H2 maj), 3.87 (d, J=7.5 Hz, 1H, H5 maj), 4.02 (d, J=6.0 Hz, 1H, H5 min), 4.71 (app. t, J=7.0 Hz, 1H, H4 maj), 4.74-4.84 (m, 2H, H3 both isomers), 4.88 (dd, J=5.5, 6.0 Hz, 1H, H4 min).

$^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ (ppm) 26.3 (C2' maj), 26.4 (C2' min), 34.1 (q, C1' both isomers), 34.6 (C2 maj), 36.1 (C2 min), 56.8 (C5 min), 57.3 (C5 maj), 76.6 (C3 min), 78.9 (C3 maj), 91.0 (C4 min), 93.5 (C4 maj).

5.4.5.7 5-(3-Benzylxy-phenyl)-4-nitro-tetrahydro-thiophen-3-ol:

\[
\begin{align*}
\text{Prepared as per procedure E using 3-benzyloxy-} & \text{-trans-} \beta \text{-nitrostyrene (0.50 g, 1.96 mmol),} \\
& 2,5\text{-dihydroxy-1,4-dithiane (0.22 g, 1.47 mmol) and triethylamine (55 } \mu \text{L, 0.39 mmol).} \\
& \text{Flash chromatography using a gradient eluant of 10-20\% EtOAc in hexane afforded compound 155 (0.49 g, 1.48 mmol, 76\% yield) as approximately a 1:3.3 mixture of diastereomers. } R_f \text{ maj (30\% EtOAc/Hexane) 0.47; } R_f \text{ min (30\% EtOAc/Hexane) 0.56.}
\end{align*}
\]
IR $\nu_{\text{max}}$ (cm$^{-1}$): 3452, 3062, 3034, 2951, 2863, 1595, 1543, 1492, 1450, 1379, 1277, 1255, 1170, 1072, 1020, 866, 855, 777, 738, 689.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (ppm) 2.73 (s broad, 1H, -OH min), 2.81 (d, $J$=5.8 Hz, 1H, -OH maj), 3.09 (dd, $J$=2.3, 12.0 Hz, 1H, H2 maj), 3.18 (dd, $J$=8.0, 11.0 Hz, 1H, H2 min), 3.32 (dd, $J$=6.5, 11.0 Hz, 1H, H2 min), 3.52 (dd, $J$=4.1, 12.0 Hz, 1H, H2 maj), 4.89-5.03 (m, 3H, H5 min, H3 min & H4 min), 5.00 (dd, $J$=3.5, 9.4 Hz, 1H, H4 maj), 5.04-5.12 (m, 1H, H3 maj), 5.08 (s, 4H, H1' both isomers), 5.23 (d, $J$=9.4 Hz, 1H, H5 maj), 6.94 (dd, $J$=2.3, 8.2 Hz, 1H, H4' maj), 6.95 (dd, $J$=2.0, 8.0 Hz, 1H, H4' min), 7.02 (d, $J$=7.5 Hz, 1H, H6' min), 7.08 (d, $J$=7.6 Hz, 1H, H6' maj), 7.07-7.15 (m, 2H, H2' both isomers), 7.29 (app t, $J$=7.6 Hz, 1H, H5' maj), 7.30 (app t, $J$=8.0 Hz, 1H, H5' min), 7.33-7.50 (m, 10H, H3''-H5'' both isomers).

$^{13}$C NMR (CDCl$_3$, 100 MHz):$\delta$ (ppm) 33.7 (C2 min), 35.7 (C2 maj), 47.9 (C5 maj), 49.3 (C5 min), 69.7 (C1'' maj), 69.8 (C1'' min), 75.0 (C3 maj), 76.6 (C3 min), 95.1 (C4 maj), 97.5 (C4 min), 113.96 (C2' min), 114.22 (C4' maj), 114.54 (C2' maj), 114.57 (C4' min), 119.8 (C6' min), 120.3 (C6' maj), 127.18 (C3'' min), 127.22 (C3'' maj), 127.67 (C5'' min), 127.71 (C5'' maj), 128.2 (C4'' min), 128.3 (C4'' maj), 129.72 (C5' maj), 129.73 (C5' min), 136.1 (q, C2' min), 136.3 (q, C2' maj), 137.9 (q, C1' min), 138.0 (q, C1' maj), 158.79 (q, C3' min), 158.80 (q, C3' maj).

HRMS: (m/z-ES) calcd. for C$_{17}$H$_{17}$NO$_4$NaS (M+Na)$^+$ 354.0776, found 354.0764. 234
5.4.5.8 4-Nitro-5-(2-trifluoromethyl-phenyl)-tetrahydro-thiophen-3-ol:

Prepared as per procedure E using compound 160 (0.43 g, 2.00 mmol), 2,5-dihydroxy-1,4-dithiane (0.23 g, 1.50 mmol) and triethylamine (56 µL, 0.40 mmol).

Flash chromatography, flushing initially with 1:1 CH$_2$Cl$_2$-hexane and then eluting with 20% EtOAc in hexane gave compound 161 (0.44 g, 1.43 mmol, 71% yield). $R_f$ maj (30% EtOAc/Hexane) 0.38; $R_f$ min (30% EtOAc/Hexane) 0.28.

IR $v_{\text{max}}$ (cm$^{-1}$): 3483, 3437, 1577, 1309, 1291, 1153, 1117, 1105, 1035, 988, 761.

$^1$H NMR (CDCl$_3$, 600 MHz); δ (ppm) 3.15 (dd, $J=3.8$, 11.9 Hz, 1H, H2 min), 3.30 (dd, $J=7.9$, 10.9 Hz, 1H, H2 maj), 3.34 (dd, $J=6.8$, 10.9 Hz, 1H, H2 maj), 3.51 (dd, $J=4.5$, 11.9 Hz, 1H, H2 min), 5.06-5.17 (m, 3H, H4 maj, H3 both isomers), 5.20 (dd, $J=4.5$, 8.7 Hz, 1H, H4 min) 5.30 (d, $J=9.0$ Hz, 1H, H5 maj), 5.62 (d, $J=8.7$ Hz, 1H, H5 min), 7.41-7.47 (m, 2H, H7’ both isomers), 7.59-7.71 (m, 4H, H3’ & H5’ both isomers), 7.80 (d, $J=7.9$ Hz, 1H, H6’ min), 7.93 (d, $J=7.9$ Hz, 1H, H6’ maj).

$^{13}$C NMR (CDCl$_3$, 150 MHz); δ (ppm) 34.1 (C2 maj), 35.7 (C2 min), 43.6 (d, $J_{C,F}=1.9$ Hz, C5 min), 44.1 (d, $J_{C,F}=1.9$ Hz, C5 maj), 74.7 (C4 min), 76.8 (C4 maj), 94.2 (C3 min), 97.6 (C3 maj), 123.7 (quartet, $J_{C,F}=274.4$ Hz, C7’ maj), 123.9 (quartet, $J_{C,F}=274.4$ Hz, C7’ min), 125.9 235
(quartet, $J_{C-F}$=5.4 Hz, C3' maj), 126.3 (quartet, $J_{C-F}$=5.4 Hz, C3' min), 128.4 (C4' min), 128.6 (C4' maj), 128.9 (C6' min), 129.0 (quartet, $J_{C-F}$=30.0 Hz, q, C2' maj), 129.3 (quartet, $J_{C-F}$=30.0 Hz, q, C2' min), 129.6 (C6' maj), 132.6 (C5' min), 132.8 (C5' maj), 135.9 (q, C1' min), 136.2 (q, C1' maj).

$^{19}$F NMR (CDCl$_3$, 377 MHz): $\delta$ (ppm) -58.65 (CF$_3$ maj), -58.82 (CF$_3$ min).

HRMS: (m/z - El) calcd. for C$_{11}$H$_{10}$NO$_3$F$_3$S (M) 293.0331, found 293.0333.

Elemental Analysis: Anal. calcd. for C$_{11}$H$_{10}$F$_3$NO$_3$S: C, 45.05; H, 3.44; N, 4.78. Found: C, 45.07; H, 3.47; N, 4.53.

5.4.5.9 5-(2-Methoxy-phenyl)-4-nitro-tetrahydro-thiophen-3-ol:

![Chemical Structure]

Prepared as per procedure E using compound 157 (0.36 g, 2.00 mmol), 2,5-dihydroxy-1,4-dithiane (0.23 g, 1.50 mmol) and triethylamine (56 µL, 0.40 mmol).

Flash chromatography, flushing initially with 1:1 CH$_2$Cl$_2$-hexane and then eluting with 20% EtOAc in hexane gave compound 158 (0.39 g, 1.53 mmol, 77% yield) as a 2:1 mixture of diastereomers. $R_f$ maj (30% EtOAc/Hexane) 0.42; $R_f$ min (30% EtOAc/Hexane) 0.36.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3413, 1542, 1368, 1246, 1044, 1021, 747.

$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 2.84 (d, $J$=6.5 Hz, 1H, -OH maj), 2.91 (d, $J$=7.5 Hz, 1H, -OH min), 3.11 (dd, $J$=3.5, 11.3 Hz, 1H, 236
H2 min), 3.18 (dd, J=7.5, 11.0 Hz, 1H, H2 maj), 3.30 (dd, J=6.0, 11.0 Hz, 1H, H2 maj), 3.46 (dd, J=4.0, 11.3 Hz, 1H, H2 min), 3.86 (s, 3H, H7' maj), 3.89 (s, 3H, H7' mm), 4.16-5.04 (m, 2H, H3 both isomers) 5.14 (dd, J=7.0, 8.0 Hz, 1H, H4 maj), 5.28-5.34 (m, 2H, H5 maj & H4 min), 5.49 (d, J=7.5 Hz, 1H, H5 min), 6.90-6.95 (m, 2H, H3' both isomers), 6.96-7.02 (m, 2H, H5' both isomers), 7.30-7.36 (m, 2H, H4' both isomers), 7.46 (dd, J=1.5, 7.5 Hz, 1H, H6' min), 7.49 (dd, J=1.5, 7.5 Hz, 1H, H6' maj).

13C NMR (CDCl3, 150 MHz): δ (ppm) 35.3 (C2 maj), 36.5 (C2 min), 44.6 (C5 min), 45.3 (C5 maj), 55.55 (C7' maj), 55.62 (C7' min), 75.6 (C3 min), 78.0 (C3 maj), 93.4 (C4 min), 96.2 (C4 maj), 111.1 (C3' maj), 111.2 (C3' min), 120.88 (C5' min), 120.98 (C5' maj), 125.32 (q, C1' min), 125.89 (q, C1' maj), 128.5 (C6' maj), 129.0 (C6' min), 129.69 (C4' min), 129.84 (C4' maj), 157.2 (q, C2' maj), 157.3 (q, C2' min).

HRMS: (m/z - ES) calcd. for C_{11}H_{12}NO_{3}S (M-H) 254.0487, found 254.0499.

5.4.5.10 4-Nitro-5-(3-nitro-phenyl)-tetrahydro-thiophen-3-ol:
Prepared as per procedure E using compound 150 (0.39 g, 2.00 mmol), 2,5-dihydroxy-1,4-dithiane (0.23 g, 1.50 mmol) and triethylamine (56 µL, 0.40 mmol).

Flash chromatography, flushing initially with 1:1 CH₂Cl₂-hexane and then eluting with 20% EtOAc in hexane gave compound 151 (0.43 g, 1.61 mmol, 80% yield) as approximately a 1.25:1 mixture of diastereomers. \( R_f \) maj (30% EtOAc/Hexane) 0.34; \( R_f \) min (30% EtOAc/Hexane) 0.28.

IR \( \nu \text{ max} \) (cm\(^{-1}\)): 3502, 2925, 1550, 1525, 1351, 1084, 1043, 728, 681.

\( ^1H \) NMR (CDCl₃, 400 MHz): \( \delta \) (ppm) 2.76 (d, \( J=4.5 \) Hz, 2H, -OH both isomers), 3.14 (dd, \( J=1.9 \), 12.2 Hz, 1H, H₂ min), 3.27 (dd, \( J=8.3 \), 10.9 Hz, 1H, H₂ maj), 3.39 (dd, \( J=6.8 \), 10.9 Hz, 1H, H₂ maj), 3.62 (dd, \( J=4.5 \), 12.2 Hz, 1H, H₂ min), 4.96 (dd, \( J=7.9 \), 9.0 Hz, 1H, H₄ maj), 5.00-5.13 (m, 3H, H₄ min, H₃ maj & H₅ maj), 5.15-5.23 (m, 1H, H₃ min), 5.37 (d, \( J=10.2 \) Hz, 1H, H₅ min), 7.58 (dd, \( J=7.5 \), 15.4 Hz, 2H, H₅' both isomers), 7.76 (d, \( J=7.5 \) Hz, 1H, H₆' maj), 7.83 (d, \( J=7.5 \) Hz, 1H, H₆' min), 8.18-8.25 (m, 2H, H₄' both isomers), 8.37-8.41 (m, 1H, H₂' maj), 8.43-8.46 (m, 1H, H₂' min).

\( ^1C \) NMR (CDCl₃, 100 MHz): \( \delta \) (ppm) 33.9 (C₂ maj), 36.0 (C₂ min), 47.7 (C₅ min), 48.5 (C₅ maj), 75.3 (C₃ maj), 76.7 (C₃ min), 95.2 (C₄ min), 97.3 (C₄ maj), 123.0 (C₂' maj), 123.3 (C₂' min) 123.7 (C₄' min), 123.9 (C₄' maj), 130.0 (C₅' min), 130.2 (C₅' maj), 134.1 (C₆' maj), 135.0 (C₆' min), 139.2 (q, C₁' min), 139.5 (q, C₁' maj), 148.64 (q, C₃' maj), 148.68 (q, C₃' min).

HRMS: \( m/z - \text{ES} \) calcd. for C₁₀H₉N₂O₅S (M-H)\(^-\) 269.0232, found 269.0244.
5.4.5.11 5'-Bromo-3-nitro-2,3,4,5-tetrahydro-[2,2']bithiophenyl-4-ol:

Prepared as per procedure E using compound 168 (0.47 g, 2.00 mmol), 2,5-dihydroxy-1,4-dithiane (0.23 g, 1.5 mmol) and triethylamine (56 µL, 0.40 mmol). Flash chromatography, flushing initially with 1:1 CH$_2$Cl$_2$-hexane and then using a gradient eluant of 10-20% EtOAc in hexane gave compound 169 (0.21 g, 0.68 mmol, 34% yield) as approximately a 1.4:1 mixture of diastereomers: $R_f$ maj (20% EtOAc/Hexane) 0.39; $R_f$ min (20% EtOAc/Hexane) 0.33.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3430, 2927, 1548, 1438, 1370, 1043, 966, 797, 751.

$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 2.72 (d, $J=5.1$ Hz, 1H, -OH maj), 2.74 (d, $J=5.1$ Hz, 1H, -OH min), 3.08 (dd, $J=1.8$, 11.7 Hz, 1H, H5 min), 3.20 (dd, $J=8.4$, 10.6 Hz, 1H, H5 maj), 3.30 (dd, $J=7.0$, 10.6 Hz, 1H, H5 maj), 3.53 (dd, $J=4.0$, 11.7 Hz, 1H, H5 min), 4.82-5.01 (m, 3H, H3 both isomers & H4 maj), 5.02-5.12 (m, 1H, H4 min), 5.17 (d, $J=9.2$ Hz, 1H, H2 maj), 5.49 (d, $J=9.5$ Hz, 1H, H2 min), 6.82 (d, $J=3.7$ Hz, 1H, H3' maj), 6.89-6.94 (m, 3H, H3' min & H4' both isomers).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 34.0 (C5 maj), 35.9 (C5 min), 44.1 (C2 min), 44.9 (C2 maj), 75.3 (C4 min), 76.8 (C4 maj), 95.8 (C3 min), 98.0 (C3 maj), 113.0 (q, C5' min), 113.4 (q, C5' maj), 127.2 (C3' maj), 127.7 (C3' min), 130.0
5.4.5.12 5-(5-Iodo-furan-2-yl)-4-nitro-tetrahydro-thiophen-3-ol:

Prepared as per procedure E using compound 165 (0.30 g, 1.13 mmol), 2,5-dihydroxy-1,4-dithiane (0.13 g, 0.85 mmol) and triethylamine (31 μL, 0.23 mmol).

Flash chromatography, flushing initially with 1:1 CH₂Cl₂-hexane and then using a gradient eluant of 10-20% EtOAc in hexane gave compound 166 (0.23 g, 0.67 mmol, 59% yield) as approximately a 1.1:1 mixture of diastereomers. $R_f$ maj (20% EtOAc/Hexane) 0.29; $R_f$ min (20% EtOAc/Hexane) 0.25.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3430, 2937, 1549, 1482, 1370, 1096, 1042, 1012, 788, 746.

$^1$H NMR (CDCl₃, 600 MHz): δ (ppm) 2.80 (d, $J$=6.0 Hz, 1H, -OH min), 2.84 (d, $J$=6.4 Hz, 1H, -OH maj), 3.07 (dd, $J$=1.1, 12.1 Hz, 1H, H2 min), 3.21 (dd, $J$=7.9, 10.9 Hz, 1H, H2 maj), 3.271 (dd, $J$=6.0, 10.9 Hz, 1H, H2 maj), 3.50 (dd, $J$=3.4, 12.1 Hz, 1H, H2 min), 4.95-5.02 (m, 1H, H3 maj), 5.03-5.15 (m, 3H, H5 maj, H3 min & H4 min), 5.24 (dd, $J$=3.8, 8.7 Hz, H4 maj), 5.32 (d, $J$=8.7 Hz, 1H, H5 min), 6.26-6.32 (m, 2H, H3' both isomers), 6.48-6.53 (m, 2H, H4' both isomers).
\[^{13}\text{C} \text{NMR (CDCl}_3, 150 \text{ MHz): } \delta \text{ (ppm)}\] 34.6 (C2 maj), 35.7 (C2 min), 41.0 (C5 min), 42.0 (C5 maj), 75.0 (C3 min), 77.0 (C3 maj), 88.3 (q, C5’ min), 85.8 (q, C5’ maj), 91.8 (C4 maj), 94.2 (C4 min), 111.5 (C3’ maj), 112.0 (C3’ min), 121.24 (C4’ maj), 121.29 (C4’ min), 154.6 (q, C2’ min), 154.9 (q, C2’ maj).

HRMS: \((m/z - \text{ES}) \text{ calcd. for C}_8\text{H}_7\text{NO}_4\text{SI (M-H)}\) 339.9141, found 339.9148.

5.4.5.13 4-Nitro-5-(4-nitro-phenyl)-tetrahydro-thiophen-3-ol:

![Chemical structure](image)

Prepared as per procedure E using compound 145 (0.39 g, 2.00 mmol), 2,5-dihydroxy-1,4-dithiane (0.23 g, 1.50 mmol) and triethylamine (56 \(\mu\)L, 0.40 mmol).

Flash chromatography, flushing initially with 1:1 CH\(_2\)Cl\(_2\)-hexane and then using a gradient eluant of 20-40% EtOAc in hexane gave compound 146 (0.40 g, 1.49 mmol, 74% yield) as approximately a 1:1 mixture of diastereomers. \(R_f\) a (20% EtOAc/Hexane) 0.25; \(R_f\) b (20% EtOAc/Hexane) 0.16.

IR \(v_{max} \text{ (cm}^{-1}\)):\n
- 3444, 1607, 1546, 1513, 1348, 1316, 1048, 862, 728, 693.

\[^1\text{H} \text{NMR (CDCl}_3, 600 \text{ MHz): } \delta \text{ (ppm)}\] 3.15 (dd, \(J=1.9, 12.0 \text{ Hz, 1H, H2a})\), 3.26 (dd, \(J=8.3, 11.1 \text{ Hz, 1H, H2b})\), 3.39 (dd, \(J=6.8, 11.1 \text{ Hz, 1H, H2b})\), 3.60 (dd, \(J=4.1, 12.0 \text{ Hz, 1H, H2a})\), 4.94 (dd, \(J=7.9, 9.4 \text{ Hz, 1H, H4b})\), 5.01 (dd, \(J=3.6, 10.2 \text{ Hz, 1H, H4a})\), 5.03 (d, \(J=9.4 \text{ Hz, 1H, H4a})\), 5.03 (d, \(J=9.4 \text{ Hz, 1H, H4a})\).
H5b), 5.08 (ddd, J=6.8, 7.9, 15.1 Hz, 1H, H3b), 5.17-5.20 (m, 1H, H3a), 5.36 (d, J=10.2 Hz, 1H, H5a), 7.65 (d, J=8.3 Hz, 2H, H2'b), 7.71 (d, J=8.3 Hz, 2H, H2'a), 8.23-8.30 (m, 4H, H3' both isomers).

$^{13}$C NMR (CDCl$_3$, 150 MHz): δ (ppm) 34.1 (C2b), 36.1 (C2a), 47.4 (C5a), 48.6 (C5b), 75.3 (C3a), 76.8 (C3b), 95.1 (C4a), 97.2 (C4b), 124.1 (C3'a), 124.3 (C3'b), 128.9 (C2'b), 129.4 (C2'a), 144.2 (q, C1'a), 144.4 (q, C1'b), 147.97 (q, C4'a), 148.13 (C4'b).

HRMS: (m/z – ES) calcd. for C$_{10}$H$_9$N$_2$O$_5$S (M-H)$^-$ 269.0232, found 269.0245.

5.4.5.14 4-Nitro-5-pyridin-3-yl-tetrahydro-thiophen-3-ol:

Prepared as per procedure E using compound 175 (0.15 g, 1.00 mmol), 2,5-dihydroxy-1,4-dithiane (0.11 g, 0.75 mmol) and triethylamine (28 µL, 0.20 mmol).

Flash chromatography using a gradient eluant of 20-50% EtOAc in CH$_2$Cl$_2$ gave compound 176 (74 mg, 0.33 mmol, 33% yield) as approximately a 1.8:1 mixture of diastereomers. $R_f$ maj (20% EtOAc/Hexane) 0.35; $R_f$ min (20% EtOAc/Hexane) 0.43.

IR $\nu_{\max }$ (cm$^{-1}$): 3041, 2852, 2746, 1542, 1432, 1270, 1323, 1051, 826, 739, 709.

$^1$H NMR (CDCl$_3$, 600 MHz): δ (ppm) 2.87 (dd, J=1.5, 11.3 Hz, 1H, H2 maj), 3.15 (dd, J=9.0, 10.4 Hz, 1H, H2 min), 3.22 (dd, J=7.5, 242
10.4 Hz, H2 min), 3.57 (dd, J=4.1, 11.3 Hz, 1H, H2 maj), 4.79-4.86 (m, 1H, H3 min), 4.97 (d, J=10.2 Hz, 1H, H5 min), 4.98-5.01 (m, 1H, H3 maj), 5.19 (d, J=10.5 Hz, 1H, H5 maj), 5.30 (dd, J=9.0, 10.2 Hz, 1H, H4 min), 5.72 (dd, J=3.8, 10.2 Hz, 1H, H4 maj), 6.26 (d, J=4.5 Hz, 1H, -OH maj), 6.33 (d, J=6.0 Hz, 1H, -OH min), 7.39 (dd, J=4.9 Hz, 7.9 Hz, 1H, H5’ maj), 7.42 (dd, J=4.5, 7.9 Hz, 1H, H5’ min), 7.99-8.05 (m, 2H, H4’ both isomers), 8.49-8.51 (m, 1H, H6’ maj), 8.52-8.54 (m, 1H, H6’ min), 8.64 (d, J=2.3 Hz, 1H, H2’ min), 8.72 (d, J=2.3 Hz, 1H, H2’ maj).

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 32.8 (C2 min), 36.1 (C2 maj), 44.3 (C5 maj), 45.2 (C5 min), 74.7 (C3 maj), 76.2 (C3 min), 93.6 (C4 maj), 96.5 (C4 min), 123.6 (C5’ maj), 123.8 (C5’ min), 133.57 (q, C3’ min), 133.61 (q, C3’ maj), 135.7 (C4’ min), 135.9 (C4’ maj), 149.1 (C6’ maj), 149.2 (C6’ min), 149.7 (C2’ min), 149.9 (C2’ maj).

HRMS: (m/z - ES) calcd. for C$_9$H$_9$N$_2$O$_3$S (M-H)$^-$ 225.0334, found 225.0323.

5.4.5.15 5-(1-Methyl-1H-indol-3-yl)-4-nitro-tetrahydro-thiophen-3-ol:
Prepared as per procedure E using compound 170 (0.20 g, 1.00 mmol), 2,5-dihydroxy-1,4-dithiane (0.11 g, 0.75 mmol) and triethylamine (28 µL, 0.20 mmol).

Flash chromatography eluting from 50% hexane in CH₂Cl₂ to 100% CH₂Cl₂ gave compound 171 (0.20 g, 0.73 mmol, 73% yield) as approximately a 2.5:1 mixture of diastereomers: \( R_f \text{ maj} (\text{CH}_2\text{Cl}_2) 0.78; \ R_f \text{ min} (\text{CH}_2\text{Cl}_2) 0.58. \)

IR \( \nu_{\text{max}} \ (\text{cm}^{-1}): \ 3500, 2938, 1540, 1476, 1372, 1335, 1084, 1064, 740. \)

\( ^1\text{H NMR (CDCl}_3, 600 \text{ MHz): } \delta \ (\text{ppm}) \)

\[
\begin{align*}
2.66 \times (d, \ J=6.0 \text{ Hz, } 1\text{H, }-\text{OH maj}), \\
2.84 \times (d, \ J=6.4 \text{ Hz, } 1\text{H, }-\text{OH min}), \\
3.16 \times (dd, \ J=3.0, 12.0 \text{ Hz, } 1\text{H, H2 maj}), \\
3.23 \times (dd, \ J=7.5, 11.2 \text{ Hz, } 1\text{H, H2 maj}), \\
3.39 \times (dd, \ J=6.8, 11.2 \text{ Hz, } 1\text{H, H2 maj}), \\
3.57 \times (dd, \ J=4.5, 12.0 \text{ Hz, } 1\text{H, H2 min}), \\
3.78 \times (s, 6\text{H, H10'- both isomers}), \\
5.03-5.10 \times (m, 2\text{H, H3 both isomers}), \\
5.25 \times (dd, \ J=7.5, 9.2 \text{ Hz, } 1\text{H, H4 maj}), \\
5.28 \times (dd, \ J=3.8, 9.0 \text{ Hz, } 1\text{H, H4 min}), \\
5.32 \times (d, \ J=9.2 \text{ Hz, } 1\text{H, H5 maj}), \\
5.59 \times (d, \ J=9.0 \text{ Hz, } 1\text{H, H5 min}), \\
7.15 \times (s, 1\text{H, H2'- maj}), \\
7.17 \times (s, 1\text{H, H2'- min}), \\
7.18-7.22 \times (m, 2\text{H, H6'- both isomers}), \\
7.27-7.32 \times (m, 2\text{H, H7'- maj }\&\ H8'- min), \\
7.32-7.36 \times (m, 2\text{H, H7'- min }\&\ H8'- maj), \\
7.76 \times (d, \ J=7.9 \text{ Hz, } 1\text{H, H5'- maj}), \\
7.78 \times (d, \ J=7.9 \text{ Hz, } 1\text{H, H5'- min}).
\end{align*}
\]
13C NMR (CDCl₃, 150 MHz): δ (ppm) 32.67 (C10’ min), 32.72 (C10’ maj), 34.3 (C2 maj), 35.7 (C2 min), 41.8 (C5 min), 42.9 (C5 maj), 75.0 (C3 min), 77.1 (C3 maj), 93.7 (C4 min), 96.7 (C4 maj), 109.3 (q, C4’ min), 109.62 (q, C4’ maj), 109.66 (C8’ maj), 109.68 (C8’ min), 118.99 (C5’ maj), 119.07 (C5’ min), 119.5 (C6’ min), 119.6 (C6’ maj), 122.2 (C7’ min), 122.3 (C7’ maj), 125.76 (q, C3’ maj), 125.82 (q, C3’ min), 127.5 (C2’ maj), 128.1 (C2’ min), 137.48 (q, C9’ maj), 137.51 (q, C9’ min).

HRMS: (m/z – ES) calcd. for C₁₃H₁₃N₂O₃S (M-H)⁻ 277.0647, found 277.0656.

5.4.5.16 5-(1-Methyl-1H-pyrrol-2-yl)-4-nitro-tetrahydro-thiophen-3-ol:

![Chemical Structure](image)

Prepared as per procedure E using compound 172 (0.30 g, 2.00 mmol), 2,5-dihydroxy-1,4-dithiane (0.23 g, 1.50 mmol) and triethylamine (56 µL, 0.40 mmol).

Flash chromatography flushing initially with 1:1 hexane-CH₂Cl₂ and then using a gradient eluant of 20-40% EtOAc in hexane gave compound 173 (0.30 g, 1.33 mmol, 67% yield) as approximately a 2.5:1 mixture of diastereomers. \( R_f \) maj (40% EtOAc/Hexane) 0.62; \( R_f \) min (40% EtOAc/Hexane) 0.56.

IR \( \nu_{\text{max}} \) (cm⁻¹): 3482, 3479, 1543, 1493, 1373, 1066, 1041, 739, 703.

245
$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 2.73 (d, $J=6.3$ Hz, 1H, -OH maj), 2.73 (d, $J=5.7$ Hz, 1H, -OH min), 3.09 (dd, $J=2.6$, 11.9 Hz, 1H, H2 min), 3.16 (dd, $J=7.5$, 11.4 Hz, 1H, H2 maj), 3.33 (dd, $J=6.7$, 11.4 Hz, 1H, H2 maj), 3.51 (dd, $J=4.3$, 11.9 Hz, 1H, H2 min), 3.622 (s, 3H, H6' maj), 3.687 (s, 3H, H6' min), 4.95-5.01 (m, 1H, H3 maj), 5.07-5.10 (m, 1H, H3 min), 5.11 (d, $J=8.8$ Hz, 1H, H5 maj), 5.17 (dd, $J=6.9$, 8.8 Hz, H4 maj), 5.19 (dd, $J=3.8$, 9.2 Hz, 1H, H4 min), 5.35 (d, $J=9.2$ Hz, 1H, H5 min), 6.10-6.12 (m, 2H, H4' both isomers), 6.20 (ddd, $J=0.9$, 1.9, 3.8 Hz, 1H, H3' min), 6.28 (dd, $J=1.9$, 3.8 Hz, 1H, H3' maj), 6.601-6.621 (m, 2H, H5' both isomers).

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 33.91 (C6' maj), 33.98 (C6' min), 34.7 (C2 maj), 35.9 (C2 min), 40.0 (C5 min), 41.5 (C5 maj), 74.7 (C3 min), 77.3 (C3 maj), 93.6 (C4 min), 96.0 (C4 maj), 106.6 (C3' min), 107.38 (C3' maj), 107.7 (C4' min), 107.76 (C4' maj), 123.7 (C5' min), 124.1 (C5' maj), 127.6 (q, C2' maj), 127.7 (q, C2' min).

HRMS: (m/z – ES) calcd. for C$_9$H$_{11}$N$_2$O$_3$S (M-H)' 227.0490, found 227.0495.
5.4.5.17 3-Nitro-2-[4-Nitro-5-phenyl-tetrahydrothiophen-3-ol-yl]-tetrahydrothiophen-3-ol:

![Structural formula](image)

Prepared as per procedure E using compound 177 (0.22 g, 1.00 mmol), 2,5-dihydroxy-1,4-dithiane (0.23 g, 1.5 mmol) and triethylamine (56 µL, 0.40 mmol).

Flash chromatography using a gradient eluant of 20-50% EtOAc in hexane gave compound 178 (0.29 g, 0.77 mmol, 77% yield) as a mixture of diastereomers (at least 8). \( R_f \) all isomers (1:1 EtOAc-Hexane) 0.56.

IR \( \nu_{	ext{max}} \) (cm\(^{-1}\)): 3390, 2939, 1542, 1369, 1077, 1040, 846, 744, 662.

\(^1\)H NMR (CDCl\(_3\), 600 MHz): \( \delta \) (ppm) 2.84 (dd, \( J=1.7 \), 11.0 Hz, 1H, H2 min), 3.07-3.13 (m, 1H, H2 maj), 3.17-3.23 (m, 1H, H2 maj), 3.54 (dd, \( J=3.7 \), 11.0 Hz, 1H, H2 min), 4.75-4.82 (m, 1H, H3 maj), 4.87-4.92 (m, 1H, H5 maj), 4.92-4.97 (m, 1H, H3 min), 5.10-5.24 (m, 2H, H5 min & H4 maj), 5.56-5.56 (m, 1H, H4 min), 6.18-6.23 (m, 1H, -OH min), 6.27-6.30 (m, 1H, -OH maj), 7.43-7.54 (m, 4H, H2' both isomers).

\(^13\)C NMR (CDCl\(_3\), 150 MHz): \( \delta \) (ppm) 32.6 (C2 maj), 36.0 (C2 min), 46.65-47.54 (C5 both isomers), 74.8 (C3 min), 76.3 (C3 maj), 93.76-93.85 (C4 min), 96.87-96.96 (C4 maj),

247
128.07-128.87 (C2' all isomers), 137.03-137.9
(C1' all isomers).

HRMS: \( m/z \) – ES calcd. for \( C_{14}H_{15}N_{2}O_{6}S_{2} \) \( (M-H)^{-} \) 371.0372, found 371.0375.

5.4.6 Synthesis of Tetrahydrothiophenes, Procedure F:

To a stirred solution of the appropriate nitroacetate (2.16 mmol) in \( CH_2Cl_2 \) (6.0 mL) were added 2,5-dihydroxy-1,4-dithiane (0.17 g, 1.08 mmol) and triethylamine (0.33 mL, 2.38 mmol) under an argon atmosphere. Stirring was continued at room temperature overnight. The reaction was diluted with \( CH_2Cl_2 \) (20 mL) and the suspended solid was removed by filtration. Water (20 mL) was added to the filtrate and the aqueous layer was extracted with \( CH_2Cl_2 \) (2 x 20 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL) and then dried over magnesium sulfate. Volatiles were removed under reduced pressure and the resulting residue was purified by column chromatography.

5.4.6.1 5-Heptyl-4-nitro-tetrahydro-thiophen-3-ol:

Prepared as per procedure F using compound 185b (0.50 g, 2.16 mmol), 2,5-dihydroxy-1,4-dithiane (0.17 g, 1.08 mmol) and triethylamine (0.33 mL, 2.38 mmol).

Flash chromatography eluting with 10% EtOAc in hexane gave compound 187 (0.38 g, 1.52 mmol, 71% yield) as approximately a 2.5:1 mixture of diastereomers. \( R_f \) maj (15% EtOAc/Hexane) 0.38; \( R_f \) min (15% EtOAc/Hexane) 0.31.

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 3419, 2924, 2854, 1547, 1369, 1080, 723.

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$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 0.90 (t, $J=7.2$ Hz, 3H, H7’ maj), 0.91 (t, $J=7.2$ Hz, 3H, H7’ min), 1.24-1.45 (m, 20H, H2’-H6’ both isomers), 1.55-1.70 (m, 2H, H1’ both isomers), 1.89-1.97 (m, 2H, H1’ both isomers), 2.60 (d, $J=6.0$ Hz, 1H, -OH maj), 2.77 (d, $J=6.4$ Hz, 1H, -OH min), 2.95 (dd, $J=7.9$, 11.0 Hz, 1H, H2 maj), 2.99 (dd, $J=3.0$, 11.9 Hz, 1H, H2 min), 3.18 (dd, $J=6.4$, 11.0 Hz, 1H, H2 maj), 3.23 (dd, $J=4.1$, 11.9 Hz, 1H, H2 min), 3.75-3.81 (m, 1H, H5 maj), 4.04-4.10 (m, 1H, H5 min), 4.59 (dd, $J=7.5$, 8.3 Hz, 1H, H4 maj), 4.63 (dd, $J=3.8$, 8.7 Hz, 1H, H4 min), 4.87 (m, 2H, H3 both isomers).

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 13.9 (C7’ both isomers), 22.4 (C6’ both isomers), 27.3 (C2’ maj), 28.2 (C2’ min), 28.82 (C4’ maj), 28.84 (C4’ min), 28.92 (C3’ maj), 29.0 (C3’ min), 31.51 (C5’ maj), 31.52 (C5’ min), 33.4 (C2 maj), 34.7 (C1’ min), 34.9 (C2 min), 35.3 (C1’ maj), 45.2 (C5 min), 46.6 (C5 maj), 75.3 (C3 min), 77.0 (C3 maj), 93.8 (C4 min), 96.4 (C4 maj).

5.4.6.2 5-Isopropyl-4-nitro-tetrahydro-thiophen-3-ol:

Prepared as per procedure F using compound 182a (0.45 g, 2.56 mmol), 2,5-dihydroxy-1,4-dithiane (0.20 g, 1.28 mmol) and triethylamine (0.39 mL, 2.82 mmol).
Flash chromatography eluting with 10% EtOAc in hexane gave compound 184 (0.29 g, 1.52 mmol, 60% yield) as approximately a 2.8:1 mixture of diastereomers. $R_f$ maj (15% EtOAc/Hexane) 0.33; $R_f$ min (15% EtOAc/Hexane) 0.42.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3411, 2963, 2873, 1547, 1369, 1075, 1041, 755, 677.

$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 1.02 (d, $J$=6.8 Hz, 3H, H7 maj), 1.03 (d, $J$=6.4 Hz, 3H, H7 min), 1.05 (d, $J$=6.4 Hz, 3H, H7' min), 1.06 (d, $J$=6.8 Hz, 3H, H7' maj), 2.01 (octet, $J$=6.4 Hz, 1H, H6 min), 2.02 (octet, $J$=6.8 Hz, 1H, H6 maj), 2.62 (d, $J$=6.4 Hz, 1H, -OH maj), 2.77 (d, $J$=7.9 Hz, 1H, -OH min), 2.90 (dd, $J$=7.9, 11.3 Hz, 1H, H2 maj), 3.12 (dd, $J$=4.0, 12.1 Hz, 1H, H2 min), 3.15 (dd, $J$=6.0, 11.3 Hz, 1H, H2 maj), 3.15 (dd, $J$=4.1, 11.7 Hz, 1H, H2 min), 3.79 (dd, $J$=6.0, 8.1 Hz, 1H, H5 maj), 4.05 (dd, $J$=6.0, 7.7 Hz, 1H, H5 min), 4.69 (dd, $J$=7.2, 8.1 Hz, 1H, H4 maj), 4.79 (dd, $J$=4.5, 7.7 Hz, 1H, H4 min), 4.84-4.92 (m, 2H, H3 both isomers).

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 18.5 (C7 maj), 18.6 (C7 min), 20.4 (C7' maj), 21.0 (C7' min), 31.7 (C6 min), 32.0 (C6 maj), 34.1 (C2 maj), 35.5 (C2 min), 52.3 (C5 min), 53.5 (C5 maj), 72.6 (C3 min), 77.8 (C3 maj), 92.0 (C4 min), 94.5 (C4 maj).

HRMS: ($m/z$ – El) calcd. for C$_7$H$_{13}$NO$_3$S (M) 191.0616, found 191.0618.
5.4.7 Synthesis of Nitrothiophenes Using Conventional Heating, Procedure G:

The desired DHT was prepared as per procedure B using the appropriate THT (0.76 mmol), trifluoroacetic anhydride (115 \( \mu \)L, 0.83 mmol) and triethylamine (230 \( \mu \)L, 1.66 mmol). The product was not further purified.

The crude product was then dissolved in CH\(_2\)Cl\(_2\) (6 mL) and dichlorodicyanobenzoquinone (0.35 g, 1.52 mmol) was introduced. The reaction was heated at reflux overnight. The reaction was diluted with EtOAc (50 mL) and washed with 10% sodium thiosulfate solution (2 x 50 mL). The aqueous layers were then extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with saturated bicarbonate solution (50 mL) and brine (50 mL) before drying over magnesium sulfate. Volatiles were removed under reduced pressure and the resulting residue was purified by column chromatography.

5.4.7.1 3-Nitro-2-phenyl-thiophene:
Prepared as per procedure G** using compound 137 (0.41 g, 1.82 mmol), trifluoroacetic anhydride (0.28 mL, 2.00 mmol), triethylamine (0.56 mL, 4.00 mmol) and dichlorodicyanobenzoquinone (0.83 g, 3.64 mmol).

Flash chromatography on neutral alumina eluting with 2% EtOAc in hexane afforded compound 138 (0.22 g, 1.09 mmol, 60% yield) as a yellow solid. M.p. 100-103 °C; lit.234 101.5-102.5 °C. $R_f$ (10% EtOAc/hexane) 0.33.

IR $\nu_{max}$ (cm$^{-1}$):  2926, 1546, 1506, 1372, 1331, 1261, 752.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm)  7.30 (d, $J=5.5$ Hz, 1H, H5), 7.45-7.54 (m, 5H, H$_2'$-H$_4'$), 7.68 (d, $J=5.5$ Hz, 1H, H4).

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm)  123.6 (C5), 124.5 (C4), 128.0 (C3'), 129.15 (C4'), 129.19 (C2'), 130.1 (q, Cl'), 142.5 (q, C3), 145.1 (q, C2).

** Also prepared as per procedure H, see below.

5.4.7.2 2-(3-Nitro-thiophen-2-yl)-furan

Prepared as per procedure G using compound 163 (0.13 g, 0.59 mmol), trifluoroacetic anhydride (90 µL, 0.65 mmol), triethylamine (185 µL, 1.30 mmol) and dichlorodicyanobenzoquinone (0.27 g, 1.18 mmol).

Flash chromatography eluting with 2% EtOAc in hexane afforded compound 197 (38 mg, 0.19 mmol, 33% yield) as a yellow oil. $R_f$ (5% EtOAc/hexane) 0.43.

IR $\nu_{max}$ (cm$^{-1}$):  3121, 2924, 2854, 1525, 1497, 1371, 1326, 1180, 1027, 838, 746, 709.

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$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 6.63 (dd, $J=1.9$, 3.8 Hz, 1H, H4'), 7.23 (d, $J=5.7$ Hz, 1H, H5), 7.55-7.58 (m, 2H, H3' & H5'), 7.66 (d, $J=5.7$ Hz, 1H, H4).

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 112.9 (C4'), 114.9 (C3'), 123.0 (C5), 125.3 (C4), 135.2 (q, C2), 140.7 (q, C3), 144.2 (C5'), 145.3 (q, C2').

HRMS: ($m/z$ – El) calcd. for C$_8$H$_5$NO$_3$S (M) 194.990, found 194.993.

5.4.7.3 2-(4-Benzyloxy-phenyl)-3-nitro-thiophene

Prepared as per procedure G using compound 148 (0.14 g, 0.42 mmol), trifluoroacetic anhydride (64 µL, 0.46 mmol), triethylamine (129 µL, 0.92 mmol) and dichlorodicyanobenzoquinone (0.19 g, 0.84 mmol).

Flash chromatography on silica eluting with 4% EtOAc in hexane afforded compound 194 (58 mg, 0.19 mmol, 44% yield) as a yellow oil. $R_f$(10% EtOAc/hexane) 0.26.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3119, 3100, 2924, 2872, 1603, 1507, 1435, 1374, 1364, 1318, 1239, 1177, 1018, 832, 747, 721, 699.

$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 5.14 (s, 2H, H1''), 7.03-7.09 (m, 2H, H3''), 7.24 (d, $J=5.5$ Hz, 1H, H5), 7.34-7.50 (m, 7H, H2'', H3'', H4'' & H5''), 7.66 (d, $J=5.5$ Hz, 1H, H4).
$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 69.7 (C1'''), 114.3 (C3'), 122.4 (q, C1'), 122.9 (C5), 124.7 (C4), 127.1 (C3''), 127.7 (C5''), 128.2 (C4''), 130.7 (C2''), 136.0 (q, C2''), 142.1 (q, C3), 145.3 (q, C2), 159.5 (q, C4').

HRMS: (m/z – El) calcd. for C$_{17}$H$_{13}$NO$_3$S (M) 311.0616, found 311.0610.

5.4.7.4 2-(4-Bromo-phenyl)-3-nitro-thiophene:

Prepared as per procedure G** using compound 153 (0.14 g, 0.49 mmol), trifluoroacetic anhydride (75 µL, 0.54 mmol), triethylamine (152 µL, 1.09 mmol) and dichlorodicyanobenzoquinone (0.22 g, 0.98 mmol).

Flash chromatography on silica eluting with 4% EtOAc in hexane afforded compound 195 (39 mg, 0.14 mmol, 30% yield) as a yellow solid: M.p. 88-90 °C. $R_f$ (10% EtOAc/hexane) 0.42.

IR $\nu_{max}$ (cm$^{-1}$): 3132, 3112, 2923, 2852, 1584, 1543, 1497, 1482, 1367, 1331, 1069, 1008, 838, 814, 782, 699.

$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 7.32 (d, $J$=5.7 Hz, 1H, H5), 7.36-7.42 (m, 2H, H2'), 7.57-7.64 (m, 2H, H3'), 7.69 (d, $J$=5.7 Hz, 1H, H4).

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 124.1 (q, C4'), 124.3 (C5), 125.1 (C4), 129.4 (q, C1'), 131.2 (C2'), 131.7 (C3'), 143.1 (q, C3), 143.9 (q, C2).
HRMS: \((m/z - \text{El})\) calcd. for \(\text{C}_{10}\text{H}_{6}\text{NO}_{2}\text{SBr} (\text{M})\ 282.9303\), found 282.9297.

**Also prepared as per procedure H, see below.

5.4.7.5 2-Isobutyl-3-nitro-thiophene:

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\begin{array}{c}
\text{O}_2\text{N} \\
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\text{S} \\
\end{array}
\]

Prepared as per procedure G using compound 181 (0.75 g, 3.63 mmol), trifluoroacetic anhydride (0.56 mL, 4.00 mmol), triethylamine (1.12 mL, 8.00 mmol) and dichlorodicyanobenzoquinone (1.65 g, 7.26 mmol).

Flash chromatography on silica eluting with 5% EtOAc in hexane afforded compound 198 (0.20 g, 1.05 mmol, 29% yield) as a yellow oil. \(R_f(10\% \text{EtOAc/hexane})\ 0.63\).

IR \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3122, 3105, 2959, 1539, 1500, 1374, 1327, 1182, 841, 703.

\(^1\)H NMR (CDCl\(_3\), 600 MHz): \(\delta\) (ppm) 0.97 (d, \(J=6.5\ Hz\), 6H, H3'), 1.94-2.09 (m, 1H, H2'), 3.07 (d, \(J=7.0\ Hz\), 2H, H1'), 7.06 (d, \(J=5.8\ Hz\), 1H, H5), 7.55 (d, \(J=5.8\ Hz\), 1H, H4).

\(^{13}\)C NMR (CDCl\(_3\), 150 MHz): \(\delta\) (ppm) 21.9 (C3'), 29.5 (C2'), 37.3 (C1'), 121.1 (C5), 124.1 (C4), 143.7 (q, C3), 148.0 (q, C2).

HRMS: \((m/z - \text{El})\) calcd. for \(\text{C}_{8}\text{H}_{11}\text{NO}_2\text{S} (\text{M})\ 185.0511\), found 185.01512.

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5.4.7.6 2-Heptyl-3-nitro-thiophene:

Prepared as per procedure G** using compound 187 (0.19 g, 0.76 mmol), trifluoroacetic anhydride (115 µL, 0.83 mmol), triethylamine (0.23 mL, 1.66 mmol) and dichlorodicyanobenzoquinone (0.35 g, 1.52 mmol).

Flash chromatography on neutral alumina using a gradient eluant of 1-4% EtOAc in hexane afforded compound 199 (72 mg, 0.32 mmol, 42% yield) as a yellow oil. $R_f$ (4% EtOAc/hexane on neutral alumina plates) 0.85.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3121, 3103, 2954, 2924, 2854, 1538, 1500, 1458, 1374, 1331, 840, 776, 701.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 0.92 (t, $J$=7.0 Hz, 3H, H7'), 1.24-1.50 (m, 8H, H3'-H6'), 1.76 (p, $J$=7.7 Hz, 2H, H2'), 3.24 (t, $J$=7.7 Hz, 2H, H1'), 7.08 (d, $J$=5.8 Hz, 1H, H5), 7.60 (d, $J$=5.8 Hz, 1H, H4).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 114.1 (C7'), 22.6 (C6'), 28.9 (C5'), 29.3 (C4'), 29.5 (C1'), 30.3 (C2'), 31.7 (C3'), 121.2 (C5), 124.6 (C4), 143.8 (q, C3), 150.4 (q, C2).

HRMS: ($m/z$-EI) calcd. for C$_{11}$H$_{17}$NO$_2$S (M) 227.0980, found 227.0984.

**Also prepared as per procedure H, see below
5.4.7.7 2-(3-Benzoyloxy-phenyl)-3-nitro-thiophene:

Prepared as per procedure G using compound 155 (0.15 g, 0.45 mmol), trifluoroacetic anhydride (69 µL, 0.49 mmol), triethylamine (137 µL, 0.98 mmol) and dichlorodicyanobenzoquinone (0.20 g, 0.90 mmol).

Flash chromatography on neutral alumina using a gradient eluant of 1-4% EtOAc in hexane afforded compound 196 (42 mg, 0.13 mmol, 30% yield) as a yellow oil: $R_f$ (4% EtOAc/hexane on neutral alumina plates) 0.38.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3116, 3065, 3033, 2926, 2870, 1542, 1506, 1371, 1328, 1288, 1265, 1190.

$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 5.13 (s, 2H, H1'), 7.09-7.13 (m, 2H, H4' & H6'), 7.15-7.17 (m, 1H, H2'), 7.29 (d, $J$=5.6 Hz, 1H, H5), 7.37 (app t, $J$=6.8 Hz, 1H, H5'), 7.39 (d, $J$=7.9 Hz, 1H, H5''), 7.47 (dd, $J$=7.2, 7.9 Hz, 2H, H4''), 7.49 (d, $J$=7.2 Hz, 2H, H3''), 7.68 (d, $J$=5.6 Hz, 1H, H4).

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 69.8 (C1'), 115.69 & 115.72 (C2' & C4'), 121.8 (C6''), 123.6 (C5), 124.5 (C4), 127.2 (C3'), 127.7
HRMS: \( m/z \) - El calcd. for \( C_{17}H_{13}NO_3S \) (M) 311.0616, found 311.0621.

5.4.8 Synthesis of Nitrothiophenes using Microwave heating, Procedure H:

The appropriate THT (0.44 mmol) and oven dried acidic alumina (2.00 g) were ground together in a mortar.\(^*\) Chloranil (0.15 g, 0.61 mmol) was introduced and the solids were further ground together. The pale yellow solids were placed in a microwave sample tube and the sample was irradiated in a chemical microwave with 200 W at 125 °C for 4 min. To the cooled solids was added \( CH_2Cl_2 \) (15 mL) and the mixture was stirred for 1 h. Ground charcoal (0.25 g) and ground potassium hydroxide powder (0.25 g, 4.46 mmol) were added and the mixture was stirred for a further 1 h. The slurry was filtered through a plug of silica (~50 mL) and the solids were washed with a further \( CH_2Cl_2 \) (150 mL). Volatiles were removed at reduced pressure.

\(^*\) Liquid THTs were adsorbed onto the oven dried acidic alumina prior to grinding.
5.4.8.1 3-Nitro-2-(4-nitro-phenyl)-thiophene:

Prepared as per procedure H using compound 146 (0.12 g, 0.44 mmol), oven dried acidic alumina (2.00 g) and chloranil (0.15 g, 0.61 mmol). Compound 205 (79 mg, 0.31 mmol, 70% yield) was isolated as a yellow solid. M.p. 153-155 °C. $R_f$ (20% CH$_2$Cl$_2$/hexane) 0.18.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3140, 3121, 1593, 1540, 1497, 1340, 1313, 1103, 851, 840, 747, 704, 691.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 7.44 (d, $J$=5.5 Hz, 1H, H5), 7.70 (d, $J$=8.0 Hz, 2H, H2'), 7.75 (d, $J$=5.5 Hz, 1H, H4), 8.34 (d, $J$=8.0 Hz, 2H, H3').

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 123.6 (C3'), 125.3 (C4), 125.6 (C5), 130.8 (C2'), 137.0 (C1'), 142.0 (C2), 143.9 (C3), 148.3 (C4').

HRMS: ($m/z$-EI) calcd. for C$_{10}$H$_8$N$_2$O$_4$NaS (M+Na) 272.9946, found 272.9939.

5.4.8.2 2-Isopropyl-3-nitro-thiophene:

[Diagram of 2-Isopropyl-3-nitro-thiophene]

[Publisher's note: Diagram of 2-Isopropyl-3-nitro-thiophene]

[Diagram of 2-Isopropyl-3-nitro-thiophene]

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Prepared as per procedure H using compound 184 (84 mg, 0.44 mmol), oven dried acidic alumina (2.00 g) and chloranil (0.15 g, 0.61 mmol).

Compound 212 (43 mg, 0.25 mmol, 57% yield) was isolated as a pale yellow oil. $R_f$ (10% EtOAc/hexane) 0.54.

IR $v_{\text{max}}$ (cm$^{-1}$): 3120, 3103, 2968, 2928, 1534, 1498, 1373, 1333, 1181, 1027, 838, 777, 705.

$^1$H NMR (CDCl$_3$, 400 MHz): δ (ppm) 1.10 (d, $J=6.5$ Hz, 6H, H2'), 4.12 (hept, $J=6.5$ Hz, 1H, H1'), 7.10 (d, $J=5.5$ Hz, 1H, H5), 7.59 (d, $J=5.5$ Hz, 1H, H4).

$^{13}$C NMR (CDCl$_3$, 150 MHz): δ (ppm) 23.6 (C2'), 28.7 (C1'), 120.5 (C5), 124.2 (C4), 142.7 (q, C3), 157.3 (q, C2).

HRMS: (m/z-El) calcd. for C$_7$H$_9$NO$_2$S (M) 171.0354, found 171.0354.

5.4.8.3 3-Nitro-2-(3-nitro-phenyl)-thiophene:

Prepared as per procedure H using compound 151 (85 mg, 0.31 mmol), oven dried acidic alumina (1.41 g) and chloranil (0.11 g, 0.43 mmol).

Compound 206 (49 mg, 0.20 mmol, 65% yield) was isolated as a yellow solid. M.p. 171-173 °C. $R_f$ (40% EtOAc/hexane) 0.79.
IR $\nu_{\text{max}}$ (cm$^{-1}$): 3122, 3105, 3092, 1525, 1501, 1370, 1342, 1319, 1091, 1073, 900, 864, 784, 732, 707, 681.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 7.43 (d, $J=5.5$ Hz, 1H, H5), 7.68 (app t, $J=8.0$ Hz, 1H, H5'), 7.76 (d, $J=5.5$ Hz, 1H, H4), 7.83-7.88 (m, 1H, H6'), 8.34-8.38 (m, 1H, H4'), 8.38-8.41 (m, 1H, H2').

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 124.4 (C4'), 124.8 (C2'), 125.19 (C5), 125.26 (C4), 129.5 (C5'), 132.1 (C1'), 135.9 (C6'), 142.0 (C2), 143.8 (C3), 148.3 (C3').

HRMS: ($m/z-\text{El}$) calcd. for C$_{10}$H$_6$O$_4$N$_2$S (M) 250.0043, found 250.0042.

5.4.8.4 3-Nitro-2-(2-trifluoromethyl-phenyl)-thiophene:

Prepared as per procedure H using compound 161 (0.14 g, 0.44 mmol), oven dried acidic alumina (2.0 g) and chloranil (0.15 g, 0.61 mmol).

Flash chromatography on neutral alumina afforded compound 208 (65 mg, 0.23 mmol, 54% yield) as a white solid. M.p. 75-77 °C. $R_F$ (5% EtOAc/hexane on neutral alumina plates) 0.45.

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3127, 3104, 1551, 1506, 1375, 1333, 1312, 1267, 1171, 1109, 1063, 1033, 767, 714.
$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 7.41 (d, $J$=5.6 Hz, 1H, H5), 7.45 (d, $J$=7.3 Hz, 1H, H6'), 7.62-7.68 (m, 2H, H4' & H5'), 7.71 (d, $J$=5.6 Hz, 1H, H4), 7.83 (d, $J$=7.3 Hz, 1H, H3').

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 123.4 (quartet, $J_{CF}$=273.6 Hz, C7'), 123.5 (C4), 124.9 (C5), 126.4 (quartet, $J_{CF}$=5.1 Hz, C3'), 129.2 (q, quartet, $J_{CF}$=2.1 Hz, C1'), 129.4 (q, quartet, $J_{CF}$=30.0 Hz, C2'), 129.5 (C4'), 131.4 (C5'), 131.7 (C6'), 140.6 (q, C2), 145.1 (q, C3).

HRMS: (m/z-El) calcd. for C$_{11}$H$_6$O$_2$NF$_3$S (M) 273.0071, found 273.0062.

5.4.8.5 2-(2-Methoxy-phenyl)-3-nitro-thiophene:

![Diagram]

Prepared as per procedure H using compound 158 (0.11 g, 0.44 mmol), oven dried acidic alumina (2.00 g) and chloranil (0.15 g, 0.61 mmol).

Compound 207 (71 mg, 0.30 mmol, 69% yield) as a green solid. M.p. 80-82 °C. $R_f$ (20% EtOAc/hexane) 0.58.

IR $\nu_{max}$ (cm$^{-1}$): 3112, 3096, 2926, 1543, 1503, 1488, 1462, 1456, 1375, 1329, 1248, 1018, 757, 724.

$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 3.81 (s, 3H, H7'), 7.00 (dd, $J$=1.1, 8.4 Hz, 1H, H3'), 7.04-7.08 (m, 1H, H5'), 7.30 (d, $J$=5.5 Hz, 1H, H5), 7.37 (dd, $J$=1.5, 7.3 Hz, 1H, H6'), 7.44-7.48 (m, 1H, H4'), 7.65 (d, $J$=5.5 Hz, 1H, H4).

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$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 55.4 (C7'), 111.0 (C3'), 119.8 (q, C1'), 120.4 (C5'), 123.8 (C5), 124.3 (C4), 130.6 (C6'), 131.0 (C4'), 140.7 (q, C2), 144.3 (q, C3), 156.7 (q, C2').

HRMS: (m/z-EI) calcd. for C$_{11}$H$_9$NO$_3$S (M) 235.0303, found 235.0298.

5.4.8.6 5'-Bromo-3-nitro-[2,2']bithiophenyl:

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\begin{array}{c}
\text{Br} \\
\text{5'} \\
\text{4'} \\
\text{3'} \\
\text{2'} \\
\text{1'} \\
\text{NO}_2
\end{array}
\]

Prepared as per procedure H using compound 166 (68 mg, 0.22 mmol), oven dried acidic alumina (1.00 g) and chloranil (75 mg, 0.31 mmol).

Compound 209 (52 mg, 0.18 mmol, 81% yield) as a brown solid. M.p. 123-125 °C. $R_f$ (40% EtOAc/hexane) 0.91.

IR $v_{\text{max}}$ (cm$^{-1}$): 3125, 3109, 1528, 1501, 1484, 1408, 1325, 1282, 1176, 834, 786, 705.

$^1$H NMR (CDCl$_3$, 600 MHz): $\delta$ (ppm) 7.11 (d, $J$=4.0 Hz, 1H, H3'), 7.24 (d, $J$=5.7 Hz, 1H, H5), 7.32 (d, $J$=4.0 Hz, 1H, H4'), 7.65 (d, $J$=5.7 Hz, 1H, H4).

$^{13}$C NMR (CDCl$_3$, 150 MHz): $\delta$ (ppm) 117.7 (q, C5'), 123.6 (C5), 125.7 (C4), 130.3 (C3'), 131.0 (C4'), 132.4 (q, C2'), 137.8 (q, C2), 142.2 (q, C3).

HRMS: (m/z-EI) calcd. for C$_8$H$_4$NO$_2$S$_2$Br (M) 288.8867, found 288.8867.
5.4.8.7 1-Methyl-3-(3-nitro-thiophen-2-yl)-1H-indole:

Prepared as per procedure H using compound 171 (0.12 g, 0.44 mmol), oven dried acidic alumina (2.00 g) and chloranil (0.15 g, 0.61 mmol).

Flash chromatography on neutral alumina afforded compound 210 (32 mg, 0.12 mmol, 28% yield) as a yellow smear. 

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)):
- 3098, 3050, 2928, 1546, 1491, 1475, 1452, 1358, 1312, 1299, 1132, 1068, 833, 737, 702.

\(^1\)H NMR (CDCl\(_3\), 600 MHz): \( \delta \) (ppm)
- 3.92 (s, 3H, H10), 7.21 (d, \( J=5.6 \) Hz, 1H, H5'),
- 7.25-7.25 (m, 1H, H6), 7.33-7.37 (m, 1H, H7),
- 7.42 (d, \( J=8.3 \) Hz, 1H, H8), 7.72 (d, \( J=5.6 \) Hz, 1H, H4'),
- 7.79 (s, 1H, H2), 7.82 (d, \( J=8.3 \) Hz, 1H, H5).

\(^13\)C NMR (CDCl\(_3\), 150 MHz): \( \delta \) (ppm)
- 33.2 (C10), 104.9 (q, C4), 109.9 (C8), 120.1 (C5),
- 121.0 (C6), 121.5 (C5'), 122.8 (C7), 125.2 (C4'),
- 126.6 (q, C3), 131.9 (q, C2), 136.8 (q, C9), 140.2 (q, C2'), 142.0 (q, C3').

HRMS: \( m/z - \text{El} \) calcd. for C\(_{13}\)H\(_{10}\)N\(_2\)O\(_2\)S (M) 258.0463, found 258.0460.
5.4.8.8 3-Nitro-2-[4-(3-nitro-thiophen-2-yl)-phenyl]-thiophene:

Prepared as per procedure H using compound 178 (82 mg, 0.22 mmol), oven dried acidic alumina (2.00 g) and chloranil (0.15 g, 0.61 mmol).

Compound 211 (34 mg, 0.09 mmol, 42% yield) was recovered as a yellow solid. M.p. 273-276 °C. Rf (30% EtOAc/hexane) 0.17.

IR νmax (cm⁻¹): 3122, 3107, 2922, 1543, 1506, 1488, 1449, 1370, 1316, 1176, 840, 824, 779, 765, 710, 682.

¹H NMR (CDCl₃, 600 MHz): δ (ppm) 7.36 (d, J=5.5 Hz, 2H, H5), 7.60 (s, 4H, H2'), 7.72 (d, J=5.5 Hz, 2H, H4).

¹³C NMR (CDCl₃, 150 MHz): δ (ppm) 124.5 (C5), 125.2 (C4), 129.8 (C2'), 131.8 (q, C1'), 143.3 (q, C3), 144.2 (q, C2).

HRMS: (m/z – El) calcd. for C₁₄H₉N₂O₅S₂ (M) 311.9926, found 311.9935.
5.4.8.9 2-Heptyl-3-nitro-thiophene:

Prepared as per procedure H using compound 187 (0.11 g, 0.44 mmol), oven dried acidic alumina (2.00 g) and chloranil (0.15 g, 0.61 mmol). Compound 213 (52 mg, 0.23 mmol, 52% yield) was recovered as a pale yellow oil: see data above.

5.4.8.10 2-(4-Bromo-phenyl)-3-nitro-thiophene:

Prepared as per procedure H using compound 187 (0.32 g, 1.05 mmol), oven dried acidic alumina (4.77 g) and chloranil (0.36 g, 1.46 mmol). Compound 195 (0.25 g, 0.86 mmol, 82% yield) was isolated as a yellow solid: see data above.
5.4.8.11 3-Nitro-2-phenyl-thiophene:

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Prepared as per procedure H using compound 137 (0.10 g, 0.44 mmol), oven dried acidic alumina (2.00 g) and chloranil (0.15 g, 0.61 mmol).

Compound 138 (82 mg, 0.40 mmol, 91% yield) was isolated as a yellow solid: see data above.
References


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