Towards the Synthesis of Novel PARP Inhibitors through Diels-Alder Chemistry

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ABSTRACT

TOWARDS THE SYNTHESIS OF NOVEL PARP INHIBITORS THROUGH
DIELS-ALDER CHEMISTRY

Irena Nikoloska  
University of Guelph, 2012

Poly(ADP-ribose) polymerase (PARP) represents a large family of enzymes that are activated upon DNA breakage, which are then involved in a cascade of reactions that eventually lead to cell death. PARPs, known to cause a variety of damage to the human body, are targets of many researches’ investigating potential inhibition mechanisms. To this point, plenty of PARP inhibitors have been synthesized and tested for potency against many diseases. Some have great potency against particular diseases, while others are already being used as drugs. The current research suggests a potential synthesis of novel PARP inhibitors, to be accomplished through Diels-Alder chemistry. The proposed compounds consist of an isoindolinone core bearing different functional groups. Three different strategies are described for the synthesis of the novel PARP inhibitors and all protocols involve sulfur dioxide extrusion chemistry to create a diene, the desired transient starting material. The diene is envisioned further to be reacted with a variety of dienophiles to give possibly potential PARP inhibitors.
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<tbody>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AIBN</td>
<td>azobisisobutyronitrile</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Bz</td>
<td>benzoyl</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DMF</td>
<td>$N,N$-dimethyl formamide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>eEF-2</td>
<td>eukaryotic Elongation factor 2</td>
</tr>
<tr>
<td>EGDE</td>
<td>ethylene glycol diglycidyl ether</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>GC-MS</td>
<td>gas chromatography/mass spectrometry</td>
</tr>
<tr>
<td>HMQC</td>
<td>heteronuclear single quantum coherence</td>
</tr>
<tr>
<td>HOMO</td>
<td>highest occupied molecular orbital</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>inhibitor concentration at 50%</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>LUMO</td>
<td>lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>NAD&lt;sup&gt;+&lt;/sup&gt;</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>nBuLi</td>
<td>normal butyllithium</td>
</tr>
<tr>
<td>NBS</td>
<td>$N$-bromosuccinamide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NMNO</td>
<td>N-methylmorpholine-N-oxide</td>
</tr>
<tr>
<td>NOE</td>
<td>nuclear Overhauser effect</td>
</tr>
<tr>
<td>PARP</td>
<td>Poly(ADP-ribose) polymerase</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>PG</td>
<td>protecting group</td>
</tr>
<tr>
<td>PMB</td>
<td>para-methoxy benzyl</td>
</tr>
<tr>
<td>ppm</td>
<td>part per million</td>
</tr>
<tr>
<td>Rf</td>
<td>retardation factor</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
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<td>trimethylsilyl</td>
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CHAPTER 1: INTRODUCTION

1.1 PARP Biochemistry

Poly(Adenosine diphosphate-ribose) polymerase-1 (PARP-1) is a nuclear enzyme, which is the most characterized and well understood protein isoform member of the PARP family.\(^1\) It is activated upon double and single stranded DNA breakage, and it catalyzes the cleavage of NAD\(^+\) into ADP-ribose and nicotinamide.\(^2\) PARP-1 is also involved in the post-translational modification of activated nuclear proteins by adding ADP-ribose unit to it, forming a poly(ADP-ribose) polymer.\(^3\) In this elongation reaction PARP-1 can add up to 200 units, forming branched and/or linear polymers through the 1,2-\(\alpha\)-glycosidic bond.\(^4\)

The discovery of PARPs dates back to the 1970s. They are very diverse family, and are found in all higher and most, lower eukaryotes.\(^3,5\) PARPs are a big super family consisting of 18 members and all perform the same functions.\(^6\) Three of the PARPs members are isoforms, where PARP-1 is the most active and most apprehended, thus scientists were led to believe for a long time, that only one isoform of PARP existed.\(^3\) Since our principal interest is focused on PARP-1 the other isoforms will not be discussed here. PARP-1 is made up of 1014 amino acids, and is a 113 kDa abundant enzyme, ranging from 0.2 – 2.0 \(\times\) 10\(^6\) molecules per cell.\(^3\) It consists of three domains: a DNA binding domain (DBD), an automodification domain and a catalytic domain (Fig. 1).\(^3,5\)

![Figure 1](Image.png)

**Figure 1.** Schematic representation of the PARP-1 enzyme, indicating its three domains. ZF = zinc finger.
The DBD domain, as the name denotes, is the part that recognizes DNA, breaks and interacts with them. More specifically the two zinc fingers, which are located at the N-terminus and are part of the DBD domain, are responsible for DNA binding. Detailed studies of deletion and mutation of both zinc fingers have supported this conclusion. For example, if the first zinc finger is absent from the enzyme the result is loss of PARP-1 activity, where both single and double stranded DNA breaks are not being recognized. On the other hand, if the second zinc finger is absent, PARP-1 is still active but it only recognizes double stranded DNA breaks. Such data clearly suggest that the N-terminus, particularly the zinc fingers, of the PARP-1 protein are necessary for its proper activity. What is noteworthy about the PARP-1 zinc fingers is that they do not recognize one particular structure in DNA, rather, they are indiscriminate towards DNA sequences. DNA ligase III, a DNA repair protein, is the only other known protein demonstrating such behaviour.

In the middle of the protein the auto-modification domain is found. It is preserved in some mammalian PARPs and is involved in protein-protein interactions. It also contains certain amino acids that are found in proteins which are involved in DNA repairs and cell-cycle checkpoints. The auto-modification domain does not contribute much towards this project, thus it will not be discussed in detail.

The third and last domain is the catalytic domain, located at the C-terminus, where the active site is found. It is the longest of all the domains and it contains a “PARP signature” sequence. The “PARP signature” sequence is a well conserved sequence containing residues 859 – 908. The active site is been intensively studied through site-directed mutagenesis. The most important discovery through site-directed mutagenesis is residue Glu-988. This is the key residue for PARP-1 activity. In the absence of Glu-988 the elongation reaction is decreased by 2000
times, and also comparative studies of PARP-1 to mono(ADP-ribosyl) transferases show that Glu-988 is the key amino acid in the polymer formation of the ADP-ribose units.\(^3\)

A. Initiation

\[
\text{Glu} \ 988 + \text{AP} \text{--OH} \rightarrow \text{Glu} \ 988 \text{--OH} + \text{NAD}^+ \text{--AMP}
\]

B. Elongation

\[
\text{Glu} \ 988 \text{--OH} + \text{AP} \rightarrow \text{Glu} \ 988 \text{--AH} + \text{NAD}^+ \text{--AMP}
\]

**Scheme 1.** Poly(ADP-ribose) polymerization reaction in PARP-1, Glu-988 acts as a general base. A) Initiation step. B) Elongation step; Note: AP = Acceptor Protein

Glu-988 is the residue responsible for the enzyme-catalyzed deprotonation of an hydroxyl group of an acceptor protein (AP), in the initiation step of poly(ADP-ribose) polymerization.
reaction (Scheme 1). It also coordinates with the hydroxyl groups of the riboses of the polymer, forming a partial negative change on the oxygen thus aiding in nucleophilic attack of the C1 on the ribose of the nicotinamide adenine dinucleotide (NAD$^+$), in the elongation step of the poly(ADP-ribose) polymer formation$^4$ (Scheme 1). Other residues around the active site also affect PARP-1 activity. Non-conservative mutation of Lys-893 and Asp-993 affect the substrate binding in the active site, which led PARP-1 to be completely inactivated.$^9$ Conservative mutations of other residues such as Asp-914 and Lys-953 led to diminished activity of PARP-1.$^9$ This is because these amino acids are further from the binding pocket, thus they impose less of an effect to the enzyme activity. Another interesting exploration is that PARP-1 is abolished in the absence of the last 45 residues of the C-terminus.$^3, ^5$

PARPs are known as multifunctional enzymes since they are involved in a variety of processes such as DNA repair, malignant transformation, gene transcription, cell cycle control, cell proliferation, cell differentiation and cell death.$^{10}$ PARP-1 also acts as a co-factor in tumorigenesis and transcription factors that regulate inflammatory and immune responses.$^{5, ^10}$ PARP-1 plays a pathophysiological role in natural immune responses. There are plenty of inflammatory diseases that PARP-1 is involved in, such as acute respiratory distress syndrome, LPS-induced septic shock, myocardial postischemic injury, hemorrhagic shock and others.$^5$ One of the biggest issues with PARP-1 activation is that with each polymer synthesis reaction 50 - 200 of the coenzyme NAD$^+$ are consumed, which will eventually lead to cell dysfunction and then necrotic cell death.$^5$ This occurs because NAD$^+$ is involved in the sixth step of the process of glycolysis, where glyceraldehydes 3-phosphate is converted into 1,3-bisphosphoglycerate, during which NAD$^+$ accepts an electron, thereby getting reduced into NADH.$^{11}$ As a result ATP formation is prohibited, thus the rate of glycolysis and electron transport in the mitochondria are
being reduced, bringing about pathophysiological changes, cellular dysfunction and eventually necrotic cell death.\textsuperscript{5} Therefore for small amounts of DNA damage PARP-1 is activated to repair the damaged DNA, but for large amounts of DNA damage PARP-1 is over-activated, leading to cell death.

1.2 Cystic Fibrosis

As mentioned above PARP-1 is structurally and functionality similar to mono-ADP-ribosyl transferases (mART), which are enzymes that function as virulence factors in pathogenic bacteria.\textsuperscript{12} mART also use NAD\textsuperscript{+} as a source of ADP-ribose units for the formation of poly(ADP-ribose) polymers. An enzyme of interest called Exotoxin A is part of the mART family. Exotoxin A is considered to be a toxin that is released by a pathogen, \textit{Pseudomonas aeruginosa}, which is found in soil, sewage and water.\textsuperscript{13} Exotoxin A is considered to be lethal (LD\textsubscript{50}) of 0.2 μg/animal, since it inhibits protein synthesis, which leads to cell death.\textsuperscript{14} It targets eukaryotic elongation factor 2 (eEF-2) by adding a ADP-ribose unit to the diphthalamide residue of eEF-2 (Fig. 2). eEF-2 is one of the factors involved in protein synthesis, hence Exotoxin A inhibits it from properly functioning.\textsuperscript{13}

Cystic fibrosis (CF) is an autosomal recessive genetic disease, commonly found among Caucasian populations.\textsuperscript{15} Every one in 216 people are born with CF and one in 25 are carriers for the disease.\textsuperscript{15} Individuals carrying a CF gene suffer from a weakened immune system response, thus leading to infections in the cardiovascular system and respiratory tract. Therefore, \textit{P. aeruginosa} is responsible for causing secondary infections in individuals with CF.\textsuperscript{16} Unfortunately CF victims do not live pass 30-40 years of age.

Even though the human genome has been completely sequenced for almost ten years, and
the gene associated with cystic fibrosis has been identified, sadly a gene therapy has not yet been accomplished. The issue with gene therapy is that it needs to be administered more than once to the individual since CF is a chronic life-long disease. Currently CF treatments are centered on easing symptoms and destroying secondary infections. Since a cure for CF has not been discovered yet, one might suggest that a reasonable treatment of CF could be dealing directly with the enzyme itself. Since PARP-1 activation leads to many pathophysiological diseases, as mentioned above, a possible treatment for such individuals could be an inhibition of over-activated PARP-1 or Exotoxin A.

**Figure 2.** Exotoxin A inactivating eEF-2.
1.3 PARP Inhibitors

The catalytic domain of PARPs have been presented to be structurally similar to Exotoxin A.\(^\text{18}\) Therefore a possible investigation of potential PARP-1 inhibitors are highly likely to aid in inhibiting Exotoxin A thereby decreasing the damage caused by \(P.\) \textit{aeruginosa} in CF patients.

![Figure 3. PARP-1 active site where Gly863 is H-bonded to 3,4-dihydro-5-methyl isoindolinone (pdb 1PAX)](image)

Plenty of already discovered PARP inhibitors are being used as drugs and the potential exists for many more. The first-generation of PARP inhibitors synthesized resembles the nicotinamide (1) functionality of the NAD\(^+\).\(^\text{19}\) Thus, nicotinamide was thought to be a good candidate for inhibiting PARP, but scientific research proved the opposite. Its IC\(_{50}\) is 100 μM,
which is not sufficient enough to even pass animal trials. Other simple molecules such as benzamide, substituted benzamides and 3-aminobenzamine (2) have also been evaluated.\textsuperscript{19,19} It was found that 2 is more potent with an IC\textsubscript{50} of 20 μM. As research about PARP inhibitors developed through the years, co-crystal structural analysis confirmed that the inhibitor prefers an \textit{s}-trans conformation of the amide for optimal binding to the enzyme.\textsuperscript{20} This can be depicted in a picture of a co-crystal structure of PARPs where Gly863 residue forms H-bonds to the inhibitor (Fig. 3). Figure 3 shows an inhibitor, 3,4-dihydro-5-methyl isoindolinone, forming H-bond to residue Gly863 of PARP-1 enzyme. And in the Exotoxin A is residue Gly441 that forms the H-bonds to the inhibitors (Fig. 2). As novel PARP inhibitor discovery has progressed it was concluded that lactam functionality is highly favorable since it adopts a more planar conformation. Hence the amide functionality is locked in its favorable \textit{s}-trans conformation for proper orientation and activity.\textsuperscript{21} Thus, second-generation PARP inhibitors such as bicyclic (3), tricyclic (4) and tetracyclic (5) analogues of nicotinamide have been synthesized (Fig. 4). Compound 5 is claimed to be a useful drug for treating peripheral neuropathy caused by ischemia, trauma and neurodegeneration, where its IC\textsubscript{50} is 0.06 μM, hence making it an effective drug.\textsuperscript{22} Other compounds like bicyclic benzamides (6) are found to be effective since they are locked in the \textit{s}-trans configuration by intramolecular hydrogen bonding.\textsuperscript{22}
Even though some compounds from the first and second-generation PARP inhibitors have proven to be effective as drugs, they lack potency and specificity. Thus, a third-generation of PARP inhibitors was created, which are highly specific and the majority of them are already used as effective drugs or are in clinical trials (Fig. 5). For example, iniparib (7) is currently in Phase III clinical trial, where it is tested against solid tumors occurring in the ovary, uterus and lungs. Cordycepin (8), a promising compound for breast and ovarian cancer, is found to give some side effects when given in large amounts. Thus, currently 8 is being tested so it can be administered in millimolar amounts which will allow it to enter into clinical trials. Another drug, olaparib (9) passed phase I trials, where it gave promising results against BRCA mutation-associated cancer. Compound 10 is currently in pre-clinical testing for cancer patients. It
gives strong results regarding improved drug biodistribution and enhanced cytotoxic effects of DNA-damaging chemotherapies.\textsuperscript{25} A possible treatment for solid tumors and lymphoid malignances has been shown by compound 11, which has entered clinical trials.\textsuperscript{19} And last but not least, compound 12 is an exciting drug candidate. It has shown to enforce a cytotoxic effects in leukemia cells, due to its potent IC\textsubscript{50} value of 0.045 μM.\textsuperscript{26,27} Its IC\textsubscript{50} value is quite remarkable and it can be attributed to the isoindolinone and adenosine groups, which mimic the nicotinamide and adenosine group found on the NAD\textsuperscript{+} respectively. In addition, the piperazine moiety acts as a linker resembling the two phosphate groups of the NAD\textsuperscript{+}. By engineering such a molecule an optimal binding to the PARP active site is achieved and great results are observed.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Third-generation PARP-1 inhibitors.}
\end{figure}
1.4 Synthetic routes to selected PARP-1 inhibitors

As suggested by the content of Figures 4 and 5, there are a variety of chemical structures to be evaluated for their suitability as PARP inhibitors, each of which has its own unique functionality and specificity for diseases caused by PARPs. One can conclude that there is an enormous field of research directed towards potential PARP inhibitors that are yet to be discovered. As this thesis is focused on synthetic chemistry, it is valuable to outline herein some of the different synthetic routes adopted for the preparation of potential PARP inhibitors.

Scheme 2 shows a synthetic pathway to benzimidazole 13, a compound which demonstrated good antiproliferation activities against breast cancer (BRCA) deficient cancer cells.28 BRCA is a tumor suppressor gene which produces a protein to fix damaged DNA or destroy damaged DNA that cannot be fixed.29 Currently 13 is in phase I clinical trials since it exhibits good pharmacokinetic properties. Even though the key H-bonding functional group of 13 is an amide which is not fixed in a ring, it nevertheless forms intramolecular hydrogen bond with the nitrogen not carrying the R group of the imidazole ring.30 This makes the molecule adopt the planar and more favourable conformation for entering into the active site of PARP-1. The strategy for synthesizing 13 begins with conversion of the carboxylic acid into an ester, then introduction of an aldehyde onto the aromatic ring. The aldehyde is then converted into an imine, and an indazole formation with the desired R group follows. The last step is a conversion of the ester group into an amide achieving the targeted compound 13.
Scheme 2: Synthetic route for 2-(4-(piperidin-3-yl)-2H-indazole-7-carboxamide (13).

Outlined in Scheme 3 is a synthesis of tricyclic quinoxalinone inhibitor 14. This compound is found to exhibit good enzymatic and cellular potency.\textsuperscript{31} This has been confirmed through X-ray co-crystal structural analysis and kinetic enzyme inhibition analysis, thus 14 has been evaluated as a good candidate for inhibiting PARP-1. This is partially due to its different molecular make up. Its uniqueness allows it to fit into the binding pocket of the active site which is normally not occupied by simple benzimidazole inhibitors.\textsuperscript{31} It is prepared by firstly protecting the carboxylic group with the Weinreb amide. Then introduction of the pyrrole species which subsequently affords the lactam formation. Following is a reduction of the Weinreb amide and placement of the amine substituent to afford compound 14.
Scheme 3: Synthetic route for 7-(pyrrolidin-1-ylmethyl)pyrrolo[1,2-a]quinoxalin-4(5H)-one.

Another example is outlined on Scheme 4, where the synthetic pathway to bioactive tricyclic compound 15 is presented. The key functional group of this molecule is a lactam, necessary for recognition into the active site of the enzyme. Also it contains a hydroxyl group and a tertiary amine. These groups aid in stronger binding of the inhibitor, by displacing unwanted water molecules from the active site, and as well by participating in hydrogen bonding with nearby amino acids. These numerous interactions results in an IC$_{50}$ value of 0.029 μM and hence, 15 is a promising candidate for treating brain ischemia. Studies have been supported by physicochemical and pharmacokinetic characterization. The assembly of 15 begins with a Suzuki coupling reaction. Following that is a conversion of the carboxylic acid moiety into an azido carbonyl compound which aids in the lactam formation accomplished via Curtius rearrangement. Subsequently a Mannich-type reaction is applied, and replacement of the chloride counter ion with a bromine anion affords the desired compound 15.
Scheme 4: Synthetic route for 1-(9-methoxy-5-oxo-4,5-dihydrothienol[2,3-c]isoquinolin-2-yl)-N,N-dimethylmethanaminium bromide (15).

The above presented synthetic routes to novel PARP inhibitors are a limited representation of the number of different PARP inhibitors that are sought by contemporary researchers. The majority of previously synthesized PARP inhibitors are patented, which clearly indicates how important the PARP inhibitors research is, particularly to the pharmaceutical industry. With this in mind, this thesis targets a new synthetic strategy for novel PARP inhibitors, one that could involve rapid assembly of a polycyclic system possessing the appropriately positioned lactam ring, yet permitting substantial variation of other substituents. Such a variety of functional groups can aid in better binding to the active site of the enzyme.

1.5 Research Objective

As discussed above, synthesis of potential PARP inhibitors is a worthy research pursuit since PARPs lead to variety of pathophysiological diseases and eventually cell death, thus not one PARP inhibitor can accommodate all the needs required. Therefore, the search for novel and more potent PARP inhibitors continues. By looking at Figures 4 and 5 one can notice that
majority of the selected PARP inhibitors contain an isoindolinone core which indicates that that is one of the key features of potentially successful PAPRs inhibitors. Therefore the synthesis of novel PARP inhibitors with an isoindolinone core holds significant promise and is the goal of this research project. The optimal goal is to synthesize a tricyclic and/or tetracyclic isoindolinone containing compounds as novel PARP inhibitors. This is envisioned to be accomplished through Diels-Alder chemistry on a particular chemical target. Figure 6 illustrates the key reactive entity required for this project, a diene which will be generated through extrusion of sulfur dioxide from the appropriate starting material. The preferences for the R group are hydrogen, methyl or a removable protecting group, and the R” group is preferred to be hydrogen or a small alkyl unit.

Two strategies, A and B, were proposed for the preparation of the diene (16). Strategy A, as shown on Scheme 5 starts with 3-methyl phthalic anhydride (17) which is converted into a phthalimide (18), via reaction with an amine. Following that is the introduction of a bromine atom on the methyl group of the aromatic ring. The third step of the synthesis is displacement of the bromine atom by a sulfur atom (20). The choice of the R’ group on the thiol is vital since the R’ must direct a subsequent oxidative fragmentation. For that event tert-butyl or para-methoxybenzyl are the groups of choice. The subsequent step is conversion of the proximal
carbonyl to an alcohol moiety (21). The R” group is either methyl or hydrogen, depending on which nucleophile is used. Sulfur oxidation is required to provide all the components for extrusion of sulfur dioxide. And the penultimate step to Diels-Alder chemistry is an oxidative fragmentation, which will be promoted by the judicious earlier selection of the R’ group. Oxidative fragmentation conditions are a recognized method of making sulfinyl chlorides, by first making a chlorosulfoxonium ion and then by cleavage of a C-S bond (Scheme 6). As mentioned above R’ is favored to be para-methoxybenzyl (22) since it will be lost upon treatment with sulfuryl chloride (Scheme 6), affording a sulfinyl chloride (23), which is unlikely to be isolable. Therefore in the same pot, the addition of base should bring about a cyclic sulfinate ester or sultine (24) which is ready for thermolysis and subsequently Diels-Alder cycloaddition chemistry. The key step of sulfinate extrusion is anticipated to be accomplished when the compound is heated in toluene in a sealed tube 180~200 °C. Sulfones have been considered instead of sulfinates but unfortunately they are known to undergo pyrolysis at even higher temperatures, at least when employing the sulfinate/sulfone precursor to 5,6-bis(methylene)-1,3-cyclohexadiene as a model.
Scheme 5. Strategy A for the synthesis of the diene 16. \( R = \text{H or Me}; R' = \text{PMB}; R'' = \text{H, Me, alkyl}; \)

Scheme 6: Oxidative fragmentation and the loss of the \( R' \) group. \( R' = \text{para-methoxy benzyl (PMB)} \).

Strategy B (Scheme 7) is designed to access the same diene (16) begins with the commercially available compound 2-bromo-3-methylbenzoic acid (25). First the carboxylic moiety is converted into an amide, via conversion to the corresponding acid chloride (26), and a
subsequent reaction with an amine to afford the desired amide (27). Next the amide is converted into an imide (28) which will serve as an electrophile after halogen-lithium exchange is executed on the ring bromine (29). The result is anticipated to be ring formation, with the alcohol position to capture a future sulfinyl chloride (30). The reminder of the synthetic steps of strategy B are same as proposed for strategy A. The only difference is that the hydroxyl group is formed before the bromination and sulfiylation reaction, thus it might require protection to facilitate the other steps.

Scheme 7. Strategy B for the synthesis of the diene 16. R = H or Me; R’ = PMB; R” = H, Me, alkyl;

Once the diene (16) is formed, it will be subjected to Diels-Alder reaction with a variety
of dienophiles as is probably not isolable. Figure 6 depicts some of the dienophiles that are envisioned to be used to make novel PARP inhibitors. A variety of starting materials with different R and R” groups are to be reacted with a different dienophile (Fig. 7). Therefore a diversity of PARP inhibitors are being targeted as suggested in Figure 8. Since a genuine problem with the PARP inhibitors is that their value may be limited by inadequate water solubility, the R groups and the dienophiles might aid with that issue. In particular, the conversion of esters to alcohols or amides may increase water solubility. Hence a variety of PARP inhibitors are sought, which further are to be tested for their PARP or Exotoxin A inhibition capability. This testing is likely to be performed by the Merrill group at the University of Guelph, or some other biochemistry group whose research interests involve enzymes encompassed by the PARP mode of action.

**Figure 7.** Some dienophiles that are envisioned to be used in the formation of novel PARP inhibitors.

**Figure 8.** Some novel PARP inhibitors to be synthesized.
1.6 Diels-Alder Chemistry

The Diels-Alder reaction, named after its two inventors, Otto Paul Hermann Diels and Kurt Alder, is a concerted [4+2]-cycloaddition reaction where two π bonds are being lost while two σ bonds are being formed. These reactions occur between a conjugated diene and a substituted alkene, called a dienophile, usually bearing an electron withdrawing group. A model reaction is represented on Scheme 8 where butadiene is reacted with maleic anhydride. As presented the transitions state consists of six delocalized π electrons which may possess aromatic character. It is believed that this is one of the reasons why Diels-Alder reactions work well since not much energy is required.

\[
\begin{align*}
\text{Butadiene} + \text{Maleic Anhydride} & \rightarrow \text{Transition State} \\
\end{align*}
\]

\[\text{Scheme 8. Diels-Alder reaction between butadiene and maleic anhydride. The transition state is presented with the six delocalized π electrons.}\]

The diene can be an open-chain or cyclic compounds that can bear a large variety of substituents. The open-chain dienes are required to adopt their less stable s-cis conformation for participation in the transition state of the reaction. In this regard the cyclic dienes are exceptionally good at Diels-Alder reactions since they do not have the limitation of open-chain dienes.

The dienophile is an alkene which commonly features a conjugated electron withdrawing group, such as carbonyl compounds, nitriles, nitro compounds, and esters, to name few.
the alkene can be a heterodienophile which means that one or both carbons can be substituted with a different atom, and that usually is nitrogen.  

To determine the feasibility of the Diels-Alder reaction one needs to look at molecular orbital theory. The energy difference between the applicable highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the two reactants, is in whichever dominate the energetic of the reaction to deliver product. The most common type interactions are manifested when the diene interacts primarily by way of its HOMO while the dienophile is presenting its LUMO as presented on Figure 9. This is favoured since the dienophiles are electron-deficient as they are conjugated with electron withdrawing groups, and the dienes are electron-rich. Such a combination of MO’s provides the lowest energy transition state.

![Figure 9](image)

**Figure 9.** Presentation of the HOMO and LUMO orbitals involved in the formation of the major product of the Diels-Alder reaction between butadiene and maleic anhydride.

Diels-Alder reactions are stereospecific, meaning particular enantiomers and diastereomers are being formed, depending on the stereochemistry of the two reactants. Hence the *cis* principle applies here which is one of the characteristics of a [4+2]-cycloaddition. The *cis* principle states that the stereochemistry of the substituents on the two reactants is retained in the cyclic product. As well Diels-Alder reactions can be regiospecific due to unsymmetrical dienes
and dienophiles, i.e. bearing different substituents, which will preferably provide cyclic products.

For cyclic reactants the *endo* principle applies when determining the stereochemistry of the product. The *endo* principle looks at the most stable transition state formed by the two reactants, which occurs when there is a maximum accumulation of double bonds. Therefore the stereochemistry of the product for different reactants varies. For Diels-Alder reactions the *endo* product is the preferred one, but that is not necessarily always the case. The ‘*endo*’ cycloadduct is a kinetic product that forms quickly; however it is typically less stable and of higher energy than the ‘*exo*’ cycloadduct. Whereas the ‘*exo*’ cycloadduct is the more stable thermodynamic product and it usually requires more time and/or harsher treatment to be formed than the ‘*endo*’ cycloadduct. For example, cyclopentadiene and maleic anhydride produce the *endo*-selective product which is less stable but typically cannot revert to the *exo*-product. On the other hand, furan and maleic anhydride produce the more stable *exo*-selective product (Fig. 10). The explanation is that in both cases *endo*-adducts are formed, but in the case of furan and maleic anhydride, reversible cleavage to the reactants and recombination in an *exo* manner delivers the more stable *exo*-cycloadduct.

The premise of the Diels-Alder reaction is also observed in reverse and that transformation is referred to as the retro-Diels-Alder reaction. Reversibility might occur at room temperature or might require thermal stimulation. One key factor involved is the nature of the reactants, since certain molecules are volatile or highly reactive. As described above, furan is more reactive in retro-DA chemistry than cyclopentadiene as seen by its willingness to form both cycloadducts. Extensive research has been done on the reversibility of Diels-Alder reactions where other parameters such solvent, temperature, concentration have been studied. Also an
interesting study has been developed by Boutelle \textit{et al.} who evaluated the effect of substituents on the 2- and 3-positions of furan in the cycloaddition with maleimide, an outcome which helps us evaluate our substrate of interest that will be discussed later on in Section 2.3.\textsuperscript{41} Retro-Diels-Alder reactions are of great interest to researchers since many natural products contain aromatic moieties, such as bioactive compounds like antibiotics. One way that retro-Diels-Alder reactions contribute to pharmaceutical chemistry is due to their ability to eliminate molecules such as carbon dioxide, nitrogen, oxygen and sulfur dioxide as represented on Scheme 9, which is a facile way to form an aromatic species.\textsuperscript{43}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure10.png}
\caption{Diels-Alder reaction between furan and maleic anhydride, representing both possible cycloadducts. The \textit{endo} conformation is less stable, kinetic product, while the \textit{exo} conformation is more stable, thermodynamic product that is favoured in this case.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{scheme9.png}
\caption{Thiophene sulfone reacts with an alkyne via Diels-Alder chemistry, which further eliminates sulfur dioxide via retro-Diels-Alder chemistry to afford an aromatic molecule.}
\end{figure}

The stereospecificity and the regiospecificity of the Diels-Alder reactions can be highly
controlled by the use of Lewis acids as catalysts since they commonly produce a single reaction product. In addition, Lewis acids speed up the rate of the reaction and enhance the preference for endo products. Such effects are observed because Lewis acids coordinate with the electron withdrawing group on the dienophile and by doing so further lower the energy of the LUMO of the dienophile. Therefore the activation energy is lowered since the Lewis acid makes the double bond of the dienophile more electron poor since it pools electrons density even further towards the electron-withdrawing group.

Diels-Alder chemistry was first introduced in organic synthesis some 80 years ago. The pioneering works were mostly based on clarifying conditions and possibilities in regards to different substrates. Over the years the reaction has grabbed many chemists’ attention since it is a facile reaction for the formation of carbocyclic and heterocyclic compounds. Thus investigations shifted towards catalytic additives such as Lewis acids and their effects, solvent influence, reaction rates, elevated pressure and temperature. Now in the modern literature, the Diels-Alder reaction remains a popular tool for synthesizing compounds that are of a biological importance, and potentially of pharmacological interest.

The chemistry investigated herein for the formation of the novel PARP inhibitors candidates involves a Diels-Alder reaction as the final step. To obtain a better understanding if Diels-Alder chemistry is being utilized in any synthetic pathways for the formation of already formed PARP inhibitors, a SciFinder cross-reference search was performed among PARPs and Diels-Alder reactions, and as well among mARTs and Diels-Alder reactions. No literature hits were found in regards to mARTs. Only one literature search is available in regards to PARPs. It

\[ a \text{ SciFinder search performed on March 20}\textsuperscript{th}, 2012. \]
utilizes $^{18}$F labelling onto a known PARP-1 inhibitor, AZD2281, where the labelling molecule is placed onto the PARP-1 inhibitor via a Diels-Alder chemistry. This type of finding does not contribute to our research of interest. On the other hand the results indicate that there is opportunity to bring this useful synthetic tool into this research realm, as it has rarely been utilized in the past for the construction of these inhibitors.
CHAPTER 2: RESULTS AND DISCUSSION

As presented above in section 1.4, there are plenty of diverse synthetic routes that have been adopted for the synthesis of a potential PARP inhibitor. The inhibitors can hold different functional groups that can mimic certain aspects of the coenzyme NAD$^+$, and as such, they target different diseases. The goal of this research project is to develop and evaluate a distinct way of synthesizing a broad family of novel PARP inhibitors. Two different strategies are studied and outlined in this thesis, but unfortunately a number of obstacles were encountered, some of which were solvable, but for others, a different pathway had to be explored.

2.1 Strategy A

The synthesis of the starting material of strategy A (Scheme 5) was first attempted by reacting (E)-penta-1,3-diene with maleic anhydride via Diels-Alder chemistry to afford cyclohexene 31 (Scheme 10). This reaction was quite successful where the desired product was obtained from the first trial, with an acceptable yield of 68%. The $^1$H NMR spectrum confirmed the presence of the two alkene peaks around 6 ppm and the rest of the peaks were observed more upfield, in the range of 3.5 – 1.5 ppm. As well the absence of the maleic anhydride alkene peak 7.10 ppm confirmed the formation of the cyclohexene 31. In the next step, this compound had to be aromatized to afford the desired starting material of strategy A. Unfortunately the aromatization step failed, confirmed by $^1$H NMR spectroscopy where only the starting material was isolated since there was no presence of aromatic peaks around 7.3 ppm. A procedure adopted from Miller et al. was followed where chloranil was used instead of DDQ as an oxidant, perhaps the chemical was not strong enough to accomplish the aromatization. Given these
initial difficulties and rather than pursuing other oxidants or conditions to create 3-methyl benzoic anhydride, it was purchased so that subsequent experiments could be investigated.

![Chemical structure](image)

**Scheme 10.** Synthesis of compound 17, starting by the cycloaddition among (E)-penta-1,3-diene maleic anhydride via Diels-Alder reaction followed by aromatization of the cyclohexene.

A fresh approach based on strategy A was commenced with 3-methyl benzoic anhydride (17). The initial step involved its reaction with two different amines to afford a phthalimide (Scheme 11). First the choice of R was a methyl group, where the reaction of 3-methyl benzoic anhydride with 40% methylamine in water gave compound 32. The $^1$H NMR spectrum confirmed the formation of compound 32 by the presence of a singlet at 3.16 ppm which is consistent for a methyl next to a nitrogen atom. This reaction was attempted under both thermal and microwave conditions.$^{48,49}$ The thermal reaction gave a yield of 88% whereas the microwave mediated conversion gave only 40% yield. The same reaction was carried out with benzylamine (R = Bn) under the same thermal and microwave treatment to afford compound 33. Formation of compound 33 was supported by the presence of the methylene group found at 4.81 ppm, and the absence of amine protons which are usually found in the range of 4.5 to 1 ppm in the $^1$H NMR spectroscopy. Also the methylene peak appeared little bit more downfield which is expected since the product is an amide which are electron withdrawing groups compared to an amine. In this instance, the reaction gave higher yield under microwave (82%) compared to thermal
conditions (71%). In the past twenty years microwave reactions have become increasingly popular within the chemistry world. This is because a microwave provides energy that increase reaction yields while lowering reaction times and providing cleaner and more reproducible reactions.\textsuperscript{50} Therefore it is believed to be ideal for imide formation since water is being eliminated, knowing that microwaves work in such a manner that water absorbs most of its power thereby fueling the reaction.\textsuperscript{51} Thus a possible explanation for the lower yield obtained in the microwave assisted reaction forming 32 could be that, since the amine is in 40% water to start with, there will be significant absorption of microwave irradiation. The resultant heating the reactants may have the greatest effect on the methylamine, which could be driven from solution by the high temperature, before it even started reacting. Since the microwave reaction was carried out in DMF, the higher boiling point gave a large temperature range for the loss of methylamine and the reduced polarity of the solution, compared to pure water, permitted the more ready release of the amine. Reasonable yields were observed for 33, where the microwave conditions were superior to the thermal treatment. The advantage of protecting the nitrogen with the benzyl group is that the nitrogen can be readily deprotected through hydrogenation. It is anticipated that removal of the benzyl group will be required since hydrogen bonding of that nitrogen is vital to any inhibitor’s interaction with the active site of the PARP enzyme.

\begin{center}
\includegraphics[width=0.5\textwidth]{Scheme11}
\end{center}

\textbf{Scheme 11.} Formation of phthalimide 32 and 33 from 3-methyl benzoic anhydride via a thermal and microwave reaction. \( R = \text{Me, Bn} \) respectively.
The next step is an introduction of a bromine (Br) atom onto the methyl group of the aromatic ring. The insertion of the bromine is accomplished via a radical reaction, a method that is well established and reactions known to be selective for the benzylic position.\textsuperscript{52,53} Such reactions require a bromine atom source, which typically would come from Br\textsubscript{2} or NBS, a radical initiator which could be AIBN, H\textsubscript{2}O\textsubscript{2}, (PhCOO)\textsubscript{2}, and a suitable neutral solvent. Usually the brominations occur as chain reactions which require light or heat to initiate the formation of the free radicals, which in turn propagate the chemistry. There are plenty of articles in the literature which offer a variety of different procedures for radical reactions. Some use CCl\textsubscript{4} as a solvent, but they were immediately disregarded right away due to the high toxicity and limited access of CCl\textsubscript{4}.\textsuperscript{54} Many protocols use NBS as a bromine source and a variety of radial initiators with different solvents. None of these procedure findings suited our needs. This is because they either required light as a source to activate the initiator, and the downside of this is the time reaction as well the requirement of unusual light bulbs like Philips HPL-N.\textsuperscript{55,56} Moreover, some suggested solvents would not solubilise our substrate.\textsuperscript{57} Alternatively a procedure adapted from a current Schwan lab member, a PhD candidate Selim Hossain, was explored (Scheme 12). The procedure used \textit{N}-bromosuccinimide (NBS) as the source of Br atom, and azobisisobutyronitrile (AIBN) as the radical initiator to afford 34. Table 1 summarizes the different conditions that were tried, with trial 5 giving the best results, even though the yield was not ideal. This was due to the formation of a side product. The TLC of the crude reaction mixture always indicated two compounds, both of which were isolated and characterized. The top spot was the desired product whereas the bottom spot was the product of dibromination of the methyl group (35). Confirmation of the structure of 35 was supported by GC-MS and HSQC. Dibromination at the
benzylic position is not an uncommon side-product, and there is plenty of evidence to support this. Factors that could lead to the dibrominated species are high temperature and use of excess equivalents of the bromine source. As well, the substituents present on the aromatic ring also affect the reactivity of the benzylic position. Also it is essential to note that no bromination occurred at the methyl attached on the nitrogen. This result was expected since a benzylic hydrogen has a bond dissociation energy of 89 kcal/mol, where as a methyl hydrogen next to a nitrogen atom is anticipated to be higher.

Table 1: Evaluation of radical conditions for the synthesis of compound 34.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Equivalents</th>
<th>Conditions</th>
<th>Yield (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s.m. 1</td>
<td>Reflux, 8.5h</td>
<td>74 – crude&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>NBS 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AIBN 0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>s.m. 1</td>
<td>Reflux, 4h</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>NBS 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AIBN 0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>s.m. 1</td>
<td>Reflux, 3h</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>NBS 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AIBN 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>s.m. 1</td>
<td>Reflux, over night</td>
<td>53 – crude&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>NBS 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AIBN 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>s.m. 1</td>
<td>Reflux, 4h</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>NBS 1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AIBN 0.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> All reactions done in benzene  
<sup>b</sup> Purified product unless otherwise noted  
<sup>c</sup> Yields was estimated by analysis of <sup>1</sup>H NMR spectra of the crude reaction mixture
Ideal bromination conditions for the benzyl protected phthalimide (33) were explored as well and the results are summarized in Table 2. The synthesis of compound 36 was more problematic compared to the synthesis of compound 34. This is believed to be due to possible cleavage of the benzyl group after the undesired bromination of the methylene group. Such a reaction would eventually lead to formation of benzaldehyde, which was supported by the observation of a diagnostic, yet small peak at 10.0 ppm in the $^1$H NMR spectrum of the crude reaction mixture. Therefore the bromination reaction of the benzyl protected nitrogen (36) gave unselective results and this chemistry was further explored with only the methyl protected nitrogen (34). Nevertheless, the structures of both compounds, 34 and 36, were confirmed by the presence of a singlet peak at around 4.97 ppm and the absence of a singlet peak at around 2.67 ppm in the $^1$H NMR spectrum. The peak at 4.97 ppm belongs to the methylene group which is found well downfield due to being next to an electronegative atom such as Br. This peak confirmed that bromination took place at the aromatic methyl group since this peak was not observed in the $^1$H NMR spectrum.
Table 2: Evaluation of radical reaction conditions for the synthesis of compound 36.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Trial</th>
<th>Equivalents</th>
<th>Conditions</th>
<th>Yield (%)\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s.m. 1, NBS 1, AIBN 0.08</td>
<td>Reflux, overnight</td>
<td>33 – crude\textsuperscript{c}</td>
</tr>
<tr>
<td>2</td>
<td>s.m. 1, NBS 1, AIBN 0.04</td>
<td>Reflux, 4h</td>
<td>33 – crude\textsuperscript{c}</td>
</tr>
<tr>
<td>3</td>
<td>s.m. 1, NBS 1, AIBN 0.06</td>
<td>Reflux, 4.5h</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>s.m. 1, NBS 1.5, AIBN 0.12</td>
<td>Reflux, 4.5h</td>
<td>43 – crude\textsuperscript{c}</td>
</tr>
<tr>
<td>5</td>
<td>s.m. 1, NBS 2, AIBN 0.24</td>
<td>Reflux, 5h</td>
<td>43 – crude\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} All reactions done in benzene
\textsuperscript{b} Purified product unless otherwise noted
\textsuperscript{c} Yields was estimated by analysis of \textsuperscript{1}H NMR spectra of the crude reaction mixture

The above carried out bromination reaction in general gave poor yields, so other methods were evaluated to improve the outcome of the reaction. In the first method two light initiated radical reactions were performed, where different strengths of light were used as a radical initiator, and again NBS was used as a bromine source. The reaction conditions were as follows: the reactants were dissolved in water in 1:1 equivalents of starting material to NBS in both reactions. One reaction used 40 W incandescent light whereas the other used 150 W incandescent light, both reactions were let to stir overnight.\textsuperscript{56} No product formation was observed in the reaction that was irradiated with a 150 W incandescent light and the starting
material was recovered. On the other hand for the reaction irradiated with a 40 W incandescent light a 10% yield of the crude mixture was observed. This was calculated from the $^1$H NMR spectrum where the majority of the crude mixture was starting material and some indeterminate impurities were observed. These conditions did not give higher yields as compared to previous conditions thus were not further explored. In the second method, starting material, 3-methyl phthalic anhydride, was first brominated and then imide formation is to follow (Scheme 13). Unfortunately under these conditions bromination gave only 20% yield, and this reaction was also not further explored. Therefore both probationary methods did not provide improved results thus the originally envisioned synthetic strategy conditions were kept as adequate.

Scheme 13. Bromination reaction of 4-Methylisobenofuran-1,3-dione.

Once the bromination step was established, a sulfur-containing group was introduced by replacing the bromine. Originally this was envisioned to be accomplished via Rongalite® (sodium hydroxymethylsulfinate). Rongalite (37) is a salt which has applications as a reducing reagent. It is commonly used in preparation of sultines in the presence of two proximal halogens, Br and Cl preferably (e.g., Scheme 14). Thorough literature research with supportive data has confirmed that sulfones (Fig. 11) are not generally formed when a dihalogenated compound is reacted with Rongalite, which was favoured for our research since we did not want the formation of a sulfone but a sultine. 

33
Scheme 14. Rongalite reactivity, formation of benzodisultine as a general sample.

Figure 11. Benzodisulfone.

Our aim was to introduce Rongalite as a *mono* nucleophile, thereby forming a sulfinic acid (38) by displacement of the bromine atom on compound 34 as Scheme 15 shows. This would permit us to circumvent the oxidative fragmentation step of the originally envisioned synthetic strategy, described in section 1.5. Hence selective reduction of the proximal carbonyl could follow where the sulfinic acid (38) could be used as a directing group (Scheme 16). Thereafter, cyclization of compound 39 should occur spontaneously, or by the application of mild heat, to afford sulfinate ester 24 (Scheme 16). Several reactions were performed where brominated phthalimide, compound 34, was treated with Rongalite at room temperature in DMF as solvent. The reaction was monitored by TLC (100% EtOAc) which showed only very polar material and complete consumption of starting material. However, the mixture provided intractable constituents.
Scheme 15. Suggested product from the treatment of the brominated phthalamide 34 with Rongalite.

Scheme 16. Different approach to strategy A by the use of Rongalite. If the sulfinic acid is managed to be synthesized, selective reduction of the proximal carbonyl followed by cyclization are envisioned.

In order to simplify the chemistry a model compound benzyl bromide was evaluated under comparable condition, where the product would be expected to be α-toluenesulfinic acid (Scheme 17). Initially, the first attempt employed same conditions as for compound 34 mentioned above, whereas another reaction used PTC (phase transfer conditions) and both were carried out at room temperature. As well, the reactions were monitored by TLC and were worked up. Unfortunately both reactions did not give α-toluenesulfinic acid, both of their $^1$H NMR spectra indicated formation of many products which were intractable.
Research shows that Rongalite works best when used in the presence of two halogen atoms in the compound of interest. In our case the plan was to take a chance on the chemistry with only one halogen atom present in the molecule. Unfortunately this proved unsuccessful. Therefore it was concluded that Rongalite would not allow direct access to a sulfinic acid by simple substitution, and an alternative means had to be considered. Returning to the original plan of oxidative fragmentation as one of the synthetic steps, the compound selected to introduce a sulfur atom would be (4-methoxyphenyl)methanethiol (40). Scheme 18 represents the plan the synthesis of 40. Initially, compound 41 was synthesized from the commercially available p-methoxyltoluene, where the source of Br came from NBS and AIBN was the radical initiator. Again \(^1\)H NMR spectrum confirmed that the bromination step was successful since a methylene singlet peak was observed at around 4.51 ppm and no presence of a methyl peak at was evident at 2.3 ppm. Then potassium thioacetate was used to displace the halogen creating the sulfur containing thioacetate 42.\(^{66}\) The \(^1\)H NMR spectrum of the thiolacetate 42 shows that the methylene singlet peak was shifted a bit upfield to 4.05 ppm, compared to the brominated species 37 consistent with the bromine being slightly more electronegative than sulfur. Also the presence of a singlet at around 2.3 ppm is consistent with an acetyl peak. Other reagents were considered for the formation of the thioacetate 42, including benzyltriethylammonium tetrathiomolybdate \([\text{[BnEt}_3\text{N}]_2\text{MoS}_4]\),\(^{67}\) but they were discarded because potassium thioacetate
works best in neutral to alkaline conditions. Such conditions are favoured for our synthesis, since the PMB group may demonstrate some acid sensitivity. As well potassium thioacetate is more cost effective and it gave high yield.

![Scheme 18. Synthetic route of compound 40.](image)

It was anticipated that the coupling reaction of thioacetate 42 with bromide 34 would afford compound 43 (Scheme 19), where the thiolate would be formed in situ thereby decreasing the number of synthetic steps. Such a reaction with different but related substrates was established by Hossain et al. and it gave good yields. The conditions attempted were an excess of 2 M NaOH in cold 95% ethanol with stirring for 30 minutes at 0 °C. Three trials were performed according to these conditions on our substrates, but they did remove the acetate group and hence no coupling of the two substrates occurred; the reaction was not further explored. Two other conditions were attempted with different bases, triethylamine and potassium carbonate. Et₃N in DCM afforded only 36% yield of the product and gave the thiol (40) as a side product. Potassium carbonate in methanol accomplished the coupling reaction with 54% yield, but gave two side-products, a disulfide and loss of the Br atom from 34 to give the phthalimide 32. As well these reaction conditions were not further explored since the side-products made the isolation of the product challenging. Since these unsuccessful trials were consuming starting
material, the two step protocol involving solvolysis of the acetate group to afford a thiol was considered more feasible. Hence thioacetate 42 was treated with aqueous NaOH and EtOH, to remove the acetate group and access thiol 40. The formation of thiol 40 was supported by the $^1$H NMR spectrum where the methylene peak was observed at 3.70 ppm and the thiol proton was observed at 1.72 ppm. The methylene peak appeared as a doublet and the thiol proton appeared as a triplet which is evident that these groups are coupling to each other with a coupling constant of 7.4 Hz.

![Scheme 19](image)

**Scheme 19. In situ synthesis of compound 43 via a direct coupling of thioacetate 42 and bromide phthalimide 34.**

To continue with the synthetic scheme sought for strategy A thiol 40 was reacted with bromide 34 (Scheme 20) to afford compound 43 which was then ready for selective reduction. $^1$H NMR spectroscopy confirms the formation of compound 43. There was neither an observable thiol proton peak nor a methylene peak at around 3.70 ppm. However there were two new singlet peaks observed at 4.14 ppm and 3.69 ppm that represent the two methylene groups.

The reduction of compound 43 required the directed and selective conversion of the proximal carbonyl into an alcohol moiety (Scheme 21). This is desired since the hydroxyl group will eventually link to the sulfinyl group during oxidative fragmentation. Research by Speckamp and Hiemstra has demonstrated that geminally disubstituted succinimides are actually more prone to reduction at the more sterically hindered carbonyl group. Not surprisingly, the
regioselectivity is based on the substituent and the reducing agent used. Those researchers performed detailed analyses of different substituents and their effect on the ratio of product isomers. They found that in some cases the use of two different reducing reagents is much more efficient. For example, initial treatment was with an aluminium hydride reagent where its two hydrides coordinate with the oxygen atoms of the substrate of interest, followed by subsequent treatment with NaBH₄, completed reduction of the carbonyl group. In a different study Henderson *et al.* used the LUMO of an anhydride of interest to create an electron density map and demonstrated that the most electron deficient carbon would be one of the carbonyl groups of the anhydride that is proximal to another carbonyl, hence it is most susceptible for nucleophilic attack.

**Scheme 20.** Coupling reaction between the brominated phtalimide 34 and the thiol 40 to afford compound 43.

**Scheme 21.** Desired selective reduction of the proximal carbonyl group of compound 43.
Having this in mind, the selective reduction of 43 was not going to be an easy step to achieve, hence a variety of reducing reagents were evaluated as outlined in Table 3. More selective reducing reagents were desired since reduction of the proximal carbonyl group is needed. Sodium borohydride (NaBH₄) is the most unselective reducing reagent used and to our disappointment it did not work at all. It is not stable at low pH and it is known to decompose in the presence of acidic functional groups on the substrate. Solvents such as methanol and ethanol are suitable as long as there are no acidic functional groups present in the molecule and the reaction time is typically 30 minutes at room temperature. Our conditions were more extreme wherein the reaction was refluxed for a day and as well our substrate contains a PMB group which may be acid sensitive. Therefore taking all of this into consideration the fact that NaBH₄ did not reduce our substrate was not surprising. Zinc dust in acetic acid or in copper (I) bromide is an excellent reducing reagent for the conversion of carbonyl groups to their corresponding alcohol moiety. The downside is that it only works in acidic conditions, and hence was not desirable for our substrate. Nevertheless the chemistry was attempted but, unfortunately no reduction was observed and only the disulfide was isolated (Fig. 12). The disulfide structure was determined only by ¹H NMR spectrum since the compound was undesired thus it was not isolated. Therefore the disulfide structure supported by ¹H NMR spectrum, by the presence of the diagnostic and consistent aromatic peaks at 7.75 ppm to 7.55 ppm. Also a methylene singlet peak at 4.26 ppm and a methyl singlet peak at 3.16 ppm, which are as well diagnostic and consistent of such structure.
Aluminum based reducing reagents are more selective, and the two that were tried out included lithium tri-tert-butoxyaluminum hydride, LiAlH(O^tBu)_3, and diisobutylaluminium hydride, DIBAL. Unfortunately both did not work, despite ample precedent in the literature. Both are bulky and at low temperatures they proved unreactive, whereas at higher temperatures both reacted with the substrate but not by reducing a carbonyl group. At reflux LiAlH(O^tBu)_3 advocated the cleavage of the PMB group, and on the other hand DIBAL gave an unidentified side-product.

All of the above mentioned reducing reagents were anticipated to effect reduction of a carbonyl group, ideally the one proximal to the sulfanyl containing substituent. As an alternative, the Grignard reagent would insert an alkyl group on a carbonyl carbon also creating the requisite hydroxyl group. Neumann et al. has demonstrated that the lower the number of equivalents of the Grignard reagent used, the more likelihood of reduction at the carbonyl proximal to substitution in a related phthalimide.\textsuperscript{77} Scheme 22 presents that reaction, which gave 100% conversion when 2 equivalents of the Grignard reagent were used, as opposed to 3 or even 4
equivalents. Hence the alternative choice of Grignard chemistry to create the desired alcohol was an attractive one. However, many factors contribute to an effective reaction. First of all our substrate differs than theirs, since we have a PMB substituted thiomethyl group attached to the aromatic ring. In addition, the Grignard reagent of choice is MeMgBr since it would create the smallest steric effect for subsequent Diels-Alder chemistry. So unfortunately the reactions performed with the Grignard reagent (MeMgBr) were not successful, they did not react with our substrate under mild or harsh conditions.

Scheme 22. Reduction of the proximal carbonyl of N-methyl-4-aminophthalimide with hexylmagnesium bromide, gave 100% conversion with 67% yield.
Table 3. Reduction of compound 43 by a variety of reducing reagents.

<table>
<thead>
<tr>
<th>Reducing reagent</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Equivalents</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaBH₄</td>
<td>MeOH</td>
<td>r.t. 16h</td>
<td>1 : 2</td>
<td>Starting material recovered</td>
</tr>
<tr>
<td>NaBH₄</td>
<td>MeOH</td>
<td>Reflux, 24h</td>
<td>1 : 2</td>
<td>Starting material recovered</td>
</tr>
<tr>
<td>1. Zn, CuBr 2. 3N HCl</td>
<td>THF</td>
<td>1. Reflux, 4h</td>
<td>1 : 3</td>
<td>Sulfur dimer</td>
</tr>
<tr>
<td>LiAlH(O²Bu)₃</td>
<td>THF</td>
<td>-78 °C, 2h → 0 °C, 2h</td>
<td>1 : 1</td>
<td>Starting material recovered</td>
</tr>
<tr>
<td>LiAlH(O²Bu)₃</td>
<td>THF</td>
<td>0 °C → r.t. 6h</td>
<td>1 : 1</td>
<td>Starting material, PMB fragment</td>
</tr>
<tr>
<td>LiAlH(O²Bu)₃</td>
<td>THF</td>
<td>0°C → r.t. 2h, reflux, 6h</td>
<td>1 : 1</td>
<td>No methylene peaks observed in ¹H NMR</td>
</tr>
<tr>
<td>DIBAL</td>
<td>THF</td>
<td>-78 °C → 0 °C, 3.5h</td>
<td>1 : 1.2</td>
<td>Starting material by TLC</td>
</tr>
<tr>
<td>DIBAL</td>
<td>THF</td>
<td>0 °C → r.t. over night</td>
<td>1 : 1.2</td>
<td>Starting material, another spot on TLC</td>
</tr>
<tr>
<td>DIBAL</td>
<td>THF</td>
<td>0 °C → reflux over night</td>
<td>1 : 1.2</td>
<td>Starting material, unknown side-product</td>
</tr>
<tr>
<td>MeMgBr</td>
<td>THF</td>
<td>-78 °C, 3h</td>
<td>1 : 1.5</td>
<td>Starting material recovered</td>
</tr>
<tr>
<td>MeMgBr</td>
<td>THF</td>
<td>-30 °C, 3.5h</td>
<td>1 : 1.5</td>
<td>Starting material recovered</td>
</tr>
<tr>
<td>MeMgBr</td>
<td>THF</td>
<td>0 °C, 3.5h</td>
<td>1 : 1.5</td>
<td>Starting material recovered</td>
</tr>
<tr>
<td>MeMgBr</td>
<td>THF</td>
<td>r.t. over night</td>
<td>1 : 1.5</td>
<td>Starting material recovered</td>
</tr>
<tr>
<td>MeMgBr</td>
<td>THF</td>
<td>Reflux, over night</td>
<td>1 : 1.5</td>
<td>Starting material recovered</td>
</tr>
</tbody>
</table>
Due to the fact that substrate 43 was not amenable to carbonyl reduction or addition, and also since it is very challenging to produce in significant amounts, model substrate 32 was chosen for the reduction and Grignard trials (Scheme 23). As such compound 32 was subjected to variety of reducing reagents as outlined on Table 4. Reduction with the LiAlH(O\text{t}Bu)_3 and DIBAL were unsuccessful and at high temperatures did react with the starting material, as with the original substrate, but the products were intractable. Reaction with the Grignard reagent gave a product where the carbonyl group was converted into an alkene (44), (Fig.13). The $^1$H NMR spectrum supported this structure by the presence of two peaks at 5.10 ppm and 4.75 ppm, which are within the range of vinylic protons. This could be due to the fact that the reaction was quenched with 3 N HCl, where the acid contributed a proton to the hydroxyl group making it a much better leaving group. Perhaps if the work-up was done in less acidic conditions and chromatography was performed under basic medium, an alcohol could have been isolated. A selective gradient NOESY NMR experiment, confirmed that the proximal carbonyl was untouched, (Appendix, Fig. 1). One of the vinyl hydrogens was irradiated at 37 ºC. The experiment showed that there were no interactions between the vinyl hydrogens and the methyl hydrogens, but there was an interaction between the vinyl hydrogens and their proximal hydrogen on the aromatic ring. Since the methyl substituent of 32 does not possess the ability to precomplex the Grignard reagent, are preferred reduction of the unwanted carbonyl was not of a great surprise, even though a $^1$H NMR spectrum of the crude reaction did show the other isomer it was clearly the minor product. Hence a Grignard reagent does deliver promising results, adjustment of the reaction conditions would be required to afford an effective reaction to occur.
Scheme 23. Selective reduction of 32, as a model compound. R = H or Methyl, depending if reducing or alkylating reagent is selected.

To our greatest surprise NaBH₄, normally the least selective reducing reagent used, gave reduction at the desired position (Fig. 14). The only difference was the solvent, methanol was used previously where 95% ethanol had been used. The structure of compound 45 was confirmed by the ¹H NMR spectrum where two doublet peaks appear at 5.70 ppm and at 2.23 ppm. These two peaks belong to the proton of the pentacyclic ring and to the proton of the hydroxyl group respectively. Clearly they couple to each other since we observe two doublets and their coupling constant is 11.4 Hz. Also the assignment was supported by 2D NMR and a selective gradient NOESY (Appendix, Fig. 2). The proton on the tertiary carbon was irradiated at room temperature and hydrogen interactions were detected between the irradiated proton with the two methyl group protons. This was not a clean reaction since a side-product was obtained but was not characterized. Also a 16% yield of compound 45 after purification is quite low, but nevertheless the reaction deserves further scrutiny.

Figure 13. Methylation of compound 32 with a Grignard reagent, MeMgBr, afforded the alkene 44.
Table 4. Reduction of compound 32 by a variety of reducing reagents.

<table>
<thead>
<tr>
<th>Reducing reagent</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Equivalents</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiAlH(OtBu)_3</td>
<td>THF</td>
<td>0 ºC, 3h</td>
<td>1 : 1.1</td>
<td>Starting material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r.t. 3h</td>
<td></td>
<td>Starting material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reflux, over night</td>
<td></td>
<td>Intractable product</td>
</tr>
<tr>
<td>MeMgBr</td>
<td>THF</td>
<td>-30 ºC, 3h</td>
<td>1 : 1.5</td>
<td>Side-product, Compound 44</td>
</tr>
<tr>
<td>DIBAL</td>
<td>THF</td>
<td>-78 ºC, 3h → r.t. over night</td>
<td>1 : 2</td>
<td>Starting material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reflux, 6h</td>
<td></td>
<td>Intractable mixture</td>
</tr>
<tr>
<td>NaBH_4</td>
<td>Ethanol</td>
<td>r.t. 1h</td>
<td>1 : 2</td>
<td>Desired product</td>
</tr>
</tbody>
</table>

Figure 14. Reduction of compound 32 with NaBH_4 afforded the desired reduction of the proximal carbonyl providing the alcohol compound 45.

2.2 Strategy B

Concomitant with strategy A, strategy B was explored. As outlined on Scheme 7 this pathway commences the synthesis with commercially available 2-bromo-3-methylbenzoic acid (25). The carboxylic acid (25) was converted to an amide via its corresponding acid chloride (26), (Scheme 24). Formation of the acid chloride was supported by the ^1^H NMR spectrum where no presence of the carboxylic acid proton was observed, which if present should have appeared somewhere in the range of 13 - 10 ppm. Such reactions are well established since acid chlorides are the most reactive carboxylic acid derivatives, thus one converts the hydroxyl group
into a better leaving group to afford facile formation of an amide. For the purpose of our project two different amines were reacted with acid chloride 26, to afford two different amides, a primary amide 46 and a secondary amide 47 where the second substituent is a methyl group. Reactions with both amines proceeded well and provided amides in high yields. The formation of the primary amide 46 was supported by the presence of two peaks in the $^1$H NMR spectrum. These peaks show as singlets at 7.82 ppm and 7.52 ppm and correspond to the amide protons. While the formation of the secondary amide 47 was supported by two peaks in the $^1$H NMR spectrum, which differ from compound 46. A broad peak is observed at 5.79 ppm corresponds to the proton of the amide. This peak is quite upfield compared to the primary amide, since it is more shielded because the nitrogen atom of the secondary amide bears a methyl group. Also a peak at 3.01 ppm corresponds to the methyl of the amide. It is observed more downfield as opposed to a alkyl methyl group since the amide is an electron withdrawing group which pools electron density away from the methyl protons thereby deshielding them.

**Scheme 24.** First two beginning steps of strategy B synthetic route.

Following amide formation is the introduction of a formyl group on the nitrogen atom to afford an imide. This step was accomplished with $N,N$–dimethyl formamide dimethyl acetal which was the source of the formyl group (Scheme 25). A procedure from a patent was adopted
and the reaction worked well when \(N,N\)-dimethyl formamide dimethyl acetal was reacted with the primary amide \(46\). However, the protocol did not work when reacted with secondary amide \(47\).\(^8\) The \(^1\)H NMR spectrum of imide \(48\) showed presence of a formyl proton at 10.4 ppm. As well the proton on the nitrogen was observed more downfield at 9.13 ppm on the imide \(48\), in contrast to that of amide \(46\), which appeared at 7.82 ppm and 7.52 ppm. This is expected since the proton of the imide is more deshielded compared to the amide protons due to the presence of two carbonyl groups which pool electron density away from it.

![Scheme 25](image)

**Scheme 25.** Imide synthesis via amide reacted with \(N, N\)-dimethyl formamide dimethyl acetal. \(R = H, \text{Me}\).

We were curious as to why one amide worked over the other and hence we investigated a plausible reaction mechanism which is represented in Scheme 26. By looking at the reagent \(N,N\)-dimethyl formamide dimethyl acetal and the fact that the reaction was first heated for 5 minutes at 120 °C, it was observed that these conditions resemble Vilsmeier-Haack reaction. In the Vilsmeier-Haack reaction the components are DMF and POCl\(_3\).\(^8\) When both are reacted a chloroiminium ion is formed which is also known as Vilsmeier reagent (Scheme 14), and as an electrophilic molecule, it is attacked by nucleophiles.\(^83,84\) As well, the Vilsmeier-Haack reaction does not occur at room temperature but requires at least 100 °C and sometimes higher temperatures near 135 °C are required.\(^84,85\) Therefore regarding \(N,N\)-dimethyl formamide dimethyl acetal, the thermal treatment of 5 minutes at 120 °C probably meant loss of one of its
methoxy groups and afforded an equilibrium between itself and an electrophilic iminium ion (Scheme 27). This iminium cation is available for nucleophilic attack from the nitrogen on the amide species (46) affording intermediate 49. This result in formation of another electrophilic iminium cation by loss of the second methoxy group, which most likely leads to an equilibrium between intermediate 49 and intermediate 50. At this point the reaction is taken from the heat and it placed at 0 °C where a solid falls from solution. After 15 minutes, the solid, which is most likely iminium salt (e.g., intermediate 50), is dissolved in 70% acetic acid and 30% water to complete conversion to the imide. This portion of the chemical transformation possesses similarities to the Bouveault aldehyde synthesis, where a disubstituted formamide, such as DMF, is reacted with Grignard reagents to prepare an aldehyde (Scheme 28). As Scheme 15 shows, DMF is reacted with a Grignard reagent to afford the intermediate which upon hydrolysis gives the aldehyde of interest. In our case, intermediate 50 is hydrolyzed by water to eventually afford the formyl containing imide (48).
Scheme 26. Proposed mechanism for the reaction of an amide 46 with N,N-dimethyl formamide dimethyl acetal towards the formation of imide 48.

Even though a plausible mechanism for the conversion of the primary amide 46 to imide 48 is been proposed, it is not clear why the secondary amide 47 did not react under the same conditions. One can only suggest that the presence of the methyl group provides steric impendence of the nitrogen attack of the initial methoxy iminium intermediate. It is also possible that the ortho bromine brought about conformational restriction slowing down the desired chemistry, a restriction that was not in place in the absence of the methyl group. The time of the
initial $N,N$–dimethyl formamide dimethyl acetal treatment was extended, but no success was achieved for the $N$-methylated substrate.

Scheme 27. Formation of Vilsmeier reagent from DMF and phosphorus oxychloride.

Scheme 28. Bouveault aldehyde synthesis reaction.

Once the imide species was isolated it was subjected to a cyclization reaction to afford an isoindolinone core with an alcohol moiety. This was envisioned to be accomplished via a lithium-halogen exchange reaction, which are well established reactions in the domain of synthetic organic chemistry. Since compound 48 contained bromine a possible Li-Br exchange was envisioned and the resulting lithiated aromatic species would attack the formyl group in an intramolecular manner to eventually lead to a cyclized product possessing an alcohol.

Halogen–metal exchange reactions work on the basis of the relative electronegativity of the halogen which allows for it to undergo a nucleophilic attack by the anion of the organometallic compound. Therefore once the halogen is removed the metal atom is
preferentially positioned on the most electronegative carbon based on the inductive effect of the aromatic ring.\textsuperscript{88} Hence the placement of the substituents on the aromatic ring plays a role in the halogen reactivity. These reactions are in equilibrium, since they occur very quickly even at low temperatures, they are reversible reactions and the organometallic intermediate is unstable.\textsuperscript{89} It is important to note that most of the research done on the regioselectivity of the BuLi species is based on dibrominated aromatic species. Consequently for compound \textit{48} the bromine atom is sought to be removed by the \textit{n}BuLi and an anion to be formed which would undergo a nucleophilic attack towards the formyl group on the imide functionality. This will lead to formation of a pentacyclic ring which is more favoured over a tricyclic rings due to ring strain. Even though lithium-halogen exchange reactions are common, no previous work has been done on compounds such as ours, where lithiation at the halogenated position will \textit{in situ} lead to a ring formation. Somewhat relevant work has been done by Smith \textit{et al.} where they used 3-(2-methoxybenzyl)-1,1-dimethylurea treated it with \textit{t}BuLi and managed to cyclize it.\textsuperscript{90} As shown on Scheme 29 there is no halogen present on this molecule hence this is a metalation reaction which mechanistically works on the same principle as the metal-halogen exchange reactions.\textsuperscript{87} But the latter reaction nevertheless provided reasonable precedent for the work at hand as there is a nucleophilic attack on the carbonyl carbon by the anion formed on the aromatic ring.

\textbf{Scheme 29.} Lithiation of 3-(2-methoxybenzyl)-1,1-dimethylurea and subsequent cyclization.
A variety of conditions were explored using conditions similar to a procedure carried out by Nicolaou et al. A number of reaction conditions were executed and the results are summarized in Table 5.91

Table 5. Lithium-bromine exchange of compound 48 and ultimately cyclization and formation of an alcohol containing species (51).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Equivalents</th>
<th>Conditions</th>
<th>Mass recovery (%)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s.m. 1 nBuLi 1</td>
<td>0 °C, 3.5h</td>
<td>84</td>
<td>Messy 1H NMR, 4 spots on the TLC</td>
</tr>
<tr>
<td>2</td>
<td>s.m. 1 nBuLi 1.3</td>
<td>-78 °C, 0.5h → -30 °C, 2.5h</td>
<td>92</td>
<td>Messy 1H NMR, 4 spots on the TLC</td>
</tr>
<tr>
<td>3</td>
<td>s.m. 1 nBuLi 1.3</td>
<td>-78 °C, 3h</td>
<td>&gt; 100</td>
<td>Messy 1H NMR, 4 spots on the TLC</td>
</tr>
<tr>
<td>4</td>
<td>s.m. 1 nBuLi 2</td>
<td>-78 °C, 0.5h → 0 °C, 1.5h</td>
<td>81</td>
<td>Messy 1H NMR, 3 spots on the TLC where one is s.m. recovered in 5%</td>
</tr>
<tr>
<td>5</td>
<td>s.m. 1 nBuLi 1 MeLi 1</td>
<td>-78 °C, 1h</td>
<td>62</td>
<td>Messy 1H NMR, 2 spots on the TLC</td>
</tr>
<tr>
<td>6</td>
<td>s.m. 1 nBuLi 1 NaH 1</td>
<td>-78 °C, 0.5h → 0 °C, 1.5h</td>
<td>88</td>
<td>Messy 1H NMR, 3 spots on the TLC where one is s.m. recovered in 6%</td>
</tr>
<tr>
<td>7</td>
<td>s.m. 1 nBuLi 1 MeLi 1</td>
<td>-78 °C, 1h → 0 °C, 3.5h</td>
<td>32 ′</td>
<td>Product isolated but not pure</td>
</tr>
</tbody>
</table>

a Mass recovery yield of crude mixture
b Mass recovery yield of product

As the first four entries of Table 5 show, this proved to be a troublesome reaction where material was not isolated. One consideration that may be preventing the success of the reaction is
the existence of the acidic proton on the nitrogen. It was viewed that a simple proton transfer to the aryl group might be preventing the desired cyclization, where such compound as presented on Figure 15 has not been observed.

![Figure 15](image)

**Figure 15.** Postulated removal of acidic proton might lead to the formation of anion species.

On the thinking that prior deprotonation of that N-H proton may circumvent the problem, initial treatment of 48 with a base such as NaH or MeLi was proposed to remove the proton from the nitrogen. Hence the organolithium formed by lithium halogen exchange would be able to perform nucleophilic attack on the formyl carbon. Indeed trial 7 is the only reaction conditions that gave the desired product but the product could not be obtained pure (Fig. 16). The isolated mixture was first recrystallized from toluene and then the solid residue obtained was purified by column chromatography. Additional purification would be required making the overall protocol a very tedious and time-consuming process, only to give product in low yield. However, the pre-treatment with base did give product and the interpretation is that the hydrogen on the nitrogen atom was interfering with the cyclization. An alternative approach would be to protect the nitrogen.
Figure 16. Lithium-bromine exchange of compound 48 afforded the desired impure product 51.

As an easy approach to the problem, treatment of amide 47 with \( N,N \)-dimethyl formamide dimethyl acetal was attempted, but the reaction failed to deliver the desired imide 52. A more typical protection approach was then considered (Scheme 30) where the proton of the nitrogen was removed by a base, and iodomethane was used to introduce the methyl group on the nitrogen.\(^9\) \( N \)-Methylations are well established reactions in the area of organic synthetic chemistry, where methyl halides or dimethyl sulfates are conventionally used as methylating agents. Recent findings also show dimethyl carbonate can be used as well, and is preferred since it is more environmentally friendly because it is not a toxic reagent.\(^9\) Regardless of such findings, for our purposes we selected MeI as the methylating agent since it was a straightforward reaction which provided us with the desired product in high yield. The choice of protecting the nitrogen with a methyl group was due to the fact that it was manageable for us since a considerable amount of chemistry has been done in strategy A with the \( N \)-methyl moiety. The structure of the \( N \)-methylated 52 was confirmed by \(^1\)H NMR where the imide proton at 9.13 ppm was lost but the presence of a singlet peak at 3.25 ppm was consistent with a methyl group on a nitrogen atom. Once the nitrogen was protected the lithium-halogen exchange reaction was tried on imide 52 (Scheme 30). Again, different conditions were explored as outlined on Table 6. As denoted in the table, the \( N \)-methylated imide gave superior results compared to the
unmethylated compound. The obtained desired product was pure but in very low yield and in the first trial a side product was obtained and it was identified as compound 47, which presumable formed by cleavage of the formyl group. The formation of the alcohol species 45 was easily observable in the $^1$H NMR spectrum where two doublet peaks appeared at 5.70 ppm and at 2.23 ppm. These two peaks belong to the proton of the pentacyclic ring and to the proton of the hydroxyl group respectively. Clearly they couple to each other since we observe two doublets and their coupling constant is 11.4 Hz. Also the $^{13}$C NMR spectrum shows absence of the formyl NMR peak which if present should appeared at around 170 ppm. The formation of the side-product was supported by an EI mass spectroscopy. Also the equivalents of the nBuLi did not matter since the product yield did not change, as well more of the starting material was recovered as presented by the ratio of product to starting material.

![Scheme 30. Methylation of imide 48 followed by halogen-metal exchange of compound 52 to afford a cyclizide species 51.](image)

**Table 6.** Lithium-bromine exchange of compound 52 and ultimately cyclization and formation of an alcohol containing species 45.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Equivalents s.m. : nBuLi</th>
<th>Conditions</th>
<th>Results</th>
<th>Ratio prod : s.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 : 1.3</td>
<td>-78 ºC, 30min → -30 ºC, 2.5h</td>
<td>Product in 4% yield, side product 47</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>1 : 1.3</td>
<td>-78 ºC, 30min → -30 ºC, 3h → 0 ºC, 3h</td>
<td>Product in 8% yield, presence of starting material</td>
<td>1 : 7</td>
</tr>
<tr>
<td>3</td>
<td>1 : 2.2</td>
<td>-78 ºC, 30min → -30 ºC, 3h → 0 ºC, 3h</td>
<td>Product in 8% yield, presence of starting material</td>
<td>1 : 9</td>
</tr>
</tbody>
</table>
Following the cyclization of the imide and thereby introduction of an alcohol at the carbon of the formyl group, the next step in the synthetic strategy B is bromination of the methyl group on the aromatic ring of the compounds 45 and 51. The conditions for bromination were same as used for the synthetic strategy A above. NBS was the bromine source and AIBN was the radical initiator. Bromination of compound 51 gave an intractable mixture as indicated by $^1$H NMR analysis. Bromination of compound 45 gave a side-product that turned out to be compound 32, which means that the hydroxyl group underwent oxidation to a carbonyl. It is well documented in the literature that NBS oxidizes secondary alcohols into their corresponding ketones. There are some kinetic analyses of the mechanism, but unfortunately these are only proposed mechanistic analyses (Scheme 31). Venkatasubramanian et al. proposed a cyclic mechanism where the Br abstracts the hydrogen from the alcohol group, which pushes the electrons towards a double bond formation, forming a carbonyl. This leads to a loss of the secondary hydrogen in the form of a hydride ion, which is believed to be the rate determining step. On the other hand Sharma et al. via a radical reaction are proposing two possible intermediates which are formed due to an abstraction of a hydrogen from either the hydroxyl group or the carbon bearing the hydroxyl group by bromine radical. Although those authors refrained from committing to a particular mechanism, the most likely radical mechanism is going via the first intermediate (Scheme 31B) since the bond dissociation enthalpy of the hydrogen-oxygen bond of a hydroxyl group is approximately 110 kcal/mol, substantially higher than the one of the hydrogen-carbon bond, which resides near 88 kcal/mol.
Scheme 31. As presented in the original articles. Two possible mechanistic analyses for the oxidation of secondary alcohols with NBS. A) Cyclic mechanism. B) Radical induced mechanism. Note: R, R’ = alkyl.

The bond enthalpy analysis presented above suggests the reason why the radical bromination chemistry of compound 45 affords a side-product. Since 45 bears a secondary alcohol, from these results we extracted that before we go ahead with the bromination step protecting the alcohol group is essential. A literature search was undertaken to determine the possible protecting groups (PG) that could be investigated. Selection of the proper protecting group also had to be congruent with the requirement to introduce the PMB group following bromination. Scheme 32 presents a general synthetic route about the way of utilizing the protecting group first (53), followed by bromination of the methyl group on the aromatic ring (54). Then subsequent introduction of the sulfur atom (55) and followed by deprotection of the alcohol. The rest of the steps would be the same as outlined on Scheme 7, section 1.5. It was
deemed logical to introduce a sulfur atom prior to deprotection of the hydroxyl group. Since it was suspected that the PMB group would be acid sensitive, choosing a PG that is removed under basic conditions, mild acid or selective redox conditions would be ideal. There are a few choices of PGs that should be explored. A tosyl group was considered since it is base sensitive so its removal would not perturb the PMB group. Another choice would be protection of the alcohol with a benzyl group. Benzyl is removed in neutral conditions with H₂/Pd-C, which as well will not interfere with the PMB group. However, given the desire to selectively perform a benzylic radical bromination, the introduction of additional benzyl hydrogens would surely introduce additional problems.

Scheme 32. Different approach to strategy B by first utilizing a protecting group followed by bromination and then introduction a sulphur. PG = Tosyl, Bn, TMS.

The trimethylsilyl (TMS) group is a common alcohol protecting group which is removed
by treatment with various fluoride sources often under aprotic conditions. Also a recent study has shown that TMS could be introduced onto a hydroxyl group when reacted with hexamethyldisilazane (HMDS) in the presence of NBS while only using the latter in catalytic amounts. It is imperative to note that none of the alcohol protecting reactions have been performed, thus for future research this will be the next step to further develop the investigation of this synthetic strategy B. As well by protecting the alcohol group it will be firmly established if indeed the hydroxyl group is the problem or the hydrogen on the carbon bearing the hydroxyl group is the problem when trying to brominate the methyl group on the aromatic ring. If it is the latter than unfortunately this pathway will be disregarded since is problematic towards the synthesis of our desired diene. If so, placement of an acetyl group instead of a formyl group on the imide species may solve the problem.

2.3 Alternative strategy

Unfortunately strategies A and B offered a number of obstacles which led to rethinking the originally envisioned synthetic plans. Selective reduction of the proximal carbonyl group in the initial plan proved difficult trouble and Li-Br exchange and behaviour of the resultant organolithium both did not proceed as intended. During that chemistry a new strategy came to light, one based on a paper by Newman et al. which led to an altered version of the two previously synthetic approaches, and is shown on Scheme 33. In this pathway the order of the steps were somewhat reversed. As a first step, the PMB group is introduced onto 2-furymethanethiol to afford compound 56, which is then subjected to a Diels-Alder chemistry with maleic anhydride (or imide) forming an oxygen bridge cycloadduct (57). That material could formally undergo loss of water to generate the desired aromatic compound (58). If an
anhydride is used for the dienophile, the aromatic compound is a phthalic anhydride with the sulfur atom already present and protected with the PMB group. This compound is therefore envisioned to parallel strategy A in its synthesis route towards the desired diene. Thus, the phthalic anhydride (58) will be treated with an amine to afford a phthalimide (43). Next will be selective reduction of the proximal carbonyl, followed by an oxidation of the sulfur atom, and subsequent oxidative fragmentation in the presence of a base to afford in situ formation of the desired diene (16), as already outlined previously.

Scheme 33. Alternative synthetic strategy. R = H or Me; R” = H, Me, alkyl

The first step of the current synthetic plan was coupling of the two substrates 2-furylethanethiol and compound 41 to afford compound 56. This reaction was accomplished in the presence of triethylamine as a base, using conditions that were well established in synthetic strategy A. The introduction of the PMB group was confirmed by the 1H NMR spectrum where the methylene peak of compound 41 that shows at 4.51 ppm was absent and it appeared more upfield at 3.60 ppm. The shift is reasonable since sulfur is less electronegative than bromine. The
next step was the Diels-Alder reaction between compound 56 and maleic anhydride. Initially, the reaction conditions as performed by Newman et al. were followed, but unfortunately those conditions were not successful for the two substrates. Therefore, varieties of conditions were explored and are outlined on Table 7.

Table 7. Different reaction conditions towards the synthesis of compounds 57.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Equivalents</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56 1 M.A. 1.1 H.Q. 0.02</td>
<td>Ether</td>
<td>r.t. 4h</td>
<td>Some presence of product</td>
</tr>
<tr>
<td>2</td>
<td>56 1 M.A. 1.1 H.Q. 0.02</td>
<td>Ether</td>
<td>r.t. 8h</td>
<td>Compound 56 recovered</td>
</tr>
<tr>
<td>3</td>
<td>56 1 M.A. 1.1 H.Q. 0.02</td>
<td>Ether</td>
<td>Reflux, 1 day</td>
<td>Compound 56 recovered</td>
</tr>
<tr>
<td>4</td>
<td>56 1 M.A. 1.5 H.Q. 0.04</td>
<td>Ether</td>
<td>r.t. 1 week</td>
<td>20% product</td>
</tr>
<tr>
<td>5</td>
<td>56 1 M.A. 1</td>
<td>Ether</td>
<td>r.t. 26h</td>
<td>Compound 56 recovered</td>
</tr>
<tr>
<td>6</td>
<td>56 1 M.A. 1</td>
<td>Ether</td>
<td>r.t. 24h → 0 °C, 1h</td>
<td>5% product</td>
</tr>
<tr>
<td>7</td>
<td>56 1 M.A. 1</td>
<td>Ether</td>
<td>Reflux, 2h → r.t. over night</td>
<td>Compound 56 recovered</td>
</tr>
<tr>
<td>8</td>
<td>56 1 M.A. 1</td>
<td>Toluene</td>
<td>80 °C, over night → Reflux, 5 days</td>
<td>5% product</td>
</tr>
<tr>
<td>9</td>
<td>56 1 M.A. 1.2 TiCl₄ 2</td>
<td>DCM</td>
<td>-78 °C, 1h → r.t. 16h</td>
<td>PMB group cleaved</td>
</tr>
</tbody>
</table>

Note: Maleic anhydride (M.A.); Hydroquinone (H.Q.)
Trials 1-4 were performed following Newman *et al.* procedure, with slight adaptations. However, since the desired cycloaddition did not occur the conditions were varied to prompt the cycloaddition. Since in the original work they stated that they never explored the reaction without hydroquinone, trial 5 was run without hydroquinone and it was determined that indeed hydroquinone is required for this reaction to occur. In organic synthesis hydroquinone is usually added as a radical interceptor.\(^{102}\) Also it is being applied to many biological and industrial processes such as paper manufacturing, photographic developing, coal-tar production and inhibits Topoisomerase II, which is a nuclear enzyme that is important in DNA replication.\(^ {103,104}\) Trial 6 was performed by combining the procedure outlined by Pelter *et al.* and Jung *et al.* The difference was the amount of equivalents used per substrate, and temperature was changed by first having it higher and afterwards cooling it, as opposed to keeping it constant throughout the full reaction.\(^ {105,106}\) Trial 7 differs from trial 6 in the sense that the reaction was first refluxed and then cooled to room temperature as suggested by Buttery *et al.*\(^ {107}\) Trial 8 followed the original conditions but had a different solvent on the premise that a low yield might be due to the solvent used. Trial 9 used a Lewis acid, TiCl\(_4\), which led to cleavage of the PMB group.\(^ {108}\) This was somewhat expected since we already suspected that the PMB group may be acid sensitive. On analysis of the results shown in Table 7, the most promising reaction conditions were given by trial 4, which produced compound 57 in 20% yield (Scheme 34). The synthesis of compound 57 was supported by the \(^1\)H NMR spectrum. First by the absence of one of the alkene peaks from compound 56 and the two alkene peaks from maleic anhydride. Second appearance of three tertiary carbon hydrogen peaks at 5.34 ppm, 3.54 ppm and 3.39 ppm. This is due to the presence of two carbonyl groups and an oxygen atom, which pool electron density away from the hydrogens thereby dishielding them.
Scheme 34. Synthesis of compound 57 via a Diels-Alder reaction between compound 56 and maleic anhydride.

The subsequent step of this synthetic procedure was an aromatization of the cyclohexene ring of compound 57 (Scheme 35). According to the Newman et al. procedure this reaction is accomplished with concentrated H$_2$SO$_4$ which is an excellent dehydrating reagent.\textsuperscript{109} This property under the appropriate conditions brings about the loss of water from the bridging cycloadduct to deliver the phthalic anhydride. Given that TiCl$_4$ treatment of the PMB-substituted furan provided problematic, there were concerns that the strong acid treatment to effect dehydration of 57 could also bring about loss of the PMB group.

Scheme 35. Aromatization of compound 57 under acidic conditions.

The reaction was nevertheless still explored, but the crude reaction residue was an intractable mixture. The TLC of this reaction mixture (1:1, hexanes/EtOAc) showed one spot at the base line. Also the $^1$H NMR spectrum supported the cleavage of the PMB group since no
peaks were observed in the aromatic region at around 7.26 ppm. Thus, probably the PMB group got cleaved contributing to some kind of undesired salt being formed. As well the $^1$H NMR spectrum did not support a recovery of the starting material. Working under the assumption that the PMB group was indeed problematic in this reaction sequence, the order of the synthetic steps was changed. The idea was first to aromatize and then to introduce the PMB group. Therefore Scheme 36 outlines the revised order of steps where there is no protecting group at all on the sulfur. As such, a Diels-Alder reaction between 2-furylmethanethiol and maleic anhydride, followed by aromatization of the cyclohexene ring and then introduction of the PMB group onto the aromatized compound was proposed.

Scheme 36. Revised order from the originally envisioned Alternative strategy.

The Diels-Alder reaction worked well following the procedure developed by Newman et al. affording compound 59 in 72% yield. The presence of the tertiary carbon alkane protons at 5.33 ppm, 3.58 ppm and 3.50 ppm in the $^1$H NMR spectrum confirmed completion of the Diels-Alder. Obviously this reaction gave a much higher yield compared to compound 57, where 20% yield was afforded by the Diels-Alder reaction between the maleic anhydride and the furan with the PMB group attached. It can be reasoned that the PMB group might add a steric barrier to the Diels-Alder reaction hence lowering the yield. Also Boutelle et al. looked at different
substituents on furan and how they affect the cycloaddition with maleimide. Among the few of the substituents they explored 2-methoxyfuran which can act as a comparison model to our substrate of interest since it is an electron donating group and both of our substituents, the thiol and the alkylthio group, are electron donating groups. 2-Methoxyfuran lowered the free energy of formation for the endo product by 4.8 kcal/mol, and for the exo product by 2.4 kcal/mol compared to the unsubstituted furan. This supports our data for obtaining low yields in the Diels-Alder reactions with the furan species since it is a reversible reaction. The retro-Diels-Alder is more favourable by looking at the data presented by Boutelle et al. therefore contributing to lower yields obtained in our research.

The subsequent aromatization of compound 59 was carried out by the same conditions as previously discussed, with concentrated H$_2$SO$_4$, and it indeed generated the desired aromatic compound 60. Synthesis of the aromatic species was confirmed by the $^1$H NMR spectrum where three peaks were observed in the aromatic region, in the range of 8.6 ppm to 7.8 ppm. These peaks appear more downfield, opposed to an unsubstituted benzene, due to resonance effect caused by the two electron withdrawing carbonyl groups. By accomplishing the aromatization of compound 59 which it does not contain a PMB group, one may conclude that it was the PMB group that was problematic with the aromatization step previously. Therefore once aromatic compound 60 is established the PMB group can be introduced, and the proceeding synthetic steps of the alternative strategy can be explored.
CHAPTER 3: CONCLUSIONS AND FUTURE RESEARCH

In conclusion, both originally envisioned strategies A and B ran into obstacles. This means that in the laboratory both of these synthetic pathways are not feasible as they were initially proposed. Our substrate of interest, the phthalimide with the PMB group attached (43), gave us substantial trouble particularly in the selective reduction step in strategy A. Even though this reaction was eventually accomplished using a model compound, the question still remains as to whether the selective reduction can be achieved on the substrate of interest. One could go about perhaps introducing different groups, for example changing the PMB group into a tert-butyl, as it would still give the same effect for the oxidative cleavage step, as outlined on Scheme 37, a reaction which takes place further down in the synthetic pathway.

It would be reasonable to explore more selective reducing reagents such as Red-Al for instance. Due to literature precedent, we only explored DIBAL, NaBH₄, LiAlH(O'Bu)₃ and zinc. But nevertheless there are plenty of other reducing reagents that should be explored.

Scheme 37. Oxidative fragmentation and the loss of the R’ group, R’ = tert-butyl.
In strategy B the lithium-bromide exchange step and the subsequent chemistry proved most troublesome. For this step, tBuLi could be used instead of nBuLi since it is known to be more selective for lithium-halogen exchange compared to nBuLi.\textsuperscript{110} Also another reagent for this purpose is the Grignard reagent isopropylmagnesium chloride (iPrMgCl) where has been used in metal-halogen exchange reactions.\textsuperscript{111} Recent work has shown that it has a clear advantage over nBuLi since it provides higher yields due to being more selective and thermally stable.\textsuperscript{112,113} As a drawback, research with iPrMgCl has been usually performed on dihalogen substituted aromatic compounds where then the regioselectivity of the Grignard reagent is being tested. Such compounds are not closely related to our compound of interest, (52) Scheme 30, and may not provide promising conditions for our purposes. Nevertheless tBuLi and iPrMgCl are two metal-halogen exchange compounds that should be explored.

Another possible area of pursuit is the protection of the alcohol group of compound 45. There are three plausible protecting groups, as discussed in Section 2.2, that are to be explored in order to understand reactivity and advance the synthetic pathway. Even though pathway B has given us promising results by synthesizing the desired compounds, the yields of these compounds were not promising. Continued variation of the reaction conditions or establishing a new synthetic route may bring about higher yields.

There is clear advantage of strategy B over strategy A in that once the PMB group is introduced onto the substrate there will be no need of selective reduction of the proximal carbonyl since we already have an alcohol moiety on that position. To recall the selective reduction is the step gave us enormous trouble in the synthetic strategy A is described in Section 2.1. The rest of the synthetic strategies A and B are envisioned to take the same steps. It is proposed for the last three steps of the synthesis that the sulfur first needs to be monoxidized in
order to provide all the components for extrusion of sulfur dioxide. This can be accomplished by an oxidative species such as meta-chloroperoxybenzoic acid (mCPBA)\textsuperscript{114} or the newly discovered H\textsubscript{2}O\textsubscript{2}/POCl\textsubscript{3}.\textsuperscript{115} Following would be an oxidative fragmentation when the sulfoxide will be reacted with SO\textsubscript{2}Cl\textsubscript{2} to afford a sulfinate. This will occur through the intermediary of a sulfinyl chloride, as discussed in Section 1.5, which after introduction of a base is anticipated to form a cyclic sulfinate ester (sultine). The formation of the desired diene would be accomplished via elimination of SO\textsubscript{2} by thermolysis of the sultine.

The alternative strategy proved progressive, albeit not according to plan. The PMB group was sensitive to strong acid during the highly acidic aromatization step. This problem was circumvented by first aromatizing without the introduction of the PMB group. It should be mentioned that the Diels-Alder cycloaddition of maleic anhydride with the 2-mercaptomethyl furan proceeded in much higher yields in the absence of the PMB protection.

Even though the introduction of the PMB group onto compound 60 was not performed, this reaction is well established and should occur without any concerns. Having the thiol at this stage would also permit variation of the protecting group in general. It is known, for instance, that disulfides can be oxidatively fragmented and converted to sulfinyl chlorides.\textsuperscript{116,117} As such, future chemistry may be able to proceed with the sulfurs in the disulfide form. For example compounds 61 and 62 could be synthesized containing a symmetric disulfide (Fig. 17). It would be ideal to have the hydroxyl group, compound (62), before the formation of the sulfinyl chloride which will induce the subsequent cyclization and \textit{in situ} formation of the desired diene (16). Reduction of the proximal carbonyl in the presence of the disulfide bond might be challenging since they are sensitive to reducing reagents such as LiAlH\textsubscript{4} in ether.\textsuperscript{117} A plausible strategy would be by using a Lewis acid which would coordinate with the disulfide bond directing the
reduction at the proximal carbonyl group. This different approach perhaps might prove more efficient since it would shorten by two steps the originally planned strategy. The sulfur oxidation and the oxidative fragmentation steps would be eliminated. It is suffice to say that further research towards novel PARP inhibitors should be principally concentrated on the furan based chemistry, the alternative strategy synthetic route.

Figure 17. Suggested different approach towards the synthetic alternative strategy where a disulfide bond might be beneficial since it could be easily converted into a sulfinyl chloride. \( R = \text{Me, Bn}; R^1 = \text{phthalimidomethyl moiety}; \)

For future exploration once the diene (16) is established one could think about varying the \( R \) group on the nitrogen, which would yield to a variety of dienes. Possible \( R \) groups that could be explored are cumyl, phenyl and other aliphatic amines. As we saw with the benzyl group a less bulky group is favoured during the course of the synthesis. Furthermore, the ease of removal of that group needs to be considered since it might be problematic via the course of the synthetic pathway or for entering into the active site of the PARP protein. If the envisioned diene is achieved it would be favourable to react it with different dienophiles to achieve tri or tetra cyclic potentially novel PARP inhibitors. Then they will be tested via biochemical analysis for efficiency towards becoming potential PARP inhibitors, and for the kind of disease they will be considered helpful.
Near the end of our research project an interesting literature article by Lubbers et al. was encountered. The aim of their research is to synthesize a group of novel 2,3-dihydroisoindol-1-ones. Along their synthetic route there are steps that might be helpful towards guiding our project. As Scheme 38 presents those researchers started the synthesis with compound 63, and a variety of anilines were co-condensed with the aldehyde and methanol/methoxide forming methylated aminal 64. Thiols could then be introduced to achieve a series of different compounds 65. Therefore this would be a reasonable pathway towards our envisioned synthesis of the diene for two reasons. First, one could utilize the amine of choice for incorporation as indicated in the Scheme 38. The second attribute is that the two functionalities of interest, a hydroxyl group and a sulfur atom, are already introduced onto the molecule. The presence of the hydroxyl group could lead to avoiding the selective reduction step. This would be optimal since that step is very troublesome. One problem to this particular synthetic route is that there is no additional carbon between the pentacyclic ring and the sulfur atom, which is required for our oxidative fragmentation step.

Scheme 38. Section of a the synthesis towards novel 2,3-dihydroisoindol-1-ones as performed by Lubbers et al.

On that note a SciFinder similarity literature search was performed for a compound (66)
presented on Scheme 39. This work done by Christov et al. seems to be the most relevant towards our synthetic strategies since the sulfur species would be introduced in an early stage as Scheme 39 shows.$^{119}$ Thus a facile Diels-Alder reaction which than could be followed by the introduction of the nitrogen species and the subsequent steps as previously described in strategy A and the alternative strategy. Therefore this approach is worth exploring further.

![Scheme 39. Synthesis of sulfonyl and sulfinyl pththalates. R = Ph, n = 1; or R = Me, n = 2;](image)

Despite the fact that the envisioned potentially novel PARP inhibitors were not synthesized in this project, the investigation towards novel PARP inhibitors still stays an exciting discipline. As presented herein PARPs can harm the human body in a variety of ways, thus a variety of PARP inhibitors are required for different types of diseases. Therefore researchers are constantly investigating possible potent PARP inhibitors for a certain disease. Hence PARP inhibitor synthesis is a very popular discipline and it shall continue with that spirit. As has been demonstrated above there are a variety of potential PARP inhibitors with different functional groups and sizes, bicyclic, tricyclic, tetracyclic which have been synthesized, where some already have entered into clinical trials and some are advanced farther, being used as drugs for treating particular diseases. As well, diversity of synthesis has been amply demonstrated for the preparation of large families of compounds. Thus synthesizing novel PARP inhibitors is of a
great importance to the medicinal chemistry and biochemistry disciplines. Also, investigating novel PARP inhibitors aids in the discovery of potentially inhibiting mono-ADP-ribosyl transferase, which is the enzyme involved in inhibiting protein synthesis in patients that are suffering from cystic fibrosis. Therefore investigating novel PARP inhibitors has a great implementation in possibly curing a variety of diseases.
CHAPTER 4: EXPERIMENTAL

4.1 Instrumentation and General Procedures

All reactions were carried out in flame dried round bottom flasks under nitrogen gas. All the solvents used were obtained dry from solvent system a LC Technology Solutions Inc solvent system. All the reagents were transferred via oven dried syringes and needles. Microwave reactions were carried out using CEM Discover S-Class reactor microwave. Flash chromatography was done on 200-425 mesh Type 60 Å silica gel. Analytical TLC was performed using 0.25 mm Merck Kieselgel 60 F254 precoated glass-backed silica gel plates. Melting points were determined using a MEL-TEMP melting point apparatus. Infrared spectra were obtained on a Bruker Alpha IR machine, with the compound applied neat to KBr salt plates unless otherwise noted. IR assignments are assigned for novel compounds. NMR spectra were acquired on either a Bruker Avance 600, 400 or 300 MHz machine and were calibrated to tetramethylsilane (0.00 ppm) in CDCl$_3$ unless otherwise noted. All NMR spectra are reported in parts per million (ppm). GC was performed on Varian 3400, column type RTX5, reagent grade DCM solvent was used to run all GC samples. GC-MS was performed on Varian Saturn 2200, reagent grade DCM solvent was used to run all GC-MS samples. Mass spectrometry was performed on "Thermo LCQ Deca" Ion trap, in the laboratory of Dr. Gabryelski by his Ph.D. candidate Daniel Beach at the University of Guelph.
4.2 Synthetic Procedures

Synthesis of 2-bromo-3-methylbenzoyl chloride (26)

2-Bromo-3-methylbenzoic acid (1 eq, 5.00 g, 23.3 mmol) was dissolved in 25 mL of toluene. Thionyl chloride (5 eq, 8.5 mL, 116.3 mmol) was added to the above mixture, and the reaction was refluxed for 3 hours under nitrogen gas. After three hours, the toluene and excess SOCl₂ were removed under reduced pressure and the solid was redissolved in 6 mL of toluene which was removed by rotary evaporation again. The crude product was further dried under high vacuum overnight. A yellow liquid 26 was obtained in 80% yield. ¹H NMR (400 MHz, CDCl₃) δ 7.75 (1H, dd, J = 7.7, 2.1 Hz, ArH, ortho to carbonyl), 7.44 (1H, dd, J = 7.7, 2.1 Hz, ArH, ortho to methyl), 7.33 (1H, t, J = 7.7 Hz, ArH, meta to carbonyl and methyl), 2.48 (3H, s, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 166.78, 140.53, 136.86, 134.92, 129.49, 127.06, 122.47, 23.81; IR (neat) ν 3073 and 3052 C(sp²)-H stretch, 2957 and 2926 C(sp³)-H stretch, 1780 C=O aromatic acid chloride stretch, 1572 C=C stretch aromatic ring system, cm⁻¹.
Synthesis of 4-methyl-3a,4,7z-tetrahydroisobenzofuran-1,3-dione (31)

\[ \text{(E)-Penta-1,3-diene (1 eq, 0.2 g, 2.01 mmol) was dissolved in 6 mL of toluene. Maleic anhydride (4 eq, 0.7876 g, 8.0317 mmol) was added to the above mixture. The reaction was stirred at 100 °C in an oil bath for 10 hours. It was quenched with 10 mL of water, and the organic layer was extracted twice with 30 mL of DCM. The combined organics were dried with MgSO}_4, concentrated under reduced pressure and placed under a high vacuum pump overnight. The crude mixture was purified by silica gel flash chromatography (3:1 hexanes/EtOAc). A white solid 31 was obtained in 68% yield, mp = 59-60°C; } \]

\[ R_f = 0.31 \text{ (3:1 hexanes/EtOAc); } \]

\[ {^1}H \text{ NMR (400 MHz, CDCl}_3\text{) } \delta 5.96 \text{ (1H, ddq, } J = 9.3, 6.4, 3.4 \text{ Hz), 5.84 (1H, ddd, } J = 9.3, 6.6, 6.4 \text{ Hz), 3.43 (1H, qd, } J = 7.7, 2.3 \text{ Hz), 3.29 (1H, dd, } J = 9.7, 6.8 \text{ Hz), 2.73 (1H, ddd, } J = 15.8, 6.8, 6.4 \text{ Hz), 2.56 – 2.47 (1H, m), 2.27 – 2.19 (1H, m), 1.37 (3H, d, } J = 7.1 \text{ Hz); } \]

\[ {^{13}}C \text{ NMR (100 MHz, CDCl}_3\text{) } \delta 174.44, 171.69, 134.79, 127.21, 44.98, 40.78, 29.82, 23.60, 16.58; \]

\[ \text{IR (neat) } \nu 2965 \text{ and } 2855 \text{ C(sp}^3\text{-H stretch, 1847 and 1708 C=O symmetric and asymmetric stretch, cm}^{-1}. \]
Synthesis of 2,4-dimethylisoindoline-1,3-dione (32)

Method A: 4-Methylisobenzofuran-1,3-dione (1 eq, 2.0 g, 12.0 mmol) was dissolved in 4.4 mL of acetic acid. To this mixture, methylamine in 40% water (1 eq, 0.37 mL, 12.0 mmol) was added. The mixture was refluxed for 3 hours. The solvent was evaporated under vacuum, and the product was recrystallized from 95% ethanol at 3 ºC. The beige crystals were collected by suction filtration and washed with cold ethanol. Product 32 was obtained in 88% yield. mp: 89 – 90.5 ºC.

Method B: 4-Methylisobenzofuran-1,3-dione (1 eq, 2.5 g, 15.4 mmol), methylamine in 40% water (1.5 eq, 0.72 mL, 23.1 mmol) and 28 mL of DMF were all mixed together in a 35 mL microwave vessel. Microwave was set at 300W for 45 minutes at 153 ºC. A dark yellow transparent liquid was obtained. It was poured over ice, and a solid formed, which was collected by suction filtration and washed with water. Beige crystals 32 were obtained in 44% yield, which were further dried under a high vacuum pump.

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.67 (1H, d, $J = 7.3$ Hz, ArH), 7.55 (1H, t, $J = 7.6, 7.3$, ArH), 7.45 (1H, d, $J = 7.6$, ArH), 3.16 (3H, s, N-CH$_3$), 2.70 (3H, s, Ar-CH$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 169.34, 168.58, 137.82, 136.26, 133.39, 132.64, 128.93, 120.82, 23.75, 17.61; IR (neat) ν 2925 and 2854 C(sp$^3$)-H stretch, 1762 and 1711 C=O symmetric and asymmetric stretch, 1606 C=C stretch aromatic ring system, cm$^{-1}$. 

77
Synthesis of 2-benzyl-4-methylisoindoline-1,3-dione (33)

Method A: 4-Methylisobenzofuran-1,3-dione (1 eq, 2.0 g, 12.3 mmol) was dissolved in 8 mL of acetic acid. To the above mixture, benzylamine (1.5 eq, 2.02 mL, 18.5 mmol) was added. The mixture was refluxed for 3 hours under nitrogen gas. The solvent was removed by rotary evaporation, and the product was recrystallized from 95% ethanol. Beige crystals 33 were obtained in 71% yield. mp: 110 – 112 ºC.

Method B: 4-Methylisobenzofuran-1,3-dione (1 eq, 2.0 g, 12.3 mmol), benzylamine (1 eq, 1.35 mL, 12.3 mmol) and 25 mL of DMF were all mixed together in a 35 mL microwave vessel. Microwave was set at 300W for 45 minutes at 153 ºC. An orange transparent liquid was obtained and poured over crushed ice to create a solid precipitate. The product was collected by suction filtration and washed with water. A beige solid 33 was obtained in 82% yield.

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.66 (1H, d, $J = 7.3$ Hz, ArH), 7.54 (1H, t, $J = 7.5$ Hz, ArH), 7.45 – 7.40 (3H, m, ArH), 7.33 – 7.22 (3H, m, ArH), 4.81 (2H, s, CH$_2$), 2.67 (3H, s, CH$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 161.34, 160.52, 138.01, 136.54 (2 peaks), 133.54, 132.52, 128.64 (2 peaks), 127.76, 121.13, 41.41, 17.59; IR (neat) ν 2925 C(sp$^3$)-H stretch , 1759 and 1710 C=O symmetric and asymmetric stretch, cm$^{-1}$. 

78
Synthesis of 4-(bromomethyl)-2-methylisoindoline-1,3-dione (34)

2,4-Dimethylisoindoline-1,3-dione (32) (1 eq, 0.99 g, 5.64 mmol), NBS (1.2 eq, 1.20 g, 6.77 mmol) and AIBN (0.25 eq, 0.23 g, 1.41 mmol) were all mixed together in 30 mL of benzene. The reaction was stirred at reflux for 4 hours under nitrogen gas and then was quenched with 15 mL of aqueous sodium thiosulphate. The aqueous layer was extracted with 3 x 15 mL of EtOAc and washed with brine. The combined organics were further dried with MgSO₄, the mixture was filtered and the solvent was removed by rotary evaporation. The residues were placed under high vacuum overnight. The crude material was purified by silica gel flash chromatography (9:1 hexanes/acetone). A white crystalline powder 34 was obtained in 75% yield; mp = 139 – 140 °C; Rf = 0.25 (9:1 hexanes/acetone). \(^1\)H NMR (400 MHz, CDCl₃) δ 7.78 (1H, dd, \(J = 1.4, 6.9\) Hz, aromatic proton), 7.72 – 7.65 (2H, m, aromatic protons), 4.97 (2H, s, CH₂), 3.17 (3H, s, CH₃); \(^1^3\)C NMR (100 MHz, CDCl₃) δ 168.20, 167.75, 136.80, 135.86, 134.19, 132.86, 128.35, 123.16, 26.14, 23.93; IR (neat) ν 2926 C(sp\(^3\))-H stretch, 1766 and 1705 C=O asymmetric and symmetric stretch, 1606 C=C stretch, aromatic ring system, cm\(^{-1}\); GC-MS(CI) \(m/z(\%) = 254(100)\).
Side-product of the above reaction is **4-(dibromomethyl)-2-methylisoindoline-1,3-dione (35)**

![Chemical Structure]

Compound 35 is a side-product from the above reaction, therefore same reactions conditions as for the formation of compound 34. Obtained in 1.5 % yield, ratio of product to side-product is 22:1.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.21 (1H, dd, $J$ = 2.0, 6.7 Hz, Ar-H), 7.89 (1H, s, next to two Br), 7.82 – 7.75 (2H, m, Ar-H’s), 3.18 (3H, s, CH$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 167.86, 167.23, 140.38, 135.21, 134.94, 131.46, 124.20, 33.10, 24.09; IR (neat) v 2947 and 2852 C(sp$^3$)-H stretch, 1770 and 1705 C=O asymmetric and symmetric stretch, 1603 C=C stretch, aromatic ring system, cm$^{-1}$; GC-MS(CI) m/z(%) = 333(8), 254(100), 252(62), 251(16).

**Synthesis of 2-benzyl-4-(bromomethyl)isoindoline-1,3-dione (36)**

![Chemical Structure]

2-Benzyl-4-methylisoindoline-1,3-dione (33) (1 eq, 0.2 g, 0.79 mmol), NBS (1 eq, 0.14 g, 0.79 mmol) and AIBN (0.06 eq, 0.0078 g, 0.048 mmol) were all mixed together in 6.5 mL of benzene. The reaction was stirred at reflux for 4.5 hours under nitrogen gas and then was quenched with 3 mL of aqueous sodium thiosulphate. The aqueous layer was extracted with 3 x 3
mL of EtOAc. The combined organics were washed with brine, and dried with MgSO₄. The solvent was removed by rotary evaporation and the mixture was further dried under high vacuum. The crude material was purified by silica gel flash chromatography (4:1 hexanes/EtOAc). A white solid 36 was obtained in 8% yield. mp: 139 – 140 ºC; Rf = 0.23 (4:1 hexanes/EtOAc). ¹H NMR (400 MHz, CDCl₃) δ 7.79 (1H, dd, J = 1.0, 6.9 Hz), 7.73 – 7.66 (2H, m), 7.44 (2H, d, J = 6.9 Hz, hydrogens on benzyl group), 7.35 – 7.26 (3H, m, hydrogens on benzyl group), 4.96 (2H, s, CH₂ next to Br), 4.84 (2H, s, CH₂ next to N); ¹³C NMR (100 MHz, CDCl₃) δ 167.81, 167.37, 136.97, 136.18, 136.04, 134.31, 132.70, 128.74, 128.21, 127.93, 123.34, 41.68, 26.09; IR (neat) v 2929 C(sp³)-H stretch, 1771 and 1712 C=O asymmetric and symmetric stretch, 1605 C=C stretch aromatic ring system, cm⁻¹.

**Synthesis of (4-methoxyphenyl)methanethiol (40)**

\[
\begin{align*}
\text{O} & \quad \text{SH} \\
\end{align*}
\]

*p-Methoxybenzyl thiolacetate* (1 eq, 10 mL, 51.0 mmol) was dissolved in 100 mL of 95% ethanol and cooled to 0 ºC. 2 M aqueous sodium hydroxide (17 eq, 35 mL, 866 mmol) was added to the above mixture dropwise. The reaction was stirred for an hour, and diluted with water. The ethanol was removed under reduced pressure and the aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organics were dried with MgSO₄, filtered, and the solvent was removed under reduced pressure. The crude material was further dried under high vacuum overnight. The crude was purified by silica gel flash chromatography (4:1, hexanes/EtOAc). A dark yellow liquid 40 was obtained in 81% yield. ¹H NMR (100 MHz, CDCl₃) δ 7.23 (2H, m, Ar-H’s meta to methoxy group), 6.84 (2H, m, Ar-H’s ortho to methoxy group), 3.77 (3H, s, CH₃), 3.70 (2H, d, J
= 7.4 Hz, CH₂), 1.72 (1H, t, \(J = 7.4\) Hz, thiol).

**Synthesis of para-methoxy benzyl bromide (41)**

\[
\begin{align*}
&\text{Br} \\
&\text{O}
\end{align*}
\]

\(p\)-Methoxytoluene (1 eq, 10 mL, 79.2 mmol) and NBS (1 eq, 14.1 g, 79.2 mmol) were dissolved in 140 mL of benzene under nitrogen gas in a three neck round bottomed flask. In one neck a thermometer was connected, in the other a condenser and in the third a septum. The mixture was placed in an oil bath and slowly heated. AIBN (0.25 eq, 3.253 g, 19.81 mmol) was added to the mixture when the internal temperature of the mixture reached 75 °C. Immediately upon addition of the AIBN the flask was removed from the oil bath and set to stir at room temperature. The mixture was stirred at room temperature until the mixture went through colour changes from orange to yellow to clear and back to orange. Then the mixture was placed back in the oil bath and refluxed for 3.5 hours. After which 70 mL of petroleum ether (boiling range 35 – 60 °C) were added and the organic layer was washed with water (2 x 60 mL). The organic mixture was dried with MgSO₄, and concentrated under pressure. The product was further dried under high vacuum for an hour. A yellow liquid 41 was obtained in 93% yield, and it was used directly in the next step without purification. \(^1\)H NMR (400 MHz, CDCl₃) \(\delta\) 7.32 (2H, m, Ar-H’s meta to methoxy group), 6.86 (2H, m, Ar-H’s ortho to methoxy group), 4.51 (2H, s, CH₂), 3.81 (3H, s, CH₃).
Synthesis of para-methoxybenzylthioacetate (42)

\[
\text{O} \quad \text{S} \quad \text{O}
\]

\( p \)-Methoxybenzyl bromide (1 eq, 13.1 g, 65.2 mmol) and potassium thioacetate (1.1 eq, 8.18 g, 71.7 mmol) were dissolved in 42 mL of DMF. The reaction was stirred at room temperature for 1.5 hours and then was diluted with water and the aqueous layer was extracted with DCM (3 x 40 mL). The combined organics were dried over MgSO\(_4\), filtered, concentrated under pressure and the crude material was further dried under high vacuum overnight. The crude mixture was purified by silica gel flash chromatography (95% : 5%, hexanes/EtOAc), to provide pure material 42 in 86% yield. \(^1\)H NMR (100 MHz, CDCl\(_3\)) \(\delta\) 7.18 (2H, m, ArH’s meta to methoxy group), 6.80 (2H, m, ArH’s ortho to methoxy group), 4.05 (2H, s, methylene), 3.75 (3H, s, CH\(_3\), methoxy), 2.32 (3H, s, CH\(_3\), methyl next to carbonyl); \(^13\)C NMR (400 MHz, CDCl\(_3\)) \(\delta\) 195.29, 158.83, 129.96, 129.60, 114.04, 55.26, 32.96, 30.36; IR (neat) \(\nu\) 2956 and 2934 C(sp\(^3\))-H stretch, 1689 C=O thioacetate stretch, 1611 and 141 C=C stretch aromatic ring system, cm\(^{-1}\).

Synthesis of 4-((4-methoxybenzylthio)methyl)-2-methylisoindoline-1,3-dione (43)

\[
\begin{array}{c}
\text{O} \\
\text{N} \\
\text{O} \\
\text{S} \\
\text{O}
\end{array}
\]

(4-Methoxyphenyl)methanethiol (1 eq, 0.19 mL, 1.21 mmol) and triethylamine (1 eq, 0.17 mL, 1.21 mmol) together with 2.0 mL of DCM were set to stir at room temperature, under a constant stream of nitrogen gas. 4-(Bromomethyl)-2-methylisoindoline-1,3-dione (1.1 eq, 0.34 g,
1.33 mmol) was dissolved in 0.1 mL of DCM and the solution was added dropwise to the above mixture. Once addition was complete, the reaction was stirred at room temperature for 4 hours. It was quenched with water and extracted with DCM (3 x 5 mL). The organic phase was dried over MgSO₄, filtered and the DCM was removed by rotary evaporation. The crude mixture was further dried under high vacuum for an hour. The crude mixture was purified by silica gel flash chromatography (4:1, hexanes/EtOAc). A white powder 43 was obtained in 59% yield. mp = 116 – 117 ºC; Rf = 0.25 (4:1, hexanes/EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 7.69 (1H, dd, J = 6.6, 1.8 Hz), 7.62 – 7.54 (2H, m, J = 7.9, 6.6, 1.8 Hz), 7.18 (2H, m, Ar-H meta to methoxy group), 6.79 (2H, m, Ar-H ortho to methoxy group), 4.14 (2H, s, CH₂), 3.78 (3H, s, methoxy CH₃), 3.69 (2H, s, CH₂ next to aromatic), 3.15 (3H, s, N-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 168.73, 168.10, 158.68, 138.81, 135.41, 133.59, 132.70, 129.99, 129.48, 128.58, 121.75, 113.83, 55.26, 36.18, 30.49, 23.82; IR (neat) ν 2999 and 2935 C(sp³)-H stretch, 1766 and 1706 C=O asymmetric and symmetric stretch, 1610 and 140 C=C stretch aromatic ring systems, cm⁻¹.

**Synthesis of 2,7-dimethyl-3-methylenoisoinodlin-1-one (44)**

![2,7-dimethyl-3-methylenoisoinodlin-1-one](image)

2,4-Dimethylisoindoline-1,3-dione (1 eq, 0.1 g, 0.571 mmol) was dissolved in 10 mL of dry THF at -30 ºC under N₂ gas. The Grignard reagent, MeMgBr 3.0 M in ether (1.5 eq. 2.6 mL, 0.856 mmol) was added slowly to the above mixture. The mixture was stirred at -30 ºC for 3 hours. The reaction was quenched with 3 M HCl until acidic pH was obtained, the Litmus paper turned red. The organic layer was extracted (3 x 10 mL) with diethyl ether and washed with water.
The combined organics were dried over MgSO₄, the solvent was removed under reduced pressure and the crude mixture was placed under vacuum overnight. The crude material was purified by silica gel flash chromatography (4:1, hexanes/EtOAc). A white solid 44 was obtained in 55% yield. mp = 83 – 85 ºC; Rf = 0.26 (4:1, hexanes/EtOAc); ¹H NMR (600 MHz, CDCl₃) δ 7.48 (1H, d, J = 7.6 Hz, ArH), 7.40 (1H, apparent t, J = 7.6, 7.5 Hz, ArH), 7.20 (1H, d, J = 7.5 Hz, ArH), 5.10 (1H, s, vinylic alkene), 4.75 (1H, s, vinylic alkene), 3.23 (3H, s, N-CH₃), 2.70 (3H, s, Ar-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 167.86, 142.83, 137.03, 136.58, 131.40, 131.27, 126.76, 117.18, 87.52, 25.45, 17.36; IR (neat) ν 2958 and 2934 C(sp³)-H stretch, 1698 C=O amide stretch, 1637 C=C alkene stretch, cm⁻¹; GC-MS(EI) m/z(%) = 144(50), 173(100).

Minor desired isomer: Partial ¹H NMR (600 MHz, CDCl₃) δ 7.73 – 7.71 (1H, m, ArH), 5.23 (1H, s, vinylic alkene), 4.99 (1H, s, vinylic alkene).

Major to minor isomer ratio is 3:1.

**Synthesis of 3-hydroxy-2,4-dimethylisoilnolin-1-one (45)**

![Chemical structure](image.png)

**Method A:** 2,4-Dimethylisoindoline-1,3-dione (1 eq. 0.1 g, 0.571 mmol) was dissolved in 0.8 mL of 95% ethanol at room temperature. NaBH₄ (2 eq, 0.043 g, 1.14 mmol) was dissolved in 0.8 mL of 95% ethanol and was added dropwise to the above mixture while stirring at room temperature. After an hour the reaction was quenched with approximately 2 mL of acetic acid. The solvent was removed by rotary evaporation and the crude was further dried under vacuum for 2 hours. The crude material was purified by silica gel flash chromatography (4:1,
hexanes/EtOAc). A faint yellow solid 45 was obtained in 16% yield. mp = 170 – 171.5 °C; Rf = 0.25 (4:1, hexanes/EtOAc).

**Method B:** 2-Bromo-N-formyl-N,3-dimethylbenzamide (1 eq, 0.1 g, 0.390 mmol) was dissolved in 6 mL of dry THF at -78 °C. To this mixture (1.3 eq, 0.03 mL, 0.38 mmol) of 1.6M nBuLi was added dropwise. The reaction mixture was stirred for 30 minutes as -78 °C, and then the temperature was increased to -30 °C where the reaction was stirred for 2.5 hours. After 3h it was taken out of the cryobath and placed at room temperature where it was quenched with 2 mL of aqueous NH₄Cl. The aqueous layer was extracted with 3 × 5 mL of EtOAc, and the combined organics were dried over MgSO₄. After filtration, the solvent was removed under reduced pressure and the crude mixture further dried under vacuum for 30 min. The crude was purified with silica gel flash chromatography (14:5:1, hexanes/EtOAc/MeOH). A faint yellow solid 45 was obtained in 4% yield. mp = 170 – 171.5 °C; Rf = 0.28 (14:5:1, hexanes/EtOAc/MeOH);

\(^1\)H NMR (400 MHz, CDCl₃) \(\delta\) 7.59 (1H, d, \(J = 7.4\) Hz, Ar-H, para to methyl), 7.40 (1H, t, \(J = 7.4\) Hz, Ar-H, ortho to methyl), 7.34 (1H, d, \(J = 7.4\) Hz, Ar-H, meta to methyl), 5.60 (1H, d, \(J = 11.4\) Hz, ), 3.10 (3H, s, N-CH₃), 2.50 (3H, s, Ar-CH₃), 2.23 (1H, d, \(J = 11.4\) Hz, hydroxyl); \(^{13}\)C NMR (150 MHz, CDCl₃) \(\delta\) 167.40, 141.24, 134.32, 133.65, 131.74, 130.06, 120.75, 83.25, 30.95, 26.18; IR (neat) \(\nu\) 3339 O-H broad stretch (alcohol group), 2957 and 2923 C(sp³)-H stretch, 1677 C=O amide stretch, 1613 C=C stretch aromatic ring system, cm⁻¹.
Synthesis of 2-bromo-3-methylbenzamide (46)

2-Bromo-3-methylbenzoyl chloride (1 eq, 2.50 mL, 10.8 mmol) was dissolved in 25 mL of DCM at 0 °C. Aqueous ammonium hydroxide (7 eq, 3.00 mL, 75.2 mmol) was added to the above mixture dropwise over a 30 minutes period. The reaction stirred for 3 hours. A white solid was collected by suction filtration and was washed with water. The solid was dried under high vacuum overnight. The product 46 was obtained in 96% yield. $^1$H NMR (400 MHz, DMSO$_{d6}$) $\delta$ 7.82 (1H, s, amide proton), 7.52 (1H, s, amide proton), 7.34 (1H, dd, $J = 7.5, 1.0$ Hz, ArH, ortho to amide group), 7.29 (1H, t, $J = 7.5$ Hz, ArH, meta to amide group), 7.15(1H, dd, $J = 7.5, 1.0$ Hz, ArH, para to amide group), 2.37 (3H, s, Ar-CH$_3$); $^{13}$C NMR (100 MHz, DMSO$_{d6}$) $\delta$ 169.54, 140.36, 138.03, 130.94, 127.22, 125.67, 120.78, 22.96; IR (Nujol) $\nu$ 3359 and 3176 (N-H stretch, primary amide), 2923 and 2854 C(sp$^3$)-H stretch, 1647 C=O amide stretch, 1571 C=C stretch aromatic ring system, cm$^{-1}$.

Synthesis of 2-bromo-N, 3-dimethylbenzamide (47)

2-Bromo-3-methylbenzoyl chloride (1 eq, 0.60 mL, 2.58 mmol) was dissolved in 18 mL of DCM, under nitrogen gas. To the above mixture methylamine in 40% water (7 eq, 0.60 mL, 19.3 mmol) was added dropwise at 0 °C. The reaction was stirred for 4 hours after which time
the organic phase was washed three times with 10 mL of water. The organic phase was then dried over MgSO₄, the organic layer was removed by rotary evaporation and the compound was further dried under high vacuum for an hour. A pale orange solid 47 was obtained in 93% yield. mp: 87.5 – 88.5 ºC; ¹H NMR (400 MHz, CDCl₃) δ 7.28 – 7.19 (3H, m, aromatic protons), 5.84 - 5.72 (1H, m, amide proton), 3.01 (3H, d, J = 5.0 Hz, amide CH₃), 2.42 (3H, s, Ar-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 169.23, 139.21, 139.18, 131.81, 127.24, 126.30, 121.61, 26.73, 23.57; IR (neat) v 3275 N-H stretch (secondary amide), 2998 and 2937 C(sp³)-H stretch, 1642 C=O stretch (amide carbonyl), 1545 C=C (aromatic ring system) cm⁻¹.

**Synthesis of 2-bromo-N-formyl-3-methylbenzamide (48)**

2-Bromo-3-methylbenzamide (1 eq, 3.71 g, 17.3 mmol) and N,N – dimethyl formamide dimethyl acetal (3 eq, 7.00 mL, 52.2 mmol) were mixed together under nitrogen gas. The mixture was stirred at 120 ºC for 5 minutes, and then was placed in ice for 10 – 15 minutes until a white precipitate formed. The precipitate was dissolved in 20 mL of 70% acetic acid. A precipitate formed again and it was left for 12h at room temperature. A white cubic crystal formed. The product 48 was collected by suction filtration and washed with water. A 53% yield was obtained, mp: 109 – 111 ºC; ¹H NMR (400 MHz, acetone-d₆) δ 10.40 (1H, s, formyl proton), 9.13 (1H, s, amide proton), 7.46 – 7.41 (1H, m, Ar-H), 7.38 – 7.35 (2H, m, Ar-H’s), 2.41 (3H, s, Ar-CH₃); ¹³C NMR (100 MHz, acetone-d₆) δ 169.33, 163.28, 139.91, 137.60, 133.55, 128.32, 127.04, 121.60, 23.21; IR (neat) v 3181 (N-H stretch, secondary amide), 2957 C(sp³)-H, 2743
C(sp²)-H stretch (formyl hydrogen), 1737 and 1693 C=O stretch, formyl and amide groups respectively, 1571 stretch aromatic ring system, cm⁻¹.

**Synthesis of 2-bromo-N-formyl-N,3-dimethylbenzamide (52)**

![Chemical Structure](image)

2-Bromo-N-formyl-3-methylbenzamide (1 eq, 1.00 g, 4.13 mmol) and powdered sodium hydride (1.5 eq, 0.15 g, 6.20 mmol) were dissolved in 60 mL of DMF under argon gas. The mixture was stirred for an hour at 50 °C in an oil bath. After an hour it was left to cool to room temperature and methyl iodide was added dropwise to the mixture. The reaction was stirred for 2.5 hours at room temperature. Methanol was added and removed under rotary evaporation. DMF was further removed by placing the reaction under high vacuum and heating it to 50 °C overnight. The crude material was purified by silica gel flash chromatography (6:1 hexanes/EtOAc). White product 52 was obtained in 70% yield; R_f = 0.21 (6:1 hexanes/EtOAc).

^1H NMR (400 MHz, CDCl₃) δ 8.68 (1H, s, formyl proton), 7.39 – 7.29 (2H, m, aromatic protons), 7.14 (1H, dd, J = 7.0 Hz, aromatic proton), 3.25 (3H, s, N-CH₃), 2.44 (3H, s, Ar-CH₃);

^13C NMR (100 MHz, CDCl₃) δ 170.95, 163.98, 139.80, 136.24, 132.66, 127.95, 126.06, 121.49, 34.83, 23.15; IR (neat) ν 2953 and 2925 C(sp³)-H stretch, 1731 and 1673 C=O stretch, formyl and amide groups respectively, 1573 C=C aromatic ring system, cm⁻¹; GC-MS(EI) 13 min; m/z (%) = 258(100) (M + H⁺).
Synthesis of 2-((4-methoxybenzylthio)methyl) furan (56)

2-Furylmethanethiol (1 eq, 5.68 g, 49.7 mmol) and triethylamine (1 eq, 7.0 mL, 49.7 mmol) together with 25 mL of DCM were set to stir at room temperature, under a constant stream of nitrogen gas. p-Methoxybenzyl bromide (1.1 eq, 11 g, 5.50 mmol) was dissolved in 5 mL of DCM and added to the above mixture dropwise. The reaction was stirred at room temperature for 2 hours during which time a precipitate formed. The precipitate was redissolved in DCM and 30 mL of 0.1 M HCl was added. The aqueous layer was extracted with DCM and washed with brine, and the combined organics were dried over MgSO₄, filtered and concentrated under reduced pressure. The crude material was further concentrated under high vacuum and purified by silica gel flash chromatography (9:1, hexanes/EtOAc). A white product 56 was obtained in 60 % yield. Rᵢ = 0.24 (9:1, hexanes/EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 7.37 (1H, dd, J = 0.85, 1.9 Hz, proton next to oxygen on furan), 7.23 (2H, m, ArH’s meta to methoxy group), 6.85 (2H, m, ArH’s ortho to methoxy group), 6.32 (1H, dd, J = 0.85, 3.1 Hz, proton on furan), 6.15 (1H, dd, J = 3.1, 1.9 Hz, proton on furan), 3.80 (3H, s, CH₃), 3.66 (2H, s, CH₂ next to furan), 3.58 (2H, s, CH₂ next to aromatic); ¹³C NMR (100 MHz, CDCl₃) δ 158.67, 151.78, 142.09, 130.11, 129.80, 113.90, 110.36, 107.46, 55.28, 35.15, 27.30; IR (neat) ν 2954 and 2912, 2834 C(sp³)-H stretch, 1609 furan ring skeletal vibrations, 140 C=C stretching aromatic ring system, 1175 C-O-C asymmetrical stretching, cm⁻¹.
Synthesis of 4,7-Epoxyisobenzoduran-1,3-dione,3a,4,7,7a-tetrahydro-(4-methoxybenzylthio) methyl (57)

Maleic anhydride (1.5 eq, 0.063 g, 0.642 mmol) was dissolved in 1 mL of dry diethyl ether. To this mixture hydroquinone (0.04 eq, 0.00398 g, 0.034 mmol) and 2-furylmethanethiol (1 eq, 0.1 g, 0.427 mmol) were added. The reaction was stirred at room temperature for one week. A white precipitate had formed which was collected by suction filtration and further dried under high vacuum pump. A single white crystal 57 was obtained in 20% yield. mp = 68.5 – 70.5 °C; R\textsubscript{f} = 0.29 (100% hexanes); \textsuperscript{1}H NMR (400 MHz, Acetone\textsubscript{d6}) \(\delta\) 7.23 (2H, dt, \(J\) = 6.6, 2.0 Hz, ArH’s, meta to methoxy group), 6.86 (2H, dt, \(J\) = 6.6, 2.0 Hz, ArH’s, ortho to methoxy group), 6.64 (1H, dd, \(J\) = 5.6, 1.3 Hz, alkene proton), 6.52 (1H, d, \(J\) = 5.6 Hz, alkene proton), 5.34 (1H, d, \(J\) = 1.3 Hz), 3.80 (2H, AB pattern, \(J\) = 13.1 Hz, methylene proton), 3.76 (3H, s, CH\textsubscript{3}), 3.54 (1H, d, \(J\) = 6.8 Hz), 3.39 (1H, d, \(J\) = 6.7 Hz), 3.18 (1H, d, \(J\) = 14.6 Hz, methylene proton), 3.01 (1H, d, \(J\) = 14.6 Hz, methylene proton); \textsuperscript{13}C NMR (150 MHz Acetone\textsubscript{d6}) \(\delta\) 166.14, 159.93, 153.26, 143.13, 137.84, 130.98, 130.92, 114.83, 111.61, 108.38, 55.46, 35.90, 28.07; IR (neat) \(\nu\) 2958 and 2933 C(sp\textsuperscript{3})-H stretch, 1857 and 1782 C=O anhydride stretch, 140 C=C stretch aromatic ring system, cm\textsuperscript{-1}; MS/EI \textit{m/z}(\%) = 348(87) (M + oxygen atom), 331(67), 242(24), 179(100), 177(22), 153(21), 133(31), 129(50).
Synthesis of 4,7-Epoxyisobenzofuran-1,3-dione, 3a,4,7,7a-tetrahydro-4-methylthiol (59)

Maleic anhydride (1.2 eq, 0.937 g, 9.55 mmol) and hydroquinone (0.08 eq, 0.070 g, 0.637 mmol) were dissolved in 2.0 mL of dry ethyl ether, and gave a yellow transparent solution. To this solution 2-furylmethanethiol (1 eq, 0.4 mL, 7.96 mmol) was added at room temperature. The reaction was let to stir at room temperature overnight. The solvent was removed under concentrated pressure and the crude was further dried under high vacuum for 4.5h. A yellow solid 59 was obtained in 72 % yield. mp = 69 – 71 ºC; Rf = 0.22 (9:1, hexanes/EtOAc); 1H NMR (400 MHz, Acetone-d6) δ 6.67 – 6.64 (2H, m, alkene protons), 5.33 (1H, d, J = 1.4 Hz), 3.58 (1H, d, J = 6.7 Hz), 3.50 (1H, d, J = 6.7 Hz), 3.18 – 3.05 (2H, m, methylene protons), 2.21 – 2.16 (1H, m, thiol); 13C NMR (150 MHz Acetone-d6) δ 166.94, 160.93, 129.92, 113.73, 110.60, 107.28, 55.56, 34.90, 27.97; IR (neat) ν 3094 C(sp2)-H stretch, 2996 C(sp3)-H stretch, 2575 S-H thiol stretch, 1846 and 1779 C=O anhydride stretch, 140 C=C alkene stretch, cm⁻¹.

Synthesis of 4-(mercaptomethyl)isobenzofuran-1,3-dione (60)

Concentrated H₂SO₄ (0.5 mL), was dissolved in 0.2 mL of freshly distilled sulfolane at -55 ºC to gave a clear colourless solution. To the above solution compound 59 (0.1 g, 0.47 mmol)
were added and the solution turned red. The mixture was let to warm up to room temperature and then was stirred for 5 h. The reaction was quenched with 0.5 mL of deionized water and the solution changed to opaque beige. The organic layer was extracted with EtOAc (3 x 1 mL) and the combined organics were washed with water (3 x 1 mL). The organic layer was dried over MgSO₄. After filtration, the solvent was removed under rotary evaporation and the product was further dried under high vacuum overnight. An off-white liquid 60 was obtained in 85 % yield. 

Rf = 0.25 (1:1, hexanes/EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 8.57 (1H, dd, J = 7.3, 1.4 Hz, ArH), 7.86 – 7.81 (2H, m, ArH’s), 4.64 (2H, s, methylene protons); ¹³C NMR (100 MHz, CDCl₃) δ 164.70, 149.81, 134.65, 134.01, 133.10, 130.82, 129.59, 35.33; IR (neat) υ 2952 and 2882 C(sp³)-H stretch, 2624 S-H thiol stretch, 1725 C=O anhydride stretch, 1584 C=C stretch aromatic ring system, cm⁻¹.
CHAPTER 5: REFERENCES


Figure 1. Selective gradient NOESY performed on compound 44 to determine which isomer was obtained.
Figure 2. Selective gradient NOESY performed on compound 45 to determine which carbonyl group got reduced.