Using the Diels-Alder Reaction in the Synthesis of Biologically Interesting Molecules: Targets of Opportunity

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Using the Diels-Alder Reaction in the Synthesis of Biologically Interesting Molecules: Targets of Opportunity

Ethan Michael DeCicco

Submitted in Partial Fulfillment
of the
Requirements for the Degree
Master of Science in Chemistry

Supervised by
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Abstract

This thesis is composed of reports on two projects, which are described separately in two chapters.

The first project described (Chapter 1) involves the synthesis of a series of molecules that contain a 1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-imide core structure. These types of molecules represent a class of analogues of the natural product cantharidin, a potent cytotoxic agent isolated from Meloidae insects. Cantharidin and cantharidin-like small molecules have been extensively studied in literature as potential leads in new anti-cancer medications. A series of 1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-imides are also being investigated by researchers at Bristol-Meyers-Squibb (BMS) as prospective treatments of prostate cancer (PC). PC is the most common malignancy among men in the United States and the second most common cause of cancer-related death worldwide. The need for novel prostate cancer medications arises from patient resistance to current PC drugs. Synthesis and biological activity of a series of cantharidin/BMS-641988 analogues will be presented. Theoretical studies to identify our next generation of synthetic targets and their potential application in the treatment of PC will also be discussed.

The second project (Chapter 2) describes new methodology studies to provide access to 9-membered lactones. This structural moiety is an unusual, yet frequently observed structural feature of natural products. The synthesis of medium sized cyclic functional groups, namely 9-membered lactones, is still a formidable challenge in synthetic organic chemistry. Studies toward a facile, three step synthesis of molecules containing 9-membered lactones is proposed and synthetic efforts towards the appropriate precursors will be discussed.
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<tr>
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<td>EtOAc</td>
<td>ethyl acetate</td>
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<td>DCM</td>
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<td>NP</td>
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<td>[4+2]</td>
<td>Diels-Alder cycloaddition</td>
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<td>Frontier Molecular Orbital</td>
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<td>LAH</td>
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Chapter 1- Synthesis of Cantharidin Based Molecules for the Discovery of New Bioactive Compounds

1- Introduction

1.1- Natural Products as Source of New Medicine

Natural products (NPs) is a term used to describe substances produced by and then isolated from all forms of life, including marine organisms, bacteria, fungi, insects and plants\(^1\) and possess enormous structural and chemical diversity\(^2\). Molecules produced by biological entities naturally have a key role in drug discovery. NPs still prove to be a reliable source of new drugs, drug targets and bioactive structures\(^3\). Accordingly, 50% of FDA approved drugs from 1981 to 2010 were natural product derived, synthetically made based on a NP skeleton or NPs themselves, where only 29% were purely synthetic based on targets from screening and the remainder were either biologic treatments or vaccines\(^3\). Combinatorial chemistry is often employed in the discovery of new drugs, however, only one \textit{de novo} discovery from combinatorial techniques has led to an FDA approved drug from 1981-2012\(^3\). Further, of the 175 small molecule cancer treatments approved since the 1940s, 74.8% are other than purely synthetic and 48.6% are either directly NPs or NP derived\(^3\). The antibacterial field shows a heavy reliance on natural products for new drugs. In the same period, 65% of new antibacterial drugs were NPs, NP based, or synthesized base on NP pharmacophore\(^3\). Only 14% of new antibiotics were produced based on screening or rational design, and the remainder were either biologic treatments or vaccines\(^3\). Deriving drugs from NPs shows advantages statistically vs. combinatorial chemistry or screening approaches. Despite showing advantages as a source for new drugs against classical techniques, other factors of drug development make them more attractive.
A desirable prospect in drug development is the possibility of oral drug delivery. The convenience of oral delivery makes it the most attractive form of drug delivery, opposed to intravenous injections and other invasive methods. The seminal work published by Lipinski in 2004 established four properties a drug target should possess for the eventual possibility of oral drug delivery\(^4\). This is known as Lipinski’s (or Pfizer’s) rule of five\(^4\). The first four rules denote that in order to be developed to an orally available drug, a drug target must: 1. Have no more than five hydrogen bond donors, 2. Have no more than ten hydrogen bond acceptors, 3. Have a molecular weight of less than 500amu and 4. Have a octanol-water partition coefficient less than five (log \(P=5\))\(^4\), describing the necessity for hydrophilicity. These limitations would serve to eliminate a large list of target molecules because their inability to be delivered orally. However, Lipinski’s 5\(^{th}\) rule states that the first four do not apply to natural products or those involved in active transport when considering “druggable chemical entities,”\(^4\). A “druggable chemical entity,” is one that has enough “druglikeness,” to be developed into an orally active drug. Thus, natural products and natural product like targets can be considered viable drug targets for oral delivery by the inherent nature of their active transport within the biological systems.

Fungi, lichens, bacteria, insects other microbial organisms are key sources of natural products\(^5,6\). Because these organisms have no phenotypic modes of defense, they have evolved to synthesize toxic molecules for defense. Such toxic molecules can be isolated and tailored for benefit. The vast degree of biological diversity that exists in environments where NPs are usually found (forests, soils and oceans) require considerable time and resources to isolate and identify new molecules.

Searching for new naturally occurring molecules has classically targeted fungi, plants, baceteria and marine organisms\(^5\). Indeed, commonly known drugs today owe their origin of
discovery to nature. The first and most commonly used antibiotic Penicillin was first isolated from the fungus *Penicillium notatum* by Alexander Fleming in 1828, later shown to be a powerful antibiotic by Howard Florey and Ernst Chain\(^7\). The total synthesis of Penicillin by John Sheehan in 1958 allowed for development of more antibiotic analogues\(^8\). Aspirin owes its development to the historical use of willow bark extracts to treat inflammation and the isolation of the active ingredient by Henri Leroux in the 1890s\(^7\). Streptomycin, the first successful treatment for Tuberculosis, was first isolated from the soil bacterium *Streptomyces griseus* in 1943 by Selman Waksman, Albert Schatz, and Elizabeth Bugie\(^9\). Halichondrin B is an anti-tumor macrolide discovered from marine sponge *Halichondria okadai* by Yoshimasa Hirata and Daisuke Uemura in 1983\(^10\). Total synthesis of halichondrin B by Thomas Aicher in 1992 led to development of the analogue Eribulin mesylate (Halaven®), currently used in the treatment of metastatic breast cancer\(^11\). Even with these invaluable contributions to medicine there has been an emerging focus on insects as a source of new natural products.
1.2- Insects as Source of New Natural Products – Cantharidin 1

![cantharidin structures](image)

**Figure 1**- Structure of cantharidin (1) (top) isolated from *Lytta vesicatoria*. Structures of naturally occurring analogues 2-6. Photo: Wikimedia Commons

For as long as plants and fungi have been used medicinally, the use of insects to treat disease (entomotherapy) has occurred for just as long. In some underdeveloped regions of the world entomotherapy is still a common practice. Historical interest in isolating natural products from insects shown is in the successful isolation of small molecules with varying bioactivity from ants, sawflies, beetles, cockroaches, grasshoppers, butterflies and moths in the modern era. Further, over one hundred new natural products have been isolated from insects in the past decade. A notable source of new molecules comes from organisms of the Coleoptera order (beetles). Among them are the Meloidae, a small group of an estimated 2500 species whose secretions can cause skin irritation and blistering, colloquially known as blister beetles. Their historical use as therapeutic agents dates to 13th century China in wart removal and anti-cancer therapy in Middle Ages Europe. The ‘spanish fly’ (*Lytta vesicatoria*), when under stress secretes a toxic hemolymph (analogous to mammalian blood) as a mode of chemical defense.
This phenomenon interested French pharmacist Pierre Jean Robiquet, who in 1838 isolated a crystalline compound from the dried and ground beetles, known today as cantharidin (1) \((\text{exo,exo}}-1,4\)-dimethyl-7-oxabicyclo\[2.2.1\]heptane-2,3-dicarboxylic acid anhydride) (Figure 1)\(^{12}\). A demethylated analogue, known as (-)-palosonin 2 and its enantiomer 3 (Figure 1), has been isolated from seeds of the Himalayan plant *Butea frondosa* and is present in dried bodies of African beetles *Hycleus oculatus* and *Hycleus tinctus*\(^{12}\). Other notable analogues also found in nature include norcantharidin (4), cantharimide (5) and norcantharimide (6).

1.3- Previous Syntheses of Cantharidin and Utility of Diels-Alder Reactions

Cantharidin historically gained notoriety as the ‘spanish fly’ aphrodisiac. Conversely, synthetic efforts toward cantharidin and structurally similar small molecules generated compounds shown to exhibit anti-tumor qualities across a variety of cell lines in 1963\(^ {14}\). It later was shown the anti-tumor activity results from strong inhibition of protein phosphatases\(^ {15,16}\), calling for analogues that possessed selective toxicity while minimizing acute toxicity.

![Scheme 1- Early proposed route to cantharidin (1) by Otto Diels and Kurt Alder\(^ {17,18}\).](image)

The simple structure of cantharidin calls for the obvious but optimal synthetic route of a Diels-Alder reaction of dimethyl-maleic anhydride (7) and furan (8), which was proposed as early as 1929 by Otto Diels and Kurt Alder themselves\(^ {17}\) (Scheme 1). The first synthesis attempts through this Diels-Alder reaction failed inexplicably. Rationale came later in the 1950s from Fukui and his molecular orbital considerations of conjugated systems, known today as frontier
molecular orbital (FMO) theory\textsuperscript{19}. The lack of reaction between furan (8) and anhydride 7 was circumvented in 1951 by Gilbert Stork and his co-workers\textsuperscript{20} achieving the first total synthesis of cantharidin (1) in poor overall yield (0.5\% overall) (Scheme 2).

**Scheme 2-** First enantioselective synthesis from Stork et al\textsuperscript{20}.

Stork and his co-workers met a formidable challenge for the time through a rather elegant series of transformations. However, the 11 total steps would later be proved exhaustive and not feasible on preparative scales. In 1976 Dauben’s group recognized that the dienophilicity (tendency of a alkene to undergo 4+2 cycloaddition) of dimethyl-maleic anhydride (7) was insufficient\textsuperscript{21}. Diels-Alder reactions proceed most rapidly by combination of electron rich dienes and electron deficient alkenes (dienophiles), which provides the necessary energies of the dienophile LUMO and diene HOMO. The presence of electron donating methyl groups of dimethyl-maleic anhydride (7) significantly decreases dienophilicity and imparts unfavorable steric in a Diels-Alder transition state. Furan (8) was also considered a poor diene due to its
aromaticity, having less tendency to transfer electrons to electron deficient systems compared to cyclopentadiene\textsuperscript{22}. The propensity of this system to undergo retro-Diels-Alder when heated was also recognized\textsuperscript{22}. Dauben \textit{et al} surpassed the limitations by employing a cyclic thioether derivative of maleic anhydride 9 in the Diels-Alder reaction with furan (8) to circumvent unfavorable sterics from the dimethyl groups of 7. Elevated pressure was employed to avoid the need for high temperatures, which avoided a retro-Diels-Alder process, pyrolysis of the thioether bridge and to overcome the steric hindrance imparted by the cyclic thioether\textsuperscript{21} (Scheme 3).

\begin{center}
\includegraphics[width=\textwidth]{Scheme3.png}
\end{center}

\textbf{Scheme 3}- Dauben \textit{et al} optimized preparative scale synthesis of cantharidin (1)\textsuperscript{21}. Under the optimized conditions (7 kbar, RT, 16eq furan (8)), full conversion of thioether anhydride 9 and furan (8) to Diels-Alder adducts 10 and 11 was observed\textsuperscript{21}. The resulting isomers were then reduced and desulfurized with catalytic Raney Ni, giving cantharidin (1) and its \textit{endo} isomer 12\textsuperscript{21}. Selective recrystallization from ethyl acetate yielded pure cantharidin (1) on a 10-15g scale in 51\% yield\textsuperscript{21}. 
Diels-Alder reactions are deceptively simple transformations with immense utility. However, variables and nuances that either rationalize reaction products or lack thereof were understood long after its application in synthesis. FMO theory has since provided explanations that provide controllable and predictable products. Diels-Alder reactions generally favor endo-substituted products due to stabilization of an endo-approach transition state by secondary orbital overlap (Figure 2).19

Figure 2- Stabilization of the endo approach transition state through secondary orbital reactions.

Woodward and Hoffman also illustrated molecular orbital (MO) considerations that accurately predict stereochemical outcomes of electrocyclic reactions23. Enantioselective Diels-Alder reactions are possible using chiral auxiliaries and chiral lewis acid activators and organocatalysis, such as camphor sulfonyl hydrazines24,25. A great deal of regioselectivity is possible based on position of electron donating (EDG) electron withdrawing (EWG) groups on dienes and dienophiles and is rationalized by MO and FMO theory26. Diastereoselectivity can be controlled when considering temperature, solvent systems, pressure and steric bulk on dienes or
dienophiles as well as the presence of Lewis acids. Because a considerable amount of control is possible through Diels-Alder transformations, its utility in synthetic chemistry is far reaching.

1.4- Previous Synthesis Biological Evaluation of Cantharidin Based Molecules

Closely related analogues of cantharidin have also seen considerable attention. The mono methyl analogues, (+)-palasonin (3) and (-)-palasonin (2) were synthesized enantioselectively by Dauben and co-workers in 1996 (Scheme 4).

Scheme 4- Synthesis and resolution of (+)/(-)-palasonin from Dauben et al.

Their work with this system revealed an optimized Diels-Alder transformation of furan (8) and methyl-maleic anhydride 13 was dependent on the molar equivalent of furan, pressure applied and reaction time. Due to the propensity of Diels-Alder products to undergo retro-Diels-Alder process (cycloaddition products revert to starting diene and dienophile), an excess of furan favors Diels-Alder products, however greater than 1.1eq of furan (8) in this system used yields diaddition products and later found 1.05eq of furan minimizes the formation of diaddition
products\textsuperscript{27}. Using 1.05 eq. of furan, investigation of reaction time and pressure employed showed optimal yields were obtained after 138h under 8 kbar of pressure, giving (+/-)-palasonin in 99% yield and no detectable amounts of endo isomer products\textsuperscript{27}. The racemic mixture was resolved by reaction with 2eq. of (S)-(−)-α-methylbenzylamine to form diastereomeric amides, that were separated and saponified to diacids and subsequent reaction with thionyl chloride yielded (−) and (+) palasonin (3) and (2), respectively\textsuperscript{27}.

Notably, a study from Lee and Herndon on the stereochemistry of the Diels-Alder reaction of furan (8) and maleic anhydride (15), it was described that the thermodynamic exo product is favored (Scheme 5)\textsuperscript{28}.

\textbf{Scheme 5-} Facile synthesis of norcantharidin 4 from Lee and Herndon\textsuperscript{28} and Hill et al\textsuperscript{29}.

Although the endo isomer forms at a faster rate it spontaneously undergoes a retro-Diels-Alder/Diels-Alder process and the resulting equilibrium results in a majority of exo isomer products\textsuperscript{12,28}. Synthesis of the non-methyl containing analogue, norcantharidin (4), is considerably easier due to minimized steric hindrance and electron donating effects of dimethyl and methyl maleic anhydrides 7 and 13. Facile synthesis is achieved with a room temp, atmospheric pressure Diels-Alder reaction of maleic anhydride and furan followed by hydrogenation with Pd/C and 4atm of H\textsubscript{2} (g)\textsuperscript{29}.

Modifications to the oxabicyclo [2.2.1] heptane core has also received attention in recent years. A thio-bicyclo analogue of cantharidin has been synthesized by McCluskey et al through
the reaction of anhydride 15 and thiophene (16) under elevated pressure (Scheme 6). The resulting analogue displayed inhibition of a protein phosphatase.

![Scheme 6](image)

**Scheme 6** - Synthesis of thio-bicyclo analogue 17 from McCluskey et al.

Fluorinated maleic anhydrides 18-20 were synthesized by Essers et al. in 2001 and used in Diels-Alder reactions with furan (8) to generate fluorinated norcantharidins 21-23 (Scheme 7).

![Scheme 7](image)

**Scheme 7** - Synthesis of fluoronorcantharidins 21-23 by Essers et al. (top). Difluoro adduct 21 rapidly undergoes saponification to diacid 24 when exposed to air moisture (bottom).

Monofluorinated derivatives 22 and 23 were reported as stable. However, the difluoro Diels-Alder adduct 21 formed by the reaction of 18 and 8 was found to be unstable and rapidly converted to diacid 24 upon exposure to air moisture. The isolated diacid was reduced to stable endothall 25 and isolated.
Modifications to the 5 position of the oxabicyclo [2.2.1] core of norcantharidin 1 were investigated with reactions of 3-substituted furans by Tatlock et al in 1997 (Scheme 8) on the basis of computational docking of cantharidin to human protein phosphatase 1 (PP1)\textsuperscript{32}.

![Scheme 8- Synthesis of norcantharidin derivatives from furanyl esters 26a-j by Tatlock et al](image)

Furanyl esters 26a-j underwent Diels-Alder addition with maleic anhydride (15) under ambient conditions and were reduced to the corresponding norcantharidin derivatives 27a-j in high yield\textsuperscript{32}. All exhibited PP1 and PP2B inhibitory activity\textsuperscript{32}.

Notably, Cott et al showed in 2005 that reactions of furfuryl compounds 28a-c and maleic anhydride (15) proceeds roughly 10 times faster in supercritical CO\textsubscript{2} than in diethyl ether (Scheme 9)\textsuperscript{33}. 

12
Scheme 9 - Synthesis of norcantharidin analogue precursors \(29a-c\) in supercritical \(\text{CO}_2\).

The accelerated rate of this reaction provided a solution to low yielding results in the reaction of similarly substituted furans with maleic anhydride (15) encountered by Hart \textit{et al}\textsuperscript{34}. The conditions also provide access to \textit{endo}-norcantharidin type structures that are difficult to isolate as their retro-Diels-Alder reaction process occurs rapidly and re-conversion to the \textit{exo} isomer is favored. More recent attention turned to modifying the anhydride ring of norcantharidin (4) from Tarleton \textit{et al} in 2012 (Scheme 10)\textsuperscript{35}.
Scheme 10- Generating alkoxy norcantharidin analogues 34 a-c and 35 a-k from hydroxylactones 32 and 33 (top). Installation of various terminal phosphate groups to alkyloxy lactone 36 (bottom) from Tarleton et al\textsuperscript{35}. 

Precursor 30 was synthesized from anhydride 15 and furan (8) (see Scheme 5)\textsuperscript{35}. Simultaneous reduction of the alkene and one carbonyl of 30 was achieved with 4 bar H\textsubscript{2} and Pd/C in ethanol of generating hydroxylactones 32 and 33 which were separated by selective recrystallization\textsuperscript{35}. Hydroxylactones 32 and 33 were then used to generate a library of alkoxy derivatives with unsaturated, straight, branched and cyclic chains by exposing the hydroxy lactone to the corresponding alcohol in the presence of catalytic para-toluenesulfonic acid (p-TsOH) under microwave (\(\mu\)W) irradiation\textsuperscript{35}. Notably, these reactions were successful with small and large alkyl chains and with alkyl substituents bearing synthetically useful alkene and alkyne moieties. Alkenes 34a, 34b, 35a and 35b were successfully epoxidized with mCPBA to the corresponding racemic epoxides\textsuperscript{35}. In the same study, various terminal phosphate groups were

*all alkenes are trans

34a R= CH\textsubscript{2}HC=CH\textsubscript{2}
34b R= CH\textsubscript{2}HC=CHCH\textsubscript{3}
34c R= (CH\textsubscript{2})\textsubscript{4}CH\textsubscript{3}
34d R= iPr

35a R= CH\textsubscript{2}HC=CH\textsubscript{2}
35b R= CH\textsubscript{2}HC=CHCH\textsubscript{3}
35c R= (CH\textsubscript{2})\textsubscript{4}C\equiv CH
35d R= CH\textsubscript{2}CH\textsubscript{3}
35e R= (CH\textsubscript{2})\textsubscript{2}CH\textsubscript{3}
35f R= (CH\textsubscript{2})\textsubscript{2}CH\textsubscript{3}
35g R= (CH\textsubscript{2})\textsubscript{5}CH\textsubscript{3}
35h R= (CH\textsubscript{2})\textsubscript{6}CH\textsubscript{3}
35i R= iPr
35j R= tBu
35k R= cyhex
1.5- Previous Syntheses and Biological Evaluation of Cantharimide and Based Molecules

The closely related imide counterpart, known as norcantharimide (5), has received most of the recent attention in developing new cantharidin analogues. The Diels-Alder reaction of furans and maleimides to prepare cantharimides are suboptimal due to lack of selectivity to form the desired bioactive exo isomers\(^\text{12}\) (Scheme 11).

\[
\begin{align*}
\text{Furan (8)} + \text{Maleimide (37)} &\xrightarrow{1. [4+2]} \text{Diels-Alder products} \\
&\xrightarrow{2. \text{reduction}} \text{Norcantharimide} (6) + \text{Exo} (38)
\end{align*}
\]

**Scheme 11**- General Diels-Alder reaction for norcantharimide (6) from furan (8) and maleimide (37).

Combining maleimide (37) and furan (8) gives significant amounts of both exo 6 and endo 38 Diels-Alder products\(^\text{29}\). The desired bioactive exo isomers then need to be isolated by chromatography. Cantharimide/norcantharimides are of interest as they still possess biological activity but exhibit less acute toxicity to mammalian enzymes\(^\text{29,36}\). The less toxic imides have been most commonly prepared in condensation of cantharidins with primary amines\(^\text{12}\). In 2001 McCluskey *et al* generated 16 norcantharimide derivates 39 a-p by treating commercially available norcantharidin (4) with various amino acids (AAs) in toluene and triethylamine at 200°C (sealed tube) with modest yields (Scheme 12)\(^\text{29}\).
Scheme 12- Synthesis of N-amino acid cantharimides by McCluskey et al.

In evaluating the inhibition of PP1 and PP2 of compounds 39a-p, tyrosine (39i and 39j), tryptophan (39m and 39n) and histidine (39k and 39l) residues showed the greatest inhibition, suggesting the greatest activity is achieved by instillation of bulky aromatic side chains\textsuperscript{36}. Detectable variation in inhibition was noticed upon the addition of D or L AAs, where the natural L-enantiomers generally showed slightly higher inhibition\textsuperscript{36}.

In 2007 Hill et al synthesized various N-alkylamines with short and long alkyl chains, cycloalkyl, unsaturated, hydroxyl and carboxylic acid substituents\textsuperscript{29} (Scheme 13). Starting material norcantharidin (4) was prepared on high scale readily by the exo-selective cycloaddition of furan (8) and anhydride 15 (rt, 48h in diethylether) that was subsequently reduced under H\textsubscript{2} (g) (4 atm) in the presence of Pd/C\textsuperscript{29}.

Scheme 13- Addition of various amines to norcantharidin (4) to generate norcantharimide derivatives 40 a-m by Hill et al\textsuperscript{29}. 
Compounds 40 a-m were then tested for activity against colon, breast, ovarian, lung, skin, prostate, neuronal and brain cancer cell lines\textsuperscript{29}. The most potent inhibitors were those bearing 8C-14C alkyl chains (40g-j) across all lines\textsuperscript{29}. N-vinyl derivative 40l showed the most potent inhibition across several lines\textsuperscript{29}. Both unsaturated chain derivatives 40l and 40m were susceptible to epoxidation (mCPBA) and dihydroxylation (OsO\textsubscript{4}/NMO)\textsuperscript{29}, providing access to even more derivatives by this method.

Two bis-cantharimides were prepared by treating norcantharidin (4) with terminal diamines, 1,3-diaminopropane and 1,12-diaminododecane (Scheme 14).

![Scheme 14](image)

**Scheme 14**- Generation of bis-cantharimides 41 a and b by Hill et al\textsuperscript{29}.

The two bis-norcantharimides 41a and 41b showed greater cytotoxicity than their monomer counterparts\textsuperscript{29}. Notably, the dodecane linked bis-cantharimide 41b showed greater cytotoxicity than norcantharidin (4)\textsuperscript{29}.

The facile addition of various amines demonstrated by the Hill and McCluskey groups allowed for preparation N-heteroatomic substituted cantharimides by Kok et al (Scheme 15)\textsuperscript{37}. 
Scheme 15- Synthesis of thiazole cantharimides 42 and 43 a-d from Kok et al\textsuperscript{37}.

Addition of aminothiazoles was achieved by the established method\textsuperscript{39}. The synthesized aminothiazole derivatives 42 and 43a-d were all shown to exhibit prominent anti-tumor activity\textsuperscript{37}. Further, installation of aminothiazoles in 43a-d were shown to increase toxicity to malignant cells while showing less toxicity to healthy cells when compared to cantharidin (1)\textsuperscript{37}. Further, 43 a-d were found to induce apoptosis in SK-Hep-1 hepatoma cells\textsuperscript{37}. Disulfide linked \(N\)-aminothiadiazole cantharimide dimers have also been prepared the Kok group\textsuperscript{38}(Scheme 16).

Scheme 16- Synthesis of disulfide linked \(N\)-thiadiazole cantharimide dimer 44 by Kok et al\textsuperscript{38}.

The dehydrative condensation of cantharidin and norcantharidins with primary amines was insufficient for anilines until an improvement from Deng et al employed catalytic manganese (II) acetate (Mn(OAc)\textsubscript{2}) synthesizing various \(N\)-aryl norcantharimides (Scheme 17)\textsuperscript{39}.  

---

\[42\]  
\[43a R=OMe\]
\[43b R=Me\]
\[43c R=OCF_3\]
\[43d R=H\]
A plethora of modifications to the [2.2.1] oxabicyclo heptane core of cantharimide and norcantharimide have been achieved. Further analogues were developed by Thaqi et al where the oxabicyclo heptane core is replaced by a 5,6 heteroatomic bridge and then further derivatized by the addition of AAs and AA esters to the anhydride ring (Scheme 18)\textsuperscript{40}.

\textbf{Scheme 17}- Synthesis of \( N \)-aryl norcantharimides \textbf{46} from dehydro-norcantharidin \textbf{45}.

\textbf{Scheme 18}- Synthesis of heteroatomic fused imide cantharimide analogues \textbf{51-54 a-p} from Thaqi \textit{et al}
The starting succinic anhydrides 47-50 were transformed to corresponding imides 51-54 with various AAs and heterocycle or alkylhydroxy substituted amines promoted by microwave (µW) irradiation. In bioactivity assays the starting anhydrides 47-50 were not active against cell lines that cantharidin (1) and norcantharidin (4) significantly inhibited. This suggests the importance of the ethyl bridge and ethereal oxygen of the oxabicyclic core of cantharidin (1) and norcantharidin (4) for bioactivity. The majority of analogues did not show increased cytotoxicity compared to cantharidin (1) and norcantharidin (4). However, it was noted that N-octyl derivative 51p showed greater cytotoxicity than 1 and 4. This finding suggests the benefit of longer N-alkyl chains, in agreement with the observed potency of N-C8-14 chained cantharimide derivatives 40 g-k from Hill’s group (Scheme 13). Analogue 48p was also found to be equipotent to norcantharidin (4) across the same cell lines.

Further derivatives from Rojas and Kouznetsov were generated through modifications of a carbonyl functionality on norcantharidin (4) (Scheme 19).

**Scheme 19-** Generation of lactam norcantharimide derivatives 57 from Rojas and Kouznetsov.

Treatment of norcantharidin 4 with ylide 55 generated olefinic lactone 56 which was susceptible to condensation with 20 different amines to generate library 57. Notably, under these careful conditions, the bicyclic core essential for bioactivity remains intact.
In 2010 Goksu *et al* exploited the double bond of 5,6-dehydro-norcantharimide 58 and methylene bridged analogue 59 to selectively generate *exo*-arylated analogues 60a-f and 61a-c through a Heck arylation process (Scheme 20)\(^{42}\).

![Scheme 20](image)

**Scheme 20** - Arylation of the [2.2.1] bicyclo heptane norcantharimide core via reductive Heck arylation from Goksu *et al*\(^{42}\)

The procedure is relatively robust as multiple aryl substituents were successfully installed, including the sterically encumbered biphenyl residue 60c and thiophene 61c.

Further modification at the 5,6-position of dehydrocantharimides came from Deng *et al* through 1,3-dipolar cycloaddition of dehydronorcantharimides and nitrile oximes (Scheme 21)\(^{43}\).

![Scheme 21](image)

**Scheme 21** - 1,3-dipolar cycloaddition of norcantharimides 62 to generate isooxazolines 64.

Deng’s group generated a library of isooxazolines 64 from 20 different aryl norcantharimides 62 by the 1,3-dipolar cycloaddition with nitrile oxide 63 in the presence of *N*-
chloro tosylamide (Chloramine T)\textsuperscript{43}. This procedure also served to install the common trimethoxyphenyl pharmacophore in a robust fashion.

Furans bearing substituents in the two position had previously gave poor yields in Diels-Alder reactions with maleic anhydride (\textbf{15}), which was subsided by reaction in supercritical CO\textsubscript{2} by Cott \textit{et al}\textsuperscript{33}. Similarly, the reaction of furfuryl amines would be expected to proceed sluggishly in Diels-Alder reactions. Galvis \textit{et al} discovered that employing boric acid can accelerate Diels-Alder reactions of furfuryl amines and maleimides (Scheme 22). Diene \textbf{65} successfully underwent Diels-Alder addition with \textit{N}-substituted maleimides \textbf{37, 66} and \textbf{67} by employing catalytic boric acid in PEG-400\textsuperscript{44} (Scheme 22).

\begin{center}
\begin{tikzpicture}
  \node (furan) at (0,0) {\includegraphics[width=0.2\textwidth]{furan.png}};
  \node (maleimide) at (1.5,0) {\includegraphics[width=0.2\textwidth]{maleimide.png}};
  \node (product) at (3.5,0) {\includegraphics[width=0.2\textwidth]{product.png}};
  \draw [->] (furan) -- node [above] {$H_2BO_3$ (10\% mol)} (maleimide);
  \draw [->] (maleimide) -- node [above] {PEG-400, 90°C 1hr} (product);
  \node at (furan) {\textbf{65}};
  \node at (maleimide) {\textbf{37} $R=H$, \textbf{66} $R=Ph$, \textbf{67} $R=4$-ClBenzyl};
  \node at (product) {\textbf{68a} $R=H$, \textbf{68b} $R=Ph$, \textbf{68c} $R=4$-ClBenzyl};
\end{tikzpicture}
\end{center}

\textbf{Scheme 22-} \textit{exo}-Selective synthesis of dihydronorcantharinimides \textbf{68 a-c} from furfuryl amide \textbf{65} and maleimides \textbf{37, 66} and \textbf{67} using catalytic boric acid from Galvis \textit{et al}\textsuperscript{44}.

We presume the coordination of boron to oxygens of carbonyls of the imide functionality favors the formation of the \textit{exo}-DA product by blocking the endo approach of furfuryl diene \textbf{65}\textsuperscript{44}. This coordination likely also accelerates the rate of Diels-Alder adducts \textbf{68 a-c} indicated by the short reaction time\textsuperscript{44}.
1.6- Summary of Biological Activity of Cantharidin and Cantharimide Based Molecules

Since its discovery, cantharidin and its analogues have been the focus of synthetic chemists in hopes of developing new analogues with selective anti-proliferative effects while minimizing the toxicity of the parent NP molecules. Several trends are apparent when comparing analogue structure to biological activity. As discussed, cantharidin acts to cause cell death by protein phosphatase inhibitory activity\textsuperscript{36}. In a recent review from Galvis \textit{et al}, the most potent PP1/PP2 inhibitors were summarized (Table 1)\textsuperscript{12}.

**Table 1-** Most potent PP1/PP2 inhibitors as summarized by Galvis \textit{et al}\textsuperscript{12}.

<table>
<thead>
<tr>
<th>Compound</th>
<th>PP1 Inhibition (IC\textsubscript{50} µM)</th>
<th>PP2 Inhibition (IC\textsubscript{50} µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.6 ± 0.42</td>
<td>0.36 ± 0.08</td>
</tr>
<tr>
<td>6</td>
<td>5.31 ± 0.36</td>
<td>2.9 ± 1.04</td>
</tr>
<tr>
<td>39l</td>
<td>3.22 ± 0.7</td>
<td>0.81 ± 0.1</td>
</tr>
<tr>
<td>39k</td>
<td>2.82 ± 0.5</td>
<td>1.35 ± 0.3</td>
</tr>
<tr>
<td>69</td>
<td>13 ± 5</td>
<td>7.0 ± 3.0</td>
</tr>
<tr>
<td>70</td>
<td>18 ± 8</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>71</td>
<td>12.5</td>
<td>426</td>
</tr>
<tr>
<td>72</td>
<td>5.9 ± 2.2</td>
<td>0.79 ± 0.1</td>
</tr>
</tbody>
</table>

*Compounds 69-72 not discussed. Primary Ref: 1, 6, 39l, 39k, 71\textsuperscript{30}, 69-72\textsuperscript{34}

Cantharimide derivatives bearing an \textit{N}-substituted aromatic species 39l, 39k and 69 were shown to be greater PP inhibitors than aliphatic derivatives\textsuperscript{12}. In imide ring opened derivatives,
strongly polarized carboxylate salt 70 and N-heterocycle amide 72 analogues proved to be potent inhibitors along with thio-bicyclo cantharidin derivative 7112.

Cantharidin based molecules cytotoxicity to human cancer cell lines has also been assessed extensively (Table 2)12,35,29,40. Across some human cancer cell lines, the alkyloxy lactone cantharidin analogues from Tarleton et al35 showed potent cytotoxicity. Cytotoxicity against more strains was observed when terminal phosphate groups were installed to the alkyloxy moiety of the alkyloxy lactone in cantharidin analogues 37 a-d35. Unsurprisingly, the same amide carboxylate salt 70 to show potent PP inhibitory activity from Hart et al34 also showed potent cytotoxicity against several cancer cell lines34. Additionally, the dodecane linked bis-norcantharidin analogue 41b from Hill et al29 showed potent cytotoxicity across several lines. Similar results were observed with long chain N-alkyl, N-propyl(1-hydroxy-2-methoxy) norcantharimide analogues from McCluskey et al36. Finally, 1,3-heteroatomic bridged norcantharimide species showed potent cytotoxicity.
Table 2- Most potent tumor cell growth inhibitors as summarized by Galvis et al\textsuperscript{12}.

<table>
<thead>
<tr>
<th>Tumor Cell Line</th>
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<th>A2780</th>
<th>G401</th>
<th>H460</th>
<th>L1210</th>
<th>HT29</th>
<th>SW480</th>
<th>MCF-7</th>
<th>A431</th>
<th>DU145</th>
<th>BE2-C</th>
<th>SJ-G2</th>
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<td>N.D.</td>
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<td>N.D.</td>
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<td>35 ± 2.3</td>
<td>50 ± 4</td>
<td>13 ± 0.3</td>
<td>33 ± 7</td>
<td>41 ± 4</td>
<td>34 ± 3</td>
<td>72 ± 3</td>
<td>90 ± 10</td>
<td>36 ± 0</td>
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<td>41 ± 9.2</td>
<td>48 ± 1.5</td>
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<td>N.D.</td>
<td>&gt;100</td>
<td>N.D.</td>
<td>42 ± 5</td>
<td>93 ± 8</td>
<td>77 ± 10</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>84 ± 5</td>
<td>55 ± 3</td>
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<td>&gt;100</td>
<td>N.D.</td>
<td>32 ± 3</td>
<td>39 ± 4</td>
<td>27 ± 6</td>
<td>68 ± 5</td>
<td>&gt;100</td>
<td>38 ± 2</td>
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<td>&gt;100</td>
<td>N.D.</td>
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<td>94 ± 1</td>
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<td>&gt;100</td>
<td>&gt;100</td>
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<td>&gt;100</td>
<td>N.D.</td>
<td>17 ± 1</td>
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<td>13 ± 0.3</td>
<td>N.D.</td>
<td>11 ± 0.3</td>
<td>10 ± 03</td>
<td>12 ± 0.9</td>
<td>12 ± 0.3</td>
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<td>9.3 ± 0.1</td>
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</tr>
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<td>40i</td>
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<td>N.D.</td>
<td>53 ± 4</td>
<td>N.D.</td>
<td>25 ± 4</td>
<td>55 ± 2</td>
<td>52 ± 8</td>
<td>35 ± 4</td>
<td>66 ± 4</td>
<td>40 ± 6</td>
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<tr>
<td>40j</td>
<td>35 ± 4</td>
<td>N.D.</td>
<td>66 ± 7</td>
<td>N.D.</td>
<td>19 ± 0</td>
<td>56 ± 4</td>
<td>43 ± 7</td>
<td>47 ± 3</td>
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<td>&lt;10</td>
<td>N.D.</td>
<td>12 ± 4</td>
<td>&lt;10</td>
<td>33 ± 3</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
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<td>31 ± 7</td>
<td>N.D.</td>
<td>8.3 ± 0.7</td>
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<td>18 ± 0</td>
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<td>N.D.</td>
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<td>29 ± 2</td>
<td>22 ± 4</td>
<td>36 ± 1</td>
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<tr>
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<td>N.D.</td>
<td>81 ± 8</td>
<td>N.D.</td>
<td>59 ± 1</td>
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<td>41 ± 1</td>
<td>61 ± 2</td>
<td>70 ± 2</td>
<td>68 ± 2</td>
<td>65 ± 5</td>
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</table>

Cpd=Compound – N.D. = Not Determined

Values expressed as lowest GI\textsubscript{50} μM

Primary Ref: 1, 4, 70\textsuperscript{36}, 34 b-d, 73, 74\textsuperscript{35}, 40i-j, 75, 41b\textsuperscript{39}, 51p and 52p\textsuperscript{40}. See Fig 3 for structures.
Figure 3- Structures of most potent tumor cell inhibitors as summarized by Galvis et al\textsuperscript{12}.

1.7- BMS-641988

A notable analogue sharing its core structure with norcantharimide (6) is BMS-641988 (75), developed from a series of [2.2.1]-oxabicyclo imide-based androgen receptor (AR) antagonists by researchers at Bristol-Meyers-Squibb\textsuperscript{45}.

Figure 4- Structures of BMS-641988 and FDA approved hydroxyflutamide and bicalutamide.

BMS-641988 is a novel rational design based AR antagonist currently in clinical development\textsuperscript{45}. Androgen receptor antagonists in combination with anti-androgens (chemical
castration) and surgical castration is the current standard of treatment for carcinoma of the prostate (CaP)\textsuperscript{46}. About 50\% of those that do not respond to initial treatment go on to develop castration resistant prostate cancer (CRPC) characterized by resistance to current androgen antagonists\textsuperscript{47}. The need for novel androgen receptor antagonists arises from this resistance to current androgen receptor antagonists, including FDA approved hydroxyflutamide (76) and bicalutamide (77)\textsuperscript{47}. BMS-641988 (75) has shown greater androgen receptor binding (K\textsubscript{i}=1.7nM), antagonist activity and anti-tumor activity (MDA-MB-453 IC\textsubscript{50}=16nM) compared to bicalutamide in \textit{in vivo} assays (Figure 4)\textsuperscript{45}. The structure of BMS-641988 (75) features a 1,4-dimethyl-N-aryl-5-\textit{endo}-sulfonamide derivation from norcantharimide (6).
The synthesis of BMS-641988 (75\textsuperscript{45}) began by generating the \(N\)-(3-trifluoromethyl-4-cyano)phenyl maleimide 78 by dehydrative condensation of anhydride 15 with the corresponding aniline 77. The diastereoselective generation of the \textit{exo} cycloadduct is achieved by a Diels-Alder reaction with sterically encumbered furan 82 at 120°C, a remarkable diastereoselective Diels-Alder without the use of a Lewis acid catalyst. Reduction of the 5,6-alkene with \(\text{H}_2\) (g) and \(\text{Pd/C}\) generates the endo ester 79 and following chiral HPLC separation gives the active enantiomer 80 from the racemic \textit{exo}-Diels-Alder adduct 79. Acid catalyzed hydrolysis from 80 gives the corresponding acid and a subsequent Curtius rearrangement in the
presence of 2-(trimethylsilyl)ethanol generates the corresponding Teoc-carbamate. TFA promoted cleavage of the resulting carbamate generates the endo-amine 81. Finally, the endo-amine is coupled to EtSO₂Cl under basic conditions to yield BMS-641988 (75).

1.8- Proposed Work

Due to the recent development of BMS-641988 (75), the plethora of modifications possible to cantharidin and cantharimide type molecules and the extensive bioactivity of cantharidin based molecules, we plan to synthesize a series of cantharidin/cantharimide based NP analogues for the discovery of new molecules with anti-proliferative properties. We employ a forward thinking synthetic strategy beginning with reactions of N-alkylmaleimides, maleic anhydride (15) and 2,5-dimethylfuran (82) due to structural similarity of corresponding Diels-Alder products and the BMS lead structure (Schemes 24 and 25).

Scheme 24- Proposed route to dimethylnorcantharidin core structure 83 from 2,5-dimethyl furan and maleic anhydride 15.

Scheme 25- Proposed route to dimethylnorcantharimide core structures from 2,5-dimethylfuran and N-alkylmaleimides.
A successful Diels-Alder reaction of furan 82 and anhydride 15 would generate a 1,4-dimethylnorcantharidin structure and would likely proceed without the need for high pressures. This represents a key intermediate in this synthetic endeavor due to the overwhelming modification possibilities namely, through the reactivity of the 5,6-double bond and anhydride ring as shown in the development of numerous cantharidin analogues as described. A Diels-Alder reaction of furan 82 with N-alkylmaleimides would produce a dimethyl-norcantharimide structure that may be modified at the alkene and by the synthesis of various N-alkylmaleimides. Once sufficient derivatives have been generated, selected compounds will be tested by a collaborator for biological activity and modified as necessary in order to discover new molecules with anti-proliferative properties.

2- Results and Discussion

2.1- Diels-Alder Reactions of anhydride 15 and furan 82

The first Diels-Alder system investigated the reaction of furan 82 and anhydride 15 to generate the 1,4-dimethyl-5,6-dehydronorcantharidin core structure 83 (Scheme 26).

![Scheme 26- Synthesis of Diels-Alder adduct 83](image-url)
Figure 5- Comparison of purified Diels-Alder adduct 83 at times t=0hr and t=16hr by $^1$HNMR.

It was first found that the Diels-Alder reaction of maleic anhydride 15 and furan 82 proceeds rapidly at room temperature (RT) in ethyl acetate (EtOAc) (Scheme 26). Fifty percent of starting anhydride 15 was converted to Diels-Alder adduct 83. The neat reaction also proceeds at RT in similar fashion. Attempts to purify Diels-Alder adduct 83 by chromatography failed due to instability on silica. Recrystallization of the crude product from diethyl ether (Et$_2$O) yielded a mixture of 80% Diels-Alder adduct 83 to 20% starting anhydride 15 by $^1$HNMR integration (Figure 5). The corresponding alkene peak of 83 is observed at 6.36ppm at time t=0hr. At time t=16hr, the alkene peak of 83 is diminished, the starting furan 82 is observed (2.25 and 5.83ppm) and the ratio of anhydride 15 (7.04ppm) to the solvent residual peak (7.26ppm) is increased, indicating the propensity of Diels-Alder adduct 83 to undergo a retro-Diels-Alder reaction at RT.
Scheme 27- Reduction of crude Diels-Alder adduct \( \text{83} \) to reduced adduct \( \text{84} \) and anhydride \( \text{85} \).

It was believed instability to silica observed during purification of Diels-Alder adduct \( \text{83} \) was due to the tendency of crude adduct \( \text{83} \) to undergo a retro-Diels-Alder reaction and hydrogenation of Diels-Alder adduct \( \text{83} \) would be necessary before purification by chromatography (Scheme 27). Purification of reduced adduct \( \text{84} \) by chromatography failed, suggesting the anhydride moiety is responsible for the instability on silica, which was confirmed by 2D TLC experiments.

Scheme 28- One pot DA/reduction reaction to isolate reduced adduct \( \text{84} \)

A one pot Diels-Alder/reduction reaction was conducted anticipating the Diels-Alder product \( \text{83} \) would be immediately reduced to adduct \( \text{84} \), thereby favoring the Diels-Alder product in the Diels-Alder/retro-Diels-Alder equilibrium (Scheme 28). However, we observed that anhydride \( \text{15} \) is also reduced to anhydride \( \text{85} \) in this process, therefore the equilibrium of the Diels-Alder reaction could not be manipulated to solely produce Diels-Alder adduct \( \text{84} \). However, we did note only \( exo \) Diels-Alder products are obtained in the reaction of anhydride \( \text{15} \).
and furan 82. This prompted investigation of exploiting the *exo* selectivity of this reaction to generate 1,4-dimethylnorcantharimides (Scheme 29).

**Scheme 29** - Synthesis of reduced *N*-benzyl Diels-Alder adduct 100 from anhydride 15.

Addition of nucleophiles to the reduced core structure 84 would serve as a route to selectively generate *exo*-1,4-dimethyl-norcanthraimides. Products 83 and 84 were used in subsequent steps without purification and the reduced *exo*-*N*-benzyl Diels-Alder adduct 100 was synthesized successfully from anhydride 15 (16% overall)\(^\text{29}\). This success opened the possibility of adding varying amine nucleophiles into the anhydride ring. This route proved advantageous not only from complete *exo* selectivity, but also that molecular diversity is added in the form of an amino nucleophile at the final stage of the route. Complete *exo* selectivity in the formation of 83 was initially surprising to our group but later rationalized from theoretical and experimental observations by Rulisek *et al* (Figure 6)\(^\text{49}\).
Figure 6- Theoretical calculations of transition state energies (kJ/mol) of the Diels-Alder reaction between furan (8) and maleic anhydride (15) from Rulisek et al\textsuperscript{49}.

Rulisek and co-workers report theoretical data that suggests a Diels-Alder reaction of anhydride 15 and furan (8) proceeds uniquely to form only exo isomer 4 (Figure 6)\textsuperscript{49}. Due to nearly identical transition state energies between endo and exo forming reactions, exo product 4 is strongly favored thermodynamically (-11.5kJ/mol) and even slightly kinetically (-0.3kJ/mol). Their theoretical calculations were supported by experimental evidence through analysis of the reaction of 15 and 8 by \textsuperscript{1}H NMR\textsuperscript{49}. We expect our system employing 2,5-dimethylfuran (82) in the reaction with anhydride 15 proceeds in a similar fashion, mainly due to no detectable formation of the endo counterpart in \textsuperscript{1}H NMR experiments and the propensity of the thermodynamic exo product 83 to undergo a retro-Diels-Alder reaction at room temperature (Figure 5). Further modifications of the core Diels-Alder structure 83 were also investigated.
2.2- Post Diels-Alder Modifications to Diels-Alder adduct 83

Due to the plethora of modification possibilities to Diels-Alder product 83 at the 5,6-alkene and labile anhydride ring, modifications to the core structure were of interest.

![Diagram showing iodolactonization of Diels-Alder adduct 83.](image)

**Scheme 30-** Attempted iodolactonization of Diels-Alder adduct 83.

Iodolactonization of Diels-Alder adduct 83 was investigated in effort to generate the synthetically useful halolactone 96 for subsequent modification (Scheme 30). Iodolactonization from 83 to 96 failed under precedented conditions\(^5^0\). Presumably, the bond angle in the \textit{exo} conformation of the fused anhydride would be too strained in the attack of the intermediary iodonium ion. This is likely considering reports where diastereomers of Diels-Alder reactions can be resolved by iodolactonization where \textit{endo} isomers are isolated as the halolactones\(^5^0\). Therefore, generating general epoxide 97 was not feasible. For \textit{endo} Diels-Alder adducts of maleimides, an iodolactamization is theoretically possible. Bromination of the 5,6-alkene was of interest as a pseudo-protecting group of the alkene (Scheme 31).

![Diagram showing synthesis of dibromo Diels-Alder adduct 98.](image)

**Scheme 31-** Synthesis of dibromo Diels-Alder adduct 98 as an alkene protecting group.
The tendency of the Diels-Alder adduct 83 to undergo retro-inversion was a concern for future modifications to the anhydride ring. Bromination proceeded as expected under the prescribed conditions. Debromination with KI was not achieved, and the reason is proposed to be that the Br atoms cannot achieve the anti-periplanar conformation required. Debromination with activated Zn in the presence of AcOH remains a possibility. Generating synthetically useful epoxides from the 5,6-alkene of 83 was also investigated.

![Scheme 32](image)

**Scheme 32** - Attempted epoxidation of Diels-Alder adduct 83.

Epoxidation of Diels-Alder adduct 83 with mCPBA for epoxide 99 was unsuccessful (Scheme 32). Alpha protons of the fused bicycle/anhydride ring were not present in $^1$HNMR of the crude product indicating the anhydride moiety does not remain intact in the presence of mCPBA. Attempted epoxidation with DMDO generated in situ from acetone and oxone resulted in no discernable $^1$HNMR signals, alluding to reactivity of the anhydride moiety to peroxides.

With valuable information gathered in the Diels-Alder reaction to form 1,4-dimethyl-norcantharidin analogues 83/84 and in the modifications of these core structures, we investigated Diels-Alder reactions of furan 82 from varying N-alkylmaleimides.

### 2.3- Diels-Alder reactions of N-alkylmaleimides

The profiling of Diels-Alder reaction systems from N-alkylmaleimides began with the reaction of N-methylmaleimide (86) and furan 82 (Scheme 33).
Scheme 33- Pathway to resolve and characterize *exo/endo* products from Diels-Alder reactions of furan 82 and maleimide 86.

Maleimide 86 was treated with furan 82 in ethyl acetate (EtOAc) at RT then the endo/exo isomers were separated by chromatography to *exo* 58 and *endo* 87 Diels-Alder adducts. Both adducts were then separately reduced with H₂ in the presence of Pd/C (Scheme 33) and the identity of *endo/exo* isomers was elucidated through NOESY experiments (Figure 7).
Figure 7- NOESY experiments to determine identity of endo 87 and exo 58 isomers from the Diels-Alder reaction of maleimide 86 and furan 82.

The identity of exo adduct 58 was assigned upon observing that α-protons of the fused maleimide moiety in reduced counterpart 88 exhibit NOE coupling to axial protons across the bicycle (Figure 7, right). The identity of endo adduct 87 was confirmed upon observing α-protons of the fused maleimide structure in reduced counterpart 89 exhibit NOE coupling to methyl protons of the bicycle (Figure 7, left). The pseudo-equatorial α-protons of endo isomers 89 and 87 were more deshielded due to a C-C single bond anisotropic effect common in conformationally fixed rings. The observable trend was applied to subsequent reaction systems to identify endo/exo isomer ratios.
Table 3- Resulting endo/exo isomer ratios from Diels-Alder reactions with furan 82 and N-alkylmaleimides with varying time, temperature and solvent (ratios by 1H NMR integration).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time</th>
<th>R=</th>
<th>Solvent</th>
<th>Endo: Exo</th>
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<tbody>
<tr>
<td>1</td>
<td>24hrs</td>
<td>Me</td>
<td>EtOAc</td>
<td>66:34</td>
</tr>
<tr>
<td>2</td>
<td>24hrs</td>
<td>Me</td>
<td>Et₂O</td>
<td>67:33</td>
</tr>
<tr>
<td>3</td>
<td>24hrs</td>
<td>Me</td>
<td>DCM</td>
<td>74:26</td>
</tr>
<tr>
<td>4</td>
<td>24hrs</td>
<td>Me</td>
<td>EtOAc (reflux)</td>
<td>56:44</td>
</tr>
<tr>
<td>5</td>
<td>24hrs</td>
<td>Ph</td>
<td>EtOAc</td>
<td>74:26</td>
</tr>
<tr>
<td>6</td>
<td>24hrs</td>
<td>Bn</td>
<td>EtOAc</td>
<td>77:23</td>
</tr>
<tr>
<td>7</td>
<td>96hrs</td>
<td>Me</td>
<td>EtOAc (reflux)</td>
<td>22:78</td>
</tr>
<tr>
<td>8</td>
<td>3hrs</td>
<td>Me</td>
<td>1,4-dioxane (30 mol % H₃BO₃)</td>
<td>23:77</td>
</tr>
<tr>
<td>9</td>
<td>3hrs</td>
<td>Ph</td>
<td>1,4-dioxane (30 mol % H₃BO₃)</td>
<td>9:91</td>
</tr>
<tr>
<td>10</td>
<td>96hrs</td>
<td>Ph</td>
<td>EtOAc (reflux)</td>
<td>25:75</td>
</tr>
</tbody>
</table>

N-alkylmaleimides were subjected to Diels-Alder reactions with furan 82 under varying conditions and the resulting isomer ratios were determined by 1H NMR integration (Table 3). It was evident that a more polar solvent aids in the formation of endo isomer 87 (entries 1-3). Applying heat increased the formation of exo adduct 58 (entry 4).

N-phenyl 90 and N-benzyl 93 maleimides were synthesized to evaluate steric effects on the corresponding Diels-Alder reaction systems. Surprisingly, Diels-Alder reactions with phenyl and benzyl maleimides showed an increase in the formation of endo isomers 92 and 95 (entries 5-6). A possible explanation is increased stabilizing secondary orbital interactions from pi orbitals of aromatic residues in the endo approach. An increased reaction time in refluxing...
EtOAc assayed at t=1hr, 24hr, 48hr, 72hr and 96hr with maleimide 86 gave an appreciable increase in the formation of exo adduct 58 (entry 7). An identical endo:exo ratio (22:78) was observed at t=72hr and t=96hr, indicating the reaction of maleimide 86 and furan 92 is in equilibrium after 72hr (entry 7). Selective generation of exo adduct 91 was achieved by treating maleimide 90 with furan 82 in the presence of catalytic boric acid\textsuperscript{12} (H\textsubscript{3}BO\textsubscript{3}) with a prominently shorter reaction time (entries 8-9).

We believed the endo:exo ratio between the reaction of maleimide 86 and furan 82 in refluxing EtOAc for 96hrs represented a thermodynamic equilibrium, as the same endo:exo ratio was obtained employing the boric acid catalyst (entries 7-8). It follows that the exo selectivity achieved by using boric acid was a result of accelerating the rate of forward and reverse Diels-Alder reactions, ultimately favoring the more stable thermodynamic product. However, employing the same conditions that generate a thermodynamic equilibrium for maleimide 90 we did not observe the same endo:exo ratios (entires 9-10). We postulate the exo selectivity of boric acid likely stems from stabilization of exo transition states, accelerating the rate of formation of exo products (entries 9-10), and not an overall acceleration of forward/reverse Diels-Alder reactions to produce the thermodynamic exo product.

Upon profiling Diels-Alder reactions of maleimides 86, 90 and 93 with furan 82, we investigated post Diels-Alder modifications of the corresponding cantharimide core structures. Although exo selectivity had been achieved (entry 9), on preparative scales exo selective conditions were not employed due to our desire to access additional molecular diversity through the isolation and modification of both endo and exo isomers.
2.4- Post Diels-Alder modifications to N-alkylmaleimide Diels-Alder adducts 91, 92 and 95

Table 4- Epoxidation of maleimide Diels-Alder adducts 91, 92 and 95.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Time</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91 <em>exo</em>, R=Ph</td>
<td>48hr</td>
<td>101</td>
<td>86% (a)</td>
</tr>
<tr>
<td>2</td>
<td>92 <em>endo</em> R=Ph</td>
<td>120hr</td>
<td>102</td>
<td>83%</td>
</tr>
<tr>
<td>3</td>
<td>95 <em>endo</em> R=Bn</td>
<td>240hr</td>
<td>103</td>
<td>92%</td>
</tr>
</tbody>
</table>

(a) Yield=95% with 120hr reaction time.

For modifications to the cantharimide core structure, generating synthetically useful epoxides were of interest. Epoxidation of Diels-Alder adducts 91, 92 and 95 proceeded normally and were isolated by chromatography (Table 4). We predicted that *endo* substrates 92 and 95 would severely hinder the mCPBA approach from the *endo* face of the bicycle and *exo* epoxidation was expected and observed (entries 2-3). We also expected a majority of *exo* epoxides to be formed in the synthesis of 101 but were surprised to observe no detectable formation of an *endo* epoxide. A possible explanation would be due to H-bonding coordination of mCPBA to the oxo bridge of the bicycle and the obvious steric consideration the α-hydrogens impart in starting adduct 91. *Exo*-epoxidation was determined by 2D $^1$HNMR experiments (Figure 8).
Figure 8- 2D NMR experiments identifying *exo* epoxidation in the synthesis of 101-103.

Epoxide 101 exhibited NOE coupling between α-protons of the fused maleimide (3.04ppm) and axial protons across the bicycle (3.40ppm), similar to *exo* isomer 88. As a result, the *exo* epoxide structure 101 was confirmed. *Exo*-epoxidation was confirmed in epoxide 102 as the protons spanning the bicycle should exhibit strong W-effect coupling. Through bond W-coupling was not observed in a COSY experiment of 102 confirming *exo*-epoxidation, an assignment that was subsequently applied to epoxy adduct 103.
**Scheme 34**- Attempted hydride reduction of epoxy Diels-Alder adduct 101 and proposed route to acyl derivatives.

With epoxide groups installed, reduction of epoxy Diels-Alder adducts was of interest in pursuit of installing acyl groups to the bicyclic core (Scheme 34). Treating Diels-Alder adduct 101 with NaBH\(_4\) under prescribed conditions showed epoxide 101 disappear from the reaction mix after 5 days (TLC). GCMS indicated appreciable formation of alcohol 104 in agreement with \(^1\)HNMR Data, albeit as a minor component of the crude mixture according to GCMS and \(^1\)HNMR integration. More rapid reduction of 101 was observed using LiBH\(_4\)\(^{55}\), however only trace amounts of alcohol 104 were detected in the crude reaction mixture by GCMS and \(^1\)HNMR. Due to the minimal amounts of desired alcohol 104 generated from reduction with NaBH\(_4\) and LiBH\(_4\), alcohol 104 was not isolated, as reduction by these means would not provide a viable route to acyl analogues 105. Stronger reducing agents such as aluminum hydrides are not a viable alternative as they are known to reduce imides\(^{56}\).
2.5- Summary and Biological Activity of BMS-641899/Cantharidin analogues

![Chemical structures](image)

**Figure 9**- Structures of BMS-641899/Cantharidin analogues synthesized. Previously reported compounds: 91-92, 58 and 87-84. Novel Compounds: 100-103, 88-89, 94-95, 111.

At this point the group was comfortable with the chemistry and had prepared our first generation of cantharidin analogues (Figure 9). Nine novel 1,4-dimethyl-norcantharimide analogues were generated (100-103, 88-89, 94-95, 111) and structures confirmed by conventional methods. Selected compounds were then tested for antibiotic activity in a preliminary bioactivity assay, completed by Dr. Hudson’s group (Figure 10).
Figure 10- Preliminary antibiotic activity assay of synthesized analogues (courtesy of Dr. Andre Hudson).

Epoxide 101 and maleimide adduct 91 did not show significant antibiotic activity.

Anhydride 83 and benzylimide 94 showed significant antibiotic activity, but the possibility for a retro-Diels-Alder process was noted. The corresponding Diels-Alder substituents 15 and 93 showed significant antibiotic activity and Diels-Alder products 83 and 94 showed detectable retro-Diels-Alder reactions in $^1$HNMR studies. No detectable retro-Diels-Alder reaction was observed with $N$-phenyl adduct 91, nor any significant antibiotic activity. These factors suggest that the antibiotic activity of DA adducts 83 and 94 may simply be due to an in vitro retro-Diels-Alder process to antibiotic substituents 15 and 93. This concern also stems from reports that $N$-phenyl 90 and $N$-benzyl 93 maleimides exhibit antifungal activity$^{61}$ and additional bioactivity$^{62}$.  

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<td>8</td>
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To verify this assumption, the reduced N-phenyl and N-benzyl maleimides will be tested (Courtesy of Dr. Andre Hudson). We then turned our attention to identifying our second generation of synthetic targets.

2.6- Covalent Modification of the Androgen Receptor Hypothesis

**Figure 11-** *In silico* docking model of BMS-641899 in the ligand binding domain of human wild type androgen receptor (used without permission from 45).

Based on *in silico* docking experiments (Figure 11) of BMS-641899 (75) binding to the human wild type androgen receptor (AR) the authors report a key interaction for its high affinity is H-bonding between the oxo bridge of 75 and an asparagine (N705) residue in the ligand binding domain of the human wild type AR45. They report the sulfonamide of 75 forces a reorientation of the phenylalanine (F876) residue, causing a significant reorientation of the receptor explaining the high antagonist activity observed *in vitro*. We noted the presence of a threonine (T877) residue adjacent to the reoriented F876 residue, which prompted the hypothesis that the AR receptor can be covalently modified using N-substitued epoxy cantharidin analogues (Figure 12).
Figure 12- (Top) Representation of BMS-641999 in the AR Ligand Binding Domain (Bottom) Predicted covalent modification mechanism of the human AR.
Figure 12 is a simplified comparison of BMS-641988 (75), former BMS-lead 106 and a cantharidin epoxide analogue in the binding pocket of the AR. Former BMS lead 106 (Figure 11) was reported to exhibit H-bonding interactions with the N705 residue through the _exo_ hydroxyl moiety along with the oxo bridge of the bicycle. Visualizing our series of 5,6-epoxy-1,4-dimethyl-norcantharimides in the AR ligand binding domain, we postulate the N705 residue can act as a Lewis acid to activate _exo_ epoxide and the nucleophilic hydroxyl of T877 can attack the activated epoxide, forming a covalent bond (Figure 12-bottom). This prompted a study to develop a facile procedure to epoxy Diels-Alder adducts with varying _N_-substituents.

2.7- Studies towards a route to Diels-Alder adducts bearing epoxides

Table 5- Results from primary amine addition to crude epoxy anhydride material.

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<td>NR</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>HOOC-COOH</td>
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</tr>
<tr>
<td>Cyclohexylamine</td>
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Amino acid substitutions of the epoxy norcantharimide structure were of initial interest due to their availability and potential bioactivity. As reduction of the alkene moiety to prevent retro-Diels-Alder of the anhydride adduct 83 is not possible, epoxidation attempted on the crude adduct gave ambiguous NMR data (Table 5). A portion of the crude product from the epoxidation was subjected to different amines using established conditions (see Scheme 29).
Addition of the amine was still attempted as mCPBA likely oxidizes the labile anhydride ring to the corresponding ester, which we expected to be displaced by an incoming amine. The crude epoxy anhydride mixture was treated with primary amines under the described conditions (Table 5). Addition of the cyclohexylamine to the epoxy anhydride moiety generates a significant hit in GCMS with agreeing NMR Data. Purification efforts showed the desired N-cyclohexyl adduct 109 co-elute with the N-cyclohexyl amide of meta-chlorobenzoic acid in poor yield (<5%).

Addition of amino acid residues did not produce detectable amounts of desired products 107 and 108.

![Scheme 35](image_url)

**Scheme 35-** Alternative route to N-substituted epoxy Diels-Alder adducts (Courtesy of Jordan Dejewski)\(^6^3\).

Due to difficulties encountered in the route described in Table 5, we decided that effort was better placed in first generating appropriately substituted maleimides. Diels-Alder reactions of the maleimides are more easily purified and readily undergo epoxidation with mCPBA (as in Table 4). As an initial study in the first two steps of the sequence, N-glycinylmaleimide 110 was synthesized from anhydride 15 and underwent a Diels-Alder reaction with furan 82 that was subsequently reduced to the corresponding Diels-Alder adduct 111 (Scheme 35). Purification of the reduced product 111 is in progress by a current Cody group member\(^6^3\).
Scheme 36- Synthesis of N-Trp Diels-Alder adduct 113.

Considering N-amino acids with large, aromatic substituents gave the best PP1/PP2 inhibition among cantharidin analogues (Table 1)\(^\text{12}\), and non-site restricted computational studies showed the epoxy-N-tryptohan-norcantharimide analogue to have high affinity to the AR\(^\text{63}\), L-tryptophan was of interest as a substituent. N-Trp maleimide 112 was synthesized according to previous methods\(^\text{64}\) and treated with 2,5-dimethylfuran which generated Diels-Alder adduct 113 (83:17 \textit{endo}:\textit{exo}) (Scheme 36). Careful chromatographic purification did not resolve \textit{endo}/\textit{exo} isomers. Diels-Alder adduct 113 was characterized as an \textit{endo}/\textit{exo} mixture, from a small sample of the crude product that was purified by chromatography. It was reasoned that if we subjected adduct 113 to \textit{m}CPBA treatment for the epoxide isomers, purification may be possible. (Scheme 37).

Scheme 37- Attempted epoxidation of N-Trp Diels-Alder adduct 113.

Initial epoxidation attempts of 113 with 1eq of \textit{m}CPBA showed no disappearance of the bicyclic vinyl protons noticed by \(^1\text{HNMR}\) analysis indicating epoxidation at the desired site had
not occurred. To accelerate the rate of epoxide formation, adduct 113 was treated with an excess of mCPBA (3eq). Crude mass spectrometry data suggested epoxidation had occurred at two sites. Upon investigation we suspect the indole alkene is epoxidized as in 114 due to the susceptibility of this functional group to oxidation. The dioxide product 114 also appeared to form with one equivalent of mCPBA employed. At this juncture we completed thorough in silico experiments to identify targets that may be produced by facile methods that also give promising data of their activity in the AR ligand binding domain.

2.8.- Studies to Identify Next Generation of Synthetic Targets

Nineteen possible N-amino acid substituents of the endo and exo epoxy cantharimide core were analyzed within the 2q7i active site of the human AR using PyRx screening software (Figure 13).
Figure 13- Results from PyRx ligand binding simulations of endo-epoxy-N-amino acid cantharimides.

Lower Y-axis values ($\Delta G$) represent a higher affinity to the active site. Each data point represents 1 of 300 simulations for each N-AA corresponding to a single position in 3D space within the active site. The blue bars represent median values. Due to the results of endo epoxy analogues tested, $N$-His, $N$-Arg, $N$-Tyr, $N$-Lys substitutions of the epoxy cantharimide core are
of interest as future synthetic targets. Under identical parameters, the *exo* epoxy *N*-amino acid analogues were also simulated (Figure 14).

**Figure 14** - Results from PyRx ligand binding simulations of *exo*-N-amino acid epoxy cantharimides.

Notably, amino acids bearing simple alkyl chain R-groups (Gly, Ala, Val, Leu) are among derivatives showing the highest affinity to the 2q7i active site. As methods to synthesize
N-glycinyl derivative 111 has been demonstrated\textsuperscript{63}, these simple amino acid substitutions are of primary interest as future synthetic targets for \textit{exo} epoxy \textit{N}-AA derivatives.

2.9- Conclusions and Future Work

We have characterized Diels-Alder reactions of 2,5-dimethylfuran with maleic anhydride and various \textit{N}-alkylmaleimides. A series of 1,4-dimethylnorcantaharidin analogues were synthesized, characterized and evaluated for biological activity. Theoretical studies were performed, and a second generation of synthetic targets has been identified. Future work will involve the synthesis of second generation targets and testing for biological activity. Based on simulation of \textit{exo}-epoxy-\textit{N}-amino acid cantharimides, those bearing simple alkyl R-groups 124-127 represent the primary targets (Figure 15). In order to evaluate our covalent modification hypothesis, Dr. Michael Gleghorn, our collaborator in the School of Chemistry of Materials Science will attempt capture the interaction of 5,6-epoxy-\textit{N}-amino acid-1,4-dimethyl-norcantharimides with the AR through conventional crystallographic methods.

![Structure Diagram](image)

**Figure 15-** \textit{Exo}-epoxy-\textit{N}-amino acid cantharimide future targets of primary interest.

In our closing synthetic efforts for Chapter 1 of this thesis, novel \textit{endo} 128 and \textit{exo} 124 \textit{N}-Gly epoxides were synthesized (Scheme 38).
Scheme 38 - Synthesis of 5,6-epoxy-N-Gly-1,4-dimethylnorcantharimide

Based on established methods and our work, N-glycinylmaleimide 128 was synthesized and underwent the expected Diels-Alder reaction with furan 82. The 48:52 endo:exo mixture underwent epoxidation with mCPBA and the resulting epoxides were resolved by chromatography to afford novel analogues 124 and 129. This newly established method will be applied in the synthesis of analogues 125-127 (Figure 15) for subsequent testing by our collaborator Dr. Michael Gleghorn.
Chapter 2- New Methodology Studies for Access to 9-Membered Lactones

3- Diels-Alder reactions of 2-methylene-cyclohexane-1,3-dione

3.1- Natural Products bearing 9-membered lactones

The proposed Diels-Alder reaction system may provide access to 9-membered lactones. 9-membered rings are a formidable challenge in many synthetic endeavors, especially in NP synthesis. For example, several classes of medium sized lactones have been reported that feature 9-membered lactone moieties (Figure 16).66,67,68,69.

![Chemical Structures]

Figure 16- Natural products bearing a 9-membered lactone or similar entity (refs. embedded).
3.2- Proposed Route to 9-membered Lactones

We anticipated the methylene dione 116 will participate in a Diels-Alder reaction with 2,5-dimethylfuran and the corresponding product can undergo an intramolecular cyclization process (Scheme 39).

Scheme 39- (a) Proposed synthetic route to 9 membered lactones. (b) Proposed mechanism.

The proposed mechanism begins with an enolization of the Diels-Alder adduct 118. The presence of the bicyclic ether as a β-leaving group could facilitate a fragmentation and ketene
formation process. Following attack from the freed alkoxy moiety and tautomerization a 9-membered lactone may be formed in one pot from the starting Diels-Alder adduct 118.

Scheme 40- Previous reports of Diels-Alder reactions of reactive intermediate 116 (top: Hoffmann and Koser\textsuperscript{70}) (bottom: Wei \textit{et al}\textsuperscript{71})

The reactive methylene species 116 has been reported in the synthesis of terpenoid natural products\textsuperscript{70, 71} (Scheme 40). Both reports detail the \textit{in situ} generation of the reactive methylene species 116 that undergoes a reverse-electron demand Diels-Alder reaction with an electron rich alkene\textsuperscript{70, 71}. To our knowledge, no studies have been reported on the use of 116 as a dienophile in a normal-electron-demand Diels-Alder reaction.
3.2.1- Hoffman and Koser Conditions

Scheme 41- Formation of Michael adduct 24 from reactive intermediate 20.

The literature described was applied to produce a normal-electron demand Diels-Alder reaction of 2,5-dimethylfuran and the reactive methylene species 116 (Scheme 41). Under Hoffman and Koser conditions, dione 115 was added in one portion to a solution of catalytic NaOAc, 2eq of 2,5-dimethylfuran (82) and 2eq of paraformaldehyde with 4A molecular sieves suspended in AcOH. We believe the use of paraformaldehyde was done by the authors for a controlled release of formaldehyde monomers and by extension controlled formation of methylene species 116. The crude product showed significant product formation in GCMS and was subsequently reduced for purification (Scheme 42). Upon in situ formation, the reactive intermediate rapidly undergoes a Michael addition to adduct 120 (Scheme 41). The Michael adduct 120 was identified by GCMS (m/z=236) with a fragmentation pattern that matched the available spectrum from SDBS.

Exhaustive chromatographic purification efforts failed to produce pure reduced adduct 118. Under the same conditions, an excess of furan was employed (10eq) but did not yield a significant increase in desired Diels-Alder adduct 117 formation compared to byproducts. An
increased reaction duration (4 days) failed to improve the formation of the Diels-Alder adduct 117 relative to the Michael adduct 120. Increased heat (90°C, 24hrs) caused decomposition of starting reagents. It was determined that tandem chromatographic purifications were necessary.

Scheme 42- Diels-Alder synthesis of 21 and reduction under Hoffman and Koser conditions.

A multigram scale reaction for 117 was prepared under the described conditions (time=7hr) and reduced to adduct 118 (Scheme 42). It was discovered that a significant portion of byproducts in crude oil 118 could be removed by suspension in hexanes. The resulting solids were further purified by column chromatography, yielding adduct 118 of an acceptable purity (6% yield from 115). Further purification was performed by radial chromatography giving a pure sample of 118 (<1% from 115) as a colorless oil. The physical properties (BP=40°C) and diminished mass returns was cause for concern as product may be lost during solvent removal under reduced pressure.

We could not verify the structure of pure 118 by conventional ¹HNMR due to the complexity of the molecule, noting its 14 magnetically inequivalent protons. The resulting spectra is a large collection of overlapping peaks. However, the signals appear in appropriate shift ranges. A conventional ¹³C CPD showed two signals from 195-200ppm a plausible region for the carbonyl carbons of 118. The remaining signals appeared in appropriate ranges for all other carbons. A ¹³C DEPT 135 experiment showed only two peaks in the positive region, corresponding to the two methyl groups of 118. The cumulative NMR data, a pure chromatogram (m/z=222) in GCMS with an agreeing fragmentation pattern, strongly suggest the
successful formation and isolation of the Diels-Alder adduct 118. Efforts to improve yield and purification methods were then investigated under Wei conditions.

### 3.2.2- Wei Conditions

![Scheme 43- Synthesis of adduct 21 under Wei conditions.](image)

Initially, Wei conditions (Scheme 43) gave crude product mixtures that contained significantly fewer by-products in comparison to Hoffmann and Koser conditions. This prompted further optimization efforts of these conditions to produce Diels-Alder product 117.

Upon reaction completion, the reaction layer is diluted with water and the organic products are extracted. It was discovered the yield is greatly affected by the extraction solvent. Extraction with Et₂O returned only Michael adduct 120, which demonstrates a way to remove the undesired byproduct before chromatographic purification. Extraction with ethyl acetate returns a mixture that contains mostly Michael adduct 120 but an appreciable amount of desired Diels-Alder adduct 117 is extracted. Extraction with DCM gave a 1:1 mixture of 120 and 117. Extraction with CHCl₃ gave a crude sample with 117 as the major component. It was concluded that more polar extraction solvents greatly increase yields of desired compound 117. Reduction of the crude oil 117 extracted with chloroform generated crude adduct 118 with significantly fewer byproducts than under Hoffman and Koser conditions, eliminating the need for the preliminary column chromatography purification. Due to the possibility of product loss during solvent removal, the use of a volatile eluent was employed so it could more easily be removed.
without exceptionally low pressures. Purification of the crude adduct 118 by radial chromatography eluted with pure Et$_2$O to afford pure adduct 118 was achieved in a 50% recovery of the material loaded.

3.3- Testing Appropriate Base for Synthesis of Lactone 119

Before extensive purification efforts for 118, partially purified portions from crude reactions under Hoffman and Koser conditions were subjected to treatment with t-BuONa and NaH (Scheme 44). Treating 118 with t-BuONa or an excess of NaH produced no detectable change in the crude mixture composition. It was discovered based on computer simulation (courtesy of D. Tusch) that the dimethyl substituents of 118 impart significant steric hindrance in the approach of a base$^{72}$. This prompted investigation for synthesis of the demethylated counterpart 121 and its role as a precursor in the transformation to lactone 123 (Scheme 45).
3.4- Synthesis of Demethylated Precursor 121

![Scheme 45](image)

Scheme 45- Investigation of demethylated analogue 121 as a precursor to lactone 123.

Both Hoffmann and Wei conditions were applied in the synthesis of 121. Attempts to reduce Diels-Alder adduct 121 gave GCMS data that suggests reduction under our precedent conditions is not chemoselective and reduction of the carbonyl occurs as in 122. As a result, adduct 121 was treated with NaH and NaNH₂ which did not produce lactone 123 in detectable amounts. However, Diels-Alder adduct 121 was consumed in treatment with both NaH and NaNH₂ according to GCMS analysis. In future work, the unknown products produced from the treatment of 121 with NaH and NaNH₂ will be isolated and characterized.

3.5- Conclusions and Future Work

Access to a 9-membered lactone was investigated via the Diels-Alder reaction of in situ generated 2-methylene-cyclohex-1,3 dione (116) with 2,5-dimethylfuran (82) and furan (8). It was found that Diels-Alder adduct 118 can be isolated by a tandem chromatographic purification procedure. The extremely poor yields of the current process require optimization studies of the current reaction system. Considerable improvements were made in the synthesis of Diels-Alder
adduct 118 under Wei conditions. Extraction with polar solvents allowed for better isolation of the Diels-Alder adduct 117. The Michael adduct 120 can be removed from the crude product by washing with Et₂O. The use of volatile solvent systems in chromatographic purification has been shown to improve recovery rates. Further studies will be identifying the appropriate conditions to reduce the alkene moiety of 121 without simultaneous reduction of its carbonyl. Additional work necessary will be to isolate and characterize products of base treatments of 121.
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(72) *Discussions with Dr. D Tusch (2018) SCMS RIT.*


General Procedures

All non-aqueous reactions were performed in flame- or oven-dried (125 °C) glassware under normal atmosphere and stirred magnetically, unless otherwise specified. Reaction temperatures other than room temperature were performed in baths (ice/water for 0 °C), and a heating mantle regulated by a variable autotransformer for temperatures greater than room temperature or a sand bath heating on a hot plate with external temperature monitoring. The phrases “concentrated in vacuo”, “concentrated under reduced pressure”, or “concentrated” refer to removal of solvent via Büchi R-3 or R-210 rotary evaporator fit with Buchi V-700 or Buchi V-100 vacuum.

Reagents and Solvents

Deionized water was used for all aqueous reactions, work-up procedures, and for the preparation of aqueous solutions. All commercially available reagents and solvents were used as obtained from the supplier without further purification unless otherwise noted below:

Dry dichloromethane- dried by reaction with calcium hydride and distilled under ambient pressure

Dry tetrahydrofuran- dried by reaction with sodium metal in the presence of benzophenone indicator and distilled under ambient pressure

Chromatography

The phrases “column chromatography”, “chromatography” or “chromatographic purification” refer to flash column chromatography using 230-400 mesh silica gel (SiliaFlash®) and standard techniques. The phrase “radial chromatography,” refers to purification on a Centrifugal Thin-Layer Chromatograph Model 7924T-01 device using standard techniques. Analytical thin-layer chromatography (TLC) was performed on pre-coated silica gel 60 F254
aluminum backed plates (Macron). Visualization was affected by short-wave (254 nm) UV illumination, and/or placing the plate in an iodine chamber for 15-30 seconds and then using a heat-gun to remove the iodine, and/or by dipping the plate in a stain solution and heating with a heat-gun for 10-15 seconds when appropriate. Stain solutions were prepared as follows:

Potassium permanganate: 3 g potassium permanganate, 20 g potassium carbonate, and 5 mL of 5% w/w sodium hydroxide in 300 mL water

*p-Anisaldehyde: 15 mL p-anisaldehyde and 2.5 mL conc. sulfuric acid in 250 mL ethanol

**Physical Data**

Proton nuclear magnetic resonance (\(^1\)H-NMR) spectra were obtained on a Bruker DRX-300 (300.13MHz) or Avance III 500 (500.13 MHz) nuclear magnetic resonance spectrometer. Chemical shifts are reported in ppm (\(\delta\)) relative to internally referenced solvent residual peak using CDCl\(_3\) as solvent (7.26 ppm), unless otherwise specified. \(^1\)H-NMR Data are reported as follows: chemical shift (multiplicity, coupling constants in Hz, number of protons)./?

Multiplicities are abbreviated as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet and/or multiple resonances), br s (broad singlet), br d (broad doublet).

Carbon nuclear magnetic resonance (\(^{13}\)C-NMR) spectra were obtained on a Bruker DRX-300 (75.48 MHz) or Avance III 500 (125.77 MHz) nuclear magnetic resonance spectrometer. Chemical shifts are reported in ppm (\(\delta\)) relative to internally referenced solvent residual peak using CDCl\(_3\) as solvent (77.16 ppm), unless otherwise specified.

Fourier-transform infrared (FT-IR) spectra were recorded using ATR on a Shimadzu IRAffinity-1 FT-IR and are reported in wavenumbers (cm\(^{-1}\)).
Low-resolution mass spectra were obtained using a Hewlett Packard 6890 Series gas chromatograph with a 5973 Mass Selective Detector using electron impact (EI) methods and dichloromethane, ethyl acetate, methanol or diethyl ether as the sample solvent. Additional low-resolution mass spectra were obtained on a Shimadzu LCMS-2020 ultra-high-performance-liquid chromatograph equipped with photo-diode array detector and Quadrupole mass spectrometer using electrospray ionization (ESI) methods with methanol, or ethyl acetate as the sample solvent.

**Theoretical Data**

All *in silico* experiments were conducted using PyRx Virtual Screening Tool docking simulator software. All ligands used in docking systems were drawn using Avagadro molecular modeling software. Simulations performed were on the 2q7i active site of the wild-type androgen receptor structure as reported from RCSB Protein Data Bank. Testosterone was removed from the active site using PyMol Molecular Visualization software.
## Compound Index

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**Experimentals**

Experimentals

\[15 \rightleftharpoons O\] 1.1 eq \[EtOAc, RT, 48hrs \]

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**exo-1,4-dimethyl-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid anhydride (83)**

To a solution of maleic anhydride (251.8mg, 2.57mmol) in EtOAc (1mL) was added 2,5-dimethylfuran (0.25mL, 0.2348mmol) and stirred at RT. After 48hrs, the solvent was removed in vacuo yielding a while solid. The crude solid was assayed by NMR in CDCl\textsubscript{3} and determined to be a 1:1 mixture of 15 and 83 (50% conversion by \textsuperscript{1}HNMR integration) and unreacted 2,5-dimethylfuran 82.

\textsuperscript{1}HNMR (300MHz, CDCl\textsubscript{3}): Compound 83 \(\delta\) 6.351 (s, 2H) 3.163 (s, 2H) 1.763 (s, 6H)

Compound 15 \(\delta\) 7.038 (s, 2H) Compound 82 \(\delta\) 5.822 (s, 2H) 2.239 (s, 6H) in agreement with previous reports\textsuperscript{48,59}.
**exo-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid anhydride (84)**

The unpurified adduct **83** (122.4mg) was dissolved in EtOAc (2mL) and degassed under vacuum and placed under N\textsubscript{2}. 5% wet Pd/C (49.2mg, cat.) was added to the solution and degassed under vacuum and placed under N\textsubscript{2}. H\textsubscript{2} was introduced with a balloon and the heterogenous mixture was stirred at RT. After 1hr, H\textsubscript{2} was removed from the vessel under vacuum and the mixture was filtered through celite, washed with EtOAc (3x 20mL) and concentrated *in vacuo* yielding a white solid. The crude solid was assayed by NMR and determined to be a mixture of **84** (45%) and anhydride **85** (55%).

\textsuperscript{1}HNMR (300MHz, CDCl\textsubscript{3}): Compound **84** δ 3.145 (s, 2H) 2.020 (s, 4H) 1.604 (s, 6H)

Compound **85** δ 2.991 (s, 4H) in agreement with previous reports\textsuperscript{48,59}. 
**exo-1,4-dimethyl-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxy-(N-methyl)imide (58)**

**endo-1,4-dimethyl-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxy-(N-methyl)imide (87)**

**EtOAC, RT, 24hrs:** To a stirred solution of maleimide 86 (270mg, 2.81mmol) in EtOAc (1mL) was added furan 83 (0.26g, 2.70mmol) and stirred at RT. After 24hrs the solvent was removed in vacuo to give a white solid which was purified via gradient flash chromatography (25% EtOAc/Hexanes → 100% EtOAc) yielding pure exo 58 and endo 87 adducts as white solids.

1HNMR (300MHz, CDCl₃): Compound 58 δ 6.302 (s, 2H) 2.959 (s, 3H) 2.831 (s, 2H) 1.706 (s, 6H) Compound 87 δ 6.192 (s, 2H) 3.226 (s, 2H) 2.803 (s, 2H) 1.780 (s, 6H) in agreement with a previous report⁵⁸.

Solvent studies performed (Table 3, entries 1-3) employed identical conditions but Et₂O and DCM were substituted for EtOAc and resulting endo:exo ratios were determined by ¹HNMR integration.

**EtOAC, reflux, 24hrs:** To solution of maleimide 86 (260mg, 2.34mmol) in EtOAc (1mL) was added 2,5-dimethylfuran (0.25mL, 2.34mmol) and heated to reflux in sand bath with external temp monitoring, then stirred at reflux for 24hrs. An aliquot of the reaction mixture was removed, concentrated in vacuo and assayed for endo/exo composition by ¹HNMR integration.
**For time study:** To solution of maleimide 86 (260mg, 2.34mmol) in EtOAc (1mL) was added 2,5-dimethylfuran (0.28mL, 2.37mmol) and stirred at reflux for 96hrs. An aliquot of the reaction mixture was removed, concentrated *in vacuo* and assayed for *endo/exo* composition by $^1$HNMR integration.

**Boric acid catalyzed:** Maleimide 86 (100mg, 1.02mmol) and boric acid (21.4mg, 0.346mmol) were dissolved in 1mL of 1,4-dioxane and stirred. 2,5-dimethylfuran was added (0.114mL, 1.07mmol) and the flask was heated to reflux for 3hrs. An aliquot of the reaction mixture was removed, concentrated *in vacuo* and assayed for *endo/exo* composition by $^1$HNMR integration.
**exo-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-(N-methyl)imide (88)**

**endo-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-(N-methyl)imide (89)**

In separate round bottom flasks, Diels-Alder adducts 58 (250mg, 1.29mmol) and 87 (340mg, 1.75mmol) were dissolved in 3:1EtOAc (16.5mL) and the mixtures were degassed by vacuum and placed under N₂. 5% wet Pd/C was added (559.3mg, cat.) was added to each flask which were degassed by vacuum and placed under N₂. H₂ (g) was introduced with a balloon. The mixtures were stirred at RT for 4hr then degassed by vacuum, filtered through celite and washed with EtOAc (3x 20 mL). The solvent was removed *in vacuo* yielding both reduced products 88 and 89.

**Compound 88**

**¹H NMR (300MHz, CDCl₃):** δ 2.950 (s, 4H) 2.853 (s, 3H) 1.753 (s, 4H) 1.591 (s, 6H)

**¹³C NMR (126MHz, CDCl₃):** δ 176.22; 85.16; 53.66; 38.13; 25.02; 18.16

**MS (EI):** Calcd. For C₁₁H₁₅NO₃ [M⁺] = 209, found = 209

**IR (neat, cm⁻¹):** 2987, 2939, 2875, 2252, 1697, 1435, 1384, 1286, 1246
Compound **89**

$^1$HNMR (300MHz, CDCl$_3$): $\delta$ 3.162 (s, 2H) 2.967 (s, 3H) 1.679 (s, 4H) 1.672 (s, 6H)

$^{13}$CNMR (126MHz, CDCl$_3$): $\delta$ 176.07; 86.10; 57.29; 33.67; 24.79; 21.50

MS (EI): Calcd. For C$_{11}$H$_{15}$NO$_3$ [M$^+$] = 209, found = 209

IR (neat, cm$^{-1}$): 2974, 2931, 2885, 2252, 1697, 1429, 1379, 1336, 1288
Anhydride 15 (5g, 50.99mmol) was dissolved in Et₂O (62.5mL) and stirred at RT. A solution of aniline (4.655mL, 50.99mmol) in Et₂O (12.5mL) was added dropwise through an addition funnel. The resulting yellow slurry was stirred at RT for 1hr then was cooled to 0°C and the solids were filtered and dried in vacuo. The intermediary yellow solid was added to a solution of NaOAc (1.65g, 3.96mmol) in Ac₂O (16.75mL), stirred and heated to 100°C for 30 min. The solution was cooled to 0°C, poured into ice cold water (200mL) and stirred vigorously for 30min. The precipitate was filtered, washed with ice cold water (3x 75mL), air-dried, then taken up in hot cyclohexane. The undissolved solids were filtered away and the filtrate was concentrated in vacuo to yield maleimide 90 (24%).

\(^1\)HNMR (300MHz, CDCl₃): 7.389 (m, 5H) 6.872 (s, 2H) in agreement with known spectrum\(^73\).
N-benzylmaleimide (93)

To a solution of anhydride 15 (5.00g, 50.99mmol) in Et₂O (67mL) was added a solution of benzylamine (5.465g, 51.00mmol) in Et₂O (25mL) dropwise through an addition funnel over 30min. The resulting slurry was stirred for 1hr then filtered and air dried. The recovered solids were added to a suspension of NaOAc (2.67g, 32.55mmol) in Ac₂O (19mL) and heated to 100°C for 30min. The resulting solution was poured into ice cold water (125mL) and stirred vigorously for 30min. The precipitate was filtered and purified by recrystallization over 2:1 EtOH:H₂O to afford maleimide 93 (49%).

¹H NMR (300MHz, CDCl₃): 7.313 (m, 5H) 6.704 (s, 2H) 4.673 (s, 2H) in agreement with a previous report⁷⁴.
**exo-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-(N-phenyl)imide (91)**

**endo-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-(N-phenyl)imide (92)**

To a solution of N-phenylmaleimide 90 (1.35g, 7.80mmol) in EtOAc (10mL) was added furan 82 (0.926mL, 8.58mmol) and stirred at RT. After 96hrs the solvent was removed in vacuo and isomers were resolved by gradient flash chromatography (25% EtOAc/Hex → 100% EtOAc) affording pure exo 91 and endo 92 adducts as white solids (53% yield, endo:exo 80:30).

\[
\begin{align*}
\text{HNMR (300MHz, CDCl}_3\text{): Compound } & 91 \delta 7.408 \text{ (m, 5H)} \ 6.369 \text{ (s, 2H)} \ 2.990 \text{ (s, 2H)} \ 2.207 \text{ (s, 6H)} \\
\text{Compound } & 92 \delta 7.387 \text{ (m, 4H)} \ 7.131 \text{ (s, 1H)} \ 7.106 \text{ (s, 1H)} \ 6.360 \text{ (s, 2H)} \ 3.385 \text{ (s, 2H)} \ 1.843 \text{ (s, 6H)}
\end{align*}
\]

in agreement with a previous report 57.

**Boric acid catalyzed:** To a solution of N-phenylmaleimide 90 (200mg, 1.15mmol) and boric acid (26.0mg, 42.0mg) in 1,4-dioxane (5mL) was added 2,5-dimethylfuran (0.114mL, 1.21mmol), stirred and heated to reflux for 3hrs. An aliquot of the reaction mixture was removed, concentrated in vacuo and assayed for endo/exo composition by \(^1\)HNMR integration.
**exo-1,4-dimethyl-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxy-(N-benzyl)imide (94)**

**endo-1,4-dimethyl-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxy-(N-benzyl)imide (95)**

To a solution of maleimide 93 (1.5730g, 8.42mmol) in EtOAc (4mL) was added furan 82 (1.00mL, 9.26mmol) and stirred at RT. After 24hrs the solvent was removed *in vacuo* and isomers were resolved by gradient flash chromatography (20% EtOAc/Hex → 100% EtOAc) affording pure *exo* 94 adduct as a pale yellow oil and *endo* 95 adduct as a white solid (90% yield, *endo:* *exo* 77:23).

**Compound 94**

$^1$HNMR (300MHz, CDCl$_3$): δ 7.303 (m, 5H) 6.305 (s, 2H) 4.647 (s, 2H) 2.835 (s, 2H) 1.695 (s, 6H)

$^{13}$CNMR (126MHz, CDCl$_3$): δ 174.43; 140.83; 128.52; 127.80; 127.56; 87.58; 52.88; 42.14; 15.84

IR (neat, cm$^{-1}$): 3066, 3034, 2987, 2981, 2935, 2358, 2335, 2252, 1697, 1386, 1342, 1178, 1109

**Compound 95**

$^1$HNMR (300MHz, CDCl$_3$): δ 7.292 (m, 5H) 5.926 (s, 2H) 4.467 (s, 2H) 3.212 (s, 2H) 3.385 (s, 2H) 1.760 (s, 6H)

$^{13}$CNMR (126MHz, CDCl$_3$): δ 174.83; 140.95, 189.08; 128.47, 127.96, 88.01, 53.40, 42.23; 18.65
IR (neat, cm$^{-1}$): 3086, 3034, 2978, 2933, 2360, 2341, 1695, 1392, 1340, 1188, 1085
exo-5,6-dibromo-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid anhydride (98)

DA adduct 83 (1.00g, 5.15mmol) was dissolved in dry DCM (25mL) in an oven dried RBF and stirred magnetically. The flask was purged and placed under N\textsubscript{2} and Br\textsubscript{2} was added dropwise via syringe. After 7hrs the mixture was quenched with sodium thiosulfate, diluted with DCM, washed with brine (2x 100mL) and the organic layer was concentrated \textit{in vacuo} affording a brown solid (86\%) that was used without further purification.

\textsuperscript{1}HNMR (300MHz, acetone-d\textsubscript{6}): Internally referenced using solvent residual peak (2.05ppm) \(\delta\) 4.508 (dd, \(J = 4.2Hz, 4.2Hz, 2\text{H}\)) 4.318 (dd, \(J = 7.5Hz, 6.0Hz, 2\text{H}\)) 1.640 (s, 3H) 1.605 (s, 3H) in agreement with a previous report\textsuperscript{75}. 
**exo-5,6-epoxy-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-(N-phenyl)imide** (101)

To a solution of Diels-Alder adduct 91 (160mg, 0.594mmol) in EtOAc (6mL) was added mCPBA (205mg, 1.19mmol) and stirred at RT until a precipitate was observed, which was filtered and washed with cold diethyl ether (2x 30mL) yielding pure epoxy adduct 101 (94%).

$^1$HNMR (300MHz, CDCl$_3$): δ 7.450 (m, 5H) 3.402 (s, 2H) 3.305 (s, 2H) 1.689 (s, 2H)

$^{13}$CNMR (126MHz, CDCl$_3$): δ 173.32; 129.32; 129.00; 126.64; 83.63; 55.15; 51.74; 14.17

MS (EI): Calcd. For C$_{16}$H$_{15}$NO$_4$ [M$^+$] = 285, found = 285

IR (neat, cm$^{-1}$): 3082, 3032, 2980, 2933, 2358, 2337, 2252, 1699, 1392, 1168
**endo-5,6-epoxy-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-(N-phenyl)imide**

(102)

To a solution of Diels-Alder adduct 91 (308mg, 0.159mmol) in EtOAc (8mL) was added mCPBA (547.5mg, 0.317mmol) and stirred at RT for 120hr. The mixture was diluted with EtOAc (25mL) and washed (5x 15mL) with 50% NaHCO$_3$ (aq), brine (1x 25mL), dried (Na$_2$SO$_4$) filtered and the filtrate was concentrated *in vacuo*. The resulting white solid was purified via gradient flash chromatography (25% EtOAc/Hexanes $\rightarrow$ 100% EtOAc) affording epoxide 102 as a white power (83%).

$^1$HNMR (300MHz, CDCl$_3$): $\delta$ 7.441 (m, 3H) 7.210 (m, 2H) 3.409 (s, 2H) 3.364 (s, 2H) 1.764 (s, 6H)

$^{13}$CNMR (126MHz, CDCl$_3$): $\delta$ 174.13; 169.48; 129.11; 129.11; 127.93; 126.04; 83.55; 55.95; 50.98; 16.93

MS (EI): Calcd. For C$_{16}$H$_{15}$NO$_4$ [M$^+$] = 285, found = 285

IR (neat, cm$^{-1}$): 3099, 3068, 2990, 2931, 1707, 1597, 1500 1381, 1182, 1145
**endo-5,6-epoxy-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-(N-benzylimide)** (103)

To a solution of Diels-Alder adduct 95 (390mg, 1.37mmol) in EtOAc (10mL) was added mCPBA (475mg, 2.75mmol) and stirred at RT for 240hr. The mixture was diluted with EtOAc (50mL) and washed (10x 20mL) with 50% NaHCO$_3$ (aq), dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo* affording a white solid (92%) of acceptable purity.

$^1$HNMR (300MHz, CDCl$_3$): $\delta$ 7.453 (m, 5H) 4.581 (s, 2H) 3.167 (s, 2H) 2.184 (s, 2H) 1.646 (s, 6H)

$^{13}$CNMR (126MHz, CDCl$_3$): $\delta$ 173.70; 129.13; 128.72; 128.40; 83.86; 55.93; 50.87; 42.67; 16.84

MS (EI): Calcd. For C$_{17}$H$_{17}$NO$_4$ [$M^+ =$ 299, found = 299

IR (neat, cm$^{-1}$): 3064, 3034, 2357, 2324, 2260, 1697, 1433, 1388, 1342, 1311, 1168
**exo-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-(N-benzyl)imide (100)**

To a solution of maleic anhydride **15** (1.05g, 10.70mmol) in EtOAc (10mL) was added 2,5-dimethylfuran (1.27mL, 11.78mmol) and stirred at RT. After 48hrs the reaction mix was diluted with EtOAc (20mL) and 5% wet Pd/C was added (450mg, cat.). The mixture was degassed by vacuum and placed under N\(_2\). H\(_2\) (g) was introduced with a balloon and stirred vigorously at RT for 20hrs. The mixture was filtered through celite and washed (3x 30mL) with EtOAc. The filtrate was concentrated \textit{in vacuo} yielding a mixture of reduced adduct **84** and reduced anhydride **85** by comparison with previous NMR data. A portion of the crude mixture (500mg) was added to a stirred solution of triethylamine (1.24mL, 8.91mmol) in toluene (12.7mL) in a three neck RBF equipped with a reflux condenser. Benzylamine (0.28mL, 2.55mmol) was added dropwise and the mixture was heated to reflux overnight. The resulting solids were filtered, and the filtrate was diluted with EtOAc (30mL) and sat. NaHCO\(_3\) (10mL) then washed with water (20mL), brine (50mL) and the organic layer was concentrated \textit{in vacuo}. The resulting solids were purified by gradient flash chromatography (15% EtOAc/Hex \rightarrow 100% EtOAc) affording compound **100** as a white solid (16% overall).

\(^1\text{HNMR (300MHz, CDCl}_3\):} \delta 7.301 (m, 5H) 4.634 (s, 2H) 2.848 (s, 2H) 1.742 (s, 4H) 1.568 (s, 6H)
$^{13}$CNMR (126MHz, CDCl$_3$): δ 173.70; 129.13; 128.72; 128.40; 83.86; 55.93; 50.87; 42.67; 16.84

MS (EI): Calcd. For C$_{17}$H$_{17}$NO$_4$ [M$^+$] = 299, found = 299

IR (neat, cm$^{-1}$): 3064, 3034, 2357, 2324, 2260, 1697, 1433, 1388, 1342, 1311, 1168
Anhydride 15 (5.00g, 50.99mmol) and glycine (3.80g, 50.61mmol) were dissolved in AcOH (80mL) and stirred at RT for 6hrs. The intermediary white solid was filtered and purified by recrystallization over MeOH. The purified intermediate was suspended in a solution of triethylamine (5mL, 35.87mmol) in toluene (110mL). The reaction vessel was equipped with a Dean-Stark apparatus and heated to reflux for 1.5hrs. The mixture was concentrated in vacuo and the residual solids were acidified with 3M HCl and extracted with EtOAc (3x 50mL). The organic layers were combined, washed with brine (50mL), dried (Na$_2$SO$_4$), filtered and concentrated in vacuo yielding an orange hygroscopic solid (1.24g). A portion of the solid (640mg) was dissolved in EtOAc (5mL) and 2,5-dimethylfuran (0.47mL) was added and the mixture was stirred at RT for 24hrs. The solvent was removed in vacuo and the crude product was dissolved in EtOAc (80mL) and combined with 5% wet Pd/C (1.00g, cat.). H$_2$ (g) was introduced with a balloon and the mixture was stirred vigorously for 7hr. The reaction mix was filtered through celite, rinsed with EtOAc (3x 50mL) and concentrated in vacuo yielding crude 111 as an off-white solid (0.4g, 3% overall). Purification and full characterization of 111 is in progress by a current Cody group member.
**exo-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-(N-L-tryptophanyl)imide (113)**

To a solution of L-tryptophan (5.00g, 24.48mmol) in AcOH (30mL) was added a solution of maleic anhydride (2.40g, 24.47mmol) in AcOH (10mL). The resulting yellow slurry was stirred at RT overnight (17hr), then the solvent was removed *in vacuo* yielding a glutinous orange solid that was taken up in THF and added to a solution of Et₃N (5mL) in toluene (500mL) in a 1L RBF equipped with a reflux condenser and Dean-Stark apparatus. The mixture was stirred at reflux for 3hrs, then cooled to RT and concentrated *in vacuo*. The crude solid was acidified with 2M HCl (100mL) and extracted with EtOAc (3x 100mL), dried (Na₂SO₄) and concentrated *in vacuo* yielding crude maleimide 112 of acceptable purity. The crude oil 112 (2.37g, 8.34mmol) was taken up in CHCl₃ (10mL) and 1mL (9.26mmol) of 2,5-dimethylfuran was added. The reaction mixture was stirred for 72hrs and assayed regularly by ¹H NMR, indicating *endo/exo* equilibrium was achieved after 2hrs. The reaction mix was concentrated *in vacuo* yielding Diels-Alder adduct 113 as a mixture of *endo:exo* isomers (23% overall, *endo:exo* 83:17). Small portions of the crude mixture were used in attempts to resolve isomers that were unsuccessful. A representative sample of a purified *endo:exo* mixture (35:65) was used for characterization of Diels-Alder product 113.
$^1$HNMR (500MHz, CDCl$_3$): $\delta$ 9.450 (br s, 1H) 8.105 (s, 1H) 8.067 (s, 1H) (7.577, d, 1H, $J = 8$Hz) (7.510, d, 1H, $J = 7.5$Hz) 7.339-7.295 (m) 7.187-7.094, (m) 6.993 (s, 1H) 6.989 (s, 1H) 6.224 (m, 2H) 5.582 (m, 2H) 5.142 (m, 2H) 5.121 (m, 2H) 3.611 (m, 2H, 2H) 3.022 (dd, $J = 8$Hz, 8Hz) 2.670 (dd, $J = 7$Hz, 7Hz) 1.674 (s, 3H) 1.646 (s, 3H) 1.624 (s, 3H) 1.318 (s, 3H).

$^{13}$CNMR (126MHz, CDCl$_3$): $\delta$ 177.34; 174.54; 174.31; 173.84; 173.67; 170.04; 141.25; 140.80; 137.74; 136.33; 134.16; 127.55; 127.16; 122.83; 122.41; 122.16; 119.82; 118.65; 111.35; 110.76; 110.28; 87.78; 53.58; 53.44; 52.88; 52.75; 52.58; 52.02

IR (neat, cm$^{-1}$): 3419, 3041, 2935, 2252, 1771, 1699, 1389, 1354, 1281, 1234, 1180, 1096, 910

MS (ESI): Calcd. For C$_{21}$H$_{20}$N$_2$O$_5$ [M$^+$] = 380, ESI$^+$ found [M-1] = 379
Paraformaldehyde (1.0712g, 35.55mmol), NaOAc (0.1464g, 1.785mmol), 2,5-dimethylfuran (3.84mL, 35.55mmol) and 4g of 4Å° mol. sieve powder were combined and stirred in AcOH (15mL). 1,3-cyclohexanedione (2.00g, 17.84mmol) was added in one portion, the flask was equipped with a reflux condenser and heated to 60°C with a sand bath with external temp. monitoring and the mixture was stirred vigorously for 5 hours. The flask was cooled to RT, diluted with DCM (100mL), filtered through celite, which was rinsed with DCM (2x 80mL). The organic layer was transferred to a 1L separatory funnel, washed with sat. NaHCO₃ (aq) (2x 80mL) then H₂O (1x 100mL), brine (1x 100mL), then dried (Na₂SO₄), filtered and concentrated in vacuo yielding a crude oil. The crude oil was taken up in EtOAc (200mL) and degassed, placed under N₂ and wet Pd/C (5%wt., 8.00g, cat.) was added. The mixture was degassed and H₂ was introduced with a balloon. The mixture was stirred vigorously under H₂ (g) overnight (24hrs) then purged, filtered through celite which was rinsed with EtOAc (2x 100mL) and concentrated in vacuo. The crude oil was purified by gradient chromatography (25% EtOAc/Hex → 100% EtOAc) then further by radial chromatography (50% EtOAc/Hex) yielding pure 118 as a colorless oil (<1%).

¹H NMR (500MHz, CDCl₃): Unresolved.

¹³C NMR (126MHz, CDCl₃): δ 168.30; 167.52; 112.13; 98.67; 38.42; 37.43; 36.79; .36.15; 29.10; 28.57; 25.37; 23.01; 20.97
MS (EI): Calcd. For $\text{C}_{13}\text{H}_{13}\text{O}_3$ $[M^+] = 222$, found = 222

IR (neat, cm$^{-1}$): 2957, 2932, 2872, 2360, 2330, 1618, 1514, 1333, 1195, 910
exo-5,6-epoxy-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-(N-glycinyl)imide (124)

endo-5,6-epoxy-1,4-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-(N-glycinyl)imide (129)

Anhydride 15 (5g, 50.99mmol) and glycine (3.83g, 51.02mmol) were dissolved in AcOH (80mL) and stirred at RT overnight (16hr). The solvent was removed in vacuo giving a white powder, that was added to a solution of NEt₃ (7mL) in toluene (500mL) in a 3-neck RBF equipped with a reflux condenser and Dean-Stark trap, then heated to reflux for 3hrs. The flask was cooled to RT and the solvent was removed in vacuo. The residual glutinous solids were taken up in 2M HCl (300mL) and extracted with EtOAc (3x 100mL), dried (MgSO₄), filtered and concentrated in vacuo yielding intermediary maleimide 128 as an orange solid that was used without further purification. The crude solids (3.84g, 24.76mmol) were dissolved in EtOAc (50mL) and furan 82 was added (3.00mL, 27.77mmol) and stirred at RT. After 24hrs, a precipitate had accumulated in the flask. The flask was cooled to 4°C in a fridge for 30min, and the precipitate was filtered, washed with cold EtOAc (3x 25mL) and identified as the endo Diels-Alder product (1.04g, 23% of crude products). The filtrate was concentrated in vacuo yielding a
48:52 endo:exo mixture of Diels-Alder products (3.4097g, 77% of crude products). A portion of
the crude endo:exo mixture (1.47g, 5.84mmol) was suspended in EtOAc (50mL) and heated
gently to dissolve. mCPBA was added (2.11g, 12.22mmol) and stirred at RT for 96hrs. The
solvent was removed in vacuo and the crude epoxide products were resolved by gradient
chromatography (2:25:73 AcOH:EtOAc:Hex → 2:98:0 AcOH:EtOAc:Hex) yielding pure
epoxides 124 (0.25g, 3.7% from 15) and 129 (0.22g, 4.3% from 15).

**Compound 124**

$^1$HNMR (500MHz, DMSO-d$_6$): Internally referenced using solvent residual peak (2.50ppm) δ
4.405 (s, 2H) 3.379 (s, 2H) 3.263 (s, 2H) 1.522 (s, 6H)

$^{13}$CNMR (126MHz, DMSO-d$_6$): Internally referenced using solvent residual peak (39.51pm) δ
173.60; 82.34; 55.68; 51.07; 16.69

MS (ESI): Calcd. For C$_{12}$H$_{13}$NO$_6$ [M$^+$] = 267, ESI$^-$ found [M-1] = 266

IR (neat, cm$^{-1}$): 3136, 2985, 2935, 1745, 1681, 1431, 1328, 1165, 1115, 932, 869

**Compound 129**

$^1$HNMR (500MHz, DMSO-d$_6$): Internally referenced using solvent residual peak (2.50ppm) δ
4.054 (s, 2H) 3.570 (s, 2H) 3.180 (s, 2H) 1.407 (s, 6H)

$^{13}$CNMR (126MHz, DMSO-d$_6$): Internally referenced using solvent residual peak (39.51pm) δ
174.03; 167.83; 82.35; 54.18; 51.24; 13.72

MS (ESI): Calcd. For C$_{12}$H$_{13}$NO$_6$ [M$^+$] = 267, ESI$^-$ found [M-1] = 266

IR (neat, cm$^{-1}$): 3107, 2980, 2962, 1699, 1418, 1329, 1182, 934, 872
Appendix: $^1$H-NMR, $^{13}$C-NMR, NOESY, COSY, GCMS and FT-IR Spectra
Exact Mass: 222.13
Exact Mass: 285.10

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