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ACCESSING THE 2-AMINO-5,5-DISUBSTITUTED-1H-IMIDAZOL-4-ONE SCAFFOLD FOR NATURAL PRODUCT SYNTHESIS AND EVALUATION FOR CHECKPOINT KINASE 2 INHIBITION

presented by

Christopher David Hupp

has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Chemistry

Major Professor's Signature

5-01-09

Date

ACCESSING THE 2-AMINO-5,5-DISUBSTITUTED-1H-IMIDAZOL-4-ONE SCAFFOLD FOR NATURAL PRODUCT SYNTHESIS AND EVALUATION FOR CHECKPOINT KINASE 2 INHIBITION

By

Christopher David Hupp

A DISSERTATION

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ABSTRACT

ACCESSING THE
2-AMINO-5,5-DISUBSTITUTED-1H-IMIDAZOL-4-ONE
SCAFFOLD FOR NATURAL PRODUCT SYNTHESIS
AND EVALUATION FOR CHECKPOINT KINASE 2 INHIBITION

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This dissertation is primarily focused on the development of a synthetic method for the preparation of 2-amino-5,5-disubstituted-1H-imidazol-4-ones through a novel rearrangement and the application toward the first total synthesis of a marine alkaloid. The first chapter identifies checkpoint kinase 2 (Chk2) as a viable drug target for adjuvant therapeutics. The second chapter elaborates on the development of a new rearrangement applicable for the synthesis of potential Chk2 inhibitors. The third chapter describes the total syntheses of a natural product and two analogs. Finally, the fourth chapter describes the biological evaluation of all synthesized compounds.

Chemotherapy and radiation therapy offer effective methods to treat various forms of cancer. However, these procedures can often lead to negative side effects resulting in the destruction of healthy cells. Developing adjuvant drugs that inhibit Chk2, a kinase part of the DNA damage response network, is believed to help ameliorate these harmful side effects by desensitizing healthy cells toward treatments such as ionizing radiation.

Indoloazepine, an analog of the natural product debromohymenialdisine, was found to be a potent inhibitor of Chk2. Additional literature evaluation led to the identification of a natural product, from the tunicate *Dendrodoa grossularia,*

which contained structural similarities to indoloazepine. The indole alkaloid contained a unique quaternary imidazolone scaffold that required the use of a novel transformation to attain.

The traditional oxazole rearrangement was discovered by Prof. Steglich in 1975. Unfortunately, this transformation is often plagued by the inability to remove an N-acyl group resulting from the ring-opened oxazolone product, thus preventing any further chemical modifications. In order to apply an oxazole rearrangement to the synthesis of the natural product, a novel rearrangement was developed. Overall, a one-pot transformation from a thiourea to a quaternary hydantoin intermediate allowed access to the imidazolone scaffold needed to complete the first total synthesis of the natural product.

The natural product was synthesized in 12 linear steps with an overall yield of 11.8%. The synthesis highlighted the use of the newly developed rearrangement and also allowed for analogs to be synthesized without a major modification of the synthetic pathway. Finally, biological evaluation of the natural product, analogs and other heterocycles revealed that all compounds prepared were inactive against the targets screened. Additionally, all compounds tested for cell cytotoxicity were found to be non-cytotoxic.

This dissertation is dedicated to my parents, David and Sandra Hupp,

who have always made my education a top priority.

I told you I'd become a doctor someday!

ACKNOWLEDGMENTS

I would first like to acknowledge my advisor, Prof. Jetze Tepe. It was truly a daunting experience to come into a large university from a small undergraduate college. Jetze showed patience (yes, he does show that quality sometimes) throughout the first years of me learning the ropes in lab and encouraged me to not just *do* chemistry, but *understand* it. I believe he always had faith in me to complete my project even though at times the road was very bumpy and the outcome looked bleak. It is with his faith and my determination that pushed me to reach my goal. I truly learned a great deal about organic synthesis and medicinal chemistry but, overall, I believe the most valuable things I learned in his lab were the lessons on dealing with struggles in lab and how to approach a problem. For all of this, I thank you Jetze.

I would also like to acknowledge Prof. Robert Maleczka, my second reader. By far, he is easily one of the best professors I have had for an Organic Chemistry course. Although his class was the hardest I have ever taken, it is also the class where I learned the most. I will always remember that class and what it has done for me on an intellectual level. I also want to acknowledge my other committee members, Professors William Wulff and Jim McCusker, whom have been an excellent source of information and guidance throughout my graduate career.

I want to extend a sincere thanks to other staff at MSU including Bob Rasico, Melissa Parsons, Bill Flick, Dr. Daniel Holmes and Kermit Johnson. The

chemistry department is very lucky to have staff such as these people, who were always willing to lend a hand. MSU is truly blessed with such talented and thoughtful staff.

The past and present Tepe group members have also shaped the type of scientist I have become and for that, I am very grateful. I wouldn't be where I am today without the help from all of the group members. It is not every day that co-workers and great friends coincide. I am blessed to have become friends with many of the people I worked with including Jason, Sam, Thu and Adam. It has made the whole process of graduate school much easier and I hope to stay in touch in the future.

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LIST OF ABBREVIATIONS

AIDS – acquired immune deficiency syndrome

Asn – asparagine

ATM - ataxia telangiectesia mutated

ATP – adenosine triphosphate

ATR - ataxia telangiectesia related

Bn – benzyl

Boc - tert-butyloxycarbonyl

BOP - Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate

Cbz – carbobenzyloxy

Cdc – cell division cycle

CDK – cyclin dependent kinase

Chk# – checkpoint kinase #

DBH - debromohymenialdisine

DCC – dicyclohexylcarbodiimide

DCM – dichloromethane

DIPEA – diisopropylethyl amine

DMAP – dimethylamino pyridine

DMDO – dimethyldioxirane

DMEM - Dulbecco's modified eagle medium

DMF – dimethylformamide

DMSO – dimethyl sulfoxide

DNA – deoxyribonucleic acid

EC_{50} – half maximal effective concentration

EDCI - 1-Ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride

EDTA - ethylenediaminetetraacetic acid

ELISA - enzyme-linked immunosorbent assay

Et – ethyl

FBS – fetal bovine serum

FDA – food and drug administration

Fmoc - 9H-fluoren-9-ylmethoxycarbonyl

Glu – glutamic acid

GSK – glycogen synthase kinase

HMDS – hexamethyl disilazane

HPLC – high performance liquid chromatography

HRMS – high resolution mass spectrometry

IBX - 2-Iodoxybenzoic acid

IC_{50} – half maximal inhibitory concentration

IL – interleukin

IR – ionizing radiation or infrared

Law. Rgt. – Lawesson's reagent

LCMS – liquid chromatography mass spectrometry

Lys – lysine

Me – methyl

Met – methionine

MCPS –

MS – ma

Necm –

NF-κB –

NHC – N

NMR – n

NOESY

OAc – a

Ph – phe

PKA – p

pom – p

ps – po

Pyr – p

RNA – r

t – "bo

SAR – s

Ser – se

Su – te

TEA – t

TFA – t

THAA –

THF – te

Thr – th

MOPS – 3-morpholinopropanesulfonic acid

MS – mass spectrometry

Napth. – naphthalene

NF-κB – nuclear transcription factor – kappa B

NMO – N-methylmorpholine-N-oxide

NMR – nuclear magnetic resonance

NOESY - nuclear overhauser enhancement spectroscopy

OAc – acetate

Ph – phenyl

PIKK - phosphoinositide 3-kinase-like kinase

ppm – parts per million

psi – pounds per square inch

Pyr. – pyridine

RNA – ribonucleic acid

rt – room temperature

SAR – structure activity relationship

Ser – serine

^{t}Bu – tert butyl

TEA – triethylamine

TFA – trifluoroacetic acid

TFAA – trifluoroacetic anhydride

THF - tetrahydrofuran

Thr – threonine

TLC – thin layer chromatography

TNF – tumor necrosis factor

Ts – tosyl

UV – ultraviolet

CHAPTER I

CHECKPOINT KINASE 2 AS A TARGET FOR ADJUVANT CANCER THERAPEUTICS

I.A Introduction to cancer

Despite the increase in knowledge and therapeutic advances, cancer is currently one of the highest causes of mortality in the United States.[1, 2] Even though the mortality rate of some forms of cancer (stomach, breast and rectum cancer) have slowly decreased, other forms of cancer (pancreatic, lung and bronchus) have seen little improvements over the last several years.[2, 3] Cancer can be defined as an unregulated growth of tissue not governed by the regulations of normal cell growth.[4] The main causes of human cancer include tobacco/tobacco products (30%), hormones (30%), diet (15%), viruses (10%), drugs, x-rays and UV light (10%) and occupational carcinogens (5%).[4]

Normal cell growth and division is systematic and necessary to replace the aging and dying cells or to repair injuries. Furthermore, normal cell growth and division can only be conducted in a manner that is cognisant of a neighboring cell's growth to efficiently and correctly form tissues and organs.[4] The process of division for a normal cell is shown below in Figure I-1. A healthy cell will grow and divide into two new daughter cells, which can each then grow and divide themselves, and so on. In the event that a cell is damaged in the process or a mutation has occurred, the cell can either repair itself or initiate cell suicide (apoptosis). The apoptotic pathway is essential for eliminating damaged DNA and preventing mutated cells from passing on the damaged genetic information.[4]

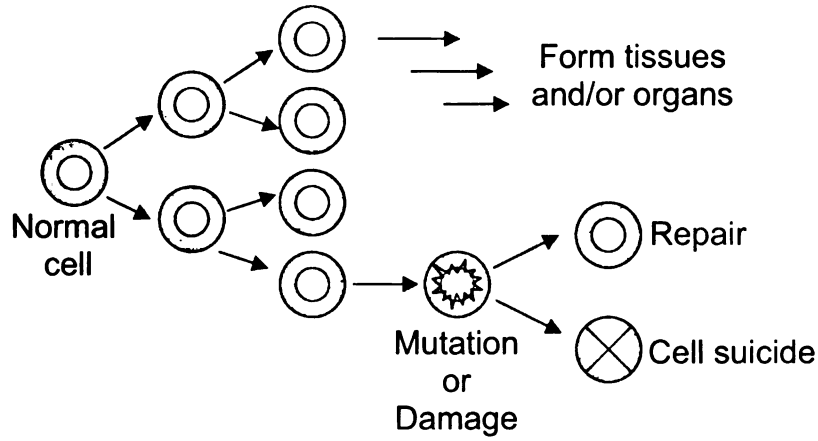

Figure I-1. Normal cell division

However, if cells stop cooperating with their neighboring cells, become autonomous in their growth and are mutated, they can form tumor cells, which could later become cancer cells. Tumors are generally classified into two categories: benign and malignant. Benign tumors are localized, do not spread to other parts of the body and are generally not lethal to their host. However, if a benign tumor is exerting pressure on a sensitive organ such as the brain, then complications can arise. Malignant tumors can destroy parts of the body from which they originate and can invade surrounding tissue. Furthermore, these tumors can form secondary tumors in new sites and eventually cause destruction of additional tissue and organs. This large difference in mobility distinguishes cancer from benign growths.[4]

Malignant tumor cells, or cancer cells, grow uncontrollably and divide in an unordered fashion. As shown in Figure I-2 a cancer cells division process proceeds in a non-systematic way leading to non-structured masses or tumors. These cells can then spread or metastasize toward other tissues and eventually cause damage at sites far from the original location.[4]

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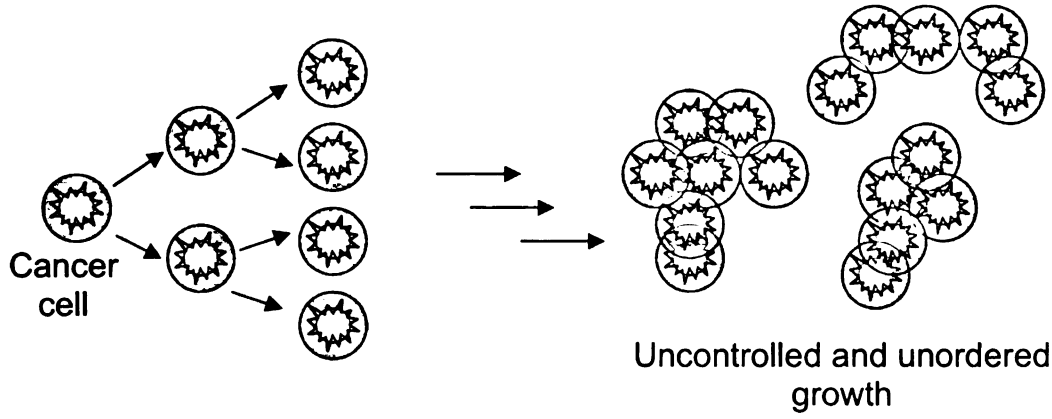

Figure I-2. Cancer cell division

I.B Chemo and radiotherapy

Although uncontrolled cancerous cell growth can lead to solid tumors (except in cases such as leukemias), cancer can be treated by a variety of methods including chemotherapeutics and/or radiation therapy.[5-18] There are many different types of chemotherapeutics used to treat cancer such as alkylating agents, antimetabolites, topoisomerase inhibitors, mitotic inhibitors as well as other targeted therapies. Alkylating agents are used to directly damage DNA by alkylating the base pairs and ultimately preventing the cell from reproducing. Some of the more common types of alkylating agents include the nitrogen mustards[10], mitomycin C[12] and the powerful platinum drugs cis-platin and carboplatin.[5] Antimetabolites are a class of drugs that interfere with the growth of DNA and RNA by essentially mimicking the natural metabolites used for the synthesis of DNA and RNA. Common modes of action for antimetabolites include inhibition of DNA synthesis enzymes or causing mismatched DNA synthesis, which could result in cell death. A few common antimetabolites that are used for chemotherapy include 5-fluorouracil[8] and 6-mercaptopurine.[15]

Topoisomerase inhibitors are compounds that interact with the enzymes called topoisomerase I and topoisomerase II, which are responsible for assisting in the unwinding of DNA for the replication process. Topoisomerase I perfoms a reversible single strand cleavage of DNA to allow relaxation of the coil and then reseals the cleaved strand after unwinding. Topoisomerase I inhibitors include, camptothecin, topotecan and irinotecan.[13] The topoisomerase II enzyme acts in a similar fashion as the topoisomerase I enzyme except that it completes a reversible double stranded DNA cleavage to aid in the unwinding of DNA for replication followed by a resealing of the cleaved strands. The most common topoisomerase II inhibitors include etoposide, teniposide, doxorubicin and daunorubicin.[9, 11, 17, 18] Anthracyclines, such as doxorubicin, intercalate between the base pairs of DNA resulting in a distortion that prevents the DNA replication process. Furthermore, metabolism of specific anthracyclines, such as doxorubicin, leads to the formation of free radicals that oxidatively damage DNA and to reactive intermediates that form covalent adducts with DNA, inhibiting the overall replication process.[18]

Mitotic inhibitors interfere with mitosis and prevent the cell from reproducing. One of the more famous mitotic inhibitors include paclitaxel (Taxol), a natural product isolated from the pacific yew tree.[14] Paclitaxel's mode of action is unique from other mitotic inhibitors due to its effect on the polymerization of tubulin, the building block of microtubules. In general, most mitotic inhibitors induce the disassembly of microtubules, a component of cells responsible for aiding in the cell division process. However, paclitaxel was found to induce the

polymerization of tubulin, producing dysfunctional microtubules leading to the death of the cell.[14]

As researchers have come to understand cancer more thoroughly, new more specific therapies have developed. Kinases have become an interesting and challenging target for cancer drug discovery in the past decade. Although extensive research has been accomplished trying to develop kinase inhibitors, only a few have been FDA approved in the last ten years (Table I-1).[7] The main complication that arises from targeted kinase chemotherapy is the formation of drug resistant tumors.

Table I-1. FDA-approved Kinase inhibitors (adapted from ref. 13)

U.S. brand name	Year approved	Cancer type	Company	Target kinase
Gleevec	2001	CML	Novartis	Abl, c-Kit, PDGFRa, PDGFRb
Iressa	2003	NSCLC	AstraZeneca	EGFR
Tarceva	2004	NSCLC, P	Genentech, OSIP	EGFR
Nexavar	2005	HC, RCC	Bayer, Onyx	Raf, VEGFR2, VEGFR3, c-Kit, PDFGRb
Sutent	2006	GIST, RCC	Pfizer	c-Kit, VEGFR, PDGFR, FLT3
Sprycel	2006	CML	BMS	Abl, c-Kit, PDGFR, Src
Tasigna	2007	CML	Novartis	Abl, c-Kit, PDGFRb, Src, Ephthrin
Tykerb	2007	BC	GSK	EGFR, Her-2

CML = chronic myeloid leukemia; NSCLC = non-small-cell lung cancer; P = pancreatic; HC = hepatocellular carcinoma; RCC = renal cell carcinoma; GIST = gastrointestinal stromal tumor; BC = breast cancer

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Radiation therapy also offers a highly sophisticated treatment of cancer. The radiation can be administered externally by a machine, internally by a catheter or taken orally. The radiation induces unsustainable DNA damage to targeted cells which results in cell death.[6, 16] Overall, one of the impediments of chemotherapeutic and ionizing radiation therapy is that these treatments are often limited in efficacy by severe side effects to healthy tissue. Strategies that address these limitations may have the potential to enhance the efficacy of current treatments.[3]

I.C DNA damage response pathways

On average, the induction of DNA damage appears to be the most common strategy in anticancer treatment and has dramatically increased the survival rate of patients.[19-22] The anticancer effects are even better when these agents are used in combination with other drugs containing different modes of action.[23] Upon treatment of cells with any of the chemotherapeutics described previously or ionizing radiation, two distinct DNA damage response pathways are induced (Figure I-3).[24] One response includes the activation of the NF-κB pathway, which is largely responsible for antiapoptotic cell signaling. The second distinct response is the activation of cell cycle checkpoint kinases, which essentially mediate the induction of cell cycle arrest and apoptosis.[25-31] Activation of the NF-κB signaling pathway induces the expression of a wide range of genes involved in cell survival responses.[32-35] Activation of the DNA checkpoint kinases results in a complex network of signaling pathways involved in the induction of apoptosis or cell cycle arrest, which allows for DNA repair and re-initiation of cell cycle

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progression.[24, 36-40] Unfortunately, the activation of these antiapoptotic signaling pathways limits the overall efficacy of cancer treatments.[3]

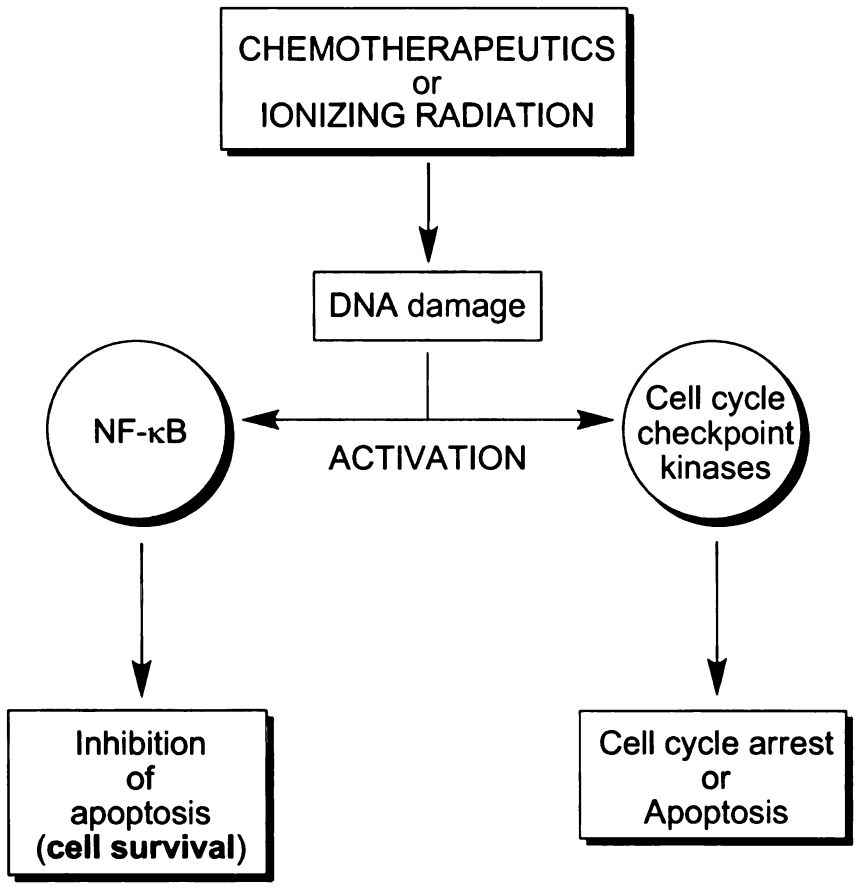


Figure I-3. DNA damage response pathways

Efficacy of treatment is often limited by the narrow therapeutic window in which drugs are effective due to their inherent toxicity to the patient. Strategies of combining traditional chemotherapeutics with cell cycle checkpoint inhibitors to either sensitize cancerous cells or desensitize healthy cells, may widen the therapeutic window and improve the overall efficacy of the cancer treatment.[3]

I.D Developing adjuvant therapeutics for cancer treatment

Adjuvant therapeutics offer a strategy to limit the negative side effects of current cancer treatment practices. It is hypothesized that by targeting a specific

checkpoint kinase, healthy cells can be selectively desensitized to radiation treatment and allow a more effective procedure to manage cancer. Developing a drug to become an adjuvant therapeutic is a long process as illustrated in Figure I-4.[41] This section (**I.D**) is meant to briefly explain each major step in the process (Figure I-4) and will be complemented by the application of this process to my project in the following sections.

As shown in Figure I-4, the first step in developing therapeutics is to identify a drug target candidate. After initial studies determine if the candidate is a viable option, the target is validated. Subsequently, efforts are made to 1) purify the drug target so that additional information can be gathered and 2) compounds from a variety of areas are screened to identify potential hits. If a crystal structure is identified for the target, it can aid in the development of additional compounds to test and other potential drug candidates through a rational drug design approach. If a lead compound is designed or found, the process of optimizing the lead drug can begin through molecular modeling, medicinal chemistry and combinatorial chemistry techniques. Once optimized, the drug is subjected through many rounds of preclinical studies and finally clinical studies before being considered for approval to go on the market.

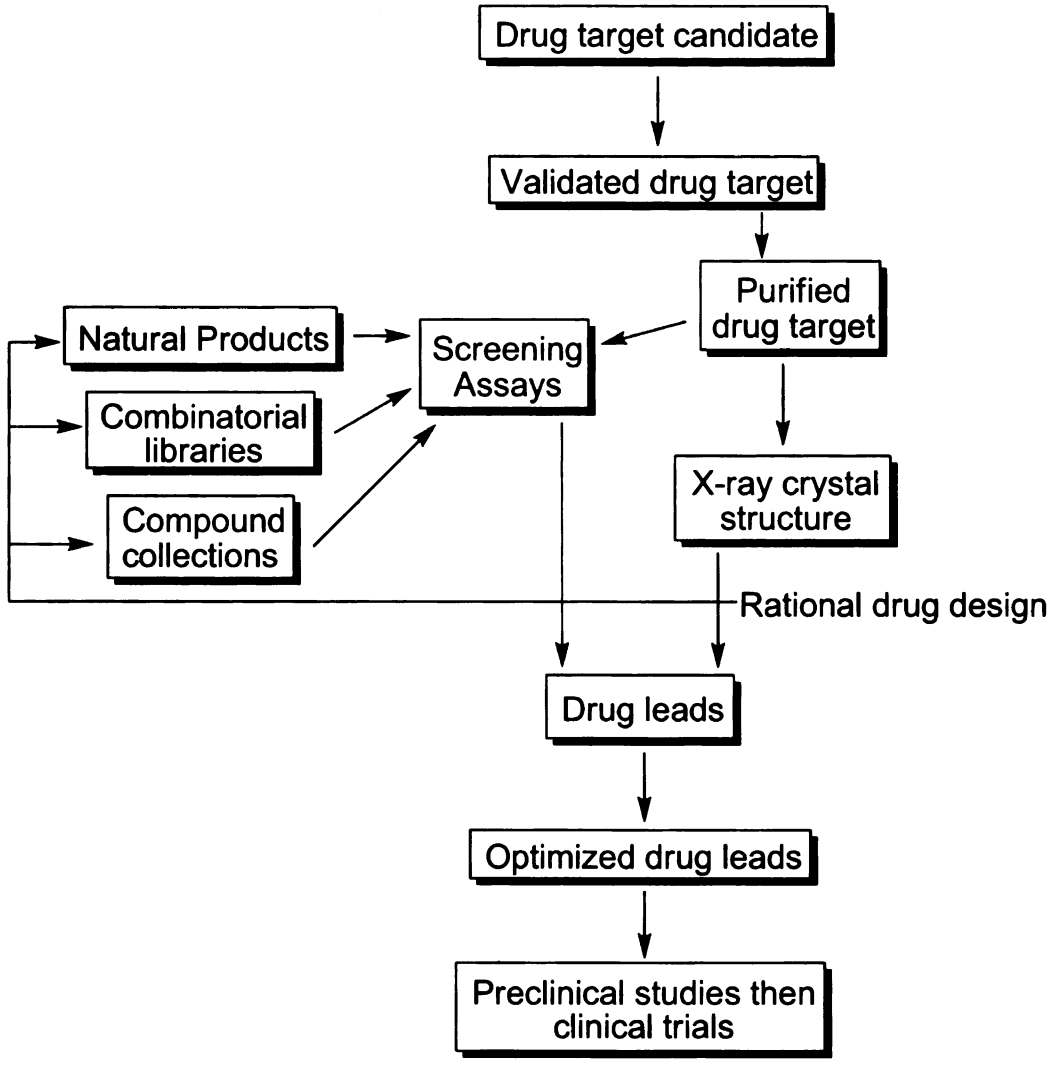


Figure I-4. Schematic for drug development (adapted from ref. 40)

I.E Chk2 as drug target candidate

The primary structure of DNA is continuously being altered by endogenous and exogenous stimuli, which cause abnormalities ranging from simple base changes to deletions. Despite these continuous DNA damaging events, nature has recruited reinforcements in the form of checkpoint pathways that maintain and monitor the integrity of the genome. These pathways were first observed when it was shown that damage induced by chemotherapeutic agents resulted in an inhibition of the cell cycle, allowing for cellular repair.[42, 43] A similar

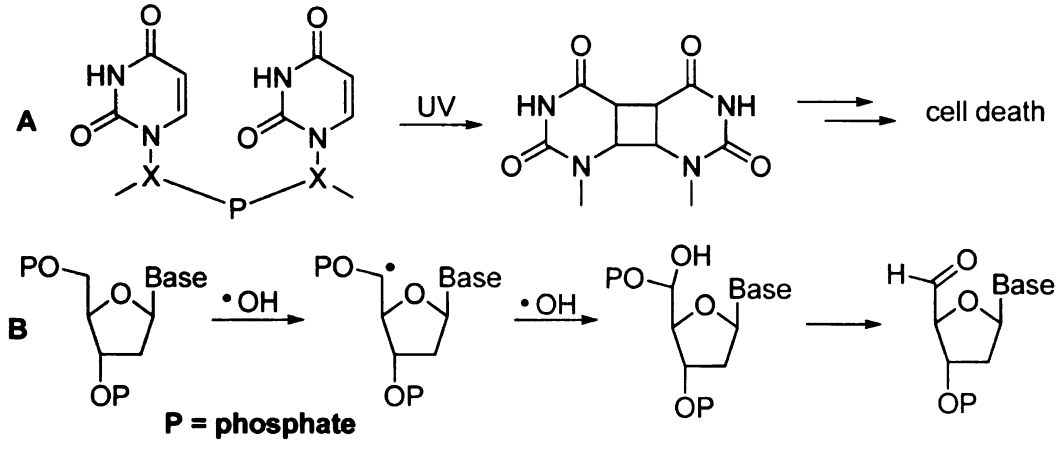
control of cell cycle progression was found in *Saccharomyces cerevisiae* and was then coined a 'checkpoint'.[44] It was originally thought that checkpoint pathways were operational for the sole purpose of regulating cell cycle transitions. Currently, it is generally accepted that the checkpoints are part of a cascade of signals that ultimately lead to DNA damage response processes.[38, 39] These response processes include removal of the DNA damaged sites and restoration of the DNA duplex. Temporary arrest of the cell cycle allows for repair and prevention of the duplication of damaged DNA or its transcriptional response. In the event of heavily damaged or seriously deregulated cells, induction of apoptosis or terminal cell cycle arrest occurs.[37, 45, 46] The DNA damage cell cycle checkpoints will repair, patch, and promote the overall survival of cells, even cancer cells, resulting in a reduction of the overall anticancer efficacy.

However, if the induction of checkpoint pathways and DNA repair could be selectively inhibited in cancerous cells, the efficacy of the treatment may be enhanced. Alternatively, if DNA repair can be selectively induced in healthy cells, the therapeutic window of the treatment could be significantly widened, allowing for a more tolerable and effective anticancer therapy. Consequently, modulating the DNA checkpoint pathways using small molecules could potentially sensitize cancer cells and desensitize healthy cells to DNA damage induced by chemotherapeutics or ionizing radiation.[42] More specifically, altering the checkpoint pathways via modulation of two of the key checkpoint kinases, Chk1

and Chk2, has become an increasingly appealing approach to broaden the therapeutic window of conventional anticancer therapies.[47]

These checkpoint pathways, rather than being thought of as a molecular switch, represent a continuous process that is amplified in the presence of DNA damage, such as from ultraviolet (UV) and ionizing radiation (IR).[37] Between UV and IR, UV radiation is lower in energy and the majority of DNA damage results from the formation of photoproducts, such as dimers of pyrimidine containing nucleotides (**A**, Scheme I-1). These dimmers can inhibit DNA transcription and replication machinery ultimately leading to cell death.[48, 49] Ionizing radiation is higher in energy and can cause more substantial damage to DNA. IR is absorbed mostly by surrounding water molecules, which subsequently form highly reactive radicals that cause severe DNA damage. These radicals can cause nucleotide damage, crosslinking and strand breaks. Reaction **B** in Scheme I-1 illustrates an example of a strand cleavage reaction that could occur due to ionizing radiation.[16]

Scheme I-1. DNA damage by UV and IR



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The DNA damage checkpoint pathways consist of three different main components: sensors, signal transducers, and effectors (Figure I-5). The sensors recognize the damaged DNA and initiate subsequent events. Two of the main sensors are ATM and ATR, which belong to the phosphoinositide 3-kinase-like kinase (PIKK) family members.[37, 50]

The first DNA damage sensor, ATM (**a**taxia **t**elangiectesia **m**utated) exhibits kinase activity when activated by agents that induce double strand breaks, such as ionizing radiation.[51] ATM will subsequently phosphorylate proteins such as Chk2 and p53[51, 52] (among others) when activated after cells have been exposed to IR. A deficiency in ATM exhibits phenotypes such as cerebellar degeneration, immunodeficiency, genome instability, clinical radiosensitivity, and cancer predisposition.[53]

The second DNA damage sensor, ATR, has a sequence homology to both ATM and SpRad3, thus comes the name ATR (**ATR**M and **R**ad3 related).[37, 54] ATR is similar to ATM since it is a kinase that essentially phosphorylates the majority of the same substrates as ATM. However, ATR is activated *in vivo* by UV radiation rather than ionizing radiation.[37, 55] Unlike ATM, it does not appear that ATR is activated by double strand breaks.[37] Thus, ATR is the main PIKK family member that initiates a signal transduction pathway after UV radiation damage.[55]

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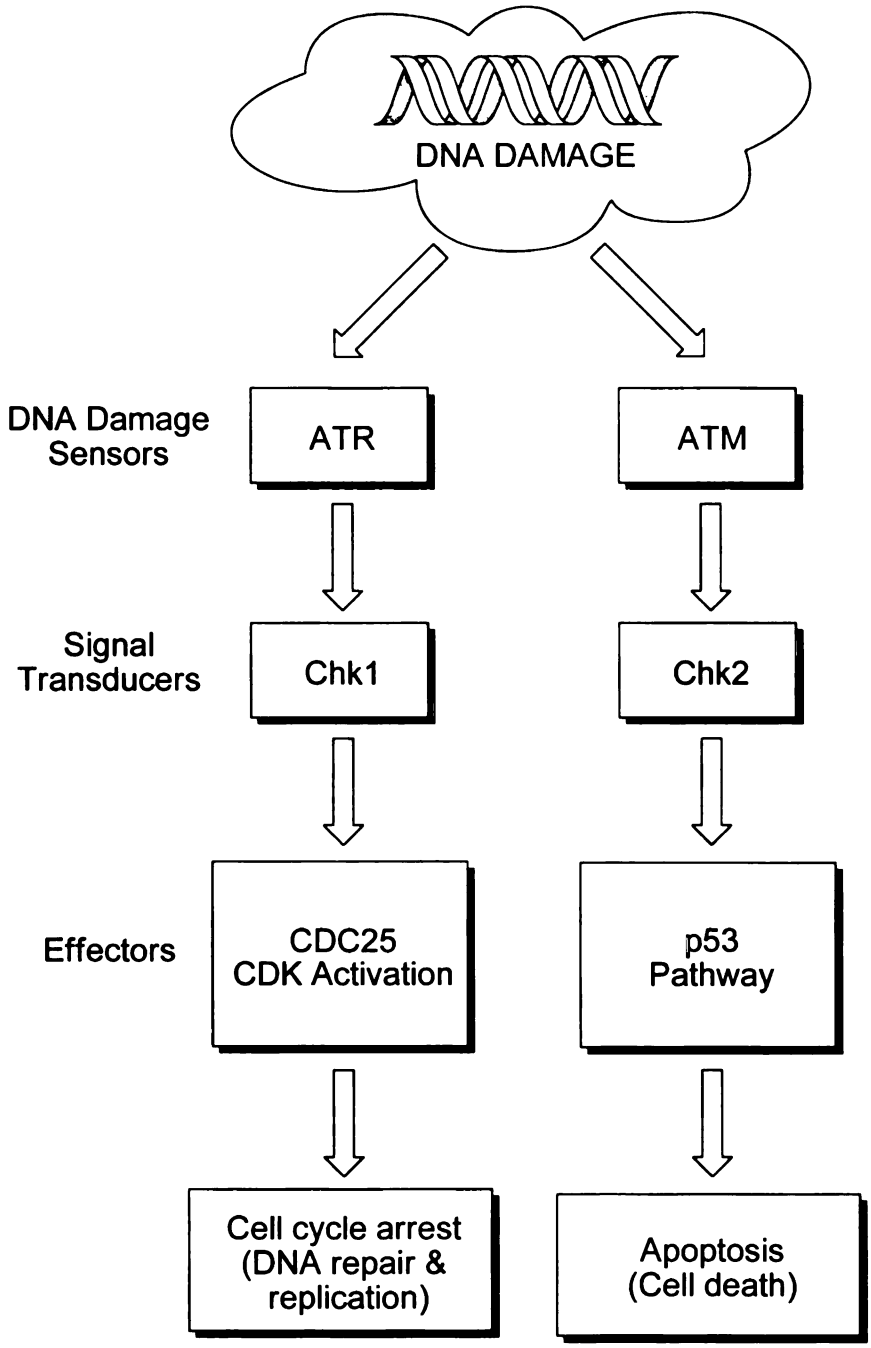


Figure I-5. Components of the damage response network

The signal transducers are components of the DNA damage pathway that essentially receive the signal from the sensors. The two signal transducers that will be discussed here in detail are Chk1 and Chk2.[56-58] Chk1 and Chk2 are structurally unrelated but are essential checkpoint kinases downstream of the

DNA damage sensors and play a critical role in determining the cell's fate.[37, 59-61] ATM is activated by double strand breaks and the signal is transduced by Chk2 [62, 63], while the UV damage signal sensed by ATR is transduced by Chk1.[55, 64] Chk1 is primarily responsible for cell arrest in response to DNA damage, allowing for the initiation of DNA repair[65], whereas Chk2 has been implicated with the phosphorylation and activation of the apoptotic transcription factor p53.[40, 62, 65] It is important to note the drastic difference in Chk1 and Chk2 null mice. Chk1 (-/-) mice are not viable and exhibit embryonic lethality[33, 66], whereas Chk2 (-/-) mice still illicit near normal checkpoint responses and are viable.[67]

The effector components, Cdc25 and p53, are involved in the arrest of the cell cycle and apoptosis, respectively. Three phosphotyrosine phosphatases, Cdc25a-c, are responsible for dephosphorylating cyclin-dependent kinases that ultimately affect proteins directly involved in cell cycle transitions.[37] Essentially, the checkpoint kinases phosphorylate one or more of the Cdc25 proteins that results in their inactivation and degradation, thus preventing the Cdc25 proteins to translocate into the nucleus and enable cell cycle progression[68] The tumor suppressor protein p53 is responsible for arresting the cell cycle and inducing apoptosis.[69]

Based on the described pathways it is possible that inhibition of these checkpoint kinases may be used to enhance the effects of chemotherapeutics by sensitizing cancer cells or desensitizing healthy cells.[60, 70-72] Inhibition of Chk1 is believed to sensitize tumor cells by blocking the cell's ability to initiate DNA repair, resulting in unsustainable DNA damage.[60, 65, 73, 74] Inhibition of the G2

DNA checkpoint Chk2 can selectively prevent apoptosis in p53 wild type cells (healthy cells), thus desensitizing them from cell death during chemotherapeutic treatment.[71, 75] Chk2 inhibitors are anticipated not to have an effect on apoptosis in cells containing mutated p53 (>50% of cancerous cells). We were interested in focusing our efforts on developing Chk2 inhibitors to desensitize healthy cells toward ionizing radiation to help widen the therapeutic window of this treatment.

I.F Validation of Chk2 as drug target

Activation of Chk2 is initiated by factors that cause DNA damage, such as ionizing radiation, chemotherapeutic agents, and telomere initiated senescence.[40, 76, 77] In response to IR induced DNA damage, signals are intercepted by ATM, which in turn directly phosphorylates Chk2 at Thr68 within the SQ/TQ rich domain.[78, 79] After the initial phosphorylation and dimerization, multiple intermolecular phosphorylation events occur to complete the activation.[40, 80] The phosphorylation of Ser516 was recently found to be of great importance as this modification is required for full activation of Chk2.[40, 81, 82] A schematic of the DNA damage checkpoint pathway involving Chk2 is shown in Figure I-6.

Once activated and all the phosphorylation events of Chk2 have transpired, Chk2 signals the effector proteins Cdc25a, Cdc25c, and p53, among others (Figure I-6). The Cdc25 family of phosphatases is responsible for the progression of the cell cycle by dephosphorylating cyclin dependent kinases cdk1 and cdk2 at specific sites.[40, 83-86] Chk2 phosphorylates Cdc25a at Ser123 and consequently renders it inactive[40, 84, 87], while Chk2 phosphorylates Cdc25c at Ser216. This results in the sequestering of Cdc25c in the cytoplasm, thus

preventing activation of the cdk1/Cyclin B complex, resulting in G2/M arrest.[40, 88-90]

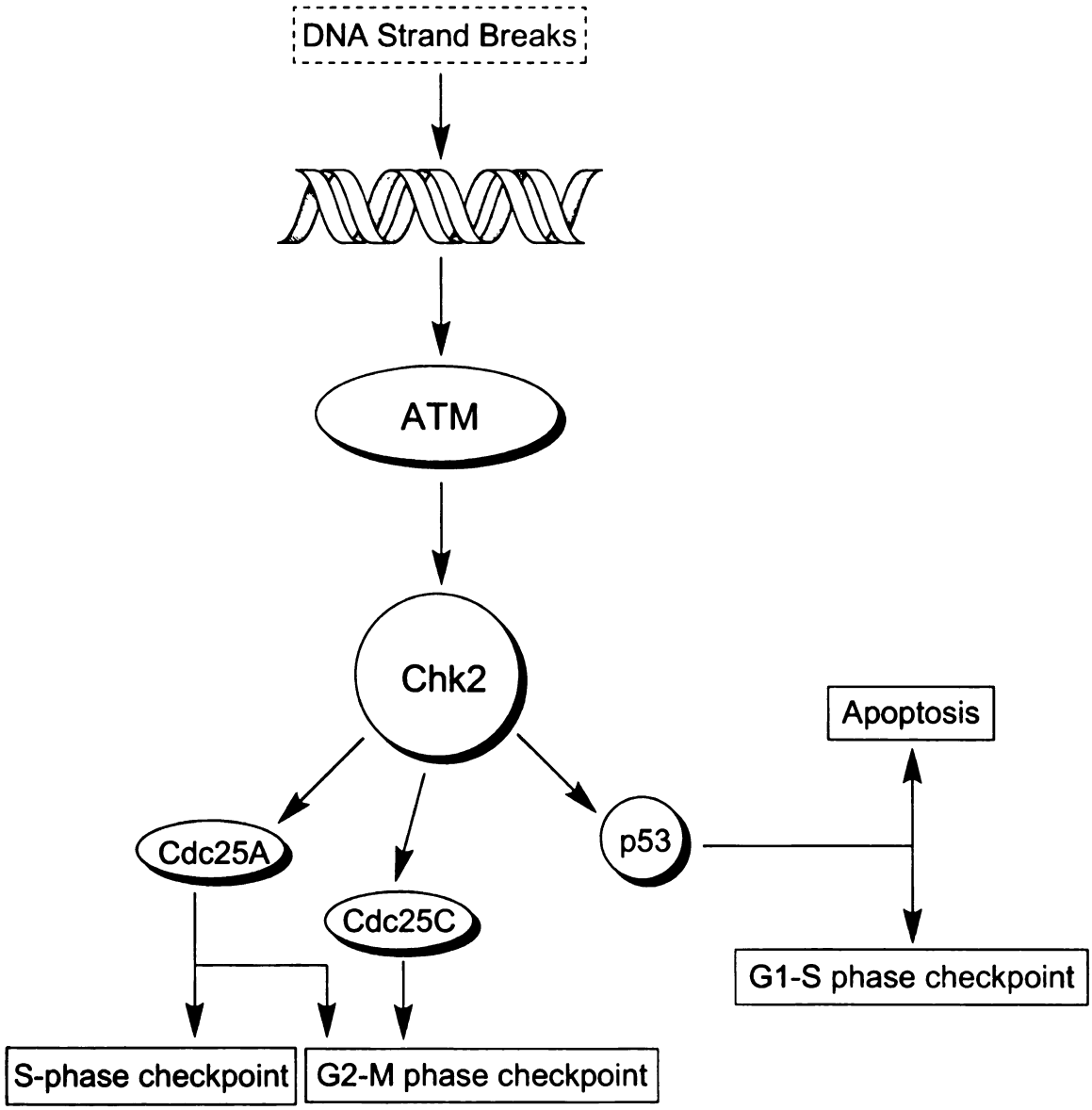


Figure I-6. DNA damage pathway involving Chk2

Another important downstream substrate of Chk2 is tumor suppressor protein p53. Although the exact relationship between p53 and Chk2 is still unclear, Chk2 is thought to be upstream of p53 in the reaction pathway that contains IR induced apoptosis.[36, 42, 91] It was reported that Chk2 phosphorylates p53 at Ser20 thereby regulating the amount of activated p53 in response to

double strand breaks.[36, 62, 92-94] However, this point has been challenged based on a few observations. First, the p53 derived peptide containing the Chk2 targeted phosphorylation sites is a poor substrate for Chk2 and does not contain the characteristic sequence for directing a phosphorylation found in other Chk2 substrates. Second, the phosphorylation of p53 induced by DNA damage was only slightly affected, if at all, by down regulation or knockout experiments of Chk2.[62, 91, 95-98] Despite these arguments, most scientific analyses of the Chk2-p53 link strongly support the role of Chk2 as a p53 kinase.[97]

The role of Chk2 in DNA damage induced apoptosis is supported by Chk2 deficient mice, which show an increased resistance to ionizing radiation and cellular defects in apoptosis.[62, 67, 95, 96] Chk2 -/- mice survived significantly longer than the wild-type mice following whole body irradiation.[96] The increased survival of those mice without Chk2 is attributed to resistance of several cell types to IR induced apoptosis.[95, 96] Furthermore, it is important to remember that Chk2 (-/-) mice still illicit near normal checkpoint responses and are viable.[67] The support given by the study where the Chk2 null mice survived whole body irradiation and the study which showed that Chk2 null mice were still viable offer validation for choosing Chk2 as the drug target.

I.G Purified drug target and X-ray crystal structure

As illustrated in Figure I-4, it can be important to obtain information from the purified drug target to aid in the development of inhibitors. Fortunately, Oliver *et al.* were able to crystallize the kinase domain of Chk2 (Figure I-7) and in effect

give an enormous amount of information to the aid in developing inhibitors of Chk2.[99]

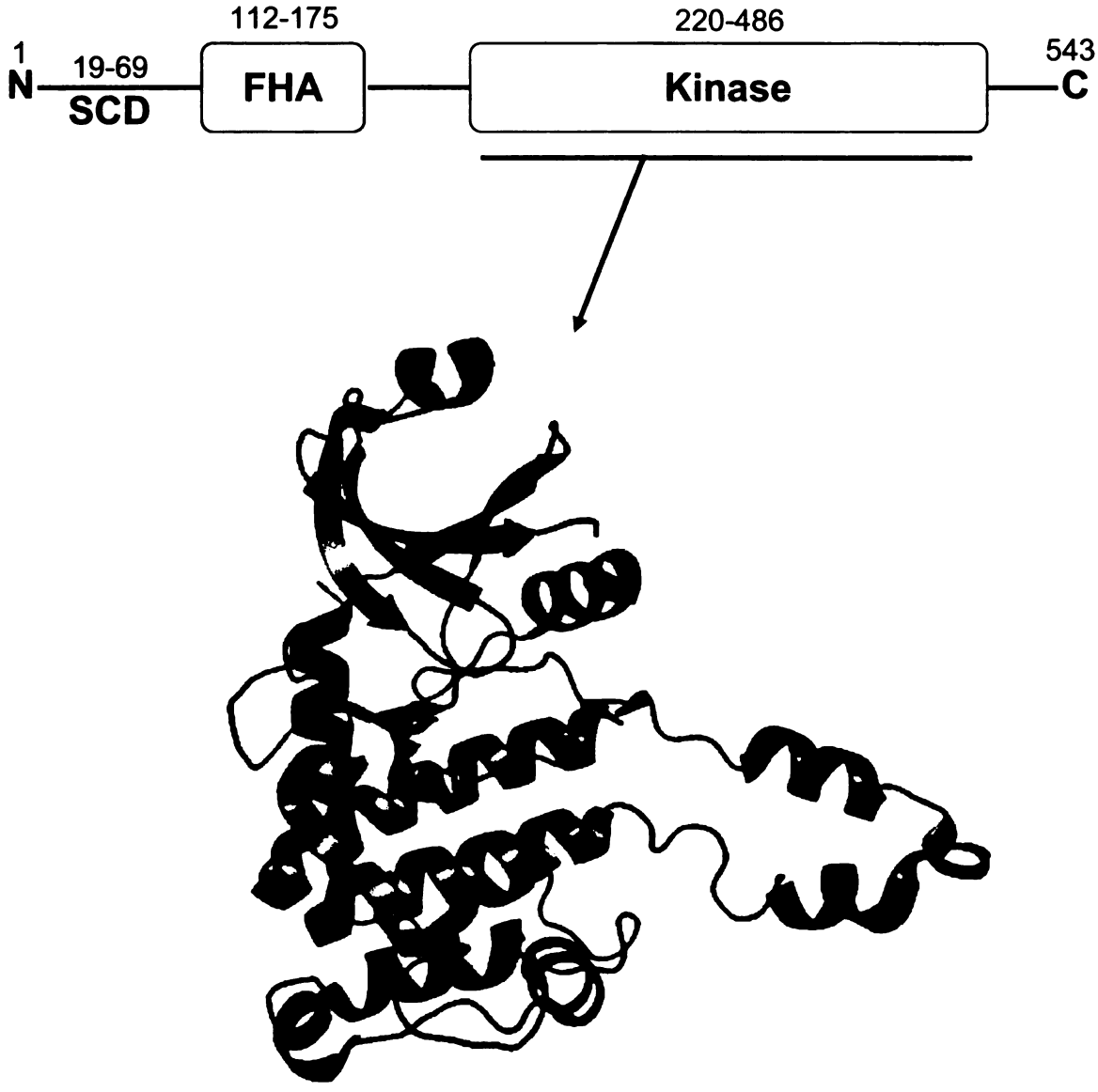


Figure I-7. Illustration of Chk2 and crystal structure of kinase domain

The top of Figure I-7 displays the linear representation of the whole checkpoint kinase 2 protein. As illustrated above, Chk2 is a 543 long amino acid protein that consists of three domains. The first domain is called a serine-glutamine/threonine-glutamine cluster domain (SCD), which is the substrate for

the upstream protein ATM, as shown in Figure I-6. The second domain is called the forkhead associated domain (FHA) which has been implicated to help modulate protein-protein interactions. The last domain, which was crystallized, is the kinase domain responsible for performing the major requirement of a kinase, that is, to phosphorylate its substrates.[40, 99] Overall, gaining access to this information helps in discovering new inhibitors of Chk2 and allows for the forward progress in obtaining an adjuvant drug for radiation therapy.

I.H Checkpoint kinase 2 inhibitors

Until recently, there were only a few reports of potent Chk2 inhibitors that show good Chk2 selectivity. Within the last decade there has been an increase into the amount of research being performed to develop checkpoint kinase 2 inhibitors. Some remain highly confidential like XL844, developed by Exelixis, which is one of the newest checkpoint kinase inhibitors to move into clinical trials.[100] Others have been kept hidden in patents, like the indazole and squaric acid derivatives shown in Figure I-8, and have not been highly publicized.[101-103]

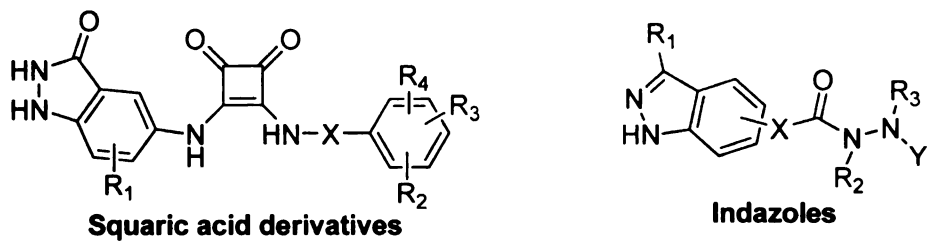
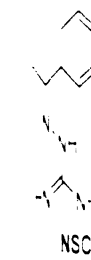


Figure I-8. Squaric acid derivatives and indazoles as Chk2 inhibitors

However, there are more and more reports of novel Chk2 inhibitors developed through high throughput screening of compound collections and combinatorial libraries.[104-110] Pommier *et al.* described a bis-guanylhydrazone (NSC 109555) identified after a high throughput screen of over 100,000



compounds (Figure I-9). The compound was found to be an ATP competitive inhibitor and displayed an IC_{50} of 0.20 μM. When screened against other kinases, including Chk1, NSC 109555 was found to be fairly selective for Chk2 with IC_{50} values for a few other kinases at least 6.5 times that for Chk2.[106]

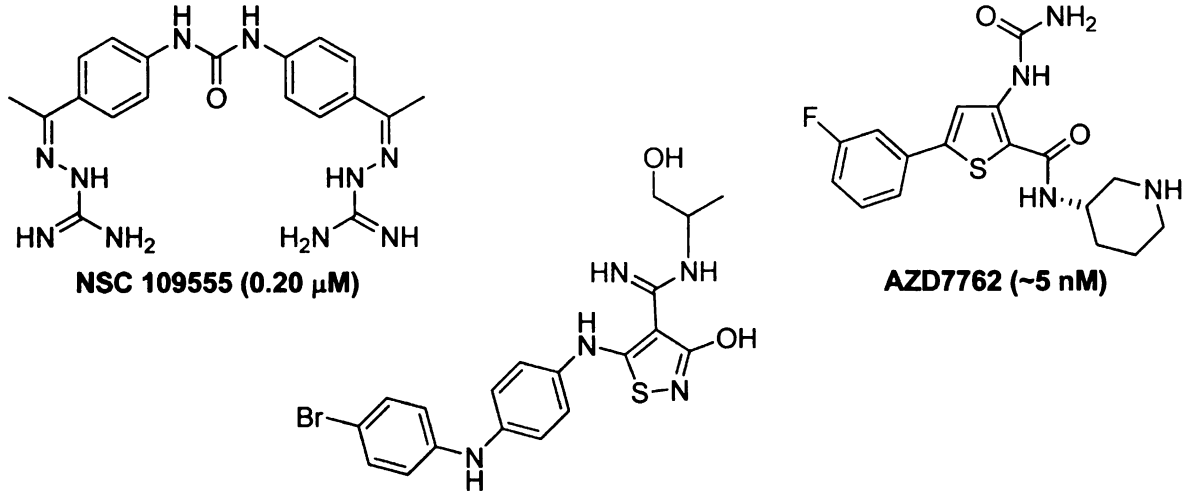


Figure I-9. Chk2 inhibitors from compound libraries

Wu and Carlessi describe a novel and selective inhibitor of Chk2, VRX0466617 (Figure 1-9).[105, 107] A series of isothiazole carboxamidine compounds were found to be potent inhibitors displaying IC_{50} values in the range of 120 nM to 40 μM. These compounds were also found to be ATP competitive inhibitors.[105, 107] Zabludoff and co-workers report a similar looking Chk2 inhibitor, AZD7762 (Figure I-9). The compound was shown to be a potent Chk2 inhibitor with IC_{50} values in the range of 5 nM.[110]

Researchers at Johnson and Johnson also developed a new class of benzimidazoles as potent and selective Chk2 inhibitors. High-throughput screening of purified human Chk2 led to the discovery of a novel series of 2-arylbenzimidazoles that exhibited potent and selective inhibition of Chk2.[104] The

most potent 2-arylbenzimidazole (Figure I-10) gave an IC_{50} value of 2 nM and showed excellent selectivity for Chk2 over 35 other kinases tested. Furthermore, it was shown that the benzimidazole inhibitors displayed effective radioprotection of human T-cells against ionizing radiation with EC_{50} values between 3-7.6 μM.[104, 109]

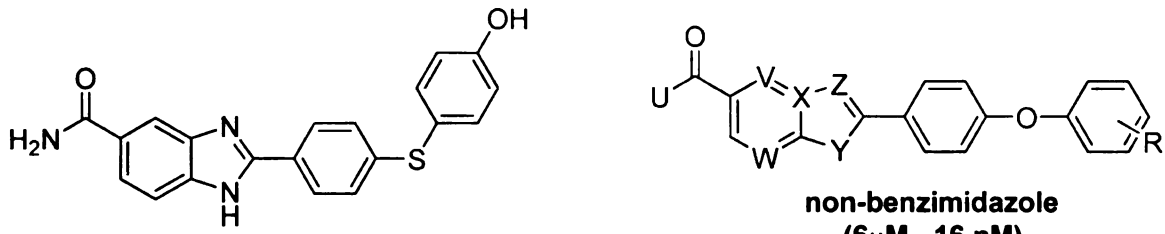


Figure I-10. Benzimidazole and non-benzimidazole Chk2 inhibitors

To help define the scope of the SAR study on the benzimidazole inhibitors, a series of non-benzimidazole Chk2 inhibitors were developed. The IC_{50} values for the more potent new inhibitors range from 5.8 μM to 16 nM.[108] Further studies are needed to determine if these inhibitors are effective at potentiating the cytotoxic abilities of current cancer therapeutics.[3]

Natural products and analogs of natural products have also contributed to the number of Chk2 inhibitors currently known. Staurosporine (Figure I-11) and analogs, such as UCN-01 (Figure I-11) have shown to be potent checkpoint inhibitors, however, the are not very selective for checkpoint kinases.[106, 111, 112] Although UCN-01 displays a potent inhibition of Chk2 (10 nM)[24] and has shown *to* potentiate the anticancer activity of a variety of therapeutics such as cis-platin, mitomycin and ionizing radiation[113-117] and augments the cytotoxicity of temozolomide in human glioblastoma cells[118], there are some concerns associated with UCN-01 such as its promiscuity as a kinase inhibitor. In addition,

UCN-01 contains some pharmacokinetic drawbacks including its strong binding to human plasma protein α_1-acid glycoprotein and low bioavailability.[119-121]

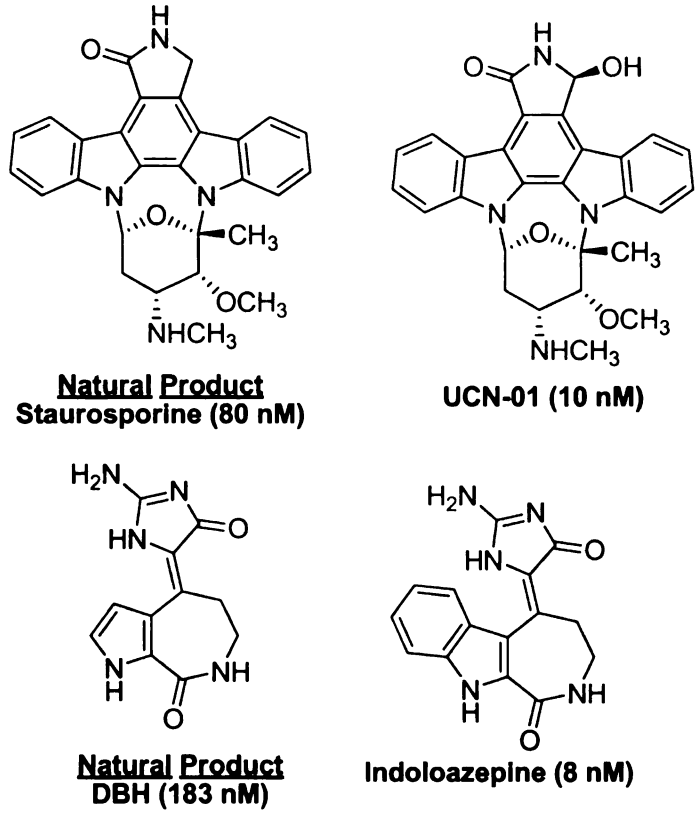


Figure I-11. Natural products and analogs used as Chk2 inhibitors

Debromohymenialdisine (DBH) (Figure I-11) is a natural product that displays moderate IC_{50} values for Chk2 inhibition (3.5 μM (cell culture), 183 nM *in vitro*).[73, 122] However, a low selectivity for Chk2 over other kinases severely limits the use of debromohymenialdisine as a Chk2 inhibitor.[3]

A new analog of debromohymenialdisine, indoloazepine (Figure I-11), was developed in our laboratory and has been shown to be a potent and selective inhibitor of Chk2.[122] It was illustrated that indoloazepine displayed an IC_{50} value of 8 nM for Chk2 kinase inhibition through an *in vitro* assay and was shown to be quite selective against a number of purified kinases including Chk1 (30 fold).[3, 122]

It is with the success of indoloazepine as a potent checkpoint kinase 2 inhibitor that the foundation of my project is built upon.

I.I Chk2 binding pocket properties

With the success of the crystallization of the kinase domain of Chk2 by Oliver and co-workers[99], information regarding the binding pocket can be examined and explored. Oliver *et al.* were also successful in crystallizing debromohymenialdisine inside of the binding pocket allowing a scrutinizing eye to identify possible residues important for binding purposes. It can be seen in Figure I-12 the important binding interactions between DBH and the Chk2 active site.

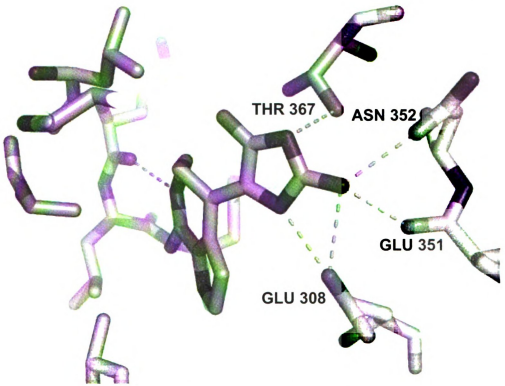


Figure I-12. DBH inside the Chk2 active site

DBH is shown in the center of Figure I-12 and the yellow dashed lines represent hydrogen bonding interactions with the side chains and main chain of Chk2. It is observed that the main interactions that make DBH a fairly potent

inhibitor include the imidazolone moiety, which shows interactions with threonine, arginine and glutamic acid residues. Furthermore, there are additional hydrogen bonding interactions that are present in the southern portion of DBH. Figure I-13 illustrates these interactions and it can be seen that the amide functional group of the seven-membered ring plays a large role in bonding to a glutamic acid and methionine residue along the main chain in Chk2.

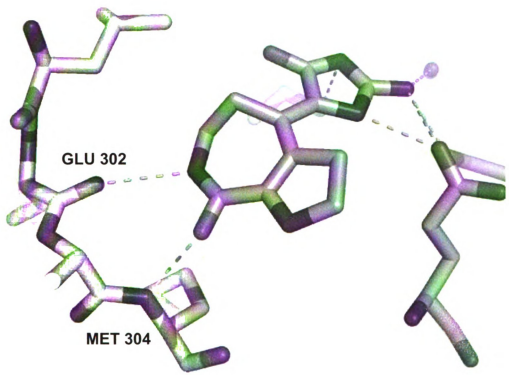


Figure I-13. Interactions between southern portion of DBH and Chk2

Interpreting this information and applying it to the DBH analog, indoloazepine, it can be imagined that indoloazepine may have similar hydrogen bonding interactions within Chk2. In fact, according to Figure I-14, indoloazepine could potentially bind in a similar fashion as DBH. The hydrogen bonding interactions between the imidazolone ring of indoloazepine and the residues

surrounding it could interact to help hold indoloazepine in the active site. Furthermore, Figure II-14 also shows that indoloazepine could have the same interactions between the amide of the seven membered ring and the same residues that were proposed to hold DBH inside the active site.

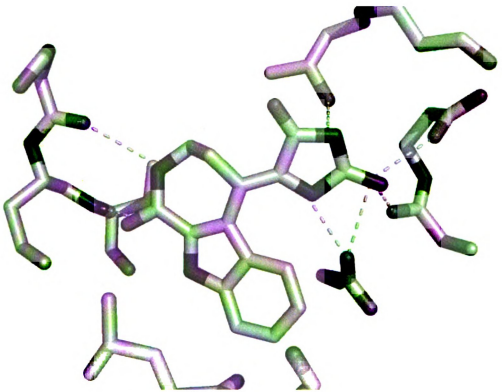


Figure I-14. Indoloazepine modeled inside the Chk2 binding pocket

Although the exact nature of the binding of indoloazepine and the active site of Chk2 is not known, it can be hypothesized based on the known binding properties of a very closely related structure (DBH). Furthermore, we can use this information to rationalize why it is thought that a new indole alkaloid, the focal point of my research, could also be a potential Chk2 inhibitor. As shown previously in Figure I-4, the point at which drug leads have been identified can lead to a rational design approach, which feeds back into the natural product tank

to uncover new natural products of interest as well as a new library of compounds.

The success of indoloazepine as a Chk2 inhibitor has led to a reevaluation of another natural product, shown in Figure I-15. This indole alkaloid possesses some structural features that are similar to that of indoloazepine. As a result, it warranted further examination to determine its potential inhibition of Chk2.

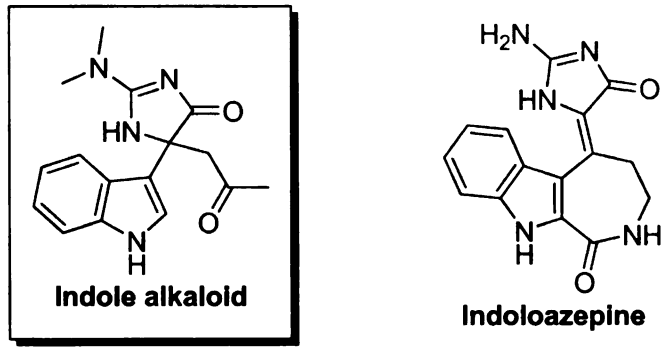


Figure I-15. New indole alkaloid and indoloazepine

At first glance the structural similarities between the indole alkaloid and indoloazepine may not be obvious, besides the fact that they both contain an imidazolone ring and indole moiety. However, after some calculations using Spartan Pro®, it can be seen that there are some more important correlations. As illustrated in Figure I-16, the dihedral angle between the plane containing the imidazolone ring and the plane containing the indole ring for indoloazepine is calculated to be 50°. Furthermore, according to the crystal structure of the indoloazepine analog where the indole nitrogen is methylated, the dihedral angle is 56°. When examining the new indole alkaloid, it was determined that the dihedral angle between the imidazolone ring and indole is also very similar with an angle of 54°. It is important to remember that spatial arrangements inside the

binding pocket are very important when considering how the molecule fits into the active site. More importantly, the imidazolone moiety seemed to be very important to the binding of DBH and indoloazepine considering the number of hydrogen bonding interactions that were present.

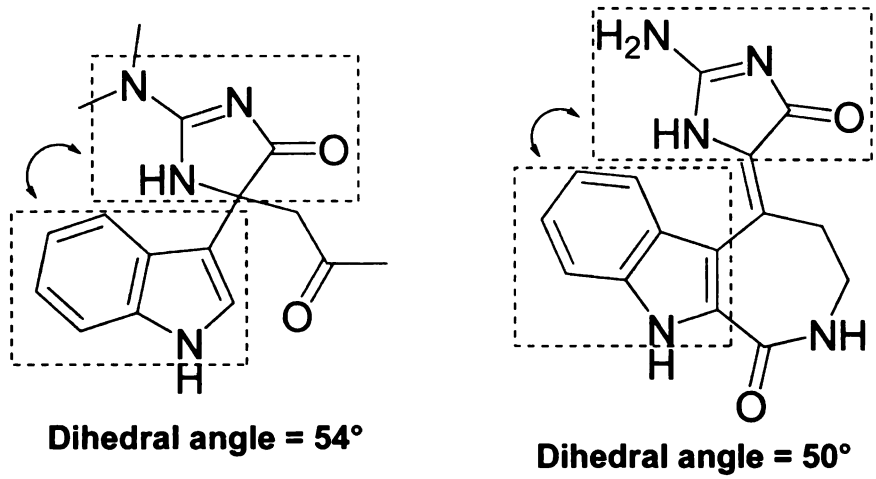


Figure I-16. Comparison of dihedral angles

Further analysis of both compounds using Spartan Pro® revealed that the carbon connected to the indole ring (at position 3) for both compounds were surprisingly similar even though the two compounds contained different hybridizations at that carbon. It is shown in Figure I-17 that the hybridization of indoloazepine at carbon 3 of the indole ring, although technically sp^2 hybridized, actually exhibits more of a sp^3 hybridization, possibly due to the partial single bond character that the bond has as a result of the donating nature of the indole nitrogen's lone pair of electrons. The angle at the indicated carbon of indoloazepine was calculated to be 111°, while the angle at the carbon on position 3 of the indole from the new indole alkaloid was calculated to be 110°.

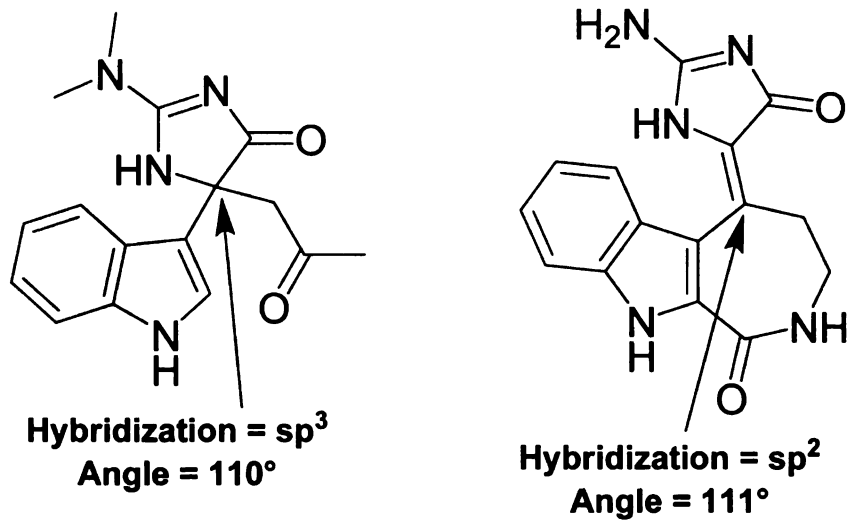


Figure I-17. Hybridization of carbon connected to indole ring

Placing the new indole alkaloid inside the binding pocket of Chk2 (Figure I-18) reveals that there is potential for the imidazolone ring of the alkaloid to hydrogen bond to the same residues responsible for binding DBH and possibly indoloazepine. Although the indole alkaloid lacks the amide functional group that seems to play a role in the binding of DBH and indoloazepine, it does contain a ketone, which was thought to potentially interact with a lysine residue to form a Schiff base and result in a covalent bonding interaction, possibly allowing for a more potent inhibition.

It is understood that these potential interactions and reason for possible potency are purely speculative. There is no definite way to know exactly which compounds are active and which are not. Our hypothesis is that this natural product has the potential and structural features that may allow it to be a checkpoint kinase 2 inhibitor. Thus, our hypothesis is the impetus for my project, which is focused on the new indole alkaloid and its potential biological activity.

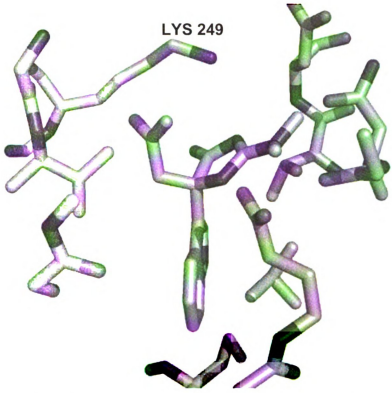


Figure I-18. New indole alkaloid modeled in Chk2 binding pocket

I.J Goal of project

The main goal of my project includes the synthesis of the natural product. Furthermore, it was thought that the synthesis of the natural product could be obtained through an oxazolone intermediate, a scaffold that our laboratory has explored quite extensively (Figure I-19).[123-135] It was thought that new methodology could be developed extending our chemistry to grant access to quaternary imidazolones, the core of the natural product. Additional goals were to synthesize analogs of the natural product, as well as other heterocyclic compounds that could be tested for Chk2 activity. The following chapters elaborate on the development of a new synthetic method that gives access to the quaternary imidazolone scaffold, the first total synthesis of the indole alkaloid and two analogs and the biological evaluation of the indole alkaloid, analogs and other heterocyclic compounds synthesized for testing.

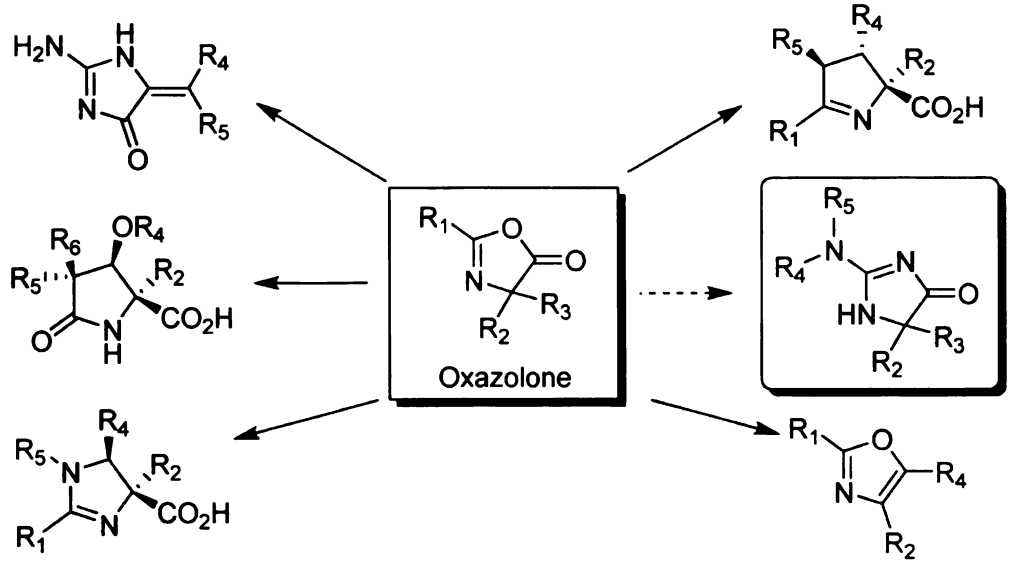


Figure I-19. Oxazolone chemistry affording various heterocycles

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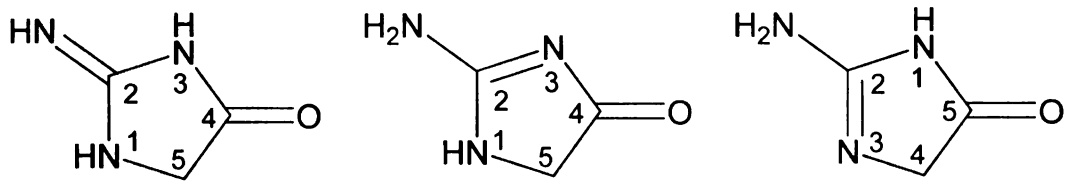
CHAPTER II

DEVELOPMENT OF A SYNTHETIC METHOD FOR THE PREPARATION OF 2-AMINO-5, 5-DISUBSTITUTED-1H-IMIDAZOL-4(5H)-ONES

II.A Nomenclature and numbering of imidazolones

The general structure for a 2-amino-imidazolone is shown in Figure II-1. It can be observed that there are actually three tautomeric forms for a 2-amino-imidazolone where the double bond is switched between the three nitrogens making up the guanidine portion of the heterocycle. The preferred tautomeric form for the imidazolone was shown by Matsumoto and co-workers[1] and in a different set of experiments by Kenyon and co-workers[2] to be tautomer **II-2**. Traditional nomenclature for these types of molecules encountered in literature has varied,[3] with names such as 2-amino-imidazol-4-ones (2-amino-imidazolone), glycocyamidines and 2-iminohydantoins being some of the more recent common terms. The numbering of the ring system varies depending on the tautomer specified as shown in Figure II-1.[3] When the double bond is exocyclic as in the case of **II-1** or when the double bond is endocyclic and in conjugation with the carbonyl (**II-2**), the numbering of the ring begins with the nitrogen furthest from the carbonyl and is given position 1. Numbering the ring clockwise from that point will result in the second nitrogen contained in the ring being in position 3, the carbonyl carbon in position 4 and the methylene carbon in position 5.[3]

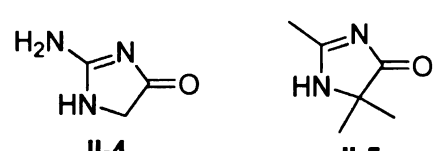


II-1 **II-2** **II-3**

Figure II-1. Numbering of the 2-amino-imdazol-4-one ring system

However, when the double bond is endocyclic and not in conjugation with the carbonyl (**II-3**), the numbering of the ring system starts with the opposite nitrogen in the ring. A counter-clockwise numbering scheme follows which puts the second nitrogen in position 3, the methylene in position 4 and the carbonyl carbon in position 5.[3]

When an amino group is not present in the 2 position, the structure is still considered an imidazolone, however, it simply contains a different substitution at the 2 position. For the purposes of consistency, the term imidazolone will be used for the nomenclature of the general ring structure. When substitution of the imidazolone ring is present, the numbering scheme will follow that for structures **II-1** and **II-2**, which is the most commonly accepted numbering format.[3] In addition, the name of the substituent will follow the number at which it is found. For example, structure **II-4** in Figure II-2, would have the name 2-aminoimidazolone where structure **II-5** (also in Figure II-2) would have the name 2, 5, 5-trimethylimidazolone.



II-4 **II-5**

Figure II-2. Representative structures of imidazolones

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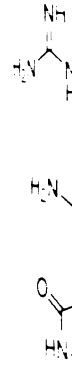

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II.B Reactivity of imidazolones

The 2-aminoimidazolone ring system can undergo a number of reactions in a variety of locations such as at the carbonyl carbon, alpha to the carbonyl and at the guanidine nitrogens (Figure II-3).[3-7]

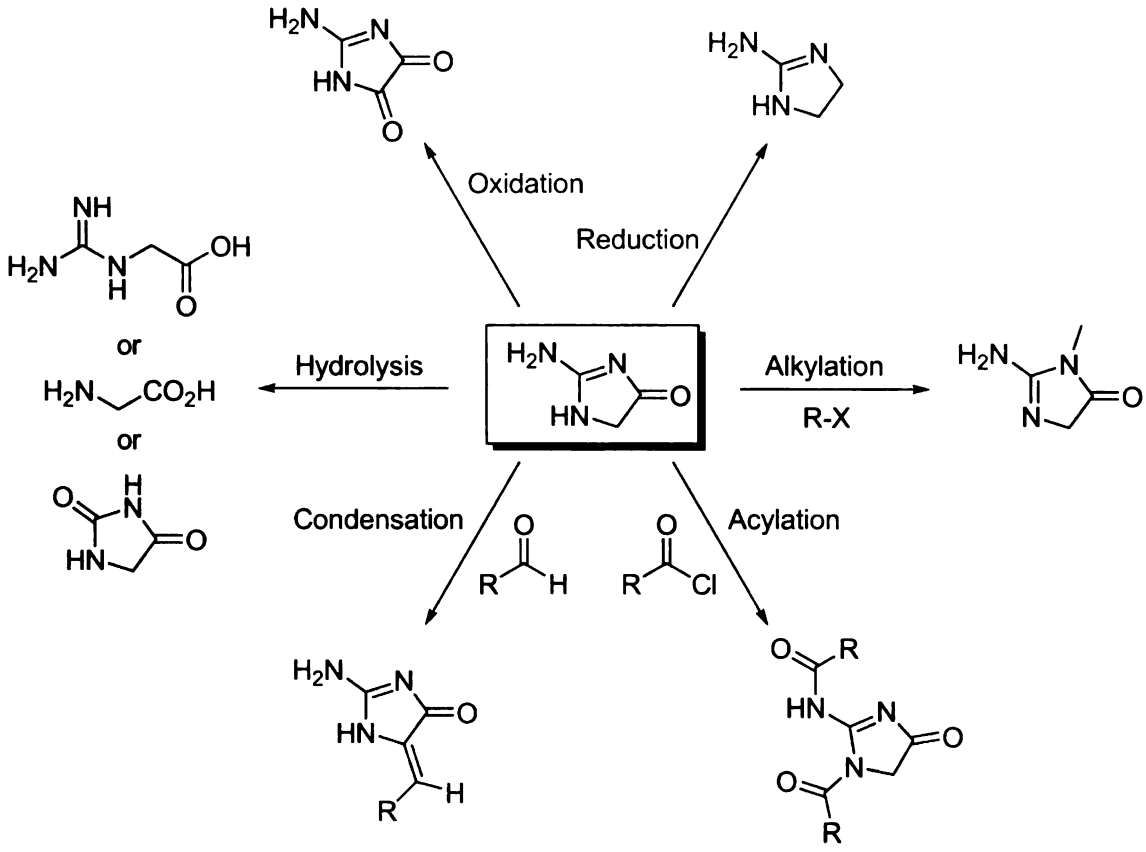
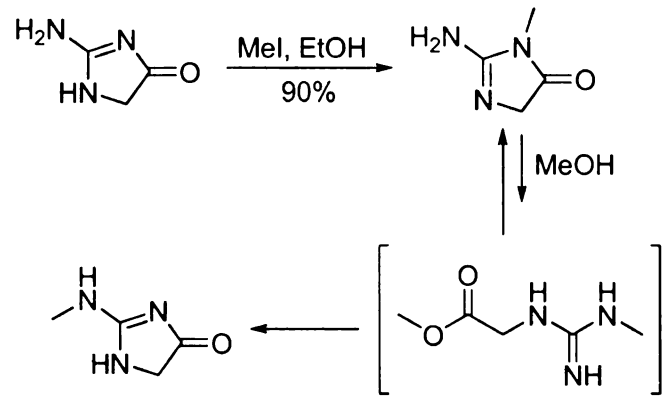


Figure II-3. Reactions of 2-aminoimidazolone

Alkylation of imidazolones using alkyl halides such as methyl iodide occurs at N-3 primarily, although if N-1 and N-3 are already alkylated, N-2 can be alkylated.[2] It was shown by Kenyon and co-workers that the nitrogen in position 3 is more reactive and the alkylated product can undergo a reversible rearrangement to put the double bond back into conjugation with the carbonyl, affording N-2 bearing the alkyl group (Scheme II-1).[2]

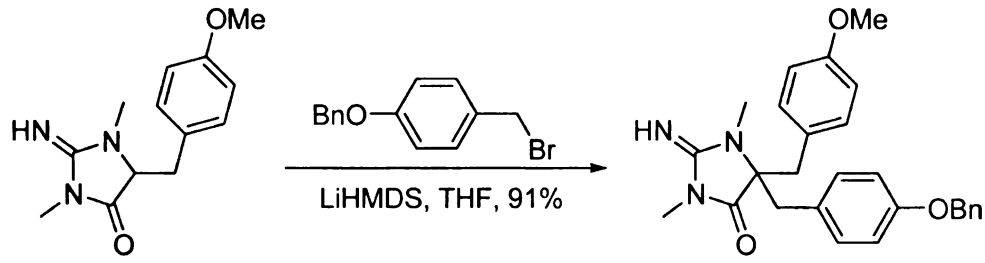
Scheme II-1. Alkylation of 2-aminoimidazol-4-one



Further support for N-3 being the most basic nitrogen, and hence most nucleophilic, comes from a study by Reddick and co-workers where they identified via ^{15}N-NMR that the nitrogen in position 3 is primarily protonated at a low pH.[8] Acylation reactions occur in a similar fashion.[3, 5] Imidazolones can also undergo a condensation reaction with aldehydes producing a 5-arylidene imidazolone (Figure II-3).[3, 4, 9-12] The success of the reaction is dependent on the nucleophilic character of the imidazolone and the electrophilic character of the aldehyde. Furthermore, the reaction often produces a mixture of *E*/*Z* isomers, some of which can isomerizes to one other depending on the product and conditions.[3, 4]

Alkylation is also possible at carbon 5 of an 2-aminoimidazolone as shown by Danishefsky and co-workers (Scheme II-2).[13] The alkylation was completed using a imidazolone core similar to **II-1** (Figure II-1) and a benzyl bromide giving the quaternary imidazolone in 91% yield.[13]

Scheme II-2. Alkylation at C-5 of an imidazolone



Hydrolysis of the 2-aminoimidazolone ring can lead to a variety of products depending on the reaction conditions (Figure II-3).[3, 4, 6] Cleavage of the amide bond of the heterocyclic ring can lead to products that resemble a glycine derivative with a guanidine moiety instead of a primary amine. It has also been observed that under certain conditions, the imidazolone ring can hydrolyze at a different position and form urea and glycine.[3] Lastly, hydrolysis can also transform the 2-aminoimidazolone into the hydantoin analog under basic conditions.[4, 6]

Oxidation of the methylene group at position 5 of the heterocyclic ring is possible and the resulting dicarbonyl compound is formed (Figure II-3). However, these reactions are often run under alkali conditions and the resulting dicarbonyl heterocycle undergoes further chemical modifications and is known to hydrolyze giving the oxalate and derivatives of guanidine.[3] Reduction of the carbonyl at position 5 can occur using hydrogenation under a platinum catalyst. The typical reaction conditions include 50 mol% of a platinum catalyst and a hydrogen pressure of 15-35 psi. The result of this reaction is the formation of cyclic guanidine structures (Figure II-3).[7]

The acidity of imidazolones have been studied by a few different groups.[1-3, 14] Chandrasekhar and co-workers compared the relative rates of a base

catalyzed deuterium exchange for an imidazolone, oxazolone and pyrrolone. It was found that the rate of deuterium exchange was greatest for the imidazolone and worst for the pyrrolone (Figure II-4),[14] leading to conclude that based on these results the imidazolone is more acidic than the other analogs tested.

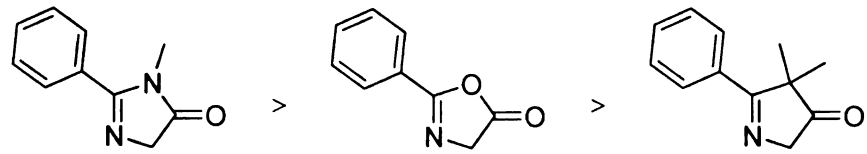


Figure II-4. Relative rates of deuterium exchange

The pKa's of multiple 2-aminoimidazolone hydrochloride compounds have been identified initially by Matsumoto and co-workers and then to a greater extent by Kenyon and co-workers.[1, 2] It was found that the pKa's of the protonated imidazolones range from 4.48-9.01 (in H_2O) depending on the substitution on the heterocyclic ring. There is a clear distinction between the pKa's of imidazolones that contain the double bond in conjugation with the carbonyl group and those that do not, such as between **II-8** and **II-9** (Figure II-5).[2]

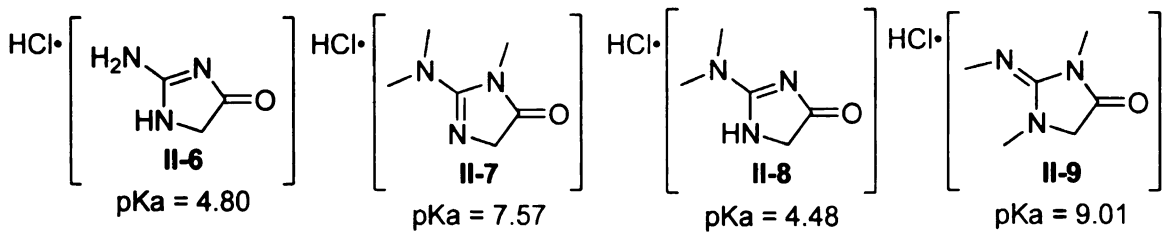


Figure II-5. pKa's of differently substituted 2-aminoimidazolones

II.C Natural products containing the imidazolone core

There are many accounts of natural products that contain the imidazolone core in a variety of different forms.[15-28] One of the most common imidazolones is an end product of nitrogen metabolism in the human body, a compound called

creatinine (Figure II-6).[8] Hymenialdisine is a natural product that contains the 2-aminoimidazolone core with an alkenic substitution at position 5 of the heterocyclic core (Figure II-6). The compound comes from the sponges *Axinella verrucosa* and *Acanthella aurantiaca* and was isolated in 1982[18] and first synthesized in 1995.[29] De-bromohymenialdisine, an analog of hymenialdisine without the bromine on the pyrrole ring, also contains the imidazolone core.

Dispacamide 1 is a novel bromo pyrrole alkaloid that was isolated from a Caribbean sponge in 1996[16] and was synthesized in 1997.[30] Debromodispacamides B and D were isolated from the marine sponge *Agelas mauritiana* and synthesized in 2008[27] , while the related compounds polyandrocarpamines A and B were isolated from the ascidian *Polyandrocarpa sp.* and synthesized in 2002.[19] All of the dispacamide and polyandrocarpamine compounds exhibit the same type of imidazolone core that hymenialdisine possesses, the 2-aminoimidazol-4-one core with an alkenic substitution pattern at carbon 5 (Figure II-6).

The last members of natural products that contain this type of heterocyclic core are the leucettamines B and C and leucettamidine (Figure II-6). Leucettamine B and leucettamidine were isolated from the sponge *Leucetta microraphis* in 1993,[17] while leucettamine C was isolated from *Leucetta avocado* in 2003.[31] To date, only leucettamine B has been synthesized with the first synthesis reported in 1994.[32]

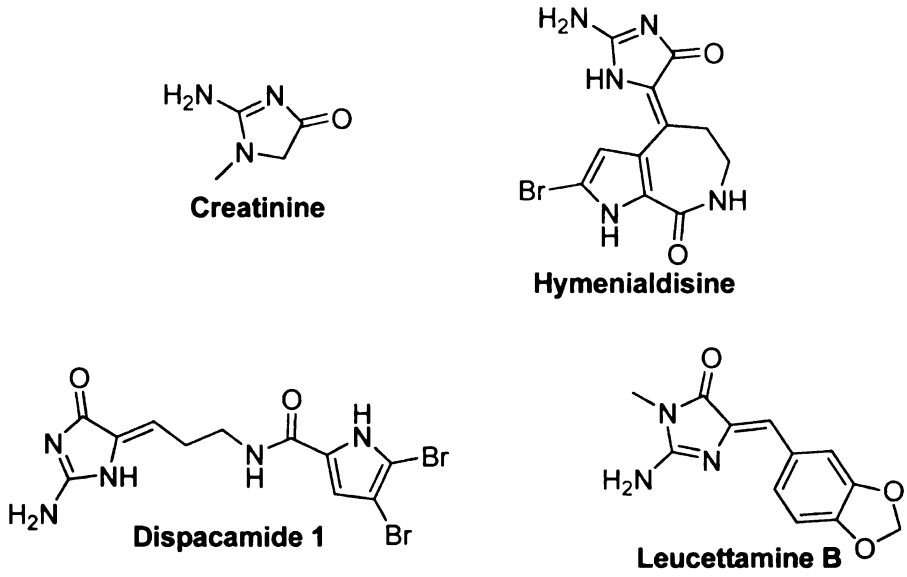


Figure II-6. Natural products containing the 2-aminoimidazolone core

Natural products that contain the imidazolone core without the 2-amino substitution have also been reported.[15, 23, 25] Rhopaladins A-D (Figure II-7) contain a unique imidazolone core consisting of an alkenic substitution at carbon 5 and an acyl group at carbon 2. These natural products come from the Okinawan tunicate *Rhopalaea sp.* and were isolated in 1998.[25] The first synthesis of rhopaladin D was completed in 2000[33], while the total synthesis of rhopaladins A-C were completed shortly after in 2002.[34]

Kottamides A-D (Figure II-7) are structurally unusual due to the quaternary substitution pattern on carbon 2. These natural products are optically active, were isolated from the New Zealand ascidian *Pycnoclavella kottae* in 2002 and have not been synthesized to date.[15] The last example of a natural product that has the imidazolone core but lacking the 2-amino functionality is a novel bis(indole) alkaloid that was isolated from the sponge Dragmacidon sp. in 1996. The structure of this compound (Figure II-7) is very unique with a quaternary

center at carbon 5 and has also not been synthesized to date. Due to the light absorbing tendencies of the compound in solution, chiroptical data was hard to establish.[23]

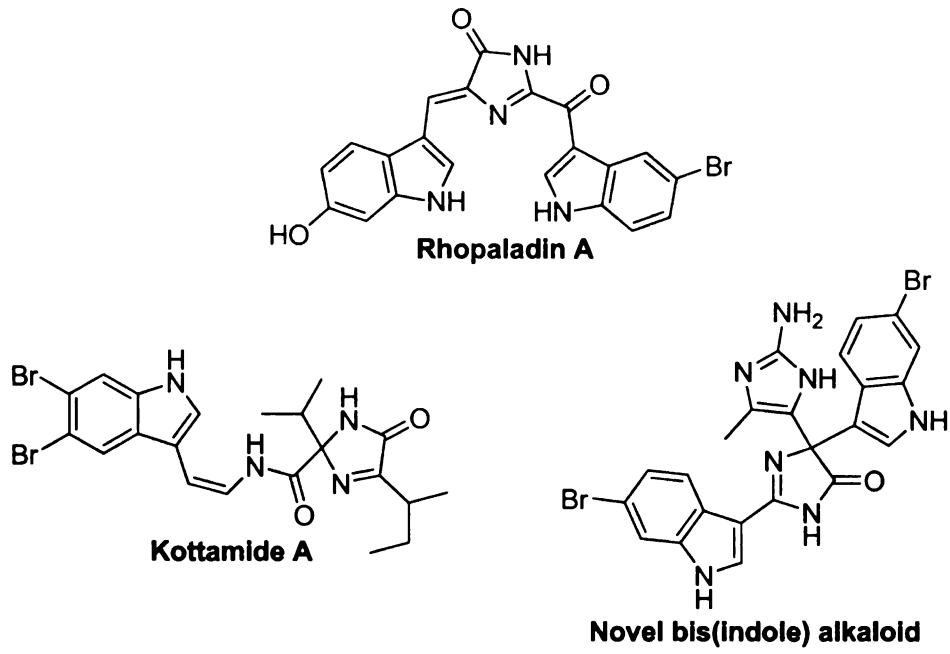
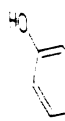


Figure II-7. Natural products containing an imidazolone core

The next group of natural products contains the imidazol-4-one heterocyclic core and also the 2-amino functionality. However, these compounds do not contain the alkenic substitution pattern at carbon 5 as those in Figure II-8. Instead, most of these natural products contain a quaternary center in the imidazolone cycle. Compound 2096A (Figure II-8) and an analog 2096B, a diastereomer of 2096A, were isolated in 2000 from extracts of Streptomyces sp.[28] It is found that these two compounds can interconvert due to the acidity of the proton at the quaternary center of the bicyclic heterocycle and as a result, the stereochemistry is not defined. Additionally, the compounds were found to decompose readily in aqueous solutions making the synthesis of these



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compounds a challenge.[28] To date, there are no reported syntheses of 2096A or B.

Oxysceptrin (Figure II-8) and related alkaloids have been isolated from the sponge *Agelas conifera* in 1991.[35, 36] Oxysceptrin contains an unique cyclobutane core flanked by imidazole, pyrrole and imidazolone appendages. The synthesis of oxysceptrin as well as other alkaloids from the same family was completed by O'Malley and co-workers in 2007.[37]

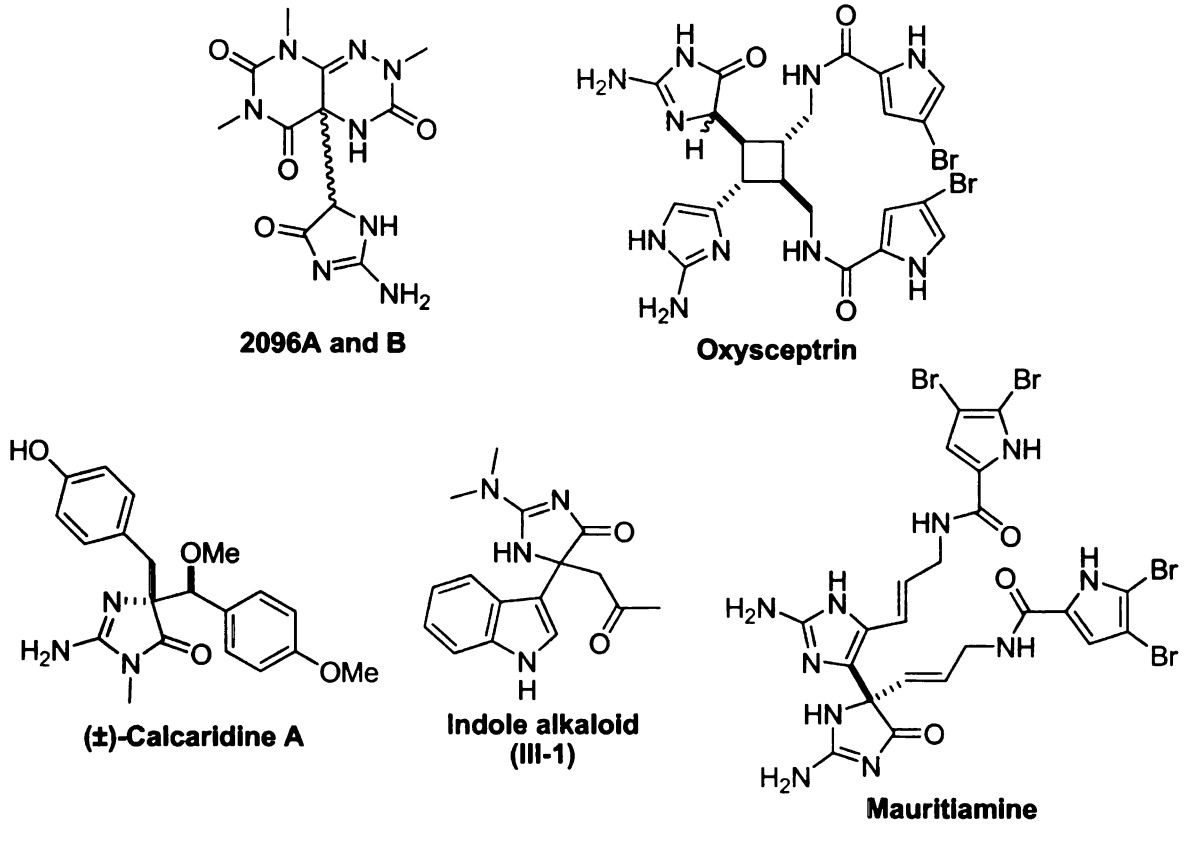


Figure II-8. Additional natural products containing 2-aminoimidazolone

Another natural product related to oxysceptrin is mauritiamine, a compound with a quaternary imidazolone containing imidazole and pyrrole appendages at position 5 (Figure II-8). The natural product comes from the marine sponge *Agelas mauritiana* and was isolated in 1996. Furthermore, the

natural product contains a quaternary center but is optically inactive and was isolated at a racemate.[26] The first racemic synthesis of mauritiamine was completed by Olofson and co-workers in 1997 utilizing an oxidative dimerization of two imidazole containing intermediates.[38]

Calcaridine A (Figure II-8) was isolated from a Fijian sponge *Leucetta* sp. in 2003.[20] The natural product contains a uniquely substituted 2-aminoimidazol-4-one core with a quaternary center at carbon 5. The natural product is optically active although the absolute stereochemistry is not yet defined.[20] The first racemic total synthesis of calcaridine A was completed by Koswatta and co-workers in 2008 using a novel rearrangement to afford the quaternary stereocenter.[39]

The last natural product that contains the 2-aminoimidazol-4-one core and also a quaternary center at carbon 5 is the indole alkaloid (**III-1**) shown in Figure II-8. It was isolated from the tunicate *Dendrodoa grossularia* in 1998 and the stereochemistry at the quaternary carbon is also not defined.[22] Further detail on this molecule is given in chapter III. The synthesis of this natural product is the goal of my project and thus a synthetic route to form the uniquely substituted quaternary imidazolone is needed.

II.D Synthetic routes to imidazolones

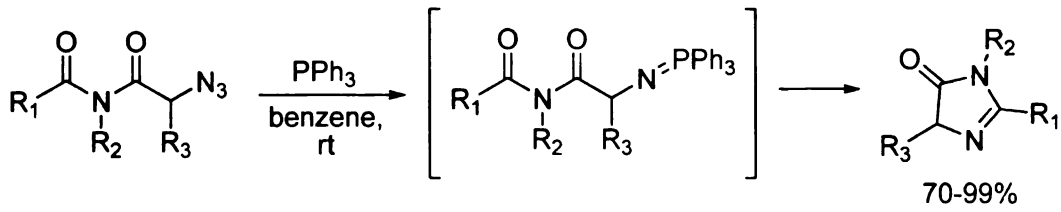
Imidazolones with and without a quaternary center at position 5 have been synthesized in a variety of ways.[39-73] The following section illustrates the various routes that have been used previously to obtain imidazolones as well as the routes that were attempted to create quaternary imidazolones for synthesizing

the indole alkaloid, the focal point of my project. Finally, the new synthetic method developed for gaining access to the quaternary imidazolone scaffold needed for the total synthesis of the indole alkaloid will be discussed.

II.D.1 Synthetic routes to imidazolones: Aza-wittig reaction

The first route that will be discussed is the synthesis of imidazolones via an intramolecular aza-wittig reaction (Scheme II-3). Takeuchi and co-workers developed an efficient aza-wittig reaction which reacts an azide and triphenylphosphine to obtain an intermediate that attacks the carbonyl of the imide to produce imidazolones in good yields.[69] They were able to use alkyl, aryl and heteroaryl groups at R_1; alkyl and aryl groups in the R_2 position; and hydrogen and aryl groups in the R_3 position. The reaction proceeds at room temperature in two hours and produces yields ranging from 70-99%.[69]

Scheme II-3. Aza-wittig reaction to produce imidazolones

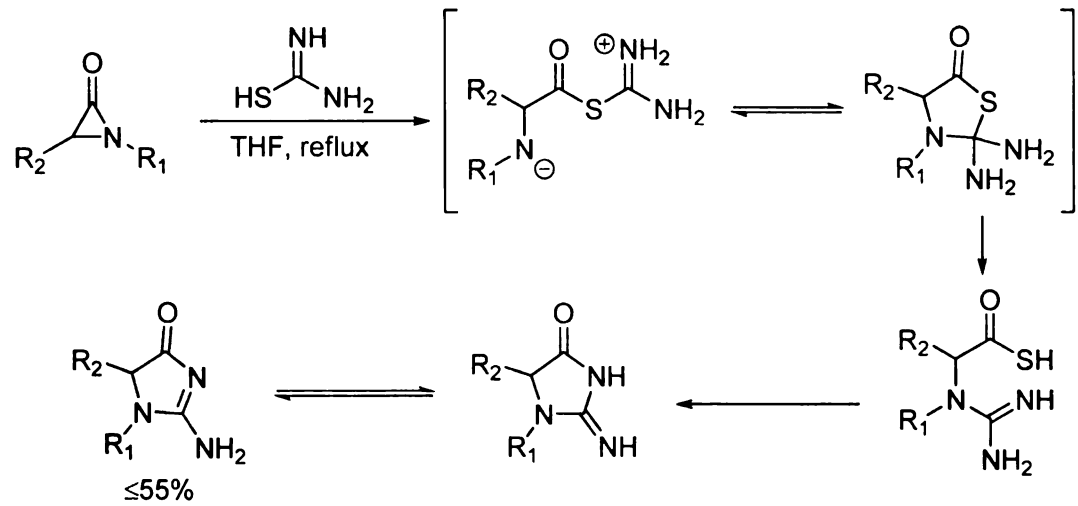


II.D.2 Synthetic routes to imidazolones: Reaction of aziridinone and thiourea

Talaty and co-workers use a reaction between an aziridinone and a thiourea to produce imidazolones in yields up to 55% (Scheme II-4).[70] The aziridinones were substituted at the R_1 and R_2 position with either a *tert*-butyl or adamantly substituent and reacted with a thiourea to attack the carbonyl of the aziridinone. After the ring opening of the aziridinone it is proposed that the reaction eventually produces a guanidine thioester intermediate.[70] After a final

attack of the guanidine on the thioester, the imidazolone product is proposed to be formed.[70]

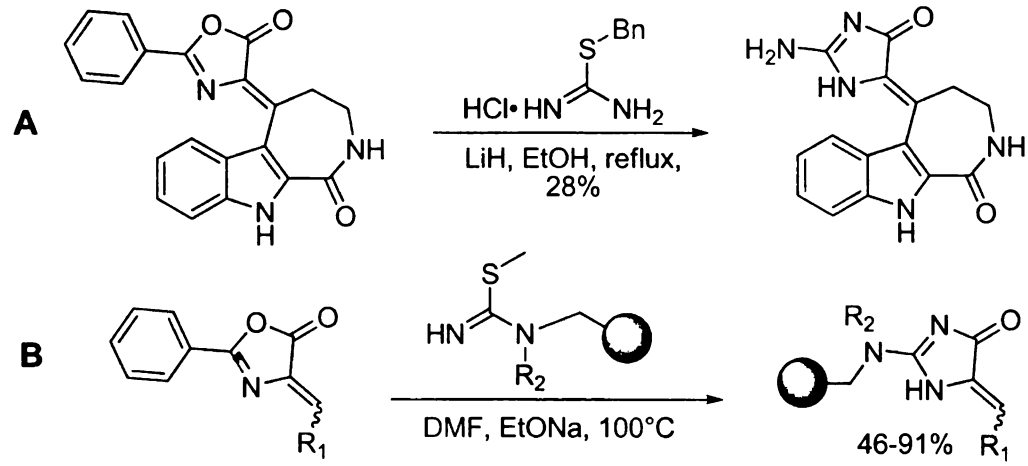
Scheme II-4. Reaction of aziridinones and thiourea to form imidazolones



II.D.3 Synthetic routes to imidazolones: Oxazolone to imidazolone

Oxazolones have been used to produce imidazolones using a thiourea as well (**A**, Scheme II-5).[49, 64, 65] Tepe and co-workers found that by treating oxazolones with a thiouronium salt under basic conditions, they were able to isolate 28% of the corresponding imidazolone.[64, 65] Flygare and co-workers developed a solid-phase synthesis of imidazolones using the same type of chemistry giving yields ranging from 46-91% yields (**B**, Scheme II-5).[49] The oxazolone consisted of aromatic R_1 substituents while the thiourea contained alkyl and benzyl R_2 substituents.[49]

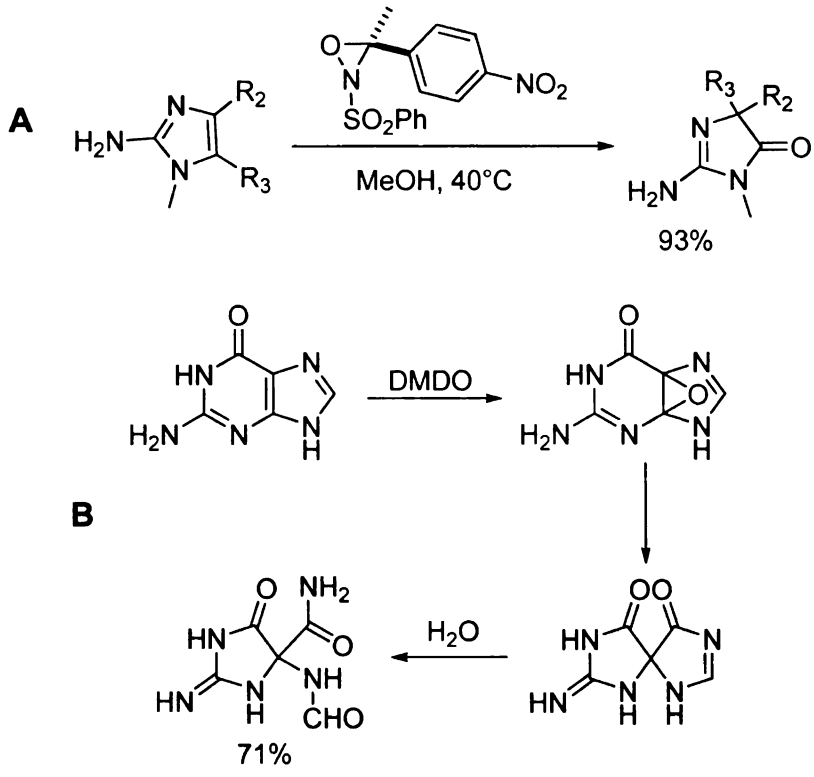
Scheme II-5. Imidazolones using oxazolones and thioureas



II.D.4 Synthetic routes to imidazolones: Oxidation of imidazoles

An oxidative rearrangement of imidazoles is also known to produce imidazolones.[39, 61, 67, 68, 73, 74] Lovely and co-workers used this type of rearrangement recently in the racemic total synthesis of calcaridine A as the key step in forming the quaternary imidazolone core. The reaction (**A**, Scheme II-6) utilized the appropriate benzyl groups in the R_2 and R_3 position and the yield was around 93%, containing a mixture of diastereomers.[39] The nucleobase guanine has also been reported to undergo a similar rearrangement under oxidative conditions.[61, 67, 68, 73] Ye and co-workers report that DMDO can oxidize guanine and produce a spiro imidazolone intermediate. Subsequent hydrolysis of the spiro imidazolone affords a final quaternary imidazolone in 71% yield (**B**, Scheme II-6).[73]

Scheme II-6. Oxidation of imidazoles to form imidazolones

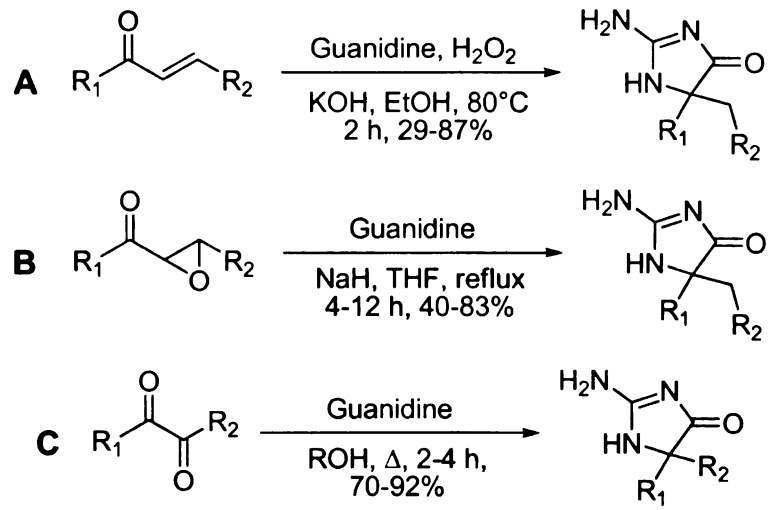


II.D.5 Synthetic routes to imidazolones: Diketone rearrangement

Chalcones, α,β-unsaturated ketones, α,β-epoxy ketones and 1,2-diketones have been reported to undergo a rearrangement with guanidine and substituted guanidines to form imidazolones.[40-43, 62, 71] Darvas and co-workers reported that α, β-unsaturated ketones (R_1 and R_2 = aryl or heteroaryl) rearrange with guanidine to form imidazolones in yields ranging from 29-87% (**A**, Scheme II-7).[71] Dhar *et al.* reported that a similar reaction occurs with α, β-epoxy ketones (R_1 and R_2 = aryl) under basic conditions with yields between 40-83% (**B**, Scheme II-7).[43] The rearrangement of 1,2-diketones with guanidines to form imidazolones has been studied quite thoroughly.[40-42, 62, 63, 75, 76] Nishimura reported the formation of imidazolones from the reaction of diketones with guanidine and 1, 1-disubstituted guanidines with yields ranging from 70-92% (**C**,

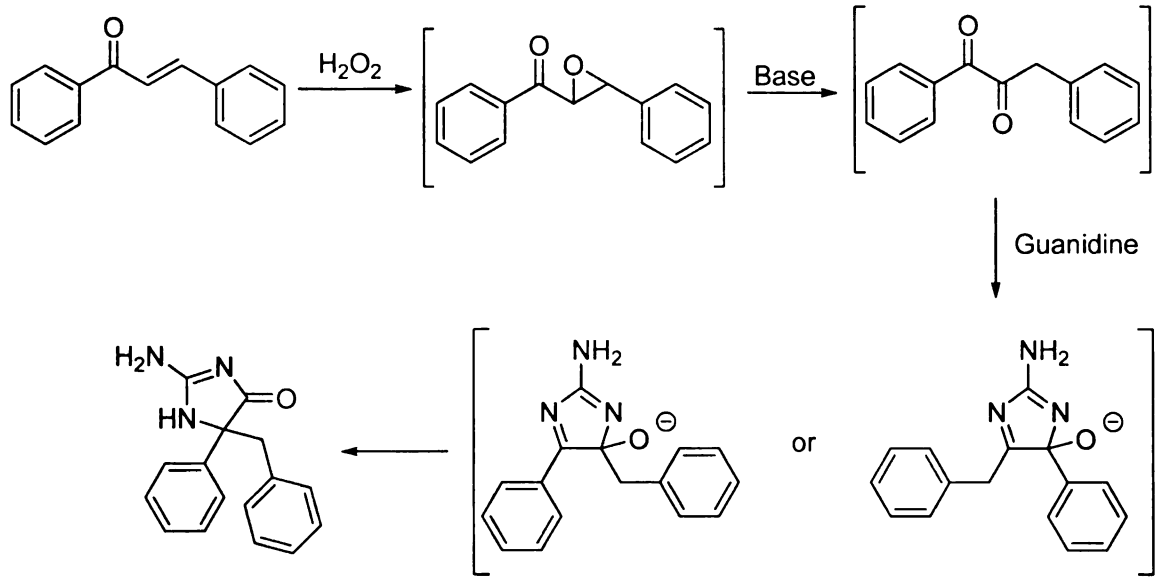
Scheme II-7).[62] Like the previous examples, R_1 and R_2 were mostly aryl.[62] However, when R_1 = methyl the rearrangement failed to produce the imidazolone and made a deep colored solution, which was thought to have gone through a completely different reaction.[62, 75]

Scheme II-7. Imidazolones from 1, 2-diketone and guanidine



The proposed mechanism of the reaction between α,β-unsaturated ketones, α,β-epoxy ketones and 1,2-diketones with guanidine is related to each other based on the intermediates proposed to be formed throughout the mechanism.[43, 62, 71] It is proposed that when the starting material is an α,β-unsaturated ketone, the mechanism starts with the formation of an α,β-epoxy ketone using hydrogen peroxide. Next, a base mediated rearrangement occurs from the epoxy ketone to afford a 1,2-diketone.[77-81] Subsequently, the guanidine then reacts with a diketone to produce intermediates that rearrange to the final imidazolone (Scheme II-8).[43, 62, 71]

Scheme II-8. Proposed mechanism for diketone rearrangement

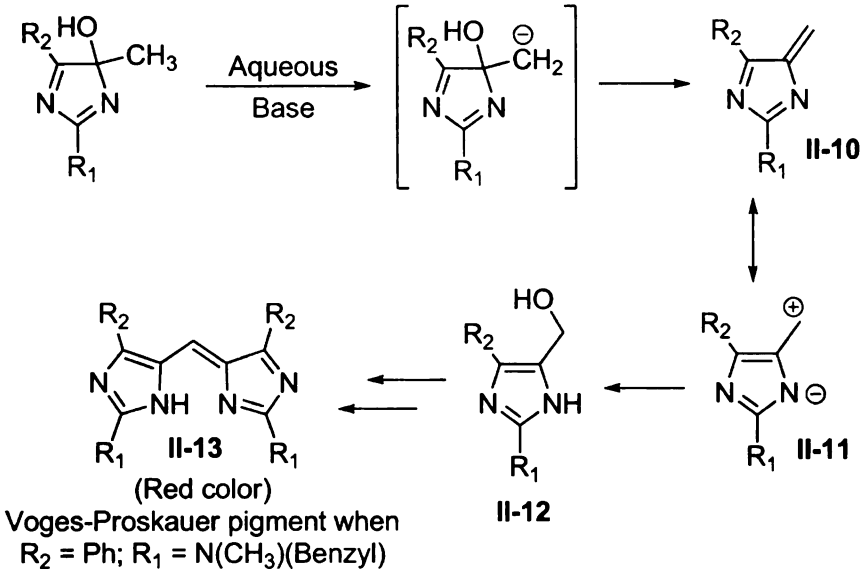


When the starting material is an α,β-epoxy ketone, the mechanism is truncated and begins with the rearrangement of the epoxy ketone to a 1,2-diketone and continues on as shown in Scheme II-8. Furthermore, when starting with the 1,2-diketone, the rearrangement occurs after the guanidine forms the heterocyclic intermediate with the diketone. In summary, whether the starting material is an α,β-unsaturated ketone, α,β-epoxy ketone or 1,2-diketone, the reaction mechanism consists of similar intermediates and simply begins at different points in the mechanism shown in Scheme II-8.

Alternatively, it is proposed by Nishimura that when an alkyl group is used in the rearrangement (R_1 = methyl; see **C**, Scheme II-7) a different reaction occurs (Scheme II-9).[62, 75] It is thought that instead of rearranging to the imidazolone, the methyl group is deprotonated under the basic conditions leading to intermediate **II-10** (Scheme II-9). After addition of water to form **II-12**, it is surmised that the imidazole is transformed into a diimidazole compound (**II-13**)

similar to the Voges-Proskauer pigment, which is red in color.[75] Although the exact mechanism of this transformation is not fully understood, it offers an explanation to the red color observed when Nishimura attempted the rearrangement with a methyl substituent.

Scheme II-9. Alternative mechanism for diketone rearrangement



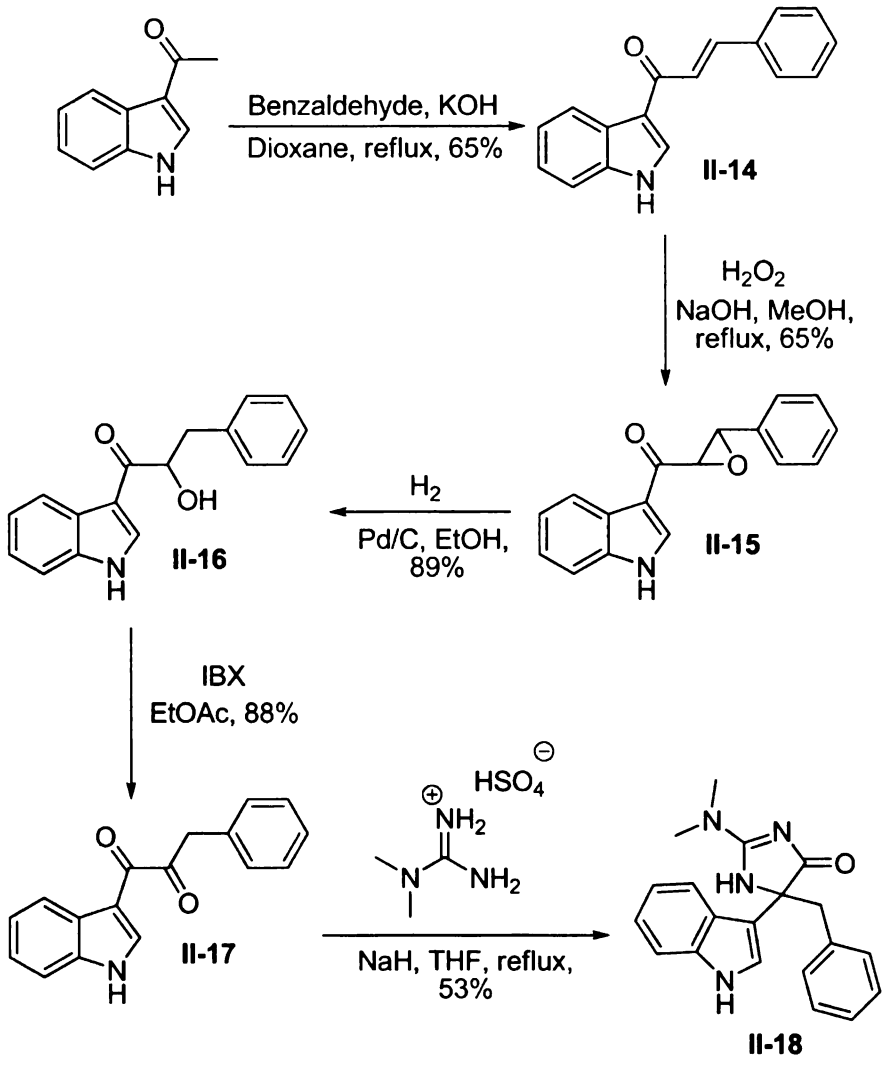
Although the 1, 2-diketone rearrangement had not been successful with substitutents other than aryl, benzyl or heteroaryl, I proposed that this rearrangement would, at the very least, provide a relatively quick access to analogs of the natural product (**III-1**). Optimistically speaking, I hypothesized that there was a possibility that the rearrangement could even afford the correct quaternary imidazolone scaffold needed for the natural product. As a result of the natural product containing an indole group at the quaternary center it was decided to first independently synthesize an analog with an indole and benzyl substitution. Having the benzyl substituent already determined to be compatible

with the rearrangement, the success of the reaction to the corresponding imidazolone would indicate that the indole moiety is also compatible.

II.D.5.a Synthetic routes to imidazolones: Using diketone rearrangement to produce imidazolone analogs of natural product

Scheme II-10 outlines the synthetic route used to create the 1,2-diketone with an indole and benzyl substitution pattern. An aldol reaction between 3-acetyl indole and benzaldehyde under basic conditions yielded the α, β-unsaturated ketone **II-14** in a 65% yield.[82, 83] Oxidation of **II-14** to the corresponding α, β-epoxy ketone **II-15** was completed using hydrogen peroxide in a 65% yield. The α, β-epoxy ketone **II-15** was transformed into the α, β-hydroxy ketone **II-16** using hydrogen and a palladium catalyst in a 89% yield, which was oxidized to the 1, 2-diketone (**II-17**) using IBX in a 88% yield. The rearrangement of **II-17** with dimethyl guanidine hydrogen sulfate under basic conditions afforded the corresponding quaternary imidazolone (**II-18**) in a moderate yield (53%). It is important to note that the rearrangement with dimethyl guanidine was also attempted with the α, β-unsaturated ketone **II-14** as well as the α, β-epoxy ketone **II-15**. However, in both cases, the reaction never produced the corresponding imidazolone in any appreciable amount. It was found that starting with the diketone efficiently reduced the amount of side products and conveniently produced the desired quaternary imidazolone in a sufficient yield to where the product would precipitate out of solution, making isolation and purification much easier.

Scheme II-10. Synthesis of imidazolone II-18



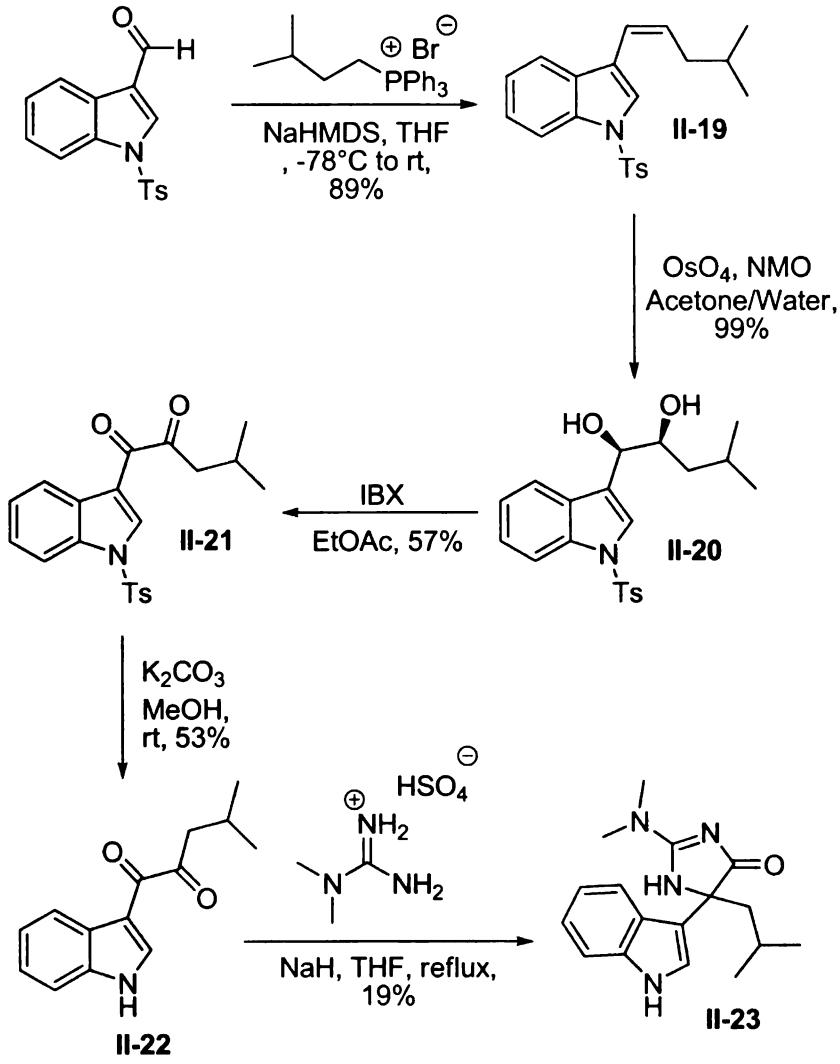
The success of this reaction was encouraging because it meant that the indole was compatible with the rearrangement (the indole had not been tested in any of the previous refs for the rearrangement). It was then decided that because the indole was compatible, the benzyl group should be replaced with another group that may allow for the formation of the 2-oxopropyl moiety needed for the natural product. However, all attempts to synthesize 1,2-diketones that provided a substituent that would give access to the 2-oxopropyl moiety needed for the natural product (**III-1**) were fruitless. The two groups attempted in the

rearrangement to access the 2-oxopropyl moiety were an allyl group and a ethylene glycol protected analog of the 2-oxopropyl moiety. Degradation of the starting materials and/or formation of multiple by-products were observed indicating that the diketone rearrangement may not be suitable for the synthesis of the natural product.

It was discovered in the process of finding successful diketones for the rearrangement that an isobutyl substituent is compatible to a small degree. The synthesis of the appropriate diketone is shown in Scheme II-11 and begins with a Wittig reaction between N-tosyl-indole-3-carboxaldehyde and isoamyltriphenylphosphine bromide to provide the *cis* alkene **II-19** in an 89% yield. The geometry of the alkene was confirmed by NOESY experiments (Figure II-15). Dihydroxylation of alkene **II-19** using osmium tetroxide and NMO led to diol **II-20** in a 99% yield. An IBX oxidation was used to oxidize both alcohols to the corresponding diketone **II-21** in a 57% yield. The next step was to deprotect the nitrogen of the indole group using potassium carbonate and methanol to yield diketone **II-22** in a 53% yield. Finally, the rearrangement of diketone **II-22** and dimethyl guanidine hydrogen sulfate under basic conditions afforded the corresponding quaternary imidazolone **II-23** in a low yield (19%, Scheme II-11). The reaction was not expected to give a high yield, if any, of the corresponding imidazolone since alkyl groups had not been previously shown to work with the rearrangement. To our delight, enough of the imidazolone was produced to have a sample to test for Chk2 inhibition, if needed. Overall, the

diketone rearrangement allowed for the synthesis of a few quaternary imidazolones that are analogs of the natural product (**III-1**).

Scheme II-11. Synthesis of imidazolone II-23



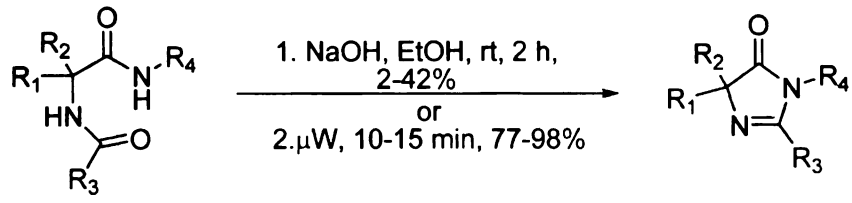
Since the diketone rearrangement was unsuccessful in the formation of an imidazolone that could be applied to the total synthesis of the natural product, other methods to prepare an imidazolone were evaluated. One of the most common synthetic routes to producing imidazolones (non-quaternary and quaternary) is through some type of ring closure.[45-48, 51-55, 58-60, 66, 72] In the case of

quaternary imidazolones, the quaternary stereocenter is formed prior to a final ring closure that forms the heterocyclic ring.

II.D.6 Synthetic routes to imidazolones: Dehydration reactions

The first type of ring closure used to produce imidazolones that will be discussed is a cyclization through a dehydration reaction. Gillman *et al.* and Liu *et al.* used the dehydration of an amide to produce various quaternary imidazolones (Scheme II-12).[53, 72] Gillman synthesized amino amides and then coupled the amine moiety to a carboxylic acid to form a diamide intermediate. Under basic conditions, he was able to dehydrate one of the amides and produce a quaternary imidazolone in yields ranging from 2-42% (Scheme II-12).[53] R_1, R_2 and R_4 remained constant throughout the study (R_1 and R_2 were phenyl groups and R_4 was a hydrogen), while R_3 was varied between aryl groups, heterocyclic groups and a few alkyl groups.[53]

Scheme II-12. Imidazolones produced by dehydration



Liu used microwave technology to speed up the dehydration process and produce imidazolones in 10-15 min,[72] where Gillman's reactions took 2 h.[53] Liu produced a diamide intermediate in a different fashion as Gillman and used the energy of microwaves to effect the dehydration and produce quaternary imidazolones (Scheme II-12). R_1 and R_2 for the majority of the examples Liu illustrated were connected through a cyclic alkane producing spiro-imidazolones

as the final product in yields ranging from 77-98%.[72] There were a few cases were R_1 and R_2 were alkyl and not tethered together and those yields were between 85-95%. Additionally, R_3 and R_4 were varied between alkyl, phenyl and benzyl and afforded imidazolones in yields between 77-98%. The yields reported were determined by HPLC from LC-MS results of the reaction mixture. The isolated yields of the imidazolones greatly decreased and were reported to be between 36-78%.[72]

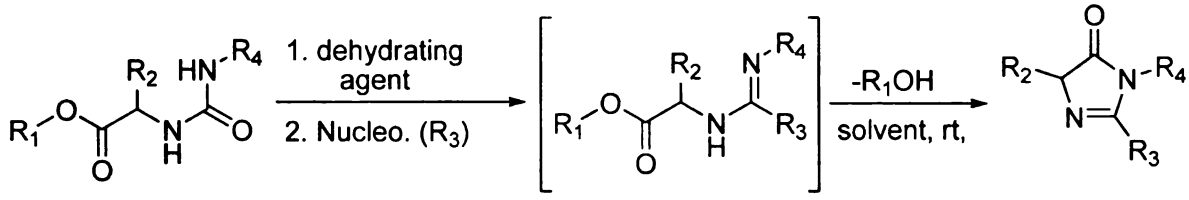
II.D.7 Synthetic routes to imidazolones: Cyclization with carbodiimide intermediates

The main type of reactions used to produce imidazolones are cyclizations where a carbodiimide intermediate is attacked internally or externally by a nucleophile, which causes a subsequent formation of the imidazolone product.[45, 46, 54, 55, 60] The main differences in most of the examples that use this type of ring closure is in the nucleophile used or if similar nucleophiles are used, such as amines to produce a guanidine moiety, then the difference is in the formation of that guanidine moiety.

Lee *et al.* and Lange *et al.* have used the dehydration of ureas with triphenylphosphine/Bromine and Burgess' reagent, respectively, to produce *in situ* a carbodiimide intermediate, which is attacked by an external nucleophile.[55, 60] Lee used alkyl and benzyl substituents for R_2; phenyl and heteroaryl grignards for the nucleophile (R_3); and benzyl and phenyl substituents for R_4 (Scheme II-13). Yields ranged from 56-99% and reactions took about 2 h to complete. There were no reports on producing any quaternary imidazolones.[55] On the other hand, Lange used a solid phase technique where R_1 was connected to a solid support,

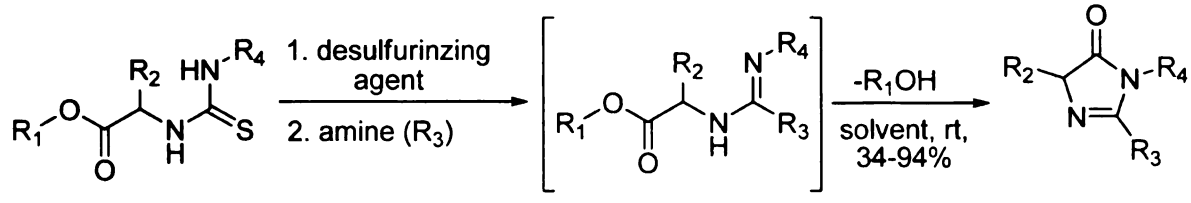
which helped in the isolation of the imidazolones (Scheme II-13).[60] Benzyl and alkyl substituents were used for R_2, while R_3 tended to be various amines giving products that were a variety of 2-aminoimidazolones. R_4 was strictly confined to aryl groups and the yields ranged from 47-93%.[60]

Scheme II-13. Cyclization from diimide: urea to imidazolone



Drewry and co-workers used a thiourea instead of a urea to form the guanidine moiety. A desulfurization of the thiourea using Mukaiyama's reagent led to a carbodiimide intermediate which was attacked by various amines to produce 2-aminoimidazolones.[46] Like Lange, Drewry used a solid support connected at R_1 allowing an easier isolation of the resulting imidazolones. Furthermore, R_2 was typically a benzyl group; R_3 was a range of primary and secondary amines; and R_4 was primarily aryl groups (Scheme II-14). The reactions took longer than solution phase reactions with times around the 24 h mark and yields ranged from 34-94%.[46]

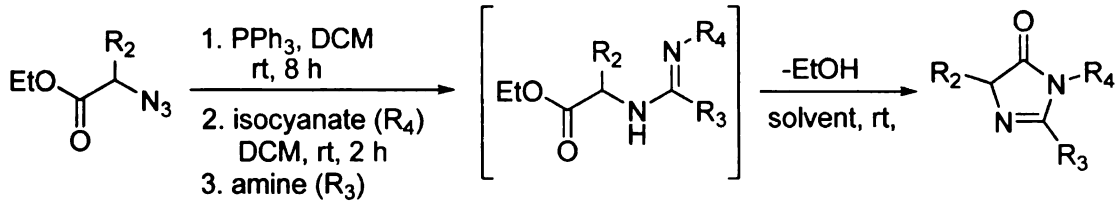
Scheme II-14. Cyclization from diimide: thiourea to imidazolone



Aza-Wittig reactions have also been used to produce a carbodiimide intermediate, which ultimately gets attacked by a nucleophile to produce the

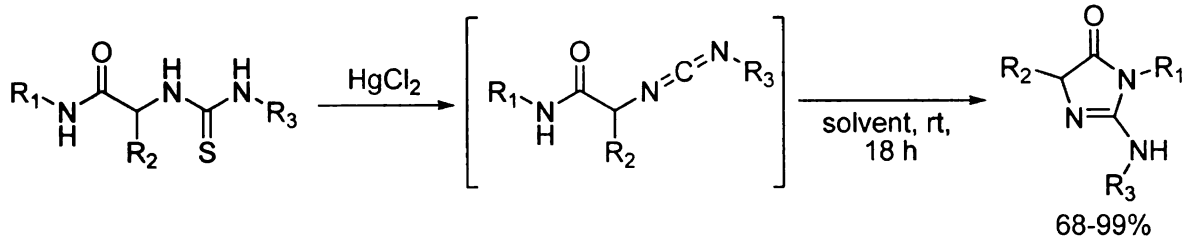
guanidine moiety (Scheme II-15).[45, 54] Villalgordo used the aza-Wittig reaction between an azide and an isocyanate to form a highly reactive carbodiimide intermediate. Subsequently, alkyl and benzyl amines, as well as, secondary amines were used to attack the carbodiimide, form the guanidine and cyclize on an ethyl ester to produce the 2-aminoimidazolone products. R_2 was typically an alkyl or aryl substituent, while R_4 was always aryl substituents. The yields for this reaction were between 72-89% and reaction times varied between 6-15 h.[54] Ding used a similar technique to produce the guanidine and yields for the 2-aminoimidazolones ranged from 40-86%. Similar substituents to Villalgordo were used, i.e. primary and secondary amines, Aryl R_4 substituents and alkyl and hydrogen at R_2.[45]

Scheme II-15. Cyclization from diimide: azide to imidazolone



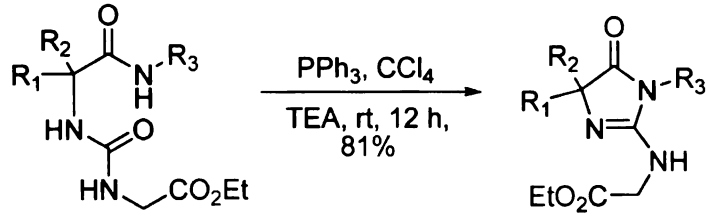
Alternatively, Batey and co-workers used an opposite approach to those described above. Batey utilized the reactivity of a thiourea to form a carbodiimide intermediate *in situ* using $HgCl_2$, which was subsequently attacked internally by an amide, closing up to form an imidazolone (Scheme II-16).[47, 52] Multiple amino acid amides were used as starting materials allowing R_2 to correspond to the appropriate amino acid. Peptides could be used in this reaction, allowing R_1 to be a long peptidic chain, as well as linked to a solid phase support ameliorating the isolation process. R_3 was typically aryl groups and yields ranged from 68-99%.[47]

Scheme II-16. Intramolecular cyclization: thiourea to imidazolone



Frutos and co-workers used a dehydration of a urea to mediate the cyclization to an imidazolone product (Scheme II-17).[48] The urea was proposed to be transformed into a carbodiimide intermediate, which was spontaneously attacked intramolecularly by the secondary amide present in the molecule. As a result, the dehydration mediated by triphenylphosphine and carbon tetrachloride afforded the substituted imidazolone in a 81% yield.[48] The quaternary substitution pattern at position 5 consisted of R_1 and R_2 being a benzyl and methyl group, while R_3 was an aryl group.[48]

Scheme II-17. Intramolecular cyclization: urea to imidazolone



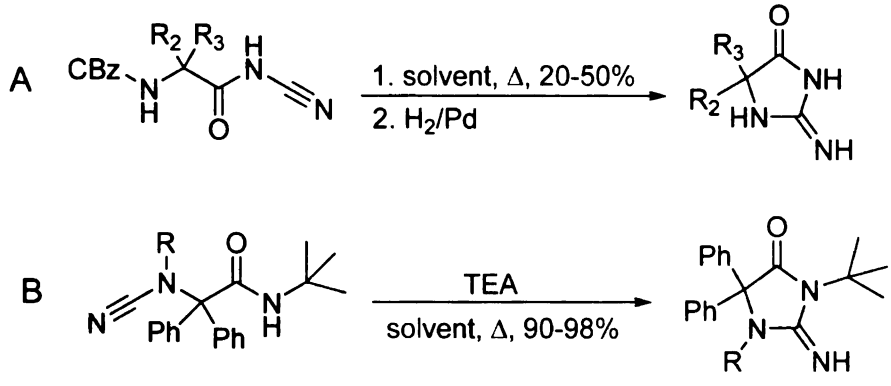
II.D.8 Synthetic routes to imidazolones: Cyclization reactions with nitriles

A second type of ring closure to form imidazolones involves the intramolecular cyclization of an amine or amide on a nitrile (Scheme II-18). Nagasawa and Kwon describe a facile synthesis of imidazolones using a ring closure between an amine and nitrile using primarily heat although sometimes a little acid was used (**A**, Scheme II-18).[58, 59] The amine moiety was protected with a carbamate group (Cbz) and the R_2 and R_3 substituents were varied between

hydrogen, alkyl, benzyl and phenyl. Quaternary imidazolones have been successfully prepared and yields for all imidazolones produced using this method ranged from 20-50%.[58] The reactive intermediate for the reaction is not clear due to IR bands observed at 2140 cm^{-1} and 2260 cm^{-1}, depending on the pH.[58] A band at 2140 cm^{-1} hints at a carbodiimide intermediate, while the band at 2260 cm^{-1} suggests more of a nitrile intermediate. The tautomeric relationship between the acylcyanamide and carbodiimide makes it hard to specify which intermediate is responsible for the cyclization. Regardless, the overall product in the reaction is variously substituted 2-aminoimidazolones.

Lempert and co-workers report a similar cyclization where an amide attacks a nitrile and forms an imidazolone product (**B**, Scheme II-18).[66] Lempert successfully produces a variety of imidazolones, where R = alkyl, benzyl and differently substituted aryl groups, in yields ranging from 90-98%. It is also possible to remove the *tert*-butyl protecting group using aqueous HCl to provide the free 2-aminoimidazolone as the final product.[66]

Scheme II-18. Imidazolone through cyclization onto nitrile



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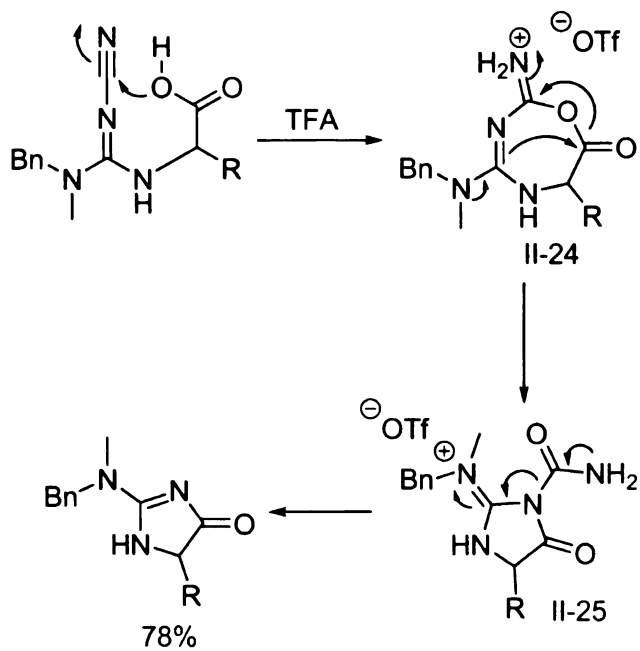
II.D.9 Synthetic routes to imidazolones: Cyclization reactions with N-cyanoguanidines

The last type of cyclization reaction to produce imidazolones involves a rarely seen cyclization between an N-cyanoguanidine and a carboxylic acid (Scheme II-19).[51] Garratt proposes a reaction mechanism where under acidic conditions, a N-cyanoguanidine is attacked by a carboxylic acid to form the seven-membered ring intermediate (**II-24**). Subsequent rearrangement of the seven-membered ring to the five-membered ring (imidazolone **II-25**), followed by elimination of the amide protecting group on N-3 results in the formation of the final imidazolone product (Scheme II-19).[51] There were only a couple imidazolones made through this route where R = ethyl and benzyl and the yields were 78% and 79%, respectively.

Scheme II-19. Imidazolone through cyclization using N-cyanoguanidines



To sum up thus far, many different types of reactions have been used to create imidazolones. Many have used unique routes to obtain quaternary (5, 5-

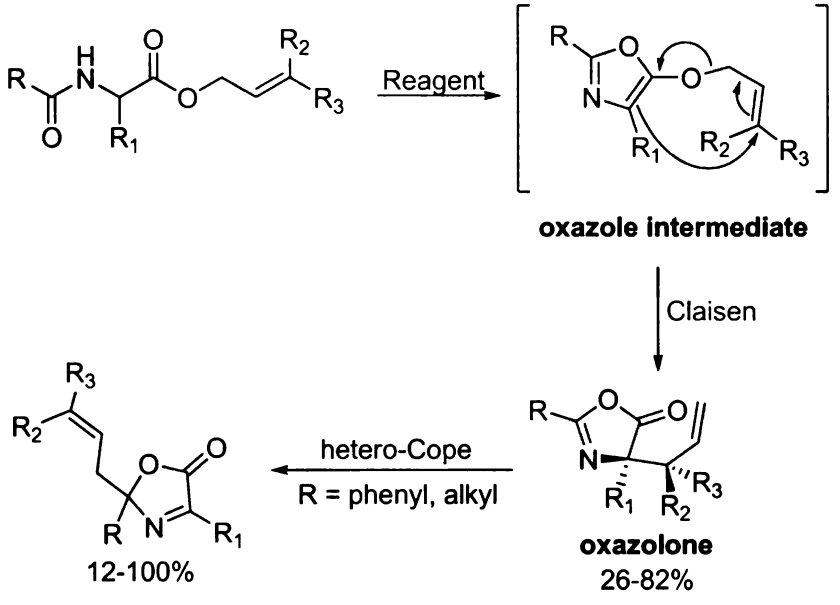
disubstitution on the imidazolone ring) and non-quaternary imidazolones. However, the entirety of the cyclization reactions that produced quaternary 2-aminoimidazolones contained the quaternary stereocenter before the cyclization. With this in mind, it was thought that the best route to obtain the desired quaternary imidazolone for the natural product (**III-1**) would be to first set the quaternary center and then cyclize to form the imidazolone.

II.E Synthesis of quaternary intermediates for the synthesis of the natural product

Steglich and co-workers first described a rearrangement in 1975 where an allyl ester of an N-acyl amino acid was converted into a quaternary oxazolone using a variety of dehydrating reagents (Scheme II-20).[84, 85] The proposed mechanism is shown below in Scheme II-20 and starts with a cyclodehydration of an allyl ester to afford an oxazole intermediate, which upon a Claisen-type reaction produces an oxazolone.[84, 85] The oxazole intermediate had not been isolated in the original reports, but is presumed based on the reaction of alkyl esters of N-acyl amino acids.[86, 87] Two acyl groups were used for the rearrangement, with R = phenyl and isopropyl, while R_1 varied between aryl and alkyl substituents. The substitution on the allyl group (R_2 and R_3) varied between hydrogen, alkyl and aryl groups with yields for the quaternary oxazolone ranging from 26-82%.[84, 85] Like the Claisen rearrangement, it was observed that the oxazole rearrangement produced only one diastereomer in cases where two stereocenters were able to be formed.[88] Steglich discusses an additional hetero-Cope rearrangement that occurred (when R_2 or $R_3 \neq H$) after the formation of the quaternary oxazolone to afford oxazolin-5-one products in yields ranging from

12-100%. Depending on the substitution of R_2 and R_3, the hetero-cope rearrangement sometimes needed additional heating to complete.[84, 85]

Scheme II-20. Rearrangement developed by Steglich



Steglich's oxazole rearrangement is a useful tool to create a quaternary stereocenter and has been used to produce a variety of different compounds.[89-97] Haufe *et al.* used the oxazole rearrangement to lead to 4-fluoropyridines (Scheme II-21).[97] Fluoro allyl esters of N-acyl amino acids were cyclodehydrated using triphenylphosphine and carbon tetrachloride to produce the corresponding quaternary oxazolones in yields ranging from 98-100%. Subsequently, Haufe exposed the oxazolones to decahydronaphthalene and air for 20 h at 160°C which transformed the oxazolones to the corresponding 4-fluoropyridines in yields between 21-55% (Scheme II-21).[97]

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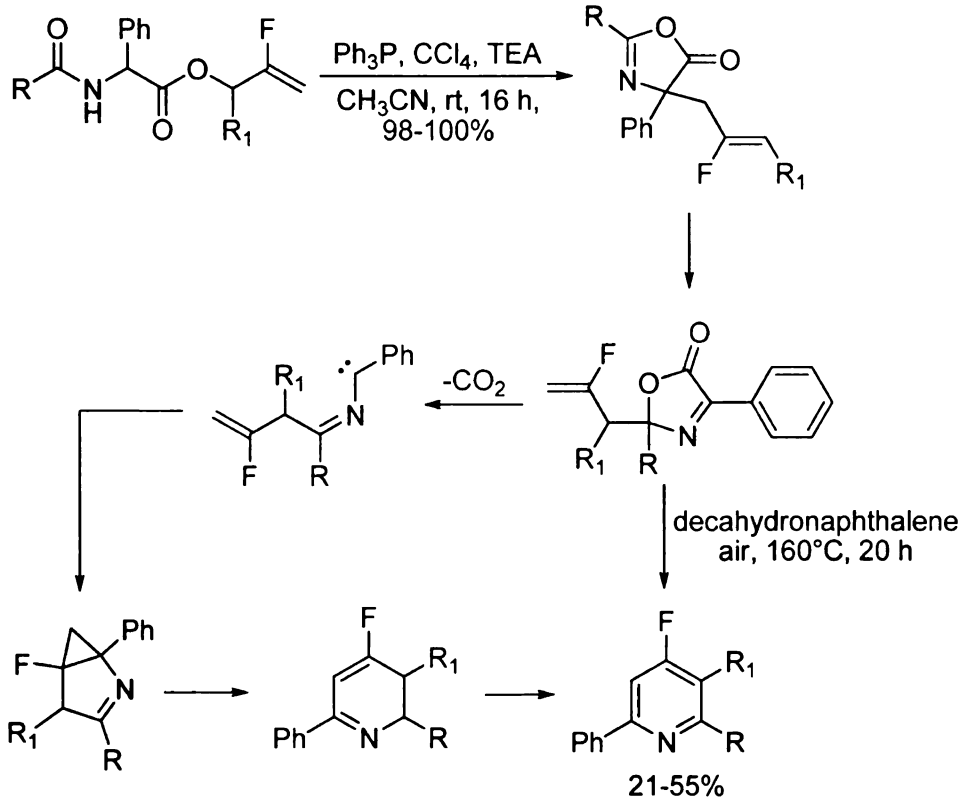
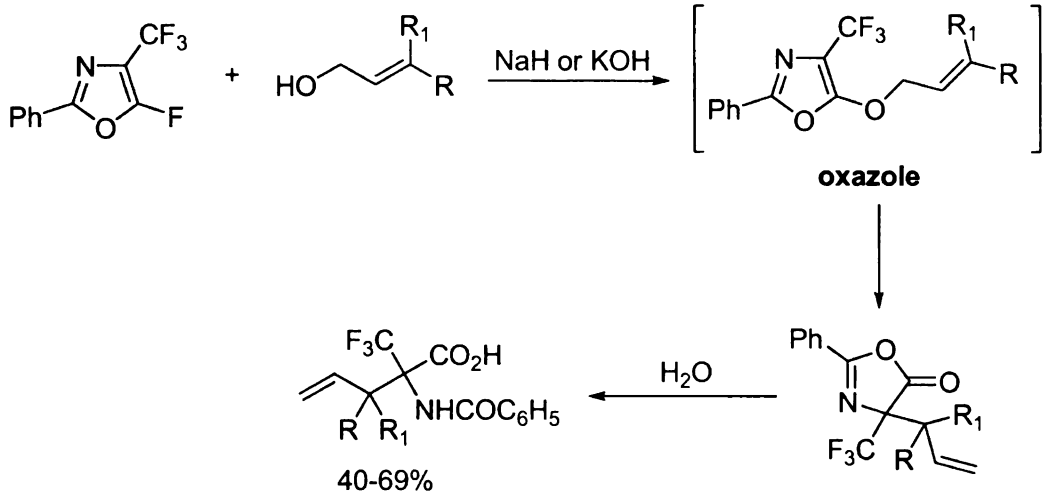
Scheme II-21. Rearrangement leading to 4-fluoropyridines



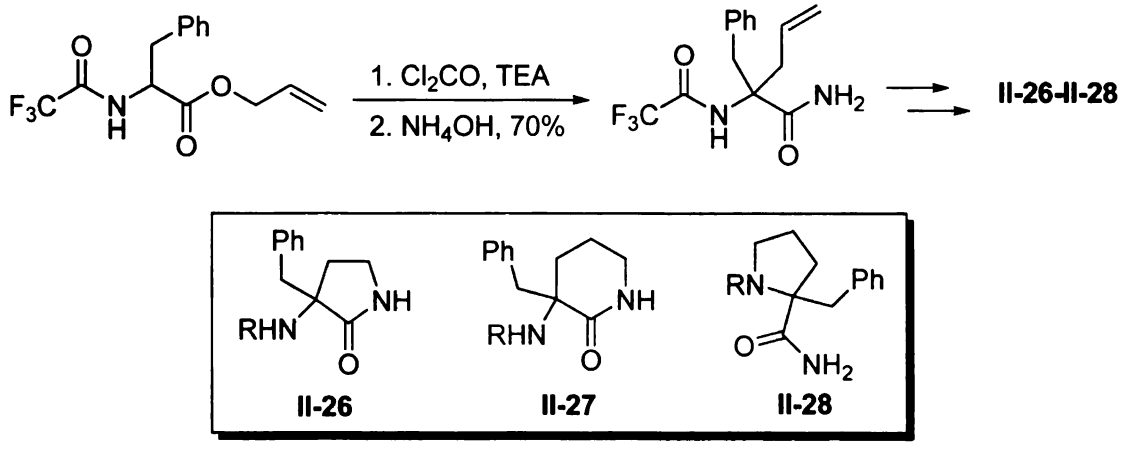
Burger *et al.* described a unique method for the formation of the allyl oxazole intermediate by coupling the fluorinated oxazole starting material with various alcohols (Scheme II-22).[89-91] Subsequently, the oxazole rearrangement produced a variety of α-trifluoromethyl α-amino acids (Scheme II-22) in yields between 40-69%.[90] Interestingly, Burger also identified that the allyl oxazole intermediate could be spectroscopically characterized when R = $(CH_3)_3Si$-CH=CH-CH_2OH. This is the first example of the allylic oxazole intermediate being observable spectroscopically.[90] Additionally, Burger showed that benzyl alcohols and hydroxymethylheterocycles, such as 2-hydroxymethylthiophene, also are compatible in the oxazole rearrangement.[89-91] Furthermore, Burger along with Krantz, illustrated that propargyl alcohols could be used in place of allylic alcohols to produce α-allenic α-amino acids.[89, 90, 92, 93]

Scheme II-22. Rearrangement affording α-trifluoromethyl α-amino acids



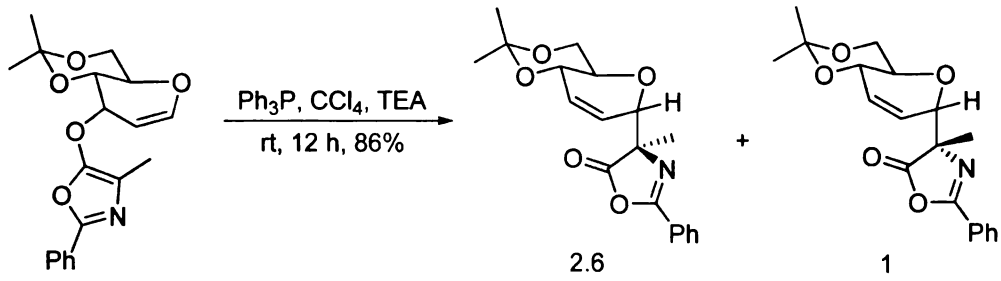
The oxazole rearrangement has been utilized in the synthesis of α-benzyl γ-lactam derivatives, α-benzyl δ-lactam derivatives and α-benzylproline derivatives (Scheme II-23, **II-26**, **II-27**, **II-28**, respectively).[96] Holladay and co-workers used phosgene to carry out the oxazole rearrangement to afford a quaternary oxazolone that is quite unstable. By treating the oxazolone formed *in situ* with ammonium hydroxide, a primary amide was formed. Subsequent treatment of the amide with a combination of reagents led to each of the desired heterocycles (Scheme II-23).[96]

Scheme II-23. Rearrangement leading to II-26-II-28



Colombo *et al.* applied the oxazole rearrangement to the synthesis of *C*-glycosyl α-amino acids, and α-D-*C*-mannosyl-(R)-alanine.[94, 95] It was found that the cyclodehydration/rearrangement reaction afforded a 2.6:1 mixture of two diastereomeric oxazolones (Scheme II-24). It was proposed that the major isomer is produced through a boat-like transition state during the oxazole rearrangement possibly due to steric interactions that would accompany a chair-like transition state for this specific case.[95]

Scheme II-24. Rearrangement to synthesize *C*-glycosyl α-amino acids



The majority of the examples of the oxazole rearrangement only produced quaternary oxazolones with one stereocenter.[84, 85, 91, 96, 97] However, it was reported that a mixture of diastereomers were observed in the examples that had the potential to produce them, as in the case of Burger and Krantz with the allenic oxazolones[89, 90, 92, 93] and Colombo with the glycosyl oxazolones.[94, 95] In the case of the allenic oxazolones, it was described that having a substitution on the methylene of the propargyl alcohol could influence the diastereomeric ratio depending on the size of the substituent.[88] Moreover, in the glycosyl oxazolone cases, the stereochemistry of the sugar moiety helps dictate the stereochemical outcome of the rearrangement.[94, 95]

We proposed that this oxazole rearrangement is a unique and efficient way to create a quaternary center and would be excellent to utilize the rearrangement in the synthesis of the natural product (**III-1**). Furthermore, it seemed to augment and highlight the theme of our group's work dealing with the development of oxazolone chemistry.[98] The oxazole rearrangement was attempted with various N-acyl groups as shown in Table II-1. The dehydrating reagents varied depending on the N-acyl group used.

Trifluoroacetic anhydride worked the best when R = phenyl (Table II-1) affording the oxazolone (**II-36**) in a 76% yield. $POCl_3$ was the dehydrating reagent used when R = methyl and dimethylamino to give the quaternary oxazolones **II-37** and **II-38** in a 69% and 86% yield, respectively. However, when the N-acyl derivative was used, a different isomer was observed as the product (Table II-1). The product isolated was actually the lactone (Isomer B, Table II-1) in which a hetero-Cope rearrangement occurred after the initial Claisen rearrangement, which had been seen before in Steglich's initial study.[85]

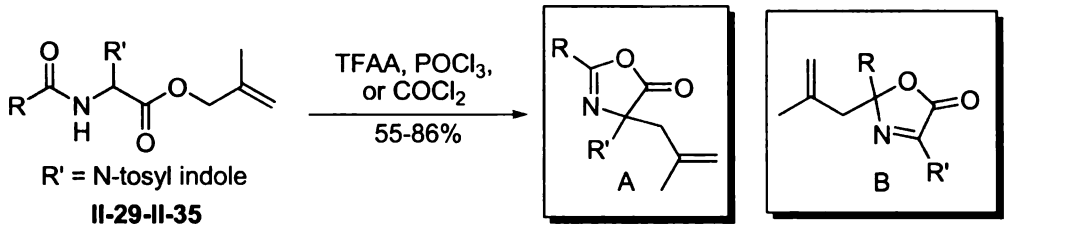
The physical evidence that supports this structural determination comes from the absorptions seen in the IR for oxazolone **II-37** compared to those reported for the oxazolone product (Isomer A) and lactone (Isomer B). For **II-37**, the signals observed in the carbonyl region are at 1779 cm^{-1} and 1627 cm^{-1}, which match very closely the reported IR signals for the lactone product (1780-1770 cm^{-1} and 1645-1610 cm^{-1})[84, 85]. The IR signals associated with the oxazolone product tend to be in the range of 1850-1810 cm^{-1} and 1660-1650 cm^{-1}.[84, 85] It is also noteworthy to mention that when the dimethylamino urea (**II-31**)

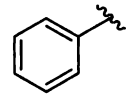
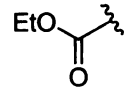
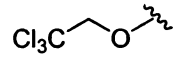
was used in the rearrangement, the resulting oxazolone was so labile that isolation could only occur after treatment with methanol. This resulted in the isolation of the quaternary ring-opened methyl ester (Structure C, Table II-1).

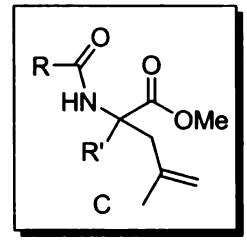
Electron withdrawing acyl groups attached to the nitrogen, such as the trifluoromethyl acetyl group and 2-ethoxy-2-oxoacetyl group (compounds **II-32** and **II-33**, respectively) require a different dehydrating reagent altogether. TFAA, $POCl_3$, PCl_3, PCl_5 and PPh_3/CCl_4 have all been evaluated and failed to effect the rearrangement. It was found that phosgene was the only reagent that could produce the corresponding quaternary products (**II-39** and **II-40**, Table II-1) in yields of 75 and 55%, respectively. However, due to the lability of the resulting oxazolones, they would readily ring-open at the carbonyl carbon even on silica gel. As a result, treatment of the quaternary oxazolones immediately with a nucleophile was necessary to be able to isolate the corresponding ring-opened products (Structure C, Table II-1).

A carbonate and pivaloyl group were also attempted in the rearrangement (entries **II-34** and **II-35**) but failed to produce the quaternary oxazolone using any of the previously described dehydrating reagents. It was observed that when a carbonate was used as the acyl group, the reaction gave back starting material after heating for 24 h with each dehydrating reagent. Conversely, when the pivaloyl group was used as the acyl group, the starting material seemed to decompose in almost every reaction condition, presumably due to the acidic nature of the reagents.

Table II-1. Oxazole rearrangement with various N-acyl groups



Compound	R	Isolated Structure	Dehydrat. Reagent	Yield	Product
II-29		A	TFAA	76%	**II-36**
II-30		B	$POCl_3$	69%	**II-37**
II-31		C	$POCl_3$	86%	**II-38**
II-32		C	$COCl_2$	75%	**II-39**
II-33		C	$COCl_2$	55%	**II-40**
II-34		NR	NA	NA	NA
II-35		NA	NA	NA	NA

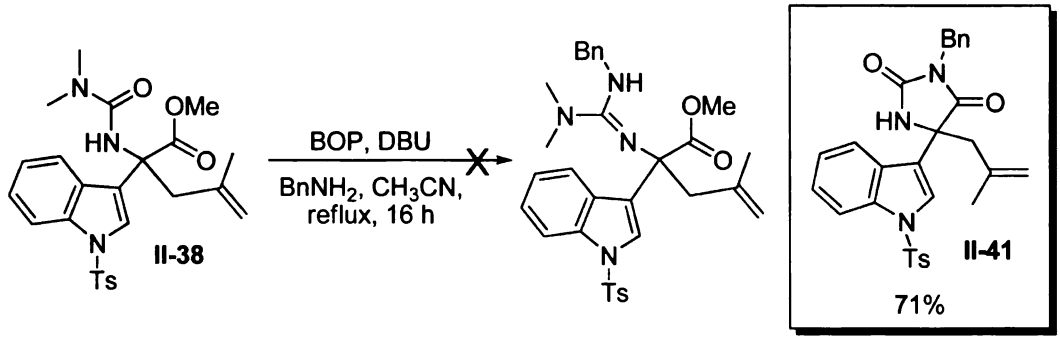


As stated before, the majority of the techniques used to create quaternary imidazolones involved the synthesis of the stereocenter prior to heterocycle formation. This technique was thought to be employed in the synthesis of the natural product. Therefore, since the quaternary center has now been achieved through the oxazole rearrangement, the next step was to form the imidazolone heterocycle.

The quaternary oxazolone products (**II-36** and **II-39**) were subjected to different reaction conditions to open the quaternary oxazolone and remove the resulting acyl group on the nitrogen to move ahead in the synthesis of the natural

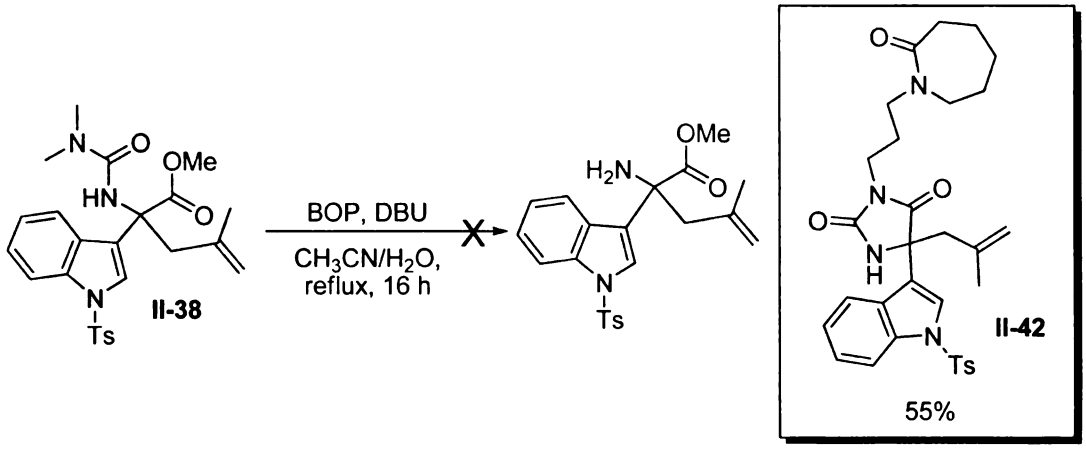
product. However, basic reaction conditions usually only opened the oxazolone and/or removed the tosyl protecting group, while acidic reaction conditions resulted in degradation of the starting material and/or formation of multiple by-products. Surprisingly, attempts to manipulate the urea quaternary product (**II-38**) provided some interesting results. When treated with reagents intended to activate the urea carbonyl and subsequently substitute with an amine[99], an unexpected result was observed (Scheme II-25). Instead of forming the intended guanidine, the final product corresponded to the quaternary hydantoin **II-41** which was isolated in good yields (71%).

Scheme II-25. Unexpected reaction producing hydantoin II-41



An even more unusual reaction was observed when the amine was left out of the reaction and water was added instead. It was believed that the unexpected reaction might have proceeded through an isocyanate intermediate and the water was intended to hydrolyze the intermediate and upon basic workup, afford an amine (Scheme II-26). However, a quaternary hydantoin with an incorporation of DBU was observed in a 55% yield (Scheme II-26).

Scheme II-26. Unexpected reaction producing hydantoin II-42



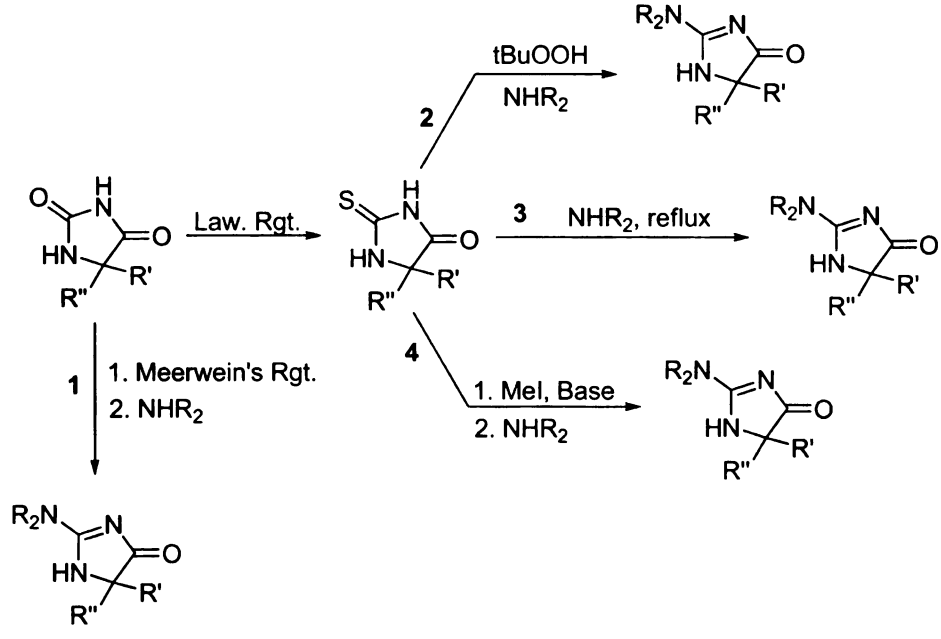
It has been reported in multiple accounts that use the oxazole rearrangement to make quaternary products that the largest downfall in the reaction is the inability to remove the acyl group (of the ring-opened oxazolone) after the rearrangement.[92, 93, 100] This main disadvantage was the driving force to create a new rearrangement in which the product could be more amenable toward synthesizing the natural product. Furthermore, the formation of the unexpected quaternary hydantoins sparked an interesting idea to create a method where an unprotected quaternary hydantoin is formed after an oxazole rearrangement. This would allow access to the imidazolone scaffold through known chemistry.[44, 50, 56, 57, 101]

II.F Development of a novel EDCI-mediated oxazole rearrangement

The restraint of the traditional oxazole rearrangement was the stimulus for developing a novel reaction that incorporates aspects of the oxazole rearrangement to afford a quaternary product capable of leading to the 5,5-(disubstituted)-imidazol-4-one scaffold of the natural product, which is the main goal of my project. It was imagined that forming a 5,5-(aryl, allyl) hydantoin after

an oxazole rearrangement[85] would be novel and ideal for the synthesis of the indole alkaloid (**III-1**) for three reasons. First, the hydantoin intermediate would allow access to the imidazolone scaffold using known chemistry either directly to the imidazolone[50] or through a thiohydantoin[19, 44, 56, 57, 101] (Scheme II-27). Gadwood and co-workers described a method in which a hydantoin was converted into the imidazolone using Meerwein's reagent to alkylate the carbonyl in the 2 position followed by replacement of the newly formed alkoxy group with an amine (Path **1**, Scheme II-27).[50]

Scheme II-27. Routes from hydantoin to imidazolone



Alternatively, a thiohydantoin can be transformed into an imidazolone using a variety of methods.[19, 44, 56, 57, 101] Ireland used an oxidation of a thiohydantoin followed by treatment of an amine to afford an imidazolone[19] (Path **2**, Scheme II-27), while Kiec-Kononowicz and co-workers simply refluxed a thiohydantoin in the presence of the desired amine to afford imidazolones (Path

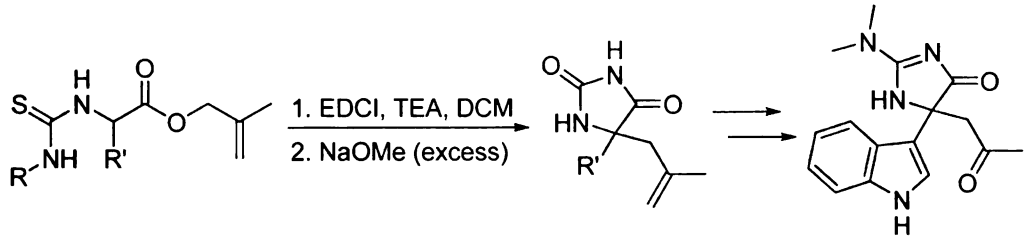
3, Scheme II-27).[57] However, the most widely used method for transforming a thiohydantoin into an imidazolone is to alkylate the thiocarbonyl first and then replace it with the desired amine as illustrated by Overman[101], Khodair[56] and Iverson[44] (Path **4**, Scheme II-27).

The second advantage of forming a 5,5-(aryl, allyl) hydantoin after an oxazole rearrangement would be that it could act as a synthetic handle with which the carbonyl in the 2-position of the hydantoin could be replaced with potentially any amine, giving access to a number of analogs of the natural product, if desired. Finally, the allyl group was thought to give access to the keto functionality found in the indole alkaloid. To achieve the formation of a quaternary hydantoin it was thought to combine certain aspects of the oxazole rearrangement and the cyclization reaction Batey[47] used to create imidazolones. In the end, a new reaction could be developed giving access to uniquely substituted quaternary hydantoins.

We chose to implement a thiourea moiety in place of the N-acyl group from the original oxazole rearrangement and use a desulfurizing agent to achieve a quaternary oxazolone product that could be manipulated into the corresponding quaternary hydantoin we desired. Overall, a novel rearrangement (Scheme II-28) that gives access to a unique marine alkaloid scaffold was developed.[102] The one-pot reaction converts a thiourea into a 5,5-(aryl, allyl) oxazolone through a new twist on the oxazole rearrangement, which upon further treatment with sodium methoxide yields a 5,5-(aryl, allyl) hydantoin. As a result, this hydantoin is

able to offer a concise synthetic route to molecules such as the natural product, indole alkaloid **III-1**.

Scheme II-28. New rearrangement applied to the synthesis of III-1



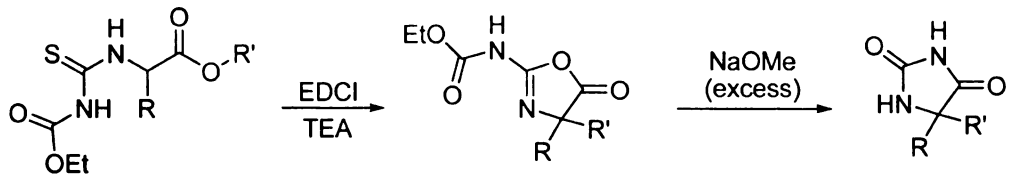
Natural Product (III-1)

II.F.1 Scope of rearrangement

It is hypothesized that the new rearrangement initiates after the thiourea is activated and converted into a carbodiimide, which undergoes a 5-*exo*-dig cyclization to form an oxazole intermediate (based on data discussed in the mechanism section). There are a variety of reagents that have been reported to transform a thiourea into a carbodiimide intermediate and subsequently allow for attack by an external or internal nucleophile.[103-107] We screened the typical reagents chosen for this type of chemistry including $HgCl_2$,[104, 108, 109] Mukaiyama's reagent,[107, 110] and EDCI[105, 111] and found that the success of the reaction was highly dependent on the reagent choice. Mercuric chloride did provide a reasonable yield of the quaternary hydantoin (49%), while Mukaiyama's reagent failed to produce any desired hydantoin. EDCI was the most effective at yielding the quaternary hydantoin in good yields (70%). Furthermore, the hazard of mercury waste removal and the ease of workup with EDCI solidified the reason to use EDCI as the reactant for the rest of the study. A more cost efficient carbodiimide, DCC, was also attempted in the rearrangement

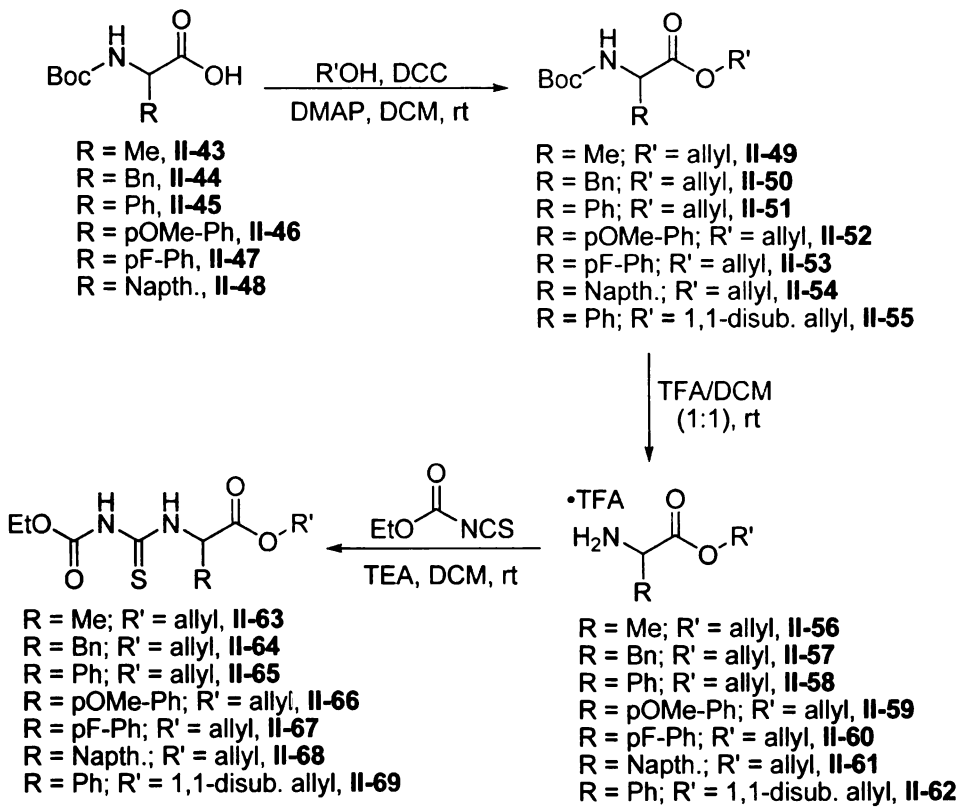
but failed to provide any reasonable yields (<5%). Polymer supported EDCI was also used although longer reaction times were necessary. A brief solvent screen revealed that dichloromethane provided the best results (70%), while solvents such as acetonitrile, benzene, tetrahydrofuran, and dichloroethane all yielded poorer results (50%, 30%, 47%, and 12%, respectively).[102]

Scheme II-29. General scheme for novel rearrangement



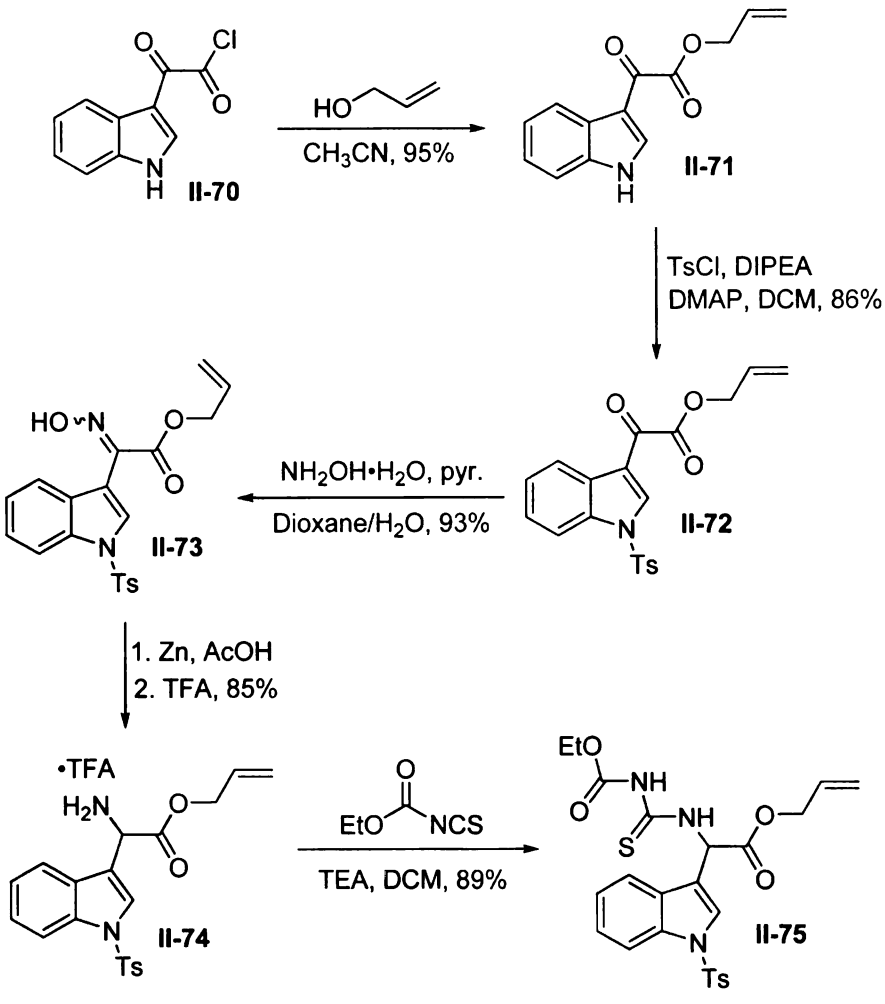
The first structural aspect we investigated in the rearrangement was the different groups compatible at the R position (Scheme II-29). The different thiourea starting materials were synthesized through standard amino acid chemistry (Scheme II-30). The Boc protected amino acids (**II-43-II-48**) (R = methyl,[112] benzyl,[113] phenyl,[114] *p*OMe-phenyl,[115] *p*F-phenyl,[116] and napthyl,[117] respectively) were esterified with the appropriate allylic alcohol using dicyclohexylcarbodiimide (DCC). Deprotection of the Boc group with a mixture of TFA and DCM (1:1) led to the TFA salt of the amino allylic esters (**II-56-II-62**). Upon treatment of the amine salts with ethyl isothiocyanatoformate under basic conditions the desired thioureas (**II-63-II-69**) were produced.[102]

Scheme II-30. General synthesis of thioureas II-63-II-69



The synthesis of thiourea (**II-75**, when R = N-tosylindole) followed a different synthetic pathway (Scheme II-31). 2-(1H-indol-3-yl)-2-oxoacetyl chloride[118, 119] (**II-70**) was treated with allyl alcohol in CH_3CN to yield keto-ester (**II-71**) in a 95% yield. Protection of the indolic nitrogen with *p*-toluene sulfonyl chloride under basic conditions afforded keto-ester (**II-72**). The transformation of the ketone functional group in (**II-72**) to oxime (**II-73**) was completed by refluxing the starting ketone with hydroxyl amine and pyridine in dioxane. The resulting oxime was a mixture of isomers and both were reduced to amine (**II-74**) using zinc and acetic acid and subsequently reacted with TFA to produce the TFA salt. Finally, treatment of the amine salt with ethyl isothiocyanatoformate led to the desired thiourea (**II-75**).[102]

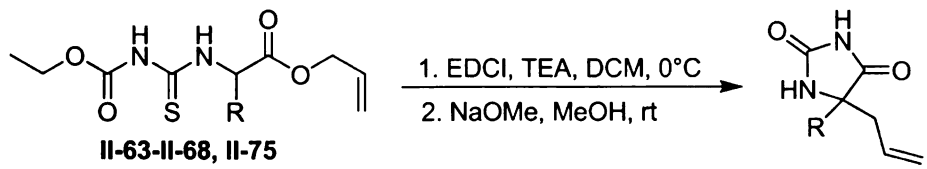
Scheme II-31. Synthesis of thiourea II-75



The results of the rearrangement with the various R groups are illustrated in Table II-2. It is shown that an alkyl group such as a methyl provided no product, while a benzyl only performed slightly better by producing the quaternary hydantoin (**II-76**) in low yields (19%). When R = aryl (**II-65-II-68**), the rearrangement occurred in good yields (Table II-2) except for when R = napthyl, which could be attributed to the steric bulk of the napthyl group. The rearrangement was also successful using a heterocycle in the R position with the N-tosyl indole thiourea (**II-75**) providing the corresponding hydantoin (**II-81**) in

good yields. This particular example is important for the synthesis of indole alkaloid (**III-1**).[102]

Table II-2. Rearrangement containing various R groups



II-63-II-68, II-75

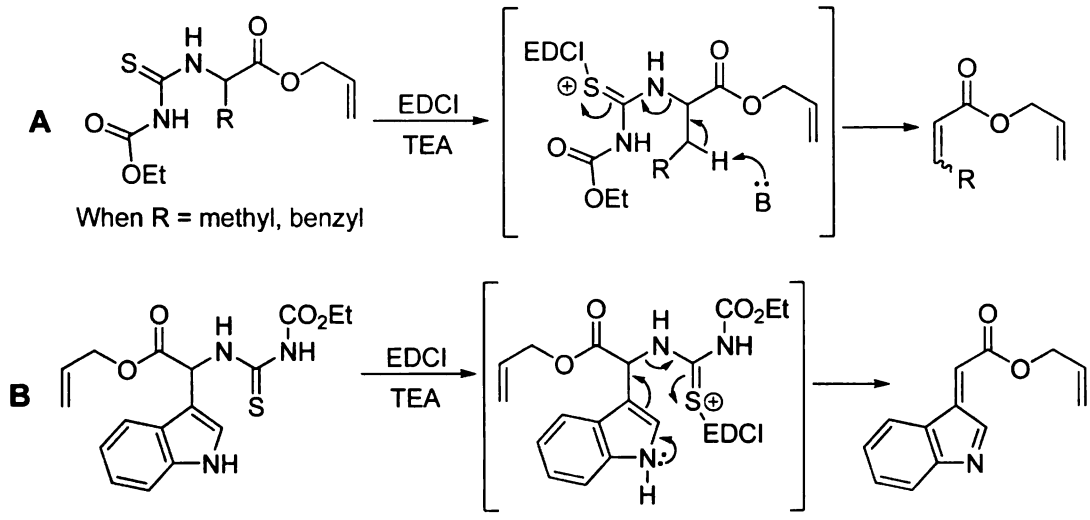
II-76-II-81

entry	R	Yield (%)	Product
II-63	Me	0	NA
II-64	Bn	19	**II-76**
II-65	Ph	70	**II-77**
II-66	*p*-OMe-Ph	67	**II-78**
II-67	*p*-F-Ph	57	**II-79**
II-68	Napth	31	**II-80**
II-75	N-tosyl-Indole	70	**II-81**

It was observed that when R = methyl and benzyl, there appeared to be the formation of byproducts. It is surmised that a potential reason for the formation of byproducts and low (if any) yield of product could be an elimination of the activated thiourea affording an intermediate that could potentially go through side reactions (Scheme II-32). Likewise, when the rearrangement was first attempted with R = indole (no nitrogen protection), the rearrangement failed to give the desired quaternary hydantoin and produced multiple byproducts. As shown in Scheme II-32, it is postulated that a similar elimination reaction could occur when R = indole giving rise to possible side reactions and byproducts. Protection of the indole nitrogen with an electron-withdrawing group (tosyl) appeared to hinder the side reactions and allow the desired rearrangement to

occur. Overall, it is believed that the reaction works well under conditions where a potential elimination reaction cannot occur and when the aromatic oxazole intermediate (discussed in the mechanism section) is stabilized by aromatic R groups.

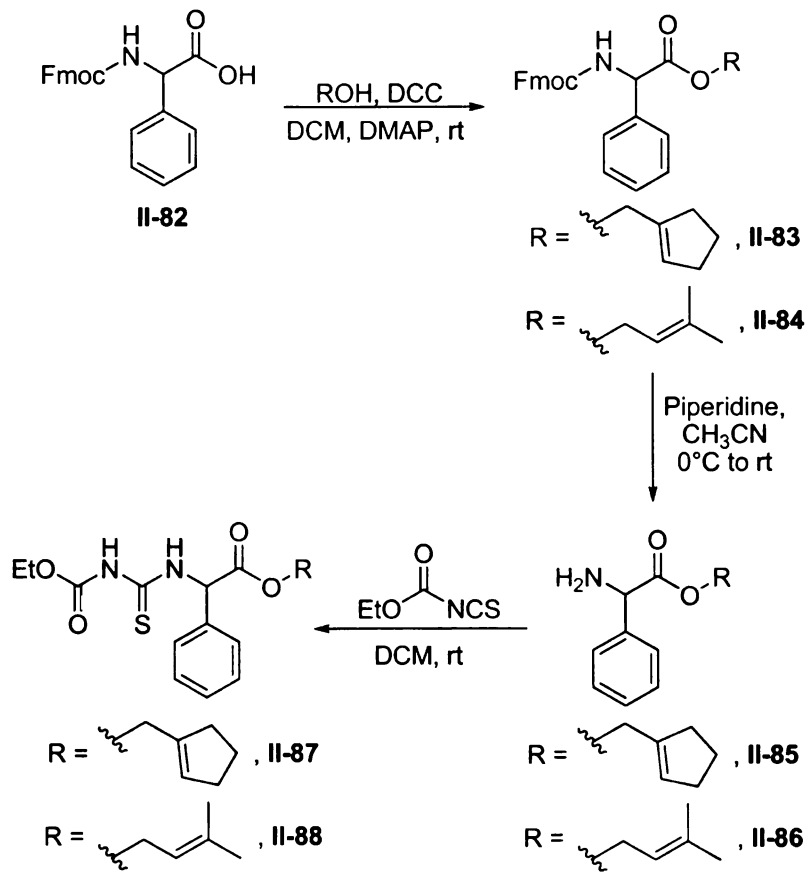
Scheme II-32. Potential byproduct pathway



We then examined the different allyl groups compatible with the rearrangement. The synthesis of the thiourea with a 1, 1-disubstituted allylic moiety (**II-69**) followed the synthetic route that is outlined in Scheme II-30. However, when trisubstituted allylic thioureas were synthesized using the corresponding trisubstituted allylic alcohols, a different route was adopted. It was found that the higher substituted allylic esters would decompose to the corresponding carboxylic acid when treated with TFA.[120] As a result, instead of using the Boc protecting group, the Fmoc group was used for the synthesis of those thioureas (Scheme II-33). Using Fmoc phenyl glycine[121] as the starting material, the allylic esters (**II-83, II-84**) were produced under the same conditions as before using DCC and DMAP. Deprotection of the Fmoc group from the amine

was completed using piperidine to afford the free amines (**II-85, II-86**). Upon treatment of the amines with ethyl isothiocyanatoformate, the corresponding thioureas (**II-87, II-88**) were formed in moderate yields.[102]

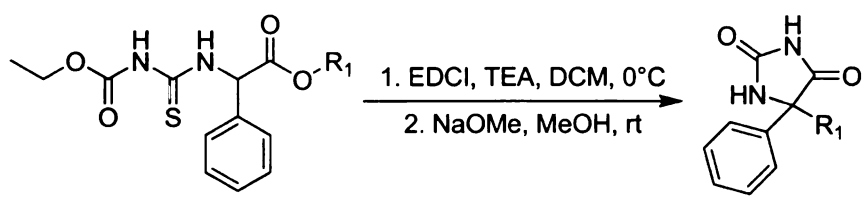
Scheme II-33. Synthesis of higher substituted allylic thioureas



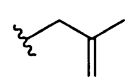
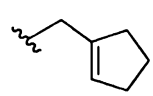
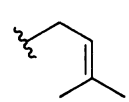
The rearrangement was successful with all of the differently substituted allylic esters that were synthesized (Table II-3). When the 1, 1-disubstituted allyl group was used (entry **II-69**) the rearrangement gave about the same yield of corresponding quaternary hydantoin as the allyl group (entry **II-65**). Entry **II-87**, which had a 1, 1, 2-trisubstitution pattern on the allylic group was also as successful as the other entries. However, unlike the two previous entries, the product from the rearrangement (**II-90**) contained two stereocenters allowing for

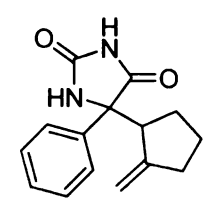
the formation of diastereomers. It was observed by ^{1}H NMR and ^{13}C NMR that two unseparatable diastereomers were formed from the reaction in about a 1:1.3 ratio. The last entry in Table II-3 includes an allyl moiety containing a 1, 2, 2-trisubstitution pattern. It is hypothesized that the lower yield resulting from this rearrangement could be attributed to the steric bulk of this particular trisubstituted allyl group. An electron withdrawing group (ethyl carbamate) on the thiourea was only used since it was previously found that electron withdrawing groups accelerate the reaction of a thiourea and desulfurizing reagent and increase the reactivity of the corresponding carbodiimide toward a nucleophile.[102, 122]

Table II-3. Rearrangement containing various allyl groups

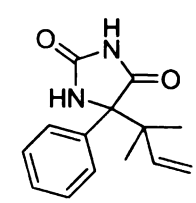


II-65. II-69, II-87, II-88 → **II-77, II-89**

entry	R_1	Yield (%)	Product
II-65		70	**II-75**
II-69		66	**II-89**
II-87		66	**II-90**
II-88		47	**II-91**



II-90



II-91

II.F.2 Proposed reaction mechanism

The proposed mechanism is illustrated below in Scheme II-34. The first step is thought to be the transformation of the starting thiourea to a carbodiimide

ntermediate (**II-92**

experimentally, it

Several reports su

such as EDCI, Hg

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with various sub

different heteroc

nucleophile on

Batey and co-w

that occured aft

by an interna

desulfurization

aminoimidazol

reasonably co

formation of a

reactive, thu

unobservable

reactivity, the

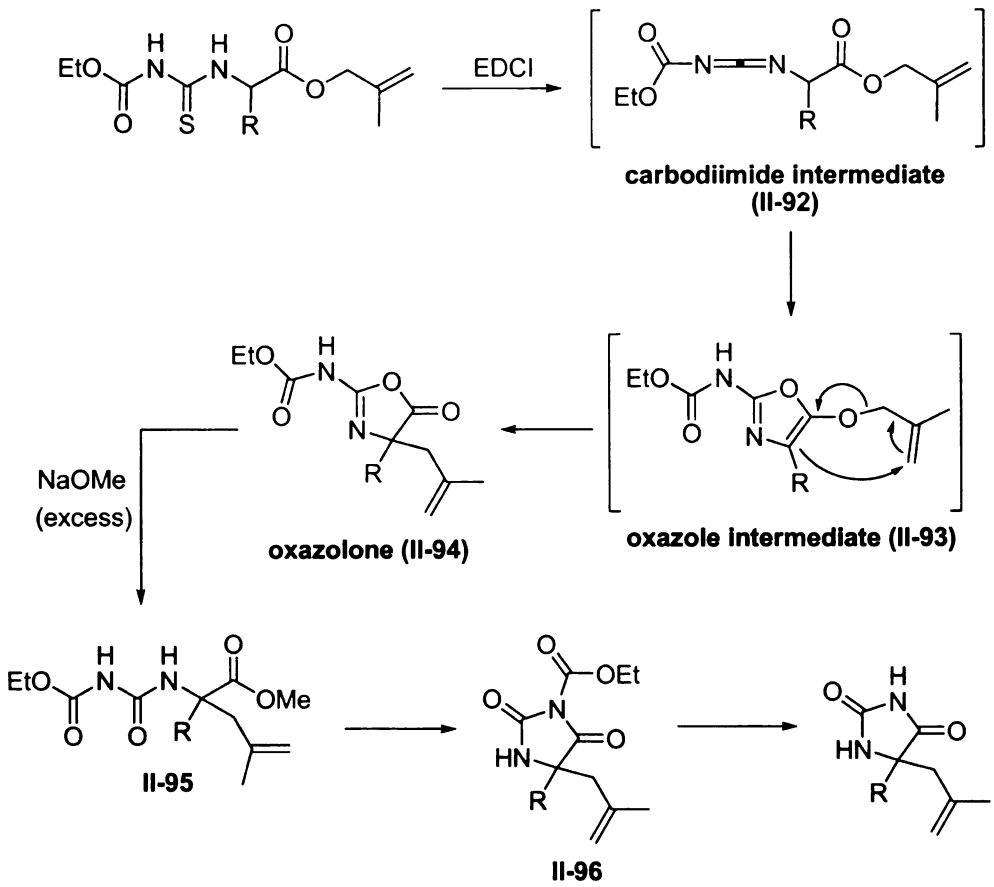
the carbodiir

cause a cycl

34).[102]

intermediate (**II-92**). Although this intermediate has not been isolated or observed experimentally, it is presumed to be formed based on previous studies.[122] Several reports suggest that thioureas undergo a desulfurization with reagents such as EDCI, $HgCl_2$, Mukaiyama's reagent, and others to afford a carbodiimide intermediate.[46, 122, 123] Subsequently, this transient carbodiimide could be attacked by an external nucleophile, such as an amine, to produce guanidines with various substitutions.[103-106, 108-111, 122, 123] Furthermore, cyclizations to form different heterocycles have also been shown to occur through an attack of a nucleophile on a carbodiimide formed from the desulfurization of a thiourea. Batey and co-workers produced various iminohydantoins through a cyclization that occured after a carbodiimide intermediate, formed using $HgCl_2$, was attacked by an internal amide.[47] Additionally, Drewry and co-workers utilized a desulfurization of a thiourea with Mukaiyama's reagent to form 2-aminoimidazolinones.[46] These examples serve as supportive evidence to reasonably conclude that the initial step in the rearrangement involves the formation of a carbodiimide intermediate. The carbodiimide is believed to be very reactive, thus giving reason to a short lifespan and consequently being unobservable spectroscopically. As a consequence of the carbodiimide's high reactivity, the carbonyl of the ester is believed to be nucleophilic enough to attack the carbodiimide, in a similar fashion as the amide in the report by Batey,[47] to cause a cyclization reaction affording an oxazole intermediate (**II-93**) (Scheme II-34).[102]

Scheme II-34. Proposed mechanism of EDCI-mediated rearrangement



After oxazole (**II-93**) undergoes a Claisen rearrangement, oxazolone intermediate (**II-94**) is formed (Scheme II-34). This intermediate was isolated and characterized with ^{1}HNMR, ^{13}CNMR and IR, which showed the characteristic absorption bands for the oxazolone (see experimental section for spectral information). It is proposed that from the oxazolone intermediate (**II-94**), the mechanism of the novel rearrangement continues with the nucleophilic opening of the oxazolone by sodium methoxide to yield urea (**II-95**). As a consequence of having excess sodium methoxide in the reaction mixture, urea (**II-95**) is deprotonated and cyclizes to form hydantoin (**II-96**), which is further modified by

sodium methoxide to yield the final quaternary hydantoin upon removal of the carbamate group (Scheme II-34).

The step in which the quaternary stereocenter was formed illustrates an oxazole rearrangement first described by Steglich and co-workers.[84, 85] Steglich discusses an additional hetero-Cope rearrangement that occurs with the oxazolone products to form oxazolin-5-ones, depending on the substitution on the allyl ester.[84, 85] However, it is noteworthy to mention that this additional hetero-Cope rearrangement was not observed in our rearrangement.

Overall, a novel rearrangement was developed that allows access to the scaffold needed for the synthesis of the natural product. The success of the N-tosyl indole and the 1, 1-disubstituted allylic moiety in the rearrangement ensures that the quaternary hydantoin formed from the pairing of those two structural features will lead to an intermediate capable of transforming into the natural product (**III-1**). The next chapter will discuss, in depth, the marine organism from which the natural product comes from as well as the first total synthesis of the natural product and two analogs.

II.G General experimental information

Reactions were carried out in flame-dried glassware under nitrogen atmosphere. All reactions were magnetically stirred and monitored by TLC with 0.25 μm pre-coated silica gel plates using either UV light or iodine to visualize the compounds. Column chromatography was carried out on Silica Gel 60 (230-400 mesh) supplied by EM Science. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. Infrared spectra were recorded on a Nicolet IR/42 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus-500 spectrometer or a Varian Inova-300, as noted in the experimental for each compound. Chemical shifts are reported relative to the residue peaks of the solvent ($CDCl_3$: 7.24 ppm for ^{1}H and 77.0 ppm for ^{13}C) (Acetone-d_6: 2.04 ppm for ^{1}H and 29.8 ppm for ^{13}C) (DMSO-d_6: 2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C). The following abbreviations are used to denote the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, and m = multiplet. HRMS were obtained with a Micromass Q-ToF Ultima API LC-MS/MS mass spectrometer. Elemental analysis data were obtained on a Perkin Elmer 2400 Series II CHNS/O analyzer. Purity of compounds, whose elemental analyses were above the ACS tolerated 0.4% deviation, were confirmed by ^{1}H NMR and ^{13}C NMR. Melting points were obtained using an Electrothermal® capillary melting point apparatus and are uncorrected. Reagents and solvents were purchased from commercial suppliers and used without further purification. Anhydrous methylene chloride and toluene were

dispensed from a delivery system which passes the solvents through a column packed with dry neutral alumina.

II.H Experimental procedures and characterization

(II-14).[124] To a flame dried 25 mL round bottom flask was added dry dioxane (10.0 mL) and KOH (0.352 g, 6.29 mmol). Then acetyl indole (1.00 g, 6.29 mmol) and benzaldehyde (1.27 mL, 12.6 mmol) were added and the mixture refluxed for 24 h under nitrogen. The precipitate was filtered off and recrystallized from EtOH to give yellow solid crystals. Yield (1.00 g, 65.0%). ^{1}H NMR (500 MHz), d-Acetone: δ 7.20-7.30 (m, 2H), 7.38-7.45 (m, 3H), 7.50-7.55 (m, 1H), 7.75-7.80 (m, 4H), 8.50 (m, 1H), 8.57 (s, 1H), 11.00 (bs, 1H); ^{13}C NMR (125 MHz), d-Acetone: δ 112.6, 119.4, 122.7, 123.2, 124.1, 125.3, 127.2, 129.0, 129.6, 130.4, 133.9, 136.6, 138.0, 140.6, 184.5. M.S: calculated for $C_{17}H_{13}NO$ (M+) = 247.1 and found (M+) = 247.1.

(II-15). To a 50 mL round bottom flask was added **II-14** (0.700 g, 2.83 mmol) and MeOH (20.0 mL). Then NaOH (1.95 mL of a 10% solution) was added to the reaction followed by H_2O_2 (1.95 mL, 17.0 mmol). The reaction refluxed for about 5 h under nitrogen. The reaction mixture was reduced to about 5.00 mL and then H_2O (20.0 mL) was added and the solid product precipitated out of solution and was filtered off. The product was recrystallized from EtOH. Yield (0.483 g, 65.0%). ^{1}H NMR (500 MHz), DMSO: δ 4.20 (d, J = 1.8 Hz, 1H), 4.49 (d, J = 1.9 Hz, 1H), 7.24 (m, 2H), 7.40 (m, 5H), 7.51 (m, 1H), 8.22 (dd, J = 1.2, 6.6 Hz, 1H), 8.65 (s, 1H), 12.20 (s, 1H); ^{13}C NMR (125MHz), DMSO: δ 57.9, 59.9, 112.2, 115.2, 121.2, 122.1, 123.3, 125.3, 126.2, 128.4, 128.5, 135.1, 136.2,

136.4, 187.3. IR (NaCl) 3190 cm^{-1}, 1625 cm^{-1}, 1612 cm^{-1}. M.S: calculated for $C_{17}H_{13}NO_2$ (M+) = 263.2 and found (M+) = 263.4. Melting Point = 232-234°C.

(II-16). To a 100 mL round bottom flask was added EtOH (30.0 mL) and **II-15** (0.250 g, 0.950 mmol). Then 10% Pd/C (1 scoop) was added and the round bottom was fitted with a hydrogen balloon and the mixture stirred at room temperature for 30 min. The mixture was filtered over celite and the filtrate concentrated. The crude material was purified by column chromatography (silica gel, 50% EtOAc; 50% hexane) affording the product as an oil. Yield (0.225 g, 89.0%). ^{1}H NMR (500 MHz), acetone: δ 2.95 (dd, J = 7.9, 13.9 Hz, 1H), 3.22 (dd, J = 4.2, 13.9 Hz, 1H), 4.24 (d, J = 6.6 Hz, 1H), 5.06 (s, 1H), 7.14-7.28 (m, 7H), 7.53 (m, 1H), 8.33 (m, 1H), 8.38 (s, 1H), 11.10 (s, 1H); ^{13}C NMR (125 MHz), Acetone: δ 43.9, 75.5, 112.8, 114.5, 122.7, 122.9, 124.1, 126.9, 127.0, 128.7, 130.4, 134.5, 137.6, 139.2, 196.6. IR (NaCl) 3389 cm^{-1}, 3261 cm^{-1}, 1633 cm^{-1}. HRMS: $[M + H]^+$ = 266.1181, calculated for $C_{17}H_{16}NO_2$, 266.1181.

(II-17). To a 50 mL round bottom flask was added **II-16** (0.169 g, 0.640 mmol) and EtOAc (20.0 mL). Then IBX (0.357 g, 1.28 mmol, synthesized according to Frigerio[125]) was added and the mixture refluxed overnight under nitrogen. The yellow mixture was filtered over celite and the filtrate concentrated. The crude material was purified by column chromatography (silica gel, 30% EtOAc; 70% hexane) affording the product as an oil. Yield (0.147 g, 88.0%). ^{1}H NMR (500 MHz), $CDCl_3$: δ 4.30 (s, 2H), 7.24-7.40 (m, 8H), 8.28 (d, J = 3.2 Hz, 1H), 8.43 (dd, J = 1.1, 8.6 Hz, 1H), 8.78 (s, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 44.1 , 111.5, 112.7, 122.4, 123.4, 124.3, 126.1, 127.0, 128.6, 129.8, 133.2, 135.8,

136.4, 183.7, 199.6. IR (NaCl) 3273 cm^{-1}, 1718 cm^{-1}, 1602 cm^{-1}. HRMS: $[M + H]^+$ = 264.1027, calculated for $C_{17}H_{14}NO_2$, 264.1025.

(II-18). To a flame dried 25 mL round bottom flask was added **II-17** (0.0740 g, 0.280 mmol) and dry THF (5.00 mL). Then dimethylguanidine hydrogen sulfate (0.114 g, 0.420 mmol) was added followed by NaH (0.0390 mg, 0.980 mmol). The reaction stirred at room temperature for 30 min and then at reflux for 2-3 h and again at room temperature overnight all under nitrogen. A precipitate was filtered off and washed with water and extracted with EtOAc (10 x 20.0 mL). The organics were combined and dried using anhydrous sodium sulfate and concentrated to give the product as a solid. Yield (0.0490 g, 53.0%). ^1H NMR (500 MHz), DMSO: δ 2.85 (s, 6H), 3.28 (d, J = 13.2 Hz, 1H), 3.46 (d, J = 12.9 Hz, 1H), 6.94 (t, J = 7.4 Hz, 1H), 7.07 (t, J = 7.3 Hz, 1H), 7.15-7.25 (m, 5H), 7.37 (d, J = 8.2 Hz, 1H), 7.41 (d, J = 7.9 Hz, 1H), 7.46 (s, 1H); ^{13}C NMR (125 MHz), DMSO: δ 37.8, 41.9, 68.8, 111.7, 115.4, 118.8, 119.9, 121.3, 123.4, 125.3, 126.5, 127.6, 130.6, 136.0, 136.8, 169.7, 187.6. IR (KBr) 3279 cm^{-1}, 1681 cm^{-1}, 1615 cm^{-1}. M.S: calculated for $C_{20}H_{20}N_4O$ (M+) = 332.4 and found (M+) = 332.2. Melting Point = 298-300°C decomposed.

(II-19). To a flame dried 25 mL round bottom flask was added isoamyltriphenylphosphonium bromide (0.690 g, 1.67 mmol) and anhydrous THF (6.00 mL). The mixture was cooled to -78°C and then NaHMDS (1.25 mL, 1.25 mmol) was added slowly and the resulting mixture stirred at -78°C for 1 h under nitrogen. Then a solution of N-tosyl-1H-indole-3-carboxaldehyde[126] (0.250 g, 0.836 mmol) in anhydrous THF (6.00 mL) was added and the mixture stirred at -

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78°C for 3 h and then warmed to room temperature overnight all under nitrogen. Water (5.00 mL) was then added and stirred for 30 min. The solvent was removed and the residue was extracted with DCM (2 x 20.0 mL) and washed with water (1 x 20.0 mL) and brine (1 x 20.0 mL). The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 50% ether; 50% hexane) affording the product as an oil. Yield (0.264 g, 89.0%). ^{1}H NMR (500 MHz), $CDCl_3$: δ 1.00 (d, J = 6.5 Hz, 6H), 1.80 (sep, J = 6.7 Hz, 1H), 2.26 (dt, J = 1.8 Hz, 6.8 Hz, 2H), 2.31 (s, 3H), 5.86-5.92 (dt, J = 7.0 Hz, 11.5 Hz, 1H), 6.46-6.49 (m, 1H), 7.20 (d, J = 8.1 Hz, 2H), 7.27 (t, J = 7.4 Hz, 1H), 7.36 (d, J = 8.3 Hz, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.58 (s, 1H), 7.80 (d, J = 8.5 Hz, 2H), 8.06 (d, J = 8.3 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 21.3, 22.4, 28.6, 38.6, 113.5, 117.9, 119.3, 119.4, 123.1, 123.3, 124.7, 126.6, 129.7, 130.8, 133.6, 134.5, 135.0, 144.7. IR: (NaCl) 1610 cm^{-1} (weak). HRMS: $[M + H]^+$ = 354.1536, calculated for $C_{21}H_{24}NO_2S$, 354.1528.

(II-20). To a 50 mL round bottom flask was added **II-19** (0.264 g, 0.748 mmol), acetone (18.0 mL) and water (2.00 mL). Then NMO (0.131 g, 1.12 mmol) and OsO_4 (0.760 mL, 0.0748 mmol) were added and the mixture stirred at RT overnight under nitrogen. The mixture was quenched with a saturated potassium sulfite solution (10.0 mL) and extracted with EtOAc (2 x 60.0 mL). The organics were washed with a brine solution (1 x 60.0 mL) and dried using anhydrous sodium sulfate and concentrated to give the product as a solid. Yield (0.288 g, 99.0%). ^{1}H NMR (500MHz), acetone: δ 0.75 (d, J = 6.6 Hz, 3H), 0.84 (d, J = 6.6 Hz, 3H), 1.24 (m, 1H), 1.32 (m, 1H), 1.80 (m, 1H), 2.32 (s, 3H), 3.68 (s, 1H), 3.98

(s, 1H), 4.32 (s, 1H), 4.84 (d, J = 4.4 Hz, 1H), 7.21 (t, J = 8.1 Hz, 1H), 7.28-7.35 (m, 3H), 7.64 (s, 1H), 7.71 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 8.3 Hz, 2H), 7.97 (d, J = 8.5 Hz, 1H); ^{13}C NMR (125MHz), acetone: δ 21.3, 21.8, 24.2, 25.1, 41.9, 72.2, 72.8, 114.3, 121.9, 123.8, 124.9, 125.2, 125.4, 127.6, 130.7, 131.0, 136.13, 136.17, 146.1. IR: (NaCl) 3425 (br) cm^{-1}. HRMS: $[M + H]^+$ = 370.1482, calculated for $C_{21}H_{24}NO_3S$, 370.1477 (**II-20** minus H_2O). Melting Point = 136-138°C.

(II-21). To a 50 mL round bottom flask was added **II-20** (0.288 g, 0.744 mmol), EtOAc (20.0 mL) and IBX (0.833 g, 2.98 mmol). The mixture was refluxed for 18 h under nitrogen and then the IBX byproduct was filtered off. The filtrate was concentrated and the crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% hexane) affording the product as an oil. Yield (0.163 g, 57.0%). ^1H NMR (500 MHz), $CDCl_3$: δ 0.99 (d, J = 6.7 Hz , 6H), 2.23 (m, 1H), 2.33 (s, 3H), 2.80 (d, J = 6.5 Hz, 2H), 7.24-7.26 (m, 2H), 7.34-7.37 (m, 2H), 7.83-7.85 (m, 2H), 7.93-7.95 (m, 1H), 8.32-8.34 (m, 1H), 8.74 (s, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 21.5, 22.6, 24.3, 45.9, 113.1, 115.2, 122.8, 125.0, 125.9, 127.2, 127.9, 130.2, 134.3, 134.4, 136.4, 146.0, 184.9, 201.1. IR: (KBr) 3175 cm^{-1}, 1712 cm^{-1}, 1651 cm^{-1}. HRMS: $[M + H]^+$ = 384.1273, calculated for $C_{21}H_{22}NO_4S$, 384.1270.

(II-22). To a 25 mL round bottom flask was added **II-21** (0.0540 g, 0.141 mmol) and MeOH (10.0 mL). Then K_2CO_3 (0.0490 g, 0.352 mmol) was added and the mixture stirred at RT for 1.5 h under nitrogen. The MeOH was taken off and the residue was put into solution with EtOAc (30.0 mL) and washed with 1%HCl (1 x 10.0 mL), water (1 x 10.0 mL) and brine (1 x 10.0 mL). The organics

were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% Hexane) affording the product as a yellow solid. Yield (0.0170 g, 53.0%). ^{1}H NMR (500 MHz), $CDCl_3$: δ 0.97 (d, J = 6.6 Hz, 6H), 2.24 (m, 1H), 2.83 (d, J = 7.0 Hz, 2H), 7.29-7.42 (m, 3H), 8.31 (d, J = 3.2 Hz, 1H), 8.42 (m, 1H), 9.15 (bs, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 22.6, 24.4, 46.3, 111.5, 112.5, 122.4, 123.3, 124.3, 126.1, 136.0, 136.1, 185.0, 203.1. IR (NaCl) 3200 cm^{-1}, 1712 cm^{-1}, 1608 cm^{-1}, 1584 cm^{-1}. M.S: calculated for $C_{14}H_{15}NO_2$ (M+) = 229.1 and found (M+) = 229.1. Melting Point = 130-132°C.

(II-23). To a flame dried 10 mL round bottom flask was added **II-22** (0.0750 g, 0.328 mmol) and dry THF (5.00 mL). Then dimethylguanidine hydrogen sulfate (0.134 g, 0.491 mmol) was added followed by NaH (0.0460 g, 1.15 mmol). The reaction stirred at room temperature for 30 min and then refluxed for 2-3 h and again at room temperature overnight all under nitrogen. A precipitate was filtered off and washed with water and extracted with EtOAc (10 x 20.0 mL). The filtrate was concentrated and the residue was extracted with EtOAc (3 x 20.0 mL) and washed with water (1 x 10.0 mL) and brine (1 x 10.0 mL). The organics were combined and dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 90% DCM; 10% MeOH) affording the product as a solid. Yield (0.0190 g, 19.0%). ^{1}H NMR (500 MHz), $CDCl_3$: δ 0.87 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.6 Hz, 3H), 1.65 (m, 1H), 2.01 (dd, J = 5.6 Hz, 11.1 Hz, 1H), 2.07 (dd, J = 5.6 Hz, 11.1Hz, 1H), 3.00-3.08 (bs, 3H), 3.09-3.14 (bs, 3H), 6.91 (t, J = 7.1 Hz, 1H), 7.03 (t,

J = 7.1 Hz, 1H), 7.24 (d, J = 2.7 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 8.26 (s, 1H), 10.9 (s, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 23.7, 24.0, 24.5, 36.1, 38.3, 44.5, 67.7, 111.3, 115.7, 118.3, 119.8, 120.8, 122.5, 124.8, 136.6, 169.7, 188.5. IR: (KBr) 3304 cm^{-1}, 1681 cm^{-1}, 1608 cm^{-1}. HRMS: $[M + H]^+$ = 299.1879, calculated for $C_{17}H_{23}N_4O$, 299.1872. Melting Point = 274-276°C decomposes.

(II-29). To a flame dried 50 mL round bottom flask was added **III-8** (0.104 g, 0.261 mmol) and anhydrous DCM (20.0 mL). Then TEA (0.0700 mL, 0.522 mmol) was added and the reaction was cooled to 0°C. Then benzoyl chloride (0.0400 mL, 0.392 mmol) was added slowly and the mixture warmed up to RT while stirring overnight under nitrogen. The mixture was washed with saturated $NaHCO_3$ (1 x 10.0 mL) and brine (1 x 10.0 mL) and the organics were dried using anhydrous sodium sulfate and concentrated. The crude material was purified using column chromatography (silica gel, 7:3 hexanes/ethyl acetate) to yield a solid (0.111 g, 85.0%). ^1H NMR (500 MHz), $CDCl_3$: δ 1.57 (s, 3H), 2.30 (s, 3H), 4.59 (q, *J* = 13.1 Hz, 2H), 4.85 (d, *J* = 7.1 Hz, 2H), 6.08 (d, *J* = 7.4 Hz, 1H), 6.96 (d, *J* = 7.3 Hz, 1H), 7.18-7.25 (m, 3H), 7.30-7.5 (m, 4H), 7.64-7.77 (m, 6H), 7.95 (d, *J* = 7.7 Hz, 1H) ; ^{13}C NMR (125 MHz), $CDCl_3$: δ 19.3, 21.5, 49.4, 69.2, 113.7, 113.9, 117.7, 119.9, 123.6, 124.9, 125.2, 126.9, 127.1, 128.5, 128.6, 129.9, 131.9, 133.4, 134.9, 135.1, 138.9, 145.2, 166.8, 170.1. IR: (NaCl) 3374 cm^{-1} , 1748 cm^{-1}, 1657 cm^{-1}. HRMS: $[M + H]^+$ = 503.1642, calculated for $C_{28}H_{27}N_2O_5S$, 503.1641. Melting Point = 118-120°C.

(II-30). To a flame dried 50 mL round bottom flask was added **III-8** (0.250 g, 0.628 mmol) and anhydrous DCM (20.0 mL). Then TEA (0.200 mL, 1.57 mmol) was added and the reaction was cooled to 0°C. Then acetyl chloride (0.0900 mL, 1.26 mmol) was added slowly and the mixture warmed up to RT while stirring overnight under nitrogen. The mixture was washed with saturated $NaHCO_3$ (1 x 10.0 mL) and brine (1 x 10.0 mL) and the organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 80% DCM; 20% EtOAc) affording the product as a solid. Yield (0.264 g, 95.0%). ^1H NMR (500 MHz), $CDCl_3$: δ 1.58 (s, 3H), 2.02 (s, 3H), 2.34 (s, 3H), 4.55 (d, J = 12.9, 1H), 4.59 (d, J = 13.0 Hz, 1H), 4.85 (s, 2H), 5.91 (d, J = 7.6 Hz, 1H), 6.40 (d, J = 6.6 Hz, 1H), 7.20-7.36 (m, 4H), 7.61 (s, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 8.3 Hz, 2H), 7.96 (d, J = 8.3 Hz, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 19.2, 21.5, 22.9, 48.9, 69.0, 113.6, 113.7, 117.6, 119.9, 123.5, 124.8, 125.2, 126.8, 128.4, 129.9, 134.9, 135.0, 138.9, 145.1, 169.4, 170.1. IR: (NaCl) 3291 cm^{-1}, 1748 cm^{-1}, 1651 cm^{-1}. HRMS: $[M + H]^+$ = 441.1486, calculated for $C_{23}H_{25}N_2O_5S$, 441.1484. Melting Point = 118-120°C.

(II-31). To a flame dried 25 mL round bottom flask was added **III-8** (1.48 g, 3.73 mmol) and anhydrous DCM (50.0 mL). Then TEA (3.29 mL, 23.8 mmol) was added and the reaction was cooled to 0°C. Then dimethylcarbamoyl chloride (1.50 mL, 16.4 mmol) was added slowly and the mixture warmed to RT overnight under nitrogen. The mixture was washed with saturated $NaHCO_3$ (1 x 10.0 mL) and brine (1 x 10.0 mL) and the organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column

chromatography (silica gel, 70% DCM; 30% EtOAc) affording the product as an oil. Yield (1.38 g, 79.0%). ^{1}H NMR (500 MHz), $CDCl_3$: δ 1.55 (s, 3H), 2.29 (s, 3H), 2.86 (s, 6H), 4.48 (d, J = 13.1 Hz, 1H), 4.57 (d, J = 13.0 Hz, 1H), 4.78 (d, J = 10.5 Hz, 2H), 5.30 (d, J = 7.5 Hz, 1H), 5.77 (d, J = 7.5 Hz, 1H), 7.15 (m, 4H), 7.55 (s, 1H), 7.65 (d, J = 8.2 Hz, 1H), 7.70 (d, J = 8.1 Hz, 2H), 7.91 (d, J = 8.5 Hz, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 19.1, 21.3, 36.0, 50.4, 68.6, 113.3, 113.4, 118.5, 120.0, 123.3, 124.4, 124.9, 126.7, 128.7, 129.7, 134.8, 134.9, 139.0, 144.9, 157.1, 171.1. IR: (NaCl) 3425 cm^{-1}, 3334 cm^{-1}, 1748 cm^{-1}, 1645 cm^{-1}. HRMS: $[M + H]^+$ = 470.1750, calculated for $C_{24}H_{28}N_3O_5S$, 470.1750.

(II-32). To a 250 mL flame dried round bottom flask was added **III-8** (2.00 g, 5.03 mmol) and anhydrous DCM (0.100 L). Then anhydrous TEA (1.60 mL, 11.6 mmol) was added and the mixture was cooled to 0°C. Trifluoroacetic anhydride (1.06 mL, 7.54 mmol) was then added dropwise and the reaction mixture was warmed to room temperature while stirring under nitrogen overnight. Saturated $NaHCO_3$ was added to quench the excess TFAA. The organics were separated from the aqueous solution and then washed with brine (1 x 50.0 mL). The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 80% hexanes; 20% EtOAc) affording the product as a solid. Yield (2.00 g, 80.0%). ^{1}H NMR (500 MHz), $CDCl_3$: δ 1.55 (s, 3H), 2.32 (s, 3H), 4.58 (d, J = 12.8 Hz, 1H), 4.64 (d, J = 12.9 Hz, 1H), 4.85 (s, 2H), 5.92 (d, J = 7.2 Hz, 1H), 7.22 (d, J = 8.7 Hz, 2H), 7.27 (t, J = 8.1 Hz, 1H), 7.36 (t, J = 8.3 Hz, 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.63 (d, J = 7.2 Hz, 1H), 7.74 (s, 1H), 7.76 (d, J = 8.5 Hz, 2H), 7.98 (d, J = 8.5 Hz, 1H);

^{13}C NMR (125 MHz), $CDCl_3$: δ 19.0, 21.3, 49.2, 69.6, 112.0, 113.6, 114.0, 114.3, 115.6, 116.5, 118.8, 119.4, 123.7, 125.3, 125.5, 126.7, 127.8, 129.8, 134.5, 134.8, 138.4, 145.3, 156.0, 156.3, 156.6, 156.9, 168.5. IR: (NaCl) 3346 cm^{-1}, 1742 cm^{-1}, 1718 cm^{-1}. HRMS: $[M + H]^+$ = 495.1206, calculated for $C_{23}H_{22}N_2O_5SF_3$, 495.1202. Melting Point = 119-121°C.

(II-33). To a flame dried 50 mL round bottom flask was added **III-8** (0.250 g, 0.628 mmol) and anhydrous DCM (20.0 mL). Then TEA (0.170 mL, 1.26 mmol) was added and the reaction was cooled to 0°C. Then ethyl (chlorocarbonyl)formate (0.100 mL, 0.942 mmol) was added slowly and the mixture stirred at 0°C for 1.5 h under nitrogen. Water (5.00 mL) was added and the organics were separated, washed with brine (1 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 70% hexane; 30% EtOAc) affording the product as an oil. Yield (0.172 g, 55.0%). ^1H NMR (500 MHz), $CDCl_3$: δ 1.32 (t, J = 7.3 Hz, 3H), 1.54 (s, 3H), 2.29 (s, 3H), 4.29 (q, J = 7.5 Hz, 2H), 4.55 (m, 2H), 4.82 (m, 2H), 5.87 (d, J = 8.1 Hz, 1H), 7.19 (d, J = 8.2 Hz, 1H), 7.21-7.31 (m, 3H), 7.59 (m, 1H), 7.64 (s, 1H), 7.74 (m, 2H), 7.93 (m, 2H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 13.8, 19.1, 21.4, 49.2, 63.3, 69.2, 113.6, 113.9, 116.3, 119.7, 123.5, 125.24, 125.26, 126.8, 128.0, 129.9, 134.8, 134.9, 138.6, 145.1, 155.8, 159.6, 168.8. IR: (NaCl) 3360 cm^{-1}, 1750 cm^{-1}, 1615 cm^{-1}. HRMS: $[M + H]^+$ = 499.1544, calculated for $C_{25}H_{27}N_2O_7S$, 499.1539.

(II-34). To a 100 mL flame dried round bottom flask was added **III-8** (0.311 g, 0.781 mmol) and anhydrous DCM (50.0 mL). Then DIPEA (0.270 mL, 1.56

mmol) was added and the mixture was cooled to 0°C. Then Troc-Cl (0.160 mL, 1.17 mmol) was then added dropwise and the reaction mixture was warmed to room temperature while stirring under nitrogen overnight. The solvent was removed and the residue was resolvated in ether (50.0 mL) and washed with 1% HCl (30.0 mL). The organics were separated and washed with brine (1 x 30.0 mL). The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 80% hexanes; 20% EtOAc) affording the product as a solid. Yield (0.264 g, 59.0%). ^{1}H NMR (500 MHz), $CDCl_3$: δ 1.57 (s, 3H), 2.34 (s, 3H), 4.57 (d, J = 12.7 Hz, 1H), 4.61 (d, J = 13.0 Hz, 1H), 4.72 (d, J = 12.2 Hz, 1H), 4.76 (d, J = 11.9 Hz, 1H), 4.85 (s, 2H), 5.70 (d, J = 7.8 Hz, 1H), 6.11 (d, J = 7.5 Hz, 1H), 7.20-7.28 (m, 3H), 7.34 (t, J = 8.3 Hz, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.66 (s, 1H), 7.76 (d, J = 8.3 Hz, 2H), 7.98 (d, J = 8.3 Hz, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 19.1, 21.4, 50.9, 69.2, 74.6, 95.1, 113.65, 113.69, 113.8, 117.1, 119.8, 123.5, 124.9, 125.2, 126.7, 126.81, 126.88, 128.1, 129.84, 129.89, 129.94, 134.8, 135.0, 138.7, 145.1, 153.6, 169.5. IR: (NaCl) 3370 cm^{-1}, 1742 cm^{-1}. HRMS: $[M + H]^+$ = 573.0430, calculated for $C_{24}H_{24}N_2O_6SCl_3$, 573.0421. Melting Point = 84-87°C.

(II-35). To a flame dried 50 mL round bottom flask was added **III-8** (0.250 g, 0.628 mmol) and anhydrous DCM (20.0 mL). Then TEA (0.200 mL, 1.57 mmol) was added and the reaction was cooled to 0°C. Then pivaloyl chloride (0.150 mL, 1.26 mmol) was added slowly and the mixture stirred at 0°C for 1.5 h under nitrogen. The mixture was washed with saturated $NaHCO_3$ (1 x 10.0 mL) and brine (1 x 10.0 mL) and the organics were dried using anhydrous sodium

sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 70% hexane; 30% EtOAc) affording the product as a solid. Yield (0.244 g, 80.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.20 (s, 9H), 1.57 (s, 3H), 2.32 (s, 3H), 4.53 (d, J = 13.0 Hz, 1H), 4.62 (d, J = 13.0 Hz, 1H), 4.84 (m, 2H), 5.87 (d, J = 7.1 Hz, 1H), 6.55 (d, J = 7.2 Hz, 1H), 7.20-7.34 (m, 4H), 7.6-7.62 (m, 2H), 7.75 (d, J = 8.5 Hz, 2H), 7.96 (d, J = 8.5 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 19.1, 21.4, 26.9, 27.2, 38.5, 49.0, 68.9, 113.6, 117.8, 119.7, 123.4, 124.6, 125.1, 126.7, 128.4, 129.8, 134.8, 135.0, 138.8, 145.0, 170.0, 177.8. IR: (NaCl) 3352 cm^{-1} , 1748 cm^{-1}, 1663 cm^{-1}. HRMS: $[M + H]^+$ = 483.1961, calculated for $C_{26}H_{31}N_2O_5S$, 483.1954. Melting Point = 114-116°C.

(II-36). To a 100 mL round bottom flask was added **II-29** (0.492 g, 0.980 mmol), dichloroethane (60.0 mL), and trifluoroacetic anhydride (1.37 mL, 9.80 mmol). The yellowish solution stirred at room temperature under nitrogen for 24 h and then was brought to reflux for 6 h and then stirred overnight at room temperature. The organics were washed with sodium bicarbonate (2 x 20.0 mL) and brine (2 x 10.0 mL), combined, dried using anhydrous sodium sulfate, and concentrated. The crude material was purified using column chromatography (silica gel, 8:2 hexanes/ethyl acetate) to yield a yellowish oil (0.360 g, 76.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.74 (s, 3H), 2.31 (s, 3H), 2.93 (dd, *J* = 5.8, 13.4 Hz, 2H), 4.80 (d, *J* = 17.8 Hz, 2H), 7.20-7.32 (m, 5H), 7.49-7.59 (m, 3H), 7.72 (s, 1H), 7.75 (d, *J* = 8.3 Hz, 2H), 7.94 (d, *J* = 9 Hz, 1H), 8.04-8.06 (m, 2H) ; ^{13}C NMR (125MHz), $CDCl_3$: δ 21.5, 24.3, 46.1, 72.8, 113.6, 116.6, 119.3, 122.0, 123.3, 123.5, 124.9, 125.7, 126.9, 127.8, 128.1, 128.8, 129.9, 132.9, 135.1, 135.4,

139.2, 145.1

495.1542, ca

(II-37

g, 0.227 mr

0.794 mmol

solvent was

and washed

the organic

crude resid

20%: EtOAc

300MHz),

22.5 Hz, 2

2H), 8.06-8

$CDCl_3$: δ 2

127.1, 127

1779 cm^{-1}

423.1379.

(II-3

g, 0.320 r

0.991 mm

The solve

(20.0 mL)

mL) and

139.2, 145.1, 160.7, 177.6. IR: (NaCl) 1821 cm^{-1}, 1657 cm^{-1}. HRMS: $[M + H]^+$ = 485.1542, calculated for $C_{28}H_{25}N_2O_4S$, 485.1535.

(II-37). To a flame dried 25 mL round bottom flask was added **II-30** (0.100 g, 0.227 mmol) and anhydrous benzene (12.0 mL). Then $POCl_3$ (0.0600 mL, 0.704 mmol) was added and the mixture refluxed overnight under nitrogen. The solvent was removed and the residue was put into solution with EtOAc (20.0 mL) and washed with saturated $NaHCO_3$ (1 x 10.0 mL) and brine (1 x 10.0 mL) and the organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 80% Hexane; 20% EtOAc) affording the product as a solid. Yield (0.0690 mg, 72.0%). ^1H NMR (300MHz), $CDCl_3$: δ 1.72 (s, 3H), 1.79 (s, 3H), 2.39 (s, 3H), 2.73 (dd, J = 14.1 Hz, 22.5 Hz, 2H), 4.82 (s, 1H), 4.97 (s, 1H), 7.28-7.46 (m, 4H), 7.91 (d, J = 8.7 Hz, 2H), 8.06-8.09 (m, 1H), 8.37-8.40 (m, 1H), 8.92 (s, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 21.5, 24.0, 24.7, 46.8, 106.6, 111.1, 113.3, 117.4, 122.5, 124.4, 125.7, 127.1, 127.7, 130.0, 131.5, 134.6, 134.7, 138.5, 145.6, 151.5, 164.7. IR: (NaCl) 1779 cm^{-1}, 1627 cm^{-1}. HRMS: $[M + H]^+$ = 423.1379, calculated for $C_{23}H_{23}N_2O_4S$, 423.1379. Melting Point = 121-123°C.

(II-38). To a flame dried 50 mL round bottom flask was added **II-31** (0.150 g, 0.320 mmol) and anhydrous benzene (20.0 mL). Then $POCl_3$ (0.0900 mL, 0.991 mmol) was added and the mixture was refluxed overnight under nitrogen. The solvent was then taken off and the residue was put into solution with EtOAc (20.0 mL) and washed with saturated $NaHCO_3$ (1 x 10.0 mL) and brine (1 x 10.0 mL) and the organics were dried using anhydrous sodium sulfate and

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$CDCl_3$: δ 1.

s, 3H), 3.7

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1H); ^{13}C N

115.8, 12

144.6, 15

=484.191

(II-

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residue

hexane

concentrated. The residue was then resuspended in benzene (20.0 mL) and MeOH (5.00 mL) and $TMSCHN_2$ (0.480 mL, 0.960 mmol) was added slowly. Once gas evolution ceased, the solvents were taken off and the crude residue was purified by column chromatography (silica gel, 90% DCM; 10% EtOAc) affording the product as an oil. Yield (0.134 g, 86.0%). ^1H NMR (500 MHz), $CDCl_3$: δ 1.63 (s, 3H), 2.27 (s, 3H), 2.84 (s, 6H), 3.10 (d, J = 13.5 Hz, 1H), 3.61 (s, 3H), 3.76 (d, J = 13.0 Hz, 1H), 4.71 (s, 1H), 4.90 (s, 1H), 5.96 (s, 1H), 7.12-7.22 (m, 4H), 7.49 (d, J = 9.1 Hz, 1H), 7.70-7.72 (m, 3H), 7.85 (d, J = 9.5 Hz, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 21.4, 23.4, 36.0, 41.3, 52.9, 61.3, 113.6, 115.8, 120.1, 122.1, 123.1, 124.2, 125.1, 126.8, 128.4, 129.7, 135.1, 140.7, 144.6, 155.8, 173.4. IR: (NaCl) 3443 cm^{-1}, 1736 cm^{-1}, 1663 cm^{-1}. HRMS: $[M + H]^+$ = 484.1915, calculated for $C_{25}H_{30}N_3O_5S$, 484.1906.

(II-39). To a flame dried 25 mL round bottom flask was added **II-32** (0.100 g, 0.202 mmol) and anhydrous acetonitrile (10.0 mL). Then anhydrous TEA (0.140 mL, 1.03 mmol) was added and the mixture was cooled down to 0°C. Then phosgene (0.240 mL, 0.465 mmol) was added and the reaction turned yellow and stirred at 0°C for 2 h. The reaction was quenched by being poured into an ice water mix (10.0 mL). EtOAc was added (3 x 20.0 mL) to extract the organics, which were dried and concentrated. The crude residue was resolvated into a basic methanolic solution and stirred for 20 min. The reaction was neutralized with a 1% HCl solution and the solvent was removed. The crude residue was purified was purified by column chromatography (silica gel, 90% hexanes; 10% EtOAc) affording the product as a solid. Yield (0.0530 mg, 55.0%).

¹H NMR (500M

3.68 (s, 3H), 3

4H), 7.32 (d, J

9.3 Hz, 1H),

6.13, 113.9, 1

129.9, 134.8,

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(II-40)

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¹H NMR (5(

3.67 (s, 3H

7.12 (d, J =

d, J = 9.5

^{1}H NMR (500MHz), $CDCl_3$: δ 1.71 (s, 3H), 2.30 (s, 3H), 3.20 (d, J = 13.4 Hz, 1H), 3.68 (s, 3H), 3.78 (d, J = 13.6 Hz, 1H), 4.81 (s, 1H), 4.99 (s, 1H), 7.14-7.27 (m, 4H), 7.32 (d, J = 8.0 Hz, 1H), 7.71 (d, J = 8.3 Hz, 2H), 7.79 (s, 1H), 7.87 (d, J = 9.3 Hz, 1H), 7.91 (s, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 21.4, 22.8, 40.2, 53.7, 61.3, 113.9, 114.1, 116.3, 117.3, 118.9, 119.2, 123.6, 124.9, 126.0, 126.8, 127.4, 129.9, 134.8, 135.1, 138.6, 145.1, 154.8, 155.1, 172.0. IR: (NaCl) 3389 cm^{-1}, 1730 (with shoulder) cm^{-1}. HRMS: $[M + H]^+$ = 509.1358, calculated for $C_{24}H_{24}N_2O_5SF_3$, 509.1358. Melting Point = 166-168°C.

(II-40). To a flame dried 25 mL round bottom flask was added **II-33** (0.0860 mg, 0.173 mmol) and anhydrous acetonitrile (10.0 mL). Then anhydrous TEA (0.120 mL, 0.882 mmol) was added and the mixture was cooled down to 0°C. Then phosgene (0.210 mL, 0.397 mmol) was added and the reaction turned yellow and stirred at 0°C for 2 h. The reaction was quenched by being poured into an ice water mix (10.0 mL). EtOAc was added (3 x 20.0 mL) to extract the organics, which were dried and concentrated. The crude residue was resolvated into a basic methanolic solution and stirred for 20 min. The reaction was neutralized with a 1% HCl solution and the solvent was removed. The crude residue was purified was purified by column chromatography (silica gel, 60% hexanes; 40% EtOAc) affording the product as a solid. Yield (0.0470 mg, 55.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.68 (s, 3H), 2.30 (s, 3H), 3.18 (d, J = 13.4 Hz, 1H), 3.67 (s, 3H), 3.79 (d, J = 11.9 Hz, 1H), 3.80 (s, 3H), 4.78 (s, 1H), 4.93 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 7.21 (d, J = 9.5 Hz, 2H), 7.39 (d, J = 8.1 Hz, 1H), 7.72 (d, J = 9.5 Hz, 2H), 7.77 (s, 1H), 7.84 (d, J = 8.3 Hz, 1H), 8.61 (s, 1H); ^{13}C NMR

125MHz).

123.4, 124

160.5, 171

499.1545.

(II-4

0.0440 g.

0.118 mmo

for 5 min.

mixture re

removed a

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sodium s

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oil. Yield

3H), 2.90

1H), 4.76

3H), 7.95

44.2, 63.

128.5, 12

3258 cm

$C_{28}H_{28}N_3$

(II

(0.0500 g

(125MHz), $CDCl_3$: δ 21.5, 23.0, 40.2, 53.4, 53.5, 61.3, 113.7, 116.8, 119.6, 119.7, 123.4, 124.7, 125.6, 126.8, 127.7, 129.9, 134.9, 135.0, 139.2, 145.0, 154.3, 160.5, 171.9. IR: (NaCl) 3388 cm^{-1}, 1742 cm^{-1}, 1712 cm^{-1}. HRMS: $[M + H]^+$ = 499.1545, calculated for $C_{25}H_{27}N_2O_7S$, 499.1539. Melting Point = 180-182°C.

(II-41). To a flame dried 25 mL round bottom flask was added **II-38** (0.0440 g, 0.0910 mmol) and anhydrous CH_3CN (10.0 mL). Then BOP (0.0520 g, 0.118 mmol) and DBU (0.200 mL, 1.37 mmol) were added and the mixture stirred for 5 min. Then benzyl amine (0.0100 mL, 0.137 mmol) was added and the mixture refluxed overnight under a nitrogen atmosphere. The solvent was removed and the residue was resolvated with EtOAc (20.0 mL) and washed with water (10.0 mL) and brine (10.0 mL). The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 4% MeOH; 96% DCM) affording the product as an oil. Yield (0.0330 g, 71.0%). ^1H NMR (500MHz), $CDCl_3$: δ 1.41 (s, 3H), 2.31 (s, 3H), 2.90 (m, 2H), 4.61 (d, J = 14.5 Hz, 1H), 4.67 (d, J = 14.5 Hz, 1H), 4.70 (s, 1H), 4.76 (s, 1H), 6.75 (s, 1H), 7.16-7.38 (m, 9H), 7.63 (s, 1H), 7.71-7.78 (m, 3H), 7.95 (d, J = 9.0 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 21.5, 23.7, 42.5, 44.2, 63.8, 113.7, 117.3, 120.2, 121.5, 123.5, 123.9, 125.1, 126.8, 127.4, 127.8, 128.5, 128.6, 129.9, 134.9, 135.5, 135.6, 138.3, 145.2, 156.6, 173.1. IR: (NaCl) 3258 cm^{-1}, 1774 cm^{-1}, 1715 cm^{-1}. HRMS: $[M + H]^+$ = 514.1807, calculated for $C_{29}H_{28}N_3O_4S$, 514.1801.

(II-42). To a flame dried 25 mL round bottom flask was added **II-38** (0.0500 g, 0.104 mmol) and anhydrous CH_3CN (10.0 mL). Then BOP (0.0590 g,

0.135 mmo

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oil. Yield (0

m, 6H), 1.

d, J = 13.

1H), 4.92

Hz, 2H), 7

$CDCl_3$: δ

114.0, 11

135.9, 13

cm^{-1}, 162

577.2485

(II-

g, 5.29 m

mmol) ar

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The whit

0.135 mmol) and DBU (0.0230 mL, 0.156 mmol) were added and the mixture stirred for 5 min. Then water (0.400 mL, 22.2 mmol) was added and the mixture refluxed overnight under a nitrogen atmosphere. The solvent was removed and the residue was resolvated with EtOAc (20.0 mL) and washed with water (10.0 mL) and brine (10.0 mL). The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 4% MeOH; 96% DCM) affording the product as an oil. Yield (0.0330 g, 55.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.58 (s, 3H), 1.59-1.73 (m, 6H), 1.84 (m, 2H), 2.35 (s, 3H), 2.50 (m, 2H), 2.93 (d, J = 13.7 Hz, 1H), 2.99 (d, J = 13.7 Hz, 1H), 3.28 (m, 2H), 3.41 (m, 2H), 3.53 (t, J = 7.1 Hz, 2H), 4.82 (s, 1H), 4.92 (s, 1H), 6.96 (s, 1H), 7.22-7.36 (m, 4H), 7.67 (s, 1H), 7.78 (d, J = 8.5 Hz, 2H), 7.87 (d, J = 7.8 Hz, 1H), 7.97 (d, J = 8.3 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 21.8, 23.6, 24.3, 26.9, 28.8, 30.1, 37.0, 37.4, 44.4, 45.8, 49.7, 64.0, 114.0, 117.4, 120.5, 121.8, 123.8, 124.2, 125.3, 127.1, 127.7, 130.2, 135.1, 135.9, 138.9, 145.5, 157.0, 173.6, 176.1. IR: (NaCl) 3243 cm^{-1}, 1779 cm^{-1}, 1718 cm^{-1}, 1621 cm^{-1}. HRMS: $[M + H]^+$ = 577.2490, calculated for $C_{31}H_{37}N_4O_5S$, 577.2485.

(II-49). To a flame dried 100 mL round bottom flask was added **II-43** (1.00 g, 5.29 mmol) and anhydrous DCM (50.0 mL). Then allyl alcohol (0.720 mL, 10.6 mmol) and DMAP (0.0650 g, 0.529 mmol) were added and the mixture was brought down to 0°C. DCC (1.63 g, 7.94 mmol) was then added and the mixture was allowed to warm to room temperature overnight while stirring under nitrogen. The white precipitate that formed was filtered off and the DCM filtrate was

washed with brine (1 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% Hexane) affording the product as an oil. Yield (1.09 g, 90.0%). ^{1}H NMR (500 MHz), $CDCl_3$: δ 1.33 (d, J = 7.1 Hz, 3H), 1.37 (s, 9H), 4.26 (bs, 1H), 4.56 (m, 2H), 5.08 (bs, 1H), 5.17-5.27 (m, 2H), 5.81-5.87 (m, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 18.4, 28.2, 49.1, 65.6, 79.6, 118.4, 131.6, 155.0, 172.9. IR: (NaCl) 3380 cm^{-1}, 1750 cm^{-1}, 1710 cm^{-1}. HRMS: $[M + H]^+$ = 230.1401, calculated for $C_{11}H_{20}NO_4$, 230.1392. Anal. Calcd. For $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 58.89; H, 8.22; N, 5.90.

(II-50). To a flame dried 100 mL round bottom flask was added **II-44** (1.38 g, 5.23 mmol) and anhydrous DCM (50.0 mL). Then allyl alcohol (0.710 mL, 10.5 mmol) and DMAP (0.0640 g, 0.523 mmol) were added and the mixture was brought down to 0°C. DCC (1.62 g, 7.85 mmol) was then added and the mixture was allowed to warm to room temperature overnight while stirring under nitrogen. The white precipitate that formed was filtered off and the DCM filtrate was washed with brine (1 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% Hexane) affording the product as a whitish solid. Yield (1.34 g, 84.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.39 (s, 9H), 3.02-3.14 (m, 2H), 4.58 (d, J = 6.6 Hz, 2H), 4.96 (bs, 1H), 5.20-5.30 (m, 2H), 5.80-5.88 (m, 1H), 7.12 (m, 2H), 7.20-7.29 (m, 3H); ^{13}C NMR (125MHz), $CDCl_3$: δ 28.2, 38.3, 54.4, 65.8, 79.8, 118.8, 126.9, 128.4, 129.3, 131.5, 135.9, 155.0, 171.5. IR: (NaCl) 3380 cm^{-1}, 1750 cm^{-1}, 1710 cm^{-1}. HRMS: $[M + H]^+$ = 306.1703, calculated for $C_{17}H_{24}NO_4$,

506.1705. A

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Anal. Calc

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(II-

10.992 g.

mL, 7.06

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306.1705. Anal. Calcd. For $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 68.29; H, 7.41; N, 4.62. Melting Point = 62-64°C.

(II-51). To a flame dried 100 mL round bottom flask was added **II-45** (1.50 g, 5.97 mmol) and anhydrous DCM (50.0 mL). Then allyl alcohol (0.820 mL, 12.0 mmol) and DMAP (0.0730 g, 0.597 mmol) were added and the mixture was brought down to 0°C. DCC (1.85 g, 8.96 mmol) was then added and the mixture was allowed to warm to room temperature overnight while stirring under nitrogen. The white precipitate that formed was filtered off and the DCM filtrate was washed with brine (1 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% Hexane) affording the product as a whitish solid. Yield (1.49 g, 86.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.41 (s, 9H), 4.58-4.60 (m, 2H), 5.12-5.17 (m, 2H), 5.32 (d, J = 7.3 Hz, 1H), 5.56 (bs, 1H), 5.75-5.83 (m, 1H), 7.26-7.36 (m, 5H); ^{13}C NMR (125MHz), $CDCl_3$: δ 28.2, 57.6, 65.9, 80.0, 118.3, 127, 128.3, 128.7, 131.3, 136.8, 154.7, 170.7. IR: (NaCl) 3390 cm^{-1} , 1750 cm^{-1}, 1710 cm^{-1}. HRMS: $[M + H]^+$ = 292.1559, calculated for $C_{16}H_{22}NO_4$, 292.1549. Anal. Calcd. For $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81. Found: C, 66.78; H, 7.14; N, 4.78. Melting Point = 40-42°C.

(II-52). To a flame dried 100 mL round bottom flask was added **II-46** (0.992 g, 3.53 mmol) and anhydrous DCM (50.0 mL). Then allyl alcohol (0.480 mL, 7.06 mmol) and DMAP (0.0430 g, 0.353 mmol) were added and the mixture was brought down to 0°C. DCC (1.09 g, 5.30 mmol) was then added and the mixture was allowed to warm to room temperature overnight while stirring under

itrogen. The

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J = 5.3 Hz,

8.7 Hz. 2H

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NaCl) 339

for $C_{17}H_{24}$N

Found: C,

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(m. 2H

7.31-7

nitrogen. The white precipitate that formed was filtered off and the DCM filtrate was washed with brine (1 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% hexane) affording the product as a whitish solid. Yield (1.07 g, 94.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.32 (s, 9H), 3.63 (s, 3H), 4.48 (d, J = 5.3 Hz, 2H), 5.14 (m, 2H), 5.19 (d, J = 7.5 Hz, 1H), 5.69 (m, 2H), 6.74 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H); ^{13}C NMR (125MHz), $CDCl_3$: δ 27.9, 54.7, 56.7, 65.4, 79.4, 113.8, 117.8, 128.0, 128.5, 131.1, 154.5, 159.2, 170.6. IR: (NaCl) 3395 cm^{-1}, 1743 cm^{-1}, 1711 cm^{-1}. HRMS: $[M + H]^+$ = 322.1653, calculated for $C_{17}H_{24}NO_5$, 322.1654. Anal. Calcd. For $C_{17}H_{23}NO_5$: C, 63.54; H, 7.21; N, 4.36. Found: C, 63.69; H, 7.14; N, 4.61. Melting Point = 60-62°C.

(II-53). To a flame dried 100 mL round bottom flask was added **II-47** (0.625 g, 2.32 mmol) and anhydrous DCM (50.0 mL). Then allyl alcohol (0.320 mL, 4.65 mmol) and DMAP (0.0280 g, 0.232 mmol) were added and the mixture was brought down to 0°C. DCC (0.717 g, 3.48 mmol) was then added and the mixture was allowed to warm to room temperature overnight while stirring under nitrogen. The white precipitate that formed was filtered off and the DCM filtrate was washed with brine (1 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% hexane) affording the product as an oil. Yield (0.626 g, 87.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.40 (s, 9H), 4.59 (d, J = 5.5 Hz, 2H), 5.15 (m, 2H), 5.29 (d, J = 6.9 Hz, 1H), 5.57 (s, 1H), 5.79 (m, 1H), 6.09-7.02 (m, 2H), 7.31-7.33 (m, 2H); ^{13}C NMR (125MHz), $CDCl_3$: δ 28.2, 56.9, 66.1, 80.2, 115.7 (d,

J = 11.7 Hz), 118.6, 128.83 (d, J = 8.3 Hz), 131.1, 132.8, 154.7, 162.6 (d, J = 247.5 Hz), 170.5. IR: (NaCl) 3383 cm^{-1} , 1749 cm^{-1}, 1711 cm^{-1}. HRMS: $[M + H]^+$ = 310.1463, calculated for $C_{16}H_{21}FNO_4$, 310.1455. Anal. Calcd. For $C_{16}H_{20}FNO_4$: C, 62.12; H, 6.52; N, 4.53. Found: C, 61.93; H, 6.29; N, 4.49.

(II-54). To a flame dried 100 mL round bottom flask was added **II-48** (1.30 g, 4.32 mmol) and anhydrous DCM (50.0 mL). Then allyl alcohol (0.590 mL, 8.64 mmol) and DMAP (0.0530 g, 0.432 mmol) were added and the mixture was brought down to 0°C. DCC (1.33 g, 6.48 mmol) was then added and the mixture was allowed to warm to room temperature overnight while stirring under nitrogen. The white precipitate that formed was filtered off and the DCM filtrate was washed with brine (1 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% hexane) affording the product as an oil. Yield (1.13 g, 77.0%). ^1H NMR (500MHz), $CDCl_3$: δ 1.42 (s, 9H), 4.61-4.64 (m, 2H), 5.08-5.15 (m, 2H), 5.49 (d, J = 7.5 Hz, 1H), 5.77 (m, 1H), 6.09 (d, J = 7.8 Hz, 1H), 7.40-7.58 (m, 4H), 7.82 (m, 1H), 7.86 (d, J = 8.7 Hz, 1H), 8.17 (d, J = 8.4 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 28.2, 54.7, 66.1, 80.2, 118.4, 123.3, 125.2, 125.4, 126.0, 126.9, 128.8, 129.3, 131.0, 131.3, 132.8, 134.0, 155.0, 171.5. IR: (NaCl) 3389 cm^{-1} , 1749 cm^{-1}, 1711 cm^{-1}. HRMS: $[M + H]^+$ = 342.1706, calculated for $C_{20}H_{24}NO_4$, 342.1705. Anal. Calcd. For $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; N, 4.10. Found: C, 67.48; H, 6.43; N, 3.85.

(II-55). To a flame dried 100 mL round bottom flask was added **II-45** (1.50 g, 5.97 mmol) and anhydrous DCM (50.0 mL). Then 2-methyl-2-propen-1-ol (1.00

mL, 12.0 mmol) and DMAP (0.0730 g, 0.597 mmol) were added and the mixture was brought down to 0°C. DCC (1.85 g, 8.96 mmol) was then added and the mixture was allowed to warm to room temperature overnight while stirring under nitrogen. The white precipitate that formed was filtered off and the DCM filtrate was washed with brine (1 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% hexane) affording the product as a whitish solid. Yield (1.39 g, 76.0%). %). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.41 (s, 9H), 1.58 (s, 3H), 4.46-4.58 (m, 2H), 4.78 (s, 1H), 4.81 (s, 1H), 5.33 (d, J = 7.0 Hz, 1H), 5.56 (s, 1H), 7.27-7.37 (m, 5H); ^{13}C NMR (125MHz), $CDCl_3$: δ 19.1, 28.2, 57.7, 68.6, 80.0, 113.2, 127.1, 128.3, 128.8, 136.9, 139.1, 154.7, 170.8. IR: (NaCl) 3389 cm^{-1}, 1743 cm^{-1}, 1711 cm^{-1}. HRMS: $[M + H]^+$ = 306.1705, calculated for $C_{17}H_{24}NO_4$, 306.1705. Anal. Calcd. For $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.95; H, 7.66; N, 4.64. Melting Point = 38-40°C.

(II-56). To a 100 mL round bottom flask was added **II-49** (1.32 g, 4.32 mmol), DCM (2.50 mL) and TFA (2.50 mL). The resulting solution stirred for 30 min. The excess TFA and DCM were removed and $CHCl_3$ (3 x 15.0 mL) was added and subsequently taken off to remove any residual TFA. The crude material (oil) was taken on without further purification. Yield (1.00 g, 88.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.56 (d, J = 7.3 Hz, 3H), 4.10 (q, J = 7.1 Hz, 1H), 4.63 (m, 2H), 5.25-5.32 (m, 2H), 5.8-5.9 (m, 1H), 8.24 (s, 3H); ^{13}C NMR (125MHz), $CDCl_3$: δ 15.6, 49.2, 67.1, 115.8 (q, J = 290.9 Hz), 119.7, 130.5, 161.8 (q, J =

36.9 Hz), 16

= 130.0872

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36.1, 53

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4.31. M

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(m,

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36.9 Hz), 169.9. IR: (KBr) 3100 (br) cm^{-1}, 1748cm^{-1}, 1675 cm^{-1}. HRMS: $[M + H]^+$ = 130.0872, calculated for $C_6H_{11}NO_2$, 130.0868.

(II-57). To a 100 mL round bottom flask was added **II-50** (1.32 g, 4.32 mmol), DCM (2.50 mL) and TFA (2.50 mL). The resulting solution stirred for 30 min. The excess TFA and DCM were removed and $CHCl_3$ (3 x 15.0 mL) was added and subsequently taken off to remove any residual TFA. The product was precipitated out of the crude residue using ether/petroleum ether to afford the product as a white solid. Yield (1.27 g, 92.0%). ^1H NMR (500MHz), DMSO: δ 3.04-3.19 (m, 2H), 4.32 (t, J = 6.1 Hz, 1H), 4.58 (m, 2H), 5.18-5.26 (m, 2H), 5.73-5.81 (m, 1H), 7.20-7.36 (m, 5H), 8.64 (bs, 3H); ^{13}C NMR (125MHz), DMSO: δ 36.1, 53.2, 65.8, 117.1 (q, J = 299.2 Hz), 118.6, 127.2, 128.5, 129.3, 131.4, 134.6, 158.4 (q, J = 31.3 Hz), 168.7. IR: (KBr) 3100 (br) cm^{-1}, 1745 cm^{-1}, 1660 cm^{-1}. HRMS: $[M + H]^+$ = 206.1188, calculated for $C_{12}H_{16}NO_2$, 206.1181. Anal. Calcd. For $C_{14}H_{16}F_3NO_4$: C, 52.67; H, 5.05; N, 4.39. Found: C, 52.69; H, 4.91; N, 4.31. Melting Point = 88-90°C.

(II-58). To a 100 mL round bottom flask was added **II-51** (1.44 g, 4.95 mmol), DCM (2.50 mL) and TFA (2.50 mL). The resulting solution stirred for 30 min. The excess TFA and DCM were removed and $CHCl_3$ (3 x 15.0 mL) was added and subsequently taken off to remove any residual TFA. The product was precipitated out of the crude residue using ether/petroleum ether to afford the product as a white solid. Yield (1.48 g, 98.0%).^1H NMR (500MHz), DMSO: δ 4.66 (m, 2H), 5.12-5.16 (m, 2H), 5.33 (s, 1H), 5.80 (m, 1H), 7.44-7.50 (m, 5H), 9.07 (bs, 3H); ^{13}C NMR (125MHz), DMSO: δ 55.4, 66.0, 117.1 (q, J = 299.7 Hz),

118.1, 128.1

3150 (br) cm

$C_{11}H_{14}NO_2$,

Found: C, 5

(II-5

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precipitate

product a

3.76 (s, 3

J = 8.9

DMSO:

131.5,

1680 c

Anal.

4.64;

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118.1, 128.1, 129.0, 129.5, 131.5, 132.6, 158.3 (q, J = 31.3 Hz), 168.1. IR: (KBr) 3150 (br) cm^{-1}, 1745 cm^{-1}, 1675 cm^{-1}. HRMS: $[M + H]^+$ = 192.1027, calculated for $C_{11}H_{14}NO_2$, 192.1025. Anal. Calcd. For $C_{13}H_{14}F_3NO_4$: C, 51.15; H, 4.62; N, 4.59. Found: C, 50.95; H, 4.51; N, 4.45. Melting Point = 96-98°C.

(II-59). To a 100 mL round bottom flask was added **II-52** (1.02 g, 3.17 mmol), DCM (2.00 mL) and TFA (2.00 mL). The resulting solution stirred for 30 min. The excess TFA and DCM were removed and $CHCl_3$ (3 x 15.0 mL) was added and subsequently taken off to remove any residual TFA. The product was precipitated out of the crude residue using ether/petroleum ether to afford the product as a white solid. Yield (0.953 g, 90.0%). ^1H NMR (500MHz), DMSO: δ 3.76 (s, 3H), 4.66 (m, 2H), 5.14-5.18 (m, 2H), 5.26 (s, 1H), 5.82 (m, 1H), 7.01 (d, J = 8.9 Hz, 2H), 7.41 (d, J = 8.8 Hz, 2H), 8.91 (s, 3H); ^{13}C NMR (125MHz), DMSO: δ 54.8, 55.2, 65.9, 114.3, 117.2 (d, J = 300.3 Hz), 118.2, 124.4, 129.6, 131.5, 158.1 (q, J = 30.6 Hz), 160.0, 168.4. IR: (KBr) 3088 (br) cm^{-1}, 1749 cm^{-1}, 1680 cm^{-1}. HRMS: $[M + H]^+$ = 222.1140, calculated for $C_{14}H_{16}NO_3$, 222.1130. Anal. Calcd. For $C_{14}H_{16}F_3NO_5$: C, 50.15; H, 4.81; N, 4.18. Found: C, 50.29; H, 4.64; N, 4.19. Melting Point = 88-90°C.

(II-60). To a 100 mL round bottom flask was added **II-53** (0.600 g, 1.94 mmol), DCM (2.00 mL) and TFA (2.00 mL). The resulting solution stirred for 30 min. The excess TFA and DCM were removed and $CHCl_3$ (3 x 15.0 mL) was added and subsequently taken off to remove any residual TFA. The product was precipitated out of the crude residue using ether/petroleum ether to afford the product as a white solid. Yield (0.424 g, 68.0%). ^1H NMR (500MHz), DMSO: δ

4.57 (m, 2H

m, 2H), 8.9

22.1 Hz), 1

Hz), 131.4.

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$C_{11}H_{13}FNO$

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4.67 (m, 2H), 5.13-5.19 (m, 2H), 5.40 (s, 1H), 5.82 (m, 1H), 7.33 (m, 2H), 7.56 (m, 2H), 8.98 (s, 3H); ^{13}C NMR (125MHz), DMSO: δ 54.6, 66.1, 115.9 (d, J = 22.1 Hz), 117.2 (q, J = 300.1 Hz), 118.2, 128.9 (d, J = 3.2 Hz), 130.6 (d, J = 8.8 Hz), 131.4, 158.0 (q, J = 30.9 Hz), 162.6 (d, J = 246.2 Hz), 168.0. IR: (KBr) 3100 (br) cm^{-1}, 1749 cm^{-1}, 1680 cm^{-1}. HRMS: $[M + H]^+$ = 210.0933, calculated for $C_{11}H_{13}FNO_2$, 210.0930. Anal. Calcd. For $C_{13}H_{13}F_4NO_4$: C, 48.30; H, 4.05; N, 4.33. Found: C, 48.33; H, 3.77; N, 4.23. Melting Point = 68-70°C.

(II-61). To a 100 mL round bottom flask was added **II-54** (1.11 g, 3.26 mmol), DCM (2.00 mL) and TFA (2.00 mL). The resulting solution stirred for 30 min. The excess TFA and DCM were removed and $CHCl_3$ (3 x 15.0 mL) was added and subsequently taken off to remove any residual TFA. The product was precipitated out of the crude residue using ether/petroleum ether to afford the product as a white solid. Yield (0.962 g, 83.0%). ^1H NMR (500MHz), DMSO: δ 4.65 (m, 2H), 5.02-5.09 (m, 2H), 5.75 (m, 1H), 6.19 (s, 1H), 7.58-7.72 (m, 4H), 8.04 (t, J = 9.0 Hz, 2H), 8.31 (d, J = 8.6 Hz, 1H), 9.15 (s, 1H); ^{13}C NMR (125MHz), DMSO: δ 51.3, 66.1, 117.2 (q, J = 300.3 Hz), 118.0, 123.3, 125.3, 125.9, 126.5, 127.2, 128.8, 129.1, 130.2, 130.5, 131.4, 133.5, 158.2 (q, J = 31.1 Hz), 168.5. IR: (KBr) 3090 (br) cm^{-1}, 1730 cm^{-1}, 1667 cm^{-1}. HRMS: $[M + H]^+$ = 242.1192, calculated for $C_{15}H_{16}NO_2$, 242.1181. Anal. Calcd. For $C_{17}H_{16}F_3NO_4$: C, 57.47; H, 4.54; N, 3.94. Found: C, 57.33; H, 4.47; N, 3.87. Melting Point = 112-114°C.

(II-62). To a 100 mL round bottom flask was added **II-55** (1.39 g, 4.56 mmol), DCM (3.00 mL) and TFA (3.00 mL). The resulting solution stirred for 30

min. The excess TFA and DCM were removed and $CHCl_3$ (3 x 15.0 mL) was added and subsequently taken off to remove any residual TFA. The product was precipitated out of the crude residue using ether to afford the product as a white solid. Yield (1.32 g, 91.0%). ^1H NMR (500MHz), DMSO: δ 1.53 (s, 3H), 4.58 (dd, J = 13.4, 33.9 Hz, 2H), 4.76 (s, 1H), 4.82 (s, 1H), 5.35 (s, 1H), 7.42-7.53 (m, 5H), 9.00 (s, 3H); ^{13}C NMR (125MHz), DMSO: δ 18.7, 55.3, 68.4, 112.9, 117.2 (q, J = 300.1 Hz), 128.0, 129.0, 129.5, 132.6, 139.0, 158.0 (q, J = 31.1 Hz), 168.1. IR: (KBr) 3150 (br) cm^{-1}, 1743 cm^{-1}, 1680 cm^{-1}. HRMS: $[M + H]^+$ = 206.1182, calculated for $C_{12}H_{16}NO_2$, 206.1181. Anal. Calcd. For $C_{14}H_{16}F_3NO_4$: C, 52.67; H, 5.05; N, 4.39. Found: C, 52.63; H, 4.99; N, 4.34. Melting Point = 142-144°C.

(II-63). To a flame dried 100 mL round bottom flask was added **II-56** (1.00 g, 4.11 mmol) and anhydrous DCM (50.0 mL). Then the solution was brought down to 0°C and anhydrous TEA (0.630 mL, 4.52 mmol) was added followed by dropwise addition of ethoxycarbonyl isothiocyanate (0.560 mL, 4.93 mmol). The solution was allowed to warm to room temperature overnight while stirring under a nitrogen atmosphere. The solvent was removed and ether (30.0 mL) was added to the crude residue and was washed with sat. sodium bicarbonate. The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% hexane) affording the product as a whitish solid. Yield (1.04 g, 97.0%). ^1H NMR (500MHz), $CDCl_3$: δ 1.28 (t, J = 7.1 Hz, 3H), 1.54 (d, J = 7.2 Hz, 3H), 4.21 (q, J = 7.1 Hz, 2H), 4.65 (m, 2H), 5.00 (p, J = 7.2 Hz, 1H), 5.22-5.26 (m, 1H), 5.30-5.35 (m, 1H), 5.89 (m, 1H), 8.15 (s, 1H), 10.14 (d, J = 5.9 Hz, 1H); ^{13}C NMR

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(125MHz), $CDCl_3$: δ 14.0, 17.5, 53.6, 62.7, 66.0, 118.7, 131.3, 152.5, 171.4, 178.8. IR: (KBr) 3295 cm^{-1}, 3240 cm^{-1}, 1750 cm^{-1}, 1725 cm^{-1}. HRMS: $[M + H]^+$ =261.0920, calculated for $C_{10}H_{17}N_2O_4S$, 261.0909. Anal. Calcd. For $C_{10}H_{16}N_2O_4S$: C, 46.14; H, 6.20; N, 10.76. Found: C, 46.88; H, 6.06; N, 10.80. Melting Point = 43-45°C.

(II-64). To a flame dried 100 mL round bottom flask was added **II-57** (1.27 g, 3.98 mmol) and anhydrous DCM (50.0 mL). Then the solution was brought down to 0°C and anhydrous TEA (0.610 mL, 4.38 mmol) was added followed by dropwise addition of ethoxycarbonyl isothiocyanate (0.540 mL, 4.78 mmol). The solution was allowed to warm to room temperature overnight while stirring under a nitrogen atmosphere. The solvent was removed and ether (30.0 mL) was added to the crude residue and was washed with sat. sodium bicarbonate. The organics were dried using anhydrous sodium sulfate and concentrated. The product was recrystallized from the crude residue with EtOAc/hexanes affording the product as a whitish solid. Yield (1.08 g, 81.0%). ^1H NMR (500MHz), $CDCl_3$: δ 1.27 (t, J = 7.1 Hz, 3H), 3.20-3.34 (m, 2H), 4.19 (q, J = 7.1 Hz, 2H), 4.60 (m, 2H), 5.24 (m, 3H), 5.82 (m, 1H), 7.15-7.30 (m, 5H), 8.10 (s, 1H), 10.08 (d, J = 5.5 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 14.0, 37.2, 59.2, 62.8, 66.1, 118.9, 127.1, 128.5, 129.2, 131.2, 135.3, 152.3, 170.0, 179.1. IR: (KBr) 3290 cm^{-1} , 3225 cm^{-1}, 1730 cm^{-1}(with shoulder). HRMS: $[M + H]^+$ =337.1227, calculated for $C_{16}H_{21}N_2O_4S$, 337.1222. Anal. Calcd. For $C_{16}H_{20}N_2O_4S$: C, 57.12; H, 5.99; N, 8.33. Found: C, 55.80; H, 5.80; N, 8.30. Melting Point = 79-81°C.

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(II-65). To a flame dried 100 mL round bottom flask was added **II-58** (1.48 g, 4.85 mmol) and anhydrous DCM (50.0 mL). Then the solution was brought down to 0°C and anhydrous TEA (0.740 mL, 5.33 mmol) was added followed by dropwise addition of ethoxycarbonyl isothiocyanate (0.660 mL, 5.82 mmol). The solution was allowed to warm to room temperature overnight while stirring under a nitrogen atmosphere. The solvent was removed and ether (30.0 mL) was added to the crude residue and was washed with sat. sodium bicarbonate. The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% hexane) affording the product as a whitish solid. Yield (1.39 g, 89.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.29 (t, J = 7.1 Hz, 3H), 4.23 (m, 2H), 4.64 (m, 2H), 5.14-5.22 (m, 2H), 5.81 (m, 1H), 5.98 (d, J = 6.9 Hz, 1H), 7.31-7.45 (m, 5H), 8.06 (s, 1H), 10.59 (d, J = 6.4 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 14.0, 61.8, 62.8, 66.2, 118.5, 127.5, 128.7, 128.9, 131.1, 135.0, 152.5, 169.3, 178.9. IR: (KBr) 3290 cm^{-1} , 3225 cm^{-1}, 1750 cm^{-1}, 1720 cm^{-1}. HRMS: $[M + H]^+$ =323.1070, calculated for $C_{15}H_{19}N_2O_4S$, 323.1066. Anal. Calcd. For $C_{15}H_{18}N_2O_4S$: C, 55.88; H, 5.63; N, 8.69. Found: C, 55.46; H, 5.51; N, 8.68. Melting Point = 44-46°C.

(II-66). To a flame dried 100 mL round bottom flask was added **II-59** (0.900 g, 2.69 mmol) and anhydrous DCM (50.0 mL). Then the solution was brought down to 0°C and anhydrous TEA (0.410 mL, 2.96 mmol) was added followed by dropwise addition of ethoxycarbonyl isothiocyanate (0.360 mL, 3.22 mmol). The solution was allowed to warm to room temperature overnight while stirring under a nitrogen atmosphere. The solvent was removed and ether (30.0

mL) was added to the crude residue and was washed with sat. sodium bicarbonate. The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% hexane) affording the product as an oil. Yield (0.917 g, 97.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.31 (t, J = 7.2 Hz, 3H), 3.80 (s, 3H), 4.22-4.26 (m, 2H), 4.60-4.72 (m, 2H), 5.19-5.25 (m, 2H), 5.85 (m, 1H), 5.93 (d, J = 6.8 Hz, 1H), 6.90 (d, J = 8.8 Hz, 2H), 7.36 (d, J = 8.5 Hz, 2H), 8.20 (s, 1H), 10.55 (d, J = 6.5 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 14.0, 55.2, 61.2, 62.8, 66.2, 114.3, 118.5, 127.1, 128.8, 131.2, 152.5, 159.8, 169.5, 178.7. IR: (KBr) 3289 cm^{-1} , 3226 cm^{-1}, 1751 cm^{-1}, 1724 cm^{-1}. HRMS: $[M + H]^+$ = 353.1180, calculated for $C_{16}H_{21}N_2O_5S$, 353.1171. Anal. Calcd. For $C_{16}H_{20}N_2O_5S$: C, 54.53; H, 5.72; N, 7.95. Found: C, 52.68; H, 5.45; N, 7.55.

(II-67). To a flame dried 50 mL round bottom flask was added **II-60** (0.394 g, 1.22 mmol) and anhydrous DCM (20.0 mL). Then the solution was brought down to 0ºC and anhydrous TEA (0.190 mL, 1.34 mmol) was added followed by dropwise addition of ethoxycarbonyl isothiocyanate (0.160 mL, 1.46 mmol). The solution was allowed to warm to room temperature overnight while stirring under a nitrogen atmosphere. The solvent was removed and ether (30.0 mL) was added to the crude residue and was washed with sat. sodium bicarbonate. The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% hexane) affording the product as an oil. Yield (0.383 g, 92.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.29 (t, J = 7.1 Hz, 3H), 4.20-4.26 (m, 2H), 4.59-4.69 (m,

2H), 5.1

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$CDCl_3$: δ

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126.0,

179.2.

2H), 5.15-5.25 (m, 2H), 5.81 (m, 1H), 5.96 (d, J = 6.8 Hz, 1H), 7.02-7.07 (m, 2H), 7.38-7.42 (m, 2H), 8.10 (s, 1H), 10.61 (d, J = 6.3 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 14.1, 61.0, 62.9, 66.4, 115.9 (d, J = 22.1 Hz), 118.8, 129.3 (d, J = 8.8 Hz), 131.07 (d, J = 2.3 Hz), 131.1, 152.6, 162.8 (d, J = 248.1 Hz), 169.2, 178.9. IR: (KBr) 3282 cm^{-1} , 3232 cm^{-1}, 1751 cm^{-1}, 1724 cm^{-1}. HRMS: $[M + H]^+$ = 341.0974, calculated for $C_{15}H_{18}FN_2O_4S$, 341.0971. Anal. Calcd. For $C_{15}H_{17}FN_2O_4S$: C, 52.93; H, 5.03; N, 8.23. Found: C, 51.76; H, 4.89; N, 8.07.

(II-68). To a flame dried 100 mL round bottom flask was added **II-61** (0.902 g, 2.54 mmol) and anhydrous DCM (50.0 mL). Then the solution was brought down to 0°C and anhydrous TEA (0.390 mL, 2.79 mmol) was added followed by dropwise addition of ethoxycarbonyl isothiocyanate (0.340 mL, 3.05 mmol). The solution was allowed to warm to room temperature overnight while stirring under a nitrogen atmosphere. The solvent was removed and ether (30.0 mL) was added to the crude residue and was washed with sat. sodium bicarbonate. The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% hexane) affording the product as an oil. Yield (0.897 g, 95.0%). ^1H NMR (500MHz), $CDCl_3$: δ 1.24 (t, J = 7.1 Hz, 3H), 4.12-4.22 (m, 2H), 4.58-4.72 (m, 2H), 5.10-5.18 (m, 2H), 5.78 (m, 1H), 6.80 (d, J = 7.3 Hz, 1H), 7.42-7.60 (m, 4H), 7.85 (t, J = 8.6 Hz, 2H), 8.20 (m, 2H), 10.57 (d, J = 7.1 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 14.0, 58.9, 62.8, 66.3, 118.5, 123.2, 125.2, 126.0, 126.1, 127.0, 128.8, 129.7, 131.0, 131.1, 131.2, 134.0, 152.5, 169.7, 179.2. IR: (KBr) 3289 cm^{-1} , 3226 cm^{-1}, 1749 cm^{-1}, 1718 cm^{-1}. HRMS: $[M + H]^+$ =

373.1222.

C, 61.27; H

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5.8 Hz,

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127.5,

3232 cm

$C_{16}H_{21}N$

8.33. Fo

(1.55 g

mmol)

overnig

373.1222, calculated for $C_{19}H_{21}N_2O_4S$, 373.1222. Anal. Calcd. For $C_{19}H_{20}N_2O_4S$: C, 61.27; H, 5.41; N, 7.52. Found: C, 58.71; H, 4.85; N, 7.15.

(II-69). To a flame dried 100 mL round bottom flask was added **II-62** (1.00 g, 3.13 mmol) and anhydrous DCM (50.0 mL). Then the solution was brought down to 0°C and anhydrous TEA (0.480 mL, 3.45 mmol) was added followed by dropwise addition of ethoxycarbonyl isothiocyanate (0.420 mL, 3.76 mmol). The solution was allowed to warm to room temperature overnight while stirring under a nitrogen atmosphere. The solvent was removed and ether (30.0 mL) was added to the crude residue and was washed with sat. sodium bicarbonate. The organics were dried using anhydrous sodium sulfate and concentrated. The product was recrystallized from the crude residue with EtOAc/hexanes affording the product as a whitish solid. Yield (0.960 g, 91.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.28 (t, J = 7.2 Hz, 3H), 1.59 (s, 3H), 4.23 (m, 2H), 4.55 (m, 2H), 4.83 (d, J = 5.8 Hz, 2H), 5.98 (d, J = 6.9 Hz, 1H), 7.31-7.44 (m, 5H), 8.08 (s, 1H), 10.62 (d, J = 6.6 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 14.1, 19.1, 61.9, 62.9, 68.9, 113.5, 127.5, 128.8, 128.9, 135.1, 139.0, 152.5, 169.4, 178.9. IR: (KBr) 3289 cm^{-1} , 3232 cm^{-1}, 1749 cm^{-1}, 1724 cm^{-1}. HRMS: $[M + H]^+$ = 337.1230, calculated for $C_{16}H_{21}N_2O_4S$, 337.1222. Anal. Calcd. For $C_{16}H_{20}N_2O_4S$: C, 57.12; H, 5.99; N, 8.33. Found: C, 55.96; H, 5.84; N, 8.03. Melting Point = 62-64°C.

(II-71). To a flame dried 50 mL round bottom flask was added **II-70**[118, 119] (1.55 g, 7.49 mmol) and anhydrous CH_3CN. Then allyl alcohol (2.56 mL, 37.5 mmol) was added and the mixture stirred at room temperature under nitrogen overnight. The solvent was removed and the residue was put into solution with

EtOAc (0.200 L) and washed with sat. sodium bicarbonate (1 x 0.100 L). The organics were dried using anhydrous sodium sulfate and concentrated to give pure product as a solid. Yield (1.70 g, 99.0%). ^{1}H NMR (500MHz), acetone: δ 4.85 (m, 2H), 5.27-5.31 (m, 1H), 5.42-5.48 (m, 1H), 6.07 (m, 1H), 7.27-7.32 (m, 2H), 7.56-7.60 (m, 1H), 8.32 (m, 1H), 8.46 (s, 1H), 11.35 (s, 1H); ^{13}C NMR (125MHz), Acetone: δ 66.5, 113.2, 114.2, 119.0, 122.5, 123.6, 124.7, 126.8, 132.8, 137.7, 138.2, 164.0, 179.5. IR: (KBr) 3200cm^{-1} , 1743cm^{-1}, 1620 cm^{-1}. HRMS: $[M + H]^{+}$ =230.0819, calculated for $C_{13}H_{12}NO_3$, 230.0817. Anal. Calcd. For $C_{13}H_{11}NO_3$: C, 68.11; H, 4.84; N, 6.11. Found: C, 66.14; H, 4.80; N, 5.93. Melting Point = 159-161˚C.

(II-72). To a flame dried 250 mL round bottom flask was added **II-71** (1.70 g, 7.42 mmol) and anhydrous DCM (0.100 L). Then TsCl (2.82 g, 14.8 mmol), DMAP (2.26 g, 18.6 mmol), and DIPEA (3.20 mL, 18.6 mmol) were added and the mixture stirred at room temperature overnight under a nitrogen atmosphere. The resulting brown solution was washed with 5% HCl (1 x 30.0 mL) and brine (1 x 30.0 mL) and the organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% Hexanes) and recrystallized with EtOAc/hexanes to afford the product as a solid. Yield (2.46 g, 86.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 2.35 (s, 3H), 4.86 (m, 2H), 5.35 (m, 1H), 5.46 (m, 1H), 6.03 (m, 1H), 7.27 (d, J = 8.0 Hz, 2H), 7.37 (m, 2H), 7.85 (d, J = 7.4 Hz, 2H), 7.94 (m, 1H), 8.34 (m, 1H), 8.82 (s, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 21.6, 66.9, 113.1, 116.9, 120.0, 122.9, 125.2, 126.1, 127.3, 127.6, 130.3, 130.7, 134.2, 134.4, 136.8, 146.2, 161.2,

178.3. IR: (KBr) 1730cm^{-1}, 1674 cm^{-1}. HRMS: $[M + H]^+$ = 384.0914, calculated for $C_{20}H_{18}NO_5S$, 383.0906. Anal. Calcd. For $C_{20}H_{17}NO_5S$: C, 62.65; H, 4.47; N, 3.65. Found: C, 62.47; H, 4.48; N, 3.64. Melting Point = 104-106˚C.

(II-73). To a 250 mL round bottom flask was added **II-72** (2.30 g, 6.01 mmol) and dioxane (0.100 L). Then hydroxylamine hydrochloride (1.24 g, 18.0 mmol) and pyridine (1.55 mL, 19.2 mmol) were added and the mixture refluxed under nitrogen overnight (enough water to dissolve the hydroxylamine salt was added). The solvent was taken off and the residue was put into solution with EtOAc (0.100 L). The organics were washed with 1% HCl (1 x 30.0 mL) and brine (1 x 30.0 mL), then dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% Hexanes) affording the product as an oil (mixture of isomers). Yield (2.22 g, 93.0%). Isomer A: ^{1}H NMR (500MHz), $CDCl_3$: δ 2.32 (s, 3H), 4.83 (d, J = 5.9 Hz, 2H), 5.28 (d, J = 10.5 Hz, 1H), 5.38 (d, J = 17.2 Hz, 1H), 5.96 (m, 1H), 7.21 (d, J = 8.5 Hz, 2H), 7.25 (t, J = 8.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.83 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.3 Hz, 1H), 8.25 (s, 1H), 10.59 (s, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 21.4, 66.8, 109.5, 113.2, 119.6, 122.4, 123.3, 124.8, 126.9, 128.0, 129.9, 130.1, 130.9, 133.9, 134.7, 143.1, 145.3, 162.7. Isomer B: ^{1}H NMR (500MHz), $CDCl_3$: δ 2.25 (s, 3H), 4.91 (d, J = 5.9 Hz, 2H), 5.33 (d, J = 10.3 Hz, 1H), 5.45 (d, J = 17.2 Hz, 1H), 6.02 (m, 1H), 7.14-7.21 (m, 3H), 7.32 (t, J = 7.5 Hz, 1H), 7.74 (d, J = 8.4 Hz, 2H), 7.76 (s, 1H), 7.94 (d, J = 8.5 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 8.99 (s, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 21.3, 66.5, 113.2, 113.6, 119.7, 122.9, 124.1, 125.6, 126.8,

126.9, 127.3, 129.9, 130.7, 134.4, 135.0, 145.4, 146.5, 162.4. IR: (NaCl) 3276 cm^{-1}, 1730cm^{-1}. HRMS: $[M + H]^+$ = 399.1017, calculated for $C_{20}H_{19}N_2O_5S$, 399.1015. Anal. Calcd. For $C_{20}H_{18}N_2O_5S$: C, 60.29; H, 4.55; N, 7.03. Found: C, 58.91; H, 4.30; N, 6.82.

(II-74). To a 250 mL round bottom flask was added water (55.0 mL) and AcOH (55.0 mL). Then **II-73** (2.12 g, 5.33 mmol) was dissolved in THF (25.0 mL) and was added to the aqueous acid. The mixture was brought down to 0°C and zinc (3.46 g, 53.3 mmol) was then slowly added in small portions over 20 min. The suspension stirred at 0°C for 2 h. The solid was filtered off and the filtrate was reduced and then brought to a pH of 8 using concentrated ammonium hydroxide. The amine was extracted into ethyl acetate (4 x 50.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was then put into solution with DCM (5.00 mL) and TFA (3.00 mL) was added. The mixture stirred for 10 min and then the solvent and excess TFA was removed. $CHCl_3$ (3 x 15.0 mL) was added and subsequently taken off to remove any residual TFA. The product was precipitated out of the crude residue using ether/petroleum ether to afford the product as a white solid. Yield (2.25 g, 85.0%). ^1H NMR (500MHz), DMSO: δ 2.31 (s, 3H), 4.66 (m, 2H), 5.06 (m, 1H), 5.09 (m, 1H), 5.71 (s, 1H), 5.75 (m, 1H), 7.33 (t, J = 8.1 Hz, 1H), 7.40 (m, 3H), 7.75 (d, J = 8.1 Hz, 1H), 7.85 (d, J = 7.5 Hz, 2H), 7.94 (d, J = 7.3 Hz, 1H), 8.09 (s, 1H), 9.12 (s, 3H); ^{13}C NMR (125MHz), DMSO: δ 20.9, 47.8, 66.2, 113.1, 114.1, 117.2 (q, J = 299.2 Hz), 118.1, 120.3, 123.6, 125.5, 126.7, 126.8, 127.8, 130.3, 131.3, 133.7, 133.8, 145.9, 158.3 (q, J = 31.3 Hz), 167.5. IR: (KBr) 3100 (br) cm^{-1}, 1736 cm^{-1}, 1674

cm^{-1}. HRMS: $[M + H]^+$ = 385.1235, calculated for $C_{20}H_{21}N_2O_4S$, 385.1222. Anal. Calcd. For $C_{22}H_{21}F_3N_2O_6S$: C, 53.01; H, 4.25; N, 5.62. Found: C, 52.95; H, 4.05; N, 5.50. Melting Point = 170-172°C.

(II-75). To a flame dried 100 mL round bottom flask was added **II-74** (1.30 g, 2.61 mmol) and anhydrous DCM (50.0 mL). Then the solution was brought down to 0°C and anhydrous TEA (0.400 mL, 2.87 mmol) was added followed by dropwise addition of ethoxycarbonyl isothiocyanate (0.350 mL, 3.13 mmol). The solution was allowed to warm to room temperature overnight while stirring under a nitrogen atmosphere. The solvent was removed and ether (30.0 mL) was added to the crude residue and was washed with sat. sodium bicarbonate. The organics were dried using anhydrous sodium sulfate and concentrated. The product was recrystallized from the crude residue with EtOAc/Hexanes affording a whitish solid. Yield (1.19 g, 89.0%). ^1H NMR (500MHz), $CDCl_3$: δ 1.26 (t, J = 7.2 Hz, 3H), 2.31 (s, 3H), 4.20 (m, 2H), 4.64 (m, 2H), 5.13-5.22 (m, 2H), 5.78 (m, 1H), 6.26 (d, J = 7.9 Hz, 1H), 7.19-7.25 (m, 3H), 7.30 (t, J = 8.3 Hz, 1H), 7.63 (d, J = 7.5 Hz, 1H), 7.72 (s, 1H), 7.74 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 7.3 Hz, 1H), 8.04 (s, 1H), 10.59 (d, J = 6.9 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 14.1, 21.5, 54.7, 62.9, 66.6, 113.7, 115.9, 119.0, 119.9, 123.6, 125.1, 126.2, 126.9, 128.2, 129.9, 131.0, 134.8, 135.0, 145.1, 152.4, 168.7, 179.0. IR: (KBr) 3282 cm^{-1}, 3232 cm^{-1}, 1749 cm^{-1}, 1724 cm^{-1}. HRMS: $[M + H]^+$ = 516.1273, calculated for $C_{24}H_{26}N_3O_6S_2$, 516.1263. Anal. Calcd. For $C_{24}H_{25}N_3O_6S_2$: C, 55.91; H, 4.89; N, 8.15. Found: C, 55.91; H, 4.79; N, 8.09. Melting Point = 117-119°C.

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Anal. C

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(II-76). To a flame dried 50 mL round bottom flask was added **II-64** (0.150 g, 0.446 mmol) and anhydrous DCM (15.0 mL). Then anhydrous TEA (0.200 mL, 1.34 mmol) was added and the mixture was cooled to 0°C. EDCI (0.189 g, 0.982 mmol) was then added and the mixture stirred at 0°C under nitrogen for 1 h and then refluxed for 15 h. After the first step was completed, (as indicated by TLC), the solution was cooled to 0°C and a solution of NaH (0.0890 g, 2.33 mmol) in MeOH (5.00 mL) was added dropwise. The cloudy mixture stirred at 0°C for 1 h and then at room temperature for 2 h. The mixture was acidified with 5% HCl and then the solvents were removed. The aqueous mixture was extracted with EtOAc (3 x 30.0 mL) and then the organics were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% DCM) affording the product as a whitish solid. Yield (0.0200 g, 19.0%). ^{1}H NMR (500MHz), Acetone: δ 2.51 (dd, J = 7.4, 13.8 Hz, 1H), 2.61 (dd, J = 7.3, 13.9 Hz, 1H), 2.90 (d, J = 13.7 Hz, 1H), 3.06 (d, J = 13.7 Hz, 1H), 5.15 (m, 2H), 5.79 (m, 1H), 7.07 (s, 1H), 7.20-7.28 (m, 5H), 9.28 (s, 1H); ^{13}C NMR (125MHz), Acetone: δ 42.1, 42.9, 67.9, 120.2, 127.6, 128.8, 131.1, 132.3, 136.0, 156.5, 176.7. IR: (KBr) 3220 cm^{-1} (broad), 1761cm^{-1}, 1711 cm^{-1}. HRMS: $[M + H]^{+}$ = 231.1141, calculated for $C_{13}H_{15}N_2O_2$, 231.1134. Anal. Calcd. For $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 66.59; H, 6.14; N, 12.09. Melting Point = 203-205°C.

(II-77). To a flame dried 50 mL round bottom flask was added **II-65** (0.150 g, 0.466 mmol) and anhydrous DCM (15.0 mL). Then anhydrous TEA (0.200 mL, 1.40 mmol) was added and the mixture was cooled to 0°C. EDCI (0.197 g, 1.02

mmol) was then added and the mixture stirred at 0°C under nitrogen for 1 h and then refluxed for 15 h. After the first step was completed, (as indicated by TLC), the solution was cooled to 0°C and a solution of NaH (0.0930 g, 2.33 mmol) in MeOH (5.00 mL) was added dropwise. The cloudy mixture stirred at 0°C for 1 h and then at room temperature for 2 h. The mixture was acidified with 5% HCl and then the solvents were removed. The aqueous mixture was extracted with EtOAc (3 x 30 mL) and then the organics were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% DCM) affording the product as a whitish solid. Yield (0.0700 g, 70.0%). ^{1}H NMR (500MHz), acetone: δ 2.78 (dd, J = 7.4, 14.0 Hz, 1H), 2.95 (dd, J = 7.3, 13.9 Hz, 1H), 5.13 (d, J = 10.1 Hz, 1H), 5.21 (d, J = 17.1 Hz, 1H), 5.73 (m, 1H), 7.33 (t, J = 7.4 Hz, 1H), 7.40 (t, J = 8.2 Hz, 2H), 7.64 (d, J = 7.9 Hz, 2H), 7.68 (s, 1H), 9.65 (s, 1H); ^{13}C NMR (125MHz), acetone: δ 43.5, 68.6, 120.8, 126.3, 128.7, 129.3, 132.0, 139.7, 157.0, 175.9. IR: (KBr) 3245 cm^{-1} (broad), 1774cm^{-1}, 1724 cm^{-1}. HRMS: $[M + H]^{+}$ = 217.0979, calculated for $C_{12}H_{13}N_2O_2$, 217.0977. Anal. Calcd. For $C_{12}H_{12}N_2O_2$: C, 66.65; H, 5.59; N, 12.96. Found: C, 66.43; H, 5.49; N, 12.90. Melting Point = 172-174°C.

(II-78). To a flame dried 50 mL round bottom flask was added **II-66** (0.155 g, 0.440 mmol) and anhydrous DCM (15.0 mL). Then anhydrous TEA (0.180 mL, 1.32 mmol) was added and the mixture was cooled to 0°C. EDCI (0.186 g, 0.968 mmol) was then added and the mixture stirred at 0°C under nitrogen for 1 h and then refluxed for 15 h. After the first step was completed, (as indicated by TLC), the solution was cooled to 0°C and a solution of NaH (0.0880 g, 2.20 mmol) in

MeOH (5.00 mL) was added dropwise. The cloudy mixture stirred at 0°C for 1 h and then at room temperature for 2 h. The mixture was acidified with 5% HCl and then the solvents were removed. The aqueous mixture was extracted with EtOAc (3 x 30.0 mL) and then the organics were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% DCM) affording the product as a whitish solid. Yield (0.0720 mg, 67.0%). ^{1}H NMR (500MHz), acetone: δ 2.74 (dd, J = 7.3, 14.1 Hz, 1H), 2.92 (dd, J = 7.9, 14.1 Hz, 1H), 3.78 (s, 3H), 5.12 (m, 1H), 5.20 (m, 1H), 5.72 (m, 1H), 6.94 (d, J = 9.0 Hz, 2H), 7.52 (d, J = 9.0 Hz, 2H), 7.64 (s, 1H), 9.63 (s, 1H); ^{13}C NMR (125MHz), Acetone: δ 43.4, 55.5, 68.2, 114.6, 120.6, 127.6, 131.7, 132.2, 156.9, 160.3, 176.1. IR: (KBr) 3251 cm^{-1} (broad), 1774cm^{-1}, 1724cm^{-1}. HRMS: $[M + H]^{+}$ = 247.1086, calculated for $C_{13}H_{15}N_2O_3$, 247.1083. Anal. Calcd. For $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 62.74; H, 5.53; N, 11.35. Melting Point = 166-168°C.

(II-79). To a flame dried 50 mL round bottom flask was added **II-67** (0.151 g, 0.444 mmol) and anhydrous DCM (15.0 mL). Then anhydrous TEA (0.180 mL, 1.33 mmol) was added and the mixture was cooled to 0°C. EDCI (0.188 g, 0.977 mmol) was then added and the mixture stirred at 0°C under nitrogen for 1 h and then refluxed for 15 h. After the first step was completed, (as indicated by TLC), the solution was cooled to 0°C and a solution of NaH (0.0880 g, 2.20 mmol) in MeOH (5.00 mL) was added dropwise. The cloudy mixture stirred at 0°C for 1 h and then at room temperature for 2 h. The mixture was acidified with 5% HCl and then the solvents were removed. The aqueous mixture was extracted with EtOAc

(3 x 30.0 mL) and then the organics were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% DCM) affording the product as a whitish solid. Yield (0.0600 g, 57.0%). ^{1}H NMR (500MHz), acetone: δ 2.76 (dd, J = 7.3, 14.0 Hz, 1H), 2.95 (dd, J = 7.2, 14.1 Hz, 1H), 5.14 (dd, J = 2.1, 10.1 Hz, 1H), 5.21 (dd, J = 2.1, 10.2 Hz, 1H), 5.72 (m, 1H), 7.16 (m, 2H), 7.67 (m, 2H), 7.75 (s, 1H), 9.74 (s, 1H); ^{13}C NMR (125MHz), Acetone: δ 43.6, 68.2, 115.9 (d, J = 21.7 Hz), 121.0, 128.6 (d, J = 8.2 Hz), 131.8, 135.9 (d, J = 3.1 Hz), 156.8, 163.2 (d, J = 245.4 Hz), 175.8. IR: (KBr) 3220 cm^{-1} (broad), 1780cm^{-1}, 1730cm^{-1}. HRMS: $[M + H]^{+}$ = 235.0876, calculated for $C_{12}H_{12}FN_2O_2$, 235.0883. Anal. Calcd. For $C_{12}H_{11}FN_2O_2$: C, 61.53; H, 4.73; N, 11.96. Found: C, 61.48; H, 4.61; N, 11.81. Melting Point = 178-180˚C.

(II-80). To a flame dried 50 mL round bottom flask was added **II-68** (0.156 g, 0.419 mmol) and anhydrous DCM (15.0 mL). Then anhydrous TEA (0.170 mL, 1.26 mmol) was added and the mixture was cooled to 0ºC. EDCI (0.177 g, 0.922 mmol) was then added and the mixture stirred at 0ºC under nitrogen for 1 h and then refluxed for 15 h. After the first step was completed, (as indicated by TLC), the solution was cooled to 0 ºC and a solution of NaH (0.0840 g, 2.09 mmol) in MeOH (5.00 mL) was added dropwise. The cloudy mixture stirred at 0ºC for 1 h and then at room temperature for 2 h. The mixture was acidified with 5% HCl and then the solvents were removed. The aqueous mixture was extracted with EtOAc (3 x 30.0 mL) and then the organics were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column

chromatography (silica gel, 30% EtOAc; 70% DCM) affording the product as an oil. Yield (0.0340 g, 31.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 3.07 (dd, J = 7.5, 14.2 Hz, 1H), 3.26 (dd, J = 6.9, 14.1 Hz, 1H), 5.12 (d, J = 11.1 Hz, 1H), 5.21 (d, J = 18.0 Hz, 1H), 5.71 (m, 1H), 7.33 (t, J = 7.7 Hz, 1H), 7.42-7.53 (m, 3H), 7.67 (d, J = 7.3 Hz, 1H), 7.78 (d, J = 8.2 Hz, 1H), 7.84 (d, J = 8.1 Hz, 1H), 8.22 (d, J = 7.6 Hz, 1H), 9.68 (s, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 42.1, 69.7, 121.4, 124.4, 124.8, 125.6, 126.5, 129.6, 130.05, 130.08, 130.14, 132.3, 134.8, 157.6, 175.3. IR: (KBr) 3245 cm^{-1} (broad), 1774cm^{-1}, 1724cm^{-1}. HRMS: $[M + H]^+$ = 267.1134, calculated for $C_{16}H_{15}N_2O_2$, 267.1134.

(II-81). To a flame dried 50 mL round bottom flask was added **II-75** (0.150 g, 0.291 mmol) and anhydrous DCM (15.0 mL). Then anhydrous TEA (0.120 mL, 0.874 mmol) was added and the mixture was cooled to 0°C. EDCI (0.123 g, 0.640 mmol) was then added and the mixture stirred at 0°C under nitrogen for 1 h and then refluxed for 15 h. After the first step was completed, (as indicated by TLC), the solution was cooled to 0°C and a solution of NaH (0.0580 g, 1.46 mmol) in MeOH (5.00 mL) was added dropwise. The cloudy mixture stirred at 0°C for 1 h and then at room temperature for 2 h. The mixture was acidified with 5% HCl and then the solvents were removed. The aqueous mixture was extracted with EtOAc (3 x 30.0 mL) and then the organics were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% DCM) affording the product as a whitish solid. Yield (0.0830 g, 69.0%). ^{1}H NMR (500 MHz), acetone: δ 2.33 (s, 3H), 2.99 (dd, J = 7.2, 14.1 Hz, 1H), 3.05 (dd, J = 7.3, 14.2

Hz, 1H), 5.15 (d, J = 10.1 Hz, 1H), 5.22 (d, J = 17.5 Hz, 1H), 5.75 (m, 1H), 7.27 (t, J = 8.0 Hz, 1H), 7.36 (m, 3H), 7.65 (s, 1H), 7.83 (s, 1H), 7.88 (d, J = 8.3Hz, 3H), 8.01 (d, J = 8.5 Hz, 1H), 9.83 (s, 1H); ^{13}C NMR (125 MHz), Acetone: δ 21.3, 41.5, 65.7, 114.4, 121.1, 121.8, 122.4, 124.2, 125.4, 125.7, 127.8, 128.7, 130.9, 131.4, 135.6, 136.3, 146.5, 156.7, 175.0. IR: (KBr) 3232 cm^{-1}, 1780cm^{-1}, 1730 cm^{-1}. HRMS: $[M + H]^+$ = 410.1178, calculated for $C_{21}H_{20}N_3O_4S$, 410.1175. Anal. Calcd. For $C_{21}H_{19}N_3O_4S$: C, 61.60; H, 4.68; N, 10.26. Found: C, 61.59; H, 4.60; N, 9.80. Melting Point = 226-228°C.

(II-83). To a flame dried 100 mL round bottom flask was added **II-82** (1.21 g, 3.24 mmol) and anhydrous DCM (50.0 mL). Then cyclopent-1-enylmethanol[127] (0.477 g, 4.87 mmol) and DMAP (0.0400 g, 0.324 mmol) were added and the mixture was brought down to 0°C. DCC (1.00 g, 4.87 mmol) was then added and the mixture was allowed to warm to room temperature overnight while stirring under nitrogen. The white precipitate that formed was filtered off and the DCM filtrate was washed with brine (1 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% hexane) affording the product as a whitish solid. Yield (0.997 g, 68.0%). ^1H NMR (500MHz), $CDCl_3$: δ 1.82 (p, J = 7.5 Hz, 2H), 2.13 (s, 2H), 2.27 (s, 2H), 4.20 (t, J = 6.6 Hz, 1H), 4.38 (m, 2H), 4.70 (m, 2H), 5.40 (d, J = 7.4 Hz, 1H), 5.52 (s, 1H), 5.89 (d, J = 6.9 Hz, 1H), 7.28-7.40 (m, 9H), 7.57 (d, J = 7.3 Hz, 2H), 7.74 (d, J = 7.5 Hz, 2H); ^{13}C NMR (125MHz), $CDCl_3$: δ 23.1, 32.3, 32.5, 47.1, 58.0, 64.4, 67.1, 119.9, 125.0, 127.0, 127.1, 127.6, 128.5, 128.8, 129.1, 136.6, 138.1, 141.2, 143.7, 143.8, 155.3, 170.6. IR:

(NaCl) 3350 cm^{-1} , 1725 cm^{-1} (broad). HRMS: $[M + H]^+$ = 454.2020, calculated for $C_{29}H_{28}NO_4$, 454.2018. Anal. Calcd. For $C_{29}H_{27}NO_4$: C, 76.80; H, 6.00; N, 3.09. Found: C, 76.97; H, 5.84; N, 3.12. Melting Point = 96-98°C.

(II-84). To a flame dried 100 mL round bottom flask was added **II-82** (1.25 g, 3.35 mmol) and anhydrous DCM (50.0 mL). Then 3-methyl-2-buten-1-ol (0.690 mL, 6.70 mmol) and DMAP (0.0410 g, 0.335 mmol) were added and the mixture was brought down to 0°C. DCC (1.04 g, 5.03 mmol) was then added and the mixture was allowed to warm to room temperature overnight while stirring under nitrogen. The white precipitate that formed was filtered off and the DCM filtrate was washed with brine (1 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% hexane) affording the product as a whitish solid. Yield (1.15 g, 77.0%). ^1H NMR (500MHz), $CDCl_3$: δ 1.62 (s, 3H), 1.71 (s, 3H), 4.21 (t, J = 7.1 Hz, 1H), 4.35-4.44 (m, 2H), 4.54-4.72 (m, 2H), 5.27 (m, 1H), 5.40 (d, J = 7.6 Hz, 1H), 5.91 (d, J = 7.3 Hz, 1H), 7.27-7.41 (m, 9H), 7.58 (d, J = 7.3 Hz, 2H), 7.75 (d, J = 7.6 Hz, 2H); ^{13}C NMR (125MHz), $CDCl_3$: δ 17.9, 25.6, 47.1, 57.9, 62.7, 67.0, 117.7, 119.9, 125.0, 127.0, 127.1, 127.6, 128.4, 128.8, 136.7, 140.0, 141.2, 143.7, 143.8, 155.3, 170.7. IR: (NaCl) 3351 cm^{-1}, 1724 cm^{-1} (broad). HRMS: $[M + H]^+$ = 442.2019, calculated for $C_{28}H_{28}NO_4$, 442.2018. Anal. Calcd. For $C_{28}H_{27}NO_4$: C, 76.17; H, 6.16; N, 3.17. Found: C, 75.87; H, 6.10; N, 3.19. Melting Point = 108-110°C.

(II-85). To a 100 mL round bottom flask was added **II-83** (0.947 g, 2.09 mmol) and DCM (8.00 mL). The solution was brought down to 0°C and then

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piperidine (2.00 mL, 20.9 mmol) was added and the mixture stirred under nitrogen for 1 h and then 1 h at room temperature. The solvent was then removed and the crude residue was taken up in EtOAc (40.0 mL) and washed with sat. NH_4Cl (1 x 10.0 mL) and brine (1 x 10.0 mL). The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% hexane, then 90% DCM 10% MeOH) affording the product as an oil. Yield (0.434 g, 90.0%).^{1}H NMR (500MHz), $CDCl_3$: δ 1.77-1.83 (m, 2H), 1.91 (s, 2H), 2.10-2.15 (m, 2H), 2.22-2.28 (m, 2H), 4.59 (s, 1H), 4.63 (m, 2H), 5.49 (m, 1H), 7.24-7.37 (m, 5H); ^{13}C NMR (125MHz), $CDCl_3$: δ 23.4, 32.5, 32.9, 59.0, 64.1, 127.0, 128.1, 128.9, 138.8, 140.6, 174.0. IR: (KBr) 3385 (br) cm^{-1}, 3321 cm^{-1}, 1733 cm^{-1}. HRMS: $[M + H]^+$ = 232.1341, calculated for $C_{14}H_{18}NO_2$, 232.1338. Anal. Calcd. For $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.05; H, 7.30; N, 6.09.

(II-86). To a 100 mL round bottom flask was added **II-84** (1.15 g, 2.61 mmol) and DCM (10.0 mL). The solution was brought down to 0°C and then piperidine (2.60 mL, 26.1 mmol) was added and the mixture stirred under nitrogen for 1 h and then 1 h at room temperature. The solvent was then removed and the crude residue was taken up in EtOAc (50.0 mL) and washed with sat. NH_4Cl (1 x 20.0 mL) and brine (1 x 20.0 mL). The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% hexane, then 90% DCM 10% MeOH) affording the product as an oil. Yield (0.488 g, 85.0%). Characterization is for the TFA salt. ^{1}H NMR (500MHz), DMSO: δ 1.58 (s, 3H),

1.66 (s, 3H), 4.60 (dd, J = 7.3, 12.2 Hz, 1H), 4.67 (dd, J = 7.0, 11.7 Hz, 1H), 5.22 (m, 1H), 5.26 (s, 1H), 7.45 (m, 5H), 8.89 (s, 3H); ^{13}C NMR (125MHz), DMSO: δ 17.7, 25.2, 55.3, 62.6, 117.2 (q, J = 300.5 Hz), 117.5, 128.1, 128.9, 129.5, 132.5, 139.8, 157.9 (q, J = 30.9 Hz), 168.3. IR: (KBr) 3164 (br) cm^{-1}, 1736 cm^{-1}, 1675 cm^{-1}. HRMS: $[M + H]^+$ = 220.1341, calculated for $C_{13}H_{18}NO_2$, 220.1338. Anal. Calcd. For $C_{15}H_{18}F_3NO_4$: C, 54.05; H, 5.44; N, 4.20. Found: C, 53.92; H, 5.26; N, 4.18. Melting Point = 118-120°C.

(II-87). To a flame dried 100 mL round bottom flask was added **II-85** (0.406 g, 1.76 mmol) and anhydrous DCM (50.0 mL). Then the solution was brought down to 0°C and ethoxycarbonyl isothiocyanate (0.240 mL, 2.11 mmol) was added dropwise. The solution was allowed to warm to room temperature overnight while stirring under a nitrogen atmosphere. The solvent was removed and ether (30.0 mL) was added to the crude residue and was washed with sat. sodium bicarbonate. The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% hexane) and the product was recrystallized with EtOAc/hexanes affording the product as a white solid. Yield (0.518 g, 81.0%). ^1H NMR (500MHz), $CDCl_3$: δ 1.29 (t, J = 7.1 Hz, 3H), 1.81 (m, 2H), 2.13 (m, 2H), 2.26 (m, 2H), 4.23 (m, 2H), 4.69 (s, 2H), 5.53 (s, 1H), 5.97 (d, J = 6.9 Hz, 1H), 7.30-7.42 (m, 5H), 8.08 (s, 1H), 10.62 (d, J = 6.5 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 14.1, 23.1, 32.3, 32.5, 61.8, 62.8, 64.5, 127.5, 128.7, 128.9, 129.1, 135.2, 138.1, 152.5, 169.4, 178.8. IR: (KBr) 3289 cm^{-1}, 3226 cm^{-1}, 1743cm^{-1}, 1724 cm^{-1}. HRMS: $[M + H]^+$ = 363.1387, calculated for $C_{18}H_{23}N_2O_4S$, 363.1379.

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Anal. Calcd. For $C_{18}H_{22}N_2O_4S$: C, 59.65; H, 6.12; N, 7.73. Found: C, 59.36; H, 5.78; N, 7.69. Melting Point = 58-60°C.

(II-88). To a flame dried 100 mL round bottom flask was added **II-86** (0.428 g, 1.95 mmol) and anhydrous DCM (50.0 mL). Then the solution was brought down to 0°C and ethoxycarbonyl isothiocyanate (0.260 mL, 2.35 mmol) was added dropwise. The solution was allowed to warm to room temperature overnight while stirring under a nitrogen atmosphere. The solvent was removed and ether (30.0 mL) was added to the crude residue and was washed with sat. sodium bicarbonate. The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% hexane) affording the product as an oil. Yield (0.678 g, 99.0%).^{1}H NMR (500MHz), $CDCl_3$: δ 1.29 (t, J = 7.1 Hz, 3H), 1.60 (s, 3H), 1.69 (s, 3H), 4.23 (m, 2H), 4.55 (dd, J = 7.3, 12.4 Hz, 1H), 4.68 (dd, J = 7.1, 12.2 Hz, 1H), 5.26 (m, 1H), 5.95 (d, J = 6.8 Hz, 1H), 7.30-7.44 (m, 5H), 7.96 (s, 1H), 10.59 (d, J = 6.6 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 14.1, 18.0, 25.6, 61.9, 62.88, 62.89, 117.7, 127.5, 128.6, 128.9, 135.3, 140.0, 152.5, 169.6, 178.8. IR: (KBr) 3282 cm^{-1} , 3232 cm^{-1}, 1724 cm^{-1}(with shoulder). HRMS: $[M + H]^+$ = 351.1380, calculated for $C_{17}H_{23}N_2O_4S$, 351.1379. Anal. Calcd. For $C_{17}H_{22}N_2O_4S$: C, 58.27; H, 6.33; N, 7.99. Found: C, 54.58; H, 5.86; N, 7.46.

(II-89). To a flame dried 50 mL round bottom flask was added **II-69** (0.150 g, 0.446 mmol) and anhydrous DCM (15.0 mL). Then anhydrous TEA (0.190 mL, 1.34 mmol) was added and the mixture was cooled to 0°C. EDCI (0.188 g, 0.981 mmol) was then added and the mixture stirred at 0°C under nitrogen for 1 h and

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then refluxed for 15 h. After the first step was completed, (as indicated by TLC), the solution was cooled to 0°C and a solution of NaH (0.0890 g, 2.23 mmol) in MeOH (5.00 mL) was added dropwise. The cloudy mixture stirred at 0°C for 1 h and then at room temperature for 2 h. The mixture was acidified with 5% HCl and then the solvents were removed. The aqueous mixture was extracted with EtOAc (3 x 30.0 mL) and then the organics were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 10% acetone; 90% DCM) affording the product as a whitish solid. Yield (0.0680 g, 66.0%). ^{1}H NMR (500MHz), acetone: δ 1.70 (s, 3H), 2.70 (d, J = 13.2 Hz, 1H), 2.99 (d, J = 13.7 Hz, 1H), 4.85 (m, 1H), 4.91 (m, 1H), 7.30-7.42 (m, 3H), 7.66 (m, 2H), 7.69 (s, 1H), 9.64 (s, 1H); ^{13}C NMR (125MHz), acetone: δ 24.1, 46.8, 68.7, 116.7, 126.3, 128.7, 129.2, 140.4, 140.7, 156.8, 176.1. IR: (KBr) 3243 cm^{-1}, 1772 cm^{-1}, 1724 cm^{-1}. HRMS: $[M + H]^{+}$ = 231.1139, calculated for $C_{13}H_{15}N_2O_2$, 231.1134. Anal. Calcd. For $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 66.99; H, 5.86; N, 11.81. Melting Point = 169-171°C.

(II-90). To a flame dried 50 mL round bottom flask was added **II-87** (0.150 g, 0.414 mmol) and anhydrous DCM (15.0 mL). Then anhydrous TEA (0.170 mL, 1.24 mmol) was added and the mixture was cooled to 0°C. EDCI (0.175 g, 0.911 mmol) was then added and the mixture stirred at 0°C under nitrogen for 1 h and then refluxed for 15 h. After the first step was completed, (as indicated by TLC), the solution was cooled to 0°C and a solution of NaH (0.0830 g, 2.07 mmol) in MeOH (5.00 mL) was added dropwise. The cloudy mixture stirred at 0°C for 1 h

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and then at room temperature for 2 h. The mixture was acidified with 5% HCl and then the solvents were removed. The aqueous mixture was extracted with EtOAc (3 x 30.0 mL) and then the organics were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 10% acetone; 90% DCM) affording the two unseparatable diastereomers (1.3:1 ratio) as a whitish solid. Yield (0.0700 g, 66.0%). ^{1}H NMR (500MHz), acetone: δ 1.28-1.96 (m, 8H), 2.26-2.38 (m, 4H), 3.45 (t, J = 7.4 Hz, 1H), 3.56 (t, J = 7.6 Hz, 1H), 4.03 (s, 1H), 4.73 (s, 1H), 4.94 (s, 1H), 5.03 (s, 1H), 7.32-7.43 (m, 6H), 7.55 (s, 1H), 7.64-7.71 (m, 4H), 7.87 (s, 1H), 9.66 (s, 1H); ^{13}C NMR (125MHz), acetone: δ 24.9, 25.2, 35.9, 36.6, 49.9, 50.0, 71.2, 72.3, 108.4, 109.2, 126.7, 127.0, 128.63, 128.69, 129.21, 129.22, 139.7, 139.8, 150.7, 151.7, 157.2, 157.4, 175.7, 176.3. IR: (KBr) 3289 cm^{-1}, 1774 cm^{-1}, 1718 cm^{-1}. HRMS: $[M + H]^{+}$ = 257.1295, calculated for $C_{15}H_{17}N_2O_2$, 257.1290. Anal. Calcd. For $C_{15}H_{16}N_2O_2$: C, 70.29; H, 6.29; N, 10.93. Found: C, 68.27; H, 6.37; N, 10.48. Melting Point = 234-236°C.

(II-91). To a flame dried 50 mL round bottom flask was added **II-88** (0.150 g, 0.429 mmol) and anhydrous DCM (15.0 mL). Then anhydrous TEA (0.180 mL, 1.29 mmol) was added and the mixture was cooled to 0°C. EDCI (0.181 g, 0.944 mmol) was then added and the mixture stirred at 0°C under nitrogen for 1 h and then refluxed for 15 h. After the first step was completed, (as indicated by TLC), the solution was cooled to 0°C and a solution of NaH (0.0860 g, 2.15 mmol) in MeOH (5.00 mL) was added dropwise. The cloudy mixture stirred at 0°C for 1 h and then at room temperature for 2 h. The mixture was acidified with 5% HCl and

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then the solvents were removed. The aqueous mixture was extracted with EtOAc (3 x 30.0 mL) and then the organics were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 10% acetone; 90% DCM) affording the product as a whitish solid. Yield (0.0490 g, 47.0%). ^{1}H NMR (500MHz), acetone: δ 1.09 (s, 3H), 1.14 (s, 3H), 4.99 (dd, J = 1.0, 17.3 Hz, 1H), 5.07 (dd, J = 1.2, 11.0 Hz, 1H), 6.02 (dd, J = 10.7, 17.3 Hz, 1H), 7.35 (m, 3H), 7.75 (m, 2H), 7.95 (s, 1H), 9.70 (s, 1H); ^{13}C NMR (125MHz), acetone: δ 22.1, 22.8, 44.6, 72.7, 115.3, 128.1, 128.3, 128.6, 136.8, 142.9, 156.6, 175.2. IR: (KBr) 3243 cm^{-1}, 1772 cm^{-1}, 1718 cm^{-1}. HRMS: $[M + H]^{+}$ = 245.1292, calculated for $C_{14}H_{17}N_2O_2$, 245.1290. Anal. Calcd. For $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.67; H, 6.39; N, 11.26. Melting Point = 229-231°C.

(II-94). To a flame dried 50 mL round bottom flask was added **II-75** (0.150 g, 0.291 mmol) and anhydrous DCM (15.0 mL). Then anhydrous TEA (0.120 mL, 0.874 mmol) was added and the mixture was cooled to 0°C. EDCI (0.123 g, 0.640 mmol) was then added and the mixture stirred at 0°C under nitrogen for 1 h and then refluxed for 15 h. After the rearrangement was completed (as indicated by TLC), the reaction was quenched by addition of ice water (10.0 mL). The organic layer was separated, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 10% Ethyl acetate; 90% DCM; ΔR_f = 0.69) affording the product as a thick oil. ^{1}H NMR (500MHz), $CDCl_3$: δ 1.38 (t, J = 7.2 Hz, 3H), 1.52 (s, 3H), 2.36 (s, 3H), 3.00 (d, J = 13.9 Hz, 1H), 3.04 (d, J = 13.8 Hz, 1H), 4.42 (dq, J = 2.6, 7.1

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Hz, 2H), 4.85 (s, 1H), 4.98 (m, 1H), 6.46 (s, 1H), 7.24-7.38 (m, 4H), 7.64 (s, 1H), 7.79 (d, J = 8.5 Hz, 2H), 7.91 (d, J = 8.9 Hz, 1H), 8.00 (d, J = 8.3 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 13.9, 21.5, 23.8, 44.4, 63.2, 64.5, 113.7, 118.0, 119.3, 121.6, 123.7, 124.1, 125.3, 127.0, 127.1, 130.0, 134.7, 135.7, 138.2, 145.4, 147.5, 151.4, 169.4. IR: (KBr) 3340 cm^{-1}, 1812 cm^{-1}, 1770 cm^{-1}, 1726 cm^{-1}. HRMS: $[M + H]^+$ = 496.1548, calculated for $C_{25}H_{26}N_3O_6S$, 496.1542.

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116. Y
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CHAPTER III

TOTAL SYNTHESIS OF AN INDOLE ALKALOID FROM THE TUNICATE *DENDRODOA GROSSULARIA* AND TWO ANALOGS

III.A Pharmacological value of marine compounds

The biodiversity found in the marine environment offers a wealth of organisms each containing unique metabolites and secondary metabolites with chemically diverse structures.[1, 2] Efforts to explore this rich and diverse set of compounds have led to the opening of a "marine pipeline," which allows for the flow of these compounds into the pharmaceutical arena. The pharmaceutical industry has benefited from the study of these organisms and the introduction of the marine natural products they contain in many areas of research. Compounds coming from marine organisms have shown to possess biological activity for diseases such as cancer, arthritis, AIDS and have potential as anti-viral, anti-microbial, anti-parasitic, analgesic and anti-inflammatory agents.[3-5]

The extraordinary biological properties that compounds from natural products possess justify the isolation and harvesting of the sources for these compounds. Unfortunately, these secondary metablites found in the marine organisms are usually present in trace amounts making acquisition of an appropriate amount of these compounds for testing very difficult. In order to determine the biological potential of a particular compound, there are two options commonly used: 1) massive recollection of the organism and reisolation of the molecule or 2) development of a concise synthetic route to the molecule.[6] Factors concerning the availablility of the marine organism and ease of isolation

will determine which method will access the desired compound the easiest and fastest.

III.B Background on *Dendrodoa grossularia*

Invertebrate marine animals such as tunicates, which include sea squirts and sea cucumbers, have been a prosperous source of structurally unique and biologically interesting secondary metabolites. In particular, these marine organisms are known to contain indole alkaloids, nitrogen containing compounds that also posses an indole structural moiety.[7] Furthermore, the novel frameworks that these indole alkaloids possess often attracts the attention of chemists interested in developing synthetic routes to the scaffolds. The tunicate *Dendrodoa grossularia* (baked bean sea squirt), a red marine organism that grows along the coasts of Brittany and in the Baltic and North Seas, contains some heterocyclic alkaloids with unique scaffolds (Figure III-1).[8-13]

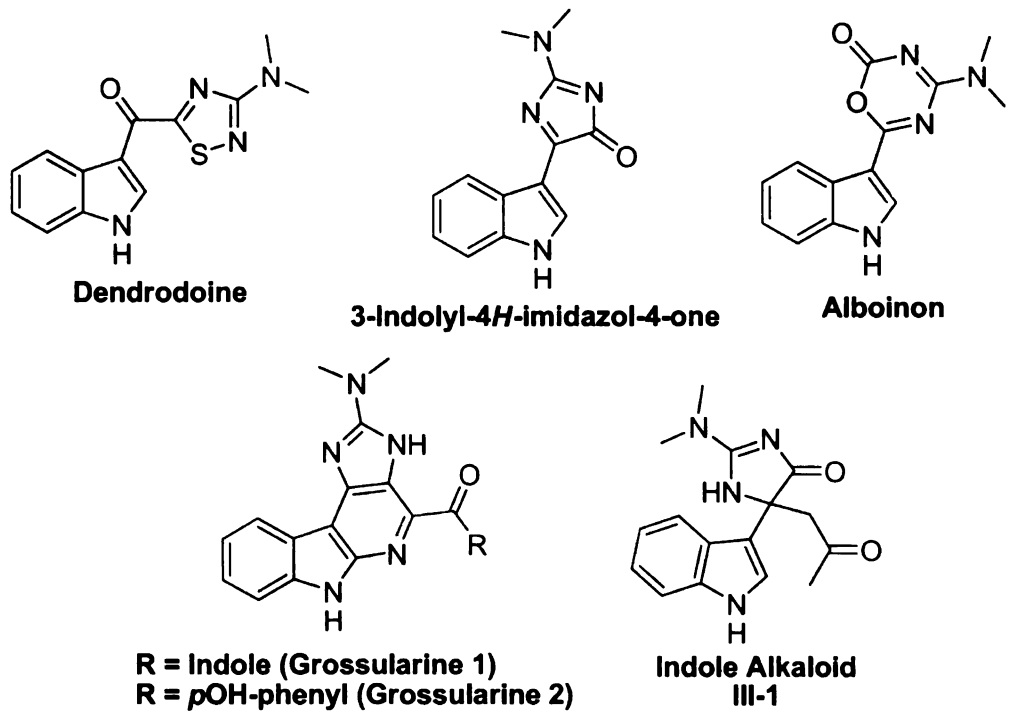


Figure III-1. Alkaloids from *Dendrodoa grossularia*

Dendrodoine was isolated by Heitz and coworkers in 1980[10] and first synthesized in 1984[14] and is a unique molecule that exhibited the first example of a divalent sulfur-nitrogen bond found in a natural product, coming from its 1, 2, 4-thiadiazole heterocyclic nucleus.[12] Results from a brief biological study on the compound revealed moderate cytotoxicity for L1210 leukemia cells with an ID_{50} of 10 μg/mL.[13] An additional report suggests that dendrodoine inhibits the synthesis of DNA in leukemia cells through preventing the incoporation of thymidine into DNA.[15]

3-Indolyl-4*H*-imidazol-4-one was isolated and then synthesized in 1986 by Guyot and coworkers.[9] The compound was found to be light sensitive in solution which resulted in the rearrangment of the molecule to yield additional byproducts.[9] Furthermore, a cytotoxicity assay revealed that the compound was void of activity.[13] Alboinon isolated in 1997 by Steffan and coworkers contains a rare 1, 3, 5-oxadiazin-2-one scaffold and represents the first natural product bearing this moiety.[8] The synthesis of alboinon was attempted by the same group using a protecting group on the indolic nitrogen, however, all attempts to remove the protecting group resulted in the attack on the oxadiazinone ring. Fortunately, it was found that oxidation of 3-Indolyl-4*H*-imidazol-4-one with m-chloroperbenzoic acid produced alboinon in a good yield.[8] This result offers insight into a potential biosynthetic route to alboinon suggesting that perhaps it is synthesized in the tunicate by oxidation of 3-Indolyl-4*H*-imidazol-4-one.

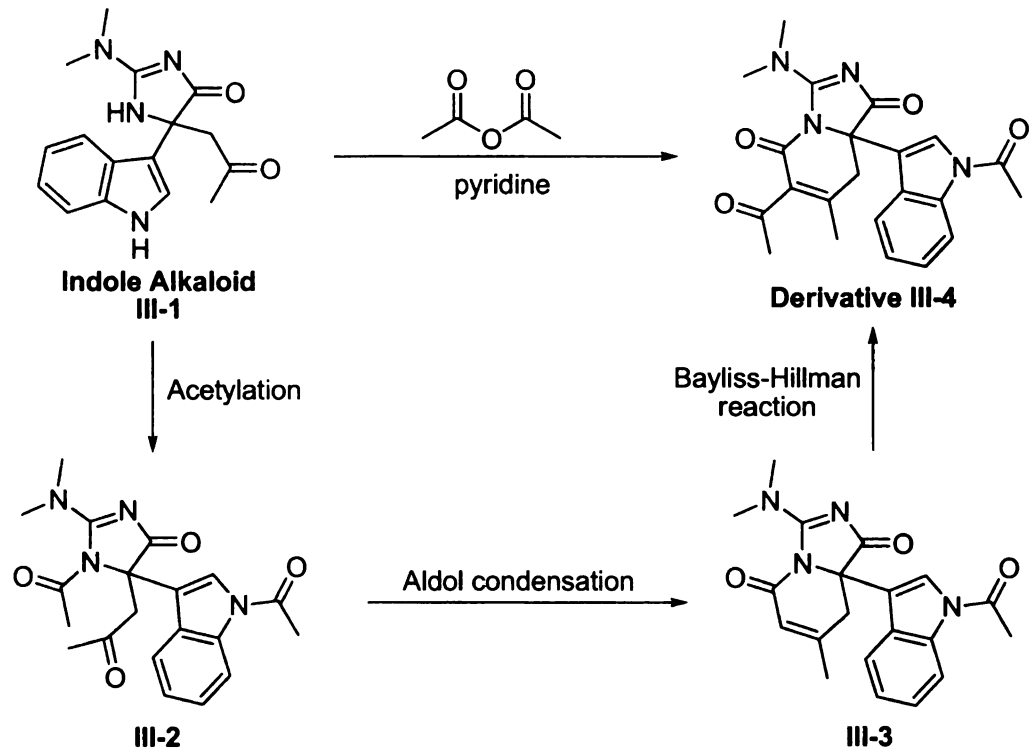
Grossularines 1 and 2 were isolated in 1989 and were originally given an alternative structure. X-ray studies and the availability of larger amounts of the

compounds allowed the definite determination of the physical structure.[12, 13, 16] Furthermore, these compounds represent the first natural products to contain an α-carboline skeleton.[13] The first total synthesis of both grossularine 1 and 2 was achieved in 1995 using a thermal electrocyclic reaction to obtain the heterocyclic core of both compounds.[17] Grossularines 1 and 2 were found to be cytotoxic toward L1210 leukemia cells displaying ID_{50} values of 6 and 4 μg/mL, respectively. A closer investigation revealed that these compounds caused accumulation of these cells in the G1 phase at concentrations of 10 μg/mL (grossularine 1) and 1.5 μg/mL (grossularine 2).[13] The mode of action for grossularine 2's cytotoxicity was examined and it was found that grossularine 2 intercalates into the DNA and also inhibits the incorporation of thymidine into DNA. Grossularine 1 was found to have a somewhat more ambiguous mode of action by giving results reminiscent of drugs that show intercalation as well as alkylation.[15] Furthermore, they were found even more cytotoxic toward the solid tumor cell lines WiDr (colon) and MCF7 (breast) showing activity in the 10 ng/mL range.[13]

The latest indole alkaloid (**III-1**) to come from *Dendrodoa grossularia* was isolated in 1998 by Guyot and coworkers and contains a unique quaternary imidazolone core.[11] The structure was determined by 1D and 2D NMR techniques as well as by information given by the X-ray structure of a derivative of the indole alkaloid (**III-4**), obtained by treating the natural product with acetic anhydride and pyridine (Scheme III-1). After an initial acetylation of the indolic nitrogen and nitrogen contained within the imidazolone ring of **III-1** affords **III-2**, it

is proposed that an aldol reaction occurs to give **III-3**. The derivative **III-4** is finally formed after a Bayliss-Hillman reaction occurs between **III-3** and acetic anhydride (Scheme III-1).[11]

Scheme III-1. Synthesis of derivative III-4



The crystal structure of **III-4** was ineffective at identifying the absolute stereochemistry at the only stereogenic center in the natural product due to the racemic nature of the crystal. It is suggested that the optical rotation observed for the indole alkaloid **III-1** and derivative **III-4** ($[\alpha]_D$ = -15 and -12, respectively) is due to the presence of an excess of one enantiomer. Furthermore, indole alkaloid **III-1** could be considered to be derived from 3-Indolyl-4*H*-imidazol-4-one another alkaloid from the same tunicate.[11] As a result of the known biological properties of other alkaloids from *Dendrodoa grossularia* and the physical

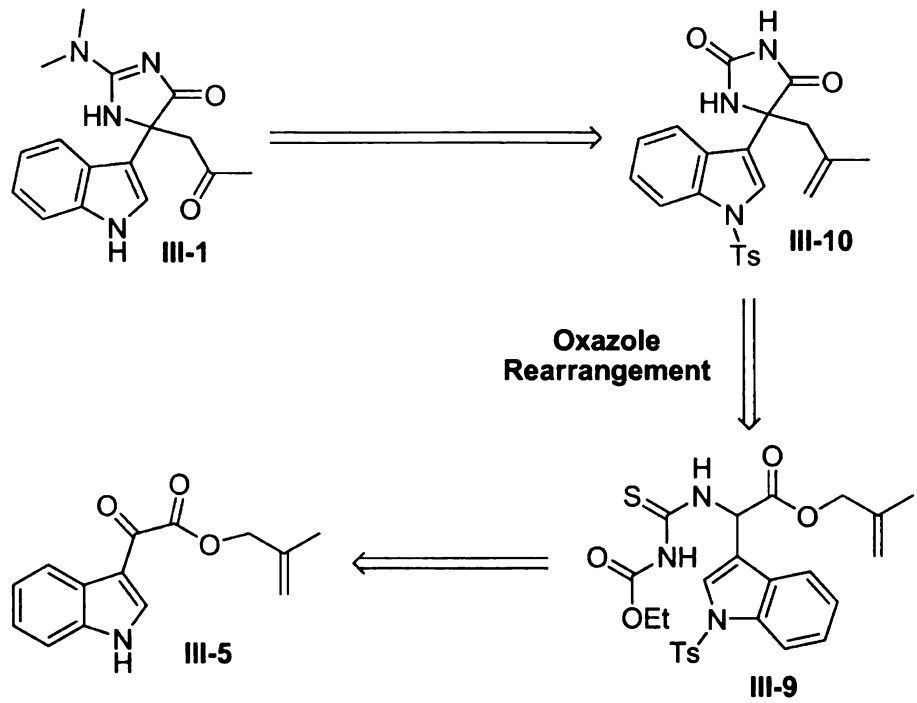
similarities the new indole alkaloid has with the Chk2 inhibitor, indoloazepine, a total synthesis for the indole alkaloid was developed.

III.C Retrosynthetic analysis of indole alkaloid III-1

As a continuation of our laboratory's focus on the development of new heterocyclic methodologies for the syntheses of pharmacologically significant scaffolds,[18] the racemic total synthesis of indole alkaloid **III-1** was completed along with two additional analogs. The key step in forming the quaternary stereocenter in the alkaloid and analogs utilizes a novel oxazole rearrangement[19] (discussed in Chapter II) producing an oxazolone intermediate, ultimately leading to a quaternary hydantoin.

Scheme III-2 illustrates our retrosynthetic strategy for the total synthesis of indole alkaloid **III-1**. It was envisioned that the imidazolone moiety of the natural product could be accessed from the hydantoin intermediate **III-10**.[20, 21] Through a newly developed EDCI-mediated oxazole rearrangement, hydantoin **III-10** was thought to be formed from thiourea **III-9**.[19, 22] In turn, thiourea **III-9** could be obtained from keto allyl ester **III-5** after a few functional group manipulations.

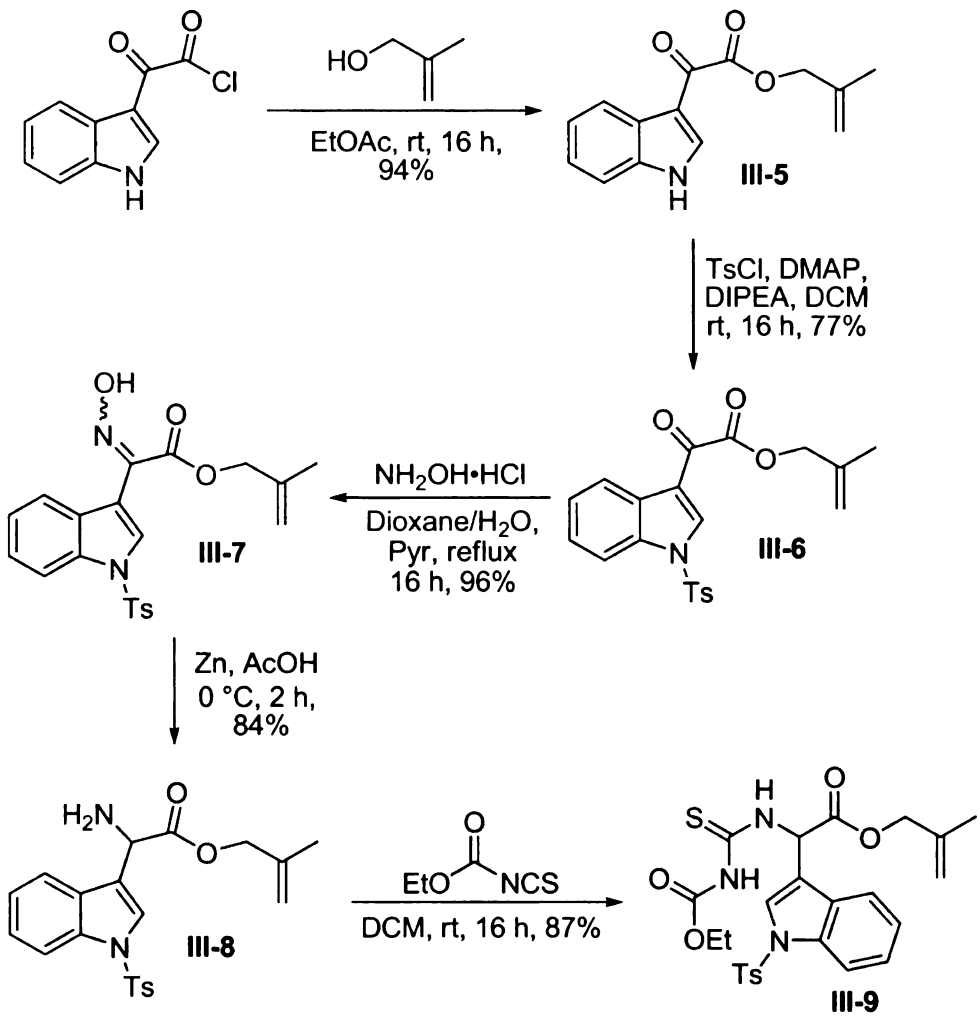
Scheme III-2. Retrosynthesis of indole alkaloid III-1



III.D Synthesis of indole alklaloid III-1

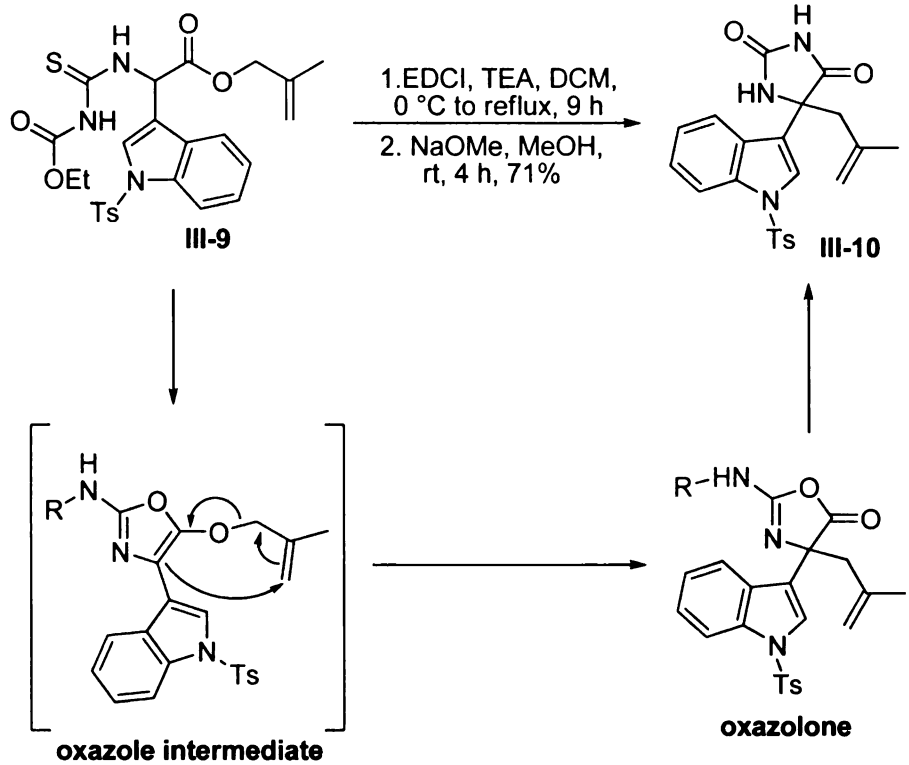
Keto allyl ester **III-5** (Scheme III-3) was synthesized in excellent yields (94%) through an esterification of 2-(1H-indol-3-yl)-2-oxoacetyl chloride, which is the product of a known reaction between indole and oxalyl chloride,[23, 24] with 2-methyl-2-propen-1-ol at room temperature for 16 h. Subsequent protection of the indolic nitrogen with *p*-toluene sulfonyl chloride under basic conditions for 16 h gave keto ester **III-6** in a 77% yield, which when treated with hydroxylamine and pyridine in dioxane, produced oxime **III-7** in a 96% yield as a mixture of *E* and *Z* isomers. Each isomer was isolated and both were carried on in the synthesis by reducing the oximes using zinc powder and aqueous acetic acid at 0°C for 2 h to amine **III-8** in a 84% yield. Thiourea **III-9** was formed in a 87% yield after treating amine **III-8** with commercially available ethoxy carbonyl isothiocyanate for 16 h at room temperature (Scheme III-3).[22]

Scheme III-3. Synthesis of thiourea III-9



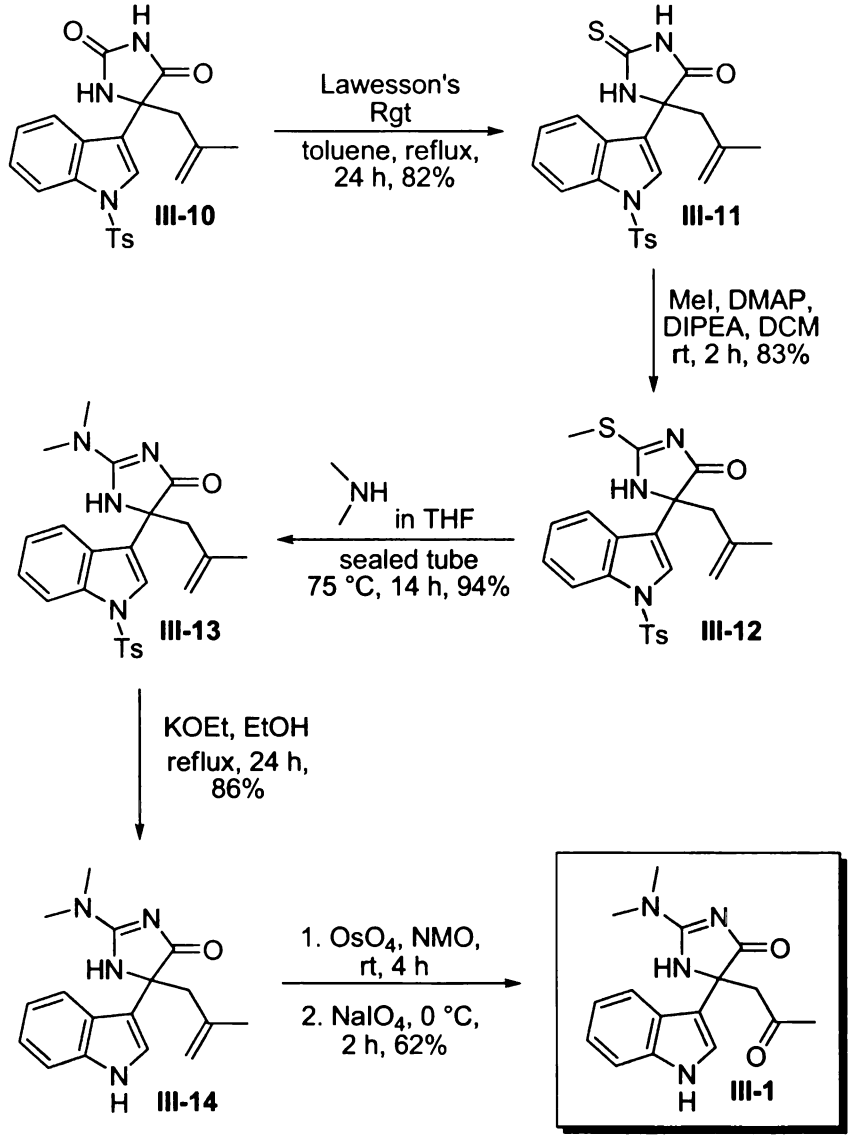
Subsequent treatment of thiourea **III-9** with EDCI followed by sodium methoxide yielded hydantoin **III-10** in a 71% yield, resulting from the EDCI-mediated oxazole rearrangement (Scheme III-4). The abbreviated proposed mechanism for the formation of hydantoin **III-10** is also highlighted in Scheme III-4. After the thiourea moiety was converted into a carbodiimide intermediate using EDCI, a 5-*exo-dig* cyclization occured between the carbonyl oxygen of the ester and the carbodiimide to form a transient oxazole intermediate.[22]

Scheme III-4. Synthesis of hydantoin III-10



Through a Claisen-type rearrangement, the oxazole was transformed into a quaternary oxazolone intermediate. Upon completion of the rearrangement, as indicated by TLC, a solution of sodium methoxide in methanol was added to complete the transformation from the oxazolone to hydantoin **III-10**.[22] Treatment of hydantoin **III-10** with Lawesson's reagent[25] for 24 h under refluxing conditions produced thiohydantoin **III-11** (Scheme III-5) in a 82% yield, which was selectively methylated at the thiocarbonyl using methyl iodide, DMAP, and diisopropylethylamine at room temperature for 2 h to furnish imidazolone **III-12** in a 83% yield. After heating imidazolone **III-12** with a THF solution of dimethylamine in a sealed tube at 75 °C for 14 h, imidazolone **III-13** was produced in a 94% yield.[22]

Scheme III-5. Synthesis of alkaloid III-1



The final two steps for the total synthesis of **III-1** began with the deprotection of the indole nitrogen of **III-13** using potassium ethoxide and ethanol under refluxing conditions to produce imidazolone **III-14** in an 86% yield. Finally, oxidation of the terminal alkene of **III-14** was achieved through a two-step/one-pot modified Johnson-Lemieux[26] reaction to give indole alkaloid **III-1** in a 62% yield (Scheme III-5). A tabulated spectral comparison of the synthesized alkaloid (**III-1**) and the isolated natural product is given below in Table III-1. Fortunately, a

suitable crystal of **III-1** was able to be formed and allowed a X-ray crystal structure of **III-1** to be determined (Figure III-2), which also confirms the structure of the natural product.[22]

Table III-1. Comparison of spectral data from synthesized natural product (III-1) and isolated natural product

Spectrum	Indole Alkaloid (III-1)	Isolated Natural Product	Δδ
^{1}H NMR (ppm in CD_3OD)	2.16 (s, 3H)	2.16 (s, 3H)	0.00
	3.05 (s, 3H)	3.04 (s, 3H)	0.01
	3.23 (s, 3H)	3.22 (s, 3H)	0.01
	3.46 (dd, J =16, 37 Hz, 2H)	3.45 (dd, J =16, 8 Hz, 2H)	0.01
	6.97 (t, J = 7 Hz, 1H)	6.9 (t, J = 8 Hz, 1H)	0.07
	7.09 (t, J = 7 Hz, 1H)	7.1 (t, J = 8 Hz, 1H)	0.01
	7.26 (s, 1H)	7.26 (s, 1H)	0.00
	7.33 (d, J = 8 Hz, 1H)	7.33 (d, J = 8 Hz, 1H)	0.00
	7.45 (d, J = 8 Hz, 1H)	7.46 (d, J = 8 Hz, 1H)	0.01
	8.24 (s, 1H, DMSO-d_6)	8.2 (s, 1H, DMSO-d_6)	0.04
	11.01 (s, 1H, DMSO-d_6)	11.01 (s, 1H, DMSO-d_6)	0.00
^{13}C NMR (ppm in CD_3OD)	31.0	31	0.0
	36.9	37	0.1
	38.8	38.8	0.0
	49.0	48	1.0
	67.0	67	0.0
	112.6	112.6	0.0
	114.5	114.6	0.1
	120.2	120.2	0.0
	120.3	120.3	0.0
	122.7	122.7	0.0
	124.1	124.1	0.0
	125.8	125.8	0.0
	138.6	138.6	0.0
	170.8	170.8	0.0
	191.7	191.7	0.0
	207.3	207.3	0.0
IR (cm^{-1})	3234, 1720, 1703, 1611	3200, 1710, 1640	NA
HRMS $[M+H]^+$	299.1508	299.1483	NA

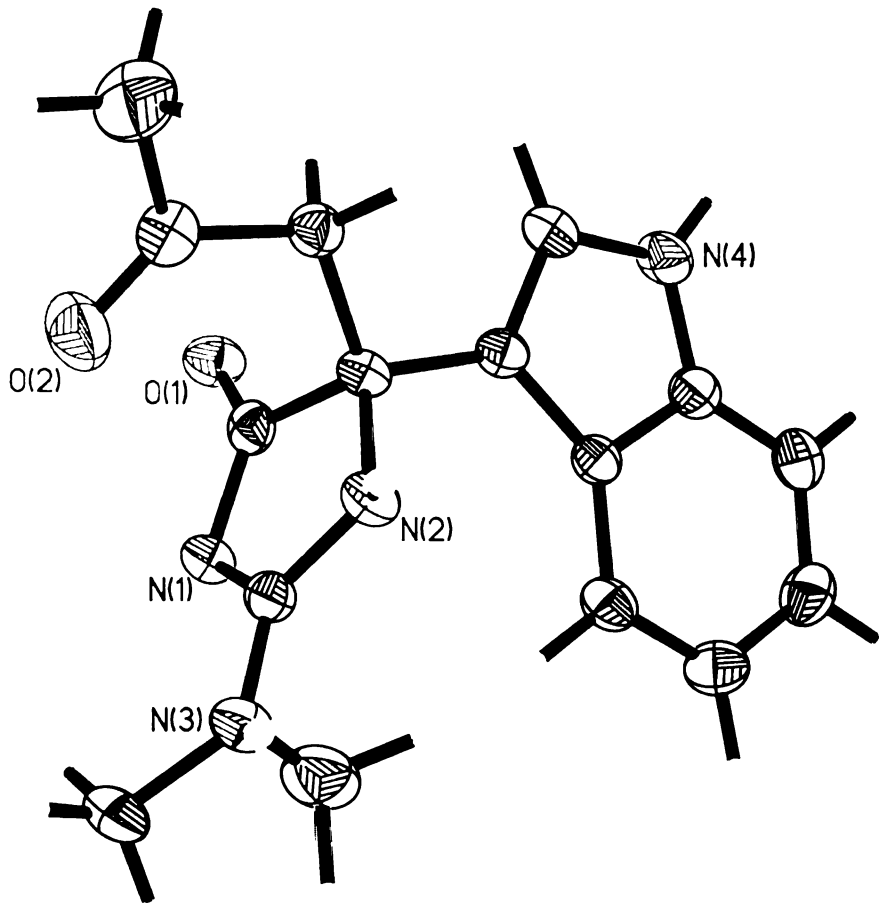


Figure III-2. X-ray crystal structure of indole alkaloid **III-1**

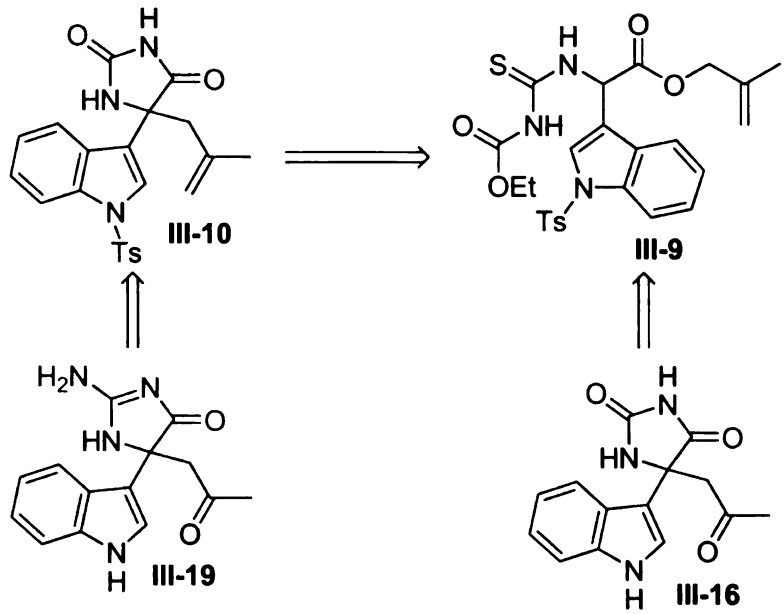
III.E Retrosynthetic analysis of analogs 1 and 2

The versatility of the synthetic pathway developed for the natural product was illustrated by the synthesis of 2 analogs that required no major modification of the original synthetic route. Hypothetically, a number of analogs could be developed with the synthetic route as a result of the diversity allowed in the

rearrangement and the variety of amines that could be used to create the final imidazolone.

Our retrosynthetic strategy for the total synthesis of analog 1 and analog 2 is illustrated in Scheme III-6. It was envisioned that the imidazolone moiety of the analog 2 (**III-19**) could be accessed from the hydantoin intermediate **III-10** utilizing classical chemical modifications.[20, 21] Hydantoin **III-10** could be formed from thiourea **III-9** using the protocol developed for the EDCI-mediated rearrangement. Analog 1 (**III-16**) was also envisioned to be formed from thiourea **III-9** by slightly modifying the rearrangement to include a removal of the tosyl protecting group.

Scheme III-6. Retrosynthesis of analog 1 (III-16) and analog 2 (III-19)

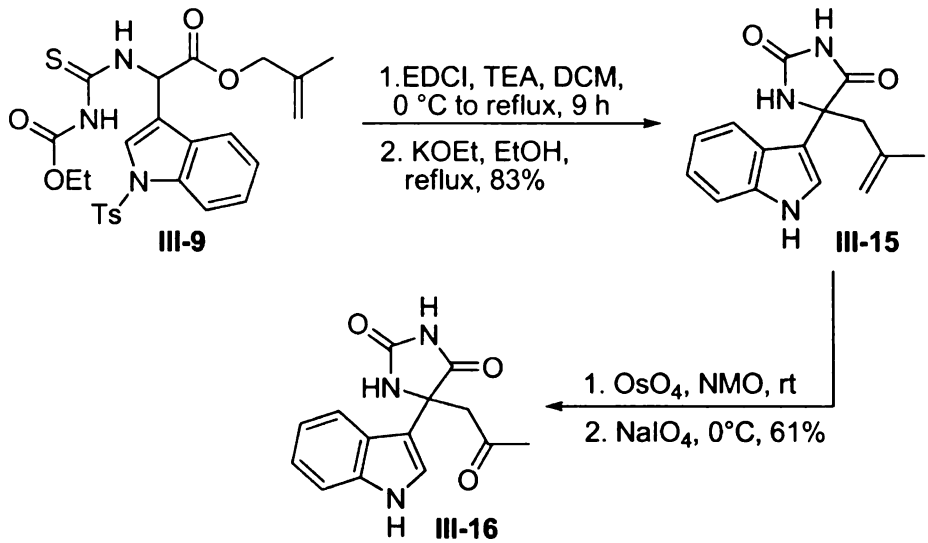


III.F Synthesis of analogs 1 and 2

The first analog, hydantoin **III-16** shown in Scheme III-7, was synthesized using the same thiourea (**III-9**) used to produce the natural product. Instead of carefully monitoring the second step of the rearrangement to avoid removal of

the tosyl protecting group, a mixture of potassium ethoxide and ethanol was used and refluxed overnight to yield hydantoin **III-15** in a 83% yield. Using the same procedure as the natural product synthesis, the terminal olefin on hydantoin **III-15** was oxidized to the ketone in a 61% yield affording hydantoin **III-16** (analog 1).[19]

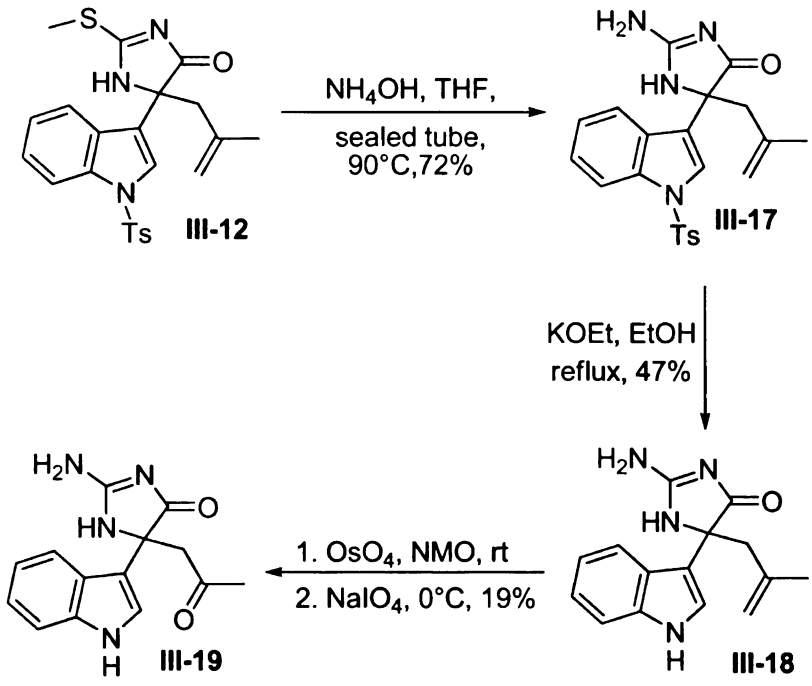
Scheme III-7. Synthesis of analog 1 (III-16)



The second analog shown in Scheme III-8, imidazolone **III-19,** took advantage of the synthetic handle that S-methylimidazolone **III-12** provided due to its reactivity and tendency to be substituted with a nucleophile. In this instance, the S-methyl group was replaced using ammonium hydroxide to give imidazolone **III-17** in a 72% yield. The deprotection method previously described with the natural product synthesis was also used to afford imidazolone **III-18** in a 47% yield. Finally, the synthesis of analog 2 (**III-19**) was completed after the terminal alkene of **III-18** was oxidized under previously determined conditions. Unfortunately, due to the difficult nature of isolating the product, only 19% of imidazolone **III-19** was recovered although the overall conversion was higher.[19] In general, the free amino imidazolones at every step were very hard to isolate

due to their tendency to stay in the aqueous phase during extractions, even after using n-butanol as an extracting solvent.

Scheme III-8. Synthesis of analog 2 (III-19)



III.G General experimental information

Reactions were carried out in flame-dried glassware under nitrogen atmosphere. All reactions were magnetically stirred and monitored by TLC with 0.25 μm pre-coated silica gel plates using either UV light or iodine to visualize the compounds. Column chromatography was carried out on Silica Gel 60 (230-400 mesh) supplied by EM Science. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. Infrared spectra were recorded on a Nicolet IR/42 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus-500 spectrometer or a Varian Inova-300, as noted in the experimental for each compound. Chemical shifts are reported relative to the residue peaks of the solvent ($CDCl_3$: 7.24 ppm for ^{1}H and 77.0 ppm for ^{13}C) (Acetone-d_6: 2.04 ppm for ^{1}H and 29.8 ppm for ^{13}C) (DMSO-d_6: 2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C). The following abbreviations are used to denote the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, and m = multiplet. Low resolution mass spectra were recorded on a Hewlet-Packard 5890 Series II gas chromatograph connected to a TRIO-1 EI mass spectrometer. HRMS were obtained with a Micromass Q-ToF Ultima API LC-MS/MS mass spectrometer. Elemental analysis data were obtained on a Perkin Elmer 2400 Series II CHNS/O analyzer. Purity of compounds, whose elemental analyses were above the ACS tolerated 0.4% deviation, were confirmed by ^{1}H NMR and ^{13}C NMR. Melting points were obtained using an Electrothermal® capillary melting point apparatus and are uncorrected. Reagents and solvents were purchased from commercial suppliers and used

without further purification. Anhydrous methylene chloride and toluene were dispensed from a delivery system which passes the solvents through a column packed with dry neutral alumina.

III.H Experimental procedures and characterization

(III-5). To a flame dried 100 mL round bottom flask was added EtOAc (50.0 mL) and 2-(1H-indol-3-yl)-2-oxoacetyl chloride[23, 24] (2.00 g, 9.66 mmol). The yellow mixture was put under an N_2 atmosphere. Then 2-methyl-2-propene-1-ol (4.00 mL, 48.3 mmol) was added. Within minutes the yellow cloudy mixture became an orange clear solution. The solution stirred overnight at room temperature under N_2. The EtOAc was evaporated by rotary evaporation and the solid was re-dissolved in EtOAc and washed with brine (2 x 30.0 mL). The aqueous layer was extracted with EtOAc (2 x 30.0 mL) and the organics were combined, dried using anhydrous sodium sulfate, and concentrated to give a the product as a dark reddish solid. Yield: (2.21 g, 94.0%). ^{1}H NMR (300MHz), Acetone: δ 1.82 (s, 3H), 4.78, (s, 2H), 4.99 (s, 1H,), 5.10 (s, 1H), 7.26-7.33 (m, 2H), 7.55-7.59 (m, 1H), 8.30-8.33 (m, 1H), 8.43 (s, 1H), 11.33 (s, 1H); ^{13}C NMR (75MHz), Acetone: δ 19.6, 69.0, 113.2, 113.7, 114.2, 122.5, 123.7, 124.8, 126.9, 137.8, 138.1, 140.7, 164.1, 179.6. IR: (NaCl) 1610 cm^{-1}, 1740 cm^{-1}, 3190 (broad) cm^{-1}. M.S: calculated for $C_{14}H_{13}NO_3$ [M+] = 243 and found [M+] = 243.0; Anal. Calcd. For $C_{14}H_{13}NO_3$: C, 69.12; H, 5.39; N, 5.76. Found: C, 67.78; H, 5.32; N, 5.26. Melting Point = 122-124˚C.

(III-6). To a 500 mL flame dried round bottom flask was added **III-5** (10.7 g, 44.0 mmol) and anhydrous DCM (0.250 L). Then TsCl (16.7 g, 88.1 mmol),

DMAP (13.4 g, 0.110 mol) and DIPEA (19.2 mL, 0.110 mol) were added and the mixture stirred at room temperature under nitrogen overnight. The solvent was removed and diethyl ether (0.200 L) was added to the residue and was washed with 1% HCl (2 x 80.0 mL) and brine (1 x 80.0 mL). The organics dried with anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% Hexane) affording the product as a whitish solid. Yield (13.6 g, 77.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.84 (s, 3H), 2.34, (s, 3H), 4.78 (s, 2H), 5.03 (s, 1H), 5.11 (s, 1H), 7.27 (d, J = 8.0 Hz, 2H), 7.36 (m, 2H), 7.84 (d, J = 8.3 Hz, 2H), 7.94 (d, J = 7.3 Hz, 1H), 8.33 (d, J = 7.33 Hz, 1H), 8.80 (s, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 19.5, 21.6, 69.4, 113.1, 114.5, 117.0, 122.9, 125.2, 126.1, 127.2, 127.6, 130.3, 134.2, 134.5, 136.7, 138.6, 146.1, 161.3, 178.4. IR: (NaCl) 1670 cm^{-1}, 1732 cm^{-1}. HRMS: $[M + H]^+$ = 398.1075, calculated for $C_{21}H_{20}NO_5S$, 398.1062. Anal. Calcd. For $C_{21}H_{19}NO_5S$: C, 63.46; H, 4.82; N, 3.52. Found: C, 63.46; H, 5.00; N, 3.39. Melting Point = 86-88°C.

(III-7). To a 250 mL round bottom flask was added **III-6** (2.10 g, 5.29 mmol) and dioxane (0.100 L). Then hydroxylamine hydrochloride (1.09 g, 15.9 mmol) was added along with a little water (5.00 mL). Then pyridine (1.36 mL, 16.9 mmol) was added and the mixture refluxed overnight under nitrogen. The solvents were removed and diethyl ether (0.100 L) was added to the residue and was washed with brine (1 x 50.0 mL). The organics were dried with anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% Hexane) affording the product as a

thick clear oil. Yield (2.10 g, 96.0%). Isomer A: ^{1}H NMR (500MHz), $CDCl_3$: δ 1.83 (s, 3H), 2.32 (s, 3H), 4.82 (s, 2H), 5.02 (s, 1H), 5.15 (s, 1H), 7.14 (d, J = 8.5 Hz, 2H), 7.17 (t, J = 8.0 Hz, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.73 (d, J = 8.5 Hz, 2H), 7.77 (s, 1H), 7.96 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 9.14 (s, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 19.4, 21.3, 69.1, 113.1, 113.7, 114.5, 123.0, 124.1, 125.6, 126.8, 126.9, 127.2, 129.9, 134.4, 135.0, 138.7, 145.4, 146.5, 162.5. Isomer B: ^{1}H NMR (500MHz), $CDCl_3$: δ 1.78 (s, 3H), 2.33 (s, 3H), 4.75 (s, 2H), 4.97 (s, 1H), 5.04 (s, 1H), 7.18 (d, J = 8.5 Hz, 2H), 7.25 (t, J = 8.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 8.5 Hz, 2H), 8.02 (d, J = 8.0 Hz, 1H), 8.28 (s, 1H), 9.78 (s, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 19.3, 21.3, 69.4, 109.6, 113.1, 114.2, 122.4, 123.2, 124.7, 126.8, 128.0, 129.8, 130.0, 133.9, 134.6, 138.8, 143.0, 145.3, 162.8. IR: (NaCl) 3460 cm^{-1}, 1736 cm^{-1}. HRMS: $[M + H]^{+}$ = 413.1167, calculated for $C_{21}H_{21}N_2O_5S$, 413.1171. Anal. Calcd. For $C_{21}H_{20}N_2O_5S$: C, 61.15; H, 4.89; N, 6.79. Found: C, 60.17; H, 4.87; N, 6.56.

(III-8). To a 1 L round bottom flask was added water (0.165 L) and AcOH (0.165 L). Then **III-7** (13.6 g, 33.0 mmol) was dissolved in THF (0.100 L) and was added to the aqueous acid. The mixture was brought down to 0°C and zinc (21.5 g, 0.330 mol) was then slowly added in small portions over 20 min. The suspension stirred at 0°C for 2 h. The solid was filtered off and the filtrate was reduced and then brought to a pH of 8 using concentrated ammonium hydroxide. The amine was extracted into ethyl acetate (4 x 0.150 L), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 30% EtOAc; 70% DCM) affording the product as a

thick oil. Yield (11.1 g, 84.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.51 (s, 3H), 1.93 (s, 2H), 2.30 (s, 3H), 4.51 (dd, J = 14.0, 19.2 Hz, 2H), 4.77 (m, 2H), 4.85 (s, 1H), 7.16-7.32 (m, 4H), 7.58 (s, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 8.0 Hz, 2H), 7.94 (d, J = 8.0 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 19.1, 21.4, 51.6, 68.5, 113.3, 113.6, 120.1, 121.5, 123.2, 123.8, 124.9, 126.7, 128.7, 129.8, 135.1, 135.2, 139.2, 144.9, 172.9. IR: (NaCl) 3389 cm^{-1}, 3350 cm^{-1}, 1740 cm^{-1}. HRMS: $[M + H]^+$ = 399.1369, calculated for $C_{21}H_{23}N_2O_4S$, 399.1379. Anal. Calcd. For $C_{21}H_{22}N_2O_4S$: C, 63.30; H, 5.56; N, 7.03. Found: C, 63.18; H, 5.70; N, 6.87.

(III-9). To a flame dried 500 mL round bottom flask was added **III-8** (10.4 g, 26.1 mmol) and anhydrous DCM (0.250 L). The solution was brought down to 0°C and ethoxycarbonyl isothiocyanate (3.54 mL, 31.4 mmol) was added dropwise. The solution was warmed to room temperature and stirred overnight under nitrogen. The solvents were removed and the product was recrystallized from EtOAc/Hexanes to give a white powder. Yield (12.1 g, 87.0%). ^{1}H NMR (500MHz), $CDCl_3$: δ 1.27 (t, J = 7.1 Hz, 3H), 1.52 (s, 3H), 2.31 (s, 3H), 4.21 (m, 2H), 4.55 (s, 2H), 4.81 (d, J = 8.0 Hz, 2H), 6.26 (d, J = 7.1 Hz, 1H), 7.18-7.33 (m, 4H), 7.62 (d, J = 7.9 Hz, 1H), 7.72-7.76 (m, 3H), 7.92 (d, J = 8.3 Hz, 1H), 8.02 (s, 1H), 10.60 (d, J = 7.1 Hz, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 14.1, 19.1, 21.5, 54.8, 62.9, 69.3, 113.7, 113.8, 116.0, 119.9, 123.6, 125.1, 126.1, 126.9, 128.2, 129.9, 134.9, 135.1, 138.8, 145.1, 152.4, 168.8, 179.0. IR: (NaCl) 3250 cm^{-1}, 3227 cm^{-1}, 1740 cm^{-1}, 1724 cm^{-1}, 1527 cm^{-1}. HRMS: $[M + H]^+$ = 530.1575, calculated for $C_{25}H_{28}N_3O_6S_2$, 530.1420. Anal. Calcd. For $C_{25}H_{27}N_3O_6S_2$: C, 56.69; H, 5.14; N, 7.93. Found: C, 56.22; H, 5.13; N, 7.80. Melting Point = 138-140°C.

(III-10). To a flame dried 50 mL round bottom flask was added **III-9** (0.265 g, 0.501 mmol), anhydrous DCM (20.0 mL), and anhydrous TEA (0.210 mL, 1.50 mmol). The solution was cooled to 0°C and then EDCI (0.212 g, 1.10 mmol) was added and the mixture stirred at 0°C for 1 h and then refluxed until disappearance of the starting material, as indicated on TLC. A solution of NaH (0.100 g, 2.51 mmol) in MeOH (10.0 mL) was then added to the mixture and stirred at room temperature for 2 h. The reaction was washed with 1% HCl (1 x 10.0 mL) and brine (1 x 10.0 mL) and the organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% EtOAc; 80% DCM) affording the product as a white solid. Yield (0.151 g, 71.0%). ^{1}H NMR (500MHz), Acetone: δ 1.69 (s, 3H), 2.32 (s, 3H), 2.92 (d, J = 14.7 Hz, 1H), 3.10 (d, J = 14.7 Hz, 1H), 4.85 (s, 1H), 4.92 (s, 1H), 7.27 (m, 1H), 7.36 (m, 3H), 7.66 (s, 1H), 7.83 (s, 1H), 7.87 (d, J = 8.3 Hz, 2H), 7.94 (d, J = 7.8 Hz, 1H), 8.01 (d, J = 8.3 Hz, 1H), 9.81 (s, 1H); ^{13}C NMR (125MHz), Acetone: δ 21.3, 24.2, 44.6, 66.0, 114.4, 117.1, 122.6, 122.8, 124.2, 125.2, 125.7, 127.8, 128.8, 130.9, 135.7, 136.4, 140.2, 146.5, 156.6, 175.2. IR: (NaCl) 3390 cm^{-1}, 3263 cm^{-1}, 1776 cm^{-1}, 1728 cm^{-1}. HRMS: $[M + H]^{+}$ = 424.1301, calculated for $C_{22}H_{22}N_3O_4S$, 424.1331. Anal. Calcd. For $C_{22}H_{21}N_3O_4S$: C, 62.40; H, 5.00; N, 9.92. Found: C, 62.08; H, 5.15; N, 9.67. Melting Point = 236-238°C.

(III-11). To a 100 mL flame dried round bottom flask was added **III-10** (0.595 g, 1.41 mmol) and anhydrous toluene (50.0 mL). Then Lawesson's reagent (0.341 g, 0.844 mmol) was added and the mixture was refluxed for 24 h.

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The toluene was then taken off and the crude residue was put into EtOAc (50.0 mL). The organic solution was then washed with brine (1 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 4% EtOAc; 96% DCM) affording the product as a solid. Yield (0.506 g, 82.0%). ^{1}H NMR (500MHz), Acetone: δ 1.77 (s, 3H), 2.32 (s, 3H), 3.01 (d, J = 13.6 Hz, 1H), 3.21 (d, J = 13.5 Hz, 1H), 4.90 (s, 1H), 4.96 (s, 1H), 7.26-7.40 (m, 4H), 7.76 (d, J = 8.0 Hz, 1H), 7.88-7.92 (m, 3H), 8.03 (d, J = 8.4 Hz, 1H), 9.46 (s, 1H), 10.80 (s, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 21.4, 24.1, 43.8, 68.8, 114.4, 117.4, 120.8, 121.8, 124.4, 125.4, 125.9, 127.8, 128.3, 130.9, 135.5, 136.1, 139.6, 146.6, 175.5, 183.0. IR: (NaCl) 3190 cm^{-1}, 1763 cm^{-1}, 1695 cm^{-1}. HRMS: $[M + H]^+$ = 440.1167, calculated for $C_{22}H_{22}N_3O_3S_2$, 440.1103. Anal. Calcd. For $C_{22}H_{21}N_3O_3S_2$: C, 60.11; H, 4.82; N, 9.56. Found: C, 60.07; H, 4.75; N, 9.58. Melting Point = 224-226°C.

(III-12). To a flame dried 25 mL round bottom flask was added **III-11** (0.105 g, 0.239 mmol), anhydrous DCM (10.0 mL), DMAP (0.00300 g, 0.0239 mmol) and DIPEA (0.210 mL, 1.20 mmol). Then MeI (0.0450 mL, 0.717 mmol) was added and the solution stirred at room temperature under nitrogen for 2 h. The DCM was taken off and diethyl ether (30.0 mL) was added to the residue. The organic solution was washed with 1% HCl (1 x 10.0 mL) and brine (1 x 10.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 5% EtOAc; 95% DCM) affording the product as an oil. Yield (0.0900 g, 83.0%). ^{1}H NMR (500MHz), Acetone: δ 1.76 (s, 3H), 2.31 (s, 3H), 2.60 (s, 3H), 2.77 (d, J = 13.2 Hz, 1H), 2.89

(d, J = 13.2 Hz, 1H), 4.66 (s, 1H), 4.72 (s, 1H), 7.22-7.35 (m, 4H), 7.70 (s, 1H), 7.83 (d, J = 8.3 Hz, 2H), 7.98 (d, J = 8.5 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 10.27 (s, 1H); ^{13}C NMR (125MHz), $CDCl_3$: δ 12.3, 21.3, 24.6, 46.2, 75.1, 114.2, 115.4, 122.3, 123.5, 123.8, 124.0, 125.4, 127.6, 129.7, 130.8, 135.8, 136.2, 141.3, 146.3, 160.8, 183.0. IR: (NaCl) 3239 cm^{-1} (broad), 1743 cm^{-1}, 1701 cm^{-1}. HRMS: $[M + H]^+$ = 454.1276, calculated for $C_{23}H_{24}N_3O_3S_2$, 454.1259. Anal. Calcd. For $C_{23}H_{23}N_3O_3S_2$: C, 60.90; H, 5.11; N, 9.26. Found: C, 59.91; H, 5.19; N, 8.90.

(III-13). To a flame dried 50 mL sealed tube was added **III-12** (0.0900 g, 0.199 mmol) and dimethylamine (10.0 mL of a 2.0M solution in THF). The tube was sealed and heated to an external sand bath temperature of 75°-90° for 14 h. Once cooled, the tube was opened and nitrogen was purged into the tube to expel any methane thiol that was formed. The whitish product was filtered off and the filtrate was concentrated and the residue was triturated with diethyl ether to obtain additional product, which was then filtered off. Yield (0.0810 g, 91.0%). ^{1}H NMR (500MHz), DMSO: δ 1.65 (s, 3H), 2.31 (s, 3H), 2.79 (dd, J = 13.1, 26.0 Hz, 2H), 3.00 (s, 3H), 3.08 (s, 3H), 4.76 (s, 1H), 4.8 (s, 1H), 7.22 (t, J = 8.1 Hz, 1H), 7.32 (t, J = 8.2 Hz, 1H), 7.39 (d, J = 8.5 Hz, 2H), 7.58 (d, J = 8.0 Hz, 1H), 7.75 (s, 1H), 7.87-7.92 (m, 3H), 8.33 (s, 1H); ^{13}C NMR (125MHz), DMSO: δ 20.9, 23.9, 36.0, 38.0, 43.2, 67.1, 113.0, 115.3, 121.5, 122.8, 123.0, 123.5, 124.6, 126.7, 127.9, 130.2, 134.1, 134.5, 139.7, 145.5, 169.8, 186.2. IR: (NaCl) 3210 cm^{-1} (broad), 1715 cm^{-1}, 1614 cm^{-1}. HRMS: $[M + H]^+$ = 451.1818, calculated for $C_{24}H_{27}N_4O_3S$, 451.1804. Anal. Calcd. For $C_{24}H_{26}N_4O_3S$: C, 63.98; H, 5.82; N, 12.44. Found: C, 62.82; H, 5.96; N, 11.76. Melting Point = 142-144°C.

(III-14). To a 50 mL round bottom flask was added **III-13** (0.200 g, 0.444 mmol) and EtOH (20.0 mL). Then KOEt (0.186 g, 2.22 mmol) was added and the solution refluxed overnight. The solvent was taken off and EtOAc (30.0 mL) was added to the residue. Then 1% HCl (30.0 mL) was added to acidify the solution. The EtOAc layer was discarded and the acidic aqueous layer was extracted again with EtOAc (1 x 10.0 mL) and that organic layer was also discarded. The acidic aqueous layer was neutralized with solid $NaHCO_3$ and then extracted with EtOAc (2 x 50.0 mL) and n-BuOH (2 x 50.0 mL). The organics were then dried using anhydrous sodium sulfate and concentrated. The crude solid was washed with acetone (10.0 mL) to remove a colored impurity to leave a white solid as product. Yield (0.113 g, 86.0%). ^{1}H NMR (500MHz), CD_3OD: δ 1.80 (s, 3H), 2.96 (d, J = 13.2 Hz, 1H), 3.03 (s, 3H), 3.07 (d, J = 13.2 Hz, 1H), 3.20 (s, 3H), 4.85 (s, 1H, hidden by MeOH), 4.90 (s, 1H), 6.96 (t, J = 8.0 Hz, 1H), 7.08 (t, J = 8.0 Hz, 1H), 7.30 (s, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 8.1 Hz, 1H); ^{13}C NMR (125MHz), CD_3OD: δ 24.6, 36.9, 38.7, 44.4, 69.7, 112.5, 115.2, 116.2, 120.1, 122.6, 124.2, 126.2, 138.5, 141.4, 170.1, 192.1. IR: (NaCl) 3321 cm^{-1} (broad), 3264 cm^{-1} (broad), 1684 cm^{-1}, 1611 cm^{-1}. HRMS: $[M + H]^+$ = 297.1723, calculated for $C_{17}H_{21}N_4O$, 297.1715. Melting Point = 267-269°C.

(III-1). To a 25 mL round bottom flask was added **III-14** (0.0500 g, 0.169 mmol), DMF (3.00 mL), THF (4.00 mL) and water (1.00 mL). Then NMO (0.0290 g, 0.253 mmol) and OsO_4 (0.171 mL of a 0.0980 M solution in toluene, 0.0169 mmol). The solution stirred at room temperature for 4 h and then was cooled to 0°C before a solution of $NaIO_4$ (0.108 g, 0.507 mmol) in water (2.00 mL) was

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added and stirred for 2 h at 0°C. The solvents were removed and EtOAc (10.0 mL) was added along with a sat. solution of K_2SO_3 (10.0 mL). This biphasic mixture stirred for 10 min and then the organic layer was separated and the aqueous layer was extracted with EtOAc (10.0 mL) again. The EtOAc layers were combined, and discarded. The aqueous layer was then extracted with n-BuOH (3 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated to give a whitish powder. The crude product was recrystallized from EtOH to give the natural product as a white powder. Yield (0.0310 g, 62.0%). ^{1}H NMR (500MHz), CD_3OD: δ 2.16 (s, 3H), 3.05 (s, 3H), 3.23 (s, 3H), 3.46 (dd, J = 16.6, 37.4 Hz, 2H), 6.97 (t, J = 7.0 Hz, 1H), 7.09 (t, J = 7.1 Hz, 1H), 7.26 (s, 1H), 7.33 (d, J = 8.2 Hz, 1H), 7.45 (d, J = 8.1 Hz, 1H); ^{13}C NMR (125MHz), CD_3OD: δ 31.0, 36.9, 38.8, 49.0, 67.0, 112.6, 114.5, 120.2, 120.3, 122.7, 124.1, 125.8, 138.6, 170.8, 191.7, 207.3. ^{1}H NMR (500MHz), DMSO: δ 2.08 (s, 3H), 2.99 (s, 3H), 3.11 (s, 3H), 3.24 (dd, J = 15.8, 38.3 Hz, 2H), 6.93 (t, J = 8.0 Hz, 1H), 7.05 (t, J = 8.0 Hz, 1H), 7.27 (s, 1H), 7.33 (d, J = 8.2 Hz, 1H), 7.42 (d, J = 7.7 Hz, 1H), 8.24 (s, 1H), 11.01 (s, 1H); ^{13}C NMR (125MHz), DMSO: δ 30.9, 36.0, 37.9, 48.7, 65.2, 111.5, 114.3, 118.5, 119.6, 121.0, 123.0, 124.5, 136.6, 170.1, 187.6, 205.1. IR: (NaCl) 3234 cm^{-1} (broad), 1720 cm^{-1}, 1703 cm^{-1}, 1611 cm^{-1}. HRMS: $[M + H]^+$ = 299.1508, calculated for $C_{16}H_{19}N_4O_2$, 299.1508. Anal. Calcd. For $C_{16}H_{18}N_4O_2$: C, 64.41; H, 6.08; N, 18.78. Found: C, 64.33; H, 6.22; N, 18.42. Melting Point = 246-248°C.

(III-15). To a flame dried 250 mL round bottom flask was added **III-9** (1.00 g, 1.89 mmol), anhydrous DCM (75.0 mL), and anhydrous TEA (0.780 mL, 5.67

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mmol). The solution was cooled to 0°C and then EDCI (0.798 g, 4.16 mmol) was added and the mixture stirred at 0°C for 1 h and then refluxed until disappearance of the starting material, as indicated on TLC. A solution of NaH (0.750 g, 18.9 mmol) in MeOH (70.0 mL) was then added to the mixture and refluxed for 8 h. Since the de-tosylation was sluggish with NaOMe the solvent was removed and ethanol (0.120 L) was added followed by KOEt (0.793 g, 9.45 mmol) and the mixture refluxed overnight. The solvent was removed and residue was acidified using 1% HCl and extracted using EtOAc (3 x 50.0 mL). The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% Acetone; 80% DCM) affording the product as a white solid. Yield (0.426 g, 83.0%). ^{1}H NMR (500MHz), DMSO: δ 1.75 (s, 3H), 2.72 (d, J = 13.4 Hz, 1H), 3.01 (d, J = 13.5 Hz, 1H), 4.82 (s, 1H), 4.91 (s, 1H), 6.99 (t, J = 6.8 Hz, 1H), 7.09 (t, J = 7.1 Hz, 1H), 7.37 (m, 2H), 7.57 (d, J = 8.1 Hz, 1H), 8.38 (s, 1H), 10.70 (s, 1H), 11.13 (s, 1H); ^{13}C NMR (125MHz), Acetone-d6: δ 23.9, 44.1, 65.9, 111.9, 114.7, 115.9, 119.5, 120.4, 121.9, 123.1, 125.2, 137.6, 140.3, 156.6, 175.8. IR: (NaCl) 3300 cm^{-1}, 1790 cm^{-1}, 1720 cm^{-1}. HRMS: $[M + H]^{+}$ = 270.1255, calculated for $C_{15}H_{16}N_3O_2$, 270.1243. Anal. Calcd. For $C_{15}H_{15}N_3O_2$: C, 66.90; H, 5.61; N, 15.60. Found: C, 66.38; H, 5.69; N, 15.11. Melting Point = 238-240°C.

(III-16). To a 25 mL round bottom flask was added **III-15** (0.172 g, 0.639 mmol), THF (8.00 mL) and water (1.00 mL). Then NMO (0.112 g, 0.959 mmol) and OsO_4 (0.650 mL of a 0.0980 M solution in THF, 0.0639 mmol) were added. The solution stirred at room temperature for 2 h and then was cooled to 0°C

before a solution of $NaIO_4$ (0.410 g, 1.92 mmol) in water (3.00 mL) was added and stirred at room temperature overnight. The solvents were removed and EtOAc (10.0 mL) was added along with a sat. solution of K_2SO_3 (10.0 mL). This biphasic mixture stirred for 10 min and then the organic layer was separated and the aqueous layer was extracted with n-BuOH (3 x 10.0 mL). The EtOAc layer and nBuOH layers were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 10% MeOH; 90% DCM) affording the product as an off white solid. Yield (0.107 g, 61.0%). Product was recrystallized over 1 week with EtOAc. ^{1}H NMR (500MHz), CD_3OD: δ 2.15 (s, 3H), 3.49 (d, J = 7.9 Hz, 1H), 3.59 (d, J = 7.9 Hz, 1H), 7.02 (t, J = 8.0 Hz, 1H), 7.11 (t, J = 8.2 Hz, 1H), 7.27 (s, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H); ^{13}C NMR (125MHz), CD_3OD: δ 30.5, 49.2, 64.1, 112.7, 113.9, 120.5, 120.6, 122.9, 123.9, 125.6, 138.8, 160.0, 179.0. IR: (KBr) 3364 (br) cm^{-1}, 1774 cm^{-1}, 1711 cm^{-1}. HRMS: $[M + H]^+$ = 272.1055, calculated for $C_{14}H_{14}N_3O_3$, 272.1035. Anal. Calcd. For $C_{14}H_{13}N_3O_3$: C, 61.99; H, 4.83; N, 15.49. Found: C, 61.10; H, 4.82; N, 15.38. Melting Point = 234-236°C.

(III-17). To a sealed tube was added **III-12** (0.850 g, 1.88 mmol) in THF (5.00 mL) and NH_4OH (15.0 mL). The mixture was heated at 90°C until the disappearance of the starting material, as indicated by TLC. The precipitate that formed was filtered, acidified with 5% HCl and extracted with nBuOH (3 x 20.0 mL), dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 10% MeOH; 90% DCM) affording the product as an off white solid. Yield (0.575 g, 72.0%). ^{1}H NMR

(500MHz), DMSO: δ 1.62 (s, 3H), 2.30 (s, 3H), 2.72 (d, J = 13.5 Hz, 1H), 2.81 (d, J = 13.5 Hz, 1H), 4.72 (s, 1H), 4.77 (s, 1H), 7.22 (t, J = 8.3 Hz, 1H), 7.32 (t, J = 8.3 Hz, 1H), 7.37 (d, J = 9.5 Hz, 2H), 7.64 (d, J = 8.1 Hz, 1H), 7.69 (s, 1H), 7.85 (d, J = 9.5 Hz, 2H), 7.90 (d, J = 8.5 Hz, 1H), 8.25 (s, 1H); ^{13}C NMR (125MHz), DMSO: δ 20.9, 23.7, 43.1, 66.0, 113.0, 115.2, 121.5, 123.0, 123.3, 124.7, 126.7, 128.0, 130.2, 134.0, 134.5, 139.9, 145.5, 170.9, 187.5. IR: (KBr) 3470 cm^{-1}, 3351 cm^{-1}, 3307 cm^{-1}, 3088 cm^{-1}, 1707 cm^{-1}, 1657 cm^{-1}. HRMS: $[M + H]^+$ = 423.1494, calculated for $C_{22}H_{23}N_4O_3S$, 423.1491. Anal. Calcd. For $C_{22}H_{22}N_4O_3S$: C, 62.54; H, 5.25; N, 13.25. Found: C, 61.21; H, 5.17; N, 13.05. Melting Point = 273-275°C.

(III-18). To a 100 mL round bottom flask was added **III-17** (0.500 g, 1.18 mmol) and EtOH (60.0 mL). Then KOEt (0.991 g, 11.8 mmol) was added and the mixture refluxed for 24 h. The EtOH was taken off and the pH of an aqueous mixture of the crude residue was adjusted to 8. The aqueous mixture was then extracted with nBuOH (3 x 40.0 mL). The organics were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% MeOH; 90% DCM) affording the product as an off white solid. The product was recrystallized from EtOH. Yield (0.150 g, 47.0%). ^1H NMR (500MHz), DMSO: δ 1.67 (s, 3H), 2.75 (d, J = 13.4 Hz, 1H), 2.81 (d, J = 13.4 Hz, 1H), 4.75 (s, 1H), 4.78 (s, 1H), 6.92 (t, J = 7.1 Hz, 1H), 7.04 (t, J = 7.0 Hz, 1H), 7.26 (d, J = 2.4 Hz, 1H), 7.33 (d, J = 8.2 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 8.03 (s, 1H), 10.96 (s, 1H); ^{13}C NMR (125MHz), DMSO: δ 23.9, 43.4, 66.3, 111.4, 114.7, 115.3, 118.3, 119.7, 120.9, 122.6, 124.8, 136.5, 140.7, 170.8, 189.1. IR: (KBr) 3470 cm^{-1}, 3390 (br) cm^{-1}, 3200 (br) cm^{-1}, 1692 cm^{-1},

1650 cm^{-1}. HRMS: $[M + H]^+$ = 269.1405, calculated for $C_{15}H_{17}N_4O$, 269.1402. Anal. Calcd. For $C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88. Found: C, 63.98; H, 5.89; N, 19.84. Melting Point = 264-266°C.

(III-19). To a 25 mL round bottom flask was added **III-18** (0.0990 g, 0.366 mmol), DMF (7.00 mL), THF (1.00 mL) and water (1.00 mL). Then NMO (0.0640 g, 0.549 mmol) and OsO_4 (0.370 mL of a 0.0980 M solution in THF, 0.0366 mmol) were added. The solution stirred at room temperature for 3 h and then was cooled to 0°C before a solution of $NaIO_4$ (0.235 g, 1.10 mmol) in water (2.00 mL) was added and stirred at room temperature overnight. The solvents were removed and EtOAc (10.0 mL) was added along with a sat. solution of K_2SO_3 (10.0 mL). This biphasic mixture stirred for 10 min and then the organic layer was separated and the aqueous layer was extracted with n-BuOH (6 x 10.0 mL). The EtOAc layer and nBuOH layers were combined, dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by column chromatography (silica gel, 20% MeOH; 90% DCM) affording the product as an off white solid. The product was recrystallized from EtOH. Yield (0.0190 g, 19.0%). ^1H NMR (500MHz), CD_3OD: δ 2.18 (s, 3H), 3.19 (d, J = 7.3 Hz, 1H), 3.64 (d, J = 7.1 Hz, 1H), 6.97 (t, J = 8.0 Hz, 1H), 7.08 (t, J = 8.0 Hz, 1H), 7.20 (s, 1H), 7.33 (d, J = 8.2 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H); ^{13}C NMR (125MHz), CD_3OD: δ 30.9, 66.5, 112.5, 114.1, 120.28, 120.29, 122.7, 124.0, 125.9, 138.5, 171.7, 192.5, 207.7. IR: (KBr) 3470 cm^{-1}, 3420 cm^{-1}, 3270 (br) cm^{-1}, 3176 (br) cm^{-1}, 1720 cm^{-1}, 1690 cm^{-1}, 1650 cm^{-1}. HRMS: $[M + H]^+$ = 271.1191, calculated for $C_{14}H_{15}N_4O_2$, 271.1195. Melting Point = 283-285°C.

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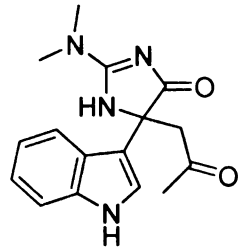
CHAPTER IV

BIOLOGICAL TESTING OF NATURAL PRODUCT, ANALOGS AND OTHER HETEROCYCLIC COMPOUNDS

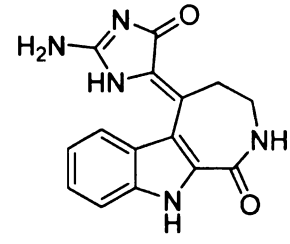
IV.A Biological target of natural product and hypothesis for activity

The biological target from the start of the project was checkpoint kinase 2 (Chk2), as discussed in chapter I. This kinase is responsible for helping to maintain the integrity of the genome by interpreting certain cellular distress signals and activating other proteins downstream to initiate a response. These responses can include cell cycle abrogation and allow for cellular repair or it can include programmed cell death, or apoptosis.[1]

It was hypothesized that the natural product (**III-1**) had some structural characteristics similar to that of a known Chk2 inhibitor, indoloazepine (Figure IV-1). Furthermore, the majority of the hydrogen bonding contacts thought to be important for indoloazepine's activity was present in the natural product. As a result, the curiosity of elucidating potential biological activity of this uniquely substituted imidazolone (**III-1**) prompted an endeavor to determine if there was any similarity in biological properties to indoloazepine, specifically, as a checkpoint kinase 2 inhibitor.



III-1



Indoloazepine

Figure IV-1. The indole natural product **III-1** and indoloazepine

IV.B Kinase screen for natural product and analogs

The natural product (**III-1**) and the two analogs synthesized were sent to a kinase screening company (Millipore). The compounds were screened for more than just Chk2 inhibition so that in the event of activity, insight into the degree of selectivity against other kinases could be evaluated. The kinases screened included Chk1, Chk2, GSK3β, IKKα and IKKβ. A general illustration of how the kinase screen works is shown in Figure IV-2. Essentially, a known substrate for the kinase is incubated with a compound, such as **III-1**, along with radio-labelled ATP. The amount of radio-activity in the end is directly related with the incorporation of ^{33}P, which can be correlated to how well the compound is at inhibiting the kinase. For example, if the compound is a good inhibitor of the kinase, there will be very little radio-activity in the end.

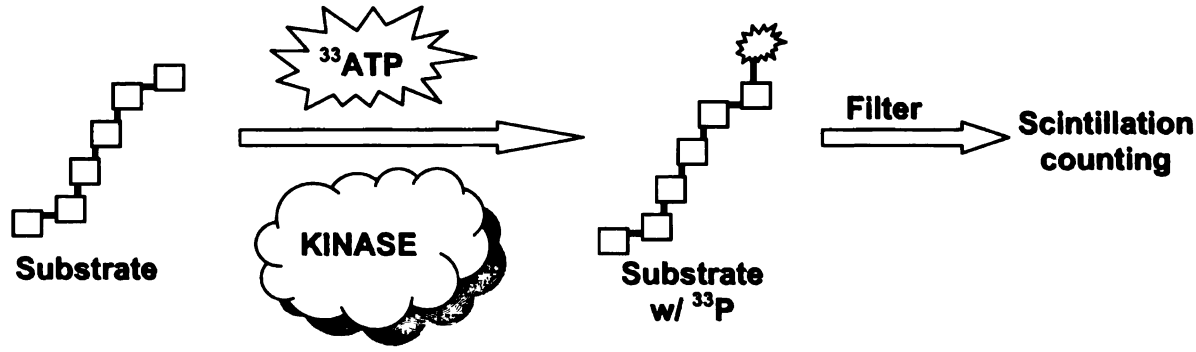
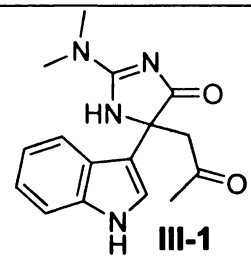
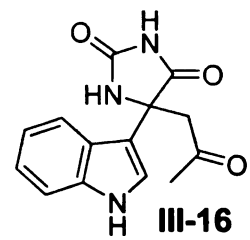
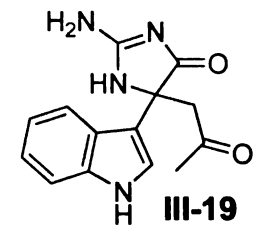


Figure IV-2. Kinase screen performed by Millipore

The results from the kinase screen are shown below in Table IV-1. The numerical value in each of the cells of the table is the percent activity of that kinase after being incubated with the corresponding compound. The lower the percent activity, the better of an inhibitor the compound is. The natural product and analogs were tested at 50 μM to identify if there was any activity of kinase

inhibition. As shown in Table IV-1, all three compounds were shown to be inactive for all five kinases tested. Taking into account the experimental error, all compounds essentially resulted in 100% activity for all kinases. Although these results are disappointing, it doesn't unequivocally prove that the compounds are inactive.

Table IV-1. Results of kinase screen for III-1, III-16 and III-19

Kinase	 III-1	 III-16	 III-19
Chk1	112	103	107
Chk2	102	106	101
GSK3β	100	94	99
IKKα	103	103	105
IKKβ	96	93	102
Biological result	**inactive**	**inactive**	**inactive**

IV.C Other biological testing for natural product and analogs

The natural product and two analogs were also tested in our laboratory in other assays to determine any biological activity. Compounds **III-1**, **III-16** and **III-19** were tested in a cell proliferation assay to determine if they were cytotoxic to MCF-7 cells, a breast cancer cell line. Furthermore, they were tested in a luciferase and whole blood assay for inhibition of NF-κB and IL-6 production, respectively, which are other biological targets of our laboratory. The two other quaternary imidazolones synthesized via the diketone rearrangement, compounds **II-18** and **II-23**, were also tested for NF-κB inhibition. Table IV-2

shows the results of all compounds for these assays. It can be seen that all compounds tested are inactive for the inhibition of NF-κB, inactive for the inhibition of IL-6 production and non-cyctotoxic for the MCF-7 cancer cell line.

Table IV-2. Results for cytotoxicity, NF-κB and IL-6 assays

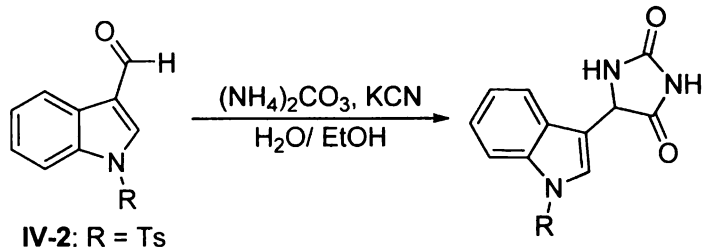
Compound	Target		
	NF-κB	IL-6	MCF-7 cells
III-1	inactive	inactive	non-cytotoxic
III-16	--	inactive	non-cytotoxic
III-19	--	inactive	non-cytotoxic
II-18	inactive	--	--
II-23	inactive	--	--

IV.D Synthesis of additional heterocycles

Throughout the process of developing a synthetic method to gain access to the imidazolone scaffold found in the natural product, other heterocycles were synthesized and were subsequently tested for biological activity. The majority of the heterocycles formed contained the indole and hydantoin moiety in various constitutions. Additionally, an indoloazepine analog was also synthesized. The following section describes the synthesis of these heterocycles.

Heterocycles such as indoles substituted in the 3-position by a hydantoin moiety were synthesized through a reaction between an aldehyde, ammonium carbonate and potassium cyanide (Scheme IV-1).[2] Hydantoin **IV-1**[3] was synthesized in a 36% yield through a reaction between indole-3-carboxaldehyde and the reagents described above, while a N-tosyl analog was synthesized in a 18% yield through a similar reaction. After the indolic nitrogen of indole-3-carboxaldehyde was protected with a tosyl group, the reaction using ammonium carbonate and potassium cyanide was used to produce the final hydantoin (**IV-3**).

Scheme IV-1. Synthesis of IV-1 and IV-3

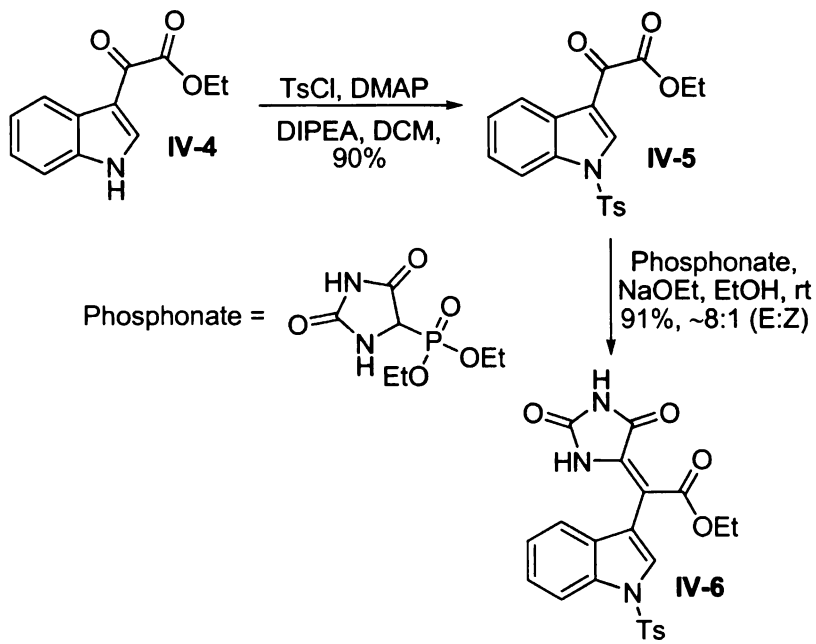


IV-2: R = Ts

IV-1: R = H, 36%
IV-3: R = Ts, 18%

A unique compound was synthesized using a hydantoin phosphonate reagent developed by Meanwell[4] (Scheme IV-2). Protection of the indolic nitrogen of **IV-4** with p-toluene sulfonyl chloride gave keto ester **IV-5** in a 90% yield. A subsequent Horner-Wadsworth-Emmons reaction produced hydantoin **IV-6** in about an 8:1 mixture of *E* and *Z* isomers in a 91% yield (Scheme IV-2). Geometric isomers were identified using NOESY experiments (Figure IV-9).

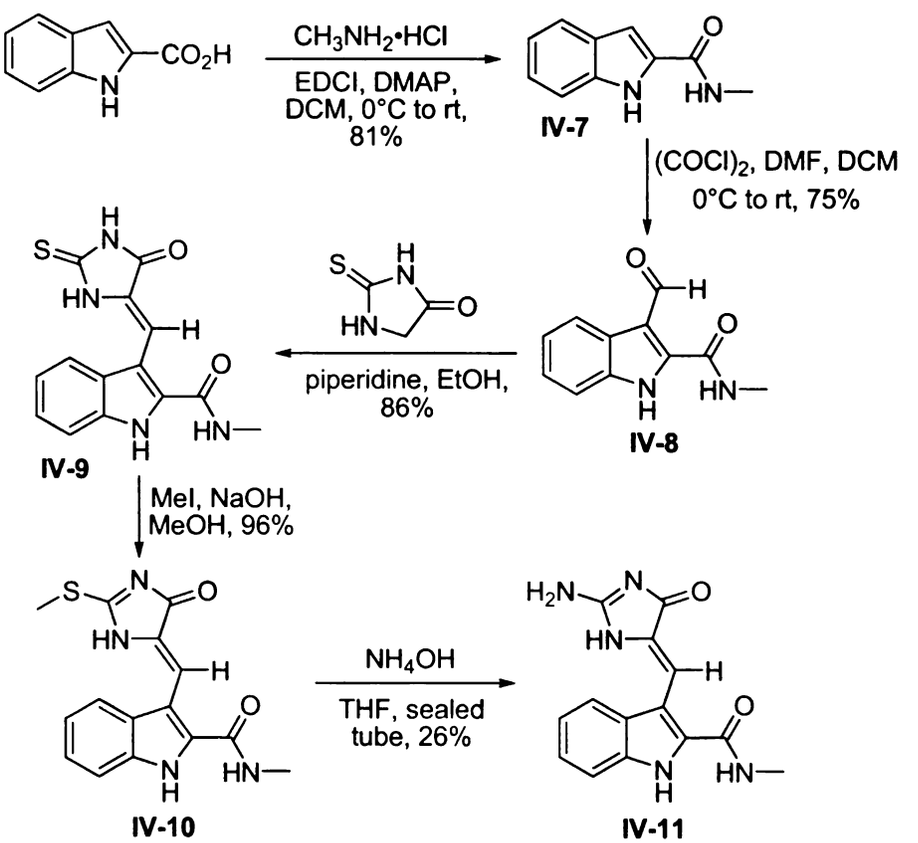
Scheme IV-2. Synthesis of hydantoin IV-6



Since indoloazepine was found to be a potent checkpoint kinase 2 inhibitor,[5] it was thought that synthesizing an analog could help identify the

structural features necessary for activity. It was decided to synthesize an analog that did not contain the seven membered ring between the indole and imidazolone moieties. To synthesize this analog, indole-2-carboxylic acid was coupled to methylamine using EDCI to afford **IV-7** in a 81% yield. Following a Vilsmeier-Haack[6, 7] reaction to produce aldehyde **IV-8**, a condensation between thiohydantoin and **IV-8** produced thiohydantoin **IV-9** in a 86% yield. Subsequently, after an S-methylation, the final imidazolone, **IV-11**, was produced in a 26% yield by replacing the S-methyl moiety with an NH_2 group using ammonium hydroxide.

Scheme IV-3. Synthesis of IV-11



IV.E Biological testing for additional heterocycles

The biological results for compounds **IV-1**, **IV-3**, **IV-6**, **IV-9** and **IV-11** are illustrated in Table IV-3. Unfortunately, all the compounds tested for kinase inhibition were found to be inactive. Additionally, none of the compounds exhibited any propensity for inhibition or abrogation of IL-6 production. Further testing is needed to determine if any of the compounds synthesized have any biological value.

Table IV-3. Biological activity results for IV-1, IV-3, IV-6, IV-9 and IV-11

Cmpd.	Target						
	Chk1	Chk2	GSK3β	IKKα	IKKβ	NF-κB	IL-6
IV-1	inactive	inactive	inactive	inactive	inactive	inactive	inactive
IV-3	--	--	--	--	--	inactive	inactive
IV-6	--	--	--	--	--	inactive	--
IV-9	--	--	--	--	--	inactive	--
IV-11	inactive	inactive	--	--	--	--	--

IV.F General experimental information

Reactions were carried out in flame-dried glassware under nitrogen atmosphere. All reactions were magnetically stirred and monitored by TLC with 0.25 μm pre-coated silica gel plates using either UV light or iodine to visualize the compounds. Column chromatography was carried out on Silica Gel 60 (230-400 mesh) supplied by EM Science. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. Infrared spectra were recorded on a Nicolet IR/42 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus-500 spectrometer or a Varian Inova-300, as noted in the experimental for each compound. Chemical shifts are reported relative to the residue peaks of the solvent ($CDCl_3$: 7.24 ppm for ^{1}H and 77.0 ppm for ^{13}C) (Acetone-d_6: 2.04 ppm for ^{1}H and 29.8 ppm for ^{13}C) (DMSO-d_6: 2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C). The following abbreviations are used to denote the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, and m = multiplet. HRMS were obtained with a Micromass Q-ToF Ultima API LC-MS/MS mass spectrometer. Elemental analysis data were obtained on a Perkin Elmer 2400 Series II CHNS/O analyzer. Purity of compounds, whose elemental analyses were above the ACS tolerated 0.4% deviation, were confirmed by ^{1}H NMR and ^{13}C NMR. Melting points were obtained using an Electrothermal® capillary melting point apparatus and are uncorrected. Reagents and solvents were purchased from commercial suppliers and used without further purification. Anhydrous methylene chloride and toluene were

dispensed from a delivery system which passes the solvents through a column packed with dry neutral alumina.

IV.G Experimental procedures and characterization

Cell Proliferation Assay

The cell proliferation study was performed by Thu Nguyen and all detailed experimental information is found within her notebook. The general procedure is as follows. Adherent cells were seeded with 500 µL of approximately 2.5 x 10^5 cells/mL in a 24-well plate. When cells were near 80% confluent, cells were treated with the appropriate drug in various doses. Cells were incubated for 12, 24 and 48 hours. At each time point, cells were released from the plate with 100 µL of 0.25% trypsin/EDTA and diluted to 500 µL, which was then counted via a cell counter (Beckman Coulter Z1 Coulter Particle Counter).

Luciferase Assay

The luciferase assay was performed by Thu Nguyen, Teri Lansdell and/or Behnaz Shafii. All detailed experimental information is located within their respective notebooks. The general experimental procedure is as follows. HeLa NF-κB cell line was cultured in DMEM, complemented with 10% Fetal Bovine Serum, 1 mM sodium pyruvate, 0.1 mg/mL Hygromycin, 2mM-L-glutamine, 1mM sodium pyruvate, 100 units/mL penicillin, 4.5 g/L D-glucose, and no phenol red. The day before the luciferase assay, the medium was switched to DMEM with 2% FBS in addition to 2mM-L-glutamine, 1mM sodium pyruvate, and 100 units/mL penicillin. The day of the experiment, the medium utilized was serum free DMEM with 2mM-L-glutamine, 1mM sodium pyruvate, and 100 units/mL

penicillin. Cells were propagated at 37°C with 5% CO_2, and ambient oxygen. Cells were seeded in a 96-well plate between 35,000-50,000 cells per well with DMEM 2% FBS. The outside rows and columns of the plate were not used. Cell were allowed to adhere and grow overnight at 37°C with 5% CO_2, and ambient oxygen. On the day of the experiment, the media was removed and replaced with serum free DMEM. Cells were pretreated with **2** (in 1 µL DMSO/well) for 30 minutes before activation with 25 ng/mL TNF-α and incubated an additional 8 hours. Steady glo reagent (Promega) was added to each well and data was collected using a luminometer.

Whole Blood IL-6 Assay

The whole blood assay was performed by Teri Lansdell and all detailed experimental information is located in her notebooks. The general experimental procedure is as follows. After obtaining the appropriate approval for de-identified human cell lines, human whole blood was obtained through the Jasper Research Clinic, Kalamazoo, MI, from a single healthy, fasted volunteer and was collected in glass citrated tubes by venipuncture. Only samples with a white blood cell count falling within the normal range (4800-10,800 per liter) were used. The blood was diluted 1:10 in RPMI-1640 media supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. Aliquots of diluted blood (1 mL) were preincubated with vehicle (0.1% DMSO, final concentration) or test agent (final concentrations were 20, 10, 5, 2.5, 1.25 and 0.625 µM) for two hours at 37 °C, 5 % CO_2. IL-1β (Roche) was added to a final concentration of 200 U/mL and the samples were further incubated for 18 hours at 37 °C, 5 %

CO_2. At the end of the incubation period, the blood samples were centrifuged at 3000 X g, 4 °C, for 10 minutes. The plasma was removed, snap frozen and stored at -80 C. IL-6 levels were determined by ELISA (R & D Systems).

Kinase Profiler (Millipore)

The kinase testing was performed by Millipore. All detailed experimental information can be found on their website.[8] The general experimental procedure for each kinase is described below.

CHK1 (h)

CHK1 (h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 200 µM KKKVSRSGLYRSPSMPENLNRPR, 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 µL of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

CHK2 (h)

CHK2 (h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 200 µM KKKVSRSGLYRSPSMPENLNRPR, 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 µL of the reaction is then spotted onto a P30 filtermat and washed

three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

GSK3β (h)

GSK3β (h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 20 μM YRRAAVPPSPSLSRHSSPHQS(p) EDEEE (phospho GS2 peptide), 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 μL of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 50 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

IKKα (h)

IKKα (h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 200 μM peptide, 10 mM MgAcetate and [γ-33PATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 μL of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

IKKβ (h)

IKKβ (h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 100 μM peptide, 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500

cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 µL of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

Experimentals for compounds IV-1-IV-11

(IV-1). To a flame dried 250 mL round bottom flask was added indole-3-carboxaldehyde (1.00 g, 6.90 mmol) and ethanol (70.0 mL). Then ammonium carbonate (2.00 g, 20.7 mmol) and water (70.0 mL) were added and the mixture was heated in an oil bath to 50°C. Once everything was in solution, KCN (0.583 g, 8.97 mmol in 10.0 mL H_2O) was added over 25 min. The light orange solution stirred at 60°C overnight and became a darker orange color. The ethanol was taken off by rotary evaporation and the solid that precipitated out was filtered off. The filtrate was neutralized with 1% HCl (aq.) and extracted with ethyl acetate (3 x 50.0 mL). The organics were dried using anhydrous sodium sulfate and concentrated to give a yellowish oil. The crude material was purified by column chromatography (silica gel, 4:1 ethyl acetate/hexanes) affording the product as an off white powder. Yield (0.537 g, 36.0%). ^{1}H NMR (500 MHz), Acetone: δ 5.47 (s, 1H), 7.03-7.06 (m, 1H), 7.12-7.16 (m, 1H), 7.32 (bs, 1H), 7.42-7.44 (m, 2H), 7.57-7.59 (m, 1H), 9.80 (bs, 1H), 10.30 (bs, 1H); ^{13}C NMR (125 MHz), Acetone: δ 57.1, 110.8, 112.6, 119.7, 120.2, 122.7, 125.4, 126.5, 137.9, 157.9, 174.8; IR (NaCl) 1710 cm^{-1}, 1770 cm^{-1}, 3300 cm^{-1}. M.S: calculated for $C_{11}H_9N_3O_2$ [M+] =

215 and found [M+] = 214.9. Anal. Calcd. For $C_{11}H_9N_3O_2$: C, 61.40; H, 4.20; N, 19.50. Found: C, 60.90; H, 3.60; N, 18.90. Melting Point = 220-222°C.

(IV-2).[9] To a flame dried 500 mL round bottom flask was added indole-3-carboxaldehyde (5.00 g, 34.5 mmol), dry DCM (0.300 L), TsCl (13.1 g, 69.0 mmol), DMAP (10.5 g, 86.2 mmol), and DIPEA (15.0 mL, 86.2 mmol). The solution stirred at room temperature overnight under nitrogen. The reaction was quenched and washed with 1% HCl (aq.) and the organics were dried using anhydrous sodium sulfate and concentrated. A silica plug was done to isolate product (silica gel, 100% ethyl acetate). Yield (10.3 g, 99.0%). ^{1}H NMR (500 MHz), $CDCl_3$: δ 2.35 (s, 3H), 7.26-7.41 (m, 4H), 7.83 (d, J = 8.9 Hz, 2H), 7.93 (d, J = 8.1 Hz, 1H), 8.21 (s, 1H), 8.23 (d, J = 8.9 Hz, 1H), 10.10 (s, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 21.6, 113.2, 122.3, 122.5, 124.9, 126.2, 127.1, 130.2, 134.2, 135.1, 136.1, 146.1, 185.2; IR (NaCl) 1710 cm^{-1}, 2750 cm^{-1}, 2850 cm^{-1}.

(IV-3). To a flame dried 250 mL round bottom flask was added **IV-2** (3.30 g, 11.0 mmol) and ethanol (75.0 mL). Then ammonium carbonate (3.18 g, 33.1 mmol) and water (75.0 mL) were added and the mixture was heated in an oil bath to 50°C. Once everything was in solution, KCN (1.08 g, 16.6 mmol in 10.0 mL H_2O) was added over 25 min. The light orange solution stirred at 60°C overnight and became a darker orange color. The ethanol was taken off by rotary evaporation and the solid that precipitated out was filtered off. The filtrate was neutralized with 1% HCl (aq.) and extracted with ethyl acetate (3 x 50.0 mL). The organics were dried using anhydrous sodium sulfate and concentrated. The crude material was purified by column chromatography (silica gel, 3:2 ethyl

acetate/hexanes) affording the product as an off white powder. Yield (0.717 g, 18.0%). ^{1}H NMR (500 MHz), Acetone: δ 2.34 (s, 3H), 5.53 (s, 1H), 7.27 (t, J = 7.3 Hz, 1H), 7.35-7.40 (m, 4H), 7.64 (d, J = 7.8 Hz, 1H), 7.84 (s, 1H), 7.89 (d, J = 8.3 Hz, 2H), 8.01 (d, J = 8.3 Hz, 1H), 9.78 (s, 1H); ^{13}C NMR (125 MHz), Acetone: δ 21.4, 56.3, 114.4, 118.5, 121.3, 124.2, 125.9, 126.0, 127.9, 129.3, 131.0, 135.8, 136.2, 146.6, 157.5, 173.3; IR (NaCl) 3249 cm^{-1}, 1772 cm^{-1}, 1724 cm^{-1}. M.S: calculated for $C_{18}H_{15}N_3O_4S$ [M+] = 369 and found [M+] = 369.0. Anal. Calcd. For $C_{18}H_{15}N_3O_4S$: C, 58.53; H, 4.09; N, 11.38. Found: C, 57.68; H, 4.16; N, 11.25. Melting Point = 226-228 °C.

(IV-5). To a 500 mL flame dried round bottom flask was added **IV-4** (4.47 g, 20.6 mmol), dry dichloromethane (0.225 L), TsCl (7.83 g, 41.2 mmol), DMAP (6.28 g, 51.5 mmol), and DIPEA (9.00 mL, 51.5 mmol), The reaction stirred under nitrogen overnight. The reaction was washed with sat. $NaHCO_3$ (2 x 50.0 mL) and brine (2 x 50.0 mL) and the organic layer was dried using anhydrous sodium sulfate and concentrated. The crude material was purified by column chromatography (silica gel, DCM) affording the product as a solid. Yield (6.80 g, 90.0%). ^{1}H NMR (500 MHz), $CDCl_3$: δ 1.43 (t, J = 7.2 Hz, 3H), 2.34 (s, 3H), 4.43 (q, J = 7.0 Hz, 2H), 7.26-7.41 (m, 4H), 7.85 (d. J = 8.5 Hz, 2H), 7.94 (m, 1H), 8.34 (m, 1H), 8.83 (s, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 14.0, 21.6, 62.5, 113.1, 116.9, 122.9, 125.2, 126.1, 127.2, 127.6, 130.3, 134.2, 134.4, 136.7, 146.1, 161.6, 178.7. IR (NaCl) 1725 cm^{-1}, 1670 cm^{-1}. HRMS: $[M + H]^+$ = 372.0907, calculated for $C_{19}H_{18}NO_5S$, 372.0906. Melting Point = 106-108°C.

(IV-6). To a flame dried 50 mL round bottom flask was added anhydrous EtOH (20.0 mL) and Na° (0.0740 g, 3.23 mmol). Once all the sodium metal reacted and the formation of bubbles ceased, diethyl 2,5-dioxoimidazolidin-4-ylphosphonate[4] (0.763 g, 3.23 mmol, borrowed from Thu Nguyen) was added and stirred at room temperature for 30 min. Then **IV-5** (1.00 g, 2.70 mmol) was added to the mixture and stirred for 18 h at room temperature under a nitrogen atmosphere. The solvent was then removed and the crude residue was neutralized with 1% HCl and extracted with EtOAc (2 x 40.0 mL). The organics were washed with brine, dried using anhydrous sodium sulfate and concentrated. The crude material was purified by column chromatography (silica gel, 96% DCM; 4% MeOH) affording the product as an oil (mixture of diastereomers (8:1; *E*:*Z*)). Yield (1.11 g, 91.0%). ^{1}H NMR (500 MHz), $CDCl_3$: (Isomer B) δ 1.28 (t, J = 7.2 Hz, 3 H), 2.28 (s, 3H), 4.35 (q, J = 7.1 Hz, 2H), 7.16-7.22 (m, 3H), 7.29 (t, J = 7.3 Hz, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.78 (d, J = 8.5 Hz, 2H), 7.87 (s, 1H), 7.89 (d, J = 8.5 Hz, 1H), 8.32 (bs, 1H), 9.01 (bs, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 13.7, 21.5, 62.4, 111.7, 113.0, 113.5, 120.4, 123.9, 125.5, 126.7, 127.0, 127.6, 127.9, 130.0, 134.5, 134.7, 145.5, 153.9, 161.6, 165.7. (Isomer A) ^{1}H NMR (500 MHz), $CDCl_3$: δ 1.07 (t, J = 7.0 Hz, 3H), 2.28 (s, 3H), 4.15 (q, J = 7.0 Hz, 2H), 7.10-7.22 (m, 5H), 7.58 (s, 1H), 7.75 (d, J = 8.3 Hz, 2H), 7.83 (d, J = 8.2 Hz, 1H), 9.70 (bs, 1H); ^{13}C NMR (125 MHz), $CDCl_3$: δ 13.8, 21.4, 61.8, 103.1, 112.4, 113.3, 120.0, 123.0, 124.3, 126.9, 127.3, 129.7, 130.5, 134.3, 135.0, 137.6, 144.7, 152.9, 161.9, 167.2. IR (NaCl) 3261 cm^{-1}, 1779 cm^{-1}, 1736 cm^{-1}, 1657 cm^{-1}. HRMS: $[M + H]^+$ = 454.1078, calculated for $C_{22}H_{20}N_3O_6S$, 454.1073.

(IV-7).[10] To a flame dried 100 mL round bottom flask was added methylamine hydrochloride (1.00 g, 14.9 mmol) and dry DCM (55.0 mL). The mixture was cooled to 0°C and then DMAP (3.79 g, 31.1 mmol), indole-2-carboxylic acid (2.00 g, 12.4 mmol) and EDCI (2.86 g, 14.9 mmol) were added. The mixture stirred at 0°C under nitrogen for 4 h and then at room temperature overnight. The clear brown solution was washed with sat. sodium bicarbonate solution (1 x 60.0 mL), 1% HCl (1 x 60.0 mL) and brine (1 x 60.0 mL). The organics were combined, dried using anhydrous sodium sulfate and concentrated. A small amount of DCM was then added and the product precipitated out of solution to give an off white solid. Yield (1.57 g, 73.0%).^{1}H NMR (500 MHz), Acetone: δ 2.94 (dd, J = 1.3, 4.7 Hz, 3H), 7.05 (t, J = 7.5 Hz, 2H), 7.20 (dd, J = 7.1, 8.3 Hz, 1H), 7.56-7.61 (m, 2H), 7.81 (s, 1H), 11.01 (s, 1H); ^{13}C NMR (125 MHz), Acetone: δ 26.2, 102.5, 113.0, 120.7, 122.3, 124.3, 128.7, 132.8, 137.6, 162.8. M.S: calculated for $C_{10}H_{10}N_2O$ (M+) = 174.2 and found (M+) = 174.3.

(IV-8). To a flame dried 50 mL round bottom flask was added anhydrous DMF (3.50 mL) and dry DCM (25.0 mL). The solution was cooled to 0°C and then oxalyl chloride (0.280 mL, 3.16 mmol) was added and the solution became a thick white mixture. **IV-7** (0.500 g, 2.87 mmol) was then added and the mixture turned from yellow to red. The solution stirred at room temperature for 8 h under nitrogen. The precipitate that formed was filtered off and washed with water (20-30.0 mL) and extracted with EtOAc (5 x 30.0 mL). The organics were combined and dried using anhydrous sodium sulfate and concentrated to give the product

as a solid. Yield (0.398 g, 69.0%). ^{1}H NMR (500 MHz), DMSO: δ 2.91 (d, J = 4.7 Hz, 3H), 7.28 (t, J = 7.1 Hz, 1H), 7.34 (t, J = 7.1 Hz, 1H), 7.55 (d, J = 8.0 Hz, 1H), 8.19 (d, J = 6.8 Hz, 1H), 9.62 (s, 1H), 10.30 (s, 1H), 12.77 (s, 1H); ^{13}C NMR (125 MHz), DMSO: δ 26.1, 113.0, 113.9, 120.5, 122.9, 124.7, 126.5, 134.9, 138.0, 159.9, 186.5. IR (NaCl) 3182 cm^{-1}, 1651 cm^{-1}, 1590 cm^{-1}. M.S: calculated for $C_{11}H_{10}N_2O_2$ (M+) = 202.2 and found (M+) = 202.4. Anal. Calcd. For $C_{11}H_{10}N_2O_2$: C, 65.34; H, 4.98; N, 13.85. Found: C, 64.08; H, 4.80; N, 13.58. Melting Point = 257-259 °C.

(IV-9). To a 25 mL round bottom flask was added **IV-8** (0.306 g, 1.51 mmol) and EtOH (8.00 mL). Then thiohydantoin (0.193 g, 1.66 mmol) and piperidine (0.900 mL, 9.10 mmol) were added and the mixture was stirred under nitrogen at room temperature overnight. The mixture was filtered off and the precipitate was stirred in acetone for 5 min and then filtered. The precipitate was collected to give the product as a solid. Yield (0.390 g, 86.0%). ^{1}H NMR (500 MHz), DMSO: δ 2.83 (d, J = 4.6 Hz, 3H), 6.95 (s, 1H), 7.17 (t, J = 7.4 Hz, 1H), 7.29 (t, J = 7.5 Hz, 1H), 7.47 (d, J = 8.3 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 8.14 (bs, 1H), 11.71 (bs, 1H), 11.98 (bs, 1H); ^{13}C NMR (125 MHz), DMSO: δ 26.2, 105.5, 109.5, 112.5, 120.6, 120.8, 124.1, 126.1, 128.8, 131.1, 135.5, 161.6, 165.4, 177.6. IR (KBr) 3370 cm^{-1}, 3194 cm^{-1}, 1730 cm^{-1}, 1663 cm^{-1}, 1615 cm^{-1}. M.S: calculated for $C_{14}H_{12}N_4O_2S$ (M+) = 300.3 and found (M+) = 300.3. Anal. Calcd. For $C_{14}H_{12}N_4O_2S$: C, 55.99; H, 4.03; N, 18.65. Found: C, 54.91; H, 4.16; N, 17.95. Melting Point = 380 °C decomposes.

(IV-10). To a 25 mL round bottom flask was added **IV-9** (0.250 g, 0.833 mmol), MeOH (5.00 mL) and a solution of NaOH (0.0370 g, 0.917 mmol in 0.300 mL H_2O). Then MeI (0.0600 mL, 0.917 mmol) was added and the reaction stirred under nitrogen over night at room temperature. The product precipitated out of solution after stirring overnight and the orange solid was filtered off. The crude product was recrystallized in MeOH to give pure product as a solid. Yield (0.252 g, 96.0%). ^{1}H NMR (500 MHz), DMSO: δ 2.66 (s, 3H), 2.84 (d, J = 4.7 Hz, 3H), 7.14 (t, J = 8.1 Hz, 1H), 7.26 (t, J = 8.1 Hz, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.55 (s, 1H), 8.42 (d, J = 5.7 Hz, 1H), 9.11 (d, J = 8.0 Hz, 1H), 11.66 (s, 1H), 12.06 (s, 1H); ^{13}C NMR (125 MHz), DMSO: δ 12.2, 26.1, 112.0, 112.5, 116.7, 120.6, 124.2, 125.1, 125.5, 135.2, 135.6, 135.9, 160.0, 161.8, 170.7. IR (KBr) 3267 cm^{-1}, 1706 cm^{-1}, 1627 cm^{-1}, 1602 cm^{-1}. HRMS: $[M + H]^+$ = 315.0916, calculated for $C_{15}H_{15}N_4O_2S$, 315.0916. Anal. Calcd. For $C_{15}H_{14}N_4O_2S$: C, 57.31; H, 4.49; N, 17.82. Found: C, 57.10; H, 4.29; N, 17.58. Melting Point = 268-270°C decomposes.

(IV-11). To a sealed tube was added **IV-10** (0.420 g, 1.34 mmol) and THF (4.00 mL). Then ammonium hydroxide (4.00 mL) was added and the mixture was sealed and heated to 90°C for 16 h. The solution was cooled and the solvent was removed. Methanol (15.0 mL) was added and the product precipitated out. Yield (0.100 g, 26.0%). ^{1}H NMR (500 MHz), DMSO: δ 2.79-2.95 (m, 3H), 6.90 (bs, 1H), 7.22 (t, J = 8.0 Hz, 1H), 7.43 (t, J = 8.2 Hz, 1H), 7.54 (d, J = 8.3 Hz, 1H), 7.74-7.80 (m, 1H), 7.90-8.18 (m, 2H), 8.32-8.72 (bs, 1H), 10.42 (bs, 1H), 12.25 (bs, 1H); ^{13}C NMR (125 MHz), DMSO: δ 27.5, 101.2, 101.5, 112.6, 120.13, 120.16,

121.3, 122.5, 123.6, 126.5, 129.6, 129.7, 132.70, 132.76, 139.4, 153.8, 161.9, 162.3, 168.2, 169.0. IR (KBr) 3468 cm^{-1}, 3304 cm^{-1}, 3218 cm^{-1}, 1639 cm^{-1}, 1608 cm^{-1}, 1578 cm^{-1}. HRMS: $[M + H]^+$ = 284.1150, calculated for $C_{14}H_{14}N_5O_2$, 284.1147. Anal. Calcd. For $C_{14}H_{13}N_5O_2$: C, 59.36; H, 4.63; N, 24.72. Found: C, 57.30; H, 4.08; N, 24.33. Melting Point = 382-386°C decomposes.

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