Title
Progress Toward a Scalable Synthesis of Azaspirene, An Angiogenesis Inhibitor and Synthesis of 2-Amino- benzimidazole Compounds Targeting Subdomain I1a of the Internal Ribosome Entry Site Inhibiting Translation of The Hepatitis C Virus

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Progress Toward a Scalable Synthesis of Azaspirene, An Angiogenesis Inhibitor

and

Synthesis of 2-Amino-benzimidazole Compounds Targeting Subdomain IIa of the Internal Ribosome Entry Site Inhibiting Translation of The Hepatitis C Virus

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in
Chemistry
by
David John Schmit

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Professor Douglas Grotjahn
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2014
The Dissertation of David John Schmit is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego
San Diego State University
2014
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LIST OF ABBREVIATIONS

Ac  Acetyl
Bn  Benzyl
BOC t-Butoxycarbonyl
BOM Benzyloxymethyl
Bu  Butyl
Bz  Benzoyle
Cbz Carboxybenzyl
Cp  Cyclopentadienyl
CSA Camphorsulfonic acid
DABCO 1,4-Diazabicyclo[2.2.2]octane
DBU 1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC Dicyclohexylcarbodiimide
DCM Dichloromethane
DDQ 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIAD Diisopropyl azodicarboxylate
DIBAL-H Diisobutylaluminum hydride
DIPEA Diisopropylethylamine
DMAP 4- N,N-dimethylaminopyridine
DMD Dimethyldioxirane
DMF N,N-dimethylformamide
DMP Dess-Martin Periodinane
DMS Dimethyl Sulfide
DMSO Dimethyl sulfoxide
EDC 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et  Ethyl
Et2O Diethyl Ether
FRET Fluorescence resonance energy transfer
HOBt Hydroxybenzotriazole
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMPA</td>
<td>Hexamethylphosphoramide</td>
</tr>
<tr>
<td>HWE</td>
<td>Horner-Wadsworth-Emmons reaction</td>
</tr>
<tr>
<td>Imid.</td>
<td>Imidazole</td>
</tr>
<tr>
<td>IRES</td>
<td>Internal ribosome entry site</td>
</tr>
<tr>
<td>KHMDS</td>
<td>Potassium hexamethyldisilazide</td>
</tr>
<tr>
<td>LAH</td>
<td>Lithium aluminum hydride</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropylamide</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>MOM</td>
<td>Methoxymethyl</td>
</tr>
<tr>
<td>Ms</td>
<td>Methanesulfonyl or mesyl</td>
</tr>
<tr>
<td>NaHMDS</td>
<td>Sodium hexamethyldisilazide</td>
</tr>
<tr>
<td>NIS</td>
<td>N-iodosuccinimide</td>
</tr>
<tr>
<td>Ni(acac)$_2$</td>
<td>Nickel (II) acetylacetonate</td>
</tr>
<tr>
<td>NMM</td>
<td>N-methylmorpholine</td>
</tr>
<tr>
<td>NMO</td>
<td>N-methylmorpholine N-oxide</td>
</tr>
<tr>
<td>NMP</td>
<td>1-Methyl-2-pyrrolidinone</td>
</tr>
<tr>
<td>PCC</td>
<td>Pyridinium chlorochromate</td>
</tr>
<tr>
<td>PDC</td>
<td>Pyridinium Dichromate</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>PMB</td>
<td>p-Methoxybenzyl</td>
</tr>
<tr>
<td>PPTS</td>
<td>Pyridinium $p$-toluenesulfonate</td>
</tr>
<tr>
<td>$i$-Pr</td>
<td>Isopropyl</td>
</tr>
<tr>
<td>Pyr</td>
<td>Pyridine</td>
</tr>
<tr>
<td>RCM</td>
<td>Ring closing metathesis</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>SADH</td>
<td>Sharpless asymmetric dihydroxylation</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>$S_{N}$Ar</td>
<td>Nucleophilic aromatic substitution</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetrabutylammonium fluoride</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Name</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>TBAI</td>
<td>Tetrabutylammonium iodide</td>
</tr>
<tr>
<td>TBDMS</td>
<td>t-Butyldimethylsilyl</td>
</tr>
<tr>
<td>TBS</td>
<td>t-Butyldimethylsilyl</td>
</tr>
<tr>
<td>Teoc</td>
<td>1-(2-Trimethylsilyl)ethoxycarbonyloxy</td>
</tr>
<tr>
<td>TES</td>
<td>Triethylsilyl</td>
</tr>
<tr>
<td>Tf</td>
<td>Trifluoromethanesulfonyl</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TIPS</td>
<td>Triisopropylsilyl</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>Tol</td>
<td>Toluene</td>
</tr>
<tr>
<td>Troc</td>
<td>2,2,2-Trichloroethoxycarbonyl</td>
</tr>
<tr>
<td>Ts</td>
<td>Toluenesulfonyl or tosyl</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. B. Mikael Bergdahl for his mentorship, leadership, and friendship over these past years. I have learned more about organic chemistry under his tutelage than I ever thought possible. Joining a laboratory with no current graduate students was a frightening and challenging undertaking, but gave me a unique perspective to watch the laboratory grow and flourish through Dr. Bergdahl’s influence and guidance. I would also like to thank all members of the Bergdahl group, past and present, for all of their chemistry discussions as well as their companionship. Special acknowledgement goes to Michael Kelly, Brent Banasik, Scott Burley, Tim Montgomery, Lee Wang, Urszula Milewicz, Lucas Fallot, and Arielle Kanner whom I’ve had the supreme pleasure of working with during my time in the laboratory. You guys have become life-long friends, we have been groomsmen in each other’s weddings, and have made sure I was never alone when I needed a cold beverage to release from the frustrations of organic chemistry. I would not have made it without you guys. Recognition must also go to Urszula Milewicz for her work on the HCV project. Another special thanks goes to Caline Abadjian who gave me my first tour of SDSU while visiting campus before deciding to come to school here, and ended up becoming one of my good friends through our year of residency at UCSD. Thanks to Dr. LeRoy Lafferty, NMR specialist, whose technical assistance was essential throughout my career as a graduate student. I appreciate all of the faculty and staff in the Department of Chemistry at SDSU and the Department of Chemistry and Biochemistry at UCSD for their critical role in my education. Much gratitude goes towards our collaborators on the HCV project, all members of the Hermann laboratory at UCSD and the Wolkowicz laboratory at SDSU. A very special thanks goes to my parents for their unconditional love and support throughout my entire life which made all of this possible. Finally, I would like to offer my deepest gratitude to my wife Jessica Schmit. We did a long distance relationship for two years from Minnesota when I started the graduate program and you never lost faith in us or what I was trying to accomplish. Thank you for always loving me, supporting me, and pushing me to be the best person/chemist I could be, even if it was to your own detriment. I love you!
Contained in chapter 3 is material that is in manuscript preparation. The following co-authors assisted with this work and should be commended: Urszula Milewicz, Mark A. Boerneke, Dr. Thomas Hermann, and of course Dr. B. Mikael Bergdahl.
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ABSTRACT OF THE DISSERTATION

Progress Toward a Scalable Synthesis of Azaspirene, An Angiogenesis Inhibitor

and

Synthesis of 2-Amino-benzimidazole Compounds Targeting Subdomain IIA of the Internal Ribosome Entry Site Inhibiting Translation of The Hepatitis C Virus

By

David John Schmit

Doctor of Philosophy in Chemistry

University of California, San Diego, 2014
San Diego State University, 2014

Professor B. Mikael Bergdahl, Chair

Chemical studies toward the synthesis of an angiogenesis inhibitor azaspirene is described. There is a need for a concise and scalable asymmetric synthesis of azaspirene. The current strategy employed can lead to the production of small libraries of azaspirene derivatives and other members of the pseurotin family, where structure activity relationship (SAR) studies can be conducted in anticipation of creating innovative and more effective anti-cancer drugs. Amino acids as well as other optically active compounds make great raw materials for the synthesis of asymmetric products with deliberate stereocontrol. Numerous synthetic routes for the synthesis of azaspirene are presented with the latest strategy starting from optically active malic acid showing the most viability. Azaspirene has a highly oxygenated complex spirocyclic structure that poses abundant synthetic challenges which are addressed herein. D-Malic acid is made by the double inversion of D-aspartic acid and the stereocenter is preserved throughout the synthesis. Cyclization to the succinimide derivative forms the lactam present in azaspirene. An aldol reaction and subsequent DMP oxidation form the 1,3-diketone necessary for an asymmetric palladium-mediated α-hydroxylation reaction. The chiral alcohol will be used as the oxygen in the β-oxygenated enone ring after Margaretha cyclization using a highly functionalized ester to add the diene tail of azaspirene. The benzyl group found in azaspirene will be added via a Grignard reaction at a late stage in the synthesis allowing for diversity.
The synthesis of a small set of 2-amino-benzimidazole compounds that bind a RNA construct of the hepatitis C virus (HCV) internal ribosome entry site (IRES) with ligand affinity in the submicro-molar range is described. The binding interaction is demonstrated by a 2.2Å resolution crystal structure of a 2-amino-benzimidazole compound bound to the RNA construct published by the Bergdahl group with collaborators. The total synthesis and crystal structure were used to guide the production of novel compounds, and the results presented herein will influence the design and construction of novel inhibitors expected to increase potency against HCV.
1 Introduction

The inhibition of tumor-angiogenesis has proven to be an effective method in the treatment of cancer. Small molecule drugs that inhibit angiogenesis have been used in chemotherapy since bevacizumab was FDA approved in 2004. Azaspirene (1), discovered in 2002, is a known inhibitor of tumor-angiogenesis and is part of the pseurotin family of compounds whose members have been shown to have a wide variety of biological properties. There are two total syntheses currently published for azaspirene. However, both routes are quite lengthy and suffer from poor overall yields and thus research into this type of cancer therapy using azaspirene has been impeded. It is absolutely imperative that a concise and high yielding synthesis be developed for azaspirene as to further manifest our knowledge of angiogenesis inhibition and potential for new drugs in chemotherapy.

The main focus of chapter 2 describes the development of a concise and high yielding asymmetric total synthesis of azaspirene. This synthesis is initiated with readily available optically active starting materials such that the chirality of the compound is preserved throughout the synthesis. Much work has been devoted to the development of this synthesis and is highlighted in this chapter.

Chapter 3 will focus on the synthesis of novel 2-amino-benzimidazole compounds that bind to the internal ribosome entry site (IRES) of the RNA of the hepatitis C virus (HCV) and thereby prevent ribosomal translation. HCV is a major human health threat and is the number one cause of death in the US from chronic viral infection. Recent discoveries have led to many new treatment options for the HCV virus. However, HCV has an extraordinarily high rate of mutation and as a consequence there is a need for additional anti-HCV drugs. The IRES is a highly conserved region of the HCV RNA and thus is a prime for anti-viral exploitation. This work will focus on the synthesis of compounds that will optimize an amine-phosphate salt bridge observed in a crystal structure obtained with collaborators showing our ligand bound inside a viral RNA construct of the HCV. These compounds will be validated by a fluorescence resonance energy transfer (FRET) assay developed by our collaborators in the Hermann laboratory at the
University of California, San Diego and by some *ex vivo* cell efficacy and toxicity studies performed by our collaborators in the Wolkowicz laboratory at San Diego State University.

Future work and additional suggestions are discussed in chapter 4. The main focus will be the completion of the total synthesis of azaspirene and to utilize this knowledge to readily produce analogs with higher biological anti-cancer properties. A proposed plan for the completion of azaspirene as well as diversification will also be presented. The focus of Part II discusses the continuation for refining the SAR study for IRES binding compounds active against HCV, and other viruses of the *Flaviviridae* and *Picornaviridae* families who have been shown to have very similar domain organization. Preparation of new compounds will be proposed as well as some preliminary data towards final targets will be shown.

All compounds experimental details as well as compound characterizations using $^1$H-NMR spectroscopy will be in chapter 5. For most of the compounds LC-MS and $^{13}$C-NMR spectroscopy are also provided.
2 Progress Toward A Scalable Synthesis of Azaspirene, An Angiogenesis Inhibitor

2.1 Introduction

According to the Centers for Disease Control and Prevention (CDC) cancer was the 2nd leading cause of death in the U.S in 2011.1 The traditional treatment of cancer is either the use of surgery, radiation, chemotherapy, or a combination of two or more treatments. Chemotherapy very simply put, is the use of drugs to kill cancer cells.2 Currently, there are over 100 drugs available for oncology use during chemotherapy treatment. However, most of these drugs lead to a number of unwanted side effects including nausea, vomiting, diarrhea, anorexia, fatigue, alopecia, insomnia, pain, and forgetfulness.3 The inhibition of angiogenesis has recently been found to be a method of treatment for cancer and other angiogenesis related diseases.4 The specificity of drugs targeting tumor angiogenesis may be able to reduce the number of unwanted side effects associated with chemotherapy. These drugs could also be used as part of a combination therapy to treat cancer.

2.2 The Role of Angiogenesis in Cancer

According to the National Cancer Institute angiogenesis is the formation of new blood vessels. This process involves the migration, growth, and differentiation of endothelial cells, which line the inside wall of blood vessels.5 Angiogenesis is a complex process controlled by specific chemical signals and signaling pathways throughout the body. This process is important for both the repair of damaged blood vessels, and the formation of new ones. The normal stimulation and inhibition of chemical signals of angiogenesis are in balance to ensure blood vessels form only when they are needed.5 A couple of factors that are responsible for regulating angiogenesis are found in Table 2.1.6 There are many diseases characterized by both the excess and scarcity of angiogenesis. Excess angiogenesis has been characterized in such diseases as cancer, atherosclerosis, obesity, endometriosis, and arthritis. Conversely, diseases characterized by insufficient angiogenesis are Alzheimer’s, hypertension, diabetes, Crohn’s, and osteoporosis to name a few.7 However, the role of angiogenesis’ in cancer will be the main focus of this
thesis. Angiogenesis plays an important role in the growth and spread of cancer. Tumors have the ability to give off chemical signals that stimulate angiogenesis and also stimulate nearby normal cells to produce angiogenesis signaling molecules. The new blood vessels in essence “feed” growing tumors with necessary oxygen and nutrients, allowing them to move throughout the body and form colonies of cancer cells called metastases. One such crucial angiogenesis inducing signal molecule is called vascular endothelial growth factor (VEGF). VEGF, along with other endothelial growth factors, bind to receptors on the surface of normal endothelial cells thereby prompting other cell signals to promote growth and survival of new blood vessels. The primary driver of angiogenesis is hypoxia (inadequate supply of oxygen) which leads to secretion of VEGF and other pro-angiogenic molecules from hypoxic cells. Figure 2.1 highlights the role of VEGF-induced angiogenesis towards tumor metastasis. VEGF family members are the most important factors that induce angiogenesis. A number of essential properties are attributed to VEGF with direct effect on promoting angiogenesis. VEGF increases the life span of endothelial cells and reduces apoptosis. VEGF enhances vascular permeability and migration of different cells, and also induces vasodilatation. Dr. Folkman published the first hypothesis of using angiogenesis as a way to prevent tumor growth in The New England Journal of Medicine in 1971.
Table 2.1. Examples of factors that regulate both positive and negative angiogenesis.

<table>
<thead>
<tr>
<th>Angiogenesis Stimulating Factors</th>
<th>Angiogenesis Inhibiting Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular endothelial growth factor</td>
<td>Thrombospondin-1,2</td>
</tr>
<tr>
<td>Acidic and basic fibroblast growth factor</td>
<td>Endostatin</td>
</tr>
<tr>
<td>Transforming growth factor-alpha/beta</td>
<td>Angiostatin</td>
</tr>
<tr>
<td>Platelet-derived endothelial cell growth factor</td>
<td>Interferon-alpha/beta</td>
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<td>Hepatocyte growth factor</td>
<td>Interleukin-1,2</td>
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<tr>
<td>Tumor necrosis factor-alpha</td>
<td>Platelet factor 4 fragment</td>
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<tr>
<td>Epidermal growth factor</td>
<td>Tissue inhibitor of metalloproteinase-1/2</td>
</tr>
<tr>
<td>Tissue factor</td>
<td>Human macrophage metalloelastase</td>
</tr>
<tr>
<td>Interleukin-6/8</td>
<td>Anti-thrombin III fragment</td>
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<tr>
<td>Angiogenin</td>
<td>Vascular endothelial growth inhibitor</td>
</tr>
<tr>
<td>Angiopoietin-1</td>
<td>Vasostatin</td>
</tr>
<tr>
<td>Cyclo-oxygenase-2</td>
<td></td>
</tr>
<tr>
<td>Macrophage migration inhibitory factor</td>
<td></td>
</tr>
<tr>
<td>Nitric oxide</td>
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</table>

### 2.3 The Use of Angiogenesis Inhibitors

Tumor growth, progression, and metastasis are dependent on angiogenesis. Given this premise, targeting tumor blood vessels is a logical approach to the treatment of a variety of different malignancies. In particular, inhibition of VEGF has been one of the most viable and effective strategies for the treatment of cancer. The drug bevacizumab (avastin) was the first angiogenesis inhibitor approved by the FDA for the treatment of cancer in 2004. Bevacizumab is a humanized anti-body that acts by inhibiting VEGF.

Since its approval in 2004, numerous anti-angiogenic therapies have also been approved and others are in late stage clinical trials. These new drugs have single, multiple, and broad spectrum molecular targets geared to increase inhibition and lower the chance of acquired resistance. These drugs include the small molecules sorafenib (nexavar), sunitinib (sutent), thalidomide, and TNP-470 (Figure 2.2). Conventional chemotherapy has been associated with significant negative side-effects such as hair loss, oral mucositis, and immunosuppression. The advantage to using drugs that have a very specific target, “smart drugs,” is that they are generally associated with fewer side effects. However, this tends to lead to increase drug resistance. Designing a drug with multiple and broad range molecular targets is essentially the opposite. They tend to have more potential side effects, but show less drug resistance.
Figure 2.1. VEGF induced angiogenesis showing tumor growth and metastasis. \(^9\)

![Diagram of VEGF-induced angiogenesis](image)

Figure 2.2. Anti-angiogenic small molecules: sorafenib (top left), sunitinib (top right), thalidomide (bottom left), TNP-470 (bottom right).

![Images of anti-angiogenic molecules](image)

2.4 The Pseurotin Family and Angiogenesis Inhibitor: Azaspirene

Azaspirene (1) was first isolated from the fungus *Neosartorya* sp. from a soil sample by Osada and co-workers in 2002. \(^{15}\) Azaspirene has a highly oxygenated 1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione skeleton structure and has been reported to inhibit endothelial migration induced by vascular endothelial
growth factor (VEGF) with an ED$_{100}$ value of 27.1 µM. Azaspirene is part of the pseurotin family of compounds (Figure 2.3) having a wide variety of biological activities.

![Chemical structures of azaspirene, pseurotin A, FD-838, and Cephalimysin A](image)

**Figure 2.3.** Pseurotin family of compounds with biological activities.

Pseurotin A has been shown to inhibit chitin synthase by Sterner in 1993 and to induce cell differentiation of PC12 cells by Komagata and co-workers in 1995. Pseurotin A was also found to inhibit immunoglobuline E production, and used as an antifungal and antifeedant by Jun Li and co-workers in a brine shrimp and armyworm larvae assay in 2012. Synerazol was found to have antifungal activity against *Candida albicans* in 1991. FD-838 has been shown to induce differentiation of leukemia in cultures, and to inhibit certain Gram-positive bacteria and fungi. Cephalimysin A has recently been found to inhibit murine P388 leukemia cells and human HL-60 leukemia cells. Cephalimysin A was recently synthesized by Rovis and coworkers utilizing a photo-isomerized-coupled asymmetric Stetter reaction to form the furanone of the γ-lactam spirocycle ubiquitous of the pseurotin family. The wide range of biological activities attributed to the pseurotin family make them an excellent target for diversity orientated
synthesis and biological screening. Highlighted in the above figure (Figure 2.3) the pseudoisin family of compounds share a highly conserved γ-lactam spirocyclic structure only differing by a benzoyl ketone, O-methoxy group, and a functionalized tail.

2.5 Mode of Action of Azaspirene

Azaspirene has been shown to inhibit human umbilical vein endothelial cells (HUVECs) migration induced by VEGF at an ED_{100} value of 27 µM without significant cell toxicity. Azaspirene has also been evaluated in a number of in vivo assay systems. In a chicken chorioallantoic membrane (CAM) assay, azaspirene administered at 30 µg/egg resulted in a 24%-45% inhibition of angiogenesis without any sign of thrombosis or hemorrhage when compared to the control (10% DMSO). Fertilized Dekalb chicken eggs were placed in an egg incubator for 4.5 days at 38 °C. The 30 µg sample of azaspirene was loaded onto a 1% solution of methylcellulose inside a silicon ring and placed onto the surface of the CAM. The eggs were further incubated for 2 days and injected with fat emulsion to intensify the vascular networks. The quantitative analysis was done with angiogenesis-measuring software and summarized in Figure 2.4.
In a tumor-induced angiogenesis renal carcinoma xenograft mouse model, azaspirene, albeit at a higher concentration, performed similarly to paclitaxel, a well-known chemotherapy drug. Although it was not explicitly demonstrated, the authors note that in general azaspirene injected mice showed less of a weight loss when compared to mice injected with paclitaxel. Female BALB/c mice were injected intradermally with $1 \times 10^6$ murine renal carcinoma (RENCA) cells at a site on the back. The mice were given vehicle or paclitaxel (6 or 20 mg/kg, daily) in 5% ethanol and 5% polyoxyethylene castor oil in normal saline. Azaspirene was dissolved in normal saline containing 10% DMSO. Azaspirene (31.6 or 100 mg/kg) was given every other day. The cancerous skin was separated and tumors quantified using a digital camera. The results are summarized in Figure 2.5.
The authors also discovered that azaspirene inhibits Raf-1 activation induced by VEGF in the MAP kinase signaling pathway of HUVECs and that it preferentially inhibits the growth of HUVECs rather than that of non-vascular endothelial cells.\textsuperscript{21}

With this knowledge, azaspirene remains an impressive target for the treatment of angiogenesis-related diseases. The treatment of endothelial cells which are directly exposed to blood borne agents, is preferential to conventional chemotherapy in which drug delivery continues to be a major obstacle. Azaspirene may have the ability to bind to other molecular targets, and thus more research into its mode of action is necessary.
2.6 Biosynthetic Pathway of Azaspirene

The initial biosynthetic pathway for azaspirene was proposed by Osada and coworkers in 2002.\textsuperscript{15} Since the culture broth that produced azaspirene also contained pseurotin A, it was thought that they may share the same biosynthetic pathway. Since azaspirene is the first example of a benzyl group in this series of compounds it was assumed that the oxidation at the benzyl position occurs after incorporation of phenylalanine. The proposal uses the starter unit propionyl-coenzyme A with 4 units of malonyl-coenzyme A and is summarized in Scheme 2.1.\textsuperscript{15}

![Scheme 2.1](image)

Scheme 2.1. Osada's proposed biosynthetic pathway of azaspirene.

A more recent elucidation using a number of knockout experiments was published by Watanabe and co-workers in 2014.\textsuperscript{22} The gene cluster responsible for biosynthesizing 1 is polyketide synthase nonribosomal peptide synthetase (PKS-NRPS) hybrid enzyme gene psoA. PsoA was shown to be responsible for the core structure of the pseurotin family of compounds.\textsuperscript{23} Azaspirene, along with the core structure of pseurotins, is produced from one unit of propionate, four units of malonate, L-phenylalanine, and SAM (S-adenosyl-L-methionine) catalyzed by the enzymes psoA and psoF. The benzyl oxidation shown in Scheme 2.1 actually occur post construction of azaspirene by the enzyme psoD. The findings by Wantanabe are highlighted in Scheme 2.2.
Scheme 2.2. Biosynthesis of Azaspirene.

2.7 Prior Total Synthesis of Azaspirene

Azaspirene has a unique and highly oxygenated 1-oxa-7-azaspiro[4,4]non-2-ene-4,6-dione skeleton. The interest in synthesizing azaspirene is rooted in the difficulty to isolate larger quantities of this compound to further determine its mode of action and its other biological properties. Chemical studies toward azaspirene will also allow for a library of analogs of this target molecule to have the basis for a SAR (Structure Activity Relationship) study to potentially find more potent anti-angiogenic compounds or other compounds with interesting biological activities. As shown previously (Figure 2.3), the pseurotin family of compounds has a wide range of biological activities, but a very similar core structure with only minor structural differences. A more practical route for the synthesis of azaspirene would also allow for syntheses of other members of the pseurotin family. The Hayashi and the Tadano groups have achieved asymmetric total syntheses of azaspirene. Furthermore, several other routes toward members of the pseurotin family have been reported.

2.7.1 Hayashi’s Total Synthesis

The first asymmetric total synthesis of (-)-azaspirene was reported by Hayashi’s group in 2002 (Scheme 2.3), and the absolute stereochemistry of the target molecule was determined. The total synthesis began with a Sharpless asymmetric dihydroxylation of methyl 2-pentenoate (II) using (DHQ)\textsubscript{2}PHAL as the chiral ligand. The product was the syn-diol which was protected as the acetal III using dimethoxypropane in the presence of catalytic amount of acid (TsOH). The next step was a Mukaiyama aldol condensation using phenylproprargyl aldehyde in the presence of MgBr\textsubscript{2}·OEt\textsubscript{2} which gave desired product IV in high yield and high diastereoselectivity. The aldol product was protected as its TIPS-
ether using TIPSOTf and the methyl ester was hydrolyzed to the carboxylic acid V using LiOH. The carboxylic acid was then converted to the amide VI by sequential steps of first using oxalyl chloride and then ammonia. The amide was next deprotonated by a strong base using NaH and the molecule readily cyclized to form benzylidene lactam with high Z-selectivity. The acetal was then opened by using TFA providing the secondary alcohol which was oxidized using DMP to form α-hydroxy ketone VII. The next step was a conventional aldol reaction using excess of LDA in THF to give secondary alcohol VIII. Successful oxidation using DMP formed 1,3-diketone IX which also was partially converted to the azaspiro[4,4]nonenedione bicycle X on thin-layer chromatography. The 1,3-diketone was fully converted to the bicycle and benzylidene hydrated to compound XI in the presence of catalytic amount of acid (TsOH). In the last step the TIPS-ether group was deprotected using ammonium fluoride to form (-)-azaspirene (I) in a 2.2% overall yield.

Scheme 2.3. Hayashi's asymmetric total synthesis of azaspirene.
2.7.2 Tadano’s Total Synthesis

The Tadano group published the total synthesis of pseurotin A and F2 in 2004.25 The synthetic route was modified to complete the total synthesis of azaspirene (Scheme 2.4). The synthesis begins with a 6-step procedure to 5,6-dideoxy-1,2-O-isopropylidene-α-D-xylo-hexofuranose (XII) from D-glucose published elsewhere.26,27 The secondary alcohol within compound XII was oxidized to the ketone using PCC, which was then allowed to react with vinyl magnesium bromide under usual Grignard conditions to give one single diastereomer. Acid hydrolysis using dilute acetic acid followed by a chemoselective oxidation using N-iodosuccinimide (NIS) in the presence of tetrabutylammonium iodide gave γ-lactone-α,β-diol. The cis-diol was then protected as its isopropylidene acetal XIII. The lactone was next opened by LiAlH₄ followed by a three-step protection/deprotection sequence via a trityl ether provided an acyclic protected alcohol as its benzyl ether. Treatment with Dess-Martin periodinane gave aldehyde XIV. This aldehyde underwent an asymmetric addition of benzylmagnesium chloride in the presence of CuBr·DMS in a solution of THF and DMS to give 1,2-benzyl addition as their major product XV. After ozonolysis and successive cleavage of the acetal, γ-lactol intermediate underwent chemoselective oxidation using NIS, and di-O-TES protection of vicinal alcohols. Removal of the benzyl protecting group and oxidation using DMP gave ketone intermediate XVI. An aldol reaction was then performed in the presence of lithium bromide using KHMDS in THF and a commercially available (2E,4E)-heptadienal XIX to give the desired compound XVII in high diastereoselectivity. The tertiary alcohol protected TES-ether XVII was then selectively removed (over the secondary TES-ether) using dilute HF-pyridine to provide the corresponding tertiary alcohol. The secondary alcohol within XVII was then oxidized using DMP to form the keto group which spontaneously rearranged to the γ-lactone hemiketal, which underwent selective dehydration with thionyl chloride in pyridine and gave the 1,7-dioxaspiro[4.4]non-2-ene-4,6-dione skeleton. In the last couple of steps it was shown necessary to use a more rigid protecting group than the remaining TES-ether group, in the final few steps of the synthesis. Thus additional HF-pyridine was used to remove the remaining TES group, and the secondary alcohol protected as the MOM-ether using dimethoxymethane and P₂O₅ to provide compound XVIII. Treatment with ammonia in isopropanol then opened the γ-lactone to the amination product. Subsequent oxidation with DMP oxidized the secondary alcohol to the
corresponding ketone. Although this oxidation step detracts from the overall stereoselective approach, the free amine appears to reset the accurate stereochemistry by a stereoselective $\pi$-face attack on the ketone to form the accurate $\gamma$-lactam as one single enantiomer. Thus, treatment with saturated aqueous sodium carbonate allowed for a stereoselective intramolecular amide-nitrogen-anion opening of the newly formed ketone- to form the $\gamma$-lactam core. In the last step, the $\gamma$-lactam-$\beta$-MOM-ether was then deprotected using 6M HCl in methanol to yield azaspirene (1) in 33 overall steps.

Scheme 2.4. Tadano's total synthesis of azaspirene.

2.8 Retrosynthetic Strategy for Azaspirene

2.8.1 Initial Retrosynthetic Strategy

It has been shown, that the likely biosynthetic building blocks used for the synthesis of the pseurotin family, which includes azaspirene, are one unit of propionate, four units of malonate, one unit of...
S-adenosyl-\textit{L}-methionine, and one unit of \textit{L}-phenylalanine.\textsuperscript{22,28} Using this knowledge, we are proposing a number of synthetic methodologies and strategies to successfully complete the total synthesis of azaspirene using the same biosynthetic precursor \textit{L}-phenylalanine (2) as an inexpensive chiral starting material. The proposed retrosynthetic strategy for azaspirene is illustrated (Scheme 2.5). Among key synthetic manipulations involved is the direct conjugate addition of either an alkenylzirconocene or a mixed alkenylalkylzincate reagent via methodologies developed in our laboratory using a copper(I) catalysis.\textsuperscript{29,30} Other notable features in this viable pathway are: i) ozonolysis, ii) Sharpless asymmetric dihydroxylation, iii) Swern oxidation, iv) aldol reaction, and v) Horner-Wadsworth-Emmons reaction of readily available intermediates to build the target molecule.

\textbf{Scheme 2.5.} Retrosynthetic scheme toward azaspirene.
2.8.2 Revised Retrosynthetic Strategy

While developing the total synthesis of the target molecule starting with $L$-phenylalanine (2) (Scheme 2.5), it was envisioned that a much more optimized route toward the target molecule could be achieved. Due to the length, and the destruction of initial chirality a much more efficient synthesis was planned. The revised retrosynthesis (Scheme 2.6) still focused on $\gamma$-lactam spirocycle core 16 or 24 (different protecting groups) (Scheme 2.5), which would continue to allow the use of the direct conjugate addition reaction developed by our laboratory.$^{29,30}$ Other notable features in this revised pathway are: i) microwave induced condensation reaction, ii) Grignard reaction, iii) aldol reaction, and iv) PCC oxidation to readily available intermediates to construct the target molecule. The second attempted synthesis begins with $L$-tartaric acid as an inexpensive starting material, and takes advantage of its natural chirality.

Scheme 2.6. Revised retrosynthetic scheme toward azaspirene.

2.8.3 Second Revised Retrosynthetic Strategy

The tertiary alcohol group on compound 20 proved difficult to protect in both acidic and basic environments, and was extremely labile and prone for dehydration. There was also a lack of literature precedence for performing the subsequent step which was a direct aldol reaction on an $\alpha$-substituted amide. Due to this revelation, a new revised synthesis for the target molecule was explored taking advantage of a copper iodide mediated silyl lithium 1,4-addition explored in our lab.$^{31}$ The key step of the newly revised
retrosynthesis (Scheme 2.7) is a stereocontrolled conjugate addition of a dimethylphenylsilyl group. In this approach the Ph$_2$MeSi group serves as a hydroxyl surrogate. The corresponding silyl group is then readily converted to an alcohol using Fleming-Tamao oxidation conditions as previously demonstrated by the Bergdahl group and others. The presumption was that after nucleophilic attack of the silyl lithium reagent the electrophilic acid chloride $^{29}$ will approach from the less hindered face of the enolate giving the desired stereoisomer $^{28}$ in high yield upon quenching. Some other key methodologies include a Dess-Martin periodinane oxidation and a Grignard reaction. This retrosynthesis focuses, as previously, on the synthesis of the azaspirene spirocyclic core to allow for the use of a vinyl zirconocene addition. The synthetic proposal begins with dimethyl acetylenedicarboxylate (31) as an inexpensive starting material.

![Scheme 2.7. Second revised retrosynthetic scheme toward azaspirene.](image)

2.8.4 Third Revised Retrosynthetic Strategy

The use of silyl cuprate reagents as nucleophiles for 1,4-conjugate additions of α,β-unsaturated esters are widely known.$^{30,32,33}$ Silyl additions to greater conjugated di-ester systems such as fumarates, compound 30, have been accomplished using AIBN activation$^{34}$, but have been mostly unexplored. The attempted silyl cuprate addition to benzyloxy fumarate $^{30}$ in the presence of copper iodide DMS was unfortunately unachievable. In light of this result, a third revised retrosynthesis was explored (Scheme 2.8). The third revised retrosynthesis is similar to the first (Scheme 2.6), but with removal of one of the initial α-hydroxy groups. The absence of this hydroxyl group should allow for a direct aldol reaction with
the unsubstituted α-carbon. Also, unlike the previous retrosyntheses, this revised scheme is proposed by forming azaspirene’s bicyclic γ-lactam core and subsequently adding the diene tail in one single step using functionalized ester 38 in a Margaretha type cyclization,\(^\text{35}\) thus eliminating the need for direct alkenylzirconocene addition suggested previously. Other key manipulations of this pathway toward the target molecule are: i) Grignard addition, ii) Dess-Martin periodinane oxidation, iii) palladium catalyzed α-hydroxylation, and iv) aldol reaction to easily available intermediates. The unnatural isomer (D-form), of aspartic acid (32) is used as an inexpensive chiral starting material. The corresponding chiral carbon is preserved throughout the entire proposed route to the target molecule and illustrated in Scheme 2.8.

Scheme 2.8. Third revised retrosynthetic scheme toward azaspirene.

### 2.9 Results and Discussion

The total synthesis of the target molecule azaspirene (1) has been revised numerous times over the course of this work. However, the synthesis of azaspirene’s 5,5-spirocyclic core containing a β-oxygenated cyclic enone has always been the top priority. The β-oxygenated enone would allow for an alkenylzirconium addition developed by our lab.\(^\text{29,30}\) More recent discoveries have allowed us to eliminate the alkenylzirconium addition, and propose cyclization using functionalized ester 38 which would effectively add azaspirene’s diene tail in one simple step according to a Margaretha type cyclization.\(^\text{35}\)
2.9.1 Synthesis of the Unsaturated Lactam

The synthetic route following the initial retrosynthesis (Scheme 2.5) to lactam 7 starts with L-phenylalanine (2), which was reduced using NaBH₄ and I₂ to give 98% of corresponding alcohol (Scheme 2.9).³⁶ L-Phenylalaninol (40) was then heated with diethyl carbonate and K₂CO₃ to afford the oxazolidinone 3 in 71% yield.³⁷ The amide was then deprotonated using NaH, and subsequently protected as its PMB amide 41 in 96% yield.³⁸ The hydrolysis of oxazolidinone 41 was done using lithium hydroxide and aqueous ethanol to give N-PMB-L-phenylalaninol (4) in 98% yield.³⁹ The protected phenylalaninol was reacted with thionyl chloride to yield oxothiazolidine 42, which was then oxidized using sodium periodate in the presence of a ruthenium catalyst to yield cyclic sulfamidate 5 in 96% yield after 2 steps.⁴⁰ Cyclic sulfamidate 5 was then reacted with triethyl phosphonoacetate under basic conditions, hydrolyzed with acid, and then forced to lactamize upon heating to yield phosphonate 6 in 70% yield in preparation for a Horner-Wadsworth-Emmons olefination.⁴¹,⁴² The Horner-Wadsworth-Emmons reaction was carried out using sodium hydride to deprotonate phosphonate 6 and then reacted with cyclohexanone to give the desired unsaturated lactam 7 in 20% yield.⁴¹ As discussed above, due to the length of the synthesis when compared with the published literature²⁴,²⁵, a much more efficient route was proposed.
**Scheme 2.9.** Synthesis of unsaturated lactam 7.

### 2.9.2 Synthesis of Substituted Lactam

The synthetic route to lactam 20 following retrosynthetic strategy **Scheme 2.6** starts with L-tartaric acid (18), which was cyclized using neat benzyl amine under microwave irradiation conditions to give the corresponding succinimide 19 in 74% yield (**Scheme 2.10**). The succinimide 19 was TBS protected using t-butyldimethylsilyl chloride and imidazole to form L-O-TBS-N-benzyl-tartarimide (43) in 95% yield. TBS protected tartarimide 43 was then reacted with freshly prepared Grignard reagent benzyl magnesium bromide to yield at best 71% of a mixture of diastereomers at roughly a 1:1 mixture of syn:anti products. Due to the C2-symmetry of the succinimide derivative only two diasteromers are possible. The diastereomers were easily separable using chromatography and resolved unambiguously by X-ray crystallography of the anti-product and 2D NMR experiments. Several Lewis acid catalysts were then explored to aid in the activation of the fairly electron rich imide in attempts to raise product yield, and to impart more chelation control to increase syn:anti selectivity. Titanium isopropoxide and lead(II)bromide were first explored. The use of PbBr2 did not improve the yield giving only 17% of the pure syn diol 20. A moderate improvement was observed with titanium isopropoxide yielding 40% of the
desired compound. Bismuth(III)chloride was then employed which gave an impressive 80% yield with 4.3:1 ratio of syn:anti diastereomers, 65% yield of pure 20.

Scheme 2.10. Synthesis of substituted lactam 20.

### 2.9.3 Protection of Triol Intermediate

The protection of the tertiary alcohol of compound 20 proved exceedingly challenging. A summary of the attempts are shown (Scheme 2.11 & Scheme 2.12).

Scheme 2.11. Attempted tertiary alcohol protection.
The first attempt was to install a benzyoyl group ($R_1$, Scheme 2.11) using benzoic anhydride, triethylamine, and magnesium bromide to which only starting material was recovered. The reactive oxygen anion after the Grignard addition was also proposed to be used directly as a nucleophile when the reaction was quenched with benzoic chloride. However, the reaction yielded no clean product. Due to the bulky nature of the vicinal $O$-TBS group, a couple of smaller protecting groups were also explored using both acidic and basic environments. A benzyloxymethyl ether ($R_2$, Scheme 2.11) protection employing basic conditions was then attempted. The tertiary alcohol 20 was deprotonated using sodium hydride, and added to a solution of benzyloxymethyl chloride and sodium iodide in dichloroethane, but the reaction gave no clean product. The reaction was also attempted using diisopropylamine as a substitute base, and/or tetrabutylammonium iodide as a substitute for sodium iodide; all with similar results. To test protection under acidic conditions a methoxymethyl ether ($R_2$, Scheme 2.11) protecting group was also attempted. Compound 20 was dissolved in dimethoxymethane and added phosphorus pentoxide. Due to the labile nature of the tertiary alcohol, the reaction gave almost exclusively the dehydrated enamine 45. A number of Lewis acids were also explored using: (a) BiCl$_3$, (b) PbBr$_2$, (c) Iodine, (d) LiCl, (e) ZnCl$_2$, (f) MgBr$_2$, (g) Bi(OTf)$_3$, (h) CuCl$_2$, and (i) AlCl$_3$. In all cases no reaction was observed, except for (g) Bi(OTf)$_3$, in which the dehydrated compound 45 was identified by TLC.

![Scheme 2.12](image.png)

**Scheme 2.12.** Acetonide alcohol protection.

It was also envisioned that triol 46 could be protected as an acetonide (Scheme 2.12), and that the syn-diol protection would be highly favorable over anti. The TBS protected triol 20 was exposed to tetrabutylammonium fluoride to yield corresponding free hydroxy compound 46, which was directly
treated with 2,2-dimethoxypropane and a catalytic amount of TsOH to yield desired acetonide protected compound 47 albeit in less than a 5% yield after 2 steps with most being the dehydrated product 45. In an attempt to push the synthesis forward enamine 45 was treated with LDA and through quenching with D₂O it was determined that the α-proton could not be cleanly removed in high yields, therefore making the subsequent aldol reaction impossible. With the difficulty of protecting the labile tertiary alcohol and inability to cleanly remove the α-proton, a new synthetic route was proposed.

2.9.4 Synthesis of Alkenoic Ester

The synthetic route to desired Z-isomer alkenoic ester 30 (Scheme 2.13), began with a DABCO catalyzed Michael addition to activated alkyne dimethyl acetylenedicarboxylate (31) with benzyl alcohol. The reaction gave 85% yield of desired compound 30 as a mixture of Z:E isomers, 57:43, respectively. The two isomers were easily separable using column chromatography.


Unfortunately, due to the high conjugation of the double bond and the vicinal benzyloxy group alkenoic ester 30 was unable to undergo nucleophilic attack by the silyl copper reagent and only starting material was recovered from the reaction. The inability to attack the alkenoic ester is potentially due to the electron donating power of the alkoxy substituent or the electron withdrawing effect of the two carbonyls which would prevent initial coordination with the copper necessary to drive the reaction forward. With these reasons and the lack of literature precedence for the silylation of fumarates a new synthetic route was proposed.
2.9.5 Synthesis of 1,3-Diketone

The synthesis of diketone 49 (Scheme 2.14) began with converting D-aspartic acid (32), a very cheap and readily available nutritional supplement, to D-malic acid (33) via the diazonium salt using an aqueous mixture of sodium nitrite and sulfuric acid.\textsuperscript{54} The reaction yielded 60% D-malic acid (33) with complete retention of the stereocenter by the presumed double inversion shown by Young and coworkers\textsuperscript{54} among others, subsequently also verified by polarimetry. D-malic acid (33) was cyclized to the corresponding N-Benzyl-D-malimide (34) via a condensation reaction with benzylamine in 60% yield.\textsuperscript{55} Malimide was deprotonated with KHMDS to form the dianion to which propanal (35) was added at -78°C to form aldol adduct 48 in 45% yield.\textsuperscript{56} The α-hydroxy group of diol 48 was then TBS protected in preference to the β-hydroxy group using tert-butyldimethylsilyl chloride and imidazole to give 82% of protected succinimide derivative 36.\textsuperscript{56} The 1,3-diketone 49 was formed by oxidizing the corresponding alcohol with Dess-Martin periodinane in dichloromethane with a 92% yield.\textsuperscript{57} It is envisioned that the α-hydroxylation could be done by using dimethyldioxirane and a chiral palladium catalyst shown by Hii and coworkers.\textsuperscript{58,59}

\begin{center}
\includegraphics[width=\textwidth]{scheme2.14.png}
\end{center}

Scheme 2.14. Synthesis of 1,3-diketone 49.
2.10 Conclusion

Angiogenesis inhibition and the recent development and use of angiogenesis inhibitors continue to be an effective strategy for the treatment of cancer in both monotherapy, and in the use of combination therapy. Angiogenesis inhibitors are generally very specific, and therefore benefit in low toxicity to non-tumor cells. Since azaspirene was found to inhibit endothelial migration induced by vascular endothelial growth factor (VEGF) by way of Raf-1 inhibition in the MAP kinase signaling pathway, this target compound has been shown to inhibit both new blood vessel formation, and tumor growth in vivo. However, more investigation into its exact mechanism of action and exact molecular target/s are necessary. Azaspirene has shown promise as a new treatment for cancer and other angiogenesis-related diseases.

Even though Hayashi’s and Tadano’s groups have reported asymmetric total syntheses for azaspirene, there is still need for a much more optimized synthetic approach. The pseurotin family has proven a rich source for biologically active compounds and as such an approach that will allow for easily accessible analogs is also needed. Our proposed synthetic strategy is more streamlined in comparison to the previously achieved total syntheses and would also allow for diversity to be installed at a late stage in the total synthesis. The current route is grounded in published literature protocols and involves an aldol reaction, palladium catalyzed asymmetric alpha-hydroxylation reaction, and a Margaretha type cyclization as a few of the key manipulations towards the target molecule.

Much work has been attempted to achieve the target molecule with the most recent proposed strategy, Scheme 2.8, showing the viability. With \((3R)-1\text{-benzyl}-3-((\text{tert-butyl}d\text{imethyl}silyl})\text{oxy})-4\text{-propionyl}p\text{yrroldine-2,5-dione (49)}\) in hand, only six synthetic steps remain for completion of the target molecule with the overall synthesis being a proposed 11 steps. Chapter 4 will discuss the future plan to progress our strategy to complete the total synthesis of azaspirene, a possible alternative route to azaspirene, and inclusion of diversity towards the development of a structure and activity relationship study (SAR).
3 Synthesis of 2-Amino-benzimidazole Compounds Targeting the Subdomain IIa of the Internal Ribosome Entry Site Inhibiting Translation of The Hepatitis C Virus

3.1 Introduction

The hepatitis C virus (HCV) is a major human health concern which affects an estimated 200 million people worldwide\textsuperscript{60} and each year a new 3 to 4 million people are diagnosed.\textsuperscript{61} According to the World Health Organization (WHO), the hepatitis C virus causes both acute and chronic infection.\textsuperscript{62} Acute infection is usually asymptomatic and in about 15-45\% of infected individuals clear the disease within 6 months without treatment. The other 55-85\% of people develop chronic HCV infection. Chronic HCV infection can potentially lead to cirrhosis, hepatocellular carcinoma (HCC), and ultimately liver failure.\textsuperscript{62,63} Cirrhosis of the liver is defined as the scarring of liver tissue and is an irreversible process.\textsuperscript{64} Such HCV-related diseases are the number one cause of liver transplantation in the United States,\textsuperscript{60} and according to the Centers for Disease Control and Prevention (CDC), chronic HCV infection currently affects more than 3 million Americans and has surpassed HIV as a leading cause of death from chronic viral infection in the US.\textsuperscript{65}

HCV is transmitted by blood contact. The most common transfer of infected blood is through contaminated needles during illicit drug use or improper procedures by the healthcare industry. In addition, HCV can also be transmitted \textit{in utero} by infected mothers to their unborn children.\textsuperscript{66}

Until recently, the treatments for HCV have been very limited and suffer from poor efficacy and many adverse side effects such as flu-like symptoms are observed.\textsuperscript{67} However, in the last couple of years many pharmaceutical companies have released new drugs and therapies to treat patients infected by the hepatitis C virus. These drugs target various proteins that inhibit a variety of cellular processes of HCV. Most of these drugs have been approved for treatment of either genotype 1 or genotype 2. In 2005, a new type of inhibitor was discovered that targeted the internal ribosome entry site (IRES) in a novel way.\textsuperscript{68} The IRES is an important section of the viral RNA and until recently it has been a largely unexplored region of the RNA of the HCV genome. Subdomain IIa, which is a crucial part of the IRES, is a highly conserved
region across all of the genotypes of HCV, and is also genetically highly conserved across the entire
*Flaviviridae* family.\(^{69}\) Targeting subdomain IIa for therapeutics may be a cross-genetic way to inhibit
HCV, and possibly other known viruses of the *Flaviviridae* family.

### 3.2 Hepatitis C Virus

The hepatitis C virus is a single-stranded RNA of positive polarity of about 10,000 nucleotides in
which the RNA encodes a long open reading frame that is flanked by two untranslated regions (UTRs).
The RNA contains signals for viral protein and RNA synthesis as well as the coordination of both
processes.\(^{70}\) In contrast to the hepatitis B virus (HBV), the HCV genome does not enter the nucleus of
infected cells. Rather, HCV RNA functions directly as an mRNA in the cytoplasm of the host cell, where
translation is initiated through an internal ribosome entry site (IRES) in the 5’ UTR.\(^{70}\) The translated
polyprotein is co- and post-translationally processed by cellular and viral proteases into structural proteins
(core, envelope protein 1 (E1) and (E2), p7 and non-structural proteins (NS2, NS3, NS4A and 4B, NS5A
and 5B). Following the synthesis and maturation, non-structural proteins and viral RNA form membrane-
associated replication complexes,\(^{70}\) which then catalyze the transcription of negative-strand RNA
intermediates which in turn form positive-strand RNA. Through intracellular membranes, the capsid
proteins and genomic RNA then assemble into a nucleocapsid, bud, and finally into cytoplasmic vesicles.\(^{70}\)
A cartoon depiction by Graham Colm illustrating the hepatitis C virus can be seen in Figure 3.1.\(^{71}\)

![Figure 3.1. Graphic depiction of HCV.](image-url)
HCV has a high genetic variability (30-35%), and is classified into seven major genotypes and over 100 subtypes (<15% variability). These subtypes can then also be distributed according to geographic distribution, risk factors, and response to treatment.\textsuperscript{70,72,73} Genotype 1, 2, and 3 are widely distributed with genotype 1 being the most prevalent accounting for 46.2% of all HCV cases.\textsuperscript{73} Genotype 3 makes up 30.1% followed by 2, 4, and 6 which together make up 22.8% of all cases.\textsuperscript{73} Genotype 4 and 5 are generally found in lower-income countries. Genotypes 1, 2, and 3 are also the most common found in the US and Canada. A graphic distribution of the HCV genotypes can be found in Figure 3.2.\textsuperscript{74} HCV also has an extremely high mutation rate occurring in 1 in 1,000 bases per year.\textsuperscript{70} To put this in perspective, the hepatitis B virus has eight different genotypes which vary by only 8%. Hepatitis Bs mutation rate is roughly 100 times less than HCV at 1 in 100,000 bases per year.\textsuperscript{70} When compared to HIV-1 (human immunodeficiency virus) pandemic HCV has an extraordinarily higher degree of genetic diversity.\textsuperscript{73} As a result, HCV is incredibly prone to mutation, and likely to develop drug resistance at a very fast rate.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{geographic_distribution_of_hcv_genotypes.png}
\caption{Geographic distribution of HCV genotypes.\textsuperscript{74}}
\end{figure}
3.3 HCV Treatment

Interferon-α (IFN-α) is a crucial mediator of the innate antiviral immune response and therefore a natural choice for the treatment of HCV. IFN-α was first shown to have beneficial effects on patients with chronic hepatitis C in 1986. HCV was first identified in 1989 and further serum blood tests were available shortly thereafter. A cure is defined as a sustained virologic response (SVR) and consists of undetectable levels of plasma HCV RNA 12 or 24 weeks after therapy completion. The effects of IFN-α were immediately marked by a rapid decline in HCV-RNA levels in the serum. Unfortunately, after 6 months of monotherapy treatment IFN-α was only 6-12% effective, and after 12-monts of treatment only improved to 16-20%. This number was greatly improved by the late 1990s by the discovery that the addition of the broad-spectrum antiviral agent ribavirin administered in combination with IFN-α nearly doubled the response rate to 35-40%. In 2000, it was discovered that by covalently attaching a large molecule of polyethylene glycol (PEG) to IFN-α the active molecule had a longer half-life, better pharmacokinetic profile, and better rate of sustained virological response. The use of PEG interferon-α and ribavirin increased the response rates seen in patients to nearly 54-56% and has since been the fundamental way of treatment of HCV.

3.4 Direct Acting Antivirals (DAAs)

The use of PEG interferon-α and ribavirin not only suffers from being only 50% effective, but it is also associated with a number of serious side effects. Interferon-α has been associated with decreased granulocytes and thrombocytes in the bone marrow, neutropenia (low white-cell count), and severe flu-like symptoms including fever, chills, headaches, and muscle and joint pain. Interferon-α can also induce autoimmune phenomena and most frequently autoimmune thyroiditis. Not only does interferon-α affect the body, but it also attacks the mind. It has been associated with increased depression, irritability, fatigue, apathy, and even suicide has been reported. The main side effect of ribavirin is that it is directly toxic to red blood cells and can cause haemolytic anaemia.

There is no vaccine to protect against HCV infection. Drug discovery research for HCV has explored the viral proteins which include structural and nonstructural (NS) targets including the NS3
protease, NS5B polymerase, and NS5A. This has led to Food and Drug Administration (FDA) approval of a new type of chronic HCV treatment in 2011. This new type of treatment was the use of direct acting antivirals (DAAs). DAAs are oral medications that specifically target various stages of the HCV lifecycle. The first such examples were boceprevir and telaprevir which inhibit the HCV NS3/NS4 serine protease responsible for processing of the HCV polyprotein and production of new infectious virions. Many other large pharmaceutical companies followed soon after introducing US FDA approved chronic hepatitis C inhibitorssimpervir and sofosbuvir; and inhibitors in late stage clinical trials daclatasvir and BMS-791325, among others.

3.4.1 Boceprevir

Boceprevir, which is sold under the trade name Victrelis is an oral direct acting antiviral that specifically inhibits the NS3 protease of the hepatitis C virus. It was originally developed by Schering-Plough which was bought by Merck in 2009. Boceprevir, shown in Figure 3.3 (left), was specifically made for the treatment of HCV of genotype 1 and forms a covalent reversible complex with the NS3 protease. Although boceprevir was shown to inhibit HCV alone, it was discovered that when administered with PEG interferon-α and ribavirin it increased SVR to 61-75% when administered three times daily. Shortened durations of 28 weeks for patients who achieved rapid viral declines within the first 12 weeks of therapy are as effective as fixed-duration therapy of 48 weeks.

Figure 3.3. Structures of boceprevir (left) and telaprevir (right).
3.4.2 Telaprevir

Telaprevir, which is sold under the trade name Incivek, is another oral direct acting antiviral that specifically inhibits the NS3/NS4 protease of HCV.\textsuperscript{77} It was co-developed by Vertex Pharmaceuticals and Johnson & Johnson, and was US FDA approved in 2011. Telaprevir, shown in Figure 3.3 (right), was also designed to be effective only against HCV genotype 1.\textsuperscript{82} Again, telaprevir was most effective when administered as triple therapy with PEG interferon-α and ribavirin, and had a similar SVR rate (61-75\%) as boceprevir when taken 2-3 times daily.\textsuperscript{77} However, shortened durations of 24 weeks for patients who achieved rapid viral declines within the first 12 weeks of therapy are as effective as fixed-duration therapy of 48 weeks.\textsuperscript{77} It is worth noting that telaprevir had a higher discontinuation rate during clinical trials because of adverse events, with rash being the most common reason for discontinuation.\textsuperscript{83}

3.4.3 Simeprevir

Simeprevir, which goes under the trade name Olysio, is an oral direct acting antiviral developed by Medivir and Johnson & Johnson Pharmaceuticals.\textsuperscript{84} Simeprevir, shown in Figure 3.4 (left), was US FDA approved in 2013 for treatment of HCV genotype 1.\textsuperscript{85} It is another inhibitor of the NS3/NS4 serine protease. When used in triple therapy SVR rates were increased to 79-86\% using 12-24 weeks of simeprevir, PEG-IFN, and ribavirin continuing PEG-IFN and ribavirin for 24 to 48 weeks.\textsuperscript{77} Simeprevir treated patients showed very similar adverse events compared to those patients in the placebo control group.\textsuperscript{85} Simeprevir is currently being evaluated using PEG-IFN-α, ribavirin, simeprevir, and another DAA. It is also under investigation if treatment without ribavirin has a drastic effect on SVR or if it can be avoided to help minimize adverse events.\textsuperscript{84}
Figure 3.4. Structures of simeprevir (left) and sofosbuvir (right).

### 3.4.4 Sofosbuvir

Sofosbuvir which goes under the brand name Solvaldi is an oral direct acting antiviral developed by Gilead. Sofosbuvir, shown in Figure 3.4 (right), is a nucleoside analog and has been recently approved by the FDA for treatment against HCV. Sofosbuvir is a NS5B polymerase inhibitor and has potential to be effective across all genotypes of HCV. Treatment with sofosbuvir, PEG-IFN, and ribavirin in patients with HCV genotype 1 show high SVR rates of 89-90% after treatment of only 12 weeks. There was almost no improvement when treatment was extended to 24 weeks. Patients with HCV genotype 2 treated with sofosbuvir and ribavirin had an SVR rate of 97% after 12 weeks and patients with genotype 3 had an SVR rate of 80%. The most common adverse events associated with sofosbuvir and ribavirin treatments were fatigue, headache, nausea, and neutropenia but were less common than with treatments incorporating PEG-IFN. It is worth noting that Gilead has recently come under much scrutiny for its incredibly high price tag for treatment using sofosbuvir. Although sofosbuvir has shown very high SVR rates, its high cost makes it inaccessible for most patients afflicted with hepatitis C.

### 3.4.5 Daclatasvir

Daclatasvir which is sold under the trade name Daklinza was approved as a medication for HCV treatment in Europe in 2014. Daclatasvir was developed by Bristol-Myers Squibb and is an inhibitor of the NS5A domain, an inhibition which is not yet well understood. Daclatasvir is a symmetric molecule and has shown activity across all genotypes of HCV and although it has yet to be approved in the US, has been
coined the “cure” for HCV. Daclatasvir has shown very high SVR rates in the high 90s across HCV genotypes 1, 2, and 3 and is also predicted to be one of the cheapest DAAs available for treatment of HCV. The most common adverse events were fatigue, headache, and nausea. The structure of daclatasvir can be seen in Figure 3.5.

![Figure 3.5. Structure of daclatasvir.](image)

### 3.5 HCV Inhibitors Targeting the IRES

The recent developments of DAAs of the hepatitis C virus have given us a good handle on the treatments available for patients with HCV. Many of the DAAs are being explored for use as combination therapy and SVR rates continue to rise. However, as discussed previously the hepatitis C virus has incredibly high genetic variability and the ability to mutate rapidly continues to pose significant challenges for the development of antiviral therapies.

The HCV genome is flanked at both 5’ and 3’ ends by the untranslated regions (UTRs), which adopt complex secondary structures. The single-stranded ~9400 base RNA genome contains a single open reading frame translated into a poly-protein which is proteolytically processed into at least ten mature proteins by viral and cellular proteases. The 5’ UTR contains an internal ribosome entry site (IRES) which initiates cap-independent viral translation. The HCV IRES directly regulates the assembly of translation initiation complexes on viral mRNA by a sequential pathway that is distinct from canonical eukaryotic initiation. The HCV IRES displays a secondary structure with two major domains, II and III, which contain all of the structural elements crucial for initiation of translation. The overall domain organization (II-IV) and several RNA structural motifs in these domains are conserved among related viruses from the *Flaviviridae* family, such as the classical swine fever virus (CSFV), the bovine viral diarrhea virus (BVDV), and GB virus B (GBV-B). This distinct domain organization has also been found in several members of the *Picornaviridae* family, such as porcine teschovirus (PTV), avian encephalitis
virus (AEV), and simian Picornavirus (SPV) suggesting an HCV-like mechanism of translation initiation. Domain III is larger than domain II, and made up of branching hairpin stem-loops subdomain (IIIa-f). Domain II is organized into a basal domain IIa and an apical domain IIb, of which only domain IIb interacts with the 40S ribosomal subunit. Cryo-electron microscopy (cryo-EM) work has revealed that domain IIa adapts a L-shaped conformation as to direct domain IIb toward the ribosomal E site in proximity of the active site. Structures within the IRES have also been implicated in the regulation of replication.

Although it will not be the focus of this thesis, it is worth noting that given the high conservation of the IRES structure across the **Flaviviridae** and **Picornaviridae** families’ small molecule drugs which bind the HCV IRES may also demonstrate strong activities against viruses in these or other closely related families.

The high conservation of the IRES sequence in HCV suggests that mutations in this functional RNA element will be difficult to reconcile with IRES function. In 2005 Seth *et al.* reported the discovery of 2-amino-benzimidazole derivatives that target the HCV IRES. In particular, the 2-amino-benzimidazole derivatives target the IIa domain of the HCV IRES. The two compounds discovered by Seth with highest affinity toward the HCV IRES are shown in Figure 3.6. The binding affinities of the compounds were determined by using a 40-mer RNA model of the IIa domain. The values were reported as 0.72 µM for compound 51 and 0.86 µM for compound 50a.

![Figure 3.6](image)

**Figure 3.6.** 2-Amino-benzimidazole compounds discovered by Seth *et al.*
The total synthesis of compound 50a was achieved by Seth and coworkers\textsuperscript{68} and is shown in Scheme 3.1. The synthesis begins with deprotonation of diethylmalonate with sodium hydride which readily attacked benzyl halide 52 to yield diol 53. The alcohols were next protected as their acetyl esters and subsequently the nitro group was introduced via an electrophilic aromatic substitution reaction using fuming nitric acid to yield nitro compound 54. A nucleophilic aromatic substitution (SNAr) reaction was then performed using 3-(dimethylamino)-propylamine (55) as the nucleophile in the presence of calcium carbonate to provide nitroaniline 56. The acetyl groups were then deprotected using potassium carbonate in methanol to yield chroman 57. The nitroaniline chroman 57 was next reduced using a Pd-catalyzed hydrogenation and both amines subsequently cyclized using benzoyl isothiocyanate and EDC to yield the 2-N-benzoyl-substituted benzimidazole. The benzyol group was then deprotected using HCl to give 2-amino-benzimidazole 58. The synthesis was completed by mesylation of the free alcohol and substitution using dimethylamine to yield 2-amino-benzimidazole compound 50a.

\textbf{Scheme 3.1.} Total synthesis of 2-amino benzimidazole 50a by Seth and coworkers.
The total synthesis published by Seth and coworkers, although relatively concise, fails to account for late stage derivatization necessary for rapidly making analogs. The total synthesis of 2-amino-benzimidazole compound 50a was improved upon by the Bergdahl group in 2011.99

3.6 Bergdahl’s Total Synthesis

The total synthesis achieved by the Bergdahl group is very streamlined, high yielding, and provided mostly crystalline intermediates which allowed for easier purification. In addition, a key variable side chain was introduced late in the synthesis, which allowed for analogs to be easily synthesized for optimization of antiviral activity.99

Scheme 3.2. Total synthesis of 2-amino-benzimidazole 50a by Bergdahl and coworkers.

The total synthesis was initiated by a nucleophilic aromatic substitution reaction (SNAr) of 2-chloro-6-fluorobenzaldehyde (59) using potassium hydroxide in DMSO to give a 75% yield of 60. 6-Chlorosalicylaldehyde (60) was then exposed to acrolein using catalytic amount of DABCO and underwent a Baylis-Hillman type cyclization reaction to give 72% of the desired chromene 61. The corresponding aldehyde was oxidized using Tollens reagent using silver oxide and sodium hydroxide to yield carboxylic acid chromene 62 in 97% yield. This chromene was then reduced to chroman 63 with sodium amalgam. A peptide coupling using dimethylamine hydrochloride, EDC, and HOBT gave chroman amide 64 in 92% yield. The chroman was then exposed to sodium nitrate in dichloroacetic acid using catalytic amounts of
TFA to provide a 55% yield of a 2:1 mixture of para:ortho nitro isomers. The isomers were separated, reduced to the corresponding aniline derivative, and assigned unambiguously using the aryl proton couplings by \textsuperscript{1}H NMR. The nitration product mixture in the presence of mono-Boc-protected N-methyl-1,3-propanediamine underwent a S\textsubscript{N}Ar reaction to give nitro chroman 66\textit{a} in 90% yield based on the para nitro isomer 65, which fortunately was the only isomer to react in the S\textsubscript{N}Ar reaction due to the nitro group being adjacent to the chlorine. The nitro group on the chroman was then reduced using a Pd-catalyzed hydrogenation to give the corresponding aniline derivative. The dianiline moiety was then cyclized to the 2-amino-benzimidazole compound 68\textit{a} in 64% yield using cyanogen bromide \textit{in situ}. In the presence of lithium aluminum hydride the amide functionality was reduced to the corresponding amine, and the N-boc protecting group reduced to the N-methyl to the 2-amino-benzimidazole 50\textit{a} respectively in 89% yield.

The Bergdahl group total synthesis gave title compound 50\textit{a} in an overall 10.7% total yield. Not only was this a vast improvement over the previously published Seth total synthesis\textsuperscript{68}, but the addition of the variable amino side chain tether introduced 3-steps away from the final compound would allow for a very easy and rapid synthesis of analogs.

### 3.7 Crystal Structure of the Bound Inhibitor

The Bergdahl group in collaboration with the Hermann laboratory were able to cocrystallize the racemic mixture of 2-amino-benzimidazole compound 50\textit{a} bound to the HCV IRES subdomain IIa.\textsuperscript{100} The ligand induced a conformational change in the RNA preventing the ribosome from docking to the IIb subdomain, and therefore inhibiting translation by the ribosome. The ligand induces an overall straightening on subdomain IIa. The cocrystallization was done in the presence of magnesium ions and X-ray crystallography was achieved at a resolution of 2.2 Å (Figure 3.7).\textsuperscript{100}

The X-ray crystallography gave a unique first look at the precise interactions between the 2-amino-benzimidazole ligand and the RNA IRES. The RNA internal loop refolds from its curved conformation in the free RNA to form a very tight and deep pocket that encompasses the ligand.\textsuperscript{100} The benzimidazole 50\textit{a} docks to the RNA pocket via a hydrogen bond to the guanine Hoogsteen edge in the cytosine58-guanine110 base pair. There are also π-stacking interactions observed between the adenine53
and guanine52–cytosine111 base pair which form the ceiling and floor of the binding pocket. Also observed, is a hydrogen bond between the protonated dimethylamino-propyl side chain of the ligand and the phosphate group of adenine109. The X-ray crystal structure did not allow for unambiguous assignment of the stereocenter, but marginally better statistics were obtained for the (R)-50a enantiomer. The conformational analysis did however suggest that the enantiomers were very similar in binding and that they would be compatible through the interactions seen in the crystal structure. The dimethylamino group at the stereocenter appears to lie outside of the pocket and does not have any observed interactions with the RNA, and therefore should not have a huge impact on binding.
Figure 3.7. X-ray crystallography of inhibitor 50a bound to the IRES subdomain IIa. The bound inhibitor forces a more linear conformation of subdomain IIa.100
3.8 FRET Determination of Binding Affinity

Given that upon ligand binding the RNA IRES undergoes a conformational change from an L-shape to a relatively straight conformation, a fluorescence resonance energy transfer (FRET) assay was developed by the Hermann group. This FRET method measured the distance of the interhelical angle between the stems flanking the internal loop in the IIa subdomain. The 5’ termini were labeled with a pair of fluorescent cyanine dyes which resulted in a RNA subdomain IIa construct (Figure 3.9). The distance between the dye pair was adjusted to be shorter than the Förster radius of the dye pair such that maximum sensitivity of the FRET assay could be observed. The wider the angle became upon ligand binding the less FRET signal was observed. The RNA construct initially free of divalent metal ions was shown to have no FRET signal. Upon addition of Mg ions the FRET signal began to appear in a dose-dependent manner as the RNA construct became more L-shaped (Figure 3.8) until the distance was representative of the IIa crystal structure. Upon addition of the benzimidazole compounds a dose-dependent quenching of the FRET signal was observed. The effector concentration for half-maximum response (EC₅₀) of FRET quenching was then calculated with a smaller EC₅₀ concentration representing better binding. To establish specificity of the ligand binding excess tRNA, with a very similar structure to subdomain IIa, was added with the dye labeled subdomain IIa construct. Upon adding the ligand, FRET quenching by the ligand was unaffected by the excess tRNA. In a complimentary experiment, many known RNA binders were added to the dye labeled subdomain IIa construct and no FRET quenching was observed.
Figure 3.8. HCV IRES IIa domain construct in the presence of magnesium ions depicting an L-shaped conformation.100

2-Amino-benzimidazole compound 50a was tested using the FRET assay described above, and yielded dose-dependent FRET quenching with an EC$_{50}$ value of 3.4 ± 0.3 µM in the presence of magnesium ions.100 This showed that binding of the ligand induced a conformational change of a widening of the interhelical angle. The ligand was also tested in the absence of magnesium ions which gave a much higher concentration, EC$_{50}$ of 117 ± 8 µM which showed that the L-conformation induced by the magnesium ions is necessary to capture the ligand.100
**3.9 Cell Efficacy and Toxicity Assay**

The efficacy and toxicity of 2-amino-benzimidazole (50a) have been tested using an *ex vivo* study performed by the Wolkowicz laboratory at San Diego State University. Hepatocytes (Huh 7.5.1 liver tissue cells), were infected with wild type JFH1 (genotype 2A) strain of the hepatitis C virus. Following infection, the cells were treated with increasing concentrations of compound 50a and incubated. After five days of infection the cells were treated with the appropriate (mouse-core and anti-mouse Alexa Fluor 488) antibodies, stained, and analyzed using fluorescence microscopy and flow cytometry to check for both the presence of GFP labeled HCV capsid protein and the number of dead cells. The percentage of GFP expressing HCV and cell number were then calculated and compared to the control which was not treated with the drug. The results of this assay are summarized in **Figure 3.10**. The percent of green core expressed decreases in a dose dependent manner displaying the efficacy of compound 50a. At a concentration of 0.5 µg/ml 101% of the cells remain expressing only 10.5% green core; compared to the untreated sample of 100% of the cells expressing 26.9% of the green core.
In another study, GFP expressing JFH1 infected cells were treated with increasing concentrations of compound 50a. The benefit of using the JFH1-GFP was that samples could be analyzed much more quickly by flow cytometry bypassing the need to stain for viral proteins. Once again, the compound was analyzed by calculating the percent GFP expressed after 5 days of infection. The results of this assay are shown in Table 3.1. The compound shows efficacy in a dose-dependent manner when compared to controls.

![Figure 3.10. Biological assay of compound 50a showing efficacy against JFH1 infected cells.](image)

The toxicity of compound 50a was also tested by treating Supt1 cells with increasing concentrations of the compound. Supt1 cells were treated with the compound 50a for 48 hours, collected, stained with propidium iodide, and run through the flow cytometer. Supt1 cells were chosen instead of Huh 7.5.1 cells because they give a better representation of cell toxicity and propidium iodide chosen because it only stains dead cells. The results of this assay are shown in Table 3.2. Anything within the gate (box) is fluorescing, and therefore considered a dead cell. Although the compound does show some toxicity, it appears that it is not very high as most of the cells appeared outside of the gate. Even at a concentration of 10 µM the compound appeared to have relatively low cell toxicity.
The assays described in this section are the basis for biological testing of all newly synthesized HCV inhibitors in this study to analyze both \textit{ex vivo} efficacy and cell toxicity.

\textbf{Table 3.1.} Graph showing efficacy of compound 50a at various concentrations using JFH1-GFP Huh 7.5.1 cells.
Table 3.2. Cell toxicity assay of compound 50a using supT1 cells.

3.10 Results and Discussion

The subdomain IIa plays an instrumental role in the function of HCV IRES-driven translation. This role is more than just purely architectural supported by the fact that the IIa subdomain has extremely high sequence conservation in this region of the viral genome. All, except for three residues >98% of the IIa subdomain, are conserved across 1,600 HCV clinical isolates across all genotypes. The residues around the ligand binding site show >99% conservation including two of the major (adenine109 and cystosine111) binding contributors which are 100% conserved across all genotypes. Given the high conservation of subdomain IIa across all genotypes of HCV, the 2-amino-benzimidazole inhibitors should in fact bind to the IRES of HCV of all genotypes with similar affinity.
As described previously, the total synthesis of 2-amino-benzimidazole compound 50a, published by the Bergdahl group,99 allows for installation of a variable amino side chain tethers at a late stage of the total synthesis, only three steps from the final compound. This approach (Scheme 3.3) was used to make analogs of active compound 50 in a structure activity relationship (SAR) study. These analogs were evaluated using the *in vitro* FRET assay described in section 3.8 and *ex vivo* cell assay described in section 3.9.

**Scheme 3.3.** Synthetic scheme for making 2-amino-benzimidazole analogs at a late stage in the synthesis.

The SAR study was planned by utilizing the information gained from the X-ray crystallography (Figure 3.7) structure of inhibitor 50a bound to the IRES in subdomain IIa of HCV. It was envisioned that by varying the dimethylamino tether we could effectively increase the binding affinity of our molecules. A close up of this interaction can been seen in Figure 3.11. The SAR study was proposed with three main strategies for increasing the binding affinity of our compounds.

The first strategy was to make conformationally restricted analogs to restrict the degrees of freedom in the diamino alkyl chain tether. Pre-constraining ligands is a very common method used to increase binding affinity, in particular in salt bridges on the surfaces of proteins.105–107 By minimizing the rotation around those bonds, we can lower the entropy of our ligand. The Gibbs free energy of binding ΔG is equal to ΔH – TΔS, and thus decreasing the initial entropy of the ligand will decrease the amount of entropy lost (ΔS) upon binding and therefore have a more favorable change in free energy upon binding. As a result, pre-constraining the side chain should increase binding affinity.
Figure 3.11. Highlight of the hydrogen bond between the ligands dimethylamino group and phosphate backbone of the RNA.

The second strategy was to optimize the amine-phosphate salt bridge (Figure 3.11) and nearby interactions by modifying the dimethyl groups on the nitrogen in our tether and to change the length of the alkyl chain tether, itself. This amino terminus lies largely outside of the hydrophobic binding pocket and therefore may be tolerant of a number of various functional groups as long as they are not highly hydrophobic. This work was done largely by Ms. Urszula Milewicz.\textsuperscript{108}

The third strategy was to make aromatic ring substitutions. The benzimidazole ring is located deep within the binding pocket and its $\pi$-stacking contributes largely to the ligand’s binding affinity. Introducing both electron donors and electron acceptors onto the aromatic ring in one or both positions, would change both the aromatic ring electronics, and possibly the way the binding pocket folds to encapsulate the ligand thereby increasing binding affinity.

A summary of the synthesized compounds as well as their EC$_{50}$ values is shown in Figure 3.12. The EC$_{50}$ values were determined using the FRET assay described earlier (section 3.8) by the Hermann group and are given in micromolar concentrations.
Figure 3.12. Summary of synthesized inhibitors and EC₅₀ values in micromolar concentrations.

3.10.1 Synthesis of Amines

The strategy for synthesizing 2-amino-benzimidazole analogs is described in Scheme 3.3. The synthesis begins with an aromatic substitution reaction using readily available nitro-chloro-chroman...
intermediate 65, previously synthesized by the Bergdahl group (section 3.6), and a primary amine dissolved in N-methyl-2-pyrrolidone. Primary amines used in the synthesis for final compounds 50a-d, f, g, i, m were purchased and used without further purification. The primary amines for compounds 50e, h, j-l were synthesized via published literature protocols (Scheme 3.4, Scheme 3.5, Scheme 3.6) and made using the Gabriel synthesis shown in Scheme 3.7.

The synthesis of primary amine 67e used in the SNAr reaction to make compound 50e is summarized in Scheme 3.4. A cis- and trans-mixture of 4-aminocyclohexanecarboxylic acid (69) was protected using Boc anhydride in the presence of sodium bicarbonate. The carboxylic acid was reformed upon addition of potassium bisulfate to give amine protected carboxylic acid 70 in 88% yield. The carboxylic acid underwent a peptide coupling with dimethyl amine to give 71 in a 92% yield under classical conditions. The Boc-protecting group was then removed using trifluoroacetic acid in DCM to yield primary amine 67e in 57% yield.

Scheme 3.4. Synthesis of primary amine 67e used in the synthesis of compound 50e.

The synthesis of the key primary amine 67l, used in the SNAr reaction to make compound 50l, is outlined in Scheme 3.5. The synthesis began with the Boc protection of the amine group of L-prolinol (72) to yield the N-Boc protected prolinol 73 in 96% yield. Activation using p-toluenesulfonyl chloride (TsCl) and following substitution using sodium azide in DMSO yielded compound 75 in 24% yield over two steps. The initial three reactions were performed using protocols published by Llop and coworkers. Finally, the azide from compound 75 was reduced to primary amine 67l using a Pd-catalyzed hydrogenation reaction in quantitative yield.
Scheme 3.5. Synthesis of primary amine 67l used to make compound 50l.

It is worth noting that in an attempt to raise the yield and reduce the number of steps an alternative route was developed using Mitsunobu conditions shown in Scheme 3.6. The primary alcohol in compound 73 was directly converted to a phthalimide protected nitrogen using DIAD and triphenylphosphine in 95% yield. Phthalimide compound 76 was deprotected in the presence of hydrazine to yield primary amine 67l in much higher yields than previously discussed in Scheme 3.5.

Scheme 3.6. Alternate synthesis of primary amine 67l.

The classic Gabriel synthesis was employed to synthesize primary amines 67j and 67k used in the $S_N$Ar reaction to make target compounds 50j and 50k. The Gabriel synthesis is summarized in Scheme 3.7. Most of this work was done by Ms. Urzsula Milewicz and is highlighted in her masters thesis and also in manuscript that is in preparation. Phthalimide (77) was reacted with dibromoalkane 80 with various chain lengths to give $N$-phthalimide alkyl bromide 78 with either a 2, 3, or 4 carbon chain.
alkyl bromide 78 was then reacted with various secondary amines to yield N-phthalimide alkyl amine compounds 79. The phthalimide group was then removed using hydrazine to yield primary amines 67.\textsuperscript{115}

Scheme 3.7. General Gabriel procedure used for the synthesis of primary amines 67.

3.10.2 Synthesis and Discussion of Target Compounds

With primary amines in hand, they were reacted in high yields with nitro-chloro-chroman 65 in an S\textsubscript{N}Ar reaction shown in Scheme 3.3. The following reactions were performed in the same manner for all compounds. The purified para-nitro chromans 66b-m were reduced using palladium on carbon and hydrogen gas, and \textit{in-situ} cyclization using cyanogen bromide to yield 2-amino-benzimidazole compounds 68b-m. The target compounds 50b-m were produced following a reduction using lithium aluminum hydride which reduced the amide(s) to an amine(s), and Boc-group to a methyl group. A summary of the primary amines 67b-m used and synthesis of the target compounds with their EC\textsubscript{50} values can be found in Table 3.3 and are results in a manuscript in preparation.
Table 3.3. Summary of the primary amines 67a-m used and target compounds 50a-m synthesized with EC$_{50}$ values.

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<th>Primary Amines (R$_1$)</th>
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Table 3.3. Cont.

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<th>Primary Amines (R₁)</th>
<th>Target Compound (R₂)</th>
<th>Target Compound #</th>
<th>EC₅₀ (µM)</th>
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</thead>
<tbody>
<tr>
<td>H₂N</td>
<td></td>
<td>50l</td>
<td>148</td>
</tr>
<tr>
<td>N</td>
<td>Boc</td>
<td></td>
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<tr>
<td>67i</td>
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<tr>
<td>H₂N</td>
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<td>50k</td>
<td>89</td>
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<td>67m</td>
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</table>

According to the data in Table 3.3, the newly synthesized inhibitors had less affinity for binding than the originally synthesized compound 50a at 3.4 µM. However, at a very early stage of our SAR study some unique and interesting trends can be extracted from the data. The crystal structure of compound 50a (Figure 3.7) bound to the RNA elucidates a hydrogen bond between the protonated dimethyl amino group in the side chain and an oxygen in the phosphate backbone. The side-chain itself is fully extended and due to the flexibility of the chain appears to be up out of the plane of the rest of the molecule to form the observed hydrogen bond with the phosphate backbone. As described earlier, the first strategy for improving binding affinity was to conformationally restrict the rotation around the diamino side chain to reduce the loss of entropy upon binding of our ligand. This is represented in compounds 50b-e, l. Compound 50b had the best EC₅₀ value of any newly synthesized compound at 21 µM. It is interesting to note that of all the inhibitors synthesized, this was the most rigid. To compare the conformations of the side-chains the MM2 energy minimized (CambridgeSoft PerkinElmer ChemBio3D ultra 14.0) compounds...
were overlaid on compound 50a bound in the pocket in the crystal structure using PyMol (open source from Schrödinger). The methyl piperidine side-chain of compound 50b adopts a chair configuration making the distance between the two nitrogens shorter than compound 50a. However, the position of the protonated nitrogen is pointing the same direction as compound 50a which could account for its activity. Compound 50c has the same methyl piperidine side-chain as 50b, but has an extra methylene separating the nitrogens. The methylene extends the distance of terminal nitrogen closer to that of compound 50a, however the protonated nitrogen responsible for hydrogen bonding points in the opposite direction and therefore could be the cause for the loss in ligand affinity to 50 µM. Compound 50d has a similar methyl piperidine tether only the methylene is bound next to the protonated nitrogen. Compound 50d was isolated as a mixture of diastereomers. The PyMol overlay showed a very similar conformation to the bound inhibitor even though the protonated nitrogen was not as extended. It is also worth noting that the conformation where the stereocenter in the side-chain is (S) positioned the nitrogen closer to that in the inhibitor over the (R) conformation. The PyMol overlay of MM2 minimized energy of compound 50a and compound 50d with S-configuration, and compound 50a bound to the RNA is shown in Figure 3.13. Compound 50d had an EC50 value of 29 µM. Compound 50e was isolated as a mixture of cis and trans isomers on the cyclohexane ring of its side-chain. Even with the cyclohexane ring chair conformation in the chain, the protonated nitrogen was further extended than compound 50a, but was left with some flexibility with the methylene group between the ring and the nitrogen. Compound 50e had an EC50 value of 31 µM and the PyMol overlay showed that the cis conformation may be favored over the trans in relation to the position of the protonated nitrogen and flexibility at that position. Compound 50l, as expected, had the largest EC50 value at 148 µM. Compound 50l has two carbons separating the nitrogens in the tether and the PyMol overlay showed the protonated nitrogen twisted away from that of compound 50a. The notion for pre-constraining the alkyl tether of these inhibitors is validated by these results, even though a compound with increased ligand affinity over the original inhibitor 50a was not discovered. However, it is important to note that even with added rigidity the distance and relative position of the protonated nitrogen plays a huge role in the formation of the observed hydrogen bond.
Figure 3.13. PyMol overlay of MM2 energy minimized compound 50a (pink) and 50d (S-configuration, yellow), and compound 50a (green) bound to the RNA from the crystal structure.

The second strategy was to adjust the length of the alkyl chain between the amine groups in the tether, and to change the substituents attached to the tertiary amine group located at terminus of the tether to try to strengthen the salt bridge between the nitrogen atom terminus and the RNA. These compounds are represented in compounds 50f-k. One of the main trends that were observed was the correlation between the number of carbons in the chain, and binding affinity. As expected, when the carbon chain was shortened to 2-carbons (compound 50h) the binding was drastically decreased, 25-fold, when directly compared to the original inhibitor 50a. The chain may not be long enough for the terminal nitrogen to reach the phosphate backbone of the RNA once docked into the pocket. A similar trend is observed in the pyrrolidine series of compounds (50f, g, i) where the two-carbon chain (50f) has the worse binding affinity of 58 µM. Increasing the carbon chain to four-carbons (50i) had little effect on the binding when compared to the 3-carbon chain (50g), 22 µM and 25 µM, respectively. The added bulk of the pyrrolidine ring as the
terminal nitrogen also had a negative effect on the affinity which was further supported by the terminal piperidine ring 50k which had an EC₅₀ value of 89 µM. Interestingly, when adding an oxygen into that 6-membered ring (morpholine-containing compound 50j) the binding increased over 2.5-fold to an EC₅₀ value of 35 µM. This is potentially due to the fact that the protonated morpholine derivative is a much stronger acid (pKa about 8.4) than the piperidine (pKa about 11.2). The presence of an oxygen atom in the ring also provides another source for potential hydrogen bonding with the HCV RNA. The phenomenon of having more than one hydrogen bond contributor in the tether is worth exploring by synthesizing new compounds with an additional atom, such as an oxygen or nitrogen, capable of forming extra hydrogen bonds in the tether. Such compounds will be proposed and discussed further in the future work.

Compound 50m with an EC₅₀ = 1066 µM lacks an atom in the tether capable of forming a hydrogen bond and was made to verify the necessity of that hydrogen bond relative to binding affinity. These results are in a manuscript in preparation for *Bioorganic & Medicinal Chemistry Letters*.

### 3.11 Conclusion

The hepatitis C virus is a major human health concern throughout the world. Between 55-85% of the people who contract the virus develop chronic infection which can ultimately lead to liver failure. HCV is the principal cause of death from liver disease and the leading cause of liver transplantation in the U.S. There has been much advancement in the treatment of HCV as of late, especially with the use of direct acting antivirals (DAAs) as monotherapy and in combination therapy. The use of new DAAs have led to SVR rates in the mid to high 90s with therapy as short as 12 weeks. However, with all of the HCV drugs to recently hit the market only the NS3/NS4 serine protease, NS5A, and NS5B polymerase have been targeted for inhibition and generally the drugs are only approved for genotypes 1 & 2. The hepatitis C virus has a high genetic variability (30-35%), and is classified into seven major genotypes and over 100 subtypes (<15% variability). HCV has an extremely high mutation rate occurring in 1 in 1,000 bases per year. Given such a high mutation rate, drug resistance by HCV has already been demonstrated in the new direct acting antivirals.
The subdomain IIa of the hepatitis C virus is >98% conserved across all genotypes. The high conservation of subdomain IIa shows that mutations around the IRES would be difficult to reconcile with IRES function. Thus, having therapeutics that target the IRES would have a high barrier to resistance and should have activity across all genotypes of HCV.

In 2005, Seth and coworkers discovered and synthesized new molecules that target the IRES of HCV and inhibit viral translation. In 2011, Bergdahl and coworkers improved upon this synthesis and were able to add diversity at a late stage of the synthesis to rapidly produce analogs that inhibit the viral translation of HCV. A small SAR study of compounds has been completed. These compounds were evaluated using an RNA construct FRET study by our collaborators in the Hermann laboratory at the University of California-San Diego and in *ex vivo* cell studies testing (currently in progress) for efficacy and toxicity by our collaborators in the Wolkowicz laboratory at San Diego State University. Although an inhibitor with a higher binding affinity than Seth’s original inhibitor has not yet been discovered, some initial trends in chain length and conformational restriction have already been illuminated. This work will continue to progress the SAR study and enhance our knowledge of the internal ribosome entry site of HCV and other closely related viruses which will be discussed in Chapter 4.

Contained in this chapter is material that is in manuscript preparation. The following co-authors assisted with this work and should be commended: Urszula Milewicz, Mark A. Boerneke, Dr. Thomas Hermann, and of course Dr. B. Mikael Bergdahl.
4 Future Work

4.1 Chapter 2 Azaspirene

4.1.1 Completion of the Total Synthesis of Azaspirene

A proposed retrosynthetic scheme toward the total synthesis of azaspirene is described previously (Chapter 2). It is important and of considerable need to obtain an efficient and scalable enantioselective synthesis of the target molecule due to its bioactivity. The proposed synthesis for the completion of the target molecule is shown in Scheme 4.1. With diketone 49 in hand it is envisioned that an asymmetric α-hydroxylation reaction can be done using the literature protocol published by Hii and coworkers\textsuperscript{59} using DMD (dimethyldioxirane) as the oxidizer. The use of nickel is also an alternative to palladium demonstrated by Smerz and coworkers.\textsuperscript{121} The cyclization will be conducted using a functionalized ester that will form azaspirene’s spirocyclic core and diene tail using a Margaretha type reaction.\textsuperscript{35} Upon dehydration the highly conjugated intermediate 39 will be formed. The conjugated ketone will then be protected as a dithiolane 82 to protect from nucleophilic attack.\textsuperscript{122} Azaspirene (1) will be completed after a Grignard reaction with benzyl magnesium bromide, and subsequent deprotection of the dithiolane and silyl ether protecting groups. The Grignard reaction will likely form a mixture of diastereomers, but at such a late stage in the synthesis the diastereomers will be isolated and tested for biological activity.
Scheme 4.1. Proposed synthesis for the completion of azaspirene 1.

The third step of the synthesis of 1,3-diketone 49 is an aldol reaction to succinimide derivative 34 which relies on the deprotonation of the α-carbon. The yield for this reaction is only 45% and has been difficult to repeat due to the relatively high pKₐ of the α-proton on the imide and with other acidic protons in the molecule. At such an early stage in the synthesis such a low yielding reaction is less than ideal. An alternative route to aid in this deprotonation would be to use a cyclic ester, similar to the total synthesis by Tadano (Scheme 2.4)²⁵, instead of the proposed imide. A proposal for the use of a lactone ring is shown in Scheme 4.2 using our laboratories knowledge of copper silyl additions as well as recent literature.²⁹,³⁰,¹²³ The aldehyde is used to quench the reaction which adds in the α-position demonstrated by Williams and coworkers who then oxidize the silyl group under Fleming-Tamao conditions to yield the corresponding alcohol with retention of stereochemistry.¹²⁴,¹²⁵

Scheme 4.2. Proposed silyl additon to an unsaturated lactone.
4.1.2 Determination of Azaspirene’s Molecular Target

It has been demonstrated that azaspirene inhibits VEGF induced angiogenesis by blocking Raf-1 activation in the MAPK signaling pathway. Azaspirene did not affect tyrosine phosphorylation of HSP90/Raf-1/MEK which suggests that its mode of action is different than some known antiangiogenic compounds such as vatalanib, which inhibits the tyrosine kinase receptor.\textsuperscript{21} Although this work is a good starting point, it does not explicitly define the molecular target of azaspirene nor does it elucidate its exact mechanism of action. A more efficient synthesis will further along these studies and a crystal structure of azaspirene bound to its molecular target will be completed. The crystal structure will enhance our knowledge of angiogenesis inhibition as well as guide the synthesis of more biologically active compounds.

4.1.3 Synthesis of Azaspirene Analogs

The proposed synthesis for the completion of the target molecule (Scheme 4.1) lends itself to the introduction of diversity at a late stage in the total synthesis. The pseurotin family of compounds share a highly conserved $\gamma$-lactam spirocyclic structure only differing by a benzoyl ketone, O-methoxy group, and a functionalized tail. Scheme 4.3 proposes a synthetic route for the synthesis of other members of the pseurotin family, as well as other analogs of azaspirene. Compound 37 is readily reacted in a Margaretha type cyclization using a previously synthesized ester which effectively adds a diversified tail (R\textsubscript{1}) to give compound 85 upon dehydration.\textsuperscript{35} The carbonyl groups on imide 85 could either be attacked by a Grignard reagent with aromatic substitution (R\textsubscript{2}), or using umpolung chemistry of a deprotonated 1,3-dithiane masked aldehyde which is easily oxidized back to the carbonyl in a variety of ways.\textsuperscript{122,126} The addition of R\textsubscript{2} as an aromatic halide gives a great chemical handle that allows for an array of aromatic coupling reactions. The quenching of both reactions can be performed with either a suitable proton source such as ammonium chloride to yield a hydroxyl group or an alkyl halide to yield an ether (R\textsubscript{3}). The other members of the pseurotin family, as well as other analogs can be synthesized following Scheme 4.3 upon a couple of deprotection steps.
Scheme 4.3. Proposed synthesis for members of the pseurotin family and other analogs of azaspirene.

4.2 Chapter 3 HCV Inhibitors that Target Subdomain IIa

4.2.1 Continue to Refine SAR Study

Using the knowledge obtained from the results of the initial compounds (Table 3.3) a few additional structures are proposed. The proposed final compounds are shown in Scheme 4.4 as well as the primary amines needed to complete the synthesis. Compounds 50n-s will be synthesized according to Scheme 3.3 in which primary amines 67n-s will be used in the S_NAr reaction to compound 65.

Compound 50n has a pyridine ring in its tether which is more conformationally restricted than the original inhibitor 50a. A pyridinium nitrogen is also much more acidic (pKa about 5.2) than an ammonium nitrogen which may contribute favorably to its binding demonstrated by the morpholine compound 50j and piperidine compound 50k discussed in chapter 3. The S_NAr reaction has been performed and the product 66n can be found in the experimental section.
Scheme 4.4. Proposed final compounds and primary amines necessary for their synthesis.

Compound 50o is a very rigid analog, and retains the optimum three-carbon chain length in between the two nitrogens. The proposed synthesis for amine 67o necessary for the S_NaR reaction is shown in Scheme 4.5. Following the procedure by Rogers-Evans and coworkers through the first four steps, \textsuperscript{127} N-Cbz-Asparagine (90) is converted to the methyl ester 91 using thionyl chloride in 95\% yield. The asparagine methyl ester 91 is cyclized using benzyl bromide following deprotonation using sodium hydride in a mixture of THF/DMF to succinimide derivative 92 in 75\% yield. The Cbz-protecting group is removed using a palladium-catalyzed hydrogenation reaction in glacial acetic acid to yield amino-succinimide derivative 93 as the ammonium acetate salt in 93\% yield. Succinimide 93 is reduced using Red-Al to pyrrolidine 94 in 55\% yield. The free amine is protected as a phthalimide to yield compound 95 in 30\% yield.\textsuperscript{128} A deprotection under hydrogenation conditions and \textit{in-situ} protection using Boc anhydride would yield N-Boc protected pyrrolidine 96,\textsuperscript{129} which upon deprotection of the phthalimide group using hydrazine would yield the desired amine 67o. Primary amine 67o will then be used in the S_NaR reaction highlighted in Scheme 3.3 to make proposed final compound 50o.
Scheme 4.5. Proposed synthesis for amine 67o.

Compound 50p has a pyrazolidine ring in its tether which would be capable for forming a second hydrogen bond with the RNA. Compound 50p retains the three atom distance between the amino groups in the side chain with the third atom being a nitrogen. Not only does the inner-nitrogen serve as a surrogate for potential hydrogen bonding, it also eliminates the formation of diastereomers upon coupling. It is worth noting that hydrazine derivatives are known to be toxic in the body, but is still worth testing in the FRET assay to assess binding. The synthesis for primary amine 67p is shown in Scheme 4.6. Alkyl bromo phthalimide 78a is formed upon reaction of phthalimide and 1,2-dibromoethane demonstrated by Nagarapu and coworkers.116 The Boc-substituted pyrazolidine ring was synthesized via procedures from Melendez and coworkers.130 Hydrazine (97) was mono Boc protected using Boc anhydride in isopropanol in 44% yield. The other nitrogen of N-Boc protected hydrazine 98 was then protected as a Cbz-group using benzyl chloroformate and N-methyl morpholine in THF to give diprotected hydrazine 99 in 60% yield. Upon deprotonation using sodium hydride di-protected hydrazine 99 was cyclized to pyrazolidine ring 100 using 1,3-dibromopropane in 80% yield. The Cbz-group was then removed using a Pd-catalyzed hydrogenation reaction to give mono-boc protected pyrazolidine 101 in 72% yield. Pyrazolidine compound 101 was then coupled to alkyl bromide 78a using microwave irradiation in the presence of catalytic amounts of potassium iodide to give compound 79a in 33% yield. The phthalimide group was then removed using hydrazine to yield primary amine 67p in 88% which will be used in the S_NAr reaction outlined in Scheme 3.3 to make proposed final compound 50p.
Scheme 4.6. Synthesis of primary amine 67p used in the SNAr reaction to make 50p.

Compound 50q is of particular interest being that it would be the first example of a secondary amine in our tether. A secondary amine is slightly more basic than a tertiary amine and also less sterically crowded. The synthesis of primary amine 67q is shown in Scheme 4.7 as well as proposed synthesis to final compound 50q as it diverges slightly from the general scheme (Scheme 3.3). The primary amine of N-Methyl-1,3-diaminopropane (102) was protected in preference to the secondary amine using ethyl trifluoroacetate to give diamine 103 in quantitative yield.131 The secondary amine was then Teoc-protected using Teoc-O-succinimide to produce di-protected amine 104 in 10% yield.132 Deprotection of the trifluoroacetamide group using potassium carbonate in aqueous methanol gave desired primary amine 67q in 90% yield.133 Primary amine 67q was immediately reacted with nitro chroman 65 in a SNAr reaction as previously shown to yield compound 66q in 82% yield. The nitro-group of compound 66q will then be reduced using a Pd-catalyzed hydrogenation reaction and cyclized using cyanogen bromide to give 2-amino-benzimidazole 68q as previously shown. The Teoc-protecting group will then be removed using a fluoride source to produce compound 68s122 whose amide group in the presence of LAH will be reduced to an amine to give the desired final compound 50q.
Scheme 4.7. Synthesis of primary amine 67q used in the proposed synthesis of final compound 50q.

Proposed compound 50r is a macrocycle of the original compound 50a. According to some recent literature\textsuperscript{134,135} the use of macrocyclization can have a large beneficial impact on inhibitors by improving potency and pharmacokinetic profiles due to the conformational restriction. Compound 50a lends itself to macrocyclization by having two dimethyl amine chains that extend largely outside of the binding pocket. The coupling of these two amine side chains by a linker could in fact increase binding affinity by increasing the inhibitors rigidity and should have little impact on the observed guanidine docking.

Compound 50s was identified as a possible target based on the PyMol overlay shown in Figure 3.13. The rigid methyl piperidine in compound 50d is conformationally very similar to inhibitor 50a bound in the pocket in both extension of the chain and position of the terminal nitrogen observed in the PyMol overlay. It does appear that it may be beneficial to extended the terminal nitrogen one atom further in order to achieve nearly identical overlap with the bound inhibitor and that the (R)-configuration would be the more favorable conformation.
4.2.2 Test Compounds Against other RNA Viruses

It was discussed in chapter 3 that the subdomain IIa of the IRES of HCV is not only highly conserved through out all genotypes of HCV, but also highly conserved across other members of the Flaviviridae family such as classical swine fever virus (CSFV), the bovine viral diarrhea virus (BVDV), and GB virus B (GBV-B). The same domain organization is also found in several members of the Picornaviridae family such as porcine teschovirus (PTV), avian encephalitis virus (AEV), and simian Picornavirus (SPV) which suggests a very similar translation type initiation as HCV. It is worth speculating that 2-amino-benzimidazole compounds with high affinity for the HCV IRES may have broad-range anti-viral activity by binding to subdomain IIa. A similar FRET based assay as the one described in chapter 3 could be conceived for other virus in these families whose RNA genomes have been elucidated. Once the assays are developed, this would allow for rapid screening of compounds across multiple RNA viruses.
5 Experimental Section

5.1 Chemicals and Instruments

**General.** All reactions were conducted under an argon atmosphere and in septum-capped oven-dried glassware unless otherwise specified. Chemical yields are based on purified material (>96% by $^1$H-NMR spectroscopy). $^1$H (400, 500, 600 MHz) and $^{13}$C (126 and 151 MHz, standard: $^{13}$CDCl$_3$, $\delta$ = 77.23 ppm) NMR spectra were recorded on a Varian 400, 500, and 600-MHz instrument using TMS as internal standard ($\delta$ = 0 ppm). Coupling patterns are abbreviated as s, singlet; d, doublet; t, triplet; q, quartet; se, sextet; qu, quintet; hep, heptet; m, multiplet; J, coupling. Mass spectra were recorded using a Thermo Finnigan LCQ Deca or an Agilent 6330 ion trap. Flash chromatography was conducted using a Biotage Isolera one instrument with prepacked silica gel columns (AnaLogix, Sepra Si 50) or self-packed Luknova and Biotage snap columns filled with silica gel (Sorbent Technologies, 60Å, 230-400 mesh) or basic alumina (60-325 mesh).

**Chemicals.** Diethyl ether (Et$_2$O) and tetrahydrofuran (THF) were distilled from sodium-benzophenone ketyl and were collected when the indicator became deep blue or purple. All reagents and solvents were purchased from Aldrich or Fisher Scientific unless otherwise specified.

5.2 Experimental Procedures

**L-Phenalaninol from reduction of L-phenylalanine. Preparation of compound 40.** To an oven dried 3-neck flask under argon L-phenylalanine (2) (2.00 g, 12.1 mmol, 1.0 equiv) was added sodium borohydride (1.05 g, 27.8 mmol, 2.3 equiv) and dissolved in 37 ml of distilled THF and cooled to 0 °C. Iodine (3.68 g, 14.5 mmol, 1.2 equiv) dissolved in 13 ml of distilled THF in a separate pear-shaped flask was then added dropwise via cannula over 20 minutes. The pear-shaped flask was rinsed with 15 ml more THF and reaction was allowed to stir overnight at 50 °C. The next day reaction was cooled and 28 ml of methanol was added to break up the white solid precipitate and solvents were removed via rotovap. To the
reaction slurry 28 ml of 20% aqueous potassium hydroxide was added, and reaction was allowed to stir for 3 hours. The aqueous phase was then extracted 3 times with 20 ml of dichloromethane. The organics were combined, dried over sodium sulfate, and concentrated \textit{in vacuo}. The product L-phenylalaninol 40 was determined to be pure and used without further purification (1.82 g, 99% yield).

\textbf{Benzyl oxazolidinone from L-phenylalaninol. Preparation of compound 3.} To L-phenylalaninol 40 (5.50 g, 35.4 mmol, 1.0 equiv) and potassium carbonate (0.455 g, 3.64 mmol, 0.10 equiv) was added diethyl carbonate (10.4 ml, 94.6 mmol, 2.6 equiv). Reaction was refluxed overnight with a short-head distillation condenser to collect the ethanol. The reaction mixture was then partitioned between 100 ml of dichloromethane and 150 ml of water. The organic layer was dried over magnesium sulfate and concentrated \textit{in vacuo}. The crude product was recrystallized with 2:3 mixture of ethyl acetate:hexane and filtered with cool hexane. The pure benzyl oxazolidinone 3 product was isolated as a white crystalline solid (4.45 g, 71% yield).

\textbf{N-PMB protected oxazolidinone from benzyl oxazolidinone. Preparation of compound 41.} Benzyl oxazolidinone 3 (2.59 g, 14.6 mmol, 1.0 equiv) was dissolved in 100 ml of distilled THF under argon. The solution was cooled to 0 °C and 60% sodium hydride (1.17 g, 29.2 mmol, 2.0 equiv) was slowly added. The reaction was allowed to warm to room temperature over 20 minutes and para-methoxybenzyl chloride (3.95 ml, 29.2 mmol, 2.0 equiv) was added. Tetrabutylammonium iodide (5.39 g, 14.6 mmol, 1.0 equiv) was added and reaction stirred at room temperature overnight. The reaction was quenched with saturated ammonium chloride and partitioned between 50 ml of diethyl ether and 50 ml of water. The organic phase was dried with magnesium sulfate and concentrated \textit{in vacuo}. The crude product was purified via flash chromatography (silica gel, 50% diethyl ether/pentane) to yield pure PMB protected oxazolidinone 41 (4.16 g, 96% yield).

\textbf{N-PMB protected phenylalaninol from hydrolysis of oxazolidinone. Preparation of compound 4.} N-PMB protected oxazolidinone 41 (3.20 g, 10.8 mmol, 1.0 equiv) was dissolved in 225 ml of ethanol. To the solution lithium hydroxide (7.76 g, 324 mmol, 30 equiv) dissolved in 100 ml of water was added. Reaction was stirred overnight at 85 °C with a drying tube. The reaction was poured into 300 ml of water
and 300 ml of dichloromethane. The two phases were separated and the aqueous phase was extracted two times more with 250 ml of dichloromethane. The organics were combined, dried over magnesium sulfate, and concentrated in vacuo. N-PMB protected L-phenylalaninol 4 was used without further purification (2.80 g, 96%).

**Oxothiazolidine from N-PMB phenylalaninol and thionyl chloride. Preparation of compound 42.** 40

Protected L-phenylalaninol 4 (2.80 g, 10.3 mmol, 1.0 equiv) dissolved in 175 ml of dichloromethane was added imidazole (2.80 g, 41.2 mmol, 4.0 equiv) and triethylamine (2.90 ml, 20.6 mmol, 2.0 equiv). The solution was cooled to 0 °C and thionyl chloride (900 µl, 12.4 mmol, 1.2 equiv) was added dropwise and allowed to stir at 0 °C for one hour. The reaction was quenched with 100 ml of water and extracted. The aqueous phase was extracted with an additional 100 ml of dichloromethane and the organics were combined. The combined organics were then washed twice with 100 ml of water, dried over magnesium sulfate, and concentrated in vacuo. The crude zwitterionic oxothiazolidine 42 was used without further purification (3.26 g, 99%).

**Cyclic sulfamidate from oxidation of oxothiazolidine. Preparation of compound 5.** 40

Crude oxothiazolidine 42 (3.26 g, 10.3 mmol, 1.0 equiv) was dissolved in 90 ml of acetonitrile and 75 ml of water and added sodium periodate (3.08 g, 14.4 mmol, 1.4 equiv). To the solution, a pinch of ruthenium (III) chloride was added and the solution stirred overnight at room temperature. The solution was diluted with 150 ml of diethyl ether and extracted. The organic phase was washed with 100 ml of saturated sodium bicarbonate and 100 ml of brine. The organic phase was dried over magnesium sulfate, filtered, and concentrated in vacuo. The cyclic sulfamidate 5 was isolated as a white solid and determined to be pure (3.00 g, 84%).

**Phosphonate for HWE from cyclic sulfamidate and triethylphosphonoacetate. Preparation of compound 6.** 41

To a round bottom flask under argon added potassium tert-butoxide (0.821 g, 7.32 mmol, 2.0 equiv) to a solution of triethylphosphonoacetate (1.45 ml, 7.32 mmol, 2.0 equiv) dissolved in 15 ml of dry tetrahydrofuran. The solution stirred at 40 °C until it was homogeneous and colorless (20 minutes). Sulfamidate 5 (1.22 g, 3.66 mmol, 1.0 equiv) was then added dissolved in 2 ml of dry THF and reaction
was stirred overnight at 40 °C. Once the reaction was cooled, 4.1 ml of 5.0M hydrochloric acid was added and reaction stirred for an additional three hours. The reaction was then made basic (pH = 8) with a saturated solution of sodium bicarbonate. The solution was then saturated with 60 ml of brine solution and extracted 3 times with 40 ml of dichloromethane. The organics were combined, dried over sodium sulfate, and concentrated in vacuo to yield 1.65g of crude compound. The crude compound was then dissolved in 45 ml of toluene and refluxed for 2 days. The reaction was then concentrated in vacuo and purified via flash chromatography (silica gel, 5% dichloromethane/ethyl acetate) to yield pure phosphonate 6 as a mixture of diastereomers (1.15 g, 68% yield).

**HWE product from phosphonate and cyclohexanone. Preparation of compound 7.** To a suspension of 60% sodium hydride (0.028 g, 0.690 mmol, 1.1 equiv) in 5 ml of dry THF was added ethyl phosphonate 6 (0.282 g, 0.650 mmol, 1.0 equiv) dissolved in 1.0 ml of dry THF via syringe. The reaction was stirred for 30 minutes at room temperature and cyclohexanone (90.0 µl, 0.866 mmol, 1.3 equiv) was added dropwise and the reaction was stirred for an additional 3.5 hours. The reaction was quenched with 25 ml of water and extracted 3 times with 25 ml of diethyl ether. The organics were combined, dried over sodium sulfate, and concentrated in vacuo. The crude material was purified via flash chromatography (silica gel, 50% ethyl acetate/hexane) to yield pure HWE product 7 as a clear oil (0.044 g, 18% yield).

**N-Benzyl tartarimide from L-Tartaric Acid and benzyl amine. Preparation of compound 19.** L-Tartaric acid (18) (2.00 g, 13.3 mmol, 1.5 equiv) and benzyl amine (971 µl, 8.89 mmol, 1.0 equiv) were added to a microwave vial and capped. The reaction was heated to 200 °C for 5 minutes in the microwave. The crude reaction was dissolved in 30 ml of THF and extracted using 20 ml of saturated ammonium chloride. The organic layer was concentrated in vacuo to give a white solid that was consecutively washed with dichloromethane, twice with water, and again dichloromethane in a filter funnel. N-benzyl tartarimide 19 was collect as a white solid (1.46 g, 74% yield).

**O-TBS-N-benzyl tartarimide from benzyl tartarimide. Preparation of compound 43.** L-benzyl tartarimide 19 (5.00 g, 22.6 mmol, 1.0 equiv) was chased 3 times with 3 ml of toluene and was immediately dissolved in 35 ml of dry N,N-dimethylformamide and was added t-butyldimethylsilyl chloride (17.0 g, 113
mmol, 5.0 equiv) and imidazole (9.26 g, 136 mmol, 6.0 equiv). The reaction was stirred overnight at room temperature. The next day the reaction was poured into 400 ml of diethyl ether and 150 ml of water and stirred until the layers were clear. The organic phase was separated and extracted 4 times with 100 ml of water, then was dried over magnesium sulfate, and concentrated \textit{in vacuo}. The crude product was purified via flash chromatography (silica gel, 10% ethyl acetate/hexane) to give \textit{O}-TBS protected \textit{N}-benzyl tartarimide \textbf{43} as a clear oil (9.66 g, 95% yield).

\textbf{Benzyl Grignard product from protected benzyl tartarimide and benzyl magnesium bromide.}

\textbf{Preparation of compound 20.} To a 2-neck flask attached a reflux condenser under argon was added magnesium turnings (0.267 g, 11.0 mmol, 5.0 equiv) and 15 ml of freshly distilled THF. The flask was cooled to 0 °C and benzyl bromide (1.22 ml, 10.2 mmol, 4.7 equiv) was added dropwise. The reaction stirred for 1 hour and began to gently reflux. The reaction was again cooled to 0 °C and \textit{O}-\textit{TBS protected \textit{N}-benzyl tartarimide \textbf{43}} (1.00 g, 2.20 mmol, 1.0 equiv) along with bismuth(III)chloride (0.001 g, 0.044 mmol, 0.02 equiv) were added dissolved in 5 ml of THF. The reaction stirred at room temperature for 6 hours and was quenched with ammonium chloride. The reaction was filtered through celite using diethyl ether and concentrated \textit{in vacuo}. The crude material was redissolved in 30 ml of diethyl ether and extracted 2 times with 20 ml of water. The organics were combined, dried over sodium sulfate, and concentrated \textit{in vacuo}. The crude material was purified via flash chromatography (silica gel, 10% ethyl acetate/hexane) to give Grignard product \textbf{20} in correct stereochemistry \textbf{RRR} as a clear oil (0.775 g, 65% isolated yield).

\textbf{Triol product from benzyl Grignard product. Preparation of compound 46.} To Grignard product \textbf{20} (0.234 g, 0.432 mmol, 1.0 equiv) dissolved in 8 ml of THF was added TBAF (1.30 ml 1.30 mmol, 3.0 equiv) and stirred for 1 hour at room temperature. The reaction was poured into 50 ml of water and 25 ml of diethyl ether and stirred until clear. The organic phase was separated and extracted with 25 ml of brine solution, dried over magnesium sulfate, and concentrated \textit{in vacuo}. The crude triol \textbf{46} was used without purification.

\textbf{Acetal product from acetonide protection of triol. Preparation of compound 47.} The crude triol \textbf{46} (0.153 g, 0.488 mmol, 1.0 equiv) was dissolved in 10 ml of 2,2-dimethoxypropane to which was added a
pinch of para-toluenesulfonic acid. The reaction was stirred overnight at room temperature and was made basic using a saturated solution of sodium bicarbonate. The reaction was concentrated in vacuo and the residue redissolved in 25 ml of dichloromethane and 25 ml of water. The aqueous phase was extracted 2 more times with 10 ml of dichloromethane and organic phases combined, dried over magnesium sulfate, and concentrated in vacuo. The crude material was purified via flash chromatography (silica gel, 40% ethyl acetate/hexane) as an oil (0.007 g, 4% yield).

Alkenoic ester from dimethyl acetylene dicarboxylate and benzyl alcohol. Preparation of compound 31. To a solution of benzyl alcohol (7.24 ml, 70.0 mmol, 1.0 equiv) and DABCO (0.785 g, 7.00 mmol, 0.10 equiv) in 400 ml of dichloromethane was added dimethyl acetylene dicarboxylate (30) (10.0 g, 70.0 mmol, 1.0 equiv) and let stir for 2 hours at room temperature. The reaction was concentrated in vacuo and purified via flash chromatography (silica gel, 15% ethyl acetate/hexane) to give fumarate 31 as isolated Z-isomer as a clear oil (8.58 g, 49%).

D-Malic acid from D-aspartic acid. Preparation of compound 33. To a stirred solution of D-aspartic acid (32) (20.0 g, 150 mmol, 1.0 equiv) in 752 ml of 0.50M sulfuric acid was added 226 ml of 30% aqueous solution of sodium nitrite by addition funnel over 1 hr. The reaction was stirred for 2 hours at room temperature and then overnight under a stream of air. The reaction was concentrated in vacuo and crude material was dissolved in boiling acetone and filtered to remove insoluble salts. Filtrate was concentrated to give D-malic acid (33) as a white solid (16.0 g, 80% yield).

N-benzyl malimide from D-malic acid and benzyl amine. Preparation of compound 34. D-Malic Acid (33) (1.00g, 7.40 mmol, 1.0 equiv) was dissolved in 800 µl of methanol and 800 µl of water. Benzyl amine (800 µl, 7.40 mmol, 1.0 equiv) was slowly added and reaction was stirred at 50 °C for 30 minutes. Methanol was then removed via rotovap. 18 ml of o-xylene was added and reaction was refluxed overnight with a Dean-Stark condenser. Once cool, the reaction was concentrated in vacuo. The crude mixture was then refluxed in toluene for 30 minutes and put on ice to crystallize. White solid was collected via filtration and washed with cold toluene. N-benzyl malimide 34 was isolated as a white solid (0.910 g, 60% yield).
Aldol product from N-benzyl malimide and propanal. Preparation of compound 48. To 33.8 ml of a 0.37M solution of NaHMDS in THF under argon at 0 °C was added malimide 34 (1.00 g, 4.90 mmol, 1.0 equiv) dissolved in 16 ml of freshly distilled THF. The reaction was stirred at 0 °C for 20 minutes. The reaction was cooled to -78 °C and propanal (0.854 g, 14.7 mmol, 3.0 equiv) dissolved in 20 ml of distilled THF was added dropwise. The reaction stirred at -78 °C for 3.5 hours and was quenched at -78°C with 20 ml of saturated ammonium chloride in 10 ml of THF. The reaction was warmed to room temperature and 100 ml of diethyl ether and 30 ml of dichloromethane was added. The organic layer was extracted 2 more times with 30 ml of saturated ammonium chloride, dried with sodium sulfate, and concentrated in vacuo. The crude product was purified by flash chromatography (silica gel, 50% diethyl ether/dichloromethane) to give aldol product 48 as a white solid of a mixture of diastereomers (0.580 g, 45% yield).

O-TBS-mono-protected diol from the aldol product. Preparation of compound 36. To the aldol product 48 (0.524 g, 2.00 mmol, 1.0 equiv) dissolved in 10 ml of dry DMF under argon was added imidazole (0.681 g, 10.0 mmol, 5.0 equiv), tert-butyldimethylsilyl chloride (0.904 g, 6.00 mmol, 3.0 equiv), and was stirred for 2.5 hours at room temperature. The reaction was poured into 50 ml of diethyl ether and 30 ml of water and stirred until the organic layer was clear. The organic layer was extracted 4 more times with 20 ml of water, dried over magnesium sulfate, and concentrated in vacuo. Crude material was purified by flash chromatography (silica gel, 15% ethyl acetate/hexane). The protected diol 36 was isolated as a yellow oil (0.610 g, 82% yield).

1,3-Diketone from mono-protected aldol product. Preparation of compound 49. To a solution of imide 36 (0.200 g, .530 mmol, 1.0 equiv) dissolved in 2.5 ml of dichloromethane was added Dess-Martin periodinane (0.247 g, 0.580 mmol, 1.1 equiv), and stirred for 2 hours at room temperature. The reaction was diluted with 40 ml of diethyl ether and poured into 50 ml of an 8% sodium thiosulfate in saturated sodium bicarbonate solution and stirred until clear. The organic layer was extracted 2 more times with 10 ml of the 8% sodium thiosulfate in saturated sodium bicarbonate solution and once with 10 ml of brine. The crude reaction was purified by flash chromatography (silica gel, 10% ethyl acetate/hexane). The 1,3-diketone product 49 was isolated as a clear oil (0.182 g, 92% yield).
Boc protected 4-aminocyclohexanecarboxylic acid from 4-aminocyclohexanecarboxylic acid.

Preparation of compound 70. To a solution of cis-trans 4-aminocyclohexanecarboxylic acid (69) (0.500 g, 3.50 mmol, 1.0 equiv) dissolved in 10 ml of 1,4-dioxane added a solution of di-tert-butyl dicarbonate (1.53 g, 7.00 mmol, 2.0 equiv) and sodium bicarbonate (1.01 g, 12.0 mmol, 3.4 equiv) dissolved in 12 ml of water. The reaction stirred for 48 hours at room temperature and was quenched with a saturated solution of potassium bisulfate until evolution of gas ceased (pH = 3). The slurry was concentrated in vacuo and partitioned between 20 ml of ethyl acetate and 20 ml of water. The organic phase was extracted 3 additional times with 20 ml of water, dried over magnesium sulfate and concentrated in vacuo. The boc-protected product 70 was isolated as a clear oil (0.749 g, 88% yield) and used without further purification.

Dimethyl amide from boc-protected 4-aminocyclohexanecarboxylic acid. Preparation of compound 71. To a solution of boc-protected 4-aminocyclohexanecarboxylic acid 70 (0.400 g, 1.90 mmol, 1.0 equiv) dissolved in 15 ml of dichloromethane added 1-hydroxybenzotriazole (0.391 g, 2.90 mmol, 1.5 equiv), dimethylamine hydrochloride (0.391 g, 4.80 mmol, 2.5 equiv), 4-methylmorpholine (1.04 ml, 9.50 mmol, 5.0 equiv), and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (0.556 g, 4.80 mmol, 1.5 equiv). The reaction stirred for 48 hours and was diluted with 20 ml of dichloromethane and quenched with 35 ml of saturated sodium bicarbonate and stirred until the organic phase was clear. The solution was extracted 3 times with 15 ml of dichloromethane and the organics were combined. The organics were dried over magnesium sulfate and concentrated in vacuo. The crude material was purified through a plug of silica gel using ethyl acetate to give dimethyl amide product 71 as a white solid (0.470 g, 92% yield).

Primary amine from boc-protected dimethyl amide. Preparation of compound 67e. To a solution of dimethyl amide 71 (0.849 g, 3.10 mmol, 1.0 equiv) dissolved in 5.5 ml of dichloromethane was added trifluoroacetic acid (5.50 ml, 68.2 mmol, 22 equiv) dropwise. The reaction was stirred at room temperature for 2 hours and concentrated in vacuo. The crude ammonium salt was dissolved in 20 ml of water and basified with a 10% solution of sodium hydroxide (pH = 10). The aqueous phase was extracted 4 times
with dichloromethane and the organics were combined, dried over sodium sulfate, and concentrated in vacuo. The crude amine product 67e was used without further purification (0.300 g, 57% yield).

**Boc-protected L-prolinol from L-prolinol. Preparation of compound 73.** A solution of L-Prolinol (72) (1.03 g, 10.1 mmol, 1.0 equiv) dissolved in 30 ml of THF and 30 ml of water was cooled to 0 °C and added sodium bicarbonate (2.55 g, 30.3 mmol, 3.0 equiv) and di-tert-butyl dicarbonate (2.64 g, 12.1 mmol, 1.2 equiv) and stirred for 24 hours at room temperature. The reaction was quenched with 10 ml of saturated ammonium chloride and extracted 3 times with 25 ml of diethyl ether. The organics were combined, dried over magnesium sulfate, and concentrated in vacuo. The crude product was purified via flash chromatography (silica gel, 35% ethyl acetate/hexane) to give N-boc-protected L-prolinol 73 as a clear oil (1.95 g, 96% yield).

**O-tosyl-protected L-prolinol from boc-protected prolinol. Preparation of compound 74.** To a solution of boc-protected L-prolinol 73 (0.715 g, 3.60 mmol, 1.0 equiv) dissolved in 6 ml of pyridine was added tosyl chloride (1.72 g, 9.00 mmol, 2.5 equiv) and stirred overnight at room temperature with a drying tube. The reaction was quenched with 50 ml of water and stirred with 15 ml of ethyl acetate until organic layer was clear. The aqueous phase was extracted 3 times with ethyl acetate and the organics combined were extracted with 20 ml of brine. The combined organics were dried over sodium sulfate and concentrated in vacuo. The crude reaction was purified by flash chromatography (silica gel, 30% ethyl acetate/hexane) and gave di-protected L-prolinol 74 as a clear oil (1.03 g, 81% yield).

**Alkyl azide from O-tosyl-protected L-prolinol. Preparation of compound 75.** To di-protected L-prolinol 74 (0.200 g, 0.560 mmol, 1.0 equiv) dissolved in 4 ml of DMSO in a 2-5 ml microwave vial was added sodium azide (0.183 g, 2.80 mmol, 5.0 equiv) and the vial was capped. The reaction was microwave irradiated at 120 °C for 5 minutes and quenched with 40 ml of saturated ammonium chloride. The solution was extracted 3 times with 40 ml of ethyl acetate. The organics were combined and extracted 4 times with 25 ml of water. The organics were dried over magnesium sulfate and concentrated in vacuo. The crude material was purified via flash chromatography (silica gel, 12% ethyl acetate/hexane) to give alkyl azide 75 as a clear oil (0.036 g, 29% yield).
Primary amine from alkyl azide. Preparation of compound 67l.\textsuperscript{112} To a solution of alkyl azide 75 (0.133 g, 0.500 mmol, 1.0 equiv) dissolved in 750 µl of dry methanol under argon was added 10% palladium on carbon (0.002 g, 0.002 mmol, 0.004 equiv). The argon was removed and the reaction stirred under an atmosphere of hydrogen gas for 6 hours. The reaction was filtered through celite using methanol and concentrated \textit{in vacuo} to give primary amine 67l in quantitative yield (0.101 g).

Alkyl phthalimide from boc-protected \textit{L}-prolinol. Preparation of compound 76.\textsuperscript{114} To a solution of boc-protected \textit{L}-prolinol 73 (1.00 g, 5.00 mmol, 1.0 equiv) dissolved in 40 ml of dry THF under argon was added triphenylphosphine (1.71 g, 6.50 mmol, 1.3 equiv) and phthalimide (0.956 g, 6.50 mmol, 1.3 equiv). The reaction was stirred until homogeneous and diisopropyl azodicarboxylate (1.28 ml, 6.50 mmol, 1.3 equiv) was added dropwise. The reaction was stirred for 24 hours at room temperature and quenched with 70 ml of water. The reaction was extracted with 3 times 55 ml of hexane and the combined organic phases extracted with 50 ml of brine. The combined organics were dried over magnesium sulfate and concentrated \textit{in vacuo}. The crude material was purified by flash chromatography (silica gel, 50% diethyl ether/pentane) to give alkyl phthalimide product 76 as a clear oil (1.57 g, 95% yield).

Primary amine from alkyl phthalimide. Alternate preparation of compound 67l.\textsuperscript{115} To a solution of alkyl phthalimide 76 (0.905 g, 2.70 mmol, 1.0 equiv) dissolved in 60 ml of methanol was added hydrazine (262 µl, 5.50 mmol, 2.0 equiv) and the reaction was stirred at reflux for 3 hours. Once cooled, the reaction was concentrated \textit{in vacuo} and redissolved in 15 ml of ethanol and 15 ml of water. The solution was made acidic with 1.0 M HCl solution (pH = 1) and the precipitate was removed by filtration through celite rinsing with water. The filtrate was made basic with 1.0 M NaOH solution (pH = 10) and extracted 4 times with 45 ml of dichloromethane and washed with 25 ml of brine. The organics were dried over sodium sulfate and concentrated \textit{in vacuo} to give primary amine 67l which was used without further purification (0.514 g, 95% yield).

Methyl ester from \textit{N}-Cbz-\textit{L}-asparagine. Preparation of compound 91.\textsuperscript{127} To a solution of \textit{N}-Cbz-\textit{L}-asparagine (90) (2.00 g, 7.50 mmol, 1.0 equiv) dissolved in 25 ml of methanol and cooled to 0 °C was added thionyl chloride (1.68 ml, 23.1 mmol, 3.1 equiv) dropwise. The reaction stirred at 0 °C for 20
minutes. The reaction was allowed to warm to room temperature and 50 ml of toluene was added. The reaction was concentrated in vacuo until 25 ml of liquid remained. The solution was diluted with 10 ml of methanol and the solution was concentrated in vacuo to form a pale yellow solid. The crude material was recrystallized using 30 ml of methyl tert-butyl ether and N-Cbz-L-asparagine methyl ester 91 was collected as a white solid (1.99 g, 95% yield).

**Benzyl succinimide from methyl ester N-Cbz-L-asparagine.** Preparation of compound 92. To a suspension of 60% sodium hydride (0.149 g, 3.70 mmol, 1.03 equiv) in 10 ml of dry THF under argon was added L-asparagine methyl ester 91 (1.00 g, 3.60 mmol, 1.0 equiv) and the reaction was stirred for 20 minutes at room temperature. To the solution benzyl bromide (476 µl, 4.00 mmol, 1.1 equiv) and 16 ml of dry DMF were added and the reaction was stirred for 3 hours at room temperature. The reaction was quenched with 20 ml of water and extracted 3 times with 16 ml of toluene. The combined organics were extracted 2 times with 20 ml of water, dried over magnesium sulfate, and concentrated in vacuo. The crude solid was recrystallized using 13.5 ml of methyl tert-butyl ether and N-Cbz-protected benzyl succinimide 92 was collected as a white solid (0.907 g, 75% yield).

**S-Amine substituted benzyl succinimide from Cbz-protected succinimide.** Preparation of compound 93. A solution of N-Cbz-protected benzyl succinimide 92 (0.400 g, 1.20 mmol, 1.0 equiv) and 10% palladium on carbon (0.040 g, 0.037 mmol, 0.03 equiv) in 8 ml of glacial acetic acid was stirred under an atmosphere of hydrogen gas for 4 hours at room temperature. The reaction was filtered through celite using acetic acid and concentrated in vacuo. The crude reaction was purified via flash chromatography (silica gel, 70% ethyl acetate/hexane to 20% methanol/ethyl acetate) to give the ammonium acetate salt of amino benzyl succinimide 93 as a white solid (0.225 g, 93% yield).

**S-Amino benzyl pyrrolidine from S-amino benzyl succinimide salt.** Preparation of compound 94. The ammonium acetate salt of S-amino benzyl succinimide 93 (0.650 g, 2.50 mmol, 1.0 equiv) was dissolved in 6 ml of dichloromethane and 6 ml of water. The solution was made basic with 1.0 M NaOH solution (pH = 10) and extracted 7 times with 8 ml of dichloromethane. The organics were combined, dried over magnesium sulfate, and concentrated in vacuo to give then free amine. The free amine was
cooled to 0 °C under argon and Red-Al (65% in toluene, 2.4 ml, 8.00 mmol, 4.0 equiv) was added dropwise. The reaction stirred for 5 minutes at 0 °C and then at 80 °C for 45 minutes when a deep red color was formed. Once cooled, the reaction was quenched with 5.5 ml of 1.0 M NaOH solution and extracted 2 times with 10 ml of toluene. The organics were combined and extracted with 10 ml of 1.0 M NaOH and 10 ml of brine. The combined organics were dried over sodium sulfate and concentrated in vacuo to give S-amino benzyl pyrrolidine 94 (.239 g, 55%) which was used without further purification.

S-Phthalimide benzyl pyrrolidine from amino benzyl pyrrolidine. Preparation of compound 95. To a solution of S-amino benzyl pyrrolidine 94 (0.239 g, 1.40 mmol, 1.0 equiv) dissolved in 5 ml of dry THF under argon was added N-carbethoxyphthalimide (0.307 g, 1.40 mmol, 1.0 equiv) and triethylamine (279 µl, 2.00 mmol, 1.4 equiv) and the reaction was stirred for 3 hours at reflux. The reaction was concentrated in vacuo and redissolved in 30 ml of dichloromethane and 30 ml of water. The aqueous phase was extracted with 15 ml more of dichloromethane and the combined organics washed with 20 ml of brine. The organics were dried over sodium sulfate and concentrated in vacuo. The crude material was purified by flash chromatography (silica gel, 65% ethyl acetate/hexane) to give S-phthalimide benzyl pyrrolidine 95 as a white solid (0.127 g, 30% yield).

Alkyl bromo phthalimide from phthalimide and dibromo ethane. Preparation of compound 78a. To a solution of 1,2-dibromoethane (1.73 ml, 20.1 mmol, 1.5 equiv) dissolved in 15 ml of DMF under argon was added potassium carbonate (3.70 g, 26.8 mmol, 2.0 equiv) and phthalimide (2.00 g, 13.4 mmol, 1.0 equiv) dissolved in 24 ml of DMF. The reaction stirred for 24 hours at room temperature and poured into 200 ml of water and 100 ml of ethyl acetate. The solution stirred until the organic layer was clear and then extracted 3 times with 60 ml of water and 60 ml of brine. The organic layer was dried over sodium sulfate and concentrated in vacuo. The crude material was purified by flash chromatography (silica gel, 20% ethyl acetate/hexane) to give bromo phthalimide 78a as a white solid (2.45 g, 72% yield).

Mono boc-protected hydrazine from hydrazine. Preparation of compound 98. To a solution of hydrazine (97) (2.06 g, 41.2 mmol, 1.0 equiv) dissolved in 8 ml of isopropanol cooled to 0 °C was added di-tert-butyl dicarbonate (4.14 g, 19.0 mmol, 0.46 equiv) dissolved in 4 ml of isopropanol. The reaction
was allowed to warm to room temperature and stirred for 25 minutes. The reaction was concentrated *in vacuo* and then redissolved in dichloromethane. The solution was dried over magnesium sulfate and concentrated *in vacuo*. The material crystallized upon cooling and gave boc-protected hydrazine 98 as a white solid which was used without further purification (2.39 g, 44% yield).

**Di-protected hydrazine from mono boc-protected hydrazine. Preparation of compound 99.** To a solution of mono boc-protected hydrazine 98 (2.19 g, 16.6 mmol, 1.0 equiv) dissolved in 15 ml of dry THF under argon added N-methylmorpholine (1.83 ml, 16.6 mmol, 1.0 equiv) and cooled to 0 °C. To the solution added benzyl chloroformate (2.37 ml, 16.6 mmol, 1.0 equiv) dropwise and the reaction was stirred for 24 hours at room temperature. The reaction was filtered through a pad of celite using THF and the filtrate was concentrated *in vacuo*. The crude material was purified via flash chromatography (silica gel, 20% ethyl acetate/hexane) to give di-protected hydrazine 99 as a white solid (2.62 g, 60% yield).

**Di-protected pyrazolidine from di-protected hydrazine and 1,3-dibromopropane. Preparation of compound 100.** To a solution of di-protected hydrazine 99 (2.48 g, 9.30 mmol, 1.0 equiv) dissolved in 50 ml of dry DMF under argon cooled to 0 °C was added 60% sodium hydride (0.784 g, 19.6 mmol, 2.1 equiv) and stirred for 1 hour at room temperature. To the solution 1,3-dibromopropane (1.00 ml, 9.80 mmol, 1.05 equiv) was added slowly and the reaction stirred for 24 hours at room temperature. The reaction was poured into 200 ml of water and 100 ml of ethyl acetate and stirred until the organic layer was clear. The solution was extracted 3 times with 70 ml of water and once with 25 ml of brine. The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The crude material was purified by flash chromatography (silica gel, 32% ethyl acetate/hexane) to produce di-protected pyrazolidine 100 as a clear oil (2.26 g, 80% yield).

**Mono boc-protected pyrazolidine from di-protected pyrazolidine. Preparation of compound 101.** A solution of di-protected pyrazolidine 100 (1.20 g, 3.90 mmol, 1.0 equiv) and 10% palladium on carbon (0.420 g, 0.390 mmol, 0.10 equiv) in 40 ml of dry methanol was stirred under an atmosphere of hydrogen gas for 6 hours. The reaction was filtered through celite using methanol and the filtrate was concentrated *in vacuo*.
The crude reaction was purified by flash chromatography (silica gel, 60% ethyl acetate/hexane to 20% methanol) to give mono protected pyrazolidine 101 as a clear oil (0.481 g, 72% yield).

**Phthalimide alkyl pyrazolidine from mono-protected pyrazolidine and bromo alkyl phthalimide.**

**Preparation of compound 79a.** Mono-boc-protected pyrazolidine 101 (0.481 g, 2.80 mmol, 1.0 equiv) dissolved in 18 ml of dry DMF was added to a 10-20 ml microwave vial which contained potassium carbonate (0.387 g, 2.80 mmol, 1.0 equiv), alkyl bromo phthalimide 78a (1.51 g, 6.00 mmol, 2.15 equiv), and potassium iodide (0.093 g, 0.560 mmol, 0.20 equiv). The vial was capped and microwave irradiated at 150 °C for 15 minutes. The bright yellow solution was poured into 80 ml of diethyl ether and 100 ml of water and stirred until the organic layer was clear. The organic phase was extracted with 3 times 80 ml of water and once with 80 ml of brine. The organic layer was dried over sodium sulfate and concentrated in vacuo. The crude material was purified by flash chromatography (silica gel, 50% ethyl acetate/hexane) to give phthalimide ethyl pyrazolidine 79a as a clear oil (0.314 g, 33% yield).

**Ethyl amino pyrazolidine from phthalimide ethyl pyrazolidine.** Preparation of compound 67p. To a solution of phthalimide pyrazolidine 79a (0.314 g, 0.909 mmol, 1.0 equiv) dissolved in 20 ml of methanol was added hydrazine (90.0 µl, 1.82 mmol, 2.0 equiv) and the reaction was stirred at reflux for 3 hours. The reaction was concentrated in vacuo and redissolved in 8 ml of ethanol and 8 ml of water. The solution was made acidic using 1.0 M HCl solution (pH = 1) and filtered through celite using water. The filtrate was made basic using 1.0 M NaOH solution (pH = 10) and extracted 4 times with 20 ml of dichloromethane. The combined organics were extracted with 20 ml of brine, dried over sodium sulfate, and concentrated in vacuo. Ethyl amino pyrazolidine 67p was used without further purification (0.171 g, 88%).

**Trifluoroacetate mono-protected diamine from N-methyl-1,3-diaminopropane.** Preparation of compound 103. To a solution of N-methyl-1,3-diaminopropane (1.00 g, 11.3 mmol, 1.0 equiv) dissolved in 4 ml of acetonitrile was added ethyltrifluoroacetate (3.10 ml, 26.0 mmol, 2.3 equiv) and the reaction was stirred at reflux for 24 hours. Once cooled, the reaction was concentrated in vacuo to give trifluoroacetamide 103 as a red oil in quantitative yield (2.08 g).
Di-protected propane diamine from trifluoroacetamide. Preparation of compound 104. To a solution of trifluoroacetamide 103 (1.00 g, 5.40 mmol, 1.0 equiv) dissolved in 26 ml of 1,4-dioxane and 26 ml of water was added triethyl amine (1.13 ml, 8.10 mmol, 1.5 equiv) and 1-(2-(trimethylsilyl)ethoxycarbonyloxy)succinimide (1.53 g, 5.90 mmol, 1.1 equiv) and the reaction was stirred for 24 hours at room temperature. The reaction was diluted with 30 ml of dichloromethane and extracted. The organic layer was extracted with 25 ml of saturated ammonium chloride and 25 ml of brine. The organic phase was dried over sodium sulfate and concentrated in vacuo. The crude reaction was purified by flash chromatography (silica gel, 30% ethyl acetate/hexane) to give di-protected propanediamine 103 as a clear oil (0.175 g, 10% yield).

Primary amine from di-protected propanediamine. Preparation of compound 67q. To a solution of di-protected propanediamine 103 (0.175 g, 0.533 mmol, 1.0 equiv) dissolved in 5.3 ml of methanol and 2.1 ml of water was added potassium carbonate (0.152 g, 1.10 mmol, 2.0 equiv) dropwise dissolved in 5.3 ml of water. The reaction was stirred for 48 hours at room temperature. Methanol was removed in vacuo and the resulting slurry was saturated with 20 ml of brine. The aqueous phase was extracted 3 times with 20 ml of dichloromethane and the organics were combined, dried over sodium sulfate, and concentrated in vacuo. The mono-protected propanediamine 67q was used without further purification (0.110 g, 89% yield).

Typical procedure for the S_NAr reaction of primary amines and nitro-chloro-chroman. Preparation of compounds 66b-n and 66p. To a solution of an isomeric mixture of nitro-chloro-chroman 65 (0.300 g, 1.10 mmol, 1.0 equiv) dissolved in 1.0 ml of 1-methyl-2-pyrrolidinone was added the appropriate primary amine 67 (5.50 mmol, 5.0 equiv) and the reaction was sealed tightly. The reaction stirred at 75 °C for 48 hours and once cooled poured into 40 ml of diethyl ether and 40 ml of water and stirred until the organic layer was clear. The organic phase was extracted 4 times with 20 ml of water, dried over sodium sulfate, and concentrated in vacuo. The crude reactions were purified via flash chromatography and alkylamino-chromans 66 were isolated in high yields based on the reactive isomer as bright yellow oils.

Typical procedure for the reduction of the aromatic nitro group and cyclization of the 2-aminobenzimidazole. Preparation of compounds 68b-m. A solution of alkylamino-chroman 66
(0.220 g, 0.476 mmol, 1.0 equiv) and 10% palladium on carbon (0.202 g, 0.190 mmol, 0.40 equiv) in 20 ml of anhydrous ethanol was stirred under an atmosphere of hydrogen gas for 3 hours. The reaction was filtered through celite using minimal amounts of anhydrous ethanol. The filtrate was returned to a round bottom under argon and cyanogen bromide (0.045 g, 0.428 mmol, 0.90 equiv) was added dissolved in 1.0 ml of anhydrous ethanol and the reaction was stirred for 24 hours. The solution was concentrated in vacuo and redissolved in 30 ml of dichloromethane and 30 ml of saturated sodium bicarbonate solution. The aqueous phase was extracted with an additional 30 ml of dichloromethane and the organics were combined, dried over sodium sulfate, and concentrated in vacuo. The crude reactions were purified via flash chromatography and 2-amino-benzimidazoles 68 were isolated as tan oils.

Typical procedure for the reduction of amides and boc-protecting groups to amines of 2-amino-benzimidazoles. Preparation of compounds 50b-m. To a solution of 2-amino-benzimidazole 68 (0.125 g, 0.273 mmol, 1.0 equiv) dissolved in 13 ml of freshly distilled THF was added lithium aluminum hydride (0.391 g, 10.3 mmol, 38 equiv) and the reaction was refluxed for 5 hours. Once cooled, the reaction was quenched with 391 µl of water, 391 µl of 15% NaOH solution, and 1.10 ml of water. The insoluble salts were filtered off though a pad of celite rinsing with freshly distilled THF. The solution was dried over sodium sulfate and concentrated in vacuo. The crude materials were purified by flash chromatography and 2-amino-benzimidazole final compounds 50 were isolated as oils in high yields.

5.3 Compounds Characterized

(S)-Phenylalaninol (40). $^1$H NMR (400 MHz, CDCl$_3$) δ ppm 7.17 - 7.37 (m, ArH, 5 H), 3.64 (dd, HCHOH, $J$=10.5, 4.0 Hz, 1 H), 3.38 (dd, HCHOH, $J$=10.5, 7.1 Hz, 1 H), 3.08 - 3.17 (m, CHNH$_2$ 1 H), 2.80 (dd, HCHC$_6$H$_5$, $J$=13.5, 5.3 Hz, 1 H), 2.54 (dd, HCHC$_6$H$_5$, $J$=13.5, 8.4 Hz, 1 H), 1.51 (br. s., NH$_2$ and OH, 3 H).
**4(S)-Benzyl-2-oxazolidinone (3).** $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.23 - 7.38 (m, ArH, 3 H), 7.15 - 7.23 (m, ArH, 2 H), 4.94 (br. s., NH, 1 H), 4.48 (t, HCHO, $J$=8.3 Hz, 1 H), 4.16 (dd, HCHO, $J$=8.6, 5.5 Hz, 1 H), 4.04 - 4.12 (m, HCNH, 1 H), 2.80 - 2.94 (m, CH$_2$C$_6$H$_5$, 2 H).

\[ \text{C}_{10} \text{H}_{11} \text{NO}_2 \]

**4(S)-4-benzyl-3-(4-methoxybenzyl)oxazolidin-2-one (41).** $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.12 - 7.35 (m, ArH, 5 H), 6.97 - 7.12 (m, ArH, 2 H), 6.84 - 6.97 (m, ArH, 2 H), 4.82 (dd, C$_6$H$_4$CHN, $J$=15.0, 2.2 Hz, 1 H), 3.94 - 4.16 (m, C$_6$H$_4$CHN and CH$_2$CH$_2$, 2 H), 3.82 (s, OCH$_3$, 3 H), 3.72 - 3.86 (m partially hidden, C$_6$H$_4$CH$_2$, 2 H), 3.11 (dt, CHCHO, $J$=13.9, 3.7 Hz, 1 H), 2.64 (ddd, CHCHO, $J$=12.5, 9.2, 3.3 Hz, 1 H).

\[ \text{C}_{18} \text{H}_{19} \text{NO}_3 \]

**4(S)-2-((4-methoxybenzyl)amino)-3-phenylpropan-1-ol (4).** $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.08 - 7.33 (m, ArH, 7 H), 6.79 - 6.87 (m, ArH, 2 H), 3.79 (s, OCH$_3$, 3 H), 3.71 (s, C$_6$H$_4$CHN, 2 H), 3.63 (dd,
CHCHO, J=10.6, 3.9 Hz, 1 H), 3.33 (dd, CHCHO, J=10.6, 5.3 Hz, 1 H), 2.91 - 3.00 (m, CH₂CHCH₂, 1 H), 2.70 - 2.85 (m, C₆H₅CH₂, 2 H), 1.51 (br. s., NH and OH, 2 H).

(4S)-4-benzyl-3-(4-methoxybenzyl)-1,2,3-oxathiazolidine 2-oxide (42). A mixture of cis and trans isomers. ¹H NMR (400 MHz, CDCl₃) δ ppm 7.19 - 7.36 (m, ArH, 10 H), 7.05 - 7.17 (m, ArH, 4 H), 6.85 - 6.95 (m, ArH, 4 H), 4.63 (m, C₆H₄CHN, 2 H), 4.26 - 4.35 (m, C₆H₄CHN, 2 H), 4.15 - 4.22 (m, CHCHO, 2 H), 4.10 - 4.15 (m, CHCHO, 2 H), 3.81 (s, OCH₃, 3 H), 3.81 (s, OCH₃, 3 H), 3.75 - 3.88 (m, C₆H₅CH₂O, 1 H), 3.53 - 3.62 (m, CHCH₂O, 1 H), 3.18 (dd, C₆H₅CH, J=13.4, 5.2 Hz, 1 H), 2.97 (dd, C₆H₅CH, J=13.6, 5.0 Hz, 1 H), 2.88 (dd, C₆H₅CH, J=13.5, 9.4 Hz, 1 H), 2.61 (dd, C₆H₅CH, J=13.5, 9.0 Hz, 1 H).

(S)-4-benzyl-3-(4-methoxybenzyl)-1,2,3-oxathiazolidine 2,2-dioxide (5). ¹H NMR (400 MHz, CDCl₃) δ ppm 7.21 - 7.33 (m, ArH, 5 H), 7.00 - 7.07 (m, ArH, 2 H), 6.84 - 6.93 (m, ArH, 2 H), 4.25 - 4.34 (m, C₆H₄CHN, 1 H), 4.15 - 4.25 (m, C₆H₄CHN and CHCH₂O, 3 H), 3.81 (s, OCH₃, 3 H), 3.69 - 3.78 (m, CH₂CHCH₂, 1 H), 3.01 (dd, C₆H₅CH, J=13.7, 5.7 Hz, 1 H), 2.72 (dd, C₆H₅CH, J=13.5, 9.2 Hz, 1 H).
diethyl ((5S)-5-benzyl-1-(4-methoxybenzyl)-2-oxopyrrolidin-3-yl)phosphonate (6). A mixture of diastereomers. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.07 - 7.32 (m, ArH, 12 H), 7.00 - 7.06 (m, ArH, 2 H), 6.80 - 6.91 (m, ArH, 4 H), 5.10 (d, C$_6$H$_4$C$_2$H$_N$, $J$=14.9 Hz, 1 H), 4.99 (d, C$_6$C$_4$CH$_N$, $J$=15.1 Hz, 1 H), 4.16 - 4.37 (m, OCH$_2$CH$_3$, 5 H), 4.04 (dd, C$_6$C$_4$CH$_N$, $J$=15.0, 2.6 Hz, 1 H), 3.96 (d, C$_6$C$_4$CH$_N$ $J$=14.9 Hz, 1 H), 3.81 (s, OCH$_3$, 3 H), 3.80 (s, OCH$_3$, 3 H), 3.78 - 3.82 (m, OCHCH$_3$, 1 H), 3.70 - 3.78 (m, CH$_2$C$_4$HN and OCH$_2$CH$_3$, 3 H), 3.61 - 3.65 (m, CH$_2$C$_4$HN, 1 H), 3.22 (dd, $J$=13.3, C$_6$H$_4$CH, 4.3 Hz, 1 H), 3.03 (dd, C$_6$C$_3$CH, $J$=13.5, 4.3 Hz, 1 H), 2.89 - 2.99 (m, CHCHCH$_3$, 1 H), 2.75 - 2.89 (m, COCHPO, 2 H), 2.68 (dd, C$_6$H$_4$CH, $J$=13.1, 10.2 Hz, 1 H), 2.52 (dd, C$_6$H$_4$CH, $J$=13.4, 8.5 Hz, 1 H), 2.17 - 2.33 (m, CHCHCH$_3$, 1 H), 2.05 - 2.16 (m, CHCHCH$_3$, 1 H), 1.93 - 2.03 (m, CHCHCH$_3$, 1 H), 1.28 - 1.43 (m, OCH$_2$CH$_3$, 12 H); LCMS: calc. for C$_{23}$H$_{30}$NO$_5$P, 431.2, found $m/z$ = 432.2 [M + H]$^+$. 

(S)-5-benzyl-3-cyclohexylidene-1-(4-methoxybenzyl)pyrrolidin-2-one (7). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.18 - 7.29 (m, ArH, 5 H), 7.05 (d, ArH, $J$=6.8 Hz, 2 H), 6.86 (d, ArH, $J$=8.6 Hz, 2 H), 5.09 (d, C$_6$H$_4$CH$_N$, $J$=14.9 Hz, 1 H), 4.01 (d, C$_6$H$_4$CH$_N$, $J$=15.1 Hz, 1 H), 3.81 (s, OCH$_3$, 3 H), 3.58 - 3.63 (m, CH$_2$CHCH$_2$C, 1 H), 3.05 (dd, C$_6$H$_4$CH$_2$, $J$=13.1, 3.9 Hz, 2 H), 2.45 - 2.53 (m, CH$_2$CHCH$_2$C, 2 H), 2.35 (d, $J$=16.6 Hz, CHCH$_2$CH$_2$, 1 H), 2.02 - 2.06 (m hidden, CH$_2$CH$_2$CH$_2$, 2 H), 1.52 - 1.58 (m, CH$_2$CHCH$_2$, 6 H), 1.26 (m hidden, CH$_2$CH$_2$CH$_2$, 1 H); LCMS: calc. for C$_{25}$H$_{26}$NO$_2$, 375.2, found $m/z$ = 376.3 [M + H]$^+$. 
(3R,4R)-1-benzyl-3,4-dihydroxypyrrolidine-2,5-dione (19). $^1$H NMR (400 MHz, DMSO) $\delta$ ppm 7.18 - 7.41 (m, ArH, 5 H), 6.20 - 6.34 (m, OH, 2 H), 4.56 (dd, C$_6$H$_5$CH$_2$N, $J$=21.9, 15.3 Hz, 2 H), 4.30 - 4.45 (m, HOCH, 2 H); LCMS: calc. for C$_{11}$H$_{11}$NO$_4$, 221.1, found m/z = 222.1 [M + H]$^+$. 

(3R,4R)-1-benzyl-3,4-bis((tert-butyldimethylsilyl)oxy)pyrrolidine-2,5-dione (43). $^1$H NMR (400 MHz, DMSO) $\delta$ ppm 7.22 - 7.38 (m, ArH, 5 H), 4.78 (s, SiOC$_3$H, 2 H), 4.59 (d, C$_6$H$_5$CH$_2$N, $J$=15.1 Hz, 1 H), 4.49 (d, C$_6$H$_5$CH$_2$N, $J$=14.9 Hz, 1 H), 0.89 - 0.95 (m, tert-butyl, 18 H), 0.18 (s, SiC$_3$H$_3$, 6 H), 0.13 (s, SiC$_3$H$_3$, 6 H).

(3R,4R,5R)-1,5-dibenzyl-3,4-bis((tert-butyldimethylsilyl)oxy)-5-hydroxypyrrolidin-2-one (20). $^1$H NMR (400 MHz, DMSO) $\delta$ ppm 7.12 - 7.34 (m, ArH, 10 H), 5.08 (d, OH, $J$=1.6 Hz, 1 H), 4.54 (d, C$_6$H$_5$CH$_2$N, $J$=15.8 Hz, 1 H), 4.34 (d, C$_6$H$_5$CH$_2$N, $J$=15.8 Hz, 1 H), 3.96 (dd, OC$_3$H$_3$, $J$=19.0, 1.4 Hz, 2 H), 2.99 (d, C$_6$H$_5$CH$_2$, $J$=13.9 Hz, 1 H), 2.71 (d, C$_6$H$_5$CH$_2$, $J$=13.8 Hz, 1 H), 0.91 - 0.97 (m, tert-butyl, 9 H),
0.71 - 0.78 (m, tert-butyl, 9 H), 0.15 - 0.22 (m, SiCH₃, 6 H), -0.18 - -0.13 (m, SiCH₃, 3 H), -0.26 - -0.21 (m, SiCH₃, 3 H); ¹³C NMR (151 MHz, CDCl₃) δ ppm 170.65, 138.64, 135.87, 131.14, 128.39, 127.97, 127.43, 126.96, 126.71, 90.93, 77.06, 74.69, 43.73, 42.18, 25.80, 25.50, 18.17, 17.88, -4.36, -4.96, -5.07, -5.75; LCMS: calc. for C₃₀H₴₇NO₄Si₂, 541.3, found m/z = 542.3 [M + H]^+.

(3R,4S)-1-benzyl-5-benzylidene-3,4-bis((tert-butyldimethylsilyl)oxy)pyrrolidin-2-one (45). ¹H NMR (600 MHz, DMSO) δ ppm 7.17 - 7.34 (m, ArH, 10 H), 6.06 (s, C₆C₅CHC, 1 H), 4.85 (dd, C₆H₅CH₂N, J=23.2, 16.4 Hz, 2 H), 4.79 (s, SiOCH, 1 H), 4.06 (s, SiOCH, 1 H), 0.86 - 0.89 (m, tert-butyl, 9 H), 0.74 - 0.78 (m, tert-butyl, 9 H), 0.17 - 0.20 (m, SiCH₃, 3 H), 0.12 - 0.15 (m, SiCH₃, 3 H), -0.04 - -0.01 (m, SiCH₃, 3 H), -0.15 - -0.12 (m, SiCH₃, 3 H).

(3aR,6R,6aR)-3a,4-dibenzyl-6-hydroxy-2,2-dimethyltetrahydro-5H-[1,3]dioxolo[4,5-b]pyrrol-5-one (47). ¹H NMR (400 MHz, DMSO) δ ppm 8.33 (t, OH, J=6.3 Hz, 1 H), 7.48 (dd, ArH, J=8.2, 1.2 Hz, 2 H), 7.17 - 7.33 (m, ArH, 7 H), 7.05 - 7.17 (m, ArH, 1 H), 5.86 (d, HOCHCHO, J=6.8 Hz, 1 H), 5.39 (d, C₆H₅CHN, J=1.4 Hz, 1 H), 5.19 (t, C₆H₅CHN, J=1.8 Hz, 1 H), 4.35 (d, HOCHCHO and C₆H₅CHC, J=6.3 Hz, 1 H), 3.86 (s, SiOC₂H₅, 1 H), 3.58 (s, tert-butyldimethylsilyl, 9 H).
Hz, 2 H), 4.27 (dd, C₆H₃CH₂, J=6.8, 2.0 Hz, 1 H), 1.60 (s, CCH₂, 3 H), 1.44 (s, CCH₂, 3 H); LCMS: calc. for C₂₁H₂₃NO₄, 353.2, found m/z = 354.1 [M + H]+.

dimethyl 2-(benzyloxy)fumarate (30). ¹H NMR (400 MHz, CDCl₃) δ ppm 7.27 - 7.50 (m, ArH, 5 H), 6.25 (s, CCH₂, 1 H), 5.19 (s, C₆H₅C₂H₂O, 2 H), 3.82 (s, CCH₂, 3 H), 3.74 (s, CH₃, 3 H).

(R)-malic acid (33). ¹H NMR (400 MHz, DMSO) δ ppm 12.34 (br. s., COOH, 2 H), 4.26 (dd, HOCH₂, J=7.8, 4.9 Hz, 1 H), 2.61 (dd, HOCH₂, J=15.7, 4.9 Hz, 1 H), 2.44 (dd, HOCH₂, J=15.7, 7.8 Hz, 1 H).
(R)-1-benzyl-3-hydroxypyrrolidine-2,5-dione (34). $^1$H NMR (400 MHz, DMSO) $\delta$ ppm 7.21 - 7.37 (m, ArH, 5 H), 6.12 (d, OH, $J$=6.7 Hz, 1 H), 4.52 - 4.61 (m, HOCH, 1 H), 4.55 (s, C$_6$H$_5$CH$_2$N, 2 H), 3.05 (dd, HOCHCH, $J$=17.7, 8.3 Hz, 1 H), 2.48 (dd, HOCHCH, $J$=17.8, 4.1 Hz, 1 H).

(3)-1-benzyl-3-hydroxy-4-(1-hydroxypropyl)pyrrolidine-2,5-dione (48r). A mixture of diastereomers A & B. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ ppm 7.22 - 7.36 (m, ArH of A & B, 10 H), 4.73 (d, HOCHCO of A, $J$=5.1 Hz, 1 H), 4.59 - 4.70 (m, C$_6$H$_5$CH$_2$N of A & B, 4 H), 4.42 (d, HOCHCO of B, $J$=5.7 Hz, 1 H), 4.28 (t, HOCHCHCO of A, $J$=6.3 Hz, 1 H), 4.88 (dd, HOCHCHCO of B, $J$=7.7, 12.9 Hz, 1 H), 3.47 (br.s., OH of B, 1 H), 3.37 (br. s., OH of A, 1 H), 2.98 (br.s., OH of B, 1 H), 2.83 (dd, HOCHCH$_2$ of A, $J$=5.4, 2.6 Hz, 1 H), 2.80 (dd partially hidden, HOCHCH$_2$ of B, 1 H), 2.13 (br. s., OH of A, 1 H), 1.70 - 1.81 (m, CH$_3$CH$_2$CH of B, 2 H), 1.63 - 1.70 (m, CH$_3$CH$_2$CH of A, 2 H), 1.02 (t partially hidden, CH$_3$CH$_2$ of B, 3 H), 1.00 (t, CH$_3$CH$_2$ of A, $J$=7.4 Hz, 3 H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ ppm 177.73, 177.02, 175.57, 175.44, 135.17, 135.03, 128.76, 128.69, 128.59, 128.33, 128.15, 127.99, 72.19, 70.07, 69.82, 67.31, 53.80, 53.11, 42.57, 42.51, 27.86, 27.62, 10.11, 9.73.

(3)-1-benzyl-3-((tert-butyldimethylsilyl)oxy)-4-(1-hydroxypropyl)pyrrolidine-2,5-dione (36r). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.24 - 7.37 (m, ArH, 5 H), 4.70 (d, SiOCH, $J$=4.7 Hz, 1 H), 4.66 (dd,
C\textsubscript{6}H\textsubscript{5}CH\textsubscript{2}N, \(J\)=26.2, 14.1 Hz, 2 H), 4.24 (tdd, HO\textsubscript{C}CH\textsubscript{2}, \(J\)=7.1, 7.1, 4.7, 2.4 Hz, 1 H), 2.80 (dd, HO\textsubscript{C}CH\textsubscript{2}, \(J\)=4.7, 2.3 Hz, 1 H), 1.86 (dd, OH, \(J\)=4.8, 0.5 Hz, 1 H), 1.58 - 1.68 (m, H\textsubscript{3}CCH\textsubscript{2}CH, 2 H), 1.00 (t, CH\textsubscript{2}CH\textsubscript{2}, \(J\)=7.4 Hz, 3 H), 0.89 (s, tert-butyl, 9 H), 0.23 (s, SiCH\textsubscript{3}, 3 H), 0.19 (s, SiCH\textsubscript{3}, 3 H).

![Chemical structure](image)

(3S)-1-benzyl-3-((tert-butyldimethylsilyl)oxy)-4-propionylpyrrolidine-2,5-dione (49S). \(^1\)H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) ppm 7.27 - 7.43 (m, ArH, 5 H), 5.06 (d, SiOCH\textsubscript{2}, \(J\)=4.9 Hz, 1 H), 4.62 (dd, C\textsubscript{6}H\textsubscript{5}CH\textsubscript{2}N, \(J\)=22.9, 14.3 Hz, 2 H), 3.81 (d, OCC\textsubscript{2}CO, \(J\)=4.9 Hz, 1 H), 2.95 - 3.12 (m, CH\textsubscript{3}CH\textsubscript{2}, 1 H), 2.55 - 2.70 (m, CH\textsubscript{2}CH\textsubscript{2}, 1 H), 1.13 (t, \(J\)=7.2 Hz, CH\textsubscript{3}CH\textsubscript{2}, 3 H), 0.88 (s, tert-butyl, 9 H), 0.19 (s, OSiCH\textsubscript{3}, 3 H), 0.11 (s, OSiCH\textsubscript{3}, 3 H).

![Chemical structure](image)

4-((tert-butoxycarbonyl)amino)cyclohexane-1-carboxylic acid (70). \(^1\)H NMR (400 MHz, DMSO) \(\delta\) ppm 12.00 (br. s., COOH, 1 H), 6.67 (br. s., NH, 1 H), 3.33 (br. s., NHCH\textsubscript{2}, 1 H), 2.31 - 2.40 (m, HO\textsubscript{2}CCH\textsubscript{2}, 1 H), 1.80 - 1.94 (m, HO\textsubscript{2}CCH\textsubscript{2}H\textsubscript{2}, 2 H), 1.39 - 1.59 (m, Cy, 6 H), 1.37 (s, tert-butyl, 9 H).
tert-butyl (4-(dimethylcarbamoylcyclohexyl)carbamate (71). \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) ppm 4.81 (br. s., NH, 1 H), 3.80 (br. s., NHCH, 1 H), 3.04 (s, NCH\(_3\), 3 H), 2.94 (s, NCH\(_3\), 3 H), 2.52 - 2.63 (m, NCOCH, 1 H), 1.80 - 1.91 (m, Cy, 2 H), 1.51 - 1.80 (m, Cy, 6 H), 1.44 (s, tert-butyl, 9 H).

4-amino-N,N-dimethylcyclohexane-1-carboxamide (67e). \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) ppm 3.00 - 3.11 (m, H\(_2\)NCH, 1 H), 3.04 (s, NCH\(_3\), 3 H), 2.93 (s, NCH\(_3\), 3 H), 2.50 - 2.65 (m, NCOCH, 1 H), 2.12 (br. s., NH\(_2\), 2 H), 1.78 - 1.99 (m, Cy, 2 H), 1.49 - 1.78 (m, Cy, 6 H).

tert-butyl (S)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (73). \( ^1H \) NMR (500 MHz, CDCl\(_3\)) \( \delta \) ppm 4.68 (br. s., OH, 1 H), 3.96 (br. s., NCH, 1 H), 3.53 - 3.69 (m, HOCH\(_2\)NCH, 1 H), 3.26 - 3.37 (m, HOCH\(_2\)NCH, 1 H), 1.94 - 2.03 (m, NCHCH, 1 H), 1.72 - 1.89 (m, NCHCHCH, 2 H), 1.55 (br. s., NCHCHCH, 1 H), 1.47 (s, tert-butyl, 9 H).
tert-butyl (S)-2-((tosyloxy)methyl)pyrrolidine-1-carboxylate (74).  $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.73 - 7.83 (m, ArH, 2 H), 7.29 - 7.40 (m, ArH, 2 H), 4.05 - 4.15 (m, OCH$_2$CH, 1 H), 3.82 - 4.05 (m, OCH$_2$, 2 H), 3.20 - 3.40 (m, NCH$_2$, 2 H), 2.44 (s, CH$_3$, 3 H), 1.86 - 2.03 (m, NCH$_2$CH, 2 H), 1.80 (t, NCH$_2$CH$_2$, $J=6.7$ Hz, 2 H), 1.38 (br. s., tert-butyl, 9 H).

tert-butyl (S)-2-(azidomethyl)pyrrolidine-1-carboxylate (75).  $^1$H NMR (599 MHz, CDCl$_3$) $\delta$ ppm 3.78 - 4.06 (m, N$_3$CH$_2$CH, 1 H), 3.22 - 3.67 (m, N$_3$CH and NCH$_2$CH$_2$, 4 H), 1.72 - 2.07 (m, NCH$_2$CH$_2$CH$_2$, 4 H), 1.47 (s, tert-butyl, 9 H).

tert-butyl (S)-2-((1,3-dioxoisindolin-2-yl)methyl)pyrrolidine-1-carboxylate (76).  $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.78 - 7.93 (m, ArH, 2 H), 7.55 - 7.79 (m, ArH, 2 H), 4.10 - 4.42 (m, NCH$_2$CH, 1 H), 3.75 - 3.90 (m, NCHCH, 1 H), 3.57 - 3.73 (m, NCHCH, 1 H), 3.27 - 3.52 (m, NCH$_2$CH, 2 H), 1.69 - 2.15 (m, NCH$_2$CH$_2$CH$_2$, 4 H), 1.16 - 1.41 (m, tert-butyl, 9 H).
tert-butyl (S)-2-(aminomethyl)pyrrolidine-1-carboxylate (67l). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 3.56 - 4.10 (m, NCHCH$_2$CH$_3$, 1 H), 3.18 - 3.55 (m, NCHCH$_2$CH$_2$ and H$_2$NCH$_2$CH$_2$N, 2 H), 2.75 - 2.94 (m, NH$_2$CHCH, 1 H), 2.67 (dd, NH$_2$CHCH, $J$=12.8, 6.9 Hz, 1 H), 1.67 - 2.10 (m, NCH$_2$CH$_2$CH$_2$, 4 H), 1.45 (s, tert-butyl, 9 H), 1.22 (br. s., NH$_2$, 2 H).

tert-butyl 4-((3-(dimethylcarbamoyl)-6-nitrochroman-5-yl)amino)piperidine-1-carboxylate (66b).

Obtained from the SNAr reaction of chroman 65 and primary amine 67b. The crude material was purified by flash chromatography (silica gel, 80% ethyl acetate/hexane) and the product was provided in 32% yield (based on the reactive isomer) as a bright yellow oil. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ ppm 7.99 (d, Ar$H$, $J$=9.4 Hz, 1 H), 7.24 (t partially hidden, NH, $J$=9.7 Hz, 1 H), 6.46 (d, Ar$H$, $J$=9.4 Hz, 1 H), 4.43 (dt, OCH, $J$=10.9, 3.1 Hz, 1 H), 4.13 (t, OCH, $J$=10.8 Hz, 1 H), 3.99 - 4.10 (m, BocNCH, 1 H), 3.78 - 3.88 (m, BocNCH, 1 H), 3.37 - 3.45 (m, OCH$_2$CHCH$_2$, 1 H), 3.14 (s, NCH$_3$, 3 H), 3.04 (s, NCH$_3$, 3 H), 2.98 - 3.03 (m, NHCHCH$_2$, 1 H), 2.88 - 2.95 (m, OCH$_2$CHCH$_2$, 1 H), 2.77 - 2.86 (m, OCH$_2$CHCH and BocNCH, 2 H), 2.04 - 2.10 (m, BocNCH, 1 H), 1.65 (d, NHCHCHCH$_2$, $J$=11.4 Hz, 1 H), 1.53 - 1.61 (m, NHCHCHCH$_2$, 1 H), 1.47 - 1.51 (m, NHCHCHCH$_2$, 1 H), 1.46 (s, tert-butyl, 9 H), 1.16 - 1.24 (m, NHCHCHCH$_2$, 1 H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ ppm 181.69, 171.33, 159.59, 154.56, 146.47, 126.37, 110.21, 79.72, 67.43, 60.38, 37.34, 35.79, 35.17, 28.43, 27.38, 21.04, 14.22.
tert-butyl 4-(2-amino-8-(dimethylcarbamoyl)-8,9-dihydrochromeno[5,6-d]imidazol-1(7H)-yl)piperidine-1-carboxylate (68b). Obtained from the hydrogenation and cyclization of chroman 66b with cyanogen bromide. The crude material was purified by flash chromatography (silica gel, 7% methanol/dichloromethane) and the product was provided in 44% yield as a tan oil. 1H NMR (599 MHz, CDCl₃) δ ppm 7.19 (d, J=8.5 Hz, ArH, 1 H), 6.71 (d, ArH, J=8.5 Hz, 1 H), 4.76 (ddd, NC₃H₂CH₂, J=16.2, 12.5, 4.3 Hz, 1 H), 4.58 (br. s., NH₂, 2 H), 4.36 (ddd, OCHCH, J=10.7, 3.1, 2.3 Hz, 1 H), 3.95 (t, OCHCH, J=10.7 Hz, 1 H), 3.62 (dd, OCH₂CHCH, J=3.3 Hz, 1 H), 3.17 (s, NCH₃, 3 H), 3.04 (s, NCH₃, 3 H), 2.98 (ddd, OCH₂CHCH, J=15.1, 5.3, 1.8 Hz, 1 H), 2.80 - 2.90 (m, BocNCH₂CH₂, 1 H), 2.33 (qd, BocNCH₂CH₂, J=12.6, 4.8 Hz, 1 H), 2.09 - 2.17 (m, BocNCH₂CH₂, 1 H), 2.04 - 2.09 (m, BocNCH₂CH₂, 1 H), 1.87 - 1.94 (m, NCH₃CH₂CH₂, 1 H), 1.84 (d, NCH₂CH₂ and NCH₂CH₂, J=11.3 Hz, 3 H), 1.49 (s, tert-butyl, 9 H); LCMS: calc. for C₂₃H₃₃N₅O₄, 443.3, found m/z = 444.3 [M + H]+.

8-((dimethylamino)methyl)-1-(1-methylpiperidin-4-yl)-1,7,8,9-tetrahydrochromeno[5,6-d]imidazol-2-amine (50b). Obtained from the reduction of amino-benzimidazole 68b with LAH. The crude material
was purified by flash chromatography (rediSep gold high performance amine column, 20% methanol/ethyl aceate) and the product was provided in 50% yield as a tan oil. $^1$H NMR (500 MHz, CDCl$_3$) δ ppm 7.14 (d, ArH, $J$=8.3 Hz, 1 H), 6.66 (d, ArH, $J$=8.5 Hz, 1 H), 4.64 (br. m., NH$_2$ and OCHCH, 3 H), 4.28 (d, $J$=9.2 Hz, NCH$_2$CH$_2$, 1 H), 3.85 (dd, OCHCH, $J$=10.6, 7.3 Hz, 1 H), 3.11 (dd, OCH$_2$CHCH, $J$=16.1, 5.1 Hz, 1 H), 3.01 - 3.08 (m, NCH$_2$CH$_2$, 2 H), 2.76 (dd, OCH$_2$CHCH, $J$=15.3, 7.6 Hz, 1 H), 2.47 (dd, NCHCH$_2$, $J$=12.6, 3.9 Hz, 1 H), 2.36 - 2.42 (2m, NCH$_2$CH$_2$ and OCH$_2$CH, 2 H), 2.35 (s, NCH$_3$, 3 H), 2.22 - 2.29 (m, (CH$_3$)$_2$NCH$_2$ and NCHCH, 3 H), 2.26 (s, N(CH$_3$)$_2$, 6 H), 2.10 (m, NCHCH, $J$=12.0, 12.0 Hz, 1 H), 1.99 (br. d, NCHCH, $J$=11.4 Hz, 1 H), 1.91 (br. d, NCHCH, $J$=11.0 Hz, 1 H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ ppm 152.54, 149.73, 135.28, 132.72, 115.41, 111.53, 104.06, 77.22, 68.18, 61.61, 55.68, 55.66, 53.54, 46.05, 46.03, 30.73, 30.64, 30.08, 27.30; LCMS: calc. for C$_{19}$H$_{29}$N$_5$O, 343.2, found m/z = 344.4 [M + H]$^+$. 

![Image](image)

tert-butyl 4-(((3-(dimethylcarbamoyl)-6-nitrochroman-5-yl)amino)methyl)piperidine-1-carboxylate (66b). Obtained from the SNAr reaction of chroman 65 and primary amine 67c. The crude material was purified by flash chromatography (silica gel, 80% ethyl acetate/hexane) and the product was provided in 71% yield (based on the reactive isomer) as a bright yellow oil. $^1$H NMR (600 MHz, CDCl$_3$) δ ppm 8.00 (d, $J$=9.4 Hz, ArH, 1 H), 7.74 (br. s., NH, 1 H), 6.38 (d, ArH, $J$=9.4 Hz, 1 H), 4.42 (dt, OCHCHCH$_2$, $J$=10.9, 3.0 Hz, 1 H), 4.12 (t, OCHCHCH$_2$ and BocNCH$_2$, $J$=10.6 Hz, 3 H), 3.28 (dt, BocNCH, $J$=12.1, 6.3 Hz, 1 H), 3.12 (s, NCH$_3$, 3 H), 2.95 - 3.08 (m, BocNCH and OCH$_2$CHCH$_2$ and OCH$_2$CHCH, 3 H), 3.03 (s, NCH$_3$, 3 H), 2.77 (dt, NHCHCH, $J$=14.2, 2.9 Hz, 1 H), 2.67 (t, OCH$_2$CHCH and NHCHCH, $J$=12.6 Hz, 2 H), 1.77 (d, NHCH$_2$CHCH$_2$, $J$=14.1 Hz, 1 H), 1.61 - 1.70 (m, BocNCH$_2$CH$_2$, 2 H), 1.45 (s, tert-butyl, 9 H),
1.06 - 1.22 (m, BocNCH$_2$CH$_2$, 2 H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ ppm 181.70, 171.38, 160.09, 154.74, 149.08, 131.48, 126.55, 110.96, 109.33, 79.47, 67.38, 54.15, 37.95, 37.31, 35.76, 35.38, 30.02, 28.47, 28.03.

tert-butyl 4-((2-amino-8-(dimethylcarbamoyl)-8,9-dihydrochromeno[5,6-d]imidazol-1(7H)-
yl)methyl)piperidine-1-carboxylate (68c). Obtained from the hydrogenation and cyclization of chroman 66c with cyanogen bromide. The crude material was purified by flash chromatography (silica gel, 7% methanol/dichloromethane) and the product was provided in 40% yield as a tan oil. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ ppm 7.18 (d, $J$=8.5 Hz, ArH, 1 H), 6.70 (d, ArH, $J$=8.5 Hz, 1 H), 4.67 (br. s, BocNCH$_2$CH$_2$, 1 H), 4.33 - 4.40 (m, OCHCH$_2$CH, 1 H), 4.12 (br. s., NH$_2$, 2 H), 3.86 - 4.00 (m, OCHCH$_2$CH, 1 H), 3.95 (t, NCH$_2$CH, $J$=10.6 Hz, 2 H), 3.44 (dd, OCH$_2$CHCH, $J$=15.4, 11.0 Hz, 1 H), 3.22 - 3.30 (m, OCH$_2$CHCH, 1 H), 3.18 (s, NCH$_3$, 3 H), 3.04 - 3.09 (m, OCH$_2$CHCH, 1 H), 3.03 (s, NCH$_3$, 3 H), 2.52 - 2.69 (m, BocNCH$_2$, 2 H), 2.19 (br. s, BocNCH$_2$CH, 1 H), 1.92 - 2.02 (m, NCH$_2$CHCH, 1 H), 1.63 (d, BocNCH$_2$CHCH, $J$=12.5 Hz, 1 H), 1.49 - 1.56 (m, BocNCH$_2$CHCH, 1 H), 1.45 (s, tert-butyl, 9 H), 1.19 - 1.31 (m, BocNCH$_2$CH, 2 H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ ppm 181.70, 171.51, 154.67, 153.18, 149.21, 131.92, 115.53, 111.60, 104.30, 79.67, 66.56, 49.84, 38.31, 37.27, 35.91, 35.86, 29.66, 28.46, 24.76.
8-((dimethylamino)methyl)-1-((1-methylpiperidin-4-yl)methyl)-1,7,8,9-tetrahydrochromeno[5,6-d]imidazol-2-amine (50c). Obtained from the reduction of amino-benzimidazole 68c with LAH. The crude material was purified by flash chromatography (rediSep gold high performance amine column, 20% methanol/ethyl acetate) and the product was provided in 95% yield as a tan oil. $^1$H NMR (600 MHz, CDCl$_3$) δ ppm 7.17 (d, Ar$H$, $J$=8.5 Hz, 1 H), 6.67 (d, Ar$H$, $J$=8.7 Hz, 1 H), 4.46 (br. s., NH$_2$, 2 H), 4.25 (dt, OCHCHCH$_2$, $J$=10.5, 1.4 Hz, 1 H), 3.94 (d, NCH$_2$CH, $J$=6.5 Hz, 2 H), 3.85 (dd, OCHCH$_2$, $J$=10.6, 7.2 Hz, 1 H), 3.13 (dd, OCH$_2$CHCH$_2$, $J$=15.6, 5.5 Hz, 1 H), 2.85 - 2.90 (m, CH$_3$NCH$_2$CH$_2$, 2 H), 2.78 (dd, OCH$_2$CHCH$_2$, $J$=15.6, 7.3 Hz, 1 H), 2.29 - 2.33 (m, OCH$_2$CHCH$_2$ and (CH$_3$)$_2$NCHCH, 2 H), 2.27 (s, NCH$_3$, 3 H), 2.25 (s, N(CH$_3$)$_2$, 6 H), 1.84 - 1.90 (m, (CH$_3$)$_2$NCHCH and CH$_3$NCHCH$_2$, 2 H), 1.78 - 1.84 (m, NCH$_2$CHCH$_2$, 1 H), 1.61 - 1.67 (m, CH$_3$NCHCH$_2$ and CH$_3$NCH$_2$CH, 2 H), 1.47 (m, CH$_3$NCH$_2$CH, $J$=3.8 Hz, 1 H), 1.40 - 1.45 (m, CH$_3$NCH$_2$CH$_2$, 2 H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ ppm 152.82, 149.62, 115.63, 111.49, 104.25, 77.22, 68.46, 61.49, 55.36, 55.32, 49.95, 46.30, 46.00, 37.64, 30.47, 30.07, 30.02, 25.81; LCMS: calc. for C$_{20}$H$_{31}$N$_5$O, 357.3, found m/z = 358.3 [M + H]$^+$. 

![Chemical Structure](image)
tert-butyl 2-(((3-(dimethylcarbamoyl)-6-nitrochroman-5-yl)amino)methyl)piperidine-1-carboxylate (66d). A mixture of diastereomers A & B. Obtained from the S_NAr reaction of chroman 65 and primary amine 67d. The crude material was purified by flash chromatography (silica gel, 70% ethyl acetate/hexane) and the product was provided in 69% yield (based on the reactive isomer) as a bright yellow oil. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) ppm 7.97 (d, \(J=9.5\) Hz, ArH of A & B, 2 H), 7.41 (br. s., NH of A, 1 H), 7.30 (br. s., NH of B, 1 H), 6.32 - 6.44 (m, ArH of A & B, 2 H), 4.39 - 4.46 (m, OCHCH\(_2\) of A & B, 2 H), 4.36 (br. s., BocNCH\(_2\) of A, 1 H), 4.14 (td, OCHCH\(_2\) of A & B, \(J=10.6, 5.6\) Hz, 2 H), 3.92 - 4.08 (m, BocNCH\(_2\) of A & B, 2 H), 3.63 (br. t., Boc NCH\(_2\) of B, \(J=9.6\) Hz, 1 H), 3.36 - 3.44 (m, BocNCH\(_2\)N of A, 1 H), 3.29 (t, BocNCH\(_2\)N of B, \(J=9.8\) Hz, 1 H), 3.13 (d, NCH\(_3\) of A & B, \(J=5.9\) Hz, 6 H), 2.92 - 3.06 (2m, NHCH\(_2\) of A and OCH\(_2\)CH of B, 3 H), 3.02 (s, NCH\(_3\) of A & B, 6 H), 2.75 - 2.89 (2m, NHCH\(_2\) of B and OCH\(_2\)CH of A, 3 H), 2.63 (t, OCH\(_2\)CHCH of A, \(J=13.0\) Hz, 1 H), 1.52 - 1.71 (3m, OCH\(_2\)CHCH\(_2\) of B and OCH\(_2\)CHCH of A and Cy, 10 H), 1.36 - 1.52 (m, tert-butyl of A and Cy, 14 H), 1.44 (s, tert-butyl of B, 9 H); \(^1\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) ppm 171.46, 171.43, 159.71, 159.70, 155.24, 148.25, 148.12, 135.83, 126.50, 125.51, 111.81, 109.43, 79.91, 79.87, 77.23, 67.41, 67.35, 47.05, 46.97, 37.34, 37.32, 35.72, 35.70, 35.33, 35.30, 30.35, 28.43, 28.37, 27.73, 27.69, 26.31, 25.24, 25.22, 19.46.

tert-butyl 2-((2-amino-8-(dimethylcarbamoyl)-8,9-dihydrochromeno[5,6-d]imidazol-1(7H)-yl)methyl)piperidine-1-carboxylate (68d). A mixture of diastereomers. Obtained from the hydrogenation and cyclization of chroman 66d with cyanogen bromide. The crude material was purified
by flash chromatography (silica gel, 12% methanol/ethyl acetate) and the product was provided in 55% yield as a tan oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.17 (d, ArH, $J$=8.6 Hz, 1 H), 6.66 (dd, ArH, $J$=8.4, 2.0 Hz, 1 H), 5.60 (br. s., NH$_2$, 2 H), 4.50 (br. s., NCHCH, 1 H), 4.31 - 4.38 (m, OCHCHCH$_2$, 1 H), 4.07 (br. s., NCHCH, 1 H), 3.45 - 3.57 (2m, OCHCHCH$_2$ and BocNCHCH$_2$, 2 H), 3.20 - 3.32 (m, OCH$_2$CHCH$_2$ and BocNCHCH$_2$, 2 H), 3.18 (s, NCH$_3$, 3 H), 3.13 (ddd, OCH$_2$CHCH, $J$=14.7, 5.0, 1.6 Hz, 1 H), 3.03 (s, NCH$_3$, 3 H), 2.81 - 2.95 (2m, OCH$_2$CHCH and NCH$_2$CH, 2 H), 1.66 - 1.95 (m, Cy, 6 H), 1.39 (br. s., tert-butyl, 9 H).

![Chemical Structure](image)

8-((dimethylamino)methyl)-1-((1-methylpiperidin-2-y1)methyl)-1,7,8,9-tetrahydrochromeno[5,6-d]imidazol-2-amine (50d). A mixture of diastereomers. Obtained from the reduction of amino-benzimidazole 68d with LAH. The crude material was purified by flash chromatography (rediSep gold high performance amine column, 10% methanol/ethyl acetate) and the product was provided in 85% yield as a tan oil. $^1$H NMR and $^{13}$C NMR is complex mixture of racemic cis and trans diastereomers. However, there is literature precedence for testing diastereomers.$^{68,102}$ LCMS: calc. for C$_{20}$H$_{31}$N$_5$O, 357.3, found m/z = 358.2 [M + H]$^+$. 
5-((4-(dimethylcarbamoyl)cyclohexyl)amino)-N,N-dimethyl-6-nitrochromane-3-carboxamide (66e). Obtained from the $S_N$Ar reaction of chroman 65 and primary amine 67e. The crude material was purified by flash chromatography (silica gel, 10% methanol/ethyl acetate) and the product was provided in 90% yield (based on the reactive isomer) as a bright yellow oil. $^1$H NMR (599 MHz, CDCl$_3$) $\delta$ ppm 7.97 (d, $\text{Ar}_H$, $J=9.4$ Hz, 1 H), 7.65 (br. s., NH, 1 H), 6.38 (d, $\text{Ar}_H$, $J=9.4$ Hz, 1 H), 4.41 (dt, OC$_H$CH$_2$, $J=10.8$, 3.1 Hz, 1 H), 4.11 (t, OCH$_2$CH$_2$, $J=10.8$ Hz, 1 H), 3.59 - 3.64 (m, OCH$_2$CH$_2$, 1 H), 3.14 (s, NCH$_3$, 3 H), 3.02 (s, NCH$_3$, 3 H), 2.99 - 3.07 (m, NHCH$_2$CH$_2$, 1 H), 3.03 (s, NCH$_3$, 3 H), 2.88 - 2.97 (m, OCH$_2$CHCH, 1 H), 2.93 (s, NCH$_3$, 3 H), 2.77 - 2.83 (m, OCH$_2$CHCH, 1 H), 2.54 - 2.61 (m, NHCH$_2$CH$_2$CH, 1 H), 2.03 - 2.07 (m, Cy, 1 H), 1.95 - 2.03 (m, Cy, 1 H), 1.75 - 1.82 (m, Cy, 1 H), 1.55 - 1.72 (m, Cy, 3 H), 1.45 - 1.54 (m, Cy, 2 H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ ppm 175.01, 171.64, 159.45, 147.22, 126.36, 112.32, 109.34, 67.29, 52.14, 38.35, 37.40, 37.21, 35.78, 35.64, 35.33, 30.64, 29.93, 27.73, 24.37, 24.19.

2-amino-1-(4-(dimethylcarbamoyl)cyclohexyl)-N,N-dimethyl-1,7,8,9-tetrahydrochromeno[5,6-d]imidazole-8-carboxamide (68e). Obtained from the hydrogenation and cyclization of chroman 66e with
cyanogen bromide. The crude material was purified by flash chromatography (silica gel, 25% methanol/ethyl acetate) and the product was provided in 25% yield as a tan oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.13 (d, $ArH$, $J$=8.4 Hz, 1 H), 6.66 (d, $ArH$, $J$=8.8 Hz, 1 H), 4.75 (br. s, $NH_2$, 2 H), 4.34 (dt, OCH$_2$CH$_2$, $J$=10.4, 2.9 Hz, 1 H), 3.93 (t, OCH$_2$CH$_2$, $J$=10.8 Hz, 1 H), 3.59 (dd, OCH$_2$CHCH$_3$, $J$=15.7, 11.3 Hz, 1 H), 3.19 - 3.28 (m, NCH$_2$CH$_2$CH$_2$, 1 H), 3.18 (s, NCH$_3$, 3 H), 3.08 (s, NCH$_3$, 3 H), 3.04 (s, NCH$_3$, 3 H), 2.93 - 3.01 (m, OCH$_2$CHCH$_3$, 1 H), 2.96 (s, NCH$_3$, 3 H), 2.65 - 2.83 (m, OCH$_2$CHCH$_2$ and NCH$_2$CH$_2$CH and Cy, 3 H), 1.92 - 2.03 (m, Cy, 2 H), 1.80 - 1.92 (m, Cy, 1 H), 1.59 - 1.80 (m, Cy, 4 H).

8-((dimethylamino)methyl)-1-(4-((dimethylamino)methyl)cyclohexyl)-1,7,8,9-tetrahydrochromeno[5,6-d]imidazol-2-amine (50e). Obtained from the reduction of amino-benzimidazole 68e with LAH. The crude material was purified by flash chromatography (rediSep gold high performance amine column, 20% methanol/ethyl acetate) and the product was provided in 90% yield as a tan oil. $^1$H NMR (599 MHz, CDCl$_3$) $\delta$ ppm 7.15 (d, $ArH$, $J$=8.7 Hz, 1 H), 6.66 (d, $ArH$, $J$=8.7 Hz, 1 H), 4.31 (br. s, $NH_2$, 2 H), 4.27 - 4.30 (m, OCH$_2$CH$_2$, 1 H), 3.82 - 3.88 (m, OCH$_2$CH$_2$ and NCH$_2$CH$_2$, 2 H), 3.13 (dd, OCH$_2$CHCH$_3$, $J$=15.5, 5.1 Hz, 1 H), 2.77 (dd, OCH$_2$CHCH$_3$, $J$=15.3, 7.6 Hz, 1 H), 2.31 - 2.39 (m, OCH$_2$CHCH$_2$ and OCH$_2$CHCH$_2$N, 3 H), 2.24 (s, all NCH$_3$, 12 H), 2.07 - 2.22 (m, NCH$_2$CHCH$_2$CH$_2$, 2 H), 1.90 - 2.00 (m, Cy, 3 H), 1.87 (d, Cy, $J$=12.6 Hz, 2 H), 1.75 - 1.82 (m, NCH$_2$CHCH$_2$ and Cy, 2 H), 1.58 - 1.67 (m, Cy, 2 H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ ppm 181.16, 167.49, 115.59, 111.44, 108.81, 104.68, 96.40, 93.90, 69.11, 68.19, 61.63, 45.99, 45.96, 30.63, 29.14, 28.07, 13.31, 1.66; ) MS: calc. for C$_{20}$H$_{31}$N$_5$O, 385.3, found m/z = 386.3 [M + H]$^+$. 
N,N-dimethyl-6-nitro-5-((2-(pyrrolidin-1-yl)ethyl)amino)chroman-3-carboxamide (66f). Obtained from the S_NAr reaction of chroman 65 and primary amine 67f. The crude material was purified by flash chromatography (silica gel, 15% methanol/ethyl acetate) and the product was provided in 96% yield (based on the reactive isomer) as a bright yellow oil. \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) ppm 7.96 (d, \(J = 9.4\) Hz, ArH, 1 H), 7.84 (br. s., NH, 1 H), 6.36 (d, \(J = 9.4\) Hz, ArH, 1 H), 4.46 – 4.37 (m, OC\(_2\)H\(_2\), 1 H), 4.16 – 4.07 (m, OC\(_2\)H\(_2\), 1 H), 3.52 (m, CHC\(_2\)H\(_2\), 1 H), 3.17 (m, CHCH\(_3\), 1 H), 3.13 (s, NCH\(_3\), 3 H), 3.07 – 2.96 (m and s, partial overlap, NCH\(_2\), HNC\(_2\)H\(_2\) and CH, 5 H), 2.87 – 2.77 (m, HNC\(_2\)H\(_2\), 1 H), 2.69 – 2.61 (m, NCH\(_2\), 2 H), 2.59 – 2.51 (m, NCH\(_2\), 2 H), 2.51 – 2.45 (m, NCH\(_2\), 2 H), 1.83 – 1.72 (m, N(CH\(_2\))\(_2\)(CH\(_2\))\(_2\), 4 H); \(^1\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) ppm 171.53, 159.69, 148.86, 132.00, 112.20, 108.93, 67.35, 55.74, 53.81, 47.05, 37.31, 35.72, 35.36, 27.77, 23.64.

2-amino-N,N-dimethyl-1-(2-(pyrrolidin-1-yl)ethyl)-1,7,8,9-tetrahydrochromeno[5,6-d]imidazole-8-carboxamide (68f). Obtained from the hydrogenation and cyclization of chroman 66f with cyanogen bromide. The crude material was purified by flash chromatography (basic alumina, 5% methanol/ethyl acetate) and the product was provided in 25% yield as a tan oil. \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) ppm 7.18 (d, \(J = 8.7\) Hz, ArH, 1 H), 6.66 (d, \(J = 8.7\) Hz, ArH, 1 H), 5.66 (br. s., NH\(_2\), 2 H), 4.37 – 4.30 (2m, OCH\(_2\) and ArNCH\(_2\), 2 H), 4.13 – 4.08 (m, ArNCH\(_2\), 1 H), 3.94 (dd, \(J = 10.7, 10.7\) Hz, OCH\(_2\), 1 H), 3.52 (dd, \(J =
15.4, 11.3 Hz, ArCH$_2$, 1 H), 3.28 – 3.22 (m, CHCO, 1 H), 3.17 (s, NCH$_3$, 3 H), 3.04 (s, NCH$_3$, 3 H), 3.03 – 2.94 (2m, partial overlap, ArCH$_2$ and NCH$_2$, 2 H), 2.90 – 2.85 (m, NCH$_2$, 1 H), 2.72 – 2.67 (m, N(CH$_2$)$_2$, 2 H), 2.58 – 2.53 (m, N(CH$_2$)$_2$, 2 H), 1.81 - 1.76 (m, N(CH$_2$)$_2$(CH$_2$)$_2$, 4 H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ ppm 171.78, 155.52, 148.61, 136.46, 132.61, 115.50, 110.73, 103.65, 66.64, 58.30, 54.80, 44.22, 37.28, 36.03, 35.80, 25.29, 23.72.

8-((dimethylamino)methyl)-1-(2-(pyrrolidin-1-yl)ethyl)-1,7,8,9-tetrahydrochromeno[5,6-d]imidazol-2-amine (50f). Obtained from the reduction of amino-benzimidazole 68f with LAH. The crude material was purified by flash chromatography (rediSep gold high performance amine column, 20% methanol/ethyl acetate) and the product was provided in 52% yield as a tan oil. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ ppm 7.16 (d, J = 8.5 Hz, ArH, 1 H), 6.64 (d, J = 8.5 Hz, ArH, 1 H), 5.46 (br. s., NH$_2$, 2 H), 4.30 – 4.25 (ddd, J = 10.2, 2.8, 1.4 Hz, OCH$_2$, 1 H), 4.28 – 4.24 (m, ArNCH$_2$, 1 H), 4.21 – 4.15 (m, ArNCH$_2$, 1 H), 3.85 – 3.81 (dd, OCH$_2$, J=10.2, 7.4 Hz, 1 H), 3.10 – 3.05 (dd, J = 15.4, 5.1 Hz, ArCH$_2$, 1 H), 3.00 – 2.95 (ddd, J = 13.2, 7.4, 3.0 Hz, NCH$_2$CH$_3$, 1 H), 2.94 – 2.89 (ddd, J = 13.2, 7.4, 3.0 Hz, NCH$_2$CH$_3$, 1 H), 2.77 – 2.72 (dd, J = 15.4, 7.4 Hz, ArCH$_2$, 1 H), 2.69 – 2.64 (m, N(CH$_2$)$_2$, 2 H), 2.64 – 2.59 (m, N(CH$_2$)$_2$, 2 H), 2.35 - 2.20 (m and s, CH$_2$CH, CH$_2$NMe$_2$, N(CH$_3$)$_2$, 9 H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ ppm 155.04, 149.33, 115.37, 110.93, 103.83, 77.22, 68.45, 62.82, 61.72, 58.07, 54.79, 46.01, 44.13, 30.60, 29.87, 26.50, 23.72; LCMS calc. for C$_{19}$H$_{29}$N$_5$O, 343.5, found m/z = 344.4 [M + H]$^+$. 
N,N-dimethyl-6-nitro-5-((3-(pyrrolidin-1-yl)propyl)amino)chromane-3-carboxamide (66g). Obtained from the SNAr reaction of chroman 65 and primary amine 67g. The crude material was purified by flash chromatography (silica gel, 25% methanol/ethyl acetate) and the product was provided in 94% yield (based on the reactive isomer) as a bright yellow oil. $^1$H NMR (600 MHz, CDCl$_3$) δ ppm 7.97 (d, $J = 9.5$ Hz, ArH, 1 H), 7.72 (br. s., NH, 1 H), 6.35 (d, $J = 9.5$ Hz, ArH, 1 H), 4.43 – 4.38 (m, OCH$_2$, 1 H), 4.15 – 4.09 (m, OCH$_2$, 1 H), 3.47 – 3.40 (m, NHCH$_3$, 1 H), 3.24 – 3.18 (m, NHCH$_3$, 1 H), 3.12 (s, NCH$_3$, 3 H), 3.05 – 2.95 (s and m, partial overlap, NCH$_3$, CHCO, CHCH$_2$, 5 H), 2.83 – 2.75 (m, CHCH$_2$, 1 H), 2.56 – 2.45 (m, N(CH$_2$)$_2$, NCH$_3$, 6 H), 1.83 – 1.73 (m, N(CH$_2$)$_2$(CH$_2$)$_2$, CH$_2$CH$_2$CH$_2$, 6 H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ ppm 171.50, 159.85, 148.77, 131.61, 126.45, 110.98, 109.02, 67.36, 54.20, 53.80, 46.64, 37.32, 35.71, 35.35, 30.01, 27.80, 23.47.

2-amino-N,N-dimethyl-1-(3-(pyrrolidin-1-yl)propyl)-1,7,8,9-tetrahydrochromeno[5,6-d]imidazole-8-carboxamide (68g). Obtained from the hydrogenation and cyclization of chroman 66g with cyanogen bromide. The crude material was purified by flash chromatography (basic alumina, 20% methanol/ethyl acetate) and the product was provided in 50% yield as a tan oil.
8-((dimethylamino)methyl)-1-(3-(pyrrolidin-1-yl)propyl)-1,7,8,9-tetrahydrochromeno[5,6-d]imidazol-2-amine (50g). Obtained from the reduction of amino-benzimidazole 68g with LAH. The crude material was purified by flash chromatography (rediSep gold high performance amine column, 20% methanol/ethyl acetate) and the product was provided in 86% yield as a tan solid. \( ^1H \) NMR (600 MHz, CDCl\(_3\)) \( \delta \) ppm 7.14 (d, \( J = 8.5 \) Hz, ArH, 1 H), 6.63 (d, \( J = 8.5 \) Hz, ArH, 1 H), 6.30 (br. s., NH\(_2\), 2H), 4.28 – 4.23 (ddd, \( J = 10.7, 2.7, 1.0 \) Hz, OCH\(_2\), 1 H), 4.23 - 4.12 (m, ArNC\(_2\)H, 2 H), 3.86 – 3.80 (dd, \( J = 10.7, 7.2 \) Hz, OCH\(_2\), 1 H), 3.25 – 3.17 (dd, \( J = 15.8, 4.8 \) Hz, ArCH\(_2\), 1 H), 2.86 – 2.77 (dd, \( J = 15.8, 7.2 \) Hz, ArCH\(_2\), 1 H), 2.58 – 2.40 (2m, N(CH\(_2\))\(_2\) and CH\(_2\)N, 6 H), 2.34 – 2.20 (m and s, CH\(_2\)CH\(_2\)N(CH\(_3\))\(_2\), 9 H), 2.06 – 1.98 (m, CH\(_2\)CH\(_2\)CH\(_2\), 2 H), 1.85 – 1.78 (m, N(CH\(_3\))\(_2\)(CH\(_2\))\(_2\), 4 H); \( ^{13}C \) NMR (151 MHz, CDCl\(_3\)) \( \delta \) ppm 155.28, 149.19, 136.35, 132.08, 114.95, 110.92, 104.00, 68.50, 61.80, 53.14, 50.44, 46.03, 40.22, 30.58, 30.29, 25.66, 23.46; LCMS calc. for C\(_{20}\)H\(_{31}\)N\(_5\)O, 357.5; found m/z = 358.4 [M + H]\(^+\).

\[ \text{C}_{20}\text{H}_{31}\text{N}_5\text{O} \]

\( N,N \)-dimethyl-6-nitro-5-((4-(pyrrolidin-1-yl)butyl)amino)chromane-3-carboxamide (66i). Obtained from the \( S_N \)Ar reaction of chroman 65 and primary amine 67i. The crude material was purified by flash chromatography (silica gel, 25% methanol/ethyl acetate) and the product was provided in 87% yield (based on the reactive isomer) as a bright yellow oil. \( ^1H \) NMR (600 MHz, CDCl\(_3\)) \( \delta \) ppm 7.99 (d, \( J = 9.4 \) Hz, ArH, 1 H), 7.78 – 7.55 (m, ArH, 4 H), 6.92 (m, ArH, 1 H), 6.02 (s, NO\(_2\), 1 H), 5.52 (s, ArH, 1 H), 4.29 – 4.14 (m, ArNC\(_2\)H, 2 H), 3.86 – 3.79 (dd, \( J = 10.7, 7.2 \) Hz, OCH\(_2\), 1 H), 3.25 – 3.17 (dd, \( J = 15.8, 4.8 \) Hz, ArCH\(_2\), 1 H), 2.86 – 2.77 (dd, \( J = 15.8, 7.2 \) Hz, ArCH\(_2\), 1 H), 2.58 – 2.40 (2m, N(CH\(_2\))\(_2\) and CH\(_2\)N, 6 H), 2.34 – 2.20 (m and s, CH\(_2\)CH\(_2\)N(CH\(_3\))\(_2\), 9 H), 2.06 – 1.98 (m, CH\(_2\)CH\(_2\)CH\(_2\), 2 H), 1.85 – 1.78 (m, N(CH\(_3\))\(_2\)(CH\(_2\))\(_2\), 4 H); LCMS calc. for C\(_{20}\)H\(_{30}\)N\(_4\)O\(_4\), 373.2; found m/z = 374.3 [M + H]\(^+\).

\[ \text{C}_{20}\text{H}_{30}\text{N}_4\text{O}_4 \]
1H, 7.68 (br. t., J = 5.4 Hz, NH, 1 H), 6.36 (d, J = 9.4 Hz, ArH, 1 H), 4.41 (dt, J = 10.7, 3.1 Hz, OCH3, 1 H), 4.12 (t, J = 10.7 Hz, OCH3, 1 H), 3.46 – 3.39 (m, CHCO, 1 H), 3.18 – 3.10 (s and m, partial overlap, N(CH3)2 and NHCH3, 4 H), 3.06 – 2.95 (s, m, N(CH3)2, NHCH3 and CHCH3, 5 H), 2.82 – 2.77 (m, CHCH3, 1 H), 2.51 – 2.40 (m, N(CH3)2 and NCH3, 6 H), 1.79 – 1.73 (m, N(CH3)2(CH2)2, 4 H), 1.69 – 1.60 (m, HNCH2CH2CH2CH2N, 2 H), 1.60 – 1.53 (m, HNCH2CH2CH2CH2N, 2 H); 13C-NMR (151 MHz, CDCl3) δ ppm 171.49, 159.99, 149.09, 126.48, 110.92, 109.10, 67.37, 56.09, 54.17, 48.38, 37.32, 35.73, 35.39, 29.34, 27.95, 26.32, 23.44.

2-amino-N,N-dimethyl-1-(4-(pyrrolidin-1-yl)butyl)-1,7,8,9-tetrahydrochromeno[5,6-d]imidazole-8-carboxamide (68i). Obtained from the hydrogenation and cyclization of chroman 66i with cyanogen bromide. The crude material was purified by flash chromatography (basic alumina, 10% methanol/ethyl acetate) and the product was provided in 17% yield as a tan solid. 1H NMR (499 MHz, CDCl3) δ ppm 7.16 (d, J = 8.5 Hz, ArH, 1 H), 6.65 (d, J = 8.5 Hz, ArH, 1 H), 5.38 (br. s., NH2, 2 H), 4.37 – 4.33 (ddd, J = 10.7, 3.1, 2.1 Hz, OCH3, 1 H), 4.16 – 4.11 (m, ArNCH3, 1 H), 4.11 – 3.96 (m, ArNCH3, 1 H), 3.95 (dd, J = 10.7, 10.7 Hz, OCH3, 1 H), 3.54 – 3.49 (dd, J = 15.4, 11.3 Hz, ArCH2, 1 H), 3.31 – 3.25 (m, CHCO, 1 H), 3.17 (s, NCH3, 3 H), 3.11 (ddd, J = 15.4, 5.4, 2.1 Hz, ArCH2, 1 H), 3.03 (s, NCH3, 3 H), 2.67 – 2.63 (m, NCH3, 1 H), 2.56 – 2.49 (m, N(CH2)2, 4 H), 2.48 – 2.44 (m, NCH3, 1 H), 1.89 – 1.81 (m, CH3, 2 H), 1.80 – 1.73 (m, (CH2)2, 4 H), 1.68 – 1.57 (m, NCH3, 2 H); 13C-NMR (126 MHz, CDCl3) δ ppm 171.91, 153.95, 148.45, 136.34, 131.92, 115.31, 110.51, 103.58, 66.69, 54.24, 54.93, 42.42, 37.30, 36.00, 35.79, 28.67, 24.50, 24.03, 23.42.
8-((dimethylamino)methyl)-1-(4-(pyrrolidin-1-yl)butyl)-1,7,8,9-tetrahydrochromeno[5,6-d]imidazole-2-amine (50i). Obtained from the reduction of amino-benzimidazole 68i with LAH. The crude material was purified by flash chromatography (rediSep gold high performance amine column, 20% methanol/ethyl acetate) and the product was provided in 70% yield as a white solid. $^1$H NMR (600 MHz, CDCl$_3$) δ ppm 7.14 (d, $J = 8.51$ Hz, ArH, 1 H), 6.62 (d, $J = 8.51$ Hz, ArH, 1 H), 5.11 (br. s., NH$_2$, 2 H), 4.28 – 4.24 (ddd, $J = 10.6$, 2.5, 1.0 Hz, OC$_2$H$_2$, 1 H), 4.11 – 4.01 (m, ArNCH$_2$, 2 H), 3.87 – 3.83 (ddd, $J = 10.6$, 7.3 Hz, OCH$_2$, 1 H), 3.18 – 3.13 (ddd, $J = 15.6$, 5.4 Hz, ArCH$_3$, 1 H), 2.82 – 2.76 (ddd, $J = 15.6$, 7.3 Hz, ArCH$_3$, 1 H), 2.63 – 2.50 (2m, CH$_2$CH$_2$N(CH$_2$)$_2$, 6 H), 2.34 – 2.24 (m and s, CHCH$_2$N(CH$_3$)$_2$, 9 H), 1.88 – 1.81 (m, CH$_2$CH$_2$CH$_2$, 2 H), 1.80 – 1.77 (m, N(CH$_2$)$_2$(CH$_2$)$_2$, 4 H), 1.67 – 1.61 (m, CH$_2$CH$_2$CH$_2$, 2 H); $^{13}$C NMR (151 MHz, CDCl$_3$) δ ppm 153.48, 149.25, 136.11, 132.00, 115.21, 110.82, 103.86, 68.46, 61.79, 54.55, 54.01, 46.04, 42.78, 30.58, 28.79, 25.64, 24.44, 23.46; LCMS calc. for C$_{21}$H$_{33}$N$_5$O, 371.5; found m/z = 372.4 [M + H]$^+$.

tert-butyl (2S)-2-(((3-(dimethylcarbamoyl)-6-nitrochroman-5-yl)amino)methyl)pyrrolidine-1-carboxylate (66l.) A mixture of diastereomers. Obtained from the S$_{N}$Ar reaction of chroman 65 and primary amine 67l. The crude material was purified by flash chromatography (silica gel, 15% diethyl ether/dichloromethane) and the product was provided in 33% yield (based on the reactive isomer) as a
bright yellow oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.96 (br. s., ArH, 1 H), 7.36 - 7.78 (br. t., NH, 1 H), 6.35 (br. s., ArH, 1 H), 4.41 (d, OCHCHCH$_2$, $J$=11.3 Hz, 1 H), 4.08 - 4.21 (m, OCHCHCH$_2$, 1 H), 3.98 (br. s., NHCH$_2$CHN, 1 H), 3.43 - 3.68 (m, BocNCH$_2$CH$_2$, 1 H), 3.25 - 3.42 (m, NHCHCHN and OCH$_2$CHCH$_2$, 2 H), 3.10 - 3.16 (m, NCH$_3$, 3 H), 3.10 - 3.16 (m, NHCHCHN, 1 H), 2.95 - 3.05 (m, BocNCH$_2$, 1 H), 3.01 (s, NCH$_3$, 3 H), 2.78 - 2.87 (m, OCH$_2$CHCH, 1 H), 1.99 (br. s., OCH$_2$CHCH, 1 H), 1.74 - 1.93 (m, BocNCH$_2$CH$_2$CHCH, 3 H), 1.69 (br. s., BocNCH$_2$CH$_2$CHCH, 1 H), 1.34 - 1.53 (m, tert-butyl, 9 H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ ppm 204.04, 189.06, 159.93, 148.73, 126.36, 125.49, 79.62, 77.21, 67.36, 57.85, 37.30, 35.67, 35.25, 34.22, 30.33, 28.48, 28.45, 27.74, 23.77.

tert-butyl (2S)-2-((2-amino-8-(dimethylcarbamoyl)-8,9-dihydrochromeno[5,6-d]imidazol-1(7H)-yl)methyl)pyrrolidine-1-carboxylate (68l). A mixture of diastereomers. Obtained from the hydrogenation and cyclization of chroman 66l with cyanogen bromide. The crude material was purified by flash chromatography (basic alumina, 4% methanol/diethyl ether) and the product was provided in 65% yield as a tan solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.40 (br. dd, ArH, $J$=8.6, 2.7 Hz, 1 H), 6.76 (br. dd, ArH, $J$=8.6, 1.4 Hz, 1 H), 4.58 (br. s., NH$_2$, 2 H), 4.33 - 4.44 (m, OCHCHCH$_2$, 1 H), 4.27 (dd, OCHCHCH$_2$, $J$=5.6, 3.4 Hz, 1 H), 4.17 (br. s., NCHCH, 1 H), 3.97 (br. s., NCHCH, 1 H), 3.39 - 3.47 (m, BocNCH$_2$CH$_2$, 2 H), 3.21 - 3.32 (m, OCH$_2$CHCH$_2$, 1 H), 3.19 (s, NCH$_3$, 3 H), 3.00 - 3.07 (m, NCH$_3$, 3 H), 2.66 (br. s., NCH$_2$CHN, 1 H), 2.46 - 2.61 (m, OCH$_2$CHCH, 1 H), 1.90 - 2.07 (m, OCH$_2$CHCH, 1 H), 1.73 - 1.89 (m, BocNCH$_2$CH$_2$, 2 H), 1.67 (br. s., BocNCH$_2$CH$_2$CH, 1 H), 1.45 (br. s., BocNCH$_2$CH$_2$CH, 1 H), 1.38 (br. s., tert-butyl, 4 H), 1.04 (br. s., tert-butyl, 5 H).
8-((dimethylamino)methyl)-1-(((S)-1-methylpyrrolidin-2-yl)methyl)-1,7,8,9-tetrahydrochromeno[5,6-
d]imidazol-2-amine (50l). A mixture of diastereomers. Obtained from the reduction of amino-
benzimidazole 68l with LAH. The crude material was purified by flash chromatography (basic alumina,
20% methanol/diethyl ether) and the product was provided in 65% yield as a tan solid. $^1$H NMR (500
MHz, CDCl$_3$) $\delta$ ppm 7.38 (d, $ArH$, $J$=8.7 Hz, 1 H), 6.74 (d, $ArH$, $J$=8.7 Hz, 1 H), 4.24 - 4.36 (m, $NH_2$, 2
H), 4.12 - 4.23 (m, OCHCHCH$_2$, 1 H), 3.82 - 3.96 (m, OCHCHCH$_2$, 1 H), 3.04 - 3.12 (m, NCHCHN, 1 H),
2.70 - 2.86 (2m, NCHCHN and OCH$_2$CHCH, 2 H), 2.61 (s, NCH$_3$, 3 H), 2.22 - 2.37 (5m and s partially
hidden, NCH$_3$ and OCH$_2$CHCH$_2$ and OCH$_2$CHCH and NCH$_2$CHN and CH$_3$NCH$_2$CH$_2$ and
(CH$_3$)$_2$NCH$_2$CH, 10 H), 2.12 (s, NCH$_3$, 1 H), 2.05 (s, NCH$_3$, 2 H), 1.77 - 1.90 (m, NCHCH$_2$, 2 H), 1.68 -
1.77 (m, NCHCH$_2$CH, 1 H), 1.53 - 1.64 (m, NCHCH$_2$CH, 1 H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ ppm
151.39, 151.37, 150.81, 150.77, 137.19, 137.13, 133.70, 133.62, 117.64, 117.63, 112.57, 112.51, 104.56,
104.51, 68.54, 68.40, 65.83, 65.71, 61.59, 61.52, 57.93, 57.80, 49.99, 49.82, 45.94, 41.94, 41.83, 30.48,

N,N-dimethyl-6-nitro-5-((pyridin-3-ylmethyl)amino)chromane-3-carboxamide (66n). Obtained from
the $S_N$Ar reaction of chroman 65 and primary amine 67n. The crude material was purified by flash
chromatography (silica gel, 15% methanol/ethyl acetate) and the product was provided in 89% yield (based
on the reactive isomer) as a bright yellow oil. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ ppm 8.57 (d, pyr, $J$=2.1 Hz, 1 H), 8.54 (dd, pyr, $J$=4.8, 1.5 Hz, 1 H), 7.99 (d, ArH, $J$=9.2 Hz, 1 H), 7.73 (t, NH, $J$=6.4 Hz, 1 H), 7.60 - 7.63 (m, pyr, 1 H), 7.27 (dd, pyr, $J$=7.8, 4.8, 0.8 Hz, 1 H), 6.45 (d, ArH, $J$=9.4 Hz, 1 H), 4.52 (dd, OCHCHCH$_3$, $J$=14.5, 6.5 Hz, 1 H), 4.42 - 4.46 (m, NHCHC, 1 H), 4.32 (dd, OCHCHCH$_3$, $J$=14.5, 6.5 Hz, 1 H), 4.13 (t, NHCHC, $J$=11.0 Hz, 1 H), 3.10 (s, NCH$_3$, 3 H), 3.06 - 3.12 (m, OCH$_2$C, 1 H), 3.00 - 3.05 (m, OCH$_2$CHCH, 1 H), 3.01 (s, NCH$_3$, 3 H), 2.88 - 2.93 (m, OCH$_2$CHCH, 1 H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ ppm 171.15, 160.04, 149.28, 149.04, 147.57, 135.05, 134.15, 126.45, 123.70, 112.18, 110.24, 67.42, 49.36, 37.26, 35.75, 35.16, 27.73.

**methyl ((benzyl)carbonyl)-L-asparaginate (91).** $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.27 - 7.39 (m, ArH, 5 H), 5.99 (br. d., NH, $J$=6.8 Hz, 1 H), 5.54 (br. s., CONH, 1 H), 5.44 (br. s., CONH, 1 H), 5.12 (s, C$_6$H$_5$CH$_2$, 2 H), 4.59 (dt, NHCHCH$_2$, $J$=8.6, 4.5 Hz, 1 H), 3.75 (s, OCH$_3$, 3 H), 2.97 (dd, COCHCH, $J$=16.2, 4.1 Hz, 1 H), 2.76 (dd, COCHCH, $J$=16.3, 4.2 Hz, 1 H).

**benzyl (S)-(1-benzyl-2,5-dioxopyrrolidin-3-yl)carbamate (92).** $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.26 - 7.42 (m, ArH, 10 H), 5.39 (br. s., NH, 1 H), 5.10 (br. s., C$_6$H$_5$CH$_2$O, 2 H), 4.69 (br. s., C$_6$H$_5$CH$_2$N, 2 H), 4.32 (br. s., NHCHCH$_2$, 1 H), 3.10 (dd, NHCHCH, $J$=17.9, 8.9 Hz, 1 H), 2.78 (d, NHCHCH, $J$=18.2 Hz, 1 H).
(S)-3-amino-1-benzylpyrrolidine-2,5-dione (93). \( ^1H \) NMR (500 MHz, CDCl\(_3\)) \( \delta \) ppm 7.24 - 7.40 (m, ArH, 5 H), 4.65 (s, C\(_6\)H\(_5\)CH\(_2\), 2 H), 3.89 (dd, NH\(_2\)CHCH\(_2\), \( J\)=8.7, 5.4 Hz, 1 H), 3.04 (dd, NH\(_2\)CHCH, \( J\)=18.2, 8.7 Hz, 1 H), 2.45 (dd, NH\(_2\)CHCH, \( J\)=18.1, 5.4 Hz, 1 H), 1.90 (br. s, NH\(_2\), 2 H).

(S)-1-benzylpyrrolidin-3-amine (94). \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) ppm 7.14 - 7.40 (m, ArH, 5 H), 3.60 (dd, C\(_6\)H\(_5\)CH\(_2\), \( J\)=20.5, 13.1 Hz, 2 H), 3.49 (ddt, NH\(_2\)CH, \( J\)=8.5, 6.3, 4.4, 4.4 Hz, 1 H), 2.65 - 2.76 (m, NCH\(_2\)CH, 2 H), 2.42 - 2.51 (m, NCH\(_2\)CHCH\(_2\), 1 H), 2.29 (dd, NCH\(_2\)CHCH\(_2\), \( J\)=9.4, 4.3 Hz, 1 H), 2.13 - 2.24 (m, NH\(_2\)CHCHCH\(_2\), 1 H), 1.42 - 1.54 (m, NH\(_2\)CHCHCH\(_2\), 1 H), 1.30 (br. s., NH\(_2\), 2 H).

(S)-2-(1-benzylpyrrolidin-3-yl)isoindoline-1,3-dione (95). \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) ppm 7.78 - 7.84 (m, pht. ArH, 2 H), 7.66 - 7.73 (m, pht. ArH, 2 H), 7.20 - 7.38 (m, ArH, 5 H), 4.91 (dt, NCH\(_2\)CH\(_2\), \( J\)=15.7, 8.6 Hz, 1 H), 3.71 (dd, C\(_6\)H\(_5\)CH\(_2\)N, \( J\)=15.7, 12.7 Hz, 2 H), 3.04 (t, NCH\(_2\)CH\(_2\)N, \( J\)=8.5 Hz, 1 H), 2.93 - 3.01 (m, NCH\(_2\)CH\(_2\), 1 H), 2.84 (q, NCH\(_2\)CH\(_2\)N, \( J\)=8.5 Hz, 1 H), 2.69 (t, NCH\(_2\)CH\(_2\)N, \( J\)=8.6 Hz, 1 H), 2.20 - 2.28 (m, NCH\(_2\)CH\(_2\), 2 H).
2-(2-bromoethyl)isoindoline-1,3-dione (78a). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.83 - 7.92 (m, pht. ArH, 2 H), 7.71 - 7.79 (m, pht. ArH, 2 H), 4.11 (t, NCH$_2$, $J$=6.7 Hz, 2 H), 3.62 (t, BrCH$_2$, $J$=6.7 Hz, 2 H).

tert-butyl hydrazinecarboxylate (98). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 5.98 (br. s., NH, 1 H), 3.69 (br. s., NH$_2$, 2 H), 1.46 (s, tert-butyl, 9 H).

1-benzyl 2-(tert-butyl) hydrazine-1,2-dicarboxylate (99). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.28 - 7.39 (m, ArH, 5 H), 6.62 (br. s., NH, 1 H), 6.37 (br. s., NH, 1 H), 5.16 (s, C$_6$H$_5$CH$_2$, 2 H), 1.45 (br. s., tert-butyl, 9 H).

1-benzyl 2-(tert-butyl) pyrazolidine-1,2-dicarboxylate (100). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 7.27 - 7.42 (m, ArH, 5 H), 5.23 (br. s., C$_6$H$_5$CH, 1 H), 5.13 (br. s., C$_6$H$_5$CH, 1 H), 3.93 (br. s., NCH$_2$, 2 H), 3.30 (br. s., NCH$_2$, 1 H), 3.20 (br. s., NCH$_2$, 1 H), 2.03 (quin, NCH$_2$CH$_2$, $J$=7.2 Hz, 2 H), 1.42 (s, tert-butyl, 9 H).
tert-butyl pyrazolidine-1-carboxylate (101). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) ppm 3.76 (br. s., NH, 1 H), 3.39 - 3.49 (m, NCH\textsubscript{2}, 2 H), 3.03 (t, \(J=6.6\) Hz, NCH\textsubscript{2}, 2 H), 1.95 - 2.11 (m, NCH\textsubscript{2}CH\textsubscript{2}, 2 H), 1.49 (s, tert-butyl, 9 H).

tert-butyl 2-(2-(1,3-dioxoisindolin-2-yl)ethyl)pyrazolidine-1-carboxylate (79a). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) ppm 7.81 - 7.86 (m, pht. ArH, 2 H), 7.68 - 7.72 (m, pht. ArH, 2 H), 3.84 - 3.91 (m, NCH\textsubscript{2}CH\textsubscript{2}N, 2 H), 3.49 - 3.56 (m, BocNCH\textsubscript{2}, 2 H), 3.07 (t, NCH\textsubscript{2}CH\textsubscript{2}N, \(J=6.7\) Hz, 2 H), 2.88 - 2.94 (m, BocNNCH\textsubscript{2}, 2 H), 2.04 - 2.12 (m, NCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}, 2 H), 1.43 (s, tert-butyl, 9 H).

tert-butyl 2-(2-aminoethyl)pyrazolidine-1-carboxylate (67p). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) ppm 3.50 (t, BocNC\textsubscript{H}\textsubscript{2}, \(J=7.4\) Hz, 2 H), 2.98 (t, NH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}, \(J=6.8\) Hz, 2 H), 2.76 (br. s., NH\textsubscript{2}CH\textsubscript{2}, 2 H), 2.60 - 2.65 (m, BocNNCH\textsubscript{2}CH\textsubscript{2}, 2 H), 2.04 - 2.13 (m, NCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}, 2 H), 1.77 (br. s., NH\textsubscript{2}, 2 H), 1.49 (s, tert-butyl, 9 H).
tert-butyl 2-(2-((3-(dimethylcarbamoyl)-6-nitrochroman-5-yl)amino)ethyl)pyrazolidine-1-carboxylate (66p). Obtained from the S_NAr reaction of chroman 65 and primary amine 67p. The crude material was purified by flash chromatography (silica gel, 7% methanol/diethyl ether) and the product was provided in 44% yield (based on the reactive isomer) as a bright yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) ppm 7.93 (d, ArH, \(J=9.4\) Hz, 1 H), 7.74 (br. s., NH, 1 H), 6.34 (d, \(J=9.4\) Hz, ArH, 1 H), 4.36 - 4.44 (m, OCHCHCH\(_2\), 1 H), 4.06 - 4.18 (m, OCHCHCH\(_2\), 1 H), 3.54 - 3.62 (m, NHCHCH\(_2\), 1 H), 3.51 (dt, OCH\(_2\)CHCH\(_2\), \(J=8.6, 4.2\) Hz, 1 H), 3.39 - 3.47 (m, NHCHCH\(_2\), 1 H), 3.14 - 3.24 (m, BocNCH, 1 H), 3.12 (s, NCH\(_3\), 3 H), 3.00 (s, NCH\(_3\), 3 H), 2.96 - 3.07 (3m, BocNCH and OCH\(_2\)CHCH and NHCH\(_2\)CH, 3 H), 2.86 - 2.96 (2m, OCH\(_2\)CHCH and NHCH\(_2\)CH, 2 H), 2.78 (dt, BocNNCHCH\(_2\)CH\(_2\), \(J=12.1, 5.1\) Hz, 1 H), 2.56 - 2.69 (m, BocNNCHCH\(_2\)CH\(_2\), 1 H), 2.07 (quin, NCH\(_2\)CH\(_2\)CH\(_2\)N, \(J=7.1\) Hz, 2 H), 1.47 (s, tert-butyl, 9 H); \(^1\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) ppm 171.47, 159.57, 148.04, 126.23, 110.92, 108.67, 80.26, 67.29, 57.86, 54.25, 49.39, 46.63, 37.22, 35.61, 35.25, 30.64, 29.53, 28.40, 27.48, 24.43, 17.65.

\(\text{C}_{22}\text{H}_{33}\text{N}_{5}\text{O}_{6}\)

\(2,2,2\text{-trifluoro-N-(3-(methylamino)propyl)acetamide (103).}\) \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) ppm 7.49 (br. s., NH, 1 H), 3.53 (t, CONHCH\(_2\), \(J=6.4\) Hz, 2 H), 3.33 (q, CH\(_3\)NHCH\(_2\), \(J=6.3\) Hz, 2 H), 3.16 (q, NHCH\(_3\), \(J=1.6\) Hz, 3 H), 1.88 (quin, CH\(_2\)CH\(_2\)CH\(_2\), \(J=6.3\) Hz, 2 H).

\(\text{C}_{6}\text{H}_{11}\text{F}_{3}\text{N}_{2}\text{O}\)
2-(trimethylsilyl)ethyl methyl(3-(2,2,2-trifluoroacetamido)propyl)carbamate (104). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 8.07 (br. s., NH, 1 H), 4.16 - 4.22 (m, OCH$_2$CH$_2$, 2 H), 3.30 - 3.40 (2m, NHCH$_2$CH$_2$N, 4 H), 2.89 (s, NCH$_3$, 3 H), 1.74 (br. s., NHCH$_2$CH$_2$CH$_2$, 2 H), 0.98 - 1.04 (m, SiCH$_2$CH$_2$, 2 H), 0.04 - 0.07 (m, Si(CH$_3$)$_3$, 9 H).

2-(trimethylsilyl)ethyl (3-aminopropyl)(methyl)carbamate (67q). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm 4.13 - 4.21 (m, OCH$_2$CH$_2$, 2 H), 3.34 (br. s., CH$_3$NCH$_2$, 2 H), 2.87 (br. s., NCH$_3$, 3 H), 2.70 (br. s., NH$_2$CH$_2$, 2 H), 1.66 (quin, NH$_2$CH$_2$CH$_2$CH$_2$, $J$=6.8 Hz, 2 H), 1.30 (br. s., NH$_2$, 2 H), 0.97 - 1.04 (m, SiCH$_2$, 2 H), 0.01 - 0.07 (m, Si(CH$_3$)$_3$, 9 H).

2-(trimethylsilyl)ethyl (3-((3-(dimethylcarbamoyl)-6-nitrochroman-5-yl)amino)propyl)(methyl)carbamate (66q). Obtained from the SNAr reaction of chroman 65 and primary amine 67q. The crude material was purified by flash chromatography (silica gel, 2% methanol/diethyl ether) and the product was provided in 82% yield (based on the reactive isomer) as a bright yellow oil. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ ppm 7.99 (d, ArH, $J$=9.5 Hz, 1 H), 7.62 (br. s., NH, 1 H), 6.38 (d, $J$=9.4 Hz, ArH, 1 H), 4.37 - 4.46 (m, OCHCHCH$_2$, 1 H), 4.05 - 4.25 (2m partially hidden, OCHCHCH$_2$ and
OCH₂CH₂Si, 3 H), 3.37 - 3.47 (m, OCH₂CHCH₂, 1 H), 3.24 - 3.37 (m, NHCH₂CH₂, 2 H), 3.14 - 3.21 (m, OCH₂CHCH, 1 H), 3.13 (s, NCH₃, 3 H), 3.02 (s, NCH₃, 3 H), 2.95 - 3.01 (m, CH₃NCH₃, 2 H), 2.86 (s, CH₃NCH₃, 3 H), 2.80 (br. s., OCH₂CHCH, 1 H), 1.74 - 1.88 (m, NHCH₂CH₂CH₂, 2 H), 0.90 - 1.07 (m, OCH₂CH₂Si, 2 H), 0.01 - 0.09 (m, Si(CH₃)₃, 9 H). ¹³C NMR (126 MHz, CDCl₃) δ ppm 172.80, 161.48, 150.17, 127.89, 112.61, 110.80, 78.71, 68.82, 65.04, 47.10, 38.76, 37.19, 36.77, 31.81, 30.70, 29.30, 19.39, 14.89, 1.46.
C_{18}H_{19}NO_3
Chemical Shift (ppm)

Normalized Intensity

Ph

PMB

C_{12}H_{19}NO_{3}S

42
Ph
PMB - O

C_{17}H_{19}NO_{4}S

N O
Ph
S
PMB
O
C_{17}H_{19}NO_{4}S

Chemical Shift (ppm)
Normalized Intensity
Chemical Shift (ppm) vs. Normalized Intensity

Compound: C_{23}H_{30}NO_{5}P

Structural formula:

N4_20090226_djs_097_Proton_01.esp
Chemical Shift (ppm)

Normalized Intensity

HO
HO
O
N
O
Ph

C₁₁H₁₁NO₄

19
Chemical Shift (ppm)

Normalized Intensity

O
O
Ph
TBSO

C_{29}H_{29}NO_4Si

49S
Chemical Shift (ppm)

Normalized Intensity

BocHN

C_{14}H_{26}N_{2}O_{3}

71
Chemical Shift (ppm)

Normalized Intensity

H2N
N
O
67e

C9H18N2O

0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

0.5
1.0
2.0
2.5
3.0
3.5
4.0
4.5
5.0

Chemical Shift (ppm)
Chemical Shift (ppm)

Normalized Intensity

\[
\text{C}_{18}\text{H}_{22}\text{N}_{2}\text{O}_{4}
\]

76

Boc
Chemical Shift (ppm)

Normalized Intensity

H₂N

Boc

C₁₈H₃₈N₂O₂

671
Chemical Shift (ppm)

Normalized Intensity

O
N
68b

C$_{23}$H$_{33}$N$_{5}$O$_{4}$
68b
Chemical Shift (ppm)

Normalized Intensity

H₂N
O

Ph

C₁₁H₁₂N₂O₂

93
Chemical Shift (ppm)

Normalized Intensity

\[
\text{C}_{10}\text{H}_{8}\text{BrNO}_2
\]

78a
Chemical Shift (ppm)

Normalized Intensity

H₂N – N<sub>Boc</sub>

C₇H₁₂N₂O₂

98
HN - NH
Cbr' Boc
C_{13}H_{18}N_{2}O_{4}
99
Chemical Shift (ppm)

Normalized Intensity

Cbz

Boc

C₁₆H₂₂N₂O₄

100
Chemical Shift (ppm)

Normalized Intensity

H$_2$N

C$_{18}$H$_{21}$N$_3$O$_2$

67p
Chemical Shift (ppm)

Normalized Intensity

N
H
NH
F3C
O
C6H11F3N2O
103
Chemical Shift (ppm)

Normalized Intensity

N\(3\)C\(\text{H}_{23}\text{F}_3\text{N}_2\text{O}_3\text{Si}\)
$\text{C}_{10}\text{H}_{24}\text{N}_2\text{O}_2\text{Si}$

$\text{H}_2\text{N} - \text{O} - \text{O} - \text{Si}$

Normalized Intensity

Chemical Shift (ppm)
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