BCL-2 INHIBITOR SCAFFOLDS THAT ARE PREORGANIZED ALONG AN AXIS OF CHIRALITY; THE EFFECT OF ATROPISOMERISM ON PROTEIN BINDING

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Mari B. Ishak Gabra
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The Undersigned Faculty Committee Approves the

Thesis of Mari B. Ishak Gabra:

Bcl-2 Inhibitor Scaffolds that are Preorganized Along an Axis of Chirality; the

Effect of Atropisomerism on Protein Binding

Jeffery L. Gustafson, Chair
Department of Chemistry and Biochemistry

Byron W. Purse
Department of Chemistry and Biochemistry

Kelly S. Doran
Department of Biology

5-6-2015
Approval Date
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by

Mari B. Ishak Gabra

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DEDICATION

For my family and friends who supported me every step of the way and kept me focused to get here and go beyond.
ABSTRACT OF THE THESIS

Bcl-2 Inhibitor Scaffolds that are Preorganized Along an Axis of Chirality; the Effect of Atropisomerism on Protein Binding

by

Mari B. Ishak Gabra
Master of Science in Chemistry
San Diego State University, 2015

Atropisomerism is a form of chirality that arises from hindered rotation around a bond resulting in rotational isomers that are non-superimposable mirror images (enantiomers). Even though atropisomerism is currently a point of contention in pharmaceutical pursuits since it can have an intrinsic effect on the biological activity of molecules, no one has purposely rigidified an atropisomeric axis as part of the drug design process. Biologically active scaffolds that display atropisomerism are ubiquitous throughout drug discovery. For example, various small molecule inhibitors of B-cell lymphoma 2 (Bcl-2) family proteins contain one or more instances of atropisomeric axes. Bcl-2 is a member of a family of proteins that regulate cellular apoptosis. The intrinsic apoptotic pathway acts via the mitochondria and is dependent on several pro-and anti-apoptotic Bcl-2 proteins and is regulated by key protein-helical interactions. The expression of these proteins is tightly regulated and overexpression of these proteins is found throughout oncology, resulting in a large research effort towards Bcl-2 inhibition. We successfully developed a synthetic route towards atropisomerically stable analogs of a class of small molecule α-helix mimics that are known to inhibit Bcl-2 anti-apoptotic proteins but exist as interconverting atropisomers, with the hypothesis that such analogs will possess greater efficacy and selectivity. This work will allow for the study of biological implications of atropisomeric preorganization and the ability to access a variety of chemical modifications in order to study the effect of atropisomerism in other biologically relevant scaffolds.
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CHAPTER 1

ATROPISOMERISM AND DRUG DISCOVERY

Atropisomerism is a stereochemical phenomenon that arises from hindered rotation around a bond (axis of chirality) that results in a molecule whose ‘rotational’ isomers are enantiomers (Figure 1.1).\textsuperscript{1} When the magnitude of the barrier to rotation around the axis of chirality is high, the molecule exists as a mixture of stable isolable atropisomers. Stable atropisomerism is ubiquitous throughout the natural product world, as in the glycopeptide antibiotic Vancomycin,\textsuperscript{2} and can prove to be decisive towards the observed biological activity of a compound. For instance, Telenzepine, a selective muscarinic antagonist used to treat peptic ulcers, possesses a half-life of racemization of 1000 years due to the restricted rotation around the axis of chirality caused by macrocyclic constraints (Figure 1.2).\textsuperscript{3} Separation of each atropisomer revealed a 500-fold difference in activity towards the muscarinic receptor with the (R)-atropisomer possessing all activity.\textsuperscript{4}

![Figure 1.1. An example of interconverting atropisomers in an amide.](image)

Differential activity among traditional point chiral enantiomers is well documented, in part due to the consequences of racemic thalidomide, which was given to pregnant women in the 1950s to treat morning sickness. It was later shown that the (R)-enantiomer was responsible of alleviating morning sickness while the (S)-enantiomer caused teratogenic effects. Thalidomide possesses a half-life of racemization of 4.7 hours at physiological condition via keto/enol tautomerization.\textsuperscript{5} This highlights the severe consequences of a freely racemizing drug, and as a result the FDA now requires \textit{in vitro} and \textit{in vivo} studies of the
biological effect of both enantiomers of a racemic drug. Despite this, there are no regulations on atropisomer drugs, even though free racemization is a hallmark of atropisomeric drugs with low barriers to rotation.

The majority of biologically active molecules have barriers to rotation that are less than 25 kcal/mol and racemize on the scale of minutes or seconds under physiological conditions yet bind their target in an enantiospecific manner. As a result, LaPlante et al. have developed computational tools to predict the stereochemical stabilities of compounds in the pharmaceutical industry. They have recently applied these tools to a larger database of over 10,000 compounds that included registered or published drugs from 2005 to 2010. The study revealed many unexpected atropisomeric molecules with varying stereochemical stabilities, and they proceeded to divide them into three classes. Class 1 atropisomers, with fast axial rotation rates on the order of seconds or less, have barriers to rotation ($\Delta E_{rot}$) of less than 20 kcal/mol and do not display chirality. Class 2 atropisomers possess a $\Delta E_{rot}$ between 20 to 30 kcal/mol and can display stable atropisomerism, however can potentially racemize on the scale of minutes to days. Lastly, they identified class 3 compounds with a $\Delta E_{rot}$ of 30 kcal/mol or more that are atropisomerically stable indefinitely at physiological conditions, and can be treated in a manner similar to the traditional point chirality as is done with Telenzepine mentioned previously. Because of the factors discussed above, the Gustafson group has set out to study the biological and structural effects of rigidifying biologically active compounds into their target binding conformations.

Figure 1.2. Slow interconversion of Telenzepine.
1.1 IMPORTANCE OF ATROPISOMERISM

While molecules that exhibit atropisomerism exist as a racemic mixture, one conformation will likely possess greater affinity toward the target, as the chiral active site will preferentially interact with one atropisomeric conformation. For example, the well-characterized B-cell lymphoma 2 (Bcl-2) inhibitor, Gossypol, exists as a mixture of separable atropisomers with the (S)-enantiomer exhibiting tenfold more potency than the (R)-enantiomer towards cancer cell lines that overexpress Bcl-2 family proteins. Efforts have been made towards stereoselective synthesis of the (S)-enantiomer only.

Figure 1.3. CCR5 inhibitor (GSK214096) with two atropisomeric axis. Axial chirality assignment of (R)- and (S)-atropisomers.

Recently, a research group at GlaxoSmithKline was able to use chiral chromatography to characterize a preclinical HIV entry inhibitor and CCR5 inhibitor GSK214096 (Figure 1.3). They have shown that this compound exists as a mixture of four separable atropisomers with interconvertable rotamers. They have assigned the axial chirality for the fast interconverting diastereomeric pairs A-B as the (R)-atropisomer and the pairs C-
D as the (S)-atropisomer. Both atropisomers have been shown to belong to class 2 compounds and have $\Delta E_{\text{rot}}$ of about 24.4 kcal/mol. The (R)-atropisomer is the biologically active form that is relevant towards HIV treatment and inhibits CCR5 while the (S)-atropisomer is inactive. The authors mentioned the need for structural modifications that would lead to more conformationally stable CCR5 antagonists.

The existence of potential drug candidates in multiple atropisomeric or diastereomeric forms can complicate drug development and recognizing the existence of atropisomerism in compounds is a crucial step. One of the first complications is whether the drug candidate will be developed as a purified enantiomer or a racemic mixture, that depends on the rate at which the enantiomers interconvert. For example, a group from AstraZeneca identified a potential compound (Figure 1.4) that showed high potency and selectivity for NK$_1$ receptor. However, this compound exited as a mixture of four interconverting atropisomers since the amide bond can exist in cis or trans form and the aryl-CO can exist in the (S)- or (R)-enantiomer. The half-life to racemization was determined to be 1.8 days, which allowed for the separation and characterization of these atropisomers using HPLC and NMR. Further studies showed that only the (S)-trans-isomer had the biological activity towards the NK$_1$ receptor. Their early decision to pursue an enantiopure drug led to the development of a structurally rigidified compound where the amide and the naphthyl group were linked in an eight-membered ring, thereby locking it in the desired (S)-trans form. However, the compound was still able to rotate rapidly about the aryl-CO axis, thus they needed to modify the structure even further to stabilize it in a single conformation and eliminate all concerns about atropisomerism. This work highlights the complications associated with developing a potential drug candidate that is atropisomeric since it requires separation, characterization and complicated developments to finally obtain the desired atropisomeric conformation.

The synthesis of atropisomerically stable analogs can also improve the selectivity and potency of each atropisomer towards its target. This is important as the non-binding conformations may possess activities towards other proteins leading to side effects. Yoshida and coworkers have recently synthesized lamellarin analogs, discovering that methyl substitution adjacent to a chiral axis resulted in the formation of stable atropisomers (Figure 1.5). Upon separation, they evaluated each atropisomer against a panel of kinases and found each atropisomer to possess strikingly different kinase selectivity profiles. The
(R)-enantiomer was potent but not selective showing inhibition of nearly all the kinases in the panel while the (S)-enantiomer was shown to selectively inhibit only three of the tested kinases: GSK-3α/βm PIM1 and DYRK1A. This illustrates the effect that atropisomerism can have on target selectivity.

LaPlante et al studied a series of anthranilic acids that were developed to inhibit the NS5B polymerase of hepatitis C virus. The parent compound interconverts between the (R)- and (S)-enantiomers with a half-life to racemization of approximately 70 min, making it a class 2 compound. Similar to the case of thalidomide, this compound could not be separated since it rapidly interconverts under physiological conditions. This study shows the potential off target effect of atropisomers with the (R)-enantiomer binding to the HCV polymerase target, and the (S)-enantiomer binding an unrelated HIV matrix target.

We have hypothesized that the selective rigidification (or preorganization) of rapidly interconverting axis of chirality in biologically active scaffolds may lead to analogs that possess fewer side effects due to the removal of off-target effects from non-relevant conformations. We have performed calculations that predict atropisomeric rigidification
around an axis restricts rotation to 60-80° of rotational freedom, meaning that the other conformers that do not bind the relevant target and might possess off target activities, are now precluded from the system. Additionally the effective concentration of the active conformation will be increased (compared to the rapidly racemizing mixture) resulting in modest gains in affinity. Finally the preorganization of these scaffolds may reduce the entropic penalty of binding.\textsuperscript{15}

Because of this, we sought out to define the utility of atropisomerism as a selectivity factor in medicinal chemistry. While the discussed literature illustrates the benefits of enantiopure stable atropisomers, these examples have largely been serendipitous, with most medicinal chemists considering atropisomerism as ‘a lurking menace’.\textsuperscript{3} We seek to demonstrate that atropisomeric preorganization can be a general strategy to obtain more selective chemical probes. Consequently, this work focuses on studying this hypothesis on therapeutically relevant compounds, specifically terephthalamide α-helix mimics and Bcl-2 small molecule inhibitors.

\textbf{1.2 Achieving Atropisomeric Preorganization}

One of the primary goals of this project is to achieve selective rigidification or preorganization of a rapidly interconverting axis of chirality in biologically active scaffolds via atropisomer selective synthesis so we can test the biological and biophysical effects of atropisomerism on protein binding. One way to achieve sufficient rigidification or preorganization is to introduce steric hindrance at strategic places around the axis of chirality to raise the barrier to rotation around the bond and yield stable analogs. A study of the steric effects on barriers to rotation done by Sternhell systematically demonstrated that the increase in effective radii correlates to an increase in the barrier to rotation around a bond.\textsuperscript{16} For instance, the presence of iodine or bromine ortho to the axis of rotation increases the barrier to rotation by about 10.9 or 10.2 kcal/mol respectively. This almost doubles the barrier to rotation when compared with fluorine, which increases the barrier to rotation by 4.6 kcal/mol, due to its smaller radius.
These structural modifications can lead to both conformational rigidification as well as the incorporation of new interactions with the target, such as hydrogen and halogen bonding. Moreover, this ortho steric effect has been shown to be an effective way to isolate atropisomers. For instance, Yang and coworkers showed that polychlorinated biphenyls (PCBs) display axial chirality. Even though they were not intentionally aiming for the atropisomeric preorganization in this work, they have studied the different neurodevelopmental effect of PCB 136 (Figure 1.7). PCB 136 atropisomers were stable enough to separate and test for biological activity but the energy of the barrier to rotation was not determined. Their study showed that (R)-PCB 136 altered dendritic growth while (S)-PCB 136 had no effect.

Current work by members of the Gustafson group has shown that introducing halogens ortho to the axis of chirality in a pyrrolopyrimidine (PPY) scaffold increased the barrier to rotation to 29.2 kcal/mol making the half-life of racemization of these molecules around 1.2 years (Figure 1.8).
In the absence of enantioselective chemistry, synthesized scaffolds can be purified through the use of chiral semi-prep HPLC to be evaluated for difference in affinity and selectivity from the parent compound in various biological assays. While this approach is reliable, and can furnish both enantiomers, it requires a large degree of effort and can be unsustainable when only one enantiomer is needed. Therefore the Gustafson group is striving to also develop enantioselective routes to these analogs through asymmetric catalysis using chiral catalysts.

1.3 RESEARCH GOALS

While the previously mentioned compounds were synthesized in a racemic fashion and separated by chiral HPLC, it would be empowering to develop an enantioselective route towards the synthesis of these atropisomers. The work discussed in this thesis is divided between two goals. The first goal was to develop a direct method for coupling a hindered benzoic acid derivative with an amine to form these scaffolds via either asymmetric catalysis or chiral auxiliary strategy. In parallel, this thesis focused on the synthesis of a specific α-helix mimic, known to inhibit the Bcl-2 anti-apoptotic proteins, in an atropisomerically stable conformation.
CHAPTER 2

RESEARCH AND SYNTHESIS APPROACH

2.1 AMIDE ATROPISOMERISM

Since atropisomerism is ubiquitous in various biological scaffolds, the work presented in this thesis is mainly focused on the effect of atropisomerism on biologically active molecules that display amide atropisomerism (Figure 2.1). For instances, the BMS Bcl-2 antagonist contain three axes of atropisomerism, each possessing limited hindrance to rotation, resulting in rapidly interconverting enantiomers, however, only one conformation actually binds Bcl-2. One of these axes was found to racemize on the hour time scale and proved to be decisive towards Bcl-2 activity. Each atropisomer was successfully isolated and tested in vitro clearly demonstrating that only one of the atropisomers inhibited Bcl-2.

Figure 2.1. Amide containing compounds that exhibit an axis of chirality.
2.2 ASYMMETRIC CATALYTIC CHEMISTRY

2.2.1 Dual Catalytic Approach

The first goal of this project was to develop a direct method for coupling a hindered benzoic acid derivative with an amine to access key biologically active benzamide scaffolds such as the BMS Bcl-2 inhibitor (Figure 2.2). We initially proposed to achieve this by utilizing both a Lewis base catalyst (e.g. DMAP) and a chiral Brönsted acidic thiourea derivative in a dual catalytic strategy inspired by seminal studies from Siedel et al. who were able to achieve kinetic resolution in amines.\(^\text{20}\) His work presents a new approach for asymmetric nucleophilic catalysis that usually relied on chiral 4-(dimethylamino)pyridine (DMAP) to achieve kinetic resolution on point chiral molecules and we sought to extend it to atropisomeric benzamides.

![Figure 2.2. Representative reaction using dual catalysis approach.](image)

The hypothesis behind this approach is that the anion of the resultant acyl pyridinium/X-ion pair will interact with the urea via H-bonding (Figure 2.3).\(^\text{21}\) This interaction will decrease any coulombic stabilization from the anion pair, destabilizing the acyl pyridinium while also bringing the thiourea’s chirality into the complex.\(^\text{22}\)

To obtain hindered carboxylic acid derivatives, we directed our attention towards C-H functionalization methods developed by Yu’s lab.\(^\text{23}\) The Yu group was able to perform monoselective ortho iodination of aryl carboxylic acids by C-H activation, using Pd(II) catalysis with (diacetoxyiodo)benzene and elemental iodine (Figure 2.4). Utilizing this chemistry, we were able to prepare a series of different diortho-substituted benzoic acids in order to test the enantioselective amidation through the dual catalysis approach (Figure 2.5).
Figure 2.3. Hypothesized interactions in a dual catalyzed system.

![Chemical Structure]

**Figure 2.4.** Pd(II)-catalyzed ortho-iodination of carboxylic acids.

![Chemical Reaction]

Commercially available ortho-substituted benzoic acids

**Figure 2.5.** Different diortho-substituted benzoic acid derivatives synthesized.

<table>
<thead>
<tr>
<th>Bennzoic Acid</th>
<th>Product</th>
<th>Temperature (°C)</th>
<th>t (day)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure" /></td>
<td><img src="image2" alt="Structure" /></td>
<td>100</td>
<td>1</td>
<td>85%</td>
</tr>
<tr>
<td><img src="image3" alt="Structure" /></td>
<td><img src="image4" alt="Structure" /></td>
<td>100-110</td>
<td>1-1.5</td>
<td>81-61%</td>
</tr>
<tr>
<td><img src="image5" alt="Structure" /></td>
<td><img src="image6" alt="Structure" /></td>
<td>130-140</td>
<td>7</td>
<td>56%</td>
</tr>
</tbody>
</table>
The trend observed in the synthesis of the various benzoic acids illustrates the effect of the group ortho to the carboxylic group on the reaction duration as well as the yields. As the groups vary from activating to deactivating, the reaction takes longer to proceed and the yields are lower.

The bis-thiourea catalyst was chosen based on its effectiveness in Siedel’s work given by its selectivity factor (s-factor). The s-factor is a measurement for the effectiveness of kinetic resolution in the formation of amines (i.e. the performance of a chiral catalyst to promote the formation of one enantiomer over the other). The synthesized bis-thiourea catalyst 7 (Figure 2.6) has an s-factor of 8.4, which was determined by HPLC analysis, making it an efficient candidate for the proposed enantioselective amidation reaction. The catalyst was synthesized from commercially available chiral diamine and isothiocynate in dry tetrahydrofuran (THF).

![Figure 2.6. Synthesis of the bis-urea catalyst.](image)

We then proceeded to evaluate the enantioselective synthesis of atropisomeric amides. The benzoic acids were activated by forming the corresponding acid chloride (Figure 2.7A). Following the conditions developed by Seidel, we evaluated a series of substrates, however, all reactions yielded racemic product mixtures (Figure 2.7B).

No selectivity was observed as well when Fu’s chiral DMAP was used or when anhydrides were employed. We hypothesized this to be due to the fact that unmodified amines tend to have high reactivity resulting in high background reaction rates with the activated acyl group. We validated this by performing the reaction with no catalyst, observing full conversion at 78ºC. In light of these results, a new approach was needed to allow the reaction to proceed through an enantioselective route and prevent the background reaction. Therefore, we proceeded to investigate the effect on enantioselectivity through the use of chiral auxiliary groups such as oxazolidinone.
2.2.2 Use of Chiral Auxiliary

Chiral oxazolidinone is a highly versatile functional group and is easily synthesized from amino acids. Its importance has been prevalent in the synthesis of biologically active natural products\(^\text{28}\) as well as asymmetric synthesis by acting as a chiral acyl transfer source.\(^\text{29}\) Thus \(N\)-acyloxazolidinones, with the use of a Lewis acid, were used to form esters, amides and hydroxamic acids.\(^\text{30}\) Interested in the acyl transfer capabilities of chiral oxazolidinones, we proceeded to implement this chemistry as a way to control the background reaction of amines. Yokomatau et al. showed the aminolysis of \(N\)-acyloxazolidinone using Lewis acids such as titanocene dichloride and zirconocene dichloride.\(^\text{31}\)

Using the previously synthesized \textit{diortho}-substituted acid chloride, we synthesized the corresponding acyloxazolidinone (Figure 2.8). Both phenyl and isopropyl substituted oxazolidinone were evaluated. While the phenyl oxazolidinone could be acylated using acid chlorides and DMAP, a strong base such as \(n\)-butyllithium was needed for the acylation of isopropyl substituted oxazolidinone due to the pKa differences between the two. The aminolysis of these acyloxazolidinones were evaluated using various amines (e.g. piperidine, benzylamine and diisopropylamine) in the presence of Lewis acids including titanocene dichloride, zirconocene dichloride and samarium triflate.\(^\text{32}\) However, instead of forming the desired amide product, the amine catalyzed the endocyclic cleavage of the oxazolidinone ring (Figure 2.9).
Figure 2.8. Synthesis of acylphenyloxazolidinone 8 and acylisopropyloxazolidinone 9.

Figure 2.9. Aminolysis of acyloxazolidinone using Lewis acid catalysis.

Davies et al. shows that the nucleophilic cleavage in the acyloxazolidinone is dependent on sterics and electronics of the attached acyl group. If the system is unhindered (i.e., the acyl group is small), exocyclic cleavage occurs to form the desired product. On the other hand, if the acyl group is large, sterics governs the unwanted endocyclic cleavage of the oxazolidinone ring. Consequently, the Davies group proposed the synthesis and use of 3,3-dimethyl-5-substituted-2-pyrrolidinone as an alternative chiral auxiliary group. The presence of the two methyl groups at the three position prevents the endocyclic cleavage and allow for the access to more hindered acyl groups. The 3,3-dimethyl-5-hydroxymethyl-pyrrolidinone 14 was synthesized over four steps (Figure 2.10). In order to be able to use the auxiliary group 14, the hydroxyl group had to be converted to an inert group by protection or further synthesis. We chose to protect the hydroxyl group using tert-butyldimethylsilylchloride
(TBSCI). 5-tert-butyldimethylsiloxymethyl-3,3-dimethyl-pyrrolidinone 16 was synthesized from 14 in one step with moderate yield (Figure 2.11A).³⁴

The synthesized chiral auxiliary group 16 was used to form the corresponding acylpyrrolidinone 17 using previously published conditions for the synthesis of acylisopropoxazolidinone (Figure 2.11B).³⁴ While a product was formed in low yields, the silane protecting appeared to be unstable under these conditions (Figure 2.12).

Figure 2.11. (A) Synthesis of 5-tert-butyldimethylsiloxymethyl-3,3-dimethyl-pyrrolidinone. (B) Synthesis of the acylpyrrolidinone.
Figure 2.12. $^1$H NMR of 5-tert-Butyldimethylsiloxymethyl-3,3-dimethyl-pyrrolidinone (A) and acypyrrolidinone (B).

2.3 Future Work

Future work will focus on investigating the use of different protecting groups or removal of the alcohol that would yield a more stable intermediate under the acylation conditions. For instance, literature precedence shows the successful conversion of the hydroxyl group to a methyl group forming 5-ethyl-3,3-dimethyl-pyrrolidin-2-one. This would offer a less reactive auxiliary group and allow for the transfer of the acyl group to an amine enantioselectivity.
CHAPTER 3

ALPHA HELIX MIMETICS

Atropisomerism has proven key in numerous Bcl-2 inhibitors. In recent years, there has been a growing interest in the synthesis of polybenzamides and terephthalamides based foldamers, known to inhibit Bcl-2 proteins (Figure 3.1), which can display amide atropisomerism. These foldamers mimic the protein secondary structure, specifically that of an α-helix, while avoiding the metabolic liability of peptides. The growing interest in these small molecules as a treatment for a wide variety of cancers render them an ideal starting point for studying the biological implications of atropisomeric preorganization.

![Figure 3.1. Small molecule atropisomeric α-helix mimetics](image)

3.1 BIOLOGICAL BACKGROUND AND RELEVANCE

Programmed cell death, apoptosis, is a highly regulated process that is important for tissue homeostasis and its deregulation contributes to various diseases including cancer. Apoptosis is controlled through two major pathways: the extrinsic death receptor pathway or
the intrinsic mitochondrial pathway. This intrinsic pathway is mainly regulated by members of the Bcl-2 family proteins, which either promote apoptosis through the pro-apoptotic proteins or inhibit apoptosis through the anti-apoptotic proteins (Figure 3.2).

Figure 3.2. Bcl-2 anti-apoptotic proteins bound to OMM (Left). Intrinsic activated apoptosis through the inhibition of Bcl-2 anti-apoptotic protein by the pro-apoptotic protein (Right).

The pro-apoptotic members of the Bcl-2 family are composed of “effectors,” such as Bcl-2 associated X protein (BAX), and “BH3-only” proteins that include Bcl-2 interacting domain (BID) and Bcl-2 interacting mediator of death (BIM). The anti-apoptotic proteins, like Bcl-A1, Bcl-xL and myeloid cell leukemia sequence 1 (MCL-1), keep the outer mitochondrial membrane (OMM) intact by preventing the pro-apoptotic proteins from forming pores in the OMM and initiating the release of caspases that lead to apoptosis. In other words, in normal cells, apoptosis is mediated through the inhibition of the anti-apoptotic Bcl-2 family proteins by the action of the BH3-only proteins and the expression of activators of the pro-apoptotic proteins.

The interaction between anti- and pro-apoptotic proteins has been the target of small molecule design for various cancer treatments. This system is regulated by key protein-helical interactions (PHIs). The anti-apoptotic proteins, which are overexpressed in cancer cells, contain a surface groove that binds to the α-helical BH3 domains of the pro-apoptotic
proteins to sequester their activity.\textsuperscript{39} The NMR structure of Bcl-xL bound to the BH3 domain of Bak shows the hydrophobic interaction between Bcl-xL and residues Val74, Leu78, Ile81 and Ile85 corresponding to the \(i, i+4, i+7\) and \(i+11\) of the \(\alpha\)-helix domain.\textsuperscript{32}

The cell regulates this process by activating the BH3 only proteins, BID and BIM, which sequester the anti-apoptotic members and allow for cell apoptosis. Small molecules that mimic this BH3 domain can bind the groove of the anti-apoptotic proteins and restore the death pathway in cancer cells.\textsuperscript{40} A series of BH3 mimetic drugs, such as ABT-199, TW-37, or BMS inhibitor mentioned previously, have been developed to specifically bind and inhibit anti-apoptotic proteins.\textsuperscript{41} Furthermore, polybenzamides or terephthalamides are recently shown to mimic the \(\alpha\)-helix domain but they possess one or several rapidly interconverting atropisomeric axes and an enantioselective method for the synthesis of these molecules has not been developed yet.

### 3.2 Alpha Helix Mimetics as Small Molecule Inhibitors

Hamilton and co-workers have designed small molecule peptide mimetics that display side chain mimics that overlap with the \(i, i+4, i+7\) positions of an \(\alpha\)-helix side chains (Figure 3.3).\textsuperscript{42} These \(\alpha\)-helix mimetics have an immense pharmaceutical potential as they play a role in inhibiting protein-protein interactions.\textsuperscript{43}

![Figure 3.3](image.png)
Evaluation of these terephthalamide scaffolds, using in vitro fluorescence polarization assays as well as cell assays, show effective pan Bcl-2 anti-apoptotic protein inhibition with nanomolar potency. Hamilton et al. observed that the terephthalamide exists as a mixture of interconverting atropisomers through NMR studies, with only the (S)-atropisomer displaying affinity. The (S)-atropisomer is the Bcl-2 relevant conformation as it mimics the \( i, i+4, i+7 \) of the \( \alpha \)-helix (Figure 3.4A). Interestingly, the (R)-atropisomer displays a different facial orientation switching the aryl side chain to the \( i+4 \) to the \( i+5 \) (6) position, mimicking an orientation that is currently not represented in the foldamers literature (Figure 3.4). In addition, the synthesis of the non-relevant atropisomer that mimics a completely different helical sequence will allow the access to novel foldamers for biological testing that is unprecedented in literature. Therefore this system is ideal to study the effect of atropisomer preorganization on protein binding. Previous literature and our preliminary molecular modeling (MMFF94 force field) predict that the addition of two bulky substituents ortho to the amide atropisomeric axis, such as bromine or a trifluoromethyl group, will sufficiently rigidify the barrier to rotation around the axis rendering the system conformationally stable (Figure 3.5). Importantly as we will be separating diastereomeric atropisomers, each rigidified analog will mimic only one helical sequence, improving the selectivity of these foldamers. Finally the (R)-atropisomer is predicted to mimic a multifacial interaction, a feat that is unprecedented in the foldamers literature, even though multi facial interactions account for almost 40 percent of documented protein-helical interactions.
3.3 ALPHA HELIX MIMETICS SYNTHESIS

With this in mind, we set out to develop a modular and scalable synthesis of atropisomerically rigidified terephthalamides. We initially focused on analogs of some of Hamilton’s most potent analogs (Figure 3.6A),\(^{44}\) planning to rigidify the axis at a late stage via Lewis base catalyzed bromination (Figure 3.6B).

The synthesis of the terephthalamide derivative is shown in Figure 3.7. First the 4-amino-3-hydroxybenzoic acid is converted to the corresponding methyl ester by Fisher Esterification (compound 19). The isopropoxy group was introduced by alkylation using 2-bromopropane to form 4-amino-3-isopropoxybenzoic acid methyl ester 20. The iodo
substituent 22 was formed using the Sandmeyer reaction after the demethylation of the benzoic acid followed by fisher esterification again to perform Stille coupling to form 3-isopropoxy-4-vinyl-benzoic acid 24. The work up post the Stille coupling reaction involved the use of sodium hydroxide solution which cleaved the methyl ester group. Finally the amide bond was formed by having the diisopropyl amine attacking the acid chloride to form compound 25.\textsuperscript{45}

After synthesizing compound 25, the goal was to dibrominate before proceeding further with the synthesis. After a literature search, a method to brominate benzanides selectively was found through the use of 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) to brominate at the 6 position and n-butyllithium and elemental bromine to brominate at the 3 position.\textsuperscript{45} However, these methods yielded the mono-brominated product only or a mixture of the 3- and 6-brominated benzamide. We then turned to the group’s Lewis base catalyzed halogenation.\textsuperscript{47} However, a small scale reaction monitored by NMR showed that this system was also reactive towards the vinyl group. The reaction was repeated again after the oxidation of the vinyl group but the dibrominated product did not form and a new synthesis route was needed.

We proceeded to synthesize the terephthalamide through a new synthesis route that would allow for late stage bromination using our catalytic bromination chemistry (Figure 3.8). We started by iodinating inexpensive 3-hydroxybenzoic acid 26, followed by Fisher esterification to form the corresponding methyl ester compound 28.

Bromination of compound 29 yielded a 1:1 mixture of the mono- and dibrominated product, which required separation by flash column chromatography. The purified dibrominated compound, identified by NMR, was used for the synthesis of the benzamide but the reaction did not proceed since the carbonyl carbon is now too hindered.

Using the intermediate, 3-hydroxy-4-iodo-benzoate methyl ester 28, we decided to synthesize the benzamide 33 through the alkylation of the free hydroxyl group (Figure 3.9). Saponification followed by formation of the acid chloride and amidation with diisopropyl amine gave 33 with high yield. The bromination reaction proceeded faster in the presence of the hydroxyl group so the 4-Iodo-3-isopropoxy-benzamide was then dealkylated using boron tribromide (BBr\textsubscript{3}) to obtain the free hydroxyl group 34. A solvent scan of chloroform, methanol, dichloromethane (DCM), acetonitrile and benzene showed that the bromination
Figure 3.7. Part 1 of the synthesis of terephthalamide skeleton before bromination.

reaction in methanol yielded a 2:1 ratio of the 2,6- and 2,5-dibromo-3-hydroxy-4-iodo-N,N-diisopropylbenzamide (Figure 3.10). Despite the low yield of compound 35 and the necessity for separation, we proceeded to form the vinylated product using the Stille chemistry, but the reaction yielded a mixture of undesired products. Thus another modification was needed to form the desired compound selectively and with better yields.

In order to avoid the mixture of product formed after the Stille reaction, a literature search yielded a method that allows for the carbonylation of the iodine in one pot procedure. Barret et al. adopted a Pd catalyzed method to selectively carbonylate a tribromominated benzamide scaffold. Inspired by this chemistry, we attempted the
carbonylation of the previously synthesized compound 34 and successfully substituting the iodine for a methyl acetate group (Figure 3.11). This not only saved three steps of synthesis but added a *meta*-directing group that would facilitate the formation of the desired dibrominated product upon addition of NBS and the phosphine sulfide catalyst. Surely, repeating the bromination chemistry on compound 37 yielded one product 38. 2D NMR analysis confirmed the presence of the bromines ortho to the amide axis (Figure 3.12, 3.13). The heteronuclear multiple bond correlation (HMBC) study correlates the only remaining hydrogen atom at 8.02 ppm (A) with both the hydroxyl hydrogen (B) and the methyl ester (C) (Figure 3.10 indicated by blue arrows) confirming the structure of compound 38.
Figure 3.9. Second synthesis route for the terephthalamide scaffold.

Figure 3.10. 2,6-Dibromo-3-hydroxy-4-iodo-N,N-diisopropylbenzamide (Right) versus 2,5-dibromo-3-hydroxy-4-iodo-N,N-diisopropylbenzamide (Left).
Figure 3.11. Final synthesis of the atropisomeric terephthalamide scaffold.

Figure 3.12. $^1$H and $^{13}$C NMR shifts for Compound 38.
O-alkylation of compound 38 yielded methyl 3,5-dibromo-4-(diisopropylcarbamoyl)-2-isopropoxybenzoate, compound 39, which was coupled to L-leucine methyl ester to generate the terephthalamide 40. The NMR and HPLC spectra of compound 40 showed two diastereomers that were easily separated by flash column chromatography for further studies (Figure 3.14, 3.15).
Figure 3.15. (A) $^1$H NMR of Compound 38 showing the diastereomeric aromatic Hs (circled). (B) $^1$H NMR overlay of the separated diastereomers (shown in green and red).
3.4 Future Work

Utilizing the developed chemistry and the separated terephthalamide scaffolds, we aim to pursue further characterization and modifications of these scaffolds to understand the effect of atropisomer preorganization. Ongoing work includes kinetic studies that will determine the barrier to rotation of the separated diastereomers. Also, each terephthalamide diastereomer will be evaluated for potency and selectivity across a panel of protein-helical interactions. Isothermal titration calorimetry will be used for validation, as well as to probe changes in the enthalpic and entropic contribution to binding of the analog. Finally, we are also pursuing modern palladium cross coupling to access analogs with diverse conformational locking groups.

3.5 Experimental Procedure

All reactions were carried out under an argon balloon unless otherwise stated. $^1$H, and $^{13}$C NMR spectra were recorded on Varian VNMRS 400 MHz and Varian Inova 500 MHz spectrometers at 24 °C. All chemical shifts are reported in ppm (δ) referenced to solvent resonances ($^1$H NMR: 7.27 for CDCl$_3$, 3.31 for CD$_3$OD, 2.052 for acetone-$d_6$, and 2.50 for (CD$_3$)$_2$SO and $^{13}$C NMR: 77.1 for CDCl$_3$, 49.0 for CD$_3$OD, 30.81, 206.55 for acetone-$d_6$, and 39.52 for (CD$_3$)$_2$SO). All starting materials were purchased from Acros Organics, Sigma Aldrich or Fisher Scientific. All flash column chromatography (FCC) was performed using 60 Silica Gel purchased from Fisher Scientific.

3.5.1 Synthesis of Compound 27

To a solution of 3-hydroxybenzoic acid (10g, 72.4 mmol) in ammonium hydroxide (25.3ml) was added drop wise a solution of I$_2$ (14.7g, 57.9 mmol), and KI (11.5g, 69.5 mmol) in water (72.6 ml, 0.96M). The mixture was stirred at room temperature for 1hr. The mixture was concentrated under reduced pressure to half its volume and the pH was adjusted to 1 with a 6N aqueous solution of hydrochloric acid. The white solid was collected by filtration and washed by small amount of water. (7.63g, 44%)

$^1$H NMR (DMSO-$d_6$, 400 MHz) δ 10.68 (s, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.42 (d, J = 1.9 Hz, 1H), 7.13 (dd, J = 8.1, 1.9 Hz, 1H); $^{13}$C NMR (DMSO-$d_6$, 400 MHz) δ 167.38, 157.24, 139.49, 132.71, 121.94, 115.47, 91.30. MS (ESI): Calculated C$_7$H$_3$IO$_3^{-}$ [M-H] 263.02. Found: 263.27 m/z.
3.5.2 Synthesis of Compound 28

To a solution of 3-hydroxy-4-iodobenzoic acid (7.63g, 28.9 mmol) in methanol (72.3 ml), thionyl chloride (2.3 ml, 31.8 mmol) was added drop wise and the reaction was mixed at 60°C for 3hr. The reaction mixture was cooled down to room temperature and the solvent was evaporated under vacuum to yield the intermediate 28.

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 7.75 (d, $J = 8.2$ Hz, 1H), 7.64 (d, $J = 1.9$ Hz, 1H), 7.32 (dd, $J = 8.2$, 1.9 Hz, 1H), 5.84 (s, 1H), 3.91 (s, 3H);

$^{13}$C NMR (CDCl$_3$, 500 MHz)
166.51, 155.17, 138.61, 132.18, 122.99, 115.81, 91.64, 52.43.

3.5.3 Synthesis of Compound 32

To a solution of methyl 3-hydroxy-4-iodobenzoate 28 (7.5 g, 27 mmol) in acetone (90 ml), 2-bromopropane (5.1 ml, 54 mmol) and potassium carbonate (7.5g, 54 mmol) were added. The mixture was refluxed overnight and 30 ml of ammonium hydroxide was added. The solution was refluxed for an additional 30 min and then cooled to room temperature. The mixture was extracted with water and ethyl acetate. The organic layer was dried over Na$_2$SO$_4$. The crude mixture was purified by column chromatography on silica gel (hexanes: EtOAc/8:1) to yield 32 (5.12g, 62%).

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 7.89 (d, $J = 8.1$ Hz, 1H), 7.49 (s, 1H), 7.41 (d, $J = 9.9$ Hz, 1H), 4.86 - 4.58 (m, 1H), 1.42 (d, $J = 6.0$ Hz, 6H); $^{13}$C NMR (CDCl$_3$, 500 MHz) 171.04,
157.01, 139.81, 130.45, 123.65, 114.39, 96.33, 72.40, 21.98. **MS (ESI): Calculated**

C$_{10}$H$_{11}$IO$_3$ [M-H] 305.10. **Found:** 305.20 m/z

![Figure 3.18. Compound 32.](image)

### 3.5.4 Synthesis of Compound 33

4-Iodo-3-isopropoxybenzoic acid was 32 (5.56 g, 18.2 mmol) was dissolved in dichloromethane (276 ml); then 3.1 ml oxalyl chloride (36.4 mmol) was slowly added in an ice bath. Drops of DMF were added and the mixture was stirred under room temperature for 2h. The solvent was evaporated under vacuum and the crude material was kept under vacuum for 1h. To the solution of 4-iodo-3-isopropoxy-benzoic acid chloride in CH$_2$Cl$_2$ (276 ml) at 0°C, N,N-diisopropylamine (6.4 ml, 45.5 mmol) was added slowly and the resulting mixture was stirred at room temperature overnight. The solution was extracted with 10% citric acid and CH$_2$Cl$_2$. The crude mixture was purified by column chromatography on silica gel (hexanes: EtOAc/ 6:1) to yield 33 (5.74 g, 81%).

$^1$H NMR (CDCl$_3$, 500 MHz) δ 7.76 (dd, $J = 7.9$ Hz, 1H), 6.76 (s, 1H), 6.62 (dd, $J = 7.9$ Hz, 1H), 4.64 – 4.54 (m, 1H), 3.97 – 3.45 (m, 2H), 1.61 – 1.41 (m, 6H), 1.39 (dd, $J = 3.9$ Hz, 6H), 1.35 – 1.15 (m, 6H): $^{13}$C NMR (CDCl$_3$, 500 MHz) 169.94, 156.89, 140.13, 139.55, 119.31, 111.51, 88.76, 72.28, 47.52, 22.07, 20.71, 19.31. **MS (ESI): Calculated**

C$_{16}$H$_{24}$INO$_2$$^+$ [M+H] 390.27. **Found:** 390.13 m/z.

![Figure 3.19. Compound 33.](image)
3.5.5 Synthesis of Compound 34

To 4-Iodo-3-isopropoxy-\(N,N\)-diisopropoxybenzamide 33 (0.900g, 2.3 mmol) in dry CH\(_2\)Cl\(_2\) (11.5 ml), 1.0 M BB\(_3\) solution in CH\(_2\)Cl\(_2\) (11.5 ml, 11.5 mmol) was added dropwise at -78°C.\(^{49}\) After stirring overnight at room temperature, the reaction was quenched with water and extracted with ethyl acetate. The organic layer was dried over Na\(_2\)SO\(_4\) and concentrated to yield 34 (0.65 g, 82%).

\(^1\)H NMR (CDCl\(_3\), 500 MHz) \(\delta\) 9.31 (s, 1H), 7.58 (d, \(J = 8.0\) Hz, 1H), 6.69 (d, \(J = 1.8\) Hz, 1H), 6.44 (dd, \(J = 8.0, 1.8\) Hz, 1H), 3.93 – 3.43 (m, 2H), 1.61 – 1.37 (m, 6H), 1.24 – 1.00 (m, 6H): \(^{13}\)C NMR (CDCl\(_3\), 500 MHz) 171.45, 156.31, 147.57, 138.09, 117.90, 113.47, 86.15, 51.37, 46.23, 20.50.

![Figure 3.20. Compound 34.](image)

3.5.6 Synthesis of Compound 37

3-Hydroxy-4-iodo-\(N,N\)-diisopropylbenzamide 34 (0.300g, 0.86 mmol), palladium acetate (0.019g, 0.09 mmol) and XPhos (0.041g, 0.09 mmol) were combined in a flask and placed under an inert argon atmosphere.\(^{48}\) Degassed methanol (17.2 ml) and trimethylamine (2.4 ml, 17.2 mmol) were then added to the flask. A balloon filled with carbon monoxide was placed on the flask. The reaction was heated at 60°C overnight. The reaction was then allowed to cool to room temperature and diluted with dichloromethane (30 ml). The organic layer was washed with 0.1 M hydrochloric acid, separated and dried over Na\(_2\)SO\(_4\). The crude residue was purified by column chromatography on silica gel (hexanes: EtOAc/ 8:1) to yield 37 (0.177 g, 74%).

\(^1\)H NMR (CDCl\(_3\), 500 MHz) \(\delta\) 10.81 (s, 1H), 7.85 (d, \(J = 8.1\) Hz, 1H), 6.88 (d, \(J = 1.3\) Hz, 1H), 6.80 (dd, \(J = 8.1, 1.5\) Hz, 1H), 3.95 (s, 3H), 3.84 – 3.41 (m, 2H), 1.52 (s, 6H), 1.13 (s, 6H): \(^{13}\)C NMR (CDCl\(_3\), 500 MHz) 170.12, 169.30, 161.69, 145.88, 130.52, 116.34, 114.41, 112.33, 52.38, 50.86, 45.95, 20.61. MS (ESI): Calculated C\(_{15}\)H\(_{21}\)NO\(_4\)\(^+\) [M+H] 280.33. Found: 280.17 m/z.
3.5.7 Synthesis of Compound 38

To a solution of methyl 4-(diisopropylcarbamoyl)-2-hydroxybenzoate 37 (0.449g, 1.6 mmol) in chloroform (27 ml), tributylphosphine sulfide (51µl, 0.32 mmol) and N-bromosuccinimide (0.954g, 4.8 mmol) were added. The reaction was stirred at room temperature overnight. The solvent was evaporated and the crude mixture was purified by column chromatography on silica gel (hexanes: EtOAc/ 8:1) to yield 38 (0.59g, 84%).

$^{1}$H NMR (CDCl$_3$, 500 MHz) δ 11.49 (s, 1H), 8.02 (s, 1H), 3.99 (s, 3H), 3.59 – 5.50 (m, 2H), 1.60 (t, $J$ = 6.3 Hz, 6H), 1.24 (dd, $J$ = 6.6, 1.2 Hz, 6H); $^{13}$C NMR (CDCl$_3$, 500 MHz) 168.94, 164.86, 157.85, 146.51, 132.80, 113.43, 109.69, 108.03, 53.15, 51.75, 46.56, 21.05 20.86, 20.08, 20.05. MS (ESI): Calculated C$_{15}$H$_{19}$Br$_2$NO$_4$ $^+\ [M+H]$ 437.12. Found: 438.00 m/z.

3.5.8 Synthesis of Compound 39

To a solution of 2,6-dibromo-3-hydroxy-4-iodo-N,N-diisopropylbenzamide 38 (0.580g, 1.3 mmol) in DMF (3 ml), potassium carbonate (0.373g, 2.7 mmol) and 2-iodopropane (0.27 ml, 2.7 mmol) were added. The reaction was stirred at 80°C for 4hrs. The reaction mixture was extracted with 1M HCl solution and ethyl acetate. The organic layer was combined, dried over Na$_2$SO$_4$ and concentrated under vacuum to yield 39 (0.58g, 94%).
$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 7.89 (s, 1H), 4.38 (hept, $J$ = 6.2 Hz, 1H), 3.90 (s, 3H), 3.52 (dt, $J$ = 13.6, 6.8 Hz, 1H), 3.45 (dt, $J$ = 13.2, 6.6 Hz, 1H), 1.57 (dd, $J$ = 6.8, 1.8 Hz, 6H), 1.32 (d, $J$ = 6.1 Hz, 3H), 1.29 (d, $J$ = 6.1 Hz, 3H), 1.20 (dd, $J$ = 8.5, 6.7 Hz, 6H): $^{13}$C NMR (CDCl$_3$, 500 MHz) 164.99, 164.83, 154.24, 144.71, 134.25, 127.29, 118.27, 113.10, 79.17, 52.60, 51.73, 46.49, 22.26, 21.94, 20.91, 20.86, 20.07, 20.00.

Figure 3.23. Compound 39.

3.5.9 Synthesis of Compound 40

To a solution of methyl 3,5-dibromo-4-(diisopropylcarbamoyl)-2-isopropoxybenzoate 39 (0.140g, 0.3 mmol), N,N'-dicyclohexylcarbodiimide (0.0619g, 0.3 mmol), hydroxybenzotriazole (0.0446g, 0.33 mmol) in anhydrous CH$_2$Cl$_2$ (10 ml), trimethylamine (0.05 ml, 0.33 mmol) and L-leucine methyl ester (0.0479g, 0.33 mmol) were added. The reaction was stirred at room temperature overnight. The reaction mixture was washed with saturated NaHCO$_3$ solution. The organic layer was combined, dried over Na$_2$SO$_4$ and concentrated under vacuum. The crude mixture was purified by column chromatography on silica gel (CH$_2$Cl$_2$: Acetone/ 98:2, 95:5) to yield two diastereomers of 39 (0.110g, 62%).

$^1$H NMR (CDCl$_3$, 500 MHz) of Compound 39.1 $\delta$ 8.18 (s, 1H), 7.88 (d, $J$ = 7.6 Hz, 1H), 4.80 – 4.73 (m, 1H), 4.71 – 4.64 (m, 1H), 3.77 (s, 3H), 3.56 (dd, $J$ = 13.6, 6.8 Hz, 1H), 3.50 (dd, $J$ = 13.2, 6.6 Hz, 1H), 1.78 – 1.69 (m, 2H), 1.69 – 1.63 (m, 1H), 1.60 (dd, $J$ = 6.8, 2.7 Hz, 6H), 1.40 (d, $J$ = 6.2 Hz, 3H), 1.34 (d, $J$ = 6.2 Hz, 3H), 1.24 (dd, $J$ = 11.7, 6.6 Hz, 6H), 0.99 (dd, $J$ = 5.9, 5.0 Hz, 6H): $^{13}$C NMR (CDCl$_3$, 500 MHz) 172.09, 164.03, 162.18, 151.35, 143.39, 133.67, 128.64, 115.77, 113.60, 77.81, 51.28, 50.78, 50.51, 45.53, 40.80, 24.02, 21.64, 21.06, 20.73, 20.43, 19.92 (d, $J$ = 2.7 Hz), 19.06 (d, $J$ = 2.6 Hz). MS (ESI): Calculated C$_{24}$H$_{36}$Br$_2$N$_2$O$_5$$^+$ [M+H] 593.36. Found: 593.13 m/z.

$^1$H NMR (CDCl$_3$, 500 MHz) of Compound 39.2 $\delta$ 8.21 (s, 1H), 8.04 (d, $J$ = 7.6 Hz, 1H), 4.79 (td, $J$ = 8.1, 5.5 Hz, 1H), 4.71 (dt, $J$ = 12.4, 6.2 Hz, 1H), 3.78 (s, 3H), 3.56 (dd, $J$ =
$13.6, 6.8 \text{ Hz, } 1\text{H}), 3.50 \text{ (dt, } J = 13.2, 6.6 \text{ Hz, } 1\text{H}), 1.72 \text{ (m, } 2\text{H}), 1.67 – 1.62 \text{ (m, } 1\text{H}), 1.60 \text{ (dd, } J = 6.8, 2.1 \text{ Hz, } 6\text{H}), 1.49 \text{ (d, } J = 6.2 \text{ Hz, } 3\text{H}), 1.27 \text{ (d, } J = 6.3 \text{ Hz, } 3\text{H}), 1.26 – 1.21 \text{ (m, } 6\text{H}), 0.97 \text{ (dd, } J = 6.1, 3.2 \text{ Hz, } 6\text{H}): \text{ } ^{13}\text{C NMR (CDCl}_3, 500 \text{ MHz)} 171.98, 164.03, 161.94, 151.34, 143.48, 133.62, 128.53, 115.66, 113.61, 77.95, 51.25, 50.74, 50.39, 45.55, 40.90, 23.88, 21.68, 21.07, 21.00, 19.98 \text{ (d, } J = 5.2 \text{ Hz), 19.73, 19.05 \text{ (d, } J = 1.8 \text{ Hz). MS (ESI): Calculated } \text{C}_{24}\text{H}_{36}\text{Br}_2\text{N}_2\text{O}_5^+ [M+H] 593.36. \text{ Found: 593.13 m/z.}$

Figure 3.24. Compound 40.
REFERENCES

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APPENDIX A

NMR APPENDIX
Figure A.1. $^1$H of 3-Hydroxy-4-IodoBenzoic Acid.
Figure A.2. $^{13}$C of 3-Hydroxy-4-Iodobenzoic Acid.
Figure A.3. $^1$H of Methyl 3-Hydroxy-4-Iodobenzoate.
Figure A.4. $^{13}$C of Methyl 3-Hydroxy-4-Iodobenzoate.
Figure A.5. $^1$H of 4-Iodo-3-IsopropoxyBenzoic Acid.
Figure A.6. $^{13}\text{C}$ of 4-Iodo-3-IsopropoxyBenzoic Acid.
Figure A.7. $^1$H of 4-Iodo-3-Isopropoxy-N,N-Diisopropylbenzamide.
Figure A.8. $^{13}$C of 4-Iodo-3-Isopropoxy-N,N-Diisopropylbenzamide.
Figure A.9. $^1$H of 3-Hydroxy-4-Iodo-$N,N$-Diisopropylbenzamide.
Figure A.10. $^{13}$C of 3-Hydroxy-4-Iodo-N,N-Diisopropylbenzamide.
Figure A.11. $^1$H of Methyl 4-(Diisopropylcarbamoyl)-2-Hydroxybenzoate.
Figure A.12. $^{13}$C of Methyl 4-(Diisopropylcarbamoyl)-2-Hydroxybenzoate.
Figure A.13. $^1$H of Methyl 3,5-Dibromo-4-(Diisopropylcarbamoyl)-2-Hydroxybenzoate.
Figure A.14. $^{13}$C of Methyl 3,5-Dibromo-4-(Diisopropylcarbamoyl)-2-Hydroxybenzoate.
Figure A.15. $^1$H of Methyl 3,5-Dibromo-4-(Diisopropylcarbamoyl)-2-Isoproxybenzoate.
Figure A.16. $^{13}$C of Methyl 3,5-Dibromo-4-(Diisopropylcarbamoyl)-2-Isopropoxybenzoate.
Figure A.17. $^1$H of Methyl 2-(3,5-Dibromo-4-(Disopropylcarbamoyl)-2-Isopropoxybenzamido)-4-
Methylpentanoate (1).
Figure A.18. $^{13}$C of Methyl 2-(3,5-Dibromo-4-(Diisopropylcarbamoyl)-2-Isopropoxybenzamido)-4-Methylpentanoate (1).
Figure A.19. $^1$H of Methyl 2-(3,5-Dibromo-4-(Diisopropylcarbamoyl)-2-Isoproxybenzamido)-4-Methylpentanoate (2).
Figure A.20. $^{13}$C of Methyl 2-(3,5-Dibromo-4-(Diisopropylcarbamoyl)-2-Isopropoxybenzamido)-4-Methylpentanoate (2).