Synthesis of Sulfonamides towards Down-Modulation of CD4, gp160, and TSHR

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Abstract

The macrocyclic polyamine, cyclotriazadisulfonamide (CADA), is a small molecule that is known to inhibit the entry of human immunodeficiency virus (HIV). The mechanism of action involves the binding of CADA to the signal peptide (SP) of human CD4 (hCD4) inside the membrane of the endoplasmic reticulum (ER), blocking the translocation of the protein into the ER lumen, and ultimately preventing the transport of the mature protein to the cell membrane. Through an alanine scan experiment, it was determined that the glutamine at position 15, lysins at positions 26 and 27, and a proline at position 20 of hCD4 were important for CADA’s potency. The most potent CADA analogue synthesized was CK147 which contains a para-N,N-dimethylbenzenesulfonyl side arm that is believed to interact with glutamine 15 of the hCD4 through dipole-dipole interactions. This study has created CADA analogues that retain the para-N,N-dimethylbenzenesulfonyl side arm, while modifying the tosyl side arm of CK147 to better understand the interaction of CADA with CD4. The goal of this study is to create disulfonamides that target signal peptides to down-modulate the expression of CD4, gp160, and TSHR. Of the compounds tested for CD4 down-modulation, TL020 and TL029 were found to be the most potent CADA analogues with IC_{50} values of 0.053 ± 0.001 μM. Several gold containing compounds were proposed to be synthesized to target gp160 due to the presence of methionines found in the hydrophobic region of gp160’s SP. Compounds containing hydrogen bond donor, hydrogen bond acceptor, and guanidino side arms were proposed for activity towards TSHR since the SP of TSHR contains charged amino acids.
Dedicated to

My parents, Duyen and Anna

and

my wife, Karen

*For their unconditional support and always believing in me no matter what*
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As a refugee from Vietnam, I’ve had my fair share of hardships and difficulties. As a child I would tell people that someday I would obtain my Ph.D., which at the time seemed impossible. This dissertation reflects the culmination of the last 5 years’ worth of hard work and determination to make my dream come true. It is a testament of my willingness to fight against all odds to reach my goals, despite suffering from illnesses that has been a constant hinderance in my education. I could not have done this without special people in my life that have helped me realize my dream in many ways.

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Chapter 1

Introduction
1.1 Drug Targets and Signal Peptides

Drug targets can be sorted into various classes and families, with most of them being receptors and enzymes. The largest percentage of drug targets were found to be associated with synaptic and neuroeffector junctions and the central nervous system.\(^1\) Drug target identification and validation are important steps in drug development. For a target to be considered, it must be accessible to the drug molecule and a biological reaction must be induced because of the drug interacting with the target. Once a target has been identified, validation of the target is then done through functional studies. These can be done through gene knockout studies, or inducing inhibition with small molecules, peptides, antibodies, or other inhibitors. These reactions can be measured *in vitro* and *in vivo*. Figure 1.1.1 shows the distribution of first-in-class drugs according to the molecular type and target family.\(^1\)

![Figure 1.1.1. Distribution of first-in-class drugs according to molecular type and target family.](image)

Distribution of first-in-class drugs according to the molecular type and target family:

- G-protein coupled receptors: 13.0%
- Kinase: 10.0%
- Protease: 8.0%
- Ion channel: 7.0%
- Nuclear Hormone Receptor: 1.0%
- Other Receptor: 7.0%
- Cytokine: 4.0%
- Other Mechanism: 19.0%
- Other Enzyme: 23.0%
- Unknown: 8.0%
A new drug target has been identified through interaction of a small molecule inhibiting expression of a glycoprotein. The small molecule cyclotriazadisulfonamide (CADA) has a novel mechanism of action, which targets the signal peptide of the glycoprotein CD4. No other small molecule has been shown to inhibit expression of a protein selectively through the signal peptide.

The signal peptide (SP) is a short peptide, roughly 16-32 amino acids long, that is present in the N-terminus of proteins that undergo the secretory pathway. Newly translated secretory proteins move from the endoplasmic reticulum (ER) to the Golgi apparatus through transport vesicles, then to the Golgi cisternae, which leads to the secretory transport vesicles. They are then secreted to the cell exterior surface by protein conducting channels. Transmembrane proteins, such as CD4, are transported from the ER membrane to the plasma membrane by the same mechanism. Signal peptides have three regions: a hydrophilic N-region, a central, hydrophobic alpha helical H-region, and a polar C-region

![General signal peptide sequence](image)

**Figure 1.1.2.** General signal peptide sequence.

with the cleavage site as shown in Figure 1.1.2. In the case of CD4, the hydrophobic H region is very important for binding of CADA. It was found that swapping out the glutamine in the hydrophobic H-region negatively impacted the potency of CADA. This was confirmed by insertion of the hydrophobic alpha helix in the human CD4 (hCD4)
signal peptide with non CADA-sensitive signal peptides. There was a significant increase in interaction between CADA and the modified signal peptide, indicating the importance of the hydrophobic H region of CD4.\(^5\)

1.2 Cyclotriazadisulfonamide (CADA)

CADA, shown in Figure 1.2.1, is a small macrocyclic molecule that has a broad spectrum of anti-HIV activity against strains of HIV as well as SIV.\(^6\)

CADA has been determined to be a promising entry inhibitor due to its ability to down-modulate the expression of CD4, preventing HIV from binding to the host cell and inhibiting the viral replication process. The \(IC_{50}\) value, or concentration at which 50% of the maximal down-modulation of CD4 expression measured in CHO-CD4-YFP cells after 24 hours of treatment with the compound, was found to be 0.56 ± 0.05 µM for CADA.\(^7\) CADA has been found to be soluble in human, rat, and mouse plasma at a concentration of 1.9-3.1 µM. It has also been found to be stable in plasma for >200 hours.\(^6\) Studies have shown that CADA affects
human and macaque CD4+ T cells and had no effect on mouse CD4+ T cells (Figure 1.2.2). CADA has also been shown selectively down-modulate CD4 when tested on a set of 20 different cellular surface antigens (Figure 1.2.3). It was determined that the CD4 down-modulation activity of CADA is correlated with its anti-HIV potency. CADA has also been found to be synergistic with different anti-HIV drugs that utilize various mechanisms \textit{in vitro}. Down-modulation of CD4 with CADA is also reversible and very selective towards primate CD4.

It is known that CADA compounds down-modulate the expression of cell surface and intracellular CD4 receptors at the post-transcriptional level. A paper published by Vermeire \textit{et al.} detailed the novel mechanism of action for how CADA down-modulates the expression of CD4 through flow cytometry, \textit{in vitro} translation, metabolic labelling, and surface plasmon resonance analysis. CD4 has been shown to play a central role in immune response through T-cell activation. However, African green monkeys infected with the simian immunodeficiency virus (SIV) have been found to naturally down-modulate the expression of CD4 to avoid progression to acquired immune deficiency
syndrome (AIDS).\textsuperscript{11} It has also been shown to be involved with the development of autoimmune conditions such as asthma, rheumatoid arthritis, and diabetes.\textsuperscript{12}

CD4 is also the main receptor for HIV to infect host cells. It is a type I transmembrane glycoprotein which is expressed through co-translational translocation (Figure 1.2.4). These types of proteins contain a signal peptide (SP) that is near the N-terminus. The signal sequence is first recognized by a protein-RNA complex known as the signal recognition particle (SRP). This pauses the translation of the protein and allows for the complex to dock to the translocon by binding it to the SRP receptor (SR), which is located on the membrane of the endoplasmic reticulum (ER). The binding of the SRP and SR releases the SRP, which allows translation to continue. As the translation continues, the polypeptide is inserted into the translocon channel and into the ER lumen and the SP
remains within the translocon. Signal peptidase finally cleaves off the SP and the nascent chain enters into the lumen.

Structure-activity relationship studies have been conducted in order to produce more potent CADA analogs, while probing the mechanism of action, in order to further understand the interactions between CADA and its biological target. CADA is a 12-membered macrocyclic compound that can be broken up into four parts (Figure 1.2.5). The first part is the isobutylene head group, then there are the two arenesulfonamide side arms (Figure 1.2.5, X and Y), and the tail group (Figure 1.2.5, Z). Modifications of these groups have given a variety of activity and vital information into the mechanism of action of CADA and CD4.

1.3 CADA Tail Modifications

Initial studies conducted utilized symmetrical CADA analogues with identical arenesulfonyl side arms. The tail group size and properties were also modified. CADA compounds were given various tail groups including alkyl, acyl, alkoxy carbonyl, and aminocarbonyl substituents to be tested for CD4 down-modulation. It was determined through these early studies that a bulky, hydrophobic tail was necessary to improve the

![Figure 1.2.5. General structure of CADA analogs.](image-url)
potency of CADA, with the cyclohexylmethylene group being ideal (Figure 1.2.1). The cyclohexylmethyl tail gave the greatest potency to the CADA analog QJ028 in this series.

**Figure 1.3.1.** CADA tail modifications and CD4 down-modulation activities in MT-4 cells.\(^\text{13, 14}\)

### 1.4 Symmetrical Side Arm Modifications of CADA

Symmetrical side arms modifications were done initially due to the nature of the synthetic pathway for these early analogs as shown in Scheme 1.4.1. The structures were built up symmetrically so various symmetrical analogs were synthesized and compared to CADA in order to observe the different activities of these modifications. In these variations, arenesulfonamide side arms were substituted with various alkyl, aryl, and polar substituents (Figure 1.4.1).
Figure 1.4.1. CADA side arm modifications and down-modulation activities in MT-4 cells.\textsuperscript{14,16}
It was determined that the presence of at least one arenesulfonamide side arm was necessary for potency of the CADA analog. Removal of the aromatic rings showed a significant decrease in activity, as shown for compound MFS105. Increasing the steric bulk of the toluene groups, as in MFS117 and ES-KKD024 showed a large decrease in activity. The use of \( p \)-methoxybenzenesulfonamide side arms gave improved activity for compound KKD023.

1.5 Unsymmetrical Side Arm Modifications of CADA

\[
\text{Scheme 1.5.1. New synthesis pathway for CADA analogs.}^{17}
\]

Modifications to the synthesis route of CADA analogs, as shown in Scheme 1.5.1, allowed for compounds with two different side arms to be synthesized. This produced a large series of unsymmetrical CADA analogs. The \( pIC_{50} \) values for these compounds were
calculated for each structural change to the CADA analog. Assuming that the relative CD4 down-modulation potencies are determined strictly by the degree of drug binding to the target, this value is directly proportional to the negative free energy of binding.\textsuperscript{17}

This study showed that the replacement of one of the toluenesulfonamide side arms in CADA with a 4-methoxybenzenesulfonyl group (\textbf{VGD027}) increased the potency as shown in Figure 1.5.1. Replacement of the benzyl tail with a cyclohexylmethyl tail (\textbf{QJ028}) previously showed an increase in potency.\textsuperscript{13}

![Chemical structures of CADA analogs](image)

\textbf{Figure 1.5.1.} Comparison of energy effects of substituents for CD4 down-modulation relative to CADA.\textsuperscript{17}

The modification of both the 4-methoxybenzenesulfonyl side arm and the cyclohexylmethyl tail gave a further increase in potency, as shown for \textbf{VGD020}, however
the symmetrical analog VGD045 showed a significant decrease in potency relative to VGD020.\textsuperscript{17} This confirmed the idea that the cyclohexylmethyl tail is necessary for improved potency and that an unsymmetrical compound gives higher potency than its symmetrical counterpart.

Solid state structural analysis of CADA analogs via X-ray crystallography showed that CADA compounds take on an unsymmetrical conformation that is believed to be their biologically active conformation.\textsuperscript{13} It was shown that the 12-membered ring system is twisted about the isobutylene head group. One arenesulfonyl side arm is shown to be in the plane of the head group while the other is out of the plane.\textsuperscript{13,17} This places the two arenesulfonyl side arms at different orientations relative to the macrocyclic ring, resulting in a two-site binding model (Figure 1.5.2).\textsuperscript{13,17}

Synthesis and evaluation of a series of unsymmetrical CADA analogs containing the cyclohexylmethyl tail was conducted (Figure 1.5.3).\textsuperscript{18} Among these compounds, CK147 was found to be the most potent analog. Prior to this, VGD020 was the most potent analog. It was determined that substituted aromatic side arms, \textit{N,N}-dimethyl for CK147 and \textit{p}-methoxy for VGD020 are necessary for improved potency. In both cases, an electron donating substituent in the para position of the arenesulfonamide increased CD4 down-modulation activity. It was also shown that the presence of a hydrogen bond donor group (NCP001) apparently weakens the binding of the molecule, decreasing potency. Increase
in CD4 down-modulation was also noticed when electron rich arylsulfonyl groups were added as shown in VGD020, CK137, and CK147 (Figure 1.5.3). However, electron
richness alone does not properly predict increased CD4 down-modulation abilities of the CADA analog. Both CK094 and CK175 are CADA analogs that have electron rich side arms but have lower CD4 down-modulation abilities than CADA. A series of CADA analogs with an electron donating and electron withdrawing group was synthesized (Figure 1.5.4). The positioning of the substitution on the aromatic ring was shown to influence the CD4 down-modulation activity. It was discovered that CADA analogs with large dipole moments oriented in one direction (Figure 1.5.5) with an electron rich arenesulfonyl side arm had higher CD4 down-modulation activity. A correlation between the dipole moment in the plane of the aromatic ring and the pIC\textsubscript{50} value was found (Figure 1.5.6). The sulfonamide group produces a dipole moment along the x axis and is increased by positioning a strong electron donor group.

![Figure 1.5.5.](image1.png) Relative sizes of in-plane dipole moment and orientations in arenesulfonamide side arms.\textsuperscript{18}

![Figure 1.5.6.](image2.png) pIC\textsubscript{50} values versus calculated x component of dipole moments for 16 CADA analogs.\textsuperscript{18}
in the para position, or an electron-withdrawing group in the ortho position.

1.6 CADA Mechanism of Action

To understand the mechanism of action of CADA with CD4, Vermeire and company conducted a series of experiments to identify the biomolecular target. The biogenesis of CD4 in the presence of CADA was initially investigated. Through pulse-chase experiments and immunoprecipitation, it was found that CADA inhibited CD4 biogenesis in CHO·CD4+ cells without general inhibition of protein synthesis. CADA did not seem to alter the expression of cytosolic proteins in both CD4 negative and CD4 positive CHO cells. It also did not affect the expression of membrane proteins in CD4 negative cells, as well as the secretion of proteins into the membrane. It was determined that CD4 down-modulation happened after transcription since CD4 messenger RNA levels remained unaffected.

The translation and translocation of CD4 was then studied using in vitro experiments with cell-free rabbit reticulocyte lysate with or without pancreatic rough microsomes (RMs). In the absence of RMs, the normal translation of full-length and truncated CD4 proteins remained unaffected by CADA. However, in the presence of RMs, translocation of CD4 into the RM lumen was inhibited by CADA in a dose-dependent manner. Chimeric constructs were made to help identify what the specific biomolecular target for CADA, which was determined to be the CD4 SP. Through in vivo studies, it was shown that CADA affects the orientation of the growing CD4 nascent chain, causing it to point out towards the cytosol where it is degraded by proteolytic enzymes. This occurs
after the targeting and transfer of the nascent chain into the translocon but prior to the peptides reaching the luminal side of the channel. It is proposed that the N-terminal signal sequence usually inserts into the translocon tail-first, with a hairpin-looped topology and the N-terminus pointing toward the cytosol. Signal sequences with hydrophobic residues can also insert into the translocon with the N-terminus pointing towards the ER lumen as well and can then change their orientation inside the channel.

To understand the exact behavior of the signal sequence for CD4, the movement of the CD4 nascent chain inside of the translocon was investigated by glycosylation tags. Glycosylation of the N-terminus of the SP would create glycosylated products if the SP entered the channel headfirst. If the C-terminus entered in first, tail-first, the SP would be cleaved. Vermeire and company used truncated and extended hCD4 SPs to investigate the stepwise interactions of the SP within the translocon. The full-length proteins with extended hCD4 SP translocated with or without glycosylation sites and were affected by CADA in a dose-dependent manner. Varying the lengths of the proteins gave different responses to glycosylation, with shorter chains gradually decreasing in glycosylation efficacy. Vermeire et al. extended the N-terminal hydrophilic domain of hCD4 SP with 17 residues. This length was the minimum length required for optimal glycosylation to occur at the N-terminus. The fraction of glycosylated nascent chains with

![Figure 1.6.1. Glycosylation in absence or presence of CADA.](image-url)
the absence or presence of CADA is shown in Figure 1.6.1. When the C-terminus of the polypeptide was extended (17+71 residues and up), inhibition activity of CADA increased resulting in non-detectable glycosylation levels. This suggested that the nascent chain requires a minimum length for CADA to have an inhibitory effect on glycosylation. It was also determined that regardless of lengths where SP cleavage could occur, translocation with the SP cleavage was inhibited. Therefore, CADA was found to affect the vertical positioning of the growing polypeptide with the N-terminus pointing towards the ER lumen. This would then disrupt the completion of the SP inversion to the hairpin looped structure that would be cleaved. CADA apparently stabilizes a folded confirmation of the nascent chain within the translocon channel, preventing the polypeptide from reaching the luminal side of the ER. With this locked confirmation, as translation continues, translocation is stopped and the polypeptide continues to grow, looping the polypeptide out into the cytosol where it is degraded by proteolytic enzymes.

CD4 biogenesis by co-translational translocation is illustrated in Figure 1.6.2. This process begins with the recognition of the hydrophobic N-terminal SP by the SRP as the SP emerges from the ribosome. This complex then docks to a protein-conducting channel in the ER membrane (Sec61). With the SP bound to the lateral gate of the channel facing the lumen, translation by the ribosome continues. As translation continues, the SP undergoes a flip turn and binds fully to the lateral gate. The SP continues to stay bound in the lateral gate and the polypeptide is cleaved by the signal peptidase enzyme. Translation
continues until the hydrophobic membrane domain emerges. This causes the release of the ribosome from the ER after insertion of the polypeptide into the ER lumen. CADA has been found to interrupt this process, as illustrated in Figure 1.6.3. The SRP recognizes the SP and then docks onto the lateral gate of the channel. The SP binds to the hydrophobic pocket of the lateral gate. CADA binds to the SP as translation continues and stabilizes a folded
conformation, preventing translocation from occurring. As translation continues the protein chain loops out into the cytosol where it is degraded by proteolytic enzymes. It is proposed that CADA binds to two key amino acid residues of the CD4 SP, Gln-15 and Pro-20, and some lysine residues as shown in Figure 1.6.4. A comprehensive alanine scan mutagenesis of the CD4 N-terminus was conducted which identified these as important residues for CADA potency. This evidence further correlates the previous results that the dipole moment of the side arm of CADA plays a critical role in the binding interaction between the SP and CADA. Groups that enhance the dipole moment induced by the sulfonyl groups of the side arms help stabilize the CADA-CD4 SP conformation. It is believed that the Gln-15 interacts with the side arm of CK147 forming a dipole-dipole interaction. It is hypothesized that the Lys-26 or 27 interacts with the tertiary amine in the tail region of CADA or uses negatively-charged amino acid residues in the translocon.

It was recently discovered that CADA also can affect expression of the neurotensin receptor 3, sortilin. Sortilin is known to be involved in sorting proteins and targeting ligands toward endosomes and lysosomes. Sortilin contains a 33 amino acid long signal peptide, which is 8 amino acids longer than CD4, as shown in Figure 1.6.5. Much like
CD4, sortilin contains a glutamine residue in the hydrophobic H region at position 25. In CD4 the glutamine is located at position 15 of the hydrophobic H region. There are two prolines 3 to 4 amino acids away from the glutamine in sortilin, like the proline of CD4, located 5 amino acids away from the glutamine. The presence of the two lysine residues at positions 26 and 27 just past the cleavage site of the CD4 SP, have been shown to be important for the binding of CADA to CD4. Sortilin however, does not contain lysine residues after the cleavage site but has a glutamine and aspartate residues there. This could possibly be one of the reasons for the decreased inhibition levels of sortilin compared to hCD4 (Figure 1.6.6).
The relative $IC_{50}$ value of CADA for sortilin down-modulation was found to be 0.88 µM and the absolute $IC_{50}$ was 2.15 µM. While the relative $IC_{50}$ value for CD4 down-modulation of CADA was 0.24 µM and the absolute $IC_{50}$ value was 0.21 µM. Since the maximal down-modulation of hCD4 was closer to 100%, the relative and absolute $IC_{50}$ values were almost similar. The maximal down-modulation of sortilin by CADA is roughly 50% indicating that CADA is a weaker inhibitor of sortilin translocation compared to hCD4.

Modifications to CADA analogs could be conducted to optimize the down-modulation of sortilin through analysis of the signal peptide. CADA analogs containing hydrogen bond accepting substituents could possibly bind to the residues just past the cleavage site in the SP of sortilin. An analog containing a negatively charged substituent could be utilized to bind with the arginines at positions 13 or 36. It is proposed that modifications to the small molecule CADA could potentially target signal peptides for other specific proteins, therefore not only preventing HIV entry but could also be used in applications of other illnesses such as treatment of atherosclerosis, coronary artery disease, and breast cancer. Understanding these interactions between CADA and the signal peptide could lead to designs of small-molecules that utilize the signal peptide as a new therapeutic target for other diseases and illnesses.

### 1.7 Summary

CADA compounds have been found to be effective against HIV by down-modulating CD4 expression of CD4$^+$ T cells. CADA has been discovered to selectively bind to the SP of hCD4 at the post-transcriptional level. It is known that CADA binds to
the SP of hCD4 and forms a locked conformation that prevents translocation from occurring, which causes the nascent chain to loop out into the cytosol where it is degraded by proteolytic enzymes, therefore inhibiting the co-translational translocation and expression of CD4.

A variety of symmetrical and unsymmetrical CADA analogs have been made in the past. Structure-activity relationship studies of CADA compounds have determined that a hydrophobic tail group on CADA analogs increase the potency, with the cyclohexylmethyl tail being the best option so far. It is generally thought that unsymmetrical CADA compounds with two differing arenesulfonyl groups tend to be more potent than their symmetrical counterparts, indicating that CADA has two different binding sites for the arenesulfonyl groups. Currently, CK147 is the most potent analog produced by Dr. Reena Chawla. Although a large library of CADA analogs have been synthesized, these analogs contain highly hydrophobic properties, which limits their solubilities.

New CADA analogs with increased hydrophilicity and increased potency is still needed in order to continue with in vivo experimentation. The binding sites of CADA with the SP of hCD4 also needs to be further investigated. Development of new CADA analogs to determine the amino acids required for binding is necessary. Testing of CADA analogs with other signal peptides could begin a new era for drug targeting therapies. CADA’s ability to also down-modulate the expression of sortilin allows us to analyze the interactions between CADA and the signal peptide. Understanding the difference between CD4 and sortilin could be key to making small molecules that can target the signal peptide as a new drug target. New signal peptide targets have been discovered through analysis of
various signal peptides. The signal peptides of the thyroid stimulating hormone receptor and the envelope protein of HIV, gp160, have been selected as new targets for CADA analogs. The novel mechanism of action for CADA could potentially be applied to help treat illnesses such as rheumatoid arthritis, multiple sclerosis, and type I diabetes.
1.8 References


Chapter 2

Synthesis and CD4 Down-Modulation Potency of Head Group

Modified CADA Analogs
2.1 Introduction

Several series of CADA analogs have been synthesized with two identical and different side arms. Structure-activity relationship studies have been conducted with a variety of modifications to CADA, including various tail groups and side arms. The cyclohexylmethyl tail, a hydrophobic group, generally increases the potency of analogs relative to smaller allyl or benzyl groups. Some unsymmetrical CADA compounds have been shown to have higher potency than their symmetrical counterparts, which is consistent with the proposed two-site binding model for CADA. Currently **CK147** is the most potent CADA analog produced (Figure 2.1.1). It contains an isobutylene headgroup, with a tosyl side arm and an \(N,N\)-dimethylaminosulfonamide side arm, with a cyclohexylmethyl tail. Various ring sizes have been tested for CD4 down-modulation and are currently being studied. Previous X-ray crystal structures have shown that the isobutylene head group places CADA in a conformation that favors a two-site binding mode for the 12-membered macrocycle. The ability to control this bioactive conformation using various head groups could lead to potentially more potent CADA analogs. The effects of varying the head group have been previously studied and tested for CD4 down-modulation.
A series of compounds without the isobutylene headgroup was initially created by Dr. Sreenivasa Anugu. In this series, it was shown that the absence of the exocyclic double bond decreased the activity of CADA analog 95-210 (Figure 2.1.2). Many of the compounds made in this series were shown to be inactive for CD4 down-modulation or have very low activity. The most potent analog was the mesyl analog SA01. These compounds were all symmetrical CADA analogs with benzyl tails. A second series of head group
modifications was recently conducted by Dr. Rameez Ali. The compounds investigated contained cyclohexylmethyl tails and two different side arms. At the time VGD020 was the most potent analog, which contained a tosyl side arm, a para-methoxybenzenesulfonyl side arm and a cyclohexylmethyl tail (Figure 2.1.3). A series of CADA analogs were made that included compounds with modified isobutylene headgroups, consisting of halides, alkyl chains, and polar head groups (Figure 2.1.4).

It was found that all modifications to the head group lowered activity when compared to VGD020. The most potent analog of the series was RA016. These head group modifications typically contained hydrophilic groups that would have increased the polarity of the CADA analogue, except for RA016. This showed that the more polar the

![Chemical Structures](image_url)

*Figure 2.1.4. Head group modifications by Dr. Ali.*
head group was on the analog, the lower the activity of the CADA compound was observed.\textsuperscript{6}

Even though modifications of the isobutylene head group tended to decrease potency, the high potency of RA016 formed the basis for newly proposed head group modifications on CADA (Figure 2.1.5). The new head groups continued to contain the olefin of the isobutylene head group while also having more hydrophobic attachments. These modifications would include both the benzyl and cyclohexylmethyl versions to compare with CADA. Symmetrical compounds were proposed in order to alleviate the need to separate E/Z isomers. It is proposed that the addition of these alkyl chains could optimize the twist about the isobutylene unit and the orientation of the two side arms, thus increasing the potency of the analog.

\textbf{2.2 Results and Discussion}

\textbf{2.2.1 Synthesis of CK147}

At the time of its synthesis by Dr. Reena Chawla, CK147 was the most potent CADA analog to date. In order to compare the new CADA analogs, more CK147 was needed. Further biological studies of CK147 were also being conducted, so more was synthesized. The synthesis is a six-step sequence, starting with 1,3-diaminopropane
(Scheme 2.2.1). The tosyl side arm is first added by the reaction of 1,3-diaminopropane with tosyl chloride to give the intermediate 4. A large excess of 1,3-diaminopropane and slow addition of the tosyl chloride were necessary to induce monotosylation and decrease the chances of tosylation on both primary nitrogens. Addition of the cyclohexylmethyl tail is done by treating compound 4 with cyclohexanecarboxaldehyde in toluene. A Dean-Stark apparatus was used to remove water generated in-situ. The resulting imine is then reduced with NaBH₄ to give the secondary amine 5. Chain extension is done by reaction of secondary amine 5 with N-(3-bromopropyl)pthalimide to form the pthalimide product 6. Removal of the pthalimide is done with hydrazine monohydrate at r.t. to form the primary amine 7. In order to add the second side arm in CK147, 4-(dimethylamino)benzenesulfonyl chloride needed to be synthesized. Chlorotrimethylsilane is treated with concentrated
H$_2$SO$_4$ to form bis(trimethylsilyl) sulfate (Scheme 2.2.2).$^7$ Bis(trimethylsilyl) sulfate is then treated with $N,N$-dimethylaniline to produce 4-(dimethylamino)benzenesulfonic acid,

\[
\begin{align*}
\text{conc. H}_2\text{SO}_4 & \quad \text{cyclohexane} \\
\text{reflux} & \quad 45 \text{ min} \\
& \quad 65\%
\end{align*}
\]

which is treated with PCl$_5$ to form the resulting sulfonyl chloride.$^8$ The second arm of CK147 is then added by reaction of primary amine 7 with 4-(dimethylamino)benzenesulfonyl chloride under biphasic conditions to form the disulfonamide 8 (Scheme 2.2.3). Palladium catalyzed macrocyclization is conducted to produce CK147.

The palladium catalyzed macrocyclization forms a $\pi$-allyl intermediate and generates an alkoxide (Figure 2.2.1).$^9$ This alkoxide serves as a base for the sulfonamide, and with an unsymmetrical open-chain disulfonamide, the more acidic amine (NH) would be allylated. The less acidic NH must then be deprotonated to undergo macrocyclization through intramolecular $N$-allylation. There is competition between intermolecular reactions with the more acid NH group of a second open-chain disulfonamide.$^9$ The

Scheme 2.2.2. Synthesis of 4-(dimethylamino)benzenesulfonyl chloride.$^7,^8$
dimethylamino substituent changes the pK\(_a\) of the NH group which could make the intermolecular reaction more favored, lowering the yield of **CK147**.

![Scheme 2.2.3. Synthesis of CK147](image)

**Figure 2.2.1.** Proposed mechanism for Pd-catalyzed macrocyclization.
2.2.2 Synthesis of Boc-Protected Diols

The current synthetic pathway for the synthesis of CADA analogs allows for easy modifications to be conducted, giving various analogs. In order to change the head groups on the CADA analogs, Boc-protected diols of the head groups had to be synthesized. The initial proposed pathway for the synthesis of **TL010** starts with THP protected ketone 9.

![Scheme 2.2.4. Initial proposed synthesis pathway for TL010.](image)

(Scheme 2.2.4). The initial approach was to change the head groups by changing the alkyl portion of the Wittig salt 10. The first step was THP protection of the alcohol groups of dihydroxyacetone dimer to give the ketone 9 (Scheme 2.2.5). The Wittig salt was then
synthesized by reaction of triphenylphosphine with bromoethane. A Wittig reaction was then conducted between ketone 9 and the Wittig salt 10 with t-BuOK as the base. Removing the resulting product from the triphenylphosphine oxide proved to be very difficult, so a different synthetic pathway was investigated.

The new proposed route utilized a Knoevenagel condensation to form the olefin. By varying the ketone used, the different head groups could be synthesized. The formation of TL001 was initially done by reaction of diethyl malonate and reagent grade acetone in the presence of ZnCl₂ (Scheme 2.2.6). This formed the resulting diester 14, which was

**Scheme 2.2.5.** Initial synthesis of THP protected diol 11.\(^{10,11}\)
reduced using LAH. The diol intermediate was difficult to purify, so Boc protection of the crude diol was conducted to give TL001.

To obtain the benzylidene diol TL004, diethyl malonate was first treated with benzaldehyde to form the diester 15 (scheme 2.2.7). This was then reduced using DIBALH to produce the diol compound 16. Compound 16 was then Boc protected to form TL004. The synthesis of TL008 involved some difficulties initially. Reaction of diethyl malonate with acetaldehyde gave the diester 17 (Scheme 2.2.8). Reduction was attempted using DIBALH to form compound 18, however ¹H NMR spectroscopy and TLC showed the

---

**Scheme 2.2.6. Synthesis of TL001.**

1.) LAH  
2.) H₂O, KNaC₄H₆O₆·4H₂O  
3.) Boc₂O, DMAP, Et₂O, rt  

Reduced using LAH. The diol intermediate was difficult to purify, so Boc protection of the crude diol was conducted to give TL001.

---

**Scheme 2.2.7. Synthesis of TL004.**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Diethyl malonate + benzaldehyde</td>
<td>Diester 15</td>
</tr>
<tr>
<td>Reduction using DIBALH</td>
<td>Diol 16</td>
</tr>
<tr>
<td>Boc₂O, DMAP, Et₂O, rt</td>
<td>TL004</td>
</tr>
</tbody>
</table>

---

**Scheme 2.2.7. Synthesis of TL004.**
presence of compound 19 as a side product, due to over reduction. Attempts to separate the two compounds largely failed. A new strategy for TL008 was developed.

A more recent paper showed the synthesis of TBS protected 2-ethylidene propane-1,3-diol formed from a Wittig reaction. So, the dihydroxyacetone dimer was TBS protected in high yield to form compound 20 (Scheme 2.2.9). Ketone 20 was reacted with ethyltriphenylphosphonium bromide (10) using n-BuLi as a base to form TBS-protected ethyldiene diol 21. Removal of the TBS group was done using TBAF, however purification
of the resulting diol was difficult. Boc protection of the crude product gave compound TL008. Syntheses of the three Boc-protected head groups were completed, allowing for the next steps to occur.

2.2.3 Synthesis of the Disulfonamide Intermediates

With the head groups formed and ready for the cyclization step, the intermediate disulfonamides needed to be synthesized. Initially, VGD020 was the most potent analog at the beginning of this project. Since the isopropylidene head group would not give rise to E/Z isomers, an unsymmetrical disulfonamide was made containing a p-methoxybenzene sulfonamide and a tosyl side arm. A symmetrical ditosyl compound was also made containing a cyclohexylmethyl tail. The synthesis starts off the same first 4 steps of CK147, as previously described,\(^3\) to form the primary amine compound 7 (Scheme 2.2.1). The side arms were then added by reaction of compound 7 with p-methoxybenzenesulfonyl chloride or 4-methylbenzenesulfonyl chloride, to form disulfonamide compounds 22 and 23 (Scheme 2.2.10).

\[
\text{Scheme 2.2.10. Synthesis of compounds 22 and 23.}
\]
2.2.4 Synthesis of TL007 and TL005

The successful syntheses of the Boc protected diols and the disulfonamides allowed for the cyclization steps to be conducted. Compound \textbf{TL004} reacted with compounds 23 and 24 by palladium-catalyzed macrocyclization to form compounds \textbf{TL007} and \textbf{TL005} (Scheme 2.2.11). Purification was done by column chromatography on neutral alumina.

![Scheme 2.2.11. Synthesis of compounds TL005 and TL007.]

2.2.5 Synthesis of TL002

Since \textbf{VGD020} was the most potent CADA analog at the time, an attempt was made to cyclize the isopropylidene Boc reagent \textbf{TL001} with compound 22. The resulting unsymmetrical CADA analog would not have the issue of E/Z isomerization from the symmetrical head group. So \textbf{TL001} was treated with compound 22 under palladium-catalyzed macrocyclization conditions.\(^9\) The predicted product 25 was not isolated from the cyclization step (Scheme 2.2.12). The isolated product resulted in a double addition
reaction, rather than a macrocyclization to give compound TL002. Due to this unexpected product formation, TL001 was not cyclized with compound 23 or 24 since the resulting compounds would likely produce the double addition products rather than the cyclized product.

Scheme 2.2.12. Synthesis of compound TL002.

2.2.6 Synthesis of Compounds TL009 and TL010

Palladium catalyzed macrocyclization⁹ was conducted with compound TL008 and compound 24 (Scheme 2.2.13). This resulted in the formation of compounds TL010 and TL009. The reaction produced both the cyclized product as well as the double addition product, which were separated using an alumina chromatotron plate.

The problem with the cyclization of the ethylidene Boc reagent TL008 and the isopropylidene Boc reagent TL001 is related to the mechanism of the palladium catalyzed reaction (Figure 2.2.1). The initial step of the catalytic cycle begins with the oxidative addition of Pd⁰ to the carbonate C-O bond. This produces a π-allyl Pd(II) species.⁹ The carbonate is displaced irreversibly, losing CO₂ and forming tert-butoxide anion. The generated basic t-BuO⁻ deprotonates the NH group of one of the sulfonamides,
forming the nucleophilic sulfonamide anion ARSO$_2$N$^-$.\(^9\) The ArSO$_2$N$^-$ then reacts with the $\pi$-allylpalladium complex generating an intermediate containing the sulfonamide covalently bonded to the isobutylene head group. The step is then repeated to replace the remaining carbonate ester with the second sulfonamide group to complete the macrocyclization. The intramolecular addition of the second sulfonamide is favored by entropy, but it could react with an additional $\pi$-allylpalladium species of a different molecule which would lead to oligomeric byproduct.\(^9\)

As previously described, the more acidic sulfonamide NH is deprotonated and becomes basic. The deprotonated NH does a nucleophilic attack forming the monoallylated product and the cycle is repeated, and the $\pi$-allyl palladium complex is
formed. Steric hindrance from the methyl groups of the isopropylidene and ethylidene Boc reagents prevent the π-allyl palladium complex from nucleophilic attack, and a base-mediated E2´ elimination of the allylic BocO´ group from the basic nitrogen forms the diene. This entire process is repeated a second time and adds a second diene to the disulfonamide.

2.2.7 CD4 Down-Modulation Activities

The newly synthesized CADA analogs were tested for their CD4 down-modulation and anti-HIV activities. The number of head group modifications was limited due to the results of the palladium macrocyclization. Of the compounds generated, TL006, TL005, TL007, and TL010 (Figure 2.2.2) were tested for CD4 down-modulation. The CD4 down-modulation activities were reported as IC_{50} values, which is the concentration at which 50% of the maximal down-modulation of CD4 expression was measured in CHO-CD4-YFP cells after 24 h of treatment with the CADA...
Table 2.2.1 shows the CD4 down-modulation activities of the TL compounds in CHO-CD4-YFP cells. The addition of the benzylidene head group to CADA made it inactive. It is believed that the bulky aryl group could change the conformation of the macrocycle, moving the two side arms out of the original binding sites, thus decreasing the activity of the compound. The lack of an isobutylene headgroup for TL006 also greatly decreases the CD4 down-modulation activity, correlating with previous results. The addition of the ethylidene head group gives some CD4 down-modulation activity but TL010 is significantly less potent than CADA. The limitations of the cyclization prevented further synthesis of longer chained aryl groups as new head group modifications.

Table 2.2.1. Newly synthesized TL compounds tested for CD4 down-modulation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$IC_{50}$ (µM)$^b$ mean ± SDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADA</td>
<td>0.36 ± 0.05$^a$</td>
</tr>
<tr>
<td>CK147</td>
<td>0.058 ± 0.006$^a$</td>
</tr>
<tr>
<td>TL005</td>
<td>&gt;50$^a$</td>
</tr>
<tr>
<td>TL006</td>
<td>&gt;10$^a$</td>
</tr>
<tr>
<td>TL007</td>
<td>&gt;50$^a$</td>
</tr>
<tr>
<td>TL010</td>
<td>0.65 ± 0.08$^a$</td>
</tr>
</tbody>
</table>

$^a$These compounds were tested as HCl salts. $^b$Inhibitory concentration 50%, concentration at which 50% down-modulation of CD4 expression was measured in CHO-CD4-YFP cells after 24 hours of treatment with CADA compound. Values are mean ± STDEV from 3 independent experiments.$^{19}$

Cytotoxicity and anti-HIV activities of compounds TL005, TL006, and TL010 were also tested. Compound TL010 had a very low cytotoxicity similarly to CADA as
shown in Table 2.2.2. **TL006** was found to be the most cytotoxic in this series of compounds. Their anti-HIV activity was found to be too low to measure.

**Table 2.2.2.** Newly synthesized TL compounds tested for cytotoxicity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CC&lt;sub&gt;50&lt;/sub&gt; (µM)&lt;sup&gt;*&lt;/sup&gt; mean ± SDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADA</td>
<td>&gt;100</td>
</tr>
<tr>
<td>TL005</td>
<td>66.86 ± 0.64</td>
</tr>
<tr>
<td>TL006</td>
<td>25.80 ± 4.91</td>
</tr>
<tr>
<td>TL010</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

These compounds were tested as HCl salts. *CC<sub>50</sub>: Inhibitory concentration 50%, concentration of the compound required to reduce the viability of MT-4 cells by 50%.

2.3 Conclusion and Outlook

Three new CADA macrocycles, **TL005**, **TL007**, and **TL010**, were synthesized containing the benzylidene and ethylidene head groups. Isobutylene, ethylidene, and benzylidene head group modifications started with utilization of commercially available products to form **TL001**, **TL004**, and **TL008** (Figure 2.3.1). The initial synthetic approach to **TL001**, **TL004**, and **TL008** was not efficient due to the difficult removal of the triphenylphosphine oxide in the Wittig step. An alternative route using the Knoevenagel condensation allowed for the synthesis of **TL001** and **TL004**. **TL008** was successfully synthesized using a Wittig reaction with TBS-protected dihydroxy acetone. Starting from the commercially available 1,3-diaminopropane and ultimately conducting a palladium-catalyzed macrocyclization, compounds **TL002**, **TL005**, **TL006**, **TL007**, **TL009**, and
were synthesized (Figure 2.3.1). Compounds TL002 and TL009 were unexpected side products caused by competitive E2’ elimination in the mechanism of the palladium-catalyzed cyclization. This side reaction limited synthesis of CADA analogs with longer alky substituents on the isobutylene head group, as well as cyclization of isopropylidene and ethylidene diBoc reagents with alternative disulfonamides.

The addition of the benzylidene and ethylidene head groups for TL005, TL007, and TL010 produced decreased activity. The benzylidene head group could change the conformation of the CADA analog, moving the two side arms away from the binding site. The ethylidene head group is not as bulky as the benzylidene and shows some level of

---

**Figure 2.3.1.** Newly synthesized TL compounds.

**TL010** were synthesized (Figure 2.3.1). Compounds **TL002** and **TL009** were unexpected side products caused by competitive E2’ elimination in the mechanism of the palladium-catalyzed cyclization. This side reaction limited synthesis of CADA analogs with longer alky substituents on the isobutylene head group, as well as cyclization of isopropylidene and ethylidene diBoc reagents with alternative disulfonamides.

The addition of the benzylidene and ethylidene head groups for **TL005**, **TL007**, and **TL010** produced decreased activity. The benzylidene head group could change the conformation of the CADA analog, moving the two side arms away from the binding site. The ethylidene head group is not as bulky as the benzylidene and shows some level of
activity but the potency of TL010 is still lower than that of CADA. The lack of an isobutylene head group in TL006 dramatically decreased activity as well, which correlates with previous SAR studies of open-chain CADA analogs without an isobutylene head group. Currently, the isobutylene group seems to be the most active head group for CADA compounds. Additional analogs with saturated alkyl chains in the head groups should be considered for further head group modification studies of CADA. Hydrogenation of TL005, TL007, and TL010 could also be considered due to the potency of the RA016.

2.4 Experimental

Flow Cytometry

These studies were conducted by Vermeire et al. at the Rega Institute for Medical Research Katholieke Universiteit Leuven, Belgium. To study the effect of CADA on CD4 expression, CHO cells, stably expressing CD4-YFP (huCD4 fused at its COOH-terminus to the yellow fluorescent protein [YFP]), were treated for 24 h with different concentrations of CADA or its analogs at 37 °C. Cells were then washed, fixed in 1% formaldehyde and analyzed immediately. Data were acquired with a FACSCalibur flow cytometer (BD Biosciences) using the 488 nm laser line and CellQuest software (BD Biosciences). YFP was measured with the FL-1 detector and data were analyzed with FLOWJO software (Tree Star, San Carlos, CA). Down-modulation of CD4 was evaluated by the decrease in fluorescence intensity on CADA-treated cells relative to matched, untreated cells. To calculate the efficiency of CD4 down-modulation, the median fluorescence intensity (MFI)
for YFP for each sample was expressed as a percentage of the MFI of control cells (after subtracting the background MFI of the non-transfected control cells).

**General Methods**

All reactions were performed under an atmosphere of dry nitrogen, unless specified otherwise. Reagents and solvents purchased from Aldrich Chemical Company, Acros Organics, or Fisher Scientific were of ACS reagent grade or better and were used without purification, unless indicated otherwise. Anhydrous acetonitrile used in the macrocyclization step was distilled from CaH₂. For macrocyclization reactions, the disulfonamide intermediates, 2-methylene-1,3-propanebis(tert-butylcarbonate), anhydrous sodium carbonate, dppb, and Pd₂(dba)₃ were dried *in vacuo* (ca. 0.1 mm) for at least 16 h. All the equipment required for macrocyclization reaction including a magnetic stir bar, spatula, syringe and needle were also dried overnight in the oven (110 ºC). Solutions of 2 N HCl in methanol were created by placing 165 mL of 12.1 M HCl into a 1 L volumetric flask. The flask is then filled with 835 mL of methanol. Column chromatography was performed with Sorbent Technologies neutral alumina (50-200 μm) or Sorbent Technologies standard grade silica (32-63 μm), unless noted otherwise. Chromatotron chromatography was performed with Sorbent Technologies neutral alumina with gypsum and UV254. Automated chromatography was performed on the Yamazen Smart Flash AKROS RE-X10 with Sorbet Technologies neutral alumina (50-200 μ) or Sorbent Technologies standard grade silica (32-63 μm) and HPLC grade ethyl acetate, hexane, and dichloromethane (DCM). Compounds dried *in vacuo* were connected to a vacuum
manifold with a Welch 1402 vacuum pump and vacuumed dried for at least 18 h at ca. 0.1 mm. Melting points were measured on a Thomas-Hoover or Mel-Temp apparatus and are uncorrected. $^1$H NMR (400 MHz or 500 MHz) and $^{13}$C NMR (75 MHz or 125 MHz) spectra were acquired on a Varian 400 or Varian Unity + 500 spectrometer. All chemical shifts ($\delta$) are reported in ppm units relative to solvent resonances, as follows: $^1$H, CDCl$_3$/TMS = 0.00, DMSO-d$_6$ = 2.50, CD$_3$OD = 3.31; $^{13}$C, CDCl$_3$ = 77.23, DMSO-d$_6$ = 39.7, CD$_3$OD = 49.15. Infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrometer. Mass spectra (MS) were acquired on a Waters Micromass ZQ electrospray ionization quadrupole mass spectrometer with positive ion detection (capillary voltage = 3.5 kV). High-resolution mass spectra (HRMS) were acquired on an Agilent 6230 TOF mass spectrometer. Samples for elemental analysis were dried at 78 ºC (0.1 mm) for 2 days, unless stated otherwise, and microanalysis was performed by NuMega Resonance Labs, Inc.

**Synthesis of $N$-(3-aminopropyl)-4-methylbenzenesulfonamide (4)**

![Chemical Structure](image)

Into a 2 L round bottom flask, a solution of 68.0 mL (0.815 mol) of 1,3-diaminopropane in 100 mL of toluene was added and stirred at r.t. An addition funnel was attached with a nitrogen gas inlet containing a solution of 51 g (0.27 mol) of $p$-toluenesulfonyl chloride in 200 mL of toluene. The $p$-toluenesulfonyl chloride solution was added to the round bottom flask dropwise over a period of 4 h. After completion of the
addition, the mixture was stirred for 24 h. The white precipitate was then collected by filtration and washed with toluene. The product was dried in vacuo for 17 h. It was then stirred with a solution of 1:1 (v/v) methanol/water for 2 h. The suspension was then filtered, and the filtrate was concentrated by rotary evaporation and placed in the refrigerator to recrystallize. This was then filtered, and the filtrate was concentrated by boiling the mixture to one-third of the volume to allow further recrystallization in the refrigerator. The resulting crystals from both recrystallization steps were combined to produce 43.8 g (71%) of \( N-(3\text{-aminopropyl})-4\text{-methylbenzenesulfonamide} \) as a white solid. 

\[ ^1H \text{ NMR (400 MHz, CD}_3\text{OD)} \delta 7.71 \text{ (d, 8.4 Hz, 2 H, o-Ts), 7.36 \text{ (d, 8.6 Hz, 2H, m-Ts), 2.86 \text{ (t, 7.3 Hz, 2 H, CH}_2\text{NH), 2.62 \text{ (t, 7.3 Hz, 2 H, CH}_2\text{NH}_2), 2.40 \text{ (s, 3 H, ArCH}_3), 1.56 \text{ (quint., 7.4 Hz, 2 H, CCH}_2\text{C).}} \]

\[ \text{Synthesis of } N-(3\text{-((cyclohexylmethyl)amino)propyl}-4\text{-methylbenzenesulfonamide (5)} \]

\[ \text{[Diagram of the molecule]} \]

Into a 500 mL round bottom flask with a reflux condenser attached, 16.8 g (74.2 mmol) of \( N-(3\text{-aminopropyl})-4\text{-methylbenzenesulfonamide} \), 200 mL of methylene chloride, 9.33 g (83.2 mmol) of cyclohexanecarboxaldehyde, and 26.0 g (0.216 mol) of MgSO\(_4\) were added. The resulting solution was then stirred and boiled under reflux under nitrogen for 24 h. The solution was then allowed to cool to r.t. and the white mixture was
then filtered through a fine sintered glass funnel. The white residue was washed with 60 mL of DCM and filtered. The filtrate was then concentrated by rotary evaporation and the residue was dried *in vacuo* for 18 h. The resulting liquid was then dissolved in 60 mL of absolute ethanol. Then 3.15 g (83.3 mmol) of NaBH₄ was added in portions over 15 minutes. This mixture was stirred at r.t. for 24 h. The mixture was then filtered through a fine sintered glass filter and the filtrate was then concentrated by rotary evaporation. Deionized H₂O (90 mL) was added and the product was extracted using DCM (3 x 30 mL). The combined organic solutions were then dried (Na₂SO₄) and filtered. The filtrate was then concentrated by rotary evaporation and the resulting residue was dried *in vacuo*. This produced 22.5 g (93.5% yield) of \( N-(3-((\text{cyclohexylmethyl})\text{amino})\text{propyl})\text{-4-} \)methylbenzenesulfonamide as a white waxy solid. \(^1\text{H NMR} (400 \text{ MHz, CDCl}_3/\text{TMS}) \delta \)

7.73 (m, 2 H, \text{o-Ts}), 7.30 (m, 2 H, \text{m-Ts}), 3.06 (t, 6.4 Hz, 2 H, CH₂NHTs), 2.63 (t, 6.3 Hz, 2 H, CCH₂NH), 2.43 (s, 3 H, ArCH₃), 2.35 (d, 7.6 Hz, 2 H, CH₂Cy), 1.70 (m, 5 H, CCH₂C, Cy, NH), 1.59 (m, 2 H, Cy), 1.39 (m, 1 H, Cy), 1.20 (m, 4 H, Cy), 0.91 (m, 2 H, Cy).
Synthesis of 1-phthalamido-4-cyclohexylmethyl-7-(p-toluenesulfonamido)-4-azaheptane (6)²

Into a 500 mL round bottom flask with a condenser attached, 19.9 g (61.4 mmol) of \( N-(3-((\text{cyclohexylmethyl})\text{amino})\text{propyl}-4\text{-methylbenzenesulfonamide} \), 39.7 g (148 mmol) of \( N-(3\text{-bromopropyl})\text{phthalimide} \), 7.06 g (70.5 mmol) of anhydrous \( \text{CaCO}_3 \), and 2.34 g (17.5 mmol) of lithium iodide were added with 75 mL of acetonitrile. The solution was then stirred and heated under reflux overnight for 18 h. The solution was cooled to r.t. and vacuum filtered through a fine sintered glass funnel. The residue was washed with 100 mL of acetonitrile, the filtrate was concentrated by rotary evaporation, and the resulting residue was dried \textit{in vacuo}. The product was purified by alumina filter column chromatography. A slurry of 900 g of alumina in 500 mL of 5:2 (v/v) hexane/ethyl acetate was added to a 2 L medium porosity sintered glass funnel to form a uniform bed, which was not allowed to dry. A solution of the crude product was dissolved in 30 mL of ethyl acetate and carefully added to the top of the alumina bed. Once the product was absorbed into the alumina, the alumina was then washed with 3 L of 5:2 (v/v) hexane/ethyl acetate, followed by 2 L of ethanol. The ethanol fraction was collected and concentrated by rotary evaporation, and the resulting residue was dried \textit{in vacuo}. This produced 17.7 g (56\%) of 1-phthalamido-4-cyclohexylmethyl-7-(p-toluenesulfonamido)-4-azaheptane as a yellow
viscous oil. $^1$H NMR (400 MHz, CDCl3/TMS) $\delta$ 7.86 (m, 2 H, NPhth), 7.74 (m, 4 H, o-Ts, NPhth), 7.26 (m, 2 H, m-Ts), 6.32 (bs, 1 H, NH), 3.62 (t, 8.3 Hz, 2 H, CH$_2$NPhth), 3.06 (t, 6.2 Hz, 2 H, CH$_2$NHTs), 2.39 (m, 4 H, CCH$_2$N), 2.34 (s, 3 H, ArCH$_3$), 2.09 (d, 7.4 Hz, 2 H, CH$_2$Cy), 1.69 (m, 10 H, CCH$_2$C, Cy), 1.38 (m, 1 H, Cy), 1.17 (m, 2 H, Cy), 0.84 (m, 2 H, Cy).

**Synthesis of N-(3-aminopropyl)-N-(3-p-toluenesulfonamidopropyl)cyclohexylmethylamine (7)$^2$**

![Chemical Structure](image)

Into a 500 mL round bottom flask equipped with a condenser, 17.7 g (34.6 mmol) of 1-phthalamido-4-cyclohexylmethyl-7-(p-toluenesulfonamido)-4-azaheptane, 40.5 mL of hydrazine monohydrate, and 300 mL of ethanol were added under N$_2$ gas. The solution was stirred and heated under reflux for 3 h and then cooled to r.t. The mixture was concentrated by rotary evaporation. Then 250 mL of a solution of 2 N aq. HCl was added slowly and stirred until white fumes were no longer present. The mixture was then made basic (pH 10) using a solution of 6 N NaOH (90 mL) and then extracted with DCM (3 x 60 mL). The combined extractions were dried (Na$_2$SO$_4$) and filtered. The filtrate was then concentrated by rotary evaporation and the resulting residue was dried *in vacuo*. This
produced 13.2 g (100%) of N-(3-aminopropyl)-N-(3-p-toluenesulfonamidopropyl) cyclohexylmethylamine as a light yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$/TMS) $\delta$ 7.78 (d, 8 Hz, 2 H, o-Ts), 7.29 (d, 2 H, m-Ts), 5.76 (bs, 3 H, NH), 3.03 (t, 6.4 Hz, 2 H, CH$_2$NHTs), 2.96 (t, 6.6 Hz, 2 H,CH$_2$NH$_2$), 2.49 (t, 6.3 Hz, 2 H, CH$_2$N), 2.45 (t, 7.2 Hz, 2 H, CH$_2$N), 2.41 (s, 3 H, ArCH$_3$), 2.12 (d, 7.4 Hz, 2 H, CH$_2$Cy), 1.80 (m, 2 H, CCH$_2$C), 1.66 (m, 8 H, CCH$_2$C, Cy), 1.40 (m, 1 H, Cy), 1.17 (m, 4 H, Cy), 0.82 (m, 2 H, Cy).

**Synthesis of bis(trimethylsilyl)sulfate**

To a 1 L round bottom flask with a condenser attached, 132 mL (1.04 mol) of chlorotrimethylsilane, 300 mL of cyclohexane, and 27 mL (0.50 mol) of conc. H$_2$SO$_4$ were added and the resulting solution was heated under reflux for 45 minutes. The solution was then concentrated by rotary evaporation. The product was purified by vacuum distillation (72-82 °C at 1 mm). This produced 78.5 g (65%) of bis(trimethylsilyl)sulfate as a colorless solid, mp 57-58 °C (lit. $^6$ 57-58 °C). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.41 (s, 18 H, S(CH$_3$)$_3$).
Synthesis of 4-(dimethylamino)benzenesulfonic acid

\[
\text{N} - \begin{array}{c} \text{SO}_3^- \\ \text{HO} \end{array}
\]

In a 300 mL #36 Ace-threds pressure tube, 5.00 g (20.6 mmol) of bistrimethylsilyl sulfate and 2.50 g (20.6 mmol) of \(N,N'\)-dimethylaniline were added and the tube was closed tightly. The pressure tube was then heated at 170 °C in an oil bath for 4 h, only allowing 2” of the tube to be submerged in the oil. This produced a blue and white solid which was washed with diethyl ether (3 x 50 mL) to remove the excess \(N,N'\)-dimethylaniline. The remaining solution was diluted with 40 mL of H\(_2\)O. The water was removed through rotary evaporation and the solids were then washed with diethyl ether (3 x 50 mL) and dried \textit{in vacuo}. This produced 4.10 g (99%) of 4-(dimethylamino)benzenesulfonic acid as a blueish white solid. \(^1\)H NMR (400 MHz, D\(_2\)O) \(\delta\) 7.99 (m, 2 H, o-Ts, 7.76 (m, 2 H, m-Ts), 7.61 (s, 1 H, SOH), 3.32 (s, 6 H, N(CH\(_3\))\(_2\)).

Synthesis of 4-(dimethylamino)benzenesulfonyl chloride

\[
\text{N} - \begin{array}{c} \text{SO}_2\text{Cl}^- \\ \text{HO} \end{array}
\]

To a mixture of 49.0 g (235 mmol) of PCl\(_5\) in 580 mL of DCM at 0°C, 43.1 g (214 mmol) of 4-dimethylaminobenzenesulfonic acid was added in portions. The mixture was stirred at 0 °C for 3 h. Then 500 mL of H\(_2\)O was added slowly and stirred at 0 °C, until no further bubbling occurred. The organic layer was then separated, and the aqueous layer was
extracted with DCM (3 x 100 mL). The combined organic layers were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 42.3 g (91%) of 4-(dimethylamino)benzenesulfonyl chloride as a yellow solid, mp 110-112 °C (lit. 7 108-111 °C). $^1$H NMR (500 MHz, CDCl$_3$/TMS) $\delta$ 7.83 (d, 9.3 Hz, 2 H, 3-ArH), 6.68 (d, 9.3 Hz, 2 H, 2-ArH), 3.12 (s, 6 H, CH$_3$).

**Synthesis of N’-(p-toluenesulfonyl)-N”-(p-dimethylaminobenzenesulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] (8)**

Into a 100 mL round bottom flask with a nitrogen inlet and a magnetic stir bar, 0.55 g (2.5 mmol) of 4-(dimethylamino)benzenesulfonyl chloride, 1.01 g (2.65 mmol) of N-(3-aminopropyl)-N-(3-p-toluenesulfonamidopropyl)cyclohexylmethylamine, 16 mL of sat. aq. NaCl solution, 16 mL of sat. aq. Na$_2$CO$_3$ solution, and 16 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 20 mL), the combined extraction layers were dried (Na$_2$SO$_4$), and filtered. The filtrate was then concentrated, and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and
stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were triturated with diethyl ether (3 x 25 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 1.09 g (96%) of N’-(p-toluenesulfonyl)-N”-(p-dimethylaminobenzenesulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] as a viscous oil. ¹H NMR (500 MHz, CDCl₃/TMS) δ 7.74 (d, 8 Hz, 2 H, o-Ts) 7.68 (d, 8 Hz, 2 H, o-ArSO₂), 7.31 (d, 8 Hz, 2 H, m-Ts), 6.68 (d, 8 Hz, 2 H, m-ArSO₂) 3.05 (s, 6 H, (CH₃)₂NAr), 2.99 (t, 6 Hz, 4 H, CH₂N), 2.05 (d, 7 Hz, 2 H, CH₂Cy), 1.63 (m, 10 Hz, CCH₂C, Cy), 1.34 (m, 1 H, CH), 1.15 (m, 2 H, Cy), 0.81 (q, 12 Hz, 2 H, Cy).
Synthesis of 9-cyclohexylmethyl-1-(N,N-dimethylaminobenzenesulfonyl)-3-methylene-5-(p-toluenesulfonyl)-1,5,9-triazacyclododecane (CK147)³

Into a 250 mL round bottom flask with nitrogen inlet, 100 mL of dry acetonitrile, 1.37 g (2.42 mmol) of N’-(p-toluenesulfonyl)-N”-(p-dimethylaminobenzenesulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine], 2.65 g (9.18 mmol) of 2-methylene-1,3-propanebis(tert-butylcarbonate), 0.0559 g (0.131 mmol) of 1,4-bis(diphenylphosphino)butane (dppb), 0.247 g (0.270 mmol) of tris(dibenzylideneacetone)dipalladium(0) (Pd₂(dba)₃), 0.232 g (2.19 mmol) of Na₂CO₃, and 268 mL of anhydrous acetonitrile were stirred under N₂ gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. NaHCO₃ solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried in vacuo. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq.
2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated and the resulting residue was dried in vacuo. The product was purified by column chromatography using neutral alumina and eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 126 mg (8%) of 9-cyclohexylmethyl-1-(N,N-dimethylaminobenzenesulfonyl)-3-methylene-5-(p-toluenesulfonyl)-1,5,9-triazacyclododecane as a yellow oil. $^1$H NMR (400 MHz, CDCl$_3$/TMS) $\delta$ 7.67 (d, 8.2 Hz, 2 H, $o$-Ts), 7.59 (d, 8.8 Hz, 2 H, $o$-ArSO$_2$), 7.30 (d, 8.0 Hz, 2 H, $m$-Ts), 6.67 (d, 9.0 Hz, 2 H, $m$-ArSO$_2$), 5.17 (d, 4.9 Hz, 2 H, C=CH$_2$), 3.85 (s, 2 H, H2/4), 3.69 (s, 2 H, H4/2), 3.22 (t, 7.0 Hz, 2 H, H6/12), 3.04 (s, 6 H, N(CH$_3$)$_2$), 3.00 (m, 2 H, H12/6), 2.43 (s, 3 H, CH$_3$), 2.28 (m, 2 H, H10/8), 2.23 (m, 2 H, H8/10), 1.95 (d, 6.8 Hz, 2 H, CH$_2$Cy), 1.64 (m, 10 H, H7, 11, Cy), 1.49 (m, 2 H, Cy), 1.11 (m, 1 H, CH), 0.68 (m, 2 H, Cy).

**Synthesis of 1,3-bis((tetrahydro-2H-pyran-2-yl)oxy)propan-2-one (9)**

\[
\text{O} - \text{O} - \text{C} = \text{O} - \text{O}
\]

Into 100 mL round bottom flask were placed 2.23 g (12.4 mmol) of 1,3-dihydroxyacetone dimer, 30 mL of DCM, 1.43 g (5.69 mmol) of pyridinium p-toluenesulfonate and 7.47 g (88.8 mmol) of 3,4-dihydro-2H-pyran. The resulting mixture was stirred under N$_2$ at 30°-34°C for 4 h. The solvent was removed by rotary evaporation.
A solution of the resulting liquid in ether was washed with sat. aq. NaCl solution (3 x 20 mL), dried (MgSO₄), and filtered. The filtrate was concentrated by rotary evaporation and purification was conducted by column chromatography on silica gel eluting with 1:1 (v/v) ethyl acetate/hexane. This produced 5.1 g (80%) of 1,3-bis((tetrahydro-2H-pyran-2-yl)oxy)propan-2-one as a clear oil. ¹H NMR (500 MHz, CDCl₃/TMS) δ 4.62 (t, 3.6 Hz, 2 H, CH), 4.43 (dd, 17.8, 10.3 Hz, 2 H, OCH₂C=O), 4.28 (dd, 17.7, 8.7 Hz, 2 H, OCH₂C=O), 3.81 (ddd, 11.4, 8.3, 3.3 Hz, 2 H, CH₂O), 3.49 (dddd, 11.1, 5.1, 3.6, 1.5 Hz, 2 H, CH₂O), 1.68 (m, 12 H, CH₂CHO₂, CCH₂CH₂C); ¹³C NMR (25 MHz, CDCl₃) δ 205.7, 98.5, 70.9, 62.3, 30.2, 25.2, 19.0.

Synthesis of ethyltriphenylphosphonium bromide (10)¹¹

![Synthesis of ethyltriphenylphosphonium bromide](image)

Into a large pressure tube was placed 33.9 g (129 mmol) of triphenylphosphine, 14.0 g (129 mmol) of ethyl bromide, and 50 mL of toluene and sealed tightly. The tube was then heated to 135 °C for 18 h. The mixture was filtered and washed with ethyl acetate (3 x 25 mL) to remove excess ethyl bromide. This produced a white crystalline solid which was dried in vacuo, giving 42.7 g (89%) of ethyltriphenylphosphonium bromide as a white crystalline solid. ¹H NMR (500 MHz, CDCl₃/TMS) δ 7.76 (m, 15 H, Ph), 3.84 (m, 2 H, CH₂), 1.36 (m, 3 H, CH₃).
Synthesis of diethyl 2-isopropylidenemalonate (14)\(^{12}\)

\[
\begin{array}{c}
\text{O} \\
\text{C} \\
\text{O} \\
\text{C} \\
\end{array}
\]

Into a 500 mL round bottom flask with a reflux condenser and nitrogen inlet, 40.8 g (255 mmol) of diethyl malonate was added, with 24.1 g (415 mmol) of reagent grade acetone, 33.1 g (325 mmol) of acetic anhydride, and 5.02 g (36.8 mmol) of anhydrous zinc chloride. The mixture was heated under reflux for 24 h. After 2 h of refluxing, the solution turned crimson red. After refluxing for 24 h, the solution was cooled to r.t. and 100 mL of benzene was then added. The dark solution was then washed with H\(_2\)O (3 x 100 mL). The combined aqueous layers were extracted with benzene (3 x 50 mL). The benzene portions were combined and concentrated by rotary evaporation. The product was purified via vacuum distillation. Unreacted diethyl malonate was removed at 60-65 °C at 1 mm. Then 17.3 g (34%) of diethyl 2-isopropylidenemalonate was removed at 73-80 °C at 1 mm as a yellow liquid. \(^1\)H NMR (500 MHz, CDCl\(_3\)/TMS) \(\delta\) 4.19 (q, 6 Hz, 4 H, CH\(_2\)), 2.05 (s, 6 H, C=C(CH\(_3\))\(_2\)), 1.26 (t, 6 H, OCH\(_2\)CH\(_3\)). \(^{13}\)C NMR (500 MHz, CDCl\(_3\)) \(\delta\) 165.6, 154.8, 124.7, 60.7, 23.0, 14.0.
**Synthesis of 2-isopropylidene-1,3-propanediyl bis(t-butylcarbonate) (TL001)**

Into a two-neck 2 L round bottom flask equipped with a thermometer and a reflux condenser, 12.1 g (60.5 mmol) of diethyl 2-isopropylidenemalonate, 3.45 (90.8 mmol) of lithium aluminum hydride, and 450 mL of benzene were stirred under N₂ gas. The mixture was heated and began to bubble and foam vigorously at 30 °C. The reaction mixture was then boiled under reflux for 6 h. It was then cooled to 0 °C and 3.5 mL of deionized water was added dropwise with stirring. This was then followed by 3.5 mL of 6 N aq. NaOH solution added dropwise and the resulting mixture was stirred for 5 min before 10 mL of deionized water was added. The mixture was stirred for 10 min, and then warmed to r.t. and stirred for 3 h. The resulting cloudy mixture was then filtered, and the filtered solids were washed with an additional 1 L of benzene. The combined filtrates were concentrated by rotatory evaporation and the resulting residue was dried *in vacuo*, giving 4.6 g (46%) of crude product as a white solid. The crude product was placed in a 500 mL round bottom flask with 200 mL of diethyl ether, 24.6 g (113 mmol) of di-tert-butyl dicarbonate, and 0.55 g (4.5 mmol) of 4-dimethylanilinopyridine. The mixture was stirred for 24 h and then washed with sat. aq. CuSO₄ solution (3 x 20 mL), sat. aq. NaHCO₃ solution (3 x 25 mL) and sat. NaCl solution (3 x 25 mL). The organic layer was dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotatory evaporation and the resulting residue was dried *in vacuo*, yielding 8.0 g (42%) of a yellow oil. An attempt at purification was done by column chromatography on silica gel eluting with 3:17 (v/v) ethyl acetate/hexane, yielding 6.0 g (31%) of impure product as a yellow oil. The impure product was further purified by
vacuum distillation (0.1 mm) in a Kugelrohr apparatus which gave 2.21 g (12%) of 2-isopropylidene-1,3-propanediyl bis(t-butylcarbonate) as a clear oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)/TMS) \(\delta\) 4.65 (s, 4 H, C(CH\(_3\))\(_2\)), 1.81 (s, 6 H, C(CH\(_3\))\(_2\)), 1.45 (s, 18 H, C(CH\(_3\))\(_3\)). \(^{13}\)C (101 MHz, CDCl\(_3\)/TMS) \(\delta\) 153.6, 142.3, 122.6, 81.9, 64.7, 27.8, 20.8. IR (neat, cm\(^{-1}\)): 2979 (w), 1733 (s), 1456 (w), 1393 (w), 1367 (m), 1269 (m), 1247 (s), 1153 (s), 1079 (m), 1035 (w), 927 (w), 854 (m), 792 (m), 764 (w). MS (ESI\(^+\)) \(m/z\) 316 (M\(^+\)) 318 (MH\(^+\)). Anal. Calcd. for C\(_{16}\)H\(_{28}\)O\(_6\): C, 60.74; H, 8.92. Found: C, 60.03; H, 8.71.

**Synthesis of diethyl 2-benzylidene malonate (15)**\(^{14}\)

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

In a 500 mL round bottom flask with a reflux condenser and Dean-Stark trap, 50.7 g (317 mmol) of diethyl malonate, 36.9 g (248 mmol) of benzaldehyde, and 4 mL of piperidine were added. The solution was heated under reflux until no further water was collected, which took 11 h. The reaction mixture cooled, and 50 mL of benzene was added. The solution was then washed with H\(_2\)O (2 x 50 mL), 2 N aq. HCl (2 x 50 mL), and then sat. aq. NaHCO\(_3\) solution (2 x 50 mL). The organic layer was then dried (NaSO\(_4\)) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was purified by vacuum distillation (120-125 °C, 0.1 mm). This produced 52.9 g (91%) of diethyl 2-benzylidene malonate as a yellow viscous oil. \(^1\)H NMR (500 MHz, CDCl\(_3\)/TMS) \(\delta\) 7.74 (s, 1 H, CH), 7.45 (m, 2 H, o-Ph), 7.36 (m, 3 H, m,p-
Ph), 4.32 (q, 7 Hz, 2 H, CH₂), 4.31 (q, 7 Hz, 2 H, CH₂) 1.32 (t, 7 Hz, 3 H, CH₃), 1.28 (t, 7 Hz, 3 H, CH₃).

**Synthesis of 2-benzylidenepropane-1,3-diol (16)**

![Chemical Structure](image)

In a 2 neck 2 L round bottom flask with rubber septums, magnetic stir bar, and nitrogen inlet, 5.00 g (20.1 mmol) of diethyl benzylidennemalonate in 100 mL of anhydrous toluene was added and cooled to -40 °C. Then 100 mL of a 0.99 M (99 mmol) solution of DIBALH in toluene was added dropwise at -40 °C and stirred for 5 h. The solution was then quenched with 10 mL of methanol at -30 °C. The mixture began to solidify and then an aqueous solution of 178 g (630 mmol) of potassium sodium tartrate was added and the resulting mixture was stirred for 1 h at r.t. The mixture was then extracted with ethyl acetate (3 x 100 mL) and the combined extracts were washed with H₂O (3 x 50 mL) and sat. aq. NaCl solution (3 x 50mL). The organic layer was then dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried *in vacuo*. The crude product was then purified by automated chromatography with silica and eluting with a 3:2 (v/v) ethyl acetate/hexane solution. This produced 2.23 g (67%) of 2-benzylidenepropane-1,3-diol as a clear oil. ¹H NMR (400 MHz, CDCl₃/TMS) δ 7.38 (m, 2 H, o-Ph), 7.28 (m, 3 H, m,p-Ph), 6.65 (s, 1 H, CH), 4.44 (dd, 7.6 Hz, 1.0 Hz, 2 H, CH₂), 4.42 (dd, 7.5 Hz, 1.0 Hz, 2 H, CH₂), 2.42 (s, 2 H, OH).
Synthesis of 2-benzylidene-1,3-propanediyl bis(t-butylcarbonate) (TL004)⁶

In a 500 mL round bottom flask, 1.50 g (9.14 mmol) of 2-benzylidenepropane-1,3-diol, 0.11 g (0.91 mmol) of 4-N,N'-dimethylaminopyridine, 4.18 g (19.2 mmol) of di-tert-butyl dicarbonate, and 100 mL of diethyl ether were added and the mixture was stirred at r.t. for 24 h. The solution was then washed with sat. aq. CuSO₄ solution (3 x 20 mL), sat. aq. NaHCO₃ solution (3 x 20 mL) and sat. aq. NaCl solution (3 x 20 mL). The organic layer was dried (Na₂SO₄) and filtered. The filtrate was then concentrated by rotary evaporation and the residue was dried in vacuo. The crude product was purified by automated chromatography on silica eluting with 1:15 (v/v) ethyl acetate/hexane. The solvent was removed via rotary evaporation and the residue was dried in vacuo. This produced 3.12 g (95%) of 2-benzylidene-1,3-propanediyl bis(t-butylcarbonate) as a viscous clear oil. ¹H NMR (400 MHz, CDCl₃/TMS) δ 7.35 (m, 2 H, o-Ph), 7.27 (m, 3 H, m,p-Ph), 6.88 (s, 1 H, CH), 4.78 (d, 0.9 Hz, 2 H, CH₂), 4.76 (d, 0.9 Hz, 2 H, CH₂), 1.51 (s, 9 H, CH₃), 1.49 (s, 9 H, CH₃). ¹³C NMR (101 MHz, CDCl₃) δ 153.3, 153.2, 135.3, 134.9, 130.5, 128.8, 128.4, 127.8, 82.3, 68.6, 62.9, 27.8, 27.7. IR (neat cm⁻¹) 2979 (w), 1736 (s), 1457 (w), 1394 (w), 1367 (m), 1270 (s), 1246 (s), 1151 (s), 1083 (m), 1035 (w), 925 (w), 896 (m), 855 (m), 790 (w), 765 (m), 752 (w), 729 (m), 698 (m), 619 (m), 595 (w), 591 (w). MS (ESI⁺) m/z 387 (M⁺ + 23), 388 (MH⁺ + 23). Anal. Calcd. for C₂₀H₂₈O₆: C, 65.92; H, 7.74. Found: C, 65.82; H, 7.66.
Synthesis of diethyl 2-ethylenemalonate (17)\textsuperscript{16}

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Diethyl malonate (49.3 g, 308 mmol), 51.7 g (506 mmol) of acetic anhydride, and 29.1 g (661 mmol) of acetaldehyde were added to a pressure tube. The tube was sealed tightly and heated until a gentle reflux was seen. The solution was heated under reflux for 21 hours. The product was purified by vacuum distillation (64-66 °C at 0.1 mm). This produced 31.0 g (54%) of diethyl 2-ethylenemalonate as a clear oil. \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}/TMS) \(\delta\) 7.05 (q, 7 Hz, 1 H, CH), 4.23 (m, 4 H, CH\textsubscript{2}), 1.92 (dd, 7.3 Hz, 1.0 Hz, 3 H, CHCH\textsubscript{3}), 1.28 (t, 7.1 Hz, 3 H, CH\textsubscript{2}CH\textsubscript{3}), 1.26 (t, 7.1 Hz, 3 H, CH\textsubscript{2}CH\textsubscript{3}).

Synthesis of 2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-6-one (20)\textsuperscript{18}

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To a 250 mL round bottom flask, 3.10 g (17.2 mmol) of 1,3-dihydroxyacetone dimer, 30 mL of \(N,N\)-dimethylformamide, 11.02 g (73.1 mmol) of tert-butyldimethylsilyl chloride, and 7.02 g (103 mmol) of imidazole were added and the resulting mixture was stirred overnight at r.t. Then 20 mL of H\textsubscript{2}O was added, and the mixture was extracted with diethyl ether (4 x 40 mL). The combined organic layers were washed with H\textsubscript{2}O (3 x 30 mL) and sat. aq. NaCl solution (3 x 30 mL). The organic layer was then dried (MgSO\textsubscript{4}) and filtered. The filtrate was then concentrated by rotary evaporation and the resulting
residue was dried in vacuo. The product was then purified via automated chromatography with silica, eluting with 1:17 (v/v) ethyl acetate/hexane. This produced 10.5 g (96%) of 2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-6-one as a clear oil. $^1$H NMR (400 MHz, CDCl$_3$/TMS) $\delta$ 4.38 (s, 4 H, CH$_2$), 0.89 (s, 18 H, C(CH$_3$)$_3$), 0.08 (s, 12 H, SiCH$_3$).

**Synthesis of 6-ethylidene-2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecane (21)$^{17}$**

![TBSO][OTBS]

To a 2-neck 500 mL round bottom flask, 18.6 g (106 mmol) of ethyltriphenylphosphonium bromide was added with 100 mL of THF and cooled to -78 °C. Then 18.6 mL (50.2 mmol) of a solution of 2.7 M n-BuLi in toluene was added, the reaction mixture was warmed to r.t., and stirred for 20 min. The mixture was then cooled down to -78 °C and 8.00 g (25.1 mmol) of 2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-6-one was added dropwise. The solution was slowly warmed to r.t. over a period of 1 h. The mixture was then quenched with 80 mL of H$_2$O and extracted with pentane (3 x 50 mL). The organic layers were combined and washed with H$_2$O (3 x 50 mL) and sat. aq. NaCl solution (100 mL). The organic layer was then dried (MgSO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was purified by automated chromatography on silica gel, eluting with 1:16 (v/v) ethyl acetate/hexane. This produced 7.47 g (90%) of 6-ethylidene-2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecane as a clear oil. $^1$H NMR (500
MHz, CDCl$_3$) $\delta$ 5.59 (q, 7 Hz, 1 H, CH), 4.23 (m, 4 H, CH$_2$), 1.69 (d, 7 Hz, 3 H, CH$_3$C=C), 0.92 (d, 6 Hz, 18 H, C(CH$_3$)$_3$), 0.08 (s, 12 H, Si(CH$_3$)$_2$).

**Synthesis of 2-ethylidene-1,3-propanediyl bis(t-butylcarbonate) (TL008)$^6$**

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To a solution of 1.02 g (3.02 mmol) of 6-ethylidene-2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecane in 25 mL of THF, 7.00 mL (7.00 mmol) of a solution of 1 M TBAF in THF was added at 0 °C. The reaction was stirred at r.t. for 3 h under N$_2$. The solution was then concentrated via rotary evaporation and a purification was conducted via automated chromatography on silica, eluting with 1/10 (v/v) methanol/DCM. This produced the ammonium salt of the diol, so the product was stirred with 4 mL of a solution of 2 N HCl in methanol for 1 h and then concentrated by rotary evaporation. A solution of the residue in 20 mL of DCM was dried (Na$_2$SO$_4$) then concentrated by rotary evaporation. To the resulting clear oil, which consisted of 0.195 g (1.91 mmol) of impure 2-ethylidene-propane-1,3-diol, was added 0.884 g (4.05 mmol) of di-tert-butyl dicarbonate, 0.264 g (2.16 mmol) of 4-dimethylaminopyridine and 15 mL of diethyl ether, and stirred at r.t. for 24 h. The resulting solution was then washed with sat. aq. CuSO$_4$ solution (3 x 10 mL), sat. aq. NaHCO$_3$ solution (3 x 10 mL) and sat. aq. NaCl solution (3 x 10 mL). The organic layer was then dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation, and the resulting residue was dried *in vacuo*. The product was then purified by automated chromatography on silica gel, eluting with 1/15
(v/v) ethyl acetate/hexane. This produced 490 mg (23%) of 2-ethylidene-1,3-propanediyl bis(t-butylcarbonate) as a clear oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.91 (q, 7 Hz, 1 H, CH), 4.68 (s, 2 H, CH$_2$), 4.56 (m, 2 H, CH$_2$), 1.77 (m, 3 H, C=CHCH$_3$), 1.48 (s, 9 H, C(CH$_3$)$_3$), 1.46 (s, 9 H, C(CH$_3$)$_3$). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 153.3, 153.1, 131.8, 129.5, 81.6, 80.6, 68.9, 61.5, 27.7, 27.6, 13.2. IR (neat cm$^{-1}$) 2926 (w), 2853 (w), 2563 (w), 1738 (w), 1704 (m), 1593 (m), 1517 (m), 1448 (w), 1419 (w), 1368 (m), 1252 (w), 1221 (w), 1162 (s), 1090 (s), 996 (m), 943(m), 898 (m), 839 (m), 790 (m), 731 (m), 691 (m), 643 (m). MS (ESI$^+$) m/z 302 (M$^+$), 303 (MH$^+$). Anal. Calcd. for C$_{20}$H$_{28}$O$_6$: C, 65.92; H, 7.74. Found: C, 65.82; H, 7.66.

**Synthesis of N’-(p-toluenesulfonyl)-N”-(p-methoxybenzenesulfonyl)-[N,N-bis(3-aminopropyl)cyclohexymethylamine] (22)**

![Chemical Structure](image)

To a 500 mL round bottom flask, 7.49 g (19.6 mmol) of N-(3-aminopropyl)-N-(3-p-toluenesulphonamidopropyl)-cyclohexymethylamine, 4.06 g (19.6 mmol) of p-methoxybenzenesulfonyl chloride, 100 mL of DCM, 100 mL of sat. aq. NaCl solution, and 100 mL of sat. aq. Na$_2$CO$_3$ solution were added. The mixture stirred vigorously at r.t. for 24 h. The organic layer was separated, and the aqueous layer was extracted with DCM (3
x 50 mL). The combined organic layers were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the residue was dried in vacuo. Then 24 mL of a solution of 2 N HCl in MeOH was added and stirred for 4 h. The mixture was then concentrated by rotary evaporation and the residue was dried in vacuo. The product was then triturated with anhydrous diethyl ether (3 x 20 mL). The solid was then dried in vacuo and then converted back to free base by stirring the resulting solids vigorously in 20 mL of DCM, 24 mL of aq. 2 N NaOH solution, and 20 mL of sat. aq. NaCl solution for 4 h. The aqueous layer was then extracted with DCM (2 x 25 mL). The combined organic layers were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 9.65 g (89%) of $N'-(p$-toluenesulfonyl)-$N''-(p$-methoxybenzenesulfonyl)-[N,N-bis(3-
aminopropyl)cyclohexylmethylamine] as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$/TMS) $\delta$ 7.75 (d, 8.4 Hz, 4 H, o-Ts, o-ArSO$_2$), 7.51 (d, 8.6 Hz, 2 H, m-ArSO$_2$), 7.29 (d, 7.9 Hz, 2 H, m-Ts), 5.85 (bs, 2 H, NH), 3.00 (q, 6.4 Hz, 4 H, CCH$_2$NH), 2.42 (s, 3 H, ArCH$_3$), 2.38 (t, 6.2 Hz, 4 H, CCH$_2$N), 2.06 (d, 7.2 Hz, 2 H, CH$_2$Cy), 1.63 (m, 10 H, CCH$_2$C, Cy), 1.34 (s, 9 H, ArC(CH$_3$)$_3$), 1.14 (m, 3 H, Cy), 0.79 (m, 2 H, Cy). $^{13}$C NMR (125 MHz, CDCl$_3$/TMS) $\delta$ 156.1, 143.1, 137.1, 137.0, 129.6, 127.1, 126.9, 126.0, 61.9, 52.9, 52.8, 42.5, 35.6, 35.1, 31.8, 31.1, 26.6, 26.0, 25.9, 21.5.
Synthesis of $N'-(p$-toluenesulfonyl)$-N''-(p$-toluenesulfonyl)$-[N,N$-bis(3$-aminopropyl)cyclohexylmethylamine] (TL006)$^6$

In a 1 L round bottom flask, 8.03 g (21.0 mmol) of $N$-(3-aminopropyl)$-N$-(3-p-toluenesulfonamidopropyl)-cyclohexylmethylamine, 4.01 g of (21.0 mmol) of $p$-toluenesulfonyl chloride, 153 mL of DCM, 153 mL of sat. aq. Na$_2$CO$_3$ solution, 153 mL of sat. aq. NaCl solution were added and the resulting mixture was stirred at r.t. for 24 h. The mixture was transferred to a separatory funnel where the organic layer was separated, and the aqueous layer was extracted with DCM (3 x 50 mL). The combined organic layers were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring vigorously with 30 mL of a solution of 2 N HCl in MeOH for 3 h. The solution was then concentrated by rotary evaporation and the residue was dried in vacuo. The solids were then triturated with diethyl ether (3 x 30 mL) and the residue was dried in vacuo. The product was converted back to the free base by stirring with 30 mL of a 2 N aq. NaOH solution and 30 mL of DCM for 3 h. The product was extracted using DCM (3 x 30 mL) and the combined extracts were dried (Na$_2$SO$_4$) and filtered. The filtrate was then concentrated by rotary evaporation and the resulting residue was dried in vacuo.
vacuo. This produced 9.06 g (80%) of $N'-(p$-toluenesulfonyl)-$N''-(p$-toluenesulfonyl)-$[N,N$-bis(3-aminopropyl)cyclohexylmethylamine] as an orange viscous oil. $^1$H NMR (400 MHz, CDCl$_3$/TMS) $\delta$ 7.73 (d, 8.3 Hz, 4 H, o-Ts), 7.29 (d, 7.9 Hz, 4 H, m-Ts), 5.60 (bs, 2 H, NH) 2.96 (t, 6.3 Hz, 4 H, CH$_2$NH), 2.42 (s, 6 H, CH$_3$), 2.35 (t, 6.5 Hz, 4 H, CH$_2$N), 2.03 (d, J = 7.0 Hz, 2 H, CH$_2$Cy), 1.62 (m, 10 H, CCH$_2$C, Cy), 1.34 (m, 1 H, CH), 1.15 (m, 2 H, Cy), 0.77 (q, 12.1 Hz, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 143.1, 136.9, 129.6, 127.1, 77.2, 61.9, 52.9, 42.4, 35.6, 31.8, 26.6, 26.0, 25.9, 21.5. IR (neat cm$^{-1}$) 3277 (w) 2921 (w) 2848 (w) 2126 (w) 1709 (w) 1494 (w) 1447 (w) 1322 (w) 1221 (w) 1153 (s) 1120 (w) 1091 (s) 1018 (w) 951 (w) 813 (m) 706 (s) 658 (s). MS (ESI$^+$) m/z 537 (MH$^+$). Anal. Calcd. for C$_{27}$H$_{41}$N$_3$O$_4$S$_2$: C, 60.53; H, 7.71; N, 7.84. Found: C, 60.87; H, 7.81; N, 7.52.

Synthesis of 9-benzyl-3-benzylidene-1,5-di(p-toluenesulfonyl)-1,5,9-triazacyclododecane (TL005)$^6$

In a 250 mL round bottom flask, 500 mg (1.37 mmol) of 2-benzylidene-1,3-propanediyl bis(t-butylcarbonate), 150 mg (0.29 mmol) of the disulfonamide, 3.05 g (0.171 mmol) of anhydrous sodium carbonate, 132 mg (0.0856 mmol) of
tris(dibenzylideneacetone)dipalladium(0) (Pd$_2$(dba)$_3$), 123 mg (0.171 mmol) of 1,4-bis(diphenylphosphino)butane (dpbb), and 58 mL of anhydrous acetonitrile were stirred under N$_2$ gas and boiled under reflux for 24 h. The mixture was filtered, and the filtrate was then washed with 25 mL of sat. aq. NaHCO$_3$ solution. The aqueous layer was then extracted with DCM (3 x 20 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was then concentrated via rotary evaporation and the residue was dried in vacuo. The product was then converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the residue was dried in vacuo. The resulting solids were triturated with diethyl ether (3 x 25 mL) and the residue was dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of 2 N aq. NaOH solution, and 25 mL of sat. aq. NaCl solution for 4 h. The layers were separated and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated and the residue was dried in vacuo. The product was purified by automated chromatography on alumina eluting with 1:4 (v/v) ethyl acetate/hexane. This produced 77.4 mg (47%) of 9-benzyl-3-benzylidene-1,5-di(p-toluenesulfonyl)-1,5,9-triazacyclododecane as a yellow viscous oil.

$^1$H NMR (500 MHz, CDCl$_3$/TMS) δ 7.73 (d, 8 Hz, 2 H, o-Ts), 7.55 (d, 8 Hz, 2 H, o-Ts), 7.30 (m, 4 H, m-Ts), 7.23 (m, 5 H, Ph), 7.18 (m, 2 H, Ph), 7.12 (d, 7.3 Hz, 3 H, Ph), 6.80 (s, 1 H, C=CH), 4.27 (s, 2 H, H2/4), 3.86 (s, 2 H, H4/2), 3.48 (m, 2 H, H6/12), 3.40 (s, 2 H, CH$_2$Ph), 2.74 (m, 2 H, H12/6), 2.53 (m, 2 H, H8/10), 2.42 (s, 3 H, ArCH$_3$), 2.40 (s, 3 H, ArCH$_3$), 2.28 (s, 2H, H8/10), 1.80 (m, 2 H, H7/11), 1.48 (m, 2 H, H7/11). $^{13}$C NMR (101
MHz, CDCl₃/TMS) δ 143.7, 143.1, 139.5, 138.0, 136.0, 133.5, 130.9, 129.7, 128.9, 128.7, 128.3, 128.2, 127.5, 127.0, 127.0, 59.4, 50.3, 49.6, 48.6, 47.6, 47.2, 42.2, 26.6, 22.5, 21.5. IR (neat cm⁻¹) 3057 (w), 3027 (w), 2923 (w), 2236(w), 1712 (w), 1597 (w), 1493 (w), 1452 (w), 1377 (w), 1337 (m), 1160 (s), 1088 (m), 1017 (w), 993 (w), 925 (w), 814 (w), 746 (m) 724 (m), 699 (s), 657 (m). MS (ESI⁺) m/z 658 (M⁺), 660 (MH⁺), 661 (MH⁺ + 1). Anal. Calcd. for C₃₇H₄₃N₃O₄S₂·HCl: C, 64.00; H, 6.39; N, 6.05. Found: C, 64.26; H, 6.66; N, 5.67.

**Synthesis of 3-benzylidene-9-(cyclohexylmethyl)-1,5-di(p-toluenesulfonyl)-1,5,9-triazacyclododecane (TL007)⁶**

![Chemical Structure]

In a 250 mL round bottom flask, 777 mg (2.13 mmol) of 2-benzylidene-1,3-propanediyl bis(t-butylcarbonate), 267 mg (0.497 mmol) of the disulfonamide, 4.85 mg (45.8 mmol) of anhydrous sodium carbonate, 123 mg (0.134 mmol) of tris(dibenzylideneacetone)dipalladium(0), 118 mg (0.277 mmol) of 1,4-bis(diphenylphosphino)butane, was added with 100 mL of anhydrous acetonitrile were stirred under N₂ gas and boiled under reflux for 24 h. The mixture was filtered, and the filtrate was then washed with 25 mL of saturated aqueous NaHCO₃ solution. The aqueous
layer was then extracted with DCM (3 x 20 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was then concentrated via rotary evaporation and the residue was dried in vacuo. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the residue was dried in vacuo. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue was dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of 2 N aq. NaOH solution, and 25 mL of sat. aq. NaCl solution for 4 h. The layers were separated and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated and the residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 1:4 (v/v) ethyl acetate/hexane. This produced 52.4 mg (16%) of 3-benzylidene-9-(cyclohexylmethyl)-1,5-di(p-toluenesulfonyl)-1,5,9-triazacyclododecane as a brown viscous oil. $^1$H NMR (500 MHz, CDCl$_3$/TMS) $\delta$ 7.78 (d, 8.3 Hz, 1 H, $\alpha$-Ts), 7.57 (d, 8.3 Hz, 1 H, $\alpha$-Ts), 7.33 (m, 2 H, $\alpha$-Ts), 7.28 (m, 5 H, Ph), 7.21 (m, 2 H, Ph), 7.04 (d, 7.6 Hz, 2 H, Ph), 6.83 (s, 1 H, C=CH), 4.25 (s, 2 H, H2/4), 3.83 (s, 2 H, H4/2), 3.59 (m, 2 H, H6/12), 2.66 (t, 6 Hz, 2 H, H12/6), 2.44 (d, 13.8 Hz, 6 H, CH$_3$), 2.35 (m, 2 H, H8/10), 2.24 (m, 2 H, H10/8), 1.98 (d, 7.0 Hz, 2 H, CH$_2$Cy), 1.68 (m, 6 H, H7,11, Cy), 1.55 (m, 1 H, Cy), 1.46 (m, 1 H, Cy), 1.27 (m, 2 H, Cy), 1.14 (m, 2 H, H19), 0.86 (m, 1 H, H18), 0.70 (m, 2 H, H16, H20). $^{13}$C NMR (126 MHz, CDCl$_3$/TMS) $\delta$ 138.88, 138.36, 133.41, 131.17, 128.76, 125.80, 125.28, 125.04, 125.00, 124.16, 123.36, 122.72, 122.25, 122.18, 57.72, 46.48, 44.78, 44.19, 42.46, 37.60, 31.16,
27.20, 24.95, 22.06, 21.79, 21.32, 17.68, 16.77. IR (neat cm\(^{-1}\)) 2920 (m) 2849 (w) 2800 (w) 1597 (w) 1493 (w) 1447 (w) 1336 (m) 1304 (w) 1157 (s) 1089 (m) 1033 (w) 941 (w) 909 (w) 876 (w) 849 (w) 813 (m) 766 (w) 729 (s) 699 (m) 657 (s). MS (ESI\(^+\)) m/z 665 (MH\(^+\)), 666 (MH\(^+\) + 1), 667 (MH\(^+\) + 2). Anal. Calcd. for C\(_{37}\)H\(_{50}\)ClN\(_3\)O\(_4\)S\(_2\): C, 63.45; H, 7.20; N, 6.00. Found: C, 61.18; H, 7.51; N, 6.08.

**Synthesis of N,N-bis[N'(3-methyl-1,3-butadien-2-yl)-3-toluenesulfonamidopropyl] cyclohexylmethylamine (TL002)**

![Chemical structure of TL002] (image)

Into a 50 mL round bottom flask with nitrogen inlet and stir bar, 0.200 g (0.632 mmol) of 2-isopropylidene-1,3-propanediyl bis(t-butyldimethoxide), 5.81 mg (0.144 mmol) of N-(3-aminopropyl)-N-(3-p-toluenesulfonamidopropyl)-cyclohexylmethylamine, 1.70 mg (0.123 mmol) of Na\(_2\)CO\(_3\), 7.13 mg (0.0111 mmol) of tris(dibenzylideneacetone)dipalladium(0) (Pd\(_2\)(dba)\(_3\)), 6.27 mg (0.0511 mmol) of 1,4-bis(diphenylphosphino)butane (dppb), and 16 mL of anhydrous acetonitrile under N\(_2\) gas. The mixture was stirred and heated under reflux for 24 h under nitrogen. The solvent is then removed via rotary evaporation and 2 mL of sat. aq. NaHCO\(_3\) solution was added.
The resulting mixture was then extracted using DCM (3 x 10 mL). The organic layer was then concentrated via rotary evaporation and the residue was dried in vacuo producing 0.17 g (>100%) of a viscous brown oil. Purification was conducted by column chromatography on neutral alumina, eluting with 1:4 (v/v) ethyl acetate/hexane. This produced 66 mg (88%) of \(N,N\)-bis[\(N\prime\)-(3-methyl-1,3-butadien-2-yl)-3-ptoluenesulfonamidopropyl] cyclohexylmethylamine as a yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)/TMS) \(\delta\) 7.74 (d, 9 Hz, 2 H, \(o\)-ArSO\(_2\)), 7.69 (d, 8 Hz, 2 H, \(o\)-Ts), 7.30 (d, 8 Hz, 2 H, \(m\)-Ts), 6.97 (d, 9 Hz, 2 H, \(m\)-ArSO\(_2\)), 5.26 (s, 2 H, C=CH), 5.24 (s, 2 H, C=CH), 5.15 (s, 2 H, C=CH), 5.05 (s, 2 H, C=CH), 3.92 (s, 3 H, \(OCH_3\)), 3.86 (s, 4 H, H6, C=CCH\(_2\)NSO\(_2\)), 3.02 (m, 4 H, H8, CH\(_2\)NCH\(_2\)Cy), 2.42 (s, 3 H, ArCH\(_3\)), 2.17 (t, 7 Hz, 4 H, CH\(_2\)NCH\(_2\)Cy), 1.94 (d, 7 Hz, 2 H, CH\(_2\)Cy), 1.91 (s, 6 H, C=CCH\(_3\)), 1.67 (m, 10 H, CCH\(_2\)C, Cy), 1.13 (m, 3 H, Cy), 0.71 (m, 2 H, Cy). \(^{13}\)C (101 MHz, CDCl\(_3\)) \(\delta\) 162.7, 143.1, 142.3, 140.4, 136.3, 131.0, 129.6, 129.3, 127.2, 115.7, 115.6, 114.4, 114.4, 114.1, 61.3, 61.2, 55.5, 52.1, 52.0, 51.7, 51.7, 47.03, 47.00, 36.0, 31.8, 27.7, 26.9, 26.2, 26.1, 21.5, 21.3, 20.1. MS (ESI\(^+\)) \(m/z\) 713 (M\(^+\)). HRMS (ESI-TOF) \(m/z\): [M + H]\(^+\) calcd. For C\(_{39}\)H\(_{58}\)N\(_3\)O\(_5\)S\(_2\) 712.3818; found 712.3788.
Synthesis of 9-benzyl-3-ethylidene-1,5-di(p-toluenesulfonyl)-1,5,9-triazacyclododecane (TL010)\(^6\)

To a 250 mL round bottom flask, 696 mg (1.31 mmol) of \(N\)-(3-aminopropyl)-\(N\)-(3-p-toluenesulfonamidopropyl)-cyclohexylmethylamine, 1.23 g (4.06 mmol) of 2-ethylidene-1,3-propanediyl bis(\(t\)-butylcarbonate), 131 mg (1.23 mmol) of Na\(_2\)CO\(_3\), 272 mg (0.297 mmol) of Pd\(_2\)dba\(_3\), 264 mg (0.619 mmol) of dppb and 138 mL of anhydrous acetonitrile were stirred under N\(_2\) gas and boiled under reflux for 24 h. The mixture was filtered, and the filtrate was then washed with 25 mL of sat. aq. NaHCO\(_3\) solution. The aqueous layer was then extracted with DCM (3 x 20 mL). The combined organic solutions were dried (Na\(_2\)SO\(_4\)) and filtered. The filtrate was then concentrated via rotary evaporation and the residue was dried in vacuo. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the residue was dried in vacuo. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue was dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of 2 N aq. NaOH solution, and 25 mL of sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL).
The combined organic solutions were dried (Na₂SO₄) and filtered. The filtrate was concentrated, and the residue was dried in vacuo. The product was purified by chromatotron on neutral alumina, eluting with 1:9 (v/v) ethyl acetate/hexane. This produced 28.8 mg (4%) of 9-benzyl-3-ethylidene-1,5-di(p-toluenesulfonyl)-1,5,9-triazacyclododecane as a clear viscous oil. 

$^1$H NMR (500 MHz, CDCl₃) $\delta$ 7.66 (dd, 9.8, 8.1 Hz, 4 H, o-Ts) 7.36 (d, 8.0 Hz, 2 H, m-Ts), 7.27 (m, 5 H, Ph), 7.14 (d, 7.3 Hz, 2 H, m-Ts), 5.84 (d, 7.4 Hz, 1 H, C=CH), 3.97 (s, 2 H, H2/H4), 3.64 (s, 2 H, H4/H2), 3.38 (s, 2 H, CH₂Ph), 3.26 (t, 7.7 Hz, 2 H, H6/12), 2.91 (t, 6.2 Hz, 2 H, H12/6), 2.50 (m, 2 H, H8/10), 2.46 (s, 3 H, ArCH₃), 2.44 (s, 3 H, ArCH₃), 2.22 (t, 5.7 Hz, 2 H, H10/8), 1.84 (m, 2 H, H7/11), 1.73 (m, 3 H, C=CHCH₃) 1.41 (s, 2 H, H11/7). 

$^{13}$C NMR (126 MHz, CDCl₃) $\delta$ 143.7, 143.1, 139.6, 137.5, 133.7, 129.8, 129.7, 128.7, 128.2, 127.7, 127.5, 127.0, 127.0, 125.9, 59.4, 50.5, 48.9, 48.6, 48.2, 46.1, 42.2, 26.0, 22.5, 21.6, 21.5, 13.0. 

IR (neat cm⁻¹) 2970 (w), 2926 (w), 2323 (w), 2161 (w), 2043 (w), 1738 (m), 1597 (w), 1454 (m), 1365 (m), 1333 (m), 1227 (m), 1217 (m), 1157 (s), 1116 (m), 1088 (m), 1015 (m), 972 (m), 896 (m), 813 (m), 768 (m), 723 (m), 699 (m), 654 (s), 639 (m), 627 (m), 604 (m). 

MS (ESI⁺) m/z 597 (M⁺). 

Anal. Calcd. for C₃₂H₄₁N₅O₄S₂·HCl·1.25H₂O: C, 58.70; H, 6.85; N, 6.42. Found: C, 58.97; H, 7.23; N, 6.43.
2.5 References


Chapter 3

Synthesis and CD4 Down-Modulation Potency of Side Arm Modified CADA Analogs
3.1 Introduction

A library of symmetrical and unsymmetrical CADA analogs has been previously synthesized to better understand the interactions between CADA and the SP of CD4. Various types of side arms have been utilized to create more potent CADA compounds. The most potent CADA analog synthesized was **CK147**, created by Dr. Reena Chawla (Figure 3.1.1). Prior to this, compound **VGD020** was the most potent CADA analog, which contained a 4-methoxybenzenesulfonamide side arm instead of the 4-(dimethylamino)benzenesulfonamide side arm of **CK147**. Previously conducted SAR studies modified one of the tosyl side arms of CADA ($IC_{50}$ 0.56 µM) and determined that analogs with two different sulfonamide side arms were more potent than the symmetrical analogs which contained two identical sulfonamide side arms. It was found that electron-donating groups (EDGs) in the para position of the benzenesulfonamide side arm increases the potency of the CADA analog.¹ A strong electron withdrawing group (EWG) in the para position, such as a nitro group, was shown to be deactivating ($IC_{50}$ 5.25 µM). The absence of a phenyl ring on the sulfonamide group and substitution of a methyl or an N-morpholino group showed some significant decreases in potencies ($IC_{50}$ 1.58 and 1.08 µM, respectively).¹ The substitution of a trifluoromethoxy group from the para-methoxy increased the $IC_{50}$ value by 1 µM. The replacement of the methoxy by propargyl showed an increase of about 0.1-0.2 µM, which showed that the electronics of the group is more

![Figure 3.1.1. Structure CK147.](image-url)
important than the sterics. Replacing the para-methoxy with NMe₂ showed a great increase in potency of the analog, while substituting the para-methoxy group with hydrogen bond donor (HBD) groups, such as an OH group or NH₂ group, showed a great decrease in potency.¹

As previously mentioned in chapter 1, it was proposed that non-HBD polar substituents on the non-tosyl side arm affects the strength of the interaction between CADA and the signal peptide (SP) of CD4. It is believed that the non-tosyl side arm interacts through dipole-dipole interactions. Increasing the dipole moment of the non-tosyl side arm increases the interaction of the analog with the SP. This study was done to determine what kind of effect modifications to the tosyl side arm of CK147 would have on the CD4 down-modulation potency of the CADA analog. A series of CK147 analogs were synthesized with modifications to the tosyl side arm and their CD4 down-modulation potencies and anti-HIV activities were measured.

3.2 Results and Discussion

3.2.1 Synthesis of N-(3-Aminopropyl)-N-(3-p-dimethylaminobenzenesulfonamidopropyl)benzylamine (29)

Syntheses of this series of compounds begins with the formation of compound 29, which would allow reactions with various sulfonyl chlorides in order to modify the tosyl side arm of CK147. The synthesis involves a four-step sequence of reactions to get the primary amine 29 (Scheme 3.2.1). Prior to beginning the synthesis, the 4-(dimethylamino)benzenesulfonyl chloride needed for the first step had to be synthesized.
(Scheme 2.2.2). Addition of the 4-(dimethylamino)benzenesulfonamide side arm was done by reaction of 1,3-diaminopropane with 4-(dimethylamino)benzenesulfonyl chloride to obtain the primary amine 26. Reductive amination with cyclohexanecarboxaldehyde was conducted to add the cyclohexylmethyl tail. This was followed by chain elongation by reaction of 27 with N-(3-bromopropyl)phthalimide to form the phthalimide protected compound 28. Compound 28 was treated with hydrazine monohydrate to remove the phthalimide group followed by acid to obtain amine 29. With the primary amine successfully synthesized, the various CADA analogs could be easily made by attaching the second side arm and conducting the Pd-catalyzed macrocyclization.
3.2.2 Synthesis of TL020

Since CK147 and VGD020 showed significant CD4 down-modulation, a new CADA analog with parts of both compounds was synthesized. The compound would contain the cyclohexylmethyl tail and isobutylene head group from both compounds. It would also have a para-methoxy side arm along with a 4-(dimethylamino)benzene sulfonamide side arm, merging the two compounds together to form TL020. To start the synthesis, the primary amine 29 was treated with p-methoxybenzenesulfonyl chloride to produce compound TL016 (Scheme 3.2.2). The compound TL016 was converted to the HCl salt by treating it with 2 N HCl in MeOH and purified by trituration with diethyl ether. Conversion of TL016 back to the free base was conducted using aq. 2 N NaOH solution in order to conduct the palladium catalyzed macrocyclization.

Scheme 3.2.2. Synthesis of compound 5.
Prior to conducting the macrocyclization, the Boc protected isobutylene diol needed to be synthesized. 2-Methylenepropane-1,3-diol was treated with di-tert-butyl dicarbonate with 4-dimethylaminopyridine in diethyl ether to produce the Boc protected compound 30 (Scheme 3.2.3). With compounds TL016 and 30 completed, the palladium-catalyzed macrocyclization was conducted to produce TL020 in 31% yield (Scheme 3.2.4).

\[ \text{Scheme 3.2.3. Synthesis of compound 30.} \]

3.2.3 Synthesis of Unsymmetrical Disulfonamides with \textit{para} and \textit{ortho} Substitutions

Reaction of compound 29 with various commercially available sulfonyl chlorides containing substituents at the \textit{para} and \textit{ortho} positions produced different open chain disulfonamides (Scheme 3.2.5).

\[ \text{Scheme 3.2.5. Synthesis of unsymmetrical disulfonamides from sulfonyl chlorides.} \]
The resulting disulfonamides, shown in Figure 3.2.1, were converted to the HCl salt by treating them with 2 N HCl in MeOH. The resulting HCl salts were purified by trituration with diethyl ether and then converted back to the free base by treatment with aq. 2 N NaOH solution. The yields for this step were high, with values ranging from 73% to 99%.

![Figure 3.2.1. Structure and percent yields of new disulfonamides with ortho or para substitutions.](image)

### 3.2.4 Synthesis of Unsymmetrical CADA Analogs with para and ortho Substitutions

![Scheme 3.2.6. Palladium-catalyzed macrocyclization of disulfonamides.](image)
The unsymmetrical disulfonamides with para or ortho substituents were cyclized by the palladium-catalyzed cyclization (Scheme 3.2.6). These newly formed TL compounds, shown in Figure 3.2.2, were purified by automated chromatography. They were converted to HCl salts and triturated with diethyl ether for further purification.

![Chemical structures and yields](image)

**Figure 3.2.2.** Structure and percent yields of new CADA analogs with *ortho* or *para* substitutions.

3.2.5 Synthesis of Various Unsymmetrical Disulfonamides from Commercially Available Sulfonyl Chlorides

A series of disulfonamides containing the 4-(dimethylamino)benzenesulfonamide side arm and side arms containing various ring substitutions were synthesized. Using the same synthesis method, the varying side arms were added by treatment of compound 29 with different sulfonyl chlorides (Scheme 3.2.5) to produce disulfonamides with two different side arms, as shown in Figure 3.2.3. These compounds were converted to the HCl...
salts by treating them with 2 N HCl in MeOH. They were then purified by trituration with diethyl ether and converted back to the free base.

![Chemical structures](image)

**Figure 3.2.3.** Newly synthesized unsymmetrical disulfonamides and their percent yields.

### 3.2.6 Synthesis of TL026, TL036, and TL044

Benzo\[d\][1,3]dioxole-5-sulfonyl chloride, morpholine-4-sulfonyl chloride, and 2-methoxybenzenesulfonyl chloride had to be synthesized (Scheme 3.2.7). Reaction of morpholine with sulfuryl chloride in acetonitrile gave morpholine-4-sulfonyl chloride. Benzodioxole was treated with sulfuryl chloride in DMF to produce benzo[d][1,3]dioxole-5-sulfonyl chloride. 2-Methoxybenzenethiol was treated with N-chlorosuccinimide to form the 2-methoxybenzenesulfonyl chloride. 2-Methoxybenzenethiol was treated with N-chlorosuccinimide and isopropanol to form 2-methoxybenzenesulfonyl chloride.
The sulfonyl chlorides were then treated with compound 29 to produce the resulting disulfonamides TL026, TL036, and TL044 (Figure 3.2.4). These compounds were converted to the HCl salts by treating them with 2 N HCl in MeOH. They were then purified by trituration with diethyl ether and converted back to the free base.

![Scheme 3.2.7. Synthesis of sulfonyl chlorides using sulfuryl chloride and NCS.](image)

3.2.7 Synthesis of Various Unsymmetrical CADA Analogs

The unsymmetrical disulfonamides from commercial and non-commercial sulfonyl chlorides were cyclized by the palladium-catalyzed cyclization (Scheme 3.2.6).
These newly formed TL compounds shown in Figure 3.2.5 were purified by column chromatography. They were also converted to the HCl salt and triturated with diethyl ether for further purification.

![Unsymmetrical CADA analogs from commercial and non-commercial sulfonyl chlorides and their percent yields.](image)

**Figure 3.2.5.** Unsymmetrical CADA analogs from commercial and non-commercial sulfonyl chlorides and their percent yields.

### 3.2.8 CD4 Down-Modulation and Anti-HIV Activities

All newly synthesized CADA analogs containing a 4-dimethylaminobenzenesulfonamide side arm shown in Figure 3.2.6, were tested for their CD4 down-modulation activities. The CD4 down-modulation activities, listed in Table 3.2.1, are given as \( IC_{50} \) values as described in chapter 2.
It was found that these newly synthesized TL compounds, except for TL027, had greater activities than that of CADA. Compounds TL020, TL029, and TL039 were found to have the highest activity in this series, which were comparable to CK147. Increasing the steric bulk of the tosyl side arm, as shown in compounds TL022, TL023, and TL038, showed a decrease in activity which correlate with previous analogs containing bulky substituents on the non-tosyl side arm. The removal of the methyl group in the side arm of compound TL032 did not seem to alter the activity of the analog, however removal of the
aromatic ring in compounds TL027 and TL033 caused a decrease in activity which was also found in previous CADA analogs without an aromatic ring on the non-tosyl side arm. Increased electron density in compound TL042 in the para position showed a slight decrease in activity. Moving the para-methoxy to the ortho-methoxy position in TL045

Table 3.2.1. Newly synthesized TL compounds with a dimethylaminobenzene sulfonamide side arm tested for CD4 down-modulation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$IC_{50}$ (µM)$^*$ mean ± SDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADA</td>
<td>0.35 ± 0.06</td>
</tr>
<tr>
<td>CK147</td>
<td>0.058 ± 0.006</td>
</tr>
<tr>
<td>TL020</td>
<td>0.053 ± 0.001</td>
</tr>
<tr>
<td>TL021</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td>TL022</td>
<td>0.071 ± 0.006</td>
</tr>
<tr>
<td>TL023</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>TL027</td>
<td>0.85 ± 0.19</td>
</tr>
<tr>
<td>TL029</td>
<td>0.053 ± 0.001</td>
</tr>
<tr>
<td>TL032</td>
<td>0.052 ± 0.035</td>
</tr>
<tr>
<td>TL033</td>
<td>0.094 ± 0.035</td>
</tr>
<tr>
<td>TL038</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>TL039</td>
<td>0.043 ± 0.018</td>
</tr>
<tr>
<td>TL042</td>
<td>0.069 ± 0.015</td>
</tr>
<tr>
<td>TL043</td>
<td>0.054 ± 0.025</td>
</tr>
<tr>
<td>TL045</td>
<td>0.12 ± 0.02</td>
</tr>
</tbody>
</table>

These compounds were tested as HCl salts. $^*$IC$_{50}$: Inhibitory concentration 50%, concentration at which 50% down-modulation of CD4 expression was measured in CHO-CD4-YFP cells after 24 hours of treatment with CADA compound. Values are mean ± STDEV from 3 independent experiments.
showed a drastic decrease in activity, while the ortho, meta dimethyl compound TL043 did not show much of a change in activity.

The newly synthesized TL compounds were tested for cytotoxicity in MT-4 cells and measured as $CC_{50}$ values, as shown in Table 3.2.2. The most cytotoxic compound was found to be TL029. Compound TL020 was found to be less cytotoxic than CK147. The least cytotoxic compound was TL042. Compound TL020 had a therapeutic index (TI) of

Table 3.2.2. Newly synthesized TL compounds with a dimethylaminobenzene sulfonamide side arm tested for cytotoxicity and their therapeutic index.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$CC_{50}$ ($\mu$M)* mean ± SDV</th>
<th>Therapeutic Index**</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADA</td>
<td>$&gt;$100</td>
<td>-</td>
</tr>
<tr>
<td>CK147</td>
<td>2.6 ± 1.2</td>
<td>45</td>
</tr>
<tr>
<td>TL020</td>
<td>4.3 ± 3.7</td>
<td>81</td>
</tr>
<tr>
<td>TL021</td>
<td>27 ± 25</td>
<td>226</td>
</tr>
<tr>
<td>TL022</td>
<td>26 ± 11</td>
<td>367</td>
</tr>
<tr>
<td>TL023</td>
<td>26 ± 14</td>
<td>149</td>
</tr>
<tr>
<td>TL027</td>
<td>25 ± 2</td>
<td>29</td>
</tr>
<tr>
<td>TL029</td>
<td>2.1 ± 1.4</td>
<td>40</td>
</tr>
<tr>
<td>TL032</td>
<td>9.4 ± 1.4</td>
<td>181</td>
</tr>
<tr>
<td>TL033</td>
<td>6.5 ± 3.8</td>
<td>70</td>
</tr>
<tr>
<td>TL038</td>
<td>6.7 ± 0.1</td>
<td>47</td>
</tr>
<tr>
<td>TL039</td>
<td>2.3 ± 0.4</td>
<td>54</td>
</tr>
<tr>
<td>TL042</td>
<td>63 ± 18</td>
<td>909</td>
</tr>
<tr>
<td>TL043</td>
<td>4.1 ± 1.6</td>
<td>75</td>
</tr>
<tr>
<td>TL045</td>
<td>17 ± 7</td>
<td>145</td>
</tr>
</tbody>
</table>

These compounds were tested as HCl salts. *$CC_{50}$: Inhibitory concentration 50%, concentration of the compound required to reduce the viability of MT-4 cells by 50%.

**Therapeutic index: Ratio of $CC_{50}/IC_{50}$ for CD4 down-modulation.
whereas CK147 had a TI of 45. However, the greatest TI was found with TL042 containing the para-difluoromethoxy side arm, making it the most selective CD4 down-modulating CADA analog.

The TL compounds were also tested for their anti-HIV properties as well (Table 3.2.3). The compounds TL020 and TL029 were found to be the most potent inhibitors of Table 3.2.3. Anti-HIV activities of TL compounds in MT-4 cells and their therapeutic index.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM) $^*$ mean ± SDV</th>
<th>Therapeutic Index $^{**}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADA</td>
<td>5.2 ± 1.7</td>
<td>-</td>
</tr>
<tr>
<td>CK147</td>
<td>0.37 ± 0.04</td>
<td>7</td>
</tr>
<tr>
<td>TL020</td>
<td>0.36 ± 0.04</td>
<td>12</td>
</tr>
<tr>
<td>TL021</td>
<td>1.5 ± 0.3</td>
<td>18</td>
</tr>
<tr>
<td>TL022</td>
<td>0.44 ± 0.10</td>
<td>59</td>
</tr>
<tr>
<td>TL027</td>
<td>9.4 ± 0.1</td>
<td>3</td>
</tr>
<tr>
<td>TL029</td>
<td>0.31 ± 0.17</td>
<td>7</td>
</tr>
<tr>
<td>TL032</td>
<td>0.51 ± 0.07</td>
<td>19</td>
</tr>
<tr>
<td>TL033</td>
<td>0.65 ± 0.08</td>
<td>10</td>
</tr>
<tr>
<td>TL038</td>
<td>1.3 ± 0.5</td>
<td>5</td>
</tr>
<tr>
<td>TL039</td>
<td>0.56 ± 0.16</td>
<td>4</td>
</tr>
<tr>
<td>TL042</td>
<td>0.75 ± 0.26</td>
<td>84</td>
</tr>
<tr>
<td>TL043</td>
<td>0.68 ± 0.13</td>
<td>6</td>
</tr>
<tr>
<td>TL045</td>
<td>2.1 ± 0.4</td>
<td>8</td>
</tr>
</tbody>
</table>

These compounds were tested as HCl salts. $^*$IC$_{50}$: Inhibitory concentration 50%, concentration of the compound required to reduce viral HIV-1 replication by 50% as measured by the p24 Ag ELISA after 4 days of drug treatment. $^{**}$Therapeutic index: Ratio of CC$_{50}$/IC$_{50}$ for CD4 down-modulation.
HIV-1 replication in cell culture. They were comparable to CK147. Compound TL042 is shown to be slightly less potent than TL020 or TL029, however, TL020 and TL029 have Tls of 12 and 7 respectively, for anti-HIV activity. While TL042 has a TI of 84, which is dramatically higher than TL020 and TL029. TL042 could be considered a better drug target than TL020 or TL029 due to its increased selectivity.

3.3 Conclusion and Outlook

The 13 new unsymmetrical CADA compounds were found to have a range of CD4 down-modulation potencies. Among all the new CADA analogs, TL020, TL029, and TL039 were found to have similar CD4 down-modulation potencies with CK147. The activities of TL032 and TL027, containing a morpholine ring and a methyl sulfonamide, confirmed the belief that two aromatic side arms of the CADA are necessary for CD4 down-modulation. Activity of TL020 and TL029 shows that a second side arm containing an electron donating group in the para position gave a slight increase in potency. Polycyclic side arms were also synthesized and shown to have a wide range of activity. When the polycyclic side arm contained the dioxole ring (TL0039), activity of the compound increased unlike the naphthalene analogs (TL022 and TL023). Compound TL029 was found to be more cytotoxic than CK147. Compound TL042 was found to be the most selective CADA analog towards HIV inhibition with the lowest level of cytotoxicity (TI = 84). These conclusions are found to be interesting to the Bell group in order to understand the interactions between CADA and the biomolecular target.
A screening of **TL020** with several other viral diseases has also been conducted. Initial screening has found it to be active against the zika virus, hepatitis B virus, chikungunya virus, dengue virus, tacaribe virus, and respiratory syncytial virus. This could potentially lead to applying CADA analogs to more than just HIV. More structure activity relationship studies could be designed around different viral entities to combat some of these diseases and understand the interaction between CADA compounds and the viral inhibition.

### 3.4 Experimental

**General Methods**

All reactions were performed under an atmosphere of dry nitrogen, unless specified otherwise. Reagents and solvents purchased from Aldrich Chemical Company, Acros Organics, or Fisher Scientific were of ACS reagent grade or better and were used without purification, unless indicated otherwise. Anhydrous acetonitrile used in the macrocyclization step was distilled from CaH$_2$. For macrocyclization reactions, the disulfonamide intermediates, 2-methylene-1,3-propanebis(*tert*-butylcarbonate), anhydrous sodium carbonate, dppb, and Pd$_2$(dba)$_3$ were dried *in vacuo* (ca. 0.1 mm) for at least 16 h. All the equipment required for macrocyclization reaction including a magnetic stir bar, spatula, syringe and needle were also dried overnight in the oven (110 °C). Solutions of 2 N HCl in methanol were created by placing 165 mL of 12.1 M HCl into a 1 L volumetric flask. The flask is then filled with 835 mL of methanol. Column chromatography was performed with Sorbent Technologies neutral alumina (50-200 μm) or Sorbent
Technologies standard grade silica (32-63 μm), unless noted otherwise. Chromatotron chromatography was performed with Sorbent Technologies neutral alumina with gypsum and UV254. Automated chromatography was performed on the Yamazen Smart Flash AKROS RE-X10 with Sorbet Technologies neutral alumina (50-200 μ) or Sorbent Technologies standard grade silica (32-63 μm) and HPLC grade ethyl acetate, hexane, and dichloromethane (DCM). Compounds dried in vacuo were connected to a vacuum manifold with a Welch 1402 vacuum pump and vacuumed dried for at least 18 h at ca. 0.1 mm. Melting points were measured on a Thomas-Hooover or Mel-Temp apparatus and are uncorrected. \(^1\)H NMR (400 MHz or 500 MHz) and \(^{13}\)C NMR (75 MHz or 125 MHz) spectra were acquired on a Varian 400 or Varian Unity + 500 spectrometer. All chemical shifts (δ) are reported in ppm units relative to solvent resonances, as follows: \(^1\)H, CDCl\(_3\)/TMS = 0.00, DMSO-d\(_6\) = 2.50, CD\(_3\)OD = 3.31; \(^{13}\)C, CDCl\(_3\) = 77.23, DMSO-d\(_6\) = 39.7, CD\(_3\)OD = 49.15. Infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrometer. Mass spectra (MS) were acquired on a Waters Micromass ZQ electrospray ionization quadrupole mass spectrometer with positive ion detection (capillary voltage = 3.5 kV). High-resolution mass spectra (HRMS) were acquired on an Agilent 6230 TOF mass spectrometer. Samples for elemental analysis were dried at 78 °C (0.1 mm) for 2 days, unless stated otherwise, and microanalysis was performed by NuMega Resonance Labs, Inc.
Synthesis of N-(3-Aminopropyl)-4-(dimethylamino)benzenesulfonamide (1)^3

In a 250 mL round bottom flask with an addition funnel and nitrogen inlet, 3.84 g (51.9 mmol) of 1,3-diaminopropane was added, cooled to 0 °C, and stirred under N₂. Then 1.14 g (5.19 mmol) of 4-(dimethylamino)benzenesulfonyl chloride in 50 mL of DCM were placed into the addition funnel and added to the reaction flask dropwise over a period of 1 h. The solution was stirred for 30 min at 0 °C and then stirred at r.t. for 24 hours. The solution was then concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. Then 50 mL of 1:1 (v/v) methanol/water solution was added and stirred for 30 min at r.t. The mixture was filtered to remove ditosylated product. The filtrate was then cooled to 0 °C and recrystallized. The mixture was then filtered, and the filtrate was then concentrated by heating and then placed in 0 °C to further crystalize more monotosylated product. This produced 0.951 g (71%) of N-(3-Aminopropyl)-4-(dimethylamino)benzenesulfonamide as a white solid. $^1$H NMR (400 MHz, CDCl₃/TMS) $\delta$ 7.67 (d, 9.0 Hz, 2 H, $o$-PhSO₂), 6.66 (d, 8.9 Hz, 2 H, $m$-PhSO₂), 3.03 (s, 3 H, ArCH₃), 3.00 (m, 2 H, ArSO₂CH₂), 2.76 (t, 6.1 Hz, 2 H, NH₂CH₂), 1.56 (p, 6.1 Hz, 2 H, CH₂CH₂CH₂). $^{13}$C NMR (101 MHz, CDCl₃) $\delta$ 152.7, 128.8, 125.3, 110.9, 42.4, 40.6, 40.1, 31.4. mp 123.7-126.0 °C (dec). IR (neat cm⁻¹) 3354 (w), 3300 (w), 2858 (w), 1598 (w), 1554 (w), 1514 (w), 1442 (w), 1401 (w), 1369 (w), 1323 (w), 1308 (s), 1225 (w), 1147 (s),
1094 (m), 1071 (m), 995 (w), 957 (w), 940 (w), 819 (s). MS (ESI+) m/z 258 (MH+). Anal. Calcd for C_{11}H_{19}N_{2}O_{2}S: C, 51.34; H, 7.44; N, 16.33. Found: C, 51.31; H, 7.58; N, 16.70.

Synthesis of N-(3-((cyclohexylmethyl)amino)propyl)-4-(dimethylamino)benzenesulfonamide (2) \(^3\)

[Chemical structure image]

In a 500 mL round bottom flask with a condenser, 21.6 g (94.6 mmol) of N-(3-aminopropyl)-4-methylbenzenesulfonamide was added with 200 mL of DCM and stirred. Then 12.0 g (107 mmol) of cyclohexane carboxaldehyde and 12.3 g of MgSO\(_4\) were added. The mixture was then heated under reflux with N\(_2\) gas overnight. The mixture was then filtered through a fine sintered funnel to remove the MgSO\(_4\). The solution was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. Then 4.14 g (109 mmol) of sodium borohydride was added with 50 mL of EtOH and stirred for 18 h. The cloudy mixture was then filtered through a fine sintered funnel and the solids were washed with 50 mL of EtOH. The filtrate was concentrated via rotary evaporation and the resulting residue was dried \textit{in vacuo}. Then 100 mL of H\(_2\)O was added and the mixture was extracted with DCM (3 x 50 mL). The combined extraction layers were dried (Na\(_2\)SO\(_4\)) and filtered. The filtrate was then concentrated by rotary evaporation and the resulting residue was dried
in vacuo, to give 1.68 g (85%) of pure compound N-(3-((cyclohexylmethyl)amino)propyl)-4-(dimethylamino)benzenesulfonamide as a clear oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.67 (m, 2 H, $\alpha$-ArSO$_2$), 6.67 (m, 2 H, $m$- ArSO$_2$), 3.05 (s, 3 H, N(CH$_3$)$_2$), 3.03 (s, 3 H, N(CH$_3$)$_2$) 3.00 (m, 2 H, CH$_2$NH), 2.61 (m, 2 H, CH$_2$NH-Cy), 2.34 (d 6.6 Hz, 2 H, CH$_2$Cy), 1.70 (m, 6 H, CH$_2$C, Cy), 1.58 (m, 2 H, Cy), 1.38 (m, 1 H, Cy), 1.21 (m, 2H, Cy), 0.89 (m, 2H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.4, 128.6, 125.2, 110.7, 56.5, 48.8, 43.1, 39.9, 37.7, 31.3, 27.9, 26.6, 25.9. IR (neat cm$^{-1}$) 3295 (w), 2920 (w), 2848 (w), 1595 (m), 1556 (w), 1514 (w), 1445 (w), 1365 (w), 1312 (m), 1227 (w), 1203 (w), 1144 (s), 1093 (m), 1000 (w), 969 (w), 943 (w), 872 (w), 813 (m), 774 (w), 732 (w). MS (ESI$^+$) $m/z$ 354 (M$^+$), 355 (MH$^+$), 356 (MH$^+$ +1). Anal. Calcd for C$_{18}$H$_{31}$N$_3$O$_2$S: C, 61.15; H, 8.84; N, 11.89. Found: C, 61.10; H, 8.44; N, 11.55.

**Synthesis of N-(3-((cyclohexylmethyl)(3,1,3-dioisoindolin-2-yl)propyl)amino)propyl)-4-(dimethylamino)benzenesulfonamide (3)**

![Chemical Structure](image)

To a two neck 1 L round bottom flask with a condenser and rubber septum, 20.4 g (57.7 mmol) of N-(3-((cyclohexylmethyl)amino)propyl)-4-
(dimethylamino)benzenesulfonamide, 6.73 g (63.5 mmol) of Na$_2$CO$_3$, 1.93 g (14.4 mmol) LiI, and 300 mL of acetonitrile were added under N$_2$ gas and stirred at r.t. The mixture was heated under reflux and 37.1 g (139 mmol) of N-(3-bromopropyl)phthalimide was added slowly to the reaction vessel and stirred for 24 h under reflux. The mixture was then cooled to r.t. and filtered through a fine sintered funnel. The funnel was washed with 100 mL of acetonitrile. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. Then 800 g of alumina was placed in a 2 L medium porosity sintered funnel with 500 mL of 5:2 (v/v) hexanes/EtOAc solution to form a uniform bed, which was not allowed to dry. A solution of the product in 20 mL of ethyl acetate was added to the top of the alumina bed. The alumina was then slowly washed with 5 L of 5:2 (v/v) hexanes/EtOAc followed by 1.5 L of ethanol. The ethanol fraction was collected and concentrated by rotary evaporation, and the resulting residue was dried in vacuo. This produced 23.5 g (75%) of N-(3-((cyclohexylmethyl)(3-(1,3-dioxoisoiindolin-2-yl)propyl)amino)propyl)-4-(dimethylamino)benzenesulfonamide as a yellow viscous oil.

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.80 (m, 2 H, NPhth), 7.68 (m, 2 H, NPhth), 7.63 (m, 2 H, o-ArSO$_2$), 6.58 (m, 2 H, m-ArSO$_2$), 5.85 (bs, 1 H, NH), 3.80 (m, 2 H, CH$_2$NPhth), 3.53 (m, 2 H, CH$_2$Cy), 1.63 (m, 10 H, CCH$_2$C, Cy), 1.34 (m, 1 H, Cy), 1.13 (m, 4 H, Cy), 0.79 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 212.6, 168.1, 133.9, 131.0, 128.8, 123.2, 111.0, 77.2, 58.3, 51.7, 39.9, 31.8, 25.8, 18.3, 16.7. IR (neat cm$^{-1}$) 2926 (w), 2851 (w), 2430 (w), 1769 (w), 1704 (s), 1596 (w), 1465 (w), 1435 (w), 1395 (m), 1364 (w), 1330 (w), 1187 (w), 1163
Synthesis of \( N\)-(3-aminopropyl)-\( N\)-(3-\( p\)-dimethylaminobenzencesulfonamidopropyl)benzylamine (4)\(^3\)

To a 1 L round bottom flask with condenser and nitrogen inlet, 20.1 g (37.2 mmol) of \( N\)-(3-((cyclohexylmethyl)(3-\( 1,3\)-dioxoisindolin-2-yl)propyl)amino)propyl)-4-(dimethylamino)benzenesulfonamide, 43.3 mL (893 mmol) of hydrazine monohydrate, and 250 mL of EtOH were added and stirred. The solution was heated and stirred under reflux for 3 h. The mixture was cooled to r.t., concentrated by rotary evaporation, and the resulting residue was dried \textit{in vacuo}. Then 250 mL of aq. 2 N HCl solution was added slowly and stirred at r.t. until the white fumes were no longer forming. The solution was made basic (pH 10) by adding 80 mL of aq. 2 N NaOH solution. The solution was then placed into a separatory funnel and extracted with DCM (3 x 50 mL). The combined organic extracts were then dried (\( Na_2SO_4 \)) and filtered. The filtrate was then concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The product was purified by automated chromatography on alumina, eluting with 1:1 (v/v) EtOH/ethyl
acetate. This produced 13.2 g (86%) of $N$-(3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)-4-(dimethylamino)benzenesulfonamide as a viscous clear oil. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.67 (m, 2 H, o-ArSO$_2$), 6.63 (m, 2 H, m-ArSO$_2$), 6.31 (bs, 3 H, NH), 2.98 (s, 6 H, N(CH$_3$)$_2$), 2.87 (t, 6.2 Hz, 2 H, CH$_2$NH$_2$), 2.39 (m, 4 H, CH$_2$N), 2.05 (d, 6.9 Hz, 2 H, CH$_2$Cy), 1.73 (m, 2 H, CCH$_2$C), 1.59 (m, 9 H, CCH$_2$C, Cy), 1.33 (m, 1 H, CH), 1.12 (m, 3 H, Cy), 0.76 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.6, 128.8, 125.2, 110.9, 61.8, 52.9, 52.3, 42.0, 40.1, 35.6, 31.8, 26.6, 26.0, 25.9. IR (neat cm$^{-1}$) 2922 (m), 2849 (m), 1769 (w), 1705 (m), 1595 (m), 1514 (m), 1444 (m), 1395 (m), 1364 (m), 1310 (m), 1224 (m), 1144 (s), 1092 (s), 999 (m), 943 (m), 892 (m), 814 (m), 770 (m), 719 (s), 645 (s) 606 (m). MS (ESI$^+$) 411 (MH$^+$), 412 (MH$^+$ +1).

Anal. Calcd for C$_{21}$H$_{38}$N$_4$O$_2$S·HCl·H$_2$O: C, 54.23; H, 8.89; N, 12.05. Found: C, 54.00; H, 8.50; N, 11.94.

**Synthesis of 2-methylene-1,3-propanebis(tert-butylcarbonate) (5)$^3$**

![Diagram of 2-methylene-1,3-propanebis(tert-butylcarbonate)](image)

To a 250 mL round bottom flask, 10.0 g (113 mmol) of 2-methylenepropane-1,3-diol-2-(propan-2-ylidene)propane-1,3-diol, 59.8 g (274 mmol) of di-tert-butyl dicarbonate, 1.39 g (11.4 mmol) of DMAP, and 200 mL of anhydrous diethyl ether were added and stirred vigorously at r.t. for 24 h. The solution was then washed with sat. aq. CuSO$_4$ solution
(3 x 25 mL), sat. aq. NaHCO₃ solution (3 x 25 mL), and 50 mL of sat. aq. NaCl solution. The organic layer was dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 32.6 g (99%) of 2-methylene-1,3-propanebis(tert-butylcarbonate) as a clear oil. ¹H NMR (400 MHz, CDCl₃/TMS) δ 5.32 (s, 2 H, CH₂=C), 4.60 (s, 4 H, CH₂C=CH₂O), 1.49 (s, 6 H, (CH₃)₃C).

Synthesis of 2-methoxybenzenesulfonyl chloride⁴

![Chemical Structure]

To a 500 mL round bottom flask were added 2.15 g (15.3 mmol) of 2-methoxybenzenethiol, 1.35 mL of isopropanol, and 188 mL of DCM. The solution was cooled to 0 °C. Then 7.75 g (58.0 mmol) of N-chlorosuccinimide was added portion wise and allowed to stir at 0 °C for 1 h. The solution was diluted with cold sat. aq. NaHCO₃ solution (50 mL). The aqueous layer was extracted with ethyl acetate (3 x 50mL). The combined extraction layers were dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 1.52 g (48%) of 2-methoxybenzenesulfonyl chloride as a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 7.96 (d, 8.4 Hz, 1 H, o-Ar), 7.68 (m, 1 H, p-Ar), 7.10 (m, 2 H, m-Ar), 4.06 (s, 3 H, OCH₃).
Synthesis of morpholinesulfonyl chloride

To a 250 mL round bottom flask, 3.34 g (38.3 mmol) of morpholine, 9.95 mL (123 mmol) of sulfuryl chloride, and 100 mL of acetonitrile were added. The reaction mixture was stirred and heated under reflux for 24 h. The mixture was cooled to r.t. and was concentrated by rotary evaporation. Then 100 mL of toluene was added with 1.01 g of activated charcoal and stirred for 5 min. The mixture was then filtered, the filtrate was concentrated by rotary evaporation, and the resulting residue was dried in vacuo. This produced 7.10 g (99%) of crude morpholinesulfonyl chloride, which was pure enough for the next step. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.84 (t, 4 Hz, 4 H, CH$_2$NCH$_2$), 3.31 (t, 4 Hz, 4 H, CH$_2$OCH$_2$).

Synthesis of benzo[d][1,3]dioxole-5-sulfonyl chloride

To a 50 mL round bottom flask with nitrogen inlet and condenser, 2.25 g (30.8 mmol) of N,N-dimethylformamide was added at 0 °C. Then 4.13 g (30.6 mmol) of sulfuryl chloride was added dropwise and allowed to stir at 0 °C for 15 min. Then 3.20 g (26.2 mmol) of benzodioxole was added and the mixture was stirred at 100 °C for 2 h. The solution was cooled to r.t. and poured into ice cold water. The organic layer was separated,
and the aqueous layer was extracted with DCM (3 x 20 mL). The combined organic layers were dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was purified by automated chromatography on silica gel, eluting with 1:1 (v/v) DCM/hexanes. This produced 1.33 g (23%) of benzo[d][1,3]dioxole-5-sulfonyl chloride as yellow crystals. 'H NMR (400 MHz, CDCl₃) δ 7.62 (m, 1 H, o-ArSO₂), 5.41 (d, 2.0 Hz, 1 H, o-ArSO₂), 6.93 (d, 8.3 Hz, 1 H, m-ArSO₂), 6.14 (s, 2 H, OCH₂O).

**Synthesis of N'-(p-dimethylaminobenzenesulfonyl)-N''-(p-methoxybenzenesulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine]
( TL016)**³

To a 250 mL round bottom flask, 1.22 g (2.97 mmol) of N-(3-aminopropyl)-N-(3-p-dimethylaminobenzenesulfonamidopropyl)benzylamine, 0.613 g (3.02 mmol) of 4-methoxybenzenesulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na₂CO₃ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction
layers were dried (Na$_2$SO$_4$), and filtered. The filtrate was then concentrated, and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The solids were triturated with diethyl ether (3 x 15 mL) and the resulting residue was dried \textit{in vacuo}. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. This produced 1.64 g (95\%) of $N'$-(p-dimethylaminobenzenesulfonyl)-$N''$-(p-methoxybenzenesulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] as a yellow oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.79 (m, 2 H, o-ArSO$_2$), 7.67 (m, 2 H, o-ArSO$_2$), 6.97 (m, 2 H, m-ArSO$_2$), 6.68 (m, 2 H, m-ArSO$_2$), 3.86 (s, 3 H, OCH$_3$), 3.04 (s, 6 H, N(CH$_3$)$_2$), 2.96 (m, 4 H, CH$_2$N), 2.35 (t, 6.5 Hz, 4 H, CH$_2$N), 2.04 (d, 7.0 Hz, 2 H, CH$_2$Cy), 1.61 (m, 13 H, CCH$_2$C, Cy) 1.18 (m, 1 H, CH), 0.81 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 162.7, 152.7, 131.7, 129.23, 129.18, 129.11, 128.9, 125.1, 114.1, 114.0, 110.9, 110.7, 77.3, 77.2, 77.0, 76.7, 62.0, 55.6, 53.2, 53.0, 42.7, 42.5, 40.1, 40.0, 35.6, 31.9, 31.6, 29.7, 26.6, 26.0, 25.9. IR (neat cm$^{-1}$) 2926 (w), 2850 (w), 2432 (w), 2121 (w), 1595 (m), 1497 (w), 1448 (w), 1324 (m), 1257 (m), 1151 (s), 1092 (m), 1020 (m), 899 (w), 835 (m), 803 (w), 656 (m). MS (ESI$^+$) 581 (MH$^+$).

Anal. Calcd for C$_{28}$H$_{45}$ClN$_4$O$_4$S$_2$1.5H$_2$O: C, 52.20; H, 7.51; N, 8.70. Found: C, 52.14; H, 7.12; N, 8.41.
Synthesis of N''-(p-dimethylaminobenzenesulfonyl)-N'''-(p-bromobenzenesulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] (TL017)³

To a 250 mL round bottom flask, 780 mg (1.90 mmol) of N-(3-aminopropyl)-N-(3-p-dimethylaminobenzenesulfonamidopropyl)benzylamine, 621 mg (1.97 mmol) of 4-bromobenzenesulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na₂CO₃ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na₂SO₄), and filtered. The filtrate was then concentrated, and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were trititated with diethyl ether (3 x 15 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 1.13 g (94%) of
**Synthesis of N’-(p-dimethylaminobenzensulfonyl)-N”-(p-bromobenzensulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] (TL018)**

To a 250 mL round bottom flask, 989 mg (2.41 mmol) of N-(3-aminopropyl)-N-(3-p-dimethylaminobenzensulfonamidopropyl)benzylamine, 560 mg (2.47 mmol) of 2-
naphthalenesulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na$_2$CO$_3$ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na$_2$SO$_4$), and filtered. The filtrate was then concentrated, and the resulting residue was dried *in vacuo*. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried *in vacuo*. The solids were triturated with diethyl ether (3 x 15 mL) and the resulting residue was dried *in vacuo*. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried *in vacuo*. This produced 1.26 g (87%) of $\text{N}^\prime$-(p-dimethylaminobenzensulfonyl)-$\text{N}''$-(naphthalene-2-sulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] as a yellow viscous oil. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.42 (s, 1 H, 2-ArSO$_2$) 7.95 (m, 2 H, 4,7-ArSO$_2$), 7.90 (dt, 7.9, 1.0 Hz, 1 H, 10-ArSO$_2$) 7.83 (dd, 8.6, 1.9 Hz, 1 H, 9-ArSO$_2$) 7.65 (m, 2 H, $\alpha$-ArSO$_2$), 7.60 (m, 2 H, 5,6-ArSO$_2$) 6.64 (m, 2 H, $m$-ArSO$_2$), 5.52 (bs, 2 H, NH), 3.02 (s, 6 H, N(CH$_3$)$_2$), 2.99 (m, 2 H, CH$_2$N), 2.90 (t, 6.4 Hz, 2 H, CH$_2$N) 2.32 (m, 4 H, CH$_2$N), 2.01 (m, 2 H, CH$_2$Cy), 1.59 (m, 10 H, CCH$_2$C, Cy), 1.32 (m, 1 H, CH), 1.12 (m, 2 H, Cy), 0.74 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 172.6, 152.7, 136.9, 134.7, 132.2, 129.3, 129.22, 129.18, 129.14,
128.83, 128.79, 128.55, 128.22, 127.9, 127.8, 127.4, 127.3, 126.6, 126.2, 125.1, 122.54, 122.49, 111.0, 110.9, 110.8, 110.7, 77.3, 77.2, 77.0, 76.7, 62.0, 53.1, 52.9, 51.0, 45.8, 42.7, 42.4, 40.0, 35.8, 35.6, 31.9, 31.8, 31.6, 26.7, 26.6, 26.1, 26.0, 25.3, 25.8, 22.6. IR (neat cm⁻¹) 2926 (w), 2851 (w), 2428 (w), 2116 (w), 1595 (w), 1449 (w), 1324 (m), 1156 (s), 1130 (m), 1092 (m), 1074 (m), 1017 (w), 970 (w), 900 (w), 858 (w), 819 (m), 750 (m), 656 (s). MS (ESI⁺) m/z 601 (MH⁺). Anal. Calcd for C₃₁H₄₄N₄O₄S₂HCl 1.5H₂O: C, 56.05; H, 7.28; N, 8.43. Found: C, 56.09; H, 7.29; N, 8.02.

**Synthesis of N’-(p-dimethylaminobenzenesulfonyl)-N”-(naphthalene-1-sulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] (TL019)³**

To a 250 mL round bottom flask, 1.03 g (2.51 mmol) of N-(3-aminopropyl)-N-(3-p-dimethylaminobenzenesulfonamidopropyl)benzylamine, 0.663 g (2.73 mmol) of 1-naphthalenesulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na₂CO₃ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na₂SO₄), and filtered. The filtrate was then concentrated, and the resulting
residue was dried in vacuo. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were tritirated with diethyl ether (3 x 15 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 1.51 g (100%) of $N'$-(p-dimethylaminobenzenesulfonyl)-$N''$-(naphthalene-1-sulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.67 (dd, 8.6, 1.1 Hz, 1H, 2-ArSO$_2$), 8.18 (dd, 7.3, 1.3 Hz, 1H, 9-ArSO$_2$), 7.98 (dt, 8.3, 1.0 Hz, 1H, 3-ArSO$_2$), 7.87 (dd, 8.2, 1.4 Hz, 1H, 7-ArSO$_2$), 7.60 (m, 3H, 8-ArSO$_2$, o-ArSO$_2$), 7.49 (m, 2H, 4,6-ArSO$_2$), 6.58 (m, 2H, m-ArSO$_2$), 2.92 (m, 8H, N(CH$_3$)$_2$, CH$_2$N), 2.81 (t, 6.2 Hz, 2H, CH$_2$N) 2.15 (td, 6.7, 3.8 Hz, 4H, CH$_2$N), 1.89 (d, 6.9 Hz, 2H, CH$_2$Cy), 1.56 (m, 9H, CCh$_2$C, Cy), 1.41 (m, 3H, Cy), (1.23 ddt, 10.2, 7.4, 3.0 Hz, 1H, CH), 1.08 (m, 2H, Cy), 0.66 (q, 13.2, 12.1 Hz, 2H, Cy).$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.7, 135.0, 134.2, 133.9, 129.3, 128.9, 128.83, 128.76, 128.3, 128.2, 126.8, 125.1, 124.8, 124.1, 110.9, 61.7, 52.9, 52.8, 42.6, 42.3, 40.0, 35.6, 31.8, 26.6, 26.0, 25.9, 25.7. IR (neat cm$^{-1}$) 2927 (w), 2852 (w), 2111 (w), 1596 (m), 1508 (w), 1447 (w), 1368 (w), 1313 (m), 1146 (s), 1132 (s), 1093 (m), 982 (w), 944 (w), 900 (w), 806 (m), 772 (m), 675 (m). MS
(ESI$^+$) m/z 601 (MH$^+$). Anal. Calcd for C$_{31}$H$_{44}$N$_4$O$_4$S$_2$HCl1.5H$_2$O: C, 56.05; H, 7.28; N, 8.43. Found: C, 56.31; H, 7.57; N, 8.29.

**Synthesis of N’-(p-dimethylaminobenzenesulfonyl)-N”-(methylsulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] (TL024)**

![Chemical Structure](image)

To a 250 mL round bottom flask, 1.75 g (4.26 mmol) of N-(3-aminopropyl)-N-(3-p-dimethylaminobenzenesulfonamidopropyl)benzylamine, 0.611 g (5.33 mmol) of methane sulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na$_2$CO$_3$ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na$_2$SO$_4$), and filtered. The filtrate was then concentrated, and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were triturated with diethyl ether (3 x 15 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq.
NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 1.87 g (90%) of N-(3-((cyclohexylmethyl)(3-(methylsulfonamido)propyl)amino)propyl)-4-(dimethylamino)benzenesulfonamide as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.64 (m, 2 H, o-ArSO$_2$), 6.65 (m, 2 H, m-ArSO$_2$), 5.72 (bs, 2 H, NH), 3.16 (t, 6.4 Hz, 4 H, CH$_2$N), 3.01 (s, 6 H, N(CH$_3$)$_2$), 2.92 (s, 3 H, CH$_3$), 2.39 (dt, 13.2 Hz, 6.3 Hz, 4 H, CH$_2$N), 2.06 (d, 6.9 Hz, 2 H, CH$_2$Cy), 1.64 (m, 10 H, CCH$_2$C, Cy), 1.37 (m, 1 H, CH) 1.16 (m, 3 H, Cy), 0.80 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 152.7, 124.9, 111.0, 110.9, 62.0, 53.1, 42.6, 42.5, 40.1, 39.7, 35.7, 31.9, 26.6, 26.5, 26.0, 25.8. IR (neat cm$^{-1}$) 2926 (m), 2851 (m), 2497 (w), 2436 (w), 1596 (w), 1448 (m), 1312 (s), 1149 (s), 1093 (s), 975 (m), 900 (w), 842 (m), 770 (m), 655 (s). MS (ESI$^+$) $m/z$ 489 (MH$^+$). Anal. Calcd for C$_{22}$H$_{42}$N$_4$O$_4$S$_2$HClH$_2$O: C, 48.65; H, 7.98; N, 10.31. Found: C, 48.76; H, 8.08; N, 10.03.
Synthesis of \( N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(benzenesulfonyl)\)\([N,N\text{-bis(3-aminopropyl)cyclohexylmethylamine}\) (TL025)³

To a 250 mL round bottom flask, 1.08 g (2.63 mmol) of \( N-(3\text{-aminopropyl})-N-(3-p\text{-dimethylaminobenzenesulfonamidopropyl})\)benzylamine, 0.710 g (4.02 mmol) of methanesulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na₂CO₃ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na₂SO₄), and filtered. The filtrate was then concentrated, and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The solids were triturated with diethyl ether (3 x 15 mL) and the resulting residue was dried \textit{in vacuo}. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. This produced 1.38 g (95%) of
$N^\prime$-($p$-dimethylaminobenzenesulfonyl)-$N^\prime\prime$-(benzenesulfonyl)-[$N,N$-bis(3-aminopropyl)cyclohexylmethylamine] as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.79 (m, 2 H, $o$-ArSO$_2$), 7.61 (m, 2 H, $o$-ArSO$_2$), 7.44 (m, 3 H, $m$-ArSO$_2$, $p$-ArSO$_2$), 6.60 (m, 2 H, $m$-ArSO$_2$), 2.95 (s, 6 H, N(CH$_3$)$_2$), 2.89 (t, 6.2 Hz, 4 H, CH$_2$N), 2.84 (m, 2 H, CH$_2$N), 2.25 (m, 4 H, CH$_2$N), 1.96 (d, 6.8 Hz, 2 H, CH$_2$Cy), 1.54 (m, 10 H, CH$_2$Cy, Cy), 1.27 (m, 1 H, CHC), 1.09 (m, 4 H, Cy), 0.69 (q, 12.0 Hz, 10.3 Hz, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.7, 134.0, 132.4, 129.1, 129.0, 126.9, 124.9, 110.9, 61.6, 52.8, 52.5, 42.4, 42.2, 40.0, 35.4, 31.8, 31.7, 26.6, 25.9, 25.6. IR (neat cm$^{-1}$) 2924 (w), 2851 (w), 2425 (w), 1596 (w), 1445 (m), 1324 (m), 1156 (s), 1128 (m), 1091 (s), 1015 (m), 996 (m), 900 (m), 840 (m), 783 (m), 726 (m), 689 (s), 653 (s), 628 (m), 615 (m). MS (ESI$^+$) m/z 551 (MH$^+$), 552 (MH$^+$ +1), 554 (MH$^+$ +2). Anal. Calcd for C$_{27}$H$_{42}$N$_4$O$_4$S$_2$·HCl·CH$_3$OH: C, 54.31; H, 7.65; N, 9.05. Found: C, 54.32; H, 7.60; N, 8.69.
Synthesis of $N'(p$-dimethylaminobenzenesulfonyl)$-N''-(morpholinylsulfonyl)$-[N,N$-bis(3$-aminopropyl)cyclohexylmethylamine] (TL026)$\textsuperscript{3}$

To a 250 mL round bottom flask, 1.58 g (3.84 mmol) of $N$-(3-aminopropyl)$-N$-(3-$p$-dimethylaminobenzenesulfonyl)benzylamine, 0.683 g (3.68 mmol) of morpholine-$4$-sulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na\textsubscript{2}CO\textsubscript{3} solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na\textsubscript{2}SO\textsubscript{4}), and filtered. The filtrate was then concentrated, and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The solids were triturated with diethyl ether (3 x 15 mL) and the resulting residue was dried \textit{in vacuo}. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na\textsubscript{2}SO\textsubscript{4}) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. This produced 2.06 g (99%) of
$N'-(p$-dimethylaminobenzenesulfonyl)-$N''-(morpholinylsulfonyl)$-[N,N$-$bis(3$-$aminopropyl)cyclohexylmethylamine] as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.65 (m, 2 H, o$-$ArSO$_2$), 6.66 (m, 2 H, m$-$ArSO$_2$), 3.72 (m, 4 H, CH$_2$OCH$_2$), 3.17 (m, 4 H, CH$_2$NCH$_2$), 3.12 (t, 6.4 Hz, 2 H, CH$_2$N), 3.03 (s, 6 H, N(CH$_3$)$_2$), 2.94 (m, 2 H, CH$_2$N), 2.39 (m, 4 H, CH$_2$N), 2.07 (d, 6.9 Hz, 2 H, CH$_2$C), 1.66 (m, 8 H, CH$_2$Cy, Cy), 1.38 (m, 1 H, CHCy), 1.16 (m, 2 H, Cy), 0.81 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.7, 128.8, 128.8, 125.0, 110.9, 110.8, 66.3, 62.1, 54.2, 53.3, 52.9, 51.8, 46.1, 43.2, 42.3, 40.1, 35.8, 35.7, 31.93, 31.88, 26.6, 26.2, 26.1, 26.0, 25.9. IR (neat cm$^{-1}$) 2926 (w), 2852 (w), 2608 (w), 2112 (w), 1596 (w), 1449 (w), 1327 (m), 1261 (w), 1153 (m), 1130 (m), 1093 (m), 1073 (m), 941 (m), 899 (w), 844 (w), 784 (w), 732 (w), 655 (m). MS (ESI$^+$) $m/z$ 560 (MH$^+$). Anal. Calcd for C$_{25}$H$_{45}$N$_5$O$_5$S$_2$HCl$\cdot$0.5H$_2$O: C, 49.61; H, 7.83; N, 11.57. Found: C, 49.34; H, 8.13; N, 11.24.
Synthesis of $N'-(p$-dimethylaminobenzenesulfonyl)$-N''-(p$-dimethylaminobenzenesulfonyl)$-\left[N,N\text{-bis}(3\text{-aminopropyl})\text{cyclohexylmethylamine}\right]$ (TL028)$^3$

To a 250 mL round bottom flask, 1.04 g (2.53 mmol) of $N$-(3-aminopropyl)$-N$-(3-$p$-dimethylaminobenzenesulfonamidopropyl)benzylamine, 0.660 g (2.80 mmol) of 4-$p$-dimethylaminobenzenesulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na$_2$CO$_3$ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na$_2$SO$_4$), and filtered. The filtrate was then concentrated, and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were triturated with diethyl ether (3 x 15 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL).
The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 1.09 g (73%) of $N'$-(p-dimethylaminobenzenesulfonyl)-$N''$-(p-dimethylaminobenzenesulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] as a clear viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.67 (m, 4 H, o-ArSO$_2$), 6.67 (m, 4 H, m-ArSO$_2$), 5.56 (bs, 2 H, NHSO$_2$), 3.04 (s, 12 H, N(CH$_3$)$_2$), 2.93 (t, 6.3 Hz, 4 H, CH$_2$N), 2.34 (m, 4 H, CH$_2$N), 2.04 (m, 2 H, CH$_2$Cy), 1.64 (m, 8 H, CCH$_2$C, Cy) 1.36 (m, 1 H, CH), 1.17 (m, 4 H, Cy), 0.78 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.9, 129.0, 125.3, 111.1, 62.1, 53.2, 42.6, 40.2, 35.8, 32.0, 26.8, 26.2, 26.0. IR (neat cm$^{-1}$) 2923 (m), 2852 (m), 2543 (m), 1596 (m), 1448 (m), 1326 (m), 1161 (s), 1129 (s), 1092 (s), 994 (m), 897 (m), 841 (m), 783 (m), 653 (s), 627 (m). MS (ESI$^+$) $m/z$ 594 (MH$^+$).

Synthesis of $N'$-(p-dimethylaminobenzenesulfonyl)-$N''$-(5-(dimethylamino)naphthalene-1-sulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] (TL034)$^3$

To a 250 mL round bottom flask, 2.04 g (4.97 mmol) of $N$-(3-aminopropyl)-$N$-(3-p-dimethylaminobenzenesulfonyl)benzylamine, 1.58 g (5.86 mmol) of dansyl
chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na₂CO₃ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na₂SO₄), and filtered. The filtrate was then concentrated, and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were triturated with diethyl ether (3 x 15 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 2.30 g (99%) of \( N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(5\text{-}(dimethylamino)napththalene-1-sulfonyl)}-[N,N\text{-bis(3 aminopropyl)cyclohexylmethylamine}] as a yellow viscous oil. \(^1\)H NMR (400 MHz, CDCl₃) \( \delta \) 8.50 (m, 1 H, 4-ArSO₂), 8.32 (m, 1 H, 9-ArSO₂), 8.20 (m, 1 H, 2-ArSO₂), 7.64 (m, 2 H, o-ArSO₂), 7.49 (m, 2 H, 3-ArSO₂, 8-ArSO₂), 7.15 (m, 1 H, 7-ArSO₂), 6.63 (m, 2 H, m-ArSO₂), 3.01 (s, 6 H, N(CH₃)₂), 2.94 (m, 2 H, CH₂N), 2.86 (m, 8 H, N(CH₃)₂, CH₂N), 2.24 (t, 6.6 Hz, 4 H, CH₂N), 1.97 (d, 2 H, CH₂Cy), 1.61 (m, 6 H, CH₂Cy, Cy), 1.48 (m, 4 H, Cy), 1.29 (m, 1 H, CHC), 1.13 (m, 4 H, Cy), 0.73 (m, 2 H, Cy). \(^{13}\)C NMR (101 MHz, CDCl₃) \( \delta \) 152.7, 151.8, 135.1, 130.1, 129.9, 129.7, 129.4, 128.9, 128.2, 125.2, 123.1, 119.2,
115.1, 110.9, 61.8, 53.0, 45.4, 42.6, 42.4, 40.1, 35.6, 31.9, 26.6, 26.0, 25.7. IR (neat cm\(^{-1}\)) 2926 (w), 2851 (w), 2434 (w), 2112 (w), 1597 (w), 1513 (w), 1448 (w), 1391 (w), 1320 (m), 1141 (s), 1093 (m), 1048 (w), 988 (w), 944 (w), 898 (w), 837 (w), 792 (m), 772 (w).

MS (ESI\(^+\)) \( m/z \) 644 (MH\(^+\)). Anal. Calcd for C\(_{33}\)H\(_{49}\)N\(_5\)O\(_4\)S\(_2\)·HCl·CH\(_3\)OH·H\(_2\)O: C, 55.91; H, 7.73; N, 9.59. Found: C, 56.09; H, 7.63; N, 9.46.

**Synthesis of \( N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(4\text{-tert-butylenesulfonyl})-[N,N\text{-bis(3-aminopropyl)cyclohexylmethylamine}] \) (TL035)\(^3\)**

To a 250 mL round bottom flask, 1.80 g (4.38 mmol) of \( N-(3\text{-aminopropyl})-N-(3-p\text{-dimethylaminobenzenesulfonamidopropyl}) \)benzylamine, 1.20 g (4.82 mmol) of \( 4\text{-}(tert-butylenesulfonyl} \)benzylamine chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na\(_2\)CO\(_3\) solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na\(_2\)SO\(_4\)), and filtered. The filtrate was then concentrated, and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 15
mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were triturationated with diethyl ether (3 x 15 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 2.46 g (93%) of $N'-(p$-dimethylaminobenzenesulfonyl)-$N''-(4$-tert-butylbenzenesulfonyl)$-\left[N,N\text{-bis(3-aminopropyl)cyclohexylmethylamine}\right]$ as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.72 (m, 2 H, $m$-ArSO$_2$), 7.62 (m, 2 H, $m$-ArSO$_2$), 7.44 (m, 2 H, $o$-ArSO$_2$), 6.61 (m, 2 H, $o$-ArSO$_2$), 2.96 (s, 6 H, N(CH$_3$)$_2$), 2.88 (m, 4 H, CH$_2$N), 2.27 (t, 6.6 Hz, 4 H, CH$_2$N), 1.97 (d, 6.9 Hz, 2 H, CH$_2$Cy), 1.54 (m, 9 H, CCH$_2$C, Cy) 1.27 (s, 9 H, C(CH$_3$)$_3$), 1.08 (m, 4 H, Cy), 0.72 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 155.9, 152.7, 137.0, 128.8, 126.9, 125.9, 125.1, 110.9, 61.8, 52.9, 52.7, 42.5, 42.3, 40.0, 35.6, 35.0, 31.8, 31.1, 26.6, 26.0, 25.9, 25.8. IR (neat cm$^{-1}$) 2928 (w), 2853 (w), 2596 (w), 2114 (w), 1596 (w), 1449 (w), 1397 (w), 1366 (w), 1324 (w), 1160 (m), 1112 (w), 1088 (w), 997 (w), 944 (w), 899 (w), 837 (w), 755 (w), 654 (w). MS (ESI$^+$) $m/z$ 607 (MH$^+$). Anal. Calcd for C$_{32}$H$_{50}$N$_4$O$_4$S$_2$HClCH$_3$OH: C, 56.91; H, 8.21; N, 8.30. Found: C, 57.26; H, 8.46; N, 7.91.
Synthesis of $N'-(p$-dimethylaminobenzenesulfonyl)$-N''-(benzo[d][1,3]dioxole-5-sulfonyl)$-[N,N$-bis(3$-aminopropyl)cyclohexylmethylamine$] (TL036)$

To a 250 mL round bottom flask, 2.42 g (5.88 mmol) of $N$-(3-aminopropyl)$-N$-(3-$p$-dimethylaminobenzenesulfonylimidopropyl)benzylamine, 1.33 g (5.62 mmol) of benzo[d][1,3]dioxole-5-sulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na$_2$CO$_3$ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na$_2$SO$_4$), and filtered. The filtrate was then concentrated, and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were triturated with diethyl ether (3 x 15 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 2.61 g
(78%) of \(N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(\text{benzo}[d][1,3]\text{dioxole-5-sulfonyl})-\left[N,N\text{-bis(3-aminopropyl)cyclohexylmethylamine}\right]\) as a yellow viscous oil. \(^1\text{H NMR (400 MHz, CDCl}_3\delta 7.57\) (m, 2 H, \(o\text{-ArSO}_2\)), 7.31 (dd, 8.2 Hz, 1.9 Hz, 1 H, \(o\text{-ArSO}_2\)), 7.17 (d, 1.8 Hz, 1 H, \(o\text{-ArSO}_2\)), 6.76 (d, 8.2 Hz, 1 H, \(m\text{-ArSO}_2\)), 6.57 (m, 2 H, \(m\text{-ArSO}_2\)), 5.96 (s, 2 H, OCH\(_2\)O), 2.92 (s, 6 H, N(CH\(_3\))\(_2\)), 2.82 (m, 4 H, CH\(_2\)N), 2.21 (m, 4 H, CH\(_2\)N), 1.92 (d, 6.9 Hz, 2 H, CH\(_2\)C), 1.55 (m, 8 H, CH\(_2\)Cy, Cy), 1.23 (m, 1 H, CHCy), 1.05 (m, 4 H, Cy), 0.67 (m, 2 H, Cy). \(^1\text{C NMR (101 MHz, CDCl}_3\delta 152.6, 151.0, 148.1, 133.3, 128.7, 124.9, 122.5, 110.8, 108.1, 107.2, 102.3, 61.7, 52.8, 52.6, 42.5, 42.2, 40.0, 35.6, 31.7, 26.6, 26.0, 25.9, 25.8. IR (neat cm\(^{-1}\)) 2926 (w), 2851 (w), 2115 (w), 1596 (w), 1502 (w), 1477 (m), 1448 (w), 1425 (w), 1368 (w), 1315 (m), 1241 (m), 1144 (s), 1112 (m), 1093 (m), 1056 (w), 1033 (m), 929 (w), 898 (w), 815 (w), 772 (w), 728 (w), 704 (w). MS (ESI\(^+\)) \(m/z 595\text{ (MH}^+\text{), 596 (MH}^+\text{ +1), 597 (MH}^+\text{ +2). Anal. Calcd for C}_{28}\text{H}_{42}\text{N}_{4}\text{O}_{6}\text{S}_{2}\text{·HCl·H}_2\text{O·CH}_3\text{OH:}\text{ C, 51.13; H, 7.25; N, 8.22. Found: C, 51.31; H, 6.90; N, 7.91.}
Synthesis of \( N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(p\text{-difluoromethoxybenzenesulfonyl})-[N,N\text{-bis(3-aminopropyl)cyclohexylmethylamine}] \) (TL040)³

To a 250 mL round bottom flask, 1.39 g (3.39 mmol) of \( N-(3\text{-aminopropyl})-N-(3-p\text{-dimethylaminobenzenesulfonamidopropyl})\text{benzylamine} \), 0.685 g (2.82 mmol) of \( 4\text{-}(\text{difluoromethoxy})\text{benzenesulfonyl chloride} \), 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na₂CO₃ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (\( \text{Na}_2\text{SO}_4 \)), and filtered. The filtrate was then concentrated, and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The solids were triturated with diethyl ether (3 x 15 mL) and the resulting residue was dried \textit{in vacuo}. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL).
The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 1.51 g (87%) of $N'$-(p-dimethylaminobenzenesulfonyl)-$N''$-(p-difluoromethoxybenzenesulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 (m, 2 H, m-ArSO$_2$), 7.62 (m, 2 H, m-ArSO$_2$), 7.18 (m, 2 H, o-ArSO$_2$), 6.63 (m, 3 H, o-ArSO$_2$, CCH), 5.60 (bs, 2 H, NHSO$_2$), 3.00 (s, 6 H, N(CH$_3$)$_2$), 2.95 (t, 6.4 Hz, 2 H, CH$_2$N), 2.88 (t, 6.4 Hz, 2 H, CH$_2$N), 2.31 (t, 6.5 Hz, 4 H, CH$_2$N), 1.98 (m, 2 H, CH$_2$Cy), 1.58 (m, 8 H, CCH$_2$C, Cy), 1.31 (m, 1 H, CH), 1.13 (m, 4 H, Cy) 0.73 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 153.9, 152.7, 136.8, 129.2, 128.8, 124.8, 119.1, 117.9, 115.3, 112.7, 110.9, 61.8, 53.0, 42.6, 42.3, 40.0, 35.6, 31.8, 26.6, 26.0, 25.8. IR (neat cm$^{-1}$) 2926 (w), 2851 (w), 2599 (w), 1596 (m), 1516 (w), 1493 (w), 1447 (w), 1369 (w), 1325 (m), 1311 (m), 1227 (m), 1147 (s), 1118 (s), 1092 (s), 1042 (s), 992 (m), 943 (m), 899 (m), 833 (m), 816 (m), 771 (m), 711 (s), 646 (s), 618 (s), 608 (m). MS (ESI$^+$) m/z 617 (MH$^+$). Anal. Calcd for C$_{28}$H$_{42}$N$_4$O$_5$S$_2$F$_2$·HCl·0.5H$_2$O: C, 50.78; H, 6.70; N, 8.46. Found: C, 50.73; H, 6.97; N, 8.30.
Synthesis of \(N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(2,5\text{-dimethylbenzenesulfonyl})-[N,N\text{-bis(3-aminopropyl)cyclohexylmethylamine}]\) (TL041)³

To a 250 mL round bottom flask, 1.65 g (4.02 mmol) of \(N\text{-}(3\text{-aminopropyl})-N\text{-}(3\text{-p\text{-dimethylaminobenzenesulfonamidopropyl})benzylamine\), 0.677 g (3.31 mmol) of 2,5-dimethylbenzenesulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na\(_2\)CO\(_3\) solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na\(_2\)SO\(_4\)), and filtered. The filtrate was then concentrated, and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The solids were triturated with diethyl ether (3 x 15 mL) and the resulting residue was dried \textit{in vacuo}. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na\(_2\)SO\(_4\)) and filtered. The filtrate was concentrated by rotary
evaporation and the resulting residue was dried in vacuo. This produced 2.30 g (99%) of
\(N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(2,5\text{-dimethylbenzenesulfonyl})-[N,N\text{-bis(3-}
\text{aminopropyl)cyclohexylmethylamine}\) as a yellow viscous oil. \(^1\)H NMR (400 MHz, 
CDCl\(_3\)) \(\delta 7.62\) (m, 3 H, \(o\text{-ArSO}_2\), \(o\text{-ArSO}_2\)), 7.13 (m, 2 H, \(m\text{-ArSO}_2\), \(p\text{-ArSO}_2\)), 6.58 (d, 
8.6 Hz, 2 H, \(m\text{-ArSO}_2\)), 5.65 (bs, 2 H, NHArSO\(_2\)), 2.94 (s, 6 H, N(CH\(_3\))\(_2\)), 2.86 (m, 4 H, 
CH\(_2\)N), 2.52 (m, 2 H, CH\(_2\)N), 2.26 (m, 4 H, CH\(_2\)N, CH\(_2\)C), 1.95 (d, 2 H, CH\(_2\)Cy), 1.53 (m, 
8 H, CH\(_2\)Cy, Cy), 1.26 (s, 1 H, CHC), 1.06 (m, 4 H, Cy), 0.70 (m, 2 H, Cy). \(^13\)C NMR 
(101 MHz, CDCl\(_3\)) \(\delta 152.6, 137.7, 135.8, 133.9, 133.1, 132.4, 129.6, 128.8, 125.0, 110.8, 
61.7, 52.9, 42.4, 42.1, 40.0, 35.6, 31.8, 26.6, 26.0, 25.6, 20.8, 19.8. IR (neat cm\(^{-1}\)) 2924 
(w), 2851 (w), 2493 (w), 1596 (w), 1516 (w), 1447 (w), 1367 (w), 1312 (m), 1146 (s), 1091 
(m), 998 (w), 944 (w), 896 (w), 816 (m), 707 (m), 696 (m), 654 (m), 619 (m). MS (ESI\(^+\)) 
m/z 579 (MH\(^+\)). Anal. Calcd for C\(_{27}\)H\(_{42}\)N\(_4\)O\(_4\)S\(_2\)·HCl·CH\(_3\)OH: C, 54.31; H, 7.65; N, 9.05. 
Found: C, 54.32; H, 7.60; N, 8.69.
Synthesis of $N'-(p$-dimethylaminobenzenesulfonyl)$-N''-(2$-methoxybenzenesulfonyl)$-[N,N$-bis(3$-aminopropyl)$cyclohexylmethylamine] (TL044)$^3$

To a 250 mL round bottom flask, 1.72 g (4.19 mmol) of $N$-(3-aminopropyl)$-N$-(3-$p$-dimethylaminobenzenesulfonamidopropyl)benzylamine, 1.52 g (7.36 mmol) of 2-methoxybenzenesulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na$_2$CO$_3$ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na$_2$SO$_4$), and filtered. The filtrate was then concentrated, and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were triturated with diethyl ether (3 x 15 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 2.43 g (99%) of
N'-({p}-dimethylaminobenzenesulfonyl)-N''-({2}-methoxybenzenesulfonyl)-[N,N-bis(3-
aminopropyl)cyclohexylmethylamine] as a yellow viscous oil. ¹H NMR (400 MHz,
{CDCl₃} δ 7.82 (t, 6.9 Hz, 1 H, o-ArSO₂), 7.60 (d, 8.5 Hz, 2 H, o-ArSO₂), 7.48 (t, 7.9 Hz, 1
H, p-ArSO₂), 6.99 (t, 7.7 Hz, 1 H, m-ArSO₂), 6.60 (d, 8.6 Hz, 2 H, m-ArSO₂), 5.57 (bs, 2
H, NHSO₂), 3.92 (s, 3 H, OCH₃), 2.97 (s, 6 H, N(CH₃)$_₂$), 2.84 (m, 4 H, CH₂N), 2.25 (t, 6.2
Hz, 4 H, CH₂N), 1.95 (d, 6.9 Hz, 2 H, CH₂Cy), 1.52 (m, 8 H, CH₂C, Cy), 1.27 (m, 1 H,
CH), 1.09 (m, 4 H, Cy), 0.69 (m, 2 H, Cy). ¹³C NMR (101 MHz, {CDCl₃} δ 156.3, 152.6,
134.4, 130.2, 128.8, 127.3, 125.1, 120.4, 112.2, 110.8, 61.7, 56.4, 53.6, 51.7, 42.8, 41.9,
40.0, 35.7, 31.8, 26.7, 26.6, 26.0, 25.6. IR (neat cm$^{-1}$) 2925 (w), 2851 (w), 2601 (w), 1594
(m), 1516 (w), 1480 (m), 1434 (m), 1368 (w), 1317 (m), 1279 (m), 1248 (w), 1152 (s),
1093 (m), 1069 (m), 1015 (m), 943 (w), 899 (w), 802 (m), 758 (m), 700 (m), 646 (m), 621
(m), 614 (m). MS (ESI$^+$) $m/z$ 581 (MH$^+$), 582 (MH$^+$ +1), 583 (MH$^+$ +2). Anal. Calcd for
C$_{28}$H$_{44}$N$_4$O$_5$S$_2$·HCl·CH$_3$OH: C, 53.65; H, 7.61; N, 8.63. Found: C, 53.40; H, 7.23; N, 8.34.
Synthesis of \(N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(2\text{-nitrobenzenesulfonyl})-\[N,N\text{-bis(3\text{-aminopropyl})cyclohexylmethylamine}\] (TL046)\(^3\)

To a 250 mL round bottom flask, 3.42 g (8.33 mmol) of \(N-(3\text{-aminopropyl})-N-(3-\text{p-dimethylaminobenzenesulfonamidopropyl})\text{-benzylamine}\), 2.03 g (9.16 mmol) of 2-nitrobenzenesulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na\(_2\)CO\(_3\) solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 15 mL), the combined extraction layers were dried (Na\(_2\)SO\(_4\)), and filtered. The filtrate was then concentrated, and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The solids were tritutrated with diethyl ether (3 x 15 mL) and the resulting residue was dried \textit{in vacuo}. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na\(_2\)SO\(_4\)) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. This produced 4.25 g (99%) of
N'-({p-dimethylaminobenzenesulfonyl})-N''-(2-nitrobenzenesulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] as a yellow viscous oil. ¹H NMR (400 MHz, CDCl₃) δ 8.14 (m, 1 H, o-ArSO₂), 7.83 (m, 1 H, p-ArSO₂), 7.73 (m, 1 H, m-ArSO₂), 7.66 (m, 2 H, o-ArSO₂), 6.68 (m, 2 H, m-ArSO₂), 3.13 (t, 6.6 Hz, 2 H, CH₂N), 3.04 (s, 6 H, N(CH₃)₂), 2.93 (t, 6.3 Hz, 2 H, CH₂N), 2.37 (m, 2 H, CH₂N), 2.07 (m, 4 H, CH₂Cy, CH₂C), 1.62 (m, 6 H, Cy), 1.35 (m, 1 H, CHCy), 1.17 (m, 4 H, Cy), 0.78 (m, 2H, Cy). ¹³C NMR (101 MHz, CD₃OD) δ 148.2, 147.2, 133.9, 132.8, 132.4, 131.7, 130.2, 128.9, 124.6, 124.0, 119.5, 59.6, 51.0, 50.8, 44.2, 39.8, 39.7, 33.2, 30.4, 25.5, 25.0, 23.6, 23.5. IR (neat cm⁻¹) 2924 (w), 2850 (w), 2442 (w), 2199 (w), 2157 (w), 2048 (w), 2035 (w), 2005 (w), 1971 (w), 1962 (w), 1941 (w), 1594 (w), 1538 (m), 1449 (w), 1367 (w), 1330 (m), 1162 (s), 1126 (m), 1091 (m), 994 (w), 940 (w), 897 (w), 851 (m), 782 (m), 707 (m), 699 (m), 678 (m), 668 (m), 654 (s), 636 (m) 636 (m), 619 (m), 606 (m). MS (ESI⁺) m/z 596 (MH⁺), 597 (MH⁺ +1), 598 (MH⁺ +2). Anal. Calcd for C₂₇H₄₁N₄O₆S₂·HCl·2CH₃OH: C, 50.02; H, 7.24; N, 10.06. Found: C, 49.76; H, 7.29; N, 10.36.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzensulfonyl)-3-methylene-5-(p-methoxybenzenesulfonyl)-1,5,9-triazacyclododecane (TL020)\textsuperscript{3}

\[
\text{In a 250 mL round bottom flask, 0.890 g (1.53 mmol) of } N'-(p-
dimethylaminobenzensulfonyl)-N''-(p-methoxybenzenesulfonyl)-[N,N-bis(3-
aminopropyl)cyclohexylmethylamine], 2.43 g (8.43 mmol) of 2-methylene-1,3-
propanebis(tert-butylcarbonate), 130 mg (1.23 mmol) of Na\textsubscript{2}CO\textsubscript{3}, 96.6 mg (0.105 mmol) of Pd\textsubscript{2}dba\textsubscript{3}, 93.3 mg (0.218 mmol) of dppb and 50 mL of anhydrous acetonitrile were stirred under N\textsubscript{2} gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. NaHCO\textsubscript{3} solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na\textsubscript{2}SO\textsubscript{4}) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried in vacuo. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h.}
The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 0.296 g (31%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(p-methoxysulfonyl)-1,5,9-triazacyclododecane as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.72 (m, 2 H, o-ArSO$_2$), 7.58 (m, 2 H, o-ArSO$_2$), 6.97 (m, 2 H, m-ArSO$_2$), 6.67 (m, 2 H, m-ArSO$_2$), 5.13 (s, 2 H, C=CH$_2$), 3.85 (s, 3 H, CH$_3$), 3.67 (s, 2H, H2/4), 3.53 (s, 2 H, H4/2), 3.19 (t, 7.1 Hz, 2 H, H6/12), 3.03 (m, 8 H, N(CH$_3$)$_2$, H12/6), 2.25 (dt, 18.4, 5.8 Hz, 4 H, H8, 10), 1.94 (d, 6.9 Hz, 2 H, CH$_2$Cy), 1.55 (m, 8 H, H7,11, Cy), 1.11 (m, 3 H, Cy), 0.81 (m, 2 H, Cy). NMR (101 MHz, CDCl$_3$) δ 162.7, 152.7, 138.2, 130.8, 129.2, 129.0, 123.3, 116.3, 114.2, 110.9, 62.1, 55.6, 51.9, 50.6, 50.2, 44.5, 43.6, 40.0, 35.9, 31.9, 26.8, 26.0, 24.8, 23.9. IR (neat cm$^{-1}$) 2926 (w), 2385 (w), 2122 (w), 1594 (m), 1517 (w), 1497 (w), 1447 (w), 1370 (w), 1333 (m), 1260 (m), 1150 (s), 1091 (m), 1018 (w), 898 (w), 835 (w), 806 (m), 724 (w), 685 (m). MS (ESI$^+$) $m/z$ 633 (MH$^+$). Anal. Calcd for C$_{32}$H$_{51}$ClN$_4$O$_4$S$_2$H$_2$O: C, 55.92; H, 7.48; N, 8.15. Found: C, 56.22; H, 7.73; N, 7.97.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(4-bromobenzenesulfonyl)-1,5,9-triazacyclododecane (TL021)³

![Chemical structure of TL021](image)

To a 250 mL round bottom flask, 0.459 g (0.729 mmol) of $N'$-(p-dimethylaminobenzenesulfonyl)-$N''$-(p-bromobenzenesulfonyl)-[$N,N$-bis(3-aminopropyl)cyclohexylmethylamine], 0.459 g (.728 mmol) of 2-methylene-1,3-propanebis(tert-butylcarbonate), 1.05 mg (3.68 mmol) of Na₂CO₃, 43.9 mg (0.0479 mmol) of Pd₂dba₃, 50.7 mg (0.119 mmol) of dppb and 81 mL of anhydrous acetonitrile were stirred under N₂ gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. NaHCO₃ solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried in vacuo. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL).
The combined organic solutions were dried ($\text{Na}_2\text{SO}_4$) and filtered. The filtrate was concentrated and the resulting residue was dried \textit{in vacuo}. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 159 mg (32%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(4-bromobenzenesulfonyl)-1,5,9-triazacyclododecane as a clear viscous oil. $^1\text{H}$ NMR (400 MHz, CDCl$_3$) $\delta$ 7.64 (m, 4 H, $o$-ArSO$_2$, $m$-ArSO$_2$), 7.54 (m, 2 H, $o$-ArSO$_2$), 6.64 (m, 2 H, $m$-ArSO$_2$), 5.12 (s, 2 H, C=CH$_2$), 3.89 (s, 2 H, H2/4), 3.62 (s, 2 H, H4/2), 3.26 (t, 7.2 Hz, 2 H, H6/12), 3.01 (s, 6 H, N(CH$_3$)$_2$), 2.96 (t, 6.4 Hz, 2 H, H12/6), 2.24 (m, 4 H, H8, 10), 1.93 (m, 6.9 Hz, 2 H, CH$_2$Cy), 1.59 (m, 8 H, H7, 11, Cy), 1.21 (m, 1 H, Cy), 1.10 (m, 4 H, Cy), 0.68 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.8, 138.1, 132.4, 129.1, 128.6, 127.4, 122.7, 117.0, 116.3, 110.9, 66.8, 64.4, 62.1, 52.9, 50.8, 49.9, 48.9, 45.0, 43.3, 40.0, 35.9, 31.9, 27.7, 26.0. IR (neat cm$^{-1}$) 2924 (w), 2852 (w), 2402 (w), 2115 (w), 1595 (w), 1573 (w), 1516 (w), 1448 (w), 1369 (w), 1336 (w), 1278 (w), 1231 (w), 1152 (m), 1091 (w), 1067 (w), 1008 (w), 924 (w), 902 (w), 873 (w), 820 (w), 776 (w), 747 (w), 713 (w), 671 (w). MS (ESI$^+$) $m/z$ 681 (M$^+$), 683 (MH$^+$ +1). Anal. Calcd for C$_{31}$H$_{45}$N$_4$O$_4$S$_2$Br.HCl.H$_2$O: C, 50.57; H, 6.57; N, 7.61. Found: C, 50.67; H, 6.63; N, 7.55.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(naphthalene-2-sulfonyl)-1,5,9-triazacyclododecane (TL022)³

To a 250 mL round bottom flask, 0.610 g (1.02 mmol) of $N^\prime$-(p-dimethylaminobenzenesulfonyl)-$N^\prime$-(naphthalene-2-sulfonyl)-[$N,N$-bis(3-aminopropyl)cyclohexylmethylamine], 1.46 g (5.06 mmol) of 2-methylene-1,3-propane bis(tert-butylcarbonate), 59.4 mg (0.560 mmol) of Na$_2$CO$_3$, 82.3 mg (0.0899 mmol) of Pd$_2$dba$_3$, 94.3 mg (0.221 mmol) of dppb and 50 mL of anhydrous acetonitrile were stirred under N$_2$ gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. NaHCO$_3$ solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried in vacuo. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was
extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na₂SO₄) and filtered. The filtrate was concentrated and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 0.264 g (40%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(naphthalene-2-sulfonfonyl)-

1,5,9-triazacyclododecane as a yellow viscous oil. ¹H NMR (400 Mhz, CDCl₃) δ 8.38 (s, 1 H, 2-ArSO₂), 7.97 (m, 2 H, 4,7-ArSO₂), 7.92 (m, 1 H, 10-ArSO₂), 7.78 (dd, 8.7, 1.9 Hz, 1 H, 9-ArSO₂), 7.62, (m, 4 H, 5,6-ArSO₂, o-ArSO₂), 6.68 (m, 2 H, m-ArSO₂), 5.18 (s, 1 H, C=CH₂), 5.16 (s, 1 H, C=CH₂) 3.98 (s, 2 H, H2/4), 3.71 (s, 2 H, H4/2), 3.30 (t, 7.2 Hz, 2 H, H6/12), 3.05 (m, 8 H, N(CH₃)₂, H12/6), 2.26 (dt, 23.5, 5.8 Hz, 4 H, H8, 10), 1.95 (d, 6.9 Hz, 2 H, CH₂Cy), 1.63 (m, 10 H, H7, 11, Cy), 1.22 (m, 3 H, CH, Cy) 0.68 (q, 11.4 Hz, 2 H, Cy). ¹³C NMR (101 MHz, CDCl₃) δ 141.8, 135.0, 133.3, 132.1, 129.8, 129.5, 129.24, 129.22, 128.0, 127.9, 122.3, 119.4, 61.0, 53.0, 52.8, 48.7, 33.7, 31.6, 25.7, 25.5. IR (neat cm⁻¹) 2925 (w), 2852 (w), 2498 (w), 2112 (w), 1595 (m), 1516 (w), 1448 (w), 1371 (w), 1332 (m), 1229 (w), 1151 (m), 1130 (m), 1092 (m), 1073 (w), 1016 (w), 899 (w), 874 (w), 857 (w), 817 (w), 776 (w), 751 (w), 734 (w), 658 (w). MS (ESI⁺) m/z 653 (MH⁺). Anal. Calcd for C₃₅H₅₁N₄O₄S₂HCl1.5H₂O: C, 58.68; H, 7.32; N, 7.82. Found: C, 58.84; H, 7.43; N, 7.74.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(naphthalene-1-sulfonyl)-1,5,9-triazacyclododecane (TL023) \(^3\)

![Chemical Structure](image)

To a 250 mL round bottom flask, 0.467 g (0.777 mmol) of \(N^\prime-(p\)-dimethylaminobenzenesulfonyl)-\(N^\prime\)-(naphthalene-1-sulfonyl)-[\(N,N\)-bis(3-aminopropyl)cyclohexylmethylamine], 1.12 g (3.88 mmol) of 2-methylene-1,3-propanebis(tert-butylcarbonate), 30.2 mg (0.285 mmol) of \(\text{Na}_2\text{CO}_3\), 46.4 mg (0.0507 mmol) of \(\text{Pd}_2\text{dba}_3\), 41.5 mg (0.0973 mmol) of dppb and 86 mL of anhydrous acetonitrile were stirred under \(\text{N}_2\) gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. \(\text{NaHCO}_3\) solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (\(\text{Na}_2\text{SO}_4\)) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried \textit{in vacuo}. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL
sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 122 mg (28%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(naphthalene-1-sulfonyl)-1,5,9-triazacyclododecane as a yellow viscous oil. $^1$H NMR (400 Mhz, CDCl$_3$) δ 8.66 (d, 8.7 Hz, 1 H, 2-ArSO$_2$), 8.26 (d, 7.3 Hz, 1 H, 9-ArSO$_2$), 8.05 (d, 8.3 Hz, 1 H, 3-ArSO$_2$), 7.93 (m, 1 H, 7-ArSO$_2$), 7.67 (m, 1 H, 8-ArSO$_2$), 7.56 (m, 4 H, 4, 6-ArSO$_2$, o-ArSO$_2$), 6.67 (m, 2 H, m-ArSO$_2$) 5.14 (s, 1 H, C=CH$_2$), 5.06 (s, 1 H, C=CH$_2$), 4.13 (s, 2H, H2/4), 3.54 (s, 2 H, H4/2), 3.42 (t, 7.6 Hz, 2 H, H6/12), 3.04 (s, 6 H, N(CH$_3$)$_2$), 2.88 (t, 6.2 Hz, H12/6), 2.33 (t, 5.9 Hz, 2 H, H8/10), 2.14 (t, 5.8 Hz, H10/8), 1.94 (d, 6.9 Hz, 2 H, CH$_2$Cy), 1.77 (m, 2 H, H7/10), 1.62 (m, 6 H, Cy), 1.43 (m, 2 H, H10/7), 1.15 (m, 3 H, Cy), 0.68 (q, 11.8 Hz, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 152.8, 138.1, 135.0, 134.3, 134.1, 130.0, 129.2, 128.9, 128.6, 128.1, 126.8, 124.9, 124.1, 122.1, 116.5, 110.9, 62.2, 54.5, 51.4, 49.5, 47.2, 45.4, 42.2, 40.0, 35.9, 31.9, 26.8, 26.0, 23.7. IR (neat cm$^{-1}$) 3214 (w), 2919 (w), 2295 (w), 2216 (w), 2181 (w), 2165 (w), 2086 (w), 2055 (w), 1983 (w), 1595 (w), 1507 (w), 1446 (w), 1317 (w), 1148 (m) 1130 (m), 1090 (m), 1075 (m), 1063 (w), 994 (w), 976 (m), 942 (w), 934 (w), 902 (w), 857 (w), 795 (w), 771 (s), 730 (m), 687 (s), 672 (m), 616 (s), 608 (m). MS (ESI$^+$) $m/z$ 653 (MH$^+$). Anal. Calcd. for C$_{35}$H$_{48}$N$_4$O$_4$S$_2$: HCl: C, 60.98; H, 7.16; N, 8.13. Found: C, 60.61; H,6.92; N, 7.86.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(methylsulfonyl)-1,5,9-triazacyclododecane (TL027)³

To a 500 mL round bottom flask, 0.957 g (1.96 mmol) of \( N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(methylsulfonyl)-[N,N\text{-bis(3-aminopropyl)}\text{cyclohexylmethylamine]} \), 2.56 g (8.88 mmol) of 2-methylene-1,3-propanebis(tert-butylcarbonate), 0.243 g (2.29 mmol) of \( \text{Na}_2\text{CO}_3 \), 0.108 g (0.118 mmol) of \( \text{Pd}_2\text{dba}_3 \), 0.110 g (0.258 mmol) of dppb and 218 mL of anhydrous acetonitrile were stirred under \( \text{N}_2 \) gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. \( \text{NaHCO}_3 \) solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (\( \text{Na}_2\text{SO}_4 \)) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried in vacuo. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h.
The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 1:1 (v/v) ethyl acetate/hexane. This produced 344 mg (33%) of 4-(((9-(cyclohexylmethyl)-3-methylene-5-(methylsulfonyl)-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.59 (m, 2 H, $\omega$-ArSO$_2$), 6.69 (m, 2 H, $m$-ArSO$_2$), 5.21 (d, 37.9 Hz, 2 H, C=CH$_2$), 3.99 (s, 2 H, H2/4), 3.60 (s, 2 H, H4/2), 3.43 (t, 7.6 Hz, 2 H, H6/12), 3.06 (s, 6 H, N(CH$_3$)$_2$), 2.95 (m, 2 H, H12/6), 2.88 (s, 3 H, SO$_2$CH$_3$), 2.41 (t, 5.8 Hz, 2 H, H8/10), 2.30 (t, 5.8 Hz, 2 H, H10/8), 2.00 (d, 6.9 Hz, 2 H, CH$_2$Cy), 1.67 (m, 10 H, H7, 11, Cy), 1.49 (m, 1 H, Cy), 1.20 (m, 4 H, Cy), 0.71 (q, 11.8 Hz, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.8, 138.3, 129.2, 122.2, 116.1, 110.9, 77.3, 77.0, 76.7, 62.1, 54.1, 51.0, 49.5, 47.4, 45.6, 42.7, 40., 39.0, 35.9, 31.9, 27.7, 26.8, 26.0, 25.9, 23.5. IR (neat cm$^{-1}$) 2925 (w), 2847 (w), 2416 (w), 2283 (w), 1656 (w), 1595 (w), 1516 (w), 1449 (w), 1373 (w), 1320 (w), 1225 (w), 1146 (m), 1090 (m), 1016 (w), 966 (m), 898 (w), 877 (w), 791 (m), 756 (w), 733 (w), 707 (w), 686 (w). MS (ESI$^+$) m/z 541 (MH$^+$), 542 (MH$^+$+1), 543 (MH$^+$+2). Anal. Calcd. for C$_{26}$H$_{44}$N$_4$O$_4$S$_2$HCl·0.25H$_2$O: C, 53.68; H, 7.88; N, 9.63. Found: C, 53.43; H,7.51; N, 9.36.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(4-dimethylaminobenzenesulfonyl)-1,5,9-triazacyclododecane (TL029)³

To a 250 mL round bottom flask, 0.501 g (0.844 mmol) of \(N'-(p\)-dimethylaminobenzenesulfonyl)-\(N''-(p\)-dimethylaminobenzenesulfonyl)-[\(N,N\)-bis(3-aminopropyl)cyclohexylmethylamine], 1.11 g (3.85 mmol) of 2-methylene-1,3-propanebis(tert-butylcarbonate), 0.0107 g (0.100 mmol) of \(\text{Na}_2\text{CO}_3\), 0.0529 g (0.0559 mmol) of \(\text{Pd}_2\text{dba}_3\), 0.0417 g (0.0978 mmol) of dpbb and 94 mL of anhydrous acetonitrile were stirred under \(\text{N}_2\) gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. \(\text{NaHCO}_3\) solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (\(\text{Na}_2\text{SO}_4\)) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried \textit{in vacuo}. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was
extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na₂SO₄) and filtered. The filtrate was concentrated and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 68.9 mg (13%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(4-dimethylaminobenzenesulfonyl)-1,5,9-triazacyclododecane as a clear viscous oil. ¹H NMR (500 MHz, CDCl₃) δ 7.62 (m, 4 H, o-ArSO₂), 6.69 (m, 4 H, m-ArSO₂), 5.19 (s, 2 H, C=CH₂), 3.77 (m, 4H, H₂, 4), 3.13 (m, 2 H, H6/12), 3.06 (s, 8 H, N(CH₃)₂, H6/12), 2.27 (m, 2 H, CH₂Cy), 1.96 (t, 6.9 Hz, 4 H, H8, 10), 1.61 (m, 8 H, H7, 11, Cy) 1.19 (m, 1 H, Cy), 0.94 (m, 4 H, Cy), 0.69 (m, 2 H, Cy). ¹³C NMR (101 MHz, CDCl₃) δ 152.33, 142.26, 129.46, 118.90, 112.56, 109.99, 77.30, 76.98, 76.66, 60.97, 52.92, 48.71, 47.25, 40.95, 33.77, 31.66, 25.68, 25.54, 20.17. IR (neat cm⁻¹) 2924 (w), 2432 (w), 1595 (s), 1516 (w), 1446 (w), 1369 (w), 1333 (m), 1317 (m), 1229 (w), 1147 (s), 1091 (s), 998 (w), 942 (w), 898 (w), 816 (w), 777 (m), 721 (w), 679 (m). MS (ESI⁺) m/z 646 (MH⁺). Anal. Calcd for C₃₃H₅₁N₅O₄S₂·HCl·MeOH: C, 57.16; H, 7.90; N, 9.80. Found: C, 57.22; H, 8.23; N, 9.47.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(benzenesulfonyl)-1,5,9-triazacyclododecane (TL032)³

To a 500 mL round bottom flask, 1.34 g (2.44 mmol) of \( \text{N'}-(p\text{-dimethylaminobenzenesulfonyl)}-\text{N'}-(\text{benzenesulfonyl)}-[\text{N,N-bis(3-aminopropyl)cyclohexylmethylamine}] \), 3.58 g (12.4 mmol) of 2-methylene-1,3-propanebis(\text{tert}-butylcarbonate), 0.0960 g (0.906 mmol) of \( \text{Na}_2\text{CO}_3 \), 0.133 g (0.145 mmol) of \( \text{Pd}_2\text{dba}_3 \), 0.136 g (0.319 mmol) of dppb and 270 mL of anhydrous acetonitrile were stirred under \( \text{N}_2 \) gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. \( \text{NaHCO}_3 \) solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (\( \text{Na}_2\text{SO}_4 \)) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried \textit{in vacuo}. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM.
The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 1:1 (v/v) ethyl acetate/hexane. This produced 534 mg (36%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(benzenesulfonyl)-1,5,9-triazacyclododecane as a clear viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.79 (m, 2 H, o-ArSO$_2$), 7.56 (m, 3 H, o-ArSO$_2$, p-ArSO$_2$) 7.51 (m, 2 H, m-ArSO$_2$), 6.66 (m, 2 H, m-ArSO$_2$), 5.14 (d, 3.2 Hz, 2 H, C=CH$_2$), 3.88 (s, 2 H, H2/4), 3.67 (s, 2 H, H4/2), 3.24 (t, 7.2 Hz, 2 H, H6/12), 3.03 (m, 8 H, N(CH$_3)_2$, H12/6), 2.24 (m, 4 H, H8, 10), 1.94 (d, 6.8 Hz, 2 H, CH$_2$Cy), 1.62 (m, 10 H, H7, 11, Cy), 1.16 (m, 4 H, Cy), 0.68 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.8, 139.4, 138.2, 132.5, 129.1, 129.1, 127.1, 123.0, 117.0, 116.3, 110.9, 66.9, 64.5, 62.1, 52.3, 50.7, 50.0, 49.5, 44.7, 43.5, 40.0, 35.9, 31.9, 27.7, 26.8, 26.0, 25.0, 23.8. IR (neat cm$^{-1}$) 2924 (w), 2852 (w), 2383 (w), 2096 (w), 1595 (w), 1516 (w), 1446 (w), 1368 (w), 1334 (w), 1319 (w), 1229 (w), 1151 (m), 1124 (w), 1092 (w), 1000 (w), 942 (w), 903 (w), 875 (w), 817 (w), 777 (w), 731 (w), 704 (w), 691 (w), 667 (w). MS (ESI$^+$) m/z 603 (MH$^+$). Anal. Calcd. for C$_{31}$H$_{46}$N$_4$O$_4$S$_2$·HCl·2H$_2$O: C, 55.13; H, 7.61; N, 8.30. Found: C, 55.12; H, 7.87; N, 8.29.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(morpholinylsulfonyl)-1,5,9-triazacyclododecane (TL033)³

To a 500 mL round bottom flask, 1.20 g (2.14 mmol) of \(N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(\text{morpholinylsulfonyl})-\left[N,N\text{-bis(3-aminopropyl)cyclohexylmethylamine}\right], 3.10 g (10.8 mmol) of 2-methylene-1,3-propane\text{bis(}\text{\textit{tert}-butylcarbonate}, 0.290 g (2.73 mmol) of \(\text{Na}_2\text{CO}_3\), 0.121 g (0.132 mmol) of \(\text{Pd}_2\text{dba}_3\), 0.136 g (0.319 mmol) of dppb and 238 mL of anhydrous acetonitrile were stirred under \(\text{N}_2\) gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. \(\text{NaHCO}_3\) solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (\(\text{Na}_2\text{SO}_4\)) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried \textit{in vacuo}. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL).
The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated, and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 438 mg (33%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(morpholinylsulfonyl)-1,5,9-triazacyclododecane as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.22 (m, 2 H, o-ArSO$_2$), 6.45 (m, 2 H, m-ArSO$_2$), 4.99 (s, 1 H, C=CH$_2$), 4.88 (s, 1 H, C=CH$_2$), 3.84 (s, 2 H, H2/4), 3.49 (m, 4 H, CH$_2$OCH$_2$), 3.30 (s, 2 H, H4/2), 3.15 (m, 2 H, H6/12), 2.93 (m, 4 H, CH$_2$NCH$_2$), 2.81 (s, 6 H, N(CH$_3$)$_2$), 2.64 (m, 2 H, H12/6), 2.18 (m, 2 H, H8/10), 2.04 (t, 5.7 Hz, 2 H, H10/8), 1.76 (d, 6.9 Hz, 2 H, CH$_2$Cy), 1.47 (m, 8 H, H7, 11, Cy), 1.01 (m, 2 H, Cy), 0.88 (m, 3 H, Cy), 0.47 (q, 11.6 Hz, 11.1 Hz, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 153.0, 138.5, 129.4, 129.0, 116.0, 111.0, 66.5, 62.3, 55.2, 51.6, 49.5, 48.0, 46.5, 46.2, 46.0, 42.9, 40.2, 36.1, 32.1, 26.9, 26.5, 26.2, 24.1. IR (neat cm$^{-1}$) 2924 (w), 2854 (w), 2399 (w), 2118 (w), 1595 (m), 1517 (w), 1448 (w), 1333 (m), 1260 (w), 1229 (w), 1149 (s), 1112 (m), 1092 (m), 1071 (w), 1017 (w), 940 (m), 818 (w), 780 (w), 727 (w), 683 (w). MS (ESI$^+$) $m/z$ 612 (MH$^+$). Anal. Calcd. for C$_{29}$H$_{49}$N$_5$O$_5$S$_2$·2HCl·H$_2$O: C, 48.32; H, 7.69; N, 9.72. Found: C, 48.38; H, 7.30; N, 9.79.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(5-(dimethylamino)naphthalene-1-sulfonyl)-1,5,9-triazacyclododecane (TL037)³

To a 500 mL round bottom flask, 2.15 g (3.34 mmol) of \(N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(5\text{-}(dimethylamino)naphthalene-1-sulfonyl)}-[N,N-bis(3-aminopropyl)cyclohexylmethylamine], 4.85 g (16.8 mmol) of 2-methylene-1,3-propanebis(tert-butylcarbonate), 0.120 g (1.13 mmol) of \(\text{Na}_2\text{CO}_3\), 0.191 g (0.209 mmol) of \(\text{Pd}_2\text{dba}_3\), 0.201 g (0.471 mmol) of dppb and 370 mL of anhydrous acetonitrile were stirred under \(\text{N}_2\) gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. NaHCO\(_3\) solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (\(\text{Na}_2\text{SO}_4\)) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried \textit{in vacuo}. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL).
The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 1:1 (v/v) ethyl acetate/hexane. This produced 1.05 g (45%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(5-(dimethylamino)naphthalene-1-sulfonyl)-1,5,9-triazacyclododecane as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.52 (d, 8.5 Hz, 1 H, 3-ArSO$_2$), 8.25 (m, 2 H, 2-ArSO$_2$, 8-ArSO$_2$), 7.53 (m, 4 H, o-ArSO$_2$, 7-ArSO$_2$, 3-ArSO$_2$), 7.16 (d, 7.5 Hz, 1 H, 6-ArSO$_2$), 6.66 (m, 2 H, m-ArSO$_2$), 5.11 (d, 42.2 Hz, 2 H, C=CH$_2$), 4.10 (s, 2 H, H2/4), 3.54 (s, 2 H, H4/2), 3.40 (t, 7.6 Hz, 2 H, H6/12), 3.04 (s, 6 H, N(CH$_3$)$_2$), 2.87 (m, 2 H, H12/6), 2.32 (m, 2 H, H8/10), 2.13 (m, 2 H, H10/8), 1.93 (d, 6.8 Hz, 2 H, CH$_2$Cy), 1.53 (m, 10 H, H7, 11, Cy), 1.18 (m, 4 H, Cy) 0.82 (m, 1 H, Cy), 0.68 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 152.8, 151.7, 138.2, 135.1, 130.3, 130.0, 130.0, 129.2, 128.0, 123.1, 122.1, 119.5, 116.6, 115.1, 110.8, 62.2, 54.4, 51.4, 49.6, 47.3, 45.4, 42.2, 40.0, 35.9, 31.9, 26.8, 26.0, 23.7. IR (neat cm$^{-1}$) 3377 (w), 2927 (w), 2852 (w), 2428 (w), 2115 (w), 1595 (w), 1514 (w), 1451 (w), 1387 (w), 1323 (w), 1233 (w), 1140 (w), 1091 (w), 1046 (w), 1016 (w), 995 (w), 899 (w), 794 (w), 733 (w), 686 (w). MS (ESI$^+$) $m/z$ 696 (MH$^+$). Anal. Calcd. for C$_{37}$H$_{53}$N$_4$O$_4$S$_2$•2HCl: C, 57.80; H, 7.21; N, 9.11. Found: C, 57.95; H, 6.87; N, 9.09.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(4-tert-butylsulfonyl)-1,5,9-triazacyclododecane (TL038)³

To a 500 mL round bottom flask, 1.68 g (2.77 mmol) of $N'$-(p-dimethylaminobenzenesulfonyl)-$N''$-(4-tert-butylanaphthalene-1-sulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine], 4.00 g (13.9 mmol) of 2-methylene-1,3-propanebis(tert-butylcarbonate), 0.177 g (1.66 mmol) of Na$_2$CO$_3$, 0.150 g (0.164 mmol) of Pd$_2$dba$_3$, 0.152 g (0.356 mmol) of dppb and 300 mL of anhydrous acetonitrile were stirred under N$_2$ gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. NaHCO$_3$ solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried in vacuo. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL).
The combined organic solutions were dried (Na\textsubscript{2}SO\textsubscript{4}) and filtered. The filtrate was concentrated and the resulting residue was dried \textit{in vacuo}. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 615 mg (34\%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(4-tert-butylsulfonyl)-1,5,9-triazacyclododecane as a clear viscous oil. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 7.69 (m, 2 H, \textit{m}-ArSO\textsubscript{2}), 7.58 (m, 2 H, \textit{m}-ArSO\textsubscript{2}), 7.50 (m, 2 H, \textit{o}-ArSO\textsubscript{2}), 6.67 (m, 2 H, \textit{o}-ArSO\textsubscript{2}), 5.17 (d, 7.1 Hz, 2 H, C=CH\textsubscript{2}), 3.84 (s, 2 H, H2/4), 3.70 (s, 2 H, H4/2), 3.21 (t, 7.1 Hz, 2 H, H6/12), 3.04 (m, 8 H, H12/6, N(CH\textsubscript{3})\textsubscript{2}), 2.25 (m, 4 H, H8, 10), 1.94 (d, 6.9 Hz, 2 H, CH\textsubscript{2}Cy) 1.60 (m, 8 H, H7, 11, Cy), 1.34 (m, 11 H, C(CH\textsubscript{3})\textsubscript{3}, Cy) 1.10 (m, 3 H, Cy), 0.67 (m, 2 H, Cy). \textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 157.2, 151.7, 142.0, 133.2, 129.37, 127.42, 126.4, 119.2, 113.8, 60.9, 53.0, 52.6, 48.7, 47.3, 47.0, 41.5, 35.2, 33.6, 31.5, 31.0, 25.6, 25.5, 20.2, 20.0. IR (neat cm\textsuperscript{-1}) 2922 (w), 2850 (w), 2372 (w), 1769 (w), 1704 (w), 1596 (m), 1515 (w), 1446 (w), 1392 (w), 1364 (w), 1330 (m), 1318 (m), 1263 (w), 1230 (w), 1151 (s), 1114 (m), 1093 (s), 1016(w), 998 (m), 943 (w), 904 (w), 841 (w), 777 (m), 760 (m), 719 (s), 672 (m), 637 (s), 619 (m). MS (ESI\textsuperscript{+}) \textit{m/z} 659 (MH\textsuperscript{+}), 660 (MH\textsuperscript{+} +1), 661 (MH\textsuperscript{+} +2). Anal. Calcd for C\textsubscript{35}H\textsubscript{54}N\textsubscript{4}O\textsubscript{4}S\textsubscript{2}HCl\cdot0.5H\textsubscript{2}O: C, 59.68; H, 8.01; N, 7.95. Found: C, 59.56; H, 7.80; N, 7.90.
Synthesis of 4-((5-(benzo[d][1,3]dioxol-5-yl)sulfonyl)-9-(cyclohexylmethyl)-3-methylene-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline (TL039)³

To a 500 mL round bottom flask, 1.74 g (2.93 mmol) of N’-(p-dimethylaminobenzenesulfonyl)-N”-(benzo[d][1,3]dioxole-5-sulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine], 4.27 g (14.8 mmol) of 2-methylene-1,3-propanebis(tert-butylcarbonate), 0.150 g (1.41 mmol) of Na₂CO₃, 0.191 g (0.209 mmol) of Pd₂dba₃, 0.158 g (0.370 mmol) of dppb and 325 mL of anhydrous acetonitrile were stirred under N₂ gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. NaHCO₃ solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried in vacuo. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h.
The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated, and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 0.492 g (26%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-[(benzo[d][1,3]dioxole-5-sulfonyl)-1,5,9-triazacyclododecane as a clear viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.58 (m, 2 H, o-ArSO$_2$), 7.35 (dd, 8.2 Hz, 1.8 Hz, 1 H, 7-ArSO$_2$), 7.20 (s, 1 H, 2-ArSO$_2$), 6.99 (d, 8.2 Hz, 1 H, 6-ArSO$_2$), 6.67 (m, 2 H, m-ArSO$_2$), 6.08 (s, 2 H, OCH$_2$O), 5.16 (m, 2 H, C=CH$_2$), 3.84 (s, 2 H, H2/4), 3.68 (s, 2 H, H4/2), 3.22 (t, 7.0 Hz, 2 H, H6/12), 3.04 (m, 8 H, N(CH$_3$)$_2$, H12/6), 2.29 (m, 2 H, H8/10), 2.24 (t, 5.8 Hz, 2 H, H10/8), 1.95 (d, 6.9 Hz, 2 H, CH$_2$Cy), 1.61 (m, 10 H, H7, 11, Cy), 1.13 (m, 2 H, Cy), 0.68 (q, 11.6 Hz, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.8, 151.2, 148.2, 138.2, 132.5, 129.0, 123.1, 122.7, 116.3, 110.9, 108.3, 107.4, 102.3, 62.1, 52.1, 50.7, 50.1, 49.7, 44.6, 43.6, 40.0, 35.9, 31.9, 26.8, 26.0, 24.9, 23.9. IR (neat cm$^{-1}$) 3361 (w), 2926 (w), 2853 (w), 2483 (w), 2132 (w), 2028 (w), 1976 (w), 1595 (m), 1502 (w), 1477 (m), 1448 (w), 1425 (w), 1372 (w), 1330 (m), 1241 (m), 1174 (w), 1145 (s), 1111 (m), 1032 (m), 928 (w), 898 (m), 876 (w), 816 (w), 774 (w)m 732 (m), 706 (w), 696 (w), 681 (w), 671 (w), 636 (w), 622 (w), 608 (w). MS (ESI$^+$) m/z 647 (MH$^+$). Anal. Calcd. for C$_{32}$H$_{46}$N$_4$O$_6$S$_2$·2HCl: C, 53.40; H 6.72; N, 7.78. Found: C, 53.30; H, 6.72; N, 7.79.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(2-methoxysulfonyl)-1,5,9-triazaclododecane (TL045)

To a 500 mL round bottom flask, 1.82 g (3.13 mmol) of \( \text{N}^\prime - (p\text{-dimethylaminobenzenesulfonyl}) - \text{N}'' - (2\text{-methoxysulfonyl}) - [\text{N,N-bis(3-aminopropyl)cyclohexylmethylamine}] \), 4.58 g (15.9 mmol) of 2-methylene-1,3-propanebis(tert-buty lacarbonate), 179 mg (1.68 mmol) of Na\(_2\)CO\(_3\), 173 mg (0.189 mmol) of Pd\(_\text{2}db\_\text{a3}\), 170. mg (0.399 mmol) of dppb and 348 mL of anhydrous acetonitrile were stirred under \( \text{N}_2 \) gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. NaHCO\(_3\) solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na\(_2\)SO\(_4\)) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried in vacuo. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl
solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 831 mg (42%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(2-methoxysulfonyl)-1,5,9-triazacyclododecane as a clear viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.09 (d, 7.2 Hz, 1 H, o-ArSO$_2$), 7.73 (m, 2 H, o-ArSO$_2$), 7.64 (m, 1 H, p-ArSO$_2$), 7.15 (m, 2 H, m-ArSO$_2$), 6.83 (m, 2 H, m-ArSO$_2$), 5.36 (s, 1 H, C=CH$_2$), 5.25 (s, 1 H, C=CH$_2$), 4.36 (s, 2 H, H2/4), 4.13 (s, 3 H, OCH$_3$), 3.77 (s, 2 H, H4/2), 3.50 (t, 7.3 Hz, 2 H, H6/12), 3.19 (s, 6 H, N(CH$_3$)$_2$), 3.13 (m, 2 H, H12/6), 2.49 (m, 2 H, H8/10), 2.29 (m, 2 H, H10/8), 2.09 (d, 2 H, CH$_2$Cy), 1.81 (m, 8 H, H7, 11, Cy), 1.48 (m, 2 H, Cy), 1.27 (m, 3 H, Cy), 0.83 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 156.7, 152.8, 134.3, 131.1, 129.0, 120.1, 112.0, 110.9, 62.1, 60.3, 56.3, 55.9, 53.8, 50.8, 49.4, 45.7, 42.5, 40.0, 35.8, 31.8, 27.7, 26.7, 26.0, 25.9, 22.6, 21.0, 14.2. IR (neat cm$^{-1}$) 2924 (w), 2851 (w), 2363 (w), 1594 (m), 1516 (w), 1480 (m), 1448 (m), 1370 (w), 1320 (m), 1279 (m), 1249 (w), 1150 (s), 1091 (m), 1065 (m), 1043 (w), 1013 (m), 899 (m), 839 (w), 803 (m), 760 (m), 733 (m), 691 (m), 642 (m). MS (ESI$^+$) $m/z$ 633 (M$^+$), 634 (MH$^+$), 635 (MH$^+$ +1). Anal. Calcd for C$_{32}$H$_{46}$N$_4$O$_5$S$_2$·HCl·H$_2$O: C, 55.92; H, 7.48; N, 8.15. Found: C, 56.12; H, 7.69; N, 8.10.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(p-difluoromethoxybenzenesulfonyl)-1,5,9-triazacyclododecane (TL042)³

To a 500 mL round bottom flask, 1.19 g (1.94 mmol) of N’-(p-dimethylaminobenzenesulfonyl)-N”-(p-difluoromethoxybenzenesulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine], 2.83 g (9.81 mmol) of 2-methylene-1,3-propanebis(tert-butylcarbonate), 85.4 mg (0.806 mmol) of Na₂CO₃, 105 mg (0.115 mmol) of Pd₂dba₃, 111 mg (0.260 mmol) of dppb and 215 mL of anhydrous acetonitrile were stirred under N₂ gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. NaHCO₃ solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na₂SO₄) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried in vacuo. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried in vacuo. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl
solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 417 mg (32%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(p-difluoromethoxybenzenesulfonyl)-1,5,9-triazacyclododecane as a clear viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.77 (m, 2 H, $m$-ArSO$_2$), 7.53 (m, 2 H, $m$-ArSO$_2$), 7.19 (m, 2 H, $o$-ArSO$_2$), 6.62 (m, 2 H, $o$-ArSO$_2$), 5.11 (s, 2 H, C=CH$_2$), 3.87 (s, 2 H, H2/4), 3.62 (s, 2 H, H4/2), 3.24 (t, 7.2 Hz, 2 H, H6/12), 2.99 (s, 6 H, N(CH$_3$)$_2$), 2.95 (t, 6.5 Hz, 2 H, H12/6), 2.26 (m, 2 H, H8/10), 2.19 (t, 2 H, H10/8), 1.92 (d, 2 H, CH$_2$Cy), 1.60 (m, 10 H, H7, 11, Cy), 1.14 (m, 3 H, Cy) 0.66 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 154.0, 152.8, 138.1, 136.2, 129.2, 122.6, 119.3, 117.9, 117.0, 116.3, 115.3, 112.6, 110.9, 66.8, 64.4, 62.1, 52.7, 50.7, 49.9, 49.0, 44.8, 43.3, 40.0, 35.9, 31.9, 27.7, 26.7, 26.0, 25.3, 23.7. IR (neat cm$^{-1}$) 2924 (w), 2852 (w), 2364 (w), 1595 (m), 1516 (w), 1492 (w), 1446 (w), 1335 (m), 1318 (m), 1226 (m), 1151 (s), 1119 (s), 1092 (s), 1040 (m), 997 (m), 942 (w), 913 (w), 873 (w), 817 (w), 775 (m), 724 (m), 676 (m), 643 (m), 618 (w). MS (ESI$^+$) m/z 669 (MH$^+$), 671 (MH$^+$ +1), 672 (MH$^+$ +2). Anal. Calcd for C$_{32}$H$_{46}$F$_2$N$_4$O$_5$S$_2$HCl·H$_2$O: C, 53.14; H, 6.83; N, 7.75. Found: C, 53.50; H, 6.63; N, 7.68.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(2-nitrobenzenesulfonfonyl)-1,5,9-triazacyclododecane (TL047)³

To a 500 mL round bottom flask, 3.40 g (5.71 mmol) \( N'(p\text{-dimethylaminobenzenesulfonyl})-N''(2\text{-nitrobenzenesulfonfonyl})-\{N,N\text{-bis(3-aminopropyl)cyclohexylmethylamine]\}, 8.50 g (29.5 mmol) of 2-methylene-1,3-propanebis(\text{tert-butylcarbonate}), 0.292 g (2.75 mmol) of \( \text{Na}_2\text{CO}_3 \), 0.290 g (0.317 mmol) of \( \text{Pd}_2\text{dba}_3 \), 0.293 g (0.687 mmol) of dppb and 634 mL of anhydrous acetonitrile were stirred under \( \text{N}_2 \) gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. \( \text{NaHCO}_3 \) solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (\( \text{Na}_2\text{SO}_4 \)) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \text{in vacuo}. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried \text{in vacuo}. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried \text{in vacuo}. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL).
The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated, and the resulting residue was dried *in vacuo*. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 0.670 g (18%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(2-nitrobenzenesulfonyl)-1,5,9-triazacyclododecane as a yellow viscous oil. $^1$H NMR (400 Mhz, CDCl$_3$) $\delta$ 7.99 (m, 1 H, o-ArSO$_2$), 7.66 (m, 2 H, m-ArSO$_2$), 7.58 (m, 3 H, p-ArSO$_2$, o-ArSO$_2$), 6.68 (m, 2 H, m-ArSO$_2$), 5.13 (s, 1 H, C=CH$_2$), 5.07 (s, 1 H, C=CH$_2$), 4.17(s, 2 H, H2/4), 3.51 (m, 4 H, H4/2, OCH$_3$), 3.04 (s, 6 H, N(CH$_3$)$_2$), 2.85 (t, 6.1 Hz, 2 H, H6/12), 2.38 (m, 2 H, H12/6), 2.22 (m, 2 H, H8/10), 1.96 (m, 2 H, CH$_2$Cy), 1.82 (t, 6.1 Hz, H10/8), 1.56 (m, 10 H, H7, 11, Cy), 1.16 (m, 3 H, Cy), 0.69 (q, 11.3 Hz, 10. 7 Hz, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.9, 148.0, 137.9, 133.3, 131.7, 130.4, 129.3, 124.1, 121.6, 116.2, 110.9, 62.1, 55.1, 51.5, 49.4, 47.1, 45.8, 42.6, 41.0, 40.0, 35.9, 31.9, 27.7, 26.7, 26.3, 26.0, 23.5. IR (neat cm$^{-1}$) 2923 (w), 2851 (w), 2443 (w), 1595 (m), 1541 (m), 1516 (m), 1446 (m), 1369 (m), 1334 (m), 1229 (w), 1146 (s), 1124 (s), 1091 (m), 1060 (m), 998 (m), 938 (m), 899 (m), 851 (m), 817 (m), 778 (s), 735 (m), 680 (m), 640 (m), 621 (m). MS (ESI$^+$) $m/z$ 648 (MH$^+$), 649 (MH$^+$ +1), 650 (MH$^+$ +2).
3.5 References


Chapter 4

Synthesis of CADA Analogs towards Down-Modulation of
gp160 and TSHR
4.1 Introduction

4.1.1 Signal Peptides as Drug Targets

The mechanism by which CADA down-modulates CD4 is novel. No other small molecule has been found to specifically target the signal peptide of a protein before now. It is believed that specific amino acids in the signal peptide increases the binding of CADA compounds, ultimately causing the co-translational translocation of CD4 to be unsuccessful. The signal peptide sequence of CD4, shown in Figure 4.1.1, is 25 amino acids long. It has been found that the most important residues of the sequence are Gln15, Pro20, and the two lysine residues located at positions 26 and 27 as mentioned in chapter 1. It is hypothesized that the proline serves as a sort of hinge that brings the glutamine and the two lysine residues close to proximity, which is the site where CADA binds. This folded conformation of the signal peptide, which would only be a transition state during the reorientation of the signal peptide when it undergoes the flip-turn is stabilized by the binding of CADA. This conformation prevents the translocation of the nascent protein through the translocon into the ER lumen, and instead loops the protein out into the cytosol, causing degradation of the protein.

CADA has also been shown to interact with the protein sortilin. It was found to down-modulate the expression of sortilin, which is a protein that contains a non-cleavable signal anchor sequence and a signal peptide. Sortilin is a neurotensin receptor that is

![Figure 4.1.1. First 30 amino acid residues of CD4](image-url)
involved in shuttling between the cell surface and different organelles. It is commonly expressed on the membranes of intracellular partitions such as the Golgi, endoplasmic reticulum, and endosomes. It is involved in protein sorting and targeting of ligands towards the endosomes and lysosomes. ² The sortilin signal peptide (Figure 4.1.2) is 33 amino acids long which is 8 amino acids longer than CD4. Analyzing the difference between sortilin and CD4, as mentioned in chapter one, could help in the process of functionalizing CADA analogs to be target specific towards other signal peptides.

![Figure 4.1.2. The signal peptide sequence of sortilin.²](image)

### 4.1.2 Metal-Based Therapeutics

Metal based drugs have been utilized for treatment of diseases for decades. The discovery of cisplatin, shown in Figure 4.1.3, in the 1960s changed the way platinum compounds were utilized in new therapeutics for multiple malignancies.³ Metal complexes and compounds have unique properties that allow them to be manipulated into biologically active agents. Through an array of coordination geometries, structural modifications can be made, and various ligands can be attached, ultimately modifying the properties of these metal-based...
compounds and complexes. This gives them unique shapes and properties that conventional carbon-based compounds cannot achieve.\textsuperscript{4, 5} Metal complexes can form charged species in aqueous solutions that could potentially interact with charged amino acids, creating ionic interactions that have high binding affinity. The ability for transition metals to have varying oxidation states, due to their d shell electronics, allow them to affect the electronic and magnetic properties of the complex or metal-based compound.

Copper-based compounds for pharmaceutical applications have been growing in interest. They have been shown to have antimicrobial, anti-inflammatory, antiviral, and anti-tumor properties. Copper has been shown to be non-toxic and its trace elements have been shown to be essential to sustaining life.\textsuperscript{6} Copper ions are typically found to exist as two oxidation states, 1\textsuperscript{+} and 2\textsuperscript{+}. They are known to rapidly switch between oxidation states under biological conditions. Changes in the oxidation state can change the geometry in which the copper complex is found. Copper(I) typically takes on a tetrahedral geometry, while copper(II) can be found as square planer, trigonal bipyramidal, or octahedral. Utilizing copper(II) with various ligands allows for a variety of structures to be synthesized.\textsuperscript{7}

Copper’s diverse coordination chemistry and redox activity make it a suitable cofactor in enzymes, such as Cu/Zn superoxide dismutase (SOD). Studies have been conducted showing the anti-inflammatory properties of copper is due to its ability to neutralize reactive oxygen species and modulation of prostaglandin synthesis, much like SOD.\textsuperscript{8} Copper complexes have also been found to induce cellular apoptosis or cell death. The environment of the tumor cells selectively makes copper toxic since it reduces
copper(II) to copper(I), causing DNA breakage and oxidative stress within the cancer cell. This ultimately leads to cellular apoptosis. However, copper is an essential trace metal and leakage of copper through metal-based therapeutics is less toxic than other metals due to the ability of the body to remove excess copper, thus making it an ideal metal as a therapeutic agent.

Gold-based drugs have been used to treat illnesses such as tuberculosis, and rheumatoid arthritis. The drug auranofin (Figure 4.1.4) was introduced in 1985 for the treatment of rheumatoid arthritis. Gold complexes were also introduced as potential anticancer agents. Gold can exist in a variety of oxidation states (-I, 0, I, II, III, IV, and V). Only gold(0), gold(I), and gold(III) are stable in aqueous and biological environments.

Gold(I) and gold(III) are less stable than gold(0) and can be readily reduced by mild reducing agents. Gold(III) ions are known to interact with the sulfur in methionine and cysteine containing amino acids, as well as ribonuclease A, which could cause DNA damage similarly to cisplatin.

![Figure 4.1.4. Structure of auranofin.](image)

### 4.1.3 Targeting gp160 through Copper and Gold Based Compounds

Analysis of other signal peptides has sparked additional interest as possible new targets for CADA analogs. One specific signal peptide that has interesting characteristics is the signal peptide of gp160. The gp160 polyprotein is known as the envelope protein of
HIV. Intracellular cleavage of gp160 forms gp120 and gp41, which are integral parts needed by HIV in order to infect the host’s cells. The newly formed gp120 and gp41 is transported to the plasma membrane of the infected cell and viral budding causes the proteins to form on the outer surface of the mature virion. The signal peptide is found to be 32 amino acids long, as shown in Figure 4.1.5. It has two methionine residues that make it a potential target for drug design. The methionine residues at positions 20 and 24 are in the hydrophobic region of the signal peptide, which means they are embedded in the alpha helical segment of the signal peptide (Figure 4.1.6). In an alpha helix, a single turn is about 3.6 amino acid residues per turn. With the signal peptide of gp160 containing a methionine at position 20 and 24, they are 4 amino acids away from each other in the alpha helix. This makes it so that the two methionine residues are roughly on the same side of the alpha helix, which can be potential binding sites for a CADA compound. It is believed that a CADA type compound with a copper or gold core could form metal-sulfur bonds with the sulfur atoms of the methionine residues, possibly inhibiting the translocation, and

\[
\begin{align*}
\text{MRKVE}^{5}\text{KYQHL}^{10}\text{WRWGW}^{15}\text{R} \\
\text{N-region} \\
\text{WGTM}^{20}\text{LLGML}^{25}\text{MI} \text{ CSA}^{30}\text{TE} \text{ K LW}^{35}\text{VT} \\
\text{H-region} \quad \text{C-Region} \quad \text{Mature Protein}
\end{align*}
\]

Figure 4.1.5. The signal peptide sequence of gp160.
causing the down-modulation of gp160. These proposed compounds, shown in Figure 4.1.7, would have ligands comprised of open-chained disulfonamide compounds, which would give more access to the metal core than the macrocyclic CADA compounds. The sulfonamide nitrogens of CADA compounds are not basic enough to be good ligands for

Figure 4.1.6. positions of methionine residues (M) in the alpha helical hydrophobic region of gp160 by Tom Bell.

Figure 4.1.7. Newly proposed copper based disulfonamides for gp160 down-modulation.
metal complexing, while the open chain disulfonamide nitrogens can be deprotonated, making the resulting anions good ligands for metal complexing.

4.1.4 Targeting the Thyroid Stimulating Hormone Receptor

The thyroid stimulating hormone receptor (TSHR) is a cell surface protein found to help regulate thyroid growth and thyroid hormone production and secretion.\textsuperscript{12} It is a G-protein-coupled receptor with seven transmembrane domains.\textsuperscript{12} TSHR autoantibodies (TSHRAbs) increase cyclic adenosine monophosphate (cAMP) levels that block TSHRAbs which inhibit thyroid stimulating hormone (TSH) binding, TSH-increased adenylate cyclase activity, and stimulating TSHRAbs activity.\textsuperscript{12} The former activities can be found in patients with Graves’ disease and are often associated with hyperthyroidism and the latter activity can be found in patients with Hashimoto’s disease or idiopathic myxedema and is associated with hypothyroidism.\textsuperscript{14}

TSHR is mainly expressed in the thyroid but can be found to also be expressed in adipocytes, fibroblasts, bone cells, and other sites including the heart.\textsuperscript{14} It has a 20 amino acid signal peptide, as shown in Figure 4.1.8. There are charged amino acids, including the arginine at position 2, and the aspartic acids at positions 5, 14, and 18. The glutamine at position 8 in the hydrophobic region can be utilized to bind with one side arm of the CADA analog much like CD4. The aspartic acid at position 14 is placed in the alpha helix 6

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{signal_peptide.png}
\caption{The signal peptide sequence of TSHR.}
\end{figure}
residues away from the glutamine, which would put the two amino acids near each other, similarly to the two methionine residues of gp160. The distance from the glutamine to the aspartic acid is believed to span the distance of the two side arms of CADA. This would allow the binding of one side arm of CADA to the glutamine and the second side arm to the aspartate or aspartic acid by modifying the side arm of CADA. The aspartic acids in the signal peptide could potentially bind with a CADA analog through hydrogen bonding or form a salt bridge with the aspartate. An analog with a hydrogen bond donating side arm, as shown in Figure 4.1.9, could potentially form a salt bridge, binding CADA to the oxygens of the aspartates. Compounds containing nitrogen heterocycles, which would allow hydrogen bonding, have also been proposed in Figure 4.1.10 to possibly target TSHR. Recently, two CADA analogs have been shown to be good TSHR down-modulators but are not CD4 sensitive. The one-armed CADA analogs, \textbf{CK075} and
**Figure 4.1.10.** Nitrogen containing side arm analogs for TSHR down-modulation

VGDO40, shown in Figure 4.1.11 are thought to bind mainly with the aspartate (D14) in the hydrophobic region of the signal peptide. A one-armed CADA analog, TL048, containing the 4-dimethylaminobenzensulfonamide side arm was proposed. It is hypothesized that this analog might be a better TSHR down-modulating compound because it could also bind strongly to the glutamine (Q8) in the H-region of the signal peptide.

**Figure 4.1.11.** Structures of CK075, VGDO40, and TL048.
4.2 Results and Discussion

4.2.1 Synthesis of Copper and Gold Compounds for gp160 Down-Modulation

Synthesis of copper-containing compounds were attempted using CuBr₂ with various disulfonamides as shown in Scheme 4.2.1. Compound TL006 was treated with CuBr₂ in the presence of NH₄OH in MeOH, however the reaction did not seem to work. The open chain disulfonamide 24 was also tested and did not yield the desired product. Additional attempts at making the desired copper compounds were conducted using copper(II) perchlorate in the presence of triethylamine as a base with TL006 and compound 24, as shown in Scheme 4.2.2, however this also failed to yield the desired product.

The synthesis of the copper disulfonamide compounds through these two methods were shown to be unsuccessful. Alternative routes for making these copper complexes utilized aqueous solutions,¹⁵,¹⁶ which was unfeasible due to the poor solubilities of the starting materials.
disulfonamides in aqueous solutions. The use of copper(II) also made \(^1\)H NMR analysis difficult, since copper(II) is paramagnetic, giving extremely broad spectra. It was determined that copper(II) complexes might not be ideal target compounds for the metal-based targeting of gp160. A change in the type of metal targets were then considered, so it was determined that gold(III) would be utilized rather than copper(II).

Attempts were made to create the gold(III) complexes using gold(III) chloride. The reaction was carried out with compound \(24\) in chloroform, as shown in scheme 4.2.3, but no complex was found. It was thought that the reaction conditions were not basic enough to deprotonate the sulfonamides in order to complex with the gold. So, an attempt was conducted by adding a weak base (K\(_2\)CO\(_3\)) to the reaction mixture, but this did not seem to work either. A literature search presented reactions that were aqueous,\(^{17,18}\) however the open-chain disulfonamides \(24\) and TL006 were both insoluble in aqueous solutions. So a new disulfonamide was considered for complexing gold(III).

\[ \text{Scheme 4.2.3. Synthesis of copper compounds using AuCl}_3. \]
4.2.2 Synthesis of Carboxysulfonamide Compounds for gp160 Down-Modulation

A new disulfonamide was considered in order to increase the likely hood of gold (III) complexing to the compound. The new disulfonamide would also contain a carbonyl group alpha to the sulfonamide nitrogens, as shown in Figure 4.2.1. This would decrease the electron density and readily release the proton of the sulfonamide, allowing for the gold to complex with the new carboxydisulfonamide. The initial synthesis route, shown in Scheme 4.2.4, starts with the primary amine containing the tail group, which is treated with methyl acrylate to produce compound TL030. Tosylation using 4-methylbenzenesulfonamide and titanium(IV) chloride in 1,1,2,2-tetrachloroethane would produce the resulting disulfonamide. The formation of the diester was conducted using cyclohexylmethylamine to produce compound TL030 in 99% yield. Attempts at making the disulfonamide using 4-methylbenzenesulfonamide and titanium(IV) chloride did not yield the required product.

![Figure 4.2.1. Structure of the new disulfonamide.](image)

![Scheme 4.2.4. Initial synthesis route of the carboxydisulfonamide.](image)
Modification of the initial synthesis route was evaluated, and a new method was devised, as shown in Scheme 4.2.5. This method involves hydrolysis of the ester TL030 to the carboxylic acid, followed by reaction with \( p \)-tosyl isocyanate to produce the carboxy sulfonamide 32. Initial attempts at reducing the diester to the dicarboxylic acid using LiOH failed to produce the resulting dicarboxylic acid. So, hydrolysis using NaOH in MeOH/H\( \text{H}_2\text{O} \) (2:1, v/v) was conducted. This resulted in formation of the carboxylic salt. The carboxylic acid could not be isolated due to the basicity of the tertiary nitrogen. It was also believed that utilizing the carboxylate salt form would reduce the need to use triethylamine in the following reaction. Attempts at tosylation of the TL031 with \( p \)-tosyl isocyanate were not successful.

\[
\begin{align*}
\text{O} & \quad \text{O} & \quad \text{N} & \quad \text{O} & \quad \text{O} \\
\text{Me} & \quad \text{Me} & \quad \text{TL030} & \quad \text{LiOH} & \quad 1:1 \text{THF/H}_2\text{O} \\
\text{O} & \quad \text{OH} & \quad \text{TL031} & \quad \text{p-tosyl isocyanate} & \quad \text{TEA} \\
\text{N} & \quad \text{TL031} & \quad \text{TEA} & \quad \text{THF} & \quad 32
\end{align*}
\]

**Scheme 4.2.5.** New synthesis route for compound 32.

**4.2.3 Synthesis of Sulfonyl Chlorides**

New CADA analogs were proposed for TSHR down-modulation. These new analogs contain the N(Me)\(_2\) substituted side arm and a second side arm containing various polycyclic amino side arms, as well as guanidine and amidine side arms. Prior to synthesizing these CADA analogs, the polycyclic amino sulfonyl chlorides were needed to be synthesized. Anthranil was treated with chlorosulfuric acid to produce
benzo[c]isoxazole-5-sulfonyl chloride (34) as shown in Scheme 4.2.6. 2-Amino-1H-benzo[d]imidazole-5-sulfonyl chloride (33) was successfully synthesized by reaction of 1H-benzo[d]imidazol-2-amine with chlorosulfonic acid with a yield of 53%. 2-Aminopyridine was treated with chlorosulfonic acid at reflux to produce 6-aminopyridine-3-sulfonyl chloride (35) in 32% yield. 4-(2-Oxopyrrolidin-1-yl)benzenesulfonyl chloride was synthesized by reaction of 1-phenyl-2-pyrrolidinone with chlorosulfonic acid. This produced the sulfonyl chloride in 91% yield. The sulfonyl chloride, 1-methyl-2-oxoindoline-5-sulfonyl chloride (37), was synthesized by reaction of 1-methylindolin-2-one with chlorosulfonic acid at r.t. This produced the resulting sulfonyl chloride at 77%. The 4-cyanobenzenesulfonyl chloride (38) was synthesized from 4-aminobenzonitrile in 45% yield. With these sulfonyl chlorides synthesized, the CADA analogs could be synthesized.

Scheme 4.2.6. Synthesis of various sulfonyl chlorides for TSHR down-modulation
4.2.4 Synthesis of CADA Analogs for TSHR Down-Modulation

The synthesized sulfonyl chlorides were utilized to form open-chain disulfonamides prior to the palladium-catalyzed cyclization. To synthesize the disulfonamides, the primary amine 29 was treated with the various sulfonyl chlorides, as shown in Scheme 4.2.7. Using this method, compounds TL049, TL051, TL054, and TL055, shown in Figure 4.2.2, were successfully synthesized with yields of 70%, 92%, 95%, and 99% respectively. Attempts at making disulfonamides using 6-aminopyridine-3-
sulfonyl chloride and 2-amino-1H-benzo[d]imidazole-5-sulfonyl chloride gave no reaction when compound 29 was treated with the sulfonyl chlorides. This could be due to the presence of the primary amines in compounds 33 and 35, which could result in polymerization of the sulfonyl chlorides instead of an addition reaction to compound 29 to form the open-chain disulfonamide. Literature search showed two alternative methods for making the sulfonamide using 6-aminopyridine-3-sulfonyl chloride and 2-amino-1H-benzo[d]imidazole-5-sulfonyl chloride. An attempt was done using 6-aminopyridine-3-sulfonyl chloride, as shown in Scheme 4.2.8. Diisopropylethylamine was used as a base to deprotonate the primary amine 29, which was intended to react with the sulfonyl chloride to give compound 39, however this resulted in no reaction as well. It was initially thought that the 2-amino-1H-benzo[d]imidazole-5-sulfonyl chloride had poor solubility with DCM, so an attempt was made by using a DMF/DCM solution. The sulfonyl chloride was found to fully dissolve into the solution, however this still resulted in no reaction between compound 29 and 2-amino-1H-benzo[d]imidazole-5-sulfonyl chloride as well.
4.2.5 Synthesis of CADA Analogs Towards TSHR Down-Modulation

With the synthesis of the four open-chain disulfonamide compounds completed, the palladium catalyzed cyclization with di-tert-butyl (2-methylene-propane-1,3-diyl) bis(carbonate) was conducted. An attempt at the cyclization of TL049, as shown in Scheme 4.2.9, resulted in no reaction. Attempted cyclization of TL051 also failed to form any desired product. Cyclization of TL054 and TL055 was also successfully conducted, as shown in Scheme 4.2.10, with a yield of 20% and 28% respectively.

![Scheme 4.2.9. Attempted cyclization of TL049.]

![Scheme 4.2.10. Cyclization of TL054 and TL055.]

4.2.6 Synthesis of Guanidine CADA Analogs

With the synthesis of compound TL050 and TL047 completed, modifications to these two compounds were attempted to add on the guanidine side arms and other sulfonamide side arms. To add the side arms that were previously used in compounds unable to be cyclized by the palladium catalyzed process, TL047 would undergo deprotection of the 2-nosyl group. Deprotection was attempted using mercaptoethanol, DBU, and DMF.\textsuperscript{19} The deprotection mechanism apparently involves the formation of a Meisenheimer complex as shown in Scheme 4.2.11.\textsuperscript{20,21} This would form the one-armed CADA compound TL048. Attempts at the deprotection using the mercaptoethanol showed evidence for the formation of the product, however there was an inseparable side product that was also present. A procedure for cleavage of the nitrobenzenesulfonamide is being tested, which uses \textit{p}-mercaptobenzoic acid and K\textsubscript{2}CO\textsubscript{3}.\textsuperscript{22} The compound TL050 should undergo reduction with Raney nickel and potassium borohydride to form a primary amine side arm. The reduction with Raney nickel was attempted, however there seemed to be no reaction as the starting material was recovered after workup. Further investigation of this reduction is needed.

![Scheme 4.2.11. Deprotection of 2-nosylamides.](image)
4.3 Conclusion and Outlook

The synthesis of copper(II) complexes were unsuccessful, so gold(III) complexes were proposed. Attempts at forming the gold(III) complexes lead to the proposal of a new dicarboxysulfonamide product. Synthesis of the diester compound TL030 was successfully conducted. Reduction of the diester to the dicarboxylic acid was done with NaOH, however the isolation of the product as a free base is still being investigated. Six different sulfonyl chlorides were synthesized to make compounds that would target the signal peptide of TSHR. Of the six compounds, only four were able to be synthesized as open chain disulfonamides. From the four open chain disulfonamides, only two were successfully underwent the palladium catalyzed cyclization to form compounds TL050 and TL056. Attempts at conducting a reduction of compound TL050 from the nitrile to a primary amine were unsuccessful and is still being investigated. Once the primary amine is formed, it should undergo reactions with the Boc protected guanidine compound to form the guanidine CADA analog. Modification of TL047 to form a single sulfonamide CADA analog using mercaptoethanol was also unsuccessful. The deprotection of the nosyl group of TL047 will be attempted with p-mercaptobenzoic acid and K₂CO₃. Once the one-armed CADA analog is formed, the addition of the sulfonyl chlorides that were unsuccessful at the palladium cyclization could be added, as well as the guanidine functional group. These compounds would then be sent to collaborators for biological studies with TSHR.
4.4 Experimental

General Methods

All reactions were performed under an atmosphere of dry nitrogen, unless specified otherwise. Reagents and solvents purchased from Aldrich Chemical Company, Acros Organics, or Fisher Scientific were of ACS reagent grade or better and were used without purification, unless indicated otherwise. Anhydrous acetonitrile used in the macrocyclization step was distilled from CaH$_2$. For macrocyclization reactions, the disulfonamide intermediates, 2-methylene-1,3-propanebis(tert-butylcarbonate), anhydrous sodium carbonate, dppb, and Pd$_2$(dba)$_3$ were dried in vacuo (ca. 0.1 mm) for at least 16 h. All the equipment required for macrocyclization reaction including a magnetic stir bar, spatula, syringe and needle were also dried overnight in the oven (110 °C). Solutions of 2 N HCl in methanol were created by placing 165 mL of 12.1 M HCl into a 1 L volumetric flask. The flask is then filled with 835 mL of methanol. Column chromatography was performed with Sorbent Technologies neutral alumina (50-200 μm) or Sorbent Technologies standard grade silica (32-63 μm), unless noted otherwise. Chromatotron chromatography was performed with Sorbent Technologies neutral alumina with gypsum and UV254. Automated chromatography was performed on the Yamazen Smart Flash AKROS RE-X10 with Sorbet Technologies neutral alumina (50-200 μ) or Sorbent Technologies standard grade silica (32-63 μm) and HPLC grade ethyl acetate, hexane, and dichloromethane (DCM). Compounds dried in vacuo were connected to a vacuum manifold with a Welch 1402 vacuum pump and vacuumed dried for at least 18 h at ca. 0.1 mm. Melting points were measured on a Thomas-Hoover or Mel-Temp apparatus and are
uncorrected. $^1$H NMR (400 MHz or 500 MHz) and $^{13}$C NMR (75 MHz or 125 MHz) spectra were acquired on a Varian 400 or Varian Unity + 500 spectrometer. All chemical shifts (δ) are reported in ppm units relative to solvent resonances, as follows: $^1$H, CDCl$_3$/TMS = 0.00, DMSO-d$_6$ = 2.50, CD$_3$OD = 3.31; $^{13}$C, CDCl$_3$ = 77.23, DMSO-d$_6$ = 39.7, CD$_3$OD = 49.15. Infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrometer. Mass spectra (MS) were acquired on a Waters Micromass ZQ electrospray ionization quadrupole mass spectrometer with positive ion detection (capillary voltage = 3.5 kV). High-resolution mass spectra (HRMS) were acquired on an Agilent 6230 TOF mass spectrometer. Samples for elemental analysis were dried at 78 °C (0.1 mm) for 2 days, unless stated otherwise, and microanalysis was performed by NuMega Resonance Labs, Inc.

**Synthesis of dimethyl 3,3'-((cyclohexylmethyl)azanediyl)dipropionate (TL030)**

To a 250 mL round bottom flask, 19.2 mL (213 mmol) of methyl acrylate and 48 mL of MeOH were added and stirred at r.t. Then 6.25 g (55.2 mmol) of cyclohexylmethylamine in 8 mL of MeOH was added dropwise. The solution was stirred and heated under reflux overnight. The resulting solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 15.3 g (97%) of dimethyl 3,3'-((cyclohexylmethyl)azanediyl)dipropionate as a clear oil. $^1$H NMR (400
MHz, CDCl\textsubscript{3}) \delta 3.63 (s, 6 H, CO\textsubscript{2}CH\textsubscript{3}), 2.69 (t, 7.1 Hz, 4 H, CH\textsubscript{2}CO\textsubscript{2}), 2.39 (t, 7.1 Hz, 4 H, CH\textsubscript{2}NCH\textsubscript{2}), 2.12 (d, 7.1 Hz, 2 H, CH\textsubscript{2}Cy), 1.65 (m, 6 H, CCH\textsubscript{2}C, Cy) 1.36 (m, 1 H, CH), 1.16 (m, 3 H, Cy), 0.75 (m, 2 H, Cy). \textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) \delta 173.1, 77.3, 77.0, 76.7, 61.2, 51.4, 49.9, 35.9, 32.6, 31.6, 26.8, 26.1. IR (neat cm\textsuperscript{-1}) 2920 (m), 2848 (m), 1734 (s), 1435 (m), 1354 (m), 1192 (s), 1170 (s), 1114 (m), 1081 (w), 1038 (m), 891 (w), 840 (m), 791 (w), 707 (w), 654 (w), 610 (w). MS (ESI\textsuperscript{+}) m/z 286 (MH\textsuperscript{+}). Anal. Calcd for C\textsubscript{15}H\textsubscript{27}NO\textsubscript{4}: C, 63.13; H, 9.54; N, 4.91. Found: C, 62.76; H, 9.91; N, 5.17.

**Synthesis of benzo[c]isoxazole-5-sulfonyl chloride\textsuperscript{24}**

A 250 mL round bottom flask containing 5.60 mL of chlorosulfonic acid was cooled to 0 °C. Then 0.942 g (7.91 mmol) of anthranil was slowly added over 20 mins. The solution was heated to 100 °C and stirred at that temperature for 27 h. The solution was diluted with 25 mL of DCM and slowly poured into 50 mL of ice water. The mixture was extracted with DCM (3 x 25 mL). The combined extraction layers were washed with H\textsubscript{2}O (3 x 30 mL). The organic layer was dried (MgSO\textsubscript{4}) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 1.49 g (66%) of benzo[c]isoxazole-5-sulfonyl chloride as a red solid. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \delta 9.48 (s, 1 H, C=CH), 8.14 (dd, 7.0 Hz, 0.9 Hz, 1 H, 6- ArSO\textsubscript{2}Cl), 8.06 (dd, 8.7 Hz, 0.9 Hz, 1 H, 5- ArSO\textsubscript{2}Cl), 7.24 (m, 1 H, 1-ArSO\textsubscript{2}Cl).
**Synthesis of 2-amino-1H-benzo[d]imidazole-5-sulfonyl chloride**

To a 250 mL round bottom flask containing 1.00 g (7.51 mmol) of 2-aminobenzimidazole, was added 6.20 mL (93.1 mmol) of chlorosulfonic acid at r.t. and stirred for 24 h. The mixture was slowly poured on to a mixture of ice and NaCl. The resulting mixture was filtered and washed with 100 mL diethyl ether. The solids were dried under *in vacuo*. This produced 0.926 g (53%) of 2-amino-1H-benzo[d]imidazole-5-sulfonyl chloride as a red solid. $^1$H (400 Mhz, DMSO-$d_6$) δ 12.50 (d, 24.3 Hz, 1 H, NH), 8.48 (br s, 2 H, NH$_2$), 7.56 (dd, 1.5 Hz, 0.6 Hz, 1 H, 1-ArSO$_2$Cl), 7.46 (dd, 8.3 Hz, 1.5 Hz, 1 H, 6-ArSO$_2$Cl), 7.26 (dd, 8.3 Hz, 0.6 Hz, 1 H, 5-ArSO$_2$Cl).

**Synthesis of 6-aminopyridine-3-sulfonyl chloride**

To a 250 mL round bottom flask containing 18 mL (270. mmol) of chlorosulfonic acid at 0 °C, 3.03 g (32.2 mmol) of 2-aminopyridine was added portion wise. The mixture was heated under reflux and stirred for 2 h. The hot mixture was poured onto 600 mL of ice. The solution was carefully neutralized by slowly adding solid NaHCO$_3$. The resulting mixture was extracted with ethyl acetate (3 × 200 mL). The organic layer was dried (MgSO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting
residue was dried in vacuo. The product was purified by recrystallization using heptane/ether (1:1, v/v) mixture. This produced 2.08 g (33%) of 6-aminopyridine-3-sulfonyl chloride as a white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.67 (dd, 2.5 Hz, 0.7 Hz, 1 H, $o$-ArSO$_2$Cl), 7.93 (dd, 9.0 Hz, 2.6 Hz, 1 H, $o$-ArSO$_2$Cl), 6.54 (dd, 9.0 Hz, 0.7 Hz, 1 H, $m$-ArSO$_2$Cl), 5.37 (s, 2 H, NH$_2$).

Synthesis of 4-(2-oxopyrrolidin-1-yl)benzenesulfonyl chloride$^{25}$

To a 100 mL round bottom flask containing 1.00 g (6.20 mmol) of 1-phenyl-2-pyrrolidinone, 4.70 mL (69.8 mmol) of chlorosulfonic acid was slowly added and stirred at r.t. for 18 h. The reaction mixture was slowly added to a 500 mL beaker containing ice and NaCl and then extracted with ethyl acetate (4 x 50 mL). The combined extraction layers were dried (MgSO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 1.46 g (91%) of 4-(2-oxopyrrolidin-1-yl)benzenesulfonyl chloride as a tan solid. $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 8.02 (m, 4 H, ArSO$_2$Cl), 3.97 (m, 2 H, NC(O)CH$_2$), 2.64 (m, 2 H, NCH$_2$), 2.19 (m, 2 H, CCH$_2$C).
Synthesis of 1-methyl-2-oxoindoline-5-sulfonyl chloride\textsuperscript{25}

To a 250 mL round bottom flask, 1.00 g (6.79 mmol) of 1-methyl-2-oxindole was added and cooled to 0 °C. Then 2.25 mL of chlorosulfonic acid was added dropwise and the mixture was stirred at r.t. for 3 h. The solution was poured onto ice and the resulting mixture was filtered and washed with H\textsubscript{2}O (3 x 20 mL). The product was dried \textit{in vacuo} to produce 1.28 g (77%) of 1-methyl-2-oxoindoline-5-sulfonyl chloride. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.02 (m, 1 H, o-ArSO\textsubscript{2}Cl), 7.88 (m, 1 H, o-ArSO\textsubscript{2}Cl), 6.95 (m, 1 H, m-ArSO\textsubscript{2}Cl), 3.63 (s, 2 H, CH\textsubscript{2}), 3.27 (s, 3 H, CH\textsubscript{3}).

Synthesis of 4-cyanobenzene sulfonyl chloride\textsuperscript{27}

Formation of sulfur dioxide solution

To a 1L three-neck round bottom flask with addition funnel and glass stoppers, 236 mL of H\textsubscript{2}O was added and cooled to 0 °C. Then 40 mL (551 mmol) of thionyl chloride was placed into the addition funnel and added dropwise over 1 h. The temperature was maintained at about 0 °C. Upon completion of the addition, the solution was slowly warmed to r.t. Then 145 mg (1.46 mmol) of copper(I) chloride was added and the solution was
cooled to 0 °C and stirred. The solution was stirred for 4 h and turned to a yellow-green color.

**Diazonium salt formation and conversion to 4-cyanobenzenesulfonyl chloride**

In a 100 mL Erlenmeyer flask was added 9.5 g (112 mmol) of NaNO₃ and 38 mL of H₂O. The mixture was stirred vigorously for 10 mins. Once the solids were dissolved, the solution was placed into the refrigerator for later use. In a 500 mL two-neck round bottom flask was added 15.0 g (127 mmol) of 4-aminobenzonitrile. To this mixture was added 128 mL of conc. HCl dropwise while stirring vigorously. The mixture was heated to 54 °C in an oil bath for 40 mins. The reaction was cooled to r.t. then cooled to -5 °C. The sodium nitrite solution was then added dropwise at -5 °C. Once the addition was completed, the mixture was stirred for an additional 30 mins. The temperature of the mixture was kept at -5 °C and the sulfur dioxide solution was slowly added while stirring. The solution was agitated at 0 °C for 75 minutes after the addition was completed. The mixture was then filtered and washed with H₂O (3 x 50 mL). The solids were then dissolved in 100 mL of hot DCM, filtered, and washed with hot DCM (2 x 25 mL). Hot hexane was added to the filtrate until it became turbid. The flask containing the filtrate was placed in the freezer overnight. The mixture was filtered and washed with cold hexane. The filtrate was concentrated by rotary evaporation and the resulting residue was dried *in vacuo*. This produced 11.6 g (45%) of 4-cyanobenzene sulfonyl chloride as a tan colored solid. ¹H NMR (400 MHz, CDCl₃) δ 8.16 (m, 2 H, o-ArSO₂Cl), 7.92 (m, 2 H, m-ArSO₂Cl).
Synthesis of $N'$-(p-dimethylaminobenzenesulfonyl)-$N''$-(benzo[c]isoxazole-5-sulfonyl)-[$N,N$-bis(3-aminopropyl)cyclohexylmethylamine] (TL049)$^{28}$

![Chemical Structure]

To a 250 mL round bottom flask, 2.06 g (5.03 mmol) of $N$-(3-aminopropyl)-$N$-(3-p-dimethylaminobenzenesulfonyl)benzylamine, 2.03 g (9.16mmol) of 2-nitrobenzenesulfonyl chloride, 25 mL of sat. aq. NaCl solution, 25 mL of sat. aq. Na$_2$CO$_3$ solution, and 25 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 25 mL), the combined extraction layers were dried (Na$_2$SO$_4$), and filtered. The filtrate was then concentrated, and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were triturated with diethyl ether (3 x 25 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 2.07 g (70%) of
$N$-(3-((cyclohexylmethyl)(3-((4-(dimethylamino)phenyl)sulfonamido)propyl)amino)propyl)benzo[c]isoxazole-5-
sulfonamide as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.34 (s, 1 H, CHCO),
7.98 (dd, 6.8 Hz, 0.9 Hz, 1 H, 6-ArSO$_2$), 7.84 (dd, 8.8 Hz, 0.9 Hz, 1 H 5-ArSO$_2$), 7.66 (m,
2 H, o-ArSO$_2$), 7.16 (dd, 8.7 Hz, 6.8 Hz, 1 H, 1-ArSO$_2$), 6.88 (m, 2 H, m-ArSO$_2$), 3.04 (m,
8 H, N(CH$_3$)$_2$ NCH$_2$), 2.92 (m, 2 H, NCH$_2$), 2.32 (m, 4 H, CH$_2$N), 2.03 (d, 7.0 Hz, 2 H,
CH$_2$Cy), 1.56 (m, 2 H, Cy), 1.20 (m, 8 H, CH$_2$Cy, Cy), 0.76 (m, 2 H, Cy). $^{13}$C NMR (101
MHz, CDCl$_3$) $\delta$ 156.4, 152.7, 150.8, 133.6, 128.8, 126.7, 125.2, 125.2, 123.6, 119.4, 111.0,
110.9, 61.8, 53.3, 52.7, 42.6, 40.1, 35.6, 31.9, 26.6, 26.1, 26.0, 25.8. IR (neat cm$^{-1}$) 2925
(w), 2851 (w), 2434 (w), 1632 (w), 1596 (w), 1444 (w), 1379 (w), 1325 (m), 1216 (w),
1161 (m), 1145 (m), 1113 (m), 1092 (m), 979 (w), 898 (w), 823 (m), 746 (m), 654 (m),
635 (m), 614 (m). MS (ESI$^+$) $m/z$ 592 (MH$^+$), 593 (MH$^+$ +1). Anal. Calcd for
C$_{28}$H$_{41}$N$_5$O$_5$S$_2$·HCl·0.5H$_2$O: C, 52.77; H, 6.80; N, 10.99. Found: C, 52.93; H, 6.77; N,
10.65
Synthesis of $N'$-(p-dimethylaminobenzenesulfonyl)-$N''$-(1-methyl-2-oxoindoline-5-sulfonyl)-[$N,N$-bis(3-aminopropyl)cyclohexylmethylamine] (TL051)$^{28}$

To a 250 mL round bottom flask, 0.931 g (2.27 mmol) of $N$-(3-aminopropyl)-$N$-(3-p-dimethylaminobenzenesulfonamidopropyl)benzylamine, 0.613 g (2.50 mmol) of 1-methyl-2-oxoindoline-5-sulfonyl chloride, 25 mL of sat. aq. NaCl solution, 25 mL of sat. aq. Na$_2$CO$_3$ solution, and 25 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 25 mL), the combined extraction layers were dried (Na$_2$SO$_4$), and filtered. The filtrate was then concentrated, and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were triturated with diethyl ether (3 x 25 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated
by rotary evaporation and the resulting residue was dried \textit{in vacuo}. This produced 1.28 g (92%) of \(N'-(p\text{-dimethylaminobenzenesulfonyl})-N''-(1\text{-methyl-2-oxoindoline-5-sulfonyl})-\[N,N\text{-bis(3-aminopropyl)cyclohexylmethylamine}\] as a yellow viscous oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.83 (m, 1 H, 1-ArSO\(_2\)), 7.73 (m, 1 H, 6-ArSO\(_2\)), 7.64 (m, 2 H, o-ArSO\(_2\)), 6.89 (m, 1 H, 5-ArSO\(_2\)), 6.65 (m, 2 H, m-ArSO\(_2\)), 3.57 (m, 2 H, CCH\(_2\)C), 3.23 (s, 3 H, NCH\(_3\)), 3.03 (m, 10 H, N(CH\(_3\))\(_2\), NCH\(_2\)), 2.38 (m, 4 H, CH\(_2\)N), 2.02 (d, 6.9 Hz, 2 H, CH\(_2\)Cy), 1.61 (m, 10 H, CH\(_2\)Cy, Cy), 1.35 (m, 1 H, CH), 1.14 (m, 4 H, Cy), 0.79 (m, 2 H, Cy). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 174.8, 152.7, 148.8, 133.7, 128.8, 128.7, 128.3, 125.1, 124.9, 123.3, 110.9, 107.8, 62.0, 53.1, 42.57, 42.51, 40.1, 35.6, 35.4, 31.9, 26.6, 26.4, 26.1, 26.0, 25.8. IR (neat cm\(^{-1}\)) 2923 (w), 2850 (w), 2501 (w), 1712 (w), 1608 (w), 1595 (w), 1516 (w), 1492 (w), 1447 (w), 1368 (w), 1314 (m), 1264 (w), 1196 (w), 1147 (m), 1093 (m), 1058 (m), 997 (w), 941 (w), 917 (w), 817 (w), 750 (w), 725 (w), 649 (m), 629 (w), 607 (w). MS (ESI\(^+\)) \(m/z\) 620 (MH\(^+\)), 621 (MH\(^+\)+1).
Synthesis of N’-(p-dimethylaminobenzenesulfonyl)-N’-(4-cyanosulfonyl)-[N,N-bis(3-aminopropyl)cyclohexylmethylamine] (TL054)\textsuperscript{28}

To a 250 mL round bottom flask, 5.09 g (12.4 mmol) of N-(3-aminopropyl)-N-(3-p-dimethylaminobenzenesulfonamidopropyl)benzylamine, 2.75 g (13.6 mmol) of 4-cyanobenzenesulfonyl chloride, 35 mL of sat. aq. NaCl solution, 35 mL of sat. aq. Na\textsubscript{2}CO\textsubscript{3} solution, and 35 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 25 mL), the combined extraction layers were dried (Na\textsubscript{2}SO\textsubscript{4}), and filtered. The filtrate was then concentrated, and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The solids were tritutated with diethyl ether (3 x 25 mL) and the resulting residue was dried \textit{in vacuo}. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na\textsubscript{2}SO\textsubscript{4}) and filtered. The filtrate was concentrated by rotary
evaporation and the resulting residue was dried in vacuo. This produced 6.88 g (95%) of 
$N'$-(p-dimethylaminobenzenesulfonyl)-$N''$-(4-cyanosulfonyl)-[N,N'-bis(3-
aminopropyl)cyclohexylmethylamine] as a yellow viscous oil. $^1$H NMR (400 MHz, 
CDCl$_3$) $\delta$ 7.96 (m, 2 H, o-ArSO$_2$), 7.77 (m, 2 H, o-ArSO$_2$), 7.63 (m, 2 H, m-ArSO$_2$), 6.66 
(m, 2 H, m-ArSO$_2$), 3.02 (m, 8 H, N(CH$_3$)$_2$, CH$_2$N), 2.92 (t, 6.3 Hz, 2 H, CH$_2$N), 2.38 (t, 4 
H, CH$_2$N), 2.03 (m, 2 H, CH$_2$Cy), 1.63 (m, 8 H, CH$_2$Cy), 1.35 (m, 1 H, CH), 1.12 (m, 4 H, 
Cy), 0.77 (q, 11.4 Hz, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.8, 144.6, 133.1, 132.8, 
129.0, 128.9, 127.7, 124.7, 117.5, 115.9, 111.0, 77.3, 77.0, 76.7, 62.1, 53.2, 53.0, 42.8, 
42.4, 40.1, 35.6, 31.9, 26.5, 26.0, 25.8. IR (neat cm$^{-1}$) 2925 (w), 2851 (w), 2359 (w), 2232 
(w), 1672 (w), 1596 (w), 1447 (w), 1375 (w), 1328 (w), 1159 (m), 1129 (m), 1091 (m), 
971 (w), 944 (w), 899 (w), 836 (w), 786 (w), 699 (w), 655 (m), 629 (m), 616 (m). MS 
(ESI$^+$) $m/z$ 576 (MH$^+$), 577 (MH$^+$ +1), 578 (MH$^+$ +2). Anal. Calcd for C$_{28}$H$_{41}$N$_5$O$_4$S$_2$·HCl: 
C, 54.93; H, 6.91; N, 11.44. Found: C, 55.28; H, 7.13; N, 11.22.
Synthesis of $N'(p$-dimethylaminobenzenesulfonyl)$-N''$(4$(2$-oxopyrrolidin$1$-y)l)benzenesulfonyl)$-N,N-bis(3$-aminopropyl)cyclohexylmethylamine$ (TL055)$

To a 250 mL round bottom flask, 0.96 g (2.3 mmol) of $N$-$3$-aminopropyl)$-N$(3-$p$-dimethylaminobenzenesulfonamidopropyl)benzylamine, 0.67 g (2.6 mmol) of $4$-$2$-oxopyrrolidin$1$-y)l)benzenesulfonyl chloride, 15 mL of sat. aq. NaCl solution, 15 mL of sat. aq. Na$_2$CO$_3$ solution, and 15 mL of DCM were added. The mixture was stirred vigorously at r.t. for 24 h. The mixture was then placed in a separatory funnel and the organic layer was removed. The aqueous layer was extracted with DCM (3 x 25 mL), the combined extraction layers were dried (Na$_2$SO$_4$), and filtered. The filtrate was then concentrated, and the resulting residue was dried in vacuo. The product was converted to the HCl salt by stirring with 15 mL of a solution of 2 N HCl in MeOH and stirring for 1 h. The solution was concentrated by rotary evaporation and the resulting residue was dried in vacuo. The solids were triturated with diethyl ether (3 x 25 mL) and the resulting residue was dried in vacuo. The product was converted back to the free base by stirring vigorously with 15 mL of 2 N aq. NaOH solution, 15 mL of sat. aq. NaCl solution, and 15 mL of DCM for 1 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 15 mL). The combined organic layer was dried (Na$_2$SO$_4$) and filtered. The filtrate was
concentrated by rotary evaporation and the resulting residue was dried in vacuo. This produced 1.48 g (99%) of $N'-(p$-dimethylaminobenzenesulfonyl)$-N''$-(4-(2-oxopyrrolidin-1-yl)benzenesulfonyl)$-[N,N$-bis(3-aminopropyl)cyclohexylmethylamine] as a yellow viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.80 (m, 4 H, $o$-ArSO$_2$, $m$-ArSO$_2$), 7.63 (m, 2 H, $o$-ArSO$_2$), 6.65 (m, 2 H, $m$-ArSO$_2$), 3.88 (m, 2 H, CCH$_2$C), 3.02 (s, 6 H, N(CH$_3$)$_2$), 2.96 (m, 2 H, CH$_2$N), 2.84 (m, 2 H, CH$_2$N), 2.63 (m, 2 H, NCH$_2$C), 2.28 (m, 4 H, CH$_2$N), 2.17 (m, 2 H, CCH$_2$C), 2.02 (d, 6.9 Hz, 2 H, CH$_2$Cy), 1.62 (m, 8 H, CH$_2$Cy, Cy). 1.48 (m, 1 H, CH), 1.34 (m, 2 H, Cy), 1.13 (m, 4 H, Cy), 0.79 (m, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 175.0, 152.7, 142.8, 134.9, 128.8, 128.0, 125.1, 119.3, 119.0, 110.9, 61.8, 53.5, 52.7, 48.5, 43.0, 42.2, 40.1, 35.6, 32.8, 31.8, 26.6, 26.0, 25.6, 17.8. IR (neat cm$^{-1}$) 2923 (w), 2850 (w), 2388 (w), 1695 (w), 1592 (w), 1495 (w), 1449 (w), 1418 (w), 1387 (w), 1319 (w), 1221 (w), 1153 (w), 1128 (w), 1092 (w), 972 (w), 945 (w), 900 (w), 837 (w), 757 (w), 696 (w), 654 (w), 622 (w), 615 (w), 605 (w). MS (ESI$^+$) $m/z$ 634 (MH$^+$). Anal. Calcd for C$_{31}$H$_{47}$N$_5$O$_5$S$_2$·HCl·CH$_3$OH: C, 54.72; H, 7.46; N, 9.97. Found: C, 54.81; H, 7.19; N, 9.90.
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonfyl)-3-methylene-5-(4-cyanobenzenesulfonyl)-1,5,9-triazacyclododecane (TL050)\(^{28}\)

To a 1 L round bottom flask, 2.70 g (4.70 mmol) of \(N'(p\)-dimethylaminobenzenesulfonfyl)-\(N''\)-(4-cyanobenzenesulfonfyl)-[\(N,N\)-bis(3-aminopropyl)cyclohexylmethylamine], 6.31 g (21.9 mmol) of 2-methylene-1,3-propanebis(tert-butylcarbonate), 130 mg (1.23 mmol) of \(\text{Na}_2\text{CO}_3\), 260 mg (0.284 mmol) of \(\text{Pd}_2\text{dba}_3\), 264 mg (0.619 mmol) of dppb and 520 mL of anhydrous acetonitrile were stirred under \(\text{N}_2\) gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. \(\text{NaHCO}_3\) solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (\(\text{Na}_2\text{SO}_4\)) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried \textit{in vacuo}. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM.
(3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated, and the resulting residue was dried *in vacuo*. The product was purified by automated chromatography on neutral alumina, eluting with 3:7 (v/v) ethyl acetate/hexane. This produced 0.30 g (20%) of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(4-cyanobenzenesulfonyl)-1,5,9-triazacyclododecane as a clear viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.91 (m, 2 H, o-ArSO$_2$), 7.79 (m, 2 H, o-ArSO$_2$), 7.54 (m, 2 H, m-ArSO$_2$), 6.66 (m, 2 H, m-ArSO$_2$), 5.11 (d, 5.1 Hz, 2 H, C=CH$_2$), 3.99 (s, 2 H, H2/4), 3.58 (s, 2 H, H4/2), 3.37 (m, 2 H, H6/12), 3.03 (s, 6 H, N(CH$_3$)$_2$), 2.90 (t, 6.2 Hz, 2 H, H12/6), 2.30 (m, 2 H, H8/10), 2.19 (t, 5.8 Hz, 2 H, H10/8), 1.94 (dd, 6.9 Hz, 3.5 Hz, 2 H, CH$_2$Cy), 1.67 (m, 8 H, H7, 11, Cy), 1.45 (m, 1 H, Cy), 1.16 (m, 4 H, Cy), 0.67 (q, 11.7 Hz, 2 H, Cy). $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 151.9, 142.5, 140.7, 133.2, 129.2, 128.2, 118.7, 116.9, 116.7, 113.4, 66.8, 62.3, 60.5, 52.8, 52.2, 48.6, 48.2, 40.3, 33.0, 30.2, 25.5, 25.0, 22.4, 20.02, 19.89. IR (neat cm$^{-1}$) IR (neat cm$^{-1}$) 2924 (w), 2852 (w), 2232 (w), 1595 (m), 1516 (w), 1447 (w), 1370 (w), 1334 (m), 1229 (w), 1149 (m), 1089 (m), 1042 (w), 996 (w), 898 (w), 875 (w), 841 (w), 790 (m), 724 (m), 682 (m), 639 (m), 621 (m), 608 (w). MS (ESI$^+$) $m/z$ 628 (MH$^+$), 629 (MH$^+$ +1), 630 (MH$^+$ +2).
Synthesis of 9-cyclohexylmethyl-1-(4-dimethylaminobenzenesulfonyl)-3-methylene-5-(4-(2-oxopyrrolidin-1-yl)benzenesulfonyl)-1,5,9-triazacyclododecane (TL056)\textsuperscript{28}

To a 1 L round bottom flask, 1.06 g (1.68 mmol) of $N^\prime$-($p$-dimethylaminobenzenesulfonyl)-$N^\prime$-($4$-(2-oxopyrrolidin-1-yl)benzenesulfonyl)-[$N,N$-bis(3-aminopropyl)cyclohexylmethylamine], 2.43 g (8.42 mmol) of 2-methylene-1,3-propanebis(\textit{tert}-butylcarbonate), 118 mg (1.11 mmol) of Na$_2$CO$_3$, 105 mg (0.115 mmol) of Pd$_2$dba$_3$, 92.7 mg (0.217 mmol) of dppb and 186 mL of anhydrous acetonitrile were stirred under N$_2$ gas and boiled under reflux. The mixture was then allowed to cool to r.t. and filtered. The filtrate was washed with 50 mL of sat. aq. NaHCO$_3$ solution. The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The product was converted to the HCl salt by stirring with 25 mL of a solution of 2 N HCl in MeOH for 1 h. The solution was then concentrated by rotary evaporation and the resulting residue was dried \textit{in vacuo}. The resulting solid was triturated with diethyl ether (3 x 25 mL) and the residue dried \textit{in vacuo}. The product was then converted back to the free base by stirring vigorously with 25 mL of DCM, 25 mL of aq. 2 N NaOH solution, and 25 mL sat. aq. NaCl.
solution for 4 h. The layers were separated, and the aqueous layer was extracted with DCM (3 x 25 mL). The combined organic solutions were dried (Na$_2$SO$_4$) and filtered. The filtrate was concentrated and the resulting residue was dried in vacuo. The product was purified by automated chromatography on neutral alumina, eluting with 3:2 (v/v) ethyl acetate/hexane. This produced 0.319 g (28%) of 1-(4-((9-(cyclohexylmethyl)-3-methylene-5-tosyl-1,5,9-triazacyclododecan-1-yl)sulfonyl)phenyl)pyrrolidin-2-one as a clear viscous oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.76 (m, 4 H, o-ArSO$_2$, o-ArSO$_2$), 7.53 (m, 2 H, m-ArSO$_2$), 6.63 (m, 2 H, m-ArSO$_2$), 5.11 (s, 2 H, C=CH$_2$), 3.86 (t, 2 H, CCH$_2$C), 3.81 (s, 2 H, H2/4), 3.65 (s, 2 H, H4/2), 3.18 (m, 2 H, H6/12), 3.00 (s, 8 H, H12/6, N(CH$_3$)$_2$), 2.60 (m, 2 H, CCH$_2$C=O), 2.19 (m, 6 H, H8, 10, CH$_2$CH$_2$C=O), 1.91 (d, 6.9 Hz, 2 H, CH$_2$Cy), 1.57 (m, 10 H, H7, 11, Cy), 1.08 (m, 3 H, Cy), 0.65 (q, 11.6 Hz, 2 H, Cy). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 175.7, 175.0, 153.2, 152.4, 143.7, 142.8, 142.2, 130.8, 129.7, 129.4, 128.8, 121.3, 121.0, 119.5, 111.9, 111.1, 61.3, 53.3, 53.3, 52.7, 48.9, 40.2, 32.9, 31.7, 29.7, 25.6, 24.2, 18.0. IR (neat cm$^{-1}$) 2924 (w), 2852 (w), 2442 (w), 1699 (w), 1593 (m), 1517 (w), 1495 (w), 1448 9w), 1419 (w), 1383 (w), 1317 (m), 1221 (w), 1149 (s), 1090 (m), 996 (m), 941 (w), 897 (m), 837 (w), 778 (m), 713 (m), 689 (m), 641 (m), 602 (w). MS (ESI$^+$) m/z 686 (MH$^+$), 687 (MH$^+$ +1). Anal. Calcd for C$_{35}$H$_{51}$N$_5$O$_5$S$_2$·HCl·3.5H$_2$O: C, 53.52; H, 7.57; N, 8.92. Found: C, 53.68; H, 7.95; N, 9.13.
4.5 References


(15) Kremer, E.; Facchin, G.; Estevez, E.; Albores, P.; Baran, E. J.; Ellena, J.; Torre, M. H. Copper Complexes with Heterocyclic Sulfonamides: Synthesis, Spectroscopic Characterization, Microbiological and SOD-like Activities: Crystal
Structure of [Cu(sulfisoxazole)$_2$(H$_2$O)$_4$]·2H$_2$O. J. Inorg. Biochem. 2006, 100, 1167-1175.


Chapter 5

Conclusion
5.1 Conclusion and Future Outlook

A library of 51 compounds has been successfully synthesized and characterized. It was initially suggested that the addition of steric bulk on to the isobutylene head group of CADA could optimize the conformation of the molecule and place the two side arms in positions to improve the interactions with the binding sites. However, synthesis of compounds TL005, TL007, and TL010 (Figure 5.1.1) showed decrease CD4 down-modulation activities. This indicates that the increased steric bulk of the new head groups causes a decrease in activity which could be due to changes in the conformation. The bulky headgroups could be affecting the conformation of the compound and placing the two side arms in positions that would decrease interactions with the binding site. This is contrary to the initial hypothesis for these CADA analogs. The synthesis of CADA analogs containing an isopropylidene head group were unsuccessful due to the nature of the palladium catalyzed cyclization. The formation of the bis diene product caused by the E2’ elimination and the double addition prevented the formation of the CADA analogs with the isopropylidene head group. However, further investigation of head group modifications to CADA could still be conducted. Although the results showed decrease in activity, TL010
could be hydrolyzed to form an ethyl group and tested for CD4 down-modulation potency. Since compound RA016 (Figure 5.1.2) showed an increase in potency, it would be interesting to see if there would be an increase in potency if TL010 were hydrolyzed. The more potent analogs TL020 could also be hydrolyzed to see if the methyl group instead of an isobutylene group would be a better head group.

Modifications to the tosyl side arm of the CADA analog CK147 were successfully conducted. This produced a series of CADA analogs with various properties which were tested for CD4 down-modulation activity. It was found that the removal of the aromatic ring showed a dramatic decrease in activity as shown in compounds TL027 and TL033 which had a methyl and a morpholine group. Increased steric bulk also showed a decrease in activity as shown in compound TL038, TL023, and TL022. Increased the electron richness of the side arm also showed a slight decrease in activity as shown for TL042. Compounds TL020, TL029, and TL039 (Figure 5.1.3) were the most potent analogs synthesized in this study. TL020 was found to be just as potent, if not slightly more potent than CK147 (Figure 5.1.4). It was found to be less cytotoxic with the same anti-HIV activity, making it more selective than CK147. However, compound TL042 was found to be the lest cytotoxic analog with comparable anti-HIV activity to CK147, making it a better
candidate for *in vivo* studies. Compounds **TL029** and **TL039** were found to be more cytotoxic than **CK147**. A viral screening was conducted on **TL020** and it was found to be active against zika, hepatitis B, chikungunya, dengue, tacaribe, and respiratory syncytial viruses. This indicates that **TL020** not only has anti-HIV properties but is a good candidate for other viruses. Further investigation into the mechanism of action against these other viruses could lead to better analogs that could ultimately produce a cure to some of these infections.

**Figure 5.1.3.** Structures of **TL020**, **TL029**, **TL039**, and **TL042**.

**Figure 5.1.4.** Structure of **CK147**.
Copper (II) complexes of open-chained disulfonamides were initially proposed as potential gp160 down-modulating compounds. However, attempts at synthesizing the copper complexes proved to be unsuccessful. Due to complications with the synthesis, copper (II) was substituted for gold (III). Initial attempts at making the gold (III) complexes also seemed to be unsuccessful. It was believed that the sulfonamide nitrogens were not nucleophilic enough for the reaction to proceed. Attempts at increasing the nucleophilicity of the nitrogens were done by addition of a weak base to the reaction mixture, however this also proved to be unsuccessful. A new open-chained disulfonamide was proposed in order to increase the nucleophilicity of the nitrogen. Synthesis of the newly proposed carboxy disulfonamide was initiated. The synthesis of the diester compound TL030 (Figure 5.1.5) was successfully conducted. The reduction of the diester to the dicarboxylic acid was also done, however the product was not isolated as the free base. Attempts at tosylation of the dicarboxylate were done but were unsuccessful. Isolation of the dicarboxylate as the free base is currently being investigated. It is believed that the carboxylate salt contains excess NaOH which would affect the tosylation reaction. Once the carboxylic acid free base is successfully isolated, the tosylation reaction should work.

Analogs for TSHR down-modulation has been conducted. Six different sulfonyl chlorides were synthesized. From these six sulfonyl chlorides, four open-chain disulfonamides were created. However, of the four open-chain disulfonamides, only two were successfully cyclized to form the CADA analogs TL050 and TL056 (Figure 5.1.6).
Compound **TL050** still needs to be reduced to the primary amine. Initial attempts of the reduction did not show any evidence for the formation of the product and the starting material was recovered after the reaction. The reduction of **TL050** is still being investigated. Additional attempts at the reduction will be conducted to form the primary amine. Once the reduction is completed, the resulting compound will be tested for TSHR down-modulation activity. The reduced product will also be used to add on guanidine and amidine functional groups to also be tested for TSHR down-modulation.

Formation of the one-armed CADA analog is also being explored. **TL047** was reacted with mercaptoethanol to remove the 2-nosyl group. Evidence of the product was shown, however there were discrepancies in the data as well as evidence of an inseparable side product. The removal of the 2-nosyl group to form the one-armed CADA analog will be attempted with \( p \)-mercaptobenzoic acid and \( K_2CO_3 \). Once the one-armed CADA analog is completed, it will be tested for TSHR down-modulation along with **TL050** and **TL056**. The one-armed CADA analog is an important analog since it was determined that compounds **VGD040** and **CK075** (Figure 5.1.7), both one-armed CADA analogs, were found to be potent TSHR down-modulators. This new one-armed CADA analog containing a \( p \)-dimethylamino side arm could show similar down-modulation activity as well. These

![Figure 5.1.6. Structures of TL047, TL050, and TL056.](image)
compounds are known to be poor down-modulators of CD4 but are found to be good down-modulators of TSHR, making them selective towards TSHR. The one-armed CADA analog could also be used to add side arms that were used to make the open-chained disulfonamide but were unsuccessful in the palladium catalyzed cyclization step.

Figure 5.1.7. Structures of CK075 and VGD040.
# Appendix

### $^1$H and $^{13}$C NMR Spectra of New Compounds

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