Benzimidazole Based Anion Transporters; Incorporation of [2]Rotaxanes into Membrane Transport Studies

Emilyn Anderi
University of Windsor

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Benzimidazole Based Anion Transporters

Incorporation of [2]Rotaxanes into Membrane Transport Studies

By

Emelyn Rose Anderi

A Thesis
Submitted to the Faculty of Graduate Studies
Through the Department of Chemistry and Biochemistry
In Partial Fulfillment of the Requirements for
The Degree of Master of Science
At the University of Windsor

Windsor, Ontario, Canada
2017

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Benzimidazole Based Anion Transporters:
Incorporation of [2]Rotaxanes into Membrane Transport Studies

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January 23, 2017
DECLARATION OF CO-AUTHORSHIP

I hereby declare that this thesis incorporates material that is the result of joint research as follows: The project design was developed by Dr. Stephen Loeb and Dr. Andreea Schmitzer. Synthetic experiments were designed by the author with additional input given by Dr. Kelong Zhu and Joseph Sbrocca. All synthetic experiments and characterization were conducted by the author with the exception of high resolution mass spectrometry recorded by Dr. Janeen Auld, single crystal X-ray diffraction experiments run by Dr. Kelong Zhu, Dr. Giorgio Baggi, and Alexander Stirk. In addition, the membrane transport studies were conducted with the aid of Dr. Julie Kempf at the University of Montreal.

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thesis has not been submitted for a higher degree to any other university or
instituition.
ABSTRACT

Development of synthetic molecules that can act in similar ways to biological proteins capable of ion transport has become a topic of interest in many fields of research. This thesis investigates the possibility of ion transport through the incorporation of [2]rotaxane molecules into a lipid membrane.

Chapter 1 outlines the reasoning behind the development of a [2]rotaxane with a T-shaped axle utilizing a benzimidazolium-based recognition site as a suitable candidate for a synthetic anion transporter. It also highlights results of transport studies from related benzimidazolium salts.

Chapter 2 outlines the synthesis of the desired T-shaped axle and its related [2]rotaxane, with discussion of the various synthetic routes attempted. Results from incorporation of both molecules into anion transport studies are discussed, with a summary and future work also included.

Chapter 3 describes all of the experimental data. This includes the full synthetic procedure for the preparation of both the T-shaped axle and related [2]rotaxane, as well as the experimental setup for the transmembrane transport assays conducted.
ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Stephen Loeb, for all of his guidance and advice throughout my academic career. He has continually provided an excellent support system, and has always had the confidence in me to grow as a researcher. I am very fortunate to have learned many lessons, both in chemistry and life, from such an exceptional mentor.

I would also like to extend a tremendous thank you to my fellow Loeb group members, past and present. Whether it was teaching me lab techniques, or reaching for something that was too high up on a shelf, I have always had continual support from them. I would like to thank Joseph Sbrocca and Dr. Kelong Zhu for having the patience to teach me all the tips and tricks of synthetic chemistry, and for always being there to consult with. To Dr. Kelong Zhu, Dr. Giorgio Baggi, and Alex Stirk, thank you for taking time out of your own projects to work on the single crystal X-ray diffraction experiments, as well as solve the crystal solutions. A special thank you to Christine To and Leslie Hernandez, my lab sisters, who never let me give up and always found a way to make me smile. To the rest of the current Loeb group, Ginny, Pablo, Ghazale, Mike, Peter, and Dr. Yoshitaka Tsuchido, as well as other members of the Chemistry department, Manar, Akhil, Chris, and Mitch; thank you for making the work day more enjoyable.

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>24C6</td>
<td>[24]-crown-6-ether</td>
</tr>
<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>α-CD</td>
<td>α-cyclodextrin</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>br</td>
<td>Broad</td>
</tr>
<tr>
<td>CB7</td>
<td>Cucurbit[7]uril</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic Fibrosis</td>
</tr>
<tr>
<td>CFTR</td>
<td>Cystic Fibrosis Transmembrane Regulator</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>Chloride anion</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlation Spectroscopy</td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift</td>
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<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DFT</td>
<td>Density Functional Theory</td>
</tr>
<tr>
<td>DI H₂O</td>
<td>Distilled water</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>EC₅₀</td>
<td>Half maximal Effective Concentration</td>
</tr>
<tr>
<td>Et₂O</td>
<td>Diethyl ether</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<td>----------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
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<td>EtOH</td>
<td>Ethanol</td>
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<tr>
<td>EYPC LUV</td>
<td>Egg yolk phosphatidylcholine large unilamellar vesicles</td>
</tr>
<tr>
<td>h</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>Water</td>
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<tr>
<td>HPTS</td>
<td>8-hydroxy-1,3,6-pyrenetrisulfonate</td>
</tr>
<tr>
<td>H$_2$SO$_4$</td>
<td>Sulfuric acid</td>
</tr>
<tr>
<td>HR-MS</td>
<td>High Resolution Mass Spectroscopy</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared spectroscopy</td>
</tr>
<tr>
<td>ISE</td>
<td>Ion-selective electrodes</td>
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<tr>
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<td>Potassium iodide</td>
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<td>m</td>
<td>Multiplet</td>
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<td>Acetonitrile</td>
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<tr>
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<td>NaH</td>
<td>Sodium hydride</td>
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<tr>
<td>NaHSO$_3$</td>
<td>Sodium bisulfite</td>
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<tr>
<td>NaNO$_2$</td>
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<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
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<tr>
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<td>Description</td>
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<td>-------------</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>Sodium sulphate</td>
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<tr>
<td>nBuLi</td>
<td>n-Butyllithium</td>
</tr>
<tr>
<td>NEt₃</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance spectroscopy</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>Nitrate anion</td>
</tr>
<tr>
<td>NOESY</td>
<td>Nuclear Overhauser Effect Spectroscopy</td>
</tr>
<tr>
<td>O-NDI</td>
<td>Oligo-(p-phenylene)-N,N-napthlenediimide</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>Rᵣ</td>
<td>Retention factor</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
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<tr>
<td>TEMPO</td>
<td>2,2,6,6-Tetramethylpiperidine-N-oxyl radical</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>ZrCl₄</td>
<td>Zirconium tetrachloride</td>
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CHAPTER 1 – Supramolecular Chemistry and Synthetic Anion Transporters

1.1 Introduction to Supramolecular Chemistry

Supramolecular chemistry has emerged as a multi-disciplinary field, drawing from organic, coordination, and physical chemistry, as well as biology, to develop complex chemical systems based on intermolecular interactions that are non-covalent in nature.¹ This branch of chemistry was defined by Jean Marie Lehn in 1969 while studying inclusion complexes formed with cryptands,² and was further formalized when Lehn, Charles Pedersen, and Donald Cram were awarded the 1987 Nobel Prize in Chemistry for their discoveries in the field.³

The construction of supramolecular molecules relies on self-assembly. This is a spontaneous process that is dependent on the information organized in the structural building blocks of a molecule. It makes use of molecular recognition, which allows multiple components to be positioned by non-covalent interactions, and later potentially interlocked through covalent bond formation. Common non-covalent interactions that drive complexation include hydrogen bonding, π-π stacking, electrostatic interactions, hydrophobic interactions and metal coordination.⁴

1.1.1 Pseudorotaxanes and Rotaxanes

Pseudorotaxanes are an example of host-guest complexes prepared by threading a macrocyclic ring, known as the wheel, onto a linear molecule, known as the axle. The two components become positioned through favourable non-covalent interactions, and the formed interpenetrated complex is in equilibrium with its
constituent components, as seen in Figure 1.1. When naming pseudorotaxanes, it is important to include the sum of all the individual components that make up the complex. Therefore, when one linear axle is interpenetrated through one macrocyclic ring, the resulting complex is said to be a [2]pseudorotaxane.

![Figure 1.1: Representation of a linear axle and macrocyclic wheel in equilibrium with a [2]pseudorotaxane](image)

Pseudorotaxanes can be further modified to prevent the macrocyclic ring from sliding off, creating what is called a [2]rotaxane. These complexes are described as mechanically interlocked; they continue to be held together by non-covalent interactions, however the interaction is now permanent as a covalent bond found in one of the components would have to be broken in order to return to its constituent components. Rotaxanes can be prepared by two main synthetic methods, capping or clipping. Figure 1.2a depicts the capping method, which involves adding large bulky end groups onto the axle of a [2]pseudorotaxane to prevent the macrocyclic ring from dissociating. The clipping method can be utilized by first synthesizing an axle with bulky end groups (a dumbbell), and later
introducing an open macrocyclic precursor that can undergo a ring closing reaction to interlock the ring around a recognition site on the axle, as shown in Figure 1.2b.

**Figure 1.2:** Representation of rotaxane synthesis via a) capping method and b) clipping method

One application of [2]rotaxanes has included development of artificial molecular machines (2016 Nobel Prize in Chemistry). By synthesizing axles with multiple recognition sites, the position of the wheel can be influenced to undergo translational motion either by chemical, electrochemical, or even photochemical stimulation. Other applications include the use of [2]rotaxanes as sensors and transporters of charged ions.

### 1.1.2 The T-Shaped Benzimidazolium Axle

There are many templating motifs that can be incorporated into the axles of interpenetrated and interlocked molecules to provide recognition sites for crown ethers and other related guest molecules. Over the years, the Loeb research group
has focused on many different motifs such as 1,2-bis(pyridinium)ethane axles,\textsuperscript{14,15} N-benzylanilinium axles,\textsuperscript{16,17} and more recently, benzimidazolium axles.\textsuperscript{18,19}

As seen in Figure 1.3, the substitution pattern of a 2,4,7-triphenylbenzimidazolium ion provides a rare example of an organic molecule that displays a rigid core with a 90° turn. It has been shown by Loeb et al. that this T-shaped benzimidazolium axle provides greater enhancement between a crown ether wheel and axle when forming a [2]pseudorotaxane, compared to simple benzimidazolium or imidazolium cations.\textsuperscript{18}

1.1.3 Intermolecular Interactions between Axle and Crown Ether Rings

The intermolecular interactions observed between an axle and a macrocyclic ring in pseudorotaxanes and rotaxanes are non-covalent in nature. When crown ether rings are used as the macrocyclic wheel, two types of hydrogen bonding interactions between the wheel and axle can occur; NH⋯O interactions, as well as CH⋯O interactions. Having a cationic charge on the benzimidazolium axle also allows for ion-dipole interactions, such as N\textsuperscript{+}⋯O, to aid in promoting axle and wheel association.

One unique property of the T-shaped axle is the ability to utilize π-π interactions to increase association with decorated crown ethers. For example,
dibenzo[24]crown-8 is a crown ether functionalized with aromatic groups that allow the ring to fold and interact with the axle. As illustrated in Figure 1.4, the contributions from all of these intermolecular interactions allow for the overall enhancement of the T-shaped axle and wheel association.

![Image of chemical structure](image)

**Figure 1.4**: Noncovalent interactions between 2,4,7-triphenylbenzimidazolium axle and dibenzo[24]crown-8 wheel

Use of this T-shaped motif has provided opportunity for a variety of functionalized aromatics to be used on the axle. When $R_1$ or $R_2$ (as labelled in Figure 1.4) are electron withdrawing groups, the non-covalent interactions are strengthened due to the increase in acidity of the hydrogen bond donors, as well as the increased charge on the benzimidazolium rings. Conversely, using electron donating groups in these positions will decrease the strength of the non-covalent interactions, and therefore weaken the association between wheel and axle.

1.2 Anion Transport Across Cell Membranes

Many biological processes rely on molecular transport into or out of a cell to function. In particular, one crucial molecule that requires transport is the chloride
ion (Cl⁻), as it is one of the most abundant anions found in the body. Chloride transport across cell membranes is necessary for a variety of functions including fluid transport, cell volume regulation, muscle contractions, and neuroexcitation.²⁰

1.2.1 Cell Membranes

Cell membranes are composed of phospholipids that form bilayers which separate the interior of a cell from the exterior environment. Cell membranes are considered amphipathic due to the inherent structure of phospholipids; they are composed of a phosphate group (polar head) attached to two fatty acid hydrocarbon chains (nonpolar tails). However, due to the large composition of fatty acid chains, the majority of the cell membrane is said to be nonpolar in nature.²¹

One of the major roles of a cellular membrane is to regulate the transport of molecules required for survival. Membrane transport can be passive or active. Passive transport, by diffusion or osmosis, occurs with no energy input and allows hydrophobic or small, uncharged polar molecules to move down a concentration gradient. The driving force is simply the difference in concentration between the interior and exterior of the membrane. Passive transport is commonly experienced by solutes such as O₂, CO₂, H₂O and urea.²¹ Alternatively, active transport is coupled to a metabolic energy source (i.e. ATP hydrolysis).²¹ This occurs when certain solutes must be pumped either against a concentration or an electric potential gradient.
1.2.2 Membrane Transport by Aid of Proteins

Sometimes, carrier or channel proteins are required to aid with both passive and active membrane transport. Carrier protein transporters are usually lipophilic, monomeric units capable of shuttling ionic guests from one side of the membrane to the other side through the hydrophobic portion of the lipid bilayer. Conversely, channel proteins are generally membrane-bound and span the length of the membrane to provide passage of the small hydrophilic molecules from the hydrophobic environment of the membrane. Both types of transport proteins are essential for the transport of ions, as lipid bilayers are highly impermeable to charged species.22

![Carrier Mechanism vs Channel Mechanism]

**Figure 1.5:** Comparison of transmembrane ion transport involving ion carriers and ion channels; anions represented as red spheres22

Ion channel proteins can be classified based on their transport stoichiometry and the direction of ion movement.22 **Figure 1.6** depicts the three main modes of transport through channel proteins. A uniport process occurs when a single ion is carried across a membrane uni-directionally at a single time. Two different ions can be transported simultaneously using co-transporters. Co-transporters that move solutes in the same direction are known as symports, whereas movement of solutes
in the opposite direction is done through antiports. Co-transporters generally result in electroneutral transport due to the transfer of equal amounts of positive and negative charges.

![Comparison of channel protein transport processes](image)

**Figure 1.6: Comparison of channel protein transport processes**

1.2.3 Mutations in Chloride Anion Transport

Unfortunately, natural carrier or channel proteins can become mutated and result in negative effects on the transport of charged molecules across the membrane. For example, malfunctioning chloride ion channels produce a deficiency in the chloride transport process, and are responsible for diseases such as Bartter syndrome, Dent’s disease, and cystic fibrosis. Cystic fibrosis (CF), which affects approximately 70,000 people worldwide, is caused by a mutation in cystic fibrosis transmembrane conductance regulator (CFTR) proteins. CFTR proteins are often found embedded in the cell membranes of epithelial cells lining the lungs, pancreas, and sweat glands to transport chloride anions. Once mutated, chloride and water movement across epithelial membranes become compromised, depicted in **Figure 1.7**.
Figure 1.7: Comparison of chloride transport in normal and CF infected sweat duct cells

Epithelia with defective CFTR proteins develop thick, sticky mucus that coats and clogs ducts, collects in the lungs, and leads to lethal bacterial infections.\textsuperscript{25} To date, the average life expectancy of a patient with CF is 40 years.\textsuperscript{23}

1.2.4 The Development of Synthetic Anion Transporters

Since there is no cure for CF, the development of synthetic anion transporters that can mimic the activity of functioning CFTR proteins and compensate for chloride transport failure has become an area of focus for many research groups. It is important to understand the chemistry behind natural protein transporters in
order to develop efficient synthetic mimics. Molecules that can be considered for transport studies must be water-soluble for delivery to the cell, but also sufficiently lipophilic for insertion into the cell membrane. Most synthetic anion transporters are still only tested using liposomes as models, however the creation of transporters that can function in vitro, as well as in vivo, is the ultimate goal.

There are many examples of supramolecular assemblies that are able to form membrane-spanning channels and provide a pathway through which anions can diffuse across. These channels utilize weak interactions, such as hydrogen bonding or anion-π interactions, to transport anions across a lipid membrane.

One of the first anion-selective channels that utilized hydrogen bonding interactions involved calix[4]arenes. Calix[4]arenes are attractive frameworks due the possibility of functionalization, their rigidity, as well as their ability to bind charged or neutral species. Work conducted by Davis et al. reported that calix[4]arene tetrabutylamide was able to form ion channels in solution and effectively transport HCl across liposomal membranes. Comparison of ion binding and transport properties of calix[4]arene tetrabutylamide (Figure 1.8a) against those of its analogues (Figure 1.8b), revealed that the compound’s hydrophobicity, as well as the amide’s substitution pattern to allow for hydrogen bonding interactions with the anion, were both essential for facilitating anion transport.
Anion-π interactions have also been useful in developing synthetic anion transporters. Matile et al. were able to demonstrate how oligo-(p-phenylene)-N-N-napthalenediimide (O-NDI) rods were capable of utilizing multi-ion hopping to selectively and effectively transport anions across lipid membranes. Multi-ion hopping relies on multiple binding sites being present, and utilizes charge repulsion to push ions through each site and across a membrane down a concentration gradient. As an external chloride approaches the channel, electrostatic repulsion causes one ion to move to a central site, causing further repulsion to other internal ions. Eventually chloride ions that once occupied sites in the channel are repelled out of the channel, and therefore through a cellular membrane.
Using DFT calculations, it was determined that tetramethylphenyl spacers between three NDI acceptors would provide the appropriate length to span the hydrophobic region of a common lipid membrane (approximately 30 Å). DFT computed electrostatic potential maps also offered information about the electrostatic properties and conformational arrangements of the O-NDI rods. As seen in Figure 1.10, O-NDI rods provide continuous positive surfaces that have varying intensity of electrostatic potential along different sites of the rods when the tetramethylphenyl spacers are perpendicular to the NDI units. Therefore, O-NDI systems can accomplish multi-ion hopping through both electrostatic and ion-induced polarization forces.

Figure 1.9: Representation of multi-ion hopping

![Diagram](image-url)
Figure 1.10: a) O-NDI rods aligning as anion-π slide 
b)DFT computed electrostatic potential map for O-NDI rod; blue: electron-poor, red: electron-rich\textsuperscript{28}

Rods 1-1 – 1-5 (Figure 1.11) were tested in egg yolk phosphatidylcholine large unilamellar vesicles (EYPC LUVs) that contained the pH-sensitive fluorescent dye 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS), and were exposed to a pH gradient with a base pulse.\textsuperscript{27} Upon addition of each rod, the change in HPTS emission was observed and the velocity of decay of the pH gradient was monitored.
Figure 1.11: O-NDI rods tested for anion transport$^{27}$
Rod 1-2 was found to have the highest activity. It was believed that having one charged terminus provided the rod with the ability to be easily delivered to the membrane as it was sufficiently water-soluble. Moreover, it was able to adapt an active transmembrane structure due to the presence of a hydrophobic terminus. Rod 1-1 was found to have lower activity and precipitate prior to insertion of the membrane due to the lack of charged termini, whereas rod 1-3, with both termini charged, had excellent water-solubility but poor transmembrane orientation. Both O-NDI hairpin structures 1-4 and 1-5 had very low activity for similar reasons.

It was determined that the activity of the rods did not change in response to changing the cation, suggesting the mode of ion transport was not H⁺/M⁺ antiport. Transport activity was affected however in response to changing the external anion, and therefore believed that the mode of ion transport utilized Cl⁻/X⁻ and Cl⁻/OH⁻ antiport.

Imidazolium salts have also been able to demonstrate controlled anion transport activity, as shown by Schmitzer et al.²⁹ Salts 1-5 - 1-7 (Figure 1.12), were tested as transporters in EYPC liposomes loaded with the Cl⁻ sensitive dye, lucigenin. Upon addition of each salt, direct measurement of Cl⁻ efflux could be monitored based on the change of fluorescence emission from lucigenin.
Figure 1.1: Imidazolium salts studied for anion transport activity\textsuperscript{29}

Salt 1-8 was found to be the only active salt due to its ability to assemble in a way that formed channels that spanned the length of the hydrophobic region of the membrane. This was done by utilizing π-π stacking to induce directionality. Once in the membrane, anion-π interactions allowed for multiion hopping across hydrophobic region. Although salt 1-7 could be partitioned into the bilayer, it is believed that this salt was not able to form strong aromatic interactions to create channels that would span the length of the membrane (Figure 1.13).\textsuperscript{29} Finally, salt 1-6 was found to have the lowest transport activity, as it was extremely water-soluble, and could not be translocated into the membrane successfully.
Salt 1-8 was further studied to investigate whether transport activity could be controlled via the creation of inclusion complexes. α-Cyclodextrin (α-CD) and cucurbit[7]uril (CB7) were used to create inclusion complexes with salt 1-8 by accommodating the aromatic sidechains into their hydrophobic cavities. Both inclusion complexes were found to be more water-soluble than the salt alone. The formation of inclusion complexes allowed for the self-assembled channels to be disrupted and lead to displacement of the salt from the membrane into the aqueous phase, as seen in Figure 1.14. Therefore it was determined that anion transport...
could be regulated through the creation of inclusion complexes between the salt and nontoxic macrocycles.

![Diagram of inclusion complexes](image)

**Figure 1.14:** Representation of the formation of inclusion complexes of salt 1-8 with CB7 and α-CD, followed by displacement of transporter from the lipid bilayer.  

Schmitzer’s group has also investigated the anionophoric activity of 2,4,7-triphenylbenzimidazole. Driven by the formation of hydrogen bonds and stabilization through π-π stacking, the crystal structure of this motif shows the formation of helical rods (Figure 1.15).
Figure 1.1: Self-assembly of 2,4,7-triphenylbenzimidazole in the solid state showing a) side view and b) top view of rod.

Although no open channels were formed, molecular modeling in an EYPC bilayer confirmed the possibility of preserving the rod-like structure in the presence of phospholipids for this molecule. As seen in Figure 1.16, four monomers of the 2,4,7-triphenylbenzimidazole motif assembled in a rod-like helix which span the thickness of the hydrophobic portion of a phospholipid membrane.
Figure 1.16: Molecular modelling results of 2,4,7-triphenylbenzimidazle in an EYPC bilayer showing how a) four monomers provide the appropriate length to span the hydrophobic region of membrane and b) possible sites for anion-π interactions.

Chloride transport studies were conducted using EYPC liposomes encapsulated with lucigenin, and Cl⁻ anions present in the extravesicular solution. Upon addition of the transporter, the fluorescence of lucigenin decreased,
confirming Cl⁻ transport into the liposome. To study the mechanism of transport, studies were conducted in 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes. Phospholipids containing DPPC undergo a phase change at a temperature of 41°C; below this temperature phospholipids are in a gel phase, while above this temperature they are in a fluid phase.³¹ It is commonly observed that molecules that act as mobile carriers show a reduction in chloride movement when placed in DPPC liposomes, whereas transmembrane channels show no or slight variations in activity. Upon testing 2.4.7-triphenylbenzimidazole in DPPC liposomes, only a slight variation in transport activity was seen, therefore supporting the idea that this motif formed transmembrane channels.³⁰

### 1.2.5 Transmembrane Transport Assays for Synthetic Anion Transporters

Phospholipid vesicles provide strong models that can be used to understand and study ion transport across cell membranes. Liposomes in particular offer great versatility due to a large variety being available commercially and synthetically with varying phospholipid composition. The intravesicular solution of liposomes is dependent upon the solution that they were assembled in. The vesicles can then be separated from the non-encapsulated material via dialysis or size-exclusion chromatography, and later suspended in an extravesicular solution that contains different components. This allows for creation of a transmembrane concentration gradient, and ion transport facilitated by the addition of a transporter compound can be monitored.²²
There are many different techniques that can be employed to monitor anion transport processes. One common technique is the use of a fluorescence-based assay which monitors changes in fluorescence emission of particular dyes (Figure 1.17) based on changes in intravesicular pH, anion concentration, or electric potential. By encapsulating the probe in a liposome, the change in intravesicular anion composition can be monitored over time.

![Common dyes used to monitor ion transport in liposomal assays](image)

**Figure 1.17: Common dyes used to monitor ion transport in liposomal assays**

Lucigenin, a bis-acridium dication, is a commonly used fluorogenic probe that can directly monitor anion transfer. Since the fluorescence of this dye is quenched in the presence of halide anions but is insensitive to oxoanions such as nitrate, phosphate, or sulfate, it is extremely useful for monitoring NO$_3^-$/Cl$^-$ exchange.

Ion-selective electrodes (ISE) can also be used as a direct method to measure ion transport processes, since the internal contents of liposomes are initially not detected. Upon the addition of a transporter, efflux of ions into the extravesicular solution results in a change of voltage that can be detected by the ISE. When using a chloride selective ISE, nitrate can easily exchange with chloride due to its relatively
high lipophilicity, and therefore is often chosen as the external anion in the surrounding buffer solution. It is optimal to use ISEs to measure ion transport when synthetic transporters display fluorescence emission, especially in the range of common fluorogenic probes.

1.3 Scope of Thesis

This thesis describes the synthesis of a new benzimidazole-based T-shaped axle and its related [2]rotaxane for investigation of their anion transport capabilities. Based on anion transport studies completed on previous benzimidazolium salts, it has become evident molecules with higher lipophilicity are generally capable of being more efficient anion transporters across phospholipid membranes. This has led to the interest of developing hydrophobic molecules that are capable of interacting with anions such as chloride through hydrogen bonding and anion-π interactions.

The 2,4,7-triphenylbenzimidazole motif depicted in Figure 1.3 was originally designed by the Loeb research group as an axle template for pseudorotaxanes, but has since been tested as an anion transporter. In an effort to increase anion transport efficiency, modification of this axle to a more lipophilic molecule was believed to be a possible solution. A new dumbbell (Figure 1.18) was designed to include an extension of the previous axle by addition of a phenyl ring, as well as methyl substituents to act as bulky stopper groups. Formation of an analogous [2]rotaxane could then become possible using the clipping methodology; in
particular utilizing ring-closing metathesis (RCM) to form a 24-crown-6 macrocyclic ring developed in the Loeb group previously.

*Figure 1.18: Target axle and [2]rotaxane for anion transport studies*

Both free axle and [2]rotaxane should provide hydrophobic surfaces capable of anion-π interactions similar to the previously tested and structurally related benzimidazole molecules. The difference between these two molecules lies in the availability of the N-H of the benzimidazole, as the macrocyclic wheel of the [2]rotaxane will interact with that site on the axle, and be less available for chloride interaction. This will allow for investigation into the formation of hydrogen bonding interactions with chloride anions, and their importance in the ion transport process.
CHAPTER 2 – Results and Discussion

2.1 Organic Synthesis

Synthesis of both the axle and [2]rotaxane involved a multistep reaction pathway. Multiple pathways were tested and are discussed below.

2.1.1 Analysis of Synthetic Scheme #1

Synthetic Scheme #1 (Figure 2.1) was initially followed in hopes of obtaining compound 7 through a condensation reaction between compounds 3 and 6, and further synthesizing axle 8 via a Sonogashira cross coupling reaction.

The synthesis of 3 was first accomplished via bromination of 3,5-dimethylaniline with NBS to produce compound 1, which subsequently underwent a Sandmeyer reaction yielding compound 2. By performing a lithium-halogen exchange reaction on 2, compound 3 was obtained in moderate yields. Lithium-halogen exchanges occur between organohalide and organolithium species at extremely fast rates, with the trend of exchange favouring I > Br > Cl > F.33 Compound 2 contains two sites for possible exchange. Although the site with bromine is more sterically hindered, and not as favoured as the site with iodine, it is possible some exchange occurred here, ultimately leading to a lower yield of the desired compound 3.

Compound 6 was obtained by first brominating 2,1,3-benzothiadiazole using molecular bromine in HBr to yield the disubstituted compound 4. This was followed by a Suzuki coupling reaction using phenylboronic acid, Cs₂CO₂ and Pd(PPh₃)₄ as a
catalyst to yield compound 5, with subsequent reductive extrusion of sulfur using NaBH₄ and CoCl₂ catalyst to produce compound 6 as the diamine product.

Figure 2.1: Synthetic Scheme #1
Since the diamine product is air-sensitive, it was important for this product to be used in the condensation reaction immediately, or otherwise be stored under an inert nitrogen atmosphere. Compounds 3 and 6 underwent a condensation reaction using ZrCl$_4$ as a catalyst to yield compound 7. This molecule was designed to provide a template for a new T-shaped axle by using the bromine substituent as a site for further functionalization.

A Sonogashira cross-coupling reaction was attempted between compound 7 and phenylacetylene using CuI and PdCl$_2$(PPh$_3$)$_2$ as catalysts, and PPh$_3$ to activate the palladium catalyst. Although the crude product was subjected to column chromatography using a variety of solvent gradients, compound 8 was never isolated pure. $^1$H NMR spectroscopy showed many proton environments relating to compound 7, suggesting that a Sonogashira reaction between compound 7 and phenylacetylene was not ideal, and the crude product contained much of the original starting material. Further analysis using IR spectroscopy supported the idea that compound 8 was not successfully synthesized due to the lack of C≡C stretching vibration in the 2200cm$^{-1}$ region.

Possible complications with this reaction could involve issues with steric hindrance and competing side reactions. Firstly, compound 7 contains two methyl groups closely substituted to the site of the aryl halide participating in the cross-coupling reaction. These methyl groups could be sterically hindering the formation of the Pd$^{II}$ intermediate during oxidative addition, thus yielding a lower chance at successfully being coupled to phenylacetylene. Secondly, although the CuI co-
catalyst is added to increase the reactivity of phenylacetylene, a Glaser coupling side reaction could occur in the presence of air, which would produce the homocoupled acetylene derivative.$^{34}$

### 2.1.2 Analysis of Synthetic Scheme #2

Synthetic Scheme #2 (Figure 2.2) was designed to investigate whether a Sonogashira cross-coupling reaction could occur using compound 3 as the aryl halide source.

![Figure 2.2: Synthetic Scheme #2](image-url)

The conditions for the Sonogashira reaction were similar to those used previously in synthetic Scheme #1, and ultimately the same problems arose. A previous graduate student, Joseph Sbrocca, reported very low yields of product 9 (<10%), but despite multiple attempts the results could not be replicated.
2.1.3 Analysis of Synthetic Scheme #3

Synthetic Scheme #3 (Figure 2.3) was designed as an alternative synthetic route to avoid the previously proposed Sonogashira cross-coupling reactions.

Review of the literature showed how Studer et al. proposed a transition metal-free Sonogashira-type coupling reaction using TEMPO as an oxidant.\textsuperscript{35}
Following this method, the generation of Grignard compounds 12 and 13 was completed through a halogen-metal exchange using 1PrMgCl-LiCl. This method was based on previous work done by Knochel et al. who discovered that the presence of lithium salts with 1PrMgCl could enhance the rate of halogen/Mg exchange at much lower temperatures for the preparation of organomagnesium compounds. The reactions to synthesize compounds 12 and 13 were completed at 0°C, in less than two hours, and the products were used directly for the cross-coupling reaction with TEMPO without further purification.

Compound 14 was successfully synthesized and data obtained from 1H NMR spectroscopy was in accordance with values from literature. Compound 14 was then subjected to a lithium-halogen exchange reaction to obtain compound 9.

Compound 9 then underwent a condensation reaction with compound 6 to produce the desire neutral thread 8. Although initially low yielding, optimization of reaction and workup conditions allowed for production of the neutral axle in much higher yields.

Characterization of Compound 9 and Neutral Axle 8

Compound 9 and neutral axle 8 were both newly synthesized compounds and therefore fully characterized.

Compound 9

1H and 13C NMR spectroscopy data support the formation of compound 9, and can be viewed in Chapter 3. IR spectroscopy was used to confirm the presence of the
alkyne bond, and confirm the success of the Sonogashira-like coupling reaction with TEMPO.

**Table 1: IR Spectrum Analysis of Compound 9**

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<th>Wavenumber (cm⁻¹)</th>
<th>Corresponding Vibrational Mode</th>
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<td>C≡C Stretch</td>
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<td>1597.26</td>
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</table>

Suitable crystals for single crystal X-ray crystallography were obtained by slow evaporation of a solution of 9 in EtOH. A summary of the crystal data, solution, and refinement parameters are presented in Table 2.

![Ball-and-Stick representation of 9](image)

**Figure 2.4: Ball-and-Stick representation of 9; black= carbon, white= hydrogen, red= oxygen.**
### Table 2: Crystal Data, Solution and Refinement Parameters for Compound 9

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[^a]: R₁ = Σ | |Fo| - |Fc|| / Σ | |Fo|;[^b]: R₂w = [Σ[w(Fo² - Fc²)²]] / [Σ[w(Fo²)]²]¹/², where w = q[σ²(Fo²) + (aP)² + bP]⁻¹
***Structure solution and refinement complete, CCDC number will be obtained upon deposition.

**Neutral Axle 8**

¹H and ¹³C NMR spectroscopy data support the formation of neutral axle 8, and can be viewed in Chapter 3. Assignment of the aromatic proton environments in the neutral axle was completed with the aid of homonuclear Correlation spectroscopy (COSY) and Nuclear Overhauser Effect spectroscopy (NOESY).
Figure 2.5: Full NOESY spectrum of Neutral Axle 8
Figure 2.6: Full COSY spectrum of Neutral Axle 8
Figure 2.7: Proton environments of Neutral Axle 8

Figure 2.8: Cross-section of aromatic regions in COSY spectrum of 8
IR spectroscopy was useful to confirm the presence of the N-H in the benzimidazole, as well as the alkyne bond in the extended portion of the axle.

**Table 3: IR Spectrum Analysis of Neutral Axle 8**

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<th>Wavenumber (cm⁻¹)</th>
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Table 4: Suitable crystals for single crystal X-ray crystallography were obtained by slow evaporation of a solution of 8 in a 1:1 mixture of EtOH:THF. A summary of the crystal data, solution, and refinement parameters are presented in **Table 4**.

**Figure 2.9:** Ball-and-Stick representation of 8; black = carbon, white = hydrogen, blue = nitrogen.
Table 4: Crystal Data, Solution and Refinement Parameters for Compound 8

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*R₁ = Σ ||F₀| - |F_c|| / Σ |F₀|; R₂w = [Σ[w(F₀² - F_c²)²)] / [Σ[w(F₀²)]²]¹/², where w = qσ²(F₀²) + (aP)² + bP ¹

***Structure solution and refinement complete, CCDC number will be obtained upon deposition.
2.1.4 Analysis of Synthetic Scheme #4

Due to the initially low yields from the condensation reaction between compound 6 and compound 9, Synthetic Scheme #4 (Figure 2.10) was proposed as an alternate synthetic scheme. Previous Loeb group members had successfully synthesized T-shaped axles by completing the reductive elimination of sulfur and subsequent condensation reactions prior to any Suzuki coupling reactions.\textsuperscript{18}

![Chemical reactions and structures](image)

**Figure 2.10: Synthetic Scheme #4**

This alternate synthetic pathway produced good yields for compound 16, however isolating pure neutral axle 8 after the Suzuki coupling reaction with phenylboronic acid was extremely difficult. Due to the large amount of phenyl rings present in both compound 16 and the desired product, it was believed that π-π
stacking interactions were greatly hindering the attempts of isolating the unsubstituted axle from the di-, as well as mono-substituted products.

Ultimately, synthetic Scheme #3 (Figure 2.3) was chosen as the synthetic route of choice to synthesize pure neutral axle 8.

2.1.5 Analysis of [2]Rotaxane Formation

Figure 2.11 outlines the synthetic route followed once pure neutral axle 8 was obtained.

\[
\begin{align*}
\text{Scheme 2.3} & \quad \text{Fig. 2.11: Synthetic Scheme for [2]Rotaxane formation}
\end{align*}
\]
Neutral axle 8 was protonated through the addition of HBF₄·Et₂O to produce [8-H][BF₄], a protonated axle able to act as a recognition site for a ring-closing metathesis reaction.

**Figure 2.12: Synthetic Scheme for 24C6 Precrown formation**

The [24]-crown-6-ether (24C6) precrown was synthesized through tosylation of pentaethylene glycol, and subsequent alkylation with 4-pentene-1-ol to produce the bis olefin. Using Grubb’s I catalyst (RuCl₂(=CHPh)(PCy₃)₂), a ring-closing metathesis reaction was conducted to clip the crown ether around the axle and produce [2]rotaxane 11 as a mixture of both the E and Z isomers. This [2]rotaxane was left as a mixture of isomers and not further reduced as to ensure the alkyne bond in the extension of the axle was also not reduced.

**Characterization of [2]rotaxane 11**

Although the [2]rotaxane was synthesized as a mixture of both E and Z isomers, small amounts of each isomer were able to be isolated and fully characterized.
1H and 13C NMR spectroscopy data support the formation of [2]rotaxane 11, and can be viewed in Chapter 3. Assignment of proton environments in each [2]rotaxane was completed with the aid of COSY and NOESY spectroscopy experiments.

**Isomer 1**

*Figure 2.13: Full NOESY spectrum of [2]rotaxane 11 isomer 1*
Figure 2.14: Full COSY spectrum of [2]rotaxane 11 isomer 1
Figure 2.15: Proton environments of [2]rotaxane 11 isomer 1

Figure 2.16: Cross-section of aromatic regions in COSY spectrum of isomer 1
Figure 2.17: Cross-section of macrocyclic regions in COSY spectrum of isomer 1
Isomer 2

Figure 2.18: Full NOESY spectrum of [2]rotaxane 11 isomer 2
Figure 2.19: Full COSY spectrum of [2]rotaxane 11 isomer 2
Figure 2.20: Proton environments of [2]rotaxane 11 isomer 2

Figure 2.21: Cross-section of aromatic regions in COSY spectrum of isomer 2
Figure 2.22: Cross-section of macrocyclic regions in COSY spectrum of isomer 2
IR spectroscopy confirmed the presence of the N-H in the benzimidazole, the alkyne bond in the extended portion of the axle, and the ether functional groups present in the crown ether ring.

**Table 5: IR Spectrum Analysis of [2]rotaxane 11 isomer 1**

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<tr>
<th>Wavenumber (cm⁻¹)</th>
<th>Corresponding Vibrational Mode</th>
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<td>2936.71-2951.19</td>
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<tr>
<td>2864.01</td>
<td>Alkane C-H Stretch</td>
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<tr>
<td>2161.20</td>
<td>C≡C Stretch</td>
</tr>
<tr>
<td>1597.01</td>
<td>Aromatic C=C Bend</td>
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<tr>
<td>1091.50</td>
<td>Ether C-O Stretch</td>
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**Table 6: IR Spectrum Analysis of [2]rotaxane 11 isomer 2**

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<td>1091.06</td>
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Suitable crystals for single crystal X-ray crystallography were obtained by slow evaporation of a solution of isomer 1 in EtOH. A summary of the crystal data, solution, and refinement parameters are presented in Table 7. Suitable crystals of isomer 2 were unable to be obtained after many trials.

**Figure 2.23:** Thermal ellipsoid representation of [2]rotaxane isomer 1; blue = axle, red = wheel.
**Table 7: Crystal Data, Solution and Refinement Parameters for Compound 11**

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---

\textsuperscript{[a]} R_{1} = \Sigma | |F_o| - |F_c||/ \Sigma |F_o||; \textsuperscript{[b]} R_{2w} = [\Sigma[w(F_o^2 - F_c^2)]^2 / \Sigma[w(F_o^2)]^{1/2} , where w = q[σ^2(F_o^2) + (aP)^2 + bP]^2

***Structure solution and refinement complete, CCDC number will be obtained upon deposition.***

Based on structure solution and observation of the alkene bond found in the closed macrocyclic wheel, this isomer was determined to be the E isomer.
2.2 Membrane Transport Studies

The chloride transport properties of neutral thread 8 and [2]rotaxane 11 were evaluated using EYPC LUVs and a chloride-selective electrode. For these studies, the liposomes were encapsulated with 500 mM NaCl buffered to pH 7.2 with 5 mM phosphate buffer solution. They were then suspended at a lipid concentration of 5 mM in 500 mM NaNO₃ buffered to pH 7.2 with 5 mM phosphate buffer solution. Experiments were initiated by the addition of a small amount of a DMSO solution of the respective transporter with concentrations varied from 6.25 to 25 mol% relative to the concentration of EYPC. The chloride efflux was monitored as a function of time by the chloride-selective electrode. A detergent, Triton-X, was added at the end of each experiment to lyse all liposomes, and the final electrode reading was used to obtain the maximum of chloride efflux.

2.2.1 Monitoring Chloride Transport Properties of Neutral Axle

Using the kinetic results (Figure 2.24a), as well as a dose-response analysis (Figure 2.24b), the effectiveness of the neutral axle in the chloride transport process across the bilayer could be determined by its EC₅₀ value. An EC₅₀ value provides the effective transporter concentration required to facilitate 50% chloride efflux after a specific time period. For Cl⁻/NO₃⁻ antiport experiments, this time period is 270 s. A dose-response analysis can also determine the Hill coefficient (n), which can be used to describe the number of monomers found in the active transport system required to perform the chloride transport process.
Figure 2.24: a) Efflux of Cl⁻ in EYPC liposomes (5 mM) containing 6.25, 10, 12.5, 15, 20, and 25 mol% (relative to phospholipid) of neutral axle 8. Intravesicular: 500 mM NaCl, 5 mM phosphate buffer (pH 7.2). Extravesicular: 500 mM NaN₃, 5 mM phosphate buffer (pH 7.2). 8 was added at $t = 0$ s and Triton X was added at $t = 540$ s. Each curve is the average of three independent measurements. b) Hill plot analysis obtained from data shown in (a).
The EC$_{50}$ value for neutral axle 8 in a Cl$^-$/NO$_3^-$ antiport system was 13.6 ± 0.5 mol% and the Hill coefficient was 4.7 ± 0.9.

2.2.2 Monitoring of Chloride Transport Properties of [2]Rotaxane

Using the kinetic results (Figure 2.25a), as well as a dose-response analysis (Figure 2.25b), the effectiveness of the [2]rotaxane (mixture of both E and Z isomers) in the chloride transport process across the bilayer could be determined by its EC$_{50}$ value.
Figure 2.25: a) Efflux of Cl⁻ in EYPC liposomes (5mM) containing 6.25, 10, 12.5, 15, 20, and 25 mol% (relative to phospholipid) of [2]rotaxane 11. Intravesicular: 500 mM NaCl, 5 mM phosphate buffer (pH 7.2). Extravesicular: 500 mM NaNO₃, 5 mM phosphate buffer (pH 7.2). 11 was added at t = 0 s and Triton X was added at t = 540 s. Each curve is the average of three independent measurements. b) Hill plot analysis obtained from data shown in (a).
The EC$_{50}$ value for [2]rotaxane 11 in a Cl$^-$/NO$_3^-$ antiport system was 13.1 ± 0.7 mol% and the Hill coefficient was 7.7 ± 2.9.

2.2.3 Evaluation of Transporter Effectiveness based on EC$_{50}$ Values

Neutral axle 8 and [2]rotaxane 11 have EC$_{50}$ values of 13.6 ± 0.5 mol% and 13.1 ± 0.7 mol% respectively, almost twenty times higher than the EC$_{50}$ value reported for 2,4,7-triphenylbenzimidazole (0.67 ± 0.1 mol%). Therefore, these transporters are extremely inefficient in chloride transport across a lipid membrane, especially when compared to the efficiency of other synthetic transporters reported in the literature.

2.2.4 Comparison of Neutral Axle 8 and 2,4,7-triphenylbenzimidazole

In order to have a better understanding of the differences in chloride transport efficiencies between the neutral axle 8 and 2,4,7-triphenylbenzimidazole, self-assembly of each transporter in the solid state was compared.

Figure 2.26 shows the crystal organization of 8 in the solid state with the formation of planar sheets driven by π-π stacking interactions. These sheets create a hydrophobic area in between layers, allowing for the possibility of anion-π interactions between chloride anions and transporter. The presence of the N-H on the benzimidazole unit also provides the possibility of hydrogen bonding interactions with chloride anions.
**Figure 2.26:** Crystal organization showing formation of planar sheets between neutral axle 8. *a*) Ball-and-stick representation along side-view, and *b*) Wire representation of top-view; Blue and red are alternating sheets. Hydrogens omitted for clarity.

The formation of sheets is driven by edge-to-face π-π interactions measuring 2.9 Å between molecules in different layers. The width of these sheets was measured
to be approximately 35 Å, each sheet consisting of two molecules. This is slightly higher than the expected thickness of the hydrophobic portion of a bilayer. If the active transport system required the anion to pass in between two layers, this system would require two monomers to assemble into a sheet and interact with another sheet consisting of two monomers, thereby having four monomers assembled into the active system. The Hill number for neutral axle 8 was found to be 4.7 ± 0.9, supporting the idea of sheet formation. However, molecular modelling would be necessary to confirm the preservation of planar sheet structure in the presence of phospholipids.

Schmitzer et al. previously showed how 2,4,7-triphenylbenzimidazole self-assembles into helical rods (Figure 2.27), driven by the formation of hydrogen bonds between individual molecules, and stabilized by π-π interactions.30
Figure 2.27: Crystal organization showing formation of helical rods of 2,4,7-triphenylbenzimidazole driven by hydrogen bond formation (shown in black). Ball-and-stick representation along a) side-view, and b) top-view; red and blue are alternating subunits.

The differences between the self-assembly of the two can be attributed to the large difference in size. Neutral axle 8 is a much larger molecule, and therefore cannot compact as closely as 2,4,7-triphenylbenzimidazole. The methyl groups prevent the formation of a helical structure through steric hindrance, and therefore prevent hydrogen bonding interactions between monomers. As well, the addition of
another phenyl ring allows π-π stacking interactions to be the driving force for self-assembly.

In order to for a molecule to be an efficient synthetic transporter, it must be water-soluble for delivery to the liposome, but also sufficiently lipophilic for insertion into the phospholipid membrane. The addition of another phenyl ring does increases the lipophilicity of the neutral axle 8 compared to 2,4,7-triphenylbenzimidazole, however this also decreases its solubility in water. At high concentrations, small amounts of white precipitate were observed upon addition of 8. This suggests that the molecule was not fully delivered to the membrane for insertion, and therefore would have low transport efficiency, as seen with a high EC50 value (13.6 ± 0.5 mol%).

2.2.5 Comparison of [2]rotaxane 11 and 2,4,7-triphenylbenzimidazole

The crystal organization of [2]rotaxane 11 is remarkably different from the neutral axle 8 and 2,4,7-triphenylbenzimidazole. As shown in Figure 2.28, adding a ring to the axle disrupts the ability of the molecule to utilize π-π stacking interactions, prevents tight packing, and hinders the formation of planar sheets. The ring also interacts with the N-H on the benzimidazole, preventing any intermolecular hydrogen bonding, and hindering the formation of helical rods. Instead the molecules align offset of one another due to spatial constraints with the presence of the ring.
Figure 2.28: Crystal organization of [2]rotaxane 11. Ball-and-stick representation along a) a-axis, b) b-axis, c) c-axis; red and blue are alternating subunits. Hydrogens omitted for clarity.
During chloride transport studies with 11, it was observed that addition of the transporter produced a white precipitate in solution, suggesting that the [2]rotaxane was not inserted into the membrane for proper facilitation of chloride transport. This may be due to the interaction of the ring with the N-H on the benzimidazole, increasing the lipophilicity of the molecule, but once again, greatly hindering the transporters solubility in water. The high EC<sub>50</sub> value (13.1 ± 0.7 mol%) and the large Hill coefficient (7.7 ± 2.9) could also be attributed to low efficiency of the transporter if it was not properly inserted into the membrane.

To determine whether the minimal voltage change detected by the ISE was due to transport by molecule 11 or solvent effects of DMSO, 15 μL of pure DMSO was added as a blank and chloride transport was monitored. DMSO is a small amphiphilic molecule that has been shown to insert itself into lipid membranes and enhance membrane permeability by producing transient defects (pores) to facilitate ionic leakage. As seen in Figure 2.25a, minimal transport was reported and the percentage of chloride efflux was similar to the value obtained for 25 mol% of transporter. Therefore, the chloride efflux observed was most likely due to the solvent effects of DMSO, and supports the idea of minimal insertion of the [2]rotaxane 11 into the lipid membrane.

2.2.6 The Importance of N-H for Ion Transport

The results obtained from these studies provide important information about the mechanism of transport using synthetic molecules with benzimidazole cores as transporters. As seen with [2]rotaxane 11, having a component such as a crown
ether ring associate with the axle through the N-H of the benzimidazole inhibits the formation of hydrogen bonds with the anion, and therefore produces total inhibition of transport. Although some anion-π interactions are still available, the observation of inhibited transport supports the idea that hydrogen bond formation with anions is a strong driving force for transport.

Neutral axle 8 was later protonated with HCl to produce [8-H][Cl] to study the interaction of chloride anions with the axle. Suitable crystals for single crystal X-ray crystallography were obtained by slow diffusion of isopropyl ether vapour into a solution of [8-H][Cl] in THF. A summary of the crystal data, solution, and refinement parameters are presented in Table 8.

**Figure 2.29:** Thermal ellipsoid representation of X-ray crystal structure of [8-H][Cl]; black = carbon, blue = nitrogen, white = hydrogen, green = chlorine. Solvent omitted for clarity.
Table 8: Crystal Data, Solution and Refinement Parameters for [8-H][Cl−]

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[a] R₁ = Σ | |F_o| - | |F_c| | / Σ | |F_o|; [b] R₂w = [Σ [w(F_o^2 - F_c^2)^2] / Σ [w(F_o^2)^2]]^(1/2), where w = q(σ + aP)² + bP⁻¹

***Structure solution and refinement complete, CCDC number will be obtained upon deposition.

Figure 2.30 displays how chloride anions interact with multiple subunits between the planar sheets formed by the axle through hydrogen bonding. Although anion-π interactions are still present, without the ability to hydrogen bond with the axle, chloride anions do not seem to be transported as efficiently across a membrane. Again, molecular modelling would be necessary to confirm the preservation of planar sheet structure in a membrane to support the idea of chloride interaction via hydrogen bonding between sheets formed by neutral axle 8.
Figure 2.30: Ball-and-stick representation of chloride anion interaction between planar sheets; blue and red are alternating sheets.
2.3 Summary and Future Work

A new T-shaped axle, 8, and related [2]rotaxane, 11, were successfully synthesized and characterized. Multiple synthetic routes were designed and attempted due to problems that arose with extending the T-shaped axle from the original 2,4,7-triphenylbenzimidazole axle. Throughout the synthetic process it was established that a Sonogashira cross-coupling reaction was not optimal to introduce an alkyne bond due to the steric hindrance provided by the methyl groups found on the axle. Instead, a transition metal-free Sonogashira-like cross coupling reaction using organomagnesium compounds and TEMPO as an oxidant was completed to produce the extension of the T-shaped axle.

Chloride transport studies were completed using 8 and 11 as synthetic transporters. EC$_{50}$ values were found to be 13.6 ± 0.5 mol% and 13.1 ± 0.7 mol% respectively, almost twenty times higher than the EC$_{50}$ value reported for 2,4,7-triphenylbenzimidazole (0.67 ± 0.1 mol%). Therefore, 8 and 11 were not efficient chloride transporters. The importance of having the N-H of the benzimidazole present should be further investigated as future work using molecular modelling.

Expanding on the idea of using [2]rotaxanes as synthetic transporters, future work could also include investigating bis(benzimidazolium) axles. This type of axle provides a free N-H site available for hydrogen bonding with anions, while also incorporating a crown ether wheel to form a [2]rotaxane. Although the H-shaped axle shown below may present solubility issues in polar environments, the
bis(benzimidazolium) axle could be functionalized to assist with water-solubility and transporter delivery to cell membranes.

![Figure 2.31: H-Shaped bis(benzimidazolium) axle and related [2]rotaxane.](image)
CHAPTER 3 – Experimental

3.1 General Comments

All reagents and starting materials were purchased from Aldrich and used as received. Deuterated solvents were obtained from Aldrich or Cambridge Isotope Laboratories and used as received. THF, DCM, and MeCN were dried upon 3Å molecular sieves. Thin layer chromatography (TLC) was performed using Teledyne Silica Gel 60 F_{254} plates and viewed under UV light. Column chromatography was performed using Silicycle Ultra-Pure Silica Gel (230-400 mesh). $^1$H, $^{13}$C, and all 2-D NMR spectra were obtained on a Bruker Advance 500 instrument, with working frequencies of 500.1 MHz for $^1$H nuclei and 125.7 MHz for $^{13}$C. Chemical shifts are quoted in ppm relative to tetramethylsilane using the residual solvent peak as a reference standard. High resolution mass spectrometry (HR-MS) experiments were performed on a Waters XEVO G2-XS TOF instrument and completed in ESI positive resolution mode. All single crystal X-ray data was collected on a Bruker D8 diffractometer with a Photon 100 CCD detector operated at 50 kV and 30 mA with a MoKα radiation. All IR spectra were collected using a Bruker-Equinox Infrared microscopy and mapping instrument. Melting points were obtained using an OptiMelt Automated Melting Point System. Liposomes were prepared from L-α Phosphatidylcholine purchased from Avanti Polar Lipids. Size-exclusion chromatography was performed using Sephadex G-25.
3.2 Synthesis of 1

![Figure 3.1: Compound 1](image_url)

A solution of 3,5-dimethylaniline (10.0 g, 0.0825 mol) in 83 mL of dry MeCN was placed in a 250 mL Schlenk flask at 0°C under an inert nitrogen atmosphere. A solution of NBS (14.7 g, 0.0825 mol) in dry MeCN was added dropwise via an addition funnel over 1 h. The reaction was allowed to come to room temperature and stirred for an additional 72 h. The reaction was quenched via the addition of DI H₂O. MeCN was removed under reduced pressure, followed by extraction with EtOAc (3 x 150 mL). The remaining organic phase was isolated, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting crude orange solid was recrystallized from hexanes. The product was isolated as a light brown solid; isolated yield: (13.21 g, 80%).¹H NMR (500 MHz, Chloroform-δ): δ= 6.47 b (s, 2H), 3.56 NH (br s, 2H), 2.35 a (s, 6H). Values obtained are in accordance with literature.³⁹
3.3 Synthesis of 2

Figure 3.2: Compound 2

1 (5.00 g, 0.0250 mol) was suspended in 100 mL of DI H$_2$O. The mixture was kept at 0°C and concentrated H$_2$SO$_4$ (7.14 mL, 5 eq, 0.125 mol) was added dropwise. The mixture was stirred for 0.5 h, after which 67 mL of acetone was added and the contents stirred for an additional 0.5 h. A solution of NaNO$_2$ (5.17 g, 3 eq, 0.0750 mol) in 80.5 mL DI H$_2$O was added dropwise and the resulting mixture was stirred for 1h. While maintaining the temperature of the reaction at 0°C, a solution of KI (20.7 g, 5 eq, 0.125 mol) in 82.5 mL of DI H$_2$O was added dropwise. The solution turned brown and was allowed to stir while warming to room temperature overnight. Following completion, an extraction with EtOAc (3 x 100 mL) was performed. The organic phase was collected and washed once with 165 mL of a 1M HCl solution, and twice with 100 mL of a 20 wt% NaHSO$_3$ solution. The remaining organic phase was then collected, dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The product was purified and isolated by column chromatography (100% hexane) $R_f$= 0.71. The product was isolated as a colourless oil; isolated yield: 6.90 g (89%). $^1$H NMR (500 MHz, Chloroform-d) $\delta$ = 7.40 b (s, 2H), 2.36 a (s, 6H). Values obtained are in accordance with literature.
3.4 Synthesis of 3

2 (5.00 g, 0.0161 mol) was dissolved in 200 mL of dry THF in a 250 mL Schlenk flask under an inert nitrogen atmosphere. The reaction was cooled to -78°C, followed by dropwise addition of \textsuperscript{t}BuLi (10.0 mL of a 1.6M solution in hexanes, 1 eq, 0.0161 mol). While maintaining the temperature at -78°C, the reaction mixture was stirred for 1 h, after which DMF (3.70 mL, 3.1 eq, 0.0499 mol) was added dropwise and the reaction warmed to room temperature overnight. The reaction was then acidified with 100 mL of a 5 wt% HCl solution, followed by an extraction with Et\textsubscript{2}O (3 x 100 mL). The organic phase was collected, dried over MgSO\textsubscript{4}, filtered and concentrated under reduced pressure. The crude product was purified and isolated by column chromatography (hexane/EtOAc, 10:1 v/v) $R_f$ = 0.38. The product was isolated as a white crystalline solid; isolated yield: 2.00 g (58%). $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$= 9.92 c (s, 1H), 7.56 b (s, 2H), 2.49 a (s, 6H). Values obtained are in accordance with literature.\textsuperscript{41}
3.5 Synthesis of 4

![Compound 4](image)

**Figure 3.4: Compound 4**

2,1,3-benzothiadiazole (8.00 g, 0.0587 mol) was dissolved in 150 ml of HBr in a 500 mL two-necked round bottom flask. A solution of Br₂ (9.06 mL, 3 eq, 0.176 mol) dissolved in 100 mL of HBr was added dropwise very slowly, and the resulting mixture was stirred at reflux for 24 h. The mixture was cooled to room temperature, followed by the addition of a saturated solution of NaHSO₃ to consume excess Br₂. The solution was then filtered, washed with cold EtOH and the solvent was removed under reduced pressure. The product was isolated as a red solid; isolated yield: 14.4 g (84%). ¹H NMR (500 MHz, Chloroform-d) δ= 7.74 a (s, 2H). Values obtained were in accordance with literature.⁴²
3.6 Synthesis of 5

![Figure 3.5: Compound 5](image)

4 (4.20 g, 0.0143 mol), phenylboronic acid (5.23 g, 3 eq, 0.0429 mol), palladium tetrakis(triphenylphosphine) (0.825 g, 0.05 eq, 0.000714 mol) and cesium carbonate (14.0 g, 3 eq, 0.0429 mol) were added to a 500 mL Schlenk flask under an inert nitrogen atmosphere. A 250 mL mixture of degasses and dried DMF/toluene (1:1) was added to the reaction flask and the resulting mixture was heated to 110°C and stirred for 24 h. The mixture was cooled to room temperature, filtered and the solvent removed under reduced pressure. The resulting crude brown solid was recrystallized from EtOH. The product was isolated as a bright, yellow crystalline solid; isolated yield: 3.28 g (80%). \( ^1 \text{H NMR} \) (500 MHz, Chloroform-\( d \)) \( \delta = 8.02 - 7.91 \) b (m, 4H), 7.80 a (s, 2H), 7.62 - 7.41 c,d (m, 6H). Values obtained were in accordance with literature.\(^{42}\)
3.7 Synthesis of 6

\textbf{Figure 3.6: Compound 6}

5 (3.00 g, 0.0104 mol) was dissolved in a 330 mL mixture of EtOH/THF (3:1 v/v) and NaBH$_4$ (2.75 g, 7 eq, 0.0728 mol) was added slowly. CoCl$_2$·6H$_2$O (0.0495 g, 2 mol%, 0.000208 mol) was then added and the mixture was stirred at reflux for 2 h while monitored by TLC (in 100% DCM). Over the course of 5 h, an additional 7 eq of NaBH$_4$ and 3 mol% CoCl$_2$·6H$_2$O were added in increments while the reaction continued to stir at reflux and be monitored by TLC. Upon completion, the reaction was cooled to room temperature, filtered and the solvent removed under reduced pressure. The crude product was dissolved in 400 mL of Et$_2$O/H$_2$O (1:1 v/v), followed by an extraction of the aqueous phase with Et$_2$O (3 x 150 mL). The organic phase was collected, dried over MgSO$_4$, filtered and solvent removed under reduced pressure. The product was isolated as a white crystalline solid and immediately stored under an inert atmosphere of nitrogen; isolated yield: 2.44 g (90%). $^1$H NMR (500 MHz, Chloroform-$d$) $\delta = 7.52 - 7.35$ b,c,d (m, 10H), 6.80 a (s, 2H), 2.91 NH (br s, 4H). Values obtained are in accordance with literature.$^{43}$
3.8 Synthesis of 7

![Compound 7]

**Figure 3.7: Compound 7**

3 (0.385 g, 0.00181 mol) was dissolved in 188 mL of CHCl₃, followed by the addition of ZrCl₄ (0.0421 g, 10 mol%, 0.000181 mol) and the resulting mixture was stirred for 10 minutes. 6 (0.470 g, 0.00181 mol) was then added and the reaction was stirred for 24 h at room temperature. Upon completion, the solvent was removed under reduced pressure. The resulting crude orange solid was washed with hot EtOH. The product was isolated as a pale orange solid; isolated yield: 1.08 g (60%). ¹H NMR (500 MHz, Chloroform-d) δ = 7.89 b (d, 4H), 7.81 e (s, 2H), 7.60 – 7.53 c (m, 4H), 7.48 a (s, 2H), 7.46 – 7.42 d (m, 2H), 2.44 f (s, 6H).
3.9 Synthesis of 8 via Synthetic Scheme 1

![Neutral Axle 8](image)

**Figure 3.8: Neutral Axle 8**

7 (0.200 g, 0.000441 mol), phenylacetylene (0.0581 mL, 1.2 eq, 0.000529 mol), CuI (0.00840 g, 10mol%, 0.0000441 mol) PdCl₂(PPh₃)₂ (0.0155 g, 5 mol%, 0.0000221 mol) and PPh₃ (0.0321 g, 0.27 eq, 0.000119 mol) were added to a 250 mL Schlenk flask under an inert nitrogen atmosphere. A 125 mL mixture of degassed NEt₃/THF (3:2) was added to the reaction flask and reaction was stirred at reflux overnight. Upon completion, the reaction was cooled to room temperature, filtered and the solvent removed under reduced pressure. The crude product was attempted to be isolated by column chromatography (hexanes/DCM, 10:1 v/v). Despite multiple attempts at purification, the product was never isolated via this synthetic route.
3.10 Synthesis of 9 via Synthetic Scheme 2

![Chemical Structure](image)

*Figure 3.9: Compound 9*

3 (0.600 g, 0.00282 mol), phenylacetylene (0.371 mL, 1.2 eq, 0.00338 mol), CuI (0.0536 g, 10 mol%, 0.000282 mol), PdCl$_2$(PPh$_3$)$_2$ (0.0988 g, 5 mol%, 0.000141 mol) and PPh$_3$ (0.199 g, 0.27 eq, 0.000760 mol) were added to a 250 mL Schlenk flask under an inert nitrogen atmosphere. A 75 mL mixture of degassed NEt$_3$/THF (3:2) was added to the reaction flask and reaction was stirred at reflux overnight. Upon completion, the reaction was cooled to room temperature, filtered and the solvent removed under reduced pressure. The crude product was attempted to be isolated by column chromatography (hexanes/DCM (3:1 → 1:1 v/v). Despite multiple attempts at purification, the product was never isolated via this synthetic route.
3.11 Synthesis of 1b

![Figure 3.10: Compound 1b](image)

A solution of 2,6-dimethylaniline (5.00 g, 0.0413 mol) in 41 mL of dry MeCN was placed in a 250 mL Schlenk flask at 0°C under an inert nitrogen atmosphere. A solution of NBS (7.34 g, 0.0413 mol) in MeCN was added dropwise via an addition funnel over 1 h. The reaction was allowed to come to room temperature and stirred for an additional 72 h. The reaction was then quenched via the addition of DI H₂O. MeCN was removed under reduced pressure, followed by extraction with EtOAc (3 x 150 mL). The remaining organic phase was isolated, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting crude purple solid was recrystallized from hexanes. The product was isolated as a light brown solid; isolated yield: 4.90 g (59%). ¹H NMR (500 MHz, Chloroform-d) δ = 7.09 b (s, 2H), 3.61 NH (br s, 2H), 2.18 a (s, 6H). Values obtained are in accordance with literature.³⁹
3.12 Synthesis of 2b

![Figure 3.11: Compound 2b](image)

1b (5.00 g, 0.0250 mol) was suspended in 100 mL of DI H₂O. The mixture was kept at 0°C and concentrated H₂SO₄ (7.14 mL, 5 eq, 0.125 mol) was added dropwise. The mixture was stirred for 0.5 h, after which 67 mL of acetone was added and the contents stirred for an additional 0.5 h. A solution of NaNO₂ (5.17 g, 3 eq, 0.0750 mol) in 80.5 mL DI H₂O was added dropwise and the resulting mixture was stirred for 1 h. While maintaining the temperature of the reaction at 0°C, a solution of KI (20.7 g, 5 eq, 0.125 mol) in 82.5 mL DI H₂O was added dropwise. The solution turned brown and was allowed to stir while warming to room temperature overnight. Following completion, an extraction with EtOAc (3 x 100 mL) was performed. The organic phase was collected and washed once with 165 mL of a 1M HCl solution and twice with 100 mL of a 20 wt% NaHSO₃ solution. The remaining organic phase was then collected, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified and isolated by column chromatography (100% hexanes) Rf = 0.56. The product was isolated as a white crystalline solid; isolated yield: 6.25 g (80%). 1H NMR (500 MHz, Chloroform-d) δ = 7.20 b (s, 2H), 2.44 a (s, 6H). Values obtained are in accordance with literature.⁴⁰
3.13 Synthesis of 12

Figure 3.12: Compound 12

Phenylacetylene (0.706 mL, 0.00643 mol) was dissolved 16 mL of dry THF at 0°C under an inert nitrogen atmosphere. i-PrMgCl•LiCl in THF (1.3 M, 5.44 mL, 1.1 eq, 0.00707 mol) was added dropwise while maintaining the temperature at 0°C, and the resulting mixture was then stirred at room temperature for 1 h. This alkyne Grignard reagent was used directly for the cross coupling reaction.
### 3.14 Synthesis of 13

![Figure 3.13: Compound 13](image)

**Figure 3.13: Compound 13**

2b (5.00 g, 2.5 eq, 0.0161 mol) was dissolved 25 mL of dry THF at 0°C under an inert nitrogen atmosphere. i-PrMgCl•LiCl in THF (1.3 M, 13.6 mL, 1.1 eq, 0.0177 mol) was added dropwise while maintaining the temperature at 0°C, and the resulting mixture was then stirred at room temperature for 1.7 h. This aryl Grignard reagent was used directly for the cross coupling reaction.
3.15 Synthesis of 14

![Figure 3.14: Compound 14](image)

The solution containing 13 was transferred to the flask containing a solution of 12 via syringe the resulting mixture was stirred for 5 to 10 min at room temperature. TEMPO (3.87 g, 3.85 eq, 0.0248 mol) was added and the mixture was stirred at reflux for 2.5 h. The reaction mixture was then treated with hexanes (35 mL) and saturated aqueous NH₄Cl solution (65 mL). The aqueous layer was extracted with hexanes (2 x 60 mL). The remaining organic phase was then collected, washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The product was purified and isolated by column chromatography (100% hexane) Rᵢ= 0.34. The product was isolated as a white crystalline solid; isolated yield: 1.68 g (91%). ¹H NMR (500 MHz, Chloroform-d) δ 7.56 – 7.51 c (m, 2H), 7.39 – 7.32 a, b (m, 3H), 7.24 e (s, 2H), 2.48 d (s, 6H). Values obtained are in accordance with literature.³⁵
3.16 Synthesis of 9 via Synthetic Scheme 3

![Figure 3.15: Compound 9](image)

14 (2.75 g, 0.00964 mol) was dissolved in 150 mL of dry THF in a 500 mL Schlenk flask under an inert nitrogen atmosphere. The reaction was cooled to -78°C, followed by dropwise addition of nBuLi (6.63 mL of a 1.6M solution in hexanes, 1.1 eq, 0.0106 mol). While maintaining the temperature at -78°C, the reaction mixture was stirred for 1 h, after which DMF (2.44 mL, 3.4 eq, 0.0328 mol) was added dropwise and the reaction warmed to room temperature overnight. The reaction was then acidified with 35 mL of a 5 wt% HCl solution, followed by an extraction with Et₂O (3 x 60 mL). The organic phase was collected, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified and isolated by column chromatography (hexane/CHCl₃, 10:1 v/v) Rf = 0.23. The product was isolated as a white crystalline solid; isolated yield: 1.87 g (83%). M.P. 78°C. HR-MS: Calculated for [M+H]+ [C₁₇H₁₅O]+ m/z = 235.1123; found m/z = 235.1121.

¹H NMR (500 MHz, Chloroform-d) δ = 9.95 a (s, 1H), 7.58 b (s, 2H), 7.57 – 7.55 d (m, 2H), 7.41 – 7.37 e,f (m, 3H), 2.58 c (s, 6H).

¹³C NMR (126 MHz, CDCl₃) δ= 192.06, 141.04, 135.00, 131.63, 129.46, 128.90, 128.56, 127.85, 123.06, 101.80, 86.56, 21.12.
3.17 Synthesis of 8 via Synthetic Scheme 3

![Figure 3.16: Neutral Axle 8](image)

6 (0.389 g, 0.00149 mol) and 9 (0.350 g, 1 eq, 0.00149 mol) were dissolved in 15 mL of CHCl₃. ZrCl₄ (0.0418 g, 10 mol%, 0.000149 mol) was added and the reaction was stirred for 24 h at room temperature. Upon completion, NEt₃ (0.5 mL) was added to neutralize any protonated compound and the solvent was removed under reduced pressure. The resulting crude orange solid was recrystallized from EtOH. The product was isolated as an off white solid; isolated yield: 0.388 g (54%). M.P. 233°C.

HR-MS: Calculated for [M+H]+ [C₃₅H₂₆N₂]⁺ m/z = 475.2174; found m/z = 475.2174.

¹H NMR (500 MHz, DMSO-d₆) δ = 12.67 NH (s, 1H), 8.17 b’ (d, J = 7.2 Hz, 2H), 8.12 e (s, 2H), 7.74 d (d, J = 7.2 Hz, 2H), 7.60 – 7.58 g (m, 2H), 7.58 – 7.54 c’ (m, 2H), 7.53 a’ (d, J = 3.6 Hz, 1H), 7.51 – 7.48 c (m, 2H), 7.48 – 7.43 h,i (m, 3H), 7.41 d,d’ (t, J = 7.4 Hz, 2H), 7.32 a (d, J = 7.7 Hz, 1H), 2.56 f (s, 6H).

¹³C NMR (126 MHz, DMSO) δ = 151.95, 142.03, 139.94, 138.20, 137.94, 133.53, 131.25, 129.87, 129.33, 128.92, 128.83, 128.76, 128.52, 128.33, 127.56, 127.13, 125.62, 125.29, 123.47, 122.54, 121.36, 99.30, 86.75, 45.55, 20.79, 8.60.
3.18 Synthesis of 15

![Figure 3.17: Compound 15](image)

4 (1.00 g, 0.00340 mol) was dissolved in 50 mL of absolute EtOH in a 250 mL Schlenk flask under an inert nitrogen atmosphere at 0°C. NaBH$_4$ (1.29 g, 10 eq, 0.0340 mol) was then added slowly over 1 h. The reaction was allowed to stir at 0°C for 1 h and warmed to room temperature overnight. Upon completion, H$_2$O (10 mL) was added and EtOH removed under reduced pressure followed by an extraction with DCM (3 x 20 mL). The organic phase was collected, dried over MgSO$_4$, filtered and solvent removed under reduced pressure. The product was isolated as a light yellow crystalline solid; isolated yield: 0.83 g (92%). $^1$H NMR (500 MHz, Chloroform-$d$) $\delta = 7.74$ ppm a (s, 2H). Values obtained are in accordance with literature.$^{43}$
3.19 Synthesis of 16

![Compound 16](image)

**Figure 3.18: Compound 16**

15 (0.750 g, 0.00282 mol) and 9 (0.661 g, 1 eq, 0.00282 mol) were dissolved in 60 mL of CHCl₃. ZrCl₄ (0.0657 g, 10 mol%, 0.000282 mol) was added and the reaction was stirred for 24 h at room temperature. Upon completion, NEt₃ (0.5 mL) was added to neutralize any protonated compound and the solvent was removed under reduced pressure. The resulting crude orange solid was washed with MeOH. The product was isolated as a pale yellow solid; isolated yield: 0.979 g (72%). ¹H NMR (500 MHz, Methylene Chloride-\(d_2\)) \(\delta\) 9.70 NH (br s, 1H), 7.84 b (s, 2H), 7.62 – 7.55 d (m, 2H), 7.43 – 7.38 e,f (m, 3H), 7.33 a (s, 2H), 2.62 c (s, 6H).
3.20 Synthesis of Precrown, Pentaethyleneglycol-dipent-4-enyl ether

![Diagram of Pentaethyleneglycol-dipent-4-enyl ether](image)

**Figure 3.19:** Pentaethyleneglycol-dipent-4-enyl ether

Ditosylated pentaethylene glycol was prepared according to literature procedure.\(^4\) NaH in 60% mineral oil (15.7 g, 0.393 mol) was slowly added to 200 mL of dry THF in a 500 mL Schlenk flask under an inert nitrogen atmosphere at 0°C. 4-pentene-1-ol (33.8 g, 1 eq, 0.393 mol) was dissolved in 40 mL of THF, added to the reaction mixture dropwise and allowed to stir for 1 h. Ditosylated pentaethylene glycol (21.45 g, 0.1 eq, 0.0393 mol) was dissolved in 125 ml of THF and added slowly to the reaction mixture via a syringe. The reaction mixture was allowed to warm to room temperature and stirred for 48 h after which the reaction was quenched with 500 mL of water. The non-aqueous solvent was removed under reduced pressure and the aqueous phase was extracted with CHCl₃ (3 x 250 mL). The organic phase was collected, washed with 1 M HCl (2 x 100 mL), dried over MgSO₄, and the solvent removed under reduced pressure. The crude product was purified and isolated by column (EtOAc/Hexanes, 3:2 v/v) \(R_f = 0.4\). The product was isolated as a colourless
oil; isolated yield: 6.45 g (64%).$^1$H NMR (500 MHz, Chloroform-$d$) $\delta =$ 5.81 c (br m, 2H), 5.02 b (m, 2H), 4.96 a (m, 2H), 3.65 h (s, 16H), 3.56 g (br m, 4H), 3.46 f (t, 4H), 2.11 d (br m, 4H), 1.68 e (br m, 4H). Values are in accordance with literature.$^{45}$
3.21 Synthesis of [8-H][BF₄⁻]

**Figure 3.20: Compound [8-H][BF₄⁻]**

8 (0.263 g, 0.000554 mol) was suspended in MeCN (5 mL) and HBF₄·Et₂O (75.4 μL, 1 eq, 0.000554 mol) was added dropwise. The reaction was allowed to stir for 1 h. The solvent was removed under reduced pressure and the resulting solid was washed with Et₂O (2 x 20 mL) to remove excess acid, filtered and collected. The product was isolated as a white solid; isolated yield: 0.286 g (92%). ¹H NMR (500 MHz, Acetonitrile-d₃) δ = 12.26 NH (s, 2H), 7.82 e (s, 2H), 7.76 – 7.73 b (m, 4H), 7.71 a (s, 2H), 7.67 – 7.63 c,d (m, 6H), 7.61 – 7.57 g (m, 2H), 7.48 – 7.44 h,i (m, 3H), 2.63 f (s, 6H).

Figure 3.21: [2]Rotaxane 11, E/Z isomers

[8-H][BF₄] (0.230 g, 0.000421 mol) and pentaethylene glycol-dipent-4-enyl ether (0.237 g, 1.5 eq, 0.000632 mol) were added in a 100 mL Schlenk flask under an inert nitrogen atmosphere. Dry DCM (30 mL) was added to the flask and reaction stirred for 10 min. Grubbs I catalyst [[RuCl₂(=CHPh)(PCy₃)₂] (0.0345 g, 10 mol%, 0.0000421 mol) was dissolved in DCM (1 mL) and added to the reaction. The reaction was stirred at reflux for 18 h after which additional catalyst (0.0173 g, 5 mol%, 0.0000211 mol) was added. The reaction was monitored by ¹H NMR and 5 mol% of catalyst was added in 24 h intervals until a total of 25 mol% catalyst was added. Upon completion, the solvent was removed under reduced pressure, and the crude solid was washed and sonicated in Et₂O. The solid was re-dissolved in DCM and washed with saturated solution of NaHCO₃. The crude product was purified and isolated by column (Hexanes/ EtOAc, 7:1 → 2:1 v/v) Rᵢₜ₁ isomer 1 = 0.5, Rᵢₜ₂ isomer 2 = 0.22.

The product obtained from the column was further purified via recrystallization from EtOH. Isomer 1 (E) was isolated as a white crystalline solid; isolated yield:
0.0310 g. **Isomer 2 (Z)** was isolated as a grey crystalline solid; isolated yield 0.0300 g. Combined yield: 0.0610 g (18%). M.P. 186.8°C.

HR-MS: Calculated for [M+H]+ [C_{53}H_{61}N_{2}O_{6}]^+ m/z = 821.4529; found m/z = 821.4536.
3.23 Synthesis of [8-H][Cl]

Figure 3.22: Compound [8-H][Cl]

8 (0.030 g, 0.0000632 mol) was dissolved in DCM (5 mL) and HCl·Et₂O (excess) was added dropwise until a white precipitate appeared. The resulting solid was filtered and collected. The product was isolated as a white solid; isolated yield: 0.0290 g (92%). 1H NMR (500 MHz, DMSO-d₆) δ = 8.10 e (s, 2H), 7.90 b (d, J = 7.5 Hz, 4H), 7.62 a (d, J = 3.4 Hz, 2H), 7.61 - 7.56 g,h,i (m, 5H), 7.49 d (t, J = 3.8 Hz, 2H), 7.46 c (dd, J = 5.2, 1.9 Hz, 2H), 2.57 f (s, 6H).
3.24 Preparation of EYPC large unilamellar vesicles (LUVs)

A phospholipid film was formed by evaporating a 1 mL chloroform solution containing 25 mg of EYPC under reduced pressure at 25°C, over 2 hours. The lipid film was then hydrated with 750 µL of a NaCl (500 mM) and phosphate buffer solution (5 mM, pH= 7.2). The resulting suspension was subjected to at least 8 freeze/thaw/vortex cycles (1 cycle = 1 min at -78°C, followed by 1 min at 35°C, and 1 min in vortex). The solution was then extruded through a 100 nm polycarbonate membrane 21 times until the solution was transparent. The resulting solution was passed down a Sephadex G-25 column to remove any extravesicular NaCl. The liposomes were eluted with a solution containing NaNO₃ (500 mM) and phosphate buffer (5 mM, pH=7.2). 3.2 mL of liposome solution were isolated after separation and the stock solution was diluted to obtain a 5 mM lipid solution, assuming all EYPC was incorporated into the liposomes.
3.25 Chloride Transport Assays with EYPC LUVs

A solution of EYPC LUVs (60 μL, 5 mM) was added to a 1.2 mL gently stirred buffer solution containing NaNO₃ (500 mM) and phosphate buffer (5 mM, pH= 7.2). The chloride efflux was monitored as a function of time by a chloride selective-electrode. Experiments were initiated by the addition of a small amount of a DMSO solution of the respective transporter with concentrations varied from 6.25 to 25 mol% relative to the concentration of EYPC. At t= 540 s, 100 μL of a Triton-X 5% solution was added to lyse all liposomes and obtain the maximum of chloride efflux. Experiments were repeated in triplicate and all the reported traces are the average of the three identical trials.

3.26 Conversion of chloride concentration data into % of chloride efflux

Each transport assay curve is normalized using the following equation:

\[
\% Cl_{efflux} = \frac{[Cl^-] - [Cl^-]_0}{[Cl^-]_{max} - [Cl^-]_0} \times 100
\]

With \( [Cl^-]_0 = \) chloride efflux at initial time

\( [Cl^-]_{max} = \) chloride efflux at the end of experiment
REFERENCES


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**Author:** Claude-Rosny Elle, Nadim Noujeim, Christophe Perdrix, Andrea R. Schmitter  

**Publication:** Chemical Communications  

**Publisher:** Royal Society of Chemistry  

**Date:** Dec 3, 2010  

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Place of Birth: Windsor, Ontario, Canada

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  Lakeshore, Ontario, Canada

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