
by

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This thesis work is dedicated to my late father

Upendra Choudhary
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### Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Aq</td>
<td>Aqueous</td>
</tr>
<tr>
<td>CT</td>
<td>Charge-transfer</td>
</tr>
<tr>
<td>CuAAC</td>
<td>Copper catalized alkyne-azide click</td>
</tr>
<tr>
<td>DIAD</td>
<td>Diisopropyl azodicarboxylate</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethyl formamide</td>
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<tr>
<td>DMPA</td>
<td>Dimthoxy phenyl acetophenone</td>
</tr>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DNP38C10</td>
<td>1,5-Dioxynaphthalene-38-crown-10</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
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<td>ESI/APCI-MS</td>
<td>Electron spray ionisation/Atmospheric pressure chemical ionisation-mass spectroscopy</td>
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<td>ε</td>
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<td>GPC</td>
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<td>Me$_6$TREN</td>
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<td>MIM</td>
<td>Mechanically interlocked molecules</td>
</tr>
<tr>
<td>MV</td>
<td>Methyl viologen</td>
</tr>
<tr>
<td>NAD+</td>
<td>Nicotinamide adenine dinucleotide (Oxidised)</td>
</tr>
<tr>
<td>NDI</td>
<td>Naphthalene dimide</td>
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<td>Single electron transfer living radical polymerization</td>
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Dissertation Abstract

Mechanically interlocked molecules (MIMs) have attracted a significant research interest for more than five decades and still need efficient synthetic methods for industry-viable applications. The thesis work herein describes the incremental use of thiol-maleimide click chemistry to synthesize [2]rotaxanes with very good yields (Chapter 2). The use of these [2]rotaxanes was further extended to the design of novel aqueous-soluble pseudorotaxane (Chapter 4) and mechanically interlocked polymeric materials (Chapter 5).

As macrocycles are inherent part of a MIM, the synthesis of five different electron rich allyl functionalized dioxynaphthalene[38]Crown-10 macrocycles (DNP38C10) are described along with their thermodynamics of binding with electron poor methyl viologen derivatives (Chapter 3). The incorporation of a single mechanical bond in a polymer has been a highly sought after goal for some time. This thesis addresses the difficulty of synthesizing such interlocked polymers through the incorporation of some of these functionalized DNP38C10 macrocycles, enabling the synthesis of, previously unavailable, gram scale novel 3-arm[2]rotaxane polyacrylates. These polymers will pave ways for designing new materials with enhanced thermal and mechanical properties. All the results in this thesis are supported by 1H NMR, 13C NMR, HRMS, GPC, DSC and ITC analysis.
Chapter 1

Introduction
1.1 Supramolecular chemistry

Supramolecular chemistry has been defined by one of its leading pioneers Jean-Marie Lehn as the “chemistry of molecular assemblies and of the intermolecular bond.” In other words, it is an architecture where the building blocks are individual molecular assemblies that are typically held together by a variety of non-covalent interactions such as hydrogen bonding, ion-ion and ion-dipole interaction, dispersion forces, cation-π interaction, π-π interaction and/or solvophobic interactions.

Historically supramolecular chemistry is considered to involve non-covalent interaction between a ‘host’ (generally a large molecule) and a ‘guest’ (generally a small molecule). Figure 1 schematically explains the relationship between molecular and supramolecular chemistry; atoms covalently react to form molecules and molecules non-covalently interact to form supramolecular assemblies, which can be used for various applications.2

![Figure 1. Relationship between molecular and supramolecular systems.](image-url)
Conventional top down approaches to synthesize complex molecules require the synthesis of specific structures through complicated and often difficult methods. Supramolecular chemistry provides a convenient, alternative way of achieving very complex architectures using a bottom up approach. Use of supramolecular chemistry has resulted in enormous growth in diverse chemical systems. Supramolecular chemistry is now not only limited to host-guest systems but has also found its applications in nanomaterials, molecular devices and molecular machines. Self-assembly and molecular recognition play very important roles in supramolecular chemistry, and are also observed in most complex systems in nature. For example, deoxyribonucleic acid (DNA) wherein the complementary nucleotides are hydrogen bonded (Figure 2) and proteins which are folded into secondary and tertiary structures by intramolecular hydrogen bonding between amino acids.

**Figure 2.** Deoxyribonucleic acid (DNA) complementarity between base pairs.
1.1.1 Self-assembly

Self-assembly is a part of supramolecular chemistry based on complementarity and non-covalent interactions\textsuperscript{6}. Materials formed by self-assembly are often defined as an architecture in which the individual building blocks assemble in a set pattern. The individual building blocks either have specific interactions with each other or have a perfect three-dimensional arrangement. Many examples of self-assembly can be found in nature, for instance, in the lipid bilayer the individual phospholipid molecules self-assemble into bilayers. Phospholipids, which are composed of a hydrophilic head and a hydrophobic tail, arrange themselves in such a way so that only the hydrophilic end faces the water and the hydrophobic tail is protected from the hydrophilic environment (Figure 3).

![Figure 3](image)

**Figure 3.** Self-assembly of lipid bilayer due to hydrophobic effects.

The dynamic non-covalent interactions of self-assembled supramolecular assemblies can be controlled in a specific and reversible manner. Supramolecular assemblies can behave as a single unit and exhibit completely new properties as compared to their individual building blocks. Some of these features are highly
beneficial for developing new applications. In fact, self-assembly has found applications in a wide range of technologies ranging from materials science to molecular biology. Self-assembly and self-organization are dominant processes in the chemistry of biological systems and thus provide an everlasting inspiration for synthetic chemists to design new molecules and understand their properties.
1.1.2 Host-guest chemistry

Since the discovery of crown ethers by Pederson in 1967\(^9\) there has been increasing interest among scientists to understand host-guest chemistry. The concept of host guest chemistry has evolved from Emil Fisher’s\(^{10}\) lock and key model of enzyme-substrate interactions. The enzyme interacts with its substrate in a size and shape dependent manner using non-covalent interactions between the host (enzyme) and the guest (substrate)\(^{11}\).

![Diagram of α- and β-cyclodextrins with 1-decanol and toluene](image)

**Figure 4.** a) α-cyclodextrin selectively complexes with 1-decanol and β-cyclodextrin with toluene. b) 18-Crown-6 selectively complexes with potassium and 15-crown-5 with sodium cations.

Similarly, a molecule (a ‘host’) can bind to another molecule (a ‘guest’) with complementary shape and size to give rise to a ‘host–guest’ complex or supramolecular assembly. A host is generally a cyclic molecule often called as a
macrocycle and a guest can be a cation, an anion or a more sophisticated acyclic synthetic molecule. For example, 15-crown-5 and a sodium ion make a stable host-guest complex as oppose to 18-crown-6 and a sodium ion which do not complex. Similarly 18-crown-6 makes and a potassium ion make a stable host guest complex but 15-crown-5 and a potassium ion do not complex with each other.\textsuperscript{12}

In addition, $\alpha$-cyclodextrin selectively binds to 1-decanol and $\beta$-cyclodextrin selectively binds to toluene (Figure 4a).\textsuperscript{13} Because cyclodextrin macrocycles can selectively bind to specific guests they have been used in targeted drug delivery systems.\textsuperscript{14} Using this very selective, yet dynamic and reversible chemistry, highly sophisticated materials can be built for various applications.\textsuperscript{15}

1.1.3 Complementarity and molecular recognition

It was not until the end of 19\textsuperscript{th} century when chemists began to recognize a new non-covalent aspect of chemistry, namely molecular recognition. It is of central importance in a biological system such as DNA replication, as well as in chemical systems for sensors,\textsuperscript{16} analytical applications,\textsuperscript{17} separation science, and catalysis.\textsuperscript{18} Molecular recognition also plays a very important role in metal templating processes.\textsuperscript{19} The three dimensional arrangement and electronic properties of complexes determine the strength of a particular recognition. However, other factors such as the stoichiometry and the number of binding sites may also influence the recognition behavior. If the ratio of the individual components that make up the assembly changes, the number of different assemblies would change as well. Due to
the enormous scope of molecular recognition it is now widely used for assembly of complex molecular structures.\textsuperscript{20}

1.2 Mechanically interlocked molecules (MIMs)

Figure 5. a) First interlocked molecule synthesis\textsuperscript{21} b) Statistical hooplane\textsuperscript{22} c) First template directed compound.\textsuperscript{23}

Historically the nomenclature of organic compounds depends on the number of atoms, the sequence and the type of bonding between the atoms. When two or more different molecules have same number of atoms but are linked differently in space they are known as constitutional isomers. Soon it was realized that it is possible
to have isomers even if the number of atoms and the number of bonds are same, known as stereoisomers. The first molecule whose absolute structure could not be described by considering the factors mentioned above was accidently synthesized by Frisch and Wasserman\textsuperscript{21} in 1961 (\textbf{Figure 5a}) and is now considered as a catenane. Since then the term “topological isomerism” came into existence. In 1967 Harrison synthesized a rotaxane\textsuperscript{22} using Merrifield resin; it was a dumbbell shaped molecule, which he named a ‘hooplane’ shown in \textbf{Figure 5b}. It required tedious and long procedures to synthesize these molecules and in addition the yields were very poor.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6}
\caption{a) Basic types of interlocked molecules b) Complex interlocked molecules.}
\end{figure}
Clearly the synthesis of these mechanically interlocked architectures lacked structure specific interactions. During the same decade Schill\textsuperscript{23} and coworkers discovered a new method called directed synthesis, where for the first time they used a covalent template in order to synthesize interlocked structures (Figure 5c) and it gave 50\% yield. With the introduction of supramolecular chemistry in late 1980s the chemistry of mechanically-interlocked bonds has advanced profoundly.

Mechanically interlocked molecules (MIMs) are molecules that are non-covalently entangled such that they cannot be separated from each other without breaking one of the covalent bonds from the individual interlocked molecules. As shown in Figure 6a\cite{2} rotaxanes and \cite{2}catenanes are the two well-known mechanically interlocked architectures. A third type of architecture called \cite{2}pseudorotaxane, which is a thermodynamically but not kinetically stable complex, is very useful starting material in preparing \cite{2}rotaxanes. Although very challenging to synthesize, Borromean rings\textsuperscript{20b} and trefoil knots,\textsuperscript{24} have also been synthesized. The number in the parenthesis signifies the number of interlocked components in a MIM.

MIMs differ from both the supramolecular assemblies and the covalently bonded molecules in their ability to change their positions with respect to one another with or without external stimuli. This unique property of MIMs allows MIM-containing materials to undergo mechanical changes. Various oligomeric and polymeric versions\textsuperscript{25} of MIMs have also been prepared, which will be discussed later in detail. Rotaxanes have been studied more extensively as compared to catenanes.
owing to the macrocyclic movements along the thread and also the relative ease of their synthesis. Examining the properties of naturally occurring supramolecular assemblies, one can certainly think of making artificial supramolecular structures via self-assembly and efficient synthetic methods. This dissertation work describes the synthesis of new rotaxanes and their incorporation into polymeric materials and protein conjugates.

1.2.1 Rotaxanes

Rotaxanes are dumbbell-shaped assemblies where an acyclic component is threaded through a cyclic component (Figure 6a) and they cannot be separated without breaking one of the covalent bonds. The free energy of activation has to be overcome in order to separate the individual components from each other. Generally a dumbbell has a recognition unit at the center and the ends are sterically imposing bulky stoppers.

![Figure 7. Shutting of macrocycle in a [2]rotaxane with two recognition units.](Thermal/Photochemical/Electrochemical/pH)
A pseudorotaxane is an assembly where the cyclic unit can fall off the acyclic unit, as shown in Figure 6a. The motion of the cyclic unit imparts dynamic properties to the materials having rotaxane units. Figure 7 shows the shuttling motion of the macrocycle along the acyclic unit upon an external stimulus. The motion of the macrocycle can be controlled using external stimuli like photochemical, pH, electrochemical and thermal inputs. However, achieving a precise control over the motion of the macrocycle unit has been an overarching goal of scientists.

In 1983 the Sauvage\textsuperscript{30} group had a breakthrough in synthesizing a catenane using a metal ligand coordination (Scheme 1). In this metal template synthesis, Cu(I) coordinates with nitrogen of the starting components to form pseudorotaxane 3 which when reacted with tetraethylene diiodide in cesium carbonate forms interlocked catenane 4.

\textbf{Scheme 1.} First example of the catenane synthesized by templating Cu(I).
Using the same copper coordinated chemistry Gibson\textsuperscript{31} and coworkers synthesized a rotaxane with 40 % yield, (Scheme 2). They combined together the diphenol and macrocycle 2 in the presence of copper to form pseudorotaxane 3 and which when reacted with triaryl stopper 5 forms [2]rotaxane 6. Many examples of the metal template synthesis have been reported since then.\textsuperscript{32}

Later using π-π templating interactions Stoddart\textsuperscript{33} reported a synthesis of a two station [2]rotaxane which was new leap towards designing molecular machines and nanoelectronics. Since then the term “molecular shuttle” came into existence. As shown in the Scheme 3, electron poor cyclobis(paraquat-p-phenylene) (CBPQT\textsuperscript{4+}) 7 and π-electron rich hydroquinol 8 form an assembly by making use of π-π interactions during cyclization. This was the first example of a complete organic- templated
rotaxane synthesis. In addition, Sanders and coworkers efficiently made use of π-π interactions to synthesize complex water soluble systems.34

Despite tremendous progress in the synthesis of rotaxanes using active templates, the scope of this chemistry was narrow. In order to increase the horizons of the MIMs, the Leigh group synthesized a peptide rotaxane shown in Scheme 4 inspired by the complexity in biomolecules. Here the macrocycle encapsulates the peptide with hydrogen bond interactions. More recently use of hydrogen bonding interactions gave rise to various new architectures like molecular machines, molecular switches and catalysts.

Stoddart and coworkers in 1996 discovered the first rotaxane using a dialkyl ammonium guest and a dibenzo crown ether macrocycle. The synthesis utilizes the hydrogen bonding interactions between the macrocycle oxygen atoms and the ammonium hydrogen atoms.

Figure 8. Dialkyl ammonium based crown ether [2]rotaxane.
Hydrophobic interactions are very important in biomolecules. One of the very important inclusion complexes that are formed due to hydrophobic interactions are cyclodextrin inclusion complexes. Cyclodextrin has a hydrophobic core and hydrophilic outer sphere which allows it to form inclusion complex with hydrophobic guests.39

Scheme 5. Synthesis of cyclodextrin based rotaxane. α-cyclodextrin forms an inclusion complex with 10.
1.3 Common synthetic strategies used to synthesize [2]rotaxanes

The most commonly used methods for rotaxane synthesis are shown in Figure 9 of which the threading followed by capping method (Figure 9b) is widely used because of its efficiency. The threading followed by capping method involves...
formation of a thermodynamically stable pseudorotaxane and then kinetically trapping the macrocycle by reacting it with sterically imposing stoppers. An active template method, which is among the well-studied methods, involves a metal or ion that pre-organizes the individual components of the rotaxane and they can be reacted to form a rotaxane. Many new recent strategies like shrinking and expanding\(^{40}\) have also been employed for the synthesis of rotaxanes. A wide range of reactions like Williamsons ether synthesis,\(^{41}\) amide and ester bond forming reactions,\(^{42}\) Glaser and Eglinton reaction,\(^{43}\) imine-bond formation,\(^{44}\) metal ligand coordination,\(^{45}\) ring-closing metathesis,\(^{46}\) and the well know copper catalyzed alkyne-azide click chemistry\(^{47}\) have been employed to synthesize rotaxanes.

**1.4 Thiol-maleimide click chemistry.**

![Scheme 6. Generic thiol-maleimide base catalyzed reaction.](image)

Historically thiol-maleimide reactions have been used in biochemistry to prepare bio-conjugates. Polyethylene glycol substituted maleimide chains are often used as link protein molecules to the surfaces of other proteins and enzymes. The double bond in maleimide readily reacts with the cysteine found on the protein, and researchers have explored a wide range of applications of this interesting chemistry. Maleimides in organic chemistry are used in many Diels alder and Michael addition reactions. The reaction is quite specific for thiols and is very rapid, high yielding and
doesn’t require difficult workup procedure, and forms thermodynamically stable products. These properties of the reaction prompted us to explore this chemistry and its versatility to prepare rotaxanes.

1.4.1 Computational studies of thiol-maleimide click chemistry.

(This section is based on the “Thiol-maleimide “click-chemistry”: evaluating the influence of solvent, initiator, and thiols on the reaction mechanism, kinetics, and selectivity” Polymer Chemistry, 2015, 6, 3415-3430, where I contributed by computationally investigating the influence of different thiols on overall reaction energetics.)

![Diagram](image)

**Figure 10.** a) Mechanism for the thiolate- catalyzed addition of thiol to N-substituted maleimide. b) Formation of a thiolate anion from an acid-base equilibrium reaction. c) Formation of a thiolate anion following a nucleophile-mediated pathway.
As shown in Figure 10 we have explored the energetics and mechanism of base- and nucleophile-initiated thiol additions to maleimide computationally using the Gaussian 09 program. The catalytic cycle of thiolate addition to maleimide is straightforward. We have showed the mechanism leading to initial formation of catalytic thiolate can follow a combination of several potential mechanistic pathways: direct deprotonation of the thiol by an initiator, attack of the maleimide π-bond by a thiol-initiator ion pair, and/or nucleophilic attack of maleimide by the initiator.\textsuperscript{48} Computational and kinetic modeling indicate that the choice of solvent, initiator, and thiol directly influences whether product formation follows a base-, nucleophile-, or ion pair-initiated mechanism.

The result of the computational studies were applied to ternary thiol-maleimide reactions between N-methyl maleimide, thiophenol and 1-hexanol in different combinations of solvents and initiators. The experimental results supports many of the computational studies. This study will enormously help chemists better understand how reaction conditions can influence thiol-maleimide reactions and selection impacting area: ranging from small molecules to polymers to bioconjugates.
1.5 Applications of rotaxanes

As discussed previously, one of the most important properties of rotaxanes is the dynamic nature of the macrocycle and its movement, which can be controlled with external stimuli. The inventions of rotaxanes containing mesoporous silica nanoparticles, molecular muscles, molecular elevators, molecular machines and molecular switches gives excellent direction to achieve very complex processes in biomolecules and also produce new composites materials. More recently David Leigh and coworkers developed a mechanically interlocked rotaxane architecture which was used to deliver an anticancer drug autonomously. Polymeric versions of cyclodextrin based materials have found their applications in many biomedical applications. Understanding the structure-property relationships of interlocked molecules is very important in designing specific applications. Hence it is imperative to invent new synthetic routes to diverse mechanically interlocked assemblies so that they can serve predefined purposes. After all nature has had over a billion years of head start for its evolution, with sophisticated analytical tools it is possible to imagine a future with nanomachines and nanomaterials are derived from MIMs, in particular rotaxanes.
1.6 Goal and motivation

Despite tremendous advances in synthetic methodologies there is still a need for an efficient synthetic tool to incorporate mechanically interlocked molecules, in particular rotaxanes, into materials. In addition, it is surprising to find very few examples of water soluble rotaxanes which will be immensely useful in the medical field owing to the free movement of the macrocycle that can impart stimuli responsive movements. This thesis work aims to utilize thiol-maleimide click chemistry for efficient syntheses of [2]rotaxanes and rotaxane-based of materials. With the successful synthesis of [2]rotaxanes we were particularly interested in synthesizing and studying the properties of polymeric versions of the [2]rotaxanes, as integration of mechanical bonds into polymers will have profound effects on the physical properties of the polymers.

With the synthesis of a [2]pseudorotaxane using derivatized glutathione as a stopper, we were particularly interested in studying water soluble versions of protein stoppered [2]rotaxanes as well. As the motions of the macrocycle can be controlled in a rotaxane using external stimuli, we were particularly interested in using this property of the macrocycle to impart allostERIC effects in proteins.
1.7 References


Chapter-2 Rotaxanes and Biofunctionalized Pseudorotaxanes via Thiol-Maleimide Click Chemistry.

This chapter is based on the following work,

Rotaxanes and Biofunctionalized Pseudorotaxanes via Thiol-Maleimide Click Chemistry

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*Organic Letters* 2012 14 (8), 2082-2085
2.1. Abstract

Base catalyzed thiol-maleimide click chemistry has been applied to the synthesis of neutral donor-acceptor [2]rotaxanes in good yield. This method is extended further to the synthesis of a glutathione-functionalised [2]pseudorotaxane, a precursor to integrated conjugates of interlocked molecules with proteins and enzymes. The results are supported by NMR, UV-Vis and mass spectroscopy.

2.2 Introduction

Molecules possessing mechanical bonds have attracted significant research interest for more than five decades.\textsuperscript{1} Initial syntheses of mechanically interlocked catenanes and rotaxanes by Wasserman\textsuperscript{3} and Harrison,\textsuperscript{4} respectively, were achieved in very low yield (<5%) owing to the statistical formation of interlocking species. Beginning in the 1980s, however, synthetic methods for the preparation of mechanically interlocked molecules have advanced apace, largely through the use of template-directed self-assembly protocols\textsuperscript{5} used in conjunction with kinetic and thermodynamic covalent bond forming reactions\textsuperscript{6} that “lock” individual components together. Increased synthetic efficiency has allowed increasingly complex mechanically interlocked structures to be synthesized, many of which have been designed to be stimuli-responsive\textsuperscript{4b, 7} and form the basis of various molecular machines.\textsuperscript{8} Toward the aim of increasing both synthetic ease and structural complexity, one of the most notable advances in the construction of mechanically interlocked molecules has been the introduction of highly efficient “click” reactions to their synthesis.\textsuperscript{9} In 2006, the groups of Leigh,\textsuperscript{10} Sauvage,\textsuperscript{11} and Stoddart\textsuperscript{12} each
independently reported the efficient synthesis of interlocked rotaxanes utilizing copper(I) catalyzed alkyne–azide click (CuAAC) chemistry, obtaining [2] and [3]rotaxanes in up to 94% isolated yield.

The many attributes of click reactions, particularly the ability to tolerate low temperatures and high concentrations, make them particularly well suited to the threading followed by stoppering approach to mechanically interlocked molecules, as the thermodynamically controlled threading step is most favorable at low temperatures and high concentrations. While the vast majority of click chemical approaches to mechanically interlocked synthesis have used the CuAAC reaction, other click methods such as those using thiol-yne, thiol-ene, and nitrile-N-oxide procedures have been successfully applied as well.


**Scheme 1.** Noncovalent self-assembly of [2]pseudorotaxane 4 followed by thiol-maleimide click chemistry mediated interlocking To give [2]rotaxane 5 in the presence of catalytic Et$_3$N.

Here we report the application of thiol-maleimide click chemistry to the synthesis of mechanically interlocked rotaxanes and noncovalently associated
pseudorotaxanes. The highly selective reactivity of thiols for maleimides has been utilized for many years in the context of bioconjugate chemistry.\textsuperscript{18} In the presence of catalytic base the thiol-maleimide Michael addition product can be obtained rapidly (minutes) and often in quantitative yields. Indeed the extensive history of thiol-maleimide bioconjugate chemistry is what inspired us to explore the use of thiol-maleimide chemistry in the synthesis of interlocked molecules. Once suitable conditions are developed it is expected that stimuli-responsive mechanically interlocked molecules can be directly integrated with biological systems (e.g., proteins, enzymes) through thiol-maleimide click reactions involving maleimide-functionalized supramolecules and free thiol moieties of solvent assessible cysteine residues (see chapter 5). Toward this aim we demonstrate the efficient synthesis and self-assembly of a glutathione-functionalized [2]pseudorotaxane. Such integration of artificial molecular machines with biological systems may open the door to external allosteric control of the functionality of biological molecules.

The design of maleimide-functionalized naphthalene diimide (NDI) guest 1 and thiol-functionalized sterically imposing “stopper” 2 is shown in Scheme 1. Noncovalent self-assembly of the electron poor naphthalene diimide with electron rich crown ethers such as DNP38C10 has been well studied,\textsuperscript{19} particularly by Sanders\textsuperscript{2, 20} and co-workers. The interaction between NDI derivatives and DNP38C10 ($K_a \approx 10^2 \text{ M}^{-1}$, CHCl$_3$:MeOH)\textsuperscript{20d} is not typically as strong as the interactions between dibenzylammonium guests with dibenzo-24-crown-8 hosts ($K_a = 10^2$–$10^4 \text{ M}^{-1}$, CHCl$_3$:CH$_3$CN)\textsuperscript{21} or those between bipyridinium derivatives with
**DNP38C10** hosts \((K_a = 10^4 - 10^5 \text{ M}^{-1}, \text{CH}_3\text{CN})\).\(^{21b,22}\) NDI derivatives are, however, stable to base and therefore suitable for base catalyzed thiol-maleimide reactions whereas dialkylammonium and N-benzylbipyridinium guests are susceptible to deprotonation\(^{7b}\) or nucleophilic attack,\(^{23}\) respectively.

Mixing a 1:1 molar ratio of 1 and **DNP38C10** macrocycle 3 in CHCl₃ at ambient temperature results in the instantaneous formation of a deep red solution. \(^1\text{H}\) NMR spectroscopy of this solution (Figure 1C) indicated the formation of thermodynamically stable [2]pseudorotaxane 4 through changes in the chemical shifts of diagnostic proton signals \(H_b\)–\(H_e\). At 300 MHz and 298 K dethreading of

![Figure 1. Partial \(^1\text{H}\) NMR spectra (CDCl₃, 298 K, 1mM) of thread 1 (A), macrocycle 3 (B), [2]pseudorotaxane 4 (C) (263 K), and [2]rotaxane 5 (D). Protons are labeled as in Scheme 1. Uncomplexed peaks are designated as “(uc)”.

Uncomplexed peaks are designated as “(uc)”.
macrocycle 3 onto and off of thread 1 is comparable to the $^1$H NMR time scale, leading to significant broadening of resonances for both compounds. A better resolved spectrum of [2]pseudorotaxane 4 is obtained at 263 K (Figure 1C) where the dethreading rate is decreased. Further evidence of a self-assembling host–guest complex is obtained from UV/vis spectroscopy (Figure 2). A prominent charge-transfer (CT) band is observed at 487 nm for a 0.001 M CHCl$_3$ solution of 1 and 3 (apparent $\varepsilon = 143$ M$^{-1}$ cm$^{-1}$).

Adding 2.2 equiv of thiol-functionalized stopper 2 to a 0.1 M CDCl$_3$ solution of [2]pseudorotaxane 4 at 273 K in the presence of 0.03 equiv of Et$_3$N results in the formation of mechanically interlocked [2]rotaxane 5 (Scheme 1) in 65% isolated yield. The formation of mechanically interlocked [2]rotaxane 5 was confirmed.
(see materials and methods) by accurate mass APCI mass spectrometric analysis: $m/z = 2653.28$ [M + Na]$^+$ and 1338.13 [M + 2Na]$^{2+}$ compared with calculated values of 2653.28 and 1338.14, respectively. It is interesting to note that the relative intensity of the [M + 2Na]$^{2+}$ peak of [2]rotaxane 5 is twice that of the [M + Na]$^+$ peak. Super- and supramolecular assemblies of NDI derivatives with DNP38C10 are known\textsuperscript{7c,20g} to interact with alkali metals in solution. Mass spectrometric analysis of [2]rotaxane 5 suggests these interactions persist in the gas phase as well. Mass spectra of the macrocycle and thread components in isolation show relatively weak intensities of [M + 2Na]$^{2+}$ peaks.

The $^1$H NMR spectrum of 5 (Figure 1D) displays well-resolved, sharp peaks commensurate with a kinetically stable, interlocked species. Characteristic upfield shifts of protons $H_c$, $H_d$, and $H_e$ (0.92, 0.52, and 0.42 ppm, respectively) are observed and are indicative\textsuperscript{19b,19d,20f} of [π···π] stacking interactions. An upfield shift of 0.52 ppm is also observed for the aromatic proton $H_b$ of the NDI guest. The disappearance of maleimide proton $H_a$ and the formation of new signals in the 3.2–2.5 ppm region indicate the formation of a thiol-maleimide Michael adduct. Investigation of [2]rotaxane 5 by UV/vis spectroscopy reveals a charge-transfer (due to π-π interaction) band at 497 nm with a apparent molar extinction coefficient of $\varepsilon = 714$ M$^{-1}$ cm$^{-1}$, which is consistent\textsuperscript{19c,20d} with other interlocked crown ether–naphthalene diimide systems.

One of the primary motivations of this work is the development of efficient methods for integrating stimuli-responsive interlocked molecules with biological systems. As an initial, though important, first step toward this goal, glutathione-functionalized thread 6 (Scheme 2) was synthesized and its self-assembly with 3 was investigated.


A solvent mixture capable of solubilizing both L-glutathione and macrocycle 3 could not be found; therefore N'-Boc-L-glutathione dimethyl ester,\textsuperscript{24} which is soluble in a range of organic solvents, was prepared. Reacting this soluble glutathione derivative with thread 1 in CHCl\textsubscript{3} and catalytic Et\textsubscript{3}N gave glutathione-functionalized thread 6. Addition of 1.0 equiv of 3 to thread 6 in
CDCl$_3$ resulted in a red solution indicating the formation of [2]pseudorotaxane 7 (Scheme 2). [2]Pseudorotaxane 7 can also be obtained in one pot by adding catalytic Et$_3$N to a solution of 1:1 thread 1 and DNP34C10 macrocycle 3. UV/Vis analysis of 7 Figure 2 reveals a CT band at 492 nm ($\varepsilon = 137$ M$^{-1}$ cm$^{-1}$).

Diagnostic protons of thread 7 and macrocycle 3 were shifted upfield relative to their free components as observed by $^1$H NMR spectroscopy (Figure 3A). Shifts of 0.51, 0.96, 0.53, and 0.37 ppm were observed for protons H$_b$, H$_c$, H$_d$, and H$_e$, respectively. Notably, signals corresponding to both complexed and uncomplexed species are well resolved in the $^1$H NMR spectrum obtained at 298 K and broadening is essentially nonexistent at 268 K (Figure 3A). This contrasts sharply with the

Figure 3. (A) Partial $^1$H NMR spectrum (CDCl$_3$, 268 K, 1mM) of glutathione-functionalized [2]pseudorotaxane 7. Protons are labeled as in Scheme 1. Uncomplexed peaks are designated as “(uc)”. (B) Experimental (black solid line) and theoretical (dashed red lines) ESI/APCI-MS isotopic distribution of 7.
significant line broadening observed for [2]pseudorotaxane 4. It is hypothesized that the bulkier glutathione moieties of thread 7 impose a higher free energy barrier to threading/dethreading in [2]pseudorotaxane 7 than the maleimide moieties of thread 1 in the case of [2]pseudorotaxane 4. Furthermore, the greater kinetic stability of [2]pseudorotaxane 7 enabled analysis by ESI/APCI mass spectrometry which indicated a peak of $m/z = 2305.88 \ [M + Na]^+$ compared to a calculated value of 2305.87 (Figure 3B). Peaks for both macrocycle 3 and thread 6, however, could also be observed in the same spectrum (see materials and methods for full spectrum).

2.5 Conclusion

The results reported here highlight the utility of thiol-maleimide click chemistry in the synthesis of mechanically interlocked molecules. Some of the greatest prospects for this chemistry stem from the potential to use thiol-maleimide click chemistry as a means to incorporate mechanically interlocked molecules into biological systems, opening the possibility of using stimuli-responsive interlocked molecules to impart allosteric control over the functions of proteins and enzymes. The demonstration of glutathione-functionalized [2]pseudorotaxane 7 represents a first step toward this goal. Another key step to extending the chemistry presented herein to the facile hybridization of interlocked molecules with biological systems is the synthesis of aqueous-soluble derivatives of macrocycle 3. For appropriate use of this rotaxane in polymeric or biological systems, it is imperative to have functionalized macrocycles which will be discussed in the following chapters.
2.6 Materials and methods

2.6.1 General methods

Chemicals were purchased from Aldrich, Acros, TCI America, or Cambridge Isotope Labs and used as received. Solvents were dried using an Innovative Technologies SPS-400-5 solvent purification system. All reactions were carried out under an anhydrous nitrogen atmosphere unless otherwise noted. Thin layer chromatography (TLC) was performed on alumina-backed sheets coated with silica gel 60 F254. TLC plates were visualized using a UV/Vis lamp and/or by staining with iodine or p-anisaldehyde solution. Column chromatography was performed using glass columns over Dynamic Absorbents 60 Å, 32-63 μm silica gel. APCI and MALDI high resolution mass spectrometric analysis of compounds were performed at the UC Riverside Mass Spec Facility.
2.6.2 Synthetic procedures

Scheme 3 Synthesis of 1. Synthesis of unlabeled compounds followed by literature procedures.\textsuperscript{2, 25}

A

\[ \text{Scheme 3 Synthesis of 1. Synthesis of unlabeled compounds followed by literature procedures.} \textsuperscript{2, 25} \]
**Scheme 4 Synthesis of 2.** Synthesis of unlabeled compounds followed by literature procedures²⁻²⁵

**Synthesis of 9.**

To a solution of 8 (1.9 g, 3.0 mmol) in dry DMF (40 mL) at room temperature was added phosphorous tribromide (1.8 g, 6.6 mmol) dropwise over 15 min. The reaction mixture was heated to 70 °C and stirred for 12 hours. The reaction mixture was cooled down to room temperature, water (100 mL) was added and the aqueous layer was extracted with dichloromethane (50 x 2 mL). The combined organic layers were washed with brine, dried over MgSO₄ and concentrated under reduced pressure. To the crude residue was added 30 mL ethanol and the resulting precipitate was gravity filtered to yield 9 (1.9g, 93%). APCI-MS (m/z) [M+Na]⁺ Calculated for C₃₀H₃₆Br₂N₂O₁₀Na, 767.41; found 767.0596. ¹H NMR (300 MHz, CDCl₃, 289K) δ =
8.72 (4H, S), 4.44 (4H, t, J = 8 Hz), 3.84 (4H, t, J = 8 Hz), 3.77-3.49 (20H, m, -O-CH2-CH2-O-), 3.49 (4H, t, J = 8 Hz). $^{13}$C NMR (75 MHz, CDCl$_3$) δ = 163, 131.2, 126.9, 126.8, 71.38, 70.78, 70.69, 70.35, 68, 39.83, 30.55.

Synthesis of 1

To a solution of 9 (2.06 g, 3.5 mmol) in anhydrous N,N-dimethylformamide (50 mL) was added cesium carbonate (2.2 g, 7.0 mmol). The reaction mixture was heated to 40 °C and allowed it to stir for 15 min. A solution of 10 (1.27 g, 7.7 mmol) in anhydrous N, N-dimethylformamide (2 mL) was then added and the reaction mixture was allowed to stir for 12 hr at 50 °C. After cooling down the reaction to room temperature, water (100 mL) was added and the aqueous layer was extracted with dichloromethane (2x50 mL). The combined organic layers were washed with 2x50 mL 1N HCl followed by 1x50 mL brine, dried under MgSO$_4$ and then concentrated under reduced pressure. The resulting residue was purified by column chromatography using a gradient starting with CH$_2$Cl$_2$ (100%) and increasing to CH$_2$Cl$_2$/MeOH (95:5), giving 2.34 g of 11 (70%, crude yield), which was directly taken up in 40 mL of dry toluene and stirred at reflux for 8 hours. The reaction mixture was then cooled down to room temperature and concentrated under reduced pressure to yield NDI thread 1 (1.88 g, quantitative). ESI-HRMS (m/z) [M+Na]$^+$

Calculated for C$_{38}$H$_{40}$N$_4$O$_{14}$Na, 799.2433; found 799.2424. $^1$H NMR (300 MHz, CDCl$_3$, 298K) δ 8.74 (4H, S), 6.7 (4H, S), 4.45 (4H, t, J = 8Hz), 3.83 (4H, t, J = 8
Hz), 3.7-3.6 (8H, m), 3.62-3.49 (16H, m). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 170.8, 163.1, 134.3, 131.2, 126.8, 70.8, 70.7, 70.30, 70.1, 68, 39.8, 37.2.

**Synthesis of 13**

To a solution of 12 (6.5 g, 9.93 mmol) in 100 mL acetonitrile was added potassium thioacetate (2.27 g, 19.86 mmol) and catalytic potassium iodide (20 mg, 0.1 mmol). The reaction mixture was heated to reflux and allowed to stir for 12 hours, during which time the reaction turned from yellow to brown. The reaction mixture was then concentrated under reduced pressure and 100 mL of water was added. This aqueous layer was extracted with dichloromethane (2 x 100 mL). The combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated under reduced pressure. The crude residue was subjected to column chromatography, eluting with a 1:1 mixture of dichloromethane and hexane to yield 13 (4.0 g, 62%).

ESI-HRMS (m/z) [M+Na]$^+$ Calculated for C$_{43}$H$_{54}$O$_3$NaS, 673.3686; found 673.3688.

$^1$H NMR (400 MHz, CDCl$_3$, 298K) δ 7.24 (6H, m, Ar-H), 7.11 (8H, m, Ar-H), 6.79 (2H, d, J = 8Hz), 4.09 (2H, t, J = 8Hz), 3.82 (2H, t, J = 8Hz), 3.69 (2H, t, J = 8Hz), 3.14 (2H, t, J = 8Hz), S4 2.32 (3H, s), 1.32 (27H, s). $^{13}$C NMR (400 MHz, CDCl$_3$) δ 195.7, 156.7, 148.5, 144.4, 140, 132, 131, 124.3, 113.3, 77.68, 77.36, 77, 70.1, 69.7, 67.4, 63.3, 34.5, 31.6, 30.8, 29.1

**Synthesis of 2**

To a solution of 13 (2.0 g, 3.0 mmol) in dry dichloromethane (20 mL) was degassed with nitrogen for 15 min. Hydrazine monohydrate (1.85 g, 46.12 mmol) was
then added under inert atmosphere and the reaction mixture was allowed to stir for 12 hours. Following the addition of 20 mL water, the reaction mixture was extracted with dichloromethane (2 x 15 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, concentrated under reduced pressure and the residue was subjected to column chromatography (dichloromethane) to yield 2 (1.2 g, 66%). ESI-HRMS (m/z) [M+Na]+ Calculated for C₄₁H₅₂O₂NaS, 631.3580; found 631.3568. \(^1\)H NMR (400 MHz, CDCl₃, 298K) δ 7.24 (6H, m, Ar-H), 7.06 (8H, m, Ar-H), 6.78 (2H, d, J =Hz), 4.10 (2H, t, J = 8Hz ), 3.81 (2H, t, J = 8Hz ), 3.68 (2H, t, J = 8Hz), 2.71 (2H, m), 1.60 (1H, t, J = 16.4Hz), 1.27 (27H, s). \(^{13}\)C NMR (400 MHz, CDCl₃) δ 156.7, 148.5, 144.4, 140.1, 132.5, 131, 124.3, 113.37, 73.3, 69.7, 67.4, 63.3, 34.5, 31.7, 24.6.

Into two separate vials were weighed 3 (20.0 mg, 0.031 mmol) and 1 (24.4 mg, 0.031 mmol) separately. Compound 3 was then transferred into the vial containing 1 using a minimum amount of chloroform, yielding a deep purple solution of [2]pseudorotaxane 4. The solvent was then removed under reduced pressure and the resulting solid dried under high vacuum (10⁻³ torr). To this solid was added 2 (42.0 mg, 0.069 mmol) and chloroform (150 µL). The solution was cooled down to 0 °C. Triethylamine (150 µL) was added and the mixture was stirred for 2 hr at the 0 °C. The resulting solution was concentrated under reduced pressure and the residue was
purified by preparative TLC, eluting with a 98:2 mixture of ethyl acetate and isopropanol to yield 5 (53 mg, 65%). ESI-HRMS (m/z) [M+Na]⁺ Calculated for C_{156}H_{188}N_{4}O_{28}NaS_{2}, 2652.274; found 2652.269. ¹H NMR (400 MHz CDCl₃, 298K) δ 8.23 (4H, S), 7.22 (12H, m), 7.04 (16H, m), 6.83 (4H, d, J = 8Hz), 6.75 (4H, d, J = 8Hz), 6.64 (4H, t, J = 16Hz), 6.08 (4H, d, J = 8Hz), 4.30 (4H, t, J = 12Hz), 4.06 (4H, t, J = 12), 3.99 (8H, m), 4.0-3.6 (32H, m), 4.0-3.6 (8H, m), 4.0-3.6 (4H, m), 4.0-3.6 (12H, m), 4.0-3.6 (4H, m), 3.2-3.06 (4H, dd, J =16), 2.88 (4H, m), 2.5 (2H, dd, J = 20Hz), 1.25 (54H, s). ¹³C NMR (100 MHz, CDCl₃) δ 176.9, 174.9, 163.3, 156.6, 153.1, 148.5, 144.3, 140.1, 132.4, 130.9, 124.9, 124.2, 123.8, 114.3, 113.2, 103.6, 71.5, 71.2, 70.9, 70.4, 70, 69.7, 68.1, 67.5, 67.2, 39.5, 38.4, 36.4, 34.5, 31.6.
**Synthesis of 16:** To a solution of 1 (12.2 mg, 0.0157 mmol) and 2 (21 mg, 0.03457 mmol) in 75 µL CHCl₃ was added triethylamine (75 µL, 0.5 mmol) and the reaction mixture was allowed to stir at room temperature for 2 hr. The solvent was then evaporated under reduced pressure and the resulting residue was purified by preparative TLC (eluting with a 1:1 mixture of dichloromethane and ethyl acetate) to yield 16 (28 mg, 99%). ESI-HRMS (m/z) [M+Na]+ Calculated for C₁₂₀H₁₄₄N₄O₁₈Na₂S₂, 2015.981; found 2015.984. \(^1\)H NMR (400 MHz CDCl₃, 298K) δ 8.75 (4H, S), 7.22 (12H, m), 7.06 (16H, m), 6.75 (4H, d, J = 8Hz), 4.30 (4H, t, J = 8Hz), 4.06 (4H, t, J = 8Hz), 3.82 (16H, m, -O-CH₂-CH₂-O-), 3.72-3.65 (4H, t), 3.64-3.54 (8H, m), 3.53-3.45 (8H, m) 3.2-3.06 (4H, m), 2.88 (4H, m), 2.5 (2H, dd, J = 

**Scheme 6.** Synthesis of control dumbbell 16.
20Hz), 1.25 (54H, s). $^1$C NMR (100 MHz, CDCl$_3$) δ 163.1, 156.6, 148.5, 144.3, 140.1, 132.4, 131.2, 130, 126.8, 124.2, 113.2, 108.7, 71.2, 70.8, 70.7, 70.3, 70.1, 69.7, 68, 67.3, 67.1, 39.8, 39.5, 38.4, 36.4, 34.5, 31.0.

**Scheme 7 Synthesis of protected glutathione 14.**
**Synthesis of 15**

To a solution of 1 (200 mg, 0.257 mmol) and 14 (235 mg, 0.539 mmol) in 10 mL CHCl$_3$ was added triethylamine (360 mg, 3.5 mmol) and the solution was stirred at room temperature for 0.5 hours. The reaction was then concentrated under reduced pressure and purified by column chromatography eluting with a 1:9 mixture of methanol and dichloromethane. Yield: 250 mg (60%). ESI (m/z) [M+Na]$^+$ Calculated for C$_{72}$H$_{98}$N$_{10}$O$_{30}$Na$_2$, 1669.58; found 1669.53. $^1$H NMR (400 MHz CDCl$_3$, 298 K) $\delta$ 8.75 (4H, s), 7.49-7.40 (2H, br), 7.37-7.26 (2H, br), 7.02-6.91 (2H, br), 4.82 (2H, dd, J = 16Hz), 4.71 (2H, t, J = 8Hz), 4.1-4.0 (4H, s), 4.06 (4H, t, J = 8Hz), 3.9-2.91 (4H, m) 3.82 (16H, m), 3.7-3.6 (4H, t), 3.6-3.55 (4H, t), 3.6-3.5 (6H, S) 3.51 (4H, t), 3.2-3.06 (4H, dd, J = 16Hz), 2.88 (4H, m), 2.5 (2H, dd, J = 20Hz), 2.18 (4H, q, J = 16Hz), 2.15 (4H, t, J = 8Hz) 1.39 (18H, S). $\delta$ $^{13}$C NMR (400 MHz, CDCl$_3$) 178, 174.65, 173.07, 172.42, 170.60, 169.99, 163.13, 131.24, 126.95, 126.82, 80.32, 70.78, 70.70, 70.30, 67.97, 67.06, 52.96, 52.69, 52.58, 52.22, 41.50, 39.81, 38.67, 36.32, 36.11, 32.24, 28.52.

**Synthesis of 7**

Into two separate vials were weighed macrocycle 3 (10 mg, 0.006 mmol) and glutathione-functionalized thread 15 (3.87 mg, 0.006 mmol). Macrocycle 3 was transferred into the vial containing 15 using minimum amount of CHCl$_3$, yielding a red solution of [2]pseudorotaxane 7. ESI/APCI (m/z) [M+Na]$^+$ Calculated for C$_{108}$H$_{142}$N$_{10}$O$_{40}$Na$_2$ 2305.87; found 2305.88. $^1$H NMR (400 MHz CDCl$_3$, 268 K)
showed a mixture of complexed and uncomplexed species characteristic of a thermodynamically stable [2]pseudorotaxane (see the partial $^1$H spectrum in Figure 2 of the main text of the chapter as well as the full $^1$H spectrum below).
2.7. References and notes


Chapter 3-Synthesis of allyl functionalized DNP38C10 macrocycle

This chapter is based on the following article

Allyl-functionalized dioxynaphthalene[38]crown-10 macrocycles: synthesis, self-assembly, and thiol-ene functionalization

3.1 Abstract

Five dioxynaphthalene[38]-crown-10 (DNP38C10) macrocycles bearing one, two, three, or four allyl moieties have been synthesized and their ability to spontaneously self-assemble with methyl viologen to form [2]pseudorotaxanes has been evaluated. Association constants between methyl viologen and several of the allyl-functionalized DNP38C10 macrocycles are found to be comparable to that of methyl viologen and unfunctionalized DNP38C10, however the enthalpic and entropic factors that underlie overall binding free energy vary systematically with increasing allyl substitution. These variations are explained through a combination of solution phase and solid-state analysis of the macrocycles and their complexes. The utility of endowing DNP38C10 macrocycles with allyl moieties is further demonstrated by the ease with which they can be functionalized through thiol-ene click chemistry.

3.2 Introduction

Crown ether macrocycles\(^1\) have played a leading role in the study of molecular recognition and self-assembly processes for several decades, broadly impacting the synthesis and development of supramolecular assemblies,\(^2\) mechanically interlocked molecules,\(^3\) and many different molecular materials and devices.\(^4\) A wide variety of structurally diverse crown ether macrocycles have been prepared since Pedersen’s initial synthesis\(^5\) of dibenzo[18]-crown-6 in the late 1960s. Synthetic modifications of crown ether structures have provided the means to tune\(^6\) their molecular recognition and binding properties toward a variety of alkali,
ammonium, transition metal, pyridinium, halide, aromatic, and other guest compounds.\textsuperscript{1a, 1b, 6a} The variety of structurally distinct crown ether macrocycles that have been synthesized constitute a rich collection of host molecules that researchers can draw from to affect particular self-assembly processes or tailor specific supramolecular functions. Given the increasing interest in integrating noncovalently associated assemblies and mechanically interlocked molecules with materials of increasing complexity, for example, polymers,\textsuperscript{7} solid supports and thin films,\textsuperscript{8} reticular materials,\textsuperscript{9} biological systems,\textsuperscript{10} etc., there continues to be a need to develop new, facile syntheses of functionalized crown ether macrocycles. Of particular interest are crown ether macrocycles bearing functionalities that allow straightforward and reliable means of further synthetic modification(s).

One of the most prominent classes of crown ether macrocycles that act as π-electron rich hosts for π electron-poor guests are those that incorporate dioxynaphthalene units. The most widely studied macrocycle in this class is $1,5$-dioxynaphthalene[38]crown-10 (\textbf{DNP38C10}).\textsuperscript{11} Over the past 25 years multiple functionalized variations of dioxynaphthalene-containing crown ether macrocycles have been synthesized, nearly all of which contain one dioxynaphthalene unit linked to a different functional moiety, for example, tetrathiafulvalenes,\textsuperscript{12} porphyrins,\textsuperscript{13} azobenzenes,\textsuperscript{14} phenanthrolines,\textsuperscript{15} fluorenones,\textsuperscript{16} terephthalates,\textsuperscript{17} phthalimides\textsuperscript{18} benzoates,\textsuperscript{19} and \textit{para}-phenylene ethynyles.\textsuperscript{20} By contrast, there have been surprisingly few examples of dioxynaphthalene crown ether macrocycles wherein the naphthalene units themselves are functionalized. Liu et al. have recently
reported\textsuperscript{10g} the synthesis of water-soluble tetratsulfonated derivatives of DNP\textsubscript{38}C\textsubscript{10} and 1,5-dioxynaphthalene[32]crown-8 and shown them to be capable of binding electron-poor bipyridinium, diimide, and NAD\textsuperscript{+} guests in aqueous environments. At present, to the best of our knowledge, no organic soluble derivatives of DNP\textsubscript{38}C\textsubscript{10} containing functionalized dioxynaphthalene units have been reported in the literature.

In this study we report the straightforward synthesis of five new allyl-functionalized derivatives of DNP\textsubscript{38}C\textsubscript{10} along with solution-phase investigations of their ability to form 1:1 complexes with methyl viologen. The solid-state structures of two of the allyl-functionalized macrocycles and two of the 1:1 complexes with methyl viologen have been solved. The combined solution and solid-state studies enable us to draw conclusions as to the influence of dioxynaphthalene functionalization on self-assembly processes. Furthermore, the straightforward functionalization of allyl-DNP\textsubscript{38}C\textsubscript{10} derivatives by radical-mediated thiol-ene click chemistry\textsuperscript{21} is demonstrated.

3.3 Synthesis and design

The syntheses of allyl-functionalized DNP\textsubscript{38}C\textsubscript{10} macrocycles are outlined in Scheme 1. Two equivalents of allyl bromide were added to 1,5-dihydroxynaphthalene (1) under basic conditions to give 2 (Scheme 1 a). Heating bis(allyloxy) naphthalene 2 to 180 °C in the absence of solvent induced a quantitative dual Claisen rearrangement to give 1,5-dihydroxy naphthalene derivative 3. Tetraethylene glycol monotosylate\textsuperscript{22} was then added to both hydroxyl groups of 3 and the resulting diol (4)
was subsequently ditosylated to give 5. Syringe-pump addition of another equivalent of 1 or diallyl intermediate 3 to ditosylate 5 gave diallyl DNP38C10 macrocycle 6 and tetraallyl DNP38C10 macrocycle 7, respectively.

**Scheme 1.** Synthesis of allyl-functionalized DNP38C10 macrocycles: a) 6, 7, and b) 11, 12, and 15. c) Structure of MV-2(PF6).

As shown in Scheme 1b, the monoaddition of allyl bromide to 1 gives 5-allyloxy-1-hydroxynaphthalene (8) and provides three additional synthetic pathways to allyl-functionalized DNP38C10 macrocycles. Heating 8 to 180 °C solvent free gave Claisen rearranged product 2-allyl-1,5-dihydroxynaphthalene (9) in
quantitative yield. Macrocyclization of known ditosylate 10 and allyl-functionalized
diol 9 gave monoallyl DNP38C10 derivative 11.

Similarly, triallyl DNP38C10 macrocycle 12 was prepared upon
macrocyclization of naphthalene diol 9 with ditosylate 5. Along an alternative route,
two equivalents of 8 were linked through a reaction with tetraethylene glycol
ditosylate 24 to give bis(allyloxy) dinaphthalene compound 13. The dual Claisen
rearrangement of 13 to give diol 14 was carried out by heating to 180 °C in DMF as
the compound decomposes if such heating is carried out in the absence of solvent.
Rearranged diol 14 was then treated with another equivalent of tetraethylene glycol
ditosylate to give diallyl DNP38C10 macrocycle 15, a structural isomer of
macrocycle 6.

3.4 Solution-phase self-assembly

Parent crown ether macrocycle DNP38C10 is known to bind electron-
poor aromatic guests, such as alkyl viologens and substituted aryl diimides. This
noncovalent self-assembly is a thermodynamic process driven by both electronic π–
π and [C-H···O] interactions, and influenced by steric factors. The allyl moieties
of DNP38C10 derivatives 6, 7, 11, 12, and 15 can be expected to impact both these
electronic and steric factors of host–guest binding. We, therefore, sought to examine
the self-assembly and binding affinity of the five new macrocycles with a typical
electron-poor guest-the hexafluorophosphate salt of methyl viologen (MV-2(PF6),
Scheme 1, c).
Macrocycles DNP38C10, 6, 7, 11, 12, and 15 were each mixed with MV-2PF6 in 1:1 molar ratios in CH3CN (1.0 mM) at 298 K and their UV/Vis spectra were recorded as an initial means to investigate their ability to form [2]pseudorotaxane host–guest complexes (Figure 1). Listed in Table 1 are the absorption maxima and extinction coefficients observed in the region between 400-600 nm for each mixture. A prominent charge-transfer (CT) band is observed at 486 nm (apparent ε=295 M\(^{-1}\) cm\(^{-1}\)) for the binding of MV-2(PF6) by unsubstituted DNP38C10. Allyl functionalized macrocycles 6, 11, 12, and 15 all display CT bands that are blue-shifted relative to parent DNP38C10, and extinction coefficients generally decrease upon increasing allyl substitution: monoallyl macrocycle 11 is observed to have the largest apparent extinction coefficient (ε=145 M\(^{-1}\) cm\(^{-1}\)) of the substituted macrocycles, followed by diallyl macrocycles 6 and 15 (apparent ε=107 and 59 M\(^{-1}\) cm\(^{-1}\), respectively), and then triallyl macrocycle 12 (22 M\(^{-1}\) cm\(^{-1}\)). The CT band for triallyl macrocycle 12 is particularly weak at this concentration and better described as a shoulder than a broad peak. No evidence of a CT band could be observed in the 1:1 mixture of MV-2(PF6) and tetraallyl DNP38C10 macrocycle 7, implying the two are not favored to form a [2]pseudorotaxane. Attempts were made to promote [2]pseudorotaxane formation by increasing the solution concentration (up to 0.5 M), letting the mixture equilibrate for extended time (>ten days), and heating (50 °C, two days); however, no change in experimental conditions gave rise to an observable CT band for the mixture of MV-(2PF6) and 7. It was preliminarily concluded that tetraallyl macrocycle 7 is unable to complex MV-2(PF6), likely as a
result of increased steric constriction of the macrocycle’s cavity by pendant allyl moieties. Allyl groups are more likely to act as electron donating groups, which should make the enthalpy of [2]pseudorotaxane formation, with the electron poor MV-2(PF₆), more favorable upon increased allyl substitution. However, we observe a decrease in binding enthalpy upon increasing allyl functionalization. It is likely, therefore that the steric interactions imposed by allyl substituents disrupts host-guest binding, leading to the observed trend in decreased apparent ε upon allyl substitution.

Figure 1. UV/Vis spectra of 1:1 mixtures of MV-2(PF₆) with DNP38C10 (red), mono-allyl macrocycle 11 (purple), di-allyl macrocycles 6 (dark blue) and 15 (maroon), tri-allyl macrocycle 12 (orange), and tetra-allyl macrocycle 7 (green), as well as MV-2(PF₆) by itself (blue). Spectra were recorded as 1.0 mM CH₃CN solutions at 25 °C.

Table 1. UV/Vis spectroscopic data[a] for charge-transfer interactions between MV-2(PF₆) and macrocycles DNP38C10, 6-7, 11-12, and 15.

<table>
<thead>
<tr>
<th>Macrocycle</th>
<th>DNP38C10</th>
<th>6</th>
<th>7ᵇ</th>
<th>11</th>
<th>12ᶜ</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ max [nm]</td>
<td>486</td>
<td>461</td>
<td>-</td>
<td>464</td>
<td>443</td>
<td>456</td>
</tr>
<tr>
<td>ε [M⁻¹cm⁻¹]</td>
<td>295</td>
<td>59</td>
<td>-</td>
<td>145</td>
<td>22</td>
<td>107</td>
</tr>
</tbody>
</table>

[a] The spectra were recorded as 1.0 mM CH₃CN solutions at 25 °C. [ᵇ] No charge-transfer band was observed. [ᶜ] At 1.0 mM the charge-transfer interaction was observed as a shoulder rather than a distinct peak, however a more easily discernable peak is observed at higher concentrations (e.g. > 10mM).
The noncovalent self-assembly of macrocycles 6, 11, 12, and 15 with MV-2PF$_6$ was further investigated by $^1$H NMR spectroscopy. Mixing MV-2(PF$_6$) with mono-, di-, and triallyl functionalized DNP38C10 macrocycles in 1:1 molar ratios in CD$_3$CN at 298 K resulted in the formation of red-colored solutions. Analysis of these

![Figure 2](image-url)

**Figure 2.** Schematic representation (a) and spectroscopic characterization (b-d) of the self-assembly of [2]pseudorotaxane MV-2(PF$_6$)$\subset$6 from di-allyl DNP38C10 macrocycle 6 and MV-2(PF$_6$). Partial $^1$H NMR spectra (298 K, CD$_3$CN, 300 MHz, 1 mM concentration) of macrocycle 6 (b), [2]pseudorotaxane MV-2(PF$_6$)$\subset$6 (c), and MV-2(PF$_6$) (d) indicating diagnostic shifts of proton signals H$_{a-g}$ and H$_{a,b}$. 
solutions by $^1$H NMR spectroscopy revealed prominent upfield shifts of protons $H_a$ and $H_b$ of MV-2(PF$_6$), upfield shifts of the naphthalene protons of each allylfuctionalized DNP38C10 macrocycle, and slight downfield shifts of the allyl proton signals of each functionalized DNP38C10 macrocycle.

A representative example of these spectroscopic shifts is provided by the partial $^1$H NMR spectra of diallyl DNP38C10 macrocycle 6, MV-2(PF$_6$), and the 1:1 host–guest complex MV-2(PF$_6$)⊂6 shown in Figure 2b-d. Naphthalene proton signals $H_a$ and $H_c$ are observed to experience upfield shifts of 0.32 and 0.37 ppm, respectively. Smaller upfield shifts are observed for proton signals $H_b$ (0.06 ppm), $H_e$ (0.14 ppm), and $H_d$ (0.03 ppm). The most pronounced spectral shifts are observed
for protons $H_a$ and $H_\beta$, which undergo upfield shifts of 0.30 and 0.86 ppm, respectively. Downfield shifts of allyl signals $H_f$ and $H_g$ of 0.07 and 0.05 ppm are also observed. Only one set of signals is present for each proton of 6 and MV-2(PF$_6$), indicating that the kinetics of [2]pseudorotaxane formation are fast on the NMR time scale at 298 K in CD$_3$CN. As a result the peaks in Figure 2c represent an average of complexed and uncomplexed species. The [2]pseudorotaxane formed between parent macrocycle DNP38C10 and MV-2(PF$_6$) is known$^{25b}$ to display similarly fast host–guest complexation/decomplexation.

Host–guest complexes of macrocycles 11, 12, and 15 with MV-2(PF$_6$) displayed similar shifts in their $^1$H NMR spectra (Figures 3–6).

**Figure 5.** Partial $^1$H NMR spectra (298 K, CD$_3$CN, 300 MHz, 1 mM concentration) of macrocycle 12 and MV-2(PF$_6$)⊂12.

**Figure 6.** Partial $^1$H NMR spectra (298 K, CD$_3$CN, 300 MHz, 1 mM concentration) of macrocycle 15 and MV-2(PF$_6$)⊂15.
In each case the protons $H_\alpha$ and $H_\beta$ of MV-2(PF$_6$) and those at positions 4 and 8 of naphthalene exhibited the largest upfield shifts upon complexation. It is likely that these protons experience the greatest amount of shielding as a result of $\pi$-stacking upon host–guest complexation. The kinetics of host–guest complexation and decomplexation are rapid relative to the NMR time scale for each [2]pseudorotaxane, as each of their $^1$H NMR spectra show averages of complexed and uncomplexed peaks. Contrasting these results, a 1:1 mixture of MV-2(PF$_6$) and tetraallyl DNP38C10 macrocycle 7 gave a colorless solution. The $^1$H NMR spectrum of this mixture (Figure 3) was found to be the sum of the individual spectra of MV-2(PF$_6$) and 7, indicating that no host–guest complex was formed.

3.5 ITC studies

Isothermal titration calorimetry (ITC) was used to quantitatively investigate the thermodynamic binding parameters for the host–guest complexation between MV-2(PF$_6$) and crown ether macrocycles DNP38C10, 6, 11, 12, and 15. ITC measurements were performed at 298 K using CH$_3$CN as the solvent (Materials and Methods 3.9.3). No ITC data were collected for tetraallyl macrocycle 7 as both UV/Vis and $^1$H NMR spectroscopic results indicate it is incapable of forming a 1:1 complex with MV-2(PF$_6$). Collected ITC results for the other five macrocycles are summarized in Table 2. The association constant between unsubstituted DNP38C10 and MV-2(PF$_6$) was measured to be 560 M$^{-1}$ ($\Delta G^o$=−3.75 kcal mol$^{-1}$). This value is within reasonable agreement with the previously reported value.
of $k_a$=670 M$^{-1}$ ($\Delta G^\circ$=−3.85 kcal mol$^{-1}$) measured by spectrophotometric titration. The binding free energy of MV-2(PF$_6$) within allyl-functionalized DNP38C10 macrocycles was found to decrease, in general, with increasing allyl substitution. The only exception to this trend is monoallyl macrocycle 11, which was observed to bind MV-2(PF$_6$) more strongly than the parent macrocycle ($k_a$=662 M$^{-1}$, $\Delta G^\circ$=−3.85 kcal mol$^{-1}$). Association constants for diallyl macrocycles 6 and 15 were found to be equal within experimental error ($k_a$=434 and 425 M$^{-1}$, respectively) and displayed weaker binding of MV-2(PF$_6$) than parent macrocycle DNP38C10 or monoallyl macrocycle 11. Binding of MV-2(PF$_6$) by triallyl macrocycle 12 was observed to be especially weak: $k_a$=57 M$^{-1}$, $\Delta G^\circ$=−2.39 kcal mol$^{-1}$.

As mentioned above, tetraallyl macrocycle 7 shows no binding of MV-2(PF$_6$) in CH$_3$CN.

**Table 2.** Thermodynamic data obtained from ITC measurements$^{[a]}$ of the strength of binding between MV-2(PF$_6$) and macrocycles DNP38C10, 6, 11-12, and 15.

<table>
<thead>
<tr>
<th></th>
<th>$K_a$ [M$^{-1}$]</th>
<th>$\Delta G^\circ$ [kcal mol$^{-1}$]</th>
<th>$\Delta H^\circ$ [kcal mol$^{-1}$]</th>
<th>$-T\Delta S^\circ$ [kcal mol$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNP38C10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>560±14</td>
<td>-3.75±0.01</td>
<td>-10.68±0.1</td>
<td>6.91</td>
</tr>
<tr>
<td>11</td>
<td>435±35</td>
<td>-3.60±0.05</td>
<td>-7.87±0.4</td>
<td>4.26</td>
</tr>
<tr>
<td>12</td>
<td>662±21</td>
<td>-3.85±0.02</td>
<td>-6.46±0.1</td>
<td>2.62</td>
</tr>
<tr>
<td>13</td>
<td>57±1.7</td>
<td>-2.39±0.02</td>
<td>-4.21±0.1</td>
<td>1.82</td>
</tr>
<tr>
<td>15</td>
<td>425±21</td>
<td>-3.58±0.03</td>
<td>-4.95±0.1</td>
<td>1.36</td>
</tr>
</tbody>
</table>

$^{[a]}$ The data was collected in CH$_3$CN at 25 °C. For macrocycles DNP38C10, 6, 11, and 15 a 10 mM solution of MV-2(PF$_6$) was titrated into a 1.0 mM solution of macrocycle. In the case of the weaker binding macrocycle 12, a 64 mM solution of MV-2(PF$_6$) was titrated into a 3.0 mM solution of macrocycle.
Although macrocycles **DNP38C10, 6, 11, 12, and 15** were found to complex MV-2(PF$_6$) with similar binding free energies ($\Delta G^\circ$=−2.39 to −3.85 kcal mol$^{-1}$) the individual enthalpic and entropic contributions to host–guest complexation reveal notable differences between the macrocycles. The enthalpy of binding MV-2(PF$_6$) is strongest for the unsubstituted parent macrocycle **DNP38C10** ($\Delta H^\circ$=−10.68 kcal mol$^{-1}$). Similarly the entropic penalty of forming a 1:1 host–guest complex with MV-2(PF$_6$) is greatest in the case of parent macrocycle 1 ($T\Delta S^\circ$=−6.91 kcal mol$^{-1}$). Two general trends relating differences in thermodynamic binding parameters to differences in the number of allyl functionalities were observed within the series of five macrocycles **DNP38C10, 6, 11, 12, and 15**: 1) $\Delta H^\circ$ tends to become more positive upon increasing allyl functionalization, and 2) $T\Delta S^\circ$ tends to increase (become less negative) with greater allyl functionalization. That is, the introduction of allyl moieties on the 1,5-dioxynaphthalene ring(s) of **DNP38C10** decreases the enthalpy of binding MV-2(PF$_6$) but also decreases the entropic penalty of forming a host–guest complex. Being electron donating, allyl groups should increase the enthalpy of formation, however we see a decrease in binding enthalpy with increase in allyl substitution which suggests these changes are not dominated by electronic changes. This trend is not perfect as reflected in the $\Delta H^\circ$ and $T\Delta S^\circ$ values measured for the more substituted diallyl macrocycle 6 compared to those of the less substituted monoallyl macrocycle 11: $\Delta H^\circ(6)$=−7.87 kcal mol$^{-1}$ versus $\Delta H^\circ(11)$=−6.46 kcal mol$^{-1}$; $T\Delta S^\circ(6)$=−4.26 kcal mol$^{-1}$ versus $T\Delta S^\circ(11)$=−2.62 kcal mol$^{-1}$. This, however, is the only pair of macrocycles that does not fit the trend.
It is likely that allyl functionalization of the 1,5-dioxynaphthalene unit(s) of DNP38C10 sterically disrupts the macrocycles’ ability to maximize $\pi-\pi$ interactions with MV-2(PF$_6$), leading to a decrease in binding enthalpies. Indeed Liu et al. have noted$^{10g}$ that host–guest complexation between tetrasulfonated DNP38C10 and bipyridinium guests is driven largely by hydrophobic interactions in water and not $\pi-\pi$ interactions as the sulfonate groups twist the naphthalene $\pi$ surface away from planarity. Allyl functionalization of DNP38C10 also directly influences the entropy of binding of MV-2(PF$_6$). It is likely that allyl moieties have the effect of restricting the conformational flexibility of DNP38C10 macrocycles. The binding of MV-2(PF$_6$) by a more conformationally constrained allyl-functionalized macrocycle would be less entropically unfavorable than when MV-2(PF$_6$) is bound by the more conformationally flexible, unsubstituted parent macrocycle DNP38C10. Triallyl macrocycle 12, for example, displays the weakest binding enthalpy ($\Delta H^\circ=-4.21$ kcal mol$^{-1}$), less than half that of unsubstituted parent macrocycle DNP38C10. The entropic penalty of forming a host–guest complex between triallyl macrocycle 12 and MV-2(PF$_6$), however, is found to be $T\Delta S^\circ=-1.82$ kcal mol$^{-1}$, approximately one quarter the entropic penalty of forming a host–guest complex between unsubstituted DNP38C10 and MV-2(PF$_6$). In the case of tetraallyl macrocycle 7 it appears the balance of binding enthalpy and entropy are not sufficient to allow the formation of a thermodynamically stable host–guest complex with MV-2(PF$_6$). The general trends in binding data observed across the collection of six different DNP38C10-based macrocycles (DNP38C10, 6, 7, 11, 12, 15) fit well with
the conclusion that increased allyl-functionalization adversely influences their enthalpy of binding MV-2(PF₆) but favorably influences their entropy of binding the electron-poor guest.

3.6 Solid state structures

Single crystals of tetraallyl DNP38C10 derivative 7 and diallyl DNP38C10 derivative 15 were grown by slow evaporation techniques (materials and methods). Macrocycle 7 was obtained by slow evaporation from a dilute solution of ethanol at 0°C whereas macrocycle 15 was obtained by slow evaporation from a 0.1 mM solution of methyl tert-butyl ether (MTBE) and dichloromethane (1:1) at room temperature. Attempts to grow X-ray quality single crystals of macrocycles 6, 11, and 12 proved unsuccessful. Shown in Figure 7 are the solid-state structures of allyl-functionalized macrocycles 7 and 15 as well as that of parent macrocycle DNP38C10 for comparison. The solid-state structures of DNP38C10 macrocycles 7 and 15 provide additional insights into the steric influence of allyl groups that complement conclusions drawn from solution phase host–guest studies. As can be seen in Figure 7a and b unsubstituted and tetrasubstituted macrocycles DNP38C10 and 7 adopt similar conformations in the solid state. There are, however, notable differences between the two structures. The planes of the 1,5-dioxynaphthalene units of parent macrocycle DNP38C10 are separated by 3.45 Å, a value typical of π–π stacking interactions. The planes of the 2,6-diallyl-1,5-dioxynaphthalene units of tetraallyl macrocycle 7, however, are separated by 4.84 Å indicating they are not involved in π–π stacking interactions. The
allyl moieties of 7 sterically prohibit intramolecular π–π stacking as any conformation that enables π–π stacking would also force opposing allyl groups to sterically overlap.

The overall solid-state packing of tetraallyl macrocycle 7 (Figure 7d) reveals extended intermolecular π–π stacking wherein the naphthalene rings of adjacent macrocycles are separated by 3.59 Å. This observation is in contrast with the solid-

![Figure 7. Solid-State X-ray structures of DNP38C10 (a), tetra-allyl macrocycle 7 (b), and di-allyl macrocycle 15 (c). Hydrogen atoms are omitted for clarity with the exception of those involved in noncovalent [C-H•••π] interactions, which are indicated by dashed lines. Also indicated are the intramolecular separations between naphthalene π-systems that are oriented parallel to each other (a and b) as well as the angle of naphthalene π-systems oriented perpendicular to each other (c), solid-state packing of tetra-allyl macrocycle 7 indicating intermolecular π–π stacking interactions between naphthyl moieties of neighbouring molecules(d).]
state structure of unsubstituted macrocycle \textbf{DNP38C10}, which does not exhibit intermolecular π–π interactions in its overall solid-state packing.\textsuperscript{11} Lastly it should be noted that two of the allyl groups of each macrocycle 7 are involved in intramolecular [C-H⋯π] interactions (dashed lines C of \textbf{Figure 7b}) with the macrocycle’s naphthalene rings. Similar [C-H⋯π] interactions involving ethylene glycol C-H groups are observed in the parent \textbf{DNP38C10} macrocycle (dashed lines A, \textbf{Figure 7a}).

Although the four allyl groups of 7 serve to increase the internal cavity of the macrocycle, it is still unable to act as a host for MV-2(PF\textsubscript{6}). We postulate that this inability is the result of either: 1) the allyl groups sterically block MV-2(PF\textsubscript{6}) from entering the internal cavity of 7, 2) steric interactions between the allyl groups and MV-2(PF\textsubscript{6}) are too disruptive to allow favorable host–guest complexation, or 3) a combination of both. We suspect the primary reason is unfavorable steric interactions between MV-2(PF\textsubscript{6}) and the allyl groups of 7 because the first postulate is more an issue of kinetics than thermodynamics. The solid-state structure of diallyl macrocycles 15 (\textbf{Figure 7c}) differs considerably from those of macrocycles \textbf{DNP38C10} and 7. The 2-allyl-1,5-dioxynaphthalene units of 16 are oriented perpendicular (ca. 91°) to each other in an edge-to-face interaction\textsuperscript{30} rather than parallel as in macrocycles \textbf{DNP38C10} and 7. The solid-state structure of 15 is more similar to that of 1,5-dioxynaphthalene[35]crown-9 (\textbf{DNP35C9})\textsuperscript{11} than \textbf{DNP38C10}. The only notable noncovalent interaction stabilizing the structure of 15 in the solid-state is a [C-H⋯π] interaction indicated by dashed line E in \textbf{Figure 7c}. It is likely that
the allyl moieties of opposing naphthalene rings disfavor intramolecular π–π stacking within macrocycle 15 for the same reason as discussed above for 7: intramolecular π–π stacking would force these allyl groups to sterically overlap. The lack of allyl moieties at position 6 of each naphthalene ring allows the two naphthene units to rotate and adopt a perpendicular conformation that would not be sterically feasible in tetraallyl macrocycle 7 because of its allyl substituents at the naphthyl position 6. The above discussion is supportive of the notion that allyl groups sterically restrict the conformations of macrocycles 7 and 15 relative to parent macrocycle DNP38C10. This conclusion complements the solution phase ITC investigations discussed earlier, which showed that increased allyl functionalization of DNP38C10 macrocycles decreases their enthalpy of binding MV-2(PF₆) and also decreases the entropic penalty (TΔS°) of host–guest self-assembly. To further investigate the influence of allyl functionalization on host–guest self-assembly multiple attempts were made to grow crystals of [2]pseudorotaxanes formed between MV-2(PF₆) and macrocycles 6, 11, 12, and 15. X-ray quality single crystals of only two host–guest complexes could be grown and their solid-state superstructures solved: those of MV-2(PF₆)⊂6 and MV-2(PF₆)⊂15.

X-ray quality single crystals of MV-2(PF₆)⊂6 were grown by slow evaporation from a 1:1 mixture of CH₃CN and MTBE. The solid-state superstructure of the resulting [2]pseudorotaxane is shown in Figure 8. MV-2(PF₆) is bound within the interior cavity of macrocycle 6 by a combination of noncovalent π–π and [C-H⋯O] interactions. Center-to-center distances between the π system of MV-2(PF₆)
and each 1,5-dioxynaphthalene unit of 6 measure 3.34 Å indicating favorable π–π interactions. Hydrogen atoms of the terminal methyl groups of MV-2(PF6) are involved in noncovalent [C-H⋯O] interactions (Figure 8a) with the ethylene glycol chains of diallyl macrocycle 6. Four such [C-H⋯O] interactions, all within 3.0 Å, are present for each methyl group. The allyl moieties of 6 are both oriented away from both MV-2(PF6) and the central cavity of the macrocycle, supportive of the notion that allyl functionalities place steric restrictions on host–guest complexation, possibly contributing to the decrease in binding enthalpy observed by ITC. The overall solid-state packing of MV-2(PF6)⊂6 (Figure 8b) shows that the host–guest complexes are arranged into sheets along the crystallographic ab plane with allyl substituents of each macrocycle within a given sheet oriented in the same direction. Host–guest complexes of adjacent sheets (i.e., along the crystallographic c axis) are flipped 180°
with respect to each other. Hexafluorophosphate (PF$_6^-$) counterions occupy the spaces between host–guest complexes and are involved in multiple [C-H⋯F] interactions (Figure 8c) that likely stabilize the overall superstructure.

Single crystals of MV-2(PF$_6$)⊂15 were grown by dissolving equimolar amounts of diallyl macrocycle 15 and MV-2(PF$_6$) in a 1:1 mixture of CH$_3$CN and CH$_3$CH$_2$OH and cooling to 0 °C for 36 h (Figure 9). The resulting solid-state superstructure is shown in Figure 9. Binding of MV-2(PF$_6$) within the interior cavity of 15 is also driven by a combination of noncovalent π–π and [C-H⋯O] interactions.

Figure 8. (a) Solid-State X-ray structures of MV-2(PF$_6$)⊂6. Allyl groups of macrocycle 6 are coloured purple for clarity. Noncovalent [C–H•••O] interactions A, B, C, and D measure 2.86, 2.29, 2.98, and 2.95 Å, respectively. Hydrogen atoms not involved in [C–H•••O] interactions are omitted for clarity. Intramolecular separation between the pyridinium and naphthyl π-systems is measured to be 3.34 Å, indicative of π–π stacking. (b) View down the crystallographic b axis showing the alternating arrangement of allyl groups in the overall packing of the [2]pseudorotaxane.
The allyl moieties of 15 result in a desymmetrization\textsuperscript{33} of the hosts’ interior binding pocket, with the N-CH\textsubscript{3} group of MV-2(PF\textsubscript{6}) \textit{syn} to the allyl moieties of 15 residing in a tighter binding pocket than the N-CH\textsubscript{3} group \textit{anti} to the allyl moieties. This asymmetry is easily observed when comparing the interatomic distances between the two nitrogen atoms of MV-2(PF\textsubscript{6}) and their closest naphthyloxy oxygen atoms: [N···O] of 2.96 Å (\textit{syn}) versus [N···O] of 4.18 Å (\textit{anti}; Figure \textbf{9a}). Eight noncovalent [C-H···O] contacts of 3.0 Å or less (labeled A–D) are observed between the methyl groups of MV-2(PF\textsubscript{6}) and the glycols 15. Intermolecular separation of the aromatic rings of 15 and MV-2(PF\textsubscript{6}) ranges from 3.65-3.35 Å---values typical of π–π stacking interactions.
Figure 9. (a) Solid-State X-ray structures of MV-2(PF₆)₁₅. Allyl groups of 15 are highlighted in purple for clarity. Noncovalent [C–H•••O] interactions A, B, C, and D measure 2.46, 2.98, 2.60, and 2.84 Å, respectively. Hydrogen atoms not involved in [C–H•••O] interactions are omitted for clarity. Views of the overall packing along the crystallographic $b$ and $c$ axes are given in (b) and (c), respectively, showing the interdigitation of allyl groups and the packing of PF₆⁻ counterions.
The overall solid-state packing of MV-2(PF$_6$)$_{15}$ is shown in Figure 9b and c. Allyl moieties of adjacent [2]pseudorotaxanes are observed to interdigitate along the crystallographic $b$ axis. No intermolecular $\pi$--$\pi$ stacking between [2]pseudorotaxanes can be observed as nearest neighbor [2]pseudorotaxanes adopt a staggered orientation (Figure 9c). Noncovalent [C--H···$\pi$] interactions, however, can be observed between the glycol and naphthalene units of neighboring [2]pseudorotaxanes (Figure 10). These [C--H···$\pi$] interactions and a multitude of [C--H···F] interactions (Figure 11) between 15 and interstitial PF$_6^-$ counterions are believed to be the dominant factors that give rise to the long-range order of the solid-state superstructure.

**Figure 10.** [C--H···$\pi$] interactions between two hydrogen atoms of an ethylene glycol chain of one macrocycle and the naphthyl $\pi$-system of its nearest neighboring macrocycle.

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Distance (Å)</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C--H···$\pi$]$_A$</td>
<td>2.41</td>
<td>146</td>
</tr>
<tr>
<td>[C--H···$\pi$]$_B$</td>
<td>2.76</td>
<td>134</td>
</tr>
</tbody>
</table>
3.7 Thiol-ene functionalization

The allyl moieties of macrocycles 6, 7, 11, 12, and 15 offer the particular advantage that they readily react with thiols under radical-mediated thiol-ene “click” conditions. Over the past decade, thiol-ene methodologies have been increasingly applied throughout polymer, materials, and bioorganic chemistries. As representative examples of the thiol-ene functionalization of new DNP38C10 macrocycles reported herein we have treated diallyl macrocycle 15 with three different thiols under radical-initiated thiol-ene reaction conditions (Scheme 2). The thiols chosen were methyl-3-mercaptopropionate (16), 1-thio-β-D-glucose tetraacetate (17), and 4-bromo-1-
mercapto-4-methylpentan-3-one (18). This selection of thiols was chosen to
demonstrate the utility of endowing DNP38C10 macrocycles with allyl groups,
which allow direct access to a variety of small molecule transformations,
functionalization with biologically relevant derivatives such as carbohydrates, and
routes for integrating macrocycles with polymeric materials, respectively.

Scheme 2. Radical-initiated thiol-ene functionalization of di-allyl DNP38C10
macrocycles 15 with methyl-3-mercaptopropionate (16), 1-thio-β-D-glucose
tetraacetate (17), and 4-bromo-1-mercapto-4-methylpentan-3-one (18) to give di-
functionalized macrocycles 19-21 in good yields. Macrocycle 21 was subsequently
used as a difunctional initiator for the polymerization of methyl acrylate DNP38C10
derivative 22 (n ≈ 19).
Thiol-ene reactions were carried out by irradiating solutions containing diallyl macrocycle 15 and thiols 16, 17, or 18 in the presence of radical initiator 2,2-dimethoxy-2-phenylacetophenone (DMPA, see Fig 2). No attempts were made to exclude oxygen from these reactions. The choice of solvent, however, was found to influence product yields, with thiol-ene reactions to give macrocycles 19, 20, and 21 being most efficient in CHCl₃, CHCl₃/MeOH (1:1), and CHCl₃/MeOH/THF (1:1:1), respectively. The resulting thiol-ene functionalized macrocycles 19–21 were obtained in 75, 90, and 90% isolated yields following purification by precipitation or passage through a short pad of silica to remove excess thiol and trace quantities of initiator. Macrocyle 21 was subsequently used to initiate the growth of poly(methyl acrylate) DNP38C10 macrocycle 22 by single-electron transfer living radical polymerization (SET-LRP) techniques. The resulting macrocycle-centered polymer was isolated at 78% conversion. The target molecular weight of 22 was intentionally kept low ($M_w \sim 4440$ g mol$^{-1}$, $n \approx 19$) to facilitate $^1$H NMR characterization of the polymer and its host–guest complex with MV-2(PF$_6$). Equimolar mixtures of MV-2(PF$_6$) and thiol-ene functionalized macrocycles 19, 20, and 22 were prepared in CD$_3$CN. Shifts of characteristic aromatic proton signals observed by $^1$H NMR spectroscopic analysis of these solutions indicated the formation of host–guest complexes. UV/Vis spectroscopic analysis of equimolar CH$_3$CN solutions (1.0 mM, 298 K) of MV-2(PF$_6$) with ester-functionalized macrocycle 19 and sugar-
functionalized macrocycle 20 revealed charge-transfer bands at 448 nm (apparent $\varepsilon$=68 M$^{-1}$ cm$^{-1}$) and 453 nm (apparent $\varepsilon$=55 M$^{-1}$ cm$^{-1}$), respectively (Figure 12).

Figure 12. UV/Vis spectra of [2]pseudorotaxanes formed between MV-2(PF$_6$) and di-allyl macrocycle 15 (red), di-propionate macrocycle 19 (blue), di-β-D-glucose tetraacetate macrocycle 20 (green), and polymethacrylate macrocycle 22 (black and purple). Spectra of MV-2(PF$_6$)⊂15, MV-2(PF$_6$)⊂19 and MV-2(PF$_6$)⊂20 were obtained at 298 K and consisted of 1.0 mM CH$_3$CN solutions containing equimolar amounts of host and guest. The polydispersity of macrocycle 22 (PDI = 1.22) precluded an exact calculation of either its solution concentration or exact equivalents of MV-2(PF$_6$). The black trace represents a solution containing macrocycle 22 diluted to approximately 1.0 mM in CH$_3$CN along with ~1 equivalent MV-2(PF$_6$). As can be seen above, this solution absorbed weakly in the 425-550 nm region. For a mixture of MV-2(PF$_6$)⊂22 to absorb similarly to the other [2]pseudorotaxanes required the addition of 4 total equivalents of MV-2(PF$_6$). The UV/Vis results are summarized in the Table below:

<table>
<thead>
<tr>
<th>[2]Pseudorotaxane</th>
<th>$\lambda_{max}$ (nm)</th>
<th>Apparent $\varepsilon$ (M$^{-1}$ cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV-2(PF$_6$)⊂15</td>
<td>461</td>
<td>59</td>
</tr>
<tr>
<td>MV-2(PF$_6$)⊂19</td>
<td>448</td>
<td>68</td>
</tr>
<tr>
<td>MV-2(PF$_6$)⊂20</td>
<td>453</td>
<td>55</td>
</tr>
<tr>
<td>MV-2(PF$_6$)⊂22 (1:1)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MV-2(PF$_6$)⊂22 (4:1)</td>
<td>449</td>
<td>59</td>
</tr>
</tbody>
</table>
These $\lambda_{\text{max}}$ and extinction coefficient values nearly match those of their parent macrocycle 15 under the same conditions ($\lambda_{\text{max}}=453 \text{ nm}, \varepsilon=59 \text{ M}^{-1} \text{ cm}^{-1}$). No identifiable charge-transfer band was observed in the approximately 1:1 mixture of polymer macrocycle 22 and MV-2(PF$_6$). Upon increasing the molar ratio of 22/MV-2(PF$_6$) from approximately 1:1 to about 1:4, however, a charge-transfer band comparable to the other functionalized macrocycles was observed ($\lambda_{\text{max}}=449 \text{ nm}, \varepsilon=59 \text{ M}^{-1} \text{ cm}^{-1}$). It is possible that the multitude of polar ester functionalities along the poly(methyl acrylate) chains of 22 interact favorably, albeit weakly, with MV-2(PF$_6$) thus requiring greater equivalents of the guest to observe appreciable host–guest complexation. The demonstration of host–guest complexation by DNP38C10 macrocycles bearing functionalities of varying complexity is important given the desire to integrate such crown ether macrocycles into a variety of organic and bioorganic materials. Although only three examples of thiol-ene functionalization of diallyl macrocycle 15 are shown, the selectivity and reliability afforded by thiol-ene click chemistry provides near limitless flexibility in the synthesis of additional functionalized DNP38C10 macrocycles. In this regard allyl functionalized macrocycles 6, 7, 11, 12, and 15 are expected to play a prominent role in the development of increasingly complex self-assembling systems and mechanically interlocked molecules. We have been exploring the exciting prospects that allyl-functionalized DNP38C10 macrocycles 6, 7, 11, 12, and 15 open in the
areas of mechanically interlocked polymers (see chapter 5), and water-soluble protein–rotaxane conjugates (see chapter 4).

3.8 Conclusion

Five new DNP38C10 macrocycles bearing one (11), two (6, 15), three (12), or four (7) allyl functionalities off their naphthalene ring(s) have been synthesized. Self-assembly studies between each macrocycle and an electron-poor methyl viologen guest compound (MV-2(PF₆)) provide insight into the influence of allyl groups on the macrocycles’ ability to undergo host–guest complexation in solution. Increasing allyl substitution appears to decrease the enthalpic favorability of host–guest complexation, likely by sterically disrupting the ability of functionalized macrocycles to adopt the most favorable supramolecular conformation with a MV-2(PF₆) guest. At the same time, allyl functionalities appear to decrease the entropic penalty of host–guest complexation, likely by rigidifying the macrocycle and restricting its conformational space when uncomplexed. As a result overall binding decreases upon increasing allyl substitution, with monoallyl macrocycle 11 binding most strongly and tetraallyl macrocycle 7 unable to bind MV-(2PF₆) at all. Solid-state structures of two of the functionalized macrocycles and two of their host–guest complexes with MV-2(PF₆) help support these conclusions.

Allyl functionalities provide the added benefit of further synthetic modification through radical-initiated thiol-ene click chemistry. The ease of such transformations has been demonstrated through the thiol-ene functionalization of one
of the newly reported diallyl macrocycles (15) with three representative thiols. These allyl-functionalized DNP38C10 macrocycles allow easy and direct access to electron-rich naphthalene crown ether hosts that are capable of binding electron-poor viologen guests and can be easily modified through thiol-ene click chemistry, thus providing straightforward routes to integrating such macrocycles with a variety of different supramolecular, macromolecular, and mechanically interlocked materials.

Incorporation of these DNP38C10 is studied in the following chapters.
3.9 Materials and methods

3.9.1 General methods

Unless otherwise stated chemicals were purchased from commercial suppliers and used as received. Tetraethyleneglycol monotosylate,\textsuperscript{22} compound 10,\textsuperscript{23} tetraethyleneglycol ditosylate,\textsuperscript{23} and thiol 18\textsuperscript{35} were prepared according to literature procedures. Solvents were dried using an Innovative Technologies SPS-400-5 solvent purification system. All reactions were carried out under an anhydrous N\textsubscript{2} atmosphere unless otherwise noted. Thin layer chromatography (TLC) was performed on alumina-backed sheets coated with silica gel 60 F254. Preparative TLC was performed on 20x20cm plates coated with a 1000 \(\mu\)m thick layer of 150 \(\AA\) silica with F254 indicator. TLC plates were visualized using a UV/Vis lamp and/or by staining with iodine or p-anisaldehyde solution. Column chromatography was performed using glass columns over Dynamic Absorbents 60 \(\AA\), 32-63 \(\mu\)m silica gel. Melting points were determined on a Mettler Toledo Mel-Temp II melting point apparatus and are uncorrected. All \(^1\)H and \(^{13}\)C NMR spectra were recorded on with a Varian Mercury (300 MHz and 75 MHz, respectively) or Varian Unity Plus (400 MHz and 100 MHz, respectively) spectrometer using residual solvent as the internal standard. All chemical shifts are quoted using the \(\delta\) scale and all coupling constants are expressed in Hertz (Hz). UV/Vis data were recorded on a Varian CAR Y-100 Bio spectrophotometer. Isothermal titration calorimetry (ITC) was performed on a Microcal VPITC. Gel Permeation Chromatography (GPC) was performed on a
Viscotek TDA 305 eluting with THF at 35 °C at 1.0 mL/min. Mn and Mw were determined using the Viscotek RI detector and OmniSEC software, as well as by ¹H NMR spectroscopy. APCI and MALDI high-resolution mass spectrometric analysis was performed at the University of California, Riverside Mass Spec Facility. X-ray crystallographic analysis was performed at the J.D. McCullough X-Ray Crystallography Laboratory of the University of California, Los Angeles Molecular Instrumentation Center and the X-ray crystallographic facility at Yale University.

3.9.2 Synthetic procedures

Synthesis of 6

A mixture of compounds 5 (5.13 g, 6.25 mmol) and 1 (1.5 g, 6.25 mmol) was dissolved in acetone (50 mL) and taken up in a plastic syringe. This mixture was added slowly via syringe pump to a refluxing solution of potassium carbonate (44 g, 0.31 mol) in acetone (800 mL) at a rate of 0.5 mL h⁻¹. Following complete addition the reaction mixture was allowed to continue refluxing for a further 12 h. The resulting solution was cooled to room temperature and filtered through Celite. The filtrate was concentrated and the resulting residue was purified by flash column chromatography using a mixture of CH₂Cl₂/MeOH (8:2) as the mobile phase. Yield: 1.25 g (28 %); ¹H NMR (300 MHz, CD₃CN): δ=7.86 (2 H, d, J=8.4 Hz), 7.67 (2 H, d, J=8.4 Hz), 7.17 (1 H, d, J=8.4 Hz), 7.13 (2 H, t, J=16.2 Hz), 7.10 (1 H, d, J=8.7 Hz), 6.67 (2 H, d, J=7.8 Hz), 5.89 (2 H, ddd, J=18.0, 4.5, 1.8 Hz), 4.99 (2 H, dd, J=1.08, 1.8 Hz), 4.91 (2 H, dd, J=11.1, 4.5 Hz), 4.17 (4 H, t, J=9.3 Hz), 3.943–
3.685 (28 H, m, -OCH₂CH₂O-), 3.39 ppm (4 H, d, J=6.6 Hz); $^{13}$C NMR (100 MHz, CDCl₃): δ=154.4, 152.4, 137.6, 129.0, 128.4, 128.0, 126.8, 125.1, 118.6, 115.8, 114.7, 105.8, 73.9, 71.3, 71.2, 70.7, 68.2, 22.8 ppm; APCI-MS $[M+NH_4]^+$ calculated for C₄₂H₅₆NO₁₀: 734.3899; found 734.3914.

**Synthesis of 7**

A mixture of compounds 5 (4.0 g, 4.44 mmol) and 3 (1.05 g, 4.44 mmol) was dissolved in acetone (35 mL) and taken up in a plastic syringe. This mixture was added to a refluxing solution of potassium carbonate (30 g, 0.222 mol) in acetone (700 mL) at a rate of 0.5 mL h⁻¹ via syringe pump. Following complete addition the reaction mixture was allowed to continue refluxing for a further 12 hours. The resulting solution was cooled to room temperature and filtered through Celite. The filtrate was concentrated and the resulting residue was purified by flash chromatography using a mixture of CH₂Cl₂/MeOH (8:2) to yield 1.0 g (30 %) of 7 as a pale yellow solid. M.p. 81 °C; $^1$H NMR (300 MHz, CD₃CN): δ=7.85 (4 H, d, J=8.7 Hz), 7.13 (4 H, d, J=8.7 Hz), 5.82 (4 H, ddd, J=17.1, 11.7, 3.6 Hz), 4.95 (4 H, dd, J=17.1, 1.8 Hz), 4.91 (4 H, dd, J=11.7, 3.6 Hz), 3.94 (32 H, m, -OCH₂CH₂O-), 3.37 ppm (8 H, d, J=6.3 Hz); $^{13}$C NMR (100 MHz, CDCl₃): δ=152.4, 137.5, 128.9, 128.3, 128.0, 118.6, 115.9, 73.9, 71.3, 71.2, 70.6, 33.8 ppm; APCI-MS $[M+NH_4]^+$ calculated for C₄₈H₆₄NO₁₀: 814.4525; found 814.4530.
Synthesis of 11

A mixture of compounds 9 (500 mg, 2.49 mmol) and 10 (2.041 g, 2.49 mmol) was dissolved in acetone (20 mL) and taken up in a plastic syringe. This mixture was added to a refluxing solution of potassium carbonate (17 g, 125 mmol) in acetone (500 mL) via syringe pump at a rate of 0.5 mL h⁻¹. Following complete addition the reaction mixture was allowed to continue refluxing for a further 12 hours. The resulting solution was cooled to room temperature and filtered through Celite. The filtrate was concentrated and the resulting residue was purified by flash chromatography using a mixture of CH₂Cl₂/MeOH (8:2) to yield 480 mg (29 %) of 11 as a pale yellow solid. M.p. 82–84 °C; ¹H NMR (300 MHz, CD₃CN): δ=7.87 (1 H, d, J=8.1 Hz), 7.57 (1 H, d, J=6.6 Hz), 7.71 (1 H, d, J=8.1 Hz), 7.28 (1 H, d, J=8.1 Hz), 7.25 (1 H, t, J=7.5 Hz), 7.17 (1 H, d, J=8.1 Hz), 6.72 (2 H, dd, J=8.1, 7.5 Hz), 6.35 (1 H, d, J=7.5 Hz), 5.93 (1 H, ddd, J=15.6, 11.4, 3.0 Hz), 5.03 (1 H, dd, J=15.6, 2.1 Hz), 5.01 (1 H, d, J=11.4, 1.5 Hz), 4.19–3.66 (32 H, OCH₂CH₂O-), 3.46 ppm (2 H, d, J=6.3 Hz); ¹³C NMR (100 MHz, CDCl₃): δ=154.7, 154.5, 154.5, 151.9, 137.7, 130.0, 129.7, 128.8, 127.4, 126.9, 126.2, 126.0, 125.3, 118.4, 115.8, 115.0, 114.8, 114.7, 105.8, 104.8, 73.7, 71.4, 71.2, 71.2, 71.0, 70.7, 70.0, 68.1, 67.8, 33.9 ppm; APCI-MS [M+NH₄]⁺ calculated for (%) for C₃₉H₅₂NO₁₀: 694.3586; found 694.3604.
Synthesis of 12

A mixture of compounds 5 (6.79 g, 7.49 mmol) and 9 (1.5 g, 7.49 mmol) was dissolved in acetone (50 mL) and taken up in a plastic syringe. This mixture was added via syringe pump to a refluxing solution of potassium carbonate (51 gm, 0.37 mol) in acetone (800 mL) at a rate of 0.5 mL h⁻¹. Following complete addition the reaction mixture was allowed to continue refluxing for a further 12 hours. The resulting solution was cooled to room temperature and filtered through Celite. The filtrate was concentrated and the resulting residue was purified by flash chromatography using a mixture of CH₂Cl₂/MeOH (8:2) to yield 1.3 g (23 %) of 12 as a light yellow viscous liquid. ¹H NMR (300 MHz, CDCl₃): δ=7.89 (4 H, d, J=9.0 Hz), 7.86 (1 H, d, J=8.7 Hz), 7.83 (1 H, d, J=7.8 Hz), 7.69 (1 H, d, J=8.7 Hz), 7.22 (1 H, d, J=9.9 Hz), 7.18 (1 H, d, J=9.0 Hz), 7.08 (1 H, t, J=8.7 Hz), 7.07 (1 H, d, J=4.5 Hz), 6.53 (1 H, d, J=7.5 Hz), 5.85 (3 H, m), 4.97 (6 H, m), 4.09 (32 H, m, -OCH₂CH₂O-), 3.44 (2 H, d, J=6.6 Hz), 3.38 (2 H, d, J=6.6 Hz), 3.36 ppm (2 H, d, J=6.3 Hz); ¹³C NMR (100 MHz, CDCl₃): δ=154.8, 152.4, 152.4, 151.9, 137.5, 129.7, 129.0, 128.8, 128.5, 128.4, 128.1, 128.0, 127.4, 126.1, 126.0, 118.7, 118.4, 115.9, 114.9, 104.8, 73.9, 73.8, 71.3, 71.2, 70.7, 69.9, 68.1, 33.9, 33.7 ppm; APCI-MS [M+NH₄]⁺ calculated for C₄₅H₆₀NO₁₀: 774.4212; found 774.4210.

Synthesis of 15

A mixture of 14 (400 mg, 0.716 mmol) and tetraethylene glycol ditosylate (383 mg, 0.716 mmol) was dissolved in acetone (20 mL) and taken up in a plastic
syringe. This mixture was then added to a refluxing solution of potassium carbonate (5.0 g, 35 mmol) in acetone (150 mL) via syringe pump at a rate of 0.5 mL h\(^{-1}\). After complete addition the reaction mixture was allowed to continue refluxing for a further 12 h. The resulting solution was cooled to room temperature and filtered through Celite. The filtrate was concentrated and the resulting residue was purified by flash column chromatography using a mixture of CH\(_2\)Cl\(_2\)/CH\(_3\)OH (98:2) to yield 1.1 g (31%) of 15 as an off white solid. M.p. 72–74 °C; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta=7.86\) (2 H, d, \(J=8.4\) Hz), 7.76 (2 H, d, \(J=8.4\) Hz), 7.26 (2 H, t, \(J=8.1\) Hz), 7.19 (2 H, d, \(J=8.4\) Hz), 6.52 (2 H, d, \(J=7.5\) Hz), 5.93 (2 H, ddt, \(J=9.0, 8.7, 6.3\) Hz), 5.04–4.97 (4 H, m), 4.00 (4 H, m), 3.86 (4 H, m), 3.75 (4 H, m), 3.67 (4 H, m), 3.49 ppm (4 H, d, \(J=6.3\) Hz); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta=154.98, 152.22, 129.98, 129.40, 127.76, 127.71, 126.08, 118.25, 117.81, 117.5, 115.70, 115.03, 105.26, 91.73, 86.01, 74.16, 71.07, 71.03, 70.93, 70.87, 70.56, 69.72, 68.39, 67.73, 33.89, 1.86, 1.38, 1.10, 0.83, 0.55, 0.28 ppm; APCI-MS \(m/z\) [M+NH\(_4\)]\(^+\) calculated for C\(_{42}\)H\(_{56}\)NO\(_{10}\): 734.3899; found 734.3914.

Synthesis of 19

2,2-Dimethoxy-2-phenylacetophenone (3.5 mg, 0.0139 mmol, 10 mol%) was added to a solution of 15 (100 mg, 0.139 mmol) and methyl 3-mercaptopropionate (16, 66 mg, 0.556 mmol) in chloroform (5 mL). The mixture was allowed to react for 2.5 h under 365 nm UV light. The resulting solution was concentrated, run through a thin pad of silica eluting with a 2% solution of CH\(_3\)OH in CH\(_2\)Cl\(_2\) to yield 100 mg
of 19 (75%). $^1$H NMR (300 MHz, CD$_3$CN): $\delta = 300.786$ (2 H, d, $J = 6$ Hz), 7.76 (2 H, d, $J = 6$ Hz), 7.28 (4 H, m), 6.48 (2 H, d, $J = 6$ Hz), 4.00 (8 H, m), 3.87 (8 H, m), 3.76 (8 H, m), 3.67 (8 H, m), 3.63 (6 H, S), 2.75 (8 H, m), 2.55 (8 H, m), 1.86 ppm (4 H, q, $J = 9$ Hz); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 172.6$, 154.8, 152.1, 130.5, 129.7, 127.2, 126.3, 125.9, 118.5, 114.9, 104.8, 73.8, 71.4, 71.3, 71.0, 70.8, 69.9, 67.9, 52.0, 34.9, 32.0, 30.52, 29.0, 27.1 ppm; APCI-MS $m/z$ [M+NH$_4$]$^+$ calculated for C$_{50}$H$_{72}$NO$_{14}$S$_2$: 974.4389; found 974.4396.

**Synthesis of 20**

2,2-Dimethoxy-2-phenylacetophenone (2.8 mg, 0.0112 mmol, 5 mol%) was added to a solution of macrocycle 15 (70 mg, 0.097 mmol) and thiol 17 (82 mg, 0.225 mmol) in a 1:1 mixture of CHCl$_3$/CH$_3$OH (2 mL total). The reaction was irradiated at 365 nm for 5.5 h. The reaction solution was then concentrated under reduced pressure and the product was precipitated from the resulting residue using a 3:2 mixture of CH$_3$CH$_2$OH/CH$_2$Cl$_2$, yielding 123 mg of 20 (90%). M.p. 63–66 °C; $^1$H NMR (300 MHz, CD$_3$CN): $\delta = 7.87$ (2 H, d, $J = 9.0$ Hz), 7.76 (2 H, d, $J = 8.4$ Hz), 7.28 (2 H, t, $J = 8.1$ Hz), 7.23 (2 H, d, $J = 9.0$ Hz), 6.40 (2 H, d, $J = 4.8$ Hz), 5.22 (2 H, t, $J = 9.3$ Hz), 5.03 (2 H, t, $J = 9.6$ Hz), 4.95 (2 H, t, $J = 9.6$ Hz), 5.03–4.88 (2 H, m), 4.18 (2 H, d, $J = 5.1$ Hz), 4.14 (2 H, d, $J = 4.8$ Hz), 4.00–3.65 (32 H, m), 2.81–2.74 (4 H, m, Ar-CH$_2$), 2.69–2.60 (4 H, m, -S-CH$_2$-), 2.21 (24 H, s), 1.97–1.89 ppm (4 H, m); $^{13}$C NMR (75 MHz, CD$_3$CN): $\delta = 170.512$, 170.13, 169.79, 169.64, 154.76, 152.15, 130.92, 129.72, 127.44, 126.49, 125.75, 118.06, 117.60, 114.78, 104.93, 83.57, 75.52, 73.90, 73.61, 70.83,
70.71, 70.60, 70.45, 70.24, 69.50, 68.501, 68.17, 62.189, 30.98, 30.24, 28.52, 20.24, 20.16, 20.08 ppm; APCI-MS \( m/z \) \([M+NH_4]^+\) calculated for C\(_{70}\)H\(_{96}\)NO\(_{28}\)S\(_2\): 1462.5555; found 1462.5588.

**Synthesis of 21**

2,2-Dimethoxy-2-phenylacetophenone (2.0 mg, 0.0139 mmol, 10 mol%) was added to a solution of macrocycle 15 (50 mg, 0.0698 mmol) and thiol 18\(^{35} \) (23 mg, 0.139 mmol) in a mixture of CHCl\(_3\)/CH\(_3\)OH/THF (1:1:1; 1 mL total). The reaction mixture was irradiated at 365 nm for 5 h. The resulting mixture was then concentrated, the residue was passed through a short pad of silica, eluting with a mixture of CH\(_2\)Cl\(_2\)/MeOH (9:1) to yield 65 mg of 21 (90%). \(^1\)H NMR (300 MHz, CD\(_3\)CN): \( \delta = 7.94 \) (2 H, d, \( J=8.7 \) Hz), 7.78 (2 H, d, \( J=8.7 \) Hz), 7.19 (2 H, t, \( J=7.8 \) Hz), 7.18 (2 H, d, \( J=8.4 \) Hz), 6.38 (2 H, d, \( J=7.5 \) Hz), 4.26 (4 H, t, \( J=6.9 \) Hz), 4.04–3.79 (32 H, m), 2.79–2.71 (8 H, m), 2.54 (4 H, t, \( J=7.2 \) Hz), 1.91 (12 H, s), 1.85 ppm (4 H, m); \(^{13}\)C NMR (75 MHz, CD\(_3\)CN): \( \delta = 171.68, 154.81, 152.08, 130.48, 129.72, 127.22, 126.38, 125.96, 118.67, 114.95, 104.77, 77.72, 77.30, 76.88, 73.75, 71.26, 71.04, 70.79, 69.96, 67.92, 65.26, 55.93, 32.29, 30.67, 30.98, 30.22, 28.99 ppm; APCI-MS \( m/z \) \([M+NH_4]^+\) calculated for C\(_{54}\)H\(_{78}\)NO\(_{14}\)S\(_2\)Br\(_2\): 1186.3225; found 1186.3253.

**Synthesis of 22**

A dry three-neck round-bottom flask was charged with macrocycle 21 (90 mg, 0.0865 mmol), methyl acrylate (1.24 gm, 14.36 mmol), tris[2-(dimethylamino)ethyl]amine (3.2 mg, 0.0138 mmol), CuBr\(_2\) (0.96 mg, 0.004 mmol),
Cu wire (2.55 mg, 0.04 mmol; the Cu wire was wrapped around a magnetic stir bar and initially suspended above the reaction mixture) in dry DMSO (0.6 mL) under inert atmosphere. The resulting mixture was subjected to three freeze-pump-thaw cycles and then allowed to warm to room temperature. The Cu wire was then allowed to drop into the mixture and the reaction solution was allowed to stir for 4.5 h. Conversion was monitored by NMR spectroscopy and the polymerization was stopped at approximately 78 % conversion. The reaction mixture was passed through a short plug of neutral alumina, cooled to −78 °C in a dry ice/acetone bath, and methanol (150 mL) was used to precipitate the polymer, yielding 700 mg of 22; 4440 $M_n$ (determined by NMR spectroscopy) with 1.2 polydispersity index (PDI; determined by GPC); $^1$H NMR (300 MHz, CD$_3$CN): $\delta$=7.86 (2 H, d, $J$=8.7 Hz), 7.76 (2 H, d, $J$=8.4 Hz), 7.27 (2 H, t, $J$=8.1 Hz), 7.24 (2 H, d, $J$=9.0 Hz), 6.49 (2 H, d, $J$=7.5 Hz), 4.12 (2 H, t, $J$=6.6 Hz, -$CH$-Br), 4.00–3.76 (32 H, m), 3.63 (114 H, s), 2.85–2.74 (4 H, m, -$CH_2$-Ar), 2.70–2.68 (8 H, m, -$CH_2$-S-$CH_2$-), 2.27 (62 H, m), 1.96–1.82 (22 H, m), 1.67 (36 H, m), 1.54 ppm (18 H, m).
3.9.3 Isothermal titration calorimetry (ITC).

Thermodynamic binding data were collected for the association of macrocycles 6, 11, 12, 15, and parent macrocycle DNP38C10 with methyl viologen (MV-2(PF₆)) at 25 °C. In the cases of macrocycles 6, 11, 15, and DNP38C10 a 10 mM acetonitrile solution of MV-2(PF₆) was titrated via syringe (5 µL injections with a time interval of 250 s per injection) into a sample cell containing a 1 mmol acetonitrile (2 mL) solution of macrocyclic host compound. For macrocycle 12, which was observed to bind weakly, a 63 mM acetonitrile solution of MV-2(PF₆) was titrated via syringe (16.5 µL injections with a time interval of 250 s per injection) into a sample cell containing a 3.0 mM acetonitrile solution of macrocyclic host compound. In each case the enthalpy of dilution of MV-2(PF₆) was subtracted from the observed heats of combination before fitting each data set. The thermodynamic parameters were calculated using the inbuilt software in the MicroCal system.
(a) MV-2(PF₆)·DNP₃₈C₁₀ binding isotherm:  
(b) MV-2(PF₆)·6 binding isotherm:
(c) MV-2(PF_6)⊂11 binding isotherm:

(d) MV-2(PF_6)⊂12 binding isotherm:
(e) MV-2(PF₆)₁₅ binding isotherm:
3.9.4 X-Ray crystallographic data

X-ray quality single crystals of macrocycles 7 and 15 as well as [2]pseudorotaxanes MV-2(PF₆)⊂6 and MV-2(PF₆)⊂15 were grown as described in the main text.

Crystallographic diffraction data for macrocycles 7 and 15 was collected at the X-ray crystallography center at Yale University by Dr. Michael K. Takase (now at the California Institute of Technology). Crystallographic diffraction data for [2]pseudorotaxanes MV-2(PF₆)⊂6 and MV-2(PF₆)⊂15 was collected at the X-ray crystallography center at the University of California, Los Angeles, by Dr. Saeed J. Khan.

Collection parameters for macrocycles 7 and 15: Low-temperature diffraction data (ω-scans) were collected on a Rigaku MicroMax-007HF diffractometer coupled to a Saturn994+ CCD detector with Cu Kα (λ = 1.54178 Å) for the structure of 12085 and 12089. All structures were solved by direct methods using SHELXS¹⁶ and refined against $F^2$ on all data by full-matrix least squares with SHELXL-97³⁷ using established refinement techniques.³⁸ All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included into the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the $U$ value of the atoms they are linked to (1.5 times for methyl groups). All disorders were refined with the help of similarity restraints on the 1,2- and 1,3-distances and displacement parameters as well as rigid bond restraints for anisotropic displacement parameters.
**Macrocycle 7**: The macrocycle crystallizes in the monoclinic space group \( P2_1/n \) with half a molecule in the asymmetric unit. The two alkyl groups were disordered over two positions. One was modeled as a two component disorder, 0.81(4):0.19(4), and the other as a three component disorder, 0.483(4):0.392(6):0.125(5). The CIF file for macrocycle 7 has been deposited with the Cambridge Crystallographic Data Center: [CCDC 962524](https://doi.org/10.1107/S1744309115002830).

**Macrocycle 15**: The macrocycle crystallizes in the triclinic space group \( P\bar{1} \) with one molecule in the asymmetric unit. The two alkyl groups were modeled as two component disorders. The occupancies of the two components were refined freely and converged at, 0.58(5):0.42(5) and 0.526(5):0.474(5), respectively. One of the carbon-oxygen chains linking the two aryl groups was disordered over two positions with occupancies 0.508(8):0.492(8). The CIF file for macrocycle 15 has been deposited with the Cambridge Crystallographic Data Center: [CCDC 962525](https://doi.org/10.1107/S1744309115002830).

**[2]Pseudorotaxane MV-2(PF₆)⊂6**: The crystals of [2]pseudorotaxane MV-2(PF₆)⊂6 grow as non-merohedral twins. The selected crystal, a red platelet of approximate size of 0.3 X 0.2 X 0.1 mm, was a two-component twin. As the compound loses solvent rather quickly, the crystal was coated with a paratone oil and quickly mounted and centered on a Bruker SMART 1000 APEX2 Diffractometer. Intensity measurements were performed using a monochromated Mo-K\(_α\)-radiation (0.71073 Å) from a sealed tube. Generator settings were 50 kV, 30 mA. Data collection temperature was 100K. Data were acquired using three sets of Omega scans at different Phi settings, and one
Phi scan. The frame width was 0.5°. A total of 1800 frames were collected. The total exposure time was 45.00 hours.

CELL_NOW software was used for the determination of the unit cell and the twin law. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. The integration of the data using a triclinic unit cell yielded a total of 36498 reflections to a maximum θ angle of 26.74° (0.79 Å resolution). The final cell constants of a = 15.527(6) Å, b = 19.205(8) Å, c = 21.620(9) Å, α = 98.271(5)°, β = 112.190(4)°, γ = 100.123(5)°, volume = 5718.(7) Å³, are based upon the refinement of the XYZ-centroids of 3241 reflections above 20 σ(I) with 7.637° < 2θ < 52.65°. Data were corrected for absorption effects using the multi-scan method (TWINABS). The ratio of minimum to maximum apparent transmission was 0.848.

The structure was solved and refined using the Bruker SHELXTL Software Package, in the space group P1bar. There were two independent molecules per asymmetric unit (Z = 4). The twin components refined at a ratio of 86:14. The final refinements were done with all the data and no equivalent reflections were merged. The final anisotropic full-matrix least-squares refinement on F2 converged at R1 = 9.24%, wR2= 23.20% and a goodness–of–fit of 0.927. The CIF file for [2]pseudorotaxane MV-2(PF₆)⊂₆ has been deposited with the Cambridge Crystallographic Data Center: CCDC 962003.
[2]Pseudorotaxane MV-2(PF₆)⊂15: A deep red prism of [2]pseudorotaxane MV-2(PF₆)⊂15, approximate dimensions 0.30 mm x 0.22 mm x 0.12 mm, was quickly mounted from a paratone oil on to a Mitgen loop and centered on a Bruker SMART APEX2 CCD-based X-ray diffractometer system equipped with a Mo-Kα radiation (λ = 0.71073 Å) operated at 1500 watts power. Data collection temperature was 100(2) K with the detector placed at a distance of 6 cm from the crystal.

A total of 1800 frames were collected with a scan width of 0.5° in ω, and an exposure time of 75 seconds per frame. Data were acquired using three sets of Omega scans at different Phi settings, and one Phi scan. The crystal diffracted poorly and the data obtained was week.

Apex2 software was used for preliminary determination of the unit cell. Determination of integral intensities and unit cell refinement were performed using SAINT⁴⁰ employing a narrow-frame integration algorithm. The integration of the data using an Orthorhombic unit cell yielded a total of 73982 reflections to a maximum θ angle of 28.29°, of which 7822 were independent (R_{int} = 4.18%) and 5621 were greater than 4σ (F). The final cell constants of a = 39.125(18) Å, b = 14.275(6) Å, c = 21.747(9) Å, volume = 12146(9) Å³, are based upon the refinement of the xyz centroids of 9996 reflections. Data was corrected for absorption effects and scaled with SADABS using multi-scan technique.
The structure was solved and refined using the Bruker SHELXTL Software Package, in the space group Cmcm, with \( Z = 4 \). The structure was highly disorder with two independent conformations of the compound almost overlapping resulting in a full molecule disorder. Due to such an extensive disorder the crystals of \( \text{MV-2(PF}_6)\subset 15 \) diffracted very poorly. The model was refined with extensive geometrical and thermal parameter restraints. The final anisotropic full-matrix least-squares refinement on \( F^2 \) converged at \( R_1 = 8.10\% \), \( wR_2 = 23.35\% \) and a goodness–of–fit of 1.055. Refinements were tried using lower symmetry non-centric space groups but no improvement in the model was observed.

The CIF file for [2]pseudorotaxane \( \text{MV-2(PF}_6)\subset 15 \) has been deposited with the Cambridge Crystallographic Data Center: **CCDC 932190.**
3.10 References and notes


organization of two heteroditopic monomers to supramolecular alternating


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27. (a) Houk, K. N.; Menzer, S.; Newton, S. P.; Raymo, F. M.; Stoddart, J. F.; Williams, D. J., Molecular meccano. 47. [C-H - O] interactions as a control element
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affinities for the binding of bipyridinium-based guests by catenated hosts. *J. Am.
Chem. Soc.* **1999**, *121* (7), 1479-1487; (b) Raymo, F. M.; Bartberger, M. D.; Houk, K.
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31. Crystal data for MV-2(PF6)⊂6: C_{54}H_{66}F_{12}N_{10}O_{10}P_{2}, F_w=1193.04, triclinic, space group P-1, a=15.27(6), b=19.205(8), c=21.620(9) Å, α = 98.271(5)°, β =112.190(4)°, γ =110.123(5)°, V=5718.4 Å³, ρ =1.386, T=100(2) K, Z=4, 36502 independent reflections measured, final R1=0.0924 and wR2=0.2997.

32. Crystal data for MV-2(PF6)⊂15: C_{112}H_{134}F_{24}N_{6}O_{20}P_{4}, Mr=2464.15, orthorhombic, space group Cmcm, a = 39.125(18), b = 14.275(6), c = 21.747(9) Å, α =β =γ =90°, V = 12145.9 Å³, ρ = 1.348, T = 100(2) K, Z = 4, 7822 independent reflections measured, final R1=0.0810 and wR2=0.02335.

33. In contrast to the solid-state structure of [2]pseudorotaxane MV-2(PF6)⊂15, the X-ray superstructure of patent [2]pseudorotaxane MV-2(PF6)⊂DNP38C10 possesses a center of symmetry (space group P1,Z=1) and reveals symmetric binding of MV-2(F6) within its interior cavity; see ref. [11].

34. The molecular weight of 22 was determined by ^1HNMR by integrating proton peaks for the methyl group of the acrylate units against aromatic protons in the
macrocycle. spectrum. The Mn measured by GPC was significantly higher at 18763 g mol$^{-1}$ as compared to poly(methyl methacrylate) standards. The significant steric bulk of the central DNP38C10 ring of polymer 22 accounts for the prediction of a much higher molecular weight polymer by GPC relative to the Mn measured by 1H NMR spectroscopy.


39 CELL_NOW Version 2008/4 (Sheldrick, Bruker AXS Inc.)

40 SAINT Version 7.68a (Bruker AXS Inc., 2007)

41 TWINLABS Version 2007/2 (Sheldrick, Bruker AXS Inc.)
Chapter 4-Synthesis of Functionalized Water Soluble DNP38C10 Macrocycles and [2]Pseudorotaxane Formation in Water

4.1 Abstract

Water soluble maleimide functionalized imidazolium bromide DNP38C10 macrocycle salts have been synthesized. The macrocycles bind with a newly synthesized water soluble naphthalene diimide (NDI) derivative and form [2]pseudorotaxanes. The formation of a representative, water soluble [2]pseudorotaxane was demonstrated by \(^1\)H NMR and UV-Vis spectroscopy. Once suitable conditions are optimized these [2]pseudorotaxane will be conjugated with a mutated green fluorescent protein which has a reactive cysteine residue. The synthesis of this water soluble functionalized macrocycle opens up new frontiers for carrying out cysteine/thiol-maleimide click chemistries on host-guest and mechanically interlocked species in water.
4.2 Introduction

Water is an essential part of our daily lives. Some of the most intricate processes that require chemical energy to be converted into mechanical energy, for example ATP synthase and the actions of motor proteins (e.g. kinesin and myosin), take place in aqueous environments\(^1\). Scientific efforts to mimic some of these processes in organic solutions and insights into the design and function of both motor proteins and their artificially synthesized analogues have produced interesting results\(^2\). However, the synthesis of water soluble synthetic molecular machines still remains a challenge for scientists due to the poor solubility of many synthetic systems.

Mechanically interlocked molecules (MIMs) present an excellent opportunity to perform mechanical tasks in either aqueous or non-aqueous environments. Despite enormous progress towards understanding complex MIMs, synthesizing water soluble MIMs as prototypical molecular machines is an overarching goal. One of the main obstacles is the water solubility of individual components of a particular MIM. Despite the obstacles, however numerous water soluble macrocycles have been incorporated into MIMs. Cyclodextrin,\(^3\) cucurbituril,\(^4\) calixaranes\(^5\) and coordination cage\(^6\) based macrocycles are among the well-studied water soluble macrocycles. Since Pederson’s crown ether\(^7\) discovery, crown ethers have attracted a lot of attention for their host properties for various organic and inorganic guests. Furthermore, electron rich crown ether macrocycles with their donor-acceptor properties have found their use in MIMs such as 1,5-dioxynaphthalene[38]crown-10.
(DNP38C10) macrocycles which are well studied by Sanders\textsuperscript{8} and coworkers for their charge transfer properties with electron deficient guests. In non-aqueous environments DNP38C10 binds to naphthalene diimide (NDI) with relatively low binding strength ($K_a \approx 10^2 \text{ M}^{-1}$)\textsuperscript{9} in CHCl$_3$:MeOH (98:2) at 298K. In an effort to make water soluble electron rich macrocycles, Yu Liu and coworkers successfully synthesized the first electron rich tetrasulfonated DNP38C10 water soluble macrocycle\textsuperscript{10} with very high binding affinity for a water soluble NDI derivative ($K_a \approx 10^6 \text{ M}^{-1}$, Figure 1).\textsuperscript{11} The tetra-sulfonated version of the macrocycle, although very encouraging in terms of binding, has very little utility for further development into molecular machines as the macrocycle lacks easily transformable functionality.

In 2012 we investigated\textsuperscript{12} (chapter 2) use of a small peptide, a glutathione derivative, to prepare mechanically interlocked molecules. However, there were two problems with the design 1) the glutathione derivative was not large enough to

\textbf{Figure 1.} First water soluble tetra-sulfonated DNP38C10 macrocycle.
prevent the macrocycle from slipping on and off the NDI guest, and 2) the glutathione
derivative had to be methylated for it to be soluble in organic media (e.g. chloroform).

To address these problems we aim to use proteins as a stoppering unit. We hypothesize that proteins as stoppering units will be more than sufficient, sterically, to prevent the macrocycle from slipping on and off the NDI guest. The second problem is being tackled by synthesizing water soluble DNP38C10 derivatives and a water soluble, maleimide functionalized NDI guest derivative.

The initial aim has been to synthesize water soluble DNP38C10 macrocycles
and a water soluble maleimide NDI guest. Once the synthesis of these water soluble components is optimized, they will be investigated for use in preparing protein conjugated MIMs in collaboration with Prof. Rich Olson (Department of Molecular Biology and Biochemistry, Wesleyan University). Prof. Rich Olson and coworkers have recently prepared a mutated green fluorescent protein (GFP) bearing a short peptide chain terminated with a reactive cysteine group. Conditions to incorporate these proteins with our DNP38C10 macrocycle and NDI guest will be evaluated. This research will provide a valuable proof-of-concept to integrate synthetically designed MIMs with biologically relevant systems.

Our long-term objective is to integrate stimuli-responsive MIMs with proteins and enzymes. Stimuli-responsive MIMs have already been shown to function in drug delivery systems,\textsuperscript{13} as nanovalves,\textsuperscript{14} as molecular muscles\textsuperscript{2b} and in a variety of other nanoscale applications. We hypothesize that the ability to use an external stimulus (e.g. pH, redox reactions, light, etc.) to initiate a change in the relative position of the
macrocyle along its guest can be used to induce allosteric effects in proteins that are attached to the stimuli responsive rotaxane. This is admittedly an ambitious goal, which can only be achieved if and when initial methods of integrating water soluble rotaxanes with entire proteins optimized. Determining such optimized methods requires that suitable aqueous soluble host-guest [2]pseudorotaxanes can be prepared, as is the goal of the research presented in this chapter.
4.3 Synthesis of a water soluble [2]pseudorotaxane 3


Imidazole and imidazolium salts are known for their versatility and compatibility with many applications,\textsuperscript{15} which inspired us to use this chemistry. Many functional groups can be readily added to the 1-position imidazole, as reported in the literature.\textsuperscript{16} The proposed design of a water soluble [2]pseudorotaxane 3 is shown in Scheme 1, which consists of a noncovalent assembly of water soluble electron poor NDI derivative 1a or 1b and water soluble DNP38C10 dervatized macrocycle 2.
4.3.1 Synthesis of a water soluble macrocycle 2

The synthesis of the water soluble macrocycle 2 is shown in Scheme 2. The bis-allyl macrocycle 4, when reacted with 2-mercaptoethanol using photo initiated thiol-ene click reaction conditions, forms dihydroxy macrocycle 5. Reacting 5 with CBr₄ under Apple reaction conditions gives dibrominated product 6. Compound 6 was then reacted with an excess amount of 1-methy imidazole to obtain water soluble compound 2.

Scheme 2. Synthesis of the water soluble bis-functionalized imidazolium macrocycles 2. Tetra-functionalized imidazolium macrocycle 7 has been synthesized as well along the same synthetic route starting from a tetraalkyl analogue of macrocycle 7.
We have also synthesized the water soluble tetrasubstituted DNP38C10 macrocycle 7, which is also being studied currently for its complexation with guests 1a and 1b.

4.3.2 Synthesis of water soluble guest

Scheme 3. Synthesis of the water soluble NDI guest.

Water soluble maleimide functionalized guests 1a and 1b were synthesized as shown in Scheme 3. Naphthalene dianhydride 13 was heated in N, N-dimethyl ethylamine to form the diamine adduct 14. When 14 was heated to 80°C in acetonitrile in the presence of furan protected maleimide derivative 15 it forms 16.
Deprotection of the furan moieties was achieved by heating 16 to 110°C in DMF to obtain 1a. Similarly 1b was prepared by reacting 14 with excess propargyl bromide.

4.4 Characterization of the [2]pseudorotaxane 3 [1a+2]

Mixing 1a and 2 in D₂O in a 1:1 molar ratio at ambient temperature results in the instantaneous formation of a deep red solution. ¹H NMR spectroscopy of this solution (Figure 2) indicates the formation of thermodynamically stable [2]pseudorotaxane 3 [1a+2] through changes in the chemical shifts of diagnostic proton signals. At 500 MHz and 298 K dethreading of macrocycle 2 onto and off the

![Chemical Structure](image)

**Figure 2.** Partial ¹H NMR spectra (298 K, D₂O, 500 MHz, 2mM) of macrocycle 2 (Scheme 1.), NDI guest 1a and [2]pseudorotaxane 3 [1a+2] (2 mM concentration) indicating diagnostic shifts of proton signals. The peak H₆ is an imidazole alkene proton which exchanges with the D₂O, which over a period diminishes completely.
thread 1a is comparable to the $^1$H NMR time scale, leading to significant broadening of resonances for both compounds.

Further evidence of the formation of a host-guest assembly was obtained from UV–Vis spectroscopy (Figure 3). A prominent charge transfer band is observed for 3 [1a+2] at 500 nm for a 0.01 M H$_2$O solution with an apparent $\varepsilon = 328$ M$^{-1}$ cm$^{-1}$ and 3 [1b+2] for a 0.01 M H$_2$O solution with an apparent $\varepsilon = 110$ M$^{-1}$ cm$^{-1}$ respectively.

![Absorbance vs. Wavelength](image)

**Figure 3.** UV-Vis spectra of 1:1 mixtures of [2]pseudorotaxane 3 [1a+2] and [2]pseudorotaxane from [1b+2] at 10 mM concentration. (H2O, 298 K)

An apparent $\varepsilon$ for [2]pseudorotaxane 3 [1a+2] is one and half times greater than the apparent $\varepsilon$ of [2]pseudorotaxane 3 [1b+2]. The increase in the apparent $\varepsilon$ is hypothesized to be result of additional $\pi$-$\pi$ interactions between the electron poor maleimide moieties of guest 1a and the naphthalene units of host 2. This possibility will be a subject of future investigation including computational and additional
experimental work, such as, further functionalization of the maleimide groups, and through concentration studies.


As discussed in the first chapter rotaxanes have a unique property such that the macrocycle can be moved with external stimuli in a specific direction. Once the conditions for the synthesis of the [2]pseudorotaxane shown in Scheme 1 are optimized the maleimide functionality on the imidazoles can be efficiently used to introduce various functional groups. The thiol-maleimide chemistry can be used to introduce proteins with reactive cysteine into rotaxane architectures.

Hypothetically, allosteric effects can be imparted to a protein that is conjugated to a bistable, stimuli responsive rotaxane which may be of great importance in drug delivery. Numerous drugs for diseases like diabetics, cancer, arthritis and many more use HSA as a drug carrier.\(^\text{17}\) Reportedly, there are three domains in HSA where most of the drugs bind\(^\text{17b, 18}\) and these domains carry hydrophobic fatty acids and drug molecules to different parts of the body. Despite enormous research the controlled release of drugs precisely at a predefined location remains a significant challenge. Hypothetically, a macrocycle unit in a HSA-rotaxane conjugate carrying a drug can be moved upon some external stimuli (pH, chemical etc.) so that the drug molecules can be released precisely.

In collaboration with Dr. Rich Olson’s group at Wesleyan university, we are currently optimizing conditions to introduce mutated GFP proteins in to rotaxanes.
As Dr. Rich Olson’s group finds working with GFP is relatively easier to HSA and may give early insights into our proposed rotaxane protein conjugate. However once experimental conditions are optimized for incorporating GFP in to rotaxane we will target the albumin proteins for conjugate studies. [2]Pseudorotaxane 3 has a bis-imidazolium bromide DNP38C10 macrocycle 2 and a NDI maleimide functionalized guest 1a. To our knowledge, this will be the first example of a [2]rotaxane that has protein stoppering units.

Currently, the mutated GFP bearing a small peptide with a reactive cysteine is being purified in the Olson lab. Once a pure sample of the protein is obtained it will be evaluated for conjugation with [2]pseudorotaxane 3 [1a+2].

**Scheme 4.** Synthesis of a proposed GFP stoppered rotaxane.

Once the [2]pseudorotaxane synthesis is optimized our larger aim is to conjugate these [2]pseudorotaxanes with active cysteine proteins containing one
accessible cysteine residue, such as human serum albumin (HSA), bovine serum albumin (BSA), ubiquitin and other modified green fluorescent protein (GFP).

Towards this aim we are in the process of optimizing reaction conditions to efficiently synthesize the above mentioned [2]pseudorotaxane 3.

4.6 Summary

The result here highlight the utility of combining π-π interactions and hydrophobic forces in the formation of water soluble [2]pseudorotaxanes. We have successfully utilized imidazolium chemistry in order to prepare water soluble DNP38C10 macrocycles. Further functionalization of the 1-position of imidazole is being investigated for the synthesis of water soluble multifunctional DNP38C10 macrocycles. We are working towards synthesizing the first protein stoppered rotaxane conjugate using thiol maleimide click chemistry.

4.7 Future direction

Imidazolium functionalized macrocycles 2 and 7 (Scheme 2) are highly soluble in water however they lack useful functionalities for further chemical transformations. The introduction of easily functionalized imidazole moieties onto DNP38C10 derivatives opens synthetic routs to other functionalized yet water soluble macrocycles, such as macrocycles 11 and 12 (Scheme 5). Currently we are optimizing reaction conditions to react maleimide functionalized imidazole 8 and propargyl functionalized imidazole 9 with 6 (8 see materials and methods, 9[16c]) in
order to obtain 11 and 12 respectively (Scheme 5). The alkyne 9 and maleimide derivative 8 are being used simply for their utility in efficient CuAAC and thiol-maleimide click reactions respectively. Similar experiments will be carried out with the tetrasubstituted macrocycle 7.

**Scheme 5** Proposed imidazole 8 and 9 when react with 6 (Scheme 2) form 11 and 12 respectively.

Previously (Chapter 3) we have shown that a tetra-allyl DNP38C10 macrocycle do not form host-guest complexes with either viologen or NDI derivatives in organic solutions. This is contrary to the preliminary results that we
observe in water, where the tetrasubstituted macrocycle 7 binds water soluble NDI
derivative 1b. The complex formation is likely to be attributable to the hydrophobic
interactions along with the π-π interactions. However further investigations are
necessary to determine the thermodynamic parameters (ΔH, ΔS and ΔG) of binding
and the roles of hydrophobic versus π-π versus other noncovalent interactions.
4.8 Materials and methods

4.8.1 General method

Chemicals were purchased from Aldrich, Acros, TCI America, or Cambridge Isotope Labs and used as received. Solvents were dried using an Innovative Technologies SPS-400-5 solvent purification system. All reactions were carried out under an anhydrous nitrogen atmosphere unless otherwise noted. Thin layer chromatography (TLC) was performed on alumina-backed sheets coated with silica gel 60 F254. TLC plates were visualized using a UV/Vis lamp and/or by staining with iodine or p-anisaldehyde solution. Column chromatography was performed using glass columns over Dynamic Absorbents 60 Å, 32-63 μm silica gel.

4.8.2 Synthetic procedures

Synthesis of 5 (Scheme 2)

To a solution of macrocycle 4 (0.5 g, 0.62 mmol) and 2-mercaptoethanol (0.783 g, 10 mmol) in 20 mL dry DMF was added 2,2-dimethoxy-2-phenylacetophenone (7.9 mg, 0.031 mmol, 5 mol%). The reaction was irradiated at 365 nm for 5.5 hours. The reaction mixture was concentrated to get rid of excess DMF. The residue was subjected to column chromatography using ethyl acetate: methanol (9:1) to yield 5 (434 mg, 70%). $^1$H NMR (300 MHz, CDCl$_3$, 298K) δ 7.80 (4H, d, $J$=9 Hz), 7.07(4H, d, $J$=9 Hz), 4.1(4H, q, $J$=9 Hz), 3.9-3.6 (36H, m, -O-CH$_2$-CH$_2$-O-), 2.62(8H, t, $J$=6 Hz), 2.39(8H, t, $J$=6 Hz), 1.73(8H, d, $J$=9 Hz), 1.25(4H, d,
J=6 Hz). $^{13}$CNMR (300 MHz, CDCl$_3$, 298K) δ 152.4, 129.6, 128.7, 128.2, 118.7, 118.6, 71.2, 71.0, 70.6, 60.6, 35.3, 31.5, 31.5, 30.7, 28.7, 28.6.

**Synthesis of 6 (Scheme 2)**

To a solution of 5 (0.4 g, 0.36 mmol) in dry dichloromethane 30 mL, PBr$_3$ (487 mg, 1.8 mmol) was added at 0°C under nitrogen atmosphere. The reaction was allowed to stir for 4 hours at room temperature. The reaction was cooled to room temperature and extracted with 1N NaOH 50 mL. The organic layer was dried over MgSO$_4$ and concentrated under reduced pressure. The residue was purified by column chromatography using dichloromethane to get 6 (341 g, 70%). $^1$H NMR (300 MHz, CDCl$_3$, 298K) δ 7.84(4H, d, J=6 Hz), 7.11(4H, d, J=6 Hz), 4.38-3.38(24H, m), 3.42(4H, t, J=9 Hz), 2.89(8H, t, J=9 Hz), 2.66(8H, t, J=9 Hz), 2.46(8H, t, J=9 Hz), 1.78(8H, t, J=9 Hz), 1.25(8H, t, J=9 Hz).

**Synthesis of 2 (Scheme 3)**

To a solution containing compound 6 (0.3 g, 0.3 mmol) in 30 mL of acetonitrile was added 1-methyl imidazole (0.35 g, 4.4 mmol). The reaction was heated to 80°C for 30 hours. The reaction was cooled to room temperature, filtered the solid and washed with 20 mL acetonitrile. The filtrate was concentrated under reduced pressure to get rid of excess 1-methyl imidazole to get product 2 (305 mg, 90%). $^1$H NMR (300 MHz, D$_2$O, 298K) δ 7.64(4H, d, J=9 Hz), 7.31(4H, s), 7.0(4H, d, J=9 Hz), 4.65(12H, s), 4.20(8H, t, J=6 Hz), 3.8-3.6(24H, m), 2.81(8H, t, J=9 Hz), 2.47(8H, t, J=9 Hz), 2.26(8H, t, J=6 Hz), 1.52(8H, t, J=9 Hz).
Synthesis of 16 (Scheme 3)

To a solution of compound 14 (0.5 g, 1.22 mmol) in 50 mL acetonitrile was added compound 15 (1.4 g, 6.1 mmol) under nitrogen. The reaction was heated to 80°C for 24 hours. Then the reaction was cooled to room temperature, followed by filtration of solid and the solid was washed with 20 mL acetonitrile. The filtered product was dried under vacuum to get product 16 (830 mg, 90%). $^1$H NMR (500 MHz, D$_2$O, 298K) δ 8.73 (4H, s), 7.9 (1H, s), 6.86 (1H, s), 6.55(2H, s), 5.30(2H, s), 4.57 (2H, m), 4.52 (2H, m), 3.66(4H, m), 3.58(4H, m), 3.24(12H, s), 2.98(3H, s), 2.82(3H, s), 2.19 (4H, m).

Synthesis of 1a

A solution of 16 (0.5 g, 0.51 mmol) in DMF 30 mL was heated to 110°C for 24 hrs in open atmosphere. The reaction was cooled to room temperature and the product was precipitated with ethanol 20 mL and filtre to yield 1a (390 mg, 90 %). $^1$H NMR (500 MHz, D$_2$O, 298K) δ 8.75 (4H, s), 6.89 (4H, s), 4.6 (4H, m), 3.6 (8H, m), 3.58 (4H, m), 3.28 (12H, s), 2.23 (4H, m).

Synthesis of 1b

A mixture of 14 (0.5 g, 1.2 mmol) and propargyl bromide (0.73 g, 6.1 mmol) in CH$_3$CN 30 mL was heated to 80°C for 24 hours under nitrogen atmosphere. The reaction was cooled to room temperature and the product was precipitated by adding
100 mL ethanol to yield 1b (0.69 g, 90%). $^1$H NMR (300 MHz, D$_2$O, 298K) $\delta$ 8.75 (4H, s), 4.6 (4H, m), 3.69 (4H, m), 3.58 (4H, m), 3.28 (12H, s), 3.1(2H, s).

Synthesis of 8

**Scheme 6 Synthesis of 8**

Synthesis of 18$^{20}$

To a solution of 2-chloroethanol (12.07 g, 0.15 mol) and 1-H-imidazole (10.2 g, 0.15 mol) in 1,4-dioxane 50 mL was added N-benzyl-N,N,N-triethylammonium chloride (1.13 g, 0.004 mol) followed by NaOH (6 g, 0.15 mol). The reaction was heated to refluxing at 100°C for 12 hours. The reaction was cooled to room temperature filtered and the solid was washed with 1,4-dioxane 30 mL. The combined filtrate was concentrated under reduced pressure and purified by column chromatography using methanol: ethyl acetate (1:9) to yield 18 (8 g, 50%). $^1$H NMR
(300 MHz, CDCl₃, 298K) δ 7.32 (1H, s), 6.89 (2H, d, J=12Hz), 3.99 (2H, t, J=3Hz), 3.84 (2H, t, J=6Hz).

Synthesis of 8

A solution of 18 (1 g, 0.0089 mol), 15 (1.84 g, 0.011 mol) and PPh₃ (2.9 g, 0.0111 mol) in dry THF 70 mL was cooled to -78°C under inert atmosphere. A solution of DIAD (2.2 g, 0.0111 mol) was mixed in 5 mL dry THF and was added dropwise in 10 min. The reaction was heated the reaction to room temperature and allowed to stir overnight. The reaction was filtered and washed with cold THF. The filtrate was dried to get product 8 in 70% yield. ¹H NMR (300 MHz, D₂O, 298K) δ 7.42 (1H, s), 7.04 (1H, s), 6.93 (1H, s), 6.51 (2H, s), 5.25 (2H, s), 4.14 (2H, t, J= 9Hz), 3.83 (2H, t, J=9Hz), 2.83(2H, s), 1.65 (2H, s).
4.9 References


Chapter 5

Synthesis of Mechanically Interlocked 3-Arm Star Polymers

5.1. Abstract

3-arm star polymers were synthesized using a novel [2]rotaxane macroinitiator. Gram scale poly-methyl acrylate interlocked polymers were prepared using SET-LRP conditions. These 3-arm polymers were studied using DSC, GPC and NMR. The effect of incorporation of a mechanical bond on the glass transition temperature of polymers was studied. Progress is being made to understand the relationship between lengths of a mechanically interlocked polymer to their glass transition temperature (Tg).
5.2 Introduction

Microstructural properties and architecture play an important role in determining the structure-property relationship of polymers. Since their inception by Herman Staudinger\(^1\), synthetic polymers have paved the way for the invention of new polymeric materials with numerous applications. Under stress the conventional covalent polymers can exhibit a recoverable viscoelastic response but with great enough stress they may undergo unrecoverable mechanical failure\(^2\). Polymers respond to the application of stress by disentanglement, bond rupture, and polymeric chains slippage relative to each other (Figure 1). Individual monomeric units and their topology determine behavior of polymers under stress. Enormous research has been focused on changing the monomeric units in order to understand the physical properties of polymers.

![Stress/Strain](image)

**Figure 1.** Deformation in a polymeric materials under stress/strain accompanied by microscopic crazing/fracture, macromolecular chain slippage and atomistic bond breakage.
Mechanical response to stress depends on the micro and macro structural architecture of a bulk polymer. Similarly, a polymer’s thermal response depends on the length of the polymer, the different chemical functional units and the amount of crosslinking in a polymer. As shown in Figure 2 polymers can be divided into two categories, thermoplastic and thermoset polymers. Thermoplastic polymers exhibit reversible deformation whereas thermoset polymers undergo irreversible changes upon deformation. Glass transition temperatures and viscoelasticities are among the most important and interrelated properties of polymers. The glass transition temperature (Tg) is the temperature above which a polymer behaves in an elastic manner. Materials that are viscous and also possess elasticity are known as viscoelastic materials. The relationship between crosslinking and Tg is quite evident from the observation that as the temperature of cross-linked polymers rises above their Tg, the polymers remain rigid and finally decompose above their melting temperature (Tm). By contrast, crystalline polymers (which are mostly linear) transform from crystalline to viscous state when their temperature rises above Tg.

**Figure 2.** Effect of temperature and cross-linking on the nature of polymers. Temp-Temperature, Tm-Melting temperature, Tg-Glass transition temperature.
Understanding the structure-property relationships of polymers will help us design smart materials with desired properties like tunable glass transition temperature and responsive mechanical properties.

5.3. Mechanically interlocked polymers

Mechanically interlocked polymers are polymers that contain two or more interlocking molecular units. As discussed in chapter 1, rotaxanes and catenanes have attracted a lot of attention not only for their structural features but also for the extra degrees of freedom they can impart on the structures of the polymers. As polymer chains can slide relative to each other before undergoing mechanical failure, the mechanical bonds in the mechanically interlocked polymers provide new modes for the individual polymer chains to respond under stress. The free movement of the macrocycle along the polymeric guest can have profound effect on the properties of the polymers as opposed to their linear counterparts. Similarly, it is possible to produce stimuli-responsive motion in the polymeric versions of rotaxanes or catenanes. The incorporation of MIM units into polymers may lead to changes in their mechanical, rheological, dynamic and thermal properties. In a quest to explore new properties that can be imparted to the polymers by incorporation of MIMs, numerous polycatenane and polyrotaxane architectures have been synthesized.
Figure 3. Polymeric versions of catenanes and rotaxanes. 

Figure 3 illustrates various forms of polymeric MIMs. The transition from catenanes or rotaxanes to their polymeric forms can be achieved through reorganization of the individual interlocked units. As shown in Figure 3, not many types of polycatenanes have been designed as opposed to rotaxanes for which several types of polymeric versions can be prepared. There are mainly two types of polyrotaxanes, main chain polyrotaxanes and side chain polyrotaxanes. Another odd
type of polyrotaxane design usually called a “daisy chain,” has also been synthesized.

5.4. Design of the macroinitiator

Given the synthetic cost of preparing mechanically interlocked molecules both in time and expenditure, the number of MIM units per polymer chain needs to be reduced. Despite significant research in polyrotaxanes, the technology of introducing a polymer chain to the macrocycle (host) and thread (guest) components is still in its infancy. Fustin and coworkers reported the first mechanically linked block copolymer after which Stoddart’s and coworkers reported the synthesis of rotaxane mechanophore. Recently, Takata and coworkers used a functionalized dibenzo-24-crown-8-ether macrocycle and ammonium guest to synthesize a 3-arm polymer with a single mechanical bond. Their approach requires growing polymers at the end of a pseudorotaxane (Figure 4). However, the pseudorotaxane itself is a thermodynamically stable unit and conducting polymerization on a pseudorotaxane results in the formation of unwanted side products. Also, the polymerization requires further purification in order isolate the desired material. Here we are addressing these problems in a unique manner by introducing a sterically imposing labile stopper, we call it a temporary stopper, in the rotaxane initiator. Here we are using a tert-butyl diphenyl silyl (TBDPS) derived stopper as a temporary stopper. TBDPS is often used for the protection of phenolic hydroxyl groups and in presence of potassium fluoride in tetraethylene glycol glycol it can be easily deprotected. Here we have used both...
temporary stoppers and a permanent stoppers in a sequence so that when temporary stoppers are removed a permanent stopper is there to prevent the macrocycle in the rotaxane from falling off the guest unit, which is discussed in the following section.
Figure 4 Conventional versus our approach to synthesize 3-arm rotaxane polymers.

Conventional synthesis
• Working with pseudorotaxanes.
• Low yields of polymer.
• Undesired side products.
• Post polymerization workup

Our approach
• No side products.
• No post polymerization workup
As discussed above, polymerization initiated by pseudorotaxanes initiators does not always produce a single rotaxane unit per polymer unit, requires a restricted set of reaction conditions and entails post-polymerization purification. Thus it has been challenging to have a facile synthesis method for such materials. Here we extend the utility of the thiol-maleimide click chemistry to synthesize [2]rotaxane macroinitiators using electron rich mono functionalized DNP38C10 macrocycle 2 and electron poor NDI guest 1 for single electron transfer-living radical polymerization (SET-LRP). As illustrated in Scheme 1, 1 and 2 were mixed in dichloromethane to form a thermodynamically favored [2]pseudorotaxane 4. [2]Pseudorotaxane 4 was then kinetically reacted with the sterically imposing TBDPS derived “temporary” stopper 3, in the presence of catalytic triethylamine, to form a tris-hydroxy[2]rotaxane 4a (see Materials and Methods). Next this tris-hydroxy[2]rotaxane was reacted with 2-bromopropionyl bromide in stoichiometric triethylamine to give macroinitiator[2]rotaxane 5.

2-bromopropionyl bromide in the presence of a stoichiometric amount of triethylamine to form the [2]rotaxane macroinitiator 5.

5.4.1. Synthesis of the temporary stopper 3

**Scheme 2** Synthesis of the TBDPS derived temporary stopper.

Synthesis of the temporary stopper is one of the most crucial steps towards the synthesis of the macroinitiator 5. In the first step of synthesis, gallic acid methyl ester 7 was reacted with 4-bromo-1-butene to obtain the corresponding monoether of gallic acid methyl ester 8. At this stage compound 8 was subjected to TBDPS protection in the presence of imidazole to synthesize compound 9, which is the basic skeleton of
the temporary stopper. Upon further reducing the methyl ester 9 to alcohol 10 using LAH followed by bromination the corresponding bromide 11 was synthesized. Bromide 11 was then reacted with potassium thioacetate to give rise to compound 12 that has a thioacetate on one end and alkene on the other end. At this point compound 12 was reacted with 2-mercaptoethanol using a thiol-ene UV (λ=365nm) catalyzed click reaction to form thioacetate 13 followed by reducing it with LAH to the final thiol 3. The major advantage of this stopper is that numerous functional groups can be introduced along with the reactive thiol.

**5.4.2. Single electron transfer living radical polymerization (SET-LRP)**

Since the evolution of living radical polymerization (LRP), various new materials have been prepared using this polymerization technique. Reversible addition-fragmentation chain transfer (RAFT),

\(^{10}\) atom transfer living radical polymerization \(^{11}\) (ATRP) and single electron transfer living radical polymerization \(^{12}\) (SET-LRP) controlled living radical polymerization are among the most well-studied LRP processes. In the SET-LRP mechanism an electron donating Cu(0) donates electron to alkyl halide followed by instantaneous disproportionation of Cu(0) to Cu(I) and Cu(II). The formed nascent Cu(II) reversibly deactivates the radical to form dormant alkyl halide species. These polymerization processes are therefore able to allow reversible activation-deactivation equilibria between the dormant species and the propagating chain radicals. SET-LRP has been preferably known for its efficiency, fast reaction rates with low polydispersity, low polymerization
temperature and more importantly for its chain retention capability.\textsuperscript{12b} Here we have utilized SET-LRP of methyl acrylates using Cu(0) in the presence of a Me\textsubscript{6}TReN ligand in DMSO at 25°C to prepare [2]rotaxane initiated 3-arm star polymer 15.

**Scheme 3** Complete process from SET-LRP to final 3-arm rotaxane polymer.
As shown in Scheme 3, the macroinitiator 5 was subjected to SET-LRP of methyl acrylate (MA) to synthesize a polymer with more than 95% bromide chain end retention as was confirmed by 1H NMR and GPC studies. A typical SET-LRP of MA initiated with macroinitiator 5 in dimethyl sulfoxide (DMSO) at 25 °C ([MA]/[5]/[CuBr2]/[Me6-TREN]/Cu (0) =222/1/0.15/0.6/0.1) takes place in 1-3 hours for a particular size of a polymer (See Materials and Methods). As discussed previously, SET-LRP is often used for block polymerization because of reactive bromide chain ends. We efficiently utilized the bromide chain ends of polymer 14 to introduce bulky permanent stoppers 15 at the end of the polymer chain. The results of this permanent stopper incorporation using an efficient thiol-bromide reaction are supported by NMR spectroscopy Figure 5. However, several attempts to get matrix assisted laser desorption/ionization – time of flight (MALDI-TOF) data for the polymers has been unsuccessful. Once permanent stoppers were incorporated, polymer 16 contained both temporary (TBDPS) and permanent stoppers and the macrocycle was trapped between the two temporary stoppers. When the temporary stoppers were removed using mild silyl deprotecting conditions (KF/TEG), the macrocycle is free to move along the polymeric thread of polymer 17 until it is reaches the polymeric ends where the permanent stoppers don’t allow the macrocycle to slip over it. We are particularly interested in understanding the effect of the free movement of the macrocycle on the properties of the polymers.
5.4.3 Model [2]rotaxane studies

Scheme 4 Partial $^1$H NMR (CDCl$_3$, 600 MHz, 298 K, 1mM) of the a model system synthesizing a 3-arm[2]rotaxane 19. Corresponding NMR peaks are color coded

In order to have a better understanding of the complex [2]rotaxane polymer 17, we synthesized a model [2]rotaxane 19 as shown in Scheme 4. The $^1$H NMR spectrum of all the interlocked polymers displays well-resolved sharp peaks that are commensurate with a kinetically stable interlocked species. This model system further supports our polymeric system (Scheme 3) and paves the way for making new polymeric materials using this system and variety of different polymeric backbones.

To best of our knowledge this is the only system to date which allows the
incorporation of a single [2]rotaxane unit in a polymer chain without requiring post-polymerization purification, which can be tedious and inefficient.

**5.4.4. NMR analysis**

![NMR spectrum](image)

**Figure 5** Partial $^1$H NMR spectrum (CDCl$_3$, 600 MHz, 298 K) of the synthesis of 3-arm [2]rotaxane polymer. Uniquely distinct naphthalene diimide aromatic proton is shown when complexed (a, b, c) and uncomplexed (d). a) $^5$ b) $^{14}$ c) $^{16}$ d) $^{17}$.

Relaxation delay d1=5

The $^1$H NMR spectrum of all the interlocked polymers display well-resolved sharp peaks that are commensurate with a kinetically stable interlocked species
(Figure 5). Characteristic upfield shifts of the NDI proton (8.24 ppm) are observed and are indicative of [π···π] stacking interactions, which have been explained in chapter 2. The interlocked polymer with a free moving macrocycle shows characteristic peaks corresponding to a mixture of some complexed and uncomplexed peaks. A complexed peak corresponds to 17 with its macrocycle bound to the NDI guest whereas an uncomplexed peak corresponds to 17 with macrocycle not bound to NDI unit but, locked somewhere between two sterically imposing stoppers (15). It is interesting to note that the peak at 8.24 ppm remains unchanged until the deprotection step. Once deprotection of the labile TBDPS groups is carried out the peaks corresponding to the NDI proton gets distributed between complexed and uncomplexed peaks of 17. This observation suggests that some macrocycle units remain complexed and some of the macrocycle polymer crawls over the polymeric thread and gets entangled and trapped in polymeric chains.
5.4.5 Gel permeation chromatography (GPC) analysis

GPC is a powerful technique used for the characterization of polymers. We have synthesized a wide range of polymers ranging from 10K to 80K molecular weight. A representative bimodal GPC curve was observed for a 36500 (Mn) polymer 17 with polydispersity of 1.37 and their GPC traces are shown in Figure 6 which may be attributed to the presence of a mixture of complexed and uncomplexed but completely interlocked polymers 17. A lower molecular weight version of polymer 17 (Mn=11868) showed a perfectly monomodal behavior throughout the process from 14 to 17 (Figure 6b). In addition, a phloroglucinol control polymer also showed a bimodal curve in the presence of tetra-phenyl permanent stoppers 15 (Figure 7b). These observation suggest that either there is incomplete end functionalization or...
these 3-arm polymers behave differently on GPC. In order to verify perfect chain end retention during SET-LRP to synthesize 15 and complete thiol-bromo click reaction to obtain 16, NMR spectroscopy and chain extension experiments were conducted.

5.4.6 Chain end functionality

![Chemical structures](image)

**Figure 7.** a) Synthesis Control phloroglucinol polymer 20 and thiol-bromide click reaction to obtain 21. b) GPC traces.

A critical step during the synthesis of the 3-arm [2]rotaxane polymer 17 is the incorporation of permanent stopper 15 using thiol-bromide\(^{13}\) click chemistry. Incomplete conversion during this step can lead to unwanted side products and
subsequent deprotection of the TBDPS groups would result in the formation of some pseudorotaxane polymers.

One of the most powerful analytical techniques to monitor the conversion of a secondary bromide into a corresponding thioether is NMR spectroscopy. Specially NMR with high magnetic field (500MHz and 600 MHz) has shown reliable results for macromolecular structures in the past with proper relaxation time delays\textsuperscript{14} (D1 5-10s).

**Figure 8** illustrates the complete reaction of the thiol stoppers with the end functionalized bromides. The presence of >95% of the bromide at the end of polymer chain is confirmed by integrating the proton count of the α- proton of bromide chain ends to the α- proton of NDI.

**Figure 8** Partial \textsuperscript{1}H NMR spectrum (CDCl\textsubscript{3}, 600 MHz, 298 K) of permanent stopper incorporation. Uniquely distinct α-hydrogen atom of the chain end bromide \textsuperscript{14} (blue peaks) and naphthalene diimide (pink peaks) are compared. Relaxation delay d1=5. Mn=12695.
A phloroglucinol control reaction as shown in Figure 7a also further validates the reactions conditions required to get complete conversion in the thiol-bromide reaction. The integration of three protons corresponding to phloroglucinol (6.8 ppm) of 20 polymer shows 3 α- proton of bromide polymer chain (4.2 ppm). A typical procedure involves the addition of excess thiol-stopper to a solution of polymer macroinitiator in acetonitrile and dichloromethane mixture (60:40) under a nitrogen atmosphere for 30 hours at 25°C. The reaction shows complete disappearance of α-proton of bromide polymer chain.

![Figure 9](image.png)

**Figure 9.** Chain extension experiment with Mn=31324 to 61601 acrylate polymers.

To further validate the complete chain end retention, chain extension experiments were carried out. A polymer with only temporary stoppers 14 of Mn=31324, with a PDI 1.3, was subjected to a second round of SET-LRP conditions to synthesize a homo polymer of Mn=61601. The GPC traces in Figure 9 show two distinct peaks for Mn=31324 and 61601 polymers. The absence of any other peaks clearly suggests quantitative chain end retention during the synthesis of 14.
5.4.7 Effect on the glass transition temperature

As discussed previously one of the most important properties of polymers is their glass transition temperature (Tg). This property is attributed to the movement of individual polymer chains in the lattice of a polymeric material. As the temperature increases the individual polymer chains move with respect to each other, which may reduce the mechanical strength in the polymers. Introducing a MIM would compensate for the loss of mechanical strength.

Figure 10 DSC plot for Mn=80410 (14) - black line, Mn=92719 (16) - Red Line and Mn=88893 (17) blue line.
Table 1. Glass transition studies on polymers ranging from 12k to 80k in degree Celsius. Mn is number average molecular weight of polymer, PDI is polydispersity, Tg is glass transition.

<table>
<thead>
<tr>
<th>Mn</th>
<th>PDI Mn/Mw</th>
<th>Tg in °C</th>
<th>Mn</th>
<th>PDI Mn/Mw</th>
<th>Tg in °C</th>
<th>Mn</th>
<th>PDI Mn/Mw</th>
<th>Tg in °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>12695</td>
<td>1.07</td>
<td>19.00</td>
<td>14792</td>
<td>1.13</td>
<td>24.00</td>
<td>11868</td>
<td>1.18</td>
<td>6.42</td>
</tr>
<tr>
<td>34419</td>
<td>1.27</td>
<td>19.83</td>
<td>31324</td>
<td>1.30</td>
<td>20.51</td>
<td>25846</td>
<td>1.35</td>
<td>13.9 (17.2)</td>
</tr>
<tr>
<td>51020</td>
<td>1.13</td>
<td>19.00</td>
<td>46844</td>
<td>1.30</td>
<td>22.24</td>
<td>36671</td>
<td>1.38</td>
<td>17.95</td>
</tr>
<tr>
<td>80410</td>
<td>1.35</td>
<td>20.22</td>
<td>92719</td>
<td>1.33</td>
<td>19.83</td>
<td>88893</td>
<td>1.39</td>
<td>14.73</td>
</tr>
</tbody>
</table>

Table 1 shows that the glass transition temperature does not change much if the temporary stoppers are intact from the initial macro initiators. Polymers with only permanent stoppers show a significant change in the glass transition temperature that can only be attributed to three possibilities 1) the macrocycle polymer is bound to the NDI guest 2) the macrocycle polymer is stuck at the end of the NDI gust polymer chain and 3) the macrocycle polymer is stuck at some position between the NDI and the stopper 15. Deprotection of the TBDPS groups of 14 in the absence of permanent stopper can lead to a thermodynamic pseudorotaxane polymer shows an intermediate Tg of 17.22 for a Mn=31419 polymer.
Synthesis of a dumbbell 24 followed by attaching a permanent stopper and deprotection to obtain 26 (Scheme 5) suggests the rigidity caused by temporary stoppers play an important role on the Tg of polymers. The Tg of dumbbell polymers (Figure 11) show a similar trend going from 25 (19.5°C) to 26 (14.5°C). Preliminary
comparison between 26 and 18 suggests the change in the Tg going from 16 to 17 is due to the rigidity of the TBDPS groups. Higher molecular weight polymers (200k and above) are being studied currently for their effects on Tg.

The GPC traces of dumbbell 26 do not show a bimodal curve (Figure 11) as opposed to GPC traces of interlocked polymers 17. This observation suggests that the bimodal curve for 17 is a unique kind of trace of a new polymer. A polymer similar to 17 but with a non-functionalised macrocycle is being studied for its effect on the GPC traces as well as on Tg.

![Figure 11. GPC traces of polymers 24 to 26.](image-url)
The above results highlight the utility of thiol-maleimide click chemistry and it’s utilization in preparing a novel macroinitiator 5. The poly (methyl acrylate) polymers synthesized using macroinitiatitor 5 show thermal changes depending on the length/size of the polymer. Mechanical properties relate to strength, toughness, Young’s modulus, amount of elongation required to break and tensile strength of a particular polymer. We hypothesize more than one mechanical bond in a polymer would have a profound effect on glass transistion temperature (Tg) and mechanical properties of the polymer. Mechanical properties are governed by the intermolecular and intramolecular interaction in polymer chains. For example cross linked polymers are stronger than their linear counterparts. With great enough stress the liner polymers chains in liner polymers move relative to each other to compensate for the stress applied, however they lose their toughness. On the other hand cross linked polymers are more strong because of the cross links but with great enough stress they tend to break. Preliminary result from the synthesis of 17 show that a single mechanical bond can have a significant impact on the glass transition temperature of a 3-arm star polymer. We decided to extend the utility of this temporary stopper 3 for preparing mechanically cross linked polymers.
5.5. Cross linked polymers

Elastomers are polymers that can recover to their original shape upon stretching. Sulfur containing polymers have attracted lot of attention for their elasticity and optical properties. Hoyle and coworkers reported the synthesis of a highly elastic polyurethane networks where they used thiol terminated prepolymers. Inspired by the synthesis of these thiol terminated polymers, we employed them in the synthesis of mechanically interlocked cross-linked polymers (Scheme 6).
As shown in Scheme 6, tris–acrylate [2]rotaxane 27 can be incorporated into a random network of thiol terminated prepolymer synthesized using hexane diacrylate and hexane dithiol to get 28. A thiol terminated 28 was reacted with acrylate terminated stopper 29 in catalytic amount of dimethyl phenyl phosphine in N,N-dimethyl acetamide (DMAC) to get 30. The disappearance of the free thiol was monitored by IR spectroscopy. Once acrylate stoppers are incorporated the temporary TBDPS stoppers can be deprotected using KF/tetraethylene glycol or tetrabutyl ammonium fluoride (TBAF). Currently we are working towards optimizing the deprotection of the temporary stoppers. Once the cross-linked polymer 31 is prepared it will be analyzed by dynamical mechanical analysis (DMA).

5.6. Conclusion

We have successfully synthesized a novel macrorinitiator 5 using thiol-maleimide click chemistry that can be used to accurately prepare polymers with a single rotaxane per polymer unit. SET-LRP was successfully employed on the macro initiator to polymerize mechanically interlocked methyl acrylate polymers. Sterically bulky protecting groups (TBDPS) are highly useful for ensuring thermodynamic and kinetic stability of [2]rotaxanes throughout the polymerization process, thus simplifying the synthesis of MIM polymers. Initial results shows that polymer chains are able to translate relative to each other. 3-arm [2]rotaxane polymers were prepared on a gram scale, which was difficult earlier. To our knowledge this represents the first large scale synthesis of well-defined mechanically interlocked polymer. Presence of
mechanical bonds in a polymer has been observed through changes in the glass transition temperature of the polymers. Preliminary observations suggest that macroinitiator 5 is thermally stable up to 90°C, above which the ring may fall off, indicating that macroinitiator route to interlocked polymers has potential to be the only method by which high temperature polymerization techniques will be accessible.

5.7. Future directions

With the successful synthesis of a unique and versatile macroinitiator 5 we have opened up new frontiers to make polymer materials. The immediate next step would be to introduce different polymer backbones, such as polyglycols, polystyrene and polycaprolactam, and study the effect of these polymer backbones on the physical properties of the polymers. Additionally, a bis substituted DNP38C10 macrocycle can be introduced in the polymer instead of the mono-macrocycle 2 to study 4-arm star polymers. Moreover, introducing bis DNP38C10 macrocycles into the crosslinked polymers would increase the cross-linked density of the polymers and would have profound effects on the properties of 4-arm star polymers.
5.8. Materials and methods

5.8.1 General Methods

Solvents were dried using an Innovative Technologies SPS-400-5 solvent purification system. All reactions were carried out under an anhydrous N\textsubscript{2} atmosphere unless otherwise noted. Thin layer chromatography (TLC) was performed on alumina-backed sheets coated with silica gel 60 F254. Preparative TLC was performed on 20x20cm plates coated with a 1000 µm thick layer of 150 Å silica with F254 indicator. TLC plates were visualized using a UV/Vis lamp and/or by staining with iodine or p-anisaldehyde solution. Column chromatography was performed using glass columns over Dynamic Absorbents 60 Å, 32-63 µm silica gel.

GPC analysis of the polymer samples were done on a Viscotek GPCmax, TDA 305 with RI detector. Mixed bed column was used for eluting the samples at 35\textdegree C at 1 mL/min with THF (HPLC grade). The number average (Mn) and weight average (Mw) molecular weights of the poly(methyl acrylates) samples were determined with polymethyl(methyl acrylates) PMMA standards purchased from Polymer Standard Service-USA. DSC was performed on a TA instruments-DSC-Q20 machine.
5.8.2 Synthetic procedures

Synthesis of 8

To a solution of gallic acid methyl ester 7 (10 g, 0.054 mol) in dry DMF (250 mL), potassium carbonate (37 g, 0.271 mol) was added at RT under nitrogen. The reaction was allowed to stir for 15 min at room temperature. A solution of 4-bromo-1-butene (7.33 g, 0.0054 mol) was added dropwise in minimum acetone over a period of 15-20 min. and allowed the reaction to stir overnight at room temperature. Then the reaction was cooled to 0°C and was quenched with 5N HCl solution until pH 4. The aqueous layer was washed with chloroform 2 x 200 mL. The organic layer was separated and washed with 200 mL 1N HCl and separated the organic layer. The organic layer was dried over MgSO\textsubscript{4} and concentrated under reduced pressure. The crude product was purified by column chromatography using hexane: ethyl acetate (80:20) to yield 8 (3.85 g, 30 %). ESI-HRMS (m/z) [M+H]\textsuperscript{+} Calculated for C\textsubscript{12}H\textsubscript{15}O\textsubscript{5}, 239.0914, found 239.0924.\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}, 298K) \(\delta\) 7.24(2H, s, Ar-H), 6.27 (2H, s, Ar-OH), 5.90 (1H, m), 5.23 (2H, dd, \(J\)=9 Hz), 4.21 (2H, t, \(J\)=9 Hz), 3.87 (2H, s), 2.49 (2H, q, \(J\)=6 Hz). \textsuperscript{13}C NMR (300 MHz, CDCl\textsubscript{3}, 298K) \(\delta\) 167.5, 149.2, 137.9, 135.0, 125.8, 118.7, 109.9, 72.5, 52.7, 52.6, 34.5.
**Synthesis 9**

To a solution of 8 (2.0 g, 8.3 mmol) in 200 mL of dry DMF was added imidazole (2.4 g, 35.2 mmol) under nitrogen at room temperature. TBDPS (5.05 g, 18.4 mmol) was added to the mix under nitrogen at room temperature. The reaction was allowed to stir for 48 hours at room temperature. To the reaction was added 200 mL water and extracted with diethyl ether 2X 100 mL. The organic layer was separated and was given brine wash and dried over MgSO₄. The organic layer was concentrated under reduced pressure to yield 9 (5.34 g, 90%). ESI-HRMS (m/z) [M+H]+ Calculated for C₄₄H₅₁O₅Si₂, 715.327, found 715.329. ¹H NMR (300 MHz, CDCl₃, 298K) δ 7.73 (8H, m, Ar-H), 7.39 (12H, m, Ar-H), 6.76 (2H, S), 5.80 (1H, m, alkene), 5.00 (2H, dd, J=9.6Hz), 4.11 (2H, t, J=7 Hz), 3.47 (2H, S), 2.48 (2H, q, J=7 Hz), 1.12 (18H, S). ¹³C NMR (300 MHz, CDCl₃, 298K) δ 149.4, 144.4, 135.5, 134.8, 132.5, 129.9, 129.5, 127.7, 127.6, 124.1, 116.8, 115.0, 71.9, 71.80, 51.5, 34.6, 26.7, 19.6.

**Synthesis of 10**

To a dry THF 200 mL in round bottom flask was charged LAH (0.398 g, 10.767 mmol) under positive flow of nitrogen. In a separate flask 9 (7 g, 9.789 mmol) was added in 20 mL dry THF under nitrogen and the solution was added to LAH solution via cannula at 0°C. The reaction was allowed to run at room temperature for 4 hours. The reaction was cooled to room temperature and quenched the reaction with
a mixture of Na₂SO₄·10H₂O: diatomaceous earth (1:1) until the disappearance of effervesced. The reaction was filtered and was concentrated under reduced pressure to yield 10 (6 g, 90%). ESI-HRMS (m/z) [M+H]+ Calculated for C₄₃H₅₀O₄Si₂, 687.332, found, 687.331. ¹H NMR (300 MHz, CDCl₃, 298K) δ 7.75 (8H, m), 7.39 (12H, m), 6.03 (2H, s), 5.91 (1H, m), 5.09 (2H, dd, J=9 Hz), 4.16 (2H, t, J=7.62 Hz), 3.87 (2H, m), 2.59 (2H, q, J=7.02 Hz), 1.16 (18H, s). ¹³C NMR (300 MHz, CDCl₃, 298K) δ 176.2, 149.8, 139.8, 135.6, 135.2, 132.9, 129.8, 127.7, 116.7, 112.2, 72.1, 64.6, 34.8, 26.6, 19.67.

**Synthesis of 11**

A solution of 10 (6 g, 8.73 mmol) in dry dichloromethane 250 mL was prepared. CBr₄ (3.474 g, 10.476 mmol) was added and the reaction was degassed with nitrogen for 10 min. At 10°C a solution of PPh₃ (2.747 g, 10.476 mmol) in 10 mL dry dichloromethane in a separate flask was added via cannula. The reaction was allowed to stir at room temperature for 4 hours. To the reaction 200 mL 5% lithium bromide solution was and separated the organic layer. The organic layer was concentrated and purified using column chromatography using dichloromethane: hexane (3:7) to yield 11 (5.225 g, 80%). ESI-HRMS (m/z) [M+H]+ Calculated for C₄₃H₄₉BrO₃Si₂, 749.2476, found 749.2469. ¹H NMR (300 MHz, CDCl₃, 298K) δ 7.72 (8H, m), 7.38 (12H, m), 6.04 (2H, s), 5.86 (1H, m), 5.05 (2H, dd, J=9 Hz), 4.10 (2H, t, J=7.62 Hz), 3.72 (2H, s), 2.52 (2H, q, J=7.0 Hz), 1.11 (18H, s). ¹³C NMR (300
MHz, CDCl$_3$, 298K) $\Delta$ 149.8, 135.8, 135.7, 135.7, 132.9, 130.1, 128.0, 127.9, 127.9, 114.7, 110.3, 34.8, 33.7, 26.9, 26.8, 19.8.

Synthesis of 12

To a round bottom flask 11 (5 g, 6.7 mmol) was added followed by 250 mL acetone under nitrogen atmosphere. Potassium acetate (0.919 g, 8 mmol) was added under nitrogen and heated the reaction for refluxing for 12 hours. The reaction was cooled to room temperature mixture concentrated and added 100 mL dichloromethane. The reaction was then washed with 200 mL water and separated the organic layer. The organic layer was dried over MgSO$_4$ and was concentrated under reduced pressure. The residue was further purified by column chromatography using dichloromethane: hexane (3:7) to yield 12 (3.9 g, 80%). ESI-HRMS (m/z) [M+H]$^+$

Calculated for C$_{45}$H$_{52}$SO$_3$Si$_2$, 745.3198, found 745.3201. $^1$H NMR (300 MHz, CDCl$_3$, 298K) $\Delta$ 7.62 (8H, m), 7.30 (12H, m), 5.87 (2H, S, Ar-), 5.77 (1H, m), 4.97 (2H, dd, $J$=9 Hz), 4.01 (2H, t, $J$=8 Hz), 3.29 (2H, m), 2.46 (2H, q, $J$= 7 Hz), 1.97 (3H, s), 1.03 (18H, S). $^{13}$C NMR (300 MHz, CDCl$_3$, 298K) $\Delta$ 149.8, 135.8, 135.8, 135.7, 133.0, 131.4, 131.0, 127.9, 127.8, 127.7, 141.1, 35.0, 33.0, 30.2, 29.9, 27.0, 26.94, 26.82, 19.8.

Synthesis of 13

A solution of 12 (5.0 g, 6.7 mmol) was prepared in 30 mL of dry 1,4-dioxane. To the reaction mixture 2-mercaptoethanol (2.093 g, 26.8mmol) was added followed by 5 mol% of 2,2-dimethoxy-2-phenyl-acetophenone (0.086 g, 0.335 mmol). The
mixture was left for degassing for 10 min with positive flow of nitrogen. The mixture was then subjected to 356 nm UV radiations for 5-8 hours until the disappearance of starting alkene. 200 mL water was added to the reaction mixture and followed by 100 mL diethyl ether. The mixture was separated and the organic layer was concentrated under reduced pressure. The product was purified by column chromatography using hexane:ethylacetate (1:4) to yield 13 (3.5 g, 65%). ESI-HRMS (m/z) [M+Na]+ Calculated for C_{47}H_{59}O_{5}NaS_{2}Si_{2}, 845.315, found 845.3136. \(^1\)H NMR (300 MHz, CDCl$_3$, 298K) \(\delta\) 7.70 (8H, m), 7.39 (12H, m), 5.96 (2H, s), 4.07 (2H, \(J=6\) Hz), 3.69 (2H, \(J=6\) Hz), 3.37 (2H, s), 2.70 (2H, m), 2.52 (2H, \(J=6\) Hz), 2.02 (3H, s), 1.80 (2H, m), 1.70 (2H, m), 1.11 (18H, s). \(^{13}\)C NMR (300 MHz, CDCl$_3$, 298K) \(\delta\) 149.7, 135.9, 135.8, 135.8, 135.7, 133.0, 131.4, 130.1, 130.0, 128.0, 127.9, 127.8, 127.77, 114.28, 114.2, 114.1, 60.3, 35.4, 32.9, 31.8, 30.1, 29.8, 27.0, 26.9, 26.9, 26.8, 19.8.

**Synthesis 3**

To a round bottom flask containing dry THF 200 mL LAH (0.271 g, 7.2 mmol) was added under positive flow of nitrogen. In a separate flask 13 (5 g, 6 mmol) was prepared in 20 mL dry THF under nitrogen atmosphere and the solution was added to LAH solution via cannula at 0°C. The reaction was allowed to run at room temperature for 4 hours. The reaction was cooled to room 0°C and quenched the reaction with a mixture of Na$_2$SO$_4$.10H$_2$O: diatomaceous earth (1:1) until the disappearance of effervescence. The product was purified by column chromatography
using dichloromethane to yield 3 (3 g, 65%). ESI-HRMS (m/z) [M+Na]^+ Calculated for C_{46}H_{56}O_4NaS_2Si_2, 803.300, found 803.3056. \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}, 298K) δ 7.73 (8H, m), 7.40 (12H, m), 5.98 (2H, s), 4.11 (2H, t, J=4.3Hz), 3.72 (2H, m), 2.96 (2H, d, J=7 Hz), 2.72 (2H, t, J=6Hz), 2.45 (2H, t, J=7 Hz), 1.86 (2H, m), 1.76(2H, m), 1.16 (18H, s), 0.91 (1H, J=7.4Hz). \textsuperscript{13}CNMR (500 MHz, CDCl\textsubscript{3}, 298K) δ 151.3, 149.5, 135.6, 135.6, 135.5, 132.9, 132.9, 129.9, 129.8, 127.7, 127.6, 127.5, 127.5, 113.3, 109.9, 72.6, 60.14, 35.25, 31.6, 29.6, 28.3, 26.7, 26.4, 26.4, 19.6.

**Synthesis of tris-hydroxy [2]rotaxane 4a**

A mixture of macrocycle 2 (0.2 g, 0.25 mmol), naphthalene diimide 1 (NDI) guest (0.148 g, 0.19 mmol) and stopper 3 (0.326 g, 0.418 mmol) was mixed in 1 mL dichloromethane. The reaction was allowed to stir at 0 °C for 12 hours. The mixture was concentrated under reduced pressure at 50°C so that the total volume reduced to 25% of the original one. At 5°C a drop of trimethylamine was added and the reaction was allowed to run overnight in the cold room. The product was carefully purified with column chromatography using dichloromethane: methanol (98:2) to get 4a (0.57 g, 65 %). (The column has to be done very precisely preferably using silica adsorption and with gravity). ESI-HRMS (m/z) [M+Na]^+ Calculated for
C_{169}H_{206}N_{43}Na_{5}S_{4}Si_{14}, 3114.2137, found 3115.2175. $^1$H NMR (300 MHz, CDCl$_3$, 298K) δ 8.25 (4H, s), 7.7 (16H, m), 7.3 (24H, s), 6.90 (2H, d, $J$=9 Hz), 6.70 (2H, t, 2H, d, $J$=9 Hz), 6.60 (3H, m), 6.50 (1H, t, $J$=9 Hz), 6.12 (2H, m), 6.09 (4H, s, Ar-), 6.00 (1H, d, $J$=6 Hz), 4.56 (4H, t, $J$=4 Hz), 4.54(4H, t, $J$=4 Hz), 4.20 (6H, m), 3.80 (40H, m), 3.50 (2H, d, $J$=14 Hz), 3.03(2H, d, $J$=12.3 Hz), 2.73 (6H, t, $J$=6 Hz, -S-CH$_2$), 2.51(6H, m, -S-CH$_2$), 2.34 (2H, m), 2.2(1H, d, $J$=6 Hz), 2.15 (1H, d, $J$=6 Hz), 2.00 (2H, s), 1.90 (6H, m), 1.86 (12H, m), 1.7 (1H, t, $J$=7 Hz), 1.25 (4H, m), 1.05 (36H, s). $^{13}$CNMR (500 MHz, CDCl$_3$, 298K) δ 176.1, 174.7, 163, 149.6, 139.9, 135.5, 132.7, 132.5, 129.9, 129.7, 127.7, 127.6, 124.7, 72.7, 70.79, 70.04, 67.8, 67.0, 60.6, 60.2, 39.1, 38.8, 38.5, 35.2, 26.5, 26.4, 19.5.

**Synthesis of the macro initiator 5**

To a solution of tris-hydroxy[2]rotaxane 4a (0.4 g, 0.13 mmol) and trimethylamine (0.052 g, 0.52 mmol) in 50 mL dry dichloromethane was added a solution of 2-bromopropionyl bromide (0.11 g, 0.52 mmol) in 1 mL of dry DCM at 0°C. The reaction was allowed to stir at room temperature overnight. The reaction was concentrated and purified using dichloromethane: methanol (98:2) to yield 5 (0.3 g, 65 %) of product. ESI-HRMS (m/z) [M+2Na]$^+$ Calculated for C$_{178}$H$_{215}$Br$_3$N$_4$O$_{36}$Na$_2$S$_5$Si$_4$, 1772.50, found 1772.5057. $^1$H NMR (300 MHz, CDCl$_3$, 298K) δ 8.25 (4H, s), 7.67 (16H, m), 7.3 (24H, s), 7.00 (2H, d, $J$=8 Hz), 6.90 (2H, d, J=9 Hz), 6.70 (2H, t, 2H, d, $J$=9 Hz), 6.60 (3H, m), 6.5 (1H, t, $J$=9 Hz), 6.12 (2H, m), 6.09 (4H, s, Ar-), 6.00 (1H, d, 2H, d, $J$=6 Hz), 4.56 (4H, t, $J$=4 Hz), 4.54(4H, t, $J$=4 Hz), 4.54(4H, t, $J$=4 Hz), 4.54(4H, t, $J$=4 Hz), 4.54(4H, t, $J$=4 Hz), 4.54(4H, t, $J$=4 Hz), 4.54(4H, t, $J$=4 Hz).
Hz), 4.2(6H, m), 3.8-3.4 (40H, m), 3.501(2H, d, \( J=14 \) Hz), 3.03(2H, d, \( J=12.3 \) Hz), 2.73(2H, t, \( J=6 \) Hz), 2.51(4H, m), 2.347(2H, m), 2.2(1H, d, \( J=6 \) Hz), 2.15(1H, d, \( J=6 \) Hz), 2(2H, s), 1.9(4H, m), 1.83(2H, m), 1.05 (36H, s). $^{13}$CNMR (300 MHz, CDCl$_3$, 298K) δ 178.4, 176.3, 176.3, 171.3, 163.2, 163.1, 153.7, 153.2, 149.8, 145.8, 139.5, 135.5, 135.9, 135.8,135.7, 135.6, 132.9, 132.6, 132.0, 128.2, 128.0, 127.9, 127.8, 127.65, 124.95, 124.3, 114.8, 114.8, 71.0, 70.9, 70.4, 70.2, 64.0, 55.8, 55.2, 35.9, 30.9, 30.7, 30.7, 26.8, 26.7, 26.6, 19.7.

**Synthesis of 18**

A solution of 5 (0.1 g, 0.000029 mmol) was prepared in 10 mL of mixture of acetonitrile and dichloromethane (6:4). Triethylamine (0.015 g, 0.00014 mmol) was added followed by thiol stopper 15 (0.105 g, 0.00016 mmol) under nitrogen and the reaction was allowed to stir for 35-40 hours. Concentration of the reaction mixture under reduced pressure and purification by column chromatography using a mixture of dichloromethane and methanol (98:2) yielded 18 (0.98 g, 70%). ESI-HRMS (m/z) [M+2Na]$^+$ Calculated for C$_{301}$H$_{568}$N$_4$O$_{32}$Na$_2$S$_8$Si$_4$, 2564.1772, found 2564.1740. $^1$H NMR (500 MHz, CDCl$_3$, 298K) δ 8.23 (4H, s), 7.7 (16H, m), 7.3(24H, m), 7.21(18H, m), 7.06(24H, m), 6.97 (2H, d, \( J=5 \) Hz), 6.91 (2H, d, \( J=15 \)Hz), 6.78 (8H, m), 6.66 (1H, t, \( J=5 \) Hz), 6.62 (2H, d, \( J=5 \) Hz), 6.56 (1H, t, \( J=5 \) Hz), 6.10 (1H, d, \( J=5 \) Hz), 6.08 (1H, t, \( J=5 \) Hz), 5.97 (1H, d, \( J=5 \) Hz), 4.4-3.5 (96H, m), 3.47 (3H, q, \( J=5 \) Hz), 3.40 (2H, d, \( J=14 \) Hz), 3.03 (2H, q, \( J=10 \) Hz), 2.93-2.2 (20H, m, -S-CH$_2$-), 2.11 (2H, dd, \( J=5 \) Hz), 2(2H, s), 1.89 (6H, m), 1.63 (9H, s), 1.83(2H, m), 1.27 (81H, s), 1.05
(36H, s). $^{13}$CNMR (500 MHz, CDCl$_3$, 298K) $\delta$ 176.1, 174.6, 172.9, 162.9, 156.4, 152.9, 1.49, 148.2, 144.1, 139.8, 135.5, 132.2, 130.7, 127.7, 127.6, 124.0, 123.8, 114.4, 113.0, 71.3, 70.8, 70.75, 70.66, 70.05, 67.16, 64.05, 63.04, 53.47, 41.24, 38.03, 34.29, 31.41, 26.56, 26.42, 26.34, 19.55, 17.34, 17.26.

**Synthesis of [2]rotaxane 19**

Tetraethylene glycol (TEG) (0.143 g, 0.00072 mmol) and potassium fluoride (0.005 g, 0.000072 mmol) were added to a solution of 18 (0.2 g, 0.000036 mmol) in 0.5 mL of dry THF (keep volume of THF as small as possible) under nitrogen. The reaction was allowed to stir for 2 hours. (More than 10 eq. of TEG and potassium fluoride may gave rise to unwanted impurities). Water 20 mL was added followed by 20 x 2 mL dichloromethane. The organic layer was separated and dried over MgSO$_4$ and concentrated under reduced pressure. (Extra KF and TEG gives rise to difficult purification) The crude residue was purified by column chromatography using dichloromethane and methanol (98.5:1.5) mixture to yield 19 (0.132 g, 90%). $^1$H NMR (500 MHz, CDCl$_3$, 298K) $\delta$ 8.24 (4H, s), 7.20 (18H, m), 7.06(24H, m), 6.98 (2H, $J=5$ Hz), 6.74 (6H,d, m), 6.66 (2H, t, $J=5$ Hz), 6.62 (2H, t, $J=5$Hz), 6.5 (2H, d, $J=5$ Hz), 6.5 (4H, s), 6.11 (2H, m), 5.97 (1H, d, $J=5$Hz), 4.2-3.5 (96 H, m, -O-CH$_2$-), 3.47 ( 2H, q, $J=5$Hz), 2.9-2.3 (20H, m, -S-CH$_2$-), 1.84 (4H, t, $J=10$ Hz), 1.75 (2H, t, $J=5$ Hz), 1.65 (9H, s), 1.27 (81H, s). $^{13}$C NMR (500 MHz, CDCl$_3$, 298K) $\delta$ 163.0, 156.4, 149.4, 148.3, 144.1, 139.8, 133.0, 132.2, 130.7, 130.5, 124.7, 124.2, 124.15, 124.0, 123.9, 113.0, 108.7, 71.2, 70.8, 70.7, 70.0, 69.5, 70.8, 70.7, 69., 69, 4, 67.9,
Typical procedure for the polymerization (Synthesis of polyacrylates)

The macroinitiator 5 (0.15 g, 0.0425 mmol), methyl acrylate (2.92 g, 34 mmol, 80 eq), tris-(2-aminomethyl amine, 0.6 eq) Me₆TREN, (0.0058 g, 0.0255 mmol, 0.15 eq, 0.05 per arm), CuBr₂ (0.0014 g, 0.0063 mmol) were added to 1.5 mL DMSO under nitrogen. After 4 freeze-pump-thaw cycles Cu(0) was added and the reaction was allowed to stir at room temperature. The reaction was monitored by ¹H NMR to check the conversion of the methyl acrylate monomer. The reaction was stopped at 80% conversion after 2.5 hours. Reaction was exposed to air for 15 min and filtered through a basic alumina to get rid of copper impurities. The reaction mixture was concentrated and precipitated with excess methanol. (Usually lower molecular weight polymers require external cooling). The polymer was analyzed using GPC, NMR and DSC.

Generic al procedure for synthesis of 16

A solution of 14 in (60:40, Me₃CN:CH₂Cl₂, 40 volumes) was prepared. 15 (6-8 eq.) was added at room temperature and the mixture was allowed to stir for 36 hours under nitrogen atmosphere. The reaction was concentrated under reduced pressure and the product was precipitated using ethyl acetate and hexane mixture (1:1) by dry ice-acetone cold bath. Alternatively, especially for low molecular weight polymers a neutral alumina column chromatography can be employed to get rid of
excess 15 using (CH$_2$Cl$_2$-hexane to pure THF). The polymer was analyzed using GPC, NMR and DSC.

**General procedure for synthesis of 17**

Tetraethylene glycol (20 eq.) and potassium fluoride (6-10 eq.) were added to a solution of 16 in minimum amount of THF (Only enough to make the solution homogeneous). The reaction was allowed to stir for 2 hours. (More than 10 eq. of TEG and potassium fluoride may gave rise to unwanted impurities). Water was added until the precipitation of polymer was complete. The water layer was decanted and the residue was dissolved in minimum 10 volumes of ethyl acetate. Hexane 20 equivalence was wadded to the ethyl acetate solution and cooled with dry ice-acetone bath. The solvents were decanted and the product was subjected to vacuum to get rid of solvent traces. The polymer was analysed using GPC, NMR and DSC.

**Synthesis of compound 27**

To a solution of tris-hydroxy[2]rotaxane 4a (0.4 g, 0.13 mmol) and trimethylamine (0.052 g, 0.52 mmol) in 50 mL dry dichloromethane was added a solution of acryloyl chloride (0.038 g, 0.429 mmol) in 1 mL of dry DCM at 0°C. The reaction was allowed to stir at room temperature overnight. The reaction was concentrated and purified by column chromatography using dichloromethane: methanol (98:2) to yield 27 (0.3 g, 65 %). $^1$H NMR (500 MHz, CDCl$_3$, 298K) δ 8.26 (4H, s), 7.71 (16H, m), 7.37 (24H, m), 7.00 (1H, d, $J$=5 Hz), 6.94 (1H, d, $J$=5 Hz), 6.68-6.57 (4H, m), 6.39 (2H, t, $J$= 25 Hz), 6.16-5.98 (8H, m), 5.81 (2H, d, $J$=5 Hz),
4.28 (8H, m), 4.1 (4H, m), 4.02-3.58 (64H, m), 3.44 (2H, d, $J=20$ Hz), 2.99 (2H, d, $J=15$ Hz), 2.78 (6H, m), 2.6-2.5 (8H, m), 2.35 (2H, m), 1.95-1.71 (12H, m), 1.11 (36H, s).
5.9 References and notes


