REVEALING THE ART AND SCIENCE OF SELF-REPLICATING ROTAXANES

Nurul Izzaty Hassan

A Thesis Submitted for the Degree of PhD at the University of St Andrews

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Revealing the art and science of self-replicating rotaxanes

by

Nurul Izzaty Hassan

A Thesis presented for the Degree of Doctor of Philosophy in the School of Chemistry University of St Andrews

December 2011
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Synopsis

This Thesis reveals the strategies for the construction and replication of mechanically interlocked molecules, particularly rotaxanes, which consist of a macroring that encircles a linear component terminated with bulky groups. The work highlights our recent research activities in exploring the recognition-mediated synthesis of this class of interlocked molecule and its amplification by replication. Our starting point is the minimal model of self-replication.

The introductory chapters (Chapter 1 and 2) provide some background and significance to the study, which presents comprehensive review of the published work carried out in the area of self-replication with existing examples from biomimetic and discrete synthetic assemblies. In Chapter 1, we mainly discuss the do and the don’ts in designing successful self-replicating systems based on our own experience in previous work. Our chief concerns in Chapter 2 are the understanding of the chemistry of the mechanical bond and the synthesis of rotaxanes by three means of approaches (clipping, threading and stoppering, and slippage). Attractive and useful examples are illustrated for each mechanism. Moreover, the definition and the roles of templated-synthesis of interlocked molecules are described. Recent advances in the understanding of the nature of the mechanical bond have also been introduced into molecular electronic devices.

Emphasis is placed in Chapter 3 upon the essential requirements for the design of self-replicating rotaxanes, namely a recognition site, a reactive site and a binding site. These aspects are explained in the designed minimal model chosen in the past (Replication model 1) and the alternate proposed models (Replication model 2 and Replication model 3). The importance of high association constant to provide substantial amount of pseudorotaxane [L-M] precursors is exemplify in the simple kinetic model of rotaxane formation. The advantages and disadvantages of each independent minimal replication model are also summarized.

In the self-replicating rotaxane frameworks, the principal strategy involves a selection of an efficient macrocycle to accommodate the guest unit. Thus, Chapter 4 exclusively describes the design, synthesis and binding properties of a series of...
macrocycle incorporating the hydrogen bond donors and/or hydrogen bond acceptors motif. In particular, the guests were designed and synthesised based on the mutual interactions with the macrocycle framework and the binding experiments is described in details. An account is provided of the problems faced in the synthetic attempts towards the formation of these macrocycles. The novel macrocycle MEU demonstrated a deficient binding performance with amide and urea compounds, and thus abandoned in later stages. The developed macrocycle MDG and MP have been selected as our workhorse macrocycles, which successfully increase the binding strength in the pseudorotaxanes formation. We have learnt that the association constant, $K_a$ can be manipulated by the changing the binding site of the guest or redesign the framework of the macrocycle itself.

An exhaustive investigation of the performance of self-replicating rotaxanes focuses on Replication model 1 is demonstrated in Chapter 5. It was evident now that as a consequence of low $K_a$, a substantial amount of thread is present over rotaxane. The implementation of the simple kinetic model of rotaxane formation is prevailed throughout this chapter. The position of the central reversible equilibrium in this model effectively resulted in a different reactivity of thread and rotaxane. Therefore, it is concluded that the ratio of rotaxane and thread is sensitive to both the association constant for the $[L\cdot M]$ complex and to the ratio of $k_{\text{rotaxane}}/k_{\text{thread}}$.

The key marker for the efficiency of the rotaxane-forming protocol is the ratio of rotaxane, $R$ to thread, $T$. In previous chapter, the $K_a$ for the $[L\cdot M]$ complex was around $100 \text{ M}^{-1}$ and $k_T = 3 \times k_R$, which led to an unacceptably small $[R]/[T]$ ratio. We demonstrated for the first time in Chapter 6, that it is possible to manipulate the $K_a$ for the $[L\cdot M]$ complex by means of a change in temperature. Yields of a rotaxane can be improved by employing a two-step capture protocol. Cooling a solution of the linear and macrocyclic components required for the rotaxane increases the population of the target pseudorotaxane, which is then captured by a rapid capping reaction between an azide and PPh$_3$. The resulting iminophosphorane rotaxane can then be manipulated synthetically at elevated temperatures. Following this, these imines could be reduced readily to afford the stable amine rotaxane.
Replication model 2 is subsequently proposed as alternate replication framework in Chapter 7, which realised significant advantages over the first model. A number of designs of a potential self-replicating rotaxane have been fabricated in order to integrate self-replication with the formation of rotaxanes. An account is provided of the problems faced with the unanticipated larger cavity of the newly prepared acid recognition macrocycles, and hence, force us to search for a new scaffold of the nitrone structures. Pleasingly, a substantial amount of rotaxane was present, mostly as trans diastereoisomer. It is concluded that the resulting rotaxane structures may be self-replicating through the recognition-mediated pathways from the preliminary kinetic experiments. Nonetheless, the remainder of the full kinetic analysis are prevented given a small quantity of the necessary building block.

Chapter 8 reveals our recent efforts to demonstrate the notions behind the final replication scheme, Replication model 3. We have become aware that the reactive site must be placed sufficiently far away from the binding site to inhibit the remote steric effect through the proximity of the macrocyclic component. The design of novel nitrone structures is described in details. We bring together conclusions that can be drawn from three designated replication models in Chapter 9. Experimental and synthetic procedures of the target compounds and appropriate spectroscopic analysis of the products are elaborated in Chapter 10.
Acknowledgements

Praise be to Allah, the most Beneficient and the most Merciful and salutations be upon His messenger Muhammad (peace upon him), his family and his companions.

I have greatly enjoyed the years (2008 – 2011) I have spent at University of St Andrews and it will be hard to express my thanks to the many people to whom I am indebted to for such unforgettable experiences.

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When I joined the DP group, I was working closely on the experiments with Dr Annick Vidonne about mechanically interlocked architectures and learned many useful things about lab management and experimental techniques. The DP group was an interesting place to work and I would like to thank some of its former and current members for providing a friendly working environment, and for lending a hand or a good word when I needed them: Dr Jürgen Huck, Dr Ilaria Bottero, Dr Craig Robertson, Dr Vicente del Amo, Dr Jan Sadownik and Harry Mackenzie.

Technical staffs of the School of Chemistry for their quality work on the parts of my experiment, Caroline Horsburgh for mass spectrometry analyses, Catherine Botting for the MALDI spectrometry, Dr Tomas Lebl and Melanja Smith was helpful with all the NMR experiments. With (Dr) Noor Akmal Shareela Ismail, (Dr) Nurul Liyana Berahim, (Dr) Siti Marwanis Anuar and (Dr) Mazlina Musa, my long time friends, I had many discussions about science and life in general. And to my St Andrews’s junior doctors and physiologist - (Dr) Eddy Shah Razdi Baharuddin, (Dr) Leyla Nadia Kamil, (Dr) Nur Atiqah Ramli, (Dr) Nasrul Asyraf Saiful Yazan and Siti Sabariah Ahmad Murtazam : The hope, the struggle and the hard work towards a goal itself is part of the rewards. Achieving goal itself is not the whole rewards. All best wishes!!
Finally, I would like to my family, especially my parents, Hj Hassan Puteh and Hjh Che Puteh Mohd Noor and my three brothers, Mohd Fahmi, Mohd Fairus Naqim and Mohd Fauzal Hassan. They always provided me with freedom of choice and supported my interest in science, even when that interest led me to distant shores.

And it is to my parents that I dedicate this thesis.
### Abbreviations

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<thead>
<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Hydrogen bond acceptor</td>
</tr>
<tr>
<td>B3LYP</td>
<td>Becke, 3 parameters, Lee, Yang and Parr</td>
</tr>
<tr>
<td>C</td>
<td>Cytidine</td>
</tr>
<tr>
<td>CBPQT&lt;sup&gt;4+&lt;/sup&gt;</td>
<td>cyclobis (paraquat-p-phenylene) tetracation</td>
</tr>
<tr>
<td>CI</td>
<td>Chemical Ionisation</td>
</tr>
<tr>
<td>conc.</td>
<td>concentrated</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>D</td>
<td>Hydrogen bond donor</td>
</tr>
<tr>
<td>DB24C8</td>
<td>dibenzo-24-crown-8</td>
</tr>
<tr>
<td>decomp.</td>
<td>decomposed</td>
</tr>
<tr>
<td>DEPTQ</td>
<td>Distorsionless Enhancement by Polarization Transfer including the detection of Quarternary nuclei</td>
</tr>
<tr>
<td>DFT</td>
<td>Density Functional Theory</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNP</td>
<td>1,5-dioxynaphthalene</td>
</tr>
<tr>
<td>EDC/EDCI</td>
<td>N'- (3-dimethylaminopropyl)-N-ethyl-carbodiimide</td>
</tr>
<tr>
<td>EI</td>
<td>Electron Impact</td>
</tr>
<tr>
<td>ES</td>
<td>Electrospray</td>
</tr>
<tr>
<td>EXSY</td>
<td>Exchange Spectroscopy</td>
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<tr>
<td>g</td>
<td>gram(s)</td>
</tr>
<tr>
<td>G</td>
<td>Guanosine</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>HMDS</td>
<td>Hexamethydisilazane</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High Resolution Mass Spectrometry</td>
</tr>
<tr>
<td>(K_a)</td>
<td>Association constant</td>
</tr>
<tr>
<td>(K_d)</td>
<td>Dissociation constant</td>
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<tr>
<td>lit.</td>
<td>literature</td>
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<tr>
<td>M</td>
<td>Molar</td>
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</table>
m     multiplet
m     meta
MALDI  Matrix Assisted Laser Desorption/Ionization
Me     Methyl
mg     miligram
MHz    Megahertz
min    minute(s)
 mL    milileter
 mM    milimolar
mmol   milimol
M.p.   Melting point
MPTTF  Monopyrrolo Tetrathiafulvalene
MS     Mass Spectroscopy
NMR    Nuclear Magnetic Resonance
NOE    Nuclear Overhauser Effect
o      ortho
p      para
Pd/C   Palladium on activated carbon
Rh/C   Rhodium on activated carbon
ROESY  Rotating frame Overhauser Effect Spectroscopy
RNA    Ribonucleic acid
rt     room temperature
s      second(s) / singlet
sat.   saturated
t      time / triplet
TFA    Trifluoroacetic Acid
THF    Tetrahydrofuran
TLC    Thin Layer Chromatography
TS     transition state
TTF    Tetrathiafulvalene
UV     Ultraviolet
wt.    weight
1.1  Supramolecular chemistry

Supramolecular chemistry has been defined as chemistry beyond the molecule\textsuperscript{1,2} more than three decades ago as one of the most active fields of science, and has grown enormously in importance. The initial motivation in the early history\textsuperscript{3,4} of supramolecular chemistry was to design chemical systems that mimic\textsuperscript{5} biological processes. Biological phenomenal exploited the wide range of weak, non-covalent interactions\textsuperscript{6,7} which are responsible for the selective transport of ions and small molecules across membranes, nucleic acid's transcription and translation, virus assembly and action, and the tertiary and quaternary structure of proteins. Nowadays, much work in supramolecular chemistry has been focusing on molecular design to achieve complementarity between molecule hosts and their guests.

The principle of complementarity follows that to complex, a host must have binding sites that can simultaneously contract and attract the binding sites of guest without generating internal strains or strong non-bonded repulsions\textsuperscript{4,8}. Thus, complementarity is the key determinant of structural recognition. Besides complementarity, self-assembly\textsuperscript{9}, recognition\textsuperscript{10,11}, preorganisation\textsuperscript{12-14} and even self-replication\textsuperscript{15} represent important key words in the armoury of the supramolecular chemist\textsuperscript{16}.

Self-assembly may be defined as the process by which a supramolecular entity forms spontaneously from its components. For the majority of synthetic systems it appears to be a beautifully simple convergent process which give rise to the assembled target in a straightforward\textsuperscript{9} manner. Like self-assembly, molecular recognition are widespread phenomena both in nature and synthetic systems. The birth of molecular recognition in the supramolecular sense can be traced back to Pedersen’s work on
crown ethers and the discovery\textsuperscript{17} of their affinities for Group 1 metal cations. In that case, selectivity was determined\textsuperscript{18} by the size of the metal cation and the diameter of the crown ether. It needs to be noted that it is the degree of electronic and steric complementarity between host and guest that dictates the magnitude of any molecular recognition that occurs for a given supramolecular system. Essentially, it is instructive to consider the relationship between molecular preorganisation\textsuperscript{4,12-14}, recognition and self-assembly in relation to the formation of supramolecular complex. Fundamentally, all three will play a sequential role in complexation. Specifically, appropriate preorganisation of the binding sites in the host to receive the guest thus predisposes the former for guest recognition. This predisposition, in turn, promotes spontaneous self-assembly of the required supramolecular entity. There is no doubt that self-assembly can be utilized to construct\textsuperscript{5} a wide diversity of structural types with apparent ease.

In addition to the above, this new chemistry has provided synthetic systems capable of self-replication\textsuperscript{19-21} by examining the way nature accomplishes these objectives and demonstrated these in a chemical context. The ability of nucleic acids to act as templates for self-replication is a fundamental process in Nature’s chemistry. Synthetic self-replicating system are of special interest in bioorganic and supramolecular chemistry since these fields may contribute\textsuperscript{15} experimentally to the understanding of the Origin of Life. This fascinating idea that a molecule can generate or catalyse its own formation by acting the same way as nucleic acids\textsuperscript{22} to act as templates for self-replication are well introduced in both the natural and synthetic models in recent years.

Additionally, an understanding of molecular replication may be also helpful to simulate self-replication, mutation, and evolution in non-natural environments\textsuperscript{15} such as computers or other technical devices. In the following section, the two elementary models of replication are described and evaluated. We will then mainly focus on development and design of synthetic replication processes to produce artificial self-replicating systems in order to provide a sufficient background in this thesis.
1.2. Minimal model of self-replication

The simplest published\textsuperscript{23} framework of non-enzymatic systems can be conceptualized in a simple three pathways model. The initial pathway can be regarded as the uncatalysed bimolecular reaction composed of two substrate molecules, A and B, that become joined to form the template molecule, T (\textbf{Figure 1.1}). This reaction is facilitated by random collisions and, hence, slow and unselective pathway in the system. However, as a consequence of the presence of the recognition sites (green cartoons), reagents A and B can associate with each other to form a binary complex, [A•B]. The formation of this complex open the second pathway, the binary complex [A•B] pathway, in which this complex has the ability to bring the reactive sites (blue cartoons) in A and B into close proximity and the reaction is rendered pseudointramolecular.

\textbf{Figure 1.1.} The minimal model of self-replication. Molecules A and B can react through three pathways, namely an uncatalysed bimolecular reaction, a recognition-mediated pseudounimolecular pathway mediated by a binary complex [A•B] and a recognition-mediated pseudounimolecular autocatalytic cycle mediated by a ternary complex [A•B•T].

Consequently, reaction through this pathway leads to the closed template T\textsubscript{inactive} in which the recognition sites used to assemble the binary complex remain associated together. Therefore, although rate acceleration\textsuperscript{24,25} is achieved by this mechanism
relative to the background bimolecular rate, this product is catalytically inert. The third reaction channel available in the system is the autocatalytic cycle. In contrast to $T_{ inactive}$, the template $T$ formed through the bimolecular reaction has its recognition sites open to the solution. In this channel, $A$ and $B$ binds reversibly to the open template $T$ to form a catalytic ternary complex $[A\cdot B\cdot T]$. In a manner similar to the $[A\cdot B]$ complex, the reaction between $A$ and $B$ is also rendered pseudointramolecular. Bond formation occurs between $A$ and $B$ to give the product duplex $[T\cdot T]$, which then dissociates to return two molecules of $T$ to initiate a new autocatalytic cycle. The dissociation of $[T\cdot T]$ duplex is a key consideration in the design of replicating systems and for efficient autocatalytic turnover as its stability must be lower or comparable to that of the ternary complex $[A\cdot B\cdot T]$. Thus, the autocatalytic function of self-replicating template is only completed, when the product duplex dissociates to return two free templates to the reaction mixture. The autocatalytic cycle can potentially lead to exponential growth of $T$ as two templates makes four, four make eight and so on, until reagents $A$ and $B$ are consumed. Thus, the important property of the template $T$ is to catalyse the formation of additional template, which results in an autocatalytic behavior.

In this respect, the template $T$ is capable of transmitting the molecular information such as stereo or regiochemistry through the formation of identical template molecules. Even so, such ideal acceleration is hardly illustrated in real closed systems and remained to be challenging. In essence, a system in which self-replication is operating can be defined as an autocatalytic reaction. The characteristic of self-replicating reaction is apparent from the concentration vs time profile, which displays a non-linear sigmoidal growth profile consisting of a lag period in the early stages of the reaction (Figure 1.2a). The product is formed slowly through the bimolecular process because there is no product $T$ available to catalyse the reaction.

As soon as the critical concentration of $T$ is reached, the autocatalytic cycles can start to operate and the reaction achieve the maximum autocatalytic rate before the starting reagents are fully consumed (reactant limiting stage). Accordingly, the rate vs time profile can be constructed (Figure 1.2b) by extracting the first derivative of the concentration vs time profile. Whilst the minimal self-replication scheme in Figure 1.1
illustrates the basic action of this type of system, more intricate model is required to illustrate the many other interactions inherent in this system. These additional considerations will be highlighted in the next section.

![Diagram](image)

**Figure 1.2.** (a) Concentration vs time profile for the formation of replicators (solid line) and the corresponding bimolecular rate reaction (dashed line) and (b) Rate vs time profile of replicators (solid line) and the corresponding bimolecular reaction (dashed line). Red dashed line point out the maximum autocatalytic rate in each profile.

### 1.3 Reciprocal model of self-replication

The intrinsic concept in this type of replication is a crosscatalytic reaction, in which one strand acts as a catalyst for the formation of another strand and vice versa. Therefore, two interlinked crosscatalytic cycles in this reciprocal model rely on each other, in which the two templates catalyse the formation of each other. The implementation model of such crosscatalytic self-replicating system can be illustrated in Figure 1.3.

It is, however, important to note that the complexity of this system can increase dramatically depending on the nature of the chemical reaction that forms the two templates. In the case where the reaction between C and D and the reaction between E and F are orthogonal, only one reciprocal replication cycle is present in the system. However, if C can react with E and D with F, four replication cycles can be envisaged, namely two minimal replication cycles – \( T_{CE} \) and \( T_{DF} \) may self-replicate and the original reciprocal replication cycle, \( T_{CD} \rightarrow T_{EF} \) and \( T_{EF} \rightarrow T_{CD} \). Eventually, the minimal replicators \( T_{CE} \) and \( T_{DF} \) may cross-catalyse the formation of each other.
Figure 1.3. The reciprocal model of self-replication comprised two interlinked crosscatalytic cycles. In these systems, compounds C and D can react to form the template $T_{CD}$, and, similarly, compounds E and F can react to form template $T_{EF}$. Since the four reactive partners bear recognition sites (green coloured cartoons) and since $T_{CD}$ and $T_{EF}$ are mutually complementary, $T_{CD}$ is capable of assembling E and F into the ternary complex $[E\cdot F\cdot T_{CD}]$. This ternary complex intramolecularizes the reaction between E and F and hence, catalyses the formation of $T_{EF}$. Similarly, $T_{EF}$ is capable of assembling C and D into the ternary complex $[C\cdot D\cdot T_{EF}]$ and catalyse the formation of $T_{CD}$.

1.4 The do’s and the don’ts in self-replication

Self-replicating systems usually are limited in their ability to undergo efficient autocatalytic self-replication. One of the factors relies on the design of the replicator itself. Therefore, we will try to justify the ideal conditions attempted to improve the systems. All these observations are demonstrated from the self-replicating systems developed in our laboratory. We have investigated different reactions, building block geometries and substitution patterns which leads to a concise understanding of the mechanism of self-replication and successfully discover some efficient replicators.

1.4.1 What usually goes wrong?

We noticed that there are two recognition-mediated pathways exist in the minimal model of self-replication (see Figure 1.1), namely the $[A\cdot B]$ complex pathway and the autocatalytic cycle accomplishing the same target, that is the acceleration of a chemical reaction. In principle, given sufficient conformational freedom and the location of complementary recognition sites on two reagents A and B permits the association of A and B through their mutually compatible recognition site to form a complex, $[A\cdot B]$. Within the $[A\cdot B]$ complex, the chemical reaction between A and B is effectively intramolecular, and thus, we would then expect the formation of the $[A\cdot B]$
complex to accelerate the transformation involving reagents A and B significantly.

Figure 1.4. (a) i. Scheme reaction between diene 1 and maleimide 2 in CDCl₃ at 50 °C and ii. Scheme reaction between diene 4 and maleimide 5 in CDCl₃ at 50 °C. In both cases, the starting concentrations of the reactants were 100 mM; (b) The filled squares represent the concentration of the exo cycloadduct 6 and the open squares represent the concentration of the exo cycloadduct 3. Figure taken from reference 27.

The [A•B] complex pathway however differ from latter pathway, as such it does not involve any form of recognition-mediated catalysis because the closed template Tinactive does not play any further role in the reaction process. In comparison, the autocatalytic cycle in the self-replicating system has become an appealing aim to amplify a targeted structure over others in the design systems. We have described a
recognition-based systems which can accelerate the rate and control the stereochemical outcome of Diels-Alder reaction between a maleimide and furan (Figure 1.4). When a 100 mM solution of diene 1 and maleimide 2 in CDCl₃ was heated at 50 °C, only very small amounts of the exo cycloadduct 3 could be detected after 15 hours. In order to investigate the recognition-mediated facilitation of this cycloaddition reaction, diene 4 bearing an amidopyridine recognition site, and maleimide 5 bearing a complementary carboxylic acid recognition site were then prepared. When a 100 mM solution of diene 4 and maleimide 5 was heated at 50 °C in CDCl₃, a significant quantity of the disfavoured exo cycloadducts 6 are detected after 15 hours. From these results, it is clear that the introduction of the recognition elements within the diene 4 and dienophile 5 has increased the extent of reaction dramatically. From the concentration profile, it is clearly visible that the maximum rate can be found at the start of the reaction (Figure 1.4b). This enhancement is indicative of the [A•B] complex pathway. Since the template is in its closed structure, the recognition sites are associated through intramolecular hydrogen bonds and hence, the template does not possess any catalytic activity. Moreover, adding pre-synthesised product template 6 at the start of the reaction has no effect on the shape of the curve. For that reason, the suppression of the binary complex [A•B] pathway is an important limitation within the minimal model to ensure an efficient self-replicating enterprise.

This problem can be resolved by careful design of the building blocks. The angle at which the reactive site and the recognition site relatively to each other is one of the main factors determining whether the reaction will follow the [A•B] complex or the autocatalytic pathway. If the bond angles in the product structure allow for intramolecular recognition of the complementary recognition sites, than the resulting closed template will be inactive for autocatalysis. We have demonstrated that by introduction of length segregation using substrates with sufficiently different length of linkers connecting the recognition and reactive sites, we enable to preclude this reaction pathway. Thus, upon recognition the reactive sites are inaccessible to stabilize the transition state.

The second significant problem associated with the catalytic efficiency of ternary complex [A•B•T] (see Figure 1.1). The template molecule T must accelerate the rate
of reaction between the substrates A and B substantially compared to the rate of reaction in the absence of T. The presence of pre-synthesised template T at the beginning of the reaction should result in the loss of the initial lag period, providing evidence for the existence of a self-replicating system. Additionally, the maximum rate of the reaction should instantly occur at the start of the reaction. In order to demonstrate this expectation, we reacted nitrone 7 and maleimide 8 at starting concentration of 25 mM in CDCl$_3$ at 10 °C, giving rise to major formation of trans 9 in a ratio 6:1 relative to cis 9 (Figure 1.5b) demonstrating the sigmoidal growth$^{31}$.

**Figure 1.5.** (a) Scheme reaction between nitrone 7 and maleimide 8 in CDCl$_3$ at 10 °C at 25 mM starting concentration; (b) Concentration vs time profile for nitrone 7 and maleimide 8 demonstrating the sigmoidal growth of the trans 9 (blue filled diamonds) and cis 9 (red filled circles). The template directed nature of the reaction was demonstrated by addition of preformed trans 9 to reagents 7 and 8 removed the lag period and the maximum autocatalytic rate was achieved at $t = 0$ (green filled boxes). By contrast, the addition of pre-synthesised template cis 9 at the beginning of the reaction has no effect on the rate of production of trans 9 or cis 9 (green open circles). Figure adapted from reference 31.
In the presence of 40 mol\% preformed template \textit{trans} 9, the reaction revealed the absence of the lag period. It is clear from the experimental results presented here that \textit{trans} 9 exerts a significant level of stereocontrol on the reaction between 7 and 8 in the [7-8-\textit{trans} 9] complex. By contrast, the addition of pre-synthesised template \textit{cis} 9 at the beginning of the reaction has no effect on the rate of formation of \textit{trans} 9, indicating that this diastereoisomer is inactive to influence self-replication either in an autocatalytic or crosscatalytic context.

In other cases, if the product molecules bind the component molecules too tightly, the product duplex [T-T] (see Figure 1.1) may be slow to dissociate and raise the product inhibition, reducing the number of available product molecules and eventually hampered the autocatalytic cycle. We have demonstrated that the reaction between azide 10 and maleimide 11 at 30 °C in CDCl3 at 25 mM starting concentration forms a template 12 (Figure 1.6a) that is capable of self-replication\textsuperscript{32} but form a strong template duplex [12-12] in the solid state (Figure 1.6c). The autocatalytic behavior of this system can be described using von Kiedrowski analysis\textsuperscript{23} to determine the type of the autocatalytic growth curve using the reaction order $p$.

In this case, the kinetic simulation revealed a $p$ value of 0.4, which indicates that the replicating system obeys the square root law. These results suggest strongly that the [12-12] duplex is very stable in our system, in which case leads to almost no turnover in the autocatalytic cycle. Such a system may be termed self-replicating because it constructs one copy of itself, but not autocatalytic, as it does not return significant amounts of free template to the reaction mixture.

This effect can be reduced by reducing the alkyl spacer placed between the carboxylic acid and the maleimide, the reactive sites are prohibited to accelerate within the binary [A-B] complex pathway. Despite that, if the product duplex [T-T] dissociates too readily, then the product template may not bind the components very well. Either case reduces the number of functional products that can serve as the template. Therefore, a delicate balance has to be achieved between strong binding of the components and facile release of the newly formed template.
Figure 1.6. (a) Template 12 can assemble maleimide 10 and azide 11 in CDCl₃ at 30°C and accelerate the reaction between them; (b) Concentration vs time profiles for the formation of 12 in the recognition-mediated reaction (red open circles) between and in the presence of 50 mol% preformed 12 (red filled circles); (c) Solid-state structure of the [12•12] duplex as determined by single-crystal X-ray diffraction. Figure taken from reference 32.

1.4.2 What happens if it works well?

In an efficient self-replicating system, the template T molecules will contribute to the formation of new product template by binding the available reagent components, A and B (see Figure 1.1). After the reaction, the product duplex [T•T] comes apart readily so that additional component molecules can be assembled. If the template molecules capable to participate in multiple replication cycles, sustained exponential growth can occur, before reaching the steady state when all the starting reactants has been consumed.
In principal, we should be able to observe a sigmoidal or ‘S’ shaped growth in the concentration vs time profile (Figure 1.7a) in the investigated systems. Theoretically, the behavior of the replicators can be evaluated by the reaction order, $\rho$ as such the value normally lies between 0.5 and 1.0, depending on how close the reaction is to exponential growth and the extent of product inhibition$^{23,33}$. In the case of rapid dissociation of the template duplex [T-T], an exponential growth would be observed from concentration vs time profile with reaction order of 1.0. However, when the product inhibition is a significant problem, the concentration vs time profile displays a parabolic growth with reaction order of 0.5 as shown in Figure 1.7b.

![Figure 1.7. Simulated concentration vs time (solid line) and rate vs time (dashed line) profiles for a reaction with reaction order of (a) $\rho = 1.0$ (exponential growth) and (b) $\rho = 0.5$ (parabolic growth). Figure adapted from reference 33.](image)

In consequence, we have to make sure that the reaction preferentially went through the autocatalytic pathway. Our own approach to distinguish a replicator’s behavior whether it is exponential or parabolic is by following the formation of template T in the presence of three different amounts of preformed template at the start of the reaction. In the case of exponential growth, the initial rate of formation of T scales as 1:2:4 whereas the initial rate of formation of T scales as 1:$\sqrt{2}$:$\sqrt{4}$ in parabolic growth.

### 1.4.3 The need for control experiments

On that account, it is essential to perform several control experiments to indicate that the formation of a product template is a consequence of self-replicating behavior. We will discuss in detail the previous cycloaddition reaction (Figure 1.5a) to illustrate the...
example of how we carried out the control experiments. Initially, a control compound 13 is identified, which possess the same chemical functionality as the building blocks of the replicator. Practically, the recognition site is removed in this control compound. In this case, the carboxylic group of maleimide 8 is substituted with the corresponding methyl ester, giving the maleimide 13, which eventually obstruct the formation of the hydrogen bonds with nitrone 7 and thus, incapable to participate in any recognition-mediated processes (Figure 1.8a).

![Scheme reaction between nitrone 7 and maleimide 13](image)

**Figure 1.8.** (a) Scheme reaction between nitrone 7 and maleimide 13 at 25 mM at 10 °C in CDCl$_3$. The formation of trans 14 is shown as filled diamonds and of cis 14 as open diamonds. Figure adapted from reference 31.

The kinetic data measured using this control compound provide a comparative baseline for kinetic studies and will be termed the background reaction. The rate
enhancement in the formation of one of the diastereoisomer in comparison with the background reaction will indicate the dominant pathway whether it is replicating cycle or the [A·B] complex. Subsequently, it is also crucial to establish the dependency of the reaction on molecular recognition since the efficient enterprise of a self-replicating system revolves around the reversible recognition events that occur in the autocatalytic cycle. Secondly, the previous developed replicating systems shown in Figure 1.5a were allowed to react in the presence of an unreactive carboxylic acid, for example benzoic acid.

![Figure 1.9](image)

**Figure 1.9.** (a) Scheme reaction between nitrone 7 and maleimide 8 in CDCl₃ in the presence of 100 mM benzoic acid at 10 °C at 25 mM starting concentration as shown in Figure 1.5a; (b) Concentration vs time profile for the formation of *trans* 9 (blue open diamonds) and *cis* 9 (red open circles) at 10 °C in the presence of 100 mM benzoic acid in CDCl₃. Figure adapted from reference 31.
This competitive inhibitor is capable of binding to the recognition sites present in compound 7 and, hence, interfere the crucial recognition processes. Consequently, the autocatalytic cycle is disrupted, resulting in a decrease in the rate and/or selectivity in the rate of the reaction, which often accompanied by disappearance of the sigmoidal curve (Figure 1.9b).

The final experiment that provides the evidence of self-replicating operating within a system can be obtained by seeding the reaction with the product T itself. The presence of pre-synthesised template T at the beginning of the reaction should result in a loss of the initial lag period in the concentration vs time profile for the reaction, indicating the reaction is now template-directed (as demonstrated in Figure 1.5b). The rate of the reaction should be highest from the start of the experiment and comparable to the maximum rate of the formation of the products in the absence of template T. Such behavior is only observed for the self-replicating pathway. If the reaction proceeds via [A•B] complex channel (as depicted in Figure 1.4b), the addition of the template to such system will not affect the rate of the product formation.

1.4.4 How to optimize the conditions?

The conditions chosen must suppress the bimolecular pathway but still allow for efficient autocatalytic turnover of the replicator. The involvement of this reaction can only be influenced by the changes in concentration and temperature. For instance, by reducing the concentration by a half reduces the rate of bimolecular reaction by four times. However, if the concentration drops below the dissociation constant, \( K_d \) for the recognition process used to assemble the ternary complex, replication will effectively be shut down. It is also noteworthy that a reduction in the reaction temperature will strengthen the binding and facilitate the formation of the product through recognition. Nonetheless, the association constant of the product duplex will also increased, resulting in the increased product inhibition. Therefore, the lowering of either of the parameters will slow down the bimolecular channel to a much greater extent than any of the recognition-mediated pathways.
1.5 Examples of self-replicating systems

Over the last twenty-five years, a various investigations on chemical self-replicating systems has make use of oligonucleotide analogues, polypeptides and small organic molecules as templates. However, a common problem of these systems is product inhibition. In the next section, we will illustrate the examples of each replicating systems including our own work based on small organic molecules.

1.5.1 Self-replicating systems based on oligonucleotides

The successful evidence for self-replication in non-enzymatic reaction utilizing oligonucleotides was demonstrated by von Kiedrowski in 1986 (Figure 1.10). 5'-terminally protected trideoxynucleotide 15 with base sequence 5' - CGG - 3' and complementary 3'-protected 16 with base sequence 5' - GCC - 3' were reacted in the presence of EDC to yield the self-complementary hexadeoxynucleotide 17, 5'-CCGCGG-3'. In addition, the CDI activated intermediate 15b underwent rapid hydrolysis to unreactive 15a which was itself reactive with 15b forming the side product, pyrophosphate 5'-MeCCGpp3'GCC-Me5' (pp) through self-condensation.

The course of the reaction was followed by HPLC and revealed two parallel pathways, both autocatalytic and non-autocatalytic pathway contributing to the formation of the hexameric templates. The latter pathway has been found to be predominant. In order to demonstrate autocatalysis in template production, the reaction mixtures differing in the initial concentration of template were prepared as shown in Figure 1.11.
Figure 1.10. Reaction of two trinucleotide substrates at 10 mM in aqueous solution is initiated by EDC activation of 15a to form the intermediate 15b. Subsequent condensation reaction with the 5' hydroxyl group on 16 leads to phosphoester formation and yields the self-complementary hexanucleotide 17 with base sequence 5'-CCGCGG-3' with the 5'-terminus protected as a methyl ester and the 3' terminus protected with the o-chlorophenyl group.
When the preformed template product was added at the start of the reaction, it did not increase the rate of autocatalytic template formation in a linear sense. Instead, the initial rate of autocatalytic synthesis was found to be proportional to the square root of the template concentration with the reaction order was found to be 0.48. They expected that most of the product template remained in their double helical forming a strong association and prevents the systems from an exponential growth\(^{37}\). It was first pointed out that the rate of formation of \(\text{pp}\) is almost independent of the initial concentration of template 17. However, it was revealed that the presence of \(\text{pp}\) template in solution is in similar concentrations to template 17. The increase of the initial concentration of template 17 would slightly decrease the rate of formation of \(\text{pp}\), simply because template \(\text{T}\) and \(\text{pp}\) compete for the same activated trinucleotide. Further investigations were directed to the base pairs sequence dependency\(^{38}\) in the self-replication of hexamer which led to significant decrease of catalytic activity when one of the bases in trimer 15 is varied from C to G and vice versa. The effect is most pronounced if the missmatch base pair is closer to the reactive site.

The first recognition of sigmoidal growth\(^{39}\) of template molecules in non-enzymatic reaction has been achieved by reacting trinucleotide 18 and 5'-amino group trinucleotide 19 which led to the formation of a 3'-5'-phosphoramidate bond in hexamer template 20 (Figure 1.12a).

\[\text{Figure 1.11.}\quad\text{The progress of the reaction without pre-synthesised template 17 at the start of the reaction (i) 0 mM and with the presence of template 17 (ii), (iii) and (iv), respectively. In the absence of template 17, the reaction proceeds slowly and by increasing the initial concentration of preformed template 17 (0.2, 0.4 and 0.8 mM) had the effect on the rate formation of template 17. Figure taken from reference 19.}\]
Figure 1.12. (b) Concentration vs time profile for the formation of the (a) hexameric 3'-5' phosphoamidate 20 from trinucleotide 18 and 19 in the absence of preformed template 20 (red solid line) and in the presence of 32 mol% preformed template 20 (red dashed line) as measured by HPLC. The yield of 20 is 50% after 2 hours. Figure taken from reference 39.
The time course for template formation was followed by HPLC (Figure 1.12b). The rate formation of template 20 is higher than template 17 with almost 50% conversion after 2 hours with sigmoidal rate profile. Addition of 32 mol% preformed template 20 at the start of the reaction increase the yield of template 20, which indicates that the autocatalytic process is operating in this system. The author later described a parabolic growth with the reaction order, \( p \) was found to be 0.5 as a consequence of product inhibition. By far, the reported self-replicating system only composed of two constituents. Therefore, more complex systems with three or more starting materials are also envisioned\(^{40}\). Further developments of oligonucleotide self-replicating system aimed on the development of crosscatalytic\(^ {41}\) replication systems.

1.5.2 Self-replicating systems based on RNA

Some scientists believe that the early forms of life may be hiding in our cells, in molecules known as RNA. RNA, DNA and other nucleic acid like compounds are informational macromolecules that have inherent template properties and so lend themselves in a straightforward way to both storing information and replicating\(^ {42}\). The discovery\(^ {43}\) of ribozymes as both genetic material and catalyst provided an empirical basis for the concept of the RNA world. An example of self-replication developed based on ribozyme\(^ {44}\), catalyses the assembly of additional copies of itself through an RNA-catalysed RNA ligation reaction (Figure 1.13a). The ribozyme catalyses attack of a 3'-hydroxyl of the substrate on the \( \alpha \)-phosphate of the 5'-triphosphate of the ribozyme, with concomitant release of inorganic pyrophosphate. The R3C ligase 23 was redesigned as a symmetrical dimer, so that it would ligate two substrates 21 and 22 to generate an exact copy of itself (Figure 1.13b).

The main consideration is to ensure the template and substrates interact with minimal base pairing so that the ligated product could easily dissociate from the template and, thus be available to catalyse subsequent ligation reactions. The ligation reaction involving 21 and 22 in the absence of 23 was very slow, with less than 1% product formed after 3 hours (Figure 1.14).
In the search for the origin of life based on DNA as we know it, the existence of an exponential replicator. The peptide replicators described by Ghadri and Chmielewski were used as the basis of extended replicating systems and logical operations which will be discussed later.

Joyce, an advocate of the RNA world scenario, RNA, is considered as a plausible scenario. Drawing on inspiration from this theory, one which used the information storage and catalytic ligase that binds two oligonucleotides through Watson–Crick base pairing and demonstrated a self–replicating system based on RNA.

The ribozyme triphosphate (ppp) group of the template molecule 23 binds the two substrate molecules 21 and 22, which then undergo RNA-catalyzed ligation (black arrow) to generate another copy of template 23.

Figure 1.14. Concentration vs time profile for the production of template 23 in reaction mixtures consisting the 21 and 22 substrates in the absence of 23 (red open boxes) and in the presence of template 23 (red filled boxes). Figure adapted from reference 44.

Figure 1.13. (a) Secondary structure of the self-replicating ligase ribozyme. The template molecule 23 binds the two substrate molecules 21 and 22, which then undergo RNA-catalyzed ligation (black arrow) to generate another copy of template 23; (b) Schematic presentation of the ribozyme-based self-replicating cycle. Figure adapted from reference 44.
When 0.5 equivalents of 23 were added to a reaction mixture, 400 folds increase in the initial rate of ligation was observed. Therefore, the template dependence of the reaction between 21 and 22 was considered to be significant because the rate enhancement achieved by the introduction of template 23 could not be achieved by the use of more substrates alone. A linear dependency between the initial rate of reaction and the starting concentration of template consistent with the reaction order of 1.0, which indicates that the availability of template 23 at the start of the reaction is not limited by dissociation of [23•23] complexes, allowing for product turnover. Despite the fact that the ternary complex [21•22•23] dissociated readily, exponential amplification was limited by a tendency for the substrate molecules to form a binary complex [21•22]. The authors reasoned that the remaining free substrates 21 and 22 capable of forming [21•22] complex before template 23 becomes available to initiate a new autocatalytic cycle and hence, further disrupted the recognition-mediated reaction.

1.5.3 Self-replicating systems based on polypeptides

The feasibility of peptide self-replication system has been established experimentally such that the preliminary report based on the peptide replicator utilized the α-helical coiled coils with 32-residue polypeptide as the template T in the replication strategy. These structures are distinguished by their amphiphilic primary sequence of heptad repeats (abcdefg)_n.

The molecular recognition is based on hydrophobic interface (residues at a and d positions) and electrostatic interactions (residues at e and g positions) in the coiled-coil structure. Residues at the b, c, and f positions are exposed to the solvent and do not participate directly in the self-assembly of the coiled-coil structure and capable of tolerating a substantial variation of amino acid substitutions. The ligation site was chosen to lie on the solvent exposed surface of the α-helical structure to avoid interference with the hydrophobic recognition surface (Figure 1.15).
Figure 1.15. Helical-wheel diagram of the template peptide in the dimeric α-helical coiled-coil configuration emphasising the heptad repeat motif. The interhelical recognition surface consists of amino acids allowing for hydrophobic packing interactions (positions a and d) and electrostatic interactions (positions e and g). Amino acids at positions b, c and f lie on the solvent-exposed surface of the helical structure and do not participate in the molecular recognition processes. Red arrows indicate the ligation site between a cysteine residue 18 at position c and alanine residue 17 at position b on the solvent exposed face. Figure adapted from reference 45.

Ghadiri et al. employed the Kent ligation as the peptide fragment coupling strategy. The coupling reaction proceeds through an initial trans thioesterification between C terminal thioester of electrophile polypeptide E and the N terminal cysteine sulfhydryl group of nucleophile polypeptide N. The resulting thioester intermediate rapidly rearranges via intramolecular S to N acyl transfer to give the final amide bond. The association of polypeptide E and N to the template T produce the ternary complex [E•N•T] (illustrated in Figure 1.16). The reactive sites are now in close proximity to allow the Kent ligation reaction pseudo-intramolecular to form duplex [T•T]. Subsequent dissociation of two molecules of T to the start of the reaction completed the autocatalytic cycle.
Figure 1.16. Schematic representation of the minimal replication model for self-replicating α-helical peptide. The electrophilic and nucleophilic peptide fragments E and N are recognised by template T through interhelical hydrophobic interactions to form catalytic complex, [T·E·N]. Subsequent chemical ligation produces an identical copy of the template, which remains bound in the product duplex [T·T]. Dissociation allows the two templates to undergo further catalytic cycles. The inset depicts the amide bond forming process based on thioester promoted peptide fragment condensation strategy of Kent.

An equimolar mixture of both electrophile and nucleophilic fragments were reacted in the absence or presence of various amounts of template (Figure 1.17). Autocatalysis in template production is established with pronounced increases as the initial concentration of the template is increased. At higher concentrations of peptides, an increase in autocatalytic growth was observed with reaction order, $p$ of 0.63. It was also attributed from the contribution of quaternary complex $[E·N·T·T]$ (the association of two substrates $E$ and $N$ with the duplex $[T·T]$) to the autocatalytic pathway despite the inhibition from duplex $[T·T]$ complex. Therefore, the reaction order as well as the autocatalytic efficiency is strongly dependent on the relative thermodynamic stability.

$E : \text{ArCONH–RMKQLEEKVYELLSKVA–COSBn}$

$N : \text{H}_2\text{N–CLEYEVARLKKLVGE–CONH}_2$

$T : \text{ArCONH–RMKQLEEKVYELLSKVA–CLEYEVARLKKLVGE–CONH}_2$
of the ternary complex \([T\cdot E\cdot N]\) with respect to the template duplex \([T\cdot T]\). Control reactions with single mutant peptide support the contribution of the autocatalytic pathways to the overall product formation, which must proceeds through the ternary and/or quarternary complex. Furthermore, these experiments reveal a remarkable sequency selectivity, as evidenced by the loss of the autocatalytic activity as a result of a single replacement of leucine or valine residue with an alanine at the recognition interface.

![Figure 1.17](image)

**Figure 1.17.** Concentration vs time profile for the formation of template \(T\) in the absence of preformed template \(T\) (i) and in the presence of different initial concentrations of template (ii), (iii), (iv) and (v). Figure adapted from reference 46.

Further progress towards exponential growth in self-replicating peptides was aimed through the destabilisation of template duplex. Chmielewski et al. have implemented two strategies to achieve a reaction order of 0.91, indicative of exponential amplification. In 2001, this was accomplished by shortening the coiled coil from 35 to its minimum length of 26 amino acids\(^{51}\). In shortening the peptide, the number of leucine residues was reduced and the overall hydrophobicity of the templating surface was decreased. These resulted in more efficient product release, therefore facilitating successive cycles of replication\(^{52}\).

Alternatively, in 2003, they documented that the insertion of one proline amino acid for the change of glutamate (Glu20) in the building blocks of the replicating peptides leads to a 30° kink in the \(\alpha\)-helix (**Figure 1.18**). The position of the proline substitution, located on the hydrophilic face of the helix, was found to be critical for
this improved behaviour. The substitution did not disrupt the hydrophobic templating face, allowing efficient complexation between the template and substrates, while the kink results in the reduced coiled-coil stability\(^{53}\).

Figure 1.18. The effect of proline substitution in the replicating peptide-based template with respect to the hydrophobic surface of leucine residues. The 30° kink destabilises the template duplex and thus enhancing replication.

1.5.4 Self-replicating systems based on small organic molecules

A further exciting field in bioorganic chemistry is development of synthetic replicators. It seems feasible to invent new replicators based on truly artificial replicators since molecular recognition and catalysis are common features among organic molecules. Rebek and co-workers first reported wholly synthetic system, which involves the formation of amide bond with relatively small organic molecules for the production of an assembled system. This system could promote replication by employing both templating and base pairing effects. They have set out to prepare two components bearing both complementary reactive functionality and a complementary hydrogen bonding surfaces. Their initial attempts\(^{36}\) employed the Kemp’s triacid 24 incorporating a phenyl spacer and adenosine 25 through the formation of amide bond by aminolysis of a pentafluorophenolate ester by the primary amine (Figure 1.19). An unfavourable [A•B] complex with subsequent intramolecular binding resulted in the product, which remain closed as a consequence of the short phenyl spacer and the
flexible chain. Alternative template was subsequently developed to include the naphthyl-ribose spacer to separate the recognition sites and destabilise the product duplex.

![Figure 1.19](image1.png)

**Figure 1.19.** Rebek’s initial design of a minimal replicator utilised a phenyl spacer in Kemp’s substrate 24 was too short as well as flexible chain in adenosine substrate 25, which resulted in the formation of unfavourable [A-B] complex.

Accordingly, a new replicator consisting of the amino adenosine 28 and its coupling reactions to imides bearing 2,6-naphthalene surfaces 27a were examined to form template 29’ in its cis amide formation. Isomerisation to the more stable trans amide 29 occurs rapidly allowing hydrogen-bonding surfaces to be exposed for dimerisation or autocatalysis.

![Figure 1.20](image2.png)

**Figure 1.20.** Synthetic self-replicating system illustrated by Rebek. Template 29 incorporates a longer naphthalene spacer on the Kemp’s acid 27 and the amino adenosine 28 now contain a ribose ring.
As shown in Figure 1.20, the reactive ends of the 27a and 28 come into spatial proximity when the reactants interact with the template 29. Nucleophilic attack of the primary amine of 28 on the activated carboxyl ester of 27a leads to amide bond formation, generating the template 29. Dissociation of the resulting template duplex closes the first replication cycle with the released free template. The control experiments rate with N-methyl imide 27b is substantially slower the formation of the product, than that of the parent NH imide, 27a under the same conditions, thus provides strong evidence for base pairing. The inhibition of the reaction 28 and 27a by the competitive inhibitor, 2,6-bis(acetylamino)pyridine provides additional evidence. A decrease in the rate of reaction corresponds with the decrease in the percentage of base paired complex (Figure 1.21).

**Figure 1.21.** Initial rates of template formation as determined by HPLC. All reactions were performed with initial concentration of [27a] = [28] = 8.2 mM in CHCl3 with four equivalent of Et3N added as a general base. (i) Reaction of 28 and the N-methylated 27b, (ii) Reaction of 28 and 27a with one equivalent of 2,6-bis(acetylamino)pyridine, (iii) Reaction of 28 and 27a. Data taken from reference 54.

The authors demonstrate “autocatalysis” by the rate enhancement of the initial reaction upon seeding the prefabricated template 29 to coupling mixture, although the observation of a sigmoidal curve was hampered by the action of reactive [A•B] complex (Figure 1.22). The formation of [A•B] complex is as a result of the association of both substrates 27a and 28 which promotes the formation of cis 29' by intramolecular reaction before cis to trans amide isomerisation furnish the open template 29. Thus, the principal step in producing a predominantly autocatalytic
system would therefore lie in removing the possibility of binary complex. In coexistence with the reactive \([A\cdot B]\) complex described in the system, Rebek groups computed that only 2% of the building blocks are assembled in the ternary complex. Therefore their estimation of the population of the ternary complex is highly questionable and posed significant controversial feedback.

![Figure 1.22](image)

**Figure 1.22.** Initial rates of template formation as determined by HPLC. All reactions were performed with initial concentration of \([27a] = [28] = 8.2 \text{ mM}\) in CHCl\(_3\) with 4 equivalent of Et\(_3\)N added as a general base. (i) Reaction of 28 and 27a, (ii) Reaction of 28 and 27a with 0.2 equivalent of preformed template 29 and (iii) Reaction of 28 and 27a with 0.5 equivalent of preformed template 29. Data taken from reference 54.

Menger and co-workers reinvestigated\(^{55}\) the claims of autocatalytic self-replication in these systems. Menger *et al.* proposed a mechanism (**Figure 1.23**) explaining the rate enhancement found in this system, based on a bimolecular reaction between the bound amine building block and the non-binding ester, which led to a tetrahedral intermediate stabilised by the amide bond of the template. This mechanism directly questioned the replicative nature of Rebek’s system as there was no obvious purpose for simultaneous recognition of both the ester and amide moieties, and, therefore the turnover could not be attributed to the ternary complex.

Thus, the methylated imide control compound 27b used in original studies to prove the need for molecular recognition would not be expected to behave as a catalyst for this reaction. Whilst the scheme presented in this case might not be considered as autocatalytic, it is not self-replication in the sense of a selective autocatalyst as it
could, as it theoretically catalyse the reaction between amine building block and any activated ester. Rebek and co-authors discredited\textsuperscript{56} this new evidence and the two groups participated in an open discussion by publishing new evidence\textsuperscript{57,58} to support their own theories. The final comment on the controversial discussion was published by Reinhoudt \textit{et al.} They utilized an intricate and detailed kinetic scheme\textsuperscript{59} to quantify various different species and the reaction paths available to the components of a self-replicating system.

![Diagram of amide catalysis mechanism](image)

\textbf{Figure 1.23.} Menger’s proposed amide catalysis mechanism. The mechanism involves the assembly of the amine building block upon the template followed by a simple bimolecular reaction with non-bonding ester to produce the tetrahedral intermediate, which is stabilised by the amide bond of the template.

The study demonstrates that these different pathways could obscure the simple picture of a single pathway as the only responsible of rate enhancement. They concluded that both the Rebek and Menger interpretations are correct as such the mechanism of self-replication originally proposed by Rebek was operating in the system however its observation was also hampered by alternative pathway described by Menger.

Although the reaction between 27a and 28 in the presence of preformed template 29 can operate through self-replication, the major pathway in its absence is the [A\cdot B]
complex. Given the debate from the initial publication of synthetic replicating molecules, this event serve to demonstrate and emphasize the usefulness of full kinetic modeling in characterising the autocatalytic reaction.

Throughout the time that these discussions were taking place, the Rebek research group improved its system to present evidence of sigmoidal growth and render the \([A\cdot B]\) pathway less important by preparing a longer spacer, 4,4'-biphenyldiyld spacer\(^{60}\) in compound 30. This adaptation separates the reactive centres in the \([A\cdot B]\) complex rendering it less reactive in the intramolecular sense (Figure 1.24).

![Figure 1.24](image)

Figure 1.24. Compound 31 was prepared by acylation of the 5'-aminoadenosine derivative 28 with the biphenyl ester 30 in CHCl\(_3\).

This adenine-imide conjugate 31 possesses self-complementary that can acts as templates for their production (Figure 1.25). It was clear that the sigmoidality of the autocatalysed reaction curve is a contribution of the self-replicating pathway via ternary complex even though the mechanism involving bimolecular base paired species is still operative.
Figure 1.25. Initial concentration of template production in second generation of Rebek’s replicator with a clear sigmoidal character (filled squares). Original data taken from reference 60.

Furthermore, the Rebek laboratory has also reported a novel design based around a xanthene scaffold with recognition between a diaminotriazine and a thymine with amide formation for the bond forming\textsuperscript{61}. Then, they combined the imide/adenosine and thymine/diaminotriazine systems to generate two hybrid systems, both capable of self-replication\textsuperscript{62}.

Majority of the systems demonstrated earlier follow a half reaction order, which display a parabolic growth. Attempts to realise this principle have utilized the reversible covalent reaction with even simpler organic constituents. Terfort and von Kiedrowski\textsuperscript{63} used the amidinium-carboxylate salt bridge to replace the base pairing for molecular recognition. Condensation of aniline 32 with aldehyde 33 yielded imine 34, which can exist in conformations in which the recognition sites are aligned roughly parallel to each other (Figure 1.26).

These molecules should act as autocatalysts since they should be able to bind components 32 and 33 more easily due to preorganisation effect within the ternary complex [32\cdot33\cdot34]. In this case, the autocatalytic cycle was observed as such the higher addition of the initial concentration of 34 in the reaction mixture, the faster its formation. From the experimental evidence, the condensation follows the square-root law.
maximum rate upon initiation (Figure 1.41 filled circles). With a racemic substrate resulted in the removal of the lag period allowing the reaction proceeded at its characteristic of autocatalytic self–replication. Autocatalysis was demonstrated with NMR spectroscopy. The concentration maleimide employed was a [4+2] Diels–Alder cycloaddition of a 1,3–cyclohexadiene naphthalene provided the molecular recognition in the system. The reaction based on Kelly produced by Wang and Sutherland Another example of a carefully crafted non–natural self–replicating system was relatively simple organic molecules were able to be used for self–replication. The system demonstrated that the faster the initial rate of its formation was. The higher the initial concentration of preformed imine added to a fresh reaction progress was monitored by condensation of an amine with an aldehyde. This was followed by a triple hydrogen bonding motif of von Kiedrowski and aldehyde as the bisubstrate template, studied by Section 1.4.3, of a pyridone and a pyridone. This was followed by condensation with a bisubstrate template, studied by Section 1.4.3, of a pyridone and a pyridone. This was followed by condensation.

1.5.5 Self-replicating systems based on Diels–Alder reaction
Other examples have been developed based on Diels–Alder reaction, studied by Wang and Sutherland in 1997. The 2-acylaminonaphthyridine 35 and the 6-acylamino-2-pyridone derivative 36 were selected as target ene and diene systems with both the naphtyridine and 2-pyridone as the complementary recognition site (Figure 1.27).

![Figure 1.26](image1.png)

**Figure 1.26.** Template 34 bind amine 32 and aldehyde 33 within the ternary complex to give the subsequent template duplex [34·34].

![Figure 1.27](image2.png)

**Figure 1.27.** Diels–Alder cycloadduct 37 can assemble the diene 35 and maleimide 36 in a catalytically-active ternary complex in CD₂Cl₂.

The course of the product formation 37 were followed by ¹H NMR spectroscopy and revealed an autocatalytic reaction order of 0.8 with sigmoidal character. This was
further confirmed by treatment of up to 10% of preformed template 37, which validate that the reaction was template-dependent with the loss of induction (Figure 1.28) period. They remarked that this reaction is the very first example of nearly exponential growth and could offers the basis of efficient self-replicating systems capable of chemical information transfer in terms of regio- and stereoselectivity of template catalysed bond formation.

![Concentration vs time profile for the formation of 37 in CD2Cl2 at 25 °C (open circles in graph). The addition of 5 mol% preformed template 37 to the reaction mixture (filled circles in graph) removes the lag period from the initial concentrations demonstrating that 37 is a catalyst for its own formation. Figure taken from reference 64.]

It also has the potential to exhibit homochiral autocatalysis and heterochiral cross-catalysis, because a chiral diene 35 was employed as a racemic mixture, this opens the possibility of many diastereoisomeric reaction pathways. However, consideration of the stereochemical implications of the bond forming reaction and the presence of a chiral building block reveals some unexplained aspects and have not yet been completed in their paper. The [4+2] cycloaddition reaction is reported to produce an endo stereochemistry but no unambiguous assignment of the stereochemistry of the template has been made.

On that account, Kinderman and Stahl from the von Kiedrowski lab in 2005 has decided to reassess the Wang-Sutherland system with respect to its stereochemical aspect. It is evident that their variants resembled Wang-Sutherland system with a further increase of reaction order of 0.89 and 0.9 denoting the operation of homochiral autocatalytic and heterochiral crosscatalytic reaction channels operating
in the systems. Self-replication with the ability of chiral instruction has recently received considerable attention from its relevance to the question of breaking of chiral symmetry on the early earth. Further examples of self-replication developed within our research groups also focused on the cycloaddition reactions. In previous example, the Philp laboratory exploited the Diels-Alder reaction between furan and maleimide as the means of forming covalent bonds while maintaining the similar recognition motif. The Diels-Alder reaction gives two diastereoisomer products, namely the endo and exo cycloadduct. Only one of two possible diastereoisomers formation is being accelerated through the reactive binary [A•B] complex as described previously in Section 1.4.1.

We then became interested to employ this reaction and carried out a series of potential replicators with structurally related system in von Kiedrowski replicator. We examined the role that systematic structural variation plays in determining the recognition-mediated reactivity in order to probe the relationship between structure and function in minimal self-replicating systems. A clear take home message is that a small structural changes can make huge differences of function that are not entirely foreseeable.

In addition, the design and characterization of a minimal self-replicating system based on 1,3-dipolar cycloaddition has also been well investigated. Typically, this reaction affords a pair of racemic diastereoisomeric cycloadducts, trans and cis, depending on the relative orientation of the nitrone and the maleimide. The two products provide the means of exposing the efficiency of information transfer in any replicating system as the trans to cis ratio observed is always close to 3:1 in the absence of molecular recognition. These two products have markedly different geometries. The trans diastereoisomer has an open geometry compatible with acceleration through the ternary complex and the cis diastereoisomer has a closed geometry compatible with acceleration through the [A•B] complex pathway.

The initial reaction between nitrene and maleimide reacted at 10 °C in CDCl₃ affording the corresponding trans and cis (as depicted in Figure 1.5a). It is clear that the introduction of the recognition motif has significantly increased the rate of the reaction with sigmoidal growth, particularly for the formation of the major, trans.
(blue filled diamonds in Figure 1.5b) with reaction order, $p$ of 0.9. The result clearly shows that the high $p$ value is a result of efficient autocatalytic cycle. Further optimization on the performance of replicating system was carried out by screening a series of compounds computationally and we identified nitrone 38 (para position) and maleimide 39 as suitable precursors (Figure 1.29). The calculated structure on the two possible diastereoisomers reveals that trans 40 has an open structure, in which the two recognition sites are available to interact with other complementary species in solution, while the structure of cis 40 is folded with the recognition sites are placed in proximity to each other, thus rendering this product inert in a catalytic sense. These computational studies indicate that trans 40 should be capable of templating its own formation through the transition state of the ternary complex [38•39•trans 40].

![Autocatalytic Cycle](image)

**Figure 1.29.** Reaction of nitrone 38 with maleimide 39 in CDCl$_3$ at $-10$ °C gives rise to two diastereoisomeric cycloadducts – trans 40 and cis 40. The exclusive formation of cycloadduct trans 40 is mediated by the formation of ternary complex [38•39•trans 40].

Experiments conducted with 38 and 39 in CDCl$_3$ at $-10$ °C revealed that the recognition-mediated reaction were uniformly fast, reaching more than 85% overall
conversion after 16 hours and the diastereoselectivity was 115:1 with the formation of the major product \textit{trans} 40 (blue filled diamonds in Figure 1.30) in comparison to the \textit{cis} diastereoisomer (red filled circles in Figure 1.30). Furthermore, the concentration \textit{versus} time profile reveals the classical sigmoidal shape characteristics of an autocatalytic process and the rate of reaction are higher than that observed in the absence of recognition.

![Figure 1.30](image-url)

\textbf{Figure 1.30.} Concentration vs time profile for the reaction between nitrone 38 and maleimide 39 demonstrating the sigmoidal growth for exclusive formation of \textit{trans} 40 (blue filled diamonds) with a ratio of 115:1 to \textit{cis} 40 (red filled circles) at –10 °C in CDCl$_3$.

The injection of substoichiometric amounts (10 mol\%) of \textit{trans} 40 at the start of the reaction between 38 and 39 accelerate the maximum autocatalytic rate formation of \textit{trans} 40 immediately at $t = 0$. The \textit{trans} to \textit{cis} ratio is now at least 250:1. By contrast, the addition of 10 mol\% of \textit{cis} 40 has no effect on the rate profile. Therefore, the \textit{cis} cycloadduct is completely incapable of competing with the efficient replication cycle for \textit{trans} 40.

As part of the ongoing studies on self-replicating systems, our focus has shifted toward exploiting the implications of replication events in dynamic and reactive chemical systems and networks. We became intrigued by the possibility of introducing a reversible covalent bond forming reaction into our own replicating systems. The central design involved the condensation reaction 74 between an aniline derivative 41 and an aromatic aldehyde 42 to form an imine 43. Imine 43 is capable of directing its own formation through recognition-mediated pathways (Figure 1.31).
Imine 43 capable to assemble amine 41 and aldehyde 42 in CDCl₃ at 25 °C and accelerate the reaction between them through the autocatalytic cycle, which is mediated by the ternary complex [41•42•43].

Concentration vs time profile for (a) non-recognition-mediated reaction between 41 and 4-methylbenzaldehyde (open circles); (b) formation of imine 43 (filled circles) and (c) in the presence of 15 mol% of 43 at the start of the experiment (filled diamonds). All experiments were conducted in dry CDCl₃ at 25 °C from starting concentrations of the appropriate reagents of 15 mM. Figure adapted from reference 74.
In the case of recognition-mediated reaction between 41 and 42, the rate of reaction was relatively fast with more than 50% conversion, which clearly manifested the sigmoidal characteristics for the rate profile (Figure 1.32b). In the absence of recognition (Figure 1.32a), the rate of the reaction was very slow with lower conversions. Addition of 15 mol% of preformed imine 43 at the start of the reaction results in the disappearance of the lag period and the maximum rate was reached at the beginning of the reaction (Figure 1.32c).

1.6 Outlook
The design and implementation of chemical entities capable of replication by either autocatalytic or crosscatalytic pathways has engaged and challenged synthetic chemistry for the past twenty-five years. The demonstration of molecular replication implied that a replication is possible for chemical information transfer and amplification with notable obstacles have been disclosed in Section 1.4. The idea that a molecule could catalyse its own formation has been long associated with the Origin of Life. In order to reach the ultimate goal of creating functional chemical systems using synthetic tools that is capable of directing its own synthesis and cooperating with other similar systems to create an organized network, a much higher level of design is therefore required. These ideas will be explored in subsequent chapters.
In general, chemical topology\textsuperscript{75} describes the connectivity and linkage of atoms and molecules in space. This connectivity and linkage play vital roles in determining\textsuperscript{76} the chemical and physical characteristics of the molecules. The concept of chemical topology is highly relevant\textsuperscript{77} when considering interlocked\textsuperscript{78} structures. A compound regarded\textsuperscript{79} as significantly topological, if, by a continuous deformation of the bonds between the atoms, it cannot be converted into another isomer of the compound albeit having the similar connectivity and occupy a non-planar molecular graph when embedded in three-dimensional space.

For instance, a [2]-catenane (Figure 2.1a) needs at least two crossing points when drawn on a pieces of paper, which illustrates the structure has a non-planar graph when embedded in three-dimensional space. Thus, a [2]-catenane cannot be converted into the constituent rings without physically breaking one of the macrocycles and reforming the ring in the absence of others. Conversely, deformation of the macrocyclic component of a [2]-rotaxane (Figure 2.1b) allows it to be removed from the dumbbell component. Therefore, the [2]-rotaxane can be drawn as a planar graph when embedded in three-dimensional space and, hence, are not topological isomers\textsuperscript{80} of their component parts.

![Figure 2.1](image)

**Figure 2.1.** Cartoon representation of mechanically interlocked molecules : (a) [2]-catenane and (b) [2]-rotaxane.
In other words, if we could imagine stretching out the ring component of the [2]-rotaxane with both hands more than its average width, at some extent the ring is going to slip over the thread, but not in the case of [2]-catenane. The astonishing developments in synthetic methodology in the field of supramolecular chemistry have allowed the creation and characterization of complex molecular topologies which are the supreme targets for chemical synthesis\(^ {81}\) of artificial products.

Over the past 40 years, chemists have sought to create complex interlocking molecular structure, in which the synthesis of intricate interwoven molecules has been taken to an advanced level from rudimentary structure\(^ {82}\) of rotaxane and catenane to trefoil knot\(^ {83}\), Solomon link\(^ {84}\), Borromean ring\(^ {85,86}\), the synthesis of the molecular equivalent of the Olympic rings, olympiadane\(^ {87}\) and ultimately to the molecules\(^ {88}\) in a suits, the suit[5]ane. The flurry of activity directed toward new topologically complex molecules will not only provide aesthetically attractive structure, but may exploit their functional potential in applications such as nanotechnology. The area of interlocked molecules is broad and therefore in the light of part of the research carried out in this thesis, focus will be lay down on recent advances in research into [2]-catenane and in particular, [2]-rotaxane molecular architectures which has been chosen to integrate the replication processes.

The first reported attempt in catenane synthesis were discussed\(^ {89}\) by Lüttringhaus \textit{et al.} in 1958, whereas rotaxanes were put forward\(^ {79}\) in 1961 and shown to exist\(^ {90}\) in 1967. The introduction of template synthesis of catenanes and rotaxanes by Sauvage \textit{et al.} in 1984 and by Stoddart \textit{et al.} in 1989 opened up a new era. According to the IUPAC compendium of chemical terminology, rotaxane was defined as molecules in which a ring encloses another rod-like molecule having end groups too large to pass through the ring opening, which holds the rod-like molecule in position without covalent bonding. Generally speaking, a rotaxane is a chemical substance comprising one or more macrocyclic species threaded onto one or more linear species.

2.1 Nomenclature for mechanically interlocked system

In 1971, Schill was the first to introduce a nomenclature\(^ {82}\) system for rotaxane, which latterly offer a good basis to develop a more comprehensive nomenclature system.
The nomenclature employed with interlocked molecules involves placing in square brackets the total number of components before the name of the compounds or complexes. Thus, a [3]-catenane is comprised of three interlocked macrocycles, and a [3]-rotaxane is comprised of one dumbbell component and two macrocycles. The nomenclature for polymeric systems is described in Figure 2.2.

![Figure 2.2](image)

**Figure 2.2.** Cartoon representation of higher order rotaxane and catenane species. For examples: (a) [3]-rotaxane; (b) [3]-catenane; (c) [8]-rotaxane; and (d) [9]-catenane.

The extended version of nomenclature system proposed by Vögtle and co-workers included the information about mechanical or covalent linkages between the components of rotaxane. As the authors themselves imply, creation of a complete systematic nomenclature system for these substances will be a formidable task.

### 2.2 The chemistry of the mechanical bond

This type of chemical bond found exclusively in mechanically interlocked molecules architectures in which two or more molecular components become mechanically interlocked, one with another, and so on, in some manner or another such as catenanes and rotaxanes. Each component are not connected by conventional covalent bond but intrinsically linked to each other, resulting in mechanical bond, which prevents dissociation without cleavage of one or more covalent bonds. The first reported synthesis, by Wasserman in 1960, of a catenane in a yield of 1% by statistical threading introduced mechanical bonds into chemical compounds.

Some years later, the Harrison and Harrison described the synthesis of a polymer-bound rotaxane in a 6% yield after numerous treatment of column chromatography. Nevertheless, the absence of any appearance of templation was evidenced in the very low yield of these first catenanes and rotaxanes. Mechanical
bonds present\textsuperscript{93} chemists with both the worlds of the molecular and supramolecular, since they contain all the intricacies and subtleties associated with coordinative and covalent bonding, rendering the molecules ideal for the control of function at single molecule to the macroscopic levels.

Therefore, the presence of geometrically precise coordination and non-covalent bonds are key\textsuperscript{94,95} to the introduction of mechanical bonds via template effects into molecules with any kind of proficiency and efficiency. In addition, the fact that these weaker bonds live on inside these mechanically interlocked molecules after their template-directed synthesis\textsuperscript{96} give the molecules much added value when it comes to exploiting their physical and chemical properties. Therefore, the fundamental understanding of the nature of the mechanical bond is paramount in the challenge to make more sophisticated compounds and can be employed to do complex systems chemistry in very different environments where emergent phenomena\textsuperscript{97} become the order of the day.

2.3 Pseudorotaxanes
A common retrosynthetic disconnection shared by generic [2]-rotaxane and [2]-catenane structures revolves around [2]-pseudorotaxane precursor\textsuperscript{98}, in which a linear molecule is threaded through a macrocyclic unit. Thus, they are similar to rotaxane structure with the exception that the linear molecule not terminated by the two bulky stoppers. Further modification of the this precursor can possibly proceed in two ways, by virtue of (a) stoppering the end linear component with sufficiently large groups, results in the formation of [2]-rotaxane or (b) macrocyclisation of the linear component to affords to [2]-catenane (Figure 2.3).
It should be noted that a considerable number of [2]-pseudorotaxane\(^99,^{100}\) have been investigated. The successful directed self-assembly of the [2]-pseudorotaxane utilized cyclobis (paraquat-\(p\)-phenylene) tetracation 44 and linear polyether 45, incorporating two tetraethyleneglycol units alternating between three hydroquinone derivatives was carried out and isolated\(^{101}\) (Scheme 2.1). The structure of this 1:1 product displays a large degree of self-ordering in the solid state, largely based around \(\pi\)-\(\pi\) stacking.

Scheme 2.1. Self-assembly of the [2]-pseudorotaxanes utilized tetracationic cyclophane 44 and linear polyether 45.

Similar ordering process also has been established by the further synthesis of higher pseudorotaxanes such as the [3]-pseudorotaxane\(^{102}\) with the formation of a
tightly-packed, π−π structure. Alternatively, an anion-directed pseudorotaxanes has been reported by Beer and co-workers\textsuperscript{103}. The zwitterion \textsuperscript{46} comprised Crabtree-type pyridinium cleft binding a chloride\textsuperscript{104,105} with macrocycle \textsuperscript{47} results in the formation of a pseudorotaxane \textsuperscript{[46•47]} in acetone-\textit{d}_6 with an association constant, \(K_a\) of 2400 M\textsuperscript{-1} (Scheme 2.2).

\begin{center}
\textbf{Scheme 2.2.} An anion-directed [2]-pseudorotaxane \textsuperscript{[46•47]} consists of a Crabtree-type pyridinium cleft \textsuperscript{46} binding a chloride with macrocycle \textsuperscript{47} reported by Beer and co–workers.
\end{center}

The pseudorotaxane is stabilized by chloride–NH hydrogen bonds, π–π stacking interaction and C-H···O hydrogen bonds. A vast consideration has been given to the potential use of pseudorotaxanes in molecular devices\textsuperscript{106} such as switches by means of external stimulus such as light. We will look into more detail on the switching experiment later in this chapter.

2.4 How to synthesise a [2]-rotaxane?

Theoretically, [2]-rotaxane can be constructed by three\textsuperscript{107} different approaches. The first clipping strategy\textsuperscript{108-110} (Figure 2.4a) initiated when an acyclic precursor is cyclised around a linear dumbbell-shaped template. This mode requires a recognition site within the linear component acting as a template for the formation of the macrocyclic component. The macrocycle is assembled in the correct formation to the binding site by means of non-covalent interactions.

Threading and stoppering\textsuperscript{111-115} (as previously depicted in Figure 2.3a), involves a two steps strategy. Initially, a [2]-pseudorotaxane complex is formed between the
linear component and the macrocyclic component, whereas a sufficient interaction between both hydrogen bond donors and acceptors takes place. Subsequently, a bulky stopper will be inserted at both ends of the linear component by covalent bond formation preventing the dissociation of the macrocyclic ring (Figure 2.4b).

The stoppering groups are usually larger in size than the inner diameter of the ring to assure it did not slip off from the linear component. An alternative approach\textsuperscript{116,117} to construct rotaxanes, called slippage (Figure 2.4c). In this procedure, the macrocycle and the dumbbell were prepared separately prior to self-assembly before heated together in an appropriate solvent permitting the macrocycle slips over the dumbbell stopper by overcoming the associated free energy of activation. The resulting [2]-rotaxane is stabilised by non-covalent bonding interactions. The solution is then cooled down to ambient temperature, whereby the rotaxane comes into existence as the free energy barrier to its dissociation back into its components becomes insurmountable. It was reasoned\textsuperscript{118} that careful size adjustment of the stoppers could permit the synthesis of the corresponding rotaxane directly at elevated temperature.

![Figure 2.4](image)

**Figure 2.4.** Three possible approaches in the formation of [2]-rotaxane: (a) clipping, (b) threading and stoppering and (c) slippage.
2.4.1 Clipping mechanism

The first [2]-rotaxane prepared to act as a molecular shuttle (Scheme 2.3) involved the clipping route\(^{119}\) starting with the preformed triisopropyl silylated 48 polyether together with tetracation cyclophane 44 (from tetracationic precursor 44a and \(p\)-dibromoxylene 44b) as illustrated in Scheme 2.3. The dynamic \(^1\)H NMR 500 MHz behaviour of the resulting [2]-rotaxane [44•48] and [44•48]' indicated that the cyclophane bead shuttles to and fro between the two hydroquinone derivative sites approximately 500 times a second at room temperature.

![Scheme 2.3](image)

Scheme 2.3. A [2]-rotaxane prepared by the clipping mechanism, [44•48] and [44•48]’ act as molecular shuttles. The tetracationic cyclophane 44 exchanges between two hydroquinone derivative sites approximately 500 times a second at room temperature.

2.4.2 Threading and stoppering mechanism

An example\(^{120}\) which illustrates this methodology is the synthesis of [2]-rotaxane illustrated in Scheme 2.4 which exploited the supramolecular assistance provided by
hydrogen bond interactions between secondary dialkylammonium centers and the macrocyclic polyether dibenzo-24-crown-8 (DB24C8). As a result of these hydrogen bond interactions, the functionalised dialkylammonium cation 49\(^+\) infiltrates the DB24C8 cavity to give the pseudorotaxane [DB24C8·49]\(^+\), which subsequently stoppered when its azido termini react with a bulky acetylenedicarboxylate by means of 1,3-dipolar cycloaddition to afford [2]-rotaxane 50\(^+\). The first amide-based rotaxanes were also have been described\(^{121}\) employing the similar strategies.

![Diagrams of DB24C8, 49\(^+\), [DB24C8·49]\(^+\), and 50\(^+\)]

**Scheme 2.4.** Formation of [2]-rotaxane 50\(^+\) via threading and stoppering mechanism, which incorporates both DB24C8 and secondary dialkylammonium cation 49\(^+\).

### 2.4.3 Slippage mechanism

The slippage procedure lends itself to the preparation of [\(\eta\)]-rotaxanes\(^{122}\) that are based on longer linear fragments incorporating more than one dicationic binding site and fitted with stoppers of appropriate size. A kinetic investigation revealed\(^{123,124}\) that the free energies of activation associated with the slipping on and slipping off processes are correlated with the size of cavity of the macrocyclic component, as well as with the size of the stoppers attached to the dumbbell shaped component. The free energy of activation was observed to increase upon reducing the size of the cavity of the macrocycle and/or enlarging the bulk of the stoppers\(^{125}\).
The successful synthesis of the [3]-rotaxane 51 has been achieved by this means\textsuperscript{126} and provides a useful technique for the preparation of oligo- and poly-rotaxanes including ones in which many recognition sites along the linear component are encircled by different macrocyclic components (shown in Scheme 2.5).

![Scheme 2.5](image)

**Scheme 2.5.** Synthesis of [3]-rotaxane 51 by slippage mechanism based on longer linear fragments incorporating two dicationic binding site and fitted with stoppers of appropriate size (denoted by black circle).

In particular, the slippage approach leads to rotaxane that only stable under certain conditions as a consequence of elevated temperature imposed during the process. As a matter of fact, an increase in temperature, solvent polarity or acidity can substantially affect the stability\textsuperscript{118} of rotaxanes.

### 2.5 Template synthesis of interlocked molecules

The term template\textsuperscript{96} effect was first defined\textsuperscript{127} and used in the beginning of late 1960s. A chemical template organises\textsuperscript{128} an assembly of atoms with respect to one or more geometric point, in order to achieve a particular linking of atoms. The template provides instructions for the formation of a single product from a substrate, which otherwise have the potential to assemble and react in a variety of ways. By changing the template resulted in a different substrate assembly and consequently a different product. After the template has directed the formation of the product, it is removed to yield the template-free product. The emergence of supramolecular chemistry has allowed the exploitation of metal-ligand binding, hydrogen bonding and π–π interaction in the synthesis of larger molecules with a remarkable degree of
control. For instance, mechanically interlocked molecules can be synthesised in high yield using template-directed assistance to covalent synthesis.

Schill et al. have introduced\textsuperscript{129} the first directed synthesis of catenanes, rotaxanes and knots with the use of covalent template. The multiple-step synthesis were employed in the preparation of [2]-catenane\textsuperscript{130}, [2]-rotaxane\textsuperscript{131} as well as of molecular knots\textsuperscript{132}. The covalent-directed synthesis also capable of delivering characterized mechanically interlocked molecular compounds although in small yields as a consequence of the many steps required in the synthesis. Furthermore, this protocol also give rise to the use of metal templates and subsequently, noncovalent templates for the efficient and practical synthesis of molecular catenanes, rotaxanes and knots in one or two steps in good yields from available precursors.

The seminal contribution\textsuperscript{133} made by Sauvage in 1983 of the use of copper(I) cations as a template to form mechanically interlocked macrocyclic phenanthroline derivatives provided a facile and efficient means of making metallo-catenanes. A range of different templates has been utilized such as transition metals\textsuperscript{134-137}, π-donor or π-acceptors\textsuperscript{138-140} and hydrogen bonding motifs\textsuperscript{141-147}. The following section highlighted the formation of catenanes and rotaxanes under kinetic regimes.

### 2.5.1 Transition metal templates

Seeing the first reported metal-templated protocols\textsuperscript{133}, the efficient synthesis of catenanes and rotaxanes has now been well demonstrated\textsuperscript{148,149} by employing a wide range of transition metals as synthetic templates. Sauvage and co-authors reported\textsuperscript{150} a Cu(I)-templated [2]-rotaxane 55•BF\textsubscript{4} with the aid of threading and stoppering approach (Scheme 2.6). In this case, a 2,2'-bipyridine derivative 52 is treated with a phenanthroline terpyridine containing macrocycle 53 in the presence of Cu(I) ions, resulting in the formation of a tetrahedral coordination complex.

The subsequent alkylation of the complex with the stoppering reagent 54 affords the desired Cu(I) templated rotaxane 55•BF\textsubscript{4} in 20\% yield for the final two steps. It was pointed out that 55•BF\textsubscript{4} capable to undergo controllable rotary motion (pirouetting) of the macrocycle around the dumbbell axis following the oxidation and reduction of the
Cu metal center. Cu(I) ion opts for four coordinate ligand set in a tetrahedral geometry with the 2,2'-bipyridine and phenanthroline ligands while Cu(II) ion favors a five coordinate geometry with 2,2'-bipyridine and terpyridine ligands (as illustrated in Scheme 2.6).

![Scheme 2.6](image)

Scheme 2.6. Synthesis of the metal-templated [2]-rotaxane 55•BF$_4$ by means of threading and stoppering approach.

### 2.5.2 π–donor or π–acceptor templation

Stoddart et al. initiated the templating effects of charged π–donor or π–acceptor$^{151}$ non-covalent bonding interaction in the synthesis of a [2]-catenane. The preparation of catenanes and rotaxanes$^{94,152}$ has benefited from charged π–donor or π–acceptor templates$^{153}$ on account of their high self-assembling efficiency. By far, the most effective π–donors for the preparation of charged catenanes and rotaxanes are those$^{154}$ based on tetrathiafulvalene (TTF) 56 and mutual recognition by the π–electron deficient tetracationic cyclophane 44, (CBPQT$^{44+}$).

The binding affinities of 1:1 pseudorotaxane complex was determined to be approximately 10 000 M$^{-1}$ in CH$_3$CN at 298 K, which is driven by π–π stacking$^{155}$ and charge transfer interactions$^{156}$. The mutual recognition was incorporated$^{157}$ in a bistable, electrochemically switchable, [2]-rotaxane 57•4PF$_6$ in 23% yield by
employing the template directed clipping process (Scheme 2.7) with the dicationic salt 44a•2PF₆ and dibenzylbromide 44b around the dumbbell component, consist of two alternative donors site, monopyrrolo tetrathiafulvalene (MPTTF) and 1,5-dioxynaphthalene (DNP). Interestingly, when the clipping reaction is performed under high pressure (for example, 10 kbar), the yield of [2]-rotaxanes increased dramatically to 50%. Contemporaneously, the Sanders group¹⁵⁸ constructed the [2]-catenanes structure with high efficiencies by employing the neutral π–stacking motifs.

Scheme 2.7. Template directed synthesis of the charged π–donor or π–acceptor [2]-rotaxanes 57•4PF₆ by virtue of clipping strategies around MPTTF units.

2.5.3 Hydrogen bond templation

Hunter¹⁵⁹ and Vögtle¹⁶⁰ independently reported the synthesis of [2]-catenanes in a satisfactory yield using amide forming reactions back in 1992. The intermediates¹⁶¹ are held in place by strong [C=O•••H-N] hydrogen bond. Next, Leigh published¹⁶²-¹⁶⁸ a various amide-based [2]-rotaxanes. One example¹⁶² is that [2]-rotaxane 61 can be synthesised in a 59% yield from succinamide and naphthalimide-containing compound 58 with a standard one pot formation of the macrocyclic lactam ring from both compound 59 and 60. The [2]-rotaxane 61 displays bistable switching behaviours in which the macrocyclic lactam ring moves from the succinamide station to the naphthalimide station (Scheme 2.8).
Template directed synthesis of the amide-based \([2]\)-rotaxane 61 from succinamide and naphthalimide-containing thread 58 with one-pot formation of the macrocyclic ring from the acyclic starting materials 59 and 60.

Furthermore, a strong \([\text{N}^+\cdot\cdot\cdot\text{H}\cdots\text{O}]\) hydrogen bonding, resulting from the interaction between the published crown ethers, such as 18-crown-6 and 24-crown-8 with primary alkyl and secondary dialkylammonium ions can also be exploited in the hydrogen-bonding motif templates. Stoddart laboratory has published\(^{169}\) that ammonium-containing \([2]\)-rotaxanes 63-\(\cdot\text{H}\cdot\text{2PF}_6\) and 65-\(\cdot\text{H}\cdot\text{PF}_6\) can be prepared by templation between DB24C8 and dialkylammonium salts 62-\(\cdot\text{H}\cdot\text{PF}_6\).

The synthesis involves\(^{170,171}\) threading and stoppering approach, whereas the stopper-end dialkylammonium salt 62-\(\cdot\text{H}\cdot\text{PF}_6\) is threaded into DB24C8, followed by stoppering with triphenylphosphine, \(\text{PPh}_3\) to afford \([2]\)-rotaxane 63-\(\cdot\text{H}\cdot\text{2PF}_6\) in 80% yield. The subsequent Wittig reaction with aldehyde 64 modified the triphenylphosphonium stoppers to an aryl stoppers. Such reactions, followed by catalytic hydrogenation provide new \([2]\)-rotaxane 65-\(\cdot\text{H}\cdot\text{PF}_6\) in about 50% yield (outlined in Scheme 2.9).

Alternatively, it was pointed out that the use of phenoxy anion templates effectively directs the synthesis of [2]-rotaxanes in excellent yield. Vögtle has demonstrated that the macrocyclic lactam 66 is a good acceptor for a $p$-triphenoxy anion as a result of forming [N-H•••O]$^-$ hydrogen bonds. As shown in Scheme 2.10, an intermediate complex [66•67]$^-$ is subsequently reacted with a trityl end-stoppered alkylbromide 68, affording an amide-containing [2]-rotaxane 69 in 95% yield.

In conclusion, mechanically interlocked molecules, such as catenanes and rotaxanes can be efficiently prepared by utilizing organic or inorganic templates under kinetic control. The efficiency of these template directed strategies immensely depend upon the supramolecular approach that combine the cooperative non-covalent bonding interactions such as metal-ligand interactions, π–π stacking and hydrogen bonding motif to form intermediates, result in the irreversible formation of mechanically interlocked molecules by the formation of a final covalent bond under kinetic control. However, as a result of the irreversible nature of this approach, unbound dumbbell components and unbound macrocycles formed during the final step invariably reduce the yield of the mechanically interlocked compounds.
Scheme 2.10. Anion-templated synthesis of the [2]-rotaxane 69 using threading and stoppering approach.

2.6 Applications in devices

Research on supramolecular chemistry has shown that molecules are convenient nanometre scale building blocks in a bottom-up approach, to construct ultra miniaturised devices and machines capable of storing information at the molecular level. Performing macroscopic mechanical tasks with the early generations of synthetic molecular machines has proved to be quite elusive. Molecules with mechanically interlocked architectures are particularly suited for these types of applications because they permit the controlled, large amplitude movement and positioning of one mechanically interlocked component with respect to another. Through the pioneering work of Stoddart, Sauvage and Vögtle, it has become clear that rotaxanes have the ability to change the relative positions of their interlocked components by means of external stimulus such as chemical, electrochemical or photochemical.
A bistable light driven switch\(^{162}\) in the form of a [2]-rotaxane 61 has been designed so that the hydrogen-bond accepting ability of a photoactive naphthalimide is turned on by a photochemical reaction with a secondary electron donor (Scheme 2.11). The benzylic amide macrocycle is located preferentially at the succinamide site in the electronic state in CH\(_3\)CN at 298 K. Electrochemical reduction drives the translocation of the macrocycle to encircle the newly reduced naphthalimide unit. The reduced form of the rotaxane can be now regarded as a possible candidate for photo-induced electron transfer. In this case, the photo-excited (355 nm) naphthalimide unit of 70 accepts an electron from the triplet transfer agent 1,4-diazabicyclo[2.2.2]octane (D), generating the reduced state and turn on the contemporary site for the macrocycle.

Thus, the photo-induced translational motion is achieved. The linear motions of the moving components typically were distributed randomly in the solution state.
Additional organization and integration onto surfaces either as self-assembled monolayers or Langmuir-Blodgett films are presently being addressed to express the motions coherently in a mechanical context. By taking advantage of the intramolecular disulfide bonding motif, [2]-rotaxane 71\textsuperscript{4+} was prepared. The rotaxane structure displays\textsuperscript{192,193} a redox controlled motion of the tetracationic cyclophane 44 from the tetrathiafulvalene (TTF) unit to the DNP ring system (Scheme 2.12).

Over the past few years, the progress toward devices for molecular electronics\textsuperscript{194} based computing systems has become increasingly important to overcome the fundamental limitations of top-down lithographic techniques. Molecular switches\textsuperscript{195-197} based on mechanically interlocked and movable components have been fabricated into nanowire crossbar arrays, producing working, defect tolerant memory devices of revolutionary densities. The amphiphilic bistable [2]-rotaxane 72\textsuperscript{198} has also been utilized in molecular electronic devices, which consist of similar cyclophane CBPQT\textsuperscript{4+}, encircling a linear component containing π–electron rich MPTTF and DNP units and terminated with the hydrophilic dendron stopper (Figure 2.5).
Scheme 2.12. The interlocked macrocycle of a surface-bound bistable [2]-rotaxane 71 displays electrochemically driven linear motions between two recognition sites – tetrathiafulvalene (TTF) and 1,5-dioxynaphthalene (DNP). The binding of the macrocycle to the weaker donor, DNP in the metastable state is sufficiently strong to prevent the reformation of the ground state, following electrochemical reset.
Figure 2.5. The amphiphilic bistable [2]-rotaxane 72 has been integrated into a crossbar device between two electrodes as Langmuir-Blodgett monolayers. The ON and OFF states of the device are accessed at +2 V and −2 V, respectively, and are assigned to two different translational isomers, the metastable and the ground states, respectively.
The molecular device depicted in Figure 2.5 displays binary switching between high and low conductance states. The ON state is accessed at +2 V, which is equivalent to net oxidizing conditions, whereas the OFF state is triggered at −2 V, or net reducing conditions. The OFF state corresponds to the translational isomer, with the tetracationic cyclophane 44 encircling the MPTTF unit. In the device, application of a +2 V bias generates an oxidize form of the MPTTF unit in the rotaxane. The resulting charge-charge repulsive force drives a linear movement of the cyclophane along the dumbbell component to a position encircling the DNP ring system. This new metastable translational isomer is responsible for the high-conductance ON state. Alternatively, applying a reverse bias of −2 V causes the electrochemical reduction of the tetracationic cyclophane 44, allowing the facile reformation to the most stable translational isomer.

Additionally, an effort in the Heath group have been focusing on the fabrication and testing of a 160-kbit memory\textsuperscript{199} at $10^{11}$ bits cm\textsuperscript{-2}, a density that correlates to the 2020 node of the International Technology Roadmap for Semiconductors published in 2005. This challenge have been met, consequently prove that a simple demonstration of a molecular electronics involves logic and memory talking to each other and to the outside world are thus possible.
3

Integrating self-replication process within rotaxane formation

3.1 Introduction
With the previous literature review in hand, we are now in position to experiment using our own expertise in the design and evaluation of highly efficient self-replicating system in the mechanically-interlocked molecules architectures. The research described in this thesis is a part of an extension of the preceding investigation on integrating self-replication process within the [2]-rotaxane structure. In the course of these investigations, the first minimal model of self-replicating rotaxanes has been outlined.

In that respect, the succeeding work should be built on the improvement of the previous designed replication model (Replication model 1) and work through the alternative options, namely Replication model 2 and Replication model 3. Additionally, the basic features of the self-replicators are discussed and the current knowledge in the formation of self-replicating rotaxanes is evaluated. Threading and stoppering strategy has been selected for rotaxane formation given that it is the only one that can be combined with the synthesis of a linear template through replication.

3.2 Essential requirements
Principally, in designing a self-replicating rotaxanes, the prerequisite design criterion consists of a recognition site, a reactive site and a binding site (shown in Figure 3.1). In the following section, these features are explained on each representation minimal model for self-replicating rotaxanes.
3.2.1 Recognition site

The preference for the choice of the recognition event between carboxylic acid and amidopyridine in the two building blocks of A and B (as depicted in Figure 1.1) has been selected considerably in our laboratory based on the work\textsuperscript{201-203} of Hamilton research group and utilized in both minimal and reciprocal self-replicating systems. The recognition sites between the two building blocks A and B will enable the association of the catalytically active ternary complex [A•B•T]. Despite that, a considerably weak recognition must be ensured to allow the dissociation of the product duplex [T•T] and hence, remove the product inhibition.

![Diagram of recognition site, reactive site, and binding site](image)

**Figure 3.1.** Three design criteria in designing a self-replicating rotaxane based on Replication model 1, namely the recognition site (allow the building blocks to associate to the template molecule), the reactive site (connect the two parts of the thread by forming a covalent bond) and the binding site (essential features of self-replicating rotaxanes). The green cartoons represent the recognition site, the blue cartoons represent the reactive site and the red cartoons represent the binding site.

The association takes place via hydrogen bonding of the carboxylic acid hydrogen 74 to the pyridine nitrogen 73 and between the amide proton 73 and the carbonyl group of the acid 74 (Scheme 3.1). The established design\textsuperscript{73} includes the 6-methyl substituent to prevent self-association between the amidopyridine moieties by bringing in the steric hindrance. Recently, in the development of self-replicating
rotaxanes, 4,6-dimethyl moiety is utilised for a notable steric barrier, in which case acted as blocking group as well as to regulate the strength of the recognition motif.

![Diagram](image)

**Scheme 3.1.** The recognition event between amidopyridine 73 and carboxylic acid 74 assisted via hydrogen bonding and the association constant, $K_a$, was determined in CDCl$_3$ at 273 K as 1000 M$^{-1}$.

An exhaustive investigation of the structural variations appended on the amidopyridine unit is described and evaluated in Chapter 7 of this thesis. Additionally, the utilization of polar solvent (which acts as good hydrogen bond acceptor and/or donor) consequently leads to strong solvation of the recognition sites and hence, disrupted the crucial binding processes. Thus, a solvent that is incapable of participating in hydrogen bonding must be chosen, such as chloroform.

### 3.2.2 Reactive site

The second design criteria in the self-replicating rotaxane framework is the reactive site, which is useful to attach the two building blocks A and B to give template T through the formation of covalent bond. This stage is equivalent to the stoppering reaction in the formation of [2]-rotaxanes and hence, an efficient and irreversible reaction is compulsory. During the course of prior investigations, the 1,3-dipolar cycloaddition between a maleimide and nitrone has been successfully incorporated in the self-replicating rotaxanes scheme. The preferential formation of the 5-substituted isoxazolidine structure proceeds through a concerted mechanism, in which the bonds between the dipolarophile (in this case, maleimide) and the dipole (in this case, nitrone) are created simultaneously.

As well as being regioselective, 1,3-dipolar cycloadditions can be highly stereoselective. The stereochemistry of nitrone cycloadditions has been the subject of several studies. Reaction between the asymmetric nitrone 75 and
maleimide derivatives 76 proceeds through a bimolecular pathway in the absence of any recognition events to give the diastereoisomeric products, namely the trans and cis isomer with respect to the endo and exo transition states (see Scheme 3.2). In the case of cis isomer, all protons are positioned on the same side with regards to the isoxazolidine ring while in the trans isomer, the nitrone-derived hydrogen atom is rendered trans to the two hydrogen atoms originally located on the maleimide group. Frequently, mixture of diastereoisomers are obtained with a trans:cis ratio between 1.4:1 and 3.9:1 depending on the electronic nature of the substituents appended on the two reagents. In other words, the outcome depends on the interplay of two generally opposing forces in the transition state – attractive π-orbital overlap of unsaturated substituents favouring an endo transition state (Scheme 3.2a), and repulsive van der Waals steric interactions favouring an exo transition state (Scheme 3.2b).

Scheme 3.2. 1,3-dipolar cycloaddition between the nitrone 75 and maleimide 76. (a) The endo approach leads to the trans 77 (b) The exo approach leads to the cis 77.

In order to connect the reactive site with the recognition site, careful consideration in selecting the spacer is required to control the geometry of the substrates. The suitable spacer can be altered by means of the length and angle. The spacer length could induce notable flexibility in the system and consequently render the recognition site and reactive site at a correct distance to eliminate excessively stable duplex [R·R] and to avoid the binary complex pathway. Furthermore, the angle could be modified with different substitution patterns on the benzene ring. The angle at which
the reactive site and the recognition motif are relatively to each other will eventually determine whether the reaction will follow the \([A\cdot B]\) complex or the autocatalytic pathways.

### 3.2.3 Binding site

The final prerequisite element embedded into the building block \(B\) is the binding site between the ring and the linear component (see Figure 3.1), which is the key feature in self-replicating rotaxanes. The importance of the association of the ring component \textit{via} hydrogen bond to the conserved binding site can be envisaged in\(^{207}\) the simple kinetic model of rotaxane formation (Figure 3.2). There are two parallel kinetic pathways operating either side of equilibrium. Upon addition of a guest in the \(L\) component and macrocycle \(M\) with the complementary binding motifs, the equilibrium is established. The macrocycle \(M\) is constantly being displaced from the binding site in the linear component, which resulted in two species, namely the [2]-pseudorotaxane complex \([L\cdot M]\) and guest \(L\) distinguishable \textit{in situ}. The subsequent stoppering reaction with the stopper reagent \(S\) affords the important rotaxane \(R\) alongside with the undesired thread \(T\).

![Figure 3.2.](image)

\textbf{Figure 3.2.} The simple kinetic model for rotaxane formation. The stopper \(S\) can react either with the unbound linear component \(L\) \((k_1)\) or the [2]-pseudorotaxane complex \([L\cdot M]\) \((k_2)\). The stability of the [2]-pseudorotaxane complex is defined by \(K_a\). Ratio of rotaxane \(R\) to thread \(T\) can be obtain by assuming \(k_1 = k_2\). At increased temperature, the \(K_a\) value decrease, therefore [2]-pseudorotaxane formation is disfavoured. At lower temperature, [2]-pseudorotaxane formation is favoured as the \(K_a\) value increase leading to higher yields of rotaxane \(R\). Figure taken from reference 207.
The rate constants for thread $T$ and rotaxane $R$ formation ($k_1$ and $k_2$) are assumed to be equivalent and, hence, the amount of rotaxane $R$ formed is dependent upon the amount of [2]-pseudorotaxane $[L\cdot M]$ present, which in turn related to the association constant, $K_a$. On that account, a high association constant for the $[L\cdot M]$ complex is mandatory. The association constant relates to the affinity of the macrocycle to associate the binding site of the guest and can be varied by manipulating the temperature, based on the free energy Gibbs, $\Delta G = -RT \ln K_a$.

In the case of low temperature, substantial amount of [2]-pseudorotaxane $[L\cdot M]$ are obtained as results of increase value of $K_a$ and, hence, force the equilibrium to the left-hand side. The stoppering reaction usually involves elevated temperature, thus lowering the $K_a$ value and consequently lowering the yield of pseudorotaxane. Raising the temperature will diminished the $K_a$ value for the complexes by increasing the magnitude of the $T\Delta S$ term in the free energy of binding. On the whole, this simple kinetic model heavily relies on a high association constant of $[L\cdot M]$ complex to ensure that a significant amount of rotaxane is synthesised.

### 3.3 Association constant, $K_a$

The thermodynamic stability of a complex between host and guest in a given solvent at a given temperature is quantified by measurement of the association constant, $K_a$. The binding constant is calculated using concentrations and thus has units of M$^{-1}$ for a given 1:1 complex. For the binding equilibrium for the reaction shown in Equation 3.1 (e.g. between a guest, G and host, H), the association constant is given by:

$$ H + G \rightleftharpoons [H \cdot G] $$

$$ K_a = \frac{[H \cdot G]}{[H][G]} $$

Equation 3.1

On that account, a large association constant corresponds to a high equilibrium concentration of bound guest, and hence a more stable or tighter the preorganisation of the $[H\cdot G]$ complex. Moreover, the association constants is also related to the free energy of the association process according to the Gibbs equation: $\Delta G^* = -RT \ln K$ ($R =$ gas constant, 8.314 J K$^{-1}$ mol$^{-1}$, $T =$ temperature in Kelvin). Thus, the general affinity of a host for a guest under specific conditions (solvent, temperature and etc.)
may be given either in terms of $K$ or $-\Delta G^\circ$ values as we have seen earlier in the simple kinetic model of rotaxane formation.

### 3.3.1 High association constant is crucial

Strong association is crucial to attain substantial amount of $[\text{L}\cdot\text{M}]$ complex. In the previous simple kinetic model in Figure 3.2, we assumed both linear component $\text{L}$ and [2]-pseudorotaxane $[\text{L}\cdot\text{M}]$ hold a similar reactivity, and hence, the ratio of rotaxane $\text{R}$ to thread $\text{T}$ component in the reaction mixture are evaluated across a series of association constant values for the $[\text{L}\cdot\text{M}]$ complex through kinetic simulation. Assuming the rate of $k_1$ and $k_2$ to be $1.67 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ and the concentrations of starting reagents $\text{L}$, $\text{S}$ and $\text{M}$ at concentration of 20 mM, it is clearly evident that the [rotaxane]/[thread] composition in the final mixture varies with respect to the $K_a$ values (Figure 3.3).

![Figure 3.3](image)

**Figure 3.3.** The kinetic simulation depicts the selectivity of rotaxane $\text{R}$ to thread $\text{T}$ in the reaction mixture as a function of $K_a$ by assuming the $k_1 = k_2 = 1.67 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$. Ratio of rotaxane to thread of 10:1 gives a $K_a$ value of $[\text{L}\cdot\text{M}]$ complex higher than 5000 M$^{-1}$. Figure taken from reference 207.

The kinetic simulation reveals a selectivity of rotaxane to thread of 10:1, which corresponds to a $K_a$ value of greater than 5000 M$^{-1}$ for the $[\text{L}\cdot\text{M}]$ complex. The association constant for the [2]-pseudorotaxane complex required to be as high as possible since the formation of this complex will suppress the kinetic pathways involving free compound $\text{L}$, which eventually lead to thread $\text{T}$ formation. As a
consequence of decrease free L species, thus concentrates the kinetic flux in the autocatalytic cycle involving the rotaxane R. Undoubtedly, [2]-pseudorotaxane [L•M] unit is the main concern for a success within a self-replicating rotaxane framework.

3.4 Minimal replication model of self-replicating rotaxanes

One of the main objectives of the research described in this thesis is the exploration of replicating system within the rotaxane architecture. Prior studies acknowledged the essence of the three stages required in the formation of self-replicating rotaxanes by virtue of threading and stoppering strategy, commencing from the association of the macrocycle to the guest binding site, the stoppering reaction yielding the simple rotaxane and ultimately the recognition event, which enable the rotaxane to self-replicate by autocatalytic means. In order to accomplish this aim, three different minimal replication models has been proposed and described. On that account, each minimal model employed is presented in the following section to comprehend the objective of each designed scheme. In each case, the advantages and disadvantages of each replication model are further emphasised.

3.4.1 Replication model 1

The primary model contemplated for the formation of self-replicating rotaxanes linked four mutual catalytic cycles. Both autocatalytic and crosscatalytic cycles of thread and rotaxane synthesis equally emerged as a consequence of the reversible reaction between the L component and the macrocyclic M unit (represent as central solid box in Figure 3.4). The probability that the thread and rotaxane to act as a replicator in autocatalytic and crosscatalytic manner has been kinetically investigated which suggests\(^ {200}\) that the thread and rotaxane are not mutually complementary templates. These evidence shows that rotaxane and thread are unable to catalyse the formation of each other. On that account, there are only two pathways accessible in the Replication model 1, which related to the innermost binding event, the reversible formation of the [2]-pseudorotaxane [L•M] complex (see Figure 3.5). In the case of rotaxane synthesis, the [L•M] complex react with the stoppering reagent S to afford rotaxane R. Subsequently, rotaxane R enable to enter the autocatalytic cycle by collecting the [L•M] complex and the stoppering reagent S and hence, catalyse the formation of a second molecule of rotaxane, R.
Figure 3.4. **Replication model 1 (Extended version)** – based on the threading and stoppering strategy with the placement of one recognition site (green cartoons) on both stoppers of the rotaxane. The catalytic cycles operate emanating from the reversible formation of the [L-M] pseudorotaxane complex. On the top, the synthesis of rotaxane is accomplished either through autocatalysis (right) by rotaxane R or crosscatalysis (left) by thread T. On the bottom, the synthesis of thread is achieved either through autocatalysis (right) by thread T or crosscatalysis (left) by rotaxane R. Reactions through binary complexes are not shown here for clarity. The blue cartoons represent the reactive sites, the green cartoons represent the recognition sites and the red cartoons represent the binding site.
Figure 3.5. Replication model 1 (Simplified version) - consists of two autocatalytic cycles emanating from the central binding event (boxed), the reversible formation of the [L•M] complex. (Right – Rotaxane synthesis) The association between macrocycle M with the linear component L afforded the [L•M] species. Reaction between [L•M] complex and stoppering reagent S gives the rotaxane R. [L•M] complex and S can bind reversibly to the rotaxane R to form a catalytically quaternary complex [S-L•M•R]. Bond formation occurs between S and [L•M] to give duplex [R•R] which then withdraw two molecules of R to start a new replicating cycle. (Left – Thread synthesis). Unbound linear component L react with the stoppering reagent S to form thread T. Thread T can then enter the autocatalytic cycle similarly by collecting the unbound species of linear component L and stoppering reagent S and, hence, catalyse the formation of a second molecule of thread T. In parallel, unbound linear component L also react with the stoppering reagent S to form thread T. Following this, thread T can then enter the autocatalytic cycle similarly by collecting the unbound species of linear component L and stoppering reagent S and, hence, catalyse the formation of a second molecule of thread T. It is now demonstrated that the rotaxane R and the thread T capable of replicating. Regardless that, significant quantity of thread T was present as a result of poor association between the macrocyclic unit M and the guest binding site. These outcomes substantiate the fact that the binding strength must exceed a certain threshold value in order to allow efficient autocatalytic formation of the rotaxane structure. An obvious take home message here is that the efficiency of the crucial binding processes for the [L•M] complex, ultimately determined its potential to act as competent replicator. One way to overcome this problem is to optimize the binding
motif between the macrocycle and the guest component. Supplementary investigations of how different macrocycle frameworks incorporates a range of potential guests can dramatically effect the efficiency of the respective binding process can be found in Chapter 4 of this thesis.

3.4.2 Replication model 2

Alternatively, we explored conceptually a second replication model in which one of the recognition site is located on the end of stopper reagent S and appending its complementary recognition site on to the macrocycle M (as depicted in Figure 3.6).

![Figure 3.6](image_url)

Figure 3.6. Replication model 2 – based on the placement of one of the complementary recognition site on one of the stoppers end and appending its complementary recognition site on to the macrocycle itself. The unbound linear component L react with the stoppering reagent S to gives the thread T, in which unable to enter the replicating cycle as it only possessed one recognition site (Non-recognition mediated synthesis). Oppositely, the formation of [L·M] complex with complementary recognition site on the macrocycle M further react with stoppering reagent S to afford the rotaxane R. Rotaxane R capable to access the replicating cycle since both complementary recognition sites present in the structure and, hence catalyse the formation of a second molecule of rotaxane R (Recognition mediated synthesis). The blue cartoons represent the reactive sites, the green cartoons represent the recognition sites and the red cartoons represent the binding site.
This modification exclusively favoured the formation of rotaxane $R$ structure by mean of recognition-mediated process, which in turn allow the rotaxane $R$ to undergo the autocatalytic cycle and subsequently provide the second molecule of $R$ to initiate the replicating cycle. On contrary, the thread $T$ resulting from the reaction of the unbound $L$ component with the stoppering reagent $S$ are now incapable to gain access to the replicating cycle as a consequence of possessing only one recognition site. It is noteworthy that the binding process is now function as catalyst to ensure the exclusive formation of rotaxane $R$ structure. Nevertheless, we have been struggling to control a number of factors in designing the suitable building blocks based on this replicating framework. The synthesis involving the recognition macrocycle is a significant synthetic challenge in itself and will be discussed in detailed in Chapter 7.

3.4.3 Replication model 3

The third replication model devised is shown in Figure 3.7. A fragment incorporating the binding site and one element stopper is attached to one of the building blocks constitutive of the template (either $[L\cdot M]$ or $L$ component). The corresponding element stopper of the rotaxane $R$ is formed by reaction with the stoppering reagent $S$. The complementary recognition sites are now located at only one stopper of the rotaxane $R$. As seen in previous minimal replication model, the reaction between $[L\cdot M]$ component and stoppering reagent $S$ affords the rotaxane $R$. Stoppering reagent $S$ and $[L\cdot M]$ complex can bind reversibly to the rotaxane $R$ to form the catalytic quarternary complex $[S\cdot L\cdot M\cdot R]$. Bond formation occurs between $S$ and $[L\cdot M]$ to give the product duplex $[R\cdot R]$, which then dissociates to return two molecules of $R$ to the start of the replicating cycle.

The essential advantage of this design is that we are capable of employing the known successful self-replicator design on the end of the stopper, which acts as sufficient end groups and efficiently accelerate the autocatalytic pathway to construct the rotaxane $R$ structure. Nonetheless, the binding site itself did not behave as catalyst for the rotaxane formation. Consequently, there will be a competition in the rotaxane formation because the thread is also being formed simultaneously. Once more, a high association constant is the prime concern in the model devised. Additionally, there will be more binding sites available for the macrocycle. One of them being is the nitrone binding site as the nitrone will first bind to the macrocycle.
before react with the maleimide compound. Moreover, there is a chance that
crosscatalyis pathway operating in this replicating model.

**Figure 3.7.** **Replication model 3** – based on the placement of the two recognition sites (green
cartoons) on the same stoppers. Reaction between [2]-pseudorotaxane \([L\cdot M]\) and
stoppering reagent \(S\) affords the rotaxane \(R\). Stoppering reagent \(S\) and \([L\cdot M]\) complex
can bind reversibly to the rotaxane \(R\) to form the catalytic quatermary complex
\([S\cdot L\cdot M\cdot R]\). Bond formation occurs between \(S\) and \([L\cdot M]\) to give the product duplex
\([R\cdot R]\), and return two molecules of \(R\) to the start of the autocatalytic cycle. The blue
cartoons represent the reactive sites, the green cartoons represent the recognition
sites and the red cartoons represent the binding site.
3.5 Summary

In the course of these discussions, we have gained considerable information in the design and evaluation of minimal model of self-replicating rotaxanes. In this system, three critical design criteria are prescribed, namely the recognition event between the carboxylic acid and amidopyridine unit, the chemical reaction employed to form the template $T$ (1,3-dipolar cycloaddition between nitrone and maleimide) and ultimately the binding site between macrocycle $M$ and the guest. These essential aspects are explained in three independent replication models, designated as Replication model 1, Replication model 2 and Replication model 3. In particular, Replication model 1 was first described in the group but met with limited success. The poor association strength in the [L-M] complex lessened the yield of rotaxane $R$ as exemplified in the simple kinetic of rotaxane formation. In order to arrive at a better and more solidly comprehension in our effort to integrate the replication process within the rotaxane structure, the remaining two models are further analysed. In essence, the specific advantages and disadvantages of individual minimal model implemented in this thesis were summarized below.

<table>
<thead>
<tr>
<th>Replication model</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Model 1" /></td>
<td>A known self-replicating system.</td>
<td>The crucial binding processes in the assembly of pseudorotaxane complex (require a high association constant, $K_a$) determined the efficiency of rotaxane formation.</td>
</tr>
<tr>
<td><img src="image" alt="Model 2" /></td>
<td>The binding process now function as a catalyst to ensure the exclusive formation of rotaxane. Thread incapable of accessing the autocatalytic cycle since it posses only one recognition site.</td>
<td>An unknown replicating system. Synthetically challenging in designing the building blocks based on this model scheme.</td>
</tr>
<tr>
<td><img src="image" alt="Model 3" /></td>
<td>Highly efficient replicator systems can be employed on the end of the stopper. Easier to design the building blocks based on this framework.</td>
<td>An unknown replicating system. Thread are being formed in parallel which allow the competition for rotaxane formation.</td>
</tr>
</tbody>
</table>
Design, synthesis and binding properties of macrocycles

4.1 Introduction

In the self-replicating rotaxanes framework, the paramount strategy involves the selection of an efficient macrocycle to accommodate the guest unit. A sufficient binding within the cavity of the macrocycle is crucial for the successful formation of pseudorotaxane in the threading processes. Previous study\textsuperscript{200} employed the non-covalent interactions to assemble the rotaxane architecture. The macrocycle ranged from the crown ether to simple hydrogen bond donor and/or acceptor motif for both binding motif and macrocycle. These structures will adopt a coconformation that maximizes the donor and acceptor hydrogen bonding interactions.

4.2 First generation macrocycles

In the development of a synthetic route toward the formation of macrocycle, one of the most important and challenging tasks is the key macrocyclisation step. In the absence of a suitable template, the synthesis of macrocyclic component employs the high-dilution\textsuperscript{208} conditions. The high-dilution approaches\textsuperscript{209} have been used in a large number of macrocyclic syntheses. One rationale behind this strategy is that in dilute solution, the formation of the cyclic product by intramolecular reaction is more likely and thus faster than formation of a polymer, which requires a collision between two different starting reagents by means of intermolecular reaction. Furthermore, if the cyclisation reaction is intrinsically faster than the rate of addition of the starting reagents, then the concentration of the reactants will always be small and not increase during the course of the reaction\textsuperscript{210}. These standard approaches have been taken to assemble the macrocycle skeleton in moderate yield and described in the following paragraphs. In addition to this methodology, many other approaches have
been described and engaged in the formation of wide variety of synthetic macrocyclic ligands.

4.2.1 Ethylene urea macrocycle (MEU)
A prototype amide-based macrocycles have been extensively explored within our research laboratory and others. Prospective guests such as pyridone, urea and amide have been identified to probe as the binding components. Nevertheless, the binding affinity shown in previous work is moderate as a consequence of diminutive cavity and hence, decreasing the association constant, $K_a$ in the pseudorotaxane complex. Therefore, a recent series of amide-based macrocycles are proposed to achieve a high $K_a$. The existing complementary motif between hydrogen bond donor and acceptor are maintained in the new macrocycle framework (Figure 4.1). For instance, the urea guest form primary hydrogen bond interaction between the oxygen and the two NHs of the macrocycle, whilst the second interaction occur between the hydrogen donor of the urea and the oxygen of the cyclic ethylene urea of the ring.

The synthesis of the ethylene urea macrocycle (MEU) was started by reacting 4-(bromomethyl) benzonitrile 78 with sodium in ethylene glycol to afford the alcohol 79 in excellent yield (Scheme 4.1). Subsequent tosylation leads to the formation of intermediate 80. Two equivalents of the tosylate 80 activated the $\alpha$–carbon, which undergo nucleophilic attack by the cyclic ethylene urea 81 in a suspension of sodium hydride in dry THF. The resulting nitrile 82 was further reduced to the corresponding amine derivative 83 using BH$_3$•THF complex in dry THF. The final ring closure takes

\[ \text{Figure 4.1. (a) Structural design of macrocycle ethylene urea (MEU) consists of two hydrogen donors and a single hydrogen bond acceptor. (b) Possible guest interaction with new design of macrocycle displayed by the urea derivatives. (D embedded in green circles denoted the hydrogen bond donors while A embedded in red circles denoted the hydrogen bond acceptors).} \]
place by the formation of two amide bonds with 2,6-pyridine dicarbonyl dichloride affording macrocycle MEU in 26% yield.

![Chemical reaction diagram]

Scheme 4.1. Synthesis of macrocycle MEU. Reagents and yields: (a) Na, ethylene glycol, THF, reflux, 16 h, 90%; (b) TsCl, Et$_3$N, CH$_2$Cl$_2$, 0 °C to 1 h and reflux for 16 h, 77%; (c) NaH, THF, reflux, 16 h, 51%; (d) BH$_3$$\cdot$THF, THF, 0 °C for 2 h and reflux for 16 h, 86%; (e) 2,6-pyridinedicarbonyl dichloride, Et$_3$N, CH$_2$Cl$_2$, rt, 3 days, 26%.

Crystals of macrocycle MEU were grown by vapor diffusion of hexane into a solution of macrocycle MEU in CHCl$_3$ at ambient temperature, and analysed by single crystal X-ray diffraction. A stick representation of the molecular structure is depicted in Figure 4.2. It came to our surprise, the two units of the macrocycle MEU were found to bind to each other through the proximity of the upper fragment of ring with a strong hydrogen bond from the carbonyl oxygen atoms to both of the amide NHs of the other ring. It is also important to note that the macrocycle crystallise with two molecules of chloroform used in the preparation of the crystals and, hence, reveal the inverted conformation of the ethylene urea carbonyl oxygen. This conformation is responsible for the hydrogen bonds contact with the solvent, which now acts as hydrogen bond acceptors. The O\(\cdots\)H distance is measured to be 2.03 Å. The subsequent binding studies were conducted with urea, amide and pyridone guests to
evaluate the capability of macrocycle MEU of hosting guest molecules, despite that inverted position of the carbonyl oxygen of the cyclic ethylene urea.

Figure 4.2. Stick representation of two units of macrocycle MEU in the solid state, which found to crystallise with each other and the solvent (CHCl₃) used in the preparation of the crystals is in the hydrogen bond contact with oxygen atom of the carbonyl group as determined by single crystal X-ray diffraction. Carbon atoms are coloured green, oxygen atoms in red, nitrogen atoms in blue and hydrogen atoms that are involved in hydrogen bonds (black lines) are coloured grey for the macrocycle and white for the chloroform.

4.2.2 Binding Studies – MEU

The 300.1 MHz ¹H NMR spectrum of an equimolar mixture of macrocycle MEU and guest 84 in CDCl₃ (Figure 4.3b) shows that the complex between macrocycle MEU and compound 84 is in fast exchange process on the ¹H NMR chemical shift timescale, which displays significant changes in the chemical shifts for the resonances of the complex relative to those of the free species (Figure 4.3a and Figure 4.3c). The resonance arising from the macrocycle NH proton, H¹¹R is shifted downfield (+0.76 ppm) as a result of the hydrogen bonds between these protons and the carbonyl group of urea 84. The resonances of macrocycle methylene protons are shifted upfield (H¹⁰R, H⁵R and H⁴R, −0.08, −0.04 and −0.17 ppm, respectively) and that of the diphenyl urea 84, H⁹G (−0.30 ppm). These chemical shift changes suggest that the urea 84 is located within the cavity of the macrocycle. The $K_a$ was measured by ¹H NMR titration experiment and was estimated to be only $39 \pm 4$ M⁻¹ in CDCl₃ at 25 °C.
Partial \( ^1\text{H} \) NMR spectra (300.1 MHz, 25 °C, CDCl\(_3\), 20 mM) of (a) urea 84 (b) an equimolar mixture of macrocycle MEU and urea 84 and (c) macrocycle MEU. Red dashed lines are shown to connect the resonances for specific protons in bound and unbound states. The saturation curve for the association of the macrocycle MEU and urea 84 gives an optimized \( K_a \) value of 39 ± 4 M\(^{-1}\) and \( \Delta G = -2.17 \text{ kcal mol}^{-1} \). \( R \) corresponds to the ring (in this case, macrocycle MEU) and \( G \) denoted the guest (in this case, urea 84). (\( \Delta\delta = \delta_{\text{unbound}} - \delta_{\text{bound}} \)).
Figure 4.4. Partial $^1$H NMR spectra (300.1 MHz, 25 °C, CDCl$_3$, 20mM) of (a) benzanilide 85 (b) an equimolar mixture of macrocycle MEU and guest 85 and (c) macrocycle MEU. Red dashed lines are shown to connect resonances for specific protons in bound and unbound states. The saturation curve for the association of the macrocycle MEU and benzanilide 85 gives an optimized $K_a$ value of $41 \pm 4$ M$^{-1}$ and $\Delta G = -2.20$ kcal mol$^{-1}$. R corresponds to the ring component (in this case, macrocycle MEU) and G denoted the guest (in this case, benzanilide 85). ($\Delta \delta = \delta_{\text{unbound}} - \delta_{\text{bound}}$).

<table>
<thead>
<tr>
<th>Proton resonances</th>
<th>$\delta_{\text{unbound}}$</th>
<th>$\delta_{\text{bound}}$</th>
<th>$\Delta \delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$^{1,R}$</td>
<td>8.04</td>
<td>8.53</td>
<td>+0.47</td>
</tr>
<tr>
<td>H$^{8,R}$, H$^{7,R}$</td>
<td>7.27</td>
<td>7.13</td>
<td>-0.14</td>
</tr>
<tr>
<td>H$^{10,R}$</td>
<td>4.69</td>
<td>4.60</td>
<td>-0.09</td>
</tr>
<tr>
<td>H$^{5,R}$</td>
<td>4.48</td>
<td>4.40</td>
<td>-0.08</td>
</tr>
<tr>
<td>H$^{4,R}$</td>
<td>3.60</td>
<td>3.45</td>
<td>-0.15</td>
</tr>
</tbody>
</table>
The second binding experiment was conducted using benzanilide 85 under the same conditions. The 300.1 MHz $^1$H NMR spectrum of an equimolar mixture of macrocycle MEU and benzanilide 85 exhibits (Figure 4.4b) a fast exchange process is taking place. The chemical shift changes observed in $^1$H NMR spectrum are comparable to those observed for macrocycle MEU and diphenylurea 84, for example the downfield shift for the resonances macrocycle NH protons H$^{11R}$, (+0.47 ppm), upfield shifts for the resonances of the macrocyle MEU phenylene protons H$^{8R}$ and H$^{7R}$ and that of the guest aromatic protons (H$^{2G}$, H$^{3G}$, H$^{8G}$ and H$^{9G}$), and upfield shifts of the macrocycle MEU methylene protons H$^{10R}$, H$^{5R}$ and H$^{4R}$ ($-0.09$, $-0.08$ and $-0.15$ ppm, respectively). The strength of the complex formed between the macrocycle MEU and N-phenylbenzamide 85 was estimated by using $^1$H NMR titration experiment and the $K_a$ was found to be $41 \pm 4$ M$^{-1}$ in CDCl$_3$ at 25 °C. A possible explanation for the low association constant obtained is that the crucial binding only occurred between the amide NHs proton of the macrocycle MEU and the carbonyl functional group from the guest. This evidence correlated with the molecular representation of macrocycle MEU in crystal structure as the carbonyl oxygen of the ethylene urea is in contact with the CHCl$_3$ solvent, which consequently incapable to participate as hydrogen bond donors with the guest.

We are aware that amide-based macrocycles have been used to construct many elegant interlocked molecules. In most circumstances, the threading of the guest into these macrocycles required the cooperation of two or more suitably positioned carbonyl groups or anionic templates. On that account, the presence of the carbonyl group is essential for this binding behavior. Nevertheless, the poor association constant derived for both complexes in the $^1$H NMR titration experiments was unanticipated and, hence, lead us in the quest for alternative binding site, such as thiourea. Additional binding experiment was performed under the same conditions using thiourea 86 (see Table 4.1) as guest. The $^1$H NMR spectrum of an equimolar mixture of macrocycle MEU and thiourea 86 (not shown) reveals that this complex also display a fast exchange process and the chemical shift changes for the macrocycle resonances are similar to those observed in Figure 4.3b and Figure 4.4b, and thus, demonstrate that macrocycle MEU capable of binding a thiourea binding site.
Figure 4.5. Partial $^1$H NMR spectra (400.1 MHz, 25 °C, CDCl$_3$, 25 mM) of (a) 4-pyridone 87 (b) an equimolar mixture of macrocycle MEU and 4-pyridone 87 (c) macrocycle MEU and (d) an equimolar mixture of macrocycle MEU and extended pyridone 88. Red dashed lines are shown to connect resonances for specific protons in bound and unbound states. UB stands for unbound species, and B1 and B2 corresponds to bound species 1 and bound species 2. R corresponds to the macrocycle MEU and G denoted the guest 87. ($\Delta \delta = \delta_{\text{unbound}} - \delta_{\text{bound}}$).
Following this, we investigated the ability of macrocycle MEU to complex pyridone guests by performing the similar binding experiments. The 400.1 MHz $^1$H NMR spectrum of an equimolar mixture of macrocycle MEU and 4-pyridone 87 (Figure 4.5b) in CDCl$_3$ shows that the complex between macrocycle MEU and 87 is again in fast exchange process and displays remarkable changes in the chemical shifts for the complex's resonances relative to those of the free species (Figure 4.5a and Figure 4.5c). The resonance arising from the macrocycle NH protons, NH$^{11r}$ is shifted downfield (+1.98 ppm) as a result of the hydrogen bonds between these protons and the carbonyl group of 4-pyridone 87. The upfield shifts of both phenylene and methylene MEU protons indicate that MEU bind the guest very well.

An equimolar solution of macrocycle MEU and extended guest 88 was subsequently prepared in CDCl$_3$ by heating of this mixture to improve its solubility. The $^1$H NMR spectrum (Figure 4.5d) exhibits four different sets of resonance, namely one for the free macrocycle MEU and a further three corresponding to (i) free pyridine guest 88 (ii) the first pseudorotaxane complex [MEU•88] – bound species 1 (B1) and (ii) the second pseudorotaxane complex [MEU•88]$'$ – bound species 2 (B2). Both bound and unbound species equilibrated in a slow exchange process on the $^1$H NMR chemical shift timescale.

Examining the 500.1 MHz 2D EXSY NMR spectrum recorded at 25 °C reveals a number of significant crosspeaks between the unbound, UB macrocycle MEU (NH$^{11}$) proton with the emergence of bound proton species, B1 (NH$^{11'}$) and B2 (NH$^{11''}$) (Figure 4.6a), unbound extended pyridone guest 88 phenylene proton, UB (H$_b$) and the two bound species, B1 (H$_b'$) and B2 (H$_b''$) (Figure 4.6b) and finally between the unbound macrocycle methylene proton, UB (H$_2$) and the corresponding bound species, B1 (H$_2'$) and B2 (H$_2''$) (Figure 4.6c). Fascinatingly, both bound species are shown to exchange with each other as well as exchanging with the unbound species. Important 2D NOESY crosspeaks can also be observed for both pseudorotaxane complexes. These duplicate resonances very likely originated from two alternative conformations that the macrocycle MEU accommodate during the complexation event. Efforts were pursued to investigate the possible lowest energy conformations of the two bound [2]-pseudorotaxane complexes (suggested by the calculated structure of UB, B1 and B2 as shown in Figure 4.7).
It is important to note the two separate orientations of the pyridine ring macrocycle MEU relative to the carbonyl group of the cyclic urea. In the case of B1 complexes, the pyridine ring of the macrocycle is aligned almost parallel to the carbonyl group of the cyclic urea. However, gaining a strong hydrogen bond between the hydrogen bond acceptor of the cyclic urea and the NH proton of the pyridone guest is rather obscure. On the other hand, B2 conformation reveal that the oxygen atom of the cyclic urea is pointing downward with respect to the pyridine ring which inevitably inhibited the preferred hydrogen bond contact with the NH of the guest 88. These calculated structures are in agreement with the single crystal structure obtained for macrocycle MEU in which case the inverted location of the cyclic urea with respect to the pyridine ring in the upper fragment of the macrocycle is similarly observed. On
the whole, both bound species (B1 and B2) represents 62% of the species in solution, compared to 38% of the unbound UB species in the mixture. Using the single-point method, the association constant, $K_a$ derived for the bound species [MEU-88] was estimated to be $170 \pm 20 \text{ M}^{-1}$ in CDCl$_3$ at 25 °C and, hence, discriminated nearly 1:1 ratio between the two bound species. The following table (Table 4.1) summarizes all the binding experiments carried out at 25 °C between macrocycle MEU and different guests synthesised.

**Figure 4.7.** Stick representation of the calculated structure (OPLS2005, GB/SA CHCl$_3$) for Unbound, Bound 1 and Bound 2 species. The reversible exchange event shown as red arrows occurred between unbound species (UB) and bound species (B1 and B2, respectively) in the reaction mixture.
Table 4.1. Binding experiments carried out with macrocycle MEU. The $^1$H NMR spectra of the macrocycle MEU and guest showed complexes either in fast exchange (F), or in intermediate exchange (I), or in slow exchange (S) that can be attributed to significant binding process in comparison to the free species observed in isolation. The $K_a$ usually determined by titration (*) technique in the case of fast exchange. In the case of slow exchange, the $K_a$ was determined using the single-point method (#) by $^1$H NMR resonance. Intermediate exchange normally leads to broad peaks and prevent the determination of $K_a$. The concentration used was 20 mM in CDCl$_3$ at room temperature unless otherwise stated.

<table>
<thead>
<tr>
<th>Guest</th>
<th>$K_a / \text{M}^{-1}$</th>
<th>Guest</th>
<th>$K_a / \text{M}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>84</td>
<td>F * $K_a = 39 \pm 4$</td>
<td>87</td>
<td>F $K_a = 39 \pm 4$</td>
</tr>
<tr>
<td>85</td>
<td>F * $K_a = 41 \pm 4$</td>
<td>88</td>
<td>S # $K_a = 170 \pm 20$</td>
</tr>
<tr>
<td>86</td>
<td>F $K_a = 41 \pm 4$</td>
<td>89</td>
<td>I $K_a = 39 \pm 4$</td>
</tr>
</tbody>
</table>

4.2.3 Acyclic urea macrocycle (MAU)

In order to improve the binding constant of pseduorotaxane complex, the framework of the macrocycle was subtly altered. The reasonable rationale behind the newly designed motif of host results from the weak binding displays between amide guest and macrocycle MEU previously. The structural design change is fabricated by creating an inclusive hydrogen bond donors pocket. Theoretically, the new adjustment will result in ‘all hydrogen bond acceptors’ pattern in designing the guest.
candidates. In particular, an appropriate guest comprised an equal amount of hydrogen bond acceptor, namely the 2,5-substituted piperazine-2,5-dione 90 and benzoquinone 91 (Scheme 4.2). The proposed guest, piperazine-2,5-dione 90 contained two carbonyl groups acting as hydrogen bond acceptors. The oxygen atom have the ability to form two hydrogen bonds each, with only weak angle dependence on the hydrogen bonds observed from the NH groups.

Thus, the macrocycle NH protons will provide exact complementarity. In addition, the 2,5-substituted benzoquinone 91 is an analogue of the piperazine-2,5-dione, in which case its two carbonyl groups can be chemically altered upon oxidation. The guest act as a hydrogen bond acceptor in the ground state to bind the macrocycle preferentially and the subsequent interconversion via reduction of the dione 91 to the diol 91a would displace the bound macrocycle as a conflicting donor motifs. Since this is a reversible reaction, diol 91a can be oxidized back to its conjugate dione 91 causing the macrocycle to shuttle back to its original position.

Therefore, molecular movement of this reversible nature means it is possible to control the position at which the macrocycle is bound. Ultimately, both of these suggested guest structures fundamentally increase the binding affinity between the guest and host. The synthesis of macrocycle MAU similarly begins with the alcohol substitution on addition of 78 to the sodium in the presence of ethylene glycol (Scheme 4.3).

The resultant alcohol 79 was tosylated in addition of p-toluenesulfonyl chloride to furnish a quantitative yield of intermediate 80. The tosylated product 80 then becomes susceptible for nucleophilic attack by sodium azide, NaN₃. Selective hydrogenation of azide 92 using palladium on carbon as catalyst afforded the amine derivative 93. Following that, triphosgene 94 was added neat to ensure that the conversion to the desired urea takes place.
Scheme 4.2. (a) Schematic presentation of possible interactions between macrocycle MAU and piperazine-2,5-dione 90 derivative. (b) Schematic presentation of possible interactions between macrocycle MAU and 2,5-substituted benzoquinone 91. It display reversible binding characteristic in response to external stimuli. In its ground state, benzoquinone 91 will preferentially bind the macrocycle MAU as a result of an increase in the number of hydrogen bond interactions. However, dione 91 can be reduced to the corresponding diol 91a, thus transforming the acceptor motif to a donor motif and theoretically displace the macrocycle. (D embedded in green circles denoted the hydrogen bond donors while A embedded in red circles denoted the hydrogen bond acceptors).

Nonetheless, the reduction of the nitrile group 95 with BH$_3$•THF solution at 0 °C has found to be unsuccessful. The second approach was considered using stronger base, LiAlH$_4$ in dry THF, however the corresponding amine was not been able to synthesise. Later attempt on the hydrogenation in the presence of Raney-Nickel
catalysis was also inefficient. We reasoned that it is possible that with the presence of acidic hydrogen in compound 95 further assisted in the failure of the reaction to go to completion.

![Scheme 4.3](image)

**Scheme 4.3.** Attempted synthesis of MAU. Reagents and yields: (a) Na, ethylene glycol, THF, reflux, 16 h, 90%; (b) TsCl, Et3N, CH2Cl2, 0 °C to 1h and reflux for 16 h, 77%; (c) NaN3, DMF, 80 °C, 16 h, 97%; (d) H2, Pd/C, Pyridine, CH3OH, rt, 8 h, 98%; (e) Et2O, Et3N, 0 °C followed by reflux, 6 h, 36%.

As a consequence, the synthesis of macrocycle MAU could not be completed to afford the hydrogen acceptors motif's host and its corresponding calculated structure is depicted in Figure 4.8.
4.2.4 Symmetrical macrocycle (SM)

Preliminary synthetic effort\textsuperscript{200} to construct the symmetrical macrocycle employed the alkyne dimeric coupling reaction to afford the hydrogen donors host, in which could similarly integrated the corresponding guests described in Scheme 4.2. Disappointedly, insufficient supply of the macrocycle hindered the performance of the essential binding study. On that account, it would be worth preparing the analogous symmetrical macrocycle, which include the two hydrogen bond acceptors site. An earlier attempt to synthesise the symmetrical macrocycle is described in Scheme 4.4. Addition of 2,6-pyridinedicarbonyl dichloride to propargyl alcohol in the presence of base, yielded compound 96 in a moderate yield.

The homocoupling reaction of 96 under standard and high dilution oxidative coupling conditions using copper diacetate as catalysts in the presence of \textit{N}-ethyl(diisopropyl)amine is however unsuccessful. We subsequently focused\textsuperscript{222} on the standard conditions taken from the Sonogashira coupling of 96 with PPh\textsubscript{3}, PdCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2}, Cul in triethylamine/CH\textsubscript{3}CN solvent mixture refluxed under a positive atmosphere of argon. Nonetheless, this attempt has also been inefficient in closing the ring. The lack of success is plausible to explain from the unusual conformation of
calculated structure of the macrocycle SM (shown in Figure 4.9), which reveals that the molecule is twisted by rotation of one pyridine ring unit relative to the other and hence, supported the unsuccessful attempts of the dimerisation reaction.

Scheme 4.4. Attempted synthesis of macrocycle SM. Reagents and yield: (a) 2,6-pyridinedicarbonyl dichloride, propargyl alcohol, Et$_3$N, CHCl$_3$, 0 °C to rt, 16 h, 50%.

Figure 4.9. (a) Face and (b) side views of stick representations of the calculated structure (OPLS2005, GB/SA CHCl$_3$) of macrocycle SM. Carbon atoms are coloured green, oxygen atoms red, nitrogen atoms blue and hydrogen atoms white (most hydrogen atoms have been removed for clarity).

4.3 Second generation of macrocycles – Alternative synthesis

Further investigations were conducted to explore the binding properties of the preceeding macrocycles prepared in the group as well as to improve the yield by introducing an alternative synthetic route. This will allow us to deduce significant conclusions on the strength and potential between the first and second generation macrocycles.
4.3.1 Alkyne ethyleneurea macrocycle (MAEU)

We had found\textsuperscript{200} evidence that macrocycle MAEU capable of bind 4-pyridone 87, but we never tested with other different guests. Thus, a different approach is described to simplify the synthesis of MAEU (Scheme 4.5). The alkyne 98 was initially prepared in the group by the treatment of bis $N$-alkylation of cyclic ethylene urea 81 with propargyl benzenesulfonate 97. 3-bromobenzonitrile 99 was employed as the starting material to couple the bis(alkyne) 98 under Sonogashira conditions to afford compound 100. Despite that, an attempt to reduce the nitrile groups in 100 has been unsuccessful to furnish the corresponding amine compound and thus, no further binding experiment could be carried out with macrocycle MAEU.

![Scheme 4.5](image)

**Scheme 4.5.** Attempted synthesis of macrocycle MAEU. Reagents and yields: (a) Bu$_4$NBr, KOH, THF, 0 °C to rt for 1 h, followed by reflux, 2 days, 40%; (b) PdCl$_2$(PPh$_3$)$_2$, Cul, PPh$_3$, Et$_3$N, THF, 70 °C, 48 h, 36%.

The calculated conformation macrocycle MAEU (Figure 4.10) illustrated that the oxygen of the carbonyl group is pointing downward with respect to the pyridine ring unit.
4.3.2 Diethylene glycol macrocycle (MDG)

Earlier in Section 4.2.2, macrocycle MEU exhibited such ineffective binding with simple amide and urea guests. The pursuit to readdress these insufficiencies have forced us one step back to repeat the syntheses of known macrocycles in order to provide conclusive binding studies. The hydrogen bond donor and acceptor interactions displayed by macrocycle MDG on addition of amide and urea derivatives shown in Figure 4.11. The resulting [2]-pseudorotaxane complexes are stabilised through the cooperation effects of \( \pi \) stacking of the two xylyl rings and N–H⋯O hydrogen bonding (between macrocycle NHs proton and the carbonyl oxygen atom of each simple amide and urea compounds and between the amide and urea proton(s) and the ether pocket of the ring).

The synthesis of macrocycle MDG was prepared\(^{207}\) according to the literature. 4-(bromomethyl)benzonitrile 78 was reacted with diethylene glycol 101 under basic conditions to afford the dibenzonitrile 102. BH\(_3\)•THF mediated reduction yielded the amine 103 followed by ring closure with 2,6-pyridinedicarbonyl dichloride in high dilutions with the aid of one equivalent of 4-pyridone 87, which acted as template gave rise to 34\% yield of the macrocycle MDG (Scheme 4.6).
Figure 4.11. Structural design of macrocycle MDG comprised both hydrogen bond donors and acceptors motif with amide and urea guest interaction displayed by dashed lines. (D embedded in green circles denoted the hydrogen bond donors while A embedded in red circles denoted the hydrogen bond acceptors).

Scheme 4.6. Synthesis of macrocycle MDG. Reagents and yields: (a) NaH, THF, reflux, 16 h, 90%; (b) BH$_3$•THF, THF, 0 °C for 2 h to reflux for 24 h, 52%; (c) 2,6-pyridinedicarbonyl dichloride, 4-pyridone 87, Et$_3$N, CH$_2$Cl$_2$, rt, 3 days, 34%.
4.3.3 Binding studies – MDG

In the past, we have established that macrocycle MDG can form [2]-pseudorotaxane complex, which stabilized through intermolecular hydrogen bonding with 4-pyridone derivatives 87a (see Table 4.2) positioned within the cavity of the ring in low polarity solvent such as chloroform. From the $^1$H NMR dilution experiment, the association constant was estimated around $1500 \pm 150 \text{ M}^{-1}$ from an equimolar mixture of macrocycle MDG and 87a. It was also demonstrated that diarylamides 85 are capable of associating macrocycle MDG and the strength of the complex formed was approximated to be $160 \pm 16 \text{ M}^{-1}$ in CDCl$_3$ at 25 °C (see Table 4.2). Hence, it is evident that a great discrepancy in the binding strength when we changed the binding site from pyridone to an amide guest. On the basis of this result, simple urea and amide guest derivatives were produced (Scheme 4.7), which each compound now incorporate the electron-withdrawing 3,5-bis(trifluoromethyl) substituent. Model amide 105 is synthesised starting from 4-butyl aniline 104, which was coupled to 3,5-bis(trifluoromethyl)benzoyl chloride 124. Likewise, reaction of the same aniline with 3,5-bis(trifluoromethyl)phenyl isocyanate 127 yielded urea 106 in 80% yield.

![Scheme 4.7. Synthesis of model amide 105 and urea 106. Reagents and yields: (a) 3,5-bis(trifluoromethyl)benzoyl chloride 124, Et$_3$N, CHCl$_3$, $-5$ °C to rt, 16 h, 50%; (b) 3,5-bis(trifluoromethyl)phenyl isocyanate 127, CHCl$_3$, $-5$ °C to rt, 16 h, 80%.]

The binding experiment was initially carried out with an equimolar mixture of macrocycle MDG and model amide 105 in CDCl$_3$ at 25 °C at 20 mM concentration of the starting reagents. The resulting 400.1 MHz $^1$H NMR spectrum of this association reveals a slow exchange process with significant chemical shifts changes for the bound and unbound guest 105 (see table in Figure 4.12). Such elegant prove to demonstrate the formation of the pseudorotaxane species arise from the complex pattern in the macrocycle methylene regions ($H^{8R}$, $H^{3R}$, $H^{2R}$ and $H^{1R}$) (red dashed boxes in Figure 4.12b) as a consequence of end-to-end asymmetry imposed by the amide 105. We determined the binding constant using the $^{19}$F NMR spectroscopy.
using the single-point method ($d_1 = 6\text{ sec}$)\textsuperscript{1} to be $900 \pm 90\text{ M}^{-1}$. The similar binding experiment was further conducted with model urea 106, which also exhibited a slow exchange on the $^1\text{H}$ NMR chemical shift time scale (Figure 4.13b).

A careful analysis of the 400.1 MHz $^1\text{H}$ NMR spectrum display chemical shift changes (see table in Figure 4.13) similar to those observed for the pseudorotaxane assembly, such as typical downfield shift of NH macrocycle protons, NH$^9\text{R}$, upfield shifts for the resonances for the macrocycle phenylene protons H$^5\text{R}$ and H$^6\text{R}$, and also upfield shifts of the guest 106 phenylene protons, H$^{13}\text{G}$ and H$^{14}\text{G}$. Given the asymmetry nature of the urea compound 106, apparently renders the resonances for proton H$^8\text{R}$ and H$^3\text{R}$ diastereotopic. Upon the interlocking of the guest and macrocycle MDG, H$^8\text{R}$ appears as two set of doublet of doublet at $\delta_H 4.82$ and $\delta_H 4.12$ and the H$^3\text{R}$, which appear as singlet in the unbound state gave rise to an AB system. The strength of the association between macrocycle MDG and urea 106 was found to be broadly similar with $K_a$ value of $980 \pm 100\text{ M}^{-1}$ in CDCl$_3$ at 25 °C.

\textsuperscript{1} After the first excitation, the sample returns to its equilibrium state at a rate $1/T_1$, where $T_1$ is the spin-lattice relaxation time-constant. In order to obtain the full signal intensity after the second excitation, a delay of approximately $5 \times T_1$ is thus required. A delay of this duration is necessary if accurate relative intensities and therefore signal integration are to be obtained in quantitative experiments. Hence, we determined the delay ($d_1$) of the sample by measuring the $T_1$ to be 6 sec between the two scans. See T.D.W. Claridge, in Tetrahedron Organic Chemistry Series, Volume 27, Elsevier 2009, pp. 11-34.
Figure 4.12. Partial $^1$H NMR (400.1 MHz, 25 °C, CDCl$_3$, 20 m) of (a) amide 105, (b) an equivalent molar mixture of macrocycle MDG and amide 105; and (c) free macrocycle MDG. Red dashed lines are shown to connect resonances for specific protons in the bound and unbound states. The complex patterns the macrocycle methylene region (H$_{8R}$, H$_{9R}$, H$_{12R}$ and H$_{13R}$) are shown in the red dashed boxes. R corresponds to macrocycle MDG and G denoted the guest 105. ($\Delta\delta = \delta_{\text{unbound}} - \delta_{\text{bound}}$).
Figure 4.13. Partial $^1$H NMR (400.1 MHz, 25 °C, CDCl$_3$, 20 mM) of (a) urea 106, (b) an equivalent molar mixture of macrocycle MDG and urea 106, and (c) free macrocycle MDG. Red dashed lines are shown to connect resonances for specific protons in the bound and unbound states. The complex pattern of macrocycle methylene region ($H^{1R}$, $H^{2R}$, $H^{3R}$ and $H^{4R}$) are shown in the red dashed boxes. R corresponds to the macrocycle MDG and G denotes the guest 106. ($\Delta\delta = \delta_{\text{unbound}} - \delta_{\text{bound}}$).
The slow exchange process on the $^1$H NMR chemical shift timescale manifests that some of the complexation process is characterized by constrictive binding. The constrictive binding energy represents the magnitude of sterically imposed activation energy barrier to the CF$_3$ guest entry (both guest 105 and 106) into the cavity of the macrocycle during the exchange process. This phenomena arise from the instability of the guest exchange transition state, which involves significant bond strain as the guest squeezes through the wall of macrocycle MDG. However, in the case of benzanilide guest 85 (smaller guest), the constrictive binding energy, which is generally much larger than the intrinsic binding energy (the complexation free energy for the guest binding reaction) decreases since the portal through which the guest must past gets larger. The constrictive and intrinsic nature of complexation between macrocycle MDG and urea compound 106 are further illustrated in Figure 4.14. Following this, Table 4.2 summarizes the binding experiments carried out at 25 °C between macrocycle MDG and different guests synthesised.

![Figure 4.14](image)

**Figure 4.14.** The activation energy depicted for urea 106 guest to enter the cavity of macrocycle MDG in a slow exchange process. ($\Delta G^\ddagger$ constrictive = $\Delta G^\ddagger$ dissociation − ($-\Delta G^\ddagger$)).
Table 4.2. Binding experiments carried out with macrocycle MDG. The \(^1\)H NMR spectrum of the macrocycle MDG and guest mixture showed complex either in fast exchange (F), or in slow exchange (S) that can be attributed to significant binding process in comparison to the free species observed in isolation. The \(K_a\) usually determined by titration (*) technique in the case of fast exchanges. In the case of slow exchanges, the \(K_a\) was determined using the single-point method (#) either by \(^1\)H NMR resonance or \(^19\)F NMR resonances (\(d_i = 8\) sec). The concentration used was 20 mM in CDCl\(_3\) at room temperature unless otherwise stated.

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<td>(K_a = 350 \pm 35)</td>
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4.3.3 Pyridine macrocycle (MP)

The second macrocycle developed in the group capable of complexing pyridone and acridone moiety in a [2]-pseudorotaxane type geometry. Nonetheless, limited studies have been carried out to examine the potential of this pyridine macrocycle, MP to bind urea and amide derivatives. The possible interactions of the guest compounds to associate with the hydrogen bond donors (D) and acceptors (A) of the macrocycle MP are illustrated in Figure 4.15.

![Figure 4.15](image)

Figure 4.15. Structural design of macrocycle MP comprised both hydrogen bond donors and acceptors motif with amide and urea guest interaction displayed by dashed lines. (D embedded in green circles denoted the hydrogen bond donors while A embedded in red circles denoted the hydrogen bond acceptors).

In that case, macrocycle MP was produced in three step sequences (Scheme 4.8), starting from the 4-(bromomethyl)benzonitrile 78 with 2,6-pyridinedimethanol 112 in basic conditions, affording the dibenzonitrile 113. The subsequent reduction provided the amine 114, which was submitted to cyclization with 2,6-pyridinedicarbonyl dichloride in high dilution to obtain the macrocycle MDG in 22% yield.
Scheme 4.8. Synthesis of macrocycle MP. Reagents and yields: (a) NaH, THF, reflux, 18 h, 88%; (b) BH$_3$•THF, THF, 0 °C to reflux, 18 h, 63%; (c) 2,6-pyridinedicarbonyl dichloride, Et$_3$N, CH$_2$Cl$_2$, rt, 3 days, 22%.

4.3.4 Binding Studies – MP

Binding studies were conducted with macrocycle MP in an equimolar mixture of model amide 105 compound in CDCl$_3$ at 20 mM reagent concentration at 25 °C. The 400.1 MHz $^1$H NMR spectrum reveals that the association and dissociation rate between the ring component and the amide 105 is again slow on the $^1$H NMR chemical shift timescale (see Figure 4.16b) relative to those unbound components (comparison with Figure 4.16a and Figure 4.16c). The macrocycle proton NHs resonances, NH$^{11R}$ displays the typical downfield shifts (+1.25 ppm) as a consequence of the hydrogen bond contacts between these protons and the carbonyl group of amide 105. An obvious upfield changes for the ring phenylene protons H$^{7R}$ and H$^{8R}$, (−0.56 and −0.83 ppm, respectively) and that amide guest, H$^{6G}$ and H$^{7G}$ are valuable aspects to denote that upon the interlocking, these protons are now located in the shielding zone of the aromatic ring.
Figure 4.15. Partial $^1$H NMR (400.1 MHz, 25 °C, CDCl$_3$, 20 mM) of (a) model amide 105, (b) an equimolar mixture of macrocycle MP and amide 105 and (c) macrocycle MP. Red dashed lines are shown to connect resonances for specific protons in the bound and unbound states. The complex pattern of macrocycle methylene region ($H^{11R}$, $H^{15R}$ and $H^{13R}$) are shown in the red dashed boxes. R corresponds to the macrocycle MP and G denoted the guest 105. ($\Delta\delta = \delta_{\text{unbound}} - \delta_{\text{bound}}$).
In addition, the NH resonances of the guest, NH$^G_{9G}$ moved (+1.50 ppm) to a higher field of $\delta^H_{9.35}$ in contrast to $\delta^H_{7.85}$ in its free state, in which case signify a strong interaction was achieved with the three hydrogen bond acceptors and, hence, suggested that the guest is preferentially located in the correct space in the macrocycle unit. The appearance of the diastereotopic protons of the macrocycle methylene region, namely H$^4R$, H$^5R$ and H$^{10R}$ (outlined as red dashed boxes in Figure 4.16b) have in fact corroborated these binding events and the association constant, $K_a$ for the [MP•105] complex was determined to be 1440 ± 150 M$^{-1}$ in CDCl$_3$ at 25 °C.

Likewise, the additional binding experiment was conducted with the model urea 106 compound. The 400.1 MHz $^1H$ NMR spectrum of an equimolar mixture of macrocycle MP and urea 106 in CDCl$_3$ (Figure 4.17b) also reveals the slow exchange process taking place with notable chemical shift changes in comparison with the free state of the starting components (Figure 4.17a and Figure 4.17c). The resulting resonance arising from the NHs macrocycle proton, NH$^{11R}$ was deshielded to $\delta^H_{9.05}$, which indicated the hydrogen bond contacts to the carbonyl group of urea 106.

The following chemical shift changes are also noticed in the $^1H$ NMR spectrum: upfield shifts of macrocycle phenylene protons, H$^7R$ and H$^8R$, upfield shift of the aromatic ring of the urea 106 guest, H$^{13G}$ and H$^{14G}$ and that complex pattern of the ring methylene protons, H$^{10R}$, H$^{5R}$ and H$^{4R}$ are characteristics of the emergence of the pseudorotaxane species in the solution. Accordingly, the strength of the complexation was evaluated using single-point method, giving a $K_a$ value of 1920 ± 200 M$^{-1}$. On that account, both supplementary binding experiments investigated for macrocycle MP gave a comparable high association constant relative to macrocycle, which demonstrated that urea and amide are suitable candidates for macrocycle MP. The following table (Table 4.3) summarizes the binding experiments carried out at 25 °C between macrocycle MP and different guests.
Figure 4.17. Partial $^1$H NMR (400.1 MHz, 25°C, CDCl$_3$, 20 mM) of (a) model urea 106, (b) an equivalent molar mixture of macrocycle MP and urea 106 and (c) macrocycle MP. Red dashed lines are shown to connect resonances for specific protons in the bound and unbound states. The complex pattern of macrocycle methylene region ($H_{10}$R, $H_{15}$R and $H_{14}$R) are shown in the red dashed boxes. R corresponds to the macrocycle MP and G denoted the guest 106. ($\Delta\delta = \delta_{\text{unbound}} - \delta_{\text{bound}}$).
Table 4.3. Binding experiments carried out with macrocycle MP. The $^1$H NMR spectrum of the macrocycle MP and guest showed complexes either in fast exchange (F), or in slow exchange (S) that can be attributed to significant binding process in comparison to the species observed in isolation. The $K_a$ usually determined by titration (*) technique in the case of fast exchanges. In the case of slow exchanges, the $K_a$ was determined by the single-point method (#) either by $^1$H NMR resonance or $^{19}$F NMR resonances ($\tau_h = 6$ sec). The concentration being used was 20 mM in CDCl$_3$ at room temperature unless otherwise stated.

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$K_a = 1440 \pm 150$

$K_a = 1920 \pm 200$

$K_a = 280 \pm 28$

$K_a = 170 \pm 20$

$K_a = 4500 \pm 450$

$K_a = 1950 \pm 200$
4.4 Conclusion

The development of a series of macrocycles incorporating the hydrogen bond donors and/or hydrogen bond acceptors motif were reported and these macrocycles have been evaluated as suitable receptor for a variety of guest compounds ranging from pyridone, thiourea, benzoquinone, piperazine, acridone, amide and urea derivatives. In particular, the guests were designed and synthesised based on the mutual interactions with the macrocycle’s framework and the binding experiment was described in details. The synthesis and the structure of novel macrocycle MEU containing a pyridine ring and cyclic ethylene urea demonstrated a deficient binding performances with both amide and urea compounds. The binding constant, $K_a$ is improved moderately by the complexation with an elongated pyridone guest 88, in which case demonstrated the dual mode of binding conformation of the macrocycle. The emergence of two pseudorotaxane species that exhibit an exchange event gave a justification that the threading and dethreading processes takes place simultaneously in the reaction mixture, as a consequence of the two preferential orientation of the ring associating the guest compound.

These outcomes shows that a limited interaction occurred during the binding event based on the single X-ray crystal structure of macrocycle MEU, which surprisingly crystallise the CHCl$_3$ solvent used to prepare the crystal and another unit of the ring component. Initial effort to increase the association strength between the host and the guest is carried out by optimizing the binding motif in the host framework. Nonetheless, it was found to be synthetically unsuccessful. On that account, we had produced the synthesis of second generation macrocycles to improve the preceding preparations as well as to provide a comprehensive binding investigation with a series of novel guest compounds. The incorporation of an electron-withdrawing substituent such as 3,5-bis(trifluromethyl) group in the amide 105 and urea 106 scaffolds significantly increased the binding strength of both macrocycle MDG ($K_a$ was estimated between 900 to 1000 M$^{-1}$) and macrocycle MP ($K_a$ was determined to be between 1400 to 2000 M$^{-1}$), respectively. Presumably, these guest compounds that integrate such substituent groups crucially assisted this binding process. The rationale behind this phenomenon is addressed through improved designs that exploit optimization using computational approaches, which are discussed in Chapter 8.
5

Replication model 1

5.1 Introduction

The initial performance of self-replicating rotaxanes revolves around Replication model 1 which was simplified to two parallel autocatalytic pathways (Figure 5.1).

![Thread synthesis and Rotaxane synthesis diagram]

Figure 5.1. Replication model 1 consists of two autocatalytic cycles, which emanates from the central binding event (boxed), the reversible formation of the [2]-pseudorotaxane [L•M] complex. The blue cartoons represent the reactive sites, the green cartoons represent the recognition sites and the red cartoons represent the binding unit.

In the rotaxane synthesis, the association between macrocycle M with the linear component L first afforded the [L•M] species. Reaction between [L•M] complex and stoppering reagent S gives the rotaxane R. [L•M] complex and S can bind reversibly to the rotaxane R to form a catalytically quaternary complex [S•L•M•R]. Following this, bond formation occurs between S and [L•M] to provide duplex [R•R], which then
withdraw two molecules of R to start a new replicating cycle. On the other hand, unbound linear component L react with the stoppering reagent S to form thread T. Similarly, thread T enter the autocatalytic cycle by collecting the unbound species L and S to catalyse the formation of a second molecule of thread T. In this model, the vital role of the association constant, $K_a$ entailed an appropriate comprehension in preparation to implement this model. The reactivity of linear component, L and $[\text{L-M}]$ complex to give the thread and rotaxane, respectively are preeminent in this case. On that account, we would like to further illustrate this effect within a set of real systems in conjunction with the kinetic investigation.

5.2 Structural invariances of thread component

It is essential to evaluate the reactivity influenced by thread component (reaction between linear component, L and stoppering reagent S) composed of 1,3-dipolar cycloaddition between maleimide and nitrone compound. The trans to cis ratio was determined directly from $^1$H NMR spectrum as they exhibit markedly different resonances between $\delta_H 4.0$ and $\delta_H 6.0$ region, arising from the isoxazolidine protons (Figure 5.2). In the case of cis diastereoisomer protons (H$^{1'}$, H$^{2'}$ and H$^{3'}$) are locate on the same face of the fused ring system, which allowed them to couple to each other. The coupling constants are $^3J_{H^{1'},H^{2'}} 7.8$ Hz and $^3J_{H^{2'},H^{3'}} 9.0$ Hz.

In contrast, as a result of dihedral angle between H$^2$ and H$^3$ in the trans diastereoisomer, the coupling constant is close to zero, and therefore H$^3$ exhibit as an apparent singlet resonance. In addition, the resonance of proton H$^2$ appears as a doublet, whereas H$^2$ as a doublet of doublets. On the basis of the simple rotaxane formation, the nitrone structure 115 bearing an amidopicoline recognition unit has been selected. Both ends of nitrone 115, the 4,6-dimethylpyridine and 4-tert-butylphenyl ring have exemplified its potential to act as the bulky blocking groups. The four maleimide compounds chosen for the synthesis of simple rotaxane were described in the absence of the carboxylic acid recognition site (Figure 5.3).
Figure 5.2. Partial $^1$H NMR spectrum (400.1 MHz, CDCl$_3$, 25 °C) resulting from the reaction of nitroline and maleimide derivatives. These particular resonances were used to characteristically identify the trans ($H^1$, $H^2$ and $H^3$) and cis ($H^1$, $H^2$ and $H^3$) diastereoisomer of the reaction.

**Nitrone**

[Diagram of nitroline structure]

**Maleimides**

108

110

109

111

Figure 5.3. Nitroline and maleimide building blocks used in the screening of the structure dependent reactivity of the thread.

The alternative stopper group consists of 3,5-dimethylphenyl and 3,5-bis(trifluoromethyl)phenyl ring was selected. Furthermore, the introduction of fluorine tags into the maleimide compounds allowed a direct and facile observation of the reaction progress. In order to keep the reactive site far enough from the binding
site, the spacer must have a sufficient length. The spacer length was modified by the extension of aromatic ring and methylene groups to assure a certain degree of rigidity in the maleimide structures. The benzene ring (in structure 108) was replaced with a diphenylethane spacer (in structure 109 – 111) whereas maleimide 111 bearing the urea functionality has also been envisaged.

5.3 Improving the synthesis of the nitrone 115

The synthetic route of nitrone 115 was revised starting from the formation of acid chloride derivative 116 from the 4-formylbenzoic acid 42 (Scheme 5.1). The nucleophilic attack of 2-amino-4,6-dimethylpyridine on aldehyde 116 afforded the amide 117. In parallel, the resulting hydroxylamine 119 was prepared from commercially available 1-tert-butyl-4 nitrobenzene 118 in analogy to the previous conditions in the synthesis of hydroxylamine and was reacted immediately with aldehyde 117 to give nitrone 115 in a good yield.

Scheme 5.1. Synthesis of nitrone 115. Reagents and yields: (a) SOCl₂, toluene, 100 °C, 24 h, quant.; (b) 2-amino-4,6-dimethylpyridine, Et₃N, CH₂Cl₂, 0 °C to rt, 48 h, 72%; (c) Rh/C, NH₂NH₂•H₂O, THF, rt, 3 h, followed by (d) EtOH, rt, 3 days, 85% over 2 steps.

5.4 Synthesis of the model maleimides

The first model maleimide 108 that contain 1,4-phenylenediamine spacer was used directly from previous preparation. Subsequently, the preparation of maleimide 109 was amended in four steps (Scheme 5.2) starting from 3,5-dimethylbenzoic acid 120, which was converted to an acid chloride 121. The activated intermediate 121 was coupled with an excess of 4,4'-ethylenedianiline to yield the amide 122. The isolation of 122 was carried out by column chromatography from the disubstituted product and the excess of the 4,4'-ethylenedianiline. After treatment with maleic
anhydride in THF, compound 123 was converted to maleimide 109 in the presence of ZnBr₂ and HMDS in excellent yield.

Scheme 5.2. Synthesis of maleimide 109. Reagents and yields: (a) SOCl₂, toluene, 80 °C, 16 h, quant.; (b) 4,4’-ethylenedianiline, Et₃N, CH₂Cl₂, rt, 16 h, 66%; (c) Maleic anhydride, THF, rt, 3 h, 66%; (d) ZnBr₂, HMDS, MeCN, reflux, 2 h, 96%.

Subsequently, maleimide 110 bearing a 3,5-bis(trifluoromethyl)phenyl ring was similarly constructed, starting from commercially-available 3,5-bis(trifluoromethyl)benzoyl chloride 124 as depicted in Scheme 5.3.

Scheme 5.3. Synthesis of CF₃ maleimide 110. Reagents and yields: (a) 4,4’-ethylenedianiline, Et₃N, CH₂Cl₂, −10 °C to rt, 16 h, 52%; (b) Maleic anhydride, THF, rt, 3 h, 98%; (c) ZnBr₂, HMDS, MeCN, reflux, 2 h, 44%.
The CF$_3$ urea maleimide 111 was prepared from 3,5-bis(trifluoromethyl)phenyl isocyanate 127 and converted to amine 128 in the presence of excess 4,4'ethylenedianiline. After treatment of 128 with maleic anhydride in THF, acid 129 compound afforded the maleimide 111 in 78% yield (Scheme 5.4). In the following sections, the kinetic analysis for each of the prepared model maleimides with nitrone 115 are discussed in detail.

![Scheme 5.4. Synthesis of CF$_3$ urea maleimide 111. Reagents and yields: (a) 4,4'-ethylenedianiline, Et$_3$N, CH$_2$Cl$_2$, −5 °C to rt, 16 h, 63%; (b) Maleic anhydride, THF, rt, 3 h, 98%; (c) ZnBr$_2$, HMDS, MeCN, reflux, 1 h, 78%.]

5.5 Kinetic analysis of thread

Satisfyingly, all nitrone and maleimide compounds synthesised were sufficiently soluble in CDCl$_3$, to permit the reactivity study of each pair of nitrone and maleimide by performing the kinetic experiments. Seeing that nitrone 115 is capable of reacting with one of the four maleimides to form a pair of diastereoisomeric products, a total of eight possible cycloadducts were further screened for their reactivities. The reaction between nitrone and maleimide is considered as bimolecular control, in which the recognition-mediated process is not feasible in the absence of complementary carboxylic acid recognition sites. Concentration vs time profile was then constructed by deconvolution of the resonances arising from the cycloadducts.

5.5.1 Reaction with short CH$_3$ maleimide 108

The kinetic analysis of the reaction between nitrone 115 and extra short maleimide 108 give rise to thread 130 was conducted in CDCl$_3$ at 25 °C with 20 mM
concentration of starting reagents for 16 hours. The time course of the reaction was recorded by 500 MHz $^1$H NMR spectroscopic analysis, which monitored the disappearance of the resonance that arise from the maleimide reagent protons present in 108 at $\delta_H$ 6.84 and the simultaneous appearance of the resonances that emerge from the $trans\ 130$ at $\delta_H$ 5.85 and $cis\ 130\ \delta_H$ 5.27. Reaction between nitrone 115 and maleimide 108 leads to 37% overall conversion and give a diastereoisomeric mixture of 2.8:1, in favour of the $trans$ diastereoisomer (Figure 5.4).

![Figure 5.4](image)

**Figure 5.4.** (a) 1,3-dipolar cycloaddition between nitrone 115 and maleimide 108 affording $trans\ 130$ and $cis\ 130$; (b) Concentration vs time profile for the reaction between nitrone 115 and maleimide 108 with total thread conversion of 37%, affording $trans\ 130$ and $cis\ 130$ in 2.8:1 ratio. The formation of $trans\ 130$ is shown as blue filled circles and the formation of $cis\ 130$ as red filled diamonds. The reaction was performed at 25 °C in CDCl$_3$ at 20 mM reagent concentrations for 16 hours.

### 5.5.2 Reaction with long CH$_3$ maleimide 109

Kinetic analysis of the reaction between long maleimide 109 and nitrone was similarly conducted under similar conditions to afford thread 131. The control experiments between both compounds give rise to the concentration vs time profile in Figure 5.5b. The situation is fundamentally similar to the one found for the extra short maleimide 108 with the selectivity was found to be 3:1 in favour of $trans$ isomer. Overall conversion of the starting material increased nearly to 60%.
Figure 5.5. (a) 1,3-dipolar cycloaddition between nitrone 115 and maleimide 109 affording both trans 131 and cis 131; (b) Concentration vs time profile for the reaction between nitrone 115 and maleimide 109 with total thread conversion of 60% affording trans 131 and cis 131 in 3.1:1 ratio. The formation of trans 131 is shown as blue filled circles and the formation of cis 131 as red filled diamonds. The reaction was performed at 25 °C in CDCl₃ at 20 mM reagent concentrations for 16 hours.

5.5.3 Reaction with CF₃ amide maleimide 110

Accordingly, the kinetic data between nitrone 115 and CF₃ maleimide 110 was recorded for 16 hours at 25 °C in CDCl₃ with 20 mM concentration of the starting materials. Deconvolution of the obtained 500 MHz ¹H NMR spectra allowed the construction of the concentration vs time profile as depicted in Figure 5.6b. The formation of thread 132 proceed with 24% conversion with a ratio of 2.7:1 trans isomer over cis isomer.
Figure 5.6. (a) 1,3-dipolar cycloaddition between nitrone 115 and maleimide 110 affording trans 132 and cis 132; (b) Concentration vs time profile for the reaction between nitrone 115 and maleimide 110 with total thread conversion of 24% affording trans 132 and cis 132 in 2.7:1 ratio. The formation of trans 132 is shown as blue filled circles and the formation of cis 132 as red filled diamonds. The reaction was performed at 25 °C in CDCl₃ at 20 mM reagent concentrations for 16 hours.

5.5.4 Reaction with CF₃ urea maleimide 111

The final kinetic experiment to examine is the reaction between CF₃ urea maleimide 111 and nitrone 115 forming cycloadducts 133 (Figure 5.7), which was monitored using 500 MHz ¹H NMR spectroscopy.

Figure 5.7. (a) 1,3-dipolar cycloaddition between nitrone 115 and maleimide 111 affording trans 133 and cis 33; (b) Concentration vs time profile for the reaction between nitrone 115 and maleimide 111 with total thread conversion of 39% affording trans 133 and cis 133 in 2.6:1 ratio. The formation of trans 133 is shown as blue filled circles and the formation of cis 133 as red filled diamonds. The reaction was performed at 25 °C in CDCl₃ at 20 mM reagent concentrations for 16 hours.
The bimolecular reaction again give *trans* 133 in a ratio of 2.6:1 over its isomeric partner with 39% overall conversion. As a consequence of the change in spacer length, the overall conversion of the starting materials have significantly affected from 37% in the case of short maleimide 108 to 60% for longer maleimide 109. However, in the case of CF₃ amide maleimide 110, the overall conversion (24%) is less pronounced in comparison with CF₃ urea maleimide 111 (39%). It can be seen that the efficiency of the bimolecular reaction are least influenced by the introduction of the fluorine tag.

5.6 Synthesis of the control nitrone

In order to synthesize control nitrone 134 in two steps sequence, compound 118 was similarly reduced to the corresponding hydroxylamine 119 followed by the condensation with 4-tert-butylbenzaldehyde to furnish control nitrone 134 in Scheme 5.5.

![Scheme 5.5. Synthesis of nitrone 134. Reagents and yields: (a) Rh/C, NH₂NH₂•H₂O, THF, rt, 4 h; (b) 4-tert-butylbenzaldehyde, EtOH, rt, 16 h, 44% over 2 steps.]

5.7 Kinetic analysis of simple rotaxane formation

We have realised that the ability of the system designed to self-replicate via Replication model 1 is a big disadvantage, as a result of the low reactivity of the [L-M] pseudorotaxane. There will be a competition between thread and rotaxane formation, which dependent on the \( K_a \) values of [L-M] complex. In order to substantiate this fact, a thorough kinetic analysis of the formation of the non-replicating rotaxane was conducted. This investigation will only exploit short CH₃ maleimide 108 and long CH₃ maleimide 109 in the presence of both macrocycles MDG and MP. All four experiments were followed using 500 MHz \(^1\)H NMR spectroscopy in CDCl₃ for 16 hours at 25 °C with 20 mM concentration of the starting materials.
A kinetic model was developed for all plausible interactions between these three building blocks of nitrone, maleimide and macrocycle compounds. The obtained data were fitted using the SimFit program and the bimolecular rate constants were extracted for the formation of the free thread and rotaxane cycloadducts. The association constants between the model maleimide and the selected macrocycle were taken from Chapter 4, ranging from 50 M\(^{-1}\) to 280 M\(^{-1}\). At least four different products are formed in the reaction mixture with elaborated structure of each potential diastereoisomers of thread and rotaxane component, rendering the spectra complex. The elucidation by \(^1\)H NMR spectroscopy is however, not always straightforward. Hence, the total integration for both diastereoisomers of the thread and rotaxane were taken to provide useful information for the kinetic analysis of the system (see Chapter 10 for details).

The reaction between nitrone 134 and maleimide 108 yielded almost 79% thread 135 in the absence of macrocycle MDG (Figure 5.8a). Under similar conditions, maleimide 108 and macrocycle MDG was pre-equilibrated for 1 hour to allow the formation of [2]-pseudorotaxane complex. The \(K_a\) of the [MDG\(\cdot\)108] complex was determined to be 70 ± 7 M\(^{-1}\). The [MDG\(\cdot\)108] complex can then react with nitrone 134 to form rotaxane 136. Alternately, free malemide 108 can react with the same nitrone 115 to form the thread 135. The total conversion of rotaxane 136 is only 13% yield as a mixture of both \(trans\) 136 and \(cis\) 136 together with thread 135 in 60% yield. The thread to rotaxane ratio was calculated to be 4.6:1 in favour of \(trans\) diastereoisomers. The bimolecular rate constants were extracted from the fitting procedure giving \(k_{thread} = 3.30 \times 10^{-3}\) M\(^{-1}\) s\(^{-1}\) and \(k_{rotaxane} = 6.96 \times 10^{-4}\) M\(^{-1}\) s\(^{-1}\), respectively. Thus, the formation of the thread 135 was accelerated by a factor of 4.7 in comparison to the rotaxane 136 (Figure 5.8b).

Additionally, 500.1 MHz 2D ROESY NMR experiment was carried out at 295 K (Figure 5.9) to provide structural information of the isolated \(trans\) rotaxane 136. Inspection of the 2D ROESY NMR spectrum revealed crosspeaks between the macrocycle NH protons NH\({}^{33R}\) and the thread 135 protons, H\(^{4T}\) and H\(^{9T}\). The analysis of the 2D ROESY experiment corroborated the predicted geometry of the rotaxane 136 as illustrated in Figure 5.9a.
Figure 5.8. (a) The overall reaction scheme between maleimide 108, macrocycle MDG and nitrone 134. The reaction was performed at 25 °C in CDCl₃ at 20 mM reagent concentrations for 16 h; (b) Concentration vs time profile for the reaction of maleimide 108 and nitrone 134 to give thread 135. In the absence of macrocycle MDG, thread 135 (79%) was isolated. (c) Concentration vs time profile for the reaction of maleimide 108, macrocycle MDG and nitrone 134 to give rotaxane 136. In the presence of MDG, thread conversion has drop to 60% alongside the formation of rotaxane of 13% yield. The formation of thread is shown as green filled triangles and the formation of rotaxane is shown as purple filled rectangulars. Solid lines represent the results for the fitting of the thread 135 (green line) and rotaxane 136 (purple line) product.
Accordingly, the macrocycle **MDG** was replaced with macrocycle **MP**. It was demonstrated that macrocycle **MP** can form a pseudorotaxane complex with short maleimide 108 and the $K_a$ was estimated to be $280 \pm 28 \text{ M}^{-1}$. The similar time course of the reaction was monitored by 500 MHz $^1\text{H}$ NMR spectroscopy. An equimolar solution of maleimide 108 and macrocycle **MP** in CDCl$_3$ was equilibrated at 25 °C for 1 hour, before nitrone 134 was added to the solution. The reaction gave after 16 hours rotaxane 137 in 31% yield and thread 135 in 44% yield as a mixture of its trans and cis diastereoisomers (Figure 5.10b).

However, the ratio of rotaxane to thread was low, due to the substantial amount of thread present over the rotaxane. The obtained data were fitted using the SimFit, to give the rate constants $k_{\text{thread}} = 4.75 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ and $k_{\text{rotaxane}} = 1.30 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$, respectively. Even though the ratio of rotaxane to thread has improved to 1.6:1 from previous experiment, the rate constant formation of thread 135 continue to be significantly higher than rotaxane 137 in a ratio of 3.7:1.0.
Figure 5.10. (a) The overall reaction scheme between maleimide 108, macrocycle MP and nitrone 134. The reaction was performed at 25 °C in CDCl₃ at 20 mM reagent concentration for 16 h; (b) Concentration vs time profile for the reaction of maleimide 108 and nitrone 134 to give thread 135. In the absence of macrocycle MP, thread 135 (79%) was isolated. (c) Concentration vs time profile for the reaction of maleimide 108, macrocycle MP and nitrone 134 to give rotaxane 137. In the presence of macrocycle MP, thread conversion has drop to 44% with the increase formation of rotaxane in 31% yield. The formation of thread is shown as green filled triangles and the formation of rotaxane is shown as purple filled rectangulars. Solid lines represent the results for the fitting of the thread 135 (green line) and rotaxane 137 (purple line) product.
The 2D ROESY spectrum of \textit{trans} rotaxane 137 displayed crosspeaks between the NH protons of the macrocycle, NH\textsuperscript{33R} with the aromatic proton H\textsuperscript{4T} and H\textsuperscript{9T} of the thread 135. Additional crosspeaks were also observed between the proton resonance NH of the thread 135, NH\textsuperscript{7T} and the macrocycle methylene protons, H\textsuperscript{40R}, and thus conformed the encircling of the macrocycle MP about the amide moiety of thread 135 (Figure 5.11).

\textbf{Figure 5.11.} (a) ROESY contacts observed for the \textit{trans} rotaxane 137 are shown as blue arrows. (b) Partial 2D ROESY (500.1 MHz, 294 K, CDCl\textsubscript{3}) spectrum display crosspeaks (red points) between NH\textsuperscript{33R} of the ring (R) and H\textsuperscript{4T}, H\textsuperscript{9T} of thread (T) component. NH\textsuperscript{7T} of thread also display a crosspeak with H\textsuperscript{40R} of the ring.
Subsequently, the longer CH₃ maleimide 109 was reacted with nitrone 134 in the absence of macrocycle MDG (Figure 5.12). This reaction gave, after 16 hours in CDCl₃ at 25 °C with starting concentration of the reagents of 20 mM, the thread 138 in 67% yield \( (k_{\text{thread}} = 4.19 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}). \)

![Figure 5.12](image)

**Figure 5.12.** (a) The overall reaction scheme between maleimide 109, MDG and nitrone 134. The reaction was performed at 25 °C in CDCl₃ at 20 mM reagent concentration for 16 h; (b) Concentration vs time profile for the reaction of maleimide 109 and nitrone 134 to give thread 138. In the absence of MDG, thread 138 (67%) was isolated. (c) Concentration vs time profile for the reaction of maleimide 138, macrocycle MDG and nitrone 134 to give rotaxane 139. In the presence of MDG, thread conversion has drop to 62% alongside the formation of rotaxane of 18% yield. The formation of thread is shown as green filled triangles and the formation of rotaxane is shown as purple filled rectangulars. Solid lines represent the results for the fitting of the thread 138 (green line) and rotaxane 139 (purple line) product.
Similarly, the reaction between the nitrone 134 and maleimide 109 was performed in the presence of macrocycle MDG. A mixture of maleimide 109 and macrocycle MDG was pre-equilibrated for 1 hour to allow the formation of [2]-pseudorotaxane [MDG•109]. The $K_a$ for [MDG•109] complex was determined to be $50 \pm 5 \text{ M}^{-1}$. The addition of nitrone 134 furnish the rotaxane 139 in 18% yield as a mixture of its trans and cis diastereoisomer and thread in 62% yield as a mixture of its trans and cis diastereoisomer (Figure 5.12). The overall conversion is 3.4:1 for thread to rotaxane, decrease from the one found with the extra short maleimide 108. Furthermore, the bimolecular rate constant of $k_{\text{thread}}$ is reduced to $4.42 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ as opposed to $k_{\text{rotaxane}} 1.89 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$. Nonetheless, the thread was formed 2.3 faster than the rotaxane.

![Figure 5.13.](attachment:image)

**Figure 5.13.** (a) ROESY contacts observed for the trans rotaxane 139 are shown as blue arrows. (b) Partial 2D ROESY (500.1 MHz, 294 K, CDCl$_3$) spectrum display crosspeaks (red points) between NH$^{39R}$ of the ring (R) and H$_{4T}$, H$_{9T}$ of thread (T) 138.

Direct evidence of the proposed structure for trans rotaxane 139 was supported from the 2D ROESY NMR spectrum (Figure 5.13) in which two crosspeaks were revealed between the ring proton resonances, NH$^{39R}$ with the two aromatic proton resonances, H$_{4T}$ and H$_{9T}$ of the thread components.
Figure 5.14. (a) The overall reaction scheme between maleimide 109, MP and nitrone 134. The reaction was performed at 25 °C in CDCl₃ at 20 mM reagent concentration for 16 h; (b) Concentration vs time profile for the reaction of maleimide 109 and nitrone 134 to give thread 138. In the absence of MP, thread 138 (67%) was isolated. (c) Concentration vs time profile for the reaction of maleimide 138, macrocycle MP and nitrone 134 to give rotaxane 140. In the presence of MP, thread conversion has drop to 43% with the increase formation of rotaxane in 35% yield. The formation of thread is shown as green filled triangles and the formation of rotaxane is shown as purple filled rectangulars. Solid lines represent the results for the fitting of the thread 138 (green line) and rotaxane 140 (purple line) product.
The final utilization of macrocycle MP within the rotaxane framework was conducted subject to prior conditions for the same time course (Figure 5.14). The ability of macrocycle MP to associate with longer maleimide 109 was assessed and established the $K_a$ value of $170 \pm 20$ M$^{-1}$. An equimolar solution of maleimide 109 and macrocycle MP in CDCl$_3$ was again pre-equilibrated for 1 hour before the addition of nitrone 134 to the solution. The overall conversion was found to be nearly 80% after 16 hours. In the case of rotaxane 140, the total yield was about 35% yield for both cycloadducts while the thread 138 diastereoisomers was formed in 43% yield. It is important to note that the comparable yields between thread 138 and rotaxane 140 results from the exploitation of macrocycle MP and, therefore encouraging. On that account, the ratio of thread 138 to rotaxane 140 was lessened from 3.4:1 to 1.2:1 when the macrocycle MP was employed. Despite that, bimolecular rate constant for the formation of thread 138 ($k_{\text{thread}}$ of $4.29 \times 10^{-3}$ M$^{-1}$ s$^{-1}$) was exhibited to be 1.8 faster than rotaxane 140 ($k_{\text{rotaxane}}$ of $2.41 \times 10^{-3}$ M$^{-1}$ s$^{-1}$).

The conformation of the synthesised trans rotaxane 140 was elucidated by 2D ROESY spectrum as shown in Figure 5.15. As predicted, the macrocycle MP was located around the amide moiety of thread 138, which is apparent from the two set of crosspeaks in the ROESY spectrum between the NH proton of the ring, NH$_{39R}$ and the thread aromatic protons, H$_{4T}$ and H$_{9T}$ and between the NH proton of the thread, H$_{7T}$ and the methylene proton resonance of the ring, H$_{46R}$. As detailed above, the interaction between macrocycle MP and the thread 138 was through hydrogen bonding and, hence, corroborated the expected geometry shown in Figure 5.15a.
5.7.1 Comparison of the analysed systems

It is noteworthy that the efficiency of bimolecular reaction increases with the spacer length of the maleimide compounds. For instance, the incorporation of a long spacer maleimide 109 influence the ratio of thread to rotaxane from 4.6:1 to 3.4:1 in the presence of macrocycle MDG. Additionally, more significant increase in the rotaxane formation was observed when macrocycle MP was involved in the reaction mixture, in which the ratio of rotaxane to thread has been largely improved to nearly 1:1.6 in the case of short maleimide 108 and 1:1.2 for longer maleimide 109. Thus, the reduced reactivity of thread generated the formation of [2]-pseudorotaxane complex [MP•108] and [MP•109] more reactive towards the reaction with nitrones 134.

It is apparent that there are two main obstacles to overcome. Fundamentally, this finding explain the critical role of high association constant, $K_a$ to provide sufficient
amount of pseudorotaxane precursor in the rotaxane synthesis since the formation of this complex predominate the kinetic pathways that include the free maleimides, which eventually results in the formation of undesired thread. Thus, the diminution of free maleimides consequently shifts the central equilibrium to the right hand side, directing the rotaxane formation as presented in the simple kinetic model of rotaxane in Chapter 3. As an acceptable level of selectivity for rotaxane being at least 10:1, a $K_a$ value greater than 5000 M$^{-1}$ is desired. We have shown earlier in Chapter 4 how these binding affinities between the macrocycle and the guest was varied to establish the stability of the pseudorotaxane complex to produce sufficient amount of rotaxanes.

However, the simple kinetic model is relevant only if we assumed that both the rate constants for the thread and rotaxane are the same ($k_{\text{thread}} = k_{\text{rotaxane}}$). In the current investigations, it is an obvious remark to make that the maleimide and pseudorotaxane complex in all cases did not have the same reactivity towards the stoppering reagent, the nitrone compound. Eventhough we managed to achieve a high association constant for the pseudorotaxane complex, the relative rate constant for rotaxane formation could still come behind and less reactive than the thread formation. As a consequence, the bimolecular reaction of the thread can start to operate much earlier than of the rotaxane. At this stage, it is important to emphasize the important consequence of this central equilibrium. It is evident from this evaluation that the position of the equilibrium between the free maleimide, $L$ and macrocycle, $M$ and the corresponding pseudorotaxane complex is central to the success of this enterprise.

Since our system is a second order reaction, the implementation of Curtin-Hammett principle is thus inappropriate. The reason is that the product ratio cannot only be determined on the basis of the relative heights of the activation energy barriers between the thread and the rotaxane cycloadducts because the reversible binding event between the free maleimide and the pseudorotaxane complex governs the final product composition. An overall summary of these kinetic observations was presented in Table 5.1.
Table 5.1. Overall conversion and bimolecular rate constants for the cycloadducts product of thread and rotaxane obtained from the (a) kinetic experiments of nitrone 134, short maleimide 108, macrocycle MDG or MP and (b) kinetic experiments of nitrone 134, long maleimide 109, macrocycle MDG or MP. These reactions were performed in CDCl₃ at 25 °C at 20 mM concentration of starting reagents for 16 hours.

(a) Short maleimide 108

<table>
<thead>
<tr>
<th>Macrocycle</th>
<th>MDG</th>
<th>MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_a )</td>
<td>–</td>
<td>70 ± 7 M⁻¹</td>
</tr>
<tr>
<td>( k_{\text{thread}} )</td>
<td>3.47 × 10⁻³ M⁻¹ s⁻¹</td>
<td>3.30 × 10⁻³ M⁻¹ s⁻¹</td>
</tr>
<tr>
<td>( k_{\text{rotaxane}} )</td>
<td>–</td>
<td>6.96 × 10⁻⁴ M⁻¹ s⁻¹</td>
</tr>
</tbody>
</table>

(b) Long maleimide 109

<table>
<thead>
<tr>
<th>Macrocycle</th>
<th>MDG</th>
<th>MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_a )</td>
<td>–</td>
<td>50 ± 5 M⁻¹</td>
</tr>
<tr>
<td>( k_{\text{thread}} )</td>
<td>4.19 × 10⁻³ M⁻¹ s⁻¹</td>
<td>4.42 × 10⁻³ M⁻¹ s⁻¹</td>
</tr>
<tr>
<td>( k_{\text{rotaxane}} )</td>
<td>–</td>
<td>1.89 × 10⁻⁴ M⁻¹ s⁻¹</td>
</tr>
</tbody>
</table>
5.8 Kinetic analysis of simple rotaxane formation 2

As explained earlier, the $^1$H NMR analysis of the previous kinetic experiments is not always straightforward as a consequence of the emergence of four different diastereoisomers with elaborated structures, rendering the spectra fairly crowded. In one occasion, the resonances for trans thread and trans rotaxane are partly coincide with each other in the first few hours of these experiments. Additionally, the resonances arising from the cis diastereoisomers of thread and rotaxane overlap between each other throughout the experiment. On that account, the thread diastereoisomers were initially synthesised and isolated to simplify each kinetic analysis. The new approach was outlined which integrate the fluorine tags in the preparation of nitrone building blocks and the full kinetic analysis was conducted by monitoring the $^{19}$F resonances (see Chapter 10 for more detail). These resonances were further deconvoluted to construct the concentration vs time profile.

5.8.1 Synthesis of the control nitrone 2

1-tert-butyl-4-nitrobenzene 118 was converted to hydroxylamine intermediate as seen in Scheme 5.5. In order to substitute the tert-butyl group in the control nitrone 134, 3,5-difluorobenzaldehyde and 3,5-bis(trifluoromethyl)benzaldehyde 148 were appropriately selected as the corresponding aldehyde in the condensation reaction to furnish F nitrone 141 and CF$_3$ nitrone 142 in a moderate yield.

![Scheme 5.6](image)

Scheme 5.6. Synthesis of F nitrone 141 and CF$_3$ nitrone 142. Reagents and yields: (a) Rh/C, NH$_2$NH$_2$•H$_2$O, THF, rt, 3 h; followed by 3,5-difluorobenzaldehyde, EtOH, rt, 16 h, 26% over 2 steps; (b) Rh/C, NH$_2$NH$_2$•H$_2$O, THF, rt, 3 h; followed by 3,5-bis(trifluoromethyl)benzaldehyde 148, EtOH, rt, 16 h, 49% over 2 steps.

The probability of macrocycle MDG to associate the synthesised control nitrone 141 and 142 prompted the investigation of additional binding experiments (Figure 5.16). An equimolar solution of macrocycle MDG and F nitrone 141 in CDCl$_3$ was prepared and analysed by $^1$H NMR spectroscopy. The 300.1 MHz $^1$H NMR spectrum of this mixture (Figure 5.16d) display an intermediate exchanges with broad peaks, and thus prevent the determination of $K_a$. In contrast, the binding study between CF$_3$
nitronc 142 and macrocycle MDG (Figure 5.16b) shows that the macrocyclic component does not form a pseudorotaxane with the nitrone guest, as such no noticeable changes on the ¹H NMR spectrum of macrocycle MDG upon addition of CF₃ nitrone 142. On that account, CF₃ nitrone 142 was incorporated as alternative control nitrone compound in the second design of simple rotaxane formation.

![Figure 5.16. Partial ¹H NMR spectra (300.1 MHz, 25 °C, CDCl₃, 20 mM) of (a) CF₃ nitrone 142; (b) an equimolar mixture of macrocycle MDG and CF₃ nitrone 142,(c) macrocycle MDG, (d) an equimolar mixture of macrocycle MDG and F nitrone 141 and (e) F nitrone 141.](image)

The maleimide 108 was reacted with CF₃ nitrone 142 in the absence of macrocycle MDG. This reaction was monitored by ¹⁹F NMR spectroscopy after 16 hours in CDCl₃ at 25 °C with starting concentration of 20 mM, give rise to thread 143 in 42% total conversion as a 6.2:1 mixture of its diastereoisomer, in favour of trans isomer. The bimolecular rate constant for thread 143 was extracted as 4.87 × 10⁻⁴ M⁻¹ s⁻¹.

The reaction between maleimide 108 and CF₃ nitrone 142 was subsequently conducted in the presence of the macrocycle MDG (Figure 5.17). A mixture of maleimide 108 and macrocycle MDG was pre-equilibrated for 1 hour to allow the substantial amount of pseudorotaxane complex [MDG·108]. Unfortunately, the addition of CF₃ nitrone 142 furnished rotaxane 144 in only 5% yield and thread 143 in 16% yield as a mixture of both diastereoisomers. The bimolecular rate constants for thread and rotaxane were extracted, giving $k_{\text{thread}} = 4.44 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ and $k_{\text{rotaxane}} = 2.08 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, respectively.
Figure 5.17.  (a) The overall reaction scheme between maleimide 108, macrocycle MDG and CF₃ nitrone 142. The reaction was performed at 25 °C in CDCl₃ at 20 mM reagent concentration for 16 h; (b) Concentration vs time profile for the reaction of maleimide 108 and nitrone 142 to give thread 143. In the absence of macrocycle MDG, thread 143 (42%) was isolated. (c) Concentration vs time profile for the reaction of maleimide 108, macrocycle MDG and nitrone 142 to give rotaxane 144. In the presence of macrocycle MDG, thread conversion has drop to 16% alongside the formation of rotaxane of 5% yield. The formation of thread is shown as green filled triangles and the formation of rotaxane is shown as purple filled rectangulars. Solid lines represent the results for the fitting of the thread (green line) and rotaxane (purple line) product.
Figure 5.18. (a) The overall reaction scheme between maleimide 108, macrocycle MP and nitrone 142. The reaction was performed at 25 °C in CDCl₃ at 20 mM reagent concentrations for 16 h; (b) Concentration vs time profile for the reaction of maleimide 108 and nitrone 142 to give thread 143. In the absence of macrocycle MP, thread 142 (42%) was isolated. (c) Concentration vs time profile for the reaction of maleimide 108, macrocycle MP and nitrone 142 to give rotaxane 145. In the presence of MP, thread conversion has drop to 20% with the increase formation of rotaxane in 30% yield. The formation of thread is shown as green filled triangles and the formation of rotaxane is shown as purple filled rectangles. Solid lines represent the results for the fitting of the thread (green line) and rotaxane (purple line) product.
Accordingly, macrocycle MP and maleimide 108 was again pre-equilibrated in CDCl$_3$ for one hour to help with the formation of pseudorotaxane [MP·108] complex (Figure 5.18). The addition of CF$_3$ nitrone 142 gave, after 16 hours at 25 °C, rotaxane 145 in 30% yield and thread 143 in 20% yield as a mixture of trans and cis cycloadducts. The bimolecular rate constants were extracted and fitted using the SimFit protocol, to give $k_{\text{thread}}$ of $1.12 \times 10^{-3}$ M$^{-1}$ s$^{-1}$ and $k_{\text{rotaxane}}$ of $8.66 \times 10^{-4}$ M$^{-1}$ s$^{-1}$, respectively. Likewise, the ratio of rotaxane to thread has improved compared to the previous system as a consequence of increase binding constant when macrocycle MP was employed. This is evident from the comparable rate formation of thread against the rotaxane formation with a ratio 1.3:1. Nevertheless, the overall conversion of the cycloadducts for both systems is not encouraging.

Comparing the concentration vs time profile between the rotaxane 136 and 137 from Figure 5.8 shows that the introduction of the 3,5-bis(trifluoromethyl) substituent in the previous control nitrone compound 134 had an impact towards the outcome of the final cycloadducts. In the presence of macrocycle MDG, the fluorine tagged nitrone 142 yield only 5% yield of rotaxane 144 and further improved to 30% yield of rotaxane 145 in the presence of macrocycle MP. A brief summary of the two systems are presented in Table 5.2.

Table 5.2. Overall conversion and bimolecular rate constants for the thread and rotaxane obtained from the kinetic experiments of CF$_3$ nitrone 142, short maleimide 108 in the presence of macrocycle MDG or macrocycle MP. The reactions were carried out in CDCl$_3$ at 25 °C with 20 mM concentration of starting reagents for 16 hours.

<table>
<thead>
<tr>
<th>Macrocycle</th>
<th>MDG</th>
<th>MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_a$</td>
<td>70 ± 7 M$^{-1}$</td>
<td>280 ± 28 M$^{-1}$</td>
</tr>
<tr>
<td>$K_{\text{thread}}$</td>
<td>$4.87 \times 10^{-4}$ M$^{-1}$ s$^{-1}$</td>
<td>$4.44 \times 10^{-4}$ M$^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>$k_{\text{rotaxane}}$</td>
<td>$2.08 \times 10^{-4}$ M$^{-1}$ s$^{-1}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$8.66 \times 10^{-4}$ M$^{-1}$ s$^{-1}$</td>
</tr>
</tbody>
</table>
5.9 Computational study

In order to gain a comprehensive understanding on the reduced reactivity of the CF$_3$ nitrone 142, the semi-empirical electronic structure calculations were employed to locate the transition state for the dipolar cycloaddition reaction in our systems. The transition state for a number of scenarios were located using a grid scanning method. The C–C and C–O bonds in the cycloadduct were gradually lengthened, in steps of 0.08 Å, and the RM1 energy computed at each point on the grid. These calculations allowed us to construct a contour plot (see Figure 5.19) for the transformation of nitrone and maleimide into the cycloadduct. The approximate location of the transition state determined in these grid calculations was then used as the basis for a further RM1 calculation$^{226}$ of the exact transition state structure. We have demonstrated previously that the RM1 semi-empirical method affords transition state structures in close agreement with higher level DFT calculations (for example, M06-2X or B3LYP) and are much more practical in terms of computation time given the large size of the structures under investigation here.

The reaction between the control nitrone 134 and short maleimide 108 was first computed using RM1 in the absence and the presence of macrocycle MDG. One of the feasible calculated transition state structure is illustrated in Figure 5.19a and Figure 5.19c for bis-tert-butyl thread and bis-tert-butyl rotaxane, respectively. It is visible from the calculated structure that the association of the macrocycle MDG to the amide binding site in maleimide compound exerted a remote steric effect on the transition state. The tert-butyl group of the nitrone is located close to the diethylene glycol region of the ring, issuing a pronounced van der Waals contacts. As a consequence, the transition state in the reaction between pseudorotaxane [MDG-108] and control nitrone 134 are certainly destabilized in comparison to the transition state obtained in the absence of macrocycle MDG.
Figure 5.19. Stick representation of the calculated (RM1 semi-empirical method) transition state structures for (a) the reaction between control nitrone 134 and short maleimide 108 and (c) the reaction between the control nitrone 134 and the pseudorotaxane complex between short maleimide 108 and macrocycle MDG; (b) and (d) Contour plot of the potential energy surface for transition state obtained for reaction (a) and reaction (c) and; (e) space filling model of rotaxane formation revealed that the tert-butyl group of nitrone 134 is essentially in van der Waals contacts with the diethylene glycol unit of macrocycle MDG. Carbon atoms are coloured green, oxygen atoms in red, nitrogen atoms in blue and hydrogen atoms in white although most hydrogen atoms have been removed for clarity. Bond formations are shown as dotted line.
Figure 5.20. Stick representation of the calculated (RM1 semi-empirical method) transition state structures for (a) the reaction between nitrone 142 and short maleimide 108 and (c) the reaction between the nitrone 142 and the pseudorotaxane complex between short maleimide 108 and macrocycle MDG, (b) and (d) Contour plot of the potential energy surface for transition state obtained for reaction (a) and reaction (c) and; (e) space filling model of rotaxane formation revealed that a more severe steric impact imposed by the bis-CF$_3$ group of 142 in the transition state between [MDG·108] complex and nitrone 142 as a result of the closely packed location to the diethylene glycol loop of the macrocycle. Carbon atoms are coloured green, oxygen atoms in red, nitrogen atoms in blue and hydrogen atoms in white although most hydrogen atoms have been removed for clarity. Bond formations are shown as dotted line.
The drastic changes observed in the reduced reactivity of thread (Figure 5.20a) and rotaxane (Figure 5.20c) formation when we employed the control CF$_3$ nitrone 142. This observation was similarly inspected by means of optimization of transition state calculated structure to provide a proper insight about the electronic structure and properties of CF$_3$ nitrone 142. The space filling model (Figure 5.20e) reveals more severe steric impact opposed by the 3,5-bis(trifloromethyl) substituent in the transition state between the complex of [MDG•108] and CF$_3$ nitrone 142 as a result of the closely packed location to the diethylene glycol loop of the macrocycle. The transition state of the rotaxane formation is undoubtedly deprived from the resulting steric barrier force, in which helps to justify the poor conversion in the rotaxane formation.

These calculations corroborated our recent investigations and once again suggests that an appropriate spacer length are vital to ensure that the reactive site on the thread component are sufficiently located away from the binding site of the macrocycle unit, rendering the introduction of the supramolecular steric effect and therefore help to improve the reactivity of the pseudorotaxane complex towards the stoppering reagent (the nitrone compound) to give a substantial amount of the desired rotaxane.
5.10 Conclusion

In the first part of the chapter, the analysis of the structural invariances of the thread component between nitrone 115 and the four maleimide compounds (108 – 111) were conducted. For all combinations, the reactivity of trans diastereoisomer of the cycloadducts is evident. The trans diastereoisomer dominated over the cis diastereoisomer by means of typical bimolecular reaction in 3:1 ratio. Overall, the four investigated systems demonstrated that the outcome of these control reactions can be predicted, even when a different maleimide compound was employed.

The second part of this chapter reveals the kinetic analysis for simple rotaxane formation. The relationship between a high association constant, $K_a$ and the substantial amount of rotaxane, $R$ over the thread, $T$ is described. Although the binding constant for the pseudorotaxane \([L\cdot M]\) is considerably low, the notion of our simple kinetic model of rotaxane formation in Chapter 3 are proved to be working as expected. In addition, the obtained bimolecular rate constants for the formation of thread and rotaxane compounds with two different maleimides, 108 and 109 issued two major importances. Bimolecular rate constant for rotaxane formation increase with the size of the spacer unit in the long CH$_3$ maleimide 109 in comparison with short CH$_3$ maleimide 108. In general, a high bimolecular rate constant of the desired product in the self-replicating framework could shorten the lag period in the early stage of the reaction. On the basis of the thread:rotaxane ratio, the results presented in Table 5.1 give an encouraging start as the yield of the thread:rotaxane ratio decrease from 4.6 to 3.4 when diphenylethane spacer was employed in the presence of macrocycle MDG. Hence, the incorporation of the diphenylethane spacer in the maleimide component has considerably improved the productivity of the system.

We have learnt that the association constant, $K_a$ can be manipulated by radical design of the binding site of the guest or the macrocycle. Therefore, the formation of rotaxanes are improved when employing alternative macrocycle MP, which leads to the thread: rotaxane ratio of 1.6:1 (in the case of short maleimide 108) and 1.2:1 (in the case of long maleimide 109), respectively. The introduction of the fluorine tagged compounds in one of the building blocks of non-replicating rotaxane is initially proposed to help with the deconvolution of the cycloadducts resonances arising from both thread and rotaxane. Nonetheless, the reaction between short maleimide 108
and CF\textsubscript{3} nitrene 142 in the presence of macrocycle MDG or MP suffer severe steric effects in the transition state as a consequence of the clashing location of the tert-butyl or CF\textsubscript{3} groups that is closely orientated towards the lower fragment of the macrocycle component which destabilized the transitions states for the subsequent stoppering reaction.

As discussed earlier, the implementation of the simple kinetic model of rotaxane formation is appropriate if the bimolecular rate constants of $k_{\text{thread}}$ and $k_{\text{rotaxane}}$ are equal. However, this is not what we found in our own systems. We observed different reactivity of thread and rotaxane, which emanates from the central reversible equilibrium of free maleimide compound and the pseudorotaxane complex. Thus, the position of this equilibrium is the primary concerns in the investigated systems. In conclusion, this finding reveal that the [R]/[T] ratio is sensitive to both the association constant, $K_a$ for the pseudorotaxane complex [L•M] and to the ratio of $k_{\text{rotaxane}}/k_{\text{thread}}$. 
Low temperature capture of pseudorotaxanes

6.1 Introduction

As explained earlier, our initial effort\(^{207}\) to integrate replication processes with mechanically interlocked molecules in Replication model 1 met with limited success. The reasons for our failure are again encapsulated in Figure 6.1a. A plausible kinetic model for rotaxane formation, in which we are trying to maximise the amount of rotaxane formed, involves two irreversible reactions coupled to a central equilibrium.

The linear component of the rotaxane L associates with macrocycle M to form the [L•M] complex. This complex is then captured irreversibly by reaction with S to form rotaxane R. If, however, the linear component L reacts directly with the capping reagent S, then product T, which does not contain the macrocycle, is formed. Therefore, a key marker for the efficiency of the rotaxane-forming protocol is the ratio of R to T. Kinetic simulation (Figure 6.1b) reveals that this [R]/[T] ratio is sensitive to both the association constant, \(K_a\), for the [L•M] complex and to the ratio \(k_R/k_T\). If we assume that the \(K_a\) for the [L•M] complex is 5000 M\(^{-1}\) and \(k_T = k_R\), then the [R]/[T] ratio in the final reaction mixture is 10.8 (green line, Figure 6.1b).

However, if \(k_T = 2 k_R\), i.e. the [L•M] complex is less reactive than L itself, then a \(K_a\) for the [L•M] complex of 5000 M\(^{-1}\) results in a [R]/[T] ratio of 6.1 (orange line, Figure 6.1b). Such situations are relatively common in rotaxane synthesis as there are frequently remote steric effects\(^{227-230}\) arising from the close proximity of the three species L, M and S at the transition state leading to R. The situation deteriorates as the \(k_R/k_T\) ratio diminishes – when \(k_T = 4 k_R\) the [R]/[T] ratio in the final reaction mixture is only 3.4 (red line, Figure 6.1b).
Figure 6.1. (a) The linear component of the rotaxane L associates with macrocycle M to form the [L•M] complex. This complex is then captured irreversibly by reaction with S to form rotaxane, R. The linear component L can also react directly with the capping reagent S, forming T, which does not contain the macrocycle. (b) Plot of [R]/[T] against $K_a$ for the kinetic scheme described in part (a). In the simulation, the starting concentrations of L, M and S are 25 mM. Rate constant $k_T$ is $1 \times 10^{-3}$ M$^{-1}$s$^{-1}$ and $k_R$ was varied from $4 \times 10^{-3}$ M$^{-1}$s$^{-1}$ (blue line) to $2.5 \times 10^{-4}$ M$^{-1}$s$^{-1}$ (red line) – the value of $k_R$ being halved in each step.

In our original work (see example in Chapter 5), the $K_a$ for the [L•M] complex was around 100 M$^{-1}$ and $k_T = 3 \ k_R$. This set of parameters led to unacceptably small [R]/[T] ratios. Clearly, this situation could be addressed by a radical redesign of the recognition event that creates the [L•M] complex in order to increase the strength of this association. However, in some situations, this strategy may be difficult to implement and synthetically challenging.
6.2 An alternative stoppering reaction

As an alternative strategy, we reasoned that it should be possible to manipulate the $K_a$ for the $[L \cdot M]$ complex by means of a change in temperature. By lowering the temperature, the $K_a$ should increase and leading, therefore increase the population of $[L \cdot M]$ species. Manipulating the population of $[L \cdot M]$ will, ultimately, lead to an increase in the $[R]/[T]$ ratio. However, as the temperature decreases, the efficiency of the capping reactions between either $L$ or $[L \cdot M]$ and $S$ also decreases (Figure 6.2). It is unlikely that capping chemistry that is rapid at room temperature scenario would also be fast enough at lower temperatures. Nevertheless, a limited number of stoppering reactions that could possibly occur at low temperature was proving quite a challenge.

![Diagram showing the effect of temperature on $K_a$](image)

**Figure 6.2.** Cartoon representation of the effect of temperature on $K_a$. In order to establish a larger $K_a$, either the macrocycle, $M$ or guest, $L$ can be altered to increase the binding (chemical modification). Alternately, a lower temperature (condition modification) can be employed to encourage pseudorotaxane formation by increasing the $K_a$.

Herein, we describe the rapid reaction between phosphines and organic azide at low temperature as an intermediate capping step by means of Staudinger reaction
(Figure 6.3a). The iminophosphorane formed in this process can then be captured permanently through reaction with an aldehyde in an aza-Wittig reaction as depicted in Figure 6.3b.

![Diagram](image)

Figure 6.3. Cartoon representation of the stoppering process which involves: (a) Stoppering reaction via the Staudinger reaction using a reactive stopper at low temperature to maximize the yield. Azide being placed at the terminus of the thread while phosphine group acted as a large capping molecule, respectively. Once the reactive stopper is bound to the guest at low temperature, a [2]-rotaxane will be formed. However, the newly formed bond (P=N) between the stopper and guest is labile and consequently the isolation of the rotaxane is unlikely. (b) An alternative route, additional synthetic conversion of functionality by way of an aza-Wittig reaction will form a stable bond, imine (C=N) in which isolation and characterization is feasible via an aza-Wittig reaction.

Thus far, this novel stoppering strategy of converting a pseudorotaxane to a rotaxane at low temperature ought to effectively trapped as much [2]-pseudorotaxane species as possible before sequential conversion to a stable [2]-rotaxane can takes place. It is vital for the successful formation of rotaxane that sufficient binding was achieved between both the macrocycle and guest or otherwise formation of the important pseudorotaxane precursor will be limited.

As a consequence, this stoppering method relies on a large binding constant between both the macrocycle and guest to form a substantial amount of rotaxane. However, if the $K_a$ value is small, a large percentage of unbound to bound species will be present thus lowering the overall yield of the rotaxane. In order to counteract this undesirable reduction in reactivity at lower temperature, we have identified a new strategy\textsuperscript{231} as depicted in Scheme 6.1. Accordingly, we designed azide 146 as a suitable linear component for incorporation within a rotaxane framework.
Scheme 6.1. Synthesis of rotaxane 151 via novel stoppering strategy. Reagents and conditions: (a) Macrocyle MDG, CDCl₃, rt to −10 °C, 1 h; (b) PPh₃, CDCl₃, 10 °C, 48 h; (c) 3,5-bis(trifluoromethyl)benzaldehyde 148, CDCl₃, 40 °C, 48 h; (d) diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate 150, CDCl₃, 40 °C, 48 h, 28% over 4 steps.

Previously, we and others²⁰⁷,²¹¹,²¹⁴,²³²-²³⁴ have demonstrated that diaryl amide, such as 146, can be recognised and bound by macrocycle MDG, forming a complex, in this case [MDG•146] with a pseudorotaxane geometry. The dimethyl substituted aromatic ring is of the correct size and presents suitable steric hinderance to prevent slipping of the macrocycle, and thus, acting as an inert stopper. The synthesis of azide formation of 146 is accomplished in Scheme 6.2.

Scheme 6.2. Synthesis of azide compound 146. Reagents and conditions: (a) HCl (conc.), NaNO₂ (aq.), −10 °C, 1 h; followed by NaN₃ (aq.), −10 °C, rt, 2 h, 97%.
Aniline derivative 122 is treated with nitrous acid, which is generated in situ from sodium nitrite and hydrochloric acid at −10 °C to give the diazonium salts and subsequently reacted with sodium azide in aqueous solution to afford azide 146 in excellent yield. The small cavity size present in macrocycle MDG introduces a constrictive element to the binding of diaryl amide in solvents such as CDCl₃. As a result, the 500 MHz ¹H NMR spectrum of the [MDG•146] complex in CDCl₃, recorded at 10 °C exhibits separate resonances for free 146 and bound 146, indicating that azide 146, macrocycle MDG and [MDG•146] complex and are in slow exchange process on the ¹H NMR chemical shift timescale. Therefore, it is possible to assay the $K_a$ for the [MDG•146] complex in CDCl₃ using the integrals of the CH₃ resonances. Hence, the ¹H NMR data were recorded at five temperatures between +10 °C and −10 °C (Figure 6.4).

Figure 6.4. Partial ¹H NMR (500 MHz, CDCl₃) spectra either at 283 K, 273 K or 263 K, exhibited separate resonance for free azide 146 and bound azide 146, indicating that 146, macrocycle MDG and [MDG•146] are in slow exchange process on the ¹H NMR chemical shift timescale with corresponding $\delta$, 2.37 arising from free azide 146 and the pseudorotaxane [MDG•146] complex at $\delta$, 2.16 and further show that pseudorotaxane formation is favoured at low temperature. In particular, the resonances arising from the CH₃ groups on the terminal aromatic ring of compound 146 are very well resolved.
As expected, the association constant is temperature dependent. At +10 °C, the $K_a$ for [MDG•146] complex is 67 ± 5 M$^{-1}$ in CDCl$_3$, rising to 118 ± 6 M$^{-1}$ at −10 °C. Fitting of the experimental binding data to the van't Hoff equation gives an excellent fit (Figure 6.5) and affords values of $\Delta H$ and $\Delta S$ of binding for pseudorotaxane [MDG•146] of $-17.2$ kJ mol$^{-1}$ and $-25.7$ J mol$^{-1}$ K$^{-1}$, respectively.

Figure 6.5. Plot of ln $K_a$ for the [MDG•146] complex against 1000/T. Linear regression affords the line of best fit (dashed line). The gradient of this line is $10^3 \Delta H/R$ and the intercept is $\Delta S/R$.

Having established the relationship between temperature and the stability of the [MDG•146] complex, we next sought to trap this pseudorotaxane by capping. To this end, we reacted a solution of azide 146 and macrocycle MDG, each at 20 mM concentration, with PPh$_3$ at a series of temperatures from +10 °C to −10 °C. The result was rapid conversion of the pseudorotaxane [MDG•146] into rotaxane 147 $via$ the Staudinger mechanism as illustrated in Scheme 6.3.

The progress of this reaction was monitored readily by $^{31}$P NMR spectroscopy analysis – namely, the formation of the iminophosphorane results from the emergence of a new broad resonance at around $\delta_p$ +5.5. Gratifyingly, the ratio of rotaxane 147 to capped linear component 147’ mirrored the ratio of 146 and MDG to [MDG•146] complex before the addition of PPh$_3$ to the solution. Furthermore, this ratio did not change upon heating the solution, indicating that 147 was, indeed, a rotaxane and that iminophosphorane capping group was robust (Figure 6.6).
Scheme 6.3. The Staudinger mechanism. (a) Triphenylphosphine (PPh₃) reacts with azide derivative to generate a phosphoazide; (b) The mechanism then proceeds via a four membered cyclic transition state; (c) Loss of N₂ (g) forms an iminophosphorane; (d) The resonance form of the charged species equates to the P=N bond.

Figure 6.6. Partial ³¹P NMR (121.5 MHz, CDCl₃) spectrum of P=N rotaxane 147 formation. The final stoppering process involves the addition of PPh₃ at −10 °C to maximize rotaxane formation. The P=N rotaxane gives a very broad signal. An excess of PPh₃ and triphenylphosphine oxide as side product has also been clearly identified in the same spectrum.
Thus, P=N rotaxane display a very broad resonance in the 121.5 MHz $^{31}$P spectra together with triphenylphosphine oxide as a side product. In order to maximize the total conversion of pseudorotaxane [MDG•146] to rotaxane 147, this novel stoppering method required an excess of PPh$_3$, which was clearly identifiable by $^{31}$P NMR. Once macrocycle MDG is part of the rotaxane 147, we are able to manipulate this rotaxane structure synthetically by virtue of its reactive iminophosphorane capping group. Hence, the addition of aldehyde 148 and one equivalent$^{236}$ of 4-bromophenylacetic acid to the mixture of iminophosphoranes 147 and 147' in CDCl$_3$, followed by heating at 40 °C for another 48 hours effected an aza-Wittig reaction; and thus affording a mixture of imines 149 and 149'. These imines could be reduced readily by treatment with dihydropyridine 150 in CDCl$_3$ for 48 hours at room temperature; giving rise to a mixture of the rotaxane 151 and thread 151', which could be separated readily by column chromatography. The 400.1 MHz $^1$H NMR spectrum of rotaxane 151, recorded in CDCl$_3$ at room temperature, (Figure 6.7 middle) shows the characteristic pattern of chemical shift changes associated with the coconformation of the macrocycle and linear component within the rotaxane.

![Figure 6.7](image_url)

**Figure 6.7.** Partial 400.1 MHz $^1$H NMR spectrum of rotaxane 151 (bottom) recorded in CDCl$_3$ at room temperature. The same chemical shift region is also shown for free macrocycle MDG (middle) and secondary amine 151' for comparison. Downfield chemical shift changes on rotaxane formation are marked with blue arrows and upfield chemical shift changes on rotaxane formation are marked with red arrows.
In particular, the amide NH resonance (A, Figure 6.7) exhibits a downfield shift of more than 1.3 ppm, consistent with its participation in a hydrogen bond with the carbonyl oxygen atom of the diarylamide unit. The resonances associated with the pyridine ring (B, Figure 6.7) in the macrocyclic component of MDG experience strong downfield shifts of up to 0.4 ppm as a result of their location within the deshielding region of the aromatic rings associated with the diarylamide in the linear component of 151 (D, Figure 6.7). The resonances arising from the diarylamide (D, Figure 6.7) are themselves shielded significantly by their proximity to the para-disubstituted aromatic rings (C, Figure 6.7). The location of the macrocycle at the diarylamide end of the structure is supported by the fact that the aromatic proton resonances associated with the CF₃-containing ring show no chemical shift changes on rotaxane formation (E, Figure 6.7).

Satisfyingly, there is an excellent fit (Figure 6.8) of the experimental yield of rotaxane 151, synthesised through the process shown in Scheme 6.1, to the behaviour expected from the kinetic model (Figure 6.1) on which the process is based. Thus, transiently raising the $K_a$ for pseudorotaxane formation by lowering the temperature results in an increased yield of rotaxane after conversion of the temporary iminophosphorane capping group to a secondary amine through the combination of an aza-Wittig reaction followed by reduction.

![Figure 6.8](http://pubs.acs.org)  
**Figure 6.8.** Yield of rotaxane 151 as a function of the $K_a$ for [MDG•146] complex. Filled squares represents experimental data and the dashed line represents the relationship expected based on the kinetic model shown in Figure 6.1 with $k_T = 1.7 k_R$. 

The financial support for this work was provided by the Ministry for Higher Education Malaysia (Graduate studentship to NIH) and the EPSRC (Grant EP/E017851/1).

Supporting Information Available: Synthetic procedures and characterization for compounds 1, 7, and 7′. Experimental details of kinetic simulation and van’t Hoff analysis of the stability of [1•2]. This material is available free of charge via the Internet at http://pubs.acs.org.
6.3 Conclusion
The main principles of the simple kinetic model of rotaxane formation have been established previously in Chapter 5, which highlighted the importance of the association of the macrocycle to the binding site. When considering this in more detail, the reversible formation of pseudorotaxane complex, which emanate from the central equilibrium (Figure 6.1a) plays a crucial role. As a matter of fact, the efficiency of the pseudorotaxane formation relies in a high association constant, $K_a$. It was evident that as a consequence of low $K_a$, a substantial amount of thread is present over rotaxane. Kinetic simulation revealed that for rotaxane to be present in at least 10 times the thread, an association constant, $K_a$ of 5000 M$^{-1}$ is required. We have also demonstrated that we can manipulate this by the modification of the macrocycle and/or the guest used. This approach has been found to be such a laborious job if we need to change it each time we wish to alter our rotaxane system.

Therefore, one possibility to circumvent this scenario is to manipulate the $K_a$ for the pseudorotaxane complex by means of temperature. By lowering the temperature, we will increase the $K_a$ and lead to an increase in the population of pseudorotaxanes and ultimately lead to an increase in the rotaxane to thread ratio. However, as the temperature decreases, the efficiency of the stoppering reactions will also subsequently decreases. Therefore, we identified a new strategy to counteract these issues by employing a rapid reaction between a phosphine and organic azide at low temperature as an intermediate capping step. The iminophosphorane formed can then be captured permanently with aldehyde in an aza-Wittig reaction. Following this, these imines could be reduced readily by the treatment with Hansztzh ester 150 to afford the secondary amine rotaxane 151 and thread 151' that later separated by column chromatography.

The partial $^1$H NMR spectra (Figure 6.4) of pseudorotaxane complex [MDG·146] is recorded at variable temperature from 10 °C, 0 °C to −10 °C. At 10 °C, the $K_a$ is almost 70 M$^{-1}$ rising to 120 M$^{-1}$ at −10 °C, which demonstrated that the $K_a$ is temperature dependent. The van’t Hoff analysis (Figure 6.5) gives an excellent fit and affords value of enthalpy of −17.2 kJ mol$^{-1}$ and entropy of −25.7 J mol$^{-1}$ K$^{-1}$, which clearly manifested the enthalpically driven processes which is typical in the formation of the two hydrogen bonds.
7

Replication model 2

7.1 Introduction

Replication model 2 has been proposed in Chapter 3 as an alternative replication framework to integrate replication processes within the mechanically interlocked architecture of our interest. Some minor modifications were made, in which one of the complementary recognition site (carboxylic acid in this case) is being positioned on to the macrocyclic component and the thread backbone carries the corresponding amido- pyridine recognition unit on its end group (Figure 7.1).

Figure 7.1. Replication model 2 for self-replicating rotaxanes based on the placement of one recognition site (green cartoons) on one of the stoppers and the other recognition site on to the macrocycle.
The model realised a significant advantage over Replication model 1. As a consequence of the thread possessing one recognition unit, the thread instantly become incompetent in the autocatalytic cycle, which hinges on recognition-mediated processes between the carboxylic acid and an amidopyridine unit. In other words, the linear molecule \( L \) is only capable of reacting with the stoppering reagent \( S \) at the bimolecular reaction rate. Only the pseudorotaxane species \([L\cdot M]\) that contains the recognition site will participate in the replicating cycle. Therefore, under no circumstances will there be a reactivity conflict between \( k_{thread} \) and \( k_{rotaxane} \). In fact, a substantial formation of \([L\cdot M]\) complex will ensure explicit opportunities to react with the stoppering reagent, \( S \) to assemble the rotaxane \( R \), without the competition from the free thread \( T \) species. This design element allows the rotaxane, \( R \) to take part in the autocatalytic pathway exclusively and further aid the formation of the second rotaxane, \( R \). In the present discussion, we will describe and summarize some of our recent experiments, which were directed toward establishing the design and synthetic approaches of potential self-replicating rotaxane in the case of Replication model 2.

### 7.2 Synthesis of control macrocycles

Our own work included the synthesis of several types of ring components in the first and second generation of macrocycles. The ability of both macrocycle MDG and MP to complex with a number of different guests through non-covalent interaction has been previously tested. Therefore, we concluded that both cyclic components are capable to interact and locate the guest within their cavities. Therefore, we are keen to preserve the important fragment of their building blocks before appending the recognition site on to their framework (Figure 7.2).

On that account, we began with the synthesis of preliminary control macrocycles to probe the binding performances with previous synthesised guests in comparison to the parent macrocycle MDG and MP. The first control macrocycle devised is based on the substructure of macrocycle MDG. The pyridine unit was substituted with the tert-butyl isophthaloyl building block. The control macrocycle based on MDG was constructed starting from 5-\textit{tert}-butylisophthalic acid 152 which was activated to acid chloride derivative 153 using thionyl chloride in toluene as outlined in literature.
Structure of control macrocycles relative to their parent macrocycle MDG and MP; namely (a) cMDG and (b) cMP, respectively with minimal adjustment in the upper fragment of the structure consists of tert-butyl isophthaloyl building block. Notice that nitrogen atom has been removed in the new design for some synthetic reason.

The intermediate 153 was subsequently coupled to the bis amine compound 103 to give the macrocycle cMDG (11% yield) via high dilution synthesis. All the reactions described here are carried out as outlined in Scheme 7.1. The synthesis of the analogous framework of the control macrocycles has been reported in other works215,233,238-242. An attempt was made to grow crystals of cMDG using several types of solvents, by growing them using slow diffusion, layering or slow evaporation. Unfortunately, all of these attempts failed to generate suitable crystals for single crystal structure analysis.

Scheme 7.1. Synthesis of macrocycle cMDG. Reagents and yields: (a) SOCl₂, toluene, 80 °C, 18 h, 98%; (b) Et₃N, THF, rt, 3 days, 11%.
Following this, we examined its calculated structure (Figure 7.2) to model the behaviour of its structure. There are obvious differences when comparing with the parent macrocycle MDG; such that the opposite orientation of one of the carbonyl group in the upper fragment and the position of the diethylene glycol unit give rise to a bigger cavity in this new macrocycle.

![Figure 7.2](image)

**Figure 7.2.** (a) Stick representation and (b) space filling model of the calculated structure (OPLS2005, GB/SA CHCl₃) of two preferable conformations for macrocycle cMDG. Carbon atoms are coloured green, oxygen atoms in red, nitrogen atoms in blue and hydrogen atoms in white (most hydrogen atoms has been removed for clarity).

![Scheme 7.2](image)

**Scheme 7.2.** Synthesis of control macrocycle cMP. Reagents and yields: (a) SOCl₂, toluene, 80 °C, 18 h, 98%; (b) Et₃N, THF, rt, 3 days, 11%.
Further, the synthesis of the second control macrocycle based on macrocycle MP is encapsulated in Scheme 7.2. In similar way, treatment of the diacid 152 with thionyl chloride under dry conditions led to the formation of diacid dichloride 153, which then coupled to the previous diamine derivative 114, affording the macrocycle cMP in only 11% yield. Thus, using simple molecular mechanics calculations, which are convenient for our purpose, we model the compound structure for macrocycle cMP (Figure 7.3).

Figure 7.3. (a) Stick representation and (b) space filling model of the calculated structure (OPLS2005, GB/SA CHCl₃) of two preferable conformation of macrocycle cMP. Carbon atoms are coloured green, oxygen atoms red, nitrogen atoms are coloured blue and hydrogen atoms are coloured white (most hydrogen atoms has been removed for clarity).

It is important to note that the yield of both cyclization step for macrocycle cMDG and cMP is significantly lower than their parent macrocycles, MDG (34%) and MP (22%), when the reactions were carried out in dichloromethane (4% yield). As might be expected, the possibility of the nucleophilic attack in the amide bond formation step is relatively minimal in high dilution synthesis, but the use of the isophthaloyl building block further diminishes the efficiency of the ring closure step. The question arose, however as to whether the coordination of the pyridine nitrogen in the upper fragment was necessary for pseudorotaxane formation to occur.
Our preceding report\textsuperscript{200} on the formation of MDG and MP reveals the two hydrogen atoms of the amides and the electron pair of the pyridine nitrogen of the upper fragment of the macrocycle point into the interior of the macrocycle. In contrast to benzene, the electron density of the pyridine ring is not evenly distributed over the ring, reflecting the negative inductive effect of the nitrogen atom. For this reason, pyridine has a large dipole moment, which importantly influences the formation of the amide bond between the acid chloride and the amine. After the formation of the first amide bond, the other set of the acid chloride is in ideal position to aid the following nucleophilic attack and close the ring. The possible mechanism of the ring closure is depicted in Figure 7.4.

![Figure 7.4](image_url)

**Figure 7.4.** Possible mechaism of the ring closure forming macrocycle, in comparison for (a) macrocycle MDG or MP and (b) macrocycle cMDG or cMP.

On that account, we considered another option, in which we carried out the ring closure step in THF. This approach is inspired from our observation from the single crystals\textsuperscript{200} structure solution of macrocycle MP in THF and cyclohexane, which reveals that the molecular structure crystallise with a molecule of THF located within its cavity, whereas the THF oxygen is now acting as hydrogen bond acceptor with N⋯O distances calculated as 3.06 Å and 3.10 Å (Figure 7.5).
This evidence supported the idea that in the presence of THF, it can act as template as well as the solvent in the high dilution synthesis and hence, a ring closure for both macrocycles is at least possible, albeit in the poor yield. Additionally, the representation of the molecular structure for macrocycle cMDG (Figure 7.2a) and cMP (Figure 7.3a) reveals that their cavities are substantially larger than the parent macrocycles as a result of the absence of nitrogen atom in the upper fragment of their frameworks. On that account, the necessity to provide a larger stopper groups in order to accommodate the formation of stable interlocked products is crucial and has later proven to be strenuous. Besides that, the ability of these control macrocycles to complex with the potential guests is quantitatively tested in the following section.

7.3 Binding studies – cMDG and cMP
Additional investigations must be conducted to evaluate the influence of the benzene ring in the framework of both control macrocycles in order to test its ability to complex the guests. In order to address this possibility, the following binding experiments were carried out with long maleimide 109 and CF₃ maleimide 110. An equimolar mixture of macrocycle cMDG and maleimide 109 in CDCl₃ was initially prepared and analysed by ¹H NMR spectroscopy. Some of the resonances in the 300.1 MHz ¹H NMR spectrum of [cMDG·109] (see Figure 7.6a) are rather broad at 25 °C, yet we
observed notable chemical shift changes, which corresponds to the pseudorotaxane formation.

The typical downfield shift (+1.15 ppm) of the macrocycle NH protons H\textsuperscript{8R}, the upfield shift for the macrocycle phenylene protons, H\textsuperscript{11R} and H\textsuperscript{12R} (−0.57 ppm and −0.68 ppm) and the upfield shift for the macrocycle methylene protons H\textsuperscript{14R} (−0.36 ppm) are noted. In addition, we recognized that the CH\textsubscript{3} resonance of maleimide 109 is separated into two sharp resonances, which corresponds to the unbound and bound state of maleimide 109.

This separation allows us to measure the association constant using the single-point method and we concluded that \textbf{cMDG} are capable of binding the maleimide 109 with a $K_a$ value of 190 ± 20 M\textsuperscript{−1} at 25 °C in CDCl\textsubscript{3}. An equimolar mixture of macrocycle \textbf{cMDG} and maleimide 110 in CDCl\textsubscript{3} was subsequently prepared. The 400.1 MHz $^1$H NMR spectrum (Figure 7.6c) shows that the exchange resulting from the complexation and decomplexation rate of maleimide 110 is slow on the $^1$H NMR chemical shift timescale and exhibits significant changes in chemical shifts upon the pseudorotaxane formation.
Figure 7.6. Partial $^1$H NMR spectra (300.1 MHz, 25 °C, CDCl$_3$, 20 mM) of (a) an equimolar mixture of macrocycle cMDG and maleimide 109 (b) macrocycle cMDG and (c) an equimolar mixture of macrocycle cMDG and maleimide 110. R stands for ring component and in this case the macrocycle cMDG. Red dashed lines are shown to connect resonances for specific protons in bound and unbound states. (Δδ = $\delta_{\text{unbound}} - \delta_{\text{bound}}$).

### i. Chemical shift data for (a) [cMDG•109]

<table>
<thead>
<tr>
<th>Proton resonances</th>
<th>$\delta_{\text{unbound}}$</th>
<th>$\delta_{\text{bound}}$</th>
<th>Δδ</th>
</tr>
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<tbody>
<tr>
<td>NH$_{8R}$</td>
<td>6.39</td>
<td>7.54</td>
<td>+1.15</td>
</tr>
<tr>
<td>$H_{11R} + H_{12R}$</td>
<td>7.30</td>
<td>6.73</td>
<td>−0.57</td>
</tr>
<tr>
<td>$H_{14R}$</td>
<td>4.51</td>
<td>4.15</td>
<td>−0.36</td>
</tr>
<tr>
<td>$H_{4R}$</td>
<td>8.08</td>
<td>8.36</td>
<td>+0.34</td>
</tr>
</tbody>
</table>

### ii. Chemical shift data for (c) [cMDG•110]

<table>
<thead>
<tr>
<th>Proton resonances</th>
<th>$\delta_{\text{unbound}}$</th>
<th>$\delta_{\text{bound}}$</th>
<th>Δδ</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_{8R}$</td>
<td>6.39</td>
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<td>+0.78</td>
</tr>
<tr>
<td>$H_{11R} + H_{12R}$</td>
<td>7.30</td>
<td>6.77</td>
<td>−0.53</td>
</tr>
<tr>
<td>$H_{14R}$</td>
<td>4.51</td>
<td>4.12</td>
<td>−0.39</td>
</tr>
<tr>
<td>$H_{4R}$</td>
<td>8.08</td>
<td>8.36</td>
<td>+0.28</td>
</tr>
</tbody>
</table>
The resonance arising from the macrocycle NH protons, H$_{8R}^8$, is again moved downfield (+0.78 ppm) as a result of the hydrogen bonding between these protons and the carbonyl group of the maleimide 110 and the characteristic upfield shifts of the macrocycle phenylene protons, H$_{11R}^{11}$ and H$_{12R}^{12}$ (−0.53 ppm and −0.69 ppm) residing in the shielding zone of an aromatic ring. Moreover, the macrocycle methylene protons H$_{14R}^{14}$ (singlet at δ$_H$ 4.51 in the free macrocycle) give rise to an AB system at δ$_H$ 4.12. From the $^{19}$F NMR spectroscopy (376.5 MHz), the association constant for [cMDG•110] complex was estimated to be 1000 ± 100 M$^{-1}$ at 25 °C in CDCl$_3$.

The next step is to conduct the similar binding experiment in the presence of macrocycle cMP. An equimolar mixture of macrocycle cMP and maleimide 109 was prepared and analysed by $^1$H NMR spectroscopy. The 300.1 MHz $^1$H NMR spectrum, (see, Figure 7.7a) shows that the [cMP•109] complex is near to a slow exchange process and we also observed the corresponding downfield shift of the macrocycle NH protons, H$_{8R}^8$ (+1.29 ppm) and the upfield shifts of the macrocycle phenylene protons, H$_{11R}^{11}$ and H$_{12R}^{12}$ (−0.41 and −0.56 ppm, respectively). The association constant for the complexation of [cMDG•109] are determined similarly using the $^1$H NMR single-point method and obtained a $K_a$ value of 160 ± 20 M$^{-1}$.

Binding experiment was performed next with maleimide 110. The 400.1 MHz $^1$H NMR spectrum of an equimolar mixture of macrocycle cMP and guest 110 in CDCl$_3$ (Figure 7.7c) again displays a slow exchange process on the $^1$H NMR chemical shift timescale. The resonances arising from NH$_{8R}^8$ is shifted downfield (+0.95 ppm), and the upfield shifts for both H$_{11R}^{11}$ and H$_{12R}^{12}$ (−0.49 and −0.56 ppm) are comparable with earlier observation in the presence of macrocycle cMDG. The macrocycle methylene protons resonances H$_{9R}^9$, H$_{14R}^{14}$ and H$_{15R}^{15}$ are rendered magnetically inequivalent as a consequence of the unsymmetrical nature of maleimide 110, leading to distinct complex patterns. The association constant for [cMP•110] complex was estimated to be 460 ± 50 M$^{-1}$ by $^{19}$F NMR spectroscopy (376.5 MHz) at 25 °C in CDCl$_3$. 

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Partial $^1$H NMR spectra (300.1 MHz, 25 °C, CDCl$_3$, 20 mM) of (a) an equimolar mixture of macrocycle cMP and maleimide 109 (b) macrocycle cMP and (c) an equimolar mixture of macrocycle cMP and maleimide 110. R stands for the ring component and in this case, the macrocycle cMP. Red dashed lines are shown to connect resonances for specific protons in bound and unbound states. UB stands for unbound species and B corresponds to bound species. ($\Delta\delta = \delta_{\text{unbound}} - \delta_{\text{bound}}$).

### i. Chemical shift data for (a) [cMP•109]

<table>
<thead>
<tr>
<th>Proton resonances</th>
<th>$\delta_{\text{unbound}}$</th>
<th>$\delta_{\text{bound}}$</th>
<th>$\Delta\delta$</th>
</tr>
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<tr>
<td>NH$^{8R}$</td>
<td>6.41</td>
<td>7.70</td>
<td>+1.29</td>
</tr>
<tr>
<td>$H^{11R} + H^{12R}$</td>
<td>7.30</td>
<td>6.89</td>
<td>-0.41</td>
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<td>$H^{4R}$</td>
<td>8.07</td>
<td>8.37</td>
<td>+0.30</td>
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</tbody>
</table>

### ii. Chemical shift data for (c) [cMP•110]

<table>
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<tr>
<th>Proton resonances</th>
<th>$\delta_{\text{unbound}}$</th>
<th>$\delta_{\text{bound}}$</th>
<th>$\Delta\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$^{8R}$</td>
<td>6.41</td>
<td>7.36</td>
<td>+0.95</td>
</tr>
<tr>
<td>$H^{11R} + H^{12R}$</td>
<td>7.30</td>
<td>6.81</td>
<td>-0.49</td>
</tr>
<tr>
<td>$H^{4R}$</td>
<td>8.07</td>
<td>8.38</td>
<td>+0.31</td>
</tr>
</tbody>
</table>

**Figure 7.7.** Partial $^1$H NMR spectra (300.1 MHz, 25 °C, CDCl$_3$, 20 mM) of (a) an equimolar mixture of macrocycle cMP and maleimide 109 (b) macrocycle cMP and (c) an equimolar mixture of macrocycle cMP and maleimide 110. R stands for the ring component and in this case, the macrocycle cMP. Red dashed lines are shown to connect resonances for specific protons in bound and unbound states. UB stands for unbound species and B corresponds to bound species. ($\Delta\delta = \delta_{\text{unbound}} - \delta_{\text{bound}}$).
We realised that the association constant, $K_a$ for [cMDG-110] complex is identical to its parent pseudorotaxane [MDG-110] (see Table 4.2). However, in the presence of cMP, the value deteriorated to $460 \text{ M}^{-1} \pm 50 \text{ M}^{-1}$ when compared to macrocycle MP (see Table 4.3), which attributed to only lower fragments of the macrocycle participating in the binding event. We reasoned that the new cavity of cMP is responsible for the insufficient preorganisation of maleimide 110 as a result of the loss of rigidity of the parent macrocycle MP. The outcomes for the binding experiments are summarized in Table 7.1.

Table 7.1. Binding assessments conducted with control macrocycle cMDG and cMP. The $^1$H NMR spectrum of the macrocycle and guest showed complex is in slow exchange (S) that can be attributed to significant binding events, compared to the free species observed in isolation. In the case of slow exchange, the $K_a$ was determined using the single-point method ($) either by $^1$H NMR or $^{19}$F NMR resonance (Relaxation time, $\tau_R$ is 8 sec). The concentration used was 20 mM in CDCl$_3$ at 25 °C unless otherwise stated.

<table>
<thead>
<tr>
<th>Maleimide guest</th>
<th>$K_a / \text{M}^{-1}$ (cMDG)</th>
<th>$K_a / \text{M}^{-1}$ (cMP)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.png" alt="image" /></td>
<td>$S$ # $K_a = 190 \pm 20$</td>
<td>$S$ # $K_a = 160 \pm 20$</td>
</tr>
<tr>
<td><img src="image.png" alt="image" /></td>
<td>$S$ # $K_a = 1000 \pm 100$</td>
<td>$S$ # $K_a = 460 \pm 50$</td>
</tr>
</tbody>
</table>

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7.4 Synthesis of a potential self-replicating rotaxane

Accordingly, a new scheme of a potential self-replicating rotaxane is envisaged utilizing both macrocycle cMDG and cMP (Scheme 7.3). Given that previous experimental results obtained, the initial design involved the use of CF$_3$ maleimide 110 and nitrone 115, which incorporates 4,6-dimethyl substituted and tert-butyl ring acted as a blocking group that are sufficiently large to prevent the complexation of these macrocycles with two possible binding sites in nitrone building blocks as well as prohibiting the ring from slipping over the thread backbone.

![Scheme 7.3. Retrosynthesis of a potential self-replicating rotaxane consists of control macrocycle (cMDG or cMP), CF$_3$ maleimide 110 and nitrone 115.](image)

7.5 Synthesis of acid recognition macrocycles

The third generation of macrocycles consists of carboxylic acid recognition site appended on variable length spacer are shown in Figure 7.8, from having no spacer to alkyne spacer and further integrate either the phenyl ring or an alkyne to maximise the length of the spacer. Despite that, due to synthetic difficulties, we managed to explore two variable spacer lengths, namely the alkynyl phenyl and phenyl ring. The key intermediate in this synthesis is the formation of iodophenyl and bromophenyl macrocycles, which helps to facilitate the Suzuki and Sonogashira coupling, respectively in later stage.
In order to prepare compound 157 (Scheme 7.4), amino diacid 154 was treated with sodium nitrate in concentrated HCl at 0 °C for an hour before the addition of potassium iodide to afford compound 155 in a yield of 44%.

Compound 155 was subsequently treated with thionyl chloride to give the corresponding acid dichloride derivative 156 and further reacted with the prepared intermediate diamine 103 in high dilution synthesis. The condensation between diamine compound 103 and acid dichloride 156 leads to the formation of iodophenyl glycol macrocycle 157, which contain identical internal cavity as control macrocycle cMDG. Purification by flash column chromatography led to the isolation of iodophenyl glycol macrocycle 157.
glycol macrocycle 157 in 21%. Following that, we prepared the iodophenyl pyridine macrocycle 158 in 18% yield after the purification (Scheme 7.5).

Scheme 7.5. Synthesis of iodophenyl pyridine macrocycle 158. Reagents and yields: (a) NaNO\textsubscript{2} (aq.), HCl (conc.), 0 °C, 1 h followed by KI neat, rt, 18 h, 44%; (b) SOCl\textsubscript{2}, DMF, 100 °C, 48 h, 98%; (c) Et\textsubscript{3}N, THF, rt, 3 days, 18%.

In order to replace the inefficient Sandmeyer reaction in preparing compound 155, we become interested in assembling analogous bromophenyl macrocycle in two consecutive steps by using the commercially available bromoacid 159.

Scheme 7.6. Synthesis of bromophenyl glycol macrocycle 161. Reagents and yields: (a) SOCl\textsubscript{2}, DMF, 100 °C, 6 h, 98%; (b) Et\textsubscript{3}N, THF, rt, 3 days, 8%.

In the first step, we prepared the diacid dichloride 160 and coupled with the diamine intermediate 103 to give 8% yield of bromophenyl glycol macrocycle 161 under the
same conditions. Despite the fact that the yield is very poor, this route supplied a sufficient amount of material to conduct the following experiments (Scheme 7.6). In common with the cyclisation of 156, treatment of dichloride 160 with amine 114 afforded limited amount of macrocycle 162 and significant quantities of other acyclic oligomeric and polymeric products (Scheme 7.7).

![Scheme 7.7](image)

Scheme 7.7. Synthesis of bromophenyl pyridine macrocycle 162. Reagents and yields: (a) SOCl₂, DMF, 100 °C, 6 h, 98%; (b) Et₃N, THF, rt, 3 days, 8%.

The advantageous of using the commercial 5-bromoisophthaloyl acid 159 to assist the cyclization of the corresponding bromophenyl macrocycles 161 and 162 was hampered by the poor conversion and, hence, the overall yield to furnish both iodophenyl and bromophenyl macrocycles are only 8% yield. Therefore, these poor yields again corroborated our postulation on the possible mechanism of the ring closure for analogous isophthaloyl macrocycles (Figure 7.4). It seems that the folding of the uncyclized oligomeric precursors of macrocycle into an ideal position to influence the formation of the second amide bonds, were enforced by highly unfavourable intramolecular interaction. Thus, the presence of a nitrogen atom in the upper fragment of the ring vitally influenced the efficiency of the cyclization processes.

### 7.5.1 Synthesis of acid recognition macrocycles with ethynylbenzene spacer

The synthesis of macrocycle AEMDG was achieved in five steps. The following procedure for the synthesis of terminal alkyne 166 was prepared as described in prior
report\textsuperscript{200} in three sequences starting from 4-bromobenzoyl chloride 163. Reaction with tert-butyl alcohol afford\textsuperscript{244} the ester 164. Subsequent Sonogashira coupling reaction with 2,2-dimethyl propargyl alcohol yielded compound 165. Final removal of the propan-2-ol group with sodium hydroxide in toluene provided the terminal alkyne 166. The remainder of the synthesis of macrocycle AEMDG is outlined in Scheme 7.8. A comparable design of macrocycles was also evident in other research groups\textsuperscript{245-250}.

Scheme 7.8. Synthesis of acid macrocycle AEMDG. Reagents and yields: (a) tert-butyl alcohol, pyridine, CH\textsubscript{2}Cl\textsubscript{2}, rt, 48 h, 91%; (b) 2,2-dimethylpropargyl alcohol, PdCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2}, CuI, PPh\textsubscript{3}, Et\textsubscript{3}N, THF, 70 °C, 48 h, 87%; (c) NaOH, toluene, 120 °C, 3 h, 69%; (d) PdCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2}, CuI, PPh\textsubscript{3}, Et\textsubscript{3}N, THF, 60 °C, 42 h, 90%; (e) Et\textsubscript{3}SiH, TFA/CH\textsubscript{2}Cl\textsubscript{2}, 0 °C to rt, 16 h, 51%.

Iodophenyl glycol macrocycle 157 was coupled to alkyne 166 under standard Sonogashira conditions to give 167. The ester macrocycle 167 was prepared to allow the study of the non-recognition mediated reaction when one of the recognition sites was blocked. The macrocycle ester 167 was next hydrolysed under the acidic conditions to afford the macrocycle AEMDG in 51% yield. Likewise, acid recognition with ethynylbenzene spacer based on pyridine macrocycle, macrocycle AEMP was
prepared next. The iodophenyl pyridine macrocycle 158 was coupled with alkyne 166 to afford the ester macrocycle 168 in 72% yield. Treatment of the ester macrocycle 168 with triethylsilane in a mixture of trifluoroacetic acid and dichloromethane afforded the acid recognition macrocycle AEMP.

Scheme 7.9. Synthesis of acid macrocycle AEMP. Reagents and yields: (a) pyridine, CH₂Cl₂, rt, 48 h, 91%; (b) 2,2-dimethylpropargyl alcohol, PdCl₂(PPh₃)₂, Cul, PPh₃, Et₃N, THF, 70 °C, 48 h, 87%; (c) NaOH, toluene, 120 °C, 3 h, 69%; (d) PdCl₂(PPh₃)₂, Cul, PPh₃, Et₃N, THF, 65 °C, 48 h, 56%; (e) Et₃SiH, TFA/CH₂Cl₂, 0 °C to rt, 16 h, 63%.

7.5.2 Synthesis of acid recognition macrocycles with phenyl spacer

In order to synthesise the acid recognition macrocycle with phenyl spacer, the bromophenyl glycol macrocycle 161 prepared earlier was coupled with 4-(methoxycarbonyl)phenylboronic acid under Suzuki coupling reaction resulting in the intermediate macrocycle 169. In accordance with the preparation in the literature²⁵¹, ester macrocycle 169 was dissolved in THF with potassium trimethyl silanolate. The nucleophilic attack of the trimethylsilanolate anion on the carbon atom of methyl group with carboxylate anion as the leaving group yielded desired acid macrocycle APMDG (Scheme 7.10).
Scheme 7.10. Synthesis of acid macrocycle APMDG. Reagents and yields; (a) 4-(methoxycarbonyl)phenylboronic acid, K$_2$CO$_3$, PdCl$_2$(PPh$_3$)$_2$, THF/H$_2$O, rt, 16 h, 87%; (b) Me$_3$SiOK, THF, 75 °C, 16 h, 70%.

161 \rightarrow \begin{align*}
\text{(a)} & \quad \text{169} \\
\text{(b)} & \quad \text{APMDG}
\end{align*}

Scheme 7.11. Synthesis of acid macrocycle APMP. Reagents and yields; (a) 4-(methoxycarbonyl)phenylboronic acid, K$_2$CO$_3$, PdCl$_2$(PPh$_3$)$_2$, THF/H$_2$O, rt, 16 h, 78%; (b) Me$_3$SiOK, THF, 75 °C, 16 h, 76%.

162 \rightarrow \begin{align*}
\text{(a)} & \quad \text{170} \\
\text{(b)} & \quad \text{APMP}
\end{align*}

The phenyl spacer analogue based on pyridine macrocycle was similarly constructed. Treatment of the bromophenyl pyridine macrocycle 162 in a Pd-catalysed Suzuki coupling to attach the phenyl spacer on to the ester control macrocycle 170 met with 78% conversion. The methyl ester group was further hydrolysed$^{251}$ to furnish acid macrocycle APMP in a moderate yield of 63%.
7.6 Binding studies with maleimides

It is clear that we must learn to thread the linear component before we can learn to perform more intricate actions with interlocked molecules in the replication processes (see Figure 7.9).

The general structure of the thread molecule has a single blocking group so that when threading is achieved, the particular kind of pseudorotaxane species requires an addition of only one end group to become a rotaxane. In the [2]-pseudorotaxane assembly, the influence of rapid threading and dethreading process is evident and can be discerned from the $^1$H NMR spectrum. If the exchange between these processes are slow on the $^1$H NMR chemical shift timescale, the spectrum will contain two populated resonances, $\nu_A$ and $\nu_B$ as the intensities of these resonances represents the relative amounts of each species (see Figure 7.9a). The linear component now incorporates a maleimide group at one side along with bulky stopper group (green circles) being appended at the other end.

For system in which exchange process is appreciably faster than the $^1$H NMR chemical shift timescale, the chemical shift is the average, $\nu_M$ of the individual species chemical shift, that give rise to single populated resonance as a consequence of small end groups on the linear component (denoted by purple circles in Figure 7.9c). However, if both A and B species is in equal concentration, there will be an intermediate exchange, where the separate lines for the two species are no longer distinguishable and give rise to a coalescence resonance as depicted in Figure 7.9b.
Figure 7.9. Influence of threading and dethreading process in the [2]-pseudorotaxane assembly on $^1H$ NMR spectrum give (a) slow exchange, (b) intermediate exchange or (c) fast exchange. Two resonances ($v_A$ and $v_B$) denoted the single atom signal corresponding to A and B species, respectively. Population of the species corresponds to the relative concentration in the reaction mixture. In the intermediate exchange, the signals observed begin to coalesce. Additionally, for a rapidly exchanging system, the average frequency $v_M$ observed is $v_M = (v_A + v_B)/2$. 
In more detail, the slow exchange process depicted in Figure 7.10 highlight the effect of the unsymmetrical nature of the maleimide component. In the free ring, the protons are regarded as chemically and magnetically equivalent, reflecting the existence plane of symmetry in the ring. Nonetheless, in the formation of pseudorotaxane complex, the plane of symmetry in the ring is broken. Consequently, the protons located on the opposite faces of the ring are rendered magnetically inequivalent, giving rise to an AB spin system. The proton α on one face of the macrocycle is directed toward the maleimide, whereas the protons β on the opposing face of the ring is orientated toward the bulky end group. Additional end-to-end asymmetry imposed by the axle component could subsequently leads to complex signal patterns and example of this behaviour was found earlier in Chapter 4 and also evident in this chapter.

Figure 7.10. The effect of the unsymmetrical nature of the maleimide component as depicted earlier in Figure 7.9a upon the assembly of [2]-pseudorotaxane rendered the Hα and Hβ of the free ring to be diasterotopic, which results in AB spin system. In the free ring, Hα and Hβ are regarded as chemically and magnetically equivalent, giving rise to a singlet resonance.
Various examples\textsuperscript{217,252,253} of amide backbones, or side chains threaded through macrocycles have been published. Our own pseudorotaxane based on amide systems have been studied in Chapter 4 and substantiate the fact that a complementary hydrogen bonding\textsuperscript{254,255} between the amide-based macrocycle and the linear amide threading group is a requirement for high yield of the rotaxane and, hence, established the pseudorotaxane as the intermediate of prime importance. Measurement of the effect of substitution on the phenyl ring of the thread component on pseudorotaxane formation\textsuperscript{256} reveals the inhibiting effect of electron-donating substituents, which reduced the hydrogen bond donating ability of the threading group. Steric effects due to substitution in other systems\textsuperscript{124,257} have also been examined and show that even small changes in steric bulk imposed deleterious effects on pseudorotaxane formation.

Here, we attempt to add answers to simple questions that provide a more complete evaluation of the effects of certain basic structural relationships on the abilities of linear molecules to thread through the cavity of our macrocycles. As in the previous example, the isophthalyl control macrocycles cMDG and cMP, binds both the CH\textsubscript{3} maleimide \textbf{109} and CF\textsubscript{3} maleimide \textbf{110}. These promising preliminary results encouraged the subsequent investigation into the assembly of a range of [2]-pseudorotaxanes with newly prepared acid macrocycles.

Based on this knowledge, we reason that the structural analogue acid macrocycles should also afford [2]-pseudorotaxane assemblies using the same maleimide compound. The preparation of new pseudorotaxane was demonstrated by employing the ester macrocycle, namely macrocycle \textbf{168} and \textbf{169} for solubility reasons and we limit our attention to CF\textsubscript{3} maleimide \textbf{110}. Discussion of these results and their significance follows.

Mixing an equimolar solution of ester macrocycle \textbf{168} and maleimide \textbf{110} in CDCl\textsubscript{3} at 25 °C reveals approximately 90% threaded species in the solution. The 400.1 MHz \textsuperscript{1}H NMR spectrum of this mixture indicated that the bound and unbound species equilibrated slowly with respect to one another on the \textsuperscript{1}H NMR chemical shift timescale. This phenomena allow the determination of the association constant, $K_a$ using the single-point method. However, the overlapping signal hampered these
assignments. $^{19}$F NMR spectroscopy (376.5 MHz, CDCl$_3$) was utilized and the concentration of the products were calculated by deconvolution of each of the characteristic resonances, which corresponds to unbound and bound species. The binding constant that quantify the threading of maleimide 110 through macrocycle 168 was determined to be $1270 \pm 130$ M$^{-1}$ at 298 K.

The chemical shift changes observed are representative of pseudorotaxane formation (Figure 7.11b) – a typical downfield shift of the macrocycle NH protons H$^{14R}$ (+0.76 ppm) and upfield shifts of the macrocycle phenylene protons (H$^{17R}$, H$^{18R}$) (−0.50 ppm). In addition, the high chemical shift of the NH proton of maleimide 110, H$^7T$ (+1.87 ppm) indicates that the amide group is participating in hydrogen bonding interactions. The binding affinity of the ring for the amide binding site was manifested from the intricate pattern of the proton resonances in the methylene regions (H$^{15R}$, H$^{20R}$ and H$^{21R}$) due to the magnetically inequivalent environments. However, these proton resonances are not very well resolved with extra broader peak beneath the H$^{15R}$, even when the temperature was lowered to 263 K.
Figure 7.11. Self-assembly of an equimolar mixture of CF$_3$ maleimide 110 with macrocycle 168 to form the pseudorotaxane complexes [168•110]. Partial $^1$H NMR spectra (400.1 MHz, CDCl$_3$, 20 mM, 298 K) of (a) Macrocycle 168, (b) an equimolar mixture of 168 and CF$_3$ maleimide compound 110 (R corresponds to ring, in this case macrocycle 168 and T denoted as thread, in this case maleimide 110) and (c) CF$_3$ maleimide compound 110. Red dashed lines and blue dashed boxes are shown to connect resonances for specific protons in bound and unbound states.
Figure 7.12. (a) The ROESY contacts observed for the complex between macrocycle 168 and maleimide 110 are shown as blue arrows; (b) and (c) Partial 2D ROESY NMR spectra (500.1 MHz, 263 K, CDCl₃, 20 mM) display crosspeaks (red points) between macrocycle 168 and maleimide compound 110.
This broadening resonance resulted from $k_{\text{on}}$ and $k_{\text{off}}$ being at the same order as the $^1$H NMR chemical shift timescale. Similar behaviour was noticed for maleimide phenylene protons with concomitant downfield changes, $H_{\text{4T}}$ (+0.28 ppm). The structural conformation of the pseudorotaxane complex [168•110] was elucidated using the 2D ROESY NMR study at 295 K as shown in Figure 7.12. Inspection of the 500.1 MHz 2D ROESY spectra revealed meaningful crosspeaks between the aromatic protons, $H_{\text{4T}}$ with macrocycle methylene protons, $H_{\text{21R}}$ and subsequently phenylene protons, $H_{\text{17R}}$ and $H_{\text{18R}}$ (see Figure 7.12c). Additionally, the macrocycle isophthaloyl bridge proton $H_{\text{12R}}$ also displayed a crosspeak with alternative phenylene proton of maleimide, $H_{\text{9T}}$ (see Figure 7.12b) confirming the encircling of the macrocyclic component about the amide moiety and therefore the pseudorotaxane has the expected geometry shown in Figure 7.12a. However, no apparent crosspeaks were observed with two NH protons of the macrocycle with maleimide 110.

The subsequent binding experiment utilized macrocycle 169 (Figure 7.13a). Upon addition of maleimide 110, the 400.1 MHz $^1$H NMR spectrum displays the existence of nearly 80% pseudorotaxane species in the solution at room temperature (not shown). Likewise, the exchange process between bound and unbound state are relatively slow on the $^1$H NMR chemical shift timescale. The exchange rate between bound and unbound state was sufficiently slow at 263 K (see Figure 7.13b) to obtain different sets of resonances for free macrocycle 169, unbound maleimide 110 and pseudorotaxane complex [169•110].

The $K_a$ value for [169•110] complex was estimated to be 5500 ± 550 M$^{-1}$ in CDCl$_3$. The formation of the [2]-pseudorotaxane [169•110] complex at 263 K give rise to similar spectral result as previously observed with [168•110] complex, that exhibited classic downfield shift of NH macrocycle, $H_{\text{12R}}$ ($\delta_H$ 7.29) and lead to substantial upfield changes in the macrocycle phenylene protons, namely $H_{\text{15R}}$ and $H_{\text{16R}}$, which become more resolved and desymmetrized. Additionally, the macrocycle 169 shift upfield towards the 3,5-bis trifluoromethyl group of maleimide 110, which is apparent from the shifts of the phenylene proton of maleimide 110, $H_{\text{4T}}$ (−0.53 ppm) and $H_{\text{1T}}$ (−0.15 pm), respectively.
Figure 7.13. Self-assembly of CF₃ maleimide 110 with macrocycle 169 to form the pseudorotaxane [169·110] complex. Partial ¹H NMR spectra (400.1 MHz, CDCl₃, 20 mM) of (a) Macrocycle 169, (b) an equimolar mixture of macrocycle 169 and CF₃ maleimide compound 110. (R corresponds to ring, in this case macrocycle 169 and T denoted as thread, in this case maleimide 110) at 263 K and (c) CF₃ maleimide compound 110. Red dashed lines and blue dashed boxes are shown to connect resonances for specific protons in bound and unbound states.
The observed changes constitute evidences from the effect of shielding in the core of the aromatic regions of the phenylene protons, H$^{15R}$ and H$^{16R}$, which demonstrated that they are participating in the parallel π–π stacking. Furthermore, the visible EXSY crosspeaks within the region of δH 7.45 – 7.65 suggests the existence of unbound and bound species in accordance with the exchange process. Protons labelled 13 and 18 on the macrocycle (Figure 7.13a) are rendered anisochronous by end-to-end asymmetry inherent in the pseudorotaxane [169•110] complex. Consequently, the resonances for these protons appear as AB pattern at 4.45 and 4.25 ppm, respectively (see Figure 7.13b). In addition, the previous overlapping signals for the methylene protons of macrocycle 169 H$^{19R}$ and H$^{20R}$ are now dispersed. These observations are consistent with the prior findings in the binding studies of macrocycle MDG in Chapter 4 and 5.

A section of 500.1 MHz 2D ROESY NMR spectra of the [2]-pseudorotaxane recorded in CDCl$_3$ at 263 K reveals important crosspeaks between the phenylene protons of the ring, H$^{15R}$ and H$^{16R}$ with the maleimide proton, H$^4T$ (Figure 7.14b). Such interaction could only possibly occur through the vacant amide moiety in a manner shown in Figure 7.14a, and thus give rise to the proposed geometry.

With the aim of investigating both the modes of the maleimide binding and its effect have on the solid state packing of these receptors, an attempt was made to grow the crystals for the two pseudorotaxane complexes, [168•110] and [169•110], using several types of solvents, by growing these complexes using slow diffusion or slow evaporation. Unfortunately, all of these failed to generate suitable crystals for single crystal structure analysis.
Figure 7.14. (a) ROESY contacts observed for the complex between macrocycle 169 and maleimide 110 are shown as blue arrows. (b) Partial 2D ROESY NMR spectrum (500.1 MHz, 263 K, CDCl₃, 20 mM) display crosspeaks (red points) between macrocycle 169 and CF₃ maleimide compound 110. UB stands for unbound species in the blue dashed boxes. EXSY crosspeaks (blue points) are shown within the region δH 7.45 – 7.65 in the red dashed boxes between the unbound species and H¹⁶T.
7.7 Design of a potential self-replicating rotaxane

A potential self-replicating rotaxane was designed accordingly to accommodate the prepared acid macrocycles and nitrone 115 compound. It has been demonstrated\textsuperscript{200} that 4,6-dimethylpyridine ring and 4\textsuperscript{-}tert-butylphenyl ring in nitrone 115 incapable of threading the cavity of both macrocycles MDG and MP, which are the structural analogues of the acid macrocycles (Scheme 7.12).

**Scheme 7.12.** Retrosynthesis of potential self-replicating rotaxanes consists of acid recognition macrocycles, nitrone 115 and CF\textsubscript{3} maleimide 110 compound.

7.7.1 Synthesis of first generation nitrones

We changed the tert-butylphenyl ring by employing different blocking group in the nitrone structure while maintaining the 4,6-dimethylpyridine ring on the other end, to provide the first generation nitrones 176 and 182 as depicted in Figure 7.15.

**Figure 7.15.** The first generation nitrone compounds with the introduction of di-tert-butylphenyl and anthracene ring as an alternative blocking groups, affording compound 176 and 182.
The synthesis of di-tert-butylphenyl nitrone 176 is included in Scheme 7.14 to illustrate the synthetic route used for this new compound. Treatment of aldehyde 171 with tetrabromomethane in the presence of triphenylphosphine and zinc afforded the intermediate 172 in good yield. Reaction of intermediate 172 with two equivalents of N-butyllithium in dry THF at −78 °C for two hours before vigorously stirring at room temperature give the terminal alkyne 173 in 52% yield. Nitro compound 174 was subsequently obtained in 81% yield, utilizing Sonogashira coupling between alkyne 173 with 1-iodo-4-nitrobenzene. The di-tert-butylphenyl nitrone 176 was successfully synthesised in 39% yield by carrying out the partial reduction of nitro group 174 followed by condensation reaction between the resultant hydroxylamine 175 with the synthesised aldehyde 117.

Recently, the research conducted by Philp group utilized a fluorescent material, namely an anthracene ring located at para position of the benzene ring of the nitrone, which works as a fluorescent tag in the development of novel self-replicating structure. This feature provides the possibility of following the reaction by the naked eyes. As a consequence of the extended π-conjugated system, the nitrone compound emits fluorescence in the yellow region of visible light. However, when the reaction
takes place, the conjugation was interrupted by the engagement of the -C=NO-double bond in the formation of the cycloadducts, giving the product a blue fluorescent colour. The dramatic colour changes visually aids our observation when the thread and/or rotaxane are formed in the solution. Therefore, the integration of anthracene scaffold in one of our building blocks is exceptionally important. Hence, it was decided that a new antharacene nitrone compound should be synthesised to incorporate into our design of potential self-replicating rotaxanes. The following synthetic scheme was described to produce the anthracene compound 182 as shown in Scheme 7.15.

![Scheme 7.15. Synthesis of anthracene nitrone 182. Reagents and yields: (a) (trimethylsilyl)acetylene, PdCl₂(PPh₃)₂, Cul, PPh₃, Et₃N, 80 °C, 24 h, 87%; (b) K₂CO₃, MeOH, THF, rt, 4 h, followed by (c) 1-iodo-4-nitrobenzene, PdCl₂(PPh₃)₂, Cul, PPh₃, Et₃N, 50 °C, 24 h, 67%; (d) Rh/C, NH₂NH₂•H₂O, THF, rt, 3 h; (e) Aldehyde 117, EtOH, rt, 3 days, 81% over 2 steps.]

There are at least five synthetic steps to yield anthracene nitrone 182, commencing from the Sonagashira coupling of 9-bromoanthracene 177 with an excess amount of (trimethylsilyl)acetylene to yield intermediate 178 in excellent yield. The acetylene 178 was deprotected under basic conditions to give the terminal alkyne 179 before the second Sonogashira coupling with 1-iodo-4-nitrobenzene to install the nitro group in compound 180. Compound 180 was further reduced with two equivalents of hydrazine monohydrate to yield hydroxylamine 181 before reacted with aldehyde 117.
to produce anthracene nitrone 182 in 81% yield of. Satisfyingly, this nitrone shows fluorescence in the yellow region of visible light.

### 7.7.2 Binding studies with first generation nitrones

There are various binding possibilities for macrocycle originated from the additional binding sites present in the nitrone structure or the amide moiety from the amidopyridine fragment of the thread. Therefore, the crucial thing is to prevent the association of the macrocycle with these competitive binding sites, which could be problematic in the construction of rotaxane since the macrocycle not only bind the maleimide but also the nitrone. Unfortunately, the binding experiment between nitrone 176 and control macrocycle, cMDG shows that the macrocycle form the undesired pseudorotaxane complex, [cMDG·176]. The chemical shift changes similar to those observed for the pseudorotaxane formation (not shown) - upfield shifts for the resonances for the macrocycle phenylene protons H^{11R} and H^{12R}, macrocycle methylene protons H^{9R} and H^{14R} and complex pattern for H^{15R} and H^{16R} (refer Figure 7.6b). The supplementary binding experiment with anthracene nitrone 182 was also carried out, which give the similar outcomes (Table 7.2).

#### Table 7.2. Chemical shift changes, ∆δ (in ppm) for selected protons of macrocycle cMDG complexing the first generation nitro compounds 176 and 182. (Note: S denoted a slow exchange process occurring on the ¹H NMR chemical shift timescale).

<table>
<thead>
<tr>
<th>Macrocycle cMDG</th>
<th>∆δ (H^{11}, H^{12})</th>
<th>∆δ H^9</th>
<th>∆δ H^{14}</th>
<th>∆δ (H^{15}, H^{16})</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Nitrone 176 (S)</td>
<td>-0.62, -0.74</td>
<td>-0.19</td>
<td>-0.35</td>
<td>complex pattern</td>
</tr>
<tr>
<td>ii. Nitrone 182 (S)</td>
<td>-0.55, -0.67</td>
<td>-0.14</td>
<td>-0.30</td>
<td>complex pattern</td>
</tr>
</tbody>
</table>

Thus, these results are surprising, demonstrating that both nitrones 176 and 182 are capable of penetrating the cavity of the macrocycle cMDG. It could be reason that the macrocyclic unit passed through the fragment of 4,6-dimethylpyridine ring. In that case, we require a larger end group to hamper the formation of this disadvantageous pseudorotaxane species. We are hoping to address this issue by replacing the 4,6-dimethylpyridine ring end with the 4-tert-butylphenyl ring in the new design.
7.7.3 Improving the design for second generation nitrones

The second strategy involves the 4-tert-butylphenyl ring as the end group in the new nitrone structure. In order to alter the other end of the blocking groups, we employed three distinct groups from naphthalene, di-tert-butylphenyl ring to anthracene ring (Figure 7.16).

Herein, we described the synthesis of nitrone compounds (192, 193 and 194) in the following paragraphs. In the preceding communications\textsuperscript{260}, the iodination of 2-amino-6-methylpyridine, 183 was achieved in the presence of periodic acid in concentrated sulfuric acid and acetic acid to yield iodoaniline derivative 184 in a good yield (Scheme 7.16). Subsequently, an amide coupling was performed with the 4-formylbenzoyl chloride 116 under standard conditions to furnish the iodo-aldehyde 185 in a fair yield. Furthermore, Suzuki coupling inserted the 4-tert-butylphenyl ring on aldehyde 186 under mild conditions in an excellent yield of 98%. Concurrently, the terminal alkyne 189 was synthesised by the oxidation and decarboxylation strategies as illustrated in earlier report\textsuperscript{261} in the group. The palladium-catalysed crosscoupling of 187 with propargyl alcohol gave the prop-2-yn-1-ol 188, which successively oxidised and decarboxylated using activated manganese (IV) oxide to afford alkyne 189 in a good yield of 82%. The subsequent Sonogashira coupling under standard conditions with 2-bromonaphthelene installed the naphthalene fragment on to the
This nitro derivative 190 compound was used directly from the work in the group and the subsequent partial reduction with hydrazine monohydrate in the presence of Rh/C catalyst allows the formation of the hydroxylamine 191.

Previous works shows that the synthetic approaches using palladium or rhodium on activated carbon with an equimolar amount of hydrazine monohydrate in THF solved the problem for the formation of highly substituted hydroxyamine derivatives. However, in some cases, we observed over reduction to an amine or an exclusive formation of the azoxy compound by the condensation of hydroxylamine and in situ formed nitroso compound. Therefore, the obtained hydroxylamine 191 was usually left to dry on the high vacuum for overnight to get rid of the excess hydrazine before reacted with aldehyde 186 to afford the corresponding nitrone 192 in 45% yield. Simultaneously, the prepared aldehyde 186 was reacted in two sequences starting from the same nitro compound 174 bearing the di-tert-butylphenyl ring to afford the
intermediate hydroxylamine 175. The resulting nitrone 193 was assembled via the condensation reaction between hydroxylamine 175 and aldehyde 186 (Scheme 7.17).

Scheme 7.17. Synthesis of nitrone 193. Reagents and yields: (a) Rh/C, \( \text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O} \), rt, 3 h; (b) EtOH, THF, rt, 3 days, 40% over 2 steps.

Scheme 7.18 depicted the synthesis of the analogous antharacene nitrone compound 194. Preparation of anthracene hydroxylamine 181 through the reduction pathway described in the preceding procedure (Scheme 7.15d) was similarly reacted with aldehyde 186 to give the second anthracene nitrone 194 in 94% yield.

Scheme 7.18. Synthesis of nitrone 194. Reagents and yields: (a) Rh/C, \( \text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O} \), rt, 3 h, followed by (b) EtOH, rt, 3 days, 94% over 2 steps.

7.7.4 Binding studies with second generation nitrones

As mentioned earlier, the binding studies with the second generation nitrones 192 and 194 were first carried out with macrocycle cMDG to examine the possibilities of pseudorotaxane formation. An equimolar mixture of the macrocycle and nitrone was monitored by \(^1\text{H} \) NMR spectroscopy at room temperature in CDCl\(_3\).
Nonetheless, the naphthalene nitrone 192 afforded pseudorotaxane assemblies with control macrocycle, cMDG. From the $^1$H NMR spectrum (not shown), the bound and unbound species equilibrate slowly with respect to the threading and dethreading process on the $^1$H NMR chemical shift timescale. Further evidence of pseudorotaxane formation was obtained from the significant downfield shift (+1.57 ppm) of the macrocycle NH protons (refer Figure 7.6b), upfield shifts for the resonances of macrocycle phenylene protons $^{11}R$ and $^{12}R$ (−0.58 and −0.70 ppm), upfield shifts for the resonance of macrocycle methylene protons, $^{9}R$ (−0.16 ppm) and $^{14}R$ (−0.31 ppm) and the emergence of the complex patterns, which corresponds to the diastereotopic methylene protons of $^{15}R$ and $^{16}R$ (Table 7.3i).

In the case of anthracene nitrone 194, the spectrum (not shown) suggests that the complex is however in fast exchange on the $^1$H NMR chemical shift timescale. Unexpectedly, thus also yielded a pseudorotaxane assembly, in which was inferred from the upfield shift of macrocycle NH protons (+1.14 ppm), downfield shifts of the macrocycle phenylene protons, $^{11}R$ and $^{12}R$ (−0.10 ppm), $^{9}R$ (−0.02 ppm) and $^{14}R$ (−0.05 ppm) (Table 7.3ii) together with similar complex pattern for $^{15}R$ and $^{16}R$.

| Table 7.3. Chemical shift changes, $\Delta \delta$ (in ppm) for selected protons of macrocycle cMDG upon complexation with nitrone compound 192 and 194. (Note : S and F denoted a slow and fast exchange process occurring on the $^1$H NMR chemical shift timescale). |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Macrocycle cMDG**             | $\Delta \delta$ ($^{11}$, $^{12}$) | $\Delta \delta$ $^{9}$ | $\Delta \delta$ $^{14}$ | $\Delta \delta$ ($^{15}$, $^{16}$) |
| i. Nitrone 192 (S)              | −0.58, −0.70    | −0.16           | −0.31           | complex pattern |
| ii. Nitrone 194 (F)             | −0.10           | −0.02           | −0.05           | complex pattern |

These observations demonstrated that macrocycle cMDG was capable of forming the interlocked species with naphthalene nitrone 194, which bring us to the final binding experiment with nitrone 193 (Table 7.4). Likewise, the formation of undesired pseudorotaxane is again favoured with significant upfield shifts from the resonance of the macrocycle 169 (refer Figure 7.13a) phenylene protons, $^{15}R$ and $^{16}R$, that give a singlet at $\delta_H$ 7.30 when the macrocycle is free in the CDCl$_3$ solution but upon pseudorotaxane formation, each of these protons dispersed as a pair of doublet at 6.73 and 6.61 ppm. The ring methylene protons $^{13}R$ and $^{18}R$ are rendered diastereotopic when the nitrone 193 was encircled by the ester macrocycle 169 and...
give rise to another set of resonances. Furthermore, the overlapping methylene protons $H^{19R}$ and $H^{20R}$ also led to the same complex patterns, and thus confirmed the pseudorotaxane complex formation. Altogether, these observations unambiguously indicated that the tert-butylphenyl ring is not sufficiently large to prevent the formation of undesired pseudorotaxane complexes.

Table 7.4. Chemical shift changes, $\Delta \delta$ (in ppm) for selected protons of ester macrocycle 169 upon complexation with the nitrone 193. (Note: $S$ denoted a slow exchange process occurring on the $^1$H NMR chemical shift timescale).

<table>
<thead>
<tr>
<th>Macrocycle 169</th>
<th>$\Delta \delta (H^{15}, H^{16})$</th>
<th>$\Delta \delta H^{13}$</th>
<th>$\Delta \delta H^{18}$</th>
<th>$\Delta \delta (H^{19}, H^{20})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrone 193 ($S$)</td>
<td>$-0.57$, $-0.68$</td>
<td>$-0.17$</td>
<td>$-0.31$</td>
<td>complex pattern</td>
</tr>
</tbody>
</table>

7.7.5 Synthesis and binding study with third generation nitrone

Having demonstrated that all nitrones prepared to date assembled into undesired pseudorotaxanes with macrocycle cMDG, we realized that 4-tert-butylphenyl stopper was responsible for this threading. Attention now focused on probing the appropriate size for alternative end groups, so it is incapable of passing through the cavity of macrocycle cMDG. In order to test this proposition, a new strategy was employed to prepare the bulky nitrone compound 197 as shown in Scheme 7.19.

Scheme 7.19. Synthesis of bulky nitrone 197. Reagents and yields: (a) Et$_3$N, CH$_2$Cl$_2$, 0 °C to rt, 48 h, 38%; (b) Alkyne 173, PdCl$_2$(PPh$_3$)$_2$, CuI, PPh$_3$, Et$_3$N, 60 °C, 24 h, 25%, (c) EtOH, rt, 48 h, 24% over 2 steps.

The iodo-aniline 194 was used directly from the work in the group and reacted with the acid chloride 116 to give the iodo-aldehyde 195, which was subsequently
functionalized with the terminal alkyne 173 via Sonogashira coupling under standard conditions affording the aldehyde 196 in a yield of 25%. The sterically di-tert-butylphenyl ring terminus from the hydroxylamine 175 was appended to produce the bulky nitrone 197. Following the replacement of the 4-tert-butylphenyl ring with the 3,5-di-tert-butylphenyl ring, subsequent binding experiment (not shown) with ester macrocycle 169 confirmed that no pseudorotaxane complexes were obtained. Pleasingly, both of terminal groups, 3,5-di-tert-butylphenyl were able to function as an effective stoppering group.

The overall outcome of the binding experiments carried out between macrocycle cMDG or ester macrocycle 169 with the first, second and third generation of nitrone compounds are presented and summarized in Table 7.5. Therefore, only the bulky nitrone compound 197 did not give the pseudorotaxane complex with the macrocyclic component (in this case, ester macrocycle 169) and will be further employed in our second design of potential self-replicating rotaxanes.
Table 7.5. Binding experiments carried out between macrocycle cMDG or ester macrocycle 169 with the first, second and third generation nitrone compounds, giving the undesired [2]-pseudorotaxane complex are listed below.

<table>
<thead>
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<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
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i. First generation nitrone

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ii. Second generation nitrone

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iii. Third generation nitrone

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<th>Compounds</th>
<th>[2]-pseudorotaxane</th>
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197
7.8 Design of a potential self-replicating rotaxane 2

Building on this work, we prepared the second design of a potential self-replicating rotaxane (Scheme 7.20), which features the acid recognition macrocycle, CF$_3$ maleimide 110, and the bulky nitrone compound 197.

Scheme 7.20. Retrosynthetic of potential self-replicating rotaxanes 2 containing bulky nitrone 197, acid recognition macrocycle and CF$_3$ maleimide 110.

However, the development of this self-replicating rotaxane was discontinued because the CF$_3$ maleimide 110 is insoluble in less polar solvent (CDCl$_3$) upon addition of the acid recognition macrocycle.

7.9 Design of a potential self-replicating rotaxane 3

In the pursuit of self-replicating rotaxanes, we refined the alternative synthetic strategies to replace the CF$_3$ maleimide 110. The final scheme comprises bulky nitrone compound 197, acid recognition macrocycle and maleimide 198 (Scheme 7.21). Maleimide component 198 has been successfully utilized to prepare a number of simple rotaxane structure, and involved particularly in the complexation of macrocycle MDG. It has been demonstrated that maleimide 198 capable of threading and the stoppers are proven to big enough to prevent dethreading. We were therefore interested to conduct a binding experiment between acid recognition macrocycle and maleimide 198.
Scheme 7.21. Retrosynthetic of potential self-replicating rotaxanes 3 comprises big bulky nitrone 197, Maleimide 198 and acid recognition macrocycle.

7.9.1 Binding study with maleimide 198

Binding experiment with acid macrocycle AEMDG was carried out with maleimide 198. The solubility of macrocycle AEMDG in chloroform was poor, but improved with the addition of the maleimide compound 198. This solubility change is an indication of the interaction between the macrocycle and maleimide guest as such the supramolecular species often being more soluble than the two separate entities. The reaction was monitored by $^1$H NMR spectroscopy, which revealed that the free and bound species equilibrate slowly with one another relative to the threading and dethreading process on the $^1$H NMR chemical shift timescale.

The 400.1 MHz $^1$H NMR spectrum of the macrocycle methylene regions is shown in Figure 7.17. These protons are all rendered magnetically inequivalent upon pseudorotaxane formation as a results of end-to-end asymmetry imposed by maleimide 110. Furthermore, using the single-point method, the binding constant was estimated to be $260 \pm 30 \text{ M}^{-1}$ in CDCl$_3$ at 25 °C.
Figure 7.17. (a) Self-assembly of macrocycle AEMDG with maleimide 198 forming pseudorotaxane complex; (b) Macrocycle methylene resonances region of $^1$H NMR spectrum (400.1 MHz, 25 °C, CDCl$_3$, 10 mM) of an equimolar mixture of macrocycle AEMDG and maleimide 198, exhibited signals that can be attributed to the intermolecular hydrogen bonding. Star symbols coincide with the diastereotopic proton in the pseudorotaxane complex.

Subsequently, we performed the kinetic simulation to assess the ratio of rotaxane to thread. As shown in Figure 7.18, the simulation reveals that [R]/[T] ratio in 1:1 stoichiometry is dependent on the ratio of $k_{\text{rot}}/k_{\text{thread}}$. If we take the $K_a$ for the [AEMDG•198] complex as 260 ± 30 M$^{-1}$ and $k_{\text{thread}} = k_{\text{rotaxane}}$, then the [R]/[T] ratio in the reaction mixture should be slightly more than 1 (dashed line).
Figure 7.18. Plot of $[R]/[T]$ against $k_{rot}/k_{thread}$. Noted that in the simulation, the starting concentrations of maleimide 198, nitrone 197 and macrocycle AEMDG are 10 mM and plotted in the logarithmic scales.

We must now determine the reaction rate for the formation of both thread and rotaxane cycloadducts in our real system, and which diastereoisomers are favoured in the reaction mixture. Therefore, the kinetic analysis for the thread and rotaxane formation in isolation must be conducted.

7.9.2 Kinetic study of the thread

The kinetic of maleimide 198 and nitrone 197 in the absence of macrocycle AEMDG was performed to investigate the rate of bimolecular reaction of resulting thread 199 (Scheme 7.22). A solution containing the nitrone 197 and maleimide 198, with starting concentrations of 10 mM each were prepared in CDCl₃.

The reaction progress was followed at 298 K over 24 hours by $^1$H NMR spectroscopy, which monitored the disappearance of the resonance that arises from the maleimide 198 present at $\delta_H$ 6.85 and the concomitant emergence of the resonances that result from the trans diastereoisomer at $\delta_H$ 5.13 – 5.80 and that from the cis diastereoisomer at $\delta_H$ 4.14 – 5.30 (Figure 7.19). The profile of concentration vs time for the formation of both cycloadducts was additionally constructed by...
deconvolution of these resonances. Reaction between nitrone 197 and maleimide 198 proceed slowly at 298 K leading to 54% overall conversion after 24 hours, which accompanied by typical diastereoisomeric ratio of 2:1 in favour of trans 199, and thus establishing the importance of recognition process in the bimolecular reaction.

![Scheme 7.22](image)

**Scheme 7.22.** 1,3-dipolar cycloaddition between nitrone 197 and maleimide 198 to give thread 199 in CDCl₃ at 298 K.

The kinetic data of the non-recognition mediated reaction were extracted and employed in the fitting protocol using the SimFit package (Figure 7.20). The quality of the fit was reflected by the solid line derived from the kinetic model compared with the experimental data points. Bimolecular rates extracted from the control experiment were determined to be 9.04 × 10⁻⁴ M⁻¹ s⁻¹ for trans thread 199 and 4.45 × 10⁻⁴ M⁻¹ s⁻¹ for cis thread 199. In addition, the formation of thread 199 cycloadducts were corroborated by MALDI mass spectrometry from the crude reaction mixture (Figure 7.21).
Figure 7.19. Partial $^1$H NMR spectra (500.1 MHz, 25 °C, CDCl$_3$, 24 h) of the cycloaddition reaction between maleimide 198 and bulky nitrone 197, illustrating spectral region associated with the resonances of the trans thread 199 and cis thread 199 protons, respectively.

Figure 7.20. Concentration vs time profile for the reaction (500 MHz, CDCl$_3$, 10 mM, 298 K, 24 h) between nitrone 197 and maleimide 198. The formation of trans thread 199 is shown as blue filled circles and the formation of cis thread 199 as red filled diamonds. Solid lines represent the results for the fitting of the trans thread 199 (blue line) and cis thread 199 (red line) product.
Calculated for C$_{94}$H$_{96}$N$_{5}$O$_{5}$ => [M + H]$^+$

Figure 7.21. MALDI mass-spectra of thread component 199. Mass spectrometry (MALDI) m/z 1374.86 (95%, [M+H]$^+$), calculated for C$_{94}$H$_{96}$N$_{5}$O$_{5}$.

7.9.3 Kinetic study of the rotaxane

The kinetic analysis of the full system in the presence of macrocycle AEMDG was investigated to determine the behaviour of rotaxane 200 as potential replicator (Scheme 7.23). At least, four possible products can be formed in this system, which emanates from the reversible binding interaction of pseudorotaxane [AEMDG·198]. The [AEMDG·198] complex reacted with nitrone 197 to form trans and cis rotaxane 200 and alternately the nitrone 197 reacted with the free maleimide 198, giving trans and cis thread 199.
Scheme 7.23. Possible reaction pathways during the formation of potential self-replicating rotaxane 200. Nitrone 197 can either react with unbound maleimide 198 to afford trans thread 199 and cis thread 199 or with pseudorotaxane [AEMDG•198] to afford trans rotaxane 200 and cis rotaxane 200.

The experimental results presented in Figure 7.20 clearly prove that the bimolecular reaction rate between maleimide 198 and nitrone 197 is relatively low in the absence of recognition. With the experimental data in hand, we turned to simulation to provide an insight how far the behaviour of the recognition-mediated process can be predicted from the behaviour in simple system. Based on the $K_a$ for [AEMDG•198] complex and assuming both this complex and maleimide 198 have the same rate of reaction with nitrone 197, we can predict the outcome of the recognition-mediated reaction. Assuming starting concentrations of the reagents of 10 mM, using the
ISOSIM mode of SimFit provides the predicted product distributions shown in Figure 7.22. The results of this simulation indicate that we should expect to see significantly more thread than rotaxane in the distribution of products. Indeed, the major product should be the trans thread.

**Figure 7.22.** Simulation of the rotaxane and thread formation between maleimide 198, nitrone 197 and macrocycle AEMDG at 10 mM starting concentrations, giving accordingly the trans thread 199 (blue line), cis thread 199 (green line), trans rotaxane 200 (red line), and cis rotaxane 200 (purple line). (T and R represents the thread and rotaxane, respectively).

In order to test whether these simulations of the thread and rotaxane formation reflect the real behaviour of the system, therefore we must explore the real scenario in the laboratory. An equimolar solution of maleimide 198 and macrocycle AEMDG in CDCl₃ was pre-equilibrated at 25 °C for one hour, before the addition of nitrone 197 to the mixture and the starting concentrations each of the three reagents thus set at 10 mM.

The course of reaction was assayed by ¹H NMR spectroscopy at 298 K for 48 hours. The 500 MHz ¹H NMR spectra of the reaction mixture are crowded, however the resonances arising from the formation of the cycloadducts can be monitored (Figure 7.23) – kinetic data for the formation of trans rotaxane 200, cis rotaxane 200, trans thread 199 and cis thread 199 can all be obtained. It is noteworthy that the appearance of the resonances for the cis diastereoisomers for both rotaxane and thread cannot be assayed accurately until four hours of the experiment.
Figure 7.23. Partial $^1\text{H}$ NMR spectra (500 MHz, 298 K, CDCl$_3$, 10 mM) of the reaction between maleimide 198, nitrone 197 and macrocycle AEMDG over 48 hours, illustrating spectral region associated with the resonances of the trans thread 199, cis thread 199, cis rotaxane 200 and trans rotaxane 200.

Deconvolution of the obtained spectra resulted in the concentration vs time profile for this experimental data and fitted using SimFit in Figure 7.24 by varying the rate of pseudorotaxane [AEMDG•198] and nitrone compound 197.
Figure 7.24. Concentration vs time profile for the reaction (500 MHz, CDCl$_3$, 298 K, 48 h) between nitrone 197, maleimide 198 and macrocycle AEMDG at 10 mM reagent concentrations. The formation of trans rotaxane 200 is shown as red filled diamonds, trans thread 199 as blue filled circles, cis thread 199 as green filled squares and cis rotaxane 200 is plotted as purple triangles filled. Solid lines represent the results for the fitting of trans rotaxane 200 (red line), trans thread 199 (blue line), cis thread 199 (green line) and cis rotaxane 200 (purple line), respectively.

The reaction resulted in 57% conversion of maleimide, macrocycle AEMDG and nitrone to cycloadducts, with the ratio of rotaxane to thread being approximately 2:1. From the beginning of the reaction, the rate constant of formation of trans rotaxane 200, $4 \times 10^{-3}$ M$^{-1}$ s$^{-1}$ is higher than trans thread 199, $9 \times 10^{-4}$ M$^{-1}$ s$^{-1}$. As a consequence of this reactivity, trans rotaxane 200 present in 4 mM after 48 hours to compare of only 1.5 mM concentration of trans thread 199. The ratio of trans rotaxane 200 to trans thread 199 was 2.5:1, i.e. there was more rotaxane present than thread. Following this, the diastereomeric ratio of the cis cycloadducts (cis thread 199 and cis rotaxane 200) were estimated to be close to 1.3:1 ratio, respectively. Hence, the trans diastereoisomers were favoured nearly about 6:1 over the cis diastereoisomers.

On average, the simulation (solid line, Figure 7.24) matches reasonably well with the experimental results for the reaction in the presence of macrocycle AEMDG even though the progression for trans rotaxane and trans thread does not correspond correctly to the simulation. The concentrations for trans diastereoisomers of rotaxane 200 is about 2.7 mM higher than that from the earlier calculated concentration from
the simulation whereas the experimental value for trans thread is 0.61 mM short of the one found in calculated concentrations. Concomitantly, the experimental data provided satisfactorily values of cis diastereoisomers for thread and rotaxane in the calculation, which were formed to minor extent, between 0.4 to 0.5 mM. All data presented clearly suggests that the formation of trans rotaxane 200 is significantly faster than the formation of trans thread 199, which can be attributed to the existence of one or more complexes that consequently enable it to facilitate its own formation. This finding is supported by space filling model of trans rotaxane 200 (Figure 7.25).

Figure 7.25. Space filling model of trans rotaxane 200. Carbon atoms are shown in dark grey, nitrogen atoms in blue, oxygen atoms in red and hydrogen atoms are coloured white. The clashing orientation of hydrogen donor from the amidopyridine and carboxylic acid recognition motif are shown in inset.

The corresponding rotaxane is predicted computationally to provide the correct geometry to support replication. As a consequence of the length mismatch between the acid building blocks on macrocycle AEMDG and the amidopyridine site of nitrone 197, the formation of a productive binary complex between the nitrone 197 and macrocycle AEMDG through the [A-B] pathway can be ruled out. Furthermore, it is hard to imagine the rigid structure of the acetylene fragment from the upper ring component is capable of bending to the contrasting orientation of hydrogen donor of
the amidopyridine recognition motif (inset, Figure 7.25). Although, an explanation for the selectivity of trans rotaxane 200 enhancement is yet unknown, the feasibility of the product duplex of trans rotaxane 200 is highlighted in the calculated structure, in which the self-complementary of the duplex structure is visible (Figure 7.26).

Figure 7.26. Molecular model (OPLS2005, GB/SA CHCl₃) of the trans rotaxane 200 duplex. The thread component are shown in dark and light blue whereas the macrocycle AEMDG are shown in dark and light red colour. Most of the hydrogen atoms were omitted for clarity.

Its rigid structure allows for the formation of a duplex structure, in which the recognition sites on both ends of the template possess the right orientation to associate through hydrogen bonding. As a consequence, template-directed synthesis of trans rotaxane 200 through a ternary complex with both reagents seems feasible. In addition, the formation of rotaxane 200 cycloadducts were corroborated by MALDI mass spectrometry from the crude ¹H NMR kinetic reaction (Figure 7.27).

Principally, in order to prove unambiguously replicating behaviour of this system, supplementary doping experiment with the product itself should be carried out. In addition of the pre-synthesised trans rotaxane 200 at the beginning of the reaction between nitrone 197, maleimide 198 and macrocycle AEMDG should resulted in an
increase in the initial rate of formation of *trans* rotaxane 200. The envisaged control experiment provide evidences of that *trans* rotaxane 200 templating and accelerating its own emergence. Moreover, in order to address the reliance of the system on molecular recognition, the remainder of the full kinetic analysis could be demonstrated by performing the inhibitor control experiment. In the presence of 4-bromophenylacetic acid, a decrease on the rate of the reaction as a consequence of the interference of the competitive inhibitor with the recognition process should be observed. Nonetheless, given small quantity of nitrone 197 available, the exhaustive kinetic investigation of the rotaxane system could not be conducted.

Figure 7.27. MALDI mass-spectra of rotaxane compound 200. Mass spectrometry (MALDI) m/z 1992.9772 (60%, [M+H]⁺), calculated for C₁₃₁H₁₃₀N₇O₁₂.
## 7.10 Conclusion

This chapter describes a potential approach to the assembly of self-replicating rotaxanes by employing the alternative Replication model 2. The formation of the replicating structure of rotaxanes relies heavily on the stronger complexation of the acid-substituted macrocycle with the desired maleimide component, in which enable them to adjust themselves to participate in the recognition-mediated pathway of the autocatalytic cycle with nitrone compound in the rotaxane formation. The pseudorotaxane complex is central element within a self-replicating framework.

A number of design potential of self-replicating rotaxanes have been devised in order to integrate self-replication with the formation of [2]-rotaxane. It is important to recognize the unanticipated larger cavity of the third generation macrocycles as a result of the insertion of acid recognition motif on the isophthaloyl building blocks of the ring component. These changes force us to modify our current nitrone compounds in order to protect the larger cavities of the newly prepared acid macrocycles. The quest for a reliable nitrone structure has intensified to integrate a bulky end group on the amidopyridine recognition site to impede the unfavourable pseudorotaxane formation species between the acid macrocycle and the nitrone compound. Satisfyingly, some of the macrocycle and guest pairs presented here have been successfully exploited to construct rotaxanes.

Nevertheless, as a consequence of solubility and synthetic difficulty, we had to compromise the macrocycle binding site, which eventually affected in a low association constant for the pseudorotaxane complex. In the system ultimately designed, pleasingly, a substantial amount of rotaxane was present, mostly as trans diastereoisomer. The initial kinetic behaviour of this system suggests that the resulting rotaxane structures are self-replicating through the recognition-mediated pathways.
8

Replication model 3

8.1 Introduction

Ultimately, we envisaged the final replication scheme of replicating rotaxanes based on the placement of the two recognition sites (green cartoons in Figure 8.1) on the same stopper.

Figure 8.1. Replication model 3 of self-replicating rotaxanes based on the placement of two recognition sites (green cartoons) on the same stopper. The blue cartoons represent the reactive sites, and the red cartoons represent the binding site.
The linear component simultaneously incorporates the binding site (red cartoons) and the recognition site (green cartoons) appended on the reactive sites stopper (blue cartoons) to allow the formation of the [L•M] complex. The subsequent reaction with the second element of the stoppering reagent S, which comprise complementary recognition site provide the rotaxane R molecule. Stoppering reagent S and pseudorotaxane [L•M] can bind reversibly to the R molecule to form the catalytic quarternary complex [S•L•M•R]. Additional bond formation takes place between reagent S and [L•M] complex to give the product duplex [R•R], which then the two molecules of R disassociates to reinstall the autocatalytic cycle. The obvious drawback from Replication model 1 yet remain – the optimisation of the binding constant between the ring component and the guest is the prime importance in order to facilitate the maximal formation of [L•M] complexes. Despite this setback, it is possible to exploit the developed replicator molecules in the group. The chosen replicator is suitable to incorporate in the self-replicating rotaxanes system such that, it proceeds readily at room temperature in maximum rate and high diastereoselectivity, and will be described in the following paragraphs.

8.2. Design of a potential self-replicating rotaxane

The parent macrocycles MDG and MP (first described in Chapter 4) were designed to incorporate the amide binding site. The pseudorotaxane assembly are thus expected, resulting from the mutual association of the macrocycle with the amide guest. The initial step in the construction of potential self-replicating rotaxane presented in Scheme 8.1 comprised either macrocycle MDG or MP as the ring component. The nitrone compound 201 consist of the carboxylic acid recognition site and di-tert-butylphenyl ring as the blocking group to furnish the pseudorotaxane complex between macrocycle MDG and nitrone compound 201 in solution.

The second stopper was attached to this complex by employing the 4,6-dimethylpyridine ring maleimide compound 202, to yield the [2]-rotaxane. It has been established so far that 4,6-dimethylpyridine and 4-tert-butylphenyl ring (as discussed in Chapter 4) are adequately large end groups to prevent macrocycle MDG from deslipping of the thread component. Both maleimide 202 and acid nitrone compound 201 have been previously developed in our laboratory for the construction of small molecule replicator.
Scheme 8.1. Retrosynthesis of potential self-replicating rotaxanes consists of an acid nitrone 201, macrocycle MDG or MP and 4,6-dimethylpyridine maleimide 202.

8.2.1 Binding study
As a result of limited solubility of nitrone compound 201 in non-polar solvents, no binding experiments were carried out between both macrocycles and the acid nitrone 201. Although nitrone 201 has greater solubility in polar solvent such as methanol and dimethylsulfoxide, such solvent can not be utilized as additive in a chloroform solution to increase its solubility since they acts as competitive inhibitors for the binding events to occur by disrupting the association of the macrocycle with the amide binding site and the recognition between the complementary recognition sites. Satisfactorily, the solubility was improved in the presence of the maleimide compound 202.

8.2.2 Kinetic study of the thread
The kinetic study was first carried out in the absence of macrocycle, in order to analyse the performance of the thread in isolation (Scheme 8.2). The course of the reaction was assayed by \(^1\)H NMR spectroscopy in CDCl\(_3\) at 298 K for 24 hours with the initial concentration of 10 mM for both starting materials. 1,3-dipolar cycloaddition reaction between the acid nitrone 201 and maleimide 202 was proved to afford thread 203 by closely monitored the disappearance of the resonance that arises from
the maleimide protons at $\delta_H 6.89$ and the concomitant appearance of the resonances that arise from the \textit{trans} 203 at $\delta_H 5.82$.

![Scheme 8.2](image1.png)

**Scheme 8.2.** 1,3-dipolar cycloaddition between acid nitrone 201 and maleimide 202 affording the highly selective \textit{trans} 203 isoxazolidines.

Accordingly, the concentration vs time profile for \textit{trans} 203 was plotted from the deconvolution of these resonances (Figure 8.2).

![Figure 8.2](image2.png)

**Figure 8.2.** Concentration vs time profile for the reaction (500 MHz, CDCl$_3$, 10 mM) between acid nitrone 201 and maleimide 202 affording \textit{trans} 203 at 298 K over 24 hours.

Reaction between acid nitrone 201 and maleimide compound 202 at 298 K result in 98% overall conversion over 24 hours, giving exclusively the \textit{trans} 203
diastereoisomer. The formation of the \textit{trans 203} denoted an efficient self-replicating behaviour with obvious sigmoidal rate profile, whereas the \textit{cis 203} revealed no recognition-mediated behaviour. The finding was supported from the molecular modelling of the monomer \textit{trans 203} (Figure 8.3).

\textbf{Figure 8.3.} Stick representation of the calculated structure (OPLS2005.GB/SA CHCl\textsubscript{3}) of the major \textit{trans 203} diastereoisomer. Carbon atoms are coloured green, oxygen atoms red, nitrogen atoms blue and hydrogen atoms white (most hydrogen atoms have been removed for clarity).

\textbf{Figure 8.4.} MALDI mass-spectra of \textit{trans 203} diastereoisomer correspond to molecular weight ions of [M+H]\textsuperscript{+}.

The feasible geometry of \textit{trans 203} permit the self-complementary duplex to slightly wrap each other in order to position the recognition units correctly, in which hydrogen
bonding between the recognition units at both ends of the template molecule can take place and self-replicating activity of trans 203 is established. It is also noteworthy that the $^1$H NMR spectrum and MALDI mass spectrometry also reveals the formation of trans 203 diastereoisomer.

### 8.2.3 Kinetic study of the rotaxane

The analogous kinetic experiment was conducted in the presence of macrocycle MDG, afforded simultaneously the [2]-rotaxane and thread component. In this experiment, the reversible formation of [MDG·201] complex reacted with acid nitrone compound 201 to yield rotaxane 204. Alternatively, thread 203 was obtained from the reaction between free maleimide 202 and acid nitrone 201 (Scheme 8.3).

![Scheme 8.3](image)

**Scheme 8.3.** Possible reaction pathways during the formation of potential self-replicating rotaxane 204. Maleimide 202 can react either with unbound acid nitrone 201 to afford thread 203 or with pseudorotaxane [MDG·201] to afford rotaxane 204, respectively.

An equimolar solution of acid nitrone 201 and macrocycle MDG in CDCl$_3$ was bring into equilibrium at 298 K for one hour followed with the addition of maleimide 202 to the mixture. As noted in the kinetic study of thread 203, the initial concentration of the
starting materials was 10 mM. The resulting product was monitored using 500 MHz \(^1\)H NMR spectroscopic analysis, which observed the development of the resonances (Figure 8.5) of proton \(H^3T\) that arise from the *trans* thread 203 at \(\delta_H 5.81\) and of proton \(H^3R\) originated from the *trans* rotaxane 204 at \(\delta_H 5.56\). It is noteworthy that no *cis* diastereoisomer was identified from the reaction mixture.

![Partial ¹H NMR spectrum](image)

**Figure 8.5.** Partial \(^1\)H NMR spectrum (500 MHz, 298 K, CDCl\(_3\), 10 mM) of the reaction between macrocycle MDG, acid nitrene 201 and maleimide 202 over 48 hours. T and R represents thread and rotaxane respectively.

Analysis of the rate profile for this new system revealed that the thread conversion dropped to 33% over 48 hours in the presence of macrocycle MDG. Unexpectedly, the reaction yielded the corresponding rotaxane 204 in reduced yield of 2% in the presence of one equivalent of macrocycle MDG (Figure 8.6).
Figure 8.6. Concentration vs time profile for the reaction (500 MHz, CDCl$_3$, 10 mM) between acid nitrone 201 and maleimide 202 in the presence of one equivalent of macrocycle MDG at 298 K over 48 hours. The formation of trans thread 203 is shown as red filled circles and trans rotaxane 204 as blue filled diamonds.

As a consequence, the ratio of thread to rotaxane was found to be at least 25:1 in favour of trans cycloadducts for each thread and rotaxane. Despite the fact that the formation of thread appear to be highly selective for the trans diastereoisomer, this selectivity was lessened in the existence of macrocycle MDG in the system. As explained in Replication model 1, the formation of the [2]-rotaxane again critically dependent on the pseudorotaxane precursor, which unambiguously demonstrated the critical binding event between the macrocycle MDG and the amide moiety of the maleimide 202.

Whilst it is presumed that macrocycle MDG bind preferably the amide moiety on the acid nitrone 201 via the formation of hydrogen bonds, the formation of pseudorotaxane complex nonetheless results in the reduced reactivity. In order to investigate the reduced reactivity of these complexes, the electronic structure calculation was subsequently applied (Figure 8.7).
Figure 8.7. Stick representations of the calculated structures (OPLS2005, GB/SA CHCl₃) of (a) [2]-rotaxane formation [MDG•201•202] displays that the carbonyl group of the macrocycle MDG and the hydroxyl group of the acid nitrone 201 are extensively hydrogen bonded (1.73 Å) and (b) The carboxylic acid of thread 203 exerts marked steric effect on the ring component as illustrated in the close-packed space filling model. Carbon atoms are coloured green, oxygen atoms red, nitrogen atoms blue and hydrogen atoms white (most hydrogen atoms have been removed for clarity).

In prior investigations, we became aware²⁰⁷ that the reactive site on the L component must be placed sufficiently far away from the binding site to prevent the introduction of a supramolecular steric effect through the proximity of the macrocyclic component M. These examples should also serve to illustrate the cause for the low reactivity of pseudorotaxane in this case.

Additionally, the carboxylic acid group from thread 203 was positioned in the vicinity of the approaching ring component, consequently exerts marked steric effect results in the unfavourable formation of the mechanically interlocked molecules. It is also visible from the calculated structure that the oxygen atom from the carbonyl group (H-acceptor) of the macrocycle MDG and the hydroxyl group (H-donor) of the acid
nitrone 201 are extensively hydrogen bonded (1.73 Å). The result of this calculation again suggests\textsuperscript{200} that in the framework of the self-replicating rotaxane, the reactive site on the \textbf{L} component must be located far from the complexation site of the macrocycle to prevent the pronounced supramolecular steric effect. Satisfyingly, Matrix Assisted Laser Desorption Ionization (MALDI) mass spectrometry confirms the formation of rotaxane 204 in the reaction mixture (Figure 8.8).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure8.8.png}
\caption{MALDI mass-spectra of rotaxane 204 compound which corresponds to molecular weight ions of [M+Na]\textsuperscript{+}.}
\end{figure}

\subsection{8.3 Modified design of a potential self-replicating rotaxane}

On the basis of designing recognition-mediated reactions, we have managed to resolve the solubility issues by reversing\textsuperscript{207} the location of recognition sites on both the \textbf{L} and \textbf{S} components of the rotaxane design. Accordingly, a new system was envisaged in which the amidopyridine recognition site is now situated on the stopper of nitrone compound (for example nitrone \textbf{C}) while the carboxylic acid recognition site is appended on the stopper of maleimide 205 (stoppering reagent \textbf{S}) as depicted in Scheme 8.4.
Scheme 8.4. Retrosynthesis of a potential self-replicating rotaxane 2. Amidopyridine recognition site (green colours) is positioned on the stopper of nitrone compound C (L component) containing the diarylamide binding site (red colour) and the carboxylic acid recognition site (green colour) on the stopper of maleimide compound 205 (S component). Both macrocycles MDG and MP are utilized in the modified design of self-replicating rotaxanes.

As noted earlier, length segregation must be established between the reactive site and the binding site to forbid any degree of supramolecular steric effect. Following this, the nitrone reactive site was placed markedly apart from the 3,5-bis(trifluoromethyl) amide binding moiety. According to preliminary binding experiments, 4,6-dimethylpyridine ring has been evaluated as sufficiently large end group for macrocycle MDG. On that account, an alternative 6-methylpyridine ring are exploited to allow the ring component to associate the diarylamide moiety of the linear component. A considerable attention was made to ensure that the ring has a higher affinity of binding the diarylamide moiety than the competitive binding sites, namely the nitrone itself and alternative amide site on the amidopyridine ring.

The selection of 3,5-bis(trifluoromethyl) substituent in the revised scheme of potential self-replicating rotaxanes (Scheme 8.4) is in accordance with prior $K_a$ values found in Chapter 4. The association strength for [MDG•110] complex was estimated to be $1000 \pm 100 \text{ M}^{-1}$ and $4500 \pm 500 \text{ M}^{-1}$ [MP•110] complex, respectively. This observation was accompanied with significant chemical shift changes upon the formation of both
[2]-pseudorotaxane complexes. In our initial attempt to influence the positioning of the ring on the thread component, we have analysed the potential of a set of 3,5-disubstituted aryl amide end groups. Additionally, the correlation between the structure and the reactivity are revealed and used as a delicate probe into the effects of which electronic perturbation produces upon interlocking process.

8.4 Synthesis of the nitrone

In order to rationalize the binding affinity of the macrocycle to the targeted amide moiety, the following set of 3,5-disubstituted phenyl ring are devised. The substituent groups are introduced from −OC₅H₁₁, −Cl and −CF₃ in which the electron-withdrawing effects brought about by this substitution were referred to a reference substituent −H, that are considered to be neutral (neither electron-donating or -withdrawing).

Hammett parameters, σᵣ for a set of substituent groups (nitrone compound A − C) and the obtained association constants are summarized below in Figure 8.9. On the whole, the differences in structure produce proportional changes in the $K_a$ values. The reason is that the changes in $K_a$ ($ΔG^\circ$) brought by the $m$-substituents are effectively change $ΔH^\circ$ since substitution does not greatly affect $ΔS^\circ$. The $K_a$ values are evidently increased to 2000 M$^{-1}$ by substituent on the $m$−OC₅H₁₁.

Additionally, relative to hydrogen in the series, the substituents of $m$-Cl and $m$-CF₃ are increasingly electron withdrawing, and consequently ordered by increasing $K_a$ of about 4600 M$^{-1}$ and 5500 M$^{-1}$, respectively. In fact, the plot of log $K_a$ for these substituents relative to that hydrogen is reasonably linear and evidence of the known linear free energy relationship. Thus, as you go through the series, only the substituent matter, which subsequently correlate with higher $K_a$ values.
**Figure 8.9.** The effect of reactivities of electron-withdrawing substituents appended on the amide moiety of the nitrone component (structures A, B, and C) relative to hydrogen with respect to the association constant, $K_a (M^{-1})$. Electron-withdrawing substituents are characterized by positive values of $\sigma$ and hydrogen as reference point has $\sigma = 0.0$. Plot of log $[K_a(X)/K_a(H)]$ against the $\sigma_m$ value.

The best way to convey this observation is by visually represent it as in an electronic potential energy maps, comparing the two model compounds incorporating $m$–CH$_3$ and $m$-CF$_3$ (Figure 8.10). Electrostatic potential energy maps are useful when illustrating the charge distributions of molecules and changes in reactive site when molecules acquire or lose different functional groups. The electrostatic potential energies for both 3,5-dimethyl and 3,5-bis(trifluoromethyl) groups of the amide model

<table>
<thead>
<tr>
<th>$X$</th>
<th>$\sigma_m$</th>
<th>$K_a/M^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+0.12</td>
<td>2000</td>
</tr>
<tr>
<td>B</td>
<td>+0.37</td>
<td>4600</td>
</tr>
<tr>
<td>C</td>
<td>+0.46</td>
<td>5500</td>
</tr>
</tbody>
</table>

$$\log \frac{K_a(X)}{K_a(H)} = 1.325\sigma + 0.8473$$
compounds were calculated. Noted that the three dimensional model of the electrostatic potential map is very similar to the fundamental structure of the two dimensional molecular structure. Area of low energy potential, red is characterize by the oxygen atom from the carbonyl group, whilst the blue region that emerge from the bottom of the molecule corresponds to the location of the hydrogen atom of the amide. However, there is a great deal of intermediary potential energy (yellow and green colour spectrum), which indicates the smaller electronegativity difference in both samples.

![Electrostatic Potential Energy](image)

**Figure 8.10.** The illustrated electrostatic potential energy for (a) 3,5-dimethylaryl benzanilide and (b) 3,5-bis(trifluoromethyl)benzyl amide, plotted on the same scale in kJ mol$^{-1}$ for comparison. The varying intensities of the electrostatic potential energy values can be conveyed with blue colour as the highest electrostatic potential energy value, red colour as the lowest and intermediary colour represent intermediary electrostatic potentials. Thus, a high electrostatic potential indicates the relative absence of electrons and a low electrostatic potential indicates an abundance of electrons.

One of the conspicuous differences is that the negative region in dimethyl substituent is considerably larger than bis(trifluoromethyl) substituent as a consequence of the electronegativity redistribution imposed by the CF$_3$ groups. These relative
distributions of electrons introduced intense positive charges on the hydrogen atom of the amide in latter group, and results in prominent feature of hydrogen donor. Therefore, the association constant is at the highest when 3,5-bis(trifluoromethyl) substituent was employed. In the pursuit of achieving high association constant in the formation of [2]-pseudorotaxane species, 3,5-di-Cl and 3,5-bis-CF₃–nitrone B and C were initially synthesised. The strategy employed to prepare 3,5-di-Cl–nitrone B was first illustrated in Scheme 8.5.

Scheme 8.5. Attempted synthesis of 3,5-di-Cl nitrone B. Reagents and yields: (a) 4-iodoaniline, PdCl₂(PPh₃)₂, PPh₃, Cul, Et₃N/THF, 65 °C, 16 h, 28%; (b) 3,5-dichlorobenzoyl chloride, Et₃N, CH₂Cl₂, 0 °C to rt, 16 h, 56%.

The nitro alkyne 189 was coupled with 4-iodoaniline under Sonogashira conditions to give 27% yield of amine 206 after purification. The nucleophilic attack of 206 on the readily obtained 3,5-dichlorobenzoyl chlorides afforded the corresponding nitro compound 207 in a yield of 56%. Nevertheless, the subsequent partial reduction to afford the hydroxylamine has been unsuccessful. Besides that, we are aware that the solubility of nitrone B in non-polar solvents is likely to be limited. Therefore, we are more interested in assembling second model nitrone C, consists of 3,5-bis(trifluoromethyl) phenyl ring as its two sterically end groups in the new scheme of potential self-replicating rotaxanes. An attempt to synthesise the analogous 3,5-bis-CF₃ nitrone C has been undertaken as depicted in Scheme 8.6. Compound 208 was obtained in a yield of 38% after precipitation and washed with cool dichloromethane. The following Sonogashira reaction of compound 208 with
synthesized terminal alkyne 189 give the corresponding nitro derivative compound 209, in a yield of 75%. Unfortunately, all efforts to reduce the nitro compound to the desired hydroxylamine has been unsuccessful and proven to be fairly troublesome.

![Scheme 8.6](image)

**Scheme 8.6.** Attempted synthesis of 3,5-bis-(CF₃) nitrone C. Reagents and yields: (a) 4-iodoaniline, Et₃N, CH₂Cl₂, 0 °C to rt, 1 h, 38%; (b) Alkyne 189, PdCl₂(PPh₃)₂, PPh₃, CuI, Et₃N/THF, 65 °C, 16 h, 75%.

The failure to obtain 3,5-bis-CF₃ nitrone C is more likely due to the formation of the azoxy compound. Furthermore, MALDI spectrometry revealed the signal that correspond to the stable azoxy compound. An attempt to circumvent the solubility limitation in the case of 3,5-di-Cl nitrone B brought us to the formation of 3,5-di-OC₈H₁₇ nitrone A. The alternative strategy in synthesising nitrone A was outlined in **Scheme 8.7**. In the alkylation of 3,5-dihydroxybenzoic acid 210, its esterification occurred concomitantly as described in the work of Tamiaki *et al*. The corresponding benzoate was hydrolysed to give the corresponding carboxylic acid 211 and quantitatively transformed to acid chloride 212 by the treatment of thionyl chloride in toluene. Subsequently, the condensation of amine 206 and acid chloride 212 resulted in the formation of nitro derivative compound 213. However, the synthetic approach to obtain the desired nitrone A was impeded by the similar reasoning, which has proven to become an unfortunate start in our early work.
Scheme 8.7. Attempted synthesis of 3,5-di-OC₈H₁₇ nitrone A. Reagents and yields: (a) i. CH₃(CH₂)₆CH₂Br, K₂CO₃, dry DMF, 65 °C, 16 h; ii. KOH/EtOH/H₂O, 88 °C, 16 h, 40% over 2 steps; (b) SOCl₂, toluene, 110 °C, 16 h, 98%; (c) Compound 206, Et₃N, CHCl₃, reflux, 10 h, 60%.

8.5 Binding study with CF₃ nitro 209

Herein, we described the formation of pseudorotaxane complex between macrocycle MDG or MP with CF₃ nitro compound 209 and monitored by ¹H NMR spectroscopy as described in the previous binding experiments. The first 400.1 MHz ¹H NMR spectrum of an equimolar mixture of macrocycle MDG and nitro compound 209 in CDCl₃ at room temperature undergo a slow exchange process on the ¹H NMR chemical shift timescale (Figure 8.11b). It is noteworthy that some significant changes in the chemical shifts of the protons of the complex (Figure 8.11b) are relative to those of its free components (Figure 8.11a and Figure 8.11c). The typical downfield shift of the macrocycle proton NH₉ (Δ=0.88 ppm), the upfield shift of macrocycle MDG phenylene protons H₅R and H₆R and the appearance of broad complex pattern resonances for macrocycle methylene protons (H₈R, H₃R, H₂R and H¹R) suggests the assembly of [MDG•209] complex.
Figure 8.11. Partial $^1$H NMR spectra (400.1 MHz, 298 K, CDCl$_3$, 20 mM) of: (a) Macrocycle MDG; (b) an equimolar mixture of macrocycle MDG and compound 209 (c) Compound 209. Red dashed lines are shown to connect resonances for specific protons in the free and complex states. Red dashed boxes shows the broad resonances of methylene protons (H$^{9R}$, H$^{10R}$, H$^{13R}$ and H$^{14R}$) upon pseudorotaxane complex [MDG•209] formation. R stands for ring (in this case macrocycle MDG) and T for thread (in this case nitro compound 209).
These observations are consistent with the appearance of new fluorine resonance from $^{19}$F NMR spectrum at $\delta_{F} = -63.1$ (not shown), which corresponds to the formation [MDG·209] complex, compared to the free species of nitro 209 in solution $\delta_{F} = -63.4$. We suspect that this result is partly due to the electron withdrawing of CF$_3$ substituents of compound 209, which essentially increase the hydrogen donor’s ability of the NH bond.

The second binding experiment was conducted in the presence of macrocycle MP (Figure 8.12) under similar condition. In contrast to the above spectra, the spectral changes were more resolved and desymmetrized, due to the fact that the face of the ring is no longer symmetrically planar. For instance, the macrocycle NH protons, H$^{11R}$ are typically shifted downfield (+1.11 ppm) upon hydrogen bonding to the oxygen of the carbonyl group of nitro compound 209. Such large downfield change was also evidenced for the NH proton resonance of nitro compound 209, H$^7T$ (+1.50 ppm), (Figure 8.12c) which signify the association strength between the two components in the pseudorotaxane assembly as illustrated in Figure 8.12b.

Furthermore, the changes in the methylene protons (H$^{10R}$, H$^{5R}$ and H$^{4R}$) of the free macrocycle MP (Figure 8.12a) are most affected – these proton resonances lead to a complex pattern between the region of $\delta_{H}$ 4.53 – 4.17 because they are now rendered magnetically inequivalent as a results of the end-to-end asymmetry imposed by compound 209. The binding constant for the pseudorotaxane [MP·209] complex was determined to be $3100 \pm 300$ M$^{-1}$ by $^{19}$F NMR spectroscopy (376.5 MHz, CDCl$_3$) using the single-point method. On the whole, we have demonstrated that both macrocycles MDG and MP are capable of forming the [2]-pseudorotaxane type geometry with nitro compound 209, which consequently stabilized via intermolecular hydrogen bonding in the low polarity solvents.
Figure 8.12. Partial $^1$H NMR spectra (400.1 MHz, 298 K, CDCl$_3$, 20 mM) of (a) Macrocycle MP; (b) an equimolar mixture of macrocycle MP and nitro compound 209 (c) 3,5-bis CF$_3$ nitro compound 209. Red dashed lines are shown to connect resonances for specific protons in the free and bound states. Red dashed boxes shows the complex pattern resonances for methylene protons ($H^{10R}$, $H^{5R}$ and $H^{6R}$) upon pseudorotaxane [MP-209] complex formation. R stands for ring (in this case the macrocycle MP) and T stands for thread (in this case compound 209).
8.6 Binding study of OC₆H₁₇ nitro 213

Similar binding behaviour was observed for nitro compound 213 as such the pseudorotaxane formation of [MDG•213] complex also encountered a slow exchange process on the ¹H NMR chemical shift timescale. The variable temperature study for the pseudorotaxane [MDG•213] assembly was carried out to evaluate the binding interaction.

The stack plots of the 500.1 MHz ¹H NMR spectra of the corresponding pseudorotaxane complex were recorded from 297 K to 250 K (Figure 8.13a to Figure 8.13e) and displays slow exchange process between the free and bound species and on all occasions, a small contribution from the intermediate species was observed from the new broad peak. Thus, this [2]-pseudorotaxane [MDG•213] was stabilized predominantly through hydrogen bonding interactions between the two components.

The conformation of the [2]-pseudorotaxane [MDG•213] was confirmed from 2D ROESY NMR spectrum as shown in Figure 8.14. As predicted, macrocycle MDG was predominantly located around the amide moiety of nitro compound 213 and the resonance of macrocycle NH⁹R proton shift toward the terminus of the compound 213 is apparent from the significant upfield shifts (up to δ_H 9.33). In addition, the spectra revealed crosspeaks between the NH proton of macrocycle MDG, H⁹R and the nitro aromatic protons H¹¹T and H¹⁶T. As detailed above, the interaction between macrocycle MDG and nitro compound 213 via hydrogen bonding and, hence, confirmed the geometry shown in Figure 8.14.
Figure 8.13. Variable temperature $^1$H NMR (500.1 MHz, CDCl$_3$, 20 mM) binding study of an equimolar mixture of macrocycle MDG and nitro compound 213.
When the binding experiment was repeated using macrocycle MP, nitro compound 213 and macrocycle MP exhibited a similar binding trend at room temperature for 1:1 binding stoichiometry. 500.1 MHz $^1$H NMR spectra of the complex when recorded at variable temperature in CDCl$_3$ are shown in Figure 8.15. As depicted from Figure 8.15, the 500.1 MHz $^1$H NMR spectra are more resolved at 248 K, which allows us to observe the dominant species of pseudorotaxane [MP•213] complex with a slow exchange process on the $^1$H NMR chemical shift timescale.

Figure 8.14. Partial 2D ROESY spectrum (500.1 MHz, 20 mM, −40 °C, CDCl$_3$) of an equimolar of macrocycle MDG and nitro compound 213. ROESY crosspeaks (red points) are observed for the complex between macrocycle MDG and nitro compound 213 are shown as black arrows.
Figure 8.15. Variable temperature of $^1$H NMR spectra (500.1 MHz, CDCl$_3$, 20 mM) of an equimolar mixture of macrocycle MP and nitro compound 213.
We observed significant chemical changes in the $^1$H NMR spectra of the corresponding [2]-pseudorotaxane [MP•213] as such the NH protons of the macrocycle MP, NH$^{11}$R are significantly shifted downfield upon binding to nitro compound 213. Furthermore, the amide proton of nitro compound 213 NH$^{14}$T was also largely affected and display pronounced downfield shift, which suggests that this proton is hydrogen bonded to the lower cavity of macrocycle MP. The slow exchange process does not only yield sharp separate resonance but also yield exchange peaks in the 2D EXSY experiment. Figure 8.16 show the 500.1 MHz 2D EXSY spectrum of the pseudorotaxane [MP•213] complex.

Figure 8.16. Partial 2D EXSY spectrum (500.1 MHz, 20 mM, 233 K, CDCl$_3$) display crosspeaks (blue points) between macrocycle MP and nitro compound 213. T and R represents thread and rotaxane compound, respectively. UB (blue dashed boxes) represents the unbound species in the reaction mixture.

It is apparent that the bound and the unbound species exchange slowly with one another from the 2D EXSY spectrum. This is evident by the three crosspeaks in the spectrum from the pseudorotaxane [MP•213] complex – H$^7$R and H$^8$R of the macrocycle MP and H$^{10}$T and H$^{11}$T of the nitro compound 213 (solid blue boxes) and that from the unbound species (dashed blue boxes). Hence, the association constant,
$K_a$ for the pseudorotaxane assembly was estimated to be 5560 ± 560 M$^{-1}$. The 2D ROESY spectra of the pseudorotaxane complex (Figure 8.17) exhibit distinct crosspeaks between the proton resonance of the macrocycle MP NH$^{11R}$ and aromatic proton of nitro compound 213, namely H$^{11T}$ and H$^{16T}$. In a similar manner, the NH proton resonance of compound 213, H$^{14T}$ exhibit a crosspeak with the methylene proton of macrocycle MP, H$^{4R}$ and thus confirmed the encircling of the macrocyclic component about the amide moiety of compound 213 with the anticipated geometry.

Figure 8.17. Partial 2D ROESY spectra (500.1 MHz, 20 mM, −40 °C, CDCl$_3$) of an equimolar of macrocycle MP and nitro compound 213. ROESY crosspeaks (red points) observed for the complex between macrocycle MP and nitro compound 213 are shown as black arrows.
8.7 Conclusion

The first and unsuccessful attempt to integrate the developed replicator molecules in Replication model 3 originated from the reduced reactivity of [2]-pseudorotaxane species. There is a close connection between this reduced circumstances and the relative position of the reactive site with the binding site. It is concluded that the reactive site must be placed sufficiently far away from the binding site to prevent the introduction of a supramolecular steric effect through the proximity of the macrocyclic component.

On that account, we improved the initial scheme of self-replicating rotaxanes, which introduced length segregation between the reactive site and binding site. In addition, we have demonstrated that we are able to influence the position of the ring component to the targeted binding site by means of high association constant. Our effort to demonstrate the fundamental idea behind the third minimal model has been properly illustrated, as such we have successfully ensured that the ring component encircled the preferable binding site of the guest compound and achieved a high association constant in the formation of the pseudorotaxane complex. All is good, except for the fact that the unsuccessful procedure to obtain the key hydroxylamine compound prevented us to explore the potential behaviour of self-replicating rotaxanes, and hence, it is necessary to consider the current synthetic approaches of the nitrone compound.
General conclusion

The concepts of artificial self-replicating system have become scientifically acknowledged over the past twenty-five years with significant number of replicator structures based on simple organic molecules have been published. In the self-replicating process, the product molecule acts as a template for its own formation by generating a catalytically active complex and exploiting the association with its building blocks via non-covalent interactions. In this context, the development of model for studying self-replication also demonstrated that the chemical information can be transmitted and amplified. These objectives required a comprehensive understanding of the recognition-mediated process that allow the product molecules to template their own formation.

The work presented here, revolves around the minimal model of replication and its incorporation into the design of mechanically interlocked molecules namely rotaxanes. Having established the fundamental properties in designing [2]-rotaxane (Chapter 3), three discrete minimal model of replication were designed. Theoretical discussions of each replication model at the start of the investigation help us to visualize both good and bad points in each framework. Initial investigations on the first replication model showed that the binding between the self-replicating thread and the macrocycle must exceed a certain threshold value in order to allow efficient autocatalytic formation of the rotaxane structure (Chapter 4, 5 and 6). Alternatively, we explored the second replication model (Chapter 7) in which one of the recognition unit was moved from the thread on to the macrocycle. This modification ensures that only the rotaxane structure undergoes the autocatalytic reaction. Ultimately, we devised conceptually the third replication model (Chapter 8), which incorporate the binding site and one element of the stopper group appended to one of the building blocks constitutive of the template, either the pseudorotaxane precursor or the linear
component. Nonetheless, we do not anticipate any significant problems beyond the prior context until the implementation of these replication models were carried out. Current approaches towards important achievements and disappointments to reach this target are summarized together in Table 9.1 within each minimal model designed. We are also investigating the underlying factors in greater details.

Table 9.1. Important achievements and disappointments within each minimal model of replicating [2]-rotaxanes.

<table>
<thead>
<tr>
<th>Observations</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. High association constant, $K_a$ is necessary to attain substantial amount of the pseudorotaxane precursor and, hence, increased the overall yield of rotaxanes.</td>
<td>A high $K_a$ is attainable and greatly improved with the modification of the guest design and by lowering the temperature. Revolves around the simple kinetic model of rotaxane formation.</td>
<td>Potential improvements in $K_a$ hampered by guest solubility.</td>
<td>Greatly improved with the modification of the guest design.</td>
</tr>
<tr>
<td>3. Synthetic approaches towards the implementation of the replication model.</td>
<td>Generally straight forward and effective.</td>
<td>Generally straight forward but less effective.</td>
<td>Generally straight forward but less effective.</td>
</tr>
<tr>
<td>4. Reduced reactivity of pseudorotaxane complex as a consequence of pronounced supramolecular steric effect.</td>
<td>Less reactive than thread. Can be modified by guest design.</td>
<td>Pseudorotaxane apparently more reactive than thread.</td>
<td>Less reactive than thread. Can be modified by guest design.</td>
</tr>
<tr>
<td>5. Effect of recognition on thread and/or pseudorotaxane reactivity.</td>
<td>Thread and pseudorotaxane rates affected.</td>
<td>Pseudorotaxane only.</td>
<td>Thread and pseudorotaxane rates affected.</td>
</tr>
</tbody>
</table>
Experimental and synthetic procedures

10.1 General experimental procedures

Chemicals were purchased from ABCR GmbH & Co, Alfa-Aesar, Apollo Scientific Ltd., Avocado Research Chemicals Ltd Fisher Scientific UK Ltd., Sigma-Aldrich Company Ltd. or VWR International Ltd. and were used as received or otherwise purified by standard techniques where required. Most of the non-aqueous reactions were carried out under an inert Ar or N₂ atmosphere with the inert gas passing through a bed of 4 Å molecular sieves and self-indicating silica gel. Brine refers to a saturated aqueous solution of sodium chloride.

Anhydrous solvents were obtained under the following conditions, such as dry MeCN was distilled from calcium hydride in a recycling still, dry CH₂Cl₂ was obtained using a MBRAUN GmbH MB SPS-800 solvent purification system, which the solvent was dried by passage through filter columns and dispensed under an atmosphere of Ar or N₂ gas, CDCl₃ was neutralised over CaCl₂ filtered and stored over 4 Å activated molecular sieves and dry THF was distilled from sodium in a recycling still using benzophenone kethyl as indicator.

Analytical Thin Layer Chromatography (TLC) analysis was performed on MACHEREY-NAGEL GmbH & Co, POLYGRAM® SIL G/UV₂₅₄ plates, plastic backed 0.2 mm silica gel plates with fluorescent indicator. Developed plates were air-dried and visualized under UV lamp (λₓᵧzx 254 or 366 nm) or incubating with iodine on sand. Where necessary, thermal development after dipping in: methanolic DNP and sulfuric acid solution and ninhydrin in n-butanol or a solution of aqueous potassium permanganate, was used to aid visualization.
Flash Column chromatography and silica plugs were carried out on Apollo Scientific Ltd. silica gel 40-63 micron or Silicycle SiliaFlash® P60 silica gel (230-400 mesh) eluting with solvents as supplied under a positive pressure of compressed air.

Melting points were measured in open capillary tubes using an Electrothermal 9200 melting point apparatus and are uncorrected.

Mass spectra were recorded on a Micromass GCT spectrometer for electron impact (EI) operating at 70 eV or chemical ionization (CI) using isobutane as the ionising gas. Electrospray ionisation spectra (ES) were performed on a Micromass LCT spectrometer operating in positive or negative mode from solutions of methanol, acetonitrile or water. m/z values are reported in Daltons and followed by their percentage abundance in parentheses. 4800 MALDI TOF/TOF™ Analyzer is capable of operating in either linear or reflectron mode allowing either high sensitivity detection over a large mass range or improved signal resolution with the associated increase mass accuracy.

10.2 General NMR spectroscopy procedures

$^1$H NMR spectra were recorded on a Bruker Avance 300 (300.1 MHz), a Bruker Avance II 400 (400.1 MHz), a Bruker Avance 500 (499.9 MHz) or a Varian UNITYplus 500 (500.1 MHz) spectrometer using the deuterated solvent as the lock and the residual spectra as the internal reference in all cases. The chemical shift information ($\delta_H$) for each resonance signal is given in units of parts per million (ppm) relative to trimethylsilane (TMS) where $\delta_H$ TMS = 0.00. The number of protons ($n$) for a reported resonances signal are indicated from their integral value and their multiplicity are represented by the symbols s, d, t, q, m and br which denote singlet, doublet, triplet, quartet, multiplet and broad singlet, respectively. Their coupling constant (J) are determined by analysis using iNMR© (Version 3.6.3, Mestrelab Research, 2010) and quoted to the nearest 0.1 Hz. Identical coupling constants are averaged in each spectrum and also reported to the nearest 0.1 Hz.

$^{13}$C NMR spectra were recorded on a Bruker Avance 300 (75.5 MHz), a Bruker Avance II 400 (100.6 MHz) or a Bruker Avance 500 (125.7 MHz) spectrometer utilizing the DEPTQ pulse sequences with broadband proton decoupling using the
deuterated solvents as the lock and the residual solvent as the internal reference in all cases. In the assignment of $^{13}$C NMR spectra, the chemical shift information ($\delta_C$) for each resonance signal are given in units of parts per million (ppm) relative to trimethylsilane (TMS) where $\delta_C$ TMS = 0.00. Mainly, the signals are singlets or unless stated, which their multiplicity is represented by the symbol d for doublet.

$^{19}$F NMR spectra were recorded using a Bruker Avance 300 (282.4 MHz), a Bruker Avance II 400 (376.5 MHz) or a Bruker Avance 500 (470.3 MHz) spectrometer using a broadband proton decoupling pulse sequence with the deuterated solvent as the internal lock. The chemical shift information ($\delta_F$) for each resonance signal are given in units of parts per million (ppm) relative to CCl$_3$F where $\delta_F$ CCl$_3$F = 0.00. The analysis of $^1$H, $^{13}$C and $^{19}$F spectra were carried out using iNMR© software (Version 3.6.3, Mestrelab Research, 2010).

10.3 $^1$H NMR spectroscopy: Preparation, kinetic measurements and deconvolution data

Masses of reagents were measured using a Sartorius BP 211D balance (± 0.01 mg) and reagent solutions were prepared by dissolving three starting materials in 0.7 mL volume of dry CDCl$_3$ at 20 mM reaction concentration. Of particular note, the maleimide and macrocycle compound were pre-equilibrated at 273 K for 1 h prior to mixing with nitrone compound. By using Hamilton gas-tight syringes, the reaction solution was transferred immediately to a 5 mM NMR tube (Wilmad 528PP) and was then fitted with a polyethylene pressure cap to minimise solvent evaporation. The tube was transferred promptly to a 500 MHz NMR spectrometer (Varian UNITYplus) regulated at reaction temperature.

The $^1$H NMR spectra were acquired automatically at a set time interval (every 1800 s) over a period of 16 h. Analysis and deconvolution of the arrayed $^1$H NMR spectra recorded in the course of the reaction was performed using iNMR© software (Version 3.6.3, Mestrelab Research, 2010). We monitored the chemical reaction of 1,3-dipolar cycloaddition between a maleimide and a nitrone, which give rise to two cycloadduct protons. These diastereoisomeric products, namely trans and cis are distinguishable in
the $^1$H NMR spectrum with different splitting pattern for each as a consequence of their different dihedral angles of H$^2$ to H$^3$ as illustrated in Figure 10.1.

![Diagram](image)

**Figure 10.1.** Partial 500.1 MHz $^1$H NMR spectrum featuring the characteristic splitting patterns for the proton resonances arising from the isoxazolidine ring for the trans and cis diastereoisomer in the reaction between nitroine and maleimide derivative compound. In the case of trans cycloadduct, the signal for proton H$^3$ is singlet and doublet for H$^1$ and H$^2$. On the other hand, the cis cycloadduct give doublets for H$^1$ and H$^2$ and doublet of doublet for H$^3$.

The concentration of reactants and products at each time point was determined by monitoring the consumption of either maleimide or nitroine proton resonances and the appearance of cycloadduct proton resonances. For instance, the area of the maleimide proton resonances and either one of the cycloadduct proton resonances were calculated through deconvolution of the spectrum. The area of maleimide protons resonances was initially divided by two and added to the area of the cycloadduct proton resonances. The resulting number indicates the area of a proton resonance of a species at fixed reaction concentration and was further employed as a reference to calculate the concentration of other detectable species in the $^1$H NMR spectra. This procedure was repeated for remaining spectra recorded at later time points. The time at which each spectrum was recorded was extracted from the log file alongside the NMR spectroscopy data. Having established the concentration of
products at each time point, the reaction profiles were plotted using the ProFit program (version 6.2.4, Quantum Soft, Switzerland). This is particularly straightforward to be employed in the kinetic analysis of thread formation as described in Section 5.5 (Chapter 5).

Figure 10.2. Partial $^1$H NMR spectra (500.1 MHz, 25 °C, CDCl$_3$, 16 h) of the reaction between nitrone 134, maleimide 108 and macrocycle MDG, illustrating spectral region associated with the resonances of the isoxazolidines proton for thread 135 and rotaxane 136 at 20 mM concentration.
Figure 10.3. Partial $^1$H NMR spectra (500.1 MHz, 25 °C, 16 h, CDCl$_3$) of the reaction between nitron 134, maleimide 108 and macrocycle MP, illustrating spectral region associated with the resonances of the isoxazolidines proton at 20 mM concentration.
Nonetheless, the elucidation by $^1$H NMR spectroscopy is however not always straightforward. For instance, in the formation of simple rotaxane formation utilizing macrocycle MDG, nitrone 134 and maleimide 108 (as described in Figure 5.8a, Chapter 5), a total integration for both diastereoisomers for each thread and rotaxane was taken to provide useful information for the kinetic analysis of the system are shown in Figure 10.2.

The partial $^1$H NMR showed that the resonances arising from the trans rotaxane 137 and of the trans thread 135 are no longer overlap between each other when we replaced the macrocycle with macrocycle MP (as illustrated in Figure 5.10a, Chapter 5). Despite that, the resonances arising from the cis cycloadducts of thread and rotaxane remains overlap over 16 hours of the experiment. Likewise, the reaction profile for the reaction was similarly constructed from the total integration of potential diastereoisomers related to thread and rotaxane, respectively and shown in Figure 10.3.

10.3 $^{19}$F NMR spectroscopy : Preparation, kinetic measurements and deconvolution data

The NMR sample was similarly prepared as described previously. The tube was transferred immediately to a 470.4 MHz Bruker Avance 500 NMR spectrometer regulated at reaction temperature. $^{19}$F($^1$H) were acquired automatically at a set time interval (every 1800 s) over a period of 16 h. Analysis and deconvolution of the arrayed $^{19}$F NMR spectra recorded in the course of the reaction was performed using iNMR© software (Version 3.6.3, Mestrelab Research, 2010). For instance, the $^{19}$F NMR spectrum shown in Figure 10.4 for the reaction between CF$_3$ nitrone 142, maleimide 108 and macrocycle MDG (as described in Figure 5.12a) provide much improved signal dispersion signals for the formation of all potential cycloadducts in comparison when analysed using $^1$H NMR spectroscopy (inset).

The area of the CF$_3$ nitrone 142 and the area of potential cycloadducts were summed up and the given number was used as reference to calculate the concentration of other species in the $^{19}$F NMR spectra. This procedure was repeated for remaining spectra recorded at later time points. The time at which each spectrum was recorded
was extracted from the log file alongside the NMR spectroscopy data. Having established the concentration of products at each time point, the reaction profiles were plotted using the ProFit program (version 6.2.4, Quantum Soft, Switzerland).

**Figure 10.2** Partial $^{19}$F NMR spectrum (470.4 MHz, 25 °C, CDCl$_3$) for the reaction between CF$_3$ nitrene 142, maleimide 108 and macrocycle MDG, illustrating distinct signals associated with the formation of cycloadducts from the thread and rotaxane at 20 mM concentration. The inset show the overlapping $^1$H NMR resonances in **Figure 10.2** for comparison.

**Figure 10.4.** Partial $^{19}$F NMR spectrum (470.4 MHz, 25 °C, CDCl$_3$) for the reaction between CF$_3$ nitrene 142, maleimide 108 and macrocycle MDG, illustrating distinct signals associated with the formation of cycloadducts from the thread and rotaxane at 20 mM concentration. The inset show the overlapping $^1$H NMR resonances in **Figure 10.2** for comparison.
10.4  Determination of binding constants
In the case of fast exchange (F) on the NMR chemical shift timescale between the complexed and uncomplexed species, the change in the observed chemical shift for discrete resonance is a weighted average between the bound and unbound species. The association constant, \( K_a \) was determined by NMR titration method using \(^1\)H NMR spectroscopy at 298 K. The stock solutions and samples were made up in dry CDCl\(_3\) using volumetric flask (accuracy ± 0.02 mL). In a typical \(^1\)H NMR titration experiment, small aliquots of guest are added to a solution of host of known concentration and the NMR spectrum of the sample monitored as a function of guest concentration. Various atomic nuclei are often reported for their changes in chemical shift. The binding constant is extracted from the titration curve, which is the plot of changes in chemical shift against added guest concentration by fitting the curve using a non-linear curve-fitting program. However, if the exchange of bound and unbound species is slow (S) on the NMR chemical shift timescale, then the association constant is estimated by simple integration of the \(^1\)H and \(^19\)F NMR resonances for bound and unbound host or guest, so called the single-point method.

10.5  Computational methods
All molecular mechanics calculations were performed on a Linux workstation using the OPLS2005 forcefield together with the GB/SA solvation model for chloroform as implemented in Macromodel (Version 9.8, Schrödinger Inc., 2010). Electronic structure calculations were carried out\(^{267,268}\) using MOPAC2009 running on a Linux cluster. Transition states were successfully located using the semi empirical RM1 methods\(^{226}\) as implemented in MOPAC2009. Version 11.038L was used in all calculations.

10.6  Kinetic simulation and fitting
All kinetic fitting and simulations were performed using the software SimFit (von Kiedrowski, 2003) and the ISOSIM package incorporated within the SimFit package. The kinetic model of all possible interactions involved in the investigated system was constructed. This model was converted into a series of rate equations whose solution determine the concentration of reactant and product species as a function of time. The program is allowed to vary the kinetic values to optimize the calculation to our experimental data. The ISOSIM procedure allowed the usage of data obtained from
the fitting to simulate scenarios under a series of different conditions, which were not experimentally available.

10.7 Synthetic procedures

The structure and the number of the compound were arranged with the description of the procedure and spectroscopic data.

4-((2-Hydroxyethoxy)methyl)benzonitrile 79

A solution of 4-(bromomethyl) benzonitrile 78 (7.50 g, 38.0 mmol) in dry THF (40 mL) was added to a solution of sodium (1.77 g, 77.0 mmol) in ethylene glycol (45 mL). The resultant mixture was heated to 60 °C for 16 h and followed by quenching with sat. solution of NH₄Cl. The product was extracted into CH₂Cl₂ and dried with MgSO₄. The solution was filtered, and concentrated in vacuo to yield 79 as a yellow oil (6.07 g, 90%). ¹H NMR (300.1 MHz, CDCl₃): δ_H 7.64 (2H, d, 3_J_HH 8.1 Hz; 2×ArH), 7.45 (2H, d, 3_J_HH 8.6 Hz; 2×ArH), 4.67 (2H, s; CH₂), 3.79 (2H, t, 3_J_HH 4.5 Hz; CH₂), 3.63 (2H, t, 3_J_HH 4.5 Hz; CH₂), 2.00 (1H, br s; OH); ¹³C NMR (75.5 MHz, CDCl₃): δ_C 143.7 (ArC), 132.3 (ArC), 127.8 (ArCH), 118.8 (C=Н), 111.4 (ArCH), 72.3 (CH₂), 72.0 (CH₂), 61.8 (CH₂). MS (ES⁺) m/z 200.1 ([M+Na]+, 100); HRMS (ES⁺) m/z calculated for C₁₀H₁₁NO₂Na [M+Na]⁺ 200.0687, found 200.0690.

2-((4-Cyanobenzyl)oxy)ethyl 4-methylbenzenesulfonate 80

The corresponding alcohol 79 (3.0 g, 17.0 mmol) was dissolved in dry CH₂Cl₂ (34 mL) and Et₃N (2.84 mL, 20.4 mmol) was added dropwise into the solution. p-toluene sulphonyl chloride (3.57 g, 18.7 mmol) was added next to the resulting mixture at 0 °C for 1 h. The reaction mixture refluxed for 16 h. The resulting solution was quenched with distilled H₂O and washed sequentially with sat. solution of NH₄Cl and extracted into CH₂Cl₂. The organic layers were combined, dried over anhydrous MgSO₄. The solution was filtered, concentrated and the crude product was subjected to the silica gel chromatography (Cyclohexane:EtOAc, 3:1). Fractions containing the product were concentrated in vacuo to give the title compound 80 as colourless solid (4.3 g, 77%); M.p. 54.3 – 56.2
°C; ¹H NMR (300.1 MHz, CDCl₃): δH 7.79 (2H, d, 3JHH 8.4 Hz; 2xArH), 7.61 (2H, d, 3JHH 8.3 Hz; 2xArH), 7.38 – 7.31 (4H, m; 4xArH), 4.55 (2H, s; CΗ₂), 4.22 (2H, t, 3JHH 4.6 Hz; CΗ₂), 3.72 (2H, t, 3JHH 4.6 Hz; CΗ₂), 2.44 (3H, s; CΗ₃); ¹³C NMR (75.5 MHz, CDCl₃): δC 148.2 (ArC), 145.4 (ArC), 143.6 (ArC), 132.6 (ArC), 130.3 (ArC), 128.4 (ArC), 128.0 (ArC), 119.1 (C≡N), 111.4 (ArC), 72.6 (CH₂), 69.5 (CH₂), 68.6 (CH₂), 22.1 (CH₃). MS (ES⁺) m/z 354.9 [M+Na]⁺ (100); HRMS (ES⁺) m/z calculated for C₁₇H₁₇NO₄NaS [M+Na]⁺ 354.0776, found 354.0775.

4,4′-(((2-Oximidazolidine-1,3-diyl)bis(ethane-2,1-diyl))bis(oxy))bis(methylene) dibazonitrile 82

NaH (0.68 g, 28.5 mmol) was added to a solution of 2-imidazoline 81 (0.25 g, 2.9 mmol) in dry THF (15 mL). The suspension was left to reflux for 1.5 h under the N₂ atmosphere. While it was allowed to cool at ambient temperature, a solution of 80 (2.0 g, 6.0 mmol) in dry THF (15 mL) was reacted slowly into the resulting solution. The reaction mixture was refluxed at 75 °C for 16 h. The resulting solution was diluted with CH₂Cl₂ and washed with distilled H₂O followed by the sat. solution of NH₄Cl. The organic layer was dried with MgSO₄, concentrated and the crude was subjected to silica gel chromatography (EtOAc:Hexane, 5:1). Fractions containing the product were concentrated in vacuo to give compound 82 as a yellow oil (0.6 g, 51%). ¹H NMR (400.1 MHz, CDCl₃): δH 7.62 (4H, d, 3JHH 8.1 Hz; 4xArH), 7.42 (4H, d, 3JHH 8.5 Hz; 4xArH), 4.57 (4H, s; 2xCH₂), 3.65 (4H, t, 3JHH 5.3 Hz; 2xCH₂), 3.44 (4H, t, 3JHH 5.4 Hz; 2xCH₂), 3.43 (4H, s; 2xCH₂); ¹³C NMR (75.5 MHz, CDCl₃): δC 161.3 (C=O), 143.8 (ArC), 132.3 (ArC), 127.6 (ArC), 118.8 (C≡N), 111.3 (ArC), 71.9 (CH₂), 69.7 (CH₂), 44.4 (CH₂), 44.2 (CH₂); MS (Cl⁺) m/z 405.2 ([M+H⁺]⁺, 100); HRMS (Cl⁺) m/z calculated for C₂₃H₂₅N₄O₃ [M+H⁺]⁺ 405.1927, found 405.1929.
1,3-Bis(2-((4-(aminomethyl)benzyl)oxy)ethyl)imidazolidin-2-one 83

BH₃·THF complex (1M solution in THF, 11 mL, 11 mmol) was slowly added to a stirred solution of 82 (1.0 g, 2.47 mmol) in dry THF (15 mL) at 0 °C under N₂ atmosphere and stirred at ambient temperature for 2 h. Following this, the reaction mixture was heated to reflux for 16 h. The reaction mixture was quenched with 1M HCl, concentrated in vacuo and extracted once with CH₂Cl₂. The aqueous layer was then adjusted to pH 12 with 5M KOH and extracted into CH₂Cl₂. The combined organic layers were dried with MgSO₄ and the solvent was removed by evaporation in vacuo. The residue was purified by silica gel chromatography (CH₂Cl₂:CH₃OH:Et₃N, 98:1:1 to 90:5:5) to furnish 83 (0.86 g, 86%) as colourless oil. ¹H NMR (400.1 MHz, CDCl₃): δH 7.31 – 7.27 (8H, m; 8 × ArH), 4.50 (4H, s; 2 × CH₂), 3.85 (4H, s; 2 × CH₂), 3.60 (4H, t, 3J_HH 5.2 Hz; 2 × CH₂), 3.41 (4H, t, 3J_HH 5.2 Hz; 2 × CH₂), 3.41 (4H, s; 2 × CH₂); ¹³C NMR (100.6 MHz, CDCl₃): δC = 161.4 (C=O), 142.5 (ArC), 136.9 (ArCH), 128.0 (ArCH), 127.3 (ArC), 73.4 (CH₂), 72.8 (CH₂), 69.4 (CH₂), 46.2 (CH₂), 44.3 (CH₂); MS (Cl⁺) m/z 413.26 ([M+H]⁺, 100); HRMS (Cl⁺) m/z calculated for C₂₃H₃₆N₄O₄ [M+H]⁺ 413.2553, found 413.2551.

Ethylene Urea Macrocycle (MEU)

Compound 83 (0.28 g, 0.68 mmol) in dry CH₂Cl₂ (10 mL) and a solution of 2,6-pyridinedicarbonyl dichloride (0.14 g, 0.68 mmol) in dry CH₂Cl₂ (10 mL) were added simultaneously over 50 min to a solution of Et₃N (0.38 mL, 2.72 mmol) in dry CH₂Cl₂ (48 mL) at ambient temperature under a N₂ atmosphere. The reaction mixture was left stirred for 3 days. The reaction mixture was then washed successively with 1M HCl and 1M KOH. The organic layer was dried with MgSO₄ and concentrated by evaporation under reduced pressure. The residue was purified by column chromatography (EtOAc:Cyclohexane, 3:1) and yielded macrocycle MEU (96 mg, 26%) as a colourless powder. M.p. = 125.5 – 127.5 °C; ¹H NMR (400.1 MHz, CDCl₃): δH 8.41 (2H, d, 3J_HH 7.8 Hz; 2xArH), 8.05 (1H, t, 3J_HH 7.8 Hz; ArH), 8.04 (2H, br s; 2xNH), 7.27 (8H, s; 8xArH), 4.69 (4H, d, 3J_HH 6.2 Hz; 2xCH₂), 4.47 (4H, s; 2xCH₂), 3.60 (4H, t, 3J_HH 5.1 Hz; 2xCH₂), 3.40
(4H, t, $^3J_{HH}$ 5.1 Hz; 2×CH$_2$), 3.32 (4H, s; 2×CH$_2$); $^{13}$C NMR (100.6 MHz, CDCl$_3$): $\delta_{C}$ 163.3 (C=O), 161.3 (C=O), 148.8 (ArC), 139.1 (ArCH), 137.9 (ArC), 137.4 (ArC), 128.2 (ArCH), 127.9 (ArCH), 125.5 (ArCH), 72.6 (CH$_2$), 69.4 (CH$_2$), 44.3 (CH$_2$), 44.0 (CH$_2$), 43.3 (CH$_2$); MS (ES$^+$) $m/z$ 566.17 ([M+Na]$^+$, 100); HRMS (ES$^+$) $m/z$ calculated for C$_{30}$H$_{33}$N$_5$O$_5$Na [M+Na]$^+$ 566.2379, found 566.2372. Crystals suitable for analysis by single crystal X-ray diffraction were grown by vapour diffusion of hexane into a solution of macrocycle MEU in CHCl$_3$ at ambient temperature.

4-((2-Azidoethoxy)methyl)benzonitrile 92

NaN$_3$ (3.9 g, 60.0 mmol) was added to a solution of 80 (6.6 g, 20.0 mmol) in DMF (100 mL). The resulting mixture was then refluxed at 80 °C for 16 h. The formation of azide was checked to be completed via TLC. DMF was removed in vacuo and the reaction mixture was diluted with CH$_2$Cl$_2$ and washed several times with 0.1M HCl and H$_2$O to dilute the acid. The organic extracts were dried over MgSO$_4$, filtered and concentrated under reduced pressure to obtain the desired product 92 as brown oil (3.9 g, 97%). $^1$H NMR (300.1 MHz, CDCl$_3$): $\delta_{H}$ 7.62 (2H, d, $^3J_{HH}$ 8.5 Hz; 2×ArH), 7.43 (2H, d, $^3J_{HH}$ 7.9 Hz; 2×ArH), 4.60 (2H, s; CH$_2$), 3.68 (2H, t, $^3J_{HH}$ 4.8 Hz; CH$_2$), 3.41 (2H, t, $^3J_{HH}$ 4.8 H; CH$_2$); $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta_{C}$ 143.9 (ArC), 132.5 (ArC), 128.1 (ArCH), 119.2 (C≡N), 111.6 (ArC), 72.5 (CH$_2$), 69.8 (CH$_2$), 51.1 (CH$_2$); MS (Cl$^+$) $m/z$ 203.09 ([M+H]$^+$, 100); HRMS (Cl$^+$) $m/z$ calculated for C$_{10}$H$_{11}$N$_4$O [M+H]$^+$ 203.0933, found 203.0936.

4-((2-Aminoethoxy)methyl)benzonitrile 93

Pyridine (0.2 mL, 2.47 mmol) was added to azide compound 92 (1.0 g, 4.95 mmol) in CH$_3$OH (20 mL) followed by Pd/C (50 mg, 0.04 mmol). The reaction mixture was hydrogenated at ambient temperature and stirred for 8 h. The resultant suspension was filtered through a pad of Celite and washed with minimum amount of CH$_3$OH. The resulting compound 93 was formed in situ and carried out to the next step without further purification. $^1$H NMR (300.1 MHz, CDCl$_3$): $\delta_{H}$ 7.74 (2H, d, $^3J_{HH}$ 8.3 Hz; 2×ArH), 7.43
(2H, d, $^3J_{HH}$ 8.5 Hz; 2×ArH), 4.63 (2H, s; CH$_2$), 3.70 (2H, t, $^3J_{HH}$ 4.7 Hz; CH$_2$), 3.44 (2H, t, $^3J_{HH}$ 4.7 Hz, CH$_2$); $^{13}$C NMR (75.5 MHz, CDCl$_3$): δ$_C$ 143.9 (ArC), 132.2 (ArCH), 127.7 (ArCH), 112.4 (ArC), 118.1 (C≡N), 72.1 (CH$_2$), 70.3 (CH$_2$), 49.3 (CH$_2$); MS (ES$^+$) m/z 177.06 ([M+H]$^+$, 100).

1,3-Bis(2-(4-cyanobenzylxoy)ethyl)urea 95

A solution of Et$_3$N (1.4 mL, 9.90 mmol) in Et$_2$O (25 mL) was added to the solution of compound 93 (1.0 g, 4.95 mmol) in minimal amount of dry CH$_2$Cl$_2$. The resulting solution was cooled at 0 °C. Triphosgene 94 (0.25 g, 0.83 mmol) was added neat under N$_2$ atmosphere and stirred for 6 h. The suspension was diluted with CHCl$_3$, acidified with 1M HCl up to pH 1 followed by H$_2$O. The resulting solution was extracted with CHCl$_3$ and the organic layers were dried over MgSO$_4$, filtered and evaporated in vacuo. The residue was further purified by recrystallisation from CHCl$_3$:Hexane to give product 95 as a yellow solid (0.34 g, 36%). M.p. = 156.5 – 158.5 °C; $^1$H NMR (300.1 MHz, CDCl$_3$): δ$_H$ 7.62 (4H, d, $^3J_{HH}$ 8.4 Hz; 4×ArH), 7.41 (4H, d, $^3J_{HH}$ 8.6 Hz; 4×ArH), 4.83 (2H, br s; 2×NHNH), 4.55 (4H, s; 2×CH$_2$), 3.58 (4H, t, $^3J_{HH}$ 5.1 Hz; 2×CH$_2$), 3.43 (4H, t, $^3J_{HH}$ 5.0 Hz; 2×CH$_2$); $^{13}$C NMR (75.5 MHz, CDCl$_3$): δ$_C$ 158.7 (C=O), 143.9 (ArC), 132.7 (ArCH), 128.2 (ArCH), 111.9 (ArC), 111.8 (C≡N), 72.5 (CH$_2$), 70.9 (CH$_2$), 40.8 (CH$_2$); MS (ES$^+$) m/z 401.2 ([M+Na]$^+$, 100); HRMS (ES$^+$) m/z calculated for C$_{21}$H$_{22}$N$_4$O$_3$Na [M+Na]$^+$ 401.1590, found 401.1580.

Diprop-2-ynyl pyridine-2,6-dicarboxylate$^{269}$ 96

A solution of 2,6-pyridinedicarbonyl dichloride (1.0 g, 4.90 mmol) and propargyl alcohol (470 μL, 8.03 mmol) were added simultaneously in CHCl$_3$ (25 mL) together with Et$_3$N (2.1 mL, 14.7 mmol) at 0 °C and left stirred for 10 min. The resulting mixtures were left stirred at ambient temperature for 16 h. Next, the reaction mixture was diluted in CHCl$_3$ and the resulting residue was purified by recrystallisation from CHCl$_3$:Hexane to give compound 96 as a beige solid (0.6 g, 50%). M.p. 116.4 –
118.5 °C (lit\textsuperscript{269}, 124 °C); \textsuperscript{1}H NMR (300.1 MHz, CDCl\textsubscript{3}): \( \delta \) \textsubscript{H} 8.34 (2H, d, \( J_{\text{HH}} \) 7.9 Hz; 2\( \times \)Ar\( H \)), 8.04 (1H, dd, \( J_{\text{HH}} \) 7.6 and \( J_{\text{HH}} \) 8.1 Hz; Ar\( H \)), 5.02 (4H, d, \( J_{\text{HH}} \) 2.5 Hz; 2\( \times \)CH\( _2 \)), 2.53 (2H, t, \( J_{\text{HH}} \) 2.5 Hz, 2\( \times \)CH\( _2 \)); \textsuperscript{13}C NMR (75.5 MHz, CDCl\textsubscript{3}): \( \delta \) \textsubscript{C} 163.7 (\( C=O \)), 147.9 (Ar\( C \)), 138.6 (Ar\( C \)), 128.6 (Ar\( C \)), 75.8 (CH), 75.6 (q\( C \)), 53.6 (CH\( _2 \)); MS (ES\textsuperscript{+}) \( m/z \) 265.98 ([M+Na]\textsuperscript{+}, 100); HRMS (ES\textsuperscript{+}) \( m/z \) calculated for C\textsubscript{13}H\textsubscript{9}NO\textsubscript{4}Na [M+Na]\textsuperscript{+} 266.0429, found 266.0439.

**3,3’-(3,3’-(2-Oximidazolidine-1,3-diyl)bis(prop-1-yn-3,1-diyl))dibenzonitrile 100**

A degassed (by bubbling Ar) solution of 3-bromobenzonitrile \textbf{99} (1.45 g, 7.7 mmol) in Et\textsubscript{3}N (15 mL) was cannulated into a degassed (by bubbling Ar) suspension of 1,3-di(prop-2-yn-1-yl)imidazolidin-2-one \textbf{98} (0.6 g, 3.70 mmol), PPh\textsubscript{3} (97 mg, 370 \( \mu \)mol), CuI (35 mg, 185 \( \mu \)mol), CuI (35 mg, 185 \( \mu \)mol), PdCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2} (130 mg, 185 \( \mu \)mol) and Et\textsubscript{3}N (10 mL) in dry THF (45 mL). The reaction mixture was heated at 70 °C under an Ar atmosphere for 48 h, and then filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was dissolved in CHCl\textsubscript{3}. The organic layer was washed with water, dried (MgSO\textsubscript{4}) and concentrated by evaporation \textit{in vacuo}. The residue was purified by column chromatography (Cyclohexane:EtOAc, 2:1) to give compound \textbf{100} as a yellow solid (490 mg, 36%).

M.p. = 121.9 – 124.9 °C; \textsuperscript{1}H NMR (300.1 MHz, CDCl\textsubscript{3}): \( \delta \) \textsubscript{H} 7.67 (2H, td, \( J_{\text{HH}} \) 1.6 and \( J_{\text{HH}} \) 0.5 Hz; 2\( \times \)Ar\( H \)), 7.62 (2H, dt, \( J_{\text{HH}} \) 7.8 and \( J_{\text{HH}} \) 1.5 Hz; 2\( \times \)Ar\( H \)), 7.58 (2H, dt, \( J_{\text{HH}} \) 7.9 and \( J_{\text{HH}} \) 1.4 Hz; 2\( \times \)Ar\( H \)), 7.41 (2H, td, \( J_{\text{HH}} \) 7.8 and \( J_{\text{HH}} \) 0.4 Hz; 2\( \times \)Ar\( H \)), 4.28 (4H, s; 2\( \times \)CH\( _2 \)), 3.52 (4H, s; 2\( \times \)CH\( _2 \)); \textsuperscript{13}C NMR (100.6 MHz, CDCl\textsubscript{3}): \( \delta \) \textsubscript{C} 159.2 (\( C=O \)), 135.9 (Ar\( C \)), 135.2 (Ar\( C \)), 131.7 (Ar\( C \)), 129.4 (Ar\( C \)), 124.2 (Ar\( C \)), 118.0 (\( C≡N \)), 112.9 (Ar\( C \)), 86.2 (q\( C \)), 82.1 (q\( C \)), 42.2 (CH\( _2 \)), 34.8 (CH\( _2 \)); MS (ES\textsuperscript{+}) \( m/z \) 387 ([M+Na]\textsuperscript{+}, 100); HRMS (ES\textsuperscript{+}) \( m/z \) calculated for C\textsubscript{23}H\textsubscript{16}N\textsubscript{4}ONa [M+Na]\textsuperscript{+} 387.1222, found 387.1211
4,4’-(2,2’-Oxybis(ethane-2,1-diyl)bis(oxy))bis(methylene)dibenzonitrile \(^{207} \text{102}\)

![Structure](image)

4-(bromomethyl)benzonitrile \(78\) (3.01 g, 15.4 mmol) was slowly added to a suspension of NaH (0.76 g, 19.0 mmol) in dry THF (60 mL) and diethylene glycol (730 μL, 7.30 mmol). The resulting mixture was refluxed at 70 °C for 16 h. The resultant solution was quenched with ethanol followed by H\(_2\)O and extracted into EtOAc. The organic extracts were dried over MgSO\(_4\), filtered and concentrated under reduced pressure. The residue was further purified via column chromatography (Cyclohexane:EtOAc, 1:1) to obtain the desired compound \(102\) as yellow oil (2.34 g, 90%). \(^1\)H NMR (300.1 MHz, CDCl\(_3\)): \(\delta H 7.61 (4H, d, ^3J_{HH} 8.4 Hz; 4\times ArH), 7.44 (4H, d, ^3J_{HH} 8.6 Hz; 4\times ArH), 4.62 (4H, s; 2\times C\(H_2\)), 3.65 – 3.73 (8H, m; 4\times C\(H_2\)); ^13\)C NMR (75.5 MHz, CDCl\(_3\)): \(\delta C 144.0 (ArC), 132.3 (ArC), 127.8 (ArCH), 118.9 (C≡N), 111.3 (ArC), 72.3 (C\(H_2\)), 70.7 (C\(H_2\)), 70.2 (C\(H_2\)); MS (Cl\(^+\)) m/z 337 ([M+H]\(^+\), 100); HRMS (Cl\(^+\)) m/z calculated for C\(_{20}\)H\(_{21}\)N\(_2\)O\(_3\) [M+H]\(^+\) 337.1552, found 337.1556.

\((4,4’-(2,2’-Oxybis(ethane-2,1-diyl)bis(oxy))bis(methylene)bis(4,1-phenylene))dimethanamine\) \(^{207} \text{103}\)

![Structure](image)

A solution of BH\(_3\)•THF complex (1M solution in THF, 35 mL, 35.0 mmol) was added dropwise to a solution of compound \(102\) (2.6 g, 7.7 mmol) in dry THF (33 mL) at 0 °C under N\(_2\) atmosphere. The reaction mixture was stirred at ambient temperature for 2 h followed by refluxing the solution for 24 h. The reaction mixture was quenched with 1M HCl and extracted once with CH\(_2\)Cl\(_2\). The separated aqueous layer was adjusted to pH 12 using 5M KOH and extracted further into CH\(_2\)Cl\(_2\). The collected organic layers were dried over MgSO\(_4\), filtered and concentrated in vacuo. The compound \(103\) (1.37 g, 52%) was isolated as a yellow oil. \(^1\)H NMR (300.1 MHz, CDCl\(_3\)): \(\delta H 7.31 (4H, d, ^3J_{HH} 8.2 Hz; 4\times ArH), 7.26 (4H, d, ^3J_{HH} 8.2 Hz; 4\times ArH), 4.55 (4H, s; 2\times C\(H_2\)), 3.85 (4H, s; 2\times C\(H_2\)), 3.70 – 3.61 (8H, m; 4\times C\(H_2\)), 1.60 (4H, br s; 2\times N\(H_2\)); ^13\)C NMR (75.5 MHz, CDCl\(_3\)): \(\delta C 142.8 (ArC), 136.9 (ArCH), 128.2 (ArCH), 127.2 (ArC), 73.1 (C\(H_2\)), 70.8 (C\(H_2\)), 69.5 (C\(H_2\)), 46.4 (C\(H_2\)); MS (Cl\(^+\)) m/z 105 (22), 119 (46), 120 (100), 328 (20), 345 ([M+H]\(^+\), 12); HRMS (Cl\(^+\)) m/z calculated for...
C_{20}H_{29}N_{2}O_{3} [M+H]^+ 345.2178, found 345.2177.

**Diethylene Glycol Macrocycle (MDG)**

A solution of 103 (1.21 g, 3.51 mmol) in dry CHCl_2 (70 mL) and 2,6-pyridinedicarbonyl dichloride (0.72 g, 3.51 mmol) in dry CHCl_2 (70 mL) were added simultaneously over a period of 1 h to the solution containing 4-pyridone 87 (0.33 g, 3.51 mmol) and Et_3N (1.96 mL, 14.1 mmol) in dry CHCl_2 (80 mL) at ambient temperature under N_2 atmosphere. The reaction mixture was left stirred for 3 days. The resulting solution was once washed with 1M HCl followed by 1M KOH. The organic extracts was dried over MgSO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography (EtOAc:CHCl_3, 9:1) to give macrocycle MDG as a colourless solid (0.54 g, 34%). M.p. = 230.6 – 232.5 °C; ^1H NMR (300.1 MHz, CDCl_3): δ_H 8.25 (2H, d, J_HH 7.8 Hz; 2×ArH), 8.10 (2H, br s; 2×N), 8.03 (1H, t, J_HH 7.8 Hz; ArH), 7.28 (4H, d, J_HH 8.0 Hz; 4×ArH), 7.21 (4H, d, J_HH 8.0 Hz; 4×ArH), 4.63 (4H, d, J_HH 5.7 Hz; 2×CH_2), 4.56 (4H, s; 2×CH_2), 3.74 – 3.68 (8H, m; 4×CH_2); ^13C NMR (75.5 MHz, CDCl_3): δ_C 163.4 (C=O), 148.6 (ArC), 139.6 (ArCH), 138.1 (ArC), 137.0 (ArC), 128.3 (ArCH), 127.5 (ArCH), 125.2 (ArCH), 72.9 (CH_2), 71.0 (CH_2), 69.8 (CH_2), 43.3 (CH_2); MS (ES^+) m/z 498.03 ([M+Na]^+, 100); HRMS (ES^+) m/z calculated for C_{27}H_{29}N_{3}O_{5}Na [M+Na]^+ 498.2005, found 498.2008.

**N-(4-Butylphenyl)-3,5-bis(trifluoromethyl)benzamide 105**

3,5-bis(trifluoromethyl)benzoyl chloride 124 (500 μL, 2.76 mmol) was added dropwise at −5 °C to the resulting solution of 4-butylaniline 104 (270 mg, 1.81 mmol) in CHCl_3 (10 mL) and Et_3N (500 μL, 3.62 mmol). The resulting solution was left stirred for 16 h at rt. The reaction mixture was diluted with CHCl_3, washed once with 1M HCl and followed by H_2O. The organic layers were dried over MgSO_4, filtered and concentrated in vacuo to furnish the compound 105 as colourless solid (350 mg, 50%). M.p. = 100.5 – 103.2 °C; ^1H NMR (300.1 MHz, CDCl_3): δ_H 8.30 (2H, s; 2×ArH), 8.03 (1H, s; ArH), 7.97 (1H, br s; N_H), 7.51 (2H, d, J_HH 8.4 Hz; 2×ArH), 7.18 (2H, d, J_HH 8.5 Hz; 2×ArH), 2.62 (2H, t, J_HH 7.7 Hz; CH_2), 1.65 – 1.55 (2H, m; CH_2), 1.42 – 1.30 (2H, m; CH_2), 0.91 (3H, t, J_HH 7.7 Hz; CH_2).
7.3 Hz; CH₃); ¹³C NMR (75.5 MHz, CDCl₃): δC 163.2 (C=O), 140.8 (ArC), 137.5 (ArC), 134.9 (ArC), 132.5 (q, ²JC 32.6 Hz; ArC), 129.5 (ArCH), 127.6 (m; ArCH₂), 125.6 (m; ArCH), 123.1 (q, ¹JC 275.0 Hz; CF₃), 121.0 (ArCH), 35.5 (CH₂), 34.0 (CH₂), 22.7 (CH₂), 14.4 (CH₃); ¹⁹F NMR (376.5 MHz, CDCl₃): δF = -62.9 (ArCF₃); MS (ES⁺) m/z 412.0 ([M+Na]⁺, 100); HRMS (ES⁺) m/z calculated for C₁₉H₁₇NONaF₆ [M+Na]⁺ 412.1112, found 412.1119.

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-butylphenyl)urea 106

3,5-bis(trifluoromethyl)phenyl isocyanate 127 (0.50 g, 1.96 mmol), was added dropwise at −5 °C to a solution of 4-butylaniline 104 (0.29 g, 1.96 mmol) in CHCl₃ (10 mL) and left to stir at ambient temperature for 16 h. The resulting precipitate was filtered and further purified by recrystallisation from CHCl₃:Petroluem ether. The desired product 106 was isolated as a colourless solid (630 mg, 80%). M.p. = 165.3 – 168.5 °C; ¹H NMR (300.1 MHz, CDCl₃): δH 8.69 (1H, br s; NH), 7.92 (1H, br s; NH), 7.89 (2H, s; 2×ArH), 7.36 (1H, s; ArH), 7.28 – 7.24 (2H, m; 2×ArH), 7.07 – 7.02 (2H, m; 2×ArH), 2.59 (2H, t, ³JHH 7.7 Hz; CH₂), 1.63 – 1.52 (2H, m; CH₂), 1.41 – 1.29 (2H, m; CH₂), 0.85 (3H, t, ³JHH 7.3 Hz; CH₃); ¹³C NMR (75.5 MHz, CDCl₃): δC 153.2 (C=O), 141.9 (ArC), 138.1 (ArC), 136.6 (ArC), 132.3 (q, ²JC 32.9 Hz, ArC), 123.0 (q, ¹JC 273.0 Hz, CF₃), 129.2 (ArCH), 124.9 (ArCH), 119.7 (ArCH), 118.2 (m; ArCH), 115.1 (m; ArCH), 35.3 (CH₂), 34.1 (CH₂), 22.6 (CH₂), 13.7 (CH₃); ¹⁹F NMR (376.5 MHz, CDCl₃): δF = -63.1 (ArCF₃); MS (ES⁺) m/z 427.03 ([M+Na]⁺, 100); HRMS (ES⁺) m/z calculated for C₁₉H₁₇NONaF₆ [M+Na]⁺ 427.1221, found 427.1220.

N-(4-(4-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenethyl)phenyl)-3,5-dimethylbenzamide 109

The acid derivative 123 (0.34 g, 0.78 mmol) was dissolved in dry CH₃CN (20 mL) and refluxed for 2 h after the subsequent addition of ZnBr₂ (0.18 g, 10.78 mmol) and hexamethyldisilazone (0.83 mL, 3.91 mmol). The reaction mixture was filtered and the filtrate was reduced to 10%. The solution was acidified with 0.5M HCl until pH 1 and extracted into CH₂Cl₂. The
organic layers were combined and dried over MgSO₄. The solvent was evaporated to give the desired product as yellow crystalline solid (0.32 g, 96%); M.p. = 229.3 – 231.4 °C; ¹H NMR (400.1 MHz, CDCl₃): δ_H 7.81 (1H, s; NH), 7.57 – 7.54 (2H, m; 2×ArH), 7.46 (2H, s; 2×ArH), 7.29 – 7.27 (2H, m; 2×ArH), 7.25 – 7.22 (2H, m; 2×ArH), 7.19 – 7.17 (3H, m; 3×ArH), 6.84 (2H, s; 2×CH), 2.97 – 2.89 (4H, m; 2×CH₂), 2.38 (6H, s; 2×CH₃); ¹³C NMR (100.6 MHz, CDCl₃): δ_C 169.8 (C=O), 166.1 (C=O), 141.8 (ArC), 138.6 (ArC), 137.7 (ArC), 136.2 (ArC), 135.2 (ArC), 134.3 (CH), 133.5 (ArCH), 129.4 (ArCH), 129.2 (ArC), 129.1 (ArCH), 126.1 (ArCH), 124.9 (ArCH), 120.4 (ArCH), 37.7 (CH₂), 37.3 (CH₂), 21.4 (CH₃); MS (ES⁺) m/z 447 ([M+Na]⁺, 75), 479 (100); HRMS (ES⁺) m/z calculated for C₂₇H₂₆N₂O₃Na [M+Na]⁺ 447.1685, found 447.1674.

N-(4-(4-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenethyl)phenyl)-3,5-bis(trifluoromethyl)benzamide 110

The acid derivative 126 (0.60 g, 1.09 mmol) was dissolved in dry CH₂CN (20 mL) and refluxed for 2 h after the subsequent addition of ZnBr₂ (0.25 g, 1.09 mmol) and hexamethyldisilazone (0.88 g, 1.2 mL, 5.45 mmol). The reaction mixture was filtered and the filtrate was reduced to 10%. The solution was acidified with 0.5M HCl until pH 1 and extracted into CH₂Cl₂. The organic layers were combined and dried over MgSO₄. The solvent was evaporated to give the desired product as yellow crystalline solid (0.25 g, 44%). M.p. = 249.5 – 251.3 °C; ¹H NMR (300.1 MHz, d₆-DMSO): δ_H 10.6 (1H, s; NH), 8.61 (2H, s; 2×ArH), 8.36 (1H, s; ArH), 7.68 (2H, d, ²JHH 8.6 Hz; 2×ArH), 7.34 (2H, d, ³JHH 8.5 Hz; 2×ArH), 7.27 (2H, d, ³JHH 8.6 Hz; 2×ArH), 7.22 (2H, d, ³JHH 8.5 Hz; 2×ArH), 7.17 (2H, s; 2×CH), 2.98 – 2.87 (4H, m; 2×CH₂); ¹³C NMR (100.6 MHz, d₆-DMSO): δ_C 170.4 (C=O), 162.7 (C=O), 141.5 (ArC), 137.8 (ArC), 137.5 (ArC), 136.7 (ArC), 135.0 (CH), 130.8 (q, ²JC 33.2 Hz, ArC), 129.3 (ArC), 128.7 (ArCH), 128.2 (ArCH), 128.1 (m; ArCH), 126.4 (ArCH), 124.7 (m; ArCH), 123.5 (q, ¹JC 276.6 Hz; CF₃), 120.2 (ArCH), 36.9 (CH₂), 36.7 (CH₂); ¹⁹F NMR (376.5 MHz, d₆-DMSO): δ_F –61.7 (ArCF₃); MS (ES⁺) m/z 531.1 ([M–H]⁻, 100); HRMS (ES⁺) m/z calculated for C₂₇H₁₇F₄O₃F₆ [M–H]⁻ 531.1143, found 531.1140.
1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenethyl)phenyl)urea 111

The acid derivative 129 (0.20 g, 0.35 mmol) was dissolved in dry CH$_3$CN (10 mL) and refluxed for 1 h after the subsequent addition of ZnBr$_2$ (80 mg, 0.35 mmol) and hexamethyldisilazane (0.38 mL, 1.77 mmol). The reaction mixture was filtered and the filtrate was reduced to 10%. The solution was acidified with 0.5M HCl until pH 1 and extracted into CH$_2$Cl$_2$. The organic layer were combined and dried over MgSO$_4$. The solvent was evaporated in vacuo to give the maleimide 111 as yellow solid (152 mg, 78%). M.p. = 205.3 – 208.3 °C; $^1$H NMR (300.1 MHz, $d_6$-DMSO) : δ$_H$ 9.36 (1H, s; N$_H$), 8.89 (1H, s, N$_H$), 8.13 (2H, s; 2×Ar$_H$), 7.61 (1H, s; Ar$_H$), 7.39 (2H, d, 3$\ J_{HH}$ 8.4 Hz; 2×Ar$_H$), 7.33 (2H, d, 3$\ J_{HH}$ 8.4 Hz; 2×Ar$_H$), 7.22 (2H, d, 3$\ J_{HH}$ 8.4 Hz; 2×Ar$_H$), 7.18 (2H, d, 3$\ J_{HH}$ 8.4 Hz; 2×Ar$_H$), 7.16 (2H, s; 2×CH$_2$), 2.94 – 2.82 (4H, m; 2×CH$_2$); $^{13}$C NMR (75.5 MHz, $d_6$-DMSO): δ$_C$ 170.0 (C=O), 152.4 (C=O), 142.0 (ArC), 141.3 (ArC), 136.8 (ArC), 135.5 (ArC), 134.6 (ArCH), 130.7 (q, $^2$J$_{CF}$ 32.7 Hz, ArC), 129.3 (ArC), 128.8 (ArCH), 128.7 (ArCH), 126.6 (CH), 123.3 (q, $^1$J$_{CF}$ 272.3 Hz, CF$_3$), 119.0 (ArCH), 117.8 (m; ArCH), 114.2 (m, ArCH), 36.7 (CH$_2$), 36.3 (CH$_2$); $^{19}$F NMR (376.5 MHz, $d_6$-DMSO) : δ$_F$ −62.2 (ArCF$_3$); MS (ES') m/z 546 ([M−H]$^-$, 100); HRMS (ES') m/z calculated for C$_{27}$H$_{18}$N$_3$O$_3$F$_6$ [M−H]$^-$ 546.1252, found 546.1257.

4,4′-((Pyridine-2,6-diylbis(methylene))bis(oxy)bis(methylene) dibenzonitrile 113

NaH (1.44 g, 36.0 mmol) was carefully added to a solution of 2,6-pyridine dimethanol 112 (2.0 g, 14.4 mmol) in dry THF (70 mL). The resulting mixture was refluxed for 1.5 h and allowed to cool to room temperature. Next, 4-bromomethyl benzonitrile 78 (5.93 g, 30.2 mmol) was added and continue to reflux for 18 h at 75 °C. H$_2$O was slowly added to quench the reaction. The organic layers were extracted into EtOAc. The combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure. The resulting residue
was purified by recrystallisation from Petroleum ether: \(\text{CHCl}_3\) to furnish a yellow solid (4.70 g, 88%). M.p. 105.8 – 107.1 °C; \(^1\)H NMR (300.1 MHz, CDCl\(_3\)): \(\delta_H 7.75\) (1H, t, \(3J_{HH} 7.7\) Hz; Ar\(H\)), 7.65 (4H, d, \(3J_{HH} 8.4\) Hz; 4×Ar\(H\)), 7.49 (4H, d, \(3J_{HH} 8.6\) Hz; 4×Ar\(H\)), 7.40 (2H, d, \(3J_{HH} 7.8\) Hz; 2×Ar\(H\)), 4.71 (4H, s; 2×C\(H_2\)), 4.70 (4H, s; 2×C\(H_2\)); \(^{13}\)C NMR (75.5 MHz, CDCl\(_3\)): \(\delta_C 157.6\) (Ar\(C\)), 143.6 (Ar\(C\)), 137.6 (Ar\(C\)), 132.4 (Ar\(C\)), 127.9 (Ar\(C\)), 120.4 (Ar\(C\)), 118.9 (C≡N), 111.6 (Ar\(C\)), 73.7 (CH\(_2\)), 72.1 (CH\(_2\)); MS (CI\(^+\)) \(m/z\) 239.0 (27), 370.2 ([M+H]\(^+\), 100); HRMS (CI\(^+\)) \(m/z\) calculated for C\(_{23}\)H\(_{20}\)N\(_3\)O\(_2\) [M+H]\(^+\) 370.1556, found 370.1563.

\((4,4'\text{-Pyridine-2,6-diylbis(methylene)})\text{(oxy)bis(methylene)bis(4,1-phenylene))dimethanamine} 114\)

A solution of BH\(_3\)•THF complex (1M solution in THF, 57 mL, 57.2 mmol) was added dropwise to a solution of 113 (4.70 g, 12.7 mmol) in dry THF (64 mL) at 0 °C under N\(_2\) atmosphere. The reaction mixture was stirred at ambient temperature for 2 h and heated to reflux for 18 h. The reaction was slowly quenched with 1M HCl and extracted once into \(\text{CH}_2\text{Cl}_2\). The aqueous layers were collected and adjusted to pH 1 using 1M KOH and continue to extract into \(\text{CH}_2\text{Cl}_2\). The organic layers were dried over MgSO\(_4\), filtered and concentrated in vacuo to yield the title compound 114 as a yellow oil (3.0 g, 63%). \(^1\)H NMR (300.1 MHz, CDCl\(_3\)): \(\delta_H 7.71\) (1H, t, \(3J_{HH} 7.7\) Hz; Ar\(H\)), 7.39 (2H, d, \(3J_{HH} 7.8\) Hz; 2×Ar\(H\)), 7.36 (4H, d, \(3J_{HH} 8.2\) Hz; 4×Ar\(H\)), 7.30 (4H, d, \(3J_{HH} 8.2\) Hz; 4×Ar\(H\)), 4.66 (4H, s; 2×C\(H_2\)), 4.63 (4H, s; 2×C\(H_2\)), 3.87 (4H, s; 2×C\(H_2\)), 1.46 (4H, br s; 2×N\(H_2\)); \(^{13}\)C NMR (75.5 MHz, CDCl\(_3\)): \(\delta_C 158.1\) (Ar\(C\)), 143.1 (Ar\(C\)), 137.4 (Ar\(CH\)), 136.5 (Ar\(C\)), 128.3 (Ar\(CH\)), 127.3 (Ar\(CH\)), 120.1 (Ar\(CH\)), 73.2 (CH\(_2\)), 72.9 (CH\(_2\)), 46.5 (CH\(_2\)); MS (ES\(^+\)) \(m/z\) 374 (50), 378 ([M+H]\(^+\), 100), 400 (47); HRMS (ES\(^+\)) \(m/z\) calculated for C\(_{23}\)H\(_{27}\)N\(_3\)O\(_2\)Na [M+Na]\(^+\) 400.2001. found 400.2005.
Pyridine Macrocycle (MP)

A solution of 114 (1.0 g, 2.65 mmol) in CH₂Cl₂ (50 mL) and a solution of 2,6-pyridinedicarbonyl dichloride (540 mg, 2.65 mmol) in CH₂Cl₂ (50 mL) was added simultaneously to a solution of Et₃N (1.50 mL, 10.6 mmol) in dry CH₂Cl₂ (133 mL) over 1 h and the resulting solution was left stirred for 3 days. The reaction mixture was diluted with CH₂Cl₂ and washed once with 1M HCl followed by sat. solution of NaHCO₃. The organic layers were further extracted into CH₂Cl₂. The combined organic layers were dried over MgSO₄ and concentrated in vacuo. The resulting residue was purified by column chromatography (EtOAc:Hexane, 2:1) to furnish the macrocycle MP as colorless solid (0.3 g, 22%). M.p. = 209.2 – 210.5 °C; ¹H NMR (300.1 MHz, CDCl₃) : δₜₜ 8.40 (2H, d, ³Jₜₜ 7.8 Hz; 2×ArH), 8.06 (1H, dd, ³Jₜₜ 7.6 and ³Jₜₜ 8.0 Hz; ArH), 7.98 (2H, t, ³Jₜₜ 5.8 Hz; 2×NH), 7.70 (1H, t, ³Jₜₜ 7.7 Hz; ArH), 7.34 – 7.31 (6H, m; 6×ArH), 7.28 – 7.26 (4H, m; 4×ArH), 4.70 (4H, d, ³Jₜₜ 6.1 Hz; 2×CH₂), 4.62 (4H, s; 2×CH₂), 4.52 (4H, s; 2×CH₂); ¹³C NMR (75.5 MHz, CDCl₃) : δₗ 163.4 (C=O), 157.6 (ArC), 148.9 (ArC), 139.3 (ArCH), 137.5 (ArC), 137.4 (ArC), 137.3 (ArCH), 129.1 (ArCH), 127.9 (ArCH), 125.5 (ArCH), 121.2 (ArCH), 72.0 (CH₂), 71.8 (CH₂), 43.3 (CH₂); MS (ES⁺) m/z 531.11 ([M+Na]⁺, 100); HRMS (ES⁺) m/z calculated for C₃₀H₂₈N₄O₄Na [M+Na]⁺ 531.2008, found 531.2009.

(Z)-4-(tert-Butyl)-N-(4-((4,6-dimethylpyridin-2-yl)carbamoyl)benzylidene)aniline oxide 115

4-tert-butyl nitrobenzene 118 (0.56 g, 3.12 mmol) was dissolved in THF (15 mL). Rh/C (0.2 g, 30 wt. %) was added and stirred for about 15 min before the addition of hydrazine monohydrate (0.19 mL, 3.74 mmol). The reaction progress was monitored by TLC until the disappearance of the starting material. The solution was then filtered through a pad of Celite and concentrated in vacuo to give the intermediate compound. The 4-tert-butyl-N-hydroxyaniline 119 (3.12 mmol) was carried out to the next step without further
purification. Hydroxylamine 119 was dissolved in EtOH (15 mL), and aldehyde 117 (0.79 g, 3.12 mmol) was added neat. The reaction mixture was left stirred at ambient temperature in the dark for 3 days. The solvent was removed by evaporation under reduced pressure. The crude product was additionally crystallised from EtOH to give a yellow solid (1.06 g, 85% over 2 steps). M.p. = 185.1 – 187.6 °C; 1H NMR (400.1 MHz, CDCl₃): δ_H 8.55 (1H, br s; NH), 8.51 – 8.48 (2H, m; 2×ArH), 8.05 (1H, s; ArH), 8.04 – 8.01 (2H, m; 2×ArH), 7.99 (1H, s; CH), 7.73 – 7.69 (2H, m; 2×ArH), 7.52 – 7.49 (2H, m; 2×ArH), 6.79 (1H, s; ArH), 2.44 (3H, s; CH₃), 2.37 (3H, s; CH₃), 1.36 (9H, s, 3×CH₃); 13C NMR (100.6 MHz, CDCl₃): δ_C 164.8 (C=O), 156.6 (ArC), 153.9 (ArC), 150.8 (ArC), 150.3 (ArC), 146.7 (ArC), 135.6 (ArC), 134.1 (ArC), 133.1 (CH), 129.1 (ArCH), 127.6 (ArCH), 126.3 (ArCH), 121.4 (ArCH), 120.9 (ArCH), 111.8 (ArCH), 35.0 (qC), 31.3 (CH₃), 23.9 (CH₃), 21.4 (CH₃); MS (Cl⁺) m/z 424 ([M+Na]⁺, 100); HRMS (ES⁺) m/z calculated for C₂₅H₂₈N₃O₂ [M+H]⁺ 402.2182, found 402.2188.

4-Formylbenzoyl chloride 116

4-formylbenzoic acid 42 (5.0 g, 33.3 mmol) in a mixture of thionyl chloride and toluene (60 mL) was refluxed at 100 °C for 24 h. A clear solution was obtained and reduced in vacuum to yield a quantitative yield of yellow solid of compound 116, which was used in the next step without further purification. 1H NMR (300.1 MHz, CDCl₃): δ_H 10.1 (1H, s; CHO), 8.26 (2H, d, 3_J_HH 8.4 Hz; 2×ArH), 8.00 (2H, d, 3_J_HH 8.4 Hz; 2×ArH); 13C NMR (75.5 MHz, CDCl₃): δ_C 191.0 (CHO), 167.8 (COCl), 140.5 (ArC), 137.6 (ArC), 131.8 (ArCH), 129.8 (ArCH).

N-(4,6-Dimethylpyridin-2-yl)-4-formylbenzamide 117

4-formylbenzoyl chloride 116 (5.61 g, 33.3 mmol) in dry CH₂Cl₂ (30 mL) was slowly reacted with a solution of 4,6-dimethylpyridin-2-amine (4.07 g, 33.3 mmol) and Et₃N (5.1 mL, 36.6 mmol) in dry CH₂Cl₂ (50 mL) at 0 °C under Ar atmosphere. The reaction was left stirred for 48 h at ambient temperature before quenched with water (50 mL). The organic layer was washed with 1M HCl (50 mL) and sat. solution of NaHCO₃ (50 mL) and extracted into CH₂Cl₂. The combined organic layers were dried over MgSO₄,
filtered and concentrated in vacuo. The crude was purified by column chromatography (Hexane:EtOAc, 4:1) to afford a colourless solid (6.0 g, 72%). M.p. = 130.8 – 133.2 °C; \(^1\)H NMR (300.1 MHz, CDCl\(_3\)): \(\delta_H\) 10.1 (1H, s; CHO), 8.50 (1H, br s; NH), 8.09 – 8.06 (2H, m; 2\(\times\)Ar\(\text{H}\)), 8.03 (1H, s; Ar\(\text{H}\)), 8.02 – 7.99 (2H, m; 2\(\times\)Ar\(\text{H}\)), 6.81 (1H, s; Ar\(\text{H}\)), 2.43 (3H, s; CH\(_3\)), 2.38 (3H, s; CH\(_3\)); \(^{13}\)C NMR (100.6 MHz, CDCl\(_3\)): \(\delta_C\) 191.5 (CHO), 164.6 (C=O), 156.7 (Ar\(\text{C}\)), 150.6 (Ar\(\text{C}\)), 150.5 (Ar\(\text{C}\)), 139.7 (Ar\(\text{C}\)), 138.7 (Ar\(\text{C}\)), 130.1 (Ar\(\text{CH}\)), 128.0 (Ar\(\text{CH}\)), 121.2 (Ar\(\text{CH}\)), 111.9 (Ar\(\text{CH}\)), 23.9 (CH\(_3\)), 21.5 (CH\(_3\)). MS (ES\(^-\)) \(m/z\) calculated for C\(_{15}\)H\(_{13}\)N\(_2\)O\(_2\) [M\(-\text{H}\)]\(^-\) 253.0977, found 253.0982.

**3,5-Dimethylbenzoyl chloride 121**

A solution of 3,5-dimethylbenzoic acid 120 (2.0 g, 13.3 mmol) in toluene (50 mL) and thionyl chloride (1.1 mL, 14.6 mmol) was heated to reflux at 80 °C for 16 h. The excess thionyl chloride was distilled off by the azeotropic distillation with toluene under reduced pressure, affording 121 as brownish oil quantitatively. The product was used in the next step without further purification. \(^1\)H NMR (400.1 MHz, CDCl\(_3\)): \(\delta_H\) 7.70 (2H, s; 2\(\times\)Ar\(\text{H}\)), 7.29 (1H, s; Ar\(\text{H}\)), 2.38 (6H, s; 2\(\times\)CH\(_3\)); \(^{13}\)C NMR (100.6 MHz, CDCl\(_3\)): \(\delta_C\) 168.5 (C=O), 138.8 (Ar\(\text{C}\)), 137.1 (Ar\(\text{C}\)), 133.1 (Ar\(\text{C}\)), 129.2 (Ar\(\text{CH}\)), 21.1 (CH\(_3\)).

**N-(4-(4-Aminophenethyl)phenyl)-3,5-dimethylbenzamidine 122**

3,5-dimethylbenzoyl chloride 121 (2.24 g, 13.3 mmol) in dry CH\(_2\)Cl\(_2\) (20 mL) was added dropwise over 1.5 h to a solution of 4,4\(^1\)-ethylenedianiline (6.20 g, 29.2 mmol) and Et\(_3\)N (3.7 mL, 26.6 mmol) in dry CH\(_2\)Cl\(_2\) (50 mL) at ambient temperature under a N\(_2\) atmosphere. The reaction mixture was stirred at ambient temperature for 16 h. The organic phase was washed successively with sat. solution of NaHCO\(_3\) and brine, dried over MgSO\(_4\) and concentrated by evaporation under reduced pressure. The residue was purified by column chromatography (Hexane:EtOAc, 2:1), affording 122 as a yellow solid (3.0 g, 66%). M.p. = 139.8 – 142.7 °C; \(^1\)H NMR (300.1 MHz, CDCl\(_3\)): \(\delta_H\) 7.72 (1H, s; CON\(\text{H}\)), 7.56 – 7.51 (2H, m; 2\(\times\)Ar\(\text{H}\)), 7.46 (2H, s; 2\(\times\)Ar\(\text{H}\)), 7.18 – 7.14 (3H, m; 3\(\times\)Ar\(\text{H}\)), 6.99 – 6.94 (2H, m;...
Maleic anhydride (0.14 g, 1.45 mmol) was added to a solution of 122 (0.5 g, 1.45 mmol) in THF (15 mL). The reaction mixture was stirred at ambient temperature under a N₂ atmosphere for 3 h. The yellow precipitate (420 mg, 66%) was filtered and carried out to the next step without further purification. \(^1\)H NMR (500.1 MHz, CDCl₃ + 3 drops of d₆-DMSO) : \(\delta_H\) 10.63 (1H, br s; NH), 9.12 (1H, br s; NH), 7.43 (2H, \(\delta_{HH}\) 8.3 Hz; 2×ArH), 7.32 (4H, m; 4×ArH), 6.94 (2H, s; 2×ArH), 6.93 (1H, s; ArH), 6.90 (2H, \(\delta_{HH}\) 8.3 Hz; 2×ArH), 6.42 (1H, d, \(\delta_{HH}\) 12.9 Hz; CH), 6.11 (1H, d, \(\delta_{HH}\) 12.9 Hz; CH), 2.68 (4H, s; 2×CH₂), 2.17 (6H, s; 2×CH₃); \(^{13}\)C NMR (125.7 MHz, CDCl₃ + 3 drops of d₆-DMSO) : \(\delta_C\) 166.7 (C=O), 166.0 (C=O), 164.7 (C=O), 138.5 (CH), 138.4 (ArC), 137.7 (ArC), 137.5 (ArC), 136.2 (CH), 135.1 (ArC), 134.8 (ArC), 134.3 (ArCH), 134.0 (ArC), 128.4 (ArCH), 127.9 (ArCH), 127.5 (ArCH), 121.5 (ArCH), 120.6 (ArCH), 37.4 (CH₂), 37.0 (CH₂), 21.2 (CH₃).

\(\text{N-(4-(4-Aminophenethyl)phenyl)-3,5-bis(trifluoromethyl)benzamide 125}\)

3,5-bis(trifluoromethyl)benzoyl chloride 124 (0.5 g, 1.81 mmol) diluted in CH₂Cl₂ (3.0 mL) was slowly added at −10 °C to the resulting mixture of 4,4′-ethylene dianiline (0.58 g, 2.72 mmol) in CH₂Cl₂ (15 mL) and Et₃N (5 mL, 3.62 mmol). The resulting mixture was allowed to warm to ambient temperature and left stirred for 16 h. Following this, the reaction mixture was diluted with CH₂Cl₂, washed once with sat.
solution of NaHCO₃, dried over MgSO₄ and the solvent was removed in vacuo. The residue was purified on silica gel column (Hexane:EtOAc, 7:3) to obtain the product as yellow solid (0.43 g, 52%). M.p. = 146.7 – 148.2 °C; ¹H NMR (300.1 MHz, CDCl₃) : δ 8.31 (2H, s; 2×ArH), 8.05 (1H, s; ArH), 7.89 (1H, s; NH), 7.52 (2H, d, 3J_HH 8.4 Hz; 2×ArH), 7.18 (2H, d, 3J_HH 8.5 Hz; 2×ArH), 6.95 (2H, d, 3J_HH 8.4 Hz; 2×ArH), 6.62 (2H, d, 3J_HH 8.4 Hz; 2×ArH), 3.57 (2H, br s; NH₂), 2.90 – 2.77 (4H, m; 2×CH₂); ¹³C NMR (100.6 MHz, CDCl₃) : δC 163.0 (C=O), 144.4 (ArC), 139.6 (ArC), 137.2 (ArC), 134.8 (ArC), 132.4 (q, 2JCF 34.1 Hz; ArC), 131.7 (ArC), 129.4 (ArCH), 129.3 (ArCH), 127.5 (m; ArCH), 125.3 (m; ArCH), 123.1 (q, 1JCF 273.1 Hz; CF₃), 121.0 (ArCH), 115.4 (ArCH), 37.8 (CH₂), 37.1 (CH₂); ¹⁹F NMR (376.5 MHz, CDCl₃) : δF –63.4 (ArCF₃); MS (Cl⁺) m/z 453.14 ([M+H]⁺, 100); HRMS (Cl⁺) m/z calculated for C₂₃H₁₉N₂O₆F₆ [M+H]⁺ 453.1402, found 453.1412.

(Z)-4-(4-(3,5-Bis(trifluoromethyl)benzamido)phenethyl)phenylamino)-4-oxobut-2-enoic acid 126

Compound 125 (0.5 g, 1.11 mmol) was dissolved in THF (15 mL). Maleic anhydride (0.11 g, 1.11 mmol) was added neat to the solution. The mixture was left stirred at ambient temperature for 3 h under positive atmosphere of Ar. The precipitate (0.59 g, 98%) was then filtered and carried out to the next step without further purification. M.p. = 207.4 – 209.3 °C; ¹H NMR (300.1 MHz, CDCl₃ + 3 drops of d₆-DMSO) : δ 11.7 (1H, s; NH), 11.4 (1H, s; NH), 9.66 (2H, s; 2×ArH), 9.43 (1H, s; ArH), 8.72 (2H, d, 3J_HH 8.6 Hz; 2×ArH), 8.58 (2H, d, 3J_HH 8.6 Hz; 2×ArH), 8.29 (2H, d, 3J_HH 8.6 Hz; 2×ArH), 8.24 (2H, d, 3J_HH 8.6 Hz; 2×ArH), 7.53 (1H, d, 3J_HH 12.1 Hz; CH), 7.37 (1H, d, 3J_HH 12.1 Hz; CH), 6.82 (1H, s, O=H), 3.92 (4H, s; 2×CH₂); ¹³C NMR (75.5 MHz, CDCl₃ + 3 drops of d₆-DMSO) : δC 165.5 (C=O), 164.1 (C=O), 162.9 (C=O), 139.2 (ArC), 137.6 (ArC), 137.1 (ArC), 136.1 (ArC), 134.7 (CH), 133.0 (CH), 132.8 (ArC), 131.5 (q, 2JCF 34.3 Hz, ArC), 129.0 (ArCH), 128.7 (ArCH), 128.4 (m, ArCH), 124.5 (m, ArCH), 121.0 (ArCH), 120.8 (ArCH), 37.2 (CH₂), 37.1 (CH₂); ¹⁹F NMR (376.5 MHz, CDCl₃) : δF –63.2 (ArCF₃); MS (ES⁻) m/z 549 ([M–H]⁻, 100); HRMS (ES⁻) m/z calculated for C₂₇H₂₆N₂O₄F₆Na [M+Na]⁺ 573.1225, found 573.1216.
1-(4-(4-Aminophenethyl)phenyl)-3-(3,5-dimethylphenyl)urea 128

4,4'-ethylene dianiline (1.08 g, 5.1 mmol) was dissolved in dry CH₂Cl₂ (20 mL) followed by the slow addition of 3,5-dimethyl phenylisocyanate 127 (0.5 g, 3.4 mmol) at −5°C and allowed to stir at ambient temperature for 16 h. The solvent was removed under reduced pressure. The residue was then purified by column chromatography (CH₂Cl₂:EtOAc, 8:1). The product 128 was obtained as yellow solid (0.58 g, 63%).

M.p. = 165.7 – 168.3 °C; ¹H NMR (400.1 MHz, d₆-DMSO) : δ_H 9.37 (1H, s; NH₂), 8.89 (1H, s; NH), 8.12 (2H, s; 2×ArH), 7.62 (1H, s; ArH), 7.37 – 7.34 (2H, m; 2×ArH), 7.13 – 7.10 (2H, m; 2×ArH), 6.86 – 6.83 (2H, m; 2×ArH), 6.48 – 6.45 (2H, m; 2×ArH), 4.80 (2H, br s; NH₂), 2.75 – 2.63 (4H, m; 2×CH₂); ¹³C NMR (100.6 MHz, d₆-DMSO) : δ_C 152.5 (C=O), 146.5 (ArC), 142.0 (ArC), 136.7 (ArC), 136.2 (ArC), 130.8 (q, ²J_CF 32.3 Hz; ArC), 128.93 (ArCH), 128.91 (ArCH), 128.6 (ArC), 123.4 (q, ¹J_CF 277.6 Hz; CF₃), 119.1 (ArCH), 118.0 (m, ArCH), 114.5 (m, ArCH), 114.1 (ArCH), 36.6 (CH₂), 37.1 (CH₂); ¹⁹F NMR (376.5 MHz, CDCl₃ + 3 drops of d₆-DMSO) : δ_F −63.0 (ArCF₃); MS (ES⁺) m/z 490 ([M+Na]⁺, 100); HRMS (ES⁺) m/z calculated for C₂₃H₂₀N₃O₆ [M+H]⁺ 468.1511, found 468.1505.

(Z)-4-(4-(3-(3,5-Bis(trifluoromethyl)phenyl)ureido)phenethyl)phenylamino)-4-oxobut-2-enoic acid 129

Maleic anhydride (82 mg, 0.84 mmol) was added to solution of 128 (0.3 g, 0.84 mmol) in THF (10 mL). The reaction mixture was stirred at ambient temperature under a Ar atmosphere for 3 h. The precipitate was filtered and the acid compound 129 (460 mg, 98%) was carried out to the next step without further purification. M.p. = 219.2 °C (decomposed); ¹H NMR (300.1 MHz, CDCl₃ + 3 drops of d₆-DMSO) : δ_H 10.7 (1H, s; NH₂), 8.77 (1H, s; NH), 7.82 (2H, s; 2×ArH), 7.39 – 7.35 (2H, m; 2×ArH), 7.26 (1H, br s; ArH), 7.19 – 7.15 (2H, m; 2×ArH), 6.99 – 6.95 (2H, m; 2×ArH), 6.92 – 6.88 (2H, m; 2×ArH), 6.47 (1H, d, ³J_HH, 3.6 Hz; 2×CH₂).
12.9 Hz; CHJ, 6.16 (1H, d, 3JHH 12.9 Hz; CHJ), 2.74 – 2.65 (4H, m; 2xCH2); 13C NMR (75.5 MHz, CDCl3 + 3 drops of d6-DMSO): δC 165.2 (C=O), 163.8 (C=O), 152.4 (C=O), 141.2 (ArC), 138.9 (ArC), 136.3 (ArC), 135.6 (ArC), 134.5 (ArC), 134.3 (CH), 132.9 (CH), 131.4 (q, 2JCF 32.9 Hz, ArC), 128.7 (ArCH), 128.6 (ArCH), 120.5 (ArCH), 118.8 (ArCH), 117.5 (m, ArCH), 114.3 (m, ArCH), 37.0 (CH2), 36.6 (CH2); 19F NMR (376.5 MHz, CDCl3) : δF -58.6 (ArCF3); MS (ES+) m/z 564 ([M-H]+, 10), 228 (100); HRMS (ES−) m/z calculated for C27H20N3O4F6 [M−H]− 564.1358, found 564.1367.

(Z)-4-tert-Butyl-N-(4-tert-butylbenzylidene)aniline oxide 134

4-tert-butylnitrobenzene 118 (0.56 g, 3.12 mmol) was dissolved in THF (15 mL). Rhodium (20 mg, 30 wt.% on carbon, wet) and hydrazine monohydrate (0.19 mL, 3.75 mmol) and the reaction was followed by TLC (Hex:EtOAc, 3:1). After completion, the solution was filtered through Celite and concentrated in vacuo to obtain the desired intermediate product 119 as a dark brown liquid, which was directly used in the next step without further purification. The obtained hydroxylamine 119 (3.12 mmol) was dissolved in EtOH (15 mL) and 4-tert-butylbenzaldehyde (0.51 mL, 3.12 mmol) was added dropwise to the solution. The reaction mixture was left stirred in the dark for 16 h at ambient temperature. The solvent was concentrated by evaporation under reduced pressure and the resulting residue was purified by column chromatography (Cyclohexane:EtOAc:Et3N, gradient from 99.5:0.5 to 94.5:5.0:0.5) to give 134 as yellow solid (0.42 g, 44% over 2 steps). M.p. = 152.5 – 154.4 °C; 1H NMR (300.1 MHz, CDCl3) : δH 8.35 – 8.30 (2H, m; 2×ArH), 7.88 (1H, s; CH), 7.72 – 7.67 (2H, m; 2×ArH), 7.52 – 7.46 (4H, m; 4×ArH), 1.35 (18H, s; 6×CH3); 13C NMR (75.5 MHz, CDCl3) : δC 154.5 (ArC), 153.3 (ArC), 146.8 (ArC), 134.2 (CH), 129.1 (ArCH), 128.3 (ArC), 126.1 (ArCH), 125.7 (ArCH), 121.4 (ArCH), 35.2 (qC), 35.0 (qC), 31.4 (CH3), 31.3 (CH3); MS (ES+) m/z 332.0 ([M+Na]+, 95), 641.0 (100); HRMS (ES+) m/z calculated for C21H27NOna 332.1990 [M+Na]+, found 332.1994.
Maleimide 108 (50.0 mg, 0.16 mmol) was dissolved in CHCl₃ (8 mL) followed by the addition of nitrone 134 (48.3 mg, 0.16 mmol). The reaction mixture was stirred at ambient temperature under Ar atmosphere in the dark for 6 days. The solvent was removed by evaporation under reduced pressure. The residue was purified by column chromatography (Cyclohexane:EtOAc; 9:1), to enable the isolation of the trans 135 as yellow solid (53 mg, 56%). Representative data for trans thread 135: M.p. = 148.7 – 149.3 °C; ¹H NMR (300.1 MHz, CDCl₃) : δH 7.82 (1H, s; NH), 7.39 – 7.32 (4H, m; 4×ArH), 7.29 – 7.26 (4H, m; 4×ArH), 7.09 – 7.04 (2H, m; 2×ArH), 6.98 (1H, s; ArH), 6.92 – 6.87 (2H, m; 2×ArH), 6.24 (2H, d, 3JHH 8.8 Hz; 2×ArH), 5.58 (1H, s, CH), 4.86 (1H, d, 3JHH 7.7 Hz, CH), 3.82 (1H, dd, 4JHH 0.8 and 3JHH 7.5 Hz, CH), 2.17 (6H, s; 2×CH₃), 1.17 (9H, s; 3×CH₃), 1.10 (9H, s; 3×CH₃); ¹³C NMR (75.5 MHz, CDCl₃) : δC 174.5 (C=O), 173.1 (C=O), 166.2 (C=O), 151.3 (ArC), 146.8 (ArC), 146.0 (ArC), 138.8 (ArC), 138.6 (ArC), 136.0 (ArC), 134.7 (ArC), 133.8 (ArCH), 127.0 (ArCH), 126.6 (ArC), 126.3 (ArCH), 126.2 (ArCH), 126.0 (ArCH), 125.0 (ArCH), 120.3 (ArCH), 114.0 (ArCH), 77.1 (CH), 69.5 (CH), 57.4 (CH), 34.7 (qC), 34.3 (qC), 31.6 (CH₃), 31.4 (CH₃), 21.2 (CH₃, C₁); MS (ES⁺) m/z 652.1 ([M+Na]+, 100); HRMS (ES⁺) m/z calculated for C₄₀H₄₃N₃O₄Na [M+Na]+ 652.3151, found 652.3135.
trans Rotaxane 136

A mixture of macrocycle MDG (30 mg, 0.06 mmol) and maleimide 108 (20 mg, 0.06 mmol) in CHCl₃ (3 mL) were stirred at ambient temperature for 2 h. Nitrone 134 (20 mg, 0.06 mmol) was then added to the resulting solution. This reaction mixture was stirred under Ar atmosphere at ambient temperature in the dark for 5 days. The solvent was removed by evaporation in vacuo. The residue was purified by column chromatography (Cyclohexane:EtOAc, gradient from 95:5 to 80:20) to yield the trans rotaxane 136 as yellow solid (20 mg, 29%). Representative data for trans rotaxane 136: M.p. = 136.0 – 139.8 °C; ¹H NMR (400.1 MHz, CDCl₃) : δH 9.19 (2H, br s; 2xNH), 8.50 (2H, d, 3JHH 7.9 Hz; 2xArH), 8.12 (1H, t, 3JHH 7.7 Hz; ArH), 7.69 (1H, s; NH), 7.54 (2H, d, 3JHH 8.3 Hz; 2xArH), 7.47 (2H, d, 3JHH 8.6 Hz; 2xArH), 7.40 (2H, d, 3JHH 8.8 Hz; 2xArH), 7.32 (2H, d, 3JHH 8.9 Hz; 2xArH), 7.16 (2H, d, 3JHH 8.9 Hz; 2xArH), 7.03 (2H, s; 2xArH), 6.99 (1H, s; ArH), 6.64 – 6.61 (4H, m; 4xArH), 6.54 – 6.51 (4H, m; 4xArH), 6.35 (2H, d, 3JHH 8.9 Hz; 2xArH), 5.78 (1H, s; CH), 5.13 (1H, d, 3JHH 7.5 Hz; CH), 4.77 – 4.70 (2H, m; CH₂), 4.31 – 4.24 (2H, m; CH₂), 4.10 – 4.09 (4H, m; 2xCH₂), 4.07 – 4.05 (1H, m; CH), 3.72 – 3.69 (4H, m; 2xCH₂), 3.50 – 3.48 (4H, m; 2xCH₂), 2.13 (6H, s; 2xCH₃), 1.35 (9H, s; 3xCH₃), 1.31 (9H, s; 3xCH₃); ¹³C NMR (100.6 MHz, CDCl₃): δC 174.5 (C=O), 173.0 (C=O), 165.4 (C=O), 164.1 (C=O), 151.3 (ArC), 149.6 (ArC), 146.9 (ArC), 145.8 (ArC), 139.5 (ArC), 138.7 (ArCH), 136.9 (ArC), 136.8 (ArC), 136.1 (ArC), 135.4 (ArC), 133.5 (ArC), 132.9 (ArCH), 128.8 (ArCH), 127.8 (ArCH), 126.4 (ArCH), 126.2 (ArCH), 126.1 (ArC), 126.1 (ArCH), 126.0 (ArCH), 125.8 (ArC), 125.5 (ArCH), 124.7 (ArCH), 119.8 (ArCH), 114.3 (ArCH), 77.6 (CH), 73.7 (CH₂), 71.1 (CH₂), 69.7 (CH), 69.1 (CH₂), 57.5 (CH), 43.7 (CH₂), 34.7 (qC), 34.4 (qC), 31.7 (CH₃), 31.5 (CH₃), 21.2 (CH₃); MS (MALDI) m/z 1106 [(M+H)+, 100); HRMS (MALDI) m/z calculated for C₇₇H₁₇₂N₉O₉Na [M+Na]+ 1127.5258, found 1127.5209.
trans Rotaxane 137

A mixture of macrocycle MP (30 mg, 0.06 mmol) and maleimide 108 (19 mg, 0.06 mmol) in CHCl₃ (3 mL) were stirred at ambient temperature for 2 h. Nitrone 134 (18.3 mg, 0.06 mmol) was then added to the resulting solution. This reaction mixture was stirred under Ar atmosphere at ambient temperature in the dark for 5 days. The solvent was removed by evaporation in vacuo. The residue was purified by column chromatography (Cyclohexane:EtOAc, gradient from 95:5 to 80:20) to yield the trans rotaxane 137 as yellow solid (20 mg, 30%). Representative data for trans rotaxane 137: M.p. = 145.8 – 148.3 °C; ¹H NMR (300.1 MHz, CDCl₃) δ₀ = 9.43 – 9.37 (2H, m; 2xNH), 9.03 (1H, s; NH), 8.51 (2H, d, ³JHH 7.8 Hz; 2xArH), 8.12 (1H, t, ³JHH 7.8 Hz; ArH), 7.79 (1H, t, ³JHH 7.7 Hz; ArH), 7.55 – 7.51 (2H, m; 2xArH), 7.49 – 7.44 (4H, m; 4xArH), 7.37 – 7.34 (2H, m; 2xArH), 7.27 – 7.24 (3H, m; 3xArH), 7.15 – 7.10 (2H, m; 2xArH), 6.96 (2H, s; 2xArH), 6.87 (1H, s; ArH), 6.74 – 6.62 (7H, m; 7xArH), 6.22 – 6.19 (2H, m; 2xArH), 5.78 (1H, s; ArH), 5.11 (1H, d, ³JHH 7.9 Hz; ArH), 4.65 – 4.58 (2H, m; 2xArH), 4.43 (2H, d, ³JHH 4.9 Hz; CH₂), 4.42 – 4.21 (8H, m; 4xCH₂), 4.04 (1H, dd, ⁴JHH 0.9 and ³JHH 7.5 Hz; CH), 1.85 (6H, s; 2xCH₃), 1.35 (9H, s; 3xCH₃), 1.12 (9H, s; 3xCH₃); ¹³C NMR (100.6 MHz, CDCl₃): δC 174.6 (C=O), 173.1 (C=O), 166.2 (C=O), 164.3 (C=O), 164.3 (C=O), 153.7 (C=Ar), 158.2 (ArC), 151.3 (ArC), 149.6 (ArC), 149.5 (ArC), 146.9 (ArC), 145.7 (ArC), 139.7 (ArC), 138.7 (ArCH), 137.8 (ArCH), 137.4 (ArC), 137.3 (ArC), 136.7 (ArC), 136.1 (ArC), 135.2 (ArC), 135.1 (ArC), 133.4 (ArC), 132.7 (ArCH), 129.1 (ArCH), 129.1 (ArCH), 127.8 (ArCH), 126.3 (ArCH), 126.2 (ArCH), 126.1 (ArCH), 126.0 (ArCH), 125.9 (ArCH), 125.9 (ArC), 125.5 (ArCH), 121.6 (ArCH), 121.5 (ArCH), 120.1 (ArCH), 114.0 (ArCH), 77.7 (CH), 73.3 (CH₂), 73.3 (CH₂), 71.4 (CH₂), 71.3 (CH₂), 69.6 (CH), 57.4 (CH), 43.6 (CH), 34.8 (qC), 34.2 (qC), 31.5 (CH₃), 31.4 (CH₃), 29.8 (qC), 20.7 (CH₃); MS (MALDI) m/z 1138.5 ([M+H]⁺, 100); HRMS (MALDI) m/z calculated for C₇₀H₇₁N₇O₈Na [M+H]⁺ 1138.5442, found 1138.5371.
**trans Thread 138**

Maleimide 109 (30 mg, 0.07 mmol) was dissolved in CHCl₃ (3 mL) followed by the addition of nitrone 134 (22 mg, 0.07 mmol). The reaction mixture was stirred at ambient temperature under Ar atmosphere in the dark for 6 days. The solvent was removed by evaporation under reduced pressure. The residue was purified by column chromatography (Cyclohexane:EtOAc, gradient from 95:5 to 75:25), which enabled the isolation of the major trans compound 138 as yellow solid (16 mg, 31%). Representative data for trans thread 138: M.p. = 174.8 – 177.6 °C; ¹H NMR (300.1 MHz, CDCl₃): δH 7.78 (1H, s; NH), 7.59 – 7.43 (8H, m; 8×ArH), 7.30 – 7.25 (2H, m; 2×ArH), 7.17 – 7.13 (2H, m; 2×ArH), 7.14 – 7.07 (4H, m; 4×ArH), 6.43 – 6.39 (2H, m; 2×ArH), 5.79 (1H, s; ArH), 5.08 (1H, d, 3JHH 7.8 Hz; ArH), 4.03 (1H, dd, 4JHH 0.9 and 3JHH 7.5 Hz; ArH), 2.92 – 2.81 (4H, m; 2×CH₂), 2.39 (6H, s; 2×CH₃), 1.35 (9H, s; 3×CH₃), 1.29 (9H, s; 3×CH₃); ¹³C NMR (75.5 MHz, CDCl₃): δC 174.6 (C=O), 173.9 (C=O), 166.2 (C=O), 151.3 (ArC), 146.9 (ArC), 145.9 (ArC), 142.9 (ArC), 138.6 (ArC), 137.6 (ArC), 136.2 (ArC), 136.1 (ArC), 135.2 (ArC), 133.5 (ArCH), 129.1 (ArCH), 129.1 (ArCH), 128.9 (ArCH), 128.9 (ArCH), 126.5 (ArCH), 126.2 (ArCH), 126.0 (ArCH), 124.8 (ArCH), 120.4 (ArCH), 113.1 (ArCH), 77.5 (CH), 69.6 (CH), 57.5 (CH), 37.6 (CH₂), 37.1 (CH₂), 34.7 (qC), 34.3 (qC), 31.6 (CH₃), 31.5 (CH₃), 21.4 (CH₃); MS (ES⁺) m/z 756.2 ([M+Na]⁺, 100); HRMS (ES⁺) m/z calculated for C₄₈H₅₁N₃O₄Na [M+Na]⁺ 756.3777, found 756.3782.
A mixture of macrocycle MDG (30 mg, 0.06 mmol) and maleimide 109 (27 mg, 0.06 mmol) in CHCl₃ (3 mL) were stirred at ambient temperature for 2 h. Nitrone 134 (20 mg, 0.06 mmol) was then added to the resulting solution. This reaction mixture was stirred under Ar atmosphere at ambient temperature in the dark for 5 days. The solvent was removed by evaporation under reduced pressure. The residue was purified by column chromatography (Cyclohexane:EtOAc, gradient from 95:5 to 80:20) to yield the trans rotaxane 139 as pale yellow solid (20 mg, 26%). M.p. = 136.2 – 139.9 °C, ¹H NMR (300.1 MHz, CDCl₃) : δH 9.36 (2H, t, 3JHH 5.7 Hz; 2×ArH), 8.49 (2H, d, 3JHH 7.9 Hz; 2×ArH), 8.12 (1H, t, 3JHH 7.9 Hz; ArH), 7.61 (1H, s; ArH), 7.54 – 7.50 (2H, m; 2×ArH), 7.47 – 7.43 (2H, m; 2×ArH), 7.30 (2H, d, 3JHH 8.5 Hz; 2×ArH), 7.26 (2H, d, 3JHH 8.9 Hz; 2×ArH), 7.13 – 7.07 (4H, m; 4×ArH), 7.05 (2H, s; 2×ArH), 6.99 (1H, s; ArH), 6.85 (2H, d, 3JHH 8.5 Hz; 2×ArH), 6.66 (4H, d, 3JHH 7.8 Hz; 4×ArH), 6.55 (4H, d, 3JHH 7.9 Hz; 4×ArH), 6.45 (2H, d, 3JHH 8.27 Hz; 2×ArH), 5.78 (1H, s; CH), 5.10 (1H, d, 3JHH 7.6 Hz; CH), 4.58 (2H, dd, 3JHH 5.90 and 2JHH 14.7 Hz; CH₂), 4.48 (2H, dd, 3JHH 5.30 and 2JHH 14.7 Hz; CH₂), 4.13 (4H, ABq, J_AB 14.7 Hz; 2×CH₂), 4.04 (1H, d, 3JHH 7.44 Hz; CH), 3.75 – 3.66 (4H, m; 2×CH₂), 3.50 – 3.39 (4H, m; 2×CH₂), 2.91 – 2.78 (4H, m; 2×CH₂), 2.17 (6H, s; 2×CH₃), 1.34 (9H, s; 3×CH₃), 1.30 (9H, s; 3×CH₃); ¹³C NMR (100.6 MHz, CDCl₃) : δC 175.4 (C=O), 173.0 (C=O), 165.8 (C=O), 164.1 (C=O), 156.8 (ArC), 150.4 (ArC), 149.5 (ArC), 146.9 (ArC), 145.9 (ArC), 142.9 (ArC), 138.7 (ArCH), 137.8 (ArCH), 137.7 (ArC), 137.1 (ArC), 136.6 (ArC), 136.1 (ArC), 135.5 (ArC), 134.0 (ArC), 132.4 (ArCH), 130.3 (ArCH), 129.2 (ArCH), 128.9 (ArCH), 128.0 (ArCH), 127.9 (ArCH), 126.4 (ArCH), 126.3 (ArCH), 126.2 (ArCH), 126.0 (ArCH), 125.4 (ArCH), 122.4 (ArCH), 119.7 (ArCH), 114.0 (ArCH), 77.7 (CH), 73.6 (CH₂), 71.1 (CH₂), 69.7 (CH), 68.8 (CH), 43.7 (CH₂), 37.7 (CH₂), 37.6 (CH₂), 34.7 (qC), 34.3 (qC), 31.6 (CH₃), 31.5 (CH₃), 21.2 (CH₃); MS (MALDI) m/z 1210 ([M+H]+, 100); HRMS (MALDI) m/z calculated for C₇₅H₈₀N₅O₅Na [M+Na]+ 1231.5884, found 1231.5194.
**trans Rotaxane 140**

A mixture of macrocycle MP (30 mg, 0.06 mmol) and maleimide 109 (25 mg, 0.06 mmol) in CHCl₃ (3 mL) were stirred at ambient temperature for 2 h. Nitrone 134 (18.3 mg, 0.06 mmol) was then added to the resulting solution. The reaction mixture was stirred under Ar atmosphere at ambient temperature in the dark for 5 days. The solvent was removed by evaporation under reduced pressure. The residue was purified by column chromatography (Cyclohexane:EtOAc, gradient from 95:5 to 80:20) to yield the trans rotaxane 140 as pale yellow solid (15 mg, 20%). M.p. = 172.4 – 178.5 °C, ¹H NMR (400.1 MHz, CDCl₃): δ H 9.56 (2H, t, ³Jₜₜₜ 5.7 Hz; 2×NH), 8.99 (1H, s; NH), 8.49 (2H, d, ³Jₜₜₜ 7.8 Hz; 2×ArH), 8.12 (1H, t, ³Jₜₜₜ 7.8 Hz; ArH), 7.78 (1H, t, ³Jₜₜₜ 7.7 Hz; ArH), 7.53 (2H, d, ³Jₜₜₜ 8.4 Hz; 2×ArH), 7.45 (2H, d, ³Jₜₜₜ 8.4 Hz; 2×ArH), 7.36 (2H, d, ³Jₜₜₜ 7.7 Hz; 2×ArH), 7.29 (2H, d, ³Jₜₜₜ 8.5 Hz; 2×ArH), 7.25 (2H, d, ³Jₜₜₜ 8.7 Hz; 2×ArH), 7.11 – 7.07 (4H, m; 4×ArH), 6.91 (2H, s; 2×ArH), 6.86 (1H, s; ArH), 6.76 – 6.69 (10H, m; 10×ArH), 6.44 (2H, d, ³Jₜₜₜ 8.3 Hz; 2×ArH), 5.78 (1H, s; CH), 5.10 (1H, d, ³Jₜₜₜ 7.6 Hz; CH), 4.64 (2H, dd, ³Jₜₜₜ 6.0 and ²Jₜₜₜ 14.8 Hz; CH₂), 4.48 (2H, dd, ³Jₜₜₜ 5.1 and ²Jₜₜₜ 20.0 Hz; CH₂), 4.38 (4H, s; 2×CH₂), 4.23 (2H, dd, ⁴Jₜₜₜ 3.1 and ²Jₜₜₜ 14.4 Hz; CH₂), 4.04 (1H, d, ³Jₜₜₜ 7.5 Hz; CH), 2.91 – 2.66 (4H, m; 2×CH₂), 1.89 (6H, s; 2×CH₃), 1.34 (9H, s; 3×CH₃); ¹³C NMR (100.6 MHz, CDCl₃): δ C 174.5 (C=O), 173.2 (C=O), 166.2 (C=O), 164.3 (C=O), 157.3 (ArC), 151.3 (ArC), 149.5 (ArC), 146.9 (ArC), 145.9 (ArC), 142.9 (ArC), 138.7 (ArCH), 137.8 (ArCH), 137.7 (ArC), 137.1 (ArC), 136.6 (ArC), 136.2 (ArC), 136.1 (ArC), 135.3 (ArC), 134.0 (ArC), 132.4 (ArCH), 129.1 (ArCH), 129.0 (ArCH), 128.9 (ArC), 128.0 (ArCH), 127.9 (ArCH), 126.4 (ArCH), 126.3 (ArCH), 126.2 (ArCH), 126.0 (ArCH), 125.9 (ArCH), 125.3 (ArCH), 122.0 (ArCH), 120.1 (ArCH), 114.0 (ArCH), 77.7 (CH), 73.2 (CH₂), 71.2 (CH₂), 69.7 (CH), 57.4 (CH), 43.5 (CH₂), 37.6 (CH₂), 37.1 (CH₂), 34.7 (qC), 34.3 (qC), 31.6 (CH₃), 31.5 (CH₃), 20.7 (CH₃); MS (MALDI) m/z 1243 ([M+H]⁺, 100); HRMS (MALDI) m/z calculated for C₇₈H₇₉N₇O₃Na
4-tert-butyl nitrobenzene 118 (0.56 g, 3.12 mmol) was dissolved in THF (15 mL). Rh/C (0.2 g, 30 wt. %) was then added and continued to stir for another 15 min before the following addition of the hydrazine monohydrate (0.19 mL, 3.75 mmol). The reaction progress was monitored by TLC (Hexane:EtOAc; 3:1) and the solution was filtered through a pad of Celite. The solvent was then removed to give the intermediate product and used for the next step without further purification. The hydroxylamine 119 (3.12 mmol) was dissolved in EtOH (15 mL) and 3,5-difluorobenzaldehyde (0.44 g, 3.12 mmol) was added. The reaction mixture was stirred for another 16 h at ambient temperature in the dark. The solvent was concentrated by evaporation under reduced pressure. The residue was purified by column chromatography (Cyclohexane:EtOAc:Et$_3$N; gradient from 99.5:0:0.5 to 94.5:5:0:0.5) to give 141 as yellow solid (0.23 g, 26% over 2 steps). M.p. = 68.8 – 70.5 °C; $^1$H NMR (400.1 MHz, CDCl$_3$) : $\delta$H 7.99 – 7.93 (2H, m; 2×ArH), 7.69 (1H, s; CH), 7.69 – 7.65 (2H, m; 2×ArH), 7.51 – 7.47 (2H, m; 2×ArH), 6.90 (1H, tt, $^2$J$_{HH}$ 2.4 and $^3$J$_{HF}$ 8.6 Hz, ArH), 1.35 (9H, s, 3×CH$_3$); $^{13}$C NMR (100.6 MHz, CDCl$_3$) : $\delta$C 163.0 (dd, $^1$J$_{CF}$ 247.7 and $^3$J$_{CF}$ 12.7 Hz; ArC-F), 154.2 (ArC), 146.1 (ArC), 133.3 (t, $^3$J$_{CF}$ 10.7 Hz; ArC), 132.2 (m; CH), 126.3 (ArCH), 121.4 (ArCH), 111.4 (dd, $^3$J$_{CF}$ 7.7 and $^2$J$_{CF}$ 18.3 Hz, ArCH), 106.2 (t, $^2$J$_{CF}$ 25.7 Hz; ArCH), 35.1 (qC), 31.4 (3×CH$_3$); $^{19}$F NMR (376.5 MHz, CDCl$_3$) : $\delta$F −109.5 (ArF); MS (ES$^+$) m/z 312.0 ([M+Na]$^+$, 100), 601.1 (12); HRMS (ES$^+$) m/z calculated for C$_{17}$H$_{17}$NOF$_2$Na [M+Na]$^+$ 312.1176, found 312.1178.

(Z)-N-(3,5-Bis(trifluoromethyl)benzylidene)-4-tert-butylaniline oxide 142

4-tert-butyl nitrobenzene 118 (0.56 g, 3.12 mmol) was dissolved in THF (15 mL). Rh/C (0.2 g, 30 wt. %) was then added and continued to stir for another 15 min before the following addition of the hydrazine monohydrate (0.19 mL, 3.75 mmol). The reaction progress was monitored by TLC (Hexane:EtOAc; 3:1) and the solution was filtered through a pad of Celite. The solvent was then removed to
give the intermediate product. The hydroxylamine 119 (3.12 mmol) was dissolved in EtOH (15 mL) and 3,5-bis(trifluoromethyl)benzaldehyde (0.52 mL, 3.12 mmol) was added. The reaction mixture was stirred for another 16 h at ambient temperature in the dark. The solvent was concentrated in vacuo and the residue was purified by column chromatography (Cyclohexane:EtOAc:Et$_3$N gradient from 99.5:0:0.5 to 94.5:5:0:0.5) to give 142 as yellow solid (0.59 g, 49% over 2 steps). M.p = 123.8 – 126.4 °C; $^1$H NMR (300.1 MHz, CDCl$_3$): $\delta$H 8.89 (2H, s; 2×ArH), 8.08 (1H, s; ArH), 7.92 (1H, s; CH), 7.72 – 7.69 (2H, m; 2×ArH), 7.53 – 7.51 (2H, m; 2×ArH), 1.37 (9H, s; 3×CH$_3$); $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta$C 154.5 (ArC), 146.3 (ArC), 132.6 (ArC), 132.2 (q, $^2$J$_{CF}$ 33.4 Hz; ArC), 131.2 (CH), 128.3 (m; ArCH), 126.5 (ArCH), 126.3 (m, ArCH), 123.4 (q, $^1$J$_{CF}$ 275.0 Hz, CF$_3$), 121.3 (ArCH), 35.1 (qC), 31.3 (3×CH$_3$); $^{19}$F NMR (376.5 MHz, CDCl$_3$): $\delta$F –63.5 (ArCF$_3$); MS (ES$^+$) m/z 412.0 ([M+Na]$^+$, 100); HRMS (ES$^+$) m/z calculated for C$_{19}$H$_{17}$NOF$_3$Na [M+Na]$^+$ 412.1112, found 412.1108.

$N$-(4-((3S,3aR,6aS)-3-(3,5-Bis(trifluoromethyl)phenyl)-2-(4-tert-butylphenyl)-4,6-dioxodihydro-2H-pyrrolo[3,4-d]isoxazol-5(3H,6H,6aH)-yl)phenyl)-3,5-dimethylbenzamide trans Thread 143

Maleimide 108 (50 mg, 0.16 mmol) was dissolved in CHCl$_3$ (12 mL) followed by the addition of CF$_3$ nitrone 142 (60.7 mg, 0.16 mmol). The reaction mixture was stirred at ambient temperature under Ar atmosphere in the dark for 6 days. The solvent was removed by evaporation in vacuo. The residue was purified by column chromatography (Hexane:EtOAc; 9:1), which enabled the isolation of the trans thread 143 as pale yellow solid in (72 mg, 65%). Representative data for trans thread 143: M.p. = 192.7 – 195.3 °C; $^1$H NMR (400.1 MHz, CDCl$_3$): $\delta$H 8.08 (2H, s; 2×ArH), 7.89 (1H, s; NH), 7.89 (1H, s; ArH), 7.59 – 7.55 (2H, m; 2×ArH), 7.43 (2H, s; 2×ArH), 7.32 – 7.29 (2H, m, 2×ArH), 7.17 (1H, s, ArH), 7.12 – 7.08 (2H, m; 2×ArH), 6.46 – 6.43 (2H, m; 2×ArH), 5.91 (1H, s; CH), 5.07 (1H, d, $^3$J$_{HH}$ 7.8 Hz; CH), 4.02 (1H, dd, $^4$J$_{HH}$ 0.8 Hz and $^3$J$_{HH}$ 7.5 Hz; CH), 2.37 (6H, s; 2×CH$_3$), 1.30 (9H, s; 3×CH$_3$); $^{13}$C NMR (100.6 MHz, CDCl$_3$): $\delta$C 173.7 (C=O), 173.1 (C=O), 166.3 (C=O), 147.1 (ArC), 145.8 (ArC), 141.8 (ArC), 139.1 (ArC), 138.9 (ArC), 134.6 (ArC), 133.9 (ArCH), 132.6 (q, $^1$J$_{CF}$ 35.4 Hz, ArC), 127.1 (m, ArCH), 127.0 (ArCH), 126.6
(ArCH), 126.4 (ArC), 125.0 (ArCH), 123.3 (q, $^1J_{CF}$ 276.1 Hz, Ar-CF$_3$), 122.4 (m, ArCH), 120.4 (ArCH), 114.1 (ArCH), 77.6 (CH), 68.8 (CH), 57.3 (CH), 34.7 (qC), 31.6 (CH$_3$), 21.3 (CH$_3$); $^{19}$F NMR (376.5 MHz, CDCl$_3$) : $\delta_F$ −63.2 (ArCF$_3$); MS (ES$^+$) m/z 731.9 ([M+Na]$^+$, 100); HRMS (ES$^+$) m/z calculated for C$_{38}$H$_{33}$N$_3$O$_4$F$_6$Na [M+Na]$^+$ 732.2273, found 732.2279.

**N-(4-(4-Azidophenethyl)phenyl)-3,5-dimethylbenzamide 146**

Aniline derivative 122 (0.2 g, 0.58 mmol) was suspended in conc. HCl (2 ml) and cooled down to −10 °C. Sodium nitrite solution (44 mg in 2 ml H$_2$O, 0.64 mmol) was added dropwise at −10 °C. Reaction mixture was stirred for 1 h. Sodium azide solution (46 mg in 2 ml H$_2$O, 0.70 mmol) was then added at −10°C. The reaction mixture was allowed to get to ambient temperature and stirred for 2 h. The resulting precipitate was filtered off and dried. The product 146 was obtained as yellow solid (207 mg, 97%). M.p. = 166.3 −169.2 °C; $^1$H NMR (400.1 MHz, CDCl$_3$) : $\delta_H$ 7.74 (1H, s; C(O)NH), 7.54 (2H, d, $^3J_{HH}$ 8.5 Hz; 2×ArH), 7.46 (2H, s; 2×ArH), 7.18 (1H, s; ArH), 7.13 (2H, d, $^3J_{HH}$ 8.5 Hz; 2×ArH), 7.12 (2H, d, $^3J_{HH}$ 8.5 Hz; 2×ArH), 6.93 (2H, d, $^3J_{HH}$ 8.5 Hz; 2×ArH), 2.89 (4H, s; 2×CH$_2$), 2.39 (6H, s; 2×CH$_3$); $^{13}$C NMR (100.6 MHz, CDCl$_3$): $\delta_C$ 166.1 (ArC), 138.7 (ArC), 138.5 (ArC), 137.8 (ArC), 137.7 (ArC), 136.2 (ArC), 135.2 (ArC), 133.6 (ArCH), 130.0 (ArCH), 129.2 (ArCH), 124.9 (ArCH), 120.3 (ArCH), 119.1 (ArCH), 37.5 (CH$_2$), 37.4 (CH$_2$), 21.4 (CH$_3$); MS (ES$^+$) m/z 393.06 ([M+Na]$^+$, 100); HRMS (Cl$^+$) m/z calculated for C$_{23}$H$_{23}$N$_4$ [M+H]$^+$ 371.187, found 371.188.
Rotaxane 151

Azide derivative 146 (0.1 g, 0.27 mmol) and macrocycle MDG (0.13 g, 0.27 mmol) were pre-equilibrated at −10 °C for 1 h in dry CDCl₃ (10 mL) before PPh₃ (142 mg, 0.54 mmol) in dry CDCl₃ (0.8 mL) was added to the reaction mixture and left to stir for 48 h under positive Ar atmosphere at 10 °C. The resulting mixture was warmed up to the ambient temperature. Subsequently, 3,5-trifluoromethylbenzaldehyde 148 (72 mg, 0.30 mmol) and 2-(4-bromophenyl)acetic acid (58 mg, 0.27 mmol), dissolved in dry CDCl₃ (0.85 mL) were added and stirred for 48 h at ambient temperature. Eventually, Hantzsch ester (0.21 g, 0.81 mmol) was added neat to the reaction mixture and allowed to heat at 40 °C. The reaction mixture was left stirred for another 48 h, before to remove the solvent by evaporation in vacuo. The residue was purified by careful column chromatography (Cyclohexane:EtOAc, 2:1), which enabled the isolation of the rotaxane 151 as yellow solid (75 mg, 28%). M.p. = 115.5 – 117.3 °C; ¹H NMR (400.1 MHz, CDCl₃): δH 9.38 (2H, t, ³JHH 5.45 Hz; 2×NH), 8.49 (2H, d, ³JHH 7.8 Hz; 2×ArH), 8.12 (1H, t, ³JHH 7.8 Hz; ArH), 7.83 (2H, s; 2×ArH), 7.77 (1H, s; ArH), 7.60 (1H, s; NH), 7.27 (2H, d, ³JHH 8.5 Hz; 2×ArH), 7.03 (2H, s; 2×ArH), 6.99 (1H, s; ArH), 6.81 (2H, d, ³JHH 8.3 Hz; 2×ArH), 6.65 (4H, d, ³JHH 7.9 Hz; 4×ArH), 6.56 (4H, d, ³JHH 8.4 Hz; 4×ArH), 6.54 (2H, d, ³JHH 7.4 Hz; 2×ArH), 4.52 (4H, d, ³JHH 3.6 Hz; 2×CH₂), 4.43 (2H, s; CH₂), 4.13 (4H, ABq, ³JAB 10.4 Hz; 2×CH₂), 3.75 – 3.66 (4H, m; 2×CH₂), 3.60 (1H, s; NH), 3.48 – 3.37 (4H, m; 2×CH₂), 2.81 (4H, s; 2×CH₂), 2.18 (6H, s; 2×CH₃); ¹³C NMR (100.6 MHz, CDCl₃): δC 165.1 (C(O)NH), 164.2 (C(O)NH), 149.6 (ArC), 145.7 (ArC), 142.9 (ArC), 138.7 (ArCH), 137.1 (ArC), 137.0 (ArC), 136.8 (ArC), 136.7 (ArC), 136.6 (ArC), 135.4 (ArC), 134.1 (ArC), 132.5 (ArCH), 131.8 (ArC), 131.7 (ArC), 129.6 (ArCH), 129.3 (ArCH), 128.9 (ArCH), 128.1 (ArCH), 127.9 (ArCH), 126.0 (ArCH), 125.4 (ArCH), 124.4 (ArCH), 119.6 (ArCH), 113.2 (ArCH), 73.6 (CH₂), 71.1 (CH₂), 68.7 (CH₂), 48.1 (CH₂), 43.7 (CH₂), 37.5 (CH₂), 37.0 (CH₂), 21.2 (CH₃); ¹⁹F NMR (376.5 MHz, CDCl₃): δF –63.3 (ArCF₃).
N-(4-(4-(3,5-Bis(trifluoromethyl)benzylamino)phenethyl)phenyl)-3,5-dimethylbenzamide Thread 151 \(^{231}\)

Compound 122 (0.2 g, 0.58 mmol) and 3,5-bis(trifluoromethyl)benzaldehyde 148 (0.14 g, 0.53 mmol) were suspended in dry MeCN (10 mL) together with 2 drops of formic acid. The mixture was heated for 3 h at 45 °C before it was allowed to cool at room temperature. NaBH\(_4\) (40 mg, 1.06 mmol) was added neat and the reaction mixture was vigorously stirred for 12 h under positive Ar atmosphere before it was quenched with 5 mL of sat. solution of NH\(_4\)Cl. The organic material was extracted into CH\(_2\)Cl\(_2\) and washed with deionised water, dried (MgSO\(_4\)) and the solvent and volatiles evacuated under reduced pressure to afford the crude. Column chromatography (Cyclohexane:EtOAc, 1:1) was used to purify the product as colorless solid (65 mg, 20\%). M.p. = 153.4 – 156.2 °C; \(^1\)H NMR (400.1 MHz, CDCl\(_3\)) : δ\(_H\) 7.85 (2H, s; 2×ArH), 7.79 (1H, s; ArH), 7.75 (1H, s; NH), 7.53 (2H, d, \(^3\)J\(_{HH}\) 8.5 Hz; 2×ArH), 7.46 (2H, s; 2×ArH), 7.46 (2H, s; ArH), 7.18 (1H, s; ArH), 7.15 (2H, d, \(^3\)J\(_{HH}\) 8.5 Hz; 2×ArH), 6.99 (2H, d, \(^3\)J\(_{HH}\) 8.5 Hz; 2×ArH), 6.54 (2H, d, \(^3\)J\(_{HH}\) 8.5 Hz; 2×ArH), 4.46 (2H, s; CH\(_2\)), 4.13 (1H, br s; NH), 2.84 – 2.83 (4H, m; 2×CH\(_2\)), 2.39 (6H, s; 2×CH\(_3\)); \(^{13}\)C NMR (100.6 MHz, CDCl\(_3\)) : δ\(_C\) 166.1 (ArC), 145.6 (ArC), 142.9 (ArC), 138.6 (ArC), 138.3 (ArC), 136.0 (ArC), 135.2 (ArC), 135.5 (ArCH), 132.1 (ArC), 131.8 (ArC), 129.5 (ArCH), 129.2 (ArCH), 127.4 (ArCH), 124.9 (ArCH), 122.1(ArC), 121.4 (ArCH), 120.2 (ArCH), 113.2 (ArCH), 48.1 (CH\(_2\)), 37.8 (CH\(_2\)), 37.2 (CH\(_2\)), 21.4 (CH\(_3\)); \(^{19}\)F NMR (376.5 MHz, CDCl\(_3\)) : δ\(_F\) –63.3 (ArCF\(_3\)); MS (ES\(^{+}\)) m/z 592.81 ([M+Na]\(^+\), 100); HRMS (ES\(^{+}\)) m/z calculated for C\(_{32}\)H\(_{28}\)N\(_2\)ONaF\(_6\) [M+Na]\(^+\) 593.200, found 593.199.

5-tert-Butylisophthaloyl dichloride\(^{237}\) 153

5-tert-butylisophthalic acid 152 (2.0 g, 9.0 mmol) was suspended in toluene (45 mL) together with thionyl chloride (0.72 mL, 18.0 mmol) and refluxed at 80°C for 18 h. An excess of thionyl chloride was
distilled off by the azeotropic distillation with toluene to furnish dark yellow oil (2.28 g, 98%) as desired product. This product was used in the next step without further purification. \(^1\)H NMR (300.1 MHz, CDCl\(_3\)) : \(\delta\)H 8.69 (1H, t, \(^4J_{HH} 1.6\) Hz; ArH), 8.40 (2H, d, \(^4J_{HH} 1.7\) Hz; 2\times ArH), 1.41 (9H, s; 3\times C\(\text{H}_3\)); \(^{13}\)C NMR (75.5 MHz, CDCl\(_3\)) : \(\delta\)C 167.8 (C\(\text{OCl}\)), 154.1 (ArC), 134.4 (ArC), 134.2 (ArCH), 131.8 (ArCH), 35.5 (qC), 31.1 (CH\(_3\)); MS (ES\(^+\)) m/z 273.1 ([M+H]\(^+\), 100), 477 (20).

**Macrocycle cMDG**

A solution of amine derivative 103 (0.5 g, 1.45 mmol) and 5-\(\text{tert}\)-butylisophthaloyl dichloride 153 (0.38 g, 1.45 mmol) in dry THF (100 mL) were added simultaneously over 1 h to a solution of Et\(_3\)N (1.25 mL, 8.71 mmol) in dry THF (82 mL) and left stirred at ambient temperature under N\(_2\) atmosphere for 3 days. The reaction mixture was concentrated by evaporation under reduced pressure and diluted with CH\(_2\)Cl\(_2\). The solution was then washed successively with 1M HCl and 1M KOH. The organic layers were dried with MgSO\(_4\) and concentrated in vacuo. The residue was purified by column chromatography (Hexane:EtOAc, 1:1) to yield macrocycle cMDG as a colorless solid (81 mg, 11%). M.p. = 247.8 – 250.4 °C; \(^1\)H NMR (400.1 MHz, CDCl\(_3\)) : \(\delta\)H 8.10 (2H, d, \(^3J_{HH} 1.5\) Hz; 2\times NH), 7.53 (1H, br s; ArH), 7.30 (8H, s; 8\times ArH), 6.43 (2H, br s; 2\times ArH), 4.57 (4H, d, \(^3J_{HH} 5.4\) Hz; 2\times CH\(_2\)), 4.50 (4H, s; 2\times CH\(_2\)), 3.58 – 3.68 (8H, m; 4\times CH\(_2\)), 1.37 (9H, s; 3\times C\(\text{H}_3\)); \(^{13}\)C NMR (75.5 MHz, CDCl\(_3\)) : \(\delta\)C 167.3 (C=O), 153.2 (ArC), 137.8 (ArC), 137.4 (ArC), 134.7 (ArC), 128.8 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 120.6 (ArC), 73.1 (CH\(_2\)), 70.7 (CH\(_2\)), 69.6 (CH\(_2\)), 44.3 (CH\(_2\)), 35.3 (qC), 31.3 (CH\(_3\)); MS (ES\(^+\)) m/z 553.1 ([M+Na]\(^+\), 100) 554.2 (30); HRMS (ES\(^+\)) m/z calculated for C\(_{39}\)H\(_{38}\)N\(_2\)O\(_5\)Na [M+Na]\(^+\) 553.2678, found 553.2664.
Macrocycle cMP

A solution of amine derivative 114 (0.6 g, 1.59 mmol) and 5-\textit{ tert-}butylisophthaloyl dichloride 153 (0.42 g, 1.59 mmol) in dry THF (100 mL) were added simultaneously over 2 h to a solution of Et\textsubscript{3}N (1.3 mL, 9.36 mmol) in dry THF (95 mL) and left stirred at ambient temperature under N\textsubscript{2} atmosphere for 3 days. The reaction mixture was concentrated \textit{in vacuo} and diluted with CH\textsubscript{2}Cl\textsubscript{2}. The solution was then washed successively with 1M HCl and 1M KOH. The organic layers were dried with MgSO\textsubscript{4} and concentrated \textit{in vacuo}. The residue was purified by column chromatography (Hexane:EtOAc, 1:1) to yield macrocycle cMP as a colorless solid (100 mg, 11%). M.p. = 256.3 – 258.9 °C; \textsuperscript{1}H NMR (500.1 MHz, CDCl\textsubscript{3}) : \textit{\delta}H 8.07 (2H, d, \textit{\textit{\delta}}J\textsubscript{HH} 1.4 Hz; 2×NH\textsubscript{2}), 7.71 (1H, t, \textit{\textit{\delta}}J\textsubscript{HH} 7.8 Hz; Ar\textit{H}), 7.57 (1H, s; Ar\textit{H}), 7.36 (2H, d, \textit{\textit{\delta}}J\textsubscript{HH} 7.8 Hz; 2×Ar\textit{H}), 7.31 (8H, s; 8×Ar\textit{H}), 6.39 (2H, br s; 2×Ar\textit{H}), 4.65 (4H, s; 2×CH\textsubscript{2}), 4.58 (4H, d, \textit{\textit{\delta}}J\textsubscript{HH} 5.5 Hz; 2×CH\textsubscript{2}), 4.42 (4H, s; 2×CH\textsubscript{2}), 1.37 (9H, s; 3×CH\textsubscript{3}); \textsuperscript{13}C NMR (100.6 MHz, CDCl\textsubscript{3}) : \textit{\delta}C 167.3 (C=O), 157.4 (ArC), 152.9 (ArC), 137.6 (ArC), 137.3 (ArCH), 137.2 (ArC), 134.6 (ArC), 129.0 (ArCH), 128.7 (ArCH), 128.1 (ArCH), 120.8 (ArCH), 120.3 (ArCH), 72.2 (CH\textsubscript{2}), 71.5 (CH\textsubscript{2}), 44.3 (CH\textsubscript{2}), 35.3 (q), 31.2 (CH\textsubscript{3}); MS (ES\textsuperscript{+}) \textit{m/z} 586.1 ([M+Na]\textsuperscript{+}, 100); HRMS (ES\textsuperscript{+}) \textit{m/z} calculated for C\textsubscript{35}H\textsubscript{37}N\textsubscript{3}O\textsubscript{4}Na [M+Na]\textsuperscript{+} 586.2682, found 586.2684.

5-Iodoisophthalic acid\textsuperscript{271} 155

5-aminoisophthalic acid 154 (3.50 g, 19.3 mmol) was suspended in 20 mL of H\textsubscript{2}O. The suspension was cooled down to 0 °C. HCl conc. (18 mL) was added dropwise followed with the solution of NaNO\textsubscript{2} (1.40 g, 20.3 mmol). The resulting solution was stirred for 1 h at 0 °C. Potassium iodide (12.74 g, 77.2 mmol) was added neat in small portions. The reaction mixture was allowed to warm to ambient temperature and stirred for 18 h. The resulting precipitate was collected by filtration and washed with H\textsubscript{2}O. Solid was tritritated with CH\textsubscript{3}OH:Hexane to give the desired product as pale orange solid (2.37 g,
5-Iodoisophthaloyl dichloride

A solution of 5-iodoisophthalic acid 155 (0.40 g, 1.37 mmol) in thionyl chloride (8 mL, 17.0 mmol) and DMF (3-5 drops) was refluxed at 100 °C for 48 h under dry condition with subsequent removal of the excess of thionyl chloride in vacuo to yield red brownish oil (0.44 g, 98%). This compound was used in the next step without further purification. 

**Iodophenyl glycol macrocycle 157**

A solution of amine derivatives 103 (0.9 g, 2.58 mmol) and 5-iodoisophthaloyl dichloride 156 (0.85 g, 2.58 mmol) in dry THF (200 mL) were added simultaneously over 1.5 h to a solution of Et3N (2.2 mL, 16.0 mmol) in dry THF (82 mL) and stirred at ambient temperature under N2 atmosphere for 3 days. The reaction mixture was concentrated by evaporation under reduced pressure and diluted with CH2Cl2. The solution was then washed successively with 1M HCl and 1M KOH solution. The organic layers were dried with MgSO4 and concentrated in vacuo. The residue was purified by column chromatography (CHCl3:EtOAc, 1:1) to yield macrocycle 157 as a pale yellow solid (110 mg, 21%). M.p. = 269.7–271.7°C; 1H NMR (300.1 MHz, CDCl3) : δH 8.25 (2H, s; 2×NH), 7.67 (1H, s; ArH), 7.30 (8H, s; 8×ArH), 6.57 (2H, t, 3Jih 3.7 Hz; 2×ArH), 4.55 (4H, d, 3Jih 5.3 Hz; 2×CH2), 4.49 (4H, s; 2×CH2), 3.56 – 3.67 (8H, m; 4×CH2); 13C NMR (75.5 MHz, CDCl3) : δC 165.2 (C=O), 140.2 (ArCH), 137.2 (ArC), 136.9 (ArC), 135.7 (ArC), 128.8 (ArCH), 128.7 (ArCH), 122.8 (ArCH), 95.0 (ArC-I), 73.5 (CH2), 70.4 (CH2), 70.1 (CH2), 44.5 (CH2); MS (ES+) m/z 622.9 ([M+Na]+, 100);
HRMS (ES⁺) m/z calculated for C_{28}H_{29}N_{2}O_{5}NaI [M+Na]^+ 623.1019, found 623.1013.

Iodophenyl pyridine macrocycle 158

A solution of amine derivative 114 (0.7 g, 1.85 mmol) and 5-iodoisophthaloyl dichloride 156 (0.61 g, 1.85 mmol) in dry THF (100 mL) were added simultaneously over 2 h to a solution of Et₃N (1.6 mL, 11.1 mmol) in dry THF (82 mL) and left stirred at ambient temperature under N₂ atmosphere for 3 days. The reaction mixture was concentrated in vacuo and diluted with CH₂Cl₂. The solution was then washed successively with 1M HCl and sat. solution of NaHCO₃. The organic layers were dried with MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (CHCl₃:EtOAc, 1:1) to yield macrocycle 158 as a colorless solid (210 mg, 18%). M.p. = 248.7 – 250.5 °C; ¹H NMR (300.1 MHz, CDCl₃ + 3 drops of d₆-DMSO) : δ H 8.44 (2H, d, ³J_HH 1.4 Hz; 2xArH), 8.24 (1H, s; ArH), 7.94 (2H, t, ³J_HH 5.4 Hz; 2xNH), 7.65 (1H, t, ³J_HH 7.6 Hz; ArH), 7.27 (2H, d, ³J_HH 6.0 Hz; 2xArH), 7.23 (8H, s; 8xArH), 4.59 (4H, d, ³J_HH 5.3 Hz; 2xC₂H₂), 4.52 (4H, s; 2xC₂H₂), 4.30 (4H, s; 2xC₂H₂); ¹³C NMR (100.6 MHz, CDCl₃ + 3 drops of d₆-DMSO) : δ C 164.7 (C=O), 139.9 (ArCH), 138.1 (ArC), 136.7 (ArC), 135.2 (ArC), 130.1 (ArCH), 129.2 (ArCH), 128.5 (ArCH), 126.7 (ArCH), 123.4 (ArCH), 120.8 (ArCH), 94.2 (ArC-I), 72.4 (CH₂), 71.4 (CH₂), 43.7 (CH₂); MS (ES⁺) m/z 656 ([M+Na]^+), 100; HRMS (ES⁺) m/z calculated for C₃₁H₃₈N₄O₄NaI [M+Na]^+ 656.1027, found 656.1027.

5-Bromoisophthaloyl dichloride 160

A solution of 5-bromoisophthalic acid 159 (0.5 g, 2.04 mmol) in thionyl chloride (10 mL, 20.0 mmol) and DMF (3-5 drops) was refluxed at 100 °C for 6 h with subsequent removal of the excess of thionyl chloride in vacuo. The residue was dried under high vacuum to furnish a brownish oil (0.56 g, 98%). The acid chloride 160 was used in the next step without further purification. ¹H NMR (400.1 MHz, CDCl₃) : δ H 8.76 (1H, t, ⁴J_HH 1.6 Hz; ArH), 8.52 (2H, d, ⁴J_HH 1.6 Hz; 2xArH); ¹³C NMR (100.6 MHz, CDCl₃) : δ C 166.2 (COCl), 139.6 (ArCH), 135.8 (ArC), 132.0 (ArCH), 124.0 (ArC-Br).
Bromophenyl glycol macrocycle 161

A solution of 5-bromoisophthaloyl dichloride 160 (0.5 g, 1.77 mmol) in dry THF (60 mL) was added simultaneously with a solution of amine derivatives 103 (0.61 g, 1.77 mmol) in dry THF (60 mL) to a solution of Et$_3$N (1.5 mL, 11.0 mmol) in dry THF (130 mL) over 1 h under a N$_2$ atmosphere. The reaction mixture was stirred at ambient temperature for 3 days and washed successively with 1M HCl and sat. solution of NaHCO$_3$. The organic layers were dried (MgSO$_4$) and the solvent was removed by evaporation in vacuo. The residue redissolved minimally in CH$_2$Cl$_2$ and purified by column chromatography (CHCl$_3$:EtOAc, 1:1) to afford macrocycle 161 (70 mg, 8%) as a colourless solid. M.p. = 219.5 – 223.7 °C; $^1$H NMR (400.1 MHz, CDCl$_3$): δ$_H$ 8.18 (2H, d, 4J$_{HH}$ 1.4 Hz; 2×ArH), 8.12 (1H, t, 4J$_{HH}$ 1.4 Hz; ArH), 7.87 (2H, br s; 2×NH), 7.22 – 7.16 (8H, m; 8×ArH), 4.48 (4H, d, 3J$_{HH}$ 4.8 Hz; 2×CH$_2$), 4.29 (4H, s; 2×CH$_2$), 3.42 – 3.43 (8H, m; 4×CH$_2$); $^{13}$C NMR (100.6 MHz, CDCl$_3$): δ$_C$ 164.9 (C=O), 137.5 (ArC), 137.2 (ArC), 135.5 (ArC), 134.2 (ArCH), 129.3 (ArCH), 128.9 (ArCH), 123.1 (ArC), 122.6 (ArCH), 73.1 (CH$_2$), 70.2 (CH$_2$), 69.1 (CH$_2$), 44.1 (CH$_2$); MS (ES$^+$) m/z 577 ([M+Na]$^+$, 100); HRMS (ES$^+$) m/z calculated for C$_{28}$H$_{29}$N$_2$O$_5$Na$_7^+$Br [M+Na]$^+$ 575.1158, found 575.1163.

Bromophenyl pyridine macrocycle 162

A solution of 5-bromoisophthaloyl dichloride 160 (1.15 g, 4.09 mmol) in dry THF (100 mL) was added simultaneously with a solution of amine derivative 114 (1.55 g, 4.09 mmol) in dry THF (100 mL) to a solution of Et$_3$N (1.72 mL, 25.0 mmol) in dry THF (140 mL) over 1 h under a N$_2$ atmosphere. The reaction mixture was left stirred at ambient temperature for 3 days, and then washed successively with 1M HCl and sat. solution of NaHCO$_3$. The organic layers were dried (MgSO$_4$) and the solvent was removed by evaporation under reduced pressure. The residue was redissolved
minimally in CH$_2$Cl$_2$ and purified by column chromatography (CHCl$_3$:EtOAc, 1:1) to afford macrocycle 162 (190 mg, 8%) as a colourless solid. M.p. = 219.5 – 223.7 °C; $^1$H NMR (300.1 MHz, CDCl$_3$): $\delta_H$ 8.23 (2H, s; 2×ArH), 8.18 (1H, s; ArH), 7.94 (2H, br t; 2×NH), 7.63 (1H, t, $^3$J$_{HH}$ 7.7 Hz; ArH), 7.25 (2H, d, $^3$J$_{HH}$ 7.8 Hz; 2×ArH), 7.22 (8H, s; 8×ArH), 4.53 (4H, d, $^3$J$_{HH}$ 5.1 Hz; 2×C$_2$H$_2$), 4.51 (4H, s; 2×C$_2$H$_2$), 4.28 (4H, s; 2×C$_2$H$_2$); $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta_C$ 165.0 (C=O), 156.9 (ArC), 138.2 (ArC), 137.0 (ArC), 135.5 (ArC), 129.8 (ArC), 129.4 (ArC), 128.7 (ArC), 123.1 (ArC-Br), 122.7 (ArC), 120.9 (ArC), 72.6 (CH$_2$), 71.7 (CH$_2$), 43.9 (CH$_2$); MS (ES$^+$) m/z 609 ([M+Na]$^+$, 75), 607 (100); HRMS (ES$^+$) m/z calculated for C$_{31}$H$_{28}$N$_3$O$_4$Na$^{79}$Br [M+Na]$^+$ 608.1161, found 608.1154 and C$_{31}$H$_{28}$N$_3$O$_4$Na$^{81}$Br [M+Na]$^+$ 610.1140, found 610.1155.

**tert-Butyl 4-bromobenzoate 164**

A solution of 4-bromobenzoyl chloride 163 (4.68 g, 21.3 mmol) in dry CH$_2$Cl$_2$ (25 mL) was added dropwise to a stirred mixture of tert-butyl alcohol (3.49 g, 47.1 mmol) and dry pyridine (3.5 mL) in dry CH$_2$Cl$_2$ (15 mL). The reaction mixture was stirred at ambient temperature under a N$_2$ atmosphere for 48 h and then diluted with CH$_2$Cl$_2$. The organic phase was washed successively with 1M HCl, water and sat. solution of NaCl, dried (MgSO$_4$) and concentrated by evaporation under reduced pressure. The residue was taken up in cyclohexane and filtered off. The filtrate was concentrated by evaporation under reduced pressure to afford 164 (5.0 g, 91%) as a pale yellow oil and was used without further purification. $^1$H NMR (400.1 MHz, CDCl$_3$): $\delta_H$ 7.85 – 7.82 (2H, m; 2×ArH), 7.56 – 7.52 (2H, m; 2×ArH), 1.58 (9H, s; 3×CH$_3$); $^{13}$C NMR (100.6 MHz, CDCl$_3$): $\delta_C$ 165.1 (C=O), 131.6 (ArCH), 131.1 (ArCH), 131.0 (ArC), 127.5 (ArC), 81.6 (qC), 28.3 (CH$_3$).

**tert-Butyl 4-(3-hydroxy-3-methylbut-1-yn-1-yl)benzoate 165**

A degassed (by bubbling Ar for 45 min) solution of 2,2-dimethylpropargyl alcohol (2.90 mL, 29.2 mmol) in Et$_3$N (50 mL) was cannulated into a degassed (by bubbling Ar for 45 min) suspension of compound 164 (5.0 g, 19.4 mmol), PPh$_3$ (0.51
g, 1.94 mmol), CuI (180 mg, 0.97 mmol), PdCl$_2$(PPh$_3$)$_2$ (68 mg, 0.97 mmol), Et$_3$N (50 mL) and dry THF (20 mL). The reaction mixture was heated at 70 °C under an Ar atmosphere for 48 h, and then concentrated by evaporation in vacuo. The residue was taken up in CH$_2$Cl$_2$. The organic phase was washed with water, dried (MgSO$_4$) and concentrated by evaporation in vacuo. The residue was purified by column chromatography (Cyclohexane:EtOAc, 7:3) to give compound 165 as brown solid (4.40 g, 87%). M.p. = 66.8 – 68.6 °C; $^1$H NMR (400.1 MHz, CDCl$_3$): $\delta$H 7.93 – 7.90 (2H, m; 2×ArH), 7.45 – 7.42 (2H, m; 2×ArH), 2.20 (1H, br s, OH), 1.63 (6H, s; 2×CH$_3$), 1.59 (9H, s; 3×C$_3$H$_7$); $^{13}$C NMR (100.6 MHz, CDCl$_3$): $\delta$C 165.3 (C=O), 131.6 (ArC), 131.5 (ArC), 129.4 (ArCH), 127.0 (ArC), 96.5 (qC), 81.7 (qC), 81.5 (qC), 65.8 (qC), 31.5 (CH$_3$), 28.3 (CH$_3$); MS (ES$^+$) m/z 283 ([M+Na]$^+$, 100); HRMS (ES$^+$) m/z calculated for C$_{16}$H$_{20}$O$_3$Na [M+Na]$^+$ 283.1310, found 283.1307.

**tert-Butyl 4-ethynylbenzoate 166**$^{244,272}$

A solution of compound 165 (2.0 g, 7.68 mmol) in toluene (70 mL) was treated with powdered NaOH (0.61 g, 15.0 mmol). The reaction mixture was heated to reflux for 3 h under a N$_2$ atmosphere, and then concentrated by evaporation under reduced pressure. The residue was taken up in CHCl$_3$. The organic phase was washed with water, dried (MgSO$_4$) and concentrated by evaporation under reduced pressure. The residue was purified by column chromatography (Cyclohexane:EtOAc, 19:1) to give terminal alkyne 166 (1.07 g, 69%) as a yellow oil. $^1$H NMR (400.1 MHz, CDCl$_3$): $\delta$H 7.95 – 7.92 (2H, m; 2×ArH), 7.54 – 7.51 (2H, m; 2×ArH), 3.14 (1H, s; CH), 1.52 (9H, s; 3×CH$_3$); $^{13}$C NMR (100.6 MHz, CDCl$_3$): $\delta$C 165.2 (C=O), 132.2 (ArC), 132.0 (ArCH), 129.4 (ArCH), 126.3 (ArC), 83.1 (qC), 81.5 (qC), 79.8 (qC), 28.3 (CH$_3$); MS (ES$^+$) m/z 225 ([M+Na]$^+$, 100); HRMS (ES$^+$) m/z calculated for C$_{13}$H$_{14}$O$_2$Na [M+Na]$^+$ 225.0891, found 225.0892.
A degassed (by bubbling Ar) solution of 166 (62 mg, 0.30 mmol) in Et$_3$N (5 mL) was cannulated into a degassed (by bubbling Ar) suspension of macrocycle 157 (122 mg, 0.23 mmol), PPh$_3$ (53 mg, 0.02 mmol), Cul (27 mg, 0.014 mmol), PdCl$_2$(PPh$_3$)$_2$ (10 mg, 0.014 mmol) and Et$_3$N (5 mL) in dry THF (10 mL). The reaction mixture was heated at 60 °C under an Ar atmosphere for 42 h, and then filtered through a pad of Celite. The filtrate was concentrated by evaporation in vacuo. The residue was dissolved in CHCl$_3$. The organic layers were washed with water, dried (MgSO$_4$) and concentrated by evaporation in vacuo. The residue was purified by column chromatography (CHCl$_3$:EtOAc, 1:1) to give macrocycle 167 as a yellow solid (140 mg, 90%). M.p. = 197.0 – 200.5 °C; $^1$H NMR (400.1 MHz, CDCl$_3$) : $\delta$H 8.19 (2H, d, $^4$J$_{HH}$ 1.5 Hz; 2×ArH), 7.97 (2H, d, $^3$J$_{HH}$ 8.6 Hz; 2×ArH), 7.75 (1H, br s, ArH), 7.56 (2H, d, $^3$J$_{HH}$ 8.6 Hz; 2×ArH), 7.31 – 7.27 (8H, m; 8×ArH), 6.71 (2H, br s; 2×NH), 4.54 (4H, d, $^3$J$_{HH}$ 5.3 Hz; 2×CH$_2$), 4.48 (4H, s; 2×CH$_2$), 3.66 – 3.59 (8H, m; 4×CH$_2$), 1.60 (9H, s; 3×CH$_3$); $^{13}$C NMR (75.5 MHz, CDCl$_3$) : $\delta$C 166.0 (C=O), 165.2 (C=O), 137.6 (ArC), 137.2 (ArC), 135.2 (ArC), 133.9 (ArCH), 132.0 (ArC), 131.7 (ArCH), , 129.6 (ArCH), 128.8 (ArCH), 128.7 (ArCH), 126.8 (ArC), 124.7 (ArC), 123.6 (ArCH), 90.9 (qC), 90.2 (qC), 81.5 (qC), 73.1 (CH$_2$), 70.5 (CH$_2$), 69.8 (CH$_2$), 44.5 (CH$_2$), 28.3 (CH$_3$); MS (ES$^+$) m/z 697 ([M+Na]$^+$, 100); HRMS (ES$^+$) m/z calculated for C$_{41}$H$_{42}$N$_2$O$_7$Na [M+Na]$^+$ 697.2890, found 697.2897.
To a solution of ester macrocycle 167 (0.27 g, 0.40 mmol), in CH$_2$Cl$_2$ (15 mL) was added at 0 °C trifluoroacetic acid in excess. Triethylsilane (0.13 mL, 0.80 mmol) was added dropwise into the resulting solution. The reaction mixture was left stirred at ambient temperature for 16 h. The solvent was reduced in vacuo, diluted with CH$_2$Cl$_2$ and washed with deionised H$_2$O. The aqueous layer was made acidic to pH 1 and extracted into CH$_2$Cl$_2$. The organic layers were dried over MgSO$_4$ and evaporated. The crude product was additionally purified by flash chromatography (EtOAc:Cyclohexane, 2:1) to yield the desired compound as yellow solid (100 mg, 51%). M.p. = 320.8 °C (decomposed); $^1$H NMR (500.1 MHz, CDCl$_3$ + 3 drops of d$_6$-DMSO): $\delta_\text{H}$ 8.23 (2H, s; 2×ArH), 8.18 (1H, br s; ArH), 7.94 (2H, d, $^3$J$_{\text{HH}}$ 8.3 Hz; 2×ArH), 7.91 (2H, t, $^3$J$_{\text{HH}}$ 4.7 Hz; 2×NH), 7.49 (2H, d, $^3$J$_{\text{HH}}$ 8.3 Hz; 2×ArH), 7.22 (8H, m; 8×ArH), 4.52 (4H, d, $^3$J$_{\text{HH}}$ 4.8 Hz; 2×CH$_2$), 4.32 (4H, s; 2×CH$_2$), 3.46 (8H, s; 4×CH$_2$); $^{13}$C NMR (100.6 MHz, CDCl$_3$ + 3 drops of d$_6$-DMSO): $\delta_\text{C}$ 167.6 (C=O), 165.6 (C=O), 137.8 (ArC), 137.2 (ArC), 134.4 (ArCH), 134.1 (ArC), 131.4 (ArCH), 130.5 (ArC), 129.8 (ArCH), 129.4 (ArCH), 128.8 (ArCH), 126.9 (ArC), 124.2 (ArCH), 123.7 (ArC), 90.6 (qC), 89.8 (qC), 73.3 (CH$_2$), 70.6 (CH$_2$), 69.2 (CH$_2$), 44.2 (CH$_2$); MS (ES$^+$) m/z 641.0 ([M+Na]$^+$, 100); HRMS (ES$^+$) m/z calculated for C$_{37}$H$_{34}$N$_2$O$_7$Na [M+Na]$^+$ 641.2264, found 641.2247.
A degassed (by bubbling Ar) solution of alkyne 166 (0.11 g, 0.55 mmol) in Et₃N (35 mL) was cannulated into a degassed (by bubbling Ar) suspension of macrocycle 158 (0.32 g, 0.50 mmol), PPh₃ (24 mg, 0.09 mmol), Cul (13 mg, 0.07 mmol), PdCl₂(PPh₃)₂ (35 mg, 0.05 mmol) and Et₃N (3 mL) in dry THF (6 mL). The reaction mixture was heated to 65 ºC under an Ar atmosphere for 48 h, and then filtered through a pad of Celite. The filtrate was concentrated by evaporation in vacuo. The residue was dissolved in CHCl₃. The organic layer was washed with water, dried (MgSO₄) and concentrated by evaporation in vacuo. The residue was purified by column chromatography (CHCl₃:EtOAc, 1:1) to give a colorless solid (0.20 g, 56%). M.p. = 255.2 ºC (decomposed); ¹H NMR (400.1 MHz, CDCl₃) : δH 8.19 (2H, br s; 2×ArH), 7.99 (2H, d, ³JHH 8.6 Hz; 2×ArH), 7.85 (1H, br s; ArH), 7.74 (1H, t, ³JHH 7.5 Hz; ArH), 7.58 (2H, d, ³JHH 8.4 Hz; 2×ArH), 7.30 (8H, s; 8×ArH), 6.53 (2H, br s; 2×NH), 4.64 (4H, s; 2×CH₂), 4.57 (4H, d, ³JHH 5.8 Hz; 2×CH₂), 4.40 (4H, s; 2×CH₂). ¹³C NMR (100.6 MHz, CDCl₃) : δC 165.9 (C=O), 165.2 (C=O), 157.5 (ArC), 137.6 (ArC), 137.4 (ArCH), 137.3 (ArC), 135.3 (ArC), 133.6 (ArCH), 132.0 (ArC), 131.6 (ArCH), 129.5 (ArCH), 129.0 (ArCH), 128.8 (ArCH), 126.7 (ArC), 124.4 (ArC), 124.1 (ArC), 120.2 (ArCH), 90.8 (qC), 90.2 (qC), 81.6 (qC), 72.4 (CH₂), 71.6 (CH₂), 44.4 (CH₂), 28.3 (CH₃); MS (ES⁺) m/z 730 ([M+Na]⁺, 100); HRMS (ES⁺) m/z calculated for C₄₄H₄₁N₃O₆Na [M+Na]⁺ 730.2893, found 730.2877.
Macrocycle AEMP

To a solution of ester macrocycle 168 (0.28 g, 0.39 mmol) in CH2Cl2 (15 mL) was added at 0 °C trifluoroacetic acid in excess. Triethylsilane (0.12 mL, 0.77 mmol) was added dropwise into the resulting mixture. The resulting solution was left stirred at ambient temperature for 16 h. The solvent was reduced in vacuo, diluted with CH2Cl2 and washed with deionised H2O. The aqueous layer was adjusted to pH 1 and extracted into CH2Cl2. The organic layers were dried over MgSO4 and evaporated. The crude product was additionally purified by flash chromatography (EtOAc:Cyclohexane, 2:1) to yield the desired compound as colourless solid (160 mg, 63%). M.p. = 294.5 °C (decomposed); 1H NMR (500.1 MHz, CDCl3 + 3 drops of d6-DMSO) : δH 8.23 (2H, s; 2×ArH), 8.19 (1H, s; ArH), 7.98 (2H, t, 3JHH 4.6 Hz; 2×NH), 7.92 (2H, d, 3JHH 8.3 Hz; 2×ArH), 7.60 (1H, t, 3JHH 7.7 Hz, ArH), 7.47 (2H, d, 3JHH 8.3 Hz; 2×ArH), 7.24 – 7.19 (10H, m; 10×ArH), 4.53 (4H, d, 3JHH 5.1 Hz; 2×CH2), 4.49 (4H, s; 2×CH2), 4.26 (4H, s; 2×CH2); 13C NMR (100.6 MHz, CDCl3+ 3 drops of d6-DMSO): δC 167.6 (C=O), 165.4 (C=O), 156.9 (ArC), 138.2 (ArC), 137.2 (ArCH), 136.8 (ArC), 134.3 (ArCH), 134.1 (ArC), 131.6 (ArC), 131.3 (ArCH), 130.4 (ArC), 129.7 (ArCH), 129.3 (ArCH), 128.9 (ArCH), 126.8 (ArC), 124.0 (ArCH), 120.8 (ArCH), 90.6 (qC), 89.7 (qC), 72.4 (CH2), 71.6 (CH2), 43.9 (CH2). MS (ES⁻) m/z 650 ([M–H]⁻, 100); HRMS (ES⁻) m/z calculated for C40H32N3O6 [M–H]⁻ 650.2291, found 650.2290.

The 4-(methoxycarbonyl)phenylboronic acid (33 mg, 0.18 mmol) and potassium carbonate (37 mg, 0.27 mmol) were dissolved in the mixture of THF and H₂O (10 mL). Following this, PdCl₂(PPh₃)₂ (7 mg, 0.009 mmol) was added together with macrocycle 161 (50 mg, 0.09 mmol), all under the Ar atmosphere. The reaction mixture was left stirred for 16 h. The reaction was filtered through a pad of Celite and the solvent was then removed in vacuo. The crude was washed with water and extracted into CHCl₃, dried (MgSO₄), filtered, and concentrated before column chromatography was used to purify to give the product as colorless solid (48 mg, 87%). M.p. = 275.6 – 279.6 °C; ¹H NMR (400.1 MHz, CDCl₃) : δ H 8.28 (2H, s; 2×ArH), 8.05 (2H, d, 3 Jₕₕ 8.5 Hz; 2×ArH), 7.86 (1H, s; ArH), 7.66 (2H, d, 3 Jₕₕ 8.4 Hz; 2×ArH), 7.24 (8H, s; 8×ArH), 6.86 (2H, br s; 2×NH₂), 4.52 (4H, d, 3 Jₕₕ 5.3 Hz; 2×CH₂), 4.45 (4H, s; 2×CH₂), 3.86 (3H, s; CH₃), 3.67 – 3.57 (8H, m; 4×CH₂); ¹³C NMR (100.6 MHz, CDCl₃) : δ C 167.0 (C=O), 166.6 (C=O), 143.6 (ArC), 141.4 (ArC), 137.8 (ArC), 137.2 (ArC), 135.5 (ArC), 130.4 (ArCH), 130.1 (ArCH), 129.9 (ArCH), 129.8 (ArC), 128.7 (ArCH), 127.4 (ArCH), 123.4 (ArCH), 73.2 (CH₂), 70.8 (CH₂), 69.7 (CH₂), 52.4 (CH₃), 44.5 (CH₂); MS (ES⁺) m/z 631 ([M+Na]⁺, 100); HRMS (ES⁺) m/z calculated for C₉₆H₇₆N₂O₇Na [M+Na]⁺ 631.2420, found 631.2410.
Macrocycle APMDG

To a suspension of ester macrocycle 169 (0.15 g, 0.24 mmol) in dry THF (10 mL), potassium trimethyl silanolate (37 mg, 0.24 mmol) was added. The reaction mixture was refluxed at 75 °C for 16 h. After the resulting solution was evaporated to dryness, distilled water was added to residue. HCl conc. was added dropwise until the pH reached 3.0. The suspension was then filtered and the residue was washed with water, and dried under high vacuum to a constant mass to give the product as colourless solid (100 mg, 70%). M.p. = 292.6 – 295.8 °C; 1H NMR (500.1 MHz, CDCl₃ + 3 drops of d₆-DMSO): δ H 8.35 (2H, s; 2×ArH); 8.19 (1H, s; ArH), 8.02 (2H, d, 3JHH 8.4 Hz; 2×ArH), 7.94 (2H, t, 3JHH 4.8 Hz; 2×N̈H), 7.66 (2H, d, 3JHH 8.4 Hz; 2×ArH), 7.24 – 7.18 (8H, m; 8×ArH), 4.53 (4H, d, 3JHH 4.7 Hz; 2×CH₂), 4.30 (4H, s; 2×CH₂), 3.45 (8H, s; 4×CH₂); 13C NMR (100.6 MHz, CDCl₃ + 3 drops of d₆-DMSO): δ C 168.1 (C=O), 166.2 (C=O), 143.5 (ArC), 140.4 (ArC), 137.8 (ArC), 137.1 (ArC), 134.4 (ArC), 130.2 (ArC), 130.1 (ArCH), 130.1 (ArCH), 129.3 (ArCH), 128.6 (ArCH), 126.8 (ArCH), 123.6 (ArCH), 73.3 (CH₂), 70.4 (CH₂), 69.2 (CH₂), 44.1 (CH₂); MS (ES⁺) m/z 617 ([M+Na]⁺, 100); HRMS (ES⁺) m/z calculated for C₃₅H₃₄N₂O₇Na [M+Na]⁺ 617.2264, found 617.2254.

Macrocycle 170

The 4-(methoxycarbonyl)phenylboronic acid (36 mg, 0.20 mmol) and potassium carbonate (41 mg, 0.30 mmol) were dissolved in the mixture of THF and H₂O (10 mL). Following this, PdCl₂(PPh₃)₂ (7 mg, 0.009 mmol) was added together with macrocycle 162 (53 mg, 0.09 mmol) all under the Ar atmosphere. The reaction mixture was left stirred for 16 h. TLC showed consumption of the starting materials and so the reaction was filtered through a pad of Celite and the solvent was
then removed in vacuo. The crude material was washed with water and extracted into CHCl₃, dried (MgSO₄), filtered, and concentrated before column chromatography was used to purify to give the product as colorless solid (45 mg, 78%). M.p. = 255.7 – 259.7 °C; ¹H NMR (400.1 MHz, CDCl₃) : δH 8.31 (2H, d, 3JH-H 1.5 Hz; 2xArH), 8.13 (2H, d, 3JH-H 8.6 Hz; 2xArH), 7.84 (1H, br s; ArH), 7.77 (1H, t, 3JH-H 7.8 Hz; ArH), 7.72 (2H, d, 3JH-H 8.6 Hz; 2xArH), 7.39 (2H, d, 3JH-H 7.7 Hz; 2xArH), 7.31 (8H, s; 8xArH), 6.58 (2H, br s; 2xNH), 4.65 (4H, s; 2xCH₂), 4.60 (4H, d, 3JH-H 5.5 Hz; 2xCH₂), 4.43 (4H, s; 2xCH₂), 3.95 (3H, s; CH₃); ¹³C NMR (100.6 MHz, CDCl₃) : δC 167.0 (C=O), 166.5 (C=O), 157.3 (ArC), 143.7 (ArC), 141.3 (ArC), 137.8 (ArCH), 137.6 (ArC), 137.2 (ArC), 135.6 (ArC), 130.0 (ArCH), 129.9 (ArCH), 129.8 (ArC), 129.4 (ArCH), 128.8 (ArCH), 127.2 (ArCH), 123.3 (ArCH), 120.4 (ArCH), 72.2 (CH₂), 71.0 (CH₂), 52.1 (CH₃), 44.2 (CH₂); MS (ES⁺) m/z 664 ([M+Na]⁺, 15), 301 (100); HRMS (ES⁺) m/z calculated for C₉₈H₅₃N₃O₆Na [M+Na]⁺ 664.2424, found 664.2429.

**Macrocycle APMP**

To a suspension of ester macrocycle 170 (0.30 g, 0.47 mmol) in dry THF (10 mL), potassium trimethyl silanolate (72 mg, 0.56 mmol) was added. The reaction mixture was refluxed at 75 °C for 16 h. After the resulting solution was evaporated to dryness, distilled water was added to residue. HCl conc. was added dropwise until the pH reached 3.0. The suspension was filtered and the residue was washed with water, and dried under high vacuum to a constant mass to give the product as colourless solid (0.22 g, 76%). M.p. = 312.8 – 315.4 °C; ¹H NMR (500.1 MHz, CDCl₃ + 3 drops of d₆-DMSO): δH 8.39 (2H, d, 3JH-H 1.3 Hz; 2xArH), 8.23 (1H, s; ArH), 8.03 (2H, d, 3JH-H 8.4 Hz; 2xArH), 7.99 (2H, t, 3JH-H 5.1 Hz; 2xNH), 7.68 (2H, d, 3JH-H 8.4 Hz; 2xArH), 7.61 (1H, t, 3JH-H 7.6 Hz; ArH), 7.25 – 7.21 (10H, m; 10xArH), 4.57 (4H, d, 3JH-H 5.1 Hz; 2xCH₂), 4.50 (4H, s; 2xCH₂), 4.28 (4H, s; 2xCH₂); ¹³C NMR (100.6 MHz, CDCl₃ + 3 drops of d₆-DMSO): δC 168.2 (C=O), 166.1 (C=O), 156.9 (ArC), 143.6 (ArC), 140.6 (ArC), 138.4 (ArC), 137.2 (ArCH), 136.9 (ArC), 134.4 (ArC), 130.2 (ArCH), 130.2 (ArC), 130.0 (ArCH), 129.3 (ArCH), 128.6 (ArCH), 126.9 (ArCH), 123.5 (ArCH), 120.7 (ArCH), 72.4 (CH₂), 71.7 (CH₂), 43.9 (CH₂); MS (ES⁺) m/z 628 ([M+H]⁺, 100); HRMS (ES⁺) m/z calculated for C₉₈H₅₃N₃O₆Na [M+Na]⁺ 664.2424, found 664.2429.
M+H]\(^+\) 628.2448, found 628.2452.

1,3-Di-tert-butyl-5-(2,2-dibromovinyl)benzene 172\(^{273}\)

PPh\(_3\) (26.2 g, 0.10 mol) and zinc powder (6.54 g, 0.10 mol) were dissolved in dry CH\(_2\)Cl\(_2\) (120 mL). Tetrabromoethane, CBr\(_4\) (33.2 g, 0.10 mol) was added at 0 °C. After 2 h, 3,5-di-tert-butylbenzaldehyde 171 (10.92 g, 50.0 mmol) was added neat to the solution and the reaction was left to stir for another 2 h at ambient temperature before cyclohexane (180 mL) was added. Filtration through a short plug of silica using cyclohexane as eluent and removal of the solvent in vacuo yielded the intermediate 172 and was used without further purification (15.2 g, 81%). \(^1\)H NMR (300.1 MHz, CDCl\(_3\)): \(\delta\)H 7.53 (1H, s; \(\text{C}H\)), 7.42 (3H, m, 3\(\times\)Ar\(\text{H}\)), 1.36 (18H, s; 6\(\times\)C\(\text{H}_3\)); \(^{13}\)C NMR (75.5 MHz, CDCl\(_3\)): \(\delta\)C 150.8 (Ar\(\text{C}\)), 137.8 (Ar\(\text{C}H\)), 134.5 (Ar\(\text{C}\)), 122.8 (Ar\(\text{CH}\)), 122.7 (Ar\(\text{CH}\)), 88.5 (\(\text{CH}\)), 34.9 (q\(\text{C}\)), 31.4 (\(\text{CH}_3\)).

1,3-Di-tert-butyl-5-ethynylbenzene 173\(^{273}\)

1,3-di-tert-butyl-5-(2,2-dibromovinyl)benzene 172 (1.0 g, 2.67 mmol) in dry THF (70 mL) was treated with \(N\)-butyllithium (2.5 M, 2.25 mL, 5.61 mmol). The solution was kept at −78 °C for 1.5 h before being allowed to warm up to ambient temperature and left stirred for 16 h. The following day, the reaction was quenched with sat. NH\(_4\)Cl solution and the mixture was extracted with CH\(_2\)Cl\(_2\). The combined organic layers were dried over MgSO\(_4\), filtered and concentrated \textit{in vacuo} to directly yield the terminal alkyne 173 as a colourless solid (0.33 g, 58%). M.p. = 88.3 – 89.2 °C (lit\(^{274}\) 84 – 85 °C). \(^1\)H NMR (300.1 MHz, CDCl\(_3\)): \(\delta\)H 7.43 (1H, dd, \(^4\)J\(\text{HH}\) 1.9 and \(^4\)J\(\text{HH}\) 1.9 Hz; Ar\(\text{H}\)), 7.37 (2H, d, \(^4\)J\(\text{HH}\) 1.9 Hz; 2\(\times\)Ar\(\text{H}\)), 3.04 (1H, s; \(\text{CH}\)), 1.33 (18H, s; 6\(\times\)C\(\text{H}_3\)). \(^{13}\)C NMR (75.5 MHz, CDCl\(_3\)): \(\delta\)C 151.0 (Ar\(\text{C}\)), 126.5 (Ar\(\text{CH}\)), 123.4 (Ar\(\text{CH}\)), 121.2 (Ar\(\text{C}\)), 85.0 (\(\text{CH}\)), 75.9 (\(\text{CH}\)), 34.9 (q\(\text{C}\)), 31.4 (\(\text{CH}_3\)). MS (EI\(^+\)) m/z 214.2 ([M+H]\(^+\), 10); HRMS (ES\(^+\)) m/z calculated for C\(_{16}\)H\(_{22}\) [M+H]\(^+\) 214.1722, found 214.1725.
1,3-Di-tert-butyl-5-((4-nitrophenyl)ethynyl)benzene 174

1-iodo-4-nitrobenzene (0.53 g, 2.12 mmol) was suspended in Et₃N (50 mL). PPh₃ (56 mg, 0.21 mmol), Cul (32 mg, 0.17 mmol) and PdCl₂(PPh₃)₂ (120 mg, 0.17 mmol) were added neat and the mixture was degassed with Ar for 40 min. Alkyne 173 (0.50 g, 2.33 mmol) was added via syringe under a protective Ar atmosphere and the mixture was heated at 50 °C for 16 h. The occurring brown solution was filtered through Celite, concentrated and purified by silica gel column chromatography (Hexane:EtOAc, 10:1) affording the nitro compound 174 as a yellow solid (0.63 g, 81%). M.p. = 161.3 – 162.9 °C; ¹H NMR (300.1 MHz, CDCl₃): δ_H 8.22 (2H, d, 3_J_HH 8.9 Hz; 2×ArH), 7.68 (2H, d, 3_J_HH 8.9 Hz; 2×ArH), 7.47 (1H, t, 4_J_HH 1.8 Hz; ArH), 7.42 (2H, d, 4_J_HH 1.8 Hz; 2×ArH), 1.35 (18H, s; 6×CH₃); ¹³C NMR (75.5 MHz, CDCl₃): δ_C 151.3 (ArC), 147.0 (ArCH), 132.4 (ArCH), 130.7 (ArC), 126.2 (ArCH), 124.0 (ArCH), 123.8 (ArCH), 121.2 (ArC), 96.2 (CH), 86.6 (CH), 35.0 (qC), 31.5 (CH₃); MS (ES⁺) m/z 358.03 ([M+Na]⁺, 100); HRMS (ES⁺) m/z calculated for C₂₂H₂₅NO₂Na [M+Na]⁺ 358.1783, found 358.1785.

(Z)-4-((3,5-Di-tert-butylphenyl)ethynyl)-N-(4-((4,6-dimethylpyridin-2-yl)carbamoyl)benzylidene)aniline oxide 176

Nitro compound 174 (100 mg, 298 μmol) was dissolved in THF (10 mL). Rhodium (36 mg, 5 wt.% on carbon, wet) and hydrazine monohydrate (20 mg, 400 μmol) were added and the reaction was followed by TLC (Hexane:EtOAC, 3:1). After completion, the solution was filtered through Celite and concentrated in vacuo to obtain the desired intermediate hydroxylamine 175 product as a yellow oil, which was directly used in the next step without further purification. The obtained hydroxylamine (286 μmol) was dissolved in EtOH (15 mL) and aldehyde 117 (58 mg, 229 μmol) was added neat. The solution was left to stand in the dark for 3 days at ambient temperature before the resulting precipitate was filtered to give the nitrone 176 as
yellow solid (50 mg, 39% over 2 steps). M.p. = 211.8 - 213.4 °C; \(^1\)H NMR (300.1 MHz, CDCl\(_3\)): \(\delta_H\) 8.52 (2H, d, \(^3\)J\(_{HH}\) 8.5 Hz; 2×Ar\(H\)), 8.49 (1H, s; NHCO), 8.05 (3H, s; 3×Ar\(H\)), 8.02 (1H, s; CH), 7.81 (2H, d, \(^3\)J\(_{HH}\) 8.7 Hz; 2×Ar\(H\)), 7.68 (2H, d, \(^3\)J\(_{HH}\) 8.7 Hz; 2×Ar\(H\)), 7.45 (1H, s; Ar\(H\)), 7.41 (2H, d, \(^4\)J\(_{HH}\) 1.7 Hz; 2×Ar\(H\)), 6.80 (1H, s; Ar\(H\)), 2.45 (3H, s; CH\(_3\)), 2.38 (3H, s; CH\(_3\)), 1.35 (18H, s; 6×CH\(_3\)); \(^13\)C NMR (100.6 MHz, CDCl\(_3\)): \(\delta_C\) 164.7 (C=O), 156.8 (Ar C), 151.2 (Ar C), 150.8 (Ar C), 150.5 (Ar C), 148.1 (Ar C), 136.0 (Ar C), 134.0 (Ar C), 133.4 (CH), 132.5 (Ar CH), 129.3 (Ar CH), 127.7 (Ar CH), 126.2 (Ar C), 126.1 (Ar CH), 123.5 (Ar CH), 121.8 (Ar CH), 121.7 (Ar C), 121.0 (Ar CH), 111.8 (Ar CH), 93.4 (q C), 86.9 (q C), 35.3 (q C), 31.3 (CH\(_3\)), 24.0 (CH\(_3\)), 21.5 (CH\(_3\)); MS (ES\(^+\)) m/z 558.29 ([M+H]\(^+\), 100); HRMS (ES\(^+\)) m/z calculated for C\(_{37}\)H\(_{40}\)N\(_3\)O [M+H]\(^+\) 558.3121, found 558.3126.

(Anthracen-9-ylyethyl)trimethylsilane 178\(^{259}\)

A solution of 9-bromoanthracene 177 (4.0 g, 15.6 mmol), Cul (237 mg, 1.24 mmol), PdCl\(_2\)(PPh\(_3\))\(_2\) (0.87 g, 1.24 mmol) and PPh\(_3\) (0.60 g, 2.63 mmol) in Et\(_3\)N (110 mL) was reacted (110 mL) with (trimethylsilyl)acetylene (7.66 g, 11.0 mL, 78.0 mmol) under Ar atmosphere at 80 °C for 24 h. The solution obtained was filtered through a pad of Celite and concentrated in vacuo. The crude was purified by column chromatography (Cyclohexane) affording a yellow solid of 178 (3.72 g, 87%). M.p. = 82.1 – 84.4 °C; \(^1\)H NMR (300.1 MHz, CDCl\(_3\)): \(\delta_H\) 8.57 (2H, dd, \(^4\)J\(_{HH}\) 1.1 and \(^3\)J\(_{HH}\) 8.7 Hz; 2×Ar\(H\)), 8.42 (1H, s; Ar\(H\)), 8.00 (2H, dt, \(^4\)J\(_{HH}\) 0.6 and \(^3\)J\(_{HH}\) 8.4 Hz; 2×Ar\(H\)), 7.59 (2H, ddd, \(^4\)J\(_{HH}\) 1.4, \(^3\)J\(_{HH}\) 6.6 and \(^3\)J\(_{HH}\) 8.6 Hz; 2×Ar\(H\)), 7.50 (2H, ddd, \(^4\)J\(_{HH}\) 1.4, \(^3\)J\(_{HH}\) 6.7, \(^3\)J\(_{HH}\) 8.2 Hz; 2×Ar\(H\)), 0.44 (9H, s; 3×CH\(_3\)); \(^13\)C NMR (75.5 MHz, CDCl\(_3\)): \(\delta_C\) 133.0 (Ar C), 131.2 (Ar C), 128.8 (Ar CH), 126.0 (Ar CH), 126.9 (Ar CH), 126.8 (Ar CH), 125.8 (Ar CH), 117.2 (Ar C), 106.3 (q C), 101.7 (q C), 0.4 (CH\(_3\)); MS (Cl\(^+\)) m/z 275.1 ([M+H]\(^+\), 100); HRMS (Cl\(^+\)) m/z calculated for C\(_{19}\)H\(_{19}\)Si [M+H]\(^+\) 275.1256, found 275.1249.

9-((4-Nitrophenyl)ethyl)anthracene 180\(^{275}\)

The protected acetylene 178 (1.20 g, 7.29 mmol) was dissolved in a 1:1 mixture of dry THF and MeOH (50 mL).
Potassium carbonate (5.04 g, 36.5 mmol) was added to the solution, which was stirred under Ar atmosphere for 4 h at ambient temperature, before quenched with distilled water (80 mL). The aqueous phase was extracted with CH$_2$Cl$_2$. The organic fractions were collected, dried (MgSO$_4$), filtered and concentrated to 10% of the original volume to give the terminal alkyne 179. Et$_3$N (100 mL) were then added as a solvent for the subsequent conversion. Cul (101 mg, 0.53 mmol), PdCl$_2$(PPh$_3$)$_2$ (372 mg, 0.53 mmol), PPh$_3$ (174 mg, 0.66 mmol) and 1-iodo-4-nitrobenzene (1.65 g, 6.63 mmol) were added to the solution simultaneously under Ar atmosphere, which was stirred at 50 °C for 24 h. The solution was filtered through a pad of Celite, concentrated and purified by column chromatography (Hexane:EtOAc, 10:1) to yield the product as a red solid (1.59 g, 67%). M.p. = 203.4 – 205.7 °C; $^1$H NMR (300.1 MHz, CDCl$_3$): $\delta$H 8.59 (2H, dd, $^4$J$_{HH}$ 1.0 and $^3$J$_{HH}$ 8.6 Hz; 2×ArH), 8.50 (1H, s; ArH), 8.28 (2H, d, $^3$J$_{HH}$ 9.0 Hz; 2×ArH), 8.05 (2H, dt, $^4$J$_{HH}$ 0.6 and $^3$J$_{HH}$ 8.4 Hz; 2×ArH), 7.86 (2H, d, $^3$J$_{HH}$ 9.0 Hz; 2×ArH), 7.64 (2H, ddd, $^4$J$_{HH}$ 1.5, $^3$J$_{HH}$ 6.8 and $^3$J$_{HH}$ 8.5 Hz; 2×ArH), 7.55 (2H, ddd, $^4$J$_{HH}$ 1.3, $^3$J$_{HH}$ 6.7 and $^3$J$_{HH}$ 8.2 Hz; 2×ArH); $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta$C 147.1 (ArC-NO$_2$), 133.0 (ArC), 132.3 (ArCH), 131.2 (ArC), 130.6 (ArC), 129.3 (ArCH), 129.1 (ArCH), 127.3 (ArCH), 126.5 (ArCH), 126.0 (ArCH), 123.9 (ArCH), 115.9 (ArC), 98.9 (qC), 92.0 (qC); MS (ES$^+$) m/z 346.84 ([M+Na]$^+$, 100).

(Z)-4-(Anthracen-9-yethynyl)-N-(4-((4,6-dimethylpyridin-2-yl)carbamoyl)benzylidene)aniline oxide 182

![Chemical structure](image-url)

9-((4-nitrophenyl)ethynyl)anthracene 180 (0.20 g, 0.62 mmol) in THF (20 mL) was reacted with Rhodium (40 mg, 10 wt. % on carbon, wet), before being slowly reacted with hydrazine monohydrate (0.12 mL, 0.74 mmol). The reaction was stirred for 2 h and monitored by TLC until the disappearance of the starting material. The solution was filtered through a pad of Celite and the solvent was then removed to give the intermediate red solid hydroxylamine (0.62 mmol), which was sufficiently pure to carry out to the next step. The hydroxylamine 181 was subsequently dissolved in EtOH (4 mL) and added to a solution of aldehyde 117 (157 mg, 0.62 mmol) in EtOH (15 mL). The reaction mixture was kept in the dark for 3 days at ambient temperature. The resulting precipitate was filtered, washed with hexane and dried in
vacuum to give nitrone 182 (0.27 g, 81%). M.p. = 196.4 – 199.2 °C; 1H NMR (400.1 MHz, CDCl3): \( \delta \text{H} 8.63 (2\text{H}, \text{d}, ^3J_{\text{HH}} 8.6 \text{ Hz}; 2\times \text{ArH}), 8.59 (1\text{H}, \text{br s}; \text{NH}), 8.55 (2\text{H}, \text{d}, ^3J_{\text{HH}} 8.5 \text{ Hz}; 2\times \text{ArH}), 8.49 (1\text{H}, \text{s}; \text{ArH}), 8.09 (1\text{H}, \text{s}; \text{CH}), 8.06 (3\text{H}, \text{s}; 3\times \text{ArH}), 8.04 (2\text{H}, \text{m}; 2\times \text{ArH}), 7.91 (2\text{H}, \text{d}, ^3J_{\text{HH}} 9.1 \text{ Hz}; 2\times \text{ArH}), 7.88 (2\text{H}, \text{d}, ^3J_{\text{HH}} 9.0 \text{ Hz}; 2\times \text{ArH}), 7.66 – 7.62 (2\text{H}, \text{m}; 2\times \text{ArH}), 7.56 – 7.53 (2\text{H}, \text{m}; 2\times \text{ArH}), 6.80 (1\text{H}, \text{s}; \text{ArH}), 2.45 (3\text{H}, \text{s}; \text{CH}_3), 2.38 (3\text{H}, \text{s}; \text{CH}_3); ^{13}\text{C NMR} (100.6 \text{ MHz}, \text{CDCl}_3): \delta \text{C} 164.7 (\text{C}=\text{O}), 156.4 (\text{ArC}), 150.5 (\text{ArC}), 150.4 (\text{ArC}), 148.3 (\text{ArC}), 136.0 (\text{ArC}), 133.9 (\text{ArC}), 133.1 (\text{CH}), 132.9 (\text{ArC}), 132.2 (\text{ArCH}), 131.3 (\text{ArC}), 128.9 (\text{ArCH}), 128.7 (\text{ArCH}), 128.2 (\text{ArCH}), 127.4 (\text{ArCH}), 126.8 (\text{ArCH}), 126.4 (\text{ArCH}), 126.2 (\text{ArC}), 125.6 (\text{ArCH}), 121.7 (\text{ArCH}), 120.6 (\text{ArCH}), 116.5 (\text{ArC}), 111.5 (\text{ArCH}), 99.3 (\text{qC}), 89.2 (\text{qC}), 23.6 (\text{CH}_3), 21.2 (\text{CH}_3); \text{MS (ES}^+\text{)} \text{m/z} 568 ([\text{M+Na}]^+, 100); \text{HRMS (ES}^+\text{)} \text{m/z} \text{calculated for C}_{37}\text{H}_{28}\text{N}_3\text{O}_2 [\text{M+H}]^+ 546.2182, \text{found 546.2183.}

5-Iodo-6-ethylpyridin-2-amine 184

2-Amino-6-picoline 183 (5.4 g, 50.0 mmol) was dissolved in CH$_3$COOH (50 mL) and H$_2$SO$_4$ conc. (7.5 mL) and stirred at 60 °C for 0.5 h before addition of HIO$_4$ (1.63 g, 7.1 mmol) and iodine (5.4 g, 21.3 mmol). The reaction solution was then stirred at 60 °C for 20 h and upon completion, the solvent was removed under reduced pressure and the residues were dissolved in water (100 mL). The pH was adjusted to 14 with 5M KOH solution and extracted into CH$_2$Cl$_2$. The combined organic fractions were dried (MgSO$_4$), filtered and the solvent was removed in vacuo to yield the crude product, which was recrystallised in CH$_2$Cl$_2$ to yield the product 184 (9.98 g, 85%) as a colourless powder. M.p. = 98.8 – 100.2 °C (lit.\textsuperscript{276} 100 °C); 1H NMR (400.1 MHz, CDCl$_3$): \( \delta \text{H} 7.66 (1\text{H}, \text{d}, ^3J_{\text{HH}} 8.7 \text{ Hz}; \text{ArH}), 6.09 (1\text{H}, \text{dd}, ^3J_{\text{HH}} 8.5 \text{ Hz and } ^4J_{\text{HH}} 0.6 \text{ Hz}; \text{ArH}), 4.75 – 4.35 (2\text{H}, \text{br s}; \text{NH}_2), 2.52 (3\text{H}, \text{s}; \text{CH}_3); ^{13}\text{C NMR} (100.6 \text{ MHz}, \text{CDCl}_3): \delta \text{C} 158.1 (\text{ArC}), 157.6 (\text{ArC}), 147.4 (\text{ArCH}), 108.0 (\text{ArCH}), 80.6 (\text{ArC}), 28.5 (\text{CH}_3); \text{MS (ES}^+\text{)} \text{m/z} 234.9 ([\text{M+H}]^+, 100); \text{HRMS (ES}^+\text{)} \text{m/z} \text{calculated for C}_{6}\text{H}_{8}\text{N}_2\text{l [M+H}]^+ 234.9732, \text{found 234.9734.}

4-Formyl-N-(5-iodo-6-methylpyridin-2-yl)benzamide 185

5-iodo-6-methylpyridin-2-amine 184 (6.56 g, 28.0 mmol) and
Et$_3$N (7.5 mL, 53.0 mmol) were dissolved in CH$_2$Cl$_2$ (20 mL) and stirred under N$_2$ atmosphere at 0 °C. A solution of 4-formylbenzoyl chloride 116 (4.5 g, 26.7 mmol) in CH$_2$Cl$_2$ (30 mL) was added in a dropwise manner and the solution was slowly allowed to rise to ambient temperature and stirred for 48 h. The reaction was quenched by addition of sat. solution of NaHCO$_3$ (100 mL) and the product was extracted into CH$_2$Cl$_2$. The combined organic fractions were washed with 1M HCl, dried (MgSO$_4$), filtered and the solvent removed to give the crude which was purified by recrystallisation in CH$_2$Cl$_2$ and yield compound 185 (9.52 g, 93 %) as a colourless solid. M.p. = 180.6 – 181.5 °C; $^1$H NMR (400.1 MHz, CDCl$_3$): δ$_H$ 10.1 (1H, s; CHO), 8.50 (1H, br s; NH), 8.11 – 8.05 (3H, m; 3×ArH), 8.02 (2H, d, 3$J_{HH}$ 8.5 Hz; 2×ArH), 7.95 (1H, dd, 3$J_{HH}$ 8.6 Hz and 4$J_{HH}$ 0.5 Hz; ArH), 2.65 (3H, s; CH$_3$); $^{13}$C NMR (100.6 MHz, d$_6$-DMSO): δ$_C$ 192.9 (CHO), 165.4 (NHCO), 157.9 (ArC), 151.1 (ArC), 147.9 (ArCH), 138.9 (ArC), 138.1 (ArC), 129.2 (ArCH), 128.8 (ArCH), 113.8 (ArCH), 89.4 (Ar-C-I), 27.9 (CH$_3$); MS (ES$^-$) m/z 365 ([M–H]$^-$, 100); HRMS (ES$^-$) m/z calculated for C$_{14}$H$_{10}$N$_2$O$_2$I [M–H]$^-$ 364.9787, found 364.9790.

**N-(5-(4-(tert-Butyl)phenyl)-6-methylpyridin-2-yl)-4-formylbenzamide 186**

**tert-butyl boronic acid** (0.48 g, 2.73 mmol) and potassium carbonate (0.57 g, 4.11 mmol) were dissolved in the solvent mixture of THF and H$_2$O (20 mL). PdCl$_2$(PPh$_3$)$_2$ (98 mg, 0.14 mmol) and aldehyde 185 (0.5 g, 1.37 mmol) were added under Ar atmosphere and heated at 70 °C for 16 h. The solution was filtered through a pad of Celite and wash thoroughly with THF and extracted into CHCl$_3$. The organic layers were washed twice with distilled water, dried (MgSO$_4$) and the solvents were evaporated under reduced pressure. The residue was purified with a column chromatography using (Hexane:EtOAC, 5:1) yielded the product 186 as yellow solid (0.5 g, 98%). M.p. = 163.7 – 167.5 °C; $^1$H NMR (300.1 MHz, CDCl$_3$): δ$_H$ 10.1 (1H, s; CHO), 8.77 (1H, s; NH), 8.24 (1H, d, 3$J_{HH}$ 8.4 Hz; ArH), 8.10 (2H, d, 3$J_{HH}$ 8.3 Hz; 2×ArH), 8.01 (2H, d, 3$J_{HH}$ 8.3 Hz; 2×ArH), 7.64 (1H, d, 3$J_{HH}$ 8.4 Hz; ArH), 7.46 (2H, d, 3$J_{HH}$ 8.3 Hz; 2×ArH), 7.26 (2H, d, 3$J_{HH}$ 8.3 Hz; 2×ArH), 2.45 (3H, s; CH$_3$), 1.37 (9H, s; 3×CH$_3$); $^{13}$C NMR (75.5 MHz, CDCl$_3$): δ$_C$ 191.5 (CHO), 164.6 (NHCO$_2$), 154.5 (ArC), 150.6 (ArC), 149.3 (ArC), 140.2 (ArCH), 139.6 (ArC), 138.7 (ArC), 136.4 (ArC), 133.9
(ArC), 130.1 (ArCH), 128.9 (ArCH), 128.1 (ArCH), 125.5 (ArCH), 111.6 (ArCH), 34.7 (qC), 31.5 (CH₃), 23.1 (CH₃); MS (ES⁻) m/z 371 ([M–H]⁻, 100); HRMS (ES⁻) m/z calculated for C₂₄H₂₃N₂O₂ [M–H]⁻ 371.1760, found 371.1752.

1-Ethynyl-4-nitrobenzene 189²⁶¹

Activated MnO₂ (4.87 g, 56.0 mmol) and KOH (1.58 g, 28.2 mmol) were added to a stirred solution of compound 188 (1.0 g, 5.64 mmol) in CH₂Cl₂ (50 mL). After completion, the mixture was filtered through a pad of Celite, and the filtrate dried over MgSO₄ and evaporated to dryness under reduced pressure affording the terminal alkyne 189 as yellow solid (0.67 g, 81%). M.p. = 138.7 – 139.6 °C (lit. 277 140 – 142 °C); ¹H NMR (400.1 MHz, CDCl₃): δH 8.20 (2H, d, 3JHH 8.9 Hz; 2×ArH), 7.64 (2H, d, 3JHH 8.9 Hz; 2×ArH), 3.36 (1H, s; qC); ¹³C NMR (75.5 MHz, CDCl₃): δC 147.5 (ArC), 133.1 (ArCH), 129.0 (ArC), 123.7 (ArCH), 82.5 (qC), 81.7 (CH); MS (Cl⁺) m/z 148.04 ([M+H]⁺, 100); HRMS (Cl⁺) m/z calculated for C₈H₆NO₂ [M+H]⁺ 148.0399, found 148.0399.

(Z)-N-(4-((5-(4-(tert-Butyl)phenyl)-6-methylpyridin-2-yl)carbamoyl)benzylidene)-4-(naphthalen-2-ylethynyl)aniline oxide 192

Nitro compound 190 (100 mg, 366 μmol) was dissolved in THF (7 mL). Rhodium (42 mg, 5 wt. % on carbon, wet) and hydrazine monohydrate (21 mg, 439 μmol) were added and the reaction was followed by TLC (Hexane:EtOAC, 3:1). After completion, the solution was filtered through Celite and concentrated in vacuo to obtain the intermediate hydroxylamine 191 as a light yellow solid, which was directly used in the next step without further purification. The obtained hydroxylamine 191 (366 μmol) was dissolved in EtOH (15 mL) and aldehyde 186 (109 mg, 293 μmol) was added neat. The solution was left to stand in the dark for 3 days at ambient temperature before the resulting precipitate was filtered to give the nitrone 192 as yellow solid (100 mg, 44% over 2 steps). M.p. = 205.6 °C (decomp.); ¹H NMR (400.1 MHz, CDCl₃): δH 8.65 (1H, s; NH), 8.54 (2H, d, 3JHH 8.46 Hz; 2×ArH), 8.26 (1H, d, 3JHH 8.34 Hz; ArH), 8.10 (1H, s; ArH), 8.07 (2H, d,
(Z)-N-(4-((5-(tert-Butyl)phenyl)-6-methylpyridin-2-yl)carbamoyl)benzylidene)-4-((3,5-di-tert-butylphenyl)ethynyl)aniline oxide 193

Nitro compound 174 (100 mg, 298 μmol) was dissolved in THF (10 mL). Rhodium (40 mg, 5 wt. % on carbon, wet) and hydrazine monohydrate (30 mg, 596 μmol) were added and the reaction was followed by TLC (Hexane:EtOAC, 3:1). After completion, the solution was filtered through celite and concentrated in vacuo to obtain the desired intermediate hydroxylamine 175 product as a yellow solid, which was directly used in the next step without further purification. The obtained hydroxylamine 175 (299 μmol) was dissolved in EtOH (15 mL) and aldehyde 186 (89 mg, 239 μmol) was added neat. The solution was left to stand in the dark for 3 days at ambient temperature before the resulting precipitate was filtered to give the nitrone 193 as yellow solid (0.2 g, 40% over 2 steps). M.p. = 225.8 – 226.7 °C; 1H NMR (300.1 MHz, CDCl3): δH 8.66 (1H, s; NHCO), 8.56 – 8.53 (2H, m; 2×ArH), 8.26 (1H, d, 3JHH 8.4 Hz; ArH), 8.08 (1H, s; CH), 8.06 (2H, s; 2×ArH), 7.82 (1H, d, 3JHH 8.7 Hz; ArH), 7.69 – 7.66 (2H, m; 2×ArH), 7.63 – 7.60 (1H, m; ArH), 7.48 – 7.41 (5H, m; 5×ArH), 7.28 (3H, m; 3×ArH), 2.48 (3H, s; CH3), 1.38 (9H, s; 3×CH3), 1.35 (18H, s; 6×CH3); 13C NMR (75.5 MHz, CDCl3): δC 164.7 (NHCO), 154.4 (ArC), 151.2 (ArC), 151.0 (ArC), 149.3 (ArC), 140.1 (ArCH), 136.5 (ArC), 132.6 (ArC), 34.8 (qC), 88.4 (qC), 31.5 (CH3), 23.1 (CH3); MS (ES+) m/z 636.16 ([M+Na]+, 100); HRMS (ES+) m/z calculated for C42H36N3O2 [M+H]+ 614.2808, found 614.2819.
\((\text{ArCH}), 139.4 (\text{ArC}), 136.5 (\text{ArC}), 134.0 (\text{ArC}), 132.8 (\text{ArCH}), 132.5 (\text{CH}), 129.3 (\text{ArCH}), 129.0 (\text{ArCH}), 127.8 (\text{ArCH}), 126.2 (\text{ArC}), 126.1 (\text{ArCH}), 125.5 (\text{ArCH}), 122.3 (\text{ArC}), 121.8 (\text{ArCH}), 121.7 (\text{ArC}), 121.3 (\text{ArCH}), 111.5 (\text{ArCH}), 91.4 (q\text{C}), 86.9 (q\text{C}), 35.0 (q\text{C}), 34.8 (q\text{C}), 31.5 (CH_3), 31.5 (CH_3), 23.1 (CH_3); \text{MS} (\text{ES}^+) \text{ m/z} 698.21 ([\text{M+Na}]^+, 100); \text{HRMS} (\text{ES}^+) \text{ m/z} \text{ calculated for C}_{46}H_{49}N_2O_2Na [\text{M+Na}]^+ 698.3722, \text{ found 698.3728.}

(Z)-4-(Anthracen-9-ylethynyl)-N-(4-((5-(4-(tert-butyl)phenyl)-6-methylpyridin-2-yl)carbamoyl)benzylidene)aniline oxide 194

Compound 180 (0.20 g, 0.62 mmol) in THF (10 mL) was reacted with Rh/C (118 mg, 50 wt. %), before being slowly reacted with hydrazine monohydrate (0.24 mL, 1.24 mmol). The reaction was stirred for 3 h and monitored by TLC until the disappearance of the starting material. The solution was filtered through a pad of Celite and the solvent was removed to give the intermediate red solid hydroxylamine, which was carried out to the next step without further purification. The hydroxylamine 181 (0.62 mmol) was subsequently dissolved in EtOH (10 mL) and added to a solution of aldehyde 186 (190 mg, 0.43 mmol) in EtOH (15 mL). The reaction mixture was left to stand in the dark for 3 days before the resulting precipitate was filtered, washed with hexane and dried in vacuo to afford nitrene 194 (0.27 g, 94% over 2 steps). M.p. = 218.3 °C (decomp.); \text{\textsuperscript{1}H NMR} (300.1 MHz, CDCl\textsubscript{3}): \(\delta\)H 8.72 (1H, s; NH\textsubscript{t}), 8.63 (2H, d, \(^3J_{\text{HH}}\) 8.4 Hz; 2\times Ar\text{H\textsubscript{t}}), 8.56 (2H, d, \(^3J_{\text{HH}}\) 8.6 Hz; 2\times Ar\text{H\textsubscript{t}}), 8.48 (1H, s; Ar\text{H\textsubscript{r}}), 8.26 (1H, d, \(^3J_{\text{HH}}\) 8.5 Hz; Ar\text{H\textsubscript{r}}), 8.09 – 8.03 (5H, m; 4\times Ar\text{H} + CH\text{t}), 7.92 – 7.87 (4H, m; 4\times Ar\text{H\textsubscript{r}}), 7.64 (2H, d, \(^3J_{\text{HH}}\) 8.5 Hz; 2\times Ar\text{H\textsubscript{r}}), 7.63 (1H, d, \(^3J_{\text{HH}}\) 7.4 Hz; Ar\text{H\textsubscript{r}}), 7.54 (2H, t, \(^3J_{\text{HH}}\) 7.2 Hz; 2\times Ar\text{H\textsubscript{r}}), 7.46 (2H, d, \(^3J_{\text{HH}}\) 8.4 Hz; 2\times Ar\text{H\textsubscript{r}}), 7.28 (2H, d, \(^3J_{\text{HH}}\) 8.4 Hz; 2\times Ar\text{H\textsubscript{r}}), 2.48 (3H, s, CH\textsubscript{3}), 1.36 (9H, s, 3\times CH\textsubscript{3}); \text{\textsuperscript{13}C NMR} (75.5 MHz, CDCl\textsubscript{3}): \(\delta\)C 164.7 (C=O), 154.4 (ArC), 150.5 (ArC), 149.6 (ArC), 148.3 (ArC), 140.1 (ArCH), 136.5 (ArC), 136.0 (ArC), 133.9 (ArC), 133.6 (ArC), 133.5 (CH), 132.9 (ArC), 132.5 (ArCH), 131.3 (ArC), 129.4 (ArCH), 129.1 (ArCH), 129.0 (ArCH), 128.6 (ArCH), 127.7 (ArCH), 127.1 (ArCH), 126.7 (ArCH), 126.2 (ArC), 126.0 (ArCH), 125.5 (ArCH), 122.1 (ArCH), 116.5 (ArC), 111.5 (ArCH), 99.3 (qC), 89.2 (qC), 34.8 (qC), 31.5 (CH\textsubscript{3}), 23.1 (CH\textsubscript{3}); \text{MS} (\text{ES}^+) \text{ m/z} 687 ([\text{M+Na}]^+, 10); HRMS
(ES$^+$) $m/z$ calculated for C$_{46}$H$_{38}$N$_3$O$_2$ [M+H]$^+$ 664.2964, found 664.21959.

4-Formyl-$N$-(5-ido-4,6-dimethylpyridin-2-yl)benzamide 195

5-ido-4,6-dimethylpyridin-2-amine 194 (1.0 g, 4.03 mmol) and Et$_3$N (1.02 g, 10.1 mmol) were dissolved in CH$_2$Cl$_2$ (30 mL) and the solution was cooled to 0 °C. 4-formylbenzoyl chloride 116 (1.02 g, 6.05 mmol) in dry CH$_2$Cl$_2$ (5 mL) was added dropwise at 0 °C and under vigorous stirring. Once the addition was finished, the reaction mixture was allowed to get to rt and was left stirred for 48 h before it was washed with 1M HCl and extracted with sat. aqueous NaHCO$_3$. The combined organic extracts were dried (MgSO$_4$) and evaporated in vacuo. The residue was purified by column chromatography (Hexane:EtOAc, 5:1) to afford the desired aldehyde 195 (0.58 g, 38%) as colorless solid. M.p.= 158.3 – 159.6 °C; $^1$H NMR (400.1 MHz, CDCl$_3$): δ$_H$ 10.1 (1H, s; CO$_H$), 8.57 (1H, s; NH$_H$), 8.10 (1H, s; Ar$_H$), 8.06 (2H, d, $^3$J$_{HH}$ 8.3 Hz; 2×Ar$_H$), 8.00 (2H, d, $^3$J$_{HH}$ 8.3 Hz; 2×Ar$_H$), 2.67 (3H, s; CH$_3$), 2.50 (3H, s; CH$_3$); $^{13}$C NMR (100.6 MHz, CDCl$_3$): δ$_C$ 191.4 (CO$_H$), 164.6 (NH$_H$CO), 159.2 (Ar$_C$), 154.1 (Ar$_C$), 150.0 (Ar$_C$), 139.3 (Ar$_C$), 138.8 (Ar$_C$), 130.1 (ArCH), 128.0 (ArCH), 112.5 (ArCH), 97.2 (ArC-1), 29.7 (CH$_3$), 29.5 (CH$_3$); MS (ES$^-$) $m/z$ 378.87 ([M$-$H]$^-$, 100); HRMS (ES$^-$) $m/z$ calculated for C$_{15}$H$_{12}$N$_2$O$_2$I [M$-$H]$^-$ 378.9944, found 378.9934.

N-(5-((3,5-Di-tert-butylphenyl)ethynyl)-4,6-dimethylpyridin-2-yl)-4-formylbenzamide 196

Aldehyde 195 (0.49 g, 1.28 mmol) was dissolved in Et$_3$N (40 mL). PPh$_3$ (37 mg, 140 μmol), Cul (21 mg, 112 μmol) and PdCl$_2$(PPh$_3$)$_2$ (79 mg, 112 μmol) were added neat and the mixture was degassed with Ar for 40 min. The previous alkyne 173 (0.33 g, 1.54 mmol) were added via syringe under a protective Ar atmosphere and the mixture was heated at 60 °C and vigorously stirred for 24 h. The occurring brown solution was filtered through a plug of Celite. The solvent was evaporated and the reaction crude was purified by flash chromatography (Hexane:EtOAc, 5:1) to afford the desired aldehyde 196 (0.15 g,
25%) as yellow solid. M.p. = 152.9 - 154.5 °C; ¹H NMR (300.1 MHz, CDCl₃): δ_H 10.1 (1H, s; CHO), 8.95 (1H, br s; NHCOC), 8.17 (1H, br s; ArH), 8.08 (2H, d, ³_JH 8.2 Hz; 2xArH), 7.98 (2H, d, ³_JH 8.2 Hz; 2xArH), 7.45 (1H, t, ⁴_JH 1.7 Hz; ArH), 7.39 (2H, d, ⁴_JH 1.7 Hz; 2xArH), 2.63 (3H, s; CH₃), 2.57 (3H, s; CH₃), 1.36 (18H, s, 6xCH₃); ¹³C NMR (75.5 MHz, CDCl₃): δ_C 191.4 (CHO), 164.5 (NHCON), 158.9 (ArC), 152.2 (ArC), 151.1 (ArC), 148.8 (ArC), 139.4 (ArC), 138.7 (ArC), 130.0 (ArCH), 128.1 (ArCH), 125.7 (ArCH), 123.2 (ArCH), 122.3 (ArC), 116.4 (ArC), 112.0 (ArCH), 100.2 (qC), 83.7 (qC), 31.4 (CH₃), 23.4 (CH₃), 21.4 (CH₃); MS (ES−) m/z 465.10 ([M−H]−, 100); HRMS (ES−) m/z calculated for C₃₁H₃₃N₂O₂ [M−H]− 465.2542, found 465.2543.

(Z)-4-((3,5-Di-tert-butylphenyl)ethynyl)-N-(4-((5-((3,5-di-tert-butylphenyl)ethynyl)-4,6-dimethylpyridin-2-yl)carbamoyl)benzylidene)aniline oxide 197

Nitro compound 174 (100 mg, 298 μmol) was dissolved in THF (10 mL). Rhodium (40 mg, 5 wt.% on carbon, wet) and hydrazine monohydrate (30 mg, 596 μmol) were added and the reaction was followed by TLC (Hexane:EtOAC, 3:1). After completion, the solution was filtered through Celite and concentrated in vacuo to obtain the desired intermediate hydroxylamine 175 as a yellow oil, which was directly used in the next step without further purification. The obtained hydroxylamine 175 (96 mg, 298 μmol) was dissolved in EtOH (7 mL) and aldehyde 196 (130 mg, 268 μmol) was added neat. The solution was left to stand in the dark for 48 h at ambient temperature before the resulting precipitate was filtered to give the desired product as yellow solid (50 mg, 24% over 2 steps). M.p. = 235.7 – 236.3 °C; ¹H NMR (400.1 MHz, CDCl₃): δ_H 8.57 (1H, br s; NHCO), 8.06 – 8.05 (2H, m; 2xArH), 8.04 (1H, s, CH₃), 7.81 (2H, d, ³_JH 8.7 Hz; 2xArH), 7.68 (2H, d, ³_JH 8.7 Hz; 2xArH), 7.45 (2H, s; 2xArH), 7.41 (2H, d, ⁴_JH 1.8 Hz; 2xArH), 7.39 (2H, d, ⁴_JH 1.8 Hz; 2xArH), 2.70 (3H, s; CH₃), 2.58 (3H, s; CH₃), 1.36 (18H, s; 6xCH₃), 1.35 (18H, s; 6xCH₃); ¹³C NMR (100.6 MHz, CDCl₃): δ_C 164.6 (NHCON), 158.6 (ArC), 152.1 (ArC), 151.2 (ArC), 147.8 (ArC), 135.7 (ArC), 134.0 (ArC), 133.4 (CH), 132.5 (ArCH), 129.3 (ArCH), 127.7
(ArCH), 126.2 (ArC), 126.1 (ArCH), 125.7 (ArCH), 123.5 (ArCH), 123.3 (ArCH), 123.2 (ArCH). 122.4 (ArC), 121.8 (ArCH), 121.7 (ArC), 116.1 (ArC), 111.9 (ArCH), 99.9 (qC), 93.2 (qC), 35.0 (qC), 31.5 (CH₃), 23.6 (CH₃), 21.7 (CH₃); MS (ES⁺) m/z 770.17 ([M+H]⁺, 100); HRMS (ES⁺) m/z calculated for C₅₃H₆₀N₃O₂ [M+H]⁺ 770.4686, found 770.4672.

4-((4-Nitrophenyl)ethynyl)aniline 206

4-iodoaniline 187 (1.0 g, 4.57 mmol) was dissolved in Et₃N (50 mL). PPh₃ (120 mg, 457 μmol), Cul (70 mg, 366 μmol) and PdCl₂(PPh₃)₂ (260 mg, 366 μmol) were added and the reaction mixture was degassed with Ar for 40 min. The alkyne 189 (0.74 g, 5.02 mmol) were added via syringe under a protective Ar atmosphere and the mixture was heated at 65 °C and vigorously stirred for 16 h. The occurring brown solution was filtered through a plug of celite. The solvent was evaporated and the reaction crude was purified by flash chromatography (Cyclohexane:EtOAc, 3:1) to afford the desired aniline 206 (0.30 g, 28%) as an orange solid. M.p. = 189.2 – 192.3 °C (lit.²⁷⁸ = 190 °C); ¹H NMR (300.1 MHz, CDCl₃): δH 8.19 (2H, d, JHH 8.9 Hz; 2×ArH), 7.60 (2H, d, JHH 8.9 Hz; 2×ArH), 7.36 (2H, d, JHH 8.6 Hz; 2×ArH), 6.66 (2H, d, JHH 8.6 Hz; 2×ArH), 3.92 (2H, br s; NH₂); ¹³C NMR (75.5 MHz, CDCl₃): δC 147.7 (ArC), 146.8 (ArC), 133.6 (ArCH), 131.9 (ArCH), 131.3 (ArC), 123.8 (ArCH), 114.8 (ArCH), 111.6 (ArC), 96.6 (qC), 86.6 (qC); MS (ES⁻) m/z 236.96 ([M–H]⁻, 100); HRMS (ES⁻) m/z calculated for C₁₄H₉N₂O₂ [M–H]⁻ 237.0664, found 237.0668.

3,5-Dichloro-N-(4-((4-nitrophenyl)ethynyl)phenyl)benzamide 207

4-((4-nitrophenyl)ethynyl)aniline 206 (100 mg, 420 μmol) and Et₃N (85 mg, 840 μmol) were dissolved in CH₂Cl₂ (30 mL). 3,5- dichlorobenzoyl chloride (85 mg, 420 μmol) was added dropwise at 0 °C under vigorous stirring. Once the addition was finished, the reaction mixture was allowed to get to room temperature and was left stirred for 16 h. The resulting precipitate formed was filtered, washed with chilled CH₂Cl₂ and dried. The title compound 207 (96 mg, 56%) was obtained as yellow solid. M.p. =

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286.2 – 287.1 °C; 1H NMR (300.1 MHz, d$_6$-DMSO): δ$_H$ 10.6 (1H, s; CONH), 8.26 (2H, d, $^3$$J_{HH}$ 8.8 Hz; 2×ArH), 7.98 (2H, d, $^4$$J_{HH}$ 1.8 Hz; 2×ArH), 7.89 (1H, s, ArH), 7.88 (2H, d, $^3$$J_{HH}$ 8.8 Hz; 2×ArH), 7.80 (2H, d, $^3$$J_{HH}$ 8.8 Hz; 2×ArH); 13C NMR (75.5 MHz, d$_6$-DMSO): δ$_C$ 163.0 (CONH), 146.7 (ArC), 139.9 (ArC), 137.8 (ArC), 134.3 (ArC), 132.5 (ArCH), 132.4 (ArCH), 131.1 (ArCH), 129.3 (ArC), 126.6 (ArCH), 123.9 (ArCH), 120.3 (ArCH), 116.5 (ArC), 94.5 (qC), 87.4 (qC); MS (ES$^-$) m/z 408.89 ([M–H]$^-$, 100); HRMS (ES$^-$) m/z calculated for C$_{21}$H$_{11}$N$_2$O$_3$Cl$_2$ [M–H]$^-$ 409.0147, found 409.0151.

**N-(4-Iodophenyl)-3,5-bis(trifluoromethyl)benzamide 208**

4-iodoaniline 187 (1.90 g, 8.68 mmol) and Et$_3$N (1.46 g, 14.46 mmol) were dissolved in CH$_2$Cl$_2$ (25 mL) and the solution was cooled at 0 °C. 3,5-bis(trifluoromethyl) benzoyl chloride 124 (2.0 g, 7.23 mmol) was added dropwise at 0 °C under vigorous stirring. Once the addition was finished, the reaction mixture was allowed to get room temperature and stirred for 1 h. The resulting precipitate formed was filtered, washed with chilled CH$_2$Cl$_2$ and dried. The title compound 208 (1.28 g, 38%) was obtained as colorless solid. M.p. = 218.6 – 219.8 °C; 1H NMR (300.1 MHz, CDCl$_3$): δ$_H$ 10.2 (1H, s; NHCO), 8.47 (2H, s; 2×ArH), 7.90 (1H, s; ArH), 7.55 (2H, d, $^3$$J_{HH}$ 8.9 Hz; 2×ArH), 7.47 (2H, d, $^3$$J_{HH}$ 8.9 Hz; 2×ArH); 13C NMR (75.5 MHz, CDCl$_3$ + 3 drops of d$_6$-DMSO): δ$_C$ 163.0 (CONH), 138.2 (ArC), 137.5 (ArCH), 136.8 (ArC), 131.0 (q, $^2$$J_{CF}$ 34.0 Hz, ArC), 128.4 (m, ArCH), 124.7 (m, ArCH), 123.1 (CF$_3$), 87.9 (ArC–I); 19F NMR (282.4 MHz, CDCl$_3$ + 3 drops of d$_6$-DMSO): δ$_F$ –63.2 (ArCF$_3$); MS (ES$^-$) m/z 457.72 ([M–H]$^-$, 100), HRMS (ES$^-$) m/z calculated for C$_{15}$H$_7$NOF$_6$I [M–H]$^-$ 457.9477, found 457.9477.

**N-(4-((4-Nitrophenyl)ethynyl)phenyl)-3,5-bis(trifluoromethyl)benzamide 209**

The iodo derivative 208 (1.25 g, 2.72 mmol) was suspended in a mixture of Et$_3$N:THF (50 mL: 10 mL). PPh$_3$ (71 mg, 272 μmol), Cul (50 mg, 218 μmol) and PdCl$_2$(PPh$_3$)$_2$ (153 mg, 218 μmol) were added neat and the mixture was degassed with Ar for 40 min. The alkyne 189 (0.48 g, 3.27 mmol) were
added via syringe under a protective Ar atmosphere and heated at 60 °C for 16 h. The brown solution was filtered through a plug of Celite, concentrated and purified by column chromatography (Hexane:EtOAc, 20:1) to afford the desired nitro compound 209 (1.0 g, 75%) as a bright yellow powder. M.p. = 223.3 – 224.0 °C; 1H NMR (300.1 MHz, CDCl3): δH 8.33 (2H, s; 2×ArH), 8.23 (2H, d, 3JHH 8.8 Hz; 2×ArH), 8.08 (1H, s; ArH), 8.02 (1H, s; NHCO), 7.68 (4H, dd, 2JHH 14.8 and 3JHH 8.7 Hz; 4×ArH), 7.60 (2H, d, 3JHH 8.6; 2×ArH); 13C NMR (75.5 MHz, CDCl3): δC 162.9 (ArCO), 147.1 (ArC), 144.2 (ArC), 138.0 (ArC), 136.8 (ArC), 133.1 (ArCH), 132.7 (q, 2JCF 34.1 Hz, ArC), 132.4 (ArCH), 130.3 (ArC), 127.5 (m, ArCH), 125.8 (m, ArCH), 123.8 (ArCH), 120.4 (ArCH), 119.1 (ArC), 94.3 (qC), 88.0 (qC); 19F (282.4 MHz, CDCl3): δF −63.4 (ArCF3); MS (ES−) m/z 477.0 ([M−H]−, 100); HRMS (ES−) calculated for C23H11N2O3F6 [M−H]− 477.0674, found 477.0672.

3,5-Bis(octyloxy)benzoic acid 211

To a dry N,N-dimethylformamide (80 mL) solution of 3,5-dihydroxybenzoic acid 210 (1.54 g, 10.0 mmol) were added 1-bromooctane (2.89 g, 2.60 mL, 15.0 mmol) and K2CO3 (13.8 g, 0.1M) and the mixture was heated at 65 °C for 16 h under N2 atmosphere. After cooling down to room temperature, aqueous 5% HCl solution (80 mL) and hexane (80 mL) were added to the reaction mixture under vigorous stirring at room temperature. The separated organic phase was washed with sat. NaHCO3 solution and brine and dried over NaSO4. Purification by flash column chromatography (CH2Cl2:Hexane, 1:1) and recrystallisation with MeOH gave the corresponding ester as white powder. Ester (0.64 g, 1.30 mmol) was dissolved in EtOH (60 mL) and water (20 mL), to which KOH (0.73 g, 13.0 mmol) was added and refluxed at 88 °C for 16 h under N2. After cooling down to ambient temperature, the solvent was removed under vacuum and the product extracted into CH2Cl2 and washed with 1M HCl. All the solvents were evaporated and the resulting solids were dried in vacuo and crystallised to give the desired corresponding acid compound 211 (1.50 g, 40% over two steps). M.p. = 53.9 – 55.2 °C; 1H NMR (300.1 MHz, CDCl3): δH 7.23 (2H, d, 4JHH 2.30 Hz; 2×ArH), 6.69 (1H, t, 4JHH 2.28 Hz; ArH), 3.98 (4H, t, 3JHH 6.54 Hz; 2×CH2), 1.79 (4H, quintet, 3JHH 7.13 Hz; 2×CH2), 1.51 – 1.41 (4H, m; 2×CH2), 1.41 – 1.29 (16H, m; 8×CH2), 0.91 – 0.87 (6H, m; 2×CH3); 13C NMR (100.6 MHz, CDCl3): δC
171.8 (COOH), 160.2 (ArC), 147.6 (ArC), 130.9 (ArC), 108.2 (ArCH), 107.5 (ArCH), 68.4 (CH$_2$), 31.8 (CH$_2$), 29.4 (CH$_2$), 29.3 (CH$_2$), 29.2 (CH$_2$), 26.0 (CH$_2$), 22.7 (CH$_2$), 14.1 (CH$_3$); MS (ES$^+$) m/z 401.40 ([M+Na]$^+$, 100); HRMS (ES$^+$) m/z calculated for C$_{23}$H$_{38}$O$_4$Na, [M+Na]$^+$ 401.2668, found 401.2665.

3,5-Bis(octyloxy)benzyol chloride 212$^{279}$

3,5-bis(octyloxy) benzoic acid 211 (1.0 g, 2.64 mmol) was dissolved in toluene (20 mL). Thionyl chloride (1.56 g, 1.02 mL, 13.0 mmol) was added and the mixture was refluxed at 110 °C for 16 h. Removing all solvents in vacuo yielded the desired product 212 as a colourless oil that was used directly without further purification (1.02 g, 97%). $^1$H NMR (300.1 MHz, CDCl$_3$): 6$_H$ 7.22 (2H, d, $^4$J$_{HH}$ 2.26 Hz; 2xArH), 6.73 (1H, t, $^4$J$_{HH}$ 2.25 Hz; ArH), 3.97 (4H, t, $^2$J$_{HH}$ 6.51 Hz; 2xCH$_2$), 1.79 (4H, quintet, $^3$J$_{HH}$ 7.13 Hz; 2xCH$_2$), 1.51 – 1.41 (4H, m; 2xCH$_2$), 1.32 – 1.29 (16H, m; 8xCH$_2$), 0.91 – 0.87 (6H, m; 2xCH$_3$); $^{13}$C NMR (75.5 MHz, CDCl$_3$): 6$_C$ 168.5 (COCl), 160.5 (ArC), 134.9 (ArC), 109.6 (ArCH), 108.8 (ArCH), 68.7 (CH$_2$), 32.0 (CH$_2$), 29.5 (CH$_2$), 29.4 (CH$_2$), 29.2 (CH$_2$), 26.1 (CH$_2$), 22.8 (CH$_2$), 14.3 (CH$_3$).

$N$-(4-((4-Nitrophenyl)ethynyl)phenyl)-3,5-bis(octyloxy)benzamide 213

4-((4-nitrophenyl)ethynyl)aniline 206 (200 mg, 839 μmol) and Et$_3$N (0.21 g, 2.10 mmol) were dissolved in CH$_2$Cl$_2$ (15 mL). 3,5-bis(octyloxy)benzyol chloride 212 (0.40 g, 1.01 mmol) was added dropwise at room temperature under vigorous stirring for 1 h. Once the addition was finished, the reaction mixture was heated under reflux for 10 h. The solvent was evaporated and the crude was purified by flash chromatography (Hexane:EtOAc, 5:1) to afford the desired nitro compound 213 (0.3 g, 60%) as yellow solid. M.p.= 147.7 – 148.2 °C; $^1$H NMR (400.1 MHz, CDCl$_3$): 6$_H$ 8.22 (2H, d, $^3$J$_{HH}$ 8.5 Hz; 2xArH), 7.88 (1H, s; CONH), 7.69 (2H, d, $^3$J$_{HH}$ 8.4 Hz; 2xArH), 7.66 (2H, d, $^3$J$_{HH}$ 8.7 Hz; 2xArH), 7.56 (2H, d, $^3$J$_{HH}$ 8.5 Hz; 2xArH), 6.95 (2H, d, $^4$J$_{HH}$ 1.5 Hz; 2xArH), 6.62 (1H, s; ArH), 3.99 (4H, t, $^3$J$_{HH}$ 6.5 Hz; 2xCH$_2$), 1.79 (4H, quintet, $^3$J$_{HH}$ 7.1 Hz; 2xCH$_2$), 1.46 (4H, dt, $^3$J$_{HH}$ 7.0 Hz and $^2$J$_{HH}$ 14.1; 2xCH$_2$), 1.42 – 1.29 (16H, m; 8xCH$_2$), 0.91 – 0.87 (6H, m; 2xCH$_3$); $^{13}$C
NMR (100.6 MHz, CDCl₃): δ_C 165.7 (CONH), 160.8 (ArC), 147.0 (ArC), 139.0 (ArC), 136.7 (ArC), 133.0 (ArCH), 132.3 (ArCH), 130.5 (ArC), 123.8 (ArCH), 119.8 (ArCH), 117.9 (ArC), 105.5 (ArCH), 104.9 (ArCH), 94.8 (qC), 87.6 (qC), 68.6 (CH₂), 31.9 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 26.2 (CH₂), 22.8 (CH₂), 14.2 (CH₃); MS (ES⁺) m/z 621.20 ([M+Na]⁺, 100); HRMS (ES⁺) m/z calculated for C_{37}H_{46}N_{2}O_{5}Na [M+Na]⁺ 621.3304, found 621.3304.
References

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Appendixes

A1. Crystal data and structure refinement for macrocycle MEU

Empirical formula $C_{94}H_{103}Cl_{12}N_{15}O_{15}$
Formula weight 2108.31
Temperature 93(2) K
Wavelength 0.71073 Å
Crystal system Triclinic
Space group P-1
Unit cell dimensions $a = 13.462(4)$ Å, $a = 84.36(3)^\circ$
$\quad b = 15.659(4)$ Å, $b = 79.435(19)^\circ$
$\quad c = 29.611(8)$ Å, $g = 80.75(3)^\circ$
Volume $6041(3)$ Å$^3$
$Z$ 2
Density (calculated) 1.159 Mg/m$^3$
Absorption coefficient 0.333 mm$^{-1}$
$F(000)$ 2192
Crystal size 0.18 x 0.06 x 0.02 mm$^3$
Theta range for data collection 1.86 to 25.36°
Index ranges $-16<=h<=14$, $-18<=k<=14$, $-29<=l<=35$
Reflections collected 37771
Independent reflections 20910 [R(int) = 0.1473]
Completeness to theta = 25.00° 95.0 %
Absorption correction Multiscan
Max. and min. transmission 1.00000 and .8763
Refinement method Full-matrix least-squares on $F^2$
Data / restraints / parameters 20910 / 834 / 1189
Goodness-of-fit on $F^2$ 0.884
Final R indices [I>2sigma(I)] $R1 = 0.1977$, $wR2 = 0.4395$
$R$ indices (all data) $R1 = 0.4225$, $wR2 = 0.5393$
Largest diff. peak and hole 0.876 and $-0.479$ e.Å$^{-3}$
A2. Publication
Low Temperature Capture of Pseudorotaxanes

Nurul Izzaty Hassan, Vicente del Amo, Ewen Calder, and Douglas Philp

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