Towards a Hydrogen Bond Mediated Directional Walker and Light Driven Molecular Shuttles

Cognitio, Sapientia, Humanitas

A thesis submitted to The University of Manchester for the degree of

Doctor of Philosophy

in the Faculty of Science and Engineering

2017

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ABSTRACT

Towards a Hydrogen Bond Mediated Directional Walker and Light Driven Molecular Shuttles

A thesis submitted to The University of Manchester for the degree of

Doctor of Philosophy

in the Faculty of Engineering and Physical Sciences

2017

Tuğrul Nalbantoğlu

School of Chemistry

This thesis reports the efforts towards the design and synthesis of a small molecule walker that would potentially move along the track directionally by exploiting the secondary interactions between the track and the walker. This thesis also reports the synthesis and operation of a light driven molecular shuttle featuring a novel acyl pyridyl hydrazone station.

Chapter One describes the biological walkers which are the source of inspiration towards the synthetic walkers, characteristics of a walker, previously described small molecule walkers and recent progress on the synthesis of molecular shuttles that operate under variety of different stimuli.

Chapter Two describes the design and synthetic efforts towards a molecular walker that has the potential to operate directionally along the track by exploiting secondary interactions between the walker and the track namely the hydrogen bonding interactions introduced by subtle incorporation of excellent hydrogen bond donor/acceptor squaramides. This chapter briefly mentions the hydrogen bonding capabilities of squaramides on which the directional operation relies. Optimization of critical reactions and attempted strategies for the assembly of the whole machine is described as well.

Chapter Three describes the synthesis and operation of 1- and 2- station [2]-rotaxanes that operate under light irradiation. 2- station [2]-rotaxane that function as a light driven molecular shuttle presents remarkable positional fidelity with high efficacy. The bistable acyl pyridyl station is incorporated as a photo active station upon which light irradiation alters the binding affinities towards the macrocycle. Series of rotaxanes featuring different amide based stations were synthesized to determine the best non-photo active station.
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For my parents, family and friends without whom
the world would lose its joy.
ACKNOWLEDGEMENTS

I would like to thank my supervisor Prof. David A. Leigh for giving me the opportunity to work in a fascinating research environment throughout my PhD. All members of the Leigh group past and present who contributed to create this amazing atmosphere are greatly appreciated. My grateful thanks go to Dr. Vanesa Marcos for her continuous support and for the fruitful discussions we had during these challenging years. Dr. Guillaume De Bo is also greatly appreciated for being there whenever I bumped into a synthetic challenge. I am grateful to Dr. Sundus Erbas Cakmak whom I had the privilege to work with for her synthetic and design related recommendations that amazed me every single time and proved how an incredibly smart and talented scientist she is. Dr. Yusuf Cakmak is also greatly appreciated for his support and help for the projects that we worked with. Tuba Yasar who is one of the most hardworking, talented and decent scientist I have ever got to know is greatly appreciated as well for her contribution to the projects that we worked together. I would also like to thank Ulvi Karaca whose company kept me going during the challenging times. I would like to thank everybody else I had the privilege of working with during my PhD years: Adrian, Alan, Alberto, Alina, Alex, Anneke, Antonio, Bartek, Charlie, Chris, Craig, Dan, Furkan, Gen, Gus, Guzman, Jack, Jason, Javier E., Javier G. Jeff A, Jeff L, John, Jon B, Leoni, Liang, Lucian, Malcolm, Marcus, Matt, Miriam, Patrick, Salma, Sau Yin, Shoufeng, Simone, Sonja, Steffen, Stephen, Steven, Sunny, Tom, Ula, Valerie, Victor.

Other than the Leigh group there are a few names I would like to thank; one of my best friends Ali from the bottom of my heart for his continuous support; Yasin for his understanding and support during the hard times; Burak and Cihad for their hospitality during my getaways to London; Alper, Aykut, Enes, Murat, Oguz, Salih, Vefa and Yasin for all the chit-chat that we had in our “whatsapp” group that kept me motivated and bright; Yusuf for his exceptional friendship; Halil, Harun, Lutfi and Mesut for all the unforgettable moments we had together; Bahar, Elif, Ethem, Melisa, Merve, Vildan B., Vildan T. for their companionship; and all the people and family members whom I had the pleasure of getting to know.

Finally, I cannot thank my mother, father and sister enough. There are no words to express my appreciation for all the support and unquestioning love that they have for me. This thesis is dedicated to you.
**ABBREVIATIONS**

(Note: conventional abbreviations for units and physical quantities are not included here)

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>aq.</td>
<td>aqueous</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butyloxycarbonyl</td>
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<tr>
<td>BPA</td>
<td>bis(2-picoly)amino</td>
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<tr>
<td>calcd.</td>
<td>calculated</td>
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<tr>
<td>cat.</td>
<td>catalytic amount</td>
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<td>CD</td>
<td>cyclodextrine</td>
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<tr>
<td>COSY</td>
<td>correlation spectroscopy</td>
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<tr>
<td>CPK</td>
<td>Corey-Pauling-Koltun space filling</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
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<tr>
<td>DEPT</td>
<td>distortionless enhancement by polarization transfer</td>
</tr>
<tr>
<td>DFT</td>
<td>density functional theory</td>
</tr>
<tr>
<td>DIBAL-H</td>
<td>diisobutylaluminium hydride</td>
</tr>
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<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
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<tr>
<td>DMAP</td>
<td>4-(dimethylamino)pyridine</td>
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<td>DMF</td>
<td>dimethylformamide</td>
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<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>dpff</td>
<td>1,1'-Bis(diphenylphosphino)ferrocene</td>
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<tr>
<td>EDCI</td>
<td>N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride</td>
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<td>equiv.</td>
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<td>Abbreviation</td>
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<tr>
<td>ESI</td>
<td>electrospray ionisation</td>
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<td><em>et al.</em></td>
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<tr>
<td>Hex.</td>
<td>Hexanes, fraction 40-60 °C</td>
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<tr>
<td>HMBC</td>
<td>heteronuclear multiple-bond correlation</td>
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<tr>
<td>HOBrt</td>
<td>1-hydroxybenzotriazole</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<td>HRMS</td>
<td>high resolution mass spectroscopy</td>
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<td>HSQC</td>
<td>heteronuclear single-quantum coherence</td>
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<td>Hz</td>
<td>hertz</td>
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<tr>
<td>ICD</td>
<td>induced circular dichroism</td>
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<td>J</td>
<td>coupling constant</td>
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<tr>
<td>LRMS</td>
<td>low resolution mass spectroscopy</td>
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<tr>
<td>M</td>
<td>molar</td>
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<tr>
<td>m/z</td>
<td>mass-to-charge ratio</td>
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<tr>
<td>MALDI</td>
<td>matrix assisted laser desorption/ionisation</td>
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<td>Me</td>
<td>Methyl</td>
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<td>megahertz</td>
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<td>mp.</td>
<td>melting point</td>
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<td>nm</td>
<td>nanometre</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOESY</td>
<td>nuclear overhauser effect spectroscopy</td>
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</table>
Ac  acetate
Tf  triflyl
p  para
pet. ether  petroleum ether (40-60 °C)
ppm  part per million
prep TLC  preparative thin layer chromatography
pTSA  para-toluenesulfonic acid
quant  quantitative
ROESY  rotating frame nuclear Overhauser effect spectroscopy
RT  room temperature
satd.  saturated
TBAF  tetra-N -butylammonium fluoride
t-Bu  tertiary butyl
TEA  triethylamine
TFA  trifluoroacetic acid
THF  tetrahydrofuran
TIPS  triisopropylsilyl
TLC  thin layer chromatography
TMS  trimethylsilyl
TsCl  4-toluenesulfonyl chloride
UV  ultraviolet
v/v  volume ratio
δ  chemical shift
µL  microlitre
Chapter One

An Introduction to Molecular Walkers and Shuttles

Synopsis: In this chapter, biological walkers, synthetic small molecule walkers and molecular shuttles are reviewed. Although, synthetic walkers are still in their infancy and yet to be further developed, there is evidently remarkable progress being made towards an ideal small molecule walker where a cargo can be transported along a polymeric track. As well, molecular shuttles have been thoroughly studied and investigated over the past several years towards the construction of complex molecular machines.

Acknowledgements

Dr. Thomas Singleton is greatly appreciated for proofreading this chapter.
An Introduction to Molecular Walkers

Characteristics of a Molecular Walker

A molecular walker can be defined as small objects that are able to walk following a variety of mechanisms on a defined track, which could be another molecule or a surface towards a direction or state that is different from the original position of the walker once the cycle is complete. There has to be some sort of interaction between the walker moiety and the “track” throughout the operation. This interaction could be a covalent bond, metal-ligand interaction or a supramolecular interaction as the system cannot rely simply on gravity in a molecular level because it is negligible compared to other interactions. Just as in the case of catalysts where small molecule analogues of enzymes are designed by following the fundamental characteristics of relatively huge enzymes such as the active pocket etc., scientists have long been trying to mimic the behaviour of the biological walkers observed in Nature. There has been a great deal of success in this area when the same idea was applied on DNA type systems and following the Nature’s way of transferring molecules from one position to another; a variety of DNA-based walkers have been described in the literature. In terms of size, artificial DNA based walkers are comparable to their natural counterparts, and in some cases they are even larger in size than natural motor proteins. One can design a small molecule walker by mimicking what is observed in Nature. Of course, the design and the way the molecule walks may significantly differ from the natural motor proteins. However, one has to keep in mind some fundamental characteristics when designing a small molecule walker. These characteristics are as follows:

- **Processivity**: The ability of the walker to remain attached to the track during the operation. As gravity cannot be relied upon while the walker is taking a step, it has to somehow keep its interaction with the track. Otherwise, it will simply diffuse away and lose its processivity.

- **Directionality**: The ability of the walker to move preferably or exclusively towards one end of the track.

- **Repetitive operation**: The ability of the system to perform similar cycles repeatedly.
- **Progressive operation**: At the end of each mechanical cycle, the system should be able to be reset without undoing the work that had been already done for the motor to operate progressively.

- **Autonomous operation**: The ability of the system to function as long as there is a fuel present. In other words, no external stimuli are required and the machine operates by using the energy source present in the media. All biological walkers operate in an autonomous fashion. In this thesis, any molecular unit connected to a track through one or more distinct points of contact and exhibiting the first four characteristics is considered a molecular walker. Autonomous operation is a desirable additional characteristic but not essential for a functional molecular walker system.
Biological Walkers

It is crucial in complex biological systems to transfer information and transport cargoes such as membranous organelles, protein complexes and mRNAs from one point to another. To that end, Nature has evolved a variety of protein-based systems that can perform complex tasks and consequently take part in cellular processes such as cell division, transporting membranous organelles, muscle contraction, mitosis and sensory transduction. Those motor proteins, namely the ones from the myosin, dynein and kinesin superfamilies, move along what are called cytoskeletal polymers that are made up of intermediate filaments, microtubules and actin microfilaments. The dynamic polymerization of actin units gives the rigid helical structure of the actin microfilaments along which myosin walks. As for the microtubules, they consist of polymeric protofilaments that subtly accumulate to give the unique cylindrical shape of microtubules. Those protofilaments are again formed through dynamic polymerization of α and β tubulin units. Kinesins moves along microtubules mostly towards the + end (cell periphery) of the cell, whereas dyneins moves towards the – end (cell centre) of the cell along microtubules. The way those motor proteins operate, how they manage near-perfect directionality and how most of them operate with excellent processivity have long inspired scientists and replicating them represents some of the most important challenges that scientists have been trying to answer. Although some motor proteins operate and function in drastically different manners from each other, they bear some similarities as well, which will be discussed next.

Despite the fact that processivity constitutes one of the fundamental characteristics of walker systems, there can be found examples in Nature where the motor protein operates directionally but not processively. For instance, conventional myosin (myosin-II) directionally but not processively performs work for muscle contraction by acting in large groups and attaching themselves on the actin track to generate force and detaching at the end of each cycle. The timing of the whole process actually depends on the motor proteins detaching themselves from the track at the end of each cycle. Therefore, it is the poor processivity in this case that gives the machine the unique function that it performs.

Not all members of myosin family operate in a non-processive manner. For example myosin-V, which is in charge of organelle transport in the cell, remains attached to
the track for several steps and both of its motor heads (whereas only one foot binds to the track in the case of myosin-II, even though it has two active feet) are involved in the binding process so that high processivity is ensured. However, it is also important to note that the number of feet associating with the track does not necessarily determine whether the protein will act processively or not. For instance, KIF1A is a motor protein belonging to the kinesin family and has only one foot, but still works processively. It is the secondary interactions between the motor and the track that ensures the processivity in this case. KIF1A slides over the track rather than actually taking discrete steps with the help of those interactions. Those having two active feet such as kinesin-I and myosin-V must still arrange themselves during the operation so that at least one foot remains attached to the track on which they are moving to exhibit high processivity.

Kinesin, myosin and dynein convert the chemical energy released from ATP hydrolysis into mechanical work and are called ATPases accordingly. During the ATP hydrolysis cycle, a small conformational change that translates into a large-amplitude motion occurs in all these proteins, and this is basically how directionality is sustained. The release of inorganic phosphate at the end of each ATP hydrolysis in myosins results in a conformational change in the motor domain that is carried all the way to the long lever arm: as a result, the arm is pushed 25 nm towards the next binding station. In this sense, kinesin and myosin are remarkably similar with virtually identical motor domains. The only two differences are that the conformational change that results in large-amplitude motion in kinesin takes place during ATP binding whereas it happens at the stage of (P_i) release in myosin, and the arm that functions as lever is a flexible one in kinesin, which implies that power-stroke mechanism observed in myosin due to the rigid arm is not indeed that crucial in kinesin.

Kinesin-I was first isolated in 1985 by Vale and his co-workers, and is seen as a model for the design of molecular walkers synthesized to date. And since it can easily be isolated in large quantities from the brain, it is the one that has been investigated and studied the most among the kinesin superfamily due to these practical reasons. Kinesin-I is a dimer with two identical chains that can be divided into three important regions: first, the tail part (Figure 1c, green), which might have a function in the motor regulation, is able to bind cargoes being transported.
Secondly, the two identical heads (feet, Figure 1c, dark blue) that bind to the microtubules play a crucial role in the enzymatic ATP hydrolysis cycle for directional motion. Lastly, the linker (Figure 1c, light blue) that connects these two, which is suspected to have a role in motor mechanism, is what gives the dimeric shape of kinesin-I.

The way kinesin-I operates is highly processive: it can take on average 100 steps, corresponding to 1 mm, before falling off the track. These measurements were made using special optical tweezers, which also showed that each step measures 8 nm, matching well with the distance between α/β tubulin units that act as binding sites.

Due to contradictory experimental results, the actual mechanism of how kinesin-I takes steps was not definitively determined until recently. Some results suggested that it was walking in a manner commonly referred to as an inchworm mechanism, where one foot is always leading and the second trails behind. This was contradicted by experiments conducted with labelled feet, revealing that each foot moves by 16 nm when taking a step while the centre of the kinesin moves 8 nm, which rules out the inchworm mechanism. All experiments suggest that the mechanism is consistent with one where the two feet exchange leading and trailing positions; namely the ‘hand-over-hand’ mechanism.
One controversial issue is how kinesin-I, having chemically-identical feet, manages to “walk” on the track with high directionality and processivity. Although no consensus has yet emerged, a proposal from Block\textsuperscript{19a} is a widely accepted one. According to this theory, when kinesin-I is in its resting state (Figure 2, I and II) where one foot is occupied by ADP and the other remains unbound, the unoccupied domain binds to the track very strongly. In conformer I, the foot occupied by ADP interacts with the track and this interaction causes a strain in the linker moiety so it relaxes into conformer II. These two conformers are actually in equilibrium with the constant determined to be roughly 1.4.\textsuperscript{19w} When all the strain is relaxed, the leading foot is able to bind to ATP, during which significant amount of energy is released, which causes a conformational change in the linker part to push it towards the motor domain. This strain-derived mechanism is a crucial step that plays an important role in the high directionality and processivity of kinesin-I. Once the rear foot is free to move, it is still possible for it to go back to the original rear binding unit that would result in no net directional movement. However, as explained above, due to the power stroke that pushes the rear foot forward, this possibility is severely disfavoured. One important feature of the overall process is that the transition from state II to III occurs much faster than the rest of the process\textsuperscript{19l}, and this helps with
high processivity. Additionally, once the rear foot is pushed forward and reaches the next binding site it makes a strong interaction with the microtubule, due to the release of the ADP sitting (Figure 2, III-IV). This release also creates strain so that another ATP is not allowed to bind until the other foot completes the hydrolysis of its ATP. Finally the catalytic cycle is completed by the release of inorganic phosphate (Figure 2, IV-I). Overall, it appears that the creation of strain plays a crucial role in the processivity of kinesin-I. Since both of the feet are identical and there needs to be an asymmetry for the system to exhibit processivity, this strain that does not allow both feet to efficiently sit on the neighbouring binding site helps create the asymmetry. The directionality is ensured by the conformational change in the system that allows the linker to push the rear foot towards the plus end of the cell.
DNA-Based Molecular Walkers

A number of walker systems which are largely or entirely composed of DNA building blocks have been introduced to the literature since 2004. Many of these DNA-walker systems can be regarded as genuine molecular motors as they exhibit all four fundamental characteristics of motors: progressive, processive, repetitive and directional transport of a molecular unit along a track. In terms of their sizes, they are comparable to the size of biological walkers and even larger in some cases.

Non-autonomous DNA Walkers

![Non-autonomous inchworm walker described by Sherman and Seeman.](image)

Figure 3 Non-autonomous inchworm walker described by Sherman and Seeman. a) Self-assembly of the system. b) Initial position of the walker. c) Foot B released from foothold 2. d) Foot B attached to foothold 3 with addition of anchor strand 3B. e) Foot A released from foothold 1. f) Foot A attached to foothold 2 with addition of anchor strand 2A. Matching colours indicate strands being complementary to each other. The lines between the strands do not represent a particular number of bases. Adapted from reference.  

The first example of a non-autonomous DNA based walker that relies on sequential addition of chemical fuels was introduced in 2004 by Sherman and Seeman (Figure3). The entire system is composed of DNA oligonucleotides with the
exception of psoralene and biotin tags. The track is a double-stranded triple-crossover DNA structure (in solid black, **Figure 3a**) from which three single-stranded footholds comprising different nucleotide sequences protrude (dark blue, green and light blue, **Figure 3a**). The biped has two double-stranded ‘legs’ (black, **Figure 3a**) with two different one-stranded feet (red and orange, psoralene tags in bright red, **Figure 3a**) and three flexible strands (black curved lines) that connect the two legs. The biped is attached to the track in a buffered solution by the addition of anchor strands that are partially complementary to the foothold/foot pair. The anchor strand 1A is complementary to the single strands of foothold 1 and foot A, whereas the anchor strand 2A is complementary to foothold 2 and foot B (**Figure 3a and 3b**). As a result of this process, a metastable aggregate is formed where instability is caused by the presence of so-called ‘toeholds’ (**Figure 3b**) that are the unpaired eight bases of the anchor strands. In order to detach foot B from the track, fuel strand 2B which is complementary to the anchor strand 2B is added. This step is powered by the formation of eight new base pairs in the stable duplex that can be removed from the media with the help of interaction of biotin-tag with magnetic streptavidin-coated beads. Once foot B is detached from the track and free to move (**Figure 3c**), another anchor strand (3B) that is complementary to foot B and foothold 3 is added so that reattachment of the leading foot to foothold 3 is ensured. Following a series of similar attachment and detachment steps leads to the directional transportation of the walker moiety via inchworm mechanism. It has also been demonstrated that the walker can walk backwards.

**Autonomous DNA Walkers**

Three examples of autonomous DNA walkers were introduced to the literature in 2004 and 2005 (**Figure 4 and 5**). In all three cases, the directional bias is ensured through enzymatic cleavage of DNA or RNA strands. The operation of the first autonomous DNA walker reported by the groups of Turberfield, Reif and Yan is shown in **Figure 4**. Double-stranded footholds are connected to a DNA duplex track through a short single-stranded hinge. The walker moiety shown in red has six DNA nucleotides and is initially attached to foothold 1 (**Figure 4**, state 1-W). The aforementioned enzymes added to the solution that are crucial to ensure directional bias are: a ligase (T4 ligase) and two restriction enzymes (PfIM I and BstAP I). Due to the flexible nature of the hinges, footholds 1 and 2 are in close proximity to each
other so that T4 ligase could connect the walker to foothold 2 through covalent bond formation (Figure 4, 1-W-2). This process creates a recognition site for PfIM I to selectively cleave the walker from foothold 1 (Figure 4, 1 W-2). The energy required for the directional bias is supplied by the ATP hydrolysis of this restriction enzyme. Repetition of similar cycles results in net directional transportation of the walker moiety to foothold 3. As there is no mechanism for walker to take a step backwards, the overall movement is directional.

Turberfield’s group reported in 2005 an autonomous burnt-bridges (where directionality arises due to the consumption of the track as the walker moves forward) DNA walker where the directionality arises from the fact that the walker moiety consumes the track as it moves forward. The system consists of three almost identical footholds, a walker moiety (Figure 5a, shown in red) and an enzyme that cleaves off the terminal side of the foothold only when the walker is fully attached to that particular foothold. The enzyme used in this process is a restriction enzyme called N.BbvC IIB that recognizes a specific sequence in the walker-foothold duplex and then hydrolyses the foothold strand. The operation begins when the walker is located on the left hand side of the track (Figure 5a). Then, enzymatic cleavage reveals a toehold region on the walker that is able to reach to foothold 2. Due to competitive hybridization, the whole walker moiety moves to the second foothold where a backward step is extremely unlikely due to a ~10 kcal mol⁻¹ gain in free energy resulting from attachment to the longer foothold. After similar cycles, the
walker reaches foothold 3 and stays there without further cleavage of the foothold by the enzyme due to a mismatch in the sequence (Figure 5a).

**Figure 5** Two other enzyme-mediated autonomous DNA walkers. a) The burnt-bridges type DNA walker by Turberfield. A walker unit (shown in red) consisting of 26 DNA bases indirectly consumes the track as it moves forward. The lines between the strands do not represent a particular number of bases. b) Burnt-bridges type DNA walker by Mao, mediated by a DNAzyme within the walker unit, the walker directly consumes the track as it moves forward. The lines between the strands do not represent a particular number of bases. Adapted from references.

A very similar concept was reported by Mao’s group where the walker is directionally transported through a burnt-bridges mechanism. In this case, no external enzyme is used as the walker itself is a DNAzyme that is able to cleave RNA strands with sequence specificity (catalytic core shown in orange in Figure 5b). Through a very similar reaction cascade to the one reported by Turberfield, the walker is transported to the final foothold. One noteworthy difference is that all footholds are susceptible to be hydrolysed by the DNAzyme, therefore the final foothold is hydrolysed as well. The directional motion was verified by monitoring the order of appearances of the short fragments released from the footholds.
Small-Molecule Walkers

In 2015 Raval’s group\(^1\) reported an on-surface bimolecular system where a small bis(imidazolyl) molecule walks directionally along a Cu surface in a manner akin to inchworm mechanism. The mobility of the walker 2 (Figure 6 ii) is restricted by the presence of oligomeric immobile porphyrin fences located on the monocrystalline copper surface. The immobile component acting as “fences” is made from porphyrin 1 (Figure 6 ii), which forms covalent organometallic oligomers when heated on a Cu [110] surface. The fences are perpendicular to the diffusion direction of the walker so that confined motion is created (Figure 6, i). DFT calculations of the walker moiety on the surface confirm that the phenyl ring does not touch the surface and stands on its imidazolyl feet (horseshoe conformation, Figure 6, iii). The imidazolyl feet are held on the surface by chemisorption of the nitrogen atoms of the feet onto the Cu atoms of the surface. Due to the specific interactions between the feet and the surface, directional motion occurs following an inchworm-type mechanism by attaching and detaching the imidazolyl feet to the surface. The direction of the motion of the walker is determined by scanning tunnelling microscopy to be along the [110] direction of the Cu surface with remarkably high selectivity. The authors claim that the basis of this directionality arises from anisotropic character of the copper surface. This remarkable study paves the way to create different and more complex walker systems such as designing non-identical

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**Figure 6** i) Representation of the system. ii) Structures of the porphyrin fence unit (1) and the walker (2). iii) Two DFT-relaxed energetically equivalent geometries (extended and contracted) of the walker on the copper surface shown as ball-and-stick model (top), and as the front (middle) and side (bottom) views. Adapted from reference.\(^1\)
stations by simply functionalising the porphyrin units, or a system that is triggered by an electrical pulse or light as opposed to the thermal activation by which this work is operated.

The very first small molecular walker comprised of 21-atoms was synthesized and published by the Leigh group in 2010.\textsuperscript{24} It features a walker unit (\textbf{Figure 7}, shown in red) consisting of two legs that is able to walk up and down a track incorporating four footholds (\textbf{Figure 7}, shown in green and blue). High processivity is ensured by the subtle use of orthogonal chemistry: that is, the functional groups on the legs become labile under different sets of conditions namely acid-base cycles in this case.

\textbf{Figure 7} Design and operation of the first small molecule walker. Condition I (reversible hydrazone exchange): 0.1 mM, TFA, CHCl\textsubscript{3}, RT, 6–96 h. Condition II (reversible disulfide exchange): 0.1 mM, DTT (10 equiv.), DBU (40 equiv.), \((\text{MeO}_2\text{CCH}_2\text{CH}_2\text{S})_2\) (20 equiv.), CHCl\textsubscript{3}, RT, 12–48 h. Adapted from reference.\textsuperscript{24}

While one of the feet is labile and free to move, the other foot is kinetically stable and fixed on the foothold acting as a pivot so that the walker unit will not diffuse away from the track. More specifically, under acidic conditions the disulfide bond
between the track and the walker is kinetically stable and locked so that the hydrazone bond between the track and the walker, which is labile, can freely move, whereas switching the environment from acidic to basic results in a smart response where the disulfide bond becomes labile and free to move while the hydrazone bond is kinetically locked. The walker walks in a non-directional and random fashion and takes one or zero steps each time the pH is switched, following a passing-leg gait mechanism. Repeatedly switching the conditions back and forth leads the system to reach a thermodynamic distribution of 39:36:19:6 (±2) for 1,2-1:2,3-1:3,4-1:1,4-1 isomers (Figure 8).

**Figure 8** Dynamic behaviour of the walker starting from both ends. a) Experimental sequence. b) Product distribution. Condition I (reversible hydrazone exchange): 0.1 mM, TFA, CHCl₃, RT, 6–96 h. Condition II (reversible disulfide exchange): 0.1 mM, DTT (10 equiv.), DBU (40 equiv.), (MeO₂CCH₂CH₂S)₂ (20 equiv.), CHCl₃, RT, 12–48 h. Adapted from reference. ²⁴
It is noteworthy to state that this distribution is reached no matter which end of the track the walking operation is started from. Even though some of the walkers reach to the opposite end of the track during the cycling of conditions, which is because the system is not yet at the minimum energy distribution, this cannot be called an intrinsically directional motion as it is simply system relaxing towards the most energetically favoured state. However, replacing the basic step with a two-stage redox reaction (Figure 9 III(i) and III(ii)) results in the walker moving down the track with inherent directionality (Figure 9c) through an information ratchet mechanism. Even though this directionality may not translate well to a polymeric track, as it relies on the fact that instantaneous and irreversible redox-mediated

![Figure 9 Directionally biased walk from 1,2-1 under cycling of acid and redox conditions.](image)

a) Redox-mediated reaction sequence illustrated for 2,3-1. III(i) trithiol is quantitatively generated by reductive ring-opening. III(ii) disulfides are regenerated through rapid oxidation. b) Reaction sequence. Condition I (reversible hydrazone exchange): 0.1 mM, TFA, CHCl₃. Condition III (kinetically controlled disulfide exchange): III(i) 1.0 mM, DTT (6 equiv.), DBU (3 equiv.), CHCl₃, reflux, 2–12 h (allowed to continue until all disulfide bonds were reduced, as monitored by ¹H NMR spectroscopy); III(ii) MeO₂CCH₂CH₂SH (8 equiv.), I₂, Et₃N, CHCl₃/MeOH 1:1, RT, 5 min. c) Evolution of the mixture of positional isomers over three acid-redox operations compared to the acid-base sequence. The right-hand column shows the percentages of each isomer at the steady state using the acid-base cycles (see Figure 8b). The difference in the amount of 3,4-1 is highlighted in bold. RSH = MeO₂CCH₂CH₂SH. Adapted from reference.

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macrocycle closure occurs at the most accessible foothold, this still provides a great advantage over the reversible acid-base cycles as 43% of the walker could reach to the end as opposed to 19%.

The Leigh group has also expanded this study and published a scope of this system where they examine how the length of the walker moiety would affect the walking process. To that end, they synthesized a series of systems with different numbers of carbon atoms on the walker unit (C_n, n= 2, 3, 4, 5 and 8). They report that the walker molecules with different lengths indeed exhibit different walking behaviours. Molecules with n=2 and 3 do not exhibit any walking behaviour as their length is too small to connect two footholds, whereas molecules n= 4, 5 and 8 are long enough to take a step and are able to move up and down (See Figure 10 for details).

Directional bias is observed when switching to acid-redox conditions only with molecules n=4 and 5.

**Figure 10** Reversible reactions connecting various pairs of the positional isomers 1,2-C_n, 2,3-C_n, 3,4-C_n, and 1,4-C_n. Condition I (reversible hydrazone exchange): 0.1 mM, TFA, CHCl_3, RT, 6-96 h (monitored by HPLC until the distribution no longer changed). Condition II (reversible disulfide exchange): 0.1 mM, DTT (10 equiv), DBU (40 equiv), dimethyl 3,3′-disulfanediyldipropanoate (20 equiv), CHCl_3, room temperature, 12-48 h (monitored by HPLC until the distribution no longer changed). Adapted from reference. 25

This work was followed by another system featuring similar moieties except for the middle part of the track where Leigh group has replaced the triazole ring with a stilbene unit. They report that the walker can be transported in either direction depending on the sequence in which the stimuli are applied. The crucial function of the stilbene unit is that it can be isomerised using light for the purpose of creating or releasing ring strain in order to achieve directionality. The way the machine operates is as follows: firstly, an acid or base stimulus is applied depending on which end the walker moiety resides on while the stilbene unit is in its Z-isomer to move the walker to the central part without creating ring strain. Then, the stilbene moiety is
isomerised to the $E$-form, which introduces a great deal of ring strain. Finally, an acid or base stimulus is applied depending on which direction the walker moiety is to be transported. The ring strain created by the $Z \rightarrow E$ transformation is released by the subsequent application of the stimulus when the walker moves away from the stilbene unit to form a less strained macrocycle, which in turn provides directionality to the system through an energy ratchet mechanism.

![Diagram of the bidirectional small molecule walker](image)

**Figure 11** Design (top) and operation (bottom) of the bidirectional small molecule walker. Conditions: (i) 0.1–10 mM, $h\nu$ (365 nm; bandwidth: 10 nm), CD$_2$Cl$_2$, RT, 5 min (0.1 mM) to 1 h (10 mM); (ii) 0.1 mM, DTT (10 equiv), DBU (40 equiv), (MeO$_2$CCH$_2$CH$_2$S)$_2$ (20 equiv), CHCl$_3$, RT, 24 h; (iii) 0.1 mm, I$_2$(ca. 10 equiv), $h\nu$ (500 nm; bandwidth: 10 nm), CD$_2$Cl$_2$, RT, 6 h; (iv) 0.1 mM, TFA (excess), CHCl$_3$, RT, 48 h. Adapted from reference. 26

As an example, the process is shown in **Figure 11**: the $E$-1,2-1 isomer is photochemically isomerized to $Z$-1,2-1 with 88% efficiency (**Figure 11, condition i**), which is then subjected to basic conditions (**Figure 11, condition ii**) to kinetically lock the hydrazine linkage and make labile the disulfide linkage so that the foot can move and either form the macrocycle with foothold 3 or rebind to foothold 1 to form the original macrocycle. This step favours the $Z$-2,3-1 positional isomer, which is then photoisomerized to $E$-2,3-1 with 75% efficiency (**Figure 11**, condition iii).
condition iii). Finally, switching the media to acidic conditions (Figure 11, condition iv) enables the hydrazone foot to move freely while the disulfide linkage is kinetically fixed and transport the walker to the opposite end to release the ring strain with a yield of $>95\%$. It is worth noting that all the required energy to fuel the directional transportation of the walker is supplied during the $E$ to $Z$ isomerization in the first step. Although this light-driven linear molecular motor system can transport the walker moiety in either direction in a unique sense not observed in nature, the flexibility of the whole molecule and folded products compromise the net directionality, and improvement of the bias is a weakness that needs to be addressed.

These small molecular walker designs published by the Leigh group satisfy all four fundamental characteristics of a walker: namely processivity, directionality (to some extent), repetitive operation and progressive operation; however they lack the ability to operate autonomously, meaning that they require external stimuli before taking any steps. The Leigh group has addressed this issue in a system that was published in 2012, where they introduce a different approach from those previously published. Taking into account the fact that so-called equilibrium transfer alkylation crosslinking (ETAC) reagents (introduced to the literature in 1970s) are able to transport themselves along proteins to accessible nucleophilic sites through reversible Michael and retro-Michael reactions (Figure 12), they designed a small molecular system where $\alpha$-methylene-4-nitrostyrene units are autonomously transported with high degree of processivity along oligomeric tracks comprising secondary amines. Under basic conditions, the $\alpha$-methylene-4-nitrostyrene moiety exchanges reversibly between the amino groups of the track through Michael and retro-Michael reactions and positions themselves somewhere along the track until the most thermodynamically favoured distribution is reached. They also introduced an anthracene unit at the opposite end of a track containing 5 amine groups and
monitored the fluorescence emission spectrum. As the walker moves along the track and approaches the anthracene unit, they observed the diminishing of fluorescence intensity by 54% over 6.5 h due to quenching by the nitrostyrene group.

The Leigh group extended this study in 2013 and published oligomeric tracks with 3, 5 and 9 footholds and replaced the anthracene unit with naphthylmethylamine group that acts as a thermodynamic sink, resulting in net directional migration of walker even in the presence of other 8 other footholds (Figure 13). In the case of tracks containing n=3 and 5 footholds, they were able to detect the percentages of each positional isomers by NMR; however, when it comes to the longer n=9 track,

![Diagram](image)

**Figure 13** a) Walking process in the presence of 10 equivalent of i-Pr₂NEt. b) Partial ¹H NMR spectra of the reaction mixture over time. 19±3 % of the walkers are positioned on the 9th foothold after 90 h. Green peaks correspond to the compound where the walker is on the 9th foothold (10-9). Adapted from reference.
they were only able to quantify the number of walkers on the ends with respect to the walkers on the inner footholds (Figure 13b). In all cases, they observed a significant bias towards the thermodynamic sink. Even though the walker migrates directionally towards a thermodynamic sink, it cannot be called intrinsically directional. This study paves the way to more complex systems where cargoes can directionally be transported along lengthy distances at the molecular level.

A similar concept was introduced by Lehn’s group in 2012, where they investigated the imine formation/exchange dynamics of salicylaldehyde, pyridine-2-carboxaldehyde, and benzaldehyde, among which salicylaldehyde (SALAL) seems to be the most promising candidate to design a small molecule walker due to its high efficiency and high reaction rates. SALAL functioning as a walker moiety is attached to a polyamine track as an imine on one end, and internal exchange process through self-transimination is observed, which leads to the migration of SALAL group through “stepping-in-place” or “single-step” to the neighbouring amines following an inchworm-type process (Figure 14). They observed that the rate of the exchange reaction decreases dramatically and rapidly as the distance from the SALAL to the amine increases. This decrease in rate is a great advantage

![Figure 14](image1.png)  
*Figure 14* Mechanism of the inchworm-type motion of the Salicylidene residue (shown in red). Adapted from reference.
to make sure that the walker does not overstep. In order to rule out overstepping, the oxa-analogue of triethylenetetraamine was synthesized and studied where the internal nitrogen atoms are replaced with oxygen. No overstepping was observed, and the walker was only transferred intermolecularly to another amine, depending on the concentration. Another concern to be addressed was whether the migration occurs processively: it was found, unsurprisingly, that the intermolecular exchange depends strongly on the concentration. Two other critical factors that influence the rate of the exchange are solvent system and the substituent on the walker unit. When a nitro benzene derivative is used as a walker, a fifty-fold increase in the rate of exchange was observed. Switching from plain acetonitrile-d$_3$ to a mixture of CD$_3$CN/D$_2$O (1:1) results in an eighty-fold increase in the rate. Overall, imines appear to be promising candidates to design small-molecule systems where cargoes can be transported by means of walking.

A follow-up paper was published by the same group in 2015,$^{30}$ reporting the design of a pH-driven bidirectional walker on a non-symmetrical polyamine track (Figure 15). The walker moiety chosen is a slightly modified version to that previously published, where they introduce a carboxylic acid unit to the benzene ring. The function of the carboxylic acid is to form a lactone at the end of the track. The hydroxyl group on the walker is retained, lowering the tendency of the machine to form aminals and maximising the favourability of imine formation. The machine operates as follows: 2-formyl-3-hydroxybenzoic acid (SAXAL) in its acid form is

![Figure 15](image_url)
attached quantitatively to the hydroxyl amine end of the polyamine track as a lactone (When SAXAL is introduced as a sodium salt, full condensation to form an imine at the primary amine site is observed). When the lactone is treated with a base (DBU or tBuOK), the walker migrates to the opposite end gradually to form an imine. A slight excess of base is used to push the equilibrium towards the imine formation, as the lactone has higher stability. In order to demonstrate the reversibility of the system, the imine is back-titrated using three equivalent of TFA to give full conversion to the lactone at the other end. If the acid is added all at once, displacement of the walker from the track as an aldehyde (20%) is observed, likely due to the generation of large localised pockets of acidity; this was prevented by gradual addition of the acid. Although the walker itself is not intrinsically directional, it represents an important step toward designing systems where cargos can be transported bidirectionally.

Although a variety of DNA based and small-molecule walkers have been reported in the literature, one of the long-standing problems that need to be addressed is the challenge of real-time monitoring of the translational motion of the walker molecules. Bayley’s group addressed this issue in 2015 and presented a system where they could monitor the motion of an organoarsenic (III) walker (Figure 16a) over a track bearing 5 footholds in real time continuously within a protein nanoreactor (Figure 16b). The walker moiety bearing thiol ligands is able to attach/detach itself from the cysteine residues that are located 6 Å apart on the α-haemolysin protein pore through a thiol exchange process. They managed to measure the duration and direction of the walker with millisecond time resolution and atomic precision at room temperature, in water and at neutral pH by recording changes in flow of ionic current through the pore, which reflects individual walker molecule taking a step by breaking/forming As-S bonds. The walker moves in a random fashion towards a well at foothold 5, with a slight bias and a weak processivity (6 ± 1 steps per outing). It moves considerably faster than the previously published covalent bond-based walkers: the authors argue that this rapid movement occurs at the expense of processivity. Interestingly, they observe disproportionality between the rate and processivity. They also note that the speed of motion can be manipulated by varying the temperature, pH and nature of the thiol ligands. Finally, they note addition of a sulphophenyl group on the walker could allow incorporation of a cargo to be transported in future designs.
As a promising alternative to the dynamic covalent chemistry approach for the synthesis and design of small-molecule walkers, the Leigh group presented a different approach in 2014, where they assembled a bimetallic biped consisting of three footholds (Figure 17a). Although this is a biped and therefore cannot be called a walker, it paves the way towards the synthesis of small-molecule walkers reminiscent of what is observed in Nature in terms of the nature of the binding. Nature cleverly and subtly controls the thermodynamics and kinetics of the binding motifs by means of manipulating the hydrogen bonding and electrostatic
interactions to create bias away from equilibrium. However, mimicking this behaviour is not straightforward, as those interactions are short-lasting and typically fairly weak. Therefore, interaction by means of metal coordination presents an advantage by combining the robustness of covalent chemistry and reversibility of what is observed in Nature. The walker

Figure 17 a) “Stepping” of a molecular biped with two metal-complex “feet”. b) Operation of stepping of a Pd(II)/Pt(II)-Complexed Molecular Biped. Adapted from reference.

moiety features two identical feet comprised of a tridentate pyridine-2,6-dicarboxamide motif, which is able to form metal complexes with derivatised pyridine-type stations (footholds) on the track. The foot with the Pd complex can be moved between pyridine and DMAP type footholds while the foot with the Pt-pyridine type complex is kinetically locked. Starting from when the Pd foot is on the DMAP-foothold, addition of 1 equivalent of methanesulfonic acid results in the
protonation of the pyridine-foothold with no discernible change in the NMR peaks of the DMAP part suggesting that the foot is still on the DMAP-foothold. Even after 30h at room temperature, no change is observed suggesting that no positional isomerization occurs and the metal complexes are kinetically locked. However, heating the solution up to 338 K, the system reaches to equilibrium after 40h and final deprotonation with K$_2$CO$_3$ results in 85% of the walker taking a step and positioning itself on the pyridine-foothold, thus creating a bias towards the pyridine-foothold. When this mixture is heated again at 338 K, a redistribution of positional isomers occurs with 95% of the walker on the DMAP-foothold after 24h (Figure 17b). These results indicate that the walker can be moved in either direction while the Pt-foot is fixed.
Aims and Objectives

Various types of motor proteins observed in Nature that are able to transport cargoes and perform different tasks in cells by means of walking on a polymeric track have been discussed so far. The fundamental characteristics comprising a “walker” and how those properties are mimicked by scientists to create small-molecule walkers have been discussed as well. Although there have been several compelling examples described in the literature, one challenging property, namely directionality, must still be addressed in greater detail as there is no example of a small molecule that operates in a true directional fashion. Although Lehn’s pH driven system and Leigh’s Michael system have the appearance of walking directionally on their tracks, the walker in fact is simply moving towards a thermodynamic sink. Each step they take is not truly directional; therefore, despite an overall movement towards one direction, they cannot be regarded as intrinsically directional. Another example published by the Leigh group is the bidirectional walker that exploits the ring strain created by the isomerization of a stilbene unit to move the walker in a preferred direction. This step is truly directional; however other steps to reach the middle part of the track to utilise ring strain effect lack directionality. It is the aim of the project discussed in Chapter 2 to design and synthesise a small molecule that is able to walk towards one direction with each step being truly directional. In order to achieve this, the machine is designed in a way that it is able to exploit secondary interactions, namely hydrogen bonding in this case, between the walker moiety and the track through squaramide motifs that are incorporated into the design.
**An Introduction to Molecular Shuttles**

Nature ubiquitously uses molecular machines constructed over billions of years of evolution in order to perform extremely complex tasks.\(^{33}\) This widespread use of molecular machines in biology has long inspired scientists to design synthetic systems\(^{34}\) that mimic Nature’s way of exploiting molecular-level motion and to create artificial architectures to perform work in the way observed in Nature, albeit not as complex. Stimuli-responsive molecular shuttles are one of the many versatile structures classified as switch-like molecular machines,\(^{35}\) which have the potential ability to progressively perform work when incorporated into a ratchet system.\(^{36}\) In particular, artificial motors powered by light or electricity are under prominent consideration in the construction of molecular devices due to the convenience of energy input and the absence of waste products.\(^{37}\) A molecular shuttle is a rotaxane that bears two (or more) binding sites (stations) for the macrocycle, which are connected via a navigable pathway. The first two-station degenerate [2] rotaxane exhibiting a temperature dependent shuttling was first published by Stoddart’s group in 1991\(^{38}\) and many including the non-degenerate ones have followed since then.

One of the most important requirements\(^{39}\) to design a stimuli-responsive molecular shuttle is that one of the stations should be able to switch between two states, one of which has higher affinity towards the macrocycle than the second (non-switchable) station, and the other of which has a lower affinity than the second. Therefore, the macrocycle populates over the stations according to the difference in binding affinities between stations and the macrocycle depending on the external stimulus.

**Figure 18** Schematic representations of: a) a [2]catenane and b) a [2]rotaxane. The arrows show the possible directions of large amplitude motion. Adapted from reference.\(^{40}\)
applied such as changing temperature, pH, oxidation state of a station, solvent polarity as well as irradiating with light, performing a chemical reaction and through anion or cation coordination.

Rotaxanes (Figure 18b) and catenanes (Figure 18a), being members of the class of mechanically interlocked molecules are important constituents of molecular machines as they provide a bottom-up approach towards building up relatively complex machineries through (typically) self-assembly. Rotaxanes are molecules where one or more macrocycles are threaded along one or more linear units (threads) and mechanically prevented from coming off these linear units by bulky units (stoppers). Catenanes are molecules where two or more macrocycles are mechanically interlocked. Although their components are not covalently connected to each other, at least one covalent bond must be broken in order to separate the components from each other. Since rotaxanes form the basis of the project discussed in Chapter 3, approaches towards making rotaxanes and their applications into designing stimuli responsive molecular shuttles are outlined below.

Synthesizing mechanically-interlocked structures was, for a long time, based on lengthy covalent-directed strategies, which represents a time-consuming and difficult approach considering their complex molecular structures. Thankfully, with the notable advances in supramolecular chemistry, their synthesis became much more straightforward, which has consequently paved the way for the construction of complex molecular machines based on rotaxanes. Chemists have exploited supramolecular interactions to develop several templated synthetic strategies, including clipping and threading. The recognition motifs that are subtly incorporated into the thread could also serve as the station in molecular shuttles.

The very first molecular shuttle (Figure 19) was designed and synthesized by Stoddart’s group in 1991. The shuttle is a rotaxane comprised of triisopropylsilyl stoppers (Figure 19, shown in green and black circles), a cyclobis (paraquat-p-phenylene, CBPQT) cyclophane macrocycle (Figure 19, shown in blue) and two identical hydroquinol stations (Figure 19, shown in black and red ribbons), which was synthesized via a clipping method. The shuttle exhibits a temperature dependent behavior: by measuring the NMR spectrum of the machine at different temperatures it was found that at elevated temperatures the representative peaks are merged into one, due to fast shuttling of the macrocycle between two stations on the NMR timescale. However, when the system is cooled down, the speed of the shuttling
decreases on the NMR timescale, and the representative peaks show up as two different sets of occupied and unoccupied stations, which is evidence of temperature-dependent shuttling. Since the two stations are identical, there is no bias towards any of the stations; however, this work is important as it is the very first molecular shuttle introduced to the literature.

Figure 19 First Molecular Shuttle. Adapted from reference.\textsuperscript{38}
Stimuli Responsive Molecular Shuttes

Motion in kinetically-stable rotaxanes can be controlled by having multiple binding sites and disrupting or changing the interaction of the macrocycle towards those binding sites through a variety of different stimuli. By weakening the existing interaction between a station and macrocycle or increasing the interaction of the macrocycle towards another station, the macrocycle can be moved to another location. Light-controlled motion was observed in a single station rotaxane comprising a “blue-box” type macrocycle (Figure 20, shown in purple and green), comprised of a bulky redox-active ferrocene type stoppers (Figure 20, shown in blue and orange) and an electron rich dioxyarene station (Figure 20, shown in red and pink). An electron transfer from dioxyarene to the macrocycle is initiated by light irradiation, which results in the generation of an intimate radical ion pair, which was subsequently followed by some of the radical ion pairs to undergo another electron transfer in which the electron hole is transferred to one of the stoppers that is in close proximity due to the secondary pi-stacking interactions. This spatial charge separation is relatively long-lived, allowing the macrocycle to shuttle away from the oxidized stopper due to electrostatic repulsion. It can therefore be called a light-induced molecular shuttle due to the fact that absorption of light results in a displacement of the macrocycle.

Figure 20 Light induced single station molecular shuttle. Adapted from reference.52

41
The hydrogen bonding in peptidic type rotaxanes shown below (Figure 21) can be controlled by changing the environment, in this case the solvent, in order to manipulate the effects of the communication between the thread and rotaxane. For example, the rotaxane in Figure 21a interestingly exists solely as the E-rotamer in C_2D_2Cl_4 due to stabilization of the E-tertiary amide rotamer; however once dissolved in [D_6] DMSO, the communication between the thread and macrocycle is severely compromised as the solvent now competes with the macrocycle for hydrogen-bonding sites, and consequently the stabilization of the E-rotamer is not as efficient, thereby allowing the rotaxane to exist in both rotamers\(^5\). A similar example is the rotaxane in Figure 21b where the achiral macrocycle is positioned in close proximity to a chiral center. In chloroform, the chirality is conveyed through the macrocycle, which in turn affects the conformation of C-terminal diphenyl methyl moiety, thereby allowing it to have an ICD (induced circular dichroism) response. However, once switched into methanol, the communication between the thread and the macrocycle is lost, thereby the chirality cannot now be conveyed through the macrocycle, thus resulting in a loss of the ICD response of the diphenyl methyl moiety that was observed in chloroform\(^5\).

Figure 21 Manipulation of the communication in single-station dipeptide [2]rotaxanes. a) In C_2D_2Cl_4, E rotamer is stabilized due to hydrogen bonding between the thread and macrocycle so that this is the sole conformation observed by NMR spectroscopy. In [D_6] DMSO, both rotamers are observed because hydrogen bonding is switched off. b) In CHCl_3, the macrocycle is in close proximity of the chiral center, thereby conferring a chiral nature on its conformation which, in turn, affects the conformation of diphenyl methyl moiety and results in an ICD response. In MeOH, the communication is lost because the hydrogen bonding is switched off. Adapted from reference\(^5\),\(^5\)
Another noteworthy example of a one-station stimuli responsive shuttle is the rotaxane in Figure 22 featuring a cyclodextrine (CD) macrocycle and a thread containing a photo-responsive stilbene unit (Figure 22, shown in purple and orange). In its $E$-form (Figure 22, left hand side), the macrocycle resides mostly over the central aromatic units; however, upon irradiation with light, the stilbene unit is isomerized into its $Z$-form (Figure 22, right hand side), and the macrocycle cannot now be positioned over the central unit due to steric concerns arising from the kinked thread, and it thus moves away from the central unit. What is interesting here is the fact that the nature of the movement is unidirectional as it is biased towards where the 6-rim of the CD is always located closer to the stilbene unit. \(^{45g}\)

![Figure 22](image)

**Figure 22** Photoresponsive stilbene-based single-station molecular shuttle. Adapted from reference.\(^{45g}\)

This is of particular interest due to the fact that it exhibits a ratchet-type behavior by moving unidirectionally. It is important to note that the bias is caused because of the asymmetric nature of the macrocycle even though the thread is a symmetrical one.

**Stimuli Responsive Molecular Shuttles with Two or More Binding Stations**

**pH-Driven Molecular Shuttles**

The very first bistable molecular shuttle with two binding sites (Figure 23) was reported by Stoddart’s group in 1994,\(^{55}\) it is overwhelmingly similar to the first molecular shuttle reported by the same group that was explained above (see Figure 19) except for the fact that the hydroquinol stations are replaced by benzidine (Figure 23, shown in light blue and pink) and biphenol (Figure 23, shown in orange) motifs. These two stations are electron-rich and act as electron-donating units to the macrocycle (Figure 23, shown in dark blue). NMR studies reveal that there exists a fast shuttling at a rate comparable to NMR time scale between two stations at room temperature resulting in broadening of many peaks in CD$_3$CN.
solution. This precludes a detailed assessment as to which station the macrocycle prefers to reside on; however, cooling the system to 229K, the rate of the shuttling is slowed down and distinct peaks arising from where the macrocycle resides on—namely translational or positional isomers—start to appear. Detailed assessments of the spectrum reveal that the macrocycle predominantly resides over the benzidine site with an occupation preference of 84% over the biphenol site.

Figure 23 First bistable molecular shuttle. Adapted from reference.55

This is in agreement with the previous binding studies conducted between model compounds and the macrocycle. The preference of the macrocycle towards the different stations can be switched by either protonation or oxidation of the benzidine unit. The electrostatic repulsion created by this switching between the positively-charged macrocycle and the benzidine unit dramatically diminishes the interaction between the two and renders the macrocycle to spend significantly more time on the other station. Quantitatively speaking, protonation of the basic nitrogen atoms of the benzidine unit with excess amount of deuterated TFA results in more than 98% of the macrocycle to reside over the biphenol station. Neutralization of the protonated rotaxane with added deuterated pyridine regenerates the original NMR spectrum with identical distribution. Changing pH is one of the most useful stimuli to initiate
and control translational motion as hydrogen bonding, electrostatic and ion-dipole interactions can be manipulated by simple protonation and deprotonation cycles. This is the first example of an acid-driven molecular shuttle based on a relocation of electron poor macrocycle. One drawback of this system is that it presents poor positional integrity in its non-protonated form as the binding constants between the macrocycle and the two stations are comparable. In order to ensure adequate bias, much higher difference in binding constants are required. Crown ether-based macrocycles are good candidates providing this opportunity. Their interaction with protonated amines and charged species inspired scientists to design rotaxane-based molecular shuttles with remarkable positional integrity in both protonated and non-protonated forms. The first example of a crown ether-based pH-driven molecular shuttle (Figure 24) reported by Stoddart group is depicted above. The Rotaxane in Figure 24 is comprised of a dibenzo crown ether macrocycle (Figure 24, shown in red) and dibenzylamine (Figure 24, shown in light blue) and bipyridinium (Figure 24, shown in dark blue) stations exhibits remarkable positional discrimination.\(^4_{6a}\)
Protonation of the dibenzylamine unit with TFA results in the macrocycle residing predominantly but not exclusively over the ammonium station mainly due to strong N\(^+\)-H…O hydrogen bonds and weak C-H…O bonds from the neighboring methylene groups next to the nitrogen. Moreover, deprotonation of the ammonium station with diisopropylethylamine reverses the discrimination and moves the macrocycle to reside predominantly over the bipyridinium station and creates remarkable positional integrity (>98%). It is important to note that although this system is a very good example of positional integrity in both states, the relatively low binding constant between the macrocycle and the bipyridinium station might get in the way when designing more complex systems especially if the macrocycle has more translational freedom. On a similar note, Balzani and Stoddart designed and published what they called a “molecular elevator” using a combination of the rotaxane-based system described above (Figure 25). The platform comprising a three-macrocyclic system based on dibenzo crown ethers (Figure 25, shown in red) moves up and down in a similar fashion to the one above upon protonation and deprotonation. Titration experiments and modelling studies reveal that the shuttling takes place in a stepwise manner and the combination of three macrocycles provides an excellent positional discrimination in both forms. Furthermore, an analogue of the platform featuring dioxynaphthalene trismacrocyclic unit exhibits even stronger binding to the bipyridinium stations. This is a good example of the application of pH-driven molecular shuttles.

**Figure 25** A molecular elevator. Adapted from reference.\(^{56}\)
A different approach to exploit hydrogen bonding other than cationic species would be through protonation of an anionic station. The first example towards that approach was published (Figure 26) by Leigh group in 2004.\textsuperscript{47a} Rotaxane in figure 26 features a commonly used benzylic tetra-amide macrocycle (Figure 26, shown in blue) shuttling between succinamide (Figure 26, shown in orange) and phenol type stations (Figure 26, shown in purple and green). In its neutral form, the macrocycle prefers the succinamide station (>95%) over the phenol station due to the strong hydrogen bonding between the succinamide oxygens and four NH protons of the macrocycle and due to phenol being a poor hydrogen bonding partner.

Upon deprotonation of the phenol in deuterated DMF using variety of bases such as LiOH, NaOH, KOH, CsOH, Bu\textsubscript{4}NOH, t-BuOK, DBU, and Schwesinger’s phosphazene P1, the macrocycle repositions itself over the phenolate station due to the creation of stronger hydrogen bonds. The fact that various bases are effective and addition of other ions up to ten equivalents does not compromise the shuttling suggests that the counter ion of the base and accompanying cations have no remarkable effect on the shuttling. However, the whole process is highly solvent dependent. Surprisingly, the system works best in DMF although it is usually expected that hydrogen bond-driven molecular shuttles work well in non-polar
solvent systems due to the fact that they do not compete for hydrogen bonding. On switching solvents to CDCl$_3$ or CD$_2$Cl$_2$, intramolecular folding of the rotaxane occurs upon deprotonation so that the phenolate ion can hydrogen bond to the macrocycle while it resides on the succinamide station. Competition by the solvent for hydrogen bonding is an advantage in this case, making the shuttling possible. The fact that shuttling occurs in DMF can be explained by the fact that the phenolate anion can only hydrogen-bond to one isophthalamide unit of the macrocycle and the remaining amides are left unsatisfied; therefore, DMF hydrogen bonds to the unsatisfied remaining amides.

**Shuttling Through Anion Recognition**

A first bis-imidazoliucontaining rotaxane synthesized via anion templated self-assembly was developed and published by Beer’s group in 2011.$^{47g}$ The shuttling is controlled through chloride anion recognition. The rotaxane features an isophthalamide macrocycle (Figure 27, shown in red), 2,6-bis-imidazolium-pyridine-cored thread (Figure 27, shown in blue) with two amide stations on the opposite sides of the imidazolium station. In the hexafluorophosphate salt, the macrocycle is confirmed to reside on the amide station next to the stoppers through N-H...O (carbonyl) hydrogen bonding interaction. Upon addition of chloride anions, the macrocycle moves to the central imidazolium station due to encapsulation of the chloride anion by both macrocycle and the track. The position of the macrocycle is confirmed by comparing the NMR spectra of both systems depicted in Figure 27.

![Figure 27](image)

**Figure 27** $^1$H NMR spectra of rotaxane 8 as its 2PF$_6$ salt and changes observed upon addition of one equivalent of TBACl (acetone-d$_6$, 293 K). Adapted from reference.$^{47g}$
Shuttling Through Redox Process

The very first bistable pH-driven molecular shuttle\textsuperscript{55} depicted above (see Figure 23) by Stoddart can also operate by means of adding or removing electrons. The benzidine station is a redox-active species, and oxidation creates an electrostatic repulsion that pushes the macrocycle to the other station. This is an example of a reagent-free shuttling using a redox process. However, it still bears the problem of not having high positional integrity in the ground state. In order to address this issue

![Image](image_url)

**Figure 28** Shuttling through redox process. Adapted from reference.\textsuperscript{46h}

and create highly biased translational isomers towards one station in the ground state, researchers have presented several different systems which have so far not produced remarkable outcomes.\textsuperscript{58} In an effort to search for better stations, redox active tetrathiafulvalene (TTF) units were incorporated into the system and have paved the way for more intensive studies towards the design of several rotaxane- and catenane-based structures. One remarkable example (Figure 28) is depicted above\textsuperscript{46h}. The Rotaxane in Figure 28, based on a blue-box type macrocycle (Figure 28, shown in dark blue), can be shuttled between a redox active TTF station (Figure 28, shown in pink and green) and dioxyarene station (Figure 28, shown in orange). Oxidation of the TTF unit creates an electrostatic repulsion that pushes the macrocycle away from the positively-charged TTF unit. One important finding is that there is a significant kinetic barrier that the macrocycle has to overcome before moving back to the original station when the reduction of the previously oxidized TTF takes place. At low temperatures this metastable state can be observed, where the macrocycle resides over the dioxyarene station in the reduced form. On the way
from TTF to dioxyarene, the kinetic barrier is relatively low due to the unfavourable interactions between the macrocycle and TTF.

A redox driven molecular shuttle with excellent translational integrity has been described by the Leigh group (Figure 29).\(^5^9\) The benzylic tetraamide-type macrocycle (Figure 29, shown in blue) is shuttled between a succinamide station (Figure 29, shown in orange) and redox active 3,6-di-tert-butyl-1,8-naphthalimide station (Figure 29, shown in green and purple), which are separated by 12 carbon atoms. The binding affinity of the succinamide station towards the macrocycle is so great that the formation of the rotaxane is carried out through templating around this station. However, the naphthalimide-type station is a very poor hydrogen-bond acceptor. Having this dramatic difference in the binding constants results in an extreme positional fidelity that the isomer where the macrocycle resides over the succinamide station is the only isomer detectable by NMR in CDCl\(_3\), CD\(_3\)CN and [D\(_8\)] THF. Even in highly polar deuterated DMSO, which can compete effectively
for hydrogen bonds, the macrocycle only spends about half the time over the succinamide station. Upon one-electron reduction of the naphthalimide station, the roles are reversed and the macrocycle is pulled to the other station due to the increased electron density on the carbonyl groups of the naphthalimide. The degree of discrimination is about 1:500. Reoxidation of the naphthalimide unit restores everything back to original and the macrocycle returns to succinamide station.

**Shuttling Through Metal Complexation**

A collaborative work (Figure 30) between the groups of Sanders and Stoddart was introduced in the literature in 2004, featuring a rotaxane system that presents a shuttling behaviour under variety of different stimuli.\(^{48a, 60}\) Characterised by NMR, the rotaxane in Figure 30 has a major translational isomer where the macrocycle (Figure 30, shown in red) predominantly resides over the naphthaldimide station (Figure 30, shown in purple).

![Figure 30](image)

**Figure 30** Shuttling induced by lithium cations. Adapted from reference\(^ {48a, 60}\).

Upon one-electron reduction of this station, the negatively-charged naphthaldimide unit pushes the macrocycle away to the other pyromellitic diimide station (Figure 30, shown in green). However, this is not the only way to induce shuttling as addition of lithium ions unexpectedly has the same shuttling effect as the reduction process. Complexation of two equivalents of lithium ions with crown ether oxygens and carbonyls of the diimide units is much stronger at the pyromellitic diimide
station than naphthalenediimide station. The macrocycle is thereby shuttled to the green station by addition of metal ions. The process can be reversed by addition of an excess of 18-crown-6, which extracts the lithium ions from the rotaxane and thereby destroys the metal complexation. Another way to induce shuttling rather than by enhancing the binding interaction of the macrocycle to a certain station is to do the opposite: weaken the interaction between the primary binding station and the macrocycle. Two examples of such a mechanism were reported by Leigh’s group in 2004 (Figure 31). Rotaxanes in Figure 31 operate in a similar fashion, but with some subtle differences. The macrocycle of rotaxane in Figure 31a (shown in blue) predominantly prefers the glycylglycine station (Figure 31a, shown in green) located next to a bis(2-picolyl)amino (BPA) stopper over the other succinic amide ester station (Figure 31a, shown in orange). Addition of one equivalent of Cd(NO$_3$)$_2$ • 4H$_2$O results in a metal complexation where three nitrogen atoms of Cd(NO$_3$)$_2$ • 4H$_2$O results in a metal complexation where three nitrogen atoms of

BPA stopper and carbonyl oxygen immediately next to the stopper are bound to the cadmium cation. The macrocycle continues to sit over the glycylglycine station as the complexation does not disrupt the system to a great extent. However, addition of

\[ \text{Cd(NO}_3\text{)}_2 \cdot 4\text{H}_2\text{O} \]

\[ \text{BPA stopper and carbonyl oxygen immediately next to the stopper are bound to the cadmium cation. The macrocycle continues to sit over the glycylglycine station as the complexation does not disrupt the system to a great extent. However, addition of} \]

\[ \text{Cd(NO}_3\text{)}_2 \cdot 4\text{H}_2\text{O} \]
a base results in deprotonation of the amide that is next to the BPA stopper, which in turn causes the complex to adopt a new coordination site, which is the freshly deprotonated nitrogen. As this change dramatically disrupts the system, it destroys the interaction of the macrocycle with the glycylglycine station and moves the macrocycle to the succinic ester amide station. The whole process is reversible, and the original state can be restored by removing the metal with cyanide and protonating the amide with ammonium chloride. In the rotaxane in Figure 31b, the macrocycle (Figure 31b, shown in blue) again predominantly prefers the succinamide station (Figure 31b, shown in green) adjacent to the BPA stopper over the succinic ester amide station (Figure 31b, shown in orange). In this case however, addition of the cadmium salt immediately causes the macrocycle to move over to the other station without the need for deprotonation. This is explained by the fact that cadmium cation in this case is only able to chelate to three nitrogen atoms of the BPA stoppers, and in order to accommodate this binding mode, the conformation that must be adopted by the system significantly distorts the 3D shape of the station, which in turn disrupts the binding interaction between the station and

---

[Figure 32] Structures of Cu$^I$ (1$_{4+}$) and Cu$^{II}$ (1$_{5+}$) rotaxanes in their thermodynamically stable forms. The subscripts 4 and 5 indicate the coordination number of the copper, excluding solvent molecules or counterions. Adapted from reference.\textsuperscript{61}
the macrocycle. Therefore, the macrocycle is directly pushed to the other station. Again, this shuttling process is fully reversible and the macrocycle can be shuttled back to its original station by removing the metal with cyanide.

Altering the oxidation state of a metal is another way to manipulate the shuttling behaviour of a rotaxane system. Sauvage’s group introduced such a system to the literature in 2009\textsuperscript{61} where the affinities of a copper cation to different stations in different oxidation states are exploited. The rotaxane features a macrocycle containing a 8,8’-diphenyl-3,3’-biisoquinoline (dpbiiq) bidentate ligand and a thread containing three different stations: 2,9-diphenyl-1,10-phenanthroline (dpp) (Figure 32, station on the left hand side), 2,2’-bipyridine (bipy) (Figure 32, station in the middle) and a 2,2’,6’,2”-terpyridine (terpy) (Figure 32, station on the right hand side) unit. The bipy unit functions as an intermediate station between the terminal stations so that the shuttling between the two is much faster than the analogous one without the intermediate station. The shuttling behaviour of the macrocycle relies on the relative stabilities of the copper(I) and copper(II) complexes formed between the macrocycle and different stations of the thread. The thermodynamic stability of the copper(I) complexes increases from $[\text{Cu(terpy)(dpbiiq)}]^+$ to $[\text{Cu(dpp)(dpbiiq)}]^+$: $[\text{Cu(terpy)(dpbiiq)}]^+ < [\text{Cu(bipy)(dpbiiq)}]^+ < [\text{Cu(dpp)(dpbiiq)}]^+$. The stability sequence is reversed for Cu(II) complexes: $[\text{Cu(dpp)(dpbiiq)}]^{2+} < [\text{Cu(bipy)(dpbiiq)}]^{2+} < [\text{Cu(terpy)(dpbiiq)}]^{2+}$. 

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure33}
\caption{Shuttling through Diel-Alder retro Diels-Alder reaction. Adapted from reference.\textsuperscript{62}}
\end{figure}
Shuttling Through Chemical Reaction

Although this type of shuttling has rather surprisingly not been extensively studied, there are a few examples introduced to the literature. Rotaxane in Figure 33 by the Leigh group features a macrocycle (Figure 33, shown in blue) that sits predominantly over a fumaramide station (Figure 33, shown in green). The double bond in the fumaramide station can be used to carry out a Diels-Alder reaction with cyclopentadiene, which gives a product (Figure 33, shown in purple) that creates a steric bulk, interfering with the interaction with the macrocycle. Consequently, the macrocycle moves over to the other station (Figure 33, shown in orange) with excellent positional discrimination. As Diels-Alder reactions are reversible, this shuttling can be reversed through a retro Diels-Alder process.

Another example (Figure 34) by Abraham et al. presents a system where photocleavage of a chemical bond induces shuttling. The blue-box type macrocycle (Figure 34, shown in purple) resides over the diarylcycloheptatriene station (Figure 34, shown in green) that acts as an electron donor unit. The rotaxane adopts a folded shape so that the macrocycle can interact with the other station as well. Upon irradiation with light, a photoheterolysis reaction takes place, where the cleavage of the methoxy group on cycloheptatriene creates a positive charge (Figure 34, shown in pink).

Figure 34 Photoheterolysis-induced shuttling. Adapted from reference.
Consequently, the macrocycle shuttles over to the anisole station (Figure 34, shown in orange). The process is reversible as the life time of the species formed as a result of light irradiation is about 15 seconds at room temperature, and the system restores back to the original state through thermal process (or through a nucleophilic attack of methoxy group to the seven membered ring to give regioisomers). Last example (Figure 35) is from Berna’s group where shuttling is induced through a Mitsunobu reaction.\(^{46j}\) One of the stations on the rotaxane is an azodicarboxamide type station (Figure 35, shown in red, light green and purple) on which the macrocycle (Figure 35, shown in dark green) predominantly sits due to the perfect hydrogen bonding caused by the double bond that preorganises the hydrogens. This station also acts as a reagent in the Mitsunobu reaction. After performing the Mitsunobu reaction, the azodicarboxamide station is reduced and the double bond that has a critical role in hydrogen bonding no longer exists, displacing the macrocycle to the other station (Figure 35, shown in orange). Upon oxidation of the station back to azodicarboxamide, the macrocycle returns to its original position.

![Diagram](image.png)

**Figure 35** Shuttling through Mitsunobu reaction. Adapted from reference.\(^{46j}\)

**Shuttling Through Light Irradiation**

Shuttling through light irradiation is a desirable stimulus, as it does not require the addition of any external chemicals. If the back reaction occurs spontaneously through thermal relaxation, the whole process becomes autonomous when the light irradiation is carried out intermittently, such that the product has enough time to go
back to the initial state. If the process involves electron transfer, one has to carefully design the system such that the back electron transfer is not too fast so that the

![Figure 36](image)

**Figure 36** Shutting through photoinduced internal charge separation. a) Chemical structure of the rotaxane. b) The operation cycle of the shuttling. Irradiation (1) of the Ruthenium complex results in electron transfer (2) to the blue station. Charge recombination process (3) is slow enough to allow approximately 10% of the molecules to undergo Brownian motion (4). When charge recombination eventually takes place (5), the macrocycle shuttles back to the original station (6). Adapted from reference.  

displacement of the macrocycle could take place before the electron is back transferred. One remarkable example (Figure 36) was introduced by Stoddart’s group, where a crown ether type macrocycle (Figure 36a, shown in red) is shuttled between viologen (Figure 36a, shown in dark blue) and dimethyl viologen (Figure 36a, shown in pink) type stations through light irradiation. Upon irradiation (Figure 36b, (1)) of the ruthenium trisbipyridine stopper (Figure 36a, shown in green), a highly reductive excited state is generated, whereby an electron transfer (Figure 36b, (2)) takes place from the stopper to the viologen station. The relatively slow enough back transfer (Figure 36b, (3)) allows the displacement of roughly 10% of the macrocycle to the dimethyl viologen station (Figure 36b, (4)). Continuous irradiation results in an equilibrium where 95% of the macrocycle is now positioned over the dimethyl viologen station, whereas before the irradiation
95% of the macrocycle was on the viologen station. Ceasing irradiation restores the
system back to the original state and the macrocycle returns to the original position
(Figure 36b, (6)).

Figure 37 Photoinduced shuttling with remarkable positional discrimination. Adapted from
reference.45h

Another example (Figure 37) that exhibits remarkable positional discrimination
between two stations was published by the Leigh group, where the macrocycle
(Figure 37, shown in blue) is shuttled between fumaramide (Figure 37, shown in
green) and succinamide (Figure 37, shown in orange) stations through light
irradiation.45h The process here relies on the photoisomerization of the fumaramide
station rather than any kind of redox process. Fumaramide is an excellent station for
benzyl amide-type macrocycle due to the fact that the amide carbonyl groups on the
thread are rigidly positioned to align almost perfectly with the hydrogen bonding
counterpart on the macrocycle. However, photoisomerization of this station into
maleamide (Figure 37, shown in purple) results in distortion of the geometry and
stabilized maleamide through internal hydrogen bonding no longer acts as a decent
station and consequently the macrocycle moves over to the other station. The
process is reversible and through thermal relaxation the macrocycle can be returned
to the original station.
A remarkable incorporation of a similar fumaramide type system into functional materials was introduced to the literature by the Leigh group (Figure 38), where microliter sized drops of diiodomethane can be transported across a surface by light irradiation. The functional material is made up of a rotaxane system involving a fumaramide (Figure 38, shown in green) and fluorinated succinamide (Figure 38, shown in orange) station. When the macrocycle (Figure 38, shown in blue) is stationed over the fumaramide station, the fluorinated side is exposed and the surface is polarophobic; however once the irradiation and subsequent displacement of the macrocycle takes place, the fluorinated side becomes concealed and the surface becomes polarophilic, attracting the polar diiodomethane droplets. The Leigh group has also managed to transport the droplet up a 12 degree incline via this method. In this experiment approximately 50% of the photons absorbed are converted into gravitation potential energy in the drop.

Figure 38 Macroscopic transport using photo induced shuttling. Adapted from reference.65
Aims and Objectives

Various types of rotaxane-based systems that serve as molecular shuttles have been discussed in this chapter. Their remarkable properties and the incorporation of them into more complex structures to design functional materials and molecular machines have been briefly explained as well. Stimuli-responsive molecular shuttles are interesting areas of research due to their versatile characteristics, the variety of stimuli and increasingly well-established synthetic routes. Among the available stimuli, light-responsive molecular shuttles are of particular interest due mainly to the advantage of waste-free operation, among others. Unfortunately, bistable light driven molecular shuttles with remarkable positional integrity and high efficacy remain scarce. It is the aim of the project discussed in Chapter Three to synthesize a light-driven molecular shuttle that presents high positional fidelity and efficacy using a novel acyl pyridyl hydrazone station.
References


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Chapter Two

Towards a Hydrogen Bond Mediated Directional Walker

Synopsis: This chapter describes the design of and synthetic efforts towards a small molecule walker that could potentially move directionally along a track by exploiting the secondary interactions introduced to the system by the remarkable hydrogen bonding capabilities of squaramide units.

Acknowledgements

Dr. Malcolm Gall is greatly appreciated for proofreading this chapter. Dr. Guillaume De Bo and Augustinas Markevicius are greatly acknowledged for their efforts in designing the previous machine. Dr. Alina Nussbaumer is gratefully recognized for her help with scaling up the walker moiety and with some of the compatibility tests run for phosphate unit. Dr. Anneke Kruger is greatly appreciated for her help for the synthesis of the tracks in the previous design. And finally Dr. Guillaume De Bo is again acknowledged for the fruitful discussion that we had throughout the project.
Towards a Hydrogen Bond Mediated Directional Walker

Introduction

Squaramides are known to exhibit excellent hydrogen bonding properties. They can form up to four hydrogen bonds by functioning as both hydrogen bond donors and acceptors. The two NH units are the H-bond donors and the two oxygens are the H-bond acceptors. The high affinity towards hydrogen bonding can be attributed to the fact that the overall aromaticity of the system increases through H-bonding. These changes in aromaticity along with its structural rigidity have long been exploited by scientists who have used them as ligands, H-bonding catalysts, ion receptors and sensors etc. Their affinity towards a series of anions has been investigated (Figure 1).

![Squaramide structure](image)

<table>
<thead>
<tr>
<th>Anion</th>
<th>log $K_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$^-$</td>
<td>8.0</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>6.05</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>4.70</td>
</tr>
<tr>
<td>I$^-$</td>
<td>3.51</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>3.68</td>
</tr>
<tr>
<td>NO$_2^-$</td>
<td>4.75</td>
</tr>
<tr>
<td>HSO$_4^-$</td>
<td>4.02</td>
</tr>
<tr>
<td>H$_2$PO$_4^-$</td>
<td>5.42</td>
</tr>
<tr>
<td>AcO$^-$</td>
<td>6.5</td>
</tr>
</tbody>
</table>

*in MeCN at 298 K*

**Figure 1** Binding affinities towards variety of anions. Adapted from reference.

As can be seen from the table above, a simple squaramide has significantly great affinity towards a selection of negatively charged species in acetonitrile. One significant drawback of using hydrogen bonding for a particular purpose is that one is generally limited to working in relatively non-polar solvents. This is because of the fact that the solvent starts to compete for hydrogen bonding as the polarity of the
solvent increases, which results in significant diminishing of the H-bonding capability of the compound to desired targets. This becomes a serious issue when solubility constraints force one to switch to polar systems. Thankfully, squaramides can still exhibit remarkable hydrogen bonding properties even in a strongly competing mixture of DMSO and water (Figure 2). The table below compares the affinity of a squaramide and its thiourea and urea counterparts to chloride. It is remarkable that squaramides allow one to work in extremely polar conditions,

\[
K_a \text{ (M}^{-1}\text{) } \text{Bu}_4\text{NCl in DMSO/0.5%water}
\]

<table>
<thead>
<tr>
<th>Compound</th>
<th>(K_a) (M(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squaramide</td>
<td>643</td>
</tr>
<tr>
<td>Urea</td>
<td>88</td>
</tr>
<tr>
<td>Thiourea</td>
<td>41</td>
</tr>
</tbody>
</table>

**Figure 2** Comparison of chloride association with squaramide (red), urea (green) and thiourea (blue). Adapted from reference.

which is very crucial for this project. As stated above they are both H-bond donors and acceptor, and this feature enables squaramides to H-bond to another squaramide molecule (Figure 3) as well, which results in stacking and usually is the reason for poor solubility. Inspired by aforementioned hydrogen bonding capabilities of squaramides and Leigh’s previously published two-legged walker systems and considering the lack of proper directionality in small-molecule walkers, the first design of hydrogen bond mediated directional small-molecule walker was proposed and depicted below (Figure 4a). However, the design had to be reconsidered and improved due to serious solubility issues, likely caused by squaramide stacking which is a complication likely to arise during the operation because of the flexible nature of the structure. During the operation, instead of the walker moiety taking the first productive step, this flexibility gives almost equal opportunity to the first
foothold to make a disulfide bond with the third foothold and form a macrocycle. One has to design the system in such a way so as to eliminate these sorts of problems and to avoid possible complications during the operation. Otherwise, the purification of the outcome of the each operation step and therefore the overall efficiency of the machine would be seriously compromised. In order to address the solubility issues, long alkylic chains were introduced to both ends of the machine. (see Figure 4b). As for the flexibility, aliphatic squaramides were replaced by biphenylic rigid aromatic squaramides. The mode of operation of the unidirectional whole walker system is as follows; when the walker moiety resides over the first and second footholds, addition of a base would create

Figure 3 Binding affinity of a squaramide towards itself. Adapted from reference.7

Figure 4 a) Previous design. b) Current design.
a negative charge on the part between the second and third footholds due to the deprotonation of the phosphate moiety. Additionally, the disulphide bond could undergo disulfide exchange to labialise the first foothold. Now the walker unit is free to pivot around the hydrazone foot as it is kinetically locked under basic conditions. According to the hydrogen bonding tendencies of squaramides, the interaction between the squaramide on the walker and the negatively charged phosphate would be much stronger than the one between two squaramides on the walker and half-track-1 (31), resulting in most of the walker moieties spending their time on the middle part of the track. Closing the macrocycle by oxidation of the free thiols into disulfides with iodine would result in most of the walker units having taken a step forwards along the machine and therefore create a directional bias. For the second step, the conditions would now be switched to acidic media to kinetically lock the disulfide foot and make the hydrazone foot labile thereby ensuring that the latter is free to pivot around the disulfide foot. Acidic media would also protonate the phosphate moiety whereby the strong interaction between the walker’s squaramide and phosphate unit is partially destroyed. Then, depending on the counter ion of the acid used, one can create a strong interaction between the walker and the half-track-2 (33). For example, if HCl is used, it is hypothesized that the chloride anion would sit between two squaramides of the walker and the half-track-2, thereby making sure that most of the walker molecules are templated on the end part of the machine. Closing the macrocycle here would then mean that a small molecule walker is directionally transported from one end of the track to the other. This concept could then be incorporated into elongated oligomeric tracks to have an overall directional bias for walking. The current system is designed to take two steps only to prove the feasibility of the approach. The same reasoning in taking the first step could be applied to the third step and subsequently, the fourth step would be as analogous to the second step and so on.

Retrosynthesis of the machine

The retrosynthesis of the whole molecular walker is shown below (Figure 5). A key point of the retrosynthesis is the final step which involves the assembly of the whole machine through introducing the phosphate unit which is crucial for directionality. There is a window of opportunity here as the two pieces could either directly be connected through phosphate ester formation or if it proves to be not feasible, the preformed phosphate unit could be introduced to the machine via a well-established
$S_N^2$ type reaction or Mitsunobu reaction. It is also important to note that the machine is divided into two main sections; one being the macrocycle and the other being the half-track-2. As the macrocyclization is one of the crucial corner stones of the design in terms of the viability of the operation (if the macrocyclization cannot be accomplished, it would then mean that the design is flawed in terms of the relative lengths of the components and the rigidity of the system), it is set to be performed before assembling the whole machine. The attachment of the walker unit to the

**Figure 5** A retrosynthetic scheme of the whole machine

half-track-1 is chosen to be done through the hydrazone foot. The reason for that is that there is only one possible outcome in this case, whereas in the case of the other foot, the possible outcomes would be the desired product, dimer of the track and dimer of the walker. One other key element of the retrosynthesis is that most of the functional groups were introduced before performing squaramide formation reactions. This is because when working on the previous design, it was perceived that squaramide units are not sufficiently compatible with the reactions required to introduce the functional groups. In the next session, detailed synthesis of the walker
moiety, half-tracks, macrocyclization, phosphate bearing units and attempts towards the assembly of the whole machine will be explained and discussed in detail.
Results and Discussion

Figure 6 Precursors for the coupling step
Starting from commercially available 3-bromo-5-hydroxybenzoic acid, compounds 3 and 4 to be used for the Suzuki coupling step were obtained in good overall yields by firstly protecting the carboxylic acid and then the phenol (Figure 6). The benzyl group was added to be removed after the Suzuki coupling step to increase the yield of the coupling. However, the hexyl group was not removed due to its solubilizing function of the whole machine in addition to helping with the Suzuki coupling. For the coupling stage, boronic ester 6 was prepared starting with the Boc protection of commercially available 3-iodoaniline and subsequent introduction of the boronic ester group (Figure 7).

Figure 7 Boronic ester formation
Although compound 6 itself is commercially available, this route, using cheaper starting materials, was preferred as a large amount of 6 was needed at the early stages of the synthesis. Coupling 6 with 4 under standard Suzuki coupling conditions proceeded smoothly with an excellent yield (Figure 8). The benzyl protecting group was then removed under hydrogenation conditions. The obtained product 8 was reduced to its corresponding benzylic alcohol, followed by bromination under Appel conditions with good yields. The transformation of the benzyl bromide units into their corresponding thioacetates generally proceed smoothly, however in this case, the presence of a free phenolic unit complicated the
reaction and formed a by-product where the phenol was also acetylated, which was confirmed by both mass spectrometry and NMR analysis. Formation of this by-product is likely to account for the low yield of the reaction. In an attempt to save this by-product for the next disulfide formation reaction, it was subjected to standard basic hydrolysis conditions, which unfortunately failed and produced unidentifiable adducts. In order to improve the yield, another strategy where compound 10 was converted into the corresponding trityl thioether was investigated as well. However, the simultaneous deprotection of both Boc and Trityl under standard acidic conditions proved to be quite tedious as the purification process required multiple runs of preparative TLCs. Therefore this strategy was abandoned and the route through thioacetate formation was retained despite low yields. Then, compound 11 was subjected to deprotection conditions using pyrrolidine and followed by in situ formation of disulfide bond using 1-butane thiol as the place holder. Considering the fact that this is a two-step reaction and chances of forming homodimers, 53% yield is acceptable. As a final step to form foothold 3, compound 12 was boc-deprotected under acidic conditions (Figure 8). Unfortunately, this reaction proceeded with a
low yield of 33%. What accounts for the low yield is that scrambling of thiols was observed, that is, homodimers of benzylic and aliphatic thiols were formed, due probably to the fact that the carbocation formed during the deprotection process initiated the thiol exchange process. Although there is no proof to support this, thiol exchange under acidic conditions is unlikely to happen, so a possible explanation is that the carbocation might play a role in this.

Figure 9 Synthesis of foothold 2

For the synthesis of foothold 2 (Figure 9), compound 9 which was also used for the synthesis of foothold 3 was boc-deprotected under acidic conditions, after which it was reacted with a squaric ester using zinc triflate as a catalyst. As a general rule-of-thumb, it is fair to state that formation of mono substituted squaramides is easier than that of disubstituted ones. And likewise, the formations of aliphatic squaramides do not require conditions as harsh as aromatic ones do. Therefore compound 15 was synthesized under mild conditions with an excellent yield. The oxidation of benzylic alcohol 15 proved to be sluggish as it required excess amount of PCC to be used. The same reaction can also be performed using Dess-Martin Periodinane (DMP) as the oxidant, however the purification step is not as straightforward as the by-products of the DMP have very similar Rf values on TLC plate to the product itself. As a final step to obtain foothold 2, the aldehyde is protected via conventional method with quantitative yield. The reason for the requirement of protection of the aldehyde is that when the following half-track formation is attempted, it is a squaramide formation reaction which involves a free amine as the second substrate. The instantaneous imine formation between the amine and the aldehyde hinders the reaction progress and produces imine related side-products along with 31, thus decreasing the yield and making the purification a
lot more tedious. One way to overcome this problem that was tried was to mix the imine products with acid and catalytic amount of water. This worked to some extent; however as soon as the residue was loaded on to preparative TLC, some of the imines were reformed. Therefore, to recover the product, multiple runs of preparative TLCs had to be performed. It is therefore crucial to protect the aldehyde before attempting the squaramide formation.

**Figure 10** Synthesis of foothold 4

For the synthesis of the foothold 4 (Figure 10), a very similar strategy to that of foothold 2 is followed. Compounds 6 and 3 were coupled via standard Suzuki coupling condition with good yield. It is important to state that the long hexyl chain has a dual function; one being a protecting group for the phenol so that Suzuki coupling could proceed smoothly and the other being a solubilizing group as the
molecule gets larger and the number of squaramide in the system increases, the solubility becomes a worrying factor. That is why this route slightly differs from the one above in terms of the functional group on the phenol oxygen. Another important difference is the use of deuterium labelling that was introduced for characterization purposes. As the system is already somewhat complex, it is crucial to simplify the characterization of each individual molecule formed during the operation at the expense of longer synthetic steps. The information that is gathered from deuterium labelling is that it helps us to understand whether the walker has reached the end of the track, because when the walker occupies foothold 2, there will not be an aldehyde peak in the $^1$H NMR spectrum. However when the walker occupies foothold 4, the unlabelled aldehyde on foothold 2 will be freed and will show up on NMR. To that effect, compound 18 was reduced to the corresponding deuterium labelled alcohol using NaBD$_4$. Use of LiAlD$_4$ makes the reduction easier and faster, however we had to switch to NaBD$_4$ because chemical companies no longer provides LiAlD$_4$ due to new regulations. Then, compound 19 was boc-deprotected with an excellent yield, followed by mono substituted squaramide formation with zinc triflate as a catalyst. The formed compound was then oxidized to corresponding benzaldehyde using PCC with a decent yield. And for the reasons explained above, the aldehyde was protected using methanol to give foothold 4. Interestingly, the yield was not as high as in the case for foothold 2. Although both structures bear similar functional groups, the reaction produced some by-products that were not identified, and this accounts for the decrease in the yield.

And finally for the synthesis of foothold 1 (Figure 11), a similar strategy to other footholds was followed with subtle modifications. Compound 18 which is also the precursor of foothold 4 was converted into the corresponding thioacetate in three steps; firstly reduction with LiAlH$_4$, secondly bromination under Appel conditions and thirdly a simple substitution reaction, with excellent overall yields. It is noteworthy how the thioacetate formation has an excellent yield (quantitative) compared to the one in the synthesis of foothold 3 due to lack of free phenol in the molecule. One important change in the strategy is that compound 26 was allowed to dimerize after the thioacetate deprotection step instead of incorporating a placeholder straight away. The reason behind this change is, as explained above, the unreliable results obtained when performing Boc-deprotection in the presence of placeholder that results in scrambling of the disulfides. Therefore, straight after the
thioacetate deprotection, the intermediate was titrated in situ with iodine and homodimer was obtained. Subsequently, Boc-deprotection was performed under standard acidic conditions, which proceeded quantitatively (instead of 33% which was the case for foothold 3). Then, in order to attach the placeholder and obtain the foothold 1, all disulfide bonds were cleaved off using TCEP.HCl and then reoxidized back to unsymmetrical disulfides with 1-butanethiol in the presence of iodine with a good total yield of 60%.

**Synthesis of Half Tracks**

Once all the footholds were obtained, the synthesis of the half-tracks was attempted. Initially, a standard procedure of obtaining squaramides using aromatic amines where the substrates are stirred in the presence of zinc triflate as a catalyst in a mixture of DMF and Toluene at elevated temperatures was tried. The reason for
using harsher conditions instead of simply using TEA as a catalyst and performing

\[ \text{Figure 12 Synthesis of half-track-1} \]

the reaction at room temperature is attributed to the low nucleophilicity of the aromatic amines compared to the aliphatic ones. However, this strategy resulted in very low yields and formation of imine related products, which made the purification tedious.

One possible explanation for imine formation is that the acetal group may have been deprotected to leave an aldehyde that allows imine formation. Given the reaction conditions, this does not seem impossible as the acetal deprotection is an acid
catalysed reaction and zinc triflate is a known Lewis acid. Therefore, in search of a better route, the same reaction was attempted using TEA as a catalyst in methanol at 80°C because TEA is known to catalyse squaramide formation, especially with aliphatic amines. Luckily, this route did not produce any imine type adducts, however, the yield was very low and the purification process was capricious. The inefficiency of the reaction and the formation of several by-products can be attributed to the fact that disulfides are not completely stable under basic conditions and the catalyst that was used is triethylamine.

Running the reaction at elevated temperatures in the presence of a base may play a role in the formation of unidentified adducts, but there is no direct evidence for this. Having gathered enough information about the nature of the reaction and the behaviour of the starting materials, it was hypothesized that using zinc triflate as the catalyst in methanol at elevated temperatures might be a promising alternative route. The reason behind the hypothesis is that even though zinc triflate activates the acetal group on the foothold, since the solvent is methanol, the deprotected molecules will be reprotected by the solvent as in the case of acid catalysed aldehyde protection by methanol. Luckily, the hypothesis proved to be reasonable and the product was obtained in an acceptable yield of 56% and the purification was significantly simpler than in the other routes as the amount of by-product was significantly diminished (Figure 12). Since the reaction was done on relatively small scales, some of the product was lost during the preparative TLC purification step, which accounts for the relatively low yield. If the reaction had been attempted on larger scales, the loss during the purification would probably have not had as much impact on the yield. And finally, the acetal group on compound 30 was deprotected into the corresponding aldehyde in order to obtain half-track-1. Based on the reasons explained above, synthesis of the half-track-2 was accomplished using the same, previously optimized conditions with a relatively high yield in the first step and almost quantitative yield in the second step (Figure 13).
Figure 13 Synthesis of half-track-2

Synthesis of the Walker Unit

The synthesis of the walker unit (Figure 14) that involves four synthetic steps was more straightforward than the synthesis of the half-tracks. Starting from commercially available materials, compound 34 was obtained in very high yield as it is a mono-substituted aliphatic type squaramide. Then, β-Alanine methyl ester hydrochloride was converted into acyl hydrazone 35 in two steps in one pot. The reason for protecting the hydrazide with 4-anisaldehyde is that the high polarity that was introduced to the system by the hydrazide formation drastically decreased the
solubility of the materials and made it impractical to work with. Then, precursor of the walker was obtained by the reaction between compounds 34 and 35 with the help of TEA catalyst. At this stage, solubility of the molecule dramatically decreased due to high stacking capabilities of squaramides, which was beneficial in terms of purification as the impurities can simply be washed off. Then a simple substitution reaction with potassium thioacetate gave the walker unit in an acceptable yield, which could also be purified by washing due to low solubility. Originally, it was contemplated to have the thiol group protected by a trityl group so that it could be deprotected and the walker unit would be introduced as free thiol to the system before the macrocyclization process as was the case in previously published walker systems. However, as soon as it was subjected to acidic deprotection conditions, what is likely to be the product crashed out of solution and was extremely insoluble: the only way to the precipitate was to heat it to elevated temperatures in DMSO, however, as soon as the system cooled down, the product precipitated again. Therefore, it was chosen to retain the thioacetate protecting group on the thiol which would be removed as the final step before closing the macrocycle. The reason for switching from trityl group to thioacetate was that trityl

**Figure 14** Synthesis of the walker unit
group is known to be acid sensitive whereas thioacetate group can be removed using basic conditions. And during the attachment of the walker unit onto half-track-1 through acid catalysed hydrazone exchange process, it was assumed that the lability of the trityl group under acidic conditions would further complicate the reaction.

**Attachment of the Walker Unit onto Half-Track-1**

Originally, what was tried to attach the walker on the half-track-1 through hydrazone exchange reaction was to mix the walker 37 with protected half-track 30 under acidic conditions, hoping that the acetal group on the track would be deprotected *in situ*. However, that did not turn out to be the case as there was significant amount of protected starting material left along with some unidentifiable adducts. Therefore, it was decided to first obtain the half-track in its aldehyde form so that an extra step of deprotection would not get in the way during the attachment process. Although several different types of acid sources were used, no promising results were obtained with the aldehyde. Then, an unconventional method of hydrazone exchange reaction where the starting materials were stirred at 60°C in a mixture of 2-(N-morpholino)ethanesulfonic acid (MES) buffer and DMSO in the presence of catalytic amount of aniline was followed. Interestingly, this strategy worked and the product was isolated, albeit with a very low yield. In an attempt to

![Figure 15](attachment.png)
improve the yield, previously tried acid sources were revisited. The solvent that was
previously used was chloroform in which the starting materials showed poor
solubility, therefore, to fully dissolve the substrates, the addition of large quantities
of acid (up to 40%) was required. It was hypothesized that maybe the usage of
excess amounts of acid at elevated temperatures was too harsh for the starting
materials, causing them to decompose; therefore, switching to another solvent in
which the materials were soluble so that excess acid could be avoided seemed like a
promising idea. Luckily, when compounds 31 and 37 were reacted in the presence
of catalytic amounts of TFA and water at room temperature in DMF, the product
was obtained with a good yield of 69% (Figure 15).

Synthesis of the Macrocycle
One very crucial step of the whole synthesis is the one where the precursor of the
macrocycle is closed. This is important not only because of synthesis point of view
but also if the macrocycle closure proves to be not viable, then the whole design and
operation would be compromised as taking a step actually means forming a
macrocycle. Therefore, the rigidity of the system and the length of each individual

**Figure 16** Attempt to form the macrocycle

cOMPONENT should be long enough to facilitate the macrocycle closure. Although,
CPK modelling suggested that the system is flexible enough and the lengths of the
walker and tracks are long enough, this modelling is never definitive and the closure
must be tested experimentally. That is why this step constitutes one of the most
important points of the project. In order to test the feasibility of the macrocycle closure, a very similar compound to 38 that was optimized for the previous design (53) which bears a mercaptoethanol unit instead of butanethiol unit was subjected to macrocycle closure condition (Figure 16). The investigated conditions involved a thioacetate deprotection step in the presence of pyrrolidine in DMF followed by an in situ closure simply by an $S_N^2$ reaction-type, where the walker thiolate substitutes the mercaptoethanol. Surprisingly, even though this strategy led to the formation of the macrocycle with full closure at room temperature in the previous design,

![Chemical Structures](image.png)

**Figure 17** Formation of the macrocycle

a mixture of open and closed forms of the macrocycle was produced here, despite longer reaction times and elevated temperatures. The mixture seemed to have reached an equilibrium with the ratio of 3/7 in favour of macrocycle 39. Unfortunately, all attempts to separate those two analogues failed. Therefore, instead of investing more time on the purification, another strategy that is able push
the reaction equilibrium all the way towards the product was investigated. It was hypothesized that if somehow all the thiol-related parts on the molecule were converted into free thiols, then the macrocycle closure could be kinetically accomplished by titration with iodine in a high dilution system. In order to test this method, the 3/7 mixture obtained from the above reaction was subjected to disulfide reduction conditions using dithiothreitol (DTT) and once all disulfides were converted into free thiols, the system was diluted and the free thiols were reoxidized to disulfides with iodine. This way full closure of the macrocycle was accomplished. Since this was tried in a very small scale, a precise yield could not be determined. And there was no more material left, therefore, in order to determine the yield and have better insight into the efficiency of the reaction; more materials had to be resynthesized. Due to some practical reasons, the precursor of the macrocycle was changed from the one bearing mercaptoethanol into the one bearing butane thiol (38). And when compound 38 was subjected to the same conditions as above at moderate scale, pleasingly, full closure was accomplished that led to the formation of the macrocycle in an acceptable yield of 48% (Figure 17). The yield is good in terms of macrocycle formation considering the potential formation of oligomeric compounds and the fact that it is a three step reaction involving deprotection, reduction and oxidation steps.

**Assembly of the Whole Machine**

In an effort towards assembling the whole machine, one can think of a variety of synthetic routes. And the way macrocycle and half-track-2 were designed do not limit the options to only one or a few, which would be really risky in case that final reaction fails. And again thanks to the design, the length of the linker that connects the macrocycle and half-track-2 does not have to be precise. According to CPK modelling, the system could accommodate a linker as short as just a phosphate unit or as long as two benzyl units as well as a phosphate unit and the operation would not become compromised. This gives a window of opportunity in terms of synthetic strategies. One obvious choice would be to take advantage of the phenol groups on both macrocycle and half-track, and directly combine them to the corresponding phosphate ester without further functionalization. One of the conventional methods of phosphorylation is the use of phosphoryl chloride (POCl₃) in the presence of a base such as TEA, pyridine etc. to neutralize the acid liberated from the reaction,
followed by an aqueous quenching of the remaining chloride atom. In terms of the negative aspects of this route, one would be concerned about the formation of by-products such as mono and tri phosphates, but this could be avoided to some extent by the use of exact equivalence of the materials, order and speed of addition. Another concern would be the highly reactive nature of POCl₃, which might produce unwanted adducts by reacting with the other functionalities on the starting material. And last but not least, there is the requirement to be extremely cautious to work under dry conditions as the reagent is highly susceptible to be hydrolysed by water. That being said, this route still gives the advantage of going from the macrocycle and half-track directly into final product in one pot. Therefore, having an excellent yield is not an immediate concern or does not have the highest priority as this is the final step of a lengthy synthetic route. One important feature of POCl₃ worth mentioning is the fact that it reacts with some of polar solvents such as DMF and DMSO. Therefore, those solvents cannot be used. However, squaramides are known to be not the best functional groups when solubility is a concern. Half-track-2 is soluble in THF, which is compatible with the reaction conditions, but the macrocycle has poorer solubility as it contains two squaramide units. It is soluble in systems with high polarity such as DMF and DMSO, but for the reason explained above; those solvents are not compatible with this reaction. It could also be solubilized in a mixture of MeOH/DCM, however alcohols are incompatible solvents with POCl₃. This leaves us with some unconventional solvent systems such as trimethylphosphate or mixtures of pyridine/DCM/THF in which the macrocycle is soluble. Unfortunately, when those systems were tried, no promising results were obtained and many adducts formed, none of which could be characterized to be the product. The same strategy was attempted with different reagents such as methyl dichlorophosphate and ethyl dichlorophosphate in the hope that replacing one of the chloride atoms with a protecting group would reduce the possibility of formation of too many by-products. However, these attempts did not afford the desired product. It was therefore decided to investigate another method so as to not waste any more material. One of the conventional methods to synthesize oligonucleotides is through a stepwise synthesis of phosphate esters. This approach proved to be quite useful in the literature for variety of phosphotriester with a 2-cyanoethyl protecting group that can easily be removed by a basic hydrolysis. The approach involves four steps; firstly one of the alcohol/phenol is reacted with 2-Cyanoethyl N,N-
diisopropylchlorophosphoramide to give an intermediate, that is to be subjected to a coupling reaction. Since there is only one chloride atom on the reagent, it only has one reactive site; it does not involve the aforementioned risk of by-product formation as is the case with POCl₃. In order to introduce the second alcohol/phenol, the phosphoramidite intermediate is activated by an acidic azole catalyst such as 1H-tetrazole and the diisopropyl amine unit is replaced by the second alcohol/phenol. This intermediate is then oxidized in situ to its corresponding phosphate using iodine. And finally the protecting group is removed by the use of a base such as triethyl amine, ammonia etc. Although this method involves four steps and can be regarded as an ambitious strategy, given the fact that our design bears so many functional groups that might get in the way, it is not an extremely risky method as all these steps can be performed in one pot, except perhaps the final step. However, one problem that arises is exactly how much reagent should be used. The reason for that concern is that if the excess amount of reagent is used, the remaining unreacted reagent will react with the second alcohol/phenol. However, if the first alcohol/phenol is not fully converted into the corresponding intermediate, then the remaining alcohol/phenol will then get in the way when the intermediate is activated by 1H-tetrazole to react with the second alcohol/phenol. These two concerns are quite crucial as they increase the possibility of formation of unwanted adducts and might make the purification significantly more complicated. Especially when the reaction is performed in very small scales, the aforementioned concerns become much more important, as the amount of the reagent that was hydrolysed or decomposed would have huge impact on the outcome of the reaction. Luckily, this method has some flexibility to work around. Instead of performing the whole steps in one pot, the intermediate that was obtained out of the reaction between the first phenol and the reagent 2-Cyanoethyl N,N-diisopropylchlorophosphoramide could indeed be isolated and used in the following step in its pure form. This allows one to use excess amount of the reagent to push the reaction and convert all the phenol into the corresponding intermediate. After performing some model studies to test if it was actually possible to isolate the intermediate and to see if the intermediate is not too susceptible to hydrolysis, it was decided that this route proved to be promising. When the same reaction was attempted on compound 33, the intermediate was indeed obtained with a poor yield of 32%. Judging by the TLC during the course of the reaction, all the starting material was converted, therefore despite our
 expectation towards a promising yield, it was not the case as there were some unidentified adducts and starting material reforming during the isolation and the NMR spectrum of the product was not very good. It turned out that the intermediate was not as stable as one would have hoped, but it was still manageable. Therefore, the remaining steps were carried out on this intermediate to assemble the machine.

The preliminary results were very promising as the mass spectrometry analysis of the crude sample showed a peak assignable to what was thought to be the product at the time (Figure 18). As can be seen from the mass spectrum, the peak that showed up at 857.48 belongs to the macrocycle, the peak at 986.40 belongs to macrocycle + iodide which is not unusual as squaramides are excellent in making hydrogen bonding to iodide, the peak at 694.30 that belongs to the half-track-2 which comes from the hydrolysis of the intermediate and finally the peak at 1781.49 that might belong to the whole machine + trifluoroacetate or the whole machine + DMF + MeCN. These two possibilities are not actually very unlikely considering the fact that the whole machine has three squaramides that has a tendency to bind anions and trifluoroacetate is not an unusual anion that shows up in our mass spectroscopy. As for the second possibility, it was encountered before that the macrocycle is able to bind some solvents as well such as DMF and pyrrolidine, and their combination.
showed up on mass-spec analysis. And this crude sample included some DMF before submitting to help with the solubility. However, despite all efforts, the product was never purified and obtained. It was assumed that maybe it was lost during the purification as the reaction scale was too small or that the product was not very stable and prone to hydrolysis due to the fact that it is a phosphotriester. And unfortunately, this method proved to be irreproducible. This could be attributed to the fact that the solvent used during the second coupling plays a crucial role. The leaving group on the reagent, namely diisopropylamine, forms a salt with acidic 1H-tetrazole and precipitates out of solution when acetonitrile is used as a solvent. And this process significantly shifts the

\[ \text{Figure 19 Assembly of the whole machine through alternative methods} \]

equilibrium towards the product. The test reactions performed revealed that this second coupling was highly solvent dependent and when performed in acetonitrile, the precipitation occurs instantly and the reaction reaches to completion in a matter of minutes. However, when performed in DMF or even in mixture of DMF/MeCN (1:9), the reaction had to be stirred overnight and results in traces of product along with hydrolysed form of the intermediate to the phenol as a major by-product. After gathering sufficient data to support the fact that assembling the whole machine through phosphate ester formation was not feasible considering there is limited amount of material, it was decided to change the strategy and pre-form the phosphate ester and assemble the whole machine around it (Figure 19). As
explained earlier, CPK modelling suggested that there is enough room to accommodate series of linkers that have varying lengths. To that end, a series of linkers that gave the opportunity to assemble the whole machine through Mitsunobu reactions or standard $S_N^2$ reactions were synthesized. In terms of the protecting group, the first choice was 2-cyanoethyl group as it was commonly used in phosphate chemistry.

![Figure 20](image_url) Preparation of the phosphate linker with 2-cyanoethyl protecting group

Starting from 3-hydroxybenzyl alcohol, the benzyl alcohol unit was converted into the corresponding benzyl bromide through an Appel reaction in good yield. Compound 40 was then reacted with 2-Cyanoethyl N,N,N′,N′-tetraisopropylphosphorodiamidite in the presence of tetrazole as a coupling reagent. The reason for choosing this reagent over the one used above is that the product is a symmetrical phosphate ester. Then the intermediate formed was oxidized *in situ* to compound 41 using Luperox® TBH70X as the oxidizing reagent with an overall yield of 39% (Figure 20). When the linker 41 was reacted with half-track-2 in the presence of potassium carbonate in dry DMF, it was observed that 2-cyanoethyl protecting group on the linker was immediately cleaved off. It was disappointing to see how unstable the linker was under basic conditions. It was then assumed that it might still be possible to perform the $S_N^2$ reaction on the unprotected linker. It was anticipated that the negative charge on the phosphonate would hinder the reaction relative to that of the neutral, protected analogue by repelling the nucleophile. In line with this hypothesis, no product formation was observed, despite using elevated temperatures and longer reaction times. At this stage, since the basic conditions cleaved off the 2-cyanoethyl group, it was considered that acidic conditions might prove to be useful as it is known that the protecting group is stable under acidic conditions. To that end, it was thought that trichloroacetimidate functional group might be a good alternative candidate as it gives the opportunity to perform the
substitution reaction under acid catalysed conditions. Some test reactions with simpler compounds were performed to see if this route was a feasible one. Trying several solvents and several acids such as TFA, HCl and TfOH; it was concluded that the reaction works best in solvents with low polarity and with TfOH. The problem here is the solubility, in order to solubilize the macrocycle, solvent systems with very high polarity must be used. And unfortunately, the test reactions performed suggested that compounds bearing trichloroacetimidate units decompose under acidic conditions when switching the highly polar solvents such as DMF. When the test reactions were performed with the increased amount of acid (because we knew that the macrocycle can be solubilized in a mixture of TFA and DCM), again the trichloroacetimidate unit did not survive. After running several test reactions, it was concluded that an acid catalysed route was not feasible either. One final thing to try with compound 41 was to see if it was possible to perform the substitution reaction under neutral conditions. It is known from literature that it is actually possible to do this using silver salts. A positive aspect of this route is that it allows the use of DMF as solvent considering solubility is an important concern in our case. To test the feasibility of this approach, commercially available compounds benzyl bromide and 4-hydroxy benzaldehyde were reacted in the presence of silver (I) oxide in DMF at room temperature and almost a quantitative yield was observed. Then before using the half-track-2, same conditions were tried as well on compound 41, however disappointingly the compound did not survive the conditions, which might be attributed to the fact that silver oxide might have reacted with the water present in the reaction medium to create basic hydroxyl anions that cleave off the 2-cyanoethyl group.

After exhausting all the options for the substitution type route, the attention was turned to a Mitsunobu approach. To that end, 3-hydroxybenzyl alcohol was first TBDMS protected followed by mono-deprotection of the phenolic ether, and finally was subjected to standard phosphorylation conditions to yield the product 44 in 20% (Figure 21). Originally it was planned to have final deprotection step of the TBDMS group, but luckily a mixture of protected and unprotected products were obtained, probably due to basic nature of the reaction conditions, accounting for the poor yield. Standard Mitsunobu conditions were applied using various temperatures, solvents and azodicarboxylate reagents on compound 44 and a simple model compound of 4-hydroxy benzaldehyde and some promising results were obtained.
However, when the optimal conditions were applied to half-track-2 and compound 44, the desired product did not form. A mixture of unreacted starting material and some unidentifiable adducts was isolated. Despite changing the reaction conditions by varying solvent compositions and coupling reagents, there was still no indication of product formation. At this point, it was concluded that 2-cyanoethyl was not the best choice despite its common use in phosphate chemistry. Two other options that were investigated were methyl and ethyl as protecting groups. It is

Figure 21 Preparation of the phosphate linker with 2-cyanoethyl protecting group

Figure 22 Preparation of the phosphate linker with methyl protecting group
indicated in the literature that they are more stable than 2-cyanoethyl under basic conditions and could be cleaved using NaI for methyl and LiBr for ethyl in DMF, which seems compatible with our system considering the solubility issues and the functional groups on the machine. In order to test if those protecting groups proved to be better than 2-cyanoethyl group, a series of compounds were synthesized. Their stability was tested in the presence of potassium carbonate in DMF, both protecting groups survived stirring overnight at room temperature, then the temperature was raised to 65°C and stirring was continued for another day. Pleasingly, both protecting groups were stable. In order to make sure that the protecting group can be removed after assembling the whole machine, deprotection conditions were tested on compounds 46 and 48 (see Figures 22 and 23 synthesis). As reported in the literature, deprotection of those compounds proceeded smoothly.

![Synthesis diagram](image)

**Figure 23** Preparation of the phosphate linker with ethyl protecting group

After being convinced that the protecting groups were neither too labile nor too stable for the reaction conditions, the Mitsunobu reaction between half-track-2 and either compound 46 or 48 was attempted. However, there was not much difference compared to the case of 2-cyanoethyl and no product formation occurred. Without wasting more material, compound 49 was synthesized to try the substitution route. It is worth mentioning that it was first attempted to synthesize compound 49 directly starting from compound 40 through POCl₃/Ethanol and ethyl dichlorophosphate.
route, however a surprising mixture of benzylic bromide and chloride type compounds was obtained. Somehow, some of the bromides were replaced by the chloride liberated from the phosphorylation reagent during the course of the reaction. In order to avoid this problem, a safer method of directly brominating compound \( 48 \) under Appel conditions was followed. Firstly reaction of compound \( 49 \) with half-track-2 in the presence of potassium carbonate in DMF was attempted, but disappointingly there was at least 16 different adducts formed that were observed on TLC (it is likely to have been more, as some of the spots on the TLC seemed to be overlapping). It was attempted to isolate the major spots through several runs of preparative TLC, but none of them unfortunately belonged to the product. Their NMRs were extremely complex suggesting that the spots isolated indeed contained more than one compound.

![Figure 24 Test reaction resulting in transesterification](image)

Mass spectroscopy analysis did not produce any reasonable explanation as to what might have occurred. For some reason, the reaction conditions were damaging the starting material. Similar results were obtained when cesium carbonate was used. The screening of different bases was carried out, investigating proton sponge, TEA and sodium hydride. However, no promising results were obtained. For example, in the case of \( \text{NaH} \), transesterification took place instead of a regular substitution reaction (Figure 24). Many of the reactions that were attempted with half-track-2 were also attempted with the macrocycle as well to see if there was any difference in reactivity, however no desired product formation was observed. In a final attempt, a neutral approach towards the substitution reaction where a silver salt was used to activate the electrophilic carbon by coordinating to bromide atom was investigated. This is because of the fact that the test reactions with this approach produced reliable and promising results. And now that the protecting group on the phosphate is much more stable compared to 2-cyanoethyl group, it was assumed that there should not be any issues concerning the phosphate moiety. The reaction was first
tested on the half-track-2 as the macrocycle is much more precious than the track in terms of synthetic efforts put forward towards its synthesis. Excess of compound 49 was used in order to push the reaction towards the mono substituted product with varying equivalences of silver (I) oxide. Unfortunately, no product formation was observed. The same approach was attempted on the macrocycle but no product formation was observed. And finally using exact equivalences of half-track-2, macrocycle and silver oxide in one pot, the same reaction was attempted. This approach was not ideal as it would produce a statistical distribution of the whole machine and dimers of both macrocycle and half-track-2 in the best case scenario, along with some by-products. However, this is the final step before the deprotection to obtain the final product, and we knew that the deprotection step was relatively straightforward based on the results obtained from test reactions. Therefore, even if the yield was anticipated to be poor, having at least some of the whole machine to try the operation was the best option one could hope for, given the circumstances. This reaction unfortunately did not produce any product as well. At this stage, there was no more material left to work with.
Conclusion

The synthesis of the macrocycle (39) and half-track-2 (33) was successfully accomplished despite multiple synthetic obstacles. However, it is disappointing to report that all the attempts and efforts towards connecting these two components directly or through a linker for the assembly of the whole machine failed. Having so many functional groups on such a large synthetically demanding molecule would significantly restrict the number of options and conditions that could be tried and are available to work with. Making sure that all the reactions performed were compatible with the functional groups on the molecule was one of the many challenges that were encountered. Another obstacle that got in the way throughout the project was the fact that solubility was poor, especially as the molecule started to have significant size and as the number of squaramides increased, due to the high polarity introduced to the system and stacking nature of squaramides mainly because of their excellent hydrogen bonding properties. The synthesis of the macrocycle and the tracks required large scale reactions to begin with in order to have only few milligrams of these advanced intermediates at the end of their synthesis. And scale-up reactions had to be repeated several times throughout because optimization of each critical step required a lot of material to find appropriate conditions. Considering the number of required synthetic steps to afford the macrocycle and the track, and the fact that some of the reactions only formed product with poor yields, one can imagine how many times the scale-up reactions had to be repeated. Despite all the efforts, introducing the phosphate unit through several different strategies as the final step of the project failed. It appears that the synthetic strategy needs to be reconsidered as the current one proved to be not working. This may require the structure of the whole machine to be redesigned. In terms of the synthetic strategy, one alternative that could be tried in the future is that the whole track containing the phosphate unit could be synthesized first and lastly the walker unit could be introduced to the system. Of course, it would require an entirely different route and a lot of efforts to optimize the conditions. On top of everything, during the attachment of the walker to the track, there is the risk of forming different positional isomers. For example if the walker is first attached to the second foothold through hydrazone exchange reaction, then during macrocycle closure, the walker could prefer either first or third foothold, which results in walker residing over different part of the track. This could be avoided by protecting one of
the footholds, however this would introduce additional steps and it is best to avoid extra synthetic steps when the molecule is nearly at the final stages. One other alternative would be to make a radical change and use thiourea or urea type motifs instead of squaramides. This would drastically compromise the hydrogen bonding ability of the system and ultimately might have a negative impact on the directionality of the walking process, which is the essential element of this project; however, it would significantly help with the solubility issues encountered throughout the project. In summary, the design needs to be reconsidered carefully.
**Experimental Procedures**

**General Methods**

Unless stated otherwise, all reagents and solvents were purchased from commercial sources and used without further purification. Dry tetrahydrofuran, N,N-dimethylformamide, dichloromethane, and acetonitrile were obtained by passing the solvent (HPLC grade) through an activated alumina column on a Phoenix SDS solvent drying system (JC Meyer Solvent Systems, CA, USA). Anhydrous methanol was purchased from Sigma-Aldrich. Flash column chromatography on silica was carried out using Aldrich Silica 60Å (particle size 40-63μm) as the stationary phase, and TLC was performed on precoated silica gel plates (0.25 mm thick, 60 F254, Merck, Germany) and visualized using UV light in combination with standard laboratory stains (acidic potassium permanganate, acidic ammonium molybdate and ninhydrin). Preparative TLC was performed using either PLC 20 x 20 cm, 60 F254 Preparative plates (Merck) or Silica Gel GF 20 x 20 cm, U254 Preparative plates (Analtech) of various thicknesses. $^1$H NMR spectra were recorded on a Bruker Avance III instrument with an Oxford AS600 magnet equipped with a cryoprobe (600 MHz). Chemical shifts are reported in parts per million (ppm) from high to low frequency referenced to the residual solvent resonance (CDCl$_3$ = 7.26 ppm, CD$_2$Cl$_2$ = 5.32 ppm, CD$_3$OD = 3.31 ppm, (CD$_3$)$_2$SO = 2.50 ppm, (CD$_3$)$_2$CO = 2.05 ppm). All 1H resonances are reported to the nearest 0.01 ppm. Coupling constants (J) are reported in hertz (Hz) and reported to the nearest 0.1 Hz. Where appropriate, averages of the signals from peaks displaying multiplicity were used to calculate the value of the coupling constant. Standard abbreviations indicating multiplicity were used as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad; or combinations of thereof. COSY, DEPT, HSQC and HMBC experiments were used to aid structural determination and spectral assignment. $^{13}$C NMR spectra were recorded on the same spectrometer with the central resonance of the solvent peak as the internal reference (CDCl$_3$ = 77.16 ppm, CD$_2$Cl$_2$ = 54.00 ppm, CD$_3$OD = 44.00 ppm, (CD$_3$)$_2$SO = 34.52 ppm, (CD$_3$)$_2$CO = 206.26 ppm). Low resolution ESI mass spectrometry was performed with a Thermo Scientific LCQ Fleet Ion Trap Mass Spectrometer or an Agilent Technologies 1200 LC system with 6130 single quadrupole MS detector mass spectrometer. High resolution ESI (electrospray ionization) and EI (electron ionization) mass spectrometry were
carried out by the EPSRC National Mass Spectrometry Service Centre (Swansea, UK). Melting points (mp.) were determined using a Büchi M-565 apparatus and are corrected.
Synthesis of (2)

To a stirring solution of 3-bromo-5-hydroxybenzoic acid (3087 mg, 14.2 mmol) in methanol (50 ml) was added concentrated sulfuric acid (1.5 ml) in a dropwise manner. The resulting solution was then stirred overnight at room temperature, after which about half of the solvent was evaporated. The concentrated solution was poured into diethyl ether (100 ml) and washed with water (100 ml). The aqueous phase was extracted with diethyl ether (2x100 ml). The combined organic layers were then washed with water (100 ml), saturated solution of NaHCO$_3$ (150 ml) and brine (100 ml), dried over MgSO$_4$ and the solvent was evaporated under reduced pressure to give 2 (3083 mg, 93%). Characterizations are in accordance with those reported in the literature. $^1$H NMR (600 MHz, CD$_2$Cl$_2$) $\delta$ 7.73 (s, 1H, H$_a$), 7.44 (s, 1H, H$_b$), 7.23 (s, 1H, H$_c$), 5.49 (s, 1H, H$_d$), 3.89 (s, 3H, H$_e$). $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$) $\delta$ 165.42, 156.51, 133.04, 124.81, 123.00, 122.66, 115.31, 52.44. HRMS (ESI): $m/z$ = 228.9495 [M-H]$^-$ (calcd. 228.9506 for C$_8$H$_6$BrO$_3$).

Synthesis of (3)

To a stirring solution of 2 (2.7 g, 11.7 mmol, 1.0 eq) and K$_2$CO$_3$ (3.3 g, 23.4 mmol, 2.0 eq) in dry DMF (8.5 ml) was added 1-Bromohexane (3.86 g, 23.4 mmol, 2.0 eq). The resulting solution was stirred at room temperature for 20 h. The mixture was then poured into diethyl ether (200 ml) and washed with first water (2x150 ml) and then brine (100 ml), dried over MgSO$_4$ and the solvent was evaporated under reduced pressure. The resulting oil was kept under high vacuum overnight to get rid of any remaining 1-Bromohexane to yield 3 (3.4 g, 92%). $^1$H NMR (600 MHz,
CD<sub>2</sub>Cl<sub>2</sub>) δ 7.68 (s, 1H, H<sub>a</sub>), 7.47 (s, 1H, H<sub>b</sub>), 7.28 − 7.20 (m, 1H, H<sub>c</sub>), 3.98 (t, J = 6.5 Hz, 2H, H<sub>d</sub>), 3.88 (s, 3H, H<sub>e</sub>), 1.82 − 1.72 (m, 2H, H<sub>f</sub>), 1.45 (m, 2H, H<sub>g</sub>), 1.34 (m, 4H, H<sub>h</sub> and i), 0.95 (t, J = 6.9 Hz, 3H, H<sub>j</sub>). <sup>13</sup>C NMR (151 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 165.53, 159.94, 132.83, 124.38, 122.52, 122.28, 114.15, 68.74, 52.31, 31.49, 28.97, 25.56, 22.58, 13.79. HRMS (ESI<sup>+</sup>): m/z = 337.0406 [M+Na]<sup>+</sup> (calcd. 337.0410 for C<sub>14</sub>H<sub>19</sub>BrO<sub>3</sub>Na).

**Synthesis of (4)**

![Diagram](http://example.com/diagram.png)

To a stirring solution of 2 (1.48 g, 6.4 mmol, 1.0 eq) and K<sub>2</sub>CO<sub>3</sub> (1.77 g, 12.8 mmol, 2.0 eq) in dry DMF (5 ml) was added benzyl bromide (2.19 g, 12.4 mmol, 2.0 eq). The resulting solution was stirred at room temperature for 14 h. The mixture was then filtered off, the filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (DCM/Hexane, 1:1) to afford compound 4 (2.04 g, 99%) as colourless oil. <sup>1</sup>H NMR (600 MHz, Acetone-<sup>d</sup>6) δ 7.70 (t, J = 1.6 Hz, 1H, H<sub>a</sub>), 7.59 (dd, J = 2.4, 1.3 Hz, 1H, H<sub>b</sub>), 7.52 (d, J = 1.6 Hz, 2H, H<sub>j</sub>), 7.48 (t, J = 2.1 Hz, 1H, H<sub>c</sub>), 7.44 − 7.39 (m, 2H, H<sub>g</sub>), 7.37 − 7.34 (m, 1H, H<sub>j</sub>), 5.24 (s, 2H, H<sub>d</sub>), 3.89 (s, 3H, H<sub>e</sub>). <sup>13</sup>C NMR (151 MHz, Acetone-<sup>d</sup>6) δ 165.74, 160.66, 137.42, 134.02, 129.40, 128.94, 128.58, 125.23, 123.25, 115.65, 71.13, 52.82. HRMS (ESI<sup>+</sup>): m/z = 338.0373 [M+NH<sub>4</sub>]<sup>+</sup> (calcd. 338.0386 for C<sub>15</sub>H<sub>17</sub>No<sub>3</sub>Br).

**Synthesis of (5)**

![Diagram](http://example.com/diagram.png)

To a stirring solution of 3-iodoaniline (1.0 g, 4.57 mmol, 1eq) in dry THF (7.5 ml) was added Di-<i>tert</i>-butyl dicarbonate (1.49 g, 6.86 mmol, 1.5 eq) in portions at 0 °C and stirred at room temperature for 16 h after which another 1 equivalent of Di-<i>tert</i>-
butyl dicarbonate was added and continued to stir for 3 more hours. Solvent was evaporated under reduced pressure and the residue was purified by column chromatography (Hexane/ EtOAc, 9:1) to afford 5 (1.25 g, 86%). Characterizations are in accordance with those reported in the literature\textsuperscript{10}. \textsuperscript{1}H NMR (600 MHz, CD\textsubscript{2}Cl\textsubscript{2}) \(\delta\) 7.90 (s, 1H, H\textsubscript{a}), 7.36 (d, \(J = 8.0\) Hz, 1H, H\textsubscript{b}), 7.29 (d, \(J = 7.9\) Hz 1H, H\textsubscript{d}), 7.01 (t, \(J = 8.0\) Hz, 1H, H\textsubscript{c}), 6.54 (s, 1H, H\textsubscript{e}), 1.49 (s, 9H, H\textsubscript{f}). \textsuperscript{13}C NMR (151 MHz, CD\textsubscript{2}Cl\textsubscript{2}) \(\delta\) 152.77, 140.43, 132.28, 130.93, 127.44, 118.00, 94.58, 81.30, 28.51. HRMS (ESI\textsuperscript{+}): \(m/z = 341.9955\) [M+Na]\textsuperscript{+} (calcd. 341.9961 for C\textsubscript{11}H\textsubscript{14}INO\textsubscript{2}Na).

**Synthesis of (6)**

A 250ml-two neck- round bottom flask containing 5 (2.7 g, 8.46 mmol, 1 eq), Bis(pinacolato)diboron (2.58 g, 10.15 mmol, 1.2 eq), K\textsubscript{2}CO\textsubscript{3} (2.49 g, 25.4 mmol, 3 eq) and Pd(dppf)Cl\textsubscript{2}· CH\textsubscript{2}Cl\textsubscript{2} (0.691 g, 0.846 mmol, 0.1 eq) was set up with a condenser on top of it and sealed off. The system was then purged with Nitrogen for 20 min after which the solvent (dry DMF, 50 ml) that had been degassed with nitrogen for 1 hour was transferred via syringe and stirred at 90 °C under nitrogen for 19 hours. The solvent was then removed under reduced pressure and the mixture was dissolved in DCM and filtered off subsequently to get rid of insoluble materials. The mixture was then partitioned between DCM and water. The organic materials were extracted with DCM, washed with brine and dried over MgSO\textsubscript{4}. The solvent was evaporated and the residue was purified by column chromatography (PET (40-60 °C)/ Et\textsubscript{2}O, 2:1) to afford 6 (2.13 g, 79%), which was clean enough to be used for the next step. Characterizations are in accordance with those reported in the literature\textsuperscript{11}. \textsuperscript{1}H NMR (600 MHz, CD\textsubscript{2}Cl\textsubscript{2}) \(\delta\) 7.71 (s, 1H, H\textsubscript{a}), 7.45 (d, \(J = 8.1\) Hz, 1H, H\textsubscript{b}), 7.41 (d, \(J = 7.4\) Hz, 1H, H\textsubscript{d}), 7.29 (t, \(J = 7.7\) Hz, 1H, H\textsubscript{c}), 6.55 (s, 1H, H\textsubscript{e}), 1.33 (s, 12H, H\textsubscript{g}), 1.23 (s, 9H, H\textsubscript{f}). \textsuperscript{13}C NMR (151 MHz, CD\textsubscript{2}Cl\textsubscript{2}) \(\delta\) 153.19, 138.63, 129.60, 128.88, 124.80, 121.74, 84.43, 83.78, 28.58, 25.40, 25.20. HRMS (ESI\textsuperscript{+}): \(m/z = 337.2290\) [M+NH\textsubscript{4}]\textsuperscript{+} (calcd. 337.2293 for C\textsubscript{17}H\textsubscript{30}BN\textsubscript{2}O\textsubscript{4}).
Synthesis of (7)

A 250ml-two neck-round bottom flask containing 4 (2.04 g, 6.37 mmol, 1 eq), 6 (2.1 g, 6.58 mmol, 1.03 eq), K$_2$CO$_3$ (1.76 g, 12.75 mmol, 2 eq) and Pd(dppf)Cl$_2$·CH$_2$Cl$_2$ (0.52 g, 0.637 mmol, 0.1 eq) was set up with a condenser on top of it and sealed off. The system was then purged with Nitrogen for 20 min after which the solvent (THF/water, 4:1, 200 ml) that had been degassed with nitrogen for 1 hour was transferred via syringe and stirred at 75 °C under nitrogen for 16 hours. The solvent was then removed under reduced pressure and the mixture was dissolved in DCM and filtered off subsequently to get rid of insoluble materials. The residue was then concentrated and purified by column chromatography (PET (40-60 °C)/Et$_2$O, 4:1) to afford 7 (2.61 g, 94%). $^1$H NMR (600 MHz, Acetone-$d_6$) δ 8.55 (s, 1H, H$_m$), 7.92 (s, 1H, H$_a$), 7.87 (t, $J = 1.5$ Hz, 1H, H$_i$), 7.65 – 7.59 (m, 2H, H$_{b+l}$), 7.59 – 7.50 (m, 3H, H$_{f+c}$), 7.41 – 7.30 (m, 5H, H$_{g+h+k+j}$), 5.29 (s, 2H, H$_d$), 5.91 (s, 3H, H$_e$), 1.51 (s, 9H, H$_n$). $^{13}$C NMR (151 MHz, Acetone-$d_6$) δ 166.96, 160.27, 153.77, 143.72, 141.34, 141.17, 137.95, 133.01, 130.26, 129.37, 128.80, 128.58, 121.70, 121.13, 119.06, 118.60, 117.54, 114.95, 80.17, 70.83, 52.55, 28.52. HRMS (ESI$^+$): $m/z = 456.1777$ [M+Na]$^+$ (calcd. 456.1781 for C$_{26}$H$_{27}$NO$_5$Na).

Synthesis of (8)

Compound 7 (2.66 g, 1 eq by mass) was dissolved in a mixture of dry THF (20ml) and dry MeOH (50ml). The mixture was then purged with Nitrogen for 20 min and
Pd/C (10%) (0.8 g, 0.3 eq by mass) was added. Resulting heterogeneous mixture was purged with hydrogen for 25 min and stirred at room temperature under hydrogen atmosphere for 3h. After the reaction was complete, the mixture was filtered through a pad of celite to get rid of the catalyst and rinsed with THF. The filtrate was evaporated to yield 8 as off-white foam (2.07 g, 98%), which was clean enough to be used without further purification for the next step. \(^1\)H NMR (600 MHz, CD\(_2\)Cl\(_2\)) \(\delta\) 7.79 (s, 1H, H\(_a\)), 7.65 (s, 1H, H\(_b\)), 7.46 (s, 1H, H\(_c\)), 7.34 – 7.26 (m, 4H, H\(_{b+g+h+i}\)), 6.70 (s, 1H, H\(_j\)), 3.89 (s, 3H, H\(_e\)), 3.46 (s, 1H, H\(_d\)), 1.52 (s, 9H, H\(_k\)). \(^{13}\)C NMR (151 MHz, CD\(_2\)Cl\(_2\)) \(\delta\) 167.14, 156.99, 153.24, 143.27, 141.13, 139.69, 132.65, 129.94, 122.14, 120.92, 119.13, 118.42, 117.55, 115.73, 81.06, 52.69, 28.59. HRMS (ESI\(^+\)): \(m/z = 366.1311\) [M+Na\(^+\)] (calcd. 366.1312 for C\(_{19}\)H\(_{21}\)NO\(_5\)Na).

**Synthesis of (9)**

To a stirring solution of 8 (2.07 g, 6.03 mmol, 1 eq) in dry THF (35 ml) was carefully added 1 M solution of LiAlH\(_4\) in THF (18ml, 18 mmol, 3 eq) in a dropwise manner at 0 °C and stirred at room temperature until the reaction reached completion (40 min). The temperature was brought back down to 0 °C and excess LiAlH\(_4\) was quenched with first MeOH then water with care. The mixture was acidified with 4M HCl until the pH is around 3. Organic solvents were evaporated and the product was extracted using ethyl acetate, and then washed with brine, dried over MgSO\(_4\) and the solvent was evaporated under reduced pressure. The resulting solid was carefully washed with DCM to afford 9 (1.57 g, 82%) and used without further purification for the next step. \(^1\)H NMR (600 MHz, Acetone-\(d_6\)) \(\delta\) 8.45 (brs, 2H, H\(_{d+k}\)), 7.89 (s, 1H, H\(_a\)), 7.55 – 7.48 (m, 1H, H\(_j\)), 7.33 (t, \(J = 7.9\) Hz, 1H, H\(_i\)), 7.24 (d, \(J = 7.7\) Hz, 1H, H\(_{h_b}\)), 7.11 (s, 1H, H\(_a\)), 6.98 (s, 1H, H\(_b\)), 6.87 (s, 1H, H\(_c\)), 4.63 (d, \(J = 5.9\) Hz, 2H, H\(_c\)), 4.21 (s, 1H, H\(_j\)), 1.50 (s, 9H, H\(_k\)). \(^{13}\)C NMR (151 MHz, Acetone-\(d_6\)) \(\delta\) 158.70, 153.78, 145.64, 143.14, 142.62, 141.09, 129.92, 121.60, 117.94, 117.47, 117.14, 113.40, 113.05, 80.00, 64.61, 28.53. HRMS (ESI\(^+\)): \(m/z = 338.1358\) [M+Na\(^+\)] (calcd. 338.1363 for C\(_{18}\)H\(_{21}\)NO\(_4\)Na).
Synthesis of (10)

A solution of 9 (822 mg, 2.61 mmol, 1 eq) and triphenylphosphine (821 mg, 3.13 mmol, 1.2 eq) in dry THF (25 ml) was cooled down to 0 °C. Tetrabromomethane (1038 mg, 3.13 mmol, 1.2 eq) was slowly added in portions to the above solution and stirred at room temperature for 3h. Then another 1.2 equivalent of triphenylphosphine and tetrabromomethane were added and continued to stir at room temperature for one more hour. Solvent was evaporated under reduced pressure and the residue was purified by column chromatography (Et₂O/ Hexane, 1:1) to afford 10 (860 mg, 87%).

\(^1\)H NMR (600 MHz, Acetone-d\(_6\)) \(\delta\) 8.69 (s, 1H, H\(_d\) or j), 8.50 (s, 1H, H\(_d\) or j), 7.93 (s, 1H, H\(_j\)), 7.55 – 7.50 (m, 1H, H\(_i\)), 7.35 (t, J = 7.9 Hz, 1H, H\(_h\)), 7.25 (d, J = 7.7 Hz, 1H, H\(_g\)), 7.20 (s, 1H, H\(_a\)), 7.05 (s, 1H, H\(_b\)), 6.94 (s, 1H, H\(_c\)), 4.64 (s, 2H, H\(_e\)), 1.50 (s, 9H, H\(_k\)).

\(^{13}\)C NMR (151 MHz, Acetone-d\(_6\)) \(\delta\) 158.85, 153.77, 143.80, 141.89, 141.19, 141.03, 130.05, 121.59, 119.90, 118.24, 117.42, 115.98, 114.72, 80.07, 34.43, 28.52. HRMS (ESI\(^+\)): \(m/z = 400.0516\) [M+Na]\(^+\) (calcd. 400.0519 for C\(_{18}\)H\(_{20}\)BrNO\(_3\)Na).

Synthesis of (11)

To a stirring solution of 10 (844 mg, 2.23 mmol, 1 eq) in dry acetonitrile (15ml) was added KSAc (764 mg, 6.69 mmol, 3eq) and the resulting solution was stirred at room temperature overnight. Solvent was evaporated and the residue was purified by preparative TLC (PET (60-80 °C)/ EtOAc, 3:1) to afford 11 (355 mg, 43 %).

\(^1\)H NMR (600 MHz, CD\(_2\)Cl\(_2\)) \(\delta\) 7.58 (s, 1H, H\(_g\)), 7.34 (m, 2H, H\(_i\) + j), 7.22 (m, 1H, H\(_b\)),
7.07 (t, J = 1.5 Hz, 1H, Hₐ), 6.93 (t, J = 1.9 Hz, 1H, Hₖ), 6.78 – 6.74 (m, 1H, Hₐ), 6.65 (s, 1H, Hₐ), 5.12 (s, 1H, Hᵢ), 4.11 (s, 2H, Hₑ), 2.35 (s, 3H, Hᵢ), 1.51 (s, 9H, Hₙ).

¹³C NMR (151 MHz, CD₂Cl₂) δ 195.40, 156.72, 153.17, 143.30, 141.68, 140.74, 139.61, 129.86, 122.10, 120.54, 118.14, 117.47, 115.21, 113.44, 80.96, 33.70, 30.72, 28.59. HRMS (ESI⁺): m/z = 396.1236 [M+Na]⁺ (calcd. 396.1240 for C₂₀H₂₃NO₄SNa).

**Synthesis of (12)**

11 (107 mg, 0.287 mmol, 1 eq) was dissolved in dry THF (6 ml) and degassed for 10 min by nitrogen. Then pyrrolidine (61.2 mg, 0.861 mmol, 3 eq) was added and stirred at room temperature under nitrogen atmosphere for 2 h. The progress of the reaction is monitored by TLC and once the deprotection reached to completion, NaI (47.3 mg, 0.316 mmol, 1.1 eq), 1-Butanethiol (258.8 mg, 2.87 mmol, 10 eq) were added. The resulting mixture was titrated with iodine while vigorously stirring until the dark purple colour persisted. The excess of iodine was quenched by addition of saturated solution of Na₂SO₃. The mixture was then partitioned between EtOAc and water. The organic materials were extracted with EtOAc, washed with brine and dried over MgSO₄. The solvent was evaporated and the residue was dissolved in small amount of DCM and loaded to column for purification by column chromatography (Hexane/ Et₂O, 1:1) to afford 12 (63.8 mg, 53%). ¹H NMR (600 MHz, CD₂Cl₂) δ 7.62 (s, 1H, Hₖ), 7.38 – 7.30 (m, 2H, Hₗ + i), 7.25 (dt, J = 7.0, 1.8 Hz, 1H, H₉), 7.13 (t, J = 1.5 Hz, 1H, Hₙ), 6.97 (dd, J = 2.5, 1.6 Hz, 1H, H₉), 6.81 (dd, J = 2.5, 1.5 Hz, 1H, H₉), 6.64 (s, 1H, Hᵢ), 5.06 (s, 1H, Hᵢ), 3.89 (s, 2H, Hₑ), 2.5 (t, J = 7.7, 2H, Hᵢ), 1.60 – 1.55 (m, 2H, Hᵢ), 1.52 (s, 9H, Hₙ), 1.37 – 1.24 (m, 2H, Hₙ), 0.86 (t, J = 7.4 Hz, 3H, Hᵢ). ¹³C NMR (151 MHz, CD₂Cl₂) δ 156.65, 153.15,
12 (63 mg, 0.15 mmol) was dissolved in dry DCM (2.7 ml) and the temperature was brought down to 0 °C with an ice bath. Trifluoroacetic acid (0.3 ml) was added in a dropwise manner and the resulting solution was stirred at room temperature for 30 min. The mixture was partitioned between EtOAc and saturated solution of NaHCO₃ and extracted with EtOAc twice. Combined organic layers were washed with water, then brine and dried over MgSO₄. After removal of the solvent, the residue was purified by column chromatography (Hexane/Et₂O, 1:1 then Et₂O MeOH/DCM, 6:94) to afford 13 (16mg, 33%) ¹H NMR (600 MHz, CD₂Cl₂) δ 7.19 (t, J = 7.8 Hz, 1H, Hₗ), 7.11 (s, 1H, Hₐ), 6.96 – 6.92 (m, 2H, Hₘ + J), 6.88 (s, 1H, Hₖ), 6.82 (s, 1H, Hₖ), 6.67 (dd, J = 8.0, 2.3 Hz, 1H, Hₖ), 3.88 (s, 2H, Hₜ), 2.50 (t, J = 7.5 Hz, 2H, H₟), 1.56 (m, 2H, Hₜ), 1.32 (m, 2H, Hₜ), 0.86 (t, J = 7.4 Hz, 3H, Hₗ).

Synthesis of (14)

9 (733 mg, 2.32 mmol, 1 eq) was dissolved in dry MeOH (22 ml). 1.25 M of HCl in methanol (22.3 ml, 27.84 mmol, 12 eq) was added slowly. The resulting solution was stirred at room temperature overnight. Solvent was evaporated and the residue was partitioned between EtOAc (150ml) and saturated solution of NaHCO₃ (150
ml). The aqueous phase was extracted with EtOAc (2x150 ml), combined organic phases was washed with brine (150 ml) and dried over MgSO₄. Solvent was evaporated under reduced pressure to afford 14 (487 mg, 97 %) which was clean enough to be used without further purification for the next step. ¹H NMR (600 MHz, Acetone-d₆) δ 8.33 (s, 1H, H_d), 7.10 (t, J = 7.7 Hz, 1H, H_i), 7.05 (s, 1H, H_a), 6.97 – 6.89 (m, 2H, H_g+i), 6.82 (m, 2H, H_b+c), 6.64 (dd, J = 8.1, 2.2 Hz, 1H, H_h), 4.69 (brs, 2H, H_k), 4.61 (s, 2H, H_e), 4.17 (s, 1H, H_f). ¹³C NMR (151 MHz, Acetone-d₆) δ 158.55, 149.70, 145.34, 143.88, 142.85, 130.14, 117.09, 116.23, 114.27, 113.65, 113.01, 112.96, 64.67. HRMS (ESI⁺): m/z = 216.1015 [M+H]⁺ (calcd. 216.1019 for C_{13}H_{14}NO₂).

Synthesis of (15)

To a stirring solution of 14 (484 mg, 2.25 mmol, 1 eq) and 3,4-Dimethoxy-3-cyclobutene-1,2-dione (383 mg, 2.7 mmol, 1.2 eq) in dry MeOH (20 ml) was added Zn(OTf)₂ (82 mg, 0.225 mmol, 0.1 eq). The mixture was stirred at room temperature for 2 days. Methanol was evaporated under reduced pressure and the resulting solid was washed with diethyl ether to afford 15 (704 mg, 96%). ¹H NMR (600 MHz, Acetone-d₆) δ 9.66 (s, 1H, H_k or d), 8.50 (s, 1H, H_k or d), 7.79 (s, 1H, H_g), 7.51 – 7.38 (m, 3H, H_h+i+j), 7.19 (s, 1H, H_d), 7.05 (s, 1H, H_b), 6.90 (s, 1H, H_c), 4.65 (s, 2H, H_e), 4.50 (s, 3H, H_l), 4.25 (brs, 1H, H_f). ¹³C NMR (151 MHz, Acetone-d₆) δ 157.91, 144.89, 142.26, 141.49, 138.63, 129.62, 122.50, 118.19, 117.98, 116.28, 112.82, 112.18, 63.65, 60.32. HRMS (ESI⁺): m/z = 348.0839 [M+Na]⁺ (calcd. 348.0842 for C_{18}H_{15}NO₅Na).
**Synthesis of (16)**

(64.3 mg, 0.2 mmol, 1 eq) was dissolved in dry THF (20 ml) and Pyridinium chlorochromate (PCC) (128 mg, 0.6 mmol, 3 eq) was added. The mixture was stirred at room temperature for 90 min after which 2 more equivalent of PCC was added. Stirring is continued for another 2.5 h. The mixture was then filtered through a pad of celite and rinsed with THF. Solvent was evaporated and the residue is purified by column chromatography (THF/DCM, 15:85) to afford 16 (30.8 mg, 48%). 1H NMR (600 MHz, Acetone-d$_6$) $\delta$ 10.05 (s, 1H, H$_e$), 7.89 (s, 1H, H$_a$), 7.76 (s, 1H, H$_b$), 7.49 (m, 4H, H$_c$ + g + h + i), 7.39 (s, 1H, H$_f$), 4.52 (s, 3H, H$_k$). 13C NMR (151 MHz, Acetone-d$_6$) $\delta$ 191.94, 158.60, 142.67, 140.70, 138.90, 129.92, 126.81, 122.49, 119.86, 119.62, 118.86, 118.02, 114.32, 60.39. HRMS (ESI$^+$): $m/z$ = 346.0682 [M+Na]$^+$ (calcd. 346.0686 for C$_{18}$H$_{13}$NO$_5$Na).

**Synthesis of (17)**

To a stirring solution of 16 (30.4 mg, 0.094 mmol, 1 eq) and Trimethyl orthoformate (99.8 mg, 0.94 mmol, 10 eq) in dry methanol (5ml) was added catalytic amount of p-Toluenesulfonic acid monohydrate (1.79 mg, 0.0094 mmol, 0.1 eq). The resulting solution was stirred under nitrogen atmosphere and reflux condition at 80 °C for 3.5 h. Solid sodium bicarbonate was added to neutralize the acid. The mixture was then partitioned between EtOAc and water, the aqueous phase was extracted with EtOAc (2x50 ml) and the combined organic phases were washed with water, brine, dried over MgSO$_4$. Evaporation of the solvent resulted in 17 (34.7 mg, quantitative) which
was clean enough to be used for the next step without further purification. $^1$H NMR (600 MHz, Acetone-$d_6$) $\delta$ 8.62 (brs, 1H, H$_d$), 7.80 (s, 1H, H$_e$), 7.54 – 7.36 (m, 3H, H$_{h+i+j}$), 7.27 (s, 1H, H$_a$), 7.14 (s, 1H, H$_b$), 6.96 (s, 1H, H$_c$), 5.40 (s, 1H, H$_e$), 4.52 (s, 3H, H$_l$), 3.31 (s, 6H, H$_f$). $^{13}$C NMR (151 MHz, Acetone-$d_6$) $\delta$ 158.70, 142.85, 142.37, 141.78, 139.56, 130.58, 123.35, 119.19, 118.83, 117.48, 114.47, 114.09, 103.56, 61.21, 52.75. HRMS (ESI$^+$): $m/z$ = 392.1098 [M+Na]$^+$ (calcd. 392.1105 for C$_{20}$H$_{19}$NO$_6$Na).

**Synthesis of (18)**

A 250ml-two neck- round bottom flask containing 3 (1896 mg, 6.02 mmol, 1 eq), 6 (2500 mg, 7.83 mmol, 1.3 eq), K$_2$CO$_3$ (1664 mg, 12.04 mmol, 2 eq) and Pd(dppf)Cl$_2$·CH$_2$Cl$_2$ (492 mg, 0.602 mmol, 0.1 eq) was set up with a condenser on top of it and sealed off. The system was then purged with Nitrogen for 20 min after which the solvent (THF/ water, 4:1, 200 ml) that had been degassed with nitrogen for 1 hour was transferred via syringe and stirred at 75 $^\circ$C under nitrogen for 18 hours. The solvent was then removed under reduced pressure and the mixture was dissolved in DCM and filtered off subsequently to get rid of insoluble materials. The residue was then concentrated and purified by column chromatography first with PET (40-60 $^\circ$C)/ Et$_2$O, 5:2 then with DCM to afford 18 (2282 mg, 89%). $^1$H NMR (600 MHz, CD$_2$Cl$_2$) $\delta$ 7.84 (s, 1H, H$_a$), 7.64 (s, 1H, H$_d$), 7.55 (s, 1H, H$_e$), 7.37 (m, 2H, H$_{h+i}$), 7.31 (s, 1H, H$_b$), 7.29 (m, 1H, H$_m$), 6.65 (brs, 1H, H$_o$) 4.06 (t, $J$ = 6.6 Hz, 2H, H$_d$), 3.91 (s, 3H, H$_j$), 1.81 (m, 2H, H$_e$), 1.52 (s, 9H, H$_p$), 1.50 – 1.46 (m, 2H, H$_f$), 1.41 – 1.29 (m, 4H, H$_{g+h}$), 0.92 (t, $J$ = 6.6 Hz, 3H, H$_i$). $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$) $\delta$ 166.71, 159.60, 152.58, 142.40, 140.88, 139.17, 131.91, 129.38, 121.62, 120.39, 118.33, 117.75, 116.99, 113.52, 80.45, 68.47, 52.10, 31.57, 29.17, 28.03, 25.67, 22.62, 13.81. HRMS (ESI$^+$): $m/z$ = 450.2246 [M+Na]$^+$ (calcd. 450.2251 for C$_{25}$H$_{33}$NO$_5$Na).
Synthesis of (19)

To a stirring solution of 18 (1114 mg, 2.6 mmol, 1 eq) in dry THF (20 ml)/MeOD (7 ml) was carefully added NaBD₄ (544 mg, 13 mmol, 5 eq) in small portions. A condenser was attached and the mixture was stirred under nitrogen atmosphere at 70 °C for 6 h, after which another 6 equivalent of NaBD₄ was added and continued to stir overnight at the same temperature. Excess NaBD₄ was carefully quenched with water. The mixture was partitioned between EtOAc (300 ml) and water (300 ml). The aqueous phase was extracted with EtOAc (2x300 ml). Combined organic phases washed with brine (200 ml), dried over MgSO₄. Solvent was evaporated and the residue was purified by column chromatography (Hexane/ Et₂O, 1:1 to 1:2) to afford 19 (969 mg, 93%). ¹H NMR (600 MHz, CD₂Cl₂) δ 7.60 (s, 1H, Hₖ), 7.35 (m, 2H, Hₘₙ), 7.28 – 7.24 (m, 1H, Hₗ), 7.14 (s, 1H, Hₐ), 7.04 (s, 1H, H₇), 6.90 (s, 1H, Hₜ), 6.64 (s, 1H, Hₙ), 4.02 (t, J = 6.6 Hz, 2H, Hₜ), 1.85 – 1.74 (m, 2H, Hₚ), 1.51 (s, 9H, Hₚ), 1.49 – 1.44 (m, 2H, H₂), 1.36 (m, 4H, Hₕₙₚ), 0.90 (t, J = 6.9 Hz, 3H, Hᵢ). ¹³C NMR (151 MHz, CD₂Cl₂) δ 160.36, 153.15, 143.71, 142.88, 142.29, 139.60, 129.81, 122.18, 118.22, 117.99, 117.57, 113.04, 112.30, 80.91, 68.71, 32.15, 29.82, 28.59, 26.26, 23.18, 14.37. HRMS (ESI⁺): m/z = 424.2423 [M+Na]⁺ (calcd. 424.2427 for C₂₄H₃₁NO₄DNa).

Synthesis of (20)

19 (969 mg, 2.41 mmol, 1 eq) was dissolved in dry MeOH (23 ml). 1.25 M of HCl in methanol (23 ml, 28.75 mmol, 12 eq) was added slowly. The resulting solution
was stirred at room temperature overnight. Solvent was evaporated and the residue was partitioned between EtOAc (150 ml) and saturated solution of NaHCO₃ (150 ml). The aqueous phase was extracted with EtOAc (2x150 ml), combined organic phases was washed with brine (150 ml) and dried over MgSO₄. Solvent was evaporated under reduced pressure to afford 20 (695 mg, 95 %) which was clean enough to be used without further purification for the next step. ¹H NMR (600 MHz, CD₂Cl₂) δ 7.19 (t, J = 7.8 Hz, 1H, Hₘ), 7.12 (t, J = 1.5 Hz, 1H, Hₖ), 7.00 (t, J = 2.0 Hz, 1H, Hₜ), 6.96 (d, J = 7.7 Hz, 1H, Hₚ), 6.90 (t, J = 2.0 Hz, 1H, Hₜ), 6.88 (t, J = 2.0 Hz, 1H, Hₜ), 6.67 (ddd, J = 7.9, 2.2, 1.0 Hz, 1H,Hₙ), 4.01 (t, J = 6.6 Hz, 2H, Hₜ), 1.83 – 1.75 (m, 2H, Hₜ), 1.47 (m, 2H, Hₜ), 1.35 (m, 4H, Hₙ+h), 0.91 (t, J = 7.0 Hz 3H, Hₜ). ¹³C NMR (151 MHz, CD₂Cl₂) δ 160.30, 147.60, 143.57, 143.40, 142.55, 130.13, 118.17, 117.72, 114.64, 114.08, 112.83, 112.20, 68.69, 32.15, 29.82, 26.26, 23.18, 14.37. HRMS (ESI⁺): m/z = 302.2077 [M+H]⁺ (calcd. 302.2084 for C₁₉H₂₄NO₂D).

**Synthesis of (21)**

To a stirring solution of 20 (688 mg, 2.28 mmol, 1 eq) and 3,4-Dimethoxy-3-cyclobutene-1,2-dione (389 mg, 2.74 mmol, 1.2 eq) in dry MeOH (20 ml) was added Zn(OTf)₂ (83 mg, 0.228 mmol, 0.1 eq). The mixture was stirred at room temperature overnight. Methanol was evaporated under reduced pressure and the residue was purified by preparative TLC (MeOH/ DCM, 4:96) to afford 21 (845 mg, 90%). ¹H NMR (600 MHz, Acetone-δ₆) δ 9.59 (brs, 1H, H₂o), 7.83 (s, 1H, Hₜ), 7.53 – 7.37 (m, 3H, Hₙ+ₖ+ₜ), 7.28 (s, 1H, Hₖ), 7.14 (s, 1H, Hₜ), 6.97 (s, 1H, Hₜ), 4.51 (s, 3H, Hₕ), 4.09 (t, J = 6.5 Hz, 2H, Hₜ), 1.88 – 1.70 (m, 2H, Hₜ), 1.51 (m, 2H, Hₜ), 1.37 (m, 4H, Hₚ+h), 0.97 (t, J = 6.7 Hz, 3H, Hₜ). ¹³C NMR (151 MHz, Acetone-δ₆) δ 159.86, 156.84, 144.73, 142.15, 141.40, 138.65, 129.65, 122.56, 118.29, 118.02, 117.17, 111.94, 111.37, 67.70, 60.32, 31.46, 29.46, 25.63, 22.43, 13.43. HRMS (ESI⁺): m/z = 434.1904 [M+Na]⁺ (calcd. 434.1907 for C₂₄H₂₅NO₃D₂Na).
Synthesis of (22)

21 (794 mg, 1.93 mmol, 1 eq) was dissolved in dry DCM (30 ml) and Pyridinium chlorochromate (PCC) (500 mg, 2.32 mmol, 1.2 eq) was added. The mixture was stirred at room temperature for 1 min after which 2.4 more equivalent of PCC was added. Stirring is continued for another 1 h. The mixture was then directly loaded to the silica column without evaporating the solvent and purified by eluting first with DCM and then EtOAc/ PET ether (40-60 °C), 1:1, to afford 22 (537 mg, 68%). 

$^1$H NMR (600 MHz, Acetone-$d_6$) $\delta$ 7.92 (s, 1H, H$_a$), 7.85 (t, $J = 1.5$ Hz, 1H, H$_i$), 7.59 (t, $J = 2.0$ Hz, 1H, H$_b$), 7.55–7.46 (m, 4H, H$_c$+$m$+$k$+$j$), 4.52 (s, 3H, H$_o$), 4.19 (t, $J = 6.5$ Hz, 2H, H$_d$), 1.89 – 1.80 (m, 2H, H$_e$), 1.56 – 1.49 (m, 2H, H$_f$), 1.38 (m, 4H, H$_g$+$h$), 0.91 (t, $J = 6.9$ Hz, 3H, H$_i$). 

$^{13}$C NMR (151 MHz, Acetone) $\delta$ 161.27, 143.41, 141.45, 139.60, 130.82, 123.44, 121.45, 121.42, 120.09, 119.79, 118.97, 113.94, 69.15, 61.27, 32.30, 30.35, 26.44, 23.29, 14.31. HRMS (ESI$^+$): $m/z$ = 431.1670 [M+Na]$^+$ (calcd. 431.1688 for C$_{24}$H$_{24}$NO$_5$DNa).

Synthesis of (23)

To a stirring solution of 22 (296 mg, 0.72 mmol, 1 eq) and Trimethyl orthoformate (764 mg, 7.2 mmol, 10 eq) in dry methanol (5ml) was added catalytic amount of $p$-Toluenesulphonic acid monohydrate (7 mg, 0.037 mmol, 0.05 eq). The resulting solution was stirred under nitrogen atmosphere and reflux condition at 80 °C for 3 h. Solid sodium bicarbonate was added to neutralize the acid. The mixture was then partitioned between EtOAc and water, the aqueous phase was extracted with EtOAc
(2x50 ml) and the combined organic phases were washed with water, brine, dried over MgSO₄. Evaporation of the solvent resulted in 23 (200 mg, 60%) which was clean enough to be used for the next step without further purification. ¹H NMR (600 MHz, Acetone-"d₆") δ 9.61 (s, 1H, H₁₀), 7.85 (s, 1H, H₉), 7.45 (m, 3H, H₆+m+n), 7.35 (s, 1H, H₈), 7.24 (s, 1H, H₇), 7.02 (s, 1H, H₆), 4.52 (s, 3H, H₇), 4.10 (t, J = 6.5 Hz, 2H, H₆), 3.32 (s, 6H, H₈), 1.86 – 1.76 (m, 2H, H₉), 1.51 (m, 2H, H₇), 1.37 (m, 4H, H₉+H₁), 0.91 (t, J = 6.8 Hz, 3H, H₇). ¹³C NMR (151 MHz, Acetone-"d₆") δ 160.64, 142.75, 142.30, 141.66, 139.57, 130.62, 123.44, 119.23, 118.87, 118.32, 113.63, 113.21, 68.71, 61.21, 52.77, 32.35, 30.35, 26.50, 23.30, 14.31. HRMS (ESI⁺): m/z = 477.2100 [M+Na⁺]⁺ (calcd. 477.2106 for C₂₆H₃₀NO₆Na). Synthesis of (24)

To a stirring solution of 18 (1184 mg, 2.77 mmol, 1 eq) in dry THF (15 ml) was carefully added 1 M solution of LiAlH₄ in THF (8.3ml, 8.3 mmol, 3 eq) in a dropwise manner at 0 °C and stirred at room temperature until the reaction reached completion (90 min). The temperature was brought back down to 0 °C and excess LiAlH₄ was quenched with first MeOH then water with care. The mixture was acidified with 4M HCl until the pH is around 3. Organic solvents were evaporated and the residue was then partitioned between EtOAc(150 ml) and water (150 ml), the aqueous phase was extracted with EtOAc (2x150 ml) and the combined organic phases was washed with water, brine, dried over MgSO₄. Evaporation of the solvent resulted in 24 (1085 mg, 98%) which was clean enough to be used for the next step without further purification. ¹H NMR (600 MHz, CD₂Cl₂) δ 7.60 (s, 1H, H₁), 7.35 (m, 2H, H₆+n), 7.29 – 7.24 (m, 1H, H₉m), 7.14 (s, 1H, H₈m), 7.02 (s, 1H, H₇m), 6.90 (s, 1H, H₈), 6.64 (s, 1H, H₇), 4.69 (s, 2H, H₆), 4.02 (t, J = 6.6 Hz, 2H, H₇), 1.92 – 1.71 (m, 2H, H₈), 1.52 (s, 9H, H₉), 1.50 – 1.45 (m, 2H, H₉), 1.40 – 1.29 (m, 4H, H₉+H₁), 0.98 (t, J = 6.4 Hz, 3H, H₇). ¹³C NMR (151 MHz, CD₂Cl₂) δ 160.36, 153.16, 143.82, 142.88, 142.30, 139.60, 129.81, 122.18, 118.18, 117.98, 117.57, 113.02, 112.25,
Synthesis of (25)

A solution of 24 (822 mg, 2.61 mmol, 1 eq) and triphenylphosphine (1075 mg, 4.1 mmol, 1.5 eq) in dry THF (30 ml) was cooled down to 0 °C. Tetrabromomethane (1360 mg, 4.1 mmol, 1.5 eq) was slowly added in portions to the above solution and stirred at room temperature for 2h. Solvent was evaporated under reduced pressure and the residue was purified by column chromatography (DCM) to afford 25 (1180 mg, 94%). \(^1\)H NMR (600 MHz, CD\(_2\)Cl\(_2\)) \(\delta\) 7.59 (s, 1H, H\(_k\)), 7.36 (m, 2H, H\(_{n+m}\)), 7.25 (m, 1H, H\(_l\)), 7.18 (s, 1H, H\(_a\)), 7.06 (s, 1H, H\(_b\)), 6.92 (s, 1H, H\(_c\)), 6.65 (s, 1H, H\(_o\)), 4.54 (s, 2H, H\(_j\)), 4.02 (t, \(J = 6.6\) Hz, 2H, H\(_d\)), 1.87 – 1.73 (m, 2H, H\(_e\)), 1.52 (s, 9H, H\(_p\)), 1.50 – 1.45 (m, 2H, H\(_f\)), 1.36 (m, 4H, H\(_g+h\)), 0.93 (t, \(J = 6.7\) Hz, 3H, H\(_i\)). \(^{13}\)C NMR (151 MHz, CD\(_2\)Cl\(_2\)) \(\delta\) 160.32, 153.13, 143.26, 141.82, 140.19, 139.66, 129.87, 122.15, 120.52, 118.16, 117.52, 114.48, 114.08, 80.95, 68.82, 34.29, 32.14, 29.77, 28.59, 26.24, 23.18, 14.37. HRMS (ESI\(^+\)): \(m/z = 484.1438\) [M+Na]\(^+\) (calcd. 484.1458 for C\(_{23}\)H\(_{32}\)BrNO\(_3\)Na).

Synthesis of (26)

To a stirring solution of 25 (1170 mg, 2.53 mmol, 1 eq) in dry acetonitrile (15ml) was added KSAc (867 mg, 7.59 mmol, 3eq) and the resulting solution was stirred at room temperature overnight. Solvent was evaporated and the residue was then
partitioned between EtOAc (150 ml) and water (150 ml), the aqueous phase was extracted with EtOAc (2x150 ml) and the combined organic phases was washed with water, brine, dried over MgSO₄ and the residue was purified by column chromatography (PET (60-80 °C)/ Et₂O, 3:1) to afford 26 (1157 mg, quantitative).

\[ ^1H \text{ NMR (600 MHz, CD}_2\text{Cl}_2 \delta 7.54 (s, 1H, H}_l, 7.38 – 7.34 (m, 1H, H}_{o+n}, 7.23 (m, 1H, H}_n), 7.06 (t, J = 1.5 Hz, 1H, H}_a), 6.97 (t, J = 2.0 Hz, 1H, H}_b), 6.81 (t, J = 1.9 Hz, 1H, H}_c), 6.64 (s, 1H, H}_p), 4.13 (s, 2H, H}_j), 3.99 (t, J = 6.6 Hz, 2H, H}_d), 2.35 (s, 3H, H}_k), 1.85 – 1.74 (m, 2H, H}_e), 1.52 (s, 9H, H}_r), 1.49 – 1.44 (m, 2H, H}_f), 1.35 (m, 4H, H}_{g+h}, 0.92 (t, J = 7.3 Hz, 3H, H}_i). \]

\[ ^{13}C \text{ NMR (151 MHz, CD}_2\text{Cl}_2 \delta 195.38, 160.27, 153.14, 143.00, 142.09, 140.28, 139.60, 129.83, 122.18, 120.29, 118.05, 117.54, 114.41, 112.71, 80.91, 68.70, 33.96, 32.15, 30.72, 29.79, 28.59, 26.25, 23.18, 14.37. \]

HRMS (ESI⁺): \[ m/z = 480.2176 [M+Na]^+ \text{ (calcd. 480.2179 for C}_{26}\text{H}_{35}\text{NO}_{4}\text{SNa})]. \]

**Synthesis of (27)**

26 (877 mg, 1.92 mmol, 1 eq) was dissolved in dry THF (25 ml) and degassed for 10 min by nitrogen. Then pyrrolidine (410 mg, 5.76 mmol, 3eq) was added and stirred at room temperature under nitrogen atmosphere for 2h. The progress of the reaction is monitored by TLC and once the deprotection reached to completion, NaI (47.3 mg, 0.192 mmol, 0.1 eq) was added. The resulting mixture was titrated with iodine while vigorously stirring until the dark purple colour persisted. The excess of iodine was quenched by addition of saturated solution of Na₂SO₃. Organic solvents were evaporated and the residue was then partitioned between EtOAc(150 ml) and water (150 ml), the aqueous phase was extracted with EtOAc (2x150 ml) and the combined organic phases was washed with water, brine, dried over MgSO₄ and the residue was purified by column chromatography (Hexane/ Et₂O, 1:1) to afford 27 (363 mg, 46%). \[ ^1H \text{ NMR (600 MHz, CD}_2\text{Cl}_2 \delta 7.50 (s, 2H, H}_k), 7.38 (m, 2H, H}_a), 7.30 (t, J = 7.8 Hz, 2H, H}_m), 7.21 (m, 2H, H}_l), 7.01 (m, 4H, H}_{o+b}, 6.79 (s, 2H, H}_c), \]
6.64 (s, 2H, H₁), 3.95 (t, J = 6.6 Hz, 4H, H₄), 3.67 (s, 4H, H₇), 1.75 (m, 4H, H₈), 1.51 (s, 18H, H₉), 1.44 (m, 4H, H₆), 1.33 (m, 8H, H₇+₈), 0.90 (t, J = 6.8, 6H, H₅). ¹³C NMR (151 MHz, CD₂Cl₂) δ 160.21, 153.13, 142.82, 142.01, 139.79, 139.58, 129.82, 122.14, 120.98, 118.06, 117.52, 114.81, 113.08, 80.88, 68.73, 43.78, 32.17, 29.79, 28.61, 26.25, 23.18, 14.38. LRMS (ES⁺): m/z = 851.6 [M+Na]⁺.

Synthesis of (28)

27 (94.3 mg, 0.114 mmol) was dissolved in dry DCM (5 ml) and the temperature was brought down to 0 °C with an ice bath. Trifluoroacetic acid (0.5 ml) was added in a dropwise manner and the resulting solution was stirred at room temperature for 30 min. Then another 0.5 ml of TFA was added and stirring was continued for another 30 min. The solvents were co-evaporated under reduced pressure with toluene (10 ml). The residue was partitioned between EtOAc (50 ml) and saturated solution of NaHCO₃ (50 ml) and extracted with EtOAc (2x50 ml). Combined organic layers were washed with water, then brine and dried over MgSO₄. Evaporation of the solvent resulted in 28 (71.5 mg, quantitative) which was clean enough to be used without further purification. ¹H NMR (600 MHz, CD₂Cl₂) δ 7.17 (t, J = 7.9, 2H, H₉), 7.02 (s, 2H, H₁), 6.99 (s, 2H, H₀), 6.92 (d, J = 7.7 Hz, 2H, H₇), 6.85 (s, 2H, H₈), 6.76 (s, 2H, H₉), 6.65 (d, J = 8.2 Hz, 2H, H₁), 3.96 (t, J = 6.5 Hz, 4H, H₄), 3.67 (s, 4H, H₇), 1.76 (m, 4H, H₈), 1.47 − 1.41 (m, 4H, H₇), 1.37 − 1.30 (m, 8H, H₈+₉), 0.90 (t, J = 6.6 Hz, 6H). ¹³C NMR (151 MHz, CD₂Cl₂) δ 160.15, 147.69, 143.36, 142.32, 139.67, 130.15, 120.87, 117.66, 114.70, 114.63, 114.00, 112.87, 68.70, 43.84, 32.18, 29.81, 26.26, 23.18, 14.38. HRMS (ESI⁺): m/z = 629.3201 [M+H]⁺ (calcd. 629.3230 for C₃₈H₄₀N₂O₂S₂).
Synthesis of (29)

A round bottom flask containing 28 (70.5 mg, 0.112 mmol, 1 eq) and Tris(2-carboxyethyl)phosphine hydrochloride (38.6 mg, 0.134 mmol, 1.2 eq) was degassed with nitrogen for 15 min. DMF (5 ml) that has already been degassed for 30 min was transferred via syringe and finally triethylamine (45.4 mg, 0.448 mmol, 4 eq) was added. The resulting mixture was stirred at room temperature under nitrogen atmosphere for 90 min. NaI (37 mg, 0.246 mmol, 2.2 eq), 1-Butanethiol (202 mg, 2.24 mmol, 20 eq) were added. The resulting mixture was titrated with iodine while vigorously stirring until the dark purple colour persisted. The excess of iodine was quenched by addition of Na$_2$SO$_3$. Solvent was evaporated and the residue was dissolved in a mixture of DCM/MeOH and dry-loaded to a silica column. 29 (54 mg, 60%) was obtained by eluting the column first with hexane/Et$_2$O (3:1) and then plain Et$_2$O. $^1$H NMR (600 MHz, Methylene Chloride-$d_2$) δ 7.19 (t, $J$ = 7.8, 1H, $H_r$), 7.10 (s, 1H, $H_a$), 7.00 (s, 1H, $H_b$), 6.96 (d, $J$ = 7.7 Hz, 1H, $H_c$), 6.90 (s, 1H, $H_d$), 6.84 (s, 1H, $H_e$), 6.67 (d, $J$ = 8.0 Hz, 1H, $H_f$), 4.01 (t, $J$ = 6.4, 2H, $H_g$), 3.90 (s, 2H, $H_h$), 2.52 (t, $J$ = 7.5 Hz, 2H, $H_i$), 1.84 – 1.75 (m, 2H, $H_j$), 1.61 – 1.44 (m, 4H, $H_{k+l}$), 1.38 – 1.29 (m, 6H, $H_{k+l+m}$), 0.91 (t, $J$ = 6.3 Hz, 3H, $H_i$), 0.86 (t, $J$ = 7.5 Hz, 3H, $H_a$). $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$) δ 160.14, 147.64, 143.37, 142.43, 139.99, 130.14, 120.81, 117.71, 114.65, 114.04, 112.73, 68.70, 44.32, 38.88, 32.15, 31.72, 29.81, 26.26, 23.18, 22.20, 14.37, 13.96. HRMS (ESI$^+$): $m/z$ = 404.2057 [M+H]$^+$ (calcd. 404.2076 for C$_{23}$H$_{34}$NO$_2$S$_2$).
Synthesis of (30)

A sealed microwave tube containing 29 (29.5 mg, 0.073 mmol, 1eq), 17 (27 mg, 0.073 mmol, 1 eq), Zn(OTf)$_2$ (13 mg, 0.037 mmol, 0.5 eq) and dry methanol (2 ml) was stirred in an oil bath at 80 °C for 20 h. Solvent was evaporated and the residue was purified by preparative TLC (Et$_2$O). 30 (30.5 mg, 0.041 mmol, 56%) was extracted out of silica using a mixture of DCM/MeOH (88/12). $^1$H NMR (600 MHz, Acetone-$d_6$) δ 9.33 (brs, 1H, H$_n$), 7.93 (s, 1H, H$_{Ar}$), 7.87 (s, 1H, H$_{Ar}$), 7.54 – 7.35 (m, 8H, H$_{Ar+o+p}$), 7.29 (s, 1H, H$_{Ar or B}$), 7.26 (s, 1H, H$_{L or N}$), 7.22 (s, 1H, H$_C$), 7.18 (s, 1H, H$_M$), 6.98 (s, 1H, H$_{A or B}$), 6.97 (s, 1H, H$_{L or N}$), 5.37 (s, 1H, H$_i$), 4.08 (t, $J = 6.5$ Hz, 2H, H$_f$), 3.97 (s, 2H, H$_g$), 3.30 (s, 6H, H$_m$), 2.51 (t, $J = 5.6$ Hz, 2H), 1.86 – 1.73 (m, 2H, H$_e$), 1.56 (m, 2H, H$_i$), 1.50 (m, 2H, H$_d$), 1.36 (m, 4H, H$_{b+c}$), 1.34 (m, 2H, H$_j$), 0.91 (t, $J = 7.1$ Hz, 3H, H$_a$), 0.84 (t, $J = 7.4$ Hz, 3H, H$_k$). $^{13}$C NMR (151 MHz, Acetone-$d_6$) δ 183.51, 183.42, 166.90, 160.74, 158.72, 142.91, 142.75, 142.69, 142.42, 141.74, 140.86, 140.24, 140.20, 140.18, 130.64, 122.83, 122.79, 122.71, 121.07, 118.79, 118.64, 118.54, 118.43, 117.50, 115.84, 114.53, 114.01, 112.68, 103.64, 68.67, 52.81, 43.84, 38.54, 32.35, 31.84, 30.34, 26.53, 23.32, 22.25, 14.34, 13.91. HRMS (ESI): $m/z$ = 739.2883 [M-H]$^{-}$ (calcd. 739.2881 for C$_{42}$H$_{77}$N$_2$O$_6$S$_2$).
**Synthesis of (31)**

To a stirring solution of 30 (30 mg) in acetone (5ml) were added Amberlyst® 15 hydrogen form (10mg) and water (2 drops). The resulting mixture was stirred at room temperature overnight and then filtered through a pad of cotton. The filtrate was concentrated to yield 31 (27.6 mg, 98%) which was clean enough to be used for the next step. $^1$H NMR (600 MHz, Acetone-$d_6$) $\delta$ 10.02 (s, 1H, H$_l$), 9.38 (brs, 2H, H$_{o+p}$), 9.17 (s, 1H, H$_m$), 8.00 (s, 1H, H$_{Ar}$), 7.92 (s, 1H, H$_{Ar}$), 7.76 (s, 1H, H$_{Ar}$), 7.54 (s, 1H, H$_{Ar}$), 7.51 – 7.36 (m, 6H, H$_{Ar}$), 7.28 (s, 1H, H$_{A or B}$), 7.22 (t, 1H, H$_c$), 6.96 (s, 1H, H$_{A or B}$), 4.07 (t, $J$ = 6.4 Hz, 2H, H$_f$), 3.96 (s, 2H, H$_g$), 2.53 (t, $J$ = 7.4 Hz, 2H, H$_h$), 1.79 (m, 2H, H$_e$), 1.55 (m, 2H, H$_i$), 1.50 (m, 2H, H$_d$), 1.41 – 1.29 (m, 6H, H$_{b+c+j}$), 0.94 (t, $J$ = 6.7 Hz, 3H, H$_a$), 0.84 (t, $J$ = 7.4 Hz, 3H, H$_k$). $^{13}$C NMR (151 MHz, Acetone) $\delta$ 192.79, 183.55, 167.08, 166.83, 160.74, 159.51, 143.64, 142.76, 142.65, 141.60, 140.85, 140.39, 140.30, 140.16, 139.77, 130.81, 130.66, 122.86, 122.72, 121.10, 121.07, 120.61, 119.21, 118.85, 118.58, 115.84, 114.81, 112.67, 68.67, 43.84, 38.54, 32.35, 31.84, 30.35, 26.53, 23.32, 22.25, 14.33, 13.91. HRMS (ESI): $m/z$ = 693.2459 [M-H]$^-$ (calcd. 693.2462 for $C_{40}H_{41}N_2O_5S_2$).
A sealed microwave tube containing 23 (78.5 mg, 0.173 mmol, 1.5 eq), 13 (36.8 mg, 0.115 mmol, 1 eq), Zn(OTf)$_2$ (31.3 mg, 0.086 mmol, 0.75 eq) and dry methanol (3 ml) was stirred in an oil bath at 80 °C for 18 h. Solvent was evaporated and the residue was purified by preparative TLC (MeOH/DCM, 4:96 to 3:97) to afford 32 (55.7 mg, 65%) which was extracted out of silica using a mixture of DCM/MeOH (88/12). $^1$H NMR (600 MHz, Acetone-$d_6$) δ 9.42 (s, 1H, H$_n$), 7.93 (s, 1H, H$_{Ar}$), 7.90 (s, 1H, H$_{Ar}$), 7.54 – 7.35 (m, 8H, H$_{Ar+h+o}$), 7.34 (s, 1H, H$_{L or N}$), 7.27 (s, 1H, H$_{L or N}$), 7.20 (s, 1H, H$_C$), 7.14 (s, 1H, H$_M$), 7.00 (s, 1H, H$_{A or B}$), 6.88 (s, 1H, H$_{L or N}$), 4.07 (t, $J = 6.4$ Hz, 2H, H$_f$), 3.93 (s, 2H, H$_i$), 3.31 (s, 6H, H$_g$), 2.55 (t, $J = 7.1$ Hz, 2H, H$_j$), 1.79 (m, 2H, H$_e$), 1.61 (m, 2H, H$_k$), 1.53 (m, 2H, H$_d$), 1.41 – 1.25 (m, 6H, H$_{b+c+l}$), 0.95 (t, $J = 6.8$ Hz, 3H, H$_a$), 0.85 (t, $J = 7.4$ Hz, 3H, H$_m$). $^{13}$C NMR (151 MHz, Acetone-$d_6$) δ 166.86, 160.63, 158.84, 142.85, 142.75, 142.71, 142.39, 141.59, 140.84, 140.25, 140.18, 140.16, 130.67, 130.58, 122.74, 122.68, 120.21, 118.68, 118.44, 118.34, 116.43, 113.68, 113.54, 113.27, 68.67, 52.82, 43.92, 38.56, 32.36, 31.84, 26.53, 23.31, 22.24, 14.34, 13.91.
Synthesis of (33)

To a stirring solution of 32 (33 mg) in acetone (5 ml) were added Amberlyst® 15 hydrogen form (10 mg) and water (2 drops). The resulting mixture was stirred at room temperature overnight and then filtered through a pad of cotton. The filtrate was concentrated to yield 33 (30.3 mg, 98%) which was clean enough to be used for the next step. \(^1\)H NMR (600 MHz, Acetone-\(d_6\)) \(\delta\) 9.31 (brs, 2H, H\(_{g+n}\)), 8.65 (s, 1H, H\(_m\)), 8.01 (s, 1H, H\(_{4r}\)), 7.87 (s, 1H, H\(_{4r}\)), 7.84 (s, 1H, H\(_L\)), 7.62 (s, 1H, H\(_{M\ or\ N}\)), 7.52 – 7.35 (m, 7H, H\(_{Ar}\)), 7.19 (s, 1H, H\(_C\)), 6.89 (s, 1H, H\(_{A\ or\ B}\)), 4.16 (t, \(J = 6.5\) Hz, 2H, H\(_f\)), 3.93 (s, 2H, H\(_h\)), 2.53 (t, \(J = 7.4\) Hz, 2H, H\(_i\)), 1.87 – 1.78 (m, 2H, H\(_e\)), 1.61 – 1.48 (m, 4H, H\(_{de}\)), 1.41 – 1.25 (m, 6H, H\(_{b+c+k}\)), 0.91 (t, \(J = 6.9\) Hz, 3H, H\(_a\)), 0.85 (t, \(J = 7.4\) Hz, 3H, H\(_l\)). \(^{13}\)C NMR (151 MHz, Acetone) \(\delta\) 183.56, 183.49, 167.03, 166.84, 161.23, 158.89, 143.51, 142.77, 142.71, 141.54, 140.84, 140.37, 140.12, 139.55, 130.85, 130.61, 122.79, 121.76, 120.22, 120.03, 119.26, 118.76, 118.61, 118.53, 118.52, 116.49, 113.73, 113.67, 69.12, 43.91, 38.56, 32.32, 31.83, 30.35, 26.46, 23.31, 22.24, 14.33, 13.91. HRMS (ESI): \(m/\ell = 694.2525\) [M-H\(^-\)] (calcd. 694.2525 for C\(_{40}\)H\(_{40}\)N\(_2\)O\(_5\)S\(_2\)D).

Synthesis of (34)

To a stirring solution of 3,4-Dimethoxy-3-cyclobutene-1,2-dione (400 mg, 2.81 mmol, 1 eq) in dry MeOH (15 ml) was added slowly a mixture of 3-Bromopropylamine hydrobromide (679 mg, 3.1 mmol, 1.1 eq) and triethylamine (709 mg, 7 mmol, 2.5 eq) in MeOH (10 ml) in a dropwise manner. The reaction was
monitored by TLC until all 3,4-Dimethoxy-3-cyclobutene-1,2-dione is consumed (2h). Solvent was evaporated and the residue was purified by column chromatography (MeOH/DCM, 6:94) to yield 34 (567 mg, 81%). $^{1}$H NMR (600 MHz, DMSO- $d_6$) $\delta$ 8.82-8.61 (brs, 1H, $H_d$), 4.41 (s, 3H, $H_e$), 3.55 (m, 2H, $H_a$), 3.65 and 3.40 (m, 2H, $H_c$), 2.05 (m, 2H, $H_b$). $^{13}$C NMR (151 MHz, DMSO) $\delta$ 189.76, 182.98, 182.74, 178.11, 177.58, 172.87, 172.58, 60.62, 60.37, 42.85, 42.46, 33.70, 33.14, 31.94. HRMS (ESI$^+$): $m/z$ = 247.9913 [M+H]$^+$ (calcd. 247.9917 for C$_8$H$_{11}$BrNO$_3$).

**Synthesis of (35)**

![Chemical Structure](image)

To a stirring solution of $\beta$-Alanine methyl ester hydrochloride (2.53 g, 18.1 mmol, 1 eq) in methanol (10ml) was added hydrazine hydrate, 50-60%, (1.35 ml, 22 mmol, 1.2 eq). The resulting solution was stirred at room temperature for 1 day after which a solution of $p$-Anisaldehyde (4.94 g, 36.2 mmol, 2 eq) in methanol (40 ml) was added. The yellow heterogeneous solution was stirred at room temperature for 6 hours. Solvent was evaporated and the remaining solid was washed first with diethylether and then ethylacetate to afford 35 (4.17 g, 90 %, as cis/trans mixture, major product is reported in the NMR section) which was clean enough to be used without further purification for the next step. $^{1}$H NMR (600 MHz, DMSO- $d_6$) $\delta$ 11.44 (s, 1H, $H_d$), 7.98 (s, 1H, $H_e$), 7.92 (brs, 3H, $H_a$), 7.6 (d, $J$ = 8.6, 2H, $H_f$), 7.0 (d, $J$ = 8.6, 2H, $H_g$), 3.79 (s, 3H, $H_h$), 3.10 (brs, 2H, $H_b$), 3.00 (m, 2H, $H_c$). $^{13}$C NMR (151 MHz, DMSO) $\delta$ 171.40, 165.60, 160.81, 160.69, 146.25, 143.44, 128.61, 128.32, 126.74, 126.66, 114.34, 55.33, 34.96, 34.59, 31.30, 29.96. HRMS (ESI$^+$): $m/z$ = 222.1235 [M+H]$^+$ (calcd. 222.1237 for C$_{11}$H$_{16}$N$_3$O$_2$).
Synthesis of (36)

To a stirring solution of **34** (1.71 g, 6.9 mmol, 1 eq) and **35** (1.98 g, 8.97 mmol, 1.3 eq) in a mixture of dry MeOH (25 ml) and dry DCM (25 ml) was added triethylamine (1.82 g, 17.94 mmol, 2.6 eq). The resulting solution was stirred at room temperature for 1 day. Solvent was evaporated and the solid was washed first with methanol and then with diethylether to afford **36** (2.69 g, 89 %, as cis/trans mixture) which was clean enough to be used without further purification for the next step. 1H NMR (600 MHz, DMSO-d$_6$) δ 11.37 – 11.32 (brs, 1H, H$_h$), 8.08 – 7.93 (s, 1H, H$_i$), 7.64 – 7.57 (m, 2H, H$_k$), 7.46 (brs, 2H, H$_{d+e}$), 7.06 – 6.90 (m, 2H, H$_l$), 3.79 (m, 5H, H$_{f+l}$), 3.62 – 3.49 (m, 4H, H$_{a+c}$), 2.93 (t, J = 6.2 Hz, 2H, H$_g$), 2.04 (m, 2H, H$_b$). 13C NMR (151 MHz, DMSO-d$_6$) δ 183.05, 161.22, 161.03, 146.51, 143.49, 129.06, 128.75, 127.21, 114.74, 55.75, 42.08, 36.08, 34.38, 34.03, 32.03. HRMS (ESI$^+$): m/z = 437.0816 [M+H]$^+$ (calcd. 437.0819 for C$_{18}$H$_{22}$BrN$_4$O$_4$).

Synthesis of (37)

To a stirring solution of **36** (1532 mg, 3.5 mmol, 1 eq) in dry DMF (80 ml) was added KSAc (1200 mg, 10.5 mmol, 3 eq). The resulting solution was stirred at room temperature for 2 days. Solvent was evaporated and the solid was washed with EtOAc, water, MeOH and Et$_2$O, respectively, to afford **37** (981 mg, 65 %) which was clean enough to be used without further purification for the next step. 1H NMR (600 MHz, DMSO-d$_6$) δ 11.38 – 11.31 (brs, 1H, H$_i$), 8.08 – 7.93 (s, 1H, H$_j$), 7.68 – 7.55 (m, 2H, H$_k$), 7.50 (brs, 2H, H$_{d+e}$), 7.05 – 6.91 (m, 2H, H$_l$), 3.79 (m, 5H, H$_{g+m}$), 3.51 (s, 2H, H$_d$), 2.93-2.5 (m, 2H, H$_b$), 2.84 (t, J = 7.2 Hz, 2H, H$_g$), 2.31 (s, 3H, H$_a$), 1.73 (m, 2H, H$_c$). 13C NMR (151 MHz, DMSO-d$_6$) δ 195.73, 182.95, 161.21, 161.03, 146.52, 143.50, 129.05, 128.75, 127.21, 114.76, 55.74, 42.59, 34.40, 31.17,
31.02, 25.90. HRMS (ESI⁺): \( m/z = 433.1520 \) [M+H]⁺ (calcd. 433.1540 for C₂₀H₂₅N₄O₅S).

**Synthesis of (38)**

To a stirring solution of 31 (71 mg, 0.102 mmol, 1 eq) and 37 (88.2 mg, 0.204 mmol, 2 eq) in dry DMF (5 ml) were added TFA (13 drops) and water (4 drops). The resulting solution was stirred at room temperature for 20 hours. It was then directly dry-loaded to silica column and eluted with first EtOAc to get rid of DMF and then switched to MeOH/DCM (6%), followed by MeOH/DCM (12%) to afford 38 (70 mg, 69%). ¹H NMR (600 MHz, DMSO-d₆) \( \delta \) 11.55 – 11.47 (s, 1H, Hₛ), 10.06 (brs, 2H, Hₑᵣ₊ₛ), 9.84 (brs, 1H, Hₑᵤ), 8.14 – 7.99 (s, 1H, Hₑᵣ), 7.93 – 7.80 (m, 2H, Hₑᵥ), 7.59 – 7.32 (m, 10H, Hₑᵣ₊ₒ₊ᵢ), 7.24 (s, 1H, Hₑᵢ or B), 7.27 – 7.07 (m, 4H, Hₑᵢ₊ᵢ), 6.94 (s, 1H, Hₑᵢ₊ᵢ), 4.04 (t, \( J = 6.4 \) Hz, 2H, Hₑᵢ), 3.97 (s, 2H, Hₑᵣ), 3.80 (br, 2H, Hₑᵣ), 3.51 (br, 2H, Hₑᵤ), 2.98-2.50 (m, 2H, Hₑᵣ), 2.84 (m, 2H, Hₑᵣ), 2.50 (m, 2H, Hₑᵣ), 2.30 (s, 3H, Hₑᵣ), 1.74 (m, 4H, Hₑᵣ₊ᵢ), 1.49 (m, 2H, Hₑᵣ), 1.43 (m, 2H, Hₑᵣ), 1.34 – 1.29 (m, 4H, Hₑᵣ₊ᵢ), 1.25 (m, 2H, Hₑᵣ), 0.88 (t, \( J = 6.9 \) Hz, 3H, Hₑᵣ), 0.80 (t, \( J = 7.4 \) Hz, 3H, Hₑᵣ). ¹³C NMR (151 MHz, DMSO) \( \delta \) 195.27, 182.52, 181.94, 165.75, 159.20, 157.95, 157.86, 157.65, 157.45, 141.65, 141.11, 141.04, 140.80, 139.83, 139.05, 139.04, 136.06, 130.00, 129.97, 121.66, 121.56, 119.98, 118.34, 117.83, 117.80, 117.06, 116.34, 115.53, 114.93, 111.50, 67.55, 54.95, 48.62, 42.15, 40.06, 36.98, 31.03, 30.70, 30.55, 28.67, 25.47, 25.25, 22.13, 21.04, 13.96, 13.47. LRMS (ES⁻): \( m/z = 1025.6 \) [M+Cl].
Synthesis of (39)

A solution of 38 (20 mg, 0.02 mmol, 1 eq) in dry DMF (4 ml) was degassed with nitrogen for 15 min. Then pyrrolidine (14.4 mg, 0.2 mmol, 10 eq) was added and the solution was stirred at room temperature overnight. In order to break all the disulfide bonds, a solution of dithiothreitol (62 mg, 0.4 mmol, 20 eq) and triethylamine (40.5 mg, 0.4 mmol, 20 eq) in dry methanol (1 ml) was added and the mixture was stirred for 2 hours after which the solution was diluted with a mixture of DCM/MeOH (1:1, 200 ml). NaI (13 mg, 0.088 mmol, 4.4 eq) and iodine (110 mg, 0.433 mmol, 22 eq) were added and it was made sure that the colour of the reaction was purple. The resulting coloured mixture was stirred for 30 min after which all of the solvents were evaporated. The residue was dissolved in small amount of DMF and loaded to a silica column. The column was first eluted with EtOAc to get rid of DMF and then switched to MeOH/DCM (6%, 10% and 14%, respectively) to afford 39 (8.3 mg, 48%). $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 11.52 – 11.40 (s, 1H, H$_m$), 10.05 (brs, 2H, H$_{6+k}$), 9.80 (brs, 1H, H$_o$), 8.15 – 7.93 (s, 1H, H$_o$), 8.02 – 6.80 (m, 16H, H$_{Ar+O+P}$), 4.02 (t, $J$ = 6.4 Hz, 2H, H$_j$), 4.01 – 3.73 (m, 4H, H$_{g+k}$), 3.53 (br, 2H, H$_i$), 3.04 (br, 2H, H$_j$), 2.50 (m, 2H, H$_b$), 1.81 (m, 2H, H$_l$), 1.72 (m, 2H, H$_c$), 1.43 (m, 2H, H$_d$), 1.32 (m, 4H, H$_{b+c}$), 0.88 (br, 3H, H$_a$). $^{13}$C NMR (151 MHz, DMF-$d_7$) $\delta$ 183.50, 172.89, 168.19, 166.30, 162.19, 159.05, 143.28, 142.13, 137.08, 130.11, 121.90, 121.00, 118.52, 118.14, 116.30, 115.05, 114.30, 112.33, 68.26, 68.23, 43.15, 42.87, 40.93, 31.77, 30.94, 25.94, 22.76, 13.91. HRMS (ESI): $m/z$ = 857.2798 [M-H]$^-$ (calcd. 857.2797 for C$_{46}$H$_{45}$N$_6$O$_7$S$_2$).
Synthesis of (40)

A solution of 3-Hydroxybenzyl alcohol (2233 mg, 18 mmol, 1 eq) and triphenylphosphine (6609 mg, 25.2 mmol, 1.4 eq) in dry THF (45 ml) was cooled down to 0 °C. Tetrabromomethane (8357 mg, 25.2 mmol, 1.4 eq) was slowly added in portions to the above solution and stirred at room temperature for 1h. Solvent was evaporated under reduced pressure and the residue was purified by column chromatography (Et₂O/ Hexane, 1:1) to afford **40** (2788 mg, 83%). Characterizations are in accordance with those reported in the literature¹². ¹H NMR (600 MHz, Acetone-d₆) δ 8.46 (s, 1H, H₁), 7.18 (t, J = 7.8 Hz, 1H, H₂), 6.95 – 6.90 (m, 2H, H₃⁺e), 6.78 (d, J = 8.2 Hz, 1H, H₄), 4.56 (s, 2H, H₅). ¹³C NMR (151 MHz, Acetone-d₆) δ 158.41, 140.52, 130.60, 121.11, 116.84, 116.26, 34.44. HRMS (ESI): m/z = 184.9594 [M-H]⁻ (calcd. 184.9608 for C₇H₆BrO).

Synthesis of (41)

To a stirring solution of **40** (653 mg, 3.49 mmol, 2.2 eq) in dry acetonitrile (5 ml) was added a solution of 2-Cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite (479 mg, 1.59 mmol, 1 eq) in dry acetonitrile (3 ml). Then tetrazole, 0.45M in acetonitrile, (14.1 ml, 6.36 mmol, 4eq) was added and the resulting solution was stirred at room temperature for 90 min after which Luperox⁰ TBH70X (2 ml) was added and stirring was continued for another 1 hour. Solvent was evaporated and the residue was then partitioned between EtOAc and water, the aqueous phase was extracted with EtOAc (2x100 ml) and the combined organic phases was washed with water, brine, dried over MgSO₄. Solvent was evaporated and the residue
(loaded to the column with DCM) was purified by column chromatography (Et$_2$O) to afford 41 (301 mg, 39%). $^1$H NMR (600 MHz, CD$_2$Cl$_2$) $\delta$ 7.37 (t, $J$ = 8.0 Hz, 2H, H$_d$), 7.30 – 7.27 (m, 4H, H$_{c,e}$), 7.19 (d, $J$ = 7.3, 2H, H$_b$), 4.49 (s, 4H, H$_a$), 4.43 (dt, $J$ = 8.2, 6.1 Hz, 2H, H$_f$), 2.79 (td, $J$ = 6.2, 1.1 Hz, 2H, H$_g$). $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$) $\delta$ 150.74, 140.78, 130.91, 126.93, 121.23, 120.58, 116.83, 64.06, 32.94, 20.24. HRMS (ESI$^+$): $m/z$ = 509.9074 [M+Na]$^+$ (calcd. 509.9076 for C$_{17}$H$_{16}$Br$_2$NO$_4$PNa).

**Synthesis of (42)**

To a stirring solution of 3-Hydroxybenzyl alcohol (3 g, 24.2 mmol, 1 eq) in dry DCM (150 ml) was added at 0 °C tert-Butyldimethylsilyl chloride (10.9 g, 72.6 mmol, 3 eq) and imidazole (9.88 g, 145.2 mmol, 6 eq), respectively. The mixture was stirred at room temperature overnight. The solution was then washed with water (3x150 ml) and then with brine (100 ml), dried over MgSO$_4$. Solvent was evaporated and the residue was dried under high vacuum overnight to afford 42 (7.65 g, 90%) which was clean enough to be used without further purification for the next step. Characterizations are in accordance with those reported in the literature.$^{13}$ $^1$H NMR (600 MHz, CD$_2$Cl$_2$) $\delta$ 7.17 (t, $J$ = 7.8 Hz, 1H, H$_d$), 6.92 – 6.86 (m, 1H, H$_b$), 6.83 (s, 1H, H$_c$), 6.71 (m, 1H, H$_e$), 4.67 (s, 2H, H$_a$), 0.98 (s, 9H, H$_g$ or i), 0.94 (s, 9H, H$_g$ or i), 0.19 (s, 6H, H$_f$ or h), 0.10 (s, 6H, H$_f$ or h). $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$) $\delta$ 156.26, 143.82, 129.59, 119.45, 119.07, 118.27, 65.13, 26.22, 25.98, 18.78, 18.63, -4.18, -5.07. HRMS (ESI$^+$): $m/z$ = 370.2586 [M+NH$_4$]$^+$ (calcd. 370.2592 for C$_{19}$H$_{40}$NO$_2$Si$_2$).
Synthesis of (43)

To a stirring solution of 42 (7.65 g, 21.7 mmol, 1 eq) in acetonitrile (45 ml) and water (1.5 ml) was added DBU (3.3 g, 21.7 mmol, 1 eq). The resulting solution was stirred at room temperature for 2.5 h after which the solution was partitioned between Et$_2$O (300 ml) and saturated solution of NH$_4$Cl (150 ml) and washed with sat. NH$_4$Cl (3x150 ml), then with water (200 ml) and brine (150 ml), dried over MgSO$_4$. Solvent was evaporated to afford 43 (4.8 g, 93%) which was clean enough to be used without further purification for the next step. Characterizations are in accordance with those reported in the literature$^{14}$. $^1$H NMR (600 MHz, CD$_2$Cl$_2$) δ 7.18 (t, J = 7.8 Hz, 1H, H$_d$), 6.86 (d, J = 7.6 Hz, 1H, H$_b$), 6.81 (s, 1H, H$_c$), 6.69 (d, J = 8.3, 1H, H$_e$), 4.97 (s, 1H, H$_f$), 4.68 (s, 2H, H$_a$), 0.94 (s, 9H, H$_h$), 0.10 (s, 6H, H$_g$). $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$) δ 156.28, 144.16, 129.90, 118.79, 114.13, 113.36, 65.09, 26.23, 18.81, -5.08. HRMS (ESI$^+$): m/z = 256.1723 [M+NH$_4$]$^+$ (calcd. 256.1727 for C$_{13}$H$_{26}$NO$_2$Si).

Synthesis of (44)

To a stirring solution of 43 (915.7 mg, 3.84 mmol, 1 eq) and triethylamine (427 mg, 4.22 mmol, 1.1 eq) in dry DCM (20 ml) was added 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (999 mg, 4.22 mmol, 1.1 eq) at 0 °C. The resulting solution was stirred at room temperature for 25 min after which 43 (1006 mg, 4.22 mmol, 1.1 eq) and tetrazole, 0.45M in acetonitrile, (18.7 ml, 8.45 mmol, 2.2 eq) were added and the resulting solution was stirred at room temperature for 3.5
h. Then, 0.165 M of iodine solution in THF/Water (5:1) was added until a colour of purple persisted and continued stirring for 1h. The excess of iodine was quenched by addition of saturated solution of Na$_2$SO$_3$. The solution was partitioned between DCM (250 ml) and water (200 ml). The organic materials were extracted with DCM (200 ml), washed with brine and dried over MgSO$_4$. The solvent was evaporated and the residue was purified by column chromatography (Hexane/ EtOAc, 3:1; Hexane/ EtOAc, 1:1; MeOH/ DCM (4%); MeOH/ DCM (6.5%), respectively) to afford 44 (270 mg, 20%). $^1$H NMR (600 MHz, Methanol-$d_4$) $\delta$ 7.38 (t, $J$ = 7.9 Hz, 2H, H$_d$), 7.31 – 7.23 (m, 4H, H$_{c+e}$), 7.16 (d, $J$ = 8.6 Hz, 2H, H$_b$), 4.62 (s, 4H, H$_a$), 4.48 (dt, $J$ = 8.1, 5.9 Hz, 2H, H$_f$), 2.92 (td, $J$ = 6.0, 1.5 Hz, 2H, H$_g$). $^{13}$C NMR (151 MHz, Methanol-$d_4$) $\delta$ 151.72, 151.67, 145.78, 131.03, 125.19, 119.78, 119.75, 119.37, 119.33, 118.22, 65.47, 65.44, 64.33, 20.12, 20.07. HRMS (ESI$^+$): $m/z$ = 386.0761 [M+Na]$^+$ (calcd. 386.0764 for C$_{17}$H$_{18}$NO$_6$PNa).

**Synthesis of (45)**

To a stirring solution of Phosphorus(V) oxychloride (98 µl, 1.05 mmol, 1 eq) and triethylamine (234 mg, 2.31 mmol, 2.2 eq) in dry MeCN (8 ml) was added a solution of 43 (500 mg, 2.1 mmol, 2 eq) in dry MeCN (10 ml) slowly at 0 °C and stirred for 5 min. Then a solution of methanol (33.6 mg, 1.05 mmol, 1 eq) and triethylamine (117 mg, 1.16 mmol, 1.1 eq) in dry MeCN (4 ml) was added at 0 °C. The resulting solution was stirred at room temperature for 90 min after which it was poured into EtOAc (250 ml) and washed with saturated solution of NH$_4$Cl (150 ml), water (150 ml), brine (150 ml) and then dry over MgSO$_4$. Solvent was evaporated and the residue was dissolved in DCM and loaded to a silica column to purify by column chromatography (Et$_2$O/ Hexane, 40:60) to afford 45 (165mg, 28%). $^1$H NMR (600 MHz, CD$_2$Cl$_2$) $\delta$ 7.31 (t, $J$ = 7.9 Hz, 2H, H$_d$), 7.20 (s, 2H, H$_b$), 7.17 (d, $J$ = 8.1 Hz, 2H, H$_e$), 7.09 (d, $J$ = 7.9 Hz, 2H, H$_f$), 4.73 (s, 4H, H$_a$), 3.93 (d, $J$ = 11.6
Hz, 3H, Hβ), 0.10 (s, 12H, Hγ). 13C NMR (151 MHz, CD2Cl2) δ 151.19, 151.14, 144.63, 130.05, 123.33, 118.83, 118.80, 118.01, 117.97, 64.75, 55.98, 55.94, 26.19, 18.77, -5.11. HRMS (ESI⁺): m/z = 570.2825 [M+NH4]⁺ (calcd. 570.2831 for C27H49NO6PSi2).

Synthesis of (46)

To a stirring solution of 45 (160 mg, 0.29 mmol, 1 eq) in methanol (10 ml) was added p-Toluenesulfonic acid monohydrate (11 mg, 0.058mmol, 0.2 eq) and stirred at room temperature for 2h. Solvent was evaporated and the residue was purified by column chromatography (starting with Et2O then switched to MeOH/DCM (5%)) to afford 46 (83.5 mg, 89 %). 1H NMR (600 MHz, CD2Cl2) δ 7.33 (t, J = 7.9 Hz, 2H, Hd), 7.21 – 7.15 (m, 4H, Hc+e), 7.12 (d, J = 8.2 Hz, 2H, Hb), 4.63 (s, 4H, Ha), 3.96 (d, J = 11.6 Hz, 3H, Hf). 13C NMR (151 MHz, CD2Cl2) δ 150.57, 150.52, 143.82, 129.78, 123.71, 118.82, 118.79, 118.34, 118.31, 64.00, 55.67, 55.63. HRMS (ESI⁺): m/z = 347.0640 [M+Na]⁺ (calcd. 347.0655 for C15H17O6PNa).

Synthesis of (47)

To a stirring solution of 43 (666 mg, 2.8 mmol, 2 eq) and triethylamine (283 mg, 2.8 mmol, 2 eq) in dry MeCN (15 ml) was added a solution of ethyl dichlorophosphate (228 mg, 1.4 mmol, 1 eq) in dry MeCN (5 ml) slowly at 0 °C and stirred at room temperature overnight. Solvent was evaporated and the residue was purified by column chromatography (Hexane/ Et2O, 4:1) to afford 47 (357 mg, 45%). 1H NMR
(600 MHz, CD$_2$Cl$_2$) δ 7.31 (t, $J = 7.9$ Hz, 2H, H$_d$), 7.20 (s, 2H, H$_c$), 7.16 (d, $J = 7.2$ Hz, 2H, H$_e$), 7.11 (d, $J = 8$ Hz, 2H, H$_b$), 4.72 (s, 4H, H$_a$), 4.30 (dq, $J = 8.3$, 7.0 Hz, 2H, H$_f$), 1.35 (td, $J = 7.1$, 1.1 Hz, 3H, H$_g$), 0.93 (s, 18H, H$_i$), 0.10 (s, 12H, H$_h$). $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$) δ 151.26, 151.21, 144.56, 130.00, 123.25, 118.89, 118.86, 118.08, 118.04, 66.10, 66.06, 64.77, 26.20, 18.78, 16.46, 16.42, -5.11. HRMS (ESI$^+$): m/z = 589.2513 [M+Na]$^+$ (calcd. 589.2541 for C$_{28}$H$_{47}$O$_6$PSi$_2$Na).

**Synthesis of (48)**

To a stirring solution of 47 (350.2 mg, 0.62 mmol, 1 eq) in methanol (10 ml) was added p-Toluenesulfonic acid monohydrate (23.5 mg, 0.124 mmol, 0.2 eq) and stirred at room temperature for 40 min. Solvent was evaporated and the residue was purified by column chromatography (MeOH/DCM (6 %)) to afford 48 (197.2 mg, 94 %). $^1$H NMR (600 MHz, Methanol-$d_4$) δ 7.37 (t, $J = 7.9$ Hz, 2H, H$_d$), 7.27 – 7.19 (m, 4H, H$_{c+e}$), 7.12 (d, $J = 7.9$ Hz, 2H, H$_b$), 4.61 (s, 4H, H$_a$), 4.36 (dq, $J = 8.7$, 7.1 Hz, 2H, H$_f$), 1.38 (td, $J = 7.1$, 1.2 Hz, 3H, H$_g$). $^{13}$C NMR (151 MHz, Methanol-$d_4$) δ 151.94, 151.90, 145.66, 130.92, 124.94, 119.73, 119.70, 119.32, 119.29, 67.31, 67.27, 64.35, 16.38, 16.34. HRMS (ESI$^+$): m/z = 361.0806 [M+Na]$^+$ (calcd. 361.0811 for C$_{16}$H$_{19}$PO$_6$Na).

**Synthesis of (49)**

A solution of 48 (170.2 mg, 0.5 mmol, 1 eq) and triphenylphosphine (328 mg, 1.25 mmol, 2.5 eq) in dry THF (5 ml) was cooled down to 0 °C. Tetrabromomethane
(415 mg, 1.25 mmol, 2.5 eq) was slowly added in portions to the above solution and stirred at room temperature for 2h. Then another 0.5 equivalent of triphenylphosphine and tetrabromomethane were added and continued to stir at room temperature for two more hours. Solvent was evaporated under reduced pressure and the residue (loaded with DCM) was purified by column chromatography (Et₂O/ Hexane, 1:1) to afford 49 (167.3 mg, 72%). ¹H NMR (600 MHz, Methanol-d₄) δ 7.39 (t, J = 7.9 Hz, 2H, H₉), 7.33 – 7.28 (m, 4H, H$_{c+e}$), 7.17 (d, J = 8.3, 2H, H$_{b}$), 4.57 (s, 4H, H$_{a}$), 4.38 (m, 2H, H$_{f}$), 1.39 (t, J = 7.1 Hz, 3H, H$_{g}$). ¹³C NMR (151 MHz, Methanol-d₄) δ 151.78, 151.73, 142.13, 131.43, 127.53, 121.87, 121.84, 121.05, 121.02, 67.59, 67.55, 32.76, 16.41, 16.37.
References


Chapter Three

Acyl Hydrazones as Hydrogen Bonding Templates of Light Driven Molecular Shuttles

Synopsis: This chapter describes the design and synthesis of a light driven molecular shuttle with remarkable positional fidelity and efficacy into which an acyl hydrazone unit is incorporated as a novel station.


Acknowledgements

Dr. Daniel Tetlow is greatly appreciated for proofreading this chapter. All the authors are greatly appreciated for their contribution and support (Dr. Xiaokang for her synthetic efforts towards the one station rotaxane, Dr. Vitorica for resolving the X-ray structure, Dr. Marcos for conducting the isomerization studies, Dr. Yasar for helping me with the synthesis of all two station rotaxanes).
Acyl Hydrazones as Hydrogen Bonding Templates of Light Driven Molecular Shuttles

Introduction

Despite the fact that a variety of external triggers have been investigated and used to promote translational movement of the macrocycle in a rotaxane system (as discussed in chapter 1), light induced molecular shuttles with high efficacy remain scarce. Three important factors should be noted in the design of a molecular shuttle that operates through irradiation with light. Firstly, having a remarkable positional discrimination between stations is what constitutes an effective shuttle. Secondly, stability of each state of the stations is crucial for the lifetime of each operation, and lastly a high interconversion ratio between two states of a station is preferable. A molecular shuttle designed to bear all three aforementioned factors is a unique advantage. A light and heat switchable bistable molecular shuttle exploiting the photochemical and thermal interconversion of fumaramide and maleamide groups that give rise to a remarkable positional discrimination caused by “matched” and “mismatched ” hydrogen bonding motifs was published by the Leigh group in 2003, with the highest photochemical interconversion ratio being 65% . Herein we report our efforts in search of a new interconvertible binding station with noteworthy positional integrity. We have focused our attention onto making a rotaxane which operates by means of H-bonding interaction, as their synthesis and functioning mechanism is well established within the literature, especially in regard towards benzylic amide rotaxanes (See Chapter 1 for detailed information). As in the case of previously reported rotaxanes which utilize various amide, ester, squarine, phenolate, urea, pyridone, azodicarboximade, nitrone, sulfoxide, phosphinamides and ion-pair templates around which the macrocycle is assembled, we need a station that bears hydrogen bond accepting units. These units should be able to be manipulated by means of light and heat in order to create relatively high and low binding affinities. To that end, we envisioned that a pyridyl acyl hydrazone type station- which, to the best of our knowledge, has never been used as a template to form rotaxanes - in which the Z isomer is stabilized through an internal hydrogen bonding could be an excellent candidate. In this motif the desired isomerization could be achieved photochemically and thermally; each isomer is stable until the next stimuli is applied and finally in the form of Z isomer, one of the
binding sites on the station is severely compromised due to aforementioned internal H-bonding, thereby, creating a station which has low affinity towards the macrocycle.


Simple amide stations and other hydrogen bond acceptor motifs are effective templates for rotaxane formation because their hydrogen bond-accepting carbonyl groups are well positioned to direct the assembly of a benzylic amide macrocycle around the template site via five-component ‘clipping’ reactions. Given the

**Figure 1** Synthesis and operation of 1-station [2]Rotaxane
geometric requirements of the multipoint hydrogen-bonded intermediate needed in this process, we were intrigued to as whether pyridyl acyl hydrazones could act as

Figure 2 HRMS (ESI') of E-10, m/z = 1178.5156 [M+H]' (calcd. 1178.5175 for C_{75}H_{68}N_{7}O_{7}).

Figure 3 Experimental (top) and theoretical (bottom) isotopic mass distribution of E-10. HRMS (ESI m/z = 1178.5156 [M+H]' (calcd. 1178.5175 for C_{75}H_{68}N_{7}O_{7}).
templates and whether their E/Z isomerization could alter the near-ideal hydrogen-bonding structure between macrocycle and thread, provoking a change in the internal dynamics governed by those interactions. *E*-pyridyl acyl hydrazone thread (*E*-9) was synthesized in six steps from commercially available compounds (**Figure 1**). Upon UV irradiation *E*-9 thread was isomerized to its corresponding *Z* isomer (*Z*-9, 91 % yield), the conversion of which could easily be determined by comparing their $^1$H NMR spectra (**Figure 5**). The most substantial difference is a downfield shift of the typical NH proton of the hydrazide moiety ($\Delta \delta_{H_h} = 5.88$ ppm), which could be rationalized by an internal H-bonding of this proton with the pyridyl nitrogen. Each thread was subjected to rotaxane-forming conditions using an eight-fold molar excess of isophthaloyl dichloride and $p$-xylylenediamine in CHCl$_3$ in the presence of Et$_3$N (**Figure 1**). Pleasingly, *E*-9 yielded the desired [2]rotaxane *E*-10.

![Figure 4](image)

**Figure 4** Partial $^1$H NMR spectra (600 MHz, CD$_2$Cl$_2$, 298 K) of: a) Thread *E*-9; b) Rotaxane *E*-10; c) Rotaxane *Z*-10 obtained from irradiation of *E*-10 with 312 nm UV light for 1 h; d) Thread *Z*-9 obtained from irradiation of *E*-9 with 312 nm UV light for 30 min. (see **Figure 2** and 3 for HRMS data) in an impressive 85 % yield (cf. 62 % glycylglycine$^{13}$ and 50 % succinamide$^{14}$ threads using similar rotaxane-forming protocols) whereas the *Z*-thread did not afford the corresponding rotaxane, presumably due to the H-bond accepting atoms on the thread not being well enough oriented to direct the kinetically-controlled interlocking reaction.
Exploiting the reversible $E$ to $Z$ isomerization of the acyl hydrazone station, rotaxane $Z$-10 was generated by UV irradiation of $E$-10 (98 %, Figure 1). In fact, the $E$-isomers of thread and rotaxane could be rapidly restored from their $Z$-isomer counterparts by heating at 40 °C with catalytic amount of trifluoroacetic acid (TFA) followed by neutralization with $K_2CO_3$ (98 %, Figures 1, 5 and 6). Both $Z$-isomers ($Z$-9 and $Z$-10) are stable in solid form and in solution, without observing isomerization to the corresponding $E$-isomers after a week at room temperature.

As predicted, the reversible $E/Z$ isomerization of the acyl hydrazone moiety alters the nature and strength of the hydrogen-bond network between macrocycle and thread and therefore also changes the internal dynamics governed by those interactions. Indeed, rotaxanes $E$-10 and $Z$-10, display noticeable differences in their $^1$H NMR spectra (CD$_2$Cl$_2$, 298 K). The most substantial differences are due to the splitting of most of the peaks corresponding to the macrocycle and thread protons in $Z$-10 (Figure 4). This is a result of restricted rotamerization of the thread and of slow rotational motion of the macrocycle.
Figure 6 Partial $^1$H NMR spectra (600 MHz, CD$_2$Cl$_2$, 298 K) of: a) Rotaxane $E$-10; b) Rotaxane $Z$-10 obtained by irradiation of $E$-10 with 365 nm UV light for 1 h; c) Solution of (b) after 2 h heating at 40 ºC with catalytic amount of TFA (20 mol%), followed by neutralization with K$_2$CO$_3$.

Also remarkable is the two distinct environments observed for the NH proton of the hydrazide moiety (δ = 13.90 and 12.68 ppm).

Figure 8 X-ray crystal structure of acyl hydrazone [2]-rotaxane Z-10. Hydrogen bond lengths [Å]: N8–H19AN, 1.91; N19–H23N, 2.35; O36–H23AN, 2.19; O36–H18N, 2.24. Hydrogen bond angles (°): N8–H19AN–N19, 142.0; N19–H23N–N23, 153.0; N23–H23N–O36, 156.0; N18–H18N–O36, 120.0.

Single crystals of each isomer of rotaxane (E-10 and Z-10) were obtained by slow evaporation of CH₂Cl₂: CH₃CN (1:1) and the solid-state structures were determined by X-ray crystallography (Figure 7 and 8). These structures demonstrate a good fit between thread and macrocycle. However, the nature of the established noncovalent interactions between the acyl hydrazone thread and the macrocycle were found to be different in both isomers. The crystal structure of rotaxane E-10 (Figure 7) shows that the macrocycle adopts a distorted boat-like conformation with one amide hydrogen bonding to the pyridyl ring nitrogen N (2.078 Å) and with the other isophthalamide unit adopting bifurcated hydrogen bonds to the single carbonyl group (2.184, 2.237 Å) of the thread. The fourth amide group of the macrocycle engages in hydrogen-bonding with a water molecule (2.059 Å) which, in turn acts as hydrogen bond acceptor to the amide NH (1.938 Å) of the hydrazide moiety. On the other hand, the crystal structure of Z-10 rotaxane (Figure 8) adopts a co-conformation, in which two of the NH groups of the macrocycle form hydrogen bonds (2.189, 2.238 Å) with the CO group and another NH group of the same ring interacts with the nitrogen atom of the hydrazone function through a NH···Nsp² hydrogen bond (2.350 Å). Additionally, a six-membered intramolecular hydrogen bond is formed between the hydrazone proton and the pyridyl nitrogen (1.908 Å); this is the cause of the high thermal stability of these isomers (Z-9 and Z-10). The difference in hydrogen-bonding behavior of these isomers suggests that it might be
possible to modulate the strength of the intercomponent binding in such rotaxanes by photochemical and thermal isomerization.

**Synthesis and Light and Thermal Switching of Acyl Hydrazone-Containing Molecular Shuttles**

The significant difference in binding modes, the high stability and interconversion ratio shown by the acyl hydrazone station led us to translate this hydrogen bonding motif into a more complex structure, in which photochemical and thermal isomerization could be used to induce shuttling of the macrocycle between two different sites of the rotaxane thread with a remarkable positional interconversion (up to 97%), high stability and photochemical interconversion ratio (up to 91%).

In an effort to search of a second station that has binding affinities towards the macrocycle significantly stronger than Z isomer of the hydrazone station and significantly weaker than E isomer of the hydrazone station. It is not sufficient for the binding affinity just to be somewhere between those of E/Z isomers, there also has to be a significant difference between them in order to ensure excellent translational discrimination.

After carefully examining the literature, it was hypothesized that an amide type station, which is a commonly used motif in rotaxanes which feature hydrogen bonding type shuttling, would be a suitable station to provide translational fidelity. In order to test this hypothesis, a mono amide bearing station was selected to be the first candidate. To that effect, rotaxane E-19 (Figure 9) was synthesized (see the

![E-19](image)

**Figure 9** 2-Station [2]Rotaxane with monoamide station
Unfortunately, the mono amide station was too weak to attract the macrocycle onto itself. When the hydrazone was in its Z-form (which was hypothesized to be the weakest binding mode), the macrocycle would still reside over hydrazone station suggesting that mono amide is a too weak station to be used in this system. Having concluded that one amide group was not enough, it was decided to introduce another amide group into the second station in order to improve its hydrogen bonding capability. To that effect, rotaxane $E$-25 (Figure 10) was synthesized (see the experimental procedures for details). However, the succinic amide station proved to be extremely strong in terms of the strength of the hydrogen bonding affinities towards the macrocycle compared to hydrazone station. When the hydrazone was in its $E$-form (which was hypothesized to be the strongest binding mode in the system), the macrocycle would prefer to reside over the succinic amide station. These results lead to the conclusion that in order to provide remarkable positional fidelity, the station should be stronger in binding than a mono amide, but weaker than a succinic amide. From these observations a similar station where one of the amide moieties in the succinic amide station is replaced by an ester group in order to decrease the binding affinity was employed. The design of this new rotaxane thread consists of a pyridyl acyl hydrazone-based binding site and a flexible succinamide-ester group (Figures 11 and 14). The macrocycle preferentially binds to the $E$-pyridyl acyl hydrazone moiety over the succinamide-ester station due to the superior hydrogen bonding strength. Photochemical isomerization to the corresponding Z-form switches off the hydrogen bonding to the pyridyl functional group and relocates the macrocycle to the succinamide-ester binding site. The synthesis of $E$-hydrazone-succinamide-ester thread ($E$-29) was achieved in eight steps from commercially available starting materials (77:23 $E$/Z
ratio, see the experimental part and Figure 11).

Figure 11 Synthesis of 2-Station [2]Rotaxane with ester amide station
Figure 12 HRMS (ESI⁺) of E-30: m/z = 1461.7299 [M+H]⁺ (calcd. 1461.7322 for C₉₁H₉₇N₈O₁₀).

Figure 13 Experimental (top) and theoretical (bottom) isotopic mass distribution of E-30. HRMS (ESI⁺): m/z = 1461.7299 [M+H]⁺ (calcd. 1461.7322 for C₉₁H₉₇N₈O₁₀).
Thread $E$-29 could be smoothly isomerized to the $Z$-29 thread by UV irradiation (91 %, Figure 14) and restored back through thermal isomerization in the presence of catalytic amount of trifluoroacetic acid (77 %, Figure 14). Formation of the interlocked architecture using the multi-component clipping reaction furnished the rotaxane $E$-30 (see Figures 12 and 13 for HRMS data) in a 70% yield (Figures 11 and 14).

Since the xylylene rings of the macrocycle shield the encapsulated regions of the thread, the position of the macrocycle in CD$_2$Cl$_2$ could be easily determined for each pair of rotaxanes by comparing the chemical shift of the protons in the rotaxane with those of the corresponding thread. The spectra of both isomers of thread and rotaxane, $E/Z$-29 and $E/Z$-30, respectively, and their operation spectra are shown in Figures 15, 16 and 17. With the $E$-acyl hydrazone rotaxane ($E$-30), the chemical shift of the signal corresponding to the NH group of the hydrazone station ($\Delta \delta = -1.84$ ppm) and protons H$_a$ and H$_c$ ($\Delta \delta = -0.63$ and -0.83 ppm) are significantly shifted upfield, (compare Figure 15a and 15b), due to shielding from the xylylene rings of the macrocycle, whereas signals from the succinimide-ester moiety do not undergo large shifts between thread and rotaxane, consistent with the macrocycle being located over the hydrazone binding site. On the contrary, the resonances of rotaxane $Z$-30 exhibit shielding of the protons (H$_l$ and H$_m$) of the succinamide-ester unit ($\Delta \delta = -1.19$ and -1.02 ppm), whereas the signals from the acyl hydrazone station remains similar, indicating that the macrocycle is preferentially located around the succinamide-ester station (compare Figure 15c and 15d).
Figure 15 Partial $^1$H NMR spectra (600 MHz, CD$_2$Cl$_2$, 298 K) of: a) Thread E-29 (77:33 E:Z ratio); b) Rotaxane E-30; c) Rotaxane Z-30 obtained from irradiation of E-30 with 312 nm UV light for 2 h; d) Thread Z-29 obtained from irradiation of E-29 with 312 nm UV light for 1 h.

Figure 16 Partial $^1$H NMR spectra (600 MHz, CD$_2$Cl$_2$, 298 K) of: a) Thread E-29; b) Thread Z-29 obtained by irradiation of E-29 with 365 nm UV light for 1 h; c) Solution of (b) after 2 h heating at 40 ºC with catalytic amount of TFA (20 mol%), followed by neutralization with K$_2$CO$_3$. 

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Figure 17 Partial $^1$H NMR spectra (600 MHz, CD$_2$Cl$_2$, 298 K) of: a) Rotaxane $E$-$30$; b) Rotaxane $Z$-$30$ obtained by irradiation of $E$-$30$ with 365 nm UV light for 2 h; c) Solution of (b) after 2 h heating at 40 °C with catalytic amount of TFA (20 mol%), followed by neutralization with K$_2$CO$_3$. 
Conclusion

In summary, we have described the unprecedented ability of acyl hydrazones to act as a new template to assemble rotaxanes via a 5-component “clipping” method. X-ray structures and $^1$H NMR spectroscopy of $E$ and $Z$-[2]rotaxane isomers reveal important information about the nature of intramolecular hydrogen bonding in both solution and the solid state. Furthermore, their configurational dynamics can be reversibly and efficiently controlled by photochemical and thermal cis-trans isomerizations. This novel photoswitchable station helped pave the way to create a highly-efficient, bistable, stimuli-responsive rotaxane with excellent positional discrimination and high stability (up to 97%). By studying a series of stations (mono amide, succinic ester amide and succinic amide), it was attempted to find the best suitable station for positional integrity. The mono amide station was found to be too poor a station to promote macrocyclic shuttling regardless of which isomer of the hydrazone was present. Conversely, the succinic amide station proved to be an extremely strong binding site when compared to the hydrazone station, in this case the macrocycle would always reside over it regardless of which stereoisomer of the hydrazone was present. Finally, the succinic ester amide station proved to be the optimal station, which presents extremely remarkable positional discrimination. Understanding the efficacy and limitations of particular classes of photoactive molecular shuttles, such as this one, should prove useful in the development of more complex molecular devices due to the convenience of energy input and the absence of waste products.
Experimental Procedures

General Methods

Unless stated otherwise, all reagents and solvents were purchased from commercial sources and used without further purification. Dry tetrahydrofuran, N,N-dimethylformamide, dichloromethane, and acetonitrile were obtained by passing the solvent (HPLC grade) through an activated alumina column on a Phoenix SDS solvent drying system (JC Meyer Solvent Systems, CA, USA). Anhydrous methanol was purchased from Sigma-Aldrich. Flash column chromatography on silica was carried out using Aldrich Silica 60Å (particle size 40-63μm) as the stationary phase, and TLC was performed on precoated silica gel plates (0.25 mm thick, 60 F<sub>254</sub>, Merck, Germany) and visualized using UV light in combination with standard laboratory stains (acidic potassium permanganate, acidic ammonium molybdate and ninhydrin). Preparative TLC was performed using either PLC 20 x 20 cm, 60 F<sub>254</sub> Preparative plates (Merck) or Silica Gel GF 20 x 20 cm, U<sub>254</sub> Preparative plates (Analtech) of various thicknesses. <sup>1</sup>H NMR spectra were recorded on a Bruker Avance III instrument with an Oxford AS600 magnet equipped with a cryoprobe (600 MHz). Chemical shifts are reported in parts per million (ppm) from high to low frequency referenced to the residual solvent resonance (CDCl<sub>3</sub> = 7.26 ppm, CD<sub>2</sub>Cl<sub>2</sub> = 5.32 ppm, CD<sub>3</sub>OD = 3.31 ppm, (CD<sub>3</sub>)<sub>2</sub>SO = 2.50 ppm, (CD<sub>3</sub>)<sub>2</sub>CO = 2.05 ppm). All <sup>1</sup>H resonances are reported to the nearest 0.01 ppm. Coupling constants (<i>J</i>) are reported in hertz (Hz) and reported to the nearest 0.1 Hz. Where appropriate, averages of the signals from peaks displaying multiplicity were used to calculate the value of the coupling constant. Standard abbreviations indicating multiplicity were used as follows: <i>s</i> = singlet, <i>d</i> = doublet, <i>t</i> = triplet, <i>q</i> = quartet, quin = quintet, <i>m</i> = multiplet, br = broad; or combinations of thereof. COSY, DEPT, HSQC and HMBC experiments were used to aid structural determination and spectral assignment. <sup>13</sup>C NMR spectra were recorded on the same spectrometer with the central resonance of the solvent peak as the internal reference (CDCl<sub>3</sub> = 77.16 ppm, CD<sub>2</sub>Cl<sub>2</sub> = 54.00 ppm, CD<sub>3</sub>OD = 44.00 ppm, (CD<sub>3</sub>)<sub>2</sub>SO = 34.52 ppm, (CD<sub>3</sub>)<sub>2</sub>CO = 206.26 ppm). Low resolution ESI mass spectrometry was performed with a Thermo Scientific LCQ Fleet Ion Trap Mass Spectrometer or an Agilent Technologies 1200 LC system with 6130 single quadrupole MS detector mass spectrometer. High resolution ESI (electrospray ionization) and EI (electron ionization) mass spectrometry were
carried out by the EPSRC National Mass Spectrometry Service Centre (Swansea, UK). Melting points (mp.) were determined using a Büchi M-565 apparatus and are corrected.
Synthesis of 2

To a stirring solution of 3,3-diphenyl-1-propanol (1) (4.70 g, 22.13 mmol, 1.00 eq) and carbon tetrabromide (8.81 g, 26.55 mmol, 1.20 eq) in CH₂Cl₂ (60 mL) at 0 °C, triphenylphosphine (6.96 g, 26.55 mmol, 1.20 eq) was added. The resulting mixture was stirred for 4 h at room temperature and then concentrated under reduced pressure. The resulting crude residue was purified by a flash column chromatography (SiO₂, Et₂O/pet. ether, 0:100 to 20:80) to give the desired compound as a colourless oil (6.09 g, 22.13 mmol, quantitative). ¹H NMR (600 MHz, CDCl₃): δ = 7.37 – 7.19 (m, 10H, H₆Ar), 4.23 (t, J = 7.7 Hz, 1H, H₇a), 3.35 (t, J = 6.7 Hz, 2H, H₇c), 2.61 (q, J = 7.0 Hz, 2H, H₇b). ¹³C NMR (151 MHz, CDCl₃): δ = 143.55, 128.78, 128.00, 126.69, 49.23, 38.41, 32.20. HRMS (ESI⁺): m/z = 274.0365 [M]+ (calcd. 274.0357 for C₁₅H₁₅Br).

Synthesis of 5

To a stirring solution of 2-bromo-5-hydroxypyridine (3) (3.86 g, 22.20 mmol, 1 eq) and caesium carbonate (8.68 g, 26.64 mmol, 1.20 eq) in MeCN (80 mL), a solution of 2 (6.11 g, 22.20 mmol, 1.00 eq) in MeCN (40 mL) was added. The resulting mixture was stirred under reflux at N₂ atmosphere for 18 h and then concentrated under reduced pressure. The residue was partitioned between H₂O (200 mL) and CH₂Cl₂ (3 x 200 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic phases were washed with brine (300 mL), dried (MgSO₄), filtrated and concentrated under reduced pressure to give the desired product as a light brown solid (7.53 g, 20.44 mmol, 92%). ¹H NMR (600 MHz, CDCl₃): δ = 8.01 (d, J = 3.1 Hz, 1H, H₆d), 7.34 (d, J = 8.8 Hz, 1H, H₆f), 7.33 – 7.20 (m, 10H, H₆Ar), 7.02 (dd, J = 8.7, 3.2 Hz, 1H, H₆e), 4.24 (t, J = 7.9 Hz, 1H, H₆g), 3.93 (t, J = 6.3 Hz, 2H, H₆b), 2.56 (dt, J = 7.9, 6.3 Hz, 2H, H₆b). ¹³C NMR (151 MHz, CDCl₃): δ = 154.78, 143.83, 137.61, 132.05,
128.67, 128.09, 127.83, 126.55, 124.75, 66.74, 47.09, 34.70. HRMS (ESI\(^+\)): \( m/z = 368.0647 \) [M+H]\(^+\) (calcd. 368.0645 for \( C_{20}H_{18}BrNO \)).

**Synthesis of 6**

To a stirring solution of 5 (250 mg, 0.67 mmol, 1.00 eq) in dry THF (10 mL) at -78 °C was slowly added \( n \)-BuLi (0.51 mL, 1.6 M in hexanes, 0.81 mmol, 1.20 eq) under \( N_2 \) atmosphere. After stirring at -78 °C for 2 h, DMF (0.5 mL) was added and the reaction was stirred at room temperature for 4 h. The mixture was quenched by addition of saturated aqueous \( \text{NH}_4\text{Cl} \) (10 mL) and extracted with Et\(_2\)O (3 \( \times \) 30 mL). The combined organic phases were dried (MgSO\(_4\)), filtrated and concentrated under reduced pressure. The resulting crude residue was purified by a flash column chromatography (SiO\(_2\), EtOAc/hexane 20:80) to give the desired product as a brown oil (148 mg, 0.47 mmol, 70%). \(^1\)H NMR (600 MHz, CDCl\(_3\)): \( \delta = 10.01 \) (s, 1H, \( H_g \)), 8.40 (d, \( J = 2.8 \) Hz, 1H, \( H_d \)), 7.94 (d, \( J = 8.6 \) Hz, 1H, \( H_f \)), 7.35 – 7.19 (m, 11H, \( H_{Ar} \)), 4.27 (t, \( J = 8.0 \) Hz, 1H, \( H_a \)), 4.05 (t, \( J = 6.3 \) Hz, 2H, \( H_c \)), 2.62 (dt, \( J = 7.9, 6.3 \) Hz, 2H, \( H_b \)). \(^{13}\)C NMR (151 MHz, CDCl\(_3\)): \( \delta = 191.98, 158.39, 146.15, 143.68, 138.79, 128.72, 127.80, 126.63, 123.41, 120.49, 66.86, 47.13, 34.58. \) HRMS (ESI\(^+\)): \( m/z = 318.1484 \) [M+H]\(^+\) (calcd. 318.1489 for \( C_{21}H_{19}NO_2 \)).

**Synthesis of 7**

To a degassed solution of methyl-4-hydroxybenzoate (4) (552 mg, 3.63 mmol, 1.00 eq) and caesium carbonate (1.42 g, 4.36 mmol, 1.20 eq) in MeCN (40 mL), a solution of 2 (1.00 g, 3.63 mmol, 1.00 eq) in MeCN (80 mL) was added. The resulting mixture was stirred under reflux at \( N_2 \) atmosphere for 18 h and then concentrated under reduced pressure. The residue was then partitioned between H\(_2\)O (200 mL) and CH\(_2\)Cl\(_2\) (100 mL) and extracted with CH\(_2\)Cl\(_2\) (3 \( \times \) 50 mL). The combined organic phases were washed with brine (200 mL), dried (MgSO\(_4\)),
filtrated and concentrated under reduced pressure to give the desired product as a light brown solid (1.13 g, 3.26 mmol, 90%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta = 7.98$ (d, $J = 8.8$ Hz, 2H, H$_e$), 7.42–7.17 (m, 10H, H$_{Ar}$), 6.87 (d, $J = 8.9$ Hz, 2H, H$_d$), 4.27 (t, $J = 7.8$ Hz, 1H, H$_a$), 3.97 (t, $J = 6.3$ Hz, 1H, H$_c$), 3.91 (s, 3H, H$_f$), 2.58 (q, $J = 6.8$ Hz, 1H, H$_b$). $^{13}$C NMR (151 MHz, CDCl$_3$): $\delta = 166.90$, 162.70, 144.06, 131.57, 128.63, 127.89, 126.46, 122.50, 114.11, 66.07, 51.89, 47.18, 34.78. HRMS (ESI$^+$): $m/z = 347.1641$ [M+H]$^+$ (calcd. 347.1642 for C$_{23}$H$_{23}$O$_3$).

**Synthesis of 8**

![Chemical structure of 8](image)

Under N$_2$ atmosphere, 7 (500 mg, 1.44 mmol, 1.00 eq) was dissolved in dry MeOH (20 mL) and hydrazine hydrate (360 µL, 11.55 mmol, 8.00 eq) was added. The reaction mixture was refluxed for 18 h and then concentrated under reduced pressure. The residue was then partitioned between H$_2$O (50 mL) and CH$_2$Cl$_2$ (50 mL) and extracted with CH$_2$Cl$_2$ (3 × 30 mL). The combined organic phases were washed with brine (100 mL), dried (MgSO$_4$), filtrated and concentrated under reduced pressure to give the desired product as a colourless solid (493 mg, 1.42 mmol, 99%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta = 7.68$ (d, $J = 8.8$ Hz, 2H, H$_e$), 7.34–7.20 (m, 10H, H$_{Ar}$), 6.89–6.86 (m, 2H, H$_d$), 4.26 (t, $J = 7.9$ Hz, 1H, H$_a$), 4.08 (br s, 2H, H$_f$), 3.95 (t, $J = 6.4$ Hz, 1H, H$_c$), 2.57 (dt, $J = 7.8$, 6.3 Hz, 2H, H$_b$). $^{13}$C NMR (151 MHz, CDCl$_3$): $\delta = 168.36$, 161.86, 144.04, 128.62, 128.58, 127.87, 126.46, 124.76, 114.49, 66.08, 47.17, 34.76. HRMS (ESI$^+$): $m/z = 347.1755$ [M+H]$^+$ (calcd. 347.1754 for C$_{22}$H$_{23}$O$_2$N$_2$).

**Synthesis of E-9**

![Chemical structure of E-9](image)

Aldehyde 6 (100 mg, 0.31 mmol, 1.00 eq) and hydrazide 8 (110 mg, 0.31 mmol, 1.00 eq) were combined in EtOH (5 mL) and catalytic amount of acetic acid was
added. The reaction mixture was stirred for 18 h at room temperature. The reaction mixture was concentrated under reduced pressure and recrystallization in EtOH gave the desired product as a brown solid (163 mg, 0.25 mmol, 80%). \(^1\)H NMR (600 MHz, CD\(_2\)Cl\(_2\)): \(\delta\) 9.24 (s, 1H, \(H_e\)), 8.21 (br, 1H, \(H_d\)), 8.03 – 7.94 (br, 1H, \(H_b\)), 7.78 (br, 2H, \(H_i\)), 7.29 (m, 17H, \(H_{Ar+g}\)), 7.19 (m, 5H, \(H_{Ar+e}\)), 6.91 (d, \(J = 8.4\) Hz, 2H, \(H_h\)), 4.24 (m, 2H, \(H_{a+m}\)), 3.98 (m, 4H, \(H_{c+k}\)), 2.56 (m, 4H, \(H_{b+l}\)). \(^{13}\)C NMR (151 MHz, CD\(_2\)Cl\(_2\)): \(\delta\) = 167.71, 163.69, 162.14, 155.09, 145.57, 144.46, 144.18, 137.93, 136.51, 129.46, 128.80, 128.71, 127.95, 127.87, 126.65, 122.11, 114.56, 66.97, 66.31, 47.40, 47.31, 34.74, 34.61. HRMS (ESI\(^+$\)): \(m/z = 646.3051\) [M+H]\(^+\) (calcd. 646.3064 for C\(_{43}\)H\(_{40}\)N\(_3\)O\(_3\)).

**Synthesis of 11**

Hydrazine hydrate (8.2 mL, 263 mmol, 8.00 eq) was added to methyl 4-hydroxy benzoate (4) (5 g, 32.88 mmol, 1.00 eq) and the reaction mixture was refluxed for 18 h. The solid obtained was washed with hexane (100 mL) to give the desired product as a light brown solid (4.94 g, 32.47 mmol, 99%). \(^1\)H NMR (600 MHz, DMSO-\(d_6\)): \(\delta\) = 9.80 (bs, 1H, \(H_e\)), 9.49 (s, 1H, \(H_b\)), 7.68 (d, \(J = 8.6\) Hz, 2H, \(H_c\)), 6.77 (d, \(J = 8.6\) Hz, 2H, \(H_d\)), 4.38 (bs, 2H, \(H_a\)). \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)): \(\delta\) = 166.36, 160.44, 129.27, 124.41, 115.27. HRMS (ESI\(^+$\)): \(m/z = 153.0655\) [M+H]\(^+\) (calcd. 153.0659 for C\(_7\)H\(_9\)N\(_2\)O\(_2\)).

**Synthesis of 12**

Aldehyde 6 (398 mg, 1.25 mmol, 1 eq) and hydrazide 11 (190 mg, 1.25 mmol, 1 eq) were combined in EtOH (5 mL) and catalytic amount of acetic acid was added. The reaction mixture was stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure and recrystallization in EtOH gave the desired product as a brown solid (451 mg, 1.00 mmol, 80%). \(^1\)H NMR (600 MHz, DMSO-
δ = 11.70 (s, 1H, Hq), 10.20 (br s, 1H, Hs), 8.39 (s, 1H, Hg), 8.26 (d, J = 2.8 Hz, 1H, Hd), 7.87 (d, J = 8.2 Hz, 1H, Hq), 7.80 (d, J = 8.3 Hz, 2H, Hf), 7.41 (dd, J = 8.8, 2.8 Hz, 1H, Hg), 7.37 (d, J = 7.6 Hz, 4H, HAr), 7.30 (t, J = 7.6 Hz, 4H, HAr), 7.18 (t, J = 7.3 Hz, 2H, Hc), 6.86 (d, J = 8.2 Hz, 2H, Hj), 4.24 (t, J = 8.0 Hz, 1H, Ha), 3.99 (t, J = 6.4 Hz, 2H, Hc), 2.54 (q, J = 6.9 Hz, 2H, Hb).

13C NMR (151 MHz, DMSO-d6): δ = 162.90, 161.47, 155.19, 145.44, 144.743, 138.33, 137.12, 129.73, 129.02, 128.29, 128.07, 126.77, 123.12, 116.17, 66.16, 47.14, 34.22. HRMS (ESI+): m/z = 452.1962 [M+H]+ (calcd. 452.1969 for C28H26N3O3).

**Synthesis of 14**

To a stirring solution of 12-aminododecanoic acid (13) (2 g, 9.29 mmol, 1 eq) in 13 mL of THF was added 1M BH3·THF (37 mL, 37 mmol, 4 eq) under N2 atmosphere at room temperature. The reaction mixture was refluxed for 18 h and then cooled to room temperature. Next, 10% AcOH in MeOH (30 mL) was added dropwise, followed by stirring at room temperature for 30 min. The solvent was concentrated under reduced pressure, and the solid was dried subsequent addition and evaporation of MeOH (3 x 30 mL). The resulting white solid was suspended in H2O (20 mL), followed by addition of 9 M aqueous HCl (40 mL). The suspension was stirred for 48 h then it was filtrated to give the desired product as a white solid (1.9 g, 7.98 mmol, 87%). 1H NMR (600 MHz, CD3OD): δ = 5.10 (t, J = 6.7 Hz, 2H, Hg), 4.45 – 4.41 (m, 2H, Ha), 3.18 (p, J = 7.3 Hz, 2H, Hb), 3.08 (p, J = 6.8 Hz, 2H, Hb), 3.00 – 2.79 (m, 16H, Haliphatic). 13C NMR (151 MHz, CD3OD): δ = 62.98, 41.02, 33.66, 30.74, 30.69, 30.65, 30.61, 30.53, 30.29, 29.34, 27.54, 26.96. HRMS (ESI+): m/z = 202.2159 [M+H]+ (calcd.202.2165 for C12H28NO).

**Synthesis of 16**

To a stirring solution of 3,3-diphenylpropionic acid (15) (570 mg, 2.52 mmol, 1 eq), 14 (600 mg, 2.52 mmol, 1 eq), 4-dimethylaminopyridine (370 mg, 3.03 mmol, 1.2 eq) in dry CH2Cl2 (50 mL) at 0 °C, EDCI. HCl (522 mg, 2.72 mmol, 1.08 eq) was
added. The reaction mixture was stirred for 48 h at room temperature. The solution was washed with saturated solution of citric acid (2 x 50 mL) and \( \text{H}_2\text{O} \) (2 x 50 mL). The combined organic phases were dried (\( \text{MgSO}_4 \)), filtrated and concentrated under reduced pressure. The crude residue was purified by a flash column chromatography (\( \text{SiO}_2, \text{MeOH}/\text{CH}_2\text{Cl}_2 \ 3:97 \)) to give the desired product as a white solid (825 mg, 2.01 mmol, 80%). \(^1\text{H}\) NMR (600 MHz, \( \text{CDCl}_3 \)) : \( \delta = 7.43 – 7.35 \) (m, 8H, \( H_{Ar} \)), 7.33 – 7.29 (m, 2H, \( H_{Ar} \)), 5.50 (brs, 1H, \( H_c \)), 4.69 (t, \( J = 7.8 \text{ Hz} \), 1H, \( H_a \)), 3.76 (t, \( J = 6.7 \text{ Hz} \), 2H, \( H_g \)), 3.20 (q, \( J = 6.8 \text{ Hz} \), 2H, \( H_d \)), 3.00 (d, \( J = 7.9 \text{ Hz} \), 2H, \( H_b \)), 1.86 (brs, 1H, \( H_h \)), 1.73 – 1.67 (m, 2H, \( H_f \)), 1.52 – 1.15 (m, 18H, \( H_{aliphatic} \)). \(^{13}\text{C}\) NMR (151 MHz, \( \text{CDCl}_3 \)): \( \delta = 175.74, 172.09, 171.21, 143.80, 143.59, 128.71, 128.67, 127.88, 127.79, 126.66, 64.79, 63.20, 47.65, 47.23, 46.91, 43.69, 41.00, 40.57, 39.58, 32.89, 29.68, 29.61, 29.49, 29.37, 29.32, 28.64, 26.80, 25.92. HRMS (ESI\(^+\)): \( m/z = 410.3047 \ [\text{M+H}\]^+ \) (calcd. 410.3054 for \( \text{C}_{27}\text{H}_{40}\text{N}_{1}\text{O}_{2} \)).

**Synthesis of 17**

To a solution of 16 (235 mg, 0.57 mmol, 1 eq) in dry \( \text{CH}_2\text{Cl}_2 \) (15 mL) at 0 °C, \( \text{Et}_3\text{N} \) (120 µL, 0.86 mmol, 1.5 eq) was added. After 5 min, methanesulfonyl chloride (66 µL, 0.86 mmol, 1.5 eq) was added dropwise and the mixture was stirred at 0 °C for 30 min. \( \text{H}_2\text{O} \) (20 mL) was added and the layers were partitioned. The aqueous layer was extracted with \( \text{CH}_2\text{Cl}_2 \) (2 x 20 mL). The combined organic phases were washed with \( \text{H}_2\text{O} \) (50 mL), dried (\( \text{MgSO}_4 \)) and concentrated under reduced pressure to give the desired product as a colorless oil (230 mg, 0.40 mmol, 82%). \(^1\text{H}\) NMR (600 MHz, \( \text{CDCl}_3 \)) : \( \delta = 7.33 – 7.24 \) (m, 8H, \( H_{Ar} \)), 7.20 (m, 2H, \( H_{Ar} \)), 5.20 (brs, 1H, \( H_c \)), 4.57 (t, \( J = 7.8 \text{ Hz} \), 1H, \( H_a \)), 4.24 (t, \( J = 6.6 \text{ Hz} \), 2H, \( H_d \)), 3.13 – 3.05 (m, 2H, \( H_b \)), 3.02 (s, 3H, \( H_e \)), 2.89 (d, \( J = 7.9 \text{ Hz} \), 2H, \( H_b \)), 1.81 – 1.72 (m, 2H, \( H_f \)), 1.46 – 1.37 (m, 2H, \( H_b \)), 1.35 – 1.03 (m, 16H, \( H_{aliphatic} \)).
Synthesis of 18

To a stirring solution of succinic anhydride (2.53 g, 25 mmol, 1 eq), a solution of 3,3-diphenylpropylamine (20) (5.33 g, 25 mmol, 1 eq) in dry THF (25 mL) was added dropwise. The reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated under reduced pressure and the resulting oil recrystallized in CH2Cl2 to give the desired product as a white solid (6.73 g, 21.61 mmol, 86%).
NMR (600 MHz, CD$_3$Cl): $\delta = 7.28-7.16$ (m, 10H, H$_{Ar}$), 5.58 (d, $J = 5.6$ Hz, 1H, H$_d$), 3.94 (t, $J = 7.8$ Hz, 1H, H$_a$), 3.26 (q, $J = 6.7$ Hz, 2H, H$_c$), 2.82 – 2.54 (m, 2H, H$_j$), 2.38 – 2.33 (m, 2H, H$_e$), 2.28 (q, $J = 7.3$ Hz, 2H, H$_b$). $^{13}$C NMR (151 MHz, CD$_3$Cl): $\delta = 175.67, 172.44, 144.21, 128.82, 127.84, 126.66, 49.40, 39.10, 35.10, 30.71, 29.98$. HRMS (ESI$^+$): $m/z = 312.1599$ [M+H]$^+$ (calcd. 312.1594 for C$_{19}$H$_{22}$NO$_3$).

**Synthesis of 22**

To a stirring solution of 21 (1 g, 3.21 mmol, 1 eq), 14 (840 mg, 3.53 mmol, 1.1 eq) and 4-dimethylaminopyridine (391 mg, 3.21 mmol, 1 eq) in CHCl$_3$ (40 mL) at 0 °C, EDCI.HCl (498 mg, 3.21 mmol, 1 eq) was added. The reaction mixture was stirred for 16 h and then diluted with CHCl$_3$ (50 mL) and organic phase was washed with 1 N HCl (75 mL), saturated sodium bicarbonate solution (75 mL) and brine (75 mL). The combined organic phases were dried (MgSO$_4$), filtrated and concentrated under reduced pressure to give the desired product as a white solid (1.38 g, 2.79 mmol, 87%). $^1$H NMR (600 MHz, CD$_3$Cl): $\delta = 7.33 – 7.16$ (m, 10H, H$_{Ar}$), 6.16 (t, $J = 5.8$ Hz, 1H, H$_g$), 6.08 (t, $J = 5.8$ Hz, 1H, H$_d$), 3.97 (t, $J = 7.8$ Hz, 1H, H$_a$), 3.66 (t, $J = 6.7$ Hz, 2H, H$_k$), 3.26 – 3.15 (m, 4H, H$_{c+h}$), 2.52 – 2.39 (m, 4H, H$_{e+f}$), 2.28 (q, $J = 7.5$ Hz, 2H, H$_b$), 1.62 – 1.55 (m, 2H, H$_j$), 1.47 (t, $J = 7.2$ Hz, 2H, H$_i$), 1.40 – 1.21 (m, 16H, H$_{aliphatic}$). $^{13}$C NMR (151 MHz, CD$_3$Cl): $\delta = 172.35, 172.25, 144.32, 128.71, 127.86, 126.51, 63.14, 49.12, 39.77, 38.62, 35.25, 32.90, 32.07, 29.61, 29.56, 29.53, 29.48, 29.30, 26.95, 25.83. HRMS (ESI$^+$): $m/z = 495.3593$ [M+H]$^+$ (calcd. 495.3581 for C$_{31}$H$_{47}$N$_2$O$_3$).

**Synthesis of 23**

To a stirring solution of 22 (200 mg, 0.40 mmol, 1 eq) in dry CH$_2$Cl$_2$ (10 mL) at 0 °C, Et$_3$N (85 µL, 0.61 mmol, 1.5 eq) was added. After 5 min, methanesulfonyl chloride (50 µL, 0.61 mmol, 1.5 eq) was added dropwise and the mixture was stirred
at 0 °C for 30 min. H₂O (20 mL) was added and the layers were partitioned. The aqueous layer was extracted with CH₂Cl₂ (2 x 20 mL). The combined organic layers were washed with H₂O (50 mL), dried (MgSO₄) and concentrated under reduced pressure to give the desired product as a colorless oil (231 mg, 0.40 mmol, quantitative).

1H NMR (600 MHz, CD₃Cl): δ = 7.29 – 7.15 (m, 10H, H₄Ar), 4.22 (t, J = 6.6 Hz, 2H, H₂), 3.95 (t, J = 7.7 Hz, 1H, H₃), 3.21 (q, J = 6.7 Hz, 4H, H₅+₆), 3.00 (s, 3H, H₇), 2.56 – 2.45 (m, 4H, H₈+⁹), 2.28 (q, J = 7.5 Hz, 2H, H₉), 1.74 (p, J = 6.8 Hz, 2H, H₁₀), 1.43 – 1.19 (m, 16H, H₁₁₋₁₆).

13C NMR (151 MHz, CD₃Cl): δ = 177.40, 144.28, 144.25, 128.74, 127.88, 127.66, 126.56, 70.36, 49.10, 37.50, 29.60, 29.57, 29.51, 29.34, 29.24, 29.13, 26.98, 25.53.

HRMS (ESI⁺): m/z = 595.3181 [M+Na]+ (calcd. 595.3176 for C₃₂H₄₈N₂O₅SNa).

Synthesis of 24

12 (149 mg, 0.33 mmol, 1 eq), 23 (378 mg, 0.66 mmol, 2 eq) and potassium carbonate (92 mg, 0.66 mmol, 2 eq) were dissolved in dry DMF (10 mL) and the reaction mixture was heated to 70 °C for 18 h. The reaction was allowed to cool to room temperature and the solvent was concentrated under reduced pressure. The crude residue was purified by a flash column chromatography (SiO₂, MeOH/EtOAc, from 0:100 to 10:90) to give the desired product as a light yellow oil (184 mg, 0.20 mmol, 60%).

1H NMR (600 MHz, CD₂Cl₂): δ = 10.34 (s, 1H, H₃), 8.16 (d, J = 2.9 Hz, 1H, H₄), 7.93 – 7.81 (m, 2H, H₅), 7.41 – 7.08 (m, 23H, H₆₋₉+₁₁), 6.93 (d, J = 8.6 Hz, 2H, H₁₀), 6.56 (s, 1H, H₁₁), 6.12 (t, J = 5.7 Hz, 1H, H₁₂), 4.24 (t, J = 7.9 Hz, 1H, H₁₃), 4.00 (t, J = 6.4 Hz, 2H, H₁₄), 3.97 (t, J = 6.4 Hz, 2H, H₁₅), 3.92 (t, J = 7.8 Hz, 1H, H₁₆), 3.19 – 3.07 (m, 4H, H₁₇₋₁₈), 2.56 (dt, J = 7.7, 6.3 Hz, 2H, H₁₉), 2.41 (qd, J = 7.3, 6.7, 3.1 Hz, 4H, H₂₀₋₂₁), 2.24 – 2.16 (m, 2H, H₂₂), 1.85 – 1.72 (m, 2H, H₂₃), 1.49 – 1.44 (m, 2H, H₂₄), 1.41 (t, J = 6.9 Hz, 2H, H₂₅), 1.36 – 1.19 (m, 14H, H₂₆₋₃₉).

HRMS (ESI⁺): m/z = 950.5167 [M+Na]+ (calcd. 950.5191 for C₅₉H₆₉N₅O₅SNa).
Synthesis of 27

To a stirring solution of 12 (179 mg, 0.39 mmol, 1 eq) and potassium carbonate (110 mg, 0.79 mmol, 2 eq) in dry DMF (10 mL), 12-bromo-1-dodecanol (26) (210 mg, 0.79 mmol, 2 eq) was added. The resulting mixture was stirred for 18 h at 60 °C under N₂ atmosphere and then concentrated under reduced pressure. The crude product was purified by a flash column chromatography (SiO₂, MeOH/CH₂Cl₂, from 0:100 to 5:95) to give the desired compound as a colourless oil (121 mg, 0.19 mmol, 48%). ¹H NMR (600 MHz, CD₂Cl₂): δ = 15.12 (s, 1H, H₇), 8.38 (d, J = 2.9 Hz, 1H, H₈), 7.92 (d, J = 8.8 Hz, 2H, H₉), 7.44 (d, J = 8.8 Hz, 1H, H₁₀), 7.41 (s, 1H, H₁₁), 7.33 – 7.18 (m, 11H, H₁₂+e), 7.00 (d, J = 8.4 Hz, 2H, H₁₂), 4.26 (t, J = 7.9 Hz, 1H, H₁₃), 4.04 (t, J = 6.4 Hz, 4H, H₁₄+₁₅), 3.58 (t, J = 6.6 Hz, 2H, H₁₆), 2.60 (dt, J = 8.0, 6.2 Hz, 2H, H₁₇), 1.81 (dt, J = 14.7, 6.9 Hz, 2H, H₁₈), 1.55 – 1.50 (m, 2H, H₁₉), 1.49 – 1.43 (m, 2H, H₂₀), 1.41 – 1.22 (m, 14H, H₂₁₋₄₀). ¹³C NMR (151 MHz, CD₂Cl₂): δ = 155.48, 146.03, 144.71, 144.59, 136.94, 129.88, 129.19, 129.15, 128.29, 128.28, 127.58, 127.05, 126.98, 122.50, 114.94, 68.85, 67.38, 67.14, 63.36, 47.73, 35.10, 35.03, 33.43, 30.24, 30.17, 30.14, 30.11, 30.07, 30.04, 29.99, 29.90, 29.80, 29.67, 29.60, 26.50, 26.43, 26.31. HRMS (ESI⁺): m/z = 636.3783 [M+H]⁺ (calcd. 636.3796 for C₄₀H₆₀N₃O₄).

Synthesis of 28

To a stirred solution of 27 (1.21 g, 1.90 mmol, 1 eq) in CH₂Cl₂ (150 mL), Et₃N (270 µL, 1.93 mmol, 1 eq) and a solution of succinic anhydride (190 mg, 1.99 mmol, 1 eq) in CH₂Cl₂ (20 mL) were added. The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was evaporated under reduced pressure. The crude product was purified by a flash column chromatography (SiO₂, MeOH/CH₂Cl₂, from 0:100 to 10:90) to give the desired compound as a colourless
oil (1.40 g, 1.90 mmol, quantitative). $^1$H NMR (600 MHz, CD$_2$Cl$_2$): $\delta = 15.21$ (s, 1H, H$_h$), 8.42 (d, $J = 2.9$ Hz, 1H, H$_d$), 7.96 (d, $J = 8.7$ Hz, 2H, H$_i$), 7.50 (br, 1H, H$_j$), 7.48 (s, 1H, H$_e$), 7.38 – 7.24 (m, 11H, H$_{Ar+e}$), 7.04 (d, $J = 8.4$ Hz, 2H, H$_j$), 4.30 (t, $J = 7.9$ Hz, 1H, H$_f$), 4.14 – 4.03 (m, 6H, H$_c+k+o$), 2.67 – 2.59 (m, 6H, H$_b+q+p$), 1.85 (p, $J = 6.8$ Hz, 2H, H$_l$), 1.62 (q, $J = 6.7$ Hz, 2H, H$_n$), 1.51 (p, $J = 7.8$ Hz, 2H, H$_m$), 1.42 – 1.25 (m, 14H, H$_{aliphatic}$).

$^{13}$C NMR (151 MHz, CD$_2$Cl$_2$): $\delta =$ 172.36, 172.30, 162.37, 155.02, 144.09, 144.03, 136.46, 129.41, 128.64, 128.58, 127.73, 126.51, 126.42, 121.93, 114.46, 68.26, 66.85, 64.88, 47.18, 34.47, 29.44, 29.41, 29.31, 29.20, 29.17, 29.09, 29.00, 28.95, 28.55, 28.49, 28.47, 25.83, 25.81.

HRMS (ESI$^+$): $m/z =$ 734.3793 [M-H]$^-$ (calcd. 734.3811 for C$_{44}$H$_{52}$N$_3$O$_7$).

Synthesis of 29

To a stirring solution of 28 (64.0 mg, 0.09 mmol, 1.00 eq), 3,3-diphenylpropyl amine (20) (18.4 mg, 0.09 mmol, 1.00 eq) and 4-dimethylaminopyridine (13.4 mg, 0.11 mmol, 1.23 eq) in dry CH$_2$Cl$_2$ (13 mL) at 0 °C was added EDCI.HCl (18.7 mg, 0.10 mmol, 1.15 eq) and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was washed with a saturated solution of citric acid (3 x 50 mL) and H$_2$O (3 x 50 mL). The combined organic phases were dried (MgSO$_4$), filtrated and concentrated under reduced pressure. The crude product was purified by a flash column chromatography (SiO$_2$, MeOH/CH$_2$Cl$_2$, from 0:100 to 15:85) to give the desired compound as a colourless oil in a 77:33 E/Z ratio (71.3 mg, 0.08 mmol, 88%). $^1$H NMR (600 MHz, CD$_2$Cl$_2$): $\delta =$ 11.20 (s, 1H, H$_h$), 8.47 (s, 1H, H$_d$), 7.88 (br, 2H, H$_j$), 7.40 (d, $J = 8.7$ Hz, 1H, H$_j$), 7.35 – 7.16 (m, 22H, H$_{Ar+e+g}$), 6.95 (d, $J = 8.3$ Hz, 2H, H$_j$), 5.86 (s, 1H, H$_d$), 4.09 – 3.95 (m, 6H, H$_{c+k+o}$), 3.80 (m, 1H, H$_d$), 3.62 (t, $J = 7.8$ Hz, 1H, H$_e$), 3.15 – 3.12 (m, 2H, H$_l$), 2.64 – 2.56 (m, 4H, H$_{b+p}$), 2.38 (t, $J = 6.8$ Hz, 2H, H$_l$), 2.37 (t, $J = 6.8$ Hz, 2H, H$_l$), 2.25 (q, $J = 7.4$ Hz, 2H, H$_m$), 1.81 (m, 2H, H$_n$), 1.40 (m, 2H, H$_m$), 1.39 – 1.27 (m, 14H, H$_{aliphatic}$). $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$): $\delta =$ 173.47, 171.59, 164.09, 162.82, 155.47, 146.03, 145.10, 144.59, 138.22, 136.94, 129.89, 129.19, 129.08, 128.28, 128.21, 127.58, 127.05, 126.86, 126.02, 122.51, 114.94, 112.52, 68.85, 67.37, 65.28, 49.49, 47.72, 38.84, 35.61,
35.03, 31.42, 30.09, 30.06, 30.05, 29.90, 29.80, 29.15, 26.50, 26.42, 16.75. HRMS (ESI\(^+\)): \( m/z = 929.5204 \) [M+H]\(^+\) (calcd. 929.5212 for C\(_{59}\)H\(_{69}\)N\(_4\)O\(_6\)).

**General procedure for the preparation of benzylic amide macrocycle containing rotaxanes.**

The corresponding thread (1.00 eq) and Et\(_3\)N (16.00 eq) were dissolved in dry chloroform (ethanol-free, stabilized with amylenes, 100 mL) and stirred vigorously whilst solutions of \( p \)-xylylenediamine (8.00 eq) in dry chloroform (40 mL) and isophthaloyl dichloride (8.00 eq) in dry chloroform (40 mL) were simultaneously added over a period of 2 h using motor driven syringe pumps. The resulting suspension was stirred at room temperature then filtrated through a pad of Celite\(^\circledR\). The filtrate was concentrated under reduced pressure. Purification by a flash column chromatography gave the desired rotaxane product. Rotaxanes containing monoamide and succinic amide stations were clean enough to elucidate the position of the macrocycle. Therefore, they were not purified any further as they failed to be a proper station and only the peaks belonging to them were reported in the NMR section.

**Synthesis of \( E-10 \)**

Rotaxane \( E-10 \) was prepared from thread \( E-9 \) (500 mg, 0.78 mmol, 1.00 eq) according to the general procedure for the preparation of benzylic amide macrocycle containing [2]rotaxane. The crude residue was purified by a flash column chromatography (SiO\(_2\), MeOH/CH\(_2\)Cl\(_2\), from 0:100 to 2:98) to give the desired product as a yellow solid (775 mg, 0.66 mmol, 85%). \(^1\)H NMR (600 MHz, CD\(_2\)Cl\(_2\)): \( \delta = 10.18 \) (s, 1H, \( H_b \)), 8.78 (s, 2H, \( H_c \)), 8.19 (d, \( J = 7.8 \) Hz, 4H, \( H_g \)), 7.97 (d, \( J = 7.7 \) Hz, 4H, \( H_h \)), 7.89 (d, \( J = 7.8 \) Hz, 4H, \( H_j \)), 7.65 (d, \( J = 7.8 \) Hz, 4H, \( H_k \)), 7.56 (d, \( J = 7.8 \) Hz, 4H, \( H_l \)), 7.35 (d, \( J = 7.8 \) Hz, 4H, \( H_m \)), 7.29 (d, \( J = 7.8 \) Hz, 4H, \( H_n \)), 7.12 (d, \( J = 7.8 \) Hz, 4H, \( H_o \)).
Hz, 2H, H_i), 7.84 (s, 4H, H_D), 7.82 (s, 1H, H_d), 7.67 (t, J = 7.8 Hz, 2H, H_A), 7.37 (s, 1H, H_g), 7.33 – 7.21 (m, 20H, H_Ar), 7.11 (d, J = 8.8 Hz, 1H, H_j), 6.97 (d, J = 8.5 Hz, 2H, H_j), 6.81 (s, 8H, H_F), 6.74 (d, J = 7.2 Hz, 1H, H_c), 4.53 (m, 4H, H_E), 4.27 (m, 5H, H_E+a), 4.22 (t, J = 6.2 Hz, 2H, H_h), 2.63 (q, J = 6.6 Hz, 2H, H_b), 2.54 (q, J = 6.5 Hz, 2H, H_e). 13C NMR (151 MHz, CD2Cl2): δ = 166.30, 164.68, 162.60, 156.15, 144.27, 143.97, 137.37, 134.40, 130.84, 129.98, 129.23, 128.63, 128.60, 127.93, 127.78, 127.69, 126.50, 126.44, 125.70, 114.23, 66.79, 66.32, 47.27, 47.10, 43.87, 34.58, 34.31. HRMS (ESI+): m/z = 1178.5156 [M+H]+ (calcd. 1178.5175 for C75H68N7O7).

**Synthesis of E-19**

Rotaxane E-19 was prepared from thread E-18 (55 mg, 0.06 mmol, 1.00 eq) according to the general procedure for the preparation of benzylic amide macrocycle-containing [2]-rotaxane. The crude residue was purified by flash column chromatography (SiO2, MeOH/CH2Cl2, 0:100 to 2:98) to obtain the desired product as a yellow solid (58 mg, 0.04 mmol, 65%). 1H NMR (600 MHz, Acetone-d6): δ = 10.78 (s, 1H, H_h), 8.89 (s, 2H, H_C), 8.21 (m, 8H, H_B+D), 8.03 (s, 1H, H_d), 7.96 (d, J = 8.2 Hz, 2H, H_i), 7.70 (t, J = 7.8 Hz, 2H, H_A), 7.38 – 7.11 (m, 22H, H_Ar+f+g), 7.08 (d, J = 8.7 Hz, 2H, H_j), 6.99 (brs, 1H, H_e), 6.90 (d, J = 8.9 Hz, 1H, H_h), 6.86 (s, 8H, H_F), 4.62 – 4.50 (m, 5H, H_E+a), 4.27 (t, J = 8.0 Hz, 1H, H_a), 4.23 (dd, J = 14.4, 4.7 Hz, 4H, H_E), 4.13 (t, J = 6.5 Hz, 2H, H_e), 4.00 (t, J = 6.3 Hz, 2H, H_h), 3.02 (q, J = 6.6 Hz, 2H, H_b), 2.83 (m, 2H, H_q), 2.54 (dt, J = 7.7, 6.2 Hz, 2H, H_b), 1.86 – 1.79 (m, 2H, H_i), 1.52 (m, 2H, H_a), 1.44 – 1.05 (m, 16H, H_Aliphatic+n). 13C NMR (151 MHz, Acetone-d6): δ = 170.86, 166.43, 166.36, 163.63, 145.66, 145.49, 145.32, 138.75, 137.32, 135.57, 131.77, 130.89, 129.90, 129.37, 129.15, 128.92, 128.92.
Synthesis of \textit{E-25}

Rotaxane \textit{E-25} was prepared from thread \textit{E-24} (31.7 mg, 0.03 mmol, 1.00 eq) according to the general procedure for the preparation of benzylic amide macrocycle-containing [2]rotaxane. The crude residue was purified by preparative TLC (SiO$_2$, MeOH/CH$_2$Cl$_2$ 4:96) to obtain a mixture of the desired product and the track with a ratio of 40:60. $^1$H NMR (600 MHz, CD$_2$Cl$_2$): $\delta = 10.34$ (s, 1H, H$_h$), 8.49 (s, 2H, H$_c$), 8.33 (brs, 1H, H$_d$), 8.17 (d, $J = 7.5$ Hz, 4H, H$_b$), 7.92 (d, $J = 8.4$ Hz, 2H, H$_i$), 7.87 – 7.65 (brs, 4H, H$_D$), 7.63 (t, $J = 8.0$ Hz, 2H, H$_A$), 7.36 – 7.10 (m, 31H, H$_{Ar+F+e+f+g}$), 6.98 (d, $J = 8.3$ Hz, 2H, H$_j$), 6.18 (brs, 1H, H$_{p or s}$), 6.05 (brs, 1H, H$_{p or s}$), 4.47 (m, 8H, H$_e$), 4.25 (m, 1H, H$_a$), 4.05 (m, 4H, H$_{c+k}$), 3.86 (m, 1H, H$_v$), 3.05 (m, 4H, H$_{o+t}$), 2.50 (m, 2H, H$_b$), 2.2 (m, 2H, H$_u$), 1.80 (m, 2H, H$_i$), 1.59 – 1.23 (m, 22H, H$_{aliphatic+m+n+q+s}$). HRMS (ESI$^+$): $m/z = 1482.7297$ [M+Na]$^+$ (calcd. 1482.7301 for C$_{91}$H$_{97}$N$_9$O$_9$Na).
Synthesis of \textit{E-30}

Rotaxane \textit{E-30} was prepared from thread \textit{E-29} (20 mg, 0.02 mmol, 1.00 eq) according to the general procedure for the preparation of benzylic amide macrocycle containing [2]rotaxane. The crude residue was purified by a flash column chromatography (SiO$_2$, MeOH/CH$_2$Cl$_2$, from 0:100 to 10:80) to obtain the desired product as a colourless oil (24 mg, 0.016 mmol, 70%).

$^1$H NMR (600 MHz, CD$_2$Cl$_2$): $\delta = 10.26$ (s, 1H, H$_b$), 8.80 (s, 2H, H$_c$), 8.20 (d, $J = 7.6$ Hz, 4H, H$_d$), 8.01 (d, $J = 7.8$ Hz, 2H, H$_j$), 7.83 (s, 4H, H$_d$), 7.67 (t, $J = 7.7$ Hz, 2H, H$_i$), 7.45 – 7.18 (m, 23H, H$_{Ar+H_{d+e+g}}$), 7.03 (d, $J = 8.0$ Hz, 2H, H$_i$), 6.82 (s, 8H, H$_f$), 6.77 (d, $J = 8.0$ Hz, 1H, H$_j$), 5.76 (br, 1H, H$_q$), 4.53 (m, 4H, H$_E$), 4.28 (m, 4H, H$_E$), 4.22 (t, $J = 7.8$ Hz, 1H, H$_a$), 4.10 (t, $J = 6.3$ Hz, 2H, H$_k$), 4.05 (dt, $J = 29.7$, 6.3 Hz, 2H, H$_n$), 3.95 (t, $J = 7.8$ Hz, 1H, H$_t$), 3.90 (t, $J = 7.8$ Hz, 2H, H$_l$), 3.16 (m, 2H, H$_r$), 2.55 (m, 4H, H$_{b+o}$), 2.33 (m, 2H, H$_p$), 2.25 (q, $J = 7.5$ Hz, 1H, H$_m$), 1.86 (q, $J = 7.1$ Hz, 2H, H$_j$), 1.62 (q, $J = 7.0$ Hz, 2H, H$_m$), 1.40 – 1.22 (m, 16H, H$_{aliphatic}$) $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$): $\delta = 172.97$, 171.30, 166.30, 164.72, 162.87, 156.11, 155.00, 144.46, 143.99, 143.99, 137.41, 134.42, 130.88, 130.04, 129.20, 128.88, 128.65, 128.54, 127.99, 127.74, 127.69, 127.63, 126.49, 126.34, 114.19, 68.35, 66.86, 64.79, 53.82, 53.64, 53.45, 53.27, 53.10, 48.93, 47.10, 43.86, 38.36, 34.99, 34.33, 30.82, 29.39, 29.14, 29.01, 28.56, 25.87, 25.82. HRMS (ESI$^+$): m/z = 1461.7299 [M+H]$^+$ (calcd. 1461.7322 for C$_{91}$H$_{97}$N$_8$O$_{10}$).
Isomerization studies of threads and rotaxanes

General procedure for the photochemical isomerization

Irradiations were carried out in a photoreactor (furnished by Photochemical Reactors Ltd.) fitted with 6× 15 W gas discharge bulbs (Vilber-Lourmat T-15M, emission centred at 312 nm). The samples were irradiated in quartz NMR tubes and NMR spectra were recorded immediately.

Synthesis of Z-9

A solution of E-9 (1.0 mg, 1.55 µmol, 1.00 eq.) in degassed CD$_2$Cl$_2$ (0.4 mL) was irradiated for 30 min using the method described above to give the desired product Z-9 (0.91 mg, 1.41 µmol, 91%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ = 15.17 (s, 1H, H$_b$), 8.41 (d, $J$ = 2.8 Hz, 1H, H$_d$), 7.93 (d, $J$ = 8.7 Hz, 2H, H$_i$), 7.48 – 7.31 (m, 17H, H$_{Ar+e}$), 7.27 – 7.18 (m, 4H, H$_{Ar}$), 6.99 (d, $J$ = 8.5 Hz, 2H, H$_j$), 4.31 (td, $J$ = 7.9, 3.2 Hz, 2H, H$_{a+m}$), 4.08 (t, $J$ = 6.3 Hz, 1H, H$_c$), 4.02 (t, $J$ = 6.3 Hz, 2H, H$_d$), 2.63 (m,
4H, H_{b+l}).  ^{13}\text{C} \text{NMR (151 MHz, CD}_2\text{Cl}_2):} \; \delta = 167.71, 163.69, 162.14, 155.09, 145.57, 144.46, 144.18, 137.93, 136.51, 129.46, 128.80, 128.71, 127.95, 127.87, 126.65, 122.11, 114.56, 66.97, 66.31, 47.40, 47.31, 34.74, 34.61.

**Synthesis of Z-4**

A solution of E-4 (1.0 mg, 0.85 µmol, 1.00 eq.) in degassed CDCl\(_2\) (0.4 mL) was irradiated for 1 h using the method described above to give the desired product Z-4 (0.98 mg, 0.83 µmol, 98%).  ^1\text{H} \text{NMR (600 MHz, CDCl}_3):} \; \delta = 13.94, 12.74 \text{ (s, 1H, H}_h\text{), 8.54, 8.33 \text{ (s, 2H, H}_C\text{), 8.23 \text{ (d, J = 7.8 Hz, 4H, H}_B\text{), 7.92 \text{ (s, 1H, H}_d\text{), 7.65, 7.57 \text{ (t, J = 7.8 Hz, 2H, H}_A\text{), 7.48 \text{ (d, J = 8.7 Hz, 1H, H}_j\text{), 7.39 \text{ (s, 1H, H}_g\text{), 7.36 – 7.22 \text{ (m, 26H, H}_{Ar+D+i}\text{), 6.97 \text{ (d, J = 8.5 Hz, 2H, H}_j\text{), 6.93, 6.90 \text{ (s, 8H, H}_F\text{), 6.53, 6.35 \text{ (d, J = 8.5 Hz, 1H, H}_e\text{), 4.64 \text{ (m, 2H, H}_E\text{), 4.49 \text{ (m, 2H, H}_E\text{), 4.27 – 3.93 \text{ (m, 9H, H}_{E+m+a+c}\text{), 3.75 – 3.64 \text{ (m, 2H, H}_k\text{), 2.63 – 2.61 \text{ (m, 2H, H}_b\text{), 2.63 – 2.61 \text{ (m, 2H, H}_l\text{).}}\)\text{}}

**Synthesis of Z-29**

A solution of E-29 (1.0 mg, 1.08 µmol, 1.00 eq.) in degassed CDCl\(_2\) (0.4 mL) was irradiated for 1 h using the method described above to give the desired product Z-29 (0.9 mg, 0.97 µmol, 90%).  ^1\text{H} \text{NMR (600 MHz, CD}_2\text{Cl}_2):} \; \delta = 15.12 \text{ (s, 1H, H}_h\text{), 8.38 \text{ (d, J = 2.8 Hz, 1H, H}_d\text{), 7.92 \text{ (d, J = 8.7 Hz, 2H, H}_j\text{), 7.43 \text{ (d, J = 8.7 Hz, 1H, H}_j\text{), 7.40 \text{ (s, 1H, H}_g\text{), 7.33 – 7.21 \text{ (m, 17H, H}_{Ar+c}\text{), 7.21 – 7.15 \text{ (m, 4H, H}_{Ar}\text{), 7.00 \text{ (d, J = 7.8 Hz, 1H, H}_j\text{), 7.33 – 7.21 \text{ (m, 17H, H}_{Ar+c}\text{), 7.21 – 7.15 \text{ (m, 4H, H}_{Ar}\text{), 7.00 \text{ (d, J = 7.8 Hz, 1H, H}_j\text{), 7.33 – 7.21 \text{ (m, 17H, H}_{Ar+c}\text{), 7.21 – 7.15 \text{ (m, 4H, H}_{Ar}\text{), 7.00 \text{ (d, J = 7.8 Hz, 1H, H}_j\text{), 7.33 – 7.21 \text{ (m, 17H, H}_{Ar+c}\text{), 7.21 – 7.15 \text{ (m, 4H, H}_{Ar}\text{), 7.00 \text{ (d, J =}}\)\text{}}
8.3 Hz, 2H, H₂j), 5.63 (s, J = 5.7 Hz, 1H, Hl), 4.26 (t, J = 8.0 Hz, 1H, Hₐ), 4.04 (td, J = 6.5, 4.5 Hz, 6H, Hc+k+o), 3.95 (t, J = 7.8 Hz, 1H, Hj), 3.19 – 3.12 (m, 2H, Hs), 2.60 (dt, J = 8.2, 6.3 Hz, 2H, Hb), 2.56 (t, J = 6.8 Hz, 2H, Hp), 2.34 (t, J = 6.8 Hz, 2H, Hq), 2.25 (q, J = 7.4 Hz, 2H, Hz), 1.81 (m, 2H, H), 1.60 (m, 2H, Hg), 1.51 – 1.43 (m, 2H, Hm), 1.40 – 1.22 (m, 14H, H_{aliphatic}). ¹³C NMR (151 MHz, CD₂Cl₂): δ = 173.47, 171.59, 164.09, 162.82, 155.47, 146.03, 145.10, 144.59, 138.22, 136.94, 129.89, 129.19, 129.08, 128.28, 128.21, 127.58, 127.05, 126.86, 126.02, 122.51, 114.94, 112.52, 68.85, 67.37, 65.28, 49.49, 47.72, 38.84, 35.61, 35.03, 31.42, 30.09, 30.06, 30.05, 29.90, 29.80, 29.68, 29.15, 26.50, 26.42, 16.75.

**Synthesis of Z-30**

A solution of E-30 (1.0 mg, 0.68 μmol, 1.00 eq.) in degassed CD₂Cl₂ (0.4 mL) was irradiated for 2 h using the method described above to give the desired product Z-30 (0.91 mg, 0.62 μmol, 91%). ¹H NMR (600 MHz, CD₂Cl₂): δ = 15.16 (s, 1H, Hh), 8.40 (s, 1H, Hb), 8.38 (s, 2H, Hc), 8.23 (d, J = 7.8 Hz, 4H, Hg), 7.92 (d, J = 8.7 Hz, 2H, Hj), 7.60 (t, J = 7.8 Hz, 2H, Hf), 7.46 (d, J = 8.7 Hz, 1H, Hj), 7.40 (s, 1H, Hg), 7.38 (s, 4H, Hd), 7.36 – 7.12 (m, 21H, H_{Ar-e}), 7.01 (s, 8H, He), 6.98 (d, J = 8.3 Hz, 2H, Hj), 6.43 (s, 1H, Hg), 4.49 – 4.41 (m, 8H, Hc+k), 4.25 (t, J = 8.0 Hz, 1H, Hf), 4.04 – 4.02 (m, 4H, Hc+k), 3.87 (t, J = 8.0 Hz, 2H, Hf), 3.75 (t, J = 7.8 Hz, 1H, Hj), 2.74 – 2.70 (m, 2H, Hz), 2.59 (dt, J = 8.2, 6.3 Hz, 2H, Hb), 2.02 – 2.00 (m, 2H, Hj), 1.82 – 1.78 (m, 2H, Hf), 1.60 – 1.19 (m, 22H, H_{o+p+aliphatic}). ¹³C NMR (151 MHz, CD₂Cl₂): δ = 173.47, 171.59, 164.09, 162.82, 155.47, 146.03, 145.10, 144.59, 138.22, 136.94, 129.89, 129.19, 129.08, 128.28, 128.21, 127.58, 127.05, 126.86, 126.02, 122.51, 114.94, 112.52, 68.85, 67.37, 65.28, 49.49, 47.72, 38.84, 35.61, 35.03, 31.42, 30.09, 30.06, 30.05, 29.90, 29.80, 29.68, 29.15, 26.50, 26.42, 16.75.

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Figure 19 Partial 2D-COSY spectra of Z-30 (600 MHz, CD$_2$Cl$_2$)

Synthesis of Z-24

A solution of $E$-24 (1.0 mg, 1.08 µmol, 1.00 eq.) in degassed CD$_2$Cl$_2$ (0.4 mL) was irradiated for 1 h using the method described above give the desired product Z-16 (90%). $^1$H NMR (600 MHz, CD$_2$Cl$_2$): $\delta = 15.12$ (s, 1H, H$_h$), 8.38 (d, $J = 2.9$ Hz, 1H, H$_d$), 7.92 (d, $J = 8.7$ Hz, 2H, H$_i$), 7.48 – 7.11 (m, 23H, H$_{Ar}$), 7.00 (d, $J = 8.3$ Hz, 2H, H$_j$), 6.03 (brs, 1H, H$_p$ or s), 5.90 (brs, 1H, H$_p$ or s), 4.26 (t, $J = 8.0$ Hz, 1H, H$_a$), 4.04 (t, $J = 6.1$ Hz, 4H, H$_{c+k}$), 3.95 (t, $J = 7.8$ Hz, 1H, H$_b$), 3.20 – 3.09 (m, 4H, H$_{o+t}$), 2.60 (dt, $J = 7.9$, 6.2 Hz, 2H, H$_b$), 2.44 – 2.34 (m, 4H, H$_{q+r}$), 2.23 (q, $J = 7.6$ Hz, 2H, H$_u$), 1.86 – 1.77 (m, 2H, H$_i$), 1.45 (m, 2H, H$_m$), 1.40 – 1.24 (m, 16H, H$_{n+Aliphatic}$). $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$): $\delta = 172.50$, 172.38, 155.49, 145.09, 144.59, 136.96, 129.91, 129.19, 129.07, 128.28, 128.22, 127.06, 126.85, 122.51, 114.96, 68.83, 67.38, 49.42, 47.72, 40.05, 38.76, 35.57, 35.02, 32.34, 30.14, 30.10, 30.05, 29.85, 29.83, 29.63, 27.44, 26.45.
Synthesis of Z-25

A solution of E-25 (1.0 mg, 0.74 µmol, 1.00 eq.) in degassed CD$_2$Cl$_2$ (0.4 mL) was irradiated for 1 h using the method described above give the desired product Z-17 (90%). $^1$H NMR (600 MHz, CD$_2$Cl$_2$): $\delta$ = 15.13 (s, 1H, H$_h$), 8.51 (s, 2H, H$_c$), 8.38 (s, 1H, H$_d$), 8.15 (d, $J$ = 7.8, 4H, H$_b$), 7.89 (d, $J$ = 8.4 Hz, 2H, H$_j$), 7.65 (t, $J$ = 5.1 Hz, 4H, H$_D$), 7.59 (t, $J$ = 7.8 Hz, 2H, H$_A$), 7.47 – 7.08 (m, 31H, H$_{Ar+e+f+g}$), 7.00 (d, $J$ = 8.5 Hz, 2H, H$_j$), 6.02 (s, 2H, H$_g$), 5.82 (t, $J$ = 5.6 Hz, 1H, H$_o$), 4.49 (dd, $J$ = 14.2, 5.6 Hz, 4H, H$_E$), 4.44 (dd, $J$ = 14.1, 5.3 Hz, 4H, H$_E$), 4.26 (t, $J$ = 8.0 Hz, 1H, H$_e$), 4.04 (t, $J$ = 6.4 Hz, 4H, H$_c$), 3.82 (t, $J$ = 7.9 Hz, 1H, H$_i$), 3.04 (q, $J$ = 6.7 Hz, 2H, H$_a$), 2.95 (q, $J$ = 6.8 Hz, 2H, H$_i$), 2.60 (dt, $J$ = 8.3, 6.3 Hz, 2H, H$_o$), 2.17 – 2.09 (m, 2H, H$_d$), 1.84 – 1.73 (m, 2H, H$_i$), 1.51 – 1.02 (m, 22H, H$_{Aliphatic+o+p}$).

Synthesis of Z-18

A solution of E-18 (1.0 mg, 1.18 µmol, 1.00 eq.) in degassed CD$_2$Cl$_2$ (0.4 mL) was irradiated for 1 h using the method described above give the desired product Z-18 (90%). $^1$H NMR (600 MHz, CD$_2$Cl$_2$): $\delta$ = 15.13 (s, 1H, H$_h$), 8.38 (d, $J$ = 2.9 Hz, 1H, H$_d$), 7.92 (d, $J$ = 8.8 Hz, 2H, H$_j$), 7.47 – 7.14 (m, 23H, H$_{Ar+e+f+g}$), 7.00 (d, $J$ = 8.3 Hz, 2H, H$_j$), 5.40 (brs, 1H, H$_p$), 4.53 (t, $J$ = 7.8 Hz, 1H, H$_i$), 4.26 (t, $J$ = 7.9 Hz, 1H,
Hₙ), 4.04 (td, J = 6.4, 3.9 Hz, 4H, H₊k), 3.05 (td, J = 7.0, 5.6 Hz, 2H, H₀), 2.85 (d, J = 7.8 Hz, 2H, Hᵣ), 2.60 (dt, J = 7.9, 6.2 Hz, 2H, H₋), 1.87 – 1.77 (m, 2H, Hₗ), 1.48 (td, J = 9.7, 8.8, 4.8 Hz, 2H, H₋m), 1.42 – 1.04 (m, 16H, Hₐliphatic).

Synthesis of Z-19

A solution of E-19 (1.0 mg, 0.72 µmol, 1.00 eq.) in degassed CD₂Cl₂ (0.4 mL) was irradiated for 1 h using the method described above give the desired product Z-19 (90%).

¹H NMR (600 MHz, CD₂Cl₂): δ = 14.65 – 13.93 (s, 1H, H₋h), 8.38 (brs, 2H, H₋c), 8.25 (brs, 1H, H₋d), 8.16 (m, 4H, H₋b), 7.92 (d, J = 8.8 Hz, 2H, H₋i), 7.62 (t, J = 7.8 Hz, 2H, H₋a), 7.49 – 7.14 (m, 23H, H₋Ar₊f+g), 7.07 (brs, 4H, H₋D), 7.00 (d, J = 8.3 Hz, 2H, H₋j), 6.97 – 6.89 (m, 8H, H₋f), 6.28 (brs, 1H, H₋p), 4.54 (m, 4H, H₋E), 4.31 – 4.20 (m, 5H, H₋E₊a), 3.96 (m, 4H, H₋c+k), 3.76 (br, 1H, H₋r), 2.64 – 2.53 (m, 2H, H₋b), 2.46 (br, 2H, H₋o), 2.02 (br, 2H, H₋q), 1.81 (m, 2H, H₋i), 1.46 (m, 2H, H₋m), 1.40 – 0.86 (m, 18H, H₋Aliphatic+n+q).

General procedure for the thermal isomerization

A solution of the corresponding Z-thread or rotaxane (1.0 mg, 1.00 eq.) in degassed CD₂Cl₂ (0.4 mL) was heated at 40 °C with catalytic amount of trifluoroacetic acid (TFA) for 2 hours followed by neutralization with potassium carbonate. The thermal isomerization was followed by ¹H NMR.

X-Ray Crystal Structure Experimental Details

Single crystals of the E- and Z-[2]rotaxanes (E-10 and Z-10) were obtained through slow evaporation of a solution in a mixture of CH₂Cl₂ and MeCN. X-ray data for compound Z-10 was collected at a temperature of 150 K on an Agilent Technologies Supernova diffractometer with MoKα radiation, (λ = 0.71073 Å), equipped with an
Oxford Cryosystems Cobra nitrogen flow gas system. Data was measured using CrysAlisPro suite of programs. X-ray data for compound \( E\-10 \) was collected at a temperature of 100 K using a Bruker X8 Prospector diffractometer with Cu-K\( \alpha \) radiation (\( \lambda = 1.54184 \) Å), equipped with an Oxford Cryosystems Cobra nitrogen flow gas system. X-ray data were processed and reduced using CrysAlisPro suite of programs. Absorption correction was performed using empirical methods based upon symmetry-equivalent reflections combined with measurements at different azimuthal angles. The crystal structures were solved and refined against all \( F^2 \) values using the SHELXTL suite of programs. All the atoms were refined anisotropically. Hydrogen atoms were placed in calculated positions refined using idealized geometries (riding model) and assigned fixed isotropic displacement parameters. Large parts of the rotaxanes were found disordered and modelled over two positions. Bond distances were restrained using DFIX and SADI command. The atomic displacement parameters (adp) of the ligands, anions and solvent molecules have been restrained using RIGU, EADP and SIMU commands.

CCDC 1491182-1491183 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk

<p>| Table S1. Crystallographic information for ( E-10 ) and ( Z-10 ) |
|---------------------------------|--------|--------|
| Crystal colour                 | colourless | colourless |
| Crystal size (mm)              | ( 0.3 \times 0.15 \times 0.15 ) | ( 0.23 \times 0.05 \times 0.05 ) |
| Crystal system                 | Monoclinic | Monoclinic |
| Space group, ( Z )           | ( P2_1/c, 4 ) | ( C2/c, 4 ) |
| ( a ) (Å)                    | 10.0686(7) | 18.5033(9) |
| ( b ) (Å)                    | 10.337(1)  | 10.5908(5) |
| ( c ) (Å)                    | 31.789(5)  | 68.072(2)  |
| ( \alpha ) (°)               | 90       | 90       |
| ( \beta ) (°)                | 91.522(6) | 91.094(4) |
| ( \gamma ) (°)               | 90       | 90       |</p>
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<th>13337.2(9)</th>
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<tr>
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<td>100</td>
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<td>5.194 to 136.494</td>
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<tr>
<td>Independent reflns (R_int)</td>
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<td>12116 (0.0605)</td>
</tr>
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<td>L.S. parameters, p</td>
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<tr>
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<td>745</td>
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<td>S(F²),ᵃ all data</td>
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<td>1.044</td>
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ᵃ R1(F) = Σ(|Fo| - |Fc|)/|Fo|; [b] wR²(F²) = [Σw(Fo² - Fc²)²/ΣwFo⁴]¹/₂; [c] S(F²) = [Σw(Fo² - Fc²)²/(n + r - p)]¹/₂
References


