OVERCOMING LIMITED SELECTIVITY IN RECOGNITION-MEDIATED REPLICATING SYSTEMS

Harry Mackenzie

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

2012

Full metadata for this item is available in Research@StAndrews:FullText at:
http://research-repository.st-andrews.ac.uk/

Please use this identifier to cite or link to this item:
http://hdl.handle.net/10023/3633

This item is protected by original copyright
Overcoming Limited Selectivity in Recognition-Mediated Replicating Systems

by

Harry Mackenzie

A Thesis Presented for the Degree of Master of Philosophy in the School of Chemistry University of St Andrews

June 2012
1. Candidate’s declarations:

I, Harry Mackenzie, hereby certify that this thesis, which is approximately 38,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student in September 2010 and as a candidate for the degree of Master of Philosophy in September 2010; the higher study for which this is a record was carried out in the University of St Andrews between 2010 and 2011.

Date ................................ signature of candidate ................................

2. Supervisor’s declaration:

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Master of Philosophy in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date ................................ signature of supervisor ................................

3. Permission for electronic publication: (to be signed by both candidate and supervisor)

In submitting this thesis to the University of St Andrews I understand that I am giving permission for it to be made available for use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby. I also understand that the title and the abstract will be published, and that a copy of the work may be made and supplied to any bona fide library or research worker, that my thesis will be electronically accessible for personal or research use unless exempt by award of an embargo as requested below, and that the library has the right to migrate my thesis into new electronic forms as required to ensure continued access to the thesis. I have obtained any third-party copyright permissions that may be required in order to allow such access and migration, or have requested the appropriate embargo below.

The following is an agreed request by candidate and supervisor regarding the electronic publication of this thesis:

(iii) Embargo on both [all or part] of printed copy and electronic copy for the same fixed period of 1 year. (maximum five) on the following ground(s):

publication would preclude future publication

Date .......... signature of candidate ............ signature of supervisor ........
Abstract

Traditionally, synthetic chemistry has always focused on yielding a target compound from the linear application of chemical transformations. After each step, a single compound is usually required and the presence of mixtures often demands lengthy purification prior to the next synthetic step. The emerging field of systems chemistry aims to study the currently under-exploited science of networks and complex mixtures. Through the chemistry of reversible chemical bonds, dynamic covalent chemistry (DCC), the creation of networks of compounds linked through a plethora of equilibrium processes, termed dynamic combinatorial libraries (DCLs), is possible. In this research, a DCL based on imine/nitrone exchange is designed and presented. The DCL is subsequently coupled to an irreversible chemical reaction based on molecular recognition and the dramatic responses observed within the DCL are discussed.

The properties of the dynamic systems developed during the course of this research is then applied to the competition between emerging self-replicators in an attempt to demonstrate Darwinian Evolution. A through discussion of the inherent limitations placed upon a system by kinetic selection is presented in the context of self-replicators.

Finally, non-linear chemical dynamics are discussed and successfully incorporated into a competitive replication scenario. The application of reaction diffusion fronts allows a self-replicating system to break the stranglehold of kinetic selection and exhibit its dominance over weaker competitors.
Acknowledgments

First off, I would like to thank my supervisor Professor Douglas Philp for all of his help and support over the last year. His enthusiasm for research has made this a very pleasant place to work and his guidance has been invaluable.

I would like to thank the members of the Philp group: Dr. Jürgen Huck - for being so relentlessly German in his organisation that despite his departure, it was very easy to pick up where he left off. Dr. Izzaty Hassan - for actually remaining in the lab while I joined the group and invaluable day-to-day help, Dr. Craig Robertson - for constant email guidance throughout this year, and the many hundreds of project students I have looked after this year, for making me feel welcome and a part of the team. A special mention must go to Martin Peeks for not only looking almost exactly like me but for the exceptionally hard work he put in during the summer he was in our lab.

I’d also like to thank the Kay group who have invaded our lab but in a welcome way. A big ‘thank you’ must go to Dr Neil Keddie of the DOH group for his invaluable advice on all things chemistry and for explaining the British tax system to me (why are there so many?!).

I would like to warmly thank all of the GF group (and Andy and Dave) for being a lot of fun this year and although I’m sure my liver won’t be shedding any tears, I certainly will miss you all very much.

I extend my thanks for the fantastic technical staff at the School of Chemistry: Caroline Horsburgh for the performance of all mass spectrometry and Melanja Smith and Dr. Tomas Lebl for their continued and invaluable help with NMR spectroscopy.

Of course I must thank all the great people I have met here in St Andrews! Constantin in particular, one of the nicest people I’ve ever met and made our Hepburn flat a great place to live. He does insist on trooping around in a German football shirt shouting “4-1” all the time, but nobody’s perfect.

Finally, I would like to thank my parents back home for their constant support during my time up here. And Kate, now this is finished, maybe I’ll read you a bedtime story of your own choosing for a change.
# Contents

Abbreviations xiii

1. Introduction 1

1.1 Preamble 1
1.2 Complex Networks 1
1.3 Constructing Complex Systems: The Importance of Feedback 3
1.4 Introducing Feedback Using Self-Replication 6
   1.4.1 The Minimal Model of Self-Replication 6
   1.4.2 Kinetic Analysis of Replicators 8
   1.4.3 The First Example of Artificial Replication 10
   1.4.4 Small Organic Molecule Replication 12
1.5 Networks of Replicators 22
   1.5.1 The Reciprocal Model of Replication 22
   1.5.2 Multicyclic Replication Networks 23
1.6 Systems Chemistry 29
   1.6.1 Dynamic Combinatorial Chemistry 29
   1.6.2 Kinetic Selection Within a Dynamic Library 31
   1.6.3 Coupling Dynamic Libraries to Replication Processes 35

2. Design Principles and Objectives 45

2.1 The Dynamic Library 45
2.2 The Recognition Event 47
2.3 The Chemical Reaction 47
   2.3.1 Reaction via the [A•B] Pathway 49
   2.3.2 Reaction via the Self-Replication Cycle 50

3. Manipulation of a Dynamic Library Using Molecular Recognition 51

3.1 Design and Construction of the Dynamic Combinatorial Library 51
3.2 Analysis of the Irreversible Reaction 56
3.3 Kinetic Selection 60
3.4 Coupling the DCL with a Recognition-Disabled Process 63
3.5 Coupling the DCL with a Recognition-Mediated Process 67
3.6 Comparison with Kinetic Selection 72
6.9 Competition in a Closed System: Kinetic Selection 145
6.10 Competition in an Open System: Survival of the Fittest? 148
6.11 Conclusions 149

7. Conclusions 151
7.1 General Conclusions 151
7.2 Future Work 152

8. Experimental 155
8.1 General Procedures 155
8.2 General NMR Procedures 156
8.3 Kinetic Measurements and Deconvolution of NMR data 157
  8.3.1 $^1$H NMR Spectroscopy 157
  8.3.2 $^{19}$F NMR Spectroscopy 159
  8.3.3 Semi-Automatic Deconvolution 160
8.4 Construction and Analysis of Dynamic Systems 161
8.5 Chemical Waves 163
  8.5.1 Individual Waves 163
  8.5.2 Competition within the Wave 164
8.6 Kinetic Fitting, Simulation and Extraction of Rate Constants 164
  8.6.1 Effective Molarity 165
  8.6.2 Free Energy of Connection 165
8.7 Synthetic Procedures 166

9. References 187

10. Appendices 191
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>cytosine</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>DCC</td>
<td>dynamic covalent chemistry</td>
</tr>
<tr>
<td>DCL</td>
<td>dynamic combinatorial library</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>dd</td>
<td>doublet of doublets</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>EDCI</td>
<td>1-ethyl-3-(3’-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene diamine tetraacetic acid</td>
</tr>
<tr>
<td>EF</td>
<td>enhancement factor</td>
</tr>
<tr>
<td>G</td>
<td>guanine</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>HMDS</td>
<td>hexamethyldisilazane</td>
</tr>
<tr>
<td>K</td>
<td>equilibrium constant</td>
</tr>
<tr>
<td>k</td>
<td>rate constant</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>m</td>
<td>multitplet</td>
</tr>
<tr>
<td>M.p.</td>
<td>melting point</td>
</tr>
<tr>
<td>Me</td>
<td>methyl group</td>
</tr>
<tr>
<td>MHz</td>
<td>megahertz</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>mL</td>
<td>millitre(s)</td>
</tr>
<tr>
<td>mM</td>
<td>milimolar</td>
</tr>
<tr>
<td>mmol</td>
<td>milimoles</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>ppm</td>
<td>part(s) per million</td>
</tr>
<tr>
<td>ρTSA</td>
<td>para-toluenesulphonic acid</td>
</tr>
<tr>
<td>r.t.</td>
<td>room temperature</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>trimethylsilane</td>
</tr>
</tbody>
</table>
Introduction

1.1 Preamble

Life is the most complex system ever to have been studied by man. The remarkable level of biodiversity that living things on this planet display hammers the point home that Life is not simple. Whilst the search for the Origin of Life can be traced\cite{1-3} back to the 1960’s, and the study of complex systems\cite{4, 5} has long been prevalent in the fields of sociology, mathematics, physics and biology, it seems incredible that chemical research into networks is lagging so far behind. In fact, it wasn’t until as recently as 2005 that von Kiedrowski coined the phrase ‘Systems Chemistry’, announcing\cite{6} it as a potential new field in chemical research. Traditional synthetic chemists have always treated complex mixtures as unwanted necessities of their research, necessities requiring clean-up in order to progress in a linear fashion to their target compound. It comes as no surprise then that while proponents of the ‘RNA world’ hypothesis\cite{7} are well on their way to uncovering\cite{8} how nucleotides may have emerged from the primordial ooze, the mechanisms by which these nucleotides oligomerise to form nucleic acids still remains very much unknown.

1.2 Complex Networks

Complexity is all around us; think of the fluctuation of global stock markets, the infrastructure of the internet or the numerous interwoven food chains in even the simplest of ecosystems. In each of these systems, a seemingly insignificant event located at a specific point within the system can have unexpected ramifications far from the point of origin. A financial merger, power outage or disease can send shockwaves through the entire network and have a direct effect on seemingly separate events. Put simply, complex networks are formed from a group of simple
species that cooperate within a system to give rise to behaviour that could not otherwise be predicted from the isolated properties of the individual components. These system-level, or emergent, characteristics are not the properties of any one of the single components but are a result of their collective interactions and allow complex networks to display behaviour that is more than just the sum of its parts. With the advent of several hugely popular social networking sites such as Facebook and Twitter, as well as the mathematical concept of Chaos Theory being popularised in the 2004 feature film *The Butterfly Effect*, the average person’s contact with the principles of complexity has become ever more apparent.

Highly complex systems are doubled-edged swords in the eyes of a researcher. A network exhibits more interesting behaviour when the level of complexity is higher, but this behaviour becomes more and more obscured by the same increasing complexity. A greater number of connections between components results in a more intricate network but also increases the difficulties associated with data presentation. The most common way to depict networks is the wiring diagram (*Figure 1.1*), which quickly becomes an indecipherable tangle in complex cases. This makes long range connections especially hard to identify and it is unfortunately these more complex systems that exhibit the most interesting properties.

*Figure 1.1.* Examples of studied networks. a) A visualisation of the network structure of the internet. b) A food web of predator-prey interactions of species in a freshwater lake. c) A social network of sexual contacts. Figure taken from ref 11.
1.3 Constructing Complex Systems: The Importance of Feedback

Of the wide-ranging properties that interconnected networks can possess, a common feature runs among them. In each network, a change in one location will be relayed to another and will have some kind of effect on other parts of the system. This in turn will affect other locations and a type of chain reaction is initiated. This phenomenon of feedback, is an essential characteristic found in all examples of complex systems.

The impact that feedback can have on a system can be illustrated by the complex fractal shapes generated\[12\] by the Mandelbrot Set in complex mathematics (Figure 1.2). An exhaustive description of the mathematics that this set exhibits is beyond the scope of this thesis but the essential feature is the quadratic polynomial function that leads to the characteristic shape.

\[ z_{n+1} = z^2 + c \]

The variable \( z \) is on both sides of the equation so the output from one iteration becomes the input for the next. This relatively simple equation leads to highly complex shapes that can be split into parts that are reduced-size copies of the image as a whole. The realisation that the remarkable level of complexity displayed by these shapes is generated from a simple equation illustrates the profound effect a single feedback operation can have on a system.

Figure 1.2. The fractal shape generated by plotting the Mandelbrot set. Zooming in on sections reveals identical copies of the image as a whole. Image taken from ref 12.
A chemical example\textsuperscript{[13]} of a complex network is based on a mixture of replicating peptides. Combinations of electrophilic peptides $E_{1-9}$ with a common nucleophilic peptide $N$ results in a distribution of peptide products $T_{1-9}$ (Figure 1.3). The peptides $T_{1-9}$ are linked to one another by a series of auto and cross-catalytic pathways governing their formation from the smaller fragments $N$ and $E_{1-9}$. $T_1$, $T_3$, $T_4$, $T_6$, $T_7$ and $T_9$ behave as templates for the synthesis of one or more of the other products from the pool of smaller fragments. As well as these cross-catalytic pathways, peptides $T_2$, $T_5$ and $T_8$ exhibit self-replicating behaviour. These collective interactions result in a highly structured network formed from the connection of a number of simple peptide building blocks.

The links in this network are provided by a common building block required for the formation of each peptide. In a simpler scenario, if just two reactions were competing for a limited reagent, increasing the efficiency of one process would directly affect the other by reducing the amount of common reagent available to it. One can imagine a hypothetical system of this type that consisted of three interlinked components $A$, $B$ and $C$ in which $A$ can react with $B$ to form $AB$ with rate constant $k_{AB}$, and in a similar fashion, $B$ can react with $C$ to form $BC$ with rate constant $k_{BC}$ (Figure 1.4a). At the end of the reaction, if the rates of two processes remained equal throughout and assuming a 50 mM starting concentration of each of the three starting components, there would be 25 mM of each of the two products $AB$ and $BC$. However if the rate of formation of $AB$, $k_{AB}$, was selectively enhanced by a factor of ten, the final product distribution would reflect an up-regulation of $AB$ at the expense of $BC$ (Figure 1.4b). In this simple network, the two products $AB$ and $BC$ are linked to one another through a mutual dependence on building block $B$. 

Figure 1.3. Experimentally derived architecture of the replicating peptide network. Straight arrows indicate a catalytic template effect pointing from template to product. Curled arrows denote the autocatalytic ability of $T_5$, $T_8$ and $T_9$. Figure adapted from 13.
Graphical representation of hypothetical reaction networks in which two chemical entities react together to form a product molecule. All starting concentrations were set to 50 mM. All rate constants were fixed at $1 \times 10^{-3} \text{M}^{-1} \text{s}^{-1}$ except for $k_{AB}$ which was increased by a factor of 10 to $1 \times 10^{-2} \text{M}^{-1} \text{s}^{-1}$ in b) and c) as indicated by the blue arrow.

More complex scenarios are generated by increasing the number of competitors for a common reagent. Such a system is generated by extending the simple hypothetical example by adding a fourth component, D, and arranging the network into a square (Figure 1.4c). With the addition of D, which can react with both C and A, a total of four products are possible: AB, BC, AD and CD, formed with rate constants $k_{AB}$, $k_{BC}$, $k_{AD}$ and $k_{CD}$ respectively. As in the simple three component network, each of the possible products is formed to the same extent when the individual rate constants equal one another. However, if $k_{AB}$ is selectively enhanced in this system, an up-regulation of both AB and CD is observed, at the expense of AD and BC. This seemingly unexpected effect on CD can be easily explained by looking at the differential equations for the formation of the four products (Figure 1.5).

\[
\frac{d[AB]}{dt} = k_{AB} [A][B] \quad \uparrow \\
\frac{d[BC]}{dt} = k_{BC} [B][C] \quad \downarrow \\
\frac{d[AD]}{dt} = k_{AD} [A][D] \quad \downarrow \\
\frac{d[CD]}{dt} = k_{CD} [C][D] \quad \uparrow
\]

Figure 1.5. Differential equations for the formation of products AB, BC, AD and CD. An upwards arrow indicates products that are up-regulated whilst a downwards arrow indicates a product whose formation is suppressed. As a result the interconnectedness of the system, enhancing the formation of AB also enhances formation of product CD.
Increasing the rate of formation of \textbf{AB} causes the concentrations of components \textbf{A} and \textbf{B} to become depleted and consequently any process that relies on either one is suppressed. While \textbf{A} and \textbf{B} are being rapidly consumed, the equivalent processes consuming \textbf{C} and \textbf{D} are becoming less efficient, causing a reduction in the competition for them. The formation of \textbf{CD} is the only process that relies solely on \textbf{C} and \textbf{D} and is subsequently up-regulated as result of the diminishing efficiency of the competing processes. It is important to note that the rate of formation of \textbf{CD}, $k_{CD}$, is never changed during these simulations and is therefore not directly connected to the enhancement of $k_{AB}$. The up-regulation of \textbf{CD} is brought about by the interconnectedness of the four components and can be thought of as an emergent property of the system.

Further complicating the peptide network in Figure 1.3 is the presence of entities capable of replication. Self-replication can be thought of as a positive feedback loop, in that the product from a reaction behaves as a catalyst for the same reaction and therefore has a direct impact on its efficiency. The autocatalytic nature of a self-replicating reaction provides the feedback required for the formation of a complex system.

\section{1.4 Introducing Feedback Using Self-Replication}

\subsection{1.4.1 The Minimal Model of Self-Replication}

A self-replicating molecule can be defined\cite{14} as a chemical species that promotes its own synthesis from a mixture of reactants capable of a variety of reactions. This objective can be achieved by templated synthesis in which the molecule is able to assemble the required building blocks to create an exact copy of itself, which is then able to do the same. This model is sometimes referred to as the minimal model of replication and is presented graphically in Figure 1.6.

Within the model,\cite{15} there are three channels by which the two entities can react. The first channel is an uncatalysed bimolecular reaction between molecules \textbf{A} and \textbf{B} to form the template molecule \textbf{T}. The second channel of reaction is brought about by a simple recognition event. A requirement for this replication model is that the two entities \textbf{A} and \textbf{B} possess complementary recognition sites, allowing them to associate with one another during the course of the reaction. The presence of these complementary recognition sites allows \textbf{A} and \textbf{B} to form the binary complex [\textbf{A-B}] in
which the reactive sites are brought closer together. The proximity of the reactive sites renders the reaction between \textbf{A} and \textbf{B} \textit{pseudo}-intramolecular and results in the formation of an catalytically inert template molecule \textbf{T}'\!. The recognition sites are still present within this molecule \textbf{T}' but they remain intramolecularly associated and therefore unable to associate with free molecules of \textbf{A} or \textbf{B}. The third channel is the replication cycle. The template molecule \textbf{T}, formed by the bimolecular reaction between \textbf{A} and \textbf{B}, also possesses recognition sites complementary to those present on \textbf{A} and \textbf{B} and can organise them onto itself forming the ternary complex [\textbf{A}\cdot\textbf{B}\cdot\textbf{T}]\!. Analogous to the [\textbf{A}\cdot\textbf{B}] complex pathway, the reaction between \textbf{A} and \textbf{B} is rendered \textit{pseudo}\textendashintramolecular, forming the product duplex [\textbf{T}\cdot\textbf{T}]\!. This duplex can then dissociate and return two molecules of \textbf{T} to the cycle. During the cycle, molecule \textbf{T} is acting as template for its own formation and transmitting molecular information through the formation of identical copies of itself.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{replication_model.png}
\caption{The minimal model of replication. The reaction between two components, \textbf{A} and \textbf{B} can proceed by a) the bimolecular background reaction (Channel 1), b) the [\textbf{A}\cdot\textbf{B}] complex (Channel 2) or c) the replication cycle (Channel 3).}
\end{figure}

It is worth noting that for this system to be efficient, the reaction within the ternary complex [\textbf{A}\cdot\textbf{B}\cdot\textbf{T}] should result in some kind of structural change that results in the duplex [\textbf{T}\cdot\textbf{T}] being less stable than [\textbf{A}\cdot\textbf{B}\cdot\textbf{T}], thus allowing \textbf{T} to dissociate back into the cycle. Excessively stable [\textbf{T}\cdot\textbf{T}] duplexes result in product inhibition. Reaction \textit{via} the [\textbf{A}\cdot\textbf{B}] complex pathway should also be minimised for an efficient replicator. In an ideal system, at the completion of the first catalytic cycle, two molecules of \textbf{T} are released as the [\textbf{T}\cdot\textbf{T}] duplex dissociates. Each of these molecules can participate in another cycle doubling the concentration of \textbf{T} at each cycle. This would result in exponential product growth until the supply of building blocks \textbf{A} and \textbf{B} became exhausted. Currently, no artificial replicators that demonstrate truly exponential growth have been reported.
1.4.2 Kinetic Analysis of Replicators

With three channels for reaction open to even the simplest of replicators, a thorough understanding of all of the processes involved in self-replication is difficult to uncover. In 1993, in what would prove to be a seminal contribution to the field of artificial replication, von Kiedrowski introduced\(^\text{[16]}\) a series of simplified kinetic models that allowed the fitting of experimental data and the extraction of important thermodynamic and kinetic parameters. The extraction and study of these parameters, such as rate and equilibrium constants for recognition processes, would shed light on how these systems behave. The simplest of these kinetic models is a purely autocatalytic reaction between $A$ and $B$ with a variable reaction order for the product $T$, known as the autocatalytic reaction order $p$. This parameter $p$ describes the autocatalytic behaviour of the system and determines the type of the autocatalytic growth curve. In real systems, the value of $p$ is expected to lie between 0.5 and 1. At the lower extreme, where $p = 0.5$, the concentration vs. time profile shows parabolic growth (Figure 1.7a), indicating that the dissociation of the duplex $[T\cdot T]$ is the rate-limiting process. At the other extreme, when $p = 1$, the concentration vs. time profile shows exponential product growth (Figure 1.7b) indicating that the dissociation of $[T\cdot T]$ is rapid and therefore not rate-limiting.

\[
A + B + pT \xrightarrow{k_a} (1 + p)T
\]

Figure 1.7. Computed concentration vs. time (left y axis, solid curve) and rate vs. time (right y axis, dashed curve) profiles for a) a reaction with an autocatalytic reaction order of 0.5 ($p = 0.5$) and b) a reaction with an autocatalytic reaction order of 1 ($p = 1$). Figure taken from ref 16.
Taking the first derivative of a concentration vs. time plot provides the rate vs. time profile (Figure 1.7, dashed curves) for a given process. It is worth noting that the maximum rate of reaction for a self-replicating system, regardless of the value of $\rho$, does not occur at $t = 0$. It instead occurs at a later point in the reaction and is associated with a particular concentration of $T$. At $t = 0$, there is no template $T$ present in the reaction mixture and as such the replication cycle (Figure 1.6, Channel 3) is not available. Therefore $A$ and $B$ can only form $T$ through the uncatalysed and slow bimolecular reaction channel before the replication cycle can begin. This results in an induction or lag period at the start of a self-replicating reaction. As the reaction approaches completion, the depleted concentration of building blocks $A$ and $B$ become rate-limiting and the concentration vs. time profile reaches a plateau and levels out. This plateau, coupled with the lag period leads to the sigmoidal or ‘S’ shaped reaction profiles characteristic of this type of systems.

Figure 1.7 illustrates systems whose progression is purely autocatalytic and as such is rarely representative of real systems. In real systems, $T$ is formed slowly through the bimolecular channel as well as by the replication cycle, however this contribution is omitted from the present reaction profiles. Figure 1.8 shows the concentration vs. time profiles when the bimolecular contribution is taken into account.

\[
A + B + \rho T \xrightarrow{k_a} (1 + \rho) T \\
A + B \xrightarrow{k_b} T \quad \varepsilon = \frac{k_a}{k_b}
\]

Figure 1.8. Computed concentration vs. time profiles for various autocatalytic efficiencies, $\varepsilon$, for a) a reaction with an autocatalytic reaction order of 0.5 ($\rho = 0.5$) and b) a reaction with an autocatalytic reaction order of 1 ($\rho = 1$). Figure taken from ref 16.
As before, the rate constant $k_a$ describes the formation of $T$ through the template catalysed reaction, while formation of $T$ through the uncatalysed bimolecular reaction is described by the rate constant $k_b$. The ratio of these two rate constants measures the relative contribution of each pathway to the formation of $T$ and is termed the autocatalytic efficiency, $\varepsilon$. The characteristic sigmoidal shape only becomes observable in the concentration vs. time profile above a critical value of $\varepsilon$. Below this value, some systems do not display a sigmoidal profile despite containing an autocatalytic pathway.

In practice, one of the simplest ways to identify self-replicating behaviour as either parabolic or exponential is to monitor the course of the reaction in the presence of differing amounts of preformed template $T$. The presence of $T$ at the start of the reaction abolishes the lag period and allows the autocatalytic pathway to function immediately. Typically, the reaction will be performed with 2 to 10 mol % of $T$ with the amount of $T$ doubling in each successive experiment. In a parabolic system, the initial rate of formation of $T$ scales as $\sqrt{1}:\sqrt{2}:\sqrt{4}$ with addition of template demonstrating the system's adherence to the 'square root law' In exponential cases, the initial rate scales as $1:2:4$.

### 1.4.3 The First Example of Artificial Replication

Nucleic acids such as DNA have long been known to synthesise copies of themselves through a process known as replication.\cite{17} These replication processes are usually catalysed by enzymes known as DNA polymerases and proceed when two strands of complementary nucleic acids are assembled by the Watson-Crick base pairing of nucleobases. In a process that mimicked DNA replication, the first example of a non-enzymatic self-replicating system was published\cite{18} by von Kiedrowski in 1986.

He demonstrated that the hexadeoxynucleotide $3$ can catalyse its own formation from a pair of self-complementary trinucleotide precursors $1$ and $2$ (Scheme 1.1). The 3’-phosphate group on $1$ is activated in situ by EDCI before being attacked by the nucleophilic hydroxyl group attached at the 5’-terminus of trinucleotide $2$. Molecular recognition is provided by the hydrogen-bonded Watson-Crick base pairs between cytosine (C) and guanine (G), leading to the CCGCGG hexadeoxynucleotide $3$. Whilst this system responded in autocatalytic fashion to the addition of preformed $3$
at the start of the reaction, the relatively fast bimolecular reaction between 1 and 2 prevents the observance of the expected sigmoidal rate profile. A measured $p$ value of 0.48 suggests a strong interaction between two strands of 3, leading to product inhibition. As it is only the free template that is catalytically active, this interaction between molecules of 3 prevents the system from achieving exponential growth. These results underlined von Kiedrowski’s empirical law that states the rate of reaction of systems with strong product inhibition depends on the square root of the total concentration of present template.

**Scheme 1.1.** The hexadeoxynucleotides 3 and 5 are capable of templating their own formation from the combination of tri-deoxynucleotides 1 and either 2 or 4. The sigmoidal growth curve is only observed in the case of the synthesis of hexadeoxynucleotide 5, however both nucleotides exhibit autocatalytic behaviour. EDCI = 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide.
It wasn’t until von Kiedrowski replaced the nucleophilic group in 2 with an amine group that the sigmoidal reaction profile of this system was uncovered.\[^{[19]}\] The 5’-aminotrimer 4 reacts with the 3’-phosphate of 1 to form the self-complementary hexameric 3’-5’-phosphoamidate linked nucleotide 5 as the exclusive product. The rate of the recognition-mediated reaction is strengthened compared to the uncatalysed reaction, allowing the sigmoidal shape of the concentration vs. time profile to be seen for the first time ($\varepsilon = 420$) (Figure 1.9a). A comparable autocatalytic efficiency ($\varepsilon = 340$) was achieved in a similar system based on a self-replicating tetranucleotide presented\[^{[20]}\] by Orgel et al. in 1987. Work on these systems based on oligonucleotides inspired the work on small organic molecule based replicating systems.

**Figure 1.9.** Concentration vs. time plot of the reaction of 5’-aminotrimer 4 with 3’-phosphate trimer 1 forming the hexanucleotide 5 (*$\ddagger$*) in a) the absence and b) presence of 16 mol % of 5. Solid lines represent a calculated best fit using least-squares regression. Data taken from ref 19.

### 1.4.4 Small Organic Molecule Replication

In 1990, Rebek and co-workers described\[^{[21]}\] the first self-replicating system based on small organic molecules. They claimed that “at best, this can be regarded as a primitive sign of life”. Their system was based on amide bond formation between two entirely synthetic components 6 and 7 (Scheme 1.2). Recognition between 6 and 7 is provided by hydrogen bonding between an imide based on Kemp’s triacid\[^{[22]}\] of 6 and the adenine nucleobase of 7. The formation of an amide between an activated pentafluorophenyl ester on 6 and a primary amine connected to the adenine structure 7 was chosen as the chemical reaction to create the covalent bond in the self-complementary template 8.
Scheme 1.2. Rebek’s self-replicating system. The reaction between activated ester 6 and amine 7 leads to the structure 8. Molecular recognition in this system is provided by hydrogen bonding between the imide moiety of 6 and the adenine moiety of 7.

The naphthalene spacer group was incorporated into 6 to minimise reaction through a binary complex brought about by intramolecular hydrogen bonding. Initial attempts to create a self-replicating system based on 8 with a phenyl spacer resulted in reaction through this unwanted binary complex resulting in the product being folded shut and catalytically inert.[23] Rebek claimed that the system outlined in Scheme 1.2 constituted the first example of a self-replicator based on completely synthetic molecules. Indeed the reaction between activated pentafluorophenyl ester 6 and amine 7 exhibits autocatalysis, doping the reaction with preformed product 8 accelerates the initial rate, although no sigmoidal curve is observed. The reaction performs significantly slower when the imide of 6 is N-methylated, interrupting the hydrogen bond facilitated recognition event, and in the presence of a competitive inhibitor such as 2,6-bis(acylamino)pyridine. These observations are evidence that the rate enhancement is the result of molecular recognition.

Based on this, Rebek identified three pathways contributing to the formation of 8. In addition to the simple bimolecular pathway depicted in Scheme 1.2, Rebek proposed two additional pathways brought about by molecular recognition (Figure 1.10). The [A•B] pathway associates 6 and 7 together, forming the complex [6•7], and rendering the amide forming reaction pseudo-intramolecular. In addition to this, the open nature of the template 8 can assemble 6 and 7 into the ternary complex [6•7•8], again rendering the reaction pseudo-intramolecular and releasing more 8 into solution after the dissociation of the resulting [8•8] duplex. Of course only the later process is indicative of a self-replicating system and a reaction proceeding via this pathway would exhibit a characteristic sigmoidal concentration vs. time profile. Rebek claimed that the absence of the expected sigmoidal growth curve can be explained by a dominant presence of the [A•B] complex pathway. The result of the pseudo-
intramolecular reaction within the [A·B] complex leads to the formation of the unfavourable cis amide of 8. This cis amide undergoes isomerisation to the more favourable trans isomer, thus returning free open template 8 to the reaction mixture. As the open template is no longer only generated by the slow bimolecular reaction and is instead provided much faster by a recognition-mediated process, no lag period in the formation of 8 is observed.

This system, and Rebek’s claims about its self-replicating nature, became the focus of much controversy in the years following its publication. Menger shared his doubts about its authenticity as a self-replicator by demonstrating that simple amides could also catalyse the reaction between 6 and 7 as well as the template 8 was a catalyst for the reaction between 7 and substrates incapable of hydrogen bonding.[25] These observations led Menger to conclude that Rebek’s claims of self-replicative behaviour was superfluous and that the observed rate enhancement does not arise from the ternary complex but merely from the effect of simple amide acceleration. Menger proposed that the zwitterionic tetrahedral intermediate formed by aminolysis of the activated ester was stabilised by the proximity of the carbonyl of the amide group of 8. Rebek’s rebuttal[26, 27] stated that the concentrations required to demonstrate this amide catalysis was over 25 times the concentration employed by the Rebek group, and additionally the ability of 8 to catalyse any other reaction does not detract from its self-replicating potential.
Despite the ongoing public conflict between Menger and Rebek, it wasn’t until Reinhoudt et al. intervened that the controversial system was subjected to a detailed kinetic analysis. The Reinhoudt laboratory identified a total of five pathways that contribute to the formation of amide 8 and calculated the relative contributions of each. Their work shed some light on the intricacies of the chemistry involved and ultimately allowed both Rebek and Menger to save face.

In addition to the three pathways identified by Rebek, Reinhoudt confirmed the presence of two additional pathways in which only one recognition event is necessary for the observed rate enhancement (Figure 1.11). The two additional pathways come from reaction between either the activated amine (when bonded to the template) and the free ester (Figure 1.11a) or the activated ester (when bonded to the template) and the free amine (Figure 1.11b).

The kinetic analysis revealed that the performance of each pathway is highly dependent on the concentrations employed. At higher concentrations the bimolecular reaction is up-regulated at the expense of the [A•B] pathway, the ternary complex pathway (self-replicating) only contributes when template is present at the start and never more than 46%. The contribution of the reaction of complex [7•8] with free 6 increases with concentration while the corresponding reaction of [6•8] with free 7 hardly contributes at all at any concentration. Reinhoudt’s paper determined that while the reaction of 6 and 7 in the presence of preformed 8 can perform through a self-replication, the dominant mode of action is the [A•B] pathway.
At the time of Reinhoudt’s paper, Rebek had already restructured his system to incorporate a longer biphenyl spacer group into the activated ester 9 and subsequently template 10. The longer spacer group forces the separation of the two components in the binary complex, minimising the contribution of this pathway in favour of the ternary complex (Figure 1.12a) and subsequently the replication cycle. Rebek’s improved system finally exhibited a sigmoidal concentration vs. time profile (Figure 1.12) that was not present in the recognition-disabled process carried out using the N-methylated derivative of 9.

Figure 1.12  a) The ternary complex as formed during the second generation Rebek replicator. Incorporating a longer biphenyl linker into the structure of 9, reduced the contribution of the [A,B] complex pathway revealing the sigmoidal reaction profile. Concentration vs. time profile for b) the formation of 10 from 7 and 9 demonstrating the characteristic sigmoidal curve, and c) the same reaction using the N-methylated derivative of 9, interrupting the hydrogen bonding eliminates the self-replicating behaviour and no sigmoidal growth curve is observed. Data taken from ref 29.
At the same time as the Rebek group were attempting to demonstrate that their system was indeed a self-replicator, von Kiedrowski described a series of systems based on the condensation of simple amines and aldehydes to give imines capable of self-replication. Instead of utilising hydrogen bonding between nucleobases, the von Kiedrowski system exploits the amidinium-carboxylate salt bridge as a means of molecular recognition (Scheme 1.3).

The synthesis of template 13 from amine 11 and aldehyde 12 is autocatalytic and depends on the concentration of 13 in the reaction mixture. As expected, the rate of the condensation obeys the square root law and demonstrates parabolic growth.

Whilst characterising their system, von Kiedrowski’s laboratory came across an interesting result. Structurally analogous template 16 also catalysed the reaction between 11 and 12 but the reaction no longer obeyed the square root law and proceeded with an almost first order profile. First order catalysis is expected if the concentration of the ternary complex is higher than that of the catalyst duplex (Figure 1.7b). Whilst this observation comes from a crosscatalytic process as opposed to autocatalysis, it seems to suggest that exponential product growth is feasible in replicating systems in which template duplexes are less stable than the corresponding ternary complexes.
1. Introduction

In 1997, Sutherland and Wang described\cite{31} a self-replicating system based on a Diels-Alder reaction with a surprisingly high $p$ value of 0.8. Their system was based on the reaction between a maleimide bearing an amidopyridone recognition site 17 and cyclohexadiene bearing an amidonathyridine moiety 18 forming a template molecule 19 (Scheme 1.4).

![Scheme 1.4](image)

Scheme 1.4. The Diels-Alder reaction of chiral diene 18 and maleimide 17 leads to the formation of cycloadduct 19. The complementary recognition sites on 17 and 18 allow product 19 to perform as an efficient self-replicator.

The progression of the reaction showed a sigmoidal profile for the formation of the product 19 (Figure 1.13a) whilst doping a fresh mixture of the reactants with preformed template 19 confirmed the autocatalytic mechanism of the reaction by overcoming the initial lag period (Figure 1.13b). Repeating the experiments with recognition-disabled components, the conversion slowed significantly as the products formed via bimolecular pathways. Fitting of the obtained results provided a value of 0.8 for $p$, a value somewhat higher than for other systems based on oligonucleotides or simple organic molecules.

![Figure 1.13](image)

Figure 1.13. Concentration vs. time plot of the reaction of chiral diene 18 and maleimide 17 forming the cycloadduct 19 (●) in a) the absence and b) presence of 10 mol% of 19 at 40 °C. Solid lines represent a calculated best fit using least-squares regression. Data taken from ref 31.
There is a considerable degree of steric repulsion at the binding site between the methyl group of 18 and the aliphatic chain on 17. While this repulsion leads to a reasonably low association constant of 228 M\(^{-1}\) (at 23 °C), it also reduces the stability of the resulting product duplex, thus increasing the concentration of available catalyst to participate in the self-replication process. A feature of this new Diels-Alder based replicator was the possibility that, as a result of the chiral diene 18, product 19 can exist as four different diastereoisomers. This issue was not addressed in the Sutherland paper and 19 was assumed to be a mixture of endo-diastereoisomers.

The Diels-Alder reaction or indeed any other process with a well-defined transition state that can lead to a distribution of either stereo- or regioisomers, offers a platform by which efficient self-replicating systems could transfer chemical information via template catalysed bond formation. After demonstrating\(^{[32]}\) the effects of molecular recognition on the cycloaddition reaction between an azide and a maleimide, Philp et al. reported\(^{[33]}\) the rational design, synthesis and characterisation of a minimal self-replicating system based on the 1,3-dipolar cycloaddition between nitrone 20 and maleimide 23 (Scheme 1.5).

![Scheme 1.5](image)

Scheme 1.5. Reaction between nitrone 20 and maleimide 21 gives rise to two diastereoisomeric products trans-22 and cis-22 with poor selectivity and conversion. Introduction of maleimide 23 in place of 21 opens a recognition-mediated channel resulting in up-regulation of both conversion and selectivity for trans-24 at the expense of cis-24.

Usually, in the absence of molecular recognition effects, the reaction between a maleimide and a nitrone is slow and fairly unselective for the trans configuration of the resulting diastereoisomeric product. Indeed this is the case for the reaction between nitrone 20 and maleimide 21, with only 17% of the starting materials converted into product after 30 hours with trans-22 dominating cis-22 to a ratio of around 4:1. However, when nitrone 20 is reacted with maleimide 23 under identical conditions, the formation of trans-24 over cis-24 is pushed to 9:1 with over 75% of
the starting materials converted after 30 hours. The concentration vs. time profile exhibits a characteristic sigmoidal shape for the formation of trans-24 (Figure 1.14). In order to verify the autocatalytic activity of the trans isomer, 10 mol % of preformed trans-24 was added at the start of the reaction. The rate profile showed the anticipated loss of lag period and up-regulated the formation of trans-24. The addition of 10 mol % of preformed cis-24 to fresh starting materials had no effect on the progression of the reaction. In addition, when the same process is performed using four equivalents of benzoic acid as a competitive inhibitor for the binding at the amidopyridine site of 20, the reactivity and selectivity falls off dramatically. These results prove that the formation of trans-24 is autocatalytic and this autocatalysis is a consequence of molecular recognition. Computational fitting of the experimental data revealed values of $p$ and $c$ of 0.9 and 5000 respectively.

Using computational methods, the Philp group optimised[34] their previous replicator, altering the substitution pattern of the nitrone from meta to para and adding a rigid phenyl spacer to the maleimide, leading to the structures 25 and 26/28 (Scheme 1.6). Similar to their previous work, the recognition-disabled reaction between nitrone 25 and maleimide 26 proceeds slowly converting only 30% of starting material into cycloadduct 27 with a trans:cis ratio of just 3:1 after 16 hours. When the reaction is repeated using recognition-enabled maleimide 28, conversion after the same time rose to 85% with a diastereoselectivity of over 50:1 in favour of trans-29. This rate enhancement is coupled with the observation of a clear sigmoidal profile (Figure 1.15) and the expected responses to the addition of a competitive
inhibitor such as benzoic acid. The addition of preformed template \textit{trans-29} removed the lag period and lead to the almost exclusive formation of \textit{trans-29} at the expense of \textit{cis-29}. With this system, Philp \textit{et al.} had discovered a means of selecting a desired diastereoisomer by implementing a highly efficient replicating process mediated by a single recognition event.

Scheme 1.6. Reaction between nitrone 25 and maleimide 26 gives rise to two diastereoisomeric products \textit{trans-27} and \textit{cis-27} with poor selectivity and conversion. Introduction of maleimide 28 in place of 26 opens a recognition-mediated channel resulting in much greater up regulation of both conversion and selectivity for \textit{trans-29} than observed for the system in Scheme 1.5.

Figure 1.15. Concentration vs. time plot of the reaction of nitrone 25 and maleimide 28 ([25] = [28] = 25 mM) forming the cycloadducts \textit{trans-29} (●) and \textit{cis-29} (●) at -10 °C. The characteristic sigmoidal curve of a self-replicator is only observed for \textit{trans-29}. Solid lines represent a calculated best fit using least-squares regression.
1.5 Networks of Replicators

1.5.1 The Reciprocal Model of Replication

The replicating species described thus far have followed the minimal model of self-replication described in Figure 1.6 with each of two building blocks bearing recognition sites complementary to one another resulting in a self-complementary template molecule. However, template effects in a replication cycle can also operate in a reciprocal fashion. In these cases, two reciprocal templates possess complementary recognition sites and operate via a pair of interlinked crosscatalytic pathways. This is known\cite{15} as the reciprocal model of replication and is presented graphically in Figure 1.16.

Figure 1.16. The reciprocal model of replication. The reaction between two components, C and D proceeds via a bimolecular reaction to form template TCD while at the same time E and F react to form TEF. The two templates possess complementary recognition sites and engage in two crosscatalytic cycles known as reciprocal replication.

In this model, compounds C and D react together to form the template TCD, while similarly, compounds E and F can react to form template TEF. The four reactive partners bear appropriate recognition sites and result in the templates TCD and TEF being mutually complementary. Therefore, TCD is capable of assembling E and F into the ternary complex \([E\cdot F\cdot TCD]\). This ternary complex intramolecularises the reaction between E and F, thus catalysing the formation of TEF. In an analogous manner, TEF is capable of assembling C and D into the ternary complex \([C\cdot D\cdot TEF]\) catalysing the formation of TCD.
The complexity of this system can increase dramatically depending on the nature of the chemical reaction which forms the two templates. In the case where the reaction between C and D and the reaction between E and F are orthogonal, only one reciprocal replication cycle is present in the system. However, if C can react with E and D with F, three replication cycles are possible: two minimal replication cycles if $T_{CE}$ and $T_{DF}$ self-replicate, and the original reciprocal replication cycle $T_{CD} \rightarrow T_{EF}$ and $T_{EF} \rightarrow T_{CD}$. This situation would result in a multicyclic system of replicators (Figure 1.17). It is worth noting that the complexity is further increased if the minimal replicators $T_{CE}$ and $T_{DF}$ are able to crosscatalyse the formation of each other.

Figure 1.17. A multicyclic network of replicators. Four building blocks possess the same reactive sites and appropriate recognition sites to form two self-replicators and a pair of reciprocal replicators.

1.5.2 Multicyclic Replication Networks

Based on their previous work on hexanucleotides as minimal self-replicators (Scheme 1.1), von Kiedrowski et al. modified their system to furnish a set of four trinucleotides that are capable of participating in a network of independent auto and crosscatalytic cycles (Figure 1.18).[35,36] The trinucleotides contain a combination of either nucleobases in a CCG sequence (A) or the complementary CGG sequence (B) and either a electrophilic 3′-phosphate group (p) or the nucleophilic 5′-amine group (n). Nucleotides Ap and nB are identical to the self-replicating system described previously between 1 and 4 except for the azide protecting group in the 5′-terminus of Ap instead of the original methyl ether of 1.
Moving the protecting groups in both Ap and nB from one end to the other, gives rise to structures nA and Bp which can react to form an equally efficient self-replicating system BpnA. Combination of all four components opened a third reaction pathway. The reaction between nA and Ap gave rise to a template nAAp with a sequence complementary to the one found in the product nBBp formed by nB and Bp. This reciprocal relationship leads to the generation of a crosscatalytic cycle in which both templates catalyse the formation of its partner. Since all catalytic pathways proceed through ternary complexes of very similar stability, the native reaction between all four starting compounds gave rise to all four possible products in nearly equal concentrations. Doping a fresh solution of reagents with preformed product showed selfish amplification in the case of both self-complementary templates whereas the addition of a crosscatalytic template led to amplification of the corresponding complementary strand.
Another example of a multicyclic replication network is based on combining the Diels-
Alder reaction of a furan with a maleimide with the 1,3-dipolar cycloaddition between
a maleimide and a nitrone. The Philp group demonstrated\[37\] that a combination of
furan compound 32, nitrone 25 and maleimides 28 and 30 lead to the generation of
four templates (Figure 1.19) linked by a series of auto and crosscatalytic processes.

In addition to the previously reported self-replicator trans-29, furan 32 was shown to
react with maleimide 30 in a Diels-Alder process to provide 34, of which both
stereoisomers exhibit modest autocatalytic reactivity. As well as these autocatalytic
cycles, nitrone 25 can react with maleimide 30 and, in a similar manner, maleimide
28 can react with furan 32. In the absence of complementary recognition units, the
native Diels-Alder reaction and the 1,3-dipolar cycloaddition were shown to proceed
with poor diastereoselectivity giving an endo:exo ratio of only 1:1.3 and a trans: cis
ratio of 3:1, respectively. However, the resulting template trans-31 possesses
complementary recognition sites and was shown computationally to be a good fit for
Doping a fresh sample of 28 and 32 with preformed trans-31 significantly increased the rate of formation of exo-33 which in turn was shown to exhibit crosscatalytic behaviour towards the reaction of 25 and 30. However, the efficiency of the replicator 34 is no match for the remarkably efficient replicator trans-29 and as a result, the combination of 25, 28, 30 and 32 in CDCl₃ at 25 °C results in trans-29 as the major product.

The problem with the multicycle depicted in Figure 1.19 is the application of two differing reaction types used to join the templates together. The 1,3-dipolar cycloadditions proceed much faster than the respective Diels-Alder processes and cause a significant bias towards self replicator trans-29. Recent, unpublished work within the Philp group has developed a multicyclic system that utilised only the 1,3-dipolar cycloaddition reaction between a nitrone and a maleimide.[38]

Using maleimide 37 and nitrone 35 in place of 30 and 32 lead to the very efficient self-replicator trans-38. Previous nitrone 25 was outfitted with a fluorine tag to enable analysis of the system by ¹⁹F NMR spectroscopy to furnish 39 and the subsequent
replicator \textit{trans-40} retained its high efficiency. The diacid template \textit{36} was shown to be a catalyst for the otherwise slow and unselective reaction of nitrone \textit{37} and maleimide \textit{39}, while its crosscatalytic partner \textit{trans-41} similarly catalysed the reaction of \textit{28} with \textit{35}.

Unlike the previous attempt at creating a multicyclic system, the two minimal replicators, \textit{trans-38} and \textit{trans-40} utilise the same chemical reaction and more importantly, are of comparable efficiencies. The combination of the four starting materials \textit{28, 35, 37} and \textit{39} in CDCl\textsubscript{3} at 10 °C resulted in the coexistence of all four replicating templates in considerable quantities. (\textbf{Figure 1.21}). While the efficiencies of the self-replicators are well matched, the results show that the reciprocal replicators (RR) dominate the self-replicators (SR) to ratio of about 3:1.

The Philp laboratory went on to prove that the multicyclic system was capable of responding to external stimuli in the form of doping with instructional template. The native ratio of reciprocal replicators to self-replicators (RR vs. SR) was determined to be 3:1. When the reaction was repeated in the presence of 10 mol % of reciprocal template \textit{trans-41}, this ratio rose to around 4.5:1. The initial presence of \textit{trans-41} overcomes the lag period associated with the bimolecular formation of the templates and immediately catalyses the reaction of \textit{28} with \textit{35}. As a consequence of their reciprocal nature, \textit{trans-41} is also up-regulated upon addition of preformed \textit{trans-41} (\textbf{Figure 1.22a}). In a similar manner, when the reaction was repeated in the presence of 10 mol % of either self-replicating template \textit{trans-38} or \textit{trans-40}, the RR vs. SR
ratio dropped to around 1:1. The initial presence of trans-38 overcomes the lag period associated with the bimolecular formation of the templates and immediately catalyses the reaction of 35 with 37. As a consequence of the interconnectedness of the system, both trans-38 and trans-40 receive an enhancement (Figure 1.22b and 1.22c). The enhancement of trans-40 is a consequence of the decreased efficiency of the reciprocal replicators requiring 28 and 29 and is an emergent property of the system (and vice versa for the up-regulation of trans-38 upon addition of trans-40).

![Figure 1.22](image_url)

**Figure 1.22.** Concentration vs. time plots of the appearance of the replicators trans-41 (+) trans-36 (·) trans-38 (·) and trans-40 (·) in the multicyclic system from the reaction of 28, 35, 37 and 39 ([28] = [35] = [37] = [39] = 10 mM) in the presence of 10 mol % of a) trans-41, b) trans-38 and c) trans-40 in CDCl3 at 10 °C. Pie charts showing the relative concentrations of both reciprocal replicators (RR, +) and both self-replicators (SR, ·) accompany each. Data taken from ref 38.
1.6 Systems Chemistry

In recent years, the field of Systems Chemistry has emerged in modern science and in a break from tradition, concerns itself with complex chemical mixtures and gaining an understanding of the behaviour of such systems.[39-41] Although currently in its infancy, research into Systems Chemistry continues to increase our understanding of the connectivity and resulting phenomena exhibited by complex networks.

1.6.1 Dynamic Combinatorial Chemistry

Real examples of complex chemical networks have been generated through the application of dynamic combinatorial chemistry (DCC).[14] This field of chemistry exploits reversible covalent bond formation in order to generate networks of interconverting compounds, termed dynamic combinatorial libraries (DCLs). With reversible chemical bonds being under thermodynamic control, the distribution of the library components depends on the free energy of each individual member. Therefore, processes that are able to alter the free energy relationships between members are able to effect a change in the distribution of the material within the network. As an emerging field within Systems Chemistry, DCLs have been successfully employed in the design of synthetic receptors and sensors[42] and the creation of supramolecular assemblies.[43]

The nature of the reversible bond between library members can be varied and includes acyl transfer reactions, acetal exchange as well as a variety of metal catalysed reactions.[44] The reversible formation of imines from aldehydes and amines has gathered significant attention[45] in this field and has been successfully exploited[46] by the Nitschke group to generate a DCL capable of self-sorting upon the addition of specific metal cations (Scheme 1.7). Their system starts with two aldehydes 42 and 43, and two amines 44 and 45, which upon condensation, result in a library of exchanging imines. Upon the addition of Fe$^{2+}$ and Cu$^{+}$ salts, complexes 46 and 47 become the most thermodynamically stable species, resulting in the exchange process forming more of the required components from the DCL.

In a similar system, Gotor used[47] diamine 48 and dialdehyde 49 to form a library of imine based macrocycles that responded to the addition of metal ions of varying size. The addition of Ba$^{2+}$ induced the formation of the smaller macrocycle 50 while the larger Cd$^{2+}$ ion favoured the formation of larger macrocycle 51 (Scheme 1.8).
Supramolecular chemistry has been successfully combined\textsuperscript{[48]} with DCC in an elegant formation of molecular Borromean rings by the Stoddart group. Macrocycle 53 is formed from the combination of the same dialdehyde 49 as Gotor \textit{et al.} used, with diamine 52. Three of these macrocycles are assembled in an interlinked fashion around 6 Zn\textsuperscript{2+} ions to form the supramolecular structure 54 (Scheme 1.9). The interwoven nature of 54 requires the breaking of one macrocycle 53 before the disassembly of the remaining two is possible. The elegant nature of a synthesis of such a large and complex molecule in a single step from structurally simple building blocks underlines the power of dynamic combinatorial chemistry and has inspired research into similar assemblies.
1.6.2 Kinetic Selection Within a Dynamic Library

The examples illustrating the manipulation of dynamic combinatorial libraries presented thus far all rely on thermodynamic differences for target amplification. The amplification is inherently linear and as the effects are limited by the amount of receptor that is added, the achievable selectivity is limited. In order to increase selectivity within DCLs, kinetic selection through irreversible chemical bond formation is being introduced to libraries in an effort to break away from the binds of a purely thermodynamic environment.

Ramström et al. successfully coupled[49] an irreversible transesterification reaction to a DCL constructed from the reversible nitroaldol reactions between a nitroalkane 60 and a number of aldehydes 55 to 59 (Scheme 1.10). Upon condensation of the five aldehydes 55 to 59 with nitroalkane 60, a distribution of five exchanging racemic β-nitroalcohols were formed. The irreversible chemical reaction was a enzyme lipase PS-C I catalysed transesterification with a suitable acyl donor, p-chlorophenyl acetate. After a period of 14 days, ester 63 was detected as the major product with a minor amount of 64 also present, in a total conversion of over 80%. β-nitroalcohol 62 was the best substrate for the enzyme lipase and, despite being a minor component within the original DCL, was amplified from the library as 63. Of the remaining β-nitroalcohols, only the ester of 61, 64, was also detected. An additional advantage of an enzymatic selection process is the asymmetric amplification observed in this library. Each of the β-nitroalcohols is formed as a racemic mixture of both enantiomers, however the major product from the transesterification 63 was obtained in an 99% enantiomeric excess.
Using a similar DCL constructed from nitroalkane 68 and a number of aldehydes, Ramström restructured[50] his system to incorporate a intramolecular cyclisation as the kinetic selection process (Scheme 1.11). The dynamic library consists of an interconverting mixture of β-nitroalcohols of which only one, 69, bears a CN group in the 2-position. This adduct undergoes a 5-exo-dig type cyclisation to the iminolactone 70. After 24 hours, nitroalkane 68 is entirely incorporated into 70 and the remaining nitroalcohol library members are no longer detected despite being initially formed fastest (Figure 1.23).
This system was expanded further when the stereochemistry of the product 70 is taken into account. In the presence of the base, the two diastereoisomers of 70 readily interconvert. However one enantiomer displays a much greater tendency to crystallise from solution. The Ramström group demonstrated this resolution using a smaller DCL constructed from three aldehydes 58, 65 and 66 and nitronalkane 68. The intramolecular cyclisation formed a pair of diastereoisomers iminolactone 70 that were further separated by crystallisation (Scheme 1.12).

Scheme 1.12. Generation of a nitroaldol based dynamic library for resolution by intramolecular cyclisation and subsequent enantiomeric resolution by crystallisation.
Another example of crystallisation as means of resolution of a dynamic equilibrium was presented by Blackmond et al. in 2008. A basic solution-solid mixture of a rapidly racemising imine was found to crystallise into two enantiopure crystals upon constant agitation by glass beads (Figure 1.24).

![Figure 1.24](image)

Figure 1.24. Racemisation of two enantiomeric imines in basic methanol or acetonitrile can lead to the formation of two single homochiral crystals. Figure adapted from ref 52.

Solution-solid mixtures of varying enantiomeric excess (ee) were mechanically stirred in the presence of glass beads before racemisation was initiated by addition of DBU. Samples of the mixture were assayed over time for optical purity by HPLC. The authors found that only a very slight ee (as low as 2-3%) was required to induce the system into forming large homochiral crystals (Figure 1.25).

![Figure 1.25](image)

Figure 1.25. Enantiomeric enrichment of solid phase ee in acetonitrile over time at varied initial imbalances. Data taken from ref 52.
1.6.3 Coupling Dynamic Libraries to Replication Processes

In recent years, attention has turned to self-replicating processes as a means of externally influencing the composition of a dynamic library. Figure 1.26 shows a model depicting how replicating may be integrated into a DCL. Four components, a to d are in dynamic exchange with one another and four products A to D. Of these four products, one is a self-complementary template that enhances its own formation via autocatalysis forming a stable duplex [D•D]. A requirement of the system is the identification of a suitable recognition event to reversibly form the four products.

![Figure 1.26](image)

Figure 1.26. A model for the coupling of a dynamic library to a reversible replication process. Compounds a to d are in dynamic equilibrium with products A to D. Product D is a self-complementary template capable of autocatalytic amplification. Species inside the black line are within the dynamic library. Figure adapted from ref 9.

In an attempt to couple a dynamic library to a self-replicating species, Giuseppone utilised[53] reversible imine bond formation in a system that draws its inspiration from the controversial Rebek replicator. The DCL was constructed from three aldehydes, Am1, Am2 and Am3, and two amines Am1 and Am2, the combination of which generated a distribution of six imines (Scheme 1.13).

Of the aldehydes, only Al1 possesses a recognition site based around Kemp’s triacid while the other two are recognition-disabled. Of the two adenosine derived amines, only Am1 was capable of hydrogen bonding while Am2 has its recognition site blocked. Upon condensation, the product arising from the two recognition-enabled species, Al1-Am1, is a self-complementary template and subsequently forms the duplex [Al1–Am1•Al1–Am1] in solution. This duplex stabilises the structure over the other possible templates causing an 83% enhancement in production when compared to a recognition-disabled control system.
The reversible nature of the template forming reaction is the undoing of this system. Examination of the concentration vs. time profile (Figure 1.27) for this library reveals that the imine $\text{Al}^{1-}\text{Am}^{1}$ is indeed formed fastest as a result of the stability afforded by the product duplex, reaching a peak concentration in around 10 hours. However, as a consequence of the dynamic nature of the library, this imbalance is addressed over time with increasing amounts of other library members produced at the expense of $\text{Al}^{1-}\text{Am}^{1}$. This discovery again underlined the limitations of working in a wholly thermodynamic environment and resulted in a restructure of the model in Figure 1.26 to include an *irreversible* chemical reaction mediated by self-replicators.
Figure 1.27. Concentration vs. time plot of the imine products in the self-replicator assisted dynamic library following $\text{Al}^1\text{-Am}^1$ (●), $\text{Al}^1\text{-Am}^2$ (●), $\text{Al}^2\text{-Am}^2$ (●), and $\text{Al}^3\text{-Am}^2$ (●). $\text{Al}^1\text{-Am}^1$ is formed much faster than the remaining imines but its advantage is eroded over time as the dynamic equilibrium recovers. Data taken from ref 53.

The new model is depicted in Figure 1.28 and now encapsulates an irreversible reaction. In the same way as before, the library is constructed from the reversible interaction of components a to d. The difference with this model however is that the reactions forming products A to D are now irreversible. A rate enhancement of the reaction consuming D will draw material out from the dynamic library, affecting its composition. The dynamic equilibrium will work to replenish d at the expense of the remaining library members. In order to experimentally demonstrate this model, a suitable class of chemical must be identified. Imines are ideal for the construction of dynamic libraries as a consequence of their reversible formation from aldehydes and ketones, but they do not participate in any suitable irreversible reactions.

Figure 1.28. A model for the coupling of a dynamic library to a irreversible reaction. Compounds a to d are in dynamic equilibrium with one another and proceed in an irreversible manner to products D. Product D is formed faster than the other products at extracts d from the library at the expense of the other products. Species inside the black line are within the dynamic library. Figure adapted from ref 9.
Nitrones are structurally very similar to imines and recently, the Philp research laboratory has demonstrated\cite{54} that in non-polar solvents such as chloroform, nitrones are capable of undergoing dynamic exchange reactions analogous to those involving imines. They created a small library of four equilibrating nitrones 71 to 74 possessing a varying capability to participate in hydrogen bond mediated molecular recognition (Scheme 1.14). As with the replicating species previously reported, molecular recognition is provided by one or more amidopyridine moieties. Nitrone 74 features two recognition units, nitrones 71 and 72 each possess a single recognition site while nitrone 73 is bears none and is incapable of recognition.

[Scheme 1.14](#) A nitrone exchange reaction. Nitrones 71 and 72 are in equilibrium with nitrones 73 and 74 that leads to an almost equal distribution of the four nitrones after 48 hours. The addition of diacidic guest compound 75 stabilises the nitrone with two amidopyridine recognition units 74 and drives the equilibrium to the right-hand side with around 4:1 selectivity.

The combination of nitrones 71 and 72 initiates the exchange reactions and leads to an equal distribution of the four nitrones after 48 hours. When the reaction is repeated in the presence of template molecule 75, the complementary nitrone 74, bearing two recognition sites is stabilised and the equilibrium is altered. After 48 hours, a selectivity of 4:1 for the right hand side of the equilibrium was achieved (Figure 1.29).

[Figure 1.29](#) Ratio of nitrones 71/73 and 72/74 as a function of increasing diacidic guest 75 concentration. Data taken from ref 54.

1. Introduction

Nitrones are structurally very similar to imines and recently, the Philp research laboratory has demonstrated\cite{54} that in non-polar solvents such as chloroform, nitrones are capable of undergoing dynamic exchange reactions analogous to those involving imines. They created a small library of four equilibrating nitrones 71 to 74 possessing a varying capability to participate in hydrogen bond mediated molecular recognition (Scheme 1.14). As with the replicating species previously reported, molecular recognition is provided by one or more amidopyridine moieties. Nitrone 74 features two recognition units, nitrones 71 and 72 each possess a single recognition site while nitrone 73 is bears none and is incapable of recognition.

Scheme 1.14. A nitrone exchange reaction. Nitrones 71 and 72 are in equilibrium with nitrones 73 and 74 that leads to an almost equal distribution of the four nitrones after 48 hours. The addition of diacidic guest compound 75 stabilises the nitrone with two amidopyridine recognition units 74 and drives the equilibrium to the right-hand side with around 4:1 selectivity.

The combination of nitrones 71 and 72 initiates the exchange reactions and leads to an equal distribution of the four nitrones after 48 hours. When the reaction is repeated in the presence of template molecule 75, the complementary nitrone 74, bearing two recognition sites is stabilised and the equilibrium is altered. After 48 hours, a selectivity of 4:1 for the right hand side of the equilibrium was achieved (Figure 1.29).

Figure 1.29. Ratio of nitrones 71/73 and 72/74 as a function of increasing diacidic guest 75 concentration. Data taken from ref 54.
The advantage of an exchanging nitrone library compared to previously reported imine systems is the ability of a nitrone to undergo irreversible 1,3-dipolar cycloaddition reactions with suitable maleimides. Because both imines and nitrones undergo similar exchange reactions and share common building blocks, it is possible to construct a dynamic library that contains both exchanging imines and nitrones. Using a recognition-mediated chemical reaction, it is possible to select and amplify a single product from within a multitude of exchanging products.

Using this knowledge, the Philp research group demonstrated[55] that nitrones and imines can be in dynamic exchange with one another. The combination of imine 76, bearing an amidopyridine recognition moiety, with non-recognition enabled nitrone 77 in CD$_2$Cl$_2$/pTSA causes both species to hydrolyse to aldehydes 78 and 79, amine 80 and hydroxylamine 81. These building blocks can recombine to give a distribution of the original two components as well as recognition-enabled nitrone 39 and non-recognition imine 82 (Scheme 1.15).

Having demonstrated the nitrone and imine exchange is dynamic, Philp et al. went on to couple recognition-mediated irreversible chemical reactions to this simple dynamic library. Mixing nitrone 77 and imine 76 together in the presence of acid maleimide 83 opens up a recognition-mediated reaction channel (Scheme 1.16). The maleimide reacts with nitrones 39 and 77 in an irreversible 1,3-dipolar cycloaddition, thus transferring material out of the dynamic library (exchange pool) and into the product pool. Non-recognition nitrone 77 reacts via the uncatalysed bimolecular channel due
to a lack of a suitable recognition site. Nitrone 39 however is able to associate with maleimide 83 as a consequence of the hydrogen bonding recognition and reaction to cis-85 is driven through the [A•B] pathway. Despite the recognition nitrone 39 not being present in the library at the start of the reaction, after 16 hours at 0 °C, cycloadduct cis-85 accounts for over 90% of the material transferred into the product pool (Figure 1.30). The fast and selective recognition-mediated reaction dramatically alters the position of the equilibrium within the library.

Scheme 1.16. A nitrone-imine exchange pool is coupled to an irreversible chemical reaction. Nitrones 77 and 39 are able to react via a 1,3-dipolar cycloaddition to form cycloadducts 84 and 85, both of which exist as a pair of diastereoisomers. Only recognition-enabled nitrone 39 is able to react via the [A•B] pathway, rapidly and stereospecifically generating cis-85 at the expense of the remaining library members.
An autocatalytic process can also be coupled to a dynamic library (Figure 1.31). In this model, the irreversible process forming D is now autocatalytic, consuming d in a feedback loop that draws material into D at the expense of the remaining library members.

The Philp group went on to couple\textsuperscript{[56]} the same library to a self-replicating process (Scheme 1.17). By substituting maleimide 28 in place of 83, a replication cycle is opened at the expense of the [A•B] pathway. The para substituted acetic acid moiety is now too far from the amidopyridine unit in the cis isomer for intramolecular hydrogen bonding. The resulting open template afforded by the trans isomer has already been demonstrated to be a very efficient minimal replicator (Figure 1.15).
A nitrone-imine exchange pool is coupled to an irreversible chemical reaction. Nitrones 39 and 77 are able to react via a 1,3-dipolar cycloaddition to form cycloadducts 40 and 86, both of which exist as a pair of diastereoisomers. Only recognition-enabled nitrone 39 is able to react via the replication cycle, autocatalytically generating trans-40 at the expense of the remaining library members. As in the previously shown system involving meta maleimide 83, the recognition-mediated reaction rapidly consumes recognition nitrone 39 whilst the non-recognition nitrone 77 reacts via the slow bimolecular pathway. This sudden demand on 39 shifts the equilibrium to replenish it, at the expense of the other library members. After 16 hours, the self-replicating species trans-40 accounts for over 80% of the material transferred irreversibly into the product pool (Figure 1.32).
It was found that it did not matter if the library was generated by combination of nitrone 77 and imine 76 or the four smaller building blocks were combined separately. In both cases the recognition-mediated reactions had the same profound effect on both the exchange pool and the product pool.

With these results at hand, it is clear that a recognition-mediated irreversible reaction has a dramatic effect on the composition of a dynamic library, a simple replicator can amplify itself from a pool of suitable reagents. The dynamic library in question was specifically designed to accommodate a single replicator competing against a much slower, non-recognition mediated process. It would be interesting to investigate a dynamic library in which this is not the case, and two replicators are competing with one another as well as against the slower non-recognition processes.
Design Principles and Objectives

The objective of the research described in this thesis is to explore methods of achieving selectivity within dynamic covalent systems. It has already been demonstrated that a single recognition-mediated process can be used to amplify a specific compound from within a dynamic library containing a large number of components. By constructing a dynamic library containing the building blocks for more than one recognition-enabled species, this research builds on previous work in an attempt to break through the limitations of kinetic selection and provide a chemical example of Darwinian Selection.

2.1 The Dynamic Library

In recent years, research within the Philp laboratory in the area of dynamic combinatorial chemistry has lead to the successful construction and analysis of dynamic libraries based on the reversible formation of nitrones and imines from simple aldehydes, amines and hydroxylamines. Exploiting this knowledge, the dynamic library in which the proposed competition will take place will be based on a 4 × 4 matrix of aldehydes and nucleophiles, leading to a distribution of 16 possible imine or nitrone products (Figure 2.1). Within this pool of 16 exchanging products, termed the exchange pool, two species will be equipped with recognition sites and as such will be targets for amplification. When a recognition-mediated process is introduced into the dynamic library, the two species bearing recognition elements will be in direct competition. It is the aim of this research to first investigate this competition in isolation, and then allow it to perform within the dynamic library. Any difference between the outcome of the competition between the two recognition-enabled species in and out of the dynamic environment will be investigated.
2. Design Principles and Objectives

The reversible combination of four aldehydes and four nucleophiles leads to an exchange pool containing 16 different products. This particular pool, based on nucleophiles consisting of three amines and one hydroxylamine, generates 12 imines and four nitrones. The nitrones carry the reactive site and if two of the four aldehydes are capable of molecular recognition (blue and red squares), the resulting nitrones are therefore targets for amplification.

Over the last 10 years, the Philp laboratory has developed\(^{[57]}\) expertise in the design and synthesis of highly efficient artificial replicating systems based on molecular recognition. The course of these investigations has lead to the optimisation of several parameters key to the performance of such systems, such as the nature of the recognition event that associates A and B and the chemical reaction employed to form template T. In the following sections, the basic features of the recognition-mediated processes described within this thesis are explained. The reader may find it instructive to refer to the minimal model of self-replication presented previously (Figure 1.6).
2.2 The Recognition Event

The recognition event used to associate the building blocks A and B to form either the [A•B] or [A•B•T] complex is provided by the hydrogen bonding between a carboxylic acid and an amidopyridine moiety (Scheme 2.1). This recognition motif was first described\[58-60\] by the Hamilton group and has been successfully incorporated into a wide range of very efficient replicating species by the Philp group. Alteration of the substituents on the pyridine ring of the amidopyridine moiety has been shown to have an effect on the strength of the association and is therefore to be used to provide replicating species of differing strengths.

![Scheme 2.1](image)

Scheme 2.1. The recognition event between an amidopyridine and a carboxylic acid through hydrogen bonding. R = Me or H.

The 4,6-dimethyl amidopyridine provides stronger association with the carboxylic acid that the analogous 6-methyl amidopyridine due to the increased electron density provided by the addition donating methyl group.

The recognition event is highly sensitive to the solvent employed. Polar solvents are themselves hydrogen bond donors/acceptors and the choice of such a solvent would leave to solvation of the recognition site and disrupt the binding. As a consequence of their non-polar nature and the availability of deuterated isotopomers for NMR studies, chloroform or dichloromethane are ideal solvent choices.

2.3 The Chemical Reaction

The 1,3-dipolar cycloaddition reaction between a nitrone and a maleimide (Scheme 2.2) has long been employed by the Philp research group as a means of joining A and B together to form template T. This reaction is ideal for a number of reasons; it is irreversible and proceeds in the absence of a catalyst or inert conditions and the rate of the reaction is virtually insensitive to acid or base catalysis.
The reaction proceeds through a concerted mechanism with a well defined transition state in which the bonds between the dipole and the dipolarophile are formed simultaneously (Scheme 2.3).

![Scheme 2.2](image)

**Scheme 2.2.** The irreversible 1,3-dipolar cycloaddition reaction between a nitrone and a maleimide.

![Scheme 2.3](image)

**Scheme 2.3.** The 1,3-dipolar cycloaddition reaction between nitrone 87 and maleimide 88 proceeds through the endo-transition state (TS) to give isoxazolidine trans-89 and through the exo-transition state to give isoxazolidine cis-89. Complementary recognition sites are displayed as green and light-blue cartoon images. Favourable intramolecular recognition between the two recognition sites in the [A-B] complex of cis-89 is highlighted by the arrow.

The reaction forms two racemic diastereoisomeric products which, due to the characteristic protons attached to the isoxazolidine ring, enable the reaction to be easily followed by $^1$H NMR spectroscopy. If the reaction proceeds through the endo-transition state, the two protons on the isoxazolidine ring junction are on the opposite face to the remaining proton, and the product is denoted the trans isomer. However if the reaction proceeds via the exo-transition state, all three protons of the isoxazolidine ring are on the same face and the product is denoted the cis isomer. It is clear from **Scheme 2.3** how the open geometry of trans-89 could allow the
molecule to act as a template, whilst the closed geometry of \textit{cis-89} renders it catalytically inert. In the absence of any recognition effects, the reaction proceeds slowly through the bimolecular pathway with low selectivity, leading to a \textit{trans/cis} ratio of around 3:1.

Attaching recognition sites to the starting materials opens the recognition-mediated channels for reaction. Throughout this research, the nitrones will be equipped with the amidopyridine moiety and the maleimide will carry the carboxylic acid. The choice of maleimide governs the pathway by which the 1,3-dipolar cycloaddition will proceed. Both reactions that proceed predominantly via the [\textit{A•B}] pathway and reactions that are self-replicating in nature will be investigated as means of selection within the dynamic environment.

\textbf{2.3.1 Reaction via the [\textit{A•B}] Pathway}

When nitrone 90 is introduced to maleimide 83 bearing the acetic acid moiety in the \textit{meta} position, the reaction proceeds by the [\textit{A•B}] pathway and the closed template \textit{cis-91} is stereoselectively formed (\textbf{Scheme 2.4}). The extra flexibility of the \textit{meta} substituted acetic acid moiety allows the recognition sites to associate in the \textit{exo}-transition state and thus form the cycloadduct \textit{cis-91}.

\begin{center}
\textbf{Scheme 2.4.} Reaction between nitrone 90 and maleimide 83 proceeds via the [\textit{A•B}] pathway and forms \textit{cis-91}. Right is the calculated molecular structure of \textit{cis-91} showing the closed structure caused by intramolecular hydrogen bonds. The calculations were performed by molecular mechanics using the OPLS2005 forcefield and the GB/SA model for solvation.
\end{center}
2. Design Principles and Objectives

2.3.2 Reaction via the Self-Replication Cycle

When the same reaction is performed using maleimide 28, the reaction proceeds largely by the *endo* transition state and forms *trans*-92 at the expense of *cis*-92 (Scheme 2.5). When the acetic acid moiety is placed in the *para* position, it is unable to achieve intramolecular hydrogen bonding and reaction via the [A•B] pathway is eliminated. Due to its open geometry, *trans*-92 is able to template its own formation from 90 and 28 and once a small amount has accumulated, reaction via the self-replication cycle is possible. Systems such as these exhibit a lag period associated with need for preformed template to be present in order for the replication cycle to function. Once *trans*-92 has been formed by the uncatalysed bimolecular pathway, the cycle can begin and a significant rate enhancement is observed.

Scheme 2.5. Reaction between nitrone 90 and maleimide 28 proceeds via the self-replication cycle and forms *trans*-92. Right is the calculated molecular structure of *trans*-92 showing the open geometry, capable of assembling 90 and 28 onto itself. The calculations were performed by molecular mechanics using the OPLS2005 forcefield and the GB/SA model for solvation.
3.1 Design and Construction of the Dynamic Combinatorial Library

The proposed DCL will consist of an exchanging mixture of imines and nitrones and will be constructed from aldehydes A to D, amines W to Y and one hydroxylamine Z (Figure 3.1). The combination of these 8 starting materials leads to a product pool consisting of four nitrones and 12 imines. The level of connectedness exhibited by the system increases with the number of components present, however the analysis of such systems becomes more challenging due to the vast number of structurally similar components present. Starting with just four aldehydes and four nucleophiles leads to a total of 24 interconverting compounds, 8 starting materials and 16 condensation products, in the DCL and provides a sufficiently complex system that remains within the scope of NMR spectroscopy for analysis. The large number of components present in a sample makes analysis by $^1$H NMR spectroscopy impractical, so the majority of the starting materials and subsequently all of the possible products are equipped with a fluorine tag, either an aryl-F or a trifluoromethyl (-CF$_3$) group, in order to facilitate monitoring by $^{19}$F NMR.

Figure 3.1. Structures of the 8 starting materials from which the DCL is to be constructed. Some of these compounds have appeared previously in this thesis but have been renumbered to aid the reader’s understanding and maintain clarity throughout the following sections.
3. Manipulation of a Dynamic Library using Molecular Recognition

Of the 8 starting components, compounds C to Y were available commercially and used as supplied. The remaining two aldehydes were synthesised from commercially available carboxylic acid 93 by activation with thionyl chloride and subsequent amide coupling with the appropriate aminopicoline (Scheme 3.1).

![Scheme 3.1](image)

Scheme 3.1. Synthesis of the recognition aldehydes A and B. a) SOCl₂, toluene, 110°C. b) 2-amino-4,6-dimethylpyridine, DCM, 0°C → RT, 63% over two steps. c) 2-amino-6-methylpyridine, DCM, 0°C → RT, 70% over two steps.

The hydroxylamine Z was obtained in good yield by partial reduction of 4-fluorонitrobenzene 95 using hydrazine over a rhodium catalyst.

![Scheme 3.2](image)

Scheme 3.2. Synthesis of hydroxylamine Z. a) NH₂NH₂, Rd/C, THF, 82%.

The next task was to determine the reaction conditions that would give not only reproducible results, but also allow the exchange reactions to occur on a reasonable timescale. The proposed exchange reactions are highly sensitive to variables such as temperature, concentration of starting materials and the amount of water and acid present, so it was important that the employed reaction conditions gave reproducible results. A solvent system that contained a controlled amount of water and acid that was constant across every batch that was used was required. Deuterated DCM was chosen as it is not only less hygroscopic than chloroform, but it contains no acidic contaminants. The solvent system of choice was prepared by rapidly stirring fresh CD₂Cl₂ with para-toluenesulfonic acid monohydrate (pTSA) under an argon atmosphere for 30 mins, thus introducing a known and reproducible amount of both acid and water. The suspension was filtered and the solvent used immediately. The reactions were to be performed at a concentration of 20 mM and a temperature of 0 °C.
It is necessary to investigate how the DCL behaves firstly in the absence of any recognition process, so that any differences that are a consequence of recognition are identifiable immediately. In a typical experiment, two stock solutions, one containing an equimolar amount of each aldehyde A to D and one containing the nucleophiles W to Z, were prepared in CD$_2$Cl$_2$/pTSA containing a known amount of the 4-fluoro-nitrobenzene 95 as a reference. The two stock solutions were combined in an NMR tube and incubated at 0 °C until the exchange reactions reached dynamic equilibrium. A quantitative analysis of the sample was performed by 470 MHz $^{19}$F NMR spectroscopy. Each species present in the sample gives rise to a characteristic fluorine resonance and the area relative to the reference compound is used to determine the concentrations of each component of the mixture (Figure 3.2).

![Figure 3.2](image)

Figure 3.2. Partial 470 MHz $^{19}$F NMR spectra of the DCL after 24 hours at 0 °C. The depicted region contains only those resonances associated with Ar-F tags. The resonances from -CF$_3$ appear downfield at ~ -65 ppm and are not shown. Inset is the region corresponding to the nitrone resonances, demonstrating the resolution of the four species is sufficient for accurate deconvolution.

An early set-back concerning the use of the trifluoromethyl (-CF$_3$) group as an analytical tool was encountered at this time. The -CF$_3$ group is much less sensitive to changes in the surrounding electronic environment than the Ar-F group and, as a result, single resonances for individual compounds could not be resolved. Imines AY, BY and DY bear only the -CF$_3$ tag and as such their concentration in DCL could not be accurately monitored.
3. Manipulation of a Dynamic Library using Molecular Recognition

Scheme 3.3. Structures of the imine and nitrone products formed within the DCL, colour coded for clarity. Products containing aldehyde A are in blue, aldehyde B are in red, aldehyde C are in green, and aldehyde D are in purple.
A solution consisting of compounds A to Z at 20 mM in CD$_2$Cl$_2$/pTSA was prepared and the evolution of the system was monitored by 470 MHz $^{19}$F NMR spectroscopy every 15 minutes for a period of 24 hours at 0 °C. The equilibrium concentration of each of the compounds in the exchange pool was determined by comparison with the reference compound and is displayed in Figure 3.3.

When the system reaches equilibrium, each aldehyde is distributed amongst the various nucleophiles (Figure 3.3a). The equilibrium constant for the formation of a nitron from an aldehyde and hydroxylamine lies far to the right, greatly favouring the nitron product. Whilst it was expected that this would result in an even distribution of the four nitrones AZ to DZ, this is clearly not the case. In the absence of any recognition processes, the hydroxylamine Z is incorporated more readily into recognition nitrones AZ and BZ than it is into non-recognition nitrones CZ and DZ ([AZ] = 7.08 mM, [BZ] = 7.51 mM, [CZ] = 2.01 mM, [DZ] = 2.38 mM). This distribution is a consequence of the design process of the recognition aldehydes themselves. The amide functional group present in the two aldehydes A and B is strongly electron-withdrawing which causes the aldehyde group to be electron deficient and subsequently open to attack by suitably nucleophilic species, as required for the synthesis of nitrones such AZ and BZ. Deconvolution of the $^{19}$F NMR data recorded on a sample allows for the construction of concentration vs. time profiles for the hydroxylamine Z and the four nitrones AZ to DZ (Figure 3.3b). There is a clear difference between the observed concentrations of the two classes of nitrone.
3.2 Analysis of the Irreversible Reaction

The library described thus far has been designed specifically to respond, with a change in composition, to irreversible recognition-mediated reactions. There are two distinct features within the DCL, a reactive site and a recognition site that are exchanging between compounds. As previously described, the recognition site is provided by an amidopyridine group whilst the reactive site is provided by the nitrone functionality. Of the 16 products in the exchange pool, only four are nitrones, and of these four, only two bear a recognition site. It is these two compounds, \textbf{AZ} and \textbf{BZ}, that can react selectively with a maleimide in a recognition-mediated 1,3-dipolar cycloaddition and are therefore the targets for amplification. The choice of maleimide will direct the DCL to respond in a certain manner. In order to probe these responses, a number of maleimides were synthesised.

![Scheme 3.4](image)

Maleimide \textbf{83}, bearing a carboxylic acid recognition site, was obtained by treatment of commercially available amine \textbf{96} with maleic anhydride \textbf{97} in acetic acid to firstly yield the intermediate amide \textbf{98}. This amide \textbf{98} was immediately cyclised by hexamethyldisilazane (HMDS) in the presence of zinc bromide to yield to target compound \textbf{83}. Recognition-disabled maleimide \textbf{99} was obtained by subsequent methylation of \textbf{83} by methyl iodide with caesium carbonate. The methyl ester \textbf{99} is incapable of the hydrogen bonding required for recognition and can only react by the bimolecular pathway. The contribution of the bimolecular pathway to the recognition-mediated reaction can be estimated by observing the nitrones reaction with recognition-disabled maleimide \textbf{99}. 
The recognition mediated reaction chosen to induce a change in the DCL proceeds by the \([A•B]\) pathway outlined earlier (Figure 3.4). The nitrones AZ and BZ and maleimide 83 bear complementary recognition sites that allow them to pre-organise themselves, bringing the reactive sites closer together and dramatically increasing the rate of reaction. In these systems, it is the cis isomer that is up-regulated and as such is expected to dominate the reaction mixture.

![Figure 3.4](image)

The minimal model of replication. The reaction to be used for amplification proceeds by the \([A•B]\) pathway highlighted.

In order to test the selectivity of such reactions, both nitrones AZ and BZ were synthesised and their reactions with maleimides 83 and 99 was assessed. The nitrones were easily synthesised from previously prepared aldehydes A and B and the hydroxylamine Z in good yield.

![Scheme 3.5](image)

Synthesis nitrones AZ and BZ from aldehydes A and B. a) Z, EtOH, 64%. b) Z, EtOH, 60%.

A sample containing the nitrone to be assessed and maleimide 83 or 99 at 20 mM was prepared in CD$_2$Cl$_2$/pTSA and the evolution of any products was followed by 500 MHz $^1$H NMR spectroscopy at 0 °C.
3. Manipulation of a Dynamic Library using Molecular Recognition

After 16 hours, the reaction of AZ with the recognition-disabled maleimide 99 showed just 18% conversion into 100 and the \textit{trans}:\textit{cis} ratio was observed at 2.3:1. When the same reaction was monitored this time with the recognition-enabled maleimide 83, conversion after 16 hours rose to 95% with a diastereoisomeric ratio of 48:1 in favour of \textit{cis}-101. Deconvolution of the data recorded on a sample allows for the construction of concentration vs. time profiles depicted in Figure 3.5.

![Scheme 3.6](image)

\textbf{Scheme 3.6.} The 1,3-dipolar cycloaddition between nitrone AZ and maleimides 99 or 83 gives rise to two diastereoisomeric products, \textit{trans}-100 and \textit{cis}-100, or \textit{trans}-101 and \textit{cis}-101.

![Figure 3.5](image)

\textbf{Figure 3.5.} Concentration vs. time plots of the reaction of a) recognition-disabled maleimide 99 and nitrone AZ ([AZ] = [99] = 20 mM) forming products \textit{trans}-100 (●) and \textit{cis}-100 (●), b) recognition-enabled maleimide 83 and nitrone AZ ([AZ] = [83] = 20 mM) forming products \textit{trans}-101 (●) and \textit{cis}-101 (●). The solid lines represent the results of the kinetic fitting process.

\begin{table}
\centering
\begin{tabular}{|l|l|l|}
\hline
 & \textit{trans}-101 & \textit{cis}-101 \\
\hline
\text{bimolecular rate constant / M}^{-1} \text{ s}^{-1} & 1.24 \times 10^{-4} & 5.57 \times 10^{-5} \\
\text{recognition-mediated rate constant / s}^{-1} & - & 1.24 \times 10^{-4} \\
\text{effective molarity / M} & - & 2.23 \\
\text{R-value / %} & - & 3.34 \\
\hline
\end{tabular}
\caption{Table 3.1. Rate constants determined by kinetic simulation and fitting of experimental data using the SimFit program.}
\end{table}
The reaction profile was subjected to simulation and fitting to a model for a bimolecular reaction using the SimFit software package. The relevant rate constants are displayed in Table 3.1.

When the analogous reactions were performed using nitrone BZ, conversion was 20% with 2.2:1 selectivity for trans-102 for the recognition-disabled reaction and 91% with 42:1 selectivity for cis-85 in the recognition-enabled process. Deconvolution of the data recorded on a sample allows for the construction of concentration vs. time profiles depicted in Figure 3.6, with the extracted rate constants displayed in Table 3.2.

<table>
<thead>
<tr>
<th>trans-85</th>
<th>cis-85</th>
</tr>
</thead>
<tbody>
<tr>
<td>bimolecular rate constant / M⁻¹ s⁻¹</td>
<td>1.30 x 10⁻⁴</td>
</tr>
<tr>
<td>recognition-mediated rate constant / s⁻¹</td>
<td>-</td>
</tr>
<tr>
<td>effective molarity / M</td>
<td>-</td>
</tr>
<tr>
<td>R-value / %</td>
<td>-</td>
</tr>
</tbody>
</table>
3.3 Kinetic Selection

Two of the four nitrones present within the library, \textbf{AZ} and \textbf{BZ}, are equipped with amidopyridine groups capable of providing molecular recognition through hydrogen bonding. These two nitrones are the targets for amplification and are expected to be amplified over the non-recognition nitrones \textbf{CZ} and \textbf{DZ}. Whilst both \textbf{AZ} and \textbf{BZ} are capable of molecular recognition, the magnitude of the resulting amplification is not identical for both species. The differences in the electronic environment of the two pyridine moieties of the nitrones causes the strength of the hydrogen bonds formed with the carboxylic acid on \textbf{83} to differ. The additional methyl group on \textbf{AZ} donates electrons into the pyridine system, increasing the strength of the hydrogen bond between the pyridine nitrogen and acidic proton of the carboxylic acid. The association constants between the two different amidopyridine moieties and a carboxylic acid have been measured at 0 °C within the group and are shown in Table 3.3.

Table 3.3. Determination of the association constants between the carboxylic acid of \textbf{83} and the different amidopyridine moieties of nitrones \textbf{AZ} and \textbf{BZ} at 0 °C.

<table>
<thead>
<tr>
<th>Association constant</th>
<th>(K_a / \text{M}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>\includegraphics[width=0.1\textwidth]{6-methyl_amidopyridine}</td>
<td>6-methyl amidopyridine</td>
</tr>
<tr>
<td>\includegraphics[width=0.1\textwidth]{4,6-dimethyl_amidopyridine}</td>
<td>4,6-dimethyl amidopyridine</td>
</tr>
</tbody>
</table>

During the envisaged DCL experiments, the two recognition mediated processes will be competing against one another for a limited supply of the maleimide. In order to ascertain any differences in the outcome of this competition when it performs in a dynamic environment, it was first necessary to observe this competition in isolation. To rule out different reactivities of the two nitrones as a selection process, the competition for the recognition-disabled maleimide \textbf{99} was first assessed. Both nitrones \textbf{AZ} and \textbf{BZ} were combined with one equivalent of maleimide \textbf{99} ([\textbf{AZ}] = [\textbf{BZ}] = [\textbf{99}] = 20 \text{ mM}) in CD\textsubscript{2}Cl\textsubscript{2}/pTSA at 0 °C and the evolution of cycloadducts was followed by 470 MHz \textsuperscript{19}F NMR spectroscopy.
Deconvolution of the data recorded on a sample allowed for the construction of the concentration vs. time profile for the competition process (Figure 3.7).

![Figure 3.7](image)

As expected, the recognition-disabled process is slow and generates no selectivity during the monitoring window, reaching just 19% conversion after 16 hours. The bimolecular reactions for the two nitrones proceed with almost identical rates, forming trans-100 and trans-102 at concentrations of 1.30 mM and 1.35 mM respectively. The trans isomers are formed over the cis isomers at a ratio of 2.4:1, with both cis-100 and cis-102 reaching concentrations of just 0.57 mM after 16 hours. It is clear from these results that the two nitrones AZ and BZ possess very similar reactivity towards the recognition-disabled maleimide 99 and any observed selectivity is due to the differing strengths of molecular recognition.

When the competition is repeated using recognition-enabled maleimide 83 in place of recognition-disabled maleimide 99, a clear difference in formation is observed. Both nitrones AZ and BZ were combined with one equivalent of maleimide 83 ([AZ] = [BZ] = [83] = 20 mM) in CD$_2$Cl$_2$/pTSA at 0 °C and the evolution of cycloadducts was followed by 470 MHz $^{19}$F NMR spectroscopy. Deconvolution of the data recorded on a sample allowed for the construction of the concentration vs. time profile for the competition process (Figure 3.8). With both nitrones competing for the limited maleimide, the reaction proceeded much faster, reaching completion in around 11 hours. As expected, cis-101 and cis-85 dominated the reaction mixture at 10.45 mM and 8.85 mM respectively, and trans-101 and trans-85 were only formed to a minor extent (0.34 mM and 0.33 mM respectively). The total conversion was 100% and the cis isomers dominated the trans isomers at a ratio of 29:1.
The relative strengths of the two recognition processes becomes apparent during the competition for a limited supply of maleimide 83. The higher association constant for the 4,6-dimethyl nitrone AZ results in a higher concentration of the binary complex [AZ·83] over [BZ·83] in solution. The results of the kinetic fitting in Figures 3.5 and 3.6 reveal the two recognition-mediated processes have comparable effective molarities (EM), thus ruling out differences in reactivity for the observed selection. It is the formation of this binary complex that causes the rate enhancement and thus the affinity of the maleimide for one nitrone over the other causes the uneven distribution of recognition cycloadducts cis-101 and cis-85.

It is envisaged that the outcome of this competition will be altered when it is performing within a dynamic environment. It is important to realise that at the start of the reaction depicted in Figure 3.8, both nitrones are present at 20 mM. In the dynamic library, all nitrones start at 0 mM and must be formed by the exchange reactions before they can react with the maleimide. As the exchange reactions are equilibrium processes, it is hoped that the presence of the maleimide can selectively pull material from the exchange pool into the recognition cycloadducts and amplify the difference in formation between cis-101 and cis-85.
3.4 Coupling the DCL with a Recognition-Disabled Process

The DCL was first introduced to recognition-disabled maleimide 99 to see if simply allowing an irreversible reaction to perform within the exchange pool generated any significant changes in the composition. A sample containing each of the 8 starting materials A to Z and the recognition-disabled maleimide 99 ([A to Z] = [99] = 20 mM) was prepared in CD$_2$Cl$_2$/pTSA and its composition was allowed to evolve at 0 °C. The composition of the sample was followed by 470 MHz $^{19}$F NMR spectroscopy every 30 minutes for a period of 36 hours, with the concentration of each species bearing a fluorine tag determined by comparison with the internal reference, 4-fluoronitrobenzene 95.

As expected, the composition of the exchange pool is largely unaffected by the slow and unselective recognition-disabled cycloaddition reactions (Figure 3.9a). Despite the low conversion, there are significant differences in the reaction profiles of the exchange only and the recognition-disabled experiment with maleimide 99. In the recognition-disabled experiment the hydroxylamine Z is consumed faster than during the isolated formation of the DCL (Figure 3.3b). This is explained by its incorporation into not only the four nitrones but also the cycloadducts 100, 102, 103 and 104 in the

Figure 3.9. a) Bar chart showing the concentrations of each of the imine/nitrone products present in the DCL after 36 hours at 0 °C in the presence of recognition-disabled maleimide 99. b) Concentration vs. time plot following the consumption of the hydroxylamine Z (●) and the evolution of the four reactive nitrones AZ (●), BZ (●), CZ (●) and DZ (●).
The recognition nitrones \( AZ \) and \( BZ \) are both formed rapidly, reaching a peak concentration of 6.50 mM at around 10 hours into the reaction before the rate of the cycloaddition reaction becomes the faster step and the concentrations of \( AZ \) and \( BZ \) begin to fall uniformly for the remainder of the monitoring window, reaching a concentration of 5.53 mM and 5.32 mM respectively after 36 hours.

**Scheme 3.8.** Structure and \(^{19}\text{F} \) NMR chemical shifts of the aromatic fluorine resonance of the cycloadducts within the recognition-disabled product pool.
product pool. The recognition nitrones AZ and BZ are both formed rapidly, reaching a peak concentration of 6.50 mM at around 10 hours into the reaction before the rate of the cycloaddition reaction becomes the faster step and the concentrations of AZ and BZ begin to fall uniformly for the remainder of the monitoring window, reaching a concentration of 5.53 mM and 5.32 mM respectively after 36 hours.

After 36 hours, the overall conversion for all of the cycloaddition reactions was only 22%, with just 4.24 mM of the hydroxylamine Z transferred unselectively into the product pool (Figure 3.10). The recognition cycloadducts 100 and 102 are present at concentrations of 1.80 mM and 1.78 mM respectively with trans:cis ratios of 2.8:1 in both cases, while the two non-recognition cycloadducts 103 and 104 were formed to a lesser extent ([103] = 0.28 mM, trans:cis = 3:1, [104] = 0.38 mM, trans:cis = 3:1). This discrepancy in the recognition-disabled cycloadduct distribution is a result of the greater concentrations of the recognition nitrones AZ and BZ compared to nitrones CZ and DZ.

Figure 3.10.  a) Bar chart showing the concentrations of each of the cycloadducts present in the product pool after 36 hours at 0 °C in the presence of recognition-disabled maleimide 99. b) Concentration vs. time plot following the evolution of the product pool on reaction of nitrones AZ, BZ, CZ and DZ with recognition-disabled maleimide 99 generating trans-100 (●), trans-102 (●), trans-103 (◆), trans-104 (◆), cis-100 (◆), cis-102 (◆), cis-103 (◆), and cis-104 (◆).
Partial 470 MHz $^1$F NMR spectra of the evolution of nitrones AZ to DZ, hydroxylamine Z and the trans cycloadducts in the DCL in the presence of recognition-disabled maleimide 99 at 0 °C at various time points. Note the rapid consumption of Z and appearance of AZ to DZ and the much slower formation of trans cycloadducts.

**Figure 3.11.** Partial 470 MHz $^1$F NMR spectra of the evolution of nitrones AZ to DZ, hydroxylamine Z and the trans cycloadducts in the DCL in the presence of recognition-disabled maleimide 99 at 0 °C at various time points. Note the rapid consumption of Z and appearance of AZ to DZ and the much slower formation of trans cycloadducts.
3.5 Coupling the DCL with a Recognition-Mediated Process

It was clear that simply introducing an irreversible reaction to the DCL generated no selectivity due to the similar reactivity of the four nitrones with recognition-disabled maleimide 99. In order to demonstrate the effect that a recognition-mediated process can have on an exchanging DCL, a similar experiment was conducted using the recognition-enabled maleimide 83 in place of maleimide 99. The carboxylic acid functionality of 83 is capable of hydrogen-bonding with the amidopyridine moieties present on nitrones AZ and BZ and it is envisaged that the demonstrated selectivity (Figure 3.5 and 3.6) can be transferred to the DCL.

A sample containing each of the 8 starting materials A to Z and maleimide 83 ([A to Z] = [83] = 20 mM) was prepared in CD_2Cl_2/pTSA and its composition was allowed to evolve at 0 °C. The composition of the sample was followed by 470 MHz \(^{19}\)F NMR spectroscopy for a period of 36 hours, with the concentration of each species bearing a fluorine tag determined by comparison with the internal standard, 4-fluoro-nitrobenzene 95.

The effect of the introduction of a recognition-mediated process is immediately noticeable within the exchange pool (Figure 3.12a). The concentration of the four nitrones is dramatically decreased when compared with the exchange in the absence of any maleimide (Figure 3.3). By the end of the 36 hour monitoring window,
3. Manipulation of a Dynamic Library using Molecular Recognition

Scheme 3.9. Structure and $^{19}$F NMR chemical shifts of the aromatic fluorine resonance of the cycloadducts within the recognition-enabled product pool.
recognition nitrones AZ and BZ are present at concentrations of just 0.41 mM and 0.59 mM respectively, while non-recognition nitrones CZ and DZ are present at 0.45 mM and 0.75 mM respectively. Deconvolution of the $^{19}$F NMR data recorded on a sample allows for the construction of concentration vs. time profiles for the hydroxylamine Z and the four nitrones AZ to DZ (Figure 3.12b). The hydroxylamine Z is very rapidly consumed, reaching a minimal concentration in around 7 hours. This is a consequence of its rapid reaction with the aldehydes A and B and their subsequent reaction with maleimide 83. There are a number of efficient processes competing for the limited supply of Z so it is of little surprise that it becomes exhausted quickly. In this experiment, the nitrones AZ and BZ reach their peak concentrations ([AZ] = 5.18 mM, [BZ] = 5.50 mM) in just 2.5 hours before the cycloaddition reaction becomes the faster process, whilst the nitrones CZ and DZ, consumed by a far less efficient process, peak ([CZ] = 1.46 mM, [DZ] = 1.63 mM) later at around 4 hours.

The recognition-mediated process is a much more efficient means of transferring material from the exchange pool into the product pool (Figure 3.13a). In the product pool, after 36 hours, cycloadducts 105 and 106, products from the reactions of nitrones CZ and DZ are present at concentrations of 0.17 mM and 0.19 mM.
respectively \([\text{trans-105}]:[\text{cis-105}] = 3:1\), \([\text{trans-106}]:[\text{cis-106}] = 3:1\). After the same time, cycloadducts \textbf{101} and \textbf{85}, arising from the recognition-mediated reactions of nitrones \textbf{AZ} and \textbf{BZ}, are present at concentrations of 9.36 mM \([\text{trans-101}]:[\text{cis-101}] = 1:25\) and 8.44 mM \([\text{trans-85}]:[\text{cis-85}] = 1:26\). The overall conversion of all nitrones within the library is now 91% with cycloadducts \textit{cis-101} and \textit{cis-85} accounting for 94% of this figure.

The profile for the formation of the cycloadducts (\textbf{Figure 3.13b}) clearly demonstrates the dominance of \textit{cis-101} and \textit{cis-85} over their non-recognition enabled competitors. It is interesting to note the appearance of a lag period, normally associated with the behaviour of self-replicating species, between the start of the reaction and the formation of the cycloadducts. This is not an indication of the self-replicating behaviour of these cycloadducts but merely a result of there being no nitrones present at the start of the reaction to react with maleimide \textbf{83}. Once the nitrones are formed to a sufficient concentration (~3 mM), the recognition-mediated process can begin.
Partial 470 MHz $^{19}F$ NMR spectra of the evolution of nitrones $AZ$ to $DZ$, hydroxylamine $Z$ and cycloadducts in the DCL in the presence of recognition-enabled maleimide 83 at 0 °C at various time points. Note the rapid consumption of $Z$ and appearance of $AZ$ to $DZ$ as well as the formation of $cis$-101 and $cis$-85.
3.6 Comparison with Kinetic Selection

In the isolated scenario in which nitrones AZ and BZ are competing for recognition-enabled maleimide 83, it was shown that the relative strengths of the recognition units play a part in the affinity for the maleimide. It was shown that nitrone AZ, equipped with the stronger amidopyridine recognition unit, is able to associate and react with 83 to a greater extent than nitrone BZ can. The final distribution of cycloadducts in this experiment was ~1.2:1 in favour of cis-101. It was hoped that when this competition was transferred to the dynamic environment the slight imbalance between cycloadducts cis-101 and cis-85 would be amplified and the system could be driven to the exclusive formation of cis-101. Frustratingly, the formation of the two cycloadducts cis-101 and cis-85 proceeds with similar selectivity as observed in the isolated kinetic selection experiments (Figure 3.15).

![Figure 3.15](image)

Figure 3.15. Pie chart representation of the relative concentrations of cis-101 (BLUE) and cis-85 (RED) as the outcome of the competition for maleimide 83 in a) the isolated kinetic experiment and b) the dynamic library. Even when the competition is coupled to the DCL, the system is unable to depart from kinetic selection.

Within the DCL, the recognition nitrones AZ and BZ must first be formed through the exchange process between the relevant aldehyde and hydroxylamine Z. If the exchange process is slower than the recognition-mediated cycloaddition reactions, a concentration of nitrones does not build up; instead the nitrones are immediately consumed by reaction with maleimide 83. This can be thought of as the cycloaddition reaction ‘pulling’ the hydroxylamine Z into the product pool via one of the reactive nitrones. As a consequence of the higher association constant of nitrone AZ than nitrone BZ with maleimide 83, it was expected that nitrone AZ could start reacting via the [A•B] pathway at a lower concentration than nitrone BZ could. In this scenario, only the dimethyl cycloadduct cis-101 would be formed through the recognition-mediated reaction and the selectivity over the weaker associating cis-85 would be enhanced.
3.7 Stacking the Deck: A Non-Biased Exchange Pool

A possible explanation for this lack of selectivity can be traced back to the composition of the exchange pool in the absence of any irreversible reactions (Figure 3.3). It was expected that the four aldehydes A to D would react to the same extent with the hydroxylamine Z leading to an even distribution of the four nitrones. Surprisingly, the recognition aldehydes A and B demonstrated an increased reactivity towards hydroxylamine Z. This resulted in a library that was already biased towards the formation of the recognition cycloadducts cis-101 and cis-85 before any recognition-mediated processes had the chance to operate. Different behaviour is expected if the recognition nitrones AZ and BZ are not given a head start and are in fact minor components within the exchange pool.

In an attempt to overcome the increased reactivity of the aldehydes A and B, the DCL was reconstructed using an excess of the non-recognition aldehydes C and D. Whilst the concentrations of all other compounds were kept at 20 mM, the concentrations of aldehydes C and D were increased to 50 mM. Increasing the concentration further would likely result in an increase of nitrones CZ and DZ but the solubility of the starting materials in CD$_2$Cl$_2$/pTSA became problematic at concentrations higher than 50 mM. A sample containing compounds A to Z was prepared ([A] = [B] = [W to Z] = 20 mM, [C] = [D] = 50 mM) and allowed to react at 0 °C. After a period of five days, the concentration of each species present was determined by 282 MHz $^{19}$F NMR spectroscopy and comparison with the internal reference 95 (Figure 3.16).

![Figure 3.16](image-url)  
Bar chart showing the concentrations of each of the imine/nitrone products present in the reconstructed DCL after five days at 0 °C.
3. Manipulation of a Dynamic Library using Molecular Recognition

It is immediately noticeable that the distribution of the hydroxylamine Z is vastly different to the situation previously presented (Figure 3.3). The recognition nitrones AZ and BZ no longer dominate over the non-recognition nitrones CZ and DZ, and all four are present comparable concentrations ([AZ] = 4.83 mM, [BZ] = 5.69 mM, [CZ] = 5.25 mM, [DZ] = 4.00 mM). The recognition nitrones AZ and BZ are still formed to greater extent ([AZ + BZ] = 10.52 mM, [CZ + DZ] = 9.25 mM) but the difference between them is far smaller. The same distribution is also observed amongst all compounds based on amines W and X resulting in a dynamic library with an almost even distribution of components.

A sample of the new library was then prepared in the presence of recognition-disabled maleimide 99 to demonstrate how the system responds to a simple irreversible reaction ([A] = [B] = [W to Z] = [99] = 20 mM, [C] = [D] = 50 mM). Figure 3.17 shows the expected result that the introduction of a recognition-disabled process does not induce any selectivity within either the exchange pool or the product pool after five days at 0 °C.

![Figure 3.17](image_url)

**Figure 3.17.** Bar chart showing the concentrations of each of the imine/nitrone products present in the DCL and the distribution of the cycloadducts in the product pool after five days at 0 °C in the presence of recognition-disabled maleimide 99. Even after five days, there is only a small amount of material transferred into the product pool and very low selectivity within it.
After five days at 0 °C, the overall conversion for all the cycloaddition reactions was 47% with 9.31 mM of the hydroxylamine Z transferred unselectively into the product pool. Interestingly, although the four nitrones AZ to DZ are formed to comparable extents during the formation of the DCL in isolation, there is a discrepancy in the concentrations of the resulting cycloadducts. The recognition cycloadducts 100 and 102 are present at concentrations of 2.78 mM and 3.05 mM with trans:cis ratios of 2.4:1 and 2.3:1 respectively, while the two non-recognition cycloadducts 103 and 104 were formed to a lesser extent ([103] = 1.96 mM, trans:cis = 2.4:1, [104] = 1.52 mM, trans:cis = 3.3:1). This difference means that the discrepancy observed in Figure 3.10 was not only due to the distribution of the hydroxylamine Z amongst the aldehydes but also due to the slightly different reactivity of the resulting nitrones.

When recognition-disabled maleimide 99 is replaced with the recognition-enabled maleimide 83, the two competing recognition-mediated reactions are able to operate. A sample of the new library was prepared in the presence of maleimide 83 ([A] = [B] = [W to Z] = [83] = 20 mM, [C] = [D] = 50 mM) and allowed to react for five days at 0 °C. Figure 3.18 shows the expected result that the introduction of a recognition-enabled process affects the composition of both the exchange pool and the product pool dramatically.

![Figure 3.18](image-url)

**Figure 3.18.** Bar chart showing the concentrations of each of the imine/nitrone products present in the DCL and the distribution of the cycloadducts in the product pool after five days at 0 °C in the presence of maleimide 83. A significant amount of material is transferred into the product pool and is selectively incorporated into cis-101 and cis-85.
Once again, the two recognition-mediated processes outperform the slower bimolecular reactions leading to the selective formation of cycloadducts cis-101 and cis-85 at the expense of the remaining cycloadducts. Despite the reconstruction of the dynamic library to afford an equal distribution of nitrones, the outcome of the competition between the two recognition processes remains largely unchanged. After complete conversion of maleimide 83, cis-101 had been formed to a concentration of 9.39 mM and cis-85 to 8.59 mM resulting in a ratio of ~1.1:1. The outcome of the competition is once again determined by kinetic selection and the ratio is the same as both those observed in isolation and within the previously described biased library (Figure 3.15).

3.8 Redesigning the Library

Although increasing the concentrations of aldehydes C and D was shown to have a positive effect on the distribution of the nitrones AZ to DZ, it was not enough to break the stranglehold of kinetic selection and allow cis-101 to dominate over cis-85. The library allowed nitrones CZ and DZ to reach comparable concentrations with recognition nitrones AZ and BZ but they were still the minor components in the exchange pool. With larger concentrations of recognition nitrones AZ and BZ present in solution, both recognition-mediated processes can perform and no selectivity is observed between the resulting cycloadducts. A system in which the two nitrones AZ and BZ are present at lower concentrations may allow for the differing strengths of the recognition sites to play a part.

Scheme 3.10. Structure and $^19$F NMR chemical shifts of the aldehydes and nitrones to make up the screen for the new library.
In an effort to find such a system, a number of different four-nitroline libraries were constructed from combinations of four aldehydes (E to H) and hydroxylamine Z (Scheme 3.10). Stock solutions of each aldehyde were prepared in CD$_2$Cl$_2$/pTSA, combined as required with hydroxylamine Z and incubated at 0 °C for a period of five days. Analysis was performed by 282 MHz $^{19}$F NMR spectroscopy with the distribution of nitrones shown in Figure 3.19.

**Figure 3.19.** Screening of nitroline libraries constructed from A and B and combinations of aldehydes E to H with hydroxylamine Z. The distribution of the nitroline products is presented in the pie charts.
As a consequence of the observed inefficiency of aldehydes C and D in competition for the hydroxylamine Z, it was thought that decorating the aldehyde with an electron-withdrawing substituent such as the trifluoromethyl (-CF$_3$) group in D, made the resulting nitrone more susceptible to hydrolysis. The -CF$_3$ group draws electrons away from the carbon atom directly adjacent to a positively charged nitrogen atom. It is this carbon that is attacked by a water molecule during hydrolysis so it is reasonable to assume that the likeness of the two adjacent charges contributes to an increased rate of hydrolysis. For this reason, a number of ‘mild’ aldehydes were screened as possible replacements for C and D in the dynamic library.

Through the various combinations of aldehydes E to H, 6 alternative libraries were constructed (Figure 3.19). In each of the 6 new libraries, the recognition nitrones AZ and BZ dominated the pool of nitrones to various extents. In Library Two, when aldehydes E and G were introduced, the ratio of recognition nitrones to non-recognition nitrones at equilibrium was ~3:1, whilst in Library Five, containing aldehydes F and H, the ratio fell to ~2:1.

Whilst none of the new libraries produced scenarios in which the recognition aldehydes were minor components, the results nevertheless uncovered a clear trend in the reactivity of the aldehydes towards hydroxylamine Z (Figure 3.20). The observed trend is in accordance with the Hammett sigma parameters for the various substituents on the aromatic ring.[62, 63]

![Figure 3.20](image_url)  
**Figure 3.20.** Reactivity trend for the aldehydes E to G with hydroxylamine Z. The observed trend is in accordance with the Hammett sigma parameters. A chlorine atom placed in the *meta* position is the most electron-withdrawing and renders the aldehydes more electrophilic to the incoming nucleophile Z. Aliphatic substituents such as those in E and G are electron donating and thus make the aldehyde less electrophilic and therefore less susceptible to nucleophilic attack.
Aliphatic substituents such as those in E and G are electron donating and therefore have negative sigma parameters ($\sigma_p$ (-Me) = -0.17, $\sigma_p$ (-Bu) = -0.20). The electron-donating has the effect of reducing the electrophilicity of the carbonyl in the aldehyde, thus reducing the reactivity towards nucleophiles such as Z. Halogens such as those present on aldehydes F and H are electron-withdrawing and thus reduce the electron density around the carbonyl of the aldehyde, making it more susceptible to nucleophilic attack from Z. The increased reactivity of F over H can be explained by the higher sigma parameter for a meta substituted chlorine atom than a para substituted bromine ($\sigma_m$ (-Cl) = 0.37, $\sigma_p$ (-Br) = 0.23). The poor performance of aldehyde C can also be explained by the comparably weak electron-withdrawing effect of a para substituted fluorine atom $\sigma_p$ (-F) = 0.06). As a result of this observed trend, aldehydes I and J bearing strongly electron-withdrawing groups were considered (Scheme 3.11).

![Scheme 3.11. Structure and $^{19}$F NMR chemical shifts of the aldehydes I and J and the subsequent nitrones formed upon reaction with hydroxylamine Z](image)

The Hammett sigma parameters of para substituted nitro ($\sigma_p$ (-NO$_2$) = 0.78) and nitrile ($\sigma_p$ (-CN) = 0.66) groups suggest strong electron-withdrawing capabilities and should make the aldehydes I and J open to attack by the hydroxylamine Z.

![Figure 3.21. A nitrone library constructed from the reaction of recognition aldehydes A and B and non-recognition aldehydes I and J with hydroxylamine Z.](image)
A 7th library was constructed from aldehydes \( A, B, I \) and \( J \) and allowed to react at \( 0 \) °C (Figure 3.21). At equilibrium, the ratio between the recognition nitrones \( AZ \) and \( BZ \) and the non-recognition nitrones \( IZ \) and \( JZ \) was only \(~1.5:1\). The recognition nitrones still out compete their non-recognition counterparts but the difference between the two is much smaller.

*Library Seven* demonstrated that aldehydes \( I \) and \( J \) were closest in reactivity to recognition aldehydes \( A \) and \( B \) and were therefore chosen as replacements for \( C \) and \( D \) in the construction of a new dynamic library (*Scheme 3.12*). A sample containing each of the components ([\( A \]) = [\( B \]) = [\( W \) to \( Z \]) = [\( I \]) = [\( J \]) = 20 mM) was prepared and allowed to react at \( 0 \) °C until the system reached equilibrium. The concentration of each species present was determined by \(^{19}\)F NMR spectroscopy and comparison with the internal reference 95 (Figure 3.22).

![Figure 3.22. Bar chart showing the concentrations of each of the imine/nitrone products present in the reconstructed DCL after five days at 0 °C.](image)

At equilibrium, the hydroxylamine \( Z \) is distributed amongst the aldehydes \( A, B, I \) and \( J \) slightly more evenly than it was amongst the aldehydes \( A \) to \( D \) in the original library. As in the previously described system (Figure 3.3), the recognition-enabled nitrones \( AZ \) and \( BZ \) react fastest with \( Z \) and reach a total concentration of 12.57 mM ([\( AZ \]) = 5.96 mM, [\( BZ \]) = 6.61 mM) while non-recognition nitrones \( IZ \) and \( JZ \) are present at a total of 6.50 mM ([\( IZ \]) = 3.33 mM, [\( JZ \]) = 3.17 mM).
Scheme 3.12. Structures of the imine and nitrone products formed within the DCL, colour coded for clarity. Products containing aldehyde A are in blue, aldehyde B are in red, aldehyde I are in green, and aldehyde J are in purple.
3. Manipulation of a Dynamic Library using Molecular Recognition

The difference in formation of the two classes of nitrones is less pronounced in the new library with 66% of the hydroxylamine $Z$ incorporated into $AZ$ or $BZ$, compared with the 78% observed in the original library. This difference represents a decrease in the dominance of recognition nitrones $AZ$ and $BZ$ but the library remains biased towards the recognition processes.

Previously, we demonstrated that increasing the concentration of the non-recognition aldehydes resulted in a further decrease of the dominance of nitrones $AZ$ and $BZ$ (Figure 3.16). Before this principle could be applied to the new library, it was important to demonstrate that the new aldehydes $I$ and $J$ did not cause the library to exhibit any unexpected characteristics. A sample of the new library was prepared in the presence of recognition-disabled maleimide $99$ ([A] = [B] = [W to Z] = [I] = [J] = [99] = 20 mM) to demonstrate how the system responds to a simple irreversible reaction. Figure 3.23 shows the expected result that the introduction of a recognition-disabled process does not induce any selectivity within either exchange pool or the product pool after five days at 0 °C.

![Figure 3.23](image)

**Figure 3.23.** Bar chart showing the concentrations of each of the imine/nitrone products present in the DCL and the distribution of the cycloadducts in the product pool after five days at 0 °C in the presence of recognition-disabled maleimide $99$. Even after five days, there is only a small amount of material transferred into the product pool and very low selectivity within it.
Scheme 3.13. Structure and $^{19}$F NMR chemical shifts of the aromatic fluorine resonance of the cycloadducts within the recognition-disabled product pool.
After five days at 0 °C, the overall conversion for all the cycloaddition reactions was 38% with 7.61 mM of the hydroxylamine Z transferred unselectively into the product pool. As a consequence of the uneven formation of nitrones AZ, BZ, IZ and JZ and the possibly higher reactivity of AZ and BZ towards maleimide 99, cycloadducts 100 ([trans-100] = 2.06 mM, [cis-100] = 0.92 mM) and 102 ([trans-102] = 2.18 mM, [cis-102] = 0.92 mM) are present at higher concentrations than cycloadducts 107 ([trans-107] = 0.54 mM, [cis-107] = 0.18 mM) and 108 ([trans-108] = 0.57 mM, [cis-108] = 0.24 mM).

When recognition-disabled maleimide 99 is replaced with the recognition-enabled maleimide 83, the two competing recognition-mediated reactions are able to operate. A sample of the new library was prepared in the presence of maleimide 83 ([A] = [B] = [W to Z] = [I] = [J] = [83] = 20 mM) and allowed to react for five days at 0 °C. Figure 3.24 shows the expected result that the introduction of a recognition-enabled process dramatically affects the composition of both the exchange pool and the product pool.

Figure 3.24. Bar chart showing the concentrations of each of the imine/nitrone products present in the DCL and the distribution of the cycloadducts in the product pool after five days at 0 °C in the presence of maleimide 83. A significant amount of material is transferred into the product pool and is selectively incorporated into cis-101 and cis-85.
Once again, the two recognition-mediated processes outperform the slower bimolecular reactions leading to the selective formation of cycloadducts cis-\text{xx} and cis-\text{xx} at the expense of the remaining cycloadducts.

Scheme 3.14. Structure and \textsuperscript{19}F NMR chemical shifts of the aromatic fluorine resonance of the cycloadducts within the recognition-enabled product pool.
Similar to the system presented in Figure 3.18, the outcome of the competition between the two recognition processes remains largely unchanged. After five days, conversion of maleimide 83 was 86%, \( \text{cis-101} \) had been formed to 8.15 mM and \( \text{cis-85} \) to 7.68 mM resulting in the familiar ratio of ~1.1:1.

The reconstructed library containing aldehydes \( I \) and \( J \) has been shown to provide a more even distribution of nitrones than the library containing \( C \) and \( D \) first described in Figure 3.3. Both of these libraries were constructed from aldehydes present in solution at a concentration of 20 mM. It was shown that increasing the concentration of aldehydes \( C \) and \( D \) in the original library caused the disparity between the two classes of nitrones to lessen. In an attempt to further bias the library towards the non-recognition nitrones, an 8th library was constructed using the same components as Library Seven but where the concentration of each aldehyde is not the same (Figure 3.25).

![Figure 3.25](image)

A nitrone library constructed from the reaction of recognition aldehydes \( A \) and \( B \) and non-recognition aldehydes \( I \) and \( J \) with hydroxylamine \( Z \). An imbalance in the concentrations of the aldehydes induces a change in the levels of each nitrone produced.

In the simple nitrone library, increasing the starting concentration of non-recognition aldehydes \( I \) and \( J \) to 50 mM results in a library of nitrones in which \( IZ \) and \( JZ \) are formed to a greater extent than \( AZ \) and \( BZ \). The ratio of recognition to non-recognition nitrones in this scenario is ~1:1.4. Using this set of aldehydes, a dynamic library was constructed using the same nucleophiles \( W \) to \( Z \) as in all previous experiments. A sample of the DCL was prepared in CD\(_2\)Cl\(_2\)/pTSA (\([A] = [B] = [W \text{ to } Z] \) = 20 mM, \([I] = [J] = 50 \text{ mM}\)) and allowed to react at 0 °C. After a period of five days, the concentration of each species present was determined by \(^{19}\text{F}\) NMR spectroscopy and comparison with the internal reference 95 (Figure 3.26).
For the first time, the hydroxylamine $Z$ is distributed amongst the four aldehydes $A$, $B$, $I$ and $J$ in such a way that the recognition nitrones are not the major components in the DCL. At equilibrium, recognition nitrones $AZ$ and $BZ$ reach concentrations of 4.37 mM and 4.32 mM respectively, representing a total concentration of 8.69 mM. Comparison with the concentrations achieved by $IZ$ (5.36 mM) and $JZ$ (5.37 mM) totalling 10.73 mM, results in a ratio of recognition to non-recognition nitrones of $\sim 1:1.2$.

With this result at hand, a sample of the non-biased library was prepared in the presence of recognition-disabled maleimide $99$ ([A] = [B] = [W to $Z$] = [99] = 20 mM, [I] = [J] = 50 mM) to demonstrate how the system responds to a simple irreversible reaction. Figure 3.27 shows the expected result that the introduction of a recognition-disabled process does not induce any selectivity within either exchange pool or the product pool after five days at 0 °C.

The overall conversion for all the cycloaddition reactions was 44% with 8.71 mM of the hydroxylamine $Z$ transferred unselectively into the product pool. Despite the recognition nitrones $AZ$ and $BZ$ being slightly minor components within the exchange pool, their increased reactivity towards maleimide $99$ results in higher formation of cycloadducts 100 ($\textit{trans-100}$) = 1.92 mM, $\textit{cis-100}$ = 0.89 mM) and 102 ($\textit{trans-102}$ = 1.80 mM, $\textit{cis-102}$ = 0.89 mM) at the expense of the non-recognition cycloadducts 107 ($\textit{trans-107}$ = 1.09 mM, $\textit{cis-107}$ = 0.40 mM) and 108 ($\textit{trans-108}$ = 1.19 mM, $\textit{cis-108}$ = 0.53 mM).
A sample of the non-biased library was prepared in the presence of recognition-enabled maleimide 83 ([A] = [B] = [W to Z] = [83] = 20 mM, [I] = [J] = 50 mM) and allowed to react for five days at 0 °C. Allowing the recognition-mediated processes to perform within the non-biased dynamic library yields the scenario depicted in Figure 3.28. Despite the library no longer being biased towards AZ and BZ, the amplification of both the recognition cycloadducts cis-101 and cis-85 is observed once again (cis-101:cis-85 = ~1.2:1). After five days, the recognition nitrones AZ and BZ have been completely consumed and transferred into the product pool resulting in recognition cycloadducts cis-101 and cis-85 reaching concentrations of 6.04 mM and 5.21 mM respectively.

Overall conversion of the maleimide 83 within the library was only 72% (cf. 91% for original library in Figure 3.13) with a total of 14.33 mM of all cycloadducts present in the product pool. This lower figure suggests that the recognition nitrones AZ and BZ react rapidly with 83 until they are completely consumed and after this point the non-recognition nitrones IZ and JZ continue to react via the uncatalysed bimolecular channel. This would suggest that the rate of the cycloaddition between IZ or JZ and 83 is faster than the exchange reactions within the library thus nitrones AZ and BZ are unable to be replenished and pulled through to the product pool.
3. Manipulation of a Dynamic Library using Molecular Recognition

3.9 Kinetic Selection Prevails

Figure 3.29 shows that the ratio of cis-101 to cis-85 remains largely unaffected by their introduction into a dynamic library as does the composition of the library. Altering the library to provide a less biased environment by reducing the dominance of the recognition nitrones AZ and BZ did not induce an increase in selectivity for cis-101.

Whilst the ratio of cis-101 to cis-85 remained impervious to changes in the library make up, these changes had a very noticeable effect on the conversion of maleimide 83. In the isolated kinetic experiment, the maleimide 83 is consumed very rapidly by reaction with AZ or BZ. However in the least biased library (4 in Figure 3.29), where the formation of nitrones AZ and BZ is significantly suppressed compared to non-recognition counterparts IZ and JZ, the conversion is much lower. This would suggest that the cycloaddition processes are faster than the exchange processes required to form the nitrones. Once AZ and BZ are depleted, the reaction of 83 with either IZ or JZ is faster than the exchange reactions that provide more recognition nitrones and therefore material is not pulled through these reactions and into cis-101 or cis-85. The position of this rate-determining step would account for the lack of selectivity provided by the dynamic scenario.
Pie chart representation of the concentrations of cis-101 (BLUE) and cis-85 (PINK) as the outcome of the competition for maleimide 83 in the isolated kinetic experiment and each of the dynamic libraries. Even when the competition is coupled to a DCL, the system is unable to significantly depart from kinetic selection. The dotted line represents 100% conversion of maleimide 83, while the actual conversion within the library is represented by the area of the pie chart.
3.10 Conclusions

It is clear from these experiments that a simple recognition event can have a profound effect on the distribution of a dynamic library and can be used to select specific chemical species from within a multitude of exchanging components. These recognition processes have been shown to be able to select even minor components from a library of spectators. However, attempts to affect the outcome of competing processes by propagating small differences in efficiencies of the recognition events within a dynamic system have met with frustrating failure.

The recognition-mediated processes were introduced to a number of dynamic environments in which the concentration of recognition nitrones AZ and BZ varied. It was expected that a library in which the concentrations of AZ and BZ was rate-limiting would amplify the difference in efficiencies between the formation of cis-101 and cis-85. It is clear from Figure 3.30 that despite the significant changes in nitrone concentrations, the competition between cis-101 and cis-85 is largely unaffected. In the original library, recognition nitrones AZ and BZ significantly dominated the nitrones within the DCL, and the fate of maleimide 83 followed kinetic selection. A change in identity and concentration of the non-recognition aldehydes resulted in a more favourable distribution of nitrones and a library that was no longer biased towards the target compounds cis-101 and cis-85. However, the restructuring of the dynamic environment in which the recognition-mediated processes were to operate appeared to have little effect on the outcome of the competition between the two.

Figure 3.30. Bar chart representation of the ratio of a) recognition:non-recognition nitrones and b) cis-101: cis-85 in each of the four dynamic libraries investigated. Library numbering system follows Figure 3.29.
3. Manipulation of a Dynamic Library using Molecular Recognition
Increasing Complexity Using Self-Replication

A fundamental requirement of constructing complex systems is the presence of feedback. In chemical terms, this requirement can be fulfilled by an autocatalytic process, in which the product of a reaction is itself a catalyst for the very process that it was formed by. The dynamic libraries discussed thus far have been specifically designed to respond to recognition-mediated processes that, depending on the structure of the maleimide substrate, proceed via either the [A•B] pathway or the self-replication cycle.

It was shown in Chapter 3 that the strongest competitor for recognition-enabled maleimide 83, namely nitrone AZ, was unable to significantly dominate over nitrone BZ, resulting in both cycloadducts cis-101 and cis-85 evolving from the exchange pool. Small structural changes in the maleimide will direct the reaction into the replication cycle, in which two species capable of self-replication of differing efficiencies will be in competition. The idea of self-replicating species emerging from within a complex mixture or pool will be familiar to scholars searching for the Origin of Life,\(^{[64]}\) and the necessity for a stronger species to dominate and outlast a weaker competitor is of paramount importance if it is to survive.

4.1 Kinetic Analysis of the Self-Replicators

The 1,3-dipolar cycloaddition reaction of nitrones AZ and BZ with maleimide 83 proceeds predominately through the exo transition state to products cis-101 and cis-85 as a result of the intramolecular hydrogen bonding facilitated by the flexibility of the meta substituted acetic acid moiety. As discussed in Chapter 2, maleimide 28 bearing a para substituted acetic acid moiety directs the reaction into the replication cycle of the minimal model (Figure 4.1). In these systems, it is the trans isomers that are expected to benefit from autocatalytic amplification.
The synthesis of maleimide 28 starts from commercially available amine 111 which is treated with maleic anhydride 97 in acetic acid at room temperature. This reaction generates the intermediate amide 112 in situ, which is cyclised immediately by refluxing the reaction mixture (Scheme 4.1).

![Scheme 4.1. Synthesis of maleimide 28. a) 97, AcOH, RT, quantitative. b) AcOH, 120 °C, 66%.

In order to test the selectivity and efficiency of the replication processes of the proposed competitors, the reactions of nitrones AZ and BZ with maleimide 28 were assessed. A sample containing the nitrone to be assessed and maleimide 28 at 20 mM was prepared in CD$_2$Cl$_2$/pTSA and the evolution of any products was followed for a period of 16 hours by $^1$H NMR spectroscopy at 0 °C.

Data for the bimolecular reactions was taken from experiments conducted in Figures 3.5 and 3.6 using meta recognition-disabled maleimide 99.
After 16 hours, the reaction of nitrone AZ with the maleimide 28 showed 80% conversion into cycloadduct 113 - the trans-113: cis-113 ratio is 31:1. Comparison with the recognition-disabled data from Figure 3.5 of 18% conversion with a trans:cis ratio of just 2.3:1 after the same time demonstrates the profound effect the recognition event has on the course of this reaction. Deconvolution of the data recorded on a sample allows for the construction of concentration vs. time profile depicted in Figure 4.2, with the extracted rate constants displayed in Table 4.1.

Scheme 4.2. The 1,3-dipolar cycloaddition between nitrone AZ and recognition-enabled maleimide 28 gives rise to two diastereoisomeric products, trans-113 and cis-113.

Figure 4.2. Concentration vs. time plot of the reaction between maleimide 28 and nitrone AZ ([AZ] = [28] = 20 mM) forming products trans-113 (●) and cis-113 (●). The solid lines represent the results of the kinetic fitting process.

Table 4.1. Rate constants determined by kinetic simulation and fitting of experimental data using the SimFit program. Bimolecular rate constants are taken from reaction of AZ with 99 in Table 3.1.

<table>
<thead>
<tr>
<th></th>
<th>trans-113</th>
<th>cis-113</th>
</tr>
</thead>
<tbody>
<tr>
<td>bimolecular rate constant / M$^{-1}$ s$^{-1}$</td>
<td>$1.24 \times 10^{-4}$</td>
<td>$5.56 \times 10^{-5}$</td>
</tr>
<tr>
<td>recognition-mediated rate constant / s$^{-1}$</td>
<td>$1.31 \times 10^{-3}$</td>
<td>-</td>
</tr>
<tr>
<td>effective molarity / M</td>
<td>10.6</td>
<td>-</td>
</tr>
<tr>
<td>$\Delta G^\circ$ / kJ mol$^{-1}$</td>
<td>1.95</td>
<td>-</td>
</tr>
</tbody>
</table>
When the analogous reactions were performed using nitrone BZ, conversion into cycloadduct 40 was lower at 68% with 30:1 selectivity for trans-40 over cis-40. Comparison with the control data from Figure 3.6 of 20% conversion with a trans:cis ratio of just 2.2:1 after the same time once again demonstrates the profound effect the recognition event has on the course of the reaction. Deconvolution of the data recorded on a sample allows for the construction of the concentration vs. time profile depicted in Figure 4.3, with the extracted reaction constants displayed in Table 4.2.

Scheme 4.3. The 1,3-dipolar cycloaddition between nitrone BZ and recognition-enabled maleimide 28 gives rise to two diastereoisomeric products, trans-40 and cis-40.

Figure 4.3. Concentration vs. time plot of the reaction between maleimide 28 and nitrone BZ ([BZ] = [28] = 20 mM) forming products trans-40 (●) and cis-40 (●). The solid lines represent the results of the kinetic fitting process.

Table 4.2. Rate constants determined by kinetic simulation and fitting of experimental data using the SimFit program. Bimolecular rate constants are taken from reaction of BZ with 99 in Table 3.2.

<table>
<thead>
<tr>
<th></th>
<th>trans-40</th>
<th>cis-40</th>
</tr>
</thead>
<tbody>
<tr>
<td>bimolecular rate constant / M⁻¹ s⁻¹</td>
<td>1.30 × 10⁻⁴</td>
<td>5.93 × 10⁻⁶</td>
</tr>
<tr>
<td>recognition-mediated rate constant / s⁻¹</td>
<td>1.87 × 10⁻³</td>
<td>-</td>
</tr>
<tr>
<td>effective molarity / M</td>
<td>14.4</td>
<td>-</td>
</tr>
<tr>
<td>ΔG° / kJ mol⁻¹</td>
<td>4.20</td>
<td>-</td>
</tr>
</tbody>
</table>
4.2 Kinetic Selection

As outlined previously in Section 3.3, the differing strengths of the recognition sites on nitrones AZ and BZ will affect the outcome of the competition between them for maleimide 28. In order to ascertain any differences in the outcome of this competition when it performs in a dynamic environment, it was first necessary to observe this competition in isolation. A sample containing both nitrones AZ and BZ and one equivalent of maleimide 28 ([AZ] = [BZ] = [28] = 20 mM) was prepared in CD_2Cl_2/pTSA at 0 °C and the evolution of cycloadducts was followed by 470 MHz ^19F NMR spectroscopy. Deconvolution of the data recorded on a sample allowed for the construction of the concentration vs. time profile for the competition process (Figure 4.4a).

During the competition, two efficient processes are competing for a limited supply of 28, it follows that 28 is depleted faster during the competition (89% conversion after 16 hours) than during either of the individual reactions of AZ or BZ with 28. After 16 hours, the 4,6-dimethyl cycloadduct trans-113 reached a concentration of 9.42 mM while the 6-methyl product trans-40 reached 7.45 mM. Cis-113 and cis-40 reached concentrations of 0.42 mM and 0.43 mM respectively, resulting in a total trans:cis ratio of 20:1. The relative strengths of the two recognition events accounts for the difference between the two replicators, the higher association constant of AZ with 28 causes the ratio of trans-113:trans-40 to be ~1.3:1 (Figure 4.4b).
4.3 Coupling the Self-Replicators to Dynamic Libraries

As a consequence of the search for a non-biased environment in which the competition between the replicators was to be played out, four separate dynamic libraries were developed, each resulting in a different ratio of recognition to non-recognition nitrones. The self-replicators will be coupled to each of the libraries in turn in an effort to identify any deviations from the expected behaviour.

4.3.1 The Original Library

The original library, and the most biased towards nitrones containing A or B, (Figure 3.3) was constructed from equal concentrations of aldehydes A, B, C and D. In order to investigate how the introduction of self-replicators affected the distribution within the library, a sample of the original library and para recognition-enabled maleimide 28 ([A to D] = [W to Z] = [28] = 20 mM) was prepared in CD₂Cl₂/pTSA and allowed to react at 0 °C. After a period of five days, analysis was performed by ¹⁹F NMR spectroscopy with the concentration of each species determined by comparison with the internal reference 95 (Figure 4.5).

![Figure 4.5](image-url)

Bar chart showing the concentrations of each of the imine/nitrone products present in the original DCL and the distribution of the cycloadducts in the product pool after five days at 0 °C in the presence of maleimide 28. A significant amount of material is transferred into the product pool and is selectively incorporated into trans-113 and trans-40.
Scheme 4.4. Structure and $^1$H NMR chemical shifts of the aromatic fluorine resonance of the cycloadducts within the recognition-mediated product pool.

Exchange Pool

Product Pool

trans-113 - 121.85 ppm
trans-40 - 121.83 ppm
trans-114 - 121.98 ppm
trans-115 - 121.70 ppm
cis-113 - 117.58 ppm
cis-40 - 117.53 ppm
cis-114 - 117.55 ppm
cis-115 - 117.05 ppm
The introduction of a self-replicating species clearly has a dramatic effect on the distribution of both the exchange pool and the product pool. After a period of five days, the maleimide is 100% converted into cycloadducts 113, 40, 114 and 115 with trans-113 and trans-40 accounting for 93% of the material within the product pool. At the end of the reaction trans-113 has been formed to a concentration of 9.59 mM while trans-40 reached 9.26 mM. As a consequence of the biased nature of the library, both the self-replicators are able to amplify themselves from the exchange pool at comparable rates. The difference in recognition strengths appears not to play much of a role in this particular scenario.

4.3.2 The Reconstructed Library

In Chapter 3, a number of different aldehydes were screened for their ability to effectively compete with A and B for hydroxylamine Z. The most favourable distribution was achieved when aldehydes C and D from the original library were replaced with I and J. While the use of these aldehydes had a positive effect on the distribution of the nitrones, their presence in the dynamic library made little difference to the competition between the [A•B] complexes formed from nitrones AZ and BZ.

![Figure 4.6](image_url)
Scheme 4.5. Structure and $^{19}$F NMR chemical shifts of the aromatic fluorine resonance of the cycloadducts within the recognition-mediated product pool.
In order to investigate how the introduction of self-replicators affected the distribution within the library, a sample of the alternate library and maleimide 28 ([A] = [B] = [I] = [J] = [W to Z] = [28] = 20 mM) was prepared in CD$_2$Cl$_2$/pTSA and allowed to react at 0 °C. After a period of five days, analysis was performed by $^{19}$F NMR spectroscopy with the concentration of each species determined by comparison with the internal reference 95 (Figure 4.6).

Within the alternate library, conversion of 28 after the same time was 84% compared to the complete conversion demonstrated within the original library. In addition, the recognition nitrones AZ and BZ are almost completely depleted (present at 0.15 mM and 0.20 mM respectively) whilst non-recognition nitrones IZ and JZ are still present at significant concentrations (1.98 mM and 1.69 mM respectively). This would suggest that once the recognition-mediated processes have used all of the available AZ and BZ, the uncatalysed reactions of IZ and JZ with 28 are faster than the exchange processes required to produce more AZ and BZ. As no material is pulled through the exchange processes into the product pool, it is unsurprising that the 4,6-dimethyl nitrone AZ is unable to significantly outcompete BZ for 28. After five days, the ratio of trans-113 to trans-40 is ~1.1:1 with each cycloadduct reaching concentrations of 7.65 mM and 7.24 mM respectively.

4.3.3 Pushing the Original Library

In Chapter 3, we demonstrated that increasing the concentration of non-recognition aldehydes C and D resulted in an increase of nitrones CZ and DZ and a library which was less biased towards the competing nitrones AZ and BZ. A sample of this library was prepared with maleimide 28 ([A] = [B] = [W to Z] = [28] = 20 mM, [C] = [D] = 50 mM) in CD$_2$Cl$_2$/pTSA and allowed to react at 0 °C. After a period of five days, analysis was performed by $^{19}$F NMR spectroscopy with the concentration of each species determined by comparison with the internal reference 95 (Figure 4.7).

After five days, conversion of maleimide 28 was 86% with a total of 17.27 mM of cycloadducts in the product pool. The two replicating species trans-113 and trans-40 are formed to 7.58 mM and 6.86 mM respectively, and account for 84% of material in the product pool. Once again, even in a library that is no longer so biased towards the replicators, trans-113 is unable to pull away from trans-40 and the ratio between the two is a familiar ~1.1:1.
4.3.4 Pushing the Reconstructed Library

In Chapter 3, a dynamic library in which recognition nitrones AZ and BZ were formed to a lesser extent that the non-recognition counterparts was constructed. This scenario arose from the use of aldehydes I and J at a higher concentration than A and B and resulted in a distribution of recognition:non-recognition nitrones of ~1:1.2 (Figure 3.25). A sample of this non-biased library was prepared with maleimide 28 ([A] = [B] = [W to Z] = [28] = 20 mM, [I] = [J] = 50 mM) in CD$_2$Cl$_2$/pTSA and allowed to react at 0 °C. After a period of five days, analysis was performed by $^{19}$F NMR spectroscopy with the concentration of each species determined by comparison with the internal reference 95 (Figure 4.8).

After five days, conversion of maleimide 28 was much lower at 67% with a total of 13.31 mM of cycloadducts in the product pool. The two replicating species trans-113 and trans-40 are formed to 5.45 mM and 4.70 mM respectively, and account for 76% of material in the product pool. As observed in Section 4.3.2, the recognition nitrones AZ and BZ are completely depleted after five days whilst non-recognition nitrones IZ and JZ remain at significant concentrations ([IZ] = 3.21 mM, [JZ] = 2.92 mM). The apparent inability of the exchange reactions to compete with the non-catalysed reaction is characteristic of the lower concentration of recognition nitrones in the dynamic library.
cycloaddition reactions of IZ or JZ with maleimide 28 would account for both the lower conversion within this library as well as the ability of trans-40 to keep up with trans-113.

Figure 4.8. Bar chart showing the concentrations of each of the imine/nitrone products present in the DCL and the distribution of the cycloadducts in the product pool after five days at 0 °C in the presence of maleimide 28. A significant amount of material is transferred into the product pool and is selectively incorporated into trans-113 and trans-40.

4.4 Comparison with Kinetic Selection

In the isolated scenario in which nitrones AZ and BZ are competing for recognition-enabled maleimide 28, it was shown that the relative strengths of the recognition units play a part in the affinity for the maleimide. It was shown that nitrone AZ, equipped with the stronger amidopyridine recognition unit, is able to associate and react with 28 to a greater extent than nitrone BZ can (Figure 4.4). The final distribution of cycloadducts in this experiment was ~1.2:1 in favour of trans-113. It was hoped that when this competition was transferred to the dynamic environment the slight imbalance between cycloadducts trans-113 and trans-40 would be amplified and the system could be driven to the exclusive formation of trans-113. Despite the non-linear coordinate provided by an autocatalytic process, the formation of the two cycloadducts trans-113 and trans-40 proceeds with similar selectivity as observed in the isolated kinetic selection experiments (Figure 4.9).
Figure 4.9. Pie chart representation of the concentrations of trans-113 (BLUE) and trans-40 (PINK) as the outcome of the competition for maleimide 28 in the isolated kinetic experiment and each of the dynamic libraries. Even when the competition is coupled to a DCL, the system is unable to significantly depart from kinetic selection. The dotted line represents 100% conversion of maleimide 28, while the actual conversion within the library is represented by the area of the pie chart.
4.5 Conclusions

It is clear from these results that allowing a self-replicating process to operate within the confines of a dynamic environment has a profound effect on both the product pool and the compounds left behind in the exchange pool. The self-replicators trans-113 and trans-40 were able to quickly amplify themselves from within the dynamic library, even though the required nitrones AZ and BZ were not present at the start of the reaction.

The recognition-mediated self-replication processes were introduced to a number of dynamic environments in which the concentration of recognition nitrones AZ and BZ varied. It was expected that a library in which the concentrations of AZ and BZ was rate-limiting would amplify the difference in efficiencies between the two replicators. It is clear from Figure 4.10 that despite the significant changes in nitrone concentrations, the competition between trans-113 and trans-40 is largely unaffected. In the original library, recognition nitrones AZ and BZ significantly dominated the nitrones within the DCL, and the fate of maleimide 28 followed kinetic selection. A change in identity and concentration of the non-recognition aldehydes resulted in a more favourable distribution of nitrones and a library that was no longer biased towards the target compounds trans-113 and trans-40. However, the restructuring of the dynamic environment in which the self-replicators were to operate appeared to have little effect on the outcome of the competition between the two.

Figure 4.10. Bar chart representation of the ratio of a) recognition:non-recognition nitrones and b) trans-113:trans-40 in each of the four dynamic libraries investigated. Library numbering system follows Figure 4.9.
Figure 4.10b shows a very slight increase in the ratio of trans-113 to trans-40 as the DCL becomes less biased towards AZ and BZ. However, this is a very small change and even in the reconstructed library ([I] = [J] = 50 mM), where AZ and BZ do not dominate, the ratio of trans-113:trans-40 remains lower than the ratio observed in the isolated kinetic experiment (Figure 4.4).

These findings ultimately underline the fundamental selectivity limitations imposed on competing replicators by kinetic selection. The application of dynamic combinatorial chemistry as a means to drive a replicator to extinguish a less efficient competitor has proven ineffective, yet a mechanism by which the most efficient replicating species can establish its own dominance must exist. In order to provide experimental evidence for the Darwinian Evolution of self-replicators, a different approach to breaking through the kinetic barrier must be sought.
The experiments described in Chapters 3 and 4 demonstrate the inherent selectivity between two competing processes in a dynamic environment is difficult to bias. Chapter 3 described the competition between two recognition-mediated reactions that proceeded via the [A•B] pathway. This competition was unaffected by transferal to a dynamic environment. The ratio of cis-101 to cis-85 at equilibrium remained the same as the ratio observed during the isolated kinetic competition between AZ and BZ for maleimide 83.

The addition of a non-linear kinetic component to the system, provided by self-replicating species, was achieved by modifying the common building block for which nitrones AZ and BZ were competing. Chapter 4 described the competition between the two self-replicating species trans-113 and trans-40, and showed that as for the [A•B] competition, it was unaffected by its transfer to a dynamic environment. The differing strengths of the recognition site of each nitrone AZ and BZ provided two replicators of unequal efficiency, however, we demonstrated that within a dynamic library, trans-113 was unable to press home its advantage over trans-40 to its exclusive formation from the library.

Emerging from a dynamic exchange pool, neither system was able to break away from the constraints imposed by kinetic selection. In both cases, products based on nitrones AZ and BZ coexisted throughout the experiments. Put simply, everybody lives. In order to fully understand the multitude of processes occurring within the system, one can turn to computational analysis and use the kinetic data measured in Chapter 3 to build a simulation of the dynamic systems. The experiments conducted within this chapter are theoretical and are carried out using the ISOSIM feature of the SimFit software package. Further details can be found in Chapter 8.
5.1 The Rate-Limiting Step

The experiments conducted on the self-replicators operating within Library 4, where aldehydes I and J were present at concentrations of 50 mM, resulted in the same selectivity between trans-101 and trans-85 but also a significant decrease in the conversion of 83. After five days, the total concentration of all cycloadducts within the product pool was 13.31 mM representing reaction of just 67% of maleimide 83. In addition to this lower figure, neither nitrone AZ nor BZ were detected in the exchange pool. This would suggest that once AZ and BZ are depleted, they are not replenished by the exchange reactions as expected but instead the non-recognition nitrones IZ and JZ react with maleimide 83 via the bimolecular channel. As hydroxylamine Z is still being transferred into the product pool, the exchange reactions cannot address the imbalance between nitrone concentrations and no more AZ or BZ is formed.

These observations shed some light on what may be the rate-determining step within the chain of cooperating processes. There are three processes operating together at different rates within the dynamic library, the relative rates of these is clearly important. The three processes are: 1. the exchange reactions between nitrones, 2. the bimolecular reactions between nitrones and maleimide 83 and 3. the recognition-mediated reactions. We have demonstrated that the recognition-mediated reactions are significantly faster than the bimolecular reactions and this fact is observed clearly within the libraries. In these cases, it is the relative rate of the exchange reactions that becomes important.

Using a simulation allows the user to vary the relative rates of these processes and perhaps shed some light on the efficiencies of the connected process. The kinetic data collected in Chapter 3 was first used to build a simulation of the competition between the [A•B] complexes formed from nitrones AZ and BZ. Using the rate constants extracted from the obtained concentration vs. time data for the reaction of each nitrone with maleimide 83, a simulation of the competition between AZ and BZ for a limited supply of 83 was constructed. Fitting of the experimental data to the simulation resulted in a good fit (Figure 5.1a).

Within the DCL, there are numerous exchange reactions occurring simultaneously each of them with a different rate. Rather than individually measure each rate constant and subsequently simulate the DCL, the experimental data from Figure 3.3 can be used to identify trends in the reactivity of each component. As these rate
constants within the DCL are to be varied with respect to the cycloadditions, the exact values are unimportant. The rate constants used to provide the simulation can be found in the appendix. Using the trends observed in Figure 3.3, a simulation of the DCL constructed from aldehydes A to D and nucleophiles W to Z was built (Figure 5.1b).

The simulation displays a distribution in concentration for each species similar to the experimentally measured DCL in Chapter 3. The recognition aldehydes A and B prove to be the most reactive and are incorporated significantly more into the nitrones AZ and BZ and imines AW and BW than the non-recognition aldehydes C and D are.

With a reliable simulation of both the competing recognition-mediated processes and the dynamic library into which they are to be introduced, a simulation of the outcome of the effect on the DCL could be performed.
5. Why Does Everybody Live?

5.1.1 Fast Exchange

The simulation was first performed where the exchange reactions were much faster than the recognition-mediated cycloadditions. The rates of the recognition mediated cycloadditions of nitrones AZ and BZ with maleimide 83 were determined previously experimentally and set in the simulation to $1.24 \times 10^{-4} \text{ M}^{-1} \text{s}^{-1}$ and $1.18 \times 10^{-4} \text{ M}^{-1} \text{s}^{-1}$ respectively. The reactions forming all condensation products were set to $1.00 \times 10^{4} \text{ M}^{-1} \text{s}^{-1}$, significantly higher than the cycloadditions while the rates of the exchange reactions were scaled appropriately to reflect the relative stability of each species to hydrolysis and subsequent exchange. This output from this simulation is depicted in Figure 5.2.

If the exchange reactions are faster than the recognition-mediated cycloadditions, a significant concentration of each nitrone will build up in solution. As the exchange process between A and Z are matched in efficiency by the analogous process between B and Z, both nitrones build up to significant concentrations in solution. In addition, any imbalance in the depleting concentrations of AZ or BZ caused by the differing efficiencies of the [A•B] pathways is erased by the rapid exchange. This scenario becomes identical to the simple kinetic competition and no increase in selectivity for cis-101 is observed.

![Figure 5.2](image-url) Results from the simulated system in which the exchange reactions are significantly faster than the recognition-mediated cycloaddition reactions of AZ and BZ with 83. The bar chart shows the concentrations of each of the imine/nitrone products present in the simulated DCL and the distribution of the cycloadducts in the product pool.
The structure and rate constants of each of the species present in the DCL after reaction with recognition-enabled maleimide 83. The rate constant for the formation of all imines and nitrones \((k_{\text{on}})\) was set at \(1.00 \times 10^4\) M\(^{-1}\) s\(^{-1}\) in all cases.

Scheme 5.1.
5.1.2 Slow Exchange

The simulation was repeated with the exchange reactions set to be much slower than the recognition-mediated cycloadditions. The rates of the recognition mediated cycloadditions of nitrones AZ and BZ with maleimide 83 were previously determined experimentally and set as before. The reactions forming all condensation products were set to $1.00 \times 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$, significantly slower than the cycloaddition reactions. This output from this simulation is depicted in Figure 5.3.

If the exchange reactions are rate-limiting and are slower than even the uncatalysed bimolecular cycloaddition, the nitrones are consumed as soon as they are formed by reaction with 83. The recognition nitrones AZ and BZ are consumed much faster via the recognition-mediated reactions leaving the non-recognition nitrones behind. As the exchange reactions are too slow to compete with even the uncatalysed reactions of non-recognition nitrones, neither AZ nor BZ are replenished while the maleimide 83 continues to be consumed. As a consequence of the nitrones being consumed immediately and the comparable efficiency of the reactions forming them, any selectivity in the product pool is eroded and the cycloadducts cis-101 and cis-85 are formed to the same extent as each other and non-recognition cycloadducts trans-105 and trans-106.

Figure 5.3. Results from the simulated system in which the exchange reactions are significantly slower than the recognition-mediated cycloaddition reactions of AZ and BZ with 83. The bar chart shows the concentrations of each of the imine/nitrate products present in the simulated DCL and the distribution of the cycloadducts in the product pool.
5.1.3 Intermediate Exchange

The simulation was repeated once more with the rate of the exchange reactions set to a value similar to the rate of the recognition-mediated cycloadditions. The rates of the recognition-mediated cycloadditions of nitrones AZ and BZ with maleimide 83 were previously determined experimentally and set as before while the reactions forming all condensation products were set to $1.00 \times 10^{-4}$ M$^{-1}$ s$^{-1}$. This output from this simulation is depicted in Figure 5.4.

If the exchange reactions are operating at a comparable rate to the recognition-mediated cycloaddition reactions, an intermediate scenario presents itself. Selectivity within the product pool for the recognition-enabled cis-101 and cis-85 over non-recognition counterparts 105 and 106 is increased when compared to the slow exchange (Figure 5.3) but considerably less than observed during the fast exchange (Figure 5.2). The selectivity between cis-101 and cis-85 is once again eroded and both cycloadducts are formed to the same extent.

![Figure 5.4](image)

Based on the erosion of selectivity observed in the simulations in which the exchange reactions were slower or of comparable rate to the cycloadditions, it is apparent that the real system behaves akin to the fast exchange simulation in Figure 5.2.
5.2 Concentration Dependence: $K_a$ is King

When working within a dynamic library, the components required for the recognition-mediated reaction have not yet been assembled at $t = 0$. Nitrones $\text{AZ}$ and $\text{BZ}$ must be formed from the reaction of either $\text{A}$ or $\text{B}$ with hydroxylamine $\text{Z}$. Ideally, this means that as the reaction progresses, nitrones $\text{AZ}$ and $\text{BZ}$ are slowly fed into the reaction with maleimide $\text{83}$. As a consequence of the stronger recognition event provided by the 4,6-dimethyl amidopyridine present in $\text{AZ}$, the recognition-mediated cycloaddition reaction between $\text{AZ}$ and $\text{83}$ (or $\text{28}$) can begin to operate before the analogous reaction with $\text{BZ}$. As a result of $\text{AZ}$ being depleted, the dynamic equilibrium shifts to replenish $\text{AZ}$ at the expense of the other nitrones $\text{BZ}$ to $\text{DZ}$ (or $\text{IZ}$ and $\text{JZ}$ depending on the library). In this way, the reaction of $\text{AZ}$ with maleimides $\text{83}$ or $\text{28}$ proceeds rapidly via the recognition-mediated pathway leading to exclusive formation of $\text{cis-101}$ or $\text{trans-113}$.

A problem arises when one considers the starting concentration of each component. Whilst working at 20 mM provides a library at which the concentrations of even the most minor components remain detectable by NMR spectroscopy, it is also significantly above the dissociation constants, $K_d$, for the two competing recognition events. At this concentration, most of both nitrones exist as the binary complexes $[\text{AZ} \cdot \text{83}]$ or $[\text{BZ} \cdot \text{83}]$ and both recognition-mediated reactions are able to perform. Working at a concentration around the $K_d$ would allow the different association constants of $\text{AZ}$ and $\text{BZ}$ to cause an imbalance in the concentrations of the two complexes, thus leading to a different ratio of cycloadducts, however the difficulties in performing an accurate analysis would increase significantly.

The difficulties associated will analysis of a real system can be initially circumvented by using the simulation constructed in the previous section. By analysis of the results obtained by varying the relative rates of the exchange reactions, an accurate simulation of the system was established. Experimental results match the simulation in which the exchange reactions are much faster than the subsequent cycloaddition reactions. With this simulation at hand, it is a trivial matter to alter the starting concentration of each component and simulate the outcome, thus avoiding any problems associated with the dilution of the system.
Results from the simulated system at 2 mM in which the exchange reactions are significantly faster than the recognition-mediated cycloaddition reactions of AZ and BZ with 83. The bar chart shows the concentrations of each of the imine/nitrone products present in the simulated DCL and the distribution of the cycloadducts in the product pool.

The simulation depicted in Figure 5.2 was repeated at a concentration closer to the $K_d$ of AZ and BZ with 83. The output from this simulation is depicted in Figure 5.5. Working at an initial concentration of 2 mM results in a slightly different ratio between cis-101 and cis-85 (Table 5.1). At the lower concentration the ratio between the cycloadducts increases from 1.20 to 1.21. Lowering the concentration by a further order of magnitude to 0.20 mM continues to increase the ratio between the cycloadducts although the effect is very slight.

<table>
<thead>
<tr>
<th>Concentration / mM</th>
<th>[cis-101]:[cis-85]</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1.20</td>
</tr>
<tr>
<td>2</td>
<td>1.21</td>
</tr>
<tr>
<td>0.20</td>
<td>1.23</td>
</tr>
</tbody>
</table>

In order to further understand why this decrease in concentration did not have the expected effect, another simulation was conducted in which the ratio of the association constants of the competing nitrones was varied along with the concentration of the library. The results from this simulation are illustrated as a
surface plot depicted in Figure 5.6. The results show that in order for a lower concentration to enable AZ to dominate over BZ, the difference in the two association constants has to be much larger. Nitrones AZ and BZ have individual association constants ($K_a$) of 315 M$^{-1}$ and 160 M$^{-1}$ which results in a ratio of around 2. From Figure 5.6 it is clear that a ratio of just 2 between the association constants of AZ and BZ is not sufficient to allow the alteration the concentration of starting materials to make a significant difference in the concentrations of the two [A•B] complexes in solution.

![Surface plot showing how the ratio of the binary complexes [AZ•M] and [BZ•M] is dependent on not only the concentration but also the ratio of the individual association constants.](image)

Figure 5.6. Surface plot showing how the ratio of the binary complexes [AZ•83] and [BZ•83] is dependent on not only the concentration but also the ratio of the individual association constants.

The surface plot does reveal that a decrease in concentration would have the expected effect on the competition but only if the difference between the two competitor’s association constants is much larger. If the difference was closer to a ratio 20:1, a decrease in concentration to around 1 mM would result in a much larger difference between the concentrations of the two reactive complexes, in excess of 10:1.

These simulations explain the lack of increased selectivity within the dynamic library based on recognition-mediated reactions proceeding via the [A•B] pathway. It is expected that similar observations would be made on the self-replicating competition
described in Chapter 4 so these processes were not simulated. These findings demonstrate that exploiting the difference in association constants may well be capable of inducing selectivity in similar systems but the two competing nitrones AZ and BZ in this system are too well matched for this to be an appropriate selection procedure here.

5.3 Multicyclic Systems

The limited selectivity achievable within a closed system is further illustrated by recent work in our group on the development of instructable multicyclic systems.[38] A network of replicators formed from two minimal replicators trans-38 and trans-40, and two reciprocal replicators, trans-36 and trans-41, was described in detail in Chapter 1 and is illustrated in Figure 5.7 below. While this system is instructable in that the addition of a preformed template will direct the system towards either the self-replicators (SR) or reciprocal replicators (RR), selectivity is limited and in fact only temporary.

![Multicyclic System Diagram]

Figure 5.7. A multicyclic network combining the self-replicating systems trans-38 (from maleimide 37 and nitrone 35) and trans-40 (from maleimide 28 and nitrone 39) to form two additional reciprocal templates trans-36 and trans-41.
As a consequence of the interconnectedness of the system, templating either trans-38 or trans-40 results in an increase of both minimal self-replicators, while templating one of the reciprocal replicators by their very nature up-regulates both trans-36 and trans-41. As a result, the system can be instructed to form both self-replicators or both reciprocal replicators.

As the system had been shown to respond with a change in the RR:SR ratio to the addition of preformed template, a series of recycling experiments were designed in an attempt to allow the system to reach higher levels of selectivity (Figure 5.8). The four starting materials, 28, 35, 37 and 39, were combined in the presence of 20 mol % of either reciprocal template trans-41 or self-replicating template trans-38 and allowed to react until completion. A portion of the final product distribution (20 mol %) was injected into fresh starting materials and allowed to react once more. This recycling process was repeated four times with the distribution of the replicators at complete conversion determined by 19F NMR spectroscopy.

The results reveal an interesting trend in that the addition of a template initially causes the expected imbalance towards the templated species however upon recycling, this imbalance is eroded and the system reaches an equilibrium point identical in both experiments. Intense kinetic simulation and modelling revealed that saturation of the catalytically active ternary complexes are accountable for this erosion of selectivity. At the first cycle, the presence of an instructional template has a major impact on the concentration of the ternary complexes, however this impact is decreased upon each cycle and the system eventually recovers to its equilibrium point.

This saturation of competing ternary complexes is a consequence of working within a closed system and once more demonstrates the limited selectivity a system operating in a closed environment can achieve. In a closed system, a self-replicating species or indeed any chemical reaction becomes self-inhibiting as it rapidly consumes the starting materials it requires for its own synthesis. As the building blocks become exhausted, the rate of the reaction slows and its efficiency drops off. One can imagine an environment in which a given reaction is able to perform with an endless supply of reagents, and therefore operate at optimum efficiency at all times. The reaction environment in this case could be considered ‘open’ as the amount of matter contained within it does not remain constant. Real examples of such a system
could be generated through the application of flow chemistry or non-linear reaction dynamics. It is expected that the transfer of a network of competing replicators into an open system may lead to a level of selectivity within the network that is unreachable in a closed environment.

5. Why Does Everybody Live?

**Figure 5.8.**  
 a) Experimental set-up to investigate the behaviour of the multicyclic system depicted in Figure 5.7 after initial addition of 20 mol % of instructional template followed by repetitive doping of 20 mol % of the outcome of the previous cycle in a fresh batch of reagents. After full conversion of starting materials, the product distribution was determined by $^{19}$F NMR spectroscopy and the concentration of the reciprocal replicators (RR) was divided by the concentration of the self-replicator (SR) to provide values for the RR/SR ratio obtained upon initial addition of b) reciprocal template trans-41 or c) autocatalytic template trans-38.

10 mM, CDCl$_3$
The experiments presented in this thesis thus far are carried out in a closed system. In general, all reagents are dissolved in a sealed NMR tube using a suitable solvent and this solution is then incubated at a certain temperature while the progression of the reaction is followed by NMR spectroscopy. No exchange of matter takes place with the environment and the reaction set-up follows the model of the static well-stirred batch reactor (WSBR). We have demonstrated that working in a closed system places fundamental limits on the ability of one species to become dominant within such systems. Even coupling the recognition-mediated reactions to a dynamic covalent library leads to coexistence of the major species rather than extinction of the weaker ones.

The transfer of the described systems into an open system may lead to scenarios in which a weaker replicator is extinguished in favour of a more efficient one. Such a system may be envisaged as a chemical process that is initiated at a specific point within a reactor and allowed to propagate into fresh reagents as a chemical wave, leaving behind product in its wake. The field of non-linear reaction dynamics deals with the design of such reaction diffusion systems and may be applied to our replicating species.

6.1 Introduction

Research into the unusual behaviour exhibited by non-linear reactions can be traced back to the early 19th century with the first published observations of chemical oscillations.\[^{65}\] However until recently, perhaps as a result of the lack of understanding of these processes, the chemical community has been resistant in accepting such observations with a general view that chemical oscillations
constituted a violation of the Second Law of Thermodynamics.\[66\] In the early 1950s, when Soviet biophysicist Belousov first noticed the colour of a solution containing bromate and cerium ions with citric acid in sulfuric acid oscillated between colourless and pale yellow, his discoveries were widely discredited as being impossible. Despite meticulous characterisation of the oscillations, no journals would publish his findings and he left the field in the late 1950s vowing never to publish at all.\[67\] Belousov’s recipes for the oscillating reaction circulated the Universities of Moscow and Puschino for a number of years before catching the attention of Zhabotinsky in 1961. Zhabotinsky refined the reaction, substituting malonic acid for the citric acid and employing ferroin as a redox indicator.\[68\] He characterised this phenomenon and demonstrated that, in an unstirred system, the reaction spontaneously gave rise to blue spirals of oxidised ferrin in an initially homogenous red solution of reduced ferroin (Figure 6.1). This new optimised reaction became known as the Belousov-Zhabotinsky (BZ) reaction and its exotic behaviour finally gained widespread attention in the chemical community.\[69\]

Figure 6.1. Still image of the Belousov-Zhabotinsky (BZ) reaction. Blue spirals of oxidised ferrin spontaneously arise in a initially homogenous red solution of reduced ferroin.

6.2 Competitive Autocatalysis

The characteristic spiral patterns exhibited by the BZ reaction in unstirred systems are examples of propagating reaction fronts, or chemical waves.\[70\] Propagating fronts are commonplace in nature: reproducing colonies of bacteria, areas of corrosion expanding on a metal surface or infectious diseases spreading through a population all exhibit this propagating phenomenon. A chemical reaction can exhibit a similar propagating wave front if the bulk of the reaction occurs within a narrow area, converting reagents into products that are left behind. Examples of such fronts have been known to occur in a variety of autocatalytic processes.\[71, 72\]
The Showalter research laboratory have long been fascinated with the area of non-linear dynamics and chemical waves. Theoretical calculations performed by them have demonstrated that two competing autocatalytic processes can, in reaction diffusion systems, exhibit exclusive product selectivity.\[73, 74\] In their system, two autocatalytic processes proceed in competition with each other for a common resource such as fuel or food:

\[
\begin{align*}
A + B &\rightarrow 2B \\
A + C &\rightarrow 2C
\end{align*}
\]

Their work showed that in a well-stirred batch reactor, the final distribution of the two products is always governed by the ratio of the rates of each process and the initial concentrations of A, B and C. These findings fit the results presented previously in Chapter 4 and reflect the inability of trans-113 to successfully out compete trans-40 for maleimide 28.

However, when the Showalter system is transferred to a reaction diffusion system, the outcome is quite different. When the reactions are seeded at a particular point within the reactor, propagating waves develop. Provided the propagation velocity of each wave is not identical, the faster wave is able to move ahead and gain preferential access to the required reagents. As the two waves travel, the slower of the two is exposed to an ever decreasing supply of reagent and it eventually dies out (Figure 6.2).

![Figure 6.2](https://example.com/figure6_2.png)

**Figure 6.2.** Concentration vs. distance from initial graphs of a) a single autocatalytic wave and b) competitive autocatalysis. In the competitive scenario, B is the faster wave and quickly uses up all the A within range of slower wave C causing it to become wiped out. Figures taken from ref 74.
6. Breaking the Barrier: Chemical Waves

A reaction diffusion system is an example of an open chemical system. In such a scenario, the concentrations of starting materials is not rate-limiting, and our replicators trans-113 and trans-40 would be able to perform at their most efficient for the entirety of the reaction. It is hoped that these principles can be expanded and applied to our system and generate selectivity for the more efficient replicator.

6.3 Chemical Waves Mediated by Self-Replicators

All previous chemical waves have either been based on inorganic processes such as the BZ reaction and variations, or on hypothetical mathematical scenarios such as the competitive autocatalysis work of the Showalter group. It wasn’t until McCaskill et al. demonstrated[75, 76] wave fronts of strands of ribonucleic acid (RNA) variants propagating along thin capillary tubes in the late 1980s that the applications of non-linear dynamics in mainstream organic synthesis could be realised.

Seeding the catalytic enzyme at a specific point in their capillary tube reactor, they were able to observe a propagating wave of RNA species along the length of the capillaries (Figure 6.3). This was the first, and currently the only, example of a propagating chemical wave that was not based on an inorganic process such as the BZ reaction.

![Figure 6.3](image)

Figure 6.3. Space-time image of McCaskill’s replication waves. The image shows the time course of RNA replication and spread in a short 5 cm segment of a capillary. The false colour is provided by ethidium bromide fluorescence and the graded colours represent increasing RNA concentration from black to orange. Image taken from ref 76.

The success of the McCaskill group in utilising a self-replicating species to induce a chemical wave suggests that in principle, the only requirement for a chemical wave is an autocatalytic process. The self-replicating species trans-113 and trans-40 described in Chapter 4 would fulfil this requirement and could possibly be tailored to produce a chemical wave.
Recent, currently unpublished, results within our research group have lead to the successful adaption of a well-characterised replicating species suitable for the observation of a chemical wave (Scheme 6.1). Real-time monitoring becomes a necessity for the observation of a chemical wave, so an alternative to the usual application of $^1$H or $^{19}$F NMR spectroscopy for analysis must be sought. Attaching a fluorescent anthracene tag to nitrone 118 allows for the system to be monitored by fluorescence spectroscopy or even the naked eye. Reaction with maleimide 28 generates an efficient minimal replicator trans-119 and is accompanied by a shift in the emission from yellow for 118 to blue for the cycloadduct 119. It was found that the efficiency of this replicator remains high, despite the size of the bulky anthracene group.

Scheme 6.1. Nitrone 118 reacts with maleimide 28 to generate self-replicator trans-119 stereospecifically. The yellow emission of 118 is shifted to a blue emission of trans-119 allowing the progress of the reaction to be followed by the naked eye. Inset is the calculated molecular structure of the product duplex illustrating the presence of the bulky tag does not interfere with the required recognition events. The calculations were performed by molecular mechanics using the OPLS2005 forcefield and the GB/SA model for solvation.
6. Breaking the Barrier: Chemical Waves

Using this replicator, a visible propagating wave front can be induced inside a 50 µL glass syringe (Figure 6.4a). A solution containing an equimolar amount of fluorescent nitrene 118 and maleimide 28 is taken up into a syringe and seeded at one end with a small amount of preformed template trans-119. As the reaction progresses, a front of blue product can be seen propagating through the yellow syringe. For clarity, the images have been processed in Figure 6.4 so that the wave front is observed as white moving through a darkened background.

In addition to via the replication cycle, trans-119 is also formed from 118 and 28 through the slow uncatalysed bimolecular reaction. This reaction is much slower than the recognition-process but nevertheless contributes to the formation of trans-119. Although the reaction is only seeded at one end of the syringe, the autocatalytic reaction seeds itself once enough trans-119 has been formed through the bimolecular channel. For this reason the unseeded syringe (Figure 6.4b) also emits a blue colour after around 90 minutes. This process results in a rather short-lived chemical wave that will need optimisation before a competition scenario can be introduced.

![Figure 6.4](image-url)

**Figure 6.4.** Time-lapse photographs of a) the chemical wave produced by seeding a mixture of 118 and 28 with trans-119 and b) the evolution of an unseeded control syringe. A propagating front can be observed in the seeded syringe that is absent from the unseeded syringe. The background reaction to trans-119 causes fringing along the syringes and causes product to begin to build up after around 90 minutes. The images have been processed for clarity.
6.4 The Design of a Competitive Autocatalytic Wave System

The competition between nitrones AZ and BZ described in Chapter 4 for recognition-enabled maleimide 28 results in the two template molecules trans-113 and trans-40. They are structurally very similar and as a result can form hetero-duplexes and thus engage in cross-catalysis. While this was not a problem when selectivity was to be generated through the differing association constants of nitrones AZ and BZ with the carboxylic acid bearing maleimide 28, it does complicate the seeding of chemical waves. Early work based on the competition between nitrones 120 and closely related nitrone 122 for maleimide 28 was significantly hampered by the presence of cross-catalytic pathways (Scheme 6.2). The reaction of methyl nitrone 120 with maleimide 28 showed an increase in initial rate when seeded with 10 mol % of cross-catalytic partner trans-123 (Figure 6.5).

Scheme 6.2. Nitrone 120 reacts with maleimide 28 to generate self-replicator trans-121, while nitrone 122 reacts with maleimide 28 to generate self-replicator trans-123. Both replicators are formed stereospecifically.

Figure 6.5 Concentration vs. time plots of the reaction of maleimide 28 and nitrone 120 ([28] = [120] = 10 mM) forming product trans-121 (●) in the a) absence and b) presence of 10 mol % of trans-123. The cis isomers of this reaction are not catalytically active and are omitted for clarity.
6. Breaking the Barrier: Chemical Waves

It was clear that a pair of self-replicators that are also cross-catalytic partners would not be suitable for the envisaged competition. Previous work within the group on the development of instructable multicyclic systems demonstrated that a size mismatch between two templates can render them catalytically inert to substrates of incorrect size. If the template is too long or too short, the reactive sites of the species are not brought close enough together for the reaction to benefit from *pseudo*-intramolecularity and no rate enhancement occurs. With this knowledge at hand, an alternate competition was designed in which no cross-catalytic terms can exist.

Fluorescent nitrone 122, based on the more efficient of the two replicators described in Chapter 4, can react with both maleimide 28 as well as the shorter C1 maleimide 23 (Scheme 6.3). Both maleimides possess recognition sites and are expected to provide the minimal self-replicators trans-123 and trans-124. As a result of the absence of the phenyl spacer group, C1 maleimide 23 is significantly shorter than original maleimide 28, resulting in a shorter self-replicating template trans-124.

Scheme 6.3. Nitrone 122 can react with either maleimide 28 to form trans-123 or with C1 maleimide 23 to form the shorter template trans-124. The two formed templates are of different sizes and are therefore ideal components to be introduced to reaction-diffusion systems.
6.5 Synthesis and Kinetic Analysis of Long-Replicator \textit{trans}-123

It was decided that, although the previous work had been conducted using nitrone 118 bearing the 6-methyl recognition site, the proposed competition would utilise nitrone 122, which contains the stronger 4,6-dimethyl recognition site. Nitrone 122 would likely result in the subsequent replicators \textit{trans}-123 and \textit{trans}-124 being more efficient than their 6-methyl counterparts based on 119.

The synthesis of nitrone 122 started from commercially-available 9-bromoanthracene 125 which underwent Sonogashira coupling with trimethylsilylacetylene to yield compound 126 in very good yield. The Sonogashira reaction requires a zero-valent palladium catalyst, which is generated \textit{in situ} from bis(triphenylphosphine)palladium (II) dichloride. The TMS-protected acetylene 126 obtained was deprotected to 127 by treatment with potassium carbonate and coupled immediately to 4-iodo-nitrobenzene 128 \textit{via} another Sonogashira reaction to yield target nitro compound 129 in good yield.

![Scheme 6.4](image)

\textbf{Scheme 6.4.} Synthesis of fluorescent nitrone 122. i) Trimethylsilylacetylene, PdCl$_2$(PPh$_3$)$_2$, CuI, Et$_3$N, 80 °C, 86%. ii) K$_2$CO$_3$, MeOH/THF, RT. iii) PdCl$_2$(PPh$_3$)$_2$, CuI, Et$_3$N, 55 °C, 76% over two steps. iv) NH$_2$NH$_2$, Rd/C, THF, RT. v) EtOH, RT, 62% over two steps.
Nitrone 122 was then assembled in good yield from nitro compound 129 by partial reduction of 129 with hydrazine over a rhodium catalyst to afford hydroxylamine 130 before condensation with previously prepared recognition aldehyde A. The additional methyl group of 122 had a positive effect on the solubility of the compound in chloroform and did not affect the fluorescent properties of the molecule.

The synthesis of recognition-enabled maleimide 28 required to react with 122 to form self-replicator trans-123 was presented in Chapter 3 and will not be discussed again here. However, a sample of 28 was methylated using methyl iodide and caesium carbonate to furnish para recognition-disabled maleimide 26.

Scheme 6.5. Synthesis of recognition-disabled maleimide 26 from 28. i) MeI, Cs₂CO₃, DMF, RT, 56%

The experiments conducted previously on the related system shown in Figure 6.4 indicated that the background bimolecular reaction between 118 and 28 provides an additional pathway to trans-119 that disrupts the progression of the observed chemical wave. In order to reduce this contribution, the background reaction needs to be eliminated or significantly slowed. As a simple bimolecular process, the rate of the reaction depends only on the concentration of each starting material:

\[
\frac{d[\text{trans-119}]}{dt} = k_{\text{trans}}[118][28]
\]

As \( k_{\text{trans}} \) is a constant, halving the starting concentration of each 118 and 28 reduces the rate of the reaction by four. Previous work was carried out at 10 mM and suggested that the background reaction obscures the wave in around 90 minutes. Therefore, it was expected that performing the analogous experiments at a concentration of 5 mM would result in a wave that was visible for longer.
In order to demonstrate the efficiency of the proposed fluorescent replicator trans-123, a sample containing an equimolar amount of nitrone 122 and either maleimide 26 or 28 was prepared ([122] = [26 or 28] = 5 mM) in CDCl₃ and allowed to react at 4 °C. The progression of the reaction was followed by 500 MHz ¹H NMR spectroscopy, monitoring the disappearance of the maleimide protons and the appearance of the characteristic protons of the forming isoxazolidine ring system. Deconvolution of the data obtained on a sample allowed for the construction of the concentration vs. time profiles in Figure 6.6.

Scheme 6.6. The 1,3-dipolar cycloaddition between nitrone 122 and maleimides 26 or 28 gives rise to two diastereoisomeric products, trans-125 and cis-125, or trans-123 and cis-123. The cis isomers of this reaction are not catalytically active and are omitted for clarity.

Figure 6.6. Concentration vs. time plots of the reaction of a) recognition-disabled maleimide 26 and nitrone 122 ([26] = [122] = 5 mM) forming product trans-125 (●), b) recognition-enabled maleimide 28 and nitrone 122 ([28] = [122] = 5 mM) forming product trans-123 (●). The cis isomers of this reaction are not catalytically active and are omitted for clarity. The solid lines represent the results of the kinetic fitting process.
After 16 hours, the reaction between recognition-disabled maleimide 26 and nitrone 122 showed just 6% conversion into cycloadduct 125 with 3:1 selectivity for trans-125 ([trans-125] = 0.24 mM, [cis-125] = 0.08 mM). This low conversion is an expected consequence of the significantly lower starting concentrations of 26 and 122.

When the reaction is repeated using recognition-enabled maleimide 28 in place of 26, conversion after the same time rises to 82% with trans-123 reaching a concentration of 4.08 mM. In the recognition-mediated reaction, the non-replicating isomer, cis-123, is not detected at any point during the monitoring window. The reaction profile was subjected to simulation and fitting to a model for a bimolecular reaction using the SimFit software package. The relevant rate constants are displayed in Table 6.1.

<table>
<thead>
<tr>
<th>Rate constant</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>bimolecular rate constant / M⁻¹ s⁻¹</td>
<td>6.37 × 10⁻⁷</td>
</tr>
<tr>
<td>recognition-mediated rate constant / s⁻¹</td>
<td>6.23 × 10⁻²</td>
</tr>
<tr>
<td>effective molarity / M</td>
<td>92.8</td>
</tr>
<tr>
<td>ΔG° / kJ mol⁻¹</td>
<td>-62.6</td>
</tr>
</tbody>
</table>

It is clear from the characteristic sigmoidal shape of the concentration vs. time profile that trans-123 is a very efficient minimal self-replicator that is able to operate even at low concentrations.

6.6 Synthesis and Kinetic Analysis of Short-Replicator trans-124

In order to create an instructable competition for nitrone 122, a second replicator is required that possess similar fluorescent properties to those of trans-122. An early design for a synthetic replicator researched by the Philp laboratory utilised C1 maleimide 23. This maleimide bears a carboxylic acid recognition site but lacks the phenyl spacer unit of 28 and thus is correspondingly shorter. The difference in size is crucial if the two replicators are to remain separate from one another in solution, a key requirement for the design an instructable chemical network.
Recognition-enabled C1 maleimide 23 is easily obtained by treatment of amino acid glycine 126 with maleic anhydride 97 in acetic acid. This reaction yields the intermediate amide 127 which is immediately cyclised by hexamethyldisilazane (HMDS) in the presence of zinc bromide to yield to target compound 23. A sample of 23 was subsequently methylated by Steglich esterification with methanol to yield recognition-disabled C1 maleimide 21 in good yield.

Scheme 6.7 Synthesis of recognition-enabled maleimide 23 and recognition-disabled maleimide 21. i) AcOH, RT, 99%. ii) HMDS, ZnBr$_2$, MeCN, 90 °C, 81%. iii) MeOH, DCC, THF, 0 °C → RT, 69%.

In order to demonstrate the efficiency of the proposed fluorescent replicator trans-124, a sample containing an equimolar amount of nitrone 122 and either maleimide 21 or 23 was prepared ([122] = [21 or 23] = 5 mM) in CDCl$_3$ and allowed to react at 4 °C. The progression of the reaction was followed by 500 MHz $^1$H NMR spectroscopy, monitoring the disappearance of the maleimide protons and the appearance of the characteristic protons of the forming isoxazolidine ring system. Deconvolution of the data obtained on a sample allowed for the construction of the concentration vs. time profiles in Figure 6.7.

Scheme 6.8. The 1,3-dipolar cycloaddition between nitrone 122 and maleimides 21 or 23 gives rise to two diastereoisomeric products, trans-128 and cis-128, or trans-124 and cis-124. The cis isomers of this reaction are not catalytically active and are omitted for clarity.
After 16 hours, the reaction between recognition-disabled maleimide 21 and nitrone 122 showed just 8% conversion into cycloadduct 128 with 6:1 selectivity for trans-128 ([trans-128] = 0.34 mM, [cis-128] = 0.06 mM). This low conversion is an expected consequence of both the reaction only proceeding via the bimolecular pathway and the significantly lower starting concentrations of 122 and 21. It is worth noting that the unusually high trans:cis ratio of 6:1 is likely caused by the very small concentration of cis-128 detected being within experimental error.

When the reaction is repeated using recognition-enabled maleimide 23 in place of 21, conversion after the same time rises to 39% with trans-124 reaching a concentration of 1.74 mM, while cis-124 was detected at a concentration of just 0.19 mM (trans:cis = 9:1). The reaction profile was subjected to simulation and fitting to a model for a bimolecular reaction using the SimFit software package. The relevant rate constants are displayed in Table 6.2.

### Table 6.2

<table>
<thead>
<tr>
<th></th>
<th>trans-124</th>
</tr>
</thead>
<tbody>
<tr>
<td>bimolecular rate constant</td>
<td>$2.71 \times 10^{-4}$</td>
</tr>
<tr>
<td>recognition-mediated rate</td>
<td>$6.89 \times 10^{-4}$</td>
</tr>
<tr>
<td>effective molarity</td>
<td>2.54</td>
</tr>
<tr>
<td>$\Delta G^\circ$ / kJ mol^{-1}</td>
<td>-44.7</td>
</tr>
</tbody>
</table>
6.7 Comparison of the Two Self-Replicators

It is clear from the concentration vs. time profiles of the two replicators that they are of very different efficiencies. For the replicator trans-123, based on longer recognition-enabled maleimide 28, a clear sigmoidal profile is seen. The replicator trans-124 is much slower at this concentration and as the reaction never reaches the point where the starting materials become rate-limiting, a similar sigmoidal profile is not observed. The higher EM value for trans-123 reflects the very different efficiencies in the two replicators.

A possible explanation for the much lower efficiency of trans-124 is the higher free energy of connection, $\Delta G^s$, than that of trans-123. The higher $\Delta G^s$ value indicates that the [trans-124•trans-124] duplex is stronger than the corresponding [trans-123•trans-123] duplex and as a result, there is less catalytically active template in solution. As a self-replicating species is by nature self-inhibiting, a stronger duplex results in a less efficient replicator as catalytic turnover is limited. This would go someway to explaining why trans-124 experiences a much lower rate enhancement upon reaction with recognition-enabled maleimide 23, despite the fact the bimolecular reactions are at comparable rate.

Kinetic analysis of this pair of replicators revealed they are far from comparable in efficiency, thus any competition between them is biased heavily towards trans-123. Nevertheless, when a sample containing an equimolar amount of nitrone 122 and each of the two maleimides 28 and 23 ([122] = [28] = [23] = 5 mM) is prepared in CDCl$_3$ and allowed to react at 4 °C, $^1$H NMR spectroscopy reveals that trans-123 is unable to completely outcompete trans-124 for the limited nitrone 122 (Figure 6.8).

![Figure 6.8](image.png)

Figure 6.8. a) Partial 400 MHz $^1$H NMR spectra showing the characteristic isoxazolidine singlets of self-replicators trans-123 and trans-124 as the outcome of the competition between recognition-enabled maleimides 28 and 23 for nitrone 122 at 5 mM and 4 °C. b) Pie chart representation of the relative concentrations of trans-123 ($\star$) and trans-124 (•) as detected by $^1$H NMR spectroscopy.
After two days at 4 °C, the concentration of each replicator was determined by 400 MHz $^1$H NMR spectroscopy and comparison with a known concentration of tert-butylbenzene as an internal standard. The ratio of trans-123 to trans-124 was 3.7:1 with 3.02 mM of nitrone 122 being incorporated into trans-123 and 0.82 mM into trans-124. The increased efficiency of trans-123 clearly leads to a dominance over weaker replicator trans-124 but this simple system can in no way be deemed selective.

Self-replicators operate via a template effect that is only possible once a sufficient concentration of a template is present. In these systems, template is formed via the uncatalysed bimolecular reactions and is the cause of the ‘lag period’ observed in the concentration vs. time profiles. If the competition between these two replicators is to be instructed by addition of preformed trans-123 or trans-124, it is important to demonstrate that no crosscatalytic pathways exist between them and they remain linked to one another only by a shared requirement for nitrone 122. To this end, the reactions outlined in Schemes 6.6 and 6.7 were repeated in the presence of a small amount of each template (Figure 6.9).

---

**Figure 6.9.** Concentration vs. time plots of the reaction of a) recognition-enabled maleimide 28 and nitrone 122 ([28] = [122] = 5 mM) forming products trans-123 (●) in the presence of 10 mol % of preformed trans-123 and b) in the presence of trans-124, and c) recognition-enabled maleimide 23 and nitrone 122 ([23] = [122] = 5 mM) forming products trans-124 (●) in the presence of 10 mol % of preformed trans-123 and d) in the presence of trans-124.
In the presence of 10 mol % of \textit{trans}-123, the reaction between nitrone 122 and maleimide 28 is able to proceed immediately via the replication cycle and no lag period is observed in the concentration vs. time profile (Figure 6.9a). When the reaction is repeated in the presence of 10 mol % of the other self replicator \textit{trans}-124, a clear sigmoidal shape is seen (Figure 6.9b). This failure of \textit{trans}-124 to abolish the lag period demonstrates that it is unable to catalyse the reaction of 122 and 28 and the replication cycle only opens up once \textit{trans}-123 is formed through the slow bimolecular reaction.

The reaction between recognition-enabled C1 maleimide 23 and nitrone 122 exhibits similar behaviour to the introduction of either preformed template. Figure 6.9d shows that the presence of 10 mol % of \textit{trans}-124 overcomes the initial lag period while Figure 6.9c underlines the inability of the mismatched template \textit{trans}-123 to catalyse the reaction of 122 and 23. Calculating the molecular structures of both self-replicating templates \textit{trans}-123 and \textit{trans}-124 clearly shows the size difference and it is easy to understand why no crosscatalytic pathways can exist (Figure 6.10).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6_10.png}
\caption{Calculated molecular structure of a) \textit{trans}-123 and b) \textit{trans}-124. The two self-replicators are of different sizes and although they bear identical and complementary recognition sites cannot form heteroduplex c). The calculations were performed by molecular mechanics using the OPLS2005 forcefield and the GB/SA model for solvation.}
\end{figure}
6.8 Isolated Chemical Waves

6.8.1 Long-Replicator trans-123

The original system described in Scheme 6.1 was transferred successfully into an observable chemical wave depicted in Figure 6.4. The progression of a blue reaction front was clearly seen against a bright yellow background of unreacted nitrone. However the wave did not propagate along the length of the syringe for a significant distance before the background reaction obscured it from view. When the proposed competition is transferred into the wave front, it is the wave front itself that is expected to drive selectivity. A more persistent wave will provide a better platform on which the competition can operate and result in an easier analysis of the results. A further reduction in the starting material concentration is likely to further suppress the bimolecular background reaction that causes the visual obscuration of the chemical wave.

Recording the kinetic profile of the reaction of nitrone 122 and maleimide 28 at 2.5 mM and subsequent deconvolution of the acquired data results in the concentration vs. time profile for trans-123 presented in Figure 6.11. For comparison purposes, the profile recorded at 5 mM is superimposed using the right-hand side y-axis and demonstrates the differing shapes at the two concentrations. As a consequence of the fluorescent properties of both nitrone 122 and cycloadduct trans-123, the characteristic yellow (122) and blue (trans-123) colours were easily observable even at such a low concentration.

Figure 6.11. Concentration vs. time plots of the reaction of recognition-enabled maleimide 28 and nitrone 122 ([28] = [122] = 2.5 mM) forming product trans-123 (●, left-hand y-axis). Overlaid is the same reaction performed at 5 mM from Figure 6.6a ([28] = [122] = 5 mM) forming trans-123 (◆, right-hand y-axis). The cis isomers of this reaction are not catalytically active and are omitted for clarity.
At the end of the 16 hour monitoring period, the reaction at 2.5 mM reached the same level of conversion observed when the reaction was performed at 5 mM (82%). The reaction profiles however are very different. The reaction at 2.5 mM exhibits a less steep sigmoidal curve, typical of a replicating system in which the simple bimolecular reaction contributes significantly less to the total product formation. It was expected that the dilution of starting materials \textbf{28} and \textbf{122} would prevent the background reaction from undermining the doping effect and result in a chemical wave that remains visible for longer.

With a suitable concentration potentially identified, an attempt to induce a chemical wave was conducted using the self-replicating system. An equimolar amount of nitrone \textbf{122} and maleimide \textbf{28} ([\textbf{122}] = [\textbf{28}] = 2.5 mM) were taken up in a glass syringe before seeding at one end with a solution of preformed \textit{trans-123}. The reaction was performed at 4 °C and followed by time-lapse photography under ultraviolet irradiation. Further details of this experimental set-up can be found in \textbf{Chapter 8}. The progression of a chemical wave of \textit{trans-123} can be seen in selected photographs in \textbf{Figure 6.12}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure612.png}
\caption{Time-lapse photographs of the chemical wave produced by seeding a mixture of \textbf{122} and \textbf{28} ([\textbf{122}] = [\textbf{28}] = 2.5 mM) with preformed \textit{trans-123}. A propagating front of \textit{trans-123} can be observed in the syringe. The background reaction to \textit{trans-123} causes fringing along the syringes and causes product to begin to build up, obscuring the wave after around 210 minutes. Right-hand side, the images have been processed for clarity.}
\end{figure}
The images reveal a clearly visible front propagating along a significant section of the syringe. The background reaction between 28 and 122 does not cause significant problems until around 210 minutes after the reaction is initiated - considerably longer than observed for the original system at 5 mM (Figure 6.4). In this time the wave has propagated through a total volume of 15 - 20 µL. This volume is sufficient for analysis using ¹H NMR spectroscopy using a micro sample tube.

6.8.2 Short-Replicator trans-124

Recording the kinetic profile of the reaction of nitrone 122 and maleimide 23 at 2.5 mM and subsequent deconvolution of the acquired data results in the concentration vs. time profile for trans-124 presented in Figure 6.13. For comparison purposes, the profile recorded at 5 mM is superimposed using the right-hand side y-axis and demonstrates the differing shapes at the two concentrations. As a consequence of the much lower replicating efficiency of trans-124, the reaction between 23 and 122 does not reach the same end point when performed at 2.5 mM as it did when performed at 5 mM.

![Figure 6.13](image)

Concentration vs. time plots of the reaction of recognition-enabled maleimide 23 and nitrone 122 ([23] = [122] = 2.5 mM at t=0) forming product trans-124 (●, left-hand y-axis). Overlaid is the same reaction performed at 5 mM from Figure 6.6a ([23] = [122] = 5 mM) forming trans-124 (◆, right-hand y-axis). The cis isomers of this reaction are not catalytically active and are omitted for clarity.

The lower concentration of 2.5 mM considerably slows the progress of replicator trans-124. The recognition-mediated reaction is so slow that after 16 hours at 4 °C the reaction has reached just 16% conversion with 0.40 mM of trans-124 detected in solution. Despite this very low conversion, trans-124 does exhibit a non-linear reaction profile and responds in an autocatalytic fashion to the addition of preformed...
template. With this knowledge at hand, equimolar amounts of nitrone 122 and maleimide 23 ([122] = [23] = 2.5 mM) were taken up in a glass syringe before seeding at one end with a solution of preformed trans-124 in an attempt to induce a chemical wave. As before, the reaction was performed at 4 °C and followed by time-lapse photography under ultraviolet irradiation. Further details of this experimental set-up can be found in Chapter 8. The progression of a chemical wave of trans-124 can be seen in selected photographs in Figure 6.14.

![Figure 6.14. Time-lapse photographs of the chemical wave produced by seeding a mixture of 23 and 122 ([23] = [122] = 2.5 mM) with preformed trans-124. A propagating front of trans-124 can be observed in the syringe. Right-hand side, the images have been processed for clarity.](image)

The photographs reveal a clearly visible propagating reaction front along a significant section of the syringe. Intriguingly, what appears to be the background reaction forming trans-124 obscures the wave after around 210 minutes. This observation is surprising bearing in mind the kinetic profiles of these reactions indicate a window far longer than 3.5 hours before the background reaction reaches significant conversion. Investigation into this unexpected observation suggested that the nitrone 122 may undergo a photo-degradation process under prolonged exposure to ultraviolet irradiation. Nitrones are reasonably light-sensitive and as exhaustively demonstrated in Chapter 3 and 4, undergo hydrolysis in acidic conditions to give the corresponding hydroxylamine and aldehyde. It is therefore quite possible that trace amounts of HCl,
the main contaminant in chloroform, and exposure to ultraviolet light catalyses the hydrolysis of 122 to hydroxylamine 130 and aldehyde A. This hypothesis is seemingly confirmed by the observation of the blue fluorescence emitted by a sample of 130 upon UV irradiation. It is likely that the fringing observed along the syringe is a result of the combination of hydrolysis to 130 and bimolecular reaction forming trans-124.

However, the propagating blue colour can be confirmed to be autocatalytic trans-123 or trans-124 by comparing Figures 6.11 and 6.13 with the images recorded on a control experiment displayed in Figure 6.15. In this instance, a syringe was filled with an equimolar amount of nitrone 122 and maleimide 28 ([122] = [28] = 2.5 mM) in CDCl₃ and allowed to react at 4 °C. The experimental set-up is identical to those described in Figures 6.11 and 6.13 aside from the addition of either template to seed the wave. Without the addition of the instructional template it is clear that no chemical wave is formed in the control experiment.

Figure 6.15. Time-lapse photographs of the reaction of 28 and 122 ([28] = [122] = 2.5 mM). Without the addition of trans-123 to seed the wave, no propagating front of trans-123 is observed in the syringe. The blue colour observed after 210 minutes is likely from a combination of the bimolecular reaction leading to trans-123 and the hydrolysis of nitrone 122 to hydroxylamine 130. Right-hand side, the images have been processed for clarity.
6.9 Competition in a Closed System: Kinetic Selection

Before the proposed competition can be allowed to operate in an open environment, it is important to demonstrate that this system is also bound by the confines of kinetic selection in a closed system. The two replicators have been shown to respond only to addition of the correct template and demonstrate no cross-catalytic activity. In the native reaction, containing no dopant, \textit{trans-123} was shown to outcompete \textit{trans-124} to a ratio of 3.7:1 (Figure 6.8b). The nature of this system allows one to influence the outcome of the competition by input of one of the two templates at the start of the reaction. In order to demonstrate the level of selectivity achievable by such an addition, samples containing nitrone 122 and maleimides 23 and 28 ([122] = [23] = [28] = 5 mM) and 10 mol % of either \textit{trans-123} or \textit{trans-124} were prepared in CDCl$_3$ and allowed to react at 4 °C. After a period of two days, the concentration of each replicator was determined by 400 MHz $^1$H NMR spectroscopy by comparison with an internal standard (\textit{tert}-butylbenzene) (Figure 6.16).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure616.pdf}
\caption{Pie charts representing the relative concentrations of newly formed \textit{trans-123} (●) and \textit{trans-124} (●) as as the outcome of the competition between recognition-enabled maleimides 28 and 23 for nitrone 122 at 5 mM and 4 °C and after correction for the added dopant in the presence of a) no dopant, b) 10 mol % of \textit{trans-123} and c) 10 mol % of \textit{trans-124}. Partial 400 MHz $^1$H NMR spectra showing the characteristic isoxazolidine singlets of self-replicators \textit{trans-123} and \textit{trans-124} as the outcome of the competition in the presence of d) 10 mol % of \textit{trans-123} and e) 10 mol % of \textit{trans-124}.}
\end{figure}
In the presence of 10 mol % of trans-123 from the start of the reaction, the competition between maleimides 23 and 28 for nitrone 122 is pushed further towards self-replicator trans-123. After two days at 4 °C and correction for the additional 0.5 mM of trans-123 added into the reaction, trans-123 was present at 3.45 mM and trans-124 at 0.69 mM, this results in a ratio of 5:1. During the corresponding reaction where replicator trans-124 is present from the start, the ratio falls to under 2.5:1 with 2.86 mM of trans-123 and 1.15 mM of trans-124 present in solution. The output from the system can be influenced by addition of an instructional template but this instruction merely tips the scales towards one replicator, it does not induce exclusive product formation. As a consequence of the far lower efficiency of trans-124, it is unlikely that the transfer of the competition depicted in Figure 6.16b, doped with preformed trans-124, into an open environment would to lead to the extinction of more efficient trans-123, however such an environment may allow trans-123 to exterminate trans-124.

It was shown in the previous section that the distance a chemical wave of trans-123 or trans-124 travels along the syringe can be increased by halving the starting concentrations of 122 and 28 or 23 to 2.5 mM. The kinetic profiles for the individual self-replicators suggest differing behaviour at this lower concentration thus the outcome of the competition may differ from the more concentrated scenario. It is therefore important to push the closed system to the limit and demonstrate that kinetic selection still reigns at lower concentrations and that even an excess of instructional template cannot drive the system to exclusive formation of trans-123. A sample containing nitrone 122 and both maleimides 28 and 23 ([122] = [28] = [23] = 2.5 mM) was prepared in CDCl₃ and allowed to react at 4 °C. After a period of two days, the concentration of each replicator was determined by 400 MHz ¹H NMR spectroscopy by comparison with the internal standard tert-butylbenzene (Figure 6.16).

At 2.5 mM, trans-123 dominates the competition to a ratio of 5:1 reaching a concentration of 1.66 mM after two days. After the same time, self-replicator trans-124 has reached a concentration of just 0.33 mM. This ratio is different from the undoped competition at 5 mM in which the trans-123:trans-124 ratio was 3.7:1. At the lower concentration, trans-124 is even less efficient allowing trans-123 to consume even more of the shared nitrone resource 122.
In order to ascertain the absolute limit of selectivity within a closed system, the above competition was repeated in an environment containing an excess of instructional template. Ideally, the competition would be run in the presence of 100 mol % of \textit{trans-123} however the limited solubility of \textit{trans-123} in CDCl$_3$ prohibited the preparation of suitably concentrated stock solution. Therefore, a sample containing nitrone \textit{122} and both maleimides \textit{28} and \textit{23} ([\textit{122}] = [\textit{28}] = [\textit{23}] = 2.5 mM) was prepared in CDCl$_3$ and dispensed into a sample vial containing 100 mol % of solid \textit{trans-123}. This operation resulted in a solvent system saturated with instructional template that was allowed to react at 4 °C for two days. After two days the sample was concentrated and the residue dissolved in deuterated dimethylsulfoxide (DMSO) and analysed by $^1$H NMR spectroscopy. The concentration of each replicator was determined by comparison with the internal standard tert-butylbenzene and is shown in Figure 6.18.

**Figure 6.17.** a) Partial 400 MHz $^1$H NMR spectra showing the characteristic isoxazolidine singlets of self-replicators \textit{trans-123} and \textit{trans-124} as the outcome of the competition between recognition-enabled maleimides \textit{28} and \textit{23} for nitrone \textit{122} at 2.5 mM and 4 °C. b) Pie chart representation of the relative concentrations of \textit{trans-123} (●) and \textit{trans-124} (●) as detected by $^1$H NMR spectroscopy.

**Figure 6.18.** a) Partial 400 MHz $^1$H NMR spectra showing the characteristic isoxazolidine singlets of self-replicators \textit{trans-123} and \textit{trans-124} as the outcome of the competition between recognition-enabled maleimides \textit{28} and \textit{23} for nitrone \textit{122} at 2.5 mM and 4 °C, in a solution saturated with \textit{trans-123}. b) Pie chart representation of the relative concentrations of \textit{trans-123} (●) and \textit{trans-124} (●) as detected by $^1$H NMR spectroscopy and after correction for the added dopant.
After the correction of the added dopant, self-replicator trans-123 outcompeted trans-124 to a ratio of around 9:1 reaching a concentration of 1.86 mM while trans-124 reached just 0.21 mM. Despite the very low concentration of each starting material and a solvent system saturated with instruction template during the course of the reaction, trans-123 remains unable to wipe out weaker rival trans-124. It is clear from the results of the doping experiments that the two competing replicators are separate from one other, as a consequence of their differing size, and that despite a clear hierarchy in their efficiencies, exclusive trans-123 formation in a closed system simply cannot be achieved.

6.10 Competition in an Open System: Survival of the Fittest?

We have demonstrated previously that the autocatalytic nature of trans-123 enabled one to induce a chemical wave inside a glass syringe (Figure 6.12). In this scenario, only the required maleimide 28 and nitrone 122 were present in the syringe. In order to transfer the competition into an open system, the wave experiments were repeated where both maleimides 28 and 23 and nitrone 122 were drawn into the syringe ([28] = [23] = [122] = 2.5 mM). Once again, the chemical wave was initiated by carefully drawing 5 µL of a 2.5 mM solution of preformed trans-123 into the end of the syringe and allowed to propagate. To minimise the suspected photodegradation of nitrone 122, exposure to UV light was minimised (see Chapter 8). Once the wave front of trans-123 had progressed through 15 - 20 µL, this volume was carefully transferred into a micro NMR sample tube and immediately analysed by 500 MHz $^1$H NMR spectroscopy.

![Figure 6.19](image)

**Figure 6.19.** a) Partial 500 MHz $^1$H NMR spectra showing the characteristic isoxazolidine singlets of self-replicator trans-123 as the exclusive product from the competition between recognition-enabled maleimides 28 and 23 for nitrone 122 at 2.5 mM and 4°C. b) Pie chart representation of the relative concentrations of trans-123 (●) and trans-124 (●) as detected by $^1$H NMR spectroscopy.
Figure 6.19 shows that the analysis of the wave revealed the formation of a single product, \( \text{trans-123} \). The sample was subjected to an increased number of scans (1K) but there was no trace of \( \text{trans-124} \) detected. The replicator \( \text{trans-123} \) has caused nitrone \( 122 \) to exclusively react with maleimide \( 28 \) over competitor maleimide \( 23 \). In a closed system, as \( \text{trans-123} \) consumes \( 28 \), the rate of its own production is decreasing, allowing the slower \( \text{trans-124} \) to catch up. However in an open environment, the concentration of \( 28 \) never becomes rate-limiting and \( \text{trans-123} \) is able to efficiently react for longer, increasing its dominance over \( \text{trans-124} \). Moving into open systems has enabled the more efficient replicator \( \text{trans-123} \) to effectively outcompete the less efficient \( \text{trans-124} \) and has finally resulted in its own exclusive formation.

6.11 Conclusions

The phenomena associated with non-linear reaction dynamics are becoming better understood every day, but their incorporation into everyday synthetic chemistry remains in its infancy. Theoretical calculations and simulations have suggested applications for non-linear dynamics that would allow systems to break free from conventional selectivity constraints but experimental evidence backing these up is scarce. Propagating reaction fronts have been identified as one such means of achieving higher levels of selectivity that are otherwise unobtainable in closed reactions. While examples of such fronts are prevalent in nature and have been demonstrated to exist in simple inorganic systems, no chemical waves based on wholly synthetic systems currently exist.

In this chapter, we describe the rational design and synthesis of two appropriate chemical systems that are subsequently used to demonstrate chemical waves visible to the naked eye. Self-replicators \( \text{trans-123} \) and \( \text{trans-124} \) are formed from fluorescent nitrone \( 122 \) upon reaction with either maleimide \( 28 \) or \( 23 \). The autocatalytic nature of these replicators provides a means of initiating a reaction at a specific point in space and, as a consequence of the fluorescent properties of \( 122 \) and replicators \( \text{trans-123} \) and \( \text{trans-124} \), one can watch the progress of the diffusing chemical reaction in real-time.

The two replicators were designed carefully to share a common building block \( 122 \). Both \( \text{trans-123} \) and \( \text{trans-124} \) require \( 122 \) for their own synthesis and are able to
compete for a limited supply of the nitrone to different extents. We have demonstrated that in a closed system following the model of a well-stirred batch reactor (WSBR), it is not possible to direct the competing system to exclusive product formation of one replicator over the other. Despite the very different efficiencies of trans-123 and trans-124 at the conditions employed during the competition, significant input of instructional template was unable to direct the system entirely towards trans-123. Even with the very low efficiency of trans-124 and the scales tipped heavily towards trans-123, both replicators coexisted within the closed system. The highest level of selectivity towards trans-123 achieved was ~9:1.

The transfer of the competition away from a closed system into an open environment allowed trans-123 to achieve the desired higher level of selectivity. When the competition is allowed to operate within the chemical wave, trans-123 is the exclusive product detected. The ability for trans-123 to diffuse into a pool of starting materials allows the replicator to operate at its optimum efficiency for the entirety of the reaction while at the same time decreasing the efficiency of its competitor trans-124. This level of selectivity is not possible in closed systems and maybe regarded as a chemical example of evolution and demonstrates that even at a molecular level, it is survival of the fittest.
Conclusions

7.1 General Conclusions

The research presented in this thesis underlines the fact that the selectivity a replicating species can achieve in a closed system is inherently limited. Replicating species are, by their nature, self-inhibiting in the confines of a closed system and while an efficient replicator quickly exhausts its resource supply, a weaker competitor is allowed to catch up. The application of reaction diffusion fronts finally breaks the stranglehold of kinetic selection within a closed system and provides a platform on which a more efficient replicator is able to drive a weaker competitor into extinction, demonstrating Darwinian Selection at a molecular level.

This work describes the construction of a number of dynamic libraries from which target compounds are able to emerge, however selection between multiple emerging species is very difficult to bias. Molecular recognition is used to select appropriate chemical species within a library for irreversible removal from the dynamic equilibrium. The strength of the molecular recognition is altered by controlling the electron density around the recognition site and is used to provide chemical species recognition-enabled to different degrees. Using reactions whose rates are enhanced simply by molecular recognition or species capable of self-replication provided two suitable competition scenarios that could emerge from within the same library. In both cases, the recognition-enabled species were both selected from the libraries and a bias towards one of them could not be induced. Both competitions were introduced to a number of dynamic libraries in which the concentrations of each species varied considerably, however no library could induce an imbalance in the final concentration of each competitor.
Kinetic measurements and subsequent simulations revealed the intricacies of the coupled systems. Rapid exchange reactions within the dynamic library eroded any temporary imbalance in the concentration of the nitrones and kinetic selection prevails. The simulations also revealed a dependence on the concentration of each library component for the outcome of the competition. Simulations performed at decreasing concentrations were shown to positively affect the difference between the two emerging species. However, the difference in the association of each competitor with the limiting reagent was too small to provide a substantial distribution in the concentration of reactive complexes and resulting products.

The autocatalytic nature of a self-replicating compound makes them ideal targets for implementation into non-linear reaction dynamics and reaction diffusion fronts. The development of a chemical reaction that one can observe in real-time with the naked eye has allowed for the demonstration of a chemical wave. In contrast to a closed system such as a traditional reaction flask, an autocatalytic reaction exhibiting a propagating front is not self-inhibiting. As the wave of product diffuses through a solution of starting materials, the concentration of building blocks at the reactive site never decreases. In this way, an autocatalytic process is always performing at optimal efficiency. The competition between two autocatalytic processes, linked together through a mutual requirement of a specific building block, has a different outcome when allowed to play out within such an open system.

While Systems Chemistry is still in its infancy, these encouraging results identify potential applications of non-linear dynamics and the advantages of open chemical systems. As well as highlighting the shortcomings of traditional closed-flask chemistry, chemical waves have allowed a wholly synthetic replicating system to exhibit Darwinian Selection on a molecular level.

7.2 Future Work

The competition between replicators \textit{trans}-123 and \textit{trans}-124 described in Chapter 6 is heavily biased towards \textit{trans}-123 as a result of its higher efficiency. Future work will probe the application of reaction diffusion systems to the competition between better matched replicators. Based on similar replicating systems developed in our laboratory, two potential competition scenarios have been identified. The first is based on restructuring the system in such a fashion that the nitrone carries the
carboxylic acid 131 and two maleimides of differing length 37 and 133 are allowed to compete for it (Scheme 7.1). Previous work within the group has demonstrated that both maleimides 37 and 133 form efficient replicators when combined with carboxy nitrones such as 131. The competition between them is expected to result in around 2:1 selectivity for trans-132 over trans-134. This is a much better match than trans-123 and trans-124 and will further test the power of reaction diffusion systems.

Early attempts at investigating this system have been hampered by the very low solubility of 131 in CDCl₃ or CD₂Cl₂. Attaching a solubilising group such as an aliphatic chain to the anthracene tag is likely to improve the solubility of both 131 and the two replicators trans-132 and trans-134 and allow for this system to be investigated.

Scheme 7.1. Nitrone 131 can react with either maleimide 37 to form trans-132 or with maleimide 133 to form the longer template trans-134. The two formed templates are of different sizes and are therefore ideal components to be introduced to reaction-diffusion systems. In addition, their efficiencies are better matched than previous systems.
7. Conclusions

The combination of maleimide 135 with nitrone 122 would furnish template \textit{trans-136} which is expected to be an efficient replicator (Scheme 7.2). Maleimide 122 bears a aliphatic solubilising group and has been used\cite{78} within the group to construct efficient replicators. As a consequence of the \textit{meta} substituted carboxylic acid, lacking a methylene spacer group results in template \textit{trans-136} being shorter than \textit{trans-123} and able to participate in a competition for nitrone 122.

Scheme 7.2. Nitrone 122 can react with either maleimide 28 to form \textit{trans-123} or with maleimide 135 to form the longer template \textit{trans-136}. The two formed templates are of different sizes and are therefore ideal components to be introduced to reaction-diffusion systems. In addition, their efficiencies are better matched than previous systems.

Both of these systems have the potential to be transferred into open systems and further test the application of non-linear dynamics to replicating networks. Further scope for this work would be an attempt to overcome the selectivity limits encountered in the study of multicyclic replicating networks.
8 Experimental

8.1 General Procedures

All chemicals and solvents were purchased from ABCR GmbH & Co, Alfa Aesar, Apollo Scientific Ltd., Fisher Scientific UK Ltd., TCI UK Ltd., Sigma–Aldrich Company Ltd. or VWR International Ltd. and purified by standard techniques where necessary. Where appropriate, all non-aqueous reactions were carried out under an inert N₂ or Ar atmosphere with the inert gas passing through a bed of 4 Å molecular sieves and self-indicating silica gel. Brine refers to a saturated aqueous solution of sodium chloride. Anhydrous solvents were obtained under the following conditions: dry MeCN was distilled from calcium hydride in a recycling still; dry CH₂Cl₂ and THF was obtained using a MBRAUN GmbH MB SPS–800 solvent purification system, where solvent was dried by passage through filter columns and dispensed under an atmosphere of N₂ or Ar gas; Analytical Thin Layer Chromatography (TLC) analysis was performed on MACHEREY–NAGEL GmbH & Co. POLYGRAM SIL G/UV₂₅₄ plates. Developed plates were air–dried and visualised under a UV lamp (λₓₐₙₐₓ 254 or 366 nm). Flash Column chromatography and silica plugs were carried out on Apollo Scientific Ltd. silica gel 40–63 micron or Silicycle SiliaFash P60 silica gel (230–400 mesh) eluting with solvents as supplied under a positive pressure of compressed air. Melting points were determined using an Electrothermal 9200 melting point apparatus and are uncorrected. Mass spectra were recorded on a Micromass GCT spectrometer for electron impact ionisation (EI) operating at 70 eV or chemical ionisation (CI) using isobutane as the ionising gas. Electrospray ionisation spectra (ES) were performed on a Micromass LCT spectrometer operating in positive or negative mode from solutions of methanol, acetonitrile or water. m/z values are reported in Daltons and followed by their percentage abundance in parentheses.
8.2 General NMR spectroscopy Procedures

**1H NMR** spectra were recorded on a Bruker Avance 500 (500.1 MHz), a Varian UNITYplus 500 (500.1 MHz), a Bruker Avance II 400 (400.1 MHz) or a Bruker Avance 300 (300.1 MHz) spectrometer using the deuterated solvent as the lock and the residual solvent as the internal reference in all cases. In the assignment of **1H NMR** spectra the chemical shift information ($\delta_H$) for each resonance signal is given in units of parts per million (ppm) relative to trimethylsilane (TMS) where $\delta_H$ TMS = 0.00 ppm. The number of protons (n) for a reported resonance signal are indicated as nH from their integral value and their multiplicity by the symbol in parentheses. Their coupling constants ($J$) are determined by analysis using the iNMR software (Version 3.6.1, Mestrelab Research, 2007) quoted to the nearest 0.1 Hz. Identical coupling constants are averaged in each spectrum and are reported to the nearest 0.1 Hz.

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

**19F NMR** spectra were recorded using a Bruker Avance 500 (470.3 MHz), a Bruker Avance II 400 (376.5 MHz) or a Bruker Avance 300 (282.4 MHz) spectrometer using a broadband proton decoupling pulse sequence with the deuterated solvent as the internal lock. The chemical shift informations ($\delta_F$) for each resonance signal are given in units of parts per million (ppm) relative to CCl$_3$F where $\delta_F$ CCl$_3$F = 0.00 ppm. All **1H**, **13C** and **19F** spectra were analysed using iNMR software (Version 3.6.3, Mestrelab Research, 2010). Individual structural assignments were assisted by the use of **1H** COSY, HSQC and HMBC experiments.
8.3 Kinetic Measurements and Deconvolution of NMR Data

8.3.1 $^1$H NMR Spectroscopy

500.1 MHz $^1$H NMR spectroscopy was used for the kinetic analysis of reactions described in Chapters 3, 4 and 6. Stock solutions of the appropriate reagents were prepared in fresh solvent using a Sartorial BP211D balance (± 0.01 mg) and equilibrated in a thermostatically controlled water bath at the desired reaction temperature. In a typical experiment, an NMR sample was prepared in a 5 mm NMR tube (Wilmad 528PP) by mixing the appropriate volumes of stock solutions so that the total volume of the sample is 800 µL and each component is present at the desired concentration. A polyethylene pressure cap is applied to the sample to prevent solvent evaporation. The sample was transferred to an NMR spectrometer (Varian UNITYplus) regulated at the desired temperature and 500.1 MHz $^1$H NMR spectra were automatically acquired at a given time interval for a period of 16 to 36 hours (Figure 8.1). Analysis and deconvolution of the collected data was performed using iNMR software (Version 3.6.1, Mestrelab Research, 2010).

Figure 8.1. Partial stack plot of 500 MHz $^1$H NMR spectra recorded during a typical kinetic experiment. Note the appearance of a cycloadduct proton at ~5.6 ppm and the disappearance of a nitron signal at ~8.4 ppm.
The progression of the reaction was calculated by monitoring the disappearance of the maleimide protons and the appearance of the characteristic protons on the isoxazolidine ring system of the cycloadducts. The percentage completion of the reaction is used to calculate the concentration of product at each time-point and thus allows for the construction of concentration vs time profiles for the monitored process.

The irreversible chemical reaction monitored in the described systems was the 1,3-dipolar cycloaddition between a maleimide and a nitrone, giving rise to two diastereoisomeric products. The two diastereoisomers are easily distinguishable by $^1$H NMR spectroscopy due to the relative orientation of the protons on the isoxazolidine ring system (Figure 8.2). In the trans isomer, the dihedral angle between protons $H^1$ and $H^2$ is $\sim$90°. The Karplus equation predicts a coupling constant of 0 Hz between these protons, thus $H^1$ appears as a singlet in the spectrum. The angle between protons $H^2$ and $H^3$ is $\sim$20° and in accordance with the Karplus equation, both display a coupling constant of 8 Hz. In the cis isomer, all protons form dihedral angles of $\sim$20° and the expected coupling constant of 8 Hz is measured for the observed doublets.

Figure 8.2. Partial 400 MHz $^1$H NMR spectra featuring the characteristic splitting patterns observed for the a) trans- and b) cis- cycloadducts and c) a control experiment containing both isomers. The trans isomer exhibits a singlet ($H^1$), doublet ($H^3$) and doublet ($H^2$). The cis isomer exhibits a doublet ($H^1$), doublet ($H^3$) and doublet of doublets ($H^2$).
8.3.2 $^{19}$F NMR Spectroscopy

470.4 MHz $^{19}$F NMR spectroscopy was used for the kinetic analysis of the competition reactions described in Chapters 3 and 4. Stock solutions of the appropriate reagents were prepared in freshly prepared solvent using a Sartorial BP211D balance (± 0.01 mg) and equilibrated in a thermostatically controlled water bath at the desired reaction temperature. In a typical experiment, an NMR sample was prepared in a 5 mm NMR tube (Wilmad 528PP) by mixing the appropriate volumes of stock solutions so that the total volume of the sample is 800 µL and each component is present at the desired concentration. A polyethylene pressure cap is applied to the sample to prevent solvent evaporation. The sample was transferred to an NMR spectrometer (Bruker Avance 500) regulated at the desired temperature and 470.4 MHz $^{19}$F{H} NMR spectra were automatically acquired every 30 mins for a period of 16 hours. Analysis and deconvolution of the collected data was performed using iNMR software (Version 3.6.1, Mestrelab Research, 2007).

The progression of the reaction was calculated by monitoring the disappearance of the nitron fluorine signals and the appearance of the fluorine signals attributed to the cycloadduct products (Figure 8.3). The percentage completion of the reaction is used to calculate the concentration of product at each time-point and thus allows for the construction of concentration vs time profiles for the monitored process.

Figure 8.3. Partial stack plot of 470 MHz $^{19}$F NMR spectra recorded during the reaction of 83 with AZ and BZ. The disappearance of fluorine peaks of nitrones AZ and BZ and the subsequent appearance of cis-101 and cis-85 fluorine peaks are monitored.
8.3.3 Semi-automatic Deconvolution

The arrayed data recorded from either $^1$H or $^{19}$F NMR experiments was analysed using the semi-automatic deconvolution feature in the iNMR software package. Using a suitable script file, the software package opened each spectra individually and a least squares optimisation was performed on each selected signal to determine the area of the peak (Figure 8.4). The relative area of the peak attributed to the product compared to the starting material was calculated and used to determine the percentage completion of the reaction at each time-point. Based on the known concentration of the starting materials at $t = 0$, concentration data for each signal could be calculated. The resulting concentration vs. time data was plotted using the ProFit software package (Version 6.2.1, Quantum Soft, 2010). An example script can be found in the appendix.

![Deconvolution console in iNMR software package](image)

Figure 8.4. Examples of the deconvolution console in the iNMR software package as applied to a) a singlet and b) a doublet. The software runs a least-square optimisation to calculate the area of each signal in the spectrum which is then used to calculate the concentration of each species at a given time-point.
8.4 Construction and Analysis of Dynamic Systems

The analysis of the dynamic systems described in Chapters 3 and 4 requires multiple components to be present in accurate quantities. Stock solutions of the appropriate reagents were prepared in freshly prepared solvent, containing a known amount of 4-fluoronitrobenzene 95 as an internal reference, using a Sartoriel BP211D balance (± 0.01 mg) and equilibrated in a thermostatically controlled water bath at the desired reaction temperature. In a typical experiment, a 40 mM stock solution of the first aldehyde was prepared and used to dissolve an appropriate amount of the second aldehyde such that the concentration of both aldehydes was 40 mM. This solution was added an appropriate amount of the third aldehyde before the being added to the final aldehyde to obtain a stock solution containing each of the four aldehydes at 40 mM (Figure 8.5). The process was repeated using the the four nucleophiles W to Z to obtain a stock solution of nucleophiles.

![Flow diagram](image)

Figure 8.5. Flow diagrams outlining the preparation of a typical sample of a dynamic library coupled to a recognition process. Once the two stock solutions are prepared, they are combined with the maleimide and transferred to an NMR tube for analysis.
An NMR sample is prepared by combination of the two stock solutions in a vessel containing the appropriate maleimide, transferred to a 5 mm NMR tube (Wilmad 528PP) and sealed with a polyethylene pressure cap to prevent solvent evaporation. The sample is left to react in a thermostatically controlled water bath at the desired temperature for a period of 5 days before analysis by NMR spectroscopy.

The large number of structurally similar compounds present in the DCL makes the analysis of such systems by $^1$H NMR spectroscopy impossible. Each of the nucleophiles used to construct the library has been equipped with fluorine tags to facilitate analysis by $^{19}$F NMR spectroscopy. It was found that despite the structural similarities of many of the compounds, it was possible to identify and monitor each one by their characteristic fluorine signals (Figure 8.6).

Concentrations for each compound was calculated from the relative intensities of each individual signal, compared with the signal of the reference compound 4-fluoronitrobenzene 95.
8.5 Chemical Waves

8.5.1 Individual Waves

The propagating wave fronts described in chapter 6 were performed in 50 µL Hamilton 1700 series gas-tight glass syringes in a cold room regulated at 4 °C. Stock solutions of the appropriate reagents were prepared in fresh solvent using a Sartorial BP211D balance (± 0.01 mg) and equilibrated in the cold room prior to the experiment start. A sample was prepared by combination of the stock solutions in a small glass vial such that the concentration of both the nitrone 122 and either maleimide 28 or 23 was 2.5 mM and the total volume was 200 µL. 50 µL of the sample was carefully drawn into two 50 µL syringes. 5 µL of a 5 mM stock solution of either trans-123 or trans-124 was carefully drawn into one syringe to seed the chemical wave (Figure 8.7). The syringes were placed under a UV lamp (366 nm) for visualisation. Photographs of the two syringes were taken with a Pentax Optic W80 12.1 megapixel digital camera every 90 seconds using interval shoot mode for a period of 4-6 hours. As nitrone 122 has been shown to be light sensitive, an Ecotechnics Precision Second interval timer was used to synchronise the UV lamp with the camera, thus slowing the photo-degradation of 122. The photographs were processed using the Pixelmator software package (Version 1.6.7, Nucleus, 2011).

Figure 8.7. The progression of a chemical wave of trans-123 at 2.5 mM and 4 °C. The images have been processed for clarity. Additional images can be found in Chapter 6.
8.5.2 Competition within the Wave

The competition between maleimide 28 and 23 for nitrone 122 described in Chapter 6 was performed in 50 µL Hamilton 1700 series gas-tight glass syringes in a cold room regulated at 4 °C. Stock solutions of the appropriate reagents were prepared in fresh solvent using a Sartorial BP211D balance (± 0.01 mg) and equilibrated in the cold room prior to the experiment start. A sample was prepared by combination of the stock solutions in a small glass vial such that the concentration of nitrone 122 and both maleimides 28 and 23 was 2.5 mM and the total sample volume was 200 µL. 50 µL of the sample was carefully drawn into three 50 µL syringes. 5 µL of a 5 mM stock solution of trans-123 was carefully drawn into one syringe and trans-124 into another. The syringes were placed under a UV lamp (366 nm) for visualisation. Photographs of the two syringes were taken with a Pentax Optic W80 12.1 megapixel digital camera every 90 seconds using interval shoot mode for a period of 4-6 hours. As nitrone 122 has been shown to be light sensitive, an Ecotechnics Precision Second interval timer was used to synchronise the UV lamp with the camera, thus slowing the photo-degradation of 122. The length of the observed wave ~15-20 µL was dispensed into a micro NMR tube (New Era NE-H5/2-V-Br) and made up to the correct volume with fresh CDCl₃, and analysed by 500 MHz ¹H NMR spectroscopy. As a consequence of the dilute nature of the sample, an increased number of scans (>1K) was required to obtain a sufficient signal-to-noise ratio.

8.6 Kinetic Fitting, Simulation and Extraction of Rate Constants

All kinetic fitting and simulations were performed using the SimFit software package (Version 32, Günther von Kiedrowski, 2003). A detailed kinetic model of all possible interactions involved in the studied systems was constructed. This model was converted into a series of rate equations whose solution determine the concentration of reactant and product species as a function of time. The program varies experimentally inaccessible kinetic values until the calculation matches the experimental data. These rate constants are then used to calculate effective molarity (EM) and the free energy of connection ΔGₛ. Selected examples of kinetic models and simulation scripts can be found in the appendix.
8.6.1 Effective Molarity

The effective molarity, $\varepsilon$ or in more recent publications abbreviated as EM, provides a quantitative measure for the acceleration of the reaction in the ternary complex and is calculated from the rate constants obtained by the kinetic fitting process. In the case of self-replicating systems, the EM value can further be understood as the hypothetical concentration at which the background bimolecular reaction between the two reagents proceeds at the same rate as the intramolecular reaction within the ternary complex. It is calculated as follows, where $k_{\text{cat.}}$ is the recognition-mediated rate constant and $k_{\text{uncat.}}$ is the bimolecular rate constant as measure from the recognition-disabled process:

$$EM = \frac{k_{\text{cat.}}}{k_{\text{uncat.}}}$$

8.6.2 Free Energy of Connection

The free energy of connection, $\Delta G^s$, gives a direct measure of the quality of the template duplex in a self-replicating species. In any self-replicating species, duplex binding occurs by two identical recognition motifs; if the binding of the first site facilitates the binding of the second one, the free binding energy for the complex will be more negative than the sum of the two individual binding energies. This is called positive cooperativity and is typical in cases in which the templates form an optimal fit. If there is a mismatch in template geometry, the binding of the first site will inhibit the binding of the second site and the free binding energy for the complex will be more positive than the sum of the two individual binding energies, resulting in negative cooperativity. The magnitude of $\Delta G^s$ denotes the strength of the duplex, with a higher value indicating a strong duplex interaction while a lower value indicates a weaker one. Using constants obtained as a result of the fitting process allows $\Delta G^s$ to be calculated as follows, where $R$ is the gas constant, $T$ is the temperature, $K_{\text{ind}}$ is the individual association constant for the carboxylic acid and amidopyridine moieties and $K_{\text{duplex}}$ is an experimentally inaccessible association constant determined by the fitting process.

$$\Delta G^s = RT \ln \left( \frac{K_{\text{duplex}}}{K_{\text{ind}}^2} \right)$$
8.7 Synthetic Procedures

bis(Triphenylphosphine)palladium (II) dichloride PdCl$_2$(PPh$_3$)$_2$

Palladium (II) chloride (2.0 g, 11.27 mmol) and lithium chloride (1.06 g, 25.00 mmol) were dissolved in MeOH (40 mL) at room temperature. Triphenylphosphine (6.56 g, 25.00 mmol) was added and the reaction was refluxed at 80 °C under nitrogen for 1 hour. The resulting precipitate was filtered, washed with MeOH and dried under high vacuum to afford the product as a yellow solid. (6.1 g, 77%). $^{31}$P NMR (161.9 MHz, DMSO-d$_6$) δ 23.4.

4-Formylbenzoyl chloride

4-Formylbenzoic acid 93 (5.0 g, 33.30 mmol) was suspended in a mixture of toluene (80 mL) and thionyl chloride (10 mL) and refluxed at 110 °C overnight. The resulting brown solution was allowed to cool to room temperature and concentrated in vacuo. Excess thionyl chloride was removed by co-evaporating with further toluene (3 × 30 mL) and lyophilisation to yield 94 as a dark brown solid in sufficient quality for further conversion. $^1$H NMR (400.1 MHz, CDCl$_3$) δ 10.15 (1H, s, CHO), 8.28 (2H, d, $J$ = 8.1 Hz, Ar CH), 8.02 (2H, d, $J$ = 7.7 Hz, Ar CH). $^{13}$C NMR (75.5 MHz, CDCl$_3$) δ 191.1 (CHO), 167.9 (COCl), 140.5 (quat. Ar), 137.6 (quat. Ar), 131.9 (Ar CH), 129.9 (Ar CH).
**N-(4,6-Dimethylpyridin-2-yl)-4-formylbenzamide A**

Crude 4-formylbenzoyl chloride 94 (33.30 mmol) was dissolved in anhydrous DCM (60 mL) at 0 °C before triethylamine (5.6 mL, 40.00 mmol) and a solution of 2-amino-4,6-dimethylpyridine (4.9 g, 40.00 mmol) in anhydrous DCM (60 mL) were added to the reaction slowly over a period of 2 hours. The reaction was then stirred overnight while warming to room temperature. Excess amines were extracted with aqueous HCl (1M, 3 × 50 mL). The aqueous phase was washed with DCM (3 × 50 mL) and the combined organic layers were further washed with sat. aq. NaHCO$_3$ solution (2 × 50 mL). The organic phases were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude product was purified via silica gel flash chromatography (eluting with hexane:EtOAc 3:1) to yield 5.3 g (63% over two steps) of A as a white solid. Rf: 0.15 (hexane:EtOAc 3:2). M.p.: 130.5 - 131.0 °C. $^1$H NMR (400.1 MHz, CDCl$_3$) δ 10.11 (1H, s, CHO), 8.56 (1H, s, NH), 8.07 (2H, d, $J$ = 8.4 Hz, Ar CH), 8.03 (1H, s, Py CH), 8.01 (2H, d, $J$ = 6.8 Hz, Ar CH), 6.80 (1H, s, Py CH), 2.42 (3H, s, CH$_3$), 2.37 (3H, s, CH$_3$). $^{13}$C NMR (100.6 MHz, CDCl$_3$) δ 191.4 (CHO), 164.6 (CO), 156.6 (quat. Py), 150.5 (quat. Py), 150.4 (quat. Py), 139.6, (quat. Ar), 138.6 (quat. Ar), 130.0 (Ar CH), 127.9 (Ar CH), 121.1 (Py CH), 111.9 (Py CH), 23.7 (CH$_3$), 21.4 (CH$_3$). ES MS (neg. m/z) 253 (100% [M-H]$^-$). HRMS (m/z) [M+Na]$^+$ calcd. for C$_{15}$H$_{14}$N$_2$O$_2$Na, 277.0953; found, 277.0957.

**4-Formyl-N-(6-methylpyridin-2-yl)benzamide B**

Crude 4-formylbenzoyl chloride 94 (33.30 mmol) was dissolved in anhydrous DCM (60 mL) at 0 °C before triethylamine (5.6 mL, 40.00 mmol) and a solution of 2-
amino-6-methylpyridine (4.32 g, 40.00 mmol) in anhydrous DCM (60 mL) were added to the reaction slowly over a period of 2 hours. The reaction was then stirred overnight while warming to room temperature. Excess amines were extracted with aqueous HCl (1M, 3 x 50 mL). The aqueous phase was washed with DCM (3 x 50 mL) and the combined organic layers were further washed with sat. aq. NaHCO₃ solution (2 x 50 mL). The organic phases were dried over MgSO₄, filtered and concentrated in vacuo. The crude product was purified via silica gel flash chromatography (eluting with hexane:EtOAc 3:1) to yield 5.6 g (70% over two steps) of B as a white solid. Rf: 0.15 (hexane:EtOAc 3:2). M.p.: 105.5 - 106.6 °C. ¹H NMR (400.1 MHz, CDCl₃) δ 10.09 (1H, s, CHO), 8.86 (1H, s, NH), 8.17 (1H, d, J = 8.2 Hz, Py CH), 8.06 (2H, d, J = 8.3 Hz, Ar CH), 7.97 (2H, d, J = 8.3 Hz, Ar CH), 7.65 (1H, dd, J = 8.2, 7.5 Hz, Py CH), 6.94 (1H, d, J = 7.5 Hz, Ar CH), 2.42 (3H, s, CH₃). ¹³C NMR (75.5 MHz, CDCl₃) δ 191.5 (CHO), 164.7 (CO), 157.1 (quat. Py), 150.7 (quat. Py), 139.6 (quat. Ar), 139.0 (Py CH), 138.7 (quat. Ar), 130.0 (Ar CH), 128.1 (Ar CH), 120.0 (Py CH), 111.3 (Py CH), 24.0 (CH₃). ES MS (pos. m/z) 263 (100% [M+Na]⁺). HRMS (m/z) [M+Na]⁺ calcd. for C₁₄H₁₃N₂O₂, 241.0972; found, 241.0977.

\textbf{N-(4-Fluorophenyl)hydroxylamine}^{81} Z

\[ \text{HOHN} \]

Fluoro-4-nitrobenzene 95 (3.0 g, 21.26 mmol) was dissolved in THF (120 mL) and rhodium (5% wt. on carbon, 600 mg) was added. Hydrazine monohydrate (2.10 mL, 42.00 mmol) was added and the reaction was followed by TLC (cHexane: EtOAc 3:2). After completion (ca. 30 mins), MgSO₄ was added and the reaction stirred for a further 5 mins. The resulting solution was filtered through Celite and concentrated in vacuo. Recrystallisation from EtOAc/hexane afforded 2.21 g (82%) of Z as a brown solid. ¹H NMR (400.1 MHz, CDCl₃) δ 6.98-6.97 (2H, m, Ar CH), 6.96 (2H, d, J = 1.3 Hz, Ar CH). ¹³C NMR (75.5 MHz, CDCl₃) δ 158.7 (d, J = 240.2 Hz, Ar CF), 145.5 (d, J = 2.3 Hz, quat. Ar), 116.6 (d, J = 7.9 Hz, Ar CH), 115.7 (d, J = 22.7 Hz, Ar CH). ¹⁹F NMR (376 MHz, CDCl₃) δ -122.4.
(Z)-4-((3-(Carboxymethyl)phenyl)amino)-4-oxobut-2-enoic acid 98

3-Aminophenylacetic acid 96 (1.0 g, 6.62 mmol) and maleic anhydride 97 (650 mg, 6.62 mmol) were dissolved in acetic acid (40 mL) and stirred at room temperature overnight. The resulting precipitate was filtered, washed with Et₂O and dried under high vacuum to afford 98 as a white solid (1.58 g, 96%). M.p.: 190.9 - 193.2 °C. ¹H NMR (300.1 MHz, DMSO-d₆) δ 10.40 (1H, s, NH), 7.56 (1H, t, J = 1.6 Hz, Ar CH), 7.52-7.49 (1H, m, Ar CH), 7.26 (1H, t, J = 7.8 Hz, Ar CH), 6.98 (1H, d, J = 7.9 Hz, Ar CH), 6.46 (1H, d, J = 12.1 Hz, CH), 6.31 (1H, d, J = 12.0 Hz, CH), 3.54 (2H, s, CH₂).

¹³C NMR (75.5 MHz, DMSO-d₆) δ 172.6 (CO), 166.9 (CO), 163.2 (CO), 138.5 (quat. Ar), 135.7 (quat. Ar), 131.5 (quat. Ar), 130.6 (Ar CH), 128.7 (Ar CH), 125.0 (Ar CH), 120.4 (Ar CH), 117.9 (Ar CH), 40.9 (CH₂). ES MS (pos. m/z) 272 (100% [M+Na]⁺). HRMS (m/z) [M+Na]⁺ calcd. for C₁₂H₁₁NO₅Na, 272.0535; found, 272.0540.

2-(3-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetic acid 83

(Z)-4-((3-(Carboxymethyl)phenyl)amino)-4-oxobut-2-enoic acid 98 (1.0 g, 4.00 mmol) and zinc bromide (900 mg, 4.00 mmol) were dissolved in anhydrous MeCN (30 mL). Hexamethyldisilazane (4.17 mL, 20.00 mmol) was added before the reaction was refluxed at 90 °C under nitrogen for 1 hour. After cooling to room temperature, the solution was filtered and reduced in vacuo to 10% its original volume. The solution was diluted with water and adjusted to pH 1 with HCl (1M) before the product was extracted in DCM (3 × 30 mL). The organic layers were combined, washed with aqueous EDTA (2 × 30 mL, 1M), water (2 × 30 mL) and brine (50 mL), dried over MgSO₄, filtered and evaporated to dryness in vacuo to furnish 83 as a white solid.
8. Experimental

(736 mg, 80%). M.p.: 113.3 - 114.6 °C. 1H NMR (300.1 MHz, DMSO-d$_6$) δ 12.44 (1H, s, OH), 7.46-7.40 (1H, m, Ar CH), 7.30 (1H, dt, $J = 7.8$, 1.4 Hz, Ar CH), 7.23-7.19 (2H, m, Ar CH), 7.18 (2H, s, (CH)$_2$), 3.63 (2H, s, CH$_2$). 13C NMR (75.5 MHz, DMSO-d$_6$) δ 172.4 (CO), 170.0 (CO), 135.9 (quat. Ar), 134.7 ((CH)$_2$), 131.5 (quat. Ar), 129.0 (Ar CH), 128.8 (Ar CH), 127.7 (Ar CH), 125.2 (Ar CH), 40.4 (CH$_2$). ES MS (pos. m/z) 254 (100% [M+Na]$^+$). HRMS (m/z) [M+Na]$^+$ calcd. for C$_{12}$H$_9$NO$_4$Na, 254.0429; found, 254.0435.

Methyl 2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetate 99

![Methyl 2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetate 99](image)

2-(3-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetic acid 83 (1.00 g, 4.30 mmol) was dissolved in DMF (20 mL) before methyl iodide (0.98 g, 6.88 mmol) and caesium carbonate (0.70 g, 2.15 mmol) were added carefully and the reaction allowed to stir for 16 hours in the dark at room temperature. The following day, the reaction was quenched with water (50 mL) and extracted into EtOAc (3 × 50 mL). The combined organic fractions were washed with HCl (3M, 2 × 50 mL) and cold water (2 × 50 mL), dried over MgSO$_4$, filtered and concentrated in vacuo. The crude product was purified via silica gel flash chromatography (eluting with hexane:EtOAc 1:2) to yield 643 mg (61%) of 99 as a yellow solid. M.p.: 58.3 - 59.0 °C. 1H NMR (300.1 MHz, CDCl$_3$) δ 7.42 (1H, t, $J = 8.0$ Hz, Ar CH), 7.27-7.38 (3H, m, Ar CH), 6.82 (2H, s, (CH)$_2$), 3.69 (3H, s, CH$_3$), 3.68 (2H, s, CH$_2$). 13C NMR (100.6 MHz, CDCl$_3$) δ 171.9 (CO), 169.8 (CO), 135.5 ((CH)$_2$), 134.6 (quat. Ar), 131.8 (quat. Ar), 129.7 (Ar CH), 129.3 (Ar CH), 127.3 (Ar CH), 125.2 (Ar CH), 52.6 (CH$_3$), 41.3 (CH$_2$). ES MS (pos. m/z) 268 (100% [M+Na]$^+$). HRMS (m/z) [M+H]$^+$ calcd. for C$_{13}$H$_{12}$NO$_4$, 246.0766; found, 246.0771.
Crude $\text{N}$(4-fluorophenyl)hydroxylamine $\text{Z}$ (3.54 mmol) was dissolved in EtOH (5 mL) before $\text{N}$(4,6-dimethylpyridin-2-yl)-4-formylbenzamide $\text{A}$ (900 mg, 3.54 mmol) was added. The reaction was stirred at room temperature for 3 hours before being placed in the freezer at -17 °C for 2 days. The resulting precipitate was filtered and washed with hexane to yield $\text{AZ}$ as an off-white solid (723 mg, 56% over two steps). M.p.: 216.1 - 217.5 °C. $^1$H NMR (300.1 MHz, CDCl$_3$) $\delta$ 8.70 (1H, s, NH), 8.47 (2H, d, $J = 8.4$ Hz, Ar CH), 8.04 (1H, s, Py CH), 8.01 (2H, d, $J = 8.6$ Hz, Ar CH), 7.96 (1H, s, CH), 7.80 (2H, dd, $J = 9.1, 4.7$ Hz, Ar CH), 7.17 (2H, dd, $J = 9.1, 8.0$ Hz, Ar CH), 6.78 (1H, s, Py CH), 2.40 (3H, s, CH$_3$), 2.36 (3H, s, CH$_3$). $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 164.7 (CO), 163.5 (d, $J = 251.3$ Hz, Ar CF), 156.6 (quat. Py), 150.8 (quat. Py), 150.4 (quat. Py), 145.2 (d, $J = 3.0$ Hz, quat. Ar), 134.0 (quat. Ar), 133.7 (quat. Ar), 133.4 (CH), 129.1 (Ar CH), 127.6 (Ar CH), 123.8 (d, $J = 8.9$ Hz, Ar CH), 120.9 (Py CH), 116.3 (d, $J = 23.3$ Hz, Ar CH), 111.9 (Py CH), 23.9 (CH$_3$), 21.43 (CH$_3$). $^{19}$F NMR (376 MHz, CDCl$_3$-d) $\delta$ -110.1. ES MS (pos. $m/z$) 386 (100% [M+Na]$^+$). HRMS ($m/z$) [M+H]$^+$ calcd. for C$_{21}$H$_{19}$N$_3$O$_2$F, 364.1461; found, 364.1461.

Crude $\text{N}$(4-fluorophenyl)hydroxylamine $\text{Z}$ (3.54 mmol) was dissolved in EtOH (5 mL) before $\text{N}$(4,6-dimethylpyridin-2-yl)-4-formylbenzamide $\text{B}$ (900 mg, 3.54 mmol) was added. The reaction was stirred at room temperature for 3 hours before being placed
in the freezer at -17 °C for 2 days. The resulting precipitate was filtered and washed with hexane to yield **BZ** as an off-white solid (740 mg, 62% over two steps). M.p.: 219.9 - 221.0 °C. ¹H NMR (400.1 MHz, CDCl₃) δ 8.60 (1H, s, NH), 8.49 (2H, d, J = 8.5 Hz, Ar CH), 8.19 (1H, d, j = 8.2 Hz, Py CH), 8.03 (2H, d, J = 8.6 Hz, Ar CH), 7.97 (1H, s, CH), 7.80 (2H, dd, J = 9.0, 4.7 Hz, Ar CH), 7.67 (1H, t, J = 7.9 Hz, Py CH), 7.19 (2H, dd, J = 8.9, 8.0 Hz, Ar CH), 6.96 (1H, d, J = 7.5 Hz, Py CH), 2.49 (3H, s, CH₃). ¹³C NMR (100.6 MHz, CDCl₃) δ 164.7 (CO), 163.5 (d, J = 251.5 Hz, Ar CF), 157.1 (quat. Py), 150.7 (quat. Py), 145.3 (d, J = 3.1 Hz, quat. Ar), 139.0 (Py CH), 135.9 (quat. Ar), 133.9 (quat. Ar), 133.4 (CH), 129.2 (Ar CH), 127.7 (Ar CH), 123.9 (d, J = 8.9 Hz, Ar CH), 119.8 (Py CH), 116.3 (d, J = 23.3 Hz, Ar CH), 111.2 (Py CH), 24.11 (CH₃). ¹⁹F NMR (376 MHz, CDCl₃) δ -110.1. ES MS (pos. m/z) 372 (100% [M+Na]⁺). HRMS (m/z) [M+Na]⁺ calcd. for C₂₀H₁₆N₃O₂FNa, 372.1124; found, 372.1114.

2-(3-(3-(4-((4,6-Dimethylpyridin-2-yl)carbamoyl)phenyl)-2-(4-fluorophenyl)-4,6-dioxotetrahydro-2H-pyrrolo[3,4-d]isoxazol-5(3H)-yl)phenyl)acetic acid **cis-101**

![Chemical Structure](image-url)

In an NMR tube, (Z)-**N-(4-((4,6-dimethylpyridin-2-yl)carbamoyl)benzylidene)-4-fluoroaniline oxide **AZ** (5.82 mg, 16.00 µmol) and 2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetic acid **83** (3.70 mg, 16.00 µmol) were dissolved in CDCl₃ (0.8 mL) and allowed to react at 0 °C for two days. After two days, the reaction showed complete conversion of starting material into **cis-101** in sufficient purity for characterisation. ¹H NMR (300.1 MHz, CDCl₃) δ 11.35 (1H, s, H₈), 8.10 (1H, s, H₆), 8.01 (2H, d, J = 8.5 Hz, H₁₁), 7.62 (2H, d, J = 8.3 Hz, H₁₂), 7.51 (1H, t, J = 1.6 Hz, H₂₄), 7.36 (1H, t, J = 7.8 Hz, H₂₁), 7.22 (1H, ddd, J = 8.0, 1.9, 1.1 Hz, H₂₀), 7.15 (1H, ddd, J = 7.6, 1.5, 1.0 Hz, H₂₂), 7.09 (2H, dd, J = 9.2, 4.7 Hz, H₂₈), 6.94 (2H, t, J = 8.7 Hz, H₂₉), 6.79 (1H, s, H₅), 5.30 (1H, d, J = 7.7 Hz, H₁₄), 4.91 (1H, d, J = 9.8 Hz, H₁₆), 172
4.11 (1H, dd, J = 9.8, 7.7 Hz, H₁₅), 3.83-3.69 (2H, m, H₂₅), 2.44 (3H, s, H₁), 2.39 (3H, s, H₅). ¹³C NMR (75.5 MHz, CDCl₃-d) δ 176.47 (C₂₆), 173.84 (C₁₇), 171.05 (C₁₈), 167.20 (C₉), 160.16 (d, J = 244.4 Hz, C₃₀), 155.15 (C₂), 152.46 (C₄), 151.45 (C₇), 143.01 (C₂₇), 138.55 (C₁₃), 135.71 (C₁₀), 135.35 (C₂₃), 131.31 (C₁₉), 129.97 (C₂₂), 129.53 (C₁₁), 128.95 (C₂₁), 127.12 (C₁₂), 126.58 (C₂₄), 124.00 (C₂₀), 121.14 (C₃), 120.80 (d, J = 8.2 Hz, C₂₈), 115.77 (d, J = 22.5 Hz, C₂₉), 113.69 (C₆), 77.30 (C₁₄), 70.73 (C₁₆), 54.77 (C₁₅), 21.91 (C₁), 21.70 (C₅).

In an NMR tube, (Z)-4-fluoro-N-(4-((6-methylpyridin-2-yl)carbamoyl)benzylidene) aniline oxide BZ (5.58 mg, 16.00 µmol) and 2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetic acid 83 (3.70 mg, 16.00 µmol) were dissolved in CDCl₃ (0.8 mL) and allowed to react at 0 °C for two days. After two days, the reaction showed complete conversion of starting material into cis-85 sufficiently pure for characterisation. ¹H NMR (300.1 MHz, CDCl₃) δ 11.30 (1H, s, H₇), 8.25 (1H, d, J = 8.3 Hz, H₅), 8.01 (2H, d, J = 8.5 Hz, H₁₀), 7.73 (1H, dd, J = 8.3, 7.6 Hz, H₄), 7.62 (2H, d, J = 8.3 Hz, H₁₁), 7.51 (1H, t, J = 1.6 Hz, H₂₃), 7.36 (1H, t, J = 7.8 Hz, H₂₀), 7.23 (1H, ddd, J = 8.1, 2.0, 1.2 Hz, H₁₉), 7.15 (1H, ddd, J = 7.6, 1.6, 1.0 Hz, H₂₁), 7.09 (2H, dd, J = 9.3, 4.7 Hz, H₂₇), 6.97-6.91 (3H, m, H₃, H₂₈), 5.30 (1H, d, J = 7.7 Hz, H₁₃), 4.91 (1H, d, J = 9.7 Hz, H₁₅), 4.12 (1H, dd, J = 9.8, 7.7 Hz, H₁₄), 3.84-3.71 (2H, m, H₂₄), 2.49 (3H, s, H₁). ¹³C NMR (75.5 MHz, CDCl₃) δ 176.3 (C₂₅), 173.8 (C₁₆), 171.1 (C₁₇), 167.2 (C₈), 160.1 (d, J = 244.5 Hz, C₂₉), 155.9 (C₂), 151.8 (C₆), 143.0 (d, J = 2.3 Hz, C₂₆), 140.4 (C₄), 133.8 (C₁₉), 132.6 (C₁₂), 131.5 (C₁₀), 130.7 (C₉), 129.6 (C₂₈), 128.8 (C₁₉), 128.6 (C₂₇), 128.3 (C₂₆), 127.9 (C₂₁), 126.8 (C₂₄), 124.7 (C₂₀), 121.8 (C₃), 121.1 (C₅), 115.9 (C₂₉), 113.8 (C₆), 77.3 (C₁₄), 70.8 (C₁₆), 54.8 (C₁₅), 21.9 (C₁), 21.7 (C₅). ¹⁹F NMR (282 MHz, CDCl₃) δ -118.2. ES MS (pos. m/z) 617 (100% [M+Na]+). HRMS (m/z) [M+Na]+ calcd. for C₃₃H₂₇N₄O₆FNa, 617.1812; found, 617.1818.
8. Experimental

138.6 (C_{12}), 135.7 (C_9), 135.2 (C_{22}), 131.4 (C_{18}), 130.0 (C_{21}), 129.5 (C_{10}), 129.0 (C_{20}), 127.2 (C_{11}), 126.6 (C_{23}), 124.1 (C_{19}), 120.8 (d, J = 8.2 Hz, C_{27}), 119.9 (C_3), 115.8 (d, J = 22.6 Hz, C_{28}), 113.2 (C_5), 77.3 (C_{13}), 70.8 (C_{15}), 54.8 (C_{14}), 38.9 (C_{24}), 22.2 (C_1). \^{19}F\text{ NMR (282 MHz, CDCl}_3) \delta -118.2. \text{ES MS (pos. m/z) 603 (100\% [M+Na]^+). HRMS (m/z) [M+Na]^+ calcd. for C}_{32}H_{26}N_4O_6F, 581.1830; found, 581.1836.

2-(4-(2,5-Dioxo-2,5-dihydro-1\text{-}H\text{-pyrrol-1-yl})phenyl)acetic acid\^{34} 28

\[
\text{O} \quad \text{CO}_2\text{H}
\]

2-(4-Aminophenyl)acetic acid 111 (5.0 g, 33.00 mmol) and maleic anhydride (3.24 g, 33.00 mmol) were dissolved in acetic acid (150 mL) and stirred at room temperature for 5 hours, refluxed at 120 °C for 3 hours and then allowed to stirred at room temperature overnight. The resulting solution was concentrated in vacuo and the crude product recrystallised from hot chloroform to yield 28 as a yellow solid (5.0 g, 66%). M.p.: 150.1 - 152.1 °C. \text{\textsuperscript{1}H NMR (300.1 MHz, DMSO-d}_6) \delta 7.37 (2H, d, J = 8.6 Hz, Ar CH), 7.27 (2H, d, J = 8.5 Hz, Ar CH), 7.18 (2H, s, (CH)_2), 3.63 (2H, s, CH_2). \text{\textsuperscript{13}C NMR (100.6 MHz, DMSO-d}_6) \delta 172.5 (CO), 170.0 (CO), 134.74 (quat. Ar), 134.69 ((CH)_2), 130.05 (quat. Ar), 129.95 (Ar CH), 126.7 (Ar CH), 40.2 (CH_2). \text{ES MS (pos. m/z) 254 (100\% [M+Na]^+). HRMS (m/z) [M+H]^+ calcd. for C}_{12}H_7NO_4Na, 254.0429; found, 254.0435.
2-(4-((4,6-Dimethylpyridin-2-yl)carbamoyl)phenyl)-2-(4-fluorophenyl)-4,6-dioxotetrahydro-2H-pyrrolo[3,4-d]isoxazol-5(3H)-yl)phenyl)acetic acid *trans*-113

(Z)-N-(4-((4,6-Dimethylpyridin-2-yl)carbamoyl)benzylidene)-4-fluoroaniline oxide **AZ** (92.0 mg, 252 µmol) and 2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetic acid **28** (58.0 mg, 252 µmol) were dissolved in CHCl₃ (10 mL) and stirred in the dark at room temperature for 4 days. The crude product was concentrated *in vacuo*, recrystallised from DCM/hexane and dried under high vacuum to yield *trans*-113 as white solid (120 mg, 80%). M.p.: 217.5 - 220.3 °C. ¹H NMR (400.1 MHz, DMSO-d₆) δ 10.65 (1H, s, H₈), 8.05 (2H, d, J = 8.4 Hz, H₁₁), 7.88 (1H, s, H₆), 7.65 (2H, d, J = 8.4 Hz, H₁₂), 7.30 (2H, d, J = 8.4 Hz, H₂₀), 7.25 (2H, dd, J = 9.2, 4.6 Hz, H₂₆), 7.14-7.09 (2H, m, H₂₇), 6.88 (1H, s, H₃), 6.68 (2H, d, J = 8.4 Hz, H₂₁), 5.95 (1H, s, H₁₄), 5.45 (1H, d, J = 7.2 Hz, H₁₈), 4.17 (1H, d, J = 7.2 Hz, H₁₅), 3.61 (2H, s, H₂₃), 2.40 (3H, s, H₁), 2.31 (3H, s, H₅). ¹³C NMR (100.6 MHz, DMSO-d₆) δ 174.4 (C₁₇), 173.2 (C₁₈), 172.4 (C₂₄), 165.4 (C₉), 157.9 (d, J = 239.1 Hz, C₂ₘ₉), 156.2 (C₂), 151.6 (C₇), 149.0 (C₄), 145.1 (C₂₅), 142.5 (C₁₃), 135.8 (C₁₉), 133.6 (C₁₀), 130.0 (C₂₀), 129.9 (C₂₂), 128.3 (C₁₁), 127.1 (C₁₂), 126.0 (C₂₁), 120.1 (C₃), 116.3 (d, J = 7.9 Hz, C₂₆), 115.7 (d, J = 22.5 Hz, C₂₇), 112.3 (C₆), 77.7 (C₁₆), 68.5 (C₁₄), 56.4 (C₁₅), 40.1 (C₂₃), 23.4 (C₁), 20.9 (C₅). ¹⁹F NMR (376 MHz, DMSO-d₆) δ -122.1 ES MS (pos. *m/z*) 595 (100% [M+H]+). HRMS (*m/z*) [M+H]+ calcd. for C₃₃H₂₈N₄O₆F, 595.1993; found, 595.1998.

8. Experimental
2-(4-(2-(4-Fluorophenyl)-3-(4-((6-methylpyridin-2-yl)carbamoyl)phenyl)-4,6-dioxotetrahydro-2H-pyrrolo[3,4-d]isoxazol-5(3H)-yl)phenyl)acetic acid trans-40

(Z)-4-Fluoro-N-(4-((6-methylpyridin-2-yl)carbamoyl)benzylidene)aniline oxide BZ (90.27 mg, 260 µmol) and 2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetic acid 28 (59.70 mg, 260 µmol) were dissolved in CHCl₃ (10 mL) and stirred in the dark at room temperature for 4 days. The crude product was concentrated in vacuo, recrystallised from THF/hexane and dried under high vacuum to yield trans-40 as white solid (112 mg, 75%). M.p.: 203.5 - 204.8 °C. ¹H NMR (300.1 MHz, DMSO-d₆) δ 10.74 (1H, s, H₇), 8.07 (2H, d, J = 8.5 Hz, H₁₀), 8.02 (1H, d, J = 8.2 Hz, H₅), 7.72 (1H, dd, J = 8.2, 7.6 Hz, H₄), 7.66 (2H, d, J = 8.5 Hz, H₁₁), 7.30 (2H, d, J = 8.6 Hz, H₁₉), 7.25 (2H, dd, J = 9.1, 4.7 Hz, H₂₅), 7.12 (2H, t, J = 9.1 Hz, H₂₆), 7.03 (1H, d, J = 7.6 Hz, H₃), 6.69 (2H, d, J = 8.6 Hz, H₂₀), 5.96 (1H, s, H₁₃), 5.46 (1H, d, J = 7.5 Hz, H₁₅), 4.18 (1H, dd, J = 7.5, 0.8 Hz, H₁₄), 3.61 (2H, s, H₂₂), 2.45 (3H, s, H₁). ¹³C NMR (75.5 MHz, DMSO-d₆) δ 174.3 (C₁₆), 173.2 (C₁₇), 172.4 (C₂₃), 165.5 (C₈), 158.0 (d, J = 240.2 Hz, C₂₇), 156.6 (C₂), 151.5 (C₆), 145.1 (d, J = 1.8 Hz, C₂₄), 142.6 (C₁₂), 138.4 (C₄), 135.8 (C₁₈), 133.6 (C₉), 130.0 (C₁₉), 129.9 (C₂₁), 128.3 (C₁₀), 127.2 (C₁₁), 126.0 (C₂₀), 119.1 (C₃), 116.3 (d, J = 7.7 Hz, C₂₅), 115.6 (d, J = 22.6 Hz, C₂₆), 111.7 (C₅), 77.7 (C₁₅), 68.5 (C₁₃), 56.4 (C₁₄), 40.1 (C₂₂), 23.6 (C₁). ¹⁹F NMR (282 MHz, DMSO-d₆) δ -122.1. ES MS (neg. m/z) 579 (100% [M-H]⁻). HRMS (m/z) [M+H]⁺ calcd. for C₃₂H₂₄N₄O₆F, 579.1680; found, 579.1675.
9-Bromoanthracene 125 (1.2 g, 4.66 mmol) was suspended in triethylamine (80 mL). Triphenylphosphine (122 mg, 0.56 mmol), copper iodide (88 mg, 0.56 mmol) and bis(triphenylphosphine) palladium (II) dichloride (170 mg, 0.30 mmol) were added and the reaction was degassed with argon for 30 mins. Trimethylsilylacetylene (1.66 ml, 11.66 mmol) was added and the reaction was stirred at 80 °C for 16 hours under a protective argon atmosphere. The resulting solution was filtered through Celite and concentrated \textit{in vacuo}. The crude product was purified via silica gel flash chromatography (eluting with 0-2\% EtOAc in hexane) to give 1.10 g (86\%) of 126 as a bright orange solid. Rf: 0.18 (hexane). M.p.: 79.8 - 83.1 °C. $^1$H NMR (300.1 MHz, CDCl$_3$) $\delta$ 8.56 (2H, dq, $J$ = 8.6, 1.0 Hz, Ar CH), 8.43 (1H, s, Ar CH), 8.00 (2H, ddt, $J$ = 8.4, 1.3, 0.7 Hz, Ar CH), 7.61-7.56 (2H, m, Ar CH), 7.50 (2H, ddd, $J$ = 8.2, 6.7, 1.4 Hz, Ar CH), 0.42 (9H, s, SiMe$_3$). $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 133.0 (quat. Ar), 131.2 (quat. Ar), 128.8 (Ar CH), 128.0 (Ar CH), 126.9 (Ar CH), 126.8 (Ar CH), 125.8 (Ar CH), 117.2 (quat. Ar), 106.3 (C=C), 101.7 (C=C), 0.4 (SiMe$_3$). CI MS (pos. m/z) 274 (100\% [M]+), 275 (85\% [M+H]+. HRMS (m/z) [M+H]$^+$ calcd. for C$_{19}$H$_{19}$Si, 275.1256; found, 275.1249.

9-Ethynylanthracene 127

(Anthracen-9-ylythynyl)trimethylsilane 126 (1.50 g, 5.50 mmol) was dissolved in MeOH/THF (50 mL, 1:1) before potassium carbonate (3.8 g, 27.50 mmol) was added and stirred for 4 hours at room temperature. The resulting solution was diluted with
8. Experimental

water (50 mL), extracted in to DCM (2 x 50 mL), dried over MgSO₄, filtered and concentrated in vacuo to afford 127 as a red oil sufficiently pure for further transformation. ¹H NMR (300.1 MHz; CDCl₃): δ 8.58 (2H, dq, J = 8.7, 1.0 Hz, Ar CH), 8.46 (1H, s, Ar CH), 8.02 (2H, ddt, J = 8.4, 1.3, 0.7 Hz, Ar CH), 7.59 (2H, ddd, J = 8.5, 6.8, 1.6 Hz, Ar CH), 7.51 (2H, ddd, J = 8.3, 6.8, 1.4 Hz, Ar CH), 3.99 (1H, s, CH).

¹³C NMR (75.5 MHz; CDCl₃): δ 133.3 (quat. Ar), 131.2 (quat. Ar), 128.8 (Ar CH), 128.4 (Ar CH), 127.7 (quat. Ar), 127.0 (Ar CH), 126.7 (Ar CH), 125.8 (Ar CH), 116.4 (C=C), 88.3 (CH).

9-((4-Nitrophenyl)ethynyl)anthracene 129

1-Iodo-4-nitrobenzene 128 (1.23 g, 4.94 mmol), bis(triphenylphosphine)palladium (II) dichloride (175 mg, 0.25 mmol), copper iodide (95 mg, 0.5 mmol) and triphenylphosphine (131 mg, 0.50 mmol) were added to a solution of crude 9-ethynylanthracene 127 (5.50 mmol) in triethylamine (100 mL) and degassed with argon for 30 mins. The reaction was stirred at 55 °C for 48 hours. The resulting solution was concentrated in vacuo. The residue was dissolved in DCM (100 mL) and washed with HCl (1M, 2 x 50 mL) and water (2 x 50 mL), dried over MgSO₄, filtered and concentrated in vacuo. The crude product was purified via silica gel flash chromatography (eluting with hexane:EtOAc 10:1) to give 2.41 g (76%) of 129 as a red solid. Rf: 0.34 (hexane:EtOAc 10:1). M.p.: 211.1 - 213.5 °C. ¹H NMR (300.1 MHz, CDCl₃) δ 8.60 (2H, d, J = 8.5 Hz, Ar CH), 8.52 (1H, s, Ar CH), 8.32 (2H, d, J = 8.7 Hz, Ar CH), 8.06 (2H, d, J = 8.5 Hz, Ar CH), 7.90 (2H, d, J = 8.7 Hz, Ar CH), 7.65 (2H, t, J = 7.4 Hz, Ar CH), 7.55 (2H, t, J = 7.4 Hz, Ar CH). ¹³C NMR (75.5 MHz, CDCl₃) δ 147.1 (quat. Ar), 133.0 (quat. Ar), 132.3 (Ar CH), 131.2 (quat. Ar), 130.6 (quat. Ar), 129.3 (Ar CH), 129.1 (Ar CH), 127.3 (Ar CH), 126.5 (Ar CH), 126.0 (Ar CH), 123.9 (Ar CH), 115.9 (quat. Ar), 98.9 (C=C), 92.0 (C=C). CI MS (pos. m/z) 324 (100% [M+H]+). HRMS (m/z) [M+H]+ calcd. for C₂₂H₁₄NO₂, 324.1025; found, 324.1031.
9-((4-Nitrophenyl)ethynyl)anthracene 129 (1.0 g, 3.10 mmol) and rhodium (5% wt. on carbon, 400 mg) were dissolved in THF (100 mL) before hydrazine monohydrate (900 µL, 18.60 mmol) was added dropwise. The reaction was followed by TLC (hexane:EtOAc 10:1). After completion (ca. 10 mins), MgSO₄ was added and stirred for a further 5 mins. The solution was then filtered through Celite and concentrated in vacuo. The residue was redissolved in EtOAc (50 mL), washed with water (5 x 50 mL), dried over MgSO₄, filtered and concentrated in vacuo to afford 130 as a red solid. The crude product was dried under high vacuum for 16 hours and used without further purification.

1H NMR (300.1 MHz, CDCl₃) δ 8.65 (2H, d, J = 8.5 Hz, Ar CH), 8.42 (1H, s, Ar CH), 8.02 (2H, d, J = 8.5 Hz, Ar CH), 7.70 (2H, d, J = 8.5 Hz, Ar CH), 7.59 (2H, t, J = 7.2 Hz, Ar CH), 7.51 (2H, t, J = 7.2 Hz, Ar CH), 7.07 (2H, d, J = 8.5 Hz, Ar CH), 6.92 (1H, s, NHOH), 5.22 (1H, s, NHOH).
Crude \(N\)-(4-(anthracen-9-ylethynyl)phenyl)hydroxylamine \(130\) (1.55 mmol) and \(N\)-(4,6-dimethylpyridin-2-yl)-4-formylbenzamide \(A\) (395 mg, 1.55 mmol) were dissolved in EtOH (50 mL) and stirred in the dark at room temperature for 16 hours. The resulting solution was concentrated \textit{in vacuo} and the residue purified by recrystallisation from THF/hexane to afford \(122\) (522 mg, 62% over two steps) as an orange solid. M.p.: 211.0 °C (dec.). \(^1\)H NMR (400.1 MHz, CDCl\(_3\)) \(\delta\) 8.63 (2H, d, \(J = 8.7\) Hz, Ar CH), 8.61 (1H, s, NH), 8.55 (2H, d, \(J = 8.5\) Hz, Ar CH), 8.48 (1H, s, Ar CH), 8.09 (1H, s, CH), 8.08-8.03 (5H, m, Ar CH, Py CH), 7.92-7.87 (4H, m, Ar CH), 7.64 (2H, ddd, \(J = 8.6, 6.6, 1.3\) Hz, Ar CH), 7.54 (2H, ddd, \(J = 8.2, 6.7, 1.3\) Hz, Ar CH), 6.80 (1H, s, Py CH), 2.45 (3H, s, CH\(_3\)), 2.38 (3H, s, CH\(_3\)). \(^{13}\)C NMR (125.7 MHz, CDCl\(_3\)) \(\delta\) 164.7 (CO), 156.6 (quat. Py), 150.8 (quat. Py), 150.5 (quat. Ar), 148.3 (quat. Py), 136.0 (quat. Ar), 133.9 (quat. Ar), 133.6 (CH), 132.9 (quat. Ar), 132.6 (Ar CH), 131.3 (quat. Ar), 129.3 (Ar CH), 129.0 (Ar CH), 128.6 (Ar CH), 127.7 (Ar CH), 127.1 (Ar CH), 126.7 (Ar CH), 126.2 (quat. Ar), 126.0 (Ar CH), 122.1 (Ar CH), 121.0 (Py CH), 116.5 (quat. Ar), 111.9 (Py CH), 99.3 (C=C), 89.2 (C=C), 23.9 (CH\(_3\)), 21.5 (CH\(_3\)). ES MS (pos. \(m/z\)) 568 (100% [M+Na]\(^+\)). HRMS (\(m/z\)) [M+H]\(^+\) calcd. for C\(_{37}\)H\(_{28}\)N\(_3\)O\(_2\), 546.2182; found, 546.2189.
8. Experimental

**Methyl 2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetate** 26

![Chemical Structure](image)

2-(4-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetic acid 28 (1.15 g, 4.97 mmol) was dissolved in DMF (20 mL) before methyl iodide (1.14 g, 8.00 mmol) and caesium carbonate (0.81 g, 2.50 mmol) were added carefully and the reaction allowed to stir for 16 hours in the dark at room temperature. The following day, the reaction was quenched with water (50 mL) and extracted into EtOAc (3 × 50 mL). The combined organic fractions were washed with HCl (3M, 2 × 50 mL) and cold water (2 × 50 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The crude product was purified *via* silica gel flash chromatography (eluting with hexane:EtOAc 1:2) to yield 682 mg (56%) of 26 as a brown solid. M.p.: 88.0 - 88.7 °C. ¹H NMR (300.1 MHz, CDCl₃) δ 7.39 (2H, d, J = 8.5 Hz, Ar CH), 7.30 (2H, d, J = 8.5 Hz, Ar CH), 6.85 (2H, s, (CH)₂), 3.70 (3H, s, CH₃), 3.65 (2H, s, CH₂). ¹³C NMR (100.6 MHz, DMSO-d₆) δ 171.5 (CO), 170.0 (CO), 134.7 ((CH)₂), 134.0 (quat. Ar), 130.3 (quat. Ar), 129.9 (Ar CH), 126.7 (Ar CH), 51.8 (CH₃), 39.7 (CH₂). ES MS (pos. m/z) 268 (100% [M+Na]+). HRMS (m/z) [M]+ calcd. for C₁₃H₁₁NO₄, 245.0688; found, 245.0694.
2-(4-(2-(4-(Anthracen-9-ylethynyl)phenyl)-3-(4-((4,6-dimethylpyridin-2-yl)carbamoyl)phenyl)-4,6-dioxotetrahydro-2H-pyrrolo[3,4-d]isoxazol-5(3H)-yl)phenyl)acetic acid trans-123

(Z)-4-(Anthracen-9-ylethynyl)-N-(4-((4,6-dimethylpyridin-2-yl)carbamoyl)benzylidene) aniline oxide 122 (71.0 mg, 130 µmol) and 2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetic acid 28 (30.0 mg, 130 µmol) were dissolved in CHCl₃ (10 mL) and stirred in the dark at room temperature for 4 days. The resulting solution was concentrated in vacuo. The residue was recrystallised from DCM/hexane and dried under high vacuum to yield trans-123 as red solid (74 mg, 74%). M.p.: 239.0 °C (dec.). ¹H NMR (300.1 MHz, DMSO-d₆) δ 10.69 (1H, s, H₈), 8.68 (1H, s, H₃₈), 8.59 (2H, d, J = 8.7 Hz, H₃₃), 8.17 (2H, d, J = 8.4 Hz, H₃₆), 8.09 (2H, d, J = 8.5 Hz, H₁₁), 7.89 (1H, s, H₆), 7.77 (2H, d, J = 8.9 Hz, H₂₆), 7.73-7.67 (4H, m, H₁₂, H₃₄), 7.61 (2H, ddd, J = 8.1, 6.8, 1.2 Hz, H₃₅), 7.39 (2H, d, J = 8.9 Hz, H₂₇), 7.29 (2H, d, J = 8.3 Hz, H₂₀), 6.87 (1H, s, H₃), 6.71 (2H, d, J = 8.3 Hz, H₂₁), 6.13 (1H, s, H₁₄), 5.53 (1H, d, J = 7.4 Hz, H₁₆), 4.23 (1H, d, J = 7.6 Hz, H₁₅), 3.54 (2H, s, H₂₃), 2.40 (3H, s, H₁), 2.31 (3H, s, H₅). ¹³C NMR (100.6 MHz, DMSO-d₆) δ 174.3 (C₁₇), 173.1 (C₁₈), 172.3 (C₂₄), 165.3 (C₉), 156.2 (C₂), 151.6 (C₇), 149.1 (C₄), 149.0 (C₂₅), 142.4 (C₁₃), 135.9 (C₁₉), 133.7 (C₁₀), 132.6 (C₂₆), 131.7 (C₃₂), 130.8 (C₃₇), 130.0 (C₂₆), 130.0 (C₂₂), 128.9 (C₃₆), 128.3 (C₁₁), 127.8 (C₃₈), 127.3 (C₁₂), 127.1 (C₃₅), 126.2 (C₂₁), 126.1 (C₃₃), 126.0 (C₃₄), 120.1 (C₃), 116.3 (C₃₁), 116.1 (C₂₈), 114.8 (C₂₇), 112.3 (C₆), 100.7 (C₂₉), 85.0 (C₃₀), 77.9 (C₁₆), 67.9 (C₁₄), 56.4 (C₁₅), 40.1 (C₂₃), 23.4 (C₁), 20.9 (C₅). ES MS (pos. m/z) 777 (100% [M+H]+). HRMS (m/z) [M+H]+ calcd. for C₄₉H₃₇N₄O₆, 777.2713; found, 777.2719.
(Z)-4-((Carboxymethyl)amino)-4-oxobut-2-enoic acid 127

[Chemical structure image]

Glycine 126 (1.50 g, 19.97 mmol) and maleic anhydride 97 (2.35 g, 23.96 mmol) were dissolved in acetic acid (50 mL) and THF (10 mL) and stirred for 16 hours at room temperature. The resulting precipitate was filtered and washed with Et₂O to give 127 as a white solid (3.45 g, 99%). ¹H NMR (300.1 MHz, DMSO-d₆) δ 9.20 (1H, t, J = 5.8 Hz, NH), 6.42 (1H, d, J = 12.4 Hz, CH), 6.30 (1H, d, J = 12.4 Hz, CH), 3.90 (2H, d, J = 5.8 Hz, CH₂). ¹³C NMR (75.5 MHz, DMSO-d₆) δ 170.5 (CO), 166.1 (CO), 165.3 (CO), 133.1 (CH), 130.1 (CH), 41.1 (CH₂). ES MS (neg. m/z) 172 (100% [M-H]-). HRMS (m/z) [M-H]- calcd. for C₆H₆NO₅, 172.0246; found, 172.0245.

2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid 23

[Chemical structure image]

(Z)-4-((Carboxymethyl)amino)-4-oxobut-2-enoic acid 127 (3.45 g, 19.97 mmol) and zinc bromide (4.50 g, 19.98 mmol) were suspended in anhydrous MeCN (50 mL). Hexamethyldisilazane (20.8 mL, 99.75 mmol) was added and the reaction was refluxed at 90 °C for 2 hours under a nitrogen atmosphere. After 2 hours, the mixture was hot filtered and concentrated in vacuo to 10% volume. The solution was diluted with water (50 mL) and treated with conc. HCl (5 mL) before the product was extracted into EtOAc (5 x 50 mL). The organic fractions were combined and washed with sat. aq. EDTA solution (3 x 50 mL) and water (2 x 50 mL), dried over MgSO₄, filtered and concentrated in vacuo to give 23 as a white solid (2.50 g, 81%). M.p.: 114.0 - 116.0. ¹H NMR (300.1 MHz, DMSO-d₆) δ 7.11 (2H, s, CH), 4.14 (2H, s, CH₂). ¹³C NMR (75.5 MHz, DMSO-d₆) δ 170.4 (CO), 169.0 (CO), 135.0 (CH), 38.6 (CH₂). ES MS (pos. m/z) 178 (100% [M+Na]+). HRMS (m/z) [M+Na]+ calcd. for C₆H₅NO₄Na, 178.0116; found, 178.0114.
8. Experimental

**Methyl 2-(2,5-diozo-2,5-dihydro-1H-pyrrol-1-yl)acetate 21**

![Methyl 2-(2,5-diozo-2,5-dihydro-1H-pyrrol-1-yl)acetate 21](image)

2-(2,5-Diozo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid 23 (0.40 g, 2.58 mmol) was dissolved in THF (10 mL) and cooled to 0 °C before \(N,N'-\text{dicyclohexylcarbodiimide} \) (0.59 g, 2.84 mmol) was added and the solution allowed to stir for 1 hour. Methanol (0.4 g, 13.00 mmol) was added and the reaction was stirred overnight whilst warming to room temperature. The resulting solution was concentrated *in vacuo* and the residue dissolved in ether (20 mL). The urea byproduct was removed by filtration before concentration *in vacuo*. The crude product was purified by flash chromatography (eluting with hexane:EtOAc 3:1) to yield 21 as a colourless liquid (0.30 g, 69%). \(^1\text{H NMR (300.1 MHz, CDCl}_3\) \(\delta 6.78 \text{ (2H, s, CH)}, 4.28 \text{ (2H, s, CH}_2\), 3.74 \text{ (3H, s, CH}_3\) \(^{13}\text{C NMR (75.5 MHz, CDCl}_3\) \(\delta 169.7 \text{ (CO)}, 167.6 \text{ (CO)}, 134.5 \text{ (CH)}, 52.7 \text{ (CH}_3\), 38.4 \text{ (CH}_2\). El MS (pos. \text{m/z}) 169 (100% \[\text{M}^+\]). HRMS (\text{m/z}) \[\text{M}^+\] calcd. for \(\text{C}_7\text{H}_7\text{NO}_4\), 169.0375; found, 169.0382.
2-(-2-(4-(Anthracen-9-ylethynyl)phenyl)-3-(4-((4,6-dimethylpyridin-2-yl)carbamoyl)phenyl)-4,6-dioxotetrahydro-2H-pyrrolo[3,4-d]isoxazol-5(3H)-yl)acetic acid trans-124

(Z)-4-(Anthracen-9-ylethynyl)-N-(4-((4,6-dimethylpyridin-2-yl)carbamoyl)benzylidene) aniline oxide 122 (77.0 mg, 0.14 mmol) and 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid 23 (22.0 mg, 0.14 mmol) were dissolved in CHCl₃ (10 mL) and stirred in the dark at room temperature for 4 days. The resulting solution was concentrated in vacuo. The residue was recrystallised from THF/hexane and dried under high vacuum to yield trans-124 as yellow solid (61 mg, 61%). M.p.: 238.0 - 243.0 °C. ¹H NMR (500.1 MHz, DMSO-d₆) δ 10.62 (1H, s, H₈), 8.66 (1H, s, H₃₄), 8.55 (2H, d, J = 8.6 Hz, H₂₉), 8.15 (2H, d, J = 8.5 Hz, H₃₂), 8.00 (2H, d, J = 8.4 Hz, H₁₁), 7.84 (1H, s, H₆), 7.69-7.66 (4H, m, H₃₀, H₂₁), 7.61-7.58 (4H, m, H₃₁, H₁₂), 7.18 (2H, d, J = 8.8 Hz, H₂₃), 6.85 (1H, s, H₃), 5.85 (1H, s, H₁₄), 5.64 (1H, d, J = 7.3 Hz, H₁₈), 4.22 (1H, d, J = 7.5 Hz, H₁₅), 4.07 (2H, d, J = 1.7 Hz, H₁₉), 2.37 (3H, s, H₁), 2.28 (3H, s, H₅). ¹³C NMR (125.7 MHz, DMSO-d₆) δ 174.2 (C₁₇), 173.5 (C₁₈), 167.6 (C₂₀), 165.2 (C₉), 156.1 (C₂), 151.5 (C₇), 148.9 (C₄), 147.3 (C₂₁), 141.4 (C₁₃), 133.7 (C₁₀), 132.2 (C₂₂), 131.6 (C₂₉), 130.8 (C₃₃), 128.9 (C₃₂), 128.2 (C₁₁), 127.8 (C₃₄), 127.7 (C₁₂), 127.3 (C₃₁), 126.1 (C₂₉), 126.0 (C₃₀), 120.1 (C₃), 116.3 (C₂₇), 115.6 (C₂₃), 115.6 (C₂₄), 112.3 (C₆), 101.0 (C₂₅), 85.0 (C₂₆), 76.5 (C₁₆), 67.4 (C₁₄), 55.9 (C₁₅), 39.9 (C₁₉), 23.4 (C₁), 20.8 (C₅). ES MS (pos. m/z) 701 (100% [M+H]+). HRMS (m/z) [M+H]+ calcd. for C₄₃H₃₃N₄O₆, 701.2400; found, 701.2418.
8. Experimental
References

9. References

61. Some of these compounds have appeared previously in this thesis, however, they have been renumbered to aid the reader’s understanding and maintain clarity throughout the following sections. Aldehydes are denoted A to J while nucleophiles are labelled W to Z. Condensation products are labelled as combinations of the required components. For example, Nitrone 39 is made up from aldehyde B and hydroxylamine Z, and it therefore designated BZ.
References

10. Appendices

Appendices
A1. Typical Semi-Automatic Deconvolution Script

-- Dinit.lua  semi-automatic deconvolution of a kinetic experiment
-- DEFINITIONS
HOME = HOME or "~/Desktop/
filename = "HM099.txt"
NumSpectra = 96
NumRegions = 2
doDeconv = false
phased = true
baselineparams = false
baseline = true
P = 1
R = 1
F = {}
par = {}  -- parameter for the deconvolution
-- they must be obtained from a preliminary deconvolution performed on the first spectrum
dx = 0.5  -- (constant) region to zoom in, in ppm units, based upon experience and observation
local i = 1  -- progressive index, simplifies the editing of this script
-- for example, you can reorder the definitions below and they will still work
-- proton 1
F[i] = 8.414
par[i] = [[
Parameters for 1 peak
frequency (Hz) intensity width (Hz) Lorentzian %
 2498.5 10.0000 30.0700 100.0000
]]
i = i + 1
-- proton 2
F[i] = 6.88
par[i] = [[
Parameters for 1 peak
frequency (Hz) intensity width (Hz) Lorentzian %
 2498.5 10.0000 30.0700 100.0000
]]
i = i + 1
-- end of definitions

io.output(HOME..filename)  -- create/open the file where the results will be stored
io.write("\tpoint")  -- header
for i = 1,NumRegions do
  io.write("\tppm\tarea")
end
io.write("\n")
spectral = getf("x")
conversion = 1.0 / spectral.MHz  -- useful to convert from Hz to ppm
spectral = getf("y")
step = spectral.width / spectral.size
Y = spectral.start + step * (spectral.size -0.1 -P+1)  -- position of the first row on its dummy ppm scale
io.write( string.format("\n\tp%02d\t", P ) )  -- report the experiment no.
mark('h', Y )  -- choose a row
extract()  -- extract the corresponding 1D spectrum
delint()  -- we need to normalize the intensities
region(0.5, 9.5 )  -- region containing protons no. 1 and 9
press 'i'  -- first integral, automatically set to 1
intreg(1, 300 )  -- we set it to 300 to have manageable numbers (>1 and <300)
print("Fix the phasing and do the baseline correction then press prep_DSTEP")
A2. Typical SimFit Input File for a Simple Bimolecular Reaction

*==============================================================================*
* Simple bimolecular cycloaddition fit*
*==============================================================================*
*
* A = Maleimide
* B = Nitrone
*
* Best Fit ( R = 5.71 % )
*
* k(trans) = 1.301 E -3
* k(cis)   = 5.929 E -4
*
*==============================================================================*

DIM ( 2 )

* Bimolecular routes to TRANS and CIS

REACTION ( A + B --> TRANS )  CONSTANT ( 1, 1.84E-4, 1, 1, 1000 )
REACTION ( A + B --> CIS   )  CONSTANT ( 2, 1.84E-4, 2, 1, 1000 )

REACTION (COMPILE)
REACTION (SHOW)
CONSTANT (SHOW)

DEFINE   ( 1, TRANS , P, 1) SCALE (3,1)
DEFINE   ( 2, CIS   , P, 3) SCALE (3,1)

SELECT   ( TRANS, CIS )

READ     ( 46DiMeCtrl )
REACTION ( DOC  )
CONSTANT ( DOC  )

TIME     (SEC)
WIN      (0, 60000, 15000, 200, 0, 5E-3, 5E-3, 1E-4)

ASSIGN   (OBS,  TRANS = TRANS  )
ASSIGN   (OBS,  CIS   = CIS    )
ASSIGN   (SPEC, A     = #20e-3 )
ASSIGN   (SPEC, B     = #20e-3 )

CHOOSE   ( EXP1 )

INTEG    ( STIFF, 1E-9, 4, 0.05, 200, 100 )

PLOT     (OBS, RES)

* 10 rounds of Simplex optimizer without screen
* update

OPAR     (1E16)
SIMPLEX (PLOT)
SIMPLEX (PLOT)
SIMPLEX (PLOT)
SIMPLEX (PLOT)
NEWTON  (PLOT)

PLOT     (FILE)

*SCAN    (1.02, 20, 20)
A3. Typical SimFit Input File for a [A•B] Reaction

*====================================================================*
* 4,6-DiMe Nitrone + Phenylacetic Maleimide                           *
*====================================================================*

REACT  ( A  + B  --> TRANS )  CONSTANT (1,  1.236E-4, 0, 1, 1)
REACT  ( A  + B  --> CIS    ) CONSTANT (2,  5.568E-5, 0, 1, 1)
REACT  ( A  + B  ==> AB     ) CONSTANT (3,  1.000E+9, 0, 1, 1) CONSTANT (4,  9.803E+5, 1, 1, 10)
REACT  ( AB      --> CIS    ) CONSTANT (5,  1.470E-2, 2, 1, 1000)
REACT  ( AB  + B --> TRANS + B ) CONSTANT (6,  1.236E-4, 0, 1, 1)
REACT  ( AB  + A --> TRANS + A ) CONSTANT (7,  1.236E-4, 0, 1, 1)
REACT  ( AB  + B --> CIS + B ) CONSTANT (8,  5.568E-5, 0, 1, 1)
REACT  ( AB  + B --> CIS + B ) CONSTANT (9,  5.568E-5, 0, 1, 1)

REACT  (COMPILE)
REACT  (SHOW)
CONSTANT (SHOW)

DEFINE   (1, TRANS    , P, 2 ) SCALE (3,1)
DEFINE   (2, CIS      , P, 4 ) SCALE (3,1)
DEFINE   (SHOW)

ASSIGN   (SPEC, A     = #0.020 )
ASSIGN   (SPEC, B     = #0.020 )
ASSIGN   (OBS, CIS    = CIS    )
ASSIGN   (OBS, TRANS  = TRANS  )
ASSIGN   (SHOW)

READ    ( 46DiMeAB   )
CHOOSE  ( EXP1      )
SELECT   ( CIS, TRANS )

TIME     (SEC)
CONC     (M)

WIN      (0, 60000, 15000, 200, 0, 20e-3, 4e-3, 3e-4)
DIM      (2)
INTEG    (STIFF, 1e-5, 8, 0.05, 50, 1000)
PLOT     (OBS, RES, SPEC)
SIMPLEX  (PLOT)
SIMPLEX  (PLOT)
SIMPLEX  (PLOT)

PLOT     (FILE)
A4. Typical SimFit Input File for a Self-Replicating Reaction

*=============================================*
* 4,6-DiMe Nitrone + Phenylacetic Maleimide*
*=============================================*

* Replication Model including all bimolecular

*Cis   = --
* Trans = SR
*
*=============================================*

* A = Phenylacetic Maleimide
* C = 4,6-DiMe Nitrone
*
* Best Fit ( R = 2.75 %)
* k1 = 5.21 E-3
* k2 = 2.20 E-1
* EM = 8.83
* 
*=============================================*

DIM (2)

* Bimolecular routes to TRANS
REACTION (A + C ---> TRANS ) CONSTANT ( 1, 1.236E-4, 0, 1, 1 )

* Bimolecular routes to CIS
REACTION (A + C ---> CIS ) CONSTANT ( 2, 5.568E-5, 0, 1, 1 )

* Formation of binary complexes
REACTION (A + TRANS ==> ATRANS ) CONSTANT ( 3, 1E9, 0) CONSTANT ( 4, 3.17E+6, 0)
REACTION (C + TRANS ==> CTRANS ) CONSTANT ( 5, 1E9, 0) CONSTANT ( 6, 3.17E+6, 0)
REACTION (A + ATRANS ==> ACTRANS) CONSTANT ( 7, 1E9, 0) CONSTANT ( 8, 3.17E+6, 0)
REACTION (C + ATRANS ==> ACTRANS) CONSTANT ( 9, 1E9, 0) CONSTANT (10, 3.17E+6, 0)
REACTION (A + CIS ==> ACIS ) CONSTANT (11, 1E9, 0) CONSTANT (12, 3.17E+6, 0)
REACTION (C + CIS ==> CCIS ) CONSTANT (13, 1E9, 0) CONSTANT (14, 3.17E+6, 0)
REACTION (A + CCIS ==> ACCIS ) CONSTANT (15, 1E9, 0) CONSTANT (16, 3.17E+6, 0)
REACTION (C + ACIS ==> ACCIS ) CONSTANT (17, 1E9, 0) CONSTANT (18, 3.17E+6, 0)

* Ternary complex reaction
REACTION (ACTRANS ---> TRANSTRANS ) CONSTANT (19, 2E-3, 1, 1, 100)

* Product duplexes - only [TRANS*TRANS] is stable beyond one Pyr*COOH association
REACTION (TRANS + TRANS ==> TRANSTRANS ) CONSTANT (20, 1E9, 0) CONSTANT (21, 1E+2, 2, 1, 1000)
REACTION (CIS  + CIS    ==> CISCIS     ) CONSTANT (22, 1E9, 0) CONSTANT (23, 3.17E+6, 0)
REACTION (TRANS + CIS   ==> TRANSCIS   ) CONSTANT (24, 1E9, 0) CONSTANT (25, 3.17E+6, 0)

* AB reaction gives CIS
REACTION (A + C          ==> AC) CONSTANT (26, 1E9, 0) CONSTANT (27, 3.17E+6, 0)

*Bimolecular Reactions of Complexes
REACTION (ACIS + C ---> TRANS + CIS ) CONSTANT (28, 1.236E-4, 0, 1, 1 )
REACTION (CCIS + A ---> TRANS + CIS ) CONSTANT (29, 1.236E-4, 0, 1, 1 )
REACTION (ATRANS + C ---> TRANS + TRANS ) CONSTANT (30, 1.236E-4, 0, 1, 1 )
REACTION (CTRANS + A ---> TRANS + TRANS ) CONSTANT (31, 1.236E-4, 0, 1, 1 )
REACTION (AC + A ---> TRANS + A ) CONSTANT (32, 1.236E-4, 0, 1, 1 )
REACTION (AC + C ---> TRANS + C ) CONSTANT (33, 1.236E-4, 0, 1, 1 )
REACTION (AC + AC  --> TRANS + A + C      )  CONSTANT (34, 1.236E-4, 0, 1, 1)
REACTION (ACIS + CCIS --> TRANS + CIS + CIS  )  CONSTANT (35, 1.236E-4, 0, 1, 1)
REACTION (ACIS + CTRANS --> TRANS + TRANS + CIS )  CONSTANT (36, 1.236E-4, 0, 1, 1)
REACTION (ATRANS + CCIS  --> TRANS + TRANS + CIS  )  CONSTANT (37, 1.236E-4, 0, 1, 1)
REACTION (ATRANS + CTRANS --> TRANS + TRANS + TRANS )  CONSTANT (38, 1.236E-4, 0, 1, 1)
REACTION (ACIS  + C --> CIS + CIS          )         CONSTANT (39, 5.568E-5, 0, 1, 1)
REACTION (CCIS  + A       --> CIS + CIS          )         CONSTANT (40, 5.568E-5, 0, 1, 1)
REACTION (ATRANS + C      --> CIS + TRANS        )         CONSTANT (41, 5.568E-5, 0, 1, 1)
REACTION (CTRANS + A      --> CIS + TRANS        )         CONSTANT (42, 5.568E-5, 0, 1, 1)
REACTION (AC  + A          --> CIS + A             )        CONSTANT (43, 5.568E-5, 0, 1, 1)
REACTION (AC + C          --> CIS + C             )        CONSTANT (44, 5.568E-5, 0, 1, 1)
REACTION (AC + AC         --> CIS + A + C         )        CONSTANT (45, 5.568E-5, 0, 1, 1)
REACTION (ACIS  + CCIS    --> CIS + CIS + CIS    )        CONSTANT (46, 5.568E-5, 0, 1, 1)
REACTION (ACIS  + CTRANS  --> CIS + TRANS + CIS   )        CONSTANT (47, 5.568E-5, 0, 1, 1)
REACTION (ATRANS + CCIS   --> CIS + TRANS + CIS   )        CONSTANT (48, 5.568E-5, 0, 1, 1)
REACTION (ATRANS + CTRANS --> CIS + TRANS + TRANS )        CONSTANT (49, 5.568E-5, 0, 1, 1)
REACTION (AC + ACIS       --> TRANS + A + CIS     )        CONSTANT (50, 1.236E-4, 0, 1, 1)
REACTION (AC + CCIS       --> TRANS + C + CIS     )        CONSTANT (51, 1.236E-4, 0, 1, 1)
REACTION (AC + ATRANS     --> TRANS + A + TRANS   )        CONSTANT (52, 1.236E-4, 0, 1, 1)
REACTION (AC + CTRANS     --> TRANS + C + TRANS   )        CONSTANT (53, 1.236E-4, 0, 1, 1)
REACTION (AC  + ACIS       --> CIS + A + CIS     )         CONSTANT (54, 5.568E-5, 0, 1, 1)
REACTION (AC + CCIS       --> CIS + C + CIS     )         CONSTANT (55, 5.568E-5, 0, 1, 1)
REACTION (AC + ATRANS     --> CIS + A + TRANS   )         CONSTANT (56, 5.568E-5, 0, 1, 1)
REACTION (AC + CTRANS     --> CIS + C + TRANS   )         CONSTANT (57, 5.568E-5, 0, 1, 1)
REACTION (ACCIS  + A      --> CIS + A + TRANS   )         CONSTANT (58, 1.236E-4, 0, 1, 1)
REACTION (ACCIS  + C      --> CIS + C + TRANS   )         CONSTANT (59, 1.236E-4, 0, 1, 1)
REACTION (ACTRANS + A     --> TRANS + A + TRANS  )         CONSTANT (60, 1.236E-4, 0, 1, 1)
REACTION (ACTRANS + C     --> TRANS + C + TRANS  )         CONSTANT (61, 1.236E-4, 0, 1, 1)
REACTION (AC + ACCIS      --> CIS + A + C + CIS    )      CONSTANT (62, 5.568E-5, 0, 1, 1)
REACTION (AC + ACCIS      --> TRANS + A + C + CIS   )      CONSTANT (63, 1.236E-4, 0, 1, 1)
REACTION (AC + ACTRANS    --> CIS + A + C + TRANS  )      CONSTANT (64, 5.568E-5, 0, 1, 1)
REACTION (AC + ACTRANS    --> TRANS + A + C + TRANS )      CONSTANT (65, 1.236E-4, 0, 1, 1)
REACTION (AC + ACCIS      --> CIS + A + C + CIS    )      CONSTANT (66, 5.568E-5, 0, 1, 1)
REACTION (AC + ACCIS      --> TRANS + A + C + CIS   )      CONSTANT (67, 1.236E-4, 0, 1, 1)
REACTION (AC + ACTRANS    --> CIS + A + C + TRANS  )      CONSTANT (68, 5.568E-5, 0, 1, 1)
REACTION (AC + ACTRANS    --> TRANS + A + C + TRANS )      CONSTANT (69, 1.236E-4, 0, 1, 1)

REACTION (COMPILE)
REACTION (SHOW)
CONSTANT (SHOW)
DEFINE   (1, TRANS , P, 1) SCALE (3,1)
DEFINE   (2, CIS   , P, 4) SCALE (3,1)
SELECT   ( TRANS, CIS )
READ     ( 46DiMeSR   )
TIME     (SEC)
WIN      (0, 60000, 15000, 200, 0, 20E-3, 5E-3, 1E-4)
ASSIGN   (OBS, TRANS = TRANS + ATRANS + CTRANS + ACTRANS + 2 TRANSTRANS + TRANSCIS )
ASSIGN   (OBS, CIS   = CIS   + ACIS   + CCIS   + ACCIS   + CISTRANS + ACTRANS )
ASSIGN   (SPEC, A     = #20E-3 )
10. Appendices

ASSIGN (SPEC, C = #20E-3 )

CHOOSE ( EXP1 )

INTEG ( STIFF, 1E-9, 8, 0.075, 200, 100 )

PLOT ( OBS, RES )

* 10 rounds of Simplex optimizer without screen
* update

SIMPLEX (PLOT)
SIMPLEX (PLOT)
SIMPLEX (PLOT)
SIMPLEX (PLOT)
SIMPLEX (PLOT)
SIMPLEX (PLOT)
SIMPLEX (PLOT)
SIMPLEX (PLOT)
SIMPLEX (PLOT)

PLOT (FILE)

*SCAN (1.02, 20, 20)
A5. SimFit Input File for Simulation of [A•B] Competition

* Simulation of two competitive efficient AB system
  * Nitrones: NitroneA (4,6-DiMe) and NitroneB (6-Me)
  * Maleimide: Maleimide

* NitroneA: $K_a = 315 \text{ M}^{-1}$ and $EM = 2.23 \text{ M}$
* NitroneB: $K_a = 160 \text{ M}^{-1}$ and $EM = 1.99 \text{ M}$

**MODE** (ISOSIM)

* Background reactions of nitrones to form cycloadducts

REACTION (NitroneA + Maleimide --> TransA , 1.236E-4)
REACTION (NitroneA + Maleimide --> CisA , 5.568E-5)
REACTION (NitroneB + Maleimide --> TransB , 1.301E-4)
REACTION (NitroneB + Maleimide --> CisB , 5.929E-5)

* Association of recognition-enabled species

REACTION (NitroneA + Maleimide ==> AMal , 1.00E+9 , 3.174E+6)
REACTION (NitroneB + Maleimide ==> BMal , 1.00E+9 , 6.250E+6)

* Reactions of complexes

REACTION (AMal + NitroneA --> TransA + NitroneA , 1.236E-4)
REACTION (AMal + NitroneA --> CisA + NitroneA , 5.568E-5)
REACTION (AMal + NitroneB --> TransB + NitroneA , 1.301E-4)
REACTION (AMal + NitroneB --> CisB + NitroneA , 5.929E-5)
REACTION (BMal + NitroneA --> TransA + NitroneB , 1.236E-4)
REACTION (BMal + NitroneA --> CisA + NitroneB , 5.568E-5)
REACTION (BMal + NitroneB --> TransB + NitroneB , 1.301E-4)
REACTION (BMal + NitroneB --> CisB + NitroneB , 5.929E-5)

* Recognition-mediated reactions

REACTION (AMal --> CisA , 1.241E-4)
REACTION (BMal --> CisB , 1.178E-4)

REACTION (COMPILE)
REACTION (SHOW)

* Initialise concentrations

INIT (NitroneA , 0.02000 , 1)
INIT (NitroneB , 0.02000 , 2)
INIT (Maleimide , 0.02000 , 3)

INTEG (STIFF, 1E-9, 8, 0.05, 200, 100)
NUMPLOT (150)
TIME (SEC)
WIN (0, 100000, 25000, 0.1, 0, 20E-3, 5E-3, 3E-4)
PLOT (FILE)
PLOT
A6. SimFit Input File for Simulation of a Dynamic System

* Simulation of two competitive efficient AB system

Nitrones : AZ (4,6-DiMe), BZ (6-Me), CZ and DZ are ballast nitrones

Maleimide : Maleimide

AZ : Ka = 315 M⁻¹ and EM = 2.23 M

BZ : Ka = 160 M⁻¹ and EM = 1.99 M

MODE ( ISOSIM )

REACTION ( A + W ==> AW , 1.00E+9 , 1.00E+8 )
REACTION ( A + X ==> AX , 1.00E+9 , 1.00E+8 )
REACTION ( A + Y ==> AY , 1.00E+9 , 1.00E+8 )
REACTION ( A + Z ==> AZ , 1.00E+9 , 1.00E+6 )
REACTION ( B + W ==> BW , 1.00E+9 , 1.00E+8 )
REACTION ( B + X ==> BX , 1.00E+9 , 1.00E+8 )
REACTION ( B + Y ==> BY , 1.00E+9 , 1.00E+8 )
REACTION ( B + Z ==> BZ , 1.00E+9 , 1.00E+6 )
REACTION ( C + W ==> CW , 1.00E+9 , 1.00E+8 )
REACTION ( C + X ==> CX , 1.00E+9 , 1.00E+8 )
REACTION ( C + Y ==> CY , 1.00E+9 , 1.00E+8 )
REACTION ( C + Z ==> CZ , 1.00E+9 , 1.00E+6 )
REACTION ( D + W ==> DW , 1.00E+9 , 1.00E+8 )
REACTION ( D + X ==> DX , 1.00E+9 , 1.00E+8 )
REACTION ( D + Y ==> DY , 1.00E+9 , 1.00E+8 )
REACTION ( D + Z ==> DZ , 1.00E+9 , 1.00E+6 )

* Background reactions of nitrones to form cycloadducts

REACTION ( AZ + M --> TransA , 1.236E-4 )
REACTION ( AZ + M --> CisA , 5.568E-5 )
REACTION ( BZ + M --> TransB , 1.301E-4 )
REACTION ( BZ + M --> CisB , 5.929E-5 )
REACTION ( CZ + M --> TransC , 1.236E-4 )
REACTION ( CZ + M --> CisC , 5.568E-5 )
REACTION ( DZ + M --> TransD , 1.301E-4 )
REACTION ( DZ + M --> CisD , 5.929E-5 )

* Association of recognition-enabled species

REACTION ( AZ + M ==> AZMal , 1.00E+9 , 4.545E+6 )
REACTION ( BZ + M ==> BZMal , 1.00E+9 , 5.785E+6 )

* Reactions of complexes with AZ, BZ, CZ and DZ

REACTION ( AZMal + AZ --> TransA + AZ , 1.236E-4 )
REACTION ( AZMal + AZ --> CisA + AZ , 5.568E-5 )
REACTION ( AZMal + BZ --> TransB + AZ , 1.301E-4 )
REACTION ( AZMal + BZ --> CisB + AZ , 5.929E-5 )
REACTION ( AZMal + CZ --> TransC + AZ , 1.236E-4 )
REACTION ( AZMal + CZ --> CisC + AZ , 5.568E-5 )
REACTION ( AZMal + DZ --> TransD + AZ , 1.301E-4 )
REACTION ( AZMal + DZ --> CisD + AZ , 5.929E-5 )

REACTION ( BZMal + AZ --> TransA + BZ , 1.236E-4 )
REACTION ( BZMal + AZ --> CisA + BZ , 5.568E-5 )

REACTION ( BZMal + BZ --> TransB + BZ , 1.301E-4 )
REACTION ( BZMal + BZ --> CisB + BZ , 5.929E-5 )

REACTION ( BZMal + CZ --> TransC + BZ , 1.236E-4 )
REACTION ( BZMal + CZ --> CisC + BZ , 5.568E-5 )

REACTION ( BZMal + DZ --> TransD + BZ , 1.301E-4 )
REACTION ( BZMal + DZ --> CisD + BZ , 5.929E-5 )

* Recognition-mediated reactions

REACTION ( AZMal --> CisA , 1.241E-4 )
REACTION ( BZMal --> CisB , 1.178E-4 )

REACTION ( Compile )
REACTION ( Show )

* Initialise concentrations

INIT ( A , 0.02000 , 1 )
INIT ( B , 0.02000 , 2 )
INIT ( C , 0.02000 , 3 )
INIT ( D , 0.02000 , 4 )
INIT ( W , 0.02000 , 5 )
INIT ( X , 0.02000 , 6 )
INIT ( Y , 0.02000 , 7 )
INIT ( Z , 0.02000 , 8 )
INIT ( M , 0.02000 , 9 )

INTEG (STIFF, 1E-9, 8, 0.05, 200, 100)

NUMPLOT ( 150 )

TIME ( SEC )

WIN (0, 250000, 250, 0.1, 0, 20E-3, 5E-3, 3E-4)

PLOT ( FILE )

PLOT