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Making Molecules Make Themselves – the Chemistry of Artificial Replicators

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Examples of chemical systems capable of templating and catalyzing their own synthesis – so-called replicating systems – have begun to appear in the chemical literature over the last 20 years. For the biologist, these systems represent a link with the origin of life – their study can perhaps shed light on prebiotic chemical evolution. For the synthetic chemist, they represent the ultimate synthetic machine, capable of templating the production of a large number of perfect copies of themselves from a single original molecule. One of the driving forces behind this research area has been the recognition of the important role that replication plays in biology and a desire to answer a fundamental question – "Is the structural complexity of nucleic acids necessary to store and transmit information at a molecular level?" In addition, the concept of a chemical template that is capable of making billions of exact copies of itself, given appropriate starting materials, is a highly attractive one for the burgeoning field of systems chemistry. It is therefore clear that the development of a detailed understanding of the behaviour of replicating systems has important perspectives for both biology and chemistry. In the present review, we will concentrate on the design and implementation of systems based on small, synthetic organic molecules that can reproduce themselves.

1. Introduction

The progressive evolution of matter through increasing levels of complexity resulted in the emergence[1] of life on Earth. While the exact sequence of events that precipitated this process will almost certainly remain unknown, an essential property of living organisms is their ability to make copies of their genetic information through replication. The discovery of the molecular structure of DNA[2] led immediately to the understanding that its replication involves template-directed synthesis. Each strand in the double helix serves as a template for the production of a new, complementary partner strand. Although the principle of DNA replication is simple, the mechanism by which it is carried out in the cell involves the intervention of a large number of enzymes and other proteins. The DNA molecule, nevertheless, carries all the information needed for its own replication, as the enzymes are produced from the information encoded within the DNA itself.

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Douglas Philp was born on the last day of 1967 in Glasgow, Scotland. After completing his early education in the West of Scotland, he moved to the University of Aberdeen in 1985 where he completed a BSc Honours Degree in Chemistry in 1989. He then spent three years in the laboratory of Professor Sir Fraser Stoddart FRS, firstly at the University of Sheffield and then subsequently at the University of Birmingham, where he completed his PhD in 1992, with a thesis entitled Self-Assembly in Chemical Systems. He was a Royal Society European Science Exchange Programme Postdoctoral Fellow at the ETH, Zürich in the laboratories of Professor François Diederich, before returning to the University of Birmingham in 1994 to take up a post first as Lecturer, and finally as Reader, in Physical Organic Chemistry. In September 2000, he moved to his current position in the Centre for Biomolecular Sciences at the University of St Andrews. His current research interests focus on the development of self-assembling and self-replicating structures, complex systems and the application of computational methods to problem solving in chemistry.

Biological systems demonstrate that chemical replication is possible and reveals that template effects are key to its success. However, a number of fundamental questions remain. Can molecules replicate themselves efficiently without the aid of enzymes or external cofactors? Are nucleic acids unique in their ability to store and transmit information at a molecular level? During the last twenty years, chemists have devised[3] numerous non-enzymatic synthetic self-replicating systems in an attempt to answer these questions. These chemical model systems have also been designed in order to elucidate the essential principles of molecular self-replication, to identify the minimal requirements, to better understand the scope and the limitations, and to translate the principles into synthetic systems. From a chemical perspective, the emergence of a synthetic machinery that is capable of directing its own synthesis and co-operating with other systems to create an organized hierarchy is an important and challenging target. Achieving this goal could have a significant impact on the fabrication of molecular architectures at the nanometer scale. The creation of molecules that function as specific and efficient templates for the formation of themselves and others should permit the development of efficient protocols that allow us to establish and manage replication, organization and evolution within synthetic supramolecular assemblies. This approach to designed dynamic behaviour has become known[4] as systems chemistry. Ultimately, these programmed systems can be exploited in the construction, selection and amplification of large molecular and supramolecular assemblies.

In the last twenty years, a considerable body of work has emerged on non-enzymatic synthetic replicating systems based on nucleic acids,[5] peptides[6] and small organic molecules. In the present context, we wish to focus on replicating systems based on small organic molecules which exploit a range of covalent bond forming reactions and recognition elements in order to bring about replication.

2. Replication Models

In the most general sense, a replicator is a molecule, or an assembly of molecules, whose rate of formation can be increased through the action of a template bearing recognition elements which are complementary to those on the replicator. At the simplest level, this template effect can operate in one of two ways. Firstly, the replicator can act as a catalyst for its own creation from simpler building blocks – a process known as minimal or self-replication. This model requires the replicator to be an autocatalyst and, therefore, it must template its own formation. The replicator preorganizes its precursors through molecular recognition in a defined spatial arrangement permitting the transfer of key structural information allowing it to template the formation of an exact copy of itself. The processes involved in minimal replication are encapsulated schematically in three possible reaction channels (Figure 1).

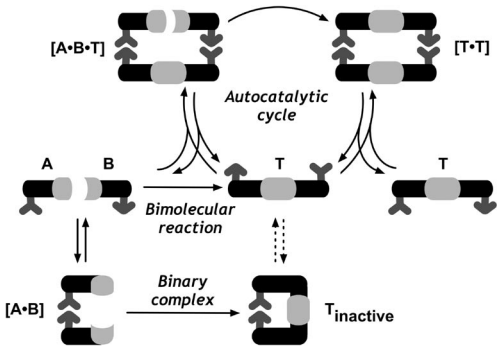

Figure 1. The minimal model of self-replication. Reagents **A** and **B** can react through three pathways – an uncatalyzed bimolecular reaction, a recognition-mediated pseudounimolecular pathway mediated by a binary complex [**A·B**] and a recognition-mediated pseudounimolecular autocatalytic cycle mediated by a ternary complex [**A·B·T**].

The first channel is the uncatalyzed bimolecular reaction between reagents **A** and **B** to afford the template **T**. A key requirement of the minimal model is that **A** and **B** bear complementary recognition sites such that **A** and **B** can associate with each other to form a binary complex, [**A·B**]. The presence of this complex opens a second reaction channel – the binary complex channel – in which **A** and **B** are preorganized with respect to each other and the reaction between them is pseudointramolecular. The product of this reaction channel is a closed template $\mathbf{T_{inactive}}$ in which the recognition used to assemble the binary complex lives on in the template. Thus, although rate acceleration is achieved by this mechanism, this template is usually[7] inert catalytically. The third reaction channel available to the system is the autocatalytic cycle. In this channel, **A** and **B** bind reversibly to the open template **T** to form a catalytic ternary complex [**A·B·T**]. In a manner similar to the [**A·B**] complex, the reaction between **A** and **B** is also rendered pseudointramolecular. Bond formation occurs between **A** and **B** to give the product duplex [**T·T**], which then dissociates to return two molecules of **T** to the start of the autocatalytic cycle. Thus, assuming the open template **T** presents its recognition sites in the correct orientation, it can act as a template for its own formation, transmitting molecular information through the formation of identical copies of itself. Self-replication is therefore a subset of autocatalytic[8] reactions. In an autocatalytic reaction, the product formed in the reaction mixture is a catalyst for the same reaction. A system in which self-replication is operating can be defined as an autocatalytic reaction capable of transmitting structural information. This information can be stored as constitution, configuration, or long-living conformation. Key design elements in this model of replication are minimization of (a) reaction flux through the binary complex reaction channel and (b) inefficient autocatalysis as a result of product inhibition arising from an excessively stable product duplex [**T·T**].

The model of self-replication discussed thus far involves self-complementary structures. However, template effects in a replication cycle can also operate in a reciprocal sense.

The paradigm is DNA replication – the two strands of the double helix are not identical; one strand acts as a template for the formation of its complementary partner. The difference in template complementarity between minimal and reciprocal self-replication is a key distinction between these two types of system. In a minimal system, the template is self-complementary, whereas in a reciprocal system a pair of templates are complementary to each other. Therefore, reciprocal replicating systems rely on two interlinked cross-catalytic cycles in which the two templates catalyze the formation of each other. The processes involved in reciprocal replication are encapsulated schematically in Figure 2.

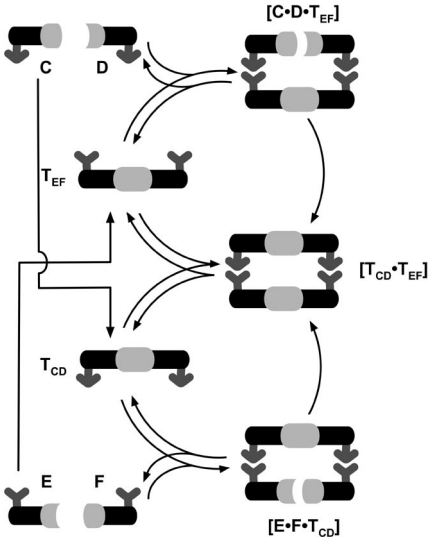

Figure 2. The reciprocal model of self-replication. Compounds **C** and **D** can react to form the template $\mathbf{T_{CD}}$, and, similarly, compounds **E** and **F** can react to form template $\mathbf{T_{EF}}$. $\mathbf{T_{CD}}$ and $\mathbf{T_{EF}}$ are mutually complementary. $\mathbf{T_{CD}}$ is capable of assembling **E** and **F** into the ternary complex [**E**·**F**·$\mathbf{T_{CD}}$] which catalyses the formation of $\mathbf{T_{EF}}$. Similarly, $\mathbf{T_{EF}}$ is capable of assembling **C** and **D** into the ternary complex [**C**·**D**·$\mathbf{T_{EF}}$] catalyzing the formation of $\mathbf{T_{CD}}$. These two interlinked cross-catalytic cycles represent a formal reciprocal replication cycle.

In these systems, compounds **C** and **D** can react to form the template $\mathbf{T_{CD}}$, and, similarly, compounds **E** and **F** can react to form template $\mathbf{T_{EF}}$. Since the four reactive partners bear appropriate recognition sites and since $\mathbf{T_{CD}}$ and $\mathbf{T_{EF}}$ are mutually complementary, $\mathbf{T_{CD}}$ is capable of assembling **E** and **F** into the ternary complex [**E**·**F**·$\mathbf{T_{CD}}$]. This ternary complex intramolecularizes the reaction between **E** and **F** and, hence, catalyzes the formation of $\mathbf{T_{EF}}$. Similarly, $\mathbf{T_{EF}}$ is capable of assembling **C** and **D** into the ternary complex [**C**·**D**·$\mathbf{T_{EF}}$] and, hence, catalyzes the formation of $\mathbf{T_{CD}}$. These two interlinked cross-catalytic cycles represent a formal reciprocal replication cycle. It is, however, important to note that 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**, four replication cycles are possible: two minimal replication cycles – $\mathbf{T_{CE}}$ and $\mathbf{T_{DF}}$ may self-replicate – and the original reciprocal replication cycle $\mathbf{T_{CD}} \rightarrow \mathbf{T_{EF}}$ and $\mathbf{T_{EF}} \rightarrow \mathbf{T_{CD}}$. Additionally, the minimal replicators $\mathbf{T_{CE}}$ and $\mathbf{T_{DF}}$ may cross-catalyze the formation of each other.

3. Analysis of Replicating Systems

One of the biggest challenges which faces a researcher investigating replicating systems is the adequate characterization of all of the processes that occur in the system under study. For a minimal replicating system, during the initial stages of the reaction, the product **T** is formed relatively slowly through the bimolecular channel. Thus, the reaction will exhibit an induction or lag period. Once the concentration of **T** reaches a level that permits the formation of a significant amount of the catalytic ternary complex [**A**·**B**·**T**], the autocatalytic cycle begins to operate. Since each turn of the cycle doubles the amount of template present in solution, the product concentration should increase exponentially, before reaching a plateau when all reactants are consumed. Thus, the concentration–time profile of a replicating system should, in principle, be a sigmoidal, or "S"-shaped, curve. Therefore, a thorough analysis of the kinetic behaviour of the system is essential to analyze a replicator fully.

In order to demonstrate that the formation of a particular product is, indeed, the result of self-replicating behaviour, it is necessary to perform several control experiments. Firstly, it is necessary to identify a control compound which possesses the same chemical functionality as the building blocks of the replicator, but which is incapable of participating in any recognition-mediated processes. In practice, this control compound usually has its recognition site obstructed or removed. Measurement of kinetic data using this control compound provides a comparative baseline for all of the other studies. Secondly, since the efficient operation of a self-replicating system hinges on the reversible binding events that occur during the autocatalytic cycle, it is important to demonstrate the reliance of the reaction on molecular recognition. Hence, the addition of a competitive inhibitor, which is unreactive but is capable of binding to the recognition sites present in **A**, **B** and **T**, to the reaction mixture will interfere with these crucial recognition processes. This interference will, in turn, disrupt the autocatalytic cycle resulting in a decrease in the rate of the reaction and/or its selectivity, often accompanied by disappearance of the sigmoidal curve. The final, critical experiment that provides evidence that self-replication is operating within a system can be obtained by doping the reaction with the product **T** itself. The presence of pre-synthesized template **T** at the beginning of the reaction should result in a loss of the initial lag period in the rate profile for the reaction, providing evidence that the reaction is templated-directed. However, it is important to point out that if the association constant of the product duplex is extremely high, adding template may not, in fact, add significant amounts of catalytically active free template to solution.

For a minimal replicator, the two recognition-mediated reaction channels – the autocatalytic cycle and the binary complex channel – accomplish the same goal, i.e. acceleration and regio- and/or stereocontrol of a chemical reaction. However, the binary complex channel does not involve any form of recognition-mediated catalysis because the closed template $\mathbf{T}_{\mathbf{inactive}}$ does not play any further role in the reaction process. By contrast, a self-replicating system is an attractive target as it offers its non-linear kinetic behaviour as a means of amplifying, in an exponential manner, a particular structure over others in the system. Therefore, the suppression, or ideally the eradication, of the binary complex route is an important parameter within the minimal model of self-replication that must be optimized to ensure an efficient system. The second parameter that must be optimized is the catalytic efficiency within the ternary complex [**A**·**B**·**T**]. The template molecule **T** must accelerate the rate of reaction between the substrates **A** and **B** substantially compared with their rate of reaction in the absence of **T**. The final requirement for efficient replication is the effective dissociation of the product duplex [**T**·**T**]. Ideally, the duplex must be intrinsically less stable than the ternary complex [**A**·**B**·**T**]. This goal may be accomplished if a slight mismatch of the alignment of the recognition sites arises upon the formation of the new covalent bond(s). If the product duplex is extremely stable, it cannot dissociate and product inhibition arises. Hence, no new template is returned to the reaction and the autocatalytic cycle stalls. This situation raises the possibility of a self-replicating molecule that cannot complete the autocatalytic cycle. Such a system may be termed self-replicating because it constructs one copy of itself, but not autocatalytic, as it does not return significant amounts of free template to the reaction mixture. Thus, a self-replicating process is efficient when the binary complex channel is eradicated, and when a balance is found between effective binding of the substrates to the template and product release. Two variables can be manipulated easily in order to optimize the reaction conditions – namely concentration and temperature. A decrease in concentration reduces the contribution from the background bimolecular channel (reducing the concentration by a half reduces the rate of the bimolecular reaction by 4×). The lowering of the reagent concentration therefore enhances the contribution from the recognition-mediated pathways. However, if the concentration drops below the K_d for the recognition process used to assemble the ternary complex, replication will effectively be shut down. A change of temperature does not simply alter the rate of chemical reaction. A reduction in the reaction temperature will increase the strength of binding (and thus facilitate the formation of product via recognition) and decrease the bimolecular rate. However, it will also increase the association constant of the product duplex resulting in increased product inhibition. Therefore, concentration and temperature must be adjusted with caution.

Kinetic simulation of the behaviour of a replicator provides some insight into the various processes which operate in a replicating system. The fitting of experimental data to kinetic models allows the determination of important thermodynamic and kinetic parameters within the system including rate constants for reactions and equilibrium constants for recognition processes. A series of simplified kinetic models to describe the behaviour of artificial self-replicating systems were introduced[9] in a seminal contribution to the field by von Kiedrowski. The simplest of these is a purely autocatalytic reaction (Figure 3) with a variable reaction order for the product – known as the autocatalytic reaction order *p*. The parameter *p* describes the autocatalytic behaviour of the system and determines the type of the autocatalytic growth curve. In real systems, its value is expected to lie between 0.5 and 1. At the two extremes, a value of *p* that is 0.5 denotes the fact that the self-replicating system obeys the "square root law". In this situation, the rate of dissociation of the template duplex [**T**·**T**] is limiting overall. The concentration-time profile of such reaction shows parabolic growth (Figure 3, a). However, if dissociation of the product duplex is not rate limiting, the value of *p* will tend to 1. In that case, the dissociation of the product duplex is rapid and the concentration-time profile of the reaction shows exponential growth (Figure 3, b). The calculated concentration time profiles shown in Figure 3 demonstrate clearly that the change of the autocatalytic reaction order *p* produces a dramatic effect on the shape of

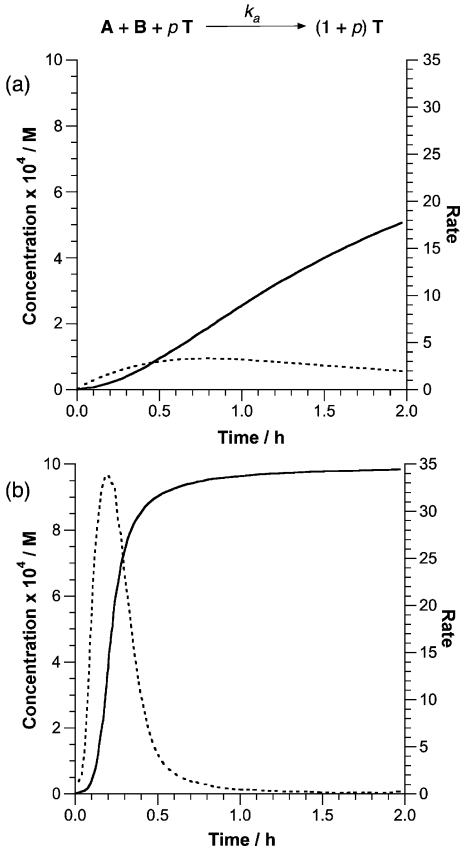

Figure 3. Concentration vs. time (left y axis, solid curve) and rate vs. time (right y axis, dashed curve) profiles for (a) a reaction with an autocatalytic reaction order of 0.5 (p = 0.5) and (b) a reaction with an autocatalytic reaction order of 1 (p = 1). Data taken from ref.[9]

the curves. It is worth noting that the maximum rate of reaction for a self-replicating system, parabolic or exponential, does not occur at $t = 0$. Instead, it occurs at some later point in the reaction and is associated with a particular concentration of **T**. These parameters can be accessed readily by exploiting a rate vs. time profile (dashed lines, Figure 3), which is simply the first derivative of the concentration vs. time profile.

The curves of the concentration–time profiles shown in Figure 3 illustrate purely autocatalytic systems, which are rarely representative of real experimental systems. Figure 4 shows the corresponding growth curves when formation of template molecules through the bimolecular channel is taken into account. The autocatalytic efficiency ε, which is the ratio of the template-catalysed rate constant, k_a, over the template-independent rate constant, k_b, measures the relative contribution of the pathway involving **T** to the observed rate of reaction. A sigmoidal shape only becomes apparent in the concentration-time profile above a critical value of ε. Indeed, some experimental systems do not display sigmoidal production of the template even though an autocatalytic pathway is present within the system.

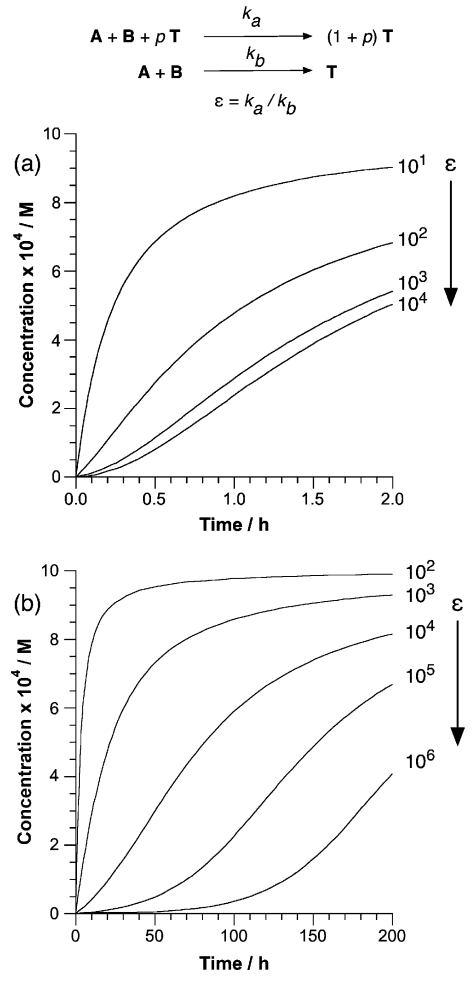

Figure 4. Concentration vs. time profiles for reactions with a contribution to their rates from an autocatalytic pathway (k_a) and a bimolecular pathway (k_b). The parameter ε (k_a/k_b) measures the relative importance of the two channels to the observed rate. For a reaction (a) with an autocatalytic reaction order of 0.5 ($p = 0.5$), increasing ε reveals a sigmoidal concentration vs. time profile with the point of inflection (maximal rate) convergent in time. For a reaction (b) with an autocatalytic reaction order of 1 ($p = 1$), increasing ε reveals a sigmoidal concentration vs. time profile with the point of inflection (maximal rate) which does not converge in time.

In practice, one of the easiest ways to discern whether a replicator is parabolic or exponential is to follow the formation of product **T** in the presence of three different amounts of preformed template at the start of the reaction (normally between 2 and 10% of the initial concentration of the precursors). Typically, the concentration of template added is doubled when proceeding to the next experiment. In the parabolic case, the initial rate of formation of **T** scales as $1:\sqrt{2}:\sqrt{4}$ upon addition of increasing amounts of **T**. In the exponential case, the initial rate of formation of **T** scales as 1:2:4.

Often, the course of a reaction is followed by NMR spectroscopy, and so kinetic data for the system is usually based on the monitoring of NMR integrals. However, the changes in the observed chemical shifts for the various species present in solution during the course of the reaction are not taken into account. By contrast, thermodynamic parameters for the supramolecular complexes involved is usually derived from independent NMR titration experiments. Von Kiedrowski recently introduced[10] a kinetic method ("kinetic NMR titration"), which combines analysis of integral and chemical shift changes, to harvest kinetic and thermodynamic information from the same experiment.

Reinhoudt and co-workers advocate[11] the use of complete kinetic modelling. In their approach, numerical integration of a complete set of differential rate laws which describe all pathways, both reversible and irreversible, within the system is used to obtain detailed information about the relative importance of each potential reaction pathway. This approach has proven extremely useful in several cases.

4. Minimal Replicating Systems

The concept of non-enzymatic artificial self-replicating systems was established by von Kiedrowski in 1986 when he demonstrated that a palindromic hexadeoxynucleotide can catalyze[12] its own formation from the self-complementary trinucleotide precursors **1** and **2** (Figure 5). The 3′-phosphate group of trimer **1** is activated in situ by EDC, and trimer **2** bears a nucleophilic group at its 5′-terminus. Initial experiments did not reveal a sigmoidal curve for the production of hexadeoxynucleotide **3** ($p = 0.48$). Nevertheless, the observation of an increase in the rate of product synthesis when template is added initially, confirmed the autocatalytic contribution to the formation of template molecule. This first demonstration of a synthetic replicating system inspired the work on small organic molecules which we will focus on for the remainder of this review.

Self-replication in small organic molecules was first described[13] by Rebek and co-workers in 1990. The Rebek laboratory developed[14] systems based upon amide bond

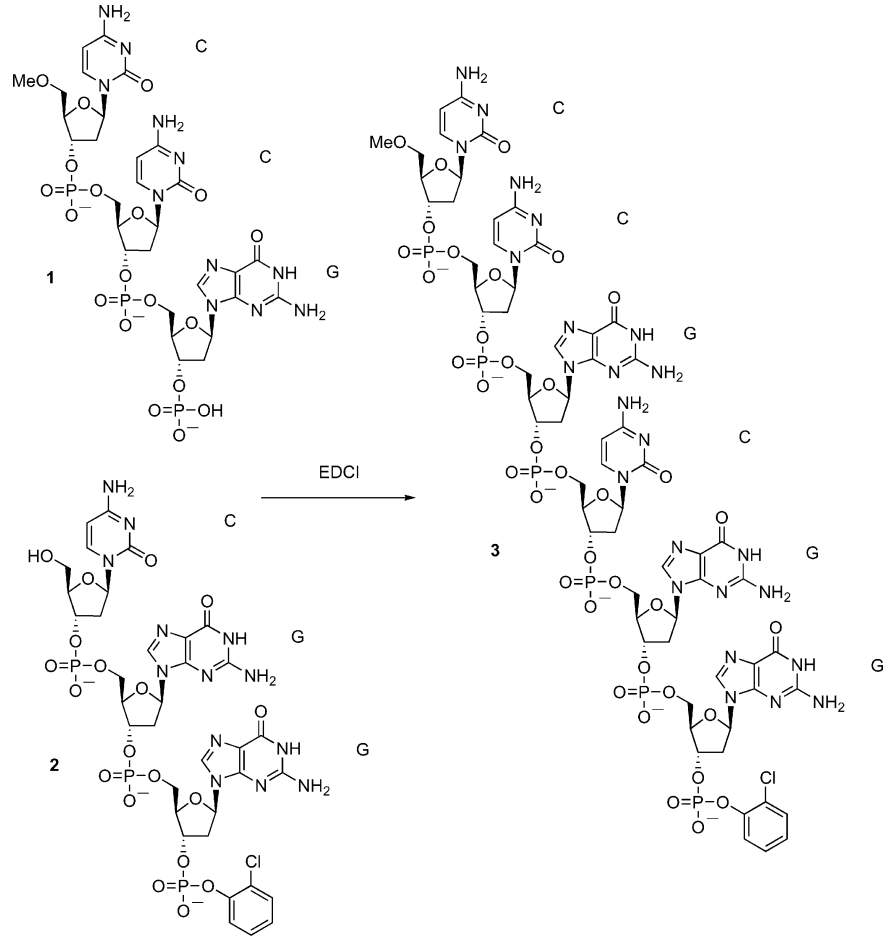

Figure 5. The palindromic hexadeoxynucleotide **3** is capable of templating its own formation from a nucleophilic trideoxynucleotide **1** and an electrophilic trideoxynucleotide **2**. EDCI = 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide.

formation as the chemical ligation step. The recognition between the components of the system is driven by the hydrogen-bonding between an adenine derivative and a Kemp's triacid imide. The design of these complementary recognition sites is reminiscent of biological systems – the imide presents a hydrogen-bond pattern similar to that of thymine. The first system constructed by Rebek in which self-replication operates is shown in Figure 6.

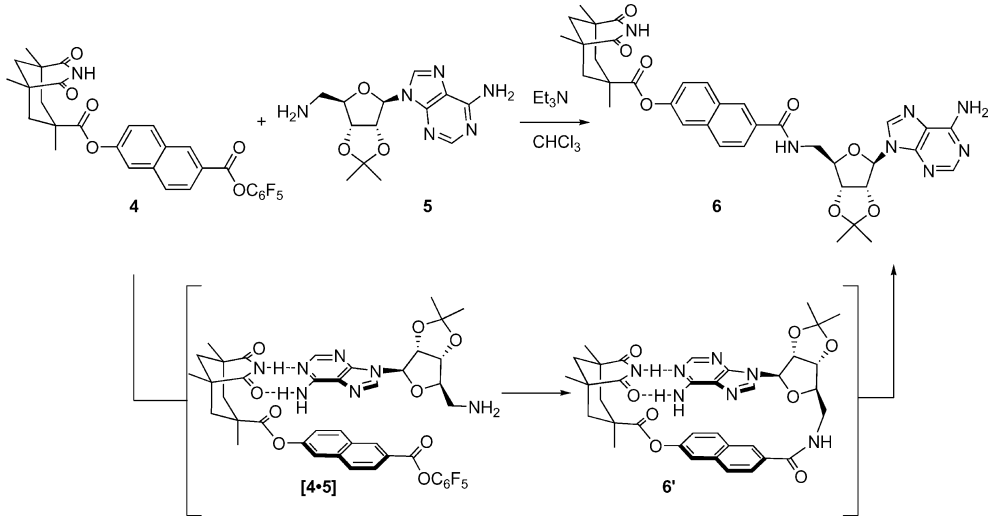

Figure 6. The recognition between the Kemp's tricaid imide in the activated ester **4** and the adenine base in **5** drives the formation of template **6** either through the binary complex [**4·5**] or through the corresponding [**4·5·6**] complex.

Rebek demonstrated the importance of the size of the spacer within the ester building block; an early attempt[15] incorporating a phenyl spacer, instead of a naphthyl spacer, failed, as a consequence of the resulting product remaining folded shut. In contrast, reaction between the activated pentafluorophenolate ester **4** and the amine **5** exhibits autocatalysis; seeding the reaction with prefabricated product **6** accelerates its initial rate, although no sigmoidal curve is observed. The reaction proceeds significantly slower when the imide nitrogen is methylated, and when one equivalent of 2,6-bis(acylamino)pyridine is added to the reaction mixture as competitive inhibitor. This observation is evidence that the rate enhancement is the result of molecular recognition. Rebek concluded that template **6** can catalyze its own formation, but that the observation of a sigmoidal growth curve is hampered by the dominant presence of the [**A·B**] complex pathway. An unfavourable *cis* amide **6′** is formed with the [**4·5**] complex; the subsequent isomerization to the more favourable *trans* linkage prevents the product retaining intramolecular hydrogen bonding and returns free template to the mixture.

Through the early 1990s, the Rebek self-replicating system became a subject of controversy. Menger reported[16] that simple amides can also catalyze the reaction between **4** and **5**. He also demonstrated[17] that **6** can accelerate the reaction between **5** and esters that are not capable of hydrogen bonding. Menger concluded from these two observations that catalysis in the Rebek system does not arise from the ternary complex, but from only part of the template, and that therefore there is no need to postulate a self-replication mechanism. However, in order to play a role, amide catalysis requires high concentrations, far higher than those used[18] in Rebek's experiments. Moreover, the ability of **6** to catalyze any other reaction does not detract[19] from its self-replicating potential.

These discussions motivated Reinhoudt and co-workers to undertake a detailed kinetic analysis[11] of this complex system. They identified five different pathways (Figure 7) that lead to the product (three are bimolecular and two are unimolecular): the background reaction (**I**), the [**A·B**] complex (**II**), the ternary complex (**III**), the reaction between the activated amine (by the bonded template) with free ester (**IV**), and the reaction between the activated ester (by the bonded template) with free amine (**V**).

Reinhoudt then calculated their individual contributions to product formation and showed that they are strongly concentration dependent. The relative contribution of the background reaction (**I**) is higher when the concentration increases, at the expense of the [**A·B**] channel (**II**). The ternary complex **III** contributes 34–46% to the product formation, in presence of template only. The contribution of the amide catalysis through the activated complex **IV** increases with increasing concentration. Pathway **V** hardly contributes over the entire concentration range. This study demonstrates that the different pathways obscure the simple picture of a single channel as the only responsible of rate enhancement. Although the reaction between **4** and **5** in the presence of preformed template **6** can operate through self-replication, the major pathway in its absence is the [**A·B**] complex.

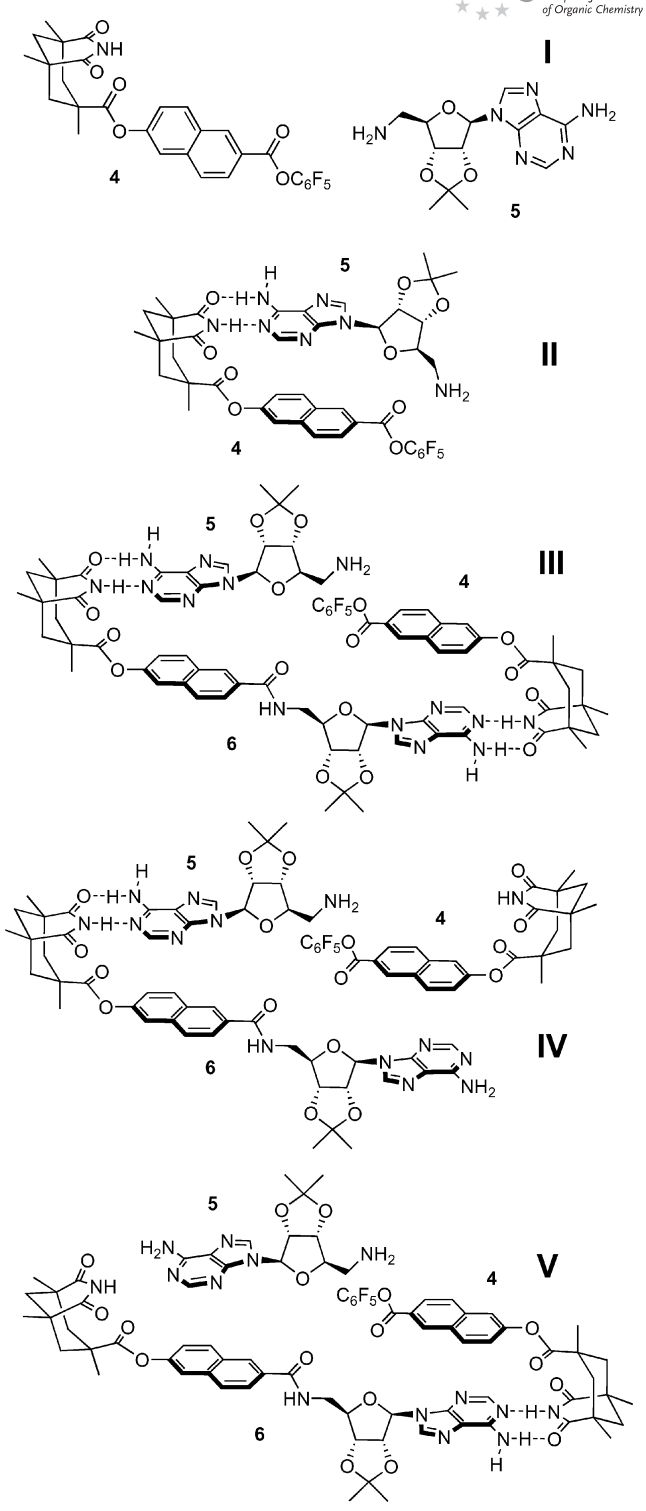

Figure 7. The five pathways which contribute to the observed kinetics of the Rebek replicator: the background reaction (**I**), the [**A·B**] complex (**II**), the ternary complex (**III**), the reaction between the activated amine (by the bonded template) with free ester (**IV**), and the reaction between the activated ester (by the bonded template) with free amine (**V**).

Rebek restructured[20] its system to render the [**A·B**] pathway less important by replacing the naphthyl spacer with a

longer biphenyl group in template **8** (Figure 8). This modification enforces the separation of ester **7** and bound amine **5** in the binary complex and this system does indeed exhibit a reaction profile with clear sigmoidal character.

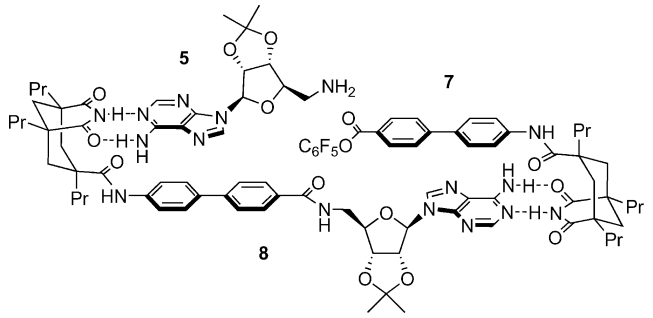

Figure 8. The longer biphenyl spacer present in activated ester **7**, when compared to the naphthyl spacer present in **4** (Figure 6), ensures that template **8** is formed principally by the reaction through the ternary complex [**5**·**7**·**8**] shown.

A further complexity present in the systems developed in the Rebek laboratory is the fact that recognition between the adenine and the imide, can occur either through the Watson–Crick or Hoogsteen binding modes. Rebek demonstrated[21] that replicators whose adenine exocyclic amine is monosubstituted are less efficient because they are limited to exploiting the Hoogsteen mode, and hence to a less favourable conformation. In order to eliminate the conformational complications from the binding of adenine in these two modes, Rebek and co-workers developed[22] a second generation of replicators that incorporate a receptor able to chelate the purine through simultaneous Watson–Crick and Hoogsteen binding modes (Figure 9). Rebek demonstrated that the reaction between activated ester **9** and amine **5** is self-replicating. The reaction through an [**A**·**B**] complex is ruled out by the biphenyl substituent on the carbazole **9**, but the efficiency of this system is impaired by strong product inhibition. Rebek also developed another class of template-directed system utilizing[23] a thymine/diaminotriazine recognition motif, based around a xanthene scaffold. Recombination[24] between this system and replicator **8** produced hybrid templates.

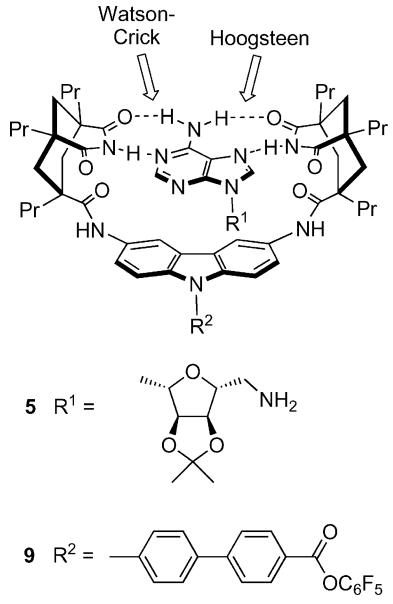

Figure 9. A combination of Watson–Crick and Hoogsteen hydrogen bonding patterns permit the recognition of **5** by **9**.

Contemporaneously with the amide-based systems reported by Rebek and co-workers, von Kiedrowski described[25] a series of systems (Figure 10) based on the condensation between structurally simple amines and aldehydes. The amidinium–carboxylate salt bridge is exploited as a substitute for base pairing. The synthesis of **12** from amine **10** and aldehyde **11** is autocatalytic: the higher the initial concentration of **12** in the reaction mixture, the faster its formation. The condensation obeys the square root law for the autocatalytic contribution, and the autocatalytic efficiency, ε, is 16 $M^{-1/2}$.

The condensation of **11** and **13** (Figure 10) also follows a square-root law. But interestingly, in the presence of the

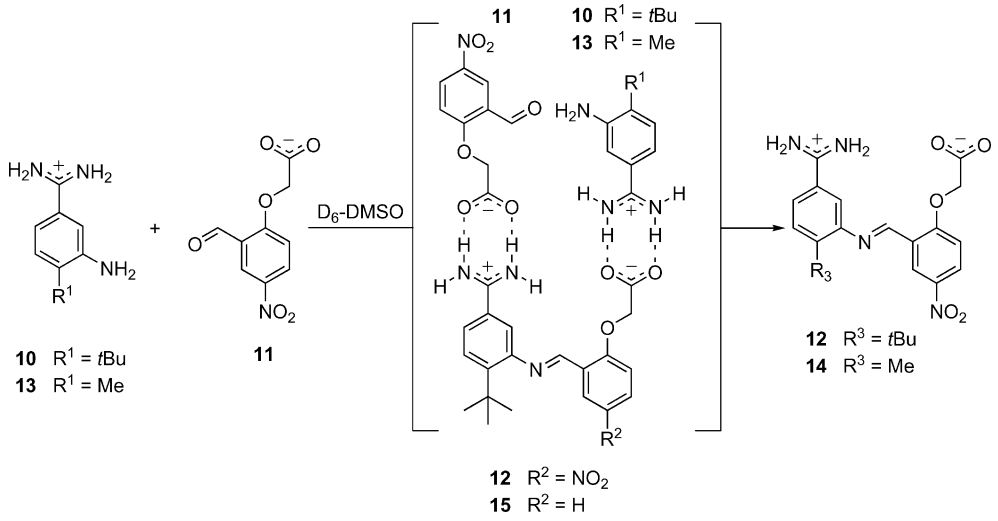

Figure 10. A series of auto- and crosscatalytic templates can be formed by combinations of amines **10** and **13** with aldehyde **11**.

template **15**, the rate of the reaction depends linearly on the concentration of template **15**. This first-order relationship indicates that product inhibition is not operative here. This result, although it originates from a crosscatalytic rather than an autocatalytic effect, suggests that self-replication with exponential autocatalysis is feasible in systems where the template forms a less stable complex with itself than with its precursors.

The condensation between an amine and an aldehyde is a reversible process. This fact has been exploited[26] very recently by Giussepone and Xu in an attempt to couple dynamic covalent chemistry[27] and replication, by exploiting a replicator based on the original Rebek design **6**, but replacing the activated ester in **4** with an aldehyde. In this situation, the system forms a template duplex reversibly and they created a system capable of a process they term duplication.

Our laboratory has also recently developed[28] an imine-based replicator (Figure 11). Reaction of amine **16** and aldehyde **17** in $CDCl_3$ at room temperature affords the imine template **18** which is capable of assembling the amine **16** and aldehyde **17** within the ternary complex [**16**·**17**·**18**]. The reaction between the aldehyde and amine is accelerated strongly (EM = 20 M) within this complex and the overall position of the equilibrium[29] is shifted significantly in favor of the formation of the duplex [**18**·**18**]. As expected from a replicator, the addition of 5 mol-% of template **18** at the start of the reaction removes the lag period observed for this system. Interestingly, the reduced template **19** is also capable of accelerating the formation of **18** through crosscatalysis. The development of a mild reduction method, compatible with the reaction conditions and recognition elements used in this system, will ultimately allow the coupling of the autocatalytic system (Figure 11, top) with the crosscatalytic system (Figure 11, bottom) through an irreversible step, thus allowing complete conversion of **16** and **17** to **19** by a combination of auto- and crosscatalysis.

One of the issues with the use of condensation reactions in replicating systems is their susceptibility to catalysis by general acids and bases. The initial conditions of the reaction can therefore have an important influence on the outcome of the reaction and the inadvertent presence of such catalysts can mask the catalytic effects of recognition. Additionally, the attack of a nucleophile on a carbonyl group has a relatively large range[30] of acceptable attack angles. Since one of the primary concerns in the design of a replicating system is the elimination of reaction through the binary complex, ideally one would wish to use a reaction that has a narrow reaction cross-section to facilitate the design process. In this respect, thermally-allowed 4π + 2π cycload-

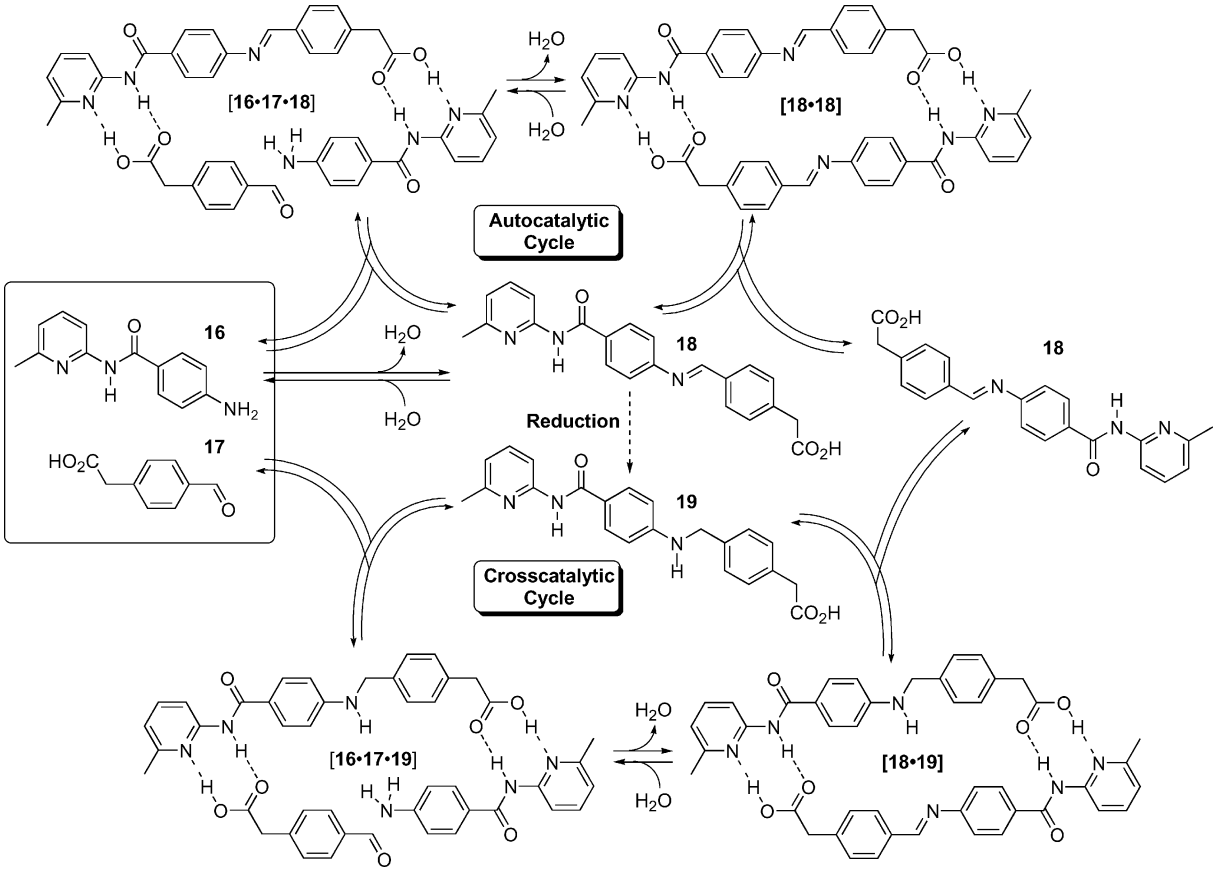

Figure 11. Imine **18** can assemble amine **16** and aldehyde **17** in $CDCl_3$ at 25 °C and accelerate the reaction between them through the autocatalytic cycle mediated by the ternary complex [**16**·**17**·**18**]. Reduction of imine **18** affords amine **19** which is also capable of crosscatalytic acceleration of imine **18** through the crosscatalytic cycle mediated by the ternary complex [**16**·**17**·**19**].

dition reactions provide an ideal alternative for chemical ligation in artificial self-replicating systems. Such reactions have very strict stereoelectronic demands, requiring the orbitals at the ends of the interacting π systems to be aligned correctly and simultaneously. Additionally, and importantly, these reactions are usually not susceptible to catalysis by general acids and bases.

In 1997, Sutherland and Wang described[31] a template-directed system (Figure 12) based on the Diels–Alder cycloaddition between diene **20** and maleimide **21**. Recognition within this system is provided by the naphthyridine present in **20** and the 2-pyridone present in **21**.

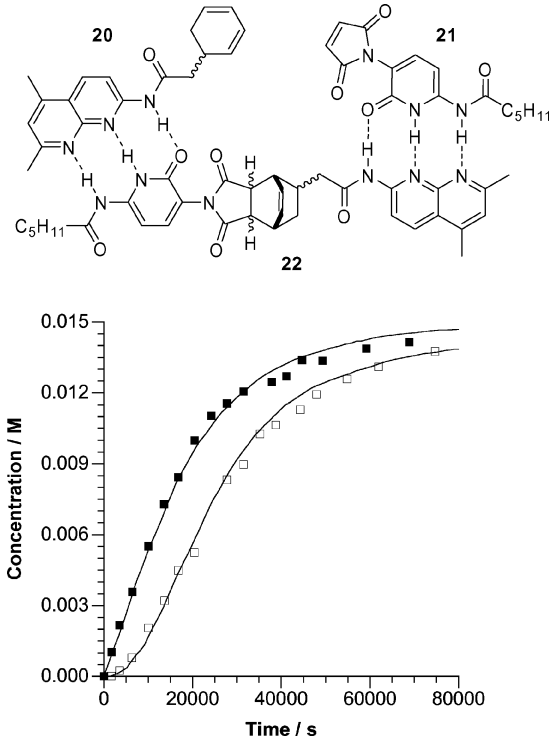

Figure 12. Diels–Alder cycloadduct **22** can assemble the chiral diene **20** and maleimide **21** in a catalytically-active ternary complex. This complex is the dominant reactive species in the formation of **22** in CD_2Cl_2 at 25 °C (open squares in graph). The addition of 5 mol-% of **22** to the reaction mixture (filled squares in graph) removes the lag period from the concentration vs. time profile demonstrating that **22** is a catalyst for its own formation. The experimental data can be fitted to a variant of the minimal model (Figure 1). Data taken from ref.[31]

The rate profile obtained for the production of **22** displays a nearly exponential growth (Figure 12) and kinetic analysis gave a value of *p* of 0.8. The treatment of the reaction with 10% of preformed template **22** shows the disappearance of the initial lag period (Figure 12), confirming that the reaction was template-directed.

Some aspects in the analysis of this system are not addressed directly in the 1997 report. The stereochemistry of the cycloadduct **22** is assumed to be *endo*, but this assignment is not confirmed unambiguously although the data does indicate that only one diastereoisomer is formed. This system is complicated further by the presence of a stereogenic centre in the diene **20**. The chiral diene is employed as a racemic mixture, thus many diastereoisomeric reaction pathways must exist. But again, no assignment of the stereochemistry of the templates found in the reaction mixture or quantification of any asymmetric induction that the recognition-mediated pathway induces over the independent bimolecular reaction pathway are presented.

In 2005, von Kiedrowski re-evaluated[32] the Sutherland system with respect to its stereochemical features, by synthesizing variants of the Sutherland replicator (Figure 13). The recognition elements were replaced by an amidopyridine and a carboxylic acid in order to increase the solubility of the system.

The monitoring of the reactions indicated no evidence for the formation of *exo* cycloadducts. Concentration-time profiles for the reactions of diene *rac*-**23** with maleimides **24** and **25** are shown in Figure 13. Kinetic analysis of these systems leads to *p* values of 0.89 for the former, and 0.9 for the latter. These data demonstrate that the von Kiedrowski variants are nearly exponential replicators and suggest that they are reasonable approximations of the Sutherland system. Studying the individual reactions between dienes (*R*)-**23** and (*S*)-**23** with maleimide **24** in the presence of 10% of template (*R*)-**26** is instructive. Analysis of the concentration-time profiles demonstrates that (*R*)-**26** can catalyze its own formation. Additionally, (*R*)-**26** can also catalyze the formation of (*S*)-**26**. The effect of the template is similar in both cases, indicating the presence of homochiral autocatalytic and heterochiral cross-catalytic channels. Kinetic modelling and fitting of reaction between diene **23** and maleimide **24** suggests that homochiral and heterochiral duplexes are nearly equally populated.

In our laboratory, we have employed the Diels–Alder reaction between maleimides and furans to create a series of potential replicators which are close structural analogues of the von Kiedrowski cyclohexadiene system. The reaction between a maleimide and a furan affords two diastereoisomeric products – the *endo* cycloadduct and the *exo* cycloadduct. We have studied extensively the subtle interplay between molecular structure and replication efficiency through comparative analyses of families of potential self-replicators involving this reaction as covalent bond forming step. The association between amidopicolines and carboxylic acids was chosen as the means of non-covalently associating the constituent building blocks. The amidopicoline functionality was connected[33] to the furan and the carboxylic acid functionality to the maleimides (Figure 14). Methylene or bismethylene chains were utilized to connect the recognition sites to the reaction sites. Thus, the structural variation inherent in this system affords three sites of variability: the number of methylene spacers between the recognition sites and the reactive sites (*m* and *n*, Figure 14), and the location of the spacer on the furan ring – either 2- or 3-substitution.

Pairwise combination of each of the dienes with each of the dienophiles gives rise to eight distinct systems – labelled **I** to **VIII** in Figure 14. In order to establish which recognition-mediated pathway is dominant in each system, the rate of reactions between pairwise combinations of maleimides

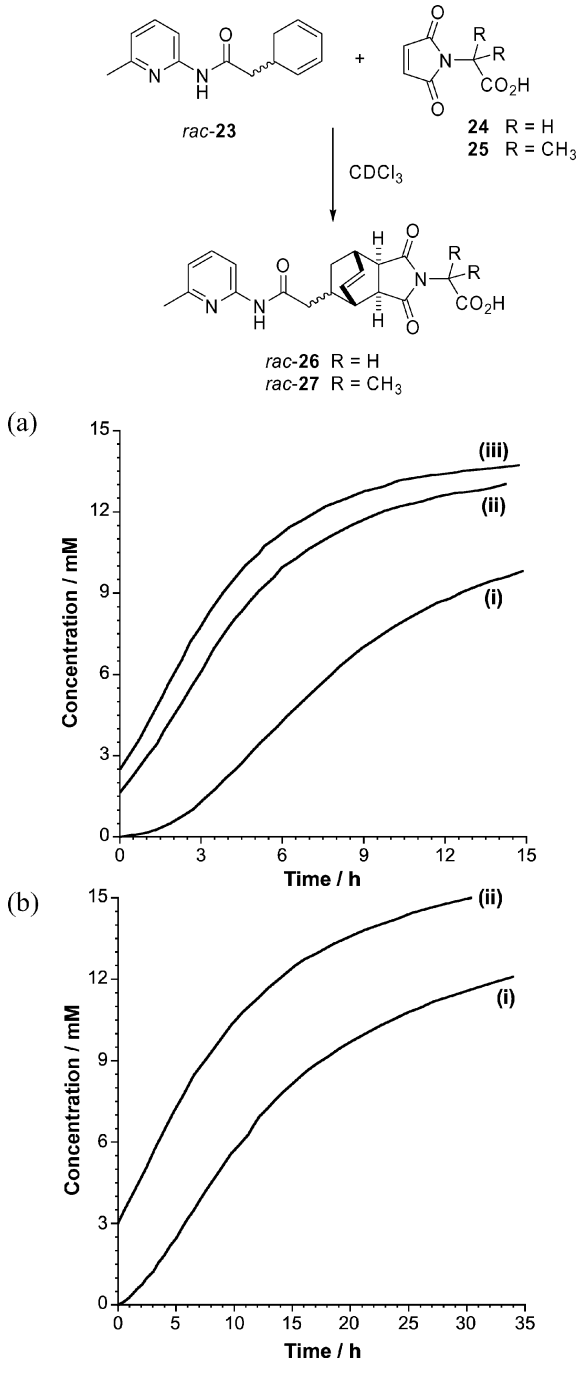

Figure 13. Diene *rac*-**23** can react with either maleimide **24** or maleimide **25** to form self-replicating templates **26** or **27**. (a) Concentration-time profile for the reaction ($CDCl_3$, 20 °C) between (i) *rac*-**23** and **24** (ii) *rac*-**23** and **24** in the presence of 10% *rac*-**26** and (iii) *rac*-**23** and **24** in the presence of 15% *rac*-**26**. (b) Concentration-time profile for the reaction ($CDCl_3$, 40 °C) between (i) *rac*-**23** and **25** and (ii) *rac*-**23** and **25** in the presence of 17% *rac*-**27**. Data taken from ref.[32]

and furans was measured along with the appropriate additional experiments. The dominant kinetic behaviours of each potential replicator are summarized in Figure 14. The diastereoisomeric pairs of *endo* and *exo* cycloadducts possess dissimilar geometries. Since the three-dimensional structure of each diastereoisomer determines its replicating

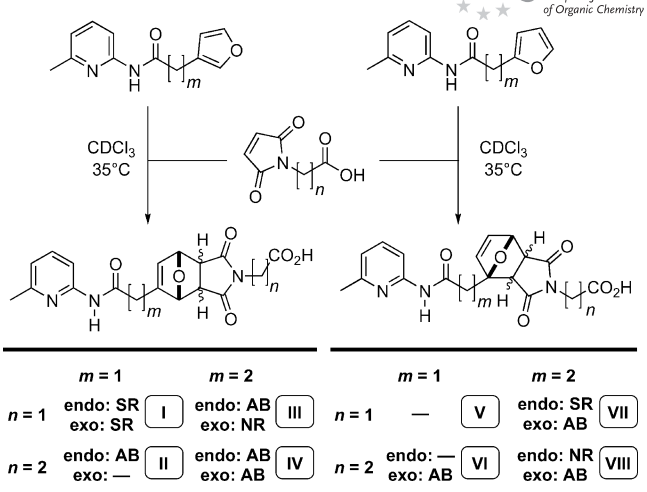

Figure 14. The combination of furan derivatives bearing an amidopyridine recognition site with maleimides bearing a carboxylic acid recognition site affords eight sets of potential replicators (systems **I** through **VIII**). The Diels–Alder reaction between the furan and maleimide give rise to two diastereoisomeric templates (*endo* and *exo*) in each system. The recognition-mediated reactivity for each system is noted as follows: SR = self-replicating; AB = reaction through binary complex ([**A**·**B**]) channel; NR = no recognition-mediated reactivity observed; – = no significant conversion under the reaction conditions employed.

capacity, this difference determines the recognition-mediated channel adopted. The data collected within these eight systems suggest that, given sufficient conformational freedom, the most likely recognition-mediated pathway for reaction is that involving the binary complex. The preference of the *exo* diastereoisomer in the case of 2-substituted furans, and of the *endo* for the 3-substituted furans is based on steric effects associated with the ring substituent. In order to generate autocatalysis, the conformational freedom must be restricted and the template must be forced to adopt an open conformation, to ensure that the undesirable pathway is shut down. This effect can be accomplished by using short and/or rigid spacers of mismatched length on the two building blocks.

The comparison of this first series with a second series in which[34] the acid recognition site is located on the diene and the amidopyridine recognition site on the dienophile is instructive. The dominant kinetic behaviours of each of the potential replicators are summarized in Figure 15.

It is clear from the data presented in Figure 15 that in three of the four systems based on 3-substituted furans (systems **X**, **XI** and **XII**), both diastereoisomers are capable of synthesizing themselves through the autocatalytic pathway. In general, the *endo* cycloadducts are slightly more efficient templates than the *exo* cycloadducts. In the case of system **IX**, the system displays no recognition-mediated reactivity at all. A dramatic change in behavior is brought about by the replacement of the diene system 3-alkylfuran with 2-alkylfuran to create systems **XIII** through **XVI**. In this second group, significant recognition-mediated reactivity is limited to the *exo* cycloadducts of systems **XV** and **XVI** and occurs exclusively through the binary reactive complex. The contrast between system **XI** and system **XIV** is striking.

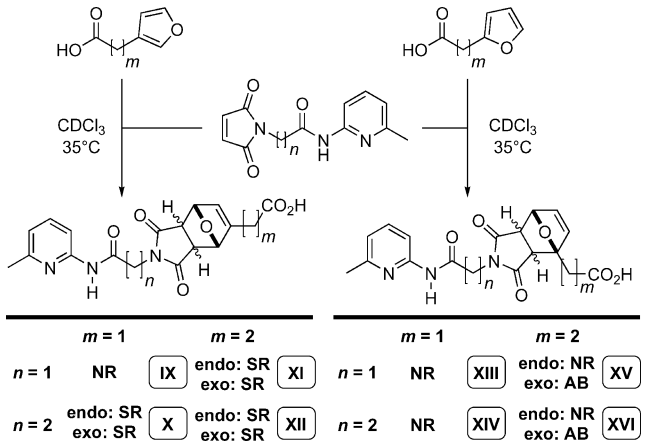

Figure 15. The combination of maleimides bearing an amidopyridine recognition site with furan derivatives bearing a carboxylic acid recognition site affords eight sets of potential replicators (systems **IX** through **XVI**). The Diels–Alder reaction between the furan and maleimide give rise to two diastereoisomeric templates (*endo* and *exo*) in each system. The recognition-mediated reactivity for each system is noted as follows: SR = self-replicating; AB = reaction through binary complex ([**A**·**B**]) channel; NR = no recognition-mediated reactivity observed; – = no significant conversion under the reaction conditions employed.

These two systems are constitutional isomers differing only in the position of substitution on the furan ring. However, whilst in system **XI** both diastereoisomers are rather efficient replicators, system **XIV** shows no recognition-mediated reactivity at all. This observation serves to emphasize the point that the structural window in which replication can be observed is rather narrow.

The relationship between these two series of replicators is simple. Examination of Figures 14 and 15 reveals that efficient self-replicating behavior observed in these structurally related systems is clustered in one region only – namely system **X**, **XI** and **XII**. Almost all of the other recognition-mediated reactivity observed occurs through a binary complex reaction channel. Out of 32 potential replicators, 9 are capable of templating their own formation (of which only 6 are relatively efficient), 9 are capable of recognition-mediated reaction through a binary complex channel and 10 show no recognition-mediated reactivity.

Data accumulated within this series show that the change in substitution pattern introduces significant geometrical change in the structure of the cycloadducts, consequently making their kinetic behaviours entirely different. The lesson of this investigation on the role that systematic structural variation plays in determining the recognition-medi-

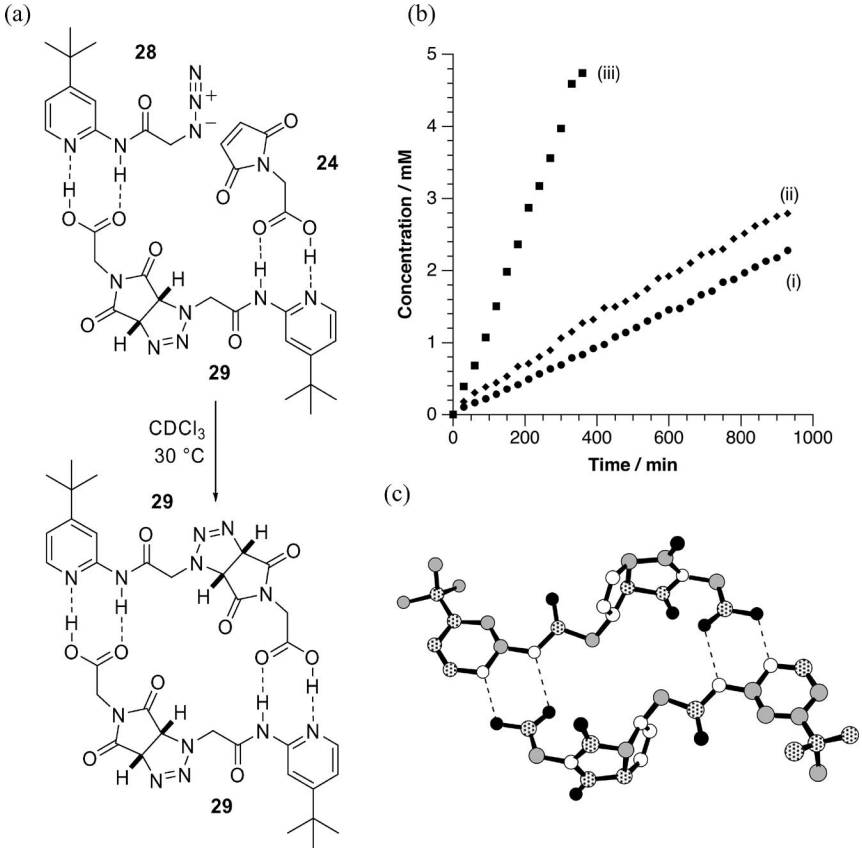

Figure 16. (a) Template **29** can assemble maleimide **24** and azide **28** in $CDCl_3$ at 30 °C and accelerate the reaction between them. (b) Concentration-time profiles for the reaction between (i) maleimide **24** and azide **28** (ii) maleimide **24** and azide **28** in the presence of 10 mol-% **29** and (iii) maleimide **24** and azide **28** in the presence of 100 mol-% **29** in $CDCl_3$ at 30 °C. Data taken from ref.[35] (c) Solid-state structure of the [**29**·**29**] duplex as determined by single-crystal X-ray diffraction.

ated reactivity displayed by a particular system is that small structural changes can make huge differences of function that are not entirely predictable. An interesting comparison can be made between von Kiedrowski's replicator **26** and our system **I**, in which the cyclohexadiene component is replaced by a furan. The cyclohexadiene-based system exhibits nearly exponential replication, whereas the furan-based system shows only weak replication signatures for the *exo* and *endo* diastereoisomers. Although the slight differences in the location of the diene termini and the different inherent reactivity of both dienes could presumably responsible for the observed effects, the striking difference in the observed kinetics of these two structurally related systems is rather intriguing.

Another class of cycloaddition reactions that has been exploited successfully in the creation of replicating systems is the reaction between a 4π 1,3-dipole and a 2π dipolarophile. In 2002, we reported[35] a structurally simple self-replicating system (Figure 16) that exploits the dipolar cycloaddition reaction between maleimide **24** and azide **28**, and the recognition between two hydrogen-bonding sites, namely an amidopyridine and a carboxylic acid.

This design is based upon a structurally related system that is capable[36] of accelerating the cycloaddition reaction between azide **28** and a maleimide with a longer spacer through an [**A**·**B**] pathway. It was thought that by reducing the alkyl spacer within the maleimide building block, the reactive centres would be too far apart to react. This modification inactivated the binary complex channel, but altered the reaction pathway to a self-replicating cycle effectively only with concomitant reduction of temperature and concentration. Kinetic simulation affords values of p and ε of 0.40 and 20, respectively. These data suggests that dissociation of the [**29**·**29**] duplex is limiting in this system. Evidence for the stability of the [**29**·**29**] duplex comes from its behaviour in the solid state, because it crystallizes as a homodimer (Figure 16, c). A series of control experiments and kinetic modelling demonstrate that this system is relatively inefficient and has a weak replicating behaviour. Thus, the reaction between azide **28** and maleimide **24** is self-replicating – the template **29** is capable of assembling **24** and **28** and accelerating the reaction between them – but not autocatalytic, as the stability of the duplex prevents turnover.

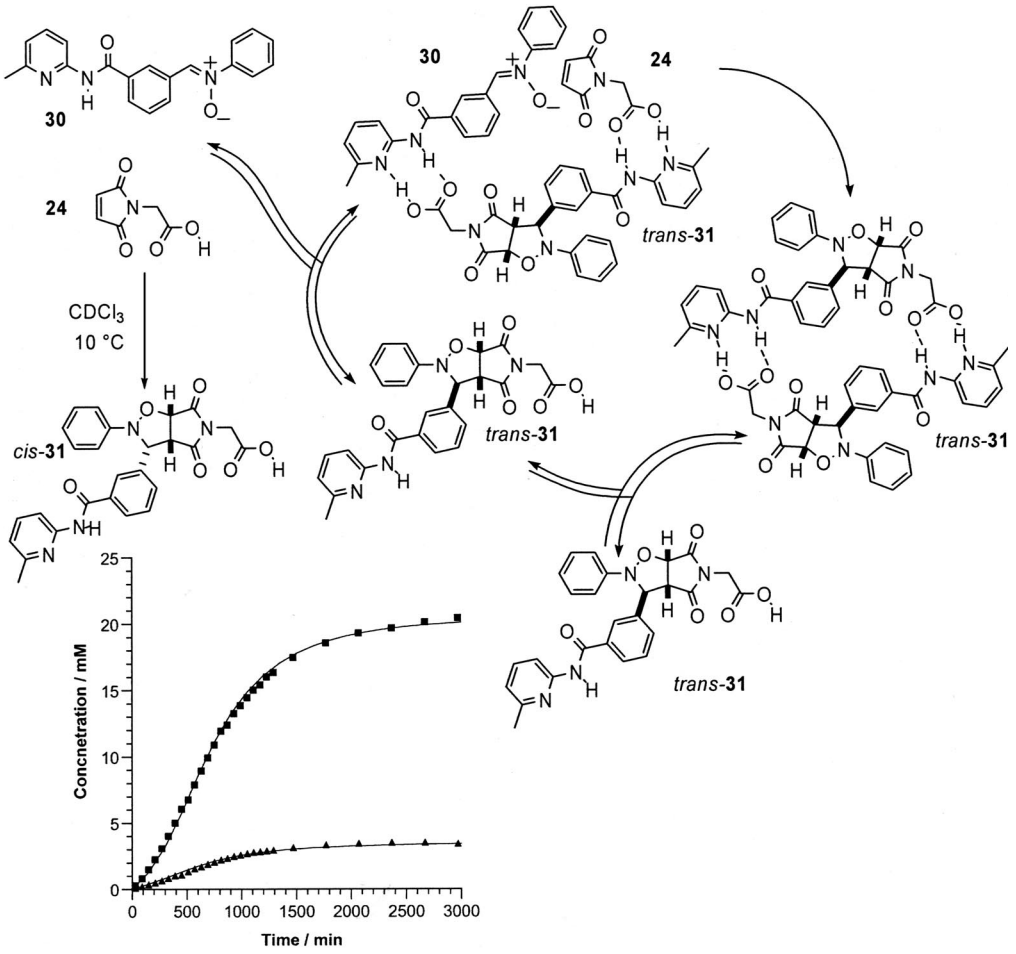

Figure 17. Reaction of nitrone **30** with maleimide **24** in $CDCl_3$ at 10 °C gives rise to two diastereoisomeric cycloadducts – *cis*-**31** and *trans*-**31**. While *cis*-**31** does not exhibit appreciable recognition-mediated reactivity (filled triangles in graph), the formation of *trans*-**31** (filled squares in graph) exhibits a sigmodial concentration-time profile and further experiments confirm the operation of the autocatalytic cycle mediated by the ternary complex [**24**·**30**·*trans*-**31**] in this system. Data taken from ref.[38]

The diastereoisomeric adducts formed by 1,3-dipolar cycloaddition between maleimides and nitrones provide scope for the investigation of stereochemical information transfer during the replication process. This reaction furnishes pairs of racemic diastereoisomeric cycloadducts – labelled[37] *trans* and *cis* – depending on the relative orientation of the nitrone and the maleimide. Symmetrical dipolarophiles are utilized to avoid further complications with regioselectivity issues. These two products have markedly different geometries. The *trans* diastereoisomer has an open geometry compatible with acceleration through the ternary complex and the *cis* diastereoisomer has a closed geometry compatible with acceleration through the [**A·B**] complex channel. We have investigated the effect that recognition-mediated processes have upon this reaction. The 1,3-dipolar cycloaddition[38] between nitrone **30** and the methyl ester of maleimide **24** is slow and reasonably unselective (*trans*/*cis* ratio = 3:1) in $CDCl_3$ at 10 °C.

However, when the same reaction is performed with the acid maleimide **24**, both the rate and the selectivity (*trans*/*cis* ratio = 6:1) of the reaction (Figure 17) are increased. The formation of the major isoxazolidine *trans*-**31** displays a sigmoidal rate profile, indicative of a self-replicating system. The addition of presynthesised template *trans*-**31** at the beginning of the reaction enhances the formation of *trans*-**31**, and not *cis*-**31**, with concomitant disappearance of the initial lag period, resulting in an improved *trans*/*cis* ratio of 9:1. By contrast, the addition of pre-synthesized template *cis*-**31** at the beginning of the reaction has no effect on the rate of production of neither *trans*-**31** or *cis*-**31**. The properties of this system demonstrate that only one of the two diastereoisomeric products of the cycloaddition reaction is capable of self-replication, acting as an efficient template for its own formation. Isoxazolidine *trans*-**31** transmits its stereochemical information effectively to the forming template copy within the [**24·30·***trans*-**31**] ternary complex.

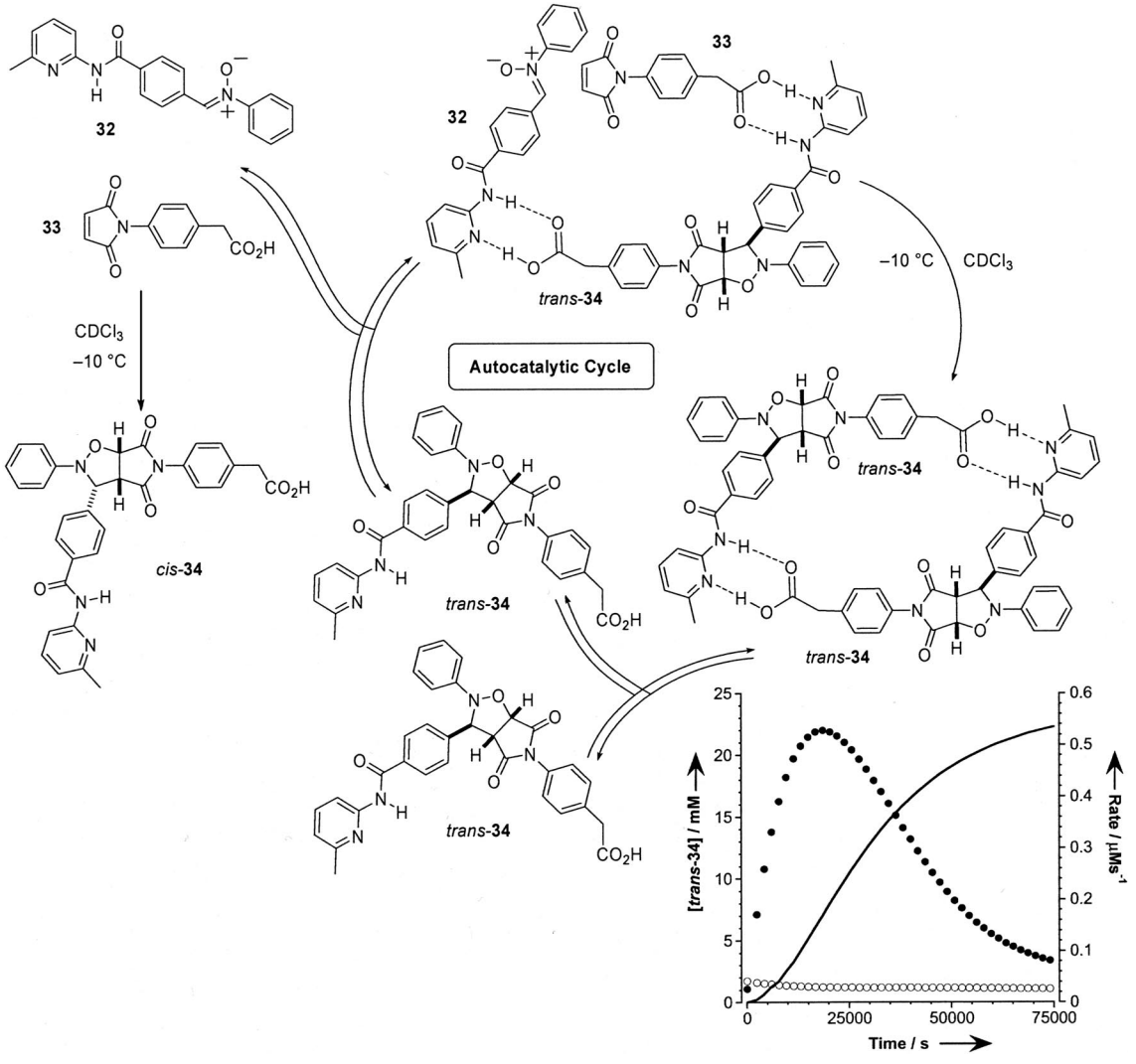

Figure 18. Reaction of nitrone **32** with maleimide **33** in $CDCl_3$ at –10 °C can potentially give rise to two diastereoisomeric cycloadducts – *cis*-**34** and *trans*-**34**. However, the efficient operation of the autocatalytic cycle mediated by the ternary complex [**32·33·***trans*-**34**] in this system ensures that *trans*-**34** is formed almost exclusively (solid line in graph, *trans*-**34**/*cis*-**34** >100:1, *cis*-**34** not shown). The rate vs. time profile for the reaction at –10 °C in $CDCl_3$ (filled circles in graph) reveals the classical bell shape characteristic of an autocatalytic process and the rate of reaction is far higher than that observed in the absence of recognition (open circles in graph). Data taken from ref.[39]

This diastereoisomer is acting as a selfish autocatalyst, enhancing the rate of formation of itself, but not its corresponding diastereoisomer. Isoxazolidine *cis*-**31** is inactive in both an autocatalytic and in a cross-catalytic sense. Fitting of experimental data affords values of p and ε of 0.9 and 5000, respectively. These results indicate that dissociation of the product duplex is not limiting and therefore the turnover in the autocatalytic cycle is relatively efficient.

Even though this self-replicating system exhibits relatively efficient autocatalysis, the levels of amplification achieved are modest. We optimized[39] the performance of this system by modifying the nitrone substitution pattern and the identity of the maleimide. Only one of the two possible products of the reaction between nitrone **32** and maleimide **33** (Figure 18) is selectively and highly amplified using a replication strategy. This reaction reaches more than 85% overall conversion after 16 h, and the diastereoselectivity is 115:1 in favour of isoxazolidine *trans*-**34**, whose rate of formation possesses a characteristic sigmoidal shape (Figure 18). By contrast, in the control reaction between nitrone **32** and the methyl ester of **33**, in which the carboxylic acid recognition site is blocked, the overall conversion is only 9% and the diastereoselectivity only 3:1. The reaction between **32** and **33** in the presence of two equivalents of benzoic acid results in a significant decrease both in the rate of the reaction and the diastereoselectivity (the final *trans*/*cis* ratio drops to 17:1), demonstrating that the formation of the cycloadduct *trans*-**34** is recognition-mediated. The injection of 10 mol-% of *trans*-**34** at the start of the reaction between **32** and **33** results in an increase in the initial rate of formation of *trans*-**34** (the *trans*/*cis* ratio is now at least 250:1), and, thus, demonstrates that *trans*-**34** is capable of templating and accelerating its own formation. By contrast, the addition of 10% of *cis*-**34** to the reaction mixture has no effect on the rate profile.

These observations show that the template *trans*-**34** is amplified selectively through an autocatalytic self-replicating cycle. The contribution of *cis*-**34** can even be removed by reducing the concentration of the reagents at the start of the reaction. Initial concentrations of 15 mM result in a *trans*/*cis* ratio of 135:1, and of 5 mM in a 250:1 ratio. Electronic structure calculations suggest[39] that the two cycloadducts have very different geometries. Isoxazolidine *trans*-**34** has an open structure in which the two recognition sites are freely available to interact with other complementary species in solution. By contrast, the structure of *cis*-**34**

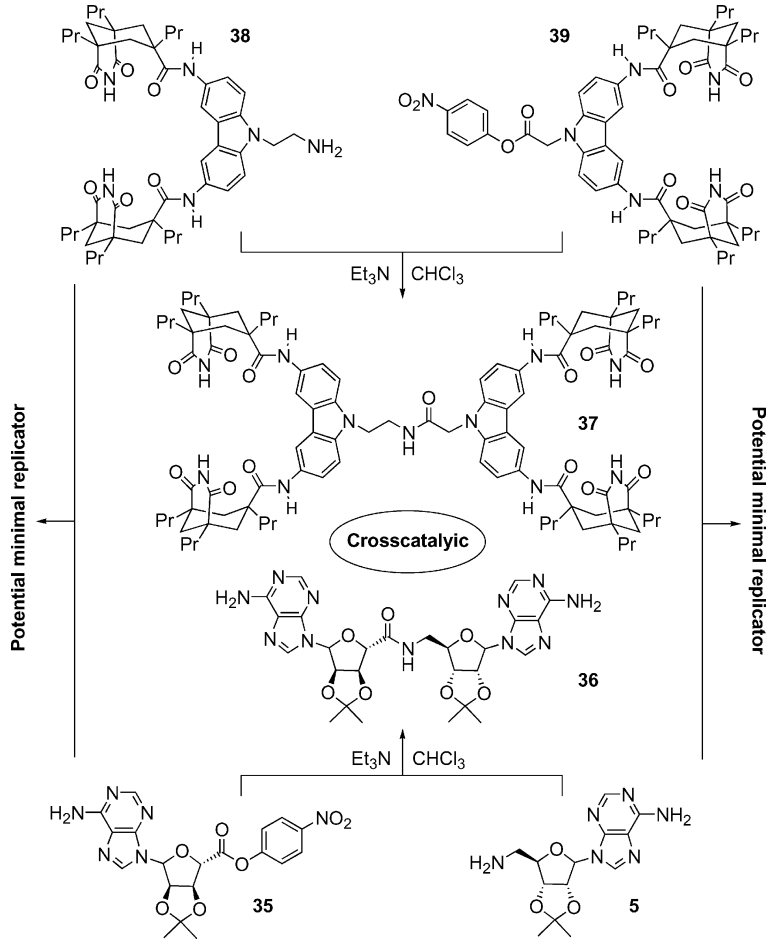

Figure 19. Template **37** can assemble amine **5** and activated ester **35** into a crosscatalytic ternary complex [**5**·**35**·**37**] in which the formation of template **36** is accelerated. Similarly, template **36** can assemble amine **38** and activated ester **39** into a crosscatalytic ternary complex [**36**·**38**·**39**] in which the formation of template **37** is also accelerated, completing a formal reciprocal replicating cycle.

is folded such that the recognition sites are placed in proximity to each other, thus rendering this product inert in a catalytic sense. It is clear that the major pathway for the formation of *cis*-**34** is simply the bimolecular reaction between **32** and **33**.

5. Reciprocal Replicating Systems

Despite the progress made in the design and implementation of minimal replicating systems, reports of reciprocal replicating systems[40] are rather sparse. Rebek and co-workers developed[41] a replication cycle in which template effects operate in a reciprocal sense. It consists (Figure 19) of a set of two reactions, in which the product of one reaction is a template for the other using building blocks incorporating recognition motifs based on the binding of adenine derivatives by carbazolediimide receptors, and bearing reactive functions for amide bond formation. The production of amide **36** from amine **5** and activated ester **35** is accelerated by a factor of ten when template **37** is present. Similarly, template **36** is able to catalyze to the coupling between amine **38** and activated ester **39**, with a fivefold rate enhancement over the uncatalyzed reaction. Although the combination of either **35** and **38** or **5** and **39** can, in principle, lead to minimal replicators, in practice, the partners in the alternate coupling combinations form active [**A**·**B**] complexes. Thus, both products are formed through the binary complex channel and remain folded shut as a consequence of the flexibility of the spacers. Therefore, investigation of the full system to determine the relative efficiencies of reciprocal replicators and minimal replicators was not possible.

In our laboratory, we have also developed[42] a system consisting of two mutually-complementary templates capable of catalyzing the formation of each other. In contrast to the system reported by Rebek and co-workers, the templates involved are formed as pairs of diastereoisomers, allowing the study of information transfer between the templates (Figure 20).

The addition of template *trans*-**41** to the Diels–Alder reaction between maleimide **33** and furan **40** results in an increase in the rate of formation of *exo*-**42** by 2.3-fold and a smaller increase in the rate of formation of *endo*-**42** of 1.5-fold. The *exo*/*endo* ratio rises from 1.3:1 for the uncatalyzed reaction to 2:1 for the templated one. The addition of the diastereoisomeric template *cis*-**41** accelerates only slightly the formation of both *exo*-**42** and *endo*-**42**. Similarly, the addition of *exo*-**42** to the 1,3-dipolar cycloaddition between nitrone **32** and maleimide **43** results in an increase in the rate of formation of *trans*-**41** of 3.6-fold and a twofold reduction in the rate of formation of *cis*-**41**. These changes generate a enhancement of the *trans*/*cis* ratio for the production of template **41** from 3:1 to 14:1. However, template

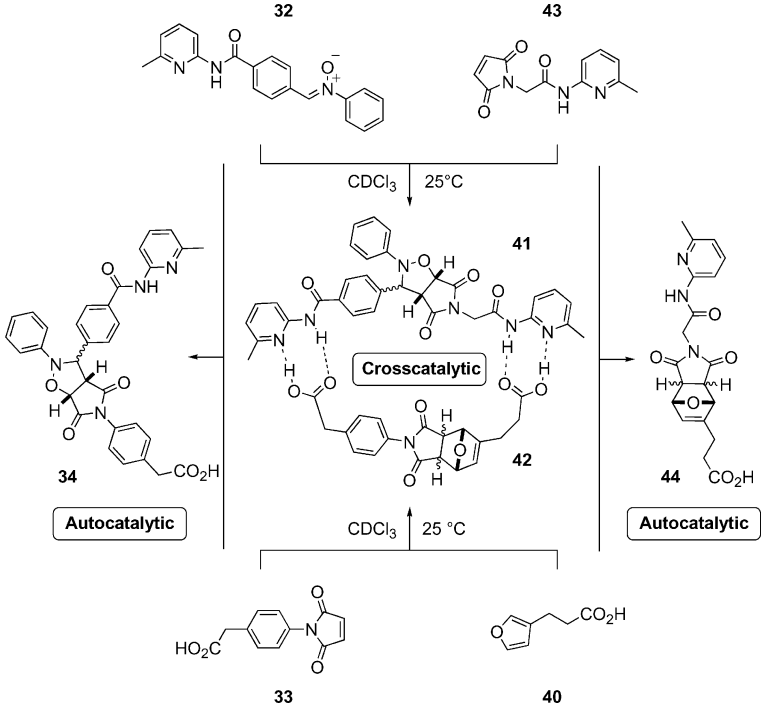

Figure 20. Template *trans*-**41** can assemble maleimide **33** and furan **40** into a crosscatalytic ternary complex [**33**·**40**·*trans*-**41**] in which the Diels–Alder reaction between **33** and **40**, forming template **42**, is accelerated. The *trans* diastereoisomer of template **41** accelerates the formation of the *exo* diastereoisomer of template **42** selectively. Similarly, template *exo*-**42** can assemble nitrone **32** and maleimide **43** into a crosscatalytic ternary complex [**32**·**43**·*exo*-**41**] in which the 1,3-dipolar cycloaddition reaction between **32** and **43**, forming template **41** is accelerated. The *exo* diastereoisomer of template **42** accelerates the formation of the *trans* diastereoisomer of template **42** selectively, completing a formal reciprocal replicating cycle. Combination of **32** with **33** and **40** with **43** gives rise to two minimal replicating templates **34** and **44**. In experiments where **32**, **33**, **40** and **43** are mixed in $CDCl_3$ at 25 °C, template *trans*-**34** is the dominant product.

endo-**42** accelerates neither the formation of *trans*-**41** nor *cis*-**41**. These results clearly show that reciprocal template effects operate between *trans*-**41** and *exo*-**42**.

Alternative combinations of the building blocks give rise to the minimal replicators **34** (Figure 18) and **44** (system XI, Figure 15). Mixing all of the four building blocks **32**, **33**, **40** and **43** in a single reaction affords a product mixture in which *trans*-**34** is the dominant species. We are currently investigating the manipulation of products ratios obtained from such experiments through the injection of instructional templates, such as **41** and **42**, into the reaction mixture.

6. Conclusion and Perspectives

To date, chemists have devised a wide range of synthetic chemical systems in which self-replication operates, demonstrating that molecules can replicate themselves without the aid of enzymes and that self-replication is not a prerogative of nucleic acids only. Self-replication is no longer an enigmatic process considered as exclusive to living matter. The development of models for studying self-replication has also demonstrated the principles by which chemical information can be transmitted and amplified.

Moving forward, the development and deployment of self-replicating molecular architectures can potentially revolutionize the assembly of complex architectures at the nanometer scale. The development of such protocols based on molecular replication – either minimal or reciprocal or a combination of the two – can deliver synthetic machinery that can be instructed by templates and is therefore capable of directing synthesis and co-operating with other similar systems to create organized system-level behavior. This goal is an ambitious one and a fundamental requirement for success is a better understanding of the recognition-mediated processes that allow molecules to template their own formation. Therefore, the study and characterization of simple replication models, delivering a clear understanding of the basic physical organic chemistry behind the recognition-mediated processes that allow molecules to template their own formation, is the correct approach. However, once design principles are well established, the opportunities for the development of networks of replicators that can co-operate and give rise to system-level behavior in response to template inputs are limited only by the imagination of the chemist who designs them. The exploitation of replicating systems in the construction, selection and amplification of large molecular and supramolecular assemblies requires a number of additional developments. The demonstration that replication can be coupled to other recognition mediated events, such as the formation[43] of mechanically interlocked systems, must be demonstrated. Dynamic reconfiguration of a reagent pool is an essential requirement in order for systems to respond to outside stimuli. Finally, control and sequencing must be included within designs in order that replication can be incorporated within more complex kinetic schemes.

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