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Integrating Replication-Based Selection Strategies in Dynamic Covalent Systems

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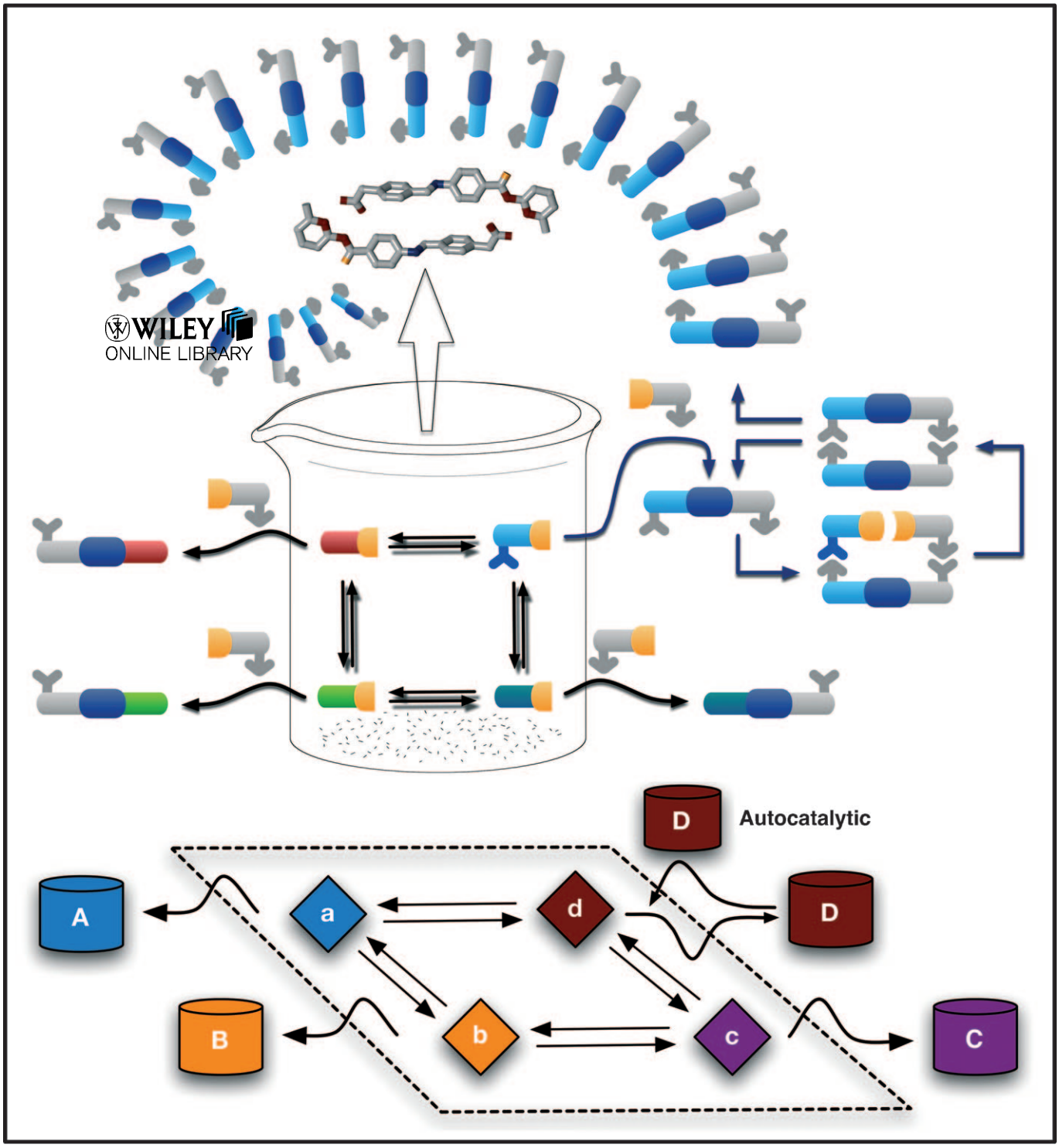

Abstract: In the past 15 years, the chemistry of reversible covalent bond formation (dynamic covalent chemistry (DCC)) has been exploited to engineer networks of interconverting compounds known as dynamic combinatorial libraries (DCLs). Classically, the distribution of library components is governed by their relative free energies, and so, processes that manipulate the free energy landscape of the DCL can influence the distribution of library members. Within the same time frame, the design and implementation of molecules capable of copying themselves—so-called replicators—has emerged from the field of template-directed synthesis. Harnessing the nonlinear kinetics inherent in replicator behavior offers an attractive strategy for amplification of a target structure within a DCL and, hence, engendering high levels of selectivity within that library. The instructional nature of replicating templates also renders the combination of replication and DCC a potential vehicle for developing complex reaction networks; a prerequisite for the development of the emerging field of systems chemistry. This Concept article explores the role of kinetically and thermodynamically controlled processes within different DCC frameworks. The effects of embedding a replicating system within these DCC frameworks is explored and the consequences of the different topologies of the reaction network for amplification and selectivity within DCLs is highlighted.

Keywords: autocatalysis · dynamic covalent chemistry · self-replication · supramolecular chemistry · template synthesis

Introduction

The development of synthetic organic chemistry during the 20th century focused on the creation and manipulation of covalent bonds with exquisite control and selectivity. This control has been exploited in the synthesis of a staggering range of chemical structures with desirable properties. These syntheses generally proceed through the programmed application of isolated chemical reactions, either individually or in a cascade, that afford the target structure. By contrast, the deliberate creation of mixtures in synthetic chemistry has only become popular with the advent of combinatorial approaches to the generation of compound libraries for screening purposes. The avoidance of mixtures in organic chemistry is somewhat surprising given the complex chemical networks that operate in biological systems that exploit a range of interconnected signaling and metabolic pathways with checkpoint controls and feedback loops. These complex networks, built on the interactions and reactions between molecules, allow biological systems to adapt and respond rapidly to external stimuli and process chemical feedstocks in defined ways as required. The emerging field of systems chemistry attempts to capture the complexity and emergent phenomena found in such networks within a wholly synthetic chemical framework.[1] In this approach, a group of synthetic chemical entities, designed to interact and react with many partners within an ensemble in programmed ways, can create a synthetic chemical system with attributes that are not simply the sum of the properties of the individual components. In contrast to traditional synthetic approaches, in which mixtures of compounds are often treated as an unwanted feature that must be eliminated, systems chemistry requires the presence of a mixture of components and the interactions between these multiple components are a necessity for the emergence of properties at an entire system level.

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Discussion

In the past 15 years, the chemistry of reversible covalent bond formation (dynamic covalent chemistry[2,3] (DCC)) has begun to be exploited in the generation of networks of interconverting compounds known as dynamic combinatorial libraries (DCLs). Since DCLs operate under thermodynamic control, the distribution of library components is governed by their relative free energies, and so, processes that are capable of manipulating the free energy relationships within the DCL can, ultimately, influence the distribution of library members.

The first necessary condition to create a DCL is to find an appropriate reversible connection process. The bond or bonds formed and broken can be both of covalent or noncovalent nature; DCC is often used to describe the first case. Either way, the reaction used in a DCL has to meet a number of requirements. First, it has to be reversible on a reasonable time scale in the given experimental conditions. Also, it cannot interfere with other processes that the library is subjected to or destroy the chemical integrity of the library building blocks. The chemistry of the reversible covalent bond has been widely exploited in the construction of DCLs: disulfide exchange reactions, acyl transfer reactions (in esters, thioesters, and amides), acetal exchange reactions, C=N exchange reactions (imines, hydrazones, oximes, nitrones), alkene and alkyne metathesis, Diels–Alder/retro-Diels–Alder reactions, Michael addition/retro-Michael reactions, and so forth are all represented in numerous examples. It has to be noted that the kinetics of the formation of the covalent bond is generally slower than that of the non-

covalent bond. Thus, to form an exchanging library, it is often necessary to make use of catalysts that enable the process to occur with ease on a reasonable time scale.

In the current context, the most relevant characteristic of DCLs is that the distribution of species within them can be influenced by outside inputs, that is, they are addressable systems. Changes in the concentration of one member of the library influences all of the other members—the final equilibrium distribution in the exchanging pool is derived from the sum of the stabilities of all species. Thus, if one component engages with a molecule present in the mixture in a noncovalent interaction, its stability and, hence, its concentration increases at the expense of other library members. This response of DCLs is referred to as amplification and this selection strategy is known as receptor-assisted DCC. Accordingly, introducing a host or a guest template into a dynamic library of potential interaction partners will result in a distribution favoring the structure that is thermodynamically most stabilized by the specific template. This process is described as molding when the target substrate is used as an *exo*-receptor (external host) and is known as casting when it utilizes a target receptor as an *endo*-receptor (external guest) to direct the assembly of the optimal substrate.[4] A third possibility, in which no external host or guest molecule is added, involves the auto selection of self-assembling library members to form a stabilized aggregate.

As a DCL grows in size, problems emerge that hamper the analysis of such complicated networks. Thus, in parallel to experimental efforts, a significant body of work targeted at the simulation of DCL behavior has been described. Severin[5] and Reek et al.[6] concluded, from the simulation of small DCLs, that in some cases the assembly with the highest affinity for the target is not necessarily the one amplified. These simulations reveal that, to prevent the DCL from selecting a structure that is not necessarily the best binder, it is advantageous to work at relatively low concentrations of the target and to design the DCL in such a way that the dominating species in the mixture are the building blocks and not their aggregates. Sanders and co-workers conducted different simulations on much larger DCLs in an attempt to assess the correlation between host–guest binding and host amplification.[7] The strength of the correlation was dependent on the distribution of the association constants between the library members. The factor that prevented amplification of the highest binding compound was competition for building blocks. Otto and co-workers examined emergent phenomena in large DCLs.[8] Under certain conditions, specific patterns appear in a correlation between an amplification factor and template affinity of DCL components. All of these theoretical results reveal that DCLs are indeed complex molecular systems in which their components are connected themselves by rather subtle reaction networks.[9] Hence, it is clear that DCC and DCLs will be central in the development of systems chemistry.[10]

Receptor-assisted strategies depend on the target being present in the library during its synthesis and amplifies the formation of the most associating component. This amplification is, however, inherently limited since the selectivity that is engendered within the DCL is directly proportional to, and limited by, the amount of template added and the difference in affinity of the template for the target compared with the other components of the mixture. To increase selectivity in DCLs and, therefore, break out of the purely thermodynamic regime, elements of kinetically controlled chemistry must be introduced into DCLs.

The term pseudo-DCL was coined by Gleason, Kazlauskas, and co-workers to describe their experimental setup (Figure 1),[11] which consisted of different dipeptide inhibitors, with carbonic anhydrase (CA) as the receptor, and a protease as a destructive enzyme.[12] The reaction vessel contains a dialysis membrane to keep the two enzymes spatially separated, but still letting the library members circulate freely. The dipeptides are in equilibrium with the CA receptor, while the protease is able to hydrolyze the dipeptides to the corresponding amino acids. The kinetic amplification is achieved when the most tightly bound dipeptides of the library are being destroyed at the slowest rate, whereas the unbound members are consumed the fastest. When a well-studied CA inhibitor and a nonbinding peptide were introduced into the system, a selectivity of over 20:1 was achieved after 12 h. The most interesting setup, consisting of two competitive dipeptide inhibitors, showed selectivities far beyond the thermodynamic limitations imposed by the binding constants between the CA and the dipeptides. One of the problems associated with the pseudo-dynamic approach is identifying the appropriate kinetic destruction component that has a broad spectrum of activity but does not bias the selectivity. Another issue is a more semantic one: During

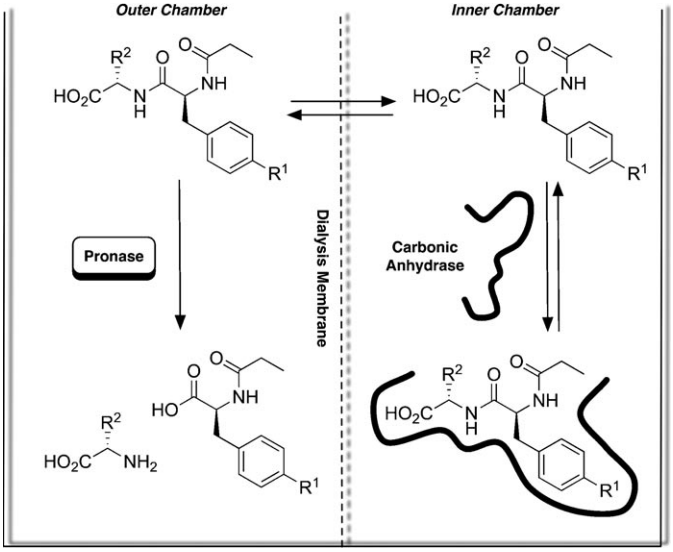
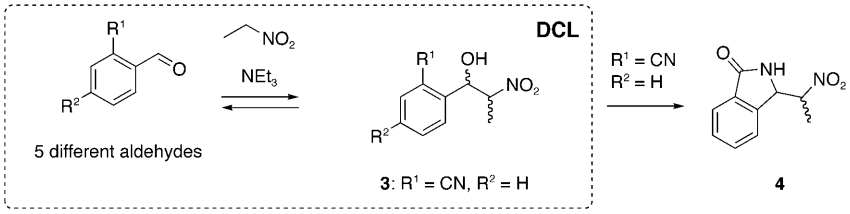


Figure 1. Design of a DCL utilizing selective destruction. The N-acyl dipeptides can diffuse across the membrane separating both chambers. Pronase hydrolyzes the dipeptides into their corresponding amino acids, while carbonic anhydrase binds them and prevents the most tightly bound constituents from being destroyed.

these experiments, most of the library is destroyed, which is rather contrary to the approach of a synthetic chemist. A more constructive approach is to develop a DCL that performs in a creative and not destructive manner and amplifies a compound kinetically by utilizing it to make a more complex and useful structure.



Scheme 2. A group of five aldehydes and nitroethane react under basic conditions to afford a DCL composed of the corresponding nitroaldol products. One member of this DCL, compound **3**, can undergo an intramolecular cyclization reaction and, hence, **4** is selected from the DCL with high efficiency.

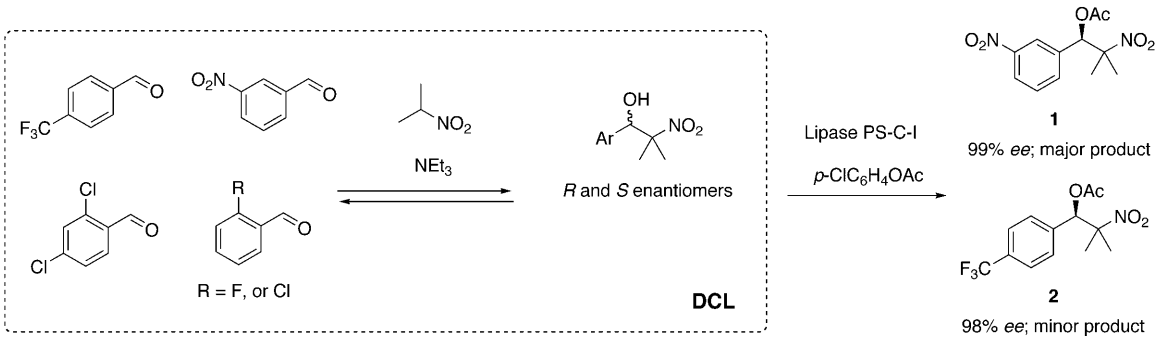
In this respect, Ramström and co-workers investigated a novel dynamic combinatorial resolution process.[13] In this system, a DCL of nitroalcohols was generated from a reversible base-catalyzed nitroaldol reaction between 2-nitropropane and five different electron-deficient aldehydes (Scheme 1). The template for the kinetic selection was an enzyme lipase PS-C-I, which catalyzes the transacetylation reaction of nitroalcohols from *p*-chlorophenyl acetate. The best substrates for the enzymatic reaction were selected from the library. The concentration of the transacetylation product **1** consisted of more than half of the mixture of the other esters. Moreover, complete asymmetric discrimination was achieved in the process, and esters **1** and **2** were obtained in 99 and 98% enantiomeric excess (*ee*), respectively.

Ramström and co-workers have also described a DCL based on an analogous reversible Henry reaction from five different benzaldehydes, with an easily differentiated substitution pattern, and nitroethane (Scheme 2).[14] In this case, the nitroalcohol **3**, with a nitrile group in the *ortho* position of the aromatic ring (R^1, Scheme 2), is capable of undergoing an irreversible intramolecular 5-*exo*-*dig* cyclization to form the corresponding iminolactone **4**. After 24 h, all of the nitroethane had been incorporated into the cyclized product **4** and the initially formed nitroalcohols had consequently disappeared from the DCL mixture.

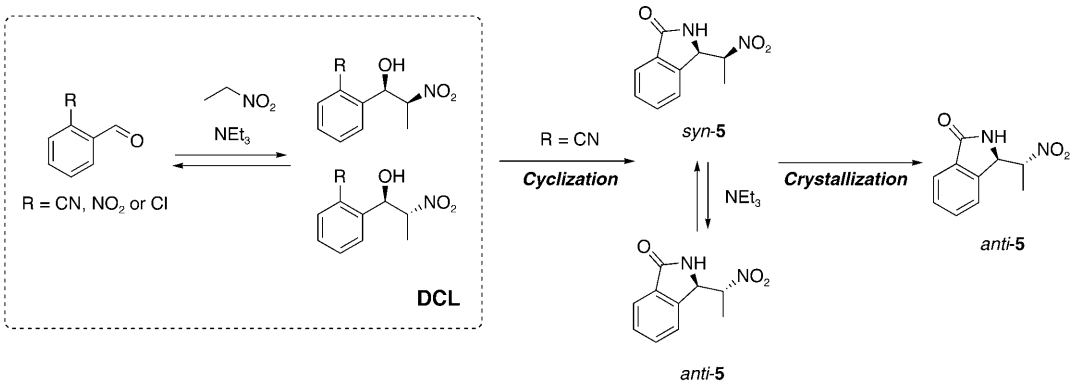
This nitroaldol system was expanded to incorporate the stereochemistry of the products.[15] Thus, a Henry reaction was again used to construct a DCL of nitroalcohols formed this time from three different aldehydes and nitroethane (Scheme 3). Of the two possible diastereoisomeric forms of the iminolactone **5**, which are in equilibrium with each other in the presence of base, one displays a much greater propensity for crystallization. This diastereoisomer (*anti*-**5**) is therefore selected kinetically and separated from the mixture of compounds by crystallization. These examples illustrate that coupling a DCL to a selective irreversible chemical process can greatly enhance the levels of selection achieved from the DCL.

The group of Ramström has engineered a double dynamic covalent system from a collection of imines that is subjected, in the same reaction vessel, to a Strecker reaction (cyanation).[16] Initial experiments confirmed that addition of isobutyl amine and catalytic amounts of acetic acid to preformed aromatic imines **AA** to **CA** gives rise to a full set of 12 different imines **AA** to **LA** (Scheme 4, Dynamic System A). Moreover, further addition of an excess of TMSCN and $ZnBr_2$ to an equilibrated mixture of imines **AA** to **LA** afforded the corresponding racemic pairs of Strecker products, α-aminonitriles **AB** to **LB** (Scheme 4, Dynamic System B), with very high conversion. Under these conditions, the racemic α-aminonitrile **AB** was the most abundant compound in the double dynamic system.

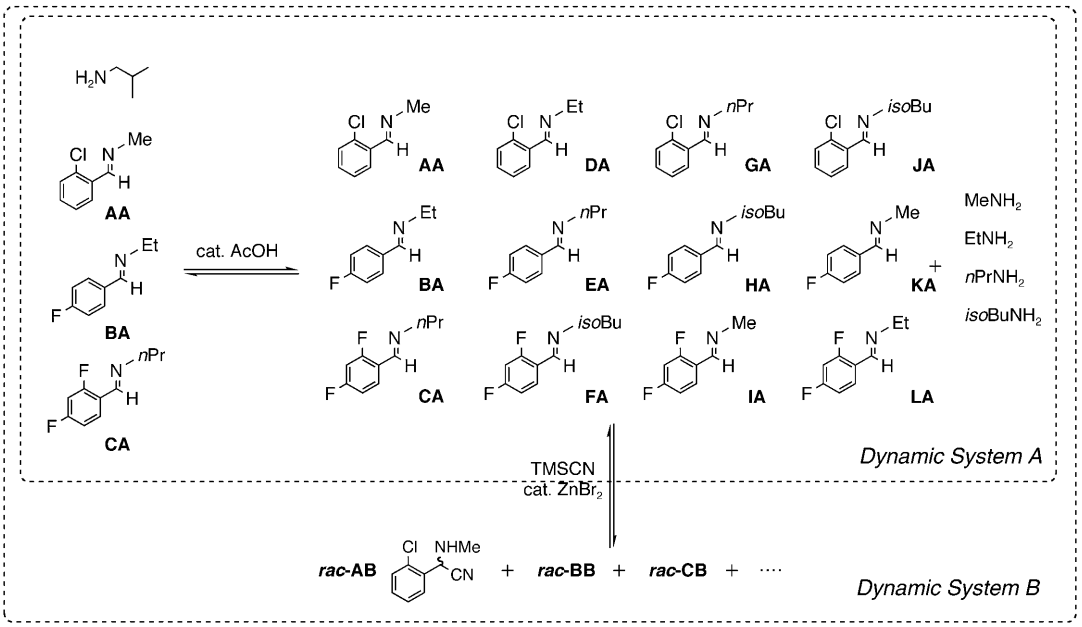
As in previous work, the authors demonstrated that such thermodynamically controlled systems could be resolved through a coupled process in the form of a kinetically controlled lipase-mediated amidation reaction. Amplification of specific chiral α-aminonitriles could in this case be efficiently achieved in a one-pot process. Accordingly, when a 150 mM mixture of imines **AA** to **CA**, isobutylamine (150 mM), acetic acid (cat.), TMSCN (450 mM), and $ZnBr_2$



Scheme 1. A group of aldehydes and 2-nitropropane react under basic conditions to afford a DCL composed of the corresponding nitroaldol products. Two components from this DCL are selected by a lipase-mediated asymmetric resolution process to afford the corresponding acetates, **1** and **2**.

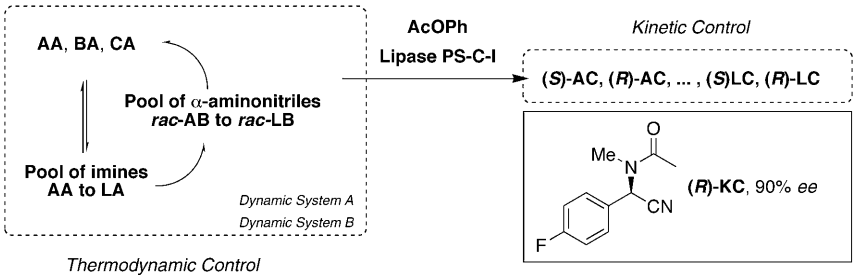


Scheme 3. An intramolecular cyclization is used to select *syn*- and *anti*-**5** from a nitroaldol-based DCL with a subsequent separation of *anti*-**5** by crystallization.



Scheme 4. The addition of isobutyl amine to preformed aromatic imines **AA** to **CA** gives rise to a full set of 12 different imines **AA** to **LA** (Dynamic System A), which afford the corresponding racemic pairs of Strecker products—α-aminonitriles **AB** to **LB** (Dynamic System B) on addition of an excess of TMSCN (TMS = trimethylsilyl) and $ZnBr_2$ to the system.

(cat.) in dry toluene was treated with a preparation of lipase PS-C-I and phenyl acetate, N-acetyl-α-aminonitriles were formed over time (Scheme 5). After 40 days, the first major product was found to be amide **KC**, derived from **KB** (37% yield from **BA**). It is notable that the relative concentration of α-aminonitrile **KB**, the corresponding substrate for the lipase reaction, was, however, not among the highest in the double dynamic system in the absence of enzyme. Further optimization of the reaction conditions also allowed the generation of high enantioselectivity in the final product **KC** (90% *ee* favoring (*R*)-**KC**). The authors concluded that the enzymatic system plays an essential role for dictating the outcome of the double dynamic covalent process, forcing



Scheme 5. Amplification of specific chiral α-aminonitriles is achieved efficiently in a one-pot process when a mixture of imines **AA** to **CA**, isobutylamine, TMSCN, and $ZnBr_2$ in dry toluene was treated with a preparation of lipase PS-C-I and phenyl acetate. The first major product was found to be amide **KC**, derived from **KB**.

the system to re-equilibrate, the reactions to continuously communicate, and select for the better substrate (*R*)-**KB**.

There are, however, other methods of exploiting recognition processes to influence a chemical reaction and, in particular, our laboratory has investigated the use of reactive binary complexes to accelerate and control cycloaddition reactions.[17] The reversible formation of a product **P** from its starting materials **A** and **B** is associated with an equilibrium constant *K*. This equilibrium constant can be expressed as a ratio of two rate constants, $k_{forward}/k_{reverse}$, for this transformation. If we preassociate the starting materials **A** and **B** in a reactive binary complex [**A**·**B**], we would expect the reaction between **A** and **B** to benefit from intramolecularization. Hence, pseudo-intramolecular reaction within [**A**·**B**] to afford **P** will be accelerated with respect to the bimolecular pathway **A**+**B**→**P**. The net effect of manipulating the forward rate constant is to increase *K* for the recognition-mediated process, which makes the formation of **P** from **A** and **B** more favorable.

Our group has exploited a reversible Diels–Alder reaction between a 2-arylfuran and a maleimide in $CDCl_3$ at 50 °C to create a small DCL (Figure 2) to test these ideas.[18] The furan derivative **6**, with an amidopyridine recognition site, was paired with a series of three maleimides **7**, **8**, and **9**, each containing a complementary carboxylic acid recognition site. In this system, recognition plays a dual role: First, the preassociation of the furan and a maleimide will serve to increase the rate of the forward Diels–Alder reaction ($k_{forward}$). Second, if the spacer in the maleimide is of the appropriate length, the recognition used to assemble the reagents will live on in the product, thus stabilizing the cycloadduct and reducing the rate of the retro-Diels–Alder reaction. These two effects have the same outcome: they both operate to shift the equilibrium towards the formation of the cycloadducts

Previously, we had demonstrated that the maleimide with a two carbon atom spacer, **8**, had an appropriate geometry to stabilize the corresponding cycloadduct **11**.[19] However, it is the reaction of the maleimide **7**, with the shorter one carbon atom spacer, that has the highest initial rate of reaction (*rfrc* in Figure 2). However, the shorter spacer lacks the flexibility to stabilize the resulting product **10** because the two hydrogen bonds can no longer be supported simultaneously. The reaction of the maleimide with the longest spacer, **8**, is disfavored both thermodynamically and kinetically. Therefore, when a DCL is prepared with the four building blocks **6** through **9**, in $CDCl_3$ at 50 °C, it is cycloadduct **10** that dominates the reaction mixture initially as a result of the strong acceleration of the forward reaction between **6** and **7**. The system evolves over time and gradually, thermodynamics takes over and the most stable product, namely, **11**, becomes dominant.

The formation of reactive binary complexes, such as those formed by the furans and maleimides in Figure 2, accelerates chemical reactions by intramolecularization the reaction. However, the product formed by this pseudo-intramolecular process has no catalytic effect on the reaction because the recognition used to assemble the reagents usually lives on in the product. By judicious design, we can create a molecule that can act as a catalyst for its own creation from simple 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, which permits the transfer of key structural information to allow it to template the formation of an exact copy of itself. The three possible reaction channels that may be active in minimal replication are encapsulated schematically in Figure 3.

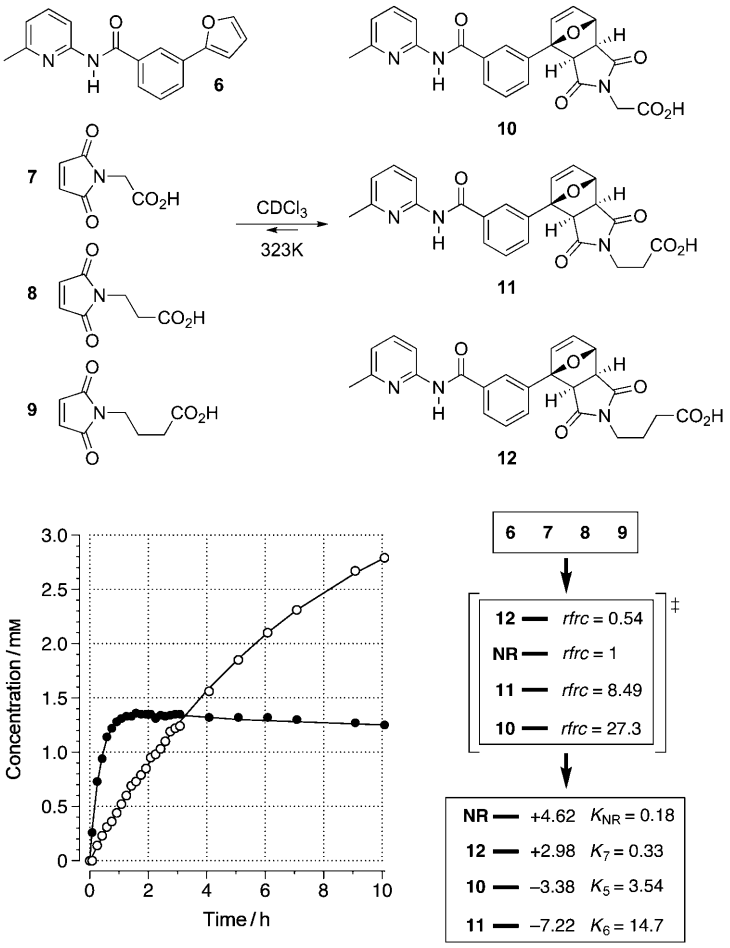


Figure 2. Relative rate constants, energies, and equilibrium constants for the conversion of diene **6** and maleimides **7**, **8**, and **9** into cycloadducts **10**, **11**, and **12**, respectively. Relative energies are in kJ mol^{-1}. **NR** represents a control reaction in which there is no molecular recognition present; *rfrc* represents the relative forward rate constant for the formation of each cycloadduct and is expressed by assuming $k_{NR}(forward)=1$. The graph illustrates the progress of a three component dynamic system as a function of time. Experimental conditions: [**6**]=[**7**]=[**8**]=25 mM; $CDCl_3$; 50 °C. Experimental concentrations of **10** are represented by the filled circles and **11** are represented by the open circles. The solid lines represent the simulated responses from the kinetic parameters shown.

The uncatalyzed bimolecular reaction between reagents **A** and **B** affords the template $\mathbf{T_{AB}}$. The complementary recognition sites located on **A** and **B** can associate with each

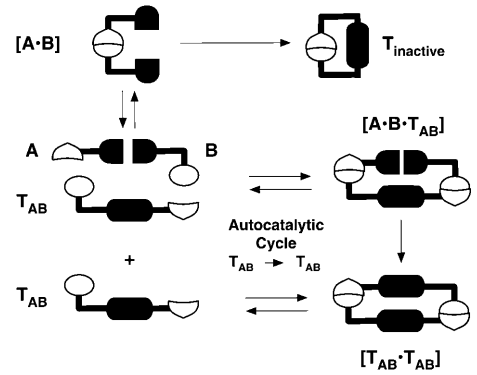


Figure 3. The minimal model of self-replication. Reagents **A** and **B** can react through three pathways: an uncatalyzed bimolecular reaction, a recognition-mediated pseudounimolecular pathway mediated by a binary complex [**A**·**B**], and a recognition-mediated pseudounimolecular autocatalytic cycle mediated by a ternary complex [$\mathbf{A}\cdot\mathbf{B}\cdot\mathbf{T_{AB}}$].

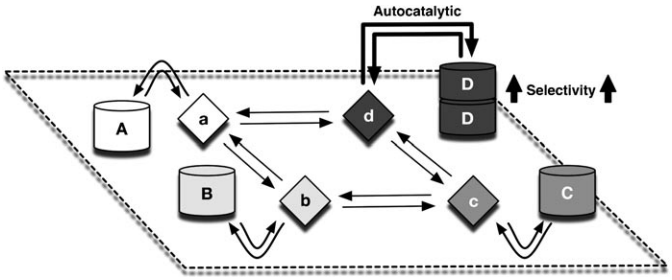


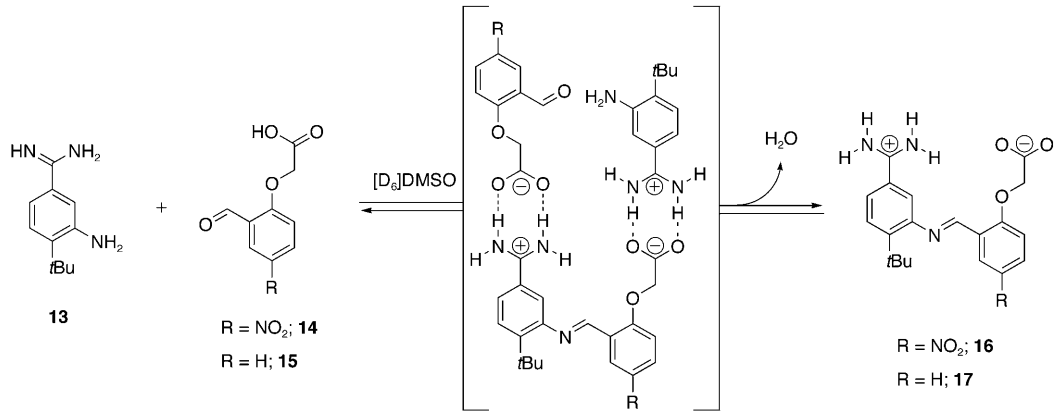
Figure 4. A model for coupling replication to a dynamic library. Building blocks **a**, **b**, **c**, and **d** can all exchange with each other. These four building blocks can be converted reversibly to four products **A**, **B**, **C**, and **D**. One of these product, **D**, forms a stable duplex and can act as a catalyst for its own formation and, as a result, should be formed preferentially.

other to permit the formation of a binary complex, [**A**·**B**]. The presence of this complex opens up the binary complex channel, in which **A** and **B** are preorganized with respect to each other and the reaction between them is pseudo-intramolecular. 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. This reaction channel is the one exploited by the system in Figure 2. If the product of the reaction between **A** and **B** generates a template $\mathbf{T_{AB}}$ in which the recognition sites are located spatially in the correct orientation, a third reaction channel becomes possible: the autocatalytic cycle. In this cycle, **A** and **B** bind reversibly to the open template $\mathbf{T_{AB}}$ to form a catalytic ternary complex [$\mathbf{A}\cdot\mathbf{B}\cdot\mathbf{T_{AB}}$]. The reaction between **A** and **B** is rendered pseudo-intramolecular and bond formation occurs at an accelerated rate between **A** and **B** to give the product duplex [$\mathbf{T_{AB}}\cdot\mathbf{T_{AB}}$]. This duplex then dissociates to return two molecules of $\mathbf{T_{AB}}$ to the start of the autocatalytic cycle. Thus, assuming the open template $\mathbf{T_{AB}}$ presents its recognition sites in the correct orientation,[20] it can act as a template for its own formation, transmitting molecular information through the formation of identical copies of itself. Key design elements in this model of replication are the minimization of 1) reaction flux through the binary complex reaction channel and 2) inefficient autocatalysis through product inhibition arising from an excessively stable product duplex [$\mathbf{T_{AB}}\cdot\mathbf{T_{AB}}$]. Self-replication guarantees nonlinear amplification of the template because each turn of the autocatalytic cycle doubles the product concentration. In a perfect situation,[21] one autocatalytic template would give two, two would give four, four would give eight, and so on, rendering product growth exponential. This exponential growth of the product concentration provides an attractive scheme for amplifying a single replicating entity from a DCL.

A potential method of integrating replication with a DCL is illustrated schematically in Figure 4. In this scenario, building blocks **a** through **d** can exchange freely with each other and with the four products **A** through **D**. One of the products, in this case **D**, forms a stable product duplex and **D** itself is formed autocatalytically from **d**. Thus, the reversible formation of **D** is associated with a nonlinear kinetic effect arising from autocatalysis and a concomitant net stabilization as a result of the stability of duplex [**D**·**D**], and, thus, experiences an increase in the equilibrium constant, K_D, for its formation. This increase in equilibrium constant derives both from duplex stability and from the selective manipulation of $k_{forward}$ (and K_D, since $K_D = k_{forward}/k_{reverse}$) for this transformation described above. The net result of autocatalysis should therefore be an increase K_D and, hence, selection of **D** from the pool.

A key requirement for the successful experimental implementation of the protocol outlined schematically in Figure 4 is the identification of a synthetic replicator in which the covalent bond forming reaction that creates the template is reversible. A suitable candidate for this reaction is the reversible formation of an imine from an amine and an aldehyde. In an early contribution to the area of synthetic replicators, von Kiedrowski and Terfort described a series of potentially dynamic replicating systems (Scheme 6) based on the condensation reaction between structurally simple amines and aldehydes.[22] The amidinium–carboxylate salt bridge is exploited as the recognition motif and the formation of imine **16**, and water, from amine **13** and aldehyde **14**, in [D_6]DMSO at 300 K was monitored by ^{1}H NMR spectroscopy for 7 h. This system was shown to be autocatalytic: the higher the initial concentration of **16** in the reaction mixture, the faster its formation and the equilibrium constant for the formation of **16** measured (5.73 M^{-1}). Interestingly, in the presence of the template **17**, the rate of the reaction between **13** and **14** depends linearly on the concentration of template **17**, demonstrating that cross-catalysis could operate successfully in this system.

The equilibrium position of fully dynamic systems, self-replicating or not, is controlled by the thermodynamic parameters for the system. The determination of the equilibrium position reached by the system given a certain set of conditions is of key relevance for the characterization of dynamic processes. Comparing a dynamic self-replicating system with that of a closely related control (a chemically



Scheme 6. Imine-based dynamic self-replicating systems formed in [D_6]DMSO by a combination of aldehydes **14** or **15** with amine **13**.

identical system that is incapable of exploiting recognition processes) provides key information on the relative stabilities of products and reagents within the reaction pools and the effects that molecular recognition has on their relative stabilities. Although the replicators in Scheme 6 are clearly autocatalytic in a kinetic sense, a complete set of thermodynamic parameters for the system is not available, making an assessment of the overall role of recognition in the formation of **16** and **17** difficult.

Giuseppone and Xu have recently exploited the reversibility of the imine bond in an attempt to couple DCC and replication.[23] Building blocks **18** to **22** (Figure 5), equipped with an aldehyde or an amine function, were designed with inspiration from the amide-based self-replicating system described by Rebek and co-workers in the early 1990s.[24,25] Aldehyde **18** and amine **21** contain complementary recognition sites. The condensation product from these two building blocks (imine **23**) is capable of forming the template duplex [**23**·**23**], and, thus, stabilize this structure over other possible products resulting from the library. The expression of the duplex [**23**·**23**] was increased more than 80% compared with a control DCL formed entirely from building blocks in which recognition had been disabled. Examination of the rate of formation of **23** and **24** (Figure 5b) illustrates clearly the different roles played by kinetics and thermodynamics. In a manner similar to the Diels–Alder-based system illustrated in Figure 2, the reaction clearly exhibits two phases. In the first 20 h, the reac-

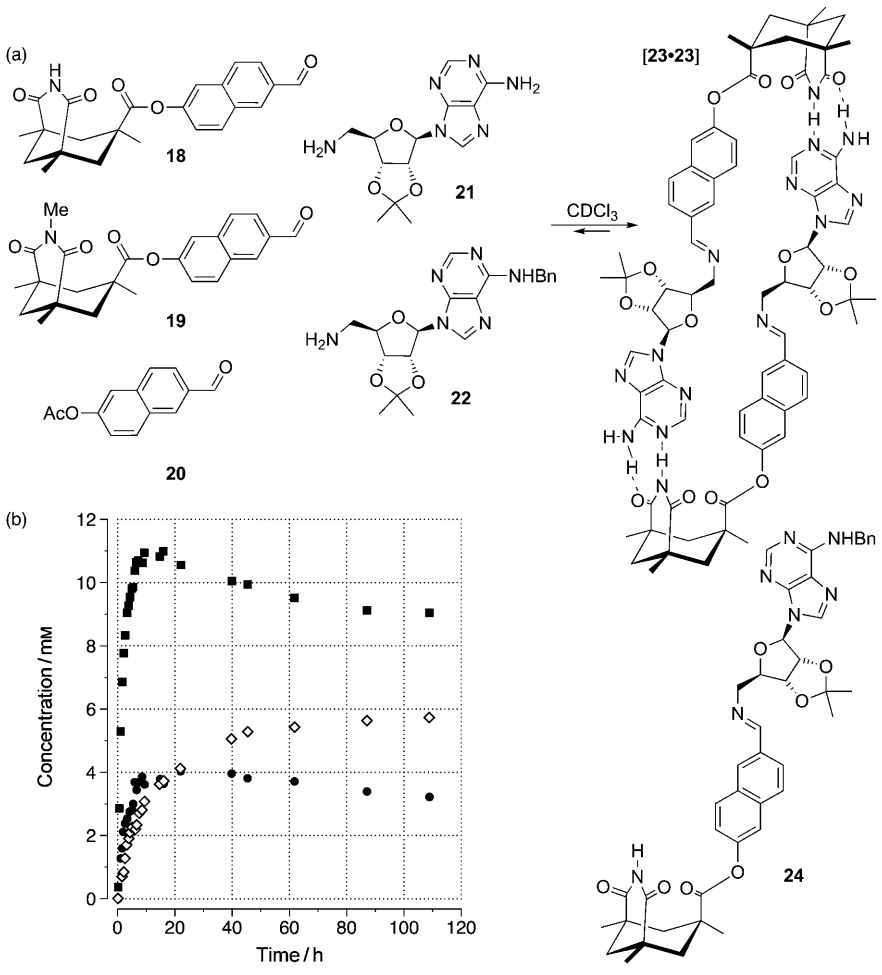


Figure 5. a) Pairwise combination of aldehydes **18**, **19**, and **20** with amines **21** and **22** generates a DCL containing six imines, including **23**, which is capable of dimerization. b) Concentration versus time profiles for compounds **23** (filled squares) and **24** (filled circles). The open diamonds show the concentrations of the imines formed by the condensation of the recognition-disabled amine **22** with the recognition-disabled aldehydes **19** and **20**.

tion kinetics dominate and we observe an overshoot in the production of **23** (filled squares, Figure 5b) and **24** (filled circles, Figure 5b). As the reaction progresses towards the equilibrium point for the system, thermodynamics dominate and this overshoot is corrected. Hence, the concentration of **23** and **24** falls between 20 and 120 h. Indeed, as thermodynamics becomes dominant within the library, another set of compounds (open diamonds, Figure 5b), the imines formed by the condensation of the recognition-disabled amine **22** with the recognition-disabled aldehydes **19** and **20**. The emergence of these compounds serves to illustrate that despite the careful design of recognition-mediated processes within a system, the thermodynamics of covalent bond formation is also an important consideration.

Motivated by our interest in the study of complex reaction networks, we became intrigued by the possibility of embedding a reversible covalent-bond-forming reaction within a replicator. The condensation reaction between an aniline derivative and an aromatic aldehyde to form an imine is central to the design (Figure 6) of our system.[26] Amine **25** and aldehyde **26** can react reversibly through a bimolecular mechanism to form imine **27**, which can potentially act as a template for its own formation. Imine **27** is capable of recognizing and binding both **25** and **26** to form the ternary complex [**25**·**26**·**27**]. Reaction of **25** and **26** within this complex leads to an additional copy of imine **27** and the formation of the product duplex [**27**·**27**]. At this point, imine **27** has completed a formal replication cycle and dissociation of the product duplex [**27**·**27**] completes the autocatalytic cycle. This autocatalytic cycle has a number of interesting features that arise from the reversibility inherent in imine formation. The reaction between **25** and **26** to form imine **27** within the ternary complex is pseudo-intramolecular and, therefore, should proceed at a faster rate than the bimolecular reaction between the same reagents. However, since the reverse reaction is still bimolecular, because it requires a molecule of water, this acceleration of the forward reaction will also affect the overall stability of imine **27**. Hence, reaction of amine **25** and aldehyde **26** at starting concentrations of 15 mM in dry[27] $CDCl_3$ at 298 K affords the imine template **27** and its formation is accelerated strongly (effective molarity (EM) = 20 M) within the complex [**25**·**26**·**27**]. The formation of the product duplex [**27**·**27**] ($K_a = 65\,000\ M^{-1}$) is also favored thermodynamically. The concentration–time profile for the formation of **27** is clearly sigmoidal and the reaction shows the characteristic rate maximum for an autocatalytic process (dashed line, Figure 6b). By contrast, to assess the effect of molecular recognition on this system, aldehyde **28**, which is incapable of recognition, was used as a model for the background bimolecular reaction between **25** and **26**. Initially, we wished to establish the position of equilibrium under the reaction conditions (8 mM, dry $CDCl_3$, 298 K) in the absence of recognition. After 16 h, the conversion of **25** and **28** to imine **29** (in the presence of equimolar amounts of 4-bromo phenylacetic acid[28]) is only around 9%.

Critically, as expected for a replicator, the addition of 15 mol% (1.2 mM) of template **27** at the start of the reaction between amine **25** and aldehyde **26** removes the lag period (squares, Figure 6c) observed for this system and shifts its maximum rate to the beginning of the reaction ($t = 0$). It is interesting to note that this addition of 15 mol% of imine **27** at $t = 0$ decreases the total amount of new imine formed[29] during the course of the reaction (Figure 6c). Intrigued by this result, we turned to kinetic simulation and fitting to gain further insight into this behavior. Figure 7a shows the response surface for the behavior of the system to varying amounts of template **27** added at the start of the reaction between **25** and **26**. This surface was generated by using the kinetic parameters derived from fitting the experimental data in Figure 6 and is corrected for $[\mathbf{27}]_i$. The addition of the template clearly affects the initial rate of reaction—increasing $[\mathbf{27}]_i$ increases the initial rate. More intriguingly, increasing $[\mathbf{27}]_i$ results in a decrease in the amount of new template formed. This fact is best appreciated by examining the projection (Figure 7b) of the back plane of the response surface. This decrease in the total amount of new template formed is simply an expression of the fact that one cannot surpass the boundaries imposed by the system equilibrium position. As the amount of **27** increases, it becomes increasingly unfavorable to form more **27**—at some point the manipulation of stability of [**27**·**27**] through autocatalysis and recognition-induced stabilization is insufficient to force the system further.

Interestingly from this system, the reduced template **30** is also capable of accelerating the formation of **27** through cross-catalysis without attenuation of the concentration of imine **27** formed in this way. The development of a mild reduction method, compatible with the reaction conditions and the recognition elements used in this system, will ultimately allow the coupling of the autocatalytic cycle with the cross-catalytic cycle through an irreversible step that drives the equilibrium formation of the imine **27** through its irreversible transformation to amine **30**, thus allowing complete conversion of **25** and **26** through **27** and, ultimately, to **30** by a combination of auto- and cross-catalysis.

It is therefore clear that in systems based on the protocol shown schematically in Figure 3, the equilibrium position reached by a dynamic system relies solely on thermodynamic parameters, the relative stability of the reagents and products within the system, and is independent of how the system reaches its equilibrium point. This observation is simply a restatement of the fact that free energy is a state function. The kinetic parameters in dynamic systems determine only how fast the equilibrium position will be reached. If the desire is to shift a certain system from its predetermined equilibrium position, an irreversible reaction must be coupled to it. This realization allows the construction of an alternative protocol, illustrated in Figure 8.

In this new scenario, building blocks **a** through **d** can, once again, exchange freely with each other. However, in this case, the transformation that forms the four products **A** through **D** is now irreversible. One of the products, in this case **D**, is a replicator and will therefore engage **d** in an autocatalytic cycle forming itself increasingly rapidly. The au-

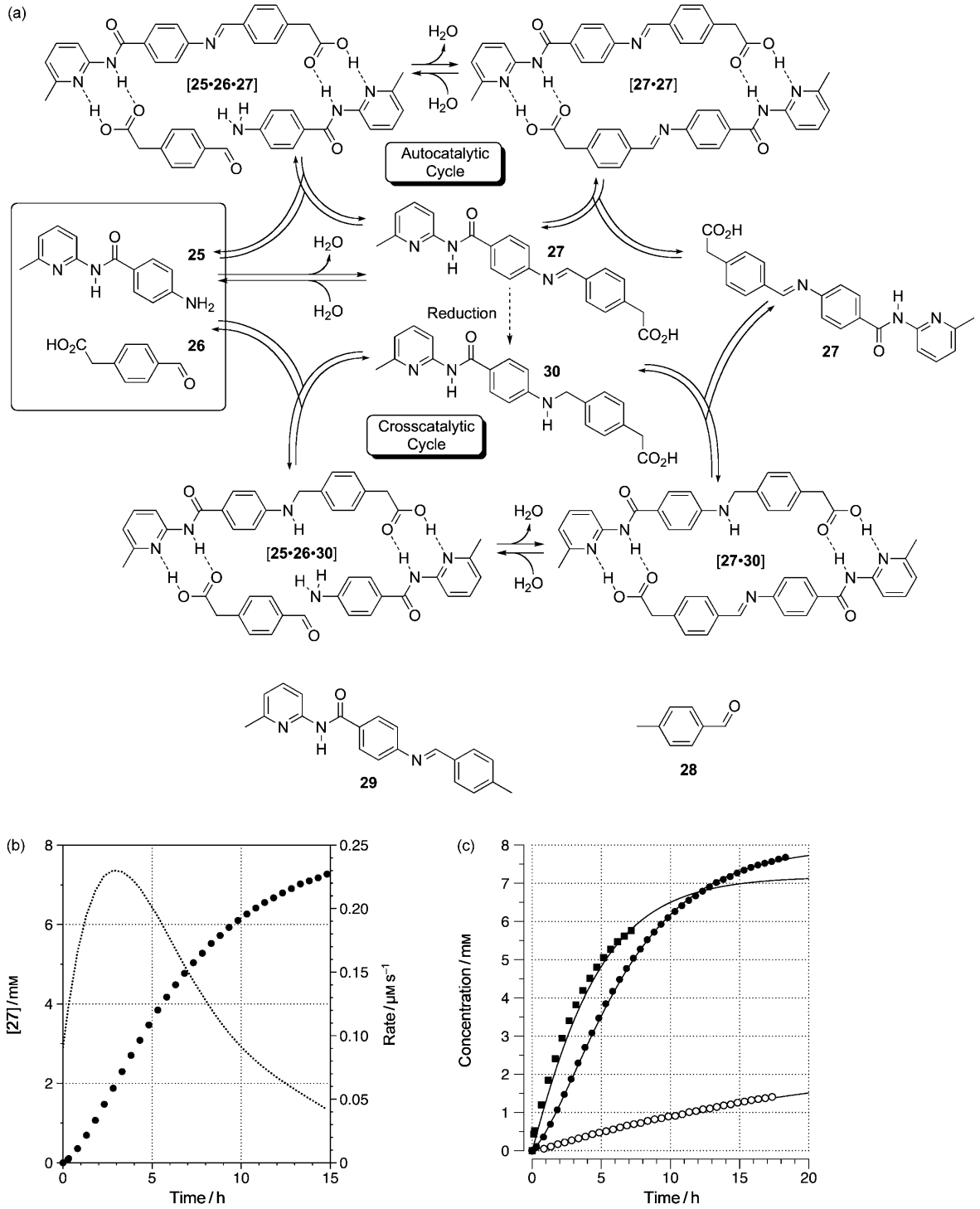


Figure 6. a) Imine **27** can assemble amine **25** and aldehyde **26** in dry $CDCl_3$ at 25 °C and accelerate the reaction between them through the autocatalytic cycle mediated by the ternary complex [**25**·**26**·**27**]. Reduction of imine **27** affords amine **30**, which is also capable of accelerating the formation of imine **27** through the cross-catalytic cycle mediated by the ternary complex [**25**·**26**·**30**]. The effect that molecular recognition played in this system was assessed by comparison with the control imine **29**, formed from building blocks **25** and **28**. b) Concentration versus time profile (filled circles, left axis) and rate versus time profile (dotted line, right axis) for the formation of **27** from **25** and **26**. c) Concentration versus time profiles for the formation of **27** from **25** and **26** (filled circles), compound **29** from **25** and **28** (open circles), and **27** from **25** and **26** in the presence of 15 mol % of **27** at the start of the experiment (filled squares). All experiments were conducted in dry $CDCl_3$ at 25 °C from starting concentrations of the appropriate reagents (**25** and **26**, or **25** and **28**) of 15 mM. In each case, the solid line represents the best fit of the experimental data to the appropriate kinetic model.

tocatalytic formation of **D** will deplete **d** from the exchanging pool at an increasing rate. This depletion of **d** will perturb the equilibrium away from the steady-state position between the four building blocks **a** through **d** and we would

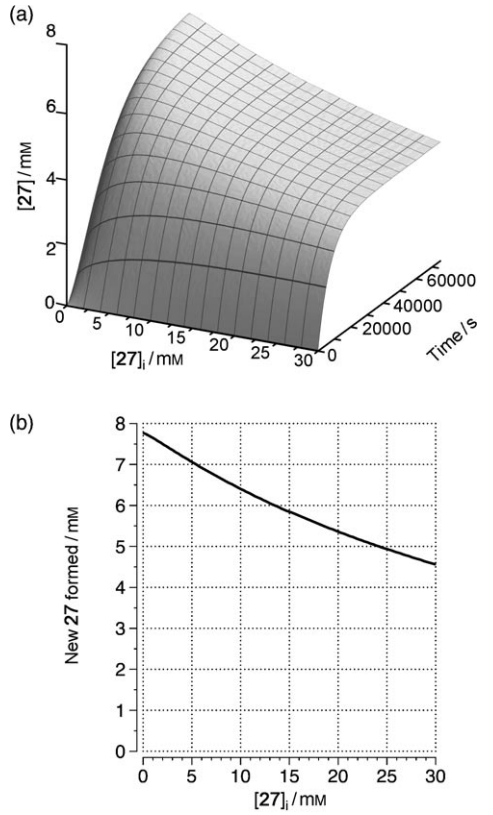


Figure 7. a) Response surface for the formation of new **27** with respect to time and the initial amount of imine **27** present in the system. At higher levels of **27** added at the start of the reaction, the initial rate of formation of **27** is enhanced significantly, however, the total amount of new **27** formed is significantly lower when compared with lower levels of imine **27** added at the start of the reaction. b) Projection of the back plane of the surface shown in (a) illustrating the dependence of the formation of new **27** with respect to the initial amount of **27** present in the system. In both cases, the data has been corrected for the amount of **27** added to the mixture initially.

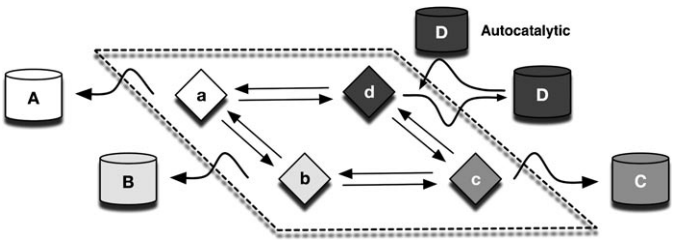


Figure 8. A model for coupling replication to a dynamic library. Building blocks **a**, **b**, **c**, and **d** can all exchange with each other. These four building blocks can be converted irreversibly to four products **A**, **B**, **C**, and **D**. One of these product, **D**, is capable of replicating and can therefore act as a catalyst for its own formation and, as a result, should be formed preferentially.

expect the system to attempt to reestablish this position through the generation of further **d** by exchange with **a**, **b**, and **c**. The autocatalytic template **D**, therefore, sets up a feedback loop that drives the exchanging pool containing **a** through **d** to satisfy its own ends, that is, the generation of **d**. To implement the strategy set out in Figure 8, it is necessary to identify a class of compounds that can participate in DCC, but, at the same time, are capable of participating in additional, irreversible, chemical processes.

Recently, we reported that nitrones undergo exchange, under mild conditions, in nonpolar solvents such as $CDCl_3$.[30] This dynamic exchange process has been demonstrated and exploited within the context of the receptor-assisted selection of a host for a dicarboxylic acid from a mixture of nitrones. A small dynamic library was set up (Figure 9) at 298 K in $CDCl_3$ consisting of four nitrones, two of them with a single aminopyridine recognition site, **31** and **32**, one incapable of recognition, **33**, and one with two amidopicoline recognition sites, **34**. The exchange process is catalyzed by 4-fluorophenylhydroxylamine (**36**). The target, in this case, is the glutaric acid derivative **35** and the presence of this target in solution should amplify the concentration of the complementary nitrone **34** with two recognition sites. As expected, after 48 h, a selectivity of 4:1 was indeed achieved

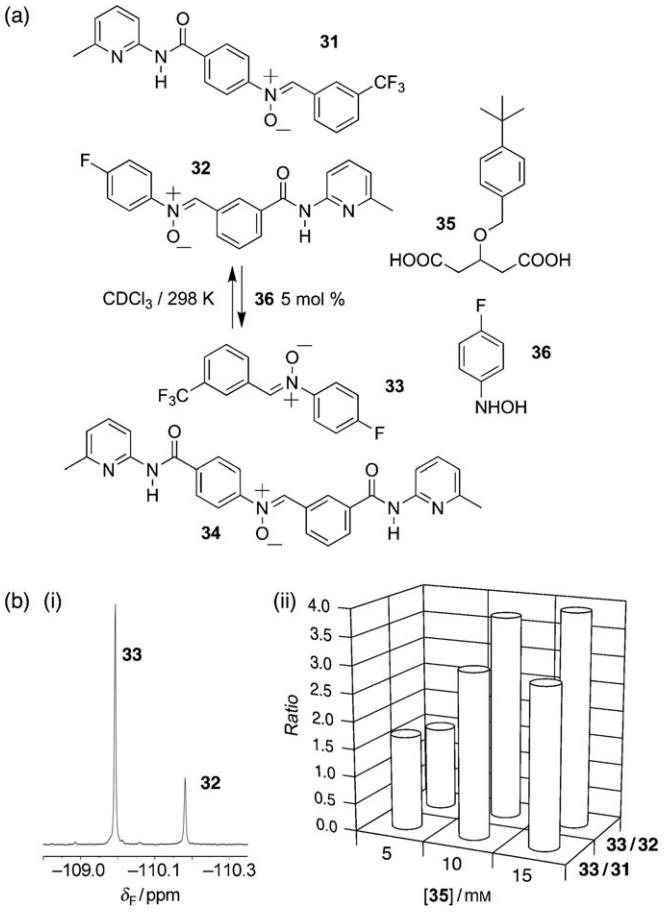


Figure 9. a) The exchange between nitrones **31**, **32**, **33**, and **34** can be biased by dicarboxylic acid **35** and is catalyzed by hydroxylamine **36**. b) In the presence of target **35** after 48 h. i) Partial 282.3 MHz ^{19}F NMR spectrum ($CDCl_3$/298 K; initial conditions: [**31**] = [**32**] = 10 mM; [**36**] = 0.5 mM; [**35**] = 10 mM) showing resonances arising from nitrones **32** and **33**. ii) Ratio of nitrones **33**/**31** and **33**/**32** as a function of diacid **35** concentration.

for the side of the equilibrium containing the targeted nitrone **34**.

Nitrones, however, are also very valuable reactive synthetic intermediates. In this context, we wished to exploit the diastereoisomeric adducts formed by the 1,3-dipolar cycloaddition reaction between nitrones and maleimides, since this feature of nitrone chemistry has allowed us to investigate, over the last decade, the transfer of stereochemical information during replication processes. This cycloaddition reaction gives pairs of racemic diastereoisomeric cycloadducts (*trans* and *cis*) depending on the relative orientation of the nitrone and the maleimide. In our most optimized system,[31] only one of the two possible products of the reaction between nitrone **37** and maleimide **38** (Figure 10) is selectively and highly amplified using a replication strategy. At −10 °C in $CDCl_3$, after 16 h this reaction reaches more than 85 % overall conversion (Figure 10) and the diastereoselectivity is 115:1 in favor of oxazolidine *trans*-**39**, the concentration–time profile (sigmoidal) and corresponding reaction rate (bell shaped) of which is characteristic of a replicator (Figure 10). By contrast, in the control reaction between nitrone **37** and methyl ester **40**, in which the carboxylic acid recognition site is blocked, the overall conversion is only 9 % and the diastereoselectivity 3:1. The reaction between **37** and **38** in the presence of two equivalents of benzoic acid, a competitive inhibitor, results in a significant decrease both in the rate of the reaction and the diastereoselectivity (*trans*/*cis* = 17:1), demonstrating that the formation of *trans*-**39** is recognition mediated. The addition of 10 mol % of *trans*-**39** at the start of the reaction between **37** and **38** results in an increase in the initial rate of formation of *trans*-**39**. Moreover, the ratio of *trans*-**39**/*cis*-**39** is now more than 250:1, demonstrating clearly that *trans*-**39** is able of templating and accelerating its own formation.

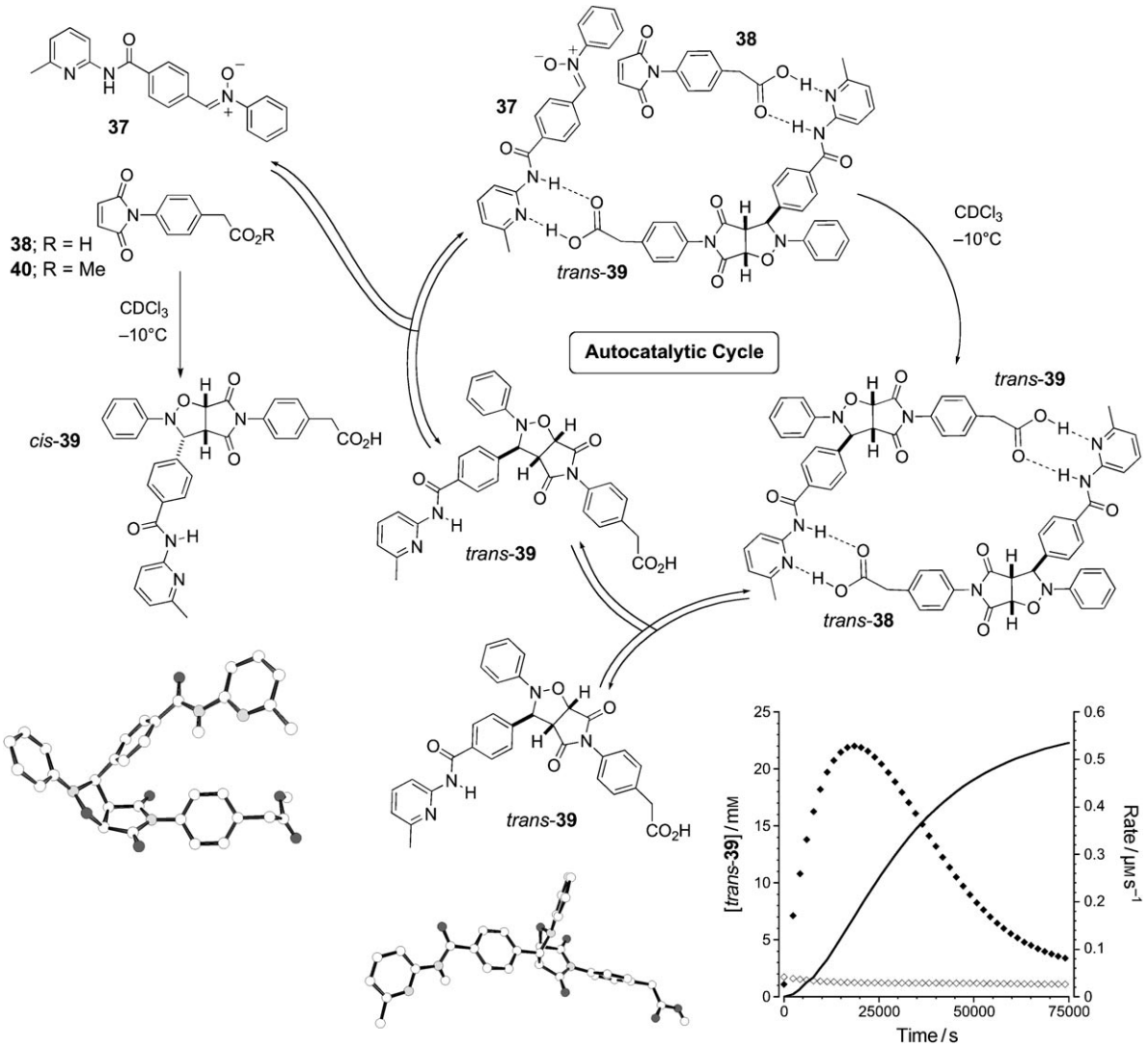


Figure 10. Reaction of nitrone **37** with maleimide **38** in $CDCl_3$ at −10 °C can potentially give rise to two diastereoisomeric cycloadducts: *cis*- and *trans*-**39**. However, the efficient operation of the autocatalytic cycle mediated by the ternary complex [**37**·**38**·*trans*-**39**] in this system ensures that *trans*-**39** is formed almost exclusively (solid line in graph, left *y* axis, *trans*-**39**/*cis*-**39** > 100:1, *cis*-**39** not shown). The rate versus time profile (right *y* axis) for the reaction at −10 °C in $CDCl_3$ (filled diamonds 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 diamonds in graph). Data taken from reference [29].

We have recently coupled this self-replicating system to a DCL built on nitrone–imine exchange to demonstrate that a synthetic replicator, by virtue of its autocatalytic properties, is capable of exploiting the entire network of reactions within a dynamic library to amplify its own formation at the expense of other species. This dynamic library (Exchange pool, Figure 11a) was assembled at 273 K in CD_2Cl_2 saturated with *p*-toluenesulfonic acid monohydrate from two imines, **41** and **44**, one of which has an amidopicoline recognition element and is therefore capable of recognition, and two nitrones, **42** and **43**. At equilibrium, this DCL contains the two imines, the two nitrones, and their corresponding precursors. Equilibration occurs within 16 h and the same equilibrium position is reached whether the starting point is imine **41** and nitrone **42** or nitrone **43** and imine **44**. By introducing a maleimide **38** or **40**, which can participate in a [3+2] dipolar cycloaddition with either nitrone **42** or nitrone **43**, material can be transferred irreversibly from the DCL to a product pool. This product pool contains two pairs of diastereoisomeric oxazolidine cycloadducts: *trans*- and *cis*-**45** and *trans*- and *cis*-**46**. If the maleimide added is designed properly and contains an appropriate recognition element, one of the cycloadducts formed in the product pool, *trans*-**46b**, is capable of self-replication.

Maleimide **40** has its carboxylic acid recognition site blocked as a methyl ester and is incapable of recognizing and binding the amidopyridine recognition sites on compounds **41** and **43**, disabling replication. After 16 h at 273 K, the coupled exchange and reaction processes result in a product pool in which *trans*-**45a** and *cis*-**45a**—the products of reaction between nitrone **42** and maleimide **40**—are present at a total concentration of 3 mM. After the same time, *trans*-**46a** and *cis*-**46b**—the products of reaction between nitrone **43** and maleimide **40**—are present at a total concentration of 1.4 mM. Thus, the total conversion through both reaction channels within the library is only 21% and within the exchange pool the composition is close to that observed in the exchange experiments in which maleimide **40** is absent. It is clear from these results, therefore, that simply coupling exchange to the irreversible cycloaddition reactions generates little selectivity in either the exchange pool or in the product pool.

The use of maleimide **38**, which possesses a carboxylic acid recognition site complementary with the amidopyridine recognition site present in nitrone **43**, allows us to exploit the $>100\times$ acceleration in the rate of reaction between maleimide **38** and nitrone **43** generated within the catalytic ternary complex [**38**·**43**·*trans*-**46b**]. This replication process should, in turn, drive the exchange process towards the formation of nitrone **43**. The results of this experiment are summarized in Figure 11b. In the product pool, after 16 h, *trans*-**45b** and *cis*-**45b**—the products of reaction between nitrone **42** and maleimide **38**—are present at a total concentration of 1.9 mM. After the same time, *trans*-**46b** and *cis*-**46b**—the products of reaction between maleimide **38** and nitrone **43**—are present at a total concentration of 7.7 mM and the selectivity for *trans*-**46b** is >20:1. Indeed, *trans*-**46b** constitutes almost 80% of the total cycloadduct in the product pool. The dominance of *trans*-**46b** is readily understood. Once exchange generates a concentration of nitrone **43** close to the K_d ($\approx$2 mM) for the carboxylic acid·amidopyridine complex, the reaction to form *trans*-**46b** will start to occur through the autocatalytic pathway mediated by the [**38**·**43**·*trans*-**46b**] ternary complex, thus removing nitrone **43** from the exchange pool rapidly. This rapid depletion of **43** from the exchange pool drives the exchange equilibria within the exchange pool, regenerating nitrone **43**. This feedback loop results in most of the reactive material in the system being funneled through pathways that lead to *trans*-**46b**. The key feature of a self-replicator is its ability to template its own formation. One might therefore view the addition of template *trans*-**46b** to the system as an informational input, instructing our dynamic system to synthesize *trans*-**46b**. This expectation can be confirmed when the reaction is seeded with *trans*-**46b** (Figure 11c), the overall conversion for all cycloaddition reactions within the library rises to 64% and cycloadduct *trans*-**46b** constitutes 88% of the total cycloadduct in the product pool.

It is instructive to take a system-level view of the experiments described above. One can view the exchange pool as containing a finite amount of a resource (the hydroxylamine), which can be converted by the exchange processes into two useable forms: nitrones **42** and **43**. These nitrones can then be converted irreversibly (metabolized) through the cycloaddition reaction with maleimides **38** or **40** to form four possible products. The total concentration of all cycloadducts that can be formed is therefore equal to the amount of resource available (20 mM). In the absence of recognition, there is no overall controlling influence within the system and exploitation of the resource through the reactions of nitrone **42** and **43** are slow (21% conversion after 16 h). When we allow the replicator to emerge within the system, it takes some time for the effect of the replicator to become evident. After 6 h, the total conversion is very similar to the control experiment (9 and 8%, respectively), but, crucially, the composition of the mixture is not: the replicator, *trans*-**46b**, now makes up 34% of the product pool as opposed to only 9% in the control experiment. When the exchanging pool is seeded with the replicator, the effects of autocatalysis are even more dramatic. After 6 h, the total conversion is much higher (29%), and the composition of the mixture is dominated by the replicator: *trans*-**46** already makes up 77% of the product pool. During the next 10 h, a further 7.6 mM of hydroxylamine is converted to cycloadducts and 92% of this conversion is the formation of *trans*-**46b** catalyzed by itself. After 16 h *trans*-**46b** constitutes 86% of the product pool and a total of 64% of the initial hydroxylamine resource has been converted. Examining the rates of formation of *trans*-**46b** in these different scenarios is instructive. In the absence of added replicator, the maximum rate of formation of *trans*-**46b** is achieved 10 h into the experiment. By contrast, in the presence of 10 mol% added *trans*-**46b**, the maximal rate for replicator formation achieved is higher and occurs earlier. Therefore, the effect of the small amount of added

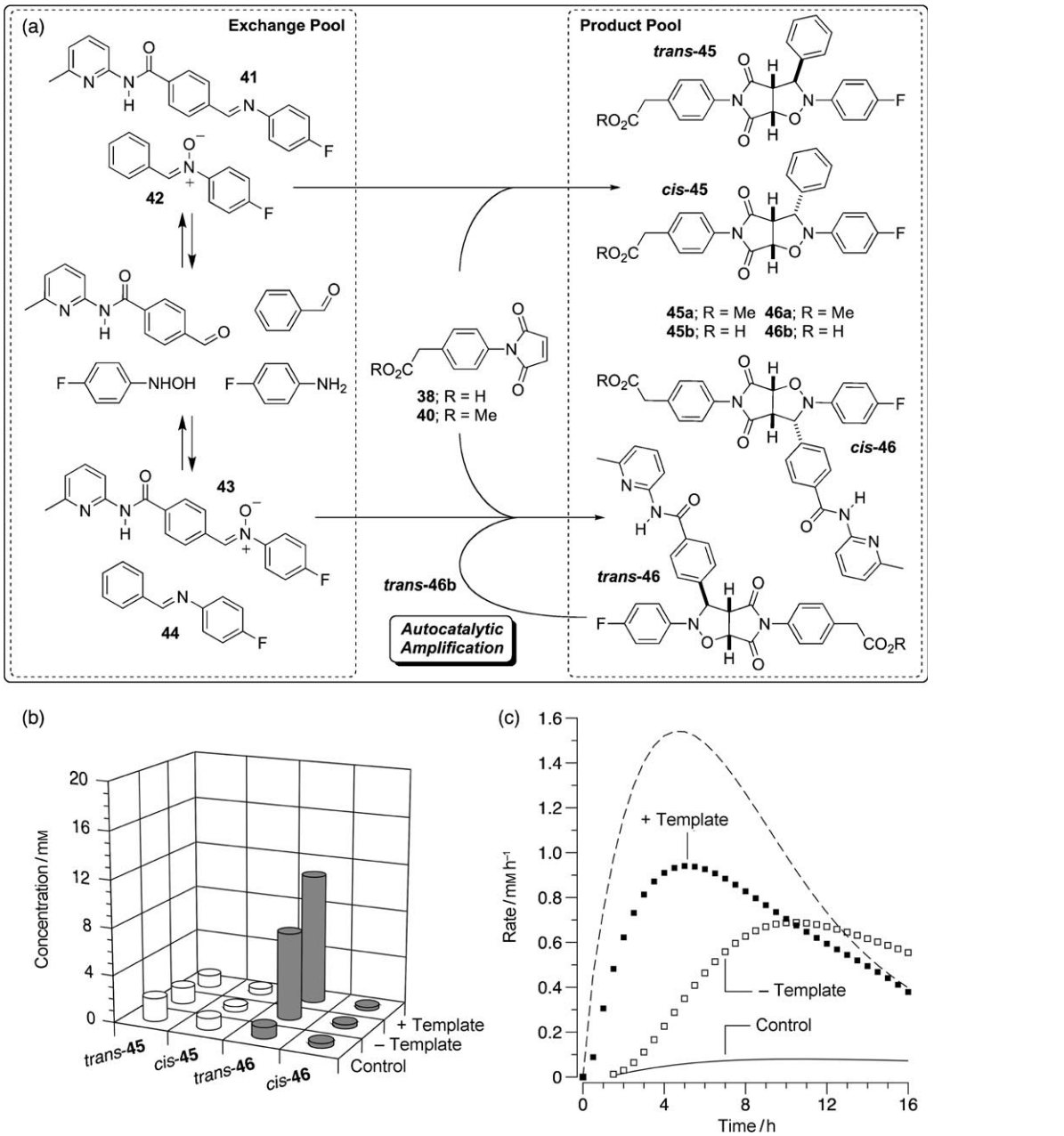


Figure 11. a) A pool of compounds containing imines **41** and **44**, and nitrones **42** and **43**, can exchange freely in CD_2Cl_2 saturated with *p*-toluenesulfonic acid monohydrate at 273 K. Material can be transferred irreversibly to a pool of products present in the same solution that cannot be interconverted or returned to the exchange pool, through reaction of nitrones **42** or **43** with an appropriate maleimide (**38** or **40**). When maleimide **38** is used as the dipolarophile, replicator *trans*-**46b** is formed in the product pool and this species can act as a catalyst for its own formation. b) Composition of the product pool after 16 h for the control experiment (starting concentrations: [**41**] = [**42**] = [**40**] = 20 mM), in the absence (−Template, starting concentrations: [**41**] = [**42**] = [**38**] = 20 mM) and in the presence (+Template, starting concentrations: [**41**] = [**42**] = [**38**] = 20 mM, [*trans*-**46b**] = 2 mM) of *trans*-**46b** (10 mol%) added at the start of the experiment. c) Rate versus time profiles in the absence (−Template) and in the presence (+Template) of *trans*-**46b** (10 mol%) added at the start of the experiment. Data for the control experiment (solid line) and for replicator *trans*-**46b** operating in isolation within a nondynamic experiment (dashed line) are shown for comparison purposes.

template is to engender selectivity, principally in the early phases of the experiment, by ensuring rapid and selective consumption of nitrone **43** through the intermediacy of the autocatalytic formation of *trans*-**46b**. Although, the replicator, *trans*-**46b**, is a relatively simple molecule in a structural sense, its recognition-mediated reactivity is still sufficient to drive the network of exchange reactions in this system. The nonlinear kinetics inherent in minimal replication mean that, despite the fact that, at the start of all of the experiments the concentration of nitrone **43**, which is required to form the replicator, is zero, replicator *trans*-**46b** is, in all cases, the dominant species found in the product pool.

Summary and Outlook

Conventionally, DCC allows for the generation of a network of interconverting compounds, in which the distribution of library members is governed by their relative free energies. It has become clear that to direct these exchanging libraries efficiently towards the desired target kinetically controlled chemistry is essential. The incorporation of template-directed processes, such as replication, is an attractive way forward, allowing, as it does, the direction of an exchanging reagent pool to synthesize and exploit a particular component using an instructional template. It is, however, essential to incorporate replication processes at the correct point within a network and in the correct manner.[32] In the systems presented herein, the key is the presence of an irreversible kinetically controlled step.[33] Although the examples presented herein are relatively simple, their successful implementation suggests that it should be possible to develop more complex recognition-mediated reaction networks, relying on multiple recognition events, such as a combination of auto- and cross-catalytic replicators, to generate and express[34] more complex programmed responses[35] to template inputs through recognition-mediated processes. The development of more complex protocols will create systems that can establish and manage replication, organization, and evolution within synthetic molecular and supramolecular assemblies which will, ultimately, be programmed by exploiting selection and amplification through emergent systems behavior.

[1] Emergent properties arise when a group of simple species cooperate within a system, giving rise to more complex behavior that is a result of their collective interactions. The system-level or emergent features are not a property of any single component of the system and cannot be predicted or deduced readily from behavior of the individual simple components. Key to the development of system-level or emergent properties is the number and type of interconnections between the simple components of the system. Although the number of interconnections in a system increases combinatorially with the number of components, simply having a large number of interconnections is not enough by itself to guarantee emergent behavior. The topology of the interconnections is also critical. Therefore, chemical networks that exhibit significant degrees of mutually catalytic relationships between their components, increasing the level of feedback within the system, are more likely to develop system-level properties than a large collection of weakly interacting compounds.
[2] a) S. Otto, R. L. E. Furlan, J. K. M. Sanders, *Curr. Opin. Chem. Biol.* **2002**, *6*, 321; b) P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders, S. Otto, *Chem. Rev.* **2006**, *106*, 3652; c) S. Ladame, *Org. Biomol. Chem.* **2008**, *6*, 219.
[3] J.-M. Lehn, *Chem. Eur. J.* **1999**, *5*, 2455.
[4] L. M. Greig, D. Philp, *Chem. Soc. Rev.* **2001**, *30*, 287.
[5] K. Severin, *Chem. Eur. J.* **2004**, *10*, 2565.
[6] B. de Bruin, P. Hauwert, J. N. H. Reek, *Angew. Chem.* **2006**, *118*, 2726; *Angew. Chem. Int. Ed.* **2006**, *45*, 2660.
[7] P. T. Corbett, S. Otto, J. K. M. Sanders, *Chem. Eur. J.* **2004**, *10*, 3139.
[8] P. T. Corbett, J. K. M. Sanders, S. Otto, *Angew. Chem.* **2007**, *119*, 9014; *Angew. Chem. Int. Ed.* **2007**, *46*, 8858.
[9] R. F. Ludlow, S. Otto, *Chem. Soc. Rev.* **2008**, *37*, 101.
[10] a) M. Kindermann, I. Stahl, M. Reimold, W. P. Pankau, G. von Kiedrowski, *Angew. Chem. Int. Ed.* **2005**, *44*, 6750; b) J. Stankiewicz, L. H. Eckardt, *Angew. Chem. Int. Ed.* **2006**, *45*, 342.
[11] J. D. Cheeseman, A. D. Corbett, J. L. Gleason, R. J. Kazlauskas, *Chem. Eur. J.* **2005**, *11*, 1708.
[12] J. D. Cheeseman, A. D. Corbett, R. Shu, J. Croteau, J. L. Gleason, R. J. Kazlauskas, *J. Am. Chem. Soc.* **2002**, *124*, 5692.
[13] P. Vongvilai, M. Angelin, R. Larsson, O. Ramström, *Angew. Chem.* **2007**, *119*, 966; *Angew. Chem. Int. Ed.* **2007**, *46*, 948.
[14] M. Angelin, P. Vongviali, A. Fischer, O. Ranström, *Chem. Commun.* **2008**, 768.
[15] M. Angelin, A. Fischer, O. Ramström, *J. Org. Chem.* **2008**, *73*, 3593.
[16] P. Vongvilai, O. Ramström, *J. Am. Chem. Soc.* **2009**, *131*, 14419.
[17] For some recent examples, see: a) R. J. Pearson, E. Kassianidis, A. M. Z. Slawin, D. Philp, *Chem. Eur. J.* **2006**, *12*, 6829; b) E. Kassianidis, R. J. Pearson, D. Philp, *Chem. Eur. J.* **2006**, *12*, 8798; c) S. M. Turega, D. Philp, *Chem. Commun.* **2006**, 3684; d) V. del Amo, D. Philp, *Org. Lett.* **2009**, *11*, 301; e) A. J. Sinclair, V. del Amo, D. Philp, *Org. Biomol. Chem.* **2009**, *7*, 3308.
[18] R. Bennes, D. Philp, *Org. Lett.* **2006**, *8*, 3651.
[19] R. Bennes, D. Philp, N. Spencer, B. M. Kariuki, K. D. M. Harris, *Org. Lett.* **1999**, *1*, 1087.
[20] a) "Templates in Chemistry I," *Top. Curr. Chem.* **2005**, *248*, whole volume; b) "Templates in Chemistry II, *Top. Curr. Chem.* **2005**, *249*, whole volume.
[21] Although strong product duplexes are a hindrance in the case of small-molecule replicators, it is unlikely that duplex dissociation will ever be rate limiting. Therefore, aggressive optimization of the acceleration of the covalent-bond-forming step, which is rate limiting, is of utmost importance.
[22] A. Terfort, G. von Kiedrowski, *Angew. Chem.* **1992**, *104*, 626; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 654.
[23] S. Xu, N. Giuseppone, *J. Am. Chem. Soc.* **2008**, *130*, 1826.
[24] a) T. Tjivikua, P. Ballester, J. Rebek Jr. , *J. Am. Chem. Soc.* **1990**, *112*, 1249; b) J. S. Nowick, Q. Feng, T. Tjivikua, P. Ballester, J. Rebek, Jr., *J. Am. Chem. Soc.* **1991**, *113*, 8831.
[25] a) F. M. Menger, A. V. Eliseev, N. A. Khanjin, *J. Am. Chem. Soc.* **1994**, *116*, 3613; b) M. M. Conn, E. A. Winter, J. Rebek, Jr., *J. Am. Chem. Soc.* **1994**, *116*, 8823; c) D. N. Reinhoudt, D. M. Rudkevich, F. de Jong, *J. Am. Chem. Soc.* **1996**, *118*, 6880.
[26] V. del Amo, A. M. Z. Slawin, D. Philp, *Org. Lett.* **2008**, *10*, 4589.
[27] The water content in $CDCl_3$ was determined to be 14 ppm by using Karl Fischer titration. For this study, we considered aldehyde **26** and imine template **27** both to crystallize with one molecule of water.
[28] 4-Bromophenylacetic acid (1 equiv) was added to the control reaction between **25** and **28** to ensure that the acid concentration was the same as in reactions involving aldehyde **26**.
[29] The concentration of new imine **27** formed is simply the total concentration of **27** at a given time point less the initial concentration of **27** at $t=0$.
[30] S. M. Turega, C. Lorenz, J. W. Sadownik, D. Philp, *Chem. Commun.* **2008**, 4076.
[31] E. Kassianidis, D. Philp, *Angew. Chem.* **2006**, *118*, 6492; *Angew. Chem. Int. Ed.* **2006**, *45*, 6344.
[32] D. G. Blackmond, *Angew. Chem.* **2009**, *121*, 392; *Angew. Chem. Int. Ed.* **2009**, *48*, 386.
[33] The irreversible process could also be a highly cooperative and, hence, nonlinear one, such as crystallization; for examples of systems that may operate in this manner, see: J. M. A. Carnall, C. A. Waudby, A. M. Belenguer, M. C. A. Stuart, J. J. P. Peyralans, S. Otto, *Science* **2010**, *327*, 1502.
[34] E. Kassianidis, R. J. Pearson, E. A. Wood, D. Philp, *Faraday Discuss.* **2010**, *145*, 235.
[35] a) G. Ashkenasy, M. R. Ghadiri, *J. Am. Chem. Soc.* **2004**, *126*, 11140; b) G. Ashkenasy, R. Jagasia, M. Yadav, M. R. Ghadiri, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10872; c) Z. Dadon, N. Wagner, G. Ashkenasy, *Angew. Chem.* **2008**, *120*, 6221; *Angew. Chem. Int. Ed.* **2008**, *47*, 6128; d) N. Wagner, G. Ashkenasy, *Chem. Eur. J.* **2009**, *15*, 1765.

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