Systems Chemistry

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A Simple Synthetic Replicator Amplifies Itself from a Dynamic Reagent Pool**

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Until very recently, synthetic chemistry has focussed on the creation of chemical entities with desirable properties through the programmed application of isolated chemical reactions, either individually or in a cascade, that afford a target compound selectively. By contrast, biological systems operate using a plethora of complex interconnected signaling and metabolic networks[1] with multiple checkpoint controls and feedback loops allowing them to adapt and respond rapidly to external stimuli. Systems chemistry[2] attempts to capture the complexity and emergent phenomena prevalent in the life sciences within a wholly synthetic chemical framework. In this approach, complex dynamic phenomena are expressed by a group of synthetic chemical entities designed to interact and react with many partners within the ensemble in programmed ways. In this manner, it should be possible to create synthetic chemical systems whose properties are not simply the linear sum of the attributes of the individual components. These new system-level properties emerge through the interactions of chemical networks[3] assembled from the many predesigned components. Unlike traditional synthetic approaches, in which mixtures of compounds are treated as an unwanted feature that must be eliminated, systems chemistry demands the presence of a mixture of components and the interactions between these multiple components are a necessity for the emergence of properties at a whole system level.

The chemistry of reversible covalent bond formation—dynamic covalent chemistry[4]—allows for 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. Hence, processes that are capable of manipulating the free energy relationships within the DCL can influence the distribution of library members. This objective can be achieved[5] using the receptor-assisted combinatorial approach and synthetic receptors and sensors,[6] supramolecular assemblies[7] and ligands[8] for proteins have been identified using this approach. Simulation[9] of large DCLs have demonstrated that emergent phenomena, such as pattern generation, may appear spontaneously within such libraries. This amplification process is limited, however, by the amount of template added and by the difference in affinity of the template for the target compared to the other members of the DCL. By contrast, biological systems often exhibit[10] highly non-linear behavior through the expression of both thermodynamic and kinetic phenomena, such as replication,[11] self-sorting,[12] autocatalysis[13] and feedback control.[14] The current existence of such frameworks raises important questions[15] concerning their emergence from much simpler systems. Indeed, it has been suggested[16] that small organic molecules can create a primitive metabolism through a system of auto- and cross-catalyzed reaction cycles that, in turn, can select and amplify[17] favored components leading to molecular evolution. A property that is undoubtedly essential for the emergence of such systems is the ability to self-replicate[18] and many theories[19] place replication before metabolism as the initially emergent process of life. At the simplest level, one can envisage the emergence of a replicating entity within a dynamic pool of building blocks. The replicator, by virtue of its autocatalytic properties,[20] should be capable of exploiting the network of reactions within the dynamic pool to maximize the production of itself to the exclusion of other similar species. Experimentally, however, the demonstration[21] of such non-linear systems-type behavior has proven elusive, since it requires both a dynamic covalent bond forming reaction and a highly efficient self-replicating system which relies on this reaction.

Recently, we reported[22] a highly efficient synthetic replicator based on the 1,3-dipolar cycloaddition between a nitrone and a maleimide. Additionally, we have demonstrated[23] that nitrones are capable of undergoing dynamic exchange in non-polar solvents such as chloroform. By coupling our nitrone-based replicator to a dynamic library based on nitrone-imine exchange, we are now in a position to demonstrate that a synthetic replicator, by virtue of its autocatalytic properties, is capable of exploiting a network of reactions within a dynamic library to amplify its own formation at the expense of other species.

We constructed our dynamic library from two aldehydes, one of which bears an amidopyridine recognition site (Figure 1). The presence of 4-fluoroaniline permits the formation of two unreactive imines **1** and **4** in our library and 4-fluorophenylhydroxylamine, in turn, permits the formation of two reactive nitrones **2** and **3**. Therefore, at equilibrium, our DCL (exchange pool, Figure 1) contains two imines and two nitrones, compounds **1** to **4**, and their respective precursors. Material can be transferred irreversibly

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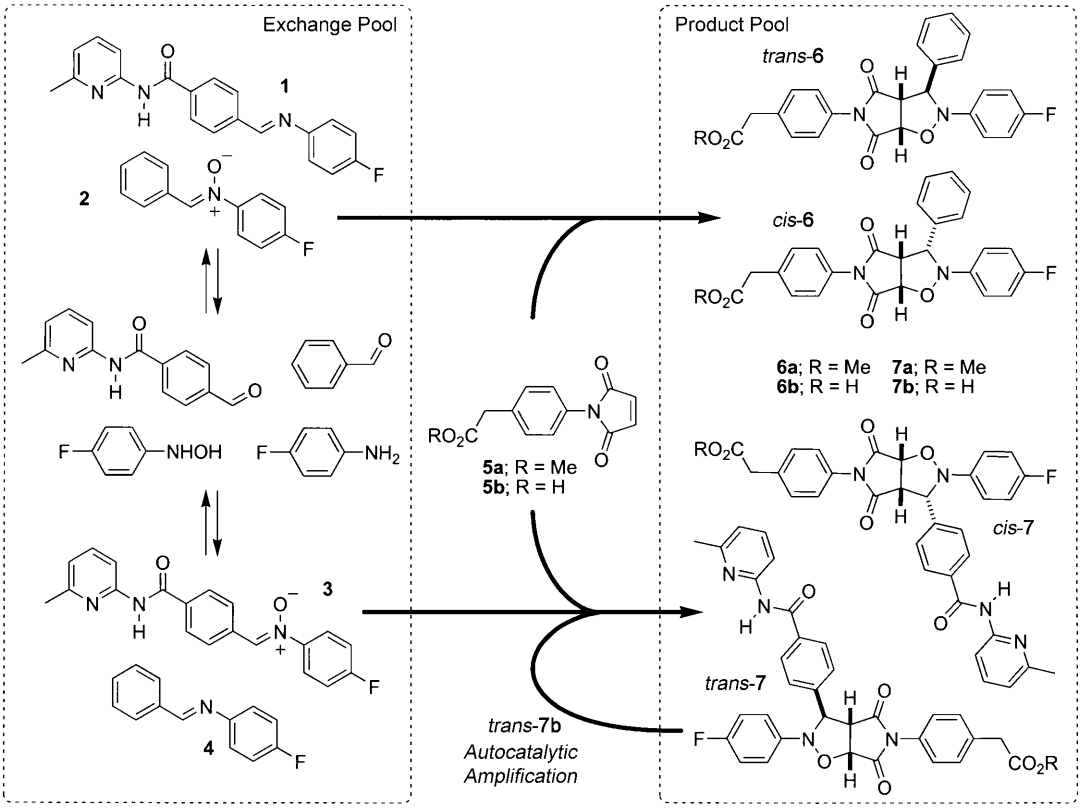

Figure 1. A pool of compounds containing imines **1** and **3** and nitrones **2** and **4** can exchange freely in CD_2Cl_2 saturated with *p*-toluenesulfonic acid monohydrate at 273 K. Material can be transferred irreversibly to a pool of products, present in the same solution, that cannot be interconverted or returned to the exchange pool, through reaction of nitrones **2** or **3** with an appropriate maleimide (**5a** or **5b**). When maleimide **5b** is used as the dipolarophile, replicator *trans*-**7b** is formed in the product pool and this species can act as a catalyst for its own formation.

from this exchange pool to the product pool through the reaction of either nitrone **2** or nitrone **3** with a maleimide (**5a** or **5b**). These dipolar cycloaddition reactions create a group of products containing two pairs of diastereoisomeric cycloadducts: *cis*- and *trans*-**6**[24] and *cis*- and *trans*-**7**. Crucially, one of the cycloadducts formed in the product pool, *trans*-**7b**, is capable of catalyzing its own formation, that is, it is capable of self-replication. This replication process relies on the ability of *trans*-**7b** to act as a template for its own formation through the recognition and binding of nitrone **3** and maleimide **5b**—the components required to form *trans*-**7b**. The catalytic ternary complex [**3**·**5b**·*trans*-**7b**] which forms, accelerates the cycloaddition reaction between the nitrone and the maleimide by more than 100× and the stereochemistry of the *trans*-**7b** template is transcribed faithfully—the *trans*-**7b**:*cis*-**7b** ratio is > 50:1 (see Supporting Information for details). In our exchange pool, only imine **1** and nitrone **2** are present initially. Since our replicating template *trans*-**7b** is formed from nitrone **3**, some exchange must occur before this replicator can be formed. It was therefore important to demonstrate the dynamic behavior of our exchange pool. When a mixture of imine **1** and nitrone **2** in CD_2Cl_2 saturated with *p*-toluenesulfonic acid monohydrate (PTSA) ([**1**] = [**2**] = 20 mM) is kept at 273 K for 16 h, equilibration occurs to give a mixture of all four compounds in the exchange pool. As expected, there is some slight selectivity for the two nitrones (**1**:**2**:**3**:**4** = 1.0:1.4:1.7:1.0) as a result of their higher stability under the exchange conditions. When the same experiment is repeated, this time starting from nitrone **3** and imine **4** ([**3**] = [**4**] = 20 mM, CD_2Cl_2/sat. PTSA, 273 K, 16 h), essentially the same equilibrium position is reached.

Having demonstrated that our mixture of imines and nitrones can, indeed, exchange, we were now in a position to attempt to couple this dynamic reagent pool to our replication process. Initially, however, we wished to perform a control experiment to determine if simply permitting two irreversible reactions involving components of the exchange pool generated any selectivity within the system. Hence, we prepared a mixture of imine **1** and nitrone **2** in CD_2Cl_2 saturated with PTSA ([**1**] = [**2**] = 20 mM) and maleimide **5a** was added immediately as the dipolarophile ([**5a**] = 20 mM). In this experiment, maleimide **5a** has its carboxylic acid recognition site blocked as a methyl ester and is incapable of recognizing and binding the amidopyridine recognition sites on compounds **1** and **3**. In other words, replication is disabled. The composition of this mixture was then allowed to evolve at 273 K for 16 h and the coupled exchange and reaction processes were monitored by ^1H and ^{19}F NMR spectroscopy every 30 min during this period. The concentrations of each of the species present in the mixture were then determined for each time point (see Supporting Information). The results of this experiment are summarized in Figure 2a. In the product pool, after 16 h, *trans*-**6a** and *cis*-**6a**—the products of reaction between nitrone **2** and maleimide **5a**—are present at a total

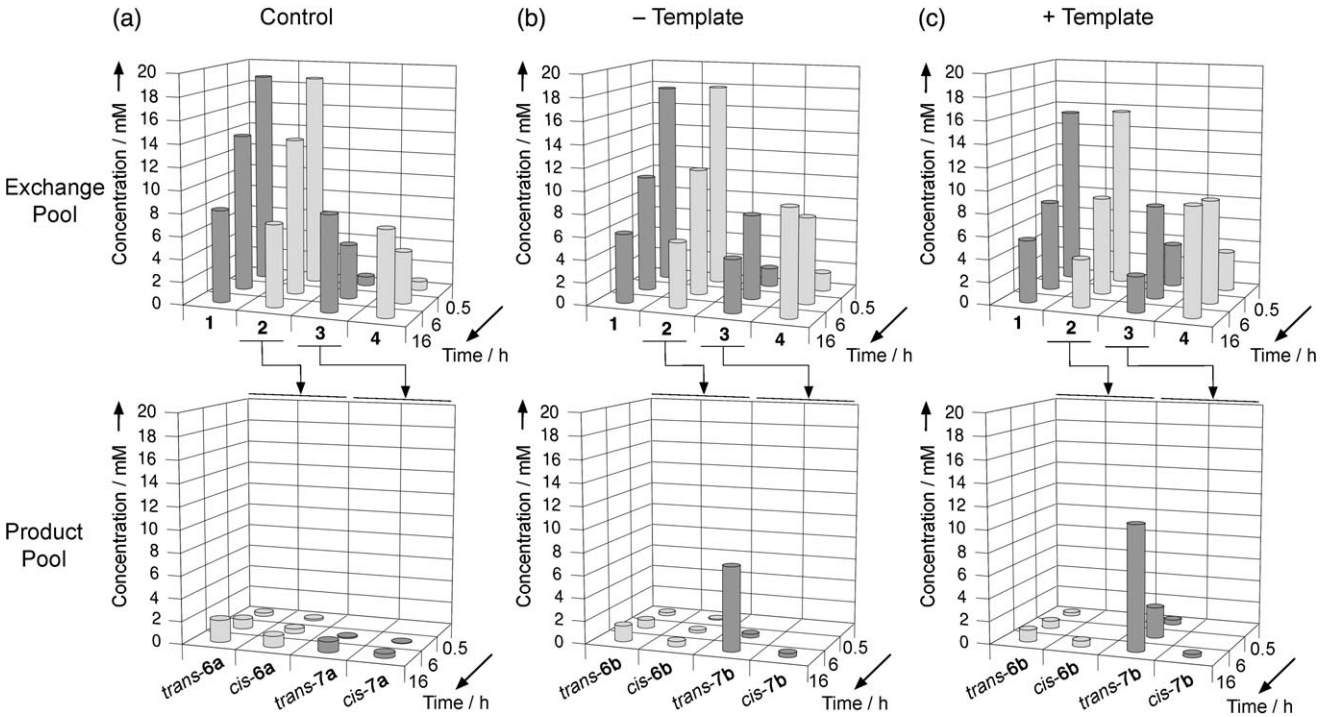

Figure 2. The composition of the exchange pool and the product pool at 0.5 h, 6 h and 16 h for a) the control exchange experiment (starting concentrations: [**1**] = [**2**] = [**5a**] = 20 mM), b) the exchange experiment performed without *trans*-**7b** added at the start of the experiment ("– Template"; starting concentrations: [**1**] = [**2**] = [**5b**] = 20 mM), and c) in the presence of *trans*-**7b** added at the start of the experiment ("+ Template"; starting concentrations: [**1**] = [**2**] = [**5b**] = 20 mM, [*trans*-**7b**] = 2 mM). All experiments were performed in CD_2Cl_2 saturated with *p*-toluenesulfonic acid monohydrate at 273 K. The concentrations of all species were determined by deconvolution of the appropriate resonances in 470.4 MHz ^{19}F NMR spectra. Dark grey cylinders represent compounds bearing an amidopyridine recognition site, light grey cylinders represent compounds bearing no recognition site. Where no cylinder is shown, the concentration of that compound is below the limit of detection (< 50 μM).

concentration of 3 mM ([*trans*-**6a**]:[*cis*-**6a**] = 2:1). After the same time, *trans*-**7a** and *cis*-**7a**—the products of reaction between nitrone **3** and maleimide **5a**—are present at a total concentration of 1.4 mM ([*trans*-**7a**]:[*cis*-**7a**] = 2.5:1). Thus, the total conversion through both cycloaddition reaction channels within the library is only 21 %. Within the exchange pool, after 16 h, compounds **1** to **4** are all present in almost equal amounts ([**1**] = 8.1 mM, [**2**] = 7.1 mM, [**3**] = 8.5 mM, [**4**] = 7.6 mM). This composition is close to that observed in the exchange experiments where maleimide **5a** is absent. The rate of the 1,3-dipolar cycloaddition reactions between **2** and **5a** and **3** and **5a** are much lower than the rate of exchange. Therefore, these irreversible reactions have little influence on the exchange processes as nitrones **2** and **3** are depleted at similar rates. Additionally, both cycloaddition reactions are rather unselective. Thus, despite the fact that nitrone **3** is not present within the starting exchange pool, after 16 h, the ratio of cycloadducts arising from nitrone **2** to those arising from nitrone **3** is only 2:1 and there is rather poor diastereoselectivity in the product pool in general. It is clear from these results that simply coupling exchange to the irreversible cycloaddition reactions generates little selectivity in either the exchange pool or in the product pool.

Next, we wished to exploit the autocatalytic behavior of *trans*-**7b** within the context of our exchanging library. The use of maleimide **5b**, which possesses a carboxylic acid recognition site complementary with the amidopyridine recognition site present in nitrone **3**, allows us to exploit the more than hundredfold acceleration in the rate of reaction between nitrone **3** and maleimide **5b** generated within the catalytic ternary complex [**3**·**5b**·*trans*-**7b**]. This replication process would, in turn, drive the exchange process towards the formation of nitrone **3**. Additionally, we envisaged that the autocatalytic behavior of *trans*-**7b** would express itself progressively, resulting in this species becoming the dominant one in the product pool at the end of the experiment. Accordingly, we prepared a mixture of imine **1**, nitrone **2** and maleimide **5b** in CD_2Cl_2 saturated with PTSA ([**1**] = [**2**] = [**5b**] = 20 mM). Once again, the composition of the mixture was allowed to evolve at 273 K for 16 h and the coupled exchange and reaction processes were monitored by ^1H and ^{19}F NMR spectroscopy as described previously. The results of this experiment are summarized in Figure 2b. In the product pool, after 16 h, *trans*-**6b** and *cis*-**6b**—the products of reaction between nitrone **2** and maleimide **5b**—are present at a total concentration of 1.9 mM ([*trans*-**6a**]:[*cis*-**6a**] = 3:1). After the same time, *trans*-**7b** and *cis*-**7b**—the products of reaction between nitrone **3** and maleimide **5b**—are present at a total concentration of 7.7 mM ([*trans*-**7b**]:[*cis*-**7b**] = 21:1). Thus, the overall conversion for all cycloaddition reactions within the library is now 48 % and cycloadduct *trans*-**7b** constitutes almost 80 % of the total cycloadduct in the product pool. The

effects of the introduction of replication into the system are equally significant in the exchange pool. After 16 h, compounds **1** through **4** are present in markedly different concentrations compared to the same exchange process in the presence of the control maleimide **5a** (Figure 2a). In particular, the concentration of the two nitrones **2** and **3** are depressed significantly ([**2**] = 5.7 mM, [**4**] = 4.7 mM). In this case, the rate of the 1,3-dipolar cycloaddition reaction between **2** and **5b** is still lower than the rate of exchange. However, the reaction between nitrone **3** and **5b** is comparable in rate to the exchange processes. Thus, once exchange generates a concentration of nitrone **3** close to the K_d (ca. 2 mM) for the carboxylic acid·amidopyridine complex, reaction to form *trans*-**7b** will start to occur through the autocatalytic process mediated by the [**3**·**5**·*trans*-**7b**] ternary complex, thereby removing nitrone **3** from the exchange pool rapidly. The depletion of **3** from the exchange pool drives the exchange equilibria involving compounds **1** to **4**, regenerating nitrone **3** which is removed by the autocatalytic reaction. As the concentration of *trans*-**7b** increases, the effect of autocatalysis is to increase the rate of the depletion of **3** until the formation of **3** through exchange becomes limiting overall. It is clear from the results presented in Figure 2b that the coupling of exchange to the formation of the autocatalytic replicator *trans*-**7b** generates significant selectivity in both the exchange pool and in the product pool.

The key feature of a self-replicator is its ability to template its own formation. Experiments in which the reaction mixture is seeded with a small amount of a replicating template[25] are usually used to demonstrate replicating behavior (see Supporting Information) and should result in a significant enhancement in the rate of formation of the replicator. One might view the addition of template *trans*-**7b** to the exchange pool as an informational input, instructing our dynamic system to synthesize *trans*-**7b**. Therefore, we prepared a mixture of imine **1**, nitrone **2**, maleimide **5b** and replicator *trans*-**7b** in CD_2Cl_2 saturated with PTSA ([**1**] = [**2**] = [**5b**] = 20 mM; [*trans*-**7b**] = 2 mM). This mixture was allowed to evolve at 273 K for 16 h and the coupled exchange and reaction processes were monitored by ^1H and ^{19}F NMR spectroscopy as described previously. The results of this experiment are summarized in Figure 2c. In the product pool, after 16 h, *trans*-**6b** and *cis*-**6b**—the products of reaction between nitrone **2** and maleimide **5b**—are present at a total concentration of 1.5 mM ([*trans*-**6a**]:[*cis*-**6a**] = 2:1). After the same time, *trans*-**7b** and *cis*-**7b**—the products of reaction between nitrone **4** and maleimide **5b**—are present at a total concentration of 11.3 mM ([*trans*-**7b**]:[*cis*-**7b**] = 38:1). Thus, the overall conversion for all cycloaddition reactions within the library is now 64% and cycloadduct *trans*-**7b** constitutes 88% of the total cycloadduct in the product pool.

It is instructive to take a system-level view (Figure 3) 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 **2** and **3**. These nitrones can then be converted irreversibly (metabolized) through the cycloaddition reaction with maleimide **5** forming 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 (Control, Figure 3), there is no overall controlling influence within the system and exploitation of the resource through the reactions of nitrone **2** and **3** is slow (conversion 21% after 16 h). When we allow the replicator to emerge within the system (− Template, Figure 3), 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*-**7b**, now makes up 34% of the product pool as opposed to only 9% in the control experiment. From this point onwards, the replicator dominates the system. Of the 8.7 mM of hydroxylamine converted to cycloadducts in the next 10 h, 7.7 mM is converted by the replicator and by 16 h *trans*-**7b** constitutes 79% of the product pool. When the exchanging pool is seeded with the replicator (+ Template, Figure 3), the effects are even more pronounced. After 6 h, the total conversion is much higher (29%), and the composition of the mixture is dominated by the replicator—*trans*-**7b** already makes up 77% of the product pool. In the next 10 h, a further 7.6 mM of hydroxylamine is converted to cycloadducts—92% of this conversion is the formation of *trans*-**7b** catalyzed by itself. After 16 h *trans*-**7b** constitutes 86% of the product pool and a total of 64% of the initial hydroxylamine resource has been converted. An explanation for the different behavior of the system in the presence of the instructional template can be found by examining the rates of formation of *trans*-**7b** in the different scenarios. In the absence of added replicator, the maximal rate of formation of *trans*-**7b** (0.69 mM h^{-1}) is

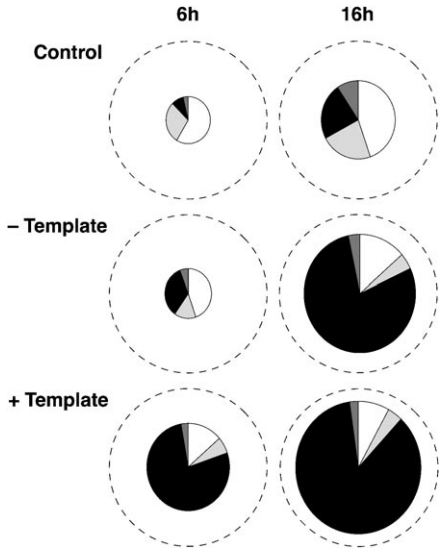

Figure 3. Composition of the product pool after 6 h and 16 h. The dashed circle represents 100% conversion to cycloadducts. The area of the pie charts indicates the actual conversion. The shading of the pie chart wedges indicates the composition of the product pool: white: *trans*-**6**, light grey: *cis*-**6**, dark grey: *cis*-**7**, black: *trans*-**7**. Starting concentrations: Control: [**1**] = [**2**] = [**5a**] = 20 mM. − Template: [**1**] = [**2**] = [**5b**] = 20 mM. + Template: [**1**] = [**2**] = [**5b**] = 20 mM, [*trans*-**7b**] = 2 mM. All experiments were performed in CD_2Cl_2 saturated with *p*-toluenesulfonic acid monohydrate at 273 K.

achieved 10 h into the experiment. By contrast, in the presence of 10 mol% added *trans*-**7b**, the maximal rate for replicator formation[26] achieved is higher (0.94 mM h^{-1}) and occurs earlier (5 h). Therefore, the effect of the small amount of added template is to engender selectivity principally in the early phases of the experiment by ensuring rapid and selective consumption of nitrone **3** through the intermediacy of the recognition processes which assemble the catalytic ternary complex [**3**·**5b**·*trans*-**7b**].

In summary, we have demonstrated that a replicating template is capable of exploiting and dominating an exchanging pool of reagents in order to amplify its own formation. Although the structural complexity and information content[27] of the replicator *trans*-**7b** are low, it is still capable of driving the network of exchange reactions by virtue of the non-linear kinetics inherent in minimal replication. Thus, despite the fact that, at the start of all of the experiments, the concentration of nitrone **3**, which is required to form the replicator, is zero, replicator *trans*-**7b** is, in all cases, the dominant species found in the product pool. Our results lend weight to hypotheses that a primitive metabolism incorporating a system of autocatalyzed reaction cycles might be able to select and amplify replicators leading to molecular evolution. The significant response of the system to a small input of instructional template is also encouraging. It suggests that it should be possible to develop of more complex recognition-mediated reaction networks, relying on multiple recognition events, such as a combination of auto- and crosscatalytic[28] replicators, to generate and express more complex programmed responses to template inputs through recognition-mediated processes.

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[24] The descriptors *cis* and *trans* identify the relative configuration of the three protons located on the bicyclic fused ring structure formed in the cycloaddition reaction. In the *cis* cycloadduct all three protons lie on the same face of the fused ring system. In the *trans* cycloadduct, the two protons originally located on the maleimide are located on the opposite face to the proton originating from the nitrone.

[25] The addition of the replicator at the start of the experiment should remove the lag or induction period associated with the necessary formation of replicator through the slow, bimolecular pathway. Therefore, seeding the reaction with replicator should permit the formation of the replicator at the maximum autocatalytic rate from $t=0$. In this case, at $t=0$, the concentration of nitrone **3** is zero and the induction period apparent in the formation of *trans*-**7b** arises from the necessary formation of nitrone **3** by exchange.

[26] The location and magnitude of maximal replicator formation (0.94 mM h^{-1} at 5 h) is close to that (1.55 mM h^{-1} at 5 h) observed in the reaction between **3** and **5b** in the absence of dynamic exchange. This result suggests that in the presence of added template the replication of *trans*-**7b** is the dominant irreversible reaction pathway in the system from the start of the experiment. See Supporting Information (Figure S8) for rate vs. time profiles.

[27] Replicator *trans*-**7b** has a molecular weight of only 580 Da, possesses only three stereocentres and the system relies on a single recognition motif for its function. Therefore, in comparison to nucleic acids, it can be considered to be structurally and informationally simple.

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