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Specific Autocatalysis in Diastereoisomeric Replicators

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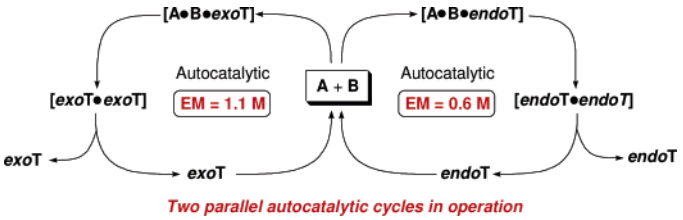
ABSTRACT



Two diastereoisomeric cycloadducts are capable of accelerating their own formation through the assembly of catalytic ternary complexes. The two cycloadducts do not have any measurable catalytic effect on the rate of formation of their diastereoisomer.

A significant current goal[1] for synthetic chemists is the formulation of a cogent conceptual framework that allows the facile assembly of complex molecular architectures. Within this broad objective, the development of efficient protocols that allow self-replication and genotypic (Darwinian) evolution within supramolecular assemblies could lead to a formidable, self-instructed synthetic apparatus, functioning in an economical and environmentally benign fashion. A fundamental understanding of recognition-mediated processes that allow molecules to template their own formation is therefore an important requirement.

In the last 15 years, several examples of self-replicating systems capable of templating and catalyzing their own synthesis have appeared[2] in the chemical literature. Almost all of the examples[3] of synthetic self-replicators reported to date are based on the minimal model[4] shown in Figure 1. Within this minimal model for self-replication, three reaction channels exist. The first is the uncatalyzed bimolecular reaction between reagents **A** and **B** to afford the template

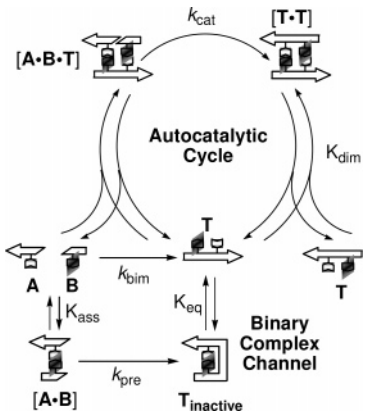


Figure 1. Minimal self-replicating system. Recognition-enabled reagents **A** and **B** can react by either random bimolecular collision or through a reactive binary complex or by means of an autocatalytic cycle mediated by the product **T** of the reaction.

T. However, a requirement of the minimal model is that **A** and **B** bear complementary recognition sites. **A** and **B** can therefore associate with each other through their complementary recognition sites to form a binary complex, [**A**•**B**]. The presence of this complex offers a second pathway, the binary complex channel, in which the reaction between **A**

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(2) For examples of complex replicable entities, see: (a) Zykov, V.; Mytilinaios, E.; Adams, B.; Lipson, H. *Nature* **2005**, *435*, 163−164. (b) Eckardt, L.-H.; Naumann, K.; Pankau, W. M.; Rein, M.; Schweitzer, M.; Windhab, N.; von Kiedrowski, G. *Nature* **2002**, *420*, 286−288. (c) Szostak, J. W.; Bartel, D. P.; Luisi, P. L. *Nature* **2001**, *409*, 387−390.

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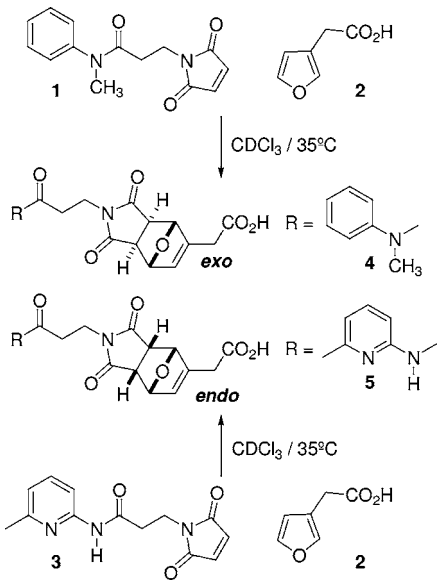
and **B** is pseudo-intramolecular and forms a closed template $\mathbf{T_{inactive}}$. The recognition used to assemble the binary complex lives on in the template ($K_{eq} \ll 1$); thus, although rate acceleration is achieved[5] by this mechanism, this product is catalytically inert.

The third reaction channel is the autocatalytic cycle. Here, **A** and **B** bind reversibly to an open template **T** to form a catalytic ternary complex [**A·B·T**] in which the reaction between **A** and **B** is also rendered pseudo-intramolecular. 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, the open template **T**, with unhindered recognition sites in the correct orientation, can potentially act as a self-replicator, transmitting molecular information[6] through the formation of identical template molecules.

We have become interested[7] in the factors that govern the choice of reaction pathway adopted by systems whose reactivity is mediated by recognition processes. Ultimately, we wish to exploit replicating systems in the construction[2] and amplification of large molecular and supramolecular assemblies. In this respect, the development of replicating systems that can operate either in isolation or in concert within the *same* reaction mixture is of paramount importance. In this work, we describe the design and synthesis of a system based on Diels−Alder chemistry whose kinetic behavior demonstrates these highly desirable features. In the system reported here, both diasteroisomeric templates are capable of specific autocatalysis. This behavior can be rationalized and explained in terms of the different recognition-mediated reaction pathways facilitated by the amidopicoline-carboxylic acid recognition sites that are present in the two templates.

The Diels−Alder reaction between alkylfurans and maleimides is a particularly suitable platform[7] upon which to conduct these studies. This reaction affords two products, the *endo* cycloadduct and the *exo* cycloadduct, which differ in the relative stereochemistry at the 6−5 ring junction, and these two cycloadducts have markedly different geometries. The reaction proceeds under mild conditions without the need for external reagents, making it ideal for study by NMR methods in nonpolar solvents.

The starting materials required for our recognition-mediated Diels−Alder cycloaddition reactions, diene **2** and maleimide **3**, were synthesized by standard methods. Additionally, maleimide **1** was also prepared to function as a control dienophile since the *N*-methyl anilide is incapable of binding to the carboxylic acid recognition site present in **2** and also in ***endo*-5** and ***exo*-5**.



Initially, the Diels−Alder reaction between the *N*-methyl maleimide **1** and diene **2** ([**1**] = [**2**] = 25.0 mM) was explored to uncover the innate kinetic characteristics and selectivity of the bimolecular reaction at 35 °C. We have established[8] previously that *N*-methylanilides do not recognize carboxylic acids. Thus, the products of this reaction, ***endo*-4** and ***exo*-4**, are incapable of recognizing the starting materials **1** and **2**. Therefore, this reaction provides a good model for the background bimolecular process in the recognition-mediated reaction. After 16 h, the total yield of cycloadduct was 19%, and the ***endo*-4**:***exo*-4** ratio was 1.6:1 (Figure 2, red points). The initial rates of formation of ***endo*-4** and ***exo*-4** were estimated as r(***endo*-4**) = 4.14×10^{-5} mM s^{-1} and r(***exo*-4**) = 2.66×10^{-5} mM s^{-1}, respectively.

When the reaction was repeated under identical conditions, but replacing **1** with recognition-enabled maleimide **3**, a significant increase in the initial rates of formation (Figure 2, green points) of the cycloadducts was observed. In this case, the initial rates of formation of ***endo*-5** and ***exo*-5** were estimated as r(***endo*-5**) = 9.79×10^{-5} mM s^{-1} (2.4 × bimolecular) and r(***exo*-5**) = 7.68×10^{-5} mM s^{-1} (2.9 × bimolecular). The increase in rates results in the combined yield of cycloadduct being 41% after 16 h. The diastereoselectivity of the reaction was also affected slightly when recognition occurred (***endo*-5**:***exo*-5** = 1.1:1).

To establish that the rate enhancement was the result of recognition-mediated processes, we performed a number of additional control experiments. Introduction of 4.0 equiv of benzoic acid to the reaction mixture at the start of the experiment ([**2**] = [**3**] = 25.0 mM, [$\mathbf{PhCO_2H}$] = 100 mM) resulted (Figure 2, blue points) in the reduction of the initial

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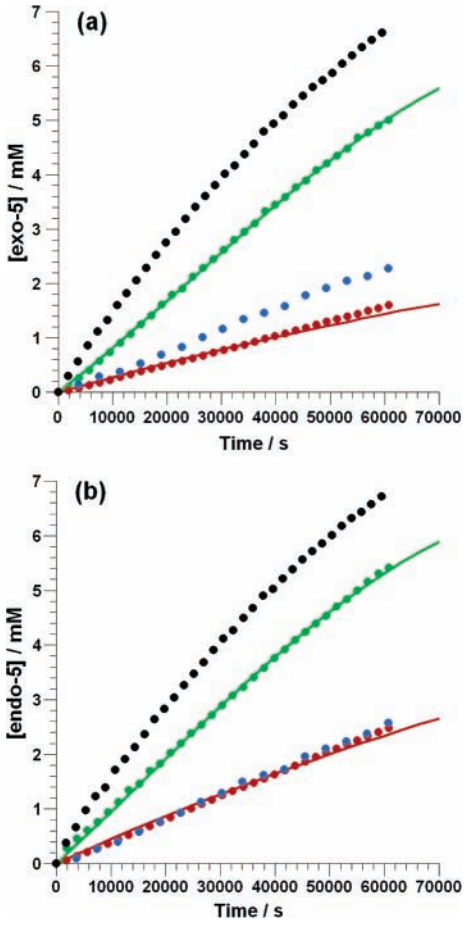

Figure 2. Rate profiles for the formation of (a) *exo* cycloadduct and (b) *endo* cycloadduct. In each case, red points denote the control reaction between **1** and **2**, green points denote the reaction between **2** and **3**, black points denote the template-directed reaction between **2** and **3** in the presence of approximately 10 mol % (a) ***exo*-5** or (b) ***endo*-5**, and blue points denote the reaction between **2** and **3** in the presence of 400 mol % benzoic acid. Solid lines represent the best fit of the appropriate kinetic model to the experimental data (green = recognition-mediated, red = bimolecular).

rates of cycloadduct formation back to the same levels as the bimolecular reaction (r(***endo*-5**) = 4.32×10^{-5} mM s^{-1}, r(***exo*-5**) = 3.85×10^{-5} mM s^{-1}). This experiment demonstrates that recognition plays a pivotal role in the rate acceleration observed within the system. The benzoic acid is acting as a competitive inhibitor of the recognition-mediated reaction by virtue of the fact that it is capable of recognizing and binding to **3** and the amidopyridine recognition sites in ***endo*-5** and ***exo*-5**. Additionally, this experiment allows us to discount rate acceleration through adventitious general acid catalysis.

These experiments indicate clearly that the formation of the two cycloadducts, ***endo*-5** and ***exo*-5**, is recognition-mediated and template-directed. Nevertheless, two mechanistic possibilities still exist. Each cycloadduct, ***endo*-5** and ***exo*-5**, could function as an isolated self-replicator, autonomously self-propagating in the absence of synergism or inhibition with its diastereoisomer. Alternatively, each diastereoisomer could, in addition to self-replication, participate in the operation of parallel, interdependent subnetworks (hypercycle), sustained by collaborative catalysis, where proliferation of ***endo*-5** is catalyzed by itself *and* by ***exo*-5** and vice versa.

A key experiment is the demonstration that ***endo*-5** and ***exo*-5** are templates for their own formation. Accordingly, we performed experiments in which we added either 2.5 mM of ***endo*-5** or 2.3 mM of ***exo*-5** (approximately 10 mol %) to the reaction mixture ([**2**] = [**3**] = 25.0 mM). In these cases, the initial rates of formation of ***endo*-5** and ***exo*-5** (r(***endo*-5**) = 15.75×10^{-5} mM s^{-1} and r(***exo*-5**) = 14.82×10^{-5} mM s^{-1}) are increased by 3.8 and 5.6 times, respectively, compared to the corresponding bimolecular reactions (Figure 2, black points).

To establish the structural basis for the observed kinetic behavior[9] of this system, we evaluated the relative stability and structural compatibility within the homodimeric and heterodimeric assemblies. We performed a series of Monte Carlo conformational searches starting from a variety of individually minimized conformations of ***endo*-5**, ***exo*-5**, [***endo*-5·*endo*-5**], [***exo*-5·*exo*-5**], and [***endo*-5·*exo*-5**] utilizing the AMBER* force field and the GB/SA solvation model[10] for $CHCl_3$. Representative coconformers of the two homodimers, [***endo*-5·*endo*-5**] and [***exo*-5·*exo*-5**] are portrayed in Figure 3. The lowest energy conformation of monomeric

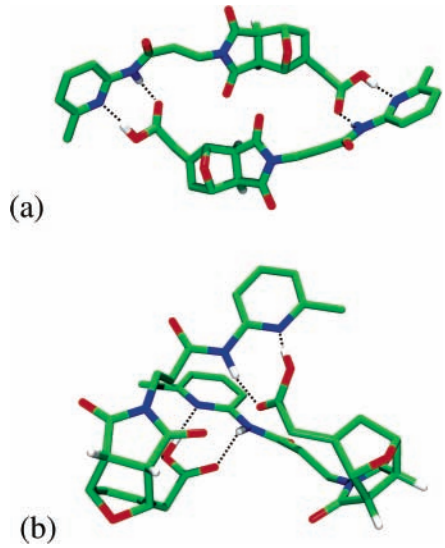

Figure 3. Stick representations of the calculated structures (AMBER*, GB/SA $CHCl_3$) of (a) ***exo*-5** and (b) ***endo*-5**. Carbon atoms are colored green, oxygen atoms are colored red, nitrogen atoms are colored blue, and hydrogen atoms are colored white. Some hydrogen atoms have been omitted for clarity. Hydrogen bonds are shown as dashed lines.

***exo*-5** is essentially linear with both recognition sites open. Severe internal strain impedes folding that would result in the intramolecular association of the amidopicoline and carboxylic acid recognition sites. The divergent recognition sites allow unhindered assembly of the catalytic termolecular

complex [**2**·**3**·***exo*-5**]. The optimal self-complementarity displayed by monomer ***exo*-5** prompted us to hypothesize that ***exo*-5** preorganizes precursors **2** and **3** for facile entrance into the *exo* transition state, generating duplex [***exo*-5**·***exo*-5**] and therefore accomplishing self-replication.

As a direct consequence of stereochemistry at the 6−5 ring junction, cycloadduct ***endo*-5** is intrinsically arched. Despite repeated attempts to bias the calculations toward intramolecularly bound structures by employing a closed starting conformation, cyclic conformations in which the recognition sites are associated intramolecularly are not represented among the 20 lowest energy conformations. Therefore, ***endo*-5** presents unoccupied recognition sites capable of complexing **2** and **3** and therefore templating its own formation by providing a facile pathway for reaction through the ***endo*-5** transition state.

From our calculations, it is evident that ***endo*-5** and ***exo*-5** are incompatible geometrically. This perception was validated by calculations that demonstrate that the heterodimeric complex [***endo*-5**·***exo*-5**] is incapable of maintaining two hydrogen bonds at each pair of recognition sites. Instead, the assembly collapses to coconformers in which only one of the two pairs of recognition sites is associated through two hydrogen bonds. It therefore seems likely that template ***endo*-5** (or ***exo*-5**) would be required to adopt an unrealistically strained conformation to align diene **2** and maleimide **3** in the correct relative orientations to enter the *exo* (or *endo*) transition state and thus accelerate the formation of its diastereoisomer by reciprocal catalysis. We therefore discount cross-catalysis as a major contributor to the rate acceleration observed in the case of ***endo*-5** and ***exo*-5**.

Seeking additional experimental evidence to support our computational studies, we turned to ^{1}H NMR spectroscopy to probe for the presence of the homo- or heterodimeric structures in solution. We therefore recorded 500 MHz ^{1}H gradient NOE experiments on equimolar solutions of ***endo*-5** and ***exo*-5** ([***endo*-5**] = [***exo*-5**] = 5 mM) in $CDCl_3$. No NOEs arising from intermolecular contacts that could be assigned to the heterodimeric assembly [***endo*-5**·***exo*-5**] were observed in any of these experiments. By contrast, there was significant evidence for the presence of both homodimeric species, [***endo*-5**·***endo*-5**] and [***exo*-5**·***exo*-5**], in solution under the experimental conditions.

To gain more accurate insight into the level of rate acceleration achieved by the template in catalyzing its own formation, we performed a series of kinetic simulations of the experimental data and simultaneous fitting to the appropriate kinetic model. Full details of these calculations are given in the Supporting Information. Excellent fits ($R < 1\%$) to the experimental data (solid lines, Figure 2) are obtained using a model in which autocatalytic pathways are the only active template-directed routes to cycloadducts ***endo*-5** and ***exo*-5**. The model predicts that ***exo*-5** generates a kinetic effective molarity of 1.10 M for its own formation within the [**2**·**3**·***exo*-5**] complex. Similarly, the model predicts that ***endo*-5** generates a kinetic effective molarity of 0.56 M for its own formation within the [**2**·**3**·***endo*-5**] complex. Contributions from reaction through a binary reactive complex, although included in the model, are, like cross-catalysis, not required to explain the observed data. These effective molarities are competitive[11] with the best reported for Diels−Alder reactions in small-molecule recognition-based systems.

We have demonstrated that it is possible to design a simple synthetic system that is capable of supporting two parallel replication pathways that operate independently. These two replication pathways are of similar efficiency, and therefore the two replicators are capable of coexisting despite their autocatalytic behavior. The results presented here demonstrate that even simple synthetic systems are capable of displaying complex kinetic behavior. This observation, coupled with a detailed knowledge of the relationship between structure and reactivity, bodes well for the design and construction of more structurally and kinetically complex systems.

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Supporting Information Available: Spectroscopic data for selected compounds, details of nOe data obtained for the ***exo*-5** and ***endo*-5** homodimers, and full description and results of kinetic simulation and fitting on the experimental rate data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) All of the experiments reported here were performed using racemic mixtures of ***exo*-5** and ***endo*-5**. We recognize that a more complex hypercyclic network involving individual enantiomers could exist in this system. However, since we have been unable to resolve the cycloadducts, we prefer to base our analysis on a model we can test experimentally.

(10) Molecular mechanics calculations were carried out using the AMBER* force field as implemented in Macromodel (version 7.1; Schrödinger, Inc.: Portland, OR, 2000) running on a Linux workstation.

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