Template Synthesis

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Design and Implementation of a Highly Selective Minimal Self-Replicating System**

*Eleftherios Kassianidis and Douglas Philp**

The development and deployment of self-replicating[1] molecular architectures[2] can potentially revolutionize the fabrication of materials on the nanometer scale. The emergence of protocols based on molecular replication will deliver synthetic machinery[3] that is capable of directing its own synthesis and cooperating with other similar systems to create an organized hierarchy. Within this broad objective, the development of efficient protocols that allow self-replication, self-organization, and evolution[4] within synthetic supramolecular assemblies is essential. This approach to predetermined dynamic behavior has been termed "systems chemistry" by von Kiedrowski and co-workers.[5] Ultimately, we wish to exploit replicating systems in the construction, selection,[6] and amplification[7] of large molecular and supramolecular assemblies.

The minimal self-replicator model[8] (Figure 1) provides the framework for our studies. Paul and Joyce highlighted[9] three parameters within this model that must be optimized to ensure an efficient system: minimization of product inhibition arising from a stable **T**·**T** complex; the suppression of the reaction[10] through **A**·**B** to form $\mathbf{T}_{inactive}$, which is inert catalytically; and the catalytic efficiency within the key ternary complex **A**·**B**·**T**. Under ideal conditions, template **T** presents its recognition sites in the correct orientation, assembles **A** and **B**, and then connects these two molecules to form an exact copy of itself by transmitting structural information to the forming template. This process completes an autocatalytic cycle. It is useful to recognize that, unlike traditional catalysts, there is no requirement for **T** to achieve particularly high turnover numbers as the inherently nonlinear kinetics should achieve amplification of the template once autocatalysis has become established. Additionally, as long as the duplex **T**·**T** is not excessively stable, propagation of the template **T** is exponential. Despite concerted efforts, there are relatively few reports of synthetic self-replicating systems.[5,11]

[*] Dr. E. Kassianidis, Dr. D. Philp
EaStCHEM and Centre for Biomolecular Sciences
University of St Andrews
North Haugh, St Andrews, Fife KY16 9ST (UK)
Fax: (+44) 1334-463-808
E-mail: d.philp@st-andrews.ac.uk

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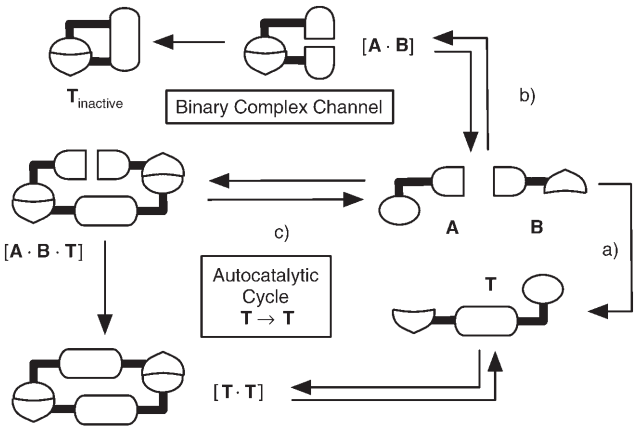
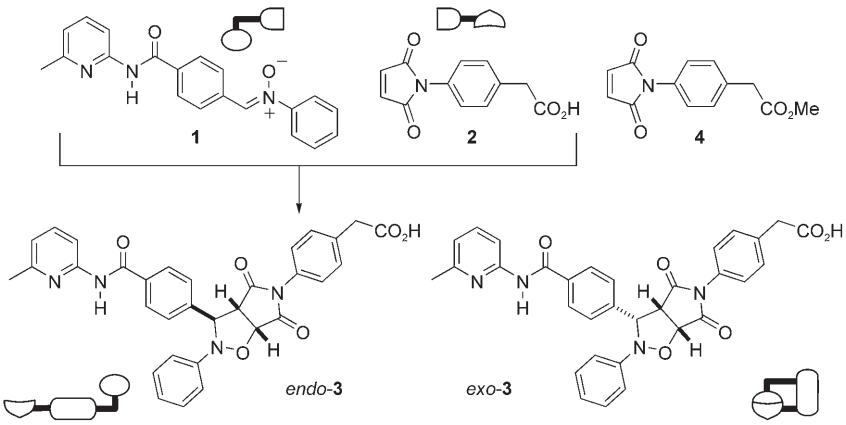


Figure 1. The minimal model of self-replication: Reagents **A** and **B** can react through three pathways: a) An uncatalyzed bimolecular reaction between **A** and **B** to give template **T**; b) a recognition-mediated reaction through the binary complex **A**·**B**; c) a recognition-mediated autocatalytic cycle through the ternary complex **A**·**B**·**T**.

In the context of our long-term goals, we must demonstrate that it is possible to exploit replication to amplify a single structure from a mixture based on its ability to guide its own formation through recognition processes, even when inherently unselective chemical reactions are used to covalently link the building blocks together. The reaction between an *N*-aryl nitrone and a maleimide is suitable for incorporation in a modular self-replicating system. The reaction proceeds readily at room temperature, and the rate and diastereoselectivity are almost immune to electronic substituent effects and adventitious catalysis by Brønsted acids. The two products (*endo* and *exo*) provide the means of exposing the efficiency of information transfer in any replicating system as the reaction is metronomic: the *endo*/*exo* ratio is always close to 3:1 in the absence of effects that arise from molecular recognition. Previous studies by us[12] demonstrated that this reaction can be incorporated into a replicating system, but that the levels of amplification achieved are poor.

We wished to develop a general protocol for optimizing the performance of replicating systems based on computational methods. Therefore, we identified **1** and **2** (Scheme 1) as suitable precursors for a successful replicating template by screening[13] a series of compounds computationally. We performed electronic structure calculations (see the Supporting Information for details) at the B3LYP/6-31G(d) level of theory on the two diastereoisomers *endo*-**3** and *exo*-**3** and the duplex *endo*-**3**·*endo*-**3**. These calculated structures reveal that *endo*-**3** has an open structure in which the two recognition sites are freely available to interact with other complementary species in solution (**T** in Figure 1). By contrast, the structure of *exo*-**3** is folded such that the recognition sites are placed in proximity to each other, thus rendering this product inert in a catalytic sense ($\mathbf{T}_{inactive}$ in Figure 1). Additionally, the calculated structure of the *endo*-**3**·*endo*-**3** duplex reveals a head-to-tail dimer whose assembly is driven by the self-complementarity of the template. We therefore expected that the *endo*-**3** template should accommodate the transition state that leads



Scheme 1. Nitrone **1** and maleimide **2** can react to form two diastereoisomeric cycloadducts *exo*-**3** (an open template) and *endo*-**3** (a closed template). Schematic representations from Figure 1 are included to highlight the role of these components in the design of the replicating system.

to *endo*-**3** readily. This expectation proved correct: Calculations revealed the transition state **1**·**2**·*endo*-**3**‡ was readily accessible and similar in geometry to that calculated for the bimolecular reaction. In summary, our computational studies indicate that the formation of *endo*-**3** by reaction through a binary complex is impossible; however, *endo*-**3** should be capable of templating its own formation from nitrone **1** and maleimide **2** through the transition state **1**·**2**·*endo*-**3**‡. Our expectation was therefore that we had designed a synthetic system that should be capable of amplifying *endo*-**3** over *exo*-**3** through autocatalysis.

Template precursors **1** and **2**, together with the control compound **4**, in which the carboxylic acid recognition site is blocked, were synthesized by using standard methods. The kinetic behavior of the system was studied by measuring the kinetics of the reaction between **1** and **2** (or **4**) in a solution of $CDCl_3$ at various temperatures between −10 and 35 °C. The time course of the reactions was evaluated by ^{1}H NMR spectroscopic analysis (500 MHz), which monitored the disappearance of the resonance that arises from the maleimide protons present in **2** (or **4**) in the region $\delta = 6.70$–7.00 ppm and the simultaneous appearance of resonances that arise from the *endo* and *exo* cycloadducts in the region $\delta =$ 3.60–5.80 ppm. Profiles of concentration versus time for the formation of the two cycloadducts were constructed by deconvolution of these resonances. No decomposition or additional reaction products were detected in any of the experiments and the reaction was shown to be under kinetic control.[14] Experiments conducted at starting concentrations of **1** and **2** of 25 mM in $CDCl_3$ revealed that the recognition-mediated reactions were uniformly fast, reaching more than 85 % overall conversion ([*endo*] + [*exo*]) at all temperatures after 16 h, and, in all cases, the diastereoselectivity was greater than 33:1 in favor of *endo*-**3**. By contrast, in the control reaction between **1** and **4**, in which recognition cannot play a role, overall conversion after 16 h was around 30 % at 35 °C and only 9 % at −10 °C and, in all cases, the diastereoselectivity was only 3:1 in favor of *endo*-**3**. Clearly, the presence of recognition has a dramatic effect in this system.

Accordingly, we undertook a detailed kinetic analysis to demonstrate that the effects mediated by molecular recognition were indeed a result of the designed replication of *endo*-**3**. In the following discussion, we focus on the results obtained at −10 °C, as this temperature was the most convenient for measuring the reaction kinetics. The measured maximum rates[15] of reaction are summarized in Table 1. It is clear from these data that the bimolecular reaction between **1** and **4** (Table 1, entry 1) is slow and unselective. By contrast, the measured rates change significantly (Table 1, entry 2) when recognition is allowed to play a role in the reaction: the formation of *endo*-**3** is accelerated 13-fold. The small rate enhancement in the formation of *exo*-**3** is consistent with the computational results that indicate that reaction through a very weak binary complex may be possible. The rate enhancement in the formation of *endo*-**3** coupled with the observation of a sigmoidal rate profile (Figure 2), characteristic of autocatalysis, warranted further investigation. The role of hydrogen bonding in the formation of *endo*-**3** and *exo*-**3** could be demonstrated readily by the introduction of a competitive inhibitor. The association between **2** and any amidopyridine-bearing species present in the reaction mixture, including the template *endo*-**3**, can be disrupted through the addition of two equivalents of benzoic acid. Reaction between **1** and **2** in the presence of benzoic acid ([benzoic acid] = 50 mM) results in a significant decrease both in the rate of reaction and the diastereoselectivity (Table 1, entry 3). The final *endo*/*exo* ratio drops from 115:1 to only 17:1. These results demonstrate that the formation of *endo*-**3** is recognition-mediated.

Table 1: Maximum observed rates of reaction.[a]

Entry	Conditions	Maximum rate [nM s^{-1}]	
		endo-**3**	*exo*-**3**
1	[**1**] = [**4**] = 25 mM	39	13
2	[**1**] = [**2**] = 25 mM	528	21
3	[**1**] = [**2**] = 25 mM [benzoic acid] = 50 mM	105	17
4	[**1**] = [**2**] = 25 mM [*endo*-**3**] = 2.5 mM	561	< 1

[a] Maximum rates measured in $CDCl_3$ at −10 °C by ^{1}H NMR spectroscopic analysis.

A crucial experiment is the demonstration that *endo*-**3** is capable of accelerating its own formation. The injection of substoichiometric amounts of *endo*-**3** at the start of the reaction between **1** and **2** should result in an increase in the initial rate of formation of *endo*-**3**. Accordingly, we performed the reaction between **1** and **2** under identical conditions to those described previously, however, the reaction mixture

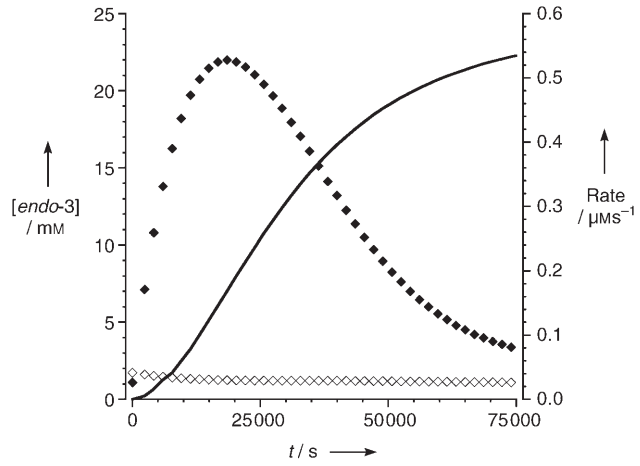


Figure 2. Variation of reaction rate for the formation of *endo*-**3** (◆, right axis) with time. The formation of the *endo* diastereoisomer in the control reaction between **1** and **4** is shown for comparison (◇, right axis). Experimental concentration–time data for *endo*-**3** (——, left axis) is also shown for comparison.

also contained 10 mol% of *endo*-**3**. The results of this experiment confirm the ability of *endo*-**3** to template its own formation (Table 1, entry 4). The maximum rate of formation of *endo*-**3** is now 14 times faster than the control and, pleasingly, almost exactly that observed for the reaction in the absence of the preformed template. This observation suggests that the maximum autocatalytic rate is reached immediately ($t=0$) on addition of this small amount of template. The *endo*/*exo* ratio is now at least 250:1,[16] compared with 115:1 in the absence of added *endo*-**3**. By contrast, the addition of 10 mol% of *exo*-**3** to the reaction mixture has no effect on the rate profile. From these observations, we conclude that the *exo*-**3** cycloadduct is completely incapable of competing with the efficient replication profile of *endo*-**3**. Therefore, it is this template that is amplified selectively through its monopolization of the precursors **1** and **2**.

The curve for the reaction rate versus time (Figure 2) confirms the autocatalytic behavior of *endo*-**3**, thus revealing that the reaction rate steadily increases, reaches a maximum, and then falls away. It is evident from Figure 2 that the maximal autocatalytic velocity is achieved at around five hours after the start of the reaction at a template concentration of around 7 mM. In fact, the maximum autocatalytic velocity is achieved in the time region in which $0.1<\rho<1.0$, where $\rho=$[template]/[precursors]. For our system, this region lies between 150 and 500 minutes, which corresponds to a concentration of *endo*-**3** between 2.5 and 12.5 mM. It is clear from this analysis of the kinetic data that autocatalyst saturation occurs at $t\approx10000$ s. The continuous increase of [*endo*-**3**] (and in turn the ρ value) during the course of the reaction does not increase reaction velocity further: within the concentration range 2.5–12.5 mM for *endo*-**3**, only a further 6% increase in reaction velocity is observed, whereas reaction velocity is considerably diminished at $\rho=1.0$ and beyond. These considerations explain the result of the template-injection experiment described above: employing an initial condition at which $\rho=0.1$ results in the system operating at the limit of its autocatalytic capacity from the start of the reaction. Indeed, exposure of reaction mixtures consisting of **1** and **2** to increasing initial concentrations of preformed *endo*-**3** (up to 7.5 mM or 30 mol%) results in rate profiles identical to those obtained when only 10 mol% of preformed *endo*-**3** is added, thus adding more template has no effect because the system is already operating at the limit of its autocatalytic capacity.

Ideally, to completely eradicate the formation of *exo*-**3** within this system, we must ensure that the contributions from the background bimolecular and binary complex modes of reactivity are removed. This objective cannot be achieved in this system by template injection. However, this goal can be accomplished by reducing the concentration of the reagents at the start of the reaction. Thus, employing initial concentrations of **1** and **2** of 15 mM results in an *endo*-**3**/*exo*-**3** ratio of 135:1, and employing initial concentrations of **1** and **2** of 5 mM results in an *endo*-**3**/*exo*-**3** ratio of more than 250:1; the concentration of *exo*-**3** is now below our limit of detection. On the basis of all of these observations, we believe that the formation of *endo*-**3** occurs principally through the iterative operation of an autocatalytic, self-replication cycle, which revolves around the assembly of the hydrogen-bonded ternary complex **1**·**2**·*endo*-**3**. In accordance with our electronic structure calculations, this assembly is capable of guiding the reaction of the nitrone and maleimide solely through the *endo* transition state, thereby allowing *endo*-**3** to create a new copy of itself.

We probed the stability of the *endo*-**3**·*endo*-**3** duplex to understand the apparent efficiency of autocatalysis in this system fully. The association constant (K_a) for the formation of the complex **1**·phenylacetic acid, a model for the binary associations in the system, was determined to be $1150\,M^{-1}$ at $-10\,^{\circ}C$ in $CDCl_3$ by using ^1H NMR titration methodology. Assuming simple additivity of interactions, we might therefore expect the *endo*-**3**·*endo*-**3** duplex to have an association constant in the region of $1.3\times10^6\,M^{-1}$.[17] We were unable, however, to directly measure the stability of the *endo*-**3**·*endo*-**3** duplex by ^1H NMR (500 MHz) dilution experiments as the ^1H NMR spectrum of *endo*-**3** showed no apparent dependence on concentration (100 μM < [*endo*-**3**] < 25 mM) at $-10\,^{\circ}C$ in $CDCl_3$). However, given the kinetic data, we reasoned that it should be possible to verify the relative stability of the *endo*-**3**·*endo*-**3** duplex through the simulation and fitting of the experimental datasets to an appropriate kinetic model (full details of this procedure are given in the Supporting Information). Our kinetic model requires that *exo*-**3** is formed exclusively through the bimolecular reaction channel and that *endo*-**3** is formed through the confluence of the bimolecular pathway and the autocatalytic self-replicating pathway. Estimates of the rate constant for the bimolecular reaction between **1** and **2** to form *exo*-**3** and *endo*-**3** were derived from experimental data for the reaction between **1** and methyl ester **4** and were used to describe all bimolecular processes that lead to the *exo* and *endo* cycloadducts, respectively. The association constant ($K_a=1150\,M^{-1}$) for the complex **1**·phenylacetic acid was used as a model for all binary complexes and the product complexes *exo*-**3**·*exo*-**3** and *exo*-

3·*endo*-**3**. By using these fixed parameters and simplex optimization of the association constant for *endo*-**3**·*endo*-**3** and the rate constant for the reaction in the ternary complex **1**·**2**·*endo*-**3** to form *endo*-**3**·*endo*-**3**, excellent fits of the experimental data to this model were obtained ($R = 0.74\,\%$; see the Supporting Information for details). The optimized value of the association constant for [*endo*-**3**·*endo*-**3**] is $4.7 \times 10^6\,\text{M}^{-1}$, close to our original estimate. The optimized rate constant for the formation of *endo*-**3** within ternary complex [**1**·**2**·*endo*-**3**] is $1.22 \times 10^{-3}\,\text{s}^{-1}$, which corresponds to an effective molarity of just over 20 M.

It is clear from the results of the kinetic simulation and fitting of the experimental data that the major pathway for the formation of *exo*-**3** is simply the bimolecular reaction between **1** and **2**. The dominant pathway for the formation of *endo*-**3** is clearly a reaction through the ternary complex **1**·**2**·*endo*-**3**, which has a rather high effective molarity ($\approx$23 M) for such a simple system.[18] The **1**·**2**·*endo*-**3** complex drives the formation of *endo*-**3** almost exclusively, and the observed maximum rate at around 20000 s (Figure 2) corresponds to the maximum concentration of **1**·**2**·*endo*-**3** (420 μM). The interplay between this catalytic prowess and the high stability of the *endo*-**3**·*endo*-**3** duplex is intriguing. Despite the fact that the concentration of free *endo*-**3** never rises above 5 μM during the reaction, this structure is still capable of amplifying itself effectively to the exclusion of *exo*-**3**. The simulation and fitting of the data allows us to explore the effect of the stability of the *endo*-**3**·*endo*-**3** duplex on the rate profile of the system. The calculated reaction profile is altered dramatically by a modest reduction in the association constant for *endo*-**3**·*endo*-**3** from 10^6 to $10^5\,\text{M}^{-1}$. This change results in the curve of rate versus time becoming sharper and the maximum rate of formation of *endo*-**3** increasing. Although we cannot test this hypothesis directly at −10 °C, increasing the temperature[19] will lower the K_a value for *endo*-**3**·*endo*-**3**, and the expected effect on the curve of rate versus time is evident as we increase the reaction temperature from −10 to 10 °C (Figure 3).

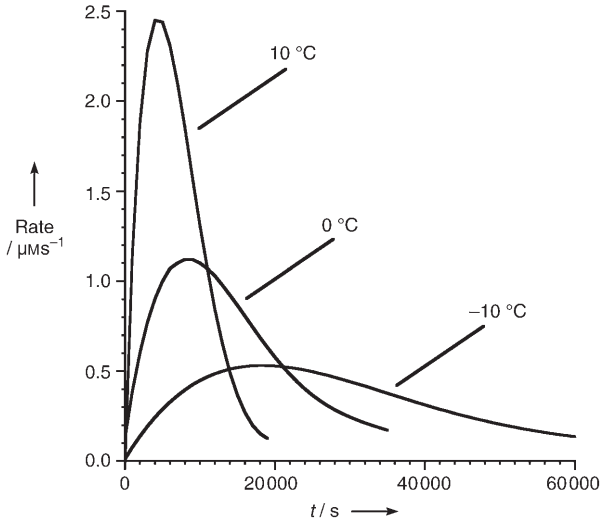


Figure 3. Observed rate versus time profiles for the reaction between **1** and **2** in $CDCl_3$ at −10, 0, and 10 °C.

Although conceptually simple, minimal self-replicating systems should require the optimization of the rate acceleration, or effective molarity, achieved by the ternary complex, the stability of the ternary complex, and the instability of the product duplex for efficient operation. We have demonstrated herein that it is possible to selectively amplify one of two possible products from a reaction mixture using a replication strategy in which only the first of these three parameters has been highly optimized through the logical application of computational methods. Therefore, although the stability of the catalytic ternary complex and product duplex are important, it is nevertheless possible to design and implement a synthetic system that is capable of highly selective amplification by focusing on templates that possess a high level of complementarity to transition states. We believe that the development of new replicating systems can be driven by aggressive optimization of the approach described herein.

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[13] We screened a series of compounds in which *ortho*-, *meta*-, or *para*-disubstituted benzene rings connected the recognition sites to the reactive sites in both the nitrone and the maleimide building blocks of the prospective template. We calculated (AMBER* forcefield, GB/SA $CHCl_3$ solvation model, Macromodel, version 7.1, Schrodinger Inc., USA, 2000) the minimum energy conformations of the both the *endo* and *exo* cycloadducts from all of the possible combinations of these six building blocks. Of the possible cycloadducts, only one combination, *endo*-**3** from the reaction between **1** with **2**, had the desired open template structure required for replication and warranted further investigation.

[14] The ratio of *endo*-**3** and *exo*-**3** did not change upon heating the reaction mixtures to 60 °C; additionally, heating *endo*-**3** and *exo*-**3** with *N*-phenylmaleimide did not result in any crossover products being formed. These results suggest that no thermodynamic equilibration between *endo* and *exo* cycloadducts occurs within the temperature range and on the timescale of our investigations.

[15] Maximum rates (velocities) of reaction were calculated by determining the largest value of the first derivative of the function that describes the concentration–time profile for each reaction; for bimolecular reactions, this metric is equivalent to the initial rate; for autocatalytic reactions, this represents the point of inflection of the sigmoidal curve.

[16] On the basis of the observed signal-to-noise ratios in the ^{1}H NMR spectra (500 MHz) of the reaction mixtures, we estimate that our limit of detection for *exo*-**3** is 100 μM. This analysis places a lower limit of 250:1 on the *endo*/*exo* selectivity generated by the recognition-mediated reaction.

[17] The simple additivity calculation described herein will underestimate the stability of the duplex somewhat (C. A. Hunter, *Angew. Chem.* **2004**, *116*, 5424–5539; *Angew. Chem. Int. Ed.* **2004**, *43*, 5310–5324); however, it gives a reasonable first estimate for the K_a value of the duplex, which is then refined by iterative fitting.

[18] The value of 22.7 M is higher than many simple synthetic systems that exploit recognition processes to achieve rate accelerations in cycloaddition reactions; for some comparison data, see: R. Cacciapaglia, S. Di Stefano, L. Mandolini, *Acc. Chem. Res.* **2004**, *37*, 113–122. The effective molarity observed in the system reported herein is of the same order of magnitude as that observed (Ref. [5]) by von Kiedrowski and co-workers in an almost exponentially replicating system based on the Diels–Alder reaction.

[19] Raising the temperature will diminish the K_a value for the product duplex by increasing the magnitude of the $T\Delta S$ term in the free energy of binding. We recognize that the change in temperature will also affect the stability of other complexes in solution; however, as the behavior of the system is sensitive to the product duplex K_a value, we wished to use these means to explore this effect qualitatively. A detailed analysis of the effect of temperature on this system will be reported elsewhere. An alternative method of achieving the same effect is to change the solvent polarity; however, in practice, it is difficult to add a polar solvent without destroying recognition between the various components within the system completely.

