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Transfer of Stereochemical Information in a Minimal Self-Replicating System

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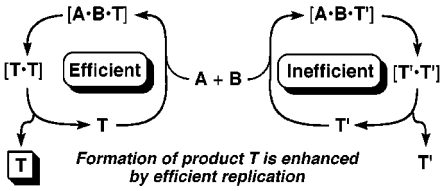
ABSTRACT



The rational design, synthesis, and characterization of a minimal self-replicating system based on a 1,3-dipolar cycloaddition between a nitrone and a maleimide is presented. The importance of molecular recognition in this system is demonstrated using a competitive inhibitor. Doping experiments demonstrate that only one of the two diastereoisomeric products of the cycloaddition reaction is capable of acting as an efficient template for its own formation, accelerating the reaction between the nitrone and maleimide and controlling the stereochemical outcome of the reaction.

Examples of chemical systems capable of templating and catalyzing their own synthesis—self-replicating systems—have begun to appear[1] in the chemical literature over the past 10 years. For the biologist, these systems represent a link with the origins of life; their study can shed light on prebiotic chemical evolution.[2] However, for the synthetic chemist, these systems represent[3] the ultimate synthetic machine, capable of templating the production of a large number of perfect copies of themselves from a single original molecule. In the present context, we will be concerned only with systems that can reproduce themselves efficiently without any requirement for external cofactors, i.e., nonenzymatic replication. Almost all of the examples of nonenzymatic self-replicators that have appeared in the literature 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 **A** and **B** to give **T**. However, a requirement of the minimal model is that **A** and **B** bear complemetary recognition sites. Thus, **A** and **B** can associate with each other in a binary complex, [**A·B**]. The presence of this complex offers a second reaction channel, the [**A·B**] complex channel,[5] in which the reaction between **A** and **B** is pseudointramolecular. The third reaction channel is the *autocatalytic* cycle. Here, **A** and **B** bind reversibly to **T** to form a catalytic ternary complex [**A·B·T**] in which the reaction between **A** and **B** is also rendered pseudointramolecular. Bond formation occurs between **A** and **B** to give

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(1) For reviews on self-replicating systems, see: (a) Robertson, A.; Sinclair, A. J.; Philp, D. *Chem. Soc. Rev.* **2000**, *29*, 141. (b) Winter, E. A.; Rebek, J., Jr. *Acta Chem. Scand.* **1996**, *50*, 469. (c) Orgel, L. E. *Nature* **1992**, *358*, 203.

(2) Bridson, P. K.; Orgel, L. E. *J. Mol. Biol.* **1980**, *144*, 567. (b) Kuhn, H.; Waser, J. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 500. (c) Inoue, T.; Orgel, L. E. *Science (Washington, D.C.)* **1983**, *219*, 859. (d) Cech, T. R. *Sci. Am.* **1986**, *255*(5), 76. (e) Joyce, G. F. *Nature (London)* **1989**, *338*, 217. (f) Cairns-Smith, A. G. In *Genetic Takeover*; Cairns-Smith, A. G., Hartman, H., Eds.; CUP: Cambridge, 1982.

(3) Philp, D.; Stoddart, J. F. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1154. (b) Lindsey, J. S. *New J. Chem.* **1991**, *15*, 153. (c) Whitesides, G. M.; Mathias, J. P.; Seto, C. T. *Science* **1991**, *254*, 1312.

(4) Von Kiedrowski, G. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 932.

(5) Tecilla, P.; Hamilton, A. D. *J. Chem. Soc., Chem. Commun.* **1990**, 1232. (b) Robertson, A.; Philp, D.; Spencer, N. *Tetrahedron* **1999**, *55*, 11365. (c) Bennes, R. M.; Philp, D.; Spencer, N.; Kariuki, B. M.; Harris, K. D. M. *Org. Lett.* **1999**, *1*, 1087.

(6) Von Kiedrowski, G. *Biorg. Chem. Front.* **1993**, *3*, 113.

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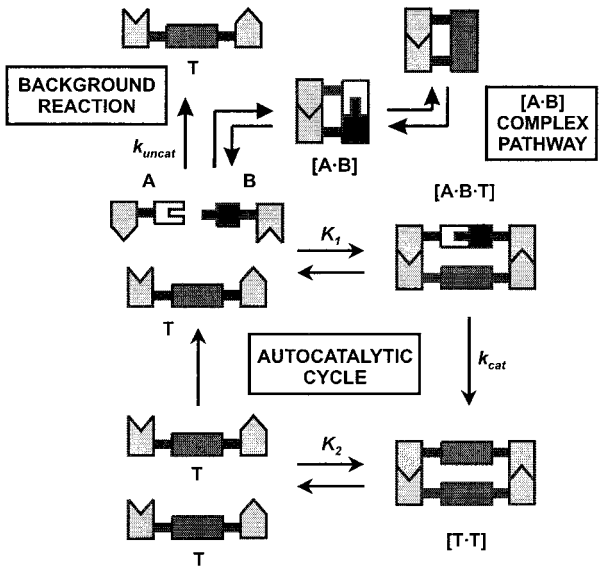


Figure 1. The minimal model for self-replication.

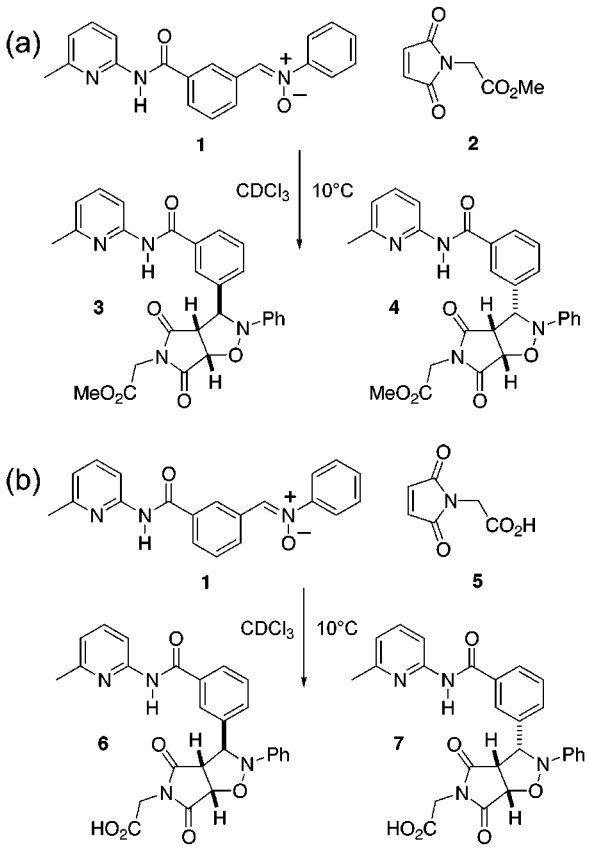
the product duplex [**T**·**T**], which then dissociates to return two molecules of **T** to the start of the autocatalytic cycle.

Thus, the reaction is autocatalytic in **T** provided K_2 is small. If this condition is met and k_{uncat} is also small, exponential growth of the concentration of **T** is predicted.[6] Thus, we should be able to exploit this behavior to achieve amplification of information, either regio- or stereochemical, present in **T**. Here, we report the design and synthesis of a system (Scheme 1) that exploits[7] the nonlinear kinetic behavior of replicating systems to achieve amplification of the relative stereochemistry of the outcome of a cycloaddition reaction between a nitrone and a maleimide. The replicating behavior of this system is demonstrated through a series of control experiments, and preliminary kinetic modeling is reported.

The reaction between *trans*-nitrone[8] **1** and maleimide ester **2** (Scheme 1a), undertaken at 10 °C at a concentration of 25 mM in $CDCl_3$, is reasonably slow, leading to 17% overall conversion to a diastereoisomeric mixture of the isoxazolidines **3** and **4** over 16 h. The reaction is also reasonably unselective with a 4:1 ratio, in favor of the *trans*-isoxazolidine **3**.[9] Kinetic simulation and fitting of the rate profile obtained for this reaction (Figure 2) gave a good fit[10] to a simple bimolecular model.

To investigate the effect that recognition-mediated processes might have upon this reaction, nitrone **1** was reacted with maleimide acid **5**, under identical conditions to those

Scheme 1



described above to afford the corresponding isoxazolidines **6** and **7**. During this reaction, nitrone **1**, bearing an amidopicoline, and maleimide **5**, bearing a carboxylic acid, are capable of associating via complementary hydrogen-bonding interactions[11] with the templates **6** and **7** to form the potentially catalytic complexes[12] [**1**·**5**·**6**] and [**1**·**5**·**7**]. From the results obtained (Figure 2) it is clear that the introduction

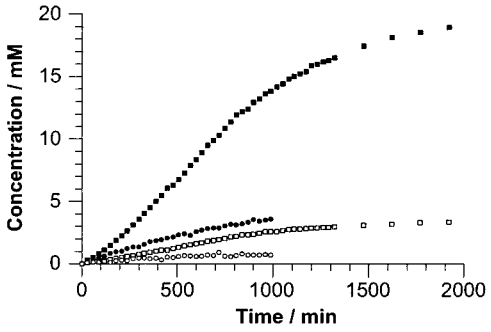


Figure 2. Rate profiles obtained for the reaction of **1** and **2** and the reaction between **1** and **5** at 25 mM, 10 °C, in $CDCl_3$. The formation of isoxazolidine **3** is shown as filled circles and that of isoxazolidine **4** as open circles. Data for the formation of **6** are shown as filled squares and for **7** as open squares. For clarity, error bars are omitted from the graphs; however, errors in concentration are estimated to be ±4%.

(7) Soai, K.; Shibata, T.; Sato, I. *Acc. Chem. Res.* **2000**, *33*, 382.

(8) The configuration about the C=N bond was determined using gradient NOE studies. Nitrone **1** was shown to possess a *Z* configuration.

(9) For a detailed explanation of the assignment of the stereochemistry of **3**, **4**, **6**, and **7**, see the Supporting Information. See also: Iwakura, Y.; Uno, K.; Hong, S.-J.; Hongu, T. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 192.

(10) Fitting of the experimental data was accomplished using SimFit 32 (Sievers, D.; von Kiedrowski, G. *Chem. Eur. J.* **1998**, *4*, 629). Best fit values of the bimolecular rate constants for the conversion of **1** and **2** to **3** and **4** were 12.8×10^{-5} M^{-1} s^{-1} and 2.98×10^{-5} M^{-1} s^{-1}, respectively.

of the recognition motif has significantly increased the rate of the reaction. Also, the classic sigmoidal-shaped curve, evident in particular for the formation of the major, *trans*-isoxazolidine **6**, is indicative of a self-replicating system.

To demonstrate that the formation of product from the reaction of nitrone **1** and maleimide **5** is indeed a result of the operation of a self-replicating system, it was necessary to demonstrate that this system possessed certain properties that are characteristic of replicating systems.

During the progress of a self-replicating reaction, the formation of product, **T**, during the initial stages of the reaction is primarily via a simple uncatalyzed bimolecular pathway. However, once the product in solution reaches a critical concentration, then the autocatalytic cycle may begin to operate. Therefore, the presence of presynthesized template, **T**, at the beginning of the reaction ($t = 0$) should result in the experimentally observable loss of the initial lag period in the rate profile for the reaction. To demonstrate this effect, nitrone **1** and maleimide **5** were reacted under conditions identical to those described previously, but with the addition of either 10 mol % or 40 mol % of added template **6**. The results of this experiment (Figure 3) clearly show the disappearance of the initial lag period, providing evidence for the existence of a self-replicating, autocatalytic system. Furthermore, the addition of presynthesized product at $t = 0$ also leads to the enhanced formation of **6** and *not* **7**, resulting in an improved product ratio of 9:1, when compared to the uncatalyzed reaction between nitrone **1** and maleimide ester **2**. This clearly illustrates that the presence of the template facilitates the reaction between **1** and **5**. Further, the selective enhancement of the rate of formation of the major cycloadduct, *trans*-isoxazolidine **6**, by the addition of presynthesized template **6** at the beginning of the reaction indicates that template **6** is transmitting its stereochemical information effectively to the forming template copy within the [**1**·**5**·**6**] complex. Hence, we conclude[13] that the *trans*-isoxazolidine is acting as a selfish autocatalyst, enhancing the rate of formation of itself but not the corresponding diastereoisomer **7**. By contrast, the addition of presynthesized template **7** at the beginning of the reaction has no effect on the rate of production of **6** or **7**, indicating that this diastereoisomer is inactive in an autocatalytic and in a crosscatalytic sense.

The efficient operation of a self-replicating system hinges on the reversible binding events that occur during the autocatalytic cycle. If this crucial factor is interfered with, to render the binding events inefficient or nonexistent, then the autocatalytic cycle is unable to operate or may only do so with minimal efficiency.

Accordingly, the reaction between nitrone **1** and maleimide **5** was performed at 10 °C at a concentration of 25 mM in $CDCl_3$ in the presence of benzoic acid at a concentration of 100 mM. Benzoic acid acts as a competitive inhibitor, binding to the amidopicoline unit present in nitrone **1**. The rate profile (Figure 4) obtained for the formation of **6** and **7** under these conditions shows a significant decrease in both the rate of reaction and the selectivity. The sigmoidal curve, indicative of a self-replicating system and observed in the absence of a competitive binding agent, completely disappears upon addition of benzoic acid. This clearly demonstrates that the reaction between **1** and **5** is indeed recognition-mediated.

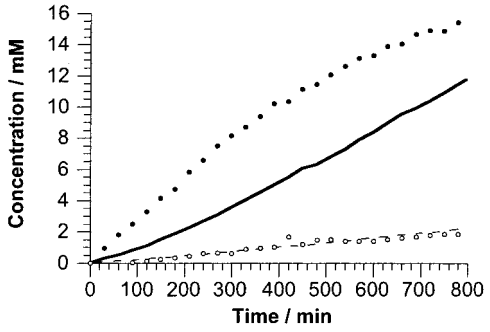


Figure 3. Rate profile obtained for the reaction of **1** and **5** at 25 mM together with presynthesized template **6** at 40 mol %, at 10 °C, in $CDCl_3$. The formation of **6** is shown as filled circles and of **7** as open circles. Experimental data for the reaction between **1** and **5** to give **6** (solid line) and **7** (dashed line) in the absence of added template are also given for comparison. For clarity, error bars are omitted from the graphs; however, errors in concentration are estimated to be ±4%.

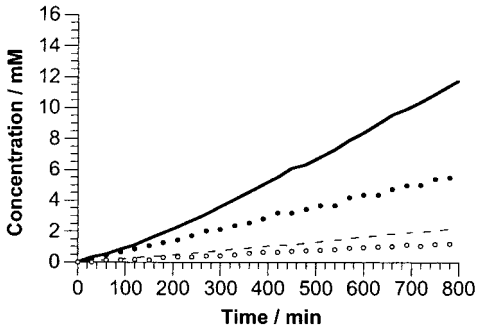


Figure 4. Rate profile obtained for the reaction of **1** and **5** at 25 mM in the presence of benzoic acid at 100 mM, at 10 °C, in $CDCl_3$. The formation of **6** is shown as filled circles and of **7** as open circles. Experimental data for the reaction between nitrone **1** and maleimide **5** to give **6** (solid line) and **7** (dashed line) in the absence of benzoic acid are shown for comparison. For clarity, error bars are omitted from the graphs; however, errors in concentration are estimated to be ±4%.

(11) Garcia-Tellado, F.; Goswami, S.; Chang, S.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1990**, *112*, 7393. (b) Yang, J.; Fan, E.; Geib, S. J.; Hamilton, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 369.

(12) Molecular modeling representations of the entire autocatalytic cycle are provided in the Supporting Information.

(13) It is clear from the experimental results presented here that **6** exerts a significant level of stereocontrol on the reaction between **1** and **5** in the [**1**·**5**·**6**] complex. However, the details of this process at a supramolecular level are likely to be complex and are currently under detailed investigation in our laboratory.

The efficient operation of the autocatalytic cycle hinges on the effective dissociation of the product duplex [**T·T**] in the autocatalytic cycle. If this duplex is too stable, no new template is returned to solution and the autocatalysis fails.

Von Kiedrowski has introduced a simple model to describe this behavior[6] (Figure 5). In this minimal model, the

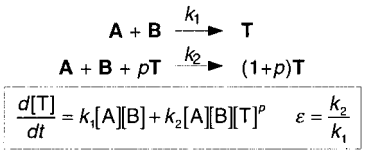


Figure 5. Minimal kinetic model for replication. For a detailed explanation of this model, see ref 6.

parameter p describes the autocatalytic behavior of the system. A value of p that is 0.5 denotes the fact that the replicating system obeys the square root law,[14] indicative of a stable [**T·T**] duplex. However, if the [**T·T**] duplex is relatively unstable, the value of p will tend to 1. An additional parameter ϵ is defined in this minimal model which describes the relative importance[15] of the bimolecular pathway with respect to the autocatalytic channel.

Fitting[10] of our experimental data to this minimal model proceeded smoothly and afforded best-fit values of p and ϵ of 0.9 and 5000, respectively.[16] These results suggest strongly that the [**6·6**] duplex is rather unstable in our system, and this leads to efficient turnover in the autocatalytic cycle. To test this hypothesis, we measured the dimerization constant for the [**6·6**] duplex directly using 400 MHz ^{1}H NMR spectroscopy. A 30 mM solution of **6** in $CDCl_3$ was diluted in a serial fashion, and the ^{1}H NMR chemical shift changes observed were fitted to the appropriate binding isotherm, affording a value for the dimerization constant of <400 M^{-1}. Since the association constant for a single carboxylic acid·amidopicoline complex is around 100 M^{-1}, in the absence of positive or negative cooperativity, we might expect the [**6·6**] duplex to have stability approaching 10 000 M^{-1}. The experimental value is clearly much lower than this and is, in fact, consistent with only one of the two possible binding sites being associated in solution. This result indicates clearly that the high p value is, indeed, the result of the inherent instability of the template duplex and that, as a result, the autocatalytic cycle involving **6** is relatively efficient.

In summary, we have demonstrated that the reaction between nitrone **1** and maleimide **5** at 10 °C in $CDCl_3$ at a concentration of 25 mM forms a template **6**, which is capable of self-replication. We have demonstrated that this reaction displays the characteristic sigmoidal rate profile for the formation of template **6**, together with the fact that the reaction is both recognition-mediated and template-directed. In addition, the results of the doping experiments demonstrate clearly that the stereochemical information present in **6** is transmitted faithfully to the forming template within the [**1·5·6**] complex. The efficient autocatalysis exhibited by this system, together with the flexibility inherent in its design are currently being exploited in our laboratory to create families of replicators that are capable of both selfish autocatalysis and promiscuous crosscatalysis.

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Supporting Information Available: Spectroscopic data for compounds **1**, **3**, **4**, and **6**. Details of the assignment of the relative stereochemistry in compounds **3**, **4**, **6** and **7**. Molecular modeling representations of the autocatalytic cycle involving **1**, **5**, and **6**. Details of the determination of stability constants for complexes involved in the autocatalytic cycle. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) There has been considerable discussion in the literature concerning the benefits and drawbacks of using a minimal model compared to full kinetic modeling (Reinhoudt, D. N.; Rudekevich, D. M.; de Jong, F. *J. Am. Chem. Soc.* **1996**, *118*, 6880). We have performed both minimal and full kinetic modeling on this system. The data from the full kinetic modeling is entirely consistent with the conclusions drawn here; however, its description is beyond the scope of this paper and will be presented elsewhere. The minimal modeling is presented here in order to set our system in the context of other replicating systems published previously.

(15) We believe that only two of the three reaction channels are open to this system. Molecular modeling indicates that the methylene spacer in **5** is too short to allow two point hydrogen bonding to the amidopicoline in **1** by the carboxylic acid in **5** (K_a = 100 M^{-1}) and reaction between the nitrone and the maleimide within the [**1·5**] complex simultaneously. Reaction is possible in a [**1·5**] complex in which there is only one hydrogen bond between the carboxylic acid proton and the pyridine nitrogen ($K_a \approx 10$ M^{-1}). The reaction is performed from a starting concentration of 25 mM, which is well below the K_d (100 mM) for this putative reactive [**1·5**] complex. We therefore discount any contribution to the overall rate of reaction from this complex.

(16) Systems based on nucleic acids, which all show strong product inhibition, have p values of around 0.5. Peptide-based systems (see, e.g., Lee, D. H.; Granja, J. R.; Martinez, J. A.; Severin, K.; Ghadiri, M. R. *Nature* **1996**, *382*, 525) have p values between 0.6 and 0.7. The Diels−Alder based system of Sutherland (Wang, B.; Sutherland, I. O. *Chem Commun.* **1997**, 1495) has a p value of 0.80. The peptide-based systems of Ghadiri have ϵ values of around 500, and the Diels−Alder based system of Sutherland has an ϵ value of around 10 000 depending on the reaction temperature.