"A Self-Replicating System": New Experimental Data and a New Mechanistic Interpretation

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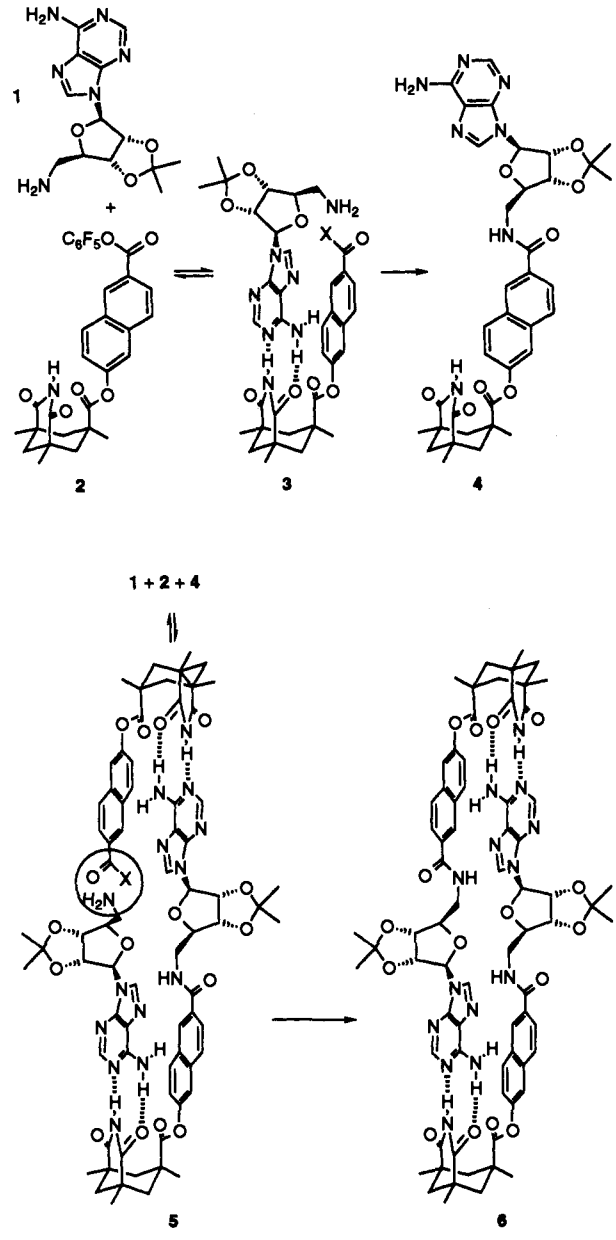
Rebek and co-workers[1] described a self-replicating system depicted in Scheme 1. Amine **1** and ester **2** in chloroform react to form amide **4** *via* complex **3**. Reactants **1** and **2** then combine with amide **4** to generate the termolecular complex **5**, in which a catalyzed production of additional **4** takes place. The system can be considered "self-replicating" in the sense that amide **4** serves as a template for its own formation. "At best", the authors write of their autocatalysis, "this can be regarded as a primitive sign of life". In the ensuing article, we show that production of **4** is catalyzed by simple amides and that there is no need to postulate a "self-replication" mechanism since **4** itself contains an amide group.

Evidence for autocatalysis came primarily from a *ca.* 40% enhancement of the initial rate when amide **4** was added externally to the reaction mixture. Strangely, a plot of [amide **4**] *vs* time taken over a long time period (1500 min) curved downward,[2,3] indicating that the aminolysis actually slowed as the "product/template" was being produced. Moreover, the plot leveled off at about 65% of the reaction. Rebek *et al.* speculated that the failure to reach completion was caused by ester hydrolysis in the chloroform solvent. Owing to these problems and to the rather small observed catalytic effect, we decided to reinvestigate the system.

Reactions between amine **1** and ester **2** in $CDCl_3$ were monitored by both ^{19}F and ^1H NMR. NMR is a simpler and, in some ways, more informative method of assessing reaction progress compared to the HPLC approach of Rebek *et al.* Kinetic curves were obtained by periodically recording ^{19}F (470 MHz) and ^1H (500 MHz) NMR spectra from solutions containing amine **1** and ester **2** (both 0.03 M in $CDCl_3$)[4] plus triethylamine (0.12 M) at 25.0 ± 0.1 °C. Signals from pentafluorophenol (10.5 and 9.6 ppm) and from amide **4** (5.26 ppm of the ribose H2′ proton), respectively, were employed.[5] Initial rates among repeat runs never deviated more than 6% from each other.[6]

The plot in Figure 1A of $[C_6F_5OH]$ *vs* time gives an initial rate of 0.18 mM/min. When the experiment was repeated with the added presence of amide **4** as a "template" (1 equiv), the rate increased 56% to 0.28 mM/min (Figure 1B). Had we terminated work at this point, our results would seemingly affirm the past conclusions of Rebek *et al.*

Scheme 1



Suspicion that matters might be more complicated than first imagined came from experiments with three control compounds (**7**, **8**, and **9** in Scheme 2).[7] Controls **7** and **8** model the "southern" and "northern" halves of amide **4**, respectively. Control **9** is identical to amide **4** except that the naphthyl ring has been replaced by a phenyl group, thereby shortening the molecule by several angstroms and, presumably, rendering it a much poorer template.

Addition of controls **7** and **8** (0.03 M, 1 equiv) to the usual mixture of amine **1** and ester **2** had no effect on the rates. Thus, the slopes of their $[C_6F_5OH]$ *vs* time plots (not shown) are within 4% of that in Figure 1A. On the other hand, control **9** displayed a 25% catalysis (Figure 1C) under the standard conditions. This acceleration is only 2-fold less than that observed for amide **4** and not too far removed from the *ca.* 40% catalysis reported by the Rebek group. The similarity between amide **4** and its shortened

(1) (a) Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 1249. (b) Nowick, J. S.; Feng, Q.; Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 8831.

(2) Von Kiedrowski, G.; Wlotzka, B.; Helbing, J.; Matzen, M.; Jordan, S. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 423. These authors discuss the sigmoidal curve with an induction period often seen with autocatalytic reactions.

(3) See Figure 2 in ref 1b.

(4) This concentration, 2-fold greater than the highest used by Rebek,[1] had several advantages: (a) the precision of the data was improved; (b) the unidentified side reaction was much less dominant than with Rebek's system (<5% *vs* 35%); and (c) template catalysis, involving a termolecular complex, should contribute more at higher concentrations.

(5) The ^{19}F NMR method has an advantage over HPLC analysis of amide **4** in that the former involves a peak not contributed by amide **4**. This is particularly important when 0.5–1.0 equiv of amide **4** is added externally to the reaction mixture.

(6) Rates for three repeat runs without additive equaled 1.78×10^{-4}, 1.72×10^{-4}, and 1.77×10^{-4} M/min. Rates for 1 equiv of **7**, 1 equiv of **8**, and 1 equiv of **7** plus 1 equiv of **8** equaled 1.66×10^{-4}, 1.61×10^{-4}, and 1.81×10^{-4} M, respectively. Rates for two repeat runs with 1 equiv of amide **4** equaled 2.76×10^{-4} and 2.90×10^{-4} M/min. Rates for two repeat runs with 1 equiv of acetamide equaled 2.68×10^{-4} and 2.43×10^{-4} M/min. A rate without additive but with 2 equiv of Et_3N (instead of 4 equiv) equaled 1.70×10^{-4} M/min.

(7) Rebek *et al.*[1] carried out controls in which formation of complex **3** was impeded either by *N*-methylation of **2** or by addition of 2,6-bis(acetylamino)-pyridine. The resulting inhibitions provide evidence for complex **3** (whose involvement prior to the presumed "template" step we readily accept).

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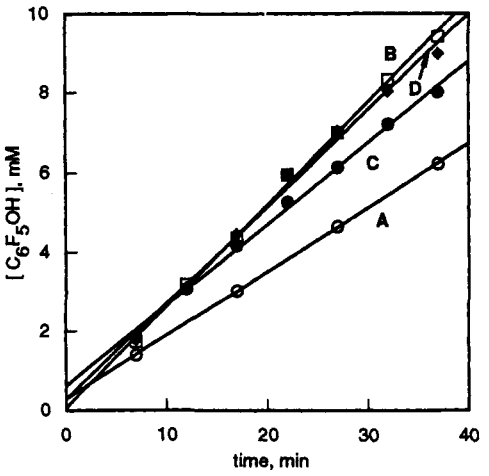


Figure 1. Initial rates of pentafluorophenol formation in the reaction of amide **1** (0.03 M) with ester **2** (0.03 M) in $CDCl_3$ in the presence of: A, no additive; B, 0.03 M amide **4**; C, 0.03 M control **9**; and D, 0.03 M acetamide. Reactions were monitored by ^{19}F NMR at 25.0 °C.

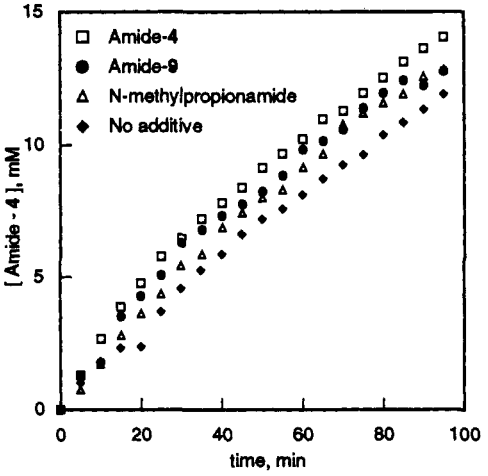
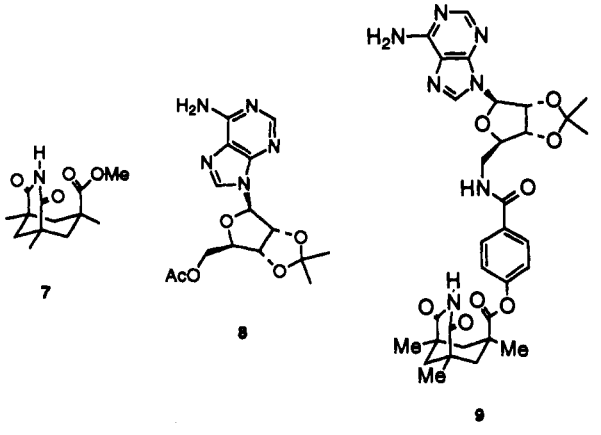


Figure 2. Time course for formation of amide **4** in the reaction between amine **1** and ester **2** in $CDCl_3$ in the presence of amide **4**, amide **9**, and *N*-methylpropionamide (all at 0.03 M). Reactions were monitored for the production of amide **4** by ^1H NMR at 25.0 °C. Error in the initial rates is estimated to be ±15%. Note that the rates in the latter part of the reaction (unexamined by Rebek[1]) are identical.

Scheme 2



analog, **9**, is seen even more strikingly in Figure 2, in which the production of amide was monitored by ^1H NMR.[8] Accurate integration was achieved with the aid of an internal standard. Hardly any observable difference exists between the amide **4** and control **9** additives over a 100-min trace. In the world of small catalytic effects, control **9** performs well. And Rebek's autocatalysis, whatever its source, does not require the precise geometric fit implied by the structure of the termolecular complex **5** in Scheme 1.

Since the "southern" control **7** and the "northern" control **8** are not catalysts, whereas control **9** does indeed accelerate the aminolysis, one might suspect that the central portion of control **9** (the amide group) plays a key role. This turned out to be the case. 2-Naphthoamide (0.5 equiv) catalyzes the reaction under standard conditions by 13%. Acetamide (1 equiv) manifests a catalysis almost as large as that of the amide **4** "template" (compare Figures 1B and 1D). *N*-Methylpropionamide (1 equiv) provides a 31% rate increase by ^{19}F NMR and a long-term profile (Figure 2) within experimental error of amide **4**. Since Rebek's catalysis can be achieved using four different amides,[9] a template-based mechanism for amide **4** becomes superfluous.

Although catalyses of less than 2-fold may be too feeble to warrant a detailed mechanistic rationale, the classic work of L. M. Litvinenko[10] seems relevant to the problem. Litvinenko showed that carboxamides are effective catalysts in the acylation of amines by acid chlorides in benzene. He favored an O-nucleophilic catalysis, although one cannot discount assisted proton transfers among ionic tetrahedral intermediates in the aprotic media. Whatever the precise mechanism, rate enhancements of 10-fold or more were obtained with amide concentrations equivalent to ours. The 20–60% catalyses observed with the Rebek system are most readily explained by a similar effect (although smaller in magnitude owing, no doubt, to the use of a less reactive carboxylic acid derivative). Note that Litvinenko found no catalysis with imides, consistent with our observation that control **7** fails to accelerate the Rebek acylations.

Our reasoning, therefore, reduces to a simple syllogism: Amides accelerate acylations. Rebek's "template" contains an amide. Therefore, the catalyzed acylations of Rebek could derive from the presence of the amide.

In summary, control **9** (a noncomplementary analog of amide **4**) shows similar kinetic characteristics to amide **4**. Moreover, acetamide duplicates the catalysis of amide **4**. It thus seems premature to assume at this point that a self-replicating system, predicated upon a template-directed autocatalysis, is in hand under the conditions of our experiments.[11,12]

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(8) It was considered desirable to utilize two independent methods of analysis. Thus, monitoring amide **4** production by ^1H NMR was carried out in addition to monitoring pentafluorophenol production by ^{19}F NMR. The latter was the more precise.

(9) The Rebek mechanism is complicated, and an additive that binds to the reactants can be perturb the rate apart from any effect on a kinetic step. This is why we studied simple amide additives, as opposed to those with multiple binding sites (see **11b** in ref 1b), to test amide catalysis.

(10) Titskii, G. D.; Litvinenko, L. M. *Zh. Obsch. Khim.* **1970**, *40*, 2680.

(11) Reactions were carried out by adding amine **1** in $CDCl_3$ to a $CDCl_3$ solution of ester **2** (plus a control compound if used) in an NMR tube. The tube was then placed in an NMR spectrometer probe thermostated at 25 ± 0.1 °C. Rebek's studies were carried out at ambient temperature (21.5–23 °C). Integrations of the ^{19}F NMR spectra were performed in an absolute intensity mode. Integrations of the ^1H NMR spectra were performed in both the absolute intensity mode and using CH_3OH in a coaxial tube as an external standard (the two methods agreeing to within the experimental error of ≤6%). Control **9**, synthesized by the general route already described,[1] gave the correct NMR and MS spectra. Anal. Calcd for $C_{32}H_{37}N_7O_8$: C, 59.43; H, 5.76; N, 15.14. Found: C, 59.37; H, 5.78; N, 15.00.

(12) Rebek *et al.*[1] found that the *ca.* 40% catalysis remained unchanged when the concentrations of **1**, **2**, and **4** were decreased 10-fold each. This is baffling because the key termolecular complex (**5**) is highly concentration-dependent. We observed no amide catalysis at 4 mM *N*-methylpropionamide and 8 mM **1** + **2**. Further kinetic work will clarify these observations.