Evidence for an Alternative Mechanism to a Previously Proposed Self-Replicating System

F. M. Menger,* A. V. Eliseev, N. A. Khanjin, and M. J. Sherrod

Department of Chemistry, Emory University, Atlanta, Georgia 30322

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Rebek *et al.*[1,2] have proposed a "self-replicative" mechanism in which the amide product of an ester aminolysis forms a termolecular complex with the ester and amine reactants. In this manner, the product catalyzes its own formation. The evidence for the mechanism lies mainly in a 40–70% acceleration when product is added externally to the reaction mixture. The system has now been reinvestigated owing, in part, to doubts created by troublesome experimental problems (*e.g.* small rate enhancements coupled to ≥35% unidentified side reactions) and by the entropic unlikelihood of the highly constrained termolecular complex. Our new experiments prove that the Rebek mechanism is unnecessary. Thus, the aminolysis of simple naphthoyl and benzoyl esters, both lacking any hydrogen-bonding sites, are catalyzed by the Rebek "template". In the latter case, the reactions were run under the identical conditions used recently by Rebek (2 mM) while monitoring the formation of the major reaction product. Although the benzoyl ester cannot hydrogen-bond to the template, the ester aminolysis is catalyzed by the template to an extent even greater than that observed by Rebek (*i.e.* 2-fold). The Rebek mechanism, predicated upon ester/template binding, is clearly invalidated by these experiments. An alternative mechanism, involving amide catalysis, is proposed and found consistent with all available data.

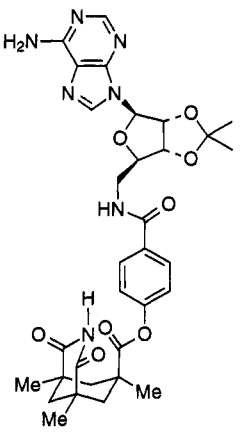
Introduction

Rebek and co-workers[1,2] 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 **4** to generate termolecular complex **5** in which a catalyzed production of additional **4** takes place. The system can be considered "self-replicating" in the sense that **4** serves as a template for its own production.

Self-replicating systems have attracted the attention of many other people including Günter von Kiedrowski,[3] Leslie Orgel,[4] and Jonathan Sessler.[5] In Rebek's words, such systems are "a primitive sign of life"[1] and a "template for life",[6] accounting in part for the wide discussion that Rebek's work has engendered in the scientific and popular press.

Evidence for the self-replicative mechanism in Scheme 1 came primarily from an observed catalysis when amide **4** was added externally to the reaction mixture. For example, 0.20 equiv of **4** added to 8.2 mM of **1** and **2** caused a 43% rate increase. A rate enhancement would indeed be expected if the termolecular association brought into proximity the amine and ester groups. Accordingly, ester aminolysis within termolecular complex **5** explains the observed catalytic effect.

We recently published[7] the observation that simple amides (*e.g.* 2-naphthamide, acetamide, and *N*-methylpropionamide) also catalyze the aminolysis of ester **2** by amine **1**. Actually, this observation was not too surprising since amine-catalyzed aminolysis of carboxylic acid derivatives in aprotic solvents is a well-documented effect.[8] Since Rebek's template **4** is itself an amide, concern arose as to whether his catalysis might arise not from a template effect but, instead, from a more mundane (and non-self-replicative) amide acceleration. Concern was further aroused by the finding that the analog drawn below can effect a 25% rate increase under our experimental conditions.[7] The analog is identical to **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. Catalytic activity of the molecule does not necessarily prove that **4** also operates via amide catalysis, but the result does engender a strong suspicion that such is the case. Our initial communication on the subject[7] discussed this possibility in detail.



In experiments inspired by our observations, Rebek *et al.*[9] detected no amide catalysis[10] when they operated at

[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1995.

(1) Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 1249.
(2) Nowick, J. S.; Feng, Q.; Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 8831.
(3) Von Kiedrowski, G. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 932. von Kiedrowski, G. *Bioorg. Chem. Frontiers* **1993**, *3*, 113. Sievers, D.; von Kiedrowski, G. *Nature* **1994**, *369*, 221.
(4) Orgel, L. E.; Lohrmann, R. *Acc. Chem. Res.* **1974**, *7*, 368. Chen, C. B.; Inone, T.; Orgel, L. E. *J. Mol. Biol.* **1985**, *181*, 271.
(5) Sessler, J. L.; Magda, D.; Hugdahl, J. *J. Inclusion Phenom.* **1989**, *7*, 19.
(6) Rebek, J., Jr. *Chem. Brit.* **1994**, *30*, 286.
(7) Menger, F. M.; Eliseev, A. V.; Khanjin, N. A. *J. Am. Chem. Soc.* **1994**, *116*, 3613.
(8) Titskii, G. D.; Litvinenko, L. M. *Zh. Obsch. Khim.* **1970**, *40*, 2680.

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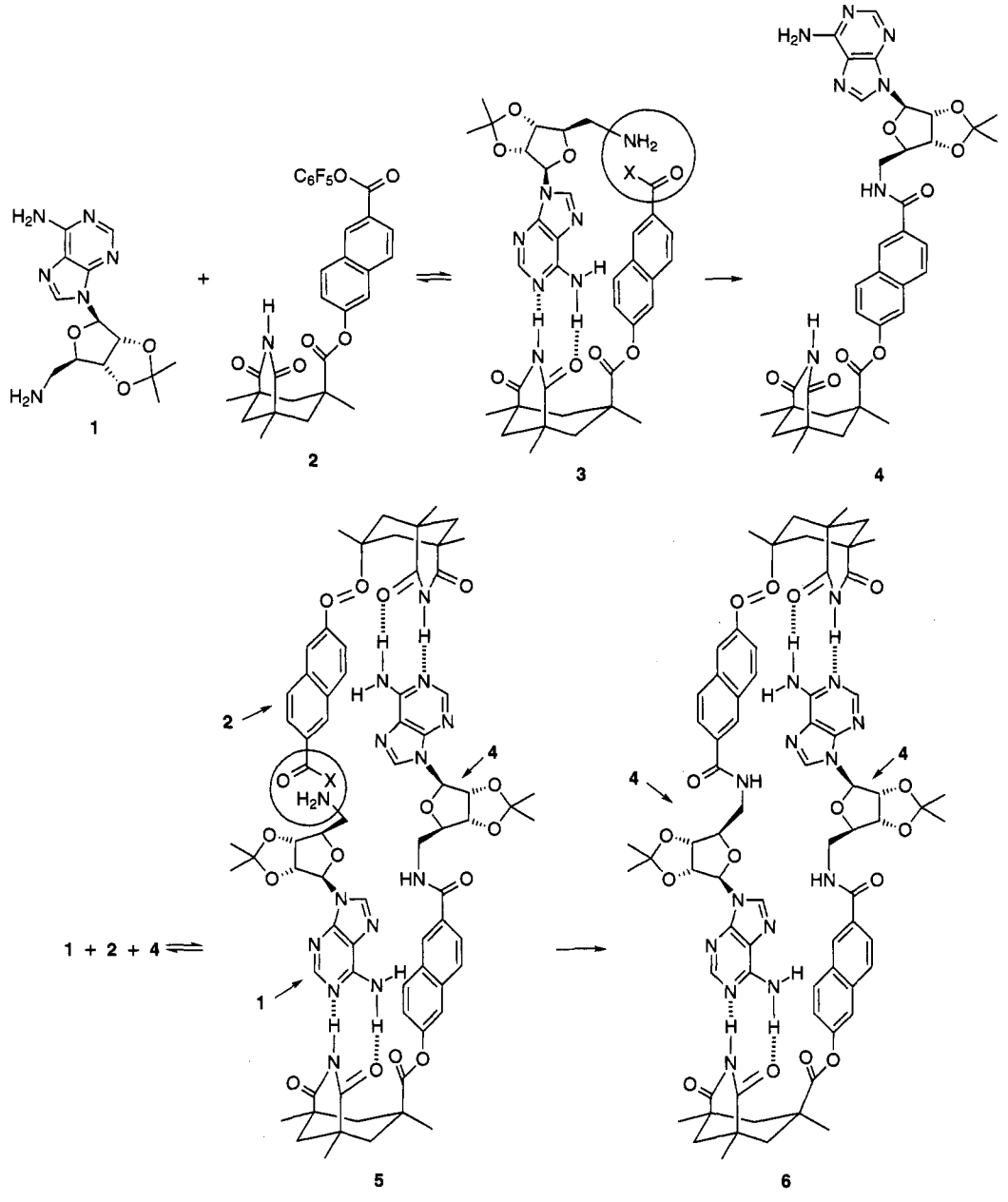
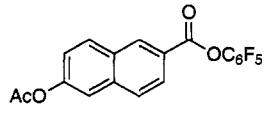
Scheme 1. Rebek's Self-Replicative Mechanism



2 mM initial concentration of **1** and **2** plus 0.5 equiv of amide. We, on the other hand, had used concentrations of 8 mM and higher (for reasons that will become clear shortly). Thus, Rebek claimed that his group was observing a self-replicating mechanism (Scheme 1) at 2.2 mM, whereas we were likely observing amide catalysis at the higher concentrations. Although a change of mechanism over a 4-fold concentration range seemed unlikely to us, it could not be excluded. This is, after all, a very complicated system. Further experimentation was deemed necessary.

Fortunately, there existed a simple and definitive test for Scheme 1. The self-replicative mechanism is predicted upon a termolecular complex (**5**) in which amine **1**, ester **2**, and template **4** are all hydrogen-bonded to each other. Thus, Rebek's mechanism demands that catalysis would be absent with esters that lack hydrogen-bonding sites. Such esters cannot, obviously, engage in the formation of a termolecular complex. On the other hand, the amide-catalysis alternative predicts that **4** might indeed catalyze the aminolysis of nonbinding esters because a termolecular complex is not invoked. These considerations prompted us, therefore, to investigate the behavior of the two non-hydrogen-bonding esters shown below. Experiments with these two esters should settle the question conclusively.



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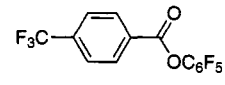

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A Critique of Scheme 1

It is instructive to examine in more detail the factors that motivated our experimental testing of the Rebek mechanism:

(9) Wintner, E. A.; Conn, M. M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1994**, *116*, 8823.
(10) Su, C.-W.; Watson, J. W. *J. Am. Chem. Soc.* **1974**, *96*, 1854.

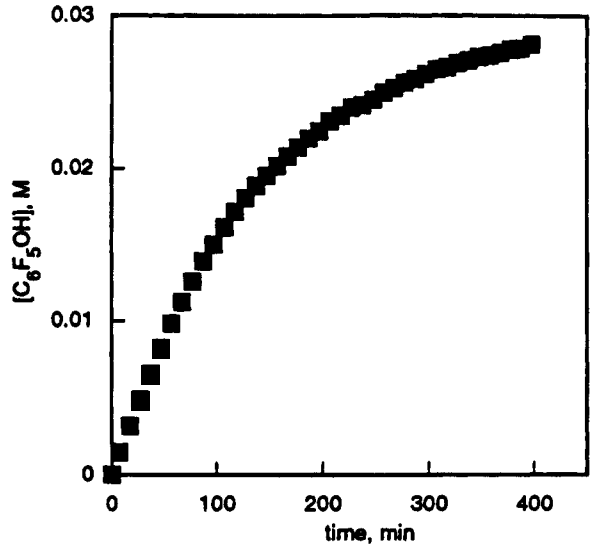


Figure 1. Production of pentafluorphenol in the reaction of 0.03 M **1** and **2**. The yield exceeds 92% at 400 min. A similar experiment, in which **4** was monitored by ^{1}H NMR, was followed to 90% yield of product with 10% of the remaining reactants still evident in the spectra. Rebek observed a 35% byproduct at 8 mM concentrations.

(1) Rebek's observed catalyses are, by any measure, small in magnitude. Accelerations of 40–70% correspond to only 0.2 kcal/mol (no larger than many solvent isotope effects). Past experience with aminolysis kinetics in organic solvents[11] has taught us how careful one must be when interpreting minor rate perturbations in the context of a complex reaction with multiple rate and equilibrium steps.

(2) The majority of Rebek's kinetics involved rate measurements spanning 10% or less of the reaction. An HPLC assay of product **4** was used to monitor these initial rates. Now consider the reaction between 2.2 mM **1** and **2** in the presence of 1.1 mM **4** which was followed to 5% completion. A kinetic point obtained at ca. 1% reaction thus corresponds to formation of only 2.2×10^{-5} M **4**. Operating at such low levels of product formation creates two problems: (a) product **4** (2.2×10^{-5} M) must be determined quantitatively by HPLC *over and above* the background level of 1.1 mM **4** that had been added to the system as a potential catalyst and (b) polar impurities in the chloroform (*e.g.* water) are an ever-present danger when initial rate studies involve extremely low concentrations. It is partly for this reason that all our previously published data[7] and some of the data herein utilize concentrations higher than 2.2 mM.

(3) Rebek *et al.*[2] published a plot of product concentration vs time taken over a long time period (1500 min). Initial concentrations of **1** and **2** equaled 8.2 mM. The plot manifested no induction period and, of considerable concern, leveled off at only 65% reaction. Rebek speculated that the 35% byproduct with 8.2 mM reactants was caused by an adventitious ester hydrolysis. Since HPLC traces have never been published, it is not possible to tell whether hydrolysis products were in fact detected. We have found (Figure 1) that by operating at 30 mM the yield increases to >95% (a fact consistent with the problems at high dilution being caused by impurities). Much of our own previous work was, therefore, carried out at 30 mM instead of 8.2 mM. The danger of complications by side reactions could become even more ominous at 2.2 mM. Ultimately, we too had to use 2.2 mM (uncertainties not withstanding) in order to duplicate the most recent Rebek conditions as closely as possible.

(11) Menger, F. M.; Smith, J. H. *J. Am. Chem. Soc.* **1972**, *94*, 3824.

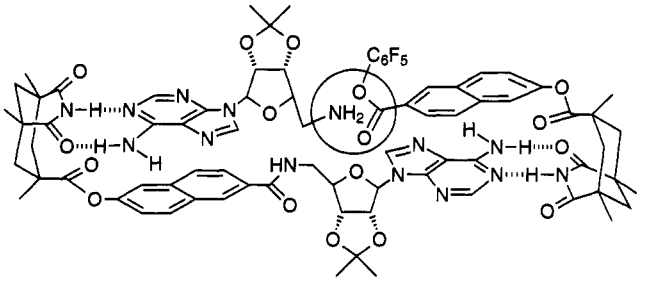


Figure 2. An exact replica of the termolecular complex taken from Wintner, E. A.; Conn, M. M.; Rebek, J., Jr. *Acc. Chem. Res.* **1994**, *27*, 198. Note how the pentafluorphenyl ester has been drawn in a high-energy *s-cis* configuration in order to avoid severe steric interactions that would otherwise result. This same misleading structure was given in Conn, M. M.; Wintner, E. A.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1994**, *116*, 8823.

(4) Rebek *et al.*[2] refer to certain systematic errors (*e.g.* temperature control no better than 21.5–23 °C and evaporate loss of solvent during prolonged runs). Anxious that such uncertainties not contribute to our <2-fold rate effects, we avoided them as explained later.

(5) Finally, we were troubled by the entropic unlikelihood of termolecular complex **5** in Scheme 1. Thus, three species from solution must be assembled, and ten single bonds must be frozen in space! Moreover, the bulky pentafluorophenyl group of ester **2** would "slam into" the template if **5** were indeed an accurate portrayal of the complex. This fact was overlooked in the Rebek papers as is apparent from two pictorial devices: (a) the pentafluorophenyl group was "diminutized" in the form of "X" as shown in Scheme 1 and (b) the ester group was imparted with an *s-cis* configuration that is ca. 6 kcal/mol higher in energy than the preferred *s-trans* configuration (Figure 2).

Exhaustive molecular mechanics calculations (see Experimental Section for details) confirm the presence of steric problems at the reactive site of **5**. Although the steric effects do not exclude intracomplex reactivity, they are decidedly inhibitory in nature. Thus, the large pentafluorophenyl group and the two *gem*-dimethyl units create an extremely crowded situation in the region where the amine and the ester groups purportedly meet. This fact tends to push the amine and ester groups away from each other in a manner not evident from Scheme 1. For example, the low-energy conformer in Figure 3 has an H_2N–C=O distance of 4.3 Å. Even worse, the nitrogen must pass "through" a *gem*-dimethyl group to reach the carbonyl carbon. In addition, there exists an entire family of low-energy conformations in which the ester **2** is bound to the wrong side of template **4** (Figure 4).

No termolecular complex was encountered that meets two reasonable criteria: (a) low energy relative to a host of other possible structures and (b) favorable relationships between the amine and ester groups that permit rapid aminolysis. Thus, arguing strictly from ground-state properties, we can state that reactivity via the Rebek complex is energetically unlikely. Of course, we cannot eliminate the possibility of some high-energy

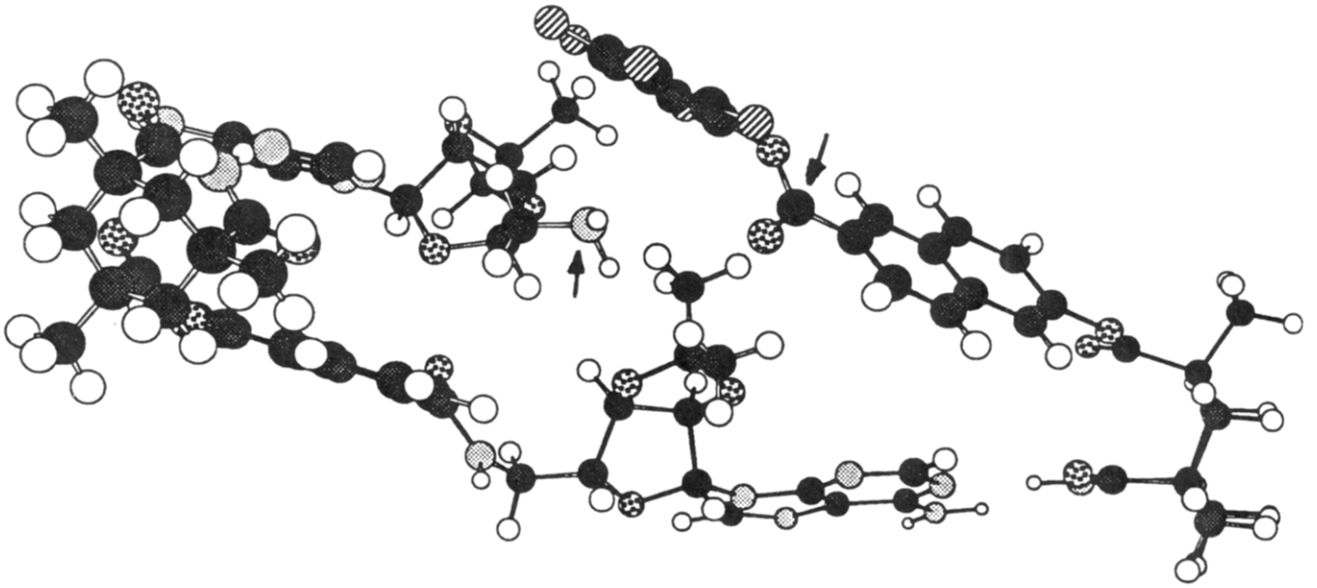


Figure 3. Low-energy conformation of the termolecular complex **4** with the amine and ester carbonyl (arrows) in a geometric disposition unfavorable for reaction.

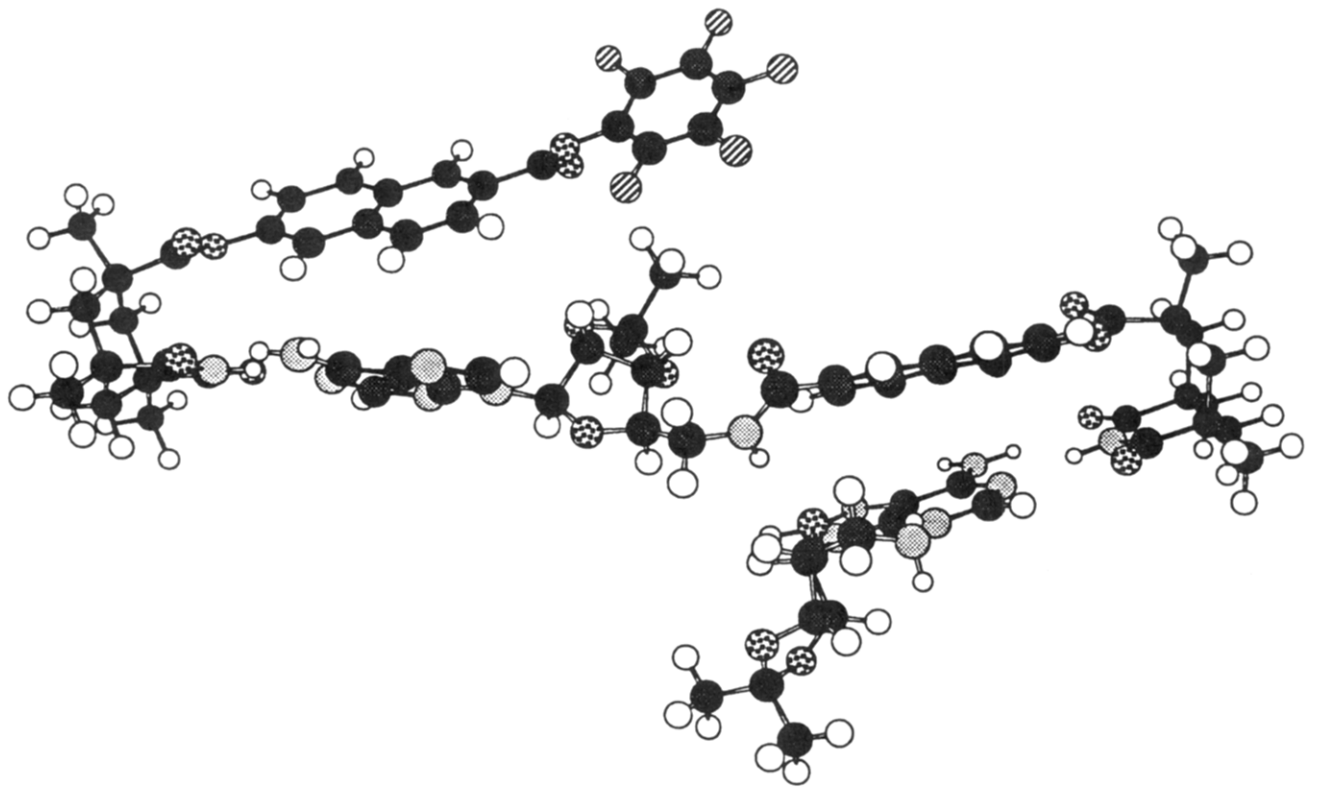


Figure 4. Low-energy conformer of the termolecular complex **4** in which the amine and ester functionalities lie on opposite sides of the "template".

structure that almost exactly compensates for its trivial concentration by possessing a prodigious rate (and leading, therefore, to only a 40−70% rate increase). For this reason, the computations are presented here only as suggestive and peripheral information. It did seem important, however, to point out the severe limitations inherent to the termolecular complex that are not readily apparent in the artistic representations of the Rebek papers.

Individually, none of the above five points conclusively negates the main conclusion of the Rebek papers, namely that the Scheme 1 explains the catalysis. Collectively, however, the list imparts an uneasy feeling, a feeling that ultimately prompted us to reinvestigate the system. Our initial discovery[7] that several amides catalyze the aminolysis of ester **2** by amine **1** intensified our belief that a deeper understanding of this chemistry is needed. The present paper describes recent experiments with nonbinding esters that support a non-self-replicating mechanism quite different from Scheme 1.

Experimental Testing of Scheme 1

In a typical kinetic run, ^{19}F NMR spectra (470 MHz) were traced from a solution containing equimolar amine

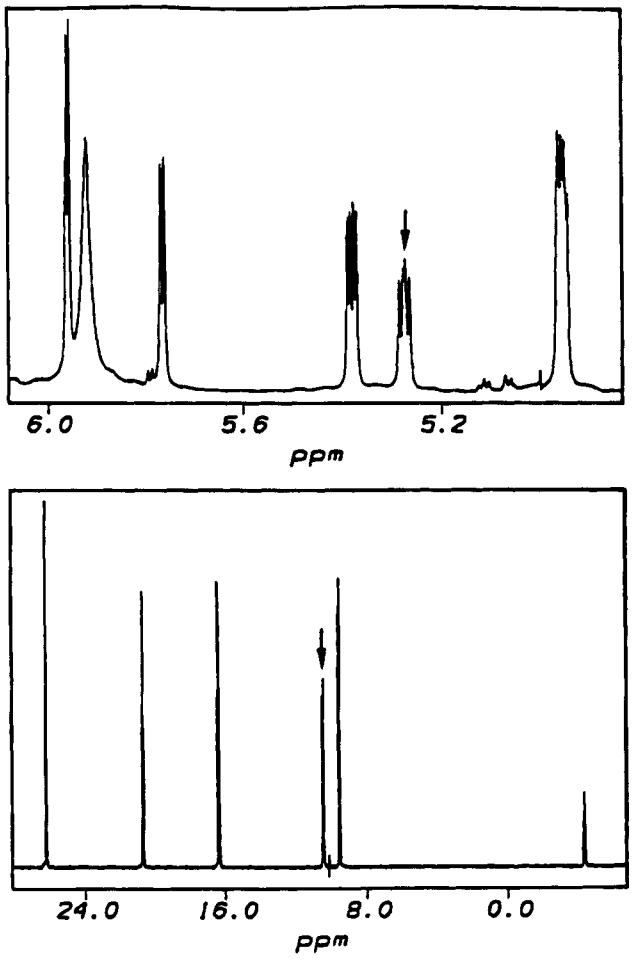


Figure 5. Top: ^{1}H NMR spectrum of 0.03 M **1** and **2** after 90 min of reaction ($CDCl_3$, 25.0 °C). Arrow points to the product peak (H2′ of ribose) monitored during the reaction. The signal to its left is the H2′ of reactant **1**. Bottom: ^{19}F NMR spectrum of 0.03 M **1** and **2** after 90 min of reaction ($CDCl_3$, 25.0 °C). Arrow points to the growing pentafluorophenol signal used for integration.

1 and ester **2** plus a 4-fold excess of triethylamine in $CDCl_3$. The temperature was controlled at 25.0 ± 0.1 °C, and evaporative loss was not a factor. Signals from one of the products, pentafluorophenol, at 9.6 and 10.5 ppm (Figure 5) were integrated to give, with the aid of a calibration plot, the initial rates in units of M/min. Rates of 1.78×10^{-4}, 1.72×10^{-4}, and 1.77×10^{-4} M/min typify our reproducibility.

Analysis of pentafluorophenol production by ^{19}F NMR has an important advantage over Rebek's HPLC analysis of product **4**: an NMR assay of pentafluorophenol is independent of **4**. This allowed us to avoid the severe background problems when 0.5−1.0 equiv of product **4** was added externally to the reaction mixture as a potential catalyst. Analyzing for pentafluorophenol is akin to the time-honored procedures for titrating tosylate in solvolyses reactions or measuring *p*-nitrophenolate in ester hydrolysis. Rebek *et al.*[9] claimed that "we had used ^{19}F NMR to follow a reaction in which the product contains no fluorine", but in our view both pentafluorophenol and amide **4** have equal claim to "product status".

Figure 6 shows kinetic plots of [pentafluorophenol] vs time for the reaction of amine **1** with nonbinding ester **7** at an initial concentration of 8.2 mM each. Plot **A** gives an uncatalyzed rate of 1.8×10^{-6} M/min. Plot **B** represents the reaction between 8.3 mM amine **1** and ester **7** in the presence of 0.5 equiv of "template" **4**. It gives a rate of 2.8×10^{-6} M/min. Clearly, esters need no hydrogen-bonding capability in order to achieve a 55% catalysis (equivalent to that observed by Rebek). The termolecular complex in Scheme 1 is thus shown to be extraneous.

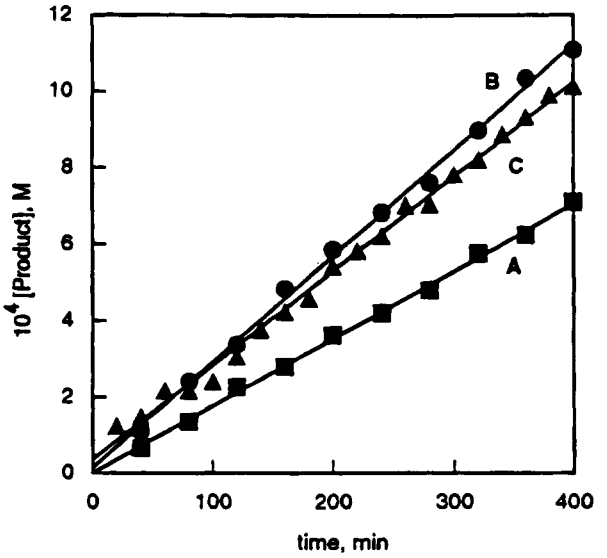


Figure 6. Plot A: initial reaction rate for 8.2 mM amine **1** and nonbinding ester **7** ($V_{uncat} = 1.8 \times 10^{-6}$ M/min) as monitored by ^{19}F NMR. Plot B: initial reaction rate for 8.2 mM **1** and **7** with 0.5 equiv of "template" **4** ($V_{cat} = 2.8 \times 10^{-6}$ M/min) as monitored by ^{19}F NMR. Plot C: initial reaction rate for 8.2 mM **1** and **7** with 0.5 equiv of **4** ($V_{cat} = 2.5 \times 10^{-6}$ M/min) as monitored by ^{1}H NMR analysis of **4**. All reactions were carried out at 25.0 °C in chloroform containing 4 equiv of triethylamine. This figure is critical because it shows that, under Rebek's conditions, an ester that cannot bind to the "template" is, nonetheless, catalyzed to an equal extent as hydrogen-bonding ester **2**.

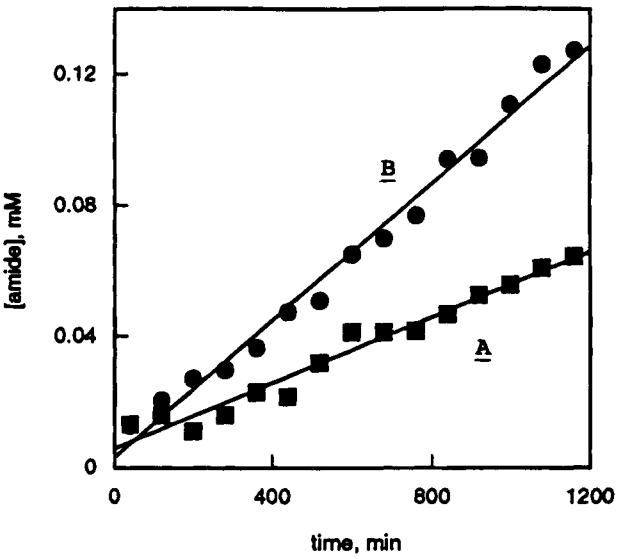
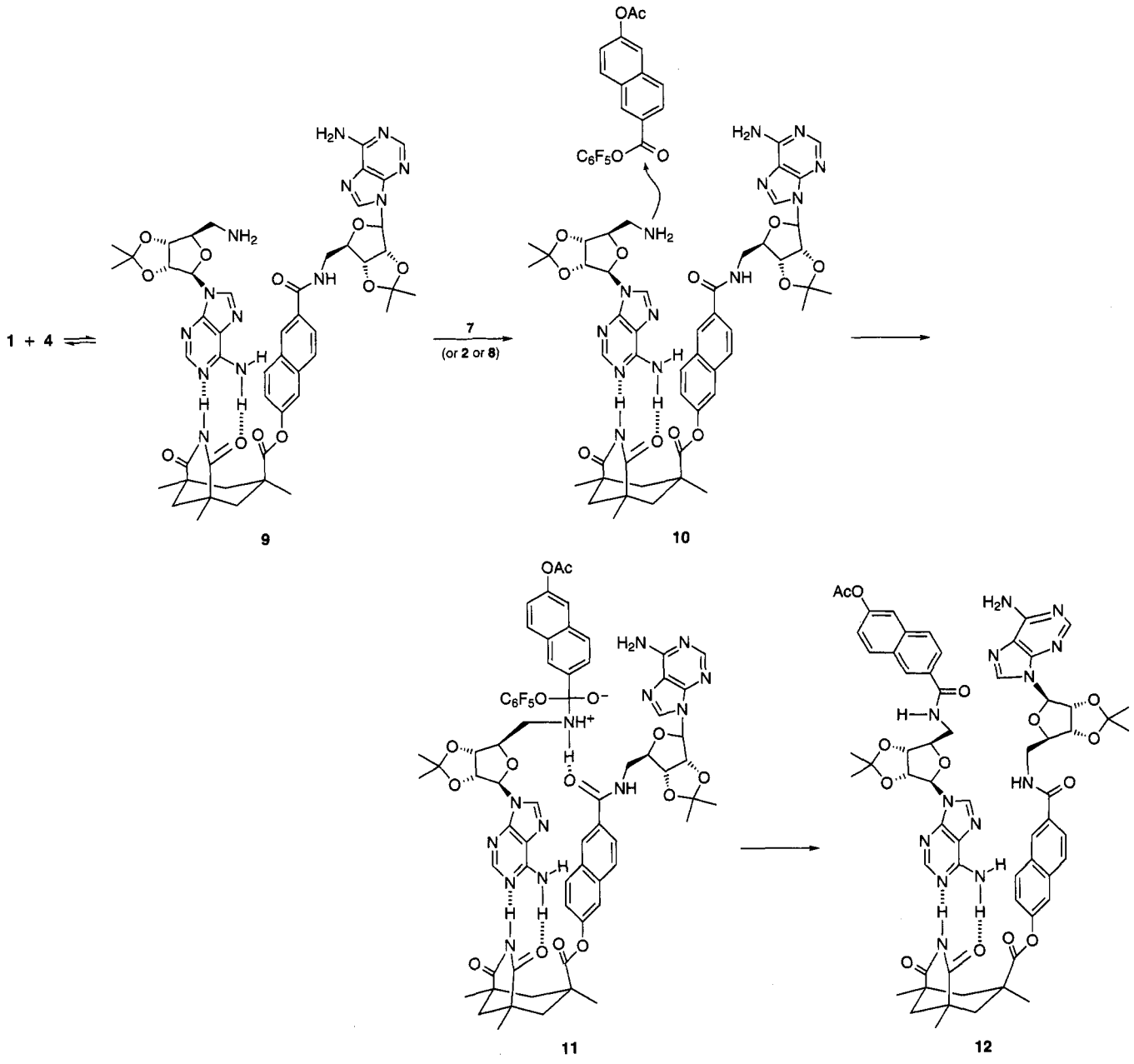


Figure 7. Initial rates of amide formation in the reaction of amine **1** (1.67 mM) with ester **8** (1.67 mM). Plot A: uncatalyzed. Plot B: catalyzed by 0.5 equiv of **4**. These plots demonstrate that the aminolysis of a non-hydrogen-bonding ester can be accelerated 2-fold by "template" **4** under exactly Rebek's conditions.

We felt it desirable to repeat the above experiment while monitoring the production of **4**. Rebek's disclaim-

Scheme 2. A Non-Self-Replicative Mechanism



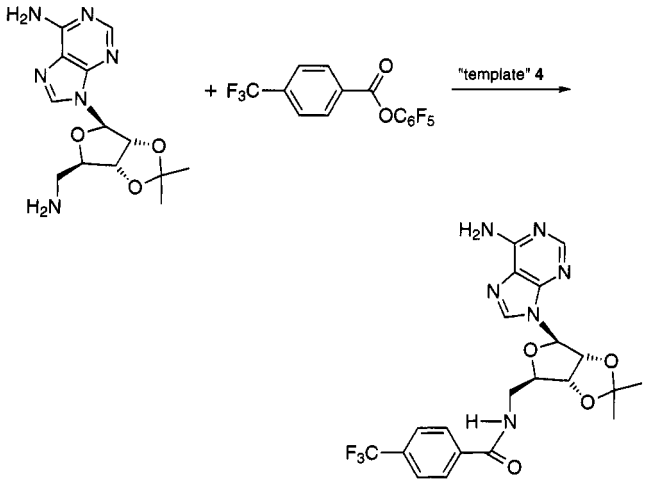
ing pentafluorophenol as a bonafide product had to be confronted directly, and in any case, a second independent analytical method is always beneficial. Thus, we followed the growth of the ribose H2′ proton signal of **4** (see Figure 5) as a function of time using an external standard (methanol). Since this method suffers from the same background problems as Rebek's HPLC analysis of **4**, the accuracy of the data is only about ±12%. Nonetheless, Figure 6C for the reaction of 8.3 mM amine **1** and nonbinding ester **7** plus 0.5 equiv of **4** shows excellent agreement with the corresponding ^{19}F NMR-based run (figure 6B). Rebek's "template" catalyzes the aminolysis of an ester that is unable to bind, and thus Scheme 1 can be discarded.

Not only did we utilize a second independent analytical method, we also examined a second nonbinding ester, ester **8**. We furthermore decided to study ester **8** at Rebek's lowest concentration of 2 mM since the argument has been made that concentration is critical. In no way does the decision to operate at 2 mM abrogate our misgivings about the problems inherent to low concentrations mentioned earlier. Rather, the experiments under the exact Rebek conditions were required to test the claim that the mechanism changes from 8 to 2 mM.

Concentrations of 1.67 mM amine **1** and ester **8** were used along with 0.5 equiv of **4** as a potential catalyst. These concentrations are slightly *less* than the lowest used by Rebek. Analysis of the amide product (Scheme 3) was accomplished with ^1H NMR. Figure 7 shows that $V_{cat}/V_{uncat} = 2.1$, a catalysis exceeding any observed by Rebek. The evidence against Scheme 1 is, therefore, decisive. Arguments by Rebek that the self-replicative mechanism operates primarily at low concentration, where until this point we had yet to explore, have been effectively eliminated.

The high impact of our experiments with esters **7** and **8** must be stressed. One can always conjure up multiple reasons for a particular control system giving negative results (*i.e.* no effect on the rate). But a tangible presence of catalysis, as observed with **7** and **8**, cannot be dismissed. Whatever the mechanism for catalysis, it must

Scheme 3. An Aminolysis of a Nonbinding Ester Catalyzed 2.1-fold by Rebek's Compound



necessarily accommodate esters **7** and **8**, and Scheme 1 fails to do so.

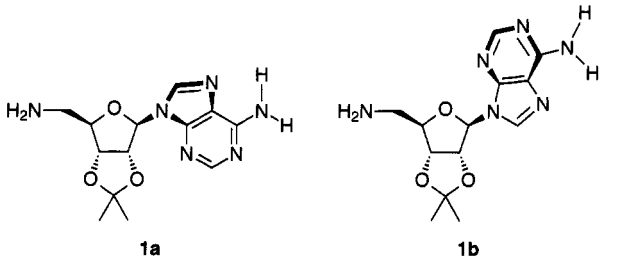
An Alternative Mechanism

Scheme 2 incorporates two key experimental findings that are not accommodated by the self-replicative mechanism: (a) catalysis by amides and (b) catalyzed aminolysis of non-hydrogen-bonding esters. Thus, amine **1** and amide **4** form a biomolecular complex (**9**). The amino group within complex **9** then attacks an unbound ester. Catalysis arises from the fact that the amide group can stabilize a zwitterionic tetrahedral intermediate in the aprotic solvent. Structure **11** shows one of several possible ways in which it can be accomplished. In effect, the amino group of **9** is slightly more nucleophilic than the amino group of free **1**, and the result is an observed catalysis.

One must be perfectly clear as to the distinction between Scheme 1 and Scheme 2. Scheme 1 is based upon a proximity effect within a termolecular complex. Scheme 2 does not invoke a termolecular complex. In fact, it is assumed that such a termolecular complex is either insignificant in concentration or inert. Catalysis in Scheme 2 is, instead, predicted upon an amide group functioning within a biomolecular complex. Although well described in the literature,[8,10] amide catalysis went unrecognized by Rebek *et al.*[1,2] and was never incorporated into their mechanism.

Scheme 2 also differs from Scheme 1 in not being "self-replicative". True, Scheme 2 allows for a trivial autocatalysis because amide **4**, formed in an ester aminolysis, can potentially catalyze subsequent reaction between **1** and **2**. But an autocatalysis could also be observed, in principle, when benzoyl chloride reacts with aniline to form an amide.[8] No one would call this "self-replication" (let alone "a primitive sign of life").

Scheme 2 is likewise consistent with three control experiments carried out by Rebek.[1,2] Thus, it was noted that 2,6-bis(acylamino)pyridine acts as a competitive inhibitor in the reaction between **1** and **2** (see structure **5** in ref 1). Moreover, methylation at the imide nitrogen of **2** does not enhance the ester aminolysis. Both observations support the involvement of complex **3** in the pathway from **1** and **2** to **4** (a sequence which we readily accept). The key question is, however, not the reaction between **1** and **2** but whether or not **4** can serve as a



1a **1b**

Figure 8. Conformational families of amine **1** used in the 64 minimizations.

template as portrayed in Scheme 1. In support of this "self-replicative" process, Rebek cites the fact that methylation of **4** on its imide nitrogen destroys the catalytic effect. But this observation is likewise consistent with Scheme 2 because Scheme 2 involves complexation between the amine and imide units (structure **9**). In summary, none of the Rebek controls distinguishes one mechanism over the other. Only the nonbinding esters resolve the question.

As mentioned, it has been shown by Rebek, and confirmed by us, that amide **4** functions as a catalyst at very low concentrations (2 mM) where simple amides (*e.g.* acetamide) have no effect. This fact was used to defend the self-replicative mechanism against the suggestions that amide catalysis plays an important role.[9] In actuality, the argument is without foundation because Scheme 2 predicts the observed concentration dependencies. Since **4** binds to amine 1 in Scheme 2, amide catalysis takes place within a bimolecular complex; catalysis persists at low concentrations via the complex. When the amides lack a binding site (*e.g.* acetamide), the catalysis occurs exclusively by an *inter*molecular process that should diminish in direct proportion to the reduction in amide concentration, as is observed. Assertions to the contrary notwithstanding,[9] Scheme 2 is not invalidated by low concentration data. Our runs with 2 mM reactants confirm this assertion.

Summary

Experiments with non-hydrogen-bonding esters, whose aminolyses are catalyzed by the Rebek "template", show that a termolecular complex is not a necessary intermediate prior to the transition state. An alternative mechanism is proposed which accommodates our two key observations: (a) catalysis by amides and (b) catalyzed aminolysis of nonbinding esters. The new mechanism accommodates a trivial autocatalysis, but it is not "self-replicative" in the usual sense of the word.

Experimental Section and Modeling

Synthesis. Compounds **1**, **2**, and **4** were synthesized as described in ref 2 and gave correct NMR and mass spectra.

Ester 7 was prepared by the reaction of the corresponding acid chloride with pentafluorophenol and triethylamine in methylene chloride and purified by chromatography on silica (EtOAc/hexanes 1:1). Yield 77.4%. ^{1}H NMR ($CDCl_3$, 500 MHz): 8.71 (s, 1H), 8.08 (dd, 1H), 7.94 (d, 1H), 7.85 (d, 1H) 7.58 (d, 1H), 7.28 (dd, 1H), 2.31 (s, 3H). Anal. Calcd for $C_{18}H_9O_4F_5$: C, 57.59; H, 2.29. Found: C, 57.89, H, 2.37.

Ester 8 was prepared by the reaction of *p*-trifluorotoluic acid with pentafluorophenol and DCC/DMAP in methylene chloride and crystallized from methanol/water. Yield 76%. ^{1}H NMR ($CDCl_3$, 500 MHz): 8.27, 7.76 (dd). Anal. Calcd for $C_{14}H_4F_8O_2$: C, 47.21; H, 1.13. Found: C, 47.25; H, 1.19.

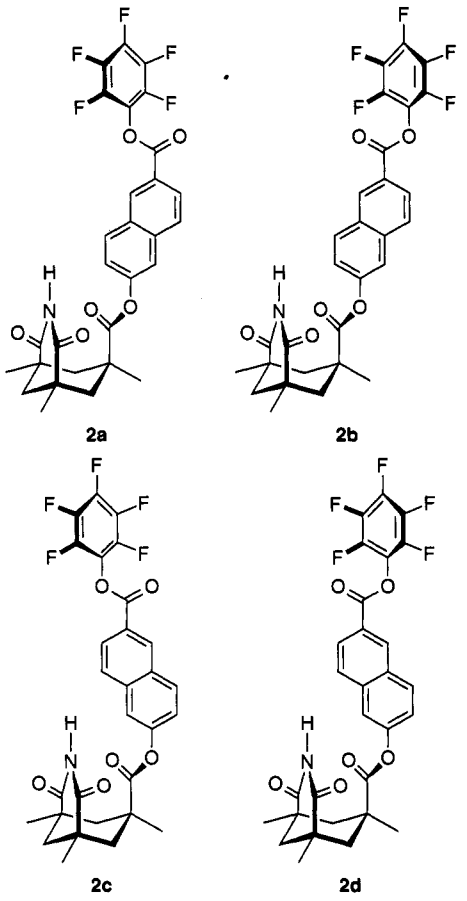


Figure 9. Conformational families of ester **2** used in the 64 minimizations.

Kinetics. Aminolysis reactions were monitored by 470 MHz ^{19}F NMR or 500 MHz ^{1}H NMR on a GN-500 spectrometer. Reactions were carried out by adding amine **1** in $CDCl_3$ to a $CDCl_3$ solution of a pentafluorophenyl ester (**2**, **14**, or **15**) and triethylamine (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. Spectra were recorded in equal time intervals after a 5 min delay needed for shimming and temperature adjustment. From 64 to 512 acquisitions were obtained for each spectrum depending on the substrate concentration. Integrations of the ^{19}F NMR spectra were performed in an absolute intensity mode. Integrations of the ^{1}H NMR spectra were performed in both the absolute intensity mode and using a $CH_3OH/CDCl_3$ solution in a coaxial tube as an external standard (the two methods agreeing to within the experimental error). Signals from pentafluorophenol (10.5 and 9.6 ppm) and from amide **4** (5.26 ppm of the ribose H2′ proton), respectively, were employed. Initial rates among repeat runs never deviated more than 6% from each other (^{19}F NMR) and 12% (^{1}H NMR).

Molecular Modeling. All calculations were performed with the MMX force field[12] 16 in PCMODEL[13] running on a 90 MHz Pentium computer. The program used an MMX default dielectric constant of 1.5 and the default method for treating dipole–dipole interactions of partial atomic charges. Aromatic atoms and the atoms of carbonyls adjacent to aromatic rings were designated as π atoms. The additional potential function for hydrogen bonds was implemented.[14]

In order to limit our conformational search, suitable geometries were restricted to energy minimization of 64 structures

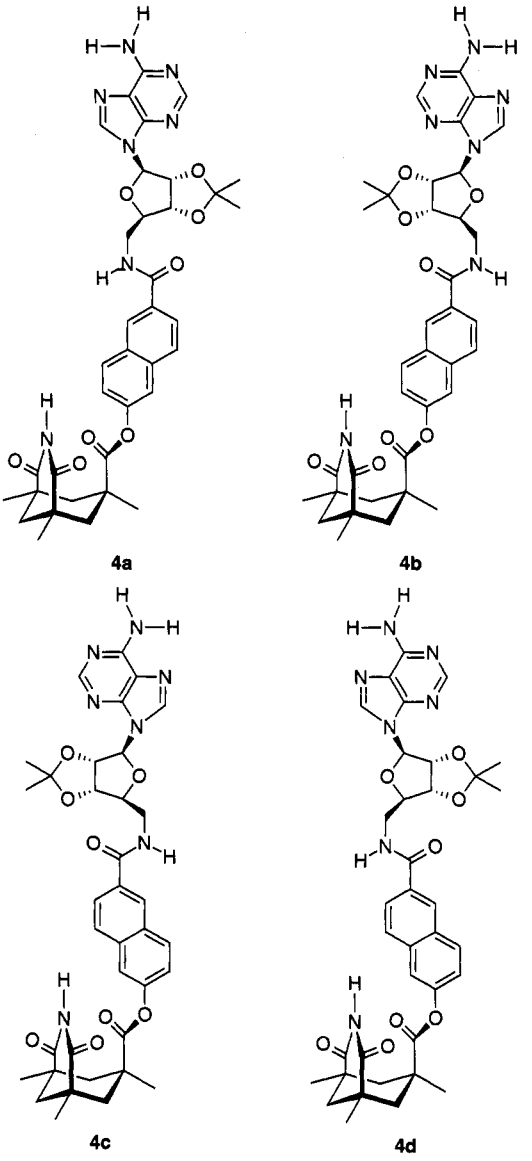


Figure 10. Conformational families of "template" **4** used in the 64 minimizations.

comprising two conformer of **1** (Figure 8), four conformers of **2** (Figure 9), and four conformers of **4** (Figure 10). These families of conformations were selected because they increased the likelihood of discovering complexes that would possess (a) low energies and (b) a short $H_2N{\cdots}C{=}O$ distance. It is important to understand this point. We deliberately used energy-minimized components of the termolecular complex that would *maximize* the chance of substantiating the structure proposed by Rebek. For example, we did not pursue a low-energy conformation of **4** which, owing to intramolecular hydrogen-bonding, is folded rather than fully extended as required for Scheme 1. In other words, if there is a bias in our computations, then it leans toward structure **5**.

In assembling the complex between **1**, **2**, and **4** for optimization, the individual substructures (taken from Figures 8, 9, and 10) were manuevered into position and given the hydrogen-bonding pattern specified by Rebek. The hydrogen-bonded atom pairs were fixed at a distance of 2.0 Å during an initial optimization. This distance constraint was then removed for the final 64 optimizations. The steric bulk of the pentafluorophenyl group and the two *gem*-dimethyl units creates an extremely crowded situation in the region where the amine and ester groups purportedly meet. This fact tends to push the amine and ester groups away from each other. The majority of conformations possess $H_2N{\cdots}C{=}O$ distances that are incompatible with a facile intracomplex reactivity. A

(12) Gajewski, J. J.; Gilbert, K. E. *Adv. Mol. Modeling* **1990**, *2*, 65.
(13) PCMODEL Version 4.0 for DOS. Serena Software, Bloomington, IN.
(14) Calculations were carried out by one of the authors (M.I.S.) whose present address is Sherrod Research Associates, 143 Main St., Farmington, ME 04938.

H_2N–C=O distance of 3.9 Å was the shortest observed. But the corresponding conformer also possessed a relatively high energy and an NH_2–O=C hydrogen bond. The latter precludes a Dunitz trajectory in which the amine attacks from a 110° N/*C*=O/C=*O* angle above the plane of the carbonyl.[15] Steric problems at the reactive center can, to some extent, be relieved by imposing an *s-cis* configuration upon the ester group. But, as already mentioned, this lactone-like configuration elevates the energies by about 6 kcal/mol, and it was not given serious consideration. Among the several structures that had relatively low energies, none had an amine that could reach the carbonyl carbon without passing "through" a *gem*-dimethyl group or grossly distorting the termolecular complex. Full details and tables of the calculations are available on request.

(15) Dunitz, J. D. *X-Ray Analysis and the Structure of Organic Molecules*; Cornell University Press: Ithaca, 1979. Menger, F. M. *Tetrahedron* **1983**, *39*, 1013.

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