The fact that an intermediate in glycolysis is an extremely potent inhibitor of this enzyme, although mechanistically reasonable, raises unexpected questions concerning the location and function of prolidase. Freeze-clamp studies have shown that the physiological concentration of P-enolpyruvate in whole rat kidney, as in other mammalian tissues, is approximately 5×10^{-5} M.[11] Thus, the physiological concentration of P-enolpyruvate is roughly 6000-fold higher than the K_i value that we observed for inhibition of prolidase. The concentration of enzyme in whole kidney, based on the purification procedure, is in the neighborhood of 10^{-7} M.[12] The K_m value observed for a typical substrate, Gly-Pro, is 1.7×10^{-3} M, so that extremely high substrate concentrations, well in excess of 1 M, would be needed to completely overcome the inhibitory effects of intracellular P-enolpyruvate.[13]

Earlier evidence suggests that prolidase is located in the cytoplasm,[4,5] but its existence at other locations does not appear to have been excluded. Recent work has identified another renal peptidase, which does not act on proline-containing dipeptides, as an ectoenzyme anchored to the cell surface through phosphatidylinositol.[14] In a similar location, prolidase would presumably escape inhibition by cytoplasmic P-enolpyruvate. The enzyme might then be susceptible to inhibition by ionized compounds approaching from the cell's exterior, simplifying the design of potent inhibitors that would be active in vivo. These possibilities remain to be explored.

Acknowledgment. We are grateful to the National Institutes of Health (Grants GM-18325 and AI-25697) for support of this work.

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(12) Davis, N. C.; Smith, E. L. *J. Biol. Chem.* **1957**, *224*, 261.
(13) An externally introduced competitive inhibitor, even if it were bound as tightly as P-enolpyruvate, would need to be present at extremely high concentrations in the cytoplasm to produce any noticeable effect.
(14) Hooper, N. M.; Low, M. G.; Turner, A. J. *Biochem. J.* **1987**, *244*, 465.

A Self-Replicating System

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The ability of nucleic acids to act as templates for self-replication has been unique. In living systems, single strands act as templates during phosphate transfer reactions. In nonenzymatic systems, autocatalysis[1] can be observed during these reactions. Here we show that base pairing can also enhance acyl transfer reactions.

The reaction involves the coupling of the amino adenosine **1** to the pentafluorophenyl ester **2a** (Scheme I), and its shows the following features:

1. *The reaction exhibits autocatalysis.* Addition of the product **3** the reaction mixture results in an increase in the initial coupling rate (Figure 1, entries c-e).

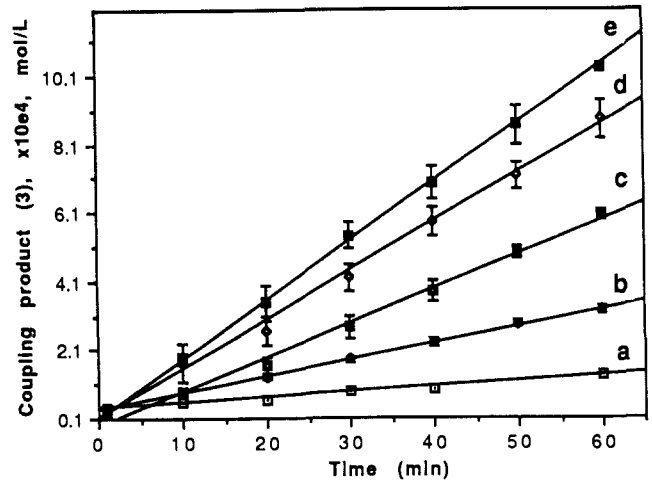
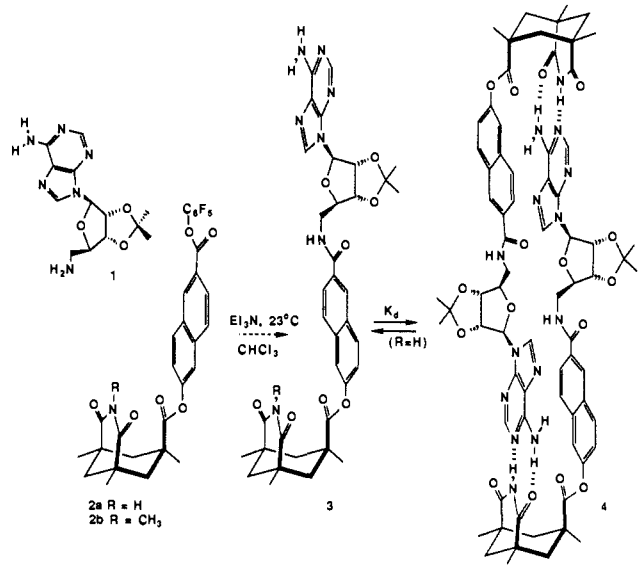


Figure 1. Initial rates of product formation (Scheme I). Plots of initial appearance of coupling products vs time as determined by HPLC. All reactions were performed with initial concentrations of [**1**] = [**2**] = 8.2 mM in $CHCl_3$ with 4 equiv of Et_3N added as a general base. Each run was independently performed in triplicate, and error bars represent standard deviations for each point. (a) Reaction of **1** and the N-methylated **2b**. (b) Reaction of **1** and **2a** with 1 equiv of 2,6-bis(acylamino)pyridine. (c) Reaction of **1** and **2a**. (d) Reaction of **1** and **2a** with 0.2 equiv of **3** added as autocatalyst. (e) As in d with 0.5 equiv of **3**.

Scheme I



2. *The product is self-complementary.* Dimerization occurs (**3** → **4**, Scheme I), and the value measured by NMR dilution studies[2] for the dimerization constant is $K_d = 630$ M^{-1}. This value is consistent with expectations involving base pairing and aryl stacking, with some attenuation by steric effects in the middle of the structure.[3]

3. *The reactions of **2a** proceed through the formation of base-paired complexes.* The imide **2a** reacts some 10 times faster than the *N*-methyl derivative **2b**, a factor that can hardly be attributed to classic steric effects at a site so remote from the reacting centers. Moreover, 2,6-bis(acylamino)pyridine acts as a competitive inhibitor by forming a nonproductive complex, **5** (Scheme II). Titration of **2a** with 5-*O*-acetyl-2′,3′-*O*-iso-

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(1) Pitha, P. M.; Pitha, J. *Nat. New Biol.* **1972**, *98*, 78-80. von Kiedrowski, G. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 932-935. von Kiedrowski, G.; Woltzka, B.; Helbing, J. *Ibid.* **1989**, *28*, 1235-1237. Zielinski, W. S.; Orgel, L. E. *Nature* **1987**, *327*, 346-347. Visscher, J.; Bakker, C. G.; van der Woerd, R.; Schwartz, A. *Science* **1989**, *244*, 329-331. For double-stranded template effects in *triple-helix* formation, see: Luebke, K. J.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 8733-8735.

(2) Wilcox, C. S.; Cowart, M. D. *Tetrahedron Lett.* **1986**, *27*, 5563-5566.
(3) Williams, K.; Askew, B.; Ballester, P.; Buhr, C.; jeong, K. S.; Jones, S.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1090-1094. Only Watson-Crick forms of base pairing are shown, but NOE experiments[4] indicate the presence of Hoogsteen forms as well.
(4) See, for example: Askew, B.; Ballester, P.; Buhr, C.; Jeong, K. S.; Jones, S.; Parris, K.; Williams, K.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1082-1090.

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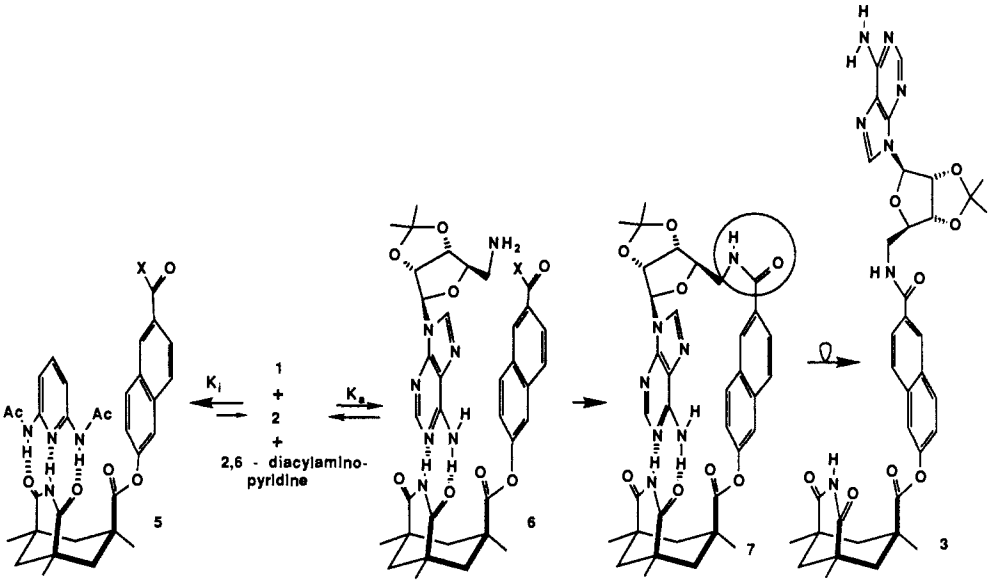
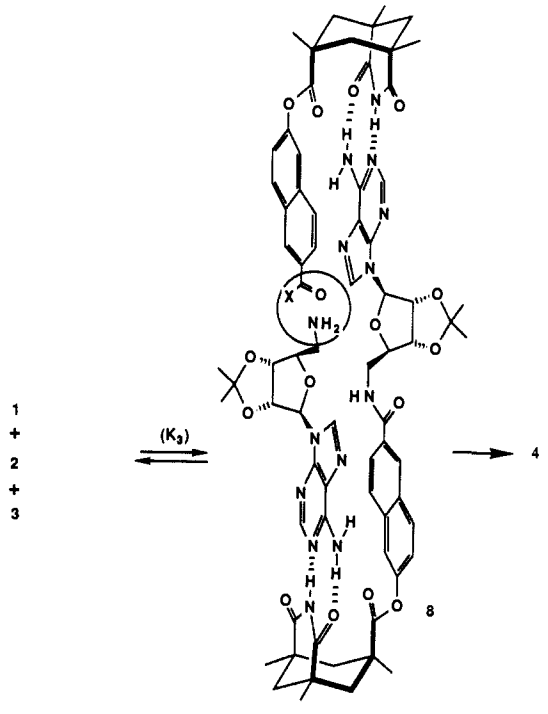
Scheme II



Scheme III

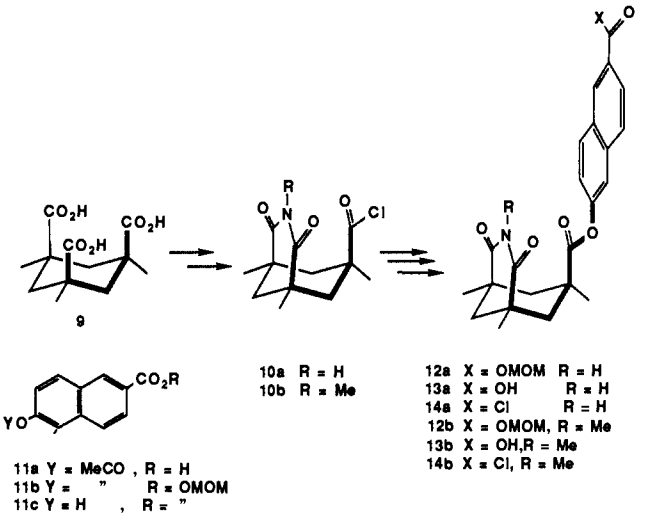


propylideneadenosine gave an association K_a of 60 M^{-1} under these conditions, a value that serves as a reasonable estimate of K_a for the formation of complex **6**.

4. *The initial product of the intramolecular acyl transfer is a cis amide.* Modeling shows that the distance between the hydrogen-bonding surface and the active ester can be spanned only by such a conformation of the amide. The product does not appear to remain in this conformation; rather, cis → trans isomerization occurs (**7** → **3**, Scheme II) and the hydrogen-bonding surfaces become exposed for dimerization or autocatalysis.

Three mechanisms are involved in the reaction of **1** with **2a**: the slow aminolysis of Scheme I, the more efficient base-paired version of Scheme II, and the autocatalytic component of Scheme III. An estimate can be made for the formation of the termolecular complex **8**. Assuming independent (noncooperative) binding sites on the template **3**, the value for K_3 should be K_a^2 or 3600 M^{-2}. Using this figure, one can calculate that only about 2% of **1** and **2a** are in the form of **8** under the autocatalytic conditions (runs d and e, Figure 1) while about 25% are in the form of the base-paired **6**. The observed enhancements (>40% in rate) caused by added **3** suggest that the intramolecular acyl transfer of Scheme III is very efficient, indeed.

Scheme IV



The synthesis of the molecules involved is outlined in Scheme IV. Kemp's[5] triacid **9** was converted to the imide acid chloride **10a** as previously described.[3] Coupling[6] of **10a** to the MOM ester **11c** followed by deblocking (H_3O^+, acetone) and activation ($SOCl_2$) gave the acid chloride **4a**, from which **2a** was prepared (HOC_6F_5/Et_3N). A parallel sequence gave **2b** from **10b**.

In summary, we have observed autocatalysis in a self-replicating system.[7] At best, this can be regarded as a primitive sign of life; at the very least, the system offers a bridge between the information of nucleic acids and the synthesis of amide bonds. It should be possible to design systems capable of peptide synthesis on a nucleic acid backbone and thereby provide models for events that occurred some time ago.

Acknowledgment. We thank the National Science Foundation for support and David Chalfoun for help with computations.

(5) Kemp, D. S.; Petrakis, K. S. *J. Org. Chem.* **1989**, *46*, 5140–5143. Commercially available from the Aldrich Chemical Co.

(6) All new compounds were characterized by a full complement of high-resolution spectra; details will be published elsewhere. The amine **1** was obtained as described by Kolb et al.: Kolb, M.; Danzin, C.; Barth, J.; Claverie, N. *J. Med. Chem.* **1982**, *25*, 550–556.

(7) For a recent, beautiful example of a template-directed substitution reaction, see: Kelly, T. R.; Zhao, C.; Bridger, G. J. *J. Am. Chem. Soc.* **1989**, *111*, 3744–3745.