HETEROCYCLES IN REPLICATION AND ASSEMBLY

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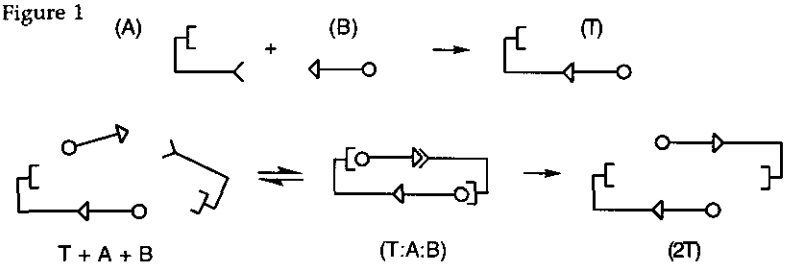
Abstract -- The role of molecular complementarity and self-complementarity in recognition, replication and assembly is reviewed.

Progress on Self-Replicating Molecules

Template effects lie at the heart of many chemical and biological processes, and they may be loosely defined as the enhancement of reactions by complementary surfaces.[1] Complementary in this sense is intended to mean of interlocking size, shape and chemical features, and this type of complementarity is involved in most molecular recognition phenomena. A very unique form of templating may occur when two complementary molecules are coupled. This is shown schematically in Figure 1.

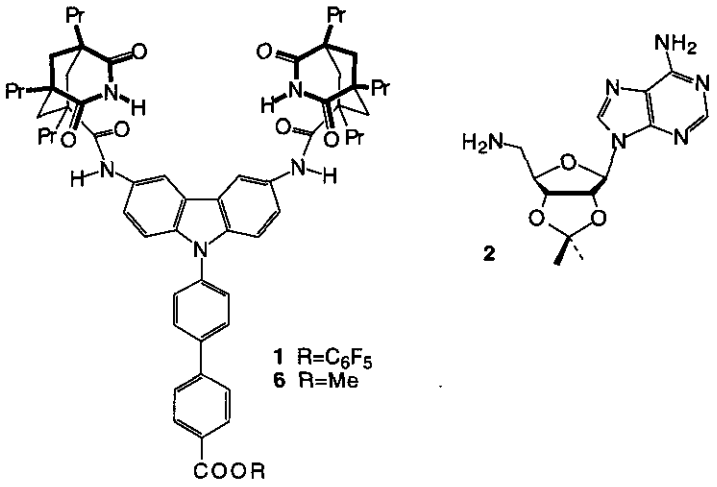
Figure 1



If the product (T) enhances its own formation from components (A) and (B), that is, if it shows autocatalysis, then *replication* is the result.[2] In earlier work we were able to show this was the case for our autocatalytic replicating systems,[3] and this had also been established for

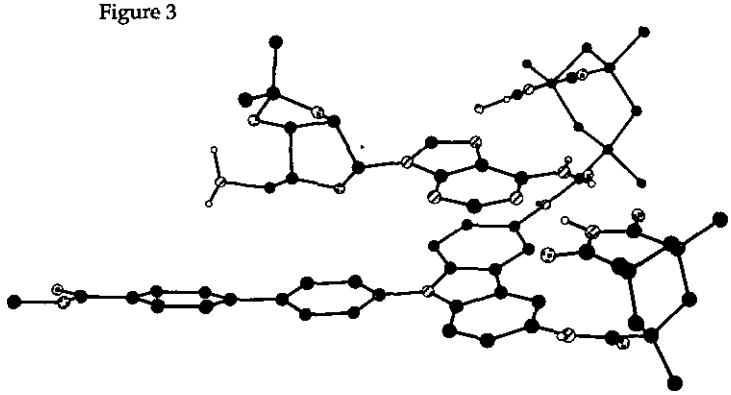
short nucleic acid replicators.[4] Here we discuss some of our second generation efforts in synthetic self-replicating molecules.

Figure 2



Templating effects place a premium on precise positioning of the reactive components, and nowhere was our knowledge of positioning as refined as in the recognition of the purine nucleus of adenine (Figure 2). Specifically, we had devised bis-imides attached to a carbazole skeleton for simultaneous Watson-Crick and Hoogsteen base-pairing to the purine nucleus.[5] These forces chelate the purine nucleus of adenine and hold it tightly; its position is well-defined in space. Functionalities attached to the purine nucleus and to the carbazole nitrogen are then on parallel trajectories. A rigid spacer element, a 4,4'- biphenyl, was attached to the carbazole nitrogen. When substituted in its terminal position by an electrophile, as in **1** , the distance between the carbazole nucleus and the electrophile remains constant regardless of rotations around single bonds. When the adenine derivative that is chelated by the imides on the carbazole is the adenosine amine (**2**), the nucleophilic amino group at the 5' position is unable to reach the electrophile at the end of the biphenyl spacer. This is illustrated in the energy minimized structure of Figure 3.

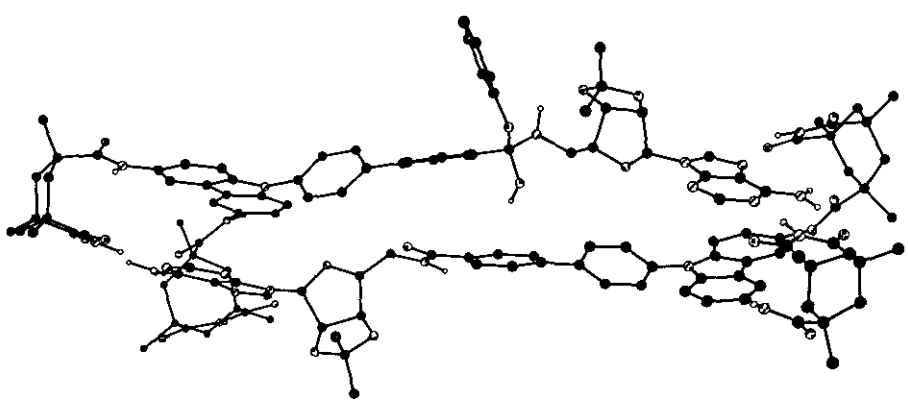
Figure 3



A corollary is that when reaction does take place between **1** and **2**, it does so in an intermolecular rather than an intracomplex way. The product (**3**) (Figure 6) cannot "fold shut". Instead, *it must stay open and express its self-complementarity* by dimerization. Very high affinities (on the order of $>10^5\ M^{-1}$) were observed for this dimerization in a non-competing solvent such as chloroform. Even in the more competitive DMSO, the dimerization constant was $>100M^{-1}$.

The monomeric form of the self-complementary product can act as a template. It can gather the two components from the bulk solution using the weak intermolecular forces of molecular recognition, and can position them such that the entropic barrier to reach the tetrahedral intermediate is lowered. This tetrahedral intermediate is modeled in Figure 4.

Figure 4



The autocatalysis in this system was established in studies involving THF-$CHCl_3$ solvent mixtures. When 0.5 equiv. product (**3**) is added to the reaction solution, a 55% increase in rate is observed (Figure 5). Control experiments with a number of likely chemical entities showed the importance of the templating effects. For example, the imide functions are not responsible for autocatalysis, since the methyl ester (**6**) (Figure 5) showed no increase in reaction rate. In fact, rate decreases are observed because **6** binds the adenine in unproductive complexes. Other imides (**4**) or amides (**5**) are also ineffective at catalysis. Previous studies[3] had already shown that neither the purine nor the adenosine contributes to the autocatalysis.

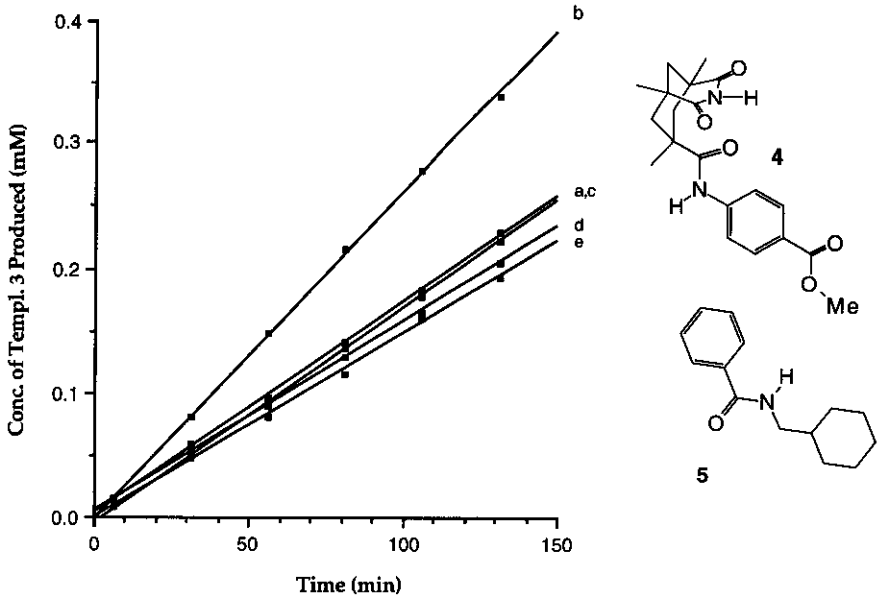
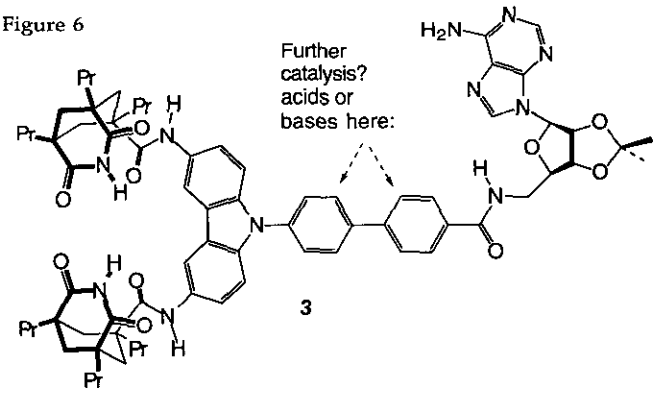


Figure 5 Generation of template (**3**) as a function of time. All reactions were performed at 6.2 mM initial concentrations of reactants (**1**) and (**2**) in 13% THF/$CHCl_3$ with 1% TEA base added. Data points are averages of multiple runs, with deviation error <5%. -- a) Baseline reaction (**1**) + (**2**); b) Baseline reaction plus 0.5 eq. Template (**3**); c) Baseline reaction plus 0.5 eq. Imide Me Ester (**4**); d) Baseline reaction plus 1.0 eq. Amide (**5**); e) Baseline reaction plus 0.5 eq. Di-imide Me Ester (**6**).

Such studies point strongly to template effects as the likely mechanism of autocatalysis: the intact template is more effective than the sum of its parts. How can further improvements be made in self-replicating molecules? In this and earlier systems, the product molecules work to reduce the entropy of activation for the coupling reaction; on the template surface, the two components find each other with greater probability then they do in bulk solution. Otherwise, the template is quite passive. It would be useful to place catalytically active functions along the mechanistic pathway of the replication step, i.e. to place acids, bases, or hydrogen-bonding elements on the molecular skeleton where they are likely to enhance the coupling step. Such functional groups are known to catalyze acyl transfers by aiding in the formation and collapse of tetrahedral intermediates.

We intend to attach such functionalities in the region where amine and ester come together on the template surface. In the biaryl skeleton this would correspond to the ortho positions indicated in Figure 6. The corresponding bipyridine derivative may also be useful to present metal ions to the intermediates of the coupling step. Another intent is to combine the high binding affinities observed between the bisimides on the carbazole skeleton with the new Kemp triacid derivatives which impart water solubility.[7] These should give a system capable of replication in that most biorelevant medium.

Figure 6



3

Assembly

One of the new directions that research in molecular recognition has taken in this decade is toward the area of supramolecular assemblies. The number of these systems is too large to review here, but includes a series of two component systems: melamine and cyanuric acid, metals with bipyridyls, diacids with aminopyridines.[8] In all of these, molecular complementarity leads to assembly. In addition, a few self-complementary structures have been devised.[9]

Figure 7

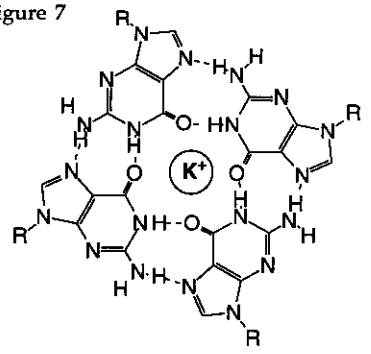
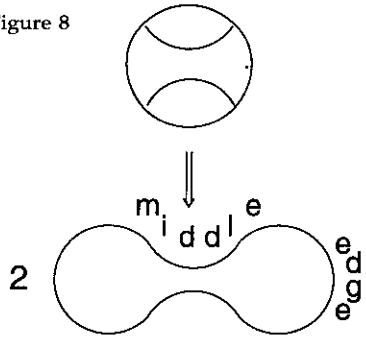


Figure 8

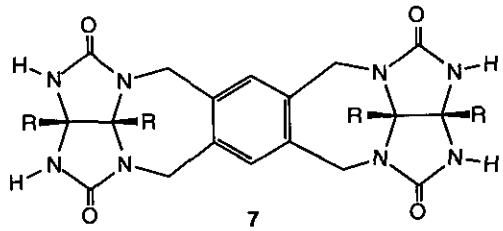


Our own interest in minimalist constructs suggested that self-complementarity is ideal for assemblies. Nature makes great use of self-complementary molecules to create cavities for transport or catalysis. Viral coat proteins are an example of the former, whereas allosteric enzymes are examples of the latter. A particularly well-studied case is the G-quartet (Figure 7), formed when guanines assemble around a metal ion.[10]

Our approach was to develop synthetic modules which can assemble reversibly to create closed-shell cavities. In this we were inspired, at least notionally, by a tennis ball. When it is cut along its seam, two identical pieces are obtained, the edges of which are complementary to the middles (Figure 8). The curvature involved in each piece then dictates the overall spherical shape of the dimeric assembly.

To reproduce these features of curvature and self-complementarity with synthetic molecules, we looked to the structure (7) shown in Figure. 9. It is prepared in a single operation from readily available materials.[10] The structure features an array of hydrogen bonding donor and acceptor sites along its edge. The *cis* fusion of the two 5- membered rings provides curvature in the width of the structure, whereas the *cis* fusion of the two 7- membered rings to the benzene spacer provides the gentle convex curvature along the length of the molecule. The rigidity of this structure positions the hydrogen bonding sites in a self-complementary manner, and dimerization occurs. The dimer is held together by eight hydrogen bonds along the "seam".

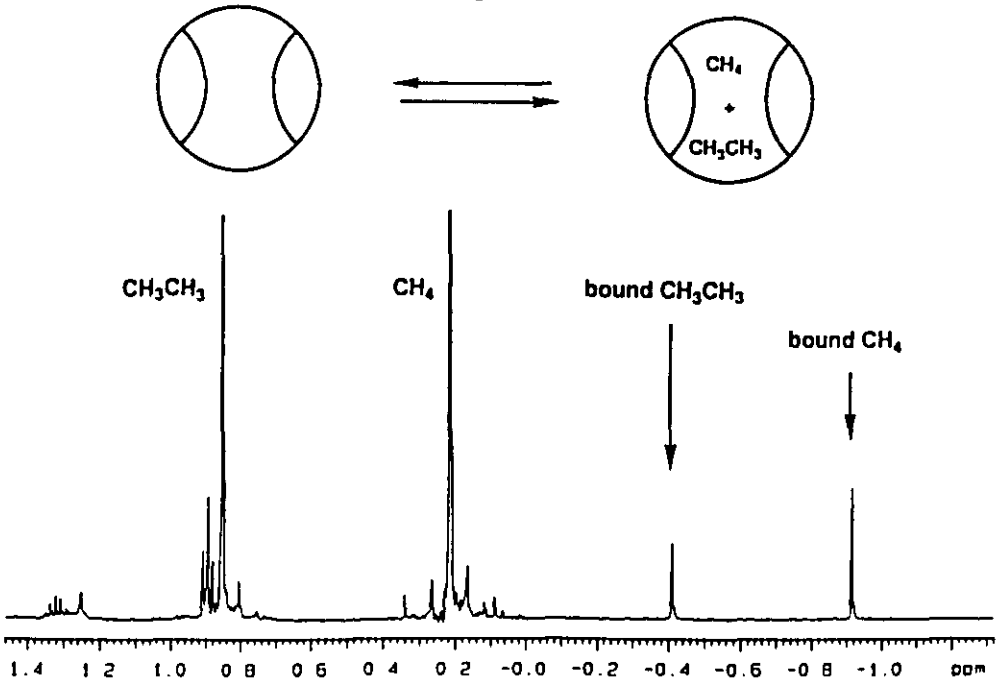
Figure 9



7

Vapor pressure osmometry and mass spectrometry under various conditions indicated that the predominant state of **7** in chloroform or in the gas phase was dimeric. Preliminary X-ray data, obtained by Dr. Carolyn Knobler at UCLA, supported this structure in the crystalline state. The binding behavior of the dimer in solution justified our premise of assembly as a strategy for inclusion. Nmr experiments established that in $CDCl_3$, two forms of the dimer are present. One is empty, (or includes dissolved gases such as N_2) while one presumably includes solvent. The exchange of these two forms is slow on the nmr time scale because separate signals are seen for several of the resonances. However, the equilibrium between empty and full forms is temperature dependent, and equilibrium constants can be obtained by integration of the spectra.

Figure 10



When small molecules are added to the $CDCl_3$ solution, signals for a third dimeric species appear. A particularly favorable case involving methane and ethane is shown in the nmr spectrum (Figure 10). The signals of the encapsulated guests can be directly observed, methane being at -0.9ppm.[11] Other molecules that have been captured are ethylene and CH_2Cl_2. Using these nmr titrations, we were able to obtain a table of binding affinities, and these are given below.[12]

Inclusion of Guest Species in the Cavity of the Dimer of (7)

Guest	K_{inc} (M^{-1}) at 273°K	range (K)	ΔH (kcal/mol)	ΔS (e.u.)	Guest vol. ($Å^3$)
$CHCl_3$	.04	243-298	-7	-31	73
CH_2Cl_2	4	248-298	-13	-45	58
$H_2C{=}CH_2$	280	263-298	-11	-30	40
CH_4	300	248-298	-9	-20	28

The following trends can be seen: 1) the inclusion of all guests involves a large entropic cost, and this cost is paid for by van der Waal's forces between the concave inner surface of the dimer and the convex outer surface of the guest species; 2) slow exchange is seen for all of the guests, and this probably reflects a common exchange mechanisms--the dimer must be dissociated before the guest can enter or escape; 3) given estimates of the guests' volume, it is probable that the volume of the cavity created in the dimer is ~ 50-60 Å^3.

Reversible assembly as a means of encapsulating small molecules in solution has now been established, and provides an alternative to macrocyclic compounds such as cryptands,[13] cryptophanes[14] and carcerands[15] that have been previously developed. Several molecular systems that are assembled with likely cavities have been devised in other laboratories,[16] and we have identified a number of other self-complementry structural modules. They feature bigger cavities and appear large enough to encapsulate some of our self-replicating molecules. We will report on these in due course.

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