Reciprocal Template Effects in a Replication Cycle**

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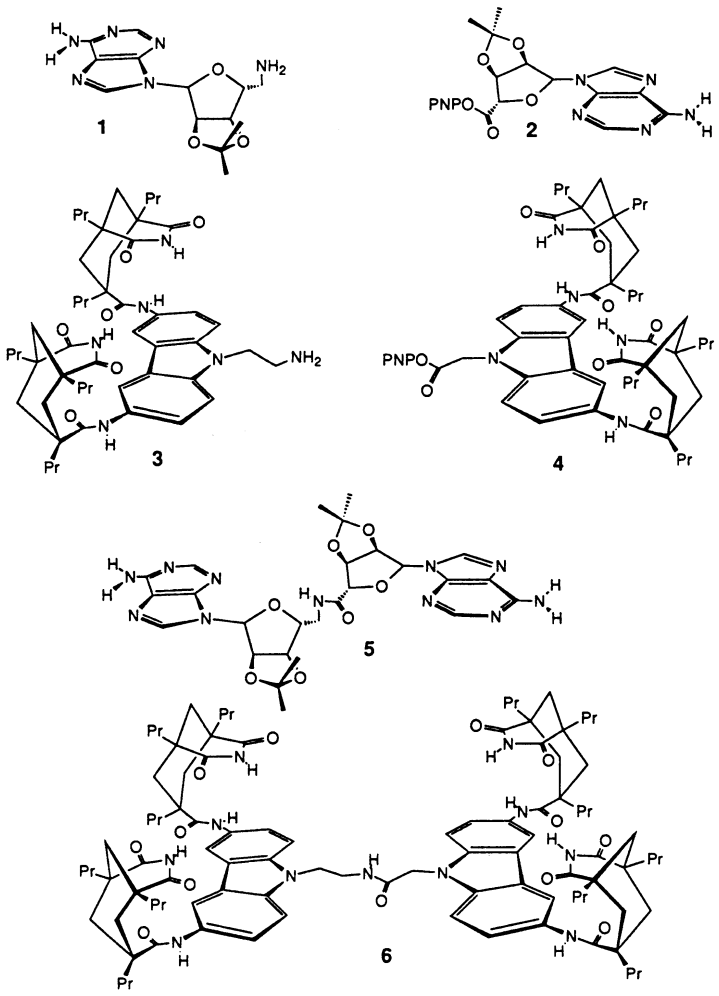
The enhancement of chemical reactions by complementary surfaces—template effects—is widespread in biological and chemical processes.[1] Nucleic acid replication is the typical example: one strand acts as a template for the other's formation. We have earlier described self-complementary structures[2] and their ability to act as templates in self-replicating systems and as subunits for molecular assemblies. Here we show that template effects operating in a reciprocal sense, in a replication cycle, can be even more efficient than the self-replicating systems.

The molecular recognition involved is the chelation of the purine nucleus of adenine by two imides attached to a carbazole surface. The imides provide simultaneous Watson–Crick and Hoogsteen base pairing, while the carbazole and the purine interact by π stacking. High binding affinities between these two components ($K_a = 10^4 - 10^5\ M^{-1}$) are observed in organic solvents.[3] We have now outfitted the two components with complementary chemically reactive functions, nucleophilic amines and electrophilic activated esters, for covalent coupling reactions. Specifically, amines **1** and **3** and *p*-nitrophenyl esters **2** and **4** were prepared (Scheme 1). The slow reaction of amine **1** with ester **2** (each 0.05 mM) in chloroform in the presence of triethylamine produced **5**.[4] The initial rate was 1.5×10^{-8} M min^{-1} (Table 1). Likewise, amine **3** and ester **4** gave **6** at an initial rate of 4.3×10^{-9} M min^{-1} under the same conditions.



Scheme 1. Amines (**1** and **3**), esters (**2** and **4**), and their coupling products (templates) **5** and **6**. PNP = *p*-nitrophenyl.

The accelerating effects of templates in these bisubstrate reactions were observed as follows: in the presence of the receptor **6**, **1** and **2** reacted to give **5** at a rate up to 12 times that of the background reaction.[5] As shown in Table 1, the acceleration was reduced by the competitive binding partner 9-ethyladenine and by the actual product **5**. Maximum rate acceleration was observed when two equivalents of the template **6** were used (Fig. 1).[6] Addition of further amounts of template led to lower coupling rates; the two reactive components form complexes on different template molecules and become increasingly separated. In control experiments with one equivalent of **7** (Scheme 2), a molecule that bears many of the structural and functional

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Table 1. Initial rates v_0 of amide formation.

Reaction[a]	c(5) [mM]	c(6) [mM]	c(7) [mM]	c(8) [mM]	c(9-Et-Ad) [mM]	v_0[b] [10^{-9} M min^{-1}]
1 + 2						15
1 + 2		0.05				150
1 + 2	0.05	0.05				42
1 + 2		0.05			0.5	30
1 + 2			0.05			14
1 + 2				0.05		11
3 + 4						4.3
3 + 4	0.05					23
3 + 4	0.05	0.05				13
3 + 4	0.05				0.5	15
3 + 2						53000
1 + 4						2200

[a] $c(1) = c(2) = c(3) = c(4) = 0.05$ mM, $c(Et_3N) = 4$ mM. Solvent: $CHCl_3$, $T = 25$ °C. [b] Averaged values from multiple independent runs; standard deviations ±15%.

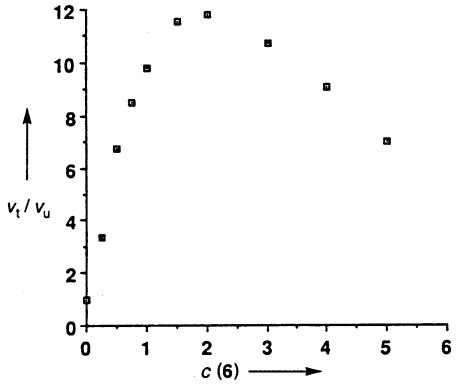
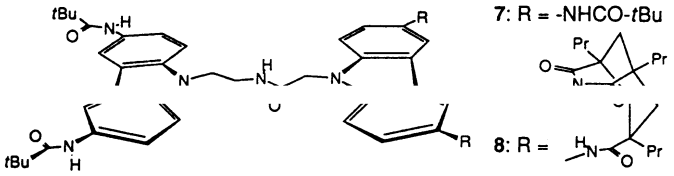


Fig. 1. The acceleration of the initial rate of the reaction between **1** and **2** (template catalyzed rate: v_t; uncatalyzed rate: v_u) as a function of the concentration of **6** (in equivalents). $c(1) = c(2) = 5 \times 10^{-5}$ M, $c(NEt_3) = 4 \times 10^{-3}$ M, solvent: $CHCl_3$, $T = 25$ °C.

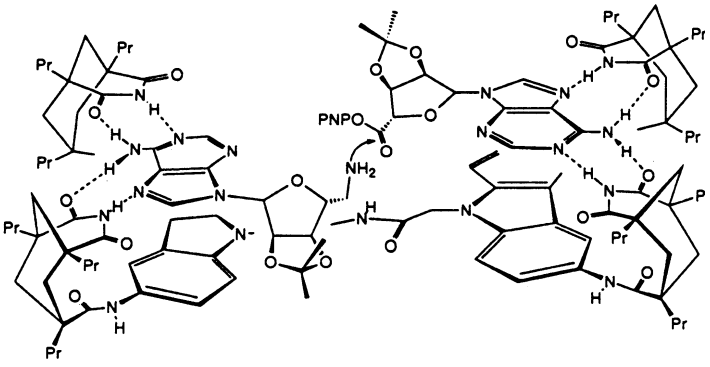


Scheme 2. Structures of molecules used as controls.

features of the template but lacks the recognition elements, no rate enhancement in the reaction of **1** with **2** was observed. Similarly, addition of one equivalent of **8**, containing one binding site, did not increase the coupling rate. These observations can be explained by the formation of a termolecular complex (Scheme 3).[7]

The reciprocal template, product **5**, proved capable of catalyzing the coupling of **3** and **4**. The observed rate enhancement over the uncatalyzed reaction was fivefold, and again, product inhibition with **6** and competitive inhibition with 9-ethyladenine was observed.

The rate enhancements here are comparable to those observed by Kelly[1d, e] for templates for bimolecular S_N2 reactions and are considerably larger (by an order of magnitude) than those observed for template effects involving *self*-complementary structures.[2] The reciprocal templating effects of **5** and **6** formally represent a replication cycle. Whether these cycles are generally more efficient than the minimalist self-complementary replicators is the subject of ongoing research.



Scheme 3. The proposed termolecular complex responsible for the enhanced rates for the reaction of **1** with **2**.

In the meantime, we note that large rate accelerations can be achieved in complexes of a bimolecular sort.[8] Thus, reaction of amine **3** with active ester **2** at concentrations of 0.05 mM was 3500 times faster than the reaction of **1** with **2**. The reaction of amine **1** with ester **4** was 500 times faster than the reaction of **3** with **4**. In both cases, the products are self-complementary, but due to the flexibility of the spacers between the reactive functions, they remain folded shut in intramolecular complexes.[9]

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[4] The rate was determined by monitoring the release of *p*-nitrophenolate spectrophotometrically at 330 nm. Typically reactions were monitored to at least 30% conversion, and initial rates were obtained for up to 10% conversion.

[5] The products were analyzed as follows: two solutions were prepared both 0.1 mM **1** and **2** and 8 mM NEt_3 (in 20 mL $CHCl_3$). One of these also contained 0.1 mM **6**, which led to an initial rate eight times faster than that without **6** (determined spectrophotometrically). After 72 h the solvent and NEt_3 were removed, and the residues were redissolved in [D_6]DMSO and analyzed by ^{1}H NMR spectroscopy. In both cases **5** was the only observed product.

[6] Due to the low concentrations of the reagents used and binding constants of $K_a = 10^4 - 10^5$ M^{-1}, the receptors are not fully saturated with guests. Therefore the maximum is not at one equivalent of template **6**.

[7] A referee suggested that these control experiments show that catalysis by simple amides as proposed by Menger et al. (F. M. Menger, A. V. Eliseev, N. A. Khanjin, *J. Am. Chem. Soc.* **1994**, *116*, 3613) can be excluded. Menger's experiments showed that high concentrations (generally 30 mM) are required for simple amides to exert their (small) catalytic effects, whereas accelerations due to a template in the same system are operative at concentrations as low as 0.33 mM [2a]. Template effects are highly dependent on the magnitude of the association served at concentrations orders of magnitude lower than required for simple amide catalysis.

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