Role of Geometrical Factors in Template Effects

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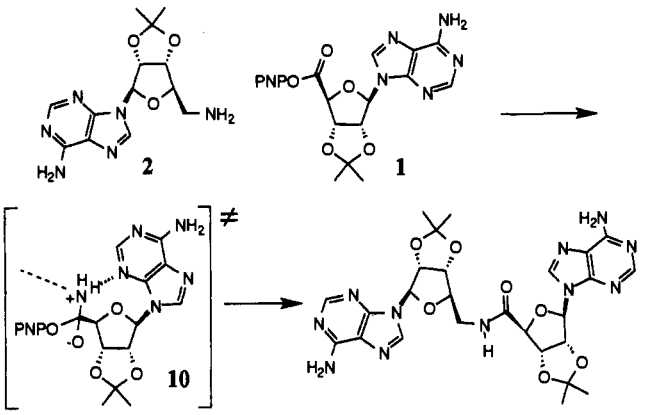
Catalysis is a popular research topic that accommodates approaches that range from stochastic to mechanism-based design.[1] One approach takes advantage of the forces of molecular recognition to position the reaction partners in proximity to each other or to catalytic functions.[2] To us, adenine recognition with synthetic receptors represents the most refined level of binding information, and we have used this in studies of catalysis in self-replicating and reciprocal replicating systems.[3] Here, we explore some geometrical factors involved in template effects with adenine derivatives.

The recognition event involves the chelation of the purine nucleus of adenosines by synthetic receptors based on Kemp's triacid and a carbazole spacer element. High affinities of these receptors for adenine in nonpolar solvents result from the additive incremental effects of hydrogen bonding and aromatic stacking.[4] Chelation of the purine nucleus between the imide functions results in an unambiguous geometry of the complex, allowing the positioning of catalytic groups or a reaction partner with some predictability.

The reaction involves the aminolysis of highly reactive *p*-nitrophenyl (PNP) ester **1** by aminoadenosine **2** in $CHCl_3$ (Scheme 1).[5] A series of carbazole derivatives bearing two receptor sites separated by various spacers was prepared (see Table 1).[3b,4,6,7] The effect of these molecules (1 equiv) on the aminolysis rate varied considerably from none to 160-fold acceleration.

When both the active ester and the amine are bound by a single template molecule and held in close proximity within the template cavity, high effective molarities within the termolecular complex result in faster reactions.[2,3] This mechanism is depicted

Scheme 1



Scheme 2

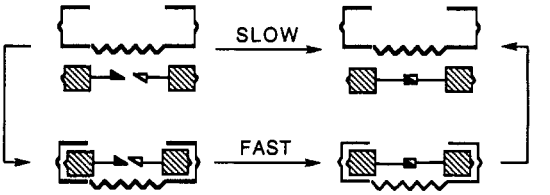
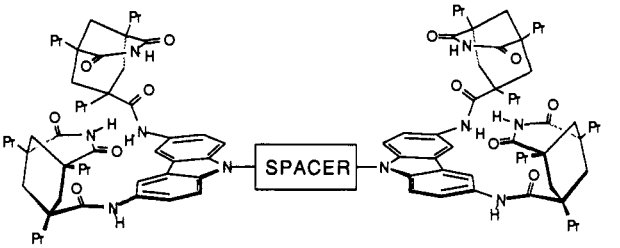
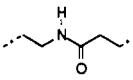
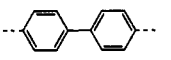
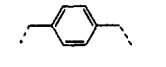
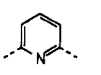
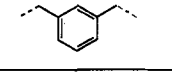
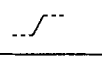


Table 1. Ratio of Initial Coupling Rates of **1** + **2** in the Presence and Absence of 1 Equiv of Potential Templates (Observed Acceleration)



	SPACER	Obs. Accel.			Obs. Accel.
			6		10
3		1.0	7		116
4		1.2	8		160
5		5.4	9		31

in Scheme 2; the zigzag represents the spacers of Table 1, and the template is arranged in the desirable C-shaped conformation.

The variable elements in the series of potential templates **3–9** are (1) the relative orientation of the two receptor sites induced by the spacer and the overall *shape* of the molecule; (2) the *distance* between the receptor sites associated with this shape; and (3) the *rigidity* of the molecule, or its propensity to stay or not to stay in a particular conformation corresponding to a shape and a distance. The nonpolar nature of the spacers allows the observed accelerations essentially to be attributed to these three elements. Control experiments established that catalysis by polar stabilization of the tetrahedral intermediate, if present, is small in comparison to the large effects discussed here.[8]

Ester **1** is about 10^3 times more reactive than *p*-nitrophenyl acetate under the same conditions. It is proposed that an

(1) For progress on several fronts, see: (a) Schultz, P. G.; Lerner, R. A. *Acc. Chem. Res.* **1993**, *26*, 391. (b) Beaudry, A. A.; Joyce, G. A. *Science* **1992**, *257*, 635. (c) Andersson, P. G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1993**, *115*, 7047. (d) Li, Z.; Conser, K. R.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1993**, *115*, 5326. (e) Kitamura, M.; Tokunaga, M.; Noyori, R. *J. Am. Chem. Soc.* **1993**, *115*, 144. (f) Burk, M. J.; Feaster, J. E.; Nugent, W. A.; Harlow, R. L. *J. Am. Chem. Soc.* **1993**, *115*, 10125. (g) Nguyen, S. T.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 9858. (h) O'Dell, R.; McConville, D. H.; Hofmeister, G. E.; Schrock, R. R. *J. Am. Chem. Soc.* **1994**, *116*, 3414.

(2) (a) Jubian, V.; Dixon, R. P.; Hamilton, A. D. *J. Am. Chem. Soc.* **1992**, *114*, 1120. (b) McCurdy, A.; Jimenez, L.; Stauffer, D. A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1992**, *114*, 10314. (c) Mackay, L. G.; Wylie, R. S.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1994**, *116*, 3141. (d) Walter, C. J.; Anderson, H. L.; Sanders, J. K. M. *J. Chem. Soc., Chem. Commun.* **1993**, 458. (e) Kelly, T. R.; Zhao, C.; Bridger, G. J. *J. Am. Chem. Soc.* **1989**, *111*, 3744. (f) Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Adhya, M. *J. Org. Chem.* **1989**, *54*, 5302. (g) Terfort, A.; von Kiedrowski, G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 654.

(3) (a) Pieters, R. J.; Huc, I.; Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1579. (b) Pieters, R. J.; Huc, I.; Rebek, J., Jr. *Tetrahedron*, in press. (c) Conn, M. M.; Wintner, E. A.; Rebek, J., Jr. *J. Am. Chem. Soc.*, in press.

(4) (a) Conn, M. M.; Deslongchamps, G.; de Mendoza, J.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 3548. (b) Pieters, R. J.; Rebek, J., Jr. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 330.

(5) All reactions were performed at 25 °C, in $CHCl_3$, in the presence of 4 mM Et_3N and with [**1**] = [**2**] = 0.05 mM, in a quartz cuvette (1 cm), and monitored spectrophotometrically at 330 nm. The reactions were generally followed to at least 80% completion. Initial rates were determined from the first 10% of reaction. Reactions were run in duplicate or triplicate, and numbers were averaged. Reactions were run with and without amine nucleophile to ensure that the *p*-nitrophenol release was due to aminolysis rather than hydrolysis from residual water or undetected impurity.

(6) Kato, Y.; Conn, M. M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1994**, *116*, 3279.

(7) All new compounds were characterized by ^1H NMR, HRMS, and IR. Experimental details will be published elsewhere.

(8) Su, C.-W.; Watson, J. W. *J. Am. Chem. Soc.* **1974**, *96*, 1854. This study showed that relatively high concentrations of hydrogen bond donors can accelerate the butylaminolysis of *p*-nitrophenyl acetate in chlorobenzene.

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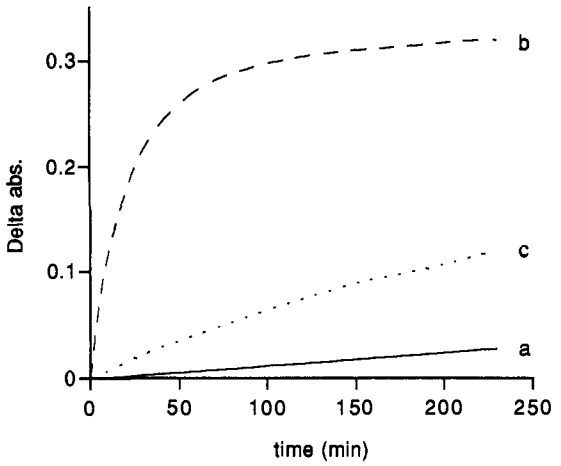

Figure 1. Coupling of **1** and **2** in $CHCl_3$ with (a) no additives, (b) 1 equiv of **8**, and (c) 1 equiv of **8** + 10 equiv of 9-ethyladenine.

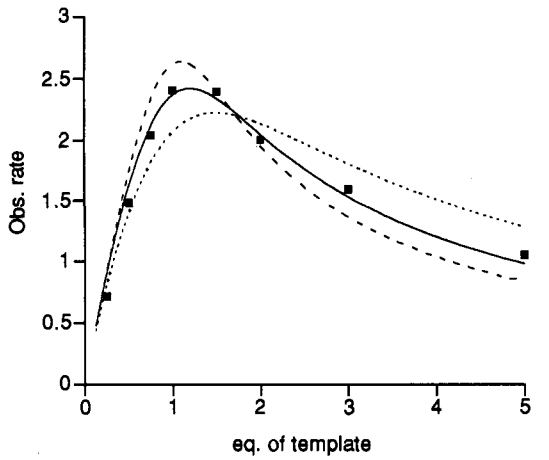

Figure 2. Observed initial rate (10^{-6} M^{-1} min^{-1}) of **1** + **2** vs the amount of template **8** (■). Calculated concentrations of productive complex (**1·2·8**) using different binding affinities: K = 65 000 M^{-1} (solid line), 22 000 M^{-1} (dotted line), and 190 000 M^{-1} (dashed line). Note that no vertical scale is represented for the calculated data as the scale is different for each line.

intramolecular stabilization of the tetrahedral intermediate **10** by hydrogen bonding to the purine N3 nitrogen is the cause. Molecular modeling suggests that **3–9** can all accommodate the intermediate in their cavities, but with conformations of the purines at the anomeric carbon that do not all allow the intramolecular stabilization. Possibly, only a few of the conformations of the intermediate result in a fast reaction rate, and only the templates complementary to these conformations can substantially accelerate the reaction.

Within experimental error, compounds **3** and **4** had no or little effect on the reaction rate. Significant catalytic activity was observed for **5** and **6**. The distance between the receptor sites for both these templates does not differ much from **3**, but they are more flexible (their spacers have fewer bonds fixed in a coplanar arrangement), and reactive conformations can be accommodated. Rate accelerations over 10^2 were observed with **7** and **8**. The spacers of these two templates are slightly shorter than the previous ones, and the bound reagents are likely to be held in closer proximity. They are also more rigid. While this feature was a drawback for the longer spacers, it becomes an advantage when the distance and shape are appropriate for a productive complexation. The rigidity of **8** is obvious: it has only two rotatable bonds. The poor flexibility of **7** arises from restricted and coupled rotations of its four single bonds. At any rate, **7** and **8** apparently have a higher degree of complementarity for the reactive conformations of the intermediate than the other templates. Finally, the shortest spacer of **9** does not lead to a larger acceleration. Here, negative cooperativity between the two binding events may be the cause due to the very short distance between the receptor sites.

The template activity of **8** was studied in further detail.[9] As illustrated in Figure 1, the 160-fold acceleration was reduced to 10-fold if 10 equiv of competitive binder 9-ethyladenine were added. Similarly, addition of 1 equiv of the coupling product reduces the initial rate acceleration to 7-fold, a feature which precludes efficient turnover in this system. Support for the presence of a termolecular complex (**1·2·8**) as responsible for the observed accelerations was obtained in experiments where more than 1 equiv of **8** was added. For concentrations of **8** beyond 1 equiv, the reaction rate *decreases*; the reagents are separated on different template molecules (Figure 2). This decrease is characteristic of the termolecular nature of the mechanism and should be expected in all such systems.[2,3]

The observed initial rate with various amounts of **8** correlated well with the calculated concentration of termolecular complex (**1·2·8**) (Figure 2).[10] The fit was optimum for an association constant of 65 000 M^{-1}, consistent with previous binding studies of the adenine/receptor interaction.[4] At 0.05 mM of each substrate, the receptor sites are not fully saturated with guests. With K = 65 000 M^{-1}, the calculated initial value of [(**1·2·8**)] is 0.0115 mM for 1 equiv of **8**, which leads to a corrected accelerating factor of 700-fold.[11]

In conclusion, large rate enhancements can result from the sole ability of a template molecule to hold two reagents in close proximity. The geometrical parameters involved in the complementarity determine the efficacy of the template effect. The next step is the functionalization of the spacers with polar groups to bring further chemical stabilization of the intermediate, and to combine template effects with chemical catalysis.

Acknowledgment. We thank NIH and NSF for supporting this work and Rhône-Poulenc for a predoctoral fellowship to I.H.

(9) The UV data were confirmed by ^1H NMR: integrations showed that, after 1 h, the aminolysis of **1** by **2** (at 0.05 mM) was over 80% completed in the presence of 1 equiv of **8** and only 3% in its absence.

(10) Calculations were based on the assumption that the template binds each substrate independently and with the same intrinsic affinity K. If $[T]_{tot}$ represents the total concentration of template, $[S]_{tot}$ represents the sum of the total concentrations of the substrates, and [R], [RS], and [S] represent the concentrations of free and occupied receptor sites and free substrate, respectively, then [S] is obtained as a solution of

$$[S]^2 + (2[T]_{tot} - [S]_{tot} + 1/K)[S] - [S]_{tot}/K = 0$$

The concentration of termolecular complex $[TS_2]$ is calculated by substitution of [S] into

$$[TS_2] = [T]_{tot}\left(\frac{K[S]}{1 + K[S]}\right)^2$$

When the concentrations of amine and esters are equal, the concentration of productive complex (containing one of each reagent) is $[TS_2]/2$.

(11) We thank a referee for an alternative description based on effective molarity. At 1 equiv of template, the initial rate is 2.4×10^{-6} M min^{-1} and a termolecular complex concentration of 1.15×10^{-5} M can be calculated,[10] which gives a "k_{cat}" of 0.21 min^{-1}. The background initial rate of 1.5×10^{-8} M min^{-1} and the initial concentrations of the reactants give k_{uncat} = 6.0 M^{-1} min^{-1}. The ratio of k_{cat}/k_{uncat} gives an effective concentration of 35 mM, which can be compared to the 0.05 mM reactant concentration.