# Catalysis of Nucleobase via Multiple Hydrogen-Bonding Interactions: Acceleration of Aminolysis of 6-Chloropurine Derivatives by Uracils

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*Received March 23, 1999*

Acceleration of chemical transformations by the use of multiple hydrogen-bonding interactions has attracted considerable attention.[1−3] Nucleobases and related compounds are capable of forming multiple hydrogen bonds in a complementary fashion.[4,5] They have been widely used as building blocks for supramolecular systems,[3,6] but their catalysis has been much less explored to date.[7,8] Herein we report the novel catalysis of a nucleobase in the aminolysis of 2-amino-6-chloropurine[9,10] and wish to highlight a crucial role of multiple hydrogen-bonding interactions in the formation of a reactive intermediate and also a possible stabilization of the transition state.

Reaction of 2-amino-6-chloro-9-hexylpurine **1** (5 mM) with diethylamine ($Et_2NH$, 125 mM)[11,12] was carried out in $C_6H_6$ at 30 °C in the presence of nucleobases **5**−**9** (Figure 1). Nucleobases **5** and **7**−**9** formed double hydrogen-bonded complexes with **1**, as observed by $^1$H NMR,[13a] and showed similar binding isotherms

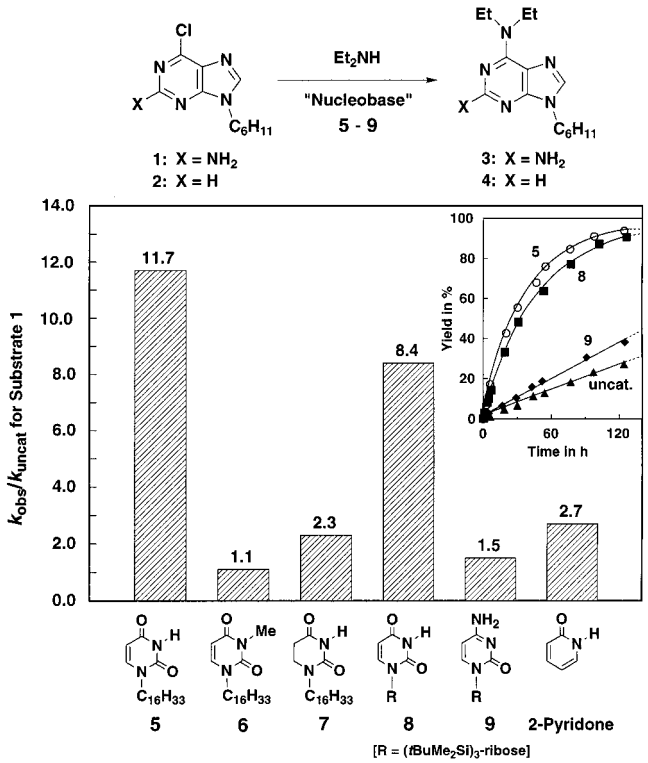


**Figure 1.** Acceleration effects ($k_{obs}/k_{uncat}$) of nucleobases **5**−**9** and 2-pyridone (15 mM) on aminolysis of **1** (5 mM) with $Et_2NH$ (125 mM) in $C_6H_6$ at 30 °C. Inset: Time courses of the reaction in the absence (▲) and presence of **5** (○), **8** (■), and **9** (◆).

to one another in $C_6D_6$ at 30 °C.[14] In the presence of 1-hexadecyluracil (**5**, 15 mM), aminolysis of **1** with $Et_2NH$ proceeded smoothly to give 2-amino-6-diethylamino-9-hexylpurine (**3**) in 92% yield in 96 h (Figure 1, ○). On the other hand, in the absence of **5** under otherwise identical conditions to the above, the aminolysis took place only very slowly to give **3** in 22% yield (Figure 1, ▲). The pseudo-first-order rate constant in the presence of **5** ($k_{obs}$) was $3.1 \times 10^{-2}$ h$^{-1}$, which is 11.7-times larger than that in the absence of **5** ($k_{uncat} = 2.6 \times 10^{-3}$ h$^{-1}$). A similar rate enhancement was observed when an uridine (uracil ribonucleoside) derivative (**8**) was used in place of **5**, where the reaction proceeded 8.4-times faster ($k_{obs} = 2.2 \times 10^{-2}$ h$^{-1}$) (Figure 1, ■) than the background reaction. In contrast, aminolysis of 6-chloro-9-hexylpurine (**2**) having no $NH_2$ functionality on C(2) was only slightly accelerated by uracil **5** with a ratio $k_{obs}/k_{uncat}$ as small as 2.8 ($k_{obs} = 2.4 \times 10^{-2}$ h$^{-1}$, $k_{uncat} = 8.8 \times 10^{-3}$ h$^{-1}$) under the same conditions as those in Figure 1.

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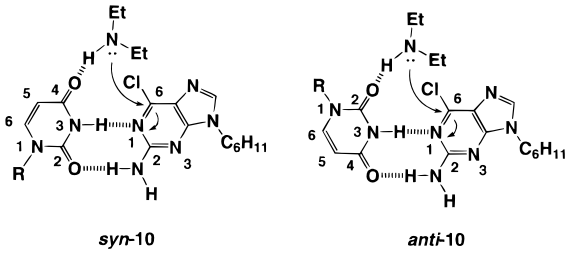
(12) The reaction was followed by HPLC, and the pseudo-first-order rate constant ($k_{obs}$) was obtained according to the equation $[\mathbf{1}]/[\mathbf{1}]_0 = e^{-kt}$. The product was identified by means of HPLC, TLC, and $^1$H NMR by comparison with the authentic sample.

(13) (a) For example, $^1$H NMR spectroscopy in toluene-$d_8$ of an equimolar mixture of **1** and **8** (5 mM each) at 30 °C showed downfield shifts for the signals due to $NH_2$ of **1** ($\delta$ 4.24 → 4.33) and 5-H ($\delta$ 5.51 → 5.52), 1′-H ($\delta$ 5.83 → 5.84), and NH ($\delta$ 8.03 → 8.21) of **8**, indicating a base-paring interaction between **1** and **8**. (b) When an equimolar amount of $Et_2NH$ was added to the above binary system, the $NH_2$ signal of **1** ($\delta$ 4.35) and the 5-H and 1′-H signals of **8** ($\delta$ 5.53 and 5.85, respectively) showed further downfield shifts. (See Supporting Information). Considering also the fact that addition of an equimolar amount of $Et_2NH$ to a toluene-$d_8$ solution of **1** or **8** resulted in smaller chemical shift changes ($NH_2$ of **1**; $\delta$ 4.25, 5-H and 1′-H of **8**; $\delta$ 5.52 and 5.84, respectively), the above observations suggest that **1**, **8**, and $Et_2NH$ form a ternary complex (**10**, Chart 1). In the ternary system, the NH signals of **1** and $Et_2NH$ were not detected, indicating a facile intra-complex proton exchange in **10**.

(14) $^1$H NMR titration of **1** (1 mM) with nucleobases **5** and **7**−**9** (1−150 mM) was performed in $C_6D_6$ at 30 °C. Binding isotherms, as obtained by chemical shift change of the signal due to $NH_2$ of **1**, were analyzed by a nonlinear curve-fitting method assuming a 1:1 complexation, to give association constants, which were all in the range 10−20 M$^{-1}$. These values are comparable to those reported for similar double hydrogen-bonded complexes such as the adenine−thymine base pair (see ref 5f).

10.1021/ja990944s CCC: $18.00 
Published on Web 08/05/1999

**Chart 1.** Plausible Ternary Complexes among 2-amino-6-chloropurine (**1**), $Et_2NH$, and Uracil

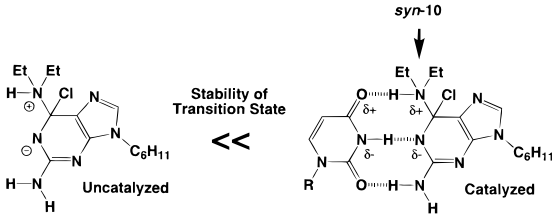


*syn*-**10** *anti*-**10**

The reaction of **1** (5 mM) with $Et_2NH$ (125 mM) was investigated at various concentrations (2−30 mM) of uracil **5**. Upon increasing the initial concentration of **5** ($[\mathbf{5}]_0$), the observed initial rates ($V_0$) showed saturation kinetics with respect to $[\mathbf{5}]_0$[15] to give a $V_{max}$ of 0.36 mM $h^{-1}$ ($k_{max} = 7.3 \times 10^{-2}$ $h^{-1}$),[16] indicating that **5** can accelerate the aminolysis of **1** by a maximum factor of 28 ($= k_{max}/k_{uncat}$). Likewise, the $k_{max}$ value of aminolysis with **8** was evaluated to be $5.2 \times 10^{-2}$ $h^{-1}$, which is 20-times larger than the rate constant of the background reaction ($k_{uncat}$). The aminolysis of **1** also showed a saturation signature when $[Et_2NH]_0$ was increased at given initial concentrations of **1** (5 mM) and **5** (15 mM).[15] These kinetic behaviors suggest that the aminolysis takes place via the formation of a ternary complex among 2-amino-6-chloropurine (**1**), $Et_2NH$, and uracil as a reactive intermediate.[13b] Accordingly, the entropy of activation for the reaction in the presence of **5** (−25.4 cal $mol^{-1}$ $K^{-1}$) was much more positive than that of the background reaction (−44.7 cal $mol^{-1}$ $K^{-1}$),[17] indicating a smaller entropy loss required for the transition state.

Chart 1 shows the most plausible ternary complexes for the aminolysis of **1** (*syn*-**10** and/or *anti*-**10**),[18] which allow a proximal orientation of the C(6)−Cl moiety of **1** to the coordinated amine due to a directional multiple hydrogen-bonding interaction between **1** and the uracil imido functionality. Such a ternary complexation is considered unfavorable for 6-chloropurine (**2**), a less reactive substrate toward the **5**-mediated aminolysis, due to the lack of $NH_2$ functionality on C(2) essential for the base-pairing interaction with **5**. Likewise, a N(3)-methylated uracil (**6**) hardly accelerated the aminolysis of **1** ($k_{obs}/k_{uncat} = 1.1$, Figure 1). Chart 1 also shows the importance of the imido C═O/N−H/C═O sequence in **5**, since it can leave one of the carbonyl functionalities for the binding of $Et_2NH$ even after the base-pairing complexation with **1** has been established: Use of a cyclic amide such as 2-pyridone bearing only a C═O/N−H sequence, in place of cyclic imide **5**, for the aminolysis of **1** resulted in a small acceleration

**Chart 2.** Possibility of Transition-State Stabilization by Multiple Hydrogen-Bonding Interactions




with a $k_{obs}/k_{uncat}$ value of 2.7 (Figure 1). Although cytosine **9** has a complementary binding site for **1**[14] and an additional carbonyl group for the interaction with $Et_2NH$, the acceleration effect of **9** on the aminolysis of **1** was again negligibly small ($k_{obs}/k_{uncat} = 1.5$, Figure 1, ◆). In this case, the most plausible ternary complex may accommodate $Et_2NH$ at the cytosine C(2)═O functionality, which is oriented away from the C(6)−Cl moiety of **1**.[15] Along the line of this mechanism, it is interesting to note that 5,6-dihydrouracil (**7**) was much less effective than **5** toward the aminolysis of **1** ($k_{obs}/k_{uncat} = 2.3$, Figure 1): As already described, **7** has a binding capability similar to **5** in complexation with **1**,[14] as it bears an imido C═O/N−H/C═O sequence. However, the basicity of the C═O oxygen atoms is not high enough to bind $Et_2NH$, due to the absence of a vinyl group.[19] Furthermore, a twisted conformation of **7** may also contribute to the catalytic activity. It should also be noted that the poor but definite acceleration effects of **7**, **9**, and 2-pyridone indicate a possible electronic contribution of the base-pairing interaction to the activation of **1** (acid catalysis), which, however, is considerably small.

The rate-determining step for the nucleophilic aminolysis of **1** must involve a zwitterionic transition state, which is considered unfavorable in aprotic solvents such as $C_6H_6$. However, in the presence of uracils **5** and **8**, such a transition state is possibly stabilized because of the multiple hydrogen-bonding interactions (Chart 2).[8] Therefore, the hydrogen-bonding interactions here are likely to play an important role not only in the formation of the reactive intermediate (*syn*-**10** and *anti*-**10**) but also in the stabilization of the transition state.

In the present paper, we have demonstrated the novel catalysis of a nucleobase such as uracil for chemical transformation of other nucleobase derivatives having a complementary hydrogen-bonding capability. This observation may provide a new strategy toward "supramolecular catalysis".

**Supporting Information Available:** Plots of initial rate ($V_0$) versus initial concentrations of **5** and $Et_2NH$, $^1H$ NMR spectra of a ternary mixture of **1**, **8**, and $Et_2NH$ and the control systems, and possible modes of hydrogen-bonded complexes between **1** and uracil and between **1** and cytosine (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) See Supporting Information.

(16) Determined from modified Lineweaver−Burk plots ($1/[V_0 - V_{uncat}]$ versus $1/[\mathbf{5}]_0$), according to the equation $1/(V_0 - V_{uncat}) = 1/(V_{max} - V_{uncat}) + K_m/[\mathbf{5}]_0/(V_{max} - V_{uncat})$.

(17) Estimated from $k_{max}$ and $k_{uncat}$ values at 20, 30, and 40 °C.

(18) Although four different hydrogen-bonding modes are possible for the ternary complexation, *syn*-**10** and *anti*-**10** are the most plausible, since the other two modes appear to suffer from a steric hindrance of the N(9) substituent (see Supporting Information).

(19) $^{13}C$ NMR signals due to the two C═O groups of **7** (30 mM in $C_6D_6$, 30 °C) at $\delta$ 155.0 (C(2)) and 168.6 (C(4)) are both downfield from those of **5** [$\delta$ 150.1 (C(2)) and 163.4 (C(4))], indicating lower electron densities at the C═O moieties in **7**.