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# Phosphodiester Cleavage of Guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine): Rate Acceleration by the 2′-Amino Function

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***Abstract:*** Hydrolytic reactions of the structural analogue of guanylyl-(3′,3′)-uridine, guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine), having one of the 2′-hydroxyl groups replaced with an amino function, have been followed by RP HPLC in the pH range 0−13 at 90 °C. The results are compared to those obtained earlier with guanylyl-(3′,3′)-uridine, guanylyl-(3′,3′)-(2′,5′-di-*O*-methyluridine), and uridylyl-(3′,5′)-uridine. Under basic conditions (pH > 8), the hydroxide ion-catalyzed cleavage of the P−O3′ bond (first-order in [$OH^-$]) yields a mixture of 2′-amino-2′-deoxyuridine and guanosine 2′,3′-cyclic phosphate which is hydrolyzed to guanosine 2′- and 3′-phosphates. Under these conditions, guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine) is 10 times less reactive than guanylyl-(3′,3′)-uridine. Under acidic and neutral conditions (pH 3−8), where the pH−rate profile for the cleavage consists of two pH-independent regions (from pH 3 to pH 4 and from 6 to 8), guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine) is considerably reactive. For example, in the latter pH range, guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine) is more than 2 orders of magnitude more labile than guanylyl-(3′,3′)-(2′,5′-di-*O*-methyluridine), while in the former pH range the reactivity difference is 1 order of magnitude. Under very acidic conditions (pH < 3), the isomerization giving guanylyl-(2′,3′)-(2′-amino-2′-deoxyuridine) and depurination yielding guanine (both first-order in [$H^+$]) compete with the cleavage. The $Zn^{2+}$-promoted cleavage ([$Zn^{2+}$] = 5 mmol $L^{-1}$) is 15 times faster than the uncatalyzed reaction at pH 5.6. The mechanisms of the reactions of guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine) are discussed, particularly focusing on the possible stabilization of phosphorane intermediate and/or transition state via an intramolecular hydrogen bonding by the 2′-amino group.

## Introduction

Transesterification and hydrolysis of RNA phosphodiester bonds by the so-called large ribozymes, that is, group I and II introns and the RNA subunit of RNase P, share a common mechanistic feature. Attack of an external nucleophile, either a nucleoside hydroxy function or hydroxide ion, on the phosphorus atom gives a pentacoordinated phosphorane-like transition state or highly unstable intermediate that is decomposed by departure of the 3′(2′)-linked nucleoside. No cleavage of the 5′-linked nucleoside has ever been observed.[1] To find out the origin of this regioselectivity, comparative studies with the wild-type group I intron and several of its structurally modified analogues have been carried out: (i) replacement of the 2′-hydroxy group with fluorine has been shown to retard the cleavage, although fluorine as a more electronegative substituent than hydroxyl group could be expected to be rate-accelerating,[2] (ii) replacement of the 2′-hydroxy group with a more acidic 2′-$NH_3^+$ group has been shown to markedly accelerate the cleavage,[3] and (iii) replacement of the leaving O3′ with sulfur, which is a weaker hydrogen-bond acceptor, has been shown to largely eliminate the rate-accelerating effect of the 2′-OH as compared to the 2′-H.[3] On the basis of the above, the 2′-hydroxy function has been suggested to stabilize the departing 3′-oxyanion as a hydrogen-bond donor, the acidity of which is increased by participation in a more extensive hydrogen-bond network.[3−7] Similar studies with group II introns do not, in turn, lend support to the significance of the neighboring hydroxyl function as a hydrogen-bond donor. The first transesterification reaction (lariat formation) is not sensitive to the presence of the 2′-OH group.[8,9] The second step (hydrolysis of the lariat) is accelerated by the 2′-OH, but the effect is similar with 3′-O and 3′-S leaving groups, although the latter should be less sensitive to hydrogen-bond stabilization.[10] With RNase P, indirect hydrogen bonding of the 2′-OH to the developing 3′-oxyanion via a $Mg^{2+}$-bound aquo ligand has been suggested.[11,12]

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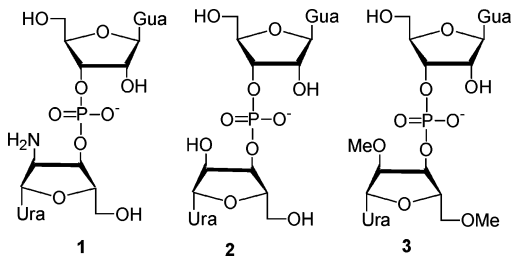
The effects that structural changes have on the catalytic activity of large ribozymes do not usually allow unambiguous mechanistic conclusions, because a given structural modification may simultaneously contribute to changes in chain-folding, hydrogen-bonding, metal-ion-binding, solvation, and electrostatic and hydrophobic interactions. Evaluation of the importance of these factors by simple chemical models may in some cases help to distinguish between alternative interpretations. Thus far, the studies on small molecule models of large ribozymes are, however, rather limited. The only example of an external nonribozymic transesterification of ribonucleotide esters is the methoxide ion-catalyzed methanolysis of ribonucleoside 3′(2′)-dimethyl phosphate, which has been shown to proceed by the cleavage of the P−O3′ bond.[13,14] In other words, the neighboring 2′-OH appears to serve as an electrophilic catalyst that facilitates the departure of the 3′-oxyanion, not as an intramolecular nucleophile as usual. In addition, it has been shown by using fully sugar O-alkylated triribonucleoside 3′,3′,5′-monophosphate as a model that the 3′-oxyanion is inherently 3 times as good a leaving-group as the 5′-oxyanion.[15] The rate-accelerating effect that hydrogen bonding of the 2′-OH to the departing O3′ and/or to a nonbridging phosphorane oxygen atom may result in has been quantified by comparing the cleavage rates of guanylyl-(3′,3′)-uridine (**2**) and guanylyl-(3′,3′)-(2′,5′-di-*O*-methyluridine) (**3**), proceeding by rate-limiting departure of uridine and 2′,5′-di-*O*-methyluridine, respectively, from a phosphorane intermediate obtained by an intramolecular attack of the 2′-oxyanion of the guanosine moiety on the phosphorus atom.[16] The overall rate acceleration is only 23-fold. As mentioned above, a larger influence may, however, be expected if the hydrogen-bond donor, the 2′-OH, simultaneously serves as a hydrogen-bond acceptor, which increases its acidity. The 2′-ammonium ion substituent may be used to mimic such a hydroxy group exhibiting markedly enhanced acidity. For this reason, we now report on studies with guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine) (3′,3′-Gp$^{NH2}$U; **1**). The effects of the 2′-amino substituent have been discussed on the basis of comparisons with previously studied guanylyl-(3′,3′)-uridine (3′,3′-GpU; **2**)[16] and guanylyl-(3′,3′)-(2′,5′-di-*O*-methyluridine) (3′,3′-Gp$^{Me}$U; **3**).[16]




## Results

**Product Distributions and pH−Rate Profiles.** Decomposition of guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine) (3′,3′-Gp$^{NH2}$U;**1**) was followed over a wide pH range at 90 °C by analyzing the compositions of the aliquots withdrawn at appropriate intervals from the reaction mixture by RP HPLC. The products were identified by spiking with authentic samples and by a mass spectrometric analysis (HPLC-ESI-MS).

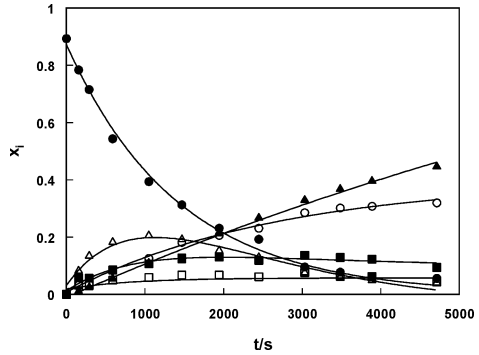



***Figure 1.*** Time-dependent product distribution for the reaction of guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine) (**1**) in a 0.1 mol L$^{-1}$ aqueous HCl at 90 °C. Notation: (●) 3′,3′-Gp$^{NH2}$U (**1**), (△) 2′,3′-Gp$^{NH2}$U (**4**), (▲) Gua (**6**), (□) 2′- GMP (**9**), (■) 3′-GMP (**8**), and (○) $^{NH2}$U (**7**). The curves are obtained by least-squares fitting and indicate the theoretical dependence of the mole fraction on time.

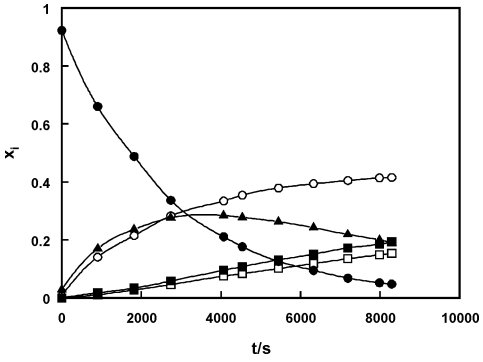



***Figure 2.*** Time-dependent product distribution for the cleavage of guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine) (**1**) in a glycine buffer at pH 9 ([HA]/[A$^-$] = 0.04/0.01 mol L$^{-1}$; $I$ = 0.1 mol L$^{-1}$ with NaCl) and 90 °C. Notation: (●) 3′,3′-Gp$^{NH2}$U (**1**), (▲) 2′,3′-cGMP (**5**), (○) $^{NH2}$U (**7**), (□) 2′-GMP (**9**), and (■) 3′-GMP (**8**).

3′,3′-Gp$^{NH2}$U (**1**) undergoes three parallel reactions under acidic conditions (pH < 3). An example of a time-dependent product distribution is given in Figure 1. The starting material (**1**) is partly isomerized to guanylyl-(2′,3′)-(2′-amino-2′-deoxyuridine) (2′,3′-Gp$^{NH2}$U; **4**) (Scheme 1; route A), and the isomeric mixture is cleaved to guanosine 2′,3′-cyclic phosphate (2′,3′-cGMP; **5**) with concomitant release of 2′-amino-2′-deoxyuridine ($^{NH2}$U; **7**) (route B). In fact, the intermediary accumulation of 2′,3′-cGMP was observed only at pH ≥ 3, because of its rapid hydrolysis to a mixture of guanosine 2′- and 3′-phosphates (2′- and 3′-GMP) (**9** and **8**) (route D). Additionally, depurination (i.e., cleavage of the *N*-glycosidic bond) yields a considerable amount of guanine (Gua; **6**) (route C).

At pH > 4, cleavage to 2′,3′-cGMP (**5**) and $^{NH2}$U (**7**) (route B) becomes so much faster than isomerization that 2′,3′-Gp$^{NH2}$U (**4**) is not formed. At pH 5−7, 2′,3′-cGMP is accumulated quantitatively, but at higher pH, subsequent hydrolysis to a 2:3 mixture of 2′- and 3′-GMP (route D) takes place. Figure 2 shows the time-dependent product distribution at pH 9.

The pH−rate profiles for the individual partial reactions of 3′,3′-Gp$^{NH2}$U (**1**) are depicted in Figure 3. The cleavage is first-

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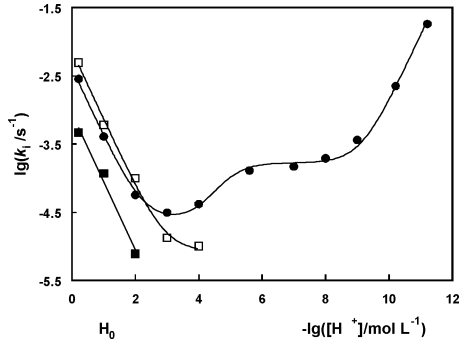
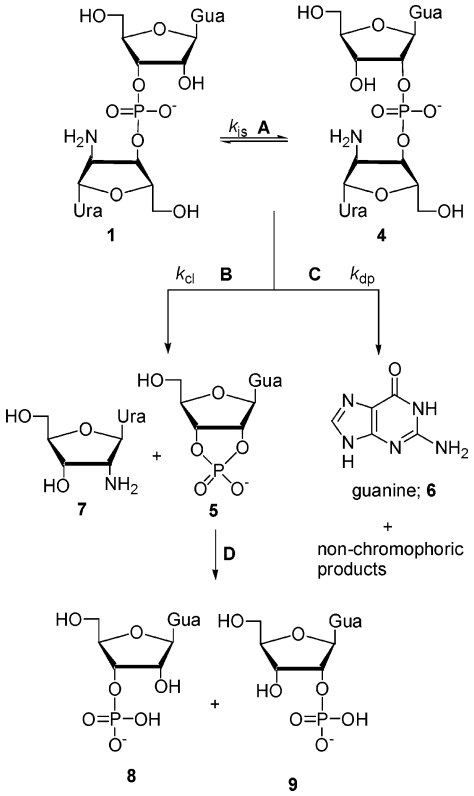



***Figure 3.*** pH−rate profile for the cleavage ($k_{cl}$; ●), isomerization ($k_{is}$; □), and depurination ($k_{dp}$; ■) of guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine) (**1**) at 90 °C. The ionic strength of the solutions was adjusted to 0.1 mol $L^{-1}$ with sodium chloride. Under conditions, where the isomerization competes with the cleavage, $k_{cl}$ refers to the cleavage of the isomeric mixture of 3′,3′-$Gp^{NH2}U$ (**1**) and 2′,3′-$Gp^{NH2}U$ (**4**). The curve refers to eq 1 when $k_1 = 4.0 \times 10^{-3}$ L $mol^{-1}$ $s^{-1}$, $k_2 = 2.4 \times 10^{-5}$ $s^{-1}$, $k_3 = 1.7 \times 10^{-4}$ $s^{-1}$, $k_4 = 0.200$ L $mol^{-1}$ $s^{-1}$, $K_a = 1.2 \times 10^{-5}$ mol $L^{-1}$, and $K_w = 6.2 \times 10^{-13}$ mol $L^{-1}$.[17,18]

***Scheme 1***




order in acidity at pH < 2 and first-order in hydroxide ion concentration at pH > 8. Between these two pH values, two pH-independent plateaus are observed: slow pH-independent cleavage at pH 3−4 and 7-fold faster cleavage at pH 6−8. The isomerization and depurination are both first-order in hydronium ion concentration at pH < 3. Under less acidic conditions, the isomerization starts to level off to a zero-order dependence of rate on hydronium ion concentration.

**$Zn^{2+}$-Promoted Cleavage.** $Zn^{2+}$-promoted cleavage of 3′,3′-$Gp^{NH2}U$ (**1**) was followed as a function of pH (5.1−5.6) and metal ion concentration (1−10 mmol $L^{-1}$ at pH 5.6). Cleavage is the only reaction observed, the first-order rate constant at [$Zn^{2+}$] 5 mmol $L^{-1}$ and pH 5.6 being $1.88 \times 10^{-3}$ $s^{-1}$, that is, 15 times as fast as that in the absence of the $Zn^{2+}$. Over the

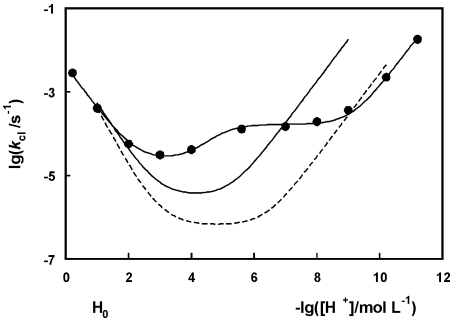



***Figure 4.*** pH−rate profile for the cleavage ($k_{cl}$) of guanylyl-(3′,3′)-(2′-amino-2′-deoxyuridine) (**1**) at 90 °C. The solid and dotted lines show the corresponding curves for 3′,3′-GpU (**2**; −) and 3′,3′-$Gp^{Me}U$ (**3**; - - -), respectively. The ionic strength of the solutions was adjusted to 0.1 mol $L^{-1}$ with sodium chloride.

narrow pH and [$Zn^{2+}$] range studied, the reaction is first-order in both the hydroxide and the metal ion concentration.

## Discussion

Figure 4 shows the pH−rate profile for the cleavage of 3′,3′-$Gp^{NH2}U$ (**1**). To illustrate the effects that replacement of the 2′-hydroxy function of the uridine moiety in 3′,3′-GpU with an amino group has on the cleavage rate, the pH−rate profiles obtained with unmodified 3′,3′-GpU (**2**)[16] and its dimethyl derivative 3′,3′-$Gp^{Me}U$ (**3**)[16] are all also included.

3′,3′-$Gp^{NH2}U$ (**1**) in all likelihood exhibits only one p$K_a$ value in the pH range studied, that is, at 0.2 < pH < 11.5. The p$K_a$ value of the 2′-ammonium group of 2′-amino-2′-deoxyuridine has been reported to be 6.2 at 25 °C.[19,20] Because p$K_a$ values of amines are usually decreased by almost 1 unit on going from 25 to 90 °C,[21] 3′,3′-$Gp^{NH2}U$ (**1**) may be expected to have a p$K_a$ value around 5 at 90 °C, that is, at the inflection point of the pH−rate profile between the two plateaus. The predominant ionic forms of 3′,3′-$Gp^{NH2}U$ (**1**) under the experimental conditions, hence, are those indicated by $SH_2$ and $SH^-$ in Scheme 2. The phosphate group most likely becomes protonated at pH < 0.2. The p$K_a$ value of 3′,5′-UpU has been reported to be 0.7 at 90 °C,[22] but interaction with the 2′-$NH_3^+$ group may be expected to lower this value. Deprotonation of the 2′-OH of the guanine moiety, in turn, takes place at pH > 12.[22,23] Accordingly, ionic forms $SH_3^+$ and $S^{2-}$, although kinetically significant intermediates, are not stoichiometrically significant species.

The shape of the pH−rate profile in Figure 4, together with the known p$K_a$ value of 5, indicates that the cleavage of 3′,3′-$Gp^{NH2}U$ (**1**) proceeds via the four different ionic forms shown in Scheme 2: (i) at pH < 2, via the monocation $SH_3^+$ of 3′,3′-$Gp^{NH2}U$ (**1**) ($H^+$-catalyzed reaction of $SH_2$), (ii) over a narrow pH range around pH 3, by spontaneous cleavage of the neutral zwitterion $SH_2$, (iii) at pH 4−8, by spontaneous reaction of the monoanion $SH^-$, and (iv) at pH > 8, via the dianion

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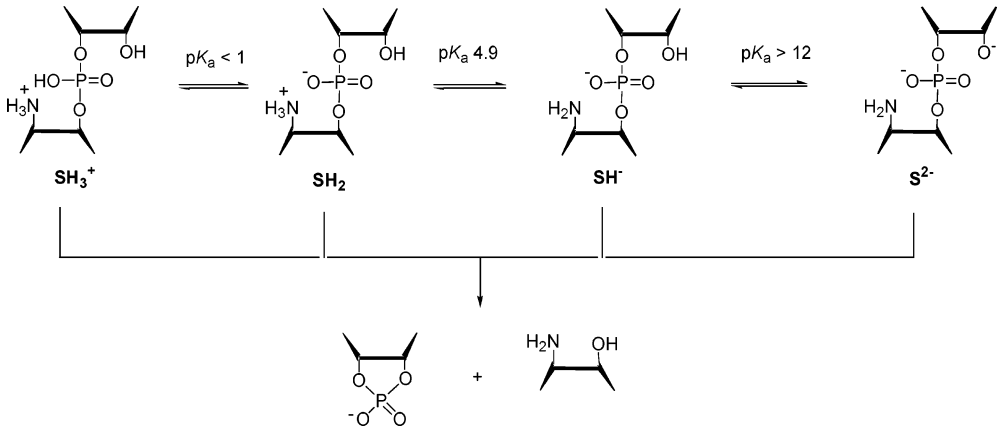
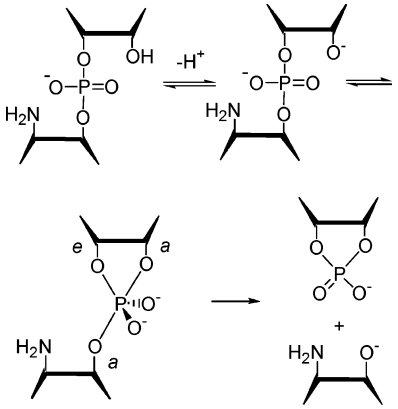
**Scheme 2**




**Scheme 3**




$S^{2-}$ (hydroxide ion-catalyzed reaction of $SH^-$). Accordingly, the observed first-order rate constant for the cleavage may be expressed by eq 1, where $k_2$ and $k_3$ are the first-order rate constants for the spontaneous cleavage of $SH_2$ and $SH^-$, respectively, $k_1$ is the second-order rate constant for the hydronium ion-catalyzed cleavage of $SH_2$, and $k_4$ is the second-order rate constant for the hydroxide ion-catalyzed cleavage of $SH^-$.

$$k_{cl} = \frac{k_1[H^+]^2 + k_2[H^+] + k_3K_a + k_4K_a(K_w/[H^+])}{K_a + [H^+]} \quad (1)$$

**Cleavage via the Dianion ($S^{2-}$) of 3′,3′-Gp$^{NH2}$U (1).** The cleavage of 3′,3′-Gp$^{NH2}$U (**1**) at pH > 8 is clearly first-order in hydroxide ion concentration and in all likelihood refers to rate-limiting departure of $^{NH2}$U 3′-oxyanion from the dianionic phosphorane intermediate obtained by the attack of guanosine 2′-oxyanion on the phosphorus atom (Scheme 3). For discussions on the role of pentacoordinated oxyphosphorane intermediate in the cleavage of ribonucleoside 3′-phosphoesters, see the studies of Westheimer,[24] Usher,[25] Taira,[26] Breslow,[27] Anslyn,[28] and Lönnberg.[29,30] As discussed recently,[31] the dianionic phosphorane really seems to be a marginally stable intermediate, not only a transition state. The second-order rate constant for this reaction is 0.200 L mol$^{-1}$ s$^{-1}$. Previously, second-order rate constants of 14.7 L and 0.45 L mol$^{-1}$ s$^{-1}$ have been reported for the hydroxide ion-catalyzed cleavage of the monoanion of 3′,3′-GpU (**2**) and 3′,3′-Gp$^{Me}$U (**3**), respectively.[16] The relative cleavage rates of the 2′-amino-, 2′-methoxy-, and 2′-hydroxy-substituted compounds, hence, are 1, 2.2, and 75, respectively. In other words, the 2′-amino group does not result in a rate acceleration similar to that of the 2′-hydroxy group (**3**). Evidently the amino group is too weakly acidic to stabilize either the phosphorane intermediate or the departing 3′-oxyanion by hydrogen bonding in a manner similar to that of the 2′-hydroxy group. Consistent with this, the p$K_a$ values of the 3′-hydroxy group of 2′-*O*-methyl-ATP and 2′-$NH_2$-dATP differ only by 0.1 units, while the respective p$K_a$ value of ATP is 0.7 units lower.[32]

**Cleavage via the Monoanion ($SH^-$) of 3′,3′-Gp$^{NH2}$U (1).** As mentioned above, the predominant ionic form of 3′,3′-Gp$^{NH2}$U (**1**) at pH > 5 is the monoanion $SH^-$. Accordingly, the pH-independent reaction at pH 6−8 refers to uncatalyzed cleavage of this species, and the hydroxide ion-catalyzed reaction in the pH range 4−6 results, that is, on passing the p$K_a$ value of the 2′-ammonium ion, indicates that this ionic form is cleaved faster than the neutral zwitterions, $SH_2$. However, the reactive species is not the major tautomer indicated in Scheme 2, but most likely a minor tautomer having the amino group protonated and the 2′-hydroxy group of the guanine moiety deprotonated (Scheme 4). Taking the difference between the p$K_a$ values of the 2′-ammonium ion and the 2′-hydroxy group into account, the mole fraction of such a species may be estimated to range from $10^{-7}$ to $10^{-6}$. This mole fraction is sufficiently high to make the minor tautomer as the reactive species, because the 2′-oxyanion is a much better nucleophile than the 2′-hydroxy group and the 2′-ammonium ion may be expected to stabilize the phosphorane intermediate either electrostatically or by hydrogen bonding and to facilitate inductively the cleavage of the P−O3′-bond. For comparison, studies with ribonucleoside 3′-phosphotriesters have shown that the attack of the 2′-hydroxy function on neutral phosphotriester

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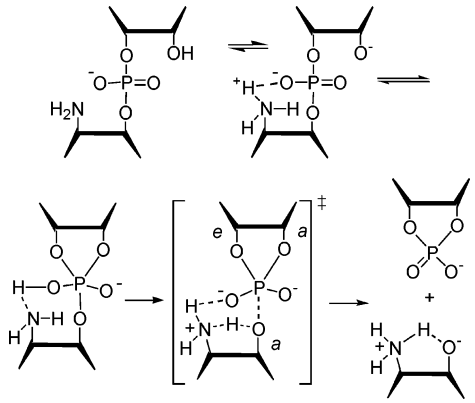
*Scheme 4*



becomes hydroxide ion catalyzed already at pH 2−3,[29,30,33,34] suggesting that the 2′-oxyanion is a 9−10 orders of magnitude better nucleophile than the 2′-hydroxy group.

A possible way to estimate how much 2′-ammonium group may facilitate the hydroxide ion-catalyzed cleavage is to compare the second-order rate constant for the hydroxide ion-catalyzed cleavage of the neutral zwitterionic form $SH_2$ of 3′,3′-Gp$^{NH2}$U (**1**) to the corresponding value for the hydroxide ion-catalyzed cleavage of the monoanion $SH^-$, where the amino group is unprotonated. The data in Figure 4 give a value of 780 L mol$^{-1}$ s$^{-1}$ for this rate constant. In other words, the relative rates as compared to the hydroxide ion-catalyzed cleavage of the 2′-amino, 2′-methoxy, and 2′-hydroxy compounds are 3900, 1700, and 50, respectively. This rate acceleration may consist of three different contributions: (i) stabilization of the phosphorane intermediate electrostatically and/or by hydrogen bonding, (ii) stabilization of the leaving group by intramolecular hydrogen bonding of the ammonium group to the departing 3′-oxygen atom, and (iii) inductive reduction of electron density of the departing 3′-oxygen atom. Choline (*N*,*N*,*N*-trimethyl-2-aminoethanol) is one p$K_a$-unit more acidic than 2-methoxyethanol.[35,36] Because the $\beta_{lg}$ value of the hydroxide ion-catalyzed cleavage of ribonucleoside 3′-phosphodiesters is known to be −1.3,[37] this means that the inductive effect of the 2′-ammonium group may result in a 20-fold acceleration as compared to the 2′-methoxy-substituted compound (**3**). Accordingly, an almost 100-fold acceleration remains to be explained by electrostatic and hydrogen-bonding interactions. The studies of Dalby et al. on N-methylated ethylenediammonium dications as general acid−base catalysts of the phosphodiester cleavage suggest that both electrostatic and hydrogen-bonding interaction with the dianionic phosphorane intermediate play a role, but the hydrogen-bonding interactions are more important.[38] Bearing these findings in mind, it appears reasonable to assume that the 2′-ammonium group is initially weakly hydrogen bonded to the phosphoryl oxyanion, because of the low basicity of the phosphodiester group. However, as the 2′-oxyanion approaches the phosphorus atom, the basicity of the nonbridging oxygen atoms is increased, the hydrogen bonding of the ammonium group is strengthened, and when the phosphorane structure is completed, the proton has actually transferred from the nitrogen atom to the oxygen atom. In principle, the amino group could also facilitate the breakdown of the phosphorane intermediate. When the leaving group starts to depart, the basicity of the nonbridging oxygen atom is decreased. Consequently, the hydrogen-bonded proton is transferred from the phosphorane oxygen atom to the amino nitrogen atom, and concomitant with strengthening of the ammonium ion character, hydrogen bonding to the departing 3′-oxygen atoms starts to play a role. The present data do not, however, allow one to conclude whether the latter proton transfer really plays a role. Possibly mere stabilization of the phosphorane intermediate offers an adequate explanation for the observed rate acceleration.

**Cleavage and Isomerization of the Neutral Zwitterions ($SH_2$) of 3′,3′-Gp$^{NH2}$U (1).** The cleavage of the zwitterion $SH_2$ of 3′,3′-Gp$^{NH2}$U (**1**) indicated in Scheme 2 is pH-independent over a narrow pH range of 3−4. Studies with ribonucleoside 3′-phosphotriesters have shown that the attack of the 2′-hydroxy function on neutral phosphotriester becomes hydroxide ion catalyzed already at pH 2−3.[29,30,33,34] In addition, a neutral phosphoester has been shown to be attacked several orders of magnitude more readily than a monoanionic one.[39,40] Accordingly, the reactivity of the rare tautomer, having the 2′-hydroxy group deprotonated and the phosphodiester linkage protonated (neutral), appears to be sufficiently high to offer the major route for formation of a monoanionic phosphorane. This tautomer is, however, highly unstable, and, hence, concerted proton transfer and nucleophilic attack constitute the plausible mechanistic alternative. Accordingly, the cleavage of zwitterionic 3′,3′-Gp$^{NH2}$U (**1**) may be expected to proceed as depicted in Scheme 5. The first-order rate constant for this spontaneous cleavage is 2.5 × 10$^{-5}$ s$^{-1}$ under the experimental conditions of the present study. For comparison, the first-order rate constants for the pH-independent cleavage of 3′,3′-GpU (**2**) and 3′,3′-Gp$^{Me}$U (**3**) have been reported to be 1.5 × 10$^{-6}$ and 5.9 × 10$^{-7}$ s$^{-1}$, respectively.[16] A 16-fold and 42-fold acceleration as compared to **2** and **3** is, hence, observed. The $\beta_{lg}$ value for the pH-independent cleavage is −0.6.[41] Accordingly, only a 4-fold acceleration as compared to the 2′-methoxy-substituted compound is expected on the basis of the inductive effect of the 2′-ammonium group. Again, the 2′-ammonium group evidently stabilizes the monoanionic phosphorane by serving as a hydrogen-bond donor. The fact that zwitterionic 3′,3′-Gp$^{NH2}$U (**1**) is isomerized at pH 3−4 5−7 times more rapidly than 3′,3′-Gp$^{Me}$U (**3**) lends additional support for stabilization of the phosphorane intermediate by electrostatic or hydrogen-bonding interaction between the phosphorane monoanion and the 2′-ammonium ion.

**Hydronium Ion-Catalyzed Cleavage and Isomerization of 3′,3′-Gp$^{NH2}$U (1).** At pH < 2, the pH−rate profile of 3′,3′-Gp$^{NH2}$U (**1**) differs from those of 3′,3′-GpU (**2**) and 3′3′-

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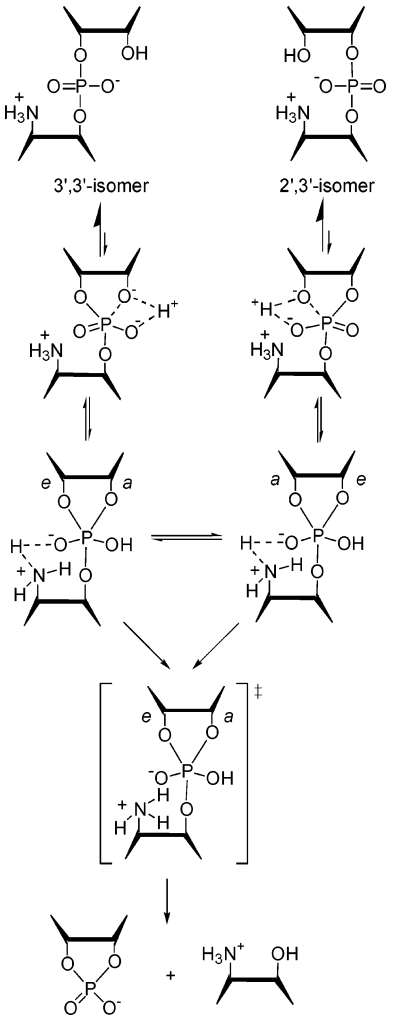
***Scheme 5***




$Gp^{Me}U$(**3**) in the sense that the cleavage and isomerization of 3′,3′-$Gp^{NH2}U$ (**1**) exhibit a first-order dependence on acidity, while the reactions of 3′,3′-GpU (**2**) and 3′,3′-$Gp^{Me}U$ (**3**) are second-order in the hydronium ion concentration. Evidently the presence of the positively charged 2′-ammonium group markedly retards protonation of the phosphodiester linkage to a monocationic form, and, hence, reaction via a monoprotonated (neutral) phosphodiester prevails over a wider pH range than with 3′,3′-GpU and 3′3′-$Gp^{Me}U$ (**3**). The phosphorane intermediate is thus obtained by an attack of the 2′-hydroxy group on neutral phosphate.[29] The leaving group departs under these conditions as an alcohol, and, hence, the rate is not sensitive to the stability of the leaving group. Consistent with this, the rates of the reactions of 3′,3′-$Gp^{NH2}U$ (**1**), 3′,3′-GpU (**2**), and 3′3′-$Gp^{Me}U$ (**3**) are comparable.

**$Zn^{2+}$-Promoted Cleavage.** The $Zn^{2+}$-promoted cleavage of 3′,3′-$Gp^{NH2}U$ (**1**) exhibits a first-order dependence on both the hydroxide ion and the metal ion concentration over the limited pH range of 5.1−5.6 studied. Accordingly, only one metal ion participates and one proton is removed on going to the transition state. It has been suggested previously[42] for the cleavage of ribonucleoside 3′-phosphodiesters that $Zn^{2+}$ facilitates as an electrophile rapid initial formation of a dianionic phosphorane intermediate and as an intramolecular general acid the rate-limiting breakdown of this intermediate. The latter step is believed to proceed by intramolecular proton transfer from an aquo ligand of the phosphorane-bound $Zn^{2+}$ to the departing oxygen concerted with the PO bond cleavage. While the same mechanism may operate in the cleavage of 3′,3′-$Gp^{NH2}U$ (**1**), binding of $Zn^{2+}$ to the amino group offers an alternative explanation for the $Zn^{2+}$-promoted reaction, as discussed below.

The $\beta_{lg}$ value of the cleavage of the 3′-alkyl esters is only slightly negative, −0.32.[42] Accordingly, the reaction is not markedly susceptible to the basicity of the leaving group. The observed first-order rate constant for $Zn^{2+}$-promoted cleavage of 3′,3′-$Gp^{NH2}U$ (**1**) at pH 5.6 ($[Zn^{2+}] = 5$ mmol $L^{-1}$) is $1.88 \times 10^{-3}$ $s^{-1}$. The rate constants reported earlier[16] for 3′,3′-GpU (**2**) and 3′,3′-$Gp^{Me}U$ (**3**) are $5.2 \times 10^{-5}$ and $2.74 \times 10^{-5}$ $s^{-1}$, respectively. The 3′-O-linked $^{NH2}U$ (**7**) is thus cleaved from 3′,3′-$Gp^{NH2}U$ (**1**) 70 and 36 times more rapidly than 3′-O-linked $^{Me}U$ from 3′,3′-$Gp^{Me}U$ (**3**) and 3′-O-linked U from 3′,3′-GpU (**2**). This rate acceleration is substantial taking the low susceptibility to the leaving group basicity into account. Tentatively, one may assume that $Zn^{2+}$ when bound to the 2′-amino group is able to stabilize the leaving group either by direct chelate formation with the departing oxygen atom or by donating a proton from its aquo ligand.

## Experimental Section

**Methods.** The NMR spectra were recorded on a Bruker AM 200 or JEOL 400 spectrometer. The $^1H$ NMR chemical shifts (400 MHz, 300 K) refer to internal TMS, and the $^{31}P$ NMR shifts (162 MHz, 300 K) refer to external orthophosphoric acid. The mass spectra were acquired using a Perkin-Elmer Sciex API 365 triple quadrupole LC/MS/MS spectrometer.

**Materials.** Guanosine 2′,3′-cyclic phosphate and guanosine 2′- and 3′-phosphates, used as reference material, were commercial products of Sigma. 2′-Amino-2′-deoxyuridine was purchased from BIORON. 2′-Amino-2′-deoxyuridine was converted to the corresponding 5′-*O*-(4,4′-dimethoxytrityl)-2′-*N*-trifluorothioacetyl-2′-deoxyuridine as described previously by Eckstein et al.[43]

**Guanosyl-(3′,3′)-(2′-amino-2′-deoxyuridine)** (**1**) was prepared by phosphoramidite methodology as described earlier.[15,16] Accordingly, $N^2$-(*p*-isopropylphenoxyacetyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)guanosine, 3′-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite), and 5′-*O*-(4,4′-dimethoxytrityl)-2′-*N*-trifluoroacetyl-2′-deoxyuridine were dissolved in a solution of tetrazole in dry acetonitrile. The phosphite ester formed was oxidized with $I_2$ in THF/$H_2O$/lutidine. The dimethoxytrityl group was removed with 80% aqueous acetic acid solution, and the cyanoethyl, $N^2$-(*p*-isopropylphenoxyacetyl), and 2′-*N*-trifluoroacetyl groups were removed with saturated methanolic ammonia. Finally, the 2′-*O*-tertbutyldimethylsilyl group was removed in a solution of tetrabutylammonium fluoride in tetrahydrofuran. The crude product was purified by reversed phase chromatography on a Lobar RP-18 column (37 × 440 mm, 40−63 mm), eluting with a mixture of water and acetonitrile (92:7%, v/v). After that, the product was passed through a $Na^+$-form Dowex 50-W (100−200 mesh) cation exchange column. $^{31}P$ NMR ($\delta_P$) (162 MHz, $D_2O$): 13.42. $^1H$ NMR ($\delta_H$) (400 MHz, $D_2O$): 7.88 (s, 1H, H8), 7.74 (d, 1H, $J = 8$, 11 Hz, H6), 6.14 (d, 1H, $J = 7.03$ Hz, H1′), 5.82 (d, 1H, $J = 5.32$ Hz, H1′), 5.78 (d, 1H, $J = 8.11$ Hz, H5), 4.87 (dd, 1H, H2′), 4.73 (m, 2H, 2 × H3′), 4.42 (d, 1H, H4′), 4.29 (d, 1H, H4′), 4.11 (dd, 1H, H2′), 3.77−3.70 (m, 4H, 2 × H5′, 2 × H5″). $ESI^-$-MS: *m*/*z* 587.3 $[M - H]^-$.

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**Kinetic Measurements.** The reactions were carried out in sealed tubes immersed in a thermostated water bath (90 ± 0.1 °C). The hydronium ion concentration of the reaction solutions was adjusted with hydrogen chloride, sodium hydroxide, and formate, acetate, (*N*-[2-hydroxyethyl]piperazine-*N*-[2-ethanesulfonic acid]) (HEPES), and glycine buffers.[36] Low buffer concentration was used (30−60 mmol $L^{-1}$). The ionic strength was adjusted to 0.1 mol $L^{-1}$ with sodium chloride. Neither of the reactions was markedly susceptible to buffer catalysis. The initial substrate concentration was ca. 0.1 mmol $L^{-1}$. The composition of the samples withdrawn at appropriate intervals was analyzed on a Hypersil ODS 5 column (4 × 250 mm, 5 mm) using mixtures of acetonitrile and a acetic acid/sodium acetate buffer (0.045/0.015 mol $L^{-1}$) containing 0.1 mol $L^{-1}$ ammonium chloride as an eluent. A good separation of the products was obtained, when a 14 min isocratic elution with buffer was followed by a linear gradient (5 min) up to 6.0% acetonitrile. After this, isocratic elution with a 6.0% content of acetonitrile (v/v) was continued. The observed retention times ($t_R$/min) for the hydrolytic products of **1** on RP HPLC (flow rate was 1 mL $min^{-1}$, 260 nm) were: 26.0 (2′,3′-isomer; **4**), 16.5 (2′,3′-cGMP; **5**), 22.0 (2′-GMP; **9**), 8.0 (2′-amino-2′-deoxyuridine; **7**), 6.6 (guanine; **6**), and 9.0 (3′-GMP; **8**). The observed retention time for starting material (**1**) was 29.5 min.

**Calculation of the Rate Constants.** At pH > 4, where the cleavage was the only reaction detected, the pseudo first-order rate constants for the disappearance of **1** ($k_{di}$) were obtained by applying the integrated first-order rate equation to the time-dependent diminution of the concentration of the starting material.

At pH 0−2, where the isomerization and depurination compete with the cleavage, the first-order rate constants ($k_{dp}$) for the depurination of an isomeric mixture of **1** were calculated by eq 2, by bisecting the rate constant ($k_{dec}$) for the decomposition of the isomeric mixture of the starting material to the rate constants of parallel first-order reactions on the basis of the product distribution at the early stages of the reaction, that is, under conditions where the formation of guanine from the cleavage products may be neglected. $[\mathbf{6}]_t$ and $[\mathbf{1} + \mathbf{4}]_t$ stand for the concentration of guanine (**6**) and the sum concentration of GpU isomers (**1** and **4**), respectively, at moment *t*. $[\mathbf{1}]_0$ denotes the initial concentration of the starting material.

$$k_{dp} = \frac{[\mathbf{6}]_t}{[\mathbf{1}]_0 - [\mathbf{1} + \mathbf{4}]_t} k_{dec} \quad (2)$$

Equation 3 was then applied to obtain the rate constants ($k_{cl}$) for the cleavage of isomeric GpUs.

$$k_{cl} = k_{dec} - k_{dp} \quad (3)$$

The first-order rate constants ($k_{is}$) for the isomerization of 3′,3′-isomer (**1**) to 2′,3′-isomer (**4**) were determined by the UFIT 1.0 program of Beckman et al.[44] using the concentrations of 3′,3′- (**1**) and 2′,3′-isomer (**4**) in the least-squares fitting.

## Conclusions

In summary, a 2′-amino group on one of 3′,3′-linked ribonucleosides markedly accelerates the cleavage of the 3′,3′-phosphodiester bond between pH 3 and 9. At pH 6−8, that is, when the amino group is not protonated, the 2′-amino group facilitates the rate-limiting breakdown of the monoanionic phosphorane intermediate by mediating intramolecularly proton transfer from the phosphorane hydroxyl ligand to the departing 3′-oxyanion. At pH 3−4, that is, when the amino group is protonated to an ammonium group, the cleavage is 5-fold slower. Under these conditions, the ammonium group may only serve as a hydrogen-bond donor. In other words, the 2′-ammonium group stabilizes the leaving group by hydrogen bonding, but is unable to assist in deprotonation of the phosphorane intermediate and, hence, the development of the cyclic phosphoester upon the cleavage. In addition, both the 2′-amino and the 2′-ammonium groups may stabilize the monoanionic phosphorane intermediate, the former by accepting a hydrogen bond from the phosphorane hydroxyl ligand and the latter by donating a hydrogen bond to the phosphorane oxyanion ligand.

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