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Hydrolytic Reactions of Guanosyl-(3′,3′)-uridine and Guanosyl-(3′,3′)-(2′,5′-di-*O*-methyluridine)

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Abstract: Hydrolytic reactions of guanosyl-(3′,3′)-uridine and guanosyl-(3′,3′)-(2′,5′-di-*O*-methyluridine) have been followed by RP HPLC over a wide pH range at 363.2 K in order to elucidate the role of the 2′-hydroxyl group as a hydrogen-bond donor upon departure of the 3′-uridine moiety. Under neutral and basic conditions, guanosyl-(3′,3′)-uridine undergoes hydroxide ion-catalyzed cleavage (first order in [OH⁻]) of the P−O3′ bonds, giving uridine and guanosine 2′,3′-cyclic monophosphates, which are subsequently hydrolyzed to a mixture of 2′- and 3′-monophosphates. This bond rupture is 23 times as fast as the corresponding cleavage of the P−O3′ bond of guanosyl-(3′,3′)-(2′,5′-di-*O*-methyluridine) to yield 2′,5′-*O*-dimethyluridine and guanosine 2′,3′-cyclic phosphate. Under acidic conditions, where the reactivity differences are smaller, depurination and isomerization compete with the cleavage. The effect of Zn²⁺ on the cleavage of the P−O3′ bonds of guanosyl-(3′,3′)-uridine is modest: about 6-fold acceleration was observed at [Zn²⁺] = 5 mmol L⁻¹ and pH 5.6. With guanosyl-(3′,3′)-(2′,5′-di-*O*-methyluridine) the rate-acceleration effect is greater: a 37-fold acceleration was observed. The mechanisms of the partial reactions, in particular the effects of the 2′-hydroxyl group on the departure of the 3′-linked nucleoside, are discussed.

Introduction

While small molecule models have been extensively used to study the underlying principles of the cleavage of RNA by the so-called small ribozymes utilizing an intramolecular mechanism,[1] the corresponding studies mimicking the intermolecular transesterification process of large ribozymes are scarce. Such a mechanism has only been found to operate in the methanolysis of ribonucleoside 2′/3′-dimethyl phosphates[2] and hydrolysis of H-phosphonodiesters[3] in organic solvents. We previously reported a kinetic study on the hydrolytic reactions of a sugar *O*-alkylated trinucleoside 3′,3′,5′-monophosphate,[4] the monoanionic pentacoordinated phosphorane intermediate/transition state of which resembles the intermediate formed in the ribozyme reaction of group I introns.[5] The results obtained suggested that the nucleophilic attack of hydroxide ion on the phosphorus atom of the phosphotriester results in only a 3 times faster departure of the 3′-hydroxy-esterified nucleoside compared to its 5′-hydroxy-esterified counterpart. In other words, nucleoside 3′-oxyanion appears to be only a three times better leaving group than the 5′-oxyanion, and hence, the difference in the inherent leaving group property is insufficient to explain the fact that with group I introns the P−O3′ bond cleavage is overwhelm-

ingly faster than the P−O5′ cleavage.[6] It has been suggested[2,3,7] that the much faster cleavage of the P−O3′ bond could be attributed to a proton transfer from the neighboring 2′-hydroxyl group to the departing 3′-oxygen atom concerted with the bond cleavage. Such rate accelerations have also been reported for the cleavage of several nonnucleosidic phosphate esters,[8] carboxylic esters,[9] and acetal derivatives.[10] To quantify the acceleration caused by intramolecular hydrogen bonding in the present case, we now report on a comparative kinetic analysis of the cleavage of two diribonucleoside 3′,3′-monophosphates, viz. guanosyl-(3′,3′)-uridine (**1a**) and guanosyl-(3′,3′)-(2′,5′-di-*O*-methyluridine) (**1b**). While these compounds are cleaved by an intramolecular attack of the 2′-hydroxy group on the phosphorus atom, they still allow assessment of the significance of transition-state stabilization by intramolecular hydrogen bonding within the leaving group. The rate-limiting step of the reaction is the departure of the 3′-linked nucleoside, either uridine or 2′-*O*-methyluridine, from the phosphorane intermediate, and hence, the rate-accelerating effect of the 2′-hydroxyl group on the cleavage (and possibly formation) of this inter-

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(9) (a) Bruice, T. C.; Fife, T. H. *J. Am. Chem. Soc.* **1962**, *82*, 1973−1979. (b) Henbest, H. B.; Lovell, B. J. *J. Chem. Soc.* **1957**, 1965−1969.
(10) (a) Capon, B. *Chem. Rev.* **1969**, *69*, 407−498. (b) Kirby, A. J. *Acc. Chem. Res.* **1997**, *30*, 290−296.

mediate can be determined. Additionally, the effect of the action of a catalytically active metal ion, Zn^{2+}, on the cleavage rate has been investigated. Mechanisms of hydrolytic reactions are discussed and compared to those obtained earlier with 3′,5′-UpU (**10**) containing a native 3′,5′-linkage.[11,12] In particular, the reactions taking place in basic and neutral solutions are of interest.
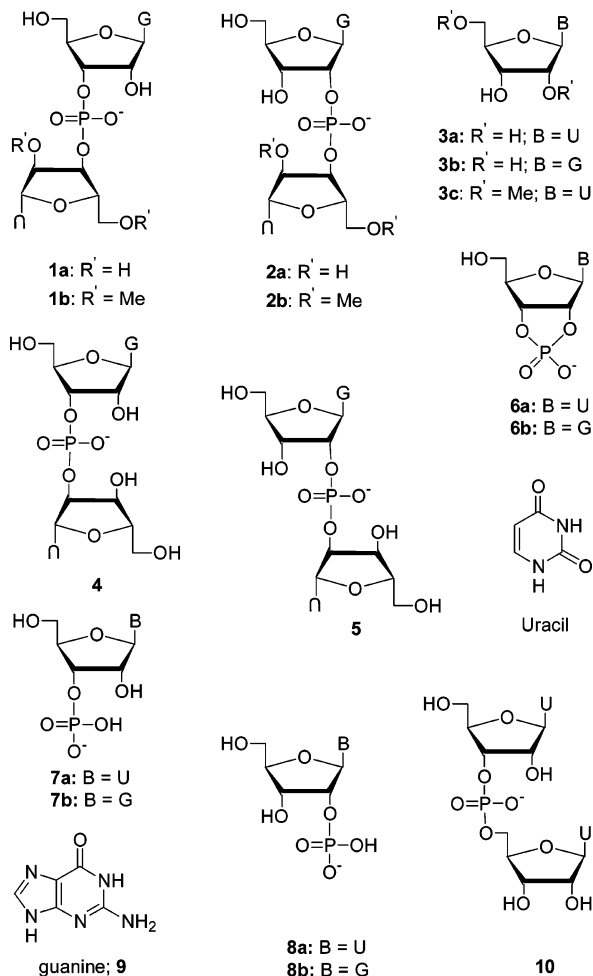
Results and Discussion

Product Distributions and Reaction Pathways. The cleavage of guanosyl-(3′,3′)-uridine (3′,3′-GpU; **1a**) and guanosyl-(3′,3′)-(2′,5′-di-*O*-methyluridine) (3′,3′-GpMe₂U; **1b**) was studied over a wide pH range at 363.2 K by determining the time-dependent product composition of the aliquots withdrawn at appropriate intervals from the reaction solution by RP HPLC. The products were characterized by either spiking with authentic samples or mass spectrometric analysis (HPLC/ESI-MS). Scheme 1 summarizes the reactions observed to take place with **1a**. Accordingly, at pH < 4, 3′,3′-GpU is cleaved to uridine 2′,3′-cyclic phosphate (2′,3′-cUMP; **6a**; reaction A) and guanosine 2′,3′-cyclic phosphate (2′,3′-cGMP; **6b**; reacion B), depurinated by loss of guanine (**9**; reaction C), and isomerized to a mixture of 2′,3′-GpU (**2a**), 3′,2′-GpU (**4**) and 2′,2′-GpU (**5**; reaction D). Under these conditions the cyclic phosphates (**6a,b**) are quite rapidly hydrolyzed to a mixture of 2′- and 3′-phosphates (**7a/**

(11) Järvinen, P.; Oivanen, M.; Lönnberg, H. *J. Org. Chem.* **1991**, *56*, 5396–5401.
(12) Kuusela, S.; Lönnberg, H. *J. Phys. Org. Chem.* **1993**, *6*, 347–356.

8a; **7b/8b**; reactions E and F) and are, hence, accumulated as intermediates only at pH 2−3. The depurination is not detected at pH > 4 and the isomerization at pH > 6. In other words, at pH 4−6, the cleavage reactions (A,B) compete with the isomerization (D), and at pH > 6, they are the only reactions observed. 3′,3′-GpMe₂U (**1b**) reacts analogously to 3′,3′-GpU, except that now only one cleavage reaction (reaction G), i.e., the one respective to reaction B in Scheme 1, may take place, and isomerization yields only 2′,3′-GpMe₂U (**2b**; reaction H; Scheme 2). Examples of time-dependent product distributions are given as Supporting Information.

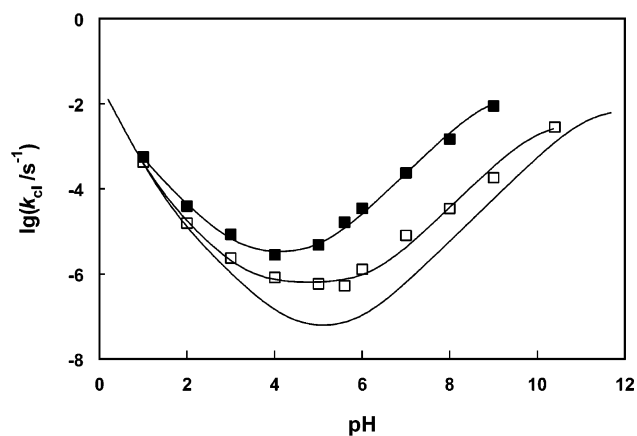**pH−Rate Profiles.** Figure 1 shows the pH−rate profiles for

Figure 1. pH−rate profile for the cleavage (k_{cl}) of 3′,3′-GpU (**1a**) (■) and guanosyl-(3′,3′)-(2′,5′-di-*O*-methyluridine) (**1b**) (□) at 363.2 K. The solid line shows the corresponding curve for 3′,5′-UpU (**10**). 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} represents the disappearance of the isomeric GpUs. The curves are calculated by least-squares fitting and indicate the theoretical dependence of the observed rate constants on H^+ concentration.

the cleavage of 3′,3′-GpU (**1a**, reactions A and B) and 3′,3′-GpMe₂U (**1b**; reaction G). The profiles consist of three regions: a hydronium-ion-catalyzed reaction at pH < 4, a nearly pH-independent reaction from pH 4 to 5, and a hydroxide-ion-catalyzed reaction at pH > 5. In addition, a slight curvature (leveling off) toward a constant value is observed on going to very basic conditions. The hydroxide-ion-catalyzed cleavage (first order in [OH^-]) of **1a** takes place 260 times as fast as that of 3′,5′-UpU (**10**).[11,13] More importantly, 3′,3′-GpU is cleaved 46 times as fast as 3′,3′-GpMe₂U. Since there are two scissile PO3′ bonds in 3′,3′-GpU which react as rapidly and only one in 3′,3′-GpMe₂U, one may conclude that methylation of the 2′- and 5′-hydroxyl groups of the uridine moiety retards the cleavage of an individual PO3′ bond by a factor of 23.

As with 3′,5′-UpU, the isomerization is hydronium-ion catalyzed and approximately as fast as the cleavage at pH < 4 and nearly pH-independent under less acidic conditions (Figure 2). The rate of pH-independent isomerization of 3′,3′-GpU is 4.5-fold compared to that of 3′,3′-GpMe₂U, which is, in turn, isomerized as fast as 3′,5′-UpU (**10**). Bearing in mind that there are two 2′-hydroxy functions in 3′,3′-GpU, the actual rate difference is only 2.3. At pH < 4, the differences between the isomerization rates are even smaller.

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Scheme 1
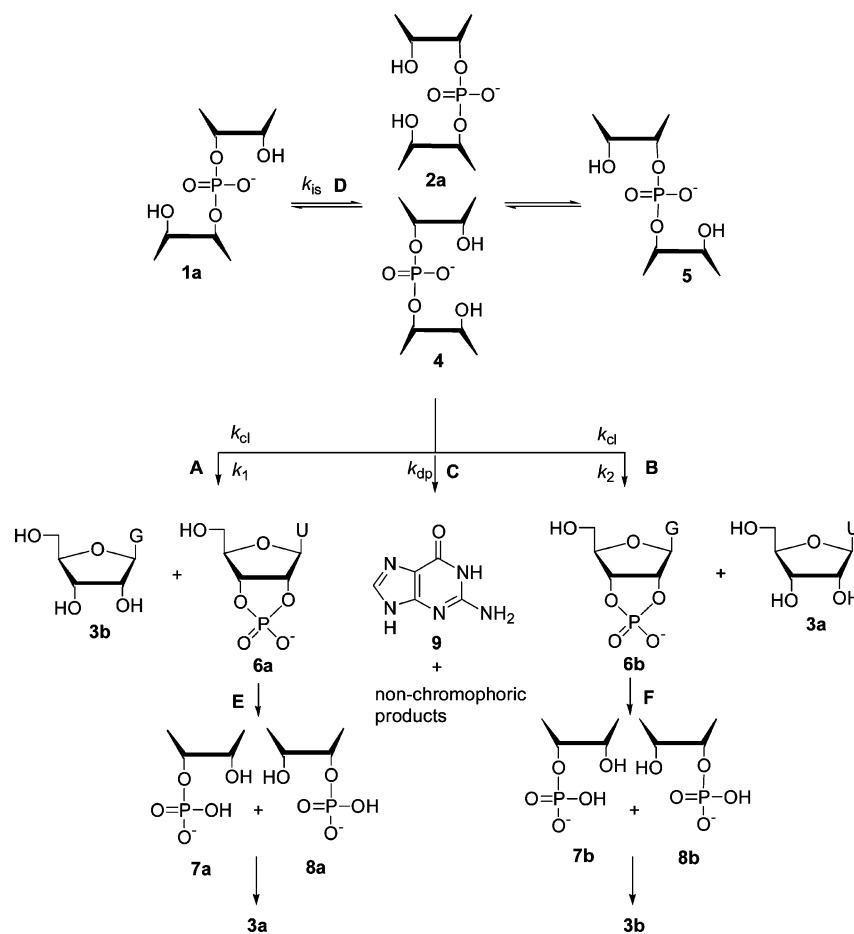
Scheme 2
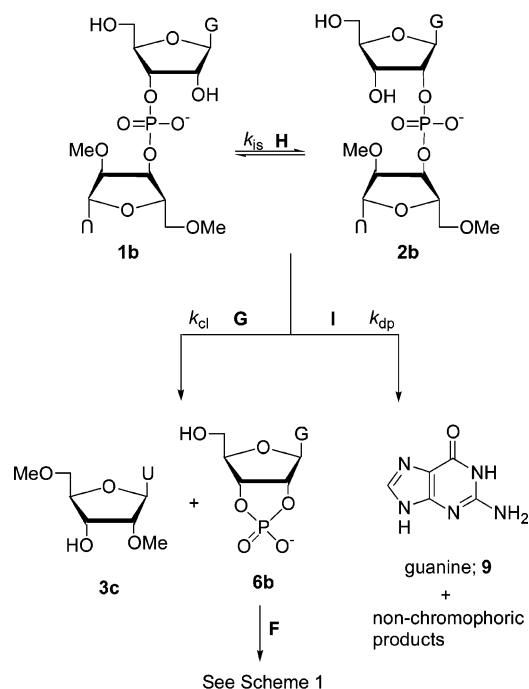
Zn²⁺-Promoted Cleavage. The rate enhancements caused by catalytically active metal ions, such as Zn²⁺, are also of interest in the present study. For this purpose, decomposition of 3′,3′-GpU and 3′,3′-GpMe₂U was studied as a function of metal-ion concentration (1−10 mmol L⁻¹) and pH (5.1−5.6). In the pH range studied, the cleavage is first order in both hydroxide-ion concentration ([Zn²⁺] = 5 mmol L⁻¹) and metal-ion concentration ([Zn²⁺] = 1−10 mmol L⁻¹ at pH 5.6 (Figure 3). The same reactions as in the absence of the metal ions take palce, viz. cleavage and isomerization, but only the cleavage is promoted by Zn²⁺.

Mechanism of the Cleavage of P−O3′ Bond under Neutral and Basic Conditions: Intramolecular Hydrogen Bonding. As mentioned above, the hydroxide-ion-catalyzed cleavage (first order in [OH⁻]) of each of the PO3' bonds in 3′,3′-GpU **1a** proceeds 23 times as fast as the PO3' bond cleavage in 3′,3′-GpMe₂U. One may ask what is the origin of this rate acceleration. The basic mechanism suggested[14] earlier for the phosphodiester cleavage of RNA in all likelihood also applies to the reactions 3′,3′-GpU and 3′,3′-GpMe₂U. Accordingly,[11] a rapid initial deprotonation of the 2′-hydroxyl group is followed by the rate-limiting attack of the resulting oxyanion on the phosphorus atom, giving a marginally stable dianionic phosphorane intermediate from which the 3′-linked nucleoside departs "in-line" with the nucleophilic attack. The considerably faster cleavage of 3′,3′-GpU compared to 3′,3′-GpMe₂U suggests the 2′-hydroxyl group exerts an additional rate-accelerating effect. This group may be expected to either stabilize the departing nucleoside 3′-oxyanion by intramolecular hydrogen

(14) Brown, D. M.; Magrath, D. I.; Neilson, A. H.; Todd, A. R. *Nature* **1956**, *177*, 1124.
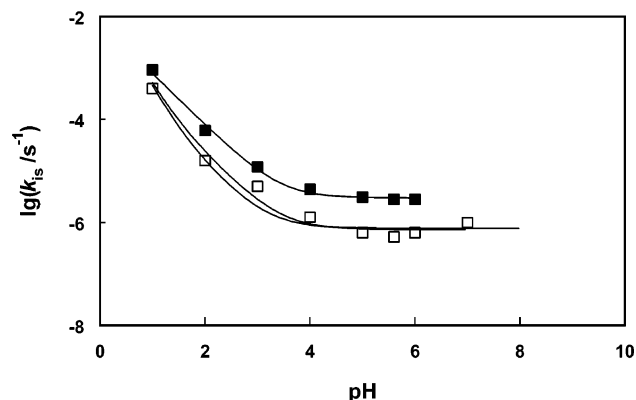
Figure 2. pH−rate profile for the isomerization (k_{is}) of 3′,3′-GpU (**1a**) (■) and guanosyl-(3′,3′)-(2′,5′-di-*O*-methyluridine) (**1b**) (□) at 363.2 K. The solid line shows the corresponding curve for 3′,5′-UpU (**10**). The ionic strength of the solutions was adjusted to 0.1 mol L^{-1} with sodium chloride.
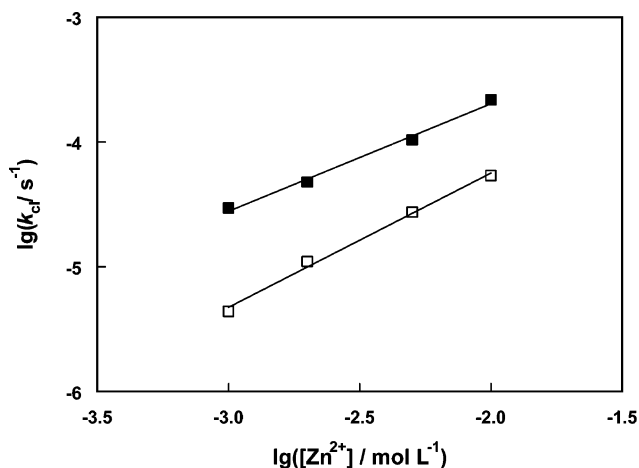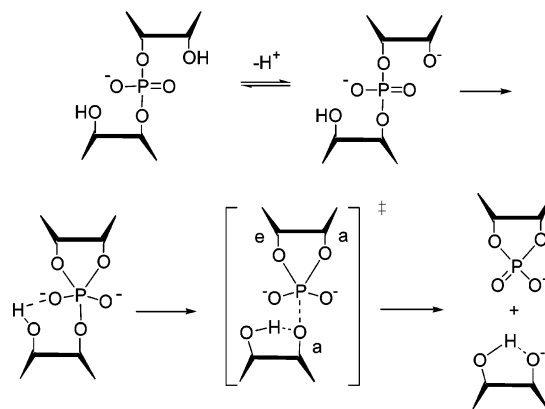

Figure 3. Effect of metal-ion concentration on the first-order rate constants of cleavage (k_{cl}) of 3′,3′-GpU (**1a**; ■) and guanosyl-(3′,3′)-(2′,5′-di-*O*-methyluridine) (**1b**; □) at pH 5.60 and 363.2 K. The pH was adjusted with HEPES buffer, and the ionic strength was maintained at 0.1 mol L^{-1} with sodium nitrate.

bonding or stabilize the dianionic phosphorane intermediate by hydrogen bonding to a phosphorane oxyanion.[2,3,7,15]

The leaving group may well be stabilized by a hydrogen-bond formation between the departing 3′-oxyanion and the neighboring 2′-hydroxyl group. The influence of a vicinal *cis*-hydroxyl group on the acidity of a sugar OH group is, however, relatively modest. It has been shown[16a] that the pK_a values of aldofuranosides having *cis*- or *trans*-2,3-diol systems differ only by about 0.5 units. Additionally, removal of the 3′-hydroxyl group from adenosine has been reported to increase the pK_a of the 2′-hydroxyl group only by 0.54 units,[16b] and part of this difference undoubtedly results from weaker electron withdrawal by hydrogen compared to a hydroxyl group. For example, with adenosine and *ara*-adenosine, the pK_a difference is not larger than 0.23 units.[16b] The most reliable estimation for the effect of the 2′-*O*-methylation is offered by the ΔpK_a value between the 3′-hydroxyl group of adenosine and 2′-*O*-methyladenosine, which has been reported to be 0.74 units.[16c] Accordingly, only

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(16) (a) Vesala, A.; Käppi, R.; Lönnberg, H. *Carbohydr. Res.* **1983**, *119*, 25−30. (b) Velikyan, I.; Acharya, S.; Trifonova, A.; Földesi, A.; Chatto-padhyaya, J. *J. Am. Chem. Soc.* **2001**, *123*, 2893−2894. (c) Strömberg, R.; Aström, H.; Limen, E. Personal communication.

Scheme 3

a 3−5-fold rate acceleration could be attributed to leaving-group stabilization by hydrogen bonding, taking into account that the transition state is very late[17] and is, hence, stabilized as efficiently as the initial product, the 3′-oxyanion. Accordingly, the observed 23-fold acceleration can hardly by entirely explained by stabilization of the leaving group by intramolecular hydrogen bonding.

As mentioned above, another possible source of rate acceleration is stabilization of the phosphorane intermediate: 2′-OH donates a hydrogen bond to one of the phosphorane oxyanions (Scheme 3).[2,3] The second pK_a of a phosphorane intermediate has been estimated to be higher than that of the 2′-hydroxyl group.[18] Accordingly, hydrogen bonding of the 2′-hydroxy function of the leaving group to one of the nonbridging phosphorane oxygens upon formation of a dianionic phosphorane appears feasible, and it may well result in an additional 3−5-fold acceleration of the cleavage. For comparison, methylation of the neighboring 2′-hydroxyl groups retards the pH-independent isomerization only by a factor of 2.3. This reaction, however, proceeds via a phosphorane monoanion,[1,19] and hence, stabilization by a similar intramolecular hydrogen bonding is less probable, since the first pK_a value of the phosphorane is lower[18] than that of the 2′-hydroxyl group. It has been previously reported that downfield shift of the 2′-hydroxyl proton in the ^{1}H NMR spectrum (in CDCl$_3$) indicates the existence of weak intramolecular hydrogen bonding even in the ground state of ribonucleoside 3′-dimethyl phosphates.[2]

Mechanism of the Zn^{2+}-Promoted Cleavage of P−O3′ Bond. As mentioned above, the cleavage of 3′,3′-GpU and 3′,3′-GpMe$_2$U is first order in the concentration of Zn^{2+} (Figure 3), indicating that only one metal ion is involved on going to the transition state. The first-order dependence of the rate on hydroxide-ion concentration, in turn, refers to deprotonation of the predominant ionic form (monoanion) of 3′,3′-GpU or metal aquo ion prior to cleavage of the P−O3′ bond (Scheme 4). Accordingly, a dianionic phosphorane may be expected to be obtained. The Zn^{2+}-promoted cleavage of 3′,3′-GpMe$_2$U through this species is approximately as fast as the corresponding reaction of 3′,5′-UpU:[12] the second-order rate constants for the hydroxide-ion-catalyzed cleavage at [Zn^{2+}] = 5 mmol L^{-1} are

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(19) Mikkola, S.; Kosonen, M.; Lönnberg, H. *Curr. Org. Chem.* **2002**, *6*, 523−538.

Scheme 4

$R' = H$ or Me
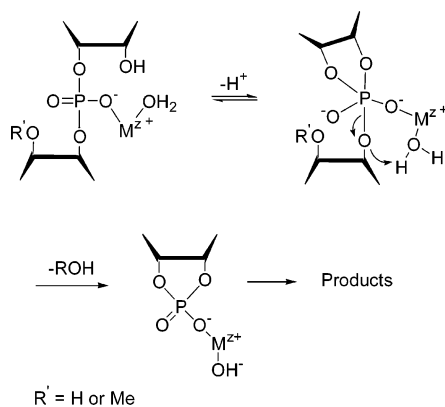
135 and 142 L mol^{-1} s^{-1}, respectively ($T = 363.2$ K and $I = 0.1$ mol L^{-1} with sodium nitrate). This is expected on the basis of the mechanism proposed earlier[20] for the Zn^{2+}-promoted cleavage of ribonucleoside 3′-phosphodiesters. The rate-limiting step is breakdown of the phosphorane intermediate, more precisely cleavage of the P−O5′ bond concerted with an intracomplex proton transfer from the aquo ligand of the phosphorane-bound zinc ion. The β_{lg} value of the reaction has been shown to be low (-0.32), and hence, the reaction is not markedly susceptible to the basicity of the leaving group. The 3′-linked uridine is cleaved 2.0 times as rapidly as the 3′-linked 2′,5′-di-*O*-methyluridine, the second-order rate constant obtained for **1a** being 280 L mol^{-1}s^{-1}. This modest rate enhancement may tentatively be attributed to the ability of the 2′-hydroxyl group to serve, in addition to the phosphorane-bound Zn^{2+} aquo ion, as an intracomplex general acid, protonating the departing 3′-oxygen atom concerted with the bond cleavage.

Mechanisms of the Acid-Catalyzed Cleavage and Isomerization. As shown by the pH−rate profiles in Figures 1 and 2, the reactivity difference between 3′,3′-GpU, 3′,3′-GpMe$_2$U, and 3′,5′-UpU is modest in acidic solutions. Under these conditions, the reactions proceed via a neutral or monocationic phosphorane intermediate and the leaving group departs as an alcohol instead of alkoxide ion.[11] Accordingly, stabilization of the phosphorane intermediate or leaving group by intramolecular hydrogen bonding does not play a role. Consistent with the observed similarity of the reaction rates, the β_{lg} value of the acid-catalyzed cleavage and isomerization of ribonucleoside 3′-phosphodiesters has been reported to be only slightly negative, -0.12 and -0.18, respectively.[17] The pH-independent cleavage, observed over a narrow pH range of 4−5, proceeds via rapid initial formation of a phosphorane monoanion that undergoes rate-limiting breakdown by concerted proton transfer from the phosphorane hydroxyl ligand to the departing oxygen.[19] This means that stabilization of neither the phosphorane intermediate nor the leaving oxygen by intramolecular hydrogen bonding with the 2′-hydroxy group is not important. As seen from Figure 1, the pH-independent cleavage is much less susceptible to the 2′-*O*-methylation than the hydroxide-ion-catalyzed cleavage.

Experimental Section

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

Materials. The preparation of guanosyl-(3′,3)-(2′,5′-di-*O*-methyluridine) has been described previously.[4] N^2-(*p*-Isopropylphenoxyacetyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)guanosine 3′-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) and N^2-acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(triisopropylsilyloxymethyl)guanosine 3′-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) were commercial products of Glen Research. Uridine monophosphates (**6a**, **7a**, and **8a**), guanosine monophosphates (**6b**, **7b,** and **8b**), guanine (**9**), guanosine (**3b**), and uridine (**3a**), all used as reference materials, were commercial products of Sigma.

Guanosyl-(3′,3′)-uridine (1a). N^2-(*p*-Isopropylphenoxyacetyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)guanosine 3′-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) and 2′,5′- bis-*O*-(*tert*-butyldimethylsilyl)uridine[4] were dissolved in a solution of 0.45 M tetrazole (0.33 mmol) in dry acetonitrile (1.5 mL). After 2 h of stirring, the phosphite ester formed was oxidized with 0.1 M I$_2$ in THF:H$_2$O:lutidine (4:2:1). The crude product was isolated by a CH$_2$Cl$_2$/aq. NaHSO$_3$ work up and purified on a silica gel column eluted with a mixture of dichloromethane and methanol (90:10%, v/v). The dimethoxytrityl group was removed with 80% aqueous acetic acid solution (7 h). The reaction mixture was evaporated to dryness, and the residue was purified on a silica gel column eluted with a mixture of dichloromethane and methanol (90:10%, v/v). The detritylated product was dissolved in saturated methanolic ammonia. After being stirred for 20 h, the solution was evaporated to dryness and purified on a silica gel column eluted with a mixture of dichloromethane and methanol (70:30%, v/v). The purified product was dissolved in 1 mol L^{-1} solution of tetrabutylammonium fluoride (0.265 g, 1.02 mmol) in tetrahydrofuran (1 mL), and the solution was stirred for 20 h at room temperature. The mixture was evaporated to dryness and 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 (92:8%, v/v). Finally, the product was passed through a Na$^+$-form Dowex 50-W (100−200 mesh) cation exchange column. ^{31}P NMR (δ_P) (162 MHz, D$_2$O): 2.13. ^{1}H NMR (δ_H) (400 MHz, D$_2$O): 7.85 (s, 1H), 7.73 (d, 1H, $J = 8.12$ Hz), 5.79 (d, 1H, $J = 4.92$ Hz), 5.77 (d, 1H, $J = 5.56$ Hz), 5.72 (d, 1H, $J = 8.12$ Hz), 4.18−4.72 (m, 6H), 3.69−3.81 (m, 4H). ESI$^+$-MS: *m/z* 588.5 [M + H]$^+$. HRMS (FAB) M$^-$ calcd 588.1091, obsd 588.1087.

Kinetic Measurements. The reactions were carried out in sealed tubes immersed in a thermostated water bath (363.2 ± 0.1 K). 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.[21] Low buffer concentration was used (30−60 mmol L^{-1}). 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 an acetic acid/sodium acetate buffer (0.045/0.015 mol L^{-1}) containing 0.1 mol L^{-1} ammonium chloride as an eluent. Good separation of the product mixture of **1a** was obtained when a 25 min isocratic elution with buffer was followed by a linear gradient (1 min) up to 5.0% acetonitrile. After this isocratic elution with a 5.0% content of acetonitrile (v/v) was continued. The observed retention times (t_R/min) for the hydrolytic products of **1a** on RP HPLC (flow rate was 1 mL min^{-1}) were 36.0 (**5**), 23.0 (**3b**), 15.0 (**6b**), 8.1 (**8b, 7b,** and **3a**), 6.7 (**8a**), 6.2 (**9**), 5.4 (**7a**), 38.0−37.0 (**2a** and **4**), and 4.9 (**6a**). Uridine (**3a**), 3′-GMP (**7b**), and 2′-GMP (**8b**) were separated by isocratic elution

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Table 1. Rate Constants for the Cleavage (Route A, k_1; and Route B, k_2) of 3′,3′-GpU (**1a**) at 363.2 K

pH	9.1	8.0	7.1	6.0	5.6	5.0
$k_1/\times 10^{-4}\ s^{-1}$	48.0	7.51	1.18	0.172	0.082	0.026
$k_2/\times 10^{-4}\ s^{-1}$	39.9	7.09	1.16	0.172	0.082	0.023

with 0.1 mol L^{-1} ammonium acetate. The observed retention times(t_R/min) were 12.2 (**6a**), 14.0 (**8b**), and 5.9 (**7b**). In the case of **1b**, samples were analyzed on a Hypersil ODS column (4 × 125 mm, 5μm). With **1b** a 5 min isocratic elution with buffer was followed by a linear gradient (5 min) up to 9% acetonitrile. The observed retention times (t_R/min) for the hydrolytic products of **1b** on RP HPLC (flow rate was 0.5 mL min^{-1}) were 13.1 (**3c**), 12.2 (**2b**), 6.6 (**3b**), 4.3 (**6b**), 2.5 (**7b**), 2.6 (**8b**), and 1.9 (**9**). The observed retention times for starting materials, **1a** and **1b**, were 42.5 and 15.6, respectively. To identify the reaction products, the mass spectra of the hydrolysis products were recorded (LC/MS). A mixture of acetonitrile and aqueous ammonium acetate (5 mmol L^{-1}) was used as an eluent. In addition, the products formed were identified by spiking with authentic reference compounds.

Calculation of the Rate Constants. The pseudo-first-order rate constants for the disappearance of **1a** and **1b** (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 > 6, where cleavage of the starting material is the only reaction detected, the rate constant (k_{cl}) for cleavage of **1a** was bisected to the rate constants of the two parallel first-order reactions (k_1, route A; and k_2, route B in Scheme 1) by eq 1 and (2) on the basis of the product distribution at the early stages of the reaction, i.e., under conditions where the decomposition of the cyclic phosphates may be neglected (see Table 1).

$$k_1 = \frac{[\text{Guo}]_t + [2',3'\text{-cUMP}]_t}{[\text{Guo}] + [2',3'\text{-cUMP}]_t + [\text{Urd}]_t + [2',3'\text{-cGMP}]_t}\, k_{di} \quad (1)$$

$$k_2 = \frac{[\text{Urd}]_t + [2',3'\text{-cGMP}]_t}{[\text{Urd}]_t + [2',3'\text{-cGMP}]_t + [\text{Guo}]_t + [2',3'\text{-cUMP}]_t}\, k_{di} \quad (2)$$

At pH 4−6, where isomerization competes with cleavage, the first-order rate constants (k_{cl}) for the cleavage of isomeric GpUs (routes A and B in Scheme 1 and route G in Scheme 2) were obtained by applying the integrated first-order rate equation to the time-dependent sum concentration of the GpU isomers. The first-order rate constants of the isomerization of the starting material, k_{is} (route D in Scheme 1 and route H in Scheme 2), were calculated by eq 3 or 4 by bisecting the rate constant of the disappearance of the starting material (k_{di}) to the rate constants of parallel first-order reactions on the basis of the product distribution at the early stages of the reaction, i.e., under conditions where the reverse reaction of the 3′ to 2′ migration may be neglected. [**1a**]$_0$ and [**1b**]$_0$ are the initial concentrations of 3′,3′-GpU and guanosyl-(3′,3)-(2′,5′-di-O-methyluridine). [**1a**]$_t$ and [**1b**]$_t$ denote the concentrations of 3′,3′GpU and guanosyl-(3′,3)-(2′,5′-di-O-methyluridine) at time

t. **2a**, **2b**, **4**, and **5** are the concentrations of the isomers of 3′,3′-GpU or guanosyl-(3′,3)-(2′,5′-di-O-methyluridine) at time t.

$$k_{is} = \frac{[2\mathbf{a} + 4 + 5]_t}{[\mathbf{1a}]_0 - [\mathbf{1a}]_t}\, k_{di} \quad (3)$$

$$k_{is} = \frac{[2\mathbf{b}]_t}{[\mathbf{1b}]_0 - [\mathbf{1b}]_t}\, k_{di} \quad (4)$$

Under more acidic conditions (pH < 4), where depurination competes with cleavage and isomerization, the first-order rate constants (k_{dp}) for the depurination of isomeric GpUs (route C in Scheme 1 or route I in Scheme 2) were calculated using eq 5 by bisecting the rate constant (k_{dec}) for the decomposition of the isomeric GpUs to the rate constants of parallel first-order reactions on the basis of the product distribution at the early stages of the reaction, i.e., under conditions where the formation of guanine from the cleavage products may be neglected. [Guanine]$_t$ and [GpUs]$_t$ are the concentration of guanine (**9**) and the sum concentration of GpU isomers (**1a**, **2a**, **4**, and **5** in the case of 3′,3′-GpU and **2a** and **2b** in the case of guanosyl-(3′,3)-(2′,5′-di-O-methyluridine)), respectively, at time t. [GpU]$_0$ denotes the initial concentration of the starting material **1a** or **1b**.

$$k_{dp} = \frac{[\text{Guanine}]_t}{[\text{GpU}]_0 - [\text{GpUs}]_t}\, k_{dec} \quad (5)$$

Equation 6 was then applied to obtain the rate constants (k_{cl}) for cleavage of isomeric GpUs (routes A and B in Scheme 1 and route G in Scheme 2).

$$k_{cl} = k_{dec} - k_{dp} \quad (6)$$

Conclusions

In summary, comparative kinetic measurements with guanosyl-(3′,3′)-uridine (**1a**) and its 2′,5′-di-O-methyluridine analogue (**1b**) have shown that the 2′-hydroxyl group of the uridine moiety, as an intramolecular hydrogen-bond donor, accelerates the cleavage of dinucleoside 3′,3′-monophosphates via a dianionic phosphorane by a factor 23. In all likelihood, stabilization of both the dianionic phosphorane intermediate and the departing nucleoside 3′-oxyanion by hydrogen bonding contributes to the observed rate enhancement. These results, together with the previous finding that a 3′-linked nucleoside inherently is cleaved 3 times as fast as its 5′-counterpart, largely explain the well-known regioselectivity governing the breakdown of the phosphorane intermediate of group I introns.

Supporting Information Available: Time-dependent product distributions for the hydrolysis of 3′,3′-GpU and 3′,3′-GpMe$_2$U. This material is available free of charge via the Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

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