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Hydrolysis of 2',3'-*O*-Methyleneadenosin-5'-yl Bis-5'-*O*-Methyluridin-3'-yl Phosphate: The 2'-Hydroxy Group Stabilizes the Phosphorane Intermediate, Not the Departing 3'-Oxyanion, by Hydrogen Bonding

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Abstract: Hydrolytic reactions of 2′,3′-*O*-methyleneadenosin-5′-yl bis-5′-*O*-methyluridin-3′-yl phosphate (**1a**) have been followed by RP HPLC over a wide pH range to elucidate the role of the 2'-OH group as an intermolecular hydrogen bond donor facilitating the cleavage of **1a**. At pH < 2, where the decomposition of **1** is first-order in hydronium-ion concentration, the P−O5′ and P−O3′ bonds are cleaved equally rapidly. Over a relatively wide range from pH 2 to 4, the hydrolysis is pH-independent and the P−O5′ bond is cleaved 1.6 times as rapidly as the P−O3′ bond. At pH 6, the reaction becomes first-order in hydroxide-ion concentration and cleavage of the P−O3′ bond starts to predominate, accounting for 89% of the overall hydrolysis in 10 mmol L^{-1} aqueous sodium hydroxide. Under alkaline conditions, the 2′-OH group facilitates the cleavage of **1** by a factor of 27 compared to the 2'-OMe counterpart, the influence on the P−O3′ and P−O5′ bond cleavage being equal. Accordingly, the 2′-hydroxy group stabilizes the phosphorane intermediate, not the departing 3'-oxyanion, by hydrogen bonding.

Introduction

The large ribozymes, viz. group I and II introns and the RNA subunit of RNase P, use an intermolecular nucleophile to displace the 3′-linked nucleoside of the scissile phosphodiester bond.[1] The reaction is highly regioselective—no cleavage of the 5′-linked nucleoside is observed. It has been suggested that the overwhelmingly faster cleavage of the P−O3′ bond is a result of a proton transfer from the neighboring 2′-hydroxyl group to the departing 3′-oxygen atom concerted with the bond cleavage.[2−4] Indeed, hydroxide-ion catalyzed cleavage of the P−O3′ bond of guanylyl-(3′,3′)-uridine (**2a**) is 23 times as fast as the corresponding cleavage of the P−O3′ bond of guanylyl-(3′,3′)−(2′,5′-di-*O*-methyluridine) (**2b**).[5] The previously reported β_{lg} value of −1.28[6] and the pK_a difference between the conjugate acids of the leaving groups of approximately 0.7[7] would predict only a 7-fold rate acceleration, so one might consider stabilization of the phosphorane intermediate as another possible source of rate acceleration. The relative importance of these two modes of acceleration cannot, however, be deduced on the basis of studies with phosphodiester models. To answer this question, a trinucleoside 3′,3′,5′-monophosphate **1a** having both of the 2′-OH functions flanking the phosphate group free has been synthesized and its decomposition studied over a wide pH range. One of the 2′-OH groups serves as an intramolecular nucleophile, while the other may donate a hydrogen bond to either the departing 3′-oxyanion or one of the phosphoryl oxygens of the pentacoordinated phosphorane intermediate. Comparison of the rate constants for the hydroxide-ion catalyzed cleavage of the P−O3′ and P−O5′ bonds of **1a** with those of 2′,3′-*O*-methyleneadenos-5′-yl 2′,5′-di-*O*-methylurid-3′-yl 5′-*O*-methylurid-3′-yl phosphate (**3**), an analogue of **1a** having the 2′-OH function of the departing 3′-linked nucleoside methylated,[8] allows breakdown of the overall acceleration to contributions of intermediate and leaving-group stabilization. If stabilization of the leaving group by hydrogen bonding plays a major role, then the product distribution should be changed considerably on methylation of the 2′-OH group of the departing 3′-linked nucleoside. If, on the other hand, stabilization of the phosphorane intermediate is the primary source of acceleration, then departure of both leaving groups should be equally facilitated.

Results

Preparation of the Fully Protected Trinucleoside 3',3',5'-Monophosphate (4). N^6-Benzoyl-2′,3′-*O*-methyleneadenosin-5′-yl bis[2′-*O*-(4,4′-dimethoxytrityl)-5'-*O*-methyluridin-3′-yl] phosphate (**5**) was obtained by tetrazole-promoted stepwise

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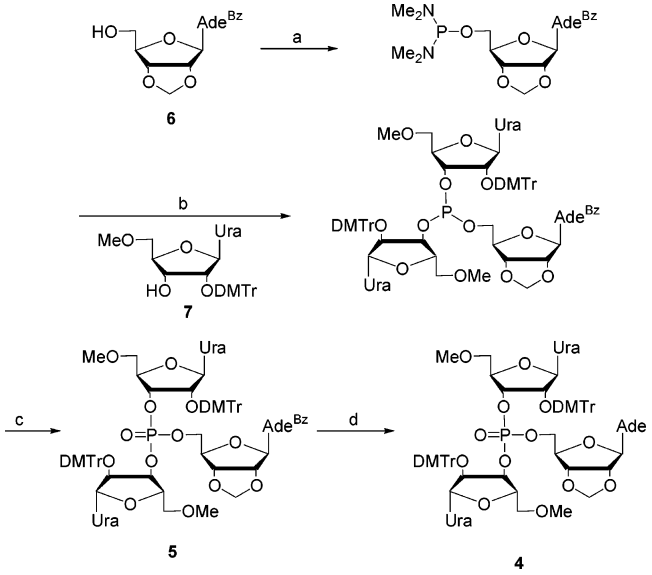
10.1021/ja050325l CCC: $30.25

Scheme 1. Preparation of the Trinucleoside 3′,3′,5′-monophosphate **4**[a]



[a] Reagents and conditions: (a) $P(NMe_2)_3$, tetrazole, MeCN, (b) tetrazole, MeCN (c) I_2, H_2O, THF, 2,6-lutidine (d) NH_3, MeOH.

displacement of the dimethylamino groups from tris(dimethylamino)phosphine with the appropriately protected nucleosides (**6**, **7**) (Scheme 1). The nucleosides were prepared as described in the literature.[8−13] Removal of the N^6-benzoyl protection with methanolic ammonia, followed by RP HPLC purification, then gave the desired triester **4**.

Product Distribution and Reaction Pathways. The hydrolysis of **1a** was followed over a wide pH range (from $H_0 = -0.1$ to pH 7) at 25 °C by analyzing the composition of the aliquots withdrawn from the reaction mixture at appropriate time intervals by RP HPLC. The 2′-*O*-(4,4′-dimethoxytrityl) groups of **4** were first removed with 99 mmol L^{-1} HCl in 1,4-dioxane to give **1a**, after which the appropriate aqueous solution was created in the reaction vessel so that the final amount of 1,4-dioxane was 5% (*v*/*v*). The aliquots were quenched by cooling to −16 °C and adjusting their pH to approximately 3 with a formic acid buffer when necessary. The products were characterized by either spiking with authentic samples or mass spectrometric analysis (HPLC/ESI−MS) and the peak areas were converted to relative concentrations by calibrating the system with uridine and adenosine solutions of known concentration.

Two distinct sets of products were observed in the entire pH range (Scheme 2). Cleavage of the P−O5’ bond (Route A) resulted in formation of 2′,3′-*O*-methyleneadenosine (m<A, **8**) and the isomeric diesters 5′-*O*-methyluridylyl-3′,3′-(5′-*O*-methyluridine) [mU(3′,3′)mU, **9a**] and 5′-*O*-methyluridylyl-2′,3′-(5′-*O*-methyluridine) [mU(2′,3′)mU, **9b**]. 5′-*O*-Methyluridylyl-2′,2′-(5′-*O*-methyluridine) [mU(2′,2′)mU, **9c**], resulting from the cleavage of the isomer **1c** of the starting material, was also observed. Cleavage of one of the P−O3′ bonds (Route B), in turn, produced 5′-*O*-methyluridine (mU, **10**) and the isomeric diesters 5′-*O*-methyluridylyl-3′,5′-(2′,3′-*O*-methyleneadenosine) [mU(3′,5′)m<A, **11a**] and 5′-*O*-methyluridylyl-2′,5′-(2′,3′-*O*-methyleneadenosine) [mU(2′,5′)m<A, **11b**]. Under acidic conditions, cleavage of the P−O3′ and P−O5′ bonds was equally rapid. Under alkaline conditions, cleavage of the P−O3′ bond predominated.

Table 1. Second-Order Rate Constants for the Buffer-Catalyzed Cleavage of **1a**,**b**,**c** (T = 25.0 °C, $I(NaNO_3)$ = 1.0 mol L^{-1})

buffer acid	[HA]/[A-]	$k_{cl}^{buf}/10^{-4}$ L mol^{-1} s^{-1}
formic acid	3:1	24.9 ± 0.8
	1:3	87 ± 9
acetic acid	1:1	189.1 ± 0.8
	1:3	316 ± 5

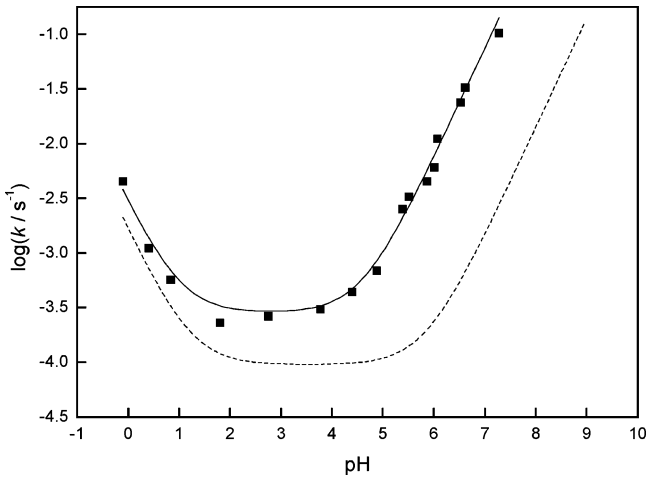


Figure 1. pH-rate profile for the decomposition and mutual isomerization of **1a**,**b**,**c** (solid line) and **3** (dotted line) at 25 °C, $I(NaNO_3)$ = 1.0 mol L^{-1}.

The pseudo first-order rate constants for the decomposition of **1a** were obtained by applying the integrated first-order rate equation to the time-dependent diminution of the concentration of the starting material. Between pH 3 and 5, a considerable buffer catalysis was observed (Table 1). Buffer-independent rate constants were obtained by performing the measurements at various buffer concentrations ranging from 0.04 to 0.24 mol L^{-1} and extrapolating the observed pseudo first-order rate constants to zero buffer concentration by linear regression.

pH-Rate Profiles. Figure 1 shows the pH-rate profile for the overall disappearance of **1**. At pH < 2, the overall reaction is first-order in $[H_3O^+]$ and the P−O5′ bond (Route A) and P−O3′ bonds (Route B) are cleaved equally rapidly. Over a relatively wide range from pH 2 to pH 4, the overall reaction is pH-independent and cleavage of the P−O5’ bond (Route A) predominates, accounting for 62% of the overall hydrolysis. At pH 5, the overall reaction becomes first-order in $[HO^-]$ and cleavage of the P−O3′ bond (Route B) gradually starts to predominate (Figure 2). In 10 mmol L^{-1} aqueous sodium hydroxide, 89% of the overall hydrolysis results from cleavage of the P−O3′ bond.

Mutual isomerization of **1a**,**b**,**c** is rapid and an equilibrium is settled already during the deprotection of **4**. This observation agrees well with previous results on the isomerization of ribonucleoside phosphotriesters.[8,14,15] For example, the half-life for the interconversion of 5′-*O*-methyluridine 2′− and 3′-dimethyl phosphates in 0.1 mol L^{-1} HCl at 25 °C is ap-

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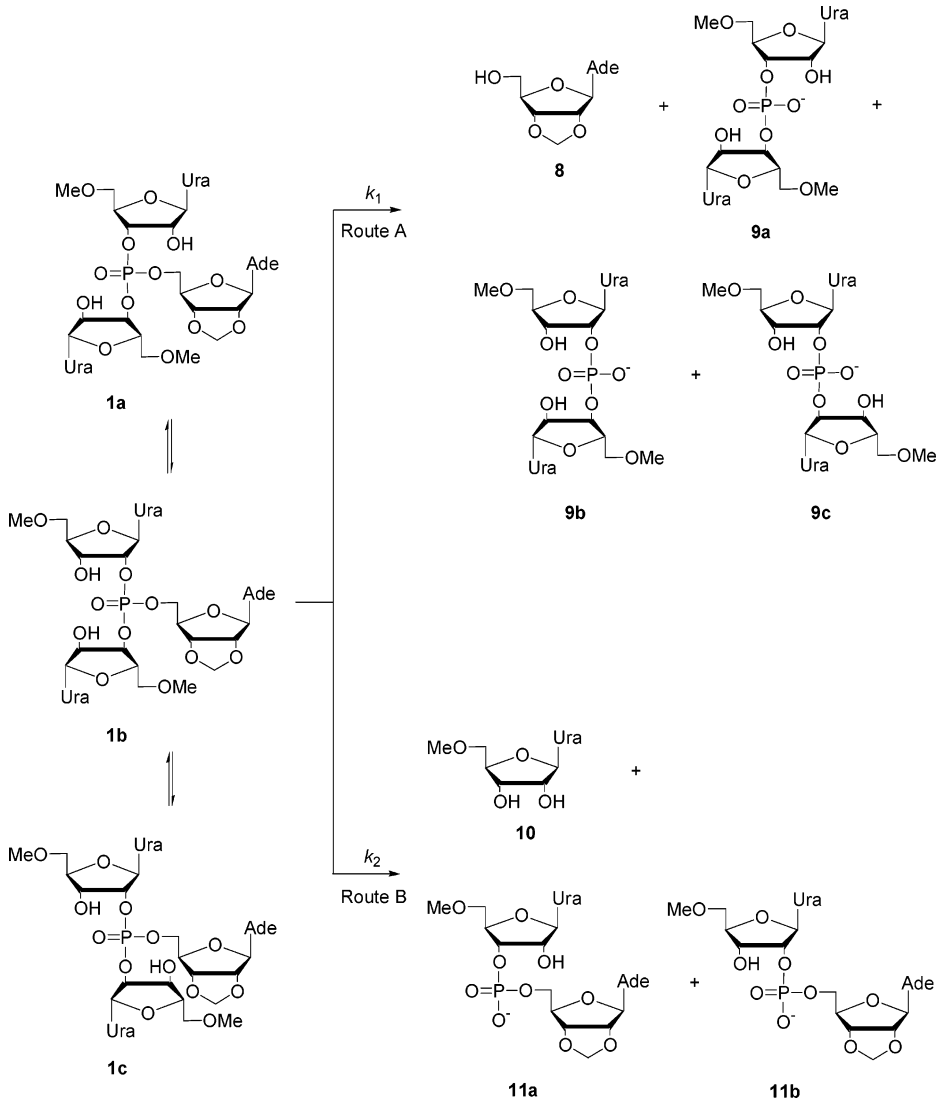
Scheme 2



proximately 11 hours,[14] while the corresponding value for the isomers of **3** is less than 14 min.[8] No kinetic data for this reaction could therefore be obtained.

Under conditions in which the overall cleavage is pH-independent, a considerable buffer catalysis was observed. Only the basic buffer constituent, viz. the carboxylate anion, contributed to the catalysis. Table 1 summarizes the second-order rate constants for the catalysis by formic and acetic acid buffers at two different ratios of [HA]/[A$^-$].

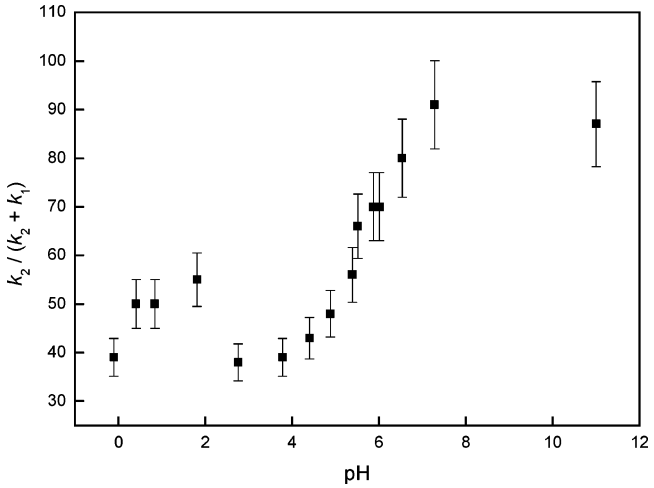


Figure 2. Ratio of k_1 (Route A) to $k_1 + k_2$ (overall cleavage) as a function of pH.

The observed rate constant for the overall cleavage of **1**, k_{cl}^{obs}, may be expressed by eq 1.

$$k_{cl}^{obs} = k_{cl}^{H} a_{H+} + k_{cl}^{W} + \frac{k_{cl}^{OH} K_W}{a_{H+}} \qquad (1)$$

k_{cl}^{H}, k_{cl}^{W}, and k_{cl}^{OH} are the rate constants for the hydronium-ion catalyzed, pH-independent and hydroxide-ion catalyzed cleavage, respectively. These rate constants were obtained by fitting the experimental values to eq 1 by a nonlinear least-squares method and dividing the results by 2 to take into account the fact that in **1** there are two potential 2′-OH nucleophiles to initiate the reaction. The rate constants for the overall cleavage of **1**, k_{cl}, can then be dissected to the rate constants for the partial reactions, viz. cleavage of P−O5′ (Route A) and P−O3′ (Route

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Scheme 3

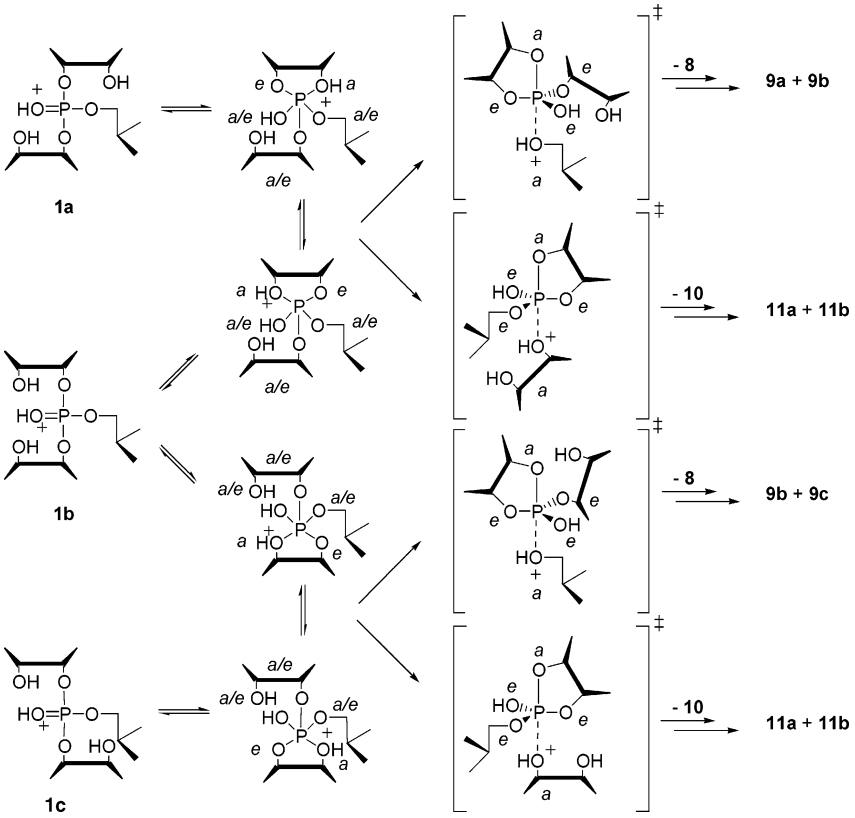

Table 2. Rate Constants for the Partial Reactions of Cleavage of **1a**,**b**,**c** and **3** (T = 25.0 °C, I($NaNO_3$) = 1.0 mol L^{-1})

		$k^H/10^{-3}$ L mol^{-1} s^{-1}	$k^N/10^{-4}$ s^{-1}	$k^{OH}/10^5$ L mol^{-1} s^{-1}
1	k_{cl}	1.4 ± 0.1	1.4 ± 0.2	2.0 ± 0.1
	k_1	0.70 ± 0.05	0.9 ± 0.1	0.22 ± 0.01
	k_2	0.70 ± 0.05	0.5 ± 0.1	1.78 ± 0.09
3	k_{cl}	1.6 ± 0.3	0.9 ± 0.09	0.075 ± 0.009
	k_1	1.0 ± 0.2	0.68 ± 0.07	0.009 ± 0.001
	k_2	0.6 ± 0.1	0.23 ± 0.02	0.066 ± 0.008

B) bonds, on the basis of the relative concentrations of the monomeric products of these competing reactions (eqs 2 and 3). The rate constants obtained are presented in Table 2.

$$k_1 = \frac{[\mathrm{m} < \mathrm{A}]}{[\mathrm{m} < \mathrm{A}] + [\mathrm{m}_2\,\mathrm{U}]} k_{cl} \qquad (2)$$

$$k_2 = \frac{[\mathrm{m} < \mathrm{U}]}{[\mathrm{m} < \mathrm{A}] + [\mathrm{m}_2\,\mathrm{U}]} k_{cl} \qquad (3)$$

Hydronium-Ion Catalyzed Cleavage. At pH < 2, the overall disappearance of **1** is first-order in [H_3O^+]. The leaving group departs as an alcohol (Scheme 3) and the rate of the reaction is, hence, only moderately sensitive to the acidity of the leaving group, the previously reported β_{lg} value for ribonucleoside 3′-(alkyl isopropyl phosphates) being −0.40.[16] The P−O5′ (Route A) and P−O3′ (Route B) bonds are cleaved equally rapidly ($k_1^H = k_2^H = 7 \times 10^{-4}$ L mol^{-1} s^{-1}), the rates being comparable to those observed with 2′,3′-*O*-methyleneadenos-5′-yl 2′,5′-di-*O*-methylurid-3′-yl 5′-*O*-methylurid-3′-yl phosphate (**3**).[8] A k_1^H/k_2^H ratio of 0.2 would be expected on the basis of the β_{lg} value and the known difference of 1.7 units between the pK_a values of **8** and **10**.[7,16,17] As suggested previously,[8] this discrepancy may probably be attributed to the fact that the 5′-*O*-methylurid-3′-yl group as a sterically demanding substituent prefers an equatorial position in the pentacoordinated phosphorane intermediate, hence making its departure less probable.[18]

Hydroxide-Ion Catalyzed Cleavage. At pH > 5, the overall disappearance of **1** is first-order in [OH^-] and the cleavage of the P−O3′ bond (Route B) is favored over the cleavage of the P−O5′ bond (Route A). Since the base-catalyzed hydrolysis proceeds by departure of an alkoxide ion, the cleavage is much more sensitive to the pK_a of the leaving group than in the case of the acid-catalyzed cleavage. The previously reported β_{lg} value for ribonucleoside 3′-(alkyl isopropyl phosphates) is −1.38.[16] This change in reaction mechanism thus explains the change in the observed product distribution. The second-order rate constant for the overall hydronium-ion catalyzed cleavage was 2.0 × 10^5 L mol^{-1} s^{-1}, 27 times greater than the respective rate constant observed with **3**.[8] It is worth noting, however, that the ratio of the second-order rate constant for the hydroxide-ion catalyzed cleavage of the P−O5′ bond (Route A), k_1^{OH} (0.22 × 10^5 L mol^{-1} s^{-1}), to the respective rate constant for the cleavage of the P−O3′ bond (Route B), k_2^{OH} (1.78 × 10^5 L mol^{-1} s^{-1}), is essentially identical to that observed with **3** (k_1^{OH}/k_2^{OH} = 0.12 and 0.14, respectively),[8] suggesting that the stabilization of the 3′-leaving alkoxide ion by hydrogen bonding from the neighboring 2′-OH group plays only a minor role in

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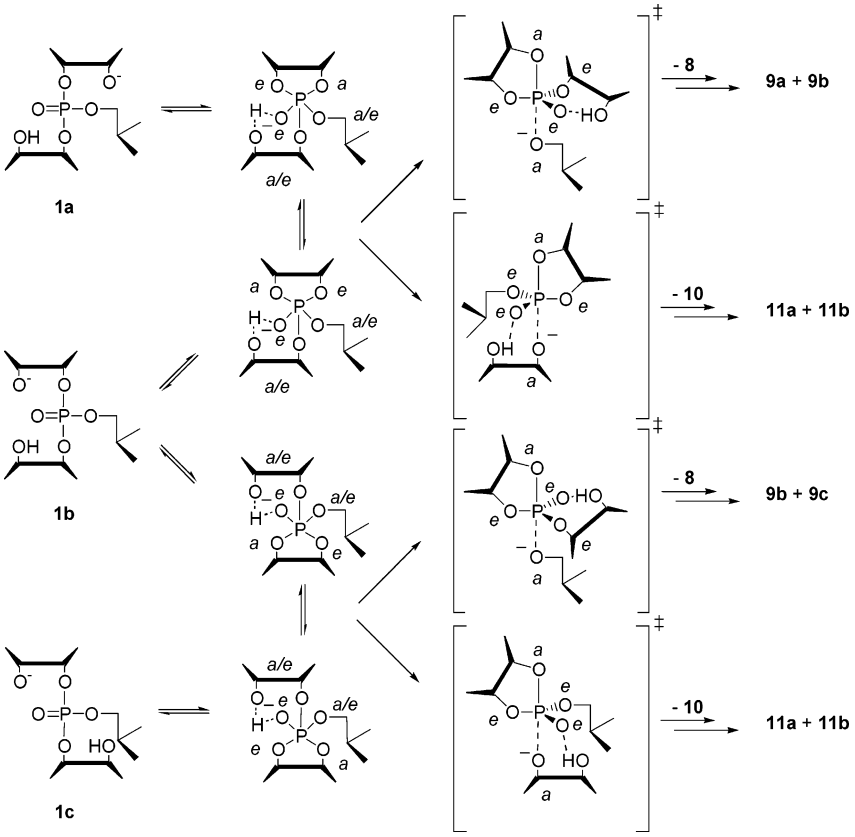
Scheme 4



determining the product distribution of the hydrolysis of **1**. In other words, although 5′-*O*-methyluridine is approximately 0.7 pK_a units more acidic than 2′,5′-di-*O*-methyluridine,[7] its 3′-oxyanion does not appear to be a significantly better leaving group. This observation can be reasoned if one assumes that the 2′-OH of the leaving 5′-*O*-methyluridine is engaged in relatively strong hydrogen bonding with a phosphoryl oxygen of the pentacoordinated phosphorane intermediate (Scheme 4) and the potential hydrogen bond to the developing 3′-oxyanion is practically nonexistent. In other words, the fact that the 2′-OH of the departing 3′-linked nucleoside accelerates the cleavage of the P−O5′ and P−O3′ bonds to an equal extent strongly suggests that the pentacoordinated phosphorane intermediate rather than the departing 3′-oxyanion is stabilized by hydrogen bonding from the 2′-OH group. Actually, stabilization of the intermediate is probably even more pronounced than the observed kinetic effect, since donation of a hydrogen bond to the phosphorane is expected to destabilize the transition state of the rate-limiting step, viz. loss of the leaving group (Figure 3). Evidently, the former effect is approximately 8 kJ mol^{-1} greater than the latter, consistent with the fact that the phosphoryl oxygen is more basic in the intermediate than in the very late transition state.[16] It is tempting to speculate that the previously reported 23-fold rate acceleration observed in the hydronium-ion catalyzed cleavage of guanylyl-(3′,3′)-uridine (**2a**) and guanylyl-(3′,3′)−(2′,5′-di-*O*-methyluridine) (**2b**) is also of the same origin.[5]

pH-Independent and Buffer Catalyzed Cleavage. In the range from pH 2 to 4, the cleavage of **1** is pH-independent but susceptible to general base catalysis by carboxylate anions. The latter reaction may be interpreted to be a sequencial specific base/general acid catalysis, where the attacking 2′-OH is deprotonated in a preequilibrium step and proton transfer from the general acid to the leaving oxyanion occurs concerted with the rate-limiting P−O bond scission.[15] The second-order rate constant for the buffer catalyzed cleavage is nearly independent of the leaving group (**8** vs **10**), probably because the polar effects of the departing nucleoside on the rate of proton transfer and bond cleavage are opposite. The previously reported β_{lg} value for this reaction in the case of ribonucleoside 3′-(alkyl isopropyl phosphates) is −0.73.[16] The ratio of the first-order rate constant for the pH- and buffer-independent cleavage of the P−O5′ bond (Route A) to the respective rate constant for the cleavage of the P−O3′ bond (Route B) is 1.6, about half of the value observed with **3**. The Brönsted β value for the observed general base catalysis is approximately 0.6 which corresponds to an α value of 0.4 for the general acid-catalyzed step of the sequential specific base/general acid catalysis. Taken together with the β_{lg} value of −0.73 these data indicate that P−O bond fission and proton transfer from the general acid are approximately equally advanced in the transition state (the previously reported β_{eq} value for this reaction is −1.83).[19]

In summary, these results, when compared with those previously obtained with **3**, suggest that stabilization of the pentacoordinated phosphorane intermediate by hydrogen bonding of the 2′-OH group of a 3′-linked nucleoside is the major source of the observed 27-fold rate acceleration and that leaving-group stabilization is only of minor importance. It is tempting to speculate that intermediate stabilization is also the cause of the rate acceleration of a similar magnitude observed with

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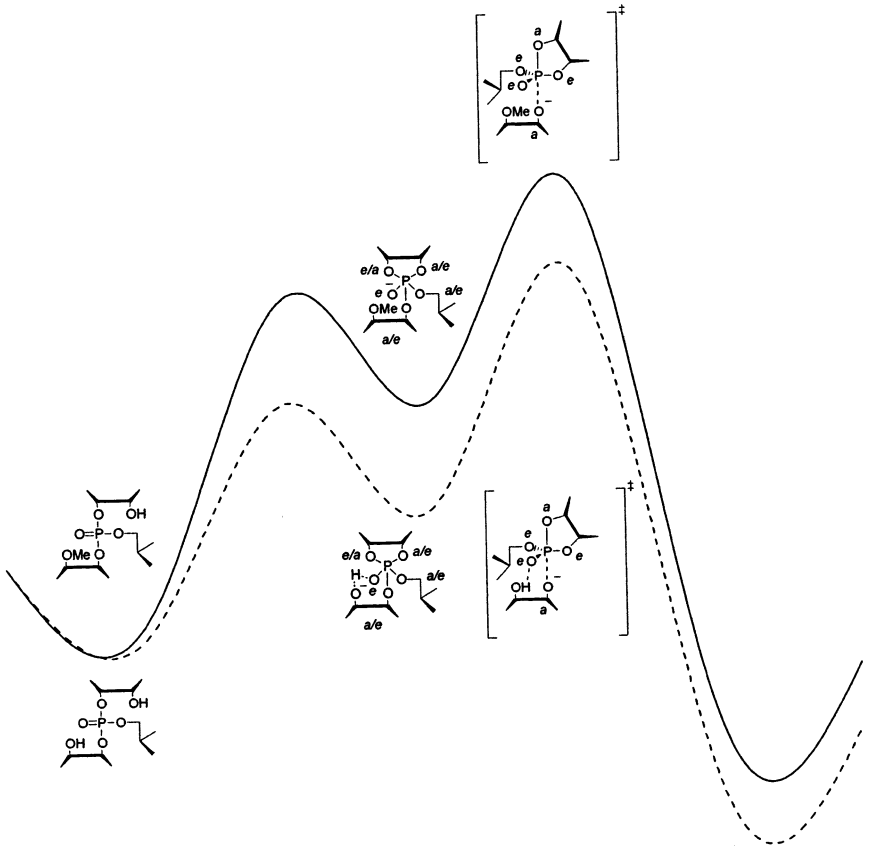
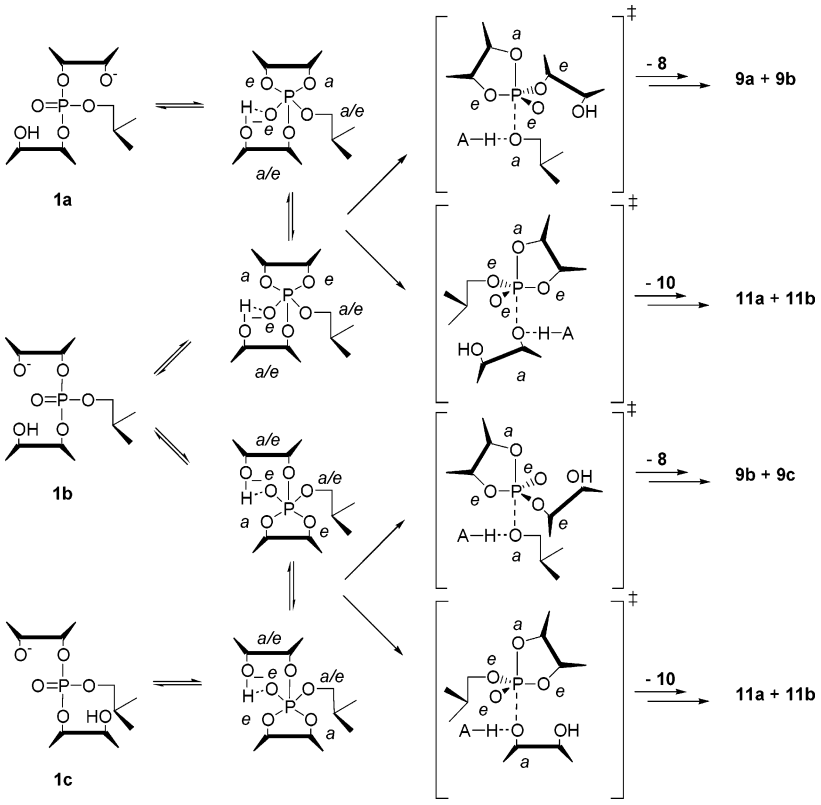


Figure 3. Free-energy profile for the hydroxide-ion catalyzed P−O3′ bond cleavage of **3** (solid line) and **1** (dashed line) (cf. Scheme 4). The transition states for the P−O5′ bond cleavage are analogous.

Scheme 5



phosphodiesters **2a** and **2b**, although the reaction with diesters proceeds via a dianionic rather than monoanionic phosphorane.[5]

Taken together, these data suggest that stabilization of the departing 3′-oxyanion by hydrogen bonding by the neighboring

2′-OH group is probably of little, if any, importance in determining the course of the reactions catalyzed by the large ribozymes.

Experimental Section

Materials. Nucleosides were commercial products and they were used as received after checking their purity by HPLC. The buffer constituents were of reagent grade.

Kinetic Measurements. Reactions were carried out in sealed tubes immersed in a thermostated water bath, the temperature of which was adjusted to 25 °C within ± 0.1 °C. The reactions were started by adding the starting material in DMSO (10 μL) to 80 μL of 99 mmol L^{-1} HCl in 1,4-dioxane. After 65 min at 45 °C, 1520 μL of the desired reaction solution (prethermostated to 25 °C) was added. The hydronium-ion concentration of reaction solutions was adjusted with nitric acid and formate, acetate, MES and HEPES buffers. The pH values of the solutions were checked with a pH meter. The ionic strength of the solutions was adjusted to 1.0 mol L^{-1} with $NaNO_3$. Between pH 3 and 5, a considerable buffer catalysis was observed (Table 1). Buffer-independent rate constants were obtained by varying the buffer concentration from 0.04 to 0.24 mol L^{-1} and extrapolating the observed pseudo first-order rate constants to zero buffer concentration by linear regression.

The initial substrate concentration in the kinetic runs was ca. 10^{-4} mol L^{-1}. The composition of the samples withdrawn at appropriate time intervals was analyzed by HPLC on a Hypersil-Keystone Aquasil C18 column (4 × 150 mm, 5 μm) using 0.06 mol L^{-1} formic acid buffer and MeCN as an eluent. The amount of MeCN was 7% for the first 15 min, after which it was increased linearly to 27% during 20 min. The observed retention times (t_R, min) for the hydrolytic products of **1** (the flow rate was 1.0 mL min^{-1}) were as follows: 31.4, 30.6, 30.1 (**1a**, **b**, **c**), 22.4, 19.5, 15.4 (**9a**, **b**, **c**), 20.3 (**11a**), 14.5 (**11b**), 7.3 (**8**), 4.4 (**10**). The products were characterized by either spiking with authentic samples or mass spectrometric analysis (HPLC/ESI−MS) and the peak areas were converted to relative concentrations by calibrating the system with uridine and adenosine solutions of known concentration. The pseudo first-order rate constants for the decomposition of **1** were obtained by applying the integrated first-order rate equation to the time-dependent diminution of the concentration of the starting material. Compound **1** decomposed somewhat during the deprotection and the product peaks for **8**, **9a**, **b**, **c**, **10** and **11a**, **b** were visible already in the first sample. To obtain the correct product distributions for the hydrolytic reactions, these initial concentrations were subtracted from the concentrations observed in the later samples.

2′,3′-*O*-Methyleneadenosin-5′-yl bis[2′-*O*-(4,4′-dimethoxytrityl)-5′-*O*-methyluridin-3′-yl] phosphate (4). N^6-Benzoyl-2′,3′-*O*-methyleneadenosine[12,13] (**6**, 0.92 mmol, 0.3522 g) was coevaporated three times from anhydrous pyridine and once from anhydrous MeCN. The residue was dissolved in anhydrous MeCN (3.0 mL) and tris(dimethylamino)-phosphine (0.92 mmol, 0.29 mL) and 1*H*-tetrazole (1.10 mmol, 76.7 mg) were added. The reaction mixture was stirred for 160 min at room temperature, after which 5′-*O*-methyl-2′-*O*-(4,4′-dimethoxytrityl)uridine[8] (**7**, 2.86 mmol, 1.60 g) dissolved in anhydrous MeCN (2.0 mL) and 1*H*-tetrazole (2.20 mmol, 154 mg) were added. After stirring for 18 h at room temperature, iodine (1.05 mmol, 0.27 g) in a mixture of water (4.2 mL), THF (8.4 mL) and 2,6-lutidine (2.1 mL) was added. The reaction mixture was stirred for 60 min at room temperature, after which it was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHSO_3$. The aqueous phase was back extracted with CH_2Cl_2. The combined organic phases were evaporated to dryness and the residue was purified on a silica gel column eluting with a mixture of MeOH, CH_2Cl_2 and Et_3N (1:98:1, v/v). The crude product thus obtained was dissolved in saturated methanolic ammonia (20 mL). After being stirred for 6 h at room temperature, the reaction mixture was evaporated to dryness. The residue was purified first on a silica gel column eluting with a mixture of MeOH, CH_2Cl_2 and Et_3N (4:95:1, v/v), then by HPLC on a Supelcosil LC-18 column (25 cm × 21.2 mm, 12 μm) eluting with a mixture of 0.05 mol L^{-1} of aqueous NH_4OAc and MeCN (30:70, *v*/*v*). Finally, the buffer salts were removed on the same column by eluting with a mixture of water and MeCN (30:70, v/v). Overall yield starting from **6** 61.5 mg (4.6%). ^1H NMR (δ_H)(500 MHz, DMSO-d_6) 11.48 (s, 1H), 11.38 (s, 1H), 8.31 (s, 1H), 8.18 (s, 1H), 6.7−7.4 (m, 28H), 7.14 (d, 1H, J = 8.1 Hz), 7.05 (d, 1H, J = 8.1 Hz), 6.28 (d, 1H, J = 7.5 Hz), 6.18 (d, 1H, J = 2.5 Hz), 6.17 (d, 1H, J = 7.5 Hz), 5.48 (d, 1H, J = 8.1 Hz), 5.38 (d, 1H, J = 8.1 Hz), 5.35 (dd, 1H, J_1 = 2.5 Hz, J_2 = 6.5 Hz), 5.20 (s, 1H), 5.20 (s, 1H), 4.93 (dd, 1H, J_1 = 3.6 Hz, J_2 = 6.5 Hz), 4.45 (m, 1H), 4.35 (m, 1H), 4.13−4.32 (m, 4H), 3.84 (m, 1H), 3.71 (s, 3H), 3.70 (s, 3H), 3.70 (s, 3H), 3.69 (s, 3H), 3.57 (m, 1H), 3.13−3.19 (m, 2H), 3.13 (s, 3H), 2.97 (s, 3H), 2.88−2.95 (m, 2H). ^{31}P NMR (δ_P)(202 MHz, DMSO-d_6) −1.96. HRMS (FAB) M^- calcd 1442.4659, obsd 1442.4647.

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