Buffer-catalyzed interconversion of ribonucleoside 2′/3′-methylphosphonates and 2′/3′-alkylphosphates

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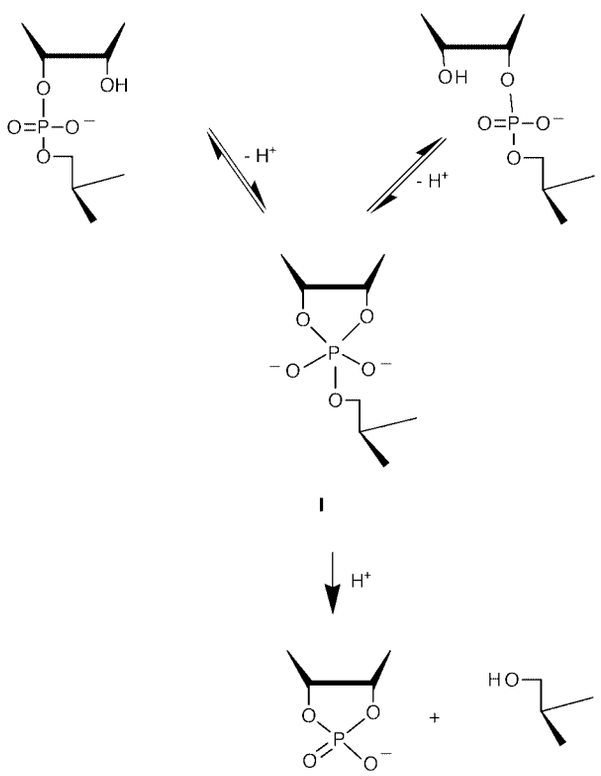
The $O^{2'} = O^{3'}$ isomerization of uridine 2′/3′-methylphosphonates, 2′/3′-isopropyl phosphates and 2′/3′-(2-ethoxyethyl) phosphates has been studied in buffer solution. In imidazole buffers, all these isomerizations exhibit only weak general acid catalysis. This low susceptibility of isomerization to buffer catalysis appears to be an inherent property of this reaction, and not a consequence of competitive buffer-catalyzed breakdown of the phosphorane intermediate to 2′,3′-cyclic phosphate: uridine 3′-(2-ethoxyethyl) phosphate and 3′-isopropyl phosphate exhibit a similar susceptibility of isomerization to buffer catalysis in spite of the fact that the former undergoes concurrent buffer-catalyzed cleavage and the latter does not. In carboxylic acid buffers (pH < 3), the 2′/3′-methylphosphonates were observed to undergo another buffer-catalyzed isomerization, which was first order in the concentration of both hydronium ion and the buffer acid. Plausible mechanistic interpretations for the buffer-catalyzed isomerizations are described.

Introduction

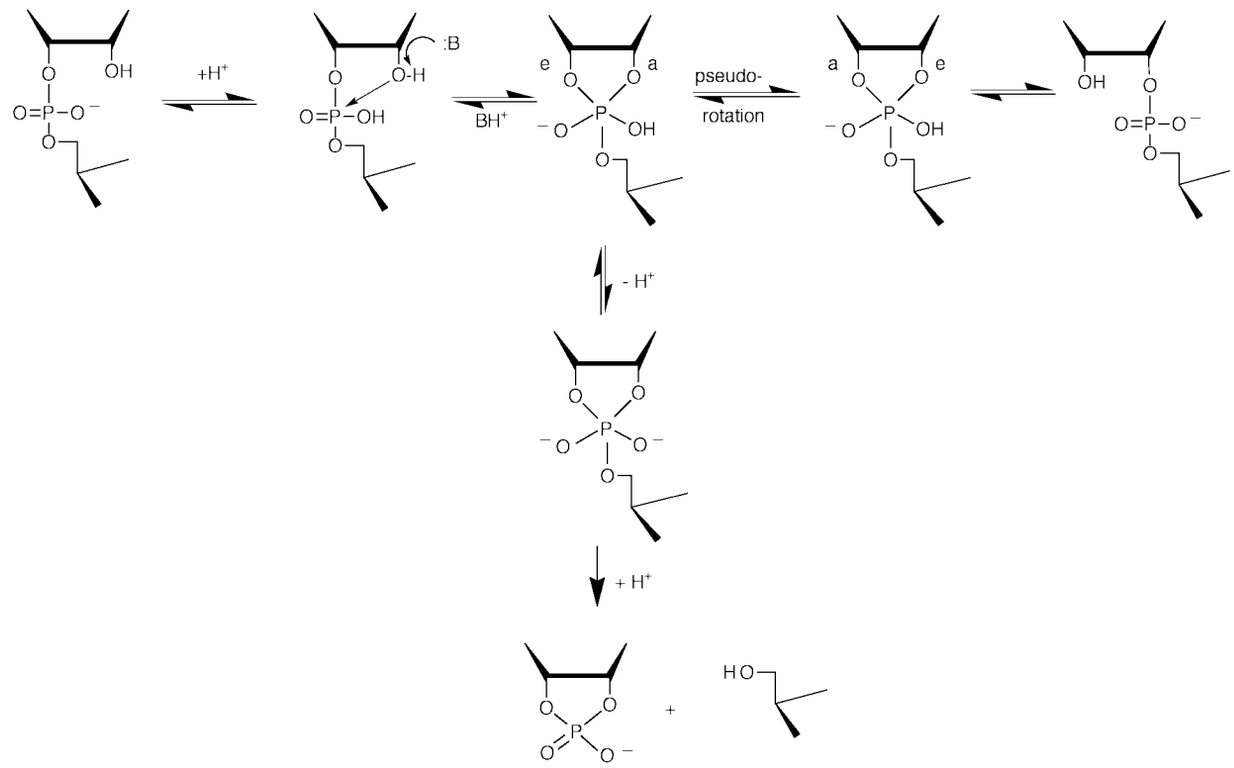
The internucleosidic 3′,5′-phosphodiester bonds of RNA are known to undergo two concurrent transesterification reactions under neutral conditions: (i) cyclization to a 2′,3′-cyclic phosphate with release of the 5′-linked nucleoside, and (ii) isomerization to a 2′,5′-bond (Scheme 1).[1] Both reactions may, at least in principle, take place *via* a common phosphorane intermediate (**I** in Scheme 1) obtained by the attack of the 2′-hydroxy



Scheme 1

function on phosphorus. The breakdown of the intermediate by cleavage of the P–O3′ bond leads to isomerization, while P–O5′ bond rupture results in cyclization to a 2′,3′-cyclic phosphate. Somewhat unexpectedly, these two reactions appear to exhibit dissimilar dependence of rate on the concentration of buffer constituents. The cyclization to a 2′,3′-cyclic phosphate is clearly buffer-catalyzed: compared to the uncatalyzed reaction, 0.7 M imidazole buffers enhance the reaction of uridylyl(3′,5′)uridine (3′,5′-UpU) by factors of 5–20, depending on the buffer ratio.[2] Both the acidic and basic buffer constituent serve as a catalyst, the contribution of general base catalysis being more important with imidazole buffers.[2,3] The isomerization reaction, in turn, has been shown to be catalyzed only by imidazolium ion, and the buffer catalysis is more modest than with the cyclization reaction.[2,3] According to Beckman *et al.*,[2] the isomerization rates in 0.7 M imidazole buffers are, depending on the buffer ratio, only 30–80% higher than the buffer-independent rates. The most recent results of Breslow *et al.* on the same reaction are similar, as far as the imidazole catalysis is concerned: a 50% increase in the isomerization rate takes place on increasing the concentration of imidazolium ion from 0.2 to 0.8 M at a constant ionic strength.[3]

Interestingly, Breslow *et al.*[3,4] have reported a decrease in the isomerization rate on increasing the buffer concentration in more basic morpholine buffers. This observation plays a central role in their mechanistic reasoning. The buffer acid is assumed to accelerate the formation of the phosphorane intermediate by a sequential specific acid/general base catalysis (Scheme 2). The intermediate is then assumed to undergo either an uncatalyzed (partly) rate-limiting pseudorotation (leading to isomerization), or general base catalyzed cyclization (actually sequential specific base/general acid catalysis). Since the buffer base catalyzes only the cyclization, not pseudorotation, the intermediate is decomposed at high concentration of the buffer base preferentially to cyclic phosphate, and this is observed experimentally as retardation of isomerization. In morpholine buffers a "negative catalysis" for isomerization is thus observed. The less basic imidazole buffers do not result in as marked a change in the partition of the phosphorane intermediate to the isomerization and cyclization products, and "the negative catalysis" hence remains hidden. The authors also argue that the observed "negative catalysis" cannot be attributed to medium effects: dioxane as a surrogate of neutral morpholine base has been observed to accelerate the isomerization. Beckman *et al.*[2] have, in turn, shown that balancing the changes in the concentration



Scheme 2

of free imidazole with DMF has only a minor effect on the rate of isomerization.

Since the "negative catalysis" discussed above plays a central role in distinguishing between various mechanistic alternatives of the transesterification reactions of RNA phosphodiester bonds, and since the buffer catalyzed contribution to the observed rate of isomerization is so small that its unequivocal distinction from medium effects is difficult, additional experimental data on the buffer catalyzed isomerization appear desirable. We have approached the subject by performing measurements with two different types of model compounds. Firstly, the buffer catalyzed interconversion of uridine 2′- (**1b**) and 3′-methylphosphonate esters (**1a**) has been studied. Owing to the marked hydrolytic stability of the P–C bond, the ribonucleoside phosphonates cannot undergo concurrent cyclization to a 2′,3′-cyclic phosphate. They are, however, isomerized approximately as readily as their phosphodiester counterparts.[5] The buffer-catalyzed isomerization can hence be studied without the interference of a competing reaction. Secondly, the isomerization of uridine 3′-isopropyl phosphate (**2a**) and uridine 3′-(2-ethoxyethyl) phosphate (**3a**) to the corresponding 2′-phosphates (**2b**, **3b**) has been examined. It is known that the 2-ethoxyethyl ester is isomerized and cyclized under neutral conditions at comparable rates.[6] The isopropyl ester, in turn, undergoes isomerization much faster than cyclization.[6] Accordingly, comparison of the buffer catalyzed isomerization of these three pairs of compounds should allow one to conclude whether the competing cyclization really markedly influences the observed buffer catalysis of the isomerization.

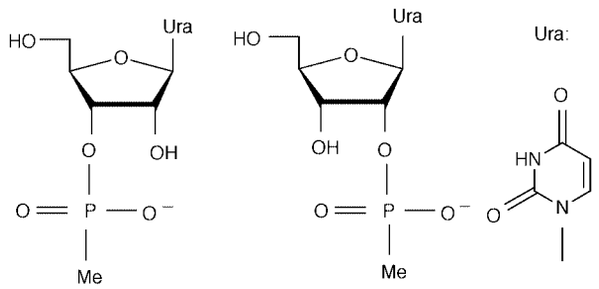
Results

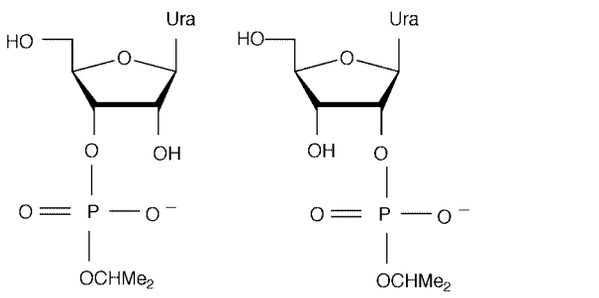
The isomerization of uridine 3′-methylphosphonate (**1a**), uridine 3′-isopropyl phosphate (**2a**), and uridine 3′-(2-ethoxyethyl) phosphate (**3a**) to their 2′-counterparts (**1b**, **2b**, and **3b**, respectively) was followed by determining the composition of aliquots withdrawn at appropriate intervals by reversed-phase (RP) HPLC. The reactions were carried out in imidazole buffers containing either 20% or 80% free imidazole. In both cases the total buffer concentration was varied from 0.050 to 0.50 M, and the ionic strength was adjusted to 1.0 M with sodium chloride. With **1a**, a similar set of measurements was also carried out in triethanolamine buffers, and the isomerization was additionally studied in acetate, formate and chloroacetate buffers, the proportion of the free acid being varied from 5 to 95% and the total buffer concentration from 0.045 to 0.80 M. The interconversion of the 2′- and 3′-isomers was the only reaction detected with the methylphosphonates (**1a**,**b**) and isopropyl phosphates (**2a**,**b**). By contrast, the uridine 3′-(2-ethoxyethyl) phosphate underwent cyclization to uridine 2′,3′-cyclic phosphate (**4**) concurrent with the isomerization. Tables 1–6 record the kinetic data obtained.

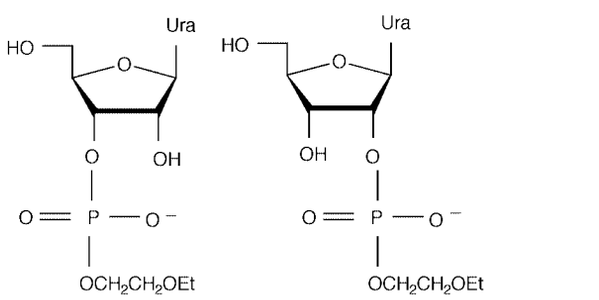
As seen from Tables 1, 5 and 6, the equilibrium of the interconversion of the 2′- and 3′-methylphosphonates (**1a**,**b**) in all the buffers studied lies slightly on the side of the 3′-isomer, the 2′→3′ migration being invariably 40% faster than the opposite reaction. The isomerization at buffer concentration zero is pH-independent over the pH range 4.7–7.6 (at 363.2 K), and shows first-order dependence on hydronium ion concentration at pH < 3. The reaction is rather insensitive to catalysis by imidazole and triethanolamine buffers. Only in the most acidic of these buffer systems, *viz*. the one containing 80% imidazolium ion, was the isomerization rate noticeably buffer-dependent: a 25% acceleration took place on increasing the buffer concentration from zero to 0.50 M ($k_1^{cat}/k_1^{\circ} = 0.49$ M^{-1}, see Table 1).

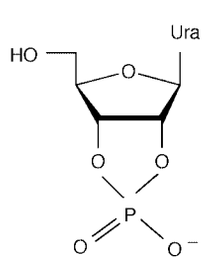
Uridine 3′-isopropyl phosphate (**2a**) behaves as the corresponding methylphosphonate (**1b**), except that the isomerization is almost one order of magnitude slower (Table 2). Again the buffer catalysis is seen only in imidazole buffers containing 80% imidazolium ion: a 44% rate acceleration was observed on increasing the buffer concentration from zero to 0.5 M ($k_1^{cat}/k_1^{\circ} = 0.88$ M^{-1}, see Table 2). Also with this compound, the buffer-independent isomerization is pH independent, and the 3′-isomer slightly predominates in the equilibrium mixture.

While the isomerization is the only reaction observed with **1a** and **2a** in imidazole buffers, uridine 3′-(2-ethoxyethyl) phosphate (**3a**) additionally undergoes cleavage to the 2′,3′-cyclic phosphate (**4**). The competition between these reactions









depends on the buffer concentration. The pH-independent isomerization is one order of magnitude faster than the cleavage, whereas at high buffer concentrations the reactions are approximately equally rapid (Table 3). Consistent with the results of Beckman *et al.*[2] and Breslow *et al.*[3] on 3′,5′-UpU, the cleavage is catalyzed by both neutral imidazole and imidazolium ion, the second-order rate constants for the general base and acid catalysis being 2.0×10^{-6} M^{-1} s^{-1} and 1.7×10^{-6} M^{-1} s^{-1}, respectively. For comparison, the second-order rate constant for the imidazolium ion catalyzed isomerization is 0.99×10^{-6} M^{-1} s^{-1}. The existence of this competition does not, however, lower the susceptibility of isomerization to buffer catalysis. In buffers containing 80% imidazolium ion a 51% rate acceleration was observed on increasing the buffer concentration from zero to 0.5 M ($k_1^{cat}/k_1^{\circ} = 1.03$ M^{-1}, see Table 3). Balancing the changes in the concentration of neutral imidazole with 1,4-dioxane only slightly decreased the rate of isomerization (Table 4). Accordingly, no clear evidence for the so-called "negative catalysis" could be obtained.

The isomerization of 2′/3′-methylphosphonates was also studied in more acidic carboxylic acid buffers. The results obtained are listed in Table 5. As with imidazole buffers, the rate of isomerization is strictly first-order in the buffer concentration, and the catalytically active buffer constituent is the buffer acid. For example, in formic acid buffers containing 95% free acid, a 12-fold acceleration took place on increasing the buffer concentration from zero to 0.8 M, whereas with buffers containing 95% formate ion no buffer catalysis could be observed. Accordingly, the reaction appears to be susceptible only to general acid catalysis, and to be of first-order in the concentration of the buffer acid. The observed second-order constant, k_{HA}^{obs}, of the general acid catalysis, however, at low pH becomes linearly related to the concentration of hydronium ion (Fig. 1). In other words, the rate of isomerization appears to depend on two distinct kinetic terms: k_a[HA] and k_b[H^+][HA] (HA stands for the buffer acid), as indicated by eqn. (1).

$$k_{HA}^{obs} = (k_a + k_b[H^+]) \quad (1)$$

The effect of electrolyte concentration on the rate of isomerization in carboxylic acid buffers is modest. Varying the salt concentration from 0.04 to 1.04 M by adding NaCl, $NaClO_4$ or Na_2SO_4 to an acetic acid buffer ([AcOH]/[AcONa] = 0.01 M/0.04 M) had virtually no effect on the isomerization rate (Table 6). This observation agrees with the results of earlier studies on isomerization of 3′,5′-UpU in imidazole buffers.[2]

Discussion

The results described above show that ribonucleoside 2′/3′-methylphosphonates undergo two different types of buffer-catalyzed isomerization reaction. At pH > 4, the reaction is pH-independent and catalyzed only by general acids. The most plausible mechanistic interpretation, sequential specific acid/general base catalysis, is indicated in Scheme 3 (Route A). In other words, the phosphoryl moiety is protonated in a rapid pre-equilibrium step and the buffer base then deprotonates the attacking 2′/3′-hydroxy function concerted with formation of the pentacoordinated intermediate. This buffer catalysis is weak, which may result from the fact that formation of the phosphorane intermediate and subsequent pseudorotation of the monoanionic pentacoordinated intermediate are both partially rate-limiting. Ribonucleoside 2′/3′-alkylphosphate diesters behave similarly under neutral conditions. Concurrent buffer-catalyzed cleavage of the starting material to the 2′,3′-cyclic phosphate does not lower the susceptibility of the isomerization to buffer catalysis: **2a** and **3a** exhibit a similar susceptibility of isomerization to buffer catalysis, although the latter undergoes competitive buffer-catalyzed cleavage and the former does not. Accordingly, the low susceptibility of the isomerization of 3′-alkylphosphates to buffer catalysis appears to be an inherent property of this reaction and does not result from competitive breakdown of the pentacoordinated intermediate to cleavage products by general base catalysis (sequential specific base/general acid catalysis).

At low pH (pH < 3), the rate of isomerization of 2′/3′-methylphosphonates is proportional to [H^+][HA], where HA stands for the buffer acid. The reaction hence is sequential specific acid/general acid catalysis, and may be interpreted as rapid pre-equilibrium protonation of the phosphonate ester monoanion, followed by the attack of 2′/3′-hydroxy function concerted with proton transfer from the buffer acid to the neutral phosphonate moiety (Route B in Scheme 3). The kinetically equivalent general base catalyzed attack of the 2′-hydroxy function on monocationic phosphorane cannot be excluded, but we regard it as a less attractive alternative. The buffer

Table 1 Isomerization of uridine 3′-methylphosphonate (**1a**) to 2′-methylphosphonate (**1b**) in imidazole and triethanolamine buffers at 363.2 K: the first-order rate constants (k_1), mole fractions of **1a** in the equilibrium mixture (x_{eq}), second-order rate constants for the buffer catalysis (k_1^{cat}), and first-order rate constants for the buffer-independent reaction (k_1^o)[a]

pH	[BH$^+$]/M	[B]/M	$k_1/10^{-6}$ s^{-1}	x_{eq}	$k_1^{cat}/10^{-6}$ M^{-1} s^{-1}	$k_1^o/10^{-6}$ s^{-1}
B = Imidazole						
5.57	0.040	0.010	2.98 ± 0.03	0.589	1.43 ± 0.02[b]	2.9 ± 0.005
	0.100	0.025	3.10 ± 0.03	0.588		
	0.200	0.050	3.27 ± 0.03	0.588		
	0.400	0.100	3.63 ± 0.04	0.584		
6.77	0.010	0.040	2.81 ± 0.03	0.590	0.49 ± 0.15	2.8 ± 0.04
	0.025	0.100	2.89 ± 0.03	0.593		
	0.050	0.200	3.00 ± 0.03	0.595		
	0.100	0.400	3.04 ± 0.02	0.587		
B = Triethanolamine						
6.42	0.040	0.010	2.85 ± 0.02	0.590	0.43 ± 0.06	2.8 ± 0.02
	0.100	0.025	2.87 ± 0.03	0.590		
	0.200	0.050	2.90 ± 0.03	0.591		
	0.400	0.100	3.04 ± 0.03	0.592		
7.62	0.010	0.040	2.77 ± 0.03	0.590	—	2.8 ± 0.04
	0.025	0.100	2.80 ± 0.03	0.591		
	0.050	0.200	2.80 ± 0.03	0.591		
	0.100	0.400	2.67 ± 0.03	0.593		

[a] The ionic strength was adjusted to 1.0 M with sodium chloride. [b] The second-order rate constant for the imidazolium ion catalysis is 1.8×10^{-6} M^{-1} s^{-1}.

Table 2 Isomerization of uridine 3′-isopropyl phosphate (**2a**) to 2′-isopropyl phosphate (**2b**) in imidazole buffers at 363.2 K: the first-order rate constants (k_1), mole fractions of **2a** in the equilibrium mixture (x_{eq}), second-order rate constants for the buffer catalysis (k_1^{cat}), and first-order rate constants for the buffer-independent reaction (k_1^o)[a]

pH	[ImH$^+$]/M	[Im]/M	$k_1/10^{-6}$ s^{-1}	x_{eq}	$k_1^{cat}/10^{-6}$ M^{-1} s^{-1}	$k_1^o/10^{-6}$ s^{-1}
5.57	0.040	0.010	0.431 ± 0.007	0.55	0.36 ± 0.11[b]	0.41 ± 0.03
	0.100	0.025	0.482 ± 0.023	0.55		
	0.200	0.050	0.455 ± 0.023	0.55		
	0.400	0.100	0.608 ± 0.009	0.55		
6.77	0.010	0.040	0.399 ± 0.002	0.55	0.12 ± 0.03	0.38 ± 0.007
	0.025	0.100	0.392 ± 0.020	0.55		
	0.050	0.200	0.410 ± 0.007	0.55		
	0.100	0.400	0.447 ± 0.008	0.55		

[a] The ionic strength was adjusted to 1.0 M with sodium chloride. [b] The second-order rate constant for the imidazolium ion catalysis is 0.45×10^{-6} M^{-1} s^{-1}.

Table 3 Isomerization of uridine 3′-(2-ethoxyethyl) phosphate (**3a**) to 2′-(2-ethoxyethyl) phosphate (**3b**) and cleavage to uridine 2′,3′-cyclic phosphate (**4**) in imidazole buffers at 363.2 K: the first-order rate constants for the isomerization (k_1) and cleavage (k_2), mole fractions of **3a** in the equilibrium mixture (x_{eq}), second-order rate constants for the buffer-catalyzed isomerization (k_1^{cat}) and cleavage (k_2^{cat})[a]

pH	[ImH$^+$]/M	[Im]/M	$k_1/10^{-6}$ s^{-1}	$k_2/10^{-6}$ s^{-1}	x_{eq}	$k_1^{cat}/10^{-6}$ M^{-1} s^{-1}	$k_2^{cat}/10^{-6}$ M^{-1} s^{-1}
5.57	0.040	0.010	0.807 ± 0.007	0.114 ± 0.003	0.572	0.79 ± 0.05[b]	1.73 ± 0.05[c]
	0.100	0.025	0.866 ± 0.016	0.268 ± 0.010	0.556		
	0.200	0.050	0.993 ± 0.006	0.448 ± 0.007	0.562		
	0.400	0.100	1.16 ± 0.02	0.902 ± 0.031	0.553		
6.77	0.010	0.040	0.815 ± 0.009	0.134 ± 0.005	0.570	—[d]	1.90 ± 0.21[e]
	0.025	0.100	0.792 ± 0.010	0.356 ± 0.014	0.559		
	0.050	0.200	0.817 ± 0.008	0.646 ± 0.013	0.561		
	0.100	0.400	0.802 ± 0.038	1.01 ± 0.03	0.537		

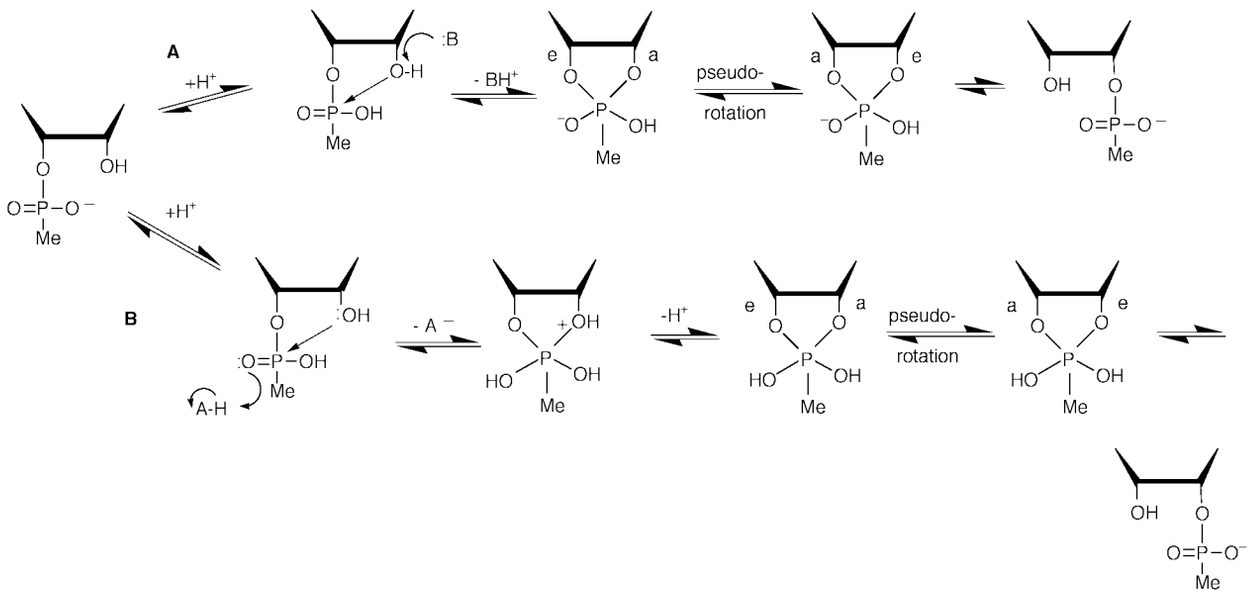
[a] The ionic strength was adjusted to 1.0 M with sodium chloride. [b] The buffer-independent rate constant for the isomerization $k_1^o = (0.77 \pm 0.1) \times 10^{-6}$ s^{-1}. The second-order rate constant for the imidazolium ion catalysis is 0.99×10^{-6} M^{-1} s^{-1}. [c] The buffer-independent rate constant for the cleavage $k_2^o = (0.03 \pm 0.01) \times 10^{-6}$ s^{-1}. The second-order rate constants for the imidazole and imidazolium ion catalysis are 2.0×10^{-6} M^{-1} s^{-1} and 1.7×10^{-6} M^{-1} s^{-1}, respectively. [d] The buffer-independent rate constant for the isomerization $k_1^o = (0.81 \times 0.01) \times 10^{-6}$ s^{-1}. [e] The buffer-independent rate constant for the cleavage $k_2^o = (0.10 \pm 0.06) \times 10^{-6}$ s^{-1}.

catalysis is more efficient than under neutral conditions, indicating that the formation of the pentacoordinated intermediate rather than its pseudorotation is rate-limiting.

Experimental

Uridine 2′- (1b) and 3′-methylphosphonates (1a)

Compounds **1a** and **1b** were prepared by phosphonylating 2′,5′- and 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)uridine[7] with methylphosphorylbis(1,2,4-triazole).[8] Methylphosphorylbis-(1,2,4-triazole) was prepared by dissolving 2 mmol (0.15 g) of recrystallized 1,2,4-triazole in dry acetonitrile (15 ml) and adding 2 mmol (300 μl) of distilled triethylamine and 2.5 mmol (0.35 g) of methylphosphonic dichloride to the resulting solution. The solution was mixed for 1 h at room temperature, and filtered onto 1 mmol (0.50 g) of dry 2′,5′- or 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)uridine. 1 mmol (150 μl) of triethylamine



Scheme 3

Table 4 Isomerization of uridine 3′-(2-ethoxyethyl) phosphate (**3a**) to 2′-(2-ethoxyethyl) phosphate (**3b**) in imidazole buffer at 363.2 K, when 1,4-dioxane was used to balance the changes in the concentration of neutral imidazole: the first-order rate constants (k_1), mole fractions of **3a** in the equilibrium mixture (x_{eq}), and first-order rate constants for the buffer-independent reaction (k_1^o)[a]

pH	[ImH$^+$]/M	[Im]/M	$k_1/10^{-6}$ s^{-1}	x_{eq}	$k_1^o/10^{-6}$ s^{-1}
6.77	0.010	0.040	0.683 ± 0.022	0.506	0.68 ± 0.5
	0.025	0.100	0.772 ± 0.008	0.569	
	0.050	0.200	0.781 ± 0.22	0.551	

[a] The ionic strength was adjusted to 1.0 M with sodium chloride.

was added, and the mixture was agitated for 44 h at room temperature. The reaction was stopped by adding water (15 ml). The crude product was isolated by conventional sodium bicarbonate workup, and purified by silica gel chromatography (MeOH–CH_2Cl_2 3 : 7, v/v). The yield of **1a** was 43% and that of **1b** was 33%.

1a: ^{1}H NMR (D_2O; ppm from external TMS): 7.73 (d, *J*(H5,H6) 8.1 Hz, H6), 5.79 (d, *J*(H1′,H2′) 5.1 Hz, H1′), 5.74 (d, H5), 4.43 (ddd, *J*(H3′,P) 9.0 Hz, *J*(H2′,H3′) 5.1 Hz, *J*(H3′,H4′) 4.9 Hz, H3′), 4.27 (dd, H2′), 4.10 (ddd, *J*(H4′,H5′) 2.7 Hz, *J*(H4′,H5″) 4.1 Hz, H4′), 3.74 (dd, *J*(H5′,H5″) 12.7 Hz, H5′), 3.66 (dd, H5″), 1.18 (d, *J*(H,P) 16.6 Hz, PCH_3); ^{31}P NMR (D_2O; ppm from H_3PO_4): 27.6. ^{13}C NMR (D_2O; ppm from external TMS): 167.9, 154.0, 144.4, 105.1, 91.2, 86.9 (d, *J* 4.1 Hz), 75.7 (d, *J* 3.3 Hz), 74.7 (d, *J* 5.4 Hz), 63.3, 15.4 (d, *J* 137.3 Hz); FAB-MS: M + 1 = 345 (42%).

1b: ^{1}H NMR (D_2O; ppm from external TMS): 7.69 (d, *J*(H5,H6) 8.1 Hz, H6), 5.84 (d, *J*(H1′,H2′) 5.1 Hz, H1′), 5.74 (d, H5), 4.16 (dd, *J*(H2′,H3′) 5.1 Hz, *J*(H3′,H4′) 5.1 Hz, H3′), 3.98 (ddd, *J*(H4′,H5′) 2.8 Hz, *J*(H4′,H5″) 4.4 Hz, H4′), 3.73 (dd, *J*(H5′,H5″) 12.8 Hz, H5′), 3.64 (dd, H5″), 1.13 (d, *J*(H,P) 16.6 Hz, PCH_3), the H2′ signal overlapped by HDO the resonance; ^{31}P NMR (D_2O; ppm from H_3PO_4): 27.4; ^{13}C NMR (D_2O from external TMS): 168.9, 154.4, 145.2, 105.5, 91.6 (d, *J* 5.0 Hz), 87.3, 77.9 (d, *J* 5.2 Hz), 72.3 (d, *J* 3.1 Hz), 63.8, 14.7 (d, *J* 137.1 Hz). FAB-MS; M + 1 = 345 (27%).

The preparation of uridine 3′-isopropyl phosphate (**2a**) and uridine 3′-(2-ethoxyethyl) phosphate (**3a**) has been described previously.[6]

Kinetic measurements

The reactions were carried out in stoppered bottles immersed in a water bath, the temperature of which was adjusted to 363.2 K. The progress of reactions was followed by analyzing the composition of the samples withdrawn at appropriate intervals by HPLC. The samples were rapidly cooled to stop the reaction, and injected onto a Hypersil ODS (250 × 4 mm, 5 μm) column. Isocratic elution with 0.025 M triethylammonium acetate containing 0.2 M tetramethylammonium chloride was used for compound **1a**. With **2a**, the eluent was an acetic acid–sodium acetate buffer (0.060 M, pH 4.3) containing 0.1 M NH_4Cl and 5.0% MeCN. With **3a**, the eluent was similar, but the MeCN content was 3.5%. The initial substrate concentration was approximately 0.1 mM.

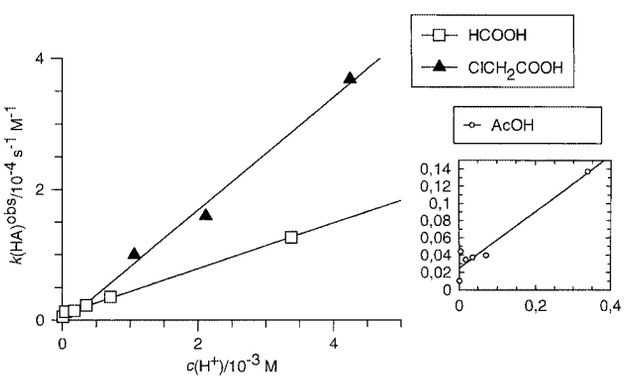


Fig. 1 The second-order rate constants, k_{HA}^{obs}, observed for the general acid catalyzed isomerization of uridine 3′-methylphosphonate (**1a**) to 2′-methylphosphonate (**1b**) in carboxylic acid buffers at 363.2 K plotted against the hydronium ion concentration. The ionic strength was adjusted to 1.0 M with sodium chloride.

The first-order rate constants, k_{obs}, for the isomerization reactions were calculated by eqn. (2), where x_t stands for the

$$k_{obs}\, t = \ln [(x_0 - x_{eq})/(x_t - x_{eq})] \qquad (2)$$

mole fraction of the 3′-isomer in the isomeric mixture at moment *t*, and x_0 and x_{eq} are the corresponding quantities at the beginning and at the equilibrium of the reaction, respectively. The areas of the HPLC signals were assumed to be proportional to concentrations, because both isomers have the same base as a chromophore and hence their molar absorptivities are virtually equal. The rate constants, k_1, for the 3′→2′ isomerization were obtained by eqn. (3).

$$k_1 = k_{obs}(1 - x_{eq}) \qquad (3)$$

Table 5 Isomerization of uridine 3′-methylphosphonate (**1a**) to 2′-methylphosphonate (**1b**) in formate, acetate and chloroacetate buffers at 363.2 K: observed first-order rate constants (k_1), mole fractions of **1a** in the equilibrium mixture (x_{eq}), second-order rate constants for the buffer catalysis (k_1^{cat}), apparent second-order rate constant for the general acid catalysis (k_{HA}^{obs}), and first-order rate constants for the buffer-independent reaction (k_1^{o})[a]

pH	[AH]/M	[A⁻]/M	$k_1/10^{-6}$ s^{-1}	x_{eq}	$k_1^{cat}/10^{-6}$ M^{-1} s^{-1}	$k_{HA}^{obs}/10^{-6}$ M^{-1} s^{-1}	$k_1^{o}/10^{-6}$ s^{-1}
A = Acetate							
6.03	0.010	0.190	2.65 ± 0.04	0.584	0.050 ± 0.001	1.0	2.6 ± 0.1
	0.02	0.380	2.66 ± 0.01	0.582			
	0.030	0.570	2.67 ± 0.04	0.583			
	0.040	0.760	2.68 ± 0.01	0.582			
5.35	0.010	0.040	2.79 ± 0.03	0.600	0.88 ± 0.28	4.4	2.7 ± 0.1
	0.025	0.100	2.72 ± 0.03	0.621			
	0.050	0.200	2.81 ± 0.03	0.607			
	0.075	0.300	3.12 ± 0.02	0.594			
	0.100	0.400	3.08 ± 0.04	0.608			
4.75	0.040	0.040	2.81 ± 0.01	0.583	1.72 ± 0.03	3.4	2.7 ± 0.1
	0.100	0.100	2.99 ± 0.01	0.580			
	0.200	0.200	3.33 ± 0.01	0.580			
	0.400	0.400	4.04 ± 0.02	0.584			
4.45	0.030	0.015	3.11 ± 0.02	0.588	2.47 ± 0.05	3.7	3.0 ± 0.1
	0.090	0.045	3.34 ± 0.02	0.585			
	0.180	0.090	3.69 ± 0.02	0.581			
	0.300	0.150	4.11 ± 0.01	0.579			
4.15	0.040	0.010	3.08 ± 0.03	0.598	3.15 ± 0.66	3.9	3.2 ± 0.2
	0.100	0.025	3.89 ± 0.04	0.593			
	0.200	0.050	3.91 ± 0.04	0.590			
	0.300	0.075	4.27 ± 0.04	0.593			
	0.400	0.100	4.75 ± 0.03	0.591			
3.47	0.190	0.010	6.31 ± 0.05	0.585	13.0 ± 0.9	13.7	3.4 ± 0.5
	0.380	0.020	8.31 ± 0.02	0.581			
	0.570	0.030	10.95 ± 0.03	0.579			
	0.760	0.040	14.1 ± 0.03	0.582			
A = Formate							
5.03	0.010	0.190	2.70 ± 0.02	0.581	0.26 ± 0.06	5.2	2.7 ± 0.1
	0.020	0.380	2.80 ± 0.04	0.581			
	0.030	0.570	2.81 ± 0.02	0.578			
	0.040	0.760	2.87 ± 0.04	0.582			
4.35	0.010	0.040	2.91 ± 0.03	0.579	2.57 ± 0.64	12.9	2.8 ± 0.2
	0.025	0.100	3.04 ± 0.01	0.577			
	0.050	0.200	3.74 ± 0.11	0.584			
	0.100	0.400	4.02 ± 0.09	0.572			
3.75	0.040	0.040	3.95 ± 0.06	0.586	7.13 ± 0.40	14.3	3.3 ± 0.2
	0.100	0.100	4.62 ± 0.04	0.578			
	0.200	0.200	6.46 ± 0.08	0.589			
	0.300	0.300	7.37 ± 0.05	0.581			
	0.400	0.400	9.13 ± 0.10	0.590			
3.45	0.030	0.015	3.95 ± 0.02	0.577	15.0 ± 0.5	22.5	3.4 ± 0.1
	0.090	0.045	5.58 ± 0.03	0.582			
	0.180	0.090	7.38 ± 0.07	0.586			
	0.300	0.150	10.12 ± 0.04	0.585			
3.15	0.040	0.010	6.53 ± 0.04	0.584	28.6 ± 0.4	35.8	5.0 ± 0.1
	0.100	0.025	8.42 ± 0.25	0.583			
	0.200	0.050	12.1 ± 0.2	0.585			
	0.400	0.100	19.3 ± 0.3	0.575			
2.47	0.190	0.010	32.3 ± 0.2	0.583	120 ± 3	126	8.6 ± 2.2
	0.380	0.020	58.1 ± 0.3	0.584			
	0.570	0.030	78.8 ± 0.8	0.580			
	0.760	0.040	105.5 ± 1.1	0.579			
A = Chloroacetate							
2.97	0.040	0.040	12.0 ± 0.3	0.592	49.6 ± 2.4	99	7.5 ± 1.1
	0.100	0.100	16.0 ± 0.3	0.592			
	0.200	0.200	28.4 ± 0.7	0.598			
	0.400	0.400	46.9 ± 1.8	0.592			
2.67	0.030	0.015	17.2 ± 0.3	0.590	106 ± 8	159	14 ± 2
	0.090	0.045	29.4 ± 0.6	0.593			
	0.180	0.090	45.2 ± 1.1	0.593			
	0.300	0.150	60.5 ± 1.4	0.594			
2.37	0.040	0.010	31.5 ± 0.4	0.589	294 ± 18	368	15 ± 5
	0.100	0.025	55.1 ± 1.1	0.593			
	0.200	0.050	80.7 ± 1.4	0.592			
	0.400	0.100	165 ± 3	0.586			

[a] The ionic strength was adjusted to 1.0 M with sodium chloride.

Table 6 The effect of electrolytes on the rate of isomerization of uridine 3′-methylphosphonate (**1a**) to 2′-methylphosphonate (**1b**): the first-order rate constants (k_1) and mole fractions of **1b** in equilibrium mixture (x_{eq}). The data refers to buffer [AcOH]/[AcONa] = 0.01 M/0.04 M at 363.2 K

Salt	*I*/M	$k_1/10^{-6}$ s^{-1}	x_{eq}
—	0.04	3.19 ± 0.11	0.579
NaCl	0.34	2.94 ± 0.10	0.588
NaCl	0.64	2.45 ± 0.11	0.571
NaCl	1.04	2.71 ± 0.02	0.578
$NaClO_4$	0.04	2.90 ± 0.03	0.584
$NaClO_4$	0.34	2.83 ± 0.03	0.584
$NaClO_4$	0.64	2.73 ± 0.03	0.587
$NaClO_4$	1.04	2.81 ± 0.04	0.591
Na_2SO_4	1.04	2.87 ± 0.02	0.587

The first-order rate constants, k_2, for the cleavage were calculated by applying the integrated first-order rate equation to the disappearance of the total concentration of the isomeric mixture. The breakdown of the second-order rate constants, k_2^{cat}, for the buffer catalysis to the contributions of general acid (BH^+) and base catalysis (B) was carried out by eqn. (4).

$$k_2^{cat} = k_B\{[B]/([B] + [BH^+])\} + k_{BH}\{[BH^+]/([B]+[BH^+])\} \quad (4)$$

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References

1 For recent reviews, see (*a*) D. M. Perreault and E. V. Anslyn, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 432; (*b*) M. Oivanen, S. Kuusela and H. Lönnberg, *Chem. Rev.*, 1998, **98**, 961.
2 C. Beckmann, A. J. Kirby, S. Kuusela and D. C. Tickle, *J. Chem. Soc., Perkin Trans. 2*, 1998, 573.
3 R. Breslow, S. D. Dong, Y. Webb and R. Xu, *J. Am. Chem. Soc.*, 1996, **118**, 6588.
4 R. Breslow and R. Xu, *J. Am. Chem. Soc.*, 1993, **115**, 10705.
5 M. Oivanen, S. N. Mikhailov, N. S. Padyukova and H. Lönnberg, *J. Org. Chem.*, 1993, **58**, 1617.
6 M. Kosonen, E. Yousefi-Salakdeh, R. Strömberg and H. Lönnberg, *J. Chem. Soc., Perkin Trans. 2*, 1998, 1589.
7 (*a*) G. H. Hakimelahi, Z. A. Proba and K. K.Ogilvie, *Can. J. Chem.*, 1982, **60**, 1106; (*b*) K. K. Ogilvie, S. L. Beaucage, A. L. Schifman, N. Y. Theriault and K. L. Sadana, *Can. J. Chem.*, 1978, **56**, 2768; (*c*) K. K. Ogilvie, A. L. Schifman and C. L. Penney, *Can. J. Chem.*, 1979, **57**, 2230.
8 A. Kraszewski and J. Stawinski, *Tetrahedron Lett.*, 1980, **21**, 2935.

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