Kinetics of Mutual Isomerization of the Phosphonate Analogs of Dinucleoside 2′,5′- and 3′,5′-Monophosphates in Aqueous Solution

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The phosphonate analogs of naturally occurring phosphoric acid esters have recently found an increasing number of applications in biological research. For example, replacing the POC fragment of phosphodiesters with a PCH_2C fragment affords a class of compounds that effectively inhibits the enzymes catalyzing the reactions of phosphoric acid derivatives.[1,2] Very recently, the effect of a 3′-methylene phosphonate moiety on the conformation of an A-DNA octamer double helix has been studied.[3] Accordingly, the knowledge of the chemical behavior of these phosphonic acid esters compared to their phosphoric acid counterparts is of considerable interest. For this purpose we now report on intermolecular transesterification of the phosphonate analogs (3′,5′-GpcU, **4**, and 2′,5′-GpcU, **5**) of guanylyl(3′–5′)uridine and guanylyl(2′–5′)-uridine, containing a PCH_2C linkage in place of the $PO^{5'}C$ linkage.

First syntheses of isosteric phosphonate analogs of nucleoside 5′-monophosphates[4] and dinucleoside monophosphates[5] were reported by the group of Moffatt. Recently, a general route to phosphonate analogs of 5′-nucleotides has been elaborated utilizing the Arbuzov reaction.[6,7] Compounds **4** and **5** were now obtained by condensing a 2′,3′-blocked 1-(5′,6′-dideoxy-6′-phosphonyl-β-D-*ribo*-hexofuranosyl)uracil (**1**) with an appropriately protected guanosine[8] (**2** or **3**) in the presence of *N,N′*-dicyclohexylcarbodiimide (DCC). Kinetics of the interconversion of **4** and **5** were determined and compared to those reported previously for dinucleoside monophosphates (**6**, **7**).[9] Special attention was paid to possible occurrence of a hydroxide ion catalyzed phosphonate migration. With dinucleoside monophosphates the only reaction taking place in aqueous alkali is hydrolysis of the phosphodiester bond. Replacement of the $O^{5'}$ atom with carbon prevents hydrolysis, and hence the interconversion of **4** and **5**, may be studied at high concentrations of hydroxide ion.

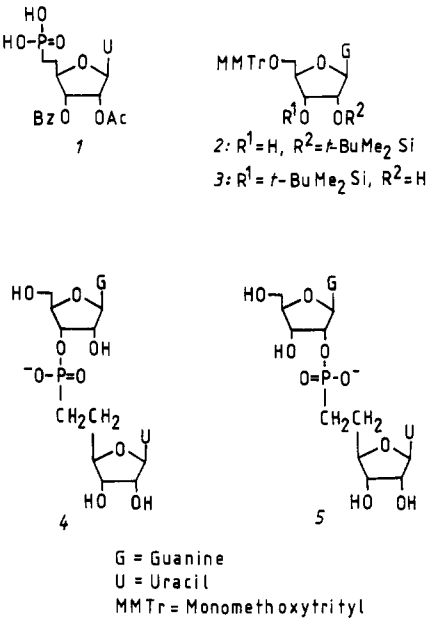

Figure 1 shows the pH–rate profile for the interconversion of **4** and **5** at 363.2 K. As with dinucleoside monophosphates (**6**, **7**),[9] the reaction is acid-catalyzed at pH < 3 and becomes pH-independent at lower hydronium ion concentrations. The acid-catalyzed isomerization yields an equilibrium mixture containing 62% 3′,5′-GpcU (**4**), whereas the 2′,5′-isomer (**5**) prevails ($x_5 = 0.58$) in the product mixture produced by the pH-independent reaction. The observed difference in the product distribution may either result from a slight difference in the acidity of the phosphonate group of **4** and **5** or from the fact that the guanine moiety becomes N7-protonated around pH 2. The only reaction that competes with the interconversion of **4** and **5** under acidic conditions is the cleavage of the guanine base. Both isomers (**4** and **5**) are depurinated approximately as rapidly, since the release of guanine strictly obeyed first-order kinetics ($v = k[4 + 5]$), in spite of the fact that the concentration ratio of the isomers markedly changed during a kinetic run. The rate constants obtained using 3′,5′- and 2′,5′-GpcU as starting material were 2.2×10^{-4} s^{-1} and 2.8×10^{-4} s^{-1}, respectively ([HCl] = 0.1 mol dm^{-3}, T = 363.2 K).

Eberhard and Westheimer[10] have shown that simple alkyl esters of alkylphosphonic acids also undergo an acid- and base-catalyzed hydrolysis. The reaction may proceed by two alternative pathways, involving either P–O or C–O cleavage. The hydrolysis of phosphonic acid esters is, however, too slow to be expected to compete with the interconversion of 3′,5′- and 2′,5′-GpcU (**4** and **5**) under acidic conditions. Under alkaline conditions (pH > 8) **4** and **5** undergo degradation concurrently with interconversion, most likely via fragmentation of the uracil residue, as described earlier for uridine.[11] A small amount of guanosine was also found to accumulate. This suggests that phosphonate ester hydrolysis may occur as a minor side reaction under alkaline conditions. At pH > 11 the phosphonate migration could not be studied, owing to rapid degradation of the starting material.

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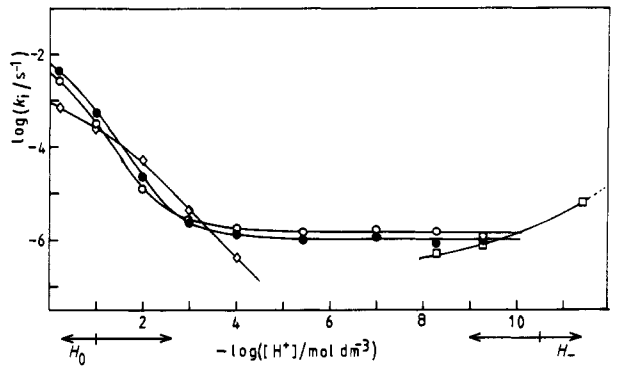
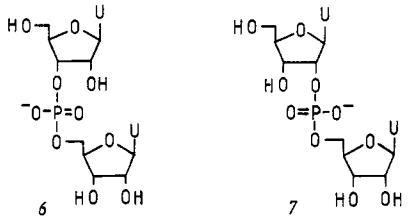


Figure 1. pH–rate profiles of reactions of 3′,5′- and 2′,5′-GpcU (4 and 5) at 363.2 K. The ionic strength adjusted at 0.1 mol/L with NaCl. The line drawn refers to least-squares fitting by eq 1; the kinetic parameters obtained are listed in Table I. Notation: (○) isomerization 3′,5′-GpcU → 2′,5′-GpcU (k_1); (●) isomerization 2′,5′-GpcU → 3′,5′-GpcU (k_{-1}); (◇) release of guanine; (□) base-catalyzed degradation.

It has been shown previously[9] that the mutual isomerization of 2′,5′- and 3′,5′-UpU (**6**, **7**) exhibits a first-order dependence of rate on acidity at pH < 1 and a second-order dependence at 1 < pH < 2, and the reaction becomes pH-independent under less acidic conditions. Although



the present kinetic data referring to the interconversion of the phosphonate dimers, **4** and **5**, are rather limited, a similar type of formal kinetics appear to be followed. Even the absolute values of the rate constants are comparable to those observed for dinucleoside monophosphates.[9] For example, the pH-independent migration is with **4**/**5** 2.1 times as fast as with **6**/**7**. Accordingly, it seems clear that the observed first-order rate constant, k_1, for the conversion of **4** to **5** may be expressed by eq 1, derived previously[9,12] for the mutual isomerization of nucleoside 2′- and 3′-alkyl phosphates.

$$k_1 = \frac{(k_a/K_a)[H^+]^2 + (k_b/K_a)[H^+] + k_c}{1 + ([H^+]/K_a)} \qquad (1)$$

In other words, the reaction is assumed to be (depending on pH) (i) a hydronium ion catalyzed transesterification of the neutral phosphonate ester (second-order term in [H$^+$]), (ii) an uncatalyzed transesterification of the neutral phosphonate ester (first-order term in [H$^+$]), and (iii) an uncatalyzed transesterification of the monoanionic phosphonate ester (zero-order term in [H$^+$]).

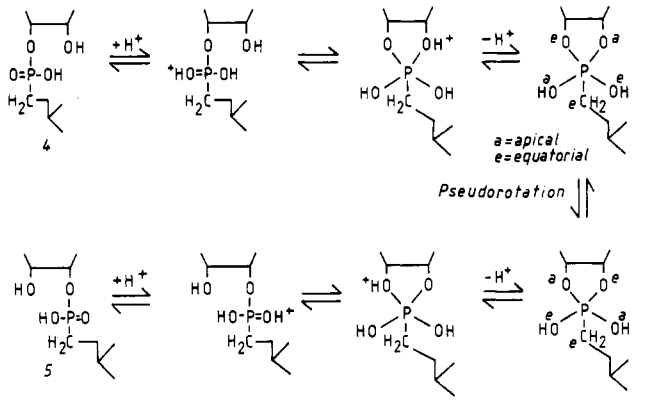
Table I records the values obtained for the partial rate and equilibrium constants (see Scheme I) by the method of least-squares fitting. For comparison, the data reported previously[9] for UpUs (**6** and **7**) are included in the same table. The following conclusions may be drawn: (i) The phosphonate dimers (**4**, **5**) are slightly less acidic than their

Table I. Partial Rate Constants for the Interconversion of 3′,5′-UpU and 2′,5′-UpU (6, 7) and 3′,5′- and 2′,5′-GpcU (4, 5) at 363.2 K[a]

	GpcU	UpU[b]
pK_a	1.3[c]	0.7[c]
$k_a/10^{-3}$ L mol^{-1} s^{-1}	4.3	13.3
$k_{-a}/10^{-3}$ L mol^{-1} s^{-1}	7.2	11.7
$k_b/10^{-3}$ s^{-1}	0.031	0.19
$k_{-b}/10^{-3}$ s^{-1}	0.052	0.16
$k_c/10^{-6}$ s^{-1}	1.7	0.66
$k_{-c}/10^{-6}$ s^{-1}	1.1	0.67

[a] The ionic strength adjusted to 0.10 mol/L with sodium chloride. [b] From ref 7. [c] For both isomers.

Scheme I



phosphate counterparts (**6**, **7**), consistent with the data reported in literature.[13,14] (ii) A neutral nucleoside phosphonate group migrates somewhat less readily than a neutral nucleoside phosphate group (compare the values of k_a, k_{-a}, k_b, and k_{-b}). The differences in reaction rates are not larger, however, than those found[9] among different dinucleoside monophosphates (ApA, ApU, UpA, UpU). (iii) A nucleoside phosphonate monoanion migrates twice as readily as a nucleoside phosphate monoanion (compare the values of k_c and k_{-c}). (iv) Neither the phosphonate (**4**, **5**) nor phosphate dimers (**6**, **7**) undergo a base-catalyzed migration. (v) The interconversion of both the phosphonate (**4**, **5**) and phosphate dimers (**6**, **7**) yields an approximately equimolar mixture of the 2′- and 3′-esterified nucleosides.

Mechanistic interpretation of the kinetic data on interconversion of the phosphonate dimers, **4** and **5**, may be based on Westheimer's[15] guidelines of pseudorotating pentacoordinated phosphorane intermediates. These intermediates exhibit a geometry of trigonal bipyramid with two apical and three equatorial ligands. Both attack of a nucleophile and departure of the leaving group will take place at an apical position only, but the position of ligands may be exchanged by a pseudorotation process. Electronegative ligands prefer an apical position and electropositive ones an equatorial position. If two of the ligands are members of a five-membered ring, one of them must be apical and the other equatorial.

Under acidic conditions the interconversion of **4** and **5** proceeds predominantly by the hydronium ion catalyzed transesterification of the neutral phosphonate ester (Scheme I). A rapid initial protonation of the neutral phosphonate ester (**4**) followed by an intramolecular nucleophilic attack of the 2′-hydroxyl group gives a

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pentacoordinated intermediate, with the entering $O^{2'}$ in an apical position and the carbon ligand in an equatorial position. Pseudorotation of the intermediate places $O^{3'}$ in an apical position, and hence after protolytic rearrangement, 3'-OH may leave giving **5**. This pseudorotation is allowed, since the carbon ligand may remain equatorial. Similarly, under neutral conditions a monoanionic pentacoordinated intermediate is obtained, which may pseudorotate without forcing the carbon ligand into an apical position.

As mentioned above, the interconversion of **4** and **5** is not catalyzed by hydroxide ion, in spite of the fact that deprotonation of the 2'(3')-hydroxyl group undoubtedly enhances its nucleophilicity. Nucleophilic attack of the 2'(3')-oxyanion on phosphorus would, however, give a pentacoordinated species having both of the unsubstituted oxygen ligands deprotonated. Accordingly, either the carbon ligand or one of the negatively charged oxygens ought to take an apical position. Obviously, this kind of a structure is too unstable to be formed. In summary, the interconversion of the phosphonate analogs (**4**, **5**) of dinucleoside monophosphates resembles in every essential respect the corresponding reaction of the latter compounds.

Experimental Section

Materials. 1-(5',6'-Dideoxy-6'-phosphonyl-β-D-*ribo*-hexofuranosyl)uracil(6'–3')guanosine (**4**). To a mixture of 320 mg (0.58 mmol) of predried pyridinium sal of 1-(2'-*O*-acetyl-3'-*O*-benzoyl-5',6'-dideoxy-6'-phosphono-β-D-*ribo*-hexofuranosyl)uracil (**1**)[6,7] and 557 mg (0.75 mmol) of N^2-isobutyryl-2'-*O*-(*tert*-butyldimethylsilyl)-5'-*O*-(monomethoxytrityl)guanosine (**2**)[8] in 5 mL of dry pyridine was added 650 mg (3.1 mmol) of *N*,*N*'-dicyclohexylcarbodiimide, and the mixture was stirred for 7 days at 20 °C. The mixture was treated with water (5 mL), stirred for 16 h at 20 °C, and extracted with chloroform (5 × 10 mL). The organic layer was concentrated in vacuo and applied to a column of silica gel (40 g). The column was washed with chloroform and eluted with a mixture of $CHCl_3$, EtOH, and Et_3N (90:5:5). The fractions containing the monomethoxytritylated product were evaporated to dryness. The residue was treated first with ammonia in methanol (5 mol/L, 10 mL, 24 h at 20 °C), then with $Bu_4NF{\cdot}3H_2O$ in THF (1 mol/L, 24 h at 20 °C), and finally with 80% acetic acid (10 mL, 10 h at 20 °C). After coevaporation with ethanol (6 × 10 mL) the deprotected product was dissolved in a mixture of water (20 mL) and chloroform (20 mL). The aqueous layer was separated, washed with chloroform (2 × 20 mL), and applied onto a column of DEAE-Toyopearl 650 M (50 mL, HCO_3^- form). The column was washed with water and eluted with a gradient of NH_4HCO_3 (from 0.01 to 0.10 mol/L). The pooled fractions were evaporated to dryness, coevaporated with water (5 × 10 mL) and freeze-dried. Yield: 45%. R_f: 0.36 (TLC, silica gel, 2-propanol–NH_4OH–H_2O (7:1:2)), 0.33 (PEI-cellulose, 0.25 mol/L NH_4HCO_3). ^1H NMR in D_2O, chemical shifts as ppm from DSS: 8.10 (s, H8); 7.65 (d, J(H5,H6) = 8.0 Hz, H6); 5.95 (d, J(H1',H2') = 5.2 Hz, H1'); 5.85 (d, J(H1',H2') = 4.6 Hz, H1'); 5.81 (d, J(H5,H6) = 8.2 Hz, H5); 4.9 (m, 2 protons, H2' and H3' of Guo); 4.4 (m, 2 protons, H2' and H3' of Urd); 4.08 (m, 2 protons, H4' of Guo and H4' of Urd); 3.90 (q, J(H5',H4') = 2.7 Hz, J(H5',H5'') = 12.8 Hz, H5' of Guo); 3.84 (q, J(H5'',H4') = 3.8 Hz, J(H5',H5'') = 12.8 Hz, H5'' of Guo); 2.1–1.7 (m, 4 protons, PCH_2CH_2). ^{31}P NMR in D_2O, chemical shift as ppm from phosphoric acid: 29.8.

1-(5',6'-Dideoxy-6'-phosphonyl-β-D-*ribo*-hexofuranosyl)uracil(6'–2')guanosine (**5**) was prepared analogously by condensation of 1-(2'-*O*-acetyl-3'-*O*-benzoyl-5',6'-dideoxy-6'-phosphono-β-D-*ribo*-hexofuranosyl)uracil (**1**) with N^2-isobutyryl-3'-*O*-(*tert*-butyldimethylsilyl)-5'-*O*-(monomethoxytrityl)guanosine (**3**)[8] in the presence of DCC. Yield: 21%. R_f 0.36 (TLC silica gel system described above). ^1H NMR in D_2O, chemical shifts as ppm from DSS: 8.0 (s, H8); 7.44 (d, J(H6,H5) = 8.2 Hz, H6); 6.00 (d, J(H1',H2') = 7.0 Hz, H1' of Guo); 5.85 (d, J(H6,H5) = 8.2 Hz, H5); 5.73 (d, J(H1',H2') = 4.6 Hz, H1' of Urd); 5.19–5.13 (m, J(H1',H2') = 7.0 Hz, J(H2',H3') = 4.8 Hz, J(H2',P) = 9.3 Hz, H2' of Guo); 4.46 (dd, J(H2',H3') = 5.1 Hz, J(H3',H4') = 2.4 Hz, H3' of Guo); 4.28 (dd, J(H2',H3') = 6.2 Hz, J(H3',H4') = 2.0 Hz, H3' of Urd); 4.16 (t, J(H2',H3') = 6.2 Hz, J(H1',H2') = 4.8 Hz, H2' of Urd); 3.9–3.7 (m, 4 protons, H4' of Urd, H4' of Guo, H5' and H5'' of Guo); 1.7–1.25 (m, 4 protons, PCH_2CH_2).

Kinetic Measurements. Reactions were followed by the HPLC technique described previously.[16] Chromatographic separations were carried out on a Hypersil ODS5 column (4.6 × 250 mm, particle size 5 μm) under isocratic conditions. The eluent employed was an acetic acid/sodium acetate buffer (0.045/0.015 mol/L, pH 4.2) containing 0.1 mol/L ammonium chloride and 3.5% acetonitrile. The retention times with flow rate 1 mL/min were 10.0, 7.0, 3.9, and 2.9 min for **4**, **5**, guanine, and a mixture of the depurinated **4** and **5**, respectively. The signals were detected by a Kontron 432 UV-detector. With the equilibrium mixture of **4** and **5** the signal areas were assumed to be proportional to concentrations, since the base moieties of both compounds are the same. Initial concentration of the starting material was of the order of 10^{-4} mol/L.

The hydronium ion concentrations of the reaction solutions were adjusted with hydrogen chloride and sodium hydroxide and formate, acetate, triethanolamine and glycine buffers. The pK_a values of the buffer acids under the experimental conditions were calculated on the basis of literature data.[17-20]

Catalysis by buffer constituents was not studied. All the rate constants were measured at buffer concentrations lower than 0.05 mol/L. Previous studies with dinucleoside monophosphates suggest that the buffer catalysis under such conditions is of minor importance.[9]

Calculation of the Rate Constants. First-order rate constants, k_1 and k_{-1}, for the interconversion of the isomers **4** and **5** were calculated by eqs 2 and 3 where x and x_e stand for the mole

$$(k_1 + k_{-1})t = \ln [(1 - x_e)/(x - x_e)] \quad (2)$$

$$k_1/k_{-1} = x_e/(1 - x_e) \quad (3)$$

fraction of 2',5'-GpcU (**5**) in the isomeric mixture at moment t and at equilibrium, respectively. $(k_1 + k_{-1})$ and x_e were obtained by a two-parameter least-squares fitting. Good correlation was obtained for each run suggesting less than 6% uncertainty in determination of the rate constants.

First-order rate constants for depurination of **4** and **5** were calculated by applying the integrated first-order rate equation both to disappearance of the isomeric mixture and, when possible, appearance of guanine. Good correlation was obtained in both ways. The rate constants calculated by different methods differed from each other by less than 10%.

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