Hydrolysis and intramolecular transesterification of ribonucleoside 3′-phosphotriesters: the effect of alkyl groups on the general and specific acid–base-catalyzed reactions of 5′-*O*-pivaloyluridin-3′-yl dialkyl phosphates

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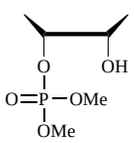
Diisopropyl, diethyl, bis(2-methoxyethyl) and isopropyl 2-methoxyethyl esters of 5′-pivaloyl-2′-(tetrahydropyran-2-yl)uridin-3′-yl phosphate have been prepared. The 2′-protecting group has been removed under acidic conditions, and the isomerization of the resulting ribonucleoside 3′-phosphotriester to its 2′-counterpart and the cleavage of the isomeric mixture to 2′- and 3′-phosphodiesters and a 2′,3′-cyclic phosphate has been followed by reverse phase HPLC in aqueous hydrogen chloride and several buffer solutions over a wide acidity range from H_0 − 1.5 to pH 8. The β_{lg} values of the buffer-independent partial reactions, and the β_{lg} and Brønsted α and β values of the buffer catalyzed reactions have been determined. The mechanisms of various partial reactions are discussed on the basis of the structural effects observed.

Introduction

The kinetics and mechanisms of the phosphodiester bonds of RNA have received increasing interest during the past decade.[1–3] As far as catalysis by Brønsted acids and bases is concerned, the motive of these studies ranges from improved protecting group strategies for oligoribonucleotide synthesis[4,5] to the development of artificial protolytic cleaving agents[6–16] and better insight into the action of ribonucleases.[1,2,17] One of the major problems encountered in mechanistic studies of ribonucleoside 3′-phosphodiesters is the reliable assignment of the reactive ionic form. In particular, several kinetically equivalent mechanisms differing only in the position of a proton in the transition state may be written for the reactions predominating under neutral conditions.[2,18–21] We have previously[22,23] attempted to solve some of the resulting ambiguities by using a ribonucleoside 3′-dimethyl phosphate **1** as a mimetic of the neutral ionic form of ribonucleoside 3′-phosphodiester **2**.



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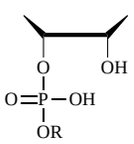

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These studies have, among other things, suggested that a monoanionic phosphorane intermediate is obtained by the attack of 2′-oxyanion on neutral phosphodiester rather than *via* the predominant ionic form, *i.e.* by the attack of 2′-hydroxy function on the phosphodiester monoanion. The recent quantum chemical calculations,[24] according to which the attack of an anionic nucleophile on protonated (neutral) phosphoester may be even faster than expected on the basis of the reactivity of its triester mimetic, lend additional support to the reasoning behind this conclusion: replacing the rapidly exchangeable proton of a neutral diester with an alkyl group leads to underestimation rather than overestimation of the reactivity of this particular ionic form. Furthermore, the previous[23] triester studies suggest that the sugar hydroxy groups depart from a monoanionic phosphorane intermediate as oxyanions much more readily than the exocyclic alkoxy group. The exocyclic bond fission thus requires general acid catalysis at pH 3 to 7, *i.e.* under conditions where the endocyclic cleavage is uncatalyzed. The present paper is an extension of our triester approach. The acidity of the esterified alcohol(s) has now been varied, and the β_{lg} values for various buffer-independent and buffer-dependent partial reactions have been determined to elucidate the transition state structures of reactions proceeding by an intramolecular nucleophilic attack on neutral or monocationic phosphoester.

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Results

Preparation of the model compounds

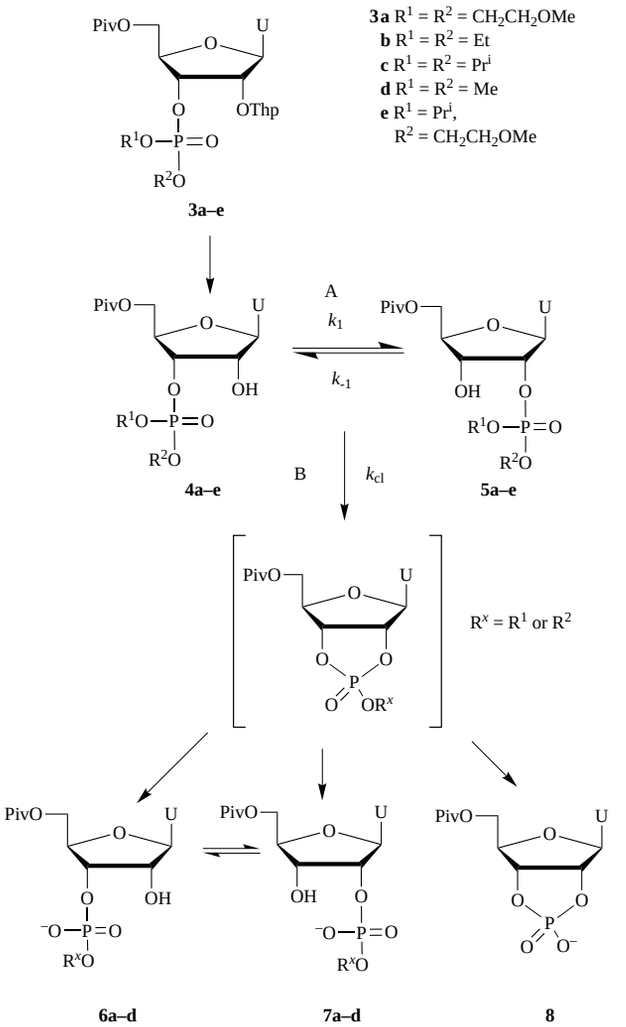
The symmetric 3′-phosphotriesters of 5′-*O*-pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridine **3a**–**c** were prepared as described previously for the corresponding dimethyl ester **3d**.[23] Accordingly, the more polar diastereomer of 5′-*O*-pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridine was phosphorylated with phosphoryl tris(1,2,4-triazolide),[25] and the remaining two triazole groups were displaced with 2-methoxyethanol, ethanol or propan-2-ol to obtain **3a**, **3b** and **3c**, respectively. The asymmetric triester, **3e**, was prepared by phosphorylating the same starting nucleoside with 2-chlorophenyl phosphoryl bis(1,2,4-triazolide), alcoholyzing the product in propan-2-ol, and displacing the 2-chlorophenoxy group with 2-methoxyethoxide ion in a mixture of 2-methoxyethanol and 1,4-dioxane. All the compounds **3a**–**c**,**e** were purified by adsorption chromatography, and when needed additionally by RP chromatography. The identity of **3a**–**c**,**e** was verified by elemental analysis, and by ^{1}H and ^{31}P NMR and FAB mass spectroscopy.

Kinetic measurements

All reactions were carried out at 298.2 K and their progress was followed by analyzing the content of the aliquots withdrawn at suitable intervals by RP HPLC. When the reactions were followed in buffer solutions at pH > 2, 2′-*O*-tetrahydropyran-2-yl group was first removed with 0.1 mol dm^{-3} aqueous hydrogen chloride to give **4a**–**c**,**e**, and the desired buffer system was then created by adding an appropriate amount of the buffer base. Under more acidic conditions, hydrolysis of the 2′-protecting group constituted the first step of the reaction sequence fol-

lowed, and equations of consecutive first-order reactions were applied to kinetic calculations.

It has been shown previously[22,23] that 5′-protected uridin-3′-yl dimethyl phosphates undergo in aqueous solution two parallel reactions: isomerization to the 2′-dimethyl phosphate (**5d**; Reaction **A** in Scheme 1) and cleavage to a mixture of 2′- and



Scheme 1

3′-methyl phosphates (**6d**, **7d**) and a 2′,3′-cyclic phosphate (**8**; Reaction **B** in Scheme 1). The latter reaction presumably proceeds *via* a 2′,3′-cyclic triester which, however, is too unstable to be detected. In all likelihood, the symmetric triesters **4a**–**c** investigated in the present study react similarly. With the asymmetric ester, **4e**, the situation is more complicated, since in principle either of the alkoxy groups, Pr^iO or $MeOCH_2CH_2O$, may be initially displaced. In fact, all possible diesters **6a**, **6c**, **7a**, **7c** and **8** were formed in aqueous hydrogen chloride and carboxylic acid buffers, the ratio of **[6a** + **7a]/[6c** + **7c]** being approximately 1 : 4. In more basic amine buffers, only the isopropyl diesters (**6c**, **7c**) were formed.

Fig. 1 shows the pH–rate profiles for the buffer-independent isomerization of **4a**–**c**,**e** to their 2′-counterparts (**5a**–**c**,**e**) and Fig. 2 those for the buffer-independent cleavage of the 2′/3′-isomeric mixture of the same compounds to a mixture of diesters. The concentration ratio of the 2′- and 3′-triesters approached unity with time, indicating that the migration of the dialkyl phosphate group between the 2′- and 3′-hydroxy functions is as fast in both directions ($k_1 \approx k_{-1}$). The buffer-independent rate constants, $k_{is}^{o} = k_1^{o} + k_{-1}^{o}$ for the isomerization and k_{cl}^{o} for the cleavage, were obtained by carrying out the kinetic measurements at four different buffer concentrations

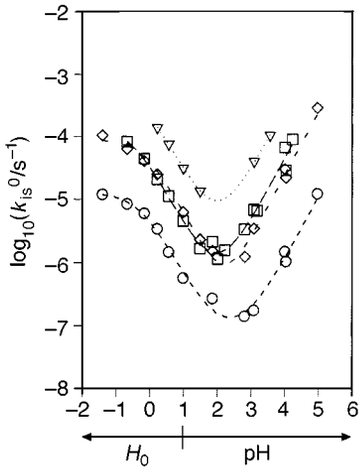


Fig. 1 pH–rate profile for the mutual buffer-independent isomerization of 5′-*O*-pivaloyluridin-3′-yl and -2′-yl dialkyl phosphates at 298.2 K. The ionic strength was adjusted to 1.0 mol dm^{-3} with NaCl, except at pH < 1.5 where aqueous HCl was used to adjust the hydronium ion concentration. Notation: **4a/5a** (△), **4b/5b** (□), **4c/5c** (○), **4e/5e** (◇).

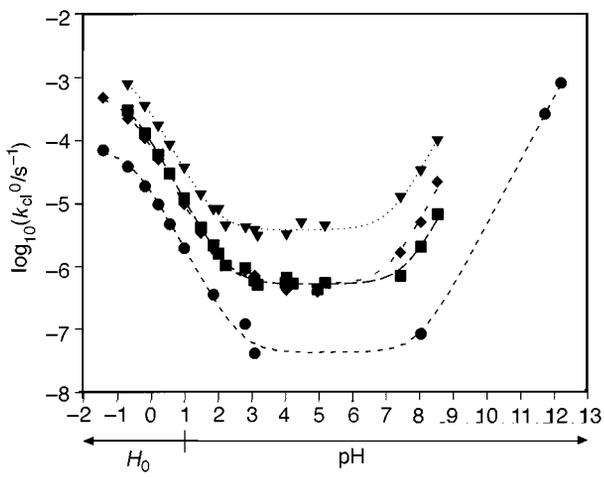


Fig. 2 pH–rate profiles for the buffer-independent cleavage of 5′-*O*-pivaloyluridin-2′-yl and -3′-yl dialkyl phosphates at 298.2 K. The ionic strength was adjusted to 1.0 mol dm^{-3} with NaCl, except at pH < 1.5 where aqueous HCl was used to adjust the hydronium ion concentration. Notation: **4a/5a** (▲), **4b/5b** (■), **4c/5c** (●), **4e/5e** (◆).

indicated in Table 1, and extrapolating the observed pseudo-first-order rate constant to zero buffer concentration by linear regression. The numerical values of k_{is}^{o} and k_{cl}^{o} are listed in Table 1. While both of these rate constants are markedly increased with the increasing electronegativity of the alkyl groups, the shape of the pH–rate profiles remains almost unchanged, being similar to that reported previously[23] for the dimethyl ester **4d**. The buffer-independent isomerization and cleavage of all the compounds are first-order in hydronium ion concentration at pH 2 to 0, and show a modest negative deviation from the linear dependence of lg (k^{o}/s^{-1}) on H_0 in concentrated solutions of hydrogen chloride. The latter downward curvature possibly results from nearly quantitative protonation of the phosphoryl oxygen, which alters the reaction order in hydronium ion activity from one to zero. If this interpretation is correct, the pK_a value of the protonated triester is about −0.7. The cleavage remains pH-independent from pH 2 to 7, and then becomes hydroxide ion catalyzed, whereas the isomerization is hydroxide ion catalyzed at pH > 3. Accordingly, k_{cl}^{o} may be expressed by eqn. (1), where the partial rate and equilibrium

$$k_{cl}^{o} = k_{cl}^{H}/(1 + K_a/a_{H^+}) + k_{cl}^{w} + k_{cl}^{OH}K_w/a_{H^+} \qquad (1)$$

constants (k_{cl}^{H}, k_{cl}^{w}, k_{cl}^{OH}, K_a) are those defined in Scheme 2,

Table 1 Rate constants for the cleavage and isomerization of 5′-*O*-pivaloyluridin-3′-yl dialkyl phosphates (**4a**–**c**,**e**) in carboxylic acid and amine buffers at 298.2 K (I = 1.0 mol dm^{-3} with sodium chloride)

Compound	Buffer acid	r [a]	$k_{cl}^{bf}/10^{-6}$ dm^3 mol^{-1} s^{-1} [b]	$k_{cl}^{o}/10^{-6}$ s^{-1} [c]	$k_{is}^{bf}/10^{-6}$ dm^3 mol^{-1} s^{-1} [d]	$k_{is}^{o}/10^{-6}$ s^{-1} [e]
4a	Cyanoacetic acid	3:1	13.7 ± 1.5	8.2 ± 0.5		
		1:3	9.8 ± 0.7	4.23 ± 0.21		
	Chloroacetic acid	3:1	9.5 ± 0.5	4.46 ± 0.14		
		1:3	13.4 ± 0.2	3.10 ± 0.07		
	Formic acid	3:1	28.5 ± 0.1	3.8 ± 0.3	61 ± 4	40.2 ± 1.2
		1:1			177 ± 6	104.1 ± 1.8
		1:3	90.5 ± 1.1	3.2 ± 0.3		
	Acetic acid	1:1	145 ± 4	5.0 ± 1.3		
	Propionic acid	1:3	268 ± 8	4.4 ± 2.4		
	Triethanolammonium ion	4:1	19.2 ± 1.4	12.3 ± 0.1		
		1:1	70 ± 50	32 ± 5		
	Diethanolammonium ion	4:1	4400 ± 400	90 ± 30		
4b	Cyanoacetic acid	3:1	5.13 ± 0.12	2.15 ± 0.04	3.4 ± 0.5	1.44 ± 0.16
		1:3	2.45 ± 0.09	0.94 ± 0.03	3.4 ± 1.1	1.9 ± 0.4
	Chloroacetic acid	3:1	2.9 ± 0.4	1.02 ± 0.12	1.8 ± 0.4	1.58 ± 0.11
		1:3	1.9 ± 0.3	0.50 ± 0.07	6.6 ± 1.6	6.0 ± 0.5
	Formic acid	3:1	3.96 ± 0.10	0.58 ± 0.03	4.6 ± 1.9	6.9 ± 0.7
		1:3	7.7 ± 0.5	0.65 ± 0.15	79 ± 12	29 ± 4
	Acetic acid	3:1	8.5 ± 0.4	0.53 ± 0.12	−1.0 ± 0.4	66.1 ± 1.1
		1:3	27.1 ± 0.9	0.4 ± 0.3		
	Propionic acid	3:1	10.8 ± 0.2	0.52 ± 0.05	−1 ± 2	89 ± 6
		1:3	32.7 ± 0.5	0.53 ± 0.15		
	Triethanolammonium ion	4:1	5.7 ± 0.2	0.677 ± 0.022		
		1:1	13.3 ± 1.3	1.96 ± 0.12		
	Diethanolammonium ion	4:1	690 ± 90	6 ± 8		
4c	Cyanoacetic acid	3:1	0.64 ± 0.04	0.347 ± 0.011	0.55 ± 0.11	0.27 ± 0.03
		1:3	0.25 ± 0.03	0.118 ± 0.011	0.34 ± 0.03	0.142 ± 0.008
	Formic acid	3:1	0.26 ± 0.03	0.040 ± 0.009	0.39 ± 0.09	0.18 ± 0.03
		1:3			0.9 ± 0.5	1.05 ± 0.15
	Acetic acid	3:1			0.70 ± 0.16	1.50 ± 0.05
		1:3	0.41 ± 0.04	0.1 ± 0.3	9.3 ± 1.9	12.2 ± 0.6
	Triethanolammonium ion	1:1	0.74 ± 0.20	0.080 ± 0.002		
4e	Cyanoacetic acid	3:1	4.1 ± 0.7	1.83 ± 0.21	5.9 ± 0.6	1.54 ± 0.21
		1:3	1.8 ± 0.3	0.86 ± 0.10	4.15 ± 0.12	1.24 ± 0.04
	Formic acid	3:1	2.51 ± 0.12	0.71 ± 0.03	5.5 ± 0.8	3.5 ± 0.2
		1:3	5.92 ± 0.18	0.41 ± 0.05	8 ± 9	22 ± 3
	Acetic acid	3:1	5.3 ± 0.4	0.62 ± 0.11	5 ± 6	30.0 ± 1.8
		1:3	15.9 ± 0.2	0.38 ± 0.07	−1 ± 1	280 ± 40
	Triethanolammonium ion	4:1	0.93 ± 0.07	1.587 ± 0.007		
		1:1	5 ± 2	4.8 ± 0.2		
	Diethanolammonium ion	4:1	6.41 ± 0.14	20.8 ± 1.3		

[a] Buffer ratio: r = [HA]/[A$^-$] and [BH$^+$]/[B] for carboxylic acids and amine buffers, respectively. [b] Second-order rate constant for the buffer catalyzed cleavage. The first-order rate constants were determined at [HA] + [A$^-$] = 0.050, 0.10, 0.30 and 0.50 mol dm^{-3}, [BH$^+$] + [B] = 0.050, 0.075, 0.100, 0.125 mol dm^{-3}. [c] The observed first-order rate constant for the cleavage at buffer concentration zero. [d] The second-order rate constant for the isomerization. For experimental conditions see footnote *b*. [e] The observed first-order rate constant for the isomerization at buffer concentration zero.

Table 2a Partial rate constants for the buffer-independent cleavage of **4a**–**c**,**e** at 298.2 K (I = 1.0 mol dm^{-3} with NaCl) [a]

Compound	K_a/mol dm^{-3}	$k_{cl}^{H}/10^{-4}$ s^{-1}	$k_{cl}^{H}/K_a/10^{-4}$ dm^{-3} mol^{-1} s^{-1}	$k_{cl}^{w}/10^{-7}$ s^{-1}	k_{cl}^{OH}/dm^3 mol^{-1} s^{-1}
4a	5.0 ± 1.8	15.5 ± 1.8	2.0 [b]	37.3 ± 2.2	14.6 ± 1.6
4b	5.0 ± 2.1	6.5 ± 2.1	1.1 [b]	5.07 ± 0.28	0.76 ± 0.09
4c	3.9 ± 1.2	0.74 ± 0.17	0.19 [b]	~0.4	0.0234 ± 0.0004
4e	6.0 ± 1.6	5.4 ± 1.2	0.90 [b,c]	5.1 ± 0.5 [d]	2.52 ± 0.32

[a] For the rate constants, see Scheme 2. [b] Estimated accuracy *ca.* ± 5%. [c] k_{cl}^{H}/K_a for the cleavage of the 2-methoxyethoxy group is 0.72 × 10^{-4} dm^{-3} mol^{-1} s^{-1}, and that for the cleavage of the isopropoxy group is 0.18 × 10^{-4} dm^{-3} mol^{-1} s^{-1}. [d] k_{cl}^{w} for the cleavage of the 2-methoxyethoxy group is 4.1 × 10^{-7} s^{-1}, and that for the cleavage of the isopropoxy group is 1.0 × 10^{-7} s^{-1}.

and K_w is the ionic product of water under the experimental conditions. A similar eqn. (2) was used to fit the values of k_{is}^{o},

$$k_{is}^{o} = k_{is}^{H}/(1 + K_a/a_{H}^{+}) + k_{is}^{w} + k_{is}^{OH}K_w/a_{H}^{+} \qquad (2)$$

although in this case the k_{is}^{w} term is not necessarily significant. The values obtained by least-squares fitting for the partial constants are given in Table 2.

Table 1 also records the second-order rate constants for the buffer catalyzed isomerization (k_{is}^{bf}) and cleavage (k_{cl}^{bf}) of **4a**–**c**, **e**. The buffer catalyzed cleavage clearly predominates over the buffer independent one at pH > 3. In 0.1 mol dm^{-3} acetic acid–sodium acetate buffer ([AcOH]/[AcO$^-$] = 1:3), for example, 87% of the cleavage of **4b/5b** is buffer catalyzed. In striking contrast, buffer catalyzed isomerization is detected only in the most acidic buffers, *i.e.* in cyanoacetic, chloroacetic and formic

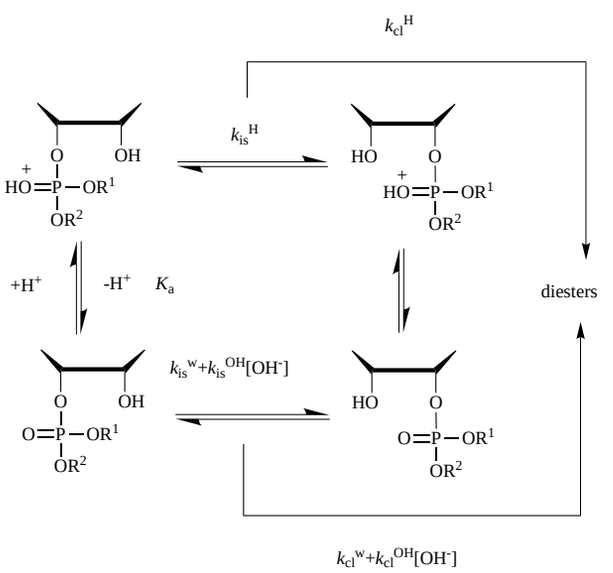
Table 2b Partial rate constants for the buffer-independent isomerization of **4a**–**c**,**e** at 298.2 K (I = 1.0 mol dm^{-3} with NaCl)[a]

Compound	$k_{is}^{H}/K_a/10^{-4}$ dm^{-3} mol^{-1} s^{-1}	$k_{is}^{w}/10^{-7}$ s^{-1}	$k_{is}^{OH}/10^{3}$ dm^{3} mol^{-1} s^{-1}
4a	2.32 ± 0.12	55 ± 11	1490 ± 80
4b	0.39[b]	6.2 ± 2.5	241 ± 29
4c	0.064[b]	0.8 ± 0.3	6.0 ± 0.9
4e	0.63[b]	1.6 ± 3.0	132 ± 16

[a] For the rate constants, see Scheme 2. [b] Estimated accuracy *ca.* ±5%.

Table 3 Second-order rate constants for the general acid and general base catalyzed cleavage at 298.2 K (I = 1.0 mol dm^{-3} with NaCl)

Compound	Buffer acid	k_{cl}^{HA}/ 10^{-6} dm^{-3} mol^{-1} s^{-1}	$k_{cl}^{A^-}$/ 10^{-6} dm^{-3} mol^{-1} s^{-1}
4a	Cyanoacetic acid	15.7	7.85
	Chloroacetic acid	7.71	15.3
	Formic acid		122
	Acetic acid		290
	Propionic acid		357
	Triethanolammonium ion		96
	Diethanolammonium ion		22 200
4b	Cyanoacetic acid	6.47	1.11
	Chloroacetic acid	3.44	1.32
	Formic acid	2.10	9.56
	Acetic acid		36.4
	Propionic acid		43.4
	Triethanolammonium ion		27.5
	Diethanolammonium ion		3500
4c	Cyanoacetic acid	0.819	0.063
	Triethanolammonium ion		1.49
4e	Cyanoacetic acid	5.33	0.59
	Formic acid	0.81	7.63
	Acetic acid	0.075	21.1
	Triethanolammonium ion		7
	Diethanolammonium ion		3200



Scheme 2

acid buffers, and even then its contribution to the observed rate constant of isomerization is rather small.

The second-order rate constants obtained for the general acid and general base catalyzed isomerization (k_{is}^{HA} and $k_{is}^{A^-}$) and cleavage (k_{cl}^{HA} and $k_{cl}^{A^-}$) by the breakdown of k_{is}^{bf} and k_{cl}^{bf} *via* eqn. (3) are listed in Table 3.

$$k_i^{bf}([HA] + [A^-]) = k_i^{HA}[HA] + k_i^{A^-}[A^-]; \text{ i = cl or is} \quad (3)$$

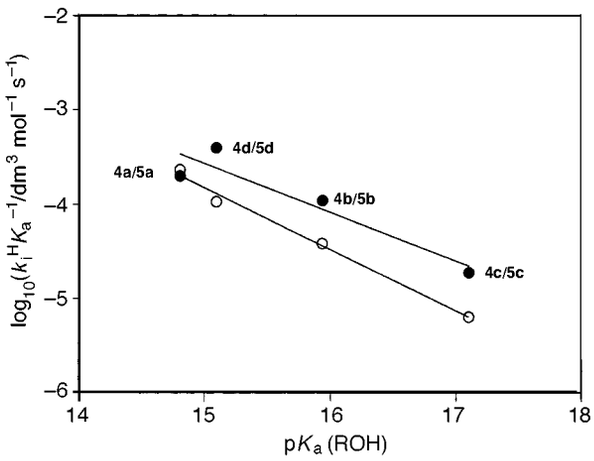


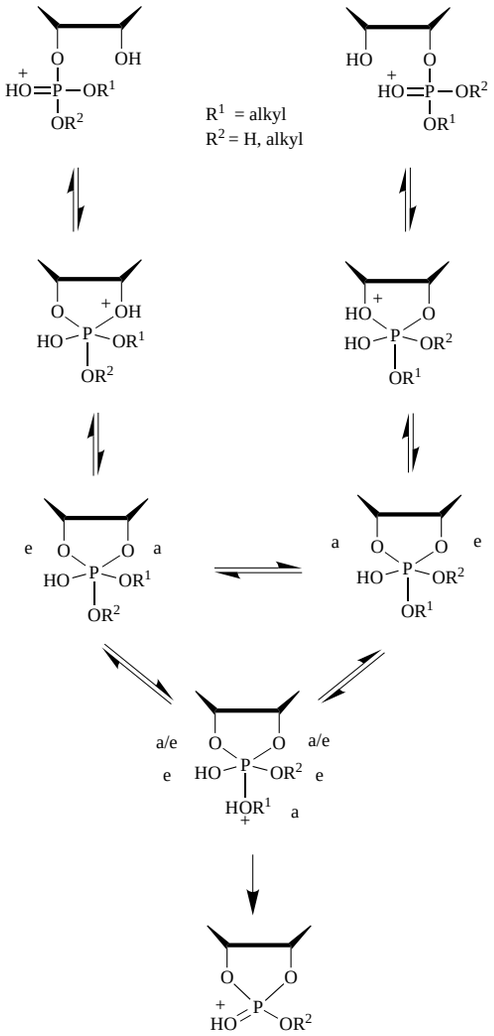
Fig. 3 Logarithmic second-order rate constants for the hydronium ion catalyzed isomerization of symmetric 5′-*O*-pivaloyluridin-3′-yl and -2′-yl dialkyl phosphates (**4a**–**d**/**5a**–**d**) and their cleavage to a mixture of diesters at 298.2 K plotted against the pK_a value of the esterified alcohol.[31] The filled circles refer to cleavage and the open circles to isomerization. The values for **4d/5d** are taken from Ref. 23. The slopes of the lines are cleavage −0.51 ± 0.13, isomerization −0.65 ± 0.04.

Discussion

Hydronium ion catalyzed cleavage and isomerization

As seen in Figs. 1 and 2, the isomerization of 5′-*O*-pivaloyluridin-3′-yl dialkyl phosphates (**4a**–**e**) to their 2′-isomers (**5a**–**e**; Reaction **A** in Scheme 1) and their cleavage to a mixture of diesters (Reaction **B**) are both acid catalyzed at pH < 2. The logarithmic rate constants of these reactions are plotted against the pK_a value of the esterified alcohol in Fig. 3. Although the acidity range of the alcohols is rather narrow and steric effects undoubtedly play a role in addition to polar effects, it is clear that both reactions are accelerated by the increasing electronegativity of the alkyl groups, the effect being more marked on isomerization than on cleavage. The β_{lg} value for the cleavage is −0.51 ± 0.13, and the β value for the isomerization −0.65 ± 0.04. Accordingly, the susceptibility to the electronegativity of the alkyl group is considerably greater than that reported for the corresponding reactions of 3′-phosphodiesters: β_{lg} = −0.12 ± 0.05 for the cleavage and β = −0.18 ± 0.02 for the isomerization.[26]

The hydronium ion catalyzed reactions of both 3′-diesters and 3′-triesters have previously been suggested to proceed by a similar mechanism. With diesters, the 2′-hydroxy function attacks on the monocationic phosphodiester obtained by preequilibrium protonation of both of the non-bridging phosphoryl oxygens (Scheme 3).[3,27,28] The resulting monocationic phosphorane intermediate then undergoes a kinetically invisible pseudorotation and protolytic rearrangement *via* the neutral ionic form. This process may bring either protonated 2′O, 3′O or OR to an apical position and hence lead to breakdown of the intermediate back to the starting material, to the isomerization product (Reaction **A**), or to the cleavage product (Reaction **B**), respectively. The pseudorotation cannot be the rate-limiting step of the isomerization as far as the reaction proceeding by initial formation of a monocationic phosphorane is concerned. This intermediate undergoes rapid thermodynamically favoured deprotonation to a neutral phosphorane. Accordingly, if the isomerization take places by rate-limiting pseudorotation, the product distribution would be pH-dependent: on increasing the hydronium ion concentration, the cleavage would become favoured over the isomerization. As seen from the pH–rate profiles (Figs. 1 and 2), this is not the case. With triester, the extra alkyl group is assumed to take the role of one of the rapidly exchangeable protons of a monocationic diester.[3,22,23] Accordingly, the structural effects ought to be



Scheme 3

rather similar with triesters and diester. The extra alkyl group on triester may, however, be expected to increase the susceptibility of the triester reaction to the acidity of the esterified alcohol. Increasing electronegativity of the alkyl group(s) lowers the electron density at phosphorus and hence facilitates the attack of the 2′-hydroxy function. Since symmetrical triesters (**4a**–**d**) bear two alkyl ligands the electronegativity of which is varied, and diesters only one, the influence is more marked with triesters. Evidently the effect on preequilibrium protonation is less important, since the inductive effect is transmitted to the phosphoryl oxygen through an additional PO bond. For the following reasons, the polar effects on the breakdown of the phosphorane intermediate may also be expected to be relatively small and comparable with triesters and diesters. As far as isomerization is concerned, the leaving group remains unchanged (3′-OH), and the nondeparting alkoxy groups have been shown to exert only a modest effect on the hydrolysis of phosphotriesters.[29] With the cleavage reaction, the increasing electronegativity of the alkyl group weakens the P–OR bond, but simultaneously retards the protonation of the leaving oxygen. These two effects at least partially cancel each other. The increasing electronegativity of the alkyl group(s), however, slightly biases the product distribution toward the isomerization products, the effect being more marked with triesters than with diesters. If only one of alkyl groups of a 3′-triester is replaced with a better leaving group, the rate-acceleration appears equal to that observed with the corresponding diesters. As seen from Table 2, the isopropyl 2-methoxyethyl 3′-phosphotriester **4e** is cleaved 3.8 times as fast as its 3′-diisopropyl counterpart **4c**, while the reactivity ratio of the

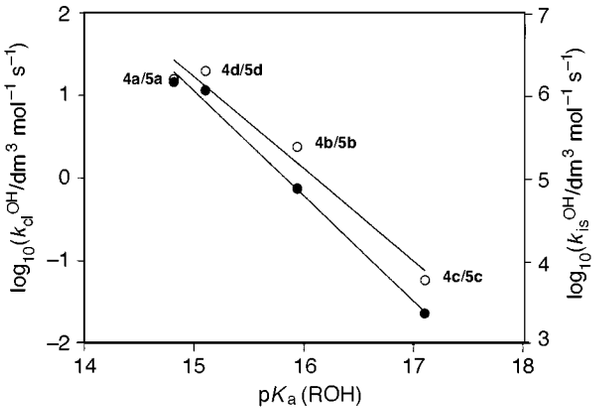
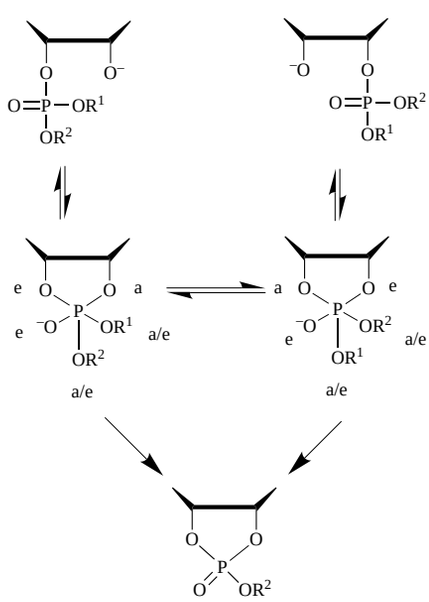


Fig. 4 Logarithmic second-order rate constants for the hydroxide ion catalyzed isomerization of symmetric 5′-*O*-pivaloyluridin-3′-yl and -2′-yl dialkyl phosphates (**4a**–**d**/**5a**–**d**) and their cleavage to a mixture of diesters at 298.2 K plotted against the pK_a value of the esterified alcohol.[31] The filled circles refer to cleavage (*y* axis to the left) and the open circles to isomerization (*y* axis to the right). The values for **4d**/**5d** are taken from ref. 23. The slopes of the lines are: cleavage -1.26 ± 0.07, isomerization -1.10 ± 0.16.

corresponding 2-ethoxyethyl and isopropyl phosphodiesters is 4.6.[26]

Hydroxide ion catalyzed cleavage and isomerization

The isomerization of **4a**–**e** becomes hydroxide ion catalyzed at pH > 3 and the cleavage at pH > 7. Both reactions are markedly susceptible to the acidity of the esterified alcohol, the β_{lg} for the cleavage being -1.26 ± 0.07 and the β for isomerization -1.10 ± 0.16 (Fig. 4). It has been suggested that the first-order dependence of rate on hydroxide ion concentration results from preequilibrium deprotonation of the 2′-hydroxy function.[22,23] The resulting 2′-oxyanion then attacks on neutral phosphotriester centre giving a monoanionic phosphorane, which may decompose in two alternative manners (Scheme 4). Either



Scheme 4

pseudorotation brings the 3′-oxygen to an apical position and the 3′-oxyanion departs, or the alkoxy group leaves as alkoxide ion. The former reaction is several orders of magnitude faster than the latter, consistent with the results of quantum chemical calculations[30] which suggest that the endocyclic cleavage of a monoanionic phosphorane intermediate is a more facile process than the exocyclic cleavage.

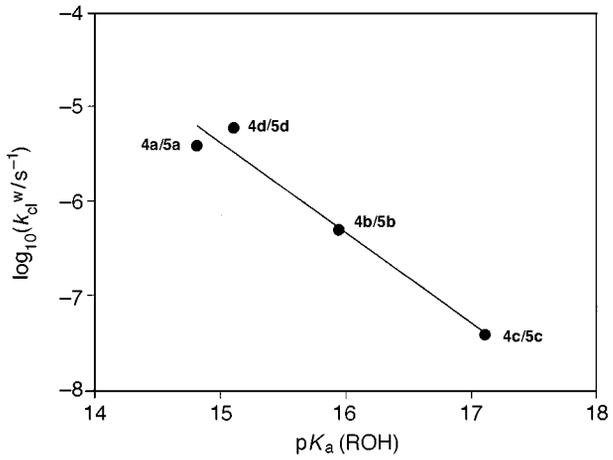


Fig. 5 Logarithmic first-order rate constants for the pH-independent cleavage of symmetric 5′-*O*-pivaloyluridin-3′-yl and -2′-yl dialkyl phosphates (**4a**–**d**/**5a**–**d**) to a mixture of diesters at 298.2 K plotted against the pK_a value of the esterified alcohol.[31] The value for **4d/5d** is taken from ref. 23. The slope of the line is: cleavage −0.94 ± 0.13.

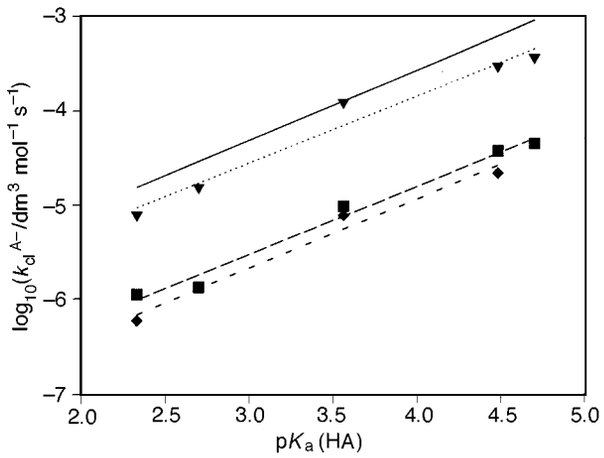


Fig. 6 Brønsted plots for the general base catalyzed cleavage of 5′-*O*-pivaloyluridin-3′-yl and -2′-yl dialkyl phosphates to a mixture of diesters at 298 K. The β values are: 0.71 ± 0.08 (**4a/5a**, ▼), 0.72 ± 0.05 (**4b/5b**, ■), 0.73 ± 0.11 (**4e/5e**, ◆). The line without experimental points refer to **4d/5d** and is taken from ref. 23. The buffer acids employed are in the order of increasing pK_a: cyanoacetic, chloroacetic, formic, acetic and propionic acid.

Since the isomerization is much faster than the cleavage, the breakdown of the intermediate is undoubtedly the rate-limiting step of the cleavage reaction. The highly negative β_{lg} value (−1.26) is consistent with this mechanism, and it may largely be attributed to a unimolecular cleavage of the P–OR bond *via* a transition state where the alkoxide ion character of the leaving group is rather well developed. In fact, the value is almost equal to that (−1.28) reported[26] for the hydroxide ion catalyzed cleavage of ribonucleoside 3′-phosphodiester, which in all likelihood takes place *via* a dianionic phosphorane-like transition state or marginally stable intermediate.[1,3] One should, however, bear in mind that with symmetric triesters the nondeparting alkyl group may exert an additional effect on both the preequilibrium concentration of the phosphorane intermediate and the rate of its breakdown, and these contributions are included in the observed β_{lg} value. When only one of the isopropyl ligands of the symmetrical diisopropyl triester **4c** is replaced with a 2-methoxyethyl group **4e**, the rate acceleration is only 110-fold, corresponding a β_{lg} value of the order of −0.9.

The isomerization takes place *via* the same phosphorane intermediate as the cleavage, but now the breakdown of the intermediate alone is not rate-limiting. The process is kinetically symmetrical, which means that the transition states for the formation and breakdown of the intermediate on the reaction path from the 3′-triester to its 2′-counterpart must be at comparable energy levels. The pseudorotation may, at least in principle, be rate-limiting. As far as symmetric triesters are concerned, the pseudorotation simply consists of an interchange of two identical alkoxy groups, one in the apical and the other in the equatorial positions, and it hence hardly contributes to the β value. The most probable explanation for the negative β value (−1.10) is that, as with the hydronium ion catalyzed isomerization, electronegative alkyl groups facilitate the formation of the phosphorane intermediate by diminishing the electron density at phosphorus. The susceptibility to this effect seems, however, to be larger than with the hydronium ion catalyzed process. Tentatively one may assume that the attack of negatively charged 2′-oxyanion on neutral phosphate is more sensitive to the electron density at phosphorus than the attack of the neutral 2′-hydroxy group on an inherently electron deficient phosphate monocation.

pH-independent cleavage

The cleavage of 3′-phosphotriesters **4a**–**e** is pH-independent over a wide pH range, 3–7, *i.e.* under conditions where the isomerization is hydroxide ion catalyzed and much faster than the cleavage. We have suggested previously[22,23] that this reaction takes place *via* the same monoanionic phosphorane intermediate as the hydroxide ion catalyzed isomerization and cleavage (Scheme 4). As discussed above, the alkoxide ion is a much worse leaving group than the 2′- and 3′-oxyanions. Accordingly, cleavage of the exocyclic P–OR bond of the monoanionic phosphorane alone is rate-limiting and requires acid catalysis under conditions where the 2′- and 3′-oxygens may depart as oxyanions. As seen from Fig. 5, the β_{lg} value for this reaction (−0.94 ± 0.13) is less negative than that of the hydroxide ion catalyzed cleavage (−1.26), but more negative than that of the hydronium ion catalyzed cleavage (−0.51). The relative magnitude of these values suggest that the bond rupture is rather advanced in the transition state and protonation of the departing alkoxide ion takes place concertedly with the bond rupture. In other words, hydronium ion acts as a general acid.

Buffer catalyzed cleavage

The cleavage of uridine 3′-phosphotriesters **4a**–**e** is susceptible to buffer catalysis in the pH range 2–7, *i.e.* under conditions where the buffer-independent cleavage is pH-independent. As seen from Table 3, the buffer catalysis is almost entirely general base catalysis, except in the most acidic buffers. In other words, general acid catalysis is only observed at pH < 3, *i.e.* under conditions where the buffer-independent reaction becomes hydronium ion catalyzed. The rate-limiting step of the buffer-independent cleavage is, as discussed above, breakdown of the monoanionic phosphorane intermediate. The most likely mechanistic interpretation for the observed general base catalysis is thus sequential specific base–general acid catalysis: rapid initial deprotonation of the 2′-hydroxy function and subsequent attack of the resulting 2′-oxyanion on phosphorus give a monoanionic phosphorane, which undergoes general acid catalyzed cleavage of one of the exocyclic alkoxy functions. The mechanism is hence essentially the same as that proposed above for the buffer-independent cleavage at pH 3–7. The β value for the observed general base catalysis by caboxylate ions is approximately 0.7 with all the triesters studies (Fig. 6), which means that the α value for the general acid catalyzed step of the sequential specific base–general acid catalysis is 0.3. The β_{lg} value is in turn about −1.0. Accordingly, the rupture of the P–OR bond seems to be considerably more advanced in the transition state than the proton transfer from the buffer acid to the leaving group.

As mentioned above, the cleavage reaction is also susceptible to general acid catalysis, but only when the pK_a value of the buffer acid is <4. The α value is approximately 0.8, and the β_{lg}

value −0.6, *i.e.* almost equal to that of the hydronium ion catalyzed cleavage (−0.51). In these markedly acidic buffers, neither the formation nor the breakdown of the phosphorane intermediate alone is rate-limiting, which makes it difficult to draw firm mechanistic conclusions. The fact that the isomerization is also buffer catalyzed in the most acidic buffer and this reaction exhibits a β_{lg} value comparable to that of cleavage, suggests that the formation of the phosphorane intermediate is buffer catalyzed. A tentative mechanistic interpretation is a sequential specific acid–general base catalysis. The phosphoryl oxygen is protonated in a rapid initial step, and the buffer base facilitates the nucleophilic attack of the 2′-hydroxy group on the phosphorus by abstracting the proton in the transition state. Departure of the alkoxy group may again be catalyzed by buffer acid, but this general catalysis is now kinetically invisible since the buffer acid formed in the first step serves as a catalyst in the second step. The α value of 0.8 suggests a β value of 0.2 for the general base catalyzed partial reaction. In other words, the transition state is rather early: neither the proton transfer nor the PO bond formation (β_{lg} −0.6) is very advanced.

Many of the β_{lg} values discussed above are markedly negative. Apart from partial bond breaking, this may be generally indicative of increased electron density on the bridging triester oxygen on going from the initial state to the phosphorane intermediate.

Buffer catalyzed isomerization

In striking contrast to the cleavage reaction, the isomerization is not markedly susceptible to general base catalysis (interpreted above as sequential specific base–general acid catalysis) at pH > 3. Our previous results suggested a modest general base catalysis also for the isomerization.[23] However, the more extensive data of the present study are in this respect more controversial. The observed rate constants of isomerization are in some cases buffer-dependent, but not always. Even in cases where an apparent buffer catalysis is observed, the buffer-dependent proportion of the observed rate constant is always less than 50%. A possible explanation for these ambiguities may be that with isomerization neither the formation nor the breakdown of the phosphorane intermediate alone is rate-limiting. In principle, the attack of the 2′-hydroxy function may be general base catalyzed, and hence the departure of the 3′-oxygen general acid catalyzed (but kinetically invisible). However, if this kind of a reaction pathway exists, it is always of minor importance compared to the hydroxide ion catalyzed reaction.

Experimental

5′-*O*-Pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridin-3′-yl bis-(2-methoxyethyl) phosphate 3a

1,2,4-Triazole (265 mg, 3.82 mmol) and triethylamine (532 mm^3, 3.83 mmol) were dissolved in dry acetonitrile (6.5 cm^3). Freshly distilled phosphoryl trichloride (117 mm^3, 1.29 mmol) was added, and the mixture was stirred for 30 min at room temp. The precipitated triethylammonium chloride was removed by filtration and the filtrate was immediately added to predried 5′-*O*-pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridine[23] (more polar diastereomer, 350 mg, 0.85 mmol). After 1 h, excess of 2-methoxyethanol (2.5 cm^3, 43 mmol) was added, and the reaction mixture was left to stand 3.5 h at room temp. The reaction mixture was evaporated to dryness, the residue was dissolved in dichloromethane (30 cm^3) and washed with an aqueous phosphate buffer (30 cm^3, KH_2PO_4–Na_2HPO_4 0.1/0.1 mol dm^{-3}). The organic phase was dried with sodium sulfate and evaporated to dryness. The crude product was first purified by adsorption chromatography, using Silica gel 60 as an adsorbent and a mixture of dichloromethane and ethanol (9 : 1, v/v) as an eluent. Further purification by RP-chromatography (LiChrospher 100 RP-18, 250 × 10 mm, 5 μm, 67% aq methanol, v/v) gave chromatographically and NMR spectroscopically homogeneous product in 13% yield. δ_H(500 MHz; $CDCl_3$; *J* values in Hz throughout) 8.43 (1H, s, H3), 7.41 (1H, d, H6, $J_{H5,H6}$ 8.1), 5.68 (1H, dd, H5), 6.11 (1H, d, H1′, $J_{H1',H2'}$ 6.54), 4.90 (1H, m, H3′), 4.80 (1H, m, thp-2), 4.51 (1H, dd, H4′, $J_{H3',H4'}$ 6.41, $J_{H4',H5'}$ 3.81), 4.37 (1H, dd, H5′, $J_{H5',H5''}$ 12.6), 4.26 (1H, dd, H5″, $J_{H4',H5''}$ 2.59), 4.22 (2H, m, CH_2OP), 3.61 (3H, m, CH_2OMe and thp-6a), 3.44 (1H, m, thp-6b), 3.38 (3H, s, CH_3O), 1.4–1.8 (6H, m, thp), 1.25 (9H, s, Piv). $\delta_P(CDCl_3)$ −1.62 from phosphoric acid. MS/FAB 631 (M + Na), 647 (M + K), 525 (M − thp + 2H). Elemental analysis: Found: C, 49.3; H, 6.6; N, 4.4. $C_{25}H_{41}N_2O_{13}P$ requires: C, 49.3; H, 6.8; N, 4.6%.

5′-*O*-Pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridin-3′-yl diethyl phosphate 3b

Compound **3b** was obtained as described for **3a** using ethanol instead of 2-methoxyethanol. The crude product was purified by silica gel chromatography, using hexane–acetone (3 : 2, v/v) as eluent, yield 42%. δ_H(400 MHz, $CDCl_3$) 7.91 (1H, m, H3), 5.73 (1H, dd, H5, $J_{H5,H6}$ 8.15), 7.40 (1H, d, H6), 6.08 (1H, d, H1′, $J_{H1',H2'}$ 6.12), 4.88 (1H, m, H3′), 4.78 (1H, m, thp-2), 4.43 (2H, m, H2′ and H4′), 4.34 (1H, dd, H5′, $J_{H5',H5''}$ 12.68, $J_{H5',H4'}$ 3.92), 4.28 (1H, dd, H5″, $J_{H5'',H4'}$ 3.16), 4.16 (2H, m, Et-CH_2), 3.63 (1H, m, thp-6a), 3.44 (1H, m, thp-6b), 1.4–1.8 (6H, m, thp), 1.36 (3H, t, Et-CH_3, $J_{CH2,CH3}$ 7.08), 1.24 (9H, s, Piv). $\delta_P(CDCl_3)$ −0.97 from phosphoric acid. MS/FAB (M + Na), 587 (M + K), 465 (100%, M − thp + 2H). Elemental analysis: Found: C, 50.7; H, 6.5; N, 5.1. $C_{23}H_{37}N_2O_{11}P$ requires: C, 50.4; H, 6.8; N, 5.1%.

5′-*O*-Pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridin-3′-yl diisopropyl phosphate 3c

Compound **3c** was obtained as described for **3a**, using propan-2-ol instead of 2-methoxyethanol. In the adsorption chromatography a mixture of dichloromethane and ethanol (19 : 1, v/v), and in RP-chromatography, a mixture of methanol and water (68 : 32) was used as the eluent. The yield was 6%. δ_H(400 MHz, $CDCl_3$) 7.89 (1H, s, H3), 7.39 (1H, d, H6, $J_{H5,H6}$ 8.10), 5.72 (1H, dd, H5), 6.07 (1H, d, H1′, $J_{H1',H2'}$ 6.3), 4.86 (1H, m, H3′), 4.78 (1H, m, thp-2), 4.69 (1H, m, Pr^i-CH, $J_{CH,CH3}$ 4.8), 4.63 (1H, m, Pr^i-CH, $J_{CH,CH3}$ 5.2), 4.46 (1H, dd, H4′), 4.42 (1H, m, H2′), 4.34 (1H, dd, H5′, $J_{H4',H5'}$ 3.20, $J_{H5',H5''}$ 9.60), 4.29 (1H, dd, H5″, $J_{H4',H5''}$ 2.4), 3.63 (1H, m, thp-6a), 3.44 (1H, m, thp-6b), 1.4–1.8 (6H, m, thp), 1.35 (6H, m, Pr^i-CH_3), 1.24 (9H, s, Piv). $\delta_P(CDCl_3)$ −2.93 from phosphoric acid. MS/FAB 599 (M + Na), 615 (M + K), 493 (100%, M − thp + 2H). Elemental analysis: Found: C, 51.3; H, 6.6; N, 4.8. $C_{25}H_{41}N_2O_{11}P$ requires: C, 52.1; H, 7.2; N, 4.9%.

5′-*O*-Pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridin-3′-yl isopropyl methoxyethyl phosphate 3e

5′-*O*-Pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridine[23] was converted to its 2-chlorophenoxy isopropyl 3′-phosphate as described for **3a**, using 2-chlorophenyl phosphoryl-bis(1,2,4-triazolide) (obtained from 2-chlorophenyl phosphorodichloridate and 1,2,4-triazole) as a phosphorylating agent, and propan-2-ol in the subsequent alcoholysis. The reaction time was 4 h in the phosphorylation and 40 h in the alcoholysis. The product was purified by silica gel chromatography, using a mixture of dichloromethane and ethanol (19 : 1, v/v) as eluent. A 5 : 2 mixture of the two phosphorus diastereomers was obtained in 51% yield. δ_H(400 MHz, $CDCl_3$, mixture of R_P and S_P isomers) 7.48 (1H, m, Ar-3), 7.41 (1H, m, Ar-5), 7.40 (1H, d, H6), 7.25 (1H, m, Ar-6), 7.14 (1H, m, Ar-4), 6.1 (1H, t, H1′), 5.73 (1H, dd, H5, $J_{H5,H6}$ 8.05), 4.99 and 5.05 (1H, m, H3′), 4.85 and 4.92 (1H, m, Pr^i-CH), 4.70 and 4.78 (1H, thp-2), 4.19 and 4.55 (1H, m, H4′), 4.45 (1H, m, H2′), 4.28–4.40 (2H, m, H5′ and H5″), 3.60 (1H, m, thp-6a), 3.42 (1H, m, thp-6b), 1.3–1.8

Table 4 Retention times and chromatographic conditions for the separation of **3**–**c**,**e** and its reaction products on Hypersil RP-18 columns[a]

Compound	Acetonitrile (%)	t_R(**3**)/min	t_R(**4**)/min	t_R(**5**)/min
a	30	21.6	6.8	6.8
b	35	19.6	6.3	5.7
c	40	20.3	7.1	5.8
e	35	21.0	6.8[b] 7.1[b]	6.2

[a] Eluent: formic acid–sodium formate buffer (each 0.05 mol dm^{-3}), containing 0.1 mol dm^{-3} ammonium chloride. The acetonitrile content of the eluent is indicated in the table. Flow rate 1 cm^3 min^{-1}. The absorptions were measured at $\lambda = 260$ nm. [b] The R_P and S_P diastereomers.

(m, thp), 1.35–1.45 (m, Pr^i-CH_3), 1.21 and 1.23 (s, Piv). $\delta_P(CDCl_3)$ +17.2 and −7.7 from phosphoric acid. MS/FAB 667 (M[^{35}Cl] + Na), 669 (M[^{37}Cl] + Na, 561 (M[^{35}Cl] − thp + 2H), 563 (M[^{37}Cl] − thp + 2H). Elemental analysis: Found: C, 51.7; H, 5.8; N, 4.4. $C_{28}H_{38}ClN_2O_{11}P$ requires: C, 52.1; H, 5.9; N, 4.3%.

The 2-chlorophenyl, isopropyl 3′-phosphate obtained (153 mg, 0.237 mmol) was dissolved in 1,4-dioxane (23 cm^3), and a solution of sodium 2-methoxyethoxide in 2-methoxyethanol (1.05 mmol in 7 cm^3) was added. The mixture was stirred for 15 min at room temp., neutralized with acetic acid and evaporated to dryness. The residue was dissolved in dichloromethane (28 cm^3), washed with aqueous phosphate buffer (40 cm^3) and dried with sodium sulfate. The product was first purified by silica gel chromatography (dichloromethane–ethanol, 9 : 1, v/v) and then by RP-chromatography (LiChrospher 100 RP-18, 250 × 10 mm, 5 μm, 65% aq methanol). A mixture of the R_P and S_P diastereomers of **3e** was obtained in 29% yield. δ_H(400 MHz, $CDCl_3$) 8.08 (1H, s, H3), 7.41 (1H, d, H6, $J_{H6,H5}$ 8.29), 5.73 (1H, dd, H5), 6.09 (1H, t, H1′), 4.88 (1H, m, H3′), 4.80 (1H, m, thp-2), 4.72 and 4.67 (1H, m, Pr^i-CH, $J_{CH,CH3}$ 6.58), 4.48 and 4.50 (1H, 2dd, H4′), 4.42 (1H, m, H2′), 4.36 and 4.35 (1H, 2dd, H5′, $J_{H4',H5'}$ 3.67, $J_{H5',H5''}$ 12.44), 4.28 and 4.27 (1H, 2dd, H5″, $J_{H4',H5''}$ 2.93), 4.16 (2H, m, CH_2O-P), 3.60 (3H, m, thp-6a and CH_2OMe), 3.49 (1H, m, thp-6b), 3.38 (3H, s, OCH_3), 1.4–1.8 (6H, m, thp), 1.37 and 1.36 (6H, 2d, Pr^i-CH_3), 1.24 (9H, s, Piv). $\delta_P(CDCl_3)$ −1.81 from phosphoric acid. MS/FAB 615 (M + Na), 509 (100%, M − thp + 2H). Elemental analysis: Found: C, 50.9; H, 6.9; N, 5.1. $C_{25}H_{41}N_2O_{12}P$ requires: C, 50.7; H, 7.0; N, 4.7%.

Kinetic measurements

The kinetic measurements were carried out and the aliquots analyzed by RP HPLC (Hypersil ODS, 250 × 4 mm, 5 μm) as described previously[23] for the dimethyl ester, **4c**. The retention times and chromatographic conditions are indicated in Table 4. The rate constants were calculated as described previously.[23]

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