# Hydrolysis and intramolecular transesterification of ribonucleoside 3′-phosphotriesters: comparison of structural effects in the reactions of asymmetric and symmetric dialkyl esters of 5′-*O*-pivaloyl-3′-uridylic acid

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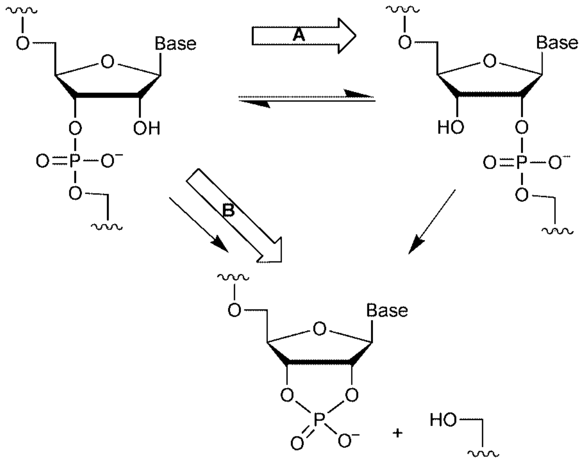
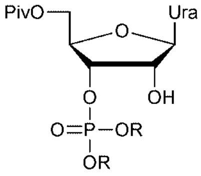
$\beta_{rg}$ Values (rg = remnant group) for the O2′↔O3′ isomerization of asymmetric dialkyl esters of 5′-*O*-pivaloyluridine 3′-phosphate and $\beta_{lg}$ values (lg = leaving group) for the cleavage of the more electronegative alkoxy group from these triesters under various conditions have been determined. The reactions studied included hydronium ion, hydroxide ion, general acid and general base, and pH- and buffer-independent cleavage. The results are compared with the corresponding values obtained earlier with the symmetric 3′-triesters and the catalysis mechanisms are discussed.

## Introduction

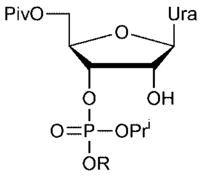
The mechanistic details of the reactions of the phosphodiester bonds of RNA have received considerable interest among the scientific community during the past decade,[1] although the general course of these reactions has been known since 1950's.[2] Two concurrent transesterifications are known to take place: the 3′,5′-phosphodiester bonds are isomerized to 2′,5′-bonds (reaction **A** in Scheme 1) and cleaved to 2′,3′-cyclic phosphates (reaction **B**) with release of the 5′-nucleoside. We have previously elucidated the mechanisms of the partial reactions proceeding *via* different ionic forms by determining the $\beta_{lg}$ values for the isomerization and cleavage of ribonucleoside 3′-monoalkylphosphates (**3a–f**) under various conditions.[3,4] The corresponding phosphotriesters, symmetrical 3′-dialkylphosphates (**1a–d**), have been used as models for phosphate protonated (neutral) phosphodiesters.[5–7] Although this approach has allowed several mechanistic conclusions to be reached concerning the distinction of kinetically equivalent pathways, it contains one inherent shortcoming. On comparing the hydrolysis rates of symmetric triesters, not only the leaving group is varied but also the alkyl group that remains bonded to phosphorus (the remnant group). Although in many cases the effect of the leaving group undoubtedly is dominant, this in any case makes the interpretation of $\beta_{lg}$ values somewhat uncertain. The present study is aimed at eliminating this source of uncertainty from the previous interpretations. $\beta_{lg}$ and $\beta_{rg}$ (lg = leaving group, rg = remnant group) values based on asymmetric triesters, *viz.* diisopropyl (**1a**), isopropyl, 2-methoxyethyl (**2b**), isopropyl, ethyl (**2a**), and isopropyl, (2,2-difluoroethyl) (**2c**) esters of 5′-pivaloyl-3′-uridylic acid, have been determined. The mechanistic conclusions are based on comparisons with the results reported earlier[7] for symmetric triesters.



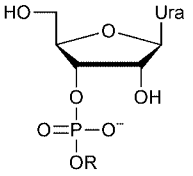
**Scheme 1**



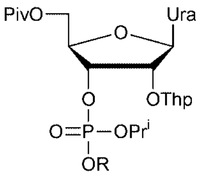
**1 a**: $R = Pr^i$
**b**: R = Et
**c**: R = Me
**d**: $R = CH_2CH_2OMe$



**2 a**: R = Et
**b**: $R = CH_2CH_2OMe$
**c**: $R = CH_2CHF_2$



**3 a**: $R = Pr^i$
**b**: R = Et
**c**: $R = CH_2CH_2OEt$
**d**: $R = CH_2CH_2Cl$
**e**: $R = CH_2CHCl_2$
**f**: $R = CH_2CCl_3$

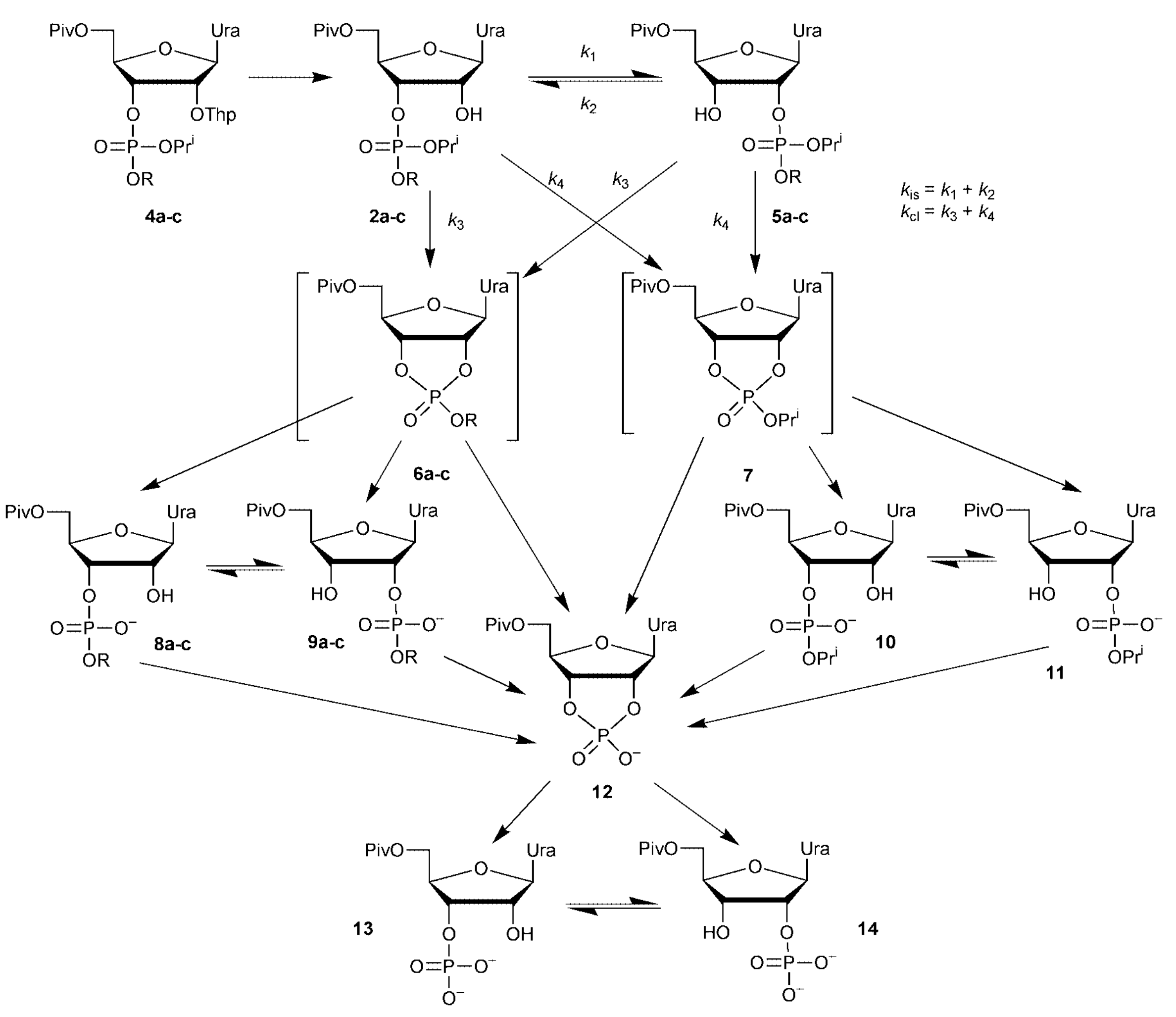


**4 a**: R = Et
**b**: $R = CH_2CH_2OMe$
**c**: $R = CH_2CHF_2$
**d**: R = 2-chlorophenyl

## Results

### Preparation of asymmetric ribonucleoside 3′-phosphotriesters

The asymmetric dialkyl esters of 5′-*O*-pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridine 3′-phosphate (**4a,c**) were prepared from 5′-*O*-pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridin-3′-yl 2-chlorophenyl isopropyl phosphate[7] (**4d**) by displacing the 2-chlorophenoxy group with an appropriate alkoxide ion in a mixture of 1,4-dioxane and the respective alcohol. On



Scheme 2

preparing **4c**, the reaction mixture additionally contained propan-2-ol. The compounds were purified by adsorption chromatography (**4a** also by RP chromatography) and their identity was verified by elemental analysis, $^1$H, $^{31}$P and $^{19}$F NMR and FAB mass spectroscopy.

### Kinetic measurements

The reactions were carried out at 298.2 K, and their progress was followed by analyzing by RP HPLC the content of the aliquots withdrawn at suitable intervals. When the reactions were followed in buffer solutions or in aqueous sodium hydroxide, the 2′-*O*-tetrahydropyran-2-yl group was first removed with 0.1 mol dm$^{-3}$ aqueous hydrogen chloride to give **2a**–**d**, and the desired buffer system was then created by adding an appropriate amount of the buffer base. In aqueous hydrogen chloride, hydrolysis of the 2′-protecting group constituted the first step of the reaction sequence, and equations of consecutive first-order reactions were applied to kinetic calculations. The subsequent conversion of the initial products **6**–**14** was not followed kinetically. Only some samples at prolonged reaction times were withdrawn to determine the final product distribution.

As shown previously,[5–7] uridin-3′-yl dialkyl phosphates (**2**) undergo in aqueous solution two parallel reactions (Scheme 2): isomerization to a 2′-dialkyl phosphate (**5**) and cleavage to a mixture of phosphodiesters (**8**–**12**). The latter reaction presumably proceeds *via* a cyclic triester (**6**/**7**), which is too unstable to be detected. With asymmetric triesters (**2a**–**c**) the situation is in fact more complicated, since either of the alkoxy groups can be displaced in the cleavage reaction, and hence two different cyclic triesters (**6a**–**c**, **7**) and two different pairs of isomeric diesters may be formed. The product signals were identified by comparing the product distributions of **2a**–**c** to those of the corresponding symmetric esters **1a**–**d**, which give only one kind of diester product.

Among the asymmetric triesters studied, the most simple product distribution was observed with **2c**: the cleavage of **2c** yields almost entirely isopropyl diesters (**10**, **11**) and monophosphates **12**–**14** under all the reaction conditions employed. In acidic solutions, however, there are traces of two unidentified products formed, probably **8c** and **9c**. With the other unsymmetric triesters (**2a**,**b**), all possible diesters **8**–**12** were formed in aqueous hydrogen chloride and carboxylic acid buffers. The ratio of [**8a** + **9a**]/[**10** + **11**] was approximately 2 : 9 and the ratio of [**8b** + **9b**]/[**10** + **11**] was approximately 1 : 4.[7] In more basic amine buffers, the ratio of [**8a** + **9a**]/[**10** + **11**] was approximately 1 : 11, while the cleavage of **2b**[7] yielded only the isopropyl esters (**10**, **11**).

The buffer-independent rate constants, $k_{is}{}^0 = k_1{}^0 + k_2{}^0$ for the isomerization and $k_{cl}{}^0 = k_3{}^0 + k_4{}^0$ for the cleavage, were obtained by carrying out the kinetic measurements at 4–5 different buffer concentrations, typically 0.05, 0.10, 0.20, 0.30 and 0.50 mol dm$^{-3}$, and extrapolating the observed pseudo first-order rate constants to zero buffer concentration by linear regression. The pH–rate profiles of the reactions are presented in Fig. 1, and the numerical values of $k_{is}{}^0$ and $k_{cl}{}^0$ are listed in Table 1. Previously[7] it has been shown that the shape of the pH–rate profiles of symmetric triesters **1a**–**d** does not markedly change from one symmetric triester to the other: the reaction order in [H$^+$] changes in the isomerization reaction from 1 to −1 at pH ~2, and in the cleavage reaction from 1 to 0 at pH ~2.5

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

| Compd. | Buffer acid | $R$ | $k_{cl}^{bf}/10^{-6}$ dm$^3$ mol$^{-1}$ s$^{-1}$ | $k_{cl}^{0}/10^{-6}$ s$^{-1}$ | $k_{is}^{bf}/10^{-6}$ dm$^3$ mol$^{-1}$ s$^{-1}$ | $k_{is}^{0}/10^{-6}$ s$^{-1}$ |
|---|---|---|---|---|---|---|
| **2a** | Cyanoacetic | 3:1 | 1.3 ± 0.4 | 0.84 ± 0.12 | 1.67 ± 0.15 | 0.63 ± 0.05 |
| | | 1:3 | 0.464 ± 0.007 | 0.181 ± 0.002 | 1.6 ± 0.4 | 0.77 ± 0.13 |
| | Acetic | 3:1 | 1.91 ± 0.07 | 0.058 ± 0.021 | 4.8 ± 1.1 | 9.9 ± 0.3 |
| | | 1:3 | 4.79 ± 0.11 | 0.14 ± 0.03 | −1.8 ± 1.0 | 90 ± 40 |
| | Triethanolammonium ion | 4:1 | 0.48 ± 0.12 | 0.269 ± 0.011 | | |
| | | 1:1 | 5.5 ± 0.6 | 0.26 ± 0.06 | | |
| | Diethanolammonium ion | 4:1 | 91 ± 3 | 0.75 ± 0.29 | | |
| | | 1:1 | 248 ± 2 | 9.94 ± 0.17 | | |
| **2a** | Cyanoacetic | 3:1 | 23.9 ± 2.7 | 14.3 ± 0.8 | 14.2 ± 1.3 | 9.7 ± 0.4 |
| | | 1:3 | 23.6 ± 0.9 | 9.7 ± 0.3 | 24 ± 4 | 16.1 ± 1.2 |
| | Formic | 3:1 | 89.4 ± 1.2 | 12.4 ± 0.4 | 128 ± 11 | 33 ± 3 |
| | | 1:3 | 242 ± 26 | 23 ± 8 | 66 ± 38 | 326 ± 12 |
| | Acetic | 3:1 | 213 ± 4 | 18.1 ± 1.2 | 270 ± 160 | 264 ± 45 |
| | | 1:3 | 761 ± 26 | 21 ± 7 | | |
| | Propionic | 3:1 | 268 ± 6 | 16.7 ± 1.5 | 320 ± 70 | 346 ± 18 |
| | | 1:3 | 1020 ± 40 | 11 ± 12 | | |
| | MES[b] | 3:1 | 469 ± 21 | 30 ± 4 | | |
| | | 1:1 | 980 ± 91 | 67 ± 17 | | |
| | Triethanolammonium ion | 9:1 | 1900 ± 2300 | 470 ± 210 | | |
| | | 4:1 | 1600 ± 500 | 1300 ± 45 | | |

[a] Rate constants refer to mutual isomerization and disappearance rates of **2a**,**c** and **5a**,**c** *i.e.* $k_{cl} = k_3 + k_4$ and $k_{is} = k_1 + k_2$; see scheme 2. [b] 4-Morpholinoethanesulfonic acid.

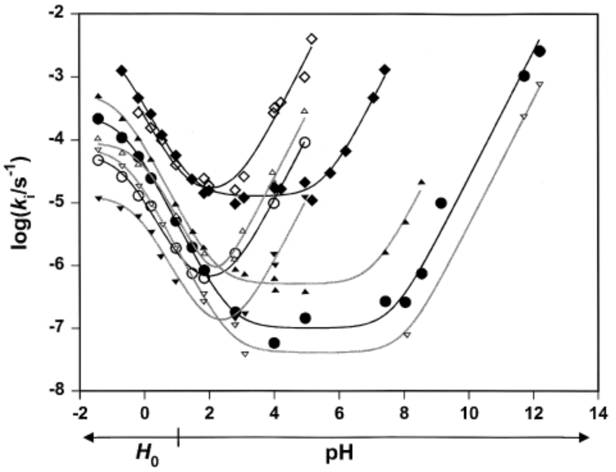
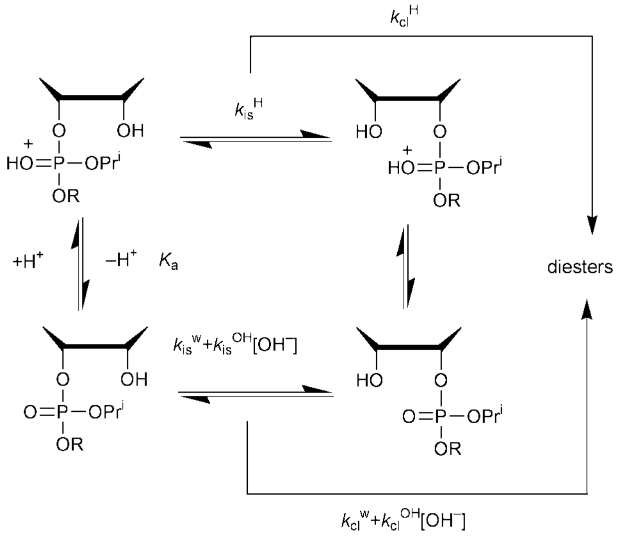


**Fig. 1** pH–rate profiles for the buffer-independent cleavage ($k_{cl}$, solid marks) and mutual isomerization ($k_{is}$, open marks) of dialkyl esters of 5′-*O*-pivaloyl-3′- and 2′-uridylic acid at 298.2 K. The ionic strength is adjusted to 1.0 mol dm$^{-3}$ with NaCl, except at pH < 1.5, where aqueous HCl was used to adjust the hydronium ion concentration. Circles refer to observed reaction rates of **2a** and diamonds to those of **2c**. Gray lines and small symbols refer to similar pH–rate profiles of **1a** (▽) and **2b** (△), and are taken from ref. 7.

and from 0 to −1 at ~7.[7] As seen from Fig. 1, the asymmetric triesters behave slightly differently: the increasing electronegativity of the leaving group considerably reduces the pH where the pH-independent cleavage becomes hydroxide-ion catalyzed. As with symmetric triesters,[7] all reactions show a modest negative deviation from the linear dependence of log($k^0$/s$^{-1}$) on $H_0$ in a concentrated solution of hydrogen chloride. This curvature is attributed, as previously, to protonation of the phosphoryl oxygen. Accordingly, the buffer-independent reaction rates $k_i$ (i = is or cl) may be expressed by eqn. (1), where the

$$k_i^0 = k_i^H/(1 + K_a/a_{H^+}) + k_i^w + k_i^{OH}K_w/a_{H^+} \quad (1)$$

partial rate and equilibrium constants are those defined in Scheme 3, and $K_w$ is the ionic product of water under the experimental conditions. The values obtained by least-squares fitting for the partial constants are given in Table 2.



**Scheme 3**

Table 1 also records the second-order rate constants for the buffer catalyzed reactions. As expected,[7] the buffer catalyzed cleavage clearly predominates over the buffer independent one at pH > 3, the proportion of the buffer-independent reaction being only a few percent of the observed reaction rate. With the isomerization reactions, the contribution of buffer catalysis to the observed rate is small and in some cases possibly nonexistent. The second-order rate constants obtained for the general acid and general base catalyzed isomerization and cleavage ($k_i^{HA}$ and $k_i^{A^-}$; i = is or cl) by breakdown of $k_{is}^{bf}$ and $k_{cl}^{bf}$ *via* eqn. (2) are listed in Table 3.

$$k_i^{bf}([HA] + [A^-]) = k_i^{HA}[HA] + k_i^{A^-}[A^-] \quad (2)$$

## Discussion

### Hydronium ion catalyzed cleavage and isomerization

It has been previously shown that the hydronium ion catalyzed cleavage and isomerization proceed *via* a common pentacoordinated phosphorane intermediate, which is obtained by an attack of the neighboring 2′-hydroxy group on protonated

**Table 2a** Partial rate constants for the buffer-independent cleavage of 5′-*O*-pivaloyluridin-3′-yl dialkyl phosphates at 298.2 K ($I = 1.0$ mol dm$^{-3}$ with sodium chloride)[a,b]

| Compd. | $K_a$/mol dm$^3$ | $k_{cl}^{H}/10^{-4}$ s$^{-1}$ | $k_{cl}^{H}K_a^{-1}/10^{-4}$ dm$^3$ mol$^{-1}$ s$^{-1}$ | $k_{cl}^{w}/10^{-7}$ s$^{-1}$ | $k_{cl}^{OH}$/dm$^3$ mol$^{-1}$ s$^{-1}$ |
|---|---|---|---|---|---|
| **2a** | 4.8 ± 2.7 | 2.4 ± 1.0 | 0.49[c] | 1.01 ± 0.27 | 0.133 ± 0.028 |
| **2c** | 9 ± 9 | 40 ± 30 | 3.8[c] | 127 ± 12 | 2030 ± 280 |

[a] For the rate constants, see Scheme 3. [b] Rate constants refer to disappearance rate of [**2a**,**c** + **5a**,**c**], *i.e.* $k_{cl} = k_3 + k_4$. [c] Estimated accuracy *ca.* 5%.

**Table 2b** Partial rate constants for the buffer-independent isomerization of 5′-*O*-pivaloyluridin-3′-yl dialkyl phosphates at 298.2 K ($I = 1.0$ mol dm$^{-3}$ with sodium chloride)[a,b]

| Compd. | $k_{is}^{H}K_a^{-1}/10^{-4}$ dm$^3$ mol$^{-1}$ s$^{-1}$ | $k_{is}^{w}/$ $10^{-7}$ s$^{-1}$ | $k_{is}^{OH}/10^4$ dm$^3$ mol$^{-1}$ s$^{-1}$ |
|---|---|---|---|
| **2a** | 0.12[c] | 4.6 ± 1.0 | 5.7 ± 0.7 |
| **2c** | 2.5[c] | 130 ± 40 | 106 ± 17 |

[a] For the rate constants, see Scheme 3. [b] Rate constants refer to mutual isomerization rate of **2a**,**c** and **5a**,**c**, *i.e* $k_{is} = k_1 + k_2$. [c] Estimated accuracy *ca.* 5%.

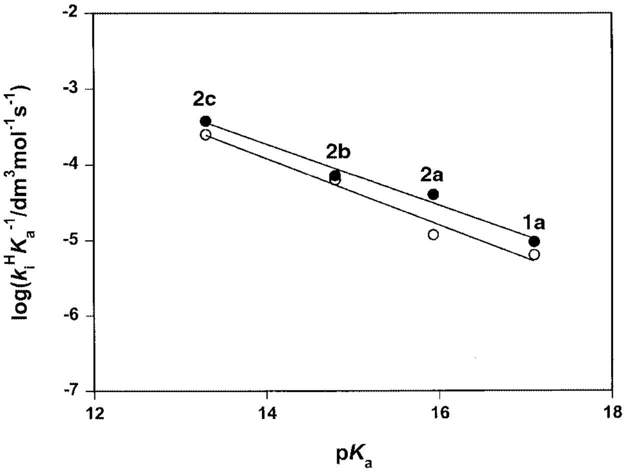


**Fig. 2** Logarithmic second-order rate constants for the hydronium ion catalyzed cleavage of **1a**, **2a**–**c** to **10**–**12** ($k_4$) and mutual isomerization of 3′- and 2′-phosphotriesters ($k_{is}$) at 298.2 K plotted against the p$K_a$ value of the esterified alcohol.[10] The $k_4$ values for **2a**–**c** were obtained by multiplying the obtained $k_{cl}$ values with the appropriate end product ratio [**10** + **11**]/[**8** + **9** + **10** + **11**]. In the case of **1a** a statistical correction was made. The slopes are: $\beta_{lg} = -0.40 \pm 0.04$, $\beta_{rg} = -0.44 \pm 0.05$. The values for **1a** and **2b** are taken from ref. 7.

**Table 3** Second-order rate constants for general acid and general base-catalyzed cleavage of 5′-*O*-pivaloyluridin-3′-yl dialkyl phosphates at 298.2 K ($I = 1.0$ mol dm$^{-3}$ with sodium chloride)[a]

| Compd. | Buffer acid | $k_{cl}^{HA}/10^{-6}$ dm$^3$ mol$^{-1}$ s$^{-1}$ | $k_{cl}^{A^-}/10^{-6}$ dm$^3$ mol$^{-1}$ s$^{-1}$ |
|---|---|---|---|
| **2a** | Cyanoacetic acid | 1.66 | 0.07 |
| | Acetic acid | 0.47 | 6.23 |
| | Triethanolammonium ion | | 7 |
| | Diethanolammonium ion | | 475 |
| **2c** | Cyanoacetic acid | 24.1 | 23.3 |
| | Formic acid | 6 | 342 |
| | Acetic acid | | 930 |
| | Propionic acid | | 1200 |
| | MES[b] | | 1920 |
| | Triethanolammonium ion | | 8200 |

[a] Rate constants refer to disappearance rate of [**2a**,**c** + **5a**,**c**], *i.e.* $k_{cl} = k_3 + k_4$. [b] 4-Morpholinoethanesulfonic acid.

(cationic) phosphate moiety.[5–7] Both of these reactions have been shown to be moderately accelerated with the increasing acidity of the esterified alcohol. With symmetric triesters (**1a**–**d**), the $\beta_{lg}$ and $\beta_{rg}$ values for the cleavage and isomerization have been reported to be $-0.51 \pm 0.13$ and $-0.65 \pm 0.04$, respectively.[7] These values are clearly more negative than those observed for the corresponding diesters: $\beta_{lg} = -0.12 \pm 0.05$ and $\beta_{rg} = -0.18 \pm 0.02$.[3] We have previously attributed the higher susceptibility of the triesters to the fact that simultaneous changing of two alkyl groups has a greater effect on the electron density at phosphorus than changing only one group, which is the case with diesters. Low electron density, in turn, favors formation of the phosphorane intermediate by facilitating the attack of 2′-hydroxy group on phosphorus and retarding the reverse reaction. The increase in the equilibrium concentration of the phosphorane intermediate then causes an increase in the reaction rate. The breakdown of the phosphorane intermediate is assumed to be rather insensitive to the acidity of the esterified alcohol, since either the leaving group remains unchanged (isomerization) or polar effects of the leaving-group on protonation of the departing oxygen and subsequent bond cleavage cancel each other (cleavage). Accordingly, asymmetric triesters could be expected to more closely mimic the behavior of diesters. The present results show that this really is the case, although the susceptibility is still higher than with diesters. Compared to the symmetric triesters, the sensitivity of both the cleavage ($\beta_{lg}$) and isomerization ($\beta_{rg}$) to the electronegativity of the alkyl group(s) is moderately lowered: $\beta_{lg} = -0.40 \pm 0.04$ and $\beta_{rg} = -0.44 \pm 0.05$ (Fig. 2).

It is also worth noting that both alkoxy groups are cleaved to a significant extent in aqueous hydrogen chloride and in carboxylic acid buffers. This may be taken as a clear indication of the leaving-groups departing as alcohols, since under basic conditions where the leaving group is alkoxide ion, the cleavage of the alkoxy group derived from the more acidic alcohol is clearly more pronounced.

### General acid-catalyzed cleavage

We have shown previously that the cleavage reaction is in acidic carboxylic acid buffers susceptible to general acid catalysis, and assigned the reaction mechanistically as a sequential specific acid/general base catalysis: carboxylate anion assists the attack of the vicinal hydroxy group on the protonated phosphate group. This type of catalysis is significant only in most acidic carboxylic acid buffers, such as cyanoacetic acid buffers. Both the absolute $\beta_{lg}$ value of this general acid catalyzed reaction and its change on going from symmetric to asymmetric esters are, consistent with the assumed mechanism, very similar to those in the specific acid catalyzed reaction: $\beta_{lg}$ decreases from $-0.58 \pm 0.09$ to $-0.46 \pm 0.01$ on going from symmetric to asymmetric triesters. As mentioned above, both alkoxy groups are cleaved to a significant extent, indicating that the alkoxy groups depart as alcohols.

### Hydroxide ion catalyzed cleavage and isomerization

The hydroxide ion catalyzed cleavage of the symmetric phosphotriesters (**1a**–**d**) has been shown to be very susceptible to the basicity of the departing alkoxide ion, the $\beta_{lg}$ value being $-1.26 \pm 0.07$. A $\beta_{lg}$ value of this magnitude may well be accounted for by departure of alkoxide ion *via* a late transition state where the alkoxide ion character is already well developed.

Rather unexpectedly, the $\beta_{rg}$ value for the isomerization has also been observed to be quite negative, $-1.10 \pm 0.16$. Accordingly, one might speculate that the highly negative $\beta_{lg}$ value of the cleavage reaction rather reflects high sensitivity of the formation than high sensitivity of the breakdown of the phosphorane intermediate to the acidity of the esterified alcohol. On using symmetric triesters as model compounds, both the departing and remaining alkoxy groups are simultaneously changed upon breakdown of the phosphorane intermediate, and this may further complicate the interpretation of the $\beta$ values. Elimination of the remnant group effect from the hydroxide ion catalyzed cleavage was actually the most important motive for the present study of asymmetric phosphotriesters.

In striking contrast to symmetric phosphotriesters, the ratio $k_{is}^{OH}/k_{cl}^{OH}$ is with the asymmetric triesters rather susceptible to the basicity of the departing alkoxide ion. As seen from Fig. 3, the $\beta_{rg}$ value for the isomerization is $-0.57 \pm 0.06$ and the $\beta_{lg}$ value for the cleavage $-1.38 \pm 0.18$. Accordingly, it appears clear that although the increasing electronegativity of the departing alkoxy function considerably facilitates the formation of the phosphorane intermediate, the main reason for the highly negative $\beta_{lg}$ value of the cleavage reaction is the high susceptibility of the breakdown of the phosphorane intermediate to the basicity of the leaving group. The transition state (**16** in Scheme 4) is late, and the alkoxide ion character of the leaving group is well developed, as we have previously suggested.

The results obtained with the asymmetric triesters suggest that the $\beta_{lg}$ value for the breakdown of the phosphorane intermediate to the cleavage products is approximately $-0.8$ [$= \beta_{lg}$(cleavage) $- \beta_{rg}$(isomerization)]. In all likelihood this must also be the case with the symmetric triesters, for which the values of $\beta_{lg}$(cleavage) $= -1.26$ and $\beta_{rg}$(isomerization) $= -1.10$ have previously been reported.[7] The fact that the $\beta_{lg}$(cleavage) in this case is only slightly more negative than $\beta_{rg}$(isomerization) must result from the fact that the $\beta_{lg}$ for the cleavage of the symmetric triesters contains a contribution of the remaining alkoxy group which partially cancels the effect of the leaving group. When the alkoxide ion starts to depart, the phosphorus atom becomes electron deficient. The more electronegative the remaining alkoxy function is, the more difficult the development of this electron deficiency on phosphorus is. Accordingly, the electronic effect of the remaining group is opposite to that of the leaving group. Although the observed $\beta_{lg}$ value of the symmetric triesters ($-1.26$) is rather negative, it in all likelihood would be even more negative without this cancellation, possibly of the order of $-1.8$.

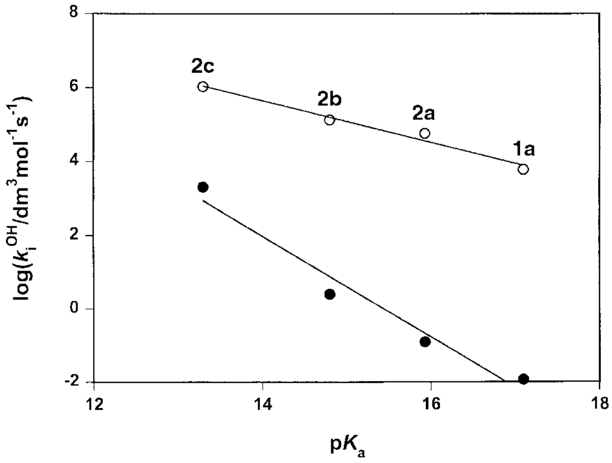
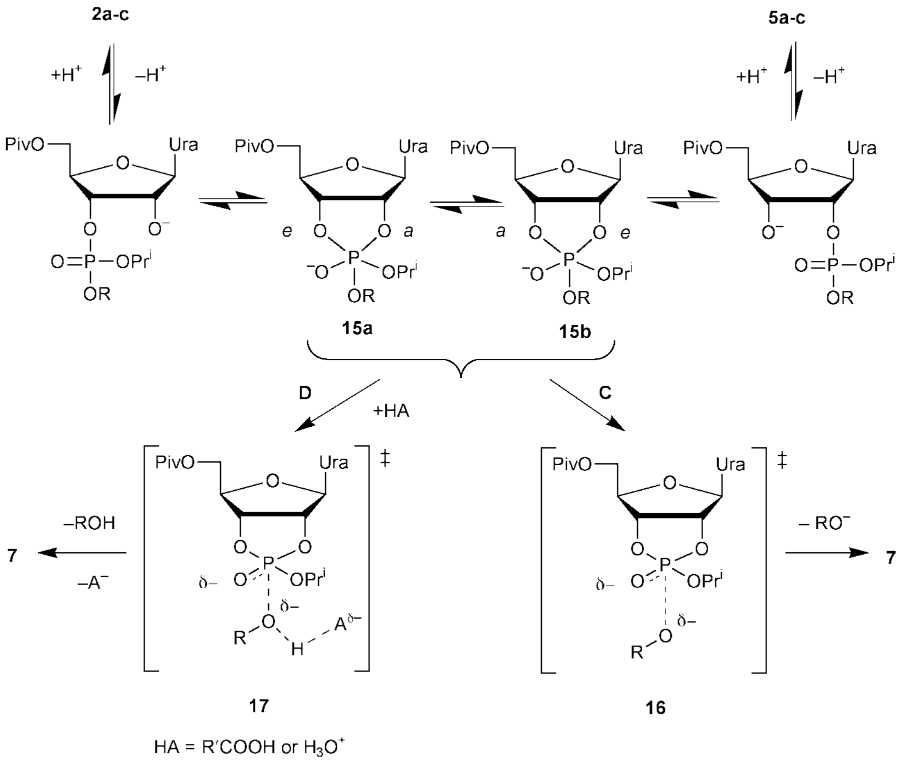


**Fig. 3** Logarithmic second-order rate constants for the hydroxide ion catalyzed cleavage of **1a**, **2a–c** to **10–12** ($k_4$) and mutual isomerization of 3′- and 2′-phosphotriesters ($k_{is}$) at 298.2 K plotted against the p$K_a$ value of the esterified alcohol.[10] The $k_4$ values for **2a–c** were obtained by multiplying the obtained $k_{cl}$ values with the appropriate end product ratio [**10** + **11**]/[**8** + **9** + **10** + **11**]. In the case of **1a** a statistical correction was made. The slopes are: $\beta_{lg} = -1.38 \pm 0.18$, $\beta_{rg} = -0.57 \pm 0.06$. The values for **1a** and **2b** are taken from ref. 7.

As mentioned above, the $\beta_{rg}$ value of the isomerization reaction decreases to one half on going from symmetric to asymmetric esters. In asymmetric esters only one of the two alkyl groups is changed, and hence the effect on rate is also decreased to one half. However, the rate of formation and the stability of the intermediate may not be the only factors affecting the isomerization rate. Unlike with symmetric triesters, the pseudorotation step from **15a** to **15b** (Scheme 4) is not a symmetric process. According to the rules of Westheimer,[8] the



**Scheme 4**

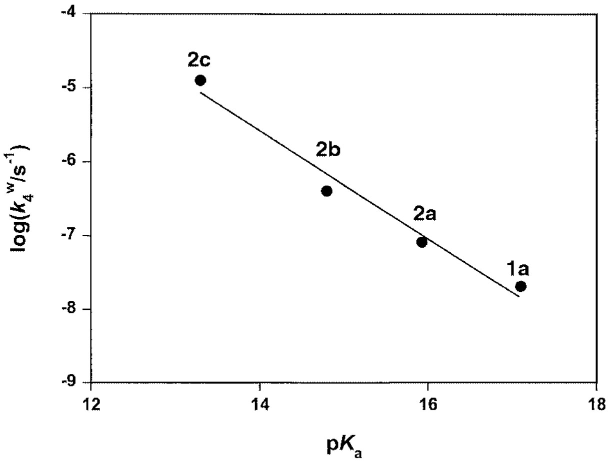


**Fig. 4** Logarithmic first-order rate constants for the pH- and buffer-independent cleavage of **1a**, **2a–c** to **10–12** ($k_4$) at 298.2 K plotted against the p$K_a$ value of the esterified alcohol.[10] The $k_4$ values for **2a–c** were obtained by multiplying the obtained $k_{cl}$ values with the appropriate end product ratio [**10** + **11**]/[**8** + **9** + **10** + **11**]. In the case of **1a** a statistical correction was made. The slope is: $\beta_{lg} = -0.73 \pm 0.08$. The values for **1a** and **2b** are taken from ref. 7.

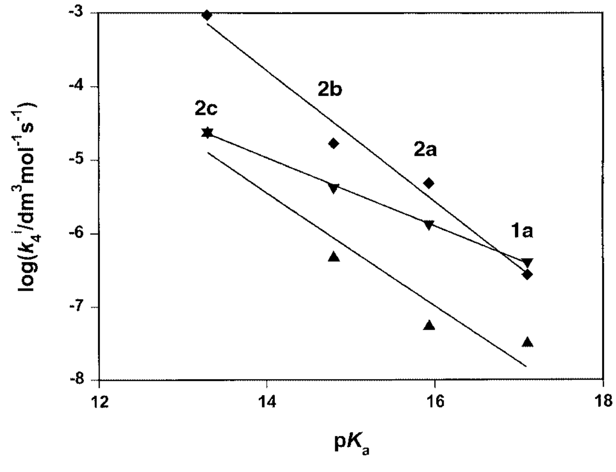


**Fig. 6** Logarithmic second-order rate constants for cyanoacetic acid (▼), cyanoacetate ion (▲) and acetate ion (◆) catalyzed cleavage of **1a**, **2a–c** to **10–12** at 298.2 K plotted against the p$K_a$ value of the esterified alcohol.[10] The $k_4$ values for **2a–c** were obtained by multiplying the obtained $k_{cl}$ values with the appropriate end product ratio [**10** + **11**]/[**8** + **9** + **10** + **11**]. In the case of **1a** a statistical correction was made. The slopes are: $\beta_{lg}^{CNAcOH} = -0.46 \pm 0.01$, $\beta_{lg}^{CNAcO^-} = -0.77 \pm 0.15$ and $\beta_{lg}^{AcO^-} = -0.89 \pm 0.09$. The values for **1a** and **2b** are taken from ref. 7 ($k_{cl}^{AcO^-}$ of **1a** is recalculated from experimental data of the reference).

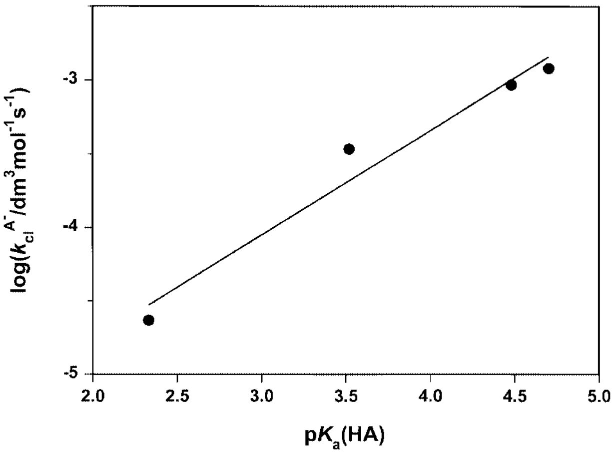


**Fig. 5** Brønsted plot for general base catalyzed cleavage of **2c** to **10–12** at 298.2 K. The slope is $\beta = 0.71 \pm 0.10$. The buffer acids employed are in order of increasing p$K_a$:[10] cyanoacetic, formic, acetic and propionic acid.

more electronegative alkoxy group tends to adopt the apical position upon the formation of the intermediate. Since pseudorotation poses the same alkyl group in the equatorial position, the pseudorotation barrier might be increased with the increasing electronegativity difference of the alkyl groups. Since the pseudorotation may at least partially limit the rate of isomerization, the rate-enhancing effect of an electronegative alkyl group may be partially cancelled.

### pH-Independent and carboxylate ion catalyzed cleavage

In the pH range from 3 to 6, where the isomerization is much faster than the cleavage, the cleavage is pH-independent and susceptible to general base catalysis by carboxylate anions. The reaction has been interpreted to be sequential specific base/general acid catalysis, where the 2′-hydroxy function is deprotonated in a pre-equilibrium step and the general acid assists the breakdown of the monoanionic phosphorane intermediate by protonating the leaving group (Path **D** in Scheme 4). Both hydronium ion and carboxylic acids may serve as proton donors (Scheme 4), and the reactions are experimentally seen as pH-independent general base catalyzed and pH-independent buffer-independent reactions, respectively. The Brønsted $\beta$ value indicating the advancement of proton transfer in the transition state is equal with symmetric and asymmetric triesters, 0.71–0.74 (Fig. 5, refs. 6 and 7). The $\beta_{lg}$ values are somewhat less negative than with the symmetric triesters: $\beta_{lg} = -0.73 \pm 0.08$ for the buffer-independent reaction (Fig. 4), $-0.77 \pm 0.15$ for the cyanoacetate ion catalysis, and $-0.89 \pm 0.09$ for the acetate ion catalysis (Fig. 6) (with the symmetric triesters $-0.94 \pm 0.13$,[7] $-1.00 \pm 0.15$[7] and $-1.29 \pm 0.21$,[9] respectively). The difference of this magnitude between the $\beta_{lg}$ values referring to asymmetric and symmetric triesters may well be accounted for by the difference that the variation of one or two alkoxy groups has on the formation of the phosphorane intermediate. As indicated above, the susceptibilities of the hydroxide ion catalyzed isomerizations of asymmetric and symmetric phosphotriesters differ even more markedly.

## Experimental

### 5′-*O*-Pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridin-3′-yl ethyl isopropyl phosphate (4a)

250 mg (0.39 mmol) 5′-*O*-pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridin-3′-yl 2-chlorophenyl isopropyl phosphate (**4d**)[7] and ethanolic sodium ethoxide (1.55 mmol in 8.2 cm$^3$ of ethanol) were dissolved in 1,4-dioxane (30 cm$^3$). The mixture was stirred for 5 min in room temperature, neutralized with acetic acid and evaporated to dryness. The residue was dissolved in dichloromethane (30 cm$^3$), washed with aqueous phosphate buffer (30 cm$^3$, $KH_2PO_4$–$Na_2HPO_4$ 0.1 : 0.1 mol dm$^{-3}$) and dried with sodium sulfate. The product was first purified by silica gel chromatography (hexane–acetone 1 : 1 v/v) and then by RP-chromatography (LiChrospher 100 RP-18, 250 × 10 mm, 5 µm, 50% aq methanol). A mixture of the $R_P$ and $S_P$ diastereomers of **4a** was obtained in 14% yield. $\delta_H$ (400 MHz, $CDCl_3$) 8.13 (1H, s, H3), 7.40 (1H, d, H6, $J_{H6,H5}$ 8.05), 6.07 (1H, d, H1′, $J_{H1',H2'}$ 5.86), 5.73 (1H, dd, H5), 4.86 (1H, m, H3′), 4.79 (1H, m, Thp-2), 4.70 and 4.64 (1H, m, Pr$^i$-CH, $J_{CH,CH3}$ 7.56), 4.44 (2H, m, H2′ and H4′), 4.34 (1H, td, $J_{H5',H5''}$ 13.5, $J_{H4',H5'}$ 4.3), 4.28 (1H, dd, H5″, $J_{H4',H5''}$ 2.3), 4.15 and 4.11 (2H, 2 × p, Et-C$H_2$, $J_{CH2,CH3}$ 6.84), 3.63 (1H, m, Thp-6a), 3.46 (1H, m, Thp-6b), 1.3–1.8 (6H, m, Thp), 1.35 (9H, m, Pr$^i$-$CH_3$, Et-$CH_3$), 1.24 (9H, s, Piv); $\delta_P$ (202.4 MHz, $CDCl_3$) −1.10 from $H_3PO_4$. MS/FAB 601 (M + K), 585 (M + Na), 563 (M + H), 479 (100%, M − Thp + 2H). Elemental analysis: C 50.7, H 7.0, N 5.0. $C_{24}H_{39}N_2O_{11}P$ requires: C 51.2, H 7.0, N 5.0%.

**Table 4** Retention times and chromatographic conditions for the separation of **2a**–**c** and its reaction products on Hypersil RP-18 columns[a]

| Compd. | Acetonitrile (%) | $t_R$(**4**)/ min | $t_R$(**2**)/ min | $t_R$(**5**)/ min |
|---|---|---|---|---|
| **2a** | 33 | 35.4 | 10.3 | 8.2[b]<br>8.4[b] |
| **2b**[7] | 35 | 21.0 | 6.8[b]<br>7.1[b] | 6.2 |
| **2c** | 38 | 22.1 | 7.5[b]<br>8.0[b] | 7.1 |
|  |  | $t_R$/min |  |  |
| **8a**, **9a** | 14 | 15.9, 18.9 |  |  |
| **8b**, **9b** | 14 | 15.1, 15.7 |  |  |
| **10**, **11** | 14 | 22.8, 33.6 |  |  |
| **13**, **14** | 14 | 7.9, 10.1 |  |  |

[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.

### 5′-*O*-Pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridin-3′-yl 2,2-difluoroethyl isopropyl phosphate (4c)

150 mg (0.23 mmol) 5′-*O*-pivaloyl-2′-*O*-(tetrahydropyran-2-yl)uridin-3′-yl 2-chlorophenyl isopropyl phosphate (**4d**)[7] was dissolved in 1,4-dioxane (3 $cm^3$), 200 $mm^3$ (3.2 mmol) 2,2-difluoroethanol and a solution of sodium isopropoxide in propan-2-ol (0.46 mmol in 3 $cm^3$) was added. The mixture was stirred for 5.5 h at room temperature, neutralized with acetic acid and evaporated to dryness. The residue was dissolved in dichloromethane (30 $cm^3$), washed with aqueous phosphate buffer (30 $cm^3$, $KH_2PO_4$–$Na_2HPO_4$ 0.1 : 0.1 mol $dm^{-3}$) and dried with sodium sulfate. The product was purified by silica gel chromatography (dichloromethane–ethanol 19 : 1 v/v). A mixture of the $R_P$ and $S_P$ diastereomers of **4c** was obtained in 67% yield. $\delta_H$ (400 MHz, DMSO-$d_6$) 11.46 (1H, s, H3), 7.62 and 7.64 (1H, 2 × d, H6, $J_{H6,H5}$ 8.05), 6.25 (1H, qt, C*H*$F_2$, $J_{H,F}$ 53.91, $J_{CH,CH2}$ 3.05), 5.92 and 5.90 (1H, 2 × d, H1′, $J_{H1',H2'}$ 7.36), 5.70 and 5.71 (1H, 2 × d, H5), 4.93 (1H, m, H3′), 4.73 (1H, m, Thp-2), 4.61 and 4.62 (1H, m, $Pr^i$-CH, $J_{CH,CH3}$ 6.6), 4.59 (1H, m, H2′), 4.32 (1H, m, H4′), 4.22 (4H, m, H5′, H5″ and C*H*$_2$CH$F_2$), 3.47 (1H, m, Thp-6a), 3.3 (1H, m, Thp 6b, overlaps with the signal of water), 1.3–1.5 (6H, m, Thp), 1.29 (6H, 2 × d, $Pr^i$-$CH_3$), 1.15 (9H, s, Piv); $\delta_P$ (202.4 MHz, DMSO-$d_6$) −2.18 and −2.35 from $H_3PO_4$ (2 × p, $J_{P,H}$ 7.45); $\delta_F$ (376 MHz, DMSO-$d_6$) −128.20 and −128.24 from $CFCl_3$ (2 × td, $J_{F,CH2}$ 15.15). MS/FAB: 1029 [2(M − Thp + H) + H], 599 (M + H), 515 (M − Thp + 2H). Elemental analysis: C 49.9, H 6.7, N 4.8. $C_{24}H_{37}F_2N_2O_{11}P$ requires: C 48.2, H 6.2, N 4.7%.

### Kinetic measurements

The kinetic measurements were carried out and the aliquots analyzed by RP HPLC (Hypersil ODS, 250 × 4, 5 μm) as described previously for the dimethyl ester **1c**.[6] The retention times and chromatographic conditions are indicated in Table 4. In the cases where the signals of the $R_P$ and $S_P$ diastereomers were separated, their area ratio remained constant during the course of the kinetic run. The rate constants were calculated as described previously.[6] In cases where both of the alkoxy groups were cleaved, the overall cleavage rate was bisected to proportions of the cleavage of each of the alkyl groups with the aid of the observed concentration ratio of the product diesters.

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