Dioxolane nucleosides and their phosphonate derivatives: synthesis and hydrolytic stability

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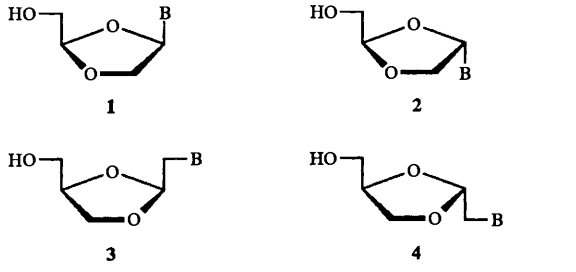
Several new nucleoside (**12–15**) and nucleoside phosphonate (**27–30**) analogues derived from (±)-*cis*- and -*trans*-2-hydroxymethyl-4-methyl-1,3-dioxolane have been prepared and their configurations assigned by ^{1}H NMR spectroscopy. First-order rate constants for the acid-catalysed hydrolysis of the dioxolane ring have been determined at different hydronium ion concentrations. The phosphonate group accelerates the hydrolysis at pH > 2 by 2 orders of magnitude, while at pH < 1 the hydrolytic stabilities of the nucleoside analogues and their phosphonate derivatives are comparable. The possible reasons for this rate-enhancement are discussed on the basis of the reaction mechanism.

Introduction

Numerous nucleoside analogues are known to possess moderate antiviral activity against human immunodeficiency virus (HIV).[1] Several structural analogues of 2',3'-dideoxyribonucleosides containing an additional heteroatom in their pentofuranosyl ring belong to this category. For example, nucleosides derived from 1,3-dioxolane (compounds **1**, **2**) have recently been shown to exhibit marked anti-HIV activity.[2–4] It is worth noting that not only are the *cis*-isomers **1**, *i.e.* the analogues of normal β-nucleosides active, but also their *trans*-counterparts **2**. We have previously[5–7] synthesized two sets of structurally modified dioxolane nucleosides, **3** and **4**, in which the nucleobase is bonded to C-2 of the dioxolane ring *via* an extra methylene group. They turned out to be inactive against HIV-1 (CEM), herpes simplex virus (HSV), human cytomegalovirus (HCMV), varicella zoster virus (VZV) and human foreskin fibroblasts (HFF) at mass concentrations up to 100 mg cm^{-3}, and were nontoxic towards CEM and HFF cells.[8] However, their triphosphates were incorporated into DNA by both DNA polymerase and reverse transcriptase.[9] Accordingly, one may speculate that the inactivity of compounds of type **3** and **4** results from the inability of cellular and viral kinases to recognize these nucleosides. Since this initial phosphorylation step may be circumvented by replacing the nucleosides with their phosphonates, we now report on the synthesis of four new dioxolane nucleosides (compounds **12–15**) and their phosphonate derivatives (**27–30**). In these compounds, the nucleobase is attached *via* a methylene group to C-4 of the dioxolane ring. In other words, they are structurally more closely related than are compounds of type **3** and **4** to those dioxolane nucleosides (**1**, **2**) that are known to be biologically active. Since hydrolytic stability plays some role in assessing the applicability of an antiviral agent, kinetic data for the acid-catalysed hydrolysis of these compounds are also given. In particular, the relatively low hydrolytic stability of the phosphonates is discussed.

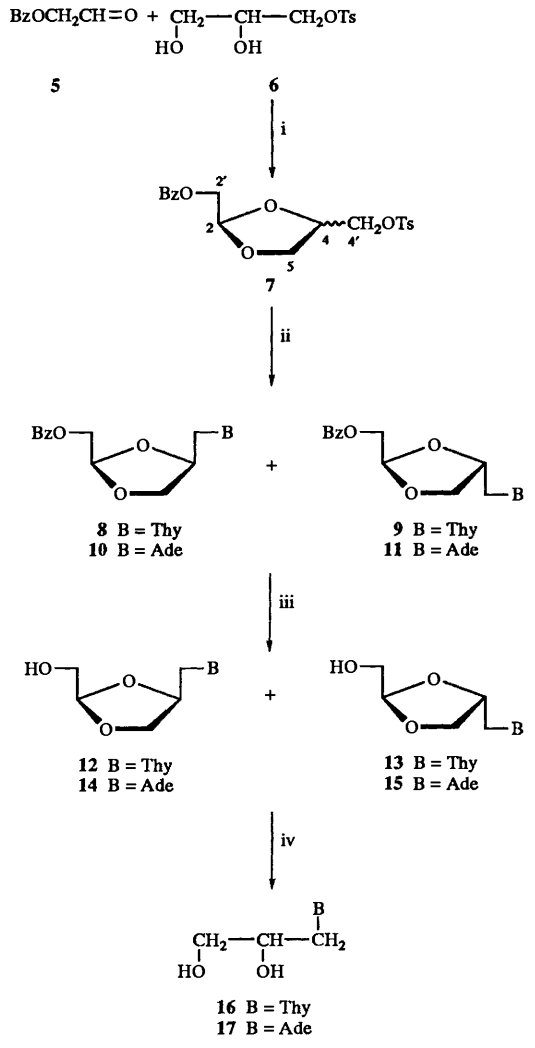


Results and discussion

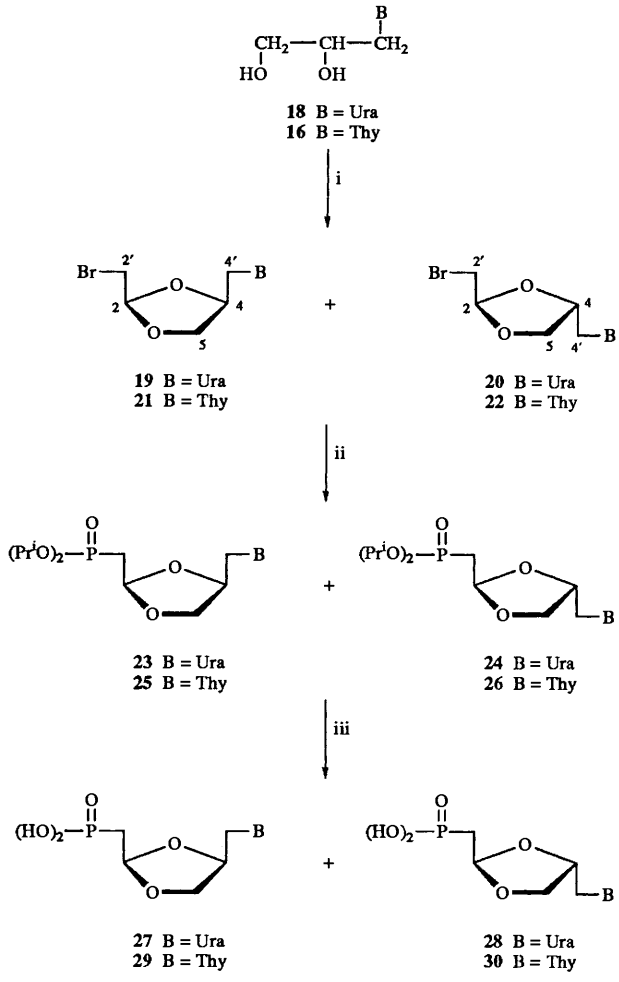
Synthetic procedure

The dioxolane nucleoside analogues **12–15** were prepared by the route depicted in Scheme 1. Accordingly, 1-*O*-benzoylglycerol[10] was initially oxidized to benzoyloxyacetaldehyde **5** with sodium periodate. Acid-catalysed acetalization of aldehyde **5** with 1-*O*-tosylglycerol[11] **6** then gave a mixture of (±)-*cis*- and (±)-*trans*-2-benzoyloxymethyl-4-tosyloxymethyl-1,3-dioxolane **7** in 57% yield. According to ^{1}H NMR spectroscopy, both isomers were produced in approximately equal amounts. Consistent with previous observations,[12,13] alkylation of the sodium salt of thymine with tosyl ester **7** gave a mixture of N^1,N^3-bis- and N^1-alkylated products, while the sodium salt of adenine was alkylated at N^3 and N^7. Separation of the regioisomers on silica gel yielded diastereoisomeric mixtures **8**/**9** (16% yield), and **10**/**11** (41% yield). With thymine derivatives the separation of (±)-*cis*- and (±)-*trans*-isomer (**8** and **9**) was achieved by reversed-phase chromatography on a Bondesil C18 column with a mixture of water and acetonitrile (4:1, v/v) as eluent. Owing to incomplete separation, the yields of compounds **8** and **9** were low. The diastereoisomeric adenine derivatives (**10** and **11**) were separated by crystallization of the (±)-*cis*-isomer **10** from the mixture of stereoisomers **10** and **11** in ethanol and then the (±)-*trans*-isomer from the residual mixture in ethyl acetate. Debenzoylation in methanolic ammonia finally gave the deprotected nucleosides **12–15** as racemic mixtures.

Scheme 2 shows the strategy applied to obtain the nucleoside phosphonate analogues **27–30**. Acid-catalysed transacetalization of 1-(2,3-dihydroxy)uracil[11] **18** and 1-(2,3-dihydroxy)-thymine[14] **16** with the diethyl acetal of bromoacetaldehyde in acetonitrile gave mixtures of the (±)-*cis*- and (±)-*trans*-isomer (**19**/**20** and **21**/**22**). The uracil derivatives **19** (32%) and **20** (27%) were separated by silica gel chromatography. Attempts to separate the thymine derivatives, **21** and **22**, by the same method failed. According to ^{1}H NMR spectroscopy the ratio of the *cis*- to *trans*-isomer in the mixture of the thymine derivatives was 2:1. Diastereoisomers **19** and **20**, and the mixture of thymines **21** and **22**, were converted into phosphonates by an Arbuzov reaction, *i.e.* by prolonged heating with triisopropyl



Scheme 1 *Reagents and conditions:* i, H^+-resin, abs. $CHCl_3$, reflux; ii, thymine or adenine, NaH, DMF, 110 °C, 10 h; iii, aq. NH_3–MeOH; iv, H^+. *Note:* The syntheses are racemic throughout. Numbering scheme applies to both *cis* and *trans* isomers **7**–**15**.



Scheme 2 *Reagents:* i, $BrCH_2CH(OEt)_2$, PTSA, MeCN; ii, $(Pr^iO)_3P$; iii, Me_3SiBr, MeCN. *Note:* The syntheses are racemic throughout. Numbering scheme applies to both *cis* and *trans* isomers **19**–**30**.

phosphite, followed by removal of the isopropyl groups with trimethylsilyl bromide[15] in acetonitrile. The products were purified by ion-exchange chromatography. The ^1H NMR spectra of the products obtained from uracils **19** and **20** revealed that the deblocking was accompanied by isomerization to a 1 : 1 mixture. Of the stereoisomeric mixtures, only the mixture of thymine phosphonates **29** and **30** (both as racemic mixtures) was successfully separated by preparative reversed-phase chromatography.

Structure elucidation by ^1H NMR spectroscopy

The structure of the compounds prepared was verified by ^1H NMR spectroscopy, as described previously[5–7] in detail for compounds **3** and **4**. Tables 1 and 2 summarize the data obtained. The assignment of *cis*/*trans* configuration was additionally verified by NOE spectroscopy.[6] Irradiation at the frequency of 2-H of compounds **8**–**11** resulted in a rather marked nuclear Overhauser effect (NOE) on the neighbouring methylene protons (total effect > 4%) and on the *cis*-oriented 5-H^b (1.5–2.1%), while practically no NOE on the *trans*-located 5-H^a could be observed. The *trans*-configuration of compounds **9** and **11** was confirmed by the absence of an NOE on 4-H. With the *cis*-derivatives, **8** and **10**, this proton exhibited an NOE of 1.7–2.3%. The structure of compound **12** was also confirmed by X-ray diffraction analysis.[16]

The structure of (±)-*cis*- and (±)-*trans*-2-bromomethyl-4-[(uracil-1-yl)methyl]-1,3-dioxolanes **19** and **20** was also confirmed by ^1H NMR spectroscopy. The configurational assignment is based on the chemical shift of the 2-H triplet. It is known that with 2,4-substituted-1,3-dioxolanes 2-H of the *cis*-isomer resonates at a higher field than that of a *trans*-isomer.[17] Introduction of a phosphonate residue in dioxolane nucleosides decreased the chemical shifts of the neighbouring methylene protons by more than 1 ppm, and resulted in appearance of $J_{2',P}$ = 18.5 Hz. In other words, the triplet of 2-H of uracil derivatives **19** and **20** was converted into a doublet of triplets with the (±)-*cis*-isomer **23** ($J_{2,P}$ = 7.8 Hz), and to quartet ($J_{2,2'a} = J_{2,2'b} = J_{2,P}$ = 4.5 Hz) with the (±)-*trans*-isomer **24**.

Acid-catalysed hydrolysis

Previous investigations on the acid-catalysed hydrolysis of 1,3-dioxolanes[18,19] and their nucleoside analogues **3** and **4** (B = Ade, Ura)[5] suggest that the hydrolysis of compounds **12**–**15** follows the mechanism depicted in Scheme 3. Pre-equilibrium protonation of one of the ring oxygens is followed by a unimolecular rate-determining opening of the dioxolane ring with concomitant formation of an acyclic oxocarbenium ion. The subsequent steps, *viz.* formation and hydrolysis of the hemiacetal, are fast. Consistent with this mechanism: (i) only one UV-absorbing product was observed when the hydrolysis of compounds **12**–**15** in hydrochloric acid was followed by HPLC; (ii) this product was chromatographically identical with an authentic sample of the corresponding 2,3-

Table 1 ^{1}H NMR chemical shifts for the dioxolane nucleosides **12–15** and their protected precursors **8–11**[a]

Compd.	Base moiety		NH	Dioxolane moiety							
				2-H	2'-H^a	2'-H^b	4-H	4'-H^a	4'-H^b	5-H^a	5-H^b
8[b]	7.03	1.72	8.45	5.21	4.43	4.38	4.36	4.06	3.52	3.78	4.02[c]
9[b]	7.05	1.85	8.52	5.35	4.32	4.28	4.42	3.96	3.67	4.17	3.66[c]
10[b]	8.19	7.85		5.16	4.36	4.30	4.42	4.37	4.15	3.80	4.00[c]
11[b]	8.21	7.88		5.29	4.25	4.24	4.51	4.35	4.23	4.17	3.64[c]
12[d]	7.40	1.74		4.89	3.56	3.52	4.37	3.88	3.73	3.77	3.94
13[d]	7.37	1.75		5.05	3.52	3.48	4.40	3.84	3.77	4.08	3.65
14[d]	8.01	7.94		4.83	3.44	3.38	4.42	4.26	4.15	3.82	3.92
15[d]	8.52	8.50		5.44	3.96	3.96	4.99	4.78	4.72	4.62	4.16

[a] Given as δ_H from $SiMe_4$ at 300 K. 5-H^a *cis* to the 2-CH_2OH; 5-H^b *trans* to the 2-CH_2OH. Designations 2'- and 4'-H^{a,b} are arbitrary. [b] In $CDCl_3$. [c] Benzoyl group: δ 8.03 (d), 7.59 (t), 7.46 (t). [d] In ^2H_2O.

Table 2 Vicinal ^{1}H,^{1}H coupling constants for the dioxolane nucleosides **12–15** and their protected precursors **8–11**[a]

Compd.	$J_{2,2'a}$	$J_{2,2'b}$	$J_{2'a,2'b}$	$J_{4,4'a}$	$J_{4,4'b}$	$J_{4'a,4'b}$	$J_{4,5a}$	$J_{4,5b}$	$J_{5a,5b}$
8[b]	3.5	3.6	−12.0	2.7	7.4	−14.3	5.6	6.8	−8.7
9[b]	3.9	3.9	−11.9	3.0	6.9	−14.5	6.3	6.8	−8.7
10[b]	3.6	3.6	−12.0	3.2	6.9	−14.5	5.2	6.7	−8.8
11[b]	3.5	4.2	−12.0	3.3	6.4	−14.7	6.1	6.3	−8.8
12[c]	3.0	3.0	−12.7	3.4	7.5	−14.6	4.7	6.8	−8.9
13[c]	3.1	3.1	−12.6	3.7	7.6	−14.6	6.4	5.7	−8.8
14[c]	2.8	2.8	−12.8	3.3	7.4	−14.8	4.2	6.8	−9.0
15[c]	3.3	3.3		3.7	6.6	−14.7	6.6	5.8	−8.8

[a] Given in Hz. For the designation of protons as a and b, see footnote a of Table 1. [b] In $CDCl_3$. [c] In ^2H_2O.

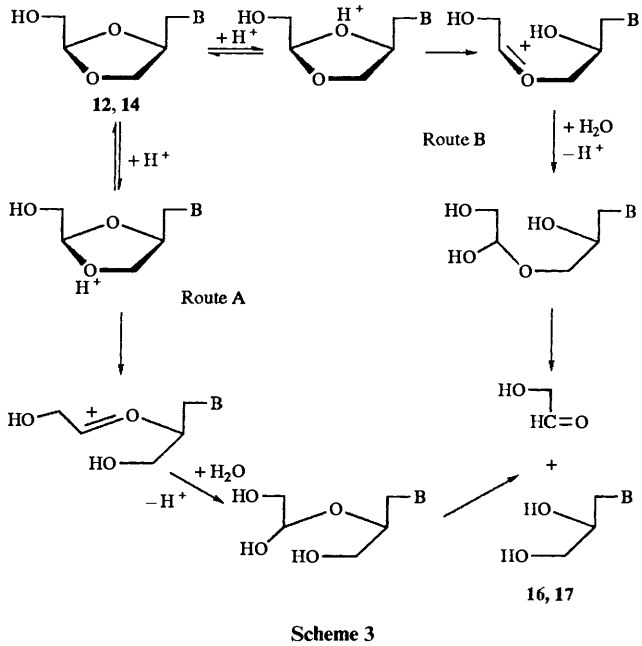
dihydroxypropyl derivative (**16**, **17**); and (iii) the entropies of activation (Table 3) were only slightly negative, as expected for a reaction having a unimolecular rate-limiting stage.[20] As reported previously,[21] reactions proceeding by unimolecular rate-limiting opening of a five-membered ring tend to exhibit slightly more negative $\Delta S^{\ddagger}$-values than do their acyclic counterparts. With both types of dioxolane nucleoside (**3**/**4**[5] and **12**/**13** or **14**/**15**) the *cis*-isomer is hydrolysed twice as readily as is the *trans*-isomer.

Dioxolanes **12**/**13** and **14**/**15** are hydrolysed considerably faster than are the corresponding derivatives of their regioisomers **3** and **4**. With adenine derivatives, for example, the reactivity difference is 60-fold. This is expected on the basis of the mechanism described. The nucleobases as electronegative substituents retard the hydrolysis of the dioxolane nucleosides compared with unsubstituted dioxolane; they decrease the basicity of the ring oxygens and destabilize the oxocarbenium ion intermediate. The distance between the nucleobase and the oxocarbenium ion is larger with compounds **12–15** than with regioisomers **3** and **4**, and hence the rate retardation is smaller. A similar reasoning, bearing in mind that the effect on oxocarbenium ion is more important than the effect on protonation,[22] suggests that the hydrolysis of compounds **12–15** favours route B (Scheme 3). It is also worth noting that the hydrolysis of compounds **12**/**14** and **13**/**15** is less susceptible to the structure of the nucleobase than is the hydrolysis of regioisomers **3** and **4**. With compounds **3** and **4** the uracil derivatives were observed to be hydrolysed one order of magnitude faster than were the adenine derivatives. This reactivity difference was attributed to the fact that protonation of the adenine moiety at pH < 4 strongly increases its electron-withdrawing effect, while the uracil moiety remains unprotonated. With compounds **12**/**14** and **13**/**15** the adenine derivatives are hydrolysed almost as readily as are the thymine derivatives. The protonation state obviously does not play such a decisive role any more, since the nucleobase is not bonded directly to the oxocarbenium ion centre.

The phosphonate derivatives **29** and **30** of the thymine dioxolane nucleosides were hydrolysed in 1 mol dm^{-3} hydro-

Table 3 First-order rate constants and the enthalpies and entropies of activation for the acid-catalysed hydrolysis of dioxolane nucleoside analogues **12–15** and their phosphonate derivatives **29**, **30** in 1.00 mol dm^{-3} hydrochloric acid at 333.2 K

Compd.	$k/10^{-4}$ s^{-1}	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}
12	5.19 ± 0.04	88 ± 2	−43 ± 2
14	2.39 ± 0.08	102 ± 3	−9 ± 7
13	2.42 ± 0.11	96 ± 4	−26 ± 9
15	1.21 ± 0.12	104 ± 7	−8 ± 19
29	14.5 ± 1.8	88 ± 9	−37 ± 26
30	7.90 ± 0.20	93 ± 2	−25 ± 5



Scheme 3

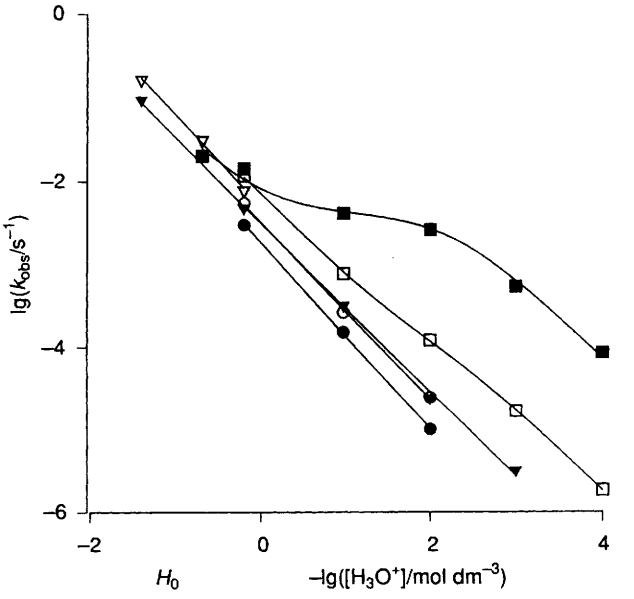
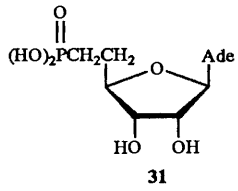


Fig. 1 pH-rate profiles for the hydrolysis of dioxolane nucleoside analogues and their phosphonate derivatives at 363.2 K. The ionic strength of the solutions was adjusted to 0.1 mol dm^{-3} with sodium chloride. Notation: (▼) **12**; (▽) **13**; (○) **14**; (●) **15**; (■) **30**; (□) **31**.

chloric acid about 3 times as fast as were the parent nucleosides (Table 3). As with compounds **12** and **13**, the only UV-absorbing product is 1-(2,3-dihydroxypropyl)thymine **16**. Even the enthalpies and entropies of activation of the hydrolysis of nucleosides and their phosphonate analogues were nearly equal (Table 3). However, a remarkable difference was observed in the pH-rate profiles of the hydrolysis (Fig. 1). While the rate of hydrolysis of dioxolane nucleosides was strictly proportional to the hydronium ion concentration of the solution, the profile obtained with the phosphonate analogue **30** showed a marked curvature in the acidity region from $H_0 = 0$ to pH 2. At pH 3 the phosphonate **30** was hydrolysed 100-times faster than was the corresponding nucleoside analogue. At $H_0 < 0$ and pH > 2, the rate of hydrolysis of compound **30** also seemed to be proportional to the acidity of the solution. The nonlinearity of the rate profile most probably arose from the change of the ionic form of the phosphonate moiety. Alkanephosphonic acids are 0.5 pK_a-units less acidic than their alkyl phosphate analogues.[23] The first pK_a-value of adenosine monophosphate (2'- and 3'-AMP) has been shown to be 1.3 under the experimental conditions used here (T = 363.2 K; I = 0.1 mol dm^{-3}).[24] Accordingly, the pK_a of alkanephosphonate **30** may be estimated to be ~1.8. At pH > pK_a the phosphonate exists mainly as monoanion, which makes the dioxolane ring oxygens more basic, and also stabilizes the oxocarbenium intermediate. Both of these factors enhance the rate of hydrolysis of the monoionic form of phosphonate **30**. Under very acidic conditions the phosphonate moiety remains protonated, and hence the reactivity difference between a nucleoside and its phosphonate derivative is small.

For comparison, we also recorded the pH-rate profile for depurination of a known[25] phosphonate analogue of 5'-AMP (compound **31**). The results (Fig. 1) are similar to those reported earlier[26] for 5'-AMP: the rate profile shows a slight curvature between pH 1 and 2, although the rate profile for depurination of adenosine is strictly linear. Accordingly, the effects of the 5'-phosphate and 5'-phosphonate groups on the hydrolysis rate of adenosine are similar, and much smaller than that observed with compound **30**. Hydrolysis of purine nucleosides and nucleotides involves rate-limiting formation of an oxocarbenium ion with positive charge localized at C(1')–O(4').[26–28] Owing to the long distance to the reaction centre, the inductive effect of the 5'-phosphate or -phosphonate group of a nucleoside is much less effectively reflected in the hydrolysis rate than is that of a 2-phosphonate group on hydrolysis of the 1,3-dioxolane **30**.



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The rate profiles of compounds **30** and **31** shown in Fig. 1 are those calculated by least-squares fitting to equation (1),

$$k_{obs} = (k_1[H^+] + k_2K_a)/(K_a + [H^+]) \quad (1)$$

where k_1 and k_2 are rate constants for the hydrolysis of the neutral and monoanionic phosphonate, respectively, and K_a is the first acidity constant of the phosphonate group. The values obtained for the rate constants are $k_1 = (4.1 \pm 1.1) \times 10^{-3}$; $k_2 = (0.75 \pm 0.14)$ with compound **30**, and $k_1 = (7.1 \pm 0.4) \times 10^{-3}$; $k_2 = (18 \pm 1) \times 10^{-3}$ dm^3 mol^{-1} s^{-1} with compound **31**. The pK_a-values obtained from the same fitting are nearly equal for both compounds: 2.2 and 2.1 (±0.2) with compounds **30** and **31**, respectively.

Experimental

General

The ^{1}H NMR spectra were recorded on a Bruker AMX 400 spectrometer, and the ^{13}C distortionless enhancement by polarization transfer (DEPT) and ^{31}P NMR spectra on a JEOL JNM-A500 spectrometer (^{13}C: 126 MHz, ^{31}P: 202 MHz) at 300 K. A 135° pulse for protons was employed to separate the CH/CH_3 and CH_2 signals in ^{13}C DEPT spectra. The ^{13}C chemical shifts were referenced to the solvent [$(CD_3)_2SO$, δ_C 39.50] and the ^{31}P shifts to external orthophosphoric acid. J-Values are in Hz. The mass spectra (EI) were recorded on a VG 7070E spectrometer with a direct inlet system. The UV spectra were recorded on a Specord UV–VIS spectrometer. TLC separations were carried out on Kieselgel 60 F_{254} (Merck) using the following developing solvents: (A) chloroform, (B) chloroform–ethanol (95:5, v/v), (C) chloroform–ethanol (90:10), (D) propan-2-ol–ammonia–water (7:1:2). Silica gel L (40–100 μm, Shemapol) and Silpearl (Shemapol) were employed for adsorption chromatography. Ion-exchange chromatography was carried out on DEAE-cellulose (Whatman). Preparative low-pressure reversed-phase chromatography was performed on Bondesil C18 (40 μm, Analitichem International). Semi-preparative reversed-phase HPLC was performed on a Merck LiChrospher RP-18 column (10 × 250 mm; 5 μm), and analytical HPLC on a Hypersil ODS column (4 × 250 mm; 5 μm). An acetic acid–sodium acetate buffer (pH 4.3; [NH_4Cl] = 0.05 mol dm^{-3}), either as such (solvent E) or with 5% (v/v) acetonitrile (solvent F), was employed as eluent. A flow rate 3 cm^3 min^{-1} was used with the semi-preparative column, and 1 cm^3 min^{-1} with the analytical one.

Benzoyloxyacetaldehyde 5

1-*O*-Benzoylglycerol[10] (14.9 g, 76 mmol) was dissolved in aq. 1,4-dioxane (1:2, v/v; 60 cm^3). Aq. sodium periodate (17.9 g, 83.6 mmol in 80 cm^3) was added and the mixture was kept at 20 °C for 3 h. The precipitate thus formed was filtered off, and washed with 1,4-dioxane (3 × 30 cm^3). The combined filtrates and washings were concentrated by evaporation to 10 cm^3, and

the additional precipitate was filtered off. The solution was evaporated to dryness, the residue was dissolved in chloroform (200 cm^3), and the solution was washed with water (3 × 20 cm^3). The organic layer was dried with Na_2SO_4, evaporated to dryness, and dried *in vacuo* over P_2O_5 to yield a syrup (11.3 g, 91%), R_f 0.83 (system C); $\delta_H(D_2O)$ 8.06–7.42 (5 H, m, Bz), 5.37 (1 H, t, *J* 5.0, CH) and 4.30 (2 H, d, CH_2).

(±)-*cis*/*trans*-2-Benzoyloxymethyl-4-toxylmethyl-1,3-dioxolanes 7

A mixture of aldehyde **5** (11.3 g, 69 mmol), 1-*O*-tosylglycerol[11] **6** (17 g, 69 mmol) and Dowex 50 ion-exchange resin (1.5 cm^3; H^+ form) in dry chloroform (100 cm^3) was heated for 2 h with a Dean–Stark adapter filled with chloroform. The resin was filtered off, and washed with chloroform. Saturated aq. sodium hydrogen carbonate (50 cm^3) was added to the combined filtrates. The organic layer was separated, washed with water (2 × 30 cm^3), dried with Na_2SO_4, and evaporated to dryness. The residue was purified on a silica gel column (100 g) eluted with chloroform. Compound **7** was obtained as a syrup (15.2 g, 57%), R_f 0.34 (solvent A); δ_H 8.04–7.19 (9 H, m, Ts, Bz ArH), 5.25 (0.5 H, t, $J_{2,2'}$ 3.5, *trans* 2-H), 5.17 (0.5 H, t, $J_{2,2'}$ 3.5, *cis* 2-H), 4.43–3.85 (7 H, m, 2 × CH_2O, 4-H and 5-H_2) and 2.41 (3 H, s, Me). Therefore, an equimolar mixture of *cis* and *trans* isomers had been obtained.

(±)-*cis* and -*trans*-2-Benzoyloxymethyl-4-[(thymin-1-yl)-methyl]-1,3-dioxolane 8 and 9

To a suspension of dry thymine (1.26 g, 10 mmol) in dry dimethylformamide (DMF) (30 cm^3) was added sodium hydride (0.5 g, 12.5 mmol; 60% in oil) and the mixture was stirred for 20 min at 100 °C. A solution of compound **7** (3.57 g, 9.1 mmol) in DMF (10 cm^3) was added, and the mixture was heated at 100 °C for 10 h. After cooling of the mixture to 20 °C and filtration, the filtrate was evaporated to dryness. The residue was dissolved in chloroform (150 cm^3), and the solution was washed with water (2 × 30 cm^3), dried with Na_2SO_4, and evaporated to dryness. The product was purified on a silica column (70 g). Elution with solvent A (chloroform) gave recovered compound **7** (2.38 g) and N^1,N^3-bis-alkylated thymine (0.5 g, 9%). Further elution with eluent B gave a mixture of compounds **8** and **9** (0.5 g, 16%), R_f 0.40 (solvent B).

The diastereoisomeric mixture of compounds **8** and **9** was separated on a Bondesil C18 column (100 g) with aq. acetonitrile (4:1) as eluent. The separation was not complete, and the mixed fractions were, after evaporation, rechromatographed in a similar manner. The products were dissolved in chloroform (50 cm^3), and the solution was dried with Na_2SO_4 and evaporated to dryness. The yield of the *cis*-isomer **8**, after crystallization from ethanol, was 150 mg (5%), mp 124.5–125 °C. Total yield of compound **9** (mp 121–123 °C) was (100 mg, 3%). The ^1H NMR data are given in Tables 1 and 2.

(±)-*cis* and -*trans*-4-[(Adenin-9-yl)methyl]-2-benzoyloxy-methyl-1,3-dioxolanes 10 and 11

Compounds **10** and **11** were prepared by alkylation of the sodium salt of adenine (1.49 g, 11 mmol) with tosyl ester **7** (3.92 g, 10 mmol), as described above for compounds **8** and **9**. Isolation on silica gel (system C) gave a mixture of title compounds **10** and **11** (1.6 g, 41%), R_f 0.60. Further elution with the same eluent gave the corresponding mixture of the N^3-isomers (0.28 g, 7%), R_f 0.42. The mixture of isomers **10** and **11** was subjected twice to crystallization from ethanol to obtain the *cis*-isomer **10** (0.42 g, 11%), mp 198–200 °C. The combined filtrates were evaporated and the residue was crystallized twice from ethyl acetate to yield the *trans*-isomer **11** (0.29 g, 7%), mp 156–158 °C. The ^1H NMR data are included in Tables 1 and 2.

(±)-*cis*-2-Hydroxymethyl-4-[(thymin-1-yl)methyl]-1,3-dioxolane 12

A solution of benzoate **8** (100 mg, 0.29 mol) in 5 mol dm^{-3} methanolic ammonia (10 cm^3) was stored for 3 days at 20 °C. The mixture was evaporated to dryness under reduced pressure, and the residue was dissolved in water (50 cm^3). The solution was extracted with chloroform (2 × 10 cm^3), and the organic layer was washed with water (10 cm^3). The combined aqueous layers were evaporated to dryness and the residue was crystallized from ethanol. The yield of title compound **12** was (50 mg, 71%), R_f 0.37 (system C); mp 160–161.5 °C. HPLC retention time (t_R) was 9.0 min (system F); λ_{max} 272 nm at pH 1–7 (ε 10 300 dm^3 mol^{-1} cm^{-1}) and 271 nm at pH 12 (ε 7840 dm^3 mol^{-1} cm^{-1}). For ^1H NMR data see Tables 1 and 2; δ_C(CH_3 and CH) 141.9, 104.2, 73.28 and 11.62; δ_C(CH_2) 66.60, 61.66 and 49.12; *m/z* 242 (M^+, 6%), 211 (100), 166 (5), 140 (8) and 122 (24).

(±)-*trans*-2-Hydroxymethyl-4-[(thymin-1-yl)methyl]-1,3-dioxolane 13

Compound **13** was prepared from benzoate **9** (50 mg, 0.145 mmol) as described above for compound **12**. The yield of product **13** was 23 mg (65%), R_f 0.37 (solvent C); mp 137–139 °C (from EtOH); t_R 7.8 min (system F); λ_{max} 272 nm at pH 1–7 (ε 9860 dm^3 mol^{-1} cm^{-1}) and 271 nm at pH 12 (ε 7560 dm^3 mol^{-1} cm^{-1}). For ^1H NMR data see Tables 1 and 2; δ_C(CH_3 and CH) 141.7, 103.4, 73.25 and 11.64; δ_C(CH_2) 66.50, 61.92 and 48.46; *m/z* 242 (M^+, 5%), 224 (6), 211 (100), 182 (3), 165 (3), 140 (9) and 122 (32).

(±)-*cis*-4-[(Adenin-9-yl)methyl]-2-hydroxymethyl-1,3-dioxolane 14

Compound **14** was obtained from benzoate **10** (300 mg, 0.85 mol) as described above for compound **12**. The yield of title compound **14** was 160 mg (75%), R_f 0.17 (system C); mp 220–221.5 °C (from EtOH); t_R 10.6 min (system F); λ_{max} 261 nm at pH 7–12 (ε 14 450 dm^3 mol^{-1} cm^{-1}) and 258 nm at pH 2 (ε 14 120 dm^3 mol^{-1} cm^{-1}). For ^1H NMR data see Tables 1 and 2; δ_C(CH_3 and CH) 152.1, 141.1, 104.3 and 73.59; δ_C(CH_2) 66.69, 61.58 and 45.23; *m/z* 251 (M^+, 22%), 220 (85), 191 (100), 176 (80), 148 (63) and 135 (87).

(±)-*trans*-4-[(Adenin-9-yl)methyl]-2-hydroxymethyl-1,3-dioxolane 15

Compound **15** was prepared from benzoate **11** (200 mg, 0.56 mol) as described above for compound **12**. The yield of title product **15** was 100 mg (71%), R_f 0.17 (system C); mp 192–193 °C (from EtOH); t_R 8.0 min (system F); λ_{max} 261 nm at pH 7–12 (ε 13 950 dm^3 mol^{-1} cm^{-1}) and 258 nm at pH 2 (ε 13 690 dm^3 mol^{-1} cm^{-1}). For ^1H NMR data see Tables 1 and 2; δ_C(CH_3 and CH) 152.2, 140.9, 103.6 and 73.51; δ_C(CH_2) 66.56, 61.90 and 44.37; *m/z* 251 (M^+, 4%), 233 (27), 220 (100), 191 (41), 176 (48), 148 (15) and 135 (15).

(±)-*cis* and -*trans*-2-Bromomethyl-4-[(uracil-1-yl)methyl]-1,3-dioxolanes 19 and 20

A mixture of 1-(2,3-dihydroxypropyl)uracil[14] **18** (0.5 g, 2.69 mmol), toluene-*p*-sulfonic acid (25 mg) and the diethyl acetal of bromoacetaldehyde (0.31 cm^3, 2.69 mmol) in acetonitrile (20 cm^3) was refluxed for more than 10 h. After evaporation, the residue was dissolved in chloroform (100 cm^3). The solution was washed successively with saturated aq. sodium hydrogen carbonate (30 cm^3) and water (2 × 30 cm^3), dried with Na_2SO_4, and evaporated to dryness. Title products **19** and **20** were separated on a Silpearl column (2 × 80 cm) eluted with chloroform.

Elution gave *cis*-isomer **19** (0.25 g, 32%), R_f 0.45 (system B); mp 164–166 °C (decomp.) (from $CHCl_3$); $\delta_H(CDCl_3)$ 7.41 (1 H, d, $J_{6,5}$ 7.9, 6-H of uracil), 5.65 (1 H, d, 5-H of uracil), 5.06 (1 H,

t, $J_{2,2'a} = J_{2,2'b}$ 3.0, 2-H), 4.42 (1 H, dddd, 4-H), 4.12 (1 H, dd, $J_{4'a,4}$ 2.7, $J_{4'a,4'b}$ −14.0, 4'-H^a), 4.05 (1 H, dd, $J_{5b,4}$ 7.0, $J_{5a,5b}$ −8.9, 5-H^b), 3.83 (1 H, dd, $J_{5a,4}$ 5.2, 5-H^a), 366 (1 H, dd, $J_{4'b,4}$ 7.9, 4'-H^b), 3.48 (1 H, dd, $J_{2'a,2'b}$ −11.6, 2'-H^a) and 3.45 (1 H, dd, 2'-H^b).

Also eluted was *trans*-isomer **20** (0.21 g, 27%), R_f 0.41 (system B); mp 143–145 °C (from EtOH); $\delta_H(CDCl_3)$ 7.30 (1 H, d, $J_{6,5}$ 7.9, 6-H of uracil), 5.74 (1 H, d, 5-H of uracil), 5.27 (1 H, t, $J_{2,2'a} = J_{2,2'b}$ = 4.0, 2-H), 4.50 (1 H, dddd, 4-H), 4.26 (1 H, dd, $J_{5a,4}$ 6.3, $J_{5a,5b}$ −8.9, 5-H^a), 4.04 (1 H, dd, $J_{4'a,4}$ 2.9, $J_{4'a,4'b}$ −14.5, 4'-H^a), 3.79 (1 H, dd, $J_{4'b,4}$ 6.7, 4'-H^b), 3.71 (1 H, dd, $J_{5b,4}$ 6.4, 5-H^b) and 3.37 (2 H, d, 2'-H_2).

(±)-*cis*/*trans*-2-Bromomethyl-4-[(thymin-1-yl)methyl]-1,3-dioxolanes 21 and 22

The mixture of products **21** and **22** was prepared analogously to the uracil analogues **19** and **20** by reaction of 1-(2,3-dihydroxypropyl)thymine[11] **16** (0.53 g, 2.64 mmol) with the diethyl acetal of bromoacetaldehyde (0.31 cm^3, 2.69 mmol). Chromatography on a silica gel column with solvent A gave a mixture of compounds **21** and **22** (0.35 g, 44%), R_f 0.50 (system B); $\delta_H(CDCl_3)$ 7.19 (0.66 H, q, $J_{6,5}$ 1.2, *cis* 6-H of thymine), 7.05 (0.33 H, q, $J_{6,5}$ 1.2, *trans* 6-H of thymine), 5.20 (0.33 H, $J_{2,2'a} = J_{2,2'b}$ = 4.0, *trans* 2-H), 5.04 (0.66 H, t, $J_{2,2'a} = J_{2,2'b}$ = 3.2, *cis* 2-H), 4.41 (1 H, m, 4-H), 4.20–3.30 (6 H, m, 2'-, 4'- and 5-H_2) and 1.86 (3 H, d, 5-Me of thymine).

(±)-*cis*-2-[(Diisopropylphosphono)methyl]-4-[(uracil-1-yl)-methyl]-1,3-dioxolane 23

A solution of compound **19** (0.1 g, 0.34 mol) in triisopropyl phosphite (3 cm^3) was heated for 7 days. The mixture was evaporated to dryness under reduced pressure, and the residue was applied to a silica column (70 g). The column was washed with chloroform, and the product was eluted with chloroform containing 2.5% (v/v) ethanol to give compound **23** (52.5 mg, 41%), R_f 0.61 (solvent C), 0.70 (solvent D); $\delta_H(CDCl_3)$ 7.47 (1 H, d, 6-H of uracil), 5.61 (1 H, d, $J_{5,6}$ 7.5, 5-H of uracil), 5.10 (1 H, dt, $J_{2,2'a} = J_{2,2'b}$ = 4.7, $J_{2,P}$ 7.8, 2-H), 4.83–4.50 (2 H, m, $J_{CH,Me}$ 7.0, $J_{CH,P}$ 5.5, Me_2CH), 4.31 (1 H, dddd, 4-H), 4.13–3.61 (4 H, m, 4' and 5-H_2), 2.16 (2 H, dd, $J_{2',P}$ 18.5, 2'-H_2) and 1.31 (12 H, d, Me_2CH).

(±)-*trans*-2-[(Diisopropylphosphono)methyl]-4-[(uracil-1-yl)-methyl]-1,3-dioxolane 24

Compound **24** was prepared, analogously to its stereoisomer **23**, from compound **20** (0.1 g, 0.34 mmol) in 56% yield (70 mg), R_f 0.56 (system C), 0.68 (system D); $\delta_H(CDCl_3)$ 7.32 (1 H, d, 6-H of uracil), 5.62 (1 H, d, $J_{5,6}$ 7.5, 5-H of uracil), 5.25 (1 H, q, $J_{2,2'a} = J_{2,2'b} = J_{2,P}$ = 4.5, 2-H), 4.80–4.47 (2 H, m, $J_{CH,Me}$ 6.0, $J_{CH,P}$ 5.0, Me_2CH), 4.37 (1 H, dddd, 4-H), 4.15 (1 H, dd, $J_{5a,4}$ 6.3, $J_{5a,5b}$ −8.5, 5-H^a), 4.09 (1 H, dd, $J_{4'a,4'b}$ −14.0, 4'-H^a), 3.59 (1 H, dd, $J_{4'b,4}$ 7.5, 4'-H^b), 3.55 (1 H, dd, $J_{5b,4}$ 6.5, 5-H^b), 2.12 (2 H, dd, $J_{2',P}$ 18.5, 2'-H_2) and 1.29 (12 H, d, Me_2CH).

(±)-*cis*/*trans*-2-[(Diisopropylphosphono)methyl]-4-[(thymin-1-yl)methyl]-1,3-dioxolanes 25 and 26

A mixture of compounds **25** and **26** was prepared as described for compound **23** from the above mixture of bromides **21** and **22** (0.15 g, 0.49 mmol) in 53% yield (100 mg), R_f 0.52 (solvent C), 0.68 (solvent D); $\delta_H(CDCl_3)$ 9.3 (1 H, NH), 7.31 (0.5 H, q, $J_{6,5}$ 1.1, *cis* 6-H of thymine), 7.13 (0.5 H, q, $J_{6,5}$ 1.1, *trans* 6-H of thymine), 5.27 (0.5 H, q, $J_{2,2'a} = J_{2,2'b} = J_{2,P}$ = 5.1, *trans* 2-H), 5.09 (0.5 H, dt, $J_{2,2'a} = J_{2,2'b}$ = 4.9, $J_{2,P}$ 7.7, *cis* 2-H), 4.65 (1 H, m, 4-H), 4.35–3.47 (6 H, m, 4'- and 5-H_2 and Me_2CH), 2.10 (2 H, m, 2'-H_2), 1.86 (3 H, d, 5-Me of thymine) and 1.25 (12 H, m, Me_2CH).

(±)-*cis*/*trans*-2-Phosphonomethyl-4-[(uracil-1-yl)methyl]-1,3-dioxolanes 27 and 28

Compound **23** (analogously **24**) (52 mg, 0.14 mmol) was dissolved in dry acetonitrile (1.5 cm^3). Trimethylsilyl bromide (0.2 cm^3, 1.5 mmol) was added under nitrogen, and the solution was stored for two days at 20 °C. The solution was evaporated to dryness, pyridine (1 cm^3) and water (2 cm^3) were added, and the mixture was incubated for 1 h at 20 °C. Water (50 cm^3) was then added and the solution was washed with ethyl acetate (2 × 10 cm^3) and evaporated to dryness under reduced pressure. The residue was dissolved in water (50 cm^3) and applied to a column of DEAE cellulose (HCO_3^- form; 150 cm^3). The column was washed successively with water (300 cm^3) and 0.05 mol dm^{-3} triethylammonium hydrogen carbonate (300 cm^3) and the product was eluted with a 0.1 mol dm^{-3} solution of the same electrolyte. Fractions containing the product were combined, evaporated to dryness and coevaporated with water (5 × 20 cm^3) to yield a mixture of title compounds **27** and **28** (54%), R_f 0.12 (solvent D); t_R 5.5 min (system E); λ_{max} 266 nm at pH 7; $\delta_H(D_2O)$ 7.65 (0.5 H, d, $J_{6,5}$ 7.9, 6-H of uracil), 7.64 (0.5 H, $J_{6,5}$ 7.9, 6-H of uracil), 5.78 (1 H, d, 5-H of uracil), 5.25 (0.5 H, m, *trans* 2-H), 5.08 (0.5 H, m, *cis* 2-H), 4.42 (1 H, m, 4-H), 4.20–3.63 (4 H, m, 4'- and 5-H_2) and 2.03–1.85 (2 H, m, 2'-H_2).

(±)-*cis* and -*trans*-2-Phosphonomethyl-4-[(thymin-1-yl)-methyl]-1,3-dioxolanes 29 and 30

A mixture of title compounds **29** and **30** (34 mg, 61%) was prepared analogously to **27**/**28** from the mixture of compounds **25** and **26** (70 mg, 0.18 mmol). The isomers were separated by reversed-phase HPLC (Merck LiChrospher RP-18; 10 × 250 mm; 5 μm). Compound **30** was eluted at 30 min and **29** at 36 min with solvent E at a flow rate of 3 cm^3 min^{-1}. Buffer salts were removed by elution of the products through the same column with water. The yield of compound **30** was 11 mg, R_f 0.14 (solvent D); t_R 13.6 min (system E); λ_{max} 266 nm at pH 7; $\delta_H(D_2O)$ 7.39 (1 H, $J_{6,5}$ 1.2, 6-H of thymine), 5.15 (1 H, dt, $J_{2,2'a} = J_{2,2'b}$ = 4.3, $J_{2,P}$ 5.8, 2-H), 4.31 (1 H, m, 4-H), 4.08 (1 H, dd, $J_{5a,4}$ 6.3, $J_{5a,5b}$ −9.3, 5-H^a), 3.85 (1 H, dd, $J_{4'a,4}$ 2.9, $J_{4'a,4'b}$ −14.7, 4'-H^a), 3.71 (1 H, dd, $J_{4'b,4}$ 8.1, 4'-H^b), 3.55 (1 H, dd, $J_{5b,4}$ 6.4, 5-H^b), 1.97–1.83 (2 H, m, 2'-H_2) and 1.72 (3 H, d, 5-Me of thymine); δ_C(CH_3 and CH) 142.1, 100.9, 72.79 and 11.57; δ_C(CH_2) 66.21, 48.26 and 34.58 (d, *J* 128); δ_P[$(CD_3)_2SO$; H_3PO_4] 15.2.

Compound **29** (~5 mg) was also obtained, R_f 0.14 (solvent D); t_R 11.6 min (system E); λ_{max} 266 nm at pH 7; $\delta_H(D_2O)$ 7.38 (1 H, q, $J_{6,5}$ 1.2, 6-H of thymine), 4.97 (1 H, dd, $J_{2,2'a} = J_{2,2'b} = J_{2,P}$ = 4.0, 2-H), 4.30 (1 H, dddd, 4-H), 3.87–3.71 (4 H, m, 4'- and 5-H_2), 1.90 (2 H, m, 2'-H_2) and 1.72 (3 H, d, 5-Me of thymine); δ_C(CH_3 and CH) 142.3, 101.9, 73.07 and 11.51; δ_C(CH_2) 66.23, 49.16 and 34.57 (d, *J* 131); δ_P[$(CD_3)_2SO$; H_3PO_4] 15.9.

Kinetic measurements

The HPLC technique utilized for kinetic runs has been described previously.[28] Chromatographic separations were carried out on a LiChroSorb RP-18 column (125 × 4 mm; 5 μm). Acetic acid–sodium acetate buffer at pH 4.2, containing 0.1 mol dm^{-3} aq. ammonium chloride and 5% (v/v) acetonitrile, was employed as eluent. The initial concentration of the dioxolane derivative studied was 2–5 × 10^{-4} mol dm^{-3} in each run. The rate constants for hydrolysis were calculated by applying the integrated first-order rate equation to the disappearance of the starting material.

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