Studies on the decomposition pathways of diastereoisomeric mixtures of aryl nucleoside α-hydroxyphosphonates under hydrolytic conditions. Synthesis of α-hydroxyphosphonate monoesters†

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Decomposition pathways of nucleoside α-hydroxyphosphonates **1** (diastereomeric mixtures) bearing different aryl groups, both in the ester and the hydroxymethine fragment, were investigated under various hydrolytic conditions. We found that in aqueous basic media, the stability and decomposition pathways of these compounds were governed by the electronic features of the aryl group in the hydroxymethine moiety (hydroxyphosphonate ⇌ H-phosphonate diester + aldehyde equilibria) and the nature of attacking nucleophiles (α-nucleophiles, *e.g.* hypoiodite or peroxide anions). The significant differences observed in the rates of hydrolysis of hydroxyphosphonates **1** *vs.* their *O*-acylated derivatives point to the importance of an intramolecular acid catalysis exerted by the adjacent hydroxyl function. Based on these findings, an efficient synthetic protocol for otherwise difficult to access hydroxyphosphonate monoesters of type **7** has been developed.

Introduction

By exploring pronucleotides[1] for AIDS therapy, we have developed aryl nucleoside α-hydroxyphosphonates of type **1** (Scheme 1) as a new type of potential drug.[2] These compounds also appear to be very interesting from a chemical point of view, since a change of aryl substituent in their phosphoester and/or hydroxymethine parts, dramatically affect their hydrolytic decomposition pathways. The decomposition studies of these compounds were carried out in cell culture media [RPMI/FBS, 9 : 1 (v/v), pH 7.4],[3] which resembles the physiological conditions that are routinely used for the *in vitro* examination of the anti-HIV potency of new compounds. Since RPMI media is a complex mixture of chemical compounds, the decomposition pathways of investigated compounds often differ significantly from those carried out in simple buffered solutions of comparable pH.[4] Due to potential importance of the title compounds in the pro-drug strategy for AIDS therapy, we undertook further studies to gain a deeper insight into their hydrolytic properties, relevant to their possible applications as anti-HIV agents. In particular, we were interested in exploring possible new decomposition pathways of these compounds, different from those observed in RPMI media, *e.g.*, a selective hydrolysis to the corresponding α-hydroxyphosphonate monoester.

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† This paper is dedicated to Prof. Dr Wojciech J. Stec, Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, Łódź, Poland, on the occasion of his 70th birthday. This article is part of a themed issue on Biophosphates.

Results and discussion

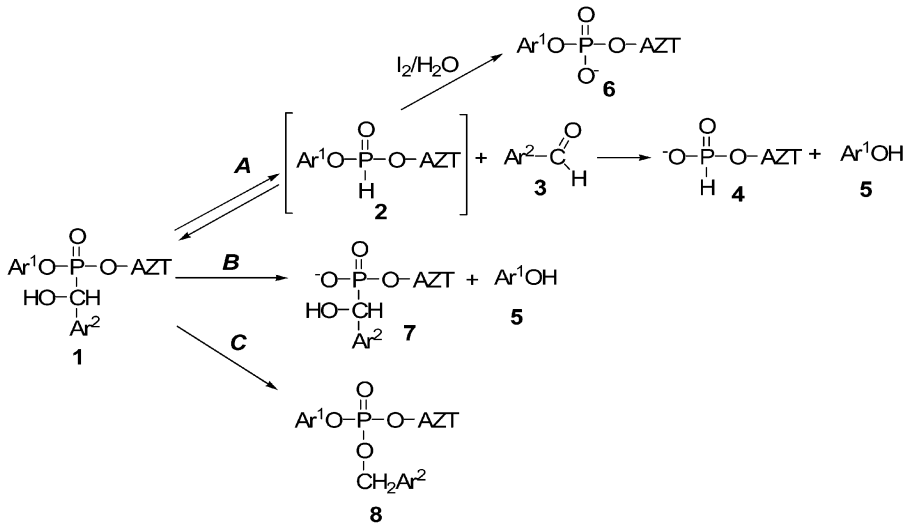
Searching for simple, well-defined hydrolytic conditions for α-hydroxyphosphonate diesters **1**,‡ we chose initially two reaction media: (i) a pyridine : water mixture (1 : 3 v/v), and (ii) acetonitrile : triethylamine : water (2 : 1 : 1 v/v/v). The choice of the systems was dictated by the solubility of the hydroxyphosphonates and their expected lability at pH > 7.

Earlier, we postulated that in solution, aryl nucleoside α-hydroxyphosphonates of type **1** exist in equilibrium with their synthetic precursors, *i.e.* their respective H-phosphonate diesters and aldehydes (of type **2** and **3**, respectively; Scheme 1),[2] and we proved this by trapping these H-phosphonate diesters *via* sulfurization with elemental sulfur. Unfortunately, since oxidation by sulfur was significantly slower than the rates of the equilibria, we could not estimate equilibrium constants for the α-hydroxyphosphonate diesters investigated. To remedy this problem, we attempted to use for the trapping experiments a rapid oxidation of H-phosphonate diesters **2** with iodine,[5,6] and for this reason we have also included in our investigation a reagent system consisting of acetonitrile : triethylamine : water (2 : 1 : 1 v/v/v) with iodine (3 equiv.) as an oxidant.

Hydrolysis of diastereoisomeric mixtures of α-hydroxyphosphonates 1 in pyridine–water and acetonitrile–triethylamine–water solvent systems

In a mixture of pyridine : water (1 : 3, v/v, pH 9.6), the investigated α-hydroxyphosphonates **1** were rather stable, and after 4 h

‡ In the ^{31}P NMR spectra of α-hydroxyphosphonates of type **1**, four signals of nearly the same intensity were observed due to the presence a mixture of 4 diastereoisomers (see also ref. 2). Since in all the experiments performed on these compounds the diastereoisomers showed the same reactivity, no attempt was made to separate these mixtures into individual compounds.



AZT = 3'-azido-3'-deoxythymidin-5'-yl

1a; Ar^1, Ar^2 = phenyl
1b; Ar^1 = phenyl; Ar^2 = 4-methylphenyl
1c; Ar^1 = phenyl; Ar^2 = 4-methoxyphenyl
1d; Ar^1 = phenyl; Ar^2 = 4-chlorophenyl
1e; Ar^1 = phenyl; Ar^2 = 4-nitrophenyl
1f; Ar^1 = 4-methoxyphenyl; Ar^2 = phenyl
1g; Ar^1 = 4-chlorophenyl, Ar^2 = phenyl

2a, **5a**, **6a**; Ar^1 = phenyl
2b, **5b**, **6b**; Ar^1 = 4-methoxyphenyl
2c, **5c**, **6c**; Ar^1 = 4-chlorophenyl
3a, **7a**; Ar^2 = phenyl
3b, **7b**; Ar^2 = 4-methylphenyl
3c, **7c**; Ar^2 = 4-methoxyphenyl
3d, **7d**; Ar^2 = 4-chlorophenyl
3e, **7e**; Ar^2 = 4-nitrophenyl
8; Ar^1 = phenyl; Ar^2 = 4-nitrophenyl

Scheme 1 Decomposition paths of AZT aryl α-hydroxyphosphonates of type **1** in various hydrolytic media.

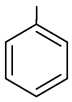
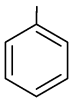
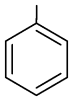
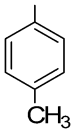
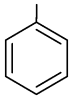
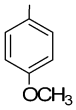
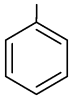
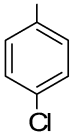
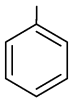
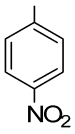
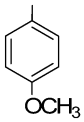
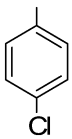
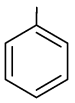
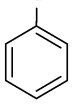
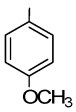
none or just a few percent of the decomposition products could be observed by ^{31}P NMR spectroscopy. These results were somewhat surprising in light of the experiments carried out in RPMI solution, in which the decomposition $t_{\frac{1}{2}}$ times of α-hydroxyphosphonates **1a–g** were between 10–30 min (Table 1). In contrast to the pyridine : water mixture, hydrolysis in the acetonitrile : triethylamine : water (2 : 1 : 1, v/v/v, pH 12.5) system occurred much faster and was conveniently monitored in ^{31}P NMR time (few minutes). Despite the differences in rates, for most of the investigated hydroxyphosphonates **1**, two decomposition pathways were observed: path **A** (the dominant one), leading ultimately to the accumulation of H-phosphonate monoester **4**, and path **B** (the minor one), affording α-hydroxyphosphonate monoesters **7** as products.

Along the series **1a–g**, the advantage of pathway **A** over pathway **B** was variable, but clearly pronounced in each case, except for hydroxyphosphonate **1e**, bearing a strong electron-withdrawing Ar^2 group (4-nitrophenyl), for which three decomposition pathways were observed, with pathway **A** being the minor one. From these data, it seemed apparent that the participation of pathway **A** in the decomposition of hydroxyphosphonates **1** paralleled the increasing electron density of Ar^2, being the highest for hydroxyphosphonate **1c**, with a 4-methoxy group as Ar^2, and the lowest for the 4-nitrophenyl derivative **1e**. Interestingly, electron-donating or electron-withdrawing substituents in the aromatic ring of Ar^1 did not affect significantly the hydrolytic decomposition pathways of hydroxyphosphonates **1**, as is apparent from a comparison of 4-methoxyphenyl (**1f**) *vs.* *p*-chlorophenyl (**1g**) derivatives. This seems to be consistent with the expected position of the equilibria of hydroxyphosphonates, which are controlled by stabilization of the carbocations formed as transient species after scission of the P–C bond[7] in **1**, and is crucial for the decomposition pathways observed for these compounds.

Because path **A** depends entirely on the equilibrium **1** ⇌ **2** + **3**, we attempted to eliminate this equilibrium by protecting the hydroxyl function of **1**, and in this way favour decomposition pathway **B**. To this end, we chose α-hydroxyphosphonate **1c**, which decomposed nearly exclusively *via* route **A** (Table 1), and protected its α-hydroxyl function by an acetyl group (Scheme 2). It was found that acetylation of the hydroxyl function of hydroxyphosphonate **1c** suppressed path **A**, and the produced acetylated hydroxyphosphonate **9** mainly underwent decomposition *via* pathway **B**. (Scheme 2). The observed formation of H-phosphonate **4** (*ca.* 30%, ^{31}P NMR) was apparently due to the incomplete stability of the acetyl group under the reaction conditions and the regeneration of the starting hydroxyphosphonate **1c** (Scheme 2).

Apart from a completely different product distribution pattern in the hydrolysis of hydroxyphosphonate **1c** *vs.* its acylated derivative **9** (pathway **A** *vs.* pathway **B**), significant differences in rates of their hydrolysis (30 min *vs.* >24 h; Table 1) were observed. These we attributed to the suppression of path **A**, leaving path type **B** as the only one acting in the decomposition of **9**. At this point, we also noticed the importance of the presence of a free hydroxyl function in an α-position to the phosphorus centre in the hydrolysis of title compounds **1** (see also later in the text for reactions in the

Table 1 The stability and decomposition pathways of diastereoisomeric mixtures of aryl nucleoside hydroxyphosphonates **1** under various hydrolytic conditions[a]

			RPMI : FBS 9 : 1 (v/v);[2] pH 7.4				$CH_3CN : H_2O : Et_3N$ 2 : 1 : 1 (v/v); pH 12.5				$CH_3CN : H_2O : Et_3N$ 2 : 1 : 1 (v/v) + I_2;[b] pH 10.4			
Cpd	Ar^1	Ar^2	$t_{\frac{1}{2}}$/min	A (%)	B (%)	C (%)	t^c/min	A (%)	B (%)	C (%)	t^c/min	A^d (%)	B (%)	C (%)
1a			27	100	0	0	30	65	35	0	<5	10	90	0
1b			19	100	0	0	45	85	15	0	<5	10	90	0
1c			13	100	0	0	30	97	3	0	<5	10	90	0
1d			13	92	8	0	10	55	45	0	<5	10	90	0
1e			9	24	47	29	5	15	25	60	<5	10	90	0
1f			26	100	0	0	40	80	20	0	10	15	85	0
1g			—	—	—	—	5	75	25	0	<5	2	98	0
9			—	—	—	—	>24 h	30[e]	70	0	>24 h	3[f]	97	0

[a] The amount of a particular compound in the reaction mixture was estimated by the ^{31}P NMR spectroscopy. [b] 3.0 molar equivalents. [c] Time for the complete disappearance of substrate **1**. [d] Estimated on the basis of the amount of aryl nucleoside phosphate **6**, a product of the oxidation of aryl nucleoside H-phosphonate **2**. [e] The presence of H-phosphonate **4** was due to the deacetylation of **9** (for details, see text). [f] Phosphodiester **6**, *vide supra*.

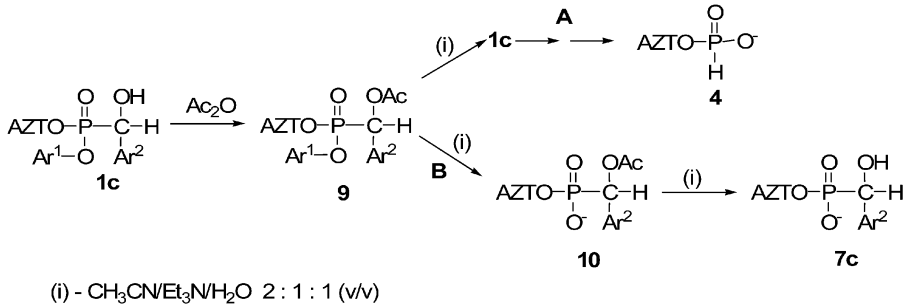
presence of iodine). Various mechanistic propositions can be put forward to explain the kinetic enhancement of path **B** in hydroxyphosphonates **1** when a free α-hydroxyl group is present, and some of them are depicted in Scheme 3.

For hydroxyphosphonates **1** in basic media, it is likely that a hydroxyl group can act as an intramolecular nucleophile towards the phosphorus center (irrespective of its configuration) and expel a leaving group OAr^1 to form, *via* an intermediate pentavalent phosphorane **II**, a cyclic oxaphosphirane of type **III**.[8] The latter species is expected to be rather reactive, and upon attack of a hydroxide ion, collapse to a hydroxyphosphonate **7**, *via* a phosphorane **IV** intermediate.[9]

A simple $S_N2(P)$ mechanism involving a nucleophilic attack of a hydroxide on the phosphorus center (Scheme 3; **1** → **V** → **7**) without participation of the α-hydroxyl function is rather unlikely, since it does not explain the observed differences in hydrolysis of hydroxyphosphonates **1** *vs.* their *O*-acylated derivatives. However, an $S_N2(P)$ process, in which a nucleophilic attack by a hydroxide is reinforced by an intramolecular acid catalysis exerted by the adjacent α-hydroxyl function (species **VI** in Scheme 3), seems a viable mechanism for the formation of phosphonate monoester **7**. Although both mechanisms in Scheme 3, *i.e.* the one proceeding *via* an oxaphosphirane **III** intermediate and that involving an intramolecular acid catalysis, are possible, the high sensitivity to hydrolysis of hydroxyphosphonates **1** to α-nucleophiles (*vide infra*) seems to lend more support to the latter.

The decomposition pathways observed during the hydrolysis of aryl nucleoside α-hydroxyphosphonate **1e** (Table 1) are also understandable in light of the above discussion.

For hydroxyphosphonate **1e**, due to the presence of a strong electron-withdrawing 4-nitro substituent in Ar^2, the



Scheme 2 Decomposition of aryl nucleoside 5′-α-acetyloxyphosphonates **9**.

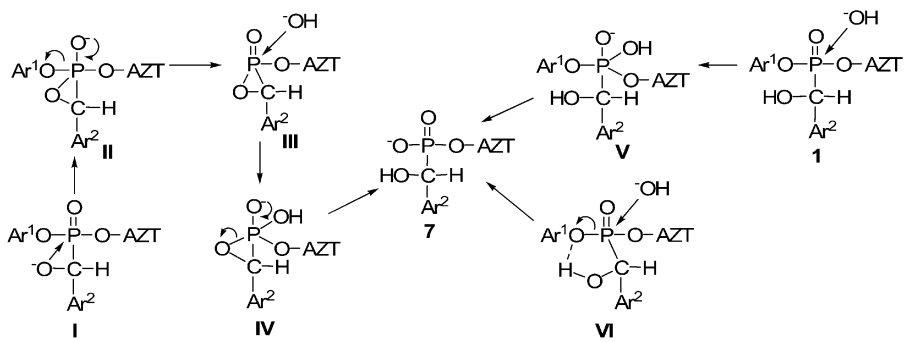
decomposition path **A** is significantly suppressed. Since the acidity of the α-hydroxyl group and the electrophilicity of the phosphorus centre in **1e** are simultaneously increased, the reaction pathway *via* a phosphirane **II** (Scheme 3 and Scheme 4) is favoured. This initially formed intermediate then undergoes decomposition in two ways: by P–C bond scission and formation of phosphotriester **8** (path **C**, phosphonate–phosphate rearrangement[7,10–12]) or with the expulsion of leaving group OAr^1, followed by hydrolysis of an oxaphosphirane intermediate **III** to form the corresponding hydroxyphosphonate **7** (path **B**). Since the former pathway proceeds apparently *via* a carbanion, whose formation should be facilitated by electron-withdrawing groups, *i.e.* 4-nitrophenyl, the decomposition of **1e** according to path **C** became the predominant one. In a separate experiment, we observed that under anhydrous reaction conditions (acetonitrile : triethylamine 2 : 1 v/v), aryl nucleoside α-hydroxyphosphonate **1e** underwent exclusively the phosphonate–phosphate rearrangement to produce phosphotriester **8**.

Hydrolysis of α-hydroxyphosphonates 1 in acetonitrile–triethylamine : water (2 : 1 : 1 v/v) containing iodine (3 molar equiv.)

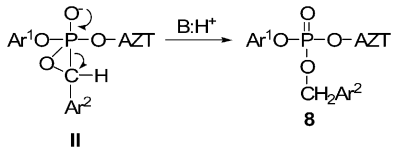
As mentioned above, we attempted to use the reagent system, acetonitrile : triethylamine : water (2 : 1 : 1 v/v) containing iodine, to kinetically quench the equilibrium **1** ⇌ **2** + **3** (Scheme 1), to obtain more quantitative data on this process. Somewhat unexpectedly, although upon treatment of hydroxyphosphonates **1** with this reagent system we observed the formation phosphodiesters **6** (due to oxidation of the corresponding *in situ*-generated H-phosphonate diesters **2**; Scheme 1), the disappearance of the starting hydroxyphosphonates **1** was significantly faster than that in the absence of iodine; in all instances, α-hydroxyphosphonates **7** were formed as the main products (85–98%, Table 1). The reactions investigated were insensitive to the electronic features of the OAr^2 groups present in hydroxyphosphonates **1**, and the amounts of phosphodiesters **6** formed correlated with the reaction times. This is consistent with kinetic competition between path **B** and path **A** (*ca.* 10% for phenyl hydroxyphosphonates **1a–e**, 15% for 4-methoxyphenyl derivative **1f** and 2% for 4-chlorophenyl derivative **1g**), indicating that the pK_a value of the leaving group OAr^1 is the main factor governing the rate of hydrolysis of hydroxyphosphonates **1** under these conditions.

As to a possible origin of the accelerating effect on hydroxyphosphonate **1** hydrolysis by using the acetonitrile–triethylamine–water–iodine reagent system, some observations are pertinent. Upon dissolving iodine in an acetonitrile : triethylamine : water (2 : 1 : 1 v/v) mixture, a rapid change in colour from a deep-violet to light-straw occurred, indicating apparently the consumption of iodine. A plausible explanation for this phenomenon could be the known disproportionation of iodine under basic aqueous conditions ($I_2 + 2HO^- \rightleftharpoons IO^- + I^- + H_2O$) to hypoiodites, which would then slowly disproportionate further to iodates and iodides ($3IO^- \rightleftharpoons IO_3^- + 2I^-$).[13] In light of this, our reagent system might contain a significant concentration of hypoiodite anions that are effective α-nucleophiles towards the phosphorus centers. Thus, a combination of efficient α-nucleophiles (hypoiodite) with an intramolecular acid catalysis provided by the adjacent hydroxyl function, could constitute a plausible mechanistic basis for the observed rapid hydrolysis of hydroxyphosphonates **1** using the acetonitrile–triethylamine–water–iodine reagent system (Scheme 5).[14]

Similarly, as discussed above, the catalytic importance of a free hydroxyl group in hydroxyphosphonate **1** hydrolysis was



Scheme 3 Possible mechanisms of nucleoside α-hydroxyphosphonate **7** formation (path **B**).



Scheme 4 A phosphonate–phosphate rearrangement of aryl nucleoside α-hydroxyphosphonates.

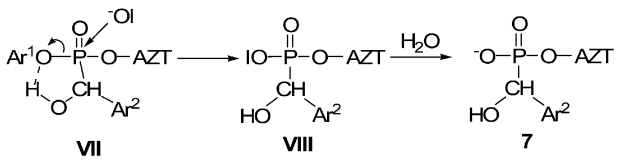
demonstrated by carrying out the hydrolysis of *O*-acetylated hydroxyphosphonate **9** using the acetonitrile–triethylamine–water–iodine reagent system. As is apparent from the data in Table 1, similar reaction times were observed for the hydrolysis of *O*-acylated hydroxyphosphonate **9** in both reaction systems (with and without iodine added), and this again (*vide supra*) emphasises the kinetic importance of a free hydroxyl function in the substitution of OAr^1 groups in hydroxyphosphonates **1**.

To substantiate the assumption about a putative involvement of powerful α-nucleophiles (hypoiodites) in hydroxyphosphonate **1** hydrolysis using the acetonitrile–triethylamine–water–iodine reagent system, we carried out experiments in which another α-nucleophile, a hydrogen peroxide ion, was used to effect the hydrolysis of hydroxyphosphonate **1a**. To this end, in the solvent system acetonitrile–triethylamine–water (2 : 1 : 1, v/v/v), the water was replaced with an equivalent volume of 30% hydrogen peroxide, and this was used instead as the reaction medium for the hydrolysis of **1a**. As expected, the hydrolysis of **1a** under these conditions was very rapid, and the first ^{31}P NMR spectrum recorded (< 5 min) showed the complete disappearance of starting material **1a**, together with the formation of the corresponding hydroxyphosphonate monoester **7a** (60%), H-phosphonate monoester **4** (20%) and an unidentified side-product (δ_P 4.35, m, no P–H bond; *ca.* 20%). We did not observe any potential oxidation products derived from the phosphorus compounds, which are known to be prone to oxidation with hydrogen peroxide (*e.g.* aryl nucleoside H-phosphonate of type **2**). Thus, hydrogen peroxide in the investigated reactions acted mainly as an α-nucleophile and not as an oxidant. The above results strongly suggest that the hydrolysis of hydroxyphosphonates **1** is significantly accelerated by α-nucleophiles, and thus lends support to a hypothesis about the involvement of hypoiodites in the previously investigated hydrolytic reactions.

Synthesis of nucleoside α-hydroxyphosphonate monoesters 7 from the corresponding aryl nucleoside α-hydroxyphosphonate diesters 1

Nucleoside α-hydroxyphosphonate monoesters **7** are often difficult to access from their synthetic precursors **1** since, due to the **1** ⇌ **2** + **3** equilibrium, other products are usually formed during the hydrolysis of hydroxyphosphonates **1** (Scheme 1). For this reason, we attempted to develop the observed highly selective hydrolysis of **1** in the acetonitrile–triethylamine–water–iodine reaction system *via* path **B** into a synthetic protocol for the preparation of hydroxyphosphonate monoesters **7**. To this end, hydrolyses of hydroxyphosphonates **1a–g** were undertaken on a preparative scale in the presence of iodine, and in all instances, high isolated yields (75–85%) of hydroxyphosphonates **7** were achieved.

To find out if it was possible to further increase the selectivity of formation of hydroxyphosphonates **7**, we carried out the hydrolysis of **1c** in the presence of excess of *p*-anisaldehyde to suppress the **1** ⇌ **2** + **3** equilibrium. Indeed, the selectivity of hydrolysis of **1c** *via* pathway **B** improved under such conditions, and reached 98% when *p*-anisaldehyde was used in a large excess (30 equiv.). It seems that such a modification of the synthetic protocol for the preparation of **7** can be used in instances when starting hydroxyphosphonates **1** are involved in unfavourable equilibria.



Scheme 5 Hypoiodite-promoted hydrolysis of aryl nucleoside α-hydroxyphosphonates **1**.

Conclusions

In conclusion, we have investigated the stability and decomposition pathways of diastereomeric mixtures of nucleoside α-hydroxyphosphonates **1** bearing different aryl groups, both in the ester and the hydroxymethine fragment. We have found essential differences in products distributions during hydrolysis under previously described physiological-like conditions (RPMI/FBS)[2] and those well defined chemical conditions used in the present studies. We have found that under aqueous basic conditions, the stability and hydrolytic decomposition pathways of these compounds were governed by the electronic nature of the aryl group in the hydroxymethine moiety of α-hydroxyphosphonates of type **1**. The more electron-donating is Ar^2, the higher the participation of path **A**, due to equilibria of α-hydroxyphosphonates with the corresponding aryl nucleoside H-phosphonate diesters and their respective aldehydes. The hydrolysis of compounds of type **1** was found to be very sensitive to the presence of α-nucleophiles, *e.g.* hypoiodite or peroxide anions, and under such conditions, reactions towards nucleoside α-hydroxyphosphonates monoesters of type **7** were favoured. Significant differences in rates of hydrolysis of hydroxyphosphonates **1** *vs.* their *O*-acylated derivatives pointed to the importance of the intramolecular acid catalysis exerted by the adjacent hydroxyl function. A preferential hydrolysis of **1** *via* path **B** in the presence of α-nucleophiles (hypoiodites) was exploited for the development of a simple and efficient protocol for the preparation of otherwise difficult to access hydroxyphosphonate monoesters of type **7**, whose biological properties are under study in our laboratory.

Experimental

General methods and materials

^1H, ^{13}C and ^{31}P NMR spectra were recorded on 300 or 400 MHz machines. The ^{31}P NMR experiments were carried out in 5 mm tubes using 0.1 M solutions of the phosphorus-containing compounds. ^{31}P NMR chemical shifts are reported in ppm

relative to 85% H_3PO_4 in water (external standard). Mass spectra were recorded by the liquid secondary ion mass technique (LSIMS) using Cs^+ (12 keV) for ionization. The amount of water in solvents was measured by Karl Fisher coulometric titration. Methylene chloride was dried over P_2O_5, distilled and kept over molecular sieves 4 Å until the amount of water was less than 10 ppm. Pyridine was stored over molecular sieves 4 Å until the amount of water was below 20 ppm. For column chromatography, Kieselgel 60 Merck was used. For TLC analysis, pre-coated plates (Merck silica gel F_{254}) were used.

Aryl nucleoside α-hydroxyphosphonates of type **1** were obtained as a mixture of four diastereoisomers by condensation of AZT H-phosphonate **4** with the respective phenol **5**, followed by the addition of aldehyde **3** (the analytical data for compounds **1a–e** were reported previously[2]).

The reference compounds used for the identification of certain reaction products or intermediates were obtained as follows: AZT H-phosphonate **4**, from the reaction of 3′-azido-3′-deoxythymidyne with pyrophosphonic acid in pyridine;[15] phosphotriester **8** from the condensation of phosphodiester **6** with 4-nitrobenzyl alcohol, aided by 2,4,6-triisopropylbenzenesulfonyl chloride in methylene chloride in the presence of *N*-methylimidazole.[16]

The purity of all compounds was >98%, as judged from ^{1}H NMR spectra. The multiplicity of signals in ^{31}P NMR spectra were due to the presence of diastereoisomers.

Syntheses

3′-Azido-3′-deoxythymidin-5′-yl 4-methoxyphenyl α-hydroxy-(phenyl)methylphosphonate 1f. Obtained as previously described[2] - 0.19 g (yield 72%). ^{31}P NMR ($CDCl_3$) δ_P 18.94, 18.97, 19.21, 19.31. ^{1}H NMR ($CDCl_3$) δ_H 1.76, 1.77, 1.79, 1.83 (4s, 3H, 5-CH_3), 1.96–2.23 (2m, 2H, 2′,2′′-H), 3.70, 3.71, 3.72 (3s, 3H, OCH_3), 3.88–3.93 (m, 1H, 4′-H), 4.04–4.20 (m, 1H, 3′-H), 4.21–4.26 (m, 2H, 5′,5′′-H), 5.22–5.25 (m, 1H, α-H), 5.60–6.06 (m, 1H, 1′-H), 6.74–7.51 (m, 10H, 6-H and ArH). ^{13}C NMR ($CDCl_3$) δ_C 12.27 (5-CH_3), 36.68 (C-2′), 55.54, 55.57 (OCH_3), 60.12–60.26 (C-3′), 66.45 (C-5′), 69.50, 69.68, 71.07, 71.23 (α-C–OH), 82.12, 82.18 (C-4′), 85.49 (C-1′), 111.41–111.52 (C-5), 114.54, 114.59, 114.69, 114.75 (C-Ar), 120.40 (C-Ar), 121.11–121.23 (C-Ar), 126.99–127.25 (C-Ar), 128.31–128.72 (2 × C-Ar), 135.40–135.86 (C-6), 143.34–143.51 (C-Ar), 150.22, 150.27 (C-2), 156.93 (C-Ar), 163.65–163.71 (C-4). HRMS $[M]^-$ *m*/*z*: 544.1586 calculated for $C_{24}H_{27}N_5O_8P$ 544.1597.

3′-Azido-3′-deoxythymidin-5′-yl 4-chlorophenyl α-hydroxy-(phenyl)methylphosphonate 1g. Obtained as previously described[2]- 0.22 g (yield 80%). ^{31}P NMR ($CDCl_3$) δ_P 18.71, 19.11. ^{1}H NMR ($CDCl_3$) δ_H 1.81, 1.82, 1.83, 1.84 (4s, 3H, 5-CH_3), 2.08–2.38 (m, 2H, 2′,2′′-H), 3.90–3.98 (m, 1H, 4′-H), 4.12–4.38 (m, 3H, 3′, 5′, 5′′-H), 5.20–5.27 (m, 1H, α-H), 5.99–6.10 (m, 1H, 1′-H), 6.98–7.50 (m, 10H, 6-H and ArH). ^{13}C NMR ($CDCl_3$) δ_C 12.32 (5-CH_3), 36.86, 36.96, 37.06 (C-2′), 60.03 (C-3′), 65.82–66.21 (C-5′), 69.79,69.88 71.47 (α-C–OH), 82.12–82.33 (C-4′), 85.48–85.70 (C-1′), 111.41–111.51 (C-5), 121.65, 121.69 (C-Ar), 126.97–127.82 (2 × C-Ar), 128.31–128.94 (2 × C-Ar), 129.64–130.11 (C-Ar), 135.10, 135.21 (C-6), 135.74, 135.84, 135.97 (C-Ar), 148.57–148.66 (C-Ar), 150.13 (C-2), 163.58, 163.65 (C-4). HRMS $[M]^-$ *m*/*z*: 548.1089 calculated for $C_{23}H_{24}N_5O_7P$ 548.1102.

General procedure for synthesis of nucleoside α-hydroxyphosphonates of type 7

Aryl nucleoside α-hydroxyphosphonate **1** (1 molar equiv., 0.5 mmol) was dissolved in a freshly prepared solution containing iodine (3 molar equiv.) in acetonitrile : triethylamine : water (2 : 1 : 1 v/v) (5 mL) (straw-coloured). After the reaction was complete (<5 min, ^{31}P NMR), the solvents were evaporated and the residue, dissolved in methylene chloride, applied to a silica gel column pre-formed with *iso*-propanol. Products **7** were isolated using a stepwise gradient of water (0–10%) in *iso*-propanol containing triethylamine (3% v/v). Fractions containing pure products **7** were collected and evaporated to dry foams. After freeze-drying from benzene–methanol, compounds **7a–e** were obtained as white amorphous solids (yellow for the *p*-nitrophenyl derivative).

3′-Azido-3′-deoxythymidin-5′-yl α-hydroxy(phenyl)methylphosphonate triethylammonium salt (7a). 0.21 g (yield 76%). ^{31}P NMR ($CDCl_3$) δ_P 16.84, 17.06. ^{1}H NMR ($CDCl_3$) δ_H 1.13, 1,16, 1.18 (t, J = 7.2 Hz, 9H, CH_2CH_3), 1.93, 1.94 (2s, 3H, 5-CH_3), 2.22–2.34 (m, 2H, 2′,2′′-H), 2.82, 2.84, 2.86, 2.89 (q, J = 7.2 Hz, 6H, CH_2CH_3), 3.98–4.40 (3m, 4H, 4′, 3′, 5′, 5′′-H), 4.88, 4.91, 4.92, 4.95 (4s, 1H, α-H), 6.14, 6.16, 6.18, 6.20 (4s, 1H, 1′-H), 7.17–7.35 (m, 3H, 6-H and ArH), 7.54–7.56 (m, 2H, ArH), 7.70–7.73 (m, 1H, ArH). ^{13}C NMR (DCl_3) δ_C 8.44 (CH_2CH_3), 12.45 (5-CH_3), 37.72, 37.80 (C-2′), 45.27 (CH_2CH_3), 60.12–61.66 (C-3′), 64.76, 64.83, 65.04, 65.12 (C-5′), 70.56, 70.78, 72.48, 72.71 (α-C–OH), 83.88, 83.93, 83.97, 84.01 (C-4′), 85.09, 85.22 (C-1′), 110.10-111.11 (C-5), 114.54, 114.59, 114.69, 114.75 (C-Ar), 120.40 (C-Ar), 121.11–121.23 (C-Ar), 126.65–128.32 (5 × C-Ar), 135.82, 135.88 (C-Ar), 140.48, 140.72 (C-6), 150.68, 150.72 (C-2), 164.44–164.60 (C-4). HRMS $[M–Et_3NH^+]^-$ *m*/*z*: 436.1032 calculated for $C_{17}H_{219}N_5O_7P$ 436.1022.

3′-Azido-3′-deoxythymidin-5′-yl α-hydroxy(4-methylphenyl)-methylphosphonate triethylammonium salt (7b). 0.24 g (yield 87%). ^{31}P NMR ($CDCl_3$) δ_P 17.04, 17.25. ^{1}H NMR ($CDCl_3$) δ_H 1.14, 1.16, 1.18 (t, J = 7.2 Hz, 9H, CH_2CH_3), 1.93 (s, 3H, 5-CH_3), 2.30 (s, 3H, CH_3), 2.22–2.32 (m, 2H, 2′,2′′-H), 2.84, 2.86, 2.87, 2.89 (q, J = 7.2 Hz, 6H, CH_2CH_3), 3.94–4.21 (3m, 3H, 3′, 5′, 5′′-H), 4.34–4.37 (m, 1H, 4′-H), 4.84, 4.86, 4.87, 4.90 (4s, 1H, α-H), 6.16, 6.18, 6.19, 6.20 (4s, 1H, 1′-H), 7.09, 7.11 (2s, 2H, ArH), 7.42, 7.44 (2s, 2H, ArH), 7.70, 7.72 (2s, 1H, 6-H). ^{13}C NMR ($CDCl_3$) δ_C 8.48 (CH_2CH_3), 12.44 (5-CH_3), 21.03 (CH_3), 37.68, 37.74 (C-2′), 45.22 (CH_2CH_3), 61.65 (C-3′), 64.82, 65.10 (C-5′), 70.71, 70.90, 72.15, 72.35 (α-C–OH), 83.92 (C-4′), 85.02, 85.11 (C-1′), 110.01, 111.10 (C-5), 126.59, 126.64, 126.75, 126.80 (2 × C-Ar), 128.44 (C-Ar), 135.87 (2 × C-Ar), 136.22 (C-Ar), 137.29, 137.51 (C-6), 150.69 (C-2), 164.40, 164.52 (C-4). HRMS $[M–Et_3NH^+]^-$ *m*/*z*: 450.1174 calculated for $C_{18}H_{21}N_5O_7P$ 450.1179.

3′-Azido-3′-deoxythymidin-5′-yl α-hydroxy(4-methoxyphenyl)-methylphosphonate ammonium salt (7c). 0.18 g (yield 75%). ^{31}P NMR ($CDCl_3$ + CD_3OD) δ_P 17.80, 17.83. ^{1}H NMR ($CDCl_3$ + CD_3OD) δ_H 1.80 (s, 3H, 5-CH_3), 2.08–2.23 (m, 2H,

2′,2″-H), 3.70 (s, 3H, OCH_3), 3.77–3.96 (m, 3H, 3′, 5′, 5″-H), 4.13–4.15 (m, 1H, 3′-H), 4.72, 4.73, 4.75, 4.76 (4s, 1H, α-H), 6.01, 6.03, 6.05 (3s, 1H, 1′-H), 6.77, 6.79 (2s, 2H, ArH), 7.31, 7.33 (2s, 2H, ArH), 7.40, 7.41, 7.42, 7.43 (4s, 1H, 6-H). ^{13}C NMR (D_2O) δ_C 11.55, 11.59 (5-CH_3), 36.27, 36.36 (C-2′), 55.26 (OCH_3), 60.36, 60.45 (C-3′), 64.02, 64.08, 64.26, 64.32 (C-5′), 69.87, 70.44, 71.44, 72.01 (α-C–OH), 83.22, 83.29 (C-4′), 84.90, 85.07 (C-1′), 110.37, 111.45 (C-5), 128.49, 128.55, 128.71, 128.77 (2 × C-Ar), 131.28, 131.30, 131.34, 131.36 (2 × C-Ar), 137.18, 137.29 (C-6), 151.48, 151.55 (C-2), 158.28, 158.31, 158.34 (2 × C-Ar), 166.38, 166.39 (C-4). HRMS $[M–NH_4^+]^-$ m/z: 466.1144 calculated for $C_{18}H_{21}N_5O_8P$ 466.1128.

3′-Azido-3′-deoxythymidin-5′-yl α-hydroxy(4-chlorophenyl)-methylphosphonate triethylammonium salt (7d). 0.25 g (yield 88%). ^{31}P NMR ($CDCl_3$) δ_P 16.10, 16.30. ^{1}H NMR ($CDCl_3$) δ_H 1.16, 1.18, 1.21 (t, J = 7.2 Hz, 9H, CH_2CH_3), 1.92, 1.93, 1.94 (3s, 3H, 5-CH_3), 2.30 (s, 3H, CH_3), 2.26–2.38 (m, 2H, 2′,2″-H), 2.88, 2.90, 2.93, 2.95 (q, J = 7.2 Hz, 6H, CH_2CH_3), 3.94–4.30 (3m, 3H, 3′, 5′, 5″-H), 4.37–4.41 (m, 1H, 4′-H), 4.85, 4.88, 4.90, 4.92 (4s, 1H, α-H), 6.12, 6.15, 6.17, 6.19 (4s, 1H, 1′-H), 7.27, 7.28 (2s, 2H, ArH), 7.47–7.52 (m, 2H, ArH), 7.66, 7.69 (2s, 1H, 6-H). ^{13}C NMR ($CDCl_3$) δ_C 8.41 (CH_2CH_3), 12.42 (5-CH_3), 37.77, 37.86 (C-2′), 45.51 (CH_2CH_3), 61.55 (C-3′), 64.87, 65.11, 65.19 (C-5′), 69.59, 70.13, 71.86, 72.05 (α-C–OH), 83.89 (C-4′), 85.22, 85.36 (C-1′), 110.96, 111.05 (C-5), 127.80 (C-Ar), 128.02, 128.08 (C-Ar), 128.22, 128.28 (C-Ar), 132.36 (C-Ar), 135.71, 135.80 (C-Ar), 139.02, 139.32 (C-6), 150.64 (C-2), 164.44, 164.61 (C-4). HRMS $[M–Et_3NH^+]^-$ m/z: 470.0646 calculated for $C_{17}H_{18}N_5O_7P$ 470.0632.

3′-Azido-3′-deoxythymidin-5′-yl α-hydroxy(4-nitrophenyl)-methylphosphonate triethylammonium salt (7e). 0.25 g (yield 85%). ^{31}P NMR ($CDCl_3$) δ_P 15.09, 15.31. ^{1}H NMR ($CDCl_3$) δ_H 1.15, 1.17, 1.20 (t, J = 7.2 Hz, 9H, CH_2CH_3), 1.93 (3s, 3H, 5-CH_3), 2.28–2.41 (m, 2H, 2′,2″-H), 2.90, 2.93, 2.95, 2.98 (q, J = 7.2 Hz, 6H, CH_2CH_3), 3.91–4.30 (3m, 3H, 3′, 5′, 5″-H), 4.37–4.40 (m, 1H, 4′-H), 5.00, 5.03, 5.06, 5.08 (4s, 1H, α-H), 6.08, 6.10, 6.13, 6.15 (4s, 1H, 1′-H), 7.62, 7.63, 7.65, 7.66 (2d, J = 0.9, 1.2 Hz, 1H, 6-H), 7.73–7.78 (m, 2H, ArH), 8.15–8.18 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$) δ_C 8.37 (CH_2CH_3), 12.40 (5-CH_3), 37.78, 37.87 (C-2′), 45.43 (CH_2CH_3), 61.39 (C-3′), 64.92, 65.00, 65.05, 65.27, 65.30 (C-5′), 70.33, 70.41, 72.21, 72.29 (α-C–OH), 83.72, 83.75, 83.81, 83.83 (C-4′), 85.32, 85.44 (C-1′), 110.96, 111.03 (C-5), 120.08, 120.16 (C-Ar), 127.25, 127.30, 127.43, 127.49 (2 × C-Ar), 135.65, 135.75 (C-6), 146.72, 146.76 (C-Ar), 148.38, 148.40 (C-Ar), 148.71 (C-Ar), 150.65, 150.67 (C-2), 164.58, 164.73 (C-4). HRMS $[M–Et_3NH^+]^-$ m/z 481.0888 calculated for $C_{17}H_{18}N_6O_9P$ 481.0873.

3′-Azido-3′-deoxythymidin-5′-yl phenyl α-acetyloxy(4-methoxyphenyl)methylphosphonate (9). 0.16 g of **1c** dissolved in 3 mL of methylene chloride containing pyridine (10% v/v) was treated with acetic anhydride (0.28 mL, 10 molar equiv.) at room temperature. After the reaction was complete (2.5 h, ^{31}P NMR), the resulting mixture was washed with water, the organic layer separated after drying over anhydrous Na_2SO_4 and evaporated. The further isolation procedure for **9** was as for the parent aryl nucleoside α-hydroxyphosphonate **1c**. Obtained 0.13 g (yield 75%). ^{31}P NMR ($CDCl_3$) δ_P 15.07, 15.11, 15.44, 15.46. ^{1}H NMR ($CDCl_3$) δ_H 1.84, 1.86 (2s, 3H, 5-CH_3), 2.01, 2.06, 2.15, 2.17 (4s, 3H, CH_3COO), 1.94–2.37 (m, 2H, 2′,2″-H), 3.78, 3.79, 3.80 (3s, 3H, OCH_3), 3.89–4.32 (4m, 4H, 4′, 3′, 5′, 5″-H), 6.06–6.21 (m, 1H, α-H), 6.27–6.35 (m, 1H, 1′-H), 6.87–7.49 (m, 10H, 6-H and ArH). ^{13}C NMR ($CDCl_3$) δ_C 12.34, 12.42 (5-CH_3), 20.62, 20.67, 20.78 (CH_3COO), 36.98, 37.06, 37.12, 37.19 (C-2′), 55.32 (OCH_3), 60.02, 60.08 (C-3′), 65.77, 65.84, 65.92, 65.99, 66.32, 66.42, 66.60, 66.69 (C-5′), 68.56, 68.67, 68.72, 68.89, 70.88, 71.01, 71.20 (α-C–OH), 81.91, 81.99, 82.06 (C-4′), 84.51, 84.57, 84.76, 84.84 (C-1′), 111.44, 111.46, 111.53, 111.56 (C-5), 120.02, 120.09, 120.14, 120.18, 120.24 (C-Ar), 129.45–130.08 (5 × C-Ar), 134.94, 135.06, 135.17 (C-6), 149.77–150.26 (C-Ar, C-2), 160.37–160.54 (C-4), 168.94–169.21 (CH_3COO). HRMS $[M]^-$ m/z 586.1711 calculated for $C_{26}H_{29}N_5O_9P$ 586.1703.

3′-Azido-3′-deoxythymidin-5′-yl α-acetyloxy(4-methoxyphenyl)-methylphosphonate triethylammonium salt (10). 0.14 g of **7c** dissolved in 3 mL of methylene chloride containing pyridine (10% v/v) was treated with acetic anhydride (0.28 mL, 10 molar equiv.) at room temperature. After the reaction was complete (0.5 h, ^{31}P NMR), the solvent was evaporated and compound **10**, after dissolving in a small volume of methylene chloride, precipitated from n-hexane. 0.16 g (yield 88%) of white solid. ^{31}P NMR ($CDCl_3$) δ_P 13.08, 13.13. ^{1}H NMR ($CDCl_3$) δ_H 1.13, 1.16, 1.18 (t, J = 7.2 Hz, 9H, CH_2CH_3), 1.90, 1.94, 2.03 (3s, 3H, CH_3COO), 2.09, 2.10 (2s, 3H, 5-CH_3), 2.23–2.31 (m, 2H, 2′,2″-H), 2.82, 2.85, 2.87, 2.91 (q, J = 7.2 Hz, 6H, CH_2CH_3), 3.76 (s, 3H, OCH_3), 3.96–4.30 (3m, 4H, 4′, 3′, 5′, 5″-H), 6.02, 6.05, 6.06, 6.09 (4s, 1H, α-H), 6.20-6.24 (m, 1H, 1′-H), 6.81, 6.84 (2s, 2H, ArH), 7.41, 7.44 (2s, 2H, ArH), 7.56, 7.59 (2s, 1H, 6-H). ^{13}C NMR ($CDCl_3$) δ_C 8.08, 8.34 (CH_2CH_3), 12.30, 12.36 (5-CH_3), 20.98, 21.03, 21.05 (CH_3COO), 37.24 (C-2′), 45.39 (CH_2CH_3), 55.25, 55.28 (OCH_3), 60.59, 60.66 (C-3′), 64.77, 64.83, 65.10, 65.16 (C-5′), 70.45, 70.61, 72.08, 72.24 (α-C–OH), 83.02, 83.09 (C-4′), 84.53 (C-1′), 111.18, 111.21 (C-5), 113.59 (2 × C-Ar), 126.07 (C-Ar), 127.54, 127.61 (C-Ar), 128.29 (C-Ar), 129.25, 129.29 (C-6), 150.31 (C-2), 159.47, 159.48 (C-Ar), 163.89 (C-4), 169.68, 169.76 (CH_3COO). HRMS $[M–Et_3NH^+]^-$ m/z 508.1229 calculated for $C_{20}H_{23}N_5O_9P$ 508.1234.

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