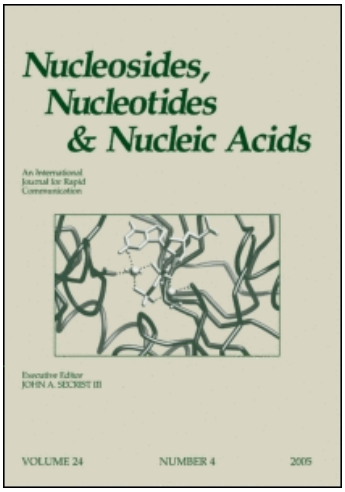

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Di-ddU-α-Hydroxyphosphonate and α-Hydroxy- α-METHYLPHOSPHONATES as Potential Prodrugs of Antiviral Nucleoside Analogues

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DI-DDU-α-HYDROXYPHOSPHONATE AND α-HYDROXY-α-METHYLPHOSPHONATES AS POTENTIAL PRODRUGS OF ANTIVIRAL NUCLEOSIDE ANALOGUES

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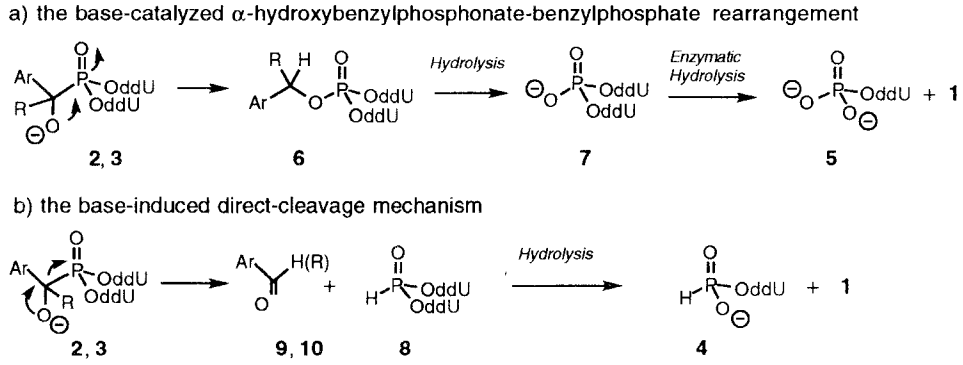
ABSTRACT: The synthesis of a potential new prodrug system for the antiviral nucleoside ddU **1** based on α-hydroxybenzylphosphonates **2** and **3** is described. In principle, **2**, **3** are able to hydrolyze via different mechanisms yielding ddU H-phosphonate **4** or ddU monophosphate **5**, respectively.

Nucleoside analogues are widely used as antiviral agents in the treatment in AIDS. After penetration through cell membranes, the conversion of the nucleoside analogues into their 5'-mono-, di- and triphosphates by cellular kinases is essential for the expression of biological activity[1]. Specially for 2',3'-dideoxyuridine **1** (ddU) the first kinase catalyzed phosphorylation is the crucial metabolization step, whereas further conversion into the triphosphate is not rate limiting.

Additionally, Krayevsky demonstrated recently that the 5'-H-phosphonates of AZT and FLT exhibit marked antiviral activity *in-vitro*[2]. But the intracellular metabolization of 5'-nucleoside H-phosphonates may be different from that of nucleoside analogues: catabolic action of pyrophosphate transferase should result in pyrophosphorylhydrogenphosphonates, which are analogues of nucleoside triphosphates. On the other hand, 5'-nucleoside H-phosphonates may be oxidized intracellularly to yield the nucleoside monophosphates.

Unfortunately, charged phosphorylated or phosphonylated nucleosides are unable to penetrate the cell membranes or the blood brain barrier due to low lipophilicity.

In this work we present 2',3'-dideoxyuridine (ddU) **1** containing homo-dinucleoside-α-hydroxybenzylphosphonates **2** and α-hydroxy-α-methylbenzylphosphonates **3** as potential prodrugs of 5'-ddU H-phosphonate **4** and 5'-ddU monophosphate **5**. We have recently shown that these two compounds are released by controlled hydrolysis via two different pathways: the phosphonate-phosphate rearrangement and the direct cleavage mechanism (scheme 1). The rearrangement leads via the benzylphosphotriesters **6** selectively to the



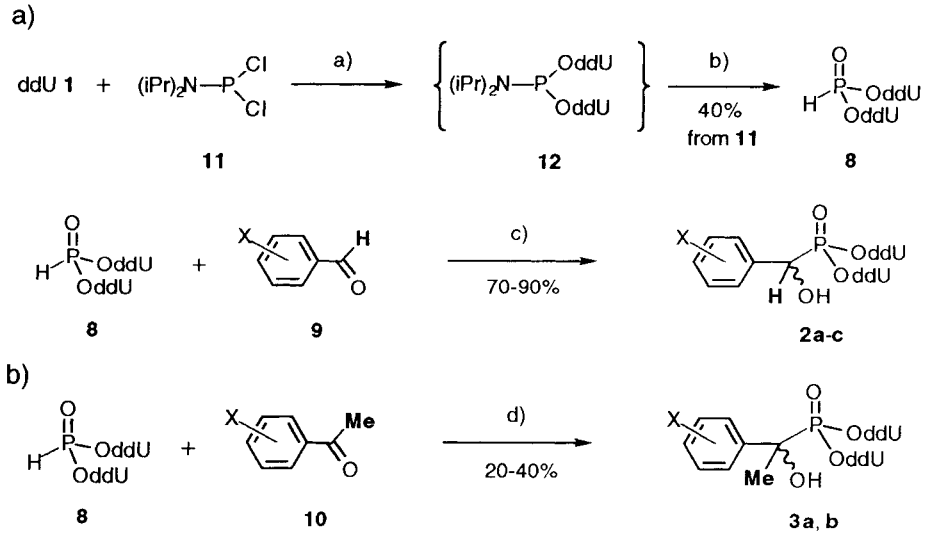
SCHEME 1: Two different hydrolysis mechanisms of the title compounds **2** and **3**

dinucleoside phosphodiesters **7**. On the other hand, the direct cleavage yields the ddU H-phosphonate diester **8** (and benzaldehyde **9** or acetophenones **10**), which decomposes rapidly into ddU H-phosphonate **4** as well as ddU **1**, respectively[3] (scheme 1). Here we studied the effect of a further substitution at the α-C-atom. For this reason we introduced a methyl group in this position.

The α-hydroxybenzylphosphonates **2** were synthesized starting from symmetric di-ddU H-phosphonate **8** as intermediate for all compounds. The synthetic approach to **2a-c** is summarized in scheme 2 and has already been described[3]. Compounds **2** were isolated after chromatography on silica gel in up to 90% yield as 1:1 diastereomeric mixtures (scheme 2a). The diastereomers were separated by means of semipreparative HPLC. The synthesis of the α-hydroxy-α-*methyl*benzylphosphonates **3a, b** was not successful using the above reaction condition. Therefore the compounds **3a, b** were synthesized as follows: Because of the low reactivity of the acetophenones in the reaction with di-ddU H-phosphonate **8** cesium fluoride (CsF) was used as base[4]. But even with CsF the yields were low (20-40%, scheme 2b). The reaction was carried out without solvent. The major limitation is the low reactivity of the ketones and the subsequent rearrangement of phosphonates **3** to give the corresponding phosphotriesters **6** under this conditions. All phosphonates **2** and **3** were characterized by means of ^{1}H, ^{13}C, ^{31}P nmr, UV as well as electrospray mass spectrometry. In some cases we were able to separate the two diastereomers concerning configuration at phosphorus atom.

The partition coefficients (PC values) of the title compounds **2, 3** were determined in 1-octanol/water as a rough estimation of the lipophilic properties. The values are summarized in table 1. As compared to ddU **1**, the lipophilicity of **2** and **3** is partly improved.

The title compounds were studied concerning their hydrolysis properties. The major question was the ability of **2** and **3** to rearrange into the phosphotriesters or to cleave to yield di-ddU H-phosphonate **8**. Then the influence of the methyl group in the α-position on the half live was of interest.



a: X = H, **b**: X = 4-OAc; **c**: X = 2-NO_2

a) DIPEA, CH_3CN, 0°C, 10 min.; b) 1H-tetrazole, H_2O, CH_3CN, 25°C, 15 min.; c) DIPEA (cat.), THF, rt, 2-10 h; d) 2 eq. cesium fluoride, rt, 3-16 h, without solvent

SCHEME 2: Synthesis of the α-hydroxybenzyl- **2** and the α-hydroxy-α-methylbenzyl-phosphonates **3**

TABLE 1: Hydrolysis of **2** and **3** in phosphate buffer, RPMI culture medium, and RPMI/FCS medium

2, **3** or **1**		Hydrolysis ($t_{1/2}$) in buffers			Hydrolysis Products		PC value
	X	phosphate pH 7.29 [h]	RPMI medium [h]	RPMI/FCS medium [h]	direct cleavage to **5** and **1**	rearrangement to **6**	
2a (**f**)	H	45.8	5.3	5.1	100	0	0.14
2a (**s**)	H	44.1	5.2	5.0	100	0	0.15
3a	H	2.6	0.5	0.4	100	0	0.29
2b (**f**)	4-OAc	22.0	2.5	2.4	100	0	0.07
2b (**s**)	4-OAc	26.3	2.4	2.6	100	0	0.09
3b	4-OAc	1.9	0.4	0.3	100	0	0.13
2c	2-NO_2	10.5	1.9	2.0	20	80	0.20
1	--	--	--	--	--	--	0.12

(**f**): fast-diastereomer; (**s**): slow-diastereomer

First, as model medium, a 50 mmol phosphate buffer, pH 7.29 was used. Additionally, the hydrolysis was investigated in RPMI 1640 culture medium with and without 10% heat-inactivated fetal calf serum (FCS). The hydrolyses were followed by means of HPLC analysis. The half lives are summarized in table 1. Independently of the studied medium and independently of the starting material **2** and **3** only the direct cleavage reaction to yield the di-ddU H-phosphonate **8** and the subsequent hydrolysis of **8** to give the ddU H-phosphonate **4** and ddU **1** was observed. The direct cleavage pathway is clearly spontaneous and is not enzymatically catalyzed. Only in the case of the 2-nitro-substituted α-hydroxyphosphonate the rearrangement was observed in 80% yield. To our surprise, the half lives of **2** and **3**, respectively, were by a factor of 5 to 10 shorter in RPMI culture medium and RPMI/FCS medium than in phosphate buffer, whereas the half lives in RPMI culture medium and RPMI/FCS medium are almost identical. Until now, we have no explanation for this very expressed effect. Furthermore, no significant difference in the half lives were detected for the two separated diastereomers of **2a** (**f**), **2a** (**s**), **2b** (**f**), and **2b** (**s**). It should be mentioned that similar results as well as similar half lives were obtained for the analogous phosphonate diesters of 2',3'-dideoxy-2',3'-didehydrothymidine (d4T; data not shown). Consequently, the hydrolysis seems to independent of the nucleosyl residue in **2** or **3**.

In summary, except of the 2-nitro-substituted derivative **2c**, the aforementioned data clearly show that the described α-hydroxybenzylphosphonates **2** and the α-hydroxy-α-methylbenzylphosphonates **3** could only serve as nucleoside and nucleoside H-phosphonate prodrugs. Noteworthy is also the high difference in half lives between **2** and **3**.

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