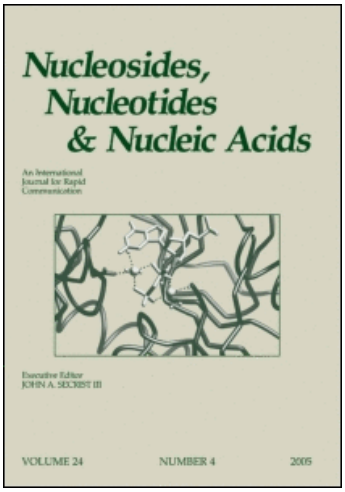

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Homo Dinucleoside-α-hydroxyphosphonate Diesters as Prodrugs of the Antiviral Nucleoside Analogues 2',3'-Dideoxythymidine and 3'-Azido-2',3'-dideoxythymidine

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BIOLOGICAL ACTIVITY

HOMO DINUCLEOSIDE-α-HYDROXYPHOSPHONATE DIESTERS AS PRODRUGS OF THE ANTIVIRAL NUCLEOSIDE ANALOGUES 2',3'-DIDEOXYTHYMIDINE AND 3'-AZIDO-2',3'-DIDEOXYTHYMIDINE

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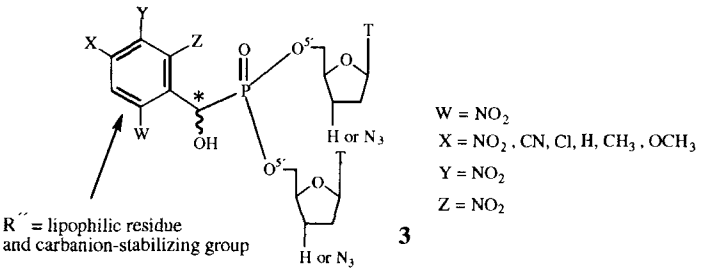
ABSTRACT: The synthesis of a new prodrug system for antiviral nucleosides AZT (**1**) and ddT (**2**) based on α-hydroxybenzylphosphonates **3** is described. **3** hydrolyze via different mechanisms yielding the H-phosphonate monoesters **4** or nucleoside monophosphates **5**, respectively. **3** were more lipophilic than **1**, **2** and showed marked activity against HIV-1/2.

Nucleoside analogues are widely used as antiviral agents in the treatment in AIDS and the AIDS related complex (ARC). After penetration through the 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]. 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 nucleoside-5′-H-phosphonates may be different from that of nucleoside analogues: catabolic action of pyrophosphate transferase should result in pyrophosphorylhydrogenphosphonates: analogues of nucleoside triphosphates. On the other hand, 5′-nucleoside H-phosphonates may be oxidized intracellulary to yield the nucleoside monophosphates.

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

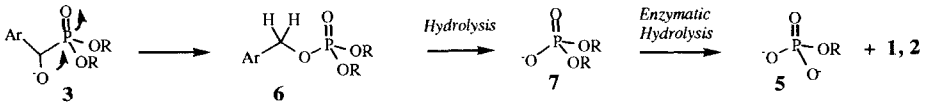
In this work we present 3′-azido-2′,3′-dideoxythymidine (AZT) (**1**) and 2′,3′-dideoxythymidine (ddT) (**2**) containing homo-dinucleoside-α-hydroxybenzylphosphonate diesters **3** as uncharged prodrugs of 5′-nucleoside H-phosphonates **4** and 5′-nucleoside monophosphates **5**. These two compounds are released by controlled hydrolysis via two different pathways: the phosphonate-phosphate rearrangement and the direct cleavage mechanism (Scheme 1). The rearrangement leads to benzylphosphotriesters **6** which are selectively cleaved to yield the dinucleoside phosphodiesters **7**. On the other hand, the direct cleavage



SCHEME 1: Two different hydrolysis mechanisms of compound **3**

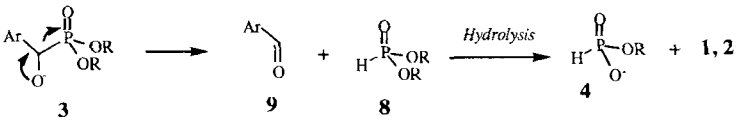
yields the dinucleoside H-phosphonate diesters **8** and the benzaldehyde **9**. Compound **8** decompose rapidly to yield the nucleoside H-phosphonate monoester **4** as well as **1** or **2**, respectively [3].

a) the base-catalysed α-hydroxybenzylphosphonate-benzylphosphate rearrangement



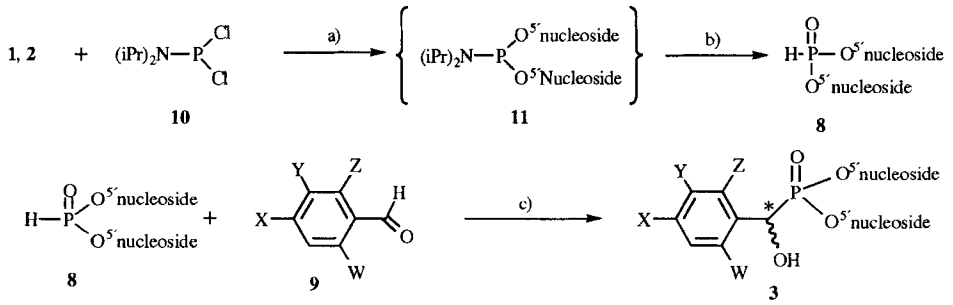
b) the base-induced direct-cleavage mechanism

ROH = AZT **1** and ddT **2**



The α-hydroxybenzylphosphonates **3** were synthesized starting from the symmetric dinucleoside H-phosphonate diesters **8** as intermediate for all compounds and was carried out as follows: **8** were obtained by reacting two equivalents of the nucleosides **1**, **2** with one equivalent of diisopropyldichlorophosphine **10** in acetonitrile in the presence of diisopropylethylamine (DIPEA) and subsequent hydrolysis of the phosphoamidites **11** after activation with tetrazole. The H-phosphonates **8** were obtained by column chromatography in 70-80% yield [3]. It is noteworthy to mention that **8** were very sensitive to aqueous alkaline conditions. The target compounds **3** were obtained by reaction of **8** with a benzaldehyde derivate **9** using catalytic amounts of a tertiary base like DIPEA or triethylamine (TEA) in tetrahydrofuran as solvent [4]. Compounds **3** were isolated after column chromatography on silica gel in up to 90% yield (Scheme 2).

All new compounds **3** were studied concerning there partition coefficients (PC values) in an octanol/water mixture as well as there hydrolysis properties using a phosphate buffer, pH 7.5 and culture medium which contains 10% heat-inactivated fetal calf serum (FCS). The PC values, the half-lives as well as the hydrolysis products of **3** are summarized in Table 1.



a) DIPEA, CH_3CN, 0°C, 10 min; b) tetrazole, H_2O, CH_3CN, 25°C, 15 min; c) DIPEA (cat.), THF, rt, 2-10h.

SCHEME 2: Synthesis of the target compounds **3**

TABLE 1: Hydrolysis of **3** in different media, the products and the PC values

3	X,Y,Z,W	Hydrolysis in ($t_{0.5}$) phosphate buffer pH 7.5 [h]	RPMI/10%FCS culture medium [h]	Products of **4** and **1**, **2**	**6**	PC value
ddT		--	--	--	--	0.14
	4-OCH_3	31	10	100%	0%	0.46
	4-CH_3	47	18	100%	0%	1.32
	H	57	22	100%	0%	0.69
	4-Cl	43	18	100%	0%	2.06
	4-CN	40	19	40%	60%	0.27
AZT		--	--	--	--	1.09
	4-OCH_3	6	2	100%	0%	5.2
	4-CH_3	7.5	3	100%	0%	15.9
	H	8	4	100%	0%	6.2
	4-Cl	7	2	100%	0%	28.0
	4-CN	6	2	75%	25%	3.1
AZT	4-NO_2	5	3	40%	60%	6.7
	3-NO_2	8	4.5	95%	5%	6.2
	2-NO_2	2.5	1.5	20%	80%	6.1
	2.4-NO_2	0.13	0.1	1%	99%	8.79
	2.6-NO_2	0.23	0.17	5%	95%	5.38

Additionally, compounds **3** were tested for their activity in HIV-1- and HIV-2-infected CEM/O cells. The antiviral activity data are shown in Table 2.

As can seen from Table 1, all new derivatives of **3** showed higher PC-values than the parent nucleosides **1** or **2**. Additionally, the breakdown mechanism could be controlled: derivatives of **3** bearing strong electron-withdrawing substituents in the 2- or/and 4-position of the aromatic system showed nearly clean rearrangement reactions, whereas derivatives with electron-donating substituents were degraded by the direct cleavage pathway. Both reactions

TABLE 2: Activity against HIV-1 and HIV-2 in CEM/0 cells

Compound **3**	W,X,Y,Z	Antiviral Activity EC_{50} (μg/ml) HIV-1	HIV-2	Toxicity CC_{50} (μg/ml)
ddT		0.60	1.40	100
	4-OCH_3	0.5	0.55	>20
	4-CH_3	3.25	10.0	>100
	H	2.93	13.3	>100
	4-Cl	4.0	4.0	>100
	4-CN	3.93	7.7	>100
AZT		0.012	0.007	100
	4-OCH_3	0.025	0.013	>100
	4-CH_3	0.004	0.018	>100
	H	0.014	0.032	>100
	4-Cl	0.009	0.018	>100
	4-CN	0.01	0.023	>100
AZT	4-NO_2	0.003	0.009	>20
	3-NO_2	0.009	0.008	86.5
	2-NO_2	0.006	0.005	>100
	2.4-NO_2	0.001	0.004	>100
	2.6-NO_2	0.001	0.004	>100

are spontaneous and are not enzymatically catalyzed. As summarized in Table 2, all the compounds **3** exhibited pronounced activity against HIV-1- and HIV-2-infected cells without toxicity. Consequently, compounds **3** can be considered as potential prodrugs of the nucleosides **1** and **2**, their H-phosphonate monoesters **4** as well as their monophosphates **5**.

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