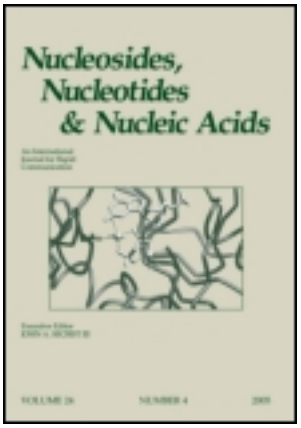


Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

New Dinucleoside Phosphonate Derivatives as Prodrugs of 3′-Azido-3′-Deoxythymidine and β-L-2′,3′-Dideoxy-3′-Thiacytidine: Synthesis and Anti-HIV Properties

Pavel N. Solyev[a], Maxim V. Jasko[a], Inna L. Karpenko[a], Yury A. Sharkin[a], Alexander V. Shipitsyn[a] & Marina K. Kukhanova[a]

[a] Engelhardt Institute of Molecular Biology RAS, Moscow, Russia

Published online: 24 Mar 2014.

To cite this article: Pavel N. Solyev, Maxim V. Jasko, Inna L. Karpenko, Yury A. Sharkin, Alexander V. Shipitsyn & Marina K. Kukhanova (2014) New Dinucleoside Phosphonate Derivatives as Prodrugs of 3′-Azido-3′-Deoxythymidine and β-L-2′,3′-Dideoxy-3′-Thiacytidine: Synthesis and Anti-HIV Properties, Nucleosides, Nucleotides and Nucleic Acids, 33:2, 64-79, DOI: 10.1080/15257770.2013.869340

To link to this article: http://dx.doi.org/10.1080/15257770.2013.869340

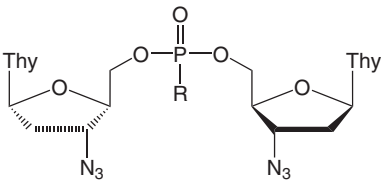
Nucleosides, Nucleotides and Nucleic Acids, 33:64–79, 2014

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2013.869340

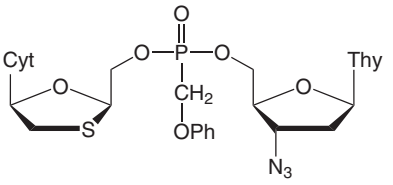
NEW DINUCLEOSIDE PHOSPHONATE DERIVATIVES AS PRODRUGS OF 3′-AZIDO-3′-DEOXYTHYMIDINE AND β-L-2′,3′-DIDEOXY-3′-THIACYTIDINE: SYNTHESIS AND ANTI-HIV PROPERTIES

Pavel N. Solyev, Maxim V. Jasko, Inna L. Karpenko, Yury A. Sharkin, Alexander V. Shipitsyn, and Marina K. Kukhanova
Engelhardt Institute of Molecular Biology RAS, Moscow, Russia

GRAPHICAL ABSTRACT



2a R = -Ph,
2b R = -CH_2OCH_3,
2c R = -CH_2OPh,
2d R = -CH_2F,
2e R = -CH_2I,
2f R = -CH_2N_3



3

□ *New phosphonate homodimers of 3′-azido-3′-deoxythymidine (AZT) and a phosphonate heterodimer of β-L-2′,3′-dideoxy-3′-thiacytidine (3TC) and AZT were synthesized. The compounds demonstrated moderate anti-HIV activity. Stability of the compounds in human blood serum was studied. A correlation between anti-HIV activity and stability was defined.*

Keywords Antiviral nucleosides; HIV nucleosides; nucleoside phosphonates; modified nucleosides

INTRODUCTION

Nucleoside analogues are widely used for the treatment of HIV infection. After penetration into cells, nucleoside analogues undergo three subsequent steps of phosphorylation by cellular kinases, followed by integration of their triphosphates into the 3′ end of the growing proviral DNA being

Received 27 August 2013; accepted 20 November 2013.
The work was supported by the Russian Foundation for Basic Research, projects 12-04-00581-a and 13-04-00829-a; Presidium of the Russian Academy of Sciences, project "Molecular and Cellular Biology."
Address correspondence to Pavel N. Solyev, Engelhardt Institute of Molecular Biology RAS, 32 Vavilov Street, Moscow, 119991, Russia. E-mail: solyev@gmail.com

catalyzed by reverse transcriptase and terminate its synthesis. Among the nucleoside-based derivatives, AZT (Retrovir®) and 3TC (Lamivudine®) are the most commonly applied drugs in anti-HIV "cocktails" that usually contain these two HIV reverse transcriptase inhibitors (Combivir®) alongside with one integrase or protease inhibitor. Despite progress in the treatment of HIV-infected patients, these drugs possess some drawbacks: AZT lifetime lasts in patients only 1 hour,[1] frequent dose administration and long-term usage of AZT cause toxic side effects, i.e., anemia, bone marrow suppression, neuropathy and the emergence of HIV resistant strains.[2–4] There have been developed various depot forms of nucleosides and nucleotides in order to reduce toxic effects of anti-HIV drugs, as well as to increase their oral bioavailability or to improve pharmacokinetic properties.[5–7] A depot form, being inactive on its own, is converted under chemical or enzymatic hydrolysis into an appropriate antiviral inhibitor. Most of depot forms employ the 5′ position for modification of nucleoside/nucleotide analogues. Out of a large number of constructed depot forms of HIV drugs, only one compound was approved by the U.S. FDA for the treatment of HIV infected patients—the prodrug of tenofovir (Viread®).[5,8] Another depot-form—a prodrug of AZT—(5′-hydrogen-phosphonate AZT, Nikavir®) was approved in Russia for the prevention and treatment of AIDS.[9–11] Phosphate/phosphonate derivatives of homo- and heterodimers of anti-HIV nucleoside drugs present a special interest. Such derivatives are depot forms of one or two HIV inhibitors joined together in one molecule. If hydrolyses of components occur at different time or with different rates, the lifetime of active drugs in vivo may be prolonged. Furthermore, two nucleoside anti-HIV drugs can give a synergistic effect that allows us to reduce concentrations of the drugs.[12] The first anti-HIV compounds of this kind, namely bis-(3′-azido-3′-deoxythymidin-5′-*O*-yl) methylphosphonate and bis-(2′,3′-dideoxycytidin-5′-*O*-yl) methylphosphonate, were obtained in 1990.[13] However, they were not readily hydrolyzed to form the active components and did not show anti-HIV activity. Later on, bis-AZT α-hydroxyphosphonates were reported to demonstrate anti-HIV activity comparable to that of AZT.[14] It was also shown that heterodimer conjugates of FLT and AZT with dicarboxylic acids possessed higher anti-HIV activity compared to the parent FLT and AZT.[15] Dinucleoside derivatives proved to be active against other viruses as well. For example, acyclovir phosphate dimer suppressed herpes simplex virus (HSV-1) replication in cell culture more effectively than acyclovir and had better bioavailability due to higher solubility than acyclovir.[16] Bis-(3′-deoxynucleoside) phosphoramidates were shown to be active inhibitors of hepatitis C virus (HCV) in replicon system.[17] We decided to investigate new dinucleoside phosphonates of AZT as prodrugs and make a coupled form of AZT and 3TC phosphonate prodrug. We aimed to define such a phosphonate substituent, which would allow one nucleoside ester bond to be hydrolyzed fast enough to reach the effective drug concentration in blood, while another

nucleoside of the phosphonate, being released slowly and gradually, could maintain the drug concentration in blood for a prolonged period of time. Earlier, we synthesized different phosphonates of AZT and evaluated their anti-HIV activity in the cell culture.[18] Apart from the successful Nikavir®, another phosphonate derivative, AZT aminocarbonylphosphonate, showed lower toxicity and had improved pharmacokinetics as compared to AZT and Nikavir.[19] Now this compound is in Phase II clinical trials.[7] Continuing the search for depot forms of anti-HIV drugs, we focused our attention on dinucleoside phosphonate derivatives.

Herein, we describe the synthesis of six new phosphonate AZT homodimers and one heterodimer containing AZT and 3TC. The stability of compounds in human blood serum, their anti-HIV activity, and toxicity in CEM-SS cell culture have been studied.

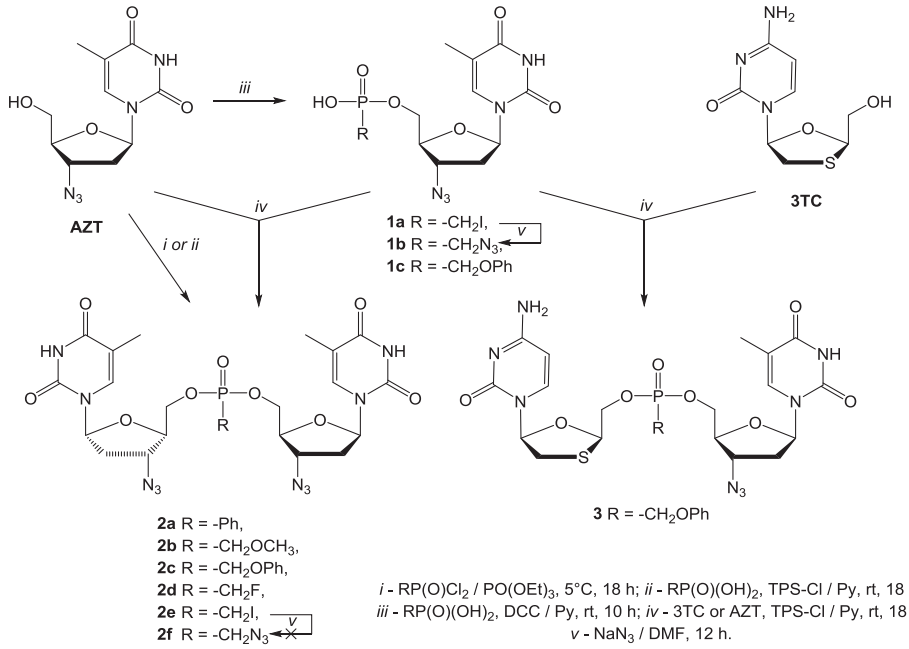
RESULTS AND DISCUSSION

Synthesis and Characterization

Synthesis was performed as described below (Scheme 1). 3′-Azido-3′-deoxythymidin-5′-*O*-yl derivatives were obtained by three different methods: by the reaction of the two-fold excess of AZT with the corresponding phosphonic dichloride (this method was used to produce **2a–b**); or by the direct condensation of the corresponding phosphonic acid with the two-fold excess of the nucleoside and TPS-Cl (synthesis of **2c–e**); as well as by the reaction of AZT with 3′-azido-3′-deoxythymidin-5′-*O*-yl -azidomethylphosphonate **1b** and DCC as a condensing agent in the case of **2f** synthesis.

Dinucleoside heterodimer **3** synthesis required step-by-step condensation of phenoxymethylphosphonic acid with equimolar amount of AZT to afford phosphonate **1c** followed by its reaction with 3TC in the presence of TPS-Cl. We did not protect amino group of 3TC since its further deprotection would cleave the 5′,5′-phosphonodiester bond. A minor product of coupling with 3TC 4-amino group was also observed and was separated by column chromatography. It was noticed that 4-*NH*-product was unstable and in a week decomposed into starting 3TC and **1c**.

This two step route is also feasible for the synthesis of bis-AZT phosphonates **2** instead of three methods described above and was used to obtain azidomethyl-phosphonate **2f**. We intended to synthesize **2f** by the substitution of the iodide adjacent to the methylene fragment by azide. It did not appear to form the desired product in bis-AZT derivatives, but mono-AZT iodomethylphosphonate **1a** successfully substituted the iodide by the azide. This has been proved by the ^{1}H NMR spectra: protons of the methylene group in the obtained **1b** are equivalent and the doublet with $^2J_{CH_2,P} = 11.2$ Hz is observed, while **1a** protons of the methylene group are



SCHEME 1 Synthesis of homodimers of AZT (**2**) and a heterodimer of AZT and 3TC (**3**).

non-equivalent and demonstrate a more complex ABX system with the geminal proton–proton coupling constant of CH_2–P fragment $^2J_{H_a, H_b} = 12.8$ Hz and geminal constants $^2J_{H, P}$ of 9.3 and 9.0 Hz (Figure 1).

Similar azidation of bis-AZT derivatives (**1e** → **1f**) led to the side process of AZT 5′,5′-phosphonodiester bond cleavage.

Determination of $^1J_{C, P}$ and $^1J_{C, F}$ in ^{13}C NMR spectrum of 3′-azido-3′-deoxythymidin-5′-*O*-yl fluoromethylphosphonate **2d** was rather complicated due to their close values, but the ^{13}C satellites in ^{31}P NMR allowed to assign $^1J_{P, C}$ unambiguously. Additionally, **2d** was characterized by ^{19}F NMR confirming all the other spin-coupling constants (Figure 2).

All the products were obtained in good yields and fully characterized by NMR spectra. Their λ_{max} in UV spectra corresponded to those for thymidine derivatives (267 nm), heterodimer **3** displayed λ_{max} of intermediate position (experimental: 268 nm) for thymidine (267 nm) and cytidine (271 nm) derivatives. Starting phosphonic acids were commercial or synthesized by the reported standard procedures.[20–23]

Anti-HIV Activity and Toxicity

Anti-HIV activity and toxicity of the compounds were estimated in HIV-1_{BRU} infected CEM-SS (human T4-lymphoblastoid) cell line as described earlier.[24] The anti-HIV activity of the compounds (Table 1) depends on the phosphonate moiety structure. Homodimer **2d** appeared to

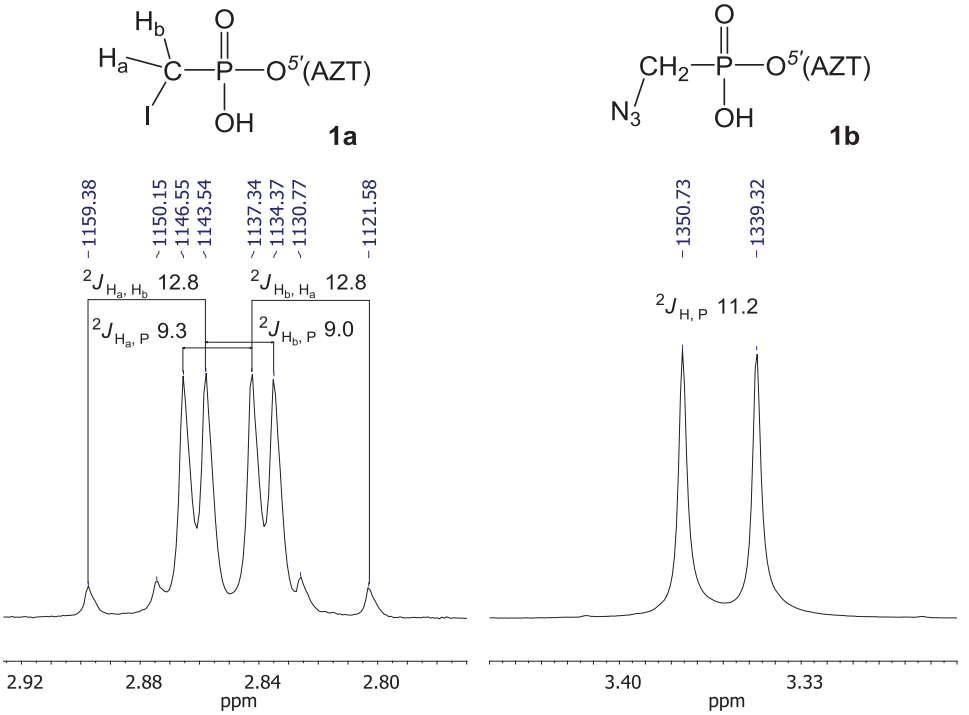


FIGURE 1 X-CH_2-P Peaks in ^{1}H NMR of **1a** and **1b**. (Color figure available online).

be the most active compound. Its inhibiting activity was twice higher than that of AZT and eight times higher than Nikavir. Heterodimer **3**, containing both AZT and 3TC, did not reveal any advantages over AZT and the rest of AZT homodimers demonstrated lower anti-HIV activity than the reference drugs. It is important to mention the augmented toxicity of the synthesized dinucleosides. Product **2d** was about five and six times more toxic than AZT

TABLE 1 Anti-HIV activity of homo- and heterodimers in CEM-SS cell culture

	Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]
2a	Ph-P(O)(AZT)$_2$	10	33	3.3
2b	CH_3OCH_2-P(O)(AZT)$_2$	0.68	31	46
2c	$PhOCH_2$-P(O)(AZT)$_2$	0.25	37	148
2d	FCH_2-P(O)(AZT)$_2$	0.015	29	1933
2e	ICH_2-P(O)(AZT)$_2$	11	39	3.5
2f	N_3CH_2-P(O)(AZT)$_2$	0.18	24.5	136
3	$PhOCH_2$-P(O)(AZT)(3TC)	0.24	60	250
	AZT	0.037	142	3837
	Nikavir	0.131	185	1405

[a]EC_{50}, effective concentration of compounds required to inhibit 50% of viral replication.
[b]CC_{50} , cytotoxic concentration of compounds required to inhibit 50% CEM-SS cell growth.
[c]SI, selectivity index, CC_{50}/ EC_{50}.

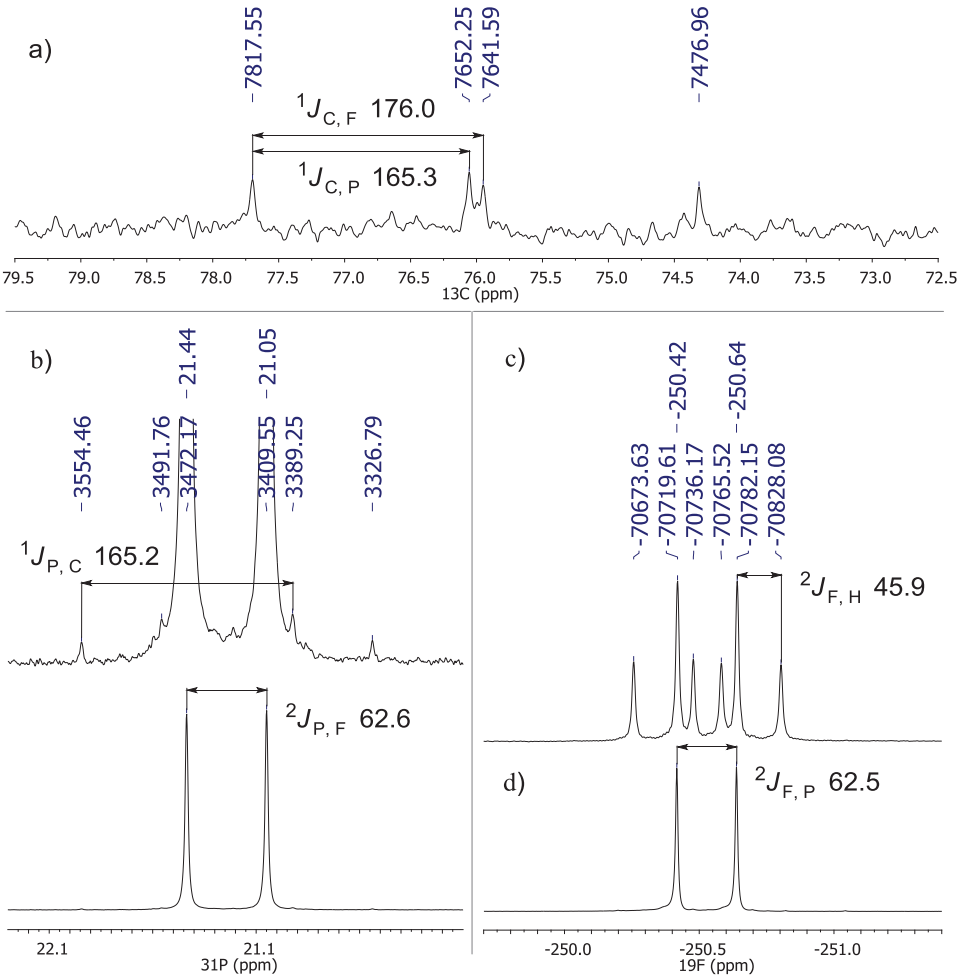


FIGURE 2 Assignments of $^1J_{C,P}$ and $^1J_{C,F}$ in ^{13}C NMR proton decoupled spectrum of **2d** (a) via the ^{13}C satellites in ^{31}P NMR proton decoupled spectrum (b); ^{19}F NMR non-decoupled (c) and proton decoupled (d) spectra of **2d**. (Color figure available online).

and Nikavir, respectively; as a result, its selectivity index was twice lower than that of AZT.

Anti-HIV properties of corresponding mono-AZT phosphonates (FCH_2-P(O)(OH)(AZT), ICH_2-P(O)(OH)(AZT) and N_3CH_2-P(O)(OH)(AZT)) bearing the same substituents as the synthesized bis-AZT phosphonates **2d–f** were studied in our laboratory and described earlier.[18]

The comparison of mono-AZT and bis-AZT phosphonates revealed that SI of **2d** was more than one order higher than that of FCH_2-P(O)(OH)(AZT) (1933 vs. 150), while SI for **2e** was much lower than that of ICH_2-P(O)(OH)(AZT) (3.5 vs. 24). Phosphonate **2f** possessed rather poor SI, as well as its mono-AZT derivative N_3CH_2-P(O)(OH)(AZT) (136 vs. 107).

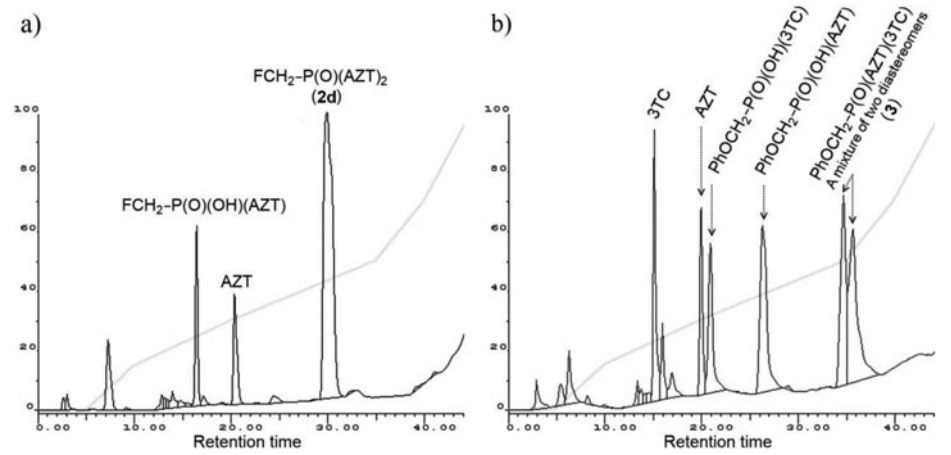


FIGURE 3 HPLC separation of the hydrolysis products of **2d** and **3** after incubation with human blood serum. Products of compound **2d** after 50 min incubation (a); products of compound **3** after 12 hours incubation (b).

Thus, antiviral properties of the anionic mono-AZT phosphonates not always correlate with that of the corresponding bis-AZT phosphonates, and their phosphonate moiety structure and antiviral activity relationship is rather complex to predict it ahead of their synthetic realization into a phosphonate depot form.

Differences in antiviral properties of dinucleoside monophosphates and corresponding mononucleotide analogues were observed earlier.[12] It was noticed that AZT homodimer (AZT)P(O)(OH)(AZT) was more toxic than heterodimers consisting of two anti-HIV drugs. Besides, toxicity varies depending on cell cultures used. The pharmacokinetic study of (AZT)P(O)(OH)(PMPA) allowed to infer that this compound was a depot form gradually releasing AZT and PMPA, and had lower toxicity. Nevertheless, its antiviral activity in cell cultures did not exceed the overall activity of AZT and PMPA combination.[25]

Hydrolysis in Human Blood Serum

Anti-HIV activity of depot forms depends on their hydrolysis rates and the conversion into an active drug form. Therefore, we evaluated the chemical stability of dinucleoside derivatives and their stability in human blood serum.

All the studied compounds were chemically stable for more than a day in pH range 4.5–7. Hydrolysis of the homodimers (**2a–f**) in blood serum resulted in the mixture of two products: AZT and AZT monophosphonate. At the same time, hydrolysis of the heterodimer **3** resulted in the mixture of four products: AZT and 3TC monophosphonate, as well as 3TC and AZT

TABLE 2 Stability of the compounds in human blood serum

	Compound	$T_{1/2}$ (hours)
2a	Ph-P(O)(AZT)$_2$	>24
2b	CH_3OCH_2-P(O)(AZT)$_2$	>24
2c	$PhOCH_2$-P(O)(AZT)$_2$	>24
2d	FCH_2-P(O)(AZT)$_2$	0.78
2e	ICH_2-P(O)(AZT)$_2$	>24
2f	N_3CH_2-P(O)(AZT)$_2$	12
3	$PhOCH_2$-P(O)(AZT)(3TC)	21
	Nikavir	6

monophosphonates. The obtained products were separated by HPLC, the retention times for starting and obtained compounds are given in the "Materials and Methods" section. Half-life data ($T_{1/2}$) were calculated according to the peak area at a different time of hydrolysis. An example of the product separation for **3** is presented in Figure 3.

Half-life data ($T_{1/2}$) of the compounds in human blood serum are shown in Table 2. We think that the hydrolysis of the compounds in human blood serum depends on cellular enzymes such as 5′-nucleotidases or other hydrolyzing enzymes that are mentioned in many publications.[26]

A correlation between hydrolysis half-life and anti-HIV activity of the synthesized dimers is evident (Tables 1 and 2). The compounds showing lower activity appeared to be more stable in human blood serum. All this suggest that the leading anti-HIV activity of **2d** can be explained by its fast hydrolysis to the parent nucleoside.

CONCLUSIONS

To sum up, we have designed and synthesized new phosphonate derivatives with different phosphonate moieties containing two nucleoside drugs in a single molecule and studied their anti-HIV activity, toxicity, and hydrolysis rates. The results confirm that the dimers acted as depot forms. Their antiviral activity in cell cultures was comparable to the referent AZT and Nikavir®, and even surpassed them twice for bis-(3′-azido-3′-deoxythymidin-5′-*O*-yl) fluoromethylphosphonate **2d**. But the increased toxicity demonstrated in CEM-SS cells remains a problem to be solved.

EXPERIMENTAL

Materials and Methods

Reagents and solvents were purchased from Acros (Belgium). The following starting phosphonic compounds were synthesized according to the

procedures previously described in the literature: methoxymethylphosphonic dichloride,[20] iodomethylphosphonic acid,[21] phenoxymethylphosphonic acid,[22] and fluoromethylphosphonic acid.[23] AZT and 3TC were a kind gift of the "AZT Association" (Moscow, Russia). The solvents were purified by standard procedures.

NMR spectra of the products (δ, ppm; J, Hz) were registered on an AMX III-400 spectrometer (Bruker BioSpin Gmbh, Karlsruhe, Germany) with the working frequencies of 400 MHz for ^{1}H NMR, 162 MHz for ^{31}P NMR (with P–H interaction decoupling) and 100.6 MHz for ^{13}C NMR (with C–H interaction decoupling). ^{19}F NMR spectra (δ, ppm; J, Hz) were registered on an Avance II-300 spectrometer (Bruker BioSpin Gmbh, Karlsruhe, Germany), with 282.4 MHz working frequency.

UV spectra were registered on a Shimadzu UV-2401PC spectrophotometer (Shimadzu Corporation, Japan) in methanol, in the range of 200–300 nm and were typical to those of thymidine and cytidine derivatives.

Mass spectra of the products (m/z,% rel. int.) were registered on an LCMS-2020 single quadrupole liquid chromatograph mass spectrometer (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan), using ESI. The measurements were acquired in a positive ion mode (interface capillary voltage, 4800 V), a syringe injection was used for solutions in acetonitrile (flow rate 200 μl/min). Nitrogen was applied as a dry gas; interface temperature was set at 350°C.

The reaction process was checked using TLC on precoated Kieselgel 60F_{254} plates (Merck, Germany) in the following systems: chloroform/methanol 19:1, chloroform/methanol 9:1 and dioxane/ammonia (25% aq.) 4:1. Column chromatography was performed on ion-exchange DEAE-Toyopearl 650 M resin (Tosoh Corporation, Japan) and on silica gel (40–63 μm; Merck, Germany).

Anti-HIV activity and toxicity assays. Anti-HIV activity of the tested compounds (0.01–100 μg/ml, three replicates for each dose) was assessed in CEM-SS cell culture by the measurement of p24 antigen amount using immunoassay after 3 days of incubation. Cellular cytotoxicity was estimated after incubation of CEM-SS cell in the presence of the compounds (0.01–500 μg/ml, three replicates for each dose) after 4 days incubation. The concentration and viability of the cells were measured by the trypan blue day exclusion colorimetric assay, and the CC_{50} for each compound was calculated.[24]

Stability in human blood serum. Tested compounds were incubated with human blood serum at 37°C in the concentration of 500 μM. After certain time intervals, aliquots were taken, and the reaction was terminated by the addition of cool methanol up to 66% (v/v). The formed precipitate was pelleted by centrifugation for 15 min at 20,000×g. The supernatant was dried in a Speed-Vac concentrator, the residue was dissolved

in 5 mM aqueous phosphate buffer (pH 5.20), and the products were analyzed by HPLC. The compound concentrations were calculated by peak areas.

Centrifugation was performed on an Eppendorf 5415 centrifuge (Eppendorf AG, Hamburg, Germany). An automatic Speed-Vac Concentrator AS260 (Savant, Osterville, MA, USA) was used for concentrating the sample.

HPLC analysis was performed using a Gilson chromatograph (Gilson Inc., Middleton, WI, USA) supplied with a digital GSIOC 506 controller and a Gilson-315 UV detector with varying wavelengths. Compounds were detected at λ 267 nm using a Nucleosil 100 C-18 column (5 μm, 4 × 150 mm) with a C-18 precolumn (10 μm, 4 × 8 mm); using mobile phase of 80% aqueous EtOH (A) in 5 mM aqueous phosphate buffer (pH 5.2). Gradient parameters: 0% A for 5 min; 0% → 15% A for 5 min; 15% → 30% A for 10 min; 30% → 70% A for 20 min; 70% → 100% A for 5 min; 100% → 0% A for 5 min; 0% A for 5 min; the flow rate of 0.5 ml/min. Retention times were as follows: AZT, 19.8 min; 3TC, 15.3 min; **2a**, 36.7 min; **2b**, 29.0 min; **2d**, 29.7 min; **2e**, 34.0 min; **2f**, 32.0 min; **3**, 32.0 min; Ph-P(O)(OH)(AZT), 23.8 min; CH_3OCH_2-P(O)(OH)(AZT), 16.3 min; $PhOCH_2$-P(O)(OH)(AZT), 25.7 min; $PhOCH_2$-P(O)(OH)(3TC), 20.6 min; FCH_2-P(O)(OH)(AZT), 16.7 min; ICH_2-P(O)(OH)(AZT), 20.3 min; N_3CH_2-P(O)(OH)(AZT), 17.8 min.

Standard Procedure for Synthesis of Anionic Phosphonate Derivatives 1a, 1c

Mono-AZT phosphonates **1a**, **1c** were synthesized similarly to the previously studied AZT alkylphosphonates.[27] A solution of the corresponding phosphonic acid (0.6 mmol) and AZT (0.6 mmol) in pyridine (3 ml) was treated with DCC (1.2 mmol). The reaction mixture was stirred at room temperature for 10 hours and then diluted with water. After 30 minutes of additional stirring the solution was separated from the precipitate, concentrated by evaporation under vacuum, and re-diluted with water. The solution was applied onto a DEAE-Toyopearl column (20 × 175 mm) and eluted with a linear gradient of NH_4HCO_3 (0 → 0.2 M, 500 ml). The target fraction was concentrated by evaporation under vacuum, the residue was diluted with water and re-evaporated.

Standard Procedure for Synthesis of Dinucleoside Phosphonate Derivatives 2 and 3

Method A

The following method was used for synthesis of **2a**, **2b**. AZT (106 mg, 0.4 mmol) was added to a solution of the corresponding phosphonic dichloride (0.2 mmol) in triethylphosphate (1 ml). The reaction mixture was

stirred at 4°C for 18 h and then diluted with water, concentrated by evaporation under vacuum, applied onto a silica gel column (20 × 250 mm) and eluted with a gradient of methanol in chloroform (0 → 15%). The target fraction was concentrated by evaporation under vacuum.

Method B

The following method was used for synthesis of **2c–2e**. AZT (106 mg, 0.4 mmol) was added to a solution of the corresponding phosphonic acid (0.2 mmol) in pyridine (3 ml), cooled to 0°C and treated with TPS-Cl (182 mg, 0.6 mmol). The reaction mixture was stirred at 4°C for 18 hours and then 1 ml of the saturated aqueous $NaHCO_3$ solution was added. The mixture was concentrated by evaporation under vacuum, applied onto a silica gel column (20 × 250 mm) and eluted with a gradient of methanol in chloroform (0 → 15%). The target fraction was concentrated by evaporation under vacuum.

Method C

The following method was used for synthesis of **2f** and **3**. To a solution of the phosphonate **1** (0.2 mmol) in pyridine (3 ml), an equimolar amount of the corresponding nucleoside was added (53 mg, 0.2 mmol of AZT for synthesis of homodimer **2f**; or 46 mg, 0.2 mmol of 3TC for synthesis of heterodimer **3**), cooled to 0°C and treated with TPS-Cl (91 mg, 0.3 mmol). The reaction mixture was stirred at 4°C for 18 hours and then 1 ml of the saturated aqueous $NaHCO_3$ solution was added. The mixture was concentrated by evaporation under vacuum, applied onto a silica gel column (20 × 250 mm) and eluted with a gradient of methanol in chloroform (0 → 15%). The target fraction was concentrated by evaporation under vacuum.

3'-Azido-3'-deoxythymidin-5'-O-yl -iodomethylphosphonate (1a)

Obtained 282 mg (yield 73%) as a yellowish viscous liquid. ^{1}H NMR, δ (CD_3OD): 7.55 (1H, s, H-6), 6.06 (1H, t, $^3J_{1',2'}$ 6.5 Hz, H-1'), 4.36 (1H, m, H-3'), 3.95–4.02 (3H, m, H-4' & H-5'), 3.31 (1H, dd, $^2J_{H_a,H_b}$ 12.8 Hz, $^2J_{H_a,P}$ 9.0 Hz, CH_a-P) 3.29 (1H, dd, $^2J_{H_b,H_a}$ 12.8 Hz, $^2J_{H_b,P}$ 9.3 Hz, CH_b-P), 2.34 (2H, m, H-2'), 1.75 (3H, s, 5-CH_3). ^{31}P NMR, δ (CD_3OD): 17.00 (s).

3'-Azido-3'-deoxythymidin-5'-O-yl -azidomethylphosphonate (1b)

A solution of **1a** (94 mg, 0.2 mmol) in DMF (2 ml) was treated with NaN_3 (20 mg, 0.3 mmol). The reaction mixture was stirred at 60°C for 12 hours, cooled to 0°C and diluted with water, concentrated by evaporation under vacuum and re-diluted with water. The solution was applied onto a DEAE-Toyopearl column (20 × 175 mm) and eluted with a linear gradient of NH_4HCO_3 (0 → 0.2 M, 500 ml). The target fraction was concentrated

by evaporation under vacuum, the residue was diluted with water and re-evaporated to result in 69 mg (yield 90%) of **1b** as a yellowish viscous liquid. ^{1}H NMR, δ (CD_3OD): 7.58 (1H, s, H-6), 6.11 (1H, t, $^{3}J_{1',2'}$ 6.5 Hz, H-1'), 4.38 (1H, m, H-3'), 4.05 (3H, m, H-4' & H-5'), 3.33 (2H, d, $^{2}J_{CH_2,P}$ 11.2 Hz, CH_2-P), 2.38 (2H, m, H-2'), 1.80 (3H, s, 5-CH_3). ^{31}P NMR, δ (CD_3OD): 17.21 (s).

3'-Azido-3'-deoxythymidin-5'-O-yl -phenoxymethylphosphonate (1c)

Obtained 223 mg (yield 85%) as a colorless viscous liquid. ^{1}H NMR, δ (CD_3OD): 7.82 (1H, q, $^{4}J_{H\text{-}6,\,5\text{-}CH_3}$ 1.2 Hz, H-6), 7.25 (2H, m, *o*-Ph), 6.96 (2H, m, *m*-Ph) 6.92 (1H, m, *p*-Ph), 6.22 (1H, dd, $^{3}J_{1',2'_a}$ 6.0 Hz, $^{3}J_{1',2'_b}$ 7.7 Hz, H-1'), 4.50 (1H, m, H-3'), 4.22 (2H, m, H-5'), 4.13 (2H, d, $^{2}J_{H,P}$ 10.0 Hz, CH_2-P), 4.09 (1H, m, H-4'), 2.41 (2H, m, H-2'), 1.86 (3H, d, $^{4}J_{5\text{-}CH_3,\,H\text{-}6}$ 1.2 Hz, 5-CH_3). ^{31}P NMR, δ (CD_3OD): 16.33 (s).

Bis-(3'-azido-3'-deoxythymidin-5'-O-yl) phenylphosphonate (2a)

Obtained 85 mg (yield 65%) as a colorless viscous liquid. ^{1}H NMR, δ ($CDCl_3$): 10.26 & 10.23 (2H, 2 × s, 3-NH), 7.82 (2H, m, *o*-Ph), 7.61 (1H, t, $^{3}J_{p\text{-Ph},\,m\text{-Ph}}$ 7.5 Hz, *p*-Ph), 7.49 (2H, m, *m*-Ph), 7.32 & 7.17 (2H, 2 × s, H-6), 6.13 & 6.07 (2H, 2 × t, $^{3}J_{1',2'}$ 6.5 Hz, H-1'), 4.35 (6H, m, H-3' & H-5'), 4.06 (2H, m, H-4'), 2.41 (4H, m, H-2'), 1.79 & 1.77 (6H, 2 × s, 5-CH_3). ^{13}C NMR, δ ($CDCl_3$): 164.29 (s, C-4), 150.65 (s, C-2), 135.92 (s, C-6), 133.90 (s, *p*-Ph), 131.83 (d, $^{2}J_{C,P}$ 10.4 Hz, *o*-Ph), 129.12 (d, $^{3}J_{C,P}$ 16.1 Hz, *m*-Ph), 126.17 (d, $^{1}J_{C,P}$ 189.5 Hz, *i*-Ph), 111.46 & 111.38 (2 × s, C-5), 85.82 (2 × s, C-1'), 82.47 (d, $^{3}J_{C,P}$ 5.6 Hz, C-4'), 65.64 & 65.68 (d, $^{2}J_{C,P}$ 4.8 Hz, C-5'), 60.79 & 60.69 (2 × s, C-3'), 37.25 & 37.12 (2 × s, C-2'), 12.40 (s, 5-CH_3). ^{31}P NMR, δ ($CDCl_3$): 15.91 (s). ESI MS, *m/z* (% rel. int.): 680.2 ($M + Na^+ + H^+$, 37.0%), 679.3 ($M + Na^+$, 100%), 658.4 ($M + 2 \times H^+$, 34.4%), 657.3 ($M + H^+$, 25.4%).

Bis-(3'-azido-3'-deoxythymidin-5'-O-yl) methoxymethylphosphonate (2b)

Obtained 87 mg (yield 70%) as a colorless viscous liquid. ^{1}H NMR, δ (DMSO-d_6): 10.99 (2H, br.s, 3-NH), 7.50 & 7.47 (2H, 2 × q, $^{4}J_{H\text{-}6,\,5\text{-}CH_3}$ 1.2 Hz, H-6), 6.12 (2H, t, $^{3}J_{1',2'}$ 6.7 Hz, H-1'), 4.44 (2H, m, H-3'), 4.25 (4H, m, H-5'), 3.99 (2H, m, H-4'), 3.88 (1H, dd, $^{2}J_{H_a,H_b}$ 15.5 Hz, $^{2}J_{H_a,P}$ 8.0 Hz, CH_a-P), 3.86 (1H, dd, $^{2}J_{H_b,H_a}$ 15.5 Hz, $^{2}J_{H_b,P}$ 8.0 Hz, CH_b-P), 3.35 (3H, d, $^{4}J_{H,P}$ 1.1 Hz, OCH_3), 2.46–2.29 (4H, m, H-2'), 1.78 (6H, br.s, 5-CH_3). ^{13}C NMR, δ (DMSO-d_6): 163.54 (s, C-4), 150.28 (s, C-2), 135.81 (s, C-6), 109.85 (s, C-5), 83.72 (s, C-1'), 81.42 & 81.38 (2 × d, $^{3}J_{C,P}$ 5.4 Hz, C-4'), 65.22 (d, $^{1}J_{C,P}$ 162.5 Hz, CH_2-P), 65.15 & 65.08 (2 × d, $^{2}J_{C,P}$ 3.1 & 4.5 Hz, C-5'), 60.42 (d, $^{3}J_{C,P}$ 13.0 Hz, OCH_3), 59.98 & 59.96 (2 × s, C-3'), 35.50 & 35.46 (2 × s, C-2'), 11.91 & 11.87 (2 × s, 5-CH_3). ^{31}P NMR, δ (DMSO-d_6): 25.62 (s). ESI MS, *m/z* (% rel. int.): 647.2 ($M + Na^+$, 100%), 625.2 ($M + H^+$, 13.9%).

Bis-(3'-azido-3'-deoxythymidin-5'-O-yl) phenoxymethylphosphonate (2c)

Obtained 103 mg (yield 75%) as a colorless viscous liquid. ^{1}H NMR, δ (DMSO-d_6): 11.30 (2H, br.s, 3-NH), 7.51 & 7.47 (2H, 2 × q, $^4J_{\text{H-6, 5-CH}_3}$ 1.2 Hz, H-6), 7.27 (2H, m, *o*-Ph), 6.96 (3H, m, *m*-Ph & *p*-Ph), 6.13 & 6.11 (2H, 2 × t, $^3J_{1', 2'}$ 7.5 Hz, H-1'), 4.55 (2H, d, $^2J_{\text{CH}_2, \text{P}}$ 9.9 Hz, CH_2-P), 4.45 (2H, m, H-3'), 4.33 (4H, m, H-5'), 4.02 (2H, m, H-4'), 2.37 (4H, m, H-2'), 1.74 & 1.72 (6H, 2 × d, $^4J_{\text{5-CH}_3, \text{H-6}}$ 1.0 & 1.3 Hz, 5-CH_3). ^{13}C NMR, δ (DMSO-d_6): 163.55 (s, C-4), 158.07 (d, $^3J_{\text{C, P}}$ 14.2 Hz, *i*-Ph), 150.29 (s, C-2), 135.84 & 135.80 (2 × s, C-6), 129.43 (s, *m*-Ph), 121.58 (s, *p*-Ph), 114.38 (s, *o*-Ph), 109.90 (s, C-5), 83.76 (s, C-1'), 81.41 & 81.35 (2 × d, $^3J_{\text{C, P}}$ 5.8 Hz, C-4'), 65.68 (d, $^2J_{\text{C, P}}$ 6.1 Hz, C-5'), 61.00 (d, $^1J_{\text{C, P}}$ 166.5 Hz, CH_2-P), 60.01 (s, C-3'), 35.52 (s, C-2'), 11.92 & 11.88 (2 × s, 5-CH_3). ^{31}P NMR, δ (DMSO-d_6): 24.12 (s). ESI MS, *m/z* (% rel. int.): 710.2 (M + Na^++H^+, 33.1%), 709.2 (M + Na^+, 100.0%), 688.2 (M + 2 × H^+, 25.4%), 687.2 (M + H^+, 47.9%).

Bis-(3'-azido-3'-deoxythymidin-5'-O-yl) fluoromethylphosphonate (2d)

Obtained 82 mg (yield 67%) as a colorless viscous liquid. ^{1}H NMR, δ (DMSO-d_6): 11.01 (2H, br.s, 3-NH), 7.48 & 7.45 (2H, 2 × q, $^4J_{\text{H-6, 5-CH}_3}$ 0.8 Hz, H-6), 6.13 (2H, dd + t, $^3J_{1', 2'_a}$ 6.7 Hz, $^3J_{1', 2'_b}$ 6.5 Hz; $^3J_{1', 2'}$ 6.7 Hz, H-1'), 5.00 (2H, dd, $^2J_{\text{CH}_2, \text{F}}$ 45.8 Hz, $^2J_{\text{CH}_2, \text{P}}$ 4.5 Hz, CH_2-P), 4.45 (2H, m, H-3'), 4.32 (4H, m, H-5'), 4.01 (2H, m, H-4'), 2.47–2.30 (4H, m, H-2'), 1.78 (6H, br.s, 5-CH_3). ^{13}C NMR, δ (DMSO-d_6): 163.55 (s, C-4), 150.28 (s, C-2), 135.82 & 135.78 (2 × s, C-6), 109.90 (s, C-5), 83.80 & 83.72 (2 × s, C-1'), 81.26 & 81.20 (2 × d, $^3J_{\text{C, P}}$ 5.4 Hz, C-4'), 76.01 (dd, $^1J_{\text{C, P}}$ 165.2 Hz, $^1J_{\text{C, F}}$ 175.9 Hz, CH_2-P), 65.56 (br.s, C-5'), 59.85 (s, C-3'), 35.51 & 35.45 (2 × s, C-2'), 11.88 & 11.84 (2 × s, 5-CH_3). ^{31}P NMR, δ (DMSO-d_6): 21.25 (d, $^2J_{\text{P, F}}$ 62.6 Hz). ^{19}F NMR, δ (DMSO-d_6): -250.53 (dt, $^2J_{\text{F, P}}$ 62.6 Hz, $^2J_{\text{F, H}}$ 45.8 Hz). ESI MS, *m/z* (% rel. int.): 635.3 (M + Na^+, 100%), 613.2 (M + H^+, 27.5%).

Bis-(3'-azido-3'-deoxythymidin-5'-O-yl) iodomethylphosphonate (2e)

Obtained 101 mg (yield 70%) as a colorless viscous liquid. ^{1}H NMR, δ (DMSO-d_6): 11.24 (2H, s, 3-NH), 7.50 & 7.46 (2H, 2 × s, H-6), 6.13 & 6.12 (2H, 2 × t, $^3J_{1', 2'}$ 6.5 Hz, H-1'), 4.45 (2H, m, H-3'), 4.26 (4H, m, H-5'), 4.02 (2H, m, H-4'), 3.39 (2H, d, $^2J_{\text{H, P}}$ 10.3 Hz, CH_2-P), 2.40 (4H, m, H-2'), 1.80 (6H, s, 5-CH_3). ^{13}C NMR, δ (DMSO-d_6): 163.53 (s, C-4), 150.27 (s, C-2), 135.78 (s, C-6), 109.92 (s, C-5), 83.87 (s, C-1'), 81.43 & 81.38 (2 × d, $^3J_{\text{C, P}}$ 5.6 & 4.8 Hz, C-4'), 65.89 & 65.83 (2 × d, $^2J_{\text{C, P}}$ 6.1 & 6.4 Hz, C-5'), 60.03 (s, C-3'), 35.54 (s, C-2'), 12.00 & 11.92 (2 × s, 5-CH_3), -14.61 (d, $^1J_{\text{C, P}}$ 151.0 Hz, CH_2-P). ^{31}P NMR, δ (DMSO-d_6): 15.58 (s). ESI MS, *m/z* (% rel. int.): 743.15 (M + Na^+, 100%), 721.2 (M + H^+, 48.4%).

Bis-(3'-azido-3'-deoxythymidin-5'-O-yl) azidomethylphosphonate (2f)

Obtained 75 mg (yield 59%) as a colorless viscous liquid. ^{1}H NMR, δ (DMSO-d_6): 11.28 (2H, s, 3-NH), 7.49 & 7.46 (2H, 2 × s, H-6), 6.13 & 6.12

(2H, 2 × t, $^{3}J_{1',2'}$ 6.5 Hz, H-1'), 4.43 (2H, m, H-3'), 4.29 (4H, m, H-5'), 4.00 (2H, m, H-4'), 3.92 (2H, d, $^{2}J_{CH_2,P}$ 11.8 Hz, CH_2-P), 2.38 (4H, m, H-2'), 1.79 (6H, s, 5-CH_3); ^{13}C NMR, δ (DMSO-d_6): 163.56 (s, C-4), 150.27 (s, C-2), 135.85 (s, C-6), 109.90 (s, C-5), 83.88 (s, C-1'), 81.33 & 81.28 (2 × d, $^{3}J_{C,P}$ 4.8 Hz, C-4'), 65.58 & 65.53 (2 × d, $^{2}J_{C,P}$ 5.6 Hz, C-5'), 59.94 (s, C-3'), 44.60 (d, $^{1}J_{C,P}$ 152.6 Hz, CH_2-P), 35.47 (s, C-2'), 11.86 & 11.82 (2 × s 5-CH_3); ^{31}P NMR, δ (DMSO-d_6): 25.06 (s). ESI MS, *m/z* (% rel. int.): 659.4 (M + Na^++H^+, 36.0%), 658.3 (M + Na^+, 100%), 636.3 (M + H^+, 48.7%)

(3'-Azido-3'-deoxythymidin-5'-O-yl) (β-L-2',3'-dideoxy-3'-thiacytidin-5'-O-yl) phenoxymethylphosphonate (3)

Obtained 58 mg (yield 45%) as a colorless viscous liquid. ^{1}H NMR, δ (DMSO-d_6): 11.31 (1H, s, 3-NH (AZT)), 7.71 & 7.70 (1H, 2 × d, $^{3}J_{H\text{-}6,H\text{-}5}$ 7.5 Hz, H-6 (3TC)), 7.51 & 7.48 (1H, 2 × q, $^{4}J_{H\text{-}6,5\text{-}CH_3}$ 1.1 Hz, H-6 (AZT)), 7.28 (2H, m, *o*-Ph), 7.20 (2H, br.s, NH_2 (3TC)), 6.98 (3H, m, *m*-Ph & *p*-Ph), 6.25 & 6.24 (1H, 2 × dd, $^{3}J_{1',2'_a}$ 5.5 Hz, $^{3}J_{1',2'_b}$ 5.1 Hz, H-1' (3TC)), 6.13 (1H, t + dd, $^{3}J_{1',2'}$ 6.7 Hz, $^{3}J_{1',2'_a}$ 6.7 Hz, $^{3}J_{1',2'_b}$ 6.9 Hz, H-1' (AZT)), 5.73 (1H, d, $^{3}J_{H\text{-}6,H\text{-}5}$ 7.5 Hz, H-5 (3TC)), 5.37 (1H, 2 × t, $^{3}J_{4',5'}$ 4.8 Hz, H-4'(3TC)), 4.55 & 4.54 (2H, 2 × d, $^{2}J_{CH_2,P}$ 9.9 Hz, CH_2-P), 4.46 (1H, m, H-3' (AZT), 4.38 (2H, m, H-5' (AZT)), 4.32 (2H, m, H-5' (3TC)), 4.03 (1H, m, H-4' (AZT)), 3.38 (1H, dd, $^{3}J_{2'_a,1'}$ 5.3 Hz, $^{2}J_{2'_a,2'_b}$ 11.5 Hz, H-2'$_a$ (3TC)), 3.09 (1H, dd, $^{3}J_{2'_b,1'}$ 6.6 Hz, $^{2}J_{2'_b,2'_a}$ 11.5 Hz, H-2'$_b$ (3TC)), 2.37 (2H, m, H-2' (AZT)), 1.74 & 1.72 (3H, 2 × d, $^{4}J_{5\text{-}CH_3,H\text{-}6}$ 1.1 Hz). ^{13}C NMR, δ (DMSO-d_6): 165.52 (s, C-4 (AZT)), 163.53 (s, C-4 (3TC)), 158.07 (d, $^{3}J_{C,P}$ 14.2 Hz, *i*-Ph), 154.48 (s, C-2 (AZT)), 150.30 (s, C-2 (3TC)), 140.49 (s, C-6 (3TC)), 135.72 (s, C-6 (AZT)), 129.43 (s, *m*-Ph), 121.55 (s, *p*-Ph), 114.48 & 114.44 (2 × s, *o*-Ph), 109.96 & 109.92 (2 × s, C-5 (AZT)), 94.43 (s, C-5 (3TC)), 87.13 & 87.09 (2 × s, C-1' (3TC)), 83.71 & 83.68 (2 × s, C-1' (AZT)), 81.41 & 81.37 (2 × d, $^{3}J_{C,P}$ 6.1 & 5.0 Hz, C-4' (3TC)), 81.17 & 81.11 (2 × d, $^{3}J_{C,P}$ 5.7 & 5.9 Hz, C-4' (AZT)), 67.18 & 67.07 (2 × d, $^{2}J_{C,P}$ 6.4 & 6.0 Hz, C-5' (3TC)), 65.49 & 65.42 (2 × d, $^{2}J_{C,P}$ 6.7 & 5.4 Hz, C-5' (AZT)), 61.16 & 61.02 (2 × d, $^{1}J_{C,P}$ 167.3 & 165.9 Hz, CH_2-P), 60.07 (s, C-3' (AZT)), 35.36 & 35.50 (2 × s, C-2' (AZT)), 35.09 & 35.07 (2 × s, C-2' (3TC)), 11.96 & 11.93 (2 × s, 5-CH_3 (AZT)). ^{31}P NMR, δ (DMSO-d_6): 26.09 (s) & 25.68 (s). ESI MS, *m/z* (% rel. int.): 672.2 (M + Na^++H^+, 31.6%), 671.2 (M + Na^+, 100.0%), 650.3 (M + 2 × H^+, 27.4%), 649.2 (M + H^+, 68.9%).

ABBREVIATIONS

HIV-1	human immunodeficiency virus type 1
AZT	3′-azido-3′-deoxythymidine
3TC	β-L-2′,3′-dideoxy-3′-thiacytidine
FLT	3′-fluoro-3′-deoxythymidine
PMPA	9-[2-(*R*)-(phosphonomethoxy)propyl]adenine

DCC N,N'-dicyclohexylcarbodiimide
TPS-Cl 2,4,6-triisopropylbenzenesulfonyl chloride.

REFERENCES

1. Cload, P.A. A review of the pharmacokinetics of zidovudine in man. *J. Infect.* **1989**, 18(Suppl. 1), 15–21.
2. Chariot, P.; Drogou, I.; de Lacroix-Szmania, I.; Eliezer-Vanerot, M.C.; Chazaud, B.; Lombès, A.; Schaeffer, A.; Zafrani, E.S. Zidovudine-induced mitochondrial disorder with massive liver steatosis, myopathy, lactic acidosis, and mitochondrial DNA depletion. *J. Hepatol.* **1999**, 30, 156–160.
3. Johnson, A.A.; Ray, A.S.; Hanes, J.; Suo, Z.; Colacino, J.M.; Anderson, K.S.; Johnson, K.A. Toxicity of antiviral nucleoside analogs and the human mitochondrial DNA polymerase. *J. Biol. Chem.* **2001**, 276, 40847–40857.
4. Cihlar, T.; Ray, A.S. Nucleoside and nucleotide HIV reverse transcriptase inhibitors: 25 years after zidovudine. *Antivir. Res.* **2010**, 85, 39–58.
5. Parang, K.; Weibe, L.I.; Knaus, E.E. Novel approaches for designing 5'-*O*-ester prodrugs of 3'-azido-2',3'-dideoxythymidine (AZT). *Curr. Med. Chem.* **2000**, 7, 995–1039.
6. Skoblov, Yu.; Karpenko, I.; Shirokova, E.; Popov, K.; Andronova, V.; Galegov, G.; Kukhanova, M. Intracellular metabolism and pharmacokinetics of 5'-hydrogen-phosphonate of 3'-azido-2',3'-dideoxythymidine, a prodrug of 3'-azido-2',3'-dideoxythymidine. *Antivir. Res.* **2004**, 63, 107–113.
7. Khandazhinskaya, A.; Matyugina, E.; Shirokova, E. Anti-HIV therapy with AZT prodrugs: AZT phosphonate derivatives, current state and prospects. *Expert Opin. Drug Metab.* **2010**, 6, 701–714.
8. Balzarini, J.; Holy, A.; Jindrich, J.; Naesens, L.; Snoeck, R.; Schols, D.; De Clercq, E. Differential antiherpesvirus and antiretrovirus effects of the (S) and (*R*) enantiomers of acyclic nucleoside phosphonates: potent and selective in vitro and in vivo antiretrovirus activities of (*R*)-9-(2-phosphonomethoxypropyl)-2,6-diaminopurine. *Antimicrob. Agents Chemother.* **1993**, 37, 332–338.
9. Khorlin, A.A.; Tarusova, N.B.; Dyatkina, N.B.; Kraevsky, A.A.; Bibilashvili, R.Sh.; Galegov, G.A.; Zhdanov, V.M.; Korneeva, M.N.; Nosik, D.N.; Maiorova, S.N.; Shobukhov, V.M. *R.F. Patent 1548182,* 1989.
10. Kukhanova, M.K.; Shirokova, E.A. *5'-O-Modified nucleoside analogues as prodrugs of anti-HIV agents, in Front. Nucleic Acids.*, eds. R. Schinazi and D. Liotta, IHL Press, Tucker, GA, USA, 2005, pp. 341–363.
11. Ivanova, E.; Shmagel, N.; Vorobeva, N. 2011. *Nikavir in chemoprevention regimens of vertical HIV transmission, in Understanding HIV/AIDS Management and Care – Pandemic Approaches in the 21st century*, ed. F.H. Kasenga, InTech, Croatia, pp. 125–148.
12. Schinazi, R.; Sommadossi, J.-P.; Saalmann, V.; Cannon, D.L.; Xie, M.-Y.; Hart, G.C.; Smith, G.A.; Hahn, E.F. Activities of 3'-azido-3'-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* **1990**, 34, 1061–1067.
13. Puech, F.; Gosselin, G.; Balzarini, J.; Good, S.S.; Rideout, J.L.; De Clercq, E.; Imbach, J.L. Synthesis and biological evaluation of dinucleoside methylphosphonates of 3'-azido-3'-deoxythymidine and 2',3'-dideoxycytidine, *Antiviral Res.* **1990**, 14, 11–23.
14. Meier, C.; Habel, L.; Laux, W.; De Clercq, E.; Balzarini, J. Homo dinucleoside-α-hydroxy-phosphonate diesters as prodrugs of the antiviral nucleoside analogues 2',3'-dideoxythymidine and 3'-azido-2',3'-dideoxythymidine. *Nucleosides Nucleotides* **1995**, 14, 759–762.
15. Agarval, H.K.; Buckheit, K.W.; Buckheit, Jr., R.W.; Parang, K. Synthesis and anti-HIV activities of symmetrical dicarboxylate esters of dinucleoside reverse transcriptase inhibitors. *Bioorg. Med. Chem. Lett.* **2012**, 22, 5451–5454.
16. Brandi, G.; Rossi, L.; Schiavano, G.F.; Millo, E.; Magnani, V. A new homodimer of aciclovir as a prodrug with increased solubility and antiviral activity. *Int. J. Antimicrob. Agents* **2009**, 34, 177–180.
17. Priet, S.; Zlatev, I.; Barvik, I.; Geertas, K.; Leyssen, P.; Neyts, J.; Dutartre, H.; Canard, B.; Vasseur, J.-J.; Morvan, F.; Alvarez, K. 3'-Deoxy phosphoramidate dinucleosides as improved inhibitors of hepatitis C virus subgenomic replicon and NS5B polymerase activity. *J. Med. Chem.* **2010**, 53, 6608–6617.
18. Krayevsky, A.A.; Watanabe, K.A. *Modified Nucleosides as Anti-AIDS Drugs: Current Status and Perspectives*, Bioinform, Moscow, 1993, pp. 98–125.
19. Khandazhinskaya, A.L.; Yanvarev, D.V.; Jasko, M.V.; Shipitsin, A.V.; Khalizev, V.A.; Shram, S.I.; Skoblov, Y.S.; Shirokova, E.A.; Kukhanova, M.K. 5'-Aminocarbonyl phosphonates as new zidovudine

depot forms: antiviral properties, intracellular transformations, and pharmacokinetic parameters. *Drug Metab. Dispos.* **2009**, 37, 494–501 (Erratum: *Drug Metab. Dispos.* **2010**, 38, 1258).
20. Grisley Jr. D.W.; Szabo, K. Alkoxymethyl-phosphonic and alkoxymethyl-phosphono-thioic derivatives. *J. Chem. Eng. Data* **1974**, 19, 175–179.
21. Crofts, P.C.; Kosolapoff, G.M. The preparation and the determination of apparent dissociation constants of some substituted aliphatic phosphonic acids. *J. Am. Chem. Soc.* **1953**, 75, 5738–5740.
22. Walsh, E.N.; Beck, T.M.; Toy, A. D. F. Phenoxymethylphosphonic acids and phosphonic acid ion-exchange resins. *J. Am. Chem. Soc.* **1956**, 78, 4455–4458.
23. Gryszkiewicz-Trochimowski, E. Acide fluorométhanephosphonique et quelques dérivés. *Bull. Soc. Chim. Fr.* **1967**, 11, 4289–4290.
24. Shirokova, E.A.; Jasko, M.V.; Khandazhinskaya, A.L.; Ivanov, A.V.; Yanvarev, D.V.; Skoblov, Y.S.; Mitkevich, V.A.; Bocharov, E.V.; Pronyaeva, T.R.; Fedyuk, N.V.; Kukhanova, M.K.; Pokrovsky, A.G. Uncharged AZT and D4T derivatives of phosphonoformic and phosphonoacetic acids as anti-HIV pronucleosides. *J. Med. Chem.* **2004**, 47, 3606–3614.
25. Rossi, L.; Serafini, S.; Franchetti, P.; Capellacci, L.;. Fraternale, A; Casabianca, A.; Brandi, G.; Pierige, F.;. Perno, C.-F.; Balestra, E.; Benatti, U.; Millo, E.; Grifantini, M.; Magnani, M. Targeting nucleotide dimers containing antiviral nucleosides. *Curr. Med. Chem.* **2005**, 4, 37–54.
26. Kukhanova, M.K.; Tarusova, N.B.; Ias'ko M.V.; Arzumanov A.A.; Gudima S.O.; Kraevskii, A.A.; Chidzhavadze, Z.G.; Bibilashvili, R.Sh. Hydrolysis of 5'-phosphonates and phosphates by phosphatases of various origin and human and calf serum. *Mol. Biol. (Moscow)* **1992**, 4, 1148–59.
27. Jasko, M.V.; Shipitsyn, A.V.; Khandazhinskaya, A.L.; Shirokova, E.A.; Sol'yev, P.N.; Plyasunova, O.A.; Pokrovskii, A.G. New derivatives of alkyl- and aminocarbonyl-phosphonic acids containing 3'-azido-3'-deoxythymidine. *Russ. J. Bioorg. Chem.* **2006**, 32, 542–546.