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Synthesis and Biological Properties of α-Thymidine 5'-Aryl Phosphonates

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Abstract—Diethyl(*N*-arylaminocarbonyl)methyl phosphonates have been obtained by the reaction of diethylphosphonoacetic acid imidazolides with methyl-4-aminobenzoate or 3,5-bis(trifluoromethyl)phenylamine. Their treatment with Me_3SiBr in DMF led to a mixture of the corresponding (*N*-arylaminocarbonylmethyl)phosphonic acids and their monoethyl esters. After separation, they were condensed with 3'-*O*-acetyl-α-thymidine, which, after the removal of the acetyl protecting group, gave (α-D-thymidine-5'-yl)-[4-aminocarbonyl-, methoxycarbonyl-, or carboxy)phenylaminocarbonyl]methyl phosphonates and (α-*D*-thymidine-5'-yl)-[3,5-bis(trifluoromethyl)phenylaminocarbonyl]methyl phosphonate and their ethyl esters. It was shown that the compounds are stable under different conditions, low toxic (in *Vero* and K-562 cell cultures), and capable of penetrating into K-562 cells. Only ethyl (α-D-thymidine-5'-yl)-[4-(methoxycarbonyl)phenylaminocarbonyl]methyl phosphonate at a high concentration (200 μg/mL) inhibited *in vitro* the growth of the laboratory strain *M. tuberculosis* H37Rv.

Keywords: nucleosides, α-thymidine, phosphonates, synthesis, cytotoxicity, stability, tuberculosis, mycobacteria, antimycobacterial activity, Mycobacterium tuberculosis

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INTRODUCTION

In the 20th century, effective antibiotics and antiviral preparations were created, which made it possible to significantly reduce the mortality from infectious diseases. However, to date, most pathogenic microorganisms and viruses have developed resistance to the main pool of drugs used for their therapy, which necessitates a search for novel classes of compounds inhibiting the growth of pathogenic bacteria and viruses [1].

Tuberculosis, which is the cause of the annual deaths of about two million people, ranks second in the world among infectious diseases next to AIDS [2]. The simultaneous development of the two diseases in man substantially aggravates the clinical picture of each disease. In 1993, WHO declared a critical global situation with tuberculosis. Of special note is the emergence of novel *Mycobacterium tuberculosis* multidrug-resistant and practically incurable "extensively drug-resistant" strains [2], which remain almost unaffected by standard chemotherapy schemes. Therefore, a search for novel antituberculosis drugs is needed.

Among modified nucleosides that exhibited a marked antimycobacterial activity in vitro, the inhibitors of *M. tuberculosis* thymidine monophosphate kinase (TMPKmt) showed a pronounced antituberculosis activity [3, 4]. A comparison of 5'-deoxy-5'-(*N*-arylthiocarbamide) derivatives of α-thymidine [3] with its arylaminocarbonyl phosphonates, performed using the Molecular Operating Environment (MOE) complex [5], demonstrated a spatial similarity of these compounds.

The goal of this study was the synthesis of α-thymidine phosphonate derivatives and the study of their stability under different conditions, the toxicity in cell cultures, and their inhibitory effect on the growth of *M. tuberculosis*.

RESULTS AND DISCUSSION

α-Thymidine phosphonate derivatives were synthesized by the method similar to [6] (Schemes 1 and 2). The first stage of the synthesis was the condensation of diethylphosphonoacetic acid (**I**) with the corresponding phenylamine. The activation of acid (**I**) through the formation of imidazolide (CDI in DMF [7]) and its subsequent reaction with methyl-4-aminobenzoate or 3,5-bis(trifluoromethyl)phenylamine led to di-*O*-ethyl-(*N*-(4-methoxycarbonyl)phenylaminocarbo-

Abbreviations: CDI, *N*,*N*'-carbonyldiimidazole; CFU, colony-forming unit; DCC, *N*,*N*'-dicyclohexylcarbodiimide; HFBA, heptafluorobutyric acid; PBS, phosphate-buffered saline.

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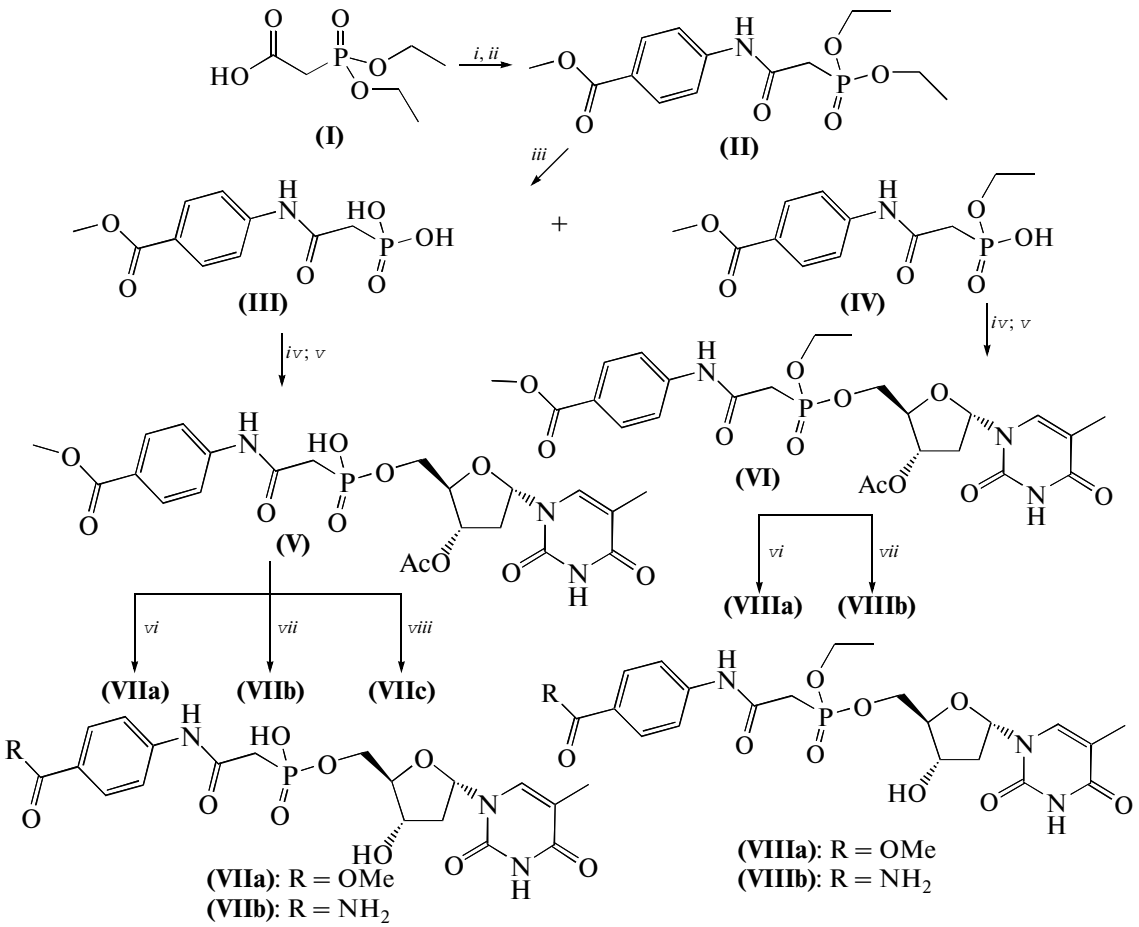
nyl)methyl phosphonate (**II**) and di-*O*-ethyl-[3,5-bis(trifluoromethyl)phenylaminocarbonyl]methyl phosphonate (**IX**), respectively.

N-Arylaminocarbonylmethylphosphonic acids (**III**) and (**X**) and their monoethyl esters (**IV**) and (**XI**) were obtained by a gentle treatment of diesters (**II**) and (**IX**) with trimethylbromosilane in DMF and isolated by chromatography on DEAE cellulose and reverse-phase silica gel. The reaction of derivatives (**III**), (**X**), (**IV**), and (**XI**) with 3'-*O*-acetyl-α-thymidine in pyridine in the presence of DCC [8] led to (3'-*O*-acetyl-α-D-thymidine-5'-yl)-[4-methoxycarbonyl)phenylaminocarbonyl]methyl phosphonate (**V**) and (3'-*O*-acetyl-α-D-thymidine-5'-yl)-[3,5-bis(trifluoromethyl)phenylaminocarbonyl]methyl phosphonate (**XII**) and the corresponding ethyl esters (**VI**) and (**XIII**) (Schemes 1 and 2).

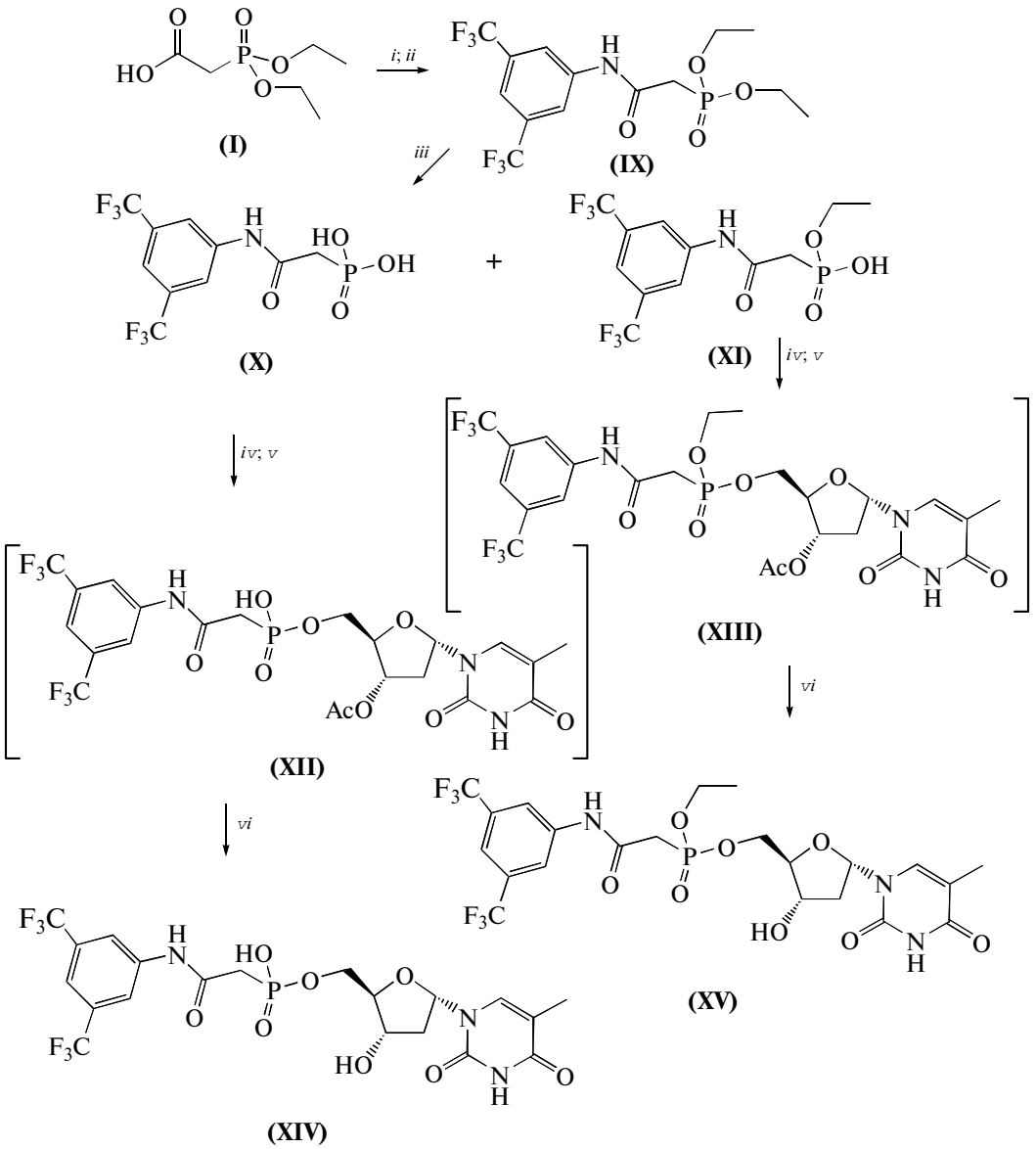


i: CDI, DMF;
ii: methyl-4-aminobenzoate
iii: Me_3SiBr, DMF, 5°C;
iv: DCC, *v*: 3'-*O*-acetyl-α-thymidine, Py;
vi: MeONa/MeOH; *vii*: NH_3/H_2O/EtOH; *viii*: $NaOH/H_2O$.

Scheme 1.

α-D-Thymidine-5'-yl)-[4-(methoxycarbonyl-, aminocarbonyl-, and carboxy)phenylaminocarbonyl]methyl phosphonates (**VIIa**)–(**VIIc**) and their ethyl esters (**VIIIa**) and (**VIIIb**) were obtained by treating compounds (**V**) or (**VI**) with sodium methylate in methanol, aqueous ammonia, or an aqueous alkaline solution [in the case of (**V**), respectively (Scheme 1). Compounds (**VIIa**)–(**VIIc**) were isolated by column chromatography on reverse-phase silica gel LiChroprep RP-8 with a yield of 53 (95%), 40 (75%), and 21 mg (80%), respectively. Compounds (**VIIIa**) and (**VIIIb**) were isolated by column chromatography on silica gel in a linear gradient of ethanol concentrations in dichloromethane with a yield of 54 (95%) and 45 mg (82%), respectively.

α-D-Thymidine-5'-yl-[3,5-bis(trifluoromethyl)phenylaminocarbonyl]methyl phosphonate (**XIV**) and its ethyl ester (**XV**) were obtained without the isolation of intermediate acetylated derivatives (**XII**) and (**XIII**). The treatment of the reaction mixture obtained by coupling compounds (**X**) and (**XI**) to 3'-*O*-acetyl-α-thymidine with a water–alcohol ammonia solution led to target products (**XIV**) and (**XV**) (Scheme 2), which were then isolated by column chromatography on silica gel in a linear gradient of ethanol concentrations in dichloromethane with a yield of 144 (81%) and 152 mg (84%), respectively.



i: CDI, DMF;
ii: 3,5-bis(trifluoromethyl)phenylamine;
iii: Me_3SiBr, DMF, 5°C;
iv: DCC, *v*: 3'-*O*-acetyl-α-thymidine, Py;
vi: NH_3/H_2O/EtOH.

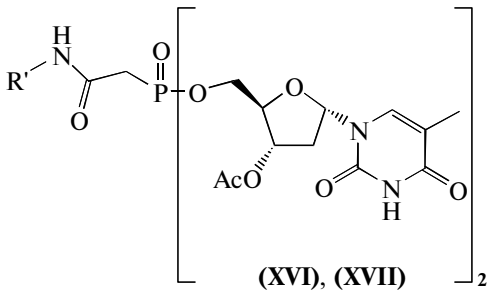
Scheme 2.

The structure of the compounds synthesized was confirmed by UV, ^1H, ^{31}P, and ^{13}C NMR spectra. NMR spectra of all compounds contain a characteristic signal of the phosphonate methylene group with the

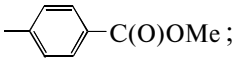
spin–spin coupling constant: $J_{CH_2P,P} = 18.0–21.0$ Hz in ^{1}H NMR spectra and $J_{CH_2P,P} = 122.0–130.0$ Hz in ^{13}C NMR spectra [9].

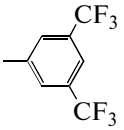
Ethyl phosphonates (**VI**), (**VIIIa**), (**VIIIb**), and (**XV**) are mixtures of diastereomers. Because they were not separated during the synthesis, the NMR spectra of these compounds contain two groups of signals, which corresponds to a mixture of two isomers in the ratio of about 1 : 1. The resolution of the NMR spectrometer made it possible to correlate chemical shifts and most of the spin–spin coupling constants of each isomer using the data of [9] (see the Experimental section). A difference in chemical shifts was observed for atoms separated by a distance of 1–3, less common 4–5 bonds from the chiral center, the phosphorus atom, and was ~0.01 ppm; the spin–spin coupling constant was ~0.1 Hz. For instance, the signals of the methylene groups of the phosphonoacetate residue of ethyl-(α-D-thymidine-5'-yl)-[4-methoxycarbonyl)phenylaminocarbonyl]methyl phosphonate (**VIIIa**) isomers are equal to 3.19 and 3.18 ppm, and the constants $J_{CH_2P,P}$ are equal to 21.6 and 21.7 Hz, respectively. The chemical shifts of the atoms of thymine and aromatic residues of the two isomers coincide.

The condensation of compounds (**III**) and (**X**) with α-thymidine also resulted in the formation of bisthymidine derivatives (**XVI**) and (**XVII**), which were isolated by preparative TLC with a yield of 6 and 8% (starting from compounds (**III**) and (**X**), respectively.



(**XVI**), (**XVII**)

(**XVI**): R' =  C(O)OMe;



In bisthymidine derivatives (**XVI**) and (**XVII**), owing to a large volume of nucleoside residues at the phosphorus atom, the latter adopt different conformations. As a result, some signals of protons (H1', H6) in ^{1}H NMR spectra are nonequivalent and are present as a double set.

The determination of the stability of compounds during the chemical and enzymatic hydrolysis is included in the stage of preclinical tests of compounds with potential medicamentosus activity. An analysis of possible products of the conversion of compounds (**VIIa**)–(**VIIc**), (**VIIIa**), (**VIIIb**), (**XIV**), and (**XV**) was carried out by reverse-phase HPLC. The compounds were stable under conditions of chemical hydrolysis for a period of more than 24 h at three different pH values: 2.2 (glycine-HCl), 7.4 (PBS), and 9.0 (glycine-NaOH).

The stability of the compounds to enzymatic hydrolysis was determined using fetal calf serum. All the compounds were stable under these conditions over a period of more than 24 h. The cytotoxicity of the compounds was determined by the methods described in [10]. Compounds (**VIIa**)–(**VIIc**), (**VIIIa**), (**VIIIb**), (**XIV**), and (**XV**) exhibited no toxicity in *Vero* and K-562 cell cultures at concentrations up to 500 and 250 μM, respectively, which is close to, or higher than, the toxicity of most of *M. tuberculosis* thymidine monophosphate kinase inhibitors described in the literature [3, 4].

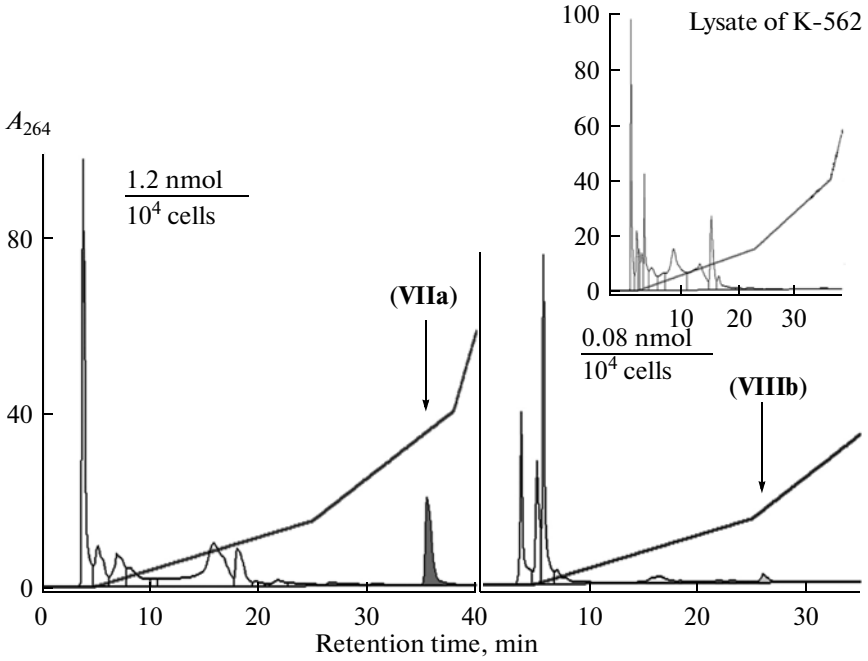
One of the factors affecting the activity of compounds is the rate of their accumulation and possible conversion inside cells. We examined the accumulation of phosphonates (**VIIa**) and (**VIIb**) in K-562 cells (human myelogenous leukemia cell line) by HPLC. After the incubation of cells with phosphonates, these compounds were identified reliably in cell lysates. As seen from the figure, the accumulation of methoxycarbonyl derivative (**VIIa**) significantly exceeds the amount of 4-aminocarbonyl derivative (**VIIb**) in the cell. It is seen from the elution profile that cell lysates contain no products of the conversion of (**VIIa**) and (**VIIb**).

The antimycobacterial effect of the compounds synthesized was studied using a Bactec MGIT960 automated system [11] by measuring the bacteriostatic activity toward the laboratory strain *M. tuberculosis* H37Rv by the method developed earlier [12]. Only phosphonate (**VIIIa**) at a high concentration (200 μg/mL) inhibited the growth of the mycobacterium, whereas the other compounds appeared to be inactive.

Thus, despite the spatial similarity of 2'-deoxy-α-D-thymidine arylaminocarbonyl phosphonates to α-thymidine 5'-deoxy-5'-(*N*-arylthiocarbamide) derivatives, they [except for (**VIIIa**)] did not inhibit *in vitro* the growth of *M. tuberculosis*.

EXPERIMENTAL

The commercial reagents of the companies Fluka (Germany), Aldrich-Sigma (United States), and Acrus (Belgium) were used. Solvents were purified by standard methods. The staring α-D-thymidine and 3'-*O*-acetyl-2'-deoxy-α-D-thymidine were obtained as described in [3, 13].



Accumulation of α-thymidine phosphonates (**VIIa**) and (**VIIb**) in K-562 myeloid leukemia cells (24 h). Elution profile of the K-562 cell lysate after a 24-h incubation with the compounds. T_{ret} of (**VIIa**) = 37 min, and T_{ret} of (**VIIb**) = 26 min. On the insert is the elution profile of the cell lysate without incubation. For the elution conditions, see the Experimental section.

Column chromatography was carried out using Kieselgel 60 silica gel (40–63 μm), LiChroprep RP-8 (25–40 μm) (Merck, Germany), and DEAE cellulose (Whatman, England); TLC was performed on Kieselgel 60 F_{254} plates (Merck, Germany) in systems: chloroform–ethanol 20 : 1 (A), chloroform–ethanol 9 : 1 (B), chloroform–ethanol 4 : 1 (C), isopropanol–25% ammonia–water 7 : 1 : 3 (D), dioxane–25% ammonia 8 : 2 (E), or dioxane–25% ammonia–water 6 : 1 : 4 (F). The preparative TLC was carried out on plates (20 × 20 cm) with Kieselgel 60 F_{254} silica gel; the thickness of the layer was 1 mm (Merck, Germany).

HPLC was performed in the pseudo ion-pair regime on a Nucleosil C-18 reverse-phase column (4 × 150 mm, 5 μm; Dr. Maisch GmbH, Germany) in a gradient of 80% EtOH in 0.1% HFBA (pH 3): 0%, 5 min; 0 → 15%, 20 min; 15 → 40%, 13 min; and 40 → 100%, 7 min.

NMR spectra (δ, ppm, spin–spin coupling constant, Hz) were recorded on an AMX III-400 spectrometer (Bruker, United States) with an working frequency of 400 MHz for ^{1}H NMR [internal standard Me_4Si for DMSO-d_6 and sodium 3-(trimethylsilyl)-1-propane sulfonate (DSS) for D_2O], 101 MHz for ^{13}C NMR (internal standard methanol), and 162 MHz for ^{31}P NMR (with the suppression of phosphorus proton spin–spin coupling; internal standard 85% phosphoric acid). UV spectra were recorded on a UV-2401P spectrophotometer (Shimadzu, Japan).

***O,O'*-Diethyl-[4-(methoxycarbonyl)phenylaminocarbonyl]methyl phosphonate (II).** CDI (486 mg, 3 mmol) was added to a solution of 2-(diethylphosphono)acetic acid (392 mg, 2 mmol) in dry DMF (5 mL). The solution was stirred for 1 h at 20°C, 4-methoxycarbonylaniline (302 mg, 2 mmol) was added, and the mixture was kept for 12 h at 37°C. The reaction was monitored by TLC in system A. Then H_2O (1 mL) was added, and the mixture was evaporated in vacuo and applied to a silica gel column (3.0 × 35 cm, 40–63 μm). The elution was carried out in a linear gradient of ethanol concentrations in dichloromethane (0 → 5%). Fractions containing compound (**II**) were evaporated in vacuo. Yield of phosphonate (**II**): 462 mg (70%); R_f 0.25 (A); UV (H_2O), λ_{max}, nm (ε, M^{-1} cm^{-1}): 269 (21850). ^{1}H NMR (DMSO-d_6) δ: 10.40 (1 H, br s, NH) 7.92 (2 H, d, $J_{m\text{-Ph},o\text{-Ph}}$ 8.7, *m*-Ph), 7.70 (2 H, d, *O*-Ph), 4.06 (4 H, m, ($C\underline{H}_2CH_3)_2$), 3.82 (3 H, s, CH_3O), 3.12 (2 H, d, $J_{CH_2P,P}$ 20.4, $C\underline{H}_2$-P), 1.24 (6 H, t, *J* 7.0, ($CH_2C\underline{H}_3)_2$); ^{31}P NMR (D_2O) δ: 24.19 s.

(4-Methoxycarbonylphenylaminocarbonyl)methyl phosphonate (III) and *O*-ethyl-[4-(methoxycarbonyl)phenylaminocarbonyl)methyl phosphonate (IV). Me_3SiBr (0.5 mL, 3.75 mmol) was added at 5°C to a solution of compound (**II**) (247 mg, 0.75 mmol) in dry DMF (5 mL), the reaction mixture was stirred for 12 h at 5°C, a 25% NH_3 solution (10 mL) was added, and the mixture was evaporated in vacuo. The resulting residue was dissolved in 10% aqueous ethanol (200 mL) and

applied to a column (7.5 × 25 cm) with DEAE cellulose (HCO_3^-). The column was washed with 10% aqueous ethanol (150 mL) and eluted in a linear gradient of $(NH_4)HCO_3$ concentrations in 10% water ethanol (0 → 0.2 M, 800 mL). Fractions containing compounds (**III**) and (**IV**) were evaporated in vacuo to dryness, dissolved in H_2O (1 mL), and applied to a LiChroprep RP-8 column (2 × 15 cm, 25–40 μm); elution was with 0.01 M $(NH_4)HCO_3$.

Fractions containing target compounds were lyophilized. The yield of the ammonium salt of (**III**) 99 mg (30%); R_f 0.49 (D); UV (H_2O), λ_{max}, nm (ε, M^{-1} cm^{-1}): 270 (22360). ^{1}H NMR (DMSO-d_6) δ: 11.34 (1 H, br s, NH) 7.82 (2 H, d, $J_{m\text{-Ph, }o\text{-Ph}}$ 8.7, *m*-Ph), 7.67 (2 H, d, *o*-Ph), 3.80 (3 H, s, CH_3O), 2.58 (2 H, d, $J_{CH_2P,\,P}$ 18.9, C$\underline{H}_2$-P); ^{31}P NMR (D_2O): 12.55 s. The yield of the ammonium salt of (**IV**) 74 mg, (22.5%); R_f 0.57 (D); UV (H_2O), λ_{max}, nm (ε, M^{-1} cm^{-1}): 272 (22150). ^{1}H NMR (DMSO-d_6) δ: 10.16 (1 H, br s, NH) 7.84 (2 H, d, $J_{m\text{-Ph, }o\text{-Ph}}$ 8.7, *m*-Ph), 7.70 (2 H, d, *o*-Ph), 3.80 (3 H, s, CH_3O), 3.73 (2 H, d q, $J_{CH_2CH_3}$ ~ $J_{POCH_2,\,P}$ 7.0 C$\underline{H}_2$CH$_3$), 2.53 (2 H, d, $J_{CH_2P,\,P}$ 18.6, C$\underline{H}_2$-P), 1.08 (3 H, t, $CH_2C\underline{H}_3$); ^{31}P NMR (DMSO-d_6) δ: 13.10 s.

***O*-(3'-*O*-Acetyl-α-D-thymidine-5'-yl)-[4-methoxycarbonyl)phenylaminocarbonyl]methyl phosphonate (V).** DCC (500 mg, 1.5 mmol) was added to a solution of compound (**III**) (100 mg, 0.3 mmol) and 3'-*O*-acetyl-α-thymidine (129 mg, 0.45 mmol) in dry pyridine. The mixture was kept at 37°C for 12 h. The reaction was monitored by TLC in system D. Then, H_2O (3 mL) was added, the mixture was evaporated in vacuo, and the residue was dissolved in 10% aqueous ethanol (200 mL) and applied to a with DEAE cellulose (HCO_3^-) (3.5 × 25 cm). The column was washed with 10% aqueous ethanol (200 mL), target compounds were eluted in a linear gradient of $(NH_4)HCO_3$ concentrations in 10% aqueous ethanol (0 → 0.15 M, 600 mL). Fractions containing compound (**V**) were combined and evaporated in vacuo to dryness. The yield of ammonium salt (**V**): 154 mg (88%); R_f 0.65 (D); UV (CH_3OH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 265 (26950). ^{1}H NMR (DMSO-d_6) δ: 11.03 (1 H, s, 3-NH), 7.87 (2 H, d, $J_{m\text{-Ph, }o\text{-Ph}}$ 8.7, *m*-Ph), 7.64 (2 H, d, *o*-Ph), 7.47 (1 H, s, H6), 5.86 (1 H, dd, *J* 7.1, 4.0, H1'), 5.19 (1 H, m, H3'), 4.54 (1 H, m, H4'), 3.84–3.78 (4 H, m, CH_3O, H5'a), 3.72 (1 H, m, H5'b), 3.33 (2 H, d, $J_{CH_2P,\,P}$ 22.5, C$\underline{H}_2$-P), 2.74 (1 H, m, H2'), 2.00–1.95 (4 H, m, $CH_3C(O)$, H2''), 1.79 (3 H, s, 5-CH_3); ^{31}P NMR (D_2O) δ: 13.27 s.

***O*-Ethyl-*O*'-(3'-*O*-acetyl-α-D-thymidine-5'-yl)-[4-(methoxycarbonyl)phenylaminocarbonyl]methyl phosphonate (VI).** DCC (500 mg, 1.5 mmol) was added to a solution of compound (**IV**) (103 mg, 0.3 mmol) and 3'-*O*-acetyl-α-D-thymidine (129 mg, 0.45 mmol) in dry pyridine. The mixture was kept at 37°C for 12 h. The reaction was monitored by TLC in system B. Then H_2O (1 mL) was added and the mixture was evaporated in vacuo. Compound (**VI**) was purified on preparative plates for TLC using ethyl acetate as an eluent. Yield of (**VI**): 164 mg (90%); R_f 0.66 (B); UV (CH_3OH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 265 (27050). ^{1}H NMR (DMSO-d_6) δ: 11.25 (1 H, br s, Ar-NH), 11.50 (1 H, s, 3-NH), 7.91 (2 H, d, $J_{m\text{-Ph, }o\text{-Ph}}$ 8.7, *m*-Ph), 7.70 (2 H, d *o*-Ph), 7.56 (1 H, s, H6), 6.18, 6.16 (1 H (dd, *J* 2.7, 1.2, isomer A; dd, *J* 2.7, 1.2, isomer B) H1'), 5.19 (1 H, m, H3'), 4.69 (1 H, m, H4'), 4.15–4.07 (4 H, m, CH_2CH_3, H5'), 3.82 (3 H, s, CH_3O), 3.22, 3.21 (2 H (d, $J_{CH_2P,\,P}$ 21.6, isomer A; d, $J_{CH_2P,\,P}$ 21.6, isomer B) CH_2-P), 2.75 (1 H, m, H2'), 2.13, 2.09 (1 H (m, isomer A; m, isomer B) H2''), 1.99 (3 H, s, $CH_3C(O)$), 1.80 (3 H, s, 5-CH_3), 1.258, 1.254 (3 H (t, *J* 6.9, isomer A; t, *J* 7.1, isomer B) $CH_2C\underline{H}_3$); ^{31}P NMR (DMSO-d_6) δ: 25.14 s, isomer A; 25.01 s, isomer B.

***O*-(α-D-Thymidine-5'-yl)-[4-(methoxycarbonyl)phenylaminocarbonyl]methyl phosphonate (VIIa).** A 1 M solution of MeONa (0.3 mL) in MeOH was added to a solution of compound (**V**) (58 mg, 0.1 mmol) in anhydrous MeOH (50 mL). The solution was stirred for 1 h, CH_3COOH (0.04 mL) was added, and the mixture was evaporated in vacuo to dryness. The residue was coevaporated with H_2O (2 × 3 mL), dissolved in H_2O (1 mL), and applied to a LiChroprep RP-8 column (2 × 25 cm, 25–40 μm). The reaction was monitored by TLC in system (D). Fractions containing compound (**VIIa**) were evaporated in vacuo. Yield of (**VIIa**): 53 mg (95%); R_f 0.59 (D); UV (H_2O), λ_{max}, nm (ε, M^{-1} cm^{-1}): 272 (27340). ^{1}H NMR (D_2O) δ: 7.84 (2 H, d, $J_{m\text{-Ph, }o\text{-Ph}}$ 8.7, *m*-Ph), 7.51 (2 H, d, *o*-Ph), 7.32 (1 H, s, H6), 5.86 (1 H, dd, *J* 7.1, 4.0, H1'), 4.49 (2 H, m, H3', H4'), 4.03 (1 H, ddd, $J5'_a$, $5'_b$, 11.2, $J5'_{a,\,P}$, 4.9, $J5'_{a,\,4}$, 2.6, H$5'_a$), 3.89 (1 H, m, H5'b), 3.85 (3 H, s, CH_3O), 2.92 (2 H, d, $J_{CH_2P,\,P}$ 20.3, C$\underline{H}_2$-P), 2.62 (1 H, m, H2'), 1.93 (1 H, ddd, *J* 14.4, 4.0, 3.9, H2''), 1.74 (3 H, s, 5-CH_3); ^{31}P NMR (D_2O) δ: 16.40 s; ^{13}C NMR (D_2O): 171.10 ($\underline{C}$(O)Ph), 169.86 (d, $J_{C(O),\,P}$ 5.3, $\underline{C}$(O)CH_2), 168.86 (C4), 153.69 (C2), 145.04 (*ipso*-Ph), 140.26 (C6), 133.11 (*m*-Ph), 127.52 (*p*-Ph), 122.52 (*o*-Ph), 112.91 (C5), 89.54 (C1'), 89.29 (d, $J_{C4',\,P}$ 7.6 C4'), 73.62 (C3'), 67.41 (d, $J_{C5',\,P}$ 5.8, C5'), 55.31 (CH_3O), 42.31 (C2'), 40.20 (d, $J_{CH_2P,\,P}$ 122.0, CH_2-P), 14.24 (5-CH_3).

***O*-(α-D-Thymidine-5'-yl)-[4-(aminocarbonyl)phenylaminocarbonyl]methyl phosphonate (VIIb).** An aque-

ous NH_3 (25%) solution (50 mL) was added to a solution of compound (**V**) (58 mg, 0.1 mmol) in ethanol (100 mL). The solution was kept for 12 h, evaporated in vacuo to dryness, and the residue was coevaporated with H_2O (2 × 3 mL), dissolved in H_2O (1 mL), and applied to a LiChroprep RP-8 column (2 × 25 cm). The reaction was monitored by TLC in system (D). Fractions containing compound (**VIIb**) were evaporated in vacuo. The yield of ammonium salt (**VIIb**): 40 mg (75%); R_f 0.55 (D); UV (H_2O), λ_{max}, nm (ε, M^{-1} cm^{-1}): 268 (20000). ^{1}H NMR (D_2O) δ: 7.67 (2 H, d, $J_{m\text{-Ph, }o\text{-Ph}}$ 8.7, *m*-Ph), 7.48 (2 H, d, *o*-Ph), 7.41 (1 H, s, H6), 5.85 (1 H, dd, *J* 7.1, 3.9, H1'), 4.37 (2 H, m, H3', H4'), 4.02 (2 H, ddd, $J5'_a$, $5'_b$, 11.2, $J5'_{a,\,P}$, 5.1, $J5'_{a,\,4'}$, 3.1, H5'a,), 3.89 (1 H, ddd, H5'b) 2.91 (2 H, d, $J_{CH_2P,\,P}$ 20.2, CH_2-P), 2.62 (1 H, m, H2'), 1.93 (1 H, ddd, *J* 14.4, 3.7, 3.7, H2''), 1.74 (3 H, s, 5-CH_3); ^{31}P NMR (D_2O): 16.40 s; ^{13}C NMR (D_2O) δ: 174.27 ($NH_2\underline{C}$(O)), 171.00 (d, $J_{C(O),\,P}$ 5.3, $\underline{C}$(O)CH_2), 169.20 (C4), 153.97 (C2), 143.86 (*ipso*-Ph), 140.47 (C6), 131.19 (*m*-Ph), 130.86 (*p*-Ph), 123.00 (*o*-Ph), 113.12 (C5), 89.64 (C1'), 89.37 (d, $J_{C4',\,P}$ 7.1 C4'), 73.46 (C3'), 67.43 (d, $J_{C5',\,P}$ 5.7, C5'), 42.17 (C2'), 40.10 (d, $J_{CH_2P,\,P}$ 122.5 CH_2-P), 14.30 (5-CH_3).

***O*-(α-D-Thymidine-5'-yl)-[4-(carboxyphenylaminocarbonyl]methyl phosphonate (VIIc).** A 1 M solution of NaOH in water (0.2 mL) was added to a solution of compound (**V**) (29 mg, 0.05 mmol) in H_2O (100 mL). The solution was stirred for 1 h, evaporated in vacuo to dryness, after which 0.04 mL of CH_3COOH was added. The solution was evaporated in vacuo to dryness, and the residue was coevaporated with H_2O (2 × 3 mL), dissolved in H_2O (1 mL), and applied to a LiChroprep RP-8 column (2 × 25 cm, 25–40 μm). The reaction was monitored by TLC in system (D). Fractions containing compound (**VIIc**) were evaporated in vacuo. The yield of product (**VIIc**): 21 mg (80%); R_f 0.48 (D); UV (H_2O), λ_{max}, nm (ε, M^{-1} cm^{-1}): 269.1 (23000). ^{1}H NMR (DMSO-d_6) δ: 10.70 (1 H, s, 3-NH), 7.81 (2 H, d, *J* 8.7 *m*-Ph), 7.73 (1 H, s, H6), 7.60 (1 H, d, *o*-Ph), 6.08 (1 H, dd, *J* 7.6, 3.1, H1'), 4.28 (1 H, m, H4'), 4.23 (1 H, m, H3'), 3.71 (2 H, m, H5'), 3.18 (2 H, d, $J_{CH_2P,\,P}$ 20.6, CH_2-P), 2.59 (1 H, m, H2'), 1.83 (1 H, m, H2''), 1.76 (3 H, s, 5-CH_3); ^{31}P NMR (DMSO-d_6) δ: 13.60 s.

***O*-Ethyl-O'-(α-D-thymidine-5'-yl)-[4-(methoxycarbonyl)phenylaminocarbonyl]methyl phosphonate (VIIIa).** A 1 M solution of MeONa (0.3 mL) in MeOH was added to a solution of compound (**V**) (61 mg, 0.1 mmol) in anhydrous MeOH (50 mL). The solution was stirred for 1 h, CH_3COOH (0.04 mL) was added, and the mixture was evaporated in vacuo to dryness. The residue was coevaporated with H_2O (2 × 3 mL). The reaction was monitored by TLC in system (B). The residue was applied to a silica gel column (2.0 × 25 cm, 40–63 μm). The elution was carried out in a linear gradient of ethanol concentrations in dichloromethane (0 → 9%). Fractions containing compound (**VIIIa**) were evaporated in vacuo. The yield of (**VIIIa**): 54 mg (95%); R_f 0.36 (B); UV (CH_3OH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 270 (22620). ^{1}H NMR (DMSO-d_6) δ: 11.21 (1 H, s, Ar-NH), 10.40, 10.41 (1 H (s, isomer A; s, isomer B) 3-NH), 7.91 (2 H, d, $J_{m\text{-Ph, }o\text{-Ph}}$ 8.5 *m*-Ph), 7.72 (1 H, s, H6), 7.69 (2 H, d, *o*-Ph), 6.14, 6.13 (1 H (dd, *J* 7.4, 3.6, isomer A; dd, *J* 7.4, 3.9, isomer B) H1'), 5.46 (1 H, d, $J_{3'OH,3'}$ 3.3, 3'-OH), 4.32 (1 H, m, H3'), 4.27 (1 H, m, H4'), 4.05 (4 H, m, H5', C$\underline{H_2}$$CH_3$), 3.82 (3 H, s, CH_3O), 3.19, 3.18 (2 H (d, $J_{CH_2P,\,P}$ 21.6, isomer A; d, $J_{CH_2P,\,P}$ 21.7, isomer B) CH_2-P), 2.57 (1 H, m, H2'), 1.93, 1.92 (1 H, m, H2''), 1.77 (3 H, s, 5-CH_3), 1.24, 1.25 (3 H (t, *J* 7.0, isomer A; t, *J* 7.0, isomer B) $CH_2C\underline{H_3}$), ^{31}P NMR (DMSO-d_6) δ: 24.92 s, isomer A; 24.79 s, isomer B.

***O*-Ethyl-O'-(α-D-thymidine-5'-yl)-[4-(aminocarbonyl)phenylaminocarbonyl]methyl phosphonate (VIIIb).** An aqueous solution (50 mL) of NH_3 (25%) was added to a solution of compound (**VI**) (61 mg, 0.1 mmol) in EtOH (100 mL). The solution was kept for 12 h, evaporated in vacuo to dryness, and coevaporated with H_2O (2 × 3 mL). The reaction was monitored by TLC in system (C). The residue was applied to a silica gel column (2.0 × 25 cm, 40–63 μm). The elution was carried out in a linear gradient of ethanol concentrations in dichloromethane (0 → 15%). Fractions containing compound (**VIIIb**) were evaporated in vacuo. The yield of (**VIIIb**): 45 mg (82%); R_f 0.25 (C); UV (CH_3OH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 272 (28050). ^{1}H NMR (DMSO-d_6) δ: 11.20 (1 H, br s, Ar-NH), 10.29, 10,28 (1 H, s, 3-NH), 7.82 (3 H, d, $J_{m\text{-Ph, }o\text{-Ph}}$ 8.7, *m*-Ph, s, H_a, NH_2), 7.74, 7.73 (1 H (s, isomer A; s, isomer B) H6), 7.61 (2 H, d, *o*-Ph), 7.19 (1 H, s, H_b, NH_2) 6.16, 6.15 (1 H (dd, *J* 7.6, 3.6, isomer A; dd, *J* 7.4, 3.9, isomer B) H1'), 5.48 (1 H, d, $J_{3'OH,3'}$ 3.4, 3'-OH), 4.27 (1 H, m, H3'), 4.32 (1 H, m, H4'), 4.05 (4 H, m, H5', O$C\underline{H_2}CH_3$), 3.17, 3.16 (2 H (d, $J_{CH_2P,\,P}$ 21.6, isomer A; d, $J_{CH_2P,\,P}$ 21.6, isomer B) CH_2-P), 2.58 (1 H, m, H2'), 1.94, 1.93 (1 H (ddd, *J* 14.3, 3.8, 3.6, isomer A; ddd, *J* 14.1, 3.6, 3.4, isomer B) H2''), 1.78 (3 H, s, 5-CH_3), 1.25, 1.24 (3 H (t, *J* 7.0, isomer A; t, *J* 7.0, isomer B) $CH_2C\underline{H_3}$); ^{31}P NMR (DMSO-d_6) δ: 25.15 s, isomer A; 25.02 s, isomer B; ^{13}C NMR (DMSO-d_6) δ: 167.29 (2 H, s, NH_2C(O)), 163.76 (C4), 163.16 (d, $J_{C4',\,P}$ 5.8, $\underline{C}$(O)CH_2), 150.44 (C2), 141.30 (*ipso*-Ph) 136.68 (C6), 129.11 (*p*-Ph), 128.37 (*m*-Ph), 118.17 (*o*-Ph), 108.85 (C5), 85.95, 85.80 ((d, $J_{C4',\,P}$ 6.7, isomer A; d, $J_{C4',\,P}$ 7.1, isomer B)

C4'), 85.17, 85.12 [(s, isomer A; s, isomer B) C1'], 70.78, 70.05 [(s, isomer A; s, isomer B) C3'], 65.48 (m, C5'), 62.12, 62.09 ((d, $J_{POCH_2,P}$ 6.2, isomer A; d, $J_{POCH_2,P}$ 5.8, isomer B) $\underline{C}H_2CH_3$), 39.70 (C2'), 35.79 (d, $J_{CH_2P,P}$ 131.9 CH_2-P), 16.14, 16.07 (1 C (s, isomer A; s, isomer B) $CH_2\underline{C}H_3$), 12.23 (5-CH_3).

***O*,*O*'-Diethyl-[3,5-bis(trifluoromethyl)phenylaminocarbonyl]methyl phosphonate (IX).** CDI (486 mg, 3 mmol) was added to a solution of 2-(diethylphosphono)acetic acid (**I**) (392 mg, 2 mmol) in dry DMF (5 mL). The solution was stirred at 20°C for 1 h, 3,5-bis(trifluoromethyl)phenylamine (458 mg, 2 mmol) was added, and the mixture was kept at 37°C for 5 h. The reaction was monitored by TLC in system (A). Then H_2O (1 mL) was added, and the mixture was evaporated in vacuo and applied to a silica gel column (3.0 × 35 cm). The elution was carried out in dichloromethane. Fractions containing compound (**IX**) were evaporated in vacuo. The yield of diester (**IX**): 612 mg (75%); R_f 0.57 (A); UV (EtOH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 242 (12650). ^{1}H NMR (DMSO-d_6) δ: 12.42 (1 H, br s, Ar-NH), 8.16 (2 H, s, *o*-Ph), 7.22 (1 H, s, *p*-Ph), 4.25 (2 H, m, $C\underline{H}_2CH_3$), 3.37 (2 H, d, $J_{CH_2P,P}$ 20.9, CH_2-P), 1.34 (3 H, t, *J* 7.1 $CH_2C\underline{H}_3$); ^{31}P NMR (DMSO-d_6) δ: 24.66 s.

[3,5-Bis(trifluoromethyl)phenylaminocarbonyl]methyl phosphonate (X) and *O*-ethyl-[3,5-bis(trifluoromethyl)phenylaminocarbonyl]methyl phosphonate (XI). To a solution of compound (**IX**) (408 mg, 1.0 mmol) in dry DMF (7 mL), Me_3SiBr (0.66 mL, 5.0 mmol) was added at 5°C, the reaction mixture was stirred at 5°C for 12 h, a 25% NH_3 solution (15 mL) was added, and the mixture was evaporated in vacuo. The reaction was monitored by TLC in system (D). The resulting residue was dissolved in 10% alcohol (300 mL) and applied to a column with DEAE cellulose (HCO_3^-) (7.5 × 25 cm). The column was washed with 10% aqueous ethanol (200 mL) and then in a linear gradient of $(NH_4)HCO_3$ concentrations in 10% aqueous ethanol (0.05 → 0.2 M, 1000 mL). Fractions containing compound (**IX**) or (**X**) were evaporated in vacuo to dryness, dissolved in H_2O (1 mL), and applied to a LiChroprep RP-8 column (2 × 15 cm). The elution was carried out with 0.01 M $(NH_4)HCO_3$. Fractions containing target compounds were lyophilized, and the ammonium salts of compounds (**X**) or (**XI**) were obtained. The yield of the ammonium salt of (**X**) 123 mg (32%); R_f 0.62 (D); UV (EtOH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 242.4 (11890). ^{1}H NMR (DMSO-d_6) δ: 12.56 (1 H, br s, Ar-NH), 8.14 (2 H, s, *o*-Ph), 7.19 (1 H, s, *p*-Ph), 2.83 (2 H, d, $J_{CH_2P,P}$ 20.7, CH_2-P), ^{31}P NMR (DMSO-d_6) δ: 13.02 s. The yield of the ammonium salt of (**XI**) 74 mg (28.0%), R_f 0.77 (D). UV (EtOH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 244 (12460). ^{1}H NMR (DMSO-d_6) δ: 12.18 (1 H, br s, Ar-NH), 8.08 (2 H, s, *O*-Ph), 7.22 (1 H, s, *p*-Ph), 3.92 (2 H, d q, $J_{CH_2CH_3}$ ~ $J_{POCH_2,P}$ 7.0 $C\underline{H}_2CH_3$), 2.78 (2 H, d, $J_{POCH_2,P}$ 20.5, CH_2-P), 1.18 (3 H, t, $CH_2C\underline{H}_3$); ^{31}P NMR (DMSO-d_6) δ: 13.57 s.

***O*-(α-D-Thymidine-5'-yl)-[3,5-bis(trifluoromethyl)phenylaminocarbonyl]methyl phosphonate (XIV).** DCC (500 mg, 1.5 mmol) was added to a solution of compound (**X**) (116 mg, 0.3 mmol) and 3'-*O*-acetyl-α-thymidine (129 mg, 0.45 mmol) in dry pyridine. The mixture was kept at 37°C for 12 h, H_2O (3 mL) was added, and the solution was evaporated in vacuo. The residue was dissolved in ethanol (150 mL), a 25% aqueous NH_3 solution (50 mL) was added, and the reaction mixture was kept at 5°C for 12 h. The reaction was monitored by TLC in system (E). Then the mixture was evaporated in vacuo, the residue was dissolved in 15% aqueous ethanol (100 mL), and the solution was applied to a column with DEAE cellulose (HCO_3^-) (3.5 × 25 cm). The column was washed with 15% aqueous ethanol (200 mL), and the target compound was eluted in a linear gradient of $(NH_4)HCO_3$ concentrations in 15% aqueous ethanol (0 → 0.25 M, 600 mL). Fractions containing compound (**XIV**) were combined and evaporated in vacuo to dryness. The yield of the ammonium salt of (**XIV**): 144 mg (81%); R_f 0.65 (E); UV (CH_3OH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 248 (14185). ^{1}H NMR (D_2O): 7.99 (2 H, s, *o*-Ph), 7.75 (1 H, s, *p*-Ph), 7.46 (1 H, s, H6), 5.86 (1 H, dd, *J* 6.8, 3.2, H1'), 4.41 (2 H, m, H3', H4'), 4.05 (1 H, m, H5'a), 3.92 (1 H, m, H5'b), 2.96 (2 H, d, $J_{POCH_2,P}$ 20.5, CH_2-P), 2.66 (1 H, m, H2'), 1.97 (1 H, m, H2''), 1.76 (3 H, s, 5-CH_3), ^{31}P NMR (D_2O): 16.10 s.

***O*-Ethyl-*O*'-(α-D-thymidine-5'-yl)-[3,5-bis(trifluoromethyl)phenylaminocarbonyl]methyl phosphonate (XV).** DCC (500 mg, 1.5 mmol) was added to a solution of compound (**X**) (116 mg, 0.3 mmol) and 3'-*O*-acetyl-α-thymidine (129 mg, 0.45 mmol) in dry pyridine. The mixture was kept at 37°C for 12 h. Then H_2O (3 mL) was added, and the reaction mixture was evaporated in vacuo. The residue was dissolved in ethanol (150 mL), a 25% aqueous NH_3 solution (50 mL) was added, and the reaction mixture was kept for 12 h at 5°C. The reaction was monitored by TLC in system (B). Compound (**XV**) was purified on preparative plates for TLC, using ethyl acetate saturated with water as an eluent. The yield of methyl phosphonate (**XV**): 152 mg (84%); R_f 0.66 (B); UV (CH_3OH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 248.6 (14185). ^{1}H NMR (DMSO-d_6) δ: 11.20 (1 H, s, Ar-NH), 10.80 (1 H, s, 3-NH), 8.20 (2 H, s, *o*-Ph), 7.75 (1 H, s, *p*-Ph), 7.71 (1 H, s, H6), 6.13, 6.12 (1 H (dd, *J* 7.6, 3.8, isomer A; dd,

J 4.3, 8.2, isomer B) H1'), 5.48 (1 H, d, $J_{3'OH,3'}$ 3.5, 3'-OH), 4.33 (1 H, m, H4'), 4.26 (1 H, m, H3'), 4.06 (4 H, m, H5', C$\underline{H}_2$CH$_3$), 3.20 (2 H, d, $J_{CH_2P,P}$ 21.6, CH_2-P), 2.57 (1 H, m, H2'), 1.93 (1 H, m, H2''), 1.76 (3 H, s, 5-CH_3), 1.26, 1.25 (3 H (t, *J* 6.9, isomer A; t, *J* 6.9, isomer B) $CH_2C\underline{H}_3$); ^{31}P NMR (DMSO-d_6): 24.39 s, isomer A; 24.27 s, isomer B; ^{13}C NMR (DMSO-d_6) δ: 164.09 (1 C, d, $J_{C(O),P}$ 5.8, $\underline{C}$(O)CH_2), 163.81 (C4), 150.44 (C2), 140.56 (*ipso*-Ph), 136.68 (C6), 130.88 (q, $J_{m\text{-}C,F}$ 33.3, *m*-Ph), 123.11 (q, J 272.8 CF_3), 118.74 (*o*-Ph), 116.42 (m, *p*-Ph), 108.87 (C5), 85.91 (1 H, m, C4'), 85.20, 85.17 (C1'), 70.71, 70.76 (C3'), 65.69, 65.62 ((d, $J_{C5',P}$ 6.7, isomer A; d, $J_{C5',P}$ 8.0, isomer B) C5'), 62.32, 62.30 ((d, $J_{POCH_2,P}$ 6.2, isomer A; d, $J_{POCH_2,P}$ 6.2, isomer B) $\underline{C}H_2CH_3$), 39.70 (C2'), 35.94 (d, $J_{CH_2P,P}$ 131.9 CH_2-P), 16.14, 16.08 ((s, isomer A; s, isomer B) $CH_2\underline{C}H_3$), 12.22 (5-CH_3).

***O,O'* -Di-(3' -O-acetyl-α-D-thymidine-5' -yl)-[4-(methoxycarbonyl)phenylaminocarbonyl]methyl phosphonate (XVI).** R_f 0.72 (D); UV (CH_3OH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 265 (36480). ^{1}H NMR (DMSO-d_6) δ: 10.61 (1 H, br s, Ar-NH), 7.89 (2 H, d, $J_{m\text{-Ph},o\text{-Ph}}$ 8.9, *m*-Ph), 7.71 (2 H, d, *o*-Ph), 7.49, 7.48 (2 H, 2 × s, H6), 6.17, 6.15 (1 H, 2 × dd, *J* 2.5, 1.7, H1'), 5.18 (2 H, m, H3'), 4.69 (2 H, m, H4'), 4.20 – 4.12 (4 H, m, H5'), 3.37 (2 H, d, $J_{CH_2P,P}$ 22.5, C$\underline{H}_2$-P), 3.81 (3 H, s, CH_3O), 2.75 (2 H, m, H2'), 2.13—2.08 (2 H, m, H2''), 1.98, 1.97 (6 H, 2 × s, CH_3C(O)), 1.79 (6 H, s, 5-CH_3); ^{31}P NMR (DMSO-d_6) δ: 25.95 s.

O,O'-Di-(3'-*O*-acetyl-(α-D-thymidine-5'-yl)-[3,5-bis(trifluoromethyl)phenylaminocarbonyl]methyl phosphonate (XVII). R_f 0.81 (D); UV (EtOH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 244 (21480). ^{1}H NMR (DMSO-d_6) δ: 11.23 (2 H, s, 3-NH), 10.95 (1 H, br s, Ar-NH), 8.22 (2 H, s, *o*-Ph), 7.74 (1 H, s, *p*-Ph), 7.50, 7.49 (2 H, 2 × s, H6), 6.15 (2 H, dd, *J* 7.1, 2.5, H1'), 5.18 (2 H, m, H3'), 4.71 (2 H, m, H4'), 4.18 (4 H, m, H5'), 3.35 (2 H, d, $J_{POCH_2,P}$ 20.2, CH_2-P), 2.74 (2 H, m, H2'), 2.11 (2 H, m, H2''), 1.97, 1.96 (6 H, 2 × s, CH_3C(O)), 1.79 (6 H, s, 5-CH_3); ^{31}P NMR (DMSO-d_6) δ: 25.26 s.

Experiments on Cell Cultures

The toxicity of compounds was determined in Vero cell culture (epithelial kidney cells of African green monkey) and K-562 cell culture (human myelogenous leukemia cells) (both from the collection of cell cultures of Ivanovskii Research Institute of Virology, Ministry of Public Health and Social Development of Russia). The cytopathic effect of compounds was tested by MTT assay after 72 h of incubation [10].

Penetration and accumulation of compounds in the K-562 cell culture. Suspension of K-562 cells (2.14 × 10^6 cells/mL, 5 mL) grown in flasks (25 cm^2) was incubated with test compounds at a concentration of 1 mM (5% CO_2, humidity 90%, 37°C) for 1, 5, and 24 h. Cells were separated from the culture medium by sedimentation on a K-23 centrifuge (1000 rpm, 10 min, 4°C), washed twice from the preparation by resuspension in PBS, and centrifuged (3500 rpm, 5 min). Then cells were resuspended in an equal volume of PBS diluted in the ratio 1 : 10, destructed by adding 6% trichloroacetic acid to a final concentration of 3% (v/v), and left at –20°C for 10 min. After centrifugation (13400 rpm, 7 min), the acid-insoluble fraction was removed, and the supernatant was neutralized by adding a saturated Na_2CO_3 solution (4.3 μL per 100 μL of a sample). The resulting samples of the K-562 cell lysate were analyzed by HPLC under conditions indicated above. Compounds were identified by the retention time specified for authentic controls. The amount of tested compound in the cell lysate was estimated from the calibration curve (mV/nmol).

Mycobacterium strain. The trials of preparations were carried out using the laboratory strain *M. tuberculosis* H37Rv, which is sensitive to antituberculosis drugs. Mycobacteria were transformed into a suspension of single cells in the same growth phase and normalized by CFU [14]. The enriched Dubois medium (Difco) was used.

Estimation of the efficiency of compounds. The effect of compounds on the growth of the mycobacterial strain was examined for 42 days on a Bactec MGIT 960 automated system for the detection of growth (BD, United States) by the standard method [11]. A mycobacterial suspension (500 μL) was inoculated in 7.9 mL of nutrient medium. The final concentration of *M. tuberculosis* in the sample was 10^5–10^6 CFU/mL. Each sample including the control samples lacking the tested compound were studied in triplicate. The antimycobacterial effect of a compound was estimated from the dynamics of growth of *M. tuberculosis* H37Rv in the presence of different concentrations of the preparation as compared with the growth of the strain in a medium containing no compound. The detection of growth was carried out every hour automatically and was recorded using the software program Epicenter (BD, United States).

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