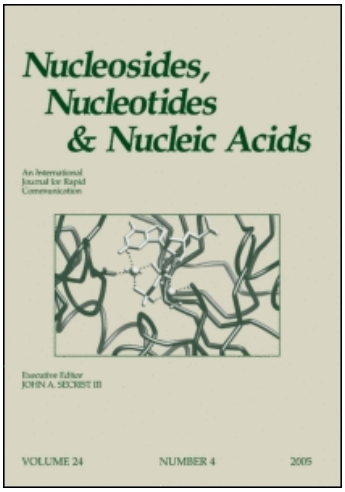

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NOVEL APPROACH TO NUCLEOSIDE-5′-(1-HYDROXYMETHYLENE-1, 1-BISPHOSPHONATES): SYNTHESIS OF NEW AZT ANALOGUES[#]

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▫ *An efficient synthetic method of nucleoside-5′-(1-hydroxymethylene-1,1-bisphosphonates) is reported here. The procedure was optimized with 3′-protected thymidine and then applied to synthesis of new AZT analogues.*

Keywords Bisphosphonate, AZT-H-phosphonate, Thymidine-H-phosphonate

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is the causative retrovirus of acquired immunodeficiency syndrome (AIDS)[1] and requires reverse transcriptase (RT) enzyme to copy its single-stranded RNA genome into a double-stranded DNA copy for integration into the host cell genome. Several members of HIV inhibitors are currently approved for the treatment of HIV-infected patients.[1,2] 3′-Azido-3′-deoxythymidine (AZT), a nucleoside reverse transcriptase inhibitor (NRTI), is one of them. It is the first clinically successful drug used for AIDS. It continues to play an important role in chemotherapy,[3,4] particularly in combination with other NRTIs, non-NRTIs, and HIV protease inhibitors, despite its toxicity to the host and the ability of the virus to mutate and gain resistance. Indeed, many undesirable side effects such as bone marrow suppression, myopathy, hepatic abnormalities,[5] neutropenia, and anemia[6–10] are frequently observed clinically. Thus, great efforts have been continuously made to develop and optimize the structure of AZT.

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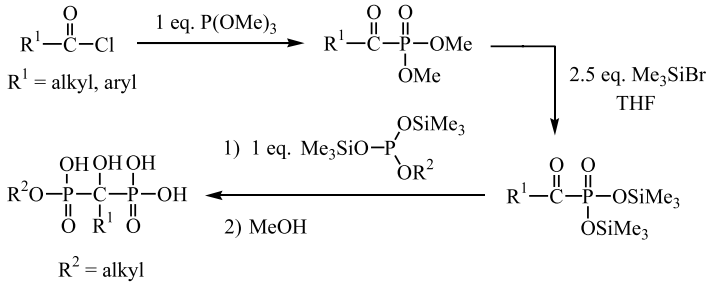
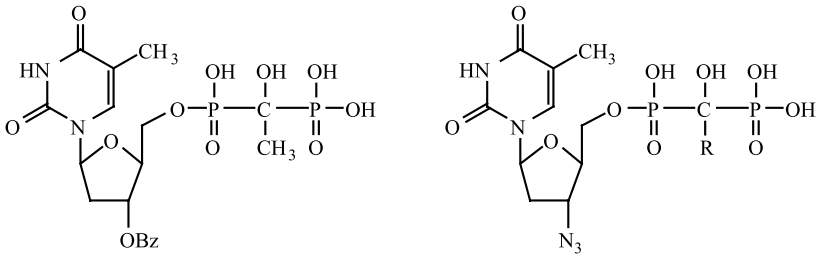
As other nucleoside analogues, AZT does not exert antiviral activity directly, but it acts rather like a prodrug of active phosphorylated metabolites. It must be successively phosphorylated in position 5′ to the corresponding mono-, di-, and triphosphates by thymidine kinases[11–14] to be recognized by HIV-1 RT. Thus, it terminates the viral DNA chain replication, since modified nucleosides do not possess the 3′-hydroxyl group necessary for chain elongation.[14–16] Unfortunately, some 2′,3′-dideoxynucleoside-5′-monophosphates are known to have a low anti-HIV activity[17] as some AZT analogues.[18] Moreover, AZT-5′-triphosphate (TP) is poorly absorbed when administered externally and readily dephosphorylated in the extracellular environment.[19] However, these drawbacks can be avoided by using an analogue of AZT that could serve as an intracellular slow-release depot to the active AZT-5′-TP. Hence, we propose to synthesize AZT-bisphosphonates (BPs) as potential prodrugs of AZT-5′-TP. Previously, it was been shown that bisphosphonates were metabolized intracellularly by *dictyostelium* amoebae into methylene-containing adenine nucleotides.[20] These AZT analogues could act as competitive inhibitors, preventing the incorporation of natural RT substrates. It would consist of AZT-5′-hydroxymethylene bisphosphonates (HMBPs) substituted with alkyl or aryl groups on the central carbon. This structural modification replaces a diphosphate moiety by a dephosphorylation-resistant methylenebisphosphonate group. It could increase lipophilicity of AZT analogues due to the presence of an aliphatic or an aromatic group. Moreover, these compounds that might still be substrates for HIV-1 RT could be encapsulated in liposomes as described previously with BPs[21] in order to cross cellular membranes more efficiently and reach intracellular targets.

To reduce toxicity of AZT, increase its anti-HIV activity, and cause fewer nucleoside-resistant mutants of HIV, a lot of work has been reported on development of 5′-*O*-ester prodrugs of AZT.[3] The 5′-*H*-phosphate of AZT (PZT) especially is one of the most significant compounds.[22] Indeed, PZT, which is less active as an anti-HIV agent than AZT itself, is nevertheless far less toxic (CC_{50} values of 2.5 mM and 210 μM for PZT and AZT, respectively). The overall selectivity for PZT (CC_{50}/IC_{50}) is superior to that of AZT,[23] and now PZT is currently in clinical trials. PZT, unlike highly acidic nucleoside 5′-phosphates, which cannot enter the cell, may penetrate the cell membrane due to its weakly acidic



SCHEME 1 General procedure for synthesis of HMBP monoalkyl esters.



3'-*O*-benzoyl thymidine-5'-HMBP **1** (Bz=benzoyl)

AZT-5'-HMBP **2a** R=CH_3 **2b** R=Ph

FIGURE 1 Structures of 3′-*O*-benzoyl thymidine-5′-HMBP **1** and AZT-5′-HMBPs **2**.

undissociated nature. These good results encouraged us to develop the synthesis of AZT-5′-HMBPs as new phosphorylated anti-HIV molecules in order to overcome AZT's drawbacks and improve its efficacy. These derivatives may be more stable in vivo and so have a longer potential antiviral activity due to the resistance of the P–C–P bond to chemical and enzymatic cleavage.

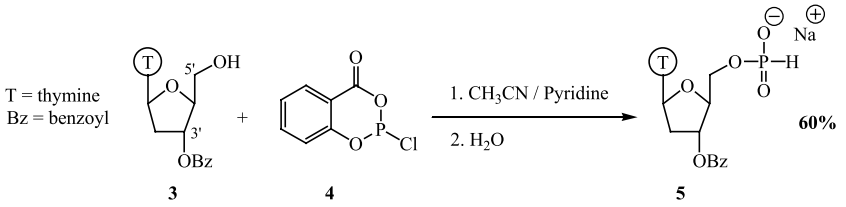
We report herein a new efficient method for preparation of these AZT-5′-HMBPs analogues. Contrary to previous works,[24] our strategy does not consist of reaction between HMBP and AZT but in mono- and then diphosphorylation of the nucleoside at the 5′-position. Our method is based on recent results obtained in our laboratory from synthesis of HMBP monoalkyl esters[25] (Scheme 1). We first describe the preliminary study with thymidine to prepare 3′-*O*-benzoyl thymidine-HMBP **1**, then synthesis of AZT analogues **2** (Figure 1). Stereochemistry will not be discussed here.

RESULTS AND DISCUSSION

Synthesis of 3′-*O*-Benzoyl Thymidine-5′-HMBP 1

The synthetic method of nucleoside 5′-HMBPs was first tested with thymidine. Before 5′-phosphorylation, thymidine was protected at the 3′-position with a benzoyl group (Bz) using intermediate dimethoxytrityl protection of 5′-OH, as described by Shott et al.[26]

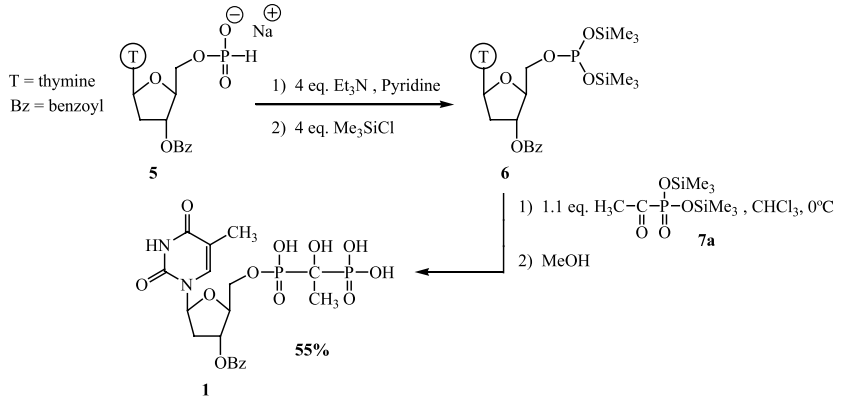
The first step consisted in the preparation of 3′-*O*-benzoyl thymidine-*H*-phosphonate **5** (Scheme 2). This compound was obtained by a reaction reported by Dreef et al.[27] between 3′-*O*-benzoyl thymidine **3** and 1.1 equivalents of 2-chlorobenzodioxaphosphorin-4-one **4** in a mixture acetonitrile/pyridine (75:25) under argon. The reaction mixture was then stirred one hour at room temperature before hydrolysis for two hours with distilled water. After evaporation under vacuum and washes of the crude product with diethyl ether in order to remove the formed salicylic acid, the pyridinium salt of thymidine-*H*-phosphonate was precipitated in cold methanol and filtered. It was then converted to the corresponding sodium salt



SCHEME 2 Preparation of 3′-*O*-benzoyl thymidine-*H*-phosphonate **5**.

by stirring in distilled water with ion-exchange resin (Dowex Na^+ 50 W × 2) to give a colorless solid with 60% yield. This monophosphate derivative **5** was identified by means of NMR spectroscopies. The ^1H NMR spectrum (in CD_3OD) showed a characteristic doublet at 6.92 ppm corresponding to the signal of the proton on the phosphorus atom, with a $^1J_{P\text{-}H}$ coupling constant of 683.6 Hz. Moreover, the ^{31}P $\{^1H\}$ NMR spectrum presented a singlet for the signal of the phosphorus atom about 8.1 ppm.

Next, thymidine-*H*-phosphonate **5** was silylated as described by Hata and Sekine[28] using four equivalents of trimethylsilyl chloride and triethylamine in pyridine under inert atmosphere (Scheme 3). The evolution of the reaction was monitored by ^{31}P $\{^1H\}$ NMR. After 1 h at room temperature, the ^{31}P $\{^1H\}$ NMR spectrum showed the appearance of a single peak around 117 ppm corresponding to compound **6** and characteristic of a bis(silylated) phosphite. This intermediate was not isolated but directly used in the last reaction. Indeed, silylated α-ketophosphonate **7a** was dissolved in chloroform and added to bis(silylated) phosphite **6** at 0°C under argon. The reaction mixture was then stirred at room temperature for 2 h. After a few hours of methanolysis and evaporation under vacuum, 3′-*O*-benzoyl thymidine-5′-HMBP **1** was obtained. The crude product was washed with diethyl ether and converted to the corresponding sodium salt by stirring in distilled water with ion-exchange resin (Dowex Na^+ 50 W × 2) to give a

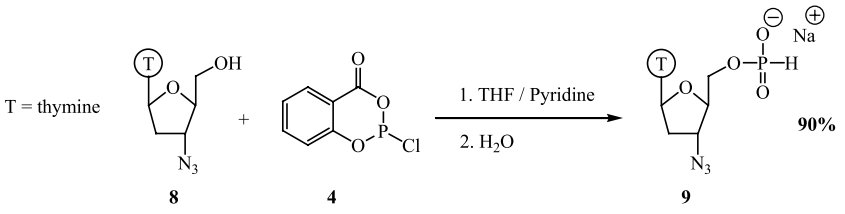


SCHEME 3 Synthesis of 3′-*O*-benzoyl thymidine-5′-HMBP **1**.

colorless solid with 55% yield. One must note that silylated α-ketophosphonate **7a** was prepared according to the procedure described in Scheme 1. The purified product **1** was characterized by NMR spectroscopies. As expected, because of the presence of two new asymmetrical centers and superposition of signals of enantiomers, the ^{31}P $\{^1H\}$ NMR spectrum showed two separate doublets at 22.5 and 21.0 ppm, respectively, with equal $^2J_{P\text{-}P}$ coupling constants corresponding to the first diastereoisomers. Two other different doublets were distinguished respectively at 22.1 and 21.5 ppm corresponding to the second diastereoisomers, formed in the same proportions. Signals about 22 ppm were assigned to phosphorus atom of phosphonic acid moiety, whereas signals about 21 ppm corresponded to phosphorus atom of phosphonate monoester fragment. On the other hand, the ^{13}C $\{^1H\}$ NMR spectrum showed a characteristic signal at 74.3 ppm (dd, $^1J_{C\text{-}P}$ = 145.0 Hz and $^1J_{C\text{-}P}$ = 145.0 Hz) for the carbon atom of the bisphosphonate moiety substituted by hydroxyl and a methyl group. Further characterizations were made by running COSY and HSQC 2D experiments.

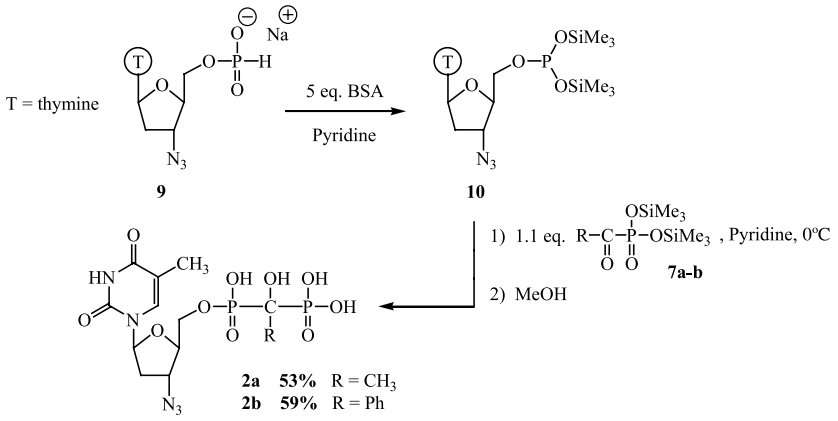
Synthesis of AZT-5′-HMBPs 2

AZT-5′-HMBPs analogues were prepared according the procedure described above and perfected with thymidine. Indeed, AZT-*H*-phosphonate **9** was first synthesized by reaction under argon between AZT **8** and 1.1 equivalents of 2-chloro-benzodioxaphosphorin-4-one **4** in a mixture THF/pyridine (75:25) (Scheme 4). THF was preferred to acetonitrile for solubility reasons. The reaction mixture was then stirred one hour at room temperature before hydrolysis for two hours with distilled water. After evaporation under vacuum, the crude product was washed with diethyl ether in order to remove the resulting salicylic acid. Next, the pyridinium salt of AZT-*H*-phosphonate was converted to the corresponding sodium salt by stirring in distilled water with ion-exchange resin (Dowex Na^+ 50 W × 2). Then, compound **9** was purified by reverse-phase column chromatography using a C-18 resin and distilled water as eluent. A colorless solid was obtained after lyophilization with 90% yield. As observed with thymidine analogue **5**, the ^1H and ^{31}P $\{^1H\}$ NMR spectra in D_2O showed the expected characteristic signals of monophosphate **9** (δ_H = 6.81 ppm, doublet for *H*-phosphonate proton and $^1J_{P\text{-}H}$ = 638.8 Hz; δ_P = 7.2 ppm, singlet).



SCHEME 4 Preparation of AZT-*H*-phosphonate **9**.

Attempts were undertaken in order to prepare the bis(silylated) analogue of monophosphate **9** with trimethylsilyl chloride and triethylamine as previously done with the thymidine derivative. Unfortunately, the expected intermediate was never obtained. However, this reaction was possible using five equivalents of N,O-bis(trimethylsilyl)acetamide (BSA) as reported in the literature by Sekine et al.[29] Distilled pyridine was used as reaction solvent instead of acetonitrile as recommended. Thus, phosphonate monoester **9** was dissolved in dry pyridine, five equivalents of BSA were added at 0°C under argon and the reaction mixture was then stirred at room temperature (Scheme 5). After 1 h, reaction completion was controlled by ^{31}P $\{^1H\}$ NMR with the appearance of a single peak (δ_P = 117.3 ppm) different from the starting product (δ_P = 7.2 ppm), characteristic of the bis(silylated) phosphite **10**. The last reaction between compound **10** and silylated α-ketophosphonate **7a** or **7b** (1.1 equivalents) was carried out in the same conditions as thymidine analogue **1**, except chloroform was replaced by pyridine. The reaction mixture was stirred at room temperature under argon overnight. After a few hours of methanolysis, a precipitate appeared and was filtered off. This product was identified by NMR spectroscopies as the expected AZT-5′-HMBP analogue **2a** or **2b** and converted to the corresponding sodium salt by stirring in distilled water with ion-exchange resin (Dowex Na^+ 50 W × 2) and lyophilized. AZT-5′-HMBP analogues **2a** and **2b** were obtained as colorless powders with 53% and 59% yields, respectively. The ^{31}P $\{^1H\}$ NMR spectrum of compound **2a** (R = CH_3) showed only two different doublets at 20.4 and 18.6 ppm ($^2J_{P\text{-}P}$ = 34.8 Hz), signals that could be attributed to the two superposed diastereoisomers. The ^{31}P $\{^1H\}$ NMR spectrum of product **2b** (R = Ph) distinguished the different diastereoisomers in 60:40 proportions. Indeed, the spectrum indicated two separate doublets at 18.1 and 14.9 ppm ($^2J_{P\text{-}P}$ = 27.7 Hz) for the first diastereoisomers and two other ones at 17.5 and 15.0 ppm ($^2J_{P\text{-}P}$ = 26.3 Hz) for the second diastereoisomers. On the ^1H NMR spectrum, diastereoisomers could not



SCHEME 5 Synthesis of AZT-5′-HMBPs **2**.

be distinguished. Only one signal was observed for each proton. However, the protons on carbon 2′ appeared as two different signals for analogue **2b**. Compounds were fully characterized by doing COSY experiments.

In conclusion, we have developed a new efficient synthetic method for preparation of AZT-5′-HMBPs analogues from AZT via AZT-*H*-phosphonate. Thus, two different aliphatic and aromatic HMBP derivatives have been synthesized in good yields. These compounds will be studied in biological tests in order to evaluate their anti-HIV activity and their toxicity.

EXPERIMENTAL SECTION

Unless otherwise noted, starting materials were purchased from commercial suppliers. 3′-*O*-benzoyl thymidine **3** was synthesized according to the procedure described by Shott et al.[26] Acetyl and benzoyl phosphonic acid dimethyl esters were prepared from the corresponding acyl chlorides and trimethyl phosphite.[25] Me_3SiCl and Me_3SiBr were distilled prior use. Commercial BSA was used without previous purification. Pyridine and triethylamine were distilled over KOH. CH_3CN and $CHCl_3$ were distilled over P_2O_5; CH_2Cl_2 and Et_2O were distilled over sodium and THF was distilled from benzophenone sodium. AZT was obtained from Retrovir capsules (250 mg) and purified by solubilization in EtOH/$CHCl_3$ (75:25), filtration to remove excipients and evaporation of the filtrate. Melting points are uncorrected. NMR spectra were recorded with a VARIAN Unity Inova 500 MHz (^{1}H: 500.6 MHz, ^{31}P: 200.7 MHz, ^{13}C: 125.9 MHz) or a VARIAN Gemini 200 MHz (^{1}H: 200 MHz, ^{31}P: 80.9 MHz, ^{13}C: 50.3 MHz) spectrometer in CD_3OD or D_2O. Chemical shifts (δ) were given in ppm. ^{1}H NMR spectra were recorded using trimethylsilane or HOD as internal standards in CD_3OD or D_2O. ^{31}P and ^{13}C NMR spectra were recorded using, respectively, phosphoric acid and methanol as external references. 1D experiments were performed at 20°C in HDO or in $CDCl_3$. To suppress water signal, presaturation in proton spectrum was made with a duration of 1.5 s after relaxation delay. On 6000 Hz, data were collected with a hard pulse proton of 8.2 μs and a power of 58 dB. 1D ^{13}C spectrum was recorded on spectral width (25,000 Hz) with a 90° nonselective carbon pulse of 31 μs at 50 dB. During acquisition, proton decoupling (Waltz 16) was used with a power of 35 dB on a duration of 0.4 s. For the 2D experiments, we collected data using the hypercomplex method. A recycle delay of 5 s was used to allow complete relaxation of all proton and carbon magnetizations.

A COSY experiment in absolute value was recorded with 512 t1 increments of 8 transients each. The total experiment time was 1.3 h. The 90° nonselective proton pulses were 8.2 μs at 58 dB and presaturation on water signal was used with a power of 2 dB during 1.5 s before the first 90° nonselective proton pulse. Data were acquired with 2048 points. Zero filling was applied in both dimensions before Fourier transformation for a final 4K*2K matrix and sine-bell apodization were

applied in both dimensions before Fourier transformation. $^{1}H^{13}C$ gHSQC experiment[30] was used with a ^{13}C natural abundance sample. The gradient pulses were in the ratio 4:1 at a power level of 72 gauss · cm^{-1}. The 90° nonselective proton pulses were 8.2 μs at 58 dB while the 90° nonselective carbon pulses were 31 μs at 50 dB. The $\frac{1}{4}{}^{1}J_{C\text{-}H}$ delay was set to a value of 1.8 ms as optimized for a corresponding $^{1}J_{C\text{-}H}$ of 140 Hz. GARP decoupling was applied during acquisition to gain sensitivity. Spectral widths were 4000 Hz and 8000 Hz for ^{1}H and ^{13}C nucleus, respectively. Zero filling up to 2K*1K and squared sine-bell apodization were applied in both dimensions before Fourier transformation. A total of 256 experiments of 8 transients each were collected. The total experiment time was 1.5 h. IR spectra were recorded with a Perkin-Elmer FT-IR model 2000 spectrophotometer in the 4000–500 cm^{-1} spectral domain. Spectral resolution was 2 cm^{-1} and usually 5 scans were accumulated. Multiple point baseline correction was performed using the PE Spectrum software. Samples were studied in H_2O and D_2O solutions placed in cells closed by ZnSe windows. Mass spectra were recorded in positive reflectron mode with DHB as a matrix on a MALDI-TOF-MS (Bruker). Microanalyses were not performed on account of hygroscopic compounds.

Synthesis of 3′-*O*-Benzoyl Thymidine-*H*-Phosphonate (5) and AZT-*H*-Phosphonate (9)

To a suspension of 3′-*O*-benzoyl thymidine **3**[26] or AZT **8** (2 mmol) in 7.5 mL of freshly distilled acetonitrile (for thymidine) or THF (for AZT) and 2.5 mL of dry pyridine was added 2-chloro-1,3,2-benzodioxaphosphorin-4-one **4** (2.2 mmol) in one portion under argon (exothermic reaction from 25°C to 35°C). The reaction mixture was stirred 1 h at room temperature and was hydrolyzed for 2 h with 2 mL of distilled water. After solvent removal in vacuum, the crude product was then washed with diethyl ether.

For 3′-*O*-benzoyl thymidine-*H*-phosphonate (5). The obtained pyridinium salt was precipitated in cold methanol and filtered. It was then converted to the corresponding sodium salt by stirring in distilled water with ion-exchange resin Dowex 50 W × 2 (Na^{+} form). Evaporation of the filtrate and lyophilization gave monophosphonate **5** as a colorless solid with 60% yield.

3′-*O*-benzoyl-3′-deoxythymidine-5′-*H*-phosphonate monosodium salt (5). mp 216°C; IR (H_2O): ν = 1728 (C = O benzoyl), 1704 (C = O thymine), 1682 (C = O thymine), 1670 (C = C), 1277 (P-O-thymidine), 1205 (P = O), 1115 (P-O); ^{1}H NMR (CD_3OD): δ 1.93 (s, 3H, CH_3), 2.47–2.60 (m, 2H, *H*2′), 4.31–4.43 (m, 3H, *H*5′ +*H*4′), 5.35–5.44 (m, 1H, *H*3′), 6.42 (t, 1H, $^{3}J_{H\text{-}H}$ = *5.8 Hz*, *H*1′), 6.92 (d, 1H, $^{1}J_{P\text{-}H}$ = *683.6 Hz*, P*H*), 7.51 (dd, 2H, $^{3}J_{H\text{-}H}$ = *7.3 Hz* and $^{3}J_{H\text{-}H}$ = *7.3 Hz*, *m*-C_6H_5), 7.63 (d, 2H, $^{3}J_{H\text{-}H}$ = *7.3 Hz*, *o*-C_6H_5), 7.71 (s, 1H, *H*6), 8.08 (d, 1H, $^{3}J_{H\text{-}H}$ = *7.3 Hz*, *p*-C_6H_5); ^{31}P NMR {^{1}H} (CD_3OD): δ 8.1 (s); ^{13}C NMR

{^{1}H} (CD_3OD): δ 13.4 (*C*H_3), 38.8 (*C*2′), 66.0 (*C*3′), 78.0 (*C*5′), 85.5 (*C*4′), 87.0 (*C*1′), 113.4 (*C*5), 130.6 (*o*-C_6H_5), 131.1 (*p*-C_6H_5), 131.4 (*m*-C_6H_5), 135.7 (OCO-C_6H_5), 138.5 (*C*6), 153.3 (*C*2), 167.4 (*C*4), 168.7 (O*C*O).

For AZT-*H*-phosphonate (9). The obtained pyridinium salt was converted to the corresponding sodium salt by stirring in distilled water with ion-exchange resin Dowex 50 W × 2 (Na^+ form). The filtrate was concentrated and the resulting salt was then purified by reverse-phase column chromatography using C-18 resin (Polygoprep 60–130, Macherey-Nagel) and distilled water as eluent. The UV-absorbing fractions (λ = 254 nm) were combined and concentrated under reduced pressure to give monophosphonate **9** as a colorless solid after lyophilization with 90% yield.

3′-Azido-3′-deoxythymidine-5′-*H*-phosphonate monosodium salt (9). mp 155°C; IR (H_2O): ν = 2114, (azido), 1695 (C = O thymine), 1663 (C = O thymine), 1639 (C = C), 1274 (P-O-AZT), 1206 (P = O), 1058 (P-O); ^{1}H NMR (D_2O): δ 1.94 (s, 3H, C*H*$_3$), 2.53 (dd, 2H, $^3J_{H\text{-}H}$ = *6.0 Hz* and $^3J_{H\text{-}H}$ = *6.0 Hz*, *H*2′), 4.08–4.17 (m, 2H, *H*5′), 4.18–4.24 (m, 1H, *H*4′), 4.51–4.54 (m, 1H, *H*3′), 6.28 (t, 1H, $^3J_{H\text{-}H}$ = *6.0 Hz*, *H*1′), 6.81 (d, 1H, $^1J_{P\text{-}H}$ = *638.8 Hz*, P*H*), 7.71 (s, 1H, *H*6); ^{31}P NMR {^{1}H} (D_2O): δ 7.2 (s); ^{13}C NMR {^{1}H} (D_2O): δ 14.9 (*C*H_3), 39.5 (*C*2′), 63.7 (*C*3′), 66.3 (*C*5′), 86.2 (*C*4′), 88.2 (*C*1′), 114.9 (*C*5), 140.5 (*C*6), 154.9 (*C*2), 169.7 (*C*4).

Synthesis of 3′-*O*-Benzoyl Thymidine Bis(trimethylsilyl) Phosphite (6) Using Me_3SiCl and Et_3N

3′-*O*-benzoyl thymidine-*H*-phosphonate **5** (205 mg, 0.5 mmol) were dissolved in 5 mL of dry pyridine. Distilled triethylamine (0.29 mL, 2 mmol) and distilled trimethylsilyl chloride (0.26 mL, 2 mmol) were successively added under argon. The reaction mixture was then stirred at room temperature for 1 h (the end of the reaction was controlled by ^{31}P {^{1}H} NMR) to furnish the expected bis(silylated) phosphite **6**.

3′-*O*-benzoyl thymidine bis(trimethylsilyl) phosphite (**6**). ^{31}P NMR {^{1}H}: δ 116.9 (s).

Synthesis of AZT Bis(trimethylsilyl) Phosphite (10) Using BSA

A suspension of AZT-*H*-phosphonate **9** (178 mg, 0.5 mmol) in 5 mL of dry pyridine was stirred under argon at room temperature for 1 h to improve the solubilization of starting material. BSA (0.62 mL, 2.5 mmol) was added drop-wise at 0°C and the reaction mixture was stirred at room temperature for 1 h (the end of the reaction was controlled by ^{31}P {^{1}H} NMR) to give the expected bis(silylated) phosphite **10**.

AZT bis(trimethylsilyl) phosphite (**10**). ^{31}P NMR {^{1}H}: δ 117.3 (s).

Synthesis of Bis(silylated) α-Ketophosphonates (7)

To acetyl phosphonic acid dimethyl ester (84 mg, 0.55 mmol) or benzoyl phosphonic acid dimethyl ester (118 mg, 0.55 mmol) in 2 mL of distilled THF at 0°C under argon, trimethylsilyl bromide (0.18 mL, 1.38 mmol) was added drop-wise. The reaction mixture was stirred at room temperature for 5 h (the end of the reaction was controlled by ^{31}P $\{^1H\}$ NMR) and evaporation of volatile fractions (0.01 Torr) at 50°C gave bis(silylated) α-ketophosphonate **7a** or **7b**, respectively.

Acetyl phosphonic acid di(trimethylsilyl) ester (**7a**). ^{31}P NMR $\{^1H\}$: δ − 17.4 (s).

Benzoyl phosphonic acid di(trimethylsilyl) ester (**7b**). ^{31}P NMR $\{^1H\}$: δ − 17.3 (s).

Synthesis of 3′-*O*-Benzoyl Thymidine-5′-Hydroxymethylene Bisphosphonate (1)

Bis(silylated) α-ketophosphonate **7a** freshly prepared was dissolved in 2 mL of distilled chloroform and added drop-wise to thymidine bis(silylated) phosphite **6** at 0°C under argon. The reaction mixture was stirred at room temperature for 2 h. Methanol was then added to the residue and the solution was stirred for 2 h. After solvent removal in vacuum, the crude product was washed with 3 × 10 mL of diethyl ether. The obtained salt was converted to the corresponding sodium salt by stirring in distilled water with ion-exchange resin Dowex 50 W × 2 (Na^+ form). Evaporation of the filtrate and lyophilization gave 3′-*O*-benzoyl thymidine-5′-HMBP **1** as a colorless solid with 55% yield.

3′-*O*-benzoyl-3′-deoxythymidine-5′-[hydroxy(methyl)methylene] bisphosphonate (1). mp >260°C; IR (H_2O): ν = 1727 (C = O benzoyl), 1703 (C = O thymine), 1664 (C = O thymine), 1643 (C = C), 1275 (P-O-thymidine), 1205 (P = O), 1102, 1074, 1062 (P-O); ^1H NMR (D_2O): δ 1.61 (dd, 3H, $^3J_{P\text{-}H}$ = *16.0 Hz* and $^3J_{P\text{-}H}$ = *16.0 Hz*, CH_3COH), 1.96 (s, 3H, CH_3), 2.60–2.69 (m, 2H, *H*2′), 3.65–3.72 (m, 2H, *H*5′), 4.31–4.42 (m, 1H, *H*4′), 5.69–5.74 (m, 1H, *H*3′), 6.49 (t, 1H, $^3J_{H\text{-}H}$ = *7.0 Hz*, *H*1′), 7.58 (dd, 2H, $^3J_{H\text{-}H}$ = *7.3 Hz* and $^3J_{H\text{-}H}$ = *7.3 Hz*, *m*-C_6H_5), 7.73 (d, 1H, $^3J_{H\text{-}H}$ = *7.3 Hz*, *p*-C_6H_5), 7.87 (s, 1H, *H*6), 8.12 (d, 2H, $^3J_{H\text{-}H}$ = *7.3 Hz*, *o*-C_6H_5); ^{31}P NMR $\{^1H\}$ (D_2O): δ 21.0 [d, $^2J_{P\text{-}P}$ = *32.4 Hz*, 5′-O-*P*(O)(OH)], 21.5 (d, $^2J_{P\text{-}P}$ = *30.9 Hz*, 5′-O-*P*(O)(OH)), 22.1 [d, $^2J_{P\text{-}P}$ = 30.9 *Hz*, *P*(O)$(OH)_2$], 22.5 (d, $^2J_{P\text{-}P}$ = 32.4 *Hz*, *P*(O)$(OH)_2$); ^{13}C NMR $\{^1H\}$ (D_2O): δ 14.9 (CH_3), 23.4 (CH_3COH), 39.5 (*C*2′), 69.0 (*C*3′), 74.3 (dd, $^1J_{C\text{-}P}$ = 145.0 *Hz* and $^1J_{C\text{-}P}$ = 145.0 *Hz*, *C*OH), 79.4 (*C*5′), 87.0 (*C*4′), 88.2 (*C*1′), 115.1 (*C*5), 132.0 (*o*-C_6H_5), 132.1 (*p*-C_6H_5), 132.8 (*m*-C_6H_5), 137.3 (OCO-C_6H_5), 140.5 (*C*6), 154.9 (*C*2), 169.7 (*C*4), 171.1 (O*C*O); MS: *m/z* 579.0 $[M + 1]^+$; 601.0 $[M + Na]^+$.

Synthesis of AZT-5′-Hydroxymethylene Bisphosphonates (2)

Bis(silylated) α-ketophosphonate **7a** or **7b** freshly prepared was dissolved in 2 mL of dry pyridine and added drop-wise to AZT bis(silylated) phosphite **10** at

0°C under argon. The reaction mixture was stirred at room temperature overnight. Methanol was then added to the residue and the solution was stirred for 2 h. A precipitate appeared and was filtered. Three successive precipitations were realized in order to recover all the expected product. The obtained pyridinium salt was converted to the corresponding sodium salt by stirring in distilled water with ion-exchange resin Dowex 50 W × 2 (Na^+ form). The filtrate was concentrated and the resulting salt was then purified by reverse-phase column chromatography using C-18 resin (Polygoprep 60–130, Macherey-Nagel) and distilled water as eluent. The UV-absorbing fractions (λ = 254 nm) were combined and concentrated under reduced pressure to give AZT-5′-HMBP **2a** or **2b** as a colorless solid after lyophilization with 53% and 59% yields, respectively.

3′-Azido-3′-deoxythymidine-5′-[hydroxy(methyl)methylene] bisphosphonate (2a). mp >260°C; IR (H_2O): ν = 2116, (azido), 1689 (C = O thymine), 1661 (C = O thymine), 1632 (C = C), 1276 (P-O-AZT), 1197 (P = O), 1106, 1072, 1061 (P-O); ^{1}H NMR (D_2O): δ 1.57 (dd, 3H, $^3J_{P\text{-}H}$ = *15.0 Hz* and $^3J_{P\text{-}H}$ = *15.0 Hz*, CH_3COH), 1.94 (s, 3H, CH_3), 2.46–2.58 (m, 2H, *H*2′), 4.16–4.22 (m, 1H, *H*4′), 4.23–4.30 (m, 2H, *H*5′), 4.51–4.59 (m, 1H, *H*3′), 6.27 (t, 1H, $^3J_{H\text{-}H}$ = *7.0 Hz*, *H*1′), 7.74 (s, 1H, *H*6); ^{31}P NMR {^{1}H} (D_2O): δ 18.6 [d, $^2J_{P\text{-}P}$ = 34.8 *Hz*, 5′-O-*P*(O)(OH)], 20.4 (d, $^2J_{P\text{-}P}$ = *34.8 Hz*, *P*(O)$(OH)_2$); ^{13}C NMR {^{1}H} (D_2O): δ 14.8 (*C*H_3), 23.3 (*C*H_3COH), 39.2 (*C*2′), 63.9 (*C*3′), 68.6 (*C*5′), 75.3 (dd, $^1J_{C\text{-}P}$ = *141.9 Hz* and $^1J_{C\text{-}P}$ = *141.9 Hz*, *C*OH), 86.6 (*C*4′), 88.1 (*C*1′), 115.1 (*C*5), 140.7 (*C*6), 155.0 (*C*2), 169.8 (*C*4); MS: *m/z* 500.0 $[M + 1]^+$; 522.0 $[M + Na]^+$.

3′-Azido-3′-deoxythymidine-5′-[hydroxy(phenyl)methylene] bisphosphonate (2b). mp >260°C; IR (H_2O): ν = 2110 (azido), 1691 (C = O thymine), 1661 (C = O thymine), 1632 (C = C), 1276 (P-O-AZT), 1202 (P = O), 1093, 1079, 1067 (P-O); ^{1}H NMR (D_2O): δ 1.94 (s, 3H, CH_3), 1.97–2.08 (m, 1H, *H*2′), 2.24–2.34 (m, 1H, *H*2′), 3.81–3.93 (m, 1H, *H*4′), 3.94–4.03 (m, 2H, *H*5′), 4.10–4.17 (m, 1H, *H*3′), 6.12 (t, 1H, $^3J_{H\text{-}H}$ = *6.5 Hz*, *H*1′), 7.24–7.32 (m, 1H, *p*-C$_6H_5$), 7.33–7.40 (m, 2H, *m*-C$_6H_5$), 7.49–7.55 (m, 1H, *H*6), 7.74–7.84 (m, 2H, *o*-C$_6H_5$); ^{31}P NMR {^{1}H} (D_2O): δ 14.9 (d, $^2J_{P\text{-}P}$ = *27.7 Hz*, 5′-O-*P*(O)(OH)), 15.0 (d, $^2J_{P\text{-}P}$ = *26.3 Hz*, 5′-O-*P*(O)(OH)), 17.5 (d, $^2J_{P\text{-}P}$ = *26.3 Hz*, *P*(O)$(OH)_2$), 18.1 (d, $^2J_{P\text{-}P}$ = *27.7 Hz*, *P*(O)$(OH)_2$); ^{13}C NMR {^{1}H} (D_2O): δ 15.0 (*C*H_3), 39.3 (*C*2′), 63.9 (*C*3′), 67.9 (*C*5′), 73.3 (dd, $^1J_{C\text{-}P}$ = *139.2 Hz* and $^1J_{C\text{-}P}$ = *139.2 Hz*, *C*OH), 86.6 (*C*4′), 88.1 (*C*1′), 114.9 (*C*5), 129.3 (*o*-*C*$_6H_5$), 129.7 (*p*-*C*$_6H_5$), 130.8 (*m*-*C*$_6H_5$), 140.7 (OCO-*C*$_6H_5$), *C*$_6H_5$), 142.4 (*C*6), 154.9 (*C*2), 169.9 (*C*4); MS: *m/z* 562.0 $[M + 1]^+$; 584.0 $[M + Na]^+$.

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