Synthesis of Polyphosphorylated AZT Derivatives for the Development of Specific Enzyme Immunoassays

Thierry Brossette,[†] Anne Le Faou,[‡] Laure Goujon,[‡] Alain Valleix,[§] Christophe Créminon,[‡] Jacques Grassi,[‡] Charles Mioskowski,*[,†,§] and Luc Lebeau*[,†]

Université Louis Pasteur de Strasbourg, Laboratoire de Synthèse Bioorganique associé au CNRS - Faculté de Pharmacie, 74, route du Rhin - BP 24 - 67 401 Illkirch Cedex - France, CEA - Service de Pharmacologie et d'Immunologie, Département de Recherche Médicale, Bât. 136, CEA-Saclay - 91 191 Gif sur Yvette - France, and CEA - Service des Molécules Marquées, Département de Biologie Moléculaire et Cellulaire, Bât. 547, CEA-Saclay - 91 191 Gif sur Yvette - France

Received December 23, 1998

The synthesis of a series of analogues of the different polyphosphorylated metabolites of AZT has been carried out. The compounds were designed in order to raise specific antibodies for the development of highly sensitive titration kits for the intracellular metabolites of AZT. The pyrophosphate moiety in AZT-DP and AZT-TP analogues is mimicked by the methylene bisphosphonate group to provide in vivo stability of the compounds. An ω-amino linker was introduced at the N^3 position on the base to allow the further anchoring of the compounds to a carrier protein before immunization, tentitatively focusing the specificity of the immune response toward the phosphate mimic moiety and the nonnatural sugar.

Introduction

The efficacy of polytherapies in the treatments of AIDS (combination of reverse transcriptase and protease inhibitors) essentially results from their ability to stop the mechanisms of genetic resistance of the virus.[1,2] Due to the poor "fidelity" of the reverse transcriptase (RT) of HIV, a large number of mutations spontaneously occur during the replication process of the virus. That hypervariability of HIV acts in favor of the emergence of variants that are resistant to the antiviral compounds used. Whatever the antiviral drug administered in the framework of monotherapies, resistance phenomena are observed within a few weeks or months. The combination of multiple drugs allows to prevent or delay the appearance of these resistances as evidenced by the lowering or even disappearance of the viral load for months or years. In these conditions, the emergence of other causes leading to therapy failure are observed. It is a common estimation that more than 30% of the patients on polytherapy are in check and this can be partly explained on account of recent pharmacological results. In 1998 a treatment escape mechanism related to the metabolism of nucleoside derivatives has been reported.[3,4] The authors suggested that a combined use of AZT and d4T could interfere with the intracellular formation of d4T-TP leading to a decrease in the treatment efficacy as seen by insufficient lowering of viral load and poor restoration of CD4+ lymphocytes. These results are supported by previous observations indicating negative metabolic interferences between antiviral nucleosides.[5−7] This highlights the importance of the intracellular metabolism for the activity of nucleoside inhibitors of HIV RT.

Nucleoside inhibitors of HIV RT (AZT, ddI, ddC, d4T, and 3TC) have been the first compounds used in large scale to fight against AIDS and a number of new nucleoside derivatives are currently under clinical evaluation. All these compounds are 2′,3′-dideoxynucleosides (ddNs) and are analogues of natural nucleosides. They inhibit the virus replication by interfering with the RT activity and by acting as chain terminators. They share the common feature to require an intracellular transformation into their corresponding dideoxynucleoside triphosphate (ddN-TP) that is the active species able to inhibit RT. Thus the ddNs are prodrugs, and their metabolism goes through the general metabolic pathway of endogenous nucleosides and nucleotides.[8] It takes place in several steps: entry of the nucleosides into the cell (passive process) and then 3 phosphorylation reactions leading successively to the monophosphorylated (ddN-MPs), diphosphorylated (ddN-DPs), and finally triphosphorylated derivatives (ddN-TPs). The nature and the activity of the enzymes involved in these transformations depend at one and the same time on the structure of the nucleoside analogues, on the cell type, and on its activation state.[9−13] Besides, the synthetic pathways to the

[†] Université Louis Pasteur de Strasbourg.
[‡] CEA - Service de Pharmacologie et d'Immunologie.
[§] CEA - Service des Molécules Marquées.

(1) Collier, A. C.; Coombs, R. W.; Schoenfeld, D. A.; Bassett, R. L.; Timpone, J.; Baruch, A.; Jones, M.; Facey, K.; Whitacre, C.; McAuliffe, V. J.; Merigan, R. C.; Reichman, R. C.; Hooper, C.; Corey, L. *New Engl. J. Med*. **1996**, *334*, 1011−1018.

(2) Gulick, R. M.; Mellors, J. W.; Havlir, D.; Eron, J. J.; Gonzalez, C.; McMahon, D.; Richman, D. D.; Valentine, F. T.; Jonas, L.; Meibohm, A.; Emini, E. A.; Chodakewitz, J. A. *New Engl. J. Med*. **1997**, *337*, 734−739.

(3) Havlir, D. Combination Zidovudine (ZDV) and Stavudine (d4T) versus other nucleosides. Report of two randomized trials (ACTG 290 and 298). Presented at Vth Conference on Retrovirus and Opportunistic Infections, Feb 1−5, 1998, Chicago, IL.

(4) Sommadossi, J. P. Impairment of Stavudine (d4T) phosphorylation in patient receiving a combination of Zidovudine (ZDV) and d4T (ACTG 290). Presented at Vth Conference on Retrovirus and Opportunistic Infections, Feb 1−5, 1998, Chicago, IL.

(5) Wu, S.; Liu, X.; Solorzano, M. M.; Kwock, R.; Avramis, V. I. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol*. **1995**, *8*, 1−9.

(6) Nyce, J.; Leonard, S.; Canupp, S.; Schulz, S.; Wong, S. *Proc. Natl. Acad. Sci. U.S.A*. **1993**, *90*, 2960−2964.

(7) Antonelli, G.; Turriziani, O.; Verri, A.; Narciso, P.; Ferri, F.; D'Offizi, G.; Dianzani, F. *AIDS Res. Hum. Retroviruses* **1996**, *12*, 223−228.

(8) Roy, B.; Fontecave, M. *Bull. Inst. Pasteur* **1993**, *91*, 89−100.

(9) Gao, W. Y.; Agbaria, R.; Driscoll, J. S.; Mitsuya, H. *J. Biol. Chem*. **1994**, *259*, 12633−12638.

10.1021/jo982502p CCC: $18.00
Published on Web 06/19/1999

different dideoxynucleotides (ddN−Ps) are not independent one from each other and some modifications in one of them can affect the others.[13−17]

There is a number of reports in the literature describing a great interindividual and longitudinal variability in the production of the phosphorylated metabolites of antiviral nucleosides in treated patients.[18−21] Today it seems obvious that the plasmatic ddNs levels do not reflect the clinical efficacy of the treatment. As for all bioprecursors, it is necessary to measure the intracellular concentration of the active principles (ddN-TPs) as well as that of their direct precursors (ddNs, ddN-MPs, and ddN-DPs) produced in vivo. The monitoring of nucleoside metabolites in treated patients requires highly sensitive analytical methods able to detect minute amounts of ddN−Ps (10−1000 fmol/10^6 cells, e.g., concentrations between 2×10^{-11} M and 2×10^{-9} M). Most of the data collected over the systemic metabolism of ddNs involved chromatography techniques.[22,23] HPLC coupled to classical detectors (UV or fluorescence spectrophotometers) is not sensitive enough to allow the determination of intracellular ddN−Ps levels. Thus the intracellular metabolism has been mainly investigated on ex vivo systems, in cells cultured in the presence of radiolabeled ddNs.[11,12,18,24−26] The method is not adapted to intracellular ddN−Ps monitoring in patients treated with ddNs.

Our efforts are directed toward the development of a strategy for direct measurement of intracellular dideoxynucleotides using specific enzyme immunoassays. The objective is to establish competitive immunoassays based on the use of specific rabbit polyclonal antibodies together with a tracer (ddN-P covalently coupled to acetylcholinesterase).[27] Immunoenzymological methods perfectly fit the problem to be solved. As a matter of fact, immunoassays are potentially very sensitive, and the femtomolar concentration range is frequently attained. Moreover these techniques present the valuable advantage to be directly applicable to routine analysis of large series of samples and protocols can be easily automated.

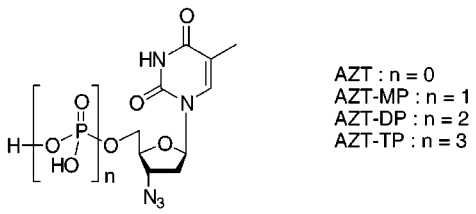


Figure 1.

Herein we describe the strategy we develop for the production of specific antibodies directed against the most widely used ddN in AIDS therapy, AZT, and its different phosphorylated metabolites, AZT-MP, AZT-DP, and AZT-TP (Figure 1). Our ultimate goal is the elaboration of the corresponding enzyme immunoassays that will allow specific monitoring of each compound in cells of treated patients and that will be suited to the clinical environment requirements.

Results and Discussion

The elaboration of enzyme immunoassays for monitoring nucleosides and nucleotides comes up against two specific difficulties. First, the molecules to assay (the haptens) are low molecular weight compounds, and their direct inoculation to rabbits would not give rise to any immune response in the animals. Thus, it is necessary to realize a covalent coupling of these haptens to an antigenic carrier (bovine serum albumin, keyhole limpet hemocyanin) prior to injections. The conjugates (the immunogens) are then capable to trigger off an immune reaction in the recipient animals susceptible to provoke the synthesis of specific antibodies directed against the haptens. The way the coupling to the antigenic carrier is realized is of crucial importance and has to be judiciously chosen to lead to the production of antibodies exhibiting the higher specificity toward the haptens. In our case, these antibodies will be required to specifically recognize AZT or one of its three metabolites in the complex pool of intracellular nucleosides and nucleotides, among which are other AZT derivatives as well as thymidine and its metabolites, structurally very close to the hapten. In that respect, the coupling to the carrier protein has been planned through the base to preserve as much as possible the structural integrity of the sugar and phosphorus-containing moieties in the different molecules (Figure 2). Another difficulty results from both the chemical and the enzymatic instability of pyrophosphate bridges, and AZT-DP and AZT-TP are rapidly hydrolyzed in recipient animals.[28] The methylene bisphosphonate group is stable under hydrolysis conditions and has been widely used to mimic pyrophosphates in nucleotides.[29,30] The corresponding nucleotide analogues are still recognized by much enzymes but are not substrate for them anymore. Thus, the transposition of that structural modification to AZT-DP and AZT-TP has been reported[31,32] and should yield haptens highly resis-

(10) Gao, W. Y.; Shirasaka, T.; John, D. G.; Broder, S.; Mitsuya, H. *J. Clin. Invest.* **1993**, *71*, 2326−2333.
(11) Balzarini, J.; Herdewijn, P.; De Clercq, E. *J. Biol. Chem.* **1989**, *264*, 6127−6133.
(12) Balzarini, J.; Pauwells, R.; Baba, M.; Herdewijn, P.; De Clercq, E.; Broder, S.; John, D. G. *Biochem. Pharmacol.* **1988**, *37*, 897−903.
(13) Sommadossi, J. P. *Clin. Infect. Dis.* **1993**, *16*, S7−S15, Suppl. 1.
(14) Gao, W. Y.; Johns, D. G.; Chokekuchai, S.; Mitsuya, H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8333−8337.
(15) Lori, F.; Malykh, A.; Cara, A.; Sun, D.; Weinstein, J. N.; Lisziewicz, J.; Gallo, R. C. *Science* **1994**, *266*, 801−805.
(16) Hartman, N. R.; Ahluwalia, G. S.; Cooney, D. A.; Mitsuya, H.; Kageyama, S.; fridland, A.; Broder, S.; Johns, D. G. *Mol. Pharmacol.* **1991**, *40*, 118−124.
(17) Bianchi, V.; Borella, S.; Calderazzo, F.; Ferraro, P.; Chieco Bianchi, L.; Reichard, P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8403−8407.
(18) Stretcher, B. N.; Pesce, A. J.; Murzai, J. A.; Hurtubise, P. E.; Vine, W. H.; Frame, P. T. *Ther. Drug Monit.* **1991**, *13*, 325−331.
(19) Kamali, F. *Clin. Investig.* **1993**, *71*, 392−405.
(20) Johnston, M. I.; Hoth, D. F. *Science* **1993**, *260*, 1286−1293.
(21) Richman, D. D.; Fischl, M. A.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Hirsch, M. S. *New Engl. J. Med.* **1987**, *317*, 192−197.
(22) Molema, G.; Jansen, R. W.; Visser, J.; Meijer, D. K. *J. Chromatogr.* **1992**, *579*, 107−114.
(23) Toyoshima, T.; Kimura, S.; Maramatsu, S.; Takahagi, H.; Shimada, K. *Anal. Biochem.* **1991**, *196*, 302−307.
(24) Stretcher, B. N.; Pesce, A. J.; Hurtubise, P. E.; Frame, P. T. *Ther. Drug Monit.* **1992**, *14*, 281−285.
(25) Furman, P. A.; Fyfe, J. A.; St. Clair, M. H.; Weinhold, K.; Rideout, J. L.; Freeman, J. I. A.; Nusinhoff-Lehman, S.; Bolognesi, D. P.; Broder, S.; Mitsuya, H.; Barry, D. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8333−8337.
(26) Tornevik, Y.; Jacobson, B.; Britton, S.; Eriksson, S. *AIDS Res. Hum. Retrov.* **1991**, *7*, 751−759.
(27) Pradelles, P.; Grassi, J.; Chabardes, D.; Guiso, N. *Anal. Chem.* **1989**, *61*, 447−453.
(28) Bredehorst, R.; Ferro, A.; Hilz, H. *Eur. J. Biochem.* **1978**, *82*, 105−113.
(29) Blackburn, G. M. *Chem. Ind.* **1981**, 134−138.
(30) Engel, R. *Chem. Rev.* **1977**, *77*, 349−367.

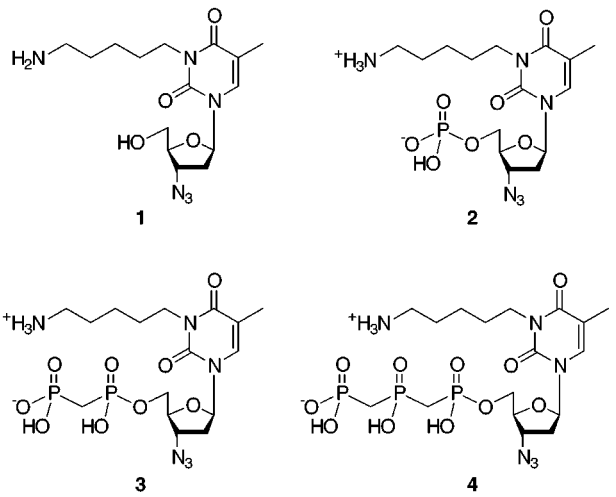
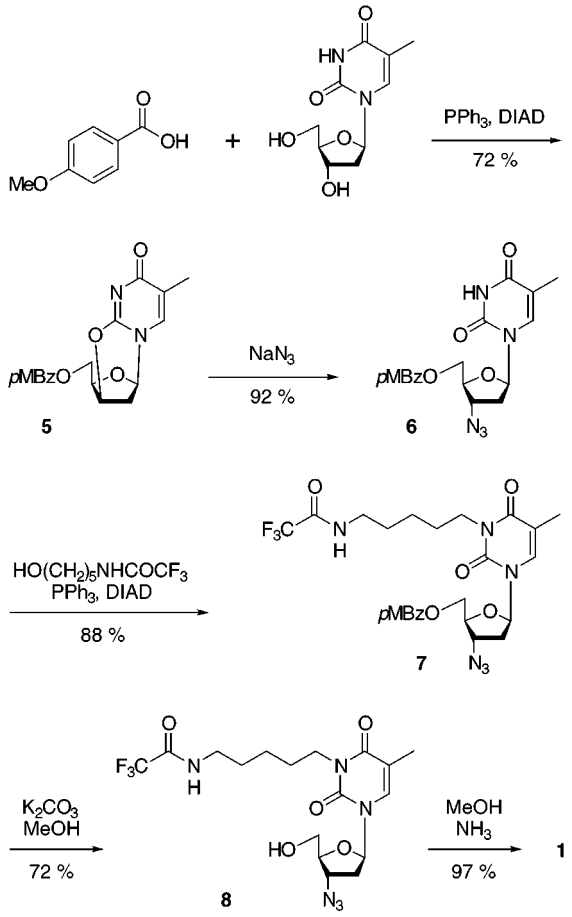


Figure 2.

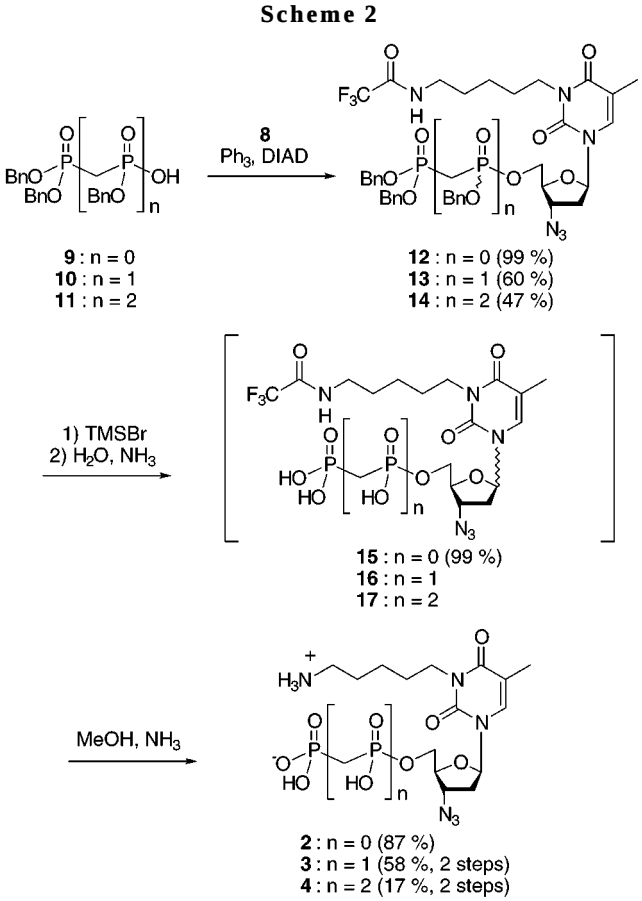
Scheme 1



tant to hydrolytic enzymes. These considerations prompted us to target compounds **1** to **4** (Figure 2).

Compound **1** was prepared from thymidine in five steps (Scheme 1). Intermediate compounds **5** and **6** were obtained following the procedure described by Czernecki and Valery.[33] The 5′-*O*-benzoyl AZT derivative **6** was regioselectively alkylated at N^3 in 88% yield via a Mitsunobu reaction with 5-(2,2,2-trifluoroacetylamino)-1-pentanol.[34] The resulting compound **7** was treated with potassium carbonate in methanol to remove the ester protective group at the 5′ position, and treatment of the subsequent compound **8** in anhydrous methanolic ammonia allowed quantitative removal of the primary amine protective group. Compound **1** was obtained in a 41% overall yield.

Scheme 2



Haptens **2**−**4** were prepared similarly from the intermediate compound **8** (Scheme 2). Phosphorylation and phosphonylations of the 5′-hydroxyl group were carried out via a Mitsunobu reaction. That procedure previously proved to be highly efficient to achieve that kind of transformation.[35,36] Reaction with phosphoric acid dibenzyl ester **9**[37] quantitatively gave compound **12**. Esterification with phosphonic acids **10** and **11**[38,39] gave **13** and **14** in lower yields. This is due to the conjugation of the reduced nucleophilicity of phosphonate anions (when compared to phosphate anions) and of an increase in steric hindrance in the close neighborhood of the nucleophilic center. Thus compound **13** was isolated as a

(31) Balzarini, J.; Herdewijn, P.; Pauwels, R.; Broder, S.; De Clercq, E. *Biochem. Pharmacol.* **1988**, *37*, 2395−2403.
(32) Labataille, P.; Pélicano, H.; Maury, G.; Imbach, J. L.; Gosselin, G. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2315−2320.
(33) Czernecki, S.; Valéry, J. M. *Synthesis* **1991**, 239−240.
(34) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Leahy, E. M.; Salvino, J.; Arison, B.; Cichy, M. A.; Spoors, P. G.; Shakespear, W. C.; Sprengeler, P. A.; Hamley, P.; Smith, A. B. I.; Reisine, T.; Raynor, K.; Maechler, L.; Donaldson, C.; Vale, W.; Freidinger, R. M.; Cascieri, M. R.; Strader, C. D. *J. Am. Chem. Soc.* **1993**, *115*, 12550−12568.
(35) Saady, M.; Lebeau, L.; Mioskowski, C. *Tetrahedron Lett.* **1995**, *36*, 2239−2242.
(36) Vincent, S.; Grenier, S.; Valleix, A.; Salesse, C.; Lebeau, L.; Mioskowski, C. *J. Org. Chem.* **1998**, *63*, 7244−7257.
(37) Baddiley, J.; Clarck, V. M.; Michalski, J. J.; Todd, A. R. *J. Chem. Soc.* **1949**, 815−821.
(38) Saady, M.; Lebeau, L.; Mioskowski, C. *J. Org. Chem.* **1995**, *60*, 2946−2947.
(39) Saady, M.; Lebeau, L.; Mioskowski, C. *Helv. Chim. Acta* **1995**, *78*, 670−678.

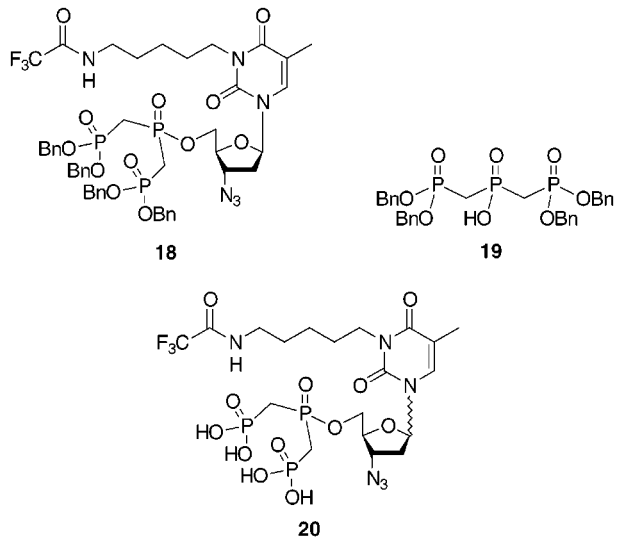
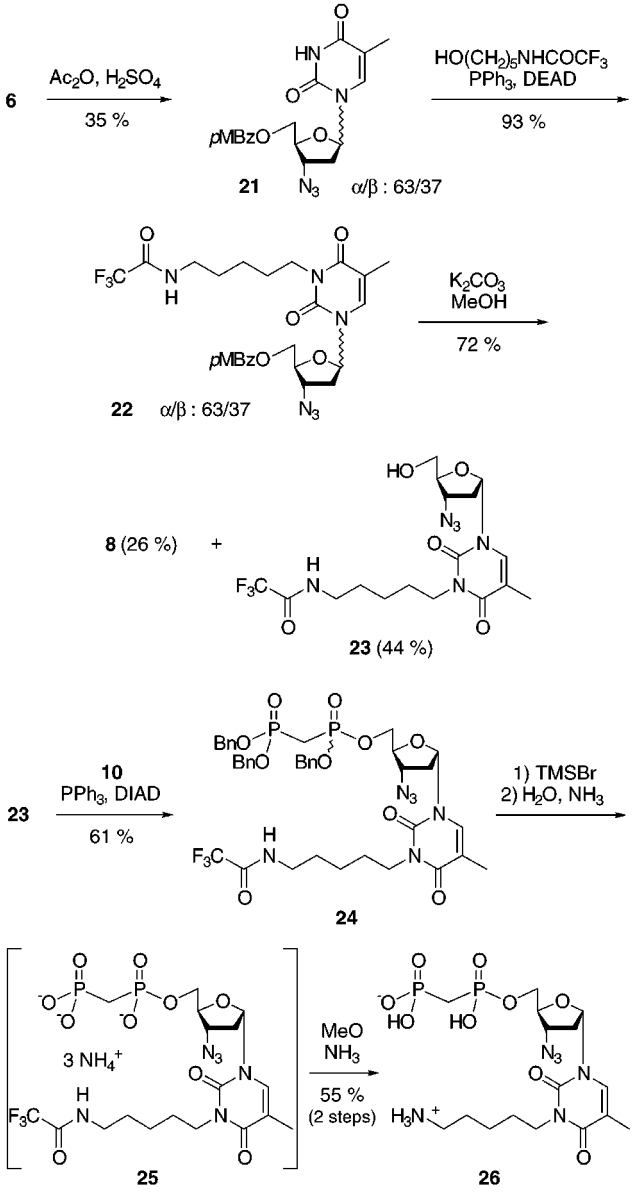


Figure 3.

mixture of two diastereomers in 60% yield whereas compound **14** was obtained as a mixture of four diastereomers, together with compound **18** that could not be separated by flash chromatography, in 47% yield (Figure 3). Compound **18** resulted from the coupling of nucleoside **8** to phosphinic acid **19** obtained as a nonseparable byproduct during the preparation of **11**. Removal of the benzyl esters in this series of compounds could not be achieved by hydrogenolysis due to the presence of the 3′-azido group. Direct saponification under basic conditions was not much suitable, resulting in partial hydrolysis of the ester bond between the phosphorus containing moiety and the deoxy sugar. We attempted to perform debenzylations using trimethylsilyl bromide according to a procedure described by McKenna and others.[40−42] The use of the experimental conditions described by these authors resulted in partial depurination and produced complex mixtures of compounds. The depurination side-reaction could be avoided by quenching the reaction mixture under slightly basic conditions. Thus compound **15** was quantitatively obtained by treating **12** with TMSBr for 3 h at room temperature, the intermediately formed bis-silyl phosphate being decomposed with diluted aqueous ammonia. Final treatment of **15** with anhydrous methanolic ammonia produced compound **2** in a high yield. The debenzylation of phosphonylated compound **13** required a longer reaction time, and a second product formed along with compound **16** that was not separated. The treatment of the previous mixture with methanolic ammonia led to a couple of compounds, and purification was realized by reversed phase HPLC. The purified compounds were analyzed by NMR (^{1}H, ^{13}C, and ^{31}P) and high-resolution mass spectrometry. The major component was identified as compound **3** and the minor one as the stereoisomer resulting from anomerization at the sugar. This was confirmed by realizing the same chemical transformations sequence in the nonnatural α-nucleoside series (Scheme 3). Compound **6** was treated according to the self-anomerization procedure described by Ward *et al.*[43,44] The separation of the resulting couple of diastereomers **21** could not be achieved by chromatography over silica gel. The two mixed isomers were then alkylated at N^3 to produce a nonseparable mixture of diastereomeric compounds **22**, and the benzoyl protective group was subsequently removed under basic conditions. The two resulting diastereomers **8** and **23** were satisfactorily separated by chromatography over silica gel. The α-isomer **23** was condensed with phosphonic acid **10**, and compound **24** was obtained as a mixture of two diastereomers in 61% yield. Subsequent treatment with TMSBr and aqueous ammonia produced **25**. Final deprotection in methanolic ammonia and purification by reversed phase HPLC provided **26** in 55% yield (from **24**) along with compound **3** (24%).

Scheme 3



Consistent with what was observed with compound **13** and **24**, treatment of the mixture of compounds **14** and **18** with TMSBr for 12 h and subsequent hydrolysis afforded **17** and **20** as two couples of diastereomers.

(40) Lazar, S.; Guillaumet, G. *Synth. Commun.* **1992**, *22*, 923−931.
(41) McKenna, C. E.; Higa, M. T.; Cheung, N. H.; McKenna, M. C. *Tetrahedron Lett.* **1977**, 155−158.
(42) McKenna, C. E.; Schmidhauser, J. *J. Chem. Soc. Chem. Commun.* **1979**, 739.
(43) Ward, I. D.; Jeffs, S. M.; Coe, P. L.; Walker, R. T. *Tetrahedron Lett.* **1993**, *34*, 6779−6782.
(44) Larsen, E.; Abdel Aleem, A. A. H.; Pedersen, E. B. *J. Heterocycl. Chem.* **1995**, *32*, 1645−1646.

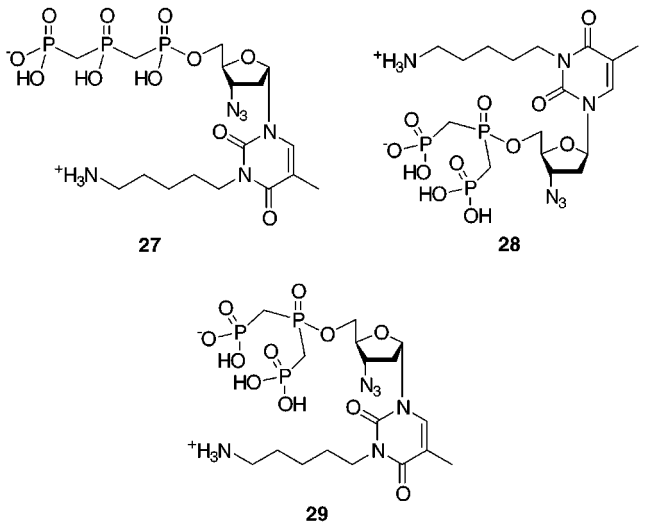


Figure 4.

Removal of the trifluoroacetamide protective group in methanolic ammonia and purification by reversed phase HPLC allowed isolation of the four major compounds **4** (17%), **27** (17%), **28** (13%), and **29** (17%) (Figure 4).

The self-anomerization of nucleosides and nucleotides catalyzed by TMSBr has not been described yet and appears to be a very interesting alternative to the previously reported procedures.[43−49] The transformation occurs in very mild conditions that can be paralleled to those reported by Kiss *et al*.[45] and Yamaguchi *et al*.[46] The first authors mentioned anomerization of a 5′-deoxy-5-fluorouridine analogue catalyzed by trimethylsilyl trifluoromethanesulfonate (TMS-OTf). The transformation however is described to be extremely slow at room temperature, and only a minute amount of the α-anomer (2%) was formed over a week. In these conditions the major product obtained (10%) resulted from deglycosylation, and, besides, the authors indicate that the anomerization reaction is not reversible, as only the β-nucleoside anomerizes. At higher temperature (70 °C) and in the presence of stoichiometric bis(trimethylsilyl)-acetamide (BSA), anomerization of deoxycytidine and deoxythymidine derivatives proceeds satisfactorily within a few hours and is fully reversible.[46] The authors propose the transformation to occur in two steps as an intermolecular reaction. First the trimethylsilylated base is released from the nucleoside, and then the liberated active sugar carbonium cation at the C^1 position is attacked again by the nucleophilic N^1 position of the pyrimidine base. An alternative pathway, however, goes through a ring-opening reaction at the sugar[43] and can account for these results as well as for ours. We actually do not have any experimental evidence for consideration of one mechanism over the other, but additional study is currently underway.

Conclusion

Functionalized analogues of AZT and of its three intracellular metabolites were prepared from thymidine. These compounds contain a spacer arm bearing a primary amine at the N^3 position in the thymine moiety to allow further coupling to a carrier protein using glutaraldehyde as cross-linking reagent. The four title compounds are actually under investigation for the production of specific polyclonal antibodies, and initial results were obtained very recently.[50]

Experimental Section

General. ^{1}H-, ^{13}C-, and ^{31}P-NMR chemical shifts δ are reported in ppm relative to an internal reference resulting from incomplete deuteration of the NMR solvent (^{1}H: $CHCl_3$ at 7.27 ppm, HDO at 4.63 ppm, CD_2HOD at 3.31 ppm; ^{13}C: $CDCl_3$ at 77.0 ppm, CD_3OD at 49.0 ppm; ^{31}P: H_3PO_4 at 0.00 ppm). IR spectra were recorded in wavenumbers (cm^{-1}). Mass spectra (MS) were recorded at chemical ionization. High-resolution mass spectra (HRMS) were recorded in the positive electrospray mode. Mass data are reported in mass units (m/z). Analytical HPLC studies were carried out in the isocratic mode using a reversed phase column (Zorbax SB C_{18}, 250 × 4.6 mm, 5 μm; flow rate 1 mL/min at 25 °C) and a photodiode array detector (LKB 2410, detection at at 267 nm). Reversed phase preparative HPLC was carried out in the isocratic mode (Hypersil BDS C_{18}, 300 × 50 mm, 12 μm; Zorbax SB C_{18}, 250 × 20 mm, 5 μm).

3′-Azido-3′-deoxy-N^3-[1-(5-aminopentyl)]thymidine (1). Compound **8** (90 mg, 0.20 mmol) is stirred for 24 h at room temperature in saturated methanolic ammonia (5 mL). Solvent is removed in vacuo, and the residue is purified by chromatography over silica gel (AcOEt/MeOH: 100/0 to 70/30) to yield **1** (68 mg, 97%) as a white powder. ^{1}H NMR (CD_3OD, 300 MHz) δ 1.28−1.40 (m, 2H); 1.48−1.62 (m, 4H); 1.90 (s, 3H); 2.40 (t, 2H, J = 6.2 Hz); 2.66 (t, 2H, J = 7.2 Hz); 3.80 (AB part of ABX syst, 2H, J_{AB} = 12.1 Hz, J_{AX} = 3.0 Hz, J_{BX} = 3.0 Hz); 3.89−4.36 (m, 3H); 4.33 (pseudo-q, 1H, J = 5.7 Hz); 6.19 (t, 1H, J = 6.2 Hz); 7.83 (s, 1H). ^{13}C NMR (CD_3OD, 50 MHz) δ 13.2; 25.0; 28.1; 29.4; 38.4; 40.5; 42.1; 61.5; 62.3; 86.2; 86.9; 110.7; 136.4; 152.2; 165.3. MS (NH_3) m/z 353 $[M + H]^+$; 370 $[M + NH_4]^+$. IR (neat) ν 1470; 2105; 3307 (br). Anal. Calcd for $C_{15}H_{24}N_6O_4$ (352.41): C 51.12, H 6.87, N 23.85. Found: C 50.96, H 6.91, N 23.61.

3′-Azido-3′-deoxy-5′-*O*-(dihydroxyphosphoryl)-N^3-[1-(5-aminopentyl)] thymidine (2). Compound **2** is obtained from **15** following the same procedure as for **1**. Purification is achieved by flash chromatography over reversed phase silica gel (Merck, RP-18, 40−63 μm, CH_3CN/H_2O: 0/100 to 15/85), and compound **2** is obtained as a white hygroscopic solid (34 mg, 87%). ^{1}H NMR (D_2O, 200 MHz) δ 1.10−1.30 (m, 2H); 1.35−1.55 (m, 4H); 1.74 (s, 3H); 2.29 (t, 2H, J = 6.9 Hz); 2.79 (t, 2H, J = 7.1 Hz); 3.71 (t, 2H, J = 6.8 Hz); 3.80−4.05 (m, 3H); 4.30 (m, 1H); 6.08 (pseudo-t, 1H, J = 6.9 Hz); 7.58 (s, 1H). ^{13}C NMR (D_2O, 50 MHz) δ 13.4; 24.0; 27.2; 27.4; 37.5; 40.3; 42.3; 61.8; 65.5; 84.3; 87.0; 112.1; 136.7; 152.8; 166.8. ^{31}P NMR (D_2O, 121.5 MHz) δ 4.90 (s). MS (NH_3) m/z 308 $[M - H_2PO_4 - N_2 + H]^+$; 325 $[M - H_2PO_4 - N_2 + NH_4]^+$. IR (Kbr) ν 1107; 1467; 2098; 3214 (br). Anal. HPLC (H_2O/CH_3CN/TFA 90/10/0.1) t_R 16.2 min.

3′-Azido-3′-deoxy-5′-*O*-[(dihydroxyphosphorylmethyl)-hydroxyphosphoryl]-N^3-1-(5-aminopentyl)thymidine (3). Trimethylsilyl bromide (67 μL, 0.55 mmol) is added dropwise to compound **13** (96 mg, 0.11 mmol) in anhydrous methylene chloride (5 mL). The reaction mixture is stirred for 6 h at room temperature before 5% aqueous ammonia (0.5 mL) is added. The solution is evaporated to dryness, and the crude residue

(45) Kiss, J.; D'Souza, R.; van Koeveringe, J. A.; Arnold, W. *Helv. Chim. Acta* **1982**, *65*, 1522−1537.
(46) Yamaguchi, I.; Saneyoshi, M. *Chem. Pharm. Bull.* **1984**, *32*, 1441−1450.
(47) Matulic-Adamic, J.; Pavela-Vrancic, M. P.; Skaric, V. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2681−2686.
(48) Seela, F.; Menkhoff, S.; Behrendt, S. *J. Chem. Soc., Perkin Trans. 2* **1986**, 525−530.
(49) Sakthivel, K.; Pathak, T.; Suresh, C. G. *Tetrahedron* **1996**, *52*, 1767−1772.
(50) Goujon, L.; Brossette, T.; Dereudre-Bosquet, N.; Creminon, C.; Clayette, P.; Dormont, D.; Mioskowski, C.; Grassi, J. *J. Immunol. Methods* **1998**, *218*, 19−30.

is stirred for 24 h at room temperature in saturated methanolic ammonia (5 mL). Solvent is removed in vacuo, and purification is achieved by reversed phase HPLC (Hypersil BDS C_{18}, 12 μm, 300 × 50 mm; isocratic mode: ammonium carbonate 0.5 M/acetonitrile 94:6, pH 8.8; flow rate: 30 mL/min). The bis-ammonium salt of compound **3** is obtained as a white hygroscopic solid (retention time: 45 min; 38 mg, 58%). ^{1}H NMR (D_2O, 300 MHz) δ 1.21−1.29 (m, 2H); 1.45−1.60 (m, 4H); 1.80 (s, 3H); 2.01−2.23 (m, 2H); 2.37 (m, 2H); 2.84 (t, 2H, J = 7.1 Hz); 3.78 (t, 2H, J = 7.1 Hz); 3.95−4.06 (m, 3H); 4.39 (m, 1H); 6.12 (pseudo-t, 1H, J = 6.0 Hz); 7.58 (s, 1H). ^{13}C NMR (D_2O, 75 MHz) δ 12.3; 22.9; 26.1; 26.3; 36.1; 39.2; 41.2; 60.6; 64.0; 83.2; 86.0; 110.9; 135.6; 151.7; 165.7. ^{31}P NMR (D_2O, 121.5 MHz) δ 16.93 (m, 1P); 19.01 (m, 1P). HRMS: calcd for $C_{16}H_{28}N_6O_9NaP_2$ $[M + Na]^+$ 533.1291, found 533.1304; calcd for $C_{16}H_{28}N_6O_9P_2K$ $[M + K]^+$ 549.1031, found 549.1039. IR (KBr) ν 1197; 1467; 2109; 3200 (br).

3′-Azido-3′-deoxy-5′-*O*-{[(dihydroxy-phosphorylmethyl)hydroxyphosphorylmethyl]hydroxyphosphoryl}-*N*^{3}-[1-(5-aminopentyl)]thymidine (4). Compound **4** is obtained as its tris-ammonium salt from **14** following the same procedure as for **3**. Purification is achieved by reversed phase HPLC (Zorbax SB C_{18}, 250 × 20 mm; isocratic mode: ammonium carbonate 0.5 M/acetonitrile 94:6, pH 8.8; flow rate: 17 mL/min), and compound **4** is obtained as a white hygroscopic solid (retention time: 37 min; 8 mg, 17%). ^{1}H NMR (D_2O, 200 MHz) δ 1.28−1.38 (m, 2H); 1.53−1.70 (m, 4H); 189 (s, 3H); 2.15−2.35 (m, 4H); 2.45 (m, 2H); 2.92 (t, 2H, J = 7.0 Hz); 3.87 (t, 2H, J = 7.0 Hz); 4.07−4.15 (m, 3H); 4.46 (m, 1H); 6.20 (pseudo-t, 1H, J = 6.1 Hz); 7.66 (m, 1H). ^{31}P NMR (D_2O, 121.5 MHz) δ 15.40 (m, 1P); 19.74 (m, 1P); 31.17 (m, 1P). HRMS: calcd for $C_{17}H_{31}N_6O_{11}NaP_3$ $[M + Na]^+$ 611.1161, found 611.1165; calcd for $C_{17}H_{31}N_6O_{11}Na_2P_3$ $[M - H+2Na]^+$ 633.0981, found 633.0953; calcd for $C_{17}H_{31}N_6O_{11}KNaP_3$ $[M - H + Na + K]^+$ 649.0720, found 649.0740. IR (neat) ν 1096; 1188; 1484; 2112; 3381 (br).

3′-Azido-3′-deoxy-5′-*O*-(4-methoxybenzoyl)-*N*^{3}-{1-[5-(2,2,2-trifluoroacetylamino)pentyl]}thymidine (7). Triphenylphosphine (1.63 g, 6.2 mmol) in anhydrous THF (8 mL) is added dropwise to a mixture of **6** (1.00 g, 2.5 mmol), 5-(2,2,2-trifluoroacetylamino)-1-pentanol (0.59 g, 3.0 mmol), and diisopropyl azodicarboxylate (1.23 mL, 6.2 mmol) in THF (5 mL) at room temperature. The reaction mixture is stirred for 30 min, solvent is removed in vacuo, and the residue is purified by chromatography over silica gel (n-C_6H_{14}/Et_2O: 25/75 to 20/80) to yield compound **7** as a white powder (1.27 g, 88%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25−1.36 (m, 2H); 1.50−1.63 (m, 7H); 2.28−2.49 (m, 2H); 3.27 (dt, 2H, J = 6.6, 5.8 Hz); 3.80 (s, 3H); 3.84 (t, 2H, J = 6.9 Hz); 4.15 (dt, 1H, J = 4.7, 3.7 Hz); 4.32 (dt, 1H, J = 7.3, 4.7 Hz); 4.53 (AB part of ABX syst, 2H, J_{AB} = 12.4 Hz, J_{AX} = 3.3 Hz, J_{BX} = 3.7 Hz, $\Delta\nu$ = 0.07); 6.14 (pseudo-t, 1H, J = 4.7 Hz); 6.87 (m, 2H); 7.17 (s, 1H); 7.33 (m, 1H); 7.91 (m, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.7; 23.6; 26.7; 27.8; 37.6; 39.6; 40.5; 55.3; 60.3; 63.1; 81.8; 85.6; 110.2; 113.7; 115.7 (q, J = 285.9 Hz); 121.1; 131.4; 133.0; 150.4; 157.1 (q, J = 36.3 Hz); 163.1; 163.7; 167.6. MS (NH_3) m/z 583 $[M + H]^+$; 600 $[M + NH_4]^+$ IR (neat) ν 1461; 2108; 2943; 3319. Anal. Calcd for $C_{25}H_{29}F_3N_6O_7$ (582.55): C 51.54, H 5.02, N 14.43. Found: C 51.63, H 5.08, N 14.31.

3′-Azido-3′-deoxy-*N*^{3}-{1-[5-(2,2,2-trifluoroacetylamino)-pentyl]} thymidine (8). Compound **7** (3.39 g, 5.8 mmol) and potassium carbonate (0.48 g, 3.5 mmol) are stirred in anhydrous methanol (40 mL) for 90 min at room temperature. Methanol is evaporated under reduced pressure, and the residue is dissolved in chloroform and washed with brine. The organic layer is dried over sodium sulfate and filtered. The filtrate is reduced in vacuo and purified by chromatography over silica gel (Et_2O) to yield **8** as a white solid (1.80 g, 72%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1. 23−1.41 (m, 2H); 1.56−1.71 (m, 4H); 1.94 (s, 3H); 2.25−2.57 (m, 2H); 3.28−3.37 (m, 3H); 3.70−3.98 (m, 5H); 4.34−4.43 (m, 1H); 6.08 (pseudo-t, J = 6.6 Hz, 1H); 7.20 (m, 1H); 7.49 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.1; 23.7; 26.9; 27.9; 37.4; 39.8; 40.6; 59.9; 61.8; 84.5; 86.9; 110.2; 115.8 (q, J = 287.7 Hz); 134.9; 150.8; 157.4 (q, J = 36.3 Hz); 163.5. MS (NH_3) m/z 424 $[M - N_3 + NH_4]^+$; 449 $[M + H]^+$; 466 $[M + NH_4]^+$. IR (neat) ν 1472; 2105; 2944; 3307 (br). Anal. Calcd for $C_{17}H_{23}F_3N_6O_5$ (448.42): C 45.53, H 5.17, N 18.74. Found: C 45.71, H 5.25, N 19.01.

3′-Azido-3′-deoxy-5′-*O*-(bis-benzyloxy-phosphoryl)*N*^{3}-{1-[5-(2,2,2-trifluoro-acetylamino)pentyl]}thymidine (12). A mixture of triphenylphosphine (146 mg, 0.56 mmol) and diidopropyl azodicarboxylate (110 μL, 0.56 mmol) in anhydrous THF (1.5 mL) is added dropwise to compound **8** (100 mg, 0.22 mmol) and phosphoric acid dibenzyl ester **9** (77 mg, 0.27 mmol) in THF (4.5 mL). The resulting solution is stirred for 1 h at room temperature, and the solvent is removed in vacuo. The crude residue is purified by chromatography over silica gel (Et_2O/$CHCl_3$/CH_3CN: 80/15/5 to 57/28/15) to yield compound **12** as a glassy solid (160 mg, 99%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.32−1.43 (m, 2H); 1.60−1.70 (m, 4H); 1.86 (s, 3H); 2.03−2.40 (m, 2H); 3.36 (dt, 2H, J = 6.0, 6.6 Hz); 3.90−4.21 (m, 6H); 5.07 (AB part of ABX syst, 4H, J_{AB} = 16.0 Hz, J_{AX} = 9.7 Hz, J_{BX} = 9.3 Hz, $\Delta\nu$ = 0.03); 6.19 (pseudo-t, 1H, J = 6.5 Hz); 6.74 (m, 1H); 7.31−7.37 (m, 11H).^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.1; 23.7; 27.0; 27.8; 37.5; 40.0; 40.5; 60.1; 66.3 (d, J = 5.6 Hz); 69.9 (m); 82.1 (d, J = 7.7 Hz); 85.5; 110.8; 128.3; 128.8; 129.0; 135.3 (d, J = 5.7 Hz); 150.8; 152.2 (q, J = 36.8 Hz); 163.5.^{31}P NMR ($CDCl_3$, 121.5 MHz) δ 0.67 (s). MS (NH_3) m/z 709 $[M + H]^+$; 726 $[M + NH_4]^+$. IR (neat) ν 1467; 2108; 2943; 3307.

3′-Azido-3′-deoxy-5′-*O*-[(bis-benzyloxyphosphoryl-methyl)benzyloxyphosphoryl)]-*N*^{3}-{1-[5-(2,2,2-trifluoro-acetylamino)pentyl]}thymidine (13). A mixture of triphenylphosphine (524 mg, 2.00 mmol) and diidopropyl azodicarboxylate (315 μL, 0.56 mmol) in anhydrous THF (2 mL) is added dropwise to compound **8** (200 mg, 0.45 mmol) and phosphonic acid **10** (219 mg, 0.49 mmol) in THF (10 mL). The resulting solution is stirred for 4 h at room temperature, and the solvent is removed in vacuo. The crude residue is purified by chromatography over silica gel (Et_2O/AcOEt/EtOH: 100/0/0 to 0/85/15) to yield compound **13** as a colorless syrup (mixture of two diastereomers, 235 mg, 60%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.19−1.29 (m, 2H); 1.51−1.59 (m, 4H); 1.86 (s, 1.5H); 1.92 (s, 1.5H); 2.21−2.40 (m, 2H); 2.48 (t, 1H, J = 21.1 Hz); 2.50 (t, 1H, J = 21.1 Hz); 3.23 (dt, 2H, J = 6.6, 6.2 Hz); 3.94 (t, 2H, J = 6.2 Hz); 4.02−4.25 (m, 4H); 4.90−5.12 (m, 6H); 6.09−6.15 (m, 1H); 6.84 (m, 1H); 7.31−7.37 (m, 16H).^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.9; 23.6; 25.7 (t, J = 138.1 Hz); 26.8; 27.5; 38.0; 39.7; 40.3; 52.9; 64.9; 68.0−68.2 (m); 84.5−85.5 (m); 86.3; 109.5; 115.9 (q, J = 289.2 Hz); 127.8−128.9 (m); 132.8; 135.6 (m); 150.4; 157.2 (q, J = 36.3 Hz); 163.4.^{31}P NMR ($CDCl_3$, 121.5 MHz) δ 20.75 (d, 0.5P, J = 5.8 Hz); 20.97 (d, 0.5P, J = 5.4 Hz); 21.05 (d, 0.5P, J = 5.4 Hz); 21.44 (d, 0.5P, J = 5.8 Hz). MS (NH_3) m/z 894 $[M + NH_4]^+$. IR (neat) ν 1437; 2108; 2919; 3237; 3495. Anal. HPLC (H_2O/CH_3CN/TFA 40/60/0.1) t_R 9.7 min.

3′-Azido-3′-deoxy-5′-*O*-{[(bis-benzyloxyphosphoryl-methyl)benzyloxyphosphorylmethyl]benzyloxyphosphoryl}-*N*^{3}-{1-[5-(2,2,2-trifluoroacetylamino)pentyl]}-thymidine (14). A mixture of triphenylphosphine (128 mg, 0.45 mmol) and diidopropyl azodicarboxylate (76 μL, 0.45 mmol) in anhydrous THF (3 mL) is added dropwise to compound **8** (49 mg, 0.11 mmol) and phosphonic acid **11** (80 mg, 0.13 mmol) in THF (4 mL). The resulting solution is stirred for 4 h at room temperature, and the solvent is removed in vacuo. The crude residue is purified by chromatography over silica gel (Et_2O/AcOEt/EtOH: 100/0/0 to 0/85/15) to yield compound **14** as a colorless syrup (mixture of 4 diastereomers, 54 mg, 47%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30−1.45 (m, 2H); 1.61−1.68 (m, 4H); 1.84−1.94 (m, 3H); 2.15−2.45 (m, 2H); 2.67−3.15 (m, 4H); 3.34 (dt, 2H, J = 6.4, 6.2 Hz); 3.93 (t, 2H, J = 7.0 Hz); 4.14−4.36 (m, 4H); 4.91−5.20 (m, 8H); 6.08−6.20 (m, 1H); 6.90 (m, 1H); 7.28−7.40 (m, 21H).^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.9; 23.6; 26.9−30.2 (m); 37.0; 39.7; 40.6; 59.8−60.1 (m); 62.6−65.4 (m); 66.9−68.5 (m); 82.0; 85.1−85.7 (m); 110.4; 115.8 (q, J = 291.8 Hz); 127.8−128.7 (m); 133.6−134.1 (m); 135.4−135.7 (m); 150.6; 157.2 (q, J = 36.3 Hz); 163.3. ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ 20.35−22.74 (m, 1P); 30.21 (s, 0.5P); 31.63 (s, 0.5P); 38.19−40.47 (m, 1P). IR (neat) ν 1461; 2108; 2355; 2943; 3237.

3′-Azido-3′-deoxy-5′-*O*-(dihydroxyphosphoryl)-*N*³-{1-[5-(2,2,2-trifluoroacetylamino)pentyl]}thymidine (15). Trimethylsilyl bromide (60 μL, 0.50 mmol) is added dropwise to compound **12** (63 mg, 0.10 mmol) in anhydrous methylene chloride (5 mL). The reaction mixture is stirred for 3 h at room temperature before 5% aqueous ammonia (0.5 mL) is added. The solution is evaporated to dryness, and the bis-ammonium salt of compound **15** is obtained as a white cristalline powder without further purification (47 mg, 99%). ^{1}H NMR (D_2O, 300 MHz) δ 1.08−1.19 (m, 2H); 1.41−1.45 (m, 4H); 1.76 (d, 3H, J = 0.9 Hz); 2.30−2.34 (m, 2H); 3.15 (t, 2H, J = 7.2 Hz); 374 (t, 2H, J = 7.2 Hz); 3.81−4.04 (m, 3H); 4.32 (m, 1H); 6.12 (pseudo-t, 1H, J = 6.4 Hz); 7.59 (d, 1H, J = 0.9 Hz). ^{13}C NMR (D_2O/CD_3OD: 95/5, 75 MHz) δ 13.3; 24.2; 27.2; 28.4; 37.5; 40.4; 42.4; 61.5; 65.5; 84.1; 86.8; 111.9; 136.4; 152.7; 166.7. ^{31}P NMR (D_2O/CD_3OD, 121.5 MHz) δ 1.50 (s). MS (NH_3) m/z 449 [M − $COCF_3$ + NH_4]$^+$; 519 [M − N_2 + NH_4]$^+$. IR (neat) ν 1402; 1467; 2108; 3119 (br). Anal. HPLC (H_2O/CH_3CN/TFA 70/30/0.1) t_R 7.3 min.

Anomerization of Compound 6 into 21. To compound **6** (1.05 g, 2.6 mmol) in CH_2Cl_2 (2 mL) is added CH_3CN/CH_2Cl_2/Ac_2O/H_2SO_4: v/v/v/v: 20/20/4/1 (11.5 mL). The resulting mixture is stirred at room temperature for 1 h before saturated aqueous $NaHCO_3$ (40 mL) is added. Solvents are removed under reduced pressure, and the residue is extracted with CH_2Cl_2. The organic layer is dried over Na_2SO_4, reduced in vacuo, and purified by chromatography over silica gel (Et_2O) to yield a mixture of the two anomers **21** (371 mg, 35%, α/β: 63/37) that could not be separated by flash chromatography. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.72 (s, 3H$_\beta$); 1.97 (s, 3H$_\alpha$); 2.14−2.59 (m, 2H$_\beta$, 1H$_\alpha$); 2.87 (m, 1H$_\alpha$); 3.88 (s, 3H$_\alpha$ and 3H$_\beta$); 4.20−4.69 (m, 4H$_\alpha$ and 4H$_\beta$); 6.19 (pseudo-t, 1H$_\beta$, J = 6.8 Hz); 6.27 (dd, 1H$_\alpha$, J = 6.8, 3.8 Hz); 6.92−6.98 (m, 2H$_\alpha$ and 2H$_\beta$); 7.22 (s, 1H$_\beta$); 7.33 (s, 1H$_\alpha$); 7.97−8.01 (m, 2H$_\alpha$ and 2H$_\beta$); 8.43 (m, 1H$_\alpha$ and 1H$_\beta$). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.0$_\beta$; 12.4$_\alpha$; 37.4$_\beta$; 37.7$_\alpha$; 55.2$_{\alpha\beta}$; 60.4$_\beta$; 61.2$_\alpha$; 63.2$_\beta$; 63.7$_\alpha$; 81.7$_\beta$; 83.2$_\alpha$; 84.9$_\beta$; 85.9$_\alpha$; 110.6$_\alpha$; 111.0$_\beta$; 113.6$_{\alpha\beta}$; 121.2$_{\alpha\beta}$; 131.5$_{\alpha\beta}$; 134.9$_{\alpha\beta}$; 150.2$_\beta$; 150.4$_\alpha$; 163.5$_\alpha$; 163.6$_\beta$; 163.9$_\beta$; 164.1$_\alpha$; 165.5$_{\alpha\beta}$. MS (NH_3) m/z 374 [M − N_2 + H]$^+$; 391 [M − N_2 + NH_4]$^+$; 419 [M + NH_4]$^+$. IR (neat) ν 1467; 2097; 2931; 3049; 3178. Anal. HPLC (H_2O/CH_3CN/TFA 70/30/0.1) t_R 36.8 min (β anomer), 40.8 min (α anomer).

3′-Azido-3′-deoxy-5′-*O*-(4-methoxybenzoyl)-*N*³-{1-[5-(2,2,2-trifluoroacetylamino)pentyl]}thymidine (mixture of α and β anomers, 22). Compound **22** is obtained from **21** following the same procedure as for **7**. The two anomers are not separated by chromatography over silica gel (502 mg, 93%, α/β: 63/37). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.21−1.40 (m, 2H$_\alpha$ and 2H$_\beta$); 1.50−1.70 (m, 4H$_\alpha$ and 7H$_\alpha$); 1.63 (s, 3H$_\beta$); 1.89 (s, 3H$_\alpha$); 2.15−2.55 (m, 1H$_\alpha$ and 2H$_\beta$); 2.74−2.89 (m, 1H$_\alpha$); 3.27 (pseudo-q, 2H$_\alpha$ and 2H$_\beta$, J = 6.2 Hz); 3.79 (s, 3H$_\alpha$ and 3H$_\beta$); 3.86 (t, 2H$_\alpha$ and 2H$_\beta$, J = 6.6 Hz); 4.15 (dt, 1H$_\beta$, J = 4.7, 3.7 Hz); 4.23−4.63 (m, 4H$_\alpha$ and 3H$_\beta$); 6.14 (pseudo-t, 1H$_\beta$, J = 6.6 Hz); 6.19 (dd, 1H$_\alpha$, J = 6.9, 3.7 Hz); 6.87 (d, 2H$_\alpha$ and 2H$_\beta$, J = 8.8 Hz); 7.17 (s, 1H$_\beta$); 7.28 (s, 1H$_\alpha$); 7.33 (m, 1H$_\alpha$ and 1H$_\beta$); 7.91 (d, 2H$_\alpha$ and 2H$_\alpha$, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.9$_\alpha$; 13.1$_\alpha$; 23.6$_{\alpha\beta}$; 26.8$_{\alpha\beta}$; 27.8$_{\alpha\beta}$; 37.5$_\beta$; 37.8$_\alpha$; 39.6$_{\alpha\beta}$; 40.4$_\alpha$; 40.5$_\beta$; 55.2$_{\alpha\beta}$; 60.3$_\beta$; 61.5$_\alpha$; 63.1$_\beta$; 63.6$_\alpha$; 81.8$_\beta$; 83.4$_\alpha$; 85.6$_\beta$; 109.7$_\alpha$; 110.2$_\beta$; 113.6$_\alpha$; 113.7$_\beta$; 115.7$_{\alpha\beta}$ (q, J = 287.7 Hz); 121.1$_{\alpha\beta}$; 131.4$_\alpha$; 131.8$_\beta$; 133.0$_\beta$; 133.1$_\alpha$; 150.4$_\beta$; 150.6$_\alpha$; 157.1$_{\alpha\beta}$ (q, J = 36.3 Hz); 163.1$_\beta$; 163.3$_\alpha$; 163.6$_\alpha$; 164.0$_\beta$; 165.6$_{\alpha\beta}$. MS (NH_3) m/z 555 [MH − N_2]$^+$; 572 [M − N_2 + NH_4]$^+$; 600 [M + NH_4]$^+$. IR (neat) ν 1261; 1467; 2108; 2355; 2943; 3307. Anal. Calcd for $C_{25}H_{29}F_3N_6O_7$ (582.55): C 51.54, H 5.02, N 14.43. Found: C 51.75, H 5.15, N 14.26.

3′-Azido-3′-deoxy-*N*³-{1-[5-(2,2,2-trifluoroacetylamino)-pentyl]}α-thymidine (23). Compound **22** (490 mg, 0.84 mmol) is stirred with potassium carbonate (70 mg, 0.51 mmol) in anhydrous methanol (10 mL) for 2 h at room temperature. Solvent is removed in vacuo before saturated aqueous $NaHCO_3$ (15 mL) is added. The solution is extracted with CH_2Cl_2, and the organic layer is dried over Na_2SO_4, filtered, and reduced in vacuo. The crude residue is purified by chromatography over silica gel (Et_2O) and yields separately compound **8** (98 mg, 26%) and its α-anomer **23** (166 mg, 44%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25−1.39 (m, 2H); 1.54−1.69 (m, 4H); 1.91 (s, 3H); 2.13 (m, 1H); 2.82 (m, 1H); 3.30 (m, 2H); 3.63−3.79 (m, 2H); 3.88 (t, 2H, J = 7.0 Hz); 4.27−4.33 (m, 2H); 6.18 (dd, 1H, J = 7.0, 3.7 Hz); 7.30−7.34 (m, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.2; 23.6; 26.8; 27.8; 38.1; 39.7; 40.6; 60.7; 62.5; 86.1; 86.8; 109.8; 115.8 (q, J = 287.7 Hz); 133.5; 150.8; 157.3 (q, J = 36.3 Hz); 163.6. MS (NH_3) m/z 421 [M − N_2 + H]$^+$; 449 [M + H]$^+$; 466 [M + NH_4]$^+$. IR (neat) ν 1467; 2097; 2943; 3307; 3448. Anal. Calcd for $C_{17}H_{23}F_3N_6O_5$ (448.42): C 45.53, H 5.17, N 18.74. Found: C 45.89, H 5.22, N 18.86.

3′-Azido-3′-deoxy-5′-*O*-[(bis-benzyloxyphosphoryl-methyl)benzyloxyphosphoryl]-*N*³-{1-[5-(2,2,2-trifluoro-acetylamino)pentyl]}-α-thymidine (24). Compound **24** is prepared starting from phosphonic acid **10** and nucleoside **23** following the same procedure as for **13** and is obtained as a mixture of two diastereomers (59 mg, 61%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30−1.40 (m, 2H); 1.60−1.70 (m, 4H); 1.93 (s, 3H); 1.94−2.04 (m, 1.5H); 2.51 (t, 1H, J = 21.1 Hz); 2.52 (t, 1H, J = 21.1 Hz); 2.60−2.75 (m, 0.5H); 3.34 (pseudo-q, 2H, J = 6.4 Hz); 3.93 (t, 2H, J = 6.8 Hz); 3.96−4.30 (m, 4H); 4.95−5.20 (m, 6H); 6.08−6.10 (m, 1H); 7.19 (s, 0.5H); 7.20 (s, 0.5H); 7.32−7.45 (m, 15H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.2; 23.7; 25.9 (t, J = 136.6 Hz); 26.9; 27.9; 37.5 and 37.7; 39.8; 40.5; 60.5 and 60.6; 65.1−65.4 (m); 67.6−68.5 (m); 83.6−83.9 (m); 87.0; 109.9; 115.9 (q, J = 287.7 Hz); 128.1−128.8 (m); 133.3; 135.4−136.0 (m); 150.7; 157.2 (q, J = 36.3 Hz); 163.6. ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ 20.71 (d, 0.5P, J = 6.6 Hz); 20.73 (d, 0.5P, J = 6.6 Hz); 22.06 (d, 0.5P, J = 6.6 Hz); 22.11 (d, 0.5P, J = 6.6 Hz). MS (NH_3) m/z 877 [M + H]$^+$, 894 [M + NH_4]$^+$. IR (neat) ν 1466; 2107; 2954; 3063; 3241.

3′-Azido-3′-deoxy-5′-*O*-[(dihydroxyphosphorylmethyl)-hydroxyphosphoryl]-*N*³-[1-(5-aminopentyl)]-α-thymidine (26). Compound **26** is obtained as its bis-ammonium salt starting from **24** following the same procedure as for **3** (retention time: 37 min; 16 mg, 55%). ^{1}H NMR (D_2O, 300 MHz) δ 1.22−1.29 (m, 2H); 1.45−1.60 (m, 4H); 1.79 (s, 3H); 2.07 (t, 2H, J = 20.3 Hz); 2.13−2.18 (m, 1H); 2.77 (m, 1H); 2.84 (t, 2H, J = 7.5 Hz); 3.78 (t, 2H, J = 7.5 Hz); 3.89 (m, 2H); 4.37 (m, 1H); 4.46 (m, 1H); 6.06 (dd, 1H, J = 6.4, J = 2.3 Hz); 7.52 (s, 1H). ^{13}C NMR (D_2O, 75 MHz) δ 12.3; 22.9; 25.3 (t, J = 142.5 Hz; 26.2; 26.3; 37.0; 39.3; 41.0; 60.8; 64.4; 85.3; 88.2; 111.0; 135.7; 151.5; 165.9. ^{31}P NMR (D_2O, 121.5 MHz) δ 16.57 (m, 1P); 19.16 (m, 1P). HRMS: calcd for $C_{16}H_{28}N_6O_9NaP_2$ [M + Na]$^+$ 533.1291, found 533.1298; calcd for $C_{16}H_{28}N_6O_9P_2K$ [M + K]$^+$ 549.1031, found 549.1037. IR (neat) ν 1455; 1922; 2108; 29.31; 3369.

3′-Azido-3′-deoxy-5′-*O*-{[(dihydroxyphosphorylmeth-yl)hydroxyphosphorylmethyl]hydroxyphosphoryl}-*N*³-[1-(5-aminopentyl)]-α-thymidine (27). Compound **27** is produced together with compound **4** from **14** and is purified by HPLC (Zorbax SB C_{18}, 250 × 20 mm; isocratic mode: ammonium carbonate 0.5 M/acetonitrile 94:6, pH 8.8; flow rate: 17 mL/min). Its tris-ammonium salt is obtained as a white hygroscopic solid (retention time: 28 min; 8 mg, 17%). ^{1}H NMR (D_2O, 200 MHz) δ 1.27−1.38 (m, 2H); 1.54−1.68 (m, 4H); 1.87 (s, 3H); 2.15−2.28 (m, 5H); 2.83 (m, 1H); 2.92 (t, 2H, J = 7.2 Hz); 3.87 (t, 2H, J = 7.0 Hz); 3.97 (m, 2H); 4.45 (m, 1H); 4.55 (m, 1H); 6.15 (m, 1H); 7.61 (m, 1H). ^{31}P NMR (D_2O, 121.5 MHz) δ 15.53 (m, 1P); 19.73 (m, 1P); 29.25 (m, 1P). HRMS: calcd for $C_{17}H_{31}N_6O_{11}NaP_3$ [M + Na]$^+$ 611.1161, found 611.1122; calcd for $C_{17}H_{30}N_6O_{11}Na_2P_3$ [M − H + 2Na]$^+$ 633.0981, found 633.0973; calcd for $C_{17}H_{32}N_6O_{11}KNaP_3$ [M − H + Na + K]$^+$ 649.0720, found 649.0687. IR (neat) ν 1471; 2110; 2955; 3377 (br).

3′-Azido-3′-deoxy-5′-*O*-[bis(dihydroxyphosphorylmeth-yl)phosphoryl]-*N*³-[1-(5-aminopentyl)]thymidine (28). Compound **28** is produced together with compound **4** from **14** and is purified by HPLC (Zorbax SB C_{18}, 250 × 20 mm; isocratic mode: ammonium carbonate 0.5 M/acetonitrile 94:6, pH 8.8; flow rate: 17 mL/min). Its tris-ammonium salt is obtained as a white hygroscopic solid (retention time: 33 min; 6 mg, 13%). ^{1}H NMR (D_2O, 200 MHz) δ 1.27−1.35 (m, 2H); 1.50−1.68 (m, 4H); 1.89 (s, 3H); 2.47−2.56 (m, 6H); 2.92 (t, 2H, J = 7.5 Hz); 3.87 (t, 2H, J = 7.0 Hz); 4.17−4.27 (m, 3H); 4.49 (m, 1H); 6.17 (pseudo-t, 1H, J = 6.4 Hz); 7.53 (m, 1H). ^{31}P NMR (D_2O, 121.5

MHz) δ 9.42 (m, 2P); 59.11 (m, 1P). HRMS: calcd for $C_{17}H_{31}N_6O_{11}NaP_3$ $[M + Na]^+$ 611.1161, found 611.1171; calcd for $C_{17}H_{31}N_6O_{11}KP_3$ $[M + K]^+$ 627.0901, found 627.0917; calcd for $C_{17}H_{30}N_6O_{11}Na_2P_3$ $[M - H + 2Na]^+$ 633.0981, found 633.0979; calcd for $C_{17}H_{32}N_6O_{11}KNaP_3$ $[M - H + Na + K]^+$ 649.0720, found 649.0719. IR (neat) ν 1458; 2106; 2928; 3364 (br).

3′-Azido-3′-deoxy-5′-*O*-[bis(dihydroxyphosphorylmethyl)phosphoryl]-N^3-[1-(5-aminopentyl)]-α-thymidine (29). Compound **29** is produced together with compound **4** from **14** and is purified by HPLC (Zorbax SB C_{18}, 250 × 20 mm; isocratic mode: ammonium carbonate 0.5 M/acetonitrile 94:6, pH 8.8; flow rate: 17 mL/min). Its tris-ammonium salt is obtained as a white hygroscopic solid (retention time: 21 min; 8 mg, 17%). ^{1}H NMR (D_2O, 200 MHz) δ 1.24−1.44 (m, 2H); 1.52−1.67 (m, 4H); 1.86 (s, 3H); 2.23−2.27 (m, 1H); 2.51 (pseudo-t, 4H, J = 19.2 Hz); 2.86 (m, 1H); 2.91 (t, 2H, J = 7.3 Hz); 3.85 (t, 2H, J = 7.0 Hz); 4.15−4.25 (m, 3H; 4.48 (m, 1H); 6.15 (dd, 1H, J = 6.8, J = 3.1 Hz); 7.59 (m, 1H). ^{31}P NMR (D_2O, 121.5 MHz) δ 9.64 (m, 2P); 59.39 (m, 1P). HRMS: calcd for $C_{17}H_{32}N_6O_{11}P_3$ $[M + H]^+$ 589.1342, found 589.1345; calcd for $C_{17}H_{31}N_6O_{11}NaP_3$ $[M + Na]^+$ 611.1161, found 611.1149.

Acknowledgment. This work was supported by a grant from ANRS (Agence Nationale de Recherche sur le Sida, France). The authors thank Eric Le Gallo for analytical work.

JO982502P