S-Acyl-2-thioethyl Aryl Phosphotriester Derivatives as Mononucleotide Prodrugs

Nathalie Schlienger, Suzanne Peyrottes, Tarek Kassem, Jean-Louis Imbach, Gilles Gosselin, Anne-Marie Aubertin,† and Christian Périgaud*

UMR 5625 CNRS-UM II, Université Montpellier II, cc 008, place E. Bataillon, 34095 Montpellier Cedex 05, France, and Laboratoire de Virologie de la Faculté de Médecine, Unité 74 I.N.S.E.R.M., Université L. Pasteur, 3, rue Koeberlé, 67000 Strasbourg, France

Received May 29, 2000

The synthesis and biological activities of phosphotriester derivatives of 3′-azido-2′,3′-dideoxythymidine (AZT) bearing a phenyl group or L-tyrosinyl residues are reported. The target compounds were obtained via either P^V or P^{III} chemistry from the appropriate aryl precursors. All the derivatives were evaluated for their in vitro anti-HIV activity, and they appeared to be potent inhibitors of HIV-1 replication in various cell culture experiments, with EC_{50} values between the micro- and nanomolar range. Furthermore, compounds incorporating an amino- and/or acid-substituted tyrosinyl residue demonstrated significant anti-HIV effects in thymidine kinase-deficient (TK^-) cells showing their ability to act as mononucleotide prodrugs. The proposed decomposition process of these mixed mononucleoside aryl phosphotriesters may involve esterase activation followed by phosphodiesterase hydrolysis.

Introduction

Among the compounds approved in the treatment of AIDS disease, 2′,3′-dideoxynucleoside analogues (ddN) represent an important class of potent inhibitors of the HIV reverse transcriptase.[1,2] Nevertheless, those ddN analogues need to be metabolized to their corresponding 5′-triphosphates (ddNTP) through the action of several intracellular kinases. In many cases, the rate-limiting step for the conversion of the ddN analogue to its active metabolite (ddNTP) is the formation of the 5′-mononucleotide from the nucleoside analogue.[3] To overcome this dependence of ddN upon nucleoside kinase activation, various research groups have focused their attention on the study of monophosphorylated prodrugs, namely pronucleotides.[4−7] Such pronucleotides should be able, after intracellular hydrolysis, to give rise to the delivery of 5′-mononucleotides, which could be further metabolized to their corresponding ddNTP analogues in a nucleoside kinase-nondependent way.

On this topic, we have previously demonstrated that the use of symmetrical mononucleoside phosphotriesters incorporating *S*-acyl-2-thioethyl (SATE) groups leads to the intracellular release of the corresponding 5′-mononucleotide.[8] The decomposition pathway of these bis(SATE) derivatives involves successively two esterase-mediated activation steps (Scheme 1).[9,10] However, the removal of the second SATE masking group could be considered as a rate-limiting step in this general process.[10,11] With the aim of the in vivo development of a pronucleotide approach, we have previously reported the effect of structural modifications of the SATE groups on kinetic parameters involved in the release of the 5′-mononucleotide from its corresponding prodrug.[12,13] All these structural modifications allowed to increase the stability of the resulting bis(SATE) phosphotriester derivatives but may have precluded the conversion of the resulting mono(SATE) phosphodiester due to inappropriate kinetic parameters (second step, Scheme 1). Therefore, we decided to evaluate the potential of a new series of mixed phosphotriesters incorporating only one SATE chain and a different protecting group on the phosphorus atom, the latter being able to be hydrolyzed by another enzymatic system than esterases.

Results and Discussion

The design of the described mixed phosphotriesters is based on literature data[14−16] which showed that nucleotide phosphodiesterases, a family of ubiquitous enzymes, have good affinity for aromatic substrates and were able to selectively hydrolyze aryl phosphodiester derivatives into the corresponding phosphomonoesters.[16,17] Thus, our idea was to generate intracellularly an aryl phosphodiester by the use of an appropriate phosphorylated precursor. This observation led us to study, as a first model, mononucleoside aryl phosphotriesters incorporating as the aryl substituent either a phenyl group or a natural aromatic amino acid such as L-tyrosine, respectively **2** and **3** (Chart 1). These preliminary investigations were published as a short note and demonstrated that the 5′-mononucleotide derivative of AZT was released intracellularly.[18] Herein, we would like to report the entire study, i.e. synthesis and anti-HIV-1 activity, of several aryl phosphotriesters of 3′-azido-2′,3′-dideoxythymidine (AZT) **2**−**5** incorporating one *S*-pivaloyl-2-thioethyl (*t*BuSATE) moiety and a phenyl group or an amino acid residue derived from L-tyrosine. The antiviral evaluations were carried out in comparison to the previously described bis(*t*BuSATE) phosphotriester derivative of AZT **1**,[9] as reference pronucleotide.

Chemistry. The synthesis of the desired mixed aryl phosphotriester derivatives could be carried out follow-

* To whom correspondence should be addressed. Tel: 33(0)-467144776. Fax: 33(0)467042029. E-mail: perigaud@univ-montp2.fr.
† Université L. Pasteur.

10.1021/jm000996o CCC: $19.00
Published on Web 10/24/2000

Scheme 1. Decomposition Pathway of Mononucleoside Bis(SATE) Phosphotriester Derivatives in Cell Extracts and Intact Cells

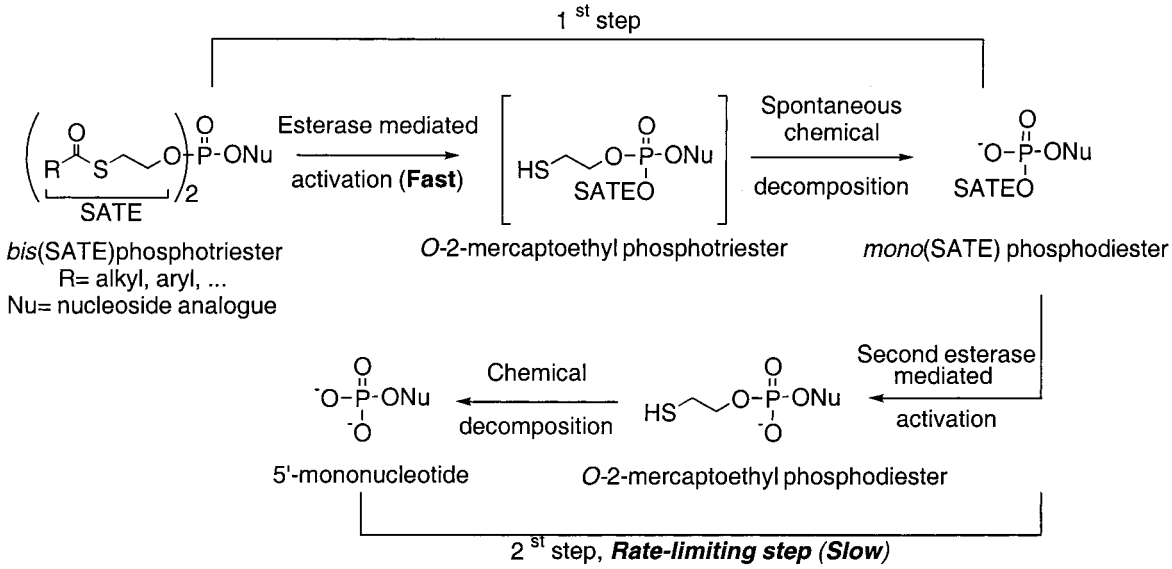
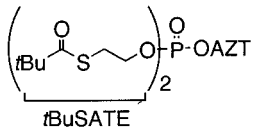
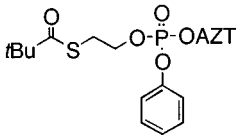


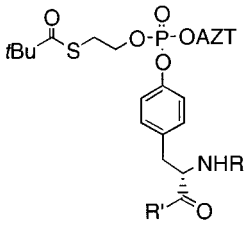
Chart 1. Structures of Studied Phosphotriester Derivatives of AZT



bis(*t*BuSATE)phosphotriester **1** as reference compound



Phenyl(*t*BuSATE)phosphotriester **2**



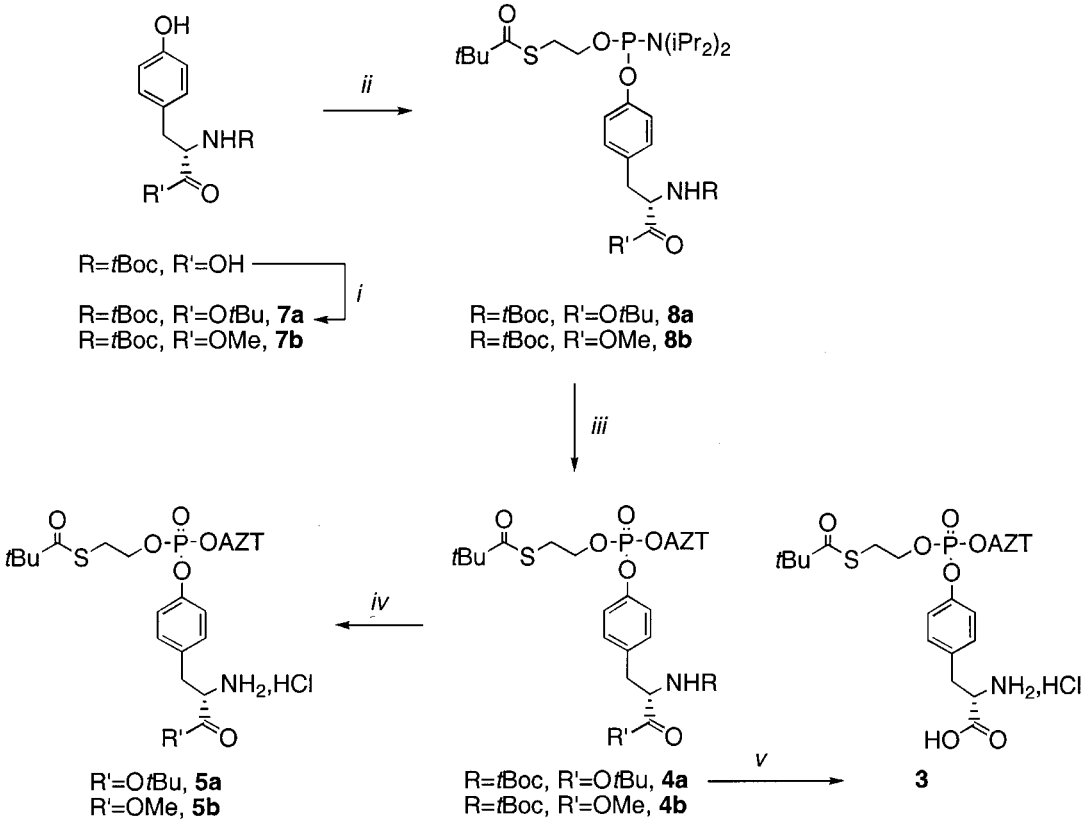
Tyrosinyl(*t*BuSATE)phosphotriesters

R=H, R'=OH **3**
R=*t*Boc, R'=O*t*Bu **4a**
R=*t*Boc, R'=OMe **4b**
R=H, R'=O*t*Bu **5a**
R=H, R'=OMe **5b**

ing two strategies using P^{III} (phosphoramidite) or P^{V} (phosphate) intermediates depending on both the availability of the starting materials and the reactivity of the intermediates. Thus, the phenyl (*t*BuSATE) phosphotriester derivative of AZT **2** was obtained using P^{V} chemistry in a one-pot procedure.[18] Another synthetic route was chosen for the preparation of the mixed phosphotriesters **4a**,**b** involving the phosphoramidite approach. The phosphytilating agents bearing the SATE moiety and the desired tyrosinyl residue could be obtained from commercially available bis(diisopropylamino)chlorophosphine with subsequent coupling of the SATE chain moiety and then the aryl residue (Scheme 2). The crucial point of these syntheses was the choice of the protecting groups borne by the amine and acid functionalities of L-tyrosine. Indeed, the cleavage conditions of such groups have to be compatible with the stability of the final derivatives which possess base- and nucleophile-sensitive functions (thioester, phosphotriester). Consequently, we decided to use acid-labile protecting groups and/or groups susceptible to being hydrolyzed by an enzymatic system during biological studies, to keep the integrity of the skeleton of the target compounds. The *tert*-butyloxycarbonyl (*t*Boc) and *tert*-butyl (*t*Bu) groups were used as amino and acid protections, respectively. Concerning the acid functionality, both *tert*-butyl and methyl esters of *N*-α-*t*Boc-L-tyrosine were selected for their ability to be potentially removed by enzymes such as esterases present in biological media. Selective esterification of the acid in the presence of the phenolic function was carried out from *N*-α-*t*Boc-L-tyrosine, using *N*,*N*-dimethylformamide dineopentyl acetal,[19,20] to obtain the *N*-α-*t*Boc-L-tyrosine *tert*-butyl ester **7a** in good yields. The tyrosinyl precursors **7a**,**b** (commercially available) were condensed in the presence of 1*H*-tetrazole with the SATE phosphorobisamidite reagent **6** obtained via a previously described procedure.[21] Then, the reaction of the adequate tyrosinyl (*t*BuSATE) phosphoramidite intermediates **8a**,**b** with AZT, followed by in situ oxidation using *tert*-butyl hydroperoxide, afforded the fully protected phosphotriester derivatives **4a**,**b** in 57% and 72% overall yields, respectively, from **6**. Treatment of compound **4a** with hydrochloric acid-saturated ethereal solution (30% in weight) gave rise directly to the tyrosinyl (*t*BuSATE) phosphotriester derivative of AZT as its hydrochloride salt **3**, in 50% yield (Scheme 2). Finally, the selective removal of the *t*Boc group from **4a**,**b** was carried out using trifluoroacetic acid/dichloromethane solution (10% in volume) in time-controlled reactions, and the partially protected entity **5a**,**b** could be isolated in good yields after their conversion to the corresponding hydrochloride salts.

Anti-HIV-1 Evaluation. The inhibitory effects on the replication of HIV-1 of the studied mixed phosphotriesters **2**−**5** were evaluated in three cell culture systems (Table 1) in comparison to the parent nucleoside AZT and to the reference pronucleotide **1**. In human T_4-lymphoblastoid CEM-SS and MT-4 cells, all the tested compounds appeared to be as potent as AZT, with 50%

Scheme 2 General Approach for the Synthesis of Aryl Phosphotriester Derivatives **3**−**5**[a]



[a] Reagents: (i) $(CH_3)_2NCH[OCH_2C(CH_3)_3]_2$, *t*BuOH, toluene; (ii) **6**, 1*H*-tetrazole, $HN(iPr)_2$, CH_3CN; (iii) AZT, 1*H*-tetrazole, CH_3CN, then *t*BuOOH, toluene; (iv) 10% TFA, CH_2Cl_2, then HCl, dioxane solution; (v) 30% HCl, Et_2O solution.

Table 1. Anti-HIV-1 Activity[a] in Three Cell Culture Systems and Apparent Partition Coefficients of Mixed Aryl Phosphotriester Derivatives **2**−**5** Compared to Parent Nucleoside AZT and Bis(*t*BuSATE) Phosphotriester Derivative **1** of AZT, as Reference Pronucleotide

	CEM-SS		MT-4		CEM/TK$^-$		
compd	EC_{50} (μM) [b]	CC_{50} (μM) [c]	EC_{50} (μM) [b]	CC_{50} (μM) [c]	EC_{50} (μM) [b]	CC_{50} (μM) [c]	log P_{app}
AZT	0.003	>1	0.015	>1	>100	>100	0.06 ± 0.003
1	0.015	>10	0.81	>10	0.45	>10	3.73 ± 0.07
2	0.002	>10	0.07	>10	3.5	>10	3.46 ± 0.04
3	0.006	>100	0.075	>10	29	>100	0.24 ± 0.015
4a	0.034	>1	0.25	>1	0.90	>1	4.29 ± 0.16
4b	0.001	>10	0.033	>10	2.3	>10	3.48 ± 0.01
5a	0.004	>10	0.042	>10	2.7	>10	2.90 ± 0.02
5b	0.002	>100	0.037	>10	1.8	>100	2.25 ± 0.04

[a] All data represent average values for at least three separate experiments. The variation of these results under standard operating procedures is below ±10%. [b] EC_{50}: effective concentration, or concentration required to inhibit the replication of HIV-1 by 50%. [c] CC_{50}: cytotoxic concentration, or concentration required to reduce the viability of uninfected cells by 50%.

effective concentration (EC_{50}) about submicromolar concentration range. In contrast to AZT, the phenyl and tyrosinyl (*t*BuSATE) phosphotriesters **2**−**5** exhibited significant anti-HIV effects in thymidine kinase-deficient CEM cells (CEM/TK$^-$) with EC_{50} values which were in the same range as that observed for the pronucleotide **1**. These results seem to demonstrate that the studied mixed aryl phosphotriesters **2**−**5** were able to give rise to the intracellular delivery of the 5′-mononucleotide of AZT (AZTMP) and can be considered as a new series of pronucleotides.

As illustrated in Table 1, the presence of lipophilic substituents or ionizable functions borne by the aryl phosphate protection influenced the anti-retroviral activity of the corresponding phosphotriester derivatives **2**−**5** in CEM/TK$^-$ cells. For instance, the phosphotriester **3** incorporating the deprotected tyrosinyl moiety appeared to be the less active with an EC_{50} value of 29 μM, while the fully protected analogue **4a** inhibited the multiplication of HIV-1 at submicromolar concentration. The behavior of the (*t*BuSATE) tyrosinyl phosphotriester **3** in total CEM cell extracts, used as mimic for the intracellular medium, has previously been reported.[18] This stability study showed that such mixed phosphotriester exerts its biological effect following a decomposition process involving, successively, esterase and phosphodiesterase systems. As a consequence, the lower anti-HIV activity observed in the CEM/TK$^-$ cell line for the zwitterionic derivative **3** could be related to its limited ability to cross the cell membrane barrier by passive diffusion due to its polarity (log P_{app} = 0.24, Table 1).

Comparison between anti-HIV activities in CEM/TK$^-$ cells and apparent partition coefficients of phosphotri-

ester derivatives **2**−**5** (Table 1) showed that other factors are involved in the observed in vitro biological effect. For example, despite marked differences in their lipophilicity, the EC_{50} values for the phosphotriesters **4b** and **5b** are in the same range. These results suggested that other factors such as the kinetics of decomposition of aryl phosphotriesters (related to their affinity for intracellular phosphodiesterases) may be involved in the observed in vitro anti-HIV activity of the corresponding phosphotriesters.

Conclusion

The present study demonstrates that (*t*BuSATE) aryl phosphotriester derivatives of AZT **2**−**5** are able to allow the efficient intracellular delivery of the parent 5′-mononucleotide and can be considered as a new series of pronucleotides. The proposed mechanism for the decomposition of these mixed phosphotriesters involves successively an esterase and a phosphodiesterase hydrolytic step. The large number of chemical modifications which could be envisaged on the aryl moiety opens the way to the search for antiviral mononucleotide prodrugs with an adequate balance between aqueous solubility, lipophilicity, and enzymatic stability in order to envisage further in vivo pharmacological studies. In this respect, the structural features of the aryl phosphotriesters **5a**,**b** appear as promising phosphate protecting groups in view of the anti-HIV activity and physicochemical properties of the resulting pronucleotides. Finally, the introduction of a tyrosinyl residue in place of the second *t*BuSATE chain, such as in phosphotriester **3**, would open novel perspectives on the specificity of 5′-mononucleotide delivery to infected cells or tissues. We are aware that several therapeutic agents[22,23] presenting mimic structures with L-phenylalanine, a natural occurring substrate for the large neutral amino acid transporter (L-system), were able to be recognized and actively transported by this carrier through biological barriers. The presence of this transporter on epithelium cell surfaces and its high capacity and medium specificity properties are particularly attractive compared to other nutrient carriers.[24−26] Work on this topic is currently in progress in our group.

Experimental Section

General Methods. See ref 27.

Biological Methods. See ref 9.

Partition Coefficient. The apparent partition coefficients (log P_{app}) were evaluated for their distribution between 1-octanol (puriss, Fluka) and potassium phosphate buffer (0.02 M, pH 7.2) using an adapted shake-flask technique.[28]

Phenyl *S*-Pivaloyl-2-thioethyl 3′-Azido-2′,3′-dideoxythymidin-5′-yl Phosphate (2). *S*-(2-Hydroxyethyl)thiopivaloate[9] (2.22 g, 13.7 mmol) was coevaporated with dry THF and the residue dissolved in the same solvent (130 mL). This solution was cooled to −78 °C and phenyl phosphorochloridate (2.1 mL, 14.0 mmol) was added dropwise over 10 min. The mixture was stirred overnight, filtered and the filtrate evaporated in vacuo. The residue was dissolved in dry CCl_4, filtered and concentrated to dryness under reduced pressure to afford the phenyl *S*-pivaloyl-2-thioethyl phosphorochloridate (4.9 g) which was directly used without further purification (^{31}P NMR in $CDCl_3$, δ −0.60). The crude phosphorochloridate (0.52 g) was coevaporated with dry THF and the residue dissolved in the same solvent (5 mL). AZT (0.137 g, 0.51 mmol) was added, followed by addition of *N*-methylimidazole (0.245 mL, 3.1 mmol). The mixture was stirred for 4 h at room temperature, then the solvent was removed in vacuo and the residue dissolved in CH_2Cl_2. This solution was washed with aqueous 1 M HCl, a saturated aqueous solution of sodium bicarbonate, and water. The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography eluting with MeOH/CH_2Cl_2 (2:98, v/v). The appropriate fractions were collected and evaporated to give pure phosphotriester **2** (0.23 g, 80%) as a colorless oil: R_f 0.47 (MeOH/CH_2Cl_2 7:93).

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-L-*tert*-butoxytyrosine (7a).** N^{α}-*tert*-Butoxycarbonyl-L-tyrosine (2.53 g, 9 mmol) was dissolved in toluene (45 mL) and *tert*-butyl alcohol (127 mmol, 11.9 mL, 14 equiv) was added. The mixture was brought to reflux and *N*,*N*-dimethylformamide dineopentyl acetal (27.2 mmol, 7.6 mL, 3 equiv) was added dropwise over 90 min. After stirring for 3 h at reflux the solution was cooled to room temperature and a saturated $NaHCO_3$ solution was added. The aqueous layer was extracted three times with CH_2Cl_2, the combined organic layers were dried over Na_2SO_4, filtered and the solvent removed in vacuo. Purification of the residue by column chromatography on silica gel, eluting with a stepwise gradient of EtOAc (0−30%) in CH_2Cl_2, and crystallization from *n*-hexane/CH_2Cl_2 afforded title compound **7a** (2.12 g) as colorless needles, 70% yield: R_f 0.63 (MeOH/CH_2Cl_2 5:95); mp 112.8−113.0 °C (lit.[29] 111.3−112.8 °C); $[\alpha]_D^{20} = 8°$ (c = 1.0, EtOH) (lit.[29] = 19.4 (c = 1.0, dioxane)). Anal. ($C_{18}H_{27}NO_5$) C, H, N.

General Procedure for Preparation of Phosphoramidites 8a,b. To a solution of the appropriate tyrosinyl precursor **7a,b** (0.50 mmol) in dry CH_3CN (5 mL) containing 3 Å molecular sieve (0.5 g) were added at 0 °C phosphorodiamidite **6** (0.29 g, 0.75 mmol, 1.5 equiv), diisopropylamine (0.14 mL, 1.00 mmol, 2 equiv) and 1-*H*-tetrazole (0.07 g, 1.00 mmol, 2 equiv). After stirring for 2 h at room temperature, the reaction mixture was diluted with acid-free EtOAc, washed with brine and water, dried with Na_2SO_4, filtered and concentrated in vacuo. Purification of the residue by flash column chromatography on silica gel eluting with EtOAc/cyclohexane (1:9, v/v) containing 1% of triethylamine afforded a diastereoisomeric mixture (1:1) of the desired phosphoramidite as a colorless oil.

***O*-(*N*$^{\alpha}$-*tert*-Butoxycarbonyl-L-*tert*-butoxytyrosinyl) *O*-(*S*-pivaloyl-2-thioethyl) *N*,*N*-diisopropylphosphoramidite (8a):** 0.29 g, 95%; R_f 0.57 (Et_3N/EtOAc/cyclohexane 1:1:8). Anal. ($C_{31}H_{53}N_2O_7PS$) C, H, N.

***O*-(*N*$^{\alpha}$-*tert*-Butoxycarbonyl-L-methoxytyrosinyl) *O*-(*S*-pivaloyl-2-thioethyl) *N*,*N*-diisopropylphosphoramidite (8b):** 0.26 g, 87%; R_f 0.55 (Et_3N/EtOAc/cyclohexane 1:1:8). Anal. ($C_{28}H_{47}N_2O_7PS$) C, H, N.

General Procedure for Preparation of Phosphotriesters 4a,b. To a solution of AZT (0.093 g, 0.35 mmol) in dry CH_3CN (3 mL) containing 3 Å molecular sieve (0.5 g) were added 1*H*-tetrazole (0.098 g, 1.40 mmol, 4 equiv) and dropwise a solution of the appropriate phosphoramidite **8a**,**b** (0.42 mmol, 1.2 equiv) in dry CH_3CN (1.5 mL). The mixture was stirred for 1 h, *tert*-butyl hydroperoxide (0.28 mL, 0.84 mmol, 3 M in toluene, 2.4 equiv) was added and the solution further stirred for 1 h. The mixture was diluted with CH_2Cl_2 and washed successively with aqueous $Na_2S_2O_3$ (10%, w/v) and water, the organic layer was dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure. Purification of the residue by silica gel column chromatography using a stepwise gradient of MeOH in CH_2Cl_2 afforded the desired phosphotriester as a white foam.

***O*-(*N*$^{\alpha}$-*tert*-Butoxycarbonyl-L-*tert*-butoxytyrosinyl) *O*-(*S*-pivaloyl-2-thioethyl) 3′-azido-2′,3′-deoxythymidin-5′-yl phosphate (4a):** 0.22 g (76%); R_f 0.50 (MeOH/CH_2Cl_2 7:93). Anal. ($C_{35}H_{51}N_6O_{12}PS$) C, H, N.

***O*-(*N*$^{\alpha}$-*tert*-Butoxycarbonyl-L-methoxytyrosinyl) *O*-(*S*-pivaloyl-2-thioethyl) 3′-azido-2′,3′-deoxythymidin-5′-yl phosphate (4b):** 0.18 g (66%); R_f 0.40 (MeOH/CH_2Cl_2 5:95). Anal. ($C_{32}H_{45}N_6O_{12}PS$) C, H, N.

General Procedure for Preparation of Phosphotriesters 5a,b. To a solution of the appropriate phosphotriester **4a**,**b** (0.06 mmol) in dry CH_2Cl_2 (1 mL) was added a solution

of trifluoroacetic acid/CH_2Cl_2 (1 mL, 20%, v/v). After stirring, the mixture was concentrated under reduced pressure. Purification of the residue by column chromatography on silica gel eluting with a stepwise gradient of MeOH in CH_2Cl_2 gave the desired phosphotriester as a white powder, after trituration with a hydrochloric acid saturated dioxane solution, evaporation and lyophilization in dioxane.

***O*-(L-*tert*-Butoxytyrosinyl) *O*-(*S*-pivaloyl-2-thioethyl) 3′-azido-2′,3′-deoxythymidin-5′-yl phosphate, hydrochloride (5a):** 0.034 g (65%); R_f 0.64 (MeOH/CH_2Cl_2 1:4). Anal. ($C_{30}H_{44}ClN_6O_{10}PS$) C, H; N: calcd, 11.25; found, 10.78.

***O*-(L-Methoxytyrosinyl) *O*-(*S*-pivaloyl-2-thioethyl) 3′-azido-2′,3′-deoxythymidin-5′-yl phosphate, hydrochloride (5b):** 0.048 g (94%); R_f 0.47 (MeOH/CH_2Cl_2 1:9). Anal. ($C_{27}H_{38}N_6ClO_{10}PS\cdot 1.25(HCl+H_2O)$) C, H, N.

***O*-L-Tyrosinyl *O*-(*S*-Pivaloyl-2-thioethyl) 3′-Azido-2′,3′-deoxythymidin-5′-yl Phosphate, Hydrochloride (3).** Phosphotriester **4a** (0.07 g, 0.086 mmol) was dissolved at 0 °C in a solution of hydrogen chloride in diethyl ether (4 mL, 30%) and the mixture stirred at room temperature for 30 min. The solvent was removed in vacuo and the residue subjected to reverse-phase C_{18} column chromatography, eluting with a linear gradient of MeOH in H_2O (0−80%). The collected product was dissolved in a solution of hydrogen chloride in dioxane (2 mL, 18%) stirred for 10 min, the solvent evaporated and the residue lyophilization in dioxane to afford the title compound **3** (0.04 g, 68%) as a white powder: R_f 0.08 (MeOH/CH_2Cl_2 1:4), 0.20 (MeOH/CH_2Cl_2 1.5:8.5). Anal. ($C_{26}H_{36}ClN_6O_{10}PS$) C, N; H: calcd, 5.25; found, 5.67.

Acknowledgment. This investigation was supported by grants from Agence Nationale de Recherches sur le SIDA (ANRS) and Fondation pour la Recherche Médicale (FRM).

Supporting Information Available: NMR, MS, HPLC, and UV data of the described compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) De Clercq, E. New perspectives for the treatment of HIV infections. *Collec. Czech. Chem. Commun.* **1998**, *63*, 449−479.
(2) De Clercq, E. Recent developments in the chemotherapy of HIV infection. *Pure Appl. Chem.* **1998**, *70*, 567−577.
(3) Gao, W.-Y.; Agbaria, R.; Driscoll, J. S.; Mitsuya, H. Divergent anti-human immunodeficiency virus activity and anabolic phosphorylation of 2′,3′-dideoxynucleoside analogues in resting and activated human cells. *J. Biol. Chem.* **1994**, *269*, 12633−12638.
(4) Arzumanov, A. A.; Dyatkina, N. B. Modified nucleoside phosphates as precursors of antiviral and antitumor compounds in cells (review). *Russ. J. Bioorg. Chem.* **1996**, *22*, 777−794.
(5) Krise, J. P.; Stella, V. J. Prodrugs of phosphates, phosphonates, and phosphinates. *Adv. Drug Deliv. Rev.* **1996**, *19*, 287−310.
(6) Périgaud, C.; Girardet, J.-L.; Gosselin, G.; Imbach, J.-L. Comments on nucleotide delivery forms. In *Antiviral Drug Design*; De Clercq, E., Ed.; JAI Press: London, 1996; Vol. 2, pp 147−172.
(7) Meier, C. Pro-Nucleotides − Recent advances in the design of efficient tools for the delivery of biologically active nucleoside monophosphates. *Synlett* **1998**, 233−242.
(8) Périgaud, C.; Gosselin, G.; Imbach, J.-L. Anti-HIV phosphotriester nucleotides. Basis for the rational design of biolabile phosphate protection. In *Biomedical Chemistry/Applying Chemical Principles to the Understanding and Treatment of Disease*; Torrence P. F., Ed.; John Wiley & Sons Inc.: New York, 2000; Vol. 5, pp 115−141.
(9) Lefebvre, I.; Périgaud, C.; Pompon, A.; Aubertin, A.-M.; Girardet, J.-L.; Kirn, A.; Gosselin, G.; Imbach, J.-L. Mononucleoside phosphotriester derivatives with *S*-acyl-2-thioethyl bioreversible phosphate protecting groups. Intracellular delivery of 3′-azido-2′,3′-dideoxythymidine 5′-monophosphate. *J. Med. Chem.* **1995**, *38*, 3941−3950.
(10) Martin, L. T.; Cretton-Scott, E.; Placidi, L.; Faraj, A.; Loi, A. G.; Schinazi, R. F.; McClure, H. M.; Gosselin, G.; Imbach, J.-L.; Sommadossi, J.-P. In vitro and in vivo metabolism and pharmacokinetics of bis-[(*tert*-butyl)-*S*-Acyl-2-ThioEthyl]-*β*-L-2′,3′-dideoxy-5-fluorocytidine monophosphate. *Nucleosides, Nucleotides Nucleic Acids* **2000**, *19*, 481−499.
(11) Placidi, L.; Périgaud, C.; Cretton-Scott, E.; Gosselin, G.; Pierra, C.; Schinazi, R. F.; Imbach, J.-L.; Sommadossi, J.-P. The intracellular pharmacology of *β*-L-ddA is responsible for the lack of potent anti-HIV activity. *Antiviral Res.* **1998**, *37*, 87.
(12) Augustijns, P. F.; Shafiee, M.; Périgaud, C.; Gosselin, G.; Villard, A.-L.; Van Gelder, J.; Van den Mooter, G.; Imbach, J.-L.; Kinget, R. Bis(SATE)ester prodrugs of AZT monophosphate: Selection of antiviral agent with the potential for oral absorption. *Pharma Sci.* **1999**, *1*.
(13) Egron, D.; Périgaud, C.; Gosselin, G.; Aubertin, A.-M.; Imbach, J.-L. Effect of the thioalkyl chain length on the anti-HIV efficiency of pronucleotides bearing *S*-acylthioalkyl phosphate protecting groups. *Bull. Soc. Chim. Belg.* **1997**, *106*, 461−466.
(14) Khorana, H. G. Phosphodiesterases. In *The Enzymes*; Boyer, P. D., Lardy, H., Myrback, K., Eds.; Academic Press: New York, 1961; Vol. 5, pp 79−94.
(15) Haugen, H. F.; Skrede, S. Nucleotide pyrophosphatase and phosphodiesterase. I. Organ distribution and activities in body fluids. *Clin. Chem.* **1977**, *23*, 1531−1537.
(16) Landt, M.; Everard, R. A.; Butler, L. G. 5′-Nucleotide Phosphodiesterase: Features of the substrate binding site as deduced from specificity and kinetics of some novel substrates. *Biochemistry* **1980**, *19*, 138−143.
(17) Kelly, S. J.; Dardinger, D. E.; Butler, L. G. Hydrolysis of phosphate esters catalyzed by 5′-nucleotide phosphodiesterase. *Biochemistry* **1975**, *14*, 4983−4988.
(18) Schlienger, N.; Beltran, T.; Périgaud, C.; Lefebvre, I.; Pompon, A.; Aubertin, A.-M.; Gosselin, G.; Imbach, J. L. Rational design of a new series of mixed anti-HIV pronucleotides. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3003−3006.
(19) Mathias, L. J. Esterification and alkylation reactions employing isoureas. *Synthesis* **1979**, 561−576.
(20) Baldwin, J. E.; Farthing, C. N.; Russel, A. T.; Schofield, C. J.; Spivey, A. C. Use of (S)-*N*-*tert*-butoxycarbonylaziridine-2-carboxylate derivatives for α-amino acid synthesis. *Tetrahedron Lett.* **1996**, *37*, 3761−3764.
(21) Alvarez, K.; Vasseur, J.-J.; Beltran, T.; Imbach, J.-L. Photocleavable protecting groups as nucleobase protections allowed the solid-phase synthesis of base-sensitive SATE-prooligonucleotides. *J. Org. Chem.* **1999**, *64*, 6319−6328.
(22) Greig, N. H.; Momma, S.; Sweeney, D. J.; Smith, Q. R.; Rapoport, S. I. Facilitated transport of Melphalan at the rat BBB by the large neutral amino acid carrier system. *Cancer Res.* **1987**, *47*, 1571−1576.
(23) Walker, I.; Nicholls, D.; Irwin, W. J.; Freeman, S. Drug delivery via active transport at the blood-brain barrier: affinity of a prodrug of phosphonoformate for the large amino acid transporter. *Int. J. Pharm.* **1994**, *104*, 157−167.
(24) Smith, Q. R.; Momma, S.; Aoyagi, M.; Rapoport, S. I. Kinetics of neutral amino acid transport across the blood-brain barrier. *J. Neurochem.* **1987**, *49*, 1651−1658.
(25) Sànchez del Pino, M. M.; Peterson, D. R.; Hawkins, R. A. Neutral amino acid transport characterization of isolated luminal and abluminal membranes of the blood-brain barrier. *J. Biol. Chem.* **1995**, *270*, 14913−14918.
(26) Oldendorf, W. H. Brain uptake of radiolabeled amino acids, amins, and hexoses after arterial injection. *Am. J. Phys.* **1971**, *221*, 1629−1639.
(27) Schlienger, N.; Périgaud, C.; Gosselin, G.; Imbach, J.-L. Synthesis and studies of mononucleoside glucosyl phosphotriester derivatives *J. Org. Chem.* **1997**, *62*, 7216−7221.
(28) Ford, H., Jr.; Merski, C. L.; Kelley, J. A. A rapid microscale method for the determination of partition coefficients by HPLC. *J. Liq. Chromatogr.* **1991**, *14*, 3365−3386.
(29) Shvests, V. I.; Pogrebnaya, L. F.; Kraevskii, A. A.; Preobrazhenskii, N. A. Investigations in the field of complex lipids XXX. Synthesis of L-α-(α′,*β*-dioleoyl)glycerylphosphoryl-L-Tyrosine. *J. Org. Khim. (USSR)* **1968**, *4*, 942−945.

JM000996O