S-Acyl-2-thioethyl Phosphoramidate Diester Derivatives as Mononucleotide Prodrugs

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The synthesis and in vitro anti-HIV activity of phosphoramidate diester derivatives of 3′-azido-2′,3′-dideoxythymidine (AZT) bearing one *S*-pivaloyl-2-thioethyl (*t*BuSATE) group and various amino residues are reported. These compounds were obtained from an *H*-phosphonate strategy using an amidative oxidation step. Most of these derivatives appeared to inhibit HIV-1 replication, with EC_{50} values at micromolar concentration in thymidine kinase-deficient (TK^-) cells, revealing a less restrictive intracellular decomposition process than previously reported for other phosphoramidate prodrugs. The proposed decomposition pathway of this new series of mixed pronucleotides may successively involve an esterase and a phosphoramidase hydrolysis.

Introduction

In an attempt to improve the therapeutic potential of nucleoside analogues, various mononucleotide prodrugs (pronucleotides) have been reported during the past decade.[1−4] Two main approaches have been envisaged requiring either structural modifications or introduction of transient groups. In the first case, a specific enzymatic system is needed to perform the bioconversion of the structurally modified phosphorylated precursor. This strategy could be illustrated by the design of nucleoside *H*-phosphonate,[5,6] fluorophosphate,[7,8] or phosphoramidate monoester[9,10] derivatives. In the second strategy, the necessity to mask the two charged phosphate oxygens of the 5′-mononucleotide in order to obtain neutral and lipophilic prodrugs has led to the development of various mononucleoside phosphotriesters.[1] The hydrolysis of phosphate-protecting groups involves either a chemical- or an enzyme-mediated (bioactivation) processes. Obviously, these two general strategies could be envisaged independently or combined as illustrated by the design of mononucleoside phenylphosphoramidate diesters containing methyl-esterified amino acids.[11,12] The delivery of 5′-mononucleotides from this promising pronucleotide series combines different decomposition mechanisms (i.e., enzymatic and chemical) as well as bioactivation and bioconversion processes (Scheme 1). Initially investigated in cell extracts,[13] metabolism studies with a radiolabeled compound[14] revealed that the decomposition pathway of such kinds of prodrugs requires, in a first step, an esterase-mediated hydrolysis of the carboxylic ester function of amino acid. This bioactivation is thought to be followed by an intramolecular nucleophilic attack of the carboxylate function to the phosphorus with spontaneous elimination of the aryl substituent after transient formation of a five-membered cyclic intermediate. Finally, bioconversion of the resulting mononucleoside phosphoramidate monoester leads to the release of the corresponding 5′-mononucleotide.[13,15,16] Nevertheless, several studies in this series displayed that the biological activity is strongly dependent on the nature of the amino acid residue. Using 3′-azido-2′,3′-dideoxythymidine (AZT) as a nucleoside model, the L-alaninyl methyl ester derivative **1** (Figure 1) appears as the most effective prodrug.[17] The variation of the acyl chain,[18] the inversion of chirality of alaninyl substituent,[19] and its replacement by another amino acid[20,21] or aminoalkyl[22] residue lead to a dramatic reduction of the antiviral potency.

We have reported preliminary investigations about a new series of phosphoramidate diester derivatives of AZT incorporating a *S*-pivaloyl-2-thioethyl (*t*BuSATE) phosphate protecting group in place of the aryl residue.[23] Such phosphorylated entities have been designed to overcome the limitations related to the decomposition pathway of mononucleoside arylphosphoramidate diesters by a direct and simple process leading to the intracellular delivery of the phosphoramidate monoester, the mononucleotide precursor (Scheme 1).

Herein, we report the full accounts of the synthesis and biological evaluation of a large number of *t*BuSATE phosphoramidate diesters of AZT bearing various methyl-esterified amino acids (**2**−**11**) and aliphatic (**12** and **13**) and aromatic (**14**) amino residues (Figure 1). The anti-HIV-1 evaluation in wild-type [thymidine kinase positive (TK^+)] cells and thymidine kinase-deficient (TK^-) cells was carried out in comparison to compound **1** as reference pronucleotide.

Results and Discussion

Chemistry. The phosphoramidate diester derivatives **2**−**14** of AZT were obtained by a one-pot procedure using a convenient hydrogenphosphonate approach (Scheme 2).[24] Oxidative coupling[25] of *H*-phosphonate diester precursor **15**, prepared according to a published procedure,[8] with the corresponding amines gave the desired

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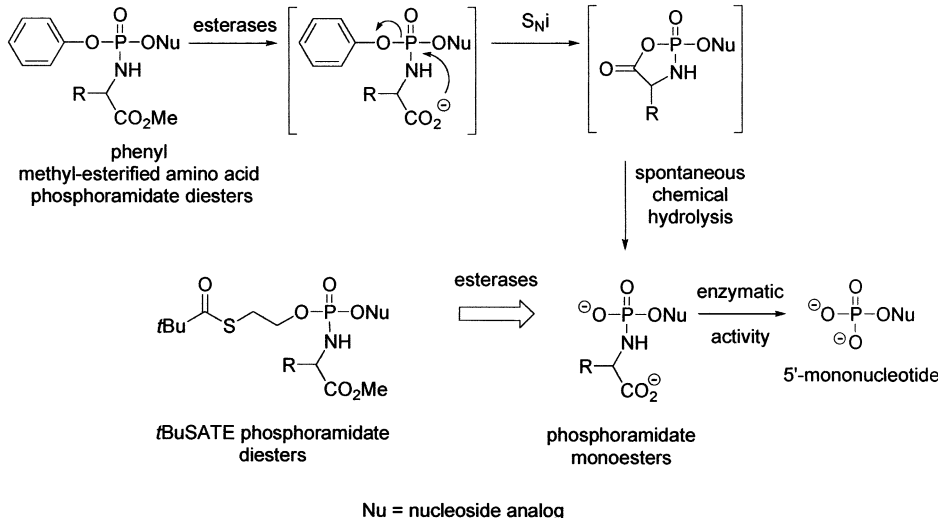
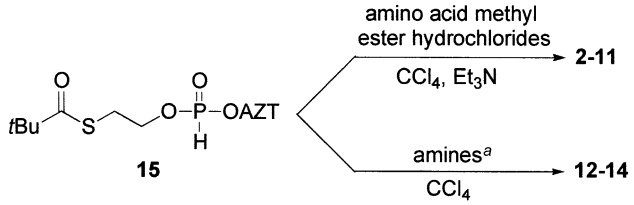
Scheme 1. Proposed Decomposition Pathways for Mononucleoside Phenylphosphoramidate Diesters Containing Methyl-Esterified Amino Acids and the Studied *t*BuSATE Phosphoramidate Diesters



Scheme 2. General Approach for the Synthesis of *t*BuSATE Phosphoramidate Diester Derivatives of AZT **2**−**14**



[a] In the case of aniline, DBU (9 equiv) was added (see Experimental Section).

mononucleotide prodrugs **2**−**14**. Since the amino acid methyl esters are commercially available as hydrochloride salts, addition of triethylamine in the medium was required, leading to the nucleophilic attack of the amino function on the putative in situ-formed intermediary phosphorochloridate.[26,27] Under these conditions, the phosphoramidate diesters **2**−**11** as well as alkylamino derivatives **12** and **13** were obtained in yields of 50−80% after purification by silica gel column chromatography. As expected, with a weak base such as aniline, no reaction occurs.[25] In the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU), however, the corresponding phosphoramidate diester **14** was obtained in moderate yield (20%).

The phosphoramidate diesters **2**−**14** displayed two closely spaced signals in ^{31}P NMR because of the presence of diastereoisomers on the phosphorus stereocenter. Similarly, distinct diastereoisomeric signals were noted on the proton-decoupled ^{13}C NMR spectra. Furthermore, the presence of diastereoisomers was apparent in some cases from ^{1}H NMR spectroscopy and analytical HPLC studies. Determined by ^{31}P NMR, the diastereoisomeric ratio seems to be affected by the presence of sterically hindered substituents in the immediate vicinity of the amino functionality. Thus, ratio deviation was observed when the number of Cα substituents increased, as illustrated by replacement of the glycinyl residue in compound **3** (1:1) with alaninyl and 2-aminoisobutyryl residues, **2** (2:3) and **9** (1:2), respectively. Unfortunately, the diastereoisomeric mixtures could not be separated by silica gel column chromatography.

Attempts to synthesize the phosphoramidate derivative by incorporating the serinyl substituent by direct condensation of the corresponding amino acid methyl ester failed. Thus, we decided to protect the hydroxyl

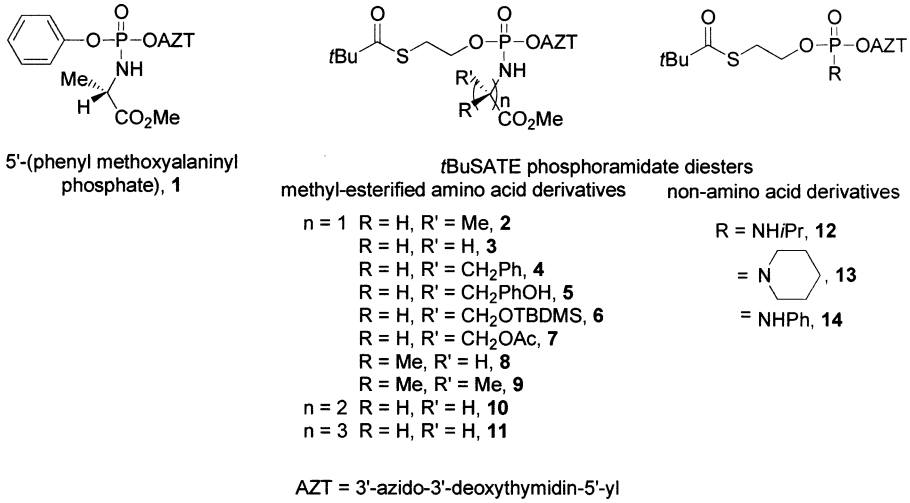


Figure 1. Structure of studied phosphoramidate derivatives of AZT.

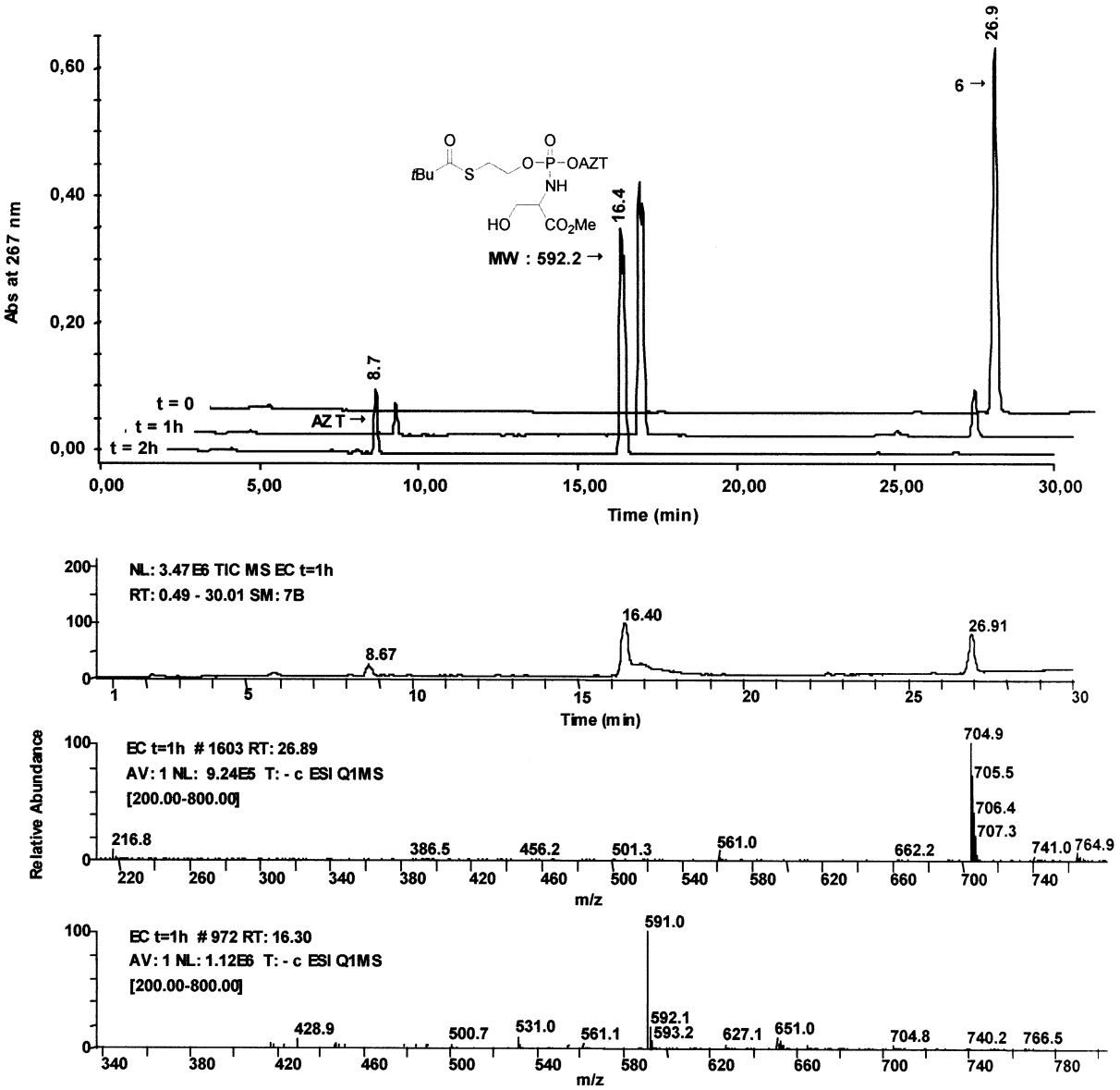


Figure 2. HPLC chromatogram and related ESI-MS spectra obtained after treatment of the *t*BuSATE phosphoramidate diester derivative of AZT **6** in the conditions used for the TBDMS group cleavage.

function with a *tert*-butyldimethylsilyl (TBDMS) group, which could be removed under conditions compatible with the presence of the phosphoramidate diester. Coupling of the resulting *O*-TBDMS-protected serine methyl ester[28] with the *H*-phosphonate diester **15**, using the described general procedure, afforded the protected phosphoramidate **6**. Cleavage of the TBDMS ether was performed by treatment of the compound with a 1% (m/v) solution of iodine in methanol at reflux temperature. The reaction was monitored by HPLC. This study showed the disappearance of starting material **6** in 2 h and a time-dependent accumulation of a major product. This product was identified as the expected phosphoramidate diester as a diastereoisomeric mixture by HPLC/MS coupling experiments (Figure 2). Unfortunately, attempts to purify the crude product under various chromatographic conditions did not afford to the desired derivative but gave almost exclusively AZT. The result could be tentatively explained by the relative chemical instability of the unprotected derivative due to a nucleophilic attack of the free hydroxyl function of serinyl residue on the phosphorus atom and subsequent release of AZT. This mechanism is in accordance with HSAB concept meaning that "hard" bases such as alcohols will react preferentially with the phosphoryl center considered as a "hard" acid. Similar data were obtained with various phosphorylated entities such as phosphorothioate mono- and diesters,[29,30] phosphotriesters,[31] and phosphoramidate diesters[15] incorporating a 2-hydroxyethyl chain. Nevertheless, we decided to evaluate the effect of this decomposition process on in vitro antiviral activity by the study of phosphoramidate diester **7** where the hydroxyl function was protected by an acetyl group that is likely to be cleaved by esterases present in biological media. The synthesis of this prodrug was carried out by reaction of the *O*-acetyl-protected serine methyl ester[32] with the *H*-phosphonate precursor **15** according to the described general procedure.

Antiviral Activity. The inhibitory effects on the replication of HIV-1 of the *t*BuSATE phosphoramidate diester derivatives **2**−**14** of AZT were evaluated, as diastereoisomeric mixtures, in three cell culture systems in comparison to the parent nucleoside AZT and to the

Table 1. Anti-HIV Activity[a] (μM) in Three Cell Culture Systems of *t*BuSATE Phosphoramidate Diesters **2**−**14** Compared to Parent Nucleoside AZT and Phenyl Alaninyl Phosphoramidate Diester **1** as Reference Pronucleotide

	CEM − SS		MT-4		CEM/TK$^-$	
compd	EC_{50}[b]	CC_{50}[c]	EC_{50}[b]	CC_{50}[c]	EC_{50}[b]	CC_{50}[c]
AZT	0.006	>100	0.017	75	>100	>100
1	0.05	>100	0.34	>10	4.3	>100
2	0.054	>100	0.34	>100	4.3	>100
3	0.009	>100	0.45	>100	1.4	>100
4	0.027	>100	0.25	>10	2.3	>10
5	0.09	>100	0.74	>100	5.3	>100
6	0.10	>10	0.4	>10	1.0	>10
7	0.007	>100	0.053	>100	16	>100
8	0.07	>100	0.45	>100	2.4	>100
9	0.08	>100	0.54	>100	2.9	>100
10	0.02	>100	0.42	>100	2.1	>100
11	0.006	>100	0.13	>100	2.4	>100
12	0.02	>100	0.13	>100	0.75	>100
13	0.02	>100	0.22	>100	5.2	>100
14	0.005	>10	0.7	>10	>10	>10

[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%.

pronucleotide **1** (Table 1). In human T_4-lymphoblastoid cells, CEM-SS and MT-4, all compounds appeared to be as potent as AZT, with 50% effective concentration (EC_{50}) about submicromolar concentration range. Striking differences were observed in the antiviral activities of the tested phosphoramidates in HIV-1 infected CEM/TK$^-$ cells. This cell line, highly deficient in thymidine kinase, should be considered as a predictive model to investigate the efficiency of nucleotide prodrugs of AZT.[1] In contrast to AZT, most of the studied phosphoramidates exhibited significant anti-HIV effects in CEM/TK$^-$ cells, demonstrating the successful release of the corresponding 5′-mononucleotide inside infected cells. The differences observed in the anti-HIV activities of the phosphoramidate derivatives of AZT in the TK$^+$ and TK$^-$ cell lines may be explained with regard to the particular metabolism of this nucleoside analogue and to the kinetic parameters involved in the decomposition of each derivatives.[33,34]

Except for derivatives **7** and **14**, micromolar values of EC_{50} were observed for all prodrugs in the same range as that obtained for pronucleotide **1**. Compound **12**, the phosphoramidate diester bearing an isopropylamino substituent, emerged as the most potent inhibitor with an EC_{50} value at 0.75 μM in this comparative evaluation. Changing the amino acid part does not lead to notable variation in antiretroviral activity of the corresponding phosphoramidate derivatives in CEM/TK$^-$ cells, except for the acetyl-protected serinyl phosphoramidate **7** (Table 1). In this case, the intramolecular nucleophilic attack of the hydroxyl function (through an esterase-activation) on the phosphorus atom, which was observed in nonenzymatic medium, probably leads to a nonselective decomposition process into the 5′-mononucleotide. In contrast, the TBDMS nonhydrolyzable analogue **6** inhibited HIV-1 multiplication at micromolar concentration. More interesting, the presence of an α-amino acid does not appear as a structural requirement for antiviral activity. Thus, compounds with β-alaninyl (**10**) and aminobutyryl (**11**) residues are equipotent with the glycinyl derivative **4**, whereas the same amino acid modifications in the phenylphosphoramidate series led to a loss of activity.[15,18] We have previously reported the stability study of the *t*BuSATE β-alaninyl phosphoramidate diester **10** in total CEM-SS cell extracts, a mimic of the intracellular medium.[35] This study demonstrated that such a pronucleotide could be transformed into the corresponding phosphoramidate monoester whereas this pivotal metabolite cannot be obtained from the phenylphosphoramidate analogue, the chain elongation limiting the intramolecular displacement of the aryl group by the carboxylate function released after enzymatic activation (Scheme 1).[15] Similar results were found through comparison of compound **2** with phosphoramidates **3** and **8**, the replacement of L-alaninyl residue by glycinyl[17] or D-alaninyl[22] methyl ester substituents, giving rise to a significant decrease of activity in the phenylphosphoramidate series. Finally, the anti-HIV evaluation of *t*BuSATE phosphoramidate diesters **12** and **13** in CEM/TK$^-$ cells clearly demonstrates that the acyl amino acid group is not required to bypass the first phosphorylation step of AZT by the intracellular delivery of its corresponding 5′-mononucleotide.

The metabolic pathway of mononucleoside phenylphosphoramidate diesters containing methyl-esterified amino acids has been studied by different approaches. Thus, we have previously demonstrated that the affinity of the amino acid methyl ester for cellular esterases and the intramolecular cyclization are prerequisites for the biological activity of this type of prodrugs.[15] The use of mononucleoside SATE phosphoramidate diesters seems to circumvent these potential limitations by a less restrictive bioactivation process (i.e., esterase hydrolysis, Scheme 1). Of course, the resulting phosphoramidate monoester has to be substrate for a cellular enzymatic system. Stability study of some of these pronucleotides has been previously reported in total CEM-SS cell extract (TCE) and in human serum.[36,37] As an example, the most active congener **12** decomposed faster in cell extracts ($t_{1/2} = 1.2$ h) than in human serum ($t_{1/2} = 10$ h). In both media, formation of the corresponding phosphoramidate monoester was observed after a first esterase activation (loss of the SATE moiety). In TCE, this metabolite accumulated to a significant level and then was very slowly converted ($t_{1/2} = 72$ h) to the 5′-mononucleotide through a putative enzyme-mediated hydrolysis. Several studies have suggested that the cleavage of the P−N bond giving rise to the formation of 5′-mononucleotide may be catalyzed by phosphodiesterase or phosphoramidase activity.[9,16,38,39] Phosphoramidases have been described in mammalian cells, and different enzymes with phosphoramidase activity have been isolated from various sources.[40−43] More recently, a partially purified rat liver enzyme, distinct from creatine kinase, alkaline phosphatase, and phosphodiesterase, was found to be able to hydrolyze mononucleoside phosphoramidate monoesters.[16] In the absence of identification, data presented here shows that the putative enzymatic activity involved in this bioconversion seems to exhibit a poor substrate specificity with regard to the significant variation of amino residues in the pronucleotides **2**−**13**. In fact, illustrated by the absence of anti-HIV activity of the anilinyl derivative

14 in CEM/TK$^-$ cells (Table), more than structural parameters (i.e., steric hindrance), the nitrogen atom basicity linked to the phosphorus center may be a critical factor in the hydrolysis of the P−N bond. This may be related to N-protonation process during the mechanism of enzymatic hydrolysis.[37,42]

Conclusion

The present study demonstrates that the studied (*t*BuSATE) phosphoramidate diester derivatives of AZT can be considered as a new series of pronucleotides, allowing the efficient intracellular delivery of the parent 5′-mononucleotide. The proposed decomposition mechanism for these mixed pronucleotides involves successively an esterase and a phosphoramidase activation step. In contrast to other phosphoramidate prodrugs, various alkylamino residues could be introduced without restrictions in the event of such a pronucleotide approach. The large number of chemical modifications that could be envisaged on the amidate moiety[37] and as well as one SATE group[44] opens the way to the search of mononucleotide prodrugs with adequate balance among aqueous solubility, lipophilicity, and enzymatic stability as candidates for in vivo pharmacological studies. Work on this topic is currently in progress in our group.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra at 100 MHz with proton decoupling at ambient temperature. Chemical shifts are given in δ-values referenced to the residual solvent peak of chloroform ($CDCl_3$) at 7.26 and 77.0 ppm and of dimethyl sulfoxide (DMSO-d_6) at 2.49 and 39.5 ppm relative to tetramethylsilane (TMS), respectively. Deuterium exchange, decoupling, and COSY experiments were performed in order to confirm proton assignments. Coupling constants, *J*, are reported in hertz. 2D ^{1}H−^{13}C heteronuclear COSY were recorded for the attribution of ^{13}C signals. ^{31}P NMR spectra were carried out at ambient temperature at 80 MHz with proton coupling and decoupling. Chemical shifts are reported relative to external H_3PO_4. FAB mass spectra were recorded in the positive-ion or negative-ion mode using thioglycerol/glycerol (1:1, v/v, G−T) as matrix. Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison (France). Thin-layer chromatography (TLC) was performed on precoated aluminum sheets of silica gel $60F_{254}$ (Merck, Art. 9385), and visualization of products was accomplished by UV absorbance followed by charring with ethanolic sulfuric acid 5% solution with heating; phosphorus-containing compounds were detected by spraying with Hanes molybdate reagent.[45] Column chromatography was carried out on silica gel 60 from Merck. Analytical HPLC studies were carried out using a reverse-phase analytical column (Nucleosil, C_{18}, 150 × 4.6 mm, 5 μm) equipped with a prefilter, a precolumn (Nucleosil, C_{18}, 5 μm), and a photodiode array detector (detection at 267 nm). Compounds were eluted using a linear gradient of 0−80% acetonitrile in 50 mM triethylammonium acetate buffer (pH 7) programmed over a 30 min period with 1 mL/min flow rate. All moisture-sensitive reactions were carried out under anhydrous conditions and argon atmosphere using oven-dried glassware. Solvents were dried and distilled prior to use, and solids were dried over P_2O_5 under reduced pressure at room temperature. All amino acid methyl ester hydrochlorides were purchased from Novabiochem except for the protected *O*-silyl and *O*-acetyl serinyl derivatives, which were prepared as previously described.[28,32] Phenyl methoxy-L-alaninyl phosphate derivative **1**[17] and 3′-azido-3′-deoxythymidin-5′-yl *S*-pivaloyl-2-thioethyl hydrogenphosphonate **15**[8] were synthesized following published procedures.

Biological Methods. The origin of the viruses and the techniques used for measuring inhibition of virus multiplication have previously been described.[46]

General Procedure for the Preparation of Phosphoramidates Diesters 2−11. To a solution of *H*-phosphonate diester **15** (0.1 g, 0.21 mmol) in dry tetrachloromethane (2 mL) was added a solution or suspension of the appropriate amino acid methyl ester hydrochloride salt (2.1 mmol, 10 equiv) in a mixture of freshly distilled triethylamine (265 μL, 1.9 mmol, 9 equiv) and pyridine (2 mL). After being stirred for 3 h at room temperature, the reaction mixture was diluted with dichloromethane and washed successively with 1 M hydrochloric acid aqueous solution, saturated sodium bicarbonate solution, and water. The organic phase was dried with Na_2SO_4, filtered, and evaporated to dryness in a vacuum. Purification of the residue by column chromatography on silica gel using a stepwise gradient of methanol (0−3%) in dichloromethane afforded a diastereoisomeric mixture of the desired phosphoramidate as a white foam.

***N*-(Methoxy-L-alaninyl)-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 2:** 0.064 g (53%); ^{31}P NMR ($CDCl_3$): δ 8.54, 8.41 (2:3); ^{1}H NMR ($CDCl_3$): δ 8.5 (sl, 1H, Thy-NH), 7.43, 7.37 (2d, 1H, J = 1.0, H-6), 6.23, 6.17 (2t, 1H, J = 6.6, H-1′), 4.43−4.34 (2m, 1H, H-3′), 4.27−4.19 (m, 2H, H-5′, H-5"), 4.14−3.93 (m, 4H, CH_2O, H-4′, CH), 3.75, 3.74 (2s, 3H, OCH_3), 3.60, 3.52 (2t, 1H, J = 10.1, NH), 3.20−3.08 (m, 2H, SCH_2), 2.46−2.28 (m, 2H, H-2′, H-2"), 1.95, 1.94 (2d, 3H, J = 1.0, Thy-CH_3), 1.42 (d, 3H, J = 7.1, CH_3), 1.23, 1.22 (2s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$): δ 205.8 (CO), 175.9, 175.8 (2d, J_{P-C} = 5.8, CO_2CH_3), 163.6 (C-4), 150.2, 150.1 (C-2), 135.1, 135.0 (C-6), 111.5, 111.4 (C-5), 84.8, 84.6 (C-1′), 82.1 (d, J_{P-C} = 7.3, C-4′), 65.5 (d, J_{P-C} = 5.2, C-5′), 65.2, 65.1 (2d, J_{P-C} = 4.8, CH_2O), 60.1, 60.0 (C-3′) 52.4, 52.3 (CO_2CH_3), 49.9 (CH), 46.5 ($C(CH_3)_3$), 37.2 (C-2′), 28.4 (d, J_{P-C} = 7.4, SCH_2), 27.1 ($C(CH_3)_3$), 20.9, 20.8 (2d, J_{P-C} = 5.8, CH_3), 12.3, 12.2 (Thy-CH_3); MS FAB$^+$ *m/z* 577 (M + H)$^+$, 551 (M − N_2 + H)$^+$, 517 (M − CO_2CH_3)$^+$, 127 $(BH_2)^+$; MS FAB$^-$ *m/z* 1151 (2M − H)$^-$, 1007 (2M − *t*BuSATE)$^-$, 575 (M − H)$^-$, 431 (M − *t*BuSATE)$^-$, 326 (M − AZT)$^-$, 125 (B)$^-$; UV λ_{max} 265 nm (ϵ 8600); Anal. ($C_{21}H_{33}N_6O_9PS$) C, H, N.

***N*-(Methoxy-L-glycinyl)-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 3:** 0.083 g (70%); ^{31}P NMR ($CDCl_3$): δ 9.52, 9.45 (1:1); ^{1}H NMR ($CDCl_3$): δ 8.95, 8.94 (2s, 1H, Thy-NH), 7.42, 7.39 (d, 1H, J = 1.0, H-6), 6.22, 6.18 (2t, 1H, J = 6.6, H-1′), 4.43−4.37 (m, 1H, H-3′), 4.32−4.22 (m, 2H, H-5′, H-5"), 4.11−4.09 (m, 2H, CH_2O), 4.06−4.03 (m, 1H, H-4′), 3.79−3.70 (m, 5H, CH_2, OCH_3), 3.65−3.56 (m, 1H, NH), 3.21−3.08 (m, 2H, SCH_2), 2.46−2.30 (m, 2H, H-2′, H-2"), 1.93 (d, 3H, J = 1.0, Thy-CH_3), 1.23, 1.22 (2s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$): δ 205.9, 205.8 (C═O), 171.3, 171.2 (2d, J_{P-C} = 6.1, CO_2CH_3), 163.6, 163.5 (C-4), 150.6, 150.5 (C-2), 135.4, 135.3 (C-6), 111.5, 111.3 (C-5), 85.0, 84.7 (C-1′), 82.4 (d, J_{P-C} = 7.7, C-4′), 65.5−65.2 (CH_2O, C-5′), 60.3, 60.2 (C-3′), 52.5 (CO_2CH_3), 46.5 ($C(CH_3)_3$), 42.8 (CH_2), 37.4 (C-2′), 28.6 (d, J_{P-C} = 7.5, SCH_2), 27.3 ($C(CH_3)_3$), 12.5 (Thy-CH_3); MS FAB$^+$ *m/z* 563 (M + H)$^+$, 537 (M − N_2 + H)$^+$, 503 (M − CO_2CH_3)$^+$, 127 $(BH_2)^+$; MS FAB$^-$ *m/z* 1123 (2M − H)$^-$, 979 (2M − *t*BuSATE)$^-$, 874 (2M − AZT)$^-$, 561 (M − H)$^-$, 417 (M − *t*BuSATE)$^-$, 312 (M − AZT)$^-$, 125 (B)$^-$; UV λ_{max} 265 nm (ϵ 8600); Anal. ($C_{20}H_{31}N_6O_9PS$) C, H, N.

***N*-(Methoxy-L-phenylalaninyl)-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 4:** 0.067 g (49%); ^{31}P NMR ($CDCl_3$): δ 8.56, 8.46 (1:1); ^{1}H NMR ($CDCl_3$): δ 8.72, 8.68 (2s, 1H, Thy-NH), 7.38−7.15 (m, 6H, H-6, Ph), 6.19, 6.14 (2t, 1H, J = 6.6, H-1′), 4.31−4.20 (m, 1H, H-3′), 4.18−3.76 (m, 6H, CH, H-5′, H-5", CH_2O, H-4′), 3.74, 3.73 (2s, 3H, OCH_3), 3.46, 3.38 (2t, 1H, J = 10.4, NH), 3.14−2.87 (m, 4H, CH_2,SCH_2), 2.42−2.15 (m, 2H, H-2′, H-2"), 1.93, 1.92 (2d, 3H, J = 1.0, Thy-CH_3), 1.22, 1.21 (2s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$): δ 205.8, 205.7 (C═O), 173.1, 173.0 (2d, J_{P-C} = 6.5, CO_2CH_3), 163.5 (C-4), 150.1, 150.0 (C-2), 135.9 (Ph ipso), 135.3, 135.2 (C-6), 129.4−127.3 (m, Ph), 111.4, 111.3 (C-5), 84.9, 84.7 (C-1′), 82.3, 82.2 (2d, J_{P-C} = 7.5, C-4′), 65.3, 65.2 (2d, J_{P-C} = 5.0, C-5′), 65.1 (d, J_{P-C} = 4.9, CH_2O), 60.4, 60.2 (C-3′), 55.8,

55.7 (CH), 52.5, 52.4 (CO_2CH_3), 46.5 ($C(CH_3)_3$), 40.4, 40.3 (2d, J_{P-C} = 6.8, CH_2), 37.4, 37.3 (C-2′), 28.5 (d, J_{P-C} = 7.5, SCH_2), 27.3 ($C(CH_3)_3$), 12.4 (Thy-CH_3); MS FAB$^+$ *m/z* 653 (M + H)$^+$, 627 (M − N_2 + H)$^+$, 593 (M − CO_2CH_3)$^+$, 127 (BH_2)$^+$; MS FAB$^-$ *m/z* 1303 (2M − H)$^-$, 1054 (2M − AZT)$^-$, 651 (M − H)$^-$, 507 (M − *t*BuSATE)$^-$, 402 (M − AZT)$^-$, 125 (B)$^-$; UV λ_{max} 265 nm (ϵ 9000); Anal. ($C_{27}H_{37}N_6O_9PS$) C, H, N.

***N*-(Methoxy-L-tyrosinyl)-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 5:** 0.080 g (57%); ^{31}P NMR (DMSO-d_6): δ 9.11, 9.04 (1:1); ^{1}H NMR (DMSO-d_6): δ 11.36 (s, 1H, Thy-NH), 9.26, 9.25 (2s, OH), 7.43, 7.41 (2d, *J* = 1.2, H-6), 7.00−6.63 (m, 4H, Ph), 6.10, 6.07 (2t, 1H, *J* = 6.7, H-1′), 5.73 (t, *J* = 11.5, NH), 4.36−4.30 (m, 1H, H-3′), 4.00−3.42 (m, 6H, H-4′, H-5′, H-5", CH, CH_2O), 3.37 (s, 3H, OCH_3), 2.98−2.63 (m, 4H, SCH_2, CH_2), 2.30−2.27 (t, 2H, *J* = 6.5, H-2′, H-2"), 1.77 (s, 3H, Thy-CH_3), 1.15, 1.14 (2s, 9H, *t*Bu); ^{13}C NMR (DMSO-d_6): δ 205.2 (C═O), 173.3 (CO_2CH_3), 163.7 (C-4), 156.1 (Ph para), 150.4 (C-2), 135.6 (C-6), 130.3 (Ph meta), 127.2 (Ph ipso), 115 (Ph ortho), 110.1, 110.0 (C-5), 83.6 (C-1′), 81.5 (d, J_{P-C} = 7.8, C-4′), 64.9 (C-5′), 63.8 (CH_2O), 60.2, 60.1 (C-3′), 56.3 (CH), 51.8 (CO_2CH_3), 46.0 ($C(CH_3)_3$), 38.3 (CH_2), 35.8 (C-2′), 28.2 (d, J_{P-C} = 7.7, SCH_2), 26.9 ($C(CH_3)_3$), 12.2, 12.1 (Thy-CH_3); MS FAB$^+$ *m/z* 669 (M + H)$^+$, 643 (M − N_2 + H)$^+$, 609 (M − CO_2CH_3)$^+$, 127 (BH_2)$^+$; MS FAB$^-$ *m/z* 1335 (2M − H)$^-$, 1191 (2M − *t*BuSATE)$^-$, 1086 (2M − AZT)$^-$, 667 (M − H)$^-$, 523 (M − *t*BuSATE)$^-$, 418 (M − AZT)$^-$, 125 (B)$^-$; UV λ_{max} 265 nm (ϵ 9100); Anal. ($C_{27}H_{37}N_6O_{10}PS$, 0.6 H_2O) C, H, N.

***N*-(Methoxy-*O*-*tert*-Butyldimethylsilyl-L-serinyl)-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 6:** 0.077 g (52%); ^{31}P NMR ($CDCl_3$): δ 8.98, 8.73 (1:1); ^{1}H NMR ($CDCl_3$): δ 8.57 (1s, 1H, Thy-NH), 7.46, 7.39 (2d, 1H, *J* = 1.1, H-6), 6.25, 6.19 (2t, 1H, *J* = 6.7, H-1′), 4.43−4.36 (m, 1H, H-3′), 4.30−3.75 (m, 9H, H-5′, H-5", CH_2O, CH, CH_2OSi, NH, H-4′), 3.73 (1s, 3H, OCH_3), 3.22−3.08 (m, 2H, SCH_2), 2.46−2.26 (m, 2H, H-2′, H-2"), 1.95, 1.94 (2d, 3H, *J* = 1.1, Thy-CH_3), 1.23, 1.22 (2s, 9H, *t*Bu), 0.85, 0.84 (2s, 9H, $(CH_3)_3CSi$), 0.03−0.00 (4s, 6H, $(CH_3)_2Si$); ^{13}C NMR ($CDCl_3$): δ 205.8, 205.7 (C═O), 171.8, 171.6 (2d, J_{P-C} = 4.9, CO_2CH_3), 163.4 (C-4), 150.1, 150.0 (C-2), 135.3, 135.2 (C-6), 111.4, 111.3 (C-5), 84.9, 84.6 (C-1′), 82.4, 82.3 (2d, J_{P-C} = 8.3, C-4′), 65.5−65.1 (CH_2O, C-5′, CH_2OSi), 60.4 (C-3′), 56.4, 56.3 (CH), 52.5, 52.4 (CO_2CH_3), 46.5 ($C(CH_3)_3$), 37.5, 37.4 (C-2′), 28.6, 28.5 (2d, J_{P-C} = 6.7, SCH_2), 27.3 ($C(CH_3)_3$), 25.6, 25.5 (($CH_3)_3CSi$), 18.2, 18.1 (($CH_3)_3CSi$), 12.5, 12.4 (Thy-CH_3), −4.6, −4.7, −4.8 (($CH_3)_2Si$); MS FAB$^+$ *m/z* 707 (M + H)$^+$, 681 (M − N_2 + H)$^+$, 647 (M − CO_2CH_3)$^+$; MS FAB$^-$ *m/z* 705 (M − H)$^-$, 561 (M − *t*BuSATE)$^-$, 456 (M − AZT)$^-$, 125 (B)$^-$; UV λ_{max} 265 nm (ϵ 9500); Anal. ($C_{27}H_{47}N_6O_{10}PSSi$) C, H, N. HRMS ($C_{27}H_{48}N_6O_{10}PSSi$) calcd 707.2633, found 707.2660.

***N*-(Methoxy-*O*-acetyl-L-serinyl)-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 7:** 0.093 g (70%); ^{31}P NMR ($CDCl_3$): δ 8.39, 8.33 (1:1); ^{1}H NMR ($CDCl_3$): δ 8.92, 8.90 (2s, 1H, Thy-NH), 7.40, 7.34 (2d, 1H, *J* = 1.1, H-6), 6.21, 6.15 (2t, 1H, *J* = 6.6, H-1′), 4.44−4.03 (m, 9H, H-3′, H-5′, H-5", CH_2O, CH, CH_2OAc, H-4′), 3.90, 3.83 (2t, 1H, *J* = 10.3, NH), 3.77 (1s, 3H, OCH_3), 3.24−3.06 (m, 2H, SCH_2CH_2O), 2.46−2.29 (m, 2H, H-2′, H-2"), 2.06, 2.05 (2s, 3H, CH_3CO), 1.94, 1.93 (2s, 3H, Thy-CH_3), 1.23, 1.22 (2s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$): δ 205.9, 205.8 (C═O), 170.8, 170.7 (2d, J_{P-C} = 5.5, CO_2CH_3), 170.4, 170.3 (CH_3CO), 163.5 (C-4), 150.1, 150.0 (C-2), 135.4, (C-6), 111.4, 111.3 (C-5), 85.2, 84.9 (C-1′), 82.3 (d, J_{P-C} = 8.0, C-4′), 65.5−65.3 (CH_2O, C-5′, CH_2OAc), 60.3, 60.2 (C-3′), 53.8, 53.7 (CH), 53.0, 52.9 (CO_2CH_3), 46.5 ($C(CH_3)_3$), 37.3, 37.2 (C-2′), 28.5, 28.4 (2d, J_{P-C} = 6.7, SCH_2), 27.2 ($C(CH_3)_3$), 20.6 (CH_3CO), 12.5, 12.4 (Thy-CH_3); MS FAB$^+$ *m/z* 635 (M + H)$^+$, 609 (M − N_2 + H)$^+$, 575 (M − CO_2CH_3)$^+$, 127 (BH_2)$^+$; MS FAB$^-$ *m/z* 1267 (2M − H)$^-$, 1123 (2M − *t*BuSATE)$^-$, 1018 (2M − AZT)$^-$, 633 (M − H)$^-$, 489 (M − *t*BuSATE)$^-$, 384 (M − AZT)$^-$, 125 (B)$^-$; Anal. ($C_{23}H_{35}N_6O_{11}PS$) C, H, N.

***N*-(Methoxy-D-alaninyl)-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 8**: 0.080 g (66%); ^{31}P NMR ($CDCl_3$): δ 8.66, 8.50 (2:3); ^{1}H NMR ($CDCl_3$): δ 8.9 (sl, 1H, Thy-NH), 7.41, 7.39 (2d, 1H, *J* = 1.0, H-6), 6.23, 6.20 (2t, 1H, *J* = 6.6, H-1′), 4.41−4.36 (m, 1H, H-3′), 4.28−4.17 (m, 2H, H-5′, H-5"), 4.15−3.88 (m, 4H, CH_2O, H-4′, CH), 3.73, 3.72 (2s, 3H, OCH_3), 3.68, 3.56 (2t, 1H, J_{P-H} = 10.1, NH), 3.19−3.08 (m, 2H, SCH_2), 2.46−2.27 (m, 2H, H-2′, H-2"), 1.95, 1.94 (2d, 3H, *J* = 1.0, Thy-CH_3), 1.42 (d, 3H, *J* = 7.0, CH_3), 1.23, 1.22 (2s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$): δ 205.8, 205.7 (C═O), 174.7, 174.5 (2d, J_{P-C} = 6.0, CO_2CH_3), 163.5, 163.4 (C-4), 150.2 (C-2), 135.4, 135.3 (C-6), 111.5, 111.4 (C-5), 84.9, 84.8 (C-1′), 82.3, 82.2 (2d, J_{P-C} = 7.5, C-4′), 65.5 (d, J_{P-C} = 5.4, C-5′), 65.2, 65.1 (2d, J_{P-C} = 4.8, CH_2O), 60.3, 60.2 (C-3′), 52.6 (CO_2CH_3), 49.9 (CH), 46.5 ($C(CH_3)_3$), 37.3, 37.2 (C-2′), 28.6 (d, J_{P-C} = 7.0, SCH_2), 27.3 ($C(CH_3)_3$), 21.1, 21.0 (2d, J_{P-C} = 5.6, CH_3), 12.5, 12.4 (2s, Thy-CH_3); MS FAB$^+$ 577 (M + H)$^+$, 551 (M − N_2 + H)$^+$, 517 (M − CO_2CH_3)$^+$, 127 (BH_2)$^+$; MS FAB$^-$ *m/z* 1007 (2M − tBuSATE)$^-$, 575 (M − H)$^-$, 431 (M − tBuSATE)$^-$, 326 (M − AZT)$^-$, 125 (B)$^-$; UV λ_{max} 264.8 nm (ϵ 9600); Anal. ($C_{21}H_{33}N_6O_9PS$) C, H, N.

***N*-(Methoxy-2-aminoisobutyryl)-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 9:** 0.077 g (62%); ^{31}P NMR ($CDCl_3$): δ 7.47, 7.27 (1:2); ^{1}H NMR ($CDCl_3$): δ 9.0 (sl, 1H, Thy-NH), 7.42, 7.38 (2d, 1H, *J* = 1.0, H-6), 6.23, 6.21 (2t, 1H, *J* = 6.6, H-1′), 4.42−4.35 (m, 1H, H-3′), 4.25−4.18 (m, 2H, H-5′, H-5"), 4.14−4.05 (m, 2H, CH_2O), 4.01 (m, 1H, H-4′), 3.92 (d, 1H, *J* = 8.5, NH), 3.74 (2s, 3H, OCH_3), 3.16−3.07 (m, 2H, SCH_2), 2.45−2.28 (m, 2H, H-2′, H-2"), 1.93, 1.92 (d, 3H, *J* = 1.0, Thy-CH_3), 1.53, 1.52 (2s, 6H, $C(CH_3)_2$), 1.22, 1.21 (2s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$): δ 205.8 (C═O), 175.9, 175.8 (2d, J_{P-C} = 5.8, CO_2CH_3), 163.6 (C-4), 150.2, 150.1 (C-2), 135.3 (C-6), 111.5, 111.4 (C-5), 84.8, 84.6 (C-1′), 82.3 (d, J_{P-C} = 7.3, C-4′), 65.3−65.1 (m, CH_2O, C-5′), 60.2, 60.1 (C-3′), 56.8, 56.7 (2d, J_{P-C} = 5.6, $C(CH_3)_2$), 52.9 (CO_2CH_3), 46.5 ($C(CH_3)_3$), 37.3 (C-2′), 28.6 (d, J_{P-C} = 7.5, SCH_2), 27.2 ($C(CH_3)_3$), 27.0, 26.8 (2d, J_{P-C} = 7.3, $C(CH_3)_2$), 12.5 (Thy-CH_3); MS FAB$^+$ *m/z* 591 (M + H)$^+$, 565 (M − N_2 + H)$^+$, 531 (M − CO_2CH_3)$^+$, 127 (BH_2)$^+$; MS FAB$^-$ *m/z* 1179 (2M − H)$^-$, 930 (2M − AZT)$^-$, 589 (M − H)$^-$, 445 (M − *t*BuSATE)$^-$, 340 (M − AZT)$^-$, 125 (B)$^-$; UV λ_{max} 265 nm (ϵ 8600); Anal. ($C_{22}H_{35}N_6O_9PS$) C, H, N.

***N*-(Methoxy-β-alaninyl)-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 10:** 0.087 g (72%); ^{31}P NMR ($CDCl_3$): δ 10.38, 10.34 (1:1); ^{1}H NMR ($CDCl_3$): δ 8.88, 8.86 (2s, 1H, Thy-NH), 7.42, 7.37 (2d, 1H, *J* = 1.0, H-6), 6.21, 6.17 (2t, 1H, *J* = 6.6, H-1′), 4.44−4.38 (m, 1H, H-3′), 4.24−4.20 (m, 2H, H-5′, H-5"), 4.12−4.04 (m, 3H, CH_2O, H-4′), 3.70 (s, 3H, OCH_3), 3.56, 3.50 (m, 1H, NH), 3.25−3.18 (m, 2H, CH_2NH), 3.14 (t, 2H, *J* = 6.4, SCH_2), 2.54 (t, 2H, *J* = 5.8, CH_2CH_2NH), 2.48−2.31 (m, 2H, H-2′, H-2"), 1.94 (d, 3H, *J* = 1.0, Thy-CH_3), 1.23, 1.22 (2s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$): δ 205.9, 205.8 (C═O), 172.5, 172.4 (CO_2CH_3), 163.5 (C-4), 150.2, 150.1 (C-2), 135.3 (C-6), 111.5, 111.4 (C-5), 85.2, 84.9 (C-1′), 82.3 (d, J_{P-C} = 7.4, C-4′), 65.4−65.1 (m, CH_2O, C-5′), 60.3, 60.2 (C-3′), 51.9 (CO_2CH_3), 46.5 ($C(CH_3)_3$), 37.4 (C-2′), 37.0 (CH_2NH), 35.6 (d, J_{P-C} = 7.5, CH_2CH_2NH), 28.6 (d, J_{P-C} = 7.5, SCH_2), 27.3 ($C(CH_3)_3$), 12.5 (Thy-CH_3); MS FAB$^+$ *m/z* 577 (M + H)$^+$, 551 (M − N_2 + H)$^+$, 517 (M − CO_2CH_3)$^+$, 127 (BH_2)$^+$; MS FAB$^-$ *m/z* 1727 (3M − H)$^-$, 1478 (3M − AZT)$^-$, 1151 (2M − H)$^-$, 1007 (2M − *t*BuSATE)$^-$, 902 (2M − AZT)$^-$, 575 (M − H)$^-$, 431 (M − *t*BuSATE)$^-$, 326 (M − AZT)$^-$, 125 (B)$^-$; UV λ_{max} 265 nm (ϵ 8600); Anal. ($C_{21}H_{33}N_6O_9PS$) C, H, N.

***N*-(Methoxy-4-aminobutyryl)-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 11:** 0.099 g (80%); ^{31}P NMR ($CDCl_3$): δ 10.51, 10.49 (1:1); ^{1}H NMR ($CDCl_3$): δ 9.21, 9.16 (2s, 1H, Thy-NH), 7.43, 7.36 (2d, 1H, *J* = 1.0, H-6), 6.21, 6.15 (2t, 1H, *J* = 6.6, H-1′), 4.44−4.39 (m, 1H, H-3′), 4.25−4.18 (m, 2H, H-5′, H-5"), 4.12−4.00 (m, 3H, CH_2O, H-4′), 3.66 (s, 3H, OCH_3), 3.28−3.25 (m, 1H, NH), 3.15−3.11 (m, 2H, SCH_2), 3.02−2.93 (m, 2H, CH_2NH), 2.47−2.28 (m, 4H, $CH_2CO_2CH_3$, H-2′, H-2"), 1.93, 1.92 (2d, 3H, *J* = 1.0, Thy-CH_3), 1.86−1.79 (m, 2H, CH_2CH_2NH), 1.22, 1.21 (2s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$): δ 205.8, 205.7 (C═O), 173.5, 173.4 (CO_2CH_3), 163.6, 163.5 (C-4), 150.2, 150.1 (C-2), 135.4 (C-6), 111.4, 111.3 (C-5), 85.3, 84.8 (C-1′), 82.4, 82.3 (2d, J_{P-C} = 7.4, C-4′), 65.3−65.0 (m, CH_2O, C-5′), 60.4, 60.3 (C-3′),51.7 (CO_2CH_3), 46.5 ($C(CH_3)_3$), 40.8, 40.7 (CH_2NH), 37.4 (C-2′), 30.9 ($CH_2CO_2CH_3$), 28.6 (d, J_{P-C} = 7.0, SCH_2), 27.3 ($C(CH_3)_3$), 26.7, 26.6 (2d, J_{P-C} = 6.0, CH_2CH_2NH), 12.5 (Thy-CH_3); MS FAB$^+$ 591 (M +

H)$^+$, 565 (M − N_2 + H)$^+$, 531 (M − CO_2CH_3)$^+$, 127 (BH_2)$^+$; MS FAB$^-$ *m/z* 1179 (2M − H)$^-$, 1035 (2M − *t*BuSATE)$^-$, 930 (2M − AZT)$^-$, 589 (M − H)$^-$ 445 (M − *t*BuSATE)$^-$, 340 (M − AZT)$^-$, 125 (B)$^-$; UV λ_{max} 264.2 nm (ϵ 9700); Anal. ($C_{22}H_{35}N_6O_9PS$) C, H, N.

General Procedure for the Preparation of Phosphoramidates Diesters 12−14. To a solution of *H*-phosphonate diester **15** (0.1 g, 0.21 mmol) in dry tetrachloromethane (2 mL) was added the appropriate amine (2.1 mmol, 10 equiv). In the case of aniline, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 1.9 mmol, 9 equiv) was added. After being stirred for 3 h at room temperature, the reaction mixture was diluted with dichloromethane and washed successively with 1 M hydrochloric acid aqueous solution, saturated sodium bicarbonate solution, and water. The organic phase was dried with Na_2SO_4, filtered, and evaporated to dryness in a vacuum. Purification of the residue by column chromatography on silica gel using a stepwise gradient of methanol (0−3%) in dichloromethane afforded a diastereoisomeric mixture of the desired phosphoramidate as a white foam.

***N*-Isopropylamino-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 12:** 0.090 g (80%); ^{31}P NMR ($CDCl_3$): δ 9.62, 9.55 (1:1); ^{1}H NMR ($CDCl_3$): δ 8.98, 8.95 (2s, 1H, Thy-NH), 7.45, 7.37 (2d, 1H, J = 1.1, H-6), 6.24, 6.18 (2t, 1H, J = 6.6, H-1′), 4.41 (m, H-3′), 4.26−4.15 (m, 2H, H-5′, H-5"), 4.14−4.00 (m, 3H, CH_2O, H-4′), 3.43−3.30 (m, 1H, NH), 3.20−3.09 (m, 2H, SCH_2), 2.83−2.77 (m, 1H, CH), 2.48−2.26 (m, 2H, H-2′, H-2"), 1.94 (d, 3H, J = 1.1, Thy-CH_3), 1.23, 1.22 (2s, 9H, *t*Bu), 1.18, 1.17 (2d, 6H, J = 6.1, CH_3CH); ^{13}C NMR ($CDCl_3$): δ 205.8, 205.7 (C═O), 163.5 (C-4), 150.2, 150.1 (C-2), 135.2, 135.1 (C-6), 111.4, 111.3 (C-5), 85.0, 84.6 (C-1′), 82.4 (d, J_{P-C} = 7.8, C-4′), 65.2, 65.1 (d, J_{P-C} = 5.0, C-5′), 64.9, 64.8 (2d, J_{P-C} = 4.5, CH_2O), 60.5, 60.4 (C-3′), 46.5 ($C(CH_3)_3$), 44.1 (CH), 37.5 (C-2′), 29.1 (d, J_{P-C} = 7.3, SCH_2), 27.3 (C(CH_3)$_3$), 25.4, 25.3 (2d, J_{P-C} = 5.5, (CH_3)$_2$CH), 12.5 (Thy-CH_3); MS FAB$^+$ 533 (M + H)$^+$, 507 (M − N_2 + H)$^+$, 127 (BH_2)$^+$; MS FAB$^-$ *m/z* 1063 (2M − H)$^-$, 919 (2M − *t*BuSATE)$^-$, 814 (2M − AZT)$^-$, 531 (M − H)$^-$, 387 (M − *t*BuSATE)$^-$, 282 (M − AZT)$^-$, 125 (B)$^-$; UV λ_{max} 264.2 nm (ϵ 9800); Anal. ($C_{20}H_{33}N_6O_7PS$) C, H, N.

***N*-Piperidino-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 13:** 0.100 g (75%); ^{31}P NMR ($CDCl_3$): δ 10.37, 10.31 (2:3); ^{1}H NMR ($CDCl_3$): δ 8.69 (s, 1H, Thy-NH), 7.46, 7.38 (2d, 1H, J = 1.0, H-6), 6.25, 6.19 (2t, 1H, J = 6.8, H-1′), 4.44−4.37 (m, 1H, H-3′), 4.21−3.95 (m, 5H, H-5′, H-5", CH_2O, H-4′), 3.15−3.09 (m, 6H, SCH_2, CH_2N), 2.49−2.21 (m, 2H, H-2′, H-2"), 1.94 (d, 3H, J = 1.0, Thy-CH_3), 1.59−1.52 (m, 6H, CH_2CH$_2$N, CH_2CH$_2$CH$_2$N), 1.23, 1.22 (2s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$): δ 205.7 (C═O), 163.4 (C-4), 150.1, 150.0 (C-2), 135.1, 135.0 (C-6), 111.4, 111.3 (C-5), 85.0, 84.6 (C-1′), 82.6, 82.5 (2d, J_{P-C} = 7.7, C-4′), 65.3, 65.1 (2d, J_{P-C} = 5.4, C-5′), 64.8 (d, J_{P-C} = 5.2, CH_2O), 60.6, 60.5 (C-3′), 46.5 ($C(CH_3)_3$), 45.4 (CH_2N), 37.6, 37.5 (C-2′), 28.7, 28.6 (2d, J_{P-C} = 7.7, SCH_2), 27.3 (C(CH_3)$_3$), 26.1 (d, J_{P-C} = 4.7, CH_2CH_2N), 24.2 ($CH_2CH_2CH_2N$), 12.6 (Thy-CH_3); MS FAB$^+$ *m/z* 559 (M + H)$^+$, 533 (M − N_2 + H)$^+$; MS FAB$^-$ *m/z* 1115 (2M − H)$^-$, 866 (2M − AZT)$^-$, 557 (M − H)$^-$, 413 (M − *t*BuSATE)$^-$, 308 (M − AZT)$^-$; UV λ_{max} 265 nm (ϵ 9500); Anal. ($C_{22}H_{35}N_6O_7PS$) C, H, N.

***N*-Anilino-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 14:** 0.024 g (20%); ^{31}P NMR ($CDCl_3$): δ 4.35,4.16 (1:1);^{31}P NMR (DMSO-d_6): δ 4.16, ^{1}H NMR (DMSO-d_6): δ 11.4, 11.3 (2s, 1H, Thy-NH), 8.23, 8.21 (2d, J = 9.7, NH), 7.48, 7.45 (2s, 1H, H-6), 7.18 (m, 2H, Ph meta), 7.01 (d, 2H, J = 8.3, Ph ortho), 6.87 (t, 1H, J = 7.3, Ph para), 6.11, 6.10 (2t, 1H, J = 6.5, H-1′), 4.45−4.38 (m, 1H, H-3′), 4.24−3.97 (m, 5H, H-5′, H-5", CH_2O, H-4′), 3.08 (t, 2H, J = 6.3, SCH_2), 2.42−2.27 (m, 2H, H-2′, H-2"), 1.74, 1.72 (2s, 3H, Thy-CH_3), 1.13, 1.12 (2s, 9H, *t*Bu); ^{13}C NMR (DMSO-d_6): δ 205.0 (C═O), 163.5 (C-4), 150.1 (C-2), 141.1, 141.0 (Ph ipso), 135.6 (C-6), 128.8 (Ph meta), 120.9 (Ph para), 117.3 (d, J_{P-C} = 7.6, Ph ortho), 109.8, 109.7 (C-5), 84.4, 83.3 (C-1′), 81.2 (d, J_{P-C} = 7.8, C-4′), 65.2 (d, J_{P-C} = 4.6, C-5′), 64.3 (d, J_{P-C} = 4.6, CH_2O), 59.8 (C-3′), 45.8 ($C(CH_3)_3$), 35.5, 35.4 (C-2′), 28.1 (d, J_{P-C} = 7.6, SCH_2CH_2O), 26.6 (C(CH_3)$_3$), 11.9 (Thy-CH_3); MS FAB$^+$ *m/z* 567 (M + H)$^+$, 541 (M − N_2 + H)$^+$, 127 (BH_2)$^+$; MS FAB$^-$ *m/z* 565 (M − H)$^-$, 421 (M − *t*BuSATE)$^-$, 316 (M − AZT)$^-$, 125 (B)$^-$; UV λ_{max} 266 nm (ϵ 11500); Anal. ($C_{23}H_{31}N_6O_7PS$) C, N. H: calcd 5.51, found 6.17.

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