S-Acyl-2-Thioethyl Aryl Phosphotriester Derivatives of AZT: Synthesis, Antiviral Activity, and Stability Study

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The synthesis, antiviral activity, and stability study of phosphotriester derivatives of 3′-azido-2′,3′-dideoxythymidine (AZT) bearing modified L-tyrosinyl residues are reported. These compounds were obtained via phosphoramidite (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, especially in thymidine kinase deficient (TK$^−$) cells, showing their ability to act as mononucleotide prodrugs. The proposed decomposition process of these mixed mononucleoside aryl phosphotriesters successively involves an esterase and a phosphodiesterase hydrolysis.

Introduction

In the past decade, an increasing number of research groups have focused their attention on the study of mononucleotide prodrugs, namely, pronucleotides.[1−4] Such pronucleotides were designed to give rise to the intracellular delivery of 5′-mononucleotides, the latter being further metabolized to the corresponding mononucleoside triphosphate analogue in order to exert antiviral activity.

In this area, we have recently reported the potentialities of mixed mononucleoside phosphotriester derivatives of 3′-azido-2′,3′-dideoxythymidine (AZT) bearing one *S*-pivaloyl-2-thioethyl (*t*BuSATE) group and an aryl residue as biolabile phosphate protection.[5] In this previous study, we have demonstrated that the antiviral activity of the related mononucleotide aryl (*t*BuSATE) phosphotriester derivatives could be influenced by the ionizable, polar, or lipophilic nature of the aryl substituents. In the case of the tyrosinyl (*t*BuSATE) phosphotriester derivative (Figure 1, compound **1**) the presence of the zwitterion, especially the free carboxylate function, hindered the cellular passive diffusion of the compound and led to a loss of the antiviral activity in CEM thymidine kinase-deficient (TK$^−$) cells. In this respect, we decided to design novel mixed phosphotriester derivatives of AZT incorporating polar but not anionic residues in order to study their influence regarding anti-HIV activities, kinetic decomposition parameters, and lipophilicity. Herein, the synthesis and the study of derivatives where the carboxylate group of L-tyrosine has been replaced by an alcohol (Figure 1, compounds **3** and **4**) or an amide (Figure 1, compounds **5**−**8**) function are reported.

Results and Discussion

Chemistry. A simple retrosynthetic route to the target compounds is presented in Scheme 1. Briefly, the mixed phosphotriester derivatives **2**−**8** could be obtained by in situ oxidation of the corresponding phosphite triesters containing AZT. Thus, the mixed phosphoramidite agents bearing the two phosphate protections consist of subsequent coupling of the *t*Bu-SATE chain moiety and the appropriate tyrosinyl residue on the commercially available bis(diisopropyl-amino) chlorophosphine. The choice of the protecting groups borne by the L-tyrosinyl analogues should be done thoughtfully. 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 (*tert*-butyloxycarbonyl, *t*Boc, and isopropylidene) and/or groups susceptible to being hydrolyzed by an enzymatic system during biological studies (i.e., acetyl group) to keep the integrity of the final compounds.

The tyrosinol precursor **10** (Scheme 2) was obtained in two steps starting from the commercially available *N*-α-Boc tyrosine methyl ester. The ester was reduced with NaBH$_4$/LiCl[6,7] to the primary alcohol **9** and then converted to the oxazolidine **10** by treatment with 2,2-dimethoxypropane in the presence of a catalytic amount of *p*-toluenesulfonic acid[7,8] in acetone. The reaction conditions of the reductive step had to be forced (overnight, solvent reflux) in order to increase the yield. Indeed, it has previously been demonstrated that esters bearing phenoxy groups are not efficiently reduced.[9] We assume that the free phenol function of tyrosine could interfere with sodium borohydride, forming various alkoxy hydroborates and uselessly consuming the reagent.

The ^{1}H NMR spectral data of the *N*-α-(Boc)oxazolidine derivative of L-tyrosine **10** were complex regarding the

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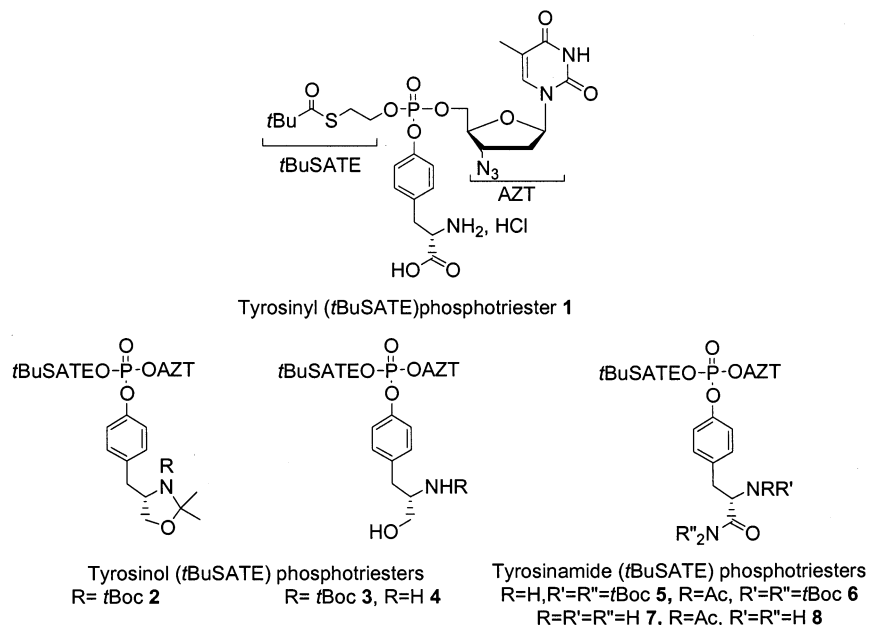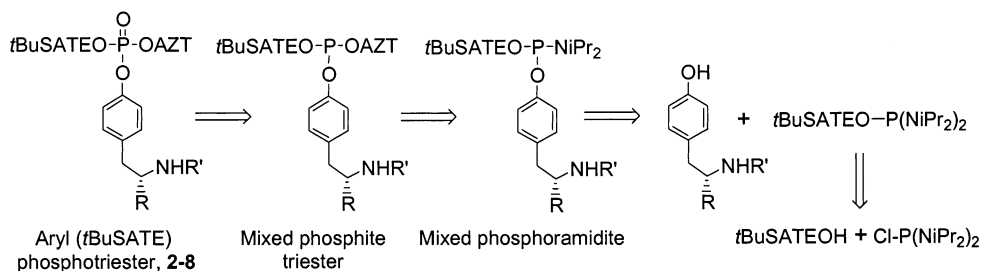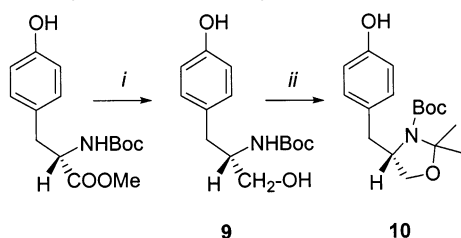   † Université Montpellier II.
   ‡ Université L. Pasteur.

Figure 1. Structures of the studied phosphotriester derivatives of AZT.

Scheme 1. Retrosynthetic Route to the Desired Derivatives **2−8**


Scheme 2. Synthesis of the Tyrosinol Precursor, **10**[a]

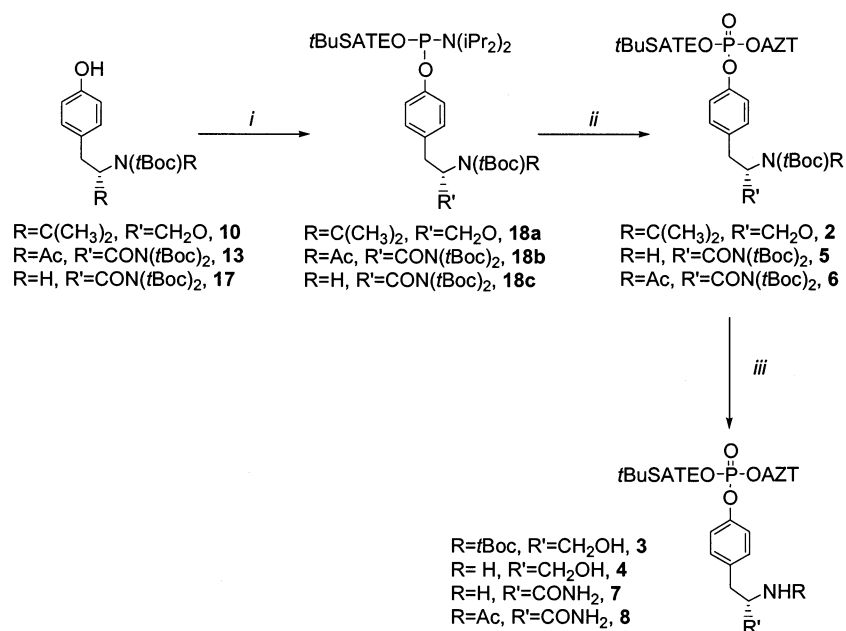[a] Reagents: (i) NaBH$_4$, LiCl, THF reflux; (ii) (CH$_3$O)$_2$C(CH$_3$)$_2$, *p*Ts acid cat., acetone.

presence of rotamers, which slowly interconvert at room temperature (20 °C), since, for example, two multiplets were observed for the H-α of the amino acid.[8] When the NMR spectrum was recorded at 55 °C, we obtained a simplified spectrum. The same doubling effect was observed for the corresponding phosphotriester **2**, which exhibits four signals in ^{31}P NMR spectrum (δ −5.11, −5.22, −5.34, −5.36 ppm in CDCl$_3$) with a ratio of 1:1:2:2 instead of the two expected (ratio, 1:1) because of the presence of diastereoisomers on the phosphorus stereocenter. Furthermore, this splitting effect disappeared after the selective hydrolysis of the *N,O*-acetal, giving rise to phosphotriesters **3** and **4**, which showed only two signals in ^{31}P NMR (δ −5.17 and −5.55 ppm in CDCl$_3$, with a ratio of 1:1), thus confirming the presence of a mixture of rotamers and not of the racemization process.

Scheme 3 shows the pathway used for the preparation of tyrosinamide precursors **13** and **17**. Attempts to directly use the commercially available *N*-α-acetyltyrosinamide or *N*-α-Boc-tyrosinamide **14** in the phosphoramidite coupling reaction (Scheme 4) only gave rise to byproducts; therefore, we decided to introduce Boc protecting groups on the amide function. The same synthetic steps were applied on both *N*-α-acetyl and Boc derivatives. Because the introduction of the Boc protecting group could not be carried out selectively on the amide in the presence of the free phenol, the phenoxy group has to be transiently masked with a *tert*-butyl-(dimethyl)silyl group,[10] giving rise to compounds **11** and **15**. These O-protected derivatives were then successively treated by Boc anhydride in the presence of DIEA and then by TBAF solution, yielding either the *N,N*-α-acetyl-Boc-*N*,*N*-diBoc-tyrosinamide **13** or to the *N*-α-Boc-*N*,*N*-diBoc-tyrosinamide **17**.

Synthesis of the desired mixed aryl phosphotriester derivatives was accomplished via P[III] (phosphoramidite) chemistry (Scheme 4), previously described for the tyrosinyl (*t*BuSATE) phosphotriester derivative of AZT.[5] The tyrosinyl precursors **10**, **13**, and **17** were condensed in the presence of 1*H*-tetrazole with the SATE phosphorobisamidite reagent.[11] Then, reaction of the adequate tyrosinyl (*t*BuSATE) phosphoramidite intermediates **18a−c** with AZT, followed by in situ oxidation using *tert*-butyl hydroperoxide, gave rise to the fully

Scheme 3. Synthesis of the Tyrosinamide Precursors **13** and **17**[a]

11   **12**   **13**

14   **15**   **16**   **17**
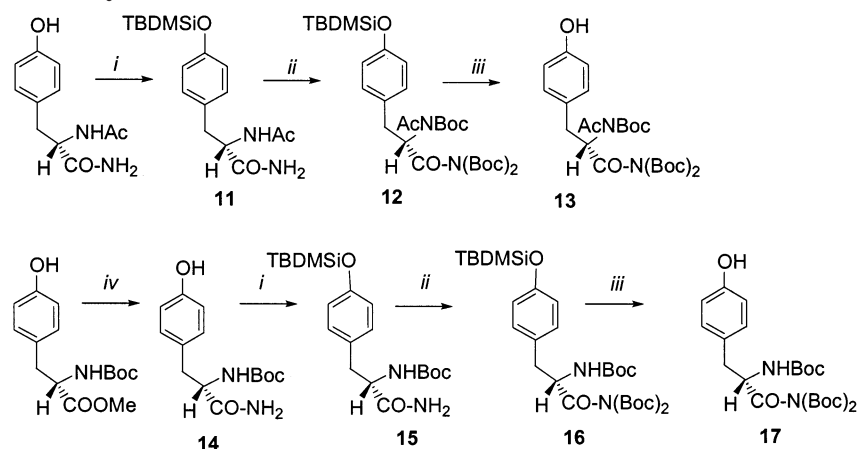
[a] Reagents: (i) TBDMSiCl, imidazole, pyridine; (ii) (Boc)$_2$O, DMAP, DIEA, CH$_2$Cl$_2$; (iii) TBAF, THF.

Scheme 4. General Approach for the Synthesis of Aryl Phosphotriester Derivatives **2**–**8**[a]

R=C(CH$_3$)$_2$, R'=CH$_2$O, **10**
R=Ac, R'=CON(tBoc)$_2$, **13**
R=H, R'=CON(tBoc)$_2$, **17**

R=C(CH$_3$)$_2$, R'=CH$_2$O, **18a**
R=Ac, R'=CON(tBoc)$_2$, **18b**
R=H, R'=CON(tBoc)$_2$, **18c**

R=C(CH$_3$)$_2$, R'=CH$_2$O, **2**
R=H, R'=CON(tBoc)$_2$, **5**
R=Ac, R'=CON(tBoc)$_2$, **6**

R=tBoc, R'=CH$_2$OH, **3**
R= H, R'=CH$_2$OH, **4**
R=H, R'=CONH$_2$, **7**
R=Ac, R'=CONH$_2$, **8**

[a] Reagents: (i) tBuSATEO-P(NiPr$_2$)$_2$, 1H-tetrazole, HN(iPr)$_2$, CH$_3$CN; (ii) AZT, 1H-tetrazole, CH$_3$CN, then tBuOOH, toluene; (iii) various acidic treatments.
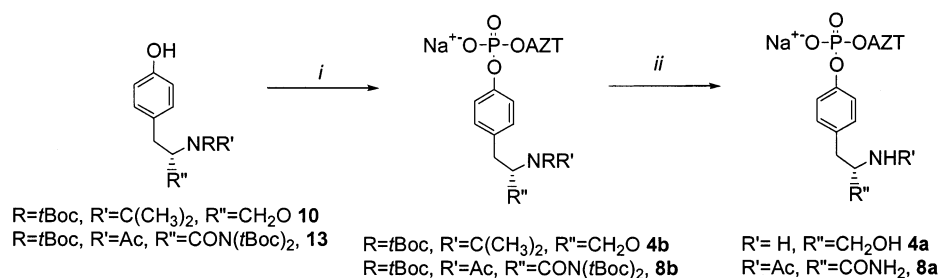
protected phosphotriester derivatives **2**, **5**, and **6** in 82%, 60%, and 63% overall yields, respectively.

Treatment of compounds **2**, **5**, and **6** in various acidic conditions allowed us to obtain the partially protected entities **3** and **8**, and also the fully deprotected aryl (tBuSATE) phosphotriester derivatives of AZT **4** and **7**. Compound **2** was reacted with 1 equiv of *p*-toluene-sulfonic acid in order to remove selectively the isopropylidene group and to give rise to the N-α-Boc-tyrosinol analogue **3**. The fully deprotected phosphotriester **4** could be obtained from **2** using a saturated solution of dry HCl in ether (35% in weight), leading directly to the hydrochloride salt, in 75% yield. Finally, the removal of the *t*Boc group from both **5** and **6** was carried out using trifluoroacetic acid/dichloromethane solution (10% in volume) in time-controlled reactions, and phosphotriesters **7** and **8** could be isolated in good yields after their conversion to the corresponding hydrochloride salts.

Compounds **2**–**8** were isolated as diastereomeric mixtures resulting from the stereochemistry on the

phosphorus atom. The diastereomeric ratio (1:1) was determined by either HPLC or ^{31}P NMR.

Antiviral Activity. The inhibitory effects on the HIV-1 replication of the mixed phosphotriesters **2**–**8** were evaluated in three cell culture systems (Table 1) in comparison to the parent nucleoside AZT and to the reference mixed pronucleotide **1**. In human T$_4$ lymphoblastoid CEM-SS and MT-4 cells, all compounds displayed activity against HIV-1 that was comparable to that of the parent nucleoside analogue AZT, with 50% effective concentration (EC$_{50}$) about submicromolar concentration range. In contrast to AZT, the tyrosinyl (tBuSATE) phosphotriesters **2**–**8** exhibited significant anti-HIV effects in CEM/TK$^-$ cells with EC$_{50}$ values in micromolar range, demonstrating the successful release of the corresponding 5'-mononucleotide (AZTMP) inside infected cells. Indeed, this cell line, highly deficient in cytosolic TK, should be considered as an ideal in vitro system to investigate the antiviral potency of thymine containing nucleotide analogues that may release the corresponding 5'-mononucleotide into cells.[3] The differ-

Scheme 5. Synthesis of Aryl Phosphodiester Derivatives **4a** and **8a**[a]

R=*t*Boc, R'=C(CH$_3$)$_2$, R"=CH$_2$O **10**
R=*t*Boc, R'=Ac, R"=CON(*t*Boc)$_2$, **13**

R=*t*Boc, R'=C(CH$_3$)$_2$, R"=CH$_2$O **4b**
R=*t*Boc, R'=Ac, R"=CON(*t*Boc)$_2$, **8b**

R'= H, R"=CH$_2$OH **4a**
R'=Ac, R"=CONH$_2$, **8a**

[a] Reagents: (i) *H*-phosphonate monoester of AZT (triethylammonium form), PivCl, C$_5$H$_5$N, CH$_3$CN, then I$_2$, H$_2$O, then Dowex Na$^+$; (ii) dry HCl in ether, then Dowex Na$^+$.

Table 1. Anti-HIV-1 Activity[a] (μM) in Three Cell Culture Systems and Apparent Partition Coefficients (log P_{app}) of Mixed Aryl Phosphotriester Derivatives **2**–**8** in Comparison to the Parent Nucleoside AZT and the Tyrosinyl (*t*BuSATE) Phosphotriester Derivative **1** of AZT as a 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]	log P_{app}
AZT	0.003	>100	0.015	>100	>100	>100	0.06 ± 0.003
1	0.006	>100	0.075	>10	29	>100	0.25 ± 0.015
2	0.031	>10	0.098	>10	9	>10	3.7[d]
3	0.017	>10	0.044	>10	4	>10	2.5[d]
4	0.007	>100	0.018	>100	7	>100	0.64 ± 0.02
5	0.049	>10	0.034	>10	4	>10	5[d]
6	0.015	>10	0.032	>10	6	>10	5.8[d]
7	0.002	>10	0.016	>10	4	>10	1.27 ± 0.005
8	0.082	>10	0.066	>10	8	>10	1.52 ± 0.006

[a] All data represent average values of 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%. [d] Calculated values. The log P determination was performed using Advanced Chemistry Development (Toronto, Canada) log P dB 4.5 calculations.

ences observed in the anti-HIV activities of the mixed phosphotriester 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 derivative.[12,13]

The log P value for each target compound was either calculated or obtained experimentally (Table 1). As expected, the replacement of the carboxylate functionality on the tyrosinyl residue and the presence of protecting groups on the amino function increased the lipophilicity of the resulting phosphotriester in a stepwise range of 0–6 log P values.

Comparison between anti-HIV activities in CEM/TK$^-$ cells and apparent partition coefficients of phosphotriester derivatives **1**–**8** (Table 1) showed that lipophilicity is not the only factor involved in the observed in vitro biological effect. Indeed, the replacement of the carboxylate by polar but not anionic groups such as alcohol or amide functions allowed us to obtain water-soluble pronucleotides without drastically losing the antiviral activity. Indeed, the tyrosinol analogue **4** exhibited a low log P value (log P_{app} = 0.64, Table 1) but retained the antiviral activity in the thymidine-kinase-deficient cell line compared to derivative **1** (log P_{app} = 0.25, Table 1). Despite marked differences in their lipophilicity, the EC$_{50}$ values for the phosphotriesters **2**–**8** are in the

same range. These results suggested that the prodrug activation kinetics of such aryl phosphotriesters also played an important role in the observed in vitro anti-HIV activity.

Stability Studies. With a previously published procedure,[14] the decomposition pathways and kinetic data of compounds **1**, **4**, **7**, and **8** were studied at 37 °C, in total CEM-SS extracts in order to mimic the behavior of such pronucleotides inside the cells, as well as in culture medium (RPMI1640 containing 10% heat inactivated fetal calf serum) to evaluate the stability of the studied phosphotriesters in the extracellular medium used during cell culture antiviral evaluation. The metabolites formed during the incubation of the phosphotriesters were identified by HPLC–ESI-MS coupling experiments and/or co-injection with authentic samples. Thus, aryl phosphodiesters **4a** and **8a** were obtained using standard *H*-phosphonate chemistry (Scheme 5). The *H*-phosphonate monoester derivative of AZT[15] was coupled with the aryl precursor **10** or **13** in the presence of pivaloyl chloride,[16,17] followed by in situ oxidation using iodine and water. The resulting aryl phosphodiesters **4b** and **8b** were treated with a saturated solution of dry HCl in ether (35% in weight) and led to **4a** and **8a** as hydrochloride forms.

The rate constants of disappearance and the half-lives of the studied phosphotriesters were calculated according to pseudo-first-order kinetic models and optimized using mono- or polyexponential regressions. These kinetic models were in accordance with the experimental data. Best-fit computed curves are shown in Figure 2, and the deduced half-lives of the starting materials and intermediates in various media are given in Tables 2 and 3.

These experiments allowed us to propose a decomposition mechanism for the tyrosinol phosphotriester **4** (Scheme 6) similar to the one previously published for the tyrosinyl entity **1**.[18] In both media (cell extracts and culture medium), the first step is an esterase-mediated activation with the loss of the *t*BuSATE chain, leading to the formation of the corresponding aryl phosphodiester derivative of AZT **4a**. This metabolite is then a substrate for a second enzymatic activity that gives rise to the formation of AZTMP. As expected, compound **4** metabolized very slowly (half-life of about a week) in culture medium compared to cell extracts.

In cell extracts, the decomposition pathways observed for the tyrosinamide phosphotriester derivatives **7** and **8** were more complex, involving two concomitant hydrolysis reactions in the first step (Scheme 6). For compound **7**, the main decomposition way (93%) results
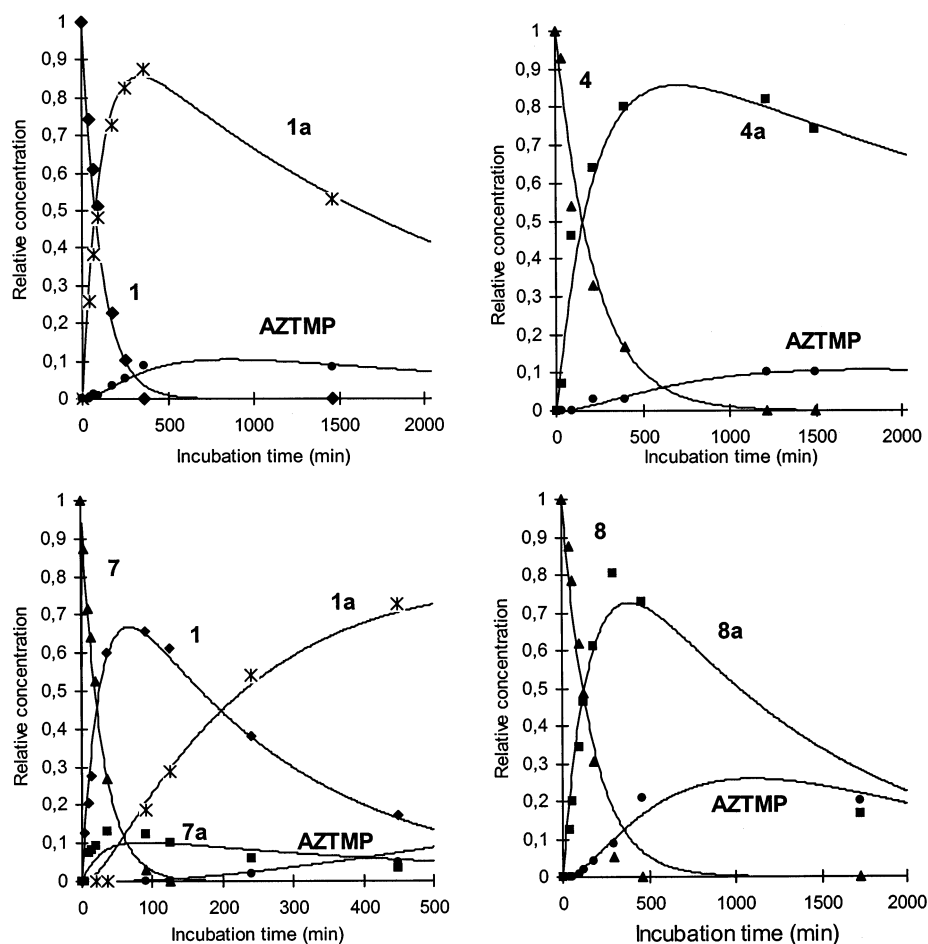
Figure 2. Decomposition kinetics in total CEM-SS cell extracts at 37 °C of phosphotriesters **1** (◆), **4**, **7**, and **8** (▲) at 50 μM initial concentration and their corresponding metabolites phosphodiesters **1a** (∗), **4a**, **7a**, **8a** (■) and AZTMP (●).

Table 2. Calculated Half-Lives of the Phosphotriester Derivatives **1**, **4**, **7**, **8** and Their Metabolites in CEM-SS Cell Extracts and Corresponding Pseudo-First-Order Rate Constants[a]

	compound 1	metabolite 1a
$t_{1/2}$ (h)	1.2	25
k (min^{-1})	0.0096	0.00046

	compound 4	metabolite 4a
$t_{1/2}$ (h)	2.5	43.1
k (min^{-1})	0.0046	0.00027

	compound		metabolite	
	7	1 (93%)	7a (7%)	1a
$t_{1/2}$	23 min	2.3 h	6 h	33.5 h
k (min^{-1})	0.030	0.005	0.0019	0.00034

	compound 8	metabolite	
		1 (<5%)	8a (>95%)
$t_{1/2}$ (h)	1.9	ND	13.1
k (min^{-1})	0.0061		0.00088

[a] All data represent average values for three separate experiments, and the accuracy was lower than 10%.

Table 3. Calculated Half-Lives of the Phosphotriester Derivatives **1**, **4**, **7**, and **8** in Culture Medium

	compound			
	1	4	7	8
$t_{1/2}$ (day)	13.9	7.4	7.7	5.5
metabolite observed	AZTMP	4a	AZTMP	AZTMP

rise to the formation of the corresponding phosphodiester **7a**. The resulting metabolites **1** and **7a** were then converted to the tyrosinyl phosphodiester **1a**, which was further hydrolyzed into AZTMP. These two ways of hydrolysis were also observed during the study of the *N*-α-acetyltyrosinamide phosphotriester **8** in total CEM-SS cell extracts (Scheme 6). Nevertheless, compared to its analogue **7**, the major decomposition pathway (>95%) for the *N*-α-acetyl derivative **8** corresponds to the removal of the *t*BuSATE chain and formation of phosphodiester **8a**. In this case, metabolite **1a** was not observed and **8a** was directly converted into AZTMP. In the culture medium, the decomposition patterns were similar for phosphotriesters **7** and **8**, the corresponding phosphodiesters could not be detected (shorter half-life and/or insufficient accumulation), and only the formation of AZTMP was observed.

Because the behavior of the tyrosinamide phosphotriesters (**7** and **8**) was puzzling, we attempted to determine which kind of enzymatic system could be involved in the hydrolysis of the amide bond. Examination of the literature[19-21] showed that several enzymes,

in the hydrolysis of the amide function to the corresponding acid and leads to phosphotriester **1** (as illustrated by the HPLC chromatogram and corresponding MS spectra, Figure 3), whereas the minor way corresponds to the loss of the *t*BuSATE chain, giving
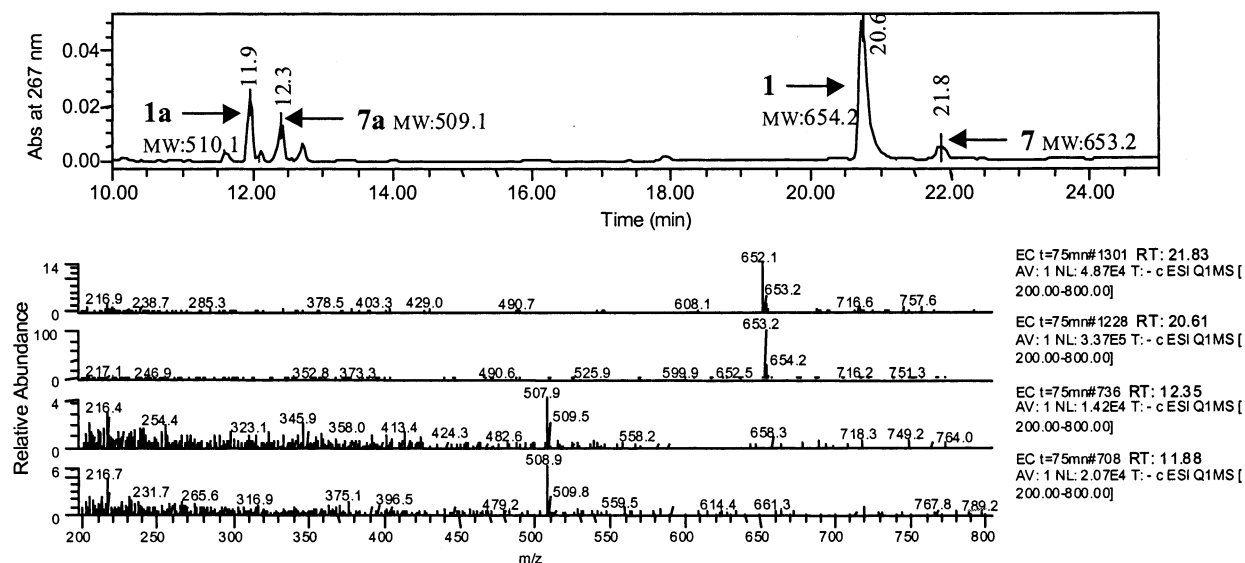
Figure 3. HPLC chromatogram and related ESI-MS spectra obtained after incubation of phosphotriester **7** (50 μM initial concentration) in CEM-SS cell extracts at 37 °C for 75 min.

Scheme 6. Proposed Decomposition Mechanism for Aryl Phosphotriester Derivatives **1**, **4**, **7**, and **8** in Total CEM-SS Cell Extracts
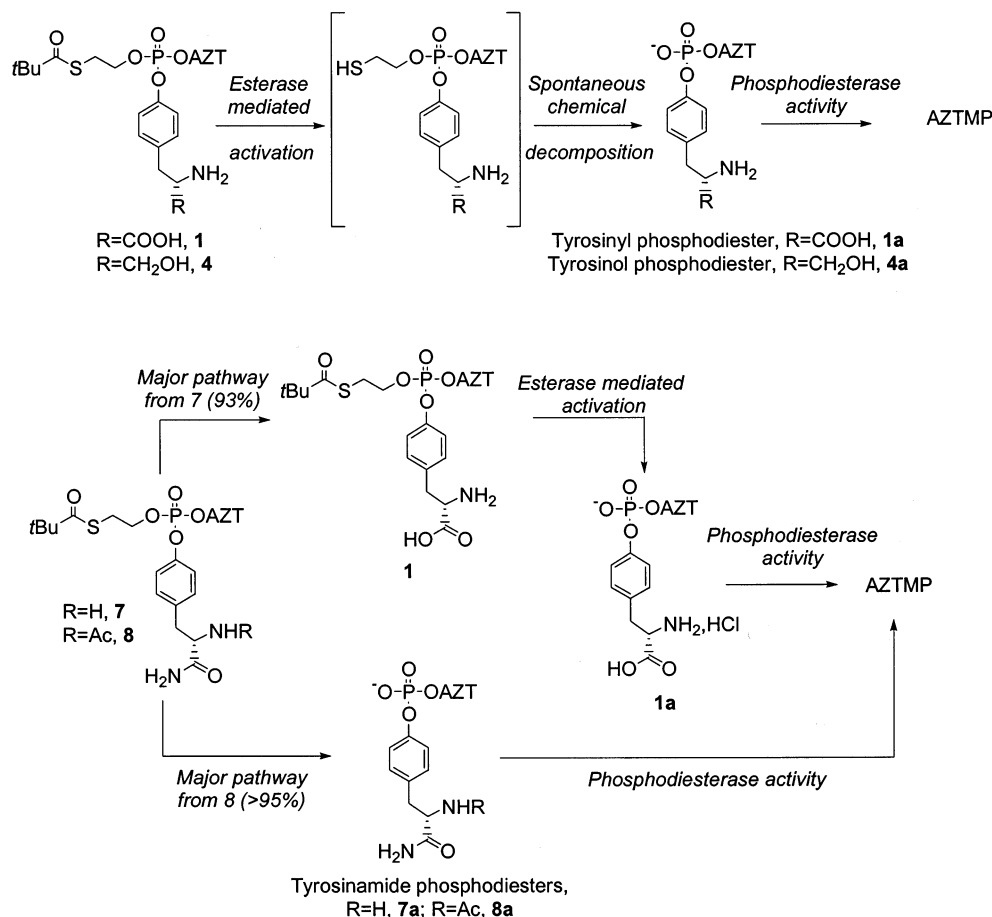
called amidases, from various organisms could be responsible for the hydrolysis of aliphatic and aromatic amides to the corresponding carboxylic acids and ammonia. However, in the amidase family, only the EC 3.5.1.4 from *Pseudomonas aeruginosa* was commercially available and catalyzed the hydrolysis of small-chain aliphatic amides. Indeed, this enzyme was not able to accept phosphotriester **7** as the substrate. We investi-

gated the behavior of **7** in the presence of various inhibitors of the amidase family such as metal ions (Hg^{2+} and Zn^{2+}) and phenylmethanesulfonyl fluoride (PMSF).[22-25] The enzyme activity responsible for the decomposition of **7** in **1** was completely inhibited by these metal ions and no degradation of **7** was observed after 3 h of incubation in cell extracts preincubated with 1 mM of either $HgCl_2$ or $ZnCl_2$, indicating that this

Table 4. Calculated Half-Lives of the Phosphodiester Derivatives **1a**, **4a**, and **8a** in Cell Extracts (CE) and Heat-Inactivated Cell Extracts

	compound		
	1a	**4a**	**8a**
CE (h)	12.8	68	19.3
CE inactivated[a]	stable	stable	stable
CE preincubated with EDTA[a]	stable	stable	6.9 days[b]

[a] Stable means no degradation observed after 5 days of incubation. [b] The half-life corresponds to the conversion of the amide function of **8a** in acid (putative amidase activity). The resulting *N*-α-acetyltyrosinyl phosphodiester is then stable.

activity was highly sensitive to such sulfhydryl reagents. However, only partial inhibition was observed with PMSF, a well-known serine protease inhibitor. In this case, the half-life of **7** was 2.5 h instead of 23 min.

The half-lives of the mononucleoside phosphotriesters and the resulting metabolites are summarized in Tables 2 and 3. In cell extracts, the rate of the *t*BuSATE moiety loss was similar for all phosphotriesters (between 2 and 3 h). However, comparison of the enzymatic stability of the aryl phosphodiesters **1a**, **4a**, and **8a** (Scheme 6) demonstrated that the nature of the functionality on the aryl residue (amino acid, amino alcohol or amino amide) of the phosphodiester derivatives seems to influence the hydrolysis rate of the phosphodiesterase-mediated step (Table 2). In this respect, we studied the behavior of **1a**, **4a**, and **8a** in cell extracts, either heat-inactivated in order to confirm that their conversion into AZTMP was not due to chemical process or preincubated with EDTA, which is known as an inhibitor of type I phosphodiesterase.[26] The corresponding kinetic data (Table 4) were in agreement with the fact that decomposition of the studied phosphodiesters was under enzymatic control, which is likely to be due to type I phosphodiesterase activity.

Conclusion

The present study demonstrates that aryl (*t*BuSATE) phosphotriester derivatives of AZT **2–5** are able to allow the efficient intracellular delivery of the parent 5′-mononucleotide and can be considered as 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 that could be envisaged on the aryl moiety opens the way to the search of antiviral mononucleotide prodrugs with an adequate balance among aqueous solubility, lipophilicity, and enzymatic stability in order to envisage further in vivo pharmacological studies. In this respect, the structural features of the aryl phosphotriester **4**, **7**, and **8** appear as promising phosphate protecting groups in view of anti-HIV activity and physicochemical properties of the resulting pronucleotides. Further work on this topic is currently in progress.

Experimental Section

General Methods. Unless otherwise stated, material and methods used were as previously described.[27] Analytical HPLC studies were carried out on an Alliance 2690 system (Waters, Milford, MA) equipped with a model 996 photodiode detector and a Millenium data workstation. A reverse-phase analytical column (Nucleosil, C_{18}, 150 mm × 4.6 mm, 5 μm), equipped with a prefilter, and a precolumn (Nucleosil, C_{18}, 5 μm) were used. Detection was monitored at 267 nm. Nucleotidic derivatives were eluted using a linear gradient of 0–80% acetonitrile in 20 mM triethylammonium acetate buffer (pH 6.9) over 30 min at 1 mL/min flow rate. For amino acids, eluents used contained 0.1% aqueous TFA, and the compounds were eluted with a linear gradient of 0–90% acetonitrile over 30 min at 1 mL/min flow rate. Electrospray ionization mass spectrometry (ESI-MS) was performed using a SSQ 7000 single quadrupole mass spectrometer (Finnigan, San Jose, CA) in the negative-ion mode with a spray voltage at − 4.5 kV. The capillary temperature was maintained at 250 °C. Nitrogen served both as sheath gas (operating pressure of 80 psi) and as auxiliary gas with a flow rate of 15 units. Under these conditions, full-scan data acquisition was performed from *m*/*z* 200 to 800 in centroid mode and using a cycle time of 1.0 s. AZT was from Brantford Chemicals Inc., bis(*N*,*N*-diisopropylamino)chlorophosphine was purchased from Aldrich, *N*-α-*tert*-butoxycarbonyl-L-tyrosine methyl ester, and *N*-α-acetyl-L-tyrosinamide were from Novabiochem. *S*-(2-Hydroxyethyl) thiopivaloate[14] and *O*-(*S*-pivaloyl-2-thioethyl) *N*,*N*,*N*,*N*-tetraisopropylphosphorodiamidite[11] were synthesized following previously published procedures.

Biological Methods. The origin of the viruses and the techniques used for measuring inhibition of virus multiplication have previously been described.[14]

Stability Studies. HPLC analyses were carried out using an improved "on-line ISRP cleaning method".[14] Briefly, the crude sample (80 μL, initial concentration 50 μM) was injected onto the precolumn, and the eluent was 20 mM triethylammonium acetate buffer (pH 6.9, 5 min). Elution of the analytes was done using a linear gradient of 0% to 80% acetonitrile over 30 min at 1 mL/min flow rate. The decomposition products were identified by HPLC−MS after calibration and/or by co-injection with authentic samples (AZTMP, AZT, phosphotriester **1**, phosphodiesters **1a**, **4a**, and **8a**). For each incubation time, the calculation of the relative concentration of each species was related to the peak areas. These data were considered as the experimental data. The rate constants for disappearance and the half-lives (kinetic data) of the phosphotriester and phosphodiester derivatives were calculated according to pseudo-first-order kinetic models and optimized using mono- or polyexponential regressions.

Total CEM-SS Cell Extracts. CEM-SS cell extracts were prepared, and stability studies were performed according to published procedures.[14] When needed, CEM-SS cell extracts were inactivated 24 h at 56 °C or preincubated for 10 min with 4 mM EDTA and 30 min with 1 mM $HgCl_2$, $ZnCl_2$, or PMSF.

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] Determinations were performed in triplicate for each compound. For the HPLC conditions, see the General Methods.

***N*-(*tert*-Butoxycarbonyl)-L-tyrosin-ol, 9.** Boc-Tyr-OMe (1.47 g, 5 mmol) was dissolved in dry THF (15 mL), and lithium chloride (0.85 g, 4 equiv) and sodium borohydride (0.76 g, 4 equiv) were added. The mixture was refluxed for 72 h, the solution was cooled to room temperature, and methanol (5 mL) was added. After the mixture was stirred for 12 h at room temperature, an aqueous acetic acid solution (10%, v/v) was added to lower pH (∼pH 5). The mixture was diluted with ethyl acetate (100 mL), and the resulting solution was washed with water (50 mL). The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and filtered, and the solvent was removed in vacuum. Purification of the residue by column chromatography on silica gel, eluting with a stepwise gradient of MeOH in CH_2Cl_2 (2:98), afforded compound **9** as a white foam that was recrystallized from chloroform, 73% yield. R_f = 0.23 (MeOH/CH_2Cl_2 5:95); mp 120 °C, lit. 112−114 °C;[29] [α]$^{20}_D$ −26° (*c* 1.0, EtOH), lit. −23° (*c* 1.2, EtOH);[7] ^{1}H NMR (CDCl₃, 200 MHz) δ 7.10−6.90 (m, 3H, OH$_{ech}$, *J* = 8.4, H$_\delta$), 6.72 (d, 2H, *J* = 8.4,

H$_\epsilon$), 5.11 (d, 1H, J = 8.4, NH$_{ech}$), 3.76−3.42 (m, 4H, CH$_2$OH, H$_\alpha$), 2.69 (d, 2H, J = 6.5, H$_\beta$), 1.39 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (CDCl$_3$) δ 155.5, 153.7 (C$_q$, CON), 129.3 (C$_\gamma$), 128.1 (C$_\delta$), 114.5 (C$_\epsilon$), 79.0 (C(CH$_3$)$_3$), 63.3 (C−OH), 52.9 (C$_\alpha$), 35.6 (C$_\beta$), 28.3 (C(CH$_3$)$_3$); MS FAB$^+$ m/z 535 (2M + H)$^+$, 268 (M + H)$^+$, 212 (M − tBu + H)$^+$; MS FAB$^-$ m/z 533 (2M − H)$^-$, 266 (M − H)$^-$; HPLC t_R 15.3 min; UV (EtOH) λ_{max} 225 nm (ϵ 7370). Anal. (C$_{14}$H$_{21}$NO$_4$) C, H, N.

N-($tert$-Butoxycarbonyl)-2,2-dimethyl-4-(4-hydroxyphenyl)-1,3-oxazolidine, 10. N-α-$tert$-Butoxycarbonyl-L-tyrosinol (0.78 g, 2.9 mmol) was dissolved in dry acetone (20 mL), and 2,2′-dimethoxypropane (0.725 mL, 2 equiv) and p-toluene-sulfonic acid (0.055 g, 0.1 equiv) were added. The mixture was refluxed for 4 h. The solution was concentrated, and the residue was dissolved in dichloromethane (25 mL). The organic layer was washed with NaHCO$_3$ solution (10% w/v, 20 mL). The aqueous layer was extracted three times with CH$_2$Cl$_2$, the combined organic layers were dried over Na$_2$SO$_4$ and filtered, and the solvent was removed in vacuum. Purification of the residue by column chromatography on silica gel, eluting with a stepwise gradient of MeOH (0−1%) in CH$_2$Cl$_2$, afforded compound **10** (0.70 g) as a white foam, 78% yield. R_f = 0.51 (MeOH/CH$_2$Cl$_2$ 5:95); mp 107 °C, lit. 106−107 °C [7]; [α]$^{20}_D$ −32.6° (c 0.98, EtOH), lit. −28° (c 1.28, HCCl$_3$).[8] Because of the presence of conformer mixture, ^{1}H NMR spectra were processed at two different temperatures (20 and 55 °C) and compared. ^{1}H NMR (CDCl$_3$, 200 MHz, 20 °C) δ 7.00−6.95 (2d, 2H, J = 8.2, H$_\delta$), 6.75−6.67 (2d, 2H, J = 8.1, H$_\epsilon$), 4.01−3.97, 3.88−3.85 (2m, 2 × 0.5H conformers, H$_\alpha$), 3.70−3.69 (2s, 2H, CH$_2$O), 3.20−2.98 (dd, 1H, J = 13.0, 10.8, H$_{\beta a}$), 2.71−2.53 (dd, 1H, J = 13.0, 10.6, H$_{\beta b}$), 1.47, 1.41 (2d, 6H, J = 11.2, C(CH$_3$)$_2$), 1.45 (s, 9H, C(CH$_3$)$_3$); ^{1}H NMR (CDCl$_3$, 200 MHz, 55 °C) δ 7.11−7.00 (d, 2H, J = 8.2, H$_\delta$), 6.82−6.70 (d, 2H, J = 8.4, H$_\epsilon$), 4.11−3.97 (br s, 1H, H$_\alpha$), 3.85, 3.72 (2s, 2H, CH$_2$O), 3.06 (d, 1H, J = 12.4, H$_{\beta a}$), 2.70, 2.55 (2d, 1H, J = 13.2, 10.2, H$_{\beta b}$), 1.57, 1.49 (2s, 6H, C(CH$_3$)$_2$), 1.53 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (CDCl$_3$) δ 155.1, 152.5 (C$_q$, CON), 130.9 (C$_\gamma$), 130.4 (C$_\delta$), 115.9 (C$_\epsilon$), 94.3 (C(CH$_3$)$_2$), 80.8 (C(CH$_3$)$_3$), 66.3 (CH$_2$O), 59.7 (C$_\alpha$), 38.5 (C$_\beta$), 28.9 (C(CH$_3$)$_3$), 27.6, 24.3 (C(CH$_3$)$_2$); MS FAB$^+$ m/z 922 (3M + H)$^+$, 615 (2M + H)$^+$, 308 (M + H)$^+$, 252 (M − tBu + 2H)$^+$; MS FAB$^-$ m/z 920 (3M − H)$^-$, 613 (2M − H)$^-$, 306 (M − H)$^-$, 250 (M − tBu)$^-$; HPLC t_R 22.7 min; UV (EtOH) λ_{max} 226 nm (ϵ 8660). Anal. (C$_{17}$H$_{25}$NO$_4$) C, H, N.

N-α-(Acetyl)-O-($tert$butyl(dimethyl)silyl)-L-tyrosinamide, 11. $tert$-Butyl(dimethyl)silyl chloride (0.88 g, 1.2 equiv) was added to a solution of N-α-(acetyl)-L-tyrosinamide (1 g, 4.5 mmol) and imidazole (0.74 g, 2.4 equiv) in dry pyridine (10 mL). After 4 h of stirring, the reaction mixture was diluted with ethyl acetate and poured into a saturated NaHCO$_3$ aqueous solution. The aqueous layer was extracted with ethyl acetate, and the combined extracts afforded after evaporation a white solid that was recrystallized from hexanes/ethyl acetate (1:9) to afford 1.40 g (93%) of white crystals. R_f = 0.45 (MeOH/CH$_2$Cl$_2$ 10:90); mp 173 °C; [α]$^{20}_D$ +6.7 (c 1.05, EtOH); ^{1}H NMR (DMSO-d_6) δ 7.82 (d, 1H, J = 8.5, NH$_{amino}$), 7.24 (s, 1H, NH$_{amido}$), 6.94 (d, 2H, J = 8.5, H$_\delta$), 6.85 (s, 1H, NH$_{amido}$), 6.56 (d, 2H, J = 6.7, H$_\epsilon$), 4.24−4.17 (m, 1H, H$_\alpha$), 2.78−2.73 (dd, 1H, J = 13.8 and 4.7, H$_{\beta a}$), 2.52−2.45 (dd, 1H, J = 13.8 and 9.7, H$_{\beta b}$), 1.59 (s, 3H, CH$_{3acetyl}$), 0.77 (s, 9H, SiC(CH$_3$)$_3$), 0.00 (s, 6H, Si(CH$_3$)$_2$); ^{13}C NMR (DMSO-d_6) δ 174.2 (CON$_{amido}$), 169.8 (COCH$_3$), 154.3 (C$_q$), 131.9 (C$_\gamma$), 131.0 (C$_\epsilon$), 120.2 (C$_\delta$), 54.8 (C$_\alpha$), 37.8 (C$_\beta$), 26.4 (C(CH$_3$)$_3$), 23.3 (CH$_3$CO), 18.8 (SiC(CH$_3$)$_3$), −3.7 (Si(CH$_3$)$_2$); MS FAB$^+$ m/z 673 (2M + H)$^+$, 337 (M + H)$^+$; MS FAB$^-$ m/z 671 (2M − H)$^-$, 335 (M − H)$^-$; HPLC t_R 15.3 min. Anal. (C$_{17}$H$_{28}$N$_2$O$_3$Si) C, H, N.

N-α-(Acetyl-$tert$butoxycarbonyl)-O-($tert$-butyl(dimethyl)silyl)-N,N-di($tert$-butoxycarbonyl)-L-tyrosinamide, 12. To a solution of **11** (1.34 g, 4 mmol) in dichloromethane (25 mL) at room temperature was added (iPr)$_2$NEt (4.1 mL, 6 equiv), DMAP (0.122 g, 0.25 equiv), and di($tert$-butyl) dicarbonate (3.45 mL, 3.75 equiv). After overnight stirring, the reaction mixture was diluted with dichloromethane and washed with 1 M aqueous KHSO$_4$ and 1 M aqueous NaHCO$_3$. The organic layer was dried over Na$_2$SO$_4$ and concentrated,

and the oily residue was purified by column chromatography on silica gel, eluting with pentane/Et$_2$O (0−10%). An amount of 1.33 g (52%) of an oil was obtained. R_f = 0.56 (Et$_2$O/pentane 20:80); [α]$^{20}_D$ +1.9 (c 1.1, EtOH); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.04 (d, 2H, J = 8.4, H$_\delta$), 6.78 (d, 2H, J = 8.4, H$_\epsilon$), 5.58−5.49 (m, 1H, H$_\alpha$), 3.35−3.21 (m, 1H, partly covered by HDO peak, H$_{\beta a}$), 2.98−2.83 (dd, 1H, J = 13.8 and 8.4, H$_{\beta b}$), 2.24 (s, 3H, CH$_{3acetyl}$), 1.45 (s, 18H, 2C(CH$_3$)$_{3Boc}$), 1.40 (s, 9H, C(CH$_3$)$_{3Boc}$), 0.94 (s, 9H, SiC(CH$_3$)$_3$), 0.17 (s, 6H, Si(CH$_3$)$_2$); ^{13}C NMR (DMSO-d_6) δ 172.6 (CON$_{amido}$), 171.0 (COCH$_3$), 154.5 (C$_q$), 152.4, 150.0 (CON$_{Boc}$), 131.5 (C$_\gamma$), 131.1 (C$_\epsilon$), 120.3 (C$_\delta$), 85.6, 84.5 (C(CH$_3$)$_{3Boc}$), 59.7 (C$_\alpha$), 34.8 (C$_\beta$), 27.9 (2s, C(CH$_3$)$_{3Boc}$), 26.9 (CH$_3$CO), 26.4 (C(CH$_3$)$_3$), 18.8 (SiC(CH$_3$)$_3$), −3.7 (Si(CH$_3$)); MS FAB$^+$ m/z 659 (M + Na)$^+$, 637 (M + H)$^+$, 537 (M − Boc + 2H)$^+$; MS FAB$^-$ m/z 1170 (2M − Boc − H)$^-$, 535 (M − Boc)$^-$. Anal. (C$_{32}$H$_{52}$N$_2$O$_9$Si) C, H, N.

N-α-(Acetyl-$tert$-butoxycarbonyl)-N,N-di($tert$-butoxycarbonyl)-L-tyrosinamide, 13. TBAF (1.1 M THF solution, 1.9 mL, 1.1 equiv) was added dropwise to a solution of **12** (1.25 g, 1.9 mmol) in anhydrous THF (25 mL) at 0 °C. After 30 min of stirring, the reaction mixture was diluted with ethyl acetate and washed with saturated aqueous NaCl solution and water. The organic layer was dried over Na$_2$SO$_4$ and concentrated, and the oily residue was purified by flash column chromatography on silica gel, eluting with pentane/Et$_2$O (10−30%). An amount of 0.78 g (79%) of an oil was obtained. R_f = 0.59 (Et$_2$O); [α]$^{20}_D$ − 105 (c 1, EtOH); ^{1}H NMR (CDCl$_3$, 200 MHz) δ 7.00 (d, 2H, J = 8.4, H$_\delta$), 6.6 (d, 2H, J = 8.4, H$_\epsilon$), 5.65−5.61 (m, 1H, H$_\alpha$), 3.37−3.21 (dd, 1H, J = 13.9 and 6.3, H$_{\beta a}$), 3.01−2.95 (dd, 1H, J = 13.9 and 8.1, H$_{\beta b}$), 2.29 (s, 3H, CH$_{3acetyl}$), 1.42 (s, 18H, 2C(CH$_3$)$_{3Boc}$), 1.39 (s, 9H, C(CH$_3$)$_{3Boc}$); ^{13}C NMR (CDCl$_3$) δ 173.2 (CON$_{amido}$), 171.3 (COCH$_3$), 155.2 (C$_q$), 152.6, 150.0 (CON$_{Boc}$), 131.3 (C$_\gamma$), 129.6 (C$_\epsilon$), 115.6 (C$_\delta$), 85.2, 84.8 (C(CH$_3$)$_{3Boc}$), 60.2 (C$_\alpha$), 35.2 (C$_\beta$), 28.0, 27.9 (2s, C(CH$_3$)$_{3Boc}$), 26.8 (CH$_3$CO); MS FAB$^+$ m/z 523 (M + H)$^+$; MS FAB$^-$ m/z 1043 (2M − H)$^-$, 521 (M − H)$^-$, 321 (M − 2Boc + H)$^-$; HPLC t_R 29.2 min. Anal. (C$_{26}$H$_{38}$N$_2$O$_9$) C, H, N.

N-α-$tert$-Butoxycarbonyl-L-tyrosinamide, 14. Boc-Tyr-OMe (0.88 g, 3 mmol) was dissolved in methanolic ammonia (10 mL), and the mixture was stirred for 60 h at 5 °C. The solvents were removed in vacuum. Purification of the residue by crystallization from ethyl acetate afforded the title compound **14** (0.75 g) as white solid in 90% yield. R_f = 0.41 (MeOH/CH$_2$Cl$_2$ 1:9); mp 160.5−161.0 °C; [α]$^{20}_D$ +1.8 (c 1.0, EtOH); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 9.19 (s, 1H, NH$_{ech}$), 7.33 (s, 1H, CONH$_{2a}$), 7.06−7.01 (m, 3H, OH$_{ech}$, H$_\delta$), 6.99−6.62 (m, 3H, CONH$_{2b}$, H$_\epsilon$), 4.06−3.96 (m, 1H, H$_\alpha$), 2.88−2.78 (m, 1H, H$_{\beta a}$), 2.67−2.54 (m, 1H, H$_{\beta b}$), 1.31 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (DMSO-d_6) δ 174.9, 174.8 (2s, CON), 156.3, 156.1 (C$_q$), 130.9 (C$_\gamma$), 129.1 (C$_\delta$), 115.7, 115.5 (C$_\epsilon$), 79.0 (C(CH$_3$)$_3$), 56.6 (C$_\alpha$), 37.5 (C$_\beta$), 28.9 (C(CH$_3$)$_3$); MS FAB$^+$ m/z 561 (2M + H)$^+$, 281 (M + H)$^+$, 225 (M − tBu + H)$^+$; MS FAB$^-$ m/z 559 (2M − H)$^-$, 279 (M − H)$^-$; HPLC t_R 13.1 min. Anal. (C$_{14}$H$_{20}$N$_2$O$_4$) C, H, N.

N-α-($tert$-Butoxycarbonyl)-O-($tert$-butyl(dimethyl)silyl)-L-tyrosinamide, 15. $tert$-Butyl(dimethyl)silyl chloride (0.45 g, 1.1 equiv) was added to a solution of **14** (0.75 g, 2.7 mmol) and imidazole (0.40 g, 2.2 equiv) in dry pyridine (10 mL). After 4 h of stirring, the reaction mixture was diluted with ethyl acetate and poured into a saturated NaHCO$_3$ aqueous solution. The aqueous layer was extracted with ethyl acetate, and the combined organic layers gave a crude product purified by column chromatography using pentane/Et$_2$O (10−60%). Resulting fractions afforded after evaporation a white solid that was recrystallized in hexanes/ethyl acetate (1:9) to afford 0.74 g (70%) of white crystals. R_f = 0.29 (Et$_2$O); mp 94.1 °C; [α]$^{20}_D$ +1.3 (c 1.1, EtOH); ^{1}H NMR (DMSO-d_6) δ 7.17 (s, 1H, NH$_{amino}$), 6.96 (d, 2H, J = 8.4, H$_\delta$), 6.84 (s, 1H, NH$_{amido}$), 6.62−6.56 (m, 3H, NH$_{amido}$, H$_\epsilon$), 3.91−3.85 (m, 1H, H$_\alpha$), 2.74−2.69 (dd, 1H, J = 13.8 and 4.0, H$_{\beta a}$), 2.51−2.44 (dd, 1H, J = 13.6 and 9.7, H$_{\beta b}$), 1.14 (s, 9H, (CH$_3$)$_{3Boc}$), 0.78 (s, 9H, SiC(CH$_3$)$_3$), 0.00 (s, 6H, Si(CH$_3$)$_2$); ^{13}C NMR (DMSO-d_6) δ 174.6 (CON$_{amido}$), 156.0 (CON$_{Boc}$), 154.3 (C$_q$), 132.0 (C$_\gamma$), 131.1 (C$_\epsilon$), 120.2 (C$_\delta$), 115.6 (C$_\epsilon$), 78.6 (C(CH$_3$)$_{3Boc}$), 56.5 (C$_\alpha$), 37.7 (C$_\beta$), 29.0 (C(CH$_3$)$_{3Boc}$), 26.4 (SiC(CH$_3$)$_3$), 18.8 (SiC(CH$_3$)$_3$), −3.7 (Si(CH$_3$)$_2$); MS FAB$^+$ m/z

789 (2M + H)$^+$, 417 (M + Na)$^+$, 395 (M + H)$^+$, 339 (M − tBu + 2H)$^+$; MS FAB$^-$ m/z 787 (2M − H)$^-$, 393 (M − H)$^-$; HPLC t_R 28.4 min. Anal. (C$_{20}$H$_{34}$N$_2$O$_4$Si) C, H, N.

N-α-(*tert*-Butoxycarbonyl)-N,N-di(*tert*-butoxycarbonyl)-O-(*tert*-butyl(dimethyl)silyl)-L-tyrosinamide, 16. To a solution of **15** (0.83 g, 2.1 mmol) in dichloromethane (20 mL) at room temperature was added (iPr)$_2$NEt (1.45 mL, 4 equiv), DMAP (0.044 g, 0.17 equiv), and di(*tert*-butyl) dicarbonate (1.21 mL, 2.5 equiv). After overnight stirring, the reaction mixture was diluted with dichloromethane and washed with 1 M aqueous KHSO$_4$ and 1 M aqueous NaHCO$_3$. The organic layer was dried over Na$_2$SO$_4$ and concentrated, and the oily residue was purified by flash column chromatography on silica gel, eluting with pentane/Et$_2$O (0−10%). An amount of 1.09 g (85%) of an oil was obtained. R_f = 0.67 (Et$_2$O); [α]^{20}$_D$ + 3.4 (*c* 1.2, EtOH); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.34−7.13 (m, 3H, NH, H$_δ$), 6.76 (d, 2H, J = 8.4, H$_ε$), 5.11 (m, 1H, H$_α$), 2.83−2.59 (m, 2H, partly covered by DMSO peak, H$_β$), 1.49 (s, 18H, (CH$_3$)$_{3Boc-amido}$), 1.31 (s, 9H, C(CH$_3$)$_{3Boc-amino}$), 0.94 (s, 9H, SiC-(CH$_3$)$_3$), 0.17 (s, 6H, Si(CH$_3$)$_2$); ^{13}C NMR (DMSO-d_6) δ 174.6 (CON), 156.2, 154.6 (C$_q$), 149.6 (CON$_{Boc}$), 131.3 (C$_γ$), 131.1 (C$_ε$), 120.3 (C$_δ$), 85.9, 80.7 (*C*(CH$_3$)$_{3Boc}$), 56.5 (C$_α$), 36.3 (C$_β$), 28.9 (s, C(CH$_3$)$_{3Boc}$), 28.0, 26.4 (C(CH$_3$)$_3$), 18.8 (SiC(CH$_3$)$_3$), −3.7 (Si-(CH$_3$)); MS FAB$^+$ m/z 617 (M + Na)$^+$, 595 (M + H)$^+$, 517 (M − Boc + Na + H)$^+$; MS FAB$^-$ m/z 593 (M − H)$^-$, 493 (M − Boc)$^-$. Anal. (C$_{30}$H$_{50}$N$_2$O$_8$Si) C, H, N.

N-α-(*tert*-Butoxycarbonyl)-N,N-di(*tert*-butoxycarbonyl)-L-tyrosinamide, 17. TBAF (1 M THF solution, 0.55 mL, 1.1 equiv) was added dropwise to a solution of **16** (0.3 g, 0.5 mmol) in anhydrous THF (5 mL) at 0 °C. After 30 min of stirring, the reaction mixture was diluted with ethyl acetate and washed with saturated aqueous NaCl solution and water. The organic layer was dried over Na$_2$SO$_4$ and concentrated, and the oily residue was purified by column chromatography on silica gel, eluting with Et$_2$O/pentane (10−30%). An amount of 0.24 g (91%) of an oil were obtained. R_f = 0.61 (Et$_2$O); [α]^{20}$_D$ − 9 (*c* 1.1, EtOH); ^{1}H NMR (CDCl$_3$) δ 7.61 (s, 1H, OH), 6.94 (d, 2H, J = 8.4, H$_δ$), 6.69 (d, 2H, J = 8.4, H$_ε$), 6.39 (bs, 1H, NH), 4.94−4.91 (m, 1H, H$_α$), 3.34−3.29 (dd, 1H, J = 14.2 and 5.2, H$_β$), 3.03−2.97 (dd, 1H, J = 14.3 and 9.8, H$_β$), 1.41 (s, 9H, C(CH$_3$)$_{3Boc}$), 1.34 (s, 18H, 2C(CH$_3$)$_{3Boc}$); ^{13}C NMR (CDCl$_3$) δ 168.9 (CON$_{amido}$), 155.6 (C$_q$), 151.9, 149.8 (CON$_{Boc}$), 131.0 (C$_γ$), 128.8 (C$_ε$), 115.9 (C$_δ$), 84.8, 83.3 (*C*(CH$_3$)$_{3Boc}$), 62.2 (C$_α$), 34.8 (C$_β$), 28.4, 28.2 (2s, C(CH$_3$)$_{3Boc}$); MS FAB$^+$ m/z 961 (2M + H)$^+$, 861 (2M − Boc + 2H)$^+$, 481 (M + H)$^+$; MS FAB$^-$ m/z 959 (2M − H)$^-$, 479 (M − H)$^-$, 379 (M − Boc)$^-$. Anal. (C$_{24}$H$_{36}$N$_2$O$_8$) H, N. C: calcd 59.98; found 59.30.

General Procedure for the Preparation of Phosphoramidites, 18a−c. To a solution of the appropriate tyrosinyl precursor **10**, **13**, or **17** (0.50 mmol) in dry CH$_3$CN (5 mL) containing 3 Å molecular sieves (0.5 g) was added at 0 °C tBuSATE phosphorodiamidite (0.29 g, 0.75 mmol, 1.5 equiv), diisopropylamine (0.14 mL, 1.00 mmol, 2 equiv), and 1H-tetrazole (0.07 g, 1.00 mmol, 2 equiv). After being stirred for 2 h at room temperature, the reaction mixture was diluted with acid-free EtOAc, washed with brine and water, dried with Na$_2$SO$_4$, filtered, and concentrated in vacuum. 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-Boc-2,2′-dimethyl-4-(4-hydroxyphenyl)-1,3-oxazolidine]-O-(S-pivaloyl-2-thioethyl)-N,N-diisopropyl Phosphoramidite, 18a. Yield, 78%; R_f = 0.64 (Et$_3$N/EtOAc/cyclohexane 1:1:8); ^{1}H NMR (CDCl$_3$) δ 7.07−6.88 (m, 4H, H$_δ$, H$_ε$), 4.02, 3.85 (2m, 2 × 0.5H, two conformers, H$_α$), 3.80−3.59 (m, 6H, 2 × N−CH, P−OCH$_2$, CH$_2$O$_{Tyr}$), 3.09−2.94 (m, 3H, CH_2S, H$_{βa}$), 2.55−2.49 (m, 1H, H$_{βb}$), 1.65, 1.64 (2s, 3H, C(CH_3)$_2$), 1.56−1.38 (m, 12H, C(CH_3)$_2$, C(CH$_3$)$_3$), 1.19−1.09 (m, 21H, C(CH$_3$)$_{3SATE}$, N−CH(CH_3)$_2$); ^{13}C NMR (CDCl$_3$) δ 206.8 (COS), 153.7, 152.6, 152.2 (CON, C$_q$), 130.7,130.5 (C$_γ$, C$_δ$), 120.5 (C$_ε$), 94.4, 93.9 (*C*(CH$_3$)$_2$), 80.5, 80.1 (*C*(CH$_3$)$_{3Boc}$), 66.4, 66.2 (*C*H$_2$−O$_{Tyr}$), 63.0 (d, $J_{P−C}$ = 17.7, POCH$_2$), 59.7 (C$_α$), 46.8 (*C*(CH$_3$)$_{3SATE}$), 43.9 (d, $J_{P−C}$ = 12.8, N*C*H(CH$_3$)$_2$), 39.3, 38.2 (C$_β$),

29.0 (d, $J_{P−C}$ = 10.4, *C*H$_2$S), 27.8, 27.3 (C(CH$_3$)$_2$, 2 × C(CH$_3$)$_3$), 24.9 (d, $J_{P−C}$ = 6.8, NCH(CH$_3$)$_2$), 24.8 (d, $J_{P−C}$ = 7.5, NCH-(CH$_3$)$_2$); ^{31}P NMR (CDCl$_3$) δ 147.0, 146.9, 146.8; MS FAB$^+$ m/z 599 (M + H)$^+$, 499 (M − Boc + 2H)$^+$; MS FAB$^-$ m/z 597 (M − H)$^-$, 513 (M − tBu)$^-$, 497 (M − Boc)$^-$, 413 (M − tBu − Boc)$^-$. Anal. (C$_{30}$H$_{51}$N$_2$O$_6$PS) C, H, N.

O-[N-(Acetyl-Boc)-L-tyrosinyl-N,N-di-Boc-amide]-O-(S-pivaloyl-2-thioethyl)-N,N-diisopropyl Phosphoramidite, 18b. Yield, 65%; R_f = 0.45 (Et$_3$N/EtOAc/cyclohexane 1:1:8); ^{1}H NMR (CDCl$_3$) δ 7.10−6.96 (m, 4H, H$_δ$, H$_ε$), 5.78−5.64 (m, 1H, H$_α$), 3.95−3.53 (m, 4H, 2 × N−CH, P−OCH$_2$), 3.50−3.37 (m, 1H, H$_{βb}$), 3.16−3.02 (m, 3H, CH_2S, H$_{βa}$), 2.37 (s, 3H, CH$_{3acetyl}$), 1.74−1.44 (m, 27H, C(CH$_3$)$_{3Boc}$), 1.28−1.16 (m, 21H, −C(CH$_3$)$_{3SATE}$, N−CH(CH_3)$_2$); ^{13}C NMR (CDCl$_3$) δ 206.7 (COS), 172.6, 171.2 (CON), 153.7 (C$_q$), 152.6, 150.0 (CON$_{Boc}$), 131.8 (C$_γ$), 131.1 (C$_ε$), 120.1 (C$_δ$), 84.9 (*C*(CH$_3$)$_{3Boc}$), 63.0, 62.9 (d, $J_{P−C}$ = 17.6, POCH$_2$), 60.0 (C$_α$), 46.8 (*C*(CH$_3$)$_{3SATE}$), 43.9, 43.8 (d, $J_{P−C}$ = 13.0, NCH(CH$_3$)$_2$), 35.2 (C$_β$), 30.4, 30.3 (d, $J_{P−C}$ = 7.4, *C*H$_2$S), 28.0−27.8 (C(CH$_3$)$_{3Boc}$), 27.3 (C(CH$_3$)$_{3SATE}$), 26.8 (CH$_{3acetyl}$), 25.0 (d, $J_{P−C}$ = 6.7, NCH(CH$_3$)$_2$), 24.8 (d, $J_{P−C}$ = 7.7, NCH-(CH$_3$)$_2$); ^{31}P NMR (CDCl$_3$) δ 146.9, 146.8; MS FAB$^+$ m/z 996 (M + H)$^+$; MS FAB$^-$ m/z 994 (M − H)$^-$. Anal. (C$_{39}$H$_{64}$N$_3$O$_{11}$-PS, 20% oxidized form) C, H, S.

O-[N-(Boc)-L-tyrosinyl N,N-di-Boc-amide]-O-(S-pivaloyl-2-thioethyl)-N,N-diisopropyl Phosphoramidite, 18c. Yield, 60%; R_f = 0.47 (Et$_3$N/EtOAc/cyclohexane 1:1:8); ^{1}H NMR (CDCl$_3$) δ 7.63 (s, 1H, NH), 7.10−6.94 (m, 4H, H$_δ$, H$_ε$), 5.05−4.98 (m, 1H, H$_α$), 3.83−3.66 (m, 4H, 2 × NCH, P−OCH$_2$), 3.47−3.38 (m, 1H, H$_{βa}$), 3.16−3.10 (m, 3H, CH_2S, H$_{βb}$), 1.51 (s, 9H, C(CH$_3$)$_{3Boc}$), 1.44, 1.43 (2s, 18H, C(CH$_3$)$_{3Boc}$), 1.29−1.16 (m, 21H, −C(CH$_3$)$_{3SATE}$, N−CH(CH_3)$_2$); ^{13}C NMR (CDCl$_3$) δ 206.7 (COS), 168.6 (CON$_{amido}$), 153.9 (C$_q$), 151.9, 149.7 (CON$_{Boc}$), 131.3, 130.9 (C$_γ$, C$_δ$), 120.3 (C$_ε$), 84.5, 83.1 (*C*(CH$_3$)$_{3Boc}$), 62.9 (d, $J_{P−C}$ = 17.5, POCH$_2$), 62.1 (C$_α$), 46.9 (*C*(CH$_3$)$_{3SATE}$), 43.9 (d, $J_{P−C}$ = 12.9, N*C*H(CH$_3$)$_2$), 34.9 (C$_β$), 30.3 (d, $J_{P−C}$ = 7.2, *C*H$_2$S), 28.4−27.8 (C(CH$_3$)$_{3Boc}$), 27.3 9 (C(CH$_3$)$_{3SATE}$), 25.0 (d, $J_{P−C}$ = 6.8, NCH-(CH$_3$)$_2$), 24.9 (d, $J_{P−C}$ = 7.6, NCH(CH$_3$)$_2$); ^{31}P NMR (CDCl$_3$) δ 146.9, 146.8; MS FAB$^+$ m/z 772 (M + H)$^+$, 686 (M − tBuCO + 2H)$^+$; MS FAB$^-$ m/z 802 (M sulfurated − H)$^-$, 770 (M − H)$^-$. Anal. (C$_{37}$H$_{62}$N$_3$O$_{10}$PS) C, H. N: calcd 5.44; found 5.96.

General Procedure for the Preparation of Phosphotriesters 2, 5, and 6. To a solution of AZT (0.093 g, 0.35 mmol) in dry CH$_3$CN (3 mL) containing 3 Å molecular sieves (0.5 g) was added 1H-tetrazole (0.098 g, 1.40 mmol, 4 equiv) and dropwise a solution of the appropriate phosphoramidite **18a**, **18b**, or **18c** (0.42 mmol, 1.2 equiv) in dry CH$_3$CN (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 was further stirred for 1 h. The mixture was diluted with CH$_2$Cl$_2$ and washed successively with aqueous Na$_2$S$_2$O$_3$ (10%, w/v) and water, and the organic layer was dried over Na$_2$SO$_4$, filtered, and evaporated to dryness under reduced pressure.

O-[N-Boc-2,2′-dimethyl-4-(4-hydroxyphenyl)-1,3-oxazolidine]-O-(S-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 2. Column chromatography of the residual syrup on silica gel using chloroform as eluent afforded phosphotriester **2** (82%) as a white foam. R_f = 0.47 (MeOH/CH$_2$Cl$_2$ 5:95); ^{1}H NMR (CDCl$_3$, 200 MHz) δ 8.69, 8.59 (2s, 1H, NH$_{AZT}$), 7.40−7.12 (m, 5H, H-6, H$_δ$, H$_ε$), 6.28−6.20 (m, 1H, H-1′), 4.41−4.38 (m, 3H, H-3′, H-5′, H-5″), 4.28−4.15 (m, 2H, POCH$_2$), 4.07 (m, 2H, H$_a$, H-4′), 3.84−3.69 (m, 2H, CH$_2$O$_{Tyr}$), 3.19−3.04 (m, 3H, H$_{βa}$, CH_2S), 2.74−2.61 (dd, J = 10.3 and 13.1 Hz, 1H, H$_{βb}$), 2.45−2.31 (m, 2H, H-2′, H-2″), 1.90 (s, 3H, CH$_{3AZT}$), 1.54−1.48 (m, 15H, C(CH$_3$)$_2$, C(CH$_3$)$_{3Boc}$), 1.24 (2s, 9H, C(CH$_3$)$_{3SATE}$); ^{13}C NMR (CDCl$_3$) δ 206.0 (COS), 163.7 (C-4), 155.2 (CON), 152.6, 152.1 (C$_q$), 150.4 (C-2), 136.5, 135.5 (C-6), 131.3−130.6 (C$_γ$, C$_δ$), 120.4 (C$_ε$), 112.0 (C-5), 94.6−94.0 (*C*(CH$_3$)$_2$), 85.2 (C-1′), 82.5 (d, $J_{P−C}$ = 7.9, C-4′), 80.9−80.1 (*C*(CH$_3$)$_3$), 67.6−67.4 (C-5′, PO*C*H$_2$), 66.4, 66.2 (*C*H$_2$O$_{Tyr}$), 60.4 (C-3′), 59.4 (C$_α$), 46.2 (*C*(CH$_3$)$_{3SATE}$), 39.2 (C$_β$), 38.0 (C-2′), 30.2−28.8 (*C*H$_2$S, C(CH$_3$)$_2$, C(CH$_3$)$_{3Boc}$), 27.7 (C(CH$_3$)$_{3SATE}$), 12.9, 12.8 (2s, CH$_{3AZT}$); ^{31}P NMR (CDCl$_3$) δ −5.11, −5.22, −5.34, −5.36; MS FAB$^+$ m/z 781 (M + H)$^+$, 725 (M − tBu + 2H)$^+$, 681 (M −

Boc + 2H)$^+$; MS FAB$^-$ *m/z* 1560 (2M)$^-$, 779 (M − H)$^-$, 635 (M − SATE)$^-$, 490 (M − Tyr)$^-$; HPLC t_R 31.4, 30.7 min; UV (EtOH) λ_{max} 265 nm (ϵ 8960). Anal. (C$_{34}$H$_{49}$N$_6$O$_{11}$PS) C, H, N.

***O*-[*N*-(Boc)-L-tyrosinyl *N,N*-di-Boc-amide]-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido −3′-deoxythymidin- 5′-yl Phosphate, 5.** Column chromatography of the residual syrup on silica gel using chloroform as eluent afforded phosphotriester **5** (63%) as a white foam. R_f = 0.39 (MeOH/CH$_2$Cl$_2$ 5:95); ^{1}H NMR (CDCl$_3$) δ 8.67 (s, 1H, NH$_{AZT}$), 7.57 (s, 1H, NH$_{Tyr}$), 7.33, 7.27 (2d, 1H, *J* = 1.0, H-6), 7.15 (m, 2H, H$_\epsilon$), 6.18−6.12 (m, 1H, H-1′), 4.99−4.94 (m, 1H, H$_\alpha$), 4.37−4.23 (m, 3H, H-3′, H-5′, H-5″), 4.18−4.10 (m, 2H, POCH$_2$), 3.98−3.95 (m, H, H-4′), 3.43−3.38 (m, 1H, H$_{\beta a}$), 3.09−2.97 (m, 3H, CH$_2$S, H$_{\beta b}$), 2.36 and 2.22 (2m, 2H, H-2′, H-2″), 1.83, 1.82 (2s, 3H, CH$_{3AZT}$), 1.41 (s, 9H, C(CH$_3$)$_{3Boc}$), 1.37 (s, 18H, C(CH$_3$)$_{3Boc}$), 1.16, 1.15 (2s, 9H, C(CH$_3$)$_{3SATE}$); ^{13}C NMR (CDCl$_3$) δ 205.9 (COS), 168.7 (CON$_{amido}$), 163.8 (C-4), 152.0 (C$_q$), 150.5, 149.8 (CON), 149.3 (C-2), 135.6 (C-6), 131.6 (C$_\gamma$), 130.5 (C$_\delta$), 120.2 (C$_\epsilon$), 112.0 (C-5), 85.3 (C-1′), 84.6, 83.2 (C(CH$_3$)$_{3Boc}$), 82.5 (C-4′), 67.6−67.4 (C-5′, POCH$_2$), 61.7 (C-3′), 60.5 (C$_\alpha$), 47.0 (C(CH$_3$)$_{3SATE}$), 37.8 (C-2′), 35.2 (C$_\beta$), 30.2 (CH$_2$S), 28.4, 28.2 (C(CH$_3$)$_{3Boc}$), 27.7 (C(CH$_3$)$_{3SATE}$), 12.9, 12.8 (2s, CH$_{3AZT}$); ^{31}P NMR (CDCl$_3$) δ −5.3, −5.4; MS FAB$^+$ *m/z* 954 (M + H)$^+$, 854 (M − Boc + 2H)$^+$, 754 (M − 2Boc + 3H)$^+$, 654 (M − 3Boc + 4H)$^+$; MS FAB$^-$ *m/z* 1906 (2M)$^-$, 952 (M − H)$^-$, 808 (M − SATE)$^-$; HPLC t_R 30.1 min; UV (EtOH) λ_{max} 264 nm (ϵ 9340). Anal. (C$_{41}$H$_{60}$N$_7$O$_{15}$PS) C, H, N, P.

***O*-[*N*-(Acetyl-Boc)-L-tyrosinyl-*N,N*-di-Boc-amide]-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 6.** Column chromatography of the residual syrup on silica gel using chloroform as eluent afforded phosphotriester **6** (60%) as a white foam. R_f = 0.39 (MeOH/CH$_2$Cl$_2$ 5:95); ^{1}H NMR (CDCl$_3$, 200 MHz) δ 8.70 (s, 1H, NH$_{AZT}$), 7.34, 7.30 (2d, 1H, H-6, *J* = 1.04 and 1.27), 7.22−7.03 (m, 4H, H$_\delta$, H$_\epsilon$), 6.19−6.14 (m, 1H, H-1′), 5.61−5.58 (m, 1H, H$_\alpha$), 4.36−4.21 (m, 3H, H-3′, H-5′, H-5″), 4.16−4.10 (m, 2H, POCH$_2$), 3.99−3.98 (m, 1H, H-4′), 3.48−3.43 (m, 1H, H$_{\beta b}$), 3.09−3.03 (m, 2H, CH$_2$S), 2.93−2.89 (m, 1H, H$_{\beta a}$), 2.40−2.17 (m, 5H, CH$_{3acetyl}$, H-2′, H-2″), 1.84 (s, 3H, CH$_{3AZT}$), 1.42 (m, 27H, C(CH$_3$)$_{3Boc}$), 1.15, 1.14 (2s, 9H, C(CH$_3$)$_{3SATE}$); ^{13}C NMR (CDCl$_3$) δ 206.0 (COS), 172.6 (CON$_{amido}$), 170.8 (CO$_{acetyl}$), 163.9 (C-4), 152.4 (C$_q$), 150.5 (CON), 149.9 (C-2), 135.6 (C-6), 131.8 (C$_\gamma$), 130.5 (C$_\epsilon$), 120.0 (C$_\delta$), 112.0 (C-5), 85.2 (C-1′), 85.0 (C(CH$_3$)$_{3Boc}$), 82.5 (C-4′), 67.6−67.4 (C-5′, POCH$_2$), 60.5 (C-3′), 59.9 (C$_\alpha$), 47.0 (C(CH$_3$)$_{3SATE}$), 35.7 (C$_\beta$), 37.8 (C-2′), 30.3 (CH$_2$S), 28.1−27.7 (C(CH$_3$)$_{3Boc}$, C(CH$_3$)$_{3SATE}$), 26.8 (CH$_{3acetyl}$), 12.9, 12.8 (2s, CH$_{3AZT}$); ^{31}P NMR (CDCl$_3$) δ −5.30, −5.37; MS FAB$^+$ *m/z* 996 (M + H)$^+$; MS FAB$^-$ *m/z* 994 (M − H)$^-$; HPLC t_R 33.1 min; UV (EtOH) λ_{max} 264 nm (ϵ 9940). Anal. (C$_{43}$H$_{62}$N$_7$O$_{16}$PS) C, H, N.

General Procedure for the Preparation of Phosphodiesters 4b and 8b. AZT-HPM[15] (triethylammonium form, 0.086 g, 0.2 mmol) and tyrosine precursor (1.1 equiv) were coevaporated in dry pyridine and dissolved in a mixture of anhydrous pyridine/acetonitrile (1:1, 1.5 mL). To this stirred solution was added dropwise pivaloyl chloride (0.033 mL, 1.3 equiv). After 30 min, iodine (0.152 g, 3 equiv) and water (0.040 mL, 10 equiv) were successively added and the reaction was allowed to proceed for a further 15 min. Then, an aqueous solution of Na$_2$S$_2$O$_4$ (0.1 M, 10 equiv) was added until the reaction mixture became colorless. The mixture was diluted with dichloromethane, and the organic layer was washed with a TEAB solution (0.5 M, 30 mL). The organic solution was dried over Na$_2$SO4 and concentrated. The oily residue was purified on a silica gel column using dichloromethane and methanol (0−20%) as eluent. The residue was dissolved in a water/ethanol mixture (5:2) and filtrated on an ion-exchange resin (Dowex Na$^+$), and the collected fractions were concentrated, dissolved in dioxane/water (1:1), and lyophilized in order to obtained the title compound as a sodium salt.

***O*-[*N*-Boc-2,2′-dimethyl-4-(4-hydroxyphenyl)-1,3-oxazolidine]-*O*-(3′-azido-3′-deoxythymidin-5′-yl) Phosphate, Sodium Salt, 4b.** Yield, 53%; R_f = 0.58 (MeOH/CH$_2$Cl$_2$ 2:8); ^{1}H NMR (D$_2$O, 200 MHz) δ 7.40 (d, *J* = 26.0, 1H, H-6), 7.00 (s, 4H, H$_\epsilon$, H$_\delta$), 6.10 (bs, 1H, H-1′), 4.30 (s, 1H, H-3′), 4.15−4.10

(m, 3H, H-4′, H-5′, H-5″), 3.92 (bs, 1H, H$_\alpha$), 3.71 (t, 2H, H$_{\beta a}$, H$_{\beta b}$), 2.84 (2s, 2H, CH$_2$O$_{Tyr}$), 2.32 (m, 2H, H-2′, H-2″), 1.61 (d, *J* = 25.3, 3H, CH$_{3AZT}$), 1.36 (s, 15H, C(CH$_3$)$_2$, C(CH$_3$)$_{3Boc}$); ^{13}C NMR (D$_2$O) δ 166.0 (s, C-4), 154.2 (CON), 152.0, 151.5 (2s, C-2, C$_q$), 138.6 (C-6), 135.7 (C$_\gamma$), 127.1 (C$_\epsilon$), 120.8 (C$_\delta$), 112.0 (C-5), 95.0 (C(CH$_3$)$_2$), 85.2 (C-1′), 83.0 (C-4′), 82.0 (C(CH$_3$)$_3$), 65.1 (C-5′), 65.0 (CH$_2$O$_{Tyr}$), 60.5 (C-3′), 58.9 (C$_\alpha$), 38.2 (C$_\beta$), 35.7 (C-2′), 27.1−25.7 (C(CH$_3$)$_{3Boc}$), 23.6−22.1 (C(CH$_3$)$_2$), 12.1 (CH$_{3AZT}$); ^{31}P NMR (D$_2$O) δ −4.15; MS FAB$^+$ *m/z* 659 (M + H)$^+$; MS FAB$^-$ *m/z* 1043 (2M − Na − H)$^-$; HPLC t_R 22.6 min; UV (EtOH) λ_{max} 265 nm (ϵ 8320); HRMS (C$_{27}$H$_{37}$N$_6$NaO$_{10}$P) calcd 659.2206, found 659.2186.

***O*-[*N*-(Acetyl-Boc)-L-tyrosinyl-*N,N*-di-Boc-amide]-*O*-(3′-azido-3′-deoxythymidin-5′-yl) Phosphate, Sodium Salt, 8b.** Yield, 41%; R_f = 0.53 (MeOH/CH$_2$Cl$_2$ 2:8); ^{1}H NMR (D$_2$O, 200 MHz) δ 7.30 (s, 1H, H-6), 6.95 (s, 4H, H$_\epsilon$, H$_\delta$), 6.00 (t, *J* = 6.6, 1H, H-1′), 4.37 (bs, 2H, H-3′, H$_\alpha$), 4.11−3.86 (m, 3H, H-4′, H-5′, H-5″), 2.83, 2.79 (2m, 2H, 2H$_\beta$), 2.37 (s, 2H, H-2′, H-2″), 1.83 (s, 3H, CH$_{3acetyl}$), 1.69 (s, 3H, CH$_{3AZT}$), 1.32 (d, 9H, C(CH$_3$)$_{3BocAmino}$), 1.18 (s, 18H, C(CH$_3$)$_{3BocAmido}$); ^{13}C NMR (D$_2$O) δ 171.7 (CON), 170.4 (CO$_{acetyl}$), 164.7 (C-4), 150.7 (C-2), 149.6 (C$_q$), 136.4 (C-6), 1.32.5 (C$_\gamma$), 121.2 (C$_\delta$), 120.5 (C$_\epsilon$), 111.8 (C-5), 84.8 (C-1′), 83.7 (C-4′), 82.9 (C(CH$_3$)$_3$), 66.4 (C-5′), 61.2 (C-3′), 59.7 (C$_\alpha$), 36.4, 36.2 (C-2′, C$_\beta$), 26.3−25.8 (C(CH$_3$)$_{3Boc}$), 24.9 (CH$_{3acetyl}$), 12.3 (CH$_{3AZT}$); ^{31}P NMR (D$_2$O) δ −4.28; MS FAB$^+$ *m/z* 874 (M + H)$^+$, 774 (M − Boc + 2H)$^+$, 674 (M − 2Boc + 3H)$^+$, 574 (M − 3Boc + 2H)$^+$; FAB$^-$ *m/z* 850 (M − Na − H)$^-$, 750 (M − Boc- Na)$^-$; HPLC t_R 22.5 min; UV (EtOH) λ_{max} 265 nm (ϵ 10010); HRMS (C$_{36}$H$_{49}$N$_7$NaO$_{15}$P) calcd 873.7797, found 873.7801.

General Procedure for the Acidic Deprotection of Compounds 2, 5, 6, 4b, and 8b. Phosphotriester or phosphodiester (0.2 mmol) was dissolved in dioxane (1 mL), and a solution of hydrogen chloride in diethyl ether (2 mL, 35%) was added at 0 °C. The mixture was stirred at room temperature for 1 or 2 h. The solvent was removed in vacuum, and the residue is either subjected to silica gel column chromatography for phosphotriester derivatives or reverse-phase C$_{18}$ column chromatography, eluting with a linear gradient of CH$_3$CN in H$_2$O (0−25%) for phosphodiesters. The desired fractions were evaporated and passed through an ion-exchange column (Dowex Na$^+$) for the corresponding phosphodiesters. The residue was lyophilized in dioxane/water (1:4) to afford the title compound as a white powder.

***O*-[*N*-Boc-2-amino-4-(4-hydroxyphenyl)propanol]yl-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 3.** Purification of the residue by column chromatography on silica gel, eluting with MeOH/HCCl$_3$ (2:98), afforded phosphotriester **3** (56%) as well as phosphotriester **4** (35%). R_f = 0.42 (MeOH/CH$_2$Cl$_2$ 8:92); ^{1}H NMR (CDCl$_3$) δ 7.28, 7.24 (2s, 1H, H-6), 7.13−7.02 (m, 4H, H$_\delta$, H$_\epsilon$), 6.17−6.11 (m, 1H, H-1′), 4.97, 4.91 (2s, 1H$_{ech}$, NH$_{Tyr}$), 4.42−4.35 (m, 1H, H-3′), 4.30−4.25 (m, 2H, H-5′, H-5″), 4.21−4.10 (m, 2H, POCH$_2$), 4.00−3.96 (m, 1H, H-4′), 3.74 (br s, 1H, H$_\alpha$), 3.55, 3.44 (2m, 2H, CH$_2$O$_{Tyr}$), 3.10−3.02 (m, 2H, CH$_2$S), 2.76 (m, 2H, H$_\beta$), 2.57−2.55 (m, 1H$_{ech}$, OH$_{Tyr}$), 2.43−2.32, 2.29−2.15 (2m, 2H, H-2′, H-2″), 1.76, 1.75 (2s, 3H, CH$_{3AZT}$), 1.37, 1.36 (2s, 9H, C(CH$_3$)$_{3Boc}$), 1.16, 1.15 (2s, 9H, C(CH$_3$)$_{3SATE}$); ^{13}C NMR (CDCl$_3$) δ 206.1 (COS), 164.0 (C-4), 156.4 (CON), 150.6−149.1 (C$_q$, C-2), 136.1 (C$_\gamma$), 135.8 (C-6), 131.1 (C$_\delta$), 120.3 (C$_\epsilon$), 111.9 (C-5), 85.4 (C-1′), 82.7−82.5 (C-4′), 77.6 (C(CH$_3$)$_3$), 67.5−67.4 (C-5′, POCH$_2$), 64.2 (CH$_2$O$_{Tyr}$), 60.4 (C-3′), 53.9 (C$_\alpha$), 47.0 (C(CH$_3$)$_{3SATE}$), 46.4 (C$_\beta$), 37.8 (C-2′), 28.9−28.8 (CH$_2$S, C(CH$_3$)$_{3Boc}$), 27.7 (C(CH$_3$)$_{3SATE}$), 12.9, 12.8 (2s, CH$_{3AZT}$); ^{31}P NMR (CDCl$_3$) δ −5.17, −5.55; MS FAB$^+$ *m/z* 741 (M + H)$^+$, 685 (M − *t*Bu + 2H)$^+$, 641 (M − Boc + 2H)$^+$; MS FAB$^-$ *m/z* 1480 (2M)$^-$, 739 (M − H)$^-$, 595 (M − SATE)$^-$, 490 (M − Tyr)$^-$; HPLC t_R 25.4 min; UV (EtOH) λ_{max} 265 nm (ϵ 9860). Anal. (C$_{31}$H$_{45}$N$_6$O$_{11}$PS) C, H, N.

***O*-[2-Amino(chlorydate)-4-(4-hydroxyphenyl)propanol]-yl-*O*-(*S*-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 4.** Purification of the residue by column chromatography on silica gel, eluting with MeOH/HCCl$_3$ (1:9), gave the phosphotriester **4** (75%) as a white foam. R_f = 0.15 (MeOH/CH$_2$Cl$_2$ 15:85); ^{1}H NMR (DMSO-d_6) δ 7.27, 7.26

(2s, 1H, H-6), 7.08, 6.95 (2d, 2 × 2H, J = 8.4, H$_\delta$, H$_\epsilon$), 5.95–5.90 (m, 1H, H-1′), 5.13 (bs, 1H, OH$_{Tyr}$), 4.30–4.24 (m, 1H, H-3′), 4.17–4.08 (m, 2H, H-5′, H-5″), 3.98–3.92 (m, 2H, POCH$_2$), 3.80 (m, 1H, H-4′), 3.30–3.27 (m, 2H, CH$_2$O$_{Tyr}$), 3.14 (m, 1H, partly covered by HDO peak, H$_\alpha$), 2.94–2.89 (m, 2H, CH$_2$S), 2.70–2.59 (2m, 2H, 2 × H$_\beta$), 2.26–2.09 (2m, 2H, H-2′, H-2″), 1.52 (2s, 3H, CH$_{3AZT}$), 0.95 (2s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (DMSO-d_6) δ 205.9 (COS), 164.5 (C-4), 151.22 (C-2), 149.7 (d, J_{P-C} = 6.5, C$_q$), 136.8 (C-6), 134.7 (C$_\gamma$), 131.6 (C$_\delta$), 120.8 (d, J_{P-C} = 4.4, C$_\epsilon$), 110.8 (C-5), 84.6 (C-1′), 81.9 (d, J_{P-C} = 7.7, C-4′), 68.2 (C-5′), 67.3 (d, J_{P-C} = 5.6, POCH$_2$), 60.7–60.4 (m, C-3′, CH$_2$O$_{Tyr}$), 54.5 (C$_\alpha$), 46.6 (C(CH$_3$)$_{3SATE}$), 36.4 (C-2′), 34.9 (C$_\beta$), 28.9 (d, J_{P-C} = 7.7, CH$_2$S), 27.7 (C(CH$_3$)$_{3SATE}$), 12.9, 12.8 (2s, CH$_{3AZT}$); ^{31}P NMR (DMSO-d_6) δ −5.3, −5.5; MS FAB$^+$ m/z 641 (M − HCl)$^+$; MS FAB$^-$ m/z 675 (M − H)$^-$, 639 (M − HCl)$^-$, 490 (M − HCl − Tyr)$^-$, 495 (M − HCl − SATE)$^-$; HPLC t_R 22.6 min; UV (EtOH) λ$_{max}$ 265 nm (ε 9300). Anal. (C$_{26}$H$_{38}$-ClN$_6$O$_9$PS) H. C calcd 46.12, found 46.62. N: calcd 12.41, found 11.34.

O-[2-Amino(chlorydate)-4-(4-hydroxyphenyl)propanol]-yl-O-(3′-azido-3′-deoxythymidin-5′-yl) Phosphate, Sodium Salt, 4a. Yield, 41%. R_f = 0.53 (MeOH/CH$_2$Cl$_2$ 2:8); ^{1}H NMR (D$_2$O, 200 MHz) δ 7.2 (s, 1H, H-6), 6.9 (s, 4H, H$_\epsilon$, H$_\delta$), 5.9 (bs, 1H, H-1′), 4.2 (m, 1H, H-3′), 4.0–3.9 (m, 3H, H-4′, H-5′, H-5″), 3.5 (d, J = 11.32, 1H, CH$_2$O$_{Tyr}$), 3.3 (m, 2H, CH$_2$O$_{Tyr}$, H$_\alpha$), 2.6 (m, 2H, H$_\beta$), 2.2 (s, 2H, H-2′, H-2″), 1.4 (s, 3H, CH$_{3AZT}$); ^{13}C NMR (D$_2$O) δ 162.1 (C-4), 148.6–147.9 (C-2, C$_q$), 134.3 (C-6), 128.6 (C$_\gamma$), 127.1 (C$_\epsilon$), 117.9 (C$_\delta$), 108.6 (C-5), 85 (C-1′), 80.7 (C-4′), 65 (C-5′), 60 (CH$_2$O$_{Tyr}$), 59.3 (C-3′), 55 (C$_\alpha$), 35,7 (C-2′), 35 (C$_\beta$), 11.4 (CH$_{3AZT}$); ^{31}P NMR (D$_2$O) δ −4.02; MS FAB$^+$ m/z 519 (M + H)$^+$, 497 (M − Na + 2H)$^+$; FAB$^-$ m/z 991 (2M − Na − H)$^-$, 495 (M − Na − H)$^-$; HPLC t_R 13.7 min; UV (EtOH) λ$_{max}$ 265 nm (ε 9200); HRMS (C$_{19}$H$_{25}$N$_6$NaO$_8$P) calcd 519.1369, found 519.1368.

O-[L-Tyrosinamide]yl-O-(S-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 7. Purification of the residue by column chromatography on silica gel, eluting with a gradient of MeOH in HCCl$_3$ (0–5%) gave the trifluoroacetate form of phosphotriester **7** (80%) as a white foam. R_f = 0.21 (MeOH/CH$_2$Cl$_2$ 15:85); ^{1}H NMR (DMSO-d_6) δ 7.72 (s, 1H, NH$_{ech}$), 7.47, 7.45 (2s, 1H, H-6), 7.38 (s, 1H, NH$_{ech}$), 7.26–7.12 (m, 5H, NH$_{ech}$, H$_\delta$, H$_\epsilon$), 6.16–6.10 (m, 1H, H-1′), 4.49–4.36 (m, 1H, H-3′), 4.35–4.26 (m, 2H, H-5′, H-5″), 4.17–4.12 (m, 2H, POCH$_2$), 4.01 (bs, 1H, H-4′), 3.74 (bs, 1H, H$_\alpha$), 3.13–3.11 (m, 2H, CH$_2$S), 3.04–2.98 (m, 2H, H$_\beta$), 2.44–2.31 (2m, 2H, H-2′, H-2″), 1.73, 1.72 (2s, 3H, CH$_{3AZT}$), 1.17, 1.12 (2s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (DMSO-d_6) δ 205.9 (COS), 164.5 (C-4), 151.2 (C-2), 149.7 (d, J_{P-C} = 6.6, C$_q$), 136.7 (C-6), 134.3 (C$_\gamma$), 131.7 (C$_\delta$), 120.6 (C$_\epsilon$), 110.8 (C-5), 84.6 (C-1′), 81.9 (d, J_{P-C} = 7.9, C-4′), 68.1 (C-5′), 67.2 (POCH$_2$), 60.6 (C-3′), 55.0 (C$_\alpha$), 46.8 (C(CH$_3$)$_{3SATE}$), 46.5 (C$_\beta$), 36.4 (C-2′), 28.9 (d, J_{P-C} = 7.5, CH$_2$S), 27.7 (C(CH$_3$)$_{3SATE}$), 12.9 (2s, CH$_{3 AZT}$); ^{31}P NMR (DMSO-d_6) δ −5.3, −5.5; MS FAB$^+$ m/z 654 (M + H)$^+$, 510 (M − SATE + 2H)$^+$; MS FAB$^-$ m/z 766 (M + CF$_3$CO$_2$)$^-$, 652 (M − H)$^-$, 490 (M − Tyr)$^-$, 403 (M − AZT)$^-$; HPLC t_R 21.7 min; UV (EtOH) λ$_{max}$ 265 nm (ε 8750). Anal. (C$_{28}$H$_{37}$F$_3$N$_7$O$_{11}$PS) C, H, N, S.

O-[N-(Acetyl)-L-tyrosinamide]yl-O-(S-pivaloyl-2-thioethyl)-3′-azido-3′-deoxythymidin-5′-yl Phosphate, 8. Purification of the residue by column chromatography on silica gel, eluting with MeOH/HCCl$_3$ (1:9), gave the phosphotriester **8** (40%) after lyophilization in dioxane as a white powder. R_f = 0.22 (MeOH/CH$_2$Cl$_2$ 1:9); ^{1}H NMR (DMSO-d_6) δ 8.06 (bs, 1H, NH$_{Tyr}$), 7.47 (m, 2H, H-6, NH$_{amido}$), 7.30–6.98 (m, 5H, NH$_{amido}$, H$_\delta$, H$_\epsilon$), 6.18–6.10 (m, 1H, H-1′), 4.52–4.23 (m, 4H, H-3′, H-5′, H-5″, H$_\alpha$), 4.22–4.09 (m, 2H, POCH$_2$), 4.06–3.97 (m, 1H, H-4′), 3.18–3.05 (m, 2H, CH$_2$S), 3.02–2.65 (2m, 2H, H$_\beta$), 2.46–2.28 (m, 2H, H-2′, H-2″), 1.76, 1.74 (2s, 3H, CH$_{3AZT}$), 1.17, 1.16 (2s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (DMSO-d_6) δ 206.1 (COS), 174 (CON), 165.0 (C-4), 151.3 (C-2), 149.5 (d, J_{P-C} = 6.5, C$_q$), 136.8 (C-6), 134.5 (C$_\gamma$), 132.0 (C$_\delta$), 121.5 (d, J_{P-C} = 4.4, C$_\epsilon$), 110.4 (C-5), 85.4 (C-1′), 81.6 (d, J_{P-C} = 7.6, C-4′), 67.4 (C-5′), 65.9 (POCH$_2$), 60.7 (m, C-3′), 55.4 (C$_\alpha$), 45.7 (C(CH$_3$)$_{3SATE}$), 48.2 (C-2′), 43.8 (C$_\beta$), 28.6 (d, J_{P-C} = 7.7, CH$_2$S), 27.5 (C(CH$_3$)$_{3SATE}$), 23.6 (CH$_{3acetyl}$), 12.7 (2s, CH$_{3AZT}$); ^{31}P NMR

(DMSO-d_6, 81 MHz) δ −5.35, −5.46; MS FAB$^+$ m/z 696 (M + H)$^+$; MS FAB$^-$ m/z 1389 (2M − H)$^-$, 694 (M − H)$^-$; HPLC t_R 24.5 min; UV (EtOH) λ$_{max}$ 265 nm (ε 9800). Anal. (C$_{28}$H$_{38}$N$_7$O$_9$-PS·0.5H$_2$O) C, H, N.

O-[N-(Acetyl)-L-tyrosinamide]yl-O-(3′-azido-3′-deoxythymidin-5′-yl) Phosphate, Sodium Salt, 8a. Yield, 43%. R_f = 0.53 (MeOH/CH$_2$Cl$_2$ 2:8); ^{1}H NMR (D$_2$O, 200 MHz) δ 7.3 (s, 1H, H-6), 6.9 (s, 4H, H$_\epsilon$, H$_\delta$), 6.0 (t, J = 6.6, 1H, H-1′), 4.3 (s, 2H, H-3′, H$_\alpha$), 4.1 (m, 1H, H-5′), 4.0 (m, 2H, H-4′, H-5″), 2.8–2.7 (m, 2H, H$_\beta$), 2.4 (t, J = 6.4, 2H, H-2′, H-2″), 1.8 (s, 3H, CH$_{3acetyl}$), 1.6 (s, 3H, CH$_{3AZT}$); ^{13}C NMR (D$_2$O) δ 177, 175 (CON), 163 (C-4), 153, 152 (C-2, C$_q$), 139 (C-6), 134 (s, C$_\gamma$), 132 (C$_\epsilon$), 122 (C$_\delta$), 117 (C-5), 86 (C-1′), 84 (C-4′), 66 (C-5′), 61 (s, C-3′), 56 (C$_\alpha$), 37 (C-2′, C$_\beta$), 23.0 (CH$_{3acetyl}$), 12.5 (s, CH$_{3AZT}$); ^{31}P NMR (D$_2$O) δ −4.06; MS FAB$^+$ m/z 1147 (2M + H)$^+$, 574 (M + H)$^+$, 552 (M − Na + 2H)$^+$, 307 (M − AZT + 2H)$^+$; FAB$^-$ m/z 1101 (2M − 2Na − H)$^-$, 572 (M − H)$^-$, 550 (M − Na − H)$^-$; HPLC t_R 14.3 min; UV (EtOH) λ$_{max}$ 268 nm (ε 14 300); HRMS (C$_{21}$H$_{26}$N$_7$NaO$_9$P) calcd 574.1427, found 574.1436.

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