Synthesis, in Vitro Anti-Breast Cancer Activity, and Intracellular Decomposition of Amino Acid Methyl Ester and Alkyl Amide Phosphoramidate Monoesters of 3′-Azido-3′-deoxythymidine (AZT)

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Received March 6, 2000

We report the synthesis and anticancer activity of a series of AZT phosphoramidate monoesters containing amino acid methyl ester (**3a**−**11a**) and *N*-alkyl amide (**3b**−**11b**, **9c**−**9f**) moieties. The aromatic amino acid methyl esters were found to be more cytotoxic than the aliphatic analogues toward MCF-7 cells (human pleural effusion breast adenocarcinoma cell line). A marked stereochemical preference for the L-amino acid stereochemistry was also observed in MCF-7 cells. There was no consistent enhancement of cytotoxicity of the methyl amides over the corresponding methyl esters. AZT and the two AZT aromatic amino acid methyl ester phosphoramidates **8a** and **9a** were found to be more cytotoxic toward MCF-7 cells than to CEM cells (human T-cell lymphoblastic leukemia). The selective cytotoxicity toward MCF-7 cells may be associated with greater intracellular levels of phosphoramidate monoester and/or phosphorylated AZT.

Introduction

Although originally designed as an antitumor agent, AZT has demonstrated far more utility as an antiviral agent.[1,2] In the past decade, however, AZT has emerged as an antitumor agent in combination with either cisplatin, methotrexate, or 5-fluorouracil, for the treatment of advanced colon cancer.[3−7] However, the role of AZT as an antineoplastic agent has not been determined. It has been postulated that the mechanism of action of AZT may be associated with its conversion to AZT-TP, followed by its incorporation into DNA by host cell polymerases.[6] Recently, we reported the first in vitro and in vivo evidence of the anti-breast cancer activity of AZT. [8] The enhanced activity of AZT toward the human breast cancer cell line MCF-7, relative to the T-leukemia cell line CEM, appears to be associated with increased levels of the intracellular AZT-MP, AZT-DP, and AZT-TP. In both cell lines, at least 89% of the mixture of AZT-5′-phosphates was shown to be AZT-MP.[3]

Unfortunately, AZT-based therapies suffer from several drawbacks. First, extensive exposure to AZT has been shown to result in decreased levels of thymidine kinase (TK1) due to a methylation-dependent inhibition of transcription.[9] Second, AZT therapy is also associated with myelosuppression, resulting in dose-dependent anemia and neutropenia.[10] Furthermore, although orally bioavailable, 75% of an oral dose of AZT is rapidly eliminated by glucuronidation of the 5′-OH group during first-pass metabolism.[11] Consequently, AZT has a short terminal half-life (1.1 ± 0.2 h) and a relatively small volume of distribution.[12]

To overcome the drawbacks associated with AZT therapy, several prodrug approaches have been investigated. Among the AZT "pronucleotides" synthesized are phospholipid conjugates,[13] dinucleoside phosphates,[14,15] dinucleoside salicylate phosphates,[16] simple alkyl phosphotriesters,[17] monophosphate diaryl esters,[18] glycolipid phosphotriesters,[19,20] *S*-acyl-2-thioethyl (SATE),[21,22] dithioethyl (DTE),[21,23] pivaloyloxymethyl (POM),[24−26] and *p*-amino oxybenzyl (PAOB) phosphotriesters[27] and phosphoramidate diesters.[28,29] Recently, we have demonstrated that AZT amino acid phosphoramidate monoesters are water-soluble, stable, and nontoxic antiviral agents that presumably release AZT-MP.[30,31] In addition, we have shown that upon intravenous dosing of rats, AZT amino acid phosphoramidate monoesters have a plasma half-life and volume of distribution that is 5 and 10 times, respectively, greater than that observed for AZT.[32] Since AZT was reported to have anti-breast cancer activity, we chose to investigate the utility of AZT phosphoramidates as anti-breast cancer agents in vitro and to evaluate the structure−activity relationships governing the role of the amino acid moiety.

Chemistry

We have previously reported the synthesis of amino acid phosproramidate monoesters of 3′-azido-3′-deoxythymidine (AZT), 3′-deoxy-2′,3′-didehydrothymidine (D4T), and 3′-fluoro-3′-deoxythymidine (FLT) utilizing phosphoramidite chemistry.[30,33] Typically, the parent nucleosides were treated with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite to give the isolated phosphoamidite intermediate. The phosphoramidites were then converted to their corresponding methyl phosphites, followed by oxidation and coupling of the appropriate amino acid methyl ester, to give the desired phosphoramidate product. Although this chemistry was used to produce many derivatives, several compounds required multiple chromatographic purification steps in order to obtain pure material. In addition, the high cost of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite

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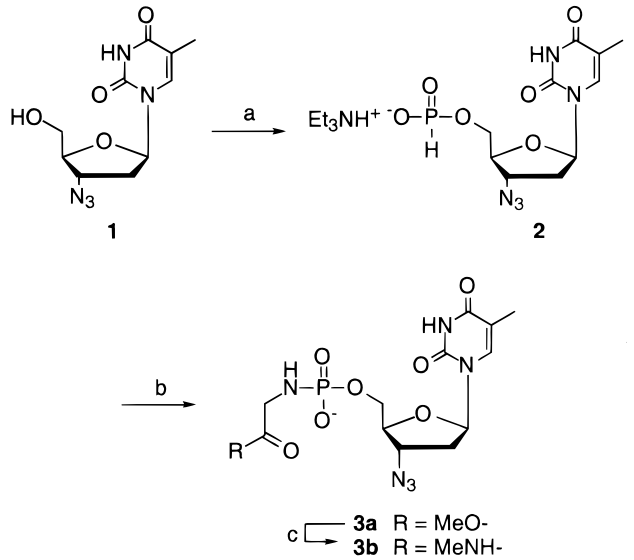
10.1021/jm000110g CCC: $19.00
Published on Web 04/21/2000

Scheme 1[a]



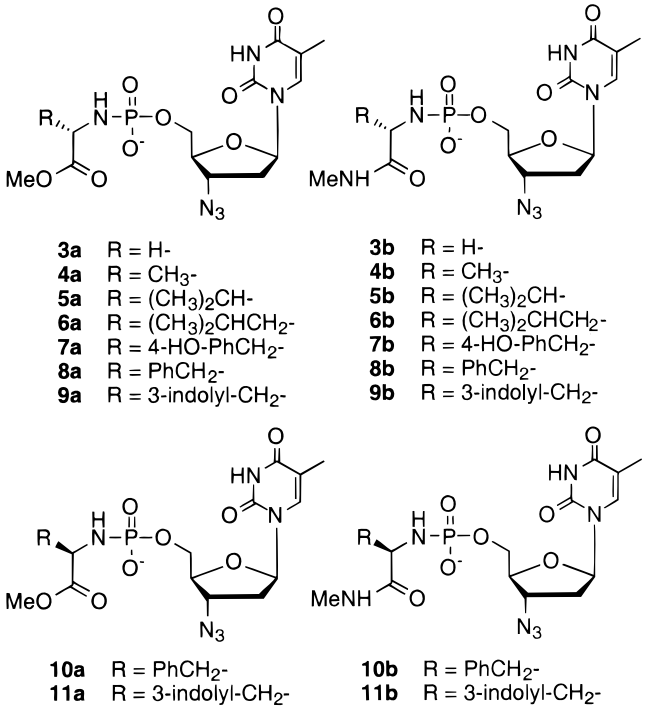
[a] Reagents: (a) diphenylphosphite, pyridine; (b) (i) TMSCI, pyridine, (ii) I_2, (iii) glycine methyl ester, Et_3N; (c) 10 M $MeNH_2$ in MeOH.

made this reagent undesirable for the synthesis of larger quantities of material. Therefore, we sought an alternate approach to the synthesis of these compounds.

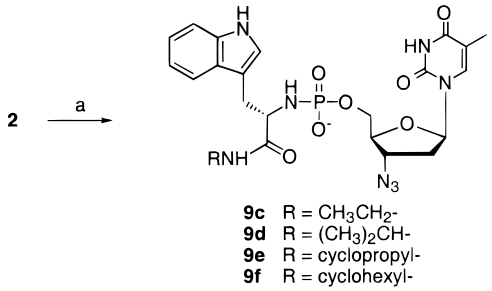
Recently, Stawinski reported the synthesis of phosphoramidate monoesters of nucleosides utilizing H-phosphonate intermediates.[34] Inspired by this approach, we treated AZT (**1**) with diphenyl phosphite in pyridine and obtained the 5′-phosphite **2** in 70% yield (Scheme 1).[35] The H-phosphonate intermediate was then treated with excess trimethylsilyl chloride, followed by oxidation with iodine. Next, glycine methyl ester was added with triethylamine and the desired phosphoramidate **3a** was isolated in 49% yield. We also desired to convert the methyl ester portion of the amino acid moiety to a methyl amide, which should be more stable in the presence of esterase activity. This was achieved by treating **3a** with 10 M methylamine in methanol to give **3b** in 84% yield. By eliminating the tedious purification required by the previously reported phosphoramidite approach to nucleoside phosphoramidates, the simplicity of the H-phosphonate chemistry allowed us to prepare a series of derivatives with hydrophobic amino acid side chains for structure−activity-relationship (SAR) studies. Reaction of **2** with L-alanine, L-valine, L-leucine, L-tyrosine, L-phenylalanine, L-tryptophan, D-phenylalanine, and D-tryptophan methyl esters gave compounds **4a**−**11a** in yields ranging from 31 to 70% (Chart 1). These compounds were all converted in high yield to their corresponding methyl amides by treatment with 10 M methylamine in methanol to give **4b**−**11b**. In addition, we also prepared a series of tryptophan derivatives with different alkyl amides (**9c**−**f** Scheme 2).

The stability of selected phosphoramidates **8a** and **9a** in culture medium (10% heat-inactivated fetal bovine serum (FBS)) and in water over the course of 6 days at 37 °C was determined before biological evaluation. The rate of decomposition for the phosphoramidates **8a** and **9a** was shown to be negligible over 6 days, ranging from 4.1×10^{-10} to 1.9×10^{-10} mol/h in fetal calf serum and from 1.4×10^{-10} to 5.1×10^{-10} mol/h in water,

Chart 1



Scheme 2[a]



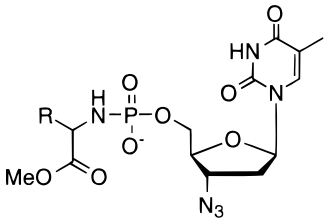
[a] (a) (i) TMSCI, pyridine, (ii) I_2, (iii) tryptophan alkyl amide, Et_3N.

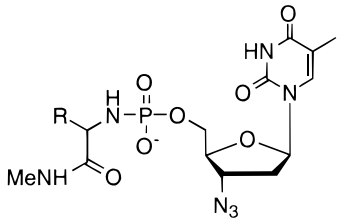
respectively. Consequently, as was observed for amino acid phosphoramidates of 5-fluoro-2′-deoxyuridine (FUdR) and 1-β-arabinofuranosylcytosine (Ara-C), >99% of the phosphoramidates of AZT remained intact over the 6 day period necessary for completion of a typical antiviral assay.[33]

Results and Discussion

Intracellularly, AZT is phosphorylated to the monophosphate (AZT-MP) and then, via a rate-limiting step, to the diphosphate (AZT-DP) and ultimately to the triphosphate (AZT-TP).[36] Exposure of H9 cells to AZT resulted in the accumulation of AZT-MP which was found to be not only a poor substrate but also an inhibitor for thymidylate kinase (TMP kinase).[37] Furthermore, it has been established that AZT-MP inhibits both thymidine kinase activity and DNA polymerase-associated 3′ to 5′ exonuclease activity, which prevents the excision of AZT already incorporated into the DNA.[37−40] It has also been reported that AZT can inhibit thymidine kinase (TK) activity and induce a defect in TK activity in AIDS patients treated with AZT. [41] The cytotoxicity of AZT toward the T-cell leukemia cell line, CEM, was shown to depend on the intracellular con-

Table 1. In Vitro Activity of Amino Acid Phosphoramidates against MCF-7 Cells





	methyl esters			methyl amides		
R	entry	CC_{50} (μM)[a]	log *P*	entry	CC_{50} (μM)[a]	log *P*
L-amino acid						
H-	**3a**	>100	−1.240	3b	5	−1.154
CH_3-	**4a**	5.8	−1.252	4b	>100	−1.205
$(CH_3)_2CH$-	**5a**	6	−1.560	5b	2.2	−1.640
$(CH_3)_2CH_2CH$-	**6a**	>100	−1.498	6b	>100	−1.524
4-HO-$PhCH_2$-	**7a**	10	−1.592	7b	>100	−1.376
$PhCH_2$-	**8a**	0.4	−1.836	8b	1.4	−1.492
3-indolyl-CH_2-	**9a**	0.059	−0.686	9b	0.016	−1.382
D-amino acid						
$PhCH_2$-	**10a**	>100	−1.329	10b	>100	−1.701
3-indolyl-CH_2-	**11a**	>100	−1.364	11b	>100	−1.894

[a] CC_{50} is the required concentration (M) to reduce MCF−7 cell growth by 50% compared to control samples.

centration of AZT-MP and not AZT-TP.[42] However, as mentioned before, the chronic administration of AZT can lead to the loss of thymidine kinase activity, which negates the therapeutic effects of AZT due to the lack of formation of phosphorylated AZT.[43] To bypass the monophosphorylation of AZT by thymidine kinase and avoid the aforementioned problem, various pronucleotide approaches, including phosphoramidate monoesters, have been pursued.[13−29] Nevertheless, the anticancer activity of AZT pronucleotides has not been reported. Consequently, we chose to investigate the anti-breast cancer actvity of AZT phosphoramidate monoesters, since we had previously demonstrated their effectiveness as anti-HIV-1 agents.[32,33,44]

AZT amino acid methyl ester (**3a**−**11a**) and alkyl amide phosphoramidates (**3b**−**11b**, **9c**−**f**) were evaluated for their in vitro cytotoxicity toward the human pleural effusion breast adenocarcinoma cell line, MCF-7. Their cytotoxicity was compared to that of the parent nucleoside, AZT (**1**). The effects of varying the α-substituents, the carboxyl end substitutions, and amino acid stereochemistry on the anti-breast cancer activity of the phosphoramidates toward MCF-7 were compared.

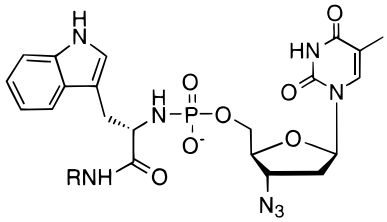
To explore the role of the amino acid α-substituent on cytotoxicity toward MCF-7 cells, a series of amino acid methyl ester (**3a**−**11a**) and methyl amide (**3b**−**11b**) phosphoramidate analogues was constructed and evaluated for in vitro anti-breast cancer activity. In Table 1, the lack of cytotoxicity of **3a** and improved activity for **4a** and **5a** suggest that the presence of an α-substituent is necessary for the methyl ester derivatives. AZT phosphoramidates with small aliphatic L-amino acid methyl esters, such as **4a** (L-alanine) and **5a** (L-valine), showed similar cytotoxicity, suggesting that the interactions may be hydrophobic in nature. Increasing the size of the α-substituent to isobutyl (**6a**) greatly diminished the cytotoxicity, suggesting that steric interactions may be dominant. Although compounds **8a** and **9a** have large aromatic α-substituents, they show remarkable cytotoxicity compared to the other amino acid methyl ester phosphoramidates in Table 1. This suggests that the hydrophobic as well as aromatic character of the α-substituents of the amino acid moiety contributes to the cytotoxicity of AZT phosphoramidates. Since the phenylalanine and the tryptophan derivatives (**8a** and **9a)** were the most active of the amino acid methyl ester phosphoramidate series, the decrease in activity observed for the tyrosine phosphoramidate **7a** may be associated with the polar nature of the hydroxyl group and not steric hindrance.

The role of the amino acid methyl ester moiety on cytotoxicity was probed by replacement with the more hydrolytically stable methyl amide. As shown in Table 1, the methyl amide of the AZT-(L)-glycyl phosphoramidate (**3b**) exhibited greater cytotoxicity relative to the methyl ester (**3a**). The same trend is seen with AZT-(L)-valyl (**5b**) and AZT-(L)-tryptophyl phosphoramidate methyl amides (**9b**). While some cytotoxicity was still retained with AZT-(L)-phenylalanyl phosphoramidate methyl amide, **8b**, a significant decrease in cytotoxicity was observed for AZT-(L)-alaninyl (**4b**) and AZT-(L)-tyrosyl (**7b**) phosphoramidate methyl amides. The L-leucyl amino acid moiety was inactive as both the methyl ester (**6a**) and the methyl amide (**6b**).

The results of the effect of stereochemistry of the α-substituent on the amino acids of the phosphoramidate analogues are also shown in Table 1. While the L-amino acid methyl ester phosphoramidates (**8a**, **9a**) and the L-amino acid methyl amide phosphoramidates (**8b**, **9b**) show significant cytotoxicity, the D-amino acid methyl ester phosphoramidates (**10a**, **11a**) and D-amino acid methyl amide phosphoramidates (**10b**, **11b**) had no significant cytotoxicity. Therefore, the amino acid stereochemistry has a dominant influence on cytotoxicity.

On the basis of the fact that the L-tryptophan methyl amide phosphoramidate **9b** was the most cytotoxic of the phosphoramidates tested, a series of L-tryptophyl alkyl amides (**9c**−**f**) was synthesized to determine the effect of larger alkylamine groups on cytotoxicity. Table 2 displays the cytotoxicity of the various alkyl amides against MCF-7 cells. Compound **9b** was the most cytotoxic in this series of phosphoramidate analogues. In fact, AZT (CC_{50} = 0.008 μM) was found to be only 2-fold more cytotoxic than **9b** (CC_{50} = 0.016 μM).

Table 2. In Vitro Activity of *N*-Alkyl Tryptophan Phosphoramidates against MCF-7 Cells



R	entry	CC_{50} (μM)[a]	log *P*
CH_3-	**9b**	0.016	−0.686
CH_3CH_2-	**9c**	>100	−1.526
$(CH3)_2CH$-	**9d**	>100	−1.296
cyclopropyl-	**9e**	90	−1.390
cyclohexyl-	**9f**	16	−0.914

[a] CC_{50} is the required concentration (μM) to reduce MCF−7 cell growth by 50% compared to control samples.

Table 3. Intracellular Levels of AZT and Phosphorylated AZT in MCF-7 and CEM Cells[a]

	AZT phosphate[b]		phosphoramidate		cytotoxicity (μM)	
entry	CEM	MCF-7	CEM	MCF-7	CEM	MCF-7
1	810.9	2658.4			14.3	0.008
8a	120.7	221	7.8	295	>100	0.4
9a	115.8	196.5	14.6	213.6	>100	0.059

[a] Amounts are expressed a pmol/million cell. [b] AZT phosphate = total amount of AZT-MP, AZT-DP, and AZT-TP.

However, the cytotoxicity of the *N*-alkyl tryptophan phosphoramidates was shown to be particularly sensitive to chain length, since the activity of **9b** is at least 6000-fold greater than the ethyl derivative, **9c**. It may be postulated that the increased size of the alkyl substituent accounts for the dramatic loss in activity. However, the compound with the largest substituent—the cyclohexyl amide **9f**—was at least 6-fold more cytotoxic than **9c**, **9d**, and **9e**, suggesting that the incorporation of aromatic or constrained hydrophobic alkyl amides may further enhance the activity of these compounds.

Partition coefficients (log *P* values) were measured to determine a possible correlation between in vitro cytotoxicity toward MCF-7 cells and lipophilicity. As observed in Tables 1 and 2, all of the AZT phosphoramidates were less lipophilic than AZT (log $P = 0.24$), with log *P* values ranging from −0.8 to −1.8. Linear regression analysis did not reveal any correlation between log *P* values and cytotoxicity toward MCF-7 cells (data not shown).

To probe the mechanism of toxicity, CEM and MCF-7 cells were incubated with either AZT, **8a**, or **9a** for 17 h and the intracellular amounts of AZT phosphate and remaining phosphoramidate were determined (Table 3). The lower cytotoxicity of AZT toward CEM cells compared to MCF-7 cells correlates with the lower levels of phosphorylated AZT in CEM cells relative to MCF-7 cells. Over 89% of the phosphorylated AZT generated from AZT was found to be AZT-MP in both cell lines.[8] For **8a** and **9a**, the greater cytotoxicity toward MCF-7 cells may be associated with higher levels of phosphoramidate and phosphorylated AZT in MCF-7 cells, relative to CEM cells. Nevertheless, the 1.7−3-fold increase in the intracellular levels of AZT phosphate and phosphoramidate found in MCF-7 cells compared to CEM cells cannot account for the 250−1787-fold increase in the cytotoxicity of AZT, **8a**, and **9a** toward MCF-7 cells. For CEM cells, the lowered toxicity of **8a** and **9a** correlates with lowered levels of AZT-MP and not increased levels of AZT-TP. (S.-L. Chang and C. R. Wagner, unpublished data) Whether the enhanced cytotoxicity of **8a** and **9a** is related to enhanced levels of AZT-MP, AZT-TP, or a unique biological activity associated with the phosphoramidate remains to be determined.

In summary, we have demonstrated that the in vitro anti-breast cancer activity of amino acid phosphoramidates is enhanced by an aromatic amino acid side chain, preferably an L-3-indolyl methyl group. Although the substitution of the amino acid methyl ester with a methyl amide moiety is tolerated, larger alkyl amides significantly reduce the activity of the AZT phosphoramidates. This represents the second example of a set of nucleoside phosphoramidate monoesters with anticancer activity.[45] Future cellular uptake and intracellular metabolism studies should provide a rationale for the impact of the amino acid structure on the anti-breast cancer activity of AZT phosphoramidate monoesters. In addition, the in vivo potency of these compounds my exceed that of AZT, since they exhibit significantly longer half-lives and larger volumes of distribution in rats than the parent nucleoside.[32] The results of ongoing experiments examining this possibility will be reported in due course.

Experimental Section

Materials. NMR (^1H and ^{31}P) spectra were recorded on a Varian VAC-200 and VAC-300 spectrometers in D_2O. An external standard of 85% H_3PO_4 was used for all ^{31}P NMR spectra. FAB mass spectra were obtained on a VG 7070E-HF mass spectrometer. Analytical TLC was performed on Analtech silica gel GHLF (0.25 mm) plates. Column chromatography was performed with grade 62, 60−200 mesh silica gel. Flash chromatography was performed with grade 60, 230−400 mesh Merck silica gel. Anhydrous pyridine was purchased from Aldrich Chemical Co. and was used without further purification. L-Amino acid methyl esters (hydrochloride salts), trimethylsilyl chloride, and diphenyl phosphite were also purchased from Aldrich. All other solvents were reagent grade and used as received. Anhydrous methylamine was bubbled through methanol to give approximately a 10 M solution. Concentration under reduced pressure refers to solvent removal on a Buchi rotary evaporator. High vacuum refers to $<10^{-2}$ psi attained with a DuoSeal mechanical pump.

MCF-7 and CCRF-CEM cells are human T-lymphoblastoid leukemia cell lines that were obtained from American Type Culture Collection, Rockville, MD. Trypan-blue stain (0.4%) in saline (0.85%) was purchased from GIBCO, Grand Island, NY.

Triethylammonium 3′-Azido-3′-deoxythymidine 5′-Phosphite. A solution of AZT (755 mg, 2.83 mmol) dissolved in 7 mL of dry pyridine was added dropwise over 40 min to a stirred solution of diphenyl phosphite (541 μL, 2.83 mmol) in 8 mL of dry pyridine under an argon atmosphere. After stirring for 2 h, Et_3N (3 mL) and H_2O (3 mL) were added, and stirring was continued for 15 min. The reaction mixture was then concentrated under reduced pressure. The resulting residue was dissolved in H_2O and extracted with CH_2Cl_2 (4×). The aqueous portion was then concentrated under reduced pressure. The resulting solid was then purified by flash chromatography (SiO_2, 9:1 $CHCl_3$/MeOH, followed by 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% NH_4OH) to give the desired product (840 mg) as a colorless solid: ^1H NMR (D_2O, 300 MHz) δ 7.522 (d, $J = 1.1$ Hz, 1 H), 6.604 (d, $J = 638$ Hz, 1 H), 6.090

(t, J = 6.7 Hz, 1 H), 4.326 (m, 1 H), 4.009 (m, 1 H), 3.933 (m, 2 H), 3.023 (q, J = 7.3 Hz, 6H), 2.328 (m, 2 H), 1.736 (d, J = 1.1 Hz, 3 H), 1.100 (t, J = 7.3 Hz, 9H); ^{31}P NMR (D_2O, 121 MHz) δ 6.856.

2-(3-Deoxy-3-azidothymidyl(hydroxy)phosphorylamino)acetic Acid Methyl Ester (3a). Triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (190 mg, 0.439 mmol) was dissolved in 7 mL of dry pyridine and treated with TMSCl (167 μL, 1.32 mmol) under Ar. After 5 min, a solution of iodine (167 mg, 0.659 mmol) in 3 mL of pyridine was added dropwise, via cannula, until the reaction color changed from yellow to a reddish-brown color. At this point, addition of the iodine solution was stopped. After 5 min, glycine methyl ester (HCl salt, 110 mg, 0.878 mmol) and Et_3N (0.43 mL, 3.08 mmol) were added. After stirring for 30 min, the reaction mixture was concentrated under reduced pressure. The resulting syrup was partitioned between 1 N NH_4OH solution and $CHCl_3$. The aqueous portion was then extracted with additional portions of $CHCl_3$ to remove unreacted amino acid methyl ester. The aqueous portion was then concentrated to give crude phosphoramidate, which was passed through a small Amberlite (IRP-64) ion exchange column, eluting with H_2O, and the desired fractions were concentrated under reduced pressure. Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (94 mg, 49%) as a slightly pink solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.550 (d, J = 1 Hz, 1 H), 6.091 (t, J = 6.7 Hz, 1 H), 4.311 (m, 1 H), 3.978 (m, 1 H), 3.935−3.787 (m, 2 H), 3.505 (s, 3 H), 3.465 (d, J = 11.5 Hz, 2 H), 2.354−2.303 (m, 2 H), 1.753 (d, J = 1 Hz, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 7.855; HRMS (FAB) m/z calcd for $C_{13}H_{20}N_6O_8P$ (M + H)$^+$ 419.1080, found 419.1103.

(1(*S*)-Methylcarbamoylmethyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (3b). 3a (35 mg) was dissolved in 2 mL of 10 M methylamine in methanol and stirred in a sealed vial for 5 d. The reaction mixture was then concentrated under reduced pressure. Flash chromatography (SiO_2, 5:2.5:0.37 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (32 mg, 92%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.538 (d, J = 1.1 Hz, 1 H), 6.089 (t, J = 6.7 Hz, 1 H), 4.302 (m, 1 H), 3.993 (m, 1 H), 3.920−3.785 (m, 2 H), 3.305 (d, J = 11.2 Hz, 2 H), 2.571 (s, 3 H), 2.323 (m, 2 H), 1.735 (d, J = 1.1 Hz, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 7.956; HRMS (FAB) m/z calcd for $C_{13}H_{20}N_7O_7P$ (M + H)$^+$ 418.1240, found 418.1247.

2(*S*)-(3-Deoxy-3-azidothymidyl(hydroxy)phosphorylamino)proprionic Acid Methyl Ester (4a). The title compound was prepared from triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (160 mg, 0.370 mmol) and alanine methyl ester (HCl salt, 103 mg, 0.740 mmol) following the procedure described for the preparation of **3a**. Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (79 mg, 48%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.557 (d, J = 1.1 Hz, 1 H), 6.085 (t, J = 6.7 Hz, 1 H), 4.298 (m, 1 H), 3.993 (m, 1 H), 3.898−3.776 (m, 2 H), 3.620 (m, 1 H), 3.536 (s, 3 H), 2.331 (m, 2 H), 1.760 (d, J = 1.1 Hz, 3 H), 1.141 (d, J = 6.9 Hz, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.626; HRMS (FAB) m/z calcd for $C_{14}H_{22}N_6O_8P$ (M + H)$^+$ 433.1237, found 433.1255.

(1(*S*)-Methylcarbamoylethyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (4b). 4a (49 mg) was dissolved in 2 mL of 10 M methylamine in methanol and stirred in a sealed vial for 5 d. The reaction mixture was then concentrated under reduced pressure. Flash chromatography (SiO_2, 5:2.5:0.37 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (41 mg, 84%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.5487 (d, J = 1.1 Hz, 1 H), 6.083 (t, J = 6.8 Hz, 1 H), 4.283 (m, 1 H), 3.988 (m, 1 H), 3.889−3.757 (m, 2 H), 3.440 (m, 1 H), 2.566 (s, 3 H), 2.313 (m, 2 H), 1.733 (d, J = 1.1 Hz, 3 H), 1.120 (d, J = 7.1 Hz, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.685; HRMS (FAB) m/z calcd for $C_{14}H_{22}N_7O_7P$ (M + H)$^+$ 432.1396, found 432.1383.

2(*S*)-(3-Deoxy-3-azidothymidyl(hydroxy)phosphorylamino)-3-methylbutyric Acid Methyl Ester (5a). The title compound was prepared from triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (204 mg, 0.471 mmol) and valine methyl ester (HCl salt, 158 mg, 0.942 mmol) following the procedure described for the preparation of **3a**. Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (154 mg, 68%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.573 (d, J = 1.1 Hz, 1 H), 6.077 (t, J = 6.7 Hz, 1 H), 4.293 (m, 1 H), 3.994 (m, 1 H), 3.907−3.752 (m, 2 H), 3.550 (s, 3 H), 3.338 (m, 1 H), 2.328 (m, 2 H), 1.778 (d, J = 1.1 Hz, 3 H), 1.772 (m, 1 H), 0.725 (d, J = 6.8 Hz, 3 H), 0.715 (d, J = 6.8 Hz, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 7.166; HRMS (FAB) m/z calcd for $C_{16}H_{26}N_6O_8P$ (M + H)$^+$ 461.1550, found 461.1550.

(2-Methyl-1(*S*)-methylcarbamoylpropyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (5b). 5a (62 mg) was dissolved in 2 mL of 10 M methylamine in methanol and stirred in a sealed vial for 50 d. The reaction mixture was then concentrated under reduced pressure. Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (40 mg, 65%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.567 (d, J = 1.1 Hz, 1 H), 6.082 (t, J = 6.8 Hz, 1 H), 4.257 (m, 1 H), 3.999 (m, 1 H), 3.895−3.738 (m, 2 H), 3.220 (m, 1 H), 2.583 (s, 3 H), 2.318 (m, 2 H), 1.828 (m, 1 H), 1.762 (d, J = 1.1 Hz, 3 H), 0.759 (d, J = 6.8 Hz, 3 H), 0.692 (d, J = 6.8 Hz, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.851; HRMS (FAB) m/z calcd for $C_{16}H_{27}N_7O_7P$ (M + H)$^+$ 460.1709, found 460.1730.

2(*S*)-(3-Deoxy-3-azidothymidyl(hydroxy)phosphorylamino)-4-methylvaleric Acid Methyl Ester (6a). The title compound was prepared from triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite and leucine methyl ester (HCl salt, 240 mg, 1.32 mmol) following the procedure described for the preparation of **3a**. Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (98 mg, 31%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.600 (d, J = 1.1 Hz, 1 H), 6.086 (t, J = 6.8 Hz, 1 H), 4.303 (m, 1 H), 3.987 (m, 1 H), 3.895−3.747 (m, 2 H), 3.538 (m, 1 H), 3.538 (s, 3 H), 2.324 (m, 2 H), 1.771 (d, J = 1.1 Hz, 3 H), 1.485 (m, 1 H), 1.335−1.278 (m, 2 H), 0.672 (d, J = 6.6 Hz, 6H); ^{31}P NMR (D_2O, 121 MHz) δ 6.701; HRMS (FAB) m/z calcd for $C_{17}H_{28}N_6O_8P$ (M + H)$^+$ 475.1706, found 475.1689.

(3-Methyl-1(*S*)-methylcarbamoylbutyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (6b). 6a (38 mg) was dissolved in 2 mL of 10 M methylamine in methanol and stirred in a sealed vial for 5 d. The reaction mixture was then concentrated under reduced pressure. Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (25 mg, 66%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.588 (d, J = 1.1 Hz, 1 H), 6.081 (t, J = 6.8 Hz, 1 H), 4.275 (m, 1 H), 3.995 (m, 1 H), 3.896−3.746 (m, 2 H), 3.403 (m, 1 H), 2.570 (s, 3 H), 2.320 (m, 2 H), 1.753 (d, J = 1.1 Hz, 3 H), 1.504 (m, 1 H), 1.352−1.225 (m, 2 H), 0.693 (d, J = 6.6 Hz, 6 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.359; HRMS (FAB) m/z calcd for $C_{17}H_{29}N_7O_7P$ (M + H)$^+$ 474.1866, found 474.1903.

2(*S*)-(3-Deoxy-3-azidothymidyl(hydroxy)phosphorylamino)-3-(4-hydroxyphenyl)proprionic Acid Methyl Ester (7a). The title compound was prepared from triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (243 mg, 0.561 mmol) and tyrosine methyl ester (219 mg, 1.12 mmol) following the procedure described for the preparation of **3a.** Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (149 mg, 49%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.401 (s, 1 H), 6.859 (d, J = 7.3 Hz, 2 H), 6.547 (d, J = 8.2 Hz, 2 H), 6.000 (t, J = 6.6 Hz, 1 H), 4.119 (m, 1 H), 3.845 (m, 1 H), 3.647 (m, 1 H), 3.620−3.540 (m, 2 H), 3.486 (s, 3 H), 2.718 (dd, J = 6.2, 13.5 Hz, 1 H), 2.591 (dd, J = 7.7, 13.5 Hz, 1 H), 2.230 (m, 1 H), 2.105 (m, 1 H), 1.662 (s, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.487; HRMS (FAB) m/z calcd for $C_{20}H_{26}N_6O_9P$ (M + H)$^+$ 525.1499, found 525.1517.

(2-(4-Hydroxyphenyl)-1(*S*)-methylcarbamoylethyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (7b). 7a (51 mg) was dissolved in 2 mL of 10 M methylamine

in methanol and stirred in a sealed vial for 5 d. The reaction mixture was then concentrated under reduced pressure. Flash chromatography (SiO_2, 5:2.5:0.37 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (36 mg, 71%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.401 (d, J = 1.0 Hz, 1 H), 6.932 (d, J = 8.6 Hz, 2 H), 6.581 (d, J = 8.6 Hz, 2 H), 5.978 (t, J = 6.6 Hz, 1 H), 4.040 (m, 1 H), 3.807 (m, 1 H), 3.550 (m, 1 H), 3.483−3.400 (m, 2 H), 2.799 (m, 1 H), 2.542 (s, 3 H), 2.534 (m, 1 H), 2.240 (m, 1 H), 2.093 (m, 1 H), 1.696 (d, J = 1.0 Hz, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 5.761; HRMS (FAB) m/z calcd for $C_{20}H_{27}N_7O_8P$ (M + H)$^+$ 524.1659, found 524.1635.

2(*S*)-(3-Deoxy-3-azidothymidyl(hydroxy)phosphoryl amino)-3-phenylproprionic Acid Methyl Ester (8a). The title compound was prepared from triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (277 mg, 0.640 mmol) and phenylalanine methyl ester (HCl salt, 276 mg, 1.28 mmol) following the procedure described for the preparation of **3a.** Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (216 mg, 64%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.424 (s, 1 H), 7.120−6.974 (m, 5 H), 5.998 (t, J = 6.7 Hz, 1 H), 4.153 (m, 1 H), 3.843 (m, 1 H), 3.728 (m, 1 H), 3.585 (m, 2 H), 3.491 (s, 3 H), 2.794 (m, 1 H), 2.699 (m, 1 H), 2.219 (m, 1 H), 2.116 (m, 1 H), 1.675 (s, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.370; HRMS (FAB) m/z calcd for $C_{20}H_{26}N_6O_8P$ (M + H)$^+$ 509.1550, found 509.1560.

(2-Phenyl-1(*S*)-methylcarbamoylethyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (8b). 4a (68 mg) was dissolved in 2 mL of 10 M methylamine in methanol and stirred in a sealed vial for 3 d. The reaction mixture was then concentrated under reduced pressure. Flash chromatography (SiO_2, 5:2.5:0.37 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (67 mg, 99%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.419 (d, J = 1.0 Hz, 1 H), 7.161−7.047 (m, 5 H), 5.978 (t, J = 6.7 Hz, 1 H), 4.083 (m, 1 H), 3.812 (m, 1 H), 3.629 (m, 1 H), 3.504−3.365 (m, 2 H), 2.866 (ddd, J = 1.8, 5.3, 13.7 Hz, 1 H), 2.669 (dd, J = 8.1, 13.7 Hz, 1 H), 2.541 (s, 3 H), 2.236 (ddd, J = 4.2, 6.4, 14.1 Hz, 1 H), 2.112 (m, 1 H), 1.707 (d, J = 1.0 Hz, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 5.723; HRMS (FAB) m/z calcd for $C_{20}H_{27}N_7O_7P$ (M + H)$^+$ 508.1709, found 508.1685.

2(*S*)-(Hydroxy(3-deoxy-3-azidothymidyl)phosphorylamino)-3-(3-indolyl)proprionic Acid Methyl Ester (9a). Triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (218 mg, 0.503 mmol) was dissolved in 7 mL of dry pyridine and treated with TMSCl (167 μL, 1.32 mmol) under Ar. After 5 min, a solution of iodine (167 mg, 0.659 mmol) in 3 mL of pyridine was added dropwise, via cannula, until the reaction color changed from yellow to a reddish-brown color. At this point, addition of the iodine solution was stopped. After 5 min, tryptophan methyl ester (HCl salt, 258 mg, 1.01 mmol) and Et_3N (0.43 mL, 3.08 mmol) were added. After stirring for 30 min, the reaction mixture was concentrated under reduced pressure. The resulting syrup was partitioned between 1N NH_4OH solution and $CHCl_3$. The aqueous portion was then extracted with additional portions of $CHCl_3$ to remove unreacted amino acid methyl ester. The aqueous portion was then concentrated to give crude phosphoramidate, which was passed through a small Amberlite (IRP-64) ion exchange column, eluting with H_2O, and the desired fractions were concentrated under reduced pressure. Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (198 mg, 70%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.222 (d, J = 7.9 Hz, 1 H), 7.103 (d, J = 8.1 Hz, 1 H), 7.072 (s, 1 H), 6.880 (s, 1 H), 6.857 (t, J = 7.7 Hz, 1 H), 6.729 (t, J = 7.5 Hz, 1 H), 5.690 (t, J = 6.6 Hz, 1 H), 3.883 (m, 1 H), 3.763 (m, 1 H), 3.635 (m, 1 H), 3.527 (m, 2 H), 3.448 (s, 3 H), 2.899 (m, 1 H), 2.765 (m, 1 H), 1.857 (m, 1 H), 1.682 (m, 1 H), 1.493 (s, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.412; HRMS (FAB) m/z calcd for $C_{20}H_{27}N_7O_8P$ (M + H)$^+$ 548.1659, found 548.1677.

(2-(3-Indolyl)-1(*S*)-methylcarbamoylethyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (9b). 3a (62 mg) was dissolved in 2 mL of 10 M methylamine in methanol and stirred in a sealed vial for 3 d. The reaction mixture was then concentrated under reduced pressure. Flash chromatography (SiO_2, 5:2.5:0.37 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (62 mg, 100%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.397 (d, J = 7.9 Hz, 1 H), 7.187 (d, J = 8.2 Hz, 1 H), 7.135 (s, 1 H), 7.011 (s, 1 H), 6.944 (dd, J = 7.0, 8.2 Hz, 1 H), 6.836 (dd, J = 7.0, 7.9 Hz, 1 H), 5.763 (t, J = 6.7 Hz, 1 H), 3.943 (m, 1 H), 3.715−3.634 (m, 2 H), 3.520−3.395 (m, 2 H), 3.050 (ddd, J = 2.4, 4.4, 14.6 Hz, 1 H), 2.780 (dd, J = 8.2, 14.6 Hz, 1 H), 2.557 (s, 3 H), 2.014 (m, 1 H), 1.770 (m, 1 H), 1.554 (s, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.049; HRMS (FAB) m/z calcd for $C_{22}H_{27}N_8O_7PNa$ (M + Na)$^+$ 569.1638, found 569.1678.

(2-(3-Indolyl)-1(*S*)-ethylcarbamoylethyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (9c). The title compound was prepared from triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (138 mg, 0.319 mmol) and tryptophan ethyl amide (111 mg, 0.479 mmol) following the procedure described for the preparation of **3a.** Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (103 mg, 56%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.433 (d, J = 7.9 Hz, 1 H), 7.221 (d, J = 8.1 Hz, 1 H), 7.207 (s, 1 H), 7.030 (s, 1 H), 6.978 (m, 1 H), 6.871 (m, 1 H), 5.832 (t, J = 6.6 Hz, 1 H), 4.012 (m, 1 H), 3.768 (m, 1 H), 3.691 (m, 1 H), 3.538 (m, 2 H), 3.053 (m, 1 H), 3.010 (q, J = 7.3 Hz, 2 H), 2.855 (dd, J = 7.5, 14.5 Hz, 1 H), 2.080 (m, 1 H), 1.868 (m, 1 H), 1.575 (s, 3 H), 0.837 (t, J = 7.3 Hz, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.140; HRMS (FAB) m/z calcd for $C_{23}H_{30}N_8O_7P$ (M + H)$^+$ 561.1975, found 561.1993.

(2-(3-Indolyl)-1(*S*)-isopropylcarbamoylethyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (9d). The title compound was prepared from triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (116 mg, 0.268 mmol) and tryptophan isopropyl amide (98 mg, 0.402 mmol) following the procedure described for the preparation of **3a.** Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (44 mg, 28%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.426 (d, J = 7.9 Hz, 1 H), 7.232 (s, 1 H),7.219 (d, J = 7.9 Hz, 1 H), 7.010 (s, 1 H), 6.975 (m, 1 H), 6.872 (m, 1 H), 5.857 (t, J = 6.7 Hz, 1 H), 4.051 (m, 1 H), 3.793 (m, 1 H), 3.713−3.624 (m, 2 H), 3.583 (m, 2 H), 3.011 (dd, J = 5.5, 14.6 Hz, 1 H), 2.882 (dd, J = 7.1, 14.6 Hz, 1 H), 2.110 (m, 1 H), 1.929 (m, 1 H), 1.566 (s, 3 H), 0.837 (d, J = 6.1 Hz, 3 H), 0.816 (d, J = 6.1 Hz, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.161; HRMS (FAB) m/z calcd for $C_{24}H_{32}N_8O_7P$ (M + H)$^+$ 575.2131, found 575.2093.

(2-(3-Indolyl)-1(*S*)-cyclopropylcarbamoylethyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (9e). The title compound was prepared from triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (166 mg, 0.383 mmol) and tryptophan cyclopropyl amide (140 mg, 0.575 mmol) following the procedure described for the preparation of **3a.** Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (98 mg, 43%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.409 (d, J = 7.9 Hz, 1 H), 7.211 (d, J = 7.3 Hz, 1 H), 7.201 (d, J = 1.0 Hz, 1 H), 6.991 (s, 1 H), 6.970 (m, 1 H), 6.865 (m, 1 H), 5.824 (t, J = 6.6 Hz, 1 H), 4.021 (m, 1 H), 3.771 (m, 1 H), 3.664 (m, 1 H), 3.554 (m, 2 H), 2.996 (m, 1 H), 2.845 (dd, J = 7.3, 14.5 Hz, 1 H), 2.341 (m, 1 H), 2.078 (m, 1 H), 1.876 (m, 1 H), 1.560 (d, J = 1.0 Hz, 3 H), 0.506 (m, 2 H), 0.179 (m, 2 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.113; HRMS (FAB) m/z calcd for $C_{24}H_{30}N_8O_7P$ (M + H)$^+$ 573.1975, found 573.1980.

(2-(3-Indolyl)-1(*S*)-cyclohexylcarbamoylethyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (9f). The title compound was prepared from triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (207 mg, 0.478 mmol) and tryptophan cyclohexyl amide (204 mg, 0.717 mmol) following the procedure described for the preparation of **3a.** Flash chromatography (SiO_2, 7:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product

(156 mg, 52%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.415 (d, J = 7.9 Hz, 1 H), 7.262 (d, J = 1.0 Hz, 1 H), 7.224 (d, J = 8.1 Hz, 1 H), 6.996 (s, 1 H), 6.976 (m, 1 H), 6.875 (m, 1 H), 5.878 (t, J = 6.7 Hz, 1 H), 4.084 (m, 1 H), 3.812 (m, 1 H), 3.694 (m, 1 H), 3.633 (m, 2 H), 3.282 (m, 1 H), 2.961 (m, 2 H), 2.133 (m, 1 H), 1.986 (m, 1 H), 1.560 (d, J = 1.0 Hz, 3 H), 1.473−1.341 (m, 5 H), 1.107−0.94 (m, 2 H), 0.924−0.745 (m, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.242; HRMS (FAB) m/z calcd for $C_{27}H_{36}N_8O_7P$ $(M + H)^+$ 615.2444, found 615.2473.

2(*R*)-(3-Deoxy-3-azidothymidyl(hydroxy)phosphorylamino)-3-phenylbutyric Acid Methyl Ester (10a). The title compound was prepared from triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (340 mg, 0.785 mmol) and D-phenylalanine methyl ester (HCl salt, 339 mg, 1.57 mmol) following the procedure described for the preparation of **3a.** Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (282 mg, 68%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.415 (s, 1 H), 7.158−6.998 (m, 5 H), 5.995 (t, J = 6.8 Hz, 1 H), 4.118 (m, 1 H), 3.808 (m, 1 H), 3.738 (m, 1 H), 3.661 (m, 1 H), 3.467 (m, 1 H), 3.403 (s, 3 H), 2.754 (m, 2 H), 2.212 (m, 2 H), 1.679 (s, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.204; HRMS (FAB) m/z calcd for $C_{20}H_{26}N_6O_8P$ $(M + H)^+$ 509.1550, found 509.1548.

(2-Phenyl-1(*R*)-methylcarbamoylethyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (10b). 4c (66 mg) was dissolved in 2 mL of 10 M methylamine in methanol and stirred in a sealed vial for 3 d. The reaction mixture was then concentrated under reduced pressure. Flash chromatography (SiO_2, 5:2.5:0.37 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (56 mg, 85%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.387 (d, J = 1.1 Hz, 1 H), 7.193−7.032 (m, 5 H), 6.022 (t, J = 6.7 Hz, 1 H), 4.066 (m, 1 H), 3.807 (m, 1 H), 3.653−3.532 (m, 2 H), 3.295 (m, 1 H), 2.822−2.687 (m, 2 H), 2.470 (s, 3 H), 2.308−2.169 (m, 2 H), 1.657 (d, J = 1.1 Hz, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 5.713; HRMS (FAB) m/z calcd for $C_{20}H_{27}N_7O_7P$ $(M + H)^+$ 508.1709, found 508.1670.

2(*R*)-(3-Deoxy-3-azidothymidyl(hydroxy)phosphorylamino)-3-(3-indolyl)proprionic Acid Methyl Ester (11a). The title compound was prepared from triethylammonium 3′-azido-3′-deoxythymidine 5′-phosphite (312 mg, 0.721 mmol) and D-tryptophan methyl ester (HCl salt, 367 mg, 1.44 mmol) following the procedure described for the preparation of **3a.** Flash chromatography (SiO_2, 5:2:0.25 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (256 mg, 63%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.299 (d, J = 7.9 Hz, 1 H), 7.182 (d, J = 8.1 Hz, 1 H), 7.141 (s, 1 H), 6.939 (s, 1 H), 6.930 (dd, J = 7.0, 8.1 Hz, 1 H), 6.816 (t, J = 7.0, 7.9 Hz, 1 H), 5.773 (t, J = 6.8 Hz, 1 H), 3.851−3.741 (m, 2 H), 3.691 (m, 1 H), 3.605 (m, 1 H), 3.357 (s, 3 H), 3.332 (m, 1 H), 2.882 (m, 2 H), 2.002 (m, 1 H), 1.815 (m, 1 H), 1.504 (s, 3 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.396; HRMS (FAB) m/z calcd for $C_{20}H_{27}N_7O_8P$ $(M + H)^+$ 548.1659, found 548.1681.

(2-(3-Indolyl)-1(*R*)-methylcarbamoylethyl)phosphoramidic Acid Mono(3-deoxy-3-azidothymidyl)ester (11b). 3c (65 mg) was dissolved in 2 mL of 10 M methylamine in methanol and stirred in a sealed vial for 3 d. The reaction mixture was then concentrated under reduced pressure. Flash chromatography (SiO_2, 5:2.5:0.37 $CHCl_3$/MeOH/H_2O containing 0.5% concentrated NH_4OH) gave the desired product (64 mg, 99%) as a white solid: ^{1}H NMR (D_2O, 300 MHz) δ 7.425 (d, J = 7.9 Hz, 1 H), 7.267 (d, J = 7.3 Hz, 1 H), 7.193 (d, J = 1.1 Hz, 1 H), 7.020 (s, 1 H), 7.007 (m, 1 H), 6.885 (m, 1 H), 5.860 (t, J = 6.8 Hz, 1 H), 3.779 (m, 1 H), 3.725 (m, 1 H), 3.647−3.552 (m, 2 H), 3.177 (m, 1 H), 3.025 (m, 1 H), 2.835 (dd, J = 7.5, 14.5 Hz, 1 H), 2.472 (s, 3 H), 2.121 (m, 1 H), 1.862 (m, 1 H), 1.525 (d, J = 1.1 Hz, 1 H); ^{31}P NMR (D_2O, 121 MHz) δ 6.028; HRMS (FAB) m/z calcd for $C_{22}H_{28}N_8O_7P$ $(M + H)^+$ 547.1818, found 547.1807.

MCF-7 Cytotoxicity Assay Procedure. Cytotoxic activity of the compounds was based on the inhibition of MCF-7 cell growth. The MCF-7 cells were maintained in medium containing RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, penicillin (124 units/ml of medium), and streptomycin (0.125 mg/mL of medium). Approximately, 5×10^4 to 6×10^4 cells were introduced per well in four six-well plates, and 25 μL of compounds were added to the cells as solutions in 1 × PBS (phosphate buffered saline) to achieve final concentrations of 0.001, 0.01, 0.1, 1, 10, and 100 μM, the final volume being adjusted to 5 mL with medium. The cells were cultured with and without compound at 37 °C in a 10% CO_2−90% air environment for 48 h, following which trypsin was added and removed after allowing it to remain in contact with the cells for 15 to 25 s. After 4 to 6 min, 500 μL of medium was added to reconstitute the cells in a suspension. The viability was determined by taking 10 μL aliquots and diluting them 10-fold with trypan blue dye, according to the trypan blue dye exclusion method.[46,47] The assay was carried out in triplicate, with six wells allotted for control (no compound).

Intracellular Concentrations of AZT and Phosphorylated AZT in CEM and MCF-7 Cells. This procedure is similar to the assay previously reported with the following modifications.[33] Approximately 1.5×10^6 MCF-7 cells and 3×10^6 CEM cells were incubated separately with 100 μM compound for 17 h, following which CEM cell cultures were centrifuged to remove medium, and medium was siphoned off MCF-7 cells. Both cell types were washed three times with 1× PBS before they were treated with 60% methanol and left to lyse overnight. The cell lysate suspensions were centrifuged; the supernatants were dried and reconstituted in 20 μL water for purification by RP-HPLC. The AZT phosphate, AZT, and AZT phosphoramidate fractions were isolated for each sample and dried. The phosphorylated AZT fractions (phosphates and phosphoramidate) were dephosphorylated using 100 μL of potato acid phosphatase and 150 μL of potassium hydrogen phthalate buffer for 4 h at 37 °C, following which they were dried. The residues and the dried AZT fractions were then analyzed by RIA after reconstituting with 1 mL of FPIA buffer to determine the amounts of AZT, from which were extrapolated the amounts of phosphorylated AZT.

Spectrophotometric Determination of Partition Coefficients. Concentration curves for each compound in octanol and water were constructed with a minimum of six data points, in duplicate, on a Beckman DU7400 spectrophotometer fitted with a 12-well microcell adapter. The concentrations for the curves, which depended on the ultraviolet absorbance of the compounds, ranged from 2 to 300 μM, but only concentrations in the linear range of the instrument (0−2.7 absorbance units) were used for the standard curves. Extinction coefficients (ϵ) were determined for each compound in octanol and water: 4 mL each of octanol and water were mixed and equilibrated in a mechanical shaker at 20 cycles/min for 6 h and allowed to stand overnight. Approximately 0.5 mg of each compound was weighed and added to the equilibrated solvent mixtures in duplicate, and then resealed with a plastic cap containing a Teflon insert. The tubes were shaken vigorously and placed on a mechanical shaker (20 cycles/minute) for 1 h at room temperature. This was followed by centrifugation for 1 h at 1000 rpm, and then the system was left to stand at room temperature for 30 h to achieve equilibrium. After this, 250 μL samples were withdrawn from each phase, in duplicate, and the ultraviolet absorbance determined with either octanol or water as blank. The concentration of compound in each phase was determined using the previously determined extinction coefficients and the Beer−Lambert equation. The log P values were determined in duplicate or triplicate and are reported as the average of the two values that were within 15% variance.

Acknowledgment. Partial financial support is gratefully acknowledged from NIH (CA61908), Advanced Magnetics, Inc., and the University of Minnesota Graduate School. We also thank Dr. Rory P. Remmel for his many helpful suggestions throughout the course of this study.

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JM000110G