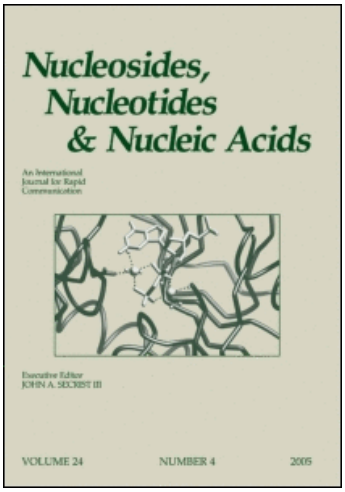

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A Phosphoramidite-Based Synthesis of Phosphoramidate Amino Acid Diesters of Antiviral Nucleosides

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A Phosphoramidite-Based Synthesis of Phosphoramidate Amino Acid Diesters of Antiviral Nucleosides

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Abstract: *A general synthetic procedure is presented for the preparation of 5'-amino acid phosphoramidates of zidovudine (AZT), 3'-deoxy-2',3'-didehydro-thymidine (D4T), and 3'-fluoro-3'-deoxythymidine (FLT) from their corresponding phosphoramidites. These water soluble amino acid phosphoramidates are more non-polar than the parent nucleoside and exhibit high stability in aqueous media.*

Due to the debilitating and ultimately fatal nature of AIDS, an intense effort has been underway to improve existing treatments and to develop new therapies. Currently, the only therapeutic agents approved by the FDA for the clinical treatment of AIDS are the HIV reverse transcriptase inhibitors, zidovudine (AZT, **1a**), dideoxyinosine (ddI), and dideoxycytidine (ddC). Recently, the nucleosides, 3'-fluoro-3'-deoxythymidine (FLT, **1b**) and 3'-deoxy-2',3'-didehydrothymidine (D4T, **1c**), have shown significant anti-HIV activity and are currently being evaluated clinically. Formally, these nucleosides are prodrugs, since upon being transported across the cellular membrane they must be converted by nucleoside kinases to their respective mono-, di-, and tri- phosphate derivatives.

Unfortunately, the efficacy of antiviral nucleosides, such as D4T, maybe limited by by the rate of intracellular phosphorylation to the active tri-phosphates.[1] Furthermore, the low levels of nucleoside kinases in monocytes and macrophages, which harbor large

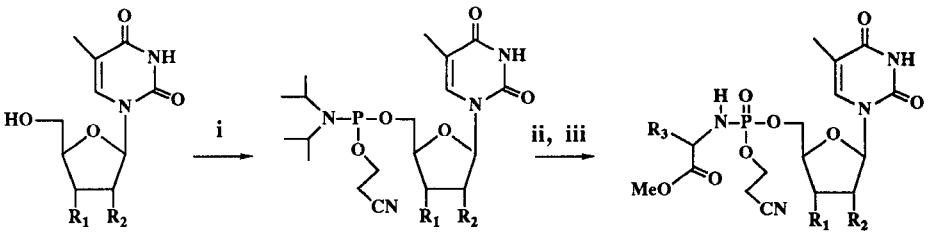
reservoirs of the virus, maybe responsible for the lack of efficacy of several antiviral nucleosides against viral proliferation in these tissues.[2-4] Although phosphate derivatives would in principle overcome this drawback, they are unable to cross cellular membranes due to their charge. Consequently, in order to increase their therapeutic index, several prodrug approaches have been explored, which rely on increasing the hydrophobicity of antiviral nucleotides.[5-10] In each of these cases a strategy was developed based on delivery of the mono-phosphate of the parent nucleoside as a hydrophobic triester. Interestingly, alkyl phosphate diesters of AZT and D4T prepared by phosphoramidite chemistry have exhibited greater activity against viral replication than the corresponding triesters.[7,10]

Recently, McGuigan and co-workers [11-14] constructed hydrophobic alkyl and aryl triesters and phosphoramidates of AZT, FLT, and D4T with phosphorochloridate chemistry. Glycine, alanine, leucine, and phenylalanine derivatives were shown to be highly effective and selective inhibitors of HIV viral replication in the T-lymphoblastoid cell lines, CEM and MT-4. Because phosphate diesters have at times exhibited a greater efficacy at inhibiting viral replication, we have chosen to construct hydrophobic amino acid diester nucleoside phosphoramidates, in order to evaluate their antiviral activity and provide useful information regarding the mechanism of phosphoramidate triester activation.

Previously, Caruthers and coworkers demonstrated that *N*-butyl amine in large excess (250/1) reacted with 2-cyano-1,1-dimethylethyldeoxydinucleoside phosphites, in the presence of iodine, to form the corresponding phosphoramidates.[15] Consequently, although this procedure might necessitate the use of large excesses of amino acid for the production of amino acid phosphoramidates, we chose to develop a synthetic strategy based on this procedure, since the synthesis of the nucleoside phosphites and their conversion to the corresponding phosphoramidates utilizes highly efficient chemistry. Our preliminary studies have focused on the construction of AZT, D4T, and FLT derivatives.

Results and Discussion

The preparation of amino acid phosphoramidate diesters of AZT, FLT, and D4T (**5a-6c**) is shown in Schemes 1 and 2. Synthesis of the cyanoethyl protected phosphoramidates, **3a-4c**, began by treating the nucleosides (**1a-1c**) with two equivalents of 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite in acetonitrile or dichloromethane. In each case the resulting phosphoramidites (**2a-2c**) were found to be more nonpolar than their parent nucleosides as judged by thin-layer chromatography (hexane:ethyl acetate, 1:1). Typically, the reaction was quenched with base, and after work-up, **2b** was purified by flash chromatography on silica gel (hexane:ethyl acetate, 1:1) and obtained in 96% yield. The phosphoramidite of D4T (**2c**) was not isolated by column chromatography due to its inherent instability.



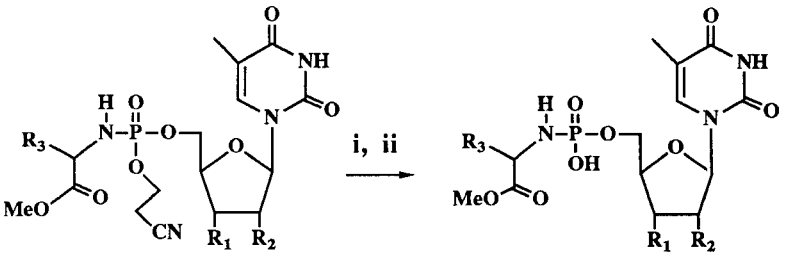
1a, R_1= N_3, R_2 = H
1b, R_1= F, R_2 = H
1c, R_1, R_2 = double bond

2a, R_1= N_3, R_2 = H
2b, R_1= F, R_2 = H
2c, R_1, R_2= double bond

3a, R_1= N_3, R_2 = H, R_3= Phe
3b, R_1= F, R_2 = H, R_3= Phe
3c, R_1, R_2= double bond, R_3= Phe
4a, R_1= N_3, R_2 = H, R_3= Trp
4b, R_1= F, R_2 = H, R_3= Trp
4c, R_1, R_2= double bond, R_3= Trp

i) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, iPr_2NEt, CH_3CN or CH_2Cl_2
ii) Methanol, tetrazole, CH_3CN
iii) L-phenylalanine or L-tryptophan methyl ester, I_2, CH_3CN or THF

Scheme 1



3a, R_1= N_3, R_2= H, R_3= Phe
3b, R_1= F, R_2= H, R_3= Phe
3c, R_1, R_2= double bond, R_3= Phe
4a, R_1= N_3, R_2= H, R_3= Trp
4b, R_1= F, R_2= H, R_3= Trp
4c, R_1,R_2= double bond, R_3= Trp

5a, R_1= N_3, R_2= H, R_3= Phe
5b, R_1= F, R_2= H, R_3= Phe
5c, R_1, R_2= double bond, R_3= Phe
6a, R_1= N_3, R_2= H, R_3= Trp
6b, R_1= F, R_2= H, R_3= Trp
6c, R_1,R_2= double bond, R_3= Trp

i) NH_3, Methanol
ii) Amberlite CG-50 (H^+)

Scheme 2

After resuspension in acetonitrile, **2b** and **2c** were converted to their corresponding methyl phosphites by treatment with tetrazole and methanol. The solvent was removed, and the crude product mixture was redissolved in dry acetonitrile or THF, followed by the addition of either phenylalanine methyl ester or tryptophan methyl ester and iodine. In the case of AZT (**1a**), the phosphoramidates (**3a** and **4a**) were prepared in one pot reactions without isolation of the intermediate phosphoramidites.[7] The reaction mixture was quenched with a sodium bisulfite solution and extracted with dichloromethane. The crude product mixture was purified by column chromatography on silica gel, to give **3a-4c** in 35-79% yield. After isolation, the cyanoethyl group was removed from **3a-4c** by treatment with ammonia and methanol (Scheme 2). The ammonium phosphoramidate salts were then passed through a cation exchange column (H^+) to give **5a-6c** in 86-100% yield.

Reverse-phase HPLC analysis of the amino acid phosphoramidates revealed that they were significantly more hydrophobic (i.e., r.t.= 10-12 mins.) than the parent nucleosides (i.e., r.t.= 7-9 mins.) or mono-phosphates (r.t.= 1-2 mins.), but less hydrophobic than the corresponding cyanoethyl triesters (i.e., r.t.= 16-18 mins.). Furthermore, when the amino acid phosphoramidate diesters, **5a** and **6a**, were incubated at 37°C for 6 hours in either distilled water or 10% fetal calf serum, no detectable degradation to the corresponding nucleosides or mono-phosphates was detectable.

In summary, a reasonable synthetic procedure has been developed for the construction of amino acid phosphoramidates of AZT, FLT, and D4T with overall yields for esters ranging from 30% to 77%. Unlike previous phosphoramidite based syntheses of nucleoside phosphoramidates, which require hundreds of equivalents of the amine, amino acid phosphoramidates were synthesized with modest excesses (i.e., 4 to 7 equivalents) of the amino acid. The anti-HIV activities of these chemically stable and hydrophobic phosphoramidates prepared in this report are currently under investigation and will be reported in due course.

Experimental

NMR (^1H and ^{31}P) spectra were recorded on Varian VXR-300 and GE Omega-300 spectrometers. An external standard of 85% H_3PO_4 was used for all ^{31}P-NMR spectra. * Refers to doubling of peaks in the NMR spectrum due to the presence of diastereoisomers. FAB mass spectra were obtained on a VG 7070E-HF mass spectrometer. Analytical TLC was performed on Analtech Silica Gel GHLF (0.25 mm) or Machery-Nagel Polygram Sil G/UV_{254} (0.2 mm) plates. Column chromatography was performed using grade 62, 60-200 mesh silica gel from Aldrich Chem. Co. Flash chromatography was performed using grade 60, 230-400 mesh Merck silica gel from Aldrich Chem. Co. MPLC was performed at a pressure of 10-12 psi using an FMI lab pump equipped with a flow meter and pulse dampener. A silica gel column (1" x 36", 230-400 mesh) was used with a solvent flow

rate of 1.5-2.0 mL/min. 2-Cyanoethyl-*N,N*-diisopropylchlorophosphoramidite was purchased from Aldrich Chem. Co. THF was distilled, under nitrogen, from sodium-benzophenone ketyl just prior to use. Methanol was distilled from magnesium methoxide and stored over 3Å molecular sieves. Dichloromethane and acetonitrile were distilled from P_2O_5 and stored over 3Å molecular sieves. All other solvents were reagent grade and used as received. 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.

3'-Azido-3'-deoxythymidine-5'-(2-cyanoethyl methoxyphenylalaninyl) phosphoramidate (3a):

AZT (**1a**) (1.40 g, 5.24 mmol) was dissolved in dry acetonitrile (20 mL) under nitrogen and cooled to 0°C in an ice bath. Diisopropylethylamine (2.74 mL, 15.72 mmol) was added to the solution by syringe followed by 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (2.34 mL, 10.48 mmol). After stirring at 0°C for 30 mins, little remaining AZT could be detected by TLC (hexane-ethyl acetate, 1/1) in the reaction mixture. Tetrazole (1.47 g, 20.96 mmol) was added and after 5 minutes methanol (0.64 mL, 15.72 mmol) was added. While stirring, the reaction mixture was allowed to warm to r.t. over the course of an hour.

Then phenylalanine methyl ester (5.50 g, 30.7 mmol) was dissolved in dry acetonitrile (10 mL) and added to the reaction mixture followed by the addition of iodine (2.66 g, 10.48 mmol). The reaction mixture was stirred at r.t. under nitrogen for 2 hrs, after which the excess iodine was quenched by the addition of ~1 mL of a saturated $NaHSO_3$ solution. Water (20 mL) was added and the resulting mixture extracted with dichloromethane (4 x 15 mL). The organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. After silica gel MPLC (chloroform-methanol gradient), the product was isolated as a colorless, crystalline solid (1.1 g, 38% yield). ^{1}H-NMR ($CDCl_3$): δ 9.4* (1H, s, H3(NH)), 7.4-7.1 (6H, m, H6', Ph), 6.1* (1H, t, H1'), 4.3 (1H, m, H3'), 4.3-3.5 (7H, m, H4', H5', OCH_2CH_2CN, $CHCO_2Me$, NHP(O)), 3.7* (3H, s, CO_2CH_3), 3.1 (1H, m, $PhCH_2$), 2.8 (1H, m, $PhCH_2$), 2.6 (2H, m, CH_2CN), 2.3 (1H, m, H2'), 2.2 (1H, m, H2'), 1.9 (3H, s, 5-Me). ^{31}P-NMR: δ 8.11 and 8.31 (diastereoisomers). FABMS : m/e $[M+H]^+$ 562.16. Anal. Calcd. for $C_{23}H_{28}N_7O_8P$: C, 49.20; H, 5.03; N, 17.46. Found: C, 48.98; H, 5.10; N, 17.53.

3'-Azido-3'-deoxythymidine-5'-methoxyphenylalaninylphosphoramidate (5a):

3'-Azido-3'-deoxythymidine-5'-(2-cyanoethyl methoxyphenylalaninyl) phosphoramidate (**3a**) (36.8 mg, 0.066 mmol) was dissolved in dry methanol (5 mL)

under nitrogen, and 3 mL of a saturated solution of NH_3 in methanol was added. The reaction mixture was allowed to stir at r.t. for 1 hour, and then concentrated under reduced pressure. The product was purified by silica gel column chromatography (chloroform-methanol-water, 5/3/0.5), yielding 3'-azido-3'-deoxythymidine-5'-(ammonium methoxyphenylalaninyl) phosphoramidate as a colorless solid (30.3 mg 88% yield). ^{1}H-NMR (CD_3OD-D_2O): δ 7.65 (1H, s, H6), 7.25-7.15 (5H, m, Phe), 6.2 (1H, t, H1'), 4.35 (1H, m, H3'), 4.0 (1H, m, H4'), 3.95 (1H, m, C<u>H</u>CO_2Me), 3.8 (2H, m, H5'), 3.65 (3H, s, CO_2CH_3), 2.95 (2H, m, PheC<u>H</u>$_2$), 2.35 (2H, m, H2'), 1.9 (3H, s, 5-CH_3). ^{31}P-NMR: δ 7.25.

3'-Azido-3'-deoxythymidine-5'-(ammonium methoxyphenylalaninyl) phosphoramidate (27 mg) was dissolved in water and passed through an Amberlite CG-50 (H^+) column (1.5 cm. x 8 cm.). After elution with water, the 5 mL fractions containing the product were combined and lyophilized, yielding the product as a colorless solid (26 mg, 100% yield). ^{1}H-NMR (D_2O): δ 7.6 (1H, s, H6), 7.3-7.1 (5H, m, Phe), 6.15 (1H, t, H1'), 4.3 (1H, m, H3'), 4.0 (1H, m, H4'), 3.85 (1H, m, C<u>H</u>CO_2Me), 3.75 (2H, m, H5'), 3.6 (3H, s, CO_2CH_3), 2.9 (2H, m, PheC<u>H</u>$_2$), 2.3 (2H, m, H2'), 1.8 (3H, s, 5-CH_3). ^{31}P-NMR: δ 6.99.

3'-Azido-3'-deoxythymidine-5'-(2-cyanoethyl methoxytryptophanyl) phosphoramidate (4a):

The same procedure was followed as for the preparation of the phenylalanine derivative **(3a)**. The product was obtained as a colorless, crystalline solid (0.1572 g, 35% yield). ^{1}H-NMR ($CDCl_3$): δ 9.7 (1H, broad s, indole NH), 8.85 (1H, broad s, H3(NH)), 7.65* (1H, s, H6), 7.5-7.1 (5H, m, indole aromatic Hs), 6.1 (1H, t, H1'), 4.4-3.9 (6H, m, H3', H4', H5', C<u>H</u>CO_2Me, NHP(O)), 3.8 (3H, s, CO_2CH_3), 3.65 (2H, m, O<u>C</u>H_2CH_2CN), 3.4 (1H, m, TrpC<u>H</u>$_2$), 3.2 (1H, m, TrpC<u>H</u>$_2$), 2.7-2.0 (4H, m, CH_2CN, H2'), 1.9 (3H, s, 5-CH_3). ^{31}P-NMR: δ 9.09. FABMS: m/e $[M+H]^+$ 601.3

3'-Azido-3'-deoxythymidine-5'-methoxytryptophanylphosphoramidate (6a):

The same procedure was followed as for the preparation of the phenylalanine derivative **(5a)**. The product was obtained as a colorless solid (53.3 mg, 86% yield). ^{1}H-NMR (CD_3OD-D_2O): δ 7.5 (1H, d, indole H4), 7.3 (1H, d, indole H7), 7.25 (1H, s, H6), 7.1 (1H, s, indole H2), 7.05 (1H, t, indole H6), 6.95 (1H, t, indole H5), 5.95 (1H, t, H1'), 4.15 (1H, m, H3'), 3.9 (2H, m, H4', C<u>H</u>CO_2Me), 3.75 (2H, m, H5'), 3.65 (3H, s, CO_2CH_3), 3.1 (1H, m, TrpC<u>H</u>$_2$), 2.95 (1H, m, TrpC<u>H</u>$_2$), 2.15 (1H, m,H2'), 1.95 (1H, m, H2'), 1.6 (3H, s, 5-CH_3). ^{31}P-NMR: δ 7.08.

3'-Fluoro-3'-deoxythymidine-5'-(2-cyanoethyl diisopropylamino) phosphoramidite (2b):

3'-Fluoro-3'-deoxythymidine **(1b)** (1.0 g, 4.1 mmol) was placed in a dry flask under nitrogen and dissolved in dry methylene chloride (50 mL). Diisopropylethylamine (2.86 mL, 16.4 mmol) was added to the solution via syringe followed by 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.83 mL, 8.2 mmol) . After stirring at r.t. for 1 hour, an ice-cold solution of 10% $NaHCO_3$ (30 mL) was added. The phases were separated and the organic phase washed with saturated NaCl (2 x 20 mL) and water (20 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product. Further purification of the product by silica gel flash chromatography (hexane-ethyl acetate, 2/3) produced a colorless, hygroscopic, crystalline solid (1.738g, 96% yield). ^{1}H-NMR ($CDCl_3$): δ ~9 (1H, broad s, H3(NH)), 7.5* (1H, s, H6), 6.3* (1H, t, H1'), 5.3* (1H, dd, H3'), 4.3* (1H, d, H4'), 3.8 (4H, m, H5', $OC\underline{H}_2CH_2CN$), 3.5 (2H, m, $C\underline{H}(CH_3)_2$), 2.6* (2H, m, CH_2CN), 2.0 (2H, m, H2'), 1.8 (3H, s, 5-CH3), 1.1* (12H, d, $(C\underline{H}_3)_2CH$). ^{31}P-NMR ($CDCl_3$): δ 149.65.

3'-Fluoro-3'-deoxythymidine-5'-(2-cyanoethyl methoxyphenylalaninyl) phosphoramidate (3b):

3'-Fluoro-3'-deoxythymidine-5'-(2-cyanoethyl diisopropylamino) phosphoramidite **(2b)** (0.736 g, 1.66 mmol) was dissolved in dry acetonitrile (20 mL) under nitrogen. Tetrazole (0.464 g, 6.63 mmol) was added and the reaction mixture stirred at r.t. for 5 mins. Next, dry methanol (0.134 mL, 3.31 mmol) was added, and the solution allowed to stir for an additional 2 hours. All the volatiles were removed under reduced pressure and the residue re-dissolved in freshly distilled THF (50 mL) under nitrogen. Phenylalanine methyl ester (1.2 g, 6.7 mmol) was added to the reaction mixture followed by iodine (0.421 g, 1.66 mmol). After stirring at r.t. for 3 hours, a solution of saturated $NaHSO_3$ (2 mL) and water (30 mL) was added and the resulting mixture extracted with methylene chloride (4 x 20 mL). The organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product. After further purification by silica gel MPLC (chloroform-methanol gradient), the product was isolated as a colorless, crystalline solid (0.626 g, 70% yield). ^{1}H-NMR ($CDCl_3$): δ 9.2* (1H, broad s, H3(NH)), 7.4 (1H, s, H6), 7.3-7.1 (5H, m, Phe), 6.3* (1H, m, H1'), 5.2* (1H, m, H3'), 4.3* (1H, m, H4'), 4.1-3.8 (4H, m, H5', $C\underline{H}CO_2Me$, NHP(O)), 3.7 (3H, s, CO_2CH_3), 3.65 (1H, m, $OC\underline{H}_2CH_2CN$), 3.15 (1H, m, $PhC\underline{H}_2$), 2.8 (1H, m, $PhC\underline{H}_2$), 2.5 (3H, m, $C\underline{H}_2CN$, H2'), 2.1 (1H, m, H2'), 1.9 (3H, s, 5-CH_3). ^{31}P-NMR: δ 7.97 and 8.06 (diastereoisomers). FABMS: m/e $[M+H]^+$ 539.22. Anal. Calcd. for $C_{23}H_{28}FN_4O_8P$: C, 51.30; H, 5.24; N, 10.41. Found: C, 51.21; H, 5.43; N, 10.25.

3'-Fluoro-3'-deoxythymidine-5'-methoxyphenylalaninylphosphoramidate (5b):

An ice-cold saturated solution of NH_3 in methanol (10 mL) was added to 3'-fluoro-3'-deoxythymidine-5'-(2-cyanoethyl methoxyphenylalaninyl)phosphoramidate **(3b)**, (100 mg, 0.186 mmol) under nitrogen. The reaction mixture was allowed to warm to r.t. while stirring for 2 hours. Next, the solution was concentrated to dryness and the product purified by silica gel column chromatography (chloroform-methanol-water, 5/3/0.5), to yield a colorless solid (90 mg, 96% yield). ^{1}H-NMR (CD_3OD): δ 7.8 (1H, s, H6), 7.4-7.2 (5H, m, Ph), 6.4 (1H, t, H1'), 5.3 (1H, m, H3'), 4.3 (1H, m, H4'), 4.1 (1H, m, C<u>H</u>CO_2Me), 3.8 (2H, m, H5'), 3.7 (3H, s, CO_2CH_3), 3.0 (2H, m, PhC<u>H</u>$_2$), 2.4 (2H, m, H2'), 1.9 (3H, s, 5-CH_3). ^{31}P-NMR: δ 6.33. Anal. Calcd. for $C_{20}H_{28}FN_4O_8P$: C, 47.81; H, 5.62; N, 11.15. Found: C, 47.71; H, 5.48; N, 11.00.

3'-Fluoro-3'-deoxythymidine-5'-(ammonium methoxyphenylalaninyl) phosphoramidate (38 mg) was dissolved in water and passed through an Amberlite CG-50 (H^+) column (1 cm. x 6 cm.). The 5 mL fractions containing the product were combined and lyophilized to provide the product as a colorless solid (37 mg, 100% yield). ^{1}H-NMR (D_2O): δ 7.6 (1H, s, H6), 7.3-7.0 (5H, m, Ph), 6.3 (1H, t, H1'), 5.2 (1H, dd, H3'), 4.3 (1H, d, H4'), 3.8 (1H, m, C<u>H</u>CO_2Me), 3.7 (2H, m, H5'), 3.6 (3H, s, CO_2CH_3), 2.9 (1H, m, PhC<u>H</u>$_2$), 2.8 (1H, m, PhC<u>H</u>$_2$), 2.4 (1H, m, H2'), 2.1 (1H, m, H2'), 1.8 (3H, s, 5-CH_3). ^{31}P-NMR: δ 6.90.

3'-Fluoro-3'-deoxythymidine-5'-(2-cyanoethyl methoxytryptophanyl) phosphoramidate (4b):

3'-Fluoro-3'-deoxythymidine-5'-(2-cyanoethyl diisopropylamino) phosphoramidite **(2b)** (1.003 g, 2.26 mmol) was dissolved in dry acetonitrile (20 mL) under nitrogen. Tetrazole (0.633 g, 9.04 mmol) was added to the stirring reaction mixture at r.t.. After 5 mins, dry methanol (0.183 mL, 4.52 mmol) was added to the solution, which was allowed to stir for 2 hours. All the volatiles were removed under reduced pressure and the residue re-dissolved in freshly distilled THF (50 mL) under nitrogen. Tryptophan methyl ester (2.0 g, 9.17 mmol) was added to the solution, followed by iodine (0.574 g, 2.26 mmol). After stirring at r.t. for 3 hours, water (30 mL) was added and the resulting mixture extracted with methylene chloride (4 x 20 mL). The organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product. After further purification by silica gel MPLC (chloroform-methanol gradient), the product was isolated as a colorless, crystalline solid (0.740 g, 57% yield). ^{1}H-NMR ($CDCl_3$): δ 9.0 (1H, s, H3(NH)), 8.5 (1H, broad s, indole NH), 7.6* (1H, s, H6), 7.4-7.0 (5H, m, indole aromatic Hs), 6.2* (1H, t, H1'), 5.0* (1H, dd, H3'), 4.2 (2H, m,

H4', H5'), 4.1-3.8 (3H, m, H5', CHCO2Me, NHP(O)), 3.8* (3H, s, CO2CH3), 3.7 (2H, m, OCH2CH2CN), 3.3 (1H, m, TrpCH2), 3.1 (1H, m, TrpCH2), 2.6-2.3 (3H, m, CH2CN, H2'), 1.9* (3H, s, 5-CH3), 1.8 (1H, m, H2'). ^{31}P-NMR: δ 8.08 and 8.12 (diastereoisomers). FABMS : m/e $[M+H]^+$ 578.16.

3'-Fluoro-3'-deoxythymidine-5'-methoxytryptophanyl phosphoramidate (6b):

An ice-cold saturated solution of NH3 in methanol (10 mL) was added to 3'-fluoro-3'-deoxythymidine-5'-(2-cyanoethyl methoxytryptophanyl)phosphoramidate (**4b**), (100 mg, 0.179 mmol) under nitrogen, and the reaction mixture allowed to warm to r.t. while stirring for 2 hours. The solution was concentrated to dryness followed by silica column chromatography (chloroform-methanol-water, 5/3/0.5). The product, 3'-fluoro-3'-deoxythymidine-5'-(ammonium methoxytryptophanyl) phosphoramidate, was isolated as a colorless solid (90.8 mg, 97% yield). ^{1}H-NMR (CD3OD): δ 7.7 (1H, s, H6), 7.6 (1H, d, indole H4), 7.4 (1H, d, indole H7), 7.15 (1H, s, indole H2), 7.1 (1H, t, indole H6), 7.0 (1H, t, indole H5), 6.35 (1H, t, H1'), 5.2 (1H, dd, H3'), 4.2 (2H, m, H4', CHCO2Me), 3.85 (2H, m, H5'), 3.7 (3H, s, CO2CH3), 3.25 (2H, m, TrpCH2), 2.4 (1H, m, H2'), 2.2 (1H, m, H2'), 1.9 (3H, s, 5-CH3). ^{31}P-NMR: δ 6.75. Anal. Calcd. for C22H29FN5O8P•H2O: C, 47.23; H, 5.77; N, 12.52. Found: C, 47.25; H, 5.63; N, 12.34.

3'-Fluoro-3'-deoxythymidine-5'-(ammonium methoxytryptophanyl) phosphoramidate (30 mg) was dissolved in water and passed through an Amberlite CG-50 (H^+) column (1 cm. x 7 cm.). The 5 mL fractions containing the product were combined and lyophilized to provide the product as a colorless solid (29 mg, 100% yield). ^{1}H-NMR (D2O): δ 7.45 (1H, d, indole H4), 7.35 (1H, d, indole H7), 7.3 (1H, s, H6), 7.1 (1H, s, indole H2), 7.05 (1H, t, indole H6), 6.9 (1H, t, indole H5), 6.15 (1H, t, H1'), 5.2 (1H, dd, H3'), 4.25 (1H, d, H4'), 3.9 (1H, m, CHCO2Me), 3.8-3.6 (2H, m, H5'), 3.65 (3H, s, CO2CH3), 3.1 (1H, m, TrpCH2), 2.9 (1H, m, TrpCH2), 2.3 (1H, m, H2'), 1.8 (1H, m, H2'), 1.6 (3H, s, 5-CH3). ^{31}P-NMR: δ 6.96.

3'-Deoxy-2',3'-didehydrothymidine-5'-(2-cyanoethyl diisopropylamino) phosphoramidite (2c):

D4T (**1c**) (0.10 g, 0.45 mmol) was placed in a dry flask under nitrogen and dissolved in dry acetonitrile (5 mL). Diisopropylethylamine (0.23 mL, 1.32 mmol) was added to the solution via syringe followed by 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.20 mL, 0.89 mmol). The reaction mixture was stirred at r.t. for 30 minutes and then quenched with an ice-cold solution of 10% NaHCO3

(15 mL). The resulting mixture was extracted with methylene chloride (3 x 15 mL) and the combined organic extracts washed with a saturated NaCl solution (10 mL) and water (10 mL). After drying over anhydrous Na_2SO_4, the organic layer was concentrated under reduced pressure to give a yellow viscous oil, which was carried forward to the next step without further purification.

3'-Deoxy-2',3'-didehydrothymidine-5'-(2-cyanoethyl methoxyphenylalaninyl) phosphoramidate (3c):

The above crude product (**2c**) was dissolved in dry acetonitrile (5 mL) under nitrogen, followed by the addition of tetrazole (0.125 g, 1.78 mmol) and stirred at r.t.. After 5 minutes, dry methanol (0.054 mL, 1.34 mmol) was added, and the reaction mixture was stirred for 2 hours. All the volatiles were removed under reduced pressure and the residue further dried under high vacuum for 30 minutes.

The crude product was re-dissolved in freshly distilled THF (5 mL) under nitrogen, and a solution of phenylalanine methyl ester (0.60 g, 3.35 mmol) in dry THF (5 mL) was added. After the addition of iodine (170 mg, 0.67 mmol), the reaction mixture was stirred at r.t. for 3 hours. The excess iodine was quenched by the addition of a saturated $NaHSO_3$ (2 mL) solution followed by water (25 mL). The resulting mixture was extracted with methylene chloride (4 x 15 mL) and the organic extracts dried over anhydrous Na_2SO_4. Concentration of the organic layer under reduced pressure gave the crude product as a viscous oil. After further purification by silica gel MPLC (chloroform-methanol gradient), the product was isolated as a colorless, crystalline solid (0.155 g, 67% yield). ^{1}H-NMR ($CDCl_3$): δ 8.8* (1H, s, H3(NH)), 7.4-7.1 (6H, m, Phe, H6), 7.0 (1H, d, H1'), 6.25 (1H, m, H2'), 5.85 (1H, broad s, H3'), 4.85 (1H, m, H4'), 4.2-3.8 (4H, m, H5', $OC\underline{H}_2CH_2CN$, NHP(O)), 3.75 (3H, s, CO_2CH_3), 3.5* (1H, m, $C\underline{H}CO_2Me$), 3.1 (1H, m, Phe$C\underline{H}_2$), 2.9 (1H, m, Phe$C\underline{H}_2$), 2.6 (2H, m, CH_2CN), 1.9* (3H, s, 5-CH_3). ^{31}P-NMR: δ 7.65 and 8.43 (diastereoisomers). FABMS: m/e $[M+H]^+$ 519.21.

3'-Deoxy-2',3'-didehydrothymidine-5'-methoxyphenylalaninylphosphoramidate (5c):

3'-Deoxy-2',3'-didehydrothymidine-5'-(2-cyanoethyl methoxyphenylalaninyl) phosphoramidate (**3c**) (100 mg, 0.193 mmol) was placed in a dry flask under nitrogen, followed by the addition of an ice cold solution of saturated NH_3 in anhydrous methanol (10 mL). While stirring, the reaction mixture was allowed to warm to r.t. over the course of an hour. After the reaction mixture was concentrated to dryness, the product was further purified by silica gel column chromatography (chloroform-methanol-water, 5/3/0.5), yielding 3'-deoxy-2',3'-didehydrothymidine-5'-(ammonium methoxyphenylalaninyl)

phosphoramidate as a colorless solid (93 mg, 100 % yield). ^{1}H-NMR (CD_3OD-D_2O): δ 7.65 (1H, s, H6), 7.35-7.15 (5H, m, Phe), 7.0 (1H, d, H1'), 6.4 (1H, d, H2'), 5.9 (1H, m, H3'), 4.9 (1H, broad s, H4'), 4.05-3.85 (3H, m, H5', C<u>H</u>CO_2Me), 3.6 (3H, s, CO_2Me), 2.95 (2H, m, PheC<u>H</u>$_2$), 1.95 (3H, s, 5-CH_3). ^{31}P-NMR: δ 6.96. Anal. Calcd. for $C_{20}H_{27}N_4O_8P$: C, 49.79; H, 5.64; N, 11.61. Found: C, 49.56; H, 5.81; N, 11.49.

3'-Deoxy-2',3'-didehydrothymidine-5'-(ammonium methoxyphenylalaninyl) phosphoramidate (20 mg, 0.041 mmol) was dissolved in water applied to an Amberlite CG-50 (H^+) column (1.5 cm. x 8 cm.) and eluted with water. The 5 mL fractions containing the product were combined and lyophilized to give the product as a colorless solid (19.2 mg, 100% yield). ^{1}H-NMR (D_2O): δ 7.50 (1H, s, H6), 7.25-7.05 (5H, m, Phe), 6.90 (1H, broad s, H1'), 6.40 (1H, d, H2'), 5.85 (1H, d, H3'), 4.95 (1H, broad s, H4'), 3.80 (2H, m, H5'), 3.70 (1H, m, C<u>H</u>CO_2Me), 3.50 (3H, s, CO_2CH_3), 2.85 (2H, m, PheC<u>H</u>$_2$), 1.80 (3H, s, 5-CH_3). ^{31}P-NMR: δ 6.69.

3'-Deoxy-2',3'-didehydrothymidine-5'-(2-cyanoethyl methoxytryptophanyl) phosphoramidate (4c):

The same procedure was followed as for the preparation of the phenylalanine derivative **(3c)**, however, the reaction was carried out with tryptophan methyl ester (0.80 g, 3.67 mmol). The product was isolated as a colorless, crystalline solid (0.196 g, 79% yield). ^{1}H-NMR ($CDCl_3$): δ 9.4* (1H, s, indole NH), 8.75 (1H, s, H3(NH)), 7.5 (1H, d, indole H4), 7.3 (1H, d, indole H7), 7.2-7.0 (4H, m, H6, indole H5, indole H6, indole H2), 6.9 (1H, broad s, H1'), 6.1 (1H, d, H2'), 5.8 (1H, m, H3'), 4.8 (1H, m, H4'), 4.2-3.6 (6H, m, H5', O<u>CH</u>$_2$$CH_2$CN, C<u>H</u>$CO_2$Me, NHP(O)), 3.7 (3H, s, CO_2CH_3), 3.25 (1H, m, TrpC<u>H</u>$_2$), 3.1 (1H, m, TrpC<u>H</u>$_2$), 2.4 (2H, m, CH_2CN), 1.8 (3H, s, 5-CH_3). ^{31}P-NMR: δ 7.89 and 8.61 (diastereoisomers). FABMS: $[M+H]^+$ 558.25.

3'-Deoxy-2',3'-didehydrothymidine-5'-methoxytryptophanyl phosphoramidate (6c):

The same procedure was followed as for the preparation of the phenylalanine derivative **(5c)**, with the exception that **(4c)** (92 mg, 0.165 mmol) was substituted for **(3c)**. 3'-Deoxy-2',3'-didehydrothymidine-5'-(ammonium methoxytryptophanyl) phosphoramidate was isolated as a colorless solid (86 mg, 100% yield). ^{1}H-NMR (CD_3OD-D_2O): δ 7.7 (1H, s, H6), 7.6 (1H, d, indole H4), 7.4 (1H, d, indole H7), 7.2 (1H, s, indole H2), 7.15 (1H, t, indole H5), 7.05 (1H, t, indole H6), 7.0 (1H, broad s, H1'), 6.35 (1H, d, H2'), 5.9 (1H, d, H3'), 4.85 (1H, broad s, H4'), 4.2 (1H, m, C<u>H</u>CO_2Me), 3.9 (2H, d, H5'), 3.65 (3H, s, CO_2CH_3), 3.2 (2H, m, TrpC<u>H</u>$_2$), 2.0 (3H, s, 5-CH_3). ^{31}P-NMR: δ 7.06.

3'-Deoxy-2',3'-didehydrothymidine-5'-(ammonium methoxytryptophanyl) phosphoramidate (16 mg, 0.031 mmol) was applied to an Amberlite CG-50 (H^+) column as previously described for **(5c)**. The product was obtained as a colorless solid (15 mg, 97% yield). ^{1}H-NMR (D_2O): δ 7.45 (1H, d, indole H4), 7.40 (1H, s, H6), 7.35 (1H, d, indole H7), 7.15 (1H, t, indole H6), 7.10 (1H, s, indole H2), 7.0 (1H, t, indole H5), 6.75 (1H, broad s, H1'), 6.30 (1H, d, H2'), 5.70 (1H, d, H3'), 4.85 (1H, broad s, H4'), 3.8 (1H, m, C<u>H</u>CO_2Me), 3.75 (2H, m, H5'), 3.6 (3H, s, CO_2CH_3), 3.0 (2H, m, TrpC<u>H</u>$_2$), 1.65 (3H, s, 5-CH_3). ^{31}P-NMR: δ 6.84.

Reversed-phase HPLC analysis

Analyses of AZT, FLT and D4T, **3a-4c**, and **5a-6c** were carried out with a 5 μ Spherisorb C-8 reversed-phase column, connected to a Spectra-Physics SP8800 ternary HPLC pump and Spectroflow 757 absorbance detector set at 255 nm. A mobile phase consisting of acetonitrile and 50 mM ammonium acetate was employed at a flow rate of 1.5 ml/min. The compounds were eluted with the following programmed gradient: 0-15 mins. (0-40% CH_3CN), 15-20 mins. (40-50% CH_3CN), 20-25 mins. (50-60% CH_3CN), and 25-30 mins. (60-0% CH_3CN).

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