Intracellular Delivery of Bioactive AZT Nucleotides by Aryl Phosphate Derivatives of AZT

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Received September 14, 1992

Novel aryl phosphate derivatives of the anti-HIV nucleoside analogue AZT have been prepared by phosphorochloridate chemistry. These materials were designed to act as membrane-soluble prodrugs of the bioactive free nucleotides. In vitro evaluation revealed the compounds to have a pronounced, selective anti-HIV activity in CEM cells; the magnitude of the biological effect varied considerably depending on the nature of the phosphate blocking group. Moreover, several of the compounds retain marked antiviral activity in TK⁻ (thymidine kinase-deficient) mutant CEM cells in which AZT was virtually inactive. These data strongly support the hypothesis that the AZT phosphate derivatives exert their biological effects via intracellular release of AZT nucleotide forms and suggest that the potential of nucleoside drugs in antiviral chemotherapy may be enhanced by suitable nucleotide delivery strategies.

Introduction

Several members of the 2′,3′-dideoxynucleoside (ddN) series are potent inhibitors of human immunodeficiency virus (HIV) in cell culture.[1-5] The 5′-triphosphates of these nucleoside analogues are potent inhibitors of HIV reverse transcriptase.[6-8] As a rule, the activation (phosphorylation) of these nucleosides is accomplished by cellular nucleoside and nucleotide kinases. Thus, in contrast to other antiviral agents (e.g. acyclovir) where (herpes simplex) virus-specific thymidine kinase mediates the first step of the conversion of the drug to the intracellular active species, the 2′,3′-dideoxynucleoside (ddN) analogues depend on cellular nucleoside kinases for their phosphorylation. However, in many cases the ddN derivatives have a poor affinity for nucleoside kinases (i.e. 2′,3′-dideoxycytidine for deoxycytidine kinase, 2′,3′-didehydro-2′,3′-dideoxythymidine and 2′,3′-dideoxyuridine for thymidine kinase, 2′,3′-dideoxyadenosine for adenosine kinase and deoxycytidine kinase, and 2′,3′-dideoxyinosine for 5′-nucleotidase).[9-13] Moreover, the dependence on phosphorylation for activation of the particular nucleoside analogue may be a problem in cells where the nucleoside kinase activity is known to be low or even lacking (i.e. monocyte/macrophages).[14] Therefore, we have sought to overcome this dependence on nucleoside kinase activation by the development of a suitable nucleotide delivery strategy. The viability of such an approach is entirely based on the ability to suitably modify the phosphate structure of a masked nucleotide to enable intracellular delivery and release of the free phosphate form. In this paper we compare the activity against HIV-1 and HIV-2 in wild-type [thymidine kinase-positive (TK⁺)] and thymidine kinase-deficient (TK⁻) CEM cells, for a series of aryloxy phosphoramidate derivatives of AZT. In marked contrast to the parent nucleoside AZT, several of the novel derivatives showed potent anti-HIV activity in the TK⁻ cells and proved superior to AZT with regard to their antiviral selectivity.

Antiretroviral Evaluation. Human immunodeficiency virus type 1 [HIV-1 (HTLV-III$_B$)] was obtained from persistently HIV-infected H9 cells as described previously.[16] Virus stocks were prepared from the supernatants of HIV-1 (III$_B$)-infected MT4 cells. HIV-2 (ROD) was provided by Dr. L. Montagnier (Pasteur Institute, Paris, France). MT-4 cells were provided by Dr. N. Yamamoto (Tokyo Medical and Dental University School of Medicine, Tokyo, Japan). CEM/O cells were obtained from the American Tissue Culture Collection (Rockville, MD), and CEM TK⁻ cells were a gift from Prof. S. Ericksson and Dr. A. Karlsson (Karolinska Institute, Stockholm, Sweden). MT-4 and CEM cells were infected with HIV-1 as previously described.[17] Briefly, 5×10^5 MT-4 or CEM cells per milliliter were infected with HIV-1 or HIV-2 at 100 CCID$_{50}$ (50% cell culture infective dose) per milliliter of cell suspension. Then, 100 μL of the infected cell suspension was transferred to microtiter plate wells and mixed with 100 μL of the appropriate dilutions of the test compounds. After 4 days giant cell formation was recorded microscopically in the HIV-infected cell cultures, and after 5 days the number of viable cells was determined by trypan blue staining in the HIV-infected MT-4 cell cultures. The 50% effective concentration (EC$_{50}$) and 50% cytotoxic concentration (CC$_{50}$) were defined as the compound concentrations required to reduce by 50% the number of viable cells (MT-4) or giant cells in the virus-infected and mock-infected cell cultures, respectively.

C3H/3T3 cells were seeded into Costar Tissue Culture Cluster plates (Costar Broadway, Cambridge, MA) at 20 000 cells/mL into 1-cm^2 wells and grown to confluency. Cell cultures were then infected by 75 foci-forming units of Moloney murine sarcoma virus (MSV) during 90 min, after which the medium was replaced by 1 mL of fresh culture medium containing different concentrations of test compound. After 6 days the transformation of the cell cultures was examined microscopically. The EC$_{50}$ was defined as the compound concentration required to inhibit MSV-induced transformation by 50%.

Results and Discussion

Chemistry. Compound **2a** was prepared entirely as described,[18] and a similar synthetic strategy was used in the preparation of the analogues **2b-g**. Thus, *p*-meth-

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0022-2623/93/1836-1048$04.00/0 © 1993 American Chemical Society

Scheme I

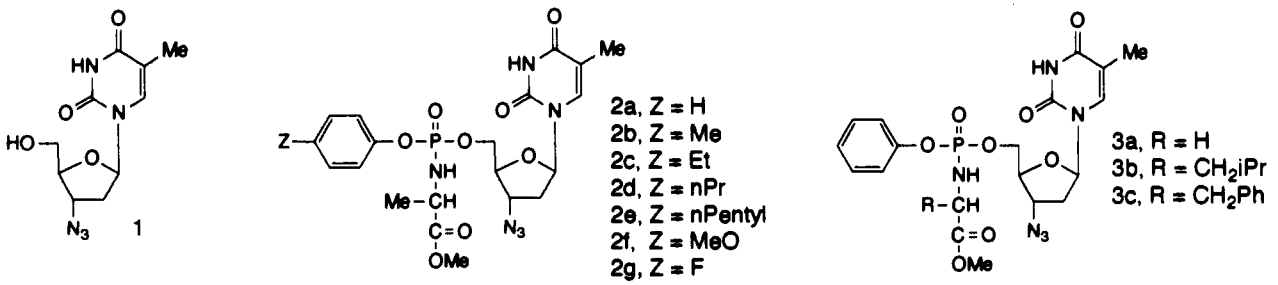

Table I. Anti-HIV-1 and Anti-HIV-2 Activity and Cytotoxicity of Test Compounds 1, **2a–g**, and **3a–c** in Vitro

	EC_{50}[a,b] (μM)					CC_{50}[b,c] (μM)	
	CEM/0		CEM/TK⁻:	MT-4			
compd	HIV-1	HIV-2	HIV-2	HIV-1	HIV-2	MT-4	CEM/0
1	0.003 ± 0	0.004 ± 0.001	>100	0.002 ± 0.0002	0.003 ± 0.00004	6.0 ± 0.02	>500
2	0.055 ± 0.021	0.070 ± 0.0	12 ± 15	0.006 ± 0.001	0.007 ± 0.003	>50	172 ± 1
2b	0.065 ± 0.021	0.056 ± 0.034	7.0 ± 4.2	0.051 ± 0.029	0.053 ± 0.020	27 ± 17	43.0 ± 4.2
2c	0.048 ± 0.024	0.060 ± 0.036	8.7 ± 1.2	0.068 ± 0.004	0.070 ± 0.009	11 ± 5	31.7 ± 0.7
2d	0.041 ± 0.013	0.051 ± 0.027	3.0 ± 0	0.043 ± 0.027	0.045 ± 0.010	28 ± 10	32.7 ± 1.1
2e	0.051 ± 0.027	0.060 ± 0.014	8.0 ± 0	0.103 ± 0.101	0.074 ± 0.053	7 ± 2	9.0 ± 1.3
2f	0.057 ± 0.032	0.065 ± 0.021	9.0 ± 6.6	0.071 ± 0.001	0.069 ± 0.002	182 ± 8	42.6 ± 3.4
2g	0.048 ± 0.029	0.065 ± 0.021	3.0 ± 1.4	0.041 ± 0.006	0.054 ± 0.002	206 ± 20	128 ± 6
3a	0.097 ± 0.065	0.230 ± 0.10	>100	0.160 ± 0.040	0.200 ± 0.122	>500	>500
3b	0.140 ± 0.120	0.210 ± 0.080	13.0 ± 8.2	0.099 ± 0.058	0.087 ± 0.013	205 ± 4	123 ± 14
3c	0.097 ± 0.065	0.160 ± 0.0	10 ± 0.0	0.080 ± 0.022	0.110 ± 0.057	208 ± 3	161 ± 1

[a] 50% effective concentration or compound concentration required to protect MT-4 or CEM cells against the cytopathogenicity of HIV by 50%. [b] Data are the mean of two to four independent experiments. [c] 50% cytotoxic concentration or compound concentration required to reduce MT-4 or CEM cell viability by 50%.

ylphenol reacted with phosphoryl chloride in diethyl ether to give the aryl phosphorodichloridate (89%, δ_P +4). This was allowed to react with alanine methyl ester hydrochloride in dichloromethane in the presence of triethylamine to give *p*-methylphenyl methoxyalaninyl phosphorochloridate (100%, δ_P +7). This reacted with AZT (1) in THF in the presence of *N*-methylimidazole to give the target compound **2b** in very good yield (Scheme I). As anticipated,[19] this material displayed two closely-spaced signals in the ^{31}P NMR corresponding to the two diastereoisomers about the phosphate center (ca. δ +2)[20] and considerable splitting in the H-decoupled ^{13}C spectrum. Similarly prepared were analogues **2c–g** which probe structural modification in the *p*-phenyl substituent. In a similar fashion, variation in the amino acid side chain was studied by the preparation of the parent phenyl derivatives in the glycine (**3a**), leucine (**3b**), and phenylalanine (**3c**) systems. Several of these compounds have been recently disclosed by us in a preliminary communication,[21] but have not been tested in this assay system before. Indeed, in no case has the activity of the masked phosphates been directly compared in TK⁺ and TK⁻ cells; this direct comparison is an important feature of the present report.

Antiviral Activity. AZT (1) and the test compounds **2a–g** and **3a–c** were evaluated for their anti-HIV-1 and anti-HIV-2 activity in human lymphocyte MT-4 and CEM cells. As a rule, all compounds proved markedly effective in inhibiting HIV replication in vitro (Table I). Also, no significant differences in the antiviral activities of the particular test compounds were observed between HIV-1 and HIV-2. The 50% effective concentrations (EC_{50}) were well below 0.5 μM (Table I). Of the AZT derivatives, compound **2a**, the unsubstituted phenyl analogue, emerged as the most potent inhibitor of HIV-1 in MT-4 cells. Its EC_{50} was only 3–4-fold higher than that of AZT. In CEM cells, compound **2a** proved ~20-fold less inhibitory against HIV-1 and HIV-2 than AZT. Introduction of a *p*-alkyl, methoxy, or fluoro substituent in the aryl moiety (**2b–g**) decreased the antiviral activity of the test compounds in MT-4 cells by 20–50-fold, as compared to AZT. Moreover, when compound **2a** was modified at the α-carbon of the alanine moiety (**3a–c**), a 40–70-fold decrease in antiviral activity was noted. The antiviral data obtained for HIV-1 and HIV-2 in MT-4 cells were in full agreement with those obtained in CEM/O cells, except for compound **2a**, which proved equally active with the rest of the series (**2b–g**) in CEM cells, while being more active than the other compounds in MT-4 cells (Table I).

Striking differences were found for the antiviral activity of the test compounds in HIV-2-infected CEM/TK⁻ cells. This cell line is highly deficient in cytosol thymidine kinase and should be considered as an ideal target cell line to investigate the potential antiviral activities of those nucleotide analogues that may release directly the 5′-monophosphate derivative into the intact cells. AZT proved completely inactive against HIV-2 replication in CEM/TK⁻ cells at a concentration as high as 100 μM. In contrast, compound **2a** and the analogues **2b–g** that were substituted in the aryl moiety proved markedly inhibitory to HIV-2 in CEM/TK⁻ cells. Their EC_{50} values varied between 3 and 12 μM. Thus, the AZT derivatives were at least >10- to >35-fold more effective than AZT in inhibiting HIV-2 replication in the thymidine kinase-deficient CEM/TK⁻ cells. Among the AZT derivatives that were substituted at the alanine moiety of **2a**, the valine compound (**3b**) and the phenylalanine compound (**3c**) were effective against HIV-2 in CEM/TK⁻ cells at 10–13 μM, whereas the glycine compound (**3a**) was devoid of anti-HIV-2 activity at 100 μM in this cell line (Table I).

When the antiviral efficacy (ratio of the EC_{50} in CEM/TK⁻ cells versus the EC_{50} in CEM/O cells) of the AZT derivatives against HIV-2 was calculated from the data in Table I, all of the test compounds proved far superior to the parent compound AZT. In fact, several

Table II. Relative Anti-HIV-2 Efficacy of Test Compounds in CEM/TK⁻ Cells and Their Anti-HIV-2 Selectivity Index in MT-4 Cells

compd	EC_{50}[a] HIV-2 (CEM/TK⁻) / EC_{50}[a] HIV-2 (CEM/0)	SI[b] HIV-2 (MT-4)
1	>28571	2000
2a	171	>5555
2b	125	509
2c	145	153
2d	59	631
2e	133	99
2f	138	2638
2g	46	3815
3a	435	>2500
3b	62	1891
3c	62	2356

[a] 50% effective concentration, or compound concentration required to inhibit HIV-2-induced cytopathicity in CEM cells. [b] Selectivity index or ratio of 50% cytotoxic concentration to 50% antivirally effective concentration.

compounds (**2d**, **2g**, **3b**, and **3c**) were only moderately (46–62-fold) less inhibitory to HIV-2 replication in CEM/TK⁻ cells than CEM/O cells, with the other derivatives being 125–435-fold less efficacious in the thymidine kinase-deficient CEM cells (Tables I and II). In contrast, AZT was more than 28 000-fold less active in CEM/TK⁻ than in CEM/O cells. These data clearly demonstrate that the test compounds, particularly **2d** and **2g**, were efficient prodrugs of AZT-MP, partially circumventing the first activation step (phosphorylation of AZT to its 5′-monophosphate) by cytosol thymidine kinase.

Also, several test compounds, i.e. **2a**, **2f**, **2g**, and **3a**, proved to be more selective anti-HIV agents than AZT, as based on the improved selectivity index (ratio of the 50% cytotoxic concentration to the 50% effective concentration) in MT-4 cells (Table II). A limited number of AZT prodrugs will now be evaluated on their efficacy against retrovirus replication in animals (i.e. mice).

In conclusion, several novel AZT-MP derivatives have been identified that proved more antivirally selective than the parent nucleoside AZT. This improved selectivity is mainly due to a much lower cytostatic activity of these AZT-MP derivatives in MT-4 cells, by comparison to AZT. Also, based on their anti-HIV activities in thymidine kinase-deficient CEM cells, the novel AZT-MP derivatives should be considered as efficient prodrugs of AZT-MP, circumventing the first phosphorylation step in the cells. It would now seem imperative to apply this synthetic approach to other antivirally active nucleoside analogues (i.e. ddC, ddI, d4T) and nucleotide analogues (i.e. PMEA), which may be hampered (in some host cells) at their first phosphorylation step. Such studies are currently in progress.

Experimental Section

All reactions were carried out under scrupulously dry conditions, using general procedures we have described.[15] ^{31}P NMR spectra were recorded on a JEOL FX90Q (36.2 MHz) instrument and are reported in units of δ relative to 85% phosphoric acid as external standard; positive shifts are downfield. ^{13}C NMR spectra were recorded on a JEOL FX270 spectrometer operating at 67.9 MHz. Shifts are expressed in units of δ relative to $CDCl_3$ at 77.000 ppm. Both phosphorus-31 and carbon-13 NMR spectra were proton noise decoupled, and all signals were singlets unless otherwise stated. ^1H NMR spectra were recorded on a JEOL FX270 spectrometer operating at 270 MHz and are reported in units of δ relative to internal $CHCl_3$ at 7.240 ppm. All NMR spectra were recorded in $CDCl_3$. Many NMR peaks are split due to the presence of diastereoisomers in the sample; these are indicated by asterisks in the text. Mass spectra were recorded by the fast atom bombardment (FAB) mode on a VG 70-250 spectrometer. HPLC data were recorded using an ACS quaternary system, with an ODS5 column and an eluent of water/acetonitrile, with 82% water 0–10 min, and then a linear gradient to 20% water at 30 min, with a flow rate of 2 mL/min and detection by UV at 265 nm.

The test compounds were isolated as mixtures of diastereoisomers, with this isomerism arising from mixed stereochemistry at the phosphate center. The resulting oils did not give useful microanalytical data but were found to be pure by high-field multinuclear NMR spectroscopy and rigorous HPLC analysis.

***p*-Methylphenyl Phosphorodichloridate.** A solution of *p*-methylphenol (10.0 g, 92.5 mmol) and triethylamine (12.9 mL, 9.35 g, 92.5 mmol) in anhydrous diethyl ether (200 mL) was added dropwise to a vigorously stirred solution of phosporyl chloride (10.3 mL, 17.0 g, 111 mmol) in diethyl ether (100 mL) at 0 °C over a period of 2 h. The mixture was allowed to warm to ambient temperature, with stirring for 15 h, and then heated under reflux for 2 h. The mixture was filtered, and the precipitate was washed with diethyl ether. The combined filtrate and washings were evaporated to dryness under reduced pressure to yield a colored oil. This was subjected to vacuum distillation to give the product as a colorless oil (bp 80–85 °C, 0.1 mmHg) (18.5 g, 89%): ^{31}P NMR 3.63; ^1H NMR 7.1 (5 H, s, Ph), 2.4 (3 H, s, Me).

***p*-Methylphenyl Methoxyalaninyl Phosphorochloridate.** A solution of triethylamine (1.4 mL, 1.0 g, 10.0 mmol) in anhydrous dichloromethane (30 mL) was added dropwise with vigorous stirring to a solution of L-alanine methyl ester hydrochloride (0.7 g, 5.02 mmol) and *p*-methylphenyl phosphorodichloridate (1.13 g, 5.02 mmol) in dichloromethane (40 mol) at −70 °C over a period of 2 h. The reaction mixture was slowly warmed to ambient temperature with stirring over 6 h, and the solvent was then removed in vacuum. The residue was treated with diethyl ether (15 mL), the mixture was filtered, and the filtrate was evaporated in vacuum to yield the product as a colorless oil (1.5 g, 100%); ^{31}P NMR 6.66; CI MS (NH_3) *m/e* 311 ($M^+ + NH_3$, ^{37}Cl, 8), 309 ($M^+ + NH_3$, 22), 294 (MH^+, ^{37}Cl, 2), 292 (MH^+, 10), 104 (100).

3′-Azidothymidine 5′-[*p*-Methylphenyl methoxyalaninyl phosphate] (2b). 3′-Azidothymidine (0.125 g, 0.41 mmol) was dissolved in THF (4 mL), and *p*-methylphenyl methoxyalaninyl phosphorochloridate (0.36 g, 1.23 mmol) and *N*-methylimidazole (0.20 mL, 0.20 g, 2.45 mmol) was added with vigorous stirring. After 12 h at ambient temperature the solvent was removed under vacuum. The residue was dissolved in chloroform (10 mL) and washed with 1 M hydrochloric acid solution (2 × 15 mL), saturated sodium bicarbonate solution (2 × 10 mL), and then water (3 × 15 mL). The organic phase was dried ($MgSO_4$) and evaporated under vacuum, and the residue was purified by chromatography on silica by elution with 5% methanol in chloroform. Pooling and evaporation of appropriate fractions gave the product (0.21 g, 86%): ^{31}P NMR 2.1, 1.8 (1:1); ^{13}C NMR (starred peak are split due to diastereoisomers) 174.2* (m, CO_2Me), 164.1 (C2), 150.6 (C4), 148.3* (d, Ph-ipso, J = 6 Hz), 135.4 (C6), 135.1 (Ph-para), 130.4 (Ph-ortho), 119.9 (d, Ph-meta, J = 5 Hz), 111.5 (C5), 84.9* (C1′), 82.4* (m, C4′), 65.8 (C5′), 60.5* (C3′), 52.7 (Ala-OMe), 50.4* (Ala-CH), 37.4 (C2′), 21.0 (d, Ala-Me, J = 5 Hz), 20.8 (*Me*PH), 12.6* (5-Me); ^1H NMR 9.8 (1 H, sb, NH), 7.4 (1 H, s, H6), 7.2 (4 H, s, Ph), 6.3 (1 H, m, H1′), 4.0–4.5 (6 H, m, H3′, H4′, H5′, Ala-CH, Ala-NH), 3.8* (3 H, s, OMe), 2.5 (1 H, m, H2′), 2.4 (3 H, s, *Me*Ph), 2.3 (1 H, m, H2′), 2.0* (3 H, s, 5-Me), 1.5 (3 H, d, Ala-Me); FAB MS *m/e* 523 (MH^+, 25), 354 (25), 154 (50), 136 (36), 81 (C_5H_5O, 100); HPLC retention time 31.21, 31.37 min (1:2); UV λ_{max} 264 nm.

***p*-Ethylphenyl Methoxyalaninyl Phosphorochloridate.** This compound was prepared by a method entirely analogous to the preparation of the *p*-methyl compound, except that addition was carried out for 3 h at −70 °C, and stirring at ambient temperature was continued for 14 h. Evaporation, treatment with diethyl ether, filtration, and evaporation gave the product as a colorless oil (95%): ^{31}P NMR 8.9, 8.7 (1:1); FAB MS *m/e* 308 (MH^+, ^{37}Cl, 10), 306 (MH^+, 30), 242 (100).

3′-Azidothymidine 5′-[*p*-Ethylphenyl methoxyalaninyl phosphate] (2c). This compound was prepared by a method entirely analogous to that used for **2b** above, except that stirring

was continued for 24 h, and the chromatographic purification utilized 6% methanol in chloroform. Thus, from 0.125 g of AZT was isolated 0.93 g (92%) of compound **2c**: ^{31}P NMR 3.6, 3.2 (3:2); ^{13}C NMR (starred peaks are split due to diastereoisomers) 174.1* (CO_2Me), 164.1 (C2), 150.6 (C4), 148.3* (d, Ph-ipso, J = 6 Hz), 141.4* (Ph-para), 135.4 (C6), 129.1 (Ph-ortho), 119.9 (d, Ph-meta, J = 5 Hz), 111.4 (C5), 85.0* (C1′), 82.4* (m, C4′), 65.7 (C5′), 60.5* (C3′), 52.6 (Ala-OMe), 50.3* (Ala-CH), 37.3 (C2′), 28.1 (CH_3CH_2), 20.8 (d, Ala-Me, J = 5 Hz), 15.6* (CH_3CH_2), 12.5* (5-Me); ^{1}H NMR 9.2* (1 H, s, NH), 7.41 (1 H, s, H6), 7.1 (4 H, m, Ph), 6.2 (1 H, m, H1′), 3.8–4.4 (6 H, m, H3′, H4′, H5′, Ala-CH, Ala-NH), 3.7* (3 H, s, OMe), 2.6 (2 H, q, CH_3CH_2), 2.4 (1 H, m, H2′), 2.2 (1 H, m, H2′), 1.9* (3 H, s, 5-Me), 1.4 (3 H, d, Ala-Me), 1.2* (3 H, t, CH_3CH_2); FAB MS m/e 537 (MH^+, 27), 368 (22), 228 (18), 81 (C_5H_5O, 100); HPLC retention time 29.30, 29.49 min (2:3); UV λ_{max} 264 nm.

p-Propylmethyl Methoxyalaninyl Phosphorochloridate. This compound was prepared by a method entirely analogous to the method used for preparing the p-methyl compound: ^{31}P NMR 8.8.

3′-Azidothymidine 5′-[p-Propylphenyl methoxyalaninyl phosphate] (2d). Compound **2d** was prepared by a method entirely analogous to that used for **2b** above, except that stirring was continued for 10 h, and the chromatographic purification utilized 4.5% methanol in chloroform. Thus, from 0.125 g of AZT was isolated 0.196 g (76%) of compound **2d**: ^{31}P NMR 3.3, 3.0 (3:2); ^{13}C NMR (starred peaks are split due to diastereoisomers) 174.2* (m, CO_2Me), 164.0 (C2), 150.5 (C4), 148.4* (d, Ph-ipso, J = 7 Hz), 140.0 (Ph-para), 135.4 (C6), 129.8 (Ph-ortho), 119.9 (d, Ph-meta, J = 5 Hz), 111.5 (C5), 84.9* (C1′), 82.5* (m, C4′), 65.8 (d, C5′, J = 5 Hz), 60.5* (C3′), 52.7 (Ala-OMe), 50.4* (Ala-CH), 37.4* (C2′), 24.7 (CH_2Ph), 20.9* (d, Ala-Me, J = 3 Hz), 13.8* (CH_3CH_2), 12.6* (5-Me); ^{1}H NMR 9.2 (1 H, sb, NH), 7.5* (1 H, s, H6), 7.3 (4 H, s, Ph), 6.3 (1 H, m, H1′), 4.0–4.5 (6 H, m, H3′, H4′, H5′, Ala-CH, Ala-NH), 3.8* (3 H, s, OMe), 2.7 (2 H, t, CH_2Ph), 2.5 (1 H, m, H2′), 2.3 (1 H, m, H2′), 2.0* (3 H, s, 5-Me), 1.8 (2 H, m, CH_2CH_2Ph), 1.5 (3 H, d, Ala-Me), 1.1 (3 H, t, CH_3-CH_2); FAB MS m/e 551 (MH^+, 33), 382 (33), 242 (28), 81 (C_5H_5O, 100); HPLC retention time 36.39, 36.50 min (4:5); UV λ_{max} 264 nm.

p-Pentylphenyl Methoxyalaninyl Phosphorochloridate. This compound was prepared by a method entirely analogous to the method used for preparing the p-methyl compound, except that addition was carried out for 2 h at −60 °C, and stirring at ambient temperature was continued for 10 h. ^{31}P NMR 14.0; CI MS (NH_3) m/e 367 ($MH^+ + NH_3$, ^{37}Cl, 38), 365 ($MH^+ + NH_3$, 100), 350 (MH^+, ^{37}Cl, 15), 348 (MH^+, 40).

3′-Azidothymidine 5′-[p-Pentylphenyl methoxyalaninyl phosphate] (2e). Compound **2e** was prepared by a method entirely analogous to that used for **2b** above, except that further portions of phosphorochloridate and heating to 45 °C were required to force the reaction to completion. Thus, from 0.125 g of AZT was isolated 0.146 g (57%) of compound **2e**; ^{31}P NMR 2.4, 2.1 (4:5); ^{13}C NMR (starred peaks are split due to diastereoisomers) 174.2* (m, CO_2Me), 164.0 (C2), 150.5 (C4), 148.4* (d, Ph-ipso, J = 7 Hz), 140.2* (Ph-para), 135.4* (C6), 129.7 (Ph-ortho), 119.9 (d, Ph-meta, J = 5 Hz), 111.5 (C5), 84.9* (C1′), 82.4* (m, C4′), 65.8* (m, C5′, J = 5 Hz), 60.5* (C3′), 52.7 (Ala-OMe), 50.4* (Ala-CH), 37.4 (C2′), 35.2 (CH_2), 31.4* (CH_2), 22.6 (CH_2), 21.0 (CH_2), 20.9* (d, Ala-Me, J = 3 Hz), 14.1 (CH_3CH_2), 12.6* (5-Me); ^{1}H NMR 9.8 (1 H, sb, NH), 7.5* (1 H, s, H6), 7.3 (4 H, s, Ph), 6.3 (1 H, m, H1′), 4.1–4.5 (6 H, m, H3′, H4′, H5′, Ala-CH, Ala-NH), 3.8* (3 H, s, OMe), 2.7 (2 H, t, CH_2Ph), 2.5 (1 H, m, H2′), 2.3 (1 H, m, H2′), 2.0* (3 H, s, 5-Me), 1.7 (2 H, m, CH_2-CH_2Ph), 1.5 (7 H, m, Ala-Me, CH_2CH_2), 1.0 (3 H, t, CH_3CH_2); FAB MS m/e 579 (MH^+, 50), 410 (14), 307 (35), 284 (37), 270 (30), 81 (100); HPLC retention time 37.16, 37.31 min (1:2); UV λ_{max} 264 nm.

p-Methoxyphenyl Methoxyalaninyl Phosphorochloridate. This compound was prepared by a method entirely analogous to that used for the p-ethyl compound above: ^{31}P NMR 9.0.

3′-Azidothymidine 5′-[p-Methoxyphenyl methoxyalaninyl phosphate] (2f). Compound **2f** was prepared by a method entirely analogous to that used for **2d** above, except that stirring was continued for 16 h. Thus, from 0.125 g of AZT was isolated 0.29 g (96%) of compound **2f**: ^{31}P NMR 4.3, 4.1 (1:2); ^{13}C NMR (starred peaks are split due to diastereoisomers) 174.1* (CO_2-Me), 164.0 (C2), 157.0 (Ph-para), 150.5 (C4), 144.0 (d, Ph-ipso, J = 7 Hz), 135.5 (C6), 121.1* (m, Ph-ortho), 114.7* (Ph-meta), 111.5 (C5), 85.0* (C1′), 82.4* (m, C4′), 65.8* (m, C5′), 60.5* (C3′), 55.8 (Ph-OMe), 52.7* (Ala-OMe), 50.4* (Ala-CH), 37.4 (C2′), 21.1 (d, Ala-Me, J = 5 Hz), 12.6* (5-Me); ^{1}H NMR 9.5 (1 H, sb, NH), 7.4 (1 H, s, H6), 6.8–7.2 (4 H, 2 × d, Ph), 6.2* (1 H, t, H1′), 3.8–4.4 (6 H, m, H3′, H4′, H5′, Ala-CH, Ala-NH), 3.8 (6 H, m, OMe), 2.4 (2 H, m, H2′), 1.8 (3 H, s, 5-Me), 1.4 (3 H, m, Ala-Me); FAB MS m/e 539 (MH^+, 17), 370 (19), 290 (22), 230 (35), 136 (35), 81 (C_5H_5O, 100); HPLC retention time 21.99, 22.17 min (1:2); UV λ_{max} 264 nm.

p-Fluorophenyl Methoxyalaninyl Phosphorochloridate. This compound was prepared by a method entirely analogous to that used for the p-methyl compound above: ^{31}P NMR 10.1.

3′-Azidothymidine 5′-[p-Fluorophenyl methoxyalaninyl phosphate] (2g). Compound **2g** was prepared by a method entirely analogous to that used for **2b** above. Thus, from 0.125 g of AZT was isolated 0.21 g (97%) of compound **2g**: ^{31}P NMR 3.5, 3.2 (1:1); ^{13}C NMR (starred peaks are split due to diastereoisomers) 174.1* (m, CO_2Me), 164.0 (C2), 159.8 (d, Ph-para, J = 244 Hz), 150.5* (C4), 146.3* (d, Ph-ipso, J = 6 Hz), 135.6 (C6), 121.6 (m, Ph-ortho), 116.3 (d, Ph-meta, J = 23 Hz), 111.5 (C5), 85.2* (C1′), 82.3* (d, C4′, J = 6 Hz), 65.8* (d, C5′, J = 5 Hz), 60.4* (C3′), 52.8 (Ala-OMe), 50.4* (Ala-CH), 37.3* (C2′), 21.0 (d, Ala-Me, J = 5 Hz), 12.6* (5-Me); ^{1}H 9.6 (1 H, sb, NH), 6.8–7.2 (5 H, m, H6, Ph), 6.1 (1 H, m, H1′), 3.8–4.3 (6 H, m, H3′, H4′, H5′, Ala-CH, Ala-NH), 3.6* (3 H, s, OMe), 2.3 (2 H, m, H2′), 1.8 (3 H, s, 5-Me), 1.3* (3 H, d, Ala-Me); FAB MS m/e 527 (MH^+, 46), 218 (25), 136 (35), 81 (C_5H_5O, 100); HPLC retention time 28.63 min; UV λ_{max} 264 nm.

Phenyl Methoxyglycinyl Phosphorochloridate. This compound was prepared by a method entirely analogous to that used for the p-methyl methoxyalaninyl compound above: ^{31}P NMR 8.2.

3′-Azidothymidine 5′-[Phenyl methoxyglycinyl phosphate] (3a). Compound **3a** was prepared by a method entirely analogous to that used for **2b** above. Thus, from 0.125 g of AZT was isolated 0.20 g (99%) of compound **3a**: ^{31}P NMR 4.1; ^{13}C NMR (starred peaks are split due to diastereoisomers) 171.3 (CO_2-Me), 164.1 (C2), 150.5 (C4), 150.4 (d, Ph-ipso, J = 4 Hz), 135.5 (C6), 129.9 (m, Ph-para), 125.5 (m, Ph-ortho), 120.0 (m, Ph-meta), 111.5 (C5), 85.0* (C1′), 82.4 (d, C4′, J = 8 Hz), 65.8 (m, C5′), 60.5* (C3′), 52.5* (Gly-OMe), 42.8* (Gly-CH_2), 37.3 (C2′), 12.5* (5-Me); ^{1}H NMR 9.6 (1 H, sb, NH), 7.2–7.4 (6 H, m, Ph, H6), 6.2* (1 H, t, H1′), 3.6–4.4 (10 H, m, H3′, H4′, H5′, Gly-CH_2, Gly-NH, OMe), 2.4 (2 H, m, H2′), 1.8* (3 H, s, 5-Me); FAB MS m/e 495 (MH^+, 46), 326 (25), 81 (C_5H_5O, 100); HPLC retention time 26.93 min; UV λ_{max} 264 nm.

Phenyl Methoxyleucinyl Phosphorochloridate. This compound was prepared by a method entirely analogous to that used for the p-methyl methoxyalaninyl compound above: ^{31}P NMR 9.1, 8.9.

3′-Azidothymidine 5′-[Phenyl methoxyleucinyl phosphate] (3b). Compound **3b** was prepared by a method entirely analogous to that used for **2b** above, except that stirring was continued for only 5 h, and chromatographic purification was achieved using 4% methanol in chloroform. Thus, from 0.125 g of AZT was isolated 0.23 g (100%) of compound **3b**; ^{31}P NMR 1.0, 0.6 (2:1); ^{13}C NMR (starred peaks are split due to diastereoisomers) 174.3* (m, CO_2Me), 164.1 (C2), 150.6 (C4), 150.5 (Ph-ipso), 135.4* (C6), 129.9 (Ph-para), 125.4 (d, Ph-ortho, J = 5 Hz), 120.2* (m, Ph-meta), 111.5* (C5), 85.0 (C1′), 82.4* (m, C4′), 65.8 (d, C5′, J = 5 Hz), 60.5* (C3′), 53.4* (Leu-OMe), 52.5 (Leu-CH*), 43.6* (m, Leu-CH_2), 37.3* (C2′), 24.5* (m, Leu-CH), 22.8* (Leu-Me), 21.8* (Leu-Me), 12.6* (5-Me); ^{1}H NMR 9.8 (1 H, sb, NH), 7.2–7.5 (6 H, m, Ph, H6), 6.2* (1 H, t, H1′), 3.8–4.5 (6 H, m, H3′, H4′, H5′, Leu-CH, Leu-NH), 3.7* (3 H, s, OMe), 2.4 (2 H, m, H2′), 1.9* (3 H, s, 5-Me), 1.6 (3 H, m, Leu-CH, Leu-CH_2), 0.9 (6 H, m, Leu-Me); FAB MS m/e 551 (MH^+, 20), 382 (15), 242 (15), 81 (C_5H_5O, 100); HPLC retention time 29.07; UV λ_{max} 264 nm.

Phenyl Methoxyphenylalaninyl Phosphorochloridate. This compound was prepared by a method entirely analogous to that used for the p-methyl methoxyalaninyl compound above: ^{31}P NMR 6.3, 6.1.

3′-Azidothymidine 5′-[Phenyl methoxyphenylalaninyl phosphate] (3c). Compound **3c** was prepared by a method entirely analogous to that used for **3b** above, except that stirring was continued for 30 h. Thus, from 0.125 g of AZT was isolated 0.15 g (66%) of compound **3b**: ^{31}P NMR 3.8; ^{13}C NMR (starred peaks are split due to diastereoisomers) 172.9* (CO_2Me), 164.0 (C2), 150.6 (C4), 150.4 (m, Ph), 135.7* (Ph), 135.4* (C6), 129.8* (Ph), 129.5* (Ph), 128.7* (m, Ph), 127.1* (m, Ph), 125.4 (Ph), 120.2* (m, Ph), 111.5* (C5), 84.9* (C1′), 82.3* (m, C4′), 65.7 (C5′), 60.5* (C3′), 56.0* (Phe-CH), 52.6 (OMe), 40.4* (m, Phe-CH_2), 37.4 (C2′), 12.6* (5-Me); ^{1}H NMR 7.2–7.6 (11 H, m, Ph, H6), 6.2* (1 H, t, H1′), 3.8–4.5 (6 H, m, H3′, H4′, H5′, Phe-CH, Phe-NH), 3.8* (3 H, s, OMe), 3.1 (2 H, m, Phe-CH_2), 2.4 (1 H, m, H2′), 2.2 (1 H, m, H2′), 1.9* (3 H, s, 5-Me); FAB MS *m/e* 585 (MH^+, 30), 391 (45), 307 (100), 289 (98), 149 (75); HPLC retention time 30.78, 30.98 (3:1); UV λ_{max} 264 nm.

Acknowledgment. The Chemistry Group thanks the AIDS Directed Programme of the UK MRC for financial support and Mr. S. Turner for HPLC analyses. The Virology Group thanks Ann Absillis and Lizette van Berckelaer for their excellent technical assistance. The virology studies were supported in part by the AIDS Basic Research Programme of the European Community and by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (projects 3.0097.87 and 3.0026.91) and the Belgian Geconcerteerde Onderzoeksacties (project 90/94-2). We thank Miss Dominique Brabants for fine editorial assistance.

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