Synthesis and Antiretroviral Evaluation of New Alkoxy and Aryloxy Phosphate Derivatives of 3′-Azido-3′-deoxythymidine

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A series of new ether lipid−3′-azido-3′-deoxythymidine (AZT) conjugates (**11a**−**g**) were synthesized and evaluated for anti-HIV activity. The effect of chirality on the antiviral activity was examined through the synthesis of AZT conjugates bearing alkoxypropanols in the lipid portion of the molecule (**11a**−**d**). In addition, the long alkyl chain of alkoxyethyl ether lipid−AZT analogs was replaced with aromatic groups (**11e**−**g**), and the effect of this structural modification on activity is reported. The results of the biological tests indicate that analogs with a methyl group α to the phosphate moiety (**11c,d**) exhibit a marked degree of stereoselectivity with regard to their anti-HIV activity. Also, replacement of the long alkyl chain with aromatic groups in the oxyalkyl ether phospholipid−AZT conjugates leads to substantially more potent compounds (**11e**−**g**) with an anti-HIV activity comparable to that of AZT.

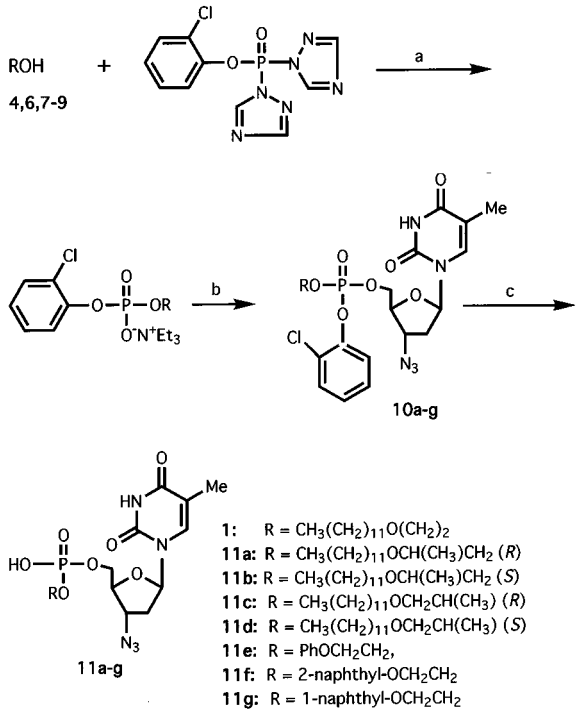
3′-Azido-3′-deoxythymidine (AZT) is a potent inhibitor of HIV replication and the first clinically successful drug for the treatment of AIDS and AIDS-related symptoms.[1] However, intensive efforts are still needed for the development of novel, more efficacious, and selective nucleoside derivatives since the therapeutic potential of AZT is limited by serious adverse reactions, particularly bone marrow suppression.[2] An attractive approach to overcome the drawbacks of AZT and improve its efficacy is the synthesis of 5′-lipophilic phosphate derivatives of this nucleoside.[3−12]

We have recently reported the synthesis and biological evaluation of a series of new AZT conjugates with alkyl and oxyalkyl ether phospholipids.[13] Among these, the alkoxyethyl analog with the 12-carbon aliphatic chain, **1** (Scheme 1), possessed the most favorable therapeutic index.

As a continuation of our efforts to explore the stereoelectronic requirements for optimal effectiveness of the lipid component of ether lipid−AZT conjugates, we now report the synthesis and biological evaluation of several new phosphate derivatives of AZT including ether lipid analogs **11a**−**d** and 2-(aryloxy)ethanols **11e**−**g** (Scheme 1), all of which are covalently linked to AZT through a phosphodiester bond.

The role of chirality on the antiviral activity was examined through the synthesis of AZT conjugates bearing optically pure alkoxypropanols in the lipid portion of the molecule. Additionally, the long alkyl chain of alkoxyethyl ether lipid−AZT analogs was

Scheme 1[a]



[a] Reagents: (a) pyridine, MeCN, H_2O; (b) MSNT, pyridine, room temperature; (c) *n*-$Bu_4N^+F^-$, THF/pyridine/H_2O (8:1:1, v/v/v).

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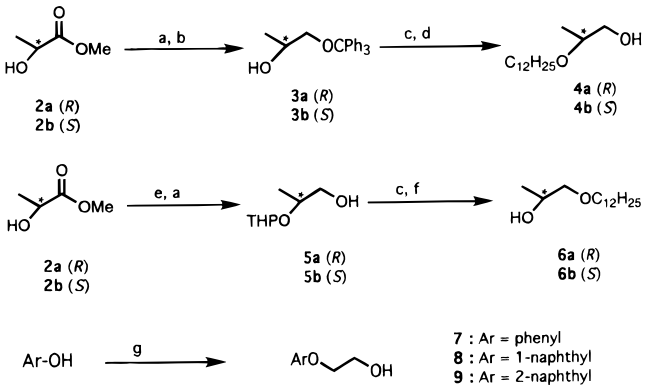
Scheme 2[a]



[a] Reagents: (a) $LiAlH_4$, ether, 0.5 h at 0 °C, 2 h at room temperature; (b) DMAP, Et_3N, DMF, room temperature; (c) $CH_3(CH_2)_{11}Br$, NaH, KI, toluene, reflux; (d) HCOOH, ether, room temperature, MeOH, K_2CO_3, room temperature; (e) PPTS (pyridinium toluene-4-sulfonate), ethyl vinyl ether, EtOH, room temperature; (f) PPTS, EtOH, 55 °C; (g) bromoethanol, K_2CO_3, acetone, reflux.

replaced by aromatic groups, and the effect of this structural modification on antiviral activity is reported.

Chemistry

The synthetic strategy followed for the preparation of the phosphodiesters **11a**−**g** is depicted in Scheme 1 and has previously been described for the preparation of **1.**[13] Thus, alcohols **4** and **6**−**9** were reacted with excess of *o*-chlorophenyl phosphodi-1,2,4-triazolide in py/MeCN and Et_3N to afford the corresponding triethylammonium salts after hydrolysis. These, in turn, were coupled with AZT in the presence of the condensing agent 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) to give the desired conjugates **11a**−**g** after removal of the *o*-chlorophenyl group with $Bu_4N^+F^-$.

The chiral alkoxypropanols **4** and **6** were synthesized as shown in Scheme 2. For the synthesis of the primary alcohols **4a**,**b**, methyl (*R*)-lactate (**2a**) and methyl (*S*)-lactate (**2b**) were reduced with $LiAlH_4$ to the corresponding diols which were, in turn, monoprotected to **3a**,**b** upon treatment with Ph_3CCl. Alkylation with 1-bromododecane followed by deprotection with formic acid afforded the desired optically pure alkoxypropanols **4a**,**b**.

The synthesis of the secondary alcohols **6a**,**b** was effected as follows: Reaction of the methyl lactates **2a**,**b** with ethyl vinyl ether gave the corresponding monoprotected esters which were then reduced with $LiAlH_4$ to the alcohols **5a**,**b**, respectively. Alkylation with 1-bromododecane followed by deprotection using PPTS/EtOH afforded the optically pure secondary alcohols **6a**,**b**.

The syntheses of phenoxyethanol **7** and naphthoxyethanols **8** and **9** were realized by *O*-alkylation of phenol or naphthol, respectively, with 1-bromoethanol in the presence of K_2CO_3.

Biological Evaluation and Discussion

The new analogs **11a**−**g** were evaluated for their anti-HIV-1 and anti-HIV-2 activities in human T-lymphocyte (CEM) cells.[14,15] In general, all compounds proved effective in inhibiting HIV replication in vitro as shown in Table 1.[16] It is notable that there is no variation in activity between the *R* and *S* isomers **11a**,**b** against HIV-1 and HIV-2. Conversely, isomers **11c**,**d** which bear the methyl group next to the phosphodiester moiety exhibit some degree of stereoselectivity with **11d** being markedly (10-fold) more active than **11c**. All the above compounds **11a**−**d** showed similar toxicity for the CEM/0 cells. The selectivity index (SI)[16] of **11a**,**b** is of the same magnitude, while the SI of the *S* isomer **11d** is 10-fold higher than that of the *R* isomer **11c**. In general, introduction of a methyl group at either the α or β position of the (dodecyloxy)ethyl lipid portion in analog **1** results in decreased anti-HIV-1 and anti-HIV-2 potency without a marked effect on cytotoxicity. However, a striking enhancement in antiviral potency accompanied by a reduction in toxicity was noted upon replacement of the long alkyl chain of analog **1** with either phenyl or α- and β- naphthyl groups. The aromatic analogs were found to be 10-fold more potent than those with long aliphatic chains, possessing comparable activity to AZT.

To obtain more detailed information on their mechanism of action, conjugates **11a**−**g** were examined in thymidine kinase deficient cells in order to evaluate their ability to act as intracellular delivery forms for the free nucleotide. To this end, CEM/TK^- cells were used. These cells are incapable of phosphorylation and, thus, are incapable of activating AZT. As shown in Table 1 none of the compounds **11a**−**g** was active in CEM/TK^- cells suggesting that they do not efficiently deliver AZT monophosphate (AZT-MP) into the cells but instead undergo rapid conversion to AZT. In order to probe this hypothesis, the hydrolytic behavior of the phosphodiester analog **11g** was examined by HPLC in RPMI-1640 and RPMI-1640 containing 10% fetal calf serum (FCS) at 37 °C. Our results indicate that the conjugates under study are partially hydrolyzed only in serum containing medium, probably through the action of phosphodiesterases and/or phosphatases present in

Table 1. EC_{50} Values for AZT and Ether Lipid−AZT Conjugates (μM)

	EC_{50} (μM)				
	CEM/0		CEM/TK^-		
	HIV-1	HIV-2	HIV-2	CC_{50} (μM), CEM/0	SI (HIV-1)
1	0.115 ± 0.037	0.133 ± 0.012	>50	76.9 ± 17	669
11a	0.50 ± 0.14	0.50 ± 0.14	>50	93.0 ± 5.7	186
11b	0.32 ± 0.25	0.35 ± 0.09	>50	94.5 ± 17.7	295
11c	2.17 ± 0.17	2.67 ± 1.15	>50	95.9 ± 10.6	44
11d	0.20 ± 0.17	0.26 ± 0.14	>50	88.0 ± 11.3	440
11e	0.020 ± 0.00	0.018 ± 0.004	>125	≥125	≥6250
11f	0.015 ± 0.00	0.020 ± 0.00	>125	≥125	≥8333
11g	0.013 ± 0.004	0.028 ± 0.018	>125	≥125	≥9615
AZT	0.006 ± 0.0	0.004 ± 0.001	>100	>100	>16666

the FCS. After 9 days of incubation in RPMI-1640 containing 10% FCS only 50% of the conjugate was hydrolyzed to afford AZT. AZT-MP was not detected during the experiment. Therefore, we believe that the test compounds are not converted intracellularly to their corresponding 5′-monophosphate derivatives but to the parent nucleoside AZT.

In conclusion, the presence of a methyl group α or β to the phosphodiester moiety in the oxyalkyl ether phospholipid−AZT conjugates leads to decreased antiviral activity. Interestingly, the compounds bearing α-methyl groups exhibit a certain degree of stereoselectivity with regard to their anti-HIV activity. Most importantly, replacement of the long alkyl chain with aromatic groups in the oxyalkyl ether phospholipid−AZT conjugates leads to substantially more potent compounds with activity comparable to that of AZT. Furthermore, these aromatic analogs are slightly less toxic than AZT in CEM/0 cells. These findings suggest that (aryloxy)ethyl phosphodiesters of AZT merit further study.

Experimental Section

Chemistry. All reactions were carried out under scrupulously dry conditions. NMR spectra were recorded on a Bruker AC 300 spectrometer operating at 300 MHz for ^{1}H, 75.43 MHz for ^{13}C, and 121.44 MHz for ^{31}P. ^{1}H NMR spectra are reported in units of δ relative to internal $CHCl_3$ at 7.24 ppm. ^{13}C NMR shifts are expressed in units of δ relative to $CDCl_3$ at 77.00 ppm, while ^{31}P NMR spectra are reported in units of δ relative to 85% phosphoric acid as external standard; positive shifts are downfield. ^{31}P and ^{13}C NMR spectra were proton noise decoupled, and all signals were singlets unless otherwise stated. All NMR spectra were recorded in $CDCl_3$. Many NMR spectra were obtained as two sets of frequencies due to the presence of two diastereoisomers in the sample; these are indicated by asterisks in the text. For chiral analogs, NMR spectral data are reported for either the *R* or *S* isomer. Silica gel plates (Merck F254) were used for thin layer chromatography. Chromatographic purification was performed with silica gel (200−400 mesh). HPLC data were recorded using a Waters quaternary system with an ODS10 column and an eluant of water/acetonitrile with a linear gradient from 100% to 65% water at 20 min and to 0% water at 25 min with a flow rate of 2 mL/min and detection by UV at 254 nm.

Optical activity values were recorded on a POLAAR 2000 automatic polarimeter instrument. Analyses indicated by the symbols of the elements were carried out by the microanalytical section of the Institute of Organic and Pharmaceutical Chemistry of the National Hellenic Research Foundation and the Microanalytical Section of the Department of Chemistry of the University College, London, U.K.

General Method for the Preparation of Optically Pure 2-(Dodecyloxy)propan-1-ols (4a,b): (*R*)- or (*S*)-1,2-Propanediol. To an ice-cooled solution of (*R*)- or (*S*)-methyl lactate (**2a**,**b**) (2.08 g, 20 mmol) in anhydrous ether (360 mL) was added $LiAlH_4$ (0.759 g, 20 mmol). The resulting mixture was stirred at 0 °C for 15 min and then at room temperature for 2 h. The reaction was quenched at 0 °C by sequential addition of a mixture of THF/H_2O (1:1) (2 mL) and H_2O (1 mL). Anhydrous Na_2SO_4 was then added, and the solid was filtered in vacuo and washed with ether (2 × 30 mL). The filtrate was evaporated in vacuo, and the residue was carried on to the next step without any purification: yield 90%; ^{1}H NMR δ 3.88−3.84 (m, 1H), 3.62−3.57 (m, 1H), 3.39−3.33 (m, 1H), 1.13 (d, J = 6.4 Hz, 3H).

(*R*)- or (*S*)-1-*O*-(Triphenylmethyl)propan-2-ol (3a,b). To a solution of (*R*)- or (*S*)-1,2-propanediol (1.26 g, 16.6 mmol) in a mixture of *N*,*N*-dimethylformamide:dichloromethane (20/170 mL) were added triethylamine (2.53 g, 25 mmol) and 4-(dimethylamino)pyridine (0.081 g, 0.7 mmol). To the resulting solution was added at 0 °C triphenylchloromethane (5.1 g, 18.3 mmol). The mixture was allowed to warm to room temperature and stirred for 8 h. The reaction mixture was diluted with dichloromethane and washed with saturated aqueous NH_4Cl, H_2O, and brine. The organic layer was dried (Na_2SO_4), and the solvent was evaporated in vacuo. Purification by flash column chromatography (petroleum ether (40−60 °C)/EtOAc, 80:20) afforded the desired monoprotected diol **3a** or **3b** in 60% yield: ^{1}H NMR δ 7.42 (d, J = 7.3 Hz, 6H), 7.32−7.21 (m, 9H), 3.96−3.82 (m, 1H), 3.12 (dd, J = 9.2, 3.5 Hz, 1H), 2.99 (dd, J = 9.2, 8.9 Hz, 1H), 2.35 (bs, 1H), 1.09 (d, J = 6.3 Hz, 3H).

(*R*)- or (*S*)-2-(Dodecyloxy)-1-*O*-(triphenylmethyl)propane. To a slurry of NaH (0.075 g, 3.12 mmol, washed twice with anhydrous hexane) in toluene (2 mL) was added at 0 °C a solution of alcohol **3a** or **3b** (0.496 g, 1.56 mmol) in toluene (2 mL). The resulting mixture was stirred at 0 °C for 0.5 h, and then 1-bromododecane (0.484 g, 1.95 mmol) and KI (0.033 g, 0.195 mmol) were added. The resulting mixture was stirred at 100 °C for 12 h. The reaction mixture was cooled to 0 °C, and excess NaH was quenched by addition of MeOH (1 mL). The mixture was diluted with ether and washed with H_2O and brine. The organic layer was dried (Na_2SO_4), and the solvent was evaporated in vacuo to afford the desired product in 78% yield after purification of the residue by flash column chromatography using petroleum ether (40−60 °C)/ethyl acetate (98:2) as eluting solvent: ^{1}H NMR δ 7.44 (d, J = 7.3 Hz, 6H), 7.29−7.21 (m, 9H), 3.54−3.49 (m, 1H), 3.47 (t, J = 6.5 Hz, 2H), 3.16 (dd, J = 9.3, 6.0 Hz, 1H), 2.94 (dd, J = 9.3, 4.8 Hz, 1H), 1.53−1.51 (m, 2H), 1.24 (bs, 18H), 1.12 (d, J = 6.2 Hz, 3H), 0.86 (t, J = 6.7 Hz, 3H); ^{13}C NMR δ 144.30, 128.92, 128.85, 128.77, 128.63, 127.69, 126.85, 86.40, 74.86, 69.61, 67.52, 31.90, 30.22, 29.64, 29.55, 29.34, 26.24, 22.68, 17.76, 14.10.

(*R*)- or (*S*)-2-(Dodecyloxy)propan-1-ol (4a,b). A solution of 2-(dodecyloxy)-1-*O*-(triphenylmethyl)propane (0.486 g, 1 mmol) in a mixture of formic acid (90%) (2 mL) and diethyl ether (2 mL) was stirred at room temperature until the completion of the reaction. Addition of ether was followed by extraction with saturated aqueous $NaHCO_3$, H_2O, and brine. The solvent was evaporated in vacuo, the resulting solid was dissolved in methanol (10 mL), and K_2CO_3 (3 mmol) was added. The reaction mixture was stirred until TLC showed the formate esters were completely hydrolyzed. The K_2CO_3 was filtered off; the filtrate was diluted with ether and washed with saturated aqueous NH_4Cl, H_2O, and brine. The organic layer was dried (Na_2SO_4), and the solvent was evaporated in vacuo. Purification by flash column chromatography (petroleum ether (40−60 °C)/ether, 75:25) afforded the desired compound **4a** or **4b** in 93% yield: ^{1}H NMR δ 3.58−3.31 (m, 5H), 2.04 (bs, 1H), 1.57−1.50 (m, 2H), 1.28−1.24 (m, 18H), 1.08 (d, J = 6.1 Hz, 3H), 0.86 (t, J = 6.4 Hz, 3H).

(*R*)-2-(Dodecyloxy)propan-1-ol (4a): $[\alpha]^{20}_D = +11.2°$ (c = 2 g/dL, MeOH). Anal. ($C_{15}H_{32}O_2$) C, H.

(*S*)-2-(Dodecyloxy)propan-1-ol (4b): $= [\alpha]^{20}_D = -11.7°$ (c = 2 g/dL, MeOH). Anal. ($C_{15}H_{32}O_2$) C, H.

General Method for the Preparation of Optically Pure 1-(Dodecyloxy)propan-2-ols (6a,b): (*R*)- or (*S*)-(1-Ethoxyethyl)methyl Lactate. To an ice-cooled solution of (*R*)- or (*S*)-methyl lactate (**2a**,**b**) (2.63 g, 25.3 mmol) in methylene chloride (143 mL) were added sequentially pyridinium toluene-4-sulfonate (0.635 g, 2.53 mmol) and ethyl vinyl ether (3.64 g, 50.6 mmol, 2 equiv), and the resulting mixture was stirred at room temperature for 45 min. The reaction mixture was cooled to 0 °C, and saturated aqueous $NaHCO_3$ was added. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Evaporation of the solvent in vacuo and purification of the residue by flash column chromatography (petroleum ether (40−60 °C)/ether, 9:1) afforded the desired protected alcohols in 75% yield: ^{1}H NMR δ 4.77−4.72* (m, 1H), 4.29* (q, J = 6.9 Hz, 1H), 4.16* (q, J = 7.1 Hz, 1H), 3.71−3.43 (m, 2H), 3.71 (s, 3H), 1.38* (d, J = 7.0 Hz, 3H), 1.35* (d, J = 7.0 Hz, 3H), 1.32* (d, J = 5.4 Hz, 3H), 1.27* (d, J = 5.2 Hz, 3H), 1.15* (t, J = 7.0 Hz, 3H), 1.14* (t, J = 7.0 Hz, 3H).

(*R*)- or (*S*)-2-(1-Ethoxyethyl)-1,2-propanediol (5a,b). The reaction was performed as above for compounds **3a**,**b** using (*R*)- or (*S*)-(1-ethoxyethyl)methyl lactate (1.76 g, 10 mmol) in anhydrous ether (184 mL) and $LiAlH_4$ (0.380 g, 10 mmol) to afford, after workup as above and distillation of the solvent, compound **5a** or **5b** in 90% yield: ^{1}H NMR δ 4.77* (q,

J = 5.4 Hz, 1H), 4.69* (q, J = 5.3 Hz, 1H), 3.79−3.42 (m, 5H), 1.32* (d, J = 5.3 Hz, 3H), 1.31* (d, J = 5.3 Hz, 3H), 1.19* (t, J = 7.1 Hz, 3H), 1.18* (t, J = 7.0 Hz, 3H), 1.15* (d, J = 6.4 Hz, 3H), 1.09* (t, J = 6.4 Hz, 3H).

(*R*)- or (*S*)-1-(Dodecyloxy)-2-*O*-(1-ethoxyethyl)propane. The reaction was performed as above for (*R*)- or (*S*)-2-(dodecyloxy)-1-*O*-(triphenylmethyl)propane using NaH (0.044 g, 1.18 mmol, washed twice with anhydrous hexane) in toluene (2 mL), alcohol **5a** or **5b** (0.140 g, 0.9 mmol) in toluene (2 mL), 1-bromododecane (0.336 g, 1.35 mmol) and KI (0.022 g, 0.135 mmol), to afford, after purification using flash column chromatography (petroleum ether (40−60 °C)/ethyl acetate, 9:1), the desired product in 62% yield: ^{1}H NMR δ 4.81* (q, J = 5.3 Hz, 1H), 4.76* (q, J = 5.3 Hz, 1H), 3.88−3.25 (m, 7H), 1.56−1.49 (m, 2H), 1.29 (d, J = 5.3 Hz, 3H), 1.24 (bs, 18 H), 1.18* (t, J = 7.0 Hz, 3H), 1.17* (t, J = 7.0 Hz, 3H), 1.15* (d, J = 6.4 Hz, 3H), 1.12 (d, J = 6.4 Hz, 3H), 0.86 (t, J = 6.8 Hz, 3H).

(*R*)- or (*S*)-1-(Dodecyloxy)propan-2-ol (6a,b). To a solution of (*R*)- or (*S*)-1-(dodecyloxy)-2-*O*-(1-ethoxyethyl)propane (0.260 g, 0.83 mmol) in anhydrous EtOH (7 mL) was added pyridinium toluene-4-sulfonate (0.021 g, 0.083 mmol), and the resulting mixture was heated at 55 °C for 1.5 h. The EtOH was then evaporated in vacuo, and the residue was taken up in ether and washed with saturated aqueous $NaHCO_3$, H_2O, and brine. The organic layer was dried over Na_2SO_4, and the solvent was evaporated in vacuo to afford, after purification of the residue by flash column chromatography (petroleum ether (40−60 °C)/ethyl acetate, 80:20), the desired product **6a** or **6b** in 80% yield: ^{1}H NMR δ 3.94−3.91 (m, 1H), 3.47−3.37 (m, 3H), 3.18 (dd, J = 9.2, 8.3 Hz, 1H), 2.35 (bs, 1H), 1.56−1.51 (m, 2H), 1.24 (bs, 18H), 1.11 (d, J = 6.3 Hz, 3H), 0.86 (t, J = 6.6 Hz, 3H); ^{13}C NMR δ 76.3, 71.5, 66.4, 31.9, 29.6, 29.5, 29.3, 26.1, 22.7, 18.6, 14.1.

(*R*)-1-(Dodecyloxy)propan-2-ol (6a): $[\alpha]^{20}_D = +8.4°$ (c = 2 g/dL, MeOH). Anal. ($C_{15}H_{32}O_2$) C, H.

(*S*)-1-Dodecyloxy-propane-2-ol (6b): $[\alpha]^{20}_D = -8.8°$ (c = 2 g/dL, MeOH). Anal. ($C_{15}H_{32}O_2$) C, H.

General Procedure for the Preparation of 2-(Aryloxy)-ethanols 7−9. To a solution of the appropriate aryl alcohol (0.05 mol) in anhydrous acetone (60 mL) were sequentially added anhydrous K_2CO_3 (0.055 mol) and bromoethanol (0.045 mol), and the resulting mixture was refluxed for 12 h. Filtration of the K_2CO_3 was followed by evaporation of the solvent from the filtrate, and the residue was added to 32% NaOH (50 mL) and stirred at room temperature for 1 h to remove unreacted aryl alcohol. The mixture was partitioned between ether and water, and the organic layer was washed with 2 N HCl, H_2O, and brine and dried (Na_2SO_4). The solvent was evaporated in vacuo, and the residue was purified by flash column chromatography (petroleum ether (40−60 °C)/ether, 70:30) to afford the desired product.

2-(Phenyloxy)ethanol (7): yield 95%; ^{1}NMR δ 7.28−7.23 (m, 2H), 6.97−6.88 (m, 3H), 4.03 (t, J = 5.1 Hz, 2H), 3.94−3.88 (m, 2H), 2.84 (t, J = 5.3 Hz, 1H).

2-(1-Naphthyloxy)ethanol (8): yield 80%; ^{1}NMR δ 8.18−8.15 (m, 1H), 7.81−7.78 (m, 1H), 7.50−7.42 (m, 3H), 7.31−7.25 (m, 1H), 6.77 (d, J = 7.4 Hz, 1H), 4.16 (t, J = 4.9 Hz, 2H), 4.03−3.99 (m, 2H), 2.30 (bs, 1H).

2-(2-Naphthyloxy)ethanol (9): yield 90%; ^{1}NMR δ 7.76−7.69 (m, 3H), 7.45−7.40 (m, 1H), 7.35−7.32 (m, 1H), 7.17−7.14 (m, 2H), 4.16 (t, J = 4.3 Hz, 2H), 4.02−3.97 (m, 2H), 2.31 (bs, 1H).

General Procedure for the Preparation of 2-Chlorophenyl 5′-(3′-Azido-2′-deoxythymidinyl) 2-Alkoxypropyl Phosphates and 2-Chlorophenyl 5′-(3′-Azido-2′-deoxythymidinyl) 2-(Aryloxy)ethyl Phosphates. Triphosphates **11a−g** were synthesized using the method previously described by us.[13] Briefly, to an ice-cooled solution of *o*-chlorophenyl phosphorodichloridate (0.35 mL, 2 mmol) in acetonitrile (8 mL) were sequentially added 1,2,4-triazole (0.310 g, 4.5 mmol) and triethylamine (0.6 mL), and the mixture was stirred at room temperature for 30 min. The appropriate alcohol (**4, 6−9**) (1 mmol) in pyridine (8 mL) was added, and after a further period of 45 min triethylamine (0.7 mL) and water (0.2 mL) were added; the mixture was stirred for 10 min. Addition of saturated aqueous $NaHCO_3$ was followed by extraction with dichloromethane. The organic phase was dried (Na_2SO_4) and evaporated in vacuo. The resulting (chlorophenyl)phosphatidic acid triethylammonium salt was used without further purification for the next step.

The (chlorophenyl)phosphatidic acid triethylammonium salt obtained above was dissolved in pyridine (5 mL). 1-(2-Mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT; 0.390 g, 1.34 mmol) and AZT (0.30 g, 1.12 mmol) were added, and the mixture was stirred at room temperature for 12 h. The reaction mixture was then worked up by addition of saturated aqueous $NaHCO_3$ and extraction with dichloromethane. The organic phase was extracted with saturated aqueous $CuSO_4$ to remove pyridine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash column chromatography using a gradient of dichloromethane/acetone (90:10−85:15).

3′-Azido-3′-deoxythymidine 5′-(*o*-chlorophenyl (*R*)-2-(dodecyloxy)propyl phosphate) (10a): yield 62%; ^{1}H NMR δ 8.47 (bs, 1H), 7.49−7.15 (m, 5H), 6.24 (t, J = 6.5 Hz, 1H), 4.49−4.07 (m, 5H), 4.06 (m, 1H), 3.65−3.62 (m, 1H), 3.46−3.41 (m, 2H), 2.42−2.26 (m, 2H), 1.88 (s, 3H), 1.54 (m, 2H), 1.25 (bs, 18H), 1.15* (d, J = 6.3 Hz, 3H), 1.16* (d, J = 6.3 Hz, 3H), 0.88 (t, J = 6.2 Hz, 3H); ^{31}P NMR δ −8.31.

3′-Azido-3′-deoxythymidine 5′-(*o*-chlorophenyl (*S*)-2-(dodecyloxy)propyl phosphate) (10b): yield 58.5%.

3′-Azido-3′-deoxythymidine 5′-(*o*-chlorophenyl (*R*)-1-methyl-2-(dodecyloxy)ethyl phosphate) (10c): yield 36%; ^{1}H NMR δ 8.44 (bs, 1H), 7.53−7.14 (m, 5H), 6.27 (t, J = 6.5 Hz, 1H), 4.46−4.07 (m, 5H), 3.65−3.37 (m, 4H), 2.41−2.25 (m, 2H), 1.89 (s, 3H), 1.63−1.5 (m, 2H), 1.34 (d, J = 6.4 Hz, 3H), 1.25 (bs, 18H), 0.88 (t, J = 6.2 Hz, 3H); ^{31}P NMR δ −8.95, −9.42.

3′-Azido-3′-deoxythymidine 5′-(*o*-chlorophenyl (*S*)-1-methyl-2-(dodecyloxy)ethyl phosphate) (10d): yield 53%.

3′-Azido-3′-deoxythymidine 5′-(*o*-chlorophenyl 2-(phenyloxy)ethyl phosphate) (10e): yield 35%; ^{1}H NMR δ 9.3 (bs, 1H), 7.44−6.8 (m, 10H), 6.17 (t, J = 6.6 Hz, 1H), 4.58−4.36 (m, 5H), 4.27−4.0 (m, 3H), 2.38−2.17 (m, 2H), 1.9 (s, 3H); ^{31}P NMR δ −8.32, −8.38. Anal. ($C_{24}H_{25}N_5O_8ClP$) C, H, N.

3′-Azido-3′-deoxythymidine 5′-(*o*-chlorophenyl 2-(2-naphthyloxy)ethyl phosphate) (10f): yield 36%; ^{1}H NMR δ 9.54 (bs, 1H), 7.81−7.72 (m, 3H), 7.53−7.12 (m, 9H), 6.21 (t, J = 6.5 Hz, 1H), 4.72−4.27 (m, 7H), 4.06−4.05 (m, 1H), 2.43−2.31 (m, 1H), 2.29−2.19 (m, 1H), 1.89 (s, 3H); ^{31}P NMR δ −8.4. Anal. ($C_{28}H_{27}N_5O_8ClP$) C, H, N.

3′-Azido-3′-deoxythymidine 5′-(*o*-chlorophenyl 2-(1-naphthyloxy)ethyl phosphate) (10g): yield 48%; ^{1}H NMR δ 9.18 (bs, 1H), 8.27−8.23 (m, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.55−7.30 (m, 7H), 7.20−7.11 (m, 2H), 6.82−6.38 (m, 1H), 6.16 (t, J = 6.5 Hz, 1H), 4.82−4.66 (m, 2H), 4.53−4.32 (m, 4H), 4.38−4.11 (m, 1H), 3.99−3.98 (m, 1H), 2.33−2.08 (m, 2H), 1.88 (s, 3H); ^{31}P NMR δ −8.19, −8.32. Anal. ($C_{28}H_{27}N_5O_8ClP$) C, H, N.

General Procedure for the Preparation of the Final Phosphodiesters. Tetra-*n*-butylammonium fluoride (3 mmol, 1 M in THF) was added to a solution of the protected conjugate in a mixture of tetrahydrofuran−pyridine−water (8:1:1, v/v/v) (10 mL). The mixture was stirred at room temperature for 5 h and then worked up by addition of saturated aqueous $NaHCO_3$ and extraction with dichloromethane. The organic phase was evaporated, and the residue was applied to a short column and eluted with dichloromethane:methanol (gradient 95:5−50:50). The appropriate fractions were concentrated and treated with DOWEX 50WX8 (H^+) in methanol to afford the final phosphodiesters.

3′-Azido-3′-deoxythymidine 5′-((*R*)-2-(dodecyloxy)propyl phosphate) (11a): yield 60%; ^{1}H NMR δ 7.46 (s, 1H), 6.22 (t, J = 6.4 Hz, 1H), 4.41−4.0 (m, 6H), 3.69−3.65 (m, 1H), 3.52−3.49 (m, 2H), 2.46−2.39 (m, 2H), 1.93 (s, 3H), 1.59−1.44 (m, 2H), 1.27 (bs, 18H), 1.19 (d, J = 6.3 Hz, 3H), 0.83 (t, J = 6.3 Hz, 3H); ^{13}C NMR δ 164.3, 150.3, 135.9, 111.3, 85.2, 82.4, 73.7, 70.5, 69.5, 66.1, 60.0, 37.5, 31.9, 29.9, 29.8 29.6, 29.4, 29.3, 26.1, 22.7, 16.4, 14.1, 12.3; ^{31}P NMR δ −1.19. Anal. ($C_{25}H_{44}N_5O_8P$) C, H, N.

3′-Azido-3′-deoxythymidine 5′-((*S*)-2-(dodecyloxy)propyl phosphate)) (11b): yield 62%. Anal. ($C_{25}H_{44}N_5O_8P$) C, H, N.

3′-Azido-3′-deoxythymidine 5′-((*R*)-1-methyl-2-(dodecyloxy)ethyl phosphate) (11c): yield 65%; ^{1}H NMR δ 7.47

(s, 1H), 6.23 (t, J = 6.1 Hz, 1H), 4.6 (m, 1H), 4.38−4.3 (m, 3H), 4.02 (m, 1H), 3.51−3.43 (m, 4H), 2.43−2.34 (m, 2H), 1.91 (s, 3H), 1.52 (m, 2H), 1.34 (d, J = 6.2 Hz, 3H), 1.25 (bs, 18H), 0.85 (t, J = 6.3 Hz, 3H); ^{13}C NMR δ 164.2, 150.3, 135.7, 111.3, 84.9, 82.5, 82.4, 74.9, 73.9, 71.8, 66.0, 60.2, 37.6, 31.9, 29.6, 29.5 29.3, 26.0, 22.6, 16.3, 14.1, 12.3; ^{31}P NMR δ −2.22. Anal. ($C_{25}H_{44}N_5O_8P$) C, H, N.

3′-Azido-3′-deoxythymidine 5′-((*S*)-1-methyl-2-(dodecyloxy)ethyl phosphate) (11d): yield 64%. Anal. ($C_{25}H_{44}N_5O_8P$) C, H, N.

3′-Azido-3′-deoxythymidine 5′-(2-(phenyloxy)ethyl phosphate) (11e): yield 69%; ^{1}H NMR δ 7.38−6.86 (m, 6H), 6.12 (t, J = 6.4 Hz, 1H), 5.44 (bs, 1H), 4.41−4.15 (m, 7H), 3.98 (m, 1H), 2.39−2.33 (m, 2H), 1.88 (s, 3H); ^{31}P NMR δ −2.6. Anal. ($C_{18}H_{22}N_5O_8P\cdot H_2O$) C, H, N.

3′-Azido-3′-deoxythymidine 5′-(2-(2-naphthyloxy)ethyl phosphate) (11f): yield: 72%; ^{1}H NMR δ 9.72 (bs, 1H), 7.86−7.78 (m, 3H), 7.55−7.39 (m, 3H), 7.24−7.20 (m, 2H), 6.19 (t, J = 5.9 Hz, 1H), 4.55−4.37 (m, 7H), 4.06 (bs, 1H), 2.42−2.40 (m, 2H), 1.99 (s, 3H); ^{13}C NMR δ 164.9, 157.0, 150.12, 136.5, 134.8, 130.0, 129.5, 127.5, 127.0, 124.1, 118.0, 112.5, 107.5, 86.1, 84.0, 83.8, 67.5, 67.3, 60.2, 38.3, 12.5; ^{31}P NMR δ −1.79. Anal. ($C_{22}H_{24}N_5O_8P\cdot H_2O$) C, H, N.

3′-Azido-3′-deoxythymidine 5′-(2-(1-naphthyloxy)ethyl phosphate) (11g): yield 77%; ^{1}H NMR δ 8.23 (dd, J = 7.1, 1.9 Hz, 1H), 7.74 (dd, J = 2.0, 6.9 Hz, 1H), 7.45−7.26 (m, 5H), 6.71−6.56 (m, 1H), 6.0 (t, J = 6.2 Hz, 1H), 4.48−4.4 (m, 2H), 4.26−4.13 (m, 4H), 3.86−3.8 (m, 1H), 2.25−2.1 (m, 2H), 1.83 (s, 3H); ^{13}C NMR δ 164.5, 153.9, 150.3, 136.1, 134.5, 127.5, 126,5, 125.7, 125.4, 125.3, 121.8, 121.0, 111.1, 105.0, 85.2, 82.4*, 82.3*, 67.1, 66.1, 60.0, 37.2, 12.2; ^{31}P NMR δ −2.29. Anal. ($C_{22}H_{24}N_5O_8P\cdot{}^1/_2H_2O$) C, H, N.

Biological Studies. HIV-1 (HTLV-III$_B$) was obtained from persistently HIV-infected H9 cells as previously described.[14] HIV-2 (ROD) was provided by Dr. L. Montagnier (Pasteur Institute, Paris, France). CEM/0 cells were obtained from the American Tissue Culture Collection (Rockville, MD), and CEM/TK$^-$ cells were a gift from Prof. S. Eriksson and Dr. A. Karlsson (Karolinska Institute, Stockholm, Sweden). CEM cells were infected with HIV-1 as previously described.[15] Briefly, 5 × 10^5 CEM cells/mL were infected with HIV-1 or HIV-2 at 100 CCID$_{50}$ (50% cell culture infective dose)/mL of cell suspension. Then, 100 mL of the infected cell suspension was transferred to microtiter plate wells and mixed with 100 mL of the appropriate dilutions of the test compounds. After 4 days giant cell formation was recorded microscopically in the HIV-infected CEM cell cultures. The 50% effective concentration (EC$_{50}$) and 50% cytototic concentration (CC$_{50}$) were defined as the compound concentrations required to reduce by 50% the number of giant cells or viable cells in the virus-infected and mock-infected cell cultures, respectively.

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References

(1) Fischl, M. A.; Richman, D. D.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Schooley, R. T.; Jackson, G. G.; Durack, D. T.; King, D. The efficacy of Complex - A double-blind, placebo-controlled trial. *N. Engl. J. Med*. **1987**, *317*, 185−191.

(2) Richman, D. D.; Fischl, M. A.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Hirsch, M. S.; Jackson, G. G.; Durack, D. T.; Nusinofflehrman, S. *N. Engl. J. Med*. **1987**, *317*, 192−197.

(3) Van Wijk, G. M. T.; Hostetler, K. Y.; Kroneman, E.; Richman, D. D.; Sridhar, C. N.; Kumar, R.; van der Bosch, H. Synthesis and antiviral activity of 3′-azido-3′-deoxythymidine triphosphate distearoylglycerol: a novel phospholipid conjugate of the anti-HIV agent AZT. *Chem. Phys. Lipids* **1994**, *70*, 213−222.

(4) Hostetler, K. Y.; Richman, D. D.; Carson, D. A.; Stuhmiller, L. M.; van Wijk, G. M. T.; van den Bosch, H. Greatly enhanced inhibition of human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 3′-deoxythymidine diphosphate dimyristoylglycerol, a lipid prodrug of 3′-deoxythymidine. *Antimicrob. Agents Chemother*. **1992**, *36*, 2025−2029.

(5) Henin, Y.; Gouyette, C.; Schwartz, O.; Debouzy, J.-C.; Neumann, J.-M.; Huynh-Dinh, T. Lipophilic glycosyl phosphotriester derivatives of AZT: Synthesis, NMR transmembrane transport study, and antiviral activity. *J. Med. Chem*. **1991**, *34*, 1830−1837.

(6) Piantadosi, C.; Marasco, C. J., Jr.; Morris-Natschke, S. L.; Meyer, K. L.; Gumus, M. E. Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV-1 activity. *J. Med. Chem*. **1991**, *34*, 1408−1414.

(7) Meier, C.; Neumann, J.-M.; Andre, F.; Hennin, Y.; Huynh-Dinh, T. *O*-Alkyl-5′,5′-dinucleoside phosphates as prodrugs of 3′-azidothymidine and cordycepin. *J. Org. Chem*. **1992**, *57*, 7300−7308.

(8) Puech, F.; Gosselin, G.; Lefebvre, I.; Pompon, A.; Aubertin, A. M.; Kirn, A.; Imbach, J. L. Intracellular delivery of nucleoside monophosphates through a reductase-mediated activation process. *Antiviral Res*. **1993**, *22*, 155−174.

(9) Sergheraert, C.; Pierlot, C.; Tartar, A.; Henin, Y.; Lemaitre, M. Synthesis and anti-HIV evaluation of D4T and D4T 5′-monophosphate prodrugs. *J. Med. Chem*. **1993**, *36*, 826−830.

(10) McGuigan, C.; Pathirana, R. N.; Davies, M. P. H.; Balzarini, J.; De Clercq, E. Diaryl phosphate derivatives act as pro-drugs of AZT with reduced cytotoxicity compared to the parent nucleoside. *Bioorg. Med. Chem. Lett*. **1994**, *4*, 427−430.

(11) McGuigan, C.; Pathirana, R. N.; Balzarini, J.; De Clercq, E. Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem*. **1993**, *36*, 1048−1052.

(12) Thomson, W.; Nicholls, D.; Irwin, W. J.; Al-Mushadani, J.; Freeman, S.; Karpas, A.; Petrik, J.; Mahmood, N.; Hay, A. J. Synthesis, bioactivation and anti-HIV activity of the bis(4-acyloxybenzyl) and mono(4-acyloxybenzyl) esters of the 5′-monophosphate of AZT. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1239−1245.

(13) Calogeropoulou, T.; Koufaki, M.; Tsotinis, A.; Balzarini, J.; De Clerq, E.; Makriyannis, A. Synthesis and anti-HIV evaluation of alkyl and alkoxyethyl phosphodiester AZT derivatives. *Antiviral Chem. Chemother*. **1995**, *6*, 43−49.

(14) Popovic, M.; Sarngadharan, M. C.; Read, E.; Gallo, R. C. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **1984**, *224*, 497−500.

(15) Balzarini, J.; Naesens, L.; Slachmuylders, J.; Niphuis, H.; Rosenberg, I.; Holy, A.; Schellekens, H.; De Clercq, E. 9-(2-Phosphonylmethoxyethyl)adenine (PMEA) effectively inhibits retrovirus replication *in vitro* and simian immunodeficiency virus-infection in rhesus-monkeys. *AIDS* **1991**, *5*, 21−28.

(16) EC$_{50}$ (50% effective concentration) is the concentration of compound that inhibits virus-induced cytopathicity in HIV-infected cells by 50%; CC$_{50}$ (50% cytotoxic concentration) is the concentration of compound which causes a 50% reduction in cell viability. The SI (selectivity index) is the ratio of 50% cytotoxic concentration to 50% antivirally effective concentration. For full details, see ref 15.

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