Synthesis, Bioactivation and Anti-HIV Activity of the Bis(4-acyloxybenzyl) and Mono(4-acyloxybenzyl) Esters of the 5'-Monophosphate of AZT[1]

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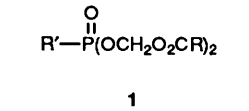
To investigate the design of prodrugs of antiviral nucleosides for targeting to the central nervous system, the bis(4-acyloxybenzyl) esters of the 5'-monophosphate of AZT **5** (R = Me, Et, Pr^i or Bu^t) have been prepared. The reaction of the appropriate bis(4-acyloxybenzyl) *N,N*-diisopropylphosphoramidite **10** (R = Me, Et, Pr^i or Bu^t) with AZT in the presence of 1*H*-tetrazole, followed by oxidation of the P^{III} intermediate with 3-chloroperoxybenzoic acid gave the required triesters **5** in good yield. The lithium salts of the mono(4-acyloxybenzyl) esters of the 5'-phosphate of AZT **7** (R = Me, Et, Pr^i or Bu^t) were prepared by treatment of the triesters **5** with lithium iodide. In the presence of porcine liver carboxyesterase the triesters **5** and diesters **7** decomposed readily to the 5'-monophosphate of AZT **9**. The anti-HIV activities of the triesters **5** and diesters **7** were, with one exception, comparable to that of AZT, but the greater cytotoxicity of certain compounds in particular types of cell significantly reduced their selectivity indices.

The AIDS dementia complex, characterised by disturbances of cognitive, motor and behavioural functions, is a serious consequence of HIV infection. This condition results from an invasion of the virus into the central nervous sytem (CNS) and is manifest in a large proportion of patients.[2,3] The effective delivery of an anti-HIV drug to the CNS would have great therapeutic potential.[4] The transport of polar drug molecules from the cerebral circulation into the brain is restricted by the blood-brain barrier (BBB). This barrier is largely due to the unique structure of brain capillaries, the endothelial cells of which are characterised by tight junctions between cells, low pinocytotic activity and a distinct lack of fenestrae. Delivery to the CNS is, therefore, generally limited to passive diffusion and active transport of lipophilic and polar molecules respectively.[5] Many potential anti-HIV drugs cannot cross the BBB due to their polar (*e.g.* nucleoside analogues) or ionic (*e.g.* phosphonates, phosphates) character and although many of these compounds are potent inhibitors of key enzymes and receptors, they seldom achieve their therapeutic potential because of poor transport across cell membranes.[6] Effective targeting of drugs to a particular tissue requires that they have access to that tissue and that they maintain a sufficient local concentration to exert their action. One way in which delivery to the CNS has been achieved is by means of the redox-carrier system developed by Bodor.[7] Here, a drug is conjugated to a redox-carrier which in its reduced form increases the conjugate's overall lipophilicity thereby facilitating passive diffusion into the CNS. Oxidative metabolism of the carrier generates a polar drug-carrier conjugate which is trapped within the CNS by virtue of its hydrophilicity. The drug-oxidised carrier conjugate is then slowly hydrolysed releasing the parent drug to exert a specific and sustained action.

A key feature of the redox system is the entrapment of an inactive drug precursor within the CNS. Biologically active phosphonates and phosphates lend themselves to a similar system of targeted delivery. Here, the parent phosphonate or phosphate dianion [RP(O)(O$^-$)$_2$] is esterified to yield the neutral ester prodrug [RP(O)(OR')$_2$]. The lipophilicity of the prodrug should facilitate passive diffusion across the BBB and into the brain. Subsequent hydrolysis would yield the mono-anion [RP(O)(O$^-$)OR'] which would then be trapped within the CNS by virtue of its hydrophilicity. Further hydrolysis would generate the parent drug *in situ*. Such derivatisation also offers the opportunity for enhanced diffusion across other biomembranes. The success of this approach is dependent upon the design of an ester protecting group which will provide suitable rates of hydrolysis. The problem of achieving this goal is highlighted by the case of simple phospho ester hydrolysis by P–O bond cleavage, where hydrolysis of the second ester group is typically 1 × 10^6 times slower than the first.[8]

Attempts at rate control have used the bis- and mono-(acyloxymethyl) esters **1** and **2** as bioreversible protecting



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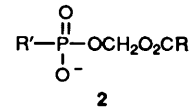

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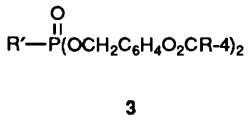

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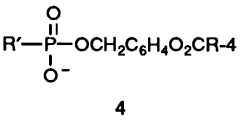

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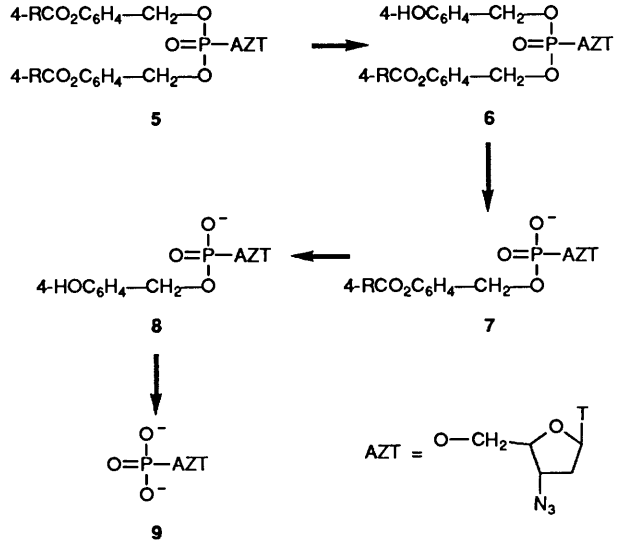
groups for the model compounds phenyl and benzyl phosphate (R' = PhO and BnO),[9,10] and more recently for phosphonoformate (R' = MeOOC),[11] the 5'-monophosphate of dideoxyuridine[12] and 9-(2-phosphonylmethoxyethyl)adenine.[13] The bis(acyloxymethyl) esters **1** were good substrates for esterases and readily gave the mono(acyloxymethyl) esters **2** together with the acylate anion and formaldehyde, without P–O bond cleavage. The monoesters **2** degrade more slowly to the phospho dianion, presumably because of the close proximity of the

anionic centre to the acyl group which hinders binding of the substrate to the active site of the esterase.

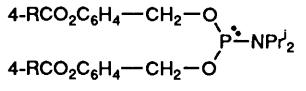
In an attempt to increase the rate of removal of the second phospho ester group, we have recently examined the bis- and mono-(4-acyloxybenzyl) esters, **3** and **4**, of methylphosphonate (R′ = Me)[14,15] and phosphonoacetate (R′ = MeO_2CCH_2).[15] Esterase catalysed the removal of the 4-acyl group and the resulting electron-donating 4-hydroxy substituent promoted cleavage of the benzyl–oxygen bond to release the phospho anion together with a short-lived intermediate with 4-hydroxybenzyl carbonium ion character. For the mono(4-acyloxybenzyl) esters **4**, the charge at phosphorus is the length of an aromatic ring plus a C–C bond (~4 Å) further removed from the site of esterase attack, when compared with the acyloxymethyl esters **2**, which improves the rate of removal of the second protecting group. For the phosphonoacetate analogues, **3** and **4** (R′ = MeO_2CCH_2), the rate of esterase hydrolysis with different acyl groups was monitored, and found to be most rapid for the 4-butyryl (R = Pr) and 4-isobutyryl (R = Pr^i) derivatives. In the present work, this approach to bioreversible protection is extended to the delivery of phosphates, specifically the 5′-monophosphate of AZT **9** (T = thymine).

The nucleoside analogue, AZT is licensed for the chemotherapy of AIDS. To exert its biological activity, it is phosphorylated by host kinases to the triphosphate, which is incorporated into DNA by reverse transcriptase; this results in chain termination as AZT lacks a 3′-hydroxy group.[16] Elimination of AZT from the body is rapid, with a half-life of only 1 h, as a result of glucuronidation of the 5′-OH.[17] Attempts to improve the pharmacokinetic properties of the drug have been explored with 5′-carboxy esters.[18–22] The problem of glucuronidation and initial phosphorylation could be avoided by using the 5′-monophosphate of AZT **9**, however, since it is dianionic at physiological pH it is unable to diffuse across cell membranes. Further, the 5′-monophosphate **9** is thought to be responsible for the toxicity associated with AZT.[23] One approach is to prepare a lipophilic derivative of the monophosphate which will slowly release **9** in a controlled manner. This strategy has been adopted by several groups for the delivery of nucleotide monophosphates. For example, McGuigan and co-workers have evaluated simple dialkyl phosphate esters,[24] phosphorodiamidate derivatives,[25] lactyl and glycolyl phosphate esters[26] and aryl phosphate esters[27] of AZT, and Huynh-Dinh and co-workers have examined lipophilic glycosyl phosphotriesters of AZT,[28] 5-fluorouridine and arabinocytidine.[29] Here, we describe the synthesis of the bis- and mono-(4-acyloxybenzyl) esters of the 5′-monophosphate of AZT **5** and **7** (R = Me, Et, Pr^i or Bu^t), together with the unsubstituted dibenzyl ester and the 5′-monophosphate **9**. Some preliminary data on their degradation and anti-HIV activity is also reported. During the course of this work some similar compounds have recently been patented[30] as a means to promote drug absorption, however, no optimisation of structural variants were declared and no degradative, kinetic or biological data were presented.



Results and Discussion

Dibenzyl *N*,*N*-diisopropylphosphoramidite[31] and di-*tert*-butyl *N*,*N*-diethylphosphoramidite[32] are convenient reagents for the phosphorylation of alcohols, and they have been used for the preparation of the dibenzyl and di-*tert*-butyl esters of the phosphate of AZT. In the present study, the bis(4-acyloxybenzyl) esters of the phosphate of AZT **5** (R = Me, Et, Pr^i, Bu^t) were synthesised by a modification of these routes: the desired P^{III} phosphorylating agents **10** (R = Me, Et, Pr^i, Bu^t) were obtained by treatment of *N*,*N*-diisopropylphosphorochloridate[33] with 2 equiv. of the appropriate 4-acyloxybenzyl alcohol[34] in the presence of triethylamine. These were purified by flash column chromatography[35] in yields of 52–87%. Coupling of AZT with **10** in the presence of 1*H*-tetrazole gave



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the P^{III} derivatives, which were oxidised without isolation with 3-chloroperoxybenzoic acid to give the required triesters **5** (R = Me, Et, Pr^i, Bu^t) in yields of 42–63% after purification by flash column chromatography. Adapting a published procedure for the dealkylation of phospho triesters,[36] the mono-(4-acyloxybenzyl) esters of AZT **7** (R = Me, Et, Pr^i, Bu^t) were prepared in yields of 55–78% by treatment of the triesters **5** with lithium iodide. The 5′-monophosphate of AZT **9** was prepared by treatment of the di-*tert*-butyl ester of the phosphate of AZT with acid. All compounds were characterised by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy, IR and low-resolution mass spectrometry, together with high-resolution mass spectrometry and/or elemental analysis.

For the chemical and esterase-catalysed hydrolyses, an ion-pair reversed-phase HPLC analysis was developed for the separation and quantification of the various components which may result from the degradation of the triester **5** (R = Me) (Fig. 1). The triester **5** (R = Me) showed little evidence of hydrolysis when incubated in a phosphate buffer (pH 7.4, 0.1 mol dm^{-3})–acetonitrile mixture (95:5 v/v) at 37 °C over a 3 h period, whereas in the presence of 0.12 U of porcine liver carboxyesterase (PLCE) it was rapidly hydrolysed to the diester **7** (Fig. 2). Equimolar amounts of 4-hydroxybenzyl alcohol and the diester **7** were produced simultaneously. 4-Acetoxybenzyl alcohol was not detected. This indicates that degradation proceeds by initial hydrolysis at the 4-acetoxybenzyl ester function to yield the 4-hydroxybenzyl derivative **6** (R = Me). Cascade elimination of a 4-hydroxybenzyl carbonium ion, perhaps as a quinone methide, generates the diester **7** (R = Me), in accordance with our previous studies.[14,15] One interesting feature of this reaction profile is that, despite the large initial rate of depletion of the triester **5** (R = Me), complete loss is not observed. This may be due to the formation of products which inhibit the PLCE. It has been suggested previously that the reactive 4-hydroxybenzyl carbonium ion may, in some way, deactivate the enzyme, although no evidence for an interaction

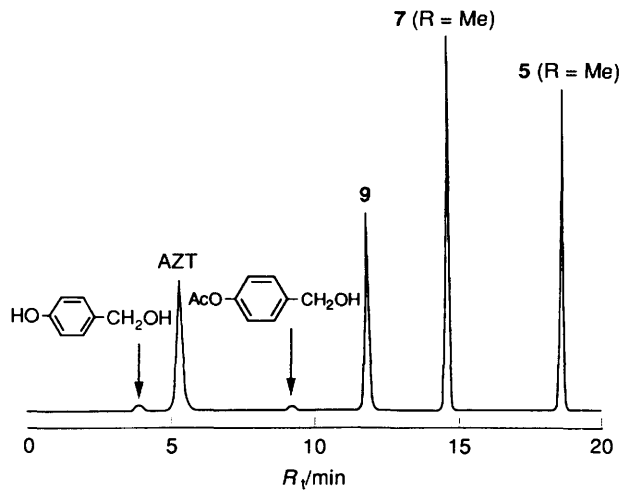


Fig. 1 High-performance chromatogram of the possible degradation products from the hydrolysis of 3'-azido-3'-deoxythymidin-5'-yl bis(4-acetoxybenzyl) phosphate **5** (R = Me).

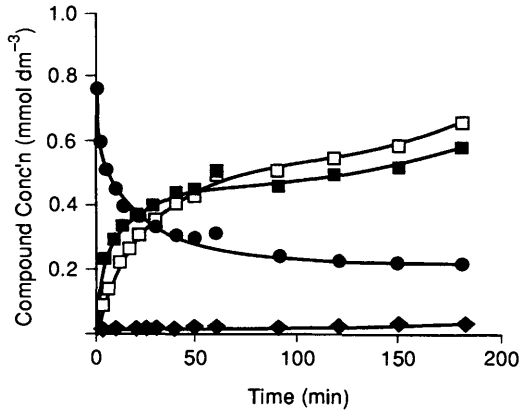
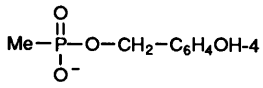


Fig. 2 Degradation profile of 3'-azido-3'-deoxythymidin-5'-yl bis(4-acetoxybenzyl) phosphate **5** (R = Me) with PLCE (0.12 units). [□, 4-hydroxybenzyl alcohol; ◆, **9**; ■, **7** (R = Me); ●, **5** (R = Me)]

has so far been demonstrated.[14,15,37-39] If a degradation product is responsible for inhibition of the enzyme then the inhibitory effect would be magnified by the low enzyme concentration used in these experiments. The design of molecules which will enable internal trapping of the carbonium ion intermediate are in progress. Using a similar strategy, Glazier has recently patented related prodrugs of AZT, the benzyl carbonium ion from which is designed to give methyl 3-(4'-hydroxyphenyl)propenoate.[30]

The above phenomenon was not observed at higher enzyme concentrations and in the presence of 12 U of PLCE the triester **5** (R = Me) was completely degraded within 5 min. After 35 min the only product detected was the 5'-monophosphate of AZT **9**. Further, treatment of the triester **5** (R = Me) with human plasma resulted in its rapid degradation so that after 5 min, the sole AZT-containing component was the diester **7**. Overnight, the diester **7** was degraded to AZT, presumably due to the presence of serum esterases and phosphatases.

For the studies using ^{31}P NMR spectroscopy, methylphosphonate [δ_P 23.7 ppm] was used as a buffer because of the similarity of the chemical shift of inorganic phosphate to those of the substrates and products. In the presence of PLCE (1 U), a solution of the triester **5** (R = Me, Et or Pr^i) (2 mmol dm^{-3}; 1 cm^3) in potassium methylphosphonate buffer (0.1 mol dm^{-3}, pD 8.0)–MeCN (9:1, v/v) [δ_P 0.0–0.05 ppm] decomposed to the diesters **7** (R = Me, Et or Pr^i) within 10 min. Experiments could not be performed with the pivaloyl triester **5** (R = Bu^t) because of the low aqueous solubility of this compound. A 5 mmol dm^{-3} solution of the diester **7** (R = Me, Et, Pr^i or Bu^t) [δ_P 1.05–1.1 ppm] was found to be stable towards chemical hydrolysis over 24 h at 37 °C. In contrast, with 5 U of PLCE, the diesters **7** (R = Me, Et, Pr^i or Bu^t) (5 mmol dm^{-3}; 1 cm^3) decomposed readily to the 5-monophosphate of AZT **9** [δ_P 4.2 ppm] with half-lives of *ca.* 16, 5, 2 and 10 min, respectively. The influence of the nature of the acyl group on the rate of hydrolysis catalysed by PLCE was similar to that observed for the phosphonoacetate analogues.[15] A peak at δ_P 1.05–1.1 ppm was also detected which is consistent with the intermediacy of the 4-hydroxybenzyl ester of the 5'-monophosphate of AZT **8**. Interestingly, in all experiments with esterase, a phosphonate was observed at δ_P 27.3 ppm which degraded with time. This is attributed to 4-hydroxybenzyl methylphosphonate **11**, formed from the reaction of methylphosphonate with the 4-hydroxybenzyl carbonium ion. This proposal is consistent with the formation of 4-hydroxybenzyl phosphate from the esterase-catalysed hydrolyses of the phosphonoacetate analogues, **3** and **4** (R = MeO_2CCH_2), in the presence of inorganic phosphate buffer.[15]



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The triesters **5** and diesters **7** were evaluated *in vitro* for anti-HIV activity in two different virus-cell systems with somewhat differing results, particularly as regards cytotoxicity.

In one, the effects of compounds upon HIV-1 and SIV infection of C8166 cells were assessed over 5–7 days by the reduction in syncytium formation, the production of gp120 and by the protection of cell viability. Toxicity to uninfected cells was assessed in parallel in the latter MTT assay. With the exception of the pivaloyl triester, the diesters **7** (R = Me, Et, Pr^i, Bu^t) and triesters **5** (R = Me, Et, Pr^i) exhibited antiviral activities, in terms of EC_{50}, comparable to that of AZT (Table 1). The greater toxicity of certain derivatives, in particular the propionyl and isobutyryl substituted triesters, reduced the selectivity indices to 12, *ca.* 1×10^3 times lower than that of AZT. Like AZT, these compounds were all ineffective in preventing HIV-1_{GB8} infection of the kinase-deficient T cell line JM.[27] This result suggests that the compounds undergo metabolism to AZT, and it was possible that the drug was simply formed in the culture medium. However, after 5 days in the culture medium, diester **7** (R = Et) showed only 30% decomposition to the 5'-monophosphate of AZT **9**, suggesting metabolism of the prodrug to AZT within the cell.

The other assay system monitored the cytopathic effect of HIV-1 infection in another T cell line, T-45, over a period of 10 days. Again, with the exception of the pivaloyl triester, the compounds were comparable to AZT in antiviral activity (Table 2). In these cells, which are more sensitive to the toxic effects of AZT, the relative toxicities of the various derivatives differed from those observed in C8166 cells, the diesters, especially R = Et, Pr^i and Bu^t, proving the most toxic, causing a substantial reduction of 10–100 fold in the selectivity indices.

The unsubstituted dibenzyl ester of the 5'-monophosphate of AZT (Bn) was also comparable in antiviral activity to AZT (Table 1). In spite of poor penetration into cells, the 5'-monophosphate of AZT **9** also exhibited antiviral activity and toxicity in infected C8166 cells similar to that of the nucleoside, as previously observed.[40] The bis(4-acetoxybenzyl) ester of benzylphosphonate was included to assess toxicity due to the 4-hydroxybenzyl carbonium ion. It exhibited minimal antiviral activity in both systems and although toxic to C8166 cells at 10 μmol dm^{-3} had little effect on T-45 cell viability below 200 μmol dm^{-3}.

Table 1 Anti-HIV-1 and SIV activities and toxicities of mono- and bis-(4-acyloxybenzyl) esters of the monophosphate of AZT in C8166 cells

Compd.	R	TC_{50}	EC_{50} HIV-1	EC_{50} SIV
5	Me	50	0.016	0.04
5	Et	1	0.08	0.08
5	Pr^i	1	0.08	0.04
5	Bu^t	> 100	8	20
7	Me	500	0.04	0.032
7	Et	500	0.032	0.08
7	Pr^i	500	0.016	0.04
7	Bu^t	100	0.016	0.016
Bn	—	> 100	0.08	0.08
9	—	> 1000	0.008	0.016
AZT	—	> 1000	0.016	0.016

EC_{50} represents the concentration (μmol dm^{-3}) of compound which reduces by 50% the production of gp120 in HIV-1_{IIIB}-infected C8166 cells. TC_{50} represents the concentration (μmol dm^{-3}) of compound which causes 50% cytotoxicity to uninfected cells.

Table 2 Anti-HIV-1 activities (EC_{50}, μmol dm^{-3}) and toxicities (TC_{50}, μmol dm^{-3}) of mono- and bis-(4-acyloxybenzyl) esters of the monophosphate of AZT in T-45 cells

Compd.	R	TC_{50}	EC_{50}
5	Me	150	0.008
5	Et	200	0.007
5	Pr^i	300	0.014
5	Bu^t	150	1
7	Me	100	< 0.002
7	Et	19	< 0.019
7	Pr^i	10	< 1.88
7	Bu^t	9	< 1.84
Bn	—	200	0.0094
9	—	100	< 2.6
AZT	—		0.0016

In summary, three of the triesters **5** and all four of the diesters **7** showed antiviral activities against HIV-1 and SIV improved or comparable to AZT, although their toxicities, which depended on the cell line, were in several instances substantially greater than that of the nucleoside.

This study demonstrates that the mono- and bis-(4-acyloxybenzyl) esters of the 5'-monophosphate of AZT, **5** and **7**, can be readily prepared, the key step involving the coupling of bis(4-acyloxybenzyl) *N,N*-diisopropylphosphoramidite **10** with AZT. In the presence of PLCE, the 4-acyloxybenzyl esters are readily removed to give the 5'-monophosphate of AZT **9**. This methodology could be applied to the design of lipophilic, bioreversible prodrugs for all medicinally important phosphates, and for the synthesis of phosphate monoesters which cannot be prepared using the routes requiring deprotection by high-pressure hydrogenation, trimethylsilyl bromide or strong acid.[15]

Experimental

NMR spectra were recorded on a Bruker 250-AC spectrometer at ^1H (250.1 MHz), ^{31}P (101.3 MHz) and ^{13}C (62.9 MHz). ^1H and ^{13}C NMR spectra were referenced to tetramethylsilane, and ^{31}P NMR spectra were referenced to 85% H_3PO_4. All ^{13}C and ^{31}P NMR spectra are ^1H decoupled (composite pulse decoupling) unless otherwise stated: positive chemical shifts are downfield from the reference. Mass spectra were recorded on a VG Micromass 12 instrument at 70 eV and a source temperature of 300 °C; accurate mass data were recorded on a VG 7070E instrument using positive ion FAB with a nitrobenzyl alcohol matrix. IR spectra were recorded on a Mattson Instruments 2020 Galaxy FT-IR Spectrophotometer. M.p.s were measured on a Gallenkamp Electrothermal Digital apparatus and are not corrected. Flash column chromatography[35] was performed using Sorbsil C60 silica gel. TLC was performed using plastic-backed Kieselgel 60 silica gel plates containing a fluorescent indicator. Spots were visualised under 254 nm UV light. Elemental analyses were performed by Butterworths Laboratories, Middlesex. Acetone and triethylamine were dried by heating under reflux followed by distillation over 4 Å molecular sieve and KOH, respectively. Chemicals were obtained from Aldrich Chemical Company and porcine liver carboxyesterase was obtained from Sigma Chemical Company. The units of enzyme added to the hydrolyses were based on the assay given by Sigma: one unit is defined as the esterase required to hydrolyse 1 μmol of ethyl butyrate to butyric acid and ethanol per min at pH 8.0 at 25 °C. The pD was calculated from the pH using the method described by Fife and Bruice.[41] High-performance liquid chromatography was performed using a Waters 600E gradient solvent delivery system fitted with a Merck reversed-phase C-18 endcapped Lichrospher 100 column (particle size 5 μm; 250 × 4 mm), Lichrocart reversed-phase C-18 endcapped guard column and monitored by UV at λ_{max} 254 nm using a Waters tunable absorbance detector. Hydrolysis samples (20 mm^3) were injected directly by way of a Waters 700 satellite WISP. Chromatograms were collected and integrated on an NEC Powermate SX Plus using Baseline software and printed on an NEC Pinwriter P5300.

Dibenzyl N,N-*diisopropylphosphoramidite*.—A solution of benzyl alcohol (4.32 g, 40 mmol) and triethylamine (4.45 g, 44 mmol) in tetrahydrofuran (100 cm^3) was added dropwise over 1 h to a stirred solution of *N,N*-diisopropylphosphorochloridate (4.04 g, 20 mmol) in tetrahydrofuran (150 cm^3) under argon at −78 °C. The mixture was stirred at room temperature for 2 h, after which time the triethylammonium hydrochloride was filtered off. The filtrate was evaporated under reduced pressure to give a pale yellow oil the analysis of which by TLC (hexane–ethyl acetate–triethylamine, 7:3:1) showed it (R_f 0.68) was contaminated with a trace of benzyl alcohol (R_f 0.28). Purification by flash column chromatography[35] gave the title compound (4.96 g, 72%); $\delta_H(CDCl_3)$ 1.20 [12 H, d, J_{HH} 6.8, $(CH_3)_2CHN$], 3.6–3.8 [2 H, m, $(CH_3)_2CHN$], 4.69 (2 H, dd, J_{gem} 12.75, J_{PH} 8.65, $PhCH_AH_BOP$), 4.78 (2 H, dd, J_{gem} 12.75, J_{PH} 8.25, $PhCH_AH_BOP$) and 7.2–7.4 (10 H, m, $PhCH_2OP$); δ_P 148.2 (s); δ_C 24.5 [d, J_{PC} 7.2, $(CH_3)_2CHN$], 42.9 [d, J_{PC} 12.4, $(CH_3)_2CHN$], 65.25 (d, J_{PC} 18.2, $PhCH_2OP$), 126.8 (s), 127.1 (s), 128.1 (s) and 139.4 (d, J_{PC} 7.4).

The following compounds were prepared from *N,N*-diisopropylphosphorochloridate and the appropriate 4-acyloxybenzyl alcohol using a method similar to that described above.

Bis(4-*acetoxybenzyl*) N,N-diisopropylphosphoramidite **10** (R = Me). (52%); $\delta_H(CDCl_3)$ 1.20 [12 H, d, J_{HH} 7.8, $(CH_3)_2CHN$], 2.28 [6 H, s, $CH_3C(O)$], 3.6–3.8 [2 H, m, $(CH_3)_2CHN$], 4.66 (2 H, dd, J_{gem} 13.0, J_{PH} 8.85, $ArCH_AH_BOP$), 4.75 (2 H, dd, J_{gem} 13.0, J_{PH} 8.4, $ArCH_AH_BOP$), 7.04 (4 H, d, J_{HH} 8.55) and 7.34 (4 H, d, J_{HH} 8.55); δ_C 21.0 [s, $CH_3C(O)$-] 24.65 [d, J_{PC} 7.2, $(CH_3)_2CHN$], 43.0 [d, J_{PC} 12.4, $(CH_3)_2CHN$], 64.7 (d, J_{PC} 18.4, $ArCH_2OP$), 121.3 (s), 127.9 (s), 137.05 (d, J_{PH} 7.6), 149.7 (s) and 169.4 (s, C=O).

Bis(4-*propionyloxybenzyl*) N,N-*diisopropylphosphoramidite* **10** (R = Et). The title compound was purified by flash column chromatography (hexane–ethyl acetate–triethylamine, 8:2:1, R_f 0.41) to give a colourless liquid, 60%; $\delta_H(CDCl_3)$ 1.19 [12 H, d, J_{HH} 6.8, $(CH_3)_2CHN$], 1.26 (6 H, t, J_{HH} 7.5, CH_3CH_2), 2.57 (4 H, q, J_{HH} 7.5, CH_3CH_2), 3.6–3.8 (2 H, m, $(CH_3)_2CHN$),

4.66 (2 H, dd, J_{gem} 13.0, J_{PH} 8.9, ArCH_AH$_B$OP), 4.75 (2 H, dd, J_{gem} 13.0, J_{PH} 8.5, ArCH$_A$$H_B$OP), 7.03 (4 H, d, J_{HH} 8.6), 7.34 (4 H, d, J_{HH} 8.6); δ_C 9.0 (s, CH_3CH_2), 24.55 [d, J_{PC} 7.2, $(CH_3)_2$CHN], 27.6 (s, CH_3CH_2), 43.0 [d, J_{PC} 12.4, $(CH_3)_2CHN$], 64.7 (d, J_{PC} 18.4, ArCH_2OP), 121.25 (s), 127.9 (s), 136.9 (d, J_{PC} 7.5), 149.8 (s) and 172.9 (s, C=O).

Bis(4-*isobutyryloxybenzyl*) N,N-*diisopropylphosphoramidite* **10** (R = Pr^i). The title compound was purified by flash column chromatography (hexane–ethyl acetate–triethylamine, 8 : 2 : 1, R_f 0.5) to give a colourless oil (81%); ν(thin film)/cm^{-1} 1757 (C=O); δ_H(CDCl$_3$) 1.20 [12 H, d, J_{HH} 6.8, $(CH_3)_2$CHN], 1.31 [12 H, d, J_{HH} 7.0, $(CH_3)_2$CHC(O)], 2.79 [2 H, sept, J_{HH} 7.0, $(CH_3)_2CH$C(O)], 3.6–3.8 [2 H, m, $(CH_3)_2CH$N], 4.68 (2 H, dd, J_{gem} 12.9, J_{PH} 8.9, ArCH_AH$_B$OP), 4.76 (2 H, dd, J_{gem} 12.9, J_{PH} 8.5, ArCH$_A$$H_B$OP), 7.03 (4 H, d, J_{HH} 8.6) and 7.35 (4 H, d, J_{HH} 8.6); δ_P 148.3 (s); δ_C 18.8 [s, $(CH_3)_2$CHC(O)], 24.5 [d, J_{PC} 7.2, $(CH_3)_2$CHN], 34.0 [s, $(CH_3)_2$CHC(O)], 42.9 [d, J_{PC} 12.3, $(CH_3)_2$CHN], 64.7 (d, J_{PC} 18.4, ArCH_2OP), 121.4 (s), 127.8 (s), 136.7 (d, J_{PC} 7.5), 149.9 (s) and 175.4 (s, C=O).

Bis(4-*pivaloyloxybenzyl*) N,N-*diisopropylphosphoramidite* **10** (R = Bu^t). The title compound was purified by flash column chromatography (hexane–ethyl acetate–triethylamine, 7:3:1, R_f 0.5) (87%); ν(thin film)/cm^{-1} 1753 (C=O); δ_H(CDCl$_3$) 1.19 [12 H, d, J_{HH} 6.9, $(CH_3)_2$CHN], 1.35 [18 H, s, $(CH_3)_3$C], 3.6–3.8 [2 H, m, $(CH_3)_2CH$N], 4.67 (2 H, dd, J_{gem} 12.6, J_{PH} 8.9, ArCH_AH$_B$OP), 4.75 (2 H, dd, J_{gem} 12.6, J_{PH} 8.4, ArCH$_A$$H_B$OP), 7.01 (4 H, d, J_{HH} 8.5) and 7.34 (4 H, d, J_{HH} 8.5); δ_P 148.4 (s); δ_C 24.5 [d, J_{PC} 7.2, $(CH_3)_2$CHN], 27.0 [s, $(CH_3)_3$C], 38.9 [s, $(CH_3)_3C$], 43.05 [d, J_{PC} 12.3, $(CH_3)_2CHN$], 64.8 (d, J_{PC} 18.3, ArCH_2OP), 121.2 (s), 127.9 (s), 136.7 (d, J_{PC} 7.4), 150.2 (s) and 176.95 (s, C=O).

3′-*Azido*-3′-*deoxythymidin*-5′-*yl Di*-tert-*butyl Phosphate*.—Adapting a published procedure,[32] 1*H*-tetrazole (0.21 g, 3 mmol) was added to a solution of AZT (0.276 g, 1 mmol) and di-*tert*-butyl *N*,*N*-diethylphosphoramidite (0.299 g, 1.2 mmol) in tetrahydrofuran (2 cm^3). After 30 min at room temperature, the reaction mixture was cooled to −40 °C and a solution of 3-chloroperoxybenzoic acid (0.23 g) in dichloromethane (3 cm^3) was added to it. The reaction mixture was worked up as described for the synthesis of 3′-azido-3′-deoxythymidin-5′-yl dibenzyl phosphate to give a pale yellow oil. Flash column chromatography eluting with ethyl acetate gave the title compound (0.41 g, 98%), R_f (EtOAc, 0.29); δ_H(CDCl$_3$) 1.48 [18 H, s, $(CH_3)_3$C], 1.93 (3 H, d, J_{HH} 1.2, thymine-CH_3), 2.25–2.45 (2 H, m, 2′-H), 3.95–4.2 (3 H, m, 3′-H and 5′-H), 4.25–4.4 (1 H, m, 4′-H), 6.28 (1 H, t, J_{HH} 6.4, 1′-H), 7.48 (1 H, q, J_{HH} 1.2, thymine-CH) and 9.4 (1 H, br s, thymine-NH); δ_P −3.5 (s); δ_C 12.45 (s, thymine-CH_3), 29.8 [d, J_{PC} 4.3, $(CH_3)_3$C-], 37.6 (s, C-2′), 60.0 (s, C-3′), 65.45 (d, J_{PC} 6.2, C-5′), 82.5 (d, J_{PC} 8.7, C-4′), 83.25 [d, J_{PC} 5.2, $(CH_3)_3C$], 83.35 [d, J_{PC} 5.05, $(CH_3)_3C$], 84.3 (s, C-1′), 111.5 (s, thymine C-5), 135.2 (s, thymine C-6), 150.3 (s, thymine C-4) and 163.8 (s, thymine C-2).

3′-*Azido*-3′-*deoxythymidin*-5′-*yl Dibenzyl Phosphate*.—1*H*-Tetrazole (0.21 g, 3 mmol) was added to a stirred solution of AZT (0.27 g, 1 mmol) and dibenzyl *N*,*N*-diisopropylphosphoramidite (0.41 g, 1.2 mmol) in tetrahydrofuran (2 cm^3) at room temperature. After 30 min, the reaction mixture was cooled to −40 °C and a solution of 3-chloroperoxybenzoic acid (0.23 g) in dichloromethane (2.5 cm^3) was added to it; the mixture was then allowed to warm to room temperature over 1 h. Sodium sulfite (10% solution; 3 cm^3) was added to the mixture to destroy excess of 3-chloroperoxybenzoic acid after which the organic layer was separated and the aqueous layer washed with dichloromethane (2 × 10 cm^3). The combined organic layer and extracts were washed with saturated aqueous sodium hydrogen carbonate (3 × 10 cm^3) and the aqueous layer back extracted with dichloromethane. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure to give a pale yellow oil, TLC (EtOAc) (R_f 0.27). Purification of this by flash column chromatography (EtOAc) gave the title compound (0.332 g, 63%) (Found: C, 53.1; H, 5.0; N, 12.2. $C_{24}H_{26}N_5O_7P$ requires C, 54.64; H, 4.97; N, 13.28%); δ_H(CDCl$_3$) 1.82 (3 H, d, J_{HH} 1.1, thymine CH_3), 2.0–2.15 (1 H, m, 2′-H), 2.25–2.35 (1 H, m, 2′-H), 3.85–4.2 (4 H, m, 3′-H, 4′-H and 5′-H), 5.02 (2 H, dd, J_{gem} 11.6, J_{PH} 9.8, ArCH_AH$_B$OP), 5.10 (2 H, dd, J_{gem} 11.6, J_{PH} 9.0, ArCH$_A$$H_B$OP), 6.17 (1 H, t, J_{HH} 6.14, 1′-H), 7.31 (1 H, q, J_{HH} 1.1, thymine CH), 7.34 (10 H, m, *Ph*CH_2OP) and 9.17 (1 H, s, thymine NH); δ_P −0.17 (s); δ_C 12.35 (s, thymine CH_3), 37.4 (s, 2′-C), 60.1 (s, 3′-C), 66.3 (d, J_{PC} 5.6, 5′-C), 69.84 (d, J_{PC} 5.2, PhCH_2OP) 69.90 (d, J_{PC} 5.5, PhCH_2OP), 82.1 (d, J_{PC} 8.2, 4′-C), 84.6 (s, 1′-C), 111.5 (s, thymine C-5), 128.2 (s), 128.7 (s), 129.0 (s), 135.1 (s, thymine C-6), 135.3 (d, J_{PC} 5.7), 150.2 (s, thymine C-4) and 163.7 (s, thymine C-2); *m*/*z* (FAB) 528 (M + H$^+$, 60%) and 550 (M + Na$^+$, 15%).

The following compounds were prepared from AZT and the appropriate bis(4-acyloxybenzyl) *N*,*N*-diisopropylphosphoramidite using a method similar to that described above.

3′-*Azido*-3′-*deoxythymidin*-5′-*yl bis*(4-*acetoxybenzyl*) *phosphate* **5** (R = Me). TLC (EtOAc) showed the major product (R_f 0.26) (52%) and a minor impurity (R_f 0.18) which was removed by flash column chromatography (Found: C, 52.1; H, 4.6; N, 10.65. $C_{28}H_{30}N_5O_{11}P$ requires C, 52.25; H, 4.70; N, 10.88%); ν(CHCl$_3$)/cm^{-1} 2110 (N_3), 1757 (ester C=O) and 1692 (C=O); δ_H(CDCl$_3$) 1.87 (3 H, d, J_{HH} 1.1, thymine CH_3), 2.0–2.35 (2 H, m, 2′-H), 2.27 [6 H, s, CH_3C(O)], 3.85–3.95 (1 H, m, 3′-H), 4.0–4.2 (3 H, m, 4′-H and 5′-H), 5.03 (2 H, dd, J_{gem} 11.7, J_{PH} 9.8, ArCH_AH$_B$OP), 5.07 (2 H, dd, J_{gem} 11.7, J_{PH} 8.3, ArCH$_A$$H_B$OP), 6.14 (1 H, t, J_{HH} 6.3, 1′-H), 7.08 (2 H, d, J_{HH} 8.6), 7.09 (2 H, d, J_{HH} 8.6), 7.25 (1 H, q, J_{HH} 1.1, thymine CH), 7.37 (2 H, d, J_{HH} 8.6), 7.38 (2 H, d, J_{HH} 8.6) and 9.23 (1 H, s, thymine NH); δ_P −0.21 (s); δ_C 12.4 (s, thymine CH_3), 21.1 [s, CH_3C(O)], 37.3 (s, 2′-C), 60.0 (s, 3′-C), 66.3 (d, J_{PC} 5.7, 5′-C), 69.18 (d, J_{PC} 4.0, ArCH_2OP), 69.25 (d, J_{PC} 5.2, ArCH_2OP), 82.0 (d, J_{PC} 8.0, 4′-C), 84.7 (s, 1′-C), 111.3 (s, thymine C-5), 122.0 (s), 129.45 (s), 132.9 (d, J_{PC} 5.8), 135.2 (s, thymine C-6), 150.1 (s, thymine 4-C), 151.1 (s), 163.5 (s, thymine C-2) and 169,3 (s, C=O); *m*/*z* (FAB) 644 (M + H$^+$, 30%) and 666 (M + Na$^+$, 20%).

3′-*Azido*-3′-*deoxythymidin*-5′-*yl bis*(4-*propionyloxybenzyl*) *phosphate* **5** (R = Et). (61%); TLC (EtOAc) R_f 0.26 (Found: C, 53.3; H, 5.2; N, 9.9. $C_{30}H_{34}N_5O_{11}P$ requires C, 53.65; H, 5.10; N, 10.43%); ν(CHCl$_3$)/cm^{-1} 2110 (N_3), 1757 (ester C=O) and 1692 (C=O); δ_H(CDCl$_3$) 1.26 (6 H, t, J_{HH} 7.0, CH_3CH_2), 1.87 (3 H, d, J_{HH} 1.1, thymine CH_3), 2.05–2.2 (1 H, m, 2′-H), 2.25–2.4 (1 H, m, 2′-H), 2.60 (4 H, q, J_{HH} 7.0, CH_3CH_2), 3.85–3.95 (1 H, m, 3′-H), 4.0–4.2 (3 H, m, 4′-H and 5′-H), 5.04 (2 H, dd, J_{gem} 11.7, J_{PH} 10.0, ArCH_AH$_B$OP), 5.07 (2 H, dd, J_{gem} 11.7, J_{PH} 7.9, ArCH$_A$$H_B$OP), 6.17 (1 H, t, J_{HH} 6.5, 1′-H), 7.08 (2 H, d, J_{HH} 8.6), 7.09 (2 H, d, J_{HH} 8.6), 7.28 (1 H, q, J_{HH} 1.1, thymine CH), 7.37 (2 H, d, J_{HH} 8.6), 7.38 (2 H, d, J_{HH} 8.6) and 9.18 (1 H, s, thymine NH); δ_P −0.20 (s); δ_C 8.9 (s, CH_3CH_2-), 12.4 (s, thymine CH_3), 27.6 (s, CH_3CH_2), 37.2 (s, 2′-C), 60.0 (s, 3′-C), 66.2 (d, J_{PC} 5.5, 5′-C), 69.2 (d, J_{PC} ~5, ArCH_2OP), 69.25 (d, J_{PC} ~5, ArCH_2OP), 82.0 (d, J_{PC} 8.1, 4′-C), 84.7 (s, 1′-C), 111.3 (s, thymine C-5), 122.0 (s), 129.4 (s), 132.7 (d, J_{PC} 5.8), 135.2 (s, thymine C-6), 150.1 (s, thymine C-4), 151.1 (s), 163.6 (s, thymine C-2) and 172.8 (s, C=O); *m*/*z* (FAB) 694 (M + Na$^+$, 25%) [Found: *m*/*z* 694.189 (M + Na$^+$). $C_{30}H_{34}N_5NaO_{11}P$ requires 694.189].

3′-*Azido*-3′-*deoxythymidin*-5′-*yl bis*(4-*isobutyryloxybenzyl*) *phosphate* **5** (R = Pr^i). (62%); TLC (EtOAc) R_f 0.28 (Found: C, 55.2; H, 5.5; N, 9.9. $C_{32}H_{38}N_5O_{11}P$ requires C, 54.93; H, 5.47; N, 10.00%); ν(CHCl$_3$)/cm^{-1} 2110 (N_3), 1753 (ester C=O) and 1692 (C=O); δ_H(CDCl$_3$) 1.31, [12 H, d, J_{HH} 7, $(CH_3)_2$CH], 1.88 (3 H, d, J_{HH} 1.0, thymine CH_3), 2.05–2.2 (1 H, m, 2′-H),

2.25–2.4 (1 H, m, 2'-H), 2.80 [2 H, sept, J_{HH} 7.0, $(CH_3)_2CH$], 3.85–3.95 (1 H, m, 3'-H), 4.0–4.2 (3 H, m, 4'-H and 5'-H), 5.04 (2 H, dd, J_{gem} 11.7, J_{PH} 10.1, $ArCH_AH_BOP$), 5.07 (2 H, dd, J_{gem} 11.7, J_{PH} 7.6, $ArCH_AH_BOP$), 6.17 (1 H, t, J_{HH} 6.5, 1'-H), 7.07 (2 H, d, J_{HH} 8.5), 7.08 (2 H, d, J_{HH} 8.5), 7.29 (1 H, q, J_{HH} 1.1, thymine CH), 7.37 (2 H, d, J_{HH} 8.5), 7.38 (2 H, d, J_{HH} 8.5) and 9.18 (1 H, s, thymine NH); δ_P −0.19 (s); δ_C 12.4 (s, thymine CH_3), 18.8 [s, $(CH_3)_2CH$], 34.1 [s, $(CH_3)_2CH$], 37.2 (s, C-2'), 60.1 (s, C-3'), 66.7 (d, J_{PC} 5.6, C-5'), 69.2 (d, J_{PC} ~ 5, $ArCH_2OP$), 69.25 (d, J_{PC} ~ 5, $ArCH_2OP$), 82.1 (d, J_{PC} 8.1, $ArCH_2OP$), 84.7 (s, C-1'), 111.3 (s, thymine C-5), 122.0 (s), 129.4 (s), 132.7 (d, J_{PC} 5.8), 135.2 (s, thymine C-6), 150.5 (s, C-4), 151.3 (s), 163.7 (s, thymine C-2) and 175.45 (s, C=O); *m/z* (FAB) 700 ($M + H^+$, 15%) and 722 ($M + Na^+$, 35%) [Found: *m/z* 700.238 ($M + H^+$). $C_{32}H_{39}N_5O_{11}P$ requires 700.238].

3'-Azido-3'-deoxythymidin-5'-yl bis (4-pivaloyloxybenzyl) phosphate **5** (R = Bu^t). (42%); TLC (EtOAc) (R_f 0.28) (Found: C, 56.8; H, 5.65; N, 9.6. $C_{34}H_{42}N_5O_{11}P$ requires C, 56.11; H, 5.81; N, 9.62%); $\nu(CHCl_3)/cm^{-1}$ 2110 (N_3), 1748 (ester C=O) and 1690 (C=O); $\delta_H(CDCl_3)$ 1.33 [18 H, s, $(CH_3)_3C$], 1.86 (3 H, d, J_{HH} 1.1, thymine CH_3), 2.02–2.2 (1 H, m, 2'-H), 2.3–2.45 (1 H, m, 2'-H), 3.85–3.95 (1 H, m, 3'-H), 4.0–4.2 (3 H, m, 4'-H and 5'-H), 5.04 (2 H, dd, J_{gem} 11.7, J_{PH} 10.2, $ArCH_AH_BOP$), 5.07 (2 H, dd, J_{gem} 11.7, J_{PH} 7.5, $ArCH_AH_BOP$), 6.14 (1 H, t, J_{HH} 6.5, 1'-H), 7.05 (2 H, d, J_{HH} 8.5), 7.06 (2 H, d, J_{HH} 8.5), 7.27 (1 H, q, J_{HH} 1.1, thymine CH), 7.367 (2 H, d, J_{HH} 8.5), 7.374 (2 H, d, J_{HH} 8.5) and 8.9 (1 H, s, thymine NH); δ_P −0.18 (s); δ_C 12.4 (s, thymine CH_3), 27.0 [s, $(CH_3)_3C$], 37.2 (s, C-2'), 39.0 [s, $(CH_3)_3C$], 60.1 (s, C-3'), 66.3 (d, J_{PC} 5.5, C-5'), 69.25 (d, J_{PC} ~ 5, $ArCH_2OP$), 69.3 (d, J_{PC} ~ 5, $ArCH_2OP$), 82.0 (d, J_{PC} 8.1, C-4'), 84.7 (s, C-1'), 111.3 (s, thymine C-5), 121.9 (s), 129.4 (s), 132.6 (d, J_{PC} 5.6), 135.2 (s, thymine C-6), 150.1 (s, thymine C-4), 151.5 (s), 163.5 (s, thymine C-2) and 176.9 (s, C=O); *m/z* (FAB) 750 ($M + Na^+$, 10%).

Lithium 3'-Azido-3'-deoxythymidin-5'-yl 4-Acetoxybenzyl Phosphate **7** (R = Me).—Lithium iodide (0.071 g, 0.53 mmol) was added to a stirred solution of the triester **5** (R = Me) (0.34 g, 0.53 mmol) in acetone (2 cm^3). After 20 h at room temperature under an argon atmosphere in the dark the white precipitate was filtered off and washed with cooled acetone–hexane (1:1). This was dried overnight *in vacuo* to give the title compound (0.20 g, 78%) (Found: C, 45.5; H, 4.4; Li, 1.2; N, 13.4; P, 6.1. $C_{19}H_{21}LiN_5O_9P$ requires C, 45.52; H, 4.22; Li, 1.38; N, 13.97; P, 6.18%); ν(KBr)/cm^{-1} 2108 (N_3), 1758 (ester C=O) and 1700 (C=O); $\delta_H(D_2O)$ 1.80 (3 H, s, thymine CH_3), 2.2–2.45 (2 H, m, 2'-H), 2.30 [3 H, s, $CH_3C(O)$], 3.94–4.04 (3 H, m, 3'-H and 5'-H), 4.25–4.35 (1 H, m, 4'-H), 4.89 (2 H, d, J_{PH} 8.4, $ArCH_2OP$), 6.13 (1 H, t, J_{HH} 6.6, 1'-H), 7.06 (2 H, d, J_{HH} 8.4), 7.41 (2 H, d, J_{HH} 8.4) and 7.51 (1 H, s, thymine CH); δ_P 0.93 (s); δ_C 14.6 (s, thymine CH_3), 23.4 [s, $CH_3C(O)$], 39.2 (s, C-2'), 63.2 (s, C-3'), 67.85 (d, J_{PC} 5.1, C-5'), 70.0 (d, J_{PC} 5.4, $ArCH_2OP$), 85.9 (d, J_{PC} 8.7, C-4' ribose), 87.9 (s, C-1'), 114.3 (s, thymine C-5), 124.6 (s), 131.9 (s), 138.5 (d, J_{PC} 6.5), 140.2 (s, thymine C-6), 152.85 (s, thymine C-4), 154.4 (s), 169.2 (s, thymine C-2) and 176.2 (s, C=O); *m/z* (FAB) 502 ($M + H^+$, 100%) and 508 ($M + Li^+$, 65%) [Found: *m/z* 502.1315 ($M + H^+$). $C_{19}H_{22}LiN_5O_9P$ requires 502.1315].

The following compounds were prepared from lithium iodide and the appropriate triester using a method similar to that described above.

Lithium 3'-azido-3'-deoxythymidin-5'-yl 4-propionyloxybenzyl phosphate **7** (R = Et). (55%); (Found: C, 47.25; H, 4.5; N, 12.95. $C_{20}H_{23}LiN_5O_9P$ requires C, 46.61; H, 4.50; N, 13.59%); ν(KBr)/cm^{-1} 2108 (N_3), 1758 (ester C=O) and 1670 (C=O); $\delta_H(D_2O)$ 1.15 (3 H, t, J_{HH} 7.4, CH_3CH_2), 1.78 (3 H, s, thymine CH_3), 2.22–2.39 (2 H, m, 2'-H), 2.60 [2 H, q, J_{HH} 7.4, CH_3CH_2], 3.9–4.05 (3 H, m, 3'-H and 5'-H), 4.2–4.3 (1 H, m, 4'-H), 4.88 (2 H, d, J_{PH} 8.4, $ArCH_2OP$), 6.10 (1 H, t, J_{HH} 6.6, 1'-H), 7.02 (2 H, d, J_{HH} 8.3), 7.38 (2 H, d, J_{HH} 8.3) and 7.48 (1 H, s, thymine CH); δ_P 0.91 (s); δ_C 11.2 [s, CH_3CH_2], 14.6 (s, thymine CH_3), 30.3 [s, CH_3CH_2], 39.2 (s, C-2'), 63.1 (s, C-3'), 67.8 (d, J_{PC} 5.0, C-5'), 70.0 (d, J_{PC} 5.5, $ArCH_2OP$), 85.8 (d, J_{PC} 8.8, C-4'), 87.8 (s, C-1'), 114.3 (s, thymine C-5), 124.6 (s), 131.9 (s), 138.45 (d, J_{PC} 6.5), 140.2 (s, thymine C-6), 152.9 (s, thymine C-4), 154.4 (s), 169.2 (s, thymine C-2) and 179.5 (s, C=O).

Lithium 3'-azido-3'-deoxythymidin-5'-yl 4-isobutyryloxybenzyl phosphate **7** (R = Pr^i). (57%) (Found: C, 47.4; H, 4.75; N, 12.6. $C_{21}H_{25}LiN_5O_9P$ requires C, 47.65; H, 4.76; N, 13.23%); ν(KBr)/cm^{-1} 2118 (N_3), 1753 (ester C=O) and 1670 (C=O); $\delta_H(D_2O)$ 1.26 [12 H, d, J_{HH} 7.4, $(CH_3)_2CH$], 1.78 (3 H, s, thymine CH_3), 2.22–2.39 (2 H, m, 2'-H), 2.78 [1 H, sept, J_{HH} 7.4, $(CH_3)_2CH$], 3.90–4.05 (3 H, m, 3'-H and 5'-H), 4.20–4.30 (1 H, m, 4'-H), 4.88 (2 H, d, J_{PH} 8.4, $ArCH_2OP$), 6.10 (1 H, t, J_{HH} 6.6, 1'-H), 7.02 (2 H, d, J_{HH} 8.3), 7.38 (2 H, d, J_{HH} 8.3) and 7.48 (1 H, s, thymine CH); δ_P 0.95 (s); δ_C 14.7 (s, thymine CH_3), 21.0 [s, $(CH_3)_2CH$], 36.9 [s, $(CH_3)_2CH$], 39.2 (s, C-2'), 63.1 (s, C-3'), 67.8 (d, J_{PC} 5.0, C-5'), 70.05 (d, J_{PC} 5.3, $ArCH_2OP$), 85.8 (d, J_{PC} 8.8, C-4'), 87.8 (s, C-1'), 114.25 (s, thymine C-5), 124.5 (s), 131.9 (s), 138.45 (d, J_{PC} 6.5), 140.2 (s, thymine C-6), 153.0 (s, thymine C-4), 154.4 (s), 169.1 (s, thymine C-2) and 182.2 (s, C=O).

Lithium 3'-azido-3'-deoxythymidin-5'-yl 4-pivaloyloxybenzyl phosphate **7** (R = Bu^t). (77%) (Found: C, 47.6; H, 5.1; N, 11.6. $C_{22}H_{27}LiN_5O_9P$ requires C, 48.63; H, 5.01; N, 12.89%); ν(KBr)/cm^{-1} 2110 (N_3), 1752 (ester C=O) and 1670 (C=O); $\delta_H(D_2O)$ 1.30 [9 H, s, $(CH_3)_3C$], 1.79 (3 H, s, thymine CH_3), 2.15–2.40 (2 H, m, 2'-H), 3.90–4.20 (3 H, m, 3'-H and 5'-H), 4.15–4.20 (1 H, m, 4'-H), 4.90 (2 H, d, J_{PH} 8.5, $ArCH_2OP$), 6.10 (1 H, t, J_{HH} 6.6, 1'-H), 7.01 (2 H, d, J_{HH} 8.3), 7.39 (1 H, s, thymine CH) and 7.43 (2 H, d, J_{HH} 8.3).

Di(ammonium) 3'-azido-3'-deoxythymidin-5'-yl phosphate **9**.—Trifluoroacetic acid (0.68 cm^3) was added to a solution of 3'-azido-3'-deoxythymidin-5'-yl di-*tert*-butyl phosphate (0.41 g, 1 mmol) in dichloromethane (2.5 cm^3). After 1 h at room temperature, the solvent was removed under reduced pressure. Ethanol (2.5 cm^3) was added to the residue and then removed *in vacuo*; this process was repeated. Concentrated ammonia (40 drops) was added to the residue followed by ethanol and the solution was left overnight at *ca.* 0 °C. The resulting white precipitate was filtered off, washed with cold ethanol and dried *in vacuo* overnight over P_2O_5 to give the title compound (0.27 g, 71%); $\delta_H(D_2O)$ 1.89 (3 H, s, thymine CH_3), 2.4–2.5 (2 H, m, 2'-H), 3.95–4.05 (2 H, m, 5'-H), 4.15–4.25 (1 H, m, 3'-H), 4.45–4.55 (1 H, m, 4'-H), 6.24 (1 H, t, J_{HH} 6.7, 1'-H) and 7.78 (1 H, s, thymine CH); δ_P 3.16 (s); δ_C 14.6 (s, thymine CH_3), 39.2 (s, C-2'), 63.9 (s, C-3'), 67.1 (d, J_{PC} 4.8, C-5'), 86.5 (d, J_{PC} 8.9, C-4'), 87.8 (s, C-1'), 114.7 (s, thymine C-5), 140.5 (s, thymine C-6), 154.7 (s, thymine C-4) and 169.6 (s, thymine C-2).

PLCE Hydrolysis Studies and HPLC Analysis of the Triester **5** (R = Me).—Hydrolyses were performed in glass screw-capped vials using a total incubation volume of 2 cm^3. A mixture of phosphate buffer (0.1 mol dm^{-3}, pH 7.4)–MeCN (95:5, v/v) was pre-incubated at 37 °C for 10 min. An acetonitrile solution of the triester **5** (R = Me) was then added to give a final concentration of 1 mmol dm^{-3}. Enzyme hydrolyses were initiated by the addition of the appropriate amount of PLCE in phosphate buffer (0.1 mol dm^{-3}, pH 7.4; 5 mm^3). Control and enzyme hydrolyses were incubated at 37 °C on a shaking water-bath. At the appropriate time, samples were removed (100 mm^3) and added to chilled MeCN (100 mm^3) and mixed on a vortex mixer. Samples were then analysed by HPLC, eluting with a linear gradient of MeCN–10 mmol dm^{-3} tetrabutylammonium hydroxide in water (pH 3.68), initial conditions 20:80 v/v; final

conditions 90:10 v/v; gradient time 20 min; flow rate 1 cm^3 min^{-1}. Retention times were: 4-hydroxybenzylalcohol, 3.9 min; AZT, 5.3 min; 4-acetoxybenzyl alcohol, 9.2 min; **9**, 11.8 min; **7** (R = Me), 14.6; **5** (R = Me), 18.5 min.

Solutions of calibration standards in phosphate buffer (0.1 mol dm^{-3}, pH 7.4)–MeCN (1:1, v/v) were prepared daily in duplicate. Curves were fitted by linear regression analysis using Baseline software and were linear over the ranges 1–1000 μmol dm^{-3} [**9**, **7** (R = Me) and **5** (R = Me)] and 100–1000 μmol dm^{-3} (AZT, 4-hydroxybenzyl alcohol and 4-acetoxybenzyl alcohol). Correlation coefficients were in the range 0.97–0.99.

Incubation of the Triester **5** (R = Me) *with Human Plasma.*—Human blood (30 cm^3) was centrifuged at 750 *g* for 10 min to give plasma as the supernatant fraction. Plasma (0.95 cm^3) was pre-incubated at 37 °C for 10 min. Hydrolysis was initiated by the addition of an acetonitrile solution of the triester **5** (50 mm^3) to give a final concentration of 1 mmol dm^{-3}. The mixture was then incubated at 37 °C on a shaking water-bath. Samples (200 mm^3) were removed and added to ice-cold MeCN (200 mm^3). Precipitated plasma proteins were removed by centrifugation at 1000 *g* for 10 min and the supernatant analysed by HPLC.

Hydrolysis Studies of the Triesters **5** *and the Diesters* **7** *by* ^{31}P *NMR Spectroscopy.*—The triesters **5** (R = Me, Et or Pr^i) [δ_P 0.0–0.05 ppm] or diesters **7** (R = Me, Et, Pr^i or Bu^t) [δ_P 1.05–1.1 ppm] were dissolved in a solution of potassium methylphosphonate buffer [δ_P 23.7 ppm] (0.1 mol dm^{-3}, pD 8.0)–MeCN (9:1, v/v) at 37 °C to give concentrations of triesters and diesters of 2 or 5 mmol dm^{-3}, respectively.

To initiate the hydrolyses with esterase, 1 U of PLCE was added to a 1 cm^3 portion of the triester solution **5** (R = Me, Et or Pr^i) and 5 U of PLCE was added to a 1 cm^3 portion of the diester solution **7** (R = Me, Et, Pr^i or Bu^t). All reactions were maintained at 37 °C and monitored by ^{31}P NMR spectroscopy at regular time intervals.

Antiviral Assay.—The anti-HIV-1 activities and toxicities of compounds were assessed in two T cell lines, C8166 and JM infected with the IIIB and GB8 strains of HIV-1, respectively.[27] Cells were grown in RPMI 1640 with 10% calf serum. 4×10^4 cells per microtitre plate-well were mixed with 5-fold dilutions of compound prior to addition of 10 $CCID_{50}$ units of virus and incubated for 5–7 days. Formation of syncytia was examined from 2 days post-infection. Culture fluid was collected at 5–7 days and gp120 antigen production measured by ELISA.[42] Cell viability of infected cells and cytotoxicity to uninfected cell controls were assessed by the MTT-Formazan method.[43] Activity against SIV was assayed similarly in C8166 cells infected with SIV_{MAC}.

Antiviral activity against HIV-1 infection of T-45 cells was assayed as described by Karpas *et al.*[44]

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References

1 A preliminary account of this work was presented at the British Pharmaceutical Society Conference, Birmingham, September 1992, W. Thomson, D. Nicholls, J. S. Al-Mushadani, W. J. Irwin and S. Freeman, *J. Pharm. Pharmacol.*, 1992, **44** (Supplement), 1080.
2 WHO Bulletin, *Neuropsychiatric aspects of HIV-1 infection*, 1990, **68**, 671.
3 T. P. Bridge and L. J. Ingraham, *Ann. Rev. Med.*, 1990, **41**, 159.
4 J. M. Gallo, J. T. Etse, K. J. Doshi, F. D. Boudinot and C. K. Chu, *Pharm. Res.*, 1991, **8**, 247.
5 W. M. Pardridge, *Ann. Rep. Med. Chem.*, 1985, **20**, 305.
6 H. Bundgaard, in *Design of Prodrugs*, ed. H. Bundgaard, Elsevier, 1985, pp. 70–74.
7 N. Bodor, *Adv. Drug Res.*, 1984, **13**, 255.
8 V. E. Bel'skii, *Russ. Chem. Rev.*, 1977, **46**, 828.
9 D. Farquhar, D. N. Srivastva, N. J. Kuttesch and P. P. Saunders, *J. Pharm. Sci.*, 1983, **72**, 324.
10 D. N. Srivastva and D. Farquhar, *Bioorg. Chem.*, 1984, **12**, 118.
11 R. P. Iyer, L. R. Phillips, J. A. Biddle, D. R. Thakker, W. Egan, S. Aoki and H. Mitsuya, *Tetrahedron Lett.*, 1989, **30**, 7141.
12 J. K. Sastry, P. N. Nehete, S. Khan, B. J. Nowak, W. Plunkett, R. B. Arlinghaus and D. Farquhar, *Mol. Pharmacol.*, 1992, **41**, 441.
13 J. E. Starrett, D. R. Tortolani, M. J. M. Hitchcock, J. C. Martin and M. M. Mansuri, *Antiviral Res.*, 1992, **19**, 267.
14 S. Freeman, W. J. Irwin, A. G. Mitchell, D. Nicholls and W. Thomson, *J. Chem. Soc., Chem. Commun.*, 1991, 875.
15 A. G. Mitchell, W. Thomson, D. Nicholls, W. J. Irwin and S. Freeman, *J. Chem. Soc., Perkin Trans. 1*, 1992, 2345, and references therein.
16 R. Yarchoan and S. Broder, *N. Engl. J. Med.*, 1987, **316**, 557.
17 R. Yarchoan, K. J. Weinhold, H. K. Lyerly, E. Gelmann, R. M. Blum, G. M. Shearer, H. Mitsuya, J. M. Collins, C. E. Myers, R. W. Klecker, P. D. Markham, D. T. Durack, S. N. Lehrman, D. W. Barry, M. A. Fischl, R. C. Gallo, D. P. Bolognesi and S. Broder, *Lancet*, 1986, **1**, 575.
18 T. Seki, T. Kawaguchi and K. Juni, *Pharm. Res.*, 1990, **7**, 948.
19 T. Kawaguchi, K. Ishikawa, T. Seki and K. Juni, *J. Pharm. Sci.*, 1990, **79**, 531.
20 T. Kawaguchi, T. Endoh, T. Seki and K. Juni, *J. Pharm. Sci.*, 1991, **80**, 404.
21 S. K. Aggarwal, S. R. Gogu, S. R. S. Rangan and K. C. Agrawal, *J. Med. Chem.*, 1990, **33**, 1505.
22 P. F. Torrence, J. Kinjo, K. Lesiak, J. Balzarini and E. De Clercq, *FEBS Lett.*, 1988, **234**, 135.
23 A. Fridland, M. C. Connelly and R. Ashmun, *Mol. Pharmacol.*, 1990, **37**, 665.
24 C. McGuigan, S. R. Nicholls, T. J. O'Connor and D. Kinchington, *Antiviral Chem. Chemother.*, 1990, **1**, 25.
25 B. C. N. M. Jones, C. McGuigan, T. J. O'Connor, D. J. Jeffries and D. Kinchington, *Antiviral Chem. Chemother.*, 1991, **2**, 35.
26 C. McGuigan, C. Nickson, T. J. O'Connor and D. Kinchington, *Antiviral Res.*, 1992, **17**, 197.
27 C. McGuigan, R. N. Pathirana, N. Mahmood, K. G. Devine and A. J. Hay, *Antiviral Res.*, 1992, **17**, 311.
28 Y. Henin, C. Gouyette, O. Schwartz, J.-C. Debouzy, J.-M. Neumann and T. Huynh-Dinh, *J. Med. Chem.*, 1991, **34**, 1830.
29 C. Le Bec and T. Huynh-Dinh, *Tetrahedron Lett.*, 1991, **32**, 6553.
30 A. Glazier, *Phosphorus Prodrugs*, International Publication Number WO 91/19721, Dec. 1991.
31 J. W. Perich and R. B. Johns, *Tetrahedron Lett.*, 1987, **28**, 101.
32 J. W. Perich and R. B. Johns, *Synthesis*, 1988, 142.
33 T. Tanaka, S. Tamatsukuri and M. Ikehara, *Tetrahedron Lett.*, 1986, **27**, 199.
34 R. Grice and L. N. Owen, *J. Chem. Soc.*, 1963, 1947.
35 W. C. Still, M. Khan and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.
36 L. Zervas and I. Dilaris, *J. Am. Chem. Soc.*, 1955, **77**, 5354.
37 P. L. Carl, P. K. Chakravarty and J. A. Katzenellenbogen, *J. Med. Chem.*, 1981, **24**, 479.
38 T. L. Amyes and J. P. Richard, *J. Am. Chem. Soc.*, 1990, **112**, 9507.
39 J. P. Richard, T. L. Amyes, L. Bei and V. Stubblefield, *J. Am. Chem. Soc.*, 1990, **112**, 9513.
40 G. Molema, R. W. Jansen, J. Visser, P. Herdewijn, F. Moolenaar and D. K. F. Meijer, *J. Med. Chem.*, 1991, **34**, 1137.
41 T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, 1961, **65**, 1079.
42 N. Mahmood and A. J. Hay, *J. Immunol. Methods*, 1992, **151**, 9.
43 R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Schols, P. Herdewijn, J. Desmeyter and E. De Clercq, *J. Virol. Methods*, 1988, **20**, 209.
44 A. Karpas, G. W. J. Fleet, R. A. Dwek, S. Petursson, S. K. Namgoong, N. G. Ramsden, G. S. Jacob and T. W. Rademacher, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 9229.

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