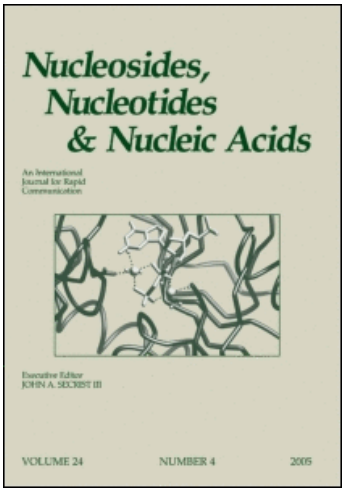

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S. I. Shimizu[a]; J. Balzarini[b]; E. De Clercq[a]; R. T. Walker[b]
[a] School of Chemistry, University of Birmingham, Birmingham, U.K. [b] Rega Institute, Katholieke Universiteit Leuven, Leuven, Belgium

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THE SYNTHESIS AND BIOLOGICAL PROPERTIES OF SOME ARYL BIS(NUCLEOSID-5'-YL) PHOSPHATES USING NUCLEOSIDES WITH PROVEN ANTI-HIV ACTIVITY#

S.I. Shimizu,[+] J. Balzarini[△], E. De Clercq[△] and R.T. Walker[+*]

[+]School of Chemistry, University of Birmingham, Birmingham B15 2TT, U.K.
[△]Rega Institute, Katholieke Universiteit Leuven,
Minderbroedersstraat 10, B-3000 Leuven, Belgium

Abstract: The synthesis of a series of aryl bis(nucleosid-5'-yl)phosphates in which the nucleosides are either 2',3'-dideoxy-(d2-) or 2',3'-didehydro-2',3'-dideoxy-(d4-) nucleosides is described. These were tested for anti-HIV activity and their efficacy and toxicity compared with the parent nucleosides. Only the 4-(methylsulphonyl)phenyl derivatives of d4T and d2A had any significant activity and had selectivity indices of the same order as the parent nucleosides. These findings can be explained by uptake of the triesters into cells followed by a slow release of nucleoside and nucleotide. In the case of some compounds (such as d2T and d2U) the 5'-monophosphate of which is known to inhibit thymidylate kinase, it is possible that the levels of nucleotide liberated are such that they are not processed into the 5'-triphosphate and hence no antiviral effect is seen.

INTRODUCTION

Over a period of many years, we[1-8] and others[9-11] have prepared a variety of pro-drugs in an attempt to get phosphates (usually nucleotides) liberated inside a cell and thus giving a method of circumventing the necessity for any nucleoside to have to be a kinase substrate before any significant biological activity can be expected.

It has previously been pointed out[12] that liberation of a nucleotide of a known active nucleoside inside a cell might be counterproductive if normally some selectivity is imposed upon the phosphorylation by a viral enzyme. However in the case of nucleosides which show activity against HIV, one has to rely upon the cellular kinase

This paper is dedicated to the memory of Professor Tohru Ueda.

for phosphorylation and it clearly might be advantageous to have the nucleotide liberated in cells and increase the specificity by targetting the drug only to those cell types which are infected.

Our first attempts involved the synthesis of nucleoside cyclic phosphoramidate analogues which needed enzymatic hydroxylation[1,2] before sufficient chemical lability was present for nucleotide to be formed under physiological conditions. The results obtained were not promising and we turned to more chemically-labile 5- and 6-membered oxazaphospha- or diazaphospha-ring systems.[3-6] These would liberate a nucleoside diester following cell penetration by the uncharged nucleoside analogue but all the evidence suggested that phosphodiesterase attack then liberated nucleoside rather than nucleotide.

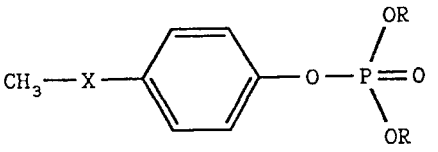
More recently, in attempts to overcome this, we reported the synthesis of some cyclic phosphoramidate nucleoside derivatives[7] which under physiological conditions and without the necessity for enzyme activity would yield exclusively nucleotide. However, the compounds were very labile and could not be prepared in sufficient quantities for use in an in vivo assay.

A new approach[8] involved the synthesis of aryl bis(nucleosid-5'-yl)phosphates, designed to penetrate cells, liberate the bis(nucleosid-5-yl)phosphate in the cell which should then yield one equivalent each of nucleoside and nucleotide upon enzymatic hydrolysis. The results using analogues of E-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) and 9[(1,3-(dihydroxy-2-propoxy)methyl]guanine (acyclovir) were of sufficient interest to encourage us to make further analogues of this sort from a range of 2',3'-dideoxy- and 2',3'-dideoxy-2',3'-didehydro-nucleoside analogues. Although some of these nucleoside analogues already show substantial activity against HIV, others, notably 2',3'-dideoxyuridine show no significant activity despite the fact that the 5'-triphosphate of the analogue is a good reverse transcriptase inhibitor because the original nucleoside is not a good nucleoside kinase substrate.[13]

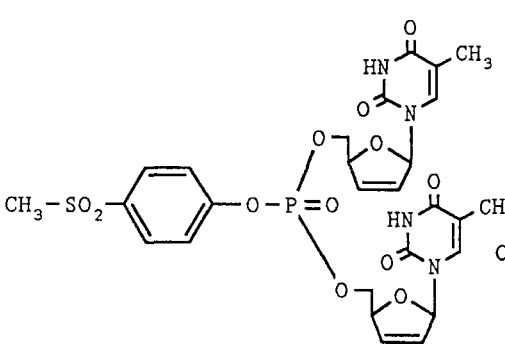
The current work describes the synthesis and biological activity of a range of aryl bis(2',3'-dideoxynucleosid-5'-yl)- (d2X-) and aryl bis(2',3'-didehydro-2',3'-dideoxynucleosid-5'-yl)- (d4X) phosphates.

RESULTS AND DISCUSSION

(a) Chemistry Previous work with aryl bis(nucleosid-5'yl)phosphates has shown that a suitable aryl group is the 4(methylsulphonyl)phenyl group.[8] The phenol liberated under physiological conditions appears not to be toxic in vitro or in vivo and the half-life of the triester is around 17 h which gives a reasonable stability to the compounds so that



	X	R		X	R
1	SO_2	d4U	9	SO_2	d4A
2	SO_2	d2U	10	SO_2	d2A
3	SO_2	d4T	11	S	d4A
4	SO_2	d2T	12	S	d2A
5	S	d4T	13	S	d4I
6	S	N^4Acd4C	14	SO_2	d4I
7	S	d4C	15	S	d2I
8	SO_2	d4C	16	SO_2	d2I



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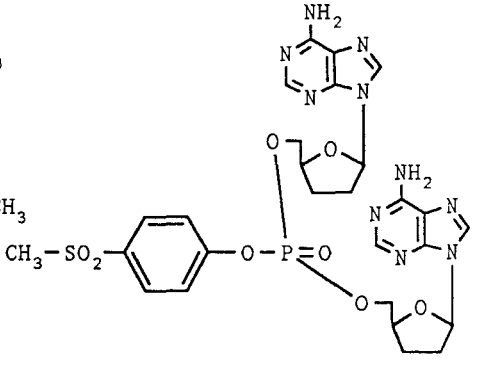

(10)

their synthesis is possible and yet within the time scale of an HIV assay, essentially all the compound would be hydrolysed. It also has the advantage that its precursor can be the 4-(methylthio)phenyl group which is very much more stable and providing the other substituents are stable to the conditions, can be oxidised by 3-chloroperoxybenzoic acid to the methylsulphonyl group at the last step.

Most of the triesters were made in the manner previously described. As the thymine (3-5), uracil (1-2) and adenine (9-12) analogues required no protection and deprotection step, the triester of 4-(methylsulphonyl)phenol could be made directly using the corresponding phosphorodichloridate rather than oxidising the corresponding methylthio derivatives, some of which were synthesised as controls for the antiviral assays.

The hypoxanthine derivatives (13-16) caused more problems as both 2',3'-dideoxyinosine and 2',3'-didehydro-2',3'-dideoxyinosine are very labile and significant quantities of hypoxanthine were produced during the attempted formation of the phosphotriesters. Although not a very sophisticated method, it was found that the 4-(methylthio)phenyl triesters of both the d2- and d4-adenosine analogues were substrates (albeit very poor ones) for the enzyme 5'-adenylic acid deaminase. To achieve a near quantitative deamination, very large quantities of enzyme were required for long incubation times but eventually the described products were formed and could be isolated because of the stability of the methythio triesters. These could then be oxidised in the usual way to the corresponding methylsulphonyl esters.

Only the triesters from 2',3'-didehydro-2',3'-dideoxy cytidine (7-8) were synthesised because of the difficulty with the synthesis of starting material and triester of d2C and because of its lack of interesting biological activity. The cytosine ring had to be acetylated selectively (6) which meant that the 4-(methylthio)phenyl triester (7) had to be synthesised, then deacetylated and finally oxidised to give the 4-(methylsulphonyl)phenyl triester (8).

(b) Antiviral assays Among the sulphone and sulphide triester derivatives of the pyrimidine 2',3'-dideoxy- and 2',3'-didehydro-2',3'-dideoxynucleotides, the d2U and d4U sulphone triesters were devoid of anti-HIV and cytostatic activity at a concentration as high as 250 μM, while d4U itself is inhibitory to MT4 cell proliferation at a 50%-cytostatic concentration (CC_{50}) of 42 μM (Table 1). In contrast to the sulphone triester derivative of d2T, the sulphide triester derivative of d4T and in particular the sulphone triester of d4T are endowed with a marked anti-HIV-1 and anti-HIV-2 activity. The concentration at which these compounds are cytostatic are $\geq$250 μM. In fact, the d4T sulphone triester inhibited HIV-1 and HIV-2 replication in MT-4 cells at an EC_{50} of 1.8 and 3.6 μM, respectively, that is at a 10-fold higher concentration that the parent free nucleoside (d4T). Considering the fact that upon hydrolysis one mole of d4T-triester releases one molecule of d4T and one molecule of d4T 5'-monophosphate, the d4T sulphone triester derivative should be considered to be 20-fold less effective than the unesterified nucleoside d4T. These antiviral properties are in agreement with the observed 20-fold lower toxicity of the d4T-triester derivatives, suggesting an effective intracellular availability of the d4T 5'-triphosphate metabolite of 5% during the time period at which the virus replication is sensitive to the test compound. A similar effect is seen with the d4C triester. The sulphide triester derivative of d4T is ~ 10-fold less effective than its sulphone triester counterpart.

For the sulphone and sulphide triester derivatives of the purine 2',3'-dideoxy- and 2',3'-didehydro-2',3'-dideoxy nucleosides, anti-HIV activity is clearly less prominent than for their corresponding parent nucleosides. None of the sulphide or sulphone triester derivatives of d2I and d4I proved antivirally effective at 250 μM, while d2I was effective against HIV-1 at an EC_{50} of 5 μM (Table 1). In contrast, the sulphone of d4A, and the sulphides of d2A and d4A proved effective against HIV-1 and HIV-2 replication at an EC_{50} of 66-133 μM, that is at a concentration that is 13-25-fold higher than d2A and d4A. These data are in agreement with our observations that the d4A-triesters were also at least 25-fold less cytostatic than d4A (CC_{50} d4A : 10 μM). Only the d2A sulphone triester proved almost equally as effective as the corresponding nucleoside analogue d2A against HIV-1 but not against HIV-2 replication. The difference in EC_{50} noted for the d2A analogues between HIV-1 and HIV-2 is unexpected but consistently found in three independent experiments. The molecular basis for this discrepancy is so far unclear.

However it is not obvious why the sulphone derivative of d2T has so little activity. It is unlikely that there is any significant difference in cellular uptake between the d2T and d4T compounds, nor should there be any significant difference in the rate of hydrolysis of the triesters to the corresponding bis(5',5'-diesters) and it is unlikely there would be any significant difference in the rates of hydrolysis of these diesters by phosphodiesterase. However, despite the parent nucleosides, d2T and d4T having somewhat similar efficacies against HIV-1 and HIV-2 (EC_{50} ratio for HIV-1 d2T/d4T = 5, for HIV-2 = 10), the corresponding figures for the sulphone triesters are HIV-1, 100 and HIV-2, 57. It is known that d2TMP inhibits TMP kinase but D4TMP does not.[14] Thus it is very likely that the concentration of D2TMP liberated in the cell, actually results in the inhibition of further phosphorylation and thus reduces the efficacy of the parent compound, whereas for d4T, this cannot occur.

As d2U is a very poor nucleoside kinase substrate[13] and d2UTP a good HIV reverse transcriptase inhibitor, it might be thought that one might see an increased efficacy for the sulphone triester of d2U. However, unfortunately d2UMP is also a thymidylate kinase inhibitor and so we see no increased efficacy for this triester. It is likely that the values for the sulphides of d4T (and d2A) are due to oxidation in the cell culture of the sulphide to the sulphone as the sulphide triesters are unlikely to hydrolyse to any significant extent.

It also shows that attempts to circumvent what appears to be a primary reason for the low efficacy of a nucleoside analogue by providing a key metabolite, can in itself raise another set of problems which were not foreseen. Attempts to design nucleoside analogues, entry of which into cells can cause incalculable effects upon nucleoside and

Table I

Anti-HIV activity of sulfone and sulfide triester derivatives of 2',3'didehydro-2',3'-dideoxy- and 2',3'-dideoxynucleoside analogues

Compound	EC_{50}[a] (μM) HIV-1	EC_{50}[a] (μM) HIV-2	CC_{50} (μM)[b]
Sulfone derivative of			
d2U (2)	>250	>250	>250
d4U (1)	>250	>250	>250
d2T (4)	177	206	>250
d4T (3)	1.8	3.6	>250
d2A (10)	8.4	84	>250
d4A (9	74	133	>250
d2I (16)	≥250	≥250	>250
d4I (14)	>250	>250	>250
d4C (8	5.3	6.0	232
Sulphide derivative of			
d4T 5)	15	45	>250
d2A (12)	67	66	>250
d4A (11)	118	89	>250
d2I (15)	≥250	≥250	>250
d4I (13)	>250	>250	>250
Parental compounds			
d2U	129	174	>500
d4U	>20	>20	42
d2T	1.1	3.9	>500
d4T	0.22	0.32	14
d2A	4.7	6.0	>500
d4A	5	-	10
d2I	4.9	-	>500
d4I	-	-	-
d4C	0.6	0.7	20

[a]50% effective concentration
[b]50% cytostatic concentration

nucleotide pools, must necessarily have a large input from serendipity, while our knowledge of the biochemistry of virally infected cells is so meagre.

EXPERIMENTAL

NMR spectra (^1H) were recorded on JEOL FX90Q (90 MHz) and JEOL GX270 (270 MHz) instruments with Me_2SO-d_6 as solvent. UV spectra were measured on a Perkin–Elmer 552 spectrophotometer and mass spectra were measured on a Kratos MS80 mass spectrometer using fast atom bombardment. Glycerol, glycerol–methanol or 3–nitrobenzyl alcohol were used as matrices, sometimes with NaCl added.

Column chromatography was carried out on silica gel, Kiesel gel 60 type 7734 (0.063–0.200 mm, 70–250 mesh) or Kieselgel 60 type 9385 (0.040–0.063 nm, 230–400 mesh). Short–column chromatography was carried out with silica gel 60 PF_{204} type 7749. Preparative thin layer chromatography plates were of the precoated silica gel type (silica gel 60 F_{254}, 2 mm thickness).

All experiments were carried out under scrupulously dry conditions unless otherwise indicated.

Aryl Phosphorodichloridates 4–(Methylthio)phenyl phosphorodichloridate and 4–(methylsulphonyl)phenyl phosphorodichloridate were prepared as previously described.[8] 2',3'–Dideoxynucleosides were prepared by catalytic hydrogenation of the corresponding 2',3'–didehydro–2',3'–dideoxynucleosides which were made in this laboratory (R. Talekar, P.L. Coe and R.T. Walker, manuscript in preparation).

Characterisation of Phosphotriesters None of the triesters was crystalline and most were hygroscopic powders or foams. As most of these compounds are deliberately rather unstable, continuous purification is a process of diminishing returns and even upon HPLC reverse–phase, considerable decomposition occurs during work–up. Thus all compounds were characterised by mass spectra (where usually a molecular ion of the parent compound and those minus one and minus two bases were the dominant peaks), by ^1H NMR and by quantitative UV spectrum with ϵ values.

Synthesis of aryl bis(nucleosid–5'–yl) phosphates. Method A To the relevant phosphorodichloridate (0.30 mmol) in dry pyridine (20 ml), was added dry 1–methylimidazole (1.4 mmol) and the solution stirred for 5 min at room temperature. The nucleoside (~ 0.4 mmol) was then added and the reaction stirred under a stream of dry nitrogen for between 18–48 h at room temperature at the end of which time between about 50–100% of the starting material had been converted to a more polar compound (TLC). The reaction mixture was then taken to dryness under reduced pressure, the residue absorbed onto silica gel and added to a column of silica gel which was eluted with dichloromethane: methanol (10:1). The required fractions were collected, evaporated to dryness and a further separation using the same solvent system was performed by preparative TLC. Method B To the relevant phosphorodichloridate (0.20 mmol) in dry acetonitrile (5 ml), was added dry 1–methylimidazole (1.00 mmol) and the solution stirred for 5 min at room temperature. The nucleoside (0.3 mmol) in dry acetonitrile (5 ml) was then added and the mixture stirred under a stream of dry nitrogen for between 18 and 36 h at room temperature until no further reaction was occurring (TLC). Phosphate buffer (0.1 M, pH 6.0, 20 ml) was then added, the solution extracted with chloroform, the organic layer separated, dried and evaporated to dryness. The residue was subjected to silica gel chromatography using diethylether: methanol (5:2) as eluent, the required fractions collected and taken to dryness.

4-(Methylsulphonyl)phenyl bis(2',3'-didehydro-2',3'-dideoxyuridin-5'-yl)phosphate (1) Method B was used with 4-(methylsulphonyl)phenyl phosphorodichloridate (0.30 mmol) and 2',3'-didehydro-2',3'-dideoxyuridine (0.5 mmol). The title compound (1) was recovered in 23% yield (23 mg) as a white foam. UV λ_{max} 257 nm, ϵ = 20,100, λ_{min} 234 nm, ϵ = 8,975. ^{1}H NMR δ 11.35 (2-H,s,2xNH), 7.97 (2-H,d,2xH-6), 7.40 (4-H,dd,phenyl), 6.85 (2-H,s,2xH-1'), 6.45 (2H,d,2xH-3'), 6.05 (2H,t,2xH-2'), 5.50 (2-H,d,2xH-5), 5.05 (2-H,s,2xH-4'), 4.35 (4-H,m,2xH-5'), 3.25 (3H,s,SO_2CH_3). Mass spectrum m/e 637 $[M+H]^+$. Elemental analysis $C_{25}H_{25}N_4O_{12}PS$ requires C, 47.2; H, 4.0; N, 8.7; found C, 46.9; H, 4.1; N, 17.2%.

4-(Methylsulphonyl)phenyl bis(2',3'-dideoxyuridin-5-yl)phosphate (2) Method B was used with 4-(methylsulphonyl)phenyl phosphorodichloridate (0.30 mmol) and 2',3'-dideoxyuridine (0.50 mmol). The title compound (2) was recovered in 60% yield (90 mg) as a white foam. UV λ_{max} 259 nm, ϵ = 18,704; λ_{min} 234 nm, ϵ = 7,000. ^{1}H NMR δ 11.36 (2-H,s,2xNH), 7.97 (2H,d,2xH-6), 7.47-7.63 (4-H,m,phenyl), 6.03 (2-H,m,2xH-1'), 5.56 (2-H,t,2xH-5), 4.23-4.41 (6-H,m,2xH-4',H-5'), 3.22 (3-H,s,SO_2CH_3), 1.79-2.33 (8H,m,2xH-2',H-3'). Mass spectrum m/e 641 $[M+H]^+$. Elemental analysis $C_{25}H_{29}N_4O_{12}PS$ requires C, 46.9; H, 4.6; N, 8.8; found C, 46.6; H, 4.9; N, 8.6%.

4-(Methylsulphonyl)phenyl bis(2',3'-didehydro-2',3'dideoxythymidin-5'-yl)phosphate (3) Method B was used with 4-methylsulphonylphenyl phosphorodichloridate (0.20 mmol) and 2',3'-didehydro-2',3'-dideoxythymidine (0.30 mmol). The title compound (3) was recovered in 34% yield (35 mg) as a white foam. UV λ_{max} 262 nm, ϵ = 19,400;λ_{min} 234 nm, ϵ = 4,719. ^{1}H NMR δ 11.35 (2-H,d,2xNH), 7.90 (2-H,m,2xH-6), 7.25-7.40 (4-H,dd,phenyl), 6.85 (2-H,s,2xH-1'), 6.40 (2-H,m,2xH-2'), 6.05 (2-H,m,2xH-3'), 4.95 (2-H,s,2xH-4'), 4.35 (4-H,m,2xH-5'), 3.25 (3-H,s,SO_2CH_3), 1.65 (6H,d,2xCH_3). Mass spec. m/e 665 $[M+H]^+$. Elemental anal. $C_{27}H_{29}N_4O_{12}PS$ requires C, 48.8; H, 4.4; N, 8.4; found C, 48.9; H, 4.6; N, 8.5%.

4-(Methylsulphonyl)phenyl bis(2',3'-dideoxythymidin-5'yl)phosphate (4) Method B was used with 4-(methylsulphonyl)phenyl phosphorodichloridate (0.10 mmol) and 2',3'-dideoxythymidine (0.20 mmol). The title compound (4) was isolated in 54% yield (40 mg) as a white foam. UV λ_{max} 264 nm, ϵ = 19,900; λ_{min} 235 nm, ϵ = 5,282. ^{1}H NMR δ 11.29 (2-H,s,2xNH), 7.95 (2-H,s,2xH-6), 7.48 (4-H,m,phenyl), 6.05 (2-H,m,2xH-1'), 4.15-4.45 (6-H,m,2xH-4',H-5'), 2.09 (3-H,s,SO_2CH_3), 1.80-2.40 (8-H,m,2xH-2',H-3'), 1.70 (6-H,s,2xCH_3). Mass spectrum m/e 669 $[M+H]^+$. Elemental analysis $C_{27}H_{33}N_4O_{12}PS.2.25\ H_2O$ requires C, 45.7; H, 5.3; N, 7.9; found C, 45.7; H, 5.1; N, 7.2%.

4-(Methylthio)phenyl bis(2',3'-didehydro-2',3'-dideoxy thymidin-5'-yl)phosphate (5) Method B was used with 4-(methylthio)phenyl phosphorodichloridate (0.2 mmol) and 2',3'-didehydro-2',3'-dideoxythymidine (0.30 mmol). The title compound (5) was recovered in 35% yield (34 mg) as a white foam. UV λ_{max} 257 nm, ϵ = 17,300;λ_{min} 232 nm, ϵ = 9,046. ^{1}H NMR δ 11.36 (2-H,s,2xNH), 7.18 (2-H,s,2xH-6), 7.07-7.24 (4H,dd,phenyl), 6.83 (2-H,s,2xH-1'), 6.40 (2-H,m,2xH-2'), 6.03 (2-H,m,2xH-3'), 4.99 (2-H,s,2xH-4'), 4.29 (4-H,s,2xH-5'), 2.41 (3-H,s,SCH_3), 1.70 (6-H,d,2xCH_3). Mass spectrum m/e 633 $[M+H]^+$, 655 $[M+Na]^+$. Elemental analysis $C_{27}H_{29}N_4O_{10}PS$, 0.67 H_2O requires C, 50.3; H, 4.5; N, 8.7; found C, 50.3; H, 4.5; N. 8.5%.

4-(Methylthio)phenyl bis(N^4-acetyl-2',3'-didehydro-2',3'-dideoxycytidin-5'-yl)phosphate (6) Method A was used with 4-(methylthio)phenyl phosphorodichloridate (0.18 mmol) and N^4-acetyl-2',3'-didehydro-2',3'-dideoxy cytidine (0.30 mmol). The title compound (6) was isolated in 47% yield (45 mg) as a white foam upon elution from a silica

column with dichloromethane: methanol (15:1). UV λ_{max} 297 nm, ϵ = 12,400; λ_{max}247 nm, ϵ = 32,500 nm; λ_{min} 272 nm, ϵ = 8,300. ^{1}H NMR δ 10.90 (2-H,s,2xNH), 7.80 (2-H,d,2xH-6), 7.15 (2-H,d,2xH-5), 7.10 (4-H,dd,phenyl), 6.90 (2-H,s,2xH-1'), 6.45 (2-H,t,2xH-2'), 6.10 (2-H,t,2xH-3'), 5.05 (2-H,bs,2xH-4'), 4.35 (4-H,m,2xH-5'), 2.45 (3-H,s,SCH_3), 2.10 (6-H,d,$COCH_3$). Mass spectrum m/e 687 $[M+H]^+$. Elemental analysis, $C_{29}H_{37}N_6O_{10}PS$. 0.67 H_2O requires C, 49.4; H, 4.7; N, 11.9, found C, 49.8; H, 5.0; N, 11.7%

4-(Methylthio)phenyl bis(2',3'-didehydro-2',3'-dideoxycytidin-5'-yl)phosphate (7) Compound 6, 30 mg (43.7 mmol) was treated with aqueous ammonia solution (d. 0.880, 0.05 ml) in methanol (3 ml) for 1 h at room temperature by which time the reaction was complete (TLC) and a more polar compound was present. The reaction mixture was concentrated to a small volume, added to a preparative TLC plate and developed in ethyl acetate: methanol (2:1). The title compound (7) was isolated by extraction from the silica gel as a white foam (14 mg, 53% yield). UV λ_{max} 273 nm, ϵ = 18,000; λ_{min} 247 nm, ϵ = 9,900. ^{1}H NMR δ 7.39 (2-H,t,2xH-6), 7.22 (4-H,bs,2xNH_2), 7.07-7.19 (4-H,dd,phenyl), 6.90 (2-H,s,2xH-1'), 6.35 (2-H,t,2xH-3'), 6.01 (2-H,m,2xH-2'), 5.66 (2-H,t,2xH-5), 4.96 (2-H,s,2xH-4'), 4.23 (4-H,m,2xH-5'), 2.46 (3-H,s,SCH_3). Mass spectrum m/e 603 $[M+H]^+$.

4-(Methylsulphonyl)phenyl bis(2',3'-didehydro-2',3'-dideoxycytidin-5'-yl)phosphate (8). To compound 7 (52 mg, 87 μmol) in methanol (30 ml), was added a solution of 3-chloroperoxybenzoic acid (32 mg, 0.2 mmol) in methanol (10 ml) dropwise over a period of 10 min at 0°C. The reaction mixture was stirred at 0°C for 4 h, concentrated, added to a preparative TLC plate which was developed in dichloromethane: methanol (4:1). The title compound (8) was isolated as a white foam (32 mg, 57% yield). UV λ_{max} 273 nm, ϵ = 18,600; λ_{min} 253 nm, ϵ = 10,300. ^{1}H NMR δ 7.68 (2-H,m,2xH-6), 7.38 (4-H,dd,phenyl), 7.26 (4-H,6s,2xNH_2), 6.91 (2-H,bs,2xH-1'), 6.36 (2-H,t,2xH-3'), 6.01 (2-H,t,2xH-2'), 5.68 (2-H,m,2xH-5), 4.97 (2-H,6s,2xH-4'), 4.28 (4-H,m,2xH-5'), 3.34 (3-H,s,SO_2CH_3). Mass spectrum m/e 657 $[M+H]^+$. Elemental analysis $C_{25}H_{27}N_6O_{10}PS$.5.5 H_2O requires C, 40.9; H, 5.2; N, 11.5; found C, 40.6; H, 4.6; N, 11.8%.

4-(Methylsulphonyl)phenyl bis(2',3'-didehydro-2',3'-dideoxyadenosin-5'-yl)phosphate (9) Method A was used with 4-(methylsulphonyl)phenyl phosphorodichloridate (0.30 mmol) and 2',3'-didehydro-2',3'-dideoxyadenosine (0.40 mmol). The title compound (9) was isolated in 16% yield (23 mg) as a white foam. UV λ_{max} 258 nm, ϵ = 27,600; λ_{min} 234 nm, ϵ = 4,700. ^{1}H NMR δ 8.16 (2-H,s,2xH-8), 8.07 (2-H,d,2xH-2), 7.22-7.77 (4-H,dd,phenyl), 7.32 (4-H,d,2xNH_2), 6.96 (2-H,s,2xH-2'), 6.42 (2-H,s,2xH-3'), 6.26 (2-H,t,2xH-1'), 5.04 (2-H,s,2xH-4'), 4.24 (2-H,s,2xH-5'), 3.19 (3-H,s,SO_2CH_3). Mass spectrum m/e 683 $[M+H]^+$. Elemental analysis $C_{27}H_{27}N_{10}O_8PS$. 3CH_3OH requires C, 46.3; H, 5.1; N, 18.0, found C, 46.3; H, 4.8; N, 17.5%.

4-(Methylsulphonyl)phenyl bis(2',3'-dideoxyadenosin-5'-yl)phosphate (10) Method A was used with 4-(methylsulphonyl)phenyl phosphorodichloridate (0.55 mmol) and 2',3'-dideoxyadenosine (0.85 mmol). The title compound (10) was isolated in 68% yield (200 mg), as a white foam. UV λ_{max} 257 nm, ϵ = 27,300; λ_{min} 234 nm, ϵ = 7,900. ^{1}H NMR δ 8.27 (2-H,s,2xH-8), 8.13 (2-H,s,2xH-2), 7.31-7.84 (4-H,m,phenyl), 7.26 (4-H,s,2xNH_2), 6.24 (2-H,m,2xH-1'), 4.26 (2-H,s,2xH-4'), 3.92 (4-H,m,2xH-5'), 3.25 (3-H,s,SO_2CH_3), 2.80 (4-H,m,2xH-3'), 2.08 (4-H,m,2xH-2'). Mass spectrum m/e 687 $[M+H]^+$. Elemental analysis $C_{27}H_{31}N_{10}O_8PS$.3.36H_2O requires, C, 43.4; H, 5.1; N, 18.7, found C, 43.4; H, 5.3, N. 18.0%.

4-(Methylthio)phenyl bis(2',3'-didehydro-2',3'-dideoxyadenosin-5'-yl)phosphate (11) Method A was used with 4-(methylthio)phenyl phosphorodichloridate (0.45 mmol) and

2',3'-didehydro-2',3'-dideoxyadenosine (0.79 mmol). The title compound (11) was isolated in 39% yield (100 mg) as a white foam. UV λ_{max} 257 nm, ϵ = 27,300; λ_{min} 225 nm, ϵ = 10,100. ^{1}H NMR δ 8.17 (2-H,s,2xH-8), 8.07 (2-H,d,2xH-2), 7.32 (4-H,d,2xNH_2), 6.99 (2-H,s,2xH-1'), 6.88-7.02 (4-H,m,phenyl), 6.39 (2-H,m,2xH-2'), 6.24 (2-H,m,2xH-3'), 5.01 (2-H,s,2xH-4'), 4.18 (4-H,m,2xH-5'), 2.40 (3-H,s,SCH_3). Mass spectrum m/e 651 $[M+H]^+$. Elemental analysis $C_{27}H_{27}N_{10}O_6PS.2H_2O$ requires C, 47.2; H, 4.5; N, 20.3; found C, 47.1; H, 4.2; N, 19.0%.

4-(Methylthio)phenyl bis(2',3'-dideoxyadenosin-5'-yl)phosphate (12) Method A was used with 4-(methylthio)phenyl phosphorodichloridate (0.63 mmol) and 2',3'-dideoxyadenosine (1.10 mmol). The title compound (12) was isolated in 97% yield (350 mg) as a white solid. UV λ_{max} 257 nm, ϵ = 27,300; λ_{min} 225 nm, ϵ = 9,400. ^{1}H NMR δ 8.25 (2-H,d,2xH-8), 8.15 (2-H,s,2xH-2), 7.25 (4-H,bs,2xNH_2), 7.05-7.15 (4-H,m,phenyl), 6.25 (2-H,m,2xH-1'), 4.05-4.30 (6-H,m,2xH-4',H-5'), 2.40 (3-H,s,SCH_3), 2.05 (4-H,m,2xH-2'). Mass spectrum m/e 655 $[M+H]^+$.

4-(Methylthio)phenyl bis(2',3'-didehydro-2',3'-dideoxyinosin-5'-yl)phosphate (13) Compound 11 (250 mg, 0.38 mmol) and 5'-adenylic acid deaminase (963 mg, 77 units, SIGMA) were suspended in phosphate buffer (0.1 M, pH 6.5, 50 ml) and stirred for 5 d at room temperature. The presence of a more polar compound (TLC) could be detected and the reaction mixture was taken to dryness and the residue pre-absorbed onto silica gel and fractionated on a silica gel column using dichloromethane: methanol (20:3) as eluent. The required fractions were collected, taken to dryness and the residue applied to a preparative TLC plate which was developed in the same solvent. The product was extracted in the usual way to give a white powder (160 mg, 64% yield). λ_{max} 247 nm, ϵ = 19,000; λ_{min} 221 nm, ϵ = 8,500. ^{1}H NMR δ 8.05 (2-H,d,2xH-8), 7.95 (2-H,d,2xH-2), 6.90-7.15 (4-H,dd,phenyl), 6.95 (2-H,s,2xH-1'), 6.45 (2-H,m,2xH-3'), 6.25 (2-H,m,2xH-2'), 5.05 (2-H,bs,2xH-4'), 4.15 (4-H,m,2xH-5'), 2.45 (3-H,s,SCH_3). Mass spectrum m/e 652 $[M+H]^+$, 675 $[M+Na]^+$.

4-(Methylsulphonyl)phenyl bis(2',3'-didehydro-2',3'-dideoxyinosin-5'-yl)phosphate (14) Compound 13 (47 mg, 0.07 mmol) was dissolved in dry methanol (40 ml) and cooled to 0°. To the solution was added a solution of 3-chloroperoxybenzoic acid (27 mg, 0.17 mmol) in dry methanol (10 ml) dropwise over a period of 10 min at 0°C. The resulting solution was stirred at 0°C for 1.5 h after which time the presence of a more polar compound was seen (TLC). The solvent was removed, pyridine (0.05 ml) added together with a little methanol and the solution applied to a preparative TLC plate which was eluted with dichloromethane: methanol (10:3) to give the title compound (36 mg, 73% yield). λ_{max} 246 nm, ϵ = 24,400; λ_{min} 232 nm, ϵ = 11,500. ^{1}H NMR δ 8.08 (2-H,s,2xH-8), 8.00 (2-H,d,2xH-2), 7.24-7.78 (4-H,dd,phenyl), 6.92 (2-H,s,2xH-1'), 6.43 (2-H,m,2xH-3'), 6.26 (2-H,m,2xH-2'), 5.06 (2-H,s,2xH-4'), 4.22 (4-H,m,2xH-5'), 3.20 (3-H,s,SO_2CH_3). Mass spectrum m/e 707 $[M+H]^+$.

4-(Methylthio)phenyl bis(2',3'-dideoxyinosin-5'-yl)phosphate (15) Compound 12 (350 mg, 0.53 mmol) and 5'-adenylic acid deaminase (2.0 g, 161 units, SIGMA) were suspended in phosphate buffer (0.1 M, pH 6.5, 80 ml) and stirred for 2 d at room temperature. The presence of a more polar compound (TLC) could be detected and the reaction mixture was taken to dryness and the residue pre-absorbed onto silica gel and fractionated on a silica gel column using dichloromethane: methanol (10:1) as eluent. The required fractions were collected, taken to dryness to give the title compound (320 mg, 91% yield) as a white powder. λ_{max} 248 nm, ϵ = 22,200; λ_{min} 224 nm, ϵ = 6,600. ^{1}H NMR δ 8.20 (2-H,d,2xH-2), 8.05 (2-H,s,2xH-8), 7.00-7.15 (4-H,dd,phenyl), 6.20 (2-H,t,2xH-1'), 4.05-4.35 (6-H,m,2xH-4',2xH-5'), 2.45 (3-H,s,SCH_3), 1.95-2.55 (8-H,m,2xH-2',H-3'). Mass spectrum m/e 679 $[M+Na]^+$. Elemental analysis, $C_{27}H_{29}N_8O_8PS.2H_2O$ requires C, 46.8; H, 4.8; N, 16.2, found C, 46.4; H, 4.5; N, 15.8%.

4-(Methylsulphonyl)phenyl bis(2',3'-dideoxyinosin-5'-yl)phosphate (16) Compound 14 (30 mg, 0.46 μmol) was dissolved in dry methanol (55 l) and treated with a solution of 3-chloroperoxybenzoic acid (25 mg, 0.16 mmol) in dry methanol as described above for compound 14. The product was isolated from a preparative TLC plate developed in dichloromethane: methanol (20:3) as a white foam (12 mg, 38% yield). UV λ_{max} 247 nm, ϵ = 25,700; λ_{min} 231 nm, ϵ = 12,100. ^{1}H NMR δ 8.20 (2-H,d,2xH-2), 8.05 (2-H,s,2xH-8), 7.35-7.85 (4-H,dd,phenyl), 6.25 (2-H,t,2xH-1'), 4.15-4.40 (6H,m,2xH-4',H-5'), 3.25 (3-H,s,SO_2CH_3), 2.00-2.45 (8-H,m,2xH-2',H-3'). Mass spectrum m/e 689 $[M+H]^+$, 711 $[M+Na]^+$. Elemental analysis, $C_{27}H_{29}N_8O_{10}PS$. $2H_2O$ requires, C, 44.7; H, 4.5; N, 15.4, found C, 45.1; H, 4.4; N, 14.9%.

Antiviral assay Human lymphocyte MT-4 cells (5 x 10^5) were infected with 100 $CCID_{50}$ HIV-1 (strain $HTLV\text{-}III_B$) or HIV-2 (strain ROD)/ml and seeded in 200 μl wells of a microtiter plate, containing appropriate dilutions of the test compounds. After 5 days incubation at 37°C, the number of viable cells was determined in a blood cell counting chamber by trypan blue dye exclusion. Solutions of test compounds were made up immediately before commencement of the assay.[15]

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