

Conversion of 2',3'-dideoxyadenosine (ddA) and 2',3'-didehydro-2',3'-dideoxyadenosine (d4A) to their corresponding aryloxyphosphoramidate derivatives markedly potentiates their activity against human immunodeficiency virus and hepatitis B virus

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Abstract 2',3'-Dideoxyadenosine (ddA), 2',3'-didehydro-2',3'-dideoxyadenosine (d4A) and their lipophilic 5'-monophosphate triester (aryloxyphosphoramidate) prodrugs were evaluated for their anti-retrovirus and anti-hepatitis B virus activity in various cell culture models. The aryloxyphosphoramidate derivatives of ddA (Cf 1093) and d4A (Cf 1001) showed markedly superior (100–1000-fold) efficacies than the parent drugs against human immunodeficiency virus type 1 (HIV-1), HIV-2, simian immunodeficiency virus (SIV), Moloney murine sarcoma virus (MSV) and human hepatitis B virus (HBV) replication regardless of the cell type in which the virus replication was studied (i.e., human T-lymphocyte CEM, MT-4, Molt/4 and C8166 cells, peripheral blood lymphocytes (PBL), monocyte/macrophages (M/M), murine embryo fibroblasts and human hepatocyte cells). Also the selectivity index (ratio of cytotoxic concentration/antivirally effective concentration) of both aryloxyphosphoramidate prodrugs was markedly increased. In particular the d4A prodrug Cf 1001 showed a selectivity index of 300–3000 as compared with 2–3 for the parental d4A in established laboratory cell lines. Also Cf 1001 had a selectivity index of 400–650 in HIV-1-infected PBL and M/M, respectively. Both Cf 1001 and Cf 1093 were equally efficient as 3TC (lamivudine) in inhibiting HBV replication in hepatocytes, and rank among the most potent HIV and HBV inhibitors reported so far in cell culture.

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Key words: HIV; HBV; Reverse transcriptase; Nucleoside analogues; AIDS; Hepatitis; Prodrugs

1. Introduction

Nucleoside analogues that are active against human immunodeficiency virus (HIV) need to be converted to their corresponding 5'-triphosphate derivatives before they can inhibit their target enzyme (reverse transcriptase). The first activation (phosphorylation) step is predominantly catalysed by nucleoside kinases and/or 5'-nucleotidases [1–6]. Except for AZT, most anti-HIV-active nucleoside analogues have a relatively poor affinity for their activating enzymes [2,3,6–10]. In an attempt to circumvent the first phosphorylation step, several types of masked (lipophilic) nucleoside 5'-monophosphate

prodrugs have been synthesized (containing two phosphate blocking groups such as bis(pivaloyloxymethyl) (bis(POM)), bis(*S*-acyl-2-thioethyl) (bis(SATE)), dioxophosphorine oxide and bis(isopropoxyloxycarbonyloxymethyl) (bis(POC))) [11–18]. Recently, we reported the synthesis and anti-retrovirus activity of the masked alaninylphosphoramidate 2',3'-didehydro-2',3'-dideoxythymidine 5'-monophosphate (d4T-MP) prodrug (designated So324), and showed that it retains full anti-HIV activity in thymidine-kinase-deficient CEM/TK[−] cells [19,20]. These findings strongly suggested that the phosphotriester prodrug of d4T efficiently released the intact phosphorylated metabolite d4T-MP inside the HIV-infected cells, thereby circumventing the first activation (phosphorylation) step [19,20]. So324 proved poorly effective against human hepatitis B virus (HBV) replication in human hepatoma cells [21]. In contrast, the corresponding 3TC aryloxyphosphoramidate derivative was very effective against HBV replication in human hepatoma cells, but only marginally effective against HIV in human T-lymphocytes [21]. Given the striking differences in antiviral efficacy of the thymine and cytosine nucleoside aryloxyphosphoramidate prodrugs in T-lymphocytes and hepatoma cells, we now investigated the antiviral activity of the 2',3'-dideoxyadenosine (ddA) and 2',3'-didehydro-2',3'-dideoxyadenosine (d4A) aryloxyphosphoramidate prodrugs. Both compounds proved exquisitely inhibitory to both HIV and HBV in cell culture and showed a markedly higher antiviral selectivity index than their parental ddA and d4A.

2. Materials and methods

2.1. Viruses

HIV-1(III_B) and HIV-2(ROD) were kindly provided by Prof. R.C. Gallo (when being at the National Cancer Institute (NCI), National Institutes of Health, Bethesda, MD, USA) and Dr. L. Montagnier (Pasteur Institute, Paris, France). HIV-1/Ba-L was a gift from Dr. M. Popovic and Dr. R.C. Gallo (NCI, NIH). SIV (MAC₂₅₁) was kindly provided by Dr. J. Slachmuylders and H. Schellekens (when at the TNO Primate Center, Rijswijk, The Netherlands).

2.2. Cells

CEM cells were obtained from the American Tissue Cell Culture Collection (Rockville, MD, USA); and MT-4, Molt 4/clone 8 and C8166 cells were kindly provided by Dr. N. Yamamoto (Nagoya Memorial Hospital, Nagoya, Japan), Dr. H. Nakashima (Kagoshima University Dental School, Kagoshima, Japan) and Dr. P. La Colla (Università degli Studi di Cagliari, Cagliari, Italy, respectively). Hep

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G2 2.2.15 hepatoblastoma cells were provided by Dr. G. Acs (Mount Sinai Medical Center, NY, USA).

2.3. Compounds

The aryloxyphosphoramidate prodrug of d4A (designated Cf 1001) has been synthesized as described before [22]. The detailed synthesis of the aryloxyphosphoramidate prodrug of ddA (designated Cf 1093) (Fig. 1) will be described elsewhere. We have designed the prodrug synthesis occurring in one high-yielding (85–95%) step under mild conditions using readily available starting materials (i.e., alanine and phenol) and resulting in d4A and ddA aryloxyphosphoramidate derivatives that should be not markedly more expensive than the de novo synthesis of d4A and ddA.

2.4. Antiviral activity of the test compounds

CEM, MT-4, Molt 4/clone 8 and C8166 cells were suspended at 250 000–350 000 cells per ml of culture medium and infected with approximately 100 CCID₅₀ (1 CCID₅₀ being the 50% cell culture infective dose) of HIV-1(III_B) or HIV-2(ROD). Then, 100 µl of the infected cell suspensions was added to 200-µl-microtiterplate wells containing 100 µl of an appropriate dilution of the test compounds (i.e., 250, 100, 20, 4, 0.8, 0.16, 0.032, 0.006, 0.001 µM). The inhibitory effect of the test compounds on HIV-1-induced syncytium formation in CEM, Molt 4 and C8166 cells was examined on day 4 post-infection, and their inhibitory effect on the viability of HIV-infected MT-4 cells was examined on day 5 post-infection, as described previously [20]. The 50% effective concentration (EC₅₀) was determined as the compound concentration required to inhibit syncytium formation (CEM, Molt 4, C8166) or to reduce cell viability (MT-4) by 50%. The 50% cytostatic concentration (CC₅₀) was defined as the compound concentration required to reduce cell viability or cell proliferation by 50%.

Peripheral blood lymphocyte cells (PBL) were isolated from a healthy HIV-1-negative human volunteer by using lymphoprep, washed twice with phosphate-buffered saline (PBS) and cultured with phytohemagglutinin (PHA, 2 µg/ml) and interleukin-2 (10 U/ml) for 3 days at 37°C in a CO₂-controlled humidified incubator. Stimulated PBMCs were washed twice with PBS and then infected with HIV-1 (HTLV-III_B) at 1000 CCID₅₀ per ml in RPMI-1640 medium supplemented with 15% heat-inactivated foetal calf serum, L-glutamine (2 mM), gentamicin (50 µg/ml), and recombinant interleukin-2 (10 U/ml). After 60 min incubation at 37°C, non-adsorbed virus was removed by successive washing steps with fresh culture medium, and the HIV-1-infected PBLs were suspended at 5 × 10⁵ cells/ml in RPMI-1640 medium and cultured in the presence of varying concentrations (20, 4, 0.8, 0.16, 0.032 and 0.006 µM) of the test compounds in 96-well culture plates (200 µl per well). At day 4, all cultures were subcultivated. HIV-1 p24 core antigen was quantified in the cell culture supernatants at 7 days after infection by an antigen-capture ELISA (DuPont).

Human primary macrophages were obtained from blood of healthy seronegative donors by countercurrent centrifugal elutriation, followed by treatment for 7 days with 1000 U/ml macrophage colony stimulating factor (M-CSF). Macrophages were then treated with various concentrations of drugs, and 30 min after being challenged with 300 50% tissue culture infectious doses (TCID₅₀/ml) of the monocytotropic BaL strain, virus production was then assessed at 14 days after infection by a commercially available p24 antigen-capture assay, and by syncytium formation. The experimental details of the technique are described elsewhere [23].

For analysis of extracellular HBV DNA Hep G2 2.2.15 hepatoblastoma cells were seeded in cell culture recipients as described before [24]. After seeding, culture medium containing the drugs was changed at days 3, 6 and 9. At day 12, the culture medium was harvested and clarified by centrifugation for determination of HBV DNA. The culture supernatants were prepared for dot blot analysis according to Korba and Milman [25] and applied to Hybon N+ membranes (Amersham Life Science Products) in the Convertible Filtration Manifold System (BRL, Life Technologies).

3. Results

3.1. Antiretroviral activity of test compounds in established laboratory cell lines

ddA, d4A, ddI and the aryloxyphosphoramidate prodrugs of ddA and d4A were evaluated for their antiviral efficacy against human immunodeficiency virus type 1 (HIV-1), HIV-2, simian immunodeficiency virus (SIV) and Moloney murine sarcoma virus (MSV) in a variety of cell culture models including MT-4, CEM, Molt 4, C8166 and C3H (Table 1). ddA and ddI were inhibitory to HIV-1, HIV-2, SIV and MSV at 50% effective concentrations that ranged between 2.7 and 10 µM in CEM, MT-4, Molt 4 and C3H cell cultures. The anti-HIV-1 and -HIV-2 activity of both reference compounds was somewhat lower in C8166 cell cultures (EC₅₀: 12–22 µM). The 2',3'-unsaturated d4A was approximately 5–10-fold less effective than ddA (EC₅₀: 21–≥50) regardless of the nature of the cell line. The aryloxyphosphoramidate derivatives of ddA (Cf 1093) and d4A (Cf 1001) were highly inhibitory to all retroviruses tested, irrespective of the nature of the cell line used. Their EC₅₀ ranged from 0.007 to 0.065 µM, that is an EC₅₀ that was at least 2 to more than 3 orders of magnitude lower than that required for the parent ddA and d4A derivatives. Whereas ddI and ddA were virtually devoid of cytostatic activity in cell culture at 250 µM, d4A was cytostatic at a CC₅₀ of 49–97 µM. Cf 1093 showed a markedly increased toxicity over ddA (CC₅₀: 6.6–15 µM), but Cf 1001 was only 3–5-fold more cytostatic to cell proliferation than d4A. Consequently, the selectivity indices (S.I. or ratio CC₅₀/EC₅₀) of the aryloxyphosphoramidate derivatives of ddA and, in particular, d4A, were substantially increased. For example, whilst d4A had an S.I. of not more than 2 or 3 for HIV-1, HIV-2 and SIV, its aryloxyphosphoramidate derivative Cf 1001 had a S.I. of 300–2500 (Table 1).

3.2. Antiretroviral activity of test compounds in freshly isolated primary peripheral blood lymphocyte cells (PBL) and monocytelmacrophage (M/M) cell cultures

The test compounds were also investigated for their antiretroviral activity in freshly isolated peripheral blood lympho-

Table 1

Cytostatic activity and inhibitory effect of ddA, Cf 1093, d4A, Cf 1001 and ddI on HIV replication in different human lymphocyte cell lines

Compound	EC ₅₀ ^a (µM)										CC ₅₀ ^b (µM)			
	MT-4		CEM		Molt 4/C8		C8166		C3H/3T3		MT-4	Molt 4/C8	C8166	C3H/3T3
	HIV-1	SIV	HIV-1	HIV-2	HIV-1	HIV-2	HIV-1	HIV-2	MSV					
ddA	5.27	5.3	4	8	4	5.5	17	22	24		> 250	> 250	> 250	> 250
Cf 1093	0.010	0.006	0.016	0.035	0.016	0.050	0.040	0.050	1.9		6.5	6.8	15	> 20
d4A	21	26	20	20	30	25	≥50	32	31		49	74	97	≥100
Cf 1001	0.007	0.008	0.006	0.018	0.030	0.040	0.055	0.065	9.4		18	16	19	> 100
ddI	2.7	4.8	2.2	—	10	4.5	12	15	—		> 250	> 250	> 250	> 250

^a50% effective concentration, or compound concentration required to inhibit virus-induced cytopathology by 50%.

^b50% cytotoxic concentration, or compound concentration required to inhibit cell proliferation by 50%.

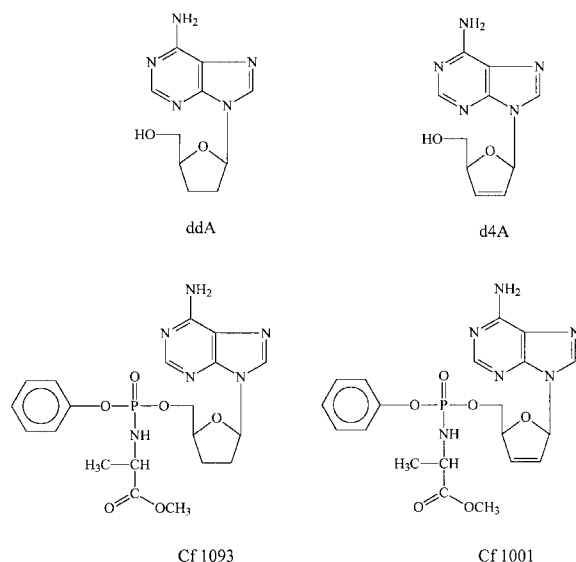


Fig. 1. Structural formulae of 2',3'-dideoxyadenosine (ddA), 2',3'-didehydro-2',3'-dideoxyadenosine (d4A), and their corresponding arylphosphoramidate derivatives, Cf 1093 and Cf 1001.

cyte cells (PBL) and monocyte/macrophage (M/M) cell cultures. Similarly striking findings were found as obtained for the HIV-1-infected established laboratory cell lines (Table 2). The ddA arylphosphoramidate derivative Cf 1093 proved 120-fold more efficient than ddA as an inhibitor of HIV-1 replication in PBLs and M/M, whilst the d4A arylphosphoramidate derivative Cf 1001 proved > 100–300-fold more inhibitory than d4A to HIV-1 in M/M and PBLs. The toxicity of Cf 1093 and Cf 1001 was increased by > 5–12-fold in M/M, but only 3–5-fold in PBLs, as compared to the parental ddA and d4A compounds, resulting in a pronounced *in vitro* selectivity index for the arylphosphoramidate prodrugs in PBL and M/M.

3.3. Antiviral activity of the test compounds against the extracellular release of HBV from HBV-transfected Hep G2 2.2.15 cell cultures

The ddA, d4A and arylphosphoramidate derivatives of ddA (Cf 1093) and d4A (Cf 1001) were also evaluated for their ability to inhibit extracellular HBV release in the supernatant of Hep G2 2.2.15 cell cultures (Fig. 2). Both ddA and d4A lacked any efficacy against HBV at a concentration as high as 10 μ M (Fig. 2, upper panels). In contrast, Cf 1093 and Cf 1001 strongly inhibited HBV DNA release at drug concentrations between 1 and 10 μ M. Even at a concentration as low as 0.1 μ M, Cf 1093 and, in particular, Cf 1001 were still able to partially suppress HBV release from the transfected cells. At 0.01 μ M, Cf 1093 and Cf 1001 had no marked inhibitory effect on HBV replication, as measured by dot-blot DNA analysis (Fig. 2, lower panels). Thus, the EC_{50} of Cf 1093 and Cf 1001 for HBV replication could be estimated at 0.1–0.01 μ M, whereas the parent drugs ddA and d4A were completely ineffective against HBV at 100–1000-fold higher concentrations.

4. Discussion

We have recently reported a successful approach to deliver directly the 5'-monophosphate derivative of the anti-HIV

drug d4T (stavudine) into intact cells by converting d4T to its arylphosphoramidate analogue [19,20]. Studies with radiolabeled test compound have revealed that substantial amounts of d4T-MP were delivered inside intact CEM cells upon exposure of the d4T arylphosphoramidate derivative to the cells [26]. In addition, we have demonstrated that the extent of d4T-MP delivery in the intact cells is highly dependent on the cell type used [20]. We have also shown that the 3TC arylphosphoramidate derivative was about 100-fold less active in HIV-infected T-lymphocytes as compared to the parent 3TC, while it retained the same efficacy as 3TC in inhibiting HBV in human hepatocytes [21].

Here, we have demonstrated that the purine nucleoside arylphosphoramidate derivatives, Cf 1093 and Cf 1001, acquire significantly increased antiviral efficacy against both HIV in T-lymphocytes and HBV in hepatocytes. These favorable properties of the purine nucleoside arylphosphoramidates are in marked contrast with our previous findings on the pyrimidine nucleoside arylphosphoramidates where the 3TC prodrug was not superior to 3TC against HBV in human hepatocytes and was even markedly inferior to 3TC against HIV in T-lymphocytes. These striking differences between the antiretroviral and anti-HBV efficacies of purine and pyrimidine nucleoside arylphosphoramidates presumably reflect differences in uptake and/or, more likely, intracellular conversion of the prodrugs to their eventual active metabolites (i.e., the 5'-triphosphate derivatives).

Indeed, we have previously shown that conversion of the arylphosphoramidate d4T prodrug to d4T-TP is highly dependent on the nature of the cell type (or species) [20]. In this respect, human and sheep cell cultures were by far more effective than murine and feline cell cultures in generating d4T-TP from the d4T prodrug. In contrast, human serum and fetal calf serum are unable to efficiently hydrolyse the d4T arylphosphoramidate derivative to its intermediate alaninyl-d4T-MP derivative, whereas murine serum fully hydrolyses the d4T prodrug to alaninyl d4T-MP within a short time period (L. Naesens, E. De Clercq, C. McGuigan and J. Balzarini, unpublished). Preliminary experiments revealed that the d4A and ddA prodrugs were efficiently converted by both human T-lymphocyte (CEM) and human hepatocyte cell extracts to their corresponding alaninyl nucleoside 5'-monophosphate derivatives (data not shown). Since esterase-catalysed conversion of the arylphosphoramidate derivatives to their alaninyl nucleoside 5'-monophosphate derivatives are thought to be a prerequisite of further efficient intracellular release of the nucleoside 5'-monophosphate

Table 2

Cytostatic activity and inhibitory effect of ddA, Cf 1093, d4A and Cf 1001 on HIV-1 replication in peripheral blood lymphocytes and monocyte/macrophages

Compound	EC_{50}^a (μ M)		CC_{50}^b (μ M)		S.I. ^c	
	PBL	M/M	PBL	M/M	PBL	M/M
ddA	0.24	1.0	24	> 50	100	> 50
Cf 1093	0.002	0.005	4.6	10	2300	2000
d4A	2.3	5	14	> 50	6.1	> 10
Cf 1001	0.008	0.010	5.2	4	650	400

^a50% effective concentration, or compound concentration required to achieve 50% reduction of the p24 antigen levels in the supernatant of HIV-1-infected peripheral blood lymphocyte cells (PBL) or monocyte/macrophages (M/M).

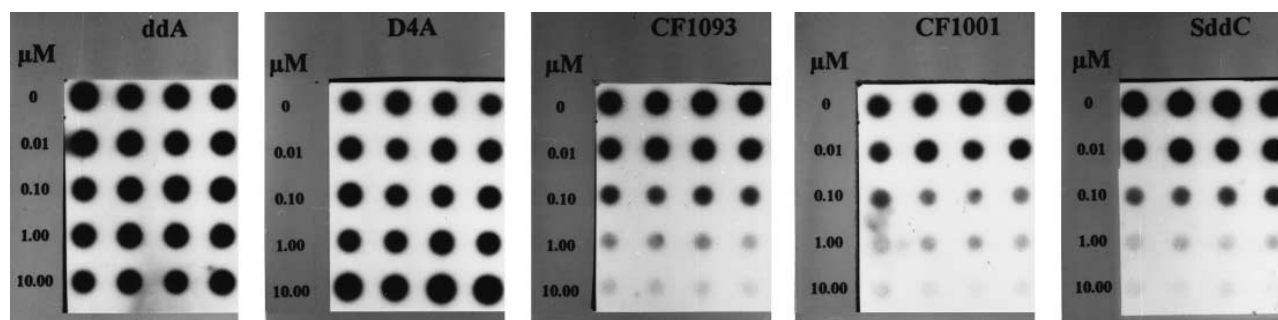


Fig. 2. Evaluation of HBV DNA by dot blot hybridisation, from cell culture supernatants of Hep G2 2.2.15 after 12 days of incubation in the presence of varying drug concentrations. The cell cultures were exposed to ddA, d4A, Cf 1001, Cf 1093 or SddC (3TC) at 0 (upper horizontal row), 0.01, 0.1, 1.0 and 10 μ M (lowest horizontal row). The experiment was performed in quadruplicate (vertical lanes).

derivatives [26], these observations may explain the exquisite and superior activity of the d4A and ddA prodrugs against HIV and HBV, as compared to the parental d4A and ddA nucleosides.

It should be noted that the experiments involving M/M were performed in the presence of M-CSF. Under these conditions, 2'-deoxyadenosine analogues (i.e., ddA and ddI) are far less active than in M/M not treated with M-CSF, because of the increased dATP pools induced by this cytokine. The results obtained with ddA and d4A are in agreement with those previously described [23]. Under these unfavourable conditions, both Cf 1093 and Cf 1001 retained antiviral activity, fully comparable, or even greater, than that shown for ddA/ddI in primary macrophages not treated with M-CSF.

Of interest is the observation that the selectivity index of the d4A aryloxyphosphoramidate prodrug is markedly increased as compared to that of d4A itself. These data suggest that the toxicity of d4A, in contrast to that of ddA, is not mainly due to its eventual conversion to its 5'-triphosphate metabolite, but presumably to d4A itself (or a non-phosphorylated catabolite of d4A). The presence and/or the intracellular formation of this toxic cellular substance must be substantially prevented by delivering the prodrug, and releasing the d4A-MP derivative directly into the intact cells. Additional studies are required to further clarify this issue. Although the antiviral activity and selectivity of the ddA and d4A aryloxyphosphoramidate prodrugs look highly promising in cell culture, no animal data on the pharmacology, potential toxicity and antiviral efficacy of the drug are available so far to confirm their pronounced therapeutic index *in vivo*.

It should also be mentioned that an improved antiviral activity of ddA has recently been observed for another prodrug approach (i.e., bis(S-acyl-2-thioethyl) or bis(SATE) derivatives of ddAMP) [15]. As for the aryloxyphosphoramidates, the bis(SATE) prodrug approach could also be successfully applied to deliver ddAMP into the intact cells. However, both the aryloxyphosphoramidate and bis(SATE) approaches virtually failed to markedly improve the antiviral activity of ddI (Ref. [15] and our unpublished data). The explanation for this phenomenon is most likely to be the different pathways that are followed for the activation of both compounds. Whereas ddA and d4A are directly metabolized to ddAMP (and d4A-MP), prior to further metabolism to the 5'-triphosphate derivatives, ddI has to be metabolised first to ddIMP, followed by a 2-step conversion to succinyl-ddAMP by succinyl-AMP synthetase and further on to ddAMP by

succinyl-AMP lyase, before it can be processed to the 5'-triphosphate. Therefore, direct delivery of ddIMP into the intact cells may only overcome the first activation (phosphorylation) step but not the subsequent two (poorly efficient) conversion steps to ddAMP.

In conclusion, we have developed a highly successful aryloxyphosphoramidate prodrug approach to markedly improve the anti-HIV and anti-HBV activity of the purine nucleoside analogues, ddA and d4A. We have also shown that the selectivity index of the ddA and d4A prodrugs can be markedly improved by such an approach, which opens up interesting and novel perspectives to optimize existing strategies and/or to develop new modalities for the treatment of both HIV and HBV infections.

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References

- [1] Hao, Z., Cooney, D.A., Hartman, N.R., Perno, C.F., Fridland, A., De Vico, A.L., Sarnagadharan, M.G., Broder, S. and Johns, D.G. (1988) *Mol. Pharmacol.* 34, 431–435.
- [2] Balzarini, J., Herdewijn, P. and De Clercq, E. (1989) *J. Biol. Chem.* 264, 6127–6133.
- [3] Balzarini, J., Cooney, D.A., Dalal, M., Kang, G.-J., Cupp, J.E., De Clercq, E., Broder, S. and Johns, D.G. (1987) *Mol. Pharmacol.* 32, 798–806.
- [4] Starnes, M.C. and Cheng, Y.-C. (1987) *J. Biol. Chem.* 262, 988–991.
- [5] Johnson, M.A., Ahluwalia, G., Connelly, M.C., Cooney, D.A., Broder, S., Johns, D.G. and Fridland, A. (1988) *J. Biol. Chem.* 263, 15354–15357.
- [6] Johnson, M.A. and Fridland, A. (1989) *Mol. Pharmacol.* 36, 291–295.
- [7] Balzarini, J., Baba, M., Pauwels, R., Herdewijn, P. and De Clercq, E. (1988) *Biochem. Pharmacol.* 37, 2847–2856.
- [8] Gray, N.M., Marr, C.L.P., Penn, C.R., Cameron, J.M. and Bethell, R. (1995) *Biochem. Pharmacol.* 50, 1043–1051.
- [9] Eriksson, S., Kierdaszuk, B., Munch-Petersen, B., Öberg, B. and Johansson, N.G. (1991) *Biochem. Biophys. Res. Commun.* 176, 586–592.
- [10] Schinazi, R.F., Lloyd Jr., R.M., Nguyen, M.-H., Cannon, D.L., McMillan, A., Ilksoy, N., Chu, C.K., Liotta, D.C., Bazmi, H.Z. and Mellors, J.W. (1993) *Antimicrob. Agents Chemother.* 37, 875–881.
- [11] Sastry, J.K., Nehete, P.N., Khan, S., Nowak, B.J., Plunkett, W.,

- Arlinghaus, R.B. and Farquhar, D. (1992) *Mol. Pharmacol.* 41, 441–445.
- [12] Pompon, A., Lefebvre, I., Imbach, J.-L., Kahn, S. and Farquhar, D. (1994) *Antiviral Chem. Chemother.* 5, 91–98.
- [13] Périgaud, C., Gosselin, G., Lefebvre, I., Girardet, J.-L., Benzaria, S., Barber, I. and Imbach, J.-L. (1993) *Bioorg. Med. Chem. Lett.* 3, 2521–2526.
- [14] Périgaud, C., Aubertin, A.-M., Benzaria, S., Pelicano, H., Girardet, J.-L., Maury, G., Gosselin, G., Kirn, A. and Imbach, J.-L. (1994) *Biochem. Pharmacol.* 48, 11–14.
- [15] Valette, G., Pompon, A., Girardet, J.-L., Cappellacci, L., Franchetti, P., Grifantini, M., La Colla, P., Loi, A.G., Périgaud, C., Gosselin, G. and Imbach, J.-L. (1996) *J. Med. Chem.* 39, 1981–1990.
- [16] Meier, C., Lorey, M., De Clercq, E. and Balzarini, J. (1997) *Nucleosides Nucleotides*, in press.
- [17] Naesens, L., Bischofberger, N., Armilli, M., Kim, C. and De Clercq, E. (1997) in: *Abstracts of the Tenth International Conference on Antiviral Research*, April 6–11, 1997, Atlanta, GA, USA. *Antiviral Res.*, in press.
- [18] Bischofberger, N., Naesens, L., De Clercq, E., Fridland, A., Srinivas, R.V., Robbins, B.L., Arimilli, M., Cundy, K., Kim, C., Lacy, S., Lee, W. and Shaw, J.-P. (1997) in: *Abstracts of the 4th Conference on Retroviruses and Opportunistic Infections*, January 22–26, 1997, Washington DC, p. 104, no. 214.
- [19] McGuigan, C., Cahard, D., Sheeka, H.M., De Clercq, E. and Balzarini, J. (1996) *J. Med. Chem.* 39, 1748–1753.
- [20] Balzarini, J., Egberink, H., Hartmann, K., Cahard, D., Vahlenkamp, T., Thormar, H., De Clercq, E. and McGuigan, C. (1996) *Mol. Pharmacol.* 50, 1207–1213.
- [21] Balzarini, J., Wedgwood, O., Kruining, J., Pelemans, H., Heijntink, R., De Clercq, E. and McGuigan, C. (1996) *Biochem. Biophys. Res. Commun.* 225, 363–369.
- [22] McGuigan, C., Wedgwood, O.M., De Clercq, E. and Balzarini, J. (1996) *Bioorg. Med. Chem. Lett.* 6, 2359–2362.
- [23] Perno, C.-F., Cooney, D.A., Gao, W.Y., Zang, H., Johns, D.G., Foli, A., Hartman, N.R., Calio, R., Broder, S. and Yarchoan, R. (1992) *Blood* 80, 995–1003.
- [24] Heijntink, R.A., De Wilde, G.A., Kruining, J., Berk, L., Balzarini, J., De Clercq, E., Holý, A. and Schalm, S.W. (1993) *Antiviral Res.* 21, 141–153.
- [25] Korba, B.E. and Milman, G. (1991) *Antiviral Res.* 15, 217–228.
- [26] Balzarini, J., Karlsson, A., Aquaro, S., Perno, C.-F., Cahard, D., Naesens, L., De Clercq, E. and McGuigan, C. (1996) *Proc. Natl. Acad. Sci. USA* 93, 7295–7299.