2-(Glucosylthio)ethyl Groups as Potential Biolabile Phosphate-Protecting Groups of Mononucleotides

by **Nathalie Schlienger**[a]), **Christian Périgaud**[a])*, **Anne-Marie Aubertin**[b]), **Christine Thumann**[b]), **Gilles Gosselin**[a]), and **Jean-Louis Imbach**[a])

[a]) Laboratoire de Chimie Organique Biomoléculaire de Synthèse, UMR CNRS 5625, Case courrier 008, Université Montpellier II, Place Eugène Bataillon, F-34095 Montpellier Cedex 5 (fax: (+33)467042029; e-mail: perigaud@univ-montp2.fr)

[b]) Institut de Virologie de la Faculté de Médecine de Strasbourg, Unité INSERM 74, 3 rue Koeberlé, F-67000 Strasbourg

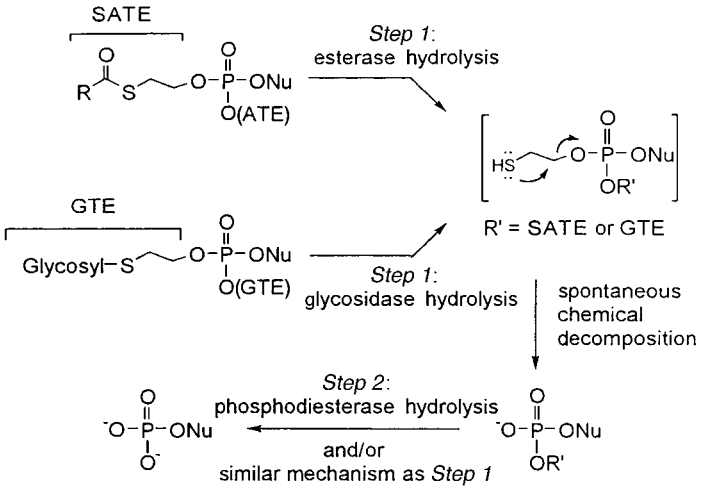
Dedicated to Prof. Dr. *Frank Seela* on the occasion of his 60th birthday

The *in vitro* anti-HIV effects and the stability studies of mononucleoside phosphotriester derivatives **1**–**3** of 3′-azido-3′-deoxythymidine (AZT) containing 2-(glucosylthio)ethyl moieties as potential biolabile phosphate-protecting groups are reported. The results of the anti-HIV evaluation demonstrate that the described compounds act *via* the release of the free nucleoside analogue and cannot be considered as mononucleotide prodrugs (pronucleotides). These data can be related to the lack of substrate affinity of these derivatives towards target-enzymes as corroborated by decomposition studies in various media and experiments with a purified β-D glucosidase.

Introduction. – In order to mask the negative charges of the phosphate function of 5′-mononucleotides (NMPs), a wide variety of protecting groups has been considered [1–3]. The resulting prodrugs (pronucleotides) were expected to cross cell membranes by passive diffusion and to liberate intracellularly the corresponding NMPs. Recent examples of pronucleotides that display *in vitro* anti-HIV-1 activity include mononucleoside phosphotriesters incorporating enzyme-labile transient phosphate-protecting groups. For instance, 2-(acylthio)ethyl (usually called SATE) groups have already been studied as esterase-mediated biolabile phosphate protections [4]. On the basis of several nucleoside models, it was demonstrated that bis[2-(acylthio)ethyl] phosphotriester derivatives were able to liberate the corresponding NMPs inside the cell [5][6]. Moreover, *in vitro* cytotoxicity studies indicated that neither the (acylthio)ethyl promoieties nor their degradation products induced additional toxicity compared to the parent nucleosides [7]. The proposed decomposition pathways of (acylthio)ethyl pronucleotides (*Scheme*) involve an esterase-mediated activation process leading to unstable phosphotriesters, which decompose spontaneously to afford the corresponding phosphodiesters. These intermediates are converted to NMPs by a similar pathway and/or phosphodiesterase activity.

However, esterases are widely distributed in organs, tissues, and body fluids of mammalian species [8][9]. In this respect, most of the ester prodrugs are rapidly hydrolyzed in human after oral absorption, since the gastrointestinal lumen, mucosal cells, and the liver are rich in these enzymes. Such a presystemic metabolism, preventing the delivery of the prodrugs to other tissues or organs, constitutes the major

Scheme. *Simplified Mechanisms Proposed for the Decomposition of (Acylthio)ethyl* (SATE) *Pronucleotides and Expected Decomposition Pathways for the Mononucleoside Bis[(glucopyranosylthio)ethyl] Phosphotriesters* **1**–**3**



limitation of the *in vivo* development of pronucleotides designed to promote a site-specific delivery by an esterase-mediated activation process [10].

These considerations led us to explore the potential of new biolabile phosphate-protecting groups, namely 2-(glycosylthio)ethyl (GTE) groups, which may involve a more specific enzymatic activation system. The resulting bis[2-(glycosylthio)ethyl] phosphotriester derivatives (*Scheme*) were designed to be hydrolyzed by glycosidases. Glycosidases being intracellular enzymes [11], a more selective delivery of the corresponding NMP should be induced. Furthermore, the carbohydrate moieties could increase the water solubility of the pronucleotides and be used not only as a site-directing moiety toward glycosyl-binding proteins [12] on cell membranes of some cells (like macrophages [13][14]), but also as substrate for monosaccharide-facilitated diffusion-transport systems at the blood/brain barrier [15].

We report herein the biological evaluation in various HIV-1-infected cell lines and the stability studies in several biological media of phosphotriester derivatives **1**–**3** of AZT (= 3′-azido-3′-deoxythymidine) (*Fig. 1*), which incorporate β-D-glucopyranosyl moieties associated with a thioethyl linker, as a first model of glycosyl-modified phosphate-protecting groups.

Results and Discussion. – *Antiviral Activity.* The (glucopyranosylthio)ethyl phosphotriesters **1**–**3** (*Fig. 1*) were evaluated, in comparison with AZT and the corresponding (acylthio)ethyl pronucleotide (^{t}BuSATE = ^{t}BuC(O)SCH$_2$CH$_2$) [16], for their *in vitro* inhibitory effects on the replication of HIV-1 in established human lymphoblastoid cell lines CEM-SS and MT-4 (*Table 1*). This antiretroviral evaluation was extended to human peripheral blood mononuclear cells (PBMC) and monocyte-derived macrophages (M/M), which are most physiologically relevant to predict *in vivo* anti-HIV activity [18]. In these four cell-culture systems, phosphotriester derivatives

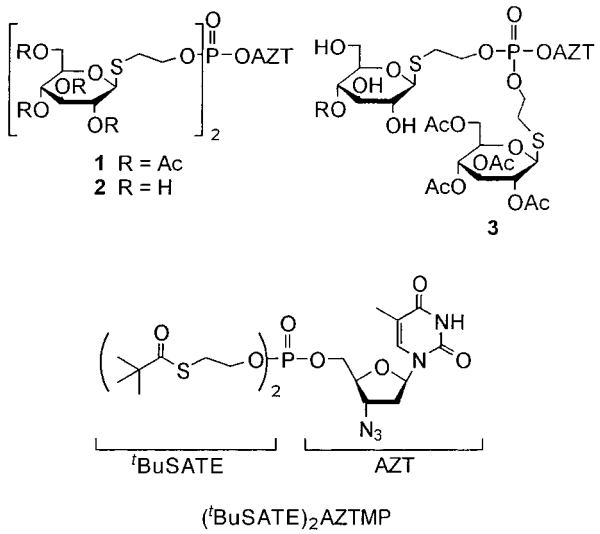


Fig. 1. *Structures of the (glucopyranosylthio)ethyl phosphotriester derivatives* **1**–**3** *of AZT and of the corresponding (acylthio)ethyl pronucleotide* (^{t}BuSATE = ^{t}BuC(O)SCH$_2$CH$_2$)

Table 1. *Antiviral Activity* [μM] *of the (Glucopyranosylthio)ethyl Derivatives* **1**–**5** *Compared to AZT and Its (Acylthio)ethyl Pronucleotide* (^{t}BuSATE = ^{t}BuC(O)SCH$_2$CH$_2$) *in Various Cell Lines Infected with HIV-1*

	CEM-SS		MT-4		PBM		M/M	
	IC_{50}	CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}
1	0.03	> 10	0.3	> 10	0.06	> 10	0.2	> 10
2	0.01	> 10	0.04	> 10	0.01	> 10	0.4	> 10
3	0.001	> 10	0.06	> 10	0.01	> 10	0.04	> 10
4	0.01	> 10	0.05	> 10	0.01	> 10	0.2	> 10
5	0.004	> 10	0.05	> 10	0.02	> 10	0.5	> 10
(^{t}BuSATE)$_2$AZTMP[a])	0.015	> 10	0.81	> 10	0.062	> 10	0.0001	> 100
AZT	0.006	> 100	0.01	75	0.001	75	0.0005	> 100

[a]) Previously published data [16][17] (^{t}BuC(O)SCH$_2$CH$_2$ = ^{t}BuSATE).

1–**3** significantly inhibited the multiplication of HIV-1 with IC_{50} values similar to or lower than those observed for AZT. This result was in agreement with data obtained from several bis[(acylthio)ethyl] phosphotriester derivatives of AZT [16], and may be related to the peculiar metabolism of this nucleoside analogue [19][20]. Studies on the metabolism of AZT in both uninfected and HIV-infected cells have shown that this nucleoside analogue, after cell uptake by a non-facilitated diffusion process (passive transport), is efficiently metabolized to AZTMP by cytosolic thymidine kinase (TK). The second phosphorylation step of AZT is catalyzed by thymidine monophosphate kinase (TMPK), but this cellular enzyme phosphorylates AZTMP inefficiently [21][22]. Thus, the intracellular concentration of AZTMP is 2–3 orders of magnitude higher than the one of AZTTP. This is consistent with the idea that TK catalyzes the rate-limiting step in the anabolism of AZT to its 5′-triphosphate. Consequently, a

mononucleotide prodrug (pronucleotide) of AZT cannot exhibit a higher *in vitro* anti-HIV activity than AZT in cells where cytosolic TK activity is expressed. In fact, contrary to the desired antiviral effect, an intracellular accumulation of AZTMP by means of a pronucleotide approach would lead to an inhibition of TMPK resulting in a blockage of the subsequent phosphorylation steps [23–25]. Concomitant cellular excretion of AZTMP [23][26–28] or dephosphorylation of AZTMP by endogenous pyrimidine nucleotidases and subsequent release of AZT by the cells [29][30] may also be related to the decreased anti-HIV-1 activity observed for an AZT pronucleotide as compared to the parent nucleoside.

More significant differences were found in the antiviral activities of the test compounds in HIV-1-infected CEM/TK$^-$ cells (*Table 2*). This cell line is highly deficient in cytosolic TK and should be considered an 'ideal' *in vitro* system to investigate the antiviral activities of nucleotide analogues of AZT that may release the corresponding NMP into the cells. As expected, AZT proved to be completely inactive against HIV-1 replication in CEM/TK$^-$ cells, whereas the corresponding ^{t}BuSATE-protected pronucleotide proved to be a potent inhibitor with a IC_{50} value of 0.45 μM. In contrast, the (glucopyranosylthio)ethyl phosphotriesters **1**–**3** showed no anti-HIV activity in this cell line at concentrations up to 10 μM. This result seems to demonstrate that the phosphotriester derivatives **1**–**3**, which incorporate (glycosylthio)ethyl (GTE) phosphate-protecting groups, were not able to deliver AZTMP inside the cells.

Table 2. *Antiviral Activity* [μM] *of (Glucopyranosylthio)ethyl Derivatives* **1**–**5** *Compared to AZT and Its (Acylthio)ethyl Pronucleotide* (^{t}BuSATE = ^{t}BuC(O)SCH$_2$CH$_2$)*, in CEM/TK$^-$ Cell Line Infected with HIV-1*

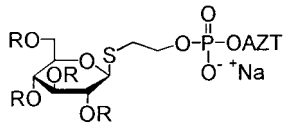
	1	**2**	**3**	**4**	**5**	(^{t}BuSATE)$_2$AZTMP[a])	**AZT**
CEM/TK$^-$ IC_{50}	> 10	> 10	> 10	> 10	> 10	0.45	> 100
CC_{50}	> 10	> 10	> 10	> 10	> 10	> 10	> 100

[a]) Previously published data [16] (^{t}BuC(O)SCH$_2$CH$_2$ = ^{t}BuSATE).

Table 3. *Half-lives of (Glucopyranosylthio)ethyl Derivatives* **1**–**5** *in RPMI 1640, Culture Medium, and Total CEM-SS Cell Extract*

	1	**2**	**3**	**4**	**5**
RPMI	35 h	4.5 h	8.3 h	6.8 d	stable (> 6 d)
Culture medium	30 h	4.5 h	8.3 h	28 h	40 h
Total CEM-SS cell extract	2.3 h	5 h	1.0 h	20 min	stable (> 2 d)

Stability and Decomposition Studies. To explain the inefficiency of the (glucopyranosylthio)ethyl moieties to act as biolabile phosphate-protecting groups of NMP, we studied the stability and the decomposition pathways of the compounds **1**–**3** in biological media by the improved 'on-line internal-surface reversed-phase (ISRP) cleaning' HPLC method previously developed in our laboratory [16]. This technique allows the direct analysis of biological samples without pretreatment. The stability of the compounds was evaluated in culture medium (RPMI 1640 containing 10% of heat-inactivated foetal calf serum), which is the extracellular medium used for antiviral evaluation in cell-culture systems, and in RPMI 1640, in order to distinguish between chemical and enzyme-controlled hydrolysis. Finally, the total cell extract from CEM-SS



4 R = Ac
5 R = H

Fig. 2. *Structure of the (glucopyranosylthio)ethyl phosphodiester derivatives* **4** *and* **5** *of AZT*

cells was used to mimic the intracellular medium. The phosphodiester derivatives **4** and **5** (*Fig. 2*) were prepared as reference samples for calibration and identification of the decomposition products.

The similar half-lives obtained in culture medium and RPMI for the decomposition of the phosphotriesters **1**–**3** seem to indicate that the hydrolysis proceeds by a chemical mechanism [31]. The faster disappearance of the phosphotriesters **1** and **3** in the total cell extract than in culture medium was essentially due to rapid cleavage of the acetyl-protecting groups by esterases, which are present in higher amounts in the total cell extract.

In the total cell extract, the phosphotriesters **1**–**3** led to a time-dependent accumulation of the phosphodiester **5**. The decomposition of phosphotriester **2** showed that phosphodiester **5** was the only detectable product; in the case of the acetylated phosphotriesters **1** and **3**, the phosphodiester **4** was the main observed metabolite, which was further deacetylated by esterases present in this medium to give **5**. Surprisingly, the phosphodiester **5** was very stable (no decomposition observed after 2 days). This might be related to either low phosphodiesterase and/or β-D-glucosidase activity in the total cell extract. In contrast, the phosphodiesters **4** and **5** were significantly hydrolyzed in culture medium, probably by phosphodiesterase activation, to give AZTMP, which was further slowly dephosphorylated ($t_{1/2}$ *ca.* 106 h) to AZT. This observation could explain the anti-HIV-1 activities observed for the compounds **4** and **5** in TK^+ cell lines, which are similar to those of the corresponding phosphotriester derivatives **1**–**3**.

To explain the stability of the phosphodiester **5** in the total cell extract, further studies were performed. An evaluation of the β-D-glucosidase activity with 4-nitrophenyl β-D-glucopyranoside as substrate showed complete absence of β-D-glucosidase activity in this medium. This may be due either to the loss of β-D-glucosidase activity during the preparation of the cell extract, like previously reported for cell homogenates [32], or to intrinsically low β-D-glucosidase activity in CEM-SS cells. Substrate-specificity studies of the phosphodiester **5** towards purified sweet-almond β-D-glucosidase, an enzyme which catalyzes the hydrolysis of a wide range of glucosides [33], showed no detectable formation of AZTMP. The phosphodiesterase activity of the cell extract was evidenced with deoxythymidine (4-nitrophenyl phosphate) as a substrate, which was cleaved with a half-life of 2 h (data not shown). Owing to its stability in the cell extract, the phosphodiester **5** seems to have no substrate affinity towards the phosphodiesterase activity present in this medium.

In contrast to (acylthio)ethyl derivatives, bis[(glucopyranosylthio)ethyl] phosphotriesters cannot be considered as pronucleotides, since they are unable to deliver the

corresponding NMP inside the cells. This fact is evidenced by the lack of antiviral activities of the AZT derivatives **1**–**3** in CEM/TK$^-$ cells. The anti-HIV-1 activity observed for these compounds in TK-expressing cell lines could be related to the release of the parent nucleoside in the extracellular medium. The hydrolysis of bis-[(glucopyranosylthio)ethyl] phosphotriesters may involve, as a first step, a chemical decomposition process [31] giving rise to the formation of the phosphodiester **5**. This metabolite, which exhibited a great stability in the total cell extract, could subsequently be converted to AZTMP in the culture medium (through a phosphodiesterase activation) and further to AZT, the latter being able to cross the cell membrane.

The results presented above do not preclude the potential interest in a pronucleotide approach with glycosyl-modified moieties as transient protecting groups of NMP analogues; but they reveal the importance of the affinity of mononucleotide prodrugs (and corresponding expected metabolites) for intracellular target enzymes. The presence of the O-atom at the anomeric position of the sugar residue appears to be a major requirement to preserve enzymatic recognition. Thus, the design of new kinds of glycosyl-modified pronucleotides will involve necessarily another linker between the P-atom and the glycosyl moiety and, as a consequence, new decomposition processes. In this respect, we have previously reported that mononucleoside *S*,*S*′-bis[(2-acyl)ethyl phosphorodithioates] allow the efficient intracellular delivery of their parent 5′-mononucleotides [34]. The synthesis and study of new potential gylcosyl-modified pronucleotides incorporating a 2-oxyethyl linker are currently in progress in our laboratory.

These investigations were supported by grants from *CNRS* and the *Agence Nationale de Recherches sur le SIDA* (*ANRS*, France). *N.S.* was supported by an *ANRS* fellowship.

Experimental Part

Chemicals. The synthesis of the phosphotriester and phosphodiester derivatives **1**–**5** has been reported elsewhere [31]. The synthesis of 3′-azido-3′-deoxy-5′-thymidylic acid bis{2-[(2,2-dimethyl-1-oxopropyl)-thio]ethyl} ester ((^{t}BuSATE)$_2$AZTMP) was published previously [16]. The synthesis of AZTMP was carried out following a general procedure with phosphoryl chloride (= phosphoric trichloride) [35]. 4-Nitrophenyl β-D-glucopyranoside and 5′-thymidylic acid 4-nitrophenyl ester (=thymidine 5′-(4-nitrophenyl hydrogen phosphate) were purchased from *Aldrich* and *Sigma*, resp.

Virology. The origin of the viruses and the techniques used for measuring inhibition of virus multiplication have been previously described [36]. Briefly, in MT-4 cells, the determination of the antiviral activity of the pronucleotides was based on a reduction of HIV-1-IIIB-induced cytopathogenicity, the metabolic activity of the cells being measured by the property of mitochondrial dehydrogenases to reduce the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a blue formazan. For CEM-SS or CEM/TK$^-$ cells, the production of virus HIV-LAI was measured by quantification of the reverse-transcriptase activity associated with the virus particle released in the culture supernatant [37]. Cells, MT-4, and CEM, were incubated with a $TCID_{50}$ of 50 or 100 or viruses during 30 min; after virus adsorption, unbound particles were eliminated by two washes, and cells were cultured in the presence of different concentrations of test compounds for 5 days, or 6 days in the case of CEM/TK$^-$ cells, before virus-production determination. The IC_{50} was derived from the computer-generated median effect plot of the dose/effect data [38]. In parallel experiments, cytotoxicity of the test compounds for uninfected cells was measured after an incubation of 5 or 6 days in their presence by means of the colorimetric MTT test. The CC_{50} is the concentration at which OD_{540} was reduced by one-half and was calculated by means of the program mentioned above. Human peripheral blood mononuclear cells (PBMC) were obtained from HIV-1 seronegative donors. Isolation and culture of human monocytes and infection of monocyte-derived macrophages (M/M) with HIV-1 BAL were performed as previously described [17]. Culture and infection with HIV-1-LAI of human PBMC have been reported elsewhere [36]. IC_{50} Values

were calculated on the basis of RT activity in the supernatant of the cell culture. Cytotoxicity of the compounds on uninfected cells was measured with the colorimetric MTT reaction.

Stability Studies. RPMI 1640 was purchased from *GibcoBRL*, and the CEM-SS cell extract was prepared according to published procedures [36]. The stability studies were performed by means of the 'on-line internal-surface reversed-phase (ISRP) cleaning' HPLC method previously described [16]. HPLC Analyses: *Waters* unit equipped with a model *600E* system controller, a model *486* detector (detection at 265 nm), and a *Millennium* data workstation; reversed-phase anal. column (*Macherey-Nagel*, *Nucleosil,* C_{18}, 150 × 4.6 mm, 5 μm), protected by a prefilter and a precolumn (*Guard-Pak* insert, *Delta-Pak* C_{18}, 100 Å) hold in a *Guard-Pak* holder; eluents *A* (50 mM triethylammonium acetate buffer (pH 7)) and *B* (*A* containing 80% of MeCN); injection of the crude sample (80 μl, initial concentration 50 μM) onto the precolumn, eluent *A* for 5 min, then connection to the column and linear gradient $A \rightarrow B$ within 35 min; calibration and identification of the decomposition products by coinjection with authentic samples (AZTMP, AZT, phosphodiesters **4** and **5**). HPLC/MS Coupling experiments: negative electrospray mode; detection of some analytes presumed to result from deacetylation of **1**, **3**, and **4**. The rate constants of disappearance of the pronucleotides were calculated according to pseudo-first-order kinetic models [16], which were in accordance with the experimental data.

Enzyme Assays. Sweet-almond β-D-glucosidase (EC 3.2.1.21) was purchased from *Fluka*. One unit of activity was determined as the amount of enzyme which liberated 1 μmol/min of 4-nitrophenol in 0.1M phosphate buffer (pH 6.8) at 37° with 4-nitrophenyl β-D-glucopyranoside as substrate. Specific activity of the enzyme was 5.6 units/mg, determined spectrophotometrically by following the release of 4-nitrophenol. A suitable enzyme concentration with 4-nitrophenyl β-D-glucopyranoside (50 μM) as substrate was found to be 3.5 μg/ml and led to 10% of hydrolysis after 30 min. Substrate-affinity determination with phosphodiester **5** under the same conditions and also with a 100-fold higher enzyme concentration was carried out. Hydrolysis of the compound was estimated with the HPLC technique described above.

REFERENCES

[1] A. A. Arzumanov, N. B. Dyatkina, *Russ. J. Bioorg. Chem*. **1996**, *22*, 777.

[2] C. Périgaud, J.-L. Girardet, G. Gosselin, J.-L. Imbach, 'Comments on Nucleotide Delivery Forms', in Antiviral Drug Design', Ed. E. De Clercq, JAI Press, London, 1996, pp. 147 – 172.

[3] J. P. Krise, V. J. Stella, *Adv. Drug Delivery Rev.* **1996**, *19*, 287.

[4] C. Périgaud, G. Gosselin, J.-L. Imbach, 'Minireview: from the Pronucleotide Concept to the SATE Phosphate Protecting Groups', in 'Current Topics in Medicinal Chemistry', Ed. J. C. Alexander, Blackwell Science Ltd., Oxford, 1997, p. 15.

[5] C. Périgaud, G. Gosselin, J.-L. Imbach, *Antiviral Ther*. **1996**, *1* (*Suppl. 4*), 39.

[6] G. Gosselin, J.-L. Girardet, C. Périgaud, S. Benzaria, I. Lefebvre, N. Schlienger, A. Pompon, J.-L. Imbach, *Acta Biochim. Pol.* **1996**, *43*, 195.

[7] C. Périgaud, J.-L. Girardet, I. Lefebvre, M.-Y. Xie, A.-M. Aubertin, A. Kirn, G. Gosselin, J.-L. Imbach, J.-P. Sommadossi, *Antiviral Chem. Chemother.* **1996**, *7*, 338.

[8] E. Heymann, 'Carboxylesterases and Amidases', in 'Enzymatic Basis of Detoxification', Eds. W. B. Jacoby and R. R. Bend, Academic Press, New York, 1980, pp. 291 – 323.

[9] T. Satoh, *Rev. Biochem. Toxicol*. **1987**, *8*, 155.

[10] D. Egron, C. Périgaud, G. Gosselin, A.-M. Aubertin, J.-L. Imbach, *Bull. Soc. Chim. Belg*. **1997**, *106*, 461.

[11] A. Esen, 'b-Glucosidases', in 'β-Glucosidases, Biochemistry and Molecular Biology', Ed. A. Esen, American Chemical Society, Washington DC, 1993, p. 1.

[12] N. Sharon, H. Lis, *Science (Washington, D.C.)* **1989**, *246*, 227.

[13] P. D. Stahl, J. Somsel Rodman, M. J. Miller, P. H. Schlesinger, *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 1399.

[14] M. Monsigny, A.-C. Roche, P. Midoux, R. Mayer, *Adv. Drug Delivery Rev.* **1994**, *14*, 1.

[15] J. N. Abbott, I. A. Romero, *Mol. Medicine Today* **1996**, *2*, 106.

[16] I. Lefebvre, C. Périgaud, A. Pompon, A.-M. Aubertin, J.-L. Girardet, A. Kirn, G. Gosselin, J.-L. Imbach, *J. Med. Chem*. **1995**, *38*, 3941.

[17] C. Thumann-Schweitzer, G. Gosselin, C. Périgaud, S. Benzaria, J.-L. Girardet, I. Lefebvre, J.-L. Imbach, A. Kirn, A.-M. Aubertin, *Res. Virol*. **1996**, *147*, 155.

[18] E. R. Kern, 'Preclinical Evaluation of Antiviral Agents: *in vitro* and Animal Model Testing', in 'Antiviral Agents and Viral Diseases of Man', Eds. G. J. Galasso, R. J. Whitley, and T. C. Merigan, Raven Press, New York, 1990, pp. 87 – 123.

[19] G. J. Veal, D. J. Back, *Gen. Pharmacol*. **1995**, *26*, 1469.
[20] J. Balzarini, *Pharm. World Sci*. **1994**, *16*, 113.
[21] A. Lavie, I. Schlichting, I. R. Vetter, M. Konrad, J. Reinstein, R. S. Goody, *Nat. Med. (N.Y.)* **1997**, *3*, 922.
[22] A. Lavie, I. R. Vetter, M. Konrad, R. S. Goody, J. Reinstein, I. Schlichting, *Nat. Struct. Biol*. **1997**, *4*, 601.
[23] L. W. Frick, D. J. Nelson, M. H. St Clair, P. A. Furman, T. A. Krenitsky, *Biochem. Biophys. Res. Commun*. **1988**, *154*, 124.
[24] P. A. Furman, J. A. Fyfe, M. H. St Clair, K. Weinhold, J. L. Rideout, G. A. Freeman, S. Nusinoff Lehrman, D. P. Bolognesi, S. Broder, H. Mitsuya, D. W. Barry, *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8333.
[25] J. A. Harrington, W. H. Miller, T. Spector, *Biochem. Pharmacol*. **1987**, *36*, 3757.
[26] R. P. Agarwal, A. M. Mian, *Biochem. Pharmacol*. **1991**, *42*, 905.
[27] R. P. Agarwal, *Int. J. Purine Pyrimidine Res.* **1991**, *2*, 1.
[28] A. Fridland, M. C. Connely, R. Ashmun, *Mol. Pharmacol*. **1990**, *37*, 665.
[29] A. De Flora, E. Zocchi, L. Guida, C. Polvani, U. Benatti, *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3145.
[30] M. Magnani, M. Bianchi, L. Rossi, V. Stocchi, *Biochem. Biophys. Res. Commun*. **1989**, *164*, 446.
[31] N. Schlienger, C. Périgaud, G. Gosselin, J.-L. Imbach, *J. Org. Chem*. **1997**, *62*, 7216.
[32] L. B. Daniels, R. H. Glew, 'β-D-Glucosidases in Tissue', in 'Methods of Enzymatic Analysis. Enzymes 2: Esterases, Glycosidases, Lyases, Ligases', Ed. H. U. Bergmeyer, J. Bergmeyer, and M. Grassl, Verlag Chemie, Weinheim, 1974, p. 217.
[33] M. P. Dale, H. E. Ensley, K. Kern, K. A. R. Sastry, L. D. Byers, *Biochemistry* **1985**, *24*, 3530.
[34] N. Schlienger, C. Périgaud, G. Gosselin, I. Lefebvre, A. Pompon, A.-M. Aubertin, A. Kirn, J.-L. Imbach, *Nucleosides Nucleotides* **1997**, *16*, 1321.
[35] L. A. Slotin, *Synthesis* **1977**, 737.
[36] F. Puech, G. Gosselin, I. Lefebvre, A. Pompon, A.-M. Aubertin, A. Kirn, J.-L. Imbach, *Antiviral Res.* **1993**, *22*, 155.
[37] C. Moog, A. Wick, P. Le Ber, A. Kirn, A.-M. Aubertin, *Antiviral Res.* **1994**, *24*, 275.
[38] J. Chou, T.-C. Chou, in 'Dose-Effect Analysis with Microcomputers: Quantitation of ED_{50}, LD_{50}, Synergism, Antagonism, Low-Dose Risk, Receptor Binding, and Enzyme Kinetics; Computer Software for Apple II Series and IBM-PC and Instruction Manual', Ed. Elsevier-Biosoft, Elsevier Science Publishers, Cambridge, U.K., 1985, p. 19.

Received July 28, 1999