*cyclo*Sal-Pronucleotides – Design of Chemical Trojan Horses

Chris Meier*

Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6; D-20146 Hamburg, Germany

Abstract: Pronucleotides represent a promising alternative to improve the biological activity of nucleoside analogues against different viral diseases. The basic idea is to achieve nucleotide delivery into cells bypassing limitations encountered during the intracellular formation of nucleotides. The *cyclo*Sal-concept is one of several pronucleotide systems reported so far. For some nucleoside analogues, the *cyclo*Sal-approach improved antiviral potency thus broadening the applicability of nucleosides. The initial design, chemistry, the proof-of-principle and different applications of the *cyclo*Sal-strategy will be discussed in this review.

"Dedicated to Professor Joachim Thiem on the occasion of his 60th birthday"

Since the discovery of 3'-azido-3'-deoxythymidine (AZT) as the first nucleoside drug for the treatment of AIDS [1], considerable efforts have been made to develop new nucleoside analogues that would be more active, less toxic inhibitors of HIV's reverse transcriptase (RT) [2]. These analogues differ from the natural nucleosides with regard to viral diseases, e.g. AIDS, herpes and hepatitis infections. The general mode of action of nucleoside analogues is through the inhibition of DNA polymerases, including reverse transcriptases by acting as competitive inhibitors and/or as DNA chain terminators. To act as DNA chain termination agents or polymerase inhibitors, intracellular

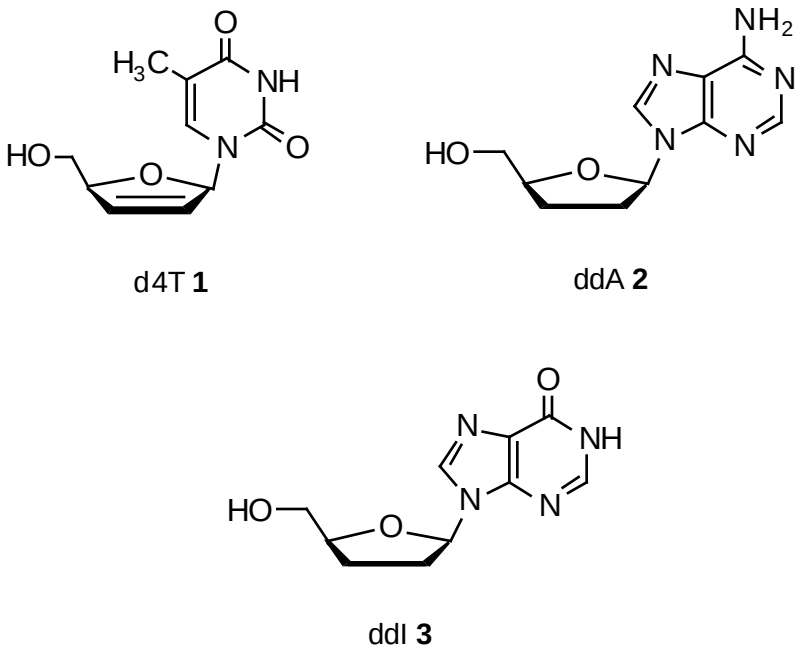


Fig. (1). Antivirally active nucleoside analogues.

modifications of the glycon and/or the aglycon residue [3]. Today, synthetic nucleoside mimetics represent a highly valuable source of antiviral agents that contribute significantly to the arsenal of agents for the treatment of conversion of the nucleoside analogues into their 5'-mono-, 5'-di- or 5'-triphosphates is a prerequisite after cell penetration [2,4]. However, the efficient anabolism to the corresponding nucleoside analogue triphosphates is often a major hurdle due to limited anabolic phosphorylation or catabolic processes as deamination or cleavage of the glycosidic bond and the therapeutic efficacy is therefore compromised [2,5].

*Address correspondence to this author at the Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6; D-20146 Hamburg, Germany; e-mail: meier@chemie.uni-hamburg.de

1389-5575/02 $35.00+.00

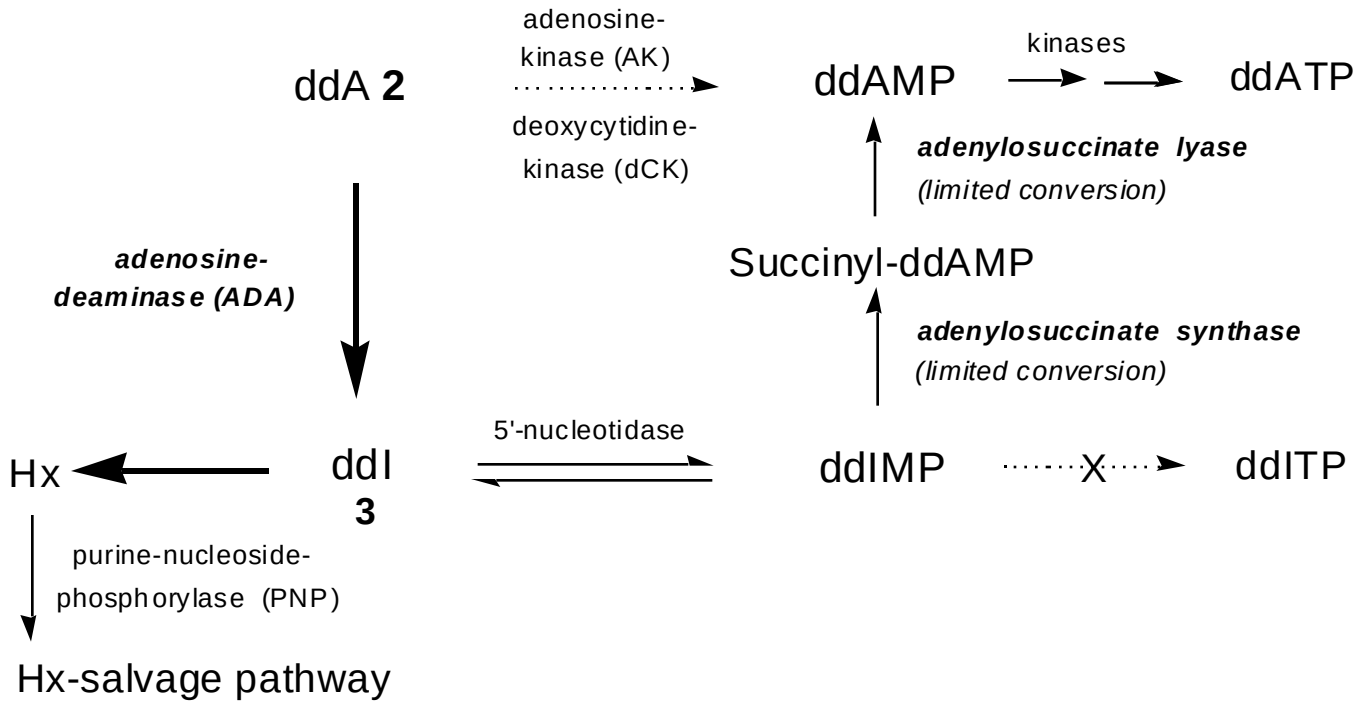


Fig. (2). Metabolism of 2',3'-dideoxyadenosine **2**.

For example, the first critical phosphorylation step of the anti-HIV active dideoxynucleoside analogue 2',3'-dideoxy-2',3'-didehydrothymidine (d4T) **1** [6,7] (Figure **1**) into the d4T 5'-monophosphate (d4TMP) catalyzed by thymidine kinase (TK) is the rate-limiting step in human cells [8,9].

Further, 2',3'-dideoxyuridine (ddU) is not converted into the nucleotide ddUMP and is therefore virtually ineffective as antiviral agent. However, its triphosphate (ddUTP) is one of the most powerful and selective inhibitors of HIV reverse transcriptase [5,10].

In addition, limited efficacy may also be due to deamination as in the case of 2',3'-dideoxyadenosine **2** (ddA) that is converted into ddI **3** by adenosine deaminase (ADA; Figure **2**). Moreover, ddA is only poorly converted directly into its monophosphate by adenosine kinase or deoxycytidine kinase [11,12] and thus only low intracellular levels of ddATP are achieved. DdI **3**, in turn, is either inactivated to hypoxanthine (Hx) by purine nucleoside phosphorylase, and/or further phosphorylated to the monophosphate ddIMP by 5'-nucleotidase [13,14]. Then, ddIMP is reaminated by adenylosuccinate synthetase and adenylosuccinate lyase to give 2',3'-dideoxyadenosine monophosphate (ddAMP) [15]. However, these enzymatic conversions are not very efficient and they represent rate-limiting steps. Further phosphorylation by cellular kinases converts ddAMP to the triphosphate level [11].

Despite of the above given examples, the intracellular fate of the majority of nucleoside analogues has not been studied in detail. Often these compounds are tested exclusively as nucleosides and discarded if found inactive. Since they are seldom studied as triphosphates against the target polymerases, uncovering where the metabolic blockade exists prevents further development. On the other hand, knowing what limitations exist during phosphorylation may offer a chance to develop compounds with improved

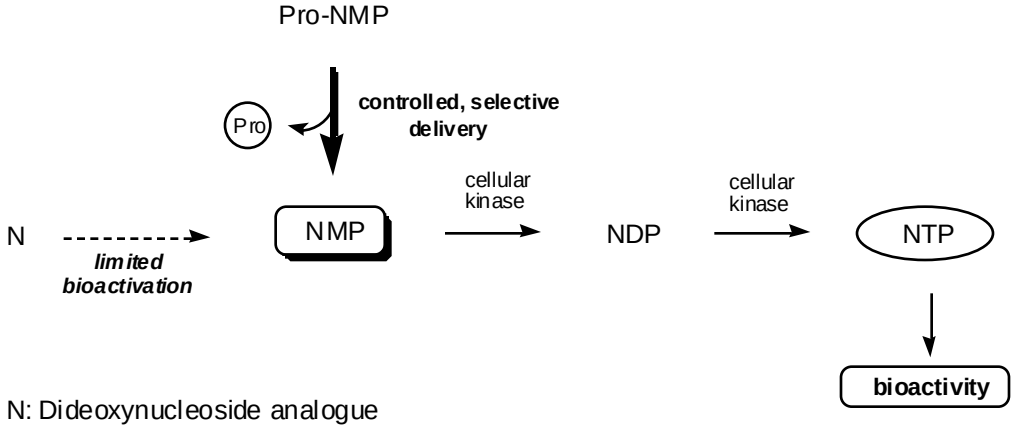


Fig. (3). Principle of the pronucleotide approach for nucleotide analogues.

biological potential. In principle, the direct administration of nucleotides should bypass the limiting step in the anabolism of some nucleosides and improve biological activity. Unfortunately, nucleotides are very polar molecules and do not easily penetrate cellular membranes. This difficulty, however, can be surmounted by chemically linking suitable degradable lipophilic carrier groups to the phosphate moiety that lead to neutral, membrane-permeable nucleotide delivery systems (***pronucleotide approach***; Figure **3**) [16-18].

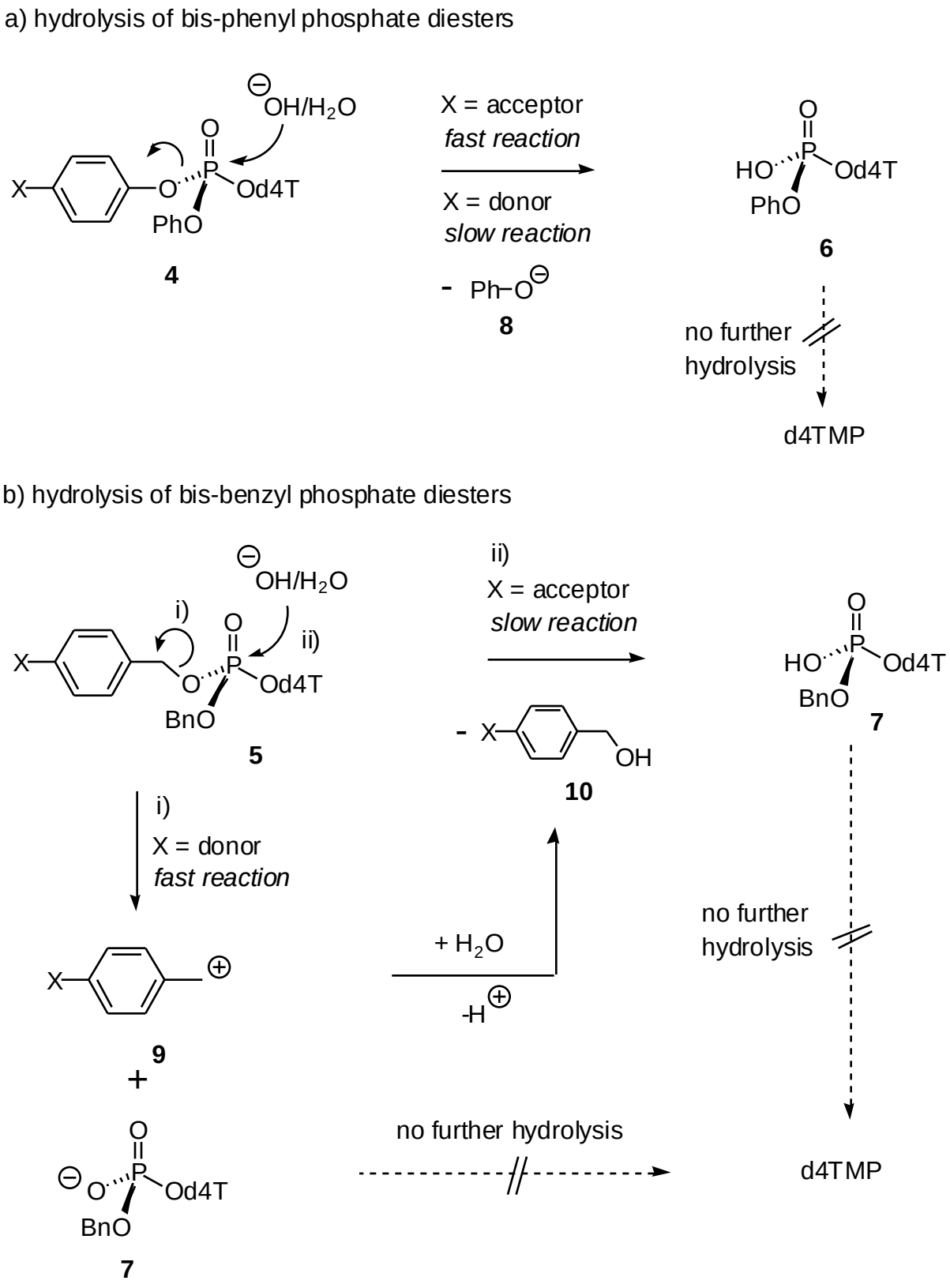


Fig. (4). Hydrolysis of bis-phenyl-(**4**) and bis-benzyl phosphate triesters **5**.

In order to obtain a neutral, lipophilic phosphate ester, two masking groups are necessary due to the presence of at least one negatively charged phosphate oxygen that exist under physiological conditions. The pK_A1 of a phosphate monoester is 1.6 and pK_A2 is 6.6, so there is an equilibrium in neutral solution between the mono- and the dianion. Moreover, the efficient intracellular delivery of nucleotides from a pronucleotide requires the design of a specific delivery mechanism. Several strategies using different nucleotide delivery mechanisms have been developed to achieve this goal [16-18].

Among these, simple systems based on pure chemical hydrolysis proved to be unsuccessful because after the first hydrolysis of the neutral phosphate triester, the resulting phosphate diester is often extremely stable to undergo against further chemical hydrolysis [19]. Several newer pronucleotide approaches are based on the concept of a tripartite prodrug system [20] and are based on the principle of selective chemical or enzymatic activation of the masking group, which leads to a second, spontaneous reaction. These approaches utilize and exploit carboxyesterase activity and pH value. The concepts working via an enzymatic trigger mechanism are the bis(POM)- [21], bis(POC)- [22], bis(DTE)- [23], bis(SATE)- [24], bis(SGTE)- [25], bis(AB)- [26], the aryloxyphosphoramidate (APA) [27], the phosphoramidate monoester [28] and a modified SATE-concept [29]. All these enzyme-triggered approaches have demonstrated that the successful intracellular delivery of nucleotides is possible. Moreover, the only successful, pH-driven nucleotide delivery strategy is the *cyclo*Sal-approach. This approach, which belongs also to the group of tripartite prodrug delivery systems and has been developed in our laboratories, will be the topic of this review.

*CYCLO*SALIGENYL-NUCLEOTIDES (*CYCLO*SAL-NMPs) [30] - DESIGN OF THE CONCEPT

In contrast to the approaches mentioned previously, the aim was the development of a highly selective delivery mechanism that is based on a pH-dependent chemical reaction involving a highly selective coupled mechanism (cascade mechanism) [31]. However, the chemically driven release of a nucleotide from a lipophilic precursor is not as easy as it seems. Studies in our and other laboratories showed that bis-phenyl- (**4**) [32,33] as well as bis-benzyl phosphate triesters (**5**) [34] hydrolyzed selectively to yield indeed the expected phenyl- (**6**) or benzyl phosphate diesters (**7**), respectively (Figure **4**).

However, no further hydrolysis was observed, thus hindering nucleotide release. The reason is the formation of a negative charge at the phosphorus atom. This prevents a second nucleophilic attack at the phosphorus atom in phenyl phosphate diesters like **6** and at the same time decreases the leaving group properties of the 5'-nucleoside phosphate fragment in benzyl phosphate diesters like **7** [19]. Nevertheless, studies with bis-phenyl phosphate triesters **4** proved the expected dependence of the hydrolytic stability of the substitution pattern in the aromatic ring: the more electron-*withdrawing* the substituent is, the more labile the phosphate triesters is to hydrolysis [32,33]. The situation was completely inverted for bis-benzyl phosphate triesters **5**: the more electron-*donating* the substituent was, the more labile was the triester to hydrolysis [34]. The mechanisms of hydrolysis were entirely different: $P\text{-}O_{phenyl}$ bond cleavage yielding phosphate diester **6** and phenolate anion **8** was observed for bis-phenyl phosphate triesters while a spontaneous C_{benzyl}-O bond cleavage leading to benzyl phosphate diester **7** and benzyl cation **9** took place for bis-benzyl phosphate triesters **5**. The cation **9** is subsequently quenched by water to give a benzyl alcohol **10**. Both reactions are quite selective but stopped at the phosphate diester level. Even enzymatic degradation of the intermediate diesters proved to be problematic [35]. Nevertheless, the different properties of phenyl- and benzyl ester hydrolysis may be used to design a nucleotide delivery system. However, the difference in the stability of the phenyl vs. the benzyl ester bond may be used for the design of a nucleotide delivery system. Important is, to have a concrete idea about the critical second hydrolysis step of the diester to give the phosphate monoester. The basis of the *cyclo*Sal concept consists of a combination of these two ester bond types as part of a cyclic bifunctional group (masking unit). Additionally, the nucleoside analogue is attached through an alkyl ester bond. Only the introduction of these three ester bonds would allow sufficient discrimination between the different phosphate ester bonds.

The designed chemically induced coupled process (tandem or cascade mechanism) which has been applied first to the nucleoside d4T **1** (*cyclo*Sal-d4TMP **11**; Figure **5**) was the following [36,37]: the phenyl ester bond is the most labile one because the negative charge could be delocalized and so cleavage should lead to 2-hydroxy*benzyl*phosphate diester **12** (step a). The alternative cleavage of the benzyl ester to yield 2-hydroxymethyl*phenyl*phosphate diester **13** is unfavorable (step c). As a consequence of the initial step, the ortho-substituent to the benzyl ester is changed from a very weak electron-donating group (phosphate ester) to a strong electron-donating group (hydroxyl). This effect of the 2-substituent intrinsically activates the remaining masking group and this induces a spontaneous rupture of diester **12** to yield the nucleotide and salicylalcohol **14** (cascade reaction; step b) presumably via formation of 2-quinone methide **15** or a zwitter-ion. So, after a first reaction involving the phosphorus atom, a cleavage mechanism may be achieved that takes place within the masking group only. This would avoid a possible pseudorotation process [38] that may partly lead to the liberation of the nucleoside instead of the nucleotide.

The major difference to the enzymatically triggered pronucleotides is that the *cyclo*Sal-strategy requires only *one* activation step to deliver the nucleotide. Moreover, due to the bifunctional character of the *cyclo*Sal group the ratio of the masking unit per nucleotide molecule is 1:1. In contrast, other pronucleotide concepts use ratios up to 4:1 [26,29]. Salicylalcohols **14** used as masking units were tested for their biological potency but showed neither antiviral activity nor cause toxicity [37]. Further *in-vivo* studies in mice showed that concentrations up to 250 mg/kg of different diols did not cause toxic side effects [39]. It should be added that salicylalcohol (saligenin) is used as part of the antirheumatic and analgetic drug Salicin (2-

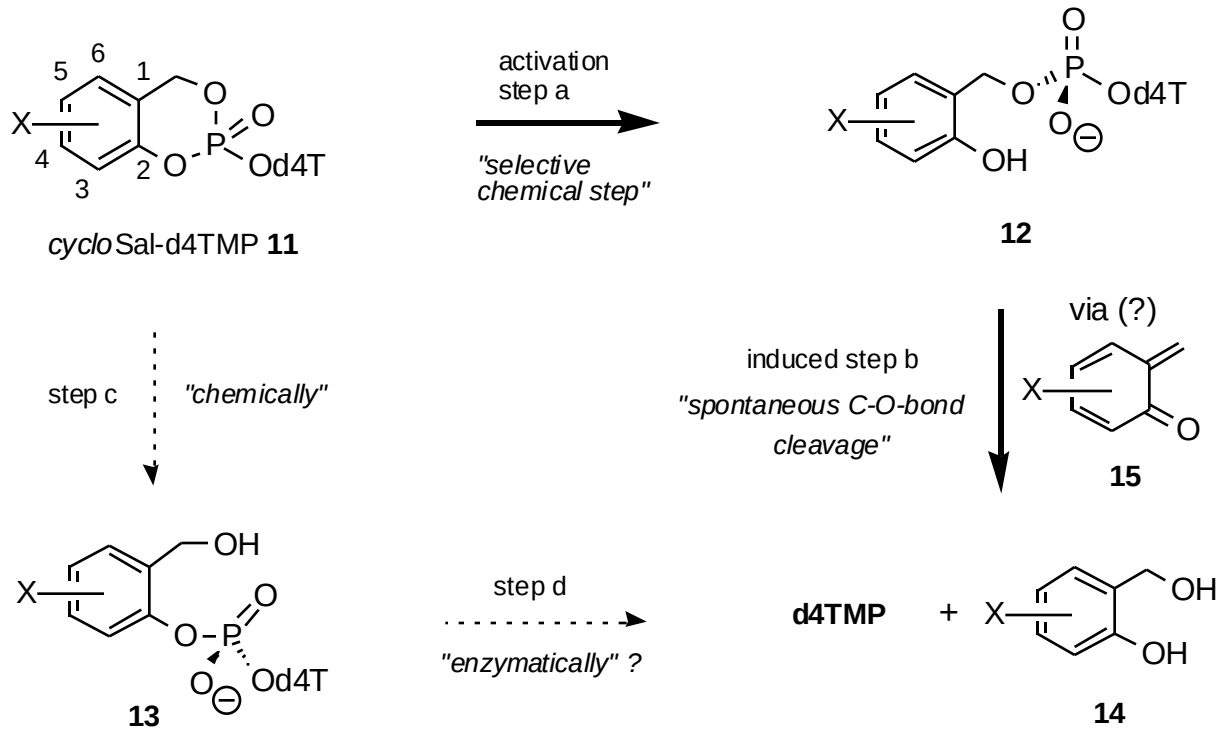


Fig. (5). Two possible hydrolysis pathways of *cyclo*Sal-d4TMP triesters **11**.

[hydroxymethyl]phenyl-β-D-glucopyranoside; Assalix®) [40]. β-Glucosidases hydrolyze Salicin to saligenin and D-glucose and the latter is then slowly oxidized by cytochrome P450 to salicylic acid in the blood and in the liver [41].

CHEMISTRY

The synthesis of the *cyclo*Sal-pronucleotides has been carried out most successfully using reactive phosphorus(III)-reagents (part a; Figure **6**) [36,37]. Therefore, diols **14** were reacted with phosphorus trichloride to give the cyclic chlorophosphites **16**. Phosphites **16** then were reacted either directly with the nucleoside analogue, e.g. d4T in the presence of diisopropylethylamine (DIPEA; Hünig`s base) to yield the cyclic phosphite triesters **17** which were oxidized in a one-pot-reaction using *t*-butylhydroperoxide (TBHP) or dimethyldioxirane. The phosphate triesters **11** were obtained in reasonable yields as diastereomeric mixtures. Alternatively, chlorophosphites **16** were treated with diisopropyl amine to yield the phosphoramidites **18** [42]. The coupling with the nucleoside analogue was carried out in acetonitrile in the presence of pyridinium chloride, 1H-tetrazole or imidazolium triflate as coupling activator. Using the latter activator yields of >90% were obtained. The latter phosphoramidite methodology has dramatic advantages for a few nucleoside analogues (e.g. 3TC) [42]. Another example is the acyclic purine nucleoside analogue acyclovir (ACV) while the use of chlorophosphites **16** gave varying yields of 25-50% of the phosphate triester, usage of phosphoramidites **18** improved not only the yield to ca. 80% but also the reproducibility of the reaction [43].

However, the use of phosphorus(V)-reagents (phosphordichloridate of the nucleoside or phosphorchloridate of salicyl alcohol) leads always to lower yields of the phosphate triesters (part b; Figure **6**) [36].

Salicylalcohols **14** have been prepared from the corresponding salicylic aldehydes **20** or -acids **21** by standard reduction protocols. In a few cases the aldehydes/acids were not commercially available. Then diols **14** have been synthesized from the phenols **22**. Selective ortho-formylation was possible by the Casiraghi-procedure [44] or the Rieche-formylation protocol [45]. Both methods lead to the salicylaldehydes, which are reduced to the corresponding **14**. An alternative is the direct hydroxymethylation according to Nagata *et al.* [46]. The latter is the mildest method of the above procedures (Figure **6**).

*CYCLO*SAL-PRONUCLEOTIDES – THE PROOF-OF-PRINCIPLE

Different studies concerning the properties of the *cyclo*Sal- d4TMP triesters **11** have been carried out. In a NMR experiment with 5-nitro-*cyclo*Sal-d4TMP **11a** in DMSO-d_6 containing 10% water the designed selective hydrolysis pathway leading to d4TMP could be confirmed. This experimental setup was chosen because the expected hydrolysis products could be detected by ^{31}P-, ^{13}C- and ^{1}H-NMR spectroscopy [36]. Later, chemical hydrolysis studies of triesters **11** in different aqueous buffers also showed the exclusive degradation to d4TMP as well as the formation of salicylalcohols **14** [36,37]. Furthermore, a clear correlation of the electronic properties introduced by the salicylalcohol substituents and the hydrolysis half-lives of the phosphate triesters was observed (Table 1). Additionally, the expected pH-dependence of the nucleotide delivery typical for the

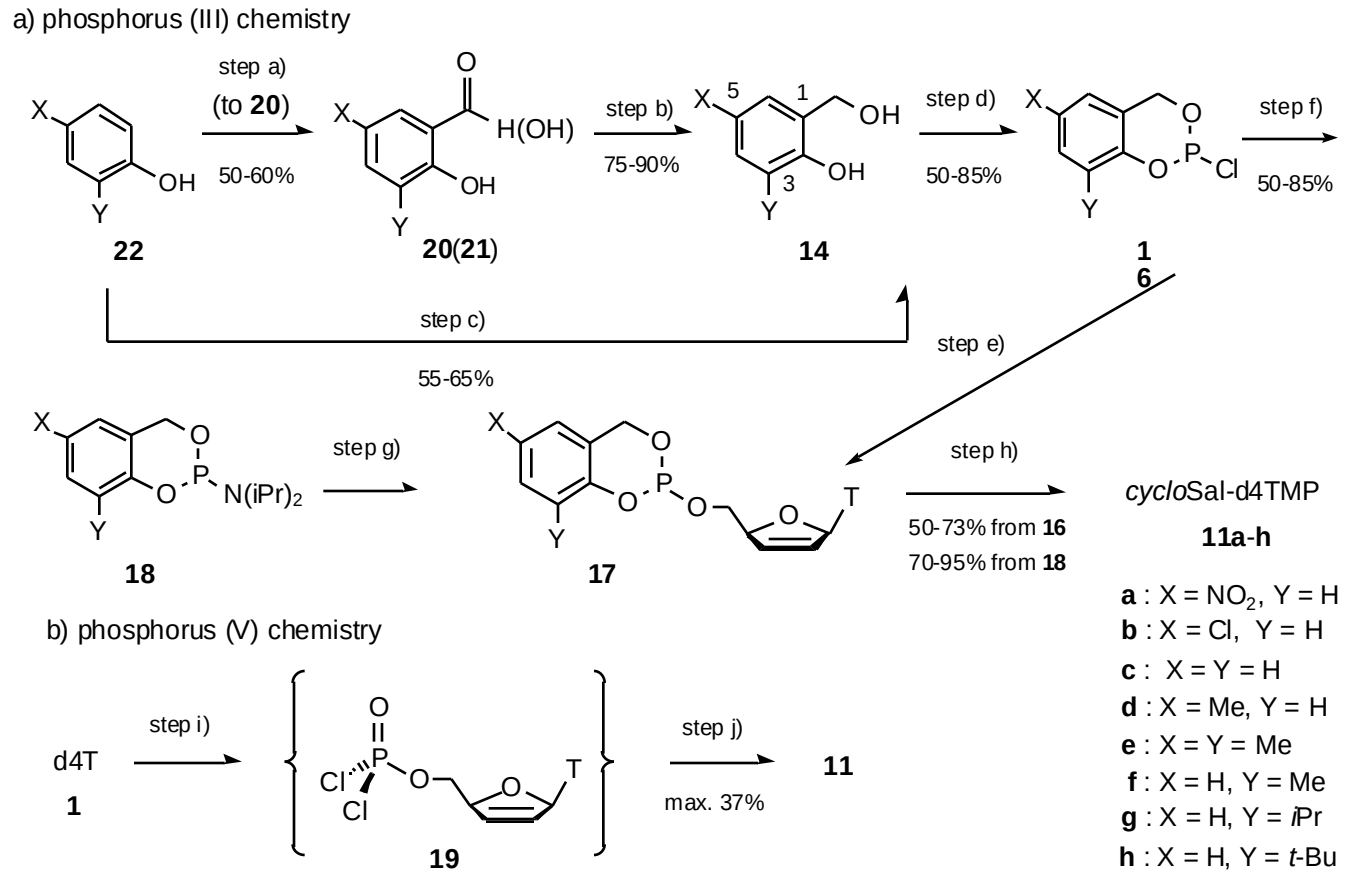


Fig. (6). Synthetic pathways to the *cyclo*Sal-d4TMP triesters **11**.
Reaction conditions: a) Casiraghi-Formylation [44]; b) $NaBH_4$ for **20** ($LiAlH_4$ or $NaBH_4/I_2$ for **21**); c) Nagata-hydroxyformylation [46]; d) PCl_3, pyridine, Et_2O, -10°C, 2h; e) d4T **1**, DIPEA, CH_3CN, 0°C, 20 min; f) Diisopropyl amine (2 eq), diethylether, 0°C, 30 min; g) pyridinium chloride, tetrazole or imidazolium triflate, CH_3CN, 0°C, 30 min; h) TBHP, CH_3CN, rt, 30 min; i) $P(O)Cl_3$, DIPEA, THF, 0°C, 12h; j) salicylalcohol **14**, DIPEA, THF, 0°C-rt, 12h.

chemical hydrolyses was observed in studies at pH 6.8, 7.3 and 8.9. As expected, studies in acidic media at pH 1.5 and pH 3 have revealed a considerable increase in stability (see discussion of the adenosine analogues below). A possible temperature dependence of the hydrolysis has not been investigated so far.

No evidence of an enzymatic degradation in RPMI-1640 medium containing 10% fetal calf serum (pH 7.3) has been observed (data not shown). Studies in CEM-cell extracts showed that the hydrolysis half-lives only slightly decreased as compared to the buffer hydrolyses [47]. Further studies in human serum (10% serum in phosphate buffer) exhibited no difference in stability as compared to the buffer hydrolysis

Table 1. Lipophilicity, Hydrolysis and Antiviral Data of *cyclo*Sal-d4TMP triesters 11

11	Subst. R	log*P*[a]	Hydrolysis ($t_{1/2}$) at 37°C			EC_{50} (μM)[f]			CC_{50} (μM)[g]
			pH 6.9[b] [h][e]	pH 7.3[c] [h][e]	pH 8.9[d] [h][e]	CEM/O HIV-1	CEM/O HIV-2	CEM/TK⁻ HIV-2	
11a	5-NO_2	0.17	4.1	0.15	0.06	0.29	0.40	40.0	75
11b	5-Cl	0.88	6.4	0.7	0.3	0.42	1.40	2.67	49
11c	5-H	0.28	24.5	4.5	1.1	0.28	0.10	0.50	47
11d	5-Me	0.73	28.3	8.0	1.3	0.18	0.34	0.18	38
11e	3,5-Me	1.18	98.2	32	3.4	0.09	0.17	0.08	21
11f	3-Me	0.70	68.5	24	1.5	0.057	0.07	0.048	26
11g	3-*i*Pr	1.60	n.d.[h]	28	n.d.[h]	0.047	0.08	0.065	18
11h	3-*t*Bu	1.75	n.d.[h]	96	n.d.[h]	0.18	0.15	0.18	49
d4T **1**	--	--	n.a.[i]	n.a.[i]	n.a.[i]	0.25	0.15	50	56

[a]partition coefficient; [b]30 mM TRIS buffer; [c]30 mM sodium phosphate buffer; [d]30 mM sodium borate buffer; [e]half-lives in hours; [f]50% antiviral activity: effective concentration; [g]50% cytotoxicity: cytotoxic concentration; [h]not determined; [i]not available

studies [43]. Thus, again no enzymatic contribution could be detected. All data found in these hydrolysis studies were in perfect agreement with the designed degradation pathway according to the cascade-reaction mechanism and all confirmed the initial idea to design a delivery mechanism that is independent to enzymatic activation (Figure **5**).

The antiviral potency of the *cyclo*Sal-nucleotides with half-lives ranging from 7-96 was assessed [36]. 3-Alkyl (**11f-h)**, 5-alkyl- (**11d**) as well as 3,5-dimethyl-*cyclo*Sal-d4TMP **11e** showed comparable or even higher antiviral potency (0.087μM) in a wild-type T-lymphocytic cell line (CEM/O) compared to d4T **1** (0.18μM, Table 1). Similar to the chemical hydrolysis studies, a correlation between the electron-donating activity of the substituent, and the antiviral activity against HIV-1 and HIV-2 in CEM cells was observed. Moreover, particularly striking is the complete retention of the antiviral potency in a mutant thymidine kinase-deficient cell line (CEM/TK$^-$) for all 3-alkyl-substituted compounds. From the antiviral data and the hydrolysis half-lives it became apparent that a certain stability is needed, but beyond this point no further improvement of activity could be observed (compare **11f** and **11h**). In contrast, very long half-lives like in the case of the 3-*t*-butyl substituent lead to a decrease in antiviral activity (compound **11h**, Table 1). Additionally, the high biological activity of the donor-substituted *cyclo*Sal-d4TMP derivatives was also observed for MT-4 and Molt4/C8 cells (data not shown). It should be added that from experiments using an isolated recombinant RT/RNA template it became clear that the *cyclo*Sal-triesters themselves have no inhibitory effect on DNA-synthesis [48], which is consistent with a mechanism of action for the *cyclo*Sal-triesters that relies on the formation of d4TTP. Taken together, these results confirm i) the cellular uptake of the compounds, ii) the highly selective intracellular delivery of d4TMP and iii) the independence of the biolocical activity on cellular thymidine kinase activation.

Nevertheless, the *in vitro* anti-HIV test gives only an indirect proof of the intracellular delivery of d4TMP. Therefore, a series of incubation experiments with wild-type CEM and CEM/TK$^-$ cells and radiolabeled 3-methyl-*cyclo*Sal-d4TMP **11f** (tritium-label in the methyl group of thymine) were conducted [49,50]. The amount of d4TMP in CEM/O cells was considerably higher (15-fold, 6 h incubations) as compared to the amount of d4TMP resulting from the metabolism of d4T in the same cell line. In addition, an increase in the concentration of d4TTP was observed (16-fold, 6 h incubation), which may explain the higher activity of the *cyclo*Sal-d4TMP triesters in wild-type CEM cells compared to d4T. These results are consistent a mechanism for the of *cyclo*Sal-d4TMPs that successfully bypasses thymidine kinase.

As can be seen from the structure of the *cyclo*Sal-d4TMP triesters, all triesters exist as a pair of diastereomers due to the fact that the chemical synthesis proceeds without any diastereoselectivity. It can not be excluded that the two diastereomers possess different hydrolytic properties as well as antiviral activities. Therefore, the diastereomeric mixtures were separated by semipreparative HPLC. Assignment of configuration was successfully done by correlation of the elution properties on a RP-18 silica gel column, ^{31}P-NMR chemical shifts, CD-spectroscopy using *cyclo*Sal-(-)-menthylmonophosphates [51] as a reference compound and, finally, antiviral activity. However, no crystal structure determination could be achieved so far. Attribution of configuration was therefore only done in an indirect manner. The chemical hydrolysis proceeded with a two-fold difference in the case of the diastereomers of 3-methyl-*cyclo*Sal-d4TMP **11f**. This may be due to the different position of the substituents at the half-chair formed by the 4H-benzodioxaphosphorin-2-oxide part of the triesters.

The antiviral evaluation of the separated diastereomers showed that both stereoisomers exhibited different antiviral activity. It was observed that the "R_p"-configurated stereoisomer was in general more potent than the "S_p"-stereoisomer. The difference in potency ranged from 5- to 10-fold either in TK-competent CEM/O and MT-4 cells or in mutant TK-deficient CEM cells. Nevertheless, even the "S_p"-stereoisomers retained some of the antiviral activity in the TK-deficient cells (Table 2).

As before, we studied both diastereomers of *cyclo*Sal-d4TMP **11f** in their tritium labeled form concerning their intracellular fate in CEM/O cells. In these studies it has been

Table 2. Antiviral Activity Dependance on the Stereochemistry of *cyclo*Sal-d4TMP Triesters 11

11	Subst	EC_{50} (μM)[a]			CC_{50} (μM)[b]	SI[c]
		CEM/O HIV-1	CEM/O HIV-2	CEM/TK$^-$ HIV-2		
R_p-**11e**	3,5-Me	0.093	0.17	0.08	18	218
S_p-**11e**	3,5-Me	0.50	0.80	0.38	22	57
R_p-**11f**	3-Me	0.08	0.067	0.063	11	190
S_p-**11f**	3-Me	0.42	1.1	0.70	76	108
R_p-**11h**	3-*t*Bu	0.13	0.44	0.19	25	190
S_p-**11h**	3-*t*Bu	0.6	3	5	79	16
d4T **1**	--	0.25	0.15	50	56	4

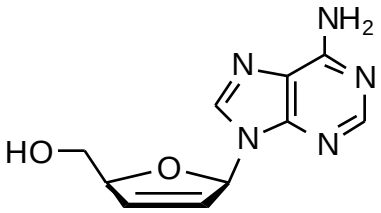
[a]50% effective concentration; [b]50% cytotoxic concentration; [c]selectivity index: ratio 50% cytotoxic concentration / 50% effective concentration

clearly shown that the "R_p"-configurated phosphate triesters forms about 10-fold higher d4TMP levels and also about five-fold higher d4TTP level as compared to the "S_p"-triester [49]. This result is consistent with the observed difference in the antiviral activity assay.

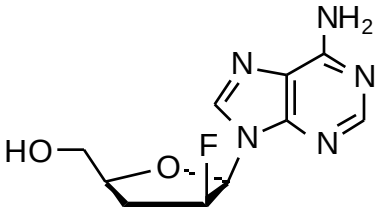
Further, the *cyclo*Sal-d4TMP triesters demonstrated significant antiviral activity in AZT-resistant $H9^rAZT^{250}$ cells. This resistant cell line was generated by continuous cultivation of H9 cells in the presence of increasing AZT concentrations. The resistance is concomitant to a five-fold lower expression of the TK gene in comparison to parental H9 cells. Thus, AZT showed an EC_{50} of >100 μM in this cell line. The EC_{50} in parental cells was found to be 0.04 μM. In addition, also the antiviral activity of d4T in $H9^rAZT^{250}$ cells is significantly lower than for the parental cell line (EC_{50}s of 26 μM and 0.9 μM, respectively). In contrast, *cyclo*Sal-d4TMP **11c** proved to be equipotent in parental and in $H9^rAZT^{250}$ cells (EC_{50} 0.3 μM and 0.5 μM) showing again the entire independence on the expressed TK levels inside the cells [52].

Finally, it should be added that *cyclo*Sal-phosphate triesters like **11** showed a marked increase in lipophilicity (log*P* [53,54]; Table 1) with respect to the parent nucleoside. This may point to a potentially higher passive diffusion through cellular membranes such as the blood-brain barrier. However, although we have determined considerable differences in the lipophilicity of the different *cyclo*Sal-d4TMPs, no correlation with the antiviral activity can be deduced.

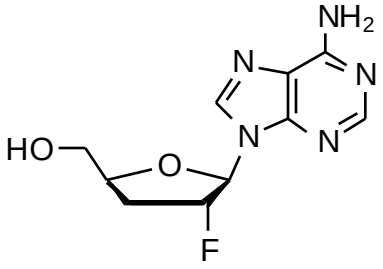
It should be mentioned that the strategy has also been applied to the delivery of the antiviral AZT [55,56] and the



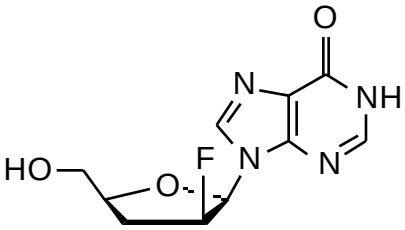
d4A **23**



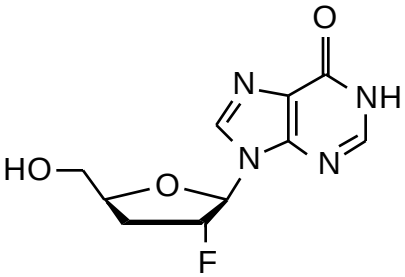
F-β-ddA **24**



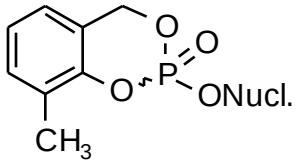
F-α-ddA **25**



F-β-ddI **26**



F-α-ddI **27**



28: Nucl. = ddA
29: Nucl. = d4A
30: Nucl. = F-β-ddA
31: Nucl. = F-β-ddI
32: Nucl. = F-α-ddA
33: Nucl. = F-α-ddI

Fig. (7). Formular of the different adenine nucleosides **23**-**27** and the general structure of 3-methyl-*cyclo*Sal-NMPs **28**-**33**.

antitumor active nucleoside analogue 5-fluoro-2'-deoxyuridine (FdU) [57]. In both examples antiviral and antitumor activity in thymidine kinase-deficient cells clearly showed the failure of the strategy. However, hydrolysis studies of the *cyclo*Sal-phosphate triesters revealed AZTMP and FdUMP as the only products. Studies using radiolabeled AZT triesters led to the conclusion that an unknown catabolic process is responsible for the efficient intracellular clearance of the liberated AZTMP to form AZT [58].

ADA-BYPASS WITH *CYCLO*SAL-ADENOSINE MONOPHOSPHATE DERIVATIVES

The *cyclo*Sal-strategy has further been applied successfully to different adenosine derivatives: ddA **2** [59-61] (Figure **1**), the unsaturated 2',3'-dideoxy-2',3'-didehydroadenosine (d4A) **23** [60] as well as 2',3'-dideoxy-2'-fluoro-β-adenosine (F-β-ddA) **24** [62,63] and 2',3'-dideoxy-2'-fluoro-α-adenosine (F-α-ddA) **25** [62,63]. Moreover, the 3-methyl-*cyclo*Sal-triesters (**31** and **33**, respectively) of the inosine derivatives F-β-ddI **26** and F-α-ddI **27** were prepared (Figure **7**).

Although the delivery mechanism of the corresponding nucleotides is identical, the biological task of the compounds is different: in contrast to the TK-bypass, the adenosine analogues were used for the ADA-bypass. The metabolic fate of ddA has been studied in detail [13-15] (Figure **2**) and most probably d4A and the fluorinated analogues follow the same metabolic pathway. Consequently, the intracellular delivery of ddAMP would bypass at least four enzymatic reaction steps starting with ADA deamination.

Chemical hydrolysis studies showed significantly higher half-lives as compared to the *cyclo*Sal-d4TMP triesters [38] and still a selective delivery of ddAMP and d4AMP from 3-methyl-*cyclo*Sal-ddAMP triester **28** and 3-methyl-*cyclo*Sal-d4AMP triester **29**, respectively [59,60]. An important aspect on the clinical use of ddA and d4A is their extreme acid sensitivity to acid catalyzed cleavage of the glycosidic bond [64]. Therefore, we studied the lability of triesters **28**,**29** at pH 3.0 and pH 1.0. To our surprise, upon introduction of the *cyclo*Sal-masking group the stability increased 10-fold as compared to the free nucleoside analogue for some unknown reasons. Moreover, we demonstrated that *cyclo*Sal-dd(4)AMP triesters were not substrates of ADA or adenosine monophosphate deaminase (AMPDA), while the nucleosides and nucleotides were efficiently deaminated by these enzymes [65].

*Cyclo*Sal-ddAMP **28** exhibited a 100-fold increase in antiviral activity in CEM/O cells (0.025μM) as compared to ddA **2** (4.3μM, Table 3) [59,60]. Moreover, *cyclo*Sal-d4AMP **29** proved to be 600-fold (0.05μM) more potent than d4A **23** (30μM). The selectivity index (SI) increased from 3 (d4A) to 746 for triester **29** (Table 3). Again, these results point to an efficient delivery of the nucleotide ddAMP as well as of d4AMP and so accomplishing successfully ADA-bypass (Figure **2**). Moreover, since the triesters were completely resistant to deamination, the biological activity could not be attributed to the delivery of ddIMP or d4IMP.

The only difference between both completely acid stable 2'-fluorinated ddA derivatives F-β-ddA **24** and F-α-ddA **25** [62] is the inverted configuration at carbon C2'. Although this may appear only a small difference at the first glance, it has tremendous impact on the biological activity of these ddA derivatives. F-β-ddA **24** is biologically active against both HIV-1 and HIV-2 in CEM/O cells while F-α-ddA **25** proved to be entirely inactive in the same cell line (Table 3) [66,67]. However, when the triphosphates of both F-ddA nucleosides **24**,**25** were tested for their potency against recombinant RT *both* triphosphates showed inhibitory activity with a difference of 3.3-fold (12μM and 40μM, respectively) [68]. This result suggested that the inability of the F-α-derivative to be metabolized was responsible for the reduced antiviral potency of the compound. The antiviral evaluation of 3-methyl-*cyclo*Sal-F-β-ddAMP triester **30** exhibited a 10-fold increase in bioactivity as compared to the parent nucleoside F-β-ddA **24** making this compounds as active as ddA **2** (Table 3). *Cyclo*Sal-F-β-ddIMP **31** was equipotent to *cyclo*Sal-F-β-ddAMP **30** proving that it was unimportant where entering the metabolic pathway. In contrast to the metabolisation of ddA **2**, the reamination seems not to be the limiting process (Figure **2**). This conclusion is supported by the identical potency of F-β-ddI **26** and F-β-ddA **24**. As a consequence, some limitations during the conversion into the monophosphate of F-β-ddA may be alleviated by the *cyclo*Sal approach, thus resulting in improved antiviral potency.

Table 3. Antiviral Data of 3-Methyl-*cyclo*Sal-adenosine/Hypoxantine Triester and the Parent Nucleosides

	EC_{50} (μM)[a]		CC_{50} (μM)[b]	SI[c]
	CEM HIV-1	CEM HIV-2		
28	0.047	0.03	28	585
ddA **2**	4.3	4.5	>250	>57
29	0.065	0.19	49	746
d4A **23**	30	50	96	3
30	3.67	3.3	146	44
F-β-ddA **24**	36.7	40.0	>250	>6.8
31	3.57	3.3	146	44
F-β-ddI **26**	41.7	30.0	>250	>8
32	11.7	12.5	118	10
F-α-ddA **25**	>250	>250	>250	n.a.[d]
33	75.0	31.7	>250	>8
F-α-ddI **27**	>250	>250	>250	n.a.[d]

[a]50% effective concentration; [b]50% cytotoxic concentration; [c]selectivity index; [d]not available

More striking were the results of 3-methyl-*cyclo*Sal-F-α-ddAMP **32** [62]. In contrast to the parent nucleoside, triester

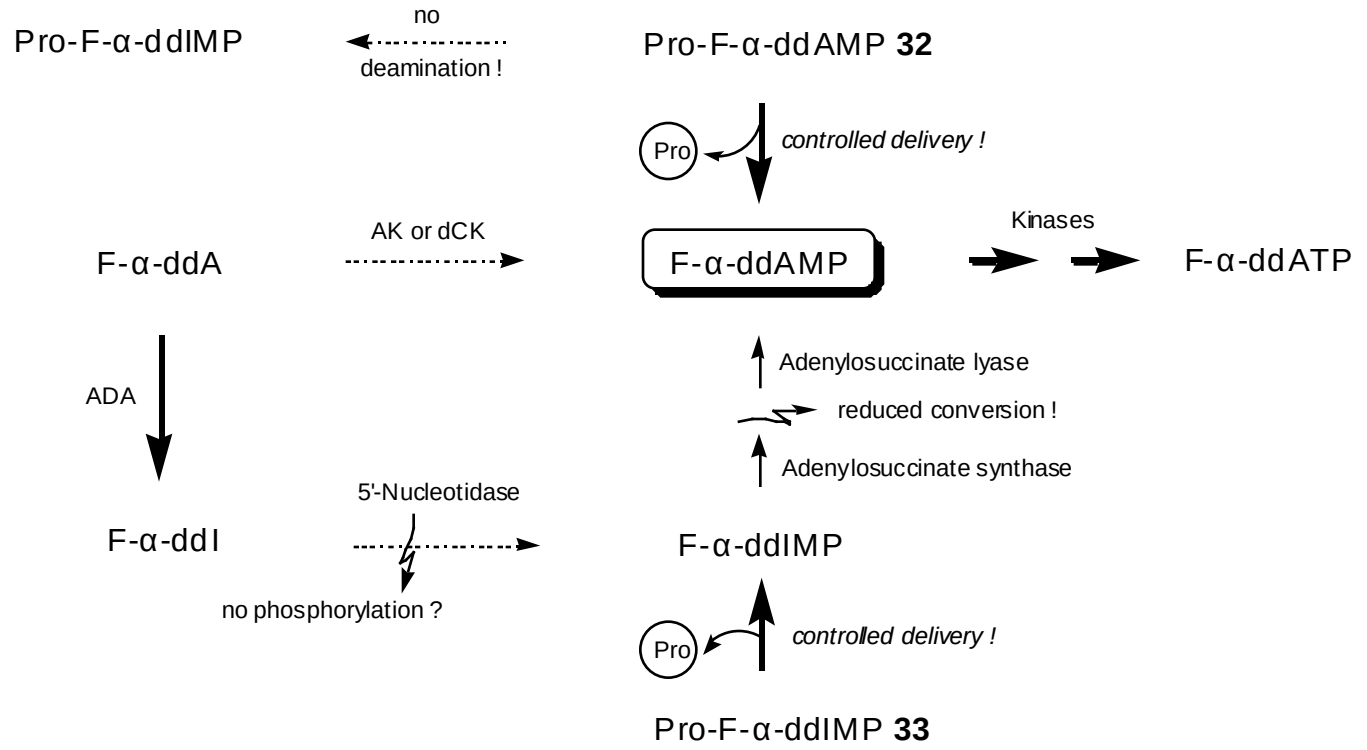


Fig. (8). Effect of *cyclo*Sal-F-α-ddAMP **32** and *cyclo*Sal-F-α-ddIMP **33**.

32 proved to be antivirally active at 12μM. This EC_{50} value was still 3-fold better than the value for F-β-ddA **24** (Table 3)! A 4-fold difference in antiviral potency was found between *cyclo*Sal-F-β-ddAMP **30** and *cyclo*Sal-F-α-ddAMP **32**, which is consistent with the difference found for inhibition of HIV-RT by the corresponding triphosphates [68]. Similar to F-α-ddA, F-α-ddI **27** proved to be completely inactive in the antiviral assay. However, 3-methyl-*cyclo*Sal-F-α-ddIMP **33** still showed some activity (32μM against HIV-2), but this EC_{50} value is three-fold higher as compared to that of *cyclo*Sal-F-α-ddAMP. This result is likely due to the limited conversion of F- α-ddIMP to F-α-ddAMP. (Figure **8**).

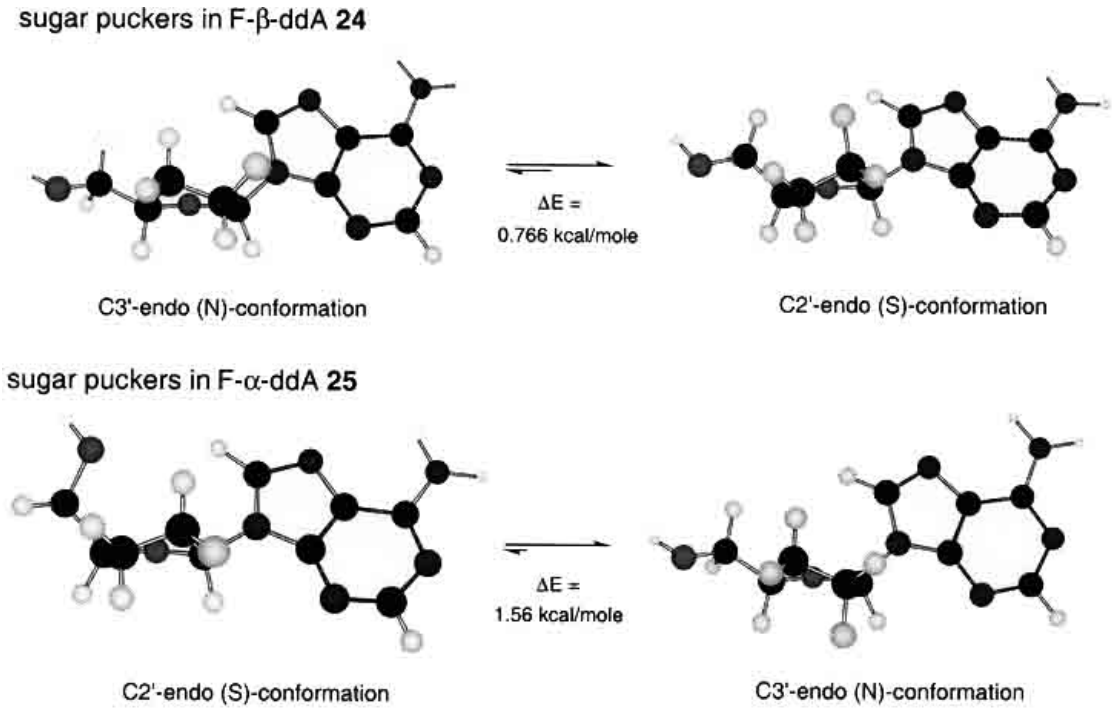


Fig. (9). Conformational preponderance of the fluorinated ddA glycon ring.

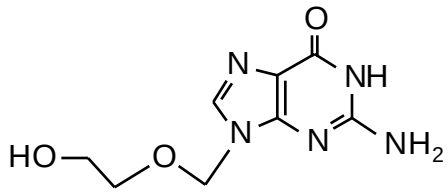
Summarizing, both nucleosides F-α-ddA and F-α-ddI have been converted into bioactive compounds by introduction of the *cyclo*Sal-masking group. Consequently, the metabolic blockade is located in the conversion of F-α-ddA **25** to F-α-ddIMP. In ADA-studies F-α-ddA proved to be the best substrate of all adenosine analogues discussed in this part and is rapidly deaminated to give F-α-ddI **27**. Thus, this can not be the limiting step and obviously the blockade is likely mono-phosphorylation of F-α-ddI (Figure **8**).

The reason for the different behavior of the two F-ddAs should be a specific intrinsic property of these nucleosides. Obviously, the different stereochemistry of the fluorine substituent is responsible for such striking biological differences because fluorine introduction at C2' is associated with a significant conformational effect on the glycon [69]. In solution, nucleoside analogues generally show a rapid equilibrium between two equally populated puckering conformations of the glycon: the 3'-endo-(North[N]) conformation and the 2'-endo-(South[S]) conformation. In contrast, a fluorine atom in a 2',3'-dideoxyribose residue leads to higher rigidity of the five-membered ring and forces the equilibrium to shift to either extreme (N- or S-form) as a function of the configuration. The origin of the fluorine-induced "stiffness" on the sugar pucker arises from a stereoelectronic effect resulting from the interaction between the ribose oxygen and the highly electronegative fluorine atom (*gauche*-effect). Due to this *gauche*-effect, the axially-oriented fluorine atom changes the sugar pucker into a preferred 3'-endo-conformation (N-form) for the F-α-configuration and a preponderant 2'-endo-conformation (S-form) in the case of the F-β-configuration [69]. This effect has been confirmed in molecular modeling calculations using the MM2 force field (Figure **9**).

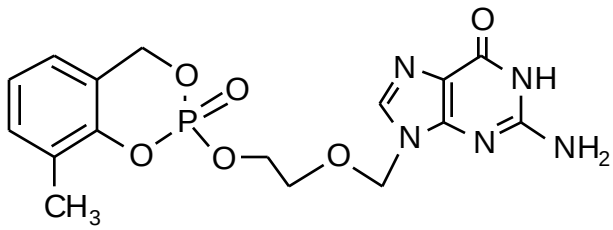
These results were further confirmed by NMR conformational analyses [70]. A phosphate group at the 5'-position has no influence on the conformation of the sugar pucker. The nucleoside monophosphates as well as the nucleoside triphosphates showed identical conformational behavior [68]. Therefore, one possible explanation for the metabolism blockade for F-α-ddA might be that the enzyme responsible for the phosphorylation of F-α-ddI to F-α-ddIMP does not accept the 3'-endo-conformation of the sugar pucker. The significance of the role of such conformational effects has been demonstrated for several enzymatic reactions before [68,71]. A preferred conformation in the glycon ring has also been observed for 2'-fluoro-2',3'-dideoxynucleoside-5'-triphosphates interacting with the HIV-1 RT active site [68]. Further studies have to be performed in order to verify this hypothesis. Nevertheless, this study validates the utility of the cycloSal-strategy for studying biosynthetic pathways utilized by antiviral nucleosides and nucleotides.

*cyclo*Sal-PRONUCLEOTIDES AGAINST DNA VIRUSES

DNA viruses are also attractive targets for the application of pronucleotides. In contrast to RNA viruses these viruses do not rely on reverse transcription of their genome prior to replication. Therefore, the target is not reverse transcriptase but a viral DNA-polymerase [72]. Moreover, some of the known antivirals against DNA viruses are not monophosphorylated by a cellular thymidine kinase but by viral-encoded thymidine kinases. These viruses have associated thymidylate kinase activity as in the cases of herpes simplex virus-type-1 (HSV-1) and varicella zoster virus (VZV) thus leading directly to specific activation of the nucleoside analogue to the corresponding diphosphates in infected. This has a tremendous advantage for the selectivity of these compounds against viruses but the disadvantage of not being activated if a cellular or virus-encoded TK is not present [73]. Moreover, not all of the virus-types belonging to the herpes virus family express thymidine kinase activity, e.g. Epstein-Barr-virus (EBV) and cytomegalovirus (CMV). Among the most active and most broadly applicable nucleoside antiviral in this area is the purine bearing acyclic nucleoside analogue acyclovir (ACV **34**, Figure **10**) [75]. ACV is a potent inhibitor of herpes virus type-1 (HSV-1), herpes virus type-2 (HSV-2), and to a lesser extent varicella-zoster virus (VZV), CMV and EBV. Acyclovir triphosphate (ACVTP) acts as a chain terminator and/or as AN inhibitor of the HSV DNA polymerase. However, ACVTP has a relatively short intracellular half-life of 0.7 hours. In addition, resistance to acyclovir has been associated with the selection of mutants deficient in TK activity or in mutants that express TKs with altered substrate specificity.



Acyclovir **34** (ACV)



3-Me-*cyclo*Sal-ACVMP **35**

Fig. (10). Formula of Acyclovir (ACV) **34** and the 3-methyl-*cyclo*Sal-ACVMP Triester **35**.

The chemical synthesis of 3-methyl-*cyclo*Sal-ACVMP triester **35** may be carried out using the chlorophosphite method [76], but superior yields results were obtained when the exocyclic amino group of the guanine residue was protected by dimethoxytritylation prior to the phosphitylation reaction using the phosphoramidite strategy [43]. The protecting group was removed by acid treatment of the N^2-blocked *cyclo*Sal-triester. *Cyclo*Sal-ACVMP triester **35** was obtained as a racemic mixture. Chemical hydrolysis showed again selective delivery of ACVMP.

Table 4. Antiviral Data of 3-Methyl-*cyclo*Sal-ACVMP 35 and ACV 34

	EC_{50} (μM)[a]							
	HSV-1/TK$^+$	HSV-1/TK$^-$	VZV/TK$^+$		VZV/TK$^-$		CMV	
	Kupka[b]	B2006[b]	YS[b]	OKA[b]	07/1[c]	YS/R[c]	AD-169[c]	Davis[c]
*cyclo*Sal-triester **35**	0.47	0.51	4.1	1.2	7.9	7.6	13	9
ACV **34**	0.62	57.7	5.2	2.6	111	191	>200	>200

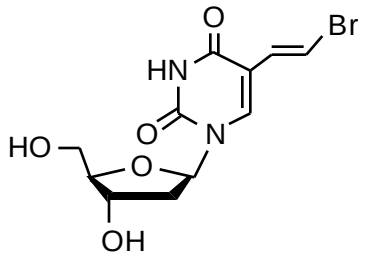
[a]Effective concentration; [b]in Vero cells; [c]in human embryonic lung (HEL) cells

Antiviral evaluation showed an EC_{50} OF 0.62μM for the parent nucleoside ACV **34** against HSV-1/TK$^+$ in Vero cells. As expected, ACV lost its activity in Vero cells infected with mutant HSV-1/TK$^-$ (EC_{50} 58μM). Strikingly, 3-methyl-*cyclo*Sal-ACVMP **35** showed identical antiviral activity values of 0.47μM and 0.51μM in the same systems, respectively (Table 4), and an EC_{90} of 1.62μM against the mutant virus strain without increasing toxicity. Again, this complete retention of activity clearly proves that ACVMP is delivered to the cells by the pronucleotide [76].

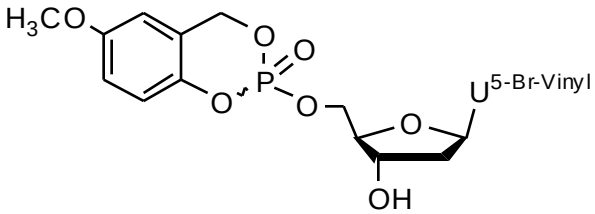
A comparable result was obtained for the antiviral activity against VZV [77]. *Cyclo*Sal-triester **35** showed antiviral activity of EC_{50} 4.1μM and 1.2μM against two wild-type virus strains (plaque reduction assay). This activity was completely retained in cells infected with VZV/TK$^-$ (7.6μM; Table 4). In contrast, ACV showed activity against the wild-type viruses but proved to be inactive in the mutant virus strain.

The situation was somewhat different when phosphate triester **35** was tested against CMV [77]. While ACV itself was completely inactive against two virus strains, the triester exhibited antiviral activity against *both* virus strains at EC_{50} 13μM and 9μM, respectively, without changing the cell morphology (MCC >200μM, Table 4). Additionally, *cyclo*Sal-triester **35** proved to be a potent and selective inhibitor of EBV DNA synthesis and EB-virus capsid antigen expression [78].

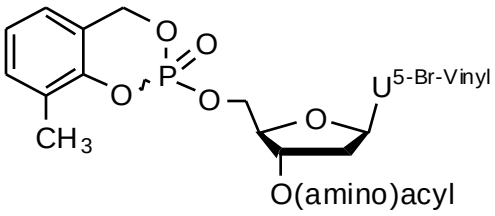
Finally, the *cyclo*Sal-strategy has been applied to the nucleoside analogue 5-[(*E*)-2-bromovinyl]-2'-deoxyuridine (BVDU or Brivudin **36**; Figure **11**) [79,80] which is a potent and highly selective inhibitor of the replication of HSV-1 and particularly VZV [79]. Interestingly, BVDU is not active against HSV-2 and EBV due to a missing HSV-1-like viral thymidine-kinase (TK). BVDU triphosphate (BVDUTP) can act either as an inhibitor of the cellular DNA polymerase or alternate substrate that after incorporation would render the DNA more prone to degradation [81]. Some limitations for the use of BVDU are known. First, there is a lack of activity during virus latency. Second, enzymatic degradation to the nucleobase 5-[(*E*)-2-bromovinyl]uracil has been observed in the bloodstream [81]. Third, drug resistant virus strains are known. Our aim was to determine the applicability of the *cyclo*Sal-strategy to the delivery of BVDUMP by examining the anti-EBV effect of BVDU. Therefore, 5-methoxy-*cyclo*Sal-BVDUMP **37** and a series of 3'-*O*-modified derivatives **38** and **39** were synthesized [83,84], bearing different lipophilic carboxylic acids (**38**) as well as α-amino acids (**39**) (Figure **11**). Originally, 3'-esterified triesters **38** were prepared in order to circumvent a possible participation of the 3'-hydroxyl group in the hydrolysis process. However, the lipophilicity was increased considerably. Therefore, α-amino acids were used because they may prevent the participation of the 3'-hydroxyl group but at the same time compensate the high lipophilicity due to the polar protonated amino group.



BVDU **36** (Brivudin)



5-methoxy-*cyclo*Sal-BVDUMP **37**



38a-c (acyl = acetyl, propanoyl, hexanoyl)
39a,b (aminoacyl = alaninyl, phenylalaninyl)

Fig. (11). Formula of Brivudin (BVDU) **36** and its *cyclo*Sal-BVDUMP triesters **37**-**39**.

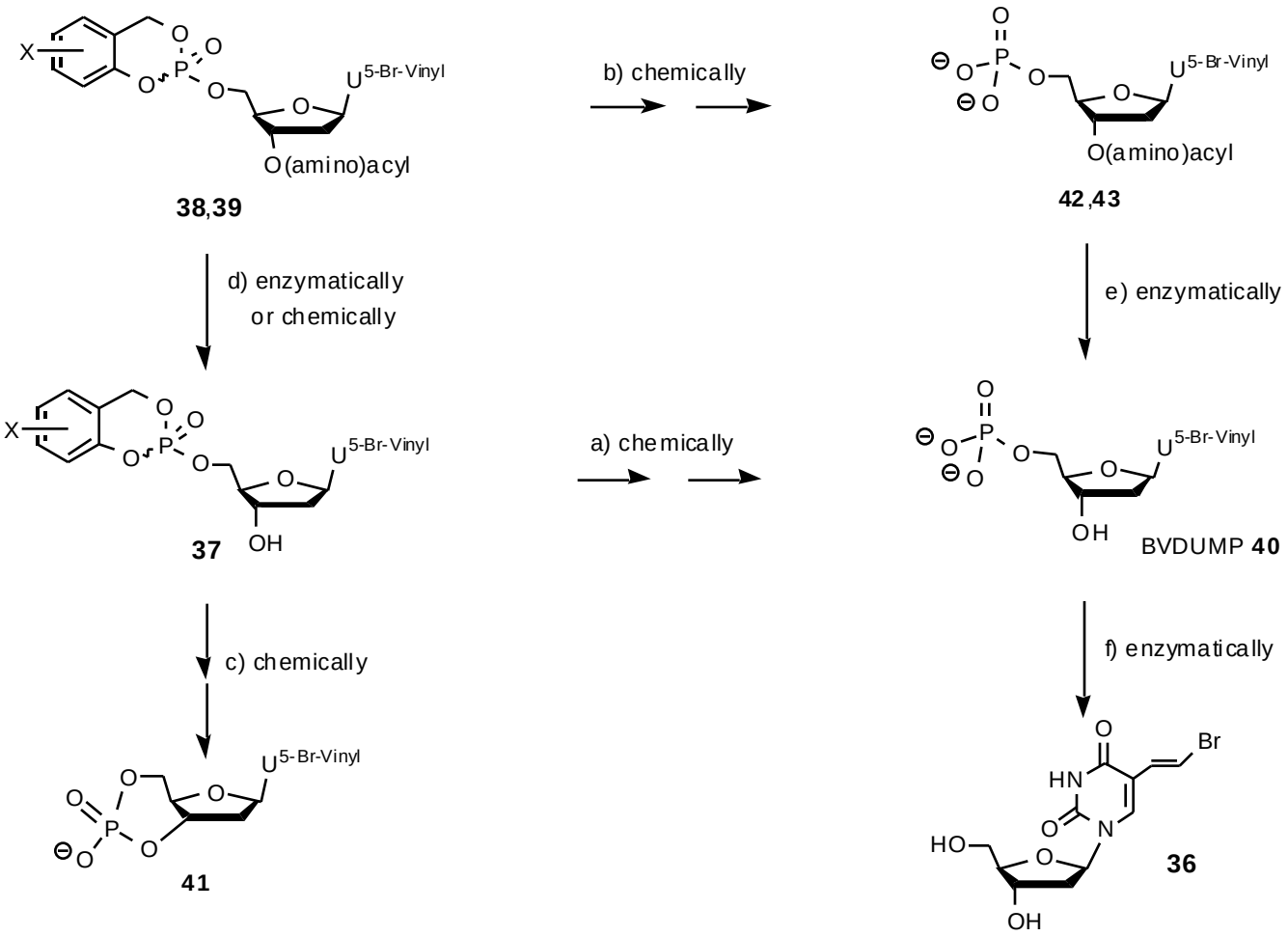


Fig. (12). Degradation pathways of the *cyclo*Sal-BVDUPs **37**-**39**.

First, hydrolysis studies demonstrated that BVDUMP **40** was the sole product. Formation of 3',5'-cyclicBVDUMP **41** (Figure **12**) was not observed. *Cyclo*Sal-BVDUMP triesters 3'-*O*-modified by a carboxylic acid (**38**) lead to the formation of the 3'-esterified BVDUMP **42** with a comparable half-live to that for 3'-unmodified derivative **37**. In contrast, the 3'-aminoacyl-esterified compounds **39** yielded BVDUMP **40** and not the corresponding 3'-aminoacyl-esterified BVDUMP **43**.

Further studies in P3HR1 cell extracts confirmed the results in aqueous buffers and showed a much higher formation of BVDUMP from triester **37** as well as the aminoacid-modified triesters, **38** and **39**, as compared to the carboxylic acid-modified derivatives [83]. Consequently, a substantial increase in the concentration of BVDUMP and thus BVDUTP should be the result in cells.

Inhibition assays of EBV replication in P3HR1 cells confirmed the complete inactivity of BVDU (EC_{50} >300μM) [83,84]. Strikingly, 5-methoxy-*cyclo*Sal-BVDUMP **37**, the D-alanine (**39a**) and D-phenylalanine modified *cyclo*Sal-BVDUMP triesters **39b** exhibited pronounced anti-EBV activity. Triester **37** was even 4-fold potent acyclovir **34**. In contrast, all derivatives bearing 3'-*O*-carboxylic acids **38** were found to be devoid of any antiviral activity (Table 5). Nevertheless, the promising antiviral data of some of the *cyclo*Sal-BVDUMP triesters proved that the *cyclo*Sal strategy is able to convert the inactive BVDU into an anti-EBV agent.

Table 5. Hydrolysis Data and Antiviral Data of *cyclo*Sal-BVDU 36

	Hydrolysis $t_{1/2}$(h) and product[a]	**EC_{50} (μM)**[b]	**CC_{50} (μM)**[b]
37	2.3 **40**	1.8	137
38a	5.8 **42a**	>85	110
38b	6.3 **42b**	>150	>300
38c	8.1 **42c**	>150	>300
D-**39a**	1.39 **40**	9.5	83
L-**39a**	1.40 **40**	22	140
D-**39b**	1.68 **40**	7.6	66
L-**39b**	1.18 **40**	>100	78
ACV **34**	--	7.2	422
BVDU **36**	--	>300	225

[a]in phosphate buffer, pH 7.3; [b]EC_{50}: concentration required to reduce EBV DNA synthesis by 50%; [c]CC_{50}: concentration required to reduce the growth of exponentially growing of exponentially growing P3HR-1 cells by 50%.

These results suggest that the reason for the inactivity of BVDU against EBV is probably an inefficient intracellular formation of BVDUMP.

CONCLUSION

Summarizing, the *cyclo*Sal approach demonstrated convincingly the intracellular delivery of antiviral active nucleotides by a non-enzymatically induced cascade reaction. It has considerably improved the antiviral activity of certain nucleoside analogues. One advantage of the *cyclo*Sal strategy compared to other pronucleotide systems is the uncomplicated synthetic excess and the reasonable solubility of the compounds in aqueous media. Moreover, the drug/masking group ratio of the *cyclo*Sal strategy is 1:1 whereas in almost all enzymatically triggered nucleotide delivery systems the ratio is 1:2 or 1:4. The 1:1 ratio may be favorable in terms of reducing potential toxicity. Additionally, we believe that a pronucleotide that can be cleaved by pH-control should have advantages in *in vivo* applications because this approach is independent of potential tissue variability in the expression of the necessary activating enzymes. Moreover, it has been shown that the *cyclo*Sal-pronucleotide system is an ideal biochemical tool to study biochemical pathways in nucleoside metabolism.

ACKNOWLEDGEMENT

This project has been carried out in collaboration with several groups. I am very grateful to Jan Balzarini and Erik De Clercq, Rega Institute, Leuven, Belgium, Peter Wutzler and Astrid Meerbach, University of Jena, Jena, Germany, Victor Marquez, NCI, NIH, Ft. Frederick, USA. Financial support by the Deutsche Forschungsgemeinschaft, Bonn, Germany, the Fonds der Chemischen Industrie, Frankfurt/Main, Germany and the Adolf-Messer-Stiftung, Frankfurt/Main, Germany is gratefully acknowledged.

REFERENCES

[1] Mitsuya, H.; Weinhold, K.J.; Furman, P.A.; St. Clair, M.H.; Nusinoff-Lehrman, S.; Gallo, R.C.; Bolognesi, D.; Barry, D.W.; Broder, S. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7096.

[2] Balzarini, J. *Pharm. World Sci.* **1994**, *16*, 113.

[3] Herdewijn, P.; Balzarini, J.; De Clercq, E. In: *Advances in Antiviral Drug Design*. (Ed. De Clercq, E.), **1993**, *Vol. 1*, 233.

[4] Furman, P.A.; Fyfe, J.A.; St. Clair, M.H.; Weinhold, K.; Rideout, J.L.; Freeman, G.A.; Lehrmann, S.N.; Bolognesi, D.P.; Broder, S. *Proc. Natl. Acad. Sci., USA* **1986**, *83*, 8333.

[5] Hao, Z.; Cooney, D.A.; Hartman, N.R.; Perno, C.-F.; Fridland, A.; DeVico, A.L.; Sarngadharan, M.G.; Broder, S.; Johns, D.G. *Mol. Pharmacol.* **1988**, *34*, 431.

[6] Riddler, S.A.; Anderson, R.E.; Mellors, J.W. *Antiviral Res.* **1995**, *27*, 189.

[7] Hitchcock, M.J.M. *Antiviral Chem. Chemother.* **1991**, *2*, 125.

[8] Balzarini, J.; Herdewijn, P.; De Clercq, E. *J. Biol. Chem.* **1989**, *264*, 6127.

[9] Sommadossi, J.-P. *J. Infect. Dis.* **1995**, *171*, S88.

[10] Hao, Z.; Cooney, D.A.; Farquhar, D.; Perno, C.-F.; Zhang, K.; Masood, R.; Wilson, Y.; Hartman, N.R.; Balzarini, J.; Johns, D.G. *Mol. Pharmacol.* **1990**, *37*, 157.

[11] Johnson, M.A.; Ahluwalia, G.; Conelly, M.C.; Cooney, D.A.; Broder, S.; Johns, D.G.; Fridland, A. *J. Biol. Chem.* **1988**, *263*, 15354.

[12] McLaren, C.; Datema, R.; Knupp, C.A.; Buroker, R. A. *Antiviral Chem. Chemother.* **1991**, *2*, 321.

[13] Johnson, M.A.; Fridland, A. *Mol. Pharmacol.* **1989**, *36*, 291.

[14] Gao, W.-Y.; Agbaria, R.; Driscoll, J.S.; Mitsuya, H. *J. Biol. Chem.* **1994**, *269*, 12633.

[15] Ahluwalia, G.; Cooney, D.A.; Mitsuya, H.; Fridland, A.; Flora, K.P.; Hao, Z.; Dalal, M.; Broder, S.; Johns, D.G. *Biochem. Pharmacol.* **1987**, *36*, 3797.

[16] C.R. Wagner, V.V. Iyer, E.J. McIntee *Med. Res. Rev*. **2000**, *20*, 417.

[17] Meier, C. *Synlett* **1998**, 233.

[18] Périgaud, C.; Gosselin, G.; Imbach, J.-L. In: *Biomedical Chemistry/Applying Chemical Principles to the Understanding and Treatment of Disease*; Torrence, P.F. (Ed.); John Wiley & Sons Inc., New York, **2000**; Vol. *55*, 115.

[19] (a) Bunton, C.A.; Mhala, M.M.; Oldham, K.G.; Vernon, C.A. *J. Chem. Soc.* **1960**, 3293. (b) Kirby, A.J.; Younas, M. *J. Chem. Soc. (B)* **1970**, 510.

[20] Carl, P.L.; Chakravarty, P.K.; Katzenellenbogen, J.A. *J. Med. Chem.* **1981**, *24*, 479.

[21] Farquhar, D.; Srivastava, D.N.; Kattesch, N.J.; Saunders, P.P. *J. Pharm. Sci.* **1983**, *72*, 324.

[22] Arimilli, M.N.; Dougherty, J.; K.C. Cundy; N. Bischofberger in: *Advances in antiviral Drug Design*, E. De Clercq (Ed.); JAI Press, Inc. Stamford, Connecticut, **1999**, Vol. *3*, 69.

[23] Périgaud, C.; Gosselin, G.; Imbach, J.-L. *Curr. Topics in Med. Chem.* **1997**, *2*, 15.

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

[25] Schlienger, N.; Périgaud, C.; Gosselin, G.; Imbach, J.-L. *J. Org. Chem.* **1997**, *62*, 7216.

[26] (a) Briggs, A.D.; Camplo, M.; Freeman, S.; Lundström, J.; Pring, B.G. *Tetrahedron*, **1996**, *47*, 14937. (b) Glazier, A.; Buckheit, R.; Yanachkova, M.; Yanachkov, I.; Wright, G.E. *Antiviral Res.* **1994**, *19*, (Suppl. 1), 57

[27] Saboulard, D.; Naesens, L.; Cahard, D.; Salgado, A.; Pathirana, R.; Velazquez, S.; McGuigan, C.; De Clercq, E.; Balzarini, J. *Mol. Pharmacol.* **1999**, *56*, 693.

[28] Chang, S.-L.; Griesgraber, G.W.; Southern, P.J.; Wagner, C.R. *J. Med. Chem.* **2001**, *44*, 223.

[29] Deferme, S.M.; Villard, A.L.; Egron, D.; Gosselin, G.; Imbach, J.-L.; Lioux, T.; Pompon, A.; Varray, S.; Aubertin, A.M.; Mooter, G.V.; Kinget, R.; Périgaud, C.; Augustijns, P. *J. Pharm. Sci*. **2001**, *90*, 448.

[30] The systematic name is 2-Nucleos-5'-O-yl-4H-1,3,2-benzodioxaphosphorinin-2-oxides. The abbreviation *cyclo*Sal-NMP has been introduced as a simplification.

[31] Meier, C. *Angew. Chem.* **1996**, *108*, 77; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 70

[32] Farrow, S.N.; Jones, A.S.; Kumar, A.; Walker, R.T.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1990**, *33*, 1400.

[33] Shimizu, S.I.; Balzarini, J.; De Clercq, E.; Walker, R.T. *Nucleosides & Nucleotides* **1992**, *11*, 583.

[34] Meier, C.; Habel, L.W.; Balzarini, J.; De Clercq, E. *Liebigs Annalen der Chemie* **1995**, 2203.

[35] Valette, G.; Pompon, A.; Girardet, J.-L.; Cappellacci, L.; Franchetti, P.; Grifantini, M.; La Colla, P.; Loi, A.G.; Périgaud, C.; Gosselin, G.; Imbach, J.-L. *J. Med. Chem.* **1996**, *39*, 1981.

[36] Meier, C.; Lorey, M.; De Clercq, E.; Balzarini, J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 99

[37] Meier, C.; Lorey, M.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **1998**, *41*, 1417.

[38] (a) Westheimer, F.H. *Acc. Chem. Res.* **1968**, *1*, 70. (b) Buchwald, S.L. Pliura, D.H.; Knowles, J.R. *J. Am. Chem. Soc.* **1984**, *106*, 4916.

[39] Balzarini, J. **2000**, unpublished results.

[40] Chen, G.; Fournier, R.L.; Varanasi, S. *Enzyme Microb. Technol*. **1997**, *21*, 491.

[41] Steinegger, E.; Hoevel, H. *Pharm. Acta Helv*. **1972**, *47*, 222.

[42] Mugnier, F.; Meier, C. *Nucleosides & Nucleotides*, **1999**, *18*, 941.

[43] Meier, C. **2000**, unpublished results.

[44] Casiraghi, G.; Casnati, G.; Puglia, G.; Satori, G.; Terenghi, G. *J. Chem. Soc., Perkin Trans. I* **1980**, 1862.

[45] Gross, H.; Rieche, H.; Matthey, G. *Chem. Ber.* **1963**, *96*, 308.

[46] Nagata, W.; Okada, K.; Aoki, T. *Synthesis* **1979**, 365.

[47] Naesens, L.; Meier, C. **1998**, unpublished results.

[48] Balzarini, J.; **2001**, unpublished results.

[49] Balzarini, J.; Aquaro, S.; Knispel, T.; Rampazzo, C.; Bianchi, V.; Perno, C.-F.; De Clercq, E.; Meier, C. *Mol. Pharmacol.* **2000**, *58*, 928.

[50] Balzarini, J.; Knispel, T.; Naesens, L.; De Clercq, E.; Meier, C. *Antiviral Res.* **2000**, *46*, A46, 36.

[51] Laux, W.; Meier, C. **1996**, unpublished results.

[52] Gröschel, B.; Meier, C.; Zehner, R.; Cinatl, J.; Doerr, H.W.; Cinatl Jr., J. *Nucleosides & Nucleotides* **1999**, *18*, 933.

[53] Lien, E.J.; Gao, H.; Prabharkar, H. *J. Pharm. Sci.* **1991**, *80*, 517.

[54] Zimmermann, T.P.; Mahony, W.B.; Prus, K.L. *J. Biol. Chem.* **1987**, *262*, 5748.

[55] Meier, C.; De Clercq, E.; Balzarini, J. *Nucleosides & Nucleotides* **1997**, *16*, 793.

[56] Meier, C.; De Clercq, E.; Balzarini, J. *Europ. J. Org. Chem.* **1998**, 837.

[57] Lorey, M.; Meier, C.; De Clercq, E.; Balzarini, J. *Nucleosides & Nucleotides* **1997**, *16*, 789; Lorey, M.; Meier, C.; De Clercq, E.; Balzarini, J. *Nucleosides & Nucleotides* **1997**, *16*, 1307.

[58] Balzarini, J.; Naesens, L.; Aquaro, S.; Knispel, T.; Perno, C.-F.; De Clercq, E.; Meier, C. *Mol. Pharmacol.* **1999**, *56*, 1354.

[59] Meier, C.; Knispel, T.; De Clercq, E.; Balzarini, J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1577.

[60] Meier, C.; Knispel, T.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **1999**, *42*, 1604.

[61] Knispel, T.; Meier, C. *Nucleosides & Nucleotides* **1999**, *18*, 945.

[62] Meier, C.; Knispel, T.; Marquez, V.E., De Clercq, E.; Balzarini, J. *Nucleosides & Nucleotides* **1999**, *18*, 907; Meier, C.; Knispel, T.; Marquez, V.E.; Siddiqui, M.A.; De Clercq, E.; Balzarini, *J. Med. Chem.* **1999**, *42*, 1615.

[63] Different names for the nucleoside analogues **24** and **25** could be found. Terms as F-up- and F-down-ddA, F-*ara*- and F-*ribo*-ddA, as well as 2',3'-dideoxy-2'-fluoro-arabinosyladenine and 2',3'-dideoxy-2'-fluororibosyl-adenine have been used. Here we use the abbreviation F-β- and F-α-ddA. The correct names are 9-(2',3'-dideoxy-2'-fluoro-β-D-*threo*-pentofuranosyl)adenine (**24**) and 9-(2',3'-dideoxy-2'-fluoro-β-D-*erythro*-pentofurano-syl)adenine (**25**)

[64] York, J.L. *J. Org. Chem.* **1981**, *46*, 2171.

[65] Bloch, A.; Robins, M.J.; McCarthy, Jr., J.R. *J. Med Chem.* **1967**, *10*, 908.

[66] Marquez, V.E.; Tseng, C.K.-H.; Mitsuya, H.; Aoki, S.; Kelley, J.A.; Ford, Jr., H.; Roth, J.S.; Broder, S.; Johns, D.G.; Driscoll, J.S. *J. Med. Chem.* **1990**, *33*, 978.

[67] Herdewijn, P.; Pauwels, R.; Baba, M.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1987**, *30*, 2132.

[68] Mu, L.; Sarafianos, G.; Nicklaus, M.C.; Russ, P.; Siddiqui, M.A.; Ford, Jr.; H.; Mitsuya, H.; Le, R.; Kodama, E.; Meier, C.; Knispel, T.; Anderson, L.; Barchi, Jr., J.J.; Marquez, V.E. *Biochemistry* **2000**, *39*, 11205.

[69] Marquez, V.E., Lim, B.B., Barchi Jr., J.J., Nicklaus M.C. in: *Nucleosides and Nucleotides as Antitumor and Antiviral Agents* (Ed. Chu, C.K., Baker, D.C.), Plenum Press, New York, **1993**, 265.

[70] Two crystal structures have been published: Liaw, V.-C.; Gao, Y.-G.; Marquez, V.E.; Wang, A.H.-J. *Nucleic Acids Res.* **1992**, *20*, 459; NMR studies: Bergstrom, D.; Swartling, D.J. in: *Fluorine containing Molecules* (Ed. Liebman, J.F.; Greenberg, A.; Dolbier Jr., W.R., Chapter 11), VCH Publishers Inc. **1988**, 259 and cited lit.; Thibaudeau, C.; Plavec, J.; Chattopadhyaya, J. *J. Org. Chem.* **1998**, *63*, 4967.

[71] Ford, H. Jr.; Dai, F.; Mu, L.; Siddiqui, M.A.; Nicklaus, M.C.; Anderson, L.; Marquez, V.E.; Barchi, J.J. Jr. *Biochemistry* **2000**, *39*, 2581.

[72] De Clercq, E. *J. Antimicrob. Chemother.* **1993**, *32* (Suppl. A), 121.

[73] De Clercq, E. *Biochemical Pharmacol.* **1984**, *33*, 2159.

[74] Fields, B.N. *Virology*, (Vol. *1*) 2nd edition, Raven Press, New York, **1990**, 450.

[75] Field, H.J.; Goldthorpe, S.E. in: *Antiviral Chemother.* (Eds. Jeffries, D.J., De Clercq, E.) John Wiley & Sons, New York, **1995**, 127.

[76] Meier, C.; Habel, L.; Haller-Meier, F.; Lomp, A.; Herderich, M.; Klöcking, R.; Meerbach, A.; Wutzler, P. *Antiviral Chem. Chemother.* **1998**, *9*, 389.

[77] De Clercq, E.; Meier, C. **1998**, unpublished results.

[78] Meerbach, A.; Klöcking, R.; Meier, C.; Lomp, A.; Helbig, B.; Wutzler, P. *Antiviral Research* **2000**, *45*, 69.

[79] De Clercq, E.; Descamps, J.; De Somer, P.; Barr, P. J.; Jones, A.S.; Walker, R.T. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 2947.

[80] Wutzler, P. *Intervirology* **1997**, *40*, 343.

[81] De Clercq, E. *Clinical Microbiol. Rev.* **1997**, *10*, 674.

[82] Anagnostopoulos, I.; Hummel, M. *Histo-Pathol.* **1996**, *29*, 297.

[83] Meier, C.; Lomp, A.; Meerbach, A.; Wutzler, P. *Chem.Bio.Chem.* **2001**, *2*, 283.

[84] Meier, C.; Lomp, A.; Meerbach, A.; Wutzler, P. *Nucleosides, Nucleotides & Nucleic Acids* **2001**, *20*, 4.