"Lock-in"-*cyclo*Sal-Pronucleotides – A New Generation of Chemical Trojan Horses?

Chris Meier*[a], Manuel F.H. Ruppel[a], Dalibor Vukadinovic[a] and Jan Balzarini[b]

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

[b]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

Abstract: The *cyclo*Sal-concept is one example of a successful nucleotide delivering system (pronucleotide). For several nucleoside analogues, the *cyclo*Sal-approach improved the antiviral potency and the applicability of the nucleosides could be broadened. Here, a conceptional extension of the original design of the *cyclo*Sal-system will be discussed.

Dedicated to Prof. Gernot Boche on the Occasion of his 65th Birthday

*CYCLO*SALIGENYL-NUCLEOTIDES (*CYCLO*SAL-NMPS) - DESIGN OF THE ORIGINAL CONCEPT

In contrast to other enzyme-triggered concepts, we have demonstrated in the past that nucleotides can be successfully delivered intracellularly by a pH-driven process. This so-called, *cyclo*Sal-pronucleotide approach belongs to the group of tripartate prodrug systems and has been developed in our laboratories. The general idea will be summarized briefly and, moreover, a conceptional extension will be discussed in this review.

In contrast to other approaches that are also presented in this hot topic issue, the aim was the development of a highly selective delivery mechanism based on a pH-dependent chemical reaction involving a highly selective, coupled, multi-step mechanism (cascade mechanism) [1]. However, the chemically driven release of a nucleotide from a lipophilic precursor is not as easy as it seems. Studies using 2',3'-dideoxy-2',3'-didehydrothymidine (d4T) **1** as the nucleoside in our and other laboratories showed that di-phenyl- (**2**) [2,3], as well as di-benzyl phosphate triesters, (**3**) [4,5] hydrolyzed selectively to yield the expected phenyl- (**4**) or benzyl phosphate diesters (**5**), respectively (Figure 1).

In both cases, there was *no* further hydrolysis of d4TMP **6** was observed due to the formed negative charge because this prevents a second nucleophilic attack on the P-atom. Nevertheless, studies using di-phenyl phosphate triesters **2** proved the expected dependence of the hydrolytic stability on the substitution pattern in the aromatic ring: the more electron-*withdrawing* the substituent was, the more labile was the phosphate triester [2,3]. The situation was completely inverse for di-benzyl phosphate triesters **3**: the more electron-*donating* the substituent was, the more labile was the compound [4]. Additionally, the mechanisms of hydrolysis were entirely different. P-O_{phenyl} bond cleavage yielding phosphate diester **4** and a phenolate anion was observed for di-phenyl phosphate triesters (S_NP reaction). By contrast, a spontaneous C_{benzyl}-O bond cleavage leading to benzyl phosphate diester **5** and a benzyl cation, which is subsequently quenched by water to give a benzyl alcohol **7**, took place for di-benzyl phosphate triesters **3** [5, 6] (S_N1-type reaction). Both reactions are quite selective, but stopped at the phosphate diester level. Even enzymatic degradation of the intermediate diesters proved to be problematic [7].

The idea was to combine the different hydrolysis properties of phenyl and benzyl phosphate triesters and the phenyl- and the benzyl ester should be part of a cyclic bifunctional group (masking unit). Thus, the idea of the *cyclo*Saligenyl- (*cyclo*Sal-) pronucleotides was born. So, three different types of ester bonds were introduced into the phosphate group: a phenyl- and a benzyl-ester to the mask and an alkyl phosphate ester resulting from the nucleoside attachment. The introduction of these three ester bonds allowed a sufficient discrimination between the different phosphate ester bonds. The alkyl ester is the most stable ester bond so that only the phenyl and the benzyl ester play a role for the delivery mechanism. Furthermore, because of the bifunctional character of the masking unit, a cleavage process could be achieved that – after a first reaction involving the phosphorus atom - takes place within the masking group only. The advantage is that avoidance of a possible pseudorotation process [8] may also lead to the liberation of the nucleoside instead of the nucleotide. Finally, this chemically induced coupled process (tandem or cascade mechanism) has been first applied to the delivery of the nucleotide d4TMP **6** (*cyclo*Sal-d4TMP **8**; Figure 2) [9, 10].

The most labile ester bond is cleaved first leading to the 2-hydroxy*benzyl*phosphate diester **9** due to resonance stabilization of the negative charge of the phenolate by the aromatic ring (Figure 3, step a). The alternative cleavage of the benzyl ester to yield the 2-hydroxymethyl*phenyl*phosphate diester **10** is unfavorable (step c). As a consequence, the substituent adjacent to the benzyl group is switched from a very weak electron-donor group (phosphate) to a very strong electron-donor group (hydroxyl) with the result of an intrinsical activation of the remaining masking group. A

*Address correspondence to this author at the Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6; D-20146 Hamburg, Germany; Tel.: +49-40-42838-4324; Fax: +49-40-42838-2495; E-mail: chris.meier@chemie.uni-hamburg.de

1389-5575/04 $45.00+.00 © 2004 Bentham Science Publishers Ltd.

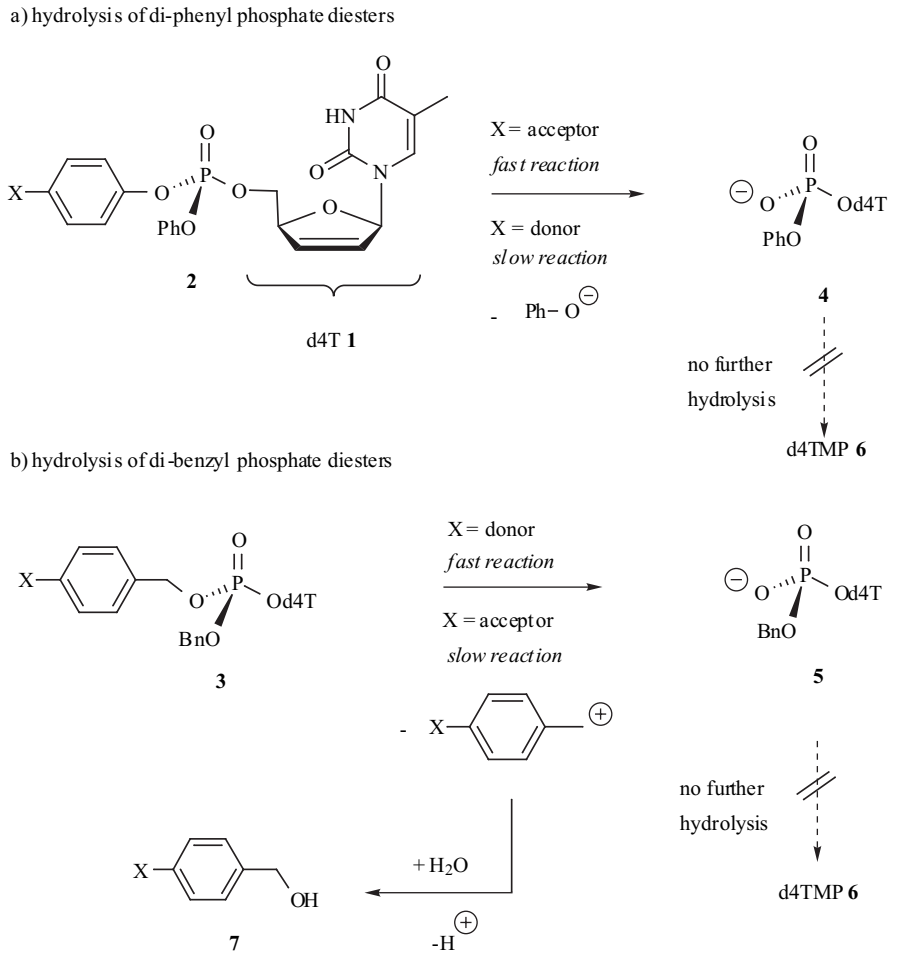
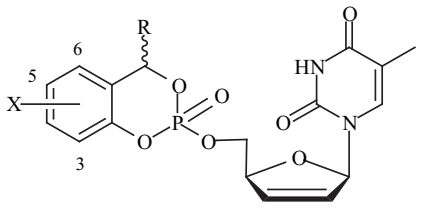


Fig. (1). Different hydrolysis of di-phenyl- and di-benzyl phosphate triesters.

following spontaneous break-down of diester **9** to yield the nucleotide d4TMP **6** and the salicyl alcohol **11** (cascade reaction; steps b) is induced presumably via formation of a zwitterion **12** or a 2-quinonemethide **13**. However, at pH-values of 7.0 to 7.4, the phenolate should be protonated to yield the phenol. Ab-initio calculations of diester **9** showed that the proton "migrates" to or forms a hydrogen bond to the benzyl-oxygen atom. This forces the heterolytic bond cleavage because of the increased leaving group tendency of the protonated phosphate in intermediate **14** (Figure 3, step b_1). Maybe this process is further assisted by an S_N2-type interaction of a water molecule leading immediately to the salicyl alcohol **11** and d4TMP **6**. In ^{31}P-NMR-experiments also the unfavorable cleavage of the benzyl ester to give the phenyl phosphate diester **10** was observed to a lower extent (Figure 3, step c; Table 1) [11]. It was interesting to note that with the increasing stability of the phenyl ester, higher amounts of phenyl diesters **10** appeared [12]. Mechanistically, the benzyl ester is cleaved via a S_N1-type reaction followed by water trapping. In our studies, a further cleavage of diester **10** by chemical or enzymatic means has not been observed yet.



*cyclo*Sal-d4TMP **8**

a: X = R = H
b: X = 3-Me; R = H
c: X = 5-Me; R = H
d: X = 3,5-Me; R = H
e: X = 3-*t*Bu; R = H
f: X = 3,5-*t*Bu; R = H
g: X = 3-Phe; R = H
h: X = 5-Phe; R = H
i: X = H; R = Me
j: X = Me; R = Me
k: X = 6-Cl; R = Me
l: X = H; R = CH_2Cl

Fig. (2). *Cyclo*Sal-phosphate triesters of the first generation.

Different studies concerning the properties of the *cyclo*Sal-d4TMP triesters **8** have been carried out. *Cyclo*Sal-phosphate triesters like **8** showed a marked increase in lipophilicity (log*P*) with respect to the parent nucleosides [1]. Hydrolysis studies in different aqueous buffers concerning the influence of different substitution patterns in the *cyclo*Sal-aromatic moiety showed the expected dependence of the chemical half-lives on the electronic properties of the substituent (Table 1) [10, 12]. From

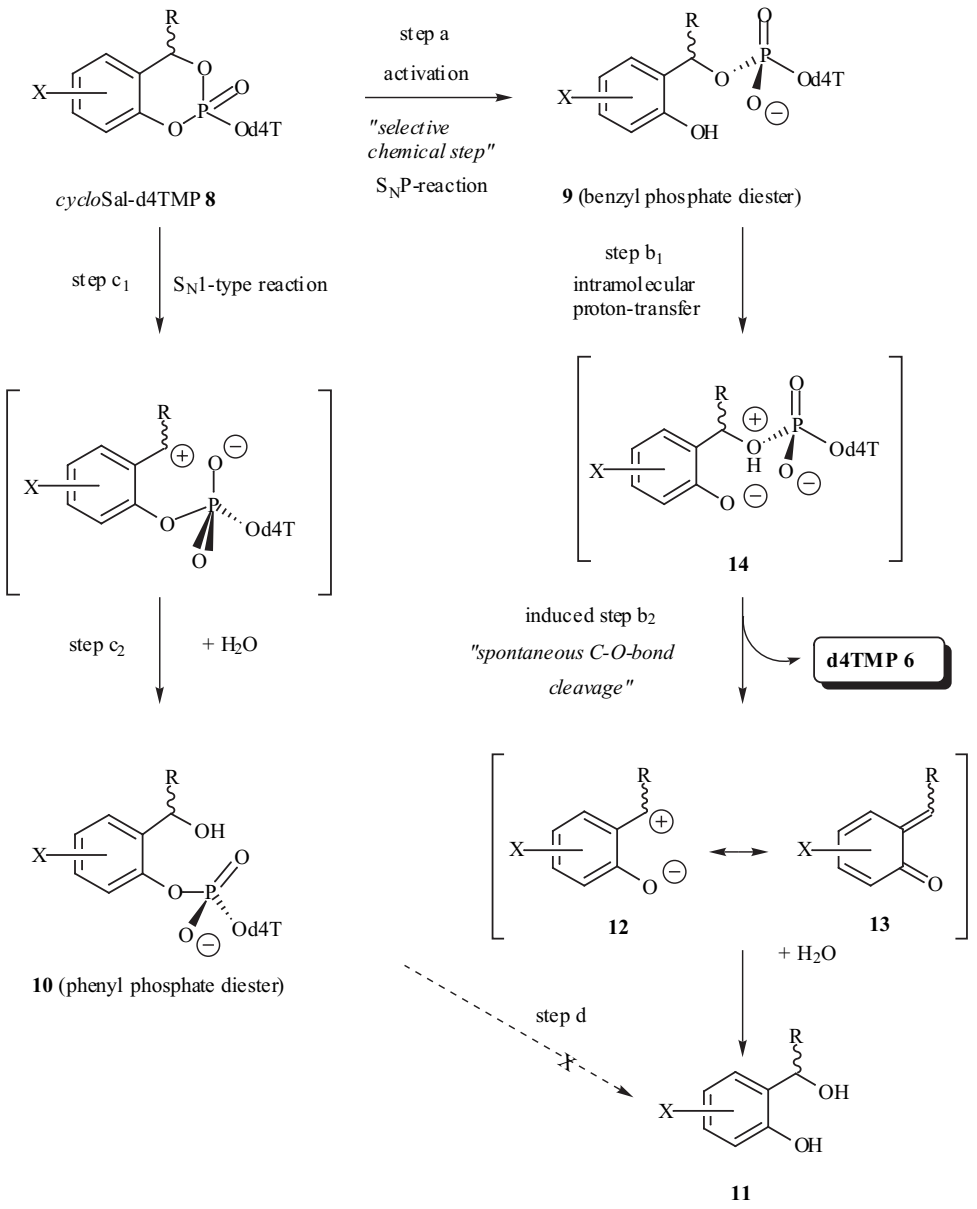


Fig. (3). Detailed hydrolysis mechanism of the prototype *cyclo*Sal-phosphate triesters.

hydrolysis studies using the corresponding PMEA/PMPA phosphonate diesters it became apparent that the second cleavage reaction to yield the nucleotide/phosphonates proceeds independently of the substitution pattern [13]. Thus, the liberated 2-hydroxyl group clearly governs the electronic properties of the different substituents. In most of the cases, d4TMP as well as the formation of salicyl alcohols **11** were observed as main products (Table 1). Moreover, studies in CEM-cell extracts showed that the hydrolysis half-lives only slightly decreased as compared to the buffer hydrolyses [14] and, finally, studies in 20% human serum in phosphate buffer exhibited no difference in stability as compared to the buffer hydrolysis studies showing that no enzymatic contribution takes place.

An interesting effect has been observed after modification of the benzyl-position in prototype triester **8a**. The introduction of a methyl group (triester **8i**) led to a predominant formation of the phenyl phosphate diester of type **10** (Table 1) [12]. This effect was even more pronounced in triester **8j**. Moreover, the chemical stability decreased enormously. Interestingly, the introduction of an electronegative chlorine atom either in the 6-position (**8k**) or in the 7-methyl group (**8l**) completely prevents the formation of diester **10** and increases the chemical half-lives (Table 1) [12]. These results proved that both reaction pathways shown in figure 3 could be selected depending on the substitution pattern in the *cyclo*Sal-fragment.

In conclusion, the major difference in the enzymatically activated pronucleotides described in the literature is that the *cyclo*Sal-concept requires only *one* activation step to deliver the nucleotide. Moreover, in contrast to all other pronucleotide systems, only *one* masking molecule per nucleotide has to be used so far due to the bifunctional character of the *cyclo*Sal group. The salicyl alcohols **11** were tested for the biological potency but neither showed antiviral activity nor caused toxicity [10]. Further, *in-vivo* studies in mice showed that concentrations up to 250 mg/kg of different diols did not cause toxic side effects [15]. Because

Table 1 Half-Lives, Product ratio and Antiviral data of the Prototype *cyclo*Sal-d4TMP Triesters 8

Triester	Substituents		$t_{1/2}$; 37°C[a]	Ratio	EC_{50} (μM)[d]			CC_{50} (μM)[e]
8	**X**	**R**	**pH 7.3[b] [h]**	**d4TMP:10[c]**	**CEM/O HIV-1 (III)**	**CEM/O HIV-2 (ROD)**	**CEM/TK⁻ HIV-2 (ROD)**	
a	H	H	4.4	99:1	0.20	0.22	0.9	50
b	3-Me	H	17.5	94:6	0.07	0.07	0.05	32
c	5-Me	H	8.1	98:2	0.18	0.34	0.18	38
d	3,5-Me	H	29	85:15	0.09	0.17	0.08	21
e	3-*t*Bu	H	96	95:5	0.18	0.15	0.18	49
f	3,5-*t*Bu	H	85	67:33	1.1	1.2	2	27
g	3-Phe	H	5.1	97:3	0.13	0.27	0.15	22
h	5-Phe	H	3.1	100:0	0.40	0.47	2	54
i	H	Me	0.25	17:83	0.22	0.34	35	152
j	3-Me	Me	0.20	6:94	4.0	4.5	20	>250
k	6-Cl	Me	2.2	100:0	0.19	0.25	7	42
l	H	CH_2Cl	2.8	100:0	0.19	0.35	22	56
d4T **1**	--	--	n.a.[f]	n.a.[f]	0.25	0.15	50	56

[a]hydrolysis half-lives in hours; [b]25 mM sodium phosphate buffer; [c]ratio of d4TMP **6** : phenyl phosphate diester **10** determined by ^{31}P-NMR; [d]antiviral activity: 50% effective concentration in wild-type (CEM/O) or thymidine kinase defiecient (CEM/TK⁻) cell cultures; [e]cytostatic concentration: 50% cytostatic activity; [f] not available

of their nature to be reactive organophosphate esters, *cyclo*Sal-d4TMP triesters were studied concerning their potential inhibitory effect against human acetylcholine esterase (AChE). However, none of the tested compounds showed inhibitory effect towards AChE [16]. In contrast, for some of the compounds inhibition of serum butyrylcholine esterase (BChE) was observed. However, neither the physiological role of this enzyme is known and nor is related to nerve signal transfer. In addition, this inhibitory effect was found to be strongly dependent on the P-stereochemistry, the attached nucleoside analogue and the substitution pattern in the aromatic ring [17].

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 of enzymatic activation (Figure 3). Particularly, the donor-substituted *cyclo*Sal-d4TMPs **8b-f** having half-lives of 8-96 hours should be interesting candidates for biological evaluation. The effectiveness of the *cyclo*Sal-d4TMP triesters was demonstrated in *in vitro* antiviral assays [10,12] in which 3- and 5-alkyl-*cyclo*Sal-d4TMPs showed comparable or even higher antiviral potency in a wild-type lymphocyte cell line (CEM/O) as compared to d4T **1** (Table 1). More important was the complete retention of the biological activity in mutant thymidine kinase-deficient CEM/TK⁻ cells for alkyl- and 3-phenyl-substituted compounds. Due to the predominant formation of diester **10** and the very short half-lives, triesters **8i**,**j** were devoid of any antiviral activity in the TK-deficient cells [12]. The half-lives of **8h**,**l** are still too short to allow efficient cellular uptake. It should be added that the *cyclo*Sal-triesters themselves have no inhibitory effect on DNA-synthesis. This was shown in experiments using a cell-free recombinant RT/RNA template. This proves that the mechanism of action of the *cyclo*Sal-triesters relies on the formation of d4TTP after d4TMP delivery. These results confirm i) the uptake of the compounds into the cells, ii) the intracellular delivery of d4TMP and iii) prove the independence of the cellular thymidine kinase activation pathway necessary for the expression of the antiviral activity: the compounds clearly achieve an efficient TK-bypass. These results were confirmed by incubation experiments of radiolabeled 3-methyl-*cyclo*Sal-d4TMP **8b** (tritium-label in the methyl group of thymine) and wild-type CEM/O and thymidine kinase-deficient CEM/TK⁻ cells [18].

Further, *cyclo*Sal-d4TMP triester **8a** demonstrated significant antiviral activity in AZT-resistant $H9^rAZT^{250}$ cells proving that the compounds seem to be able to overcome cellular resistance of a certain drug. The resistant cell line was generated by continuous cultivation of H9 cells in the presence of increasing AZT concentrations. The developed AZT resistance is concomitant to a five-fold lower expression of the TK gene in comparison to parental H9 cells. Consequently, AZT showed an EC_{50} of >100 μM in this cell-subline, while the EC_{50} in parental cells was found to be 0.04 μM. In addition, also d4T looses most of its activity in $H9^rAZT^{250}$ cells as compared to parental H9 cells (EC_{50}s of 26 μM and 0.9 μM, respectively). In contrast, *cyclo*Sal-d4TMP **8a** proved to be equipotent in parental and in $H9^rAZT^{250}$ cells (EC_{50} 0.3 μM and 0.5 μM) [19].

The *cyclo*Sal-approach has further been applied to different nucleoside analogues (Figure 4). When 2',3'-dideoxyadenosine (ddA) **15** was used, a 100-fold increase of

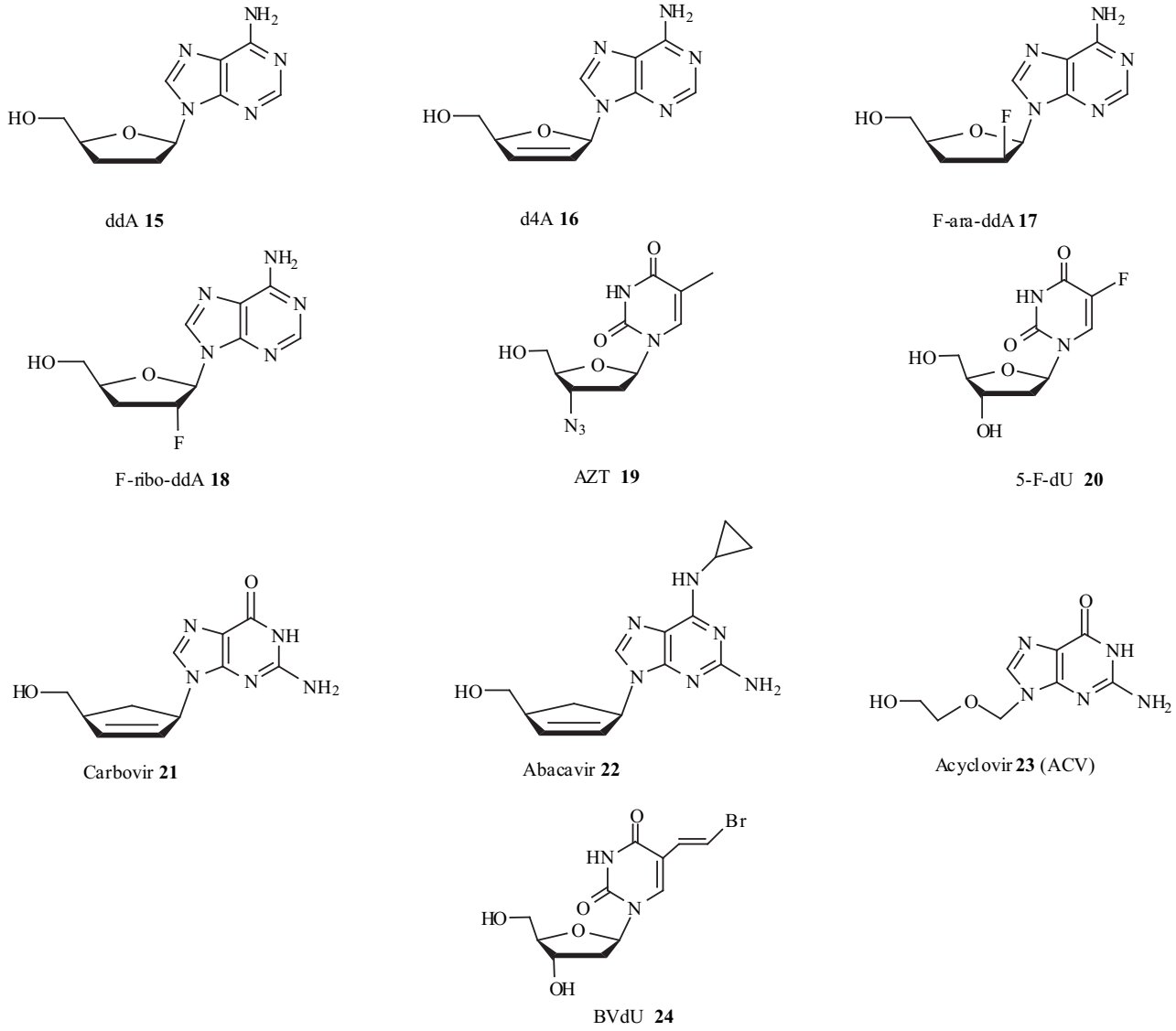


Fig. (4). Antivirally active nucleoside analogues used in the *cyclo*Sal-approach.

the anti-HIV activity has been found with respect to the parent nucleoside analogue [20-22]. The activity of 2',3'-dideoxy-2',3'-didehydroadenosine (d4A) **16** has been increased by 600-fold by the introduction of the *cyclo*Sal-mask [21]. Thus, both the *cyclo*Sal-derivatives achieved the adenosine-deaminase-bypass [23-25]. In the same context, two fluorinated ddA derivatives **17** and **18** have been used [26, 27]. The activity of 2'-*ara*-F-ddA **17** was improved (10-fold) but, more interestingly, the entirely inactive nucleoside 2'-*ribo*-F-ddA **18** [28, 29] was converted into an anti-HIV active compound after modification with the 3-methyl-*cyclo*Sal-moiety. The reason for the unexpected difference in the bioactivity of the two nucleosides may be related to a conformational difference due to the introduction of the fluorine atom [30, 31]. This was the first example of a conversion of an inactive nucleoside into a bioactive derivative by the *cyclo*Sal-approach.

The approach has also been applied to the delivery of the antiviral 3'-azidothymidine (AZT) **19** [32, 33] and the antitumor active nucleoside analogue 5-fluoro-2'-deoxyuridine (FdU) **20** [34]. In both cases, antiviral and antitumor activity in thymidine, kinase-deficient cells clearly proved the concept a failure. This result completely contrasts our expectation, because the corresponding *cyclo*Sal-phosphate triesters delivered the nucleotides AZTMP and FdUMP in hydrolysis studies. Studies using the radiolabeled AZT triesters led to the conclusion that a so far unknown catabolic process is responsible for the intracellular clearance of the liberated AZTMP with the consequence that phosphorylation into the triphosphate is prevented [35]. Most likely, 5'-nucleotidase(s) may be involved. Recently, also the purine nucleoside analogues (carbovir **21** and abacavir **22**) were used. No improvement against HIV and HSV-1/2 was observed for carbovin as compared to the parent nucleoside. However, the antiviral potency of abacavir has been improved after *cyclo*Sal-modification against HIV and the antiviral activity has been observed also against HSV-1/2 [36].

Moreover, the *cyclo*Sal-approach has been applied to nucleosides with activity against DNA-viruses like herpes viruses. In contrast to HIV, these viruses do not rely on reverse transcription of their genome prior to replication. The

target then is the viral DNA-polymerase. Interestingly, some of the known antivirals against DNA viruses are not monophosphorylated by a cellular thymidine kinase but by viral-encoded thymidine kinases (TK) [37]. Often, drug resistant virus strains are selected *in-vivo*. One reason for this resistance seems to be associated with a downregulation of the expression of viral thymidine kinase [38]. Moreover, some virus-types belonging to the herpes virus family either express no thymidine kinase (e.g. cytomegalovirus, herpes virus type 6) or a TK with different substrate specificities, e.g. Epstein-Barr-virus (EBV). Therefore, the *cyclo*Sal-approach has been applied to the broad-spectrum, acyclic, purine nucleoside acyclovir (ACV) **23** and to the pyrimidine-modified nucleoside 5-[(*E*)-2-bromovinyl]-2'-deoxyuridine (BVDU or Brivudin) **24** [39] (Figure 4).

Antiviral evaluation showed an EC_{50} for the parent nucleoside ACV **23** of 0.62 μM against HSV-1/TK^+ in Vero cells. As expected, ACV lost its activity in Vero cells infected with HSV-1/TK^- (EC_{50}: 58 μM). Strikingly, 3-methyl-*cyclo*Sal-ACVMP showed identical antiviral activity values of 0.47 μM and 0.51 μM in the same HSV-1 TK^+ and TK^- systems, respectively, and an EC_{90} of 1.62 μM against the mutant virus strain without increasing the toxicity. Again, the complete retention of activity clearly proves that ACVMP is delivered to the cells by the pronucleotide [40, 41].

A comparable result was obtained for the antiviral activity against VZV [42]. 3-Methyl-*cyclo*Sal-ACVMP showed an antiviral activity of EC_{50} 4.1 μM and 1.2 μM against two wild-type virus strains respectively. This activity was completely retained in cells infected with VZV/TK^- (EC_{50}: 7.6 μM) without changing the cell morphology (MCC >200 μM). As expected, ACV showed activity against the wild-type viruses but lost its antiviral potency in the mutant virus strain. Furthermore, 3-methyl-*cyclo*Sal-ACVMP exhibited antiviral activity against two VZV/TK^- strains (EC_{50}: 9-13 μM) while ACV was entirely inactive [42].

The nucleoside analogue BVdU **24** is a potent and highly selective inhibitor of the replication of HSV-1 and particularly VZV [43, 44]. Interestingly, BVdU is not active against HSV-2 and EBV due to a different viral thymidine kinase (TK). HSV-2 TK lacks significant thymidylate kinase activity required to convert BVdUMP to its 5'-diphosphate metabolite, explaining the lack of marked activity of BVdU against HSV-2. Our aim was to investigate whether the *cyclo*Sal-concept is able to broaden the application of BVdU to EBV caused infections that play a significant role as secondary infection e.g. in AIDS patients [45]. Therefore, different *cyclo*Sal-BVdUMPs were synthesized [46, 47].

Hydrolysis studies proved the selective delivery of BVdUMP **25** as the sole product without the formation of 3',5'-cyclicBVdUMP **26** (Figure 5). Interestingly, the half-lives found were significantly lower as compared to the corresponding *cyclo*Sal-d4TMP triesters **8** [11, 13, 47].

Further studies in P3HR1 cell extracts confirmed the results obtained for aqueous buffers [47]. In contrast to the chemical hydrolysis studies, BVdU **24** was formed from BVdUMP **25** in the cell extracts. Interestingly, this was never observed for d4TMP. Assays for the inhibition of EBV replication in P3HR1 cells proved the complete inactivity of BVdU (EC_{50} >300 μM) [46,47]. In contrast to BVdU, 5-methoxy-*cyclo*Sal-BVdUMP showed significant anti-EBV activity with an EC_{50} of 1.8 μM and was even 4-fold more active as the reference compound ACV **23**. These results clearly point to the fact that the reason for the inactivity of BVdU against EBV only relies on an inefficient intracellular formation of BVdUMP and that cellular kinases

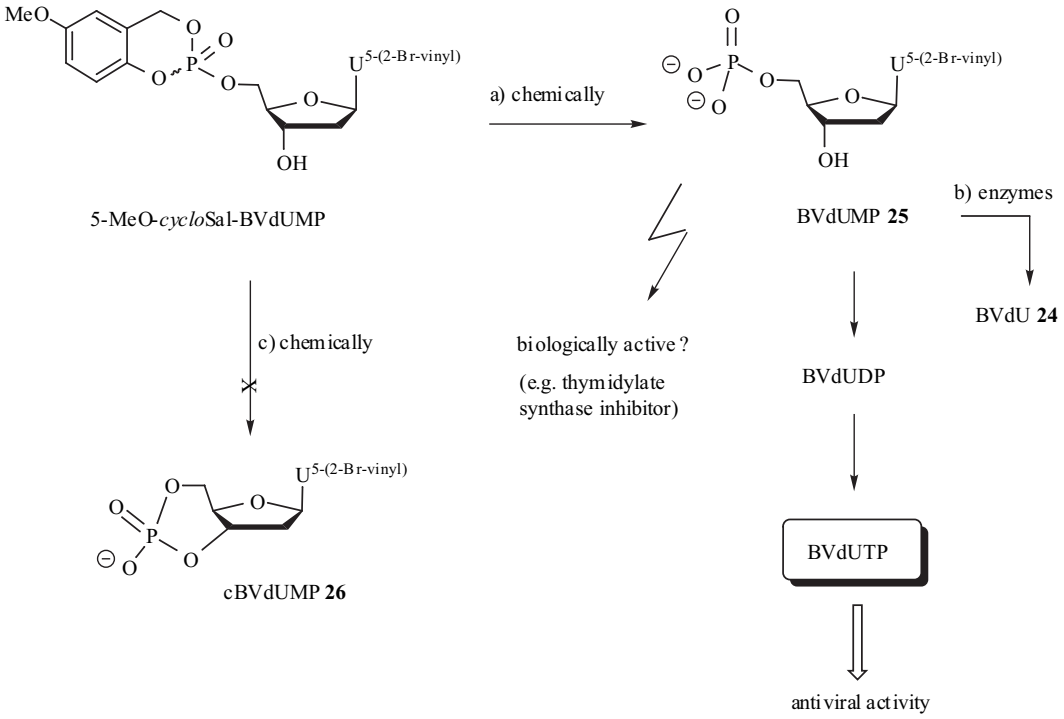


Fig. (5). Hydrolysis pathway of 5-methoxy-*cyclo*Sal-BVdUMP triesters.

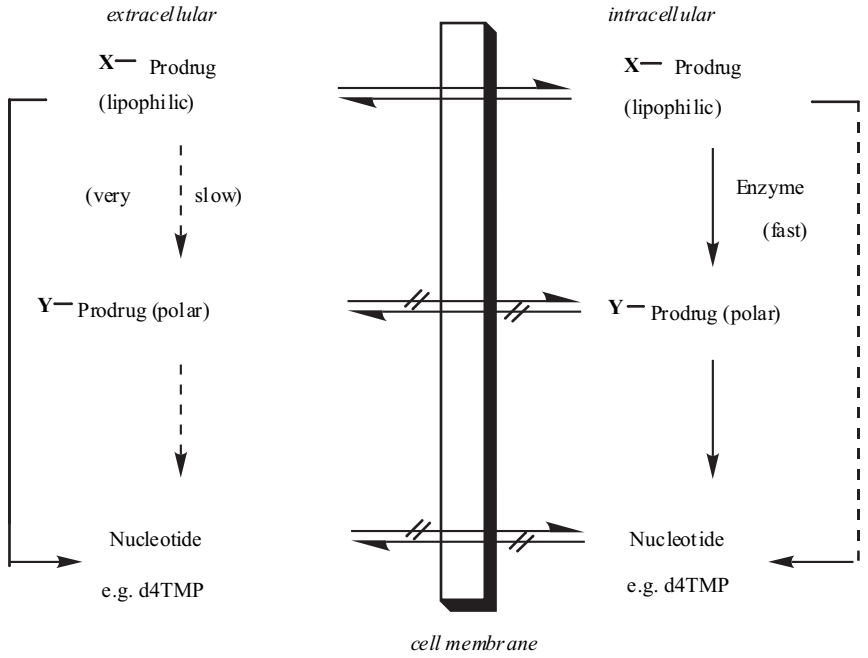
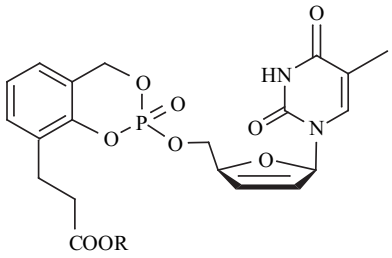


Fig. (6). Concept of the "lock-in" modified *cyclo*Sal-phosphate triesters.

seem to be able to further convert phosphorylated (activated) BVdU into the ultimate metabolite BVdUTP. Again, these examples clearly show the potential of the *cyclo*Sal-phosphate triester approach: it is able to convert nucleoside analogues that have no antiviral activity against a virus into bioactive compounds.

"LOCK-IN"- *CYCLO*SAL-D4TMPS –A CONCEPTIONAL EXTENTION OF THE TROJAN HORSE CONCEPT

The compounds described so far belong to the first generation compounds of the *cyclo*Sal-concept. Although these first-generation, *cyclo*Sal-triesters led to convincing

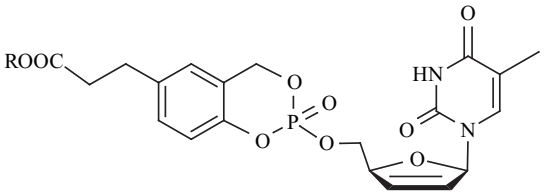


*cyclo*Sal-d4TMP acid **27**: R = H

*cyclo*Sal-d4TMP esters: **31a**: R = Me

31b: R = *i*Pr

31c: R = *t*Bu

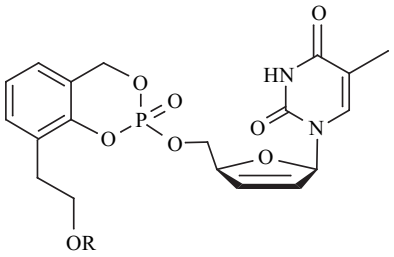


*cyclo*Sal-d4TMP acid **28**: R = H

*cyclo*Sal-d4TMP esters: **32a**: R = Me

32b: R = *i*Pr

32c: R = *t*Bu

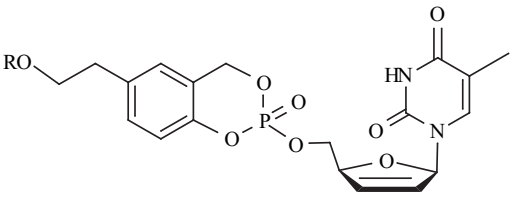


*cyclo*Sal-d4TMP alcohol **29**: R = H

*cyclo*Sal-d4TMP esters: **33a**: R = Ac

33b: R = Lev

33c: R = Piv



*cyclo*Sal-d4TMP alcohol: **30a**: R = H

*cyclo*Sal-d4TMP esters: **34a**: R = Ac

34b: R = Piv

Fig. (7). Studied ester-modified *cyclo*Sal-d4TMP triesters.

antiviral results, the use of a chemical hydrolysis mechanism may also have some limitations. We have clearly shown for several *cyclo*Sal-triesters that an enzymatic contribution to the hydrolysis could neither be found in cell extracts nor in human serum. Moreover, we have clearly proven that the lipophilic *cyclo*Sal-triesters are able to penetrate the cell membranes and deliver nucleotides *inside* the cell. However, it can not be excluded that the chemical hydrolysis also takes place outside the cells. In addition, although the compounds are lipophilic enough to migrate inside the cells, we can not exclude tha possibility that they also can diffuse in the opposite direction through the membrane. This would lead to the establishment of equilibrium. In order to avoid such a back-diffusion, a fast conversion of the triesters having a lipophilic X-residue inside the cells into a much more polar compound with a Y-group by an enzymatic reaction should be envisaged, thus preventing the efflux ("lock-in mechanism"; Figure 6).

Therefore, we used an (carboxy)esterase reaction on a carboxylic ester attached to the *cyclo*Sal-aromatic ring via a linker. As a linker, a C2-alkyl chain was introduced. The ethylene spacer should separate the ester group efficiently from the aromatic ring in order to avoid an electronic effect on the hydrolysis of the phosphate triester moiety. Due to results obtained from earlier experiments, two positions in the *cyclo*Sal-moiety have been selected for the introduction of the ester-spacer residue: the 3- and the 5-position. As an ester group, there were two possibilities were used: i) esterification of a *cyclo*Sal-acid (**27**,**28**) with an alcohol, and ii) esterification of a *cyclo*Sal-alcohol (**29**,**30**) with a carboxylic acid leading to compound series **31**,**32** and **33**,**34**, respectively (Figure 7).

After enzymatic cleavage, the former triesters **31**,**32** lead to the liberation of a free carboxylic acid residue that should be deprotonated under physiological pH-conditions and the latter triesters **33**,**34** would lead to a free alcohol group. Different esters bearing linear or branched alkyl groups were introduced and the new concept has been applied first to the nucleoside analogue d4T.

CHEMISTRY

The synthesis of the *cyclo*Sal-pronucleotides has been done most successfully using reactive phosphorus(III)-reagents (Figure 8) [1]. Therefore, diols **11** were reacted with phosphorus trichloride yielding the cyclic chlorophosphites **35**, which were used as such for the following reactions. Phosphites **35** were reacted with d4T in the presence of diisopropylethylamine (DIPEA) yielding cyclic phosphite triesters which were oxidized in a one-pot-reaction using *t*-butylhydroperoxide (TBHP). The phosphate triesters **31**-**34** were obtained in reasonable yields (30-50%) as diastereomeric mixtures.

Originally, salicyl alcohols **11** have been prepared from the corresponding salicylic aldehydes or -acids by standard reduction protocols. However, the aldehydes/acids that were used here were not commercially available. Then, diols **11** have been synthesized from the phenols **36**. Selective *ortho*-hydroxymethylation was possible according to Nagata *et al.* [48]. This method is the mildest procedure used so far in our laboratories leading to the products without any side reaction in the ester moiety. Unfortunately, there is no generally applicable method for the preparation of the substituted phenols **36** bearing the ester–spacer residue.

The 3-(2-hydroxyphenyl)alkylpropionates were prepared from dihydrocoumarin **37** by transesterification with methanol or 2-propanol in the presence of H_2SO_4 in 96% and 73% yield (step A). The *t*-butyl esters of 4- (**38**) and (2-hydroxyphenyl)propionic acid **39** can be isolated in 80% yield using DMF-dineopentylacetale and *t*-butanol (step B) [49]. The methyl ester of 4-hydroxyphenylpropionate **38** was formed by refluxing the acid, methanol and sulfuric acid in CH_2Cl_2 (step C, 93% yield). 3-(4-Hydroxyphenyl)propionic acid **38** was also the starting material for the *i*-propyl ester that has been formed in 2-propanol and with HCl-gas in 62% yield (step D) [50]. The acetyl esters 2-(hydroxyphenyl)ethanols **40**,**41** were prepared by transesterification from ethylacetate catalyzed by SiO_2•$NaHSO_4$ in 95% and 60% yield (step E) [51]. Both, 2-(2-hydroxyphenyl)ethylpivalate and 2-(4-hydroxyphenyl)ethylpivalate were prepared from 2-(hydroxyphenyl)ethanols using the "twisted"-amide method in toluene in 80% and 51% yield (step F) [52], respectively. These phenol derivatives were then converted into the corresponding *cyclo*Sal-phosphate triesters using the known procedures [11]. As reference compounds, 3- and 5-*cyclo*Sal-acids **27**,**28** were prepared from *t*-butyl esters **31c**,**32c** by treatment with trifluoroacetic acid in 85% yield (step K). 3-*Cyclo*Sal-alcohol **29** was synthesized from the corresponding levulinyl (Lev) ester **33b** prepared via methods G-I. The Lev-group has been cleaved from the triester **33b** by hydrazine-hydrate treatment in 25% yield (step J) [53]. The Lev-ester of the phenol was prepared as the acetyl esters (step E). Unfortunately, the acetyl esters could not be cleaved chemically when incorporated into the *cyclo*Sal-phosphate triester structure. All triesters obtained were then studied concerning their properties to liberate the polar group by the enzymatic reaction as well as their chemical hydrolysis to yield d4TMP.

"LOCK-IN"-*CYCLO*SAL-PRONUCLEOTIDES – PROPERTIES AND ANTIVIRAL EVALUATION

First, chemical hydrolysis studies were conducted. The results are summarized in table 2. As expected, all new triesters were cleaved to yield d4TMP at pH 7.3 in 25 mM phosphate buffer. Half-lives were found ranging from 7.3 and 13.5 hours in the case of the 3-modified triesters **31**,**33** and from 5.4 to 7.3 hours for their 5-modified counterparts **32**,**34**. A comparison with the 3- (**8b**; $t_{1/2}$ = 17.5 h) and 5-methyl-*cyclo*Sal-d4TMP triesters (**8c**; $t_{1/2}$ = 8.1 h) showed that the ethylene-spacer sufficiently separates the electron-withdrawing ester group from the *cyclo*Sal-aromatic ring. Interestingly, to note that both free acid-*cyclo*Sal-d4TMP triesters **27**,**28** showed up to two-fold, higher, half-lives as compared to the ester-modified *cyclo*Sal-triesters under identical experimental conditions. A possible explanation for this effect may be the presence of an overall negative charge on the molecule due to the formed carboxylate at pH 7.3, which slows down the nucleophilic reaction necessary for the initial cleavage step.

In contrast, 3-*cyclo*Sal-triester **29** bearing the hydroxyl group in the side chain did not show such an increase in the

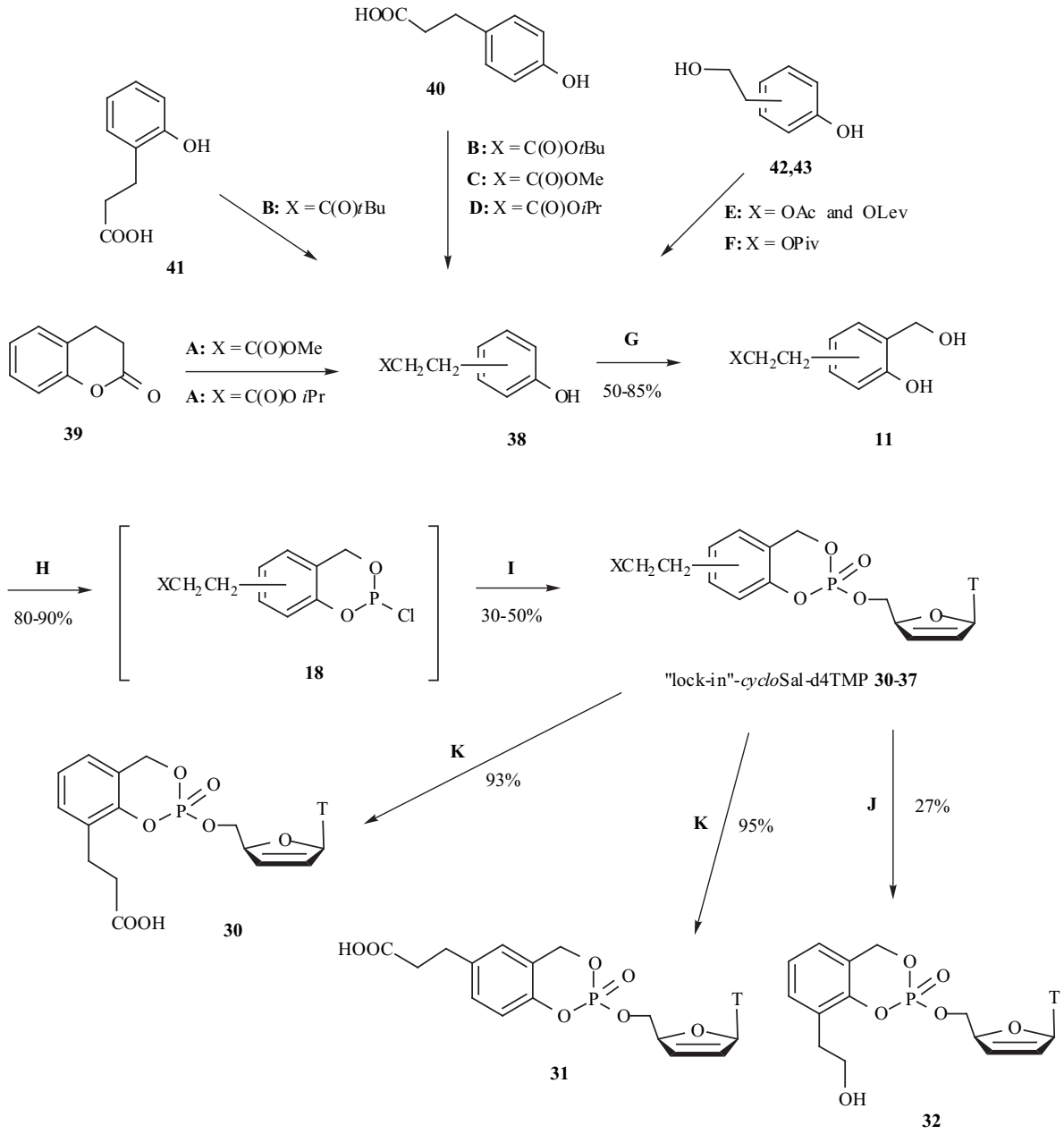


Fig. (8). Synthesis of the ester-modified *cyclo*Sal-d4TMP triesters.
Method **A**: alcohol, H_2SO_4, reflux, 5-8 h; method **B**: $(CH_3)_2NCH(OCH_2tBu)_2$, toluene, reflux, 5 h; method **C**: methanol, CH_2Cl_2, H_2SO_4, reflux, 5 h; method **D**: 2-propanol, HCl-gas, rt, 16 h; method **E**: ethylacylate, *n*-hexane, SiO_2•$NaHSO_4$, 67°C, 6-18 h; method **F**: 3-pivaloyl-1,3-thiazolidine-2-thion, toluene, 65°C, 48 h; method **G**: i. phenylboronic acid, propionic acid (cat.), *p*-formaldehyde, toluene, reflux, 6-8 h; ii) H_2O_2, THF, 0°C, 30 min; method **H**: PCl_3, pyridine, diethylether, 0-21°C, 12 h; method **I**: i. d4T **1**, AcCN, DIPEA, 0-20°C; ii. *t*BuOOH, AcCN, rt, 30 min; method **J**: hydrazine•hydrate, pyridine/acetic acid 3:2, pyridine, 0°C, 10 min; method **K**: TFA (10 equiv.), CH_2Cl_2, rt, 1h.

half-lives ($t_{1/2}$ = 12.6 h; Table 2). This value is within the same range as those of the esters **33** ($t_{1/2}$ = 13 h).

A hydrolysis experiment with the 3-MePr-*cyclo*Sal-d4TMP **31a** followed by ^{31}P-NMR showed that also the phenyl phosphate diester was formed although in a minor extend of ~2%. This amount is considerably lower as compared to 3-methyl-*cyclo*Sal-d4TMP **8b** (5.5%). In both experimental set-ups for chemical hydrolysis, no cleavage of the carboxylic ester group was observed.

Next, as a model reaction for the enzymatic cleavage of the carboxylic esters, studies in 25 mM phosphate buffer, pH 7.3, containing 50 units of pig liver esterase (PLE) were carried out (Table 2). It was observed that the half-lives of the 3-modified *cyclo*Sal-triesters **31** (methyl-, *i*-propyl and *t*-butyl-esters) were slightly lower as compared to the situation in pure phosphate buffer. However, no trace of the expected *cyclo*Sal-triester acid **27** could be detected. Thus, as observed in the chemical hydrolysis, no enzymatic cleavage took place. In contrast, the acetyl and the pivaloyl ester of triesters **29** showed a two-fold decrease in the half-lives, and alcohol **29** was observed in the HPL-chromatograms. Interestingly, the situation was different for the 5-modified *cyclo*Sal-d4TMPs. Here, none of the studied esters of the

Table 2. Antiviral Data of *cyclo*Sal-d4TMP Triesters 27-29 and 31-34

Compound	Subst.	Hydrolysis ($t_{1/2}$) at 37°C			EC_{50} (μM)[e]			CC_{50} (μM)[f]
	X	pH 7.3[a] [h][d]	PLE[b] [h][d]	CE[c] [h][d]	CEM/O HIV-1	CEM/O HIV-2	CEM/TK$^-$ HIV-2	
31a	3-MePr[g]	7.3	7.6	7.2	0.09	0.25	0.40	57
31b	3-*i*PrPr	12.5	9.3	10.1	0.14	0.80	1.50	54
31c	3-*t*BuPr	13.5	10.9	9.0	0.33	0.50	1.14	43
27	3-HPr	22.9	22.6	20.4	0.19	1.4	20	100
33a	3-AcEt[h]	13.6	8.3	1.9	0.16	0.33	0.15	40
33b	3-LevEt	12.5	n.d.[i]	1.9	0.13	0.15	0.33	58
33c	3-PivEt	13.1	8.2	6.6	0.16	0.70	0.40	55
29	3-HOEt	12.6	n.d.[i]	14.9	0.24	0.25	0.49	96
32a	5-MePr	7.0	4.0	5.7	0.33	1.05	1.20	58
32b	5-*i*PrPr	7.3	4.7	5.7	0.17	0.90	3.00	59
32c	5-*t*BuPr	7.1	5.5	5.3	0.18	2.40	4.00	42
28	5-HPr	12.5	9.1	11.4	0.14	0.80	50	76
34a	5-AcEt	6.3	5.8	2.6	0.15	0.80	0.55	44
34b	5-PivEt	5.4	4.8	5.5	0.23	0.90	0.60	22
d4T **1**	--	n.a.[j]	n.a.[j]	n.a.[j]	0.25	0.15	50	56

[a]25 mM sodium phosphate buffer; [b]25 mM phosphate buffer + 50 units pig liver esterase (PLE); [c]CEM cell extracts; [d]half-lives in hours; [e]antiviral activity: 50% effective concentration; [f]cytotoxicity: 50% cytostatic concentration; [g]X=3-MeOC(O)CH_2CH_2; [h]X=3-MeC(O)OCH_2CH_2; [i]not determined; [j]not available.

*cyclo*Sal-d4TMP acid **28** and the *cyclo*Sal-d4TMP alcohol **30** was hydrolyzed. In conclusion, the outcome of these cleavage studies using PLE was disappointing. It should be added that first experiments using the methyl ester of the 3-propionate-*cyclo*Sal-mask showed an extremely fast de-esterification under the same conditions. Therefore, we also expected a fast ester hydrolysis in most of the cases of the *cyclo*Sal-triesters **31**. Nevertheless, in all cases d4TMP **6** was formed as a result of a chemical hydrolysis of the phosphate triester entity.

Further studies were done in CEM/O cell extracts, and triesters were incubated for 10 hours at 37°C (Table 2). The acetyl- (**33a**) and the levulinyl ester of the *cyclo*Sal-d4TMP alcohol **33b** showed the most impressive result: these triesters were degraded 6- to 7-fold faster as compared to the buffer incubations, and the intermediate alcohol **29** was clearly detected in the chromatogram. So, the cleavage capacity of the extracts was markedly higher as compared to the isolated enzyme PLE. However, the enzyme responsible for the ester hydrolysis is not known. Moreover, the pivaloyl ester **33c** was also cleaved (half-life dropped two-fold) while all the other esters (**31**) were not cleaved enzymatically. In the 5-ester-modified, *cyclo*Sal-d4TMP series **32**,**34**, only the acetyl ester (**34a**) showed a two-fold decrease in $t_{1/2}$. In conclusion, the acetyl esters were proven to be good substrates for the human esterases while all alkyl esters seem not to be cleavable by the present esterases. However, as before, the final products of the complete chemical hydrolysis of all triesters were d4TMP and the salicyl alcohols **11**.

The unsusceptibility of the alkyl esters is surprising because alkyl esters are often used in prodrug strategies, and at least the phosphoramidate approach developed by McGuigan is based on an initial cleavage of such an ester group [54]. However, esters of natural α-amino acids are used in that case.

It was interesting to note that in studies of the corresponding BVdUMP triesters also the benzyl esters were cleaved in P3HR-1 cell extracts, in addition to the acetyl ester (data not shown). So, it seems that the cleavage is also dependent on the nature of the nucleoside analogue. However, we can not exclude that the extracts from P3HR-1 cells contain different esterases or different concentrations of esterases as CEM cell extracts.

Finally, the triesters were tested for their antiviral potency in CEM/O cells infected with HIV-1 and HIV-2 as well as in HIV-2-infected CEM/TK$^-$ cells. The results are given in table 2.

All *cyclo*Sal-triesters proved to be antivirally active in the wild-type cell line against both virus types. Only the 5-*t*Bu-ester **32c** was found to be 5-fold less active against HIV-2 as compared to the reference compound d4T. More interesting are the results obtained in the thymidine kinase deficient CEM cells (CEM/TK$^-$). First, both *cyclo*Sal-triesters (**27** and **28**) having the unesterified acid

functionality in the side chain lost all their antiviral activity in the mutant cell line due to the charge at the carboxylate, which prevents an efficient membrane penetration into the cells. On the other hand, this result shows that in the case of liberation of the carboxylate inside the cell by an enzymatic cleavage, the resulting polar product would stay trapped inside the cell. This identifies that the planned lock-in mechanism should work. Moreover, and in contrast to the parent nucleoside d4T, all *cyclo*Sal-triesters bearing alkyl esters in the 5- or 3-position of the *cyclo*Sal-aromatic ring retained their antiviral activity in the CEM/TK$^-$ cells (EC_{50} 1-2 μM) and thus proving at least the TK-bypass envisaged by these pronucleotides. Taking into account the results of the cell extract studies, no additional effects of the lock-in could be expected. However, the *cyclo*Sal-triesters **33a**,**34a** that were enzymatically cleaved in the cell extracts showed lower EC_{50} values in the CEM/TK$^-$ cells. Although these triesters do not release a charged carboxylate but a neutral alcohol group (**29** and **30**, respectively), it appears that these compounds provide the first evidence for a successful trapping inside the cells.

CONCLUSION

In summary, the *cyclo*Sal approach convincingly demonstrated the intracellular delivery of antivirally active nucleotides by a non-enzymatically induced cascade reaction. It has considerably improved the antiviral activity of certain nucleoside analogues using the first generation *cyclo*Sal-triesters. First attempts have been made to influence the equilibrium formed by a lipophilic phosphate triester through the membrane resulting in the development of second-generation *cyclo*Sal-triesters having an ester bearing side chain in the *cyclo*Sal-aromatic ring. So far, only acetyl- and (partly) pivaloyl-esters were found to be substrates for cellular esterases. Nevertheless, the release of a negatively charged carboxylate would be advantageous in order to get an efficient intracellular trapping (lock-in) of the triester. Work in order to achieve this goal is currently in progress in our laboratories.

ACKNOWLEDGEMENT

Financial support by the Deutsche Forschungsgemeinschaft, Bonn, Germany; the Fonds der Chemischen Industrie, Frankfurt/Main, Germany; the Adolf-Messer-Stiftung, Frankfurt/Main, Germany; and the René Descartes Prize 2001 of the European Commission is gratefully acknowledged.

REFERENCES AND NOTES

[1] (a) Meier, C. *Mini-Rev. Med. Chem.*, **2002**, *2*, 219; (b) Meier, C. *Angew. Chem.,* **1996**, *108*, 77; *Angew. Chem. Int. Ed. Engl*., **1996**, *35*, 70.

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

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

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

[5] Meier, C.; Muus, U.; Renze, J.; Naesens, L.; De Clercq, E.; Balzarini, J. *Antiviral Chem. & Chemother.,* **2002**, *13*, 101

[6] Muus, U.; De Clercq, E.; Balzarini, J.; Naesens, L.; Meier, C. *Nucleosides, Nucleotides & Nucleic Acids,* **2003**, in press.

[7] 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.

[8] (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.

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

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

[11] Ducho, C.; Balzarini, J.; Naesens, L.; De Clercq, E.; Meier, C. *Antiviral Chem. & Chemother.,* **2002**, *13*, 129

[12] Meier, C.; Renze, J.; Ducho, C.; Balzarini, J. *Current Topics in Med. Chem.,* **2002**, *2*, 1111.

[13] Müller, C.; Meier, C. **2003**, unpublished results.

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

[15] Balzarini, J. **2000**, unpublished results. It should be added that salicyl alcohol (saligenin) is used as part of the antirheumatic and analgetic drug Salicin (2-[hydroxymethyl]phenyl-β-D-glucopyranoside; Assalix®). β-Glucosidase hydrolyzes Salicin to D-glucose and saligenin and the latter is then slowly oxidized by cytochrome P450 to salicylic acid in the blood and in the liver: (a) Chen, G.; Fournier, R.L.; Varanasi, S. *Enzyme Microb. Technol.,* **1997**, *21*, 491; (b) Steinegger, E.; Hoevel, H.. *Pharm. Acta Helv*., **1972**, *47*, 222

[16] Ducho, C.; Balzarini, J.; Meier, C. *Nucleosides, Nucleotides & Nucleic Acids,* **2003**, *22*, 841.

[17] Ducho, C.; Meier, C.; Balzarini, J. *J. Med. Chem*., **2004**, in press.

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

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

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

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

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

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

[24] 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.

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

[26] 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. *J. Med. Chem.,* **1999**, *42*, 1615.

[27] Different names for the nucleoside analogues **17** and **18** could be found. Terms as F-up- and F-down-ddA, F-*ara*- and F-*ribo*-ddA, as well as 2',3'-dideoxy-2'-fluoroarabinosyladenine and 2',3'-dideoxy-2'-fluororibosyladenine 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 (**17**) and 9-(2',3'-dideoxy-2'-fluoro-β-D-*erythro*-pentofuranosyl)adenine (**18**)

[28] 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.

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

[30] 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.

[31] 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.

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

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

[34] 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.

[35] Balzarini, J.; Naesens, L.; Aquaro, S.; Knispel, T.; Perno, C.-F.; De Clercq, E.; Meier, C. *Mol. Pharmacol.,* **1999**, *56*, 1354.
[36] Balzarini, J.; Haller-Meier, F.; De Clercq, E.; Meier, C. *Antiviral Chem. & Chemother.,* **2002**, *12*, 301.
[37] De Clercq, E. *Biochemical Pharmacol.,* **1984**, *33*, 2159.
[38] Fields, B.N. *Virology*, (Vol. 1) 2nd edition, Raven Press, New York, **1990**, 450.
[39] Wutzler, P. *Intervirology,* **1997**, *40*, 343.
[40] Meier, C.; Habel, L.; Haller-Meier, F.; Lomp, A.; Herderich, M.; Klöcking, R.; Meerbach, A.; Wutzler, P. *Antiviral Chem. Chemother.,* **1998**, *9*, 389.
[41] Meerbach, A.; Klöcking, R.; Meier, C.; Lomp, A.; Helbig, B.; Wutzler, P. *Antiviral Research,* **2000**, *45*, 69.
[42] De Clercq, E.; Meier, C. **1998**, unpublished results.
[43] 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.
[44] De Clercq, E. *Clinical Microbiol. Rev.,* **1997**, *10*, 674.
[45] Anagnostopoulos, I.; Hummel, M. *Histo-Pathol.,* **1996**, *29*, 297.
[46] Meier, C.; Lomp, A.; Meerbach, A.; Wutzler, P. *ChemBioChem*, **2001**, *2*, 283.
[47] (a) Meier, C.; Lomp, A.; Meerbach, A.; Wutzler, P. *J. Med. Chem.,* **2002**, *45*, 5157; (b) Meier, C.; Lomp, A.; Meerbach, A.; Wutzler, P. *Nucleosides, Nucleotides & Nucleic Acids,* **2001**, *20*, 4.
[48] Nagata, W.; Okada, K.; Aoki, T. *Synthesis,* **1979**, 365.
[49] Brechbühler, H; Büchi, H.; Hatz, E.; Schreiber, J.; Eschenmoser, A. *Helv. Chim. Acta,* **1965**, *48*, 1746.
[50] Garber, S.B.; Kingsbury, J.S.; Gray, B.L.; Hoveyda, A.H. *J. Am. Chem. Soc.,* **2000**, *122*, 8168.
[51] Breton, G.W. *J. Org. Chem.,* **1997**, *62*, 8952.
[52] Yamada, S.; Sugaki, T.; Matsuzaki, K. *J. Org. Chem.,* **1996**, *61*, 5932.
[53] Van Boom, J.H.; Burgers, P.M.J. *Tetrahedron Lett.,* **1976**, *52*, 4875.
[54] Saboulard, D.; Naesens, L.; Cahard, D.; Salgado, A.; Pathirana, R.; Velazquez, S.; McGuigan, C.; De Clercq, E.; Balzarini, J. *Mol. Pharmacol.*, **1999**, *56*, 693.

Copyright of Mini Reviews in Medicinal Chemistry is the property of Bentham Science Publishers Ltd. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.

Copyright of Mini Reviews in Medicinal Chemistry is the property of Bentham Science Publishers Ltd. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.