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5-DIACETOXYMETHYL-*CYCLO*SAL-D4TMP—A PROTOTYPE OF ENZYMATICALLY ACTIVATED *CYCLO*SAL-PRONUCLEOTIDES

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□ *A new class of "lock-in"-modified* cyclo*Sal-pronucleotides has been synthesized. On the example of 5-diacetoxymethyl-*cyclo*Sal-d4T-monophosphate (5-di-AM-*cyclo*Sal-d4TMP), the concept of enzymatically activated* cyclo*Sal-pronucleotides is elucidated. Synthesis, hydrolysis studies, and antiviral activities against HIV are presented.*

Keywords Pronucleotides; *cyclo*Sal; anti-HIV; enzymatic activation; antiviral activity

INTRODUCTION

One established concept for the delivery of therapeutically active nucleoside monophosphates (NMPs) like 2′,3′-dideoxy-2′,3′-didehydrothymidine monophosphate (d4TMP, **1**) into cells is the *cyclo*Sal-concept.[1] This prodrug concept has been successfully applied to a multitude of nucleoside analogs.[2] Due to the chemically triggered delivery mechanism[3] and the lipophilic character of *cyclo*Sal-triester a concentration equilibrium formed through the cell membrane is supposed. This is unfavorable for antiviral efficiency because a high intracellular concentration of the pronucleotide is necessary. Therefore "lock-in"-modified *cyclo*Sal-pronucleotides have been developed. These pronucleotides bear a (carboxy)esterase-cleavable ester site attached to the aromatic ring in order to trap the *cyclo*Sal-triester inside cells by cleavage of the ester group to release a highly polar derivate. To avoid a considerable reduction of the chemical stability of the *cyclo*Sal-triester, these groups have been separated from the aromatic ring by an

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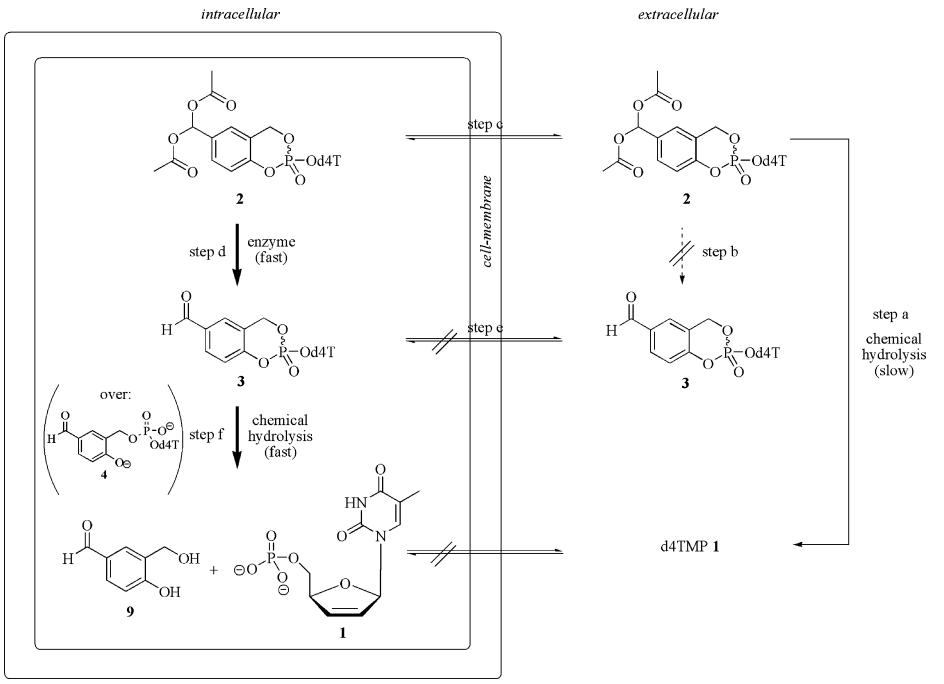
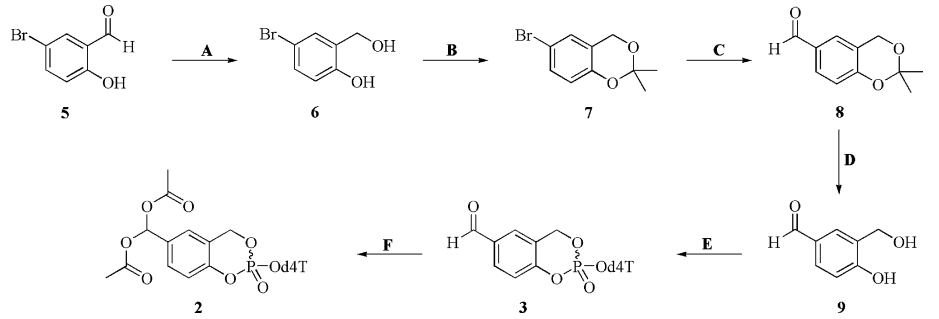


FIGURE 1 Concept of enzymatically activated *cyclo*Sal-Pronucleotides shown on the example of 5-diacetoxymethyl-*cyclo*Sal-d4TMP **2**.

alkyl spacer. It has been shown that an effective intracellular trapping should be possible, if highly polar *cyclo*Sal-d4TMP acids are released from *cyclo*Sal-d4TMP acid ester. From 5-propionyl-*cyclo*Sal-d4TMP acid, d4TMP was released by the known chemically induced pathway. However, these "lock-in"-compounds led to a delayed drug delivery due to high chemical stability.[4,5] Here, we present a prototype of the new concept of enzymatically activated *cyclo*Sal-pronucleotides, 5-diacetoxymethyl-*cyclo*Sal-d4TMP **2**. In this concept, after passive transport of **2** into cells (step c, Figure 1), the lipophilic acylal substituent having a weak electron-withdrawing effect attached to the aromatic ring is converted into a highly polar aldehyde group with a strong electron-withdrawing effect by intracellular cleavage (step d, Figure 1).

The formed acceptor group leads to a strong decrease in hydrolysis stability and the rapid formation of a charged intermediate **4** (phosphodiester),[3] so that compound **3** should not be effluxed (step e, Figure 1) and the phosphodiester intermediate **4** should not be effluxed at all due to the resulting polarity. From the phosphodiester d4TMP **1** is released subsequently (step f, Figure 1). This concept is based on the higher intracellular concentration of esterases compared to the extracellular medium[6] (Figure 1, step b should not take place) and on a considerable difference



SCHEME 1 Synthesis of 5-diacetoxymethyl-*cyclo*Sal-d4TMP **2**. Method **A**: THF, $LiAlH_4$, room temp. to reflux, 3 h, 91%; method **B**: acetone, 2,2-dimethoxypropane, *p*TsOH, Na_2SO_4, 40°C, 3 d, 95%; method **C**: THF, *n*-BuLi, DMF, –78°C, 3 h, 94%; method **D**: CH_3CN/H_2O, cat. HCl, 81%; method **E**: i) THF, PCl_3, pyridine,–20°C to room temp., 4.5 h; ii) CH_3CN, DIPEA, d4T, –20°C to room temp., 3 h; iii) CH_3CN, *t*BuOOH, –20°C to room temp., 1 h, 31%; method **F**: CH_3CN, acetic anhydride, $ZrCl_4$; room temp., 45 min, 44%.

between the extracellular hydrolysis stability of the 5-diacetoxymethyl-*cyclo*Sal-d4TMP **2** and the intracellular hydrolysis stability of the 5-formyl-*cyclo*Sal-d4TMP **3** after enzymatical cleavage (Figure 1, $t_{1/2}$ step a $\gg$ $t_{1/2}$ step d).

RESULTS

The title compound **2** was synthesized starting from the commercial available 5-bromosalicyl aldehyde **5** as outlined in Scheme 1.

Reduction of **5** gave the 4-bromosalicyl alcohol **6** in 91% yield. After protection of **6** as isopropylidene acetal (**7**, 95% yield) the formyl group was introduced via bromo-lithium exchange to yield 4-formylsalicyl alcohol isopropylidene acetal **8** in 94%. By means of acidic deprotection 4-formylsalicyl alcohol **9** could be obtained in 81% yield. Compound **9** was converted to **3** (mixture of two diastereomeres) using chlorophosphite chemistry as described before.[1] The only modification was an exchange of the solvent. The title compound **2** was synthesized by protection of the formyl group of **3** as an acylal in 44% yield.

The *cyclo*Sal-triesters **2** and **3** were studied for their stability in aqueous 25 mM phosphate buffer (pH = 7.3). As expected, 5-formyl-*cyclo*Sal-d4TMP **3** has a very short half-life of 0.18 h. The half-life of 5-diacetoxymethyl-*cyclo*Sal-d4TMP **2** is 6-fold higher ($t_{1/2}$ = 1.2 h). In the study of **2** no competing hydrolysis of the acylal ester group was observed. So, the half-life corresponds to the cleavage of the triesters and the exclusive formation of d4TMP **1** (proven by ^{31}P-NMR hydrolysis). The cleavage of the acylal group of **2** was shown in hydrolysis studies in T-lymphocyte cell extracts. The half-life was 0.08 h and the corresponding 5-formyl-*cyclo*Sal-d4TMP **3** was formed (HPLC co-elution experiments). *Cyclo*Sal-triester **2** and **3** were tested for

their anti-HIV activity in wild type CEM/0 and mutant thymidine-kinase-deficient CEM/TK$^-$ cells. As reference d4T (active against HIV-1 and HIV-2 in CEM/0 but weakly active in CEM/TK$^-$ cells) was used. Compounds **2** and **3** have the same activity against HIV-1 in wild-type cells and against HIV-2 in CEM/0 cells as d4T (**2**: 0.42±0.28 μM and 0.40±0.0 μM; **3**: 0.41±0.29 μM and 0.15±0.08 μM; d4T: 0.48±0.45 μM and 0.63±0.21 μM).

The activity of triesters **2** and **3** against HIV-2 in CEM/TK$^-$ cells is 26-fold, respectively, 33-fold lower as in CEM/0 cells (**2**: 10.5±8.3 μM; **3**: 5.0±4.6 μM). However, the activity of d4T decreases even 100-fold (d4T: 47.5±26.3 μM). So, a partial delivery of the pronucleotide takes place. The loss of antiviral activity in CEM/TK$^-$is comparable with the values for other acceptor substituted *cyclo*Sal-d4TMPs with low hydrolysis half-lives.[2]

In conclusion, a fast release of NMPs by enzymatic activation out of *cyclo*Sal-prodrugs like compound **2** seems to be possible. Further work will be done to increase the hydrolysis stability in order to achieve full retention of antiviral activity.

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