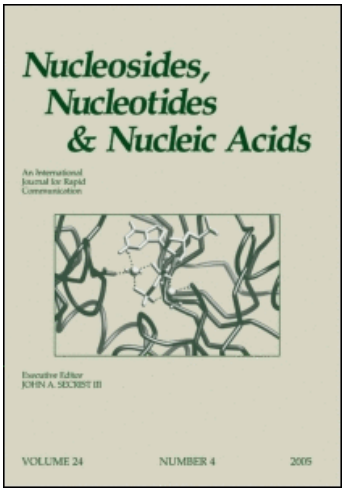

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NEW DEVELOPMENTS OF THE "LOCK-IN" MODIFIED *cyclo*Sal-d4TMPs

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□ *New developments of the "lock-in" modified* cyclo*Sal-d4TMP are reported. Novel prodrugs with variations in the linker chain were introduced. The synthesis, hydrolysis properties in different media (PBS, CEM/0- and liver extracts) and the antiviral activities against HIV are shown.*

Keywords *Cyclo*Sal-pronucleotides; prodrugs; enzymatic cleavage

INTRODUCTION

The *cyclo*Sal-pronucleotides are well-established prodrugs for a delivery of bioactive nucleotides.[1] For these lipophilic prodrugs a passive membrane transport into cells is supposed. So, it can not be excluded that an unfavorable equilibrium is formed through the membrane. Therefore, modified *cyclo*Sal-triesters were designed to trap the compounds inside cells. To achieve this goal, triesters bearing esterase-cleavable sites attached via an alkyl-linker to the *cyclo*Sal-moiety were synthesized. After metabolism a polar *cyclo*Sal-NMP, e.g., carboxylate or alcohol, will be released. This should prevent an efflux from the cells and accumulate the compounds inside ("lock-in"; Figure 1).[2] Our previous work dealt with various alkyl ester and a C2-alkyl-linker attached either in 3- or 5-position to the masking unit. The prodrugs, leading to an alcohol after enzymatic cleavage, were good substrates for esterases. Unfortunately, the formed alcohols were not polar enough for an efficient "lock-in." Esters releasing a carboxylate showed no enzymatic

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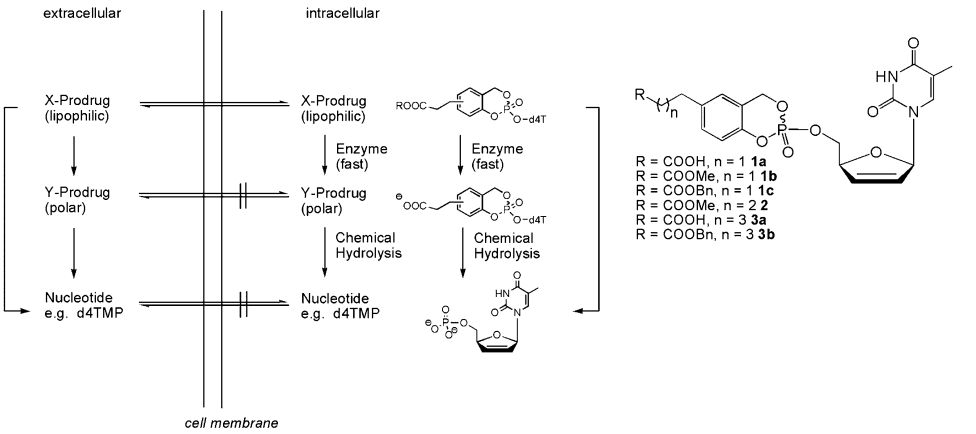

FIGURE 1 Proposed "lock-in" mechanism and "lock-in" modified *cyclo*Sal-d4TMPs.

cleavage.[3] So, the idea was to lengthen the linker chain to improve substrate properties of the ester-modified prodrugs. Spacers with C3- and C4-chains bearing methyl- and benzyl esters were introduced.

RESULTS

Starting material for the synthesis of the new "lock-in"-modified *cyclo*Sal-d4TMPs were 3-butenoic and 4-pentenoic acid and 5-bromosalicyl aldehyde. The acids were converted via DCC-activation into the corresponding methyl- and benzyl ester. The *tert*-butyl ester was prepared by reaction of lithium *tert*-butylate and 4-pentenoic acid chloride. 5-bromosalicyl aldehyde was reduced with $LiAlH_4$ to yield 5-bromosalicyl alcohol. To avoid side reactions the alcohol functions were protected as an isopropyliden acetale by transacetalisation with 2,2-dimethoxypropane. Hydroboration of the 3-butenoates and 4-pentenoates with 9-BBN gave the corresponding trialkylboranes. These compounds were used without further purification (one pot) in a Suzuki cross coupling reaction with 5-bromosalicyl alcohol isopropyliden acteale and Pd(dppf) as catalyst. The obtained ester modified salicyl alcohol isopropyliden acetals were deproctected by a catalytic amount of hydrochloric acid to yield the desired salicyl acohol derivates.[4] These diols were reacted with PCl_3 to yield cyclic chlorophosphanes, which were then used as phosphitylation agents of d4T. The resulting phosphites were oxidized to give *cyclo*Sal-triesters **1-3**. Finally, deprotection of 3-*t*-BuPe-*cyclo*Sal-d4TMP with TFA led to 3-COOHBu-*cyclo*Sal-d4TMP **3a**.

All *cyclo*Sal-prodrugs **1-3** were analyzed according to their hydrolysis half-lives in different media (PBS, CEM/0- and liver extracts) and their anti-HIV activities. In chemical hydrolysis (PBS) half-lives $t_{1/2}$ of 4-6 h for the ester

TABLE 1 Hydrolysis half-lives and anti-HIV activities

			$t_{1/2}$ [h]			EC_{50} [μM]			
Compound	n	R	pH 7.3	CEM/O	Liver	CEM/0 HIV-1	CEM/0 HIV-2	CEM/TK$^-$ HIV-2	CC_{50} [μM] CEM/0
1a	1	-COOH	12.5	11.4	n.d.	0.14 ± 0.10	0.80 ± 0.20	50.0 ± 30.0	76
1b	1	-COOMe	5.7	7.0	n.d.	0.33 ± 0.11	1.05 ± 0.35	1.20 ± 1.10	58.1 ± 1.84
1c	1	-COOBn	4.3	6.5	n.d.	0.08 ± 0.00	1.00 ± 0.28	2.00 ± 0.00	60.6 ± 3.30
2	2	-COOMe	4.3	5.7	3.8	0.26 ± 0.14	0.20 ± 0.10	3.5	74.4 ± 5.30
3a	3	-COOH	9.0	6.8	9.8	n.d.	n.d.	n.d.	n.d.
3b	3	-COOBn	4.0	6.0	0.1	0.37 ± 0.20	0.31 ± 0.08	>10	68.1 ± 11.7
d4T			n.a.	n.a.	n.a.	0.23 ± 0.04	0.24 ± 0.02	15.0 ± 7.1	58.8 ± 24.2

n.d.: not determined; n.a.: not available.

1b-c, **2**, and **3b** were determined. These values have been expected, because the negative inductive effect of the ester groups is separated by the alkyl-spacer from the aromatic moiety. The increased stabilities for the acids **1a** ($t_{1/2}$ = 12.5 hours) and **3a** ($t_{1/2}$ = 9.0 hours) may be attributed to the charge of the carboxylates at pH 7.3. An overall negative charge of the molecule may interfere with the nucleophilic attack at the phosphate group. Hydrolysis studies in human CEM/0 extracts revealed that all esters **1-3** showed no enzymatic cleavage in this medium. The product of the hydrolysis was d4TMP, not the corresponding acids **1a** and **3a**. Due to the lower pH value (6.8) the half-lives for **1-3** were higher than those found in PBS. In contrast in mouse liver extracts an enzymatic reaction for **3b** was observed. The hydrolysis half-life ($t_{1/2}$ = 0.1 hour) was 40-fold lower than in chemical media (PBS $t_{1/2}$ = 4.0 hours). HPLC-co-elution experiments showed unambiguously that the carboxylate **3a** was the main hydrolysis product. The compounds **1b-c** and **2** were not tested or showed no enzymatic reaction. Finally, the "lock-in"-*cyclo*Sal-d4TMPs were tested for their antiviral activities against HIV-1 and HIV-2 in wild-type CEM- and in mutant thymidine kinase deficient cells (CEM/TK$^-$, Table 1). The parent nucleoside d4T was found to be active against HIV-1 and HIV-2 but it lost its activity in the mutant CEM cells. The same has been found for acid **1a**, which can be explained by an incapability of membrane penetration due to the charged carboxylate. The found activity of **1a** wild-type cells (CEM/0) derives from the parent nucleoside d4T, which is generated by chemical hydrolysis of the prodrug and a subsequent dephosphorylation of d4TMP outside cells. The esters **1b**, **2**, and **3b** showed good activities against HIV-1 and HIV-2 (0.2–1.0 μM) in wild-type cells. The benzyl-ester **1c** was very potent (0.08 μM) against HIV-1 and 10-fold lower potency against HIV-2 (1.0 μM). Surprisingly, only for **1b** and **1c** full retention of the activity in the mutant cell-line was observed (TK-Bypass). The triester with C3- (**2**) or C4-alkyl-linker (**3b**) lost their antiviral activities in TK-deficient cells.

CONCLUSION

A new synthesis of "lock-in" modified *cyclo*Sal-d4TMPs has been established and applied in order to vary the linker length of the prodrugs. The introduction of C3- and C4-alkyl-linker to the *cyclo*Sal-moiety did not lead to a significant improvement of esterase affinity.

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