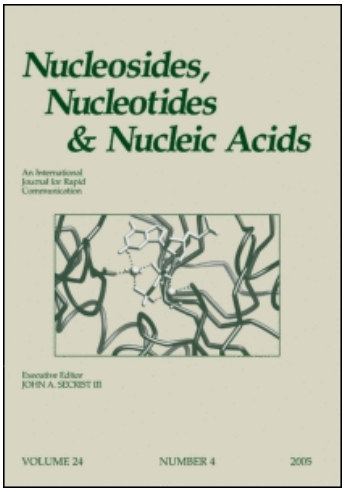

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INTRACELLULAR TRAPPING OF *CYCLO*SAL-PRONUCLEOTIDES BY ENZYMATIC CLEAVAGE

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□ *A new synthesis for cycloSal-pronucleotides bearing enzymatically cleavable triggers is presented. This trigger is introduced to trap the pronucleotide inside cells. The general concept and hydrolysis data in different media are discussed.*

Keywords *cyclo*Sal-nucleotides; pronucleotides; antiviral nucleosides; nucleoside analogues; prodrugs

INTRODUCTION

Nucleoside analogs are widely applied in antitumor and antiviral therapy. However, in many cases the active form is not the nucleoside itself but its triphosphate, which is then incorporated into DNA. Sometimes the cellular or viral kinases do not accept the analogs as substrates or the phosphorylation proceeds at low rates, leading to a decrease or loss of antiviral activity. To circumvent these problems, it would be favorable to apply at least the monophosphates, but these polar compounds do not penetrate cell membranes and are rapidly degraded in plasm. Another possibilty of enhancing antiviral activity is the application of a prodrug that releases the nucleotide analog inside the cell by pH driven or enzymatic hydrolysis. The *cyclo*Sal approach has been applied to many different nucleosides, sometimes resulting in an impressive boost of activity.[1] However, as the release of the nucleotide is pH driven, the prodrug is able to enter but also to leave the cell. This is due to the fact that the hydrolysis half-lives are typically in the range of 4 to 20 hours. A further improvement of the *cyclo*Sal approach might therefore be the attachment of a trigger, which is sensible to enzymes inside a cell. As esterase activity is much higher intracellularly, we decided to use esterase cleavable moieties that release highly polar carboxylates, trapping the prodrug inside the cell.[2] Because first experiments with simple esters attached to the *cyclo*Sal

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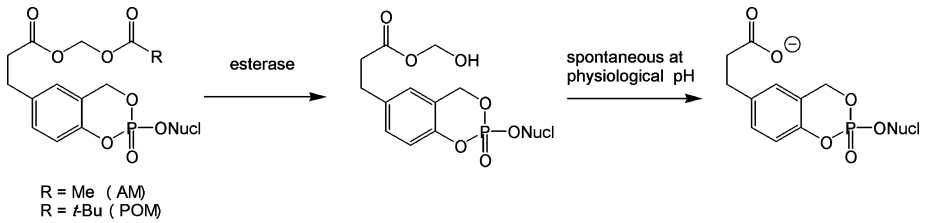
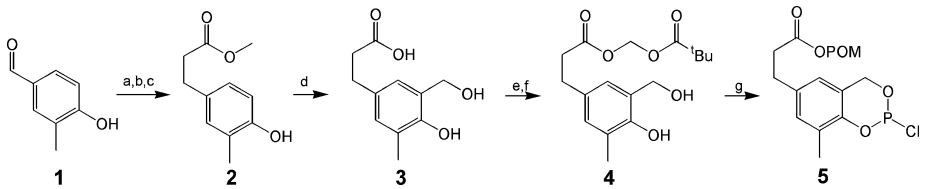


FIGURE 1 Two different triggers (AM and POM) cleaved by esterase.

prodrug did not show enzymatic cleavage, we switched to more elaborate groups. Acetoxymethyl- (AM), pivaloyloxymethyl-moieties (POM) were linked to the aromatic of the *cyclo*Sal-system via a carboxylate. Indeed, these compounds are rapidly degraded by enzymes.[3] The proposed mechanism of degradation for these compounds is given in the Figure 1.

RESULTS

To achieve an effective "lock in," the pH dependent half-life of the compound should be high in contrast to a very low half-life for the enzyme dependent step.[4] The attachment of alkyl groups to the aromatic of the *cyclo*Sal-systems enhances the pH dependent half-life but should not severely influence the stability of the trigger. Therefore, a methyl group was attached to the aromatic in position 3. The starting material 4-hydroxy-3-methylbenzaldehyde **1** was protected with benzyl bromide at the hydroxyl group and then subjected to a Wittig reaction in excellent yields (Scheme 1). The newly introduced CC-double bond was hydrogenated and the protecting group removed in a one pot reaction with Pd/C and hydrogen. The product **2** was converted into the salicylic alcohol **3** via hydroxymethylation in formalin and potassium hydroxide.[5] Under these conditions, the methyl ester was also cleaved. Protection of the hydroxyl groups as a cyclic benzylidene acetal, alkylation of the carboxylate with pivaloyloxymethyl-iodine (POM-I), and cleavage of the protecting group with catalytic amounts of HCl yielded the POM modified salicylic alcohol **4**. Conversion into the corresponding chlorophosphite **5** was achieved by treatment with PCl_3 and pyridine.



SCHEME 1 Synthesis of chlorophosphite **5**. Method a) BnBr, K_2CO_3 (100%); b) Wittig reagent $Ph_3PCHCOOMe$ (92%); c) H_2/Pd/C (100%), d) KOH, HCHO (100%); e) 1. Benzaldehydedimethylacetale, p-TsOH (91%); 2. DIPEA, POM-I (100%); f) [HCl], reflux (78%); g) PCl_3, pyridine (98%).

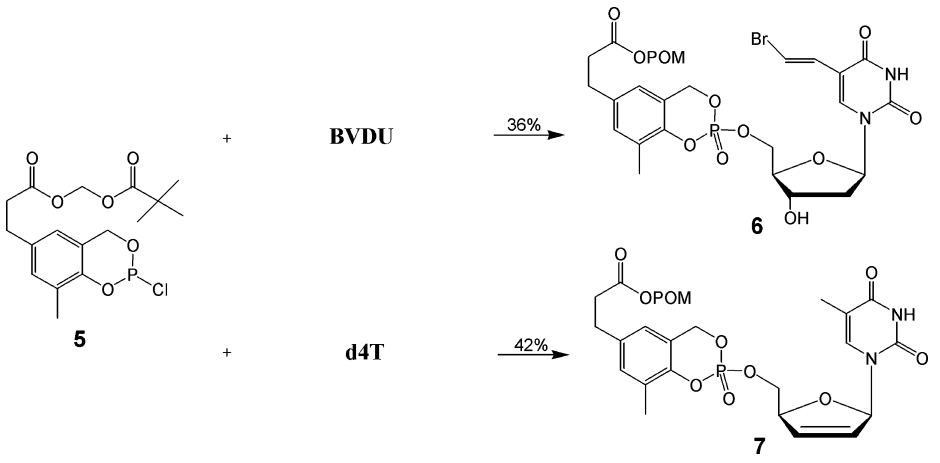


FIGURE 2 Synthesis of 3-Me-5-POM-prop-*cyclo*SalBVDUMP **6** and –d4TMP **7.**

Chlorophosphite **5** was coupled to different nucleoside analogs (BVDU, d4T) under basic conditions and oxidized with *tert*-butylhydroperoxide (Figure 2). ^{1}H-NMR data of compounds **5**, **6**, and **7** are given in the references and comments section.

Compounds **6** and **7** were hydrolyzed in an aqueous buffer (PBS, pH 7.3). The resulting products d4TMP and BVDUMP were analyzed by means of ion-pair RP-chromatography. Another experiment was conducted in CEM/0 (d4T) or P3HR1 (BVDU) cell extract to examine the cleavage of the POM group. The observed hydrolysis half-lives are given in Table 1.

Finally, the stability in plasm was analyzed. In this case, no enzymatic cleavage of the trigger was detected within a period of several hours.

CONCLUSIONS

A new and very efficient route to POM-modified *cyclo*Sal-pronucleotides has been developed. The synthesis of the masking group proceeded in excellent yields for every single step and the obtained chlorophosphite **5** could be coupled to different nucleoside analogs. As expected, striking differences in hydrolysis half-lives of **6** and **7** in aqueous versus biological media were observed. This behavior should eventually lead to drug enrichment inside the cell, trapping the pronucleotide by generating a negative charge, triggered

TABLE 1 Comparison of hydrolysis half-lives

Compound	$t_{1/2}$ [h] (pH = 7.3)	$t_{1/2}$ [h] (cell extract)	Acceleration
6	6.2	0.2	31
7	7.4	0.4	19

by enzymatic cleavage. Additionally, the compounds were not prone to enzymatic cleavage in plasm. This is the basis for an effective "lock in" mechanism: prolonged hydrolysis stability in PBS (pH 7.3), reasonable stability in plasm, and very low half-life in cell extracts, resulting in the generation of a charged intermediate that slowly releases the drug inside the cell.

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^{1}H-NMR: **5** (δ/ppm, 400 MHz, $CDCl_3$): 6.95 (1H), 6.64 (1H), 5.73(2H), 5.39 (1H), 4.97 (1H), 2.86 (2H), 2.64 (2H), 2.20 (3H), 1.18 (9H); **6** (two diastereoisomers, δ/ppm, 400 MHz, DMSO-d_6): 11.59 (1H), 7.77 (1H), 7.28 (1H), 7.11 (1H), 6.93 (1H), 6.87 (1H), 6.15 (1H), 5.68 (2H), 5.37 (2H), 4.28 (3H), 3.93 (1H), 2.77 (2H), 2.68 (2H), 2.52 (3H), 2.17 (5H), 1.09 (9H); **7** (two diastereoisomers,δ/ppm, 400 MHz, $CDCl_3$): 8.00 (1H), 7.22 (1H), 7.01 (1H), 6.99 (1H), 6.76 (1H), 6.36 (1H), 5.93 (1H), 5.74 (2H), 5.25 (2H), 5.01 (1H), 4.36 (2H), 2.88 (2H), 2.64 (2H), 2.23 (3H), 1.73 (3H), 1.19 (9H).