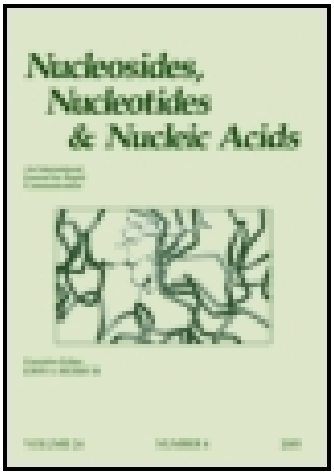

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Cyclo-saligenyl-5-fluoro-2′-deoxyuridine monophosphate (cycloSal-FdUMP) — A New Prodrug Approach for FdUMP

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CYCLO-SALIGENYL-5-FLUORO-2'-DEOXYURIDINEMONOPHOSPHATE (*CYCLO*SAL-FDUMP) - A NEW PRODRUG APPROACH FOR FDUMP -

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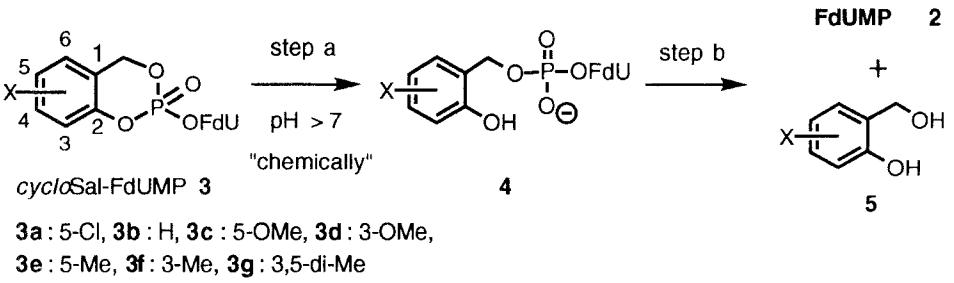
ABSTRACT: The synthesis of *cyclo*Sal-FdUMP 3**a-d** as a new prodrug approach for FdU **1** is described. Phosphotriesters **3** release the FdUMP **2** selectively by a controlled, chemically induced tandem reaction in hydrolysis studies. The biological activity (IC_{50}) of *cyclo*Sal-phosphotriesters **3** was evaluated in FM3A/O cells and FM3A/TK$^-$ cells.

5-Fluorouracil is used as antitumor agent in the treatment of cancer. *In-vivo* it is converted into 5-fluoro-2'-deoxyuridine 5'-phosphate (FdUMP), which acts as a powerful mechanism-based inhibitor of thymidylate synthetase. The major disadvantages in the clinical application of 5-fluorouracil are the low response rates and the development of resistance. The direct administration of FdUMP might circumvent these problems, but nucleotides are negatively charged at physiologic pH and unable to penetrate cell membranes or the blood brain barrier due to their low lipophilicity. In addition, nucleotides are susceptible to rapid degradation to the corresponding nucleosides in tissues by nonspecific phosphohydrolases such as phosphatases and nucleotidases. A concept to overcome this limitation is the delivery of the 5'-monophosphate from an uncharged lipophilic prodrug[1].

In this work we present the synthesis and properties of ***cyclo*sal**igenyl 5-**f**luoro-2'-**d**eoxy**u**ridine-**m**ono**p**hosphate **3** (*cyclo*Sal-FdUMP, scheme 1) as neutral prodrugs of FdUMP **2**. This prodrug concept was designed to release the nucleotide **2** *selectively* by ***controlled, chemically induced hydrolysis*** following a tandem-mechanism. In contrast to other reported prodrug concepts our approach leads to FdUMP **2** after a *coupled* cleavage of the phenyl- and the benzylester bond of the phosphotriester **3**. The rational of our new prodrug concept **3** applied here on 5-fluoro-2'-deoxyuridine **1** (FdU) is based on the already published difference in stability of the phenyl- and the benzyl phosphate ester which allows us to discriminate between the different phosphate ester bonds. The hydrolysis concept

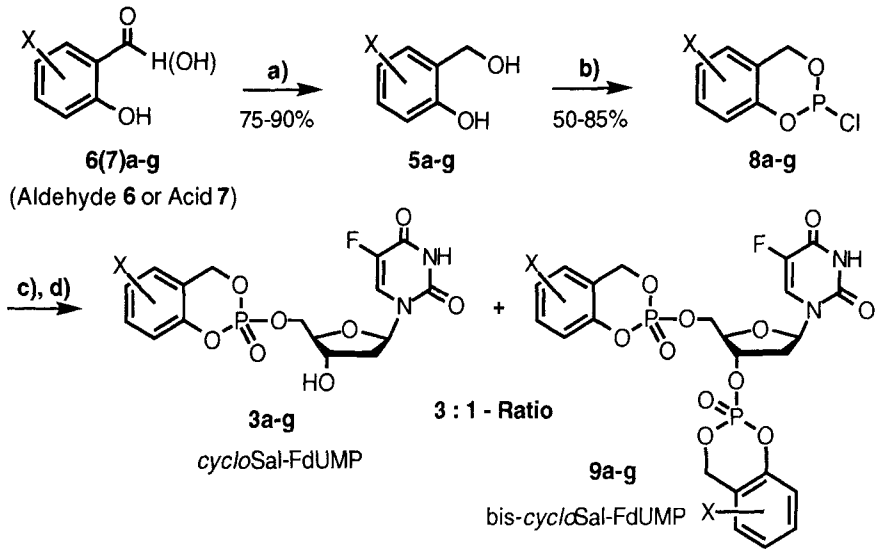


SCHEME 1: The hydrolysis pathways of *cyclo*Sal-FdUMP phosphotriesters **3**

has already been verified and is summarized in scheme 1[2]. It involves a selective first cleavage of the phenolic ester bond to give 2-hydroxybenzylphosphodiester **4** (step a) and subsequently a spontaneous cleavage of **4** releasing FdUMP **2** and the salicylalcohols **5** (step b; tandem-reaction)[2].

The title compounds **3** were synthesized as outlined in scheme 2. The salicylaldehydes **6** or salicylic acids **7** were reduced by standard procedures to give the salicylalcohols **5** in 75-90% yield. Then the salicylalcohols **5** were reacted with phosphorustrichloride to yield the cyclic saligenylchlorophosphanes **8** (50-85% yield). The subsequent synthesis of *cyclo*Sal-FdUMP's **3a-g** was first carried out using 3'-(OLev)-thymidine as model nucleoside: *cyclo*Sal-3'-(OLev)TMP was synthesized successfully following our procedure described before[2]. The deprotection of the levulinic group was achieved by treatment of *cyclo*Sal-3'-(OLev)TMP with hydrazine hydrate in almost quantitative yield. In contrast, this procedure was unsuccessful in the case of the title compounds **3**: While the reaction to *cyclo*Sal-3'-(OLev)FdUMP proceeded in good yield, the deprotection of the 3'-OLev group was unsuccessful and resulted in nearly complete degradation of the target compounds **3** (7%). Due to this result, we started to synthesize the *cyclo*Sal-FdUMP's without any protection of the 3'-OH group. FdU **1** and 1.0 equiv. chlorophosphanes **8a-g** reacted at O°C in the presence of di*iso*propylethylamine (DIPEA) and were oxidized *in-situ* with *t*-butylhydroperoxide. In addition to the desired *cyclo*Sal-FdUMP's **3a-g**, the 3',5'-bis*cyclo*Sal-FdUMP **9a-g** were obtained in a 3:1-ratio. After purification, **3a-g** and **9a-g** were characterized by means of ^{1}H, ^{13}C, and ^{31}P nmr, UV, and electrospray (ESI) mass spectrometry (negative mode). The title compounds **3a-g** were isolated as a diastereomeric mixture in 1:1-ratio and were separated by semipreparative HPLC. The configuration was assigned with the X-ray analysis of 5-Cl-*cyclo*Sal-(-)-MenthylMP as model compound (data not shown). The attribution of configuration at the P-atom of the separated *cyclo*Sal-FdUMP's was done by their CD-effect's as compared to the aforementioned model compound[3].

Hydrolysis data were obtained in different buffer systems. As a model for the physiological milieu *cyclo*Sal-FdUMP's **3** were hydrolyzed in 50 mmol phosphate buffer, pH



a) $NaBH_4$ for **6**, i-PrOH ($LiAlH_4$ for **7**, Et_2O); b) PCl_3, pyridine, Et_2O, -10°C, 2 h; c) FdU **1**, DIPEA, CH_3CN, 0°C, 10 min.; d) TBHP, CH_3CN, rt, 30 min.

SCHEME 2: Synthesis of the *cyclo*Sal-FdUMP's **3a-g**

TABLE 1: Hydrolysis in different aqueous buffers, and antitumor activity of *cyclo*Sal-FdUMP's **3**

3 or FdU **1**	Hydrolysis ($t_{1/2}$) in buffers					Antitumor Activity IC_{50} (μM/ml)	
X	TRIS pH 6.9 [h]	phosphate pH 7.29 [h]	borate pH 8.9 [h]	RPMI pH 7.4 (h)	RPMI + 10% FCS	FM3A/O	FM3A/ TK^-
5-Cl	6.8	0.9	0.4	1.5	2.2	0.05	3.25
H	17.9	1.9	0.9	5.4	2.2	0.09	3.45
5-OMe	18.6	2.8	1.0	4.6	3.6	0.09	3.95
3-OMe	11.3	1.4	0.6	3.0	2.1	0.08	3.65
5-Me	21.3	2.8	1.3	6.9	5.1	0.11	4.00
3-Me	59.2	7.0	1.8	7.4	8.3	0.12	4.06
3.5-Me	41.5	10.6	2.1	11.1	10.7	0.22	5.15
1	--	--	--			0.009	1.98

7.29 at 37°C. In order to investigate the pH dependence of hydrolysis triesters **3** were also studied in TRIS buffer (50 mmol, pH 6.8) and borate buffer (30 mmol, pH 8.9).

The hydrolyses were followed by means of HPLC analysis. At pH>7 all *cyclo*Sal-FdUMP's **3** were degraded to give only FdUMP **2** and salicylalcohols **5**. Furthermore, the expected pH dependence was observed. The half lives are summarized in table 1. All these results are in fully agreement with the designed hydrolysis pathway (scheme 1). Additionally, hydrolysis studies were carried out in RPMI culture medium with or without 10% heat-inactivated fetal calf serum (FCS). The products were identical as before (FdUMP **2** and diols **5**) but the half lives varied as can be seen in table 1. Nevertheless, the strong donor substituted compounds **3e**, **3f**, and **3g** showed half lives in FCS containing RPMI medium that should be high enough in order to serve as prodrug forms. This assumption was verified by the evaluation of the inhibitory effects of *cyclo*Sal-FdUMP's **3** on the proliferation of murine mammary carcinoma cells (FM3A/O) and the corresponding thymidine kinase deficient cell line (FM3A/TK$^-$). The results are summarized in table 1. In the wild type cell line, all *cyclo*Sal-FdUMP's **3** exhibited an inhibitory effect that was by a factor of 5 to 10 lower as compared to FdU **1**. Additionally, compounds **3** were by a factor of 2 weaker in activity than **1** in the TK$^-$ cells. Consequently, *cyclo*Sal-FdUMP's **3** do not serve as FdUMP prodrug forms. This result was not expected from the hydrolysis and previous studies[3]. Interestingly, a correlation of the inhibitory effect with the half lives was observed in both cell lines: with increasing stability, the activity decreases. Comparable low inhibitory effects were found in murine leukemia cells (L1210/O), and human T-lymphocyte cells (Molt4/C8, CEM/O, CEM/TK$^-$; data not shown). Moreover, it should be mentioned that also the 3',5'-bis*cyclo*Sal-FdUMP's **9** were completely ineffective (data not shown).

In summary, despite the selective delivery of FdUMP **2** from *cyclo*Sal-FdUMP's **3** in hydrolysis studies, triesters **3** were weakly active in FM3A/O cells and completely inactive in TK$^-$ cells. Further work is currently in progress in order to explain this unexpected results.

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