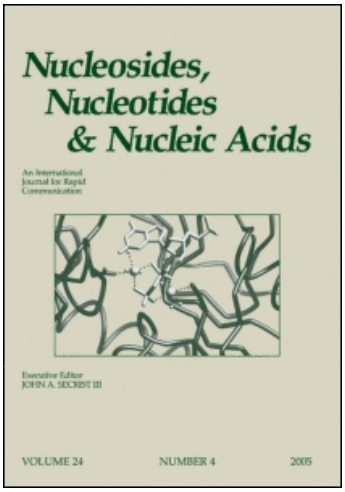

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Lorey, Martina , Meiera, Chris , De Clercq, Eric and Balzarini, Jan(1997) 'New Synthesis and Antitumor Activity of *Cyclo*Sal- Derivatives of 5-Fluoro-2'-deoxyuridinemonophosphate', Nucleosides, Nucleotides and Nucleic Acids, 16: 5, 789 — 792
To link to this Article: DOI: 10.1080/07328319708002952
URL: http://dx.doi.org/10.1080/07328319708002952

NUCLEOSIDES & NUCLEOTIDES, 16(5&6), 789-792 (1997)

NEW SYNTHESIS AND ANTITUMOR ACTIVITY OF *CYCLO*SAL-DERIVATIVES OF 5-FLUORO-2'-DEOXYURIDINEMONOPHOSPHATE

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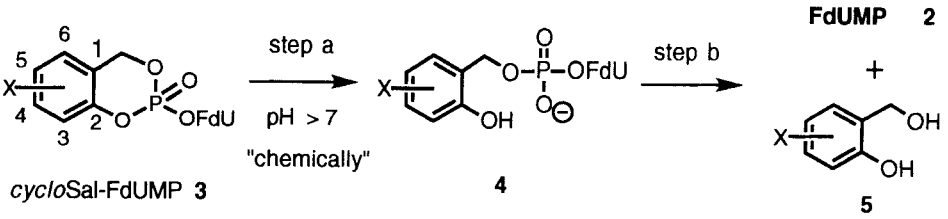
ABSTRACT: An improved synthesis of 5'-*cyclo*Sal-FdUMP 3**a-g** and 3',5'-bis-*cyclo*Sal-FdUMP **9a-g** as potential prodrugs of FdU **1** is described. In hydrolysis studies, phosphotriesters **3** released FdUMP **2** selectively by a tandem reaction. The biological activity of *cyclo*Sal-phosphotriesters **3** and **9** was evaluated in different cell lines.

Analogues of nucleosides, particularly their 5'-nucleoside mono-, di-, and triphosphates play an important role as antimetabolites in cellular metabolism. In the case of the antitumor agent 5-fluoro-2'-deoxyuridine **1** (FdU) the bioactive metabolite is 5-fluoro-2'-deoxyuridine-5'-monophosphate **2** (FdUMP), a mechanism-based inhibitor of the target enzyme thymidylate synthetase. The use of **2** as chemotherapeutic is limited due to two reasons: First, FdUMP is negatively charged at physiologic pH and the resulting low lipophilicity enables passive membrane penetration into cells. Second, nucleotides are rapidly degraded in the blood and on cell surfaces by nonspecific phosphohydrolases. To overcome these limitations, many attempts have been made to realize a prodrug system which releases the nucleotide intracellularly and selectively by chemical or enzymatic hydrolysis (***pronucleotide-approach***)[1].

In this work we present a new, improved route for the synthesis as well as the biological activity of ***cyclo*sal**igenyl 5-**f**luoro-2'-**d**eoxy**u**ridine-**m**ono**p**hosphates **3** (*cyclo*Sal-FdUMP, scheme 1) as neutral prodrugs of FdUMP **2**. The *cyclo*saligenyl pronucleotide concept was designed to release nucleotides as **2** selectively by *controlled, chemically induced hydrolysis* following a tandem-mechanism[2]. The hydrolysis concept has been verified and is summarized in scheme 1[3,4]. The proposed mechanism of hydrolysis starts with the cleavage of the phenolic ester bond to give 2-hydroxybenzylphosphodiester **4** (activating step; step a). In a spontaneous second step the decomposition of **4** yielded FdUMP **2** and the salicylalcohols **5** (step b; tandem-reaction)[2,3].

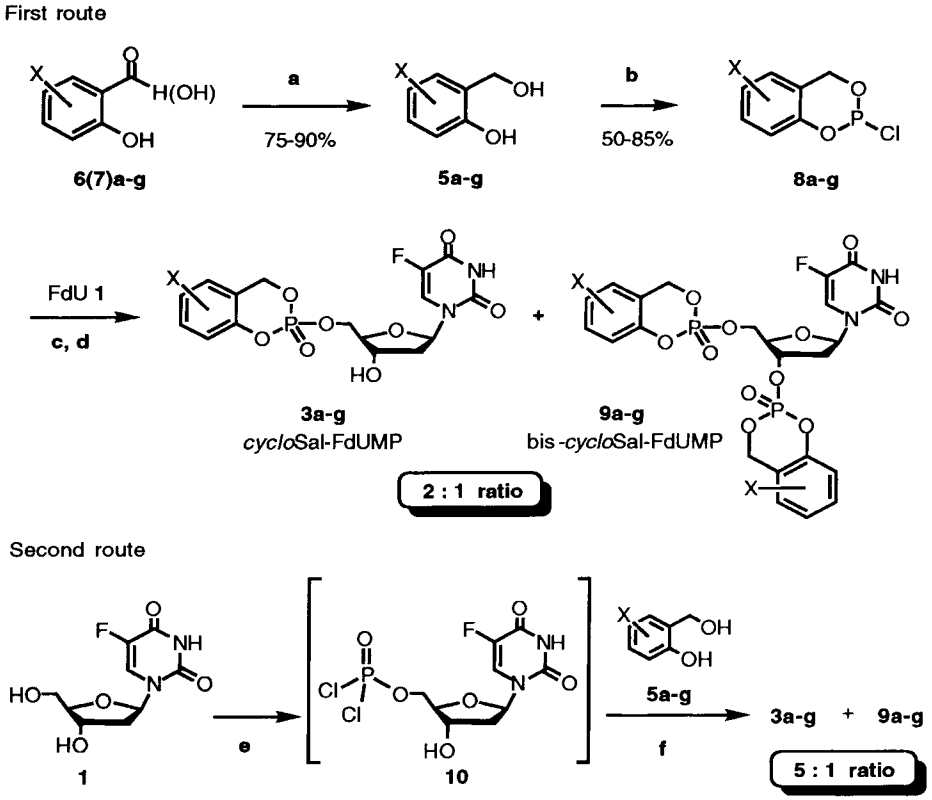


3a : 5-Cl, **3b** : H, **3c** : 5-OMe, **3d** : 3-OMe,
3e : 5-Me, **3f** : 3-Me, **3g** : 3,5-di-Me

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

The *cycloSal*-FdUMPs **3a-g** were synthesized using two different routes as outlined in scheme 2. Starting materials in both routes were the salicylalcohols **5a-g**, which were obtained by standard reduction of salicylaldehydes **6** or salicylic acids **7**. In the previously described synthesis (first route), salicylalcohols **5a-g** were reacted with phosphorustrichloride to yield the cyclic saligenylchlorophosphanes **8a-g** (50-85%)[4]. In a following step FdU **1** was reacted with 1.0 equiv. cyclic chlorophosphanes **8a-g** in the presence of distilled DIPEA to give cyclic phosphites which were subsequently oxidized to triesters **3** with *t*-butylhydroperoxid. In addition to the desired *cycloSal*-FdUMPs **3a-g**, the 3',5'-bis-*cyclo*-Sal-FdUMPs **9a-g** were obtained (**3**:**9** = 2:1 ratio). In the new synthesis (second route) FdU **1** was reacted with phosphorus oxychloride to give the 5-fluoro-2'-deoxyuridine 5'-phosphorus dichloridate **10** which was treated *in-situ* with salicylalcohols **5a-g** to yield again the *cyclo*Sal-FdU phosphotriesters **3** and **9**. The reaction was carried out in THF as solvent in contrast to acetonitrile according to the first route. In the second route we obtained a higher regioselectivity of the phosphorylation site (**3**:**9** = 5:1 ratio). The title compounds **3** and **9** were isolated via both routes as 1:1- or 1:1:1:1 diastereomeric mixtures. After purification, triesters **3** and **9** were characterized by means of ^{1}H, ^{13}C, and ^{31}P nmr, UV, and electrospray mass spectrometry. The purity of the compounds was verified by means of analytical HPLC.

Hydrolysis studies were carried out in RPMI culture medium with and without 10% heat-inactivated fetal calf serum (FCS) at 37°C. The hydrolyses were followed by means of HPLC. All *cyclo*Sal-FdUMPs **3** were degraded to give exclusively FdUMP **2** and salicyl-alcohols **5**. The half lives are summarized in table 1. The results of the hydrolysis studies in RPMI culture medium containing FCS suggest that at least the donor substituted compounds **3e**, **3f**, and **3g** should be high enough to serve as prodrug forms of FdUMP **2**. Therefore, the inhibitory effects of *cyclo*Sal-FdUMPs **3** and 3',5'-bis-*cyclo*Sal-FdUMPs **9** on the proliferation of murine L1210/0 leukemia cells, human T-lymphocyte cells (Molt4/C8, CEM/0) and a thymidine-kinase-deficient cell line (CEM/TK$^-$) were evaluated. The results are summarized in table 1.



a) $NaBH_4$ for **6**, i-PrOH, ($LiAlH_4$ for **7**, THF); b) PCl_3, pyridine, Et_2O, -10°C, 2h; c) FdU **1**, DIPEA, CH_3CN, 0°C, 10 min; d) TBHP, CH_3CN, rt, 30 min; e) DIPEA, THF, 0°C, 2h; f) **5a-g**, DIPEA, 2h, 0°C- rt

SCHEME 2: The two synthesis pathways of the *cyclo*Sal-FdUMPs **3a-g**

TABLE 1: Hydrolysis in RPMI culture medium with or without FCS and antitumor activity of *cyclo*Sal-FdUMPs **3** and 3',5'-bis-*cyclo*Sal-FdUMPs **9**

3 or **9** and FdU **1**	Hydrolysis ($t_{1/2}$) of **3** in buffers (h)		Antitumor Activity of **3** IC_{50} (μM/ml)				Antitumor activity of **9** IC_{50} (μM/ml)	
	RPMI pH 7.4	RPMI + 10% FCS	L1210/0 cells	Molt4/C8 cells	CEM cells	CEM/TK⁻ cells	CEM cells	CEM/TK⁻ cells
a	1.5	2.2	0.021	20.2	0.055	10.9	0.61	55.0
b	5.4	2.2	0.026	20.1	0.070	13.2	0.64	>100
c	4.6	3.6	0.032	16.6	0.065	15.2	0.89	>100
d	3.0	2.1	0.030	2.65	0.071	16.4	0.41	>100
e	6.9	5.1	0.042	3.96	0.049	11.4	0.73	88.6
f	7.4	8.3	0.040	3.28	0.029	4.80	0.44	84.0
g	11.1	10.7	0.074	4.63	0.072	11.5	0.46	56.8
1	-	-	0.003	15.5	0.058	3.55	0.058	3.55

In the wild-type cell lines all *cyclo*Sal-FdUMPs **3** showed inhibitory effect comparable to FdU **1**. In the TK^- cells the activity was more than 230 times weaker than in the wild-type cells. Additionally, triesters **3** were 3-4 times less active than FdU **1** in these cells. Consequently, *cyclo*Sal-FdUMPs **3** seem to serve only as FdU depot forms and serve not as FdUMP prodrugs. These results are in sharp contrast to our findings with the corresponding d4T-*cyclo*Sal-derivatives described before[3]. It seems, that a pro-nucleotide concept which is working with the antiviral active nucleoside d4T does not give *a-priori* also a working pro-nucleotide of the antitumor active nucleoside FdU **1**. This conclusion is also confirmed by the results of other groups working on pro-nucleotide concepts that are different from ours[5]. Furthermore, it should be mentioned that the 3',5'-bis-*cyclo*Sal-FdUMPs **9** were completely ineffective in wild type line and in TK^- cells.

In summary, further work is currently in progress in our laboratory in order to study the unexpected low *in-vitro* activity of triesters **3** despite the selective delivery of FdUMP **2** from *cyclo*Sal-FdUMPs **3** in FCS-containing RPMI culture medium.

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