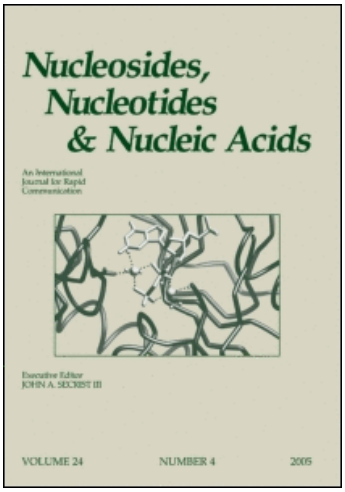


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Cyclo-saligenyl-2',3'-dideoxy-2',3'-didehydroythymidinemonophosphate (*cyclo*Sal-d4TMP) — A New Pro-Nucleotide Approach

Chris Meier[a]; Martina Lorey[a]; Eric De Clercq[b]; Jan Balzarini[b]
[a] Institute of Organic Chemistry, Johann Wolfgang Goethe-University, Frankfurt am Main, Germany [b] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

To cite this Article Meier, Chris , Lorey, Martina , De Clercq, Eric and Balzarini, Jan(1997) '*Cyclo*-saligenyl-2',3'-dideoxy-2',3'-didehydroythymidinemonophosphate (*cyclo*Sal-d4TMP) — A New Pro-Nucleotide Approach', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1303 — 1306
To link to this Article: DOI: 10.1080/07328319708006176
URL: http://dx.doi.org/10.1080/07328319708006176

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1303-1306 (1997)

CYCLO-SALIGENYL-2',3'-DIDEOXY-2',3'-DIDEHYDROYTHYMIDINEMONOPHOSPHATE (*CYCLO*SAL-D4TMP) - A NEW PRO-NUCLEOTIDE APPROACH -

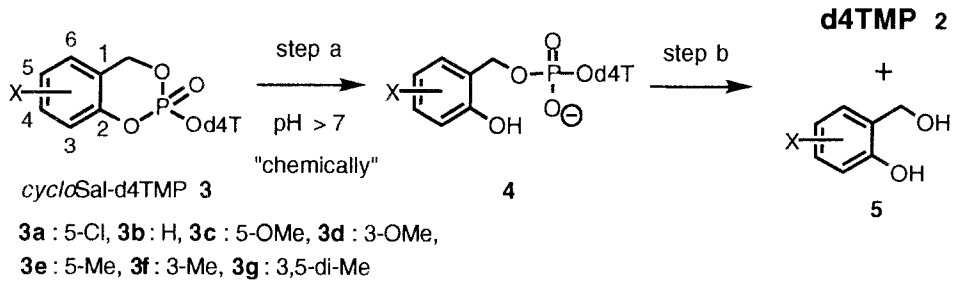
Chris Meier[a]*, Martina Lorey [a], Eric De Clercq[b], and Jan Balzarini[b]

a) Institute of Organic Chemistry, Johann Wolfgang Goethe-University, Marie-Curie-Str.11, D-60439 Frankfurt am Main, Germany
b) Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

ABSTRACT: The synthesis of *cyclo*Sal-d4TMP 3**a-g** as new pro-nucleotide approach for d4TMP **2** is described. Phosphotriesters **3** release the d4TMP **2** selectively by a controlled, chemically induced tandem reaction. *Cyclo*Sal-phosphotriesters **3** exhibited high biological activity against HIV-1/HIV-2 in CEM cells which was completely retained in CEM TK$^-$ cells.

2',3'-Dideoxy-2',3'-didehydrothymidine **1** (d4T, Stavudine, Cerit®) is used as anti-viral agent in the treatment in AIDS. As compared to the anti-HIV drug AZT, d4T **1** shows comparable selective anti-HIV activity *in-vitro*. Moreover, d4T **1** has been found to be less toxic than AZT for bone marrow stem cells and to be less inhibitory to mitochondria DNA replication. After penetration through cell membranes, intracellular conversion of **1** into the 5'-triphosphate is essential for the expression of the biological activity, but the first thymidine kinase catalyzed phosphorylation into d4T-monophosphate **2** (d4TMP) is the limiting step of the metabolization in CEM and MT-4 cells. Consequently, the direct introduction of d4TMP **2** should bypass the limiting step and have advantages for the biological activity. Unfortunately, the highly polar monophosphate **2** is not able to penetrate membranes due to low lipophilicity. One attempt to improve the therapeutic potential of d4T **1** is the use of neutral, lipophilic prodrugs of d4TMP **2** (***Pro-Nucleotide-Approach***)[1].

Here we present the synthesis and properties of ***cyclo*sal**igenyl **d4T-mo**nophosphate **3a-g** (*cyclo*Sal-d4TMP, scheme 1) as neutral prodrugs of d4TMP **2**. This prodrug concept was designed to release the nucleotide **2** *selectively* by ***controlled, chemically induced hydrolysis*** according to a tandem-mechanism. In contrast to other reported prodrug concepts, our approach leads to d4TMP **2** after a *coupled* cleavage of the phenyl- and the benzylester bond of phosphotriesters of type **3**. The rational of our new prodrug concept introduced here with **3** is based on the well known difference in stability of the phenyl- and



SCHEME 1: The hydrolysis pathways of *cyclo*Sal-d4TMP phosphotriesters **3**

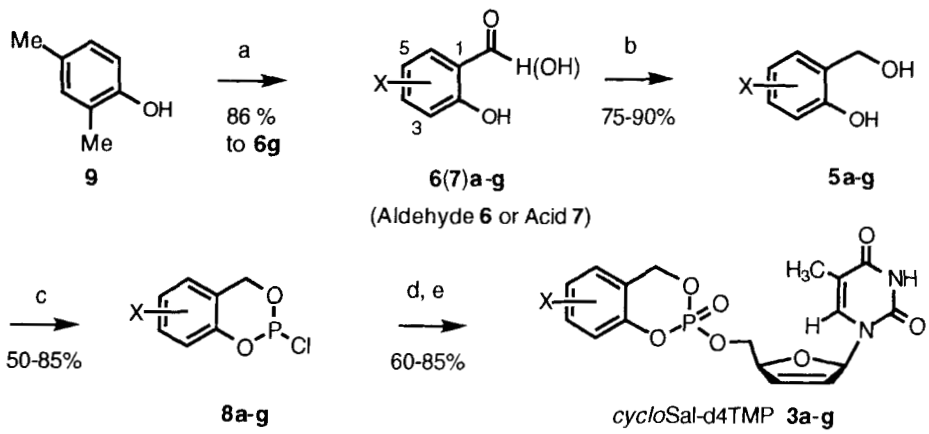
the benzyl phosphate ester that allows us to discriminate between the different phosphate ester bonds. The hydrolysis concept has already been verified and is summarized in scheme 1[2]. It involves a selective first cleavage of the phenolic ester bond to give 2-hydroxybenzyl-phosphodiester **4** (step a) and subsequently a spontaneously induced cleavage of **4** releasing d4TMP **2** and the salicylalcohols **5** (step b; tandem-reaction). Here we studied the effect of some more donor-substituted derivatives of the general structure **3**.

The title compounds **3a-g** were synthesized as outlined in scheme 2. Salicylaldehydes **6** or salicylic acids **7** were used as starting materials, which were reduced by standard procedures to give the salicylalcohols **5** in 75-90% yield. Diols **5** were reacted with PCl_3 to yield the cyclic saligenylchlorophosphanes **8a-g** (50-85% yield). The *cyclo*Sal-d4TMP's **3a-g** were obtained from d4T **1** and 1.5 equiv. chlorophosphanes **8a-g** at O°C in the presence of di-*iso*propylethylamine (DIPEA) and subsequent *in-situ* oxidation with *t*-butylhydroperoxide. After purification (60-85% yield, 1:1 diastereomeric mixtures), the *cyclo*Sal-d4TMP's **3a-g** were characterized by means of ^{1}H, ^{13}C, and ^{31}P nmr, UV, and electrospray (ESI) mass spectrometry (negative mode). In the case of the 3,5-dimethyl derivative **3g**, we started from 2,4-dimethylphenol **9**, which was formylated using dichloromethylmethylether in the presence of $TiCl_4$ to give the appropriate salicylaldehyde **7g** in 86% yield (scheme 2).

The partition coefficients (PC values) of *cyclo*Sal-d4TMP's **3a-g** were determined in 1-octanol/phosphate buffer (pH 6.5) and are listed in table 1.

As a model for the physiological milieu, *cyclo*Sal-d4TMP's **3** were hydrolyzed in 30 mmol phosphate buffer, pH 7.29 at 37°C. In order to investigate the pH dependence of hydrolysis triesters **3a-g** 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. Under the hydrolysis conditions at pH>7 all *cyclo*Sal-d4TMP's **3a-g** were degraded following pseudo-first order kinetics to give *only* d4TMP **2** and the salicylalcohols **5**. At pH<7, the 3,5-dimethyl-substituted derivative **3g** yielded also the "wrong" phenyl-d4T-phosphodiester in 30%. Furthermore, the expected pH dependence was observed. The half lives are summarized in



a) 5 equiv. $TiCl_4$, 3 equiv. Cl_2HCOCH_3, CH_2Cl_2, 0°C, 12 h, rt; b) $NaBH_4$ for **6** ($LiAlH_4$ for **7**); c) PCl_3, pyridine, Et_2O, -10°C, 2 h; d) d4T **1**, DIPEA, CH_3CN, 0°C, 20 min.; e) TBHP, CH_3CN, rt, 30 min.

SCHEME 2: Synthesis of the *cyclo*Sal-d4TMP's **3a-g**

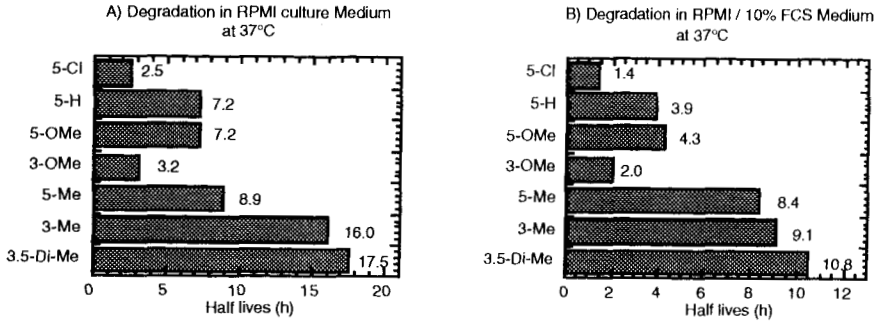


FIGURE 1: Hydrolysis of *cyclo*Sal-d4TMP's **3a-g** in RPMI 1640 culture medium (A) and RPMI 1640 containing 10% heat-inactivated FCS (B)

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 mentioned before (d4TMP **2** and diols **5a-g**) but the half lives varied as can see in figure 1. Nevertheless, at least the strong donor substituted compounds **3d**, **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 antiviral activity of *cyclo*Sal-d4TMP's **3a-g** against HIV-1 and HIV-2 infected CEM/O cells and HIV-2 infected CEM-thymidine kinase deficient (TK^-) cells (table 1). The *cyclo*Sal-d4TMP's **3a**, **3b**, **3c**, and **3d**,

TABLE 1: Hydrolysis in different aqueous buffers, PC values, and antiviral activity of *cyclo*Sal-d4TMP's **3**

3a-g, d4T **1**	Hydrolysis ($t_{1/2}$) in buffers			Antiviral Activity EC_{50} (μg/ml)			PC value
X	TRIS pH 6.9 [h]	phosphate pH 7.29 [h]	borate pH 8.9 [h]	CEM/O HIV-1	CEM/O HIV-2	CEM/TK⁻ HIV-2	
5-Cl	6.4	0.7	0.3	0.40	0.40	**0.70**	7.57
H	24.5	4.5	1.1	0.25	1.20	**1.20**	1.90
5-OMe	28.3	7.2	1.07	0.60	0.70	**0.70**	2.30
3-OMe	9.5	1.4	0.4	0.70	0.60	**4.00**	0.83
5-Me	28.3	8.0	1.3	0.25	1.00	**0.60**	5.45
3-Me	68.5	10.2	1.5	0.10	0.25	**0.25**	5.08
3,5-Me	98.2	16.1	3.4	0.25	0.40	**0.15**	15.33
1	--	--	--	0.40	1.45	**45**	0.15

which have half live in RPMI/FCS medium up to 4.3h, showed at least equal activity in the wild type cell line as d4T **1** and (partly) a somewhat lower activity in the TK⁻ cell line. In contrast, all methyl-substituted triesters **3e-g** exhibited higher activity already in the CEM/O cell line as **1**, which is completely *retained* in the TK⁻ cell line. This correlates with the higher stability of **3e-g** as compared to **3a-d** in the hydrolyses studies. No direct correlation of the antiviral activity with the lipophilicity of **3a-g** (PC values) was observed.

In summary, the described prodrug concept is suitable to deliver d4TMP **2** from *cyclo*Sal-d4TMP's **3a-g** by a non-enzymatical activation at physiological pH and donor-substituted derivatives of **3a-g** were highly active in CEM/TK⁻ cells. Further work is currently in progress in our laboratory in order to explore this *pro-nucleotide concept.*

REFERENCES

1. Sergheraert, C.; Pierlot, C.; Tartar, A.; Henin, Y.; Lemaitre, M.; *J. Med. Chem.* **1993**, *36*, 826; Girardet, J.-L.; Perigaud, C.; Aubertin, A.-M.; Gosselin, G.; Kirn, A.; Imbach, J.-L.; *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2981; McGuigan, C.; Cahard, D.; Sheeka, H.M.; De Clercq, E.; Balzarini, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1183.
2. Meier, C.; *Angew. Chem.* **1996**, *108*, 77; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 70; Meier, C; Lorey, M.; De Clercq, E.; Balzarini, J.; *Antiviral Res.* **1996**, *28*, A29.