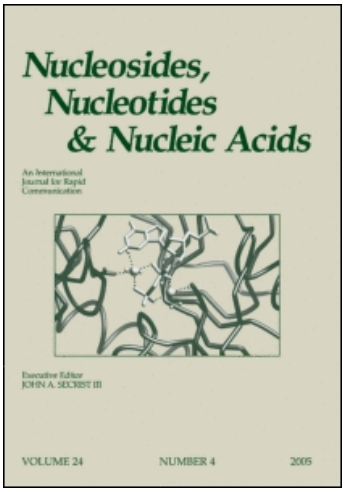

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Cyclo-Saligenyl-3'-Azido-2',3'-Dideoxy- Thymidinemonophosphate (*cyclo*Sal-AZTMP) - A New Pro-Nucleotide Approach[1] ?-

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To cite this Article Meiera, Chris , De Clercq, Eric and Balzarini, Jan(1997) '*Cyclo*-Saligenyl-3'-Azido-2',3'-Dideoxy-Thymidinemonophosphate (*cyclo*Sal-AZTMP) - A New Pro-Nucleotide Approach[1] ?-', Nucleosides, Nucleotides and Nucleic Acids, 16: 5, 793 — 796
To link to this Article: DOI: 10.1080/07328319708002953
URL: http://dx.doi.org/10.1080/07328319708002953

NUCLEOSIDES & NUCLEOTIDES, 16(5&6), 793-796 (1997)

CYCLO-SALIGENYL-3'-AZIDO-2',3'-DIDEOXY-THYMIDINEMONOPHOSPHATE (*CYCLO*SAL-AZTMP) - A NEW PRO-NUCLEOTIDE APPROACH[1] ?-

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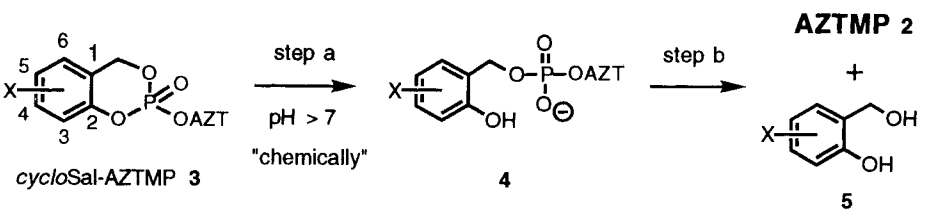
ABSTRACT: The synthesis of *cyclo*Sal-AZTMPs 3**a-h** as new pro-nucleotides of AZTMP **2** is described. Phosphotriesters **3** selectively release AZTMP **2** by a controlled, chemically induced tandem reaction. *Cyclo*Sal-AZTMPs **3** exhibited high biological activity in HIV-1/HIV-2 infected CEM/O cells but lost their activity nearly completely in CEM/TK$^-$ cells.

Nucleoside analogues, e.g. 3'-azido-2',3'-dideoxythymidine **1** (AZT, Zidovudine, Retrovir®), are widely used as antiviral agents in the treatment for AIDS and the AIDS related complex (ARC). After penetration through the cell membranes, conversion of the nucleoside analogues into their 5'-mono-, di- and triphosphates by cellular kinases is essential for the expression of their biological activity[2]. But because of their different structure compared to natural nucleosides, they are often not readily converted into the triphosphates. One attempt to improve the biological activity of the nucleoside analogues is the intracellular delivery of their monophosphates from neutral, lipophilic prodrugs in order to bypass the limiting step (*Pro-Nucleotide-Approach, Kinase-bypass*)[3].

Here we describe the synthesis and properties of ***cyclo*saligenyl AZT-monophosphates 3a-h** (*cyclo*Sal-AZTMP, scheme 1) as potential neutral prodrugs of AZTMP **2**. In contrast to other prodrug concepts[4], our prodrug approach was designed to deliver nucleotides as **2** selectively and directly by *controlled, chemically induced hydrolysis* according to a *coupled* cleavage of two phosphate ester bond of the neutral precursor **3** (tandem-mechanism). The rational of the concept presented here is based on the different stability of the phenyl- and the benzyl ester which allows us to discriminate between the different phosphate ester bonds[5]. The hydrolysis concept has already been verified and is summarized in scheme 1: It involves a selective first cleavage of the phenyl ester to give 2-hydroxybenzylphosphodiester **4** (step a) and then a spontaneously induced cleavage of **4**



SCHEME 1: The hydrolysis pathway of *cyclo*Sal-AZTMP phosphotriesters **3**

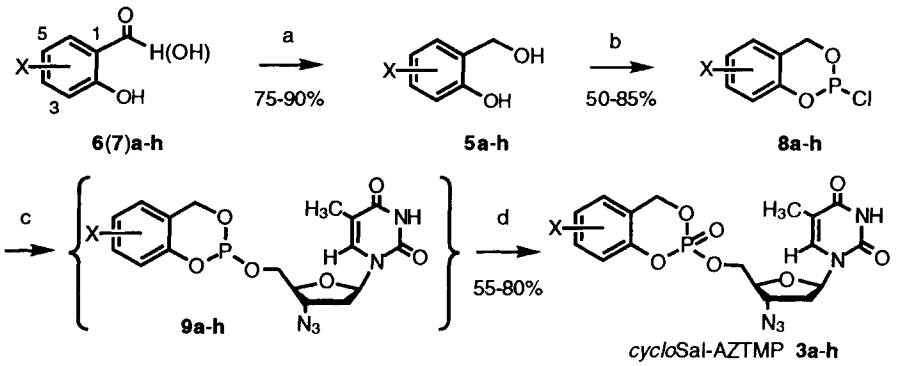
releasing AZTMP **2** and salicylalcohols **5** (step b; tandem-reaction)[6]. This approach, which was introduced with the nucleoside analogues d4T[7a] and FdU[7b], was now applied to AZT **1**.

The title compounds **3a-h** were synthesized as outlined in scheme 2. Salicylalcohols **5**, which were obtained by reduction of the corresponding salicylaldehydes **6** or salicylic acids **7** (75-90% yield), were reacted with PCl_3 to yield the cyclic saligenylchlorophosphanes **8a-h** (50-85% yield). The title compounds **3a-h** were obtained via **9a-h** by reacting AZT **1** with 1.5 equiv. chlorophosphanes **8a-h** at O°C in the presence of di-*i*-propylethylamine (DIPEA) and subsequent oxidation using *t*-butylhydroperoxide[5-7]. After purification (60-85% yield, 1:1 diastereomeric mixtures), the *cyclo*Sal-AZTMPs **3a-h** were characterized by means of ^{1}H-, ^{13}C-, ^{31}P-nmr, and UV spectroscopy as well as electrospray (ESI, negative mode) and HR mass spectrometry. The purity was checked by analytical HPLC analysis.

The partition coefficients (PC-values) of the *cyclo*Sal-AZTMPs **3a-h** were determined in 1-octanol/phosphate buffer (pH 6.5). The partition coefficients were six to sixty fold higher (PC=6.1-62.2) than the PC-value of parent nucleoside AZT **1** (PC=1.04; data not shown). Consequently the passive membrane penetration should be improved.

In order to prove the selective delivery of AZTMP **2**, *cyclo*Sal-AZTMPs **3** were hydrolyzed in 50 mmol phosphate buffer, pH 7.3 at 37°C as a model of the physiological milieu. Furthermore, the pH dependence of the hydrolysis was also studied in borate buffer (50 mmol, pH 8.9), in TRIS buffer (50 mmol, pH 6.9), and acetate buffer (50 mmol, pH 4.6). In all cases the hydrolyses were followed by means of HPLC analysis. The half lives are summarized in table 1. Using the mentioned buffers at pH>7, all *cyclo*Sal-d4TMPs **3a-h** were degraded following pseudo-first order kinetics to give d4TMP **2** and the salicylalcohols **5** as exclusive products. At pH<7, only the 3,5-dimethyl-substituted derivative **3h** yielded also the "wrong" phenyl-AZT-phosphodiester in 30%. Furthermore, the expected pH dependence was observed (table 1). Again as expected, the stability of the phosphotriesters **3** differ not much under mild acidic conditions (see hydrolyses at pH 4.6 vs. pH 6.9). 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 and without 10% heat-inactivated fetal calf serum (FCS). The products were again exclusively



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

a) $NaBH_4$ for **6** ($LiAlH_4$ for **7**); b) PCl_3, pyridine, Et_2O, -10°C, 2h; c) AZT **1**, DIPEA, CH_3CN, 0°C, 20 min; d) TBHP, CH_3CN, rt, 30 min

SCHEME 2: Synthesis of the *cyclo*Sal-AZTMPs **3a-h**

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

1, 3	Hydrolysis ($t_{1/2}$) in buffers				Antiviral Activity EC_{50} (µg/ml)		
X	acetate pH 4.6 [h]	TRIS pH 6.9 [h]	phosphate pH 7.3 [h]	borate pH 8.9 [h]	CEM/O HIV-1	CEM/O HIV-2	CEM/TK⁻ HIV-2
a 5-Nitro	1.8	1.5	0.2	0.1	0.008	0.02	**>100**
b 5-Cl	6.7	6.4	0.7	0.3	0.005	0.006	**>40**
c H	25.0	24.5	4.5	1.1	0.004	0.005	**>20**
d 5-OMe	28.7	28.3	7.2	1.07	0.006	0.021	**30.0**
e 3-OMe	10.1	9.5	1.4	0.4	0.009	0.009	**>100**
f 5-Me	29.0	28.3	8.0	1.3	0.005	0.006	**21.0**
g 3-Me	69.9	68.5	10.2	1.5	0.006	0.013	**15.0**
h 3,5-Me	102.1	98.2	16.1	3.4	0.007	0.017	**7.0**
1 (AZT)	--	--	--	--	0.006	0.005	**>100**

AZTMP **2** and the diols **5a-h** but the half lives were slightly shorter than in phosphate buffer, pH 7.3. This is certainly an effect of the more basic pH of the culture medium (pH 7.6). As judged from the obtained half lives, the donor-substituted derivatives of **3** should be stable enough to serve as intracellular depots of AZTMP **2**.

The antiviral activity of *cyclo*Sal-AZTMPs **3** was evaluated against HIV-1 and HIV-2 infected CEM/O cells and HIV-2 infected CEM-thymidine-kinase deficient (TK^-) cells (table 1). As can be seen, all *cyclo*Sal-AZTMPs proved to be active as AZT **1** in the wild-type cell line. To our surprise, the activity was nearly completely lost in the HIV-2 infected CEM/TK^-

cell line. Only in the cases of the strong donor-substituted triesters **3f**, **3g**, and **3h** some antiviral activity in the TK$^-$ cells was found (up to 12-fold more active than AZT **1**, which was completely inactive). This correlates with the hydrolytic stability of **3f-h** in phosphate buffer and in RPMI culture medium. No correlation of the antiviral activity with the lipophilicity of **3a-h** (PC values) was observed. These results are in total contrast to the observed antiviral activity of the corresponding *cyclo*Sal-d4TMP derivatives studied before[6,7]. The *cyclo*Sal-d4TMPs exhibited at least the same antiviral activity in the CEM/TK$^-$ cells as in the wild type cell line whereas d4T itself was inactive in the TK$^-$ cells. It should be mentioned that others have observed the same phenomenon before[8].

In summary, our prodrug approach obviously is suitable to deliver AZTMP **2** from *cyclo*Sal-AZTMPs **3a-h** by a non-enzymatic activation at physiological pH. Surprisingly, in contrast to the corresponding derivatives of the nucleoside analogue d4T, the good biological activity of **3a-h** found in the wild type cells could not be observed in TK$^-$ cells. Further work is currently in progress in order to find the reasons why this is the case. This is an important question with respect to the exploration of this *pro-nucleotide concept.*

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