CycloSal-2'-ara (ribo)-fluoro-2',3'-dideoxyadenosine Monophosphates—An Effort to Solve the Structure-Activity Relationship of 2'-Fluoro-dda

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CYCLOSAL-2'-ARA(RIBO)-FLUORO-2',3'-DIDEOXYADENOSINE MONOPHOSPHATES - AN EFFORT TO SOLVE THE STRUCTURE-ACTIVITY RELATIONSHIP OF 2'-FLUORO-DDA

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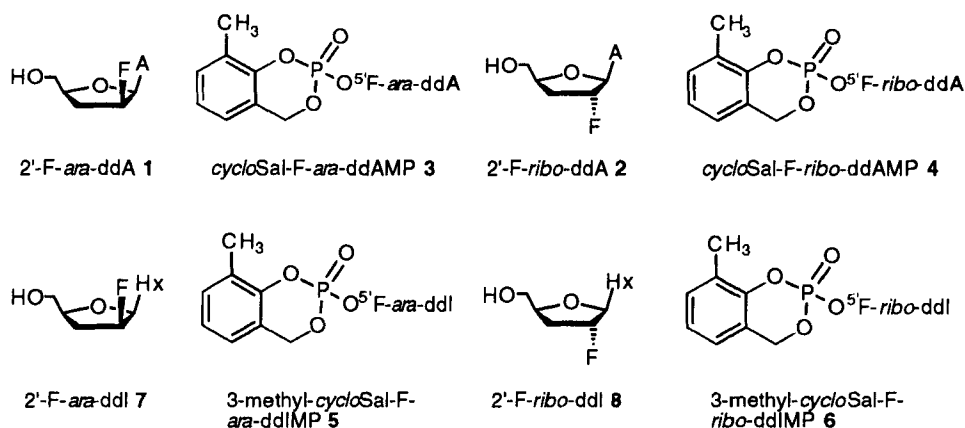[2]NCI, NIH, 37 Convent Drive MSC 4255, Bethesda, Maryland, 20892-4255, USA,
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Abstract: Novel lipophilic *cyclo*Sal-triesters **3** and **4** from the *ara*- and *ribo*-configurated 2'-fluorinated ddAs **1** and **2**, respectively, were prepared. The title compounds **3** and **4** delivered the corresponding monophosphates and thus, increasing the bioactivity or convert a formerly inactive compound into a RT inhibitor.

Fluorinated nucleoside analogues, e.g. 2'-F-*ara*-ddA **1** or 2'-F-*ribo*-ddA **2**[1] (Scheme 1) have been prepared in order to avoid the major drawback of purine 2',3'-dideoxynucleoside analogues: the extreme sensitivity against acid catalyzed depurination[2]. The introduction of the fluorine converts them into acid stable compounds. Nevertheless, 2',3'-dideoxyadenosine (ddA) and its deamination product 2',3'-dideoxyinosine (ddI; Didanosine, Videx®) are potent anti-HIV purine nucleoside analogues *in-vitro*[3]. However, before they can act as inhibitors of reverse transcriptase (RT) they have to be converted inside the cells enzymatically to the antivirally active 2',3'-dideoxyadenosine-5'-triphosphate (ddATP)[4].

A further drawback with ddA is its extensive catabolism initiated by deamination by the ubiquitous adenosine deaminase (ADA)[5] which results in an inefficient conversion to ddAMP by four different cellular enzymes[6]. However, it has been reported that the latter obstacle can be avoided by selective intracellular delivery of ddAMP using pronucleotides[7-10]. Thus, due to the delivery of the monophosphate, the ADA reaction is bypassed (ADA-bypass) and therefore avoids further necessary anabolic steps. It has been shown before that 2'-F-*ara*-ddA **1** follows essentially the same metabolic pathway than

Scheme 1: *cyclo*Sal-pro-nucleotides **3-6** of the different 2'-fluorinated ddAs

2'-F-*ara*-ddA **1**   *cyclo*Sal-F-*ara*-ddAMP **3**   2'-F-*ribo*-ddA **2**   *cyclo*Sal-F-*ribo*-ddAMP **4**

2'-F-*ara*-ddI **7**   3-methyl-*cyclo*Sal-F-
ara-ddIMP **5**   2'-F-*ribo*-ddI **8**   3-methyl-*cyclo*Sal-F-
ribo-ddIMP **6**

ddA[11]. However, no detailed studies have been published on the metabolism of its *ribo*-configurated counterpart **2**. May be the reason therefore is, that the 2'-fluorinated ddAs behave completely different in *in-vitro* antiviral evaluations: while the 2'-F-*ara*-derivative **1** is a potent RT inhibitor (also *in-vivo*)[12], the 2'-F-*ribo*-counterpart was devoid of any biological activity.

Obviously, this extremely different antiviral activity behaviour has to be related with a different metabolism and this is may be due to the opposite stereochemistry of the glycon residue of the nucleosides. The basis of this assumption is that in contrast to ddA and ddI, which show a fast equilibrium of the C2'-endo- and C3'-endo sugar puckers with respect to their sugar ring conformation (although for both compounds a predominant C3'-endo conformation has been shown in NMR analyses)[13], the two 2'-F-ddAs **1** and **2** adopt only *one* sugar pucker conformation: The introduction of fluorine at the 2'-*ara*-position forces the sugar pucker to the C2'-endo/South(S) conformation while a C3'-endo/North(N) conformation is adopted in the 2'-*ribo*-nucleoside[14,15]. In both situations the fluorine is not in a trans-diaxial but in an axial orientation to the oxygen of the five-membered glycon ring. The reason for this unusual behaviour is the *gauche*-effect[16] (Scheme 2).

In this work we applied our *cyclo*Sal-pro-nucleotide concept[17,18] to the F-ddA derivatives **1,2**. One rational therefore was that we knew from our previous work that *cyclo*Sal-ddAMP and *cyclo*Sal-d4AMP increased the biological activity of the parent nucleoside tremendously[10,19] which could only be explained by an efficient intracellular delivery of the corresponding nucleotides and thus, effecting the ADA-bypass. Consequently, the application of the *cyclo*Sal-pro-nucleotide concept to the F-ddA problem would give us the first information if the limiting step is located within the metabolism towards the

Scheme 2: Sugar pucker of 2'-fluorinated 2',3'-dideoxyadenosine
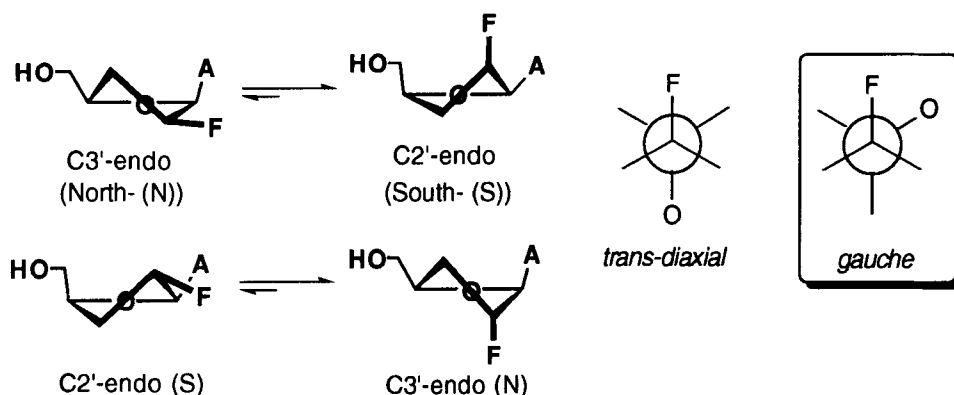
nucleotides 2'-F-*ara*-ddAMP and 2'-F-*ribo*-ddAMP or if the limitation is caused by a low conversion to the triphosphates.

Furthermore, corresponding ddA/d4A we knew that the *cyclo*Sal-triesters were not susceptible to deamination by ADA nor adenosine monophosphate deaminase (AMPDA)[19,20]. This result would in principle exclude the possibility that the *cyclo*Sal-phosphotriesters deliver 2'-F-*ara*- and 2'-F-*ribo*-ddIMP. However, these experiments have been done with isolated enzymes and hence may not reflect the cellular situation correctly. Consequently, we prepared four different *cyclo*Sal-phosphotriesters: the masked triesters bearing 2'-F-*ara*-ddAMP (**3**), 2'-F-*ribo*-ddAMP (**4**), 2'-F-*ara*-ddIMP (**5**) and 2'-F-*ribo*-ddIMP (**6**) (Scheme 1). Triester **5** and **6** were prepared from the hypoxanthine derivatives **7** and **8**, which were synthesized by ADA deamination from **1** and **2** (Scheme 1). All were prepared as their 3-methyl-*cyclo*Sal-triesters because those gave the best biological data in the case of the d4T[21], ddA[10] and d4A compounds. The synthetic approach towards the *cyclo*Sal-phosphotriesters was conducted as described previously[21,22].

*Cyclo*Sal-phosphotriesters **3-6** were subsequently tested for their stability against isolated ADA (EC 3.5.4.4.) and AMPDA (EC 3.5.4.6.) and were evaluated for their antiviral potential in HIV-1- and HIV-2-infected CEM wild-type lymphocytes (Table 1).

First, the antiviral data of 3-methyl-*cyclo*Sal-2'-F-*ara*-ddAMP **3** exhibited a 10-fold increase in antiviral activity as compared to the parent nucleoside analogue **1**. As expected, 2'-F-*ara*-ddI **7** proved as active as its adenine counterpart **1**. Thus, nucleoside **1** seems to be efficiently deaminated in the cellular system to yield **7**. To our surprise, the *cyclo*Sal-triester of **7** proved as active as the adenine analogue **3**. As a first result, assuming an efficient delivery of 2'-F-*ara*-ddIMP from **5**, there seems to be no further metabolic

Table 1:   Anti-HIV data and cytotoxicity of the *cyclo*Sal-triester **3-6** and the parent nucleosides **1,2** and **7,8**

Compound	Antiviral Activity EC$_{50}$ [μM] CEM lymphocytes		Cytotoxicity CC$_{50}$ [μM]	Selectivity Index SI
	HIV-1	HIV-2		
3	3.67	3.3	146	40
2'-F-*ara*-ddA **1**	36.7	40.0	> 250	7
5	3.57	3.57	91	25
2'-F-*ara*-ddI **7**	41.7	30.0	> 250	6
4	11.7	12.5	118	10
2'-F-*ribo*-ddA **2**	> 250	> 250	> 250	n.a.[a]
6	75.0	31.7	> 250	3
2'-F-*ribo*-ddI **8**	> 250	> 250	> 250	n.a.[a]

a) not available

limitation during the phosphorylation to the triphosphate 2'-F-*ara*-ddATP. Thus, also the enzymatic reamination of 2'-F-*ara*-ddIMP has to be carried out effectively.

Most important was the observation that the 2'-F-*ribo*-ddA *cyclo*Sal-phosphotriester **4** of exhibited now an antiviral effect while the parent 2'-F-*ribo*-nucleoside **2** was completely inactive. The antiviral potency is even higher (3-fold) as for 2'-F-*ara*-ddA **1**.

Remarkable is the result that 3-methyl-*cyclo*Sal-2'-F-*ribo*-ddIMP **6** showed pronounced decrease in antiviral activity as compared to triester **4** (3- to 6-fold). However, even compound **6** was still slightly more biologically active as the parent nucleoside 2'-F-*ribo*-ddI **8**. As a second result, we can conclude that in contrast to 2'-F-*ara*-ddAMP, the reamination of 2'-F-*ribo*-ddIMP to 2'-F-*ribo*-ddAMP seem to be influenced by the introduction of the fluorine atom.

A comparison of the biological activity of 3-methyl-*cyclo*Sal-2'-F-*ara*-ddAMP **3** and 3-methyl-*cyclo*Sal-2'-F-*ribo*-ddAMP **4** shows that the *ara*-configurated compound **3** is still 3- to 4-fold more active than the *ribo*-configurated derivative **4**. This difference may reflect a different phosphorylation kinetics to the triphosphates or a different interaction of the triphosphates with RT. Interestingly, Mitsuya has shown that the inhibitory concentration (IC$_{50}$) for the inhibition of RT by isolated 2'-F-*ara*- or 2'-F-*ribo*-ddATP differed also by a factor of 4 to 5[23]. This study gives clear evidence that the difference in biological activity observed with our compounds **3,4** is not due to a different phosphorylation to the 2'-F-ddATPs. Moreover, the IC$_{50}$s found by Mitsuya were in the same order of magnitude than

the EC_{50} reported here[23]. Furthermore, it was noted that the phosphotriesters presented here increased also the selectivity indices (SI) as compared to the parent nucleoside analogues (Table 1). From the biological data discussed so far, we conclude as a third result that the metabolic differentiation between the two fluorinated ddA derivative **1,2** have to take place during metabolism to the nucleotides 2'-F-*ara*-ddAMP and 2'-F-*ribo*-ddAMP, respectively.

In experiments with isolated ADA and AMPDA, we observed a striking difference in the deamination behaviour of 2'-F-*ara*- and 2'-F-*ribo*-ddA **1** and **2**, respectively. As reported before, 2'-F-*ara*-ddA **1** (deamination within 30 min) was found to be 10-fold more resistant to ADA deamination as ddA[1]. In contrast, 2'-F-*ribo*-ddA **2** showed complete deamination within 0.6 min (ddA was deaminated in 3 min). Thus, the fluorinated nucleoside **2** was 6-fold less stable than ddA and, more importantly, 60-fold less stable than the 2'-F-*ara*-configurated **1**. Thus, 2'-F-*ribo*-ddA **2** seems be extremely susceptible to intracellular deamination and consequently needs reamination in order to yield 2'-F-*ribo*-ddATP. On the other hand, 2'-F-*ara*-ddA **1** is markedly less susceptible to ADA deamination and the alternative direct phosphorylation to 2'-F-*ara*-ddAMP by adenosine kinase and/or deoxycytidine kinase plays a role. It seems that the limiting step in the conversions to 2'-F-*ara*-ddAMP is the phosphorylation of the deamination product 2'-F-*ara*-ddI by 5'-nucleotidase because, keeping in mind that F-*ara*-ddIMP is efficiently reaminated which was concluded from our biological data of compounds **3** and **5**, the antiviral activity should otherwise be as good as the pro-nucleotide **3**. On the other hand, from our data we can not conclude that the phosphorylation of 2'-F-*ribo*-ddI to its monophosphate is the rate limiting step because from the antiviral potencies of our *cyclo*Sal-phosphotriesters **4,5** it was deduced that the reamination by adenylosuccinate synthetase/adenylosuccinate lyase seems not to proceed very efficient.

How this can be attributed to the different sugar puckers of the parent nucleosides has to be further elucidated.

In conclusion, with our *cyclo*Sal-pro-nucleotide approach we observe an increase in antiviral activity for both 2'-F-*ara*-ddAMPs **3** and 2'-F-*ribo*-ddAMPs **4** with respect to the parent nucleosides **1** and **2**. Most striking is the fact, that we converted the inactive nucleoside analogue 2'-F-*ribo*-ddA **2** into a bioactive compound (**4**). Additionally, the *cyclo*Sal-pro-nucleotide approach proved also to be a suitable tool to get first informations for studies concerning metabolic limitations of nucleoside analogues.

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