Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 4, pp. 473-478, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00044-9

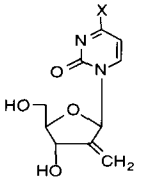
SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF 2'-*O*-ALLYL-1-β-D-ARABINOFURANOSYL-URACIL, -CYTOSINE AND -ADENINE

Stefano Manfredini,* Pier Giovanni Baraldi, Rita Bazzanini, Daniele Simoni,§ Jan Balzarini# and Erik De Clercq#

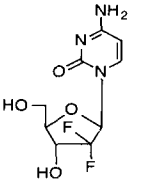
Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Via Fossato di Mortara 17-19; I-44100 Ferrara. Fax: +39.532.29.1296, E-mail: mv9@dns.unife.it. §*Istituto Farmacochimico, Università di Palermo,* #*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium.*

Abstract: With the aim to design potential inhibitors of ribonucleotide reductase (RR), 2'-O-allyl-β-D-arabinofuranosyl-uracil (**4**), -cytosine (**7**) and -adenosine (**10**) were prepared and evaluated for their cytostatic activity against Molt4/C8, CEM and L1210 cell lines. Although our preliminary data do not allow to assess if RR is the intracellular target, the results point to differences in the (anti)metabolic behavior of these compounds. This study also offers a general synthesis of 2'-O-allyl-β-D-arabinofuranosyl nucleosides for potential applications in the preparation of 2'-O-allyl-β-D-oligoarabino nucleotides.

An important step in the DNA biosynthesis, catalyzed by ribonucleotide reductase (RR), involves radical intermediates.[1,2,3]



X = NH_2: MdCyd
X = OH: MdUrd



dFdCyd

It has been reported by Thelander *et al.*[4] and Stubbe and coworkers,[3,5] that compounds featured by reactive groups capable of quenching the tyrosyl free radical, present on RR, may work as inhibitors of this enzyme. Thus, hydroxyurea (HU) and related compounds,[6] the potent mechanism-based inhibitors 2'-methylene-2'-deoxy-cytidine (MdCyd),[7] 2'-methylene-2'-deoxy-uridine (MdUrd)[8] and 2',2'-difluoro-2'-deoxy-ytidine (dFdCyd),[9] have been shown to be effective as cytostatic and/or antitumor agents.

Moreover, it has been recently observed that HU, at non-toxic doses, enhances the activity against human immunodeficiency virus type 1 (HIV-1) of both purine and pyrimidine 2',3'-dideoxynucleosides (ddNs) in human lymphocytes and macrophages.[10] Therefore, nucleosides endowed with radical scavenging moieties, that

* E-mail: mv9@dns.unife.it; Fax: +39-532-291296

might behave as mechanism-based RR inhibitors, are of interest for the development of potential antiviral and/or antitumor agents.

To this regard suitable functional groups have been proposed by Robins and Stubbe,[8,11] and we have recently investigated vinyl and alkynyl substituted nucleosides.[12] The allylic moiety is known to act as an effective radical scavenger: Chattopadhyaya et al.,[13] developing some new chemistry for the functionalization of 2' and 3' positions of nucleosides, have recently shown that 1-(5'-*O*-(MMtr)-3'-deoxy-3'-phenylseleno-2'-*O*-allyl-1-β-D-ribofuranosyl or -arabinofuranosyl)uracil readily underwent intramolecular cyclization upon generation of the free-radical at position 3' with tin hydride.

2'-*O*-Allyl-ribonucleosides have been widely studied, because of the biological importance of 2'-*O*-alkyl-oligoribonucleotides,[14] but the corresponding 2'-*O*-allyl-arabinofuranosides have, to the best of our knowledge, not been investigated. Furthermore, araC itself has been demonstrated to be a moderate RR inhibitor[15] but this intriguing aspect was not further examined.

Taking all these considerations into account, and continuing our efforts toward the study of nucleosides endowed with radical-scavenging moieties, we envisaged compounds **4**, **7** and **10** as possible candidate antitumor/antiviral agents. The potential diastereoselective radical cyclization promoted by RR on 2'-*O*-allyl-arabinofuranosyl nucleosides, through the generation of a radical species at the 3'-position, could account for the biological activity.

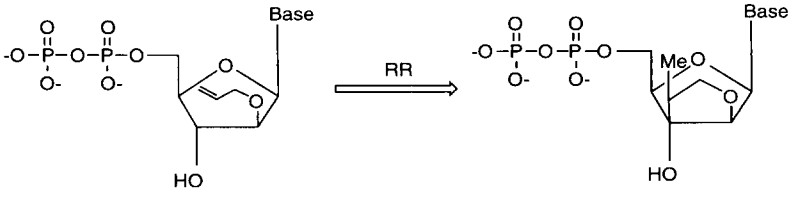


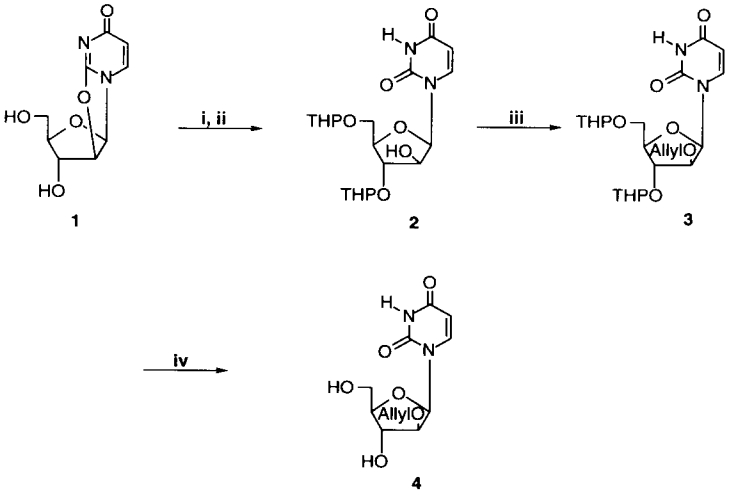
Figure 1. Diastereoselective radical cyclization possibly induced by RR in 2'-O-allyl-arabinonucleosides

Chemistry

The 2'-*O*-allylation of ribonucleosides, without protecting the 3'- and 5'-hydroxy groups, is reported to yield a mixture of 2'- or 3'-*O*-allyl derivatives that next require the conversion into the corresponding 3',5'- and 5'-protected nucleosides, respectively, in order to be resolved. These procedures also require the use of a phase transfer catalyst (tetrabutyl ammonium bromide, TBAB) and dibutyltin derivatives (i.e. dibutyltinoxide, DBTO) in DMF.[14,16] In our case we decided to simply proceed with protected precursors and a conventional base.

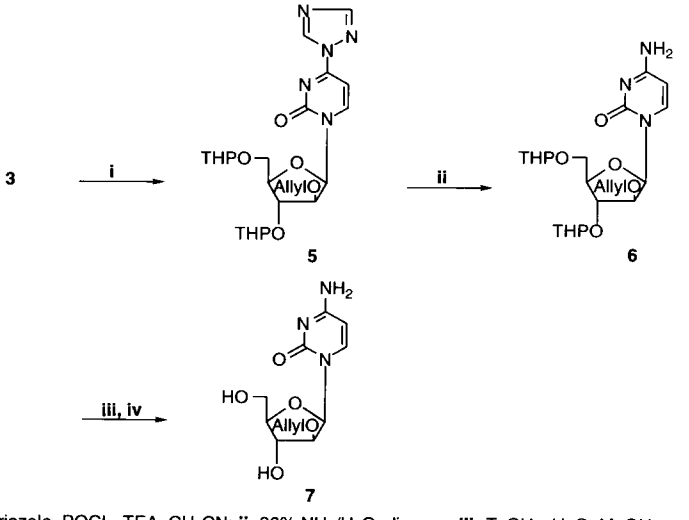
2,2'-Anhydro-(1-β-D-arabinofuranosyl)uracil (**1**) was chosen as common starting material for the preparation of **4** and **7**. This intermediate is particularly useful for our purposes because it can be easily protected in a regiospecific manner at the 5'- and 3'-hydroxy groups and also converted into the corresponding cytidine derivative by known procedures. It is worth to mention that with cytidine (either protected or not at N^4-position), direct alkylation at the base occurs rather then *O*-alkylation.[17] Thus, after having explored several protecting groups for the 3' and 5'-hydroxy functions, we selected the tetrahydropyranyl moiety (THP).

Scheme 1



i: DHP, TsOH x H_2O, CH_3CN; **ii:** KOH, EtOH; **iii:** allyl bromide, NaH 60%, THF;
iv: TsOH x H_2O, MeOH.

Scheme 2



i: triazole, $POCl_3$, TEA, CH_3CN; **ii:** 30% NH_3/H_2O, dioxane; **iii:** TsOH x H_2O, MeOH;
iv: dowex 1 (OH^- form), H_2O

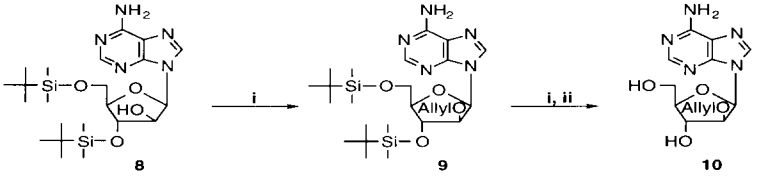
Briefly, 2,2'-anhydro-(1-β-D-arabinofuranosyl)uracil (**1**) was protected as 3', 5'-*O*-THP-derivative, treated with KOH in ethanol to give 3',5'-*O*-THP-araU (**3**) and allylated at the 2'-position with allyl bromide and sodium hydride, as the base, in CH_3CN (52% overall yield, as compared to **1**).

Compound **3** could be deprotected to give the 2'-*O*-allyl-araU (**4**) by treatment with toluenesulphonic acid (TsOH) in 63% yield or converted into the corresponding 3',5'-*O*-THP-2'-*O*-allyl-araC (**6**) in two steps, following and adapting the procedure of Divakar and Reese (75% overall yield).[18] This latter intermediate could be easily deprotected at the THP-groups, by treatment with TsOH, to give **7** in 83% yield.

In the case of araA, the use of 2',5'-*O*-THP- or 3',5'-*O*-TIPDS-protecting groups gave unsatisfactory results in terms of yields and selectivity of the 2'-*O*-allylation reaction. Better results were obtained by protecting araA as its 3',5'-*O*-*tert*-butyldimethylsilyl derivative (TBDMS-araA) (**8**).[19] This latter was then allylated with allyl bromide and sodium hydride in 32% yield (**9**), with concomitant formation of mixtures of by-products alkylated at the N^6- and/or 2'-*O*-positions.

This occurrence[17] could be avoided by employing 3',5'-*O*-TIPDS-6-*O*-(2,6-dichlorophenyl) protected purine ribonucleosides either with hindered bases or under Pd(0) catalysis.[14,16] Our yields were not optimized and estimated suitable to achieve sufficient material for the preliminary biological investigations. The TBDMS-groups were next removed by treatment with ammonium fluoride/methanol (NH_4F/MeOH) to give the final **10** in 92% yield.[20]

Scheme 3



i: allyl bromide, NaH 60%, THF; ii: NH_4F/MeOH

Finally, to assess if partial cleavage to the parent nucleosides could be at the basis of the observed activity, the stability of title compounds to hydrolytic cleavage was evaluated in water and aqueous acidic solutions.[21] All compounds proved fully stable after incubation at pH 2 and pH 7 for 24h at 37°C.

Biological Activity

Compounds **4**, **7** and **10** were evaluated for their cytostatic activity against murine leukemia cells (L1210) and human T-lymphocyte cells (Molt4/C8, CEM). The results are presented in Table 1. Whereas 2'-*O*-allyl-araU (**4**) was devoid of any marked cytostatic activity against murine and human cells, 2'-*O*-allyl-araC (**7**) and 2'-*O*-allyl-araA (**10**) inhibited tumor cell proliferation at an IC_{50} of 72-588 μM against the three tumor cell lines investigated. Interestingly 2'-*O*-allyl-araA (**10**) inhibited the proliferation of all three cell lines (IC_{50} 72-250 μM).

Table 1. Inhibitory effects of nucleoside derivatives **4**, **7**and **10** on the proliferation of murine leukemia L1210 and human T-lymphocyte Molt4/C8 and CEM cells.

	$IC_{50}(\mu M)^a$		
Compound	L1210	Molt4/C8	CEM
4	> 500	> 500	> 500
7	127 ± 30	588 ± 204	127 ± 14
10	250 ± 62	88 ± 31	72 ± 5
araU	> 200	> 200	> 200
araC	0.031 ± 0.004	0.030 ± 0.004	0.0037 ± 0.004
araA	14.2 ± 6.4	11.9 ± 7.3	24.8 ± 1.9

[a] 50% Inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%.

2'-*O*-Allyl-araC (**7**) was poorly cytostatic against human Molt4/C8 cells, but had a more pronounced inhibitory effect on human CEM and murine L1210 cell proliferation (IC_{50}: 127 μM). This result may be suggestive of a differential metabolism of these compounds in the above cited tumor cell lines. It is notewortly that the araC derivative **7** is approximately three orders of magnitude less cytostatic than the parent compound araC. This can be due to a much lower efficiency of phosphorylation by the nucleoside and/or nucleotide kinases to the 5'-mono-, di- and triphosphate derivatives, and/or to a decreased affinity of the **7** metabolite(s) for their target enzyme(s).

Our preliminary data do not allow to assess if RR is an intracellular target for the antitumor activity of these compounds. Further biological studies will be required to elucidate the molecular mechanism of the biological activity.

In conclusion, previously unreported 2'-*O*-allyl-1-β-D-arabinofuranosyl-uracil, -cytosine and -adenine were obtained and evaluated for their cytostatic activity. The results, point to differential antiproliferative activity of the novel compounds, which might be suggestive of a difference in the metabolism of these compounds in the different cell lines and/or different inhibitory activity of their metabolites against their intracellular targets. In view of the importance of 2'-*O*-alkyl-oligoribonucleotides, this study offers a facile general synthesis of 2'-*O*-allyl-arabinofuranosyl nucleosides of interest for potential applications to the preparation of 2'-*O*-allyl-oligoarabinonucleotides.

Acknowledgements. This work was supported by the Consiglio Nazionale delle Ricerche (CNR) and Ministero Ricerca Scientifica e Tecnologica (MURST) of Italy and by the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (project no. 3.0180.95). We thank Lizette van Berckelaer for excellent technical help.

References

1. Robins, M. J.; Samano, C. M. ; Samano, V. *Nucleosides, Nucleotides* **1995**, *14*, 485-493.
2. Behravan, G.; Sen, S.; Rova, U.; Thelander, L.; Eckstein, F. ; Gräslund, A. *Biochim. Biophys. Acta* **1995**, *1264*, 323-329.
3. Stubbe, J. *Nature* **1994**, *370*, 502.
4. Thelander, L.; Larsson, B.; Hobbs, J. ; Eckstein, F. *J. Biol. Chem.* **1976**, *251*, 1398-1405.
5. Ator, M. A. ; Stubbe, J. *Biochem.* **1985**, *24*, 7214-7221.
6. Ehrenberg, A. ; Reichard, P. *J. Biol. Chem.* **1972**, *247*, 3485-3488.
7. Takenuki, K.; Matsuda, A.; Ueda, T.; Sasaki, T.; Fuji, A. ; Yamagami, K. *J. Med. Chem.* **1988**, *31*, 1064-1073.
8. Baker, C. H.; Banzon, J. M.; Stubbe, J.; Samano, V.; Robins, M. J.; Lippert, B.; Jarvi, E. ; Resvick, R. *J. Med. Chem.* **1991**, *34*, 1879-1884.
9. Hertel, L. W.; Kroin, J. S.; Misner, J. W. ; Tustin, J. M. *J. Org. Chem.* **1988**, *53*, 2406-2413.
10. Gao, W. Y.; Mitsuya, H.; Driscoll, J. S. ; Johns, D. G. *Biochem. Pharmacol.* **1995**, *50*, 274-276.
11. Samano, V. ; Robins, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 4007-4008.
12. Manfredini, S.; Baraldi, P. G.; Bazzanini, R.; Marangoni, M.; Simoni, D.; Balzarini, J. ; De Clercq, E. *J. Med. Chem.* **1995**, *38*, 199-203.
13. Wu, J. C.; Xi, Z.; Gioeli, C. ; Chattopadhyaya, J. *Tetrahedron* **1991**, *47*, 2237-2254.
14. Gopalakrishnan, V.; Kumar, V. ; Ganesh, K. N. *Nucleosides, Nucleotides* **1992**, *11*, 1263-1273 and references herein quoted.
15. Chu, Y. ; Fischer, G. A. *Biochem. Pharmacol* **1969**, *17*, 753-756.
16. Sproat, B. S.; Iribarren, A. M.; Garcia, R. G. ; Beijer, B. *Nucleic Acids Res.* **1991**, *19*, 733-738 and references herein quoted.
17. Wagner, D.; Verheyden, J. P. H. ; Moffatt, J. G. *J. Org. Chem.* **1974**, *39*, 24-30.
18. Divakar, K. J. ; Reese, C. B. *J.Chem. Soc. Perkin I* **1982**, 1171-1176.
19. Ogilvie, K. K.; Hakimelahi, G. H.; Proba, Z. A. ; McGee, D. P. C. *Tetrahedron Lett.* **1982**, *23*, 1997-2000.
20. Zhang, W. ; Robins, M. J. *Tetrahedron Lett.* **1992**, *33*, 1177-1180.
21. Kano, F.; Ijichi, K.; Ashida, N.; Watanabe, Y.; Sakata, S. ; Machida, H. *Antiviral Chem. Chemother.* **1994**, *5*, 74-82.

(*Received in Belgium* 15 *November* 1996; *accepted* 14 *January* 1997)