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UNNATURAL ENANTIOMERS OF 5-AZACYTIDINE ANALOGUES: SYNTHESES AND ENZYMATIC PROPERTIES

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ABSTRACT

2′-Deoxy-β-L-5-azacytidine (L-Decitabine), β-L-5-azacytidine, and derivatives were stereospecifically prepared starting from L-ribose or L-xylose. D- and L-enantiomers of 2′-deoxy-β-5-azacytidine were weak substrates of human recombinant deoxycytidine kinase (dCK), whereas both enantiomers of β-5-azacytidine or the L-xylo-analogues were not substrates of the enzyme. None of the reported derivatives of β-L-5-azacytidine was a substrate of human recombinant cytidine deaminase (CDA).

β-D-5-Azacytidine (β-D-5-azaC), **1**, and 2′-deoxy-β-D-5-azacytidine (β-D-5-azadC, Decitabine), **2**, are important antileukemic agents used in clinical treatment [1,2]. The use of β-D-5-azaC or β-D-5-azadC suffers however from several drawbacks. The inclusion of an extra nitrogen atom into the cytosine base increases its chemical sensitivity with respect to nucleophiles and accounts for the instability of the compounds in aqueous solution [3]. Another major cause of resistance is

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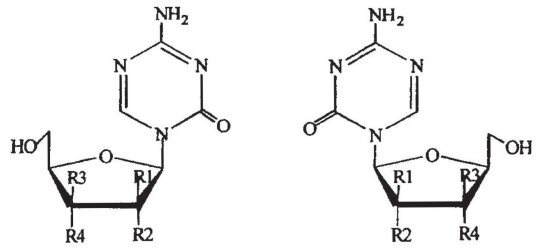

1 (R1 = R3 = H, R2 = R4 = OH) **3** (R1 = R3 = H, R2 = R4 = OH)

2 (R1 = R2 = R3 = H, R4 = OH) **4** (R1 = R2 = R3 = H, R4 = OH)

5 (R1 = R4 = H, R2 = R3 = OH)

6 (R1 = R2 = R4 = H, R3 = OH)

Figure 1. β-D- and β-L-5-azacytidine analogs studied as substrates of human dCK and human CDA.

induced by the ubiquitous cellular enzyme cytidine deaminase (CDA) since deamination of **1** or **2** results in total loss of activity [4,5]. We have previously shown that a number of cytidine analogues having the unnatural L stereochemistry are both substrates of human deoxycytidine kinase (dCK) and resistant to human cytidine deaminase [6]. We therefore stereospecifically prepared the L-enantiomers of **1**, **2** and other analogues (Fig. 1), and we studied their enzymatic properties with respect to dCK and CDA in the hope of getting phosphorylation, a lack of deamination of these compounds and possibly an efficient incorporation into DNA.

Chemistry

Most previous syntheses of 5-azacytidine analogues yield both α- and β-anomers often difficult to separate. For this reason, we used regio-and stereospecific methods to prepare the L-enantiomers **3**–**6** starting from L-ribose or L-xylose (Fig. 1). 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-L-ribofuranose was coupled with silylated 5-azacytidine in the presence of trimethylsilyltriflate. The 2′-benzoyl group controls the stereochemistry of the substitution [7] and only the β-anomeric derivative was obtained. Deprotection catalysed by sodium methanolate in methanol afforded β-L-ribofuranosyl-5-azacytosine, **3**, in good yield. The 3′- and 5′-positions of **3** were then protected using dichlorotetraisopropyldisiloxane. A Barton-McCombie elimination of the 2′-hydroxyl group of the 3′,5′-diprotected compound followed by deprotection gave L-Decitabine, **4**, in 42% overall yield from **3**. 1,2-Di-*O*-acetyl-3,5-di-*O*-benzoyl-L-xylo-furanose was similarly condensed with silylated 5-azacytosine giving exclusively the β-anomeric derivative. Deprotection with sodium

methanolate afforded β-L-xylofuranosyl-5-azacytosine, **5**, whereas deacetylation followed by a Barton-McCombie elimination of the 2′-hydroxyl group and debenzoylation yielded 2′-deoxy-β-L-*threo*-pentofuranosyl-5-azacytosine, **6**.

Biological Results and Discussion

Several studies of the action of dCK on **1** and **2** have been previously reported, and evaluations of the substrate character of β-D-5-azaC with respect to dCK have shown that it is either low or non-existent [8]. Concerning β-D-5-azadC (Decitabine), all existing studies indicate that this compound is an average substrate of human or mammal dCK, with Km ranging from 29 to 71 μM depending on the origin of the enzyme and on the conditions [9,10]. We used HPLC to follow the kinetics of phosphorylation of **1–6** in the presence of human dCK. Under our conditions, substrate reversible decomposition was held below 2% for the duration of the kinetics. With 5 mM ATP as phosphate donor, only 2′-deoxy-β-D-5-azacytidine, **2**, and 2′-deoxy-β-L-5-azacytidine, **4**, were phosphorylated (Km: 94 and 17 μM, respectively). The efficiencies of phosphorylation were similar for the two enantiomers (Vm/Km: 0.075 and 0.05, respectively, compared to 2′-deoxy-β-D-cytidine). The value of the Km constant for 2′-deoxy-β-D-5-azacytidine is similar to the constants previously determined [9,10]. In contrast, no phosphorylation was observed in the case of both enantiomers of β-5-azacytidine, **1** and **3**. The β-L-xylo- or 2′-deoxy-xylo-derivatives **5** and **6** did not display any substrate properties.

Numerous studies have evaluated the sensitivity of β-D-5-azacytidine and 2′-deoxy-β-D-5-azacytidine to deamination catalysed by cytidine deaminase [11,12]. Using a low temperature (25°C) and short kinetic durations allowed us to limit the decomposition of the substrates as observed from HPLC analysis. Only the D-enantiomers **1** and **2** were substrates of human CDA (Km values 225 and 690 μM, respectively) whereas no deamination occurred for the L-enantiomers **3–6**, under the same conditions and in the presence of increased concentrations of enzyme.

The prepared L-nucleoside analogues **3–6** were tested as inhibitors of HIV replication in CEM-SS and MT-4 cell systems or as inhibitors of HBV replication in HepG2 cells following standard protocols. None of the compounds displayed any significant antiviral or cytotoxic effect. Our study only shows that L-Decitabine may be monophosphorylated in cells and is resistant to enzymatic deamination. The substrate properties and enantioselectivities of cellular nucleotide kinases, viral DNA polymerases or other concerned enzymes with respect to the reported compounds are not known and could be unfavourable.

REFERENCES

1. Sorm, F.; Vesely, J. *Neoplasma* **1968**, *15*, 339–343.
2. Momparler, R.L.; Momparler, L.F.; Samson, J. *Leukemia Res.* **1984**, *8*, 1043–1049.
3. Pinto, A.; Zagonel, V. *Leukemia*, **1993**, *7 Suppl. Monograph 1*, 51–60.

4. Momparler, R.L.; Côté, S.; Eliopoulos, N. *Leukemia*, **1997**, *11 Suppl. 1*, 1–6.
5. Laliberté, J.; Marquez, V.E.; Momparler, R.L. *Cancer Chemother. Pharmacol.* **1992**, *30*, 7–11.
6. Shafiee, M.; Griffon, J.-F.; Gosselin, G.; Cambi, A.; Vincenzetti, S.; Vita A.; Erikson, S.; Imbach, J.-L.; Maury, G. *Biochem. Pharmacol.* **1998**, *56*, 1237–1242.
7. Gaubert, G.; Gosselin, G.; Boudou, V.; Imbach, J.-L.; Eriksson, S.; Maury, G. *Biochimie* **1999**, *81*, 1041–1047.
8. Krenitsky, T.A.; Tuttle, J.V.; Koszalka, G.W.; Chen, I.S.; Beacham III, L.M.; Rideout, J.L.; Elion, G.B. *J. Biol. Chem.* **1976**, *251*, 4055–4061.
9. Momparler, R.L. *FEBS Symp.* **1979**, *57*, 33–41.
10. Vesely, J.; Cihak, A. *Neoplasma* **1980**, *27*, 121–127.
11. Chabot, G.G.; Bouchard, J.; Momparler, R.L. *Biochem. Pharmacol.* **1983**, *32*, 1327–1328.
12. Vincenzetti, S.; Cambi, A.; Neuhard, J.; Garattini, E.; Vita, A. *Protein Expression Purif.* **1996**, *8*, 247–253.