



## HOMO-N-NUCLEOSIDES : INCORPORATION INTO OLIGONUCLEOTIDES AND ANTIVIRAL ACTIVITY.

N. Hossain, C. Hendrix, E. Lescrinier, A. Van Aerschot, R. Busson, E. De Clercq and P. Herdewijn\*.

*Rega Institute for Medical Research, K.U.Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium.*

Fax: +32 16 33.73.87, E-mail Piet.Herdewijn@Rega.KULeuven.ac.be

### Abstract

Homo-N-nucleosides can be efficiently synthesized from 2-deoxyribose. When incorporated in an oligonucleotide, the compounds have a detrimental influence on duplex stability and on the catalytic activity of hammerhead ribozymes. However, homo-N-nucleosides with a guanine or adenine base moiety do exhibit selective antiviral activity against herpes simplex virus (HSV-1 and HSV-2). Copyright © 1996 Elsevier Science Ltd

### INTRODUCTION

Nucleosides are compounds composed of a sugar part and a nitrogen-containing heterocyclic aglycon. Both moieties are connected through a N-glycosidic bond. The presence of an anomeric centre in natural nucleosides is one of the important factors which contribute to the conformational behaviour of nucleosides and, hence, to their biological properties. When replacing, for example, the furanose ring by a carbocyclic five-membered ring, the biological activity changes drastically. This can be demonstrated by the different structure activity relationship between furanose nucleosides and carbocyclic nucleosides<sup>1</sup>. Another way of removing the anomeric centre is the introduction of a methylene group between the base part and the furanose moiety leading to homo-N-nucleosides. In this case, however, more conformational flexibility is introduced and the base moiety is put further away from the other nucleoside recognition sites (ring oxygen atom and hydroxyl groups). These compounds may be easily synthesized starting from 2-deoxy-3,5-di-*O*-benzyl-D-ribose<sup>2</sup>. The ready availability of these compounds allowed us to start the study of their biological properties.

### Conformational analysis

X-ray crystallographic analysis of homo-N-nucleosides with a cytosine, adenine or thymine base moiety proved the alkylated base part to be in a pseudoequatorial conformation and the tetrahydrofuran moiety in a south conformation<sup>2</sup>. This south conformation is also populated preferentially for deoxyribose nucleosides<sup>3</sup>. However, because biological processes mostly occur in an aqueous environment, we also determined the conformation of a representative example of these homo-N-nucleosides in solution using NMR spectroscopy. Because of solubility reasons (H<sub>2</sub>O solubility) the cytosine analogue was selected for analysis. Information derived from coupling constants (Table 1), especially the rather high value for  $J_{1',2'b}$  which is characteristic for a quasi axial-axial relationship of the 1' and 2'b hydrogens, revealed that the solution state conformation resembles very well the solid state conformation.

Indeed, application of the computer program PSEUROT (version 6.2)<sup>4</sup> showed a clear preference for a South conformation (molar fraction  $X_s = 0.86$ , and puckering parameters  $P_s = 146$  and  $\psi_{ms} = 0.37$ , while keeping the parameters for the minor N-type conformer at fixed values of  $P_N = 14^\circ$  and  $\psi_{mN} = 35^\circ$ ). The analogy between the conformation of the sugar moiety of a natural 2'-deoxynucleoside and the conformation of the tetrahydrofuran moiety of homo-N-nucleosides, further prompted us to analyze their potential biological properties.

Shift values	Coupling constants
1' : 4.44 ppm (m)	$J_{1',2a} = 6.0$ Hz
2'a : 2.01 ppm (ddAB)	$J_{1',2b} = 9.7$ Hz
2'b : 1.82 ppm (ddAB)	$J_{2a,2b} = -13.6$ Hz
3' : 4.25 ppm (dt)	$J_{2a,3'} = 2.3$ Hz
4' : 3.86 ppm (m)	$J_{2b,3'} = 6.0$ Hz
5'a : 3.54 ppm (dAB)	$J_{3',4'} = 2.5$ Hz
5'b : 3.48 ppm (dAB)	$J_{4',5a} = 4.5$ Hz
6'a : 4.00 ppm (dd)	$J_{4',5b} = 5.6$ Hz
6'b : 3.83 ppm (dd)	$J_{5a,5b} = -12.1$ Hz
6 : 7.57 ppm (d)	$J_{1',6a} = 3.2$ Hz
5 : 5.94 ppm (d)	$J_{1',6b} = 7.3$ Hz
	$J_{6a,6b} = -14.4$ Hz

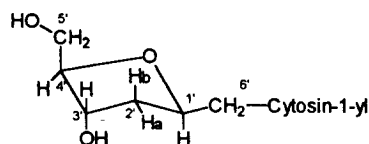


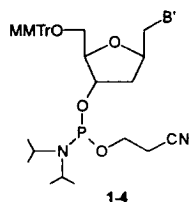
Table 1 : Preferential conformation of 1-N-[(2'-deoxy-β-D-ribo-pentofuranosyl)methyl]cytosine as derived from a 2D DQFCOSY  $^1\text{H}$  NMR in  $\text{D}_2\text{O}$ .

#### Incorporation of homo-N-nucleosides in DNA

Short oligonucleotides which are able to hybridize with complementary sequences using Watson-Crick base pairing, may potentially inhibit the transcription or translation process<sup>5</sup>. One of our research programs is to investigate which oligonucleotides are able to hybridize strongly with their target and, hence, may sterically block the targeted nucleic acids. Therefore we incorporated the homo-N-nucleosides in an oligonucleotide sequence and studied the duplex stability, primarily with DNA as target. For this purpose the monomers were converted into their homo-N-nucleoside monomethoxytritylated phosphoramidites 1-4<sup>6</sup>.

In Table 2, the  $T_m$ 's ( $^{\circ}\text{C}$ ) are given for the indicated duplexes where X represents a natural or modified (\*) nucleotide and Y a natural nucleotide. From this Table it is clear that incorporation of only one modified nucleoside into DNA destabilized the duplex by 9 to  $10^{\circ}\text{C}$ . Also no alternative base-pairing was observed. Incorporation of a second homo-N-nucleoside further destabilized the duplex by another  $10^{\circ}\text{C}$  (data not shown), while the fully modified oligonucleotide (I) did not hybridize at all with the natural complementary sequence (II) even at 1 M NaCl.

These data indicate that homo-N-nucleosides are not useful as substitutes for natural deoxynucleosides in a DNA duplex construct, most probably due to steric hindrance. Because of the large decrease in  $T_m$  noted for the DNA duplexes, we did not further examine the stability of the corresponding DNA-RNA complexes.



1-4

- 1 : B' = N<sup>6</sup>-benzoyladenine-9-yl
- 2 : B' = N<sup>2</sup>-isobutanoylguanine-9-yl
- 3 : B' = thymine-1-yl
- 4 : B' = N<sup>4</sup>-benzoylcytosine-1-yl

I : 5'-CACCGXCGGCGCC-3'

II : 3'-GTGGCYGCCGCGG-5'

X	Y	A	T	G	C
A		61.5	70.0	67.0	58.3
A*		57.8	61.8	58.4	55.9
T		70.3	59.0	65.0	56.5
T*		60.7	57.7	60.0	53.4
G		67.0	64.9	66.7	72.8
G*		57.3	60.7	62.4	63.2
C		60.1	58.8	73.5	54.9
C*		56.4	55.0	64.4	52.3

Table 2 : Structure of a homo-N-nucleoside phosphoramidite and Tm of duplexes as determined at 0.1 M NaCl, 0.02 M potassium phosphate pH 7.5, 0.1 mM EDTA; conc. : 4  $\mu$ M.

### Homo-N-nucleosides in the catalytic core of Hammerhead Ribozymes

Hammerhead ribozymes consist of hybridizing sequences, a stem loop and a catalytic core<sup>7</sup>. As homo-N-nucleosides are not able to stabilize duplexes, we examined the possibility of substituting natural nucleosides by homo-N-nucleosides in the catalytic core. The selection of the substitution sites was based on previous studies indicating which 2'-hydroxyl function (ribonucleotides) is not absolutely required for catalytic activity<sup>8</sup>.

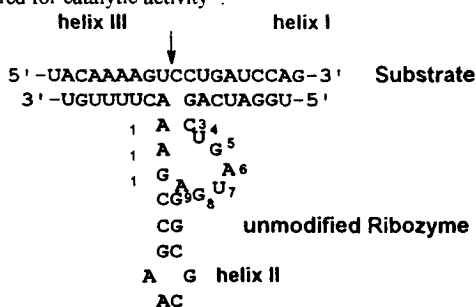


Figure 1 : Structure of a hammerhead ribozyme targeted at IL-6 mRNA, indicating the cleavage site and the position modified by homo-N-nucleosides (C<sub>3</sub>,U<sub>4</sub>); (A<sub>6</sub>,U<sub>7</sub>); (G<sub>12</sub>,A<sub>13</sub>,A<sub>14</sub>) and the combination of the three cassette replacements.

Three nucleotide cassettes (C<sub>3</sub>,U<sub>4</sub>), (A<sub>6</sub>,U<sub>7</sub>) (G<sub>12</sub>,A<sub>13</sub>,A<sub>14</sub>) and a combination of them (C<sub>3</sub>,U<sub>4</sub>,U<sub>7</sub>,G<sub>12</sub>,A<sub>13</sub>,A<sub>14</sub>) were replaced by homo-N-nucleotides in a selected hammerhead ribozyme targeted at the mRNA of interleukin 6 (IL-6). To simplify the kinetic experiments, a short synthetic RNA substrate, mimicking nucleosides 471-489 (19-mer) of human IL-6 mRNA<sup>9</sup> was used for the cleavage reactions. This sequence (Fig. 1) containing the potential cleavage site GUC, has been studied previously as a target for hammerhead ribozyme-mediated cleavage<sup>10-13</sup>. However, no cleavage was observed for any of the four modified ribozymes under the assay conditions published before<sup>11,13</sup>, indicating the detrimental influence of homo-N-nucleosides on the substitution sites.

### Homo-N-nucleosides as antiviral lead compounds

As viral enzymes often have a broader substrate specificity than mammalian enzymes and as selective inhibition of viral enzymes may lead to antiviral activity, the above mentioned nucleosides were evaluated for their antiviral activity<sup>14,15</sup>. The pyrimidine homo-N-nucleosides (with thymine or cytosine as the base moieties) did not show any activity against herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), vaccinia virus, parainfluenza-3 virus, reovirus-1,

Sindbis virus, Coxsackie virus B4, Punta Toro virus or respiratory syncytial virus (highest concentration tested 400 µg/mL). Marked activity, however, was found for the purine homo-N-nucleosides against HSV-1, HSV-2 (Table 3) and for the adenine homo-N-nucleoside against vaccinia virus (20 µg/mL), but not against other viruses. Although the activity was not very high, the results are remarkable in that similar activity was observed against thymidine kinase-deficient (TK<sup>-</sup>) strains as for the TK<sup>+</sup> strains, while no cytotoxicity was detected.

Table 3: Cytotoxicity and antiviral activity of homo-N-nucleosides with an adenine-9-yl (A) or guanine-9-yl (G) base moiety

	Minimum cytotoxic concentration <sup>a</sup> (µg/mL)	Minimum inhibitory concentration <sup>b</sup> (µg/mL)			
		HSV-1 (KOS)	HSV-2 (G)	HSV-1 TK <sup>-</sup> B2006	HSV-1 TK <sup>-</sup> VMW 1837
A	>400	20	6	10	8
G	>400	5	5	12	12
Brivudin	200	0.007	>100	20	30
Acyclovir	>400	0.02	0.03	80	4

<sup>a</sup> required to cause a microscopically detectable alteration of normal cell morphology;

<sup>b</sup> required to reduce virus-induced cytopathogenicity by 50%.

#### Acknowledgement

Authors thank M. Vandekinderen for editorial help and G. Schepers for technical assistance. A. Van Aerschot is a Research Associate of the Belgian National Fund of Scientific Research.

#### References and notes

1. Herdewijn, P. *Antiviral Res.* **1992**, *19*, 1-14.
2. Hossain, N.; Blaton, N.; Peeters, O.; Rozenski, J.; Herdewijn, P. *Tetrahedron* **1996**, *52*, 5563-5578.
3. Saenger, W. In *principles of Nucleic Acid Structure*, Springer-Verlag, **1984**, p. 55-61.
4. de Leeuw, F.A.A.M.; Altona, C. *J. Comput. Chem.* **1983**, *4*, 428.
5. Uhlman, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543-584.
6. The phosphoramidites were characterized by NMR and MS analysis, and satisfactory data were obtained. LSIMS (NBA) *m/z*: 1: 842 (MH<sup>+</sup>, 15), 273 (MMTr<sup>+</sup>, 100); 2: 824 (MH<sup>+</sup>, 5), 273 (MMTr<sup>+</sup>, 100); 3: 729 (MH<sup>+</sup>, 30), 273 (MMTr<sup>+</sup>, 100); 4: 816 [(M-H)<sup>+</sup>, 20]. <sup>31</sup>P-NMR δ (ppm, H<sub>3</sub>PO<sub>4</sub> external reference): 1: 148.97, 149.24; 2: 148.55, 149.33; 3: 148.86, 149.20; 4: 148.90, 149.27.
7. Uhlenbeck, O.C. *Nature* **1987**, *328*, 596-600.
8. Pley, H.W.; Flaherty, K.M.; McKay, D.B. *Nature* **1994**, *372*, 68-74.
9. Hirano, T.; Yasukawa, K.; Harada, H.; Taga, T.; Watanabe, Y.; Matsuda, T.; Kashiwamura, S.; Nakajima, K.; Koyama, K.; Iwamatsu, A.; Tsunasawa, S.; Sakiyama, F.; Matsui, H.; Takahara, U.; Taniguchi, T.; Kishimoto, T. *Nature* **1986**, *324*, 73-76.
10. Mahieu, M.; Deschuyteneer, R.; Forget, D.; Vandenbussche, P.; Content, J. *Blood* **1994**, *84*, 3758-3765.
11. Hendrix, C.; Mahieu, M.; Anné, J.; Van Calenbergh, S.; Van Aerschot, A.; Content, J.; Herdewijn, P. *Biochem. Biophys. Res. Commun.* **1995**, *210*, 67-73.
12. Mahieu, M.; Hendrix, C.; Ooms, J.; Herdewijn, P.; Content, J. *Biochem. Biophys. Res. Commun.* **1995**, *214*, 36-43.
13. Hendrix, C.; Anné, J.; Joris, B.; Van Aerschot, A.; Herdewijn, P. *Biochem. J.* **1996**, *314*, 655-661.
14. De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R.T.; Jones, A.S.; Torrence, P.F.; Shugar, D. *J. Infect. Dis.* **1980**, *141*, 563-574.
15. De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P.C.A. *Nature* **1986**, *323*, 464-467.
16. Note added in proof: a paper describing the synthesis of analogous homo-N-nucleosides but with the base residing in the α-conformation recently appeared. No results of incorporation studies were reported. Scremin, C.L.; Boal, J.H.; Wilk, A.; Phillips, L.R.; Beaucage, S.L. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 207-212.