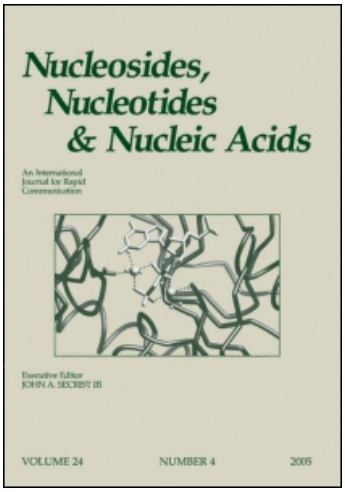

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CYCLOHEXENE NUCLEIC ACIDS (CeNA) FORM STABLE DUPLEXES WITH RNA AND INDUCE RNASE H ACTIVITY

J. Wang,[1] B. Verbeure,[1] I. Luyten,[1] M. Froeyen,[1] C. Hendrix,[1] H. Rosemeyer,[2] F. Seela,[2] A. Van Aerschot, [1] and P. Herdewijn[1,*]

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ABSTRACT

Cyclohexene nucleic acids (CeNA) were synthesized using classical phosporamidite chemistry. Incorporation of a cyclohexene nucleo-side in a DNA chain leads to an increase in stability of the DNA/RNA duplex. CeNA is stable against degradation in serum. A CeNA/RNA hybrid is able to activate *E. Coli* RNase H, resulting in cleavage of the RNA strand.

Cyclohexene nucleic acids (CeNA) are a new class of nucleic acids in which the furanose sugar moiety is replaced by a cyclohexene ring. It is general knowledge that replacement of the five-membered ring of a natural DNA and RNA by a six-membered ring increases the conformational rigidity of the oligomer. Several types of six-membered nucleic acids have been studied. The pyranose nucleic acids have an equatorially oriented base moiety at the 1′-position. These oligomers form strong self-complementary duplexes, but don't hybridize with natural nucleic acids. On the other hand, the hexitol nucleic acids (HNA) have the base moiety located at the 2′-position. They have axially oriented nucleobases and hybridize with natural nucleic acids and themselves. The RNA selective hybridization of HNA is explained

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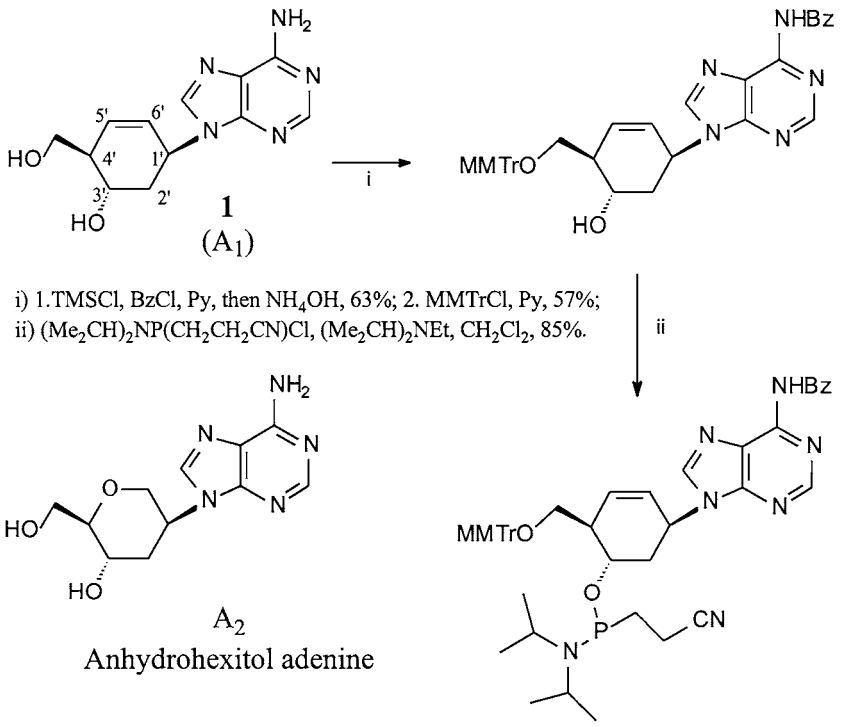
by the pre-organization of the hexitol ring in an N-type furanose conformer (3′-*endo* conformation with an axially oriented base moiety). The cyclohexane nucleic acids (CNA), however, have an equatorially oriented nucleobase at the monomeric level, but at the oligomeric level the base moiety is axially oriented. The axial orientation is required for hybridixation of CNA with natural nucleic acids. The chair-chair conformation interconversion of the cyclohexane ring is energetically highly demanding. This energy barrier is expected to be significantly reduced by the introduction of a double bond in the cyclohexane ring, resulting in cyclohexene nucleic acids (CeNA).

Cyclohexene nucleic acids were prepared by classical phosphoramidite chemistry. The cyclohexenyl adenine phosphoramidite building block was synthesized as shown in Scheme 1. The adenine base moiety of **1** was protected as a benzoate and the primary hydroxyl group as a monomethoxytrityl ether. The secondary hydroxyl group was reacted with 2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphoramidate to yield the nucleotide building block. Assembly of the monomers into oligonucleotides was accomplished using the standard phosphoramidite method. The cyclohexenyl analogue was used at a 0.12 M concentration and the coupling was allowed to proceed for 3 min (>95% coupling efficiency).

When incorporating the cyclohexenyl-A (A_1) in a mixed purine-pyrimidine DNA sequence (5′-CCAGTGATATGC-3′) and in the corresponding RNA sequence



Scheme 1.

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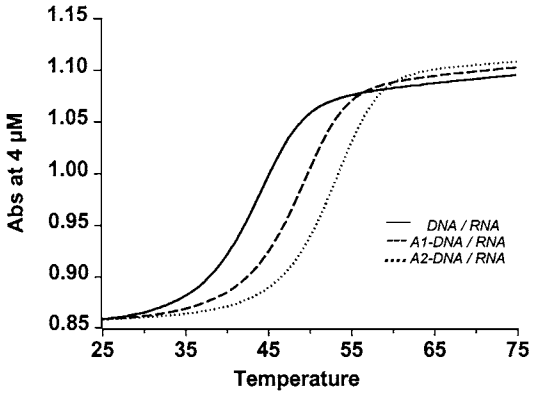

Figure 1.

(5′-CCAGUGAUAUGC-3′), a small decrease in stability of dsDNA (−0.5°C Δ Tm/mod) was observed. A similar drop in stability is observed after incorporation of anhydrohexitol-A (A_2). The influence of A_1 and A_2 on the duplex stability of dsDNA doesn't seem to be fundamentally different. Of more interest is the influence of A_1 on the stability of a DNA/RNA duplex. Incorporation of 1, 2 or 3 cyclohexenyl nucleosides in the DNA strand increases duplex stability with 1.1, 1.6, and 5.2°C, respectively, corresponding to a ΔTm/mod of $+1.1$, $+0.8$ and $+1.7$°C. As expectd, the influence on duplex stability is dependent on the incorporation site of A_1. The stabilization effect of A_2 on the DNA/RNA duplex is twice as high as of A_1. However, the sequence selective effect is the same. An overlay of the melting curves for DNA/RNA duplexes with a triple substitution of A_1 and A_2 is depicted in Figure 1. Incorporation of three A_1 in a dsRNA sequence leads to an increase in duplex stability of $+0.6$°C. The increase in duplex stability after incorporation of A_1, using RNA as the complementary strand, suggests conformational preorganization of A_1 ($^3H_2$, mimicking the 3′-endo conformation of the ribofuranose sugar). The $^3H_2$ conformation is indeed the most stable conformation of cyclohexenyl-A.

The complex formed between $(A_1)_{13}$ and poly(U) is of similar stability as between $(A_2)_{13}$ and poly(U). However, the $(A_1)_{13}$-poly(dT) complex is much more stable ($\Delta Tm = +10$°C) than the corresponding $(A_2)_{13}$-poly(dT). The titration experiment showed the nature of $(A_1)_{13}$-poly(dT) complex is of triplex origin ($(dT)_{13}.(A1)_{13}.(dT)_{13}$). The complex formed between oligo(A_1) and its DNA or RNA complement is of similar stability.

CD spectral analysis showed that the spectrum of the A_1-DNA/DNA complex (three A1 were incorporated in the above mentioned DNA mixing sequence) is intermediate between that of a DNA/DNA and a RNA/DNA complex, while the spectrum of the corresponding A_2-DNA/DNA complex is more similar to the DNA/RNA complex. The CD spectrum of all RNA containing complexes are similar and suggest that they adopt similar conformations. This result suggests that the incorporation of three A_2 in a DNA strand is able to induce a conformational change

of a dsDNA duplex in the direction of a DNA/RNA type structure. However, three A_1 residues don't seem to have this effect and the DNA/A_1-DNA conformation stays close to the typical dsDNA form.

The complex formed between the A_1 oligomer and its RNA complement is able to activate *E. Coli* RNase H, resulting in the cleavage of the RNA strand, while the HNA containing complex is devoid of this ability. CeNA has serum stability similar to HNA. Both modified oligonucleotides are much more stable against enzymatic degradation (no degradation after incubation for 3 h in serum at 37°C) than DNA (almost half degraded after 3 h incubation).

In conclusion, cyclohexene nucleic acids are much more flexible than other six-membered nucleic acids and are good mimicks of natural furanose nucleic acids. They combine the advantage of duplex stabilization and serum stability with the potential to activate RNase H. They are potential candidates for antisense application.

REFERENCES

1. Wang, J.; Verbeure, B., Luyten, I., Froeyen, M.; Hendrix, C.; Rosemeyer, H.; Seela, F.; Van Aerschot, A.; Herdewijn, P. *J. Am. Chem. Soc*. **2000**, *in press*.
2. Herdewijn, P. *Liebigs Annalen* **1996**, 1337–1348.
3. Eschenmoser, A. *Pure Applied Chem*. **1993**, *65*, 11790–1188.
4. Hendrix, C.; Rosenmeyer, H.; De Bouvere, B.; Van Aerschot, A.; Seela, F.; Herdewijn, P. *Chem. Eur. J*. **1997**, *3*, 1513–1520.
5. Maurinsh, Y.; Rosenmeyer, H.; Esnouf, R.; Medvedovici, A.; Wang, J.; Ceulemans, G.; Lescrinier, E.; Hendrix, C.; Busson, R.; Sandra, P.; Seela, F.; Van Aerschot, A.; Herdewijn, P. *Chem. Eur. J*. **1999**, *5*, 2139–2150.

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