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Conformationally-2',4'-Locked Aza-ENA and Carbocyclic *ribo*-Thymidine

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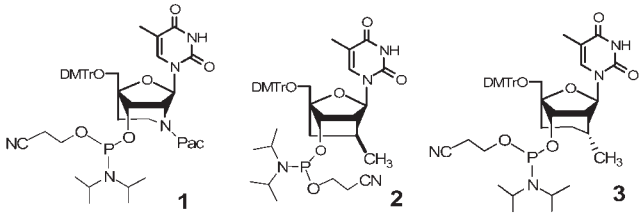
ABSTRACT

AONs containing aza-ENA (1), 5-membered (2) and 6-membered (3) carbocyclic analogs of LNA (carbocyclic-LNA-T) and ENA (carbocyclic-ENA-T) are both nuclease resistant and capable of eliciting RNase H response, very similar to that of the native.

INTRODUCTION

Three factors are perhaps most important in order to develop gene silencing agents in the antisense approach with natural phosphodiester linkages: Stability, Delivery and RNase H recruitment. In this regard, we have designed and synthesized AONs containing aza-ENA (**1**), 5-membered (**2**) and 6-membered (**3**) carbocyclic analogs of LNA (carbocyclic-LNA-T) and ENA (carbocyclic-ENA-T), which are both nuclease resistant and capable of eliciting RNase H response.



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RESULTS AND DISCUSSION

Aza-ENA-T

The 2'-deoxy-2'-*N*,4'-*C*-ethylene bridged thymidine (aza-ENA-T)[1,2] has been synthesized to give a pair of 3',5'-*bis*-OBn protected diastereomerically pure aza-ENA-Ts with the fused piperidino skeleton in the chair conformation, whereas the pentofuranosyl moiety is locked in the North-type conformation. The origin of the chirality of two diastereomerically pure aza-ENA-Ts was found to be due to the endocyclic chiral 2'-nitrogen, which has axial N-H in and the equatorial N-H. The latter is thermodynamically preferred while the former is kinetically preferred with E_a = 25.4 kcal mol^{-1}, which is so far the highest observed inversion barrier at pyramidal N-H in the bicyclic amines.

Carbocylic LNA and ENA

The synthesis of the novel conformationally constrained carbocyclic analogs of LNA and ENA have been achieved[3] on the other hand using free-radical C-C bond formation as a key step.

To ensure that the radical generated has adequate lifetime to capture the double bond before it is quenched by hydrogen radical, the concentrations of Bu_3SnH and AIBN were maintained through high dilution and slow drop-wise addition. The 5-hexenyl type *exo* mode cyclization of the radical to C4'-propenyl double-bond yielded exclusively the expected 5-membered 2',4'-*cis*-fused carbocyclic product with bicyclo[2.2.1]heptane skeleton as inseparable diastereomeric mixtures (major compound 70 %, 7'*R*, and minor compound 30 %, 7'*S*). On the other hand, for 6-exo-heptenyl type cyclization of an appropriate distant double-bond at C4' (C4'-butenyl) and the radical center at C2' of the ribofuranose ring of thymidine gave exclusively *exo*-carbocylic 6-membered fused product in 76 % yield.

The Aza-ENA-T, carbocyclic-LNA-T and carbocyclic-ENA-T were subsequently incorporated in to the antisense oligonucleotides (AONs) to show that they enhance the T_m of the modified AON/RNA heteroduplexes by 2.5 to 4 °C per modification for aza-ENA –T, and 3.5 to 5 °C and 1.5 °C /modification for carbocyclic-LNA-T, and carbocyclic-ENA-T respectively, depending upon the modification site in the AONs. Whereas the relative RNase H cleavage rates with carbocyclic-LNA-T, carbocyclic-ENA-T, aza-ENA-T and LNA-T modified AON/RNA duplexes were found to be very similar to that of the native counterpart, irrespective of the type and the site modification in the AON strand. A *single incorporation* of aza-ENA-T or carbocyclic-LNA and the carbocyclic-ENA into AONs leads to very much more enhanced nuclease stability (of the residual AON from the 3'-end of the modification site) in the blood serum (stable >48 h) as compared to that of the native (fully degraded <3 h) and the *identically* LNA-modified AONs (fully degraded < 9 h) and aza-ENA (≈85 % stable in 48 h). Clearly, remarkably enhanced life-time of these carbocyclic-modified AONs in the blood serum may produce the highly desired pharmacokinetic properties because of their unique stability, and consequently a net reduction of the required dosage. This unique quality as well as their efficient use as the AON in the RNase H promoted cleavage of the target RNA, makes our carbocyclic-LNA /-ENA modifications excellent candidates as potential antisense therapeutic agent.

CONCLUSION

The main conclusion which can be drawn from these studies[1-3] is that even though all modified AONs used recruited RNase H almost as efficiently as that of the native counterpart, but it is only the carbocyclic-LNA and the carbocyclic-ENA modified AONs which have shown, much enhanced nuclease stability in the blood serum (ca 48h) as compared to that of the native and the LNA-modified AONs (fully degraded <12 h) and aza-ENA (≈85 % stable in 48 h), when AONs used have identical modification site and condition for stability measurement.

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Erratum

Conformationally-2',4'-Locked Aza-ENA and Carbocyclic ribo-Thymidine

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