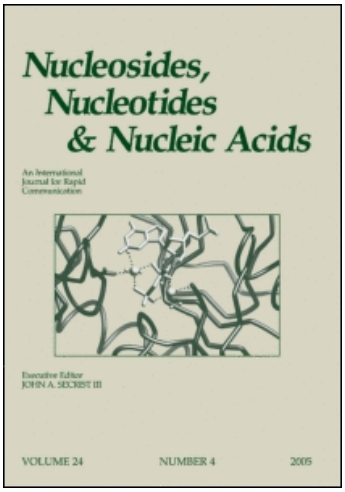

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Properties of Oligonucleotide Chimeras Containing 5'-Amino-2'-deoxy-2'-fluoroarabinonucleosides

Ekaterina Viazovkina[a]; Kyung-Lyum Min[a]; Annie Galarneau[a]; Masad J. Damha[ab]
[a] Department of Chemistry, McGill University, Montreal, Quebec, Canada [b] Otto Maass Chemistry Building, McGill University, Montreal, Quebec, Canada

Online publication date: 09 August 2003

To cite this Article Viazovkina, Ekaterina , Min, Kyung-Lyum , Galarneau, Annie and Damha, Masad J.(2003) 'Synthesis and Properties of Oligonucleotide Chimeras Containing 5'-Amino-2'-deoxy-2'-fluoroarabinonucleosides', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1335 — 1338
To link to this Article: DOI: 10.1081/NCN-120022959
URL: http://dx.doi.org/10.1081/NCN-120022959

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1335–1338, 2003

Synthesis and Properties of Oligonucleotide Chimeras Containing 5′-Amino-2′-deoxy-2′-fluoroarabinonucleosides

Ekaterina Viazovkina, Kyung-Lyum Min, Annie Galarneau, and Masad J. Damha*

Department of Chemistry, McGill University, Montreal, Quebec, Canada

ABSTRACT

Oligonucleotide analogues comprised of 2′-deoxy-2′-fluoro-β-D-arabinose units joined via P3′-N5′ phosphoramidate linkages (2′F-ANA$^{5'N}$) were prepared for the first time. Among the compounds prepared were a series of 2′OMe-RNA-[GAP]-2′OMe-RNA 'chimeras', whereby the "GAP" consisted of DNA, DNA$^{5'N}$, 2′F-ANA or 2′F-ANA$^{5'N}$ segments. The chimeras with the 2′F-ANA and DNA gaps exhibited the highest affinity towards a complementary RNA target, followed by the 5′-amino derivatives, i.e., 2′F-ANA > DNA > 2′F-ANA$^{5'N}$ > DNA$^{5'N}$. Importantly, hybrids between these chimeras and target RNA were all substrates of both human RNase HII and *E.coli* RNase HI. In terms of efficiency of the chimera in recruiting the bacterial enzyme, the following order was observed: gap DNA > 2′F-ANA > 2′F-ANA$^{5'N}$ > DNA$^{5'N}$. The corresponding relative rates observed with the human enzyme were: gap DNA > 2′F-ANA$^{5'N}$ > 2′F-ANA > DNA$^{5'N}$.

Key Words: 5′-Amino-2′-deoxy-2′-fluoroarabinonucleosides; Thermal stability; RNase H.

*Correspondence: Masad J. Damha, McGill University, Otto Maass Chemistry Building, 801 Sherbrooke Street W, H3A 2K6, Montreal, Quebec, Canada; Fax: +1 514 398 3797; E-mail: masad.damha@mcgill.ca.

DOI: 10.1081/NCN-120022959
1525-7770 (Print); 1532-2335 (Online)

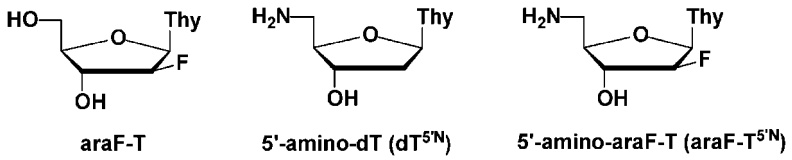

Figure 1. Structure of the nucleosides synthesized for this work.

Antisense oligonucleotides (AONs) suitable for use as therapeutics must bind tightly to their complementary RNA targets, elicit degradation of the RNA by the cellular enzyme RNase H, and have the general physiochemical characteristics of a drug-like molecule (solubility, stable to degradation, etc). A number of modified AONs have been developed, but very few of them are able to activate RNase H. We have previously shown that 2′-deoxy-2′-fluoro-β-D-arabinonucleic acids (FANA) can form stable complexes with complementary RNA and activate RNase H.[1,2] The present report describes the antisense characteristics of oligonucleotides constructed from 5′-amino-2′-deoxy-2′-fluoroarabinonucleoside units (2′F-ANA$^{5'N}$). Since substitution of the 5′-OH with a 5′-NH_2-group has very little influence on the conformation of the sugar ring,[3,4] we speculated that this modification would confer better nuclease resistance without compromising the ability of the AON to bind to RNA or elicit RNase H activity.

To test this hypothesis, the key starting material, 5′-amino-2′-deoxy-2′-fluoro-arabinothymidine (araF-T$^{5'N}$, Fig. 1) was prepared starting from araF-T. Thus, araF-T was converted to its 5′-tetrachlorophthalimide derivative, which upon treatment with ethylenediamine afforded araF-T$^{5'N}$ in good yields.[5,6] dT$^{5'N}$ was prepared by the same procedure,[5] and together with araF-T$^{5'N}$, were converted to their 5′-O-monomethoxytrityl-3′-O-cyanoethylphosphoramidite derivatives. To assess the effect of 5′O → 5′N substitutions on the stability of dT/rA hybrids, oligodeoxythymidylates containing a single araF-T$^{5'N}$ or dT$^{5'N}$ unit were prepared. T_m data obtained demonstrated a significant destabilization effect ($\Delta T_m = -2$ to -3°C/ substitution). Next, the affinity of oligonucleotide chimeras was assessed (Table 1).

Table 1. Melting temperature (T_m) of hybrids formed between RNA and oligonucleotide chimeras (gapmers).

AON #	AON composition	T_m (°C)
1	$U_{me}U_{me}U_{me}U_{me}U_{me}U_{me}$d(TTTTTT)$U_{me}U_{me}U_{me}U_{me}U_{me}U_{me}$	40
2	$U_{me}U_{me}U_{me}U_{me}U_{me}U_{me}$d($T^nT^nT^nT^nT^nT^n$)$U_{me}U_{me}U_{me}U_{me}U_{me}U_{me}$	27
3	$U_{me}U_{me}U_{me}U_{me}U_{me}U_{me}$ara($T_fT_fT_fT_fT_fT_f$)$U_{me}U_{me}U_{me}U_{me}U_{me}U_{me}$	44
4	$U_{me}U_{me}U_{me}U_{me}U_{me}U_{me}$ara($T_f{}^nT_f{}^nT_f{}^nT_f{}^nT_f{}^nT_f{}^n$)$U_{me}U_{me}U_{me}U_{me}U_{me}U_{me}$	32

U_{me} corresponds to 2′-O-methyluridine; dT, dT^n, $araT_f$, and $araT_f^n$ correspond to 2′-deoxythymidine, dT$^{5'N}$, araF-T, and araF-T$^{5'N}$ nucleosides, respectively (see Fig. 1). All melting experiments were done with rA_{18} as target, in a buffer: 140 mM KCl, 5 mM, Na_2HPO_4, 1 mM $MgCl_2$, pH 7.2.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

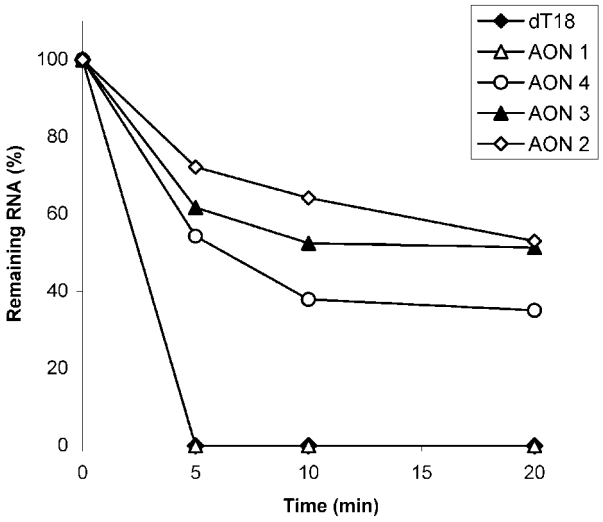

Figure 2. RNase H-mediated degradation of RNA (rA_{18}). The hybrids AON/RNA were exposed to human RNase HII as previously described.[7] The base sequence of the AONs is given in Table 1.

These AONs contained two 2′OMe-rU_6 'wings' flanking a hexathymidylate 'gap', namely dT_6, $(dT^{5'N})_6$, araF-T_6 and araF-$(T^{5'N})_6$ (Table 1). Again, T_m data demonstrated a destabilizing effect upon substituting a 5′O with a 5′NH group (ΔT_m −2°C).

Figure 2 shows that all AON chimeras induced target RNA cleavage by human RNase HII. The ability of the various AONs to elicit RNase HII activity followed the order: DNA > araF-$T^{5'N}$ > araF-T > $dT^{5'N}$ demonstrating that while 5′O → 5′N substitutions in the DNA strand is detrimental to RNase HII activity, this was not the case for 2′F-ANA strands for which a small increment in enzymatic activity was observed.

ACKNOWLEDGMENTS

We wish to thank CIHR (Canada) and Anagenis, Inc. for financial support.

REFERENCES

1. Damha, M.J.; Wilds, C.J; Noronha, A.; Brukner, I.; Borkow, G.; Arion, D.; Parniak, M.A. Hybrids of RNA and arabinonucleic acids (ANA and 2′F-ANA) are substrates of RNase H. J. Am. Chem. Soc. **1998**, *120*, 12,976–12,977.
2. Wilds, C.J.; Damha, M.J. 2′-Deoxy-2′-fluoroarabinonucleosides and oligonucleotides (2′F-ANA): synthesis and physicochemical studies. Nucl. Acids Res. **2000**, *28*, 3625–3635.

3. Nottoli, E.M.; Lambert, J.B.; Letsinger, R.L. Study of conformational changes induced on substituting NH for O at C(5′) of thymidine nucleosides and nucleotides. J. Am. Chem. Soc. **1977**, *99*, 3486–3490.
4. Gryaznov, S.M.; Letsinger, R.L. Synthesis and properties of oligonucleotides containing aminodeoxythymidine units. Nucl. Acids Res. **1992**, *20*, 3403–3409.
5. Tetzlaff, C.N; Schwope, I.; Bleczinski, C.F.; Steinberg, J.A.; Richert, C. A convenient synthesis of 5′-amino-5′-deoxythymidine and preparation of peptide-DNA hybrids. Tetrahedron Letters **1998**, *39*, 4215–4218.
6. Harada, K.; Matulic-Adamic, J.; Price, R.W.; Schinazi, R.F.; Watanabe, K.; Fox, J.J. Nucleosides. 139. Synthesis and anticytomegalovirus and antiherpes simplex virus activity of 5′-modified analogues of 2′-fluoroarabinosylpyrimidine nucleosides. J. Med. Chem. **1987**, *30*, 226–229.
7. Min, K.; Viazovkina, E.; Galarneau, A.; Parniak, M.; Damha, M. Oligonucleotides comprised of alternating 2′-deoxy-2′-fluoro-b-D-arabinonucleosides and D-2′-deoxyribonucleosides (2′F-ANA/DNA 'altimers') induce efficient RNA cleavage mediated by RNase H. Bioorg. Med. Chem. Lett. **2002**, *12*, 2651–2654.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016