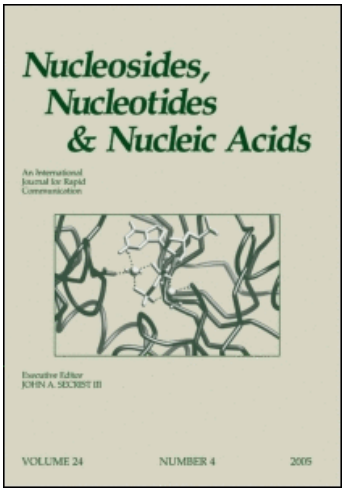

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Novopashina, D. S. , Totskaya, O. S. , Lomzov, A. A. and Venyaminova, A. G.(2005) '3'-Modified Oligo(2'-*O*-Methylribonucleotides) as Improved Probes for Hybridization with RNA', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 527 — 531
To link to this Article: DOI: 10.1081/NCN-200061795
URL: http://dx.doi.org/10.1081/NCN-200061795

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):527–531, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200061795

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3′-MODIFIED OLIGO(2′-*O*-METHYLRIBONUCLEOTIDES) AS IMPROVED PROBES FOR HYBRIDIZATION WITH RNA

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▫ *A series of octa(2′-O-methylribonucleotides) with an additional 3′-terminal deoxynucleoside (T, dC, dA or dG) linked by the 3′-3′ (inverted) bond was synthesized. The exceptional stability of these oligomers to a 3′-exonuclease (SVP) and nucleases in culture medium containing 10% heat-inactivated fetal calf serum was demonstrated. It was shown that the addition of the 3′-dangling inverted deoxynucleoside increases substantially the thermal stability of the duplexes of oligo(2′-O-methylribonucleotides) with complementary RNA and DNA in the case of a relatively weak terminal A^mU(T) pair and enhances the mismatch sensitivity.*

Keywords Oligo(2′-*O*-methylribonucleotides), 3′-Dangling "Inverted" Deoxyribonucleoside, Nuclease Resistance, Duplex Stability

INTRODUCTION

RNA is not only a unique object for the investigation of the origin of life and evolution, but also an important therapeutic target. However, its complex secondary and tertiary structure restricts RNA interaction with oligonucleotide probes. Oligo(2′-*O*-methylribonucleotides) have some important advantages as oligonucleotide probes capable to unfold highly structured RNA molecules: enhanced binding affinity to RNA, high rate of hybridization, improved nuclease resistance, and increased sensitivity to mismatches compared to oligodeoxyribonucleotides. Introduction of the "inverted" thymidine (T_{inv}) to the 3′-end of oligo(2′-*O*-methylribonucleotides) increases their resistance to 3′-exonuclease digestion and duplex stability.[1,2] The use of these modified oligonucleotides for designing NAzyme effector systems is a very perspective approach.[3,4]

This work is devoted to comparative study of the influence of different inverted deoxynucleosides on the properties of oligo(2′-*O*-methylribonucleotides). Resistance

The authors are grateful to Dr. D.V. Pyshnyi for helpful discussion and Dr. M.N. Repkova for synthesis of oligonucleotides. This work was supported by RFBR (project N 02-04-48586) and INTAS (project N 03-51-5281).

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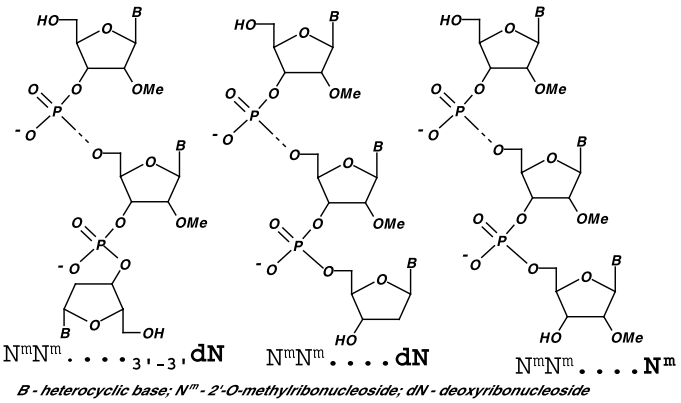

FIGURE 1 Structure of the synthesized oligo(2′-*O*-methylribonucleotides) with an additional 3′-terminal deoxynucleoside and control oligomers.

of these modified oligoribonucleotides to nuclease digestion and thermodynamic parameters of duplex formation were investigated.

RESULTS AND DISCUSSION

Solid supports with attached 3′-*O*-DMTr-deoxyribonucleosides for the synthesis of octa(2′-*O*-methylribonucleotides) with an additional 3′-terminal deoxyribonucleoside (T, dC, dA, or dG) linked by the 3′-3′ phosphodiester bond were prepared according to Ramalho Ortigao et al.[5] Standard phosphoramidite chemistry was used for solid-phase synthesis of a set of 3′-modified and unmodified oligonucleotides (Figure 1). Nucleoside composition of the oligonucleotides synthesized has been confirmed by enzymatic hydrolysis.

Stability of the oligomers to the 3′-exonuclease (SVP) and nucleases in culture medium containing 10% heat-inactivated fetal calf serum was investigated (Figures 2

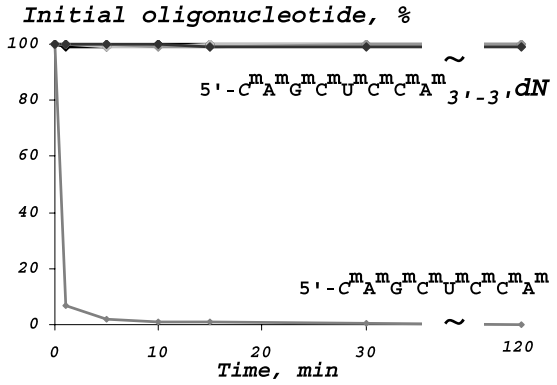

FIGURE 2 Oligonucleotides were incubated at 37°C with snake venom phosphodiesterase (0.01 units/mL) in the buffer (10 mM Tris-HCl, pH 7.8, 0.5 mM $MgCl_2$); [oligonucleotide] = 10^{-5} M.

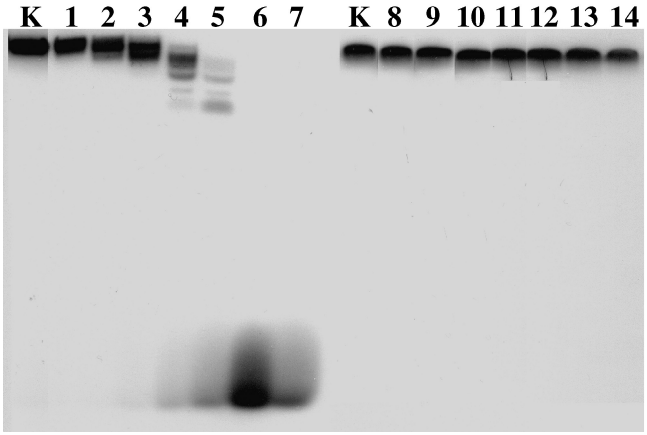

FIGURE 3 Stability of oligonucleotides at 37°C in a culture medium (IMDM) containing 10% fetal calf serum. 5′-[^{32}P]-$C^mA^mG^mC^mU^mC^mC^mA^mT$–lines (1–7), 5′-[^{32}P]-$C^mA^mG^mC^mU^mC^mC^mA^m$$_{3'-3'}$T–lines (8–14). Probes were taken after 10 min (lines 1,8), 30 min (lines 2,9), 60 min (lines 3,10), 180 min (lines 4,11), 360 min (lines 5,12), 1 day (lines 6,13), and 3 days (lines 7,14) of incubation.

and 3). The oligonucleotides containing simultaneously both types of modification, i.e. 2′-*O*-methylation and terminal 3′-3′-bond, were completely stable in these test systems for up to several days independently of the nature of inverted deoxyribonucleoside.

Three different systems were used for evaluation of the influence of a dangling inverted deoxyribonucleoside on thermal stability of the duplexes (Figure 4). The thermodynamic parameters of duplex formation have been determined (Table 1).

The 3′-dangling T_{inv} attached to ribo or deoxyribo oligomers increased T_m of duplexes with RNA rather weekly and decreased T_m of duplexes with DNA (***controls***). Surprisingly, we observed a sharp increase of duplex stability, when a 3′-dangling inverted deoxyribonucleoside was introduced in oligo(2′-*O*-methylribonucleotides) (***system A***). Analogous results were obtained using (2′-*O*-methyl) RNA as a target (***system B***). In the case of ***system C***, when G^mC pair preceded a 3′-dangling inverted deoxyribonucleoside, the effect is insignificant.

We speculate that the reason for such a phenomenon might be the delicate conformational features of the 3′-modified oligo(2′-*O*-methylribonucleotide) duplexes provoked by favorable stacking interaction of a 3′-dangling nucleoside with the terminal base pair and/or hydration of capped duplex terminus.

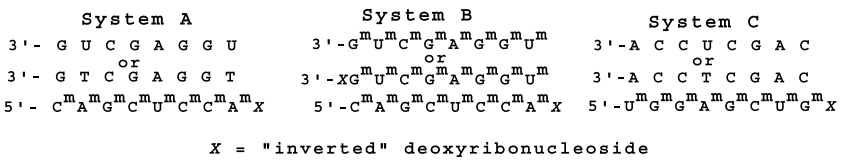

FIGURE 4 Systems of duplexes for thermal denaturation assay.

TABLE 1 Thermodynamic Parameters of Duplex Formation[a]

	−ΔH	−ΔS	−ΔG$_{37}$	T$_m$, °C	−ΔH	−ΔS	−ΔG$_{37}$	T$_m$, °C
Controls								
		3′-GUCGAGGU				3′-GTCGAGGT		
5′-CAGCUCCA	75.1	206.5	11.1	53.2	60.4	171.4	7.2	36.2
5′-CAGCUCCA$_{3'\text{-}3'}T$	67.4	182.3	10.9	54.0	50.9	141.2	7.1	35.6
5′-CAGCTCCA	67.9	192.5	8.2	41.0	56.6	159.9	7.0	35.2
5′-CAGCTCCA$_{3'\text{-}3'}T$	61.9	172.4	8.4	42.5	78.3	230.3	6.9	31.1
System A								
		3′-GUCGAGGU				3′-GTCGAGGT		
5′-C^{m}A^{m}G^{m}C^{m}U^{m}C^{m}C^{m}A^m	57.0	154.4	9.1	47.0	31.8	85.7	5.2	17.6
5′-C^{m}A^{m}G^{m}C^{m}U^{m}C^{m}C^{m}A$^m{}_{3'\text{-}3'}T$	72.6	196.1	11.8	57.3	49.5	138.4	6.6	32.5
5′-C^{m}A^{m}G^{m}C^{m}U^{m}C^{m}C^{m}A$^m T$	66.6	177.7	11.5	57.6	45.4	125.3	6.5	31.4
5′-C^{m}A^{m}G^{m}C^{m}U^{m}C^{m}C^{m}A$^m{}_{3'\text{-}3'}dC$	74.0	200.3	11.9	57.9	49.9	139.4	6.7	32.7
5′-C^{m}A^{m}G^{m}C^{m}U^{m}C^{m}C^{m}A$^m{}_{3'\text{-}3'}dG$	62.9	167.1	11.1	56.8	46.7	129.4	6.5	31.6
5′-C^{m}A^{m}G^{m}C^{m}U^{m}C^{m}C^{m}A$^m{}_{3'\text{-}3'}dA$	64.8	171.7	11.5	58.3	47.7	132.1	6.7	33.0
5′-C^{m}A^{m}G^{m}C^{m}U^{m}C^{m}C^{m}A$^m U^m$	68.3	183.1	11.5	57.0	54.0	152.1	6.8	33.7
System B								
		3′- G^{m}U^{m}C^{m}G^{m}A^{m}G^{m}G^{m}U^m				3′- $T_{3'\text{-}3'}$G^{m}U^{m}C^{m}G^{m}A^{m}G^{m}G^{m}U^m		
5′-C^{m}A^{m}G^{m}C^{m}U^{m}C^{m}C^{m}A^m	58.8	157.3	10.0	51.7	60.3	162.3	10.0	51.2
5′-C^{m}A^{m}G^{m}C^{m}U^{m}C^{m}C^{m}A$^m{}_{3'\text{-}3'}T$	89.1	242.1	14.0	62.1	91.8	249.1	14.5	63.3
System C								
		3′-ACCUCGAC				3′-ACCTCGAC		
5′-U^{m}G^{m}G^{m}A^{m}G^{m}C^{m}U^{m}G^m	67.3	182.1	10.8	53.9	59.3	166.8	7.5	38.0
5′-U^{m}G^{m}G^{m}A^{m}G^{m}C^{m}U^{m}G$^m{}_{3'\text{-}3'}T$	71.6	194.6	11.3	55.0	60.1	168.5	7.8	39.4
5′-U^{m}G^{m}G^{m}A^{m}G^{m}C^{m}U^{m}G$^m{}_{3'\text{-}3'}dC$	72.3	196.1	11.5	55.6	64.0	183.6	7.0	35.3
5′-U^{m}G^{m}G^{m}A^{m}G^{m}C^{m}U^{m}G$^m{}_{3'\text{-}3'}dG$	71.4	194.6	11.1	53.9	67.7	195.7	7.0	35.5
5′-U^{m}G^{m}G^{m}A^{m}G^{m}C^{m}U^{m}G$^m{}_{3'\text{-}3'}dA$	80.7	220.7	12.2	56.9	60.8	168.8	8.4	42.4

[a]ΔH = ΔH^{vH}(kcal/mol); ΔS = ΔS^{vH} (cal/mol·K); ΔG$_{37}$ = ΔG$_{37}$ (kcal/mol); ΔH and ΔS have been obtained by fitting procedure of UV-melting curves registered at three different wavelengths (260, 270, and 280 nm); error did not exceeded 5–10%; T$_m$ (°C)–melting temperature; buffer: 0.1 M NaCl, 10 mM Na-cacodylate (pH 7.4), 1 mM Na$_2$EDTA; [oligonucleotide] = $1.3 \cdot 10^{-5}$ M, [target] = $1.3 \cdot 10^{-5}$ M. N^m–2′-*O*-methylribonucleoside; *N*–dangling nucleoside.

TABLE 2 Dissociation Constants of Duplexes[a]

Oligomer	K_d, nM
5′- TGGAGCTG	nd[b]
5′- UGGAGCUG	195 ± 70
5′- $U^mG^mG^mA^mG^mC^mU^mG^m$	97 ± 5
5′- AUGGAGCUG	28 ± 8
5′- CUGGAGCUG	212 ± 82

[a]Dissociation constants were obtained by gel-shift assay [20% nondenaturing PAAG in TBE buffer (89 mM TBE, pH 8.3, 100 mM NaCl) at 25°C]. Total concentration of complementary oligomer 5′-γ-[^{32}P]-$C^mA^mG^m$-$C^mU^mC^mC^mA^m$$_{3'-3'}$T was 100 nM.

[b]The duplex was not detected under these conditions.

Dissociation constants of 3′-$T_{3'-3'}A^mC^mC^mU^mC^mG^mA^mC^m$ duplexes with complementary deoxyribo, ribo, and 2′-*O*-methylribo oligomers obtained by gel shift assay (Table 2) were in agreement with thermal stability data. It is worth noting that the matched duplex of ribo/2′-*O*-methylribo nonamers with a complementary rA faced an inverted T had the markedly higher stability in comparison with the mismatched one.

Taken altogether, these data demonstrate that oligo(2′-*O*-methylribonucleotides) with any 3′-terminal inverted deoxyribonucleoside are promising probes for unfolding of RNA.

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