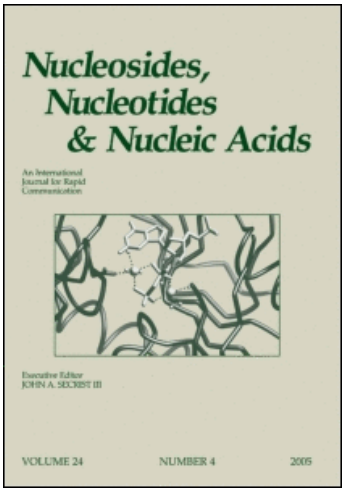

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2′-O-MODIFIED OLIGORIBONUCLEOTIDES WITH TERMINAL 3′-3′-INTERNUCLEOTIDE LINKAGE AND THEIR DERIVATIVES

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ABSTRACT

A high RNA binding affinity and nuclease resistance of 2′-O-modified (2′-O-methyl, 2′-O-tetrahydropyranyl) oligoribonucleotides containing the "inverted" T at the 3′-end have been shown. The synthesis and properties of new photoactivatable perfluoroarylazide derivatives of these oligoribonucleotides are discussed.

Looking for antisense oligonucleotides with improved chemical, biochemical and physicochemical properties, a special attention is paid now to the design of oligonucleotides with various combinations of modifications. The synthesis and comparative study of the properties of 2′-O-modified (2′-O-methyl- and 2′-O-tetrahydropyranyl) oligoribonucleotides containing the terminal 3′-3′-internucleotide linkage as well as of their photoactivatable derivatives are represented in this report.

RESULTS AND DISCUSSION

A number of 2′-O-modified oligoribonucleotides was synthesized by the solid phase H-phosphonate method according to protocols developed earlier [1,2] with

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Table. Melting Temperature of Duplexes (T_m,°C)*

Oligomer (3′ → 5′)	r-target	d-target
3′-A C C T C G A C (d)	43	41
3′-A C C U C G A C (r)	55	38
3′-$A^tC^tC^tU^tC^tG^tA^tC^t$ (t)	41	19
3′-$T_{3'-3'}A^tC^tC^tU^tC^tG^tA^tC^t$ (t-inv)	40	16
3′-$A^mC^mC^mU^mC^mG^mA^mC^m$ (m)	50	20
3′-$T_{3'-3'}A^mC^mC^mU^mC^mG^mA^mC^m$(m-inv)	60	35
3′-L_6 $pA^tC^tC^tU^tC^tG^tA^tC^t$	39	15
3′-$T_{3'-3'}A^tC^tC^tU^tC^tG^tA^tC^t$ pL_3	41	17
3′-$T_{3'-3'}A^tC^tC^tU^tC^tG^tA^tC^t$ pL_6	38	15
3′-$pT_{3'-3'}A^mC^mC^mU^mC^mG^mA^mC^m$	57	35
3′-L_2 $pT_{3'-3'}A^mC^mC^mU^mC^mG^mA^mC^m$	56	35
3′-RL_2 $pT_{3'-3'}A^mC^mC^mU^mC^mG^mA^mC^m$	57	35
3′-$T_{3'-3'}A^mC^mC^mU^mC^mG^mA^mC^mp$	57	35
3′-$T_{3'-3'}A^mC^mC^mU^mC^mG^mA^mC^mp$ L_2	56	35
3′-$T_{3'-3'}A^mC^mC^mU^mC^mG^mA^mC^mp$ L_2R	54	33

*m: 2′-O-methyl-; t: 2′-tetrahydropyranyl-; L_2: $NH_2(CH_2)_2NH$-; L_3: $NH_2(CH_2)_3NH$-; L_6: $NH_2(CH_2)_6NH$-; R: $N_3C_6F_4CO$-; r-target: 5′-r(UGGAGCUG); d-target: 5′-d(TGGAGCTG). Oligonucleotide concentration $1.3 \cdot 10^{-5}$M; buffer 100 mM NaCl, 1 mM EDTA, 10 mM sodium cacodylate, pH 7.4.

an overall yield of 10–18% after two preparative HPLC procedures. A method of 5′-phosphorylation of the polymer-bound protected oligonucleoside-H-phosphonates utilizing 2-cyanoethyl-H-phosphonate and pivaloyl chloride was applied [3]. The polymer-bound 3′-O-dimethoxytritylthymidine was used to synthesize oligomers containing "inverted" T at the 3′-end (by analogy with [4,5]). To synthesize oligomers with phosphate at the 3′-end the modified polymer bearing 2-[2-(4,4′-dimethoxytrityloxy)ethylsulfonyl]ethyl group has been used.

The main criteria of primary selection of antisense oligonucleotides are their nucleolytic stability and high affinity towards NA targets. The presence of 2′-O-modifying groups in ribo oligomers decreases significantly the thermal stability of their complexes with DNA (Table). In the case of duplexes with RNA this effect is much weaker, especially for 2′-O-methyl oligomers. "Inverted" T at the 3′-end scarcely affects the hybridization properties of 2′-O-tetrahydropyranyl octamer [6]. In the case of 2′-O-methyl octamer with the modified 3′-end a substantial increase of T_m of the duplexes with complementary ribo and deoxy octamers is observed. The same increase also takes place for the 20-mer templates (data not shown). The 2′-O-modified oligomers with 3′-"inverted" T display pronounced resistance towards nucleases (Figs. 1 and 2).

Another way to design efficient antisense reagents is the introduction of reactive groups into these modified oligoribonucleotides. A number of such 3′-"inverted" 2′-O-modified oligomers carrying perfluoroarylazide groups attached to the terminal phosphate *via* aliphatic diamine linkers has been synthesized according

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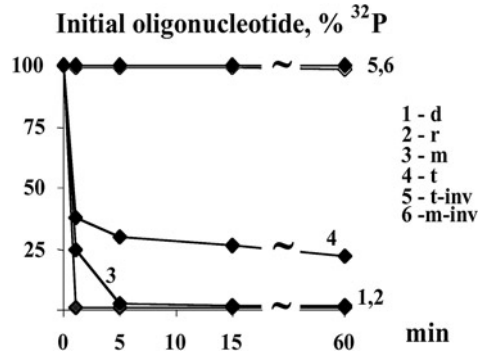

Figure 1. Kinetic curves of the oligonucleotide degradation with snake venom phosphodiesterase (0.01 U/ml, 0.5 mM $mgCl_2$, 10 mM Tris-HCl, pH 7.8, 37°C). Oligonucleotide concentration 10^{-5} M.

to [7]. The presence of aliphatic amino linker or *p*-azidotetrafluorobenzamide group at the terminal phosphate of the modified oligomers slightly affects the stability of duplexes (Table).

The ability of photoreagents (1–13) to modify NA was evaluated using ribo and deoxy 20-mers as targets, with a sequence identical to the fragment of (+)-strand of the DNA of HIV-1 (nucleotide 7516–7535, *env* gene) (Fig. 3). Using these model systems, a noticeable difference in the action of the various photoreagents was shown. Reagents (1–4) based on 2′-O-methyl oligomers modify efficiently both RNA and DNA targets independently on the position of the photoactivatable group (at the 3′- or 5′-end). Reagents (5–10) prepared from 2′-O-tetrahydropyranyl oligomers preferentially modify RNA. The reagents bearing photo group at the

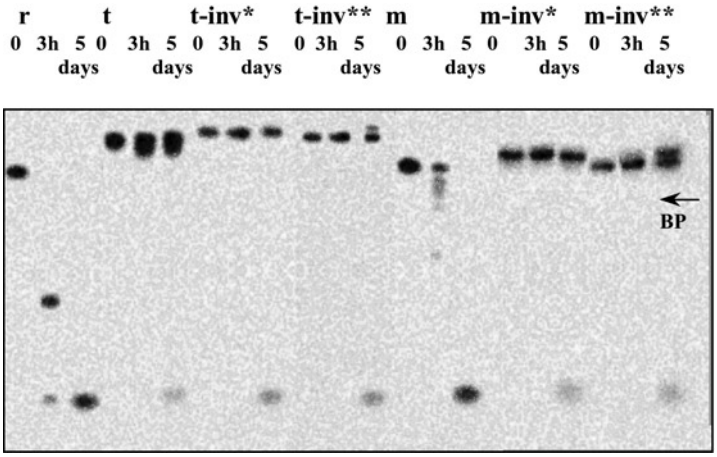

Figure 2. Electrophoretic analysis of 5′-^{32}-P-labelled oligonu-cleotides incubated at 37°C in culture medium RPMI-1640 containing 10% calf serum inactivated 1 h for at 56°C. Oligonucleotide concentration 10^{-5} M (* or** - oligonucleotides with "inverted" T at the 3′-end, containing one or two terminal 5′-^{32}P-phosphates).

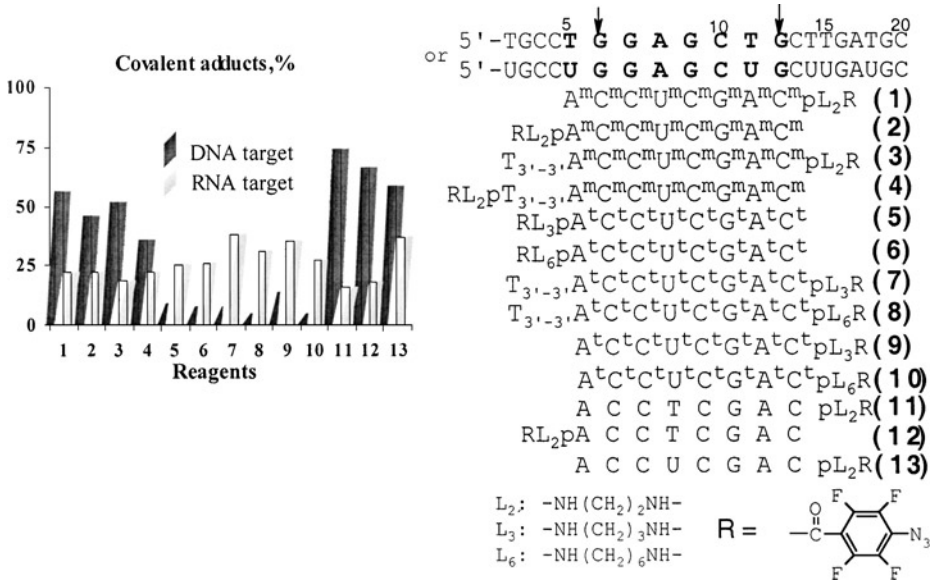

Figure 3. The extent of the formation of covalent adducts after irradiation (5°C, 10 min, $5 \cdot 10^{-4}$ $W \cdot cm^{-2}$, 303–365 nm; buffer 100 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.2; concentrations: target, 10^{-7} M; reagent, 10^{-5} M).

5′-end were more efficient in this case. The presence of 3′-"inverted" T doesn't affect the extent of modification. The main modified sites are G6 or G12 for all reagents in the case of DNA target.

The high RNA binding affinity and nuclease resistance of the synthesized modified oligoribonucleotides allow us to consider them as prospective second generation antisense oligonucleotides.

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