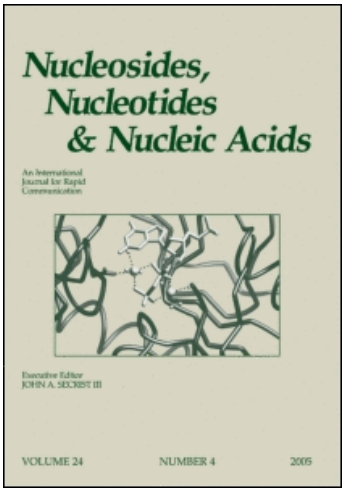

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 31 March 2001

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 819–823 (2001)

OLIGONUCLEOTIDES WITH ISOPOLAR PHOSPHONATE INTERNUCLEOTIDE LINKAGE: A NEW PERSPECTIVE FOR ANTISENSE COMPOUNDS?

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ABSTRACT

Several types of isopolar modified oligothymidylates and oligoadenylates (15 mers) with the phosphonate *-O-P-CH_2-O-* internucleotide linkage were prepared. The modified oligonucleotides were subjected to the study of their hybridization properties, resistance against nucleases, and the ability to elicit RNase H activity.

An effective *in vivo* use of oligonucleotides as antisense compounds requires their stability towards nucleases in biological fluids, sufficient affinity to the target sequences, and elicitation of RNase H activity. Of a number of modifications of internucleotide linkage, only the phosphodiester, phosphorothioate, phosphorodithioate, and boranophosphate linkage-containing (including sugar-modified) oligonucleotides (but not 2′-*O*-Me derivatives) have so far fulfilled the latter requirement. It suggests that the presence of a negatively charged sugar-phosphate backbone in modified oligonucleotides is only one of the ultimative requirements for RNase H cleavage activity. Our long-term interest in the chemistry of nucleoside phosphonic acids and their derivatives [1–5] has led us to the examination

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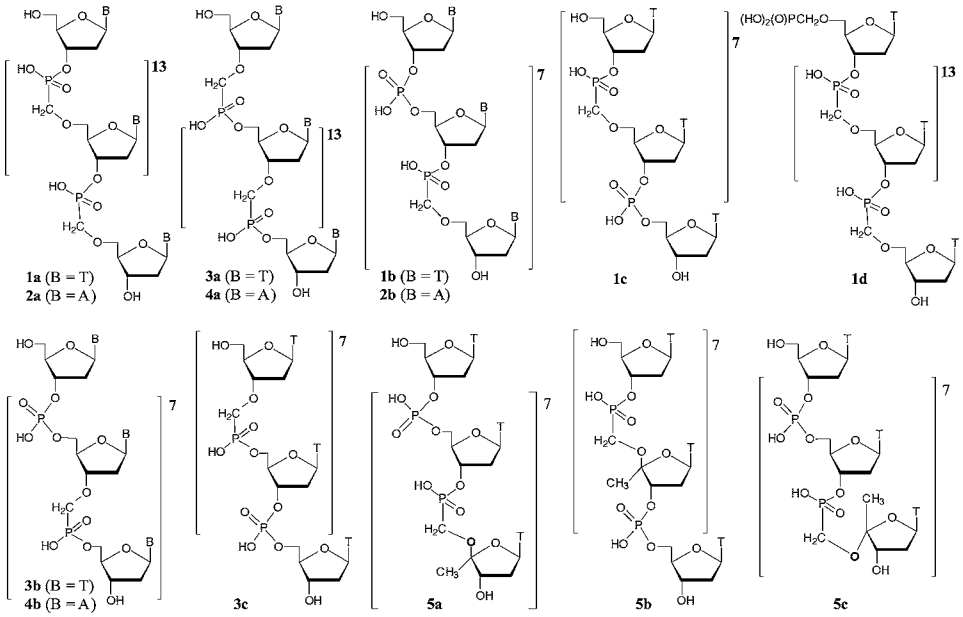
of the properties of oligonucleotides containing the bridging *-O-P-C-O-* linkage in several arrangements [6–9].

EXPERIMENTAL

The step-by-step synthesis of oligonucleotides (0.5–1.0 μmol) was performed on GeneSyn synthesizer using the combination of the phosphoramidite and phosphotriester methods. Oligonucleotides **3a–c**, **4a**, **4b** were synthesized from the 3′- to the 5′-end, and the oligomers **1a–d**, **2a**, **2b**, **5a–c** in reverse order [4]. Oligomer **1d** bearing 5′-end phosphonate moiety was prepared using solid support with attached ∃-cyanohydroxyalkyl moiety [10]. Oligonucleotides were purified by RP HPLC, first as dimethoxytrityl derivatives and then in the fully deprotected forms.



RESULTS AND DISCUSSION

Insertion of an *extra* methylene group between one of the two ester oxygen atoms and the phosphorus atom in phosphodiester linkages has led to the regioisomeric phosphonate oligonucleotides containing various number of the 3′-*O-P-CH*$_2$*-O*-5′ (**1a–d**, **2a**, **2b**) or 3′-*O-CH*$_2$*-P-O*-5′ (**3a–c**, **4a**, **4b**) internucleotide linkages [4,7]. Beside these nonisosteric, one-atom-longer types we also designed an isosteric type 3′-*O-P-C-O*-4′ (**5a–c**) derived from phosphodiester linkage by hypothetical substitution of the 5′-methylene group for 5′-oxygen atom [5].

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Table 1. Hybridization Properties of the Modified Oligonucleotides

2:1 Complexes[1]		T_m[2]	T_m[3]
py-chain	pu-chain	[°C]	[°C]
dT_{15}	dA_{15}	45	0
S-dT_{15}	dA_{15}	31	−0.4
dT_{15}	S-dA_{15}	41	−0.3
1a	dA_{15}	12	−2.4
dT_{15}	**2a**	42	−0.2
1b	dA_{15}	41	−0.6
dT_{15}	**2b**	32	−1.9
1c	dA_{15}	41	−0.6
1d	dA_{15}	18	−1.9
3a	dA_{15}	18	−1.9
3b	dA_{15}	38	−1.0
3c	dA_{15}	38	−1.0
dT_{15}	**4a**	24	−1.5
dT_{15}	**4b**	38	−1.0
5a	dA_{15}	29	−2.3
5b	dA_{15}	29	−2.3
5c	dA_{15}	no complex	

[1]4:M total strands concentration.
[2]measured in 50 mM TRIS/HCl pH 7.2, 1 mM EDTA, 10 mM Mg^{2+}.
[3]per modification.

The melting curves of complexes of modified oligonucleotides with their natural counterparts revealed considerable differences in the hybridization properties. In general, the ability of phosphonate oligonucleotides to form duplexes/triplexes with natural counterparts (in terms of T_m values) is mostly close to that of phosphorothioates (Table 1).

Complete stability against nucleases of L 1210 cell free extract was observed under conditions when half time of natural dT_{15} cleavage is less than 1 min. No cleavage of phosphonate oligomers **1a**, **1b**, **3a**, **3b** was found within 2 h (determined by HPLC).

All the prepared modified thymine-containing oligonucleotides, in the 2:1 complex with rA_{15}, were examined for their ability to elicit RNase H activity. The combination of one sole type of the phosphonate linkage with a phosphodiester one in the alternating arrangement in oligonucleotides **1b** and **1c** was able to elicit the RNase H activity as much as the natural dT_{15} (Fig. 1). Other modified oligonucleotides with alternating phosphodiester and phosphonate internucleotide linkages which failed in RNase H assay are still a challenge for their further study with this enzyme.

We anticipate that decrease in the number of phosphonate linkages and changes in their distribution along the modified oligonucleotide chain might still give the

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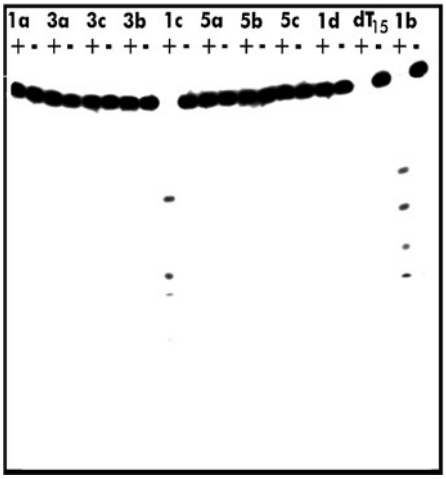

Figure 1. The RNase H activity assay [(+): experiment with RNase H; (−): experiment without RNase H)]. A mixture of 20 ml HEPES pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM MDTT, 10.4 pmol 5′-^{32}P-labeled rA_{15} and 10.4 pmol oligonucleotide was heated to 85°C and cooled. Then 1.0 U (or 0.1 U) of RNase H in the same buffer was added. After 10 min. the reaction was stopped by addition of stop-loading buffer. Samples were denatured at 90°C for 3 min. and electrophoresis on 20% poly-acrylamide gel—7M urea was performed.

chance for these modified oligonucleotides to elicit RNase H activity and to secure superior nuclease resistance. This study is underway.

Considering the exceptional nuclease resistance of the 3′-*O*-CH_2-*P*-*O*-5′ and 3′-*O*-*P*-CH_2-*O*-5′ internucleotide linkages, their protective effect on the neighbouring phosphodiester linkages, hybridization properties of the modified oligonucleotides, and ability of one type of oligomer to elicit RNase H activity, we propose the oligonucleotide constructs containing isopolar nonisosteric -*O*-*P*-CH_2-*O*- linkage and phosphodiester one for the use in antisense technology.

ACKNOWLEDGMENT

The work was supported by grants No. NL5441-3 (G.A. of Czech Ministry of Health) and No. A4055902 (G.A. of the Czech Acad. Sci.). The authors are indebted to Dr. Ivana Kavenová for a perfect technical assistance.

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