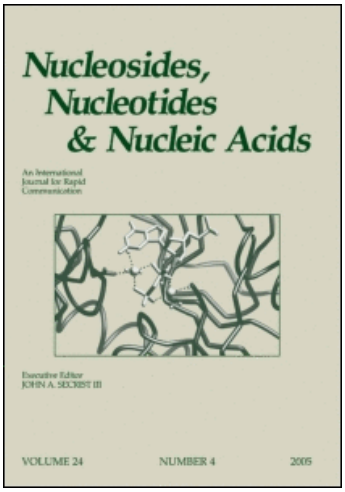


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

α-Hydroxyphosphonate Oligonucleotides: A Promising DNA Type?

Šárka Králíková[a]; Miloš Buděšínský[a]; Ivan Rosenberg[ab]
[a] Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Prague, Czech Republic [b] Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Prague 6, Czech Republic

Online publication date: 09 August 2003

To cite this Article Králíková, Šárka , Buděšínský, Miloš and Rosenberg, Ivan(2003) 'α-Hydroxyphosphonate Oligonucleotides: A Promising DNA Type?', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1061 — 1064
To link to this Article: DOI: 10.1081/NCN-120022736
URL: http://dx.doi.org/10.1081/NCN-120022736

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1061–1064, 2003

α-Hydroxyphosphonate Oligonucleotides: A Promising DNA Type?

Šárka Králíková, Miloš Buděšínský, and Ivan Rosenberg*

Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Prague, Czech Republic

ABSTRACT

The synthesis of monomers **(*S*)-1**, **(*R*)-1** and **2** derived from **(5′*S*)-**, **(5′*R*)-**2′-deoxythymidine-5′-*C*-phosphonic acids and 2′,5′-dideoxythymidine-5′-*C*-phosphonic acids was elaborated. The protection of the 5′-hydroxyl by the methoxycarbonyl group was a key step of the synthesis. Prepared monomers were used for the solid-phase assembly of several types oligothymidylate 15-mers **(*S*)-3**, **(*S*)-4**, **(*S*)-5**, **(*R*)-4** and **(*R*)-5** containing the chiral 3′-*O*-*P*-*CH(OH)*-5″ internucleotide linkage. Their hybridization properties with dA_{15} and rA_{15} were studied as well as their resistance against nuclease cleavage.

Key Words: Isopolar phosphonate oligonucleotides; Geminal hydroxyphosphonates; Nonisosteric internucleotide linkage; Nucleoside 5′-phosphonic acids; Phosphotriester method; Reversed phosphoramidites; Triplex; Hybridization.

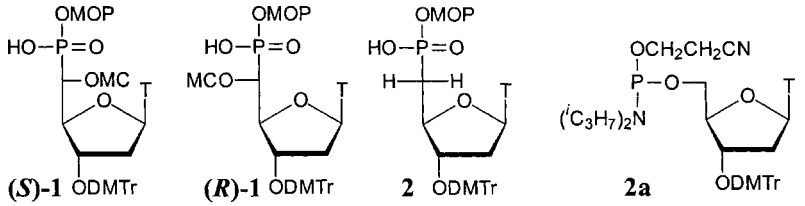
RESULTS AND DISCUSSION

The search for antisense and triplex-forming-oligonucleotides (TFO) with enhanced nuclease resistance, increased hybridisation properties as well as high

*Correspondence: Ivan Rosenberg, Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic; Fax: +420 2 3333 5268; E-mail: ivan@uochb.cas.cz.

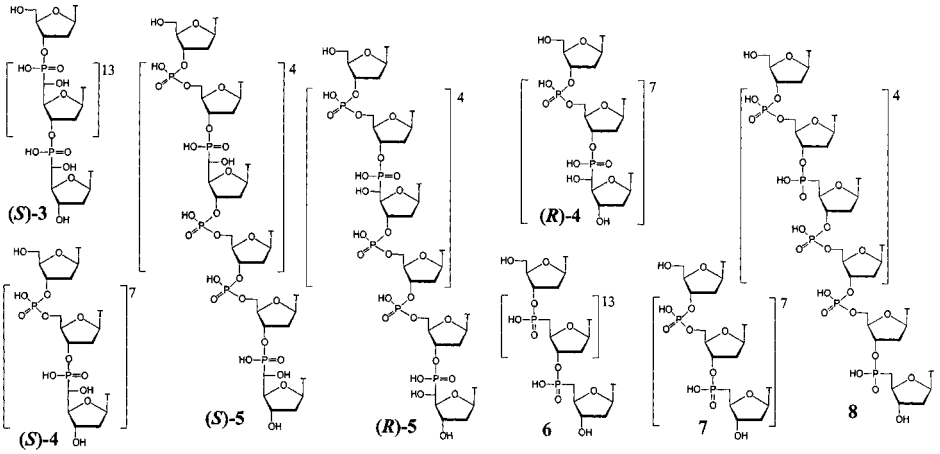
DOI: 10.1081/NCN-120022736
1525-7770 (Print); 1532-2335 (Online)

selectivity in discrimination between RNA and DNA, has led to many structural alterations of phosphodiester internucleotide linkage.[1,2] The phosphonate analogs of oligonucleotides featuring enzyme-resistant, bridging *P-C* internucleotide linkage have attracted our attention for a long time.[3–4] The recent study on hybridisation properties of r(ApA) and d(ApA) analogues, featuring one bridging atom shorter 3′-*O-P-CH(OH)* -5″ internucleotide linkage, with polyU revealed remarkable differences in stabilities of the complexes depending on the 5″-carbon atom configuration.[7] To proove the usefulness of this modification in oligonucleotide chemistry, we synthesized modified oligothymidylate 15-mers, differing in a number and distribution of the chiral 3′-*O-P-CH(OH)*-5″ internucleotide linkage along the chain, in both (*S*)- and (*R*)-series. For a comparative study, the oligothymidylate 15-mer with the non-chiral 3′-*O-P-CH*$_2$-5″ internucleotide linkage lacking the 5″-hydroxyl was also prepared.



MOP..... 4-methoxy-1-oxido-2-picolyl; MC..... methoxycarbonyl; DMTr..... dimethoxytrityl

Thus, the synthesis of monomers **(*S*)-1**, **(*R*)-1** and **2** was elaborated starting in all cases from the 3′-protected dimethyl-**(5′*RS*)-**2′-deoxythymidine-5′-*C*-phosphonate. The protection of the 5′-hydroxyl was a key step at the preparation of monomers,



and we have selected, from many others, the methoxycarbonyl protecting group as a group which does not participate at the activation of phosphorus moiety during the condensation step. Its quantitative introduction was accomplished using methoxycarbonyl tetrazole as acylating agent.[7]

The step-by-step synthesis of oligonucleotides was performed on GeneSyn DNA synthesizer using LCAA-CPG (500 Å) with attached 2′-deoxy-3′-*O*-dimethoxytrityl-

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

Table 1. Hybridization properties of modified oligothymidylates (2:1 complexes[a] with natural oligoadenylates).

	A-STRAND			
	2′-DeoxyriboA$_{15}$		RiboA$_{15}$	
	Mg^{2+}	Na^{+}	Mg^{2+}	Na^{+}
T-STRAND	T_m [°C]		T_m [°C]	
dT$_{15}$	45	36	36	34
(*S*)-3	10	—	—	—
(*S*)-4	69	53	45	44
(*S*)-5	60	47	—	—
(*R*)-4	22	—	—	—
(*R*)-5	29	—	—	—
6	17	no complex	—	—
7	49	27	—	—
8	47	27	—	—

[a]4 μM total strand concentration, measured in 50 mM TRIS/HCl pH 7.2, 1 mM EDTA, 10 mM Mg^{2+} or 100 mM Na^{+}.

5′-*O*-hemisuccinylthymidine. The synthesis was driven from the 5′- to the 3′-end using the phosphotriester method for introduction of monomers **(*S*)-1**, **(*R*)-1** and **2,** and the phosphoramidite method to introduce reversed phosphoramidite **2a**. Thus, the 15-mers **(*S*)-3**, **(*S*)-4**, **(*S*)-5**, **(*R*)-4**, **(*R*)-5**, **6, 7** and **8** were prepared and their hybridization properties studied (Table 1).

Remarkable differences in hybridization properties of the modified oligonucleotides (Table 1) depending on the configuration at the 5′-carbon atom (Table 1) were found. Whereas fully modified 15-mer **(*S*)-3** exhibits only a week hybridization with very low T_m value, the oligomer **(*S*)-4** containing equal number of both phosphonate and phosphodiester internucleotide linkages in alternating mode exhibits, surprisingly, very high T_m value. Reduction of the number of phosphonate linkages in oligomer **(*S*)-5** leads only to a little decrease of the T_m value. On the other hand, both **(*R*)-4** and **(*R*)-5** oligomers show a very week hybridization. The explanation of these phenomena could be seen in preorganization of shorter sugar phosphonate backbone in both **(*S*)-** and **(*R*)-**configured oligomers due to a suppressing of a free rotation around the C4′-C5′ linkage.[7] In the former case, the preorganization of the chain is strongly stabilizing but in the latter case strongly destabilizing for complex formation. It could be supported by hybridization properties of oligomers **7** and **8** lacking the 5′-hydroxyl; their T_m values are almost identical with the T_m value found for the natural dT$_{15}$. The total shortening of the sugar-phosphonate backbone in fully modified oligomers **(*S*)-3** and **6** leads to dramatic decreasing of hybridization abilities. Melting curves of complexes of prepared oligomers with dA$_{15}$ exhibited only a single transition profile corresponding apparently to only one type of complex (duplex or triplex). The mixing curves measured at 0°C had one minimum signalizing the 2T:1A stoichiometry of complexes (triplex structure). Despite of these findings we cannot also eliminate the presence of duplexes that can melt simultaneously

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

with triplexes. It would mean that in complexes of homooligomers the stability of Hoogsteen and Watson-Crick hydrogen bonds is very similar. In order to find out equilibrium concentration of duplexex, triplexes and free complementary oligomers in a mixture, we have applied recently published mathematical method for the quantitative analysis of Raman spectral data of ApA–polyU complexes;[8] such study with mixtures of modified dT_{15} and natural dA_{15} or rA_{15} is underway.

Concerning the nuclease resistance of oligomer **(*S*)-4**, we found complete stability of this compound against snake venom exonuclease (EC 3.1.30.1) and endonuclease P1 (EC 3.1.3.16) cleavage. From the facts mentioned above we could conclude that this (*S*)-configured type of modification of internucleotide linkage seems to be very promising for its further evaluation in TFO and also antisense oligonucleotides.

ACKNOWLEDGMENTS

Support by grants # A4055101 (Acad. Sci., Czech Rep.) and #203/01/1166 (GA, CR) under research project Z4055905 is gratefully acknowledged.

REFERENCES

1. Freier, S. M; Altmann, K.-H. The ups and downs of nucleic acid duplex stability: structure-stability studies on chemically-modified DNA:RNA duplexes. Nucleic Acid Res. **1997**, *25*, 4429.
2. Cuenoud, B.; Casset, F.; Hűsken, D.; Natt, F.; Wolf, R.M.; Altmann, K.-H.; Martin, P.; Moser, H.E. Dual recognition of double-stranded DNA by 2′-Aminoethoxy-modified oligonucleotides. Angew. Chem. Int. Ed. **1998**, *37*, 1288–1291.
3. Točík, Z.; Kavenová, I.; Rosenberg, I. Synthetic route to novel isosteric phosphonate analogs of oligonucleotides. Collect. Czech. Chem. Commun., Symp. Series. **1999**, 109–113.
4. Králíková, Š.; Budějínský, M.; Masojídková, M.; Rosenberg, I. Efficient synthesis of nucleoside 3′-*C*-phosphonates: reativity of geminal hydroxyphosphonate moiety. Nucleosides, Nucleotides & Nucleic Acids **2000**, *19* (7), 1159–1183.
5. Králíková, Š.; Budějínský, M.; Masojídková, M.; Rosenberg, I. Geminal hydroxy phosphonate derivatives of nucleosides: a novel class of nucleoside 5′-monophosphate analogs. Tetrahedron Lett. **2000**, *41*, 955–958.
6. Rejman, D.; Snášel, J.; Liboska, R.; Točík, Z.; Pačes, O.; Králíková, Š.; Rinnová, M.; Koiš, P.; Rosenberg, I. Oligonucleotides with isopolar phosphonate internucleotide linkage: a new perspective for antisense compounds? Nucleosides, Nucleotides & Nucleic Acids **2001**, *20*, 819–823.
7. Králíková, Š.; Masojídková, M.; Budějínský, M.; Rosenberg, I. Study on reactivity and protection of the α-hydroxyphosphonate moiety in 5′-nucleotide analogues: Formation of the 3′-*O*-*P*-*C*(*OH*)-*C*-4′-internucleotide linkage. Nucleosides, Nucleotides & Nucleic Acids **2003**, *22*, 329–347.
8. Hanuš, J.; Barvík, I.; Ruszová-Chmelová, K.; Štěpánek, J.; Turpin, P.-Y.; Bok, J.; Rosenberg, I.; Petrová-Endová, M. -CH2- lengthening of the internucleotide linkage in the ApA dimer can improve its conformational compatibility with its natural polynucleotide counterpart. Nucl. Acids. Res. **2001**, *29*, 5182–5194.