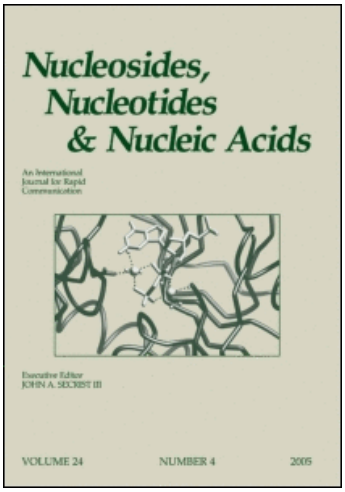

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Isosteric Phosphonate Pyrrolidine-Based Dinucleoside Monophosphate Analogues

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ABSTRACT

A novel α- and β-configured pyrrolidine nucleoside phosphonates in adenine series were synthesized from *trans*-4-hydroxy-L-proline as starting material. d(ApA) analogues were also prepared and studied with respect to their hybridization properties with polyU.

Key Words: Pyrrolidine-based phosphonate nucleotides; ApA analogues; Isopolar isosteric phosphonate internucleotide linkage; Triplex; Hybridization.

INTRODUCTION

The modified oligonucleotides represent a very promising group of compounds interfering with gene expression by duplex/triplex formation with RNA or DNA. Therefore, the search for modified oligonucleotides with increased hybridisation properties has been in a focus of attention.[1] The decreasing of repulsive forces between negatively charged strands in complexes by the introduction of a positively

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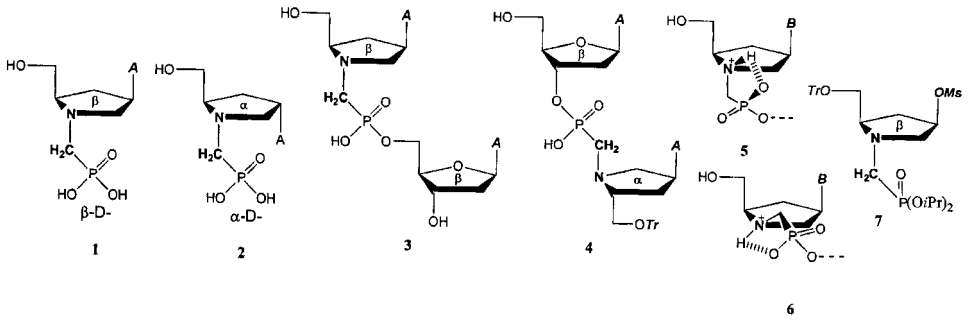
charged grouping into modified oligonucleotides could increase the hybridisation.[2] Studies on complexation properties of polyU with adenine dinucleoside monophosphate analogues as the shortest oligonucleotides showed an effective way how to find out quickly the changes in hybridisation properties due to the presence of modified internucleotide linkage.[3,4]



RESULTS AND DISCUSSION

We report here the synthesis of nucleotide analogues **1** and **2** related to α and β D-2′-deoxyadenosine 3′-phosphate, containing pyrrolidine ring instead of the sugar unit, in which the 3′-oxygen atom is replaced by CH_2 group and the pyrrolidine nitrogen atom is located in place of the C3′ of the sugar ring. Two adenine dimers **3** and **4** with highly nuclease-stable *3′-N-CH_2-P-O-5″* internucleotide linkage were synthesized from compounds **1** and **2.** Due to the chirality on the C4′ atom, these pyrrolidine derivatives could be labeled, in analogy with nucleotides, as α- and β-anomers.

The presence of nitrogen atom at the 3′-position leads to the loss of chirality at this centre. Resulting conformational flexibility could improve the hybridisation properties by the possible formation of hydrogen bond-based pseudo-spiro structures (**5**, **6**). We expected hybridisation properties to be pH-dependent due to the presence of tertiary amine in the molecule. For the preparation of pyrrolidine analogues, we have elaborated a new synthetic route based on the method reported recently.[5] The synthesis started from commercially available *trans*-4-hydroxy-L-proline, which was converted in several steps including its inversion to *cis*-4-hydroxy-D-proline,[6] protection of amine by benzyloxycarbonyl group, reduction of carboxyl by $NaBH_4$ at the level of methyl ester, and deprotection, to *cis*-4-hydroxy-D-prolinol. Phosphonomethylation of the secondary amine (via *Mannich* reaction), followed by selective tritylation of primary hydroxyl and mesylation of secondary hydroxyl, afforded the key synthon **7** for the preparation of compounds of α-series.

For the preparation of β-D-type, configuration on the C4 atom of pyrrolidine ring was inverted via nucleophilic substitution of 4-O-mesyl group of **7** with acetyl group. Subsequent acetolysis and mesylation of secondary hydroxyl gave a synthon with inverted configuration. Both α-D- and β-D-mesylates were then used for direct alkylation of adenine by heating at 90°C in the presence of Cs_2CO_3 in anhydrous

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DMF for 8 h. After partial deprotection and purification, the starting modified nucleotides **1** and **2** were obtained.

Two dimers **3** and **4** were prepared using diester condensation method, characterized and the hybridisation properties of the compound **3**, in complex with polyU, studied in neutral (pH 7.2) and slightly acidic (pH 4.2) solutions. The T_m value 7.4°C at neutral pH is comparable to that of natural d(ApA) ($T_m \approx 7$°C). Remarkable increasing of the T_m value under acidic conditions to 11.4°C for the complex of dimer **3** with polyU seems to confirm our idea concerning possible stabilisation of modified sugar-phosphate backbone of the polypurine pseudo strand(s) via structures **5** or **6**. A detailed study on hybridization of longer oligonucleotides containing modified pyrrolidine units is underway.

ACKNOWLEDGMENTS

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