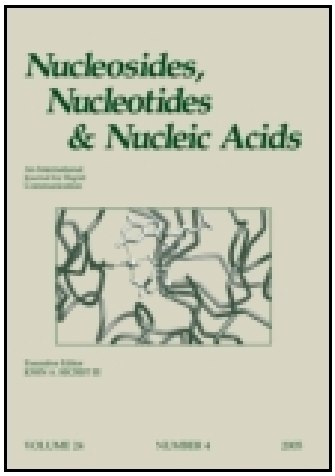

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

Pyrrolidine PNA-DNA Chimeric Oligonucleotides with Extended Backbone

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Published online: 31 Aug 2006.

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1101–1104, 2003

Pyrrolidine PNA-DNA Chimeric Oligonucleotides with Extended Backbone

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ABSTRACT

Cis-D-2-hydroxy-4-thymin-1-yl-pyrrolidine propionic acid unit is used to make PNA-DNA dimer block that is incorporated in DNA sequences at selected positions. Since the amide linkage is shorter than phosphodiester linkage, insertion of an extra atom in the backbone with amide linkage seems to be better accommodated for internucleotide distance-complementarity.

Key Words: PNA-DNA chimera; PNA.

INTRODUCTION

The combination of PNA and DNA as PNA-DNA chimera has useful biological application such as primer for DNA polymerases and as antisense oligonucleotides in therapeutics. Introduction of positive charges might increase the binding ability of such chimeric oligonucleotides due to reduction of the negatively charged phosphate interactions. In this regard cis-D-2-hydroxy-4-thymin-1-yl-pyrrolidine acetic acid unit was used to make PNA-DNA chimera and when

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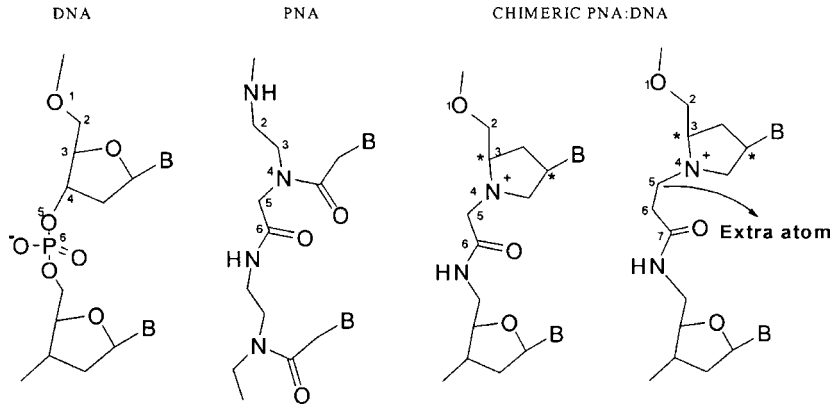
DOI: 10.1081/NCN-120022746
1525-7770 (Print); 1532-2335 (Online)

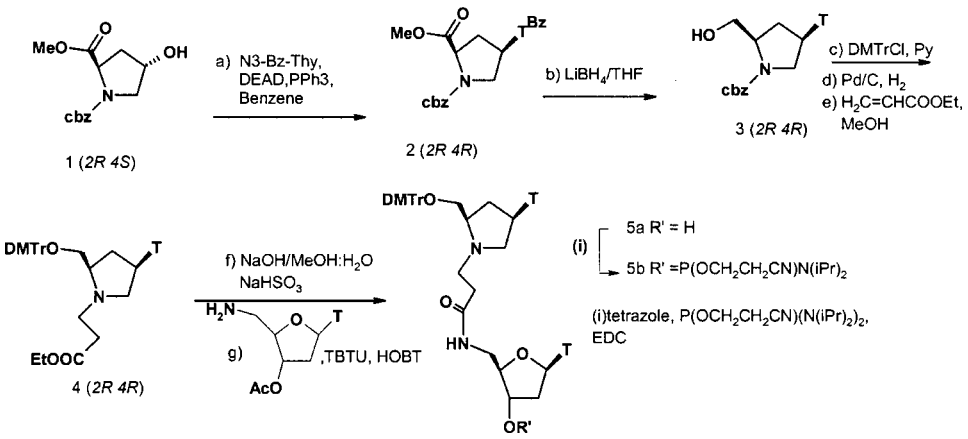


incorporated into DNA sequences at selected positions was found to destabilize the derived DNA/DNA-PNA chimera duplexes and triplexes.[1] Since amide linkage is shorter than phosphodiester linkage, replacement of the phosphodiester linkage with amide linkage may necessiate the insertion of an extra atom in the backbone[2] to retain internucleotide distance-complementarity. With this aim, study of PNA-DNA chimera where pyrrolidine propionic acid unit having one extra carbon atom in the backbone as compared to phosphodiester linkage was attempted. We present here the synthesis of the dimer building block used for chimera, synthesis of DNAs with chimeric unit at selected positions and their binding studies with complementary DNA.

RESULTS AND DISCUSSION

The pyrrolidine derivative 3 was synthesized from *trans*-4-hydroxy-D-proline according to the reported procedure.[1] After DMT protection of the primary hydroxy group, the ring nitogen was deprotected by hydrogenation using Pd-C and was then alkylated by Michael addition to ethyl acrylate to get compound 4. The ester was then hydrolyzed and coupled with 5′-amino-5′-deoxy thymidine derivative using TBTU/HOBT in dry DMF to get the amide dimer 5a. Phosphitylation of 5a gave the desired protected phosphoramidite 5b for site specific incorporation into oligonucleotides (Sch. 1). The modified and control DNA sequences synthesized are listed in Table 1. All the new compounds synthesized were characterized by spectroscopic analysis.

The stability (Table 2) of the triplex 5:6*7 containing polypyrimidine ODN 7 carrying amide linked dimer block at the center is similar to the stability of the corresponding unmodified triplex 5:6*1. This suggests that the distance between the neighboring base pairs is affected by the incorporation of this extended backbone and is better accommodated in the triplex. The duplexes are also less destabilized with this modification as compared to our earlier modification.[1]



Scheme 1.

Table 1. DNA sequences.

1	5′ TTC TTC TTC TTT TCT TTT
2	5′ AAA AGA AAA GAA GAA GAA
3	5′ CTT GTA CTT TTC CGG TTT
4	5′ AAA CCG GAA AAG TAC AAG
5	5′ TCC AAG AAG AAG AAA AGA AAA TA
6	5′ ATA TTT TCT TTT CTT CTT CTT GGA
7	5′ TTC TTC TTC tt C TCT TTT
8	5′ CTT GTA CT tt TC CGG TTT

tt represents pyrrolidine-DNA thymine dimer block.

Table 2. UV-Tm studies of DNA duplex and triplex structures.

Duplex	Tm(°C)	ΔTm	Triplex	Tm(°C)	ΔTm
1:2	46		5:6*1	23.5	
7:2	41	−5(−7)	5:6*7	23.5	0(−4)
3:4	51.8				
3:8	46.2	−5.6(−6)			

Values in parenthesis indicate the UV-Tm of duplexes and triplexes with modified pyrrolidine oligomers reported earlier by us.[1]

CONCLUSIONS

We report here the synthesis of a PNA-DNA dimer block with controlled conformational flexibility. The selected stereochemistry in the pyrrolidine PNA monomer is 2*R*, 4*R* which is similar to the ribose sugar stereochemistry. This dimer block is incorporated at predetermined positions in DNA oligomers. DNA

complementation studies indicate that the extended amide backbone shows better nucleobase distance complemetarity to target DNA as compared to the shorter amide linkage. Further studies to exploit this understanding are in progress in our laboratory.

ACKNOWLEDGMENTS

VAK thanks Department of Science and Technology, New Delhi, for financial support. Meena thanks CSIR, New Delhi, for research fellowship.

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