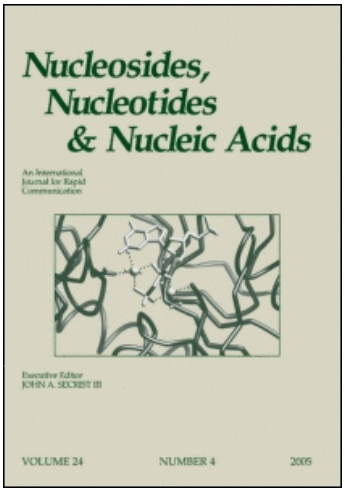

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SYNTHESIS AND PROPERTIES OF PYRROLIDINE-BASED NEGATIVELY CHARGED DNA MIMICS

Vladimir A. Efimov, Valeryi N. Klykov, and Oksana G. Chakhmakhcheva
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□ *A set of novel chiral pyrrolidine-based nucleotide mimics, in which nucleobase, hydroxyl group and phosphonic acid residue were attached to different carbon atoms of the pyrrolidine ring, was synthesized. These monomers were used for the synthesis of the corresponding oligomers, and their physico-chemical properties were evaluated.*

Keywords DNA mimics; pyrrolidine; hybridization; antisense reagents

INTRODUCTION

In the last years, a search for modified oligonucleotides and their analogues with improved physico-chemical and biological properties attracted our attention in connection with their application in molecular biology and medicine as diagnostic probes and antisense agents. With this aim in view, we have undertaken the investigations on the synthesis of several types of negatively charged oligonucleotide mimics constructed from pyrrolidine-based chiral monomer units connected with phosphonate ester bonds. Particularly, we reported pHypNA mimics containing 1-acetyl-4-hydroxypyrrolidine-2-phosphonic acid backbone[1] (Figure 1). *Trans*-L type of pHypNAs, similarly to HypNA-pPNA hetero-oligomers developed by us earlier,[2] have demonstrated high hybridization and discrimination characteristics as well as the ability to hybridize to complementary nucleic acids in low salt concentrations.[1–3] Analysis of biological properties of pHypNA oligomers has shown that this type of DNA mimics combined high biological stability with the ability to penetrate cell membranes. They do not activate

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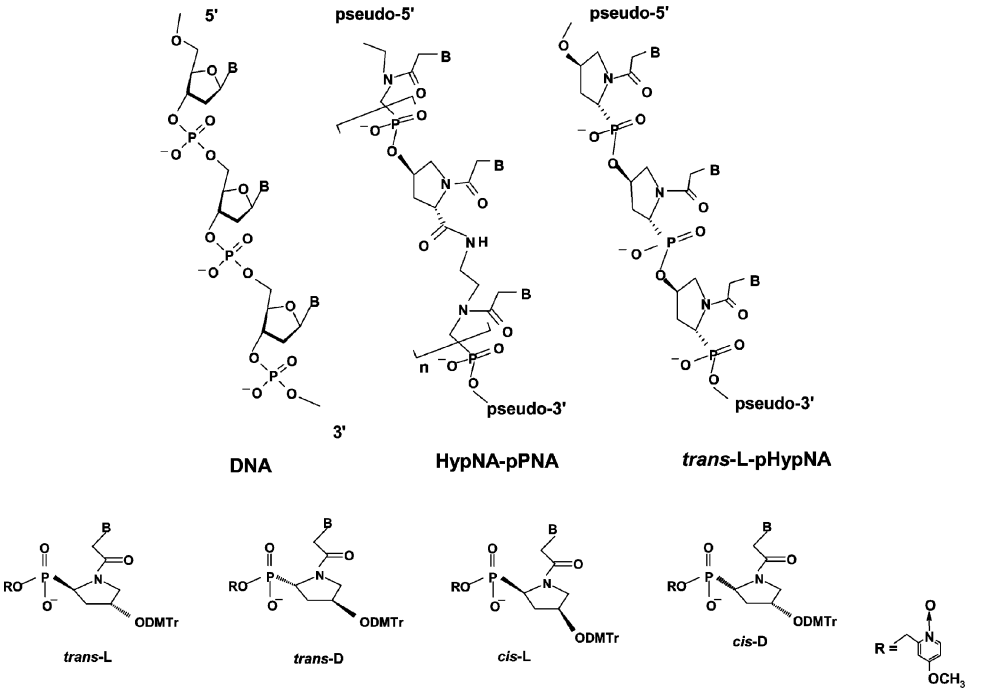


FIGURE 1 General chemical structures of HypNA-pPNA and pHypNA mimic oligomers and four chiral pHypNA monomers.

RNase H and inhibit mRNA translation by hybridization arrest. Other properties of pHypNA mimics include ability to bind RNA targets stronger than DNA, good water solubility and cellular uptake with distribution in cytosol and preferentially in nucleus as well as a long shelf-life at room temperature. Thus, these compounds can be evaluated as useful tools for application in molecular biology, particularly in nucleic acid based diagnostics, isolation and analysis of nucleic acids and in the study of gene expression.[3–5] Gene knockdown experiments in vivo revealed that their effect lasts over a period of several days. So, the use of these mimics can represent a potent technology for administering antisense-based drugs for future therapeutic applications.[3,4] To continue these investigations, we obtained several novel types of monomers on the base of pyrrolidine (PyrrNAs).

RESULTS AND DISCUSSION

In pyrrolidine-based monomers obtained, nucleobase, hydroxyl group and phosphonic acid residue were attached to different carbon atoms of the pyrrolidine ring (Figure 2). As examples, the synthetic routes to obtain *trans*-L PyrrNA-2 and PyrrNA-3 monomers are depicted in Scheme 1. The derivatives of *trans*-4-OH-*N*-acetyl-L-prolinol bearing heterocyclic bases,

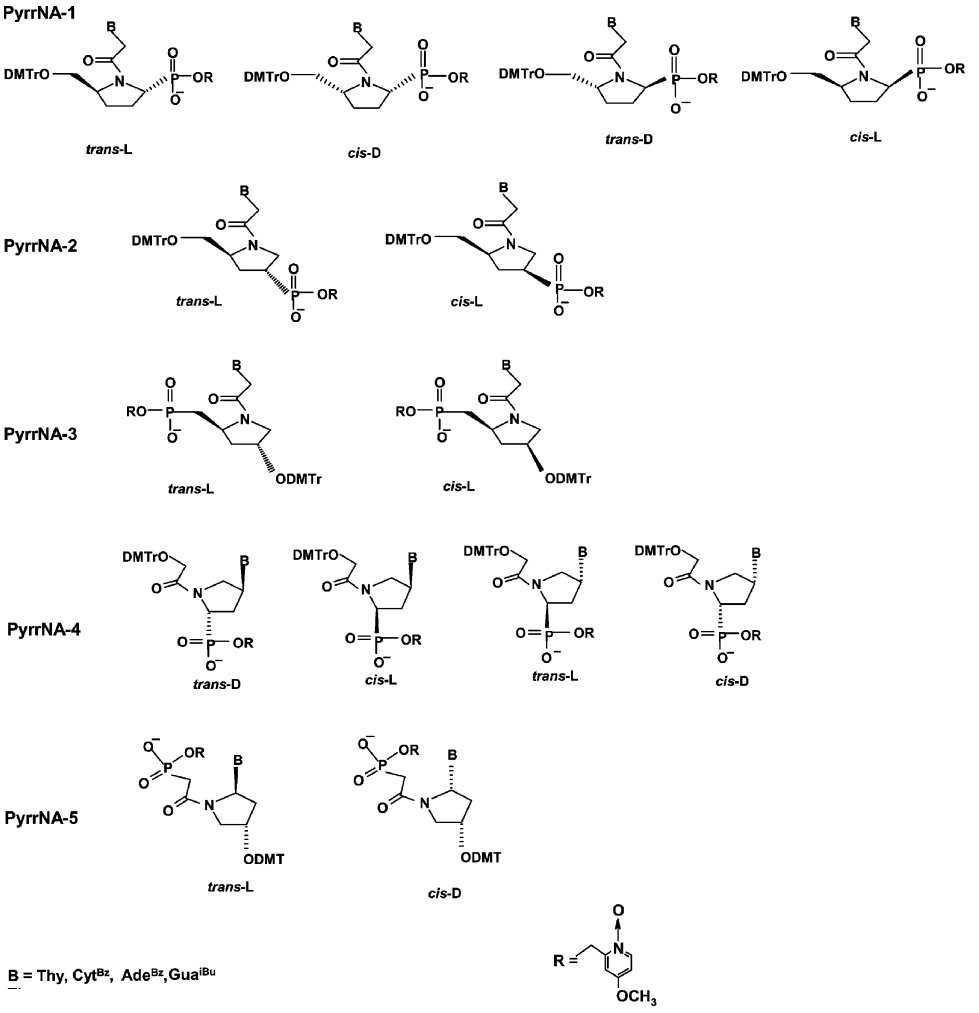
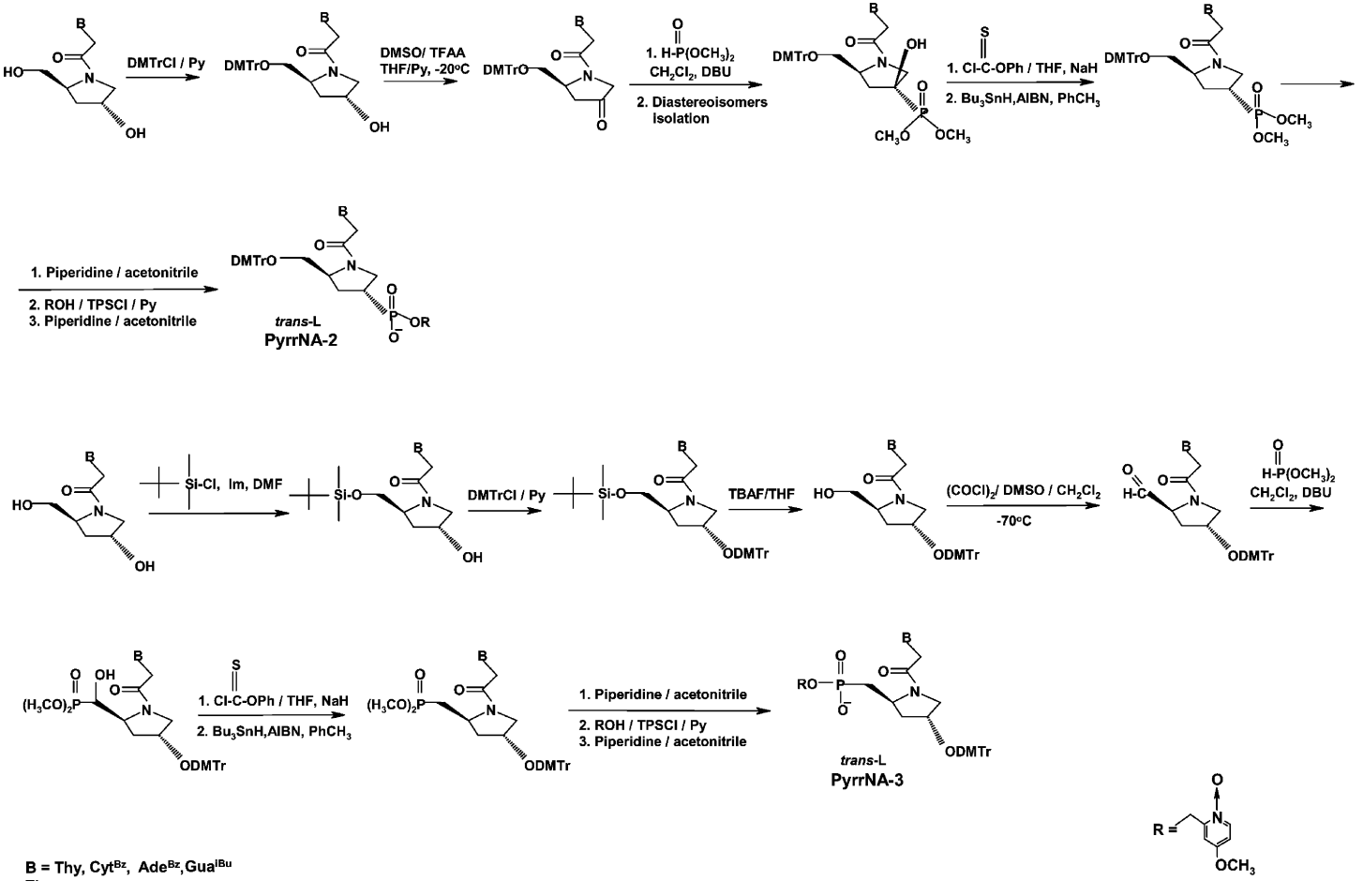


FIGURE 2 General chemical structures of chiral monomer units synthesized for the construction of the corresponding PyrrNA mimic oligomers.

which were used as starting compounds, were obtained as described.[6] Schemes for the synthesis of other PyrrNA monomers will be published elsewhere. The synthesis of the corresponding PyrrNA oligomers was carried out by the modified solid-phase phosphotriester method with O-nucleophilic intramolecular catalysis similar to the synthesis of pHypNA and HypNA-pPNA oligomers.[2,4] Preliminary results obtained in the study of the hybridization properties of PyrrNA-2 and PyrrNA-3 mimics revealed that oligomers constructed from monomers with *cis*-L configuration are not able to form stable complexes with the complementary oligonucleotides, whereas oligomers constructed from *trans*-L monomers are able to form complexes with the complementary targets, but the stability of these complexes is lower than



Q2 **SCHEME 1**

TABLE 1 Stability of complexes formed by homo-Thy_{15} PyrrNA DNA mimics with the complementary DNA, or RNA, targets in 5-10 μM concentration in 10 mM Tris-HCl (pH 7.5)/0.5 M NaCl/10 mM $MgCl_2$

Oligomer type		T_m, °C	
		DNA target	RNA target
DNA		47	35
pHypNA	*trans*-L	76	64
	trans-D	53	47
PyrrNA-1	*trans*-L	ND[a]	ND[a]
	cis-L	<20	<20
	trans-D	ND[a]	ND[a]
	cis-D	<20	<20
PyrrNA-2	*trans*-L	35	30
	cis-L	<20	<20
PyrrNA-3	*trans*-L	31	27
	cis-L	<20	<20
PyrrNA-4	*trans*-L	ND[a]	ND[a]
	cis-L	<20	<20
	trans-D	<20	<20
	cis-D	ND[a]	ND[a]
PyrrNA-5	*trans*-L	ND[a]	ND[a]
	cis-D	ND[a]	ND[a]

[a]Examination is currently in progress.

the stability of natural DNA/DNA and DNA/RNA duplexes and significantly lower than the stability of complexes between *trans*-L pHypNA oligomers and DNA (or RNA) targets (Table 1). Also, *cis*-L and *cis*-D PyrrNA-1 oligomers as well as *cis*-L and *trans*-D PyrrNA-4 oligomers were not able to form complexes with the complementary targets. The investigations on the properties of other types of PyrrNA mimics are currently in progress.

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