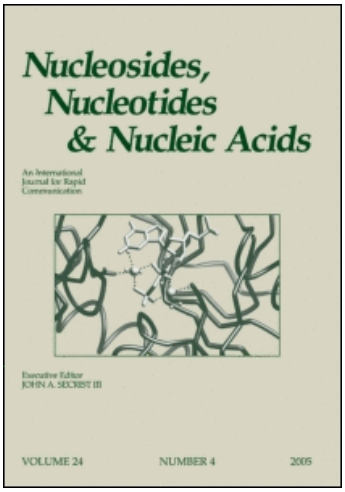

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Synthesis of *trans*-L/D-2-(Tert-butoxycarbonyl-aminomethyl)-4-(thymin-1-yl) Pyrrolidin-1-yl Acetic Acid

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ABSTRACT

To delineate the binding preferences of stereochemically divergent pyrrolidine PNAs, synthesis of all four diastreomeric monomers of **I** and the systematic complexation studies of the resultant PNAs with complementary DNA/RNA is essential. We herein report the synthesis of *trans*-L/D-2-(tert-butoxycarbonyl-aminomethyl)-4-(thymin-1-yl) pyrrolidin-1-yl acetic acids **I**, their incorporation in PNA oligomers and DNA binding studies will be presented.

Key Words: Pyrrolidine-PNA; PNA-DNA/RNA complexes.

INTRODUCTION

In the recent years cationic analogues of peptide nucleic acids are gaining attention because of their favorable properties for therapeutic applications.[1] From the interesting results of others[2,3] and our own earlier work,[4] PNAs based on

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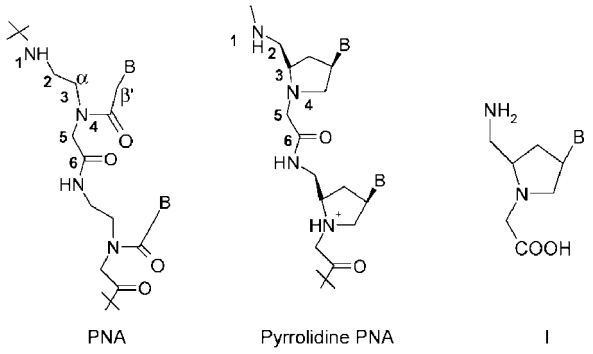
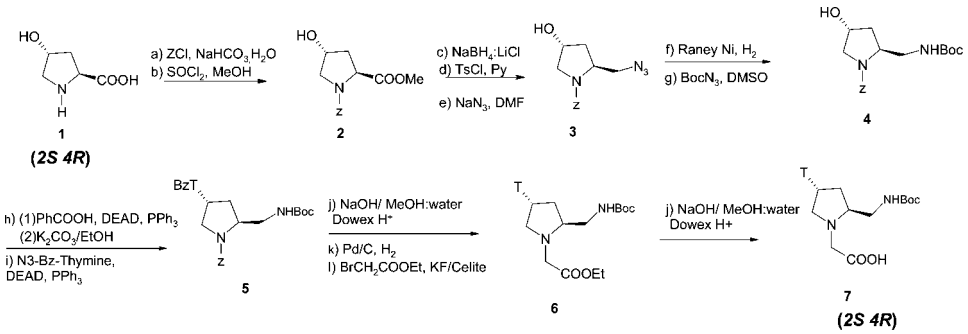
pyrrolidine acetic acid seem to hold some potential for the development as important antisense molecules. The pyrrolidine based PNAs are chiral, conformationally constrained molecules with a methylene bridge inserted between α carbon atom of ethylenediamine and β′ carbon atom of linker to nucleobase and simultaneously removing the rigid carbonyl group. Their DNA/RNA binding preferences may be dictated by the geometry of the backbone as well as the orientation of the nucleobase. To delineate the recognition preferences of stereochemically divergent pyrrolidine PNAs, synthesis of all four diastereomers of I and systematic studies of binding with complementary DNA/RNA are essential. Here, we report the synthesis of *trans*-2-(Boc-aminomethyl)-4-(thymin-1-yl)-pyrrolidine-N-acetic acid.



RESULTS AND DISCUSSION

The suitably protected *trans*-4-hydroxy proline **2** was converted to the *trans*-2-Bocaminomethyl-4-hydroxy pyrrolidine derivative **4** by employing a number of steps (Sch. 1). These sequentially consist of reduction of ester function, tosylation of the resulting primary hydroxy group and reaction with sodium azide to get **3** which was then selectively hydrogenated using Ra-Ni and Boc-protected to get the 2-aminomethyl pyrrolidine **4**. *Trans*-4-N3-benzoyl-Thymin-1-yl-2-Bocaminomethyl pyrrolidine derivative **5** was synthesized from **4** by double inversion steps under



Scheme 1.

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Table 1. PNA_2:DNA sequences and UVTm°C (ΔTm°C, % Hyperchromicity).

DNA 5′-G C A A A A A A A A C G-3′	Stereochemistry			
PNA	(2*S*,4*S*)	(2*R*,4*R*)	(2*S*,4*R*)	(2*R*,4*S*)
1. H-T T T T T T T T-NH-β-alanine-OH	Control PNA		43.5	
2. H-T T T T T T T t-NH-β-alanine-OH	37(−6.5, 10.6)	44(+0.5, 23.5)	46.5(+3, 19.3)	ND(−, 7.6) 3.
3. H-T T T T T T T t-NH-β-alanine-OH	ND(−, 4.7)	ND(−, 7.6)	ND(−, 12.4)	ND(−, 5.8)

t indicates incorporation of modified PNA unit in *aeg*PNA, ND not detected, Buffer conditions-10 mM sodium phosphate. 100 mM Nacl. 0.01 mM EDTA pH 7.0.

Mitsunobu conditions. Hydrolysis of N-3-benzoyl group in compound **5**, followed by hydrogenation using Pd-C to remove the ring nitrogen protection and alkylation with ethyl bromoacetate gave the target protected 2*S*,4*R* thymine monomer unit. **6**. It was hydrolyzed using aqueous methanolic sodium hydroxide to obtain the thymine monomer **7** that could be used for solid phase synthesis of PNA-PyrrolidinePNA oligomer/mixmers. All the new compounds were characterized using suitable spectroscopic analysis.

A similar set of reactions starting from *trans* 4-hydroxy-D-proline (2*R*,4*S*) gave the other isomer. PNA oligomers containing the unmodified *aeg*PNA and modified pyrrolidine-PNA backbone units were synthesized by SPPS using the BOC-protection strategy. DNA oligomers were synthesized on Pharmacia GA plus synthesizer gave employing phosphoramidite chemistry.

The preliminary DNA binding studies (Table 1) indicate that the 2*S*,4*R* stereochemistry of pyrrolidine PNA I induced the PNA conformation to be in agreement for binding with complementary DNA as positive changes are observed in both Tm and % Hyperchromicity when a single unit is present at the C-terminus. The effect was not found to be synergistic as an additional unit in the center of the sequence completely destabilizes the triplex. It is possible that the complementarity to the internucleobase distances in DNA could be disturbed due to the constrained pyrrolidine-amide linker as compared to the control PNA.

CONCLUSIONS

We report here the synthesis of all four diastereomers of the pyrrolidine PNA monomer **I** and their incorporation at predetermined positions in PNA oligomers. DNA complementation studies indicate that the 2*S*,4*R* stereochemistry could be well suited for better DNA binding. Extended amide backbone may be better for inter 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.

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