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Design and synthesis of conformationally frozen peptide nucleic acid backbone: chiral piperidine PNA as a hexitol nucleic acid surrogate ☆

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Abstract—The design and facile synthesis of novel chiral piperidine PNA from naturally occurring 4-hydroxy-L-proline is reported. The stereospecific ring-expansion reaction to get six-membered piperidine derivative from 5-membered pyrrolidine derivative is exploited for this synthesis. The resulting conformationally constrained PNA is utilized for the synthesis of PNA mixmers and the concept is substantiated by UV-Tm studies of the resulting PNA_2:DNA complexes.

The remarkable medicinal importance of the achiral, acyclic, and uncharged aminoethylglycyl peptide nucleic acids[1] *aeg*PNAs as DNA/RNA mimics has challenged chemists to circumvent the limitations of their in vivo efficacy.[2] The efforts are mainly directed towards further refining the *aeg*PNA properties such as water solubility, cellular uptake, and discrimination between parallel versus anti-parallel binding modes.[2,3] The conformational freedom in the nucleobase linker arm and the backbone aminoethyl and glycyl segments in the *aeg*-PNA were found to be a cause of unfavorable entropic loss during complex formation with complementary DNA/RNA.[4] Complexation with target complementary RNA by the DNA analogs that assume A-type of sugar conformations such as LNA,[5] hexitol nucleic acids[6] or 2′-modified nucleic acids[7] (Fig. 1) was found to be much stronger than natural nucleic acids. The main reason for this improved stability of the complexes is attributed to the conformational pre-organization due to either locking or freezing the sugar ring conformation that is prevalent in complexes with RNA. The structural information of the PNA:DNA/RNA complexes[8] is not as completely available as for the DNA:DNA/RNA complexes and there are possibilities for modulating the conformational features of PNA in a variety of ways

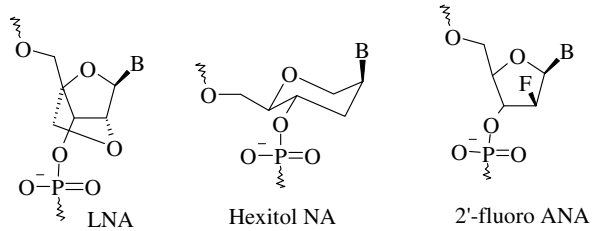

Figure 1. Conformationally locked and frozen nucleic acid analogs.

considering the acyclic nature of *aeg*PNA. Construction of the five-membered pyrrolidine-based conformationally restricted chiral PNAs as well has met with some success in this direction.[9] The introduction of the rigid chair conformations of the six-membered rings that determine the orientation of the ring substituents with respect to each other will further add to the structural diversity of PNA. The earlier reported six-membered PNA analogs[10] did not exhibit stabilization of the complexes with DNA as compared to *aeg*PNA, although limited binding selectivity for RNA over DNA was observed. In this communication, we introduce a new six-membered, chiral piperidine PNA analog that is a hexitol-NA surrogate. Hexitol NA has equatorial (hydroxymethyl), equatorial (hydroxy group) and axial (base moiety) substitutions.[11] The design of the monomer is configured in such a way that, to maintain the preferred N1-equatorial alkyl substitution on piperidine ring,[12] and 1,3 *cis* diequatorial disposition of the backbone, the orientation of nucleobase needs to be axial. As

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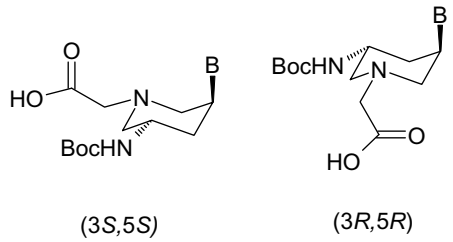
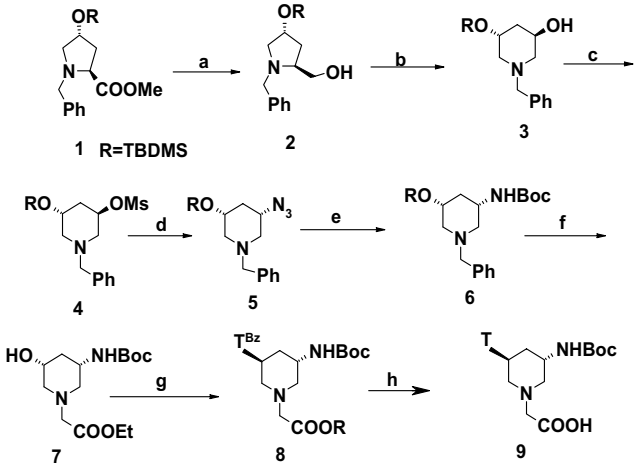


Figure 2. Proposed conformationally frozen peptide nucleic acid analogs.

in the case of hexitol-NA, the axial orientation of the nucleobase and equatorial disposition of the backbone may lead to the formation of stable duplexes with natural nucleic acids in contrast to hexose nucleic acids that assume an all equatorial hexose ring[13] substitution pattern. We report herein, the synthesis of the monomer unit (Fig. 2) *trans*-(3S,5S)-3-(thymin-1-yl)-5-*tert*·butyloxycarbonylamino-piperidin-1-yl acetic acid from naturally occurring *trans*-4-hydroxy-proline.[14] The PNA oligomer synthesis incorporating this designed monomeric unit at pre-defined positions and the DNA-binding properties of the resulting oligomers with complementary DNA is reported.

The suitably protected derivative of *trans*-(2*S*,4*R*)-4-hydroxy-L-proline **1** (Scheme 1) was converted to the *trans*-(2*S*,4*R*) pyrrolidine-2-methanol **2** by reduction of the ester function.[15] Treatment with trifluoracetic anhydride followed by diisopropylethylamine gave the six-membered rearranged product **3** (3*R*,5*R*) with retention of configuration.[16] Mesylation of the resulting unprotected hydroxy group and reaction with excess sodium azide in dry DMF at 65 °C gave *cis* azide **5** (3*S*,5*R*) with inversion of configuration at C3. Compound **5** was then selectively hydrogenated using Ra–Ni and the resulting amine was Boc-protected to get the



Scheme 1. Synthesis of the 3*S*,5*S* piperidine monomeric unit. (a) $LiBH_4$/THF; (b) (i) TFAA, (ii) DIPEA; (c) MsCl/Pyridine; (d) NaN_3/DMF; (e) Ra-Ni/H_2, Boc anhydride; (f) (i) H_2/Pd–C; (ii) $BrCH_2$ COOEt/DIPEA; (iii) TBAF/THF; (g) N-3-benzoylthymine, PPh_3/DIAD; (h) NaOH/water:MeOH.

(3*S*,5*R*) *tert*·butyloxycarbonyl piperidine derivative **6**. Compound **6** was subjected to hydrogenation to remove ring nitrogen benzyl protection. Alkylation of ring nitrogen using ethyl bromoacetate followed by removal of silyl protection of the 5-OH using TBAF gave **7**. *trans*-5*S*-(*N*3-Benzoyl-thymin-1-yl)-3*S*-*tert*·butyloxycarbonylaminomethyl piperidine derivative **8** was synthesized under Mitsunobu conditions with *N*3-benzoyl thymine. The product was purified by column chromatography and the ester function was hydrolyzed using aqueous methanolic sodium hydroxide to get the thymine-monomer **9** that could be used for solid phase synthesis of PNA-PiperidinePNA mixmers. All new compounds were characterized using suitable spectroscopic analysis. The synthesis of the other enantiomer (3*R*,5*R*) can be achieved by using appropriate starting material *trans*-(2*R*,4*S*)-4-hydroxy-D-proline.[15] The synthesis of the *cis* isomer proved to be difficult as the Mitsunobu reaction with *trans*-**7** failed.

PNA **10** is the unmodified *aeg*PNA with aminoethylglycyl backbone. PNAs **11**–**14** are the PNA oligomers with the modified PNA units incorporated at the predefined sites as represented in Table 1. The UV-T_m studies of the resulting complexes (Fig. 3) indicate that the modified PNA unit at the C-terminus in PNA **11** stabilized the complex with complementary DNA by about 7 °C. The synergistic effect was observed with one more unit in the center of the sequence PNA **12** and the PNA_2:DNA complex was further stabilized by about 4 °C. The presence of conformationally frozen six-membered piperidine at C-terminus seems to induce favorable pre-organization of PNA to interact with target DNA, leading to more stable PNA_2:DNA complexes. A single modified unit in the center of the sequence in PNA **13** seems to be accommodated in the uniform *aeg*PNA backbone causing only a minor effect. Interestingly, modification at the N-terminus destabilizes the complex although the transition is sharp with higher percent hyperchromicity. These preliminary results are encouraging and invoke the possibility of development of designed antisense oligonucleotide mimics with minimum structural modifications. The 3*S*,5*S* stereochemistry of the piperidine ring reported here might prefer axial nucleobase orientation and equatorial backbone orientation as in the case of hexitol nucleic acids.

Table 1. UV-T_m studies of PNA_2:DNA complexes[a]

	Sequences[b]	UV-T_m (°C)	Hyperchromicity (%)
PNA **10**	H-TTTTTTTT-β-ala-OH	43	10
PNA **11**	H-TTTTTTT**t**-β-ala-OH	50.7	15
PNA **12**	H-TTT**t**TTT**t**-β-ala-OH	54.4	9
PNA **13**	H-TTT**t**TTTT-β-ala-OH	41.4	6
PNA **14**	H-**t**TTTTTTT-β-ala-OH	35	15
DNA **15**	5′-GC$(A)_8$ CG-3′		

[a] Buffer: 10 mM sodiumcacodylate, 100 mM NaCl, 0.01 mM EDTA, pH 7.3.

[b] T represents *aeg*PNA unit and **t** represents piperidine PNA unit.

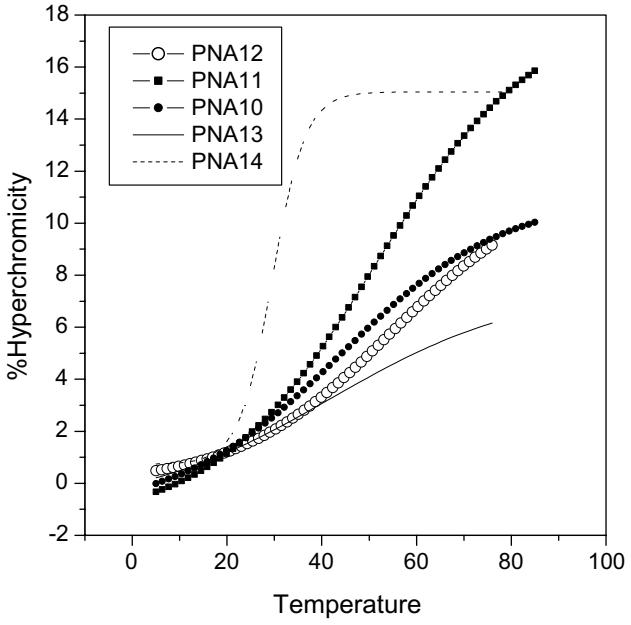


Figure 3. Percent hyperchromicity verses temperature plots of the PNA_2:DNA complexes.

In summary, this communication presents a unique example of a rationally designed PNA analog that takes advantage of the conformationally frozen six-membered ring having substituents in definite preferred orientations with respect to each other. DNA complementation studies of the modified PNAs by UV-T_m measurements indicate that these PNAs form stable PNA_2:DNA complexes. The tertiary ring nitrogen is protonable at physiological pH. The additional positive charges in the backbone may add favorable therapeutic features to the oligomers as the positive charges in the backbone are known to aid cellular uptake[3]. Further work that includes the synthesis of mixed purine/pyrimidine PNA sequences incorporating this monomer and synthesis of other stereoisomers is currently underway in our laboratory.

Supporting information

Experimental procedures and characterization for **1**–**9**, ^{13}C and mass spectra of **9**, HPLC, ESIMS, and CD spectra of **10**–**13**, and CD spectra of the complexes of **10**–**13** with DNA **15**. Binding stoichiometry by CD Job's Plot of **13:15**.

Acknowledgements

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