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A Cyclopentane Conformational Restraint for a Peptide Nucleic Acid: Design, Asymmetric Synthesis, and Improved Binding Affinity to DNA and RNA

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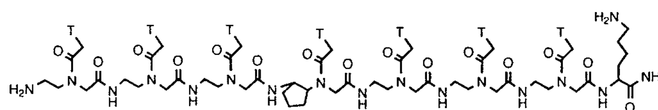
ABSTRACT



A strategy to restrict the highly flexible backbone conformation of a peptide nucleic acid (PNA) by incorporation of a cyclopentane ring is proposed. An asymmetric synthesis of cyclopentane-modified PNA is reported, and its binding properties were determined. The cyclopentane ring leads to a significant improvement in the binding properties of the resulting PNA to DNA and RNA.

The high binding affinity of peptide nucleic acids to complementary DNA and RNA has spurred development of numerous biochemical, biomedical, and medicinal applications for this class of molecules.[1] The most well-known class of peptide nucleic acids (PNAs) consists of nucleic acid bases attached to an achiral peptide backbone that is made up of *N*-(2-aminoethyl)glycine units (Figure 1).[2] These oligonucleotide mimics bind sequence specifically to DNA and RNA with higher affinity than complementary oligonucleotides. Structures of PNA−DNA and PNA−RNA duplexes have been well characterized, in addition to PNA_2DNA triplexes consisting of two polypyrimidine PNAs bound to one polypurine DNA.[3] Numerous backbone modifications to PNAs have been explored with the intention of preorganizing the highly flexible PNA backbone into the requisite conformations necessary for binding to DNA and RNA.[4] Proper preorganization could significantly increase the PNA binding affinity for oligonucleotides. Such increases in binding affinity could lower the detection limits for PNA-based diagnostic applications and improve the antisense properties for this class of molecules. Currently, there are relatively few modifications that have successfully yielded PNAs with

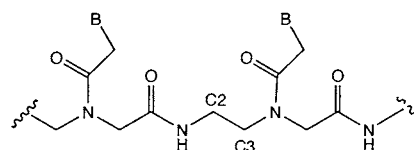


Figure 1. Structure of a peptide nucleic acid.

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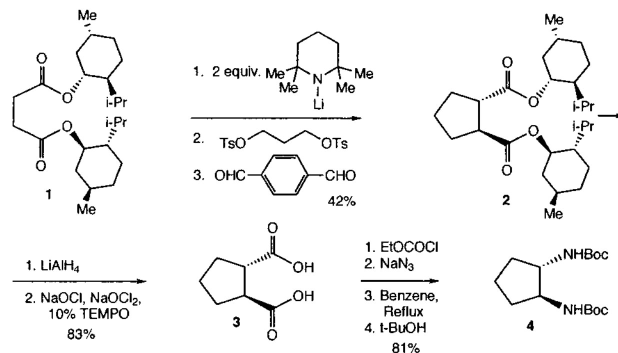
improved binding affinity over the original, unmodified backbone. The most successful modifications described to date involve incorporating proline and derivatives of 4-hydroxyproline into a PNA backbone.[4] We describe in this communication a novel conformational restraint for a PNA backbone that leads to increased binding affinity to complementary DNA and RNA.

A simple strategy for rigidifying a PNA is to incorporate a cyclic ring into the C2−C3 carbon−carbon bond of the PNA backbone (Figure 1). Nielsen and co-workers initially examined this strategy by incorporating a cyclohexane ring at this position.[5] The facile synthesis of these PNAs started from the well-known and commercially available *trans*-1,2-diaminocyclohexane (available as a single enantiomer in either the (*R*,*R*) or (*S*,*S*) form). The results of this study demonstrated that the PNAs derived from the (*R*,*R*) enantiomer bound very weakly to DNA and RNA, while the (*S*,*S*)-cyclohexyl PNAs bound with slightly weaker affinity compared to the unmodified PNA.

Analysis of the preferred PNA backbone dihedral angles when bound to DNA or RNA, in addition to molecular modeling studies (using molecular mechanics calculations with the MM3 force field), indicated to us that the cyclohexane ring does not possess the optimal dihedral angles to promote PNA binding to DNA or RNA.[6] From NMR structures of PNA−DNA and PNA−RNA duplexes, the preferred dihedral angle about C2−C3 is 130−165° and 60−80°, respectively.[3] Our molecular modeling studies indicate that the minimum energy conformation of a trans-diequatorial cyclohexane ring is about 50−65°. The conformational constraints of the cyclohexane ring prevent this dihedral angle from attaining values that are considerably outside this range. Modeling studies of a corresponding cyclopentane indicated that this ring would be better suited to adopt the requisite C2−C3 dihedral angles in PNAs. Energy-minimized conformations of a trans-diequatorial cyclopentane ring possessed dihedral angles of 70−90°. In addition, the broad potential energy well suggested that the cyclopentane could adopt dihedral angles up to 160°. Based on these predictions, we embarked on a synthesis of (*S*,*S*)-cyclopentanediamine to determine if it was a suitable conformational restraint for a PNA.

To test the effects of cyclopentane modification in a PNA, we required multigram quantities of enantiomerically pure (*S,S*)-*trans*-1,2-cyclopentanediamine. Only a few syntheses of this diamine have appeared in the literature, and most rely on resolutions of racemic *trans*-1,2-cyclopentanediamine to obtain enantiomerically enriched material.[7] An enzymatic resolution of this diamine has recently been published,[8] but in our work, we felt that an asymmetric synthesis based on Yamamoto's diasteroselective alkylation of dimenthylsuccinate (**1**) would be the most direct route.[9] We successfully scaled up (by 3 times) Yamamoto's asymmetric alkylation of (−)-dimenthylsuccinate with 1,3-propanediol ditosylate to a 70 g scale (Scheme 1). Purification of the resulting

Scheme 1. Asymmetric Alkylation, Removal of Chiral Auxiliaries, and Curtius Rearrangement



cyclopentane dimenthyl ester was enhanced by quenching the unreacted dianion of dimenthylsuccinate with terephthaldicarboxaldehyde, resulting in easily separable impurities. In contrast to the reported dr of 24:1, we routinely obtained **2** as a 9:1 mixture of diastereomers (based on GC analysis). The (1*S*,2*S*)-cyclopentane diastereomer **2** was highly crystalline and was separated from the (1*R*,2*R*)-cyclopentane diastereomer by recrystallization. The material that was obtained in this fashion had >99% de (based on GC analysis), and the absolute stereochemistry at the substituted cyclopentane carbons was confirmed as (*S,S*) on the basis of a crystal structure of **2**.

Removal of the (−)-menthyl chiral auxiliaries was problematic because **2** was resistant to hydrolysis using Yamamoto's alkaline conditions that were published for the hydrolysis of the corresponding cyclopropane dimenthyl ester (10% KOH in 9:1 MeOH/H_2O, 60 °C).[9b] Acidic conditions were also unsuccessful at hydrolyzing the menthyl esters of **2**, as were reactions using peroxy anion. These findings are in line with related hydrolyses of 1,2-disubstituted cyclohexane menthyl esters where aqueous tetrabutylammonium hydroxide (TBAH) gave low yields of mono- and dicarboxylic acids.[10] Under more vigorous conditions (1.5 N KOH in 5:1 MeOH/H_2O, reflux) hydrolysis occurred, but the product obtained was partially racemized based on a chiral HPLC analysis of a derivative of the isolated diacid (details concerning evaluation of ee are given in the Supporting Information).

Since **2** could not be hydrolyzed without racemization, alternate conditions were developed to remove the chiral

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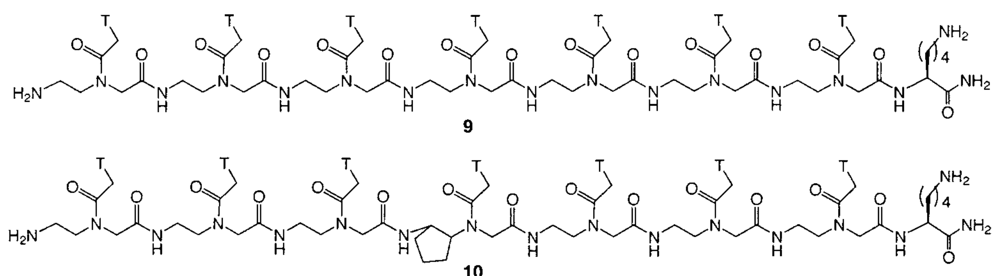
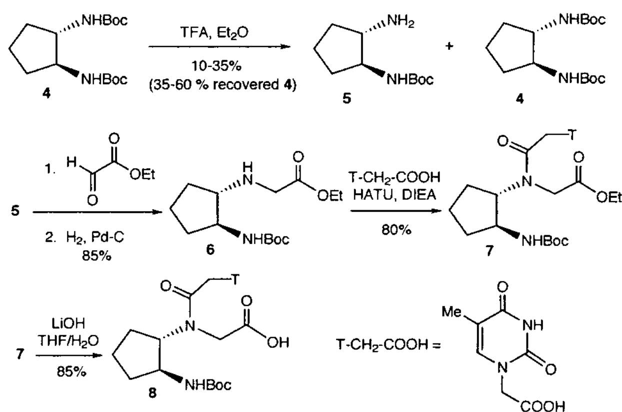


Figure 2. PNAs to explore effects of cyclopentane.

auxiliaries. Reaction with $LiAlH_4$ reduced the menthyl esters to a diol that was subsequently oxidized with catalytic TEMPO, $NaOCl_2$, and NaOCl to give **3**.[11] Overall yields for this two-step reduction−oxidation were good, and no additional purification steps were necessary. Chiral HPLC analysis showed an ee >99% for **3**. With enantiomerically pure 1,2-dicarboxylic acid in hand, the next steps focused on installing the nitrogens onto the cyclopentane ring utilizing a double Curtius rearrangement. After rearrangement to a bis-isocyanate, reaction with neat *tert*-butyl alcohol afforded di-*tert*-butyl carbamate **4** as a stable solid.[12]

To make a PNA monomer, the next step focused on differentiating the two nitrogens of **4** so that a single *tert*-butyl carbamate protecting group was present (Scheme 2).

Scheme 2. Monodeprotection and Synthesis of Cyclopentane PNA Monomer



This type of monoprotected diamine is frequently a synthetic intermediate in the synthesis of PNA monomers.[13] In the synthesis of a regular PNA monomer, monoprotected ethylenediamine is obtained by combining a 5:1 ratio of ethylenediamine to di-*tert*-butyl dicarbonate (Boc_2O).[14] The excess ethylenediamine is not recovered. While this procedure is acceptable for ethylenediamine, it was extremely low yielding when applied to cyclopentane diamine. Furthermore, the extensive loss of diamine under these conditions forced us to devise a more efficient method for obtaining a monoprotected cyclopentane diamine. After much experimentation, it was found that a solution of trifluoroacetic acid in anhydrous ethyl ether would effect a monodeprotection to give **5** in modest yields. Fortunately, 60% of unreacted starting material **4** could be recovered after the reaction by an acid−base aqueous workup and recycled for use in subsequent mono-deprotections. Repeated monodeprotections gave sufficient quantities of **5** to make the necessary PNA monomer.

The final steps in the synthesis of the cyclopentane PNA monomer involved attaching a methylenecarboxy group and a thymine base to the amine. Reaction between monoamine **5** and ethylglyoxalate afforded an intermediate imine that was immediately hydrogenated with a palladium catalyst to produce **6**. Alternative alkylation procedures using methyl bromoacetate routinely gave dialkylated material. The reductive amination was advantageous because no dialkylated byproduct was formed.

Combination of thymine carboxylic acid with **6** and HATU[15] formed the fully protected cyclopentyl-PNA monomer **7**. Hydrolysis of the ethyl ester cleanly afforded **8**, which was used directly in the solid-phase synthesis of PNAs. We have used this synthetic route to produce **8**, in eight steps with a 4% overall yield.

Using standard solid-phase peptide synthesis procedures,[16] two PNA heptamers were made to test the ability of the cyclopentane ring to promote PNA binding to DNA and RNA (Figure 2). In each PNA, a lysine was present as the first residue and the N-terminal was left unprotected in order to promote aqueous solubility and prevent aggregation.

The effects of cyclopentane substitution in PNAs were determined by examining the melting temperature for each PNA bound to a hepta-adenine oligonucleotide. PNAs **9** and **10** were compared for their ability to form stable PNA_2DNA triplexes.[17] The effects on DNA binding are dramatic. A

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single cyclopentane residue in the middle of PNA **10** increases the melting temperature by almost 6 °C relative to control PNA **9** (Table 1). In the PNA−RNA duplex, the cyclopentane ring also confers increased melting temperatures to the duplex.

Table 1. T_m Data for PNA/Oligonucleotide Complexes[a]

PNA	T_m PNA_2DNA	T_m PNA/RNA
9	44.4	48.3
10	50.3	51.4

[a] All samples were prepared in 10 mM phosphate buffer (pH 7) with 100 mM NaCl. Each strand concentration is 5 μM for RNA and 7 μM for DNA. Each melting temperature is the average of a forward and backward run from 20 to 80 °C. Data were obtained using $d(A)_7$ and $r(A)_7$ as the oligonucleotides.

In conclusion, the cyclopentane ring can be a useful conformational restraint for the C2−C3 dihedral angle of the PNA backbone. Molecular modeling and computational analyses were useful guides for designing this conformational restraint. An asymmetric synthesis of cyclopentane diamine, and a monodeprotection were important synthetic developments that allowed access to enough cyclopentane monomer in order to incorporate cyclopentane into a PNA. The cyclopentane modification improves the stability of PNA−DNA triplexes and PNA−RNA duplexes for a poly-T PNA. We are currently examining the incorporation of cyclopentane into additional PNA sequences to determine whether this modification is compatible with other bases, and we are examining whether multiple cyclopentanes will further stabilize duplexes and triplexes. We are also exploring the use of the cyclopentane ring as a scaffold for attachment of functional groups that will result in new PNAs with useful chemical and biological properties.

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Supporting Information Available: Molecular modeling information for cyclohexane vs cyclopentane PNA monomers, all synthetic procedures for preparation of cyclopentane PNA monomers, procedure for determining % ee of **3**, MALDI mass spectra for PNAs **9** and **10**, and melting curves for all duplexes and triplexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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