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Tetrahedron Letters 46 (2005) 915–917
Tetrahedron Letters

Cyclopropane PNA: observable triplex melting in a PNA constrained with a 3-membered ring

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Received 17 November 2004; revised 10 December 2004; accepted 14 December 2004
Available online 29 December 2004

Abstract—The first peptide nucleic acid (PNA) with a cyclopropane in the backbone has been synthesized, and the effects of the ring on DNA/RNA binding properties of the PNA have been examined. Well-defined triplex to duplex melting transitions of PNA_2 DNA complexes is clearly observed by variable temperature UV absorbance with the cyclopropane-constrained PNA.

Peptide nucleic acids (PNAs) are nucleic acid mimics that possess a polyamide backbone instead of the sugar–phosphate backbone of DNA/RNA. The most common PNA is derived from an (aminoethyl)glycine (*aeg*) backbone which was first introduced by Nielsen et al. (Fig. 1A).[1] The *aeg*PNAs bind to complementary DNA and RNA sequences through Watson–Crick (and in some cases Hoogsteen) hydrogen bonding with higher affinity than the corresponding DNA or RNA sequences.[2] Since the introduction of *aeg*PNA, researchers have attempted to modify the simple polyamide backbone to study the effects of different modifications on the oligonucleotide binding properties.[3] Recent efforts in this area have focused on the incorporation of cyclic rings into the PNA backbone in attempts to pre-organize the PNA for oligonucleotide binding.[4] The most successful modifications using cyclic restraints involve the introduction of 5- or 6-membered rings into various positions in the backbone.[5,6] Smaller rings have, to the best of our knowledge, not been explored for their effects on PNA binding to oligonucleotides. In previous work, we have shown that the introduction of *trans*-cyclopentane rings into a PNA backbone affords a class of PNAs (which we refer to as *t*cypPNAs) with both higher binding affinity and improved sequence specificity to complementary DNA (Fig. 1B).[6] To probe the effects of smaller carbocyclic rings on the oligonucleotide-binding properties of PNA, we have incorporated a *trans*-cyclopropane ring into the PNA backbone (Fig. 1C). Compared to cyclopentane, the cyclopropane ring is more rigid, and the dihedral angle between *trans* substituents on the 3-membered ring (approximately 145°) is larger than the corresponding 5-membered ring (approximately 70°).[6a,7] Herein, we report an asymmetric synthesis of the *trans*-cyclopropane (*t*cpr) PNA monomer and binding studies of a poly-T heptamer with one cyclopropane monomer to complementary DNA and RNA.

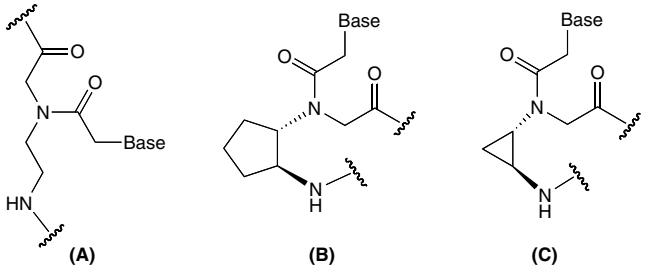

Figure 1. (A) aegPNA, (B) *t*cypPNA, (C) *t*cprPNA.

The *t*cprPNA monomer was synthesized as shown in Scheme 1. The synthesis began by making (−)-dimenthyl succinate (**1**) from succinic anhydride and (−)-menthol under Dean–Stark conditions. Then, Yamamoto's asymmetric alkylation of (−)-dimenthyl succinate with bromochloromethane afforded the (*S*,*S*)-cyclopropane dimenthyl ester **2** in 45% yield after recrystallization (>99% de).[8] Hydrolysis of **2** under basic conditions, following Yamamoto's procedure, gave (1*S*,2*S*)-cyclopropane-1,2-dicarboxylate (**3**) as a single enantiomer. A

Keywords: Peptide nucleic acid; PNA; Cyclopropane.
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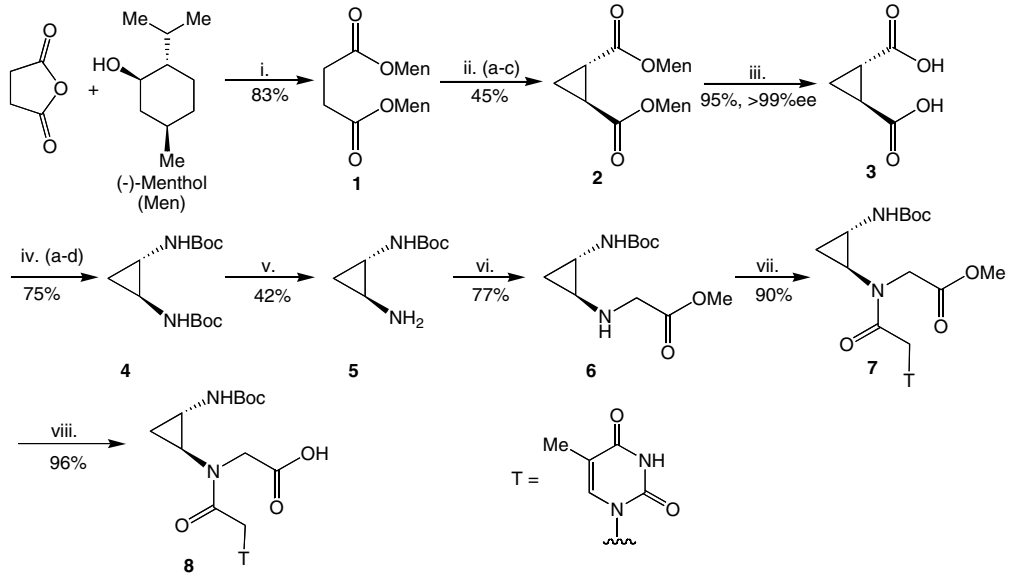
doi:10.1016/j.tetlet.2004.12.061



Scheme 1. Reagents and conditions: (i) cat. *p*-TsOH, toluene, Dean stark, 18 h; (ii) (a) LiTMP, −78 °C; (b) $BrClCH_2$; (c) terephthalaldehyde; (iii) 10% KOH, 9:1 MeOH/H_2O, 60 °C, 4 h; (iv) (a) EtOCOCl, TEA; (b) NaN_3, H_2O; (c) benzene, reflux; (d) *t*-butanol, reflux, 15 h; (v) 20 equiv TFA, Et_2O, 12 h; (vi) methylbromoacetate, DIEA, DMF, 15 h; (vii) T-CH_2COOH, HATU, DIEA, 24 h; (viii) LiOH, THF, H_2O, 4 h.

Curtius rearrangement, followed by trapping the intermediate isocyanate with *tert*-butanol, gave the di-protected *tert*-butyl carbamate **4** in 75% yield. Mono-deprotection of **4** with trifluoroacetic acid gave mono-amine **5** in 42% yield with a 30% recovery of starting material which could be resubmitted to the reaction conditions. Mono-amine **5** was alkylated with methylbromoacetate to give **6**, and then thymine acetic acid was coupled to **6** using HATU to give **7**. Hydrolysis of **7** afforded (*S*,*S*)-*t*cprPNA monomer **8**. This route yielded *t*cprPNA monomer in eight steps with a 9% overall yield and allowed access to gram quantities of material. The cyclopropane monomer was then incorporated into a PNA oligomer using modified solid phase peptide synthesis procedures.[9] All oligomers were purified by reverse phase HPLC and characterized by MALDI-TOF mass spectrometry (See supporting information for procedures and characterization data).

The oligonucleotide binding properties of *t*cprPNA heptamer **10** were compared to the corresponding *aeg*PNA **9** using variable temperature UV experiments to examine the melting temperatures (T_m's) of the complexes. The results indicate that introduction of an (*S*,*S*)-cyclopropane into the PNA backbone lowers the T_m's of the DNA and RNA complexes (Table 1). In the case of the *t*cprPNA–RNA complex, a well-defined melting transition was clearly visible in both the heating and cooling experiments. However, a comparison of the two experiments shows very significant hysteresis (Fig. 2A). The calculated T_m from the heating experiment was nearly identical to that of *aeg*PNA; however, the calculated T_m for the cooling experiment was 16 °C lower. Since this hysteresis was not observed in the heating and cooling runs of the *aeg*PNA–RNA complex, it is possible that *t*cprPNA binds to RNA more slowly than *aeg*PNA.

Table 1. Melting data for PNA: Oligonucleotide complexes. All samples were prepared in 10 mM phosphate buffer (pH 7) containing 150 mM NaCl and 0.1 mM EDTA

Entry	Sequence	PNA_2–DNA[a]		PNA–RNA[b]	
		T_m^{up} (°C)[c]	T_m^{dn} (°C)[d]	T_m^{up} (°C)[c]	T_m^{dn} (°C)[d]
9	TTTTTTT-Lys	44.8	44.8	54.2	53.5
10	TTT***T*****$_{cpr}$**TTT-Lys	21.6, 46.5	22.4, 39.0	49.2	33.0

[a] Each strand concentration is 7 μM.
[b] Each strand concentration is 15 μM.
[c] T_m^{up} refers to a melting run going from 20–80 °C in which readings were taken in 1 °C increments and held at the temperature for 5 min before readings were taken.
[d] T_m^{dn} refers to the reverse run with the same conditions as (c).

The melting curves of the *t*cprPNA–DNA complex were more interesting. First, a Job plot confirmed that *t*cprPNA **10** formed a 2:1 PNA–DNA triplex.[10] Surprisingly, the melting curves of this complex clearly show two thermal melting transitions (Fig. 2B). We speculate that the lower transition corresponds to the dissociation of the Hoogsteen strand of the triplex, while the higher transition is the melting of the duplex. Analogous biphasic transitions have been seen in triplex melting curves, but they are rarely seen in the melting of PNA triplexes.[11] For instance, the melting of *aeg*PNA **9** shows only a single broad transition, even though a similar triplex is formed. The T_m's corresponding to the melting of the *t*cprPNA–DNA duplex (46.5 °C for heating, 39.0 °C for cooling) show significant hysteresis and are similar to the T_m's of the corresponding complex with *aeg*PNA, indicating that the cyclopropane constraint has little effect on duplex stability. In contrast, the triplex T_m's (21.6 °C for heating, 22.4 °C for cooling) show significantly less hysteresis, which could signify that the cyclo-

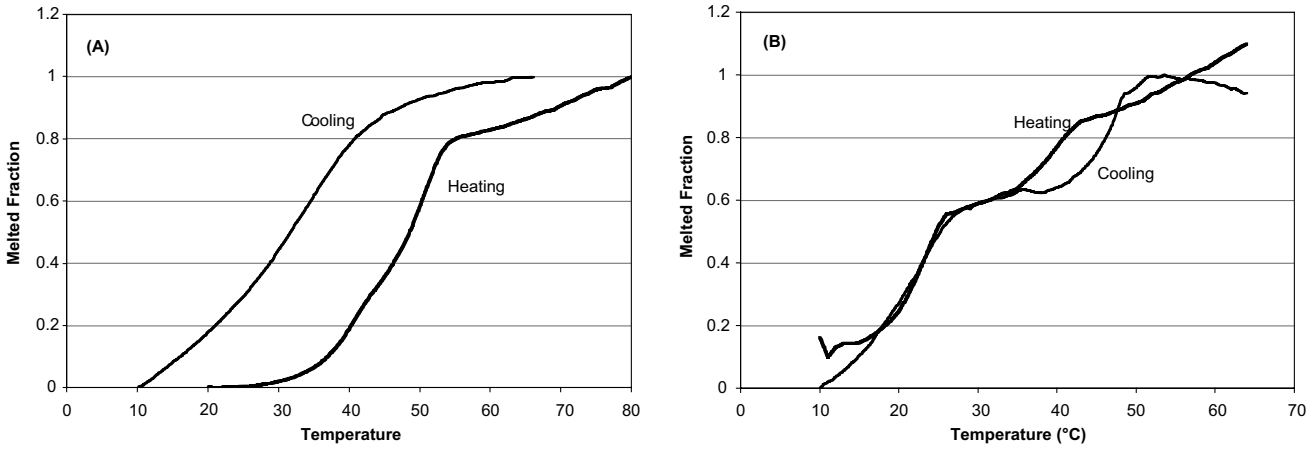


Figure 2. Heating and cooling curves of *t*cprPNA–oligonucleotide complexes. (A) *t*cprPNA–RNA, (B) *t*cprPNA$_2$–DNA.

propane constraint is more compatible in the Hoogsteen strand of a PNA$_2$DNA triplex than in the Watson–Crick strand. Unfortunately, no triplex melting was observed in the *aeg*PNA control, so no direct comparison is currently possible.

In conclusion, we have made the first PNA with an (*S*,*S*)-*trans*-cyclopropane in the backbone, and we present initial data indicating how this constraint may be useful to the preorganization of a PNA for oligonucleotide binding. A central premise in our research is that in order for a carbocyclic ring to promote PNA binding to oligonucleotides, both ring size and stereochemistry must restrict the PNA to access only the range of dihedral angles necessary for binding, while excluding conformations that are irrelevant to duplex formation. From this work, there are indications that the range of dihedral angles accessible to a cyclopropane ring are more compatible in the Hoogsteen strand of a PNA$_2$DNA triplex than in the Watson–Crick strand. Future work will focus on examination of *t*cprPNA in Hoogsteen interactions within a PNA–*t*cprPNA–DNA triplex.

Acknowledgements

We gratefully acknowledge financial support from Northwestern University's Weinberg College of Arts and Sciences, Department of Chemistry, and the VP of Research; and the American Chemical Society Petroleum Research Fund. Jon Pokorski was supported by Northwestern University's NIH Biotechnology Training Grant (T32 GM008449).

Supplementary data

Procedures for synthesis of *t*cprPNA monomers, solid phase synthesis of all PNAs, and thermal melting analysis. Mass spectra to characterize PNAs, ^{1}H spectra for all new compounds, and Job plot are also included. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2004.12.061.

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