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β-PNA: Peptide nucleic acid (PNA) with a chiral center at the β-position of the PNA backbone

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ABSTRACT

Peptide nucleic acid (PNA) monomers with a methyl group at the β-position have been synthesized. The modified monomers were incorporated into PNA oligomers using Fmoc chemistry for solid-phase synthesis. Thermal denaturation and circular dichroism (CD) studies have shown that PNA containing the *S*-form monomers was well suited to form a hybrid duplex with DNA, whose stability was comparable to that of unmodified PNA–DNA duplex, whereas PNA containing the *R*-form monomers was not.

Peptide nucleic acid (PNA) is a synthetic DNA/RNA mimic in which the sugar–phosphate backbone is replaced by a peptide backbone consisting of *N*-(2-aminoethyl)glycine units.[1] PNAs hybridize to complementary sequences by Watson–Crick base pairing with high affinity and strict sequence specificity.[2] PNAs are resistant to nucleases and proteases[3] and have a low affinity for proteins.[4] These properties make PNAs an attractive agent for biological and medical applications. To improve the antisense and antigene properties of PNAs, it is reasonable to increase binding affinity for DNA or RNA by suitable preorganization. A lot of backbone modifications of PNAs have been explored under the concept of preorganization.[5,6] However, only limited numbers of modifications improved the hybridization properties.[7–10]

A simple strategy for preorganizing a PNA is to add substituents to the PNA backbone. Several modified PNAs were obtained by incorporating substituents at either the α- or the γ-position.[9–13] Comparison of the effects of substituents at the α-position with those of the γ-position revealed that γ-modification is more effective to improve the DNA binding ability.[14] On the other hand, to our knowledge, PNAs with a single substituent only at the β-position have not been previously reported. Although some cyclic PNA analogs contain a chiral center at the β-position,[7,8,15–17] they do not directly reflect the effects of the β-substituents because the effects of cyclic structures are quite substantial. The β-position of the PNA backbone corresponds to the C4′ of the deoxyribose moiety of DNA and the C4′ is a chiral carbon atom. Thus, the introduction of a chiral center at the β-position may significantly affect the conformation and the DNA binding ability of PNA oligomers. Here we report the synthesis of PNA monomers with a methyl group at the β-position and their incorporation into oligomers as well as their DNA binding properties.

We designed a new PNA monomer possessing a methyl group of *S*-configuration at the β-position of the PNA backbone (Fig. 1) because molecular modeling, based on the crystal structure of a PNA–DNA duplex,[18] suggested this configuration is less disruptive to hybridization than the *R*-configuration (Fig. 2).[19]

The requisite chiral β-PNA thymine monomer **9** was prepared as follows (Scheme 1). Treatment of chiral diamine **1** with 2 mol

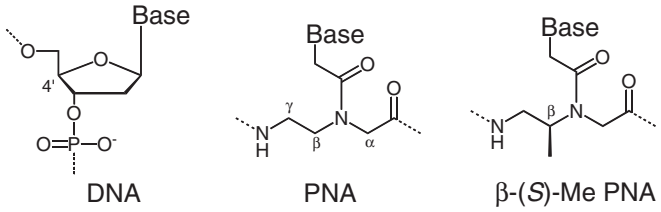

Figure 1. Structures of DNA, PNA and β-(*S*)-Me PNA.

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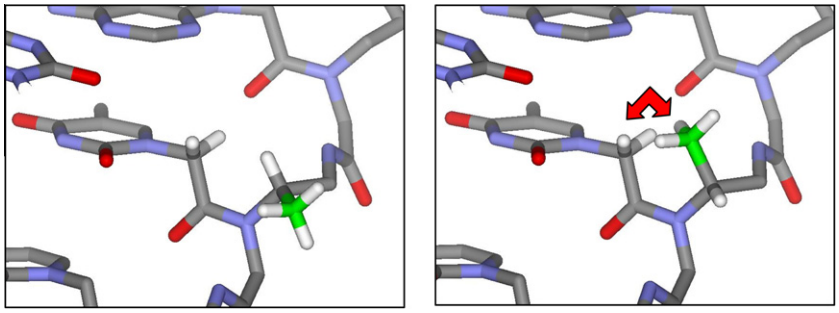
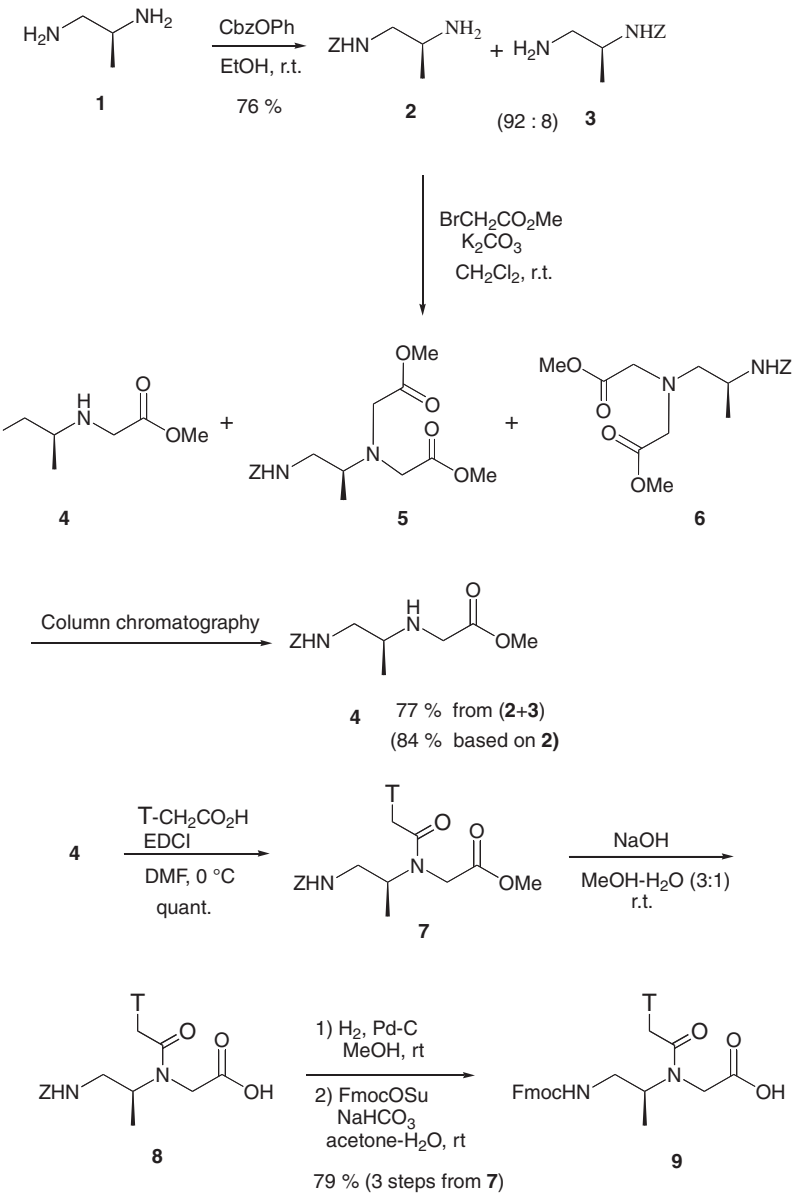


Figure 2. Molecular modeling of β-Me PNA/DNA duplex. The methyl group (green) of *S*-form β-PNA projects away from the duplex (left). The methyl group (green) of *R*-form β-PNA sterically clashes with the methylene of the thyminylacetyl moiety (right).



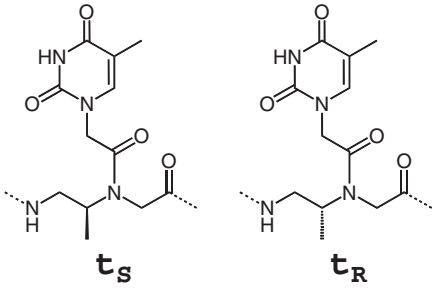
Scheme 1. Synthesis of β-(*S*)-Me PNA thymine monomer.

equiv of CbzOPh[20] in EtOH overnight gave a mixture of isomers **2** and **3** in a 92:8 ratio in 76% overall yield. The ratio was determined from the ^{1}H NMR signals for the methyl proton. Since these isomers could not be separated by usual chromatographic methods, the mixture of isomers was used directly for alkylation reaction. Treatment of the isomeric mixture with 4 mol equiv of methyl bromoacetate gave **4** in 77% yield (84% from **2**) as a pure isomer. This alkylation is noteworthy in that the minor isomer **3** was completely dialkylated under this condition and became chromatographically separable. Coupling of the thymin-1-ylacetic

Table 1
PNA sequences

PNA	Sequence[a]	
PNA 1	aeg	H-GTAGATCACT-^{L}LYS-NH_2
PNA 2	β-(*S*)-Me	H-Gt_SAGAt_SCACt_S-^{L}LYS-NH_2
PNA 3	β-(*R*)-Me	H-Gt_RAGAt_RCACt_R-^{D}LYS-NH_2
	t_S = β-(*S*)-Me PNA, t_R = β-(*R*)-Me PNA	

[a] Written from N to C terminal. PNAs terminate in a lysine amide.



acid was accomplished with EDCI in DMF in quantitative yield. Alkaline hydrolysis of the ester **7** proceeded quantitatively to afford **8**. Catalytic hydrogenation of **8** followed by its subsequent Fmoc protection with FmocOSu gave β-PNA monomer **9** in 79% yield for 3 steps from **7**. For comparison, the enantiomer of **9** was also prepared from (*R*)-1,2-diaminopropane. These monomers were used directly for the synthesis of PNA oligomers.

Using a standard manual solid-phase peptide synthesis procedure,[21] three PNA oligomers were synthesized to probe the effect of a methyl group at the β-position of the PNA backbone (Table 1). We chose a 10-residue mixed-base PNA sequence that has been well documented.[2a,22] β-PNA monomers could be successfully incorporated into the PNA sequence without difficulty. After deprotection and cleavage from the resin, each PNA was purified by reversed-phase HPLC and characterized by ESI-TOF mass spectrometry.[23] PNA oligomers synthesized here were readily soluble (more than 500 μM) in water.

To evaluate the effect of β-backbone modification on the DNA binding affinity of PNA, we measured the melting temperatures (T_ms) of the β-PNAs with the complementary DNA strand in 10 mM sodium phosphate buffer containing 1 mM EDTA (Fig. 3). β-PNA containing three *S*-form chiral units (PNA2) and unmodified PNA (PNA1) have similar T_m values. In contrast, PNA3, the enantiomer of PNA2, did not show a well-defined melting transition. Since the effect of C-terminal lysine on T_m is generally small, the melting behavior of PNA3-DNA should be dominated by the *R*-form chiral units. These results indicate that the stereochemistry of the β-position is critical to the hybridization stability of PNA and is strictly limited to *S*-configuration. This contrasts to α-modified PNAs in which chirality arising from a methyl group incorporated at the α-position hardly affects the hybridization properties.[10]

To gain structural insights, we measured the circular dichroism (CD) spectra of single-stranded PNAs (PNA1-3) and their hybrids with the complementary DNA in the same buffer used in UV-melting experiments. A comparison of the CD spectra of single-stranded PNAs (PNA1-3) is shown in Figure 4A. No exciton coupling pattern was observed for unmodified PNA (PNA1) in the nucleobase absorption regions (220–300 nm), indicating the lack of helical base-stacking. On the other hand, the β-PNAs, PNA2 and PNA3, showed distinct CD signals. The shape of the spectrum of PNA2 was similar to that of right-handed PNAs[10,13,19] and the signs of the CD bands of PNA2 and PNA3 were opposite each other. Thus it is reasonable to propose that PNA2 adopts a right-handed helical structure and PNA3 is left-handed. As shown in Figure 4B, the CD profile of PNA2-DNA hybrid duplex is very similar to that of PNA1-DNA hybrid, with a positive band at 266 nm and a

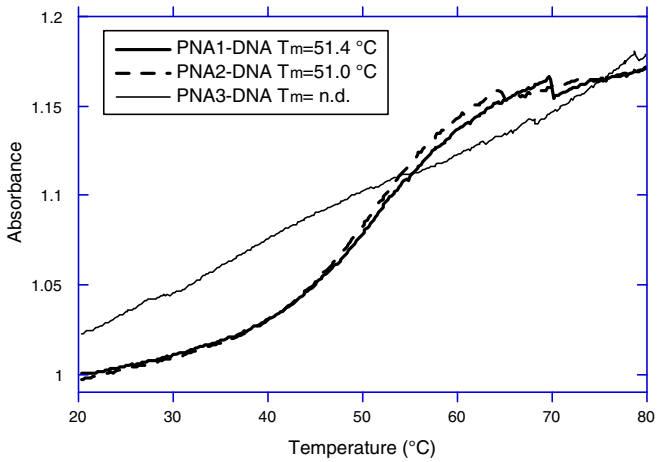


Figure 3. UV-melting curves of the PNA–DNA hybrid duplexes at 5 μM strand concentration each. Samples were prepared in 10 mM sodium phosphate buffer containing 1 mM EDTA (pH 7.4). Thermal denaturation was monitored at 260 nm at a rate of 0.2 °C per minute. The melting transitions were determined from the first derivatives of the UV-melting curves. n.d.: not determined. DNA: 5′-AGTG ATCTAC-3′.

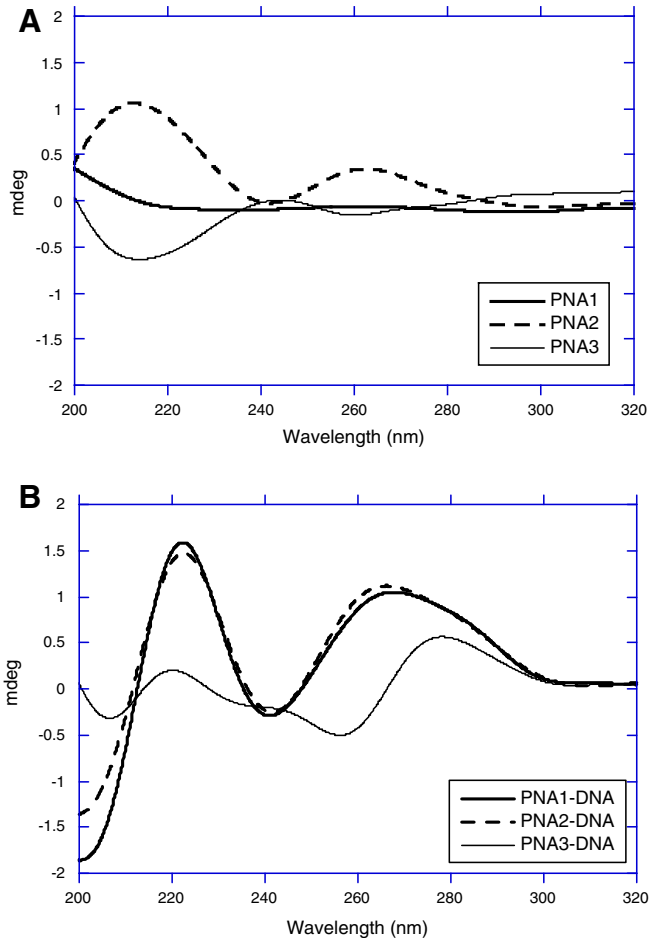


Figure 4. (A) Circular dichroism (CD) spectra of single-stranded PNA1 (thick solid curve), PNA2 (thick broken curve), and PNA3 (thin solid curve) at 5 μM strand concentration each in the same buffer used in UV-melting experiments, recorded at room temperature. (B) CD spectra of the corresponding PNA–DNA hybrids at 5 μM strand concentration each, recorded at room temperature after annealing in the same buffer. DNA: 5′-AGTGATCTAC-3′.

low-intensity negative band at 242 nm, suggesting a high degree of helical identity between PNA1/PNA2-DNA hybrids. In contrast,

the CD spectrum of PNA3-DNA hybrid is distinctly different from those of PNA1/PNA2-DNA hybrids. These results are consistent with the T_m data. Furthermore, the CD spectrum of single-stranded PNA2 is similar but somewhat different from that of the corresponding hybrid duplex, PNA2-DNA (PNA2 in Fig. 4A vs. PNA2-DNA in Fig. 4B). The most significant differences appeared in the 200–230 nm region. The positive band at 213 nm in single-stranded PNA2 was red-shifted to 222 nm in PNA2-DNA duplex and an intense negative band was observed at 200 nm only in the hybrid duplexes. These differences reflect conformational changes that occur upon DNA hybridization of PNA2. Since the induced secondary structure of β-Me PNA did not contribute to the total hybridization stability, the benefit of the induced structure in terms of DNA binding might be canceled by unfavorable steric interactions arising from β-methyl groups in the PNA–DNA hybrid duplex. To minimize the energy loss associated with conformational changes, further sophisticated design of PNA is required.

In conclusion, we have synthesized both the *S*- and the *R*-forms of β-Me PNA monomers and incorporated them individually into a 10-residue mixed-base PNA sequence. PNA containing the *S*-form chiral units was well suited to form a right-handed hybrid duplex with DNA, whereas incorporation of the *R*-form units was detrimental to hybridization with DNA. CD spectra suggested that β-backbone modification of PNA induced a right-handed helix. The results reported here would contribute to a better understanding of PNA chemistry and provide a basis for the future design of PNA with improved properties.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.017.

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