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Synthesis and Hybridization Property of Novel 2′,5′-isoDNA Mimic Chiral Peptide Nucleic Acids

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Abstract—2′,5′-isoDNA mimic chiral peptide nucleic acid (isoPNA) monomers derived from D- and L-aspartic acids were synthesized. These novel monomers were incorporated in aminoethylglycine peptide nucleic acid (aegPNA) thymine dodecamers, and the hybridization properties to RNA and DNA were demonstrated by UV thermal denaturation.

A large variety of structural modifications of oligonucleotides have been investigated as an exciting new concept for drug design to modulate the expression of genetic information by antisense and antigene oligonucleotides. Aminoethylglycine peptide nucleic acids (aegPNAs) developed by Nielsen and co-workers are oligonucleotide analogues in which the sugar phosphate backbone is replaced by a peptide chain.[1] PNA is a very potent DNA mimic capable of hybridizing to complementary DNA, RNA or PNA. Therefore many kinds of nucleotides with peptide backbone have been synthesized in order to improve the limited solubility and to investigate the structural flexibility and requirements for nucleic acid recognition (by, e.g., introduction of a certain side chain[2] and double bond[3] to peptide backbones, conversion of carbonyl group to sulfonyl group,[4] and construction of ethereal and/or cyclic structural peptide backbones[5]). However, there is no report on the peptide nucleic acids corresponding to the 'non-genetic' 2′,5′-linked oligonucleotide (2′,5′-isoDNA),[6] which is one of the important candidates to search the effective antisense oligonucleotides due to the intrinsic selective RNA-binding activity of 2′,5′-isoDNA (although the hybridization affinity of 2',5'-DNA or RNA/3′,5′-RNA is somewhat inferior to that of the 3′,5′-DNA/3′,5′-RNA duplex, 2′,5′-DNA or RNA shows binding selectivity for 3′,5′-RNA over 3′,5′-ssDNA). The favorable RNA- and DNA-binding properties of PNA and the selective binding activity of 2′,5′-isoDNA to RNA have led us to design 2′,5′-isoDNA mimic chiral peptide nucleic acids (isoPNAs) (Fig. 1). In this letter, we report on the synthesis and hybridization properties of PNAs containing novel isogaPNA monomers **4a** and **4b** with glycyl-β-alanine backbone derived from D- and L-aspartic acid.

The synthesis of glycyl-β-alanine isoPNA monomers proceeds through hydroxymethyl-β-alanine **1a**,**b**,[7] which were prepared from D- or L-aspartic acids protected with Boc and benzyl groups by reaction with ethyl chloroformate followed by $NaBH_4$ reduction in 71 and 69% yields, respectively. As shown in Scheme 1, hydroxymethylalanine **1a**,**b** was treated with 3-benzoylthymine under Mitsunobu conditions to give thyminylmethyl-β-alanine **2a**,**b**.[8] After removal of Boc group of **2a**,**b** by the addition of 3 N HCl, coupling with FmocGly in the presence of TBTU followed by deprotection of benzyl group of **3a**,**b** with 10% Pd/C and 1,4-cyclohexadiene provided thymine isogaPNA monomer T_R and T_S (**4a**,**b**).[9]

In order to assess the hybridization properties of these monomers to complementary DNA and RNA, the two monomeres **4a** and **4b** were individually introduced into aegPNA sequences in different positions to form different thymine dodecamers. The preparation of these oligomers was carried out using automated synthesis on an Expedite 8909 (Applied Biosystems, Foster City, CA, USA) and standard Fmoc-chemistry on Fmoc-XAL PEG-PS resin. L-Lysine was introduced at the C-terminus of the PNAs to reduce their self-aggregation and to ensure adequate solubility in water. The monomers were

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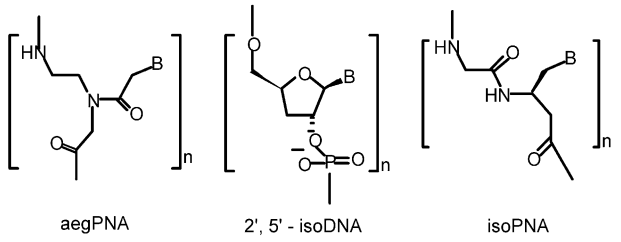
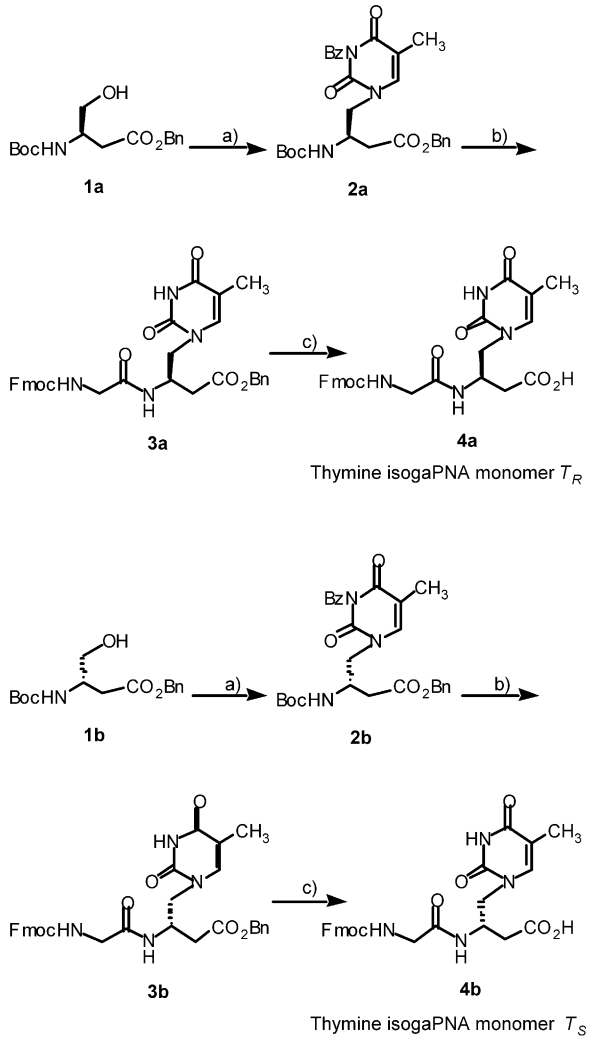


Figure 1. Design of 2′,5′-isoDNA mimic peptide nucleic acids (isoPNA).



Scheme 1. Synthesis of thymine isogaPNA monomers T_R and T_S (**4a** and **4b**): (a) DEAD (1.1 equiv), Ph_3P (1.1 equiv), 3-benzoylthymine (1 equiv), THF, −10 °C, 45% (**2a**), 44% (**2b**); (b) (i) 3 N HCl, EtOAc; (ii) FmocGly (1 equiv), TBTU (1 equiv), HoBt (1 equiv), DIEA (2 equiv), DMF, 49% (**3a**), 50% (**3b**); (c) 1,4-cyclohexadiene (20 equiv), 10% Pd/C, MeOH, 65% (**4a**), 62% (**4b**).

coupled by activation with HATU in DMF/NMP (1/1). After the final coupling, Fmoc-group was removed by 20% piperidine in DMF and cleavage of the PNAs from the resin was performed using TFA/*m*-cresol (4/1). The crude products were purified by C18-reverse-phase HPLC using gradients of 0.1% TFA in water and 0.1% TFA in acetonitrile. Also, complete isogaPNA dodecamers, which consist of only **4a** and **4b**, were prepared in the same manner, respectively. The purity of the oligomers was rechecked by HPLC and characterized by MALDI-TOF mass spectrometry.

PNA sequences

5	H-T_{12}-Lys-NH_2
6	DNA T_{12}
7	H-T_{11}-T_R-Lys-NH_2
8	H-T_R-T_{11}-Lys-NH_2
9	H-T_9-$T_RT_RT_R$-Lys-NH_2
10	H-$T_RT_RT_R$-T_9-Lys-NH_2
11	H-T_6-T_R-T_5-Lys-NH_2
12	H-T_{R12}-Lys-NH_2
13	H-T_{11}-T_S-Lys-NH_2
14	H-T_S-T_{11}-Lys-NH_2
15	H-T_9-$T_ST_ST_S$-Lys-NH_2
16	H-$T_ST_ST_S$-T_9-Lys-NH_2
17	H-T_6-T_S-T_5-Lys-NH_2
18	H-T_{S12}-Lys-NH_2

T = thymine aegPNA monomer; T_R = thymine isogaPNA monomer (**4a**); T_S = thymine isogaPNA monomer (**4b**).

The hybridization properties of PNA oligomers **7**–**18** with complementary DNA and RNA were investigated in UV-melting experiments (260 nm) and the results of these studies are shown in Table 1. The aegPNA T_{12}Lys control strand (**5**) gave T_m values of 65 and 73 °C when hybridized to dA_{12} and rA_{12}, respectively. Destabilization was observed upon incorporation of one and three isoagPNA T_R into the decamers (**7**–**11**), resulting in significant close T_m values (ΔT_m 2–3 °C) observed between the PNA **7**–**11** DNA and the corresponding PNA **7**–**11** RNA, contrary to the characters that aegPNA and isoDNA hybridize with RNA preferentially. The complexes

Table 1. Melting temperature (T_m values) of complexes between PNAs **5**–**16** and complementary DNA and RNA

Compd	T_m (°C)[a]	
	Complex with dA_{12}	Complex with rA_{12}
5	65	73
6	32	27
7	65	67
8	65	67
9	60	67
10	59 (50)[b]	62
11	53 (44)[c]	56
12	nd[d]	nd
13	61	67
14	64	68
15	56	63
16	58 (56)[b]	64
17	46 (39)[c]	53
18	nd	nd

[a]The T_m was measured at a ratio of PNA/DNA or RNA = 1:1, concentration of PNA strand = 2.5 μM, 10 mM phosphate buffer, pH 7.4, heating rate 0.5 °C/min. All of these oligomers can be dissolved easily in this buffer.
[b]The values with triple mismatching DNA A_9T_3.
[c]The values with single mismatching DNA A_5TA_6.
[d]nd, Not detected.

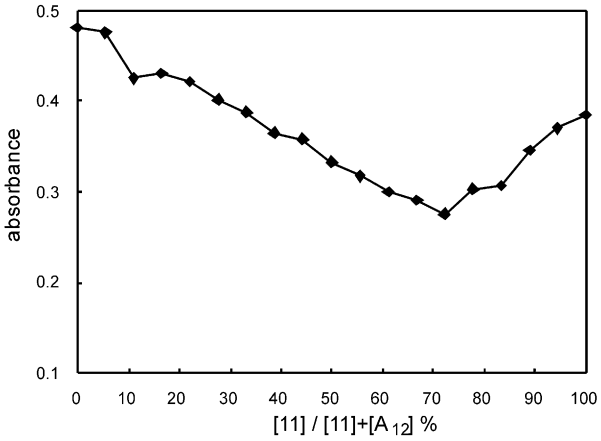


Figure 2. Job plots of absorbance at 25 °C for **11** and dA_{12} at 260 nm. The following extinction coefficients were used: T = 8.6 mL/mmol cm for PNA, A = 15.3 mL/mmol cm, [**11**] and [dA_{12}] = 3.0 μM, 10 mM phosphate buffer, pH 7.4.

between **13**–**17** incorporated of isogaPNA T_S and dA_{12} showed decreased T_m values (ΔT_m 1–7 °C) compared to the corresponding T_m values between PNA **7**–**11** incorporated T_R and DNA dA_{12}. Unfortunately, no hybridization was found for the complexes of complete isogaPNA **12** and **18** with DNA and RNA, respectively. Introduction of mismatch base T to dA_{12} at the position to hybridize with isogaPNA T_R and T_S resulted in decrease of the T_m (ΔT_m 9 °C for **10** and **11**, ΔT_m 2 °C and 7 °C for **16** and **17** respectively). These results show the conformation of backbone of T_R is more effective sterically than that of T_S. Furthermore, the binding stoichiometry was found by UV titration (Job-plot)[10] to be 2:1 (entry **11** vs dA_{12}) indicating a PNA_2-DNA triplex structure (Fig. 2).

The decreased T_m values compared to unmodified aegPNA oligomer indicate that the glycylalanine backbone of isogaPNA may not be optimal length to hybridize with DNA and RNA, and work is in progress to examine isoPNA with different backbone.

Acknowledgements

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9. **4a**: mp 144 °C; ν_{max}/cm^{-1} 1714, 1638 (C=O); δ_H (300 MHz, DMSO-d_6) 1.75 (3H, s, CH_3), 2.35–2.46 (2H, m, CH_2), 3.30 (1H, br, NH), 3.39–3.62 (2H, m, CH_2), 3.83–4.01 (2H, m, CH_2), 4.21–4.27 (3H, m, CH and CH_2), 4.40–4.56 (1H, m, CH), 7.23 (1H, s, CH-6 of T), 7.32 (2H, dd, *J* 7.5 and 7.5, Ar–H), 7.40 (2H, dd, *J* 7.5 and 7.5, Ar–H), 7.71 (2H, d, *J* 7.5, Ar–H), 7.87 (2H, d, *J* 7.5, Ar–H), 10.81 (1H, brs, NH), 12.14 (1H, br, COOH); δ_C (75 MHz, DMSO-d_6) 12.48 (CH_3), 37.03 (CH_2), 42.72 (CH_2), 43.39 (CH_2), 44.55 (CH), 46.63 (CH), 65.74 (CH_2), 107.13 (C), 120.06 (CH), 125.27 (CH), 127.06 (CH), 127.60 (CH), 136.28 (CH), 140.69 (C), 143.85 (C), 151.54 (C), 156.30 (C), 164.14 (C), 168.48 (C), 171.94 (C); FAB-MAS: *m*/*z* 507 (M + 1).
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