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THE SYNTHESIS, CO-OLIGOMERIZATION AND HYBRIDIZATION OF A THYMINE-THYMINE HETERODIMER CONTAINING PNA

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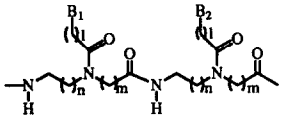
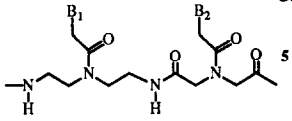
Abstract : A peptide nucleic acid (PNA) decamer in which one of the amide bonds between two units is reversed was prepared and its binding affinity to complementary DNA is shown to be preserved.

Peptide nucleic acids (PNAs, **1**) are oligonucleotide analogs in which the sugar-phosphate backbone is replaced by a backbone consisting of *N*-(2-aminoethyl)glycine units to which nucleobases are attached through methylene carbonyl linkers. We have previously demonstrated the very high binding affinity between such PNA oligomers and single- and double-stranded DNA as well as the sequence specificity of hybridization[1-6]. Thus, PNAs are potential candidates for gene-targeted drugs and for tools in molecular biology and diagnostics[7].



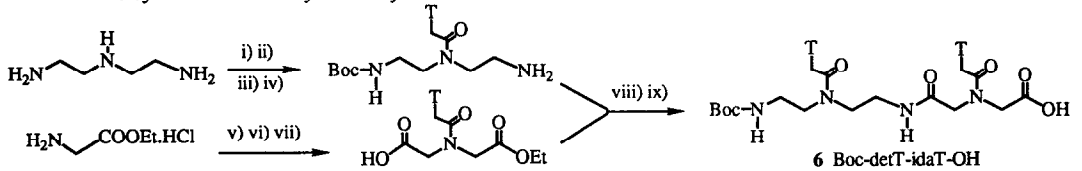
1 n = 1, m = 1, l = 1
2 n = 1, m = 1, l = 2
3 n = 2, m = 1, l = 1
4 n =1, m = 2, l = 1



5 PNA with reversed amide bond

In previous studies on the structure activity relationship of PNA we have shown the importance of having the correct interbase distance[8,9]. For example, when extending the original *N*-(2-aminoethyl)glycine backbone or the linker to the base by one methylene group (**2**, **3** and **4**), the binding affinity was lowered while the sequence specificity was preserved[8]. In order to examine further to which extent the backbone can be modified without destroying the PNA-DNA binding properties, we now report the synthesis of a PNA decamer **5** in which one of the amide bonds between two units is reversed. In this PNA, the interbase distance is the same as in the original PNA oligomer and it is shown by T_m determination that the binding affinity for DNA of this new PNA oligomer is retained (Table 1).

The synthesis of the thymine-thymine heterodimer **6** is outlined below.



6 Boc-detT-idaT-OH

i) *t*-butyl *p*-nitrophenyl carbonate / $CHCl_3$ / 55% ; ii) benzyl *p*-nitrophenyl carbonate / dioxane-water / 59% ; iii) *N*-1-carboxymethylthymine / DCC / DhbtOH / 75% ; iv) H_2 / Pd-C / 100% v) benzyl bromoacetate / triethylamine / EtOH / 39% ; vi) *N*-1-carboxymethylthymine / DCC / DhbtOH / 80% ; vii) H_2 / Pd-C / 100% ; viii) DCC / DhbtOH / 12% ix) 1M NaOH / 86%

Incorporation of **6** into a homopyrimidine PNA decamer was performed by solid phase peptide synthesis as previously described[2-3]. The crude oligomer was purified by preparative HPLC and characterized by FAB mass spectrometry {H-TT(detT-idaT) CCT CTC-Lys-NH_2 : m/z 2747.29 [M+1] (calc 2747.15)}.The thermal stability of the hybrid between this PNA oligomer and the complementary oligodeoxynucleotide was

determined by melting temperature (T_m) measurements (Table 1).

row n°	PNA	DNA	Tm(°C) *		
			pH 5	pH 7	pH 9
(1)	H-TT(detT-idaT)CC TCT C-Lys-NH_2	5'-d(AAA AGG AGA G)†	≥90	55.0	36.0
(2)	H-TT(detT-idaT)CC TCT C-Lys-NH_2	5'-d(GAG AGG AAA A)#	74.0	43.5	31.0
(3)	H-TTT TCC TCT C-Lys-NH_2	5'-d(AAA AGG AGA G)†	≥87	58.5	46.0
(4)	H-TTT TCC TCT C-Lys-NH_2	5'-d(GAG AGG AAA A)#	69.5	40.5	33.5

Table 1 : Melting temperatures (T_m) for PNA-DNA hybrids. * Determined as previously described[2]. The solutions were 10mM in phosphate, 100mM in NaCl and 0.1mM in EDTA. † parallel orientation. # antiparallel orientation.

It was previously shown that homopyrimidine PNAs recognize their complementary DNA or RNA target *via* the formation of a 2:1 complex (as determined by UV titration, CD and LD measurements[5]). Furthermore, unlike DNA, PNA can form hybrids with DNA in both orientations (with the parallel orientation defined as the amino terminal complementary to the 5'-end of the DNA). As previously shown for other sequences, the parallel orientation is the more stable as far as homopyrimidine PNAs are concerned (Table 1 : Row 3). But even the antiparallel orientation (Table 1 : Row 4) is much more stable than the corresponding DNA / DNA hybrid (the T_m of 5'-d(TTT TCC TCT C) / 5'-d(GAG AGG AAA A) is only 29°C). The very high stability of $(PNA)_2$ / DNA hybrids is believed to be due, at least partly, to the neutral nature of the PNA backbone and a triple helix structure stabilized by both Watson-Crick and Hoogsteen base pairing. The presence of 4 cytosine residues in the sequence is responsible for the strong pH-dependency observed in both the unmodified and the modified PNA, since it is known that cytosine needs to be protonated to form Hoogsteen base pairing.

In this report, we have shown that the PNA backbone structure can be modified without disturbing the recognition properties. Basically, by reversing the orientation of a single backbone unit within a decamer PNA, we have made a PNA of inherent dual orientation, and accordingly the thermal stability of the $(PNA)_2$/DNA triplex in the (preferred) parallel orientation is slighty decreased whereas that of the antiparallel orientation is slighty increased. Further structural modifications are currently under way .

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10. Boc- : *tert*-butoxycarbonyl- ; Bn : benzyl ; DCC : N, N'-dicyclohexylcarbodiimide ; DhbtOH : 2,3-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine ; det : diethylene triamine ; ida : iminodiacetic acid

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