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A PNA-DNA LINKER SYNTHESIS OF *N*-((4,4`-DIMETHOXYTRITYLOXY)ETHYL)-*N*-(THYMIN-1-YLACETYL)GLYCINE.

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Abstract: *N*-(2-benzyloxyethyl)-*N*-(thymin-1-ylacetyl)glycine was synthesised and used as a linker for coupling DNA and PNA together. Three different 10-mers composed of 6 DNA units and 4 PNA thymine units were made. The PNA-DNA chimera hybridise to complementary DNA or PNA oligomers, and both the PNA and the DNA part of the chimera are involved in the binding.

Peptide Nucleic Acids (PNA´s) are DNA mimics in which the sugar-phosphate backbone has been replaced with a backbone consisting of *N*-(2-amino-ethyl)glycine units[1]. PNA binds very efficiently and sequence specifically to DNA or RNA[2-4]. Furthermore, we have designed a series of other related PNAs with various modifications in the backbone in order to examine the structure/activity relationship[5,6].

In this paper we describe the solid phase synthesis and preliminary DNA-binding results with a chimera consisting of DNA covalently linked to PNA. Such chimera are of interest, since they might combine the high binding efficiency and specificity of PNA, with the ability of DNA to interact with enzymes for example providing RNase H activation when complexed with mRNA in antisense applications or functioning as a primer for DNA polymerases. The main concern in the design and synthesis of the PNA-DNA chimera, was to find a linker with a suitable distance between the PNA and DNA strands, while still allowing for base stacking between the neighbouring bases in the PNA and the DNA part of the chimera.

As shown in figure 1 incorporation of *N*-(2-hydroxyethyl)-*N*-(thymin-1-yl-acetyl)glycine as the last monomer in the PNA, allows continued synthesis of the DNA part without altering the distance between the bases. This was confirmed by simple model building which showed that this linker should give the correct distance between the bases. The orientation of the chimera is such that in a duplex between the chimera and DNA the preferred anti parallel orientation of both the PNA and the DNA is conserved. The C-terminal of the chimera will be opposite the 5´- of the complementary DNA, and the 5´-end of the chimera will be opposite the 3´-end of the complementary DNA.

N-(2-Benzyloxyethyl)-*N*-(thymin-1-ylacetyl)glycine (**3**) was prepared from 2,2-Dimethyl-4-hydroxy-methyl-1,3-dioxolane (**1**) which was transformed to 2-benzyloxyacetaldehyde (**2**) by a known procedure[7]. The aldehyde was then reductively aminated with methyl glycinate followed by coupling of

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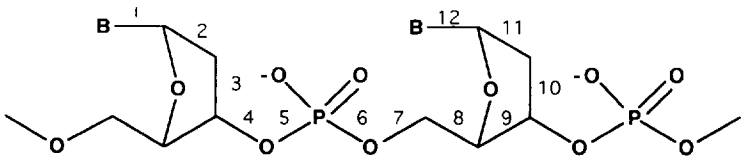
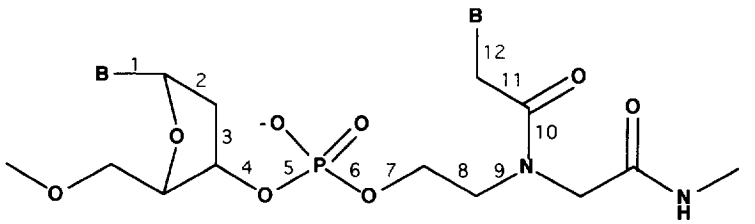


DNA



PNA-DNA

Figure 1. Comparison of the DNA phosphodiester and PNA-DNA chimera junction with the proposed linker.

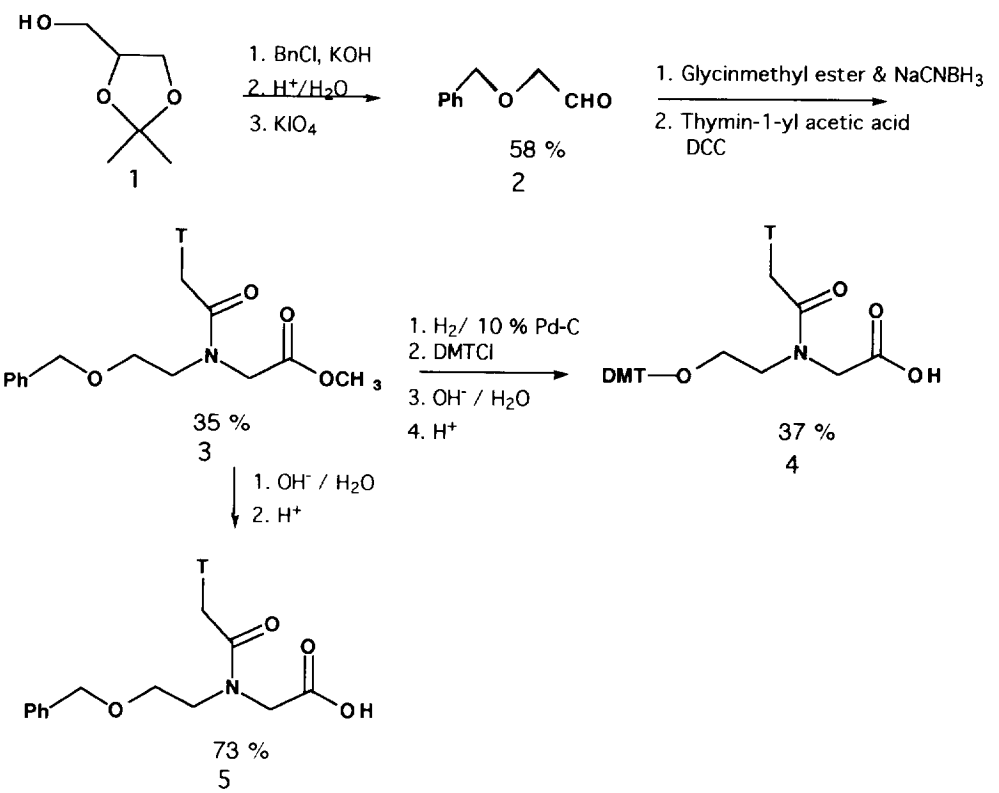


Figure 2. The synthesis of the PNA-DNA linker

thymin -1-ylacetic acid to the secondary amino group. Hydrolysis of the methyl ester (**3**) yielded the desired monomer (**5**). The monomer was incorporated at the end of a PNA 9-mer using standard PNA synthesis conditions[8]. The benzyl protection group is removed during the cleavage from the resin leaving the free hydroxy group. The PNA oligomer was purified by RP-HPLC and the identity confirmed by MS-FAB$^+$. However, the solubility of the PNA 9-mer in organic solvents was to low for the coupling of amidites to the hydroxy group.

This led us to a change in strategy, *i.e.*, to make the chimera by solid phase synthesis. However, this imposes some restrictions on the synthesis. The PNA part has to be made first which necessitates that the benzyl protection group is changed to 4,4`-Dimethoxytrityl (DMT). This was accomplished by hydrogenation of **3** to *N*-(2-hydroxyethyl)-*N*-(thymin-1-ylacetyl)glycine, followed by reaction with DMTCl and hydrolysis of the ester to give the corresponding DMT protected monomer (**4**). No reduction of the double bond in thymine was observed under the conditions of the hydrogenation as previously published[9].

The Z-group that is used in PNA oligomer synthesis for protection of cytosine, guanine and adenine is removed by strong acids such as HF or TFMSA. This treatment would degrade the DNA and we therefore chose at this stage to let the PNA strand consist of thymines only.

The polystyrene resin normally used for PNA synthesis was exchanged with CPG to allow the synthesis of the DNA part on a automated DNA synthesiser. MBHA could not be used as the linker to the resin, because the harsh acid treatment nessesary to cleave the MBHA linker depurinates DNA. The PNA-DNA chimeras was synthesised using controlled pore glass (CPG) with 4-hydroxymethylbenzoic acid as the link between the CPG and the chimera. The chimera were cleaved from the CPG by treatment with 0,1 M NaOH. The benzoyl protection groups on dC was removed by treatment over night with concentrated aq. ammonia at 55 °C.

Table 1. T_m´s for the complexes of the 2 chimeras containing only thymine with complementary DNA in comparison with the T_m of the corresponding DNA/DNA and PNA/DNA complexes. All experiments were carried out in 1 M NaCl, 10 mM Na-phosphate and 0.1 mM EDTA at pH 7 (high salt).

	5`-d$T_6$-3`-T_4-Gly-OH	3`-d$T_6$-5`-T_4-Gly-OH	PNA T_{10}	DNA dT_{10}
dCdGdCdA_{10}dCdGdC	~15°	~12°	75°	32°

The 10-mer containing only thymines was synthesised with the DNA part in each of the two possible orientations, to determine if our orientation of the DNA part in relation to the PNA part of the chimera was correct[10,11]. The results indicate that the "head to tail" orientation (3´-end linked to the "N"-terminal of the PNA) maybe slightly preferred. However the thermal stability of the complexes between the chimeras and the complementary DNA are significantly lower than that of the corresponding DNA duplex. A possible explanation could be that only the PNA part or the DNA part of the chimera had hybridised to the target DNA. However, we could not detect the formation of any complex with DNA targets containing a mismatch (a guanine) in position 4 or 5. Furthermore incorporation of cytosine in the DNA part[10] gave a higher T_m (table 2), and no complexes could be detected by thermal denaturation between either the PNA

part (H-T_4-Gly-NH_2) or the DNA part (dCdTdCdTdC$_2$) alone, or both combined, and the dA_{10} target, thus we conclude that both the DNA and PNA part of the chimera are involved in target binding.

Changing the pH from 5 to 9 had almost no effect on the T_m(table 2), indicating that the DNA part is hybridising as a duplex. However titration of the complex at pH 5 shows a 2:1 ratio of chimera to DNA (Figure 4). We believe that this apparent discrepancy can be explained if only the PNA part of the chimera participates in the triplex. This may also explain why the the melting curves for the chimeras were somewhat broader than those of the DNA/DNA or PNA/DNA complexes (Figure 3).

Table 2. T_m's for the cytosine containing chimera. The chimera generally gave rather broad melting curves (see Figure 3). All measurements done in high salt.

		dCdTdC dTdC$_2$ dT$_4$	H-CTC TC$_2$ T$_4$-LysNH$_2$	5'dCdTdCdT dC$_2$-3'-T$_4$Gly-OH
DNA	pH 5	41°	61°	~26°
dA$_4$dG$_2$dAdGdAdG	pH 7	43°	35°	~26°
	pH 9	43°	~35°	~24°
PNA: A$_4$G$_2$AGAG	pH 7	70°		59°
GAGAG$_2$A$_4$	pH 7	56°		45°

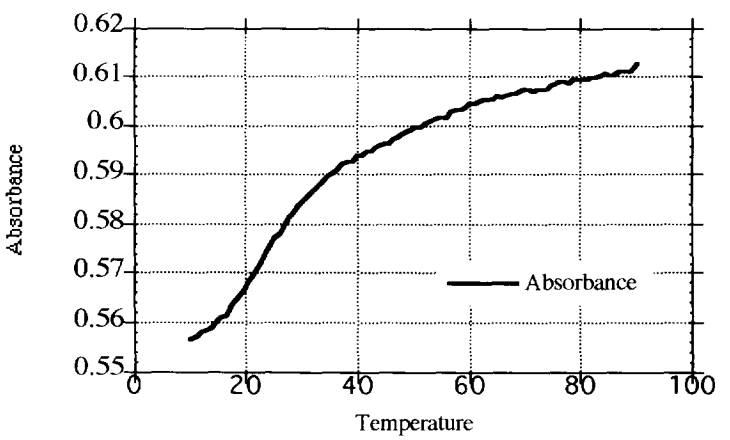


Figure 3. Thermal denaturation of dA$_4$dG$_2$dAdGdAdG with 5'dCdTdCdTdC$_2$-3'-T$_4$Gly-OH (T_m= 26 °C). OD's where calculated according to ref. 12.

The chimera also bound quite efficiently to a complementary PNA and as expected the anti parallel complex showed the highest thermal stability. The stability of the chimera/PNA complexes are also relatively higher than those of the chimera/DNA as compared to the corresponding DNA/PNA and DNA/DNA complexes. This probably reflects the greater flexibility of the PNA backbone, allowing it to better accommodate the PNA-DNA junction.

In conclusion the results show that PNA-DNA chimera in which both parts are involved in hybridisation complementary DNA can be made. However, these chimeras hybridize less efficiently to complementary DNA than the parent DNA oligomers indicating that the PNA-DNA 3'-junction is not structurally optimal despite a favourable configuration by model building. The explanation could be that although the PNA and DNA backbones are homomorphically related they need not be isomorphically so, i.e. they are positioned differently in space relative to the nucleobases. In fact, structural information from ^{1}H-NMR studies (unpublished) have hinted that PNA/DNA duplexes have a more narrow minor groove than DNA/DNA duplexes. Thus detailed structural data on PNA/DNA duplexes should be very helpful to optimize the 3'-DNA-PNA linker. Further work will show if this is possible and will also reveal the biological properties of the PNA-DNA chimeras. The chimeras made in this study consist entirely of pyrimidines, but work is in progress to extend these results to mixed sequences.

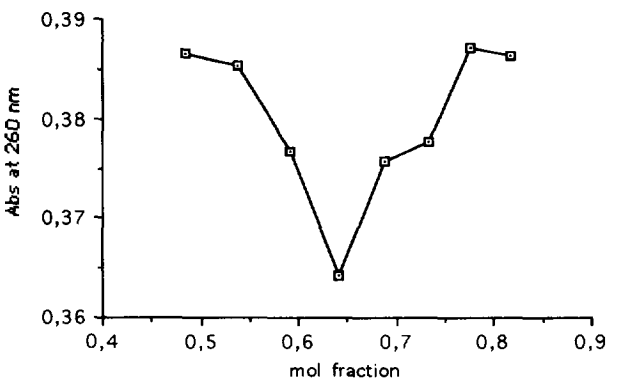


Figure 4. Titration of $dA_4dG_2dAdGdAdG$ with 5'dCdTdC dC_2-3'-T_4Gly-OH. OD's where calculated according to ref. 12.

Acknowledgement

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