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Phe and Asn Side Chains in DNA Double Strands

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Abstract—The contribution of amino acid side chains to the recognition of DNA by peptides or proteins is evaluated by substituting single nucleobases of a DNA double strand by amino acid side chains. *C*-nucleosides with the side chains of phenylalanine and asparagine were synthesized and incorporated in DNA. This modification should allow to keep the double strand conformation. Hydrogen bonds, π–π-interactions and solvation have an influence on the double strand stability.

The recognition of DNA double strands by proteins or peptides involves amino acid side chain interactions with the nucleobases. Binding to DNA can be based on hydrophobic or electrostatic contributions for non-specific interactions, whereas hydrogen bonds and aromatic interactions are responsible for sequence dependent recognition.[1] For a better understanding of the nature and specificity of these amino acid side chain interactions[2] experimental evidence is needed.

DNA binding in the major or minor groove or by intercalation is usually accompanied by conformational reorientation of the double helix. Therefore, it is difficult to isolate the individual amino acid side chain contributions to binding. Positioning amino acid side chains in the base stack of the double helix, in place of a native DNA nucleobase, yields an opportunity to observe interactions between peptide side chains and neighboring nucleobases without disturbing the helix topology. Two examples for incorporated amino acid side chains in the base stack of DNA are described. Instead of a regular nucleotide, *C*-nucleotides with the desired side chain at the anomeric center were incorporated in DNA (Fig. 1).

These experiments were further encouraged by results obtained by the linear, rigid, and well-defined model system of alanyl peptide nucleic acids.[3] The alanyl-PNA double strands are based on a regular peptide backbone with alternating configuration of alanyl nucleo amino acids. Within this system the substitution of guaninylalanine by a phenylalanine or tryptophan has a stabilizing effect, despite the missing hydrogen bonds with the complementary cytosine.[4] This unexpected stabilization is likely to be the result of the reduction of unfavorable aromatic-water interactions, which also depend on the double strand topology. In linear alanyl-PNA the

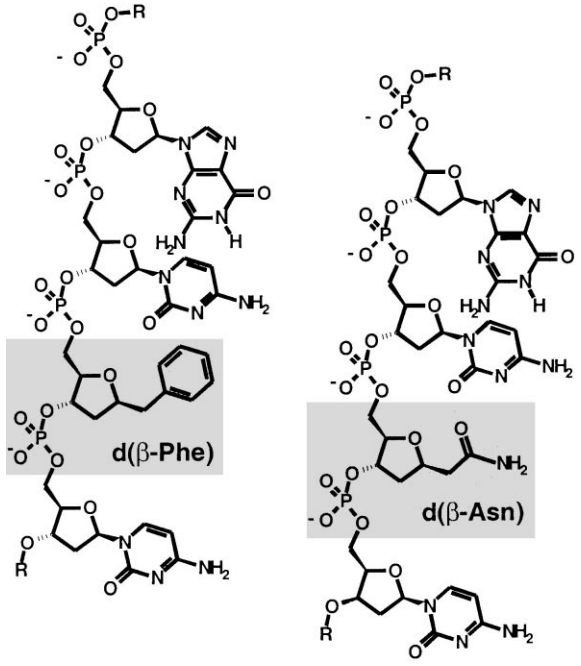

Figure 1. DNA oligomers with *C*-nucleosides having a benzyl d(β-Phe) or aminocarbonylmethyl residue d(β-Asn) at the anomeric center.

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nucleobases or amino acid side chains are less water accessible than in helical DNA. Further evidence for the influence of solvent accessibility is based on the PNA (phenyl) amino acid (Fig. 2) with a phenyl side chain incorporated in a helical PNA–DNA duplex which has also a destabilizing effect.[5]

Many *C*-nucleosidic DNA-modifications are known already.[6] Most of them contain an arene directly bound to the anomeric center in analogy to the binding of the nucleobases (Fig. 2).[7] Usually, the arene mismatch destabilizes the DNA double strand, except when positioned at the end of the sequence.[8] Larger aromatic systems like d(pyrene) only show a small mismatch effect because of a higher stacking contribution.[10] Furthermore, a homoglutamine side chain incorporated covalently to the anomeric center also induces a significant destabilization of the duplex.[9]

To begin our examinations, *C*-nucleosides with a benzyl side chain (d(Phe)) and an aminocarbonylmethyl residue (d(Asn)) linked to the anomeric center were synthesized. Both were prepared as the α- and β-anomers because the different orientation of the side chain was expected to have a significant influence within the double strand topology. They were incorporated into DNA oligomers as phosphoramidites **α/β-1** (Fig. 3) and **α/β-2** (Fig. 4) by solid phase synthesis on a CPG-resin using standard DNA chemistry. The esters **α-2** and **β-2** were used for the synthesis of DNA oligomers, but it was shown that they were converted to the amides d(Asn) under the deprotection conditions of the oligomer (NH_3, H_2O, 55 °C, 16 h).

Synthesis of the Phe and Asn Nucleotides

The synthesis of d(Phe)-epimers **α-1** and **β-1** required a benzyl side chain at the anomeric center. It was coupled using a Grignard reaction with ribosylchloride **3**[11] (Fig. 3).

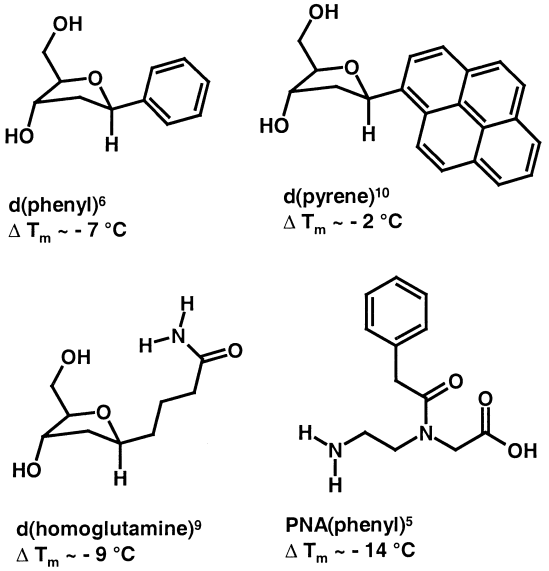

Figure 2. Examples for nucleoside analogues incorporated already in DNA–DNA or DNA–PNA double strands.

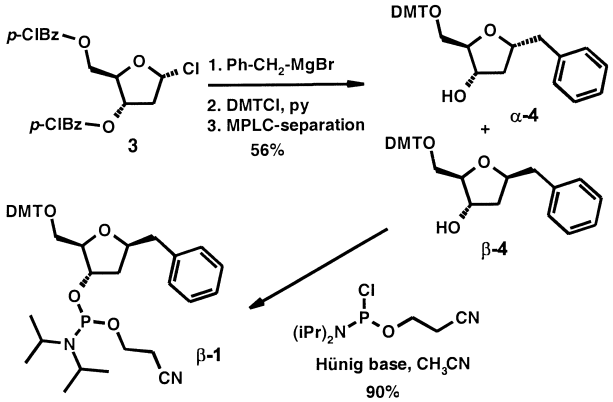

Figure 3. Synthesis of the Phe-Nucleoside **β-1**; d(α-Phe) nucleotide **α-1** was obtained in analogous manner from **α-4**.

Deprotection of the *p*-chlorobenzylesters was performed with an excess of the Grignard reagent. The α/β-epimers were obtained in a 2:1 ratio and separation of the epimers was provided by MPL chromatography of the trityl derivatives **α-/β-4** on silica gel with hexane/ethylacetate. The assignment of the epimers was performed with 2D-NMR spectroscopy.[12] From the nucleosides **α-4** and **β-4** the phosphoramidites **α-1** and **β-1** were easily obtained with 2-cyanoethyl *N,N*-diisopropylchloro-phosphoramidite.[13]

For the synthesis of the phosphoramidites **α-2** and **β-2**, leading to the d(Asn)-nucleotides in the oligomer, a procedure for the allylation of the anomeric center of acetyl-protected ribosylchloride **5** was used (Fig. 4)[14] followed by separation of the 3:1 ratio of epimers **α-6** and **β-6** by MPL chromatography. The configurations were assigned using 2D-NMR-spectroscopy.[15] The terminal double bond was oxidized with $KMnO_4$ under phase transfer conditions followed by methylation to the esters **α-7** and **β-7**. Selective deprotection of the hydroxyl groups was performed with ammonia in methanol at room temperature. At this point, formation of the amide was avoided because phosphitylation of the amide would take place as a side reaction in the final transformation to the phosphoramidites **α-2** and **β-2**. Therefore, **α-2** and **β-2** were obtained by DMT-protection of the primary hydroxyl group followed by phosphitylation.[16]

DNA Double Strands with Phe and Asn Nucleotides

Hybridization of the DNA oligomers was performed in 0.1 M NaCl, 0.01 M Na_2HPO_4/H_3PO_4, pH 7. Their stability was evaluated by thermal denaturation experiments using UV-spectroscopy. The double strand stabilities were indicated by the sigmoidal increase of absorption caused by the cooperative destacking of nucleobases. Pairing and depairing of all the double strands described was reversible. The self-pairing DNA octamer d(GCG-CGCGC) forms a double strand ($T_m = 60$ °C) with eight G–C Watson–Crick base pairs and was used as the reference. In the mismatch studies, either the central cytosinyl or the respective guaninyl nucleotide were substituted by the d(α/β-Phe) or

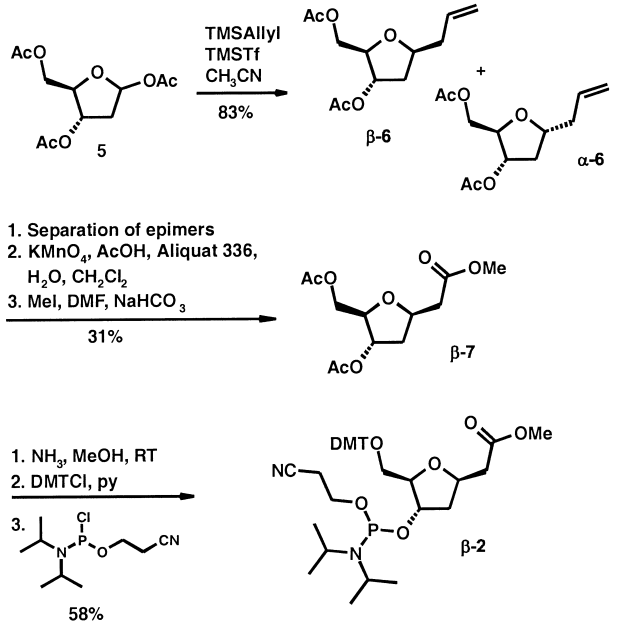

Figure 4. Synthesis of the nucleoside **β-2** leading to d(β-Asn): The ester β-2 will be converted to the amide under deprotection conditions of the oligomer. The precursor for d(α-Asn)nucleotide **α-2** was obtained in analogous manner from **α-7**.

d(α/β-Asn) *C*-nucleotides.[17] Further oligomers containing an abasic deoxy-ribose (d(Gly) represents a glycine side chain) as a mismatch were used for comparison. The stabilities for antiparallel self pairing double strands are summarized in Table 1. All mismatch sequences contain two neighboring defects.

The two abasic sites in the d(Gly) oligomers lower the stability by 14 °C in case of the purine rich duplex **9**, and by 20 °C when guanine is eliminated (**8**). In contrast to the result in alanyl-PNA, a benzylgroup destabilized a DNA double strand even more than the abasic site. The d(Phe) modified oligomers with β-configuration **12** and **13** are 5 °C more stable than the sequences containing the α-epimers **10** and **11**. The destabilizing effect is more relevant with the benzylgroup between the larger guanines. Through simple model studies we suggest a sterical conflict is responsible for this destabilization, since the only possibility to place the phenylring within the nucleobase stack of B-DNA requires ecliptic bonds at the anomeric center.[18] The CD-spectra of all oligomers are quite similar. Therefore, it seems unlikely that the *C*-nucleotides disturb the overall helix conformation.

Table 1. Stabilities of DNA double strands with benzyl d(Phe) and aminocarbonylmethyl d(Asn) mismatch compared to regular DNA sequences and abasic d(Gly) mismatch double strands

Mismatch	Sequences	
	d(GCGCGCGC) T_m = 60 °C (8% H[a], 7 μM)	
	d(GCGCXCGC)	d(GCGXGCGC)
d(Gly)	T_m = 40 °C (**8**, 9% H, 9 μM)	T_m = 46 °C (**9**, 9% H, 9 μM)
d(α-Phe)	T_m = 35 °C (**10**, 3% H, 6 μM)	T_m = 30 °C (**11**, 9% H, 9 μM)
d(β-Phe)	T_m = 40 °C (**12**, 5% H, 7 μM)	T_m = 35 °C (**13**, 6% H, 8 μM)
d(α-Asn)	T_m = 40 °C (**14**, 9% H, 7 μM)	T_m = 46 °C (**15**, 9% H, 7 μM)
d(β-Asn)	T_m = 46 °C (**16**, 9% H, 7 μM)	T_m = 40 °C (**17**, 8% H, 8 μM)

[a]H = hyper-chromicity, conditions: 0.1 M NaCl, 0.01 M Na_2HPO_4/H_3PO_4, pH 7.

As indicated by the lower destabilization of the oligomers **14**–**17** the d(Asn) nucleotides fit better into the B-DNA helix topology. On the other hand, stabilization by hydrogen bonds seems unlikely since double strand stabilities are comparable to the sequences with abasic sites. Only the slightly more stable oligomer **16** might be able to form hydrogen bonds with the Asn-side chain.[19]

DNA modification with the *C*-nucleotides d(Phe) and d(Asn) turned out to destabilize the double strands similar to, or even more than, an abasic site. Other nucleotide derivatives are currently under investigation in order to obtain a DNA double strand with preference for Z-DNA conformation. Guanosine isosters imitating the *syn* conformation should be promising. As a final comment, we note that knowledge about the influence of anomeric side chains on the DNA double strand may have implications in replication studies and the design of a universal nucleobase.

Acknowledgements

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13. Analytical data for **α-1**: ^{1}H NMR (CD_2Cl_2): 1.02–1.18 (m, 12H, *i*Pr), 1.84 (m, 1H, H2′, H2″), 2.04 (m, 1H, H2′, H2″), 2.43 (t, 1H, $J=6$ Hz, CH_2CN), 2.53 (t, 1H, $J=6$ Hz, CH_2CN), 2.91 (m, 2H, CH_2-Ph), 3.08 (m, 2H, H5′, H5″), 3.42–3.75 (m, 4H, *i*Pr, CH_2-CH_2-CN), 3.78 (s, 3H, OMe), 3.79 (s, 3H, OMe), 4.02 (m, 1H, H4′), 4.33–4.40 (m, 2H, H1′, H3′), 6.86 (m, 4H, DMT), 7.15–7.38 (m, 12H, DMT, Ph), 7.42–7.50 (m, 2H, DMT). ^{31}P NMR (CD_2Cl_2): 148.2 (both diastereomers). ESI-MS: 733.1 $(M+Na)^+$. **β-1**: ^{1}H NMR (C_6D_6): 0.96–1.20 (m, 12H, *i*Pr), 1.78 (m, 1H, H2′, H2″), 2.22 (m, 1H, H2′, H2″), 2.28 (t, 1H, $J=5$ Hz, CH_2CN), 2.46 (t, 1H, $J=5$ Hz, CH_2CN), 2.80 (m, 1H, H5′, H5″), 2.97 (m, 2H, CH_2-Ph), 3.09 (m, 1H, H5′, H5″), 3.40–3.56 (m, 4H, *i*Pr, CH_2-CH_2-CN), 3.70 (2s, 6H, OMe), 4.15 (m, 1H, H4′), 4.32 (m, 1H, H1′, H3′), 4.42 (m, 1H, H1′, H3′), 6.67 (m, 4H, DMT), 7.05–7.20 (m, 12 H, DMT, Ph), 7.34–7.38 (m, 2H, DMT). ^{31}P NMR (C_6D_6): 145.0 (both diastereomers). ESI-MS: 733.1 $(M+Na)^+$.
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