Roberto Roda Bravo (Autor)

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1. Introduction and objectives. Part A

Alternative synthesis of an isoguanine alanyl-peptide nucleic acid monomer and study of its pairing properties

Alanyl-PNA (Peptide Nucleic Acid) is based on a regular peptide backbone with alternating configuration of the amino acids and covalently linked nucleobases as side chains. The distance between side chains in peptides with β-sheet conformation is similar to the favoured base-pair stacking distance in B-DNA. Therefore, alanyl-PNA provides self-pairing linear double-strands where base pairs are formed mostly by recognition based on H-bonding. The helix topology in B-DNA allows only the Watson-Crick pairing mode between complementary strands whereas linear double-strand topology does not restrict base-pair size and geometry. Therefore, all purine-purine, purine-pyrimidine, and pyrimidine-pyrimidine combinations are allowed with all pairing modes. This property makes the alanyl-PNA an adequate system to study the factors involved in nucleobase recognition.[1] The stability for the tridentate G-C pair in hexameric double strands is much higher (T_m = 58°) when compared with the stability of hexameric double strands based on a twodentate base pair (T_m = 21 - 32°). This makes possible a base-pair complementarity in linear double strands when considering three H-bonds base pairs. The base pairs of 2,6-diaminopurine (D) with xanthine (X) and of isoguanine (I) with guanine (G) can be built over tridentate H-bonds. In previous work of the group, the pairing properties of 2,6-diaminopurine, xanthine and isoguanine in an alanyl-PNA-system have been studied with the future objective of building an information system based on G-, I-, X- and D- containing alanyl-PNA. If as expected, tridentate pairing is favoured in the double strand formation, the possibility of an analogous information system to B-DNA stays open (Figure 1).[2,3,4]

In this previous work, the oligomer H-(AlaI-<u>AlaG</u>-AlaI-<u>AlaG</u>)-Lys-NH_2 [1] showed a increased stabilized melting temperature that could be explained through an increased base stacking and three hydrogen bonds per base pair.[4] A larger system for a deeper study of the pairing properties of isoguanine in the alanyl-PNA system and the possible

[1] AlaI = β-(isoguanine-9-yl)alanine, AlaG = β-(guanine-9-yl)alanine; D-configured nucleo-amino acids are underlined.

stabilization through base stacking was needed, so the synthesis of the hexamers containing guanine and isoguanine nucleobases was planned.

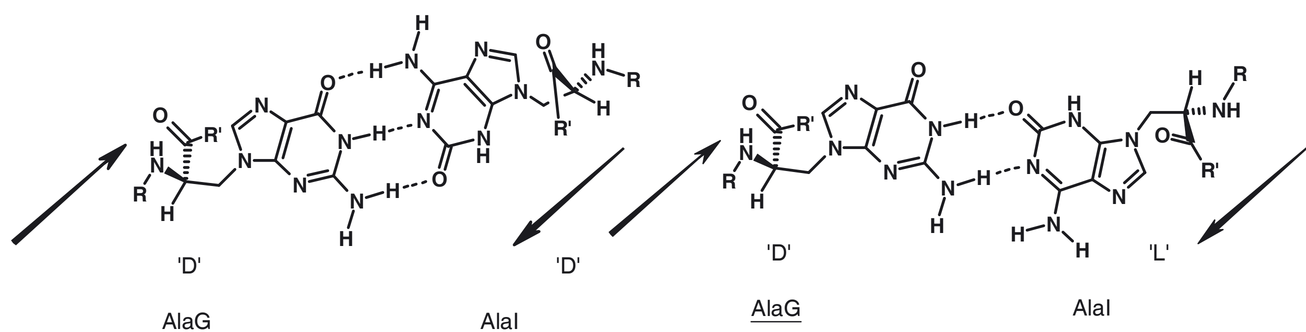


Figure 1: Expected pairing modes in the system Guanin-Isoguanin. Strand orientation is signalled by the arrows.

The standard enantioselective synthesis of the protected purinyl amino acid *N*-Boc-β-(purine-9-yl)-alanine in L and D form is usually carried out through nucleophilic ring opening of *N*-Boc-β-L-serinelactone with adequate purine bases. However, an alternative way was being developed in the group to avoid the separated synthesis of the L and D forms.[5] The synthesis of β-(isoguanine-9-yl)-alanine should succeed through a racemic synthesis, taking profit of the specificity of enzymes in reacting with different enantiomers.[6] After the enzymatic separation of the racemic product, the protection of the α-amino group should yield the *N*-Boc-β-(isoguanine-9-yl)-alanine in L- and D- form.

2. Alanyl-PNA

2.1 Peptide Nucleic Acids (PNA)

Over the past decade, there has been considerable interest in the development of synthetic structures that specifically recognize and bind selectively to DNA and RNA sequences for use as diagnostic or therapeutic agents.[7, 8, 9] PNA is an analogue of DNA and RNA in which the natural scaffold of sugars linked by phosphates is replaced by (2-aminoethyl)-glycine units linked to nucleobases by acyl groups (Figure 2).[10] This so called Nielsen-PNA contains the same number of backbone bonds between the bases and the same number of bonds from the backbone to the base as in DNA.[11] Aminoethylglycine-PNA binds with high affinity and sequence specificity to complementary RNA, DNA or PNA and exhibits sequence discrimination equal to or better than DNA. Furthermore, the resulting duplexes show higher thermal stability than the corresponding DNA duplexes at physiological ionic strength as there is no charge repulsion present in the backbone and high biological stability due to the absence of specific proteases.[10]

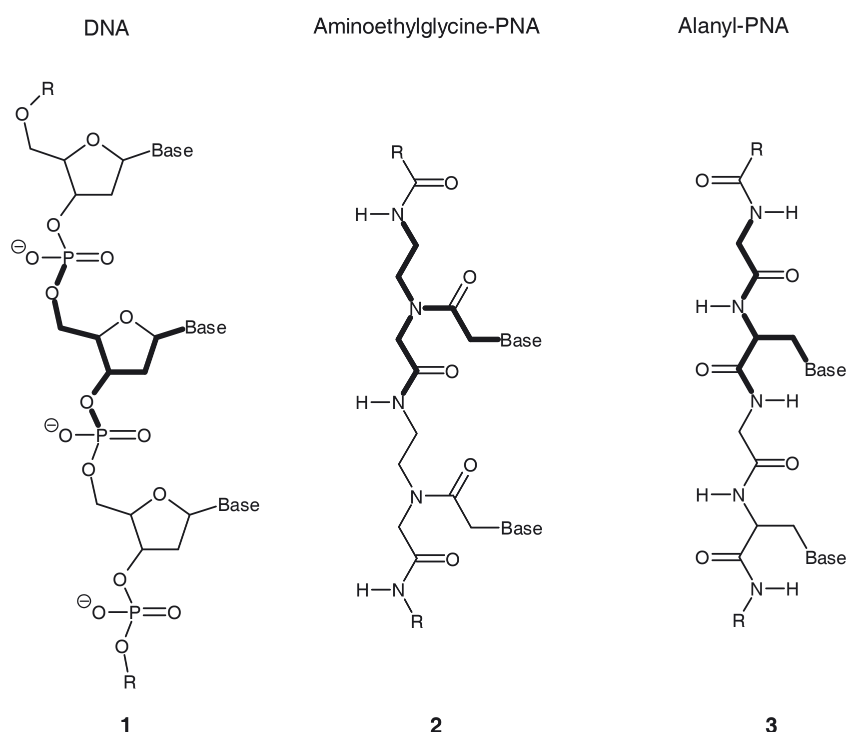


Figure 2: DNA **1** aminoethylglycin-PNA **2** and alanyl-PNA **3**. The isosteric backbone it is emphasized in bold. “Base” represents any nucleobase.

The substitution of the sugar backbone moieties by two amino acids maintains an isomorphous backbone to DNA. Based on this concept alanyl-PNA was designed alternating a glycine with a nucleobase substituted amino acid (Figure 2).[12] However, alanyl-PNA does not interact with DNA or RNA. Possibly due to the additional amide bond per monomer, when compared with aminoethylglycine-PNA, reducing the conformational possibilities to adapt to DNA or RNA conformation.[13,14] The distance between nucleobases in B-DNA is 3.4 Å. In a peptide chain with β-sheet conformation, the distance between side chains is 3.6 Å, similar to the adequate distance for base stacking interactions.[15] An alanyl-PNA with β-sheet conformation and with all side chains replaced by nucleobases fulfils all the requirements for building a linear double strand. When all amino acids have the same configuration, the nucleobases are antiperiplanar to each other. Using an alternating configuration the nucleobases point to the same side of the β-sheet like structure (Figure 3).

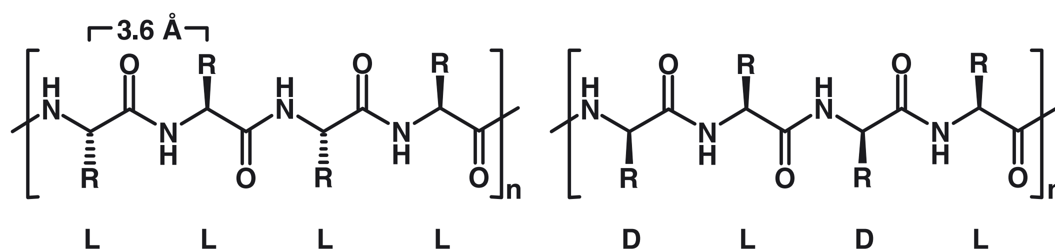


Figure 3: Linear peptide strand with constant and alternating chirality.

Due to the rigidity of the system the formation of the double strand leave the nucleobases in an almost orthogonal position to the backbone. This geometric system has a simpler topology than DNA providing a useful model for the study of molecular recognition, electron-transfer processes and the contribution of the hydrogen bonds to the stability of the double strand.[16,17]

2.2 Pairing selectivity

Because of the helix topology in B-DNA only the Watson-Crick pairing mode between complementary strands is possible (Figure 4). In the alanyl-PNA system there are more parameters that influence the pairing selectivity. Specifically, pairing mode, amino acid configuration and strand orientation play an important role in the linear double strand formation. In alanyl-PNA purine-purine or purine-pyrimidine pairing can take place, whereas in B-DNA only the unequivocal pairing between adenine-thymine and guanine-cytosine is possible.

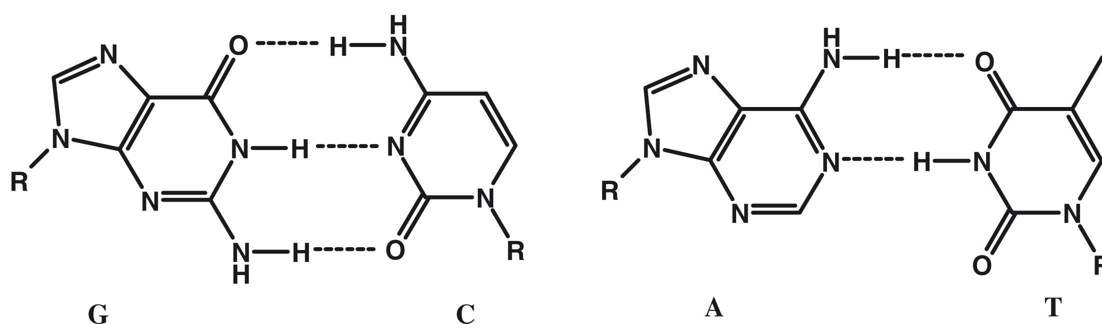


Figure 4: Watson-Crick pairing mode in B-DNA.

With purine bases in addition to the Watson-Crick pairing mode, *reverse* Watson-Crick, Hoogsteen and *reverse* Hoogsteen pairing modes are possible (Figure 5).

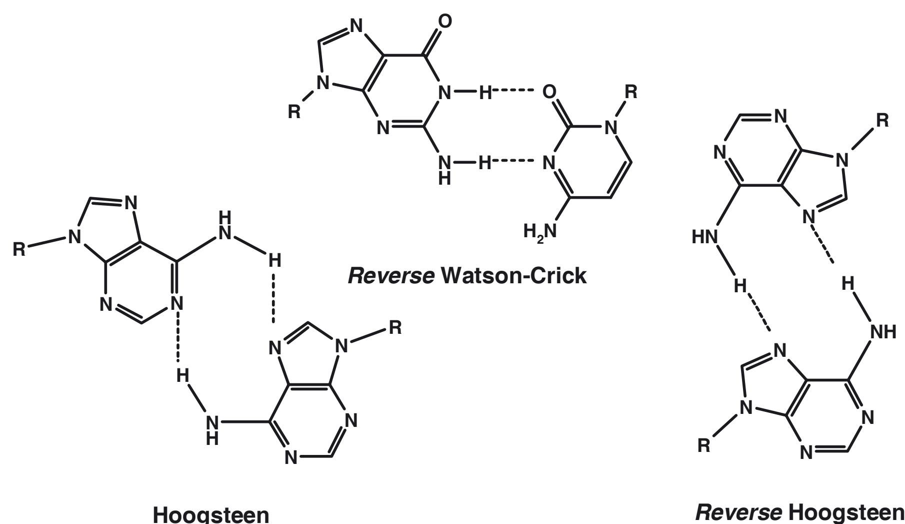


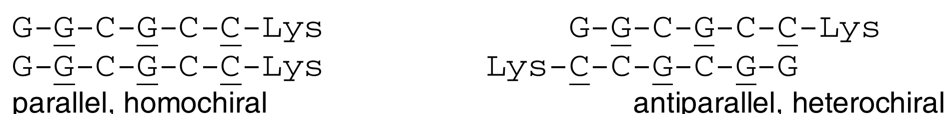
Figure 5: *Reverse* Watson-Crick pairing mode between G and C and the Hoogsteen and *reverse* Hoogsteen pairing mode between A-A.

In the Hoogsteen pairing mode, the N^7-atom of a purine base works as acceptor in the hydrogen bond formed with a H-donor of another nucleobase. In the reverse modes one of the bases is mirrored.

As peptides are oriented from the N- to the C- terminus, parallel or antiparallel orientation of the backbone in the formation of the linear double strand is possible. The alternating configuration makes possible heterochiral and homochiral pairing between nucleobases possible.[18]

The simultaneous change of the strand orientation and configuration of all amino acids results in no change of the geometry of the side chains. The consequence of the *Retro-Inverso*-principle is that, without influencing the secondary structure of the peptide, the orientation of one strand can be inverted (Figure 6).

Self pairing:



Enantiomeric pairing:

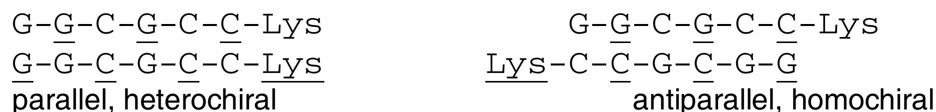


Figure 6: Pairing possibilities of the oligomer H-(AlaG-<u>AlaG</u>-AlaC-<u>AlaG</u>-AlaC-<u>AlaC</u>)-Lys-NH_2 (C = β-(cytosine-9-yl)alanine, G = β-(guanine-9-yl)alanine; D-configured nucleo amino acids are underlined) with itself and its enantiomer.

Lysine was built in because of increased PNA solubility. Because of the charge repulsion at pH 7 the presence of lysine favours the antiparallel orientation without disturbing the orthogonality of the nucleobases relative to the backbone. YYZYZZ sequences with a preference for the Y-Z pairing build antiparallel double strands. Therefore, self pairing strands give heterochiral pairing and the addition of the enantiomer yields homochiral Y-Z base pairing. Previous work has shown that for the G-C pairing in an antiparallel,

homochiral double strand only the Watson-Crick mode is possible and the *reverse* Watson-Crick in case of the antiparallel, heterochiral base pairing.[18]