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Synthesis and study of new PNA monomers and P-chiral mononucleotides

Ph.D. Thesis

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2009
Introduction and aims

Incorporation of C5-alkynyl- or heteroaryl-pyrimidine bases into oligonucleotides (ODN) enhances the stability of the DNA:DNA and DNA:RNA duplexes. It can be attributed to the increased stacking interactions. Peptide nucleic acids contain N-(2-aminoethyl)-glycine backbone instead of sugar-phosphate skeleton of the natural oligonucleotides. PNA:DNA and particularly the PNA:RNA duplexes are considerably more stable than the corresponding DNA counterparts. In addition PNA is quite resistant to peptidases and proteases.

On the basis of the thorough NMR analysis of natural dinucleotides it is known that the conformational preferences around the C3’-O3’ bond can be well-characterized by the $^3J(P, C4')$ and $(P, C2')$ vicinal coupling constants. The $\Delta J = ^3J(P,C2')- ^3J(P,C4')$ value unambiguously correlate with the preponderance of the trans or gauche conformers in the conformational equilibrium. The positive $\Delta J$ values are characteristic to the dominance of $\epsilon^t$ (trans) while the negative ones to that of the $\epsilon^g$ (gauche$^-$) conformers. In addition, these values are also correlated with the absolute P-configuration.

During my Ph.D. work I aimed to synthetize new PNA monomers containing 5-substituted pyrimidine bases (a), then to synthetize and study the structure of P-chiral-mononucleotides (b).

a) Considering that stability of PNA:DNA but especially the PNA:RNA duplexes is higher compared to that of the DNA counterparts thus incorporation of the 5-heteroaryl or 5-alkynyl pyrimidine bases into homogenous PNA is expected to result in similar or even higher duplex stabilization. On hand I intended to incorporate into PNA such new uracil 5-substituents which were not even tested as DNA modifications earlier, on the other hand some known modifications such as 5-(1-propynyl)- and 5-(2-thienyl)-uracil) were also planned to incorporate in order to compare their $T_m$ values with the corresponding DNA probes.

b) Oxidation of different P(III) compounds (phosphite triesters, phosphoramidites) into the corresponding P(V) derivatives can be accomplished by the application of a wide variety of oxidizing agents which basically determine the stereochemical course of these reactions. I wished to investigate the effect of 3 heteroatoms (O, S, and Se), double-bonded to the phosphorus, to the conformational equilibrium. Since the size as well as the electronegativity of these heteroatoms differs it surely influences the difference between the $\Delta J$ values of the individual P-diastereomers. In addition I also wished to study the chromatographic behaviour
(normal and reverse phase HPLC) of the compounds in order to establish a correlation between the absolute P-configuration and the $t_R$ values.

**Results and Conclusions:**

1. Ethyl N-(2-Boc-aminoethyl) glycinate (3) is an intermediate of peptide-nucleic acids. More efficient synthetic route was developed. Boc-ethylenediamine (2) was isolated hereby purification of (3) was easier.

2. 5-iodo-uracil PNA monomer (7), raw material of the palladium-catalysed cross coupling reactions, was synthesized from 5-iodo-uracil (4) in three steps.

3. Coupling of the base-unprotected 5-I-U PNA monomer with aryl-tributylstannanes (Stille couplings) afforded the new 5-aryl analogues with good yields.

4. Copper (I) and palladium catalysed cross-couplings (Sonogashira couplings) were implemented from (7) with acceptable overall yields, due the amount of unrequired by-products, caused the formation of ring-closure. Suzuki couplings were failed when unprotected starting material (7) was used. The protection of the lactam function was necessary, therefore N³-PMB-protected monomer (17) was synthesized.
5. Starting from the N³-PMB-protected monomer (17), the required new 5-alkynyl derivatives (18 a-c), as sole products, were isolated by nearly quantitative yields (Sonogashira couplings).

6. From acceptable to good yields were attained in the couplings of the N³-PMB-protected monomer with different aryl-boronic acids (Suzuki couplings). The new 5-(2-thienyl)-, 5-(4-biphenyl)-, 5-(benzo[b]thiophene-2-yl)- and 5-(4-dimethylaminophenyl)-uracil PNA monomers (19 a-d) were synthesised. The efficiency of couplings further increased when boronic acid pinacol esters were used instead of the free acids.
7. Boc deprotection was observed in the cleavage of PMB group. New method of Suzuki coupling of 5-iodo-uracil derivates was developed using in situ silyl protection.

8. New method was developed for synthesis of cytozine PNA monomer. 4-triazolopyrimidinone derivatives (29) was isolated and the uracil – cytozine conversion was successful.

9. \( R_p \) and \( S_p \)- diastereomers of 5’-dimethoxytrityl-thymidine-3’-O-[O-(2-cyanoethyl)-\( N,N \)-diisopropyl]-phosphoramidite (T-CED) were separated by silica gel chromatography. Oxidation of both isomers resulted in the corresponding oxidized analogues by nearly quantitative yields. All reactions were found to proceed with retention of \( P \)-configuration.
10. Retention was confirmed by thorough NMR analysis which, in addition, aimed to study the spectral properties of the diastereomers with a special respect to differences in the heteroatom effect of the O, S and Se atoms, double-bonded to the phosphorus, on the vicinal carbon-phosphorus couplings. It was found that the changes in the $\Delta J (=^3J(P,C4') - ^3J(P,C2'))$ values were basically induced by the electronegativity of the heteroatoms, rather than differences in the rotational preferences about the C3'-O3' bond.

11. The behaviour of the compounds was also investigated by chromatography. The reverse phase HPLC profiles unambiguous correlation was found between the electronegativity of the heteroatoms and the chromatographic mobility of the analogues.
Publications


Nucleosides, Nucleotides and Nucleic Acids 2007, 26 (6-7), 681-685.


Poster lectures:


F. Sipos, Gy. Sági : Synthesis of 5-substituted-uracil PNA monomers by Pd-catalyzed cross-couplings. 29th European Peptide Symposium, Gdansk, Poland, September 3-8, 2006


