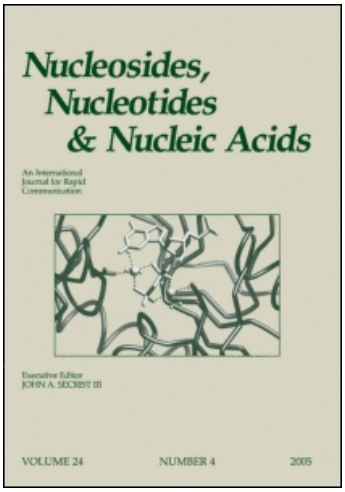

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A Novel Concept for the Combinatorial Synthesis of Peptide Nucleic Acids.

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ABSTRACT: A novel concept is presented for combinatorial pna synthesis. Novel peptide nucleic acids with improved properties can be anticipated to be generated by this method.

Peptide nucleic acids (PNA) are oligomers where the sugar phosphodiester backbone of DNA or RNA is replaced by an amide containing backbone. The idea of backbone replacement is rather old. The finding of willardiine, a natural product isolated from several *Mimosaceae*[1] stimulated their total synthesis and all possible natural and some unnatural analogous (FIG. 1).[2] These nucleobase containing α-aminoacids have been introduced in peptides and oligomerized to the first PNAs. Since the beginning of the 60th the russian group of Svachkin is publishing continuously papers[3] and reviews[4] on PNAs. Very early novel PNAs as well as their interesting biological properties were uncovered by other groups.[5]

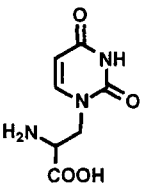

FIG. 1. Willardiine a nucleobase containing naturally occuring α-aminoacid, the starting point of PNA chemistry.

In 1981 the antisense concept was introduced.[6] Many groups proved the concept to work. Since that time many big companies establish antisense groups and several small companies emerged covering exclusively antisense technologies. That time most of the antisense molecules were phosphothioates and methylphosphonates.

In 1991 Nielsen et al. described a novel PNA backbone.[7] The resulting oligomers had especially interesting properties namely an enhanced binding to complementary DNA as well as RNA strands. Furthermore PNA can discriminate mismatches in the corresponding sense DNA strand much better than the antisense DNA is able to. Purine rich DNA strands form a triple helix with the corresponding PNA antisense strand of stoichiometry $(PNA)_2DNA$.[8] Their utility in

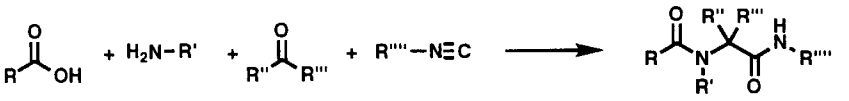

FIG. 2: The carboxylic acid variant of the Ugi four component reaction.

diagnostics as well as a tool in molecular biology has been proved in many publications[9], their *in vitro* capacity as antisense inhibitors as well.[10] The very good protease and nuclease stability makes this PNA promissing in antisense therapy. Unfortunately properties as the tendency of self aggregation, the bad solubility in water and their inability to cross the cell membrane hampers the *in vivo* applications of PNA. Therefore many scientists are trying to enhance these properties by introducing modifications in the backbone.[11]

Most approaches trace back to methods were monomers are coupled by traditional peptide chemistry.[12] Theses have the great advantage of using worked out and well known chemistry and can therefore easily be automatizised. The disadvantage is that the adequately protected monomers have to be synthesized in a tedious multi step synthesis. In order to investigate the influence of a modification in the backbone a novel monomer has to be synthesized in a rather time consuming multi step synthesis. Therefore it is impossible to investigate a larger number of backbone modifications by this approach in a reasonable time frame.

A new way to discover novel drugs in pharmaceutical industries is combinatorial chemistry. Libraries are screened for a given target and any found hits can also be improved to leads by combinatorial methods. Since many properties of compounds are hardly to predict this methodology together with high throughput screening seems to greatly accelerate the drug discovery process.

The same as for small molecular weight drugs is true for oligomers like PNA. Therefore a combinatorial approach seems to have higher chances in enhancing the properties of PNA. A novel concept for the combinatorial property improvement of PNA the first time is presented here.

Of all methods performing combinatorial chemistry multi component reactions (MCR) have distinctive advantages.[13] During a MCR at least three different starting materials react to form a product. This has preperative advantages, since very complex structures can easily be built-up from several less complex educts in an one pot reaction. The most general MCR leading to a great variety of different backbones is Ugi's four component reaction.[14] By inspection of the carboxylic acid variant of the Ugi reaction it can be seen that Nielsen's PNA and a huge variety of other PNA types should be accessible (FIG. 2).

In order to synthesize hetero oligomers in an ordered and defined manner one has to use bifunctional monoprotected building blocks. Amino protected ω-amino isocyanides are the bifunctional compounds needed to synthesize Nielsen's PNA and novel analogs thereof. These react together with a primary amine, an oxo component (aldehyde or ketone) and a carboxylic acid with a nucleobase sidechain in one Ugi reaction. After deprotecting the amino group the resulting primary amine is brought to reaction with an oxo component, a carboxylic acid and an amino protected ω-amino isocyanide. This cycle can be repeated to form PNA oligomers (FIG. 3).

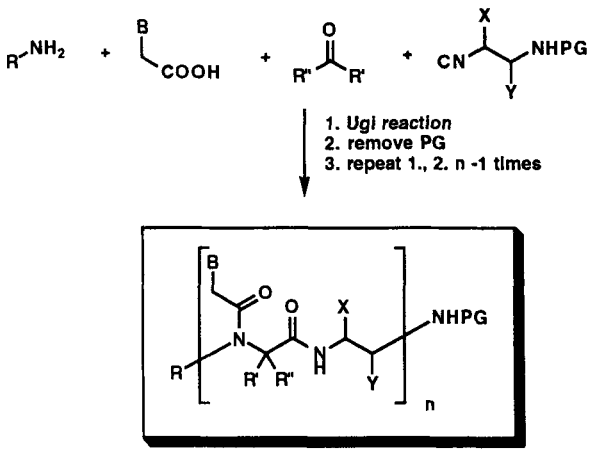

FIG. 3. New combinatorial PNA synthesis.

The advantages of this new synthesis concept are obvious. Since a submonomer approach is used the diversity of the PNA is much higher. Therefore the properties of PNA can be fine tuned and novel properties can be found. Starting materials are commercially available or easily in two to three step synthesizable starting materials can be used.[15] In one synthetic step three parts can be varied, compared to only one part in the conventional PNA synthesis. Isotopically marked PNA are becoming easily accessable and therefore the binding to the sense strand can be examined. Chimeric PNAs with other oligomers are becoming easily accessable. Novel backbones become available since not only carboxylic acids react in the Ugi reaction. Phosphonic acids, thiocarboxylic acids and a variety of other acids work as well.[16]

In conclusion, a novel concept for combinatorial PNA synthesis and their property tuning is described. It can be anticipated that this method will lead to new PNAs, enhance known PNA backbones and improve many of their poor properties for a fast application in antisense/antigene therapy and diagnostics.

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