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MODIFIED PNAS: A SIMPLE METHOD FOR THE SYNTHESIS OF MONOMERIC BUILDING BLOCKS

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Abstract

The synthesis of PNA-monomers with variations in the substitution pattern using the Ugi-Reaction is described. The one-pot procedure leads to new totally protected PNA-monomers which can be selectively cleaved to *N*-protected monomeric building blocks for PNA synthesis.

Keywords

Nucleic acid; DNA; Mimetics; Peptoids

Throughout the last years much interest was focused on oligonucleotides and analogouge structures because they are promising candidates for therapeutics and diagnostics.[1] The ability of sequence-specific binding to complementary DNA- and RNA-strands makes oligonucleotides usefull tools for developing antisense or antigene agents. Herein oligonucleotides inhibit the process of transcription (antigene-strategy) or translation (antisense-strategy) while binding to DNA or mRNA. Since naturally occuring oligonucleotides are enzymatically degraded under physiological conditions there is great interest in evaluating new synthetic oligonucleotid analogues with higher resistance against nucleases.

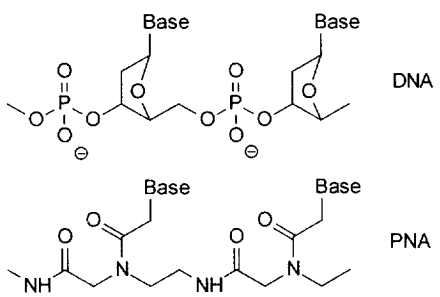

Fig. 1. Structures of DNA and PNA

Peptide Nucleic Acids (PNAs) are oligonucleotide analogues in which the sugar-phosphate backbone of DNA is substituted by a *N*-(2-aminoethyl)-glycine backbone. The naturally occuring nucleobases are attached to the peptide backbone *via* a carbonyl methylene linker (Fig. 1).[2] PNAs hybridize strongly and sequence-specifically to complementary DNA or RNA and in addition are not degraded by nucleases and proteases.[3] Such PNA-oligomers are in most cases synthesized by solid phase peptide synthesis from *N*-protected monomers (Fig. 2).[4]

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The known methods for preparation of PNA-monomers commonly require several steps to generate the *N*-(2-aminoethyl)glycine unit followed by *N*-acylation of the glycine derivative by a carboxymethylated nucleobase.[5] In recent publications rising interest was focused on PNA derivatives which are modified in the peptide backbone. The suitable combination of modified and unmodified monomeric building blocks to mixed backbone oligonucleotide analogues has led to better binding properties to DNA and RNA.[6] Another aim of this strategy is the optimize the properties of oligonucleotides (i.e. to enhance solubility or cellular uptake) by suitable variation of the substitution pattern.

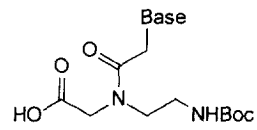
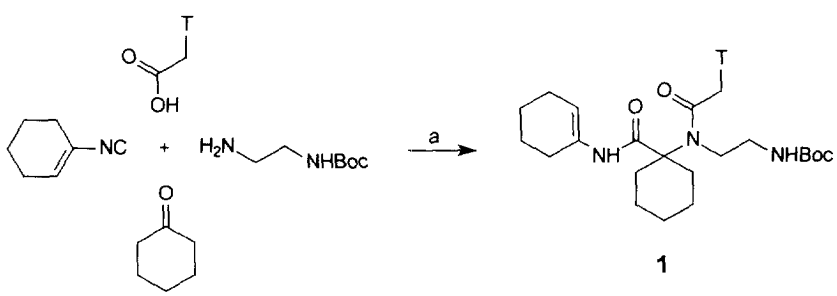


Fig. 2. *N*-protected PNA-monomer

In this work we present a new synthesis of previously unknown derivatives of protected PNA-monomers with a highly versatile substitution pattern in a one-pot process via Ugi Four Component Condensation (4CC).[7] Hydrolysis of these adducts affords *N*-protected monomers for PNA-synthesis.

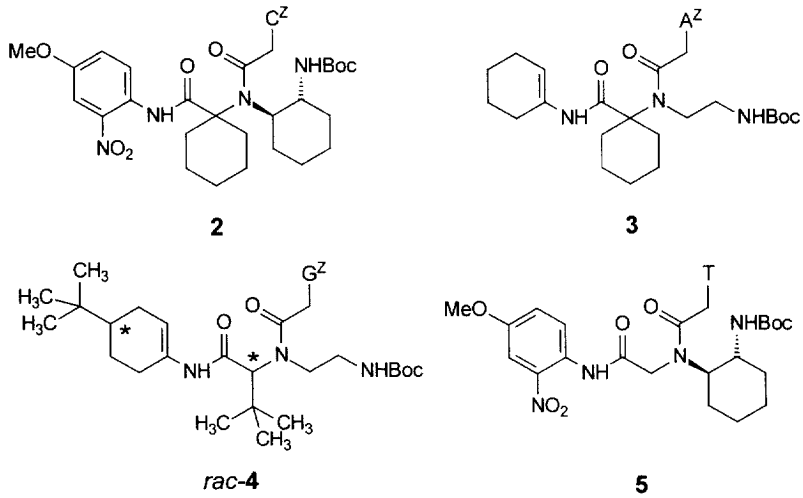
As recently described the Ugi-Reaction serves as a powerfull tool for the generation of nucleobase-peptide chimeras.[8] We used a nucleobase-acetic acid, a *mono*-protected diamine, an oxo compound (aldehyde or ketone) and an isocyanide as educts in the Ugi 4CC to generate totally protected derivatives of PNA-monomers in a one-pot process.



Scheme 1. Synthesis of the totally protected PNA-monomer **1** *via* Ugi-Reaction. a) MeOH, 20°C, 48h, 90 %.

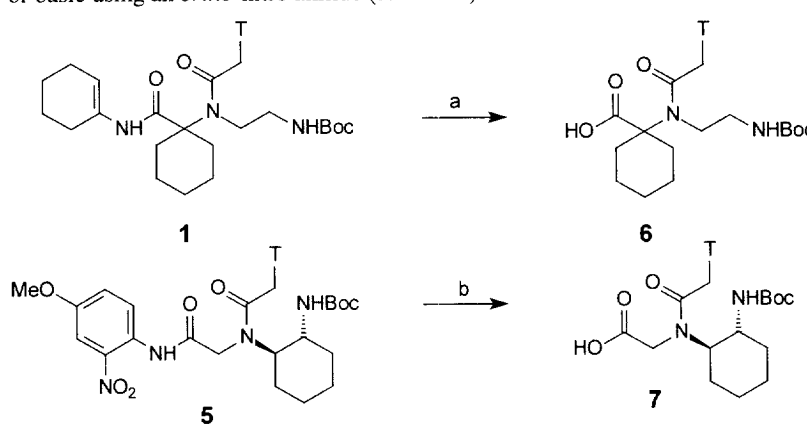
The four naturally occuring nucleobases thymine, adenine, guanine and cytosine were incorporated into the synthesized structures (**1**-**3** and *rac*-**4**). Variation of the amino compound leads to different diamino units. In compound **1**, **3** and *rac*-**4** a *β*-aminoethyl unit was introduced while the products **2** and **5** exhibit a *trans*-*β*-aminocyclohexyl unit. The isocyano and oxo compound input generates the α-amino acid end of the protected monomers. Herein the oxo compound constitutes the α-carbon atom with the corresponding substituents and the isocyanide serves as the precursor for an amide protected carboxylic acid.

The wide variability of the Ugi-Reaction and the availability of many different aldehydes and ketones allow the synthesis of various α-amino acid structures. The use of formaldehyde leads to the simple achiral glycine unit in compound **5**.



Scheme 2. Examples of totally protected PNA-monomers synthesized via Ugi 4CC corresponding to scheme 1. **Reagents and conditions:** **2** was prepared from N^4-Z-*N*-1-carboxymethylcytosine, *trans*-1,2-*mono*-Boc-cyclohexylendiamine, cyclohexanone and 4-methoxy-2-nitro-phenyl isocyanide in 73 % yield; **3** from N^6-Z-*N*-9-carboxymethyladenine, *mono*-Boc-ethylendiamine, cyclohexanone and cyclohex-1-enyl isocyanide in 61 % yield; *rac*-**4** from N^2-Z-*N*-9-carboxymethylguanine, *mono*-Boc-ethylendiamine, pivalaldehyde and 4-*tert*.-butyl-cyclohex-1-enyl isocyanide in 37 % yield; **5** from *N*-1-carboxymethylthymine, *trans*-1,2-*mono*-Boc-cyclohexylendiamine, formaldehyde and 4-methoxy-2-nitro-phenyl isocyanide in 64 % yield.

The achiral but dialkylated examples **1**, **2** and **3** were synthesized using cyclohexanone as an oxo compound in the 4CC. Chiral structures are prepared by using unsymmetric ketones or aldehydes like pivalaldehyde (*rac*-**4**). The use of a suitable isocyano input generates a secondary amide which can be cleaved selectively to the corresponding carboxylic acid. Acidic hydrolysis of 4CC adduct **1** gives the PNA monomer **6** and basic hydrolysis of compound **5** gives **7**. The required hydrolytic cleavage can be performed acidic in case of a vinylic amide[9] or basic using an *ortho*-nitro anilide (scheme 3).



Scheme 3. Hydrolysis of the totally protected PNA-monomers **1** and **5** to the *N*-protected PNA-monomers **6** and **7**.
Reagents and conditions: a) 1.7% conc HCl in THF, rt, 16 h, 82 % yield. b) 6 equivalents LiOH in MeOH, reflux, 3 h, 68 % yield.

In conclusion we present a simple and efficient method for the synthesis of PNA-monomers using the Ugi 4CC. The one-pot synthesis provides a lot of previously unknown and interesting structures of PNA-monomers. Monomeric building blocks (like 6 and 7) with high variability in the substitution pattern may be usefull tools for the target specific design of PNAs.

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