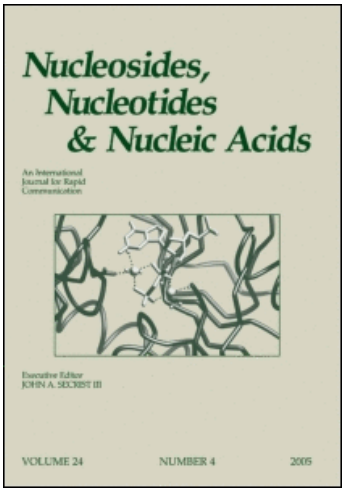


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Polyamide Nucleic Acids (Pnas), Pna /Dna-Chimeras and Phosphonic Ester Nucleic Acids (Phonas)

E. Uhlmann[a]; D. W. Will[a]; G. Breipohl[a]; A. Peyman[a]; D. Langner[a]; J. Knolle[a]; G. O'Malley[a]
[a] Hoechst AG, Central Pharma Research G 838, Frankfurt, Germany

To cite this Article Uhlmann, E. , Will, D. W. , Breipohl, G. , Peyman, A. , Langner, D. , Knolle, J. and O'Malley, G.(1997) 'Synthesis of Polyamide Nucleic Acids (Pnas), Pna /Dna-Chimeras and Phosphonic Ester Nucleic Acids (Phonas)', Nucleosides, Nucleotides and Nucleic Acids, 16: 5, 603 — 608
To link to this Article: DOI: 10.1080/07328319708002923
URL: http://dx.doi.org/10.1080/07328319708002923

NUCLEOSIDES & NUCLEOTIDES, 16(5&6), 603-608 (1997)

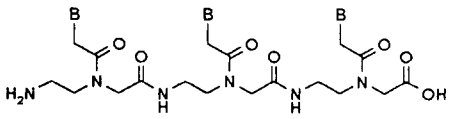
SYNTHESIS OF POLYAMIDE NUCLEIC ACIDS (PNAs), PNA / DNA-CHIMERAS AND PHOSPHONIC ESTER NUCLEIC ACIDS (PHONAs)

E. Uhlmann*, D. W. Will, G. Breipohl, A. Peyman, D. Langner, J. Knolle, G. O'Malley

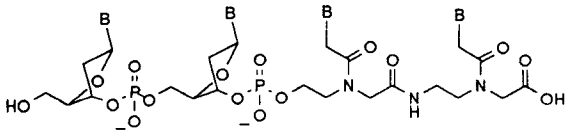
Hoechst AG, Central Pharma Research G 838, D-65926 Frankfurt, Germany

ABSTRACT. The synthesis of polyamide nucleic acids (PNAs) and derivatives thereof by different synthetic routes is described. The first strategy makes use of 9-Fluorenylmethoxycarbonyl (Fmoc)/monomethoxytrityl (Mmt) protected building blocks, whereas the second approach involves the use of Mmt/acyl protected monomers, which allows the preparation of PNA/DNA chimera. Additionally, a block coupling strategy is presented for the synthesis of novel phosphonic ester nucleic acids (PHONAs).

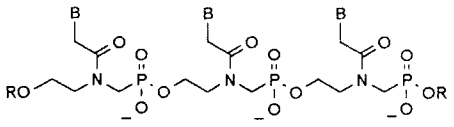
In 1991, Nielsen et al. [1] developed one of the most remarkable of non-ionic oligonucleotide analogues [2], known as the polyamide nucleic acids (PNAs) which bind to complementary DNA and RNA with higher affinity than natural oligonucleotides [3-5]. These PNAs are nucleic acid mimetics in which the entire sugar-phosphate backbone is replaced by *N*-(2-aminoethyl)glycine units. PNAs recognize complementary nucleic acid sequences by obeying the Watson-Crick base-pairing rules, but can also form triple helices *via* Hoogsteen base-pairing [4]. Furthermore, PNAs are extremely resistant to degradation by nucleases. An obstacle to their potential use as antisense agents is their tendency to self-aggregate and their extremely low cellular uptake [4].



PNA



DNA-PNA Chimera

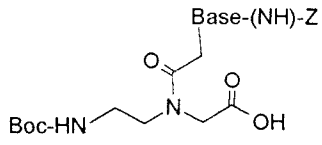


PHONA

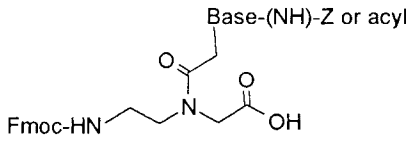
Due to the potential use of PNAs in antisense therapeutics and DNA diagnostics there is a need for efficient synthesis techniques for PNAs and PNA derivatives having improved properties, such as PNA/DNA chimeras [6] or PHONAs (phosphonic ester nucleic acids) [7]. In PHONAs the *N*-(2-aminoethyl)glycine backbone of PNAs is replaced by a *N*-(2-hydroxyethyl)aminomethyl phosphonic acid backbone. Here we report on methods for the synthesis of PNAs, PNA/DNA chimeras and PHONAs.

Synthesis of PNAs

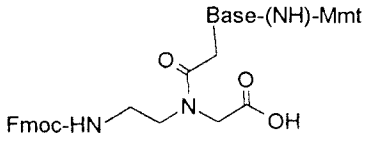
Originally, PNAs have been prepared starting from Boc/Z-protected monomeric building blocks requiring repetitive TFA deprotection during chain elongation and a final HF or trifluoromethane sulfonic acid cleavage to release the PNAs from the resin [1, 8]. Fmoc-mediated PNA synthesis has been described [9] using the benzyloxycarbonyl (Z) group for protection of the exocyclic amino groups of the nucleobases in combination with *p*-methyl-benzhydrylamine resins necessitating the use of strong acids for deprotection and product release from the solid support. We have developed a milder method for the synthesis of PNAs using Fmoc protection for the *N*-(2-aminoethyl)glycine unit in combination with acid labile 4-methoxyphenyldiphenylmethyl (Mmt) protection for the exocyclic amino function of the nucleobases [10].



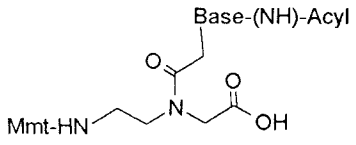
Boc / Z - Monomer
(Ref. 1, 8)



Fmoc / Z- or Fmoc / acyl - Monomer
(Ref. 9 and 14)



Fmoc / Mmt - Monomer
(Ref. 10)



Mmt / acyl - Monomer
(Ref. 11)

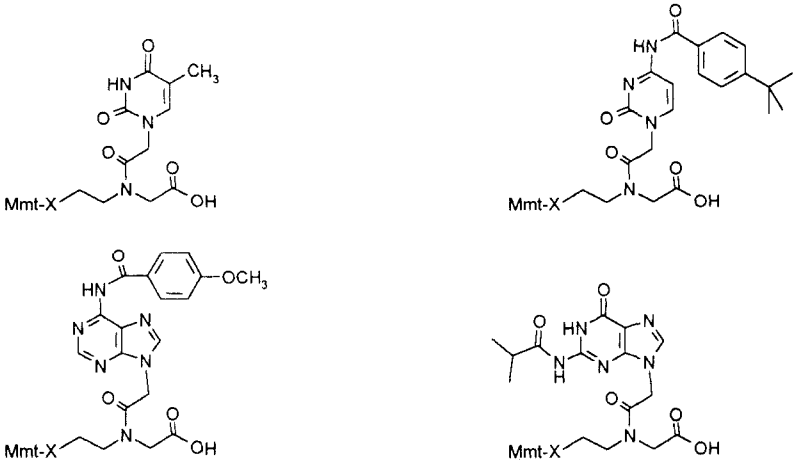
Use of Mmt group for base protection enhances the solubility of the monomers in organic solvents and allows final deprotection by mild acid treatment, such as 80% acetic acid. This synthetic strategy can also be used for convenient synthesis of PNA-peptide conjugates. In search of an alternative strategy which would open the way to a combination of PNA and DNA synthesis we have developed novel PNA monomers with orthogonal protecting groups in which the Mmt group is used as an *N*-terminal temporary protecting group and the exocyclic amino functions of the bases are protected by base-labile acyl protecting groups [11]. Similarly to standard oligonucleotide synthesis, the Mmt group can be removed with mild acids, such as 3% trichloroacetic acid (TCA), whereas the base protecting groups are cleaved off at the end of the synthesis using conc. ammonia. This synthetic procedure is milder than both the Boc- and Fmoc-strategies and is completely compatible with oligonucleotide synthesis. The thyminyl building block which needs no base protection has also been described by others [12, 13].

Synthesis of PNA/DNA Chimera

In order to overcome some of the drawbacks of PNAs, including poor solubility, parallel and antiparallel binding and very poor cellular uptake, we have developed a strategy for the synthesis of PNA/DNA chimeras using solid-phase methology. An ideal synthetic method should allow fully automated "on-line" synthesis of PNA/DNA chimeras of any desired order (5'-DNA-PNA-carboxy or amino-PNA-DNA-3') using commercially available DNA building blocks. From the above-mentioned building blocks only the Mmt/acyl-protected monomers fulfill these requirements. The repeated treatment with TFA required for Boc-deprotection, and the harsh HF or TFMSA treatment required for cleavage from the resin and deprotection, render the Boc-strategy incompatible with DNA synthesis. The Fmoc-procedure requires the availability of specially protected DNA monomers, such as those with allyl blocking groups on the phosphate instead of the normally used ß-cyanoethyl groups [14]. The only previously reported synthesis of PNA/DNA chimeras was of oligomers containing no purine bases.

We have employed the Mmt/acyl protecting group strategy in combination with suitable solid support linkers to allow the uninterrupted solid-phase synthesis of PNA-DNA chimeras [6]. Standard solid-phase peptide synthesis requires treatment with strong acid to cleave the peptide chain from the support. Since these conditions would not allow

the synthesis of PNA/DNA chimera with purine nucleosides in their sequence, we have synthesized a universal solid support. For this, controlled pore glass (CPG) was derivatized with the succinate of *N*-Mmt-aminohexan-6-ol to obtain an ammonia-cleavable linker. The PNA part of the chimera is synthesized using the monomers shown below. For linking the PNA part with the DNA part, a *N*-(2-hydroxyethyl)glycine derivative [6, 15] is coupled to the amino terminus. After detritylation with TCA, a standard nucleoside phosphoramidite can be reacted with the hydroxy group to initiate synthesis of the DNA by standard methods.



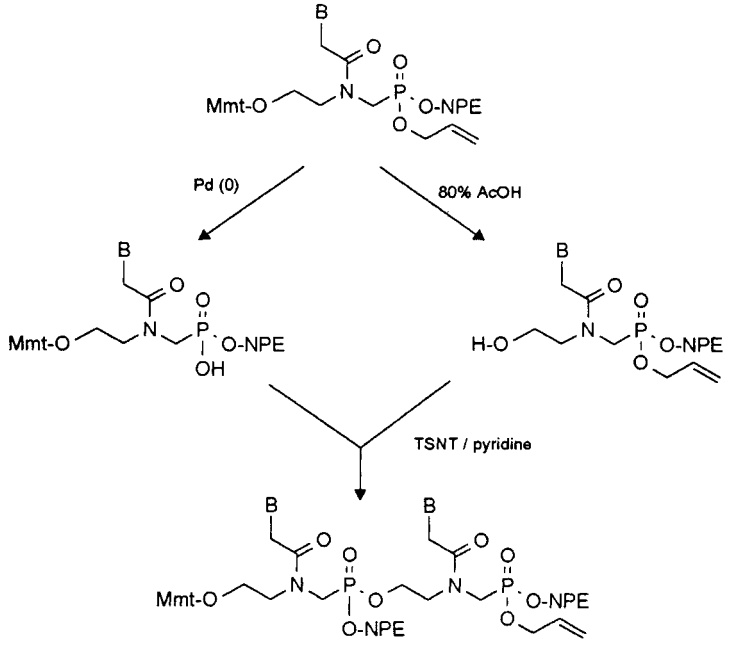
X = NH for PNA monomers; X = O for DNA-PNA linking monomers

Synthetic access to DNA-PNA chimera in which DNA constitutes the 5'-terminal part are especially interesting for *in vivo* applications [6], as the PNA part provides perfect stability against 3'-exonucleases which is the major DNA degrading component in serum. Interestingly, DNA-PNA chimera are incorporated more efficiently by living cells than pure PNAs. Although binding affinity of DNA-PNA chimera is somewhat lower than of PNAs, the chimeric oligomers bind only in the desired antiparallel orientation. Furthermore, the DNA part of the DNA-PNA chimera can induce the RNase H cleavage of a complementary mRNA [16] which is the major mechanism of action of phosphodiester and phosphorothioate oligonucleotides [17].

Synthesis of PHONAs

For the synthesis of PHONAs we have used the solution phase block synthesis strategy [7]. Similarly to phosphotriester block condensation, a monomeric building block

having the orthogonal protection groups Mmt for the hydroxy and 2-(4-nitrophenyl)ethyl [18] for the phosphonic acid functions, was employed as starting material for oligomer synthesis. The allyl group was chosen as the temporary phosphate protection group which can be cleaved selectively by Pd (0) under neutral conditions. Coupling of the phosphonate with the hydroxyethyl derivative is carried out by means of 3-nitro-1-(p-toluoylsulfonyl)-1*H*-1,2,4-triazole (TSNT) in pyridine. At the end of the synthesis the NPE groups are cleaved by treatment with 0.5 M DBU in pyridine. The resulting PHONA oligomers are perfectly soluble in aqueous medium, do not tend to aggregation, and show interesting binding properties to complementary nucleic acids [7].



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