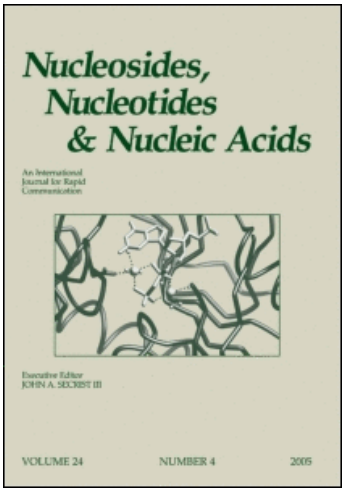

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AUTOMATED CHEMICAL SYNTHESIS OF PNA AND PNA-DNA CHIMERA ON A NUCLEIC ACID SYNTHESIZER

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ABSTRACT: Automated chemical synthesis of PNA and PNA-DNA chimera on a DNA synthesizer using the monomethoxytrityl / acyl protecting group strategy is described.

Interest in nucleic acids analogs has increased considerably in the past few years because of their potential in antisense and DNA based diagnostic studies. The development of peptide nucleic acids (PNA) has demonstrated that pseudopeptide backbones can effectively mimic the phosphodiester backbone of nucleic acids.[1] First described in 1991, PNA closely maps the spatial arrangement of nucleic acids with achiral, neutral N-(2-aminoethyl)glycine units.[2]

Several methods to synthesize PNA, PNA-peptide and PNA-DNA chimera on solid supports have been reported in the last couple of years.[3-10] We have developed syntheses of PNA using the monomethoxytrityl (MMT) protected monomer strategy.[7,8] This group is used in protecting the primary amine functionality on the (aminoethyl)glycine backbone. The exocyclic amine groups on the PNA nucleobases employ the standard DNA protecting groups: benzoyl for A and C and isobutyryl for G. The MMT group is removed under mild acidic conditions and the exocyclic amino groups can be removed using conc. ammonium hydroxide at 55 °C. We have also developed a solid support that has an amino acid residue (glycine) attached to the non-swelling, high-cross linked polystyrene beads through a hydroxymethyl benzoic acid linker. Ammonium hydroxide cleaves this support resulting in a PNA oligomer with C-terminal (3') glycine.

For synthesis of PNA on the ABI DNA/RNA synthesizer, the PNA monomers are dissolved (0.2M) in DMF : CH_3CN (1 : 1) and combined with 0.2M *O*-benzotriazol-1-yl-

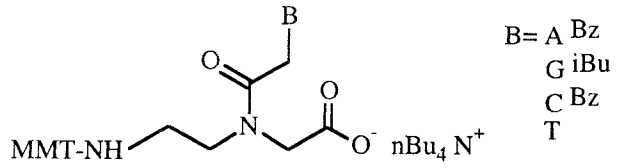

FIG. 1. PNA monomers used in solid-phase synthesis on ABI 394 DNA/RNA synthesizer.

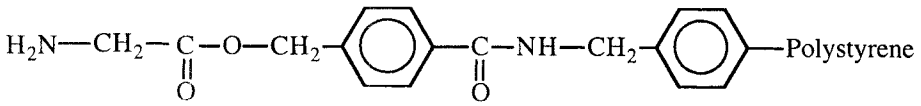

FIG. 2. Structure of Gly-HMBA-polystyrene support used in solid-phase PNA synthesis.

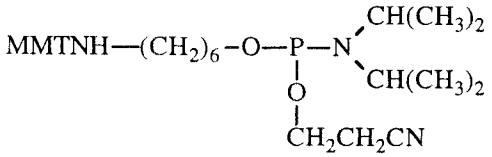

FIG. 3. Structure of the linker (c6) used in linking PNA to DNA.*

N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) in DMF : CH_3CN (1 : 1). This mixture is delivered to the synthesis column concurrently with an equal volume of diisopropylethylamine (DiPEA, 0.2M) in DMF : CH_3CN (1 : 1). After coupling for 15 minutes, capping reagents are delivered followed by detritylation of the MMT group with 3% TCA/DCM to complete the synthesis cycle. At the end of the synthesis the N-terminal end (5') of PNA is capped as acetyl. PNA is then cleaved from the support (1h, ambient temperature, ammonium hydroxide) on the instrument. Exocyclic amino groups are deprotected by heating the ammonium hydroxide solution to 55 °C for 16 h and PNA is purified by reverse phase HPLC.

For linking PNA to DNA, we have used the commercially available phosphoramidite of monomethoxytritylaminohexanol (Figure 3, Glen Research). This amine linker was

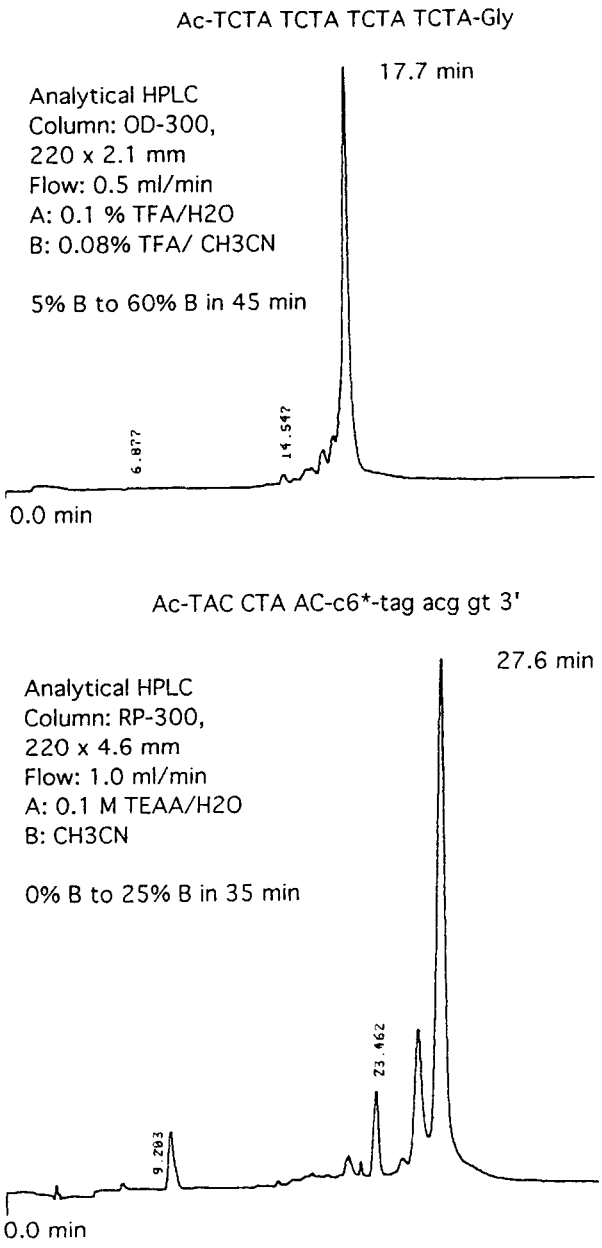

FIG. 4. Reverse phase HPLCs of a PNA (top) and a PNA-DNA chimera (bottom) obtained after removal of base protecting groups followed by gel-filtration. DNA bases are represented in small letters.

coupled to the 5' terminus of DNA via standard DNA synthesis protocols, followed by coupling of PNA monomers using the PNA synthesis protocols.

In conclusion, we have shown that synthesis and deprotection conditions used for PNA synthesis on an automated DNA synthesizer are compatible with DNA synthesis and allow for the construction of PNA-DNA chimera. For diagnostic and antisense applications these sequences could be of great interest due.to enhanced exonuclease stability, ability to induce RNase H duplex cleavage and their ability to form strong Watson-Crick base-pairing with complementary DNA and RNA strands.

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