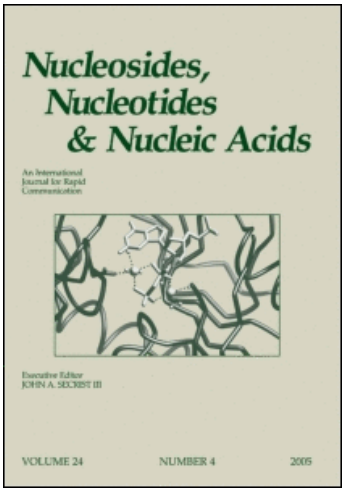

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INTRODUCTION OF CHIRALITY INTO PNA BY REPLACEMENT OF THE ACHIRAL METHYLENE CARBONYL LINKAGE TO THE NUCLEOBASE

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□ *A novel approach to the introduction of chirality into peptide nucleic acid (PNA) by replacement of the methylene carbonyl linker by an alpha-amino acid derived moiety is described. A monomer compatible with Fmoc-based oligomerization chemistry possessing an L-serine derived linker has been synthesized and incorporated into PNA oligomers. A single, central substitution in a hexathymine PNA strongly destabilized triple helix formation whereas a central substitution in a mixed sequence is much better tolerated. We have investigated the influence of this substitution on the selectivity for strand composition (DNA versus RNA complement) and strand orientation (antiparallel versus parallel) in the context of duplex formation. A PNA 11-mer with a single substitution demonstrates a preference for an antiparallel RNA complement, as judged by thermal denaturation analysis of the complexes.*

Keywords Peptide nucleic acid; PNA; chirality; L-serine; PNA oligomers

INTRODUCTION

Peptide nucleic acid (PNA) is a nucleic acid analogue constructed with a neutral polyamide backbone composed of *N*-(2-aminoethyl)glycine units and a methylene carbonyl linkage between the alpha-amino group and a nucleobase. Most modifications to PNA, including the introduction of chirality, have been achieved by modifying the backbone structure. PNA being acyclic has a significant conformational freedom in the nucleobase linker and the methylene segments of the backbone. Thus, the formation of a PNA-DNA or PNA-RNA complex results in decreased entropy and conformational freezing of PNA.[1] PNA is also achiral and as a result may bind to DNA/RNA in the parallel or antiparallel binding mode with almost equal ability.[2] These issues have been addressed by introducing chirality and cyclic conformational constraints into the PNA backbone. However, most of these modifications are synthetically challenging. To our knowledge

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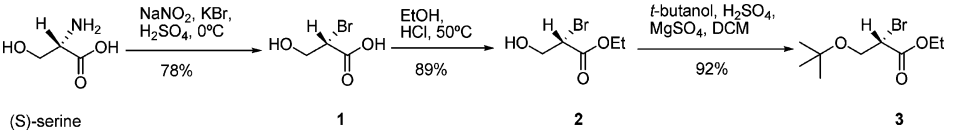
introduction of chirality via the methylene carbonyl linker has not been reported. Therefore we were motivated to replace the achiral methylene carbonyl linker by an alpha amino acid derived moiety. From a minimalist's point of view this is the simplest modification to the methylene carbonyl linker that introduces both aqueous solubilizing groups and chirality while preserving the original spacing of repeating units of 6 bonds along the backbone and 3 bonds linking the nucleobase, as first reported by Nielsen and coworkers.[3]

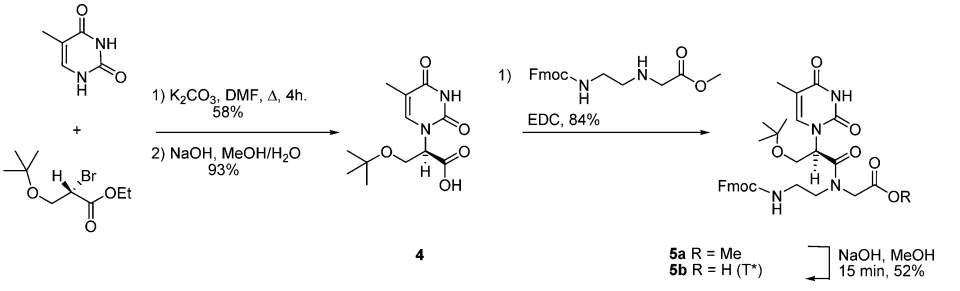
RESULTS AND DISCUSSION

The synthesis of the nucleobase linker **3** was achieved in three steps, starting with (S)-serine, as described by Scheme 1. Conversion of serine into the α-halo acid **1** was readily done on a multi-gram scale in good yield.[4] In order to alkylate thymine efficiently both the hydroxyl group and the carboxylic acid required protection. The latter was protected under Fischer esterification conditions to yield the ethyl ester **2**, whereas the *t*-butyl ether was conveniently prepared by treatment of **2** with *t*-butanol in the presence of acid catalyst.[5]



SCHEME 1

The chiral-linker containing monomer was accessed using standard chemistry as detailed in Scheme 2. Of note, slightly more forcing conditions were required for the alkylation of thymine and the product was recovered in marginally lower yield as compared to the use of primary α-bromoacetates. After ester hydrolysis, **4** was condesed with the Fmoc methyl ester backbone to give the monomer ester **5a**. Removal of the methyl ester from **5a** proved to be difficult in the presence of the base labile Fmoc group but was accomplished in 52% yield to give the monomer **5b (T*)**.



SCHEME 2

TABLE 1 Thermal denaturation dependence on strand composition and orientation

	Tm, °C (Δ Tm)* Target Strand			
PNA sequence	DNA_{AP}	DNA_P	RNA_{AP}	RNA_P
Ac-TCC-AGC-GTA-AC-K-NH_2	62.0	47.0	66.0	52.0
Ac-TCC-AGC-GT*A-AC-K-NH_2	49.1 (−12.9)	41.6 (−5.4)	58.2 (−7.8)	49.1 (−2.9)

Tm values were estimated by the first derivative method. DNA_{AP} = antiparallel oligodeoxynucleotide target: 5′-GTT-ACG-CTGGA-3′; DNA_P = parallel oligodeoxynucleotide target: 5′-AGG-TCG-CAT-TG-3′; RNA_{AP} = antiparallel oligoribonucleotide target: 5′-GTT-ACG-CTGGA-3′; RNA_P = parallel oligoribonucleotide target: 5′-AGG-TCG-CAT-TG-3′. Δ Tm = difference in the Tm value of the unmodified PNA and the PNA containing **T***.

Incorporation of **(T*)** into a PNA oligomer was done on NovaSyn TGR resin using FastMoc chemistry module on an Applied Biosystems 433A synthesizer. UV-T_m measurements of triplex (PNA_2:NA), and duplex systems (PNA:DNA_{AP}, PNA:DNA_P, PNA:RNA_{AP}, PNA:RNA_P) were performed at 2 μM in 100 mM NaCl, 10 mM PO_4^{3-}, 0.1 mM EDTA at pH 7.0.

All compounds exhibited monophasic cooperative transitions. A PNA hexamer bearing a single modification, at an internal position (Ac-TTT **T***TT-K-NH_2) was used to evaluate the effect of this substitution in the context of triple helix formation with poly(rA). A single internal substitution was found to strongly destabilize the complex ($\Delta Tm = -37°C$). However, the substitution is better tolerated in duplex formation as shown in Table 1. Although the chiral **T*** monomer is overall destabilizing when compared to the unmodified PNA, it does exhibit some interesting hybridization properties. When incorporated near the C-terminus of an 11-mer mixed sequence PNA the modified monomer (**T***) shows a 9°C preference in T_m for hybridization to an antiparallel RNA target ($T_m = 58.2°C$) versus the DNA ($T_m = 49.1°C$), whereas the unmodified PNA only demonstrates a 4°C preference for an antiparallel RNA target versus an antiparallel DNA target. The chiral monomer also has a 9°C discrimination for hybridization to the antiparallel RNA target ($T_m = 58.2°C$) versus the parallel RNA ($T_m = 49.1°C$).

In summary, the introduction of a serine-derived linker into a PNA monomer proceeded readily. An oligomer containing the modified monomer exhibits preference for RNA binding in the antiparallel orientation. We currently are pursuing the synthesis of PNA monomer starting with (R)-serine and will investigate the behaviour of the opposite enantiomer on RNA/DNA binding, and the effect of multiple substitutions and position/sequence effects. Substitution of the α-amino group of other amino acids such as glutamic acid and lysine also will be investigated in order to gain insight into why there is a preference for RNA binding and how the stability of the complex may be increased. Additionally, it has not escaped our attention that after reduction of the carboxyl group in compound **4,** we would be left with an acyclic nucleoside analog, similar to that used for

glycol nucleic acid.[6] Transformation of this compound into a monomer for DNA analog synthesis using classical phosphoramidite chemistry is also being pursued.

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