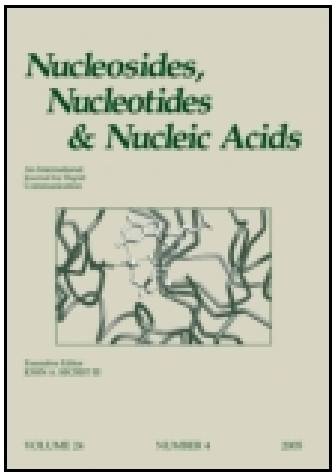


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

SYNTHESIS AND HYBRIDIZATION OF NOVEL CHIRAL PYRROLIDINE BASED PNA ANALOGUE

Andis Slaitas [a] & Esther Yeheskiely [b]
[a] Division of Organic and Bioorganic Chemistry, MBB , Scheele Laboratory, Karolinska Institute , Stockholm, S-17177, Sweden
[b] Division of Organic and Bioorganic Chemistry, MBB , Scheele Laboratory, Karolinska Institute , Stockholm, S-17177, Sweden
Published online: 07 Feb 2007.

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 1377–1379 (2001)

SYNTHESIS AND HYBRIDIZATION OF NOVEL CHIRAL PYRROLIDINE BASED PNA ANALOGUE

Andis Slaitas and Esther Yeheskiely*

Division of Organic and Bioorganic Chemistry,
MBB, Scheele Laboratory, Karolinska Institute,
S-17177 Stockholm, Sweden

ABSTRACT

Four different PNA fragments containing units of either the *R*- or *S*- isomer of *N*-(2-pyrrolidine-methyl)-*N*-(thymine-1-acetyl)-glycine (Pmg) were synthesized on a solid support. UV thermal melting experiments with complementary RNAs were performed and it was found that *R*-Pmg containing PNAs bind better to RNA than those containing the *S*-Pmg units.

INTRODUCTION

Amino acid based oligonucleotide analogues can form stable duplexes with complementary DNA (RNA) (1). Many factors influence the stability of these duplexes, e.g., it is dependent on the rigidity and bulkiness of the backbone and on whether it contains the D or the L amino acid (1).

Our objective was to prepare and investigate the properties of a new chiral PNA analogue, the backbone of which contains *N*-[(2*R*-pyrrolidine)methyl]-glycine (*R*-Pmg) or the 2S isomer (*S*-Pmg). We reasoned that the pyrrolidine moiety (Fig. 1) will introduce rigidity and chirality, while the backbone will still contain the requisite six covalent bonds as in PNA monomers (2).

*Corresponding author.

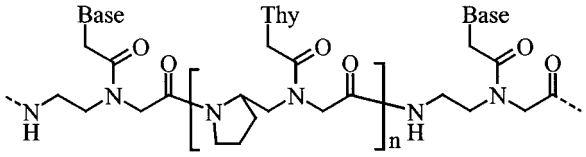
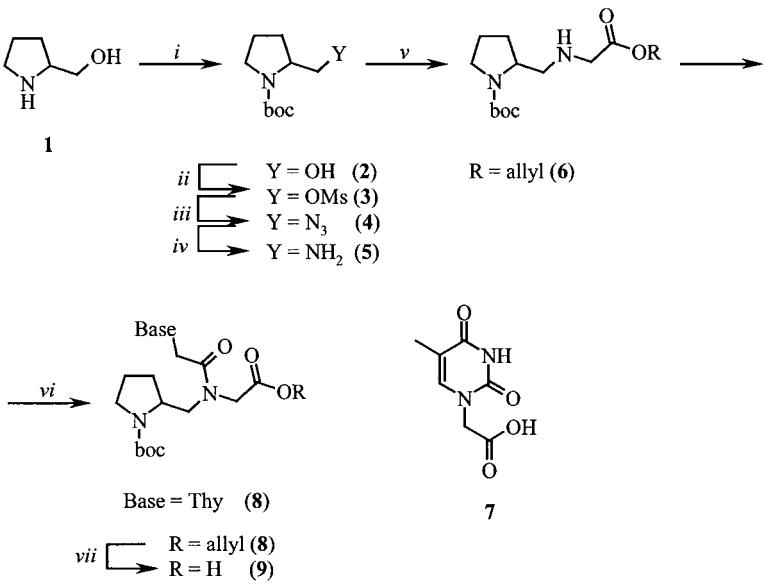


Figure 1. Structure of thymine-Pmg PNA.

RESULTS AND DISCUSSION

Construction of the Pmg backbone starting from prolinol involves the following steps: The amino function of prolinol **1** (Scheme 1) was protected with the *t*-butoxycarbonyl group (Boc) and the primary alcohol of **2** was then mesylated. Nucleophilic substitution of the mesylate **3** with azide ion in DMF and subsequent reduction of compound **4** afforded 2-(aminomethyl)-*N*-Boc-pyrrolidine **5** in 81% overall yield (3). Alkylation with allyl bromoacetate and 2-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate (HBTU) mediated acylation of the resulting secondary amino function of the backbone **6** with thymine-1-acetic acid (**7**) gave the fully protected monomeric unit **8** (4). Finally, Pd-catalyzed cleavage of the allyl ester (5) afforded the T-Pmg building block **9** in a form suitable for solid phase synthesis.



i) Boc_2O, NaOH, H_2O *ii*) MsCl, Et_3N, CH_2Cl_2 *iii*) LiN_3, DMF *iv*) Ph_3P, H_2O, THF
v) Allyl bromoacetate, Et_3N, THF *vi*) **7**, HBTU, DIEA, DMF *vii*) $[Pd(Ph_3P)_2]Cl_2$, Bu_3SnH, AcOH, CH_2Cl_2

Scheme 1. Synthesis of T-Pmg monomer.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1. UV Thermal Melting Data

#	Sequence	Tm,† °C		ΔTm/mod.,‡ °C		ΔTm (R–S)/mod.
1.	H-Lys$_2$-tcacttccat		53.0			
		S	*R*	*S*	*R*	
2.	H-Lys$_2$-tcacttccat	39.0	44.0	−14.0	−9.0	+5.0
3.	H-Lys$_2$-tcacttccat	27.0	36.0	−13.0	−8.5	+4.5

†Measured with the complementary (antiparallel) RNA strand in 100 mM NaCl.
‡Compared to Tm of entry 1.

PNA sequences 'H-(Lys)$_2$-tcacttccat-Gly-NH$_2$' containing one or two *S*- or *R*- T-Pmg units were synthesized on a solid support using mixed Fmoc/MMT/Boc strategy [N^{α}-Fmoc-N^{ε}-Boc-L-lysine, *N*-MMT-AEG-PNA and *N*-Boc-Pmg-PNA monomers] and HBTU as condensation agent. The coupling yields were up to 99% per step. The PNAs were purified on reverse-phase HPLC and their authenticity confirmed by MALDI-TOF mass spectrometry.

The ability of the above-mentioned oligomers to form stable duplexes with RNA was evaluated by UV thermal melting experiments (reference: PNA-RNA duplex). The values for *S*-Pmg fragments indicated decrease of T_m (14°C/mod.), while the *R*-Pmg containing PNA showed decrease of 8.5°C/mod (See Table 1).

CONCLUSIONS

Both *S*- and *R*- T-Pmg units were prepared in good yields and incorporated into four individual PNA decamers the hybridization properties of which were investigated. All modified sequences showed decreased thermal stability. At the moment we are engaged in the synthesis and study of fully modified *R*-Pmg decamers.

ACKNOWLEDGMENT

We wish to thank the Swedish Natural Science Research Council (NFR) for financial support.

REFERENCES

1. Laan, A.C. van der.; Amsterdam, I. van.; Tesser, G.I.; Boom, J.H. van.; Kuyl-Yeheskiely, E. *Nucleosides & Nucleotides* 1998, *17*, 219–232.
2. Nielsen, P.E.; Egholm, M.; Berg, R.M.; Buchardt, O. *Science* 1991, *254*, 1497–1500.
3. Soth, M.J.; Nowick, J.S. *J. Org. Chem.* 1999, *64*, 276–281.
4. Laan, A.C. van der.; Brill, R.; Kuimelis, R.G.; Kuyl-Yeheskiely, E.; Boom, J.H. van.; Andrus, A.; Vinayak, R. *Tetrahedron Lett.* 1997, *38*, 2249–2252.
5. Dangles, O.; Guibe, F.; Balavoine, G.; Lavielle, S.; Marquet, A. *J. Org. Chem.* **1987**, *52*, 4984–4993.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016