Synthesis of a newly peptide nucleic acid that contains tertiary amino groups

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ABSTRACT
A thymine monomer of pyrrolidine-based peptide nucleic acid that contains a tertiary amino group in the backbone has been synthesized for use in the synthesis of novel peptide nucleic acid.

INTRODUCTION
Peptide nucleic acids (PNAs) are peptides carrying nucleobases on side chains. So far, a number of PNA structures have been proposed and synthesized. Some of the PNAs have been expected to work as antisense and antagonist drugs and as molecular biology tools.

Nielsen-type PNA (Scheme 1) forms very stable duplexes and triplexes with nucleic acids (1, 2). The high hybrid stability may be attributed to its neutral backbone. However, Nielsen-type PNA shows low water solubility and low cellular uptake.

Pyrrolidine-based oxy-PNAs (POPNA) are a new type of PNA. The POPNAs have ether linkages and pyrrolidine rings in the backbones (Scheme 1). Because of the ether linkages increase flexibility of the main chains, the water solubility improves. Furthermore, because of the pyrrolidine rings restrict the side chains, hybridization between POPNAs and nucleic acids optimizes. The two chiral centers on a pyrrolidine ring allow four stereoisomers (cis-L-POPNA, trans-L-POPNA, cis-D-POPNA, trans-D-POPNA). In previous works, all adenine nonamers of POPNAs have been showed formation of duplex only with nucleic acids (6-8). Cis-L-POPNA shows a most stable duplex with DNA. Trans-L-POPNA shows a most stable duplex with RNA. We are attending to trans-L-POPNA because we will investigate hybridization of POPNAs with tRNA.

The hybrid stability of Nielsen-type PNA with nucleic acids is too high to obtain sequence specificity but POPNAs are able to optimize to hybridize with nucleic acid oligomers.

To control the stability, we synthesize a new cationic PNA monomer in this work (Scheme 1).

RESULTS AND DISCUSSION
The synthesis of the thymine monomer 11 is shown in Scheme 2. The secondary amine of trans-L-hydroxyproline 1 was Boc protected in 95 % yield. The reaction of N-Boc-trans-L-hydroxyproline 2 with ethyl bromide gave compound 3 in 94 % yield. The inversion of the C4 hydroxy function under Mitsunobu reaction condition gave the N-Boc-cis-L-hydroxyproline 4 in 82 % yield (2 steps). Treatment of the 4 with tert-butylchlorodiphenylsilane gave 5 in 77 % yield. Reduction of compound 5 with NaBH4 resulted in the corresponding alcohol 6, then, alcohol 6 was

Scheme 1 Chemical structures of DNA, Nielsen-type PNA, OPNA (3-5), POPNA and Cationic PNA.
reacted with CB₃Et to give compound 7 in 60% yield (2 steps). Compound 7 was reacted with sarcosine tert-butyl ester to give compound 8 in 36% yield. Removal of tert-butylidiphenylsilyl group in compound 8 with TBAF gave the trans-L intermediate 9 in 99% yield. Compound 9 was afforded in an overall yield 12% from trans-L-hydroxyproline 1.

The alcohol 9 was reacted with N³-benzoylthymine under standard Mitsunobu condition to give the desired N³-isomer 10, then compound 10 was treated with 30% HBr in acetic acid to remove the Boc, OBu', and the exocyclic amide protecting group. The free compound was protected again with 9-fluorenylmethyl succinimidyl carbonate to give the final product 11 in an overall yield of 60% from 9.

CONCLUSION

A thymine monomer of pyrrolidine-based peptide nucleic acid that contains a tertiary amino group in the backbone has been successfully synthesized. In future, POPNAs that mixed with the cationic PNA will utilize as molecular biology tools for RNA in vivo.

REFERENCES


![Scheme 2](image-url)

Scheme 2 Synthetic route of the thymine monomer. (i) Boc₂O (1.1 eq), NaHCO₃, water:acetonitrile (1:1), rt, over night, 95% (ii) Br₂ (8.0 eq), DMF, rt, over night, 94% (iii) I₂CO₂H (1.5 eq), DEAD (1.5 eq), Ph₃P (1.5 eq), THF, rt, over night, quant (iv) 28% NH₄aq, MeOH, rt, 1 h, 82% (v) TBDPS-Cl (1.5 eq), imidazole (2.2 eq), DIPEA (1.5 eq), DMF, rt, over night, 77% (vi) NaBH₄ (4.0 eq), EtOH, rt, over night, 70% (vii) CB₃Et (1.5 eq), Ph₃P (1.5 eq), THF, rt, 3 h, 85% (viii) H-Sar-OEt (2.0 eq), Na₂CO₃ (3.0 eq), DMF, 75°C, over night, 36% (ix) TBAF, THF, rt, over night, 99% (x) N³-benzoylthymine (2.0 eq), DEAD (2.5 eq), Ph₃P (2.5 eq), THF, rt, over night, crude (xi) 30% HBr/MeOH, rt, 30 min, crude (xii) Fmoc-OSu (1.5 eq), water/MeCN (=1/1(v/v), pH 8), rt, over night 60% (3 steps).