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Pyrrolidine PNA: A Novel Conformationally Restricted PNA Analogue

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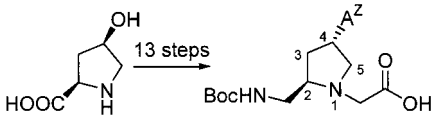
ABSTRACT



A new conformationally restricted PNA adenine monomer has been synthesized in 13 steps from *cis*-4-hydroxy-D-proline. A fully modified adenine decamer displayed improved binding affinity toward complementary DNA and RNA oligonucleotides as compared to that of the parent PNA adenine decamer.

Peptide nucleic acid (PNA) is a DNA mimic in which the nucleobases are linked to an *N*-(2-aminoethyl)glycine backbone through methylene carbonyl linkers (Figure 1).[1] PNA binds to DNA and RNA with high affinity and specificity.[2] The antisense properties of PNA have recently been reviewed.[3] In this context the binding affinity of PNA toward RNA is important. PNA/DNA and PNA/RNA duplex formation is accompanied by a decrease in entropy. This entropy loss could be reduced by using a more rigid PNA analogue.

We have recently designed and synthesized such an analogue: the pyrrolidinone PNA (Figure 1, B = adenine).[4] In this analogue, the carbonyl group of the linker is forced to point toward the carboxy terminus of the backbone. This approximates the conformation of the PNA strands found in PNA/DNA and PNA/RNA duplexes.[5] As opposed to PNA which is achiral, two new stereocenters are introduced in each monomer unit in the pyrrolidinone PNA. By synthesizing all four possible stereoisomeric monomer building blocks and incorporating these into PNA oligomers, it was found that the (3*S*,5*R*) isomer was the better binding isomer. A

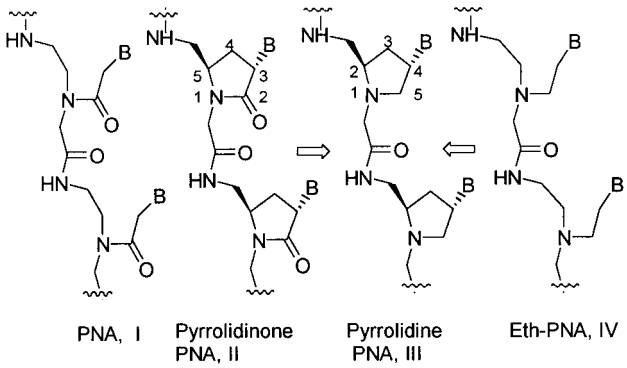


Figure 1. B = Adenine, cytosine, guanine, or thymine.

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fully modified (3*S*,5*R*)-pyrrolidinone adenine decamer displayed a T_m depression per modification of only 1 °C as compared to PNA against $r(U)_{10}$.[4b]

However, a larger destabilization (ΔT_m/mod −3.5 °C) against complementary RNA was seen when the (3*S*,5*R*)-pyrrolidinone analogue was incorporated once into a decamer PNA oligomer.

Inspired by the recent publication by D'Costa et al. of the aminoethylprolyl PNA (not shown),[6] we decided to synthesize the reduced analogue of the pyrrolidinone PNA (II): the pyrrolidine PNA (III) (Figure 1, B = adenine). III (and aminoethylprolyl PNA) is also an analogue of the flexible Eth-PNA IV.[7] Incorporation of IV (Figure 1, B = thymine) into PNA oligomers was shown to destabilize duplex and triplex formation considerably.

Monomer Synthesis. The synthesis of the protected (2*R*,4*S*) adenine pyrrolidinine monomer **12** is shown in Scheme 1. *cis*-4-Hydroxy-D-proline was synthesized by the epimerization of *trans*-4-hydroxy-L-proline in 22% yield as described.[8] The secondary amine was Boc protected in 81% yield as described.[9] *N*-Boc-*cis*-4-hydroxy-D-proline methyl ester **1** is usually prepared by the diazomethane procedure.[10] Instead we prepared **1** in 99% yield by alkylating the cesium salt of the acid with MeI in DMF. **3** was prepared from **1** via **2** as described.[11] The azide **5** was prepared via the mesyl compound **4**. The Boc protecting group was cleaved with TFA, and the resulting secondary amine **6** was alkylated with methyl bromoacetate in the presence of DIEA. The azide **7** was reduced with concomitant Boc protection.[12] Standard TBAF cleavage of the TBS group then produced the novel pyrrolidine backbone **9**. At this point it was planned to introduce the adenine base under Mitsunobu conditions. However, all attempts to substitute the secondary hydroxy group by adenine using DEAD and PPh_3 failed for yet unknown reasons.

This is surprising since adenine is easily attached to the corresponding pyrrolidinone derivative using Mitsunobu conditions.[4] Instead adenine was introduced by converting **9** into the tosyl compound **10** and then displacing the tosyl group with benzyloxycarbonyl-protected adenine. Using adenine instead of benzyloxycarbonyl-protected adenine[13] gave a very low yield. For yet unknown reasons the Z group was lost during the reaction but the adenine derivative was readily reprotected using Rapoports reagent thus producing **11**.[14] ^{13}C NMR proved that the correct N9 isomer was obtained.[15] Finally **12** was synthesized by cleaving the methyl ester with $Ba(OH)_2$ and then precipitating $BaSO_4$ with H_2SO_4. In this way **12**·H_2SO_4 was recovered by lyophilizing the aqueous phase.

Oligomer Synthesis.[16] To evaluate the DNA and RNA recognition of the pyrrolidine PNA analogue, three PNA dodecamers were synthesized:

PNA 2005: H-TAC-TCA-TAC-TCT-LysNH$_2$
PNA 2075: H-TAC-TCA*-TAC-TCT-LysNH$_2$
PNA 2104: H-TAC-TCA#-TAC-TCT-LysNH$_2$

A*= (*3S, 5R*) pyrrolidone PNA monomer
A#= (*2R, 4S*) pyrrolidine PNA monomer (12)

Binding. The binding affinity toward complementary RNA and DNA oligomers was measured by obtaining the T_m

Scheme 1

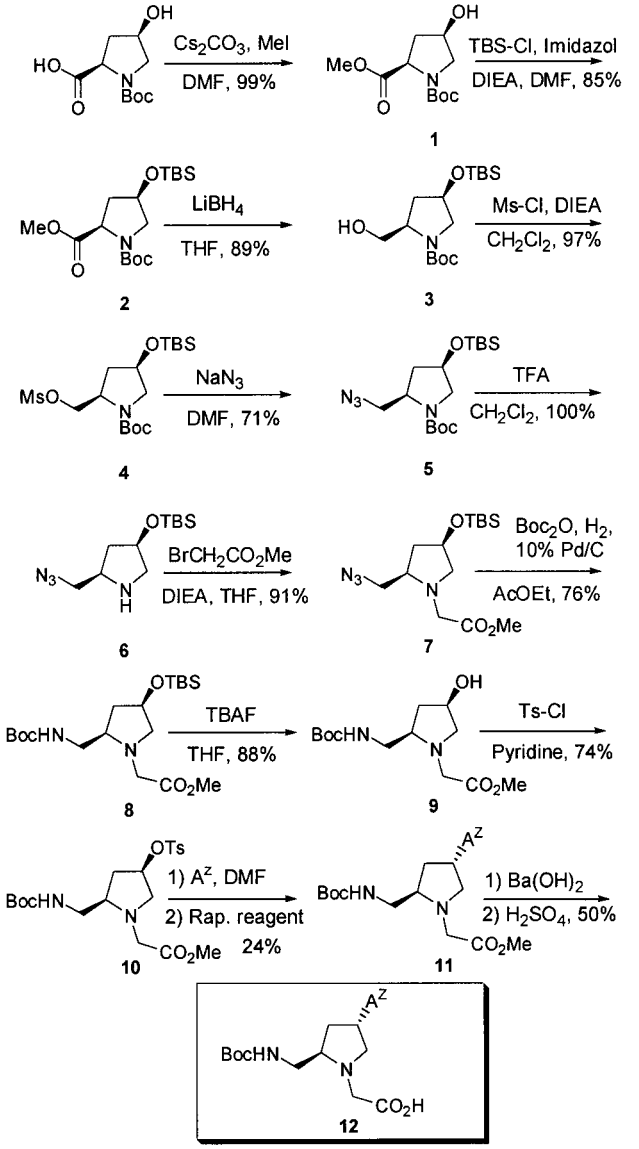

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Table 1. Melting Temperatures (T_m Values)[a]

entry	sequence	DNA	RNA
1	PNA 2005	49.5	59.5
2	PNA 2075	41.0	53.8
3	PNA 2104	28.5	44.5

[a] T_m = melting temperature (measured in medium salt buffer: 100 mM NaCl, 10 mM phosphate, 0.1 mM EDTA, pH = 7.0). Measured from 15 to 90 °C. Heating rate: 1 K/min. UV absorbance measured at 254 nm.

curves (Table 1). Not surprisingly, incorporation of both the pyrrolidinone and the pyrrolidine analogues into the PNA strand results in destabilization against DNA and RNA as compared to unmodified PNA (entry 1 vs 2 and 3). However, a larger destabilization was found in the case of the pyrrolidine analogue (entry 3) as compared to the pyrrolidinone analogue (entry 2).

Despite these unexpected and disappointing results, we decided to synthesize a fully modified decamer (PNA 2110):

PNA 186: H-Gly-$(A)_{10}$-NH_2
PNA 2020: H-$(A^*)_{10}$-LysNH_2
PNA 2110: H-$(A\#)_{10}$-LysNH_2

As can be seen (Table 2), the fully modified pyrrolidine

Table 2. Melting Temperatures (T_m Values)[a]

entry	sequence	5′-d$(T)_{10}$	5′-r$(U)_{10}$
1	PNA 186	55.5 (55.5)	35.5 (35.5)
2	PNA 2020	*b*	25.5
3	PNA 2110	79.0 (41.0)	60.0 (45.5)

[a] T_m = melting temperature (measured in medium salt buffer: 100 mM NaCl, 10 mM phosphate, 0.1 mM EDTA, pH = 7.0). Measured from 15 to 90 °C (values in parentheses are measured from 90 to 15 °C). Heating rate: 1 K/min. UV absorbance measured at 254 nm. [b] Not a sigmoid melting curve. Values in parentheses are T_m for the cooling curves.

decamer forms very stable complexes with DNA (ΔT_m/mod = +2.5 °C) and RNA (ΔT_m/mod = +2.5 °C) (compare entries 1 and 3). However, as opposed to the parent PNA 186, significant hysteresis was detected against both DNA and RNA, in the case of PNA 2110 indicating a slow rate of formation of the complex possibly due to a triplex structure. Obtaining the melting curves at pH 9 instead of pH 7 in the case of PNA 2110 only lowered the T_m values about 3.5 °C against DNA and 1.5 °C against RNA (not shown), thereby indicating only a modest electrostatic contribution to complex stability.

The complex between PNA 2110 and DNA was further evaluated. UV titration showed the complex between PNA 2110 and 5′-d$(T)_{10}$ to be a 1:2 complex. This complex may therefore be the first example of a DNA_2−PNA triplex structure with the PNA as Watson-Crick strand. Furthermore, the recognition between PNA 2110 and 5′-d$(T)_{10}$ was shown to be sequence specific (Table 3).

Table 3. Melting Temperatures (T_m Values)[a]

entry	sequence	X = T[b]	X = A[b]	X = C[b]	X = G[b]
1	PNA 186	55.5 (55.5)	nd	36.5 (36.5)	40.5 (40.5)
2	PNA 2110	79.0 (41.0)	67.5 (35.5)	71.0 (36.0)	68.5 (37.0)

[a] T_m = melting temperature (measured in medium salt buffer: 100 mM NaCl, 10 mM phosphate, 0.1 mM EDTA, pH = 7.0). Measured from 90 to 15 °C. Heating rate: 1 K/min. UV absorbance measured at 254 nm. nd = not determined. Values in parentheses are T_m for the cooling curves. [b] DNA target: 5′-d(TTT-TXT-TTT-T)-3′.

In conclusion, we have designed and synthesized a novel highly soluble PNA analogue: the pyrrolidine PNA analogue. The preliminary T_m data indicate that this analogue has strong affinity toward DNA and RNA although one should be cautious when extrapolating these data to mixed sequence complexes. Work is in progress to synthesize pyrrolidine PNA monomers other than the adenine monomer in order to synthesize fully modified mixed sequences.

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Supporting Information Available: Experimental details and characterization of compounds **1**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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