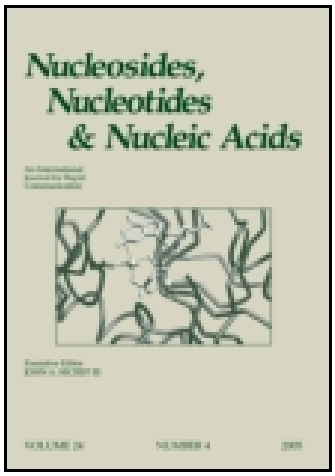

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Pyrrolidino-DNA

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Pyrrolidino-DNA

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ABSTRACT

We synthesized pyrrolidino-C-nucleosides, incorporated them into oligodeoxynucleotides and investigated their pairing properties. The thermal duplex and triplex stabilities were measured. While triplex formation is destabilized in the case of pyrrolidino-pseudo-U and -T, pyrrolidino-pseudo-iso-C leads to an increase of the T_m value for third strand dissociation. Duplexes are destabilized with all pyrrolidino-C-nucleosides.

Triplex formation between a triplex forming oligonucleotide (TFO) and a target DNA duplex can modulate gene expression on the level of transcription and is thus of interest as a tool in molecular biology and in human therapy (antigene therapy). Although TFOs bind with high specificity, their affinity is usually much weaker than that of the underlying DNA duplex. In part this is thought to be due to the charge repulsion resulting from bringing together three polyanionic DNA strands. Several studies have shown, that the introduction of positive charges in the sugar-phosphate backbone or at the bases can enhance triplex stability.[1] We reasoned that exchange of the 4′-oxygen by nitrogen in a nucleoside unit of a TFO would position a positive

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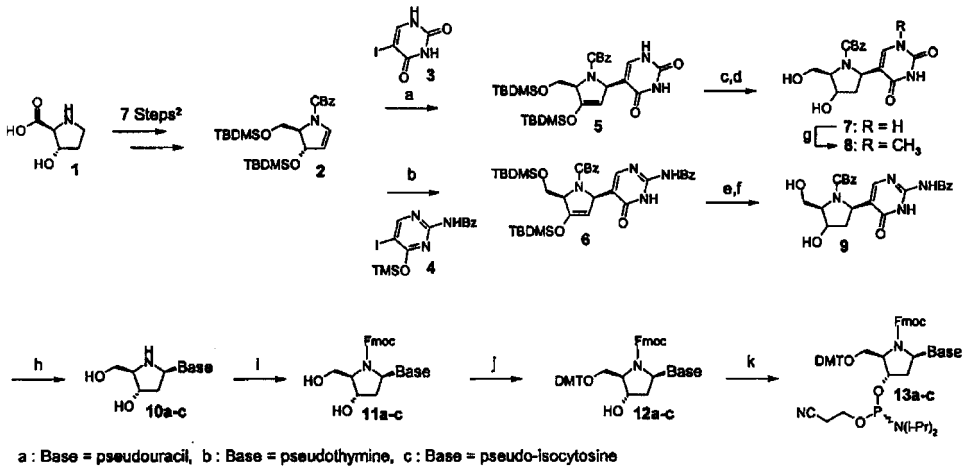

Scheme 1. (a) $Pd(OAc)_2$, $AsPh_3$, Bu_3N, DMF, 65°C, 20 h, (58%); (b) $Pd(OAc)_2$, $AsPh_3$, *N*,*N*-diisopropylethylamine, DMF, 80°C, 22 h, (63%); (c) AcOH, TBAF, THF, −15°C → rt, 37 h; (d) $NaB(OAc)_3H$, AcOH, MeCN, −15°C → rt, 20 min, (87%); (e) HF·pyridine, MeCN, rt, 6 h; (f) $NaB(OAc)_3H$, AcOH, MeCN, −15°C → rt, 5 h, (79%); (g) BSA, CH_3I, CH_2Cl_2, rt, 70 h, (77%); (h) H_2, Pd/C, MeOH, rt, 4 to 7 h, (quant.); (i) Fmoc-OSu, THF, dioxane, 5% $NaHCO_3$ soln., rt, 3 h, (65–91%); (j) DMT-Cl, pyridine, rt, 2 h, (65%); (k) (iPr$_2$N)($NCCH_2CH_2O$) PCl, iPr$_2$NEt, THF, rt, 2 h, (71–88%).

charge next to a phosphate unit of the duplex target, thus enhancing triplex stability. We therefore embarked on the synthesis of the pyrrolidino nucleosides depicted in Sch. 1.

Starting from trans-3-hydroxy-L-proline (**1**), CBz-protected enamine **2** was obtained in 7 steps in 52% overall yield.[2] The next steps were a palladium-mediated Heck coupling leading to **5** and **6**. The silyl ethers were then cleaved, and the obtained keto groups were stereoselectively reduced in one pot, to give nucleosides **7** and **9**. At this stage, CBz-protected pyrrolidino pseudo-U **7** can be converted into its N-1′-methyl derivative **8**. The pyrrolidine ring nitrogens in **7**–**9** were then reprotected with the Fmoc group which is compatible with standard oligonucleotide synthesis. Tritylation of the primary alcohol followed by reaction with the corresponding chloro phosphite reagent lead to building blocks **13a–c**. The same pathway was used as a new synthesis for pseudo-iso-C whereas pseudo-U is commercially available.

The different building blocks were incorporated into 15-mer oligonucleotides by standard oligonucleotide synthesis. The melting temperatures (T_m) of the modified oligonucleotides were measured and compared to the T_m of the natural sequence. The T_m values for triplex dissociation from UV-melting curve experiments of the strands containing pyrrolidino-pseudo-U and its N-1′-methyl derivative units are significantly lower compared to the natural oligomer.[3] We find relative destabilizations of triplex formation by ca. −13 to −1°C per mod. in a strongly sequence dependent mode. Both sugar and base modifications contribute to the destabilization. In the case of pyrrolidino-pseudo-iso-C (**Y**), the protonated pyrrolidine unit leads to a

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Table 1. T*m* values (°C) determined from UV melting experiments ($\lambda = 260$ nm). Duplex or triplex concentration = 1.2 μM; buffer: 140 mM KCl, 7 mM NaH_2PO_4, 0.5 mM $MgCl_2$; pH for duplex: 7.0.

Sequence	T_m (triplex)[a] [°C]	ΔT_m/mod [°C]	T_m (duplex)[b] [°C]	ΔT_m/mod [°C]
5′-TTTTTCTCTCTCTCT-3′	43.1	0	45.2	0
5′-TTTTTCT**Y**TCTCTCT-3′, pH 6.0	45.5	+2.4	39.1	−6.1
5′-TTTTTCT**Y**TCTCTCT-3′, pH 7.0	23.0	−20.1		
5′-TTTTTCT**Q**TCTCTCT-3′, pH 6.0	29.7	−13.4	43	−2.2
5′-TTTTTCT**Q**TCTCTCT-3′, pH 7.0	30.1	−13.0		
5′-TTTTT**Y**T**Y**T**Y**T**Y**T-3′, pH 6.0	53.0	+2.0	17.8	−5.5
5′-TTTTT**Y**T**Y**T**Y**T**Y**T-3′, pH 8.0	37.1	−1.2		
5′-TTTTT**Y**T**Y**T**Y**T**Y**T-3′, pH 9.0	28.0	−3.0		
5′-TTTTT**Q**T**Q**T**Q**T**Q**T-3′, pH 6.0	16.4	−5.3	24.6	−4.1
5′-TTTTT**Q**T**Q**T**Q**T**Q**T-3′, pH 8.0	13.5	−5.9		
5′-TTTTT**Q**T**Q**T**Q**T**Q**T-3′, pH 9.0	10.0	−6.6		
5′-TTTTT**Y**TCT**Y**TCTCT-3′, pH 6.0	46.9	+1.9	33.1	−6.1
5′-TTTTT**Y**TCT**Y**TCTCT-3′, pH 7.0	27.9	−7.6		
5′-TTTTT**Q**TCT**Q**TCTCT-3′, pH 6.0	38.3	−2.4	37.6	−3.8
5′-TTTTT**Q**TCT**Q**TCTCT-3′, pH 7.0	28.3	−7.4		

[a]Target duplex: 5′-CGTAAAAAGAGAGAGAGATCG-3′/5′-CGATCTCTCTCTCTTT-TTAGC-3′.
[b]Antiparallel complementary DNA strand: 5′-AGAGAGAGAGAAAAA-3′.

stabilization of the triplex by ca. 2°C per mod. at pH 6 (Table 1). Since the oligonucleotides containing pseudo-iso-C (**Q**) give less stable triplexes, we can conclude that the sugar modification is responsible for the stabilization in the latter case. The differential behavior between the pyrrolidino-C-nucleosides containing different bases is not clear at this point. For all the oligonucleotides containing pyrrolidino-C-nucleosides, the T_m of the hybrid duplex is lower than that of the unmodified duplex.

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