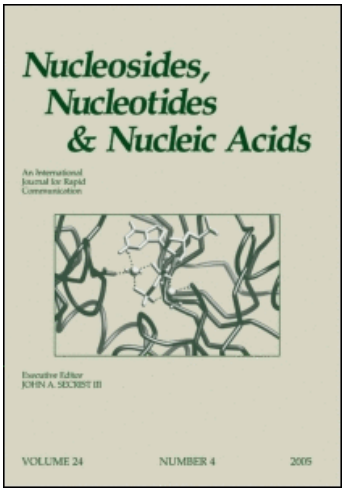

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Synthesis and Studies of Modified Oligonucleotides-Directed Triple Helix Formation at the Purine-Pyrimidine Interrupted Site

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ABSTRACT

Triple helix formation is still restricted to oligopurine-oligopyrimidine double stranded DNA target. Herein we focus on our progress achieved in nucleobase and oligonucleotide modifications area to address the chemical challenge to circumvent the recognition of a purine-pyrimidine base pair interruption in an oligopyrimidine-oligopurine DNA sequence.

Key Words: Triple helix; Artificial nucleobases; AT inversion.

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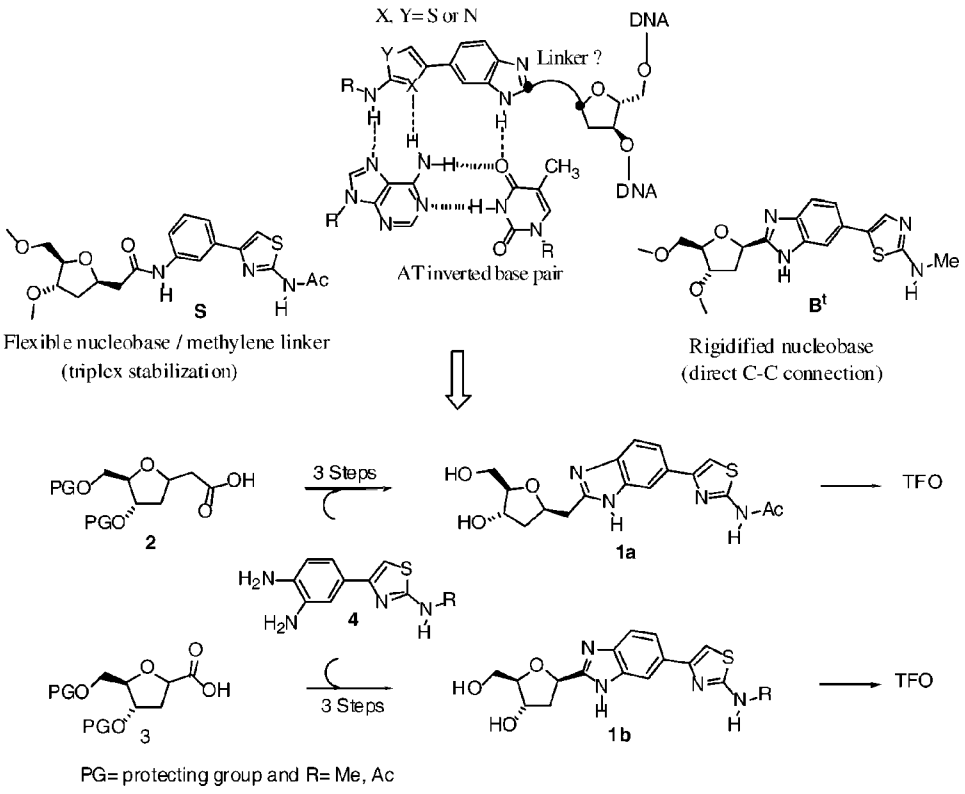
Triple helix-forming oligonucleotides bind in the major groove of oligopyrimidine-oligopurine double-stranded DNA (ds-DNA) sequences. In the pyrimidine motif, the molecular recognition process occurs between the oligonucleotide and the oligopurine strand of ds-DNA by formation of TA•T and CG•C^+ Hoogsteen base triplets.[1] Hence, this approach (antigene strategy) provides rational basis for the development of new tools in molecular biology and for therapeutic applications.[2] Unfortunately, this strategy has a major intrinsic limitation since, the interruption of polypurine tract by one or more pyrimidine bases usually results in significant reduced triplex stability.[3] Therefore, much efforts have been undertaken to overcome this sequence limitation but, no successful results have been so far reported especially in unnatural triplexes incorporating artificial nucleobases.[4]

We have recently observed that the use of an extended heterocyclic system like **S** within TFO (**S** facing the inverted AT base pair) highly stabilized the triplex formed between this TFO and the ds-DNA target (Sch. 1). Indeed, the obtained *Tm* values were found to be very close to those of canonical triplexes (Table 1).[5] Moreover, the incorporation of a rigidified nucleobase $\mathbf{B^t}$ into TFO, also induced an increased triplex stabilization[6] compared to the flexible analog (data not shown).



Scheme 1.

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```
26-mer ds-DNA    3'- CGTA-TTTTCTTCTCTTXTTCTT-AGTG-5'
target           5'- GCAT-AAAAGAAGAGAAYAAGAA-TCAC-3'
18-mer TFO            5'- TTTTCTTCTCTTZTTCTT -3'
```

Table 1. Melting temperature values (*Tm*) of all combinations of XYZ triplets (10 mM cacodylate, 100 mM NaCl, 10 mM $MgCl_2$, 0.5 mM spermine, pH 6).

	Z =	T	C	G	S	Bt
XY			*Tm* (°C)			
TA		51	31	31	42	37
CG		40	50	31	41	38
AT		33	33	45	50	43
GC		38	35	35	46	41

These findings inspired us to undertake an extensive structure-stability relationship study to assess the role of different molecular group.[a] Accordingly, we have recently synthesized, following two approaches, the modified nucleosides **1a** (**S** analog) and **1b** ($\mathbf{B^t}$ analog) featuring (i) a rigidified thiazolyl-benzimidazole nucleobase and (ii) the common aminothiazole ring in the best configuration (nitrogen-donor instead of sulfur atom). The modified nucleoside **1b** contains, compared to **1a**, a supplementary methylene group as a spacer between the sugar and the base. Incorporation of **1a** and **1b** into TFO together with triplex hybridization studies are in progress.

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