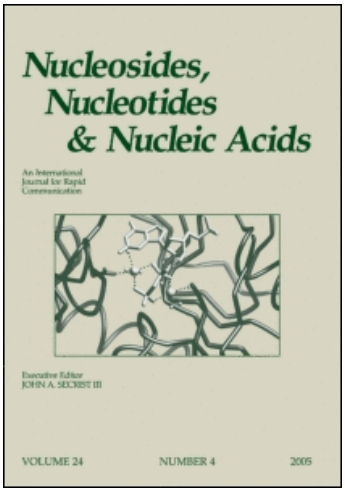

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 09 August 2003

To cite this Article Lecubin, Florence , Devys, Michel , Fourrey, Jean Louis , Sun, Jian-Sheng and Benhida, Rachid(2003) 'Synthesis and Triplex Binding Properties of Oligonucleotides Containing a Novel Nucleobase', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1281 — 1284
To link to this Article: DOI: 10.1081/NCN-120022946
URL: http://dx.doi.org/10.1081/NCN-120022946

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1281–1284, 2003

Synthesis and Triplex Binding Properties of Oligonucleotides Containing a Novel Nucleobase

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ABSTRACT

The thiazolo-indole compound **1** bearing the complementary donor-acceptor-donor sites (dad) was designed for specific recognition of an AT inverted base pair in pyrimidine triple helix motif. It was successfully incorporated into 14-mer oligonucleotide using a serinol unit as sugar derivative. The triple helix hybridization studies were examined by means of thermal denaturation experiments with a 26-mer DNA duplex containing the AT inverted base pair.

Key Words: Serinol-nucleobase; TFO; AT interruption.

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DOI: 10.1081/NCN-120022946
1525-7770 (Print); 1532-2335 (Online)

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Triple helix forming oligonucleotides (TFO) belonging to the pyrimidine motif, are subject of considerable attention as potential gene regulation agents.[1] Oligodeoxynucleotides can interact with double-stranded DNA to form local triple-helical structures. Pyrimidine TFO bind specifically in the major groove of DNA parallel to the purine strand throught the formation of Hoogsteen TA•T and CG•C^+ triplet.[2] However, the stability of the triple helix depend of numerous factors and it is dramatically reduced by the presence of one or few pyrimdine bases in the oligopurine strand of DNA duplex.[3] Hence, to date, the recognition of mixed purine/pyrimidine sequences remains a challenge.[4] Herein, we describe the synthesis of a new non-natural extended heterocyclic base **1**, which was designed on the basis of molecular modeling studies for the recognition of an AT inverted base pair as shown in Fig. 1.

For the incorporation of nucleobase **N** into TFO, we investigated in the first time to connect this aglycone base to a serinol derivative, an acyclic analogue of the natural 2′-deoxyribose. The synthesis of the targeted compound **1** is based on the preparation of three precursors: haloindole-acetate, 2-aminothiazolyltin derivative and serinol system.[5]

We used in this synthesis as key steps : an intramolecular Wittig reaction for acyl-indole construction followed by a Stille type coupling and amide formation as the last step for serinol-aglycone connection (Sch. 1).

The obtained compound **1** was phosphitylated and incorporated into 14-mer TFO using standard automated oligonucleotide synthesis. The triplex binding properties of these pyrimidine TFOs (**Z** = N, T : 2′-deoxythymidine and Ts : thyminyl-serinol) were examined by means of thermal denaturation experiments with the 26-mer ds-DNA containing the AT interruption (**XY** = AT)

26-mer ds-DNA	3′- TGTCAA-TTCTTCTTXTTTCT-AACTCG-5′
target	5′- ACAGTT-AAGAAGAAYAAAGA-TTGACG-3′
TFO	5′- TTCTTCTTZTTTCT-3′

We observed that the replacement of **T** by **Ts** (serinol instead of 2′-deoxyribose) in third strand at position **Z** caused a high destabilization of the triplex ($\Delta Tm = -17°C$, Table 1). It seems that this destabilization is due in part to the flexible serinol backbone. Furthermore, in the case of the studied **AT** inverted base pair (**XY** = AT), all TFO nucleobases at position **Z** (T, Ts and N) gave a low *Tm* value

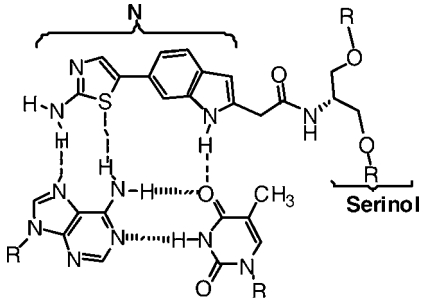

Figure 1. The proposed AT•N interaction.

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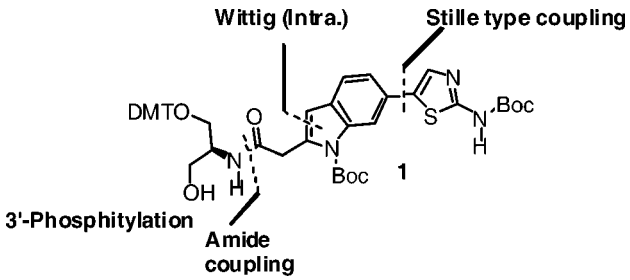

Scheme 1. The synthetic strategy.

Table 1. Melting Temperature values (*Tm*) of all combinations of XYZ triplets (20 mM cacodylate, 100 mM NaCl, 10 mM $MgCl_2$, pH 6).

XY	Z=	T	Ts *Tm* (°C)	N
TA		37	20	15
CG		24	14	13
AT		10	10	10
GC		26	15	14

(*Tm* = 10°C). Taken altogether these data indicate that the flexible serinol backbone could account for the decreased triple helice stability.

ACKNOWLEDGMENTS

We thank Dr. M. Thomas for oligonucleotide syntheses, ANRS for financial support and the Ligue Nationale pour la Recherche sur le Cancer for a doctoral fellowship to FL.

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