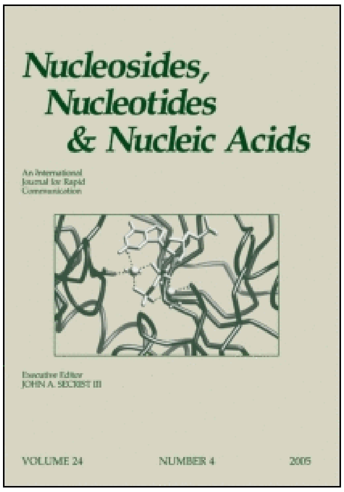

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# Oligonucleotides Containing an Acridine Group Covalently Bonded to the Nucleotide Flanking the 3'-3' Phosphodiester Junction for Alternate Strand Triple Helix Formation

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# Oligonucleotides Containing an Acridine Group Covalently Bonded to the Nucleotide Flanking the 3′-3′ Phosphodiester Junction for Alternate Strand Triple Helix Formation

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## ABSTRACT

Oligonucleotides with a 3′-3′ inversion of polarity and containing an acridine group attached to nucleotide base flanking the 3′-3′ phosphodiester bon have been synthesized, characterized and used as third strand in alternate triple helix formation. CD melting studies and molecular mechanics calculations have been carried out to investigate these triplex structures.

*Key Words:* Oligonucleotide; Acridine conjugate; Triplex; 3′-3′-phosphodiester bond.

Synthetic oligonucleotides (ODNs) can bind the major groove of double stranded DNA having oligopurine tracts, forming local triple helices by sequence specific hydrogen bonding. To extend the range of applicability of this gene control strategy, otherwise confined at the presence, on the target gene, of a long (at least

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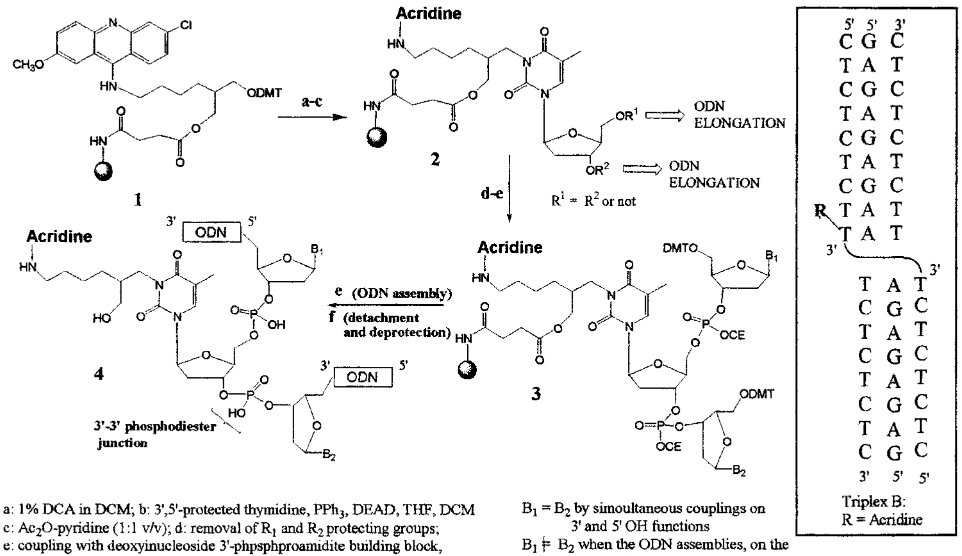
16–17 bases) homopurine sequence, it is possible to form triplex structures in which the duplex is composed of two adjacent and alternating oligopurine-oligopyrimidine tracts. In this approach called "alternate strand triple helix formation" ODNs having a 3′-3′ or 5′-5′ internucleoside junction simultaneously hybridise the adjacent purine tracts by switching strand at the junction between the oligopurine and the oligopyrimidine domains of the duplex.[1] To increase the stability of the triplex structure ODNs carrying an intercalator, covalently linked to triple helix forming oligonucleotides (TFOs) can be used. This approach has been recently proposed by Matsuda and coworkers who synthesized ODNs carrying an intercalator attached to a pentaerythritol used as 3′-3′ linker.[2]

In the last years we focused our attention on the synthesis of ODNs containing a 3′-3′ inversion polarity motif and able to hybridize the target duplex, in parallel mode, by formation of Hoogsteen triplets.[3] Our results on the stability of the so obtained triplex structures indicated the phosphodiester bond as a good 3′-3′ junction into TFOs. We report here an easy and convenient synthetic method for TFOs carrying a metoxyacridine derivative on the base of the nucleotide flanking the 3′-3′ phosphodiester bond.

Our approach uses a commercially available solid support functionalised with 2-metoxy-6-chloroacridine (**1**, Sch. 1) on which, after removal of DMT, a suitable 3′,5′-protected thymidine is attached trough the N-3 position of the pyrimidine base using Mistunobu condensing conditions. The obtained support **2** resulted stable under the chemical treatments required by the DNA chain assembly and in the final detachment/deprotection procedures. In fact, support **2**, after capping of unreacted hydroxy functions and successive removal of 3′,5′-protecting groups, reacted almost quantitatively with the 5′-DMT-thymidine-3′-phosphoramidite under standard



a: 1% DCA in DCM; b: 3',5'-protected thymidine, $PPh_3$, DEAD, THF, DCM
c: $Ac_2O$-pyridine (1:1 v/v); d: removal of $R_1$ and $R_2$ protecting groups;
e: coupling with deoxyinucleoside 3'-phpsphproamidite building block, 0.1 M tetrazole; $I_2$-THF-$H_2O$; f: NaOH 0.1M ($CH_3OH/H_2O$ 4:1 v/v)

$B_1 = B_2$ by simoultaneous couplings on 3' and 5' OH functions
$B_1 \neq B_2$ when the ODN assemblies, on the 5' and 3' functions, are performed in two distinct steps

*Scheme 1.*

automated procedure, giving support **3** (B1 = B2 = Thymine). Support **2** and **3** treated with a solution of 0.4 M of NaOH furnished respectively the expected acridine-nucleoside and the acridine-trinucleotide deivative containing a 3′-3′ phosphodiester function. Support **3**, reacting simultaneously on both 5′ OH functions can afford the ODN 5′-$(CT)_4$-$T^{Acr}$-3′-3′$(TC)_4$-5′ (**4**). Alternatively when in support **2** $R^1$ and $R^2$ are suitable orthogonal protecting groups two different ODN sequences can be sequentially synthesized on the 5′ and the 3′ OH functions.

Preliminary structural studies by mechanical and dynamic molecular calculation on Triplex A and B (respectively without and with acridine moiety) were performed.

Molecular mechanics (MM) calculations revealed no great overall structure distortion of Triplex A model, compared to the experimentally determined triple helices parameters[4] used as starting point (heavy atoms RMSD value of 5.56 Å), demonstrating that the 3′-3′ phosphodiester junction, introduced in to the third strand to give the appropriate inversion of polarity, give rise to structurally and energetically stable triple helices, according to previously obtained experimental results.[3,5] The R configuration of the linker-acridine moiety has been introduced in Triplex B replacing the appropriate thymine N3 hydrogen of TFO. The conformational space of the selected linker has been explored in order to appropriate dock the acridine moiety in to the major groove. The resulting structure was subjected to an energy minimization protocol. The energy minimized structure of Triplex B showed the acridine ring, can intercalate without further loss of hydrogen bond interactions with respect to Triplex A (heavy atoms RMSD value of 0.56 Å). Extensive MD studies will be performed on Triplex B.

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