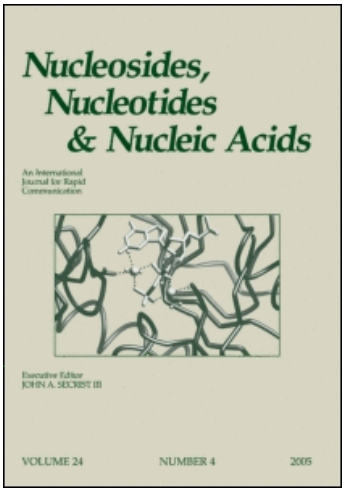

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SYNTHESIS OF CIRCULAR OLIGODEOXYNUCLEOTIDE CONJUGATES *VIA* TRANSIENT ABASIC SITES.

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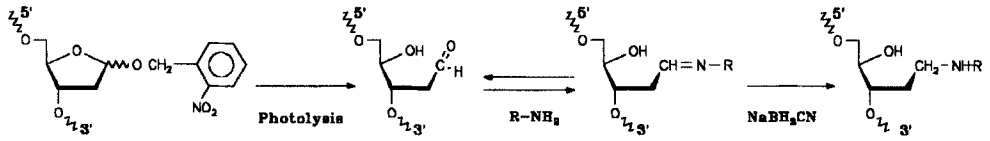
Abstract: New chemical ligation or cyclisation reactions, using high reactivity of abasic sites with amines, are reported for the synthesis of oligonucleotide clamps and single-stranded circular oligonucleotides. Thermal denaturation experiments show that these molecules display very high binding affinities for complementary DNA oligomer by forming triple-helical complexes.

There has been great interest recently in developing oligonucleotides as regulators of cellular nucleic acid function[1]. However, the potential of antisense/ antigene oligonucleotides to regulate genes involved in diseases is considerably limited by their low binding affinity, their instability due to cleavage by endogenous exo- and endonucleases and also by their low efficiency of cellular uptake.

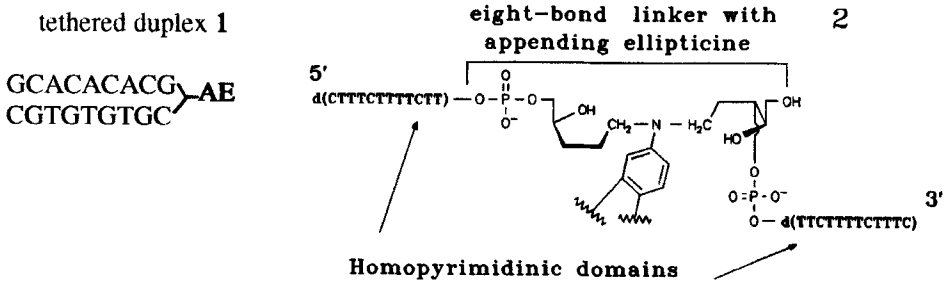
Several studies have shown that oligonucleotide clamps containing two pyrimidinic sequences display strong affinity for single-stranded polypurine oligomer targets by forming triple-helices[2,3]. Circular oligonucleotides can also bind with high affinity to complementary single-stranded RNA or DNA and display exceptional resistance to degradation by nucleases[4,5].

Thus, we have developed new chemical ligation or cyclization reaction using the high reactivity of deoxyribose residues located within abasic oligodeoxynucleotides towards various amines in reductive conditions and we have synthesized oligonucleotide clamps and circular oligonucleotides, in which the Watson-Crick and Hoogsteen base-pair-forming portions were tethered by a linker containing the intercalating 9-aminoellipticine (9-AE). The presence of an appending ellipticine residue in the linker might improve membrane permeability by increasing the lipophilicity of oligonucleotide clamps and circular oligomers.



Oligonucleotide Precursor *Abasic oligomer* *Schiff-base intermediate* *Derivatized oligonucleotide*

Scheme 1: General procedure for synthesis of conjugate oligonucleotide via abasic sites.

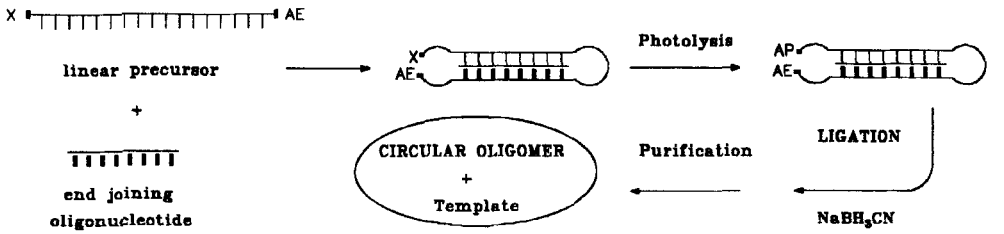


Scheme 2 *cross-linked duplex 1 and oligonucleotide clamp 2*

We have previously developed a general method for the efficient synthesis of oligodeoxynucleotides containing abasic sites in selected positions [6]. It was also demonstrated that the photolabile o-nitrobenzyl group is suitable for the protection of the anomeric hydroxyl function of deoxyribofuranoses during the synthesis of abasic oligonucleotides.

Additionally we have shown that oligonucleotides bearing an abasic residue can be easily and efficiently derivatized with various amino compounds [7]. Indeed, the high reactivity of deoxyribose residues within abasic oligonucleotides towards amines has been used successfully to create covalent linkages, by reductive amination reaction with sodium cyanoborohydride (according to *Scheme 1*).

We observed that the secondary amine function of the 9-aminoellipticine conjugates can further react with acetaldehyde in the presence of reducing sodium cyanoborohydride ($NaBH_3CN$), yielding a N9-alkylated-aminoellipticine derivatized oligonucleotide. Thus, the possibility of sequentially reacting two different aldehyde compounds with 9-AE was extended to the synthesis of an interstrand cross-linked duplex oligonucleotide **1**. UV melting experiments revealed a strong stabilizing effect



X = protected abasic site AP = abasic site

Scheme 3

Table 1: Thermal stability T_m of complexes formed between oligomers **2**, **3** and **4** and the target.

Oligonucleotides	**Tm** of complexes (°C) with 5'AAGAAAAGAAAG3'	
	pH 7	**pH 5**
3'TTCTTTTCTTTC **4**	41.6	41.4
AE– TTCTTTTCTTTC3' / TTCTTTTCTTTC5' **2**	50.8	55.7
AE– CTTCTTTTCTTTCCA C / CTTCTTTTCTTTCCA **3**	57.4	64.6

Conditions: 3 µM (each strand) oligonucleotide in 10 mM $MgCl_2$, 100mM NaCl and 10 mM Tris.HCl (pH 7.0) or 10 mM AcONa (pH 5.0).

induced by the interstrand cross-linkage when compared to similar unmodified duplex (83°C *versus* 53°C). In the same way, an oligonucleotide clamp **2** having two runs of pyrimidines and bearing a 8-bond linker which contains an 9-aminoellipticine residue was synthesized (*Scheme2*).

Using the same procedure, we have synthesized a circular hybrid molecule **3** which contains two opposed pyrimidine-rich binding domains (Watson-Crick and Hoogsteen domains) bridged by a 5-nucleotide loop at one extremity and by a 10-bond linker with an appending ellipticine residue at the other.

The 31-base circular oligonucleotide **3** was synthesized by a template-directed cyclization of the corresponding linear precursor. The role of the template was to bring the reactive 3'-abasic site (AP) and the opposite 5'-end bearing the aminoellipticine residue close to each other in order to favour an intramolecular reaction in reductive conditions (*Scheme 3*). Conversion to circular product was 65 %, as judged by HPLC analysis.

Hybridization studies of these oligonucleotide clamps and circular oligonucleotides with a short polypurine oligonucleotide target confirm that **bimolecular triple-helical complexes** were formed, and that theses complexes were considerably more stable than the comparable Watson-Crick duplex *(Table1)*. Indeed, the triple-helix formed between circular compound **3** and the purine oligonucleotide exhibited a melting transition (T_m) of 57.4°C, which is 15.8°C higher than that of the corresponding Watson-Crick duplex and 6.6°C higher than that of the triple-helical complex formed with the oligonucleotide clamp **2**. In addition to the high melting transitions, the pH dependence provide further support for the formation of triples-helices.

In conclusion, a new chemical cyclisation reaction was developed using the reactivity of abasic sites with amines and we have demonstrated that the covalent ring closure (cyclisation) of triple-helix forming oligonucleotides considerably increases their binding affinity. The binding of single-stranded RNA or DNA with circular oligomers represents a new strategy in single-stranded oligonucleotide recognition for blocking translation, splicing and reverse transcription.

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