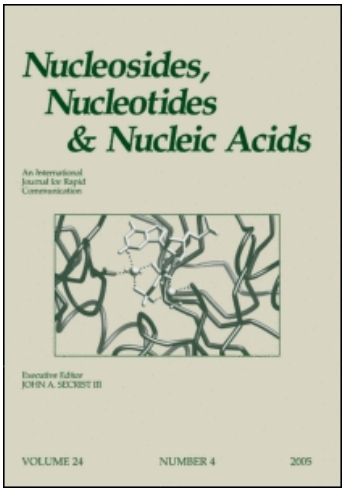

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A Solid-Phase Method for the Synthesis of Small to Medium-Sized Cyclic Oligonucleotides

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A SOLID-PHASE METHOD FOR THE SYNTHESIS OF SMALL TO MEDIUM-SIZED CYCLIC OLIGONUCLEOTIDES.

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Abstract. Cyclic oligonucleotides (2- to 30-mer) are synthesized by a solid-phase method, for both chain elongation and cyclization, employing a new linker and standard phosphoramidite chemistry. Fairly pure crude products (>90% by HPLC) are obtained.

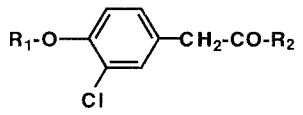
The therapeutic use of oligonucleotides as anti-gene or antisense agents is limited because of their sensitivity to degradation by nucleases, mainly exonucleases. The increased exonuclease resistance of cyclic oligonucleotides has stimulated interest in developing reliable synthetic methods for their preparation. In addition, cyclic sequences are excellent models for the study of nucleic acid structure.

The synthetic methods described so far are not universally applicable to the preparation of cyclic oligonucleotides regardless of size or sequence. On the one hand, small cyclic oligonucleotides have been obtained by chemical cyclization of protected oligonucleotides, either in solution[1] or on a solid support[2]. On the other hand, the synthesis of rather large cyclic sequences is achieved by template-directed cyclization methods of unprotected oligonucleotides, through duplex[3] or triplex[4] formation. Therefore, medium-sized cyclic oligonucleotides (10- to 30-mer) have been up to now the most difficult to prepare.

We report on a simple, general and effective method for the synthesis of small- to medium-sized cyclic oligodeoxyribonucleotides. Both the elongation of the oligonucleotide chain and the cyclization reaction are carried out on a solid support.

A new linker, 3-chloro-4-hydroxyphenylacetic acid **1**, is used to anchor the oligonucleotide chain to the solid matrix. The reaction between its 2,4,5-trichlorophenyl ester derivative **2** and a 5'-DMT-nucleoside cyanoethylphosphoramidite in the presence of tetrazole, followed by oxidation, provides the nucleotide-linker **3** (R_3=CNE; N= T, dC^{Bz}, dA^{Bz}, dG^{iBu}), which is anchored to an amino-derivatized support (polystyrene or polyethyleneglycol-polystyrene) to afford the nucleotide-resin **4** (R_3=CNE). From **4**, chain elongation takes place by the standard phosphite-triester approach using commercially

available methylphosphoramidites. After the chain assembly, removal of the 5'-DMT and CNE groups (Et_3N/Pyr, 1:1, 1h) allows the regioselective condensation of the 5'-terminal OH and the 3'-phosphate using 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (0.1M in Pyr, 12-24 h) as the cyclization reagent. Finally, the cyclic oligonucleotide is obtained after the following steps: the phosphate methyl protecting groups are removed first ($PhSH/Et_3N$), the product is then cleaved from the solid support with TMG *syn*-pyridine-2-aldoximate (0.1M in dioxane/water, 8-16 h) and the nucleobases are deprotected with conc. NH_3. After Sephadex gel-filtration the crude cyclic oligonucleotides are analyzed, purified and characterized by usual procedures. The synthesized cyclic structures show the correct nucleoside composition upon complete digestion, increased resistance to exonuclease degradation (calf spleen phosphodiesterase) and the expected mass when analyzed by mass spectrometry (negative electrospray or MALDI-TOF).



1	R_1=H	R_2=OH
2	R_1=H	R_2=O-$Cl_3C_6H_2$
3	R_1=DMT-N-O-P(O)-OR_3	R_2=O-$Cl_3C_6H_2$
4	R_1=DMT-N-O-P(O)-OR_3	R_2=NH-Ⓟ

Other phosphate-protecting schemes have been explored with fairly similar results. For instance, the synthesis can start with a **4** analogue (R_3=Me) and the chain elongation can be carried out using CNE-phosphoramidites. In a different approach, all the phosphates may be protected with the CNE group, but only if the 3'-terminal CNE protecting group (in **4**) is removed before assembling the rest of the chain.

Using these strategies, several cyclic oligonucleotides within the range of 2- to 30-mer have been prepared, containing all the nucleobases and without sequence restrictions. Yields of crude products depend on the size of the cyclic structure (up to 50% for the smallest cycles, 10% or less for the largest ones). However, highly pure crude cyclic oligonucleotides are obtained (typically >90% by HPLC). This fact illustrates the power of this solid-phase synthetic method, since during the cleavage step the non-cyclisized product and other impurities remain anchored to the support through a phosphate diester bond, whereas the phosphate triester-linked cyclic molecule is removed from the resin.

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