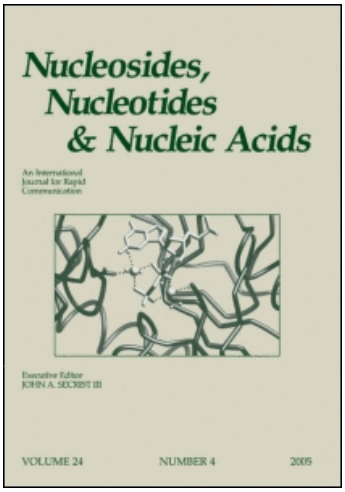

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Progress in the Synthesis of Cyclic Deoxyribo- and Oligoribonucleotides

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PROGRESS IN THE SYNTHESIS OF CYCLIC DEOXYRIBO- AND OLIGORIBONUCLEOTIDES

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ABSTRACT: The preparation of purine-rich sequences of cyclic DNA, up to a 28-mer, has been achieved. The products were purified by HPLC and PAGE (larger circles) and fully characterized. Cyclic RNA synthesis can be carried out using the same methodology as for cyclic DNA, provided that a single deoxynucleoside or a 2'-O-methylribonucleoside is placed at the 3'-end of the linear precursor.

We have recently shown that cyclic oligodeoxyribonucleotides can be efficiently obtained by a procedure in which both the chain elongation and the cyclization reaction take place on a solid support (polystyrene or polyethyleneglycol-polystyrene)[1]. The key innovation of the procedure was a new linker, 3-chloro-4-hydroxyphenylacetic acid, that forms a phosphate diester with the 3'-end of the oligonucleotide sequence and an amide with the amino groups on the solid matrix. We report now on our recent progress in the development of this methodology for the preparation of cyclic DNA fragments as well as for the synthesis of cyclic oligoribonucleotides.

Cyclic DNA. In order to explore the limits of our synthesis method we have prepared several short to medium size cyclic oligodeoxyribonucleotides (8 to 28-mer) with purine-rich sequences (up to 82%). Yields of crude products vary from 35% for the 8-mer to 7% for the 28-mer. HPLC profiles show a main peak for the smallest circles (90% in the case of the 8-mer), but complex and non-reproducible HPLC traces are observed for the larger circles. For the analysis and purification of such products PAGE proved to be superior to HPLC, showing a major band in all cases. Purified cyclic oligonucleotides give the expected mass when analyzed by MALDI-TOF mass spectrometry.

We have already mentioned that the oximate treatment used to detach the cyclic product from the support is able to cleave phosphate triesters but not phosphate diesters.

Therefore, an interesting property of the method is that the detached cyclic products exhibit a high degree of homogeneity. However, we have seen that the oximate treatment partially cleaves the amide bond that links the oligonucleotide to the resin. Thus, when a non-cyclized oligonucleotide-resin is treated with oximate a fairly pure product is detached, typically in 10% yield, that has the structure of the linear oligonucleotide-linker and which is completely digested by SpPD and has a correct nucleoside composition. With respect to the cyclic oligonucleotide, the mass of this product is 187 units higher and the mobility on PAGE is generally lower. In this respect, it is of the greatest importance that the desired cyclization reaction take place in reasonably good yield because, otherwise, the linear oligonucleotide bound to the linker may be detached from the support.

Cyclic RNA. Of the two commercially available and most popular ribonucleoside phosphoramidites, which have the 2'-OH protected with TBDMS or Fpmp groups, the former was discounted for the preparation of cyclic RNA because TBDMS groups are known to be unstable to the oximate treatment used to detach the cyclic product from the support. This undesired early deprotection of 2'-hydroxyl groups may then result in phosphate migration and the formation of products with 2'-5' phosphate diester linkages.

Our first unsuccessful attempts to obtain cyclic RNA using 2'-O-Fpmp ribonucleoside phosphoramidites led us to consider whether steric hindrance in the vicinity of the 3'-phosphate diester group prevents cyclization. In order to evaluate if the bulky 2'-O-Fpmp substituent at the 3' ribonucleoside was the reason for cyclization failure, we prepared three nucleotide-resins with differently-hindered phosphate groups: T-*R*, U_{OMe}-*R* and U_{OFpmp}-*R* (T=thymidine, U_{OMe}=2'-O-methyluridine, U_{OFpmp}=2'-O-Fpmp-uridine, *R*=TentaGel resin). The following dimers were assembled on these nucleotide-resins and dinucleotide-resins T-T-*R*, U_{OFpmp}-T-*R*, T-U_{OMe}-*R*, T-U_{OFpmp}-*R* and U_{OFpmp}-U_{OFpmp}-*R* were submitted to cyclization, deprotection and cleavage (yields of crude product were 30%, 48%, 43%, 14% and 3%, respectively). Cyclic dinucleotides c(TT), c(UT) [which is obviously identical to c(TU)] and c(TU_{OMe}) were analyzed and purified by HPLC and gave the exact expected mass when analyzed by electrospray mass spectrometry. c(UU) could not be isolated. In our opinion these results prove the validity of our hypothesis, so that with the current synthetic scheme, cyclic RNA can only be obtained if the linear precursor attached to the support has a 2'-O-methylribonucleoside or a deoxynucleoside at the 3'-end. Work is in progress to prepare larger RNA chimeras using this approach and to allow "all-ribonucleoside" cyclic RNA to be obtained.

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