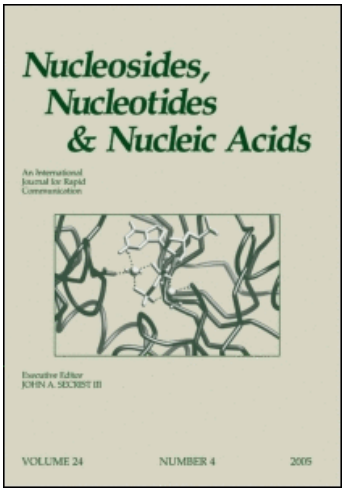

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SYNTHESIS AND ^{1}H-NMR STUDIES OF DNA-RNA HYBRIDS FOR STRUCTURAL ANALYSIS

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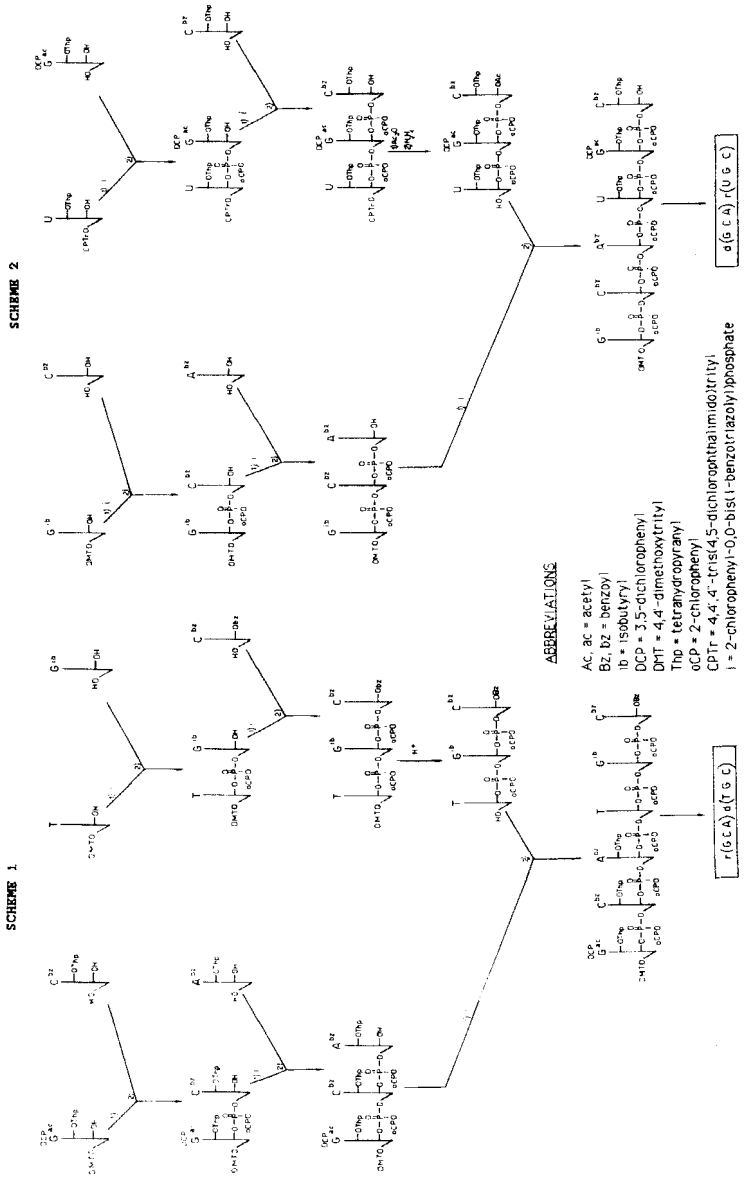
ABSTRACT. We report on the chemical synthesis of two short DNA-RNA hybrids in solution. Examples of the sequential assignment of the resonances of their two-dimensional NOESY spectra are presented.

DNA-RNA hybrid molecules occur naturally at different stages of the transfer of biological information, for example in the initiation of DNA replication and during transcription or reverse transcription.

It is well known that DNA-DNA duplexes preferentially adopt a B-type conformation while RNA-RNA duplexes form an A-type helix. We are studying the conformation of nucleic acids in solution with a combined use of nuclear magnetic resonance and restrained molecular dynamics. For this purpose we synthesized two short DNA-RNA hybrids, 5'[d(GCA)r(UGC)]$_2$ and 5'[r(GCA)d(TGC)]$_2$. These sequences were chosen to allow a direct comparison with the analogue DNA and RNA oligonucleotides 5'd(GCATGC)$_2$ and 5'r(GCAUGC)$_2$ whose three-dimensional structures had previously been determined[1,2].

Quite large amounts (10 μmoles) of very pure material are necessary to carry out high-resolution ^{1}H-NMR studies. After the good results obtained with the synthesis in solution of the hexaribonucleotide 5'r(GCAUGC)$_2$[3], we decided to approach the preparation of the two hybrid sequences in a similar way (schemes 1 and 2).

Standard N-benzoyl and isobutyryl protection was used for the deoxyribonucleosides as well as for adenosine and cytidine. Guanosine was double protected[4] with acetyl at the exocyclic amino function and 3,5-dichlorophenyl (DCP) at the O^6-position to increase the lipophilicity of the intermediates and therefore simplify their chromatographic purification.



The 2'-OH position of the ribonucleosides was blocked by the non migrating tetrahydropyranyl group. Temporary protection of the 5'-OH was achieved with DMTr in the case of deoxyribonucleosides and with 4,4',4''-tris(4,5-dichlorophthalimido)trityl (CPTr)[5,3] for the uridine moiety. The two 3'-terminal nucleosides were acylated at the 3'-OH in order to enhance the lipophilicity of the final products.

Trimer blocks were constructed in a stepwise manner by the benzotriazolyl-phosphotriester approach[6] and the products isolated in good yields (75-90%) after column chromatography. Lower yields were obtained for intermediates containing deoxyguanosine, due to a partial loss of material during chromatographic purification.

After phosphorylation of the 5'-terminal trimers and removal of the temporary 5'-OH protecting group of the 3'-terminal trimers, appropriate blocks were condensed[7] in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride and 1-methylimidazole to yield the fully protected hexamers. The products were deblocked in three steps as described previously[3]. After extraction with diethylether, the crude products were purified by ion-exchange chromatography on DEAE-Sephadex A25 under denaturing conditions using a linear gradient of NaCl (0.1-0.4M); the hexamers were thereby converted into their sodium salts. Removal of urea and salt and concentration were achieved by solid phase extraction on a Baker-10 SPE reverse phase C18 column. A final desalting on Sephadex G25 gave compounds suitable for 500 MHz ^{1}H-NMR studies. The overall yields after deblocking and purification were 30-50%.

500 MHz ^{1}H-NMR spectra were recorded at 20°C in either 99.995% D_2O or 90% H_2O/10% D_2O. Oligonucleotides were 4-5mM in single strand and found double stranded under the experimental conditions employed

The NOESY spectra show connectivities between all protons that are separated by short distances (≤5Å) within the spatial structure. The sequential assignment of the NOE connectivities along the H1'(i-1)<-->H8/H6(i)<-->H1'(i) pathway unambiguously confirmed the correct sequence of both hybrid hexamers. An example of this sequential assignment is given in Fig.1. The pathway along the sugar protons in a guanosine residue is shown in Fig.2.

The complete assignment of the resonances of the nonexchangeable protons of 5'[r(GCA)d(TGC)]$_2$ will be reported elsewhere. Assignment

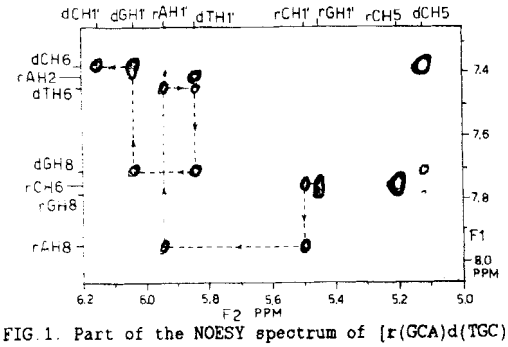


FIG. 1. Part of the NOESY spectrum of $[r(GCA)d(TGC)]_2$ showing cross peaks between the H1' and the nucleobase protons.

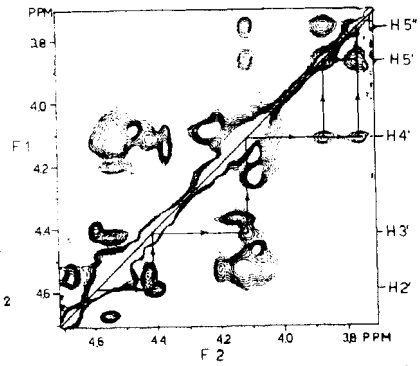


FIG. 2. Pathway of the sugar protons of the 5'-terminal guanosine of $[r(GCA)d(TGC)]_2$.

of the resonances of 5'$[d(GCA)r(UGC)]_2$ and refinement of the three-dimensional structures in solution of the two hybrid sequences by restrained molecular dynamics are currently carried out.

ACKNOWLEDGEMENTS

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