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OLIGONUCLEOTIDE SYNTHESIS ON A CROSSLINKED POLYACRYLMORPHOLIDE SUPPORT

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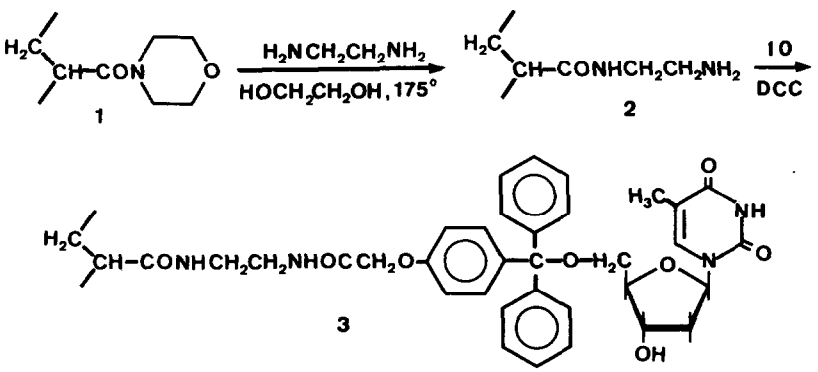
We wish to report the convenient stepwise synthesis of the pentanucleotide d-TpTpTpTpT, using commercially available crosslinked polyacrylmorpholide as an insoluble support. The conversion in each step ranged between 84 and 90% based on the next lower homolog. The polymer, which was designed as a column packing material for organic gel filtration[1], swells both in water and in polar organic solvents such as pyridine.

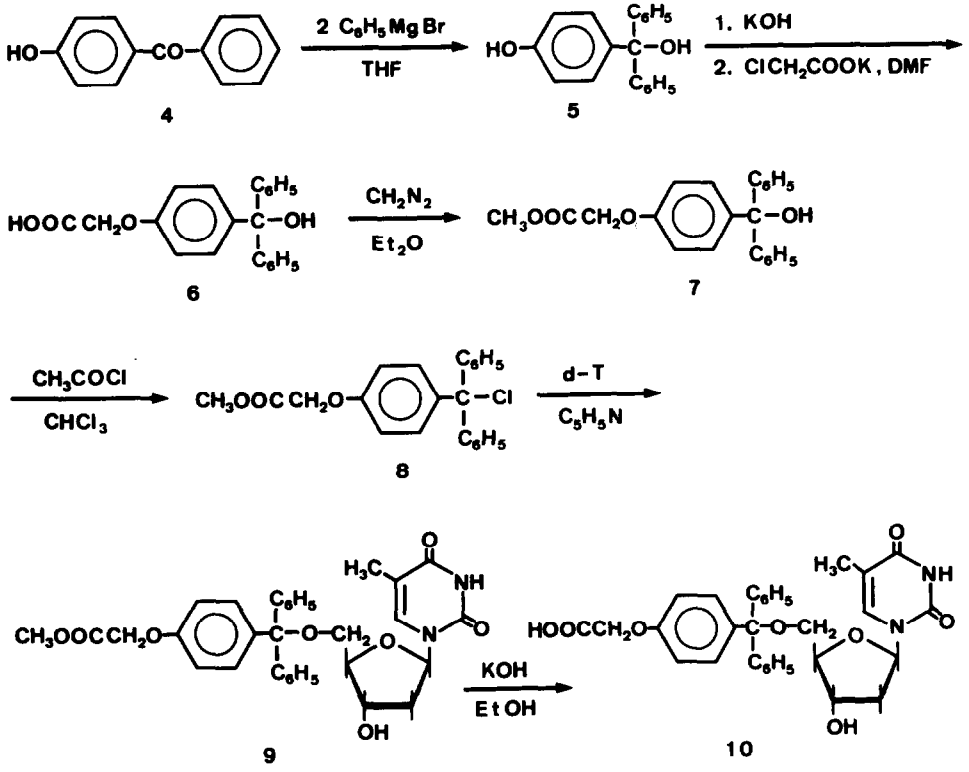
While the present work was carried out, a method was published[2] which describes the use of a crosslinked polydimethylacrylamide as a support for the synthesis of oligodeoxynucleotides carrying 5'-phosphate end groups. We have chosen to aim at the synthesis of oligodeoxynucleotides with 5'-hydroxyl groups, thereby keeping the number of phosphate groups for a given chain length at a minimum. If desired, a 5'-phosphate group can easily be introduced into the oligonucleotide after cleavage from the polymer and purification, by reaction with ATP (eventually γ-^{32}P-ATP) and the enzyme T4 polynucleotide kinase. Furthermore, the method here described, has the obvious advantage, that a commercially available polymer is used as a support.

Crosslinked polyacrylmorpholide (Enzacryl Gel K2, fine, from Koch Light Laboratories, Ltd., England) was derivatized by partial aminolysis with ethylenediamine as described in scheme I. In this way a polymer containing free amino groups, 2, was produced.



Scheme I

The resulting polymer was reacted with 5'-O-[p-carboxymethyloxytrityl]-thymidine, 10, and N,N'-dicyclohexylcarbodiimide (scheme I). Unreacted amino groups were blocked by acetylation, whereafter acetylated 3'-hydroxyl groups were selectively saponified as described below. The synthesis of the reactive thymidine derivative, 10, is described in scheme II.



4 5 6 7 8 9 10

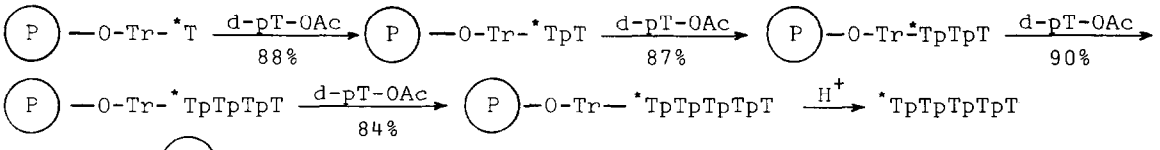
Scheme II

Carboxymethylation of p-hydroxytrityl alcohol,5, gave p-carboxymethyloxytrityl alcohol, 6, which was converted into the corresponding methyl ester, 7. Reaction of 7 with acetyl chloride gave the trityl chloride derivate, 8, which was reacted with thymidine in anhydrous pyridine to afford the 5'-protected thymidine derivative, 9. After saponification of 9 with alkali,5'-O-[p-carboxymethyloxytrityl]-thymidine, 10, was isolated as the dicyclohexylammonium salt.
In order to facilitate the analysis of the different steps of the oligonucleotide synthesis, [methyl-^{3}H] thymidine of a known specific activity was built into the polymer as described in scheme I. The labelling of the polymer makes it possible to keep the main portion of the polymer swollen in pyridine between two condensation cycles. Samples which have to be analyzed are withdrawn,dried and weighed. The radioactivity of a weighed part of the sample is determined

after burning in a sample oxidizer, allowing a determination of how much the entire sample amounts to of the swollen polymer. The remaining part of the sample is subjected to a cleavage reaction. The radioactive labelling also makes it easy to follow the cleavage reaction. Another advantage of the labelling is, that after separation of the cleavage products by paper chromatography or ion exchange chromatography[3] the molar ratios of the products are easily found by scintillation counting, and that the chain length of each compound can be determined from a combination of UV-spectrophotometry and scintillation counting.

The conditions during the condensation reaction and the saponification of the 3'-O-acetyl group was kept as close as possible to those which are normally used for the 'classical' synthesis of oligonucleotides in solution, as developed by H.G.Khorana[4]. Thus 500 mg of thymidine polymer, 3, containing 0,130 mmole [methyl-^{3}H]thymidine with a specific activity of 8,98.10^7 dpm/mmole was condensed with 0,650 mmole 3'-O-acetylthymidine 5'-phosphate for 5½ hours in 3 ml of anhydrous pyridine using 1,30 mmole of 2,4,6-triisopropylbenzenesulfonyl chloride as the coupling agent. After an overnight treatment with diisopropylethylamine in aqueous pyridine, the 3'-O-acetyl group was quantitatively saponified by treatment of the polymer with a mixture of 2M aqueous tetramethylammonium hydroxide, pyridine,and ethanol (2:1:1) for 5 min at 0°. After neutralization with acetic acid in pyridine, the polymer was washed several times with pyridine. A sample of the polymer was subjected to cleavage with a 1% solution of trifluoroacetic acid in methylene chloride. Scintillation counting of the acidolyzed polymer after burning showed that the cleavage reaction was complete. Analysis of the filtrate from the cleavage reaction by paper chromatography in ethanol/1M ammonium acetate (7:3) showed that 88% of the thymidine of polymer 3 had been converted into d-TpT.

A reaction vessel was designed[5], which allowed for evaporation and filtration to be carried out without removal of the main part of the polymer during all synthetic steps. The reaction vessel was siliconized in order to minimize adhesion of the polymer particles to the glass surface. For the synthesis of the longer oligonucleotides, the same conditions as described above were employed. The synthesis of the pentanucleotide, d-TpTpTpTpT, is described in scheme III.



Scheme III. (P)—O-Tr-T is a symbol for the structure 3 described in scheme I. *T is [methyl-^{3}H]thymidine. In the synthesis of the polymer bound pentanucleotide the condensation reaction was repeated before saponification of the 3'-O-acetyl group.

The acidolytic cleavage reaction with trifluoroacetic acid in methylene chloride was not satisfactory from the trinucleotide stage and on. However treatment of the polymer bound oligonucleotide with a 1% solution of trifluoroacetic acid in 50% aqueous acetonitrile at room temperature for two hours gave essentially quantitative cleavage.

The identity of the oligonucleotides was established by comparison with a set of standard markers, prepared by chemical polymerization of d-pT[6], followed by purification and removal of 5'-terminal phosphate with bacterial alkaline phosphatase.

The fact that long double stranded DNA molecules of defined sequence can be constructed enzymatically from appropriate octa- to dodecadeoxyribonucleotides produced by chemical synthesis[4,7] has made it highly desirable to develop quick and efficient methods for the synthesis of such short chains. The results obtained in the present work indicate that crosslinked polyacrylmorpholide is a suitable support for synthesis of oligodeoxyribonucleotides. Extension of the methodology to the synthesis of somewhat longer oligonucleotides containing all four nucleotides with the aim of automation of the process is currently under investigation.

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