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RAPID SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES

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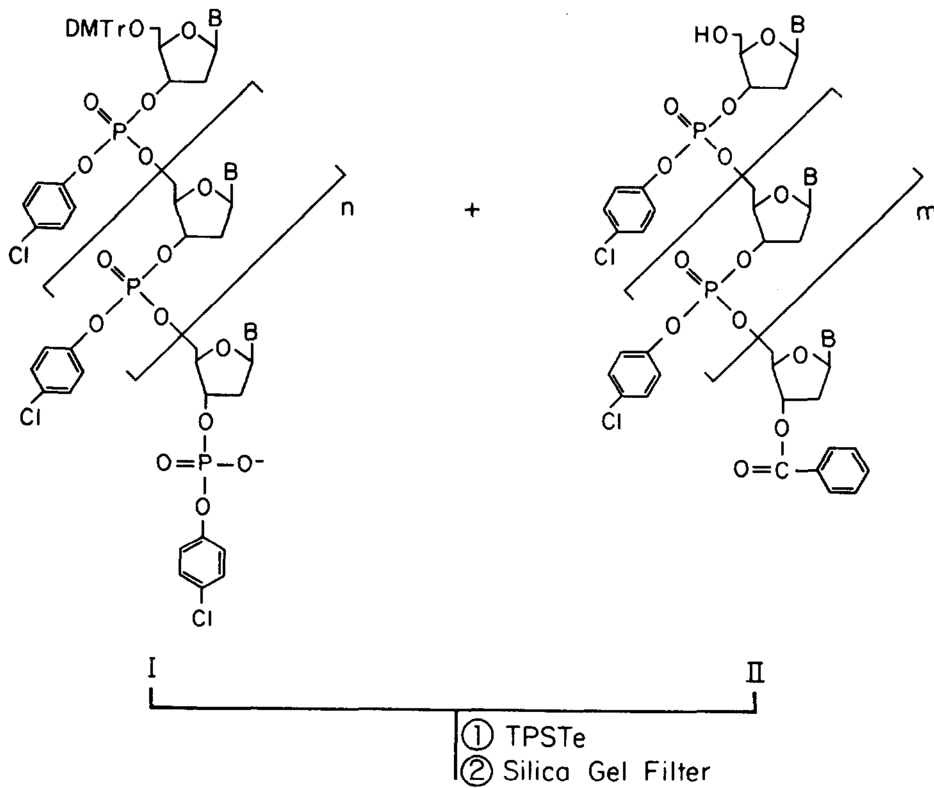
In the preceding paper we reported the rapid synthesis of trideoxyribonucleotides which are the basic starting blocks in our strategy to construct relatively long oligodeoxyribonucleotides[1].

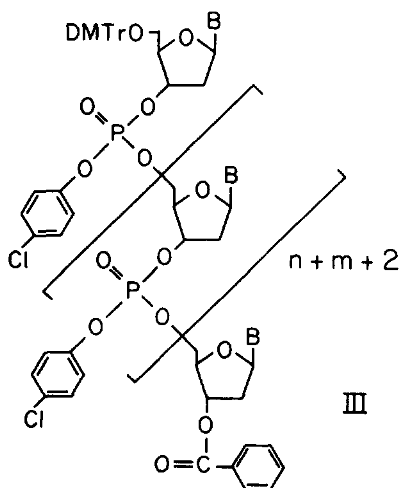
In this communication we wish to describe a rapid procedure for the synthesis of pentadecamer oligonucleotides starting from the basic trimer blocks in solution. Perhaps the most difficult problem, which we have encountered in the construction of the somewhat long and fully protected oligonucleotides by the phosphotriester approach with the block coupling method, is the separation of the products from the starting material by chromatography on silica gel. The separation gets more difficult with increasing chain length and is dependent upon the base composition of the oligonucleotides.

There could be two ways to overcome this problem: one is to develop a better separation method and the other is to force the coupling reaction to completion. We took the latter approach which made it possible to synthesize pentadeca-oligonucleotides without extensive purification steps.

The coupling reaction of an excess of the 3'-phosphodiester trimer block I (n = 1, 1.0 mmole) with the 5'-hydroxyl trimer block II (m = 1, 0.5 mmole) in the presence of 2,4,6-triisopropylbenzenesulfonyl tetrazolide[2] (TPSTe, 3 mmole) could be forced almost to completion in 3 hrs.

In order to remove the excess of the 3'-phosphodiester block I from the product III (hexamer), the reaction mixture was shaken with ion-exchange resin such as Dowex 1-X8 or DEAE-cellulose in various aqueous organic solvents and then filtered, but it was not possible to remove I completely. Thus the





B = Thymine
N – Benzoylated Adenine
N – Benzoylated Cytosine
N – Isobutyrulated Guanine

n = 1~5
m = 1~10

reaction mixture was passed through a very short (3 cm long) and wide (7 cm diameter) silica gel column set up in a sintered glass filter to remove the charged component I. The column was first washed with $CHCl_3$ (150 ml) to elute some side products, and then with $CHCl_3$-MeOH (95:5 v/v, 250 ml) in which almost all of the fully protected hexamer III was eluted. Under these conditions the charged compound I remained in the column. The second fraction was evaporated to give a glass which was treated with 2% benzenesulfonic acid solution in $CHCl_3$-MeOH (7:3 v/v)[2]. The resulting 5'-hydroxyl hexamer II (m = 4) was precipitated from ether and used for the next coupling reaction without further purification. Under this purification procedure the 5'-hydroxyl trimer, if still present, could not be removed. However, after the construction of the pentadecamer and the removal of all protecting groups, shorter fragments at least 3 bases shorter than the desired product, can be separated very easily by high-performance liquid chromatography (HPLC) on Permaphase AAX[3].

Repetition of this cycle four times or a coupling between the hexamer I and the nonamer II, which were made from trimer blocks as mentioned above, gave the fully protected pentadecamer III in a very short time. The final product was purified by HPLC after removal of the all protecting groups. The main peak, about 30 ∿ 50% of the total optical absorption material, was the desired pentadecamer. By this approach, $d(Tp)_{14}T$, *d*ApCpApCpCpCpApApGpApCpCp-CpGpT and *d*TpTpTpGpTpCpApApTpCpApGpGpApC were synthesized in a very short time.

Finally, confirmatory evidence for the structure of pentadecamers was obtained by homochromatography on thin-layer DEAE-cellulose and by gel electrophoresis on a 20% acrylamide slab after incubation of the pentadecamers with [γ-^{32}P]-adenosine 5'-triphosphate in the presence of T4-phosphokinase[4]. One major labelled product was obtained from each substrate. Furthermore, the labelled products were partially digested with venom phosphodiesterase and the digests were submitted to two-dimentional homochromatography[5] which showed the expected sequence pattern of the two pentadecamers of defined sequences.

The result obtained in the present work indicates that the coupling reactions between the short oligomer blocks (trimer ∿ hexamer) and long oligomer blocks (nona ∿ dodecamer) go almost to completion using an excess of the short blocks and that pentadecamers can be synthesized very quickly without extensive purification steps of the intermediates. Moreover, a combination of this approach with the method for the rapid synthesis of trimer blocks mentioned in the preceding paper[1] has tremendously increased the speed of the triester approach for the synthesis of oligodeoxyribonucleotides of defined sequences.

A further improvement in the oligodeoxyribonucleotide synthesis could be done by solid phase synthesis. Some authors have reported trials of solid phase oligonucleotide synthesis[6], but methods as successful as those with polypeptides have not been developed. One reason for this lack may be

the coupling reagent: if the coupling reaction does not go to completion, the final product will be a mixture of different oligonucleotides. However, we have shown in this and in the preceding paper that an excess of one component drives the coupling reaction almost to completion in the presence of a powerful coupling reagent (TPSTe)[2]. Thus solid phase synthesis of oligonucleotides by triester approach seems to be very promising to synthesize rapidly biologically important DNA sequences.

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