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RAPID SYNTHESIS OF TRIDEOXYRIBONUCLEOTIDE BLOCKS

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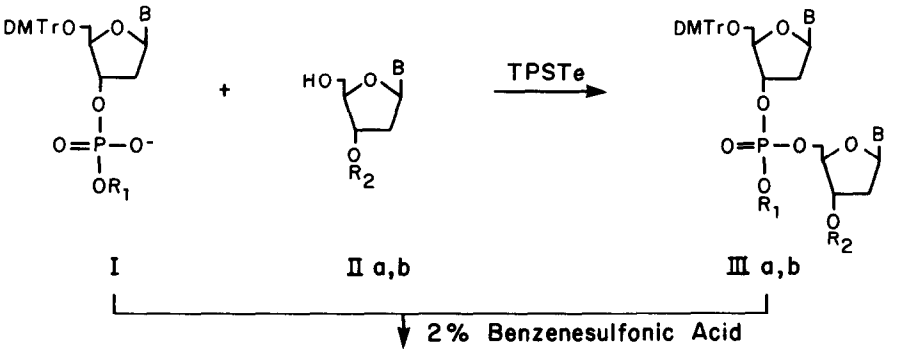
Polydeoxyribonucleotides of defined sequences have proven to be very useful as tools for studies of molecular biology that are not attainable in any other foreseeable way[1]. Although the triester approach has been developed to the stage of the synthesis of 21-base DNA with a defined sequence[2], problems still remain to be solved before polynucleotide synthesis becomes as efficient a process as peptide synthesis. A reduction of the number of steps per coupling would represent a real gain.

In this and the following papers, we wish to report a rapid synthetic method for oligodeoxyribonucleotides of defined sequences using the phosphotriester approach in solution. To synthesize relatively long oligomers, we chose the block coupling approach rather than the step by step approach. Trinucleotides are the basic construction blocks in our modified triester approach[3].

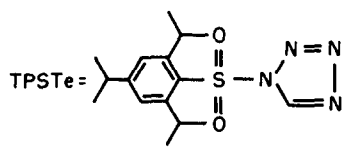
The present modified triester strategy for the synthesis of trimer blocks is time-consuming and low in overall yield. Since the coupling reaction using stoichiometric amounts of the 3'-phosphodiester component I and the 5'-hydroxyl component II does not go to completion, the extensive purification of the product (III, dimer) by silica gel column chromatography is very important to carry out the subsequent coupling. In some cases, one more chemical reaction and purification steps are required to remove the unreacted 5'-hydroxyl component II[4]. After removal of 4,4'-dimethoxytrityl group from III, the purified dimer V (column chromatography on silica gel) is further coupled with I to afford a trimer VI which is again extensively purified by column chromatography. Thus, $3 \sim 4$ times purification by column chromatography on silica gel is necessary to get the pure trimer blocks. Currently, we are using dimethoxytrityl for 5'-hydroxyl and β-cyanoethyl for 3'-end phosphate protecting groups. Both protecting groups are very sensitive to acid and base respectively and some degradation of the products during purification by column chromatography on silica gel could be expected[5].

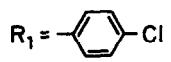
We have modified and simplified the process using an excess of the 3'-component I with respect to the 5'component II. As shown in the chart, using an excess of I (2 mmole), the coupling reaction with II (1 mmole) went almost to completion[6] in 60 minutes with the

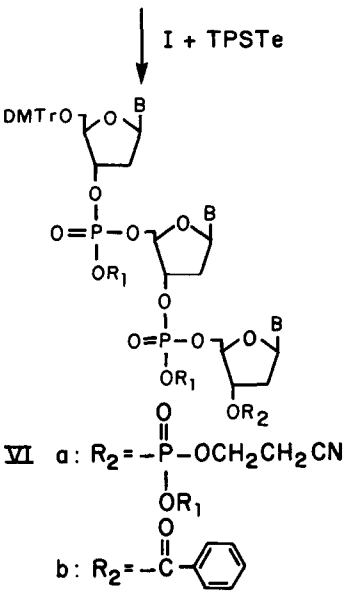


B = Thymine, N-Benzoylated Adenine
N-Benzoylated Cytosine
N-Isobutyrulated Guanine

DMTr = 4,4'-di-methoxytrityl







aid of a powerful coupling reagent, 2,4,6,-triisopropylbenzenesulfonyl tetrazolide (TPSTe, 4 mmole)[4]. The reaction mixture then was treated with 2% benzenesulfonic acid (BSA)[4] solution for ten minutes at 0°C to give IV and V. Since IV has a charged phosphate, it can be removed from V by simple extraction with aqueous $NaHCO_3$ solution in $CHCl_3$. The 5'-hydroxyl dinucleotide V was precipitated from ether to give a homogeneous product (T.l.c. on silica gel). The fully protected trimer block VI was prepared successively from the precipitates V, I (2 mmole) and TPSTe (4 mmole) and isolated by short column chromatography[7] on silica gel. The fully protected product was shown to be homogeneous on silica gel t.l.c. and its unprotected nucleotide after the removal of all protecting groups showed one peak with high-performance liquid chromatography on permaphase AAX[8]. The extensive final purification of each fully protected trimer block is very important, because even a small amount of contaminated shorter fragments nullifies the following block coupling approach to construct a long chain of oligodeoxyribonucleotides. The yields of fully protected trimers VI based on the 5'-hydroxyl monomers II are listed in the following table.

TABLE

Yields of Fully Protected Trimers (VI a and b)

Sequence	Yield	Sequence	Yield	Sequence	Yield
TTT(b)	81%	ATG(b)	69%	AAG(a)	51%
TTT(a)	75%	GCC(a)	61%	GTC(a)	57%
GGA(a)	41%	CCA(a)	72%	AAT(a)	62%
AGA(a)	49%	CAA(a)	72%	CAG(a)	56%
ATC(a)	71%	TTA(a)	71%	GAC(b)	59%
CCT(a)	61%	CAT(a)	52%		
ACA(a)	63%	CCC(a)	73%		
ACC(a)	65%	AAC(a)	59%		
CGT(b)	51%	GAT(a)	60%		

The table shows that the overall yields of trimers are much higher than those published before and that the yields of sequences with guanosine base are often lower than those without the base[9].

These modifications of the triester approach for the synthesis of the trimers using an excess of the 3'-phosphodiester component I to complete the coupling reaction and subsequently simplifying the purification step lead to an increase in the overall yield as well as to a decrease in the working time by at least a factor of 2.

Currently we are synthesizing all possible sixty-four blocks in large quantities to allow a rapid and efficient synthesis of polynucleotides with biologically important sequences.

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