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SOLID PHASE SYNTHESIS OF NONADECATHYMIDYLIC ACID BY THE PHOSPHOTRIESTER APPROACH

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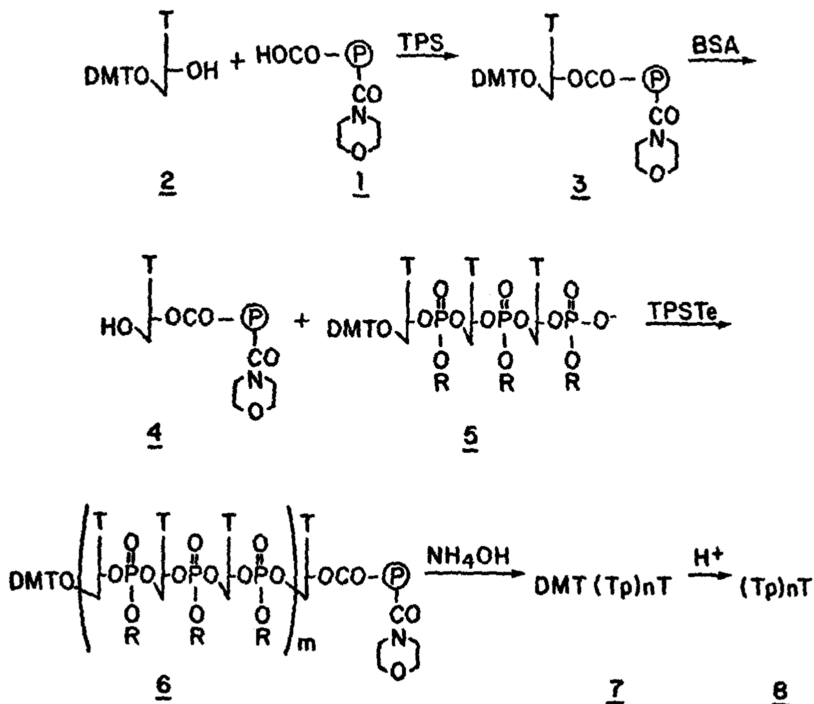
ABSTRACT Nonadecathymidylic acid was synthesized by the block coupling phosphotriester approach on a polymer support.

Solid phase synthesis of oligonucleotides has not been as successful as that of peptide synthesis. There may be three reasons for this relative lack of success. 1) The attention has focused almost entirely on the phosphodiester method[1], with its inherent disadvantages, such as low yields in coupling reaction. 2) The commonly used supports have been polystyrene and its derivatives. These non-polar polymers do not swell very well in the polar solvents required for oligonucleotide synthesis[2], and 3) the stepwise coupling approach[3] gives a series of oligonucleotides with one nucleotide difference and, therefore, it is very difficult to isolate the desired product from the final product mixture.

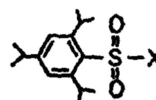
In this communication, we wish to report the synthesis of nonadecathymidylic acid by the block coupling phosphotriester approach on a well swollen polymer support. The polymer 1 (1 g, 1 mmole equivalent of carboxyl group), the partially hydrolyzed derivative of cross linked polyacrylmorpholide (Enzacryl Gel K2), was shaken with 5'-O-dimethoxytritylthymidine (2, 4 mmoles) and 2,4,6-triisopropylbenzenesulfonyl chloride (8 mmoles) in a mixture of acetonitrile and pyridine (1:1 $^{v}/_{v}$) for 24 hours at room temperature. The unreacted activated carboxyl groups were blocked by shaking with an excess of methanol. The dimethoxytrityl group of the resulting polymer 3 was removed by treatment with a 2% solution of benzenesulfonic acid in $CHCl_3$-MeOH (7:3 $^{v}/_{v}$)[4] to give the polymer 4 containing 0.140 mmole of thymidine. For the extension of oligonucleotide chain, the trithymidylic acid block 5 (5 equivalent) was coupled to the polymer 4 with the aid of a coupling reagent, 2,4,6-triisopropylbenzenesulfonyl tetrazolide[4] (TPSTe, 10 equivalent) for 3 hours. The polymer was washed with pyridine and, subsequently, with methanol.

FIGURE 1.



DMT = dimethoxytrityl
BSA = benzenesulfonic acid
TPS (X=-Cl)
TPSTe (X=-N tetrazolyl)
R = -C$_6$H$_4$-Cl

T = thymidine



After the removal of the dimethoxytrityl group from the oligothymidyl polymer 6, the coupling cycle was repeated (see Table 1).[5] After each coupling cycle, a sample of the polymer 6 (m = 1,2,3,4,5 and 6) was subjected to hydroly

TABLE 1.

STEP	REAGENT OR SOLVENT	MINUTES SHAKING	NUMBER OF OPERATIONS
1	2% BSA in $CHCl_3$-MeOH (7:3 $^v/_v$)	5	3
2	$CHCl_3$-MeOH (7:3 $^v/_v$)	2	3
3	Pyridine	2	3
4	Trinucleotide and TPSTe in pyridine	180	1
5	Pyridine	2	3
6	MeOH	2	3

sis with concentrated ammonium hydroxide solution for 24 hours at 50°C to remove the p-chlorophenyl protecting groups and also to cleave the oligonucleotides from the polymer. The reaction mixture was filtered and the filtrate was concentrated and treated with 80% aqueous acetic acid for 10 minutes at room temperature to give each oligothymidylic acid, 8, (n = 3,6,9,12,15 and 18). The isolation and analysis of each product was carried out by high performance liquid chromatography (HPLC) on Permaphase AAX.[6] The yield of each oligomer is shown in Table 2, and the HPLC pattern of the final product, dT_{19}, is presented in Figure 2.

TABLE 2.

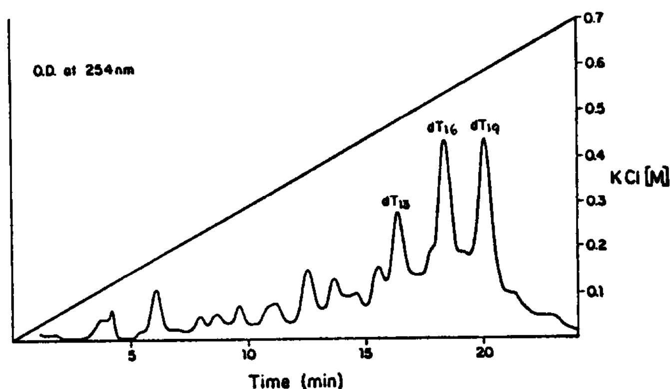
The conversion yields are estimated by comparison of the molar ratios of the product peak (chain length n) to the peak corresponding to chain length n-3 after the separation of the product mixture by HPLC.

Oligonucleotide 8	dT_4	dT_7	dT_{10}	dT_{13}	dT_{16}	dT_{19}
Yield (%)	77	75	79	71	58	48

The purity of each oligomer was analyzed by gel electrophoresis on 20% polyacrylamide gel after incubation of each oligomer with [γ-^{32}P]-adenosine 5'-triphosphate in the presence of T_4 polynucleotide kinase.[7] One major labelled product was obtained from each substrate. Finally, the identity of nonadecathymidylic acid was established by partial digestion of the labelled product with venom phosphodiesterase which showed nineteen spots on homochromatography[8].

FIGURE 2.

The chromatography was performed on a Permaphase AAX column with a linear gradient of KCl as published[10].



This study shows that the phosphotriester method to construct oligothymidylic acid on the polymer support 1 is simple and rapid. The complete cycle for the addition of one trinucleotide block can be accomplished in less than five hours. Although each coupling reaction did not go to completion, the major advantage of our approach using a trinucleotide block instead of a mononucleotide

as an incoming component lies in the isolation of the final product. The desired products are easier to separate from the by-products lacking a trinucleotide block than those from the by-products lacking only a mononucleotide residue. With the improvement of the rapid synthesis of trinucleotide block by the phosphotriester approach[9], extension of the methodology to the synthesis of oligonucleotides containing all four bases is currently under investigation.

Acknowledgements

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