products were chromatographed on a DEAE-cellulose column. The chromatographic pattern and the conditions are given in Figure 6. Selected fractions were checked by DEAE-cellulose paper chromatography as described above and in solvent system C after removal of the N-protecting groups. Peak A contained excess trinucleotide d-pTpC^{An}pT; peak B contained the unreacted hexanucleotide; peak C contained the symmetrical pyrophosphate of the trinucleotide; and peak D contained the desired nonanucleotide, which had λ_{max} 280 mμ, $\varepsilon_{280}/\varepsilon_{260}$ 1.16. The yield of the nonanucleotide was 35%. R_f values of the unprotected nonanucleotide are given in Table III and the results of snake venom phosphodiesterase degradation are given in Table IV.

Preparation of the Dodecanucleotide, d-MMTr-A^{Bz}pTpG^{iBu}pC^{An}-pA^{Bz}pC^{An}pTpC^{An}pTpTpA^{Bz}pG^{iBu}. An anhydrous mixture of the nonanucleotide (5 μmol, 600 OD_{280}), pyridinium d-pTpA^{Bz}pG^{iBu}-OAc (300 μmol), and MsCl (0.900 mmol) was kept in pyridine (1 ml) for 2 hr at room temperature. After the usual work-up, the reaction mixture was applied on a DEAE-cellulose column. The condition and chromatographic properties are given in Figure 7. Selected fractions were checked in solvent C after taking off the N-protecting groups. Peak A contained excess trinucleotide d-pTpA^{Bz}pG^{iBu}; peak B contained the symmetrical pyrophosphate of the trinucleotide; peak C was not identified; peak D contained unreacted nonanucleotide; and peak E contained the dodecanucleotide. The fractions shown by dotted line in Figure 7 were pooled, concentrated, and further purified by preparative paper chromatography using solvent systems C and D. The yield of the dodecanucleotide was 25%. R_f values and results of enzymatic degradation are given in Tables III and IV. The protected dodecanucleotide had λ_{max} 277 mμ, $\varepsilon_{280}/\varepsilon_{260}$ 1.08.

Studies on Polynucleotides. XCIII.[1] A Further Study of the Synthesis of Deoxyribopolynucleotides Using Preformed Oligonucleotide Blocks[2]

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Abstract: In a further study of polynucleotide synthesis by the use of preformed oligonucleotide blocks, the condensation between the hexanucleotide Tr-TpTpTpTpTpT and the thymidine di-, tri-, tetra-, and pentanucleotides carrying 5′-phosphate end groups (pTpT-OAc and homologs) has been investigated. Mesitylenesulfonyl chloride was used as the condensing agent. The molar excess of the oligonucleotide blocks bearing phosphomonoester groups, the proportion of the condensing agent, and the reaction time were varied. The rates of condensation reaction as well as the *isolated* yields of the desired products were determined. In addition, attention was paid to the extent of polynucleotide chain degradation during the activation process and the nature of the resulting side products. The use of a 10:1 ratio of the oligonucleotide block to Tr-T_6P_5, a lower amount of the condensing agent, and shorter reaction times than those used previously gave the best yields with minimal side reactions. The condensations using di- and trinucleotide blocks showed almost the same rate, and the optimal yields of the desired polynucleotides were, respectively, 78 and 75%. The condensations using the tetra- and pentanucleotides also proceeded at about the same rate, but this rate was lower than that observed for the di- and trinucleotide reactions; the maximum yields obtained in these condensations were close to 50%.

The stepwise synthesis of deoxyribopolynucleotides containing specific sequences may be approached in two alternative ways. In the first approach, suitably protected mononucleotides may be added one at a time to a developing polynucleotide chain. In the second approach, preformed oligonucleotides bearing phosphomonoester end groups may be used in condensation reactions with oligo- or polynucleotidic components bearing hydroxyl end groups. The first approach was developed[3] and used successfully in the earlier work on synthetic deoxyribopolynucleotides.[4] The second approach is more attractive for at least two reasons. If each of the successive block condensations proceed in reasonably good yield, it is possible to increase the overall yield of the desired polynucleotide as based on the increasingly valuable developing chain. Thus, the growing polynucleotide chain is subjected to fewer condensation steps, and even though the yields in the individual reactions may be lower than those for mononucleotide condensations, the yield of the final product can be higher.[5] The second feature of the block approach is

(1) Paper XCII: A. Kumar and H. G. Khorana, *J. Am. Chem. Soc.*, **91**, 2743 (1969).

(2) This work has been supported by grants from the Life Insurance Medical Research Fund (Grant No. 65-44), the National Science Foundation (Grant No. GB-7484X), and the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service (Grant No. CA-05178).

(3) T. M. Jacob and H. G. Khorana, *J. Am. Chem. Soc.*, **87**, 368 (1965).

(4) T. M. Jacob and H. G. Khorana, *ibid.*, **87**, 2971 (1965); S. A. Narang and H. G. Khorana, *ibid.*, **87**, 2981 (1965); S. A. Narang, T. M. Jacob, and H. G. Khorana, *ibid.*, **87**, 2988 (1965).

(5) The oligonucleotidic blocks can be prepared usually in satisfactory yields; however, the steps involved in their preparation and the question of yields, which are well below 100% at individual steps, are being omitted from the present argument for over-all efficiency in the synthesis of relatively long deoxyribopolynucleotide chains.

the expected easier separation of the product from the unreacted starting material (3′-hydroxylic component) on anion-exchange chromatography because of more appreciable difference in total negative charge. This feature becomes more important as the length of the polynucleotide chain increases. Because of these considerations, this approach has been preferred in more recent syntheses of deoxyribopolynucleotides.[1,6,7] The preformed oligonucleotide blocks bearing 5′-phosphate end groups have been di-, tri-, and tetranucleotides. The yields using tri- and especially tetranucleotide blocks have been moderate, and the extent of side reactions was often high. Thus, in the experimental conditions used in the synthesis of a hexadecanucleotide by condensation of the dodecanucleotide with the appropriate tetranucleotide block, the yield of the desired product was about 20% and the unreacted dodecanucleotide was recovered only in the amount of 23%.[6b]

Because of the ever-increasing interest in the deoxyribopolynucleotides with specific sequences[7,8] and because of our conclusion that at present the above approach is as attractive as any alternative ones that are currently being investigated[9] we have subjected this approach to a more systematic study. An investigation of the use of preformed thymidine dinucleotide in block condensations has been reported previously;[10] in relation to the present demands on synthesis; however, this study appeared to be restricted in scope. In the present work, the hexanucleotide Tr-TpTpTpTpTpT was chosen as the standard component carrying the 3′-hydroxyl end group; the oligonucleotide has five phosphodiester bonds so that, in its susceptibility to chain-cleavage reactions[3,6b,11] due to activation of the phosphodiester linkage, it represents a reasonable size. The oligonucleotide blocks bearing 5′-phosphate end groups used were thymidine di-, tri-, tetra-, and pentanucleotides and the condensing agent used throughout was mesitylenesulfonyl chloride (MsCl[12]). Reaction conditions were varied with respect to the concentration of MsCl, the excess of the oligonucleotide block containing the phosphomonoester group, and reaction time. The effects of these different sets of conditions on the rate of reaction, the yield of the desired product, and the extent of side reactions were studied. The nature of some of the side products formed in these reactions was also partially investigated. The total approach is discussed in the light of the present findings.

Experimental Section

Reagents. Pyridine was distilled over potassium hydroxide and dried over Linde Type 4A Molecular Sieves. Mesitylenesulfonyl chloride (Aldrich Chemical Co., Milwaukee, Wis.) was recrystallized twice from *n*-pentane. This preparation was stored in a desiccator over phosphorus pentoxide until use. All other reagents and solvents were reagent grade, commercially available materials and were used without further purification.

Paper Chromatography. Unless otherwise noted, this was carried out using the descending technique on Whatman No. 1 paper. Thin layer chromatography (tlc) was performed on E. Merck cellulose F plates by the ascending method. The solvent systems used were: solvent A, 1-propanol–concentrated ammonia–water (55:10:35, v/v); solvent B, ethanol–1 *M* ammonium acetate, pH 7.5 (7:3, v/v). The R_f values for various compounds used in these experiments are given in Table IX. Trityl group containing compounds were detected on chromatograms by spraying with 10% aqueous perchloric acid; they developed a yellow color on drying in warm air.

Paper Electrophoresis. This was performed with a high-voltage apparatus in which the paper was immersed in Varsol. Potassium phosphate buffer (0.03 *M*, pH 7.1) was used.

Oligonucleotides.[13] The hexanucleotide Tr-T_6P_5[14] was prepared as described previously.[3] The preparations of the dinucleotide pTpT-OAc,[14] the trinucleotide, pTpTpT-OAc,[14] and the cyanoethyl derivative CE-pTpT[14] have also been described previously.[1,6b]

The Tetranucleotide pTpTpTpT-OAc. An anhydrous solution of CE-pTpT (2.16 mmol) and pTpT-OAc (6.3 mmol) in pyridine (30 ml) was treated with 3.80 g (17.4 mmol) of MsCl. The solution was allowed to stand for 2 hr at room temperature. At that time 30 ml of water was added to stop the reaction and the resulting solution was allowed to stand overnight at room temperature. Removal of the protecting groups was accomplished by treatment with 60 ml of 2 *N* NaOH for 30 min at room temperature. After neutralization with an excess of pyridinium Dowex 50W-X8 ion-exchange resin the solution was passed through a column (300-ml bed volume) of the same resin to ensure complete removal of sodium ions. The column was washed with 1 l. of 30% aqueous pyridine. The solution and washings were made up to 3 l. by the addition of 20% aqueous ethanol, and this solution was applied to a DEAE-cellulose column (4.5 × 100 cm) which had been previously equilibrated with 0.01 *M* triethylammonium bicarbonate buffer (pH 7.5) in 10% aqueous ethanol. After application of the oligonucleotides, the column was washed with the same buffer until the ultraviolet absorption at 253 mμ returned to near zero. A linear gradient of $(C_2H_5)_3NH^+HCO_3^-$ buffer concentration was then applied. The gradient was from 0.01 to 0.50 *M* $(C_2H_5)_3NH^+$-HCO_3^-, a total of 8 l. being used. The eluting buffer solution contained 20% ethanol throughout. Five major peaks were obtained; the last one contained 32,600 OD_{267} (0.850 mmol; 39.4%) of pure pTpTpTpT. After evaporation of the buffer, the tetranucleotide fraction was dissolved in pyridine and rendered anhydrous by repeated coevaporation with the same solvent. A solution of this material in 30 ml of dry pyridine was treated with 7 ml of acetic anhydride for 4 hr at room temperature. The reaction was stopped by the addition of water (30 ml) and the solution was allowed to stand overnight at room temperature. After evaporation, this material was rendered anhydrous again and dissolved in 30 ml of pyridine. The tetranucleotide was precipitated by dropwise addition of the pyridine solution to 1 l. of dry ethyl ether. The precipitate was collected by centrifugation, washed five times with ether (250 ml), and dried overnight in a vacuum desiccator (P_2O_5). The yield of pTpTpTpT-OAc was 29,000 OD_{267} (0.76 mmol, 90%). Paper chromatography in solvent B showed that the acetylation was complete.

The Pentanucleotide pTpTpTpTpT-OAc. Cyanoethylation of pTpTpT[15] (1.2 mmol) was accomplished by a procedure similar to those previously described.[6b] An anhydrous pyridine (10 ml) solution of CE-pTpTpT (0.994 mmol) and pTpT-OAc (2.95 mmol) was treated with 1.55 g (7.10 mmol; MsCl:P = 0.60) of MsCl. The clear solution was kept sealed for 2 hr at room temperature and then treated with 10 ml of water. The aqueous pyridine

(6) (a) H. Kössel, H. Büchi, and H. G. Khorana, *J. Am. Chem. Soc.*, **89**, 2185 (1967); (b) E. Ohtsuka and H. G. Khorana, *ibid.*, **89**, 2195 (1967).

(7) H. G. Khorana, H. Büchi, M. H. Caruthers, S. H. Chang, N. K. Gupta, A. Kumar, E. Ohtsuka, V. Sgaramella, and H. Weber, *Cold Spring Harbor Symp. Quant. Biol.*, in press.

(8) H. G. Khorana, Proceedings of the 5th International Symposium on the Chemistry of Natural Products, London, in press.

(9) For references and more comprehensive discussion of alternative possible approaches to polynucleotide synthesis see ref 8.

(10) H. Kössel, M. W. Moon, and H. G. Khorana, *J. Am. Chem. Soc.*, **89**, 2148 (1967).

(11) E. Ohtsuka, M. W. Moon, and H. G. Khorana, *ibid.*, **87**, 2956 (1965).

(12) T. M. Jacob and H. G. Khorana, *ibid.*, **86**, 1630 (1964).

(13) For the mononucleotide pT, a molar extinction coefficient of 9600 (267 mμ) has been used. For the thymidine oligonucleotides, any hypochromicity has been ignored for calculation of molar concentrations.

(14) The system of abbreviations is as has been described and used in previous papers [see, *e.g.*, H. G. Khorana and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **83**, 675 (1961); H. Schaller and H. G. Khorana, *ibid.*, **85**, 3841 (1963)]. Tr-TpTpTpTpTpT is further abbreviated in the present work to Tr-T_6P_5 and the higher homologs of this compound prepared by condensation of Tr-T_6P_5 with oligonucleotides such as pTpT have been similarly abbreviated.

(15) Generous gift of Dr. Ashok Kumar.

solution was allowed to stand for 2.5 days at room temperature. The solution was then treated with 10 ml of 2 *N* sodium hydroxide (a further amount of water had to be added in order to obtain a homogeneous solution) for 45 min at room temperature. Neutralization was effected by passing the solution through a column (3 × 26 cm) of Dowex 50W-X8 cation-exchange resin in the pyridinium form. The column was washed with 1 l. of 30% aqueous pyridine. The eluate was diluted to 2 l. with water and applied to a DEAE-cellulose column (4.5 × 87 cm) which had been previously equilibrated with 0.01 *M* $(C_2H_5)_3NH^+HCO_3^-$ in 20% aqueous ethanol. After washing with several liters of the same buffer to remove pyridine, a $(C_2H_5)_3NH^+HCO_3^-$ gradient was applied. The gradient went from 0.01 *M* to 0.6 *M* $(C_2H_5)_3NH^+HCO_3^-$. A total of 12 l. was used, the ethanol concentration in the eluent being 20% throughout. Four major peaks were collected. The fourth of these contained 20,200 OD_{267} (0.430 mmol, 45%) of pure pTpTpTpTpT.

All of the pentanucleotide obtained above was rendered anhydrous with pyridine and treated with 5 ml of acetic anhydride in 20 ml of pyridine for 4 hr at room temperature. Then water (20 ml) was added and the solution was allowed to stand overnight at room temperature. The product was rendered anhydrous again, dissolved in 30 ml of dry pyridine, and precipitated by addition of the pyridine solution to 1.2 l. of dry ethyl ether. The precipitate was collected by centrifugation and washed with five 200-ml portions of dry ether. It was then dried in a vacuum desiccator (P_2O_5) overnight. The yield for the acetylation step was 0.400 mmol (93.0%).

Acetylation of Tr-T_6P_5. An anhydrous solution of Tr-T_6P_5 (0.02 mmol) in 0.5 ml of pyridine was treated with 0.19 ml (2 mmol) of acetic anhydride. After standing for 4 hr at room temperature the reaction mixture was treated with 0.5 ml of methanol under cooling. After keeping the solution for 0.5 hr the product was precipitated by addition of the solution to 50 ml of ethyl ether. The precipitate was collected by centrifugation and washed with five 20-ml portions of ether. It was then dried in a vacuum desiccator (P_2O_5) overnight. Thin layer chromatography in solvent B showed that the reaction was complete and that only one product was formed.

Reaction of Tr-TpTpTpTpTpT-OAc with MsCl. All of the material obtained in the above reaction was rendered anhydrous and treated with 109 mg (0.50 mmol; MsCl:P = 5.0) of MsCl in pyridine (2 ml). The mixture was kept for 2 hr at room temperature and treated with 2 ml of water, and the solution was further allowed to stand overnight at room temperature. The solution was then treated with 3 ml of 2 *N* sodium hydroxide for 1 hr at room temperature. The solution was neutralized by passing it through a column (50 ml) of Dowex 50W-X8 cation exchange resin in the pyridinium form. The column was washed with 300 ml of 30% aqueous pyridine. The total eluate was made up to 500 ml by adding more 30% aqueous pyridine and chromatographed on a column under the conditions given in the legend to Figure 8. The elution pattern is shown in Figure 8.

General Method of Condensation between Tr-T_6P_5 and Oligonucleotide Blocks. Anhydrous solutions of the two nucleotidic components were prepared by evaporating their pyridine solutions to dryness at least three times with added dry pyridine. After every evaporation, the flask was opened to the atmosphere of a drybox (anhydrous conditions were maintained inside the drybox by a positive dry nitrogen atmosphere and phosphorus pentoxide). Just prior to the final addition of pyridine suction of the gummy residue in the vacuum was continued for at least 0.5 hr. The additions of pyridine and MsCl were also performed inside the drybox. All experiments were carried out at a standard scale in which 0.01 mmol of Tr-T_6P_5 was used per experiment, and the amount of pyridine finally added to the gum was 0.5 ml. The concentrations of pTpT-OAc and the higher oligonucleotides were as shown in appropriate tables. The appropriate weighed amount of MsCl was also dried in a small vial in a vacuum desiccator over Drierite for at least 0.5 hr before use. After a homogeneous solution of the nucleotidic components was obtained following the last addition of dry pyridine, the vial containing MsCl was dropped into the reaction flask. For kinetic studies, aliquots of about 10 μl were removed from the sealed reaction vessels kept in the drybox at different time intervals. For removal of the aliquots 10-μl micropipets attached to a syringe top were used, all manipulations being again inside the drybox.

Kinetic Analysis by Anion-Exchange Chromatography on DEAE-Cellulose Paper Strips. Each of the 10-μl aliquots taken from the reaction mixtures was treated immediately with 0.05–0.1 ml of water to stop the reaction. After standing overnight the solution was treated with 0.1 ml of concentrated ammonia. After 30 min the solution was evaporated, the residue dissolved in 50% aqueous pyridine, and the solution applied to Whatman DE81 DEAE-cellulose paper. The solvent systems that were used for separation of the products in different series were as follows: for the dinucleotide reactions, 0.3 *M* ammonium bicarbonate–7 *M* urea; for the trinucleotide reactions, 0.2 *M* ammonium acetate–7 *M* urea; for the tetranucleotide reactions, 0.25 *M* ammonium acetate–7 *M* urea.

In trial runs with each reaction mixture, the location of the two major trityl-containing polynucleotides, Tr-T_6P_5 and the product, was determined by perchloric acid spray; a marker of Tr-T_6P_5 was run along side. In reaction products from pTpTpT-OAc and Tr-T_6P_5 it was found that the symmetrical pyrophosphate, O-(pTpTpT)$_2$, and Tr-T_6P_5 did not separate. Therefore, for this analysis, the symmetrical pyrophosphate was first cleaved to the trinucleotide as described below. In the reaction products from Tr-T_6P_5 and pTpTpTpTpT-OAc, DEAE-cellulose paper strip chromatography did not separate Tr-T_6P_5 from the pentanucleotide. The pentanucleotide was, however, removed by electrophoresis on DEAE-cellulose paper at pH 7.1. Thus, at an applied potential of 17.4 V/cm, the pentanucleotide migrated 5.1 cm in 1 hr, while Tr-T_6P_5 stayed at the origin. The paper was cut into strips which were washed with distilled water and dried. The trityl-containing products were now eluted with 1 *M* $(C_2H_5)_3NH^+HCO_3^-$ solution and after removal of the buffer the compounds were separated by paper chromatography on Whatman No. 1 paper in solvent A.

For ultraviolet absorption measurements, the compounds were eluted from DEAE-cellulose paper with 1 *M* sodium chloride–7 *M* urea. The compounds were made up to standard volumes with the same solvent. Compounds were eluted from Whatman No. 1 paper with water. In all cases appropriate paper blanks were used in the reference cuvette.

Analysis by Chromatography on DEAE-cellulose Columns. At appropriate times, after removal of the 10-μl aliquots, 0.5 ml of water was added to the remainder of the solution to stop the reaction. The solution was allowed to stand overnight at room temperature and the acetyl group was then removed by treatment with 1 ml of concentrated ammonia for 30 min at room temperature. The solution was then evaporated to near dryness and the residue immediately made up to 50 ml in aqueous ethanol of the same concentration as was to be used for column chromatography. The pH was adjusted to 7.0 by the addition of one or two drops of triethylamine. The OD_{267} units in this solution were measured after evaporation of a small aliquot in the presence of ammonium bicarbonate to remove traces of pyridine. The solution was then applied to a DEAE-cellulose column for chromatography. The conditions used for chromatography are given in the legends to figures. For each series of experiments using the same block, the conditions for chromatography were identical throughout. The yields listed in the tables were calculated by multiplying the OD_{267} units of the products obtained by the ratio of the number of OD_{267} units calculated to be present in the reaction[16] to the number of OD_{267} units actually applied to the column.

The products in different peaks were further identified by paper or thin layer chromatography in solvents A or B.

Identification of Symmetrical Pyrophosphates of Oligonucleotide Blocks. When a product was suspected to be the symmetrical pyrophosphate of the oligonucleotide block, it was subjected to the acetic anhydride–pyridine procedure for conversion to the parent oligonucleotide.[17] Thus 10 OD_{267} was rendered anhydrous in the usual manner. A solution of the residue in 1 ml of dry pyridine was treated with 0.1 ml of acetic anhydride for 6 hr at room temperature. After addition of 1 ml of water the solution was allowed to stand overnight at room temperature. The solution was next evaporated and made anhydrous again. The residue was dissolved in one drop of pyridine and precipitated by addition of 5 ml of dry ethyl ether. The precipitate was collected by centrifugation and washed with three 5-ml portions of ether. It was then dissolved in 1 ml of concentrated ammonia and allowed to

(16) The calculations necessitated allowance for the molar extinction of mesitylenesulfonate ion. This value for the ammonium salt was found to be 505 OD at 267 mμ in water.

(17) H. G. Khorana and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **81**, 4660 (1959).

Table I. Conditions and Results of Experiments on the Condensation of the Dinucleotide pTpT-OAc with the Hexanucleotide Tr-T_6P_5[a]

Expt	MsCl:P	pTpT-OAc, molar equiv	Time, hr	Octanucleotide yield, %	Unreacted Tr-T_6P_5, %	Calcd[b] degradation, %
1	0.43	10	4	52	20	28
2	0.57	10	2	78	14	8
3	0.57	20	2	75	10	15
4	0.75	10	1[c]	58	32	10
	0.75	10	2[c]	62	14	24
5	0.75	20	1	67	23	10
6	1.00	10	2	62	10	28
7	2.00	2	2	48	25	27

[a] All seven experiments were performed using 0.01 mmol of the hexanucleotide in 0.5 ml of pyridine. For further details see text. [b] The numbers in this column indicate the loss of Tr-T_6P_5 or the desired product, presumably, by cleavage of internucleotide bonds during the condensation reactions. These numbers were calculated by subtracting the isolated desired product and recovered hexanucleotide from 100%. [c] Both results came from one experiment, a 100-μl aliquot being removed after 1 hr for analysis by column chromatography.

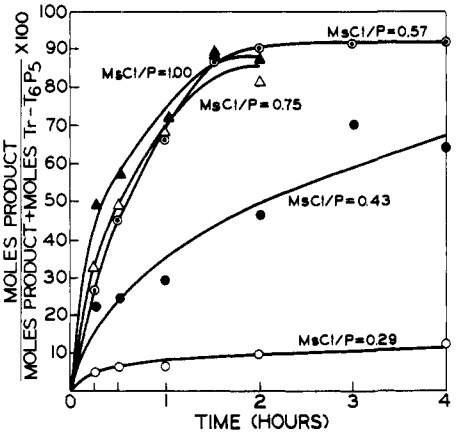


Figure 1. Studies of the rate of synthesis of the octanucleotide Tr-T_8P_7. The reaction mixtures contained Tr-T_6P_5 (0.01 mmol) and pTpT-OAc (0.10 mmol), in 0.5 ml of pyridine in each case. The MsCl:P ratio was varied as shown in the figure. For further details see Experimental Section.

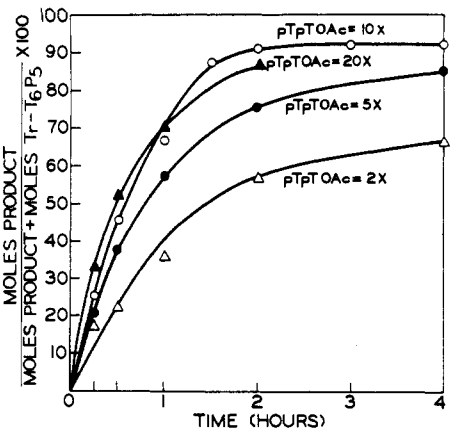


Figure 2. Studies of the rate of synthesis of the octanucleotide Tr-T_8P_7. All reaction mixtures contained Tr-T_6P_5 (0.01 mmol) in 0.5 ml of pyridine. The MsCl:P ratio was maintained at 0.57. The molar ratio of pTpT-OAc was varied as shown in the figure. For further details see Experimental Section.

stand at room temperature for 1 hr. This solution was evaporated and applied for paper chromatography in solvent A. Under these conditions all of the pyrophosphates were found to have been cleaved to the parent oligonucleotides with 5′-phosphomonoester end groups.

Results

Condensations Using the Dinucleotide pTpT-OAc Block. The condensation between the dinucleotide pTpT-OAc and the hexanucleotide Tr-T_6P_5 to form the octanucleotide is the one that has been investigated the most for different parameters.

In previous studies using arylsulfonyl chlorides as the condensing agents, the molar proportions of these to be used have been variously calculated. In view of the expected participation of every phosphoryl dissociation in the anhydride-forming reactions the rational approach is to relate the amount of the sulfonyl chloride to the total molar concentration of the phosphoryl dissociations including both Tr-T_6P_5 and the oligonucleotide component bearing the phosphate end group. This has been systematically done in the present work, and the expression MsCl:P refers to the ratio of molar equivalents of MsCl used to the total molar equivalents of phosphoryl dissociations present in the reaction mixture.

Figure 1 shows the results of a kinetic study in which a constant excess (molar ratio of pTpT-OAc to Tr-T_6P_5 10:1) of pTpT-OAc was used and the amount of MsCl was varied. The extent of reaction as a function of time is plotted in this and the following kinetic studies by using the term (moles of Tr-T_8P_7)/(moles of Tr-T_8P_7 + moles of unreacted Tr-T_6P_5). The values thus obtained are not accurate yields at different times, because the consumption of nucleotidic material by side reactions is not taken into account. The loss due to the latter reactions has, however, been relatively small in these studies, and therefore the method used in plotting the data is essentially valid. Analyses of selected experiments were carried out by the more comprehensive and quantitative method of column chromatography, and the absolute yields obtained in these experiments are shown in several tables (see below).

It is seen in Figure 1 that in the reaction where MsCl:P was 0.29, which is the minimum amount of the reagent theoretically required for specific activation of the phosphomonoester end groups, the rate and extent of synthesis was very low. Increasing the MsCl:P ratio to 0.43 increased both the rate and the extent of the synthesis. A maximum in the rate of synthesis and yield was reached with a MsCl:P ratio of 0.57 and a further increase in MsCl was essentially without effect. As

discussed below (Table I), at higher MsCl:P ratios the isolated yields of the octanucleotide actually decreased due to an increase in the extent of polynucleotide chain degradation. Finally, in these experiments, the time in which the yields reached a maximum was about 2 hr.

In Figure 2 are shown the results of a study in which the concentration of the dinucleotide block was varied while keeping the MsCl:P ratio constant at 0.57. The rate of synthesis and the final yield (see also Table I) increased with a increase in the dinucleotide:hexanucleotide ratio. The rate and the yield were at a maximum with the use of 10 *M* proportions of the dinucleotide; further increasing the dinucleotide to 20 *M* proportions gave a slightly diminished rate and perhaps a slightly lower yield (see Table I) due to an increase in the chain degradation.

Further kinetic experiments were also carried out in an attempt to increase the extent of reaction by using a small excess (2 *M* proportions) of dinucleotide and a relatively large proportion (MsCl:P = 2) of the condensing agent. While an increase in the rate of synthesis over that obtained at a MsCl:P ratio of 0.57 (Figure 2) was observed, the yield (see Table I) was low and the extent of chain degradation was markedly high. The results are similar to those obtained in the previous work where, in general, a high MsCl:P ratio was used.[10]

Finally, in seven selected experiments, the reaction products were chromatographed on DEAE-cellulose columns, a procedure which was more demanding but was the most reliable, quantitative, and most informative about side products. In these experiments, which are listed in Table I, the MsCl:P ratio, the excess of the dinucleotide, and the time of reaction were varied and the yields of the products based on the starting material (see Experimental Section) used were recorded. Figure 3 gives a typical separation pattern obtained in this series of experiments. As seen in Figure 3 and in Table II, a rather large number of minor side products were also formed. In character, the bulk of these appeared to be similar to those reported earlier,[3] and the mechanism of their formation has also been discussed previously.[3,6b] While general comments on the results in Table I are made in the Discussion section, it should be noted here that the highest yield of the desired octanucleotide was 78%, and, in agreement with the kinetic results presented above, this result was obtained using tenfold molar equivalents of pTpT-OAc, a ratio of MsCl:P of 0.57, and a 2-hr time period.

Condensations Using the Trinucleotide pTpTpT-OAc Block. Following the results of the above experiments with the dinucleotide block, the condensation between the trinucleotide and Tr-T_6P_5 was investigated in a selected number of experiments. Figure 4 compares the rate of condensation using the trinucleotide block with those observed for the other oligonucleotide blocks under a standard set of conditions. Table III lists the conditions used and the yields of the nonanucleotide Tr-T_9P_8 obtained in a further set of experiments, while Figure 5 and Table IV give the detailed chromatographic analysis of one of the typical experiments. As seen in Figure 4, the rate of condensation was slightly lower than that with the dinucleotide block but, under similar conditions, the isolated yields of the nonanucleotide (Figure 5 and Table IV) were comparable and the extent of degradation through side reactions was no higher.

Table II. Analysis of Nucleotidic Products Obtained in the Condensation of pTpT-OAc with Tr-T_6P_5[a]

Peak no. (of Figure 3)	Total OD_{267}	R_f[b]	Test for trityl group	Identification remarks
I	67.5	0.23	–	Cyclo-pTpT
II	13.94	0.35	–	
		0.95	–	
		0.60	+	
III	1222	0.17	–	pTpT
		0.38	+	
		0.10	–	Trace
IV	254	0.11	–	Pyrophosphate of pTpT
V	9.19	0.26	+	
		0.07	–	
VI	64.5	0.56	+	
		0.06	–	
		0.08	–	
VII	84.0	0.33	+	Hexanucleotide Tr-T_6P_5
VIII	15.7	0.33	+	
		0.04	–	
		0.29	+	
		0.05	–	
IX	40.04	0.23	+	
X	416	0.12	+	Octanucleotide Tr-T_8P_7
XI	46.8	0.10	+	
		0.08	+	
1 *M* eluate	40.8			

[a] The separation was by DEAE-cellulose column chromatography. The elution pattern is shown in Figure 3 and the conditions for the condensation are in the legend to Figure 3. [b] R_f values determined by thin layer chromatography in solvent A on cellulose powder plates.

Thus, by using a 10:1 ratio of pTpTpT-OAc to the hexanucleotide, the nonanucleotide was obtained in yields of around 75% and the loss through degradation was around 11%. From these results, therefore, it is concluded that the use of the trinucleotide block was essentially as effective as that of the dinucleotide block.

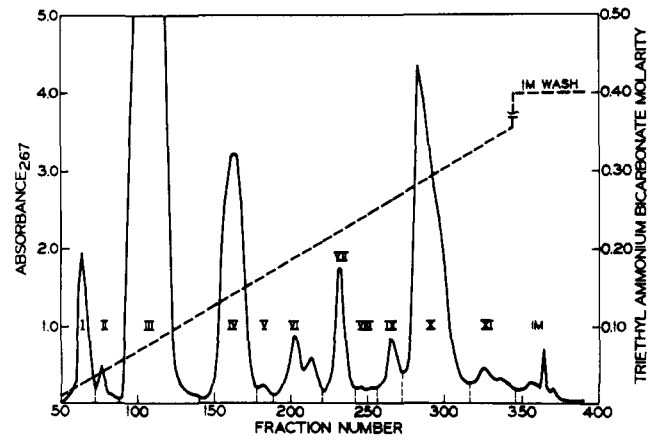


Figure 3. Elution pattern of the total nucleotidic products obtained in a condensation reaction between Tr-T_6P_5 and the dinucleotide pTpT-OAc. The reaction conditions were as given in expt 2 of Table I. A DEAE-cellulose column of 60 × 1 cm diameter was used. A total of 2531 OD_{267} units of the reaction mixture was applied to the column. After application, the column was washed with 0.01 *M* $(C_2H_5)_3NH^+HCO_3^-$ in 30% ethanol until the absorbance at 253 mμ was near zero. The effluents at this point were found to contain 255 OD_{267} units after removal of pyridine. This was mostly the triethylammonium salt of mesitylenesulfonic acid. A linear gradient of $(C_2H_5)_3NH^+HCO_3^-$ in 30% ethanol was then applied as shown in the figure. Fractions of approximately 3.5 ml were collected every 20 min. The analysis of the products is given in Table II.

Table III. Conditions and Results of Experiments on the Condensation of the Trinucleotide pTpTpT-OAc with the Hexanucleotide Tr-T_6P_5[a]

Expt	MsCl:P	pTpTpT-OAc, molar equiv	Time, hr	Nonanucleotide yield, %	Unreacted Tr-T_6P_5, %	Calcd[b] degradation, %
1	0.57	10	2	75	14	11
2	0.57	10	2	73	15	12
3	0.75	20	1	63	29	8

[a] All the experiments were in 0.5 ml of pyridine at 0.02 *M* concentration of Tr-T_6P_5. For further details, see text. [b] The numbers in this column indicate the loss of Tr-T_6P_5 or the desired product, presumably by cleavage of internucleotide bonds during the condensation reactions. These numbers were calculated by subtracting the isolated desired product and recovered hexanucleotide from 100%.

Table IV. Analysis of Nucleotidic Products Obtained in Condensation of pTpTpT-OAc with Tr-T_6P_5[a]

Peak no. (of Figure 5)	Total OD_{267}	R_f[b]	Test for trityl group	Identification remarks
I (fractions 20–65 inclusive)	86.8		–	Minimum of ten compounds (unidentified)
II	38.2	0.57	–	Trace
		0.44	–	Trace
		0.34	–	Trace
		0.24	–	Trace
III	1480.0	0.42	–	Trace
		0.33	–	Mostly pTpTpT
		0.23	–	Trace, unidentified
IV	51.3	0.94	+	Trace, unidentified
		0.76	+	Trace, unidentified
		0.57	–	Trace, unidentified
		0.37	–	Trace, unidentified
V	48.8	0.70	+	Mostly Tr-T_6P_5
		0.61	+	Trace
		0.29	–	Trace
VI	189.0	0.34	–	Symmetrical pyrophosphate of pTpTpT
VII	41.7	0.58	+	Trace
		0.28	–	Trace
VIII	391.0	0.54	+	TrT_9P_8
IX	51.5	0.45	+	Unidentified
1 *M* eluate	16.8			Unidentified

[a] The separation was by DEAE-cellulose column chromatography. The elution pattern is shown in Figure 5, conditions for the condensation are in Table III, and conditions for chromatography are in the legend to Figure 5. [b] On thin layer cellulose chromatography in solvent A.

Condensations Using the Tetranucleotide pTpTpTpT-OAc Block. The rate of reaction using this block is compared with those for other blocks in Figure 4 and the yields obtained in a series of comparative experiments are given in Table V. The detailed analysis of the reaction products obtained in a typical condensation is given in Figure 6 and Table VI. As seen, the rate of condensation using this block was markedly lower than those with the di- and trinucleotides (Figure 4) and, in all experiments (Table V), the yields were also lower than those obtained with the shorter blocks. Thus, the use of a MsCl:P ratio of 0.57, 10 equiv of the tetranucleotide block, and a 2-hr reaction time gave the decanucleotide in a yield close to 40%; however, the extent of degradation was similar to that obtained in di- and trinucleotide condensations under similar conditions. The use of a 20-fold excess of the tetranucleotide did result in an increase in the yield, but a further attempt in which the time of reaction was increased gave mainly an increase in the extent of degradation (Table V). An increase in the MsCl:P ratio (expt 4 of Table V) in addition to the use of a 20-fold excess of the block resulted in no advantage.

Condensation Using the Pentanucleotide pTpTpTpTpT-OAc Block. Figure 4 compares the rate of this condensation with those obtained in the preceding condensations. It was surprising that the rate and the yield of

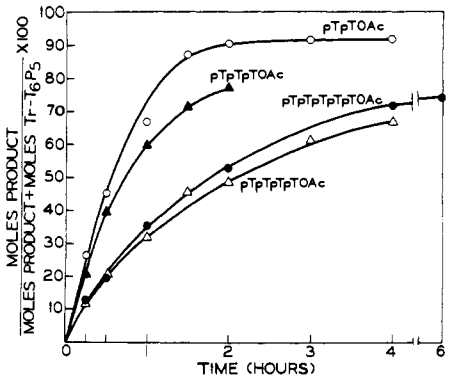


Figure 4. Comparative study of the rates of condensation of the hexanucleotide Tr-T_6P_5 with oligonucleotide blocks of increasing chain length. Each reaction mixture contained 0.01 mmol of Tr-T_6P_5 and 0.10 mmol of the acetylated oligonucleotide block in 0.5 ml of pyridine. The MsCl:P ratio was maintained at 0.57 throughout. For further details see Experimental Section.

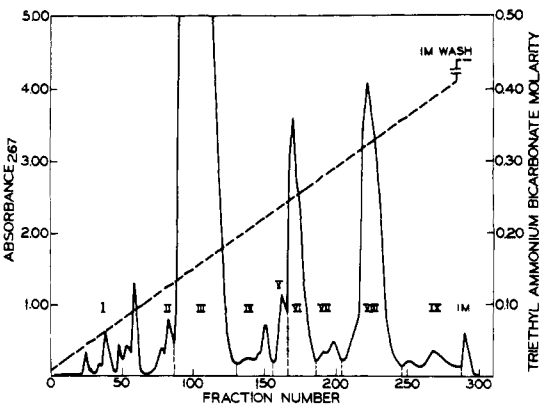


Figure 5. Elution pattern of the total nucleotidic products obtained in a condensation of Tr-T_6P_5 with the trinucleotide pTpTpT-OAc. The reaction conditions were as in expt 1 of Table III. A DEAE-cellulose column of 60 × 1 cm diameter was used. A total of 2390 OD_{267} units was applied. After application the column was washed with 0.01 *M* $(C_2H_5)_3NH^+HCO_3^-$ in 30% ethanol. The effluent contained 153 OD_{267} units after removal of pyridine. This was mostly triethylammonium mesitylenesulfonate. A linear gradient of $(C_2H_5)_3NH^+HCO_3^-$ in 30% ethanol was applied as shown in the figure. Fractions of approximately 3.7 ml were collected every 20 min. The analysis of the products is given in Table IV.

Table V. Conditions and Results of Experiments on the Condensation of the Tetranucleotide pTpTpTpT-OAc with the Hexanucleotide Tr-T_6P_5[a]

Expt	MsCl:P	pTpTpTpT-OAc, molar equiv	Time, hr	Decanucleotide yield, %	Unreacted Tr-T_6P_5, %	Calcd[b] degradation, %
1[b]	0.57	10	2	37	56	7
1[c]	0.57	10	2	38	51	11
2	0.57	10	4	50	33	17
3	0.57	20	2[c]	49	34	17
	0.57	20	4[c]	53	22	25
4	0.75	20	1[d]	41	42	17
	0.75	20	2[d]	48	33	19

[a] All of the experiments were in 0.5 ml of pyridine using 0.01 mmol of Tr-T_6P_5. For further details see text. [b] The numbers in this column indicate the loss of Tr-T_6P_5 or the desired product, presumably by cleavage of internucleotide bonds during the condensation reactions. These numbers were calculated by subtracting the isolated desired product and recovered hexanucleotide from 100%. [c] These analyses represent two time period aliquots of expt 3. [d] These analyses represent two time period aliquots of expt 4.

Table VI. Analysis of Nucleotidic Products Obtained in the Condensation of pTpTpTpT-OAc with Tr-T_6P_5[a]

Peak no. (of Figure 6)	Total OD_{267}	R_f[b]	Test for trityl group	Identification remarks
I	57.4			
II	143	0.41	−	
III	2960	0.38	−	pTpTpTpT
IV	244	0.64	+	Mostly Tr-T_6P_5
		0.23	−	Trace
V	54.3	0.59	−	
		0.27	−	
VI	139	0.57	+	Trace
		0.28	−	Mostly the symmetrical pyrophosphate of pTpTpTpT
VII	48.0	0.55	+	
		0.20	−	
VIII	311	0.51	+	Tr-$T_{10}P_9$
IX	62.9	0.79	−	Trace
		0.40	+	
		0.96	−	Trace
		0.77	−	
		0.43	+	
1 *M* eluate	15.1			

[a] The separation was by DEAE-cellulose chromatography. The elution pattern is shown in Figure 6 and the conditions for the condensation are in the legend to Figure 6. [b] On thin layer cellulose chromatography in solvent A.

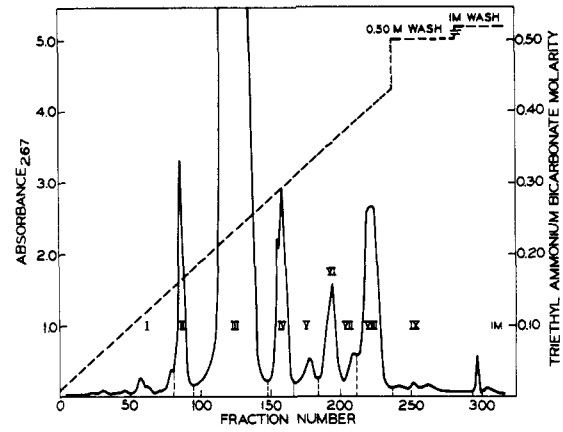


Figure 6. Elution pattern of the total nucleotidic products of a condensation reaction of Tr-T_6P_5 with the tetranucleotide pTpTpTpT-OAc. The reaction conditions were as in expt 1a in Table V. A DEAE-cellulose column of 84 × 1.5 cm diameter was used. A total of 4220 OD_{267} units of the reaction mixture was applied to the column. After application, washing with 0.01 *M* $(C_2H_5)_3NH^+HCO_3^-$ in 10% ethanol gave an effluent which contained 197 OD_{267} units after removal of pyridine. This was mainly triethylamomonium mesitylenesulfonate. A linear gradient of $(C_2H_5)_3NH^+HCO_3^-$ in 10% ethanol was then applied as shown in the figure. Fractions of approximately 9.2 ml were collected every 15 min. The analysis of the products is given in Table VI.

Table VII. Analysis of Nucleotidic Materials Obtained in the Condensation of pTpTpTpTpT-OAc with Tr-T_6P_5[a]

Peak no. (of Figure 7)	Total OD_{267}	R_f[b]	Test for trityl group	Identification remarks
I	106			Mesitylenesulfonic acid
II	112			
		0.59	+	Trace
		0.42	−	
III	142	0.58	+	Tr-T_6P_5 (46%)
IV	346	0.30	−	pTpTpTpTpT
V	1660	0.30	−	pTpTpTpTpT
VI	47.6			
VII	296	0.44	+	Tr-$T_{11}P_{10}$ and the symmetrical pyrophosphate of pTpTpTpTpT[c]
		0.26	−	
1 *M* eluate	25.1			

[a] The separation was by DEAE-cellulose column chromatography. The elution pattern is shown in Figure 7. [b] On thin layer cellulose chromatography in solvent A. [c] This product was separated from the pyrophosphate as described in the Experimental Section; the yield of Tr-$T_{11}P_{10}$ was 49%.

the product were, if anything, higher than that obtained in the tetranucleotide condensation. The detailed analysis of an experiment carried out using a tenfold excess of the pentanucleotide block, an MsCl:P ratio of 0.57, and a 2-hr time is shown in Figure 7 and Table VII. As seen in Table VII, the isolated yield of the undecanucleotide product was 49% and the recovery of the unreacted Tr-T_6P_5 was about 46%; the extent of degradation observed was only 5%.

The Reaction of the Hexanucleotide Tr-TpTpTpTpTpT-OAc with MsCl. In an attempt to probe further the cause of oligonucleotide chain cleavage during condensation reactions, a control experiment was performed in which the 3′-O-acetylated hexanucleotide Tr-TpTpTpTpTpT-OAc was treated for 4 hr with a large excess (MsCl:P = 5) of MsCl. The products formed were separated on a DEAE-cellulose column and the results are given in Figure 8 and Table VIII. As expected, a number of 5′-O-trityl oligonucleotides bearing phosphomonoester and groups (presumably 3′-phosphates)

Table VIII. Analysis of the Products Obtained by Treatment of Tr-T_6P_5-OAc with Mesitylenesulfonyl Chloride[a]

Peak no. (of Figure 8)	Total OD_{267}	R_f[b]	Test for trityl group	R_f[b] of trityl-containing spots after alkaline phosphatase treatment	Identification remarks
I	5.58	0.94	+		
		0.82	−		
		0.43	−		
		0.18	−		
II	3.45	0.90	−	0.92	Tr-Tp[d]
		0.77	−	0.62 (trace)	
		0.50	+		
		0.14 (trace)	−		
		0.09	−		
III[c]	2.22				
IV	6.60	0.62	−		
		0.08 (trace)	−		
V	5.58	0.24	−		
		0.10 (trace)	−		
VI	6.79	0.82 (trace)	+	0.82	Tr-TpTp[d]
		0.32	+	0.50	
VII	10.1	0.23	+	0.59	Tr-TpTpTp[d]
		0.13 (trace)	−	0.30	
		0.05 (trace)	−		
VIII	8.99	0.36	+	0.73	
		0.20	+	0.40	
		0.03 trace)	−		
IX	25.3	0.39	+	0.75	Tr-TpTpTpTp[d]
		0.23	+	0.48	
				0.23	
X	813.0	0.28	+		Tr-T_6P_5
XI	13.3	0.06	+	0.45	Tr-TpTpTpTpTp[d]
1 *M* eluate[c]	2.17				

[a] The separation was by DEAE-cellulose column chromatography. The elution pattern is shown in Figure 8. [b] On thin layer cellulose chromatography in solvent B. [c] These fractions were shown not to contain any nucleotidic material by ultraviolet spectral data. [d] These structures were assigned on the basis of relative R_f values. The mobilities of the trityl-containing compounds after alkaline phosphatase treatment do not correspond exactly to the values of the expected products of the digest as shown in Table IX. This may have been due to the presence of salts in the reaction mixture.

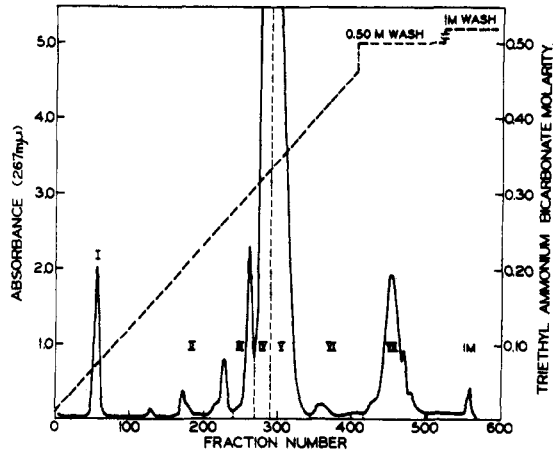


Figure 7. Elution pattern of the total nucleotidic products of a condensation reaction of Tr-T_6P_5 with the pentanucleotide pTpTpTpTpT-OAc. A standard amount (0.01 mmol) of Tr-T_6P_5 in 0.5 ml of pyridine was used. The amount of the pentanucleotide was tenfold, the ratio of MsCl:P was 0.57, and the reaction time was 2 hr. The work-up was as in the Experimental Section. A DEAE-cellulose column (86 × 1.5 cm diameter) was used. After application, a wash with 0.01 *M* $(C_2H_5)_3NH^+HCO_3^-$ in 40% ethanol eluted 28 OD_{267} units of an unidentified material after removal of pyridine. A linear gradient of $(C_2H_5)_3NH^+HCO_3^-$ in 40% ethanol was then applied as shown in the figure. Fractions of approximately 5.0 ml were collected every 15 min. The analysis of the products is given in Table VII.

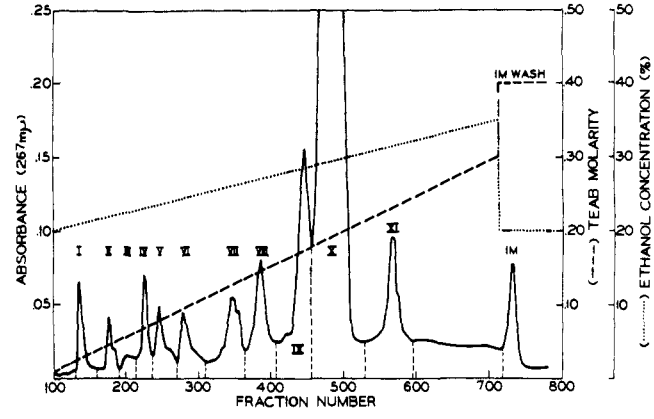


Figure 8. Elution pattern of the total nucleotidic products of the reaction of Tr-T_6P_5-OAc with mesitylenesulfonyl chloride. The reaction conditions are given in the Experimental Section. For chromatography, a DEAE-cellulose column (73 × 1.5 cm diameter) was used. The total reaction mixture was applied and the column was washed with 0.01 *M* $(C_2H_5)_3NH^+HCO_3^-$ in 20% ethanol. The effluent at this point contained 372 OD_{267} units after removal of pyridine. This was triethylammonium mesitylenesulfonate. A linear gradient of $(C_2H_5)_3NH^+HCO_3^-$ and ethanol was then applied as shown in the figure. Fractions of approximately 4.0 ml were collected every 15 min. The analysis of the products is given in Table VIII.

were obtained (Table VIII). While the finding of these products further supported the previously proposed mechanism for chain cleavage,[3,6b] the extent of degradation (about 20%) appeared to be less than that which

Table IX. R_f Values of Different Compounds on Paper and Thin Layer Chromatography

Compound	R_f value: Solvent A, Paper	Solvent B, Paper	Solvent B, Thin layer
Tr-T			0.93
Tr-T_2P			0.85
Tr-T_3P_2			0.78
Tr-T_4P_3			0.63
Tr-T_5P_4			0.44
Tr-T_6P_5	0.70	0.52	0.23
Tr-T_6P_5-OAc			0.35
Tr-T_8P_7	0.57	0.29	
Tr-T_9P_8	0.47	0.15	
Tr-$T_{10}P_9$	0.46	0.11	
Tr-$T_{11}P_{10}$	0.43		
pTpT	0.43	0.39	
pTpT-OAc		0.45	
pTpTpT	0.38	0.29	
pTpTpT-OAc		0.35	
pTpTpTpT	0.32	0.22	
pTpTpTpT-OAc		0.28	
pTpTpTpTpT	0.31	0.17	
pTpTpTpTpT-OAc		0.25	

might have been expected in a block condensation reaction in which a comparable time and MsCl were to be used.

Discussion

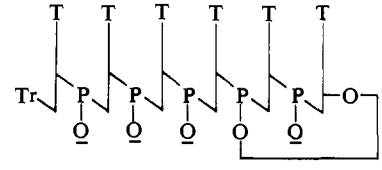
The present work has reported on a further systematic study of the approach to polynucleotide synthesis which involves the use of preformed oligonucleotide blocks. The two aspects of this approach which merited attention are (a) optimal yields of the product and (b) minimal degree of side reactions leading to cleavage of polynucleotide chains. By kinetic studies and by detailed chromatographic analysis, attention was focussed on both of these aspects and, as a result, quite precise conditions have been formulated for performing block condensations. The over-all recommendations are the use of a large (about tenfold) excess[18] of the oligonucleotide block bearing the phosphomonoester group, a rather low molar proportion (MsCl:P $\sim$ 0.6) of the sulfonyl chloride, and a short reaction period (about 2 hr). Using these conditions, yields of 75–78% were obtained in condensations with di- and trinucleotide blocks, and the total extent of degradative reactions was around 10%. These results are a marked improvement over those reported in a previous study[10] (Table VIII of ref 10) and the main reasons would appear to be that, previously, a smaller excess of the dinucleotide and a much larger proportion of the reagent[19] were used. As again found in the present work, in experiments in which only a small excess of the dinucleotide was used, the yield of the product was low (Table I and Figure 1) and attempts to increase the yield by using a larger proportion of MsCl led to a concomitant increase in the extent of degradative reactions (Table I).

(18) The excess for optimal results would of course vary with the size of the component bearing the 3′-hydroxyl end group. It is likely that, with chain lengths longer than the hexanucleotide used in the present work, a larger excess would be desirable.

(19) Triisopropylbenzenesulfonyl chloride was used mostly in the previous work. However, while MsCl is known to give higher rates of condensation, the change in the reagents is not believed to be a major reason for the differences in the results.

The maximum yields and the rates of reaction observed with the tetra- and pentanucleotide blocks were lower than those found for the di- and trinucleotide blocks. On the whole, therefore, the use of di- and trinucleotide blocks will be preferred but, on the other hand, there can well be situations[20] where the use of larger blocks offers outstanding advantages. In particular, the present work has demonstrated for the first time the feasibility of using a pentanucleotide block; the yield of the expected product was around 50%, the recovery of the unreacted hexanucleotide was good, and the extent of degradative reactions was only about 5%. In addition, these results, point up the desirability of testing the use of even larger blocks in condensations.

Partial characterization of many of the side products, especially those that contained the trityl group at one end and a phosphomonoester group at the other end, was carried out. A plausible mechanism for their formation has previously been proposed.[3,6b] However, all the factors involved in the degradation of the polynucleotide chains are obviously not understood. Thus, in an experiment in which the 3′-O-acetyl derivative of Tr-T_6P_5 was treated with a high proportion of MsCl in dry pyridine, the total extent of degradation was less than might have been expected in a condensation reaction carried out under comparable conditions. At least two possibilities for the observed difference can be considered. One is that the nature of the activated internucleotide bonds undergoing cleavage is different when a large excess of the oligonucleotide component with a phosphomonoester group is also present in the reaction mixture. The second possibility is that the component bearing a 3′-hydroxyl group, *e.g.*, Tr-T_6P_5, is more vulnerable than its 3′-O-acetyl derivative. This is easy to understand because an additional facile reaction could be the involvement of the 3′-OH end group in reaction with an activated internucleotidic linkage in the same molecule to form a triester linkage as shown in I. Thus, the formation of cyclothymidine di- and



I

trinucleotides occurs with great ease,[21] and this clearly involves the esterification of the 3′-terminal hydroxyl group. Intermediates of this type would break down readily by attack of pyridine to form a 5′-C-pyridinium group at one end, and phosphodiester linkage now produced at the site would be of the $C_{3'}$–$C_{3'}$ variety. This mechanism, while leading to consumption of the oligonucleotide component with the 3′-hydroxyl group, does not, however, lead to the formation of products with phosphomonoester end groups.

(20) The lower yields could be compensated for by the fact that although the yield for an individual condensation might be low, the total number of steps in the synthesis are fewer than if smaller blocks were used. Further, as mentioned already, the use of a larger block may be necessary for the separation of the product from the starting materials.

(21) G. M. Tener, H. G. Khorana, R. Markham, and E. H. Pol, *J. Am. Chem. Soc.*, **80**, 6223 (1958).