pA^{Bz}-OAc (432 OD_{260} units) in anhydrous pyridine (1 ml) was polymerized under standard conditions (2 hr) using mesitylenesulfonyl chloride (0.15 g). After the standard work-up involving treatment with water and triethylamine, the reaction mixture was concentrated to a gum and treated with a 1:1 mixture of methanol and *n*-butylamine (10 ml) for 5 days to remove the protecting groups; 80% of the product was concentrated, taken up in 0.1 *M* triethylammonium bicarbonate solution (2 ml) and chromatographed on a Sephadex G-50 (fine) column (2.5 × 300 cm) using 0.1 *M* triethylammonium bicarbonate as the eluent. The elution pattern is given in Figure 13. The distribution of the polymers is given in Table XXVII. The R_f values and yields of the polymers are given in Table XXVIII.

Acknowledgment. The technical assistance of Harry Radloff is acknowledged with appreciation.

Studies on Polynucleotides. LXIV.[1] The Synthesis of Deoxyribopolynucleotides Containing Repeating Tetranucleotide Sequences (1)[2]

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Abstract: Syntheses of three homologous series of deoxyribopolynucleotides containing the following repeating tetranucleotide sequences are described: (1) thymidylylthymidylylthymidylyldeoxycytidylate, (2) deoxyadenylyldeoxyadenylyldeoxyadenylyldeoxyguanylate, and (3) deoxyadenylylthymidylyldeoxycytidylyldeoxyguanylate. The synthesis of the first series was accomplished by the stepwise method which involved repetitive condensation of a suitably protected mononucleotide to the 3′-hydroxyl end group of a growing oligonucleotide chain. Syntheses of deoxyribopolynucleotides belonging to the other two series were accomplished by the polymerization of suitably protected tetranucleotides.

The usefulness of ribopolynucleotides with repeating tetranucleotide sequences in studies of the genetic code has been reviewed in an accompanying paper.[3] As with ribopolynucleotides containing repeating di- and trinucleotide sequences, the first requirement in the preparation of ribopolynucleotides with repeating tetranucleotide sequences was the preparation of short-chain deoxyribopolynucleotides containing the appropriate repeating nucleotide sequences. The work described in this paper formed the first phase of the total synthetic work dealing with the preparation of deoxyribonucleotides containing repeating tetranucleotide sequences. Syntheses of the following three series of deoxyribopolynucleotides have been accomplished: (1) a dodecanucleotide containing the repeating thymidylylthymidylylthymidylyldeoxycytidylate sequence, (2) octa- and dodecadeoxyribonucleotides containing the repeating deoxyadenylyldeoxyadenylyldeoxyadenylyldeoxyguanylate sequence, and (3) octa-, dodeca-, and hexadecanucleotides containing all four mononucleotides in the repeating deoxyadenylylthymidylyldeoxycytidylyldeoxyguanylate sequence. Two accompanying papers[4,5] describe continuation of the work dealing with the synthesis of deoxyribopolynucleotides containing repeating tetranucleotide sequences.

The choice of the repeating tetranucleotide sequences synthesized in this paper was determined by the following considerations. At the start of the present work, DNA-dependent RNA polymerase was the only enzyme which had been used successfully in preparation of a long ribopolynucleotide with a repeating trinucleotide sequence.[6] Thus the use of the previously synthesized nonanucleotide containing the repeating thymidylylthymidylyldeoxycytidylate sequence as template for this enzyme had afforded a long ribopolynucleotide containing the complementary ribonucleotides in repeating sequence. In contrast, attempts to use several other series of short deoxyribopolynucleotides containing purine nucleotides as templates for the above enzyme had given negative results.[7] In extending the use of DNA-dependent RNA polymerase to the preparation of long ribopolynucleotides containing repeating tetranucleotide sequences, it was therefore concluded that deoxyribopolynucleotides containing only pyrimidine nucleotide units in them had the maximum chance of serving as templates. The synthesis of the deoxyribopolynucleotide containing the repeating tetranucleotide sequence thymidylylthymidylylthymidylyldeoxycytidylate sequence was therefore undertaken. Further considerations in favor of this particular sequence were that it required the minimum of protecting groups and that the methodology for chromatography and isolation at successive synthetic steps had been satisfactorily worked

(1) Preceding paper in this series: S. A. Narang, T. M. Jacob, and H. G. Khorana, *J. Am. Chem. Soc.*, **89**, 2167 (1967).

(2) This work has been supported by grants from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service (Grant No. CA-05178), the National Science Foundation (Grant No. GB-3342), and the Life Insurance Medical Research Fund (Grant No. G-62-54).

(3) H. G. Khorana, H. Büchi, T. M. Jacob, H. Kössel, S. A. Narang, and E. Ohtsuka, *J. Am. Chem. Soc.*, **89**, 2154 (1967).

(4) H. Kössel, H. Büchi, and H. G. Khorana, *ibid.*, **89**, 2185 (1967).

(5) E. Ohtsuka and H. G. Khorana, *ibid.*, **89**, 2195 (1967).

(6) S. Nishimura, T. M. Jacob, and H. G. Khorana, *Proc. Natl. Acad. Sci. U. S.*, **52**, 1494 (1964).

(7) Thus, for example, short-chain deoxyribopolynucleotides containing alternating deoxyadenylate and deoxyguanylate units and another series containing alternating thymidylate and deoxyguanylate units had failed to serve as templates for RNA polymerase: unpublished results of Drs. S. Nishimura and B. D. Mehrotra.

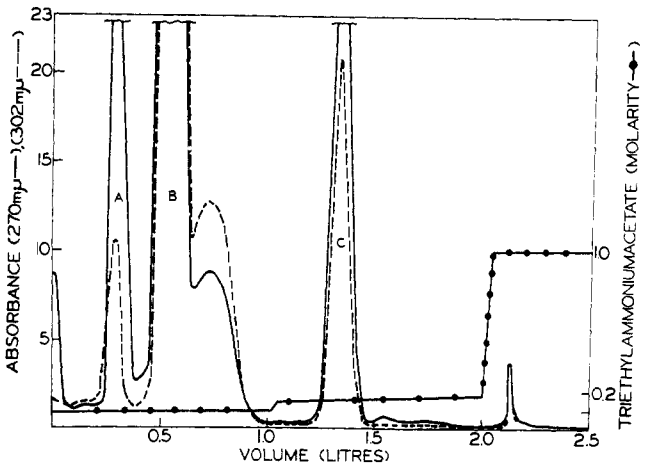


Figure 1. Chromatography of the reaction products in the preparation of the tetranucleotide d-Tr-TpTpTpC^{An} on a DEAE-cellulose (acetate) column. Peak C contained d-Tr-TpTpTpC^{An}.

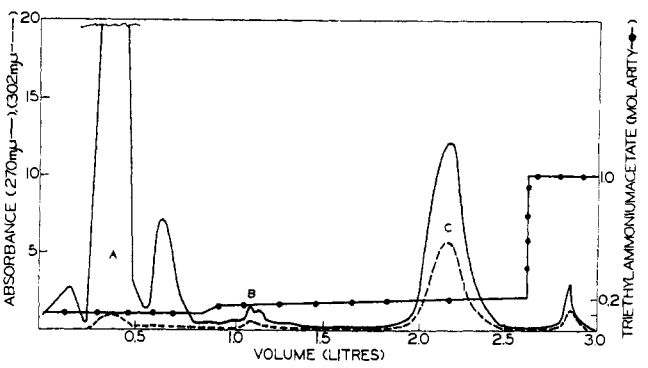


Figure 2. Chromatography of the reaction products in the preparation of the pentanucleotide d-Tr-TpTpTpC^{An}pT on a DEAE-cellulose (acetate) column. Peak C contained d-Tr-TpTpTpC^{An}pT.

out previously for a related series (deoxyribopolynucleotides containing repeating thymidylylthymidylyldeoxycytidylate sequence).[8]

In studies with RNA polymerase, the synthetic octa- and dodecadeoxyribonucleotides containing the repeating tetranucleotide sequence described above were, in fact, shown to bring about the synthesis of a ribopolynucleotide containing the expected repeating adenylyladenylyladenylylguanylate sequence. The yield of the product, however, was low and a careful control of experimental conditions was necessary for faithful reproduction of the repeating tetranucleotide sequence in the product.[9] On the other hand, in concurrent studies with DNA polymerase short deoxyribopolynucleotides with repeating trinucleotide sequences were successfully used as templates, and the products were characterized as DNA-like polymers containing repeating trinucleotide sequences. It therefore appeared promising to attempt to prepare DNA-like polymers with repeating tetranucleotide sequences. (The use of the latter as templates for RNA polymerase would then afford, as before, an efficient route to the preparation of ribopolynucleotides with repeating tetranucleotide sequences.) Because DNA polymerase catalyzed reactions invariably have required short deoxyribopolynucleotide chains corresponding to both strands of the resulting DNA-like polymer, the synthesis of the deoxyribopolynucleotide containing the repeating sequence deoxyadenylyldeoxyadenylyldeoxyadenylyldeoxyguanylate, complementary to the repeating sequence discussed above, was undertaken.

The synthesis of the third series of deoxyribopolynucleotides containing the repeating tetranucleotide sequence deoxyadenylylthymidylyldeoxycytidylyldeoxyguanylate was undertaken in further attempts to explore the DNA polymerase catalyzed synthesis of interesting DNA-like polymers. It is to be noted that this particular sequence is complementary to itself in the antiparallel Watson–Crick base-pairing sense and it would therefore be necessary to carry out the chemical synthesis of one homologous series only for its potential use as a template for the corresponding DNA-like polymer. The resulting DNA-like polymer containing all four bases in strictly defined sequence could be of especial interest for study of DNA secondary structure.

Synthesis by the Stepwise Method. The dodecanucleotide d-T(pTpTpCpT)$_2$pTpTpC[10] was synthesized by the addition of one mononucleotide unit at a time to the 3′-hydroxyl end group of a growing oligonucleotide chain. The starting material was 5′-O-tritylthymidylylthymidylylthymidine, Tr-TpTpT, which was available from previous work,[11] and the protected mononucleotides used were pT-OAc[10] and d-pC^{An}-OAc.[10] The condensing agents used were either mesitylenesulfonyl chloride[12] or triisopropylbenzenesulfonyl chloride,[13] the reactions being run at room temperature in pyridine for about 4 hr as was done in the previous work.[8] Following each condensation reaction, an alkaline treatment, which was completely selective for the removal of the terminal 3′-O-acetyl group, was given and the products were then separated. The method of separation used up to the preparation of the protected octanucleotide, d-Tr-TpTpTpC^{An}pTpTpTpC^{An}, involved anion-exchange chromatography on DEAE-cellulose (acetate) columns using triethylammonium acetate (in 50% ethyl alcohol) as the eluting agent. The elution patterns obtained at different steps are shown in Figures 1–5.

The technique of gel filtration on Sephadex G-50 columns was used during the four subsequent synthetic steps (nona- to dodecanucleotide) for separation of the condensation product from the unused mononucleotide, sulfonic acid, and chloride ions. The elution patterns obtained are shown in Figures 6–9. This technique, which provided a simple, satisfactory, and rapid method for removal of the mononucleotide, was not expected to separate the unreacted polynucleotide from the condensation product which differed in chain length by only one nucleotide unit. Direct use of the polynucleotidic products thus obtained in further condensation reactions, therefore, required assurance that the yield at each step was practically quantitative. Careful examination of the products, with and without protecting groups, by paper chromatography did, in

(8) T. M. Jacob and H. G. Khorana, *J. Am. Chem. Soc.*, 87, 2971 (1965).
(9) Unpublished work of Dr. Richard Morgan in this laboratory.
(10) For the basic system of abbreviations see footnote 3 in S. A. Narang, T. M. Jacob and H. G. Khorana *J. Am. Chem. Soc.*, 89, 2158 (1967). For designation of the polymers with repeating sequences, further extension of this system of abbreviations has been described in the preceding paper.[1]
(11) T. M. Jacob and H. G. Khorana, *ibid.*, 87, 368 (1965).
(12) T. M. Jacob and H. G. Khorana, *ibid.*, 86, 1630 (1964).
(13) R. Lohrmann and H. G. Khorana, *ibid.*, 88, 829 (1966).

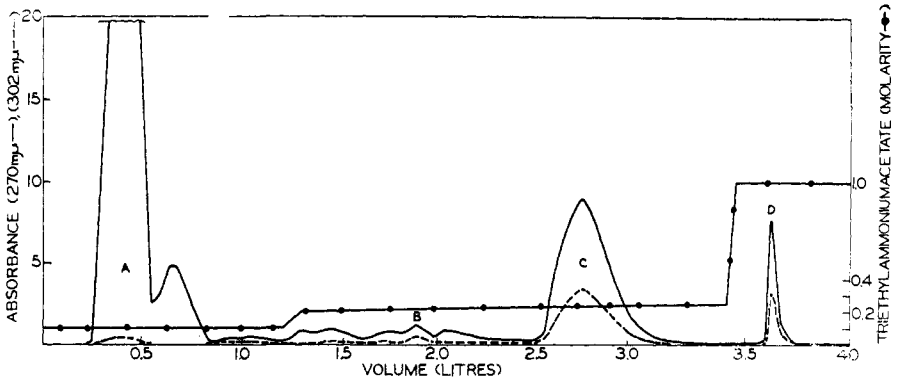
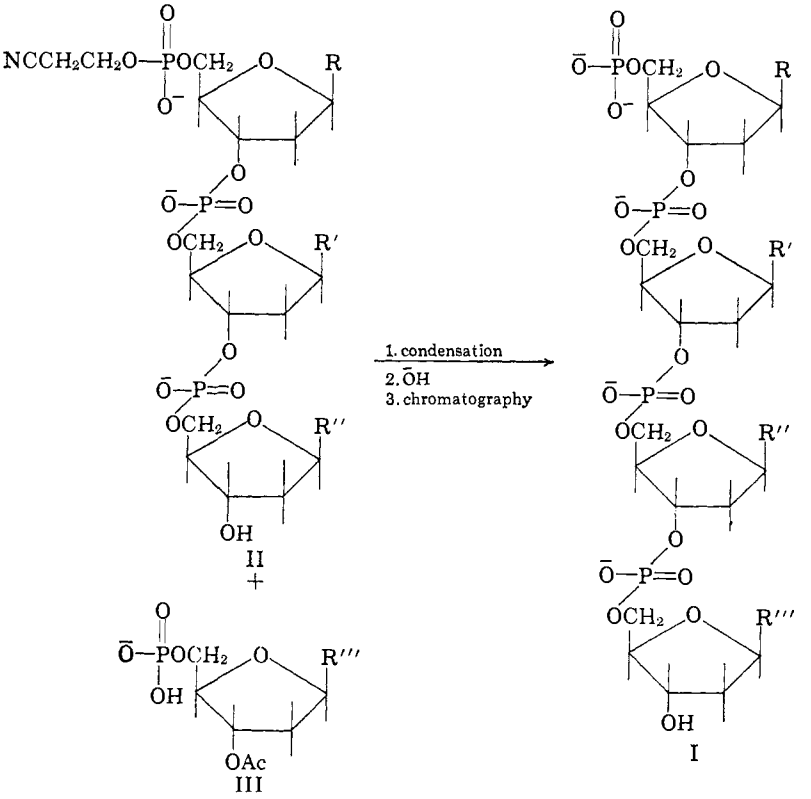


Figure 3. Chromatography of the reaction products in the preparation of the hexanucleotide d-Tr-TpTpTpC^{An}pTpT on a DEAE-cellulose (acetate) column. Peak C contained d-Tr-TpTpTpC^{An}pTpT.

fact, show that the yield at the individual step was about 90%. Furthermore, it should be noted that, in this particular series, contamination from the unreacted polynucleotidic starting materials was not expected to cause ambiguity in nucleotide sequence at least at three out of the four final steps (nonanucleotide, decanucleotide, and undecanucleotide) because the mononucleotide component was pT-OAc in all of these steps. The solvent systems used for paper chromatography (solvents B and D) clearly resolved the homologous products expected, and the protected undecanucleotide could thus be ascertained to be pure before use for the final step, which required condensation with d-pC^{An}-OAc.



Syntheses by the Polymerization Method. Because of the encouraging results obtained in concurrent work on the polymerization method, this method was used for the synthesis of the two remaining series of deoxyribopolynucleotides. The protected tetranucleotides (I), which served as starting materials for polymerization, were prepared by extension of the stepwise method described in an accompanying paper[14] for the synthesis of protected deoxyribotrinucleotides. Protected trinucleotides bearing a 3′-hydroxyl group at one end and a 5′-phosphomonoester group at the other end were converted to the monocyanoethyl derivatives II. These were condensed with an excess of the protected mononucleotide of type III and an alkaline treatment was then given to remove the acetyl and cyanoethyl groups. The required products, I, which were obtained in high yield, were isolated by anion-exchange chromatography. Thus in one series, the protected trinucleotide d-pA^{Bz}-pA^{Bz}pA^{Bz}, which was available from work described in an accompanying paper,[14] was converted to the cyanoethyl ester d-CE-pA^{Bz}pA^{Bz}pA^{Bz}, and the latter was condensed with d-pG^{Ac}-OAc. After an alkaline treatment the required product, d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac}, was isolated by chromatography on a DEAE-cellulose column

(14) See ref 1, and also article cited in ref 10.

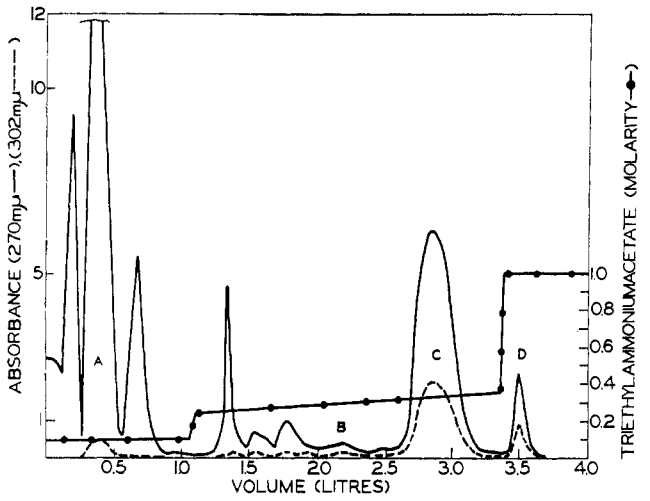


Figure 4. Chromatography of the reaction products in the preparation of the heptanucleotide d-Tr-TpTpTpC^{An}pTpTpT on a DEAE-cellulose (acetate) column. Peak C contained d-Tr-TpTpTpC^{An}pTpTpT.

All of the synthetic products described above were checked for purity by chromatography in solvents B and D (Tables I, IV–VI) at different stages of purification. Finally, selected compounds were tested with spleen phosphodiesterase for complete degradation and for an analysis of the ratios of the different nucleosides and nucleotides produced as products. The results are given in Table VII.

The synthetic deoxyribopolynucleotide d-T(pT-pTpCpT)$_2$pTpTC, in combination with the complementary deoxyribopolynucleotides containing the repeating d-ApApApG sequence, has already been shown to serve as a template for the DNA polymerase of *Escherichia coli*, and the product has been characterized as a DNA-like polymer containing the expected repeating tetranucleotide sequences in the complementary strands.[15–17]

Experimental Section

General Methods and Materials. Paper chromatography and paper electrophoresis were performed as described earlier.[14] Tr-

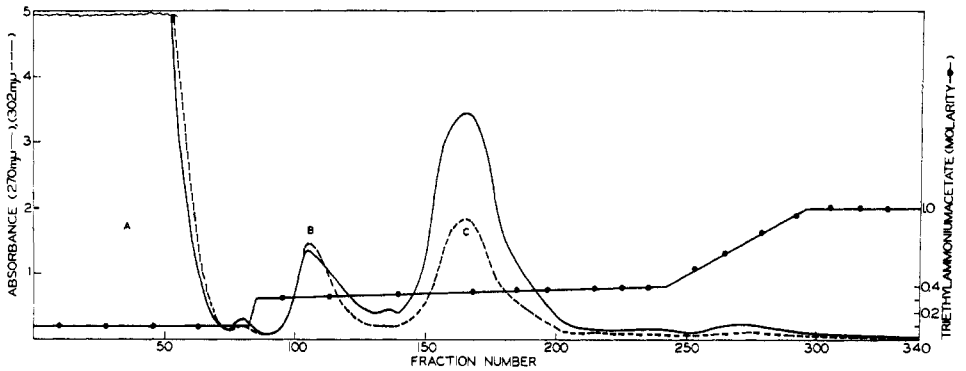


Figure 5. Chromatography of the reaction products in the preparation of the octanucleotide d-Tr-TpTpTpC^{An}pTpTpTpC^{An} on a DEAE-cellulose (acetate) column. Peak C contained d-Tr-TpTpTpC^{An}pTpTpTpC^{An}.

(elution pattern in Figure 10). In the second series, the protected tetranucleotide used was d-pA^{Bz}pTpC^{An}-pG^{Ac}. The starting protected trinucleotide for this intermediate was d-pA^{Bz}pTpC^{An}, the preparation of which has also been recorded.[14] This was converted to the monocyanoethyl ester which was then condensed with d-pG^{Ac}-OAc. Separation of the products resulting after standard work-up is shown in Figure 11.

The general polymerization procedure developed in the preceding paper was used, a portion of the protected tetranucleotide being present as the corresponding 3′-O-acetyl derivative at the start of the polymerization reaction. The polymerization of d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac} was carried out in dilute pyridine solution in an attempt to reduce the danger of the depurination reaction. The final yields (Table I) of the homologous polynucleotides were low. The separation of the polymerization products, after removal of the protecting groups, was effected by chromatography on a Sephadex G-50 column (Figure 12 and Table II). The polymerization of the protected tetranucleotide d-pA^{Bz}pTpC^{An}pG^{Ac} was carried out in a concentrated pyridine solution using mesitylenesulfonyl chloride as the condensing agent. After standard work-up, the products were separated on a Sephadex G-50 column (Figure 13 and Tables III and IV).

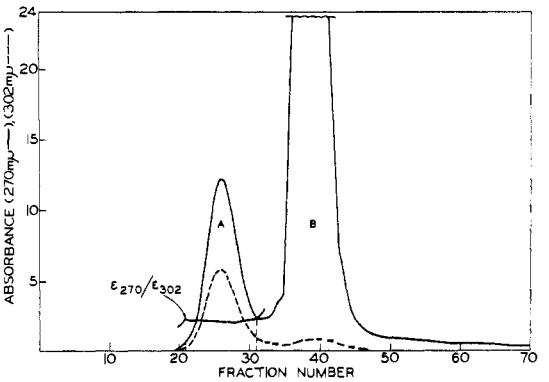


Figure 6. Chromatography of the reaction products in the preparation of the nonanucleotide d-Tr-TpTpTpC^{An}pTpTpTpC^{An}pT on a Sephadex G-50 (coarse) column. Peak A contained the nonanucleotide.

TpTpT was prepared as described earlier.[11] Preparation of d-pA^{Bz}pA^{Bz}pA^{Bz} is given under the preparation of d-pA^{Bz}pA^{Bz}

(15) R. D. Wells, T. M. Jacob, H. Kössel, A. R. Morgan, S. A. Narang, E. Ohtsuka and H. G. Khorana, *Federation Proc.*, **25**, 404 (1966).

(16) H. G. Khorana in "Proceedings of the Third Meeting of the European Biochemists, Warsaw, 1966," in press.

(17) H. G. Khorana, H. Büchi, H. Ghosh, N. Gupta, T. M. Jacob, H. Kössel, R. Morgan, S. A. Narang, E. Ohtsuka, and R. D. Wells, *Cold Spring Harbor Symp. Quant. Biol. (June 1966)*, in press.

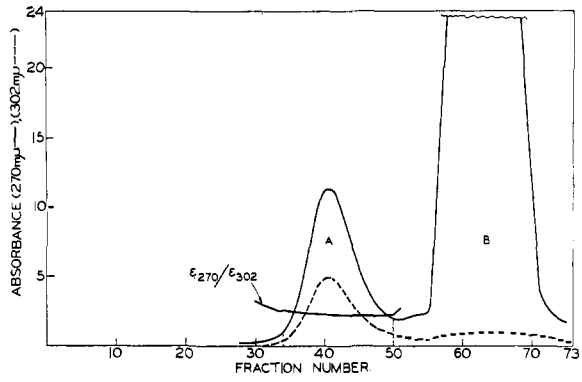


Figure 7. Chromatography of the reaction products in the preparation of the decanucleotide d-Tr-T(pTpTpC^{An}pT)$_2$pT on a Sephadex G-50 (coarse) column. Peak A contained the decanucleotide.

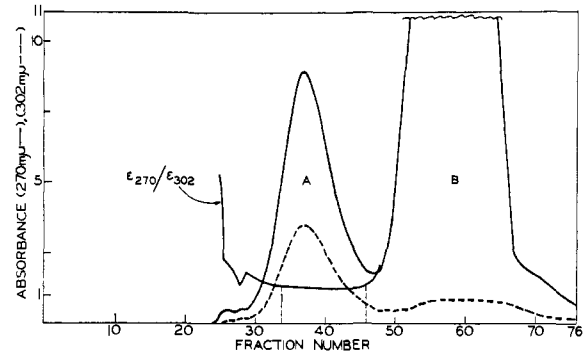


Figure 8. Chromatography of the reaction products in the preparation of the undecanucleotide d-Tr-T(pTpTpC^{An}pT)$_2$pTpT on a Sephadex G-50 (coarse) column. Peak A contained the undecanucleotide.

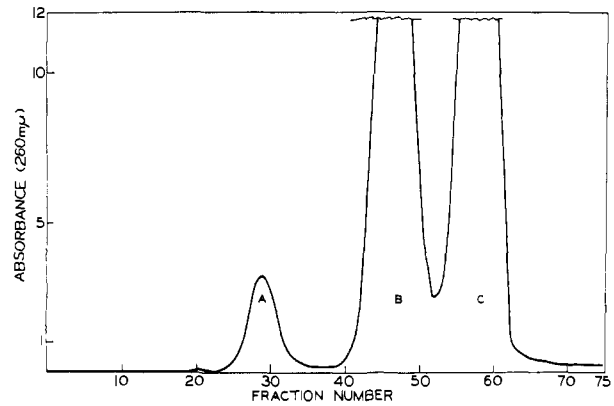


Figure 9. Chromatography of the reaction products in the preparation of the dodecanucleotide d-Tr-T(pTpTpCpT)$_2$pTpTpC on a Sephadex G-50 (coarse) column. Peak A contained the dodecanucleotide.

in the preceding paper.[14] Preparation of d-pA^{Bz}pTpC^{An} is given in the preceding paper.[14]

Enzymatic degradation of the synthetic products was carried out by using bacterial alkaline phosphatase and spleen phosphodiesterase as described previously.[18] The general method of condensation and work-up used for the elongation of an oligonucleotide chain containing a 5′-O-trityl protecting group by the addition

(18) H. G. Khorana, A. F. Turner, and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **83**, 686 (1961).

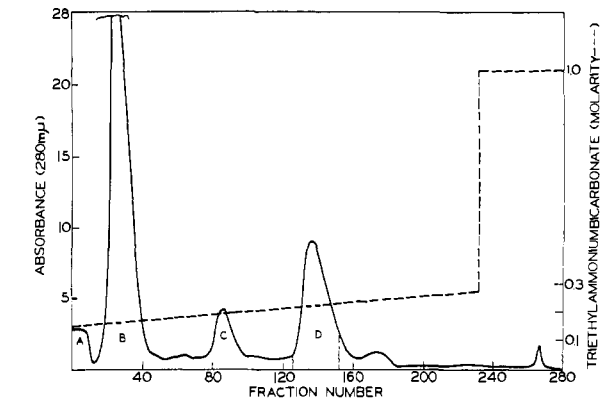


Figure 10. Chromatography of the reaction products in the preparation of the tetranucleotide d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac} on a DEAE-cellulose (carbonate) column. Peak D contained the tetranucleotide.

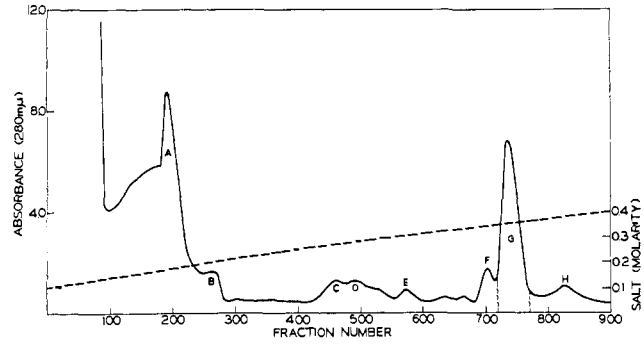


Figure 11. Chromatography of the reaction products in the preparation of the tetranucleotide d-pA^{Bz}pTpC^{An}pG^{Ac} on a DEAE-cellulose (carbonate) column. Fractions 720–765 contained the tetranucleotide.

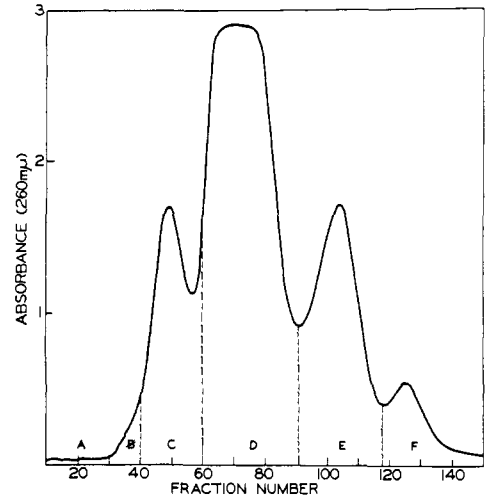


Figure 12. Chromatography of the reaction products in the polymerization of a mixture of d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac} and d-pA^{Bz}pA^{Bz}-pA^{Bz}pG^{Ac}-OAc on a Sephadex G-50 (coarse) column.

of a protected mononucleotide to the 3′-hydroxyl group is as described earlier.[8] Triethylammonium acetate buffer used contained 50% alcohol. The isolation of protected oligonucleotides from triethylammonium acetate buffer was carried out as described earlier.[8] Triethylammonium bicarbonate buffer was removed by evaporation under reduced pressure in the presence of added pyri-

Table I. Yields and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Tetranucleotide Sequence Deoxyadenylyldeoxyadenylyldeoxyadenylyldeoxyguanylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group	R_f in solvent C[a] With 5′-phosphate end group	R_f in solvent C[a] Without 5′-phosphate end group
d-Cyclo-pApApApG	14				
d-pApApApG	54	1	1.9	1	1.8
d-pApApApGpApApApG	11	0.2	0.46	0.64	0.9
d-pA(pApApGpA)$_2$pApApG	1	0.03	0.08	0.31	0.6

[a] R_f relative to d-pApApApG.

dine. The removal of protecting groups was done as described earlier.[8,14]

The Tetranucleotide d-Tr-TpTpTpC^{An}. An anhydrous pyridine solution (5 ml) of the triethylammonium salts of the protected trinucleotide d-Tr-TpTpT (3187 OD_{267} units, 0.125 mmole) and pC^{An}-OAc (1 mmole) was treated with mesitylenesulfonyl chloride (0.46 g, 2 mmoles) at room temperature for 3.5 hr. After the usual work-up the mixture was chromatographed at room temperature on a DEAE-cellulose (acetate) column (75 × 2 cm). The conditions of chromatography and the elution pattern obtained are shown in Figure 1. Peak C had a constant $\epsilon_{270}/\epsilon_{302}$ ratio of 1.8 and contained pure d-Tr-TpTpTpC^{An} (2124 OD_{302} units, 76%).

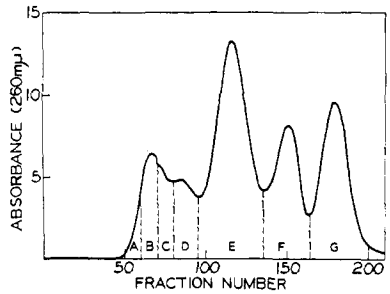


Figure 13. Chromatography of the reaction products in the polymerization of a mixture of d-pA^{Bz}pTpC^{An}pG^{Ac} and d-pA^{Bz}pTpC^{An}-pG^{Ac}-OAc on a Sephadex G-50 (coarse) column.

The Pentanucleotide d-Tr-TpTpTpCp^{An}T. An anhydrous pyridine solution (3 ml) of the triethylammonium salts of d-Tr-TpTpTpC^{An} (1800 OD_{302} units, 0.08 mmole) and pT-OAc (1 mmole) and mesitylenesulfonyl chloride (0.44 g, 2 mmoles) was kept at room temperature for 4 hr. After the usual work-up the mixture was chromatographed at room temperature on a DEAE-cellulose (acetate) column (75 × 2 cm). The conditions of chromatography and the elution pattern obtained are shown in Figure 2. Peak C (630 ml) had a constant $\epsilon_{270}/\epsilon_{302}$ of 2.3 and contained pure d-Tr-TpTpTpC^{An}pT (1436 OD_{302} units, 80%).

Table II. Separation on a Sephadex G-50 Column of the Deoxyribopolynucleotides Containing the Repeating Tetranucleotide Sequence Deoxyadenylyldeoxyadenylyldeoxyadenylyldeoxyguanylate (Elution Pattern in Figure 12)

Peak	Fraction	Total OD_{260} units	Total eluted, %	Identification (%[a])
A	11–30	1.0		Nonnucleotidic material
B	31–40	4.5	1.2	Dodecanucleotide (90)
C	41–60	50.8	13.6	Octanucleotide (80)
D	61–90	251.0	67.4	Tetranucleotide (80)
E	91–118	65.8	17.7	Cyclotetranucleotide (80)
F				Triisopropylbenzenesulfonic acid and benzoic acid

[a] Numbers in brackets show the content of the polynucleotides in the appropriate peaks.

Table III. Separation on a Sephadex G-50 Column of Deoxyribopolynucleotides Containing the Repeating Tetranucleotide Sequence Deoxyadenylylthymidylyldeoxycytidylyldeoxyguanylate (Elution Pattern in Figure 13)

Peak	Fraction	Total OD_{260} units	Total eluted, %	Identification (%[a])
A	49–60	80	5.8	Compounds higher than dodecanucleotide and pyrophosphates
B	61–70	215	15.3	Octanucleotide (16), dodecanucleotide (20)
C	71–80	164	11.7	Octanucleotide (30), dodecanucleotide (20)
D	81–95	185	13.2	Tetranucleotide (18), octanucleotide (50)
E	96–136	756	54	Cyclotetranucleotide (25), tetranucleotide (50)
F	137–164	250	...	Nonnucleotidic material
G	165–200	382	...	Nonnucleotidic material

[a] Numbers in brackets show the percentages of the polynucleotides in the appropriate peaks.

Table IV. Yield and Paper Chromatography of Deoxyribopolynucleotides Containing the Repeating Tetranucleotide Sequence Deoxyadenylylthymidylyldeoxycytidylyldeoxyguanylate

Compd	Yield, %	R_f in solvent D[a] With 5′-phosphate end group	R_f in solvent D[a] Without 5′-phosphate end group	R_f in solvent C[b] Without 5′-phosphate end group
Cyclo-d-pApTpCpG	12.5	1.5	1.5	
d-pApTpCpG	29.5	1	2	1.0
d-pApTpCpGpApTpCpG	11.5	0.2	0.4	0.21
d-pA(pTpCpGpA)$_2$pTpCpG and Higher polynucleotides	6.5	0.05	0.12	0.06

[a] R_f relative to d-pApTpCpG. [b] R_f relative to d-ApTpCpG.

The Hexanucleotide d-Tr-TpTpTpC^{An}pTpT. An anhydrous pyridine solution (3 ml) of the triethylammonium salts of d-Tr-TpTpTpC^{An}pT (1300 OD_{302} units, 0.058 mmole) and pT-OAc (1 mmole) was treated with mesitylenesulfonyl chloride (0.444 g, 2 mmoles) for 4 hr at room temperature. After the usual work-up the mixture was chromatographed at room temperature on a DEAE-cellulose (acetate) column (75 × 2 cm). The conditions of chromatography and the elution pattern obtained are shown in Figure 3. Peak C (770 ml) had a constant $\epsilon_{270}/\epsilon_{302}$ of 2.7 and contained pure d-Tr-TpTpTpC^{An}pTpT (1009 OD_{302} units, 77.6%).

The Heptanucleotide d-Tr-TpTpTpC^{An}pTpTpT. An anhydrous pyridine solution (2 ml) of the triethylammonium salts of d-Tr-TpTpTpC^{An}pTpT (900 OD_{302} units, 0.04 mmole) and pT-OAc (1

Table V. Paper Chromatography of Deoxyribopolynucleotides

Compd	R_f in solvent D[a] With trityl group	R_f in solvent D[a] Without trityl group	R_f in solvent B[a] With trityl group
d-Tr-TpTpTpC			1.4
d-Tr-TpTpTpCpT			0.99
d-Tr-TpTpTpCpTpT			0.64
d-Tr-TpTpTpCpTpTpT			0.35
d-Tr-TpTpTpCpTpTpTpC	0.97	0.43	0.15
d-Tr-TpTpTpCpTpTpTpCpT	0.71	0.32	0.06
d-Tr-T(pTpTpCpT)$_2$pT	0.67	0.25	0.03
d-Tr-T(pTpTpCpT)$_2$pTpT	0.6	0.19	0.016
d-Tr-T(pTpTpTpCpT)$_2$pTpTpC	0.45	0.13	...

[a] R_f with respect to pT.

Table VI. Paper Chromatography and Electrophoresis of Tri- and Tetranucleotides and Their Derivatives

Compd	R_f in solvent B	R_f in solvent D	R_f in solvent C	Electrophoretic mobility, pH 7.1
d-pA^{Bz}pA^{Bz}pA^{Bz}	1.6[a]			0.94[a]
d-CE-pA^{Bz}pA^{Bz}pA^{Bz}	2.4[a]			0.78[a]
d-pApApA	0.18[a]			
d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac}	0.95[a]			
d-pApApApG		0.37[a]		
d-CE-pA^{Bz}pTpC^{An}	2[b]			0.73[b]
d-pA^{Bz}pTpC^{An}pG^{Ac}	0.52[b]			
d-pA^{Bz}pTpC^{An}pG^{Ac}-OAc	0.63[b]			
d-pApTpCpG		0.3[b]	0.49	
d-ApTpCpG		0.61[b]	0.88	

[a] R_f relative to d-pA. [b] R_f relative to pT.

column (1.5 × 200 cm) preequilibrated with 0.1 *M* triethylammonium bicarbonate. Elution was carried out using the same buffer. The conditions of chromatography and the elution pattern obtained are shown in Figure 6. Peak B contained pT. Peak A (72 ml, 212 OD_{302} units, 447 OD_{270} units, 83%) had a constant $\epsilon_{270}/\epsilon_{302}$ of 2.1, except at the tail ends, and contained mainly (95%) the nonanucleotide d-Tr-TpTpTpC^{An}pTpTpTpTC^{An}pT. It was directly used for the next condensation step.

The Decanucleotide d-Tr-T(pTpTC^{An}pT)$_2$pT. An anhydrous pyridine solution (1 ml) of the triethylammonium salts of d-Tr-TpTpTpC^{An}pTpTpTpC^{An}pT (200 OD_{302} units) and pT-OAc (0.17 mmole) and triisopropylbenzenesulfonyl chloride (0.104 g, 0.34 mmole) was kept at room temperature for 4 hr. After the usual work-up the mixture was concentrated to 5 ml with the addition of triethylammonium bicarbonate buffer (1 *M*) and chromatographed on a Sephadex G-50 column as described under the preparation of the nonanucleotide. The elution pattern obtained is shown in Figure 7. Peak B contained pT. Peak A (85 ml, 165 OD_{302} units, 388 OD_{270} units, 82.5%) had a constant $\epsilon_{270}/\epsilon_{302}$ of 2.3, except at the tail ends, and contained mainly (90%) the decanucleotide d-Tr-T-(pTpTpC^{An}pT)$_2$pT. It was directly used for the next condensation step without further purification.

The Undecanucleotide, d-Tr-T(pTpTpC^{An}pT)$_2$pTpT. An anhydrous pyridine solution (1 ml) of the triethylammonium salts of d-Tr-T(pTpTpC^{An})$_2$pT (156 OD_{302} units) and pT-OAc (0.17 mmole) and triisopropylbenzenesulfonyl chloride (0.104 g, 0.34 mmole) was kept at room temperature for 4 hr. After the usual work-up the mixture was concentrated to 5 ml with the addition of triethylammonium bicarbonate buffer (1 *M*) and chromatographed on a Sephadex G-50 column as described under the preparation of the nonanucleotide. The elution pattern obtained is shown in Figure 8. Peak B contained pT. Peak A (80 ml, 139 OD_{302} units, 359 OD_{270} units, 89%) had a constant $\epsilon_{270}/\epsilon_{302}$ of 2.55, except at both the tail ends, and contained mainly (80%) the undecanucleotide d-Tr-T(pTpTpC^{An}pT)$_2$pTpT. The product, which had a constant $\epsilon_{270}/\epsilon_{302}$ of 2.55, was used for the next condensation step without further purification.

The Dodecanucleotide d-Tr-T(pTpTpCpT)$_2$pTpTpC. An anhydrous pyridine solution (1 ml) of the triethylammonium salts of d-Tr-T(pTpTpC^{An}pT)$_2$pTpT (40 OD_{302} units, from the center of peak A in Figure 8) and d-pC^{An}-OAc (0.05 mmole) and triisopropylbenzenesulfonyl chloride (0.037 g, 0.11 mmole) was kept at room temperature for 4 hr. Water (1 ml) was added under cooling and then excess (0.5 ml) triethylamine and the mixture was kept at room temperature for 16 hr. It was then treated with excess concentrated ammonia for 4 days at room temperature. The solution was evaporated to a gum with the addition of pyridine. Pyridine was finally removed by evaporation with 0.1 *M* triethylammonium bicarbonate solution (2 ml). Finally the product was taken up in 0.1 *M* triethylammonium bicarbonate (5 ml) and chromatographed on a Sephadex G-50 column as described under the preparation of the nonanucleotide. The elution pattern obtained is shown in Figure 9. Peak B contained d-pC and peak C contained anisic acid. Peak A (81 OD_{260}) contained mainly (90%) the required dodecanucleotide d-Tr-T(pTpTpCpT)$_2$pTpTpC, which was further purified by paper chromatography; yield 75%.

d-CE-pA^{Bz}pA^{Bz}pA^{Bz}. An anhydrous pyridine solution (5 ml) of pyridinium d-pA^{Bz}pA^{Bz}pA^{Bz} (4600 OD_{280} units, 0.085 mmole), hydracrylonitrile (2 ml), and DCC (1 g) was kept at room temperature for 16 hr in the presence of pyridinium Dowex 50 ion-exchange resin (1 g). Water (5 ml) was added and the mixture was filtered through glass wool to remove the resin and dicyclohexylurea. Excess DCC was extracted with ether and the reaction mixture was allowed to stand at room temperature for 16 hr. It was again extracted with ether (two 50-ml portions) as the aqueous layer was concentrated with the addition of pyridine. The resultant

Table VII. Spleen Phosphodiesterase Degradation of Deoxyribopolynucleotides Containing Repeating Tetranucleotide Sequences

Compd	OD_{260} units degraded	Nucleotides:nucleoside	Molar ratio of products — Found	Molar ratio of products — Theor
d-TpTpTpCpTpTpTpC	9.0	Tp:d-Cp:d-C	5.8:1:1	6:1:1
d-ApApApGpApApApG	7.5	d-Ap:d-Gp:d-G	6.1:1:0.9	6:1:1
d-ApTpCpGpApTpCpG	8.5	d-Ap:Tp:d-Cp:d-Gp:d-G	2.1:2.05:2:0.9:1	2:2:2:1:1

mmole) and mesitylenesulfonyl chloride (0.33 g, 1.5 mmoles) was kept at room temperature for 5 hr. After the usual work-up the mixture was chromatographed at room temperature on a DEAE-cellulose (acetate) column (75 × 2 cm). The condition of chromatography and the elution pattern obtained are shown in Figure 4. Peak C (640 ml) had a constant $\epsilon_{270}/\epsilon_{302}$ of 3 and contained pure d-Tr-TpTpTpC^{An}pTpTpT (628 OD_{302} units, 70%).

The Octanucleotide d-Tr-TpTpTpC^{An}pTpTpTpC^{An}. An anhydrous pyridine solution (2 ml) of the triethylammonium salts of d-Tr-TpTpTpC^{An}pTpTpT (550_{302} OD units, 0.0245 mmole) and d-pC^{An}-OAc (1 mmole) and mesitylenesulfonyl chloride (0.383 g, 1.7 mmoles) was kept at room temperature for 2 hr. After the usual work-up the mixture was chromatographed at room temperature on a DEAE-cellulose (acetate) column (75 × 2 cm). The conditions of chromatography and the elution pattern obtained are shown in Figure 5. Peak C had a constant $\epsilon_{270}/\epsilon_{302}$ of 1.9 and contained pure d-Tr-TpTpTpC^{An}pTpTpTpC^{An} (810 OD_{302} units, 73.6%).

The Nonanucleotide d-Tr-TpTpTpC^{An}pTpTpTpC^{An}pT. An anhydrous pyridine solution (1 ml) of the triethylammonium salts of d-Tr-TpTpTpC^{An}pTpTpTpC^{An} (255 OD_{302} units, 0.0057 mmole) and pT-OAc (0.171 mmole) and triisopropylbenzenesulfonyl chloride (0.104 g, 0.34 mmole) was kept at room temperature for 4 hr. After the usual work-up the mixture was concentrated to 5 ml with the addition of triethylammonium bicarbonate (1 *M*) buffer and chromatographed at room temperature on Sephadex G-50 (coarse)

anhydrous pyridine solution (10 ml) was dropped into anhydrous ether (500 ml). The precipitate was collected by centrifugation and washed with ether. The product showed mainly one spot on chromatography in solvent C: yield, 4250 OD_{280} units (92%).

The Tetranucleotide d-pA^{Bz}pA^{Bz}pA^{Bz}G^{Ac}. An anhydrous pyridine solution (4 ml) of the triethylammonium salts of d-CE-pA^{Bz}pA^{Bz}pA^{Bz} (3000 OD_{280} units, 0.055 mmole) and d-pG^{Ac}-OAc (0.5 mmole) and triisopropylbenzenesulfonyl chloride (0.3 g, 1 mmole) was kept at room temperature for 2 hr. Water (6 ml) was added under cooling and the solution was treated with sodium hydroxide (2 *N*, 10 ml) for 20 min at 0°. The alkali was neutralized with Dowex 50 pyridinium resin, and the resin was removed by filtration (pyridine–water washing). The solution was chromatographed on a DEAE-cellulose (carbonate) column (83 × 2 cm) at 5° using a gradient of triethylammonium bicarbonate containing 25% alcohol for elution. The conditions of chromatography and the elution pattern obtained are shown in Figure 10. Peak D contained d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac} (2495 OD_{280} units, $\epsilon_{280}/\epsilon_{260}$ = 1.27, 69%).

d-CE-pA^{Bz}pTpC^{An}. An anhydrous pyridine solution (5 ml) of pyridinium d-pA^{Bz}pTpC^{An} (9000 OD_{280} units, 0.25 mmole), hydracrylonitrile (5 ml, 75 mmoles), and DCC (1 g) was shaken at room temperature for 3 days. Water (20 ml) was added and the mixture was extracted with ether (three 50-ml portions). After 1 day at room temperature the aqueous pyridine solution was adjusted to pH 8.5 with 2 *N* ammonium hydroxide and the solution was allowed to stand at room temperature for 18 hr. It was passed through a column of pyridinium Dowex 50 ion-exchange resin. The resulting solution was evaporated to a gum with the addition of pyridine. The anhydrous residue was dissolved in dry pyridine (10 ml) and added dropwise to dry ether (200 ml) with stirring. The white precipitate was collected after centrifugation and again washed with ether several times. The yield was quantitative and the product was homogeneous as shown by paper chromatography in solvent B and by electrophoresis.

d-pA^{Bz}pTpC^{An}pG^{Ac}. To an anhydrous pyridine solution (5 ml) of the tri-*n*-hexylammonium salts of d-CE-pA^{Bz}pTpC^{An} (4500 OD_{280} units, 0.13 mmole) and d-pG^{Ac}-OAc (1 mmole) was added mesitylenesulfonyl chloride (0.4 g) and the reaction mixture was kept at room temperature for 4 hr. Water (2 ml) was added under cooling and the resulting solution was treated with sodium hydroxide (2 *N*, 10 ml) for 20 min at 0°. The alkali was neutralized with pyridinium Dowex 50 ion-exchange resin. The resin was removed by filtration and washed with aqueous pyridine. The reaction mixture was chromatographed on a DEAE-cellulose (bicarbonate) column at 4° using triethylammonium bicarbonate for elution. The conditions of chromatography and the elution pattern are shown in Figure 11. Fractions 720–765 contained the pure tetranucleotide d-pA^{Bz}-pTpC^{An}pG^{Ac} (3000 OD_{280} units, $\epsilon_{250}/\epsilon_{302}$ = 1.9, 54%).

d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac}-OAc. An anhydrous pyridine solution (2 ml) of d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac} (440 OD_{280} units) was kept with acetic anhydride (0.5 ml) at room temperature for 4 hr. Ethanol (0.5 ml) was added with cooling. After 0.5 hr water (1 ml) was added and the solution was kept for 7 hr. The mixture was evaporated to a gum with the addition of pyridine. Finally it was taken up in dry pyridine (2 ml) and dropped into ether (100 ml). The precipitate was collected after centrifugation and several washings with ether. The yield of d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac}-OAc was 394 OD_{280} units (89.5%).

d-pA^{Bz}pTpC^{An}pG^{Ac}-OAc. The tetranucleotide d-pA^{Bz}pTpC^{An}pG^{Ac} (500 OD_{280} units, 0.01 mmole) was acetylated in anhydrous pyridine (1 ml) with acetic anhydride (0.5 ml) for 4 hr and worked up as described above. The yield of d-pA^{Bz}pTpC^{An}pG^{Ac}-OAc was quantitative as determined spectrophotometrically.

Polymerization of a Mixture of d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac} and d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac}-OAc. An anhydrous pyridine solution (2.5 ml) of the tri-*n*-hexylammonium salts of d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac} (400 OD_{280} units) and d-pA^{Bz}pA^{Bz}pA^{Bz}pG^{Ac}-OAc (100 OD_{280} units) was treated with triisopropylbenzenesulfonyl chloride (0.16 g) for 2 hr. Water (2 ml) was added with cooling, followed by triethylamine (1 ml). The mixture was kept at room temperature for 1 day and then treated with concentrated ammonia (10 ml) for 3 days at room temperature. The mixture was evaporated to dryness with the addition of pyridine. Pyridine was finally removed by evaporation with 0.1 *M* triethylammonium bicarbonate solution (2 ml). The product was taken up in triethylammonium bicarbonate (0.1 *M*, 5 ml) and the solution was chromatographed on a Sephadex G-50 (coarse) column (2.5 × 69 cm) using 0.1 *M* triethylammonium bicarbonate for elution. The elution pattern obtained is given in Figure 12. The distribution of the polymers in the elution pattern is given in Table II. The yields and paper chromatographic mobilities of the polymers are given in Table I.

Polymerization of a Mixture of d-pA^{Bz}pTpC^{An}pG^{Ac} and d-pA^{Bz}pTpC^{An}pG^{Ac}-OAc. To an anhydrous pyridine solution (1 ml) of a mixture of the tri-*n*-hexylammonium salts of d-pA^{Bz}pTpC^{An}pG^{Ac} (2000 OD_{280} units, 0.04 mmole) and d-pA^{Bz}pTpC^{An}pG^{Ac}-OAc (500 OD_{280} units, 0.01 mmole) was added mesitylenesulfonyl chloride (0.11 g) under exclusion of moisture. The reaction mixture was evaporated to about 0.3 ml *in vacuo* and kept at room temperature for 1.5 hr. Aqueous pyridine (1.5 ml) was added with cooling, followed by triethylamine (0.2 ml), and the resulting solution was left overnight. It was next treated with concentrated ammonia (5 ml) for 2 days at room temperature for the removal of the protecting groups. A portion (150 OD_{260} units) of the polymeric mixture was chromatographed in solvent D for 5 days. Clearly separated bands corresponding to the cyclotetranucleotide, tetranucleotide, and octanucleotide were observed. The rest of the reaction mixture was concentrated and chromatographed on a Sephadex G-50 column (93 × 2 cm) using triethylammonium bicarbonate for elution. The elution pattern obtained is given in Figure 13. The distribution of the nucleotidic material is given in Table III. The yields and the R_f values of the products are given in Table IV.

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