peared was removed by centrifugation and the supernatant was applied at 2° on top of a column (3.5 × 45 cm.) of DEAE-cellulose (carbonate). Elution was carried out in the cold by a linear gradient of ammonium bicarbonate (pH 8.5) solution (3 l. of water in the mixing vessel and an equal volume of 0.3 *M* salt in the reservoir); 20-ml. fractions were collected at a flow rate of 2.6 ml./min. After a pyridine peak (20–30 fractions), mononucleotides appeared in fractions 45–145. The desired dinucleotide was present in fractions 172–196. Fractions 177–194 were pooled and evaporated in the standard way to give 6500 O.D. units (302 mμ, 0.29 mmole, 48%) of pure dinucleotide. The product was pure by paper electrophoresis and in solvent C and identical with the sample prepared by method A.

N-Acetyldeoxyadenosine-5′ Phosphate.—N,O$^{3'}$-Diacetyldeoxyadenosine-5′ phosphate was prepared as described previously.[37] The rate of removal of the N-acetyl group was followed by measuring the decrease in ultraviolet absorption at 290 mμ in 1 *N* sodium hydroxide (adenosine has virtually no absorption at 290 mμ, whereas the N-acetyl derivative has λ_{max} 286 mμ in alkali). The half-life of the N-acetyl group was thus found to be 160 min. at room temperature. For preparation of N-acetyldeoxyadenosine-5′ phosphate, N,O$^{3'}$-diacetyldeoxyadenosine-5′ phosphate was kept in 1 *N* sodium hydroxide at 0° for 3 min. N-Acetyldeoxyadenosine-5′ phosphate, the sole product, was isolated by the procedure described for the preparation of N-acetyldeoxyguanosine-5′ phosphate[8]; R_f's in solvent C: N-acetyldeoxyadenosine-5′ phosphate, 0.51; 3′-O-acetyldeoxyadenosine-5′ phosphate,[37] 0.46; N,O^{3}-diacetyldeoxyadenosine-5′ phosphate, 0.62; deoxyadenosine-5′ phosphate, 0.35.

Cleavage of P^1,P^2-3′-O-Acetylthymidine-5′ Pyrophosphate by Reaction With Hydroacrylonitrile and DCC.—P^1,P^2-3′-O-Acetylthymidine-5′ pyrophosphate (0.2 mmole) was treated in dry pyridine (2 ml.) with hydroacrylonitrile (0.29 ml., 2 mmoles) and DCC (2 mmoles) for 3 days at room temperature. Water (1 ml.) was then added and the mixture extracted twice with 10-ml. portions of ether. The aqueous layer was made up to 10 ml. with pyridine and the solution kept at room temperature. Chromatography of the solution in solvent C at the start showed the major product to be 3′-O-acetylthymidine-5′ β-cyanoethyl phosphate (R_f 0.63), but in addition a minor faster traveling product (R_f 0.86) was also present. The latter product lacked any phosphoryl dissociation as indicated by its zero mobility on paper electrophoresis (pH 7.1). Chromatography of the aqueous pyridine solution after 1 week showed the disappearance of the neutral side product, the yield of the desired β-cyanoethyl thymidine-5′ phosphate being quantitative.

In another experiment, the neutral product was present initially in the amount of 18%. On maintaining the total reaction mixture in dilute aqueous ethanolic ammonia solution at around pH 8.5 at room temperature, the neutral product could be hydrolyzed to give the cyanoethyl thymidine-5′ phosphate as the sole product.

Alkaline Hydrolysis of 3′-O-Acetylthymidine-5′ β-Cyanoethyl Phosphate.—A solution of this ester (0.2 mmole; see foregoing preparation) in 5 ml. of pyridine was treated at 0° with an equal volume of 1 *M* sodium hydroxide. Aliquots were removed at different intervals and treated with an excess of pyridinium Dowex-50 ion exchange resin in the cold, the neutralization being complete within 0.5 min. The products were analyzed by paper chromatography in solvent C using Whatman 40 paper. The half-life of the acetyl group was too short to be measured (under 1 min.), while the half-life of the cyanoethyl group was about 5 min.

In another experiment, the same reactions were studied in 1 *M* sodium hydroxide at 0°. The half-life of the cyanoethyl group was about 2.5 min.

(37) Prepared by acetylation of deoxyadenosine-5′ phosphate with acetic anhydride–pyridine for a period of about 1 hr.

[CONTRIBUTION FROM THE INSTITUTE FOR ENZYME RESEARCH, THE UNIVERSITY OF WISCONSIN, MADISON 6, WIS.]

Studies on Polynucleotides. XXVI.[1] The Stepwise Synthesis of Specific Deoxyribopolynucleotides (6).[2] The Synthesis of Thymidylyl-(3′ → 5′)-deoxyadenylyl-(3′ → 5′)-thymidylyl-(3′ → 5′)-thymidylyl-(3′ → 5′)-thymidine and of Polynucleotides Containing Thymidine and Deoxyadenosine in Alternating Sequence[3]

BY G. WEIMANN, H. SCHALLER, AND H. G. KHORANA

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5′-O-Dimethoxytritylthymidylyl-(3′ → 5′)-N-benzoyldeoxyadenosine was prepared by the condensation of N-benzoyl-3′-O-acetyldeoxyadenosine-5′ phosphate with 5′-O-dimethoxytritylthymidine in the presence of dicyclohexylcarbodiimide (DCC), followed by alkaline treatment. Condensation of this protected dinucleoside phosphate with 5′-O-phosphorylthymidylyl-(3′ → 5′)-thymidylyl-(3′ → 5′)-3′-O-acetylthymidine followed by a work-up inclusive of chromatography gave a 12% isolated yield of the pentanucleotide thymidylyl-(3′ → 5′)-deoxyadenylyl-(3′ → 5′)-thymidylyl-(3′ → 5′)-thymidylyl-(3′ → 5′)-thymidine. Condensation of thymidine-5′ β-cyanoethyl phosphate with N-benzoyl-3′-O-acetyldeoxyadenosine-5′ phosphate followed by an alkaline treatment gave the protected dinucleotide 5′-O-phosphorylthymidylyl-(3′ → 5′)-N-benzoyldeoxyadenosine in 30–40% yield. The treatment of the latter in dry pyridine with DCC for 6 days followed by a work-up inclusive of ammoniacal treatment gave a polymeric mixture containing polynucleotides with alternating thymidine and deoxyadenosine residues. Products up to the dodecanucleotide d-pT-(pApT)$_5$-pA were characterized, higher polynucleotides being present in detectable amounts. The synthesis of 5′-O-dimethoxytritylthymidylyl-(3′ → 5′)-thymidine-3′ phosphate and the corresponding unprotected dinucleotide is recorded. 5′-O-Trimethylacetylthymidylyl-(3′ → 5′)-3′-O-acetyl-N-benzoyldeoxyadenosine was prepared.

In continuation of the work reported in the two accompanying papers,[1,2d] the synthesis of larger oligonucleotide chains by condensation of preformed "blocks" of di- and trinucleotides[4,5] was undertaken and the results are presented in this and the succeeding paper.[6] The synthesis of the pentanucleotide thymidylyl-(3′ → 5′)-deoxyadenylyl-(3′ → 5′)-thymidylyl-(3′ → 5′)-thymidylyl-(3′ → 5′)-thymidine and of polynucleotides containing thymidine and deoxyadenosine residues in alternating sequence, which were prepared by polymerization of the suitably protected thymidine–deoxyadenosine dinucleotide, are reported in this paper. The following paper deals mainly with parallel investigations of polynucleotides containing deoxycytidine and deoxyguanosine.[6] A preliminary report of some of this work has been published.[7]

Thymidylyl-(3′ → 5′)-deoxyadenylyl-(3′ → 5′)-thymidylyl-(3′ → 5′)-thymidylyl-(3′ → 5′)-thymidine (VI).—

(1) Paper XXV: H. Schaller and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 3828 (1963).

(2) Previous papers which deal directly with this topic: (a) P. T. Gilham and H. G. Khorana, *ibid.*, **80**, 6212 (1958); (b) *ibid.*, **81**, 4647 (1959); (c) G. Weimann and H. G. Khorana, *ibid.*, **84**, 419 (1962); (d) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *ibid.*, **85**, 3821 (1963); (e) H. Schaller and H. G. Khorana, ref. 1.

(3) This work has been supported by grants from the Life Insurance Medical Research Fund, New York, N. Y., the National Science Foundation, Washington, D. C., and the National Cancer Institute of the National Institutes of Health, Bethesda, Md.

(4) H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," John Wiley and Sons, Inc., New York, N. Y., 1961, Chapter 5.

(5) The only previous study along these lines involved thymidine oligonucleotides. Notably, the synthesis of a tetranucleotide by condensation of 5′-O-tritylthymidylyl-(3′ → 5′)-thymidine-3′ phosphate with thymidylyl-(3′ → 5′)-3′-O-acetylthymidine was recorded (G. Weimann and H. G. Khorana, ref. 2c).

(6) H. Schaller and H. G. Khorana, *ibid.*, **85**, 3841 (1963).

(7) H. Schaller, G. Weimann, and H. G. Khorana, *ibid.*, **85**, 355 (1963).

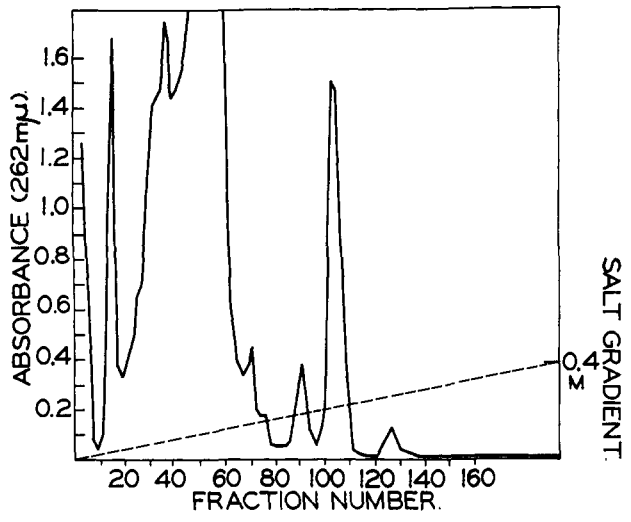
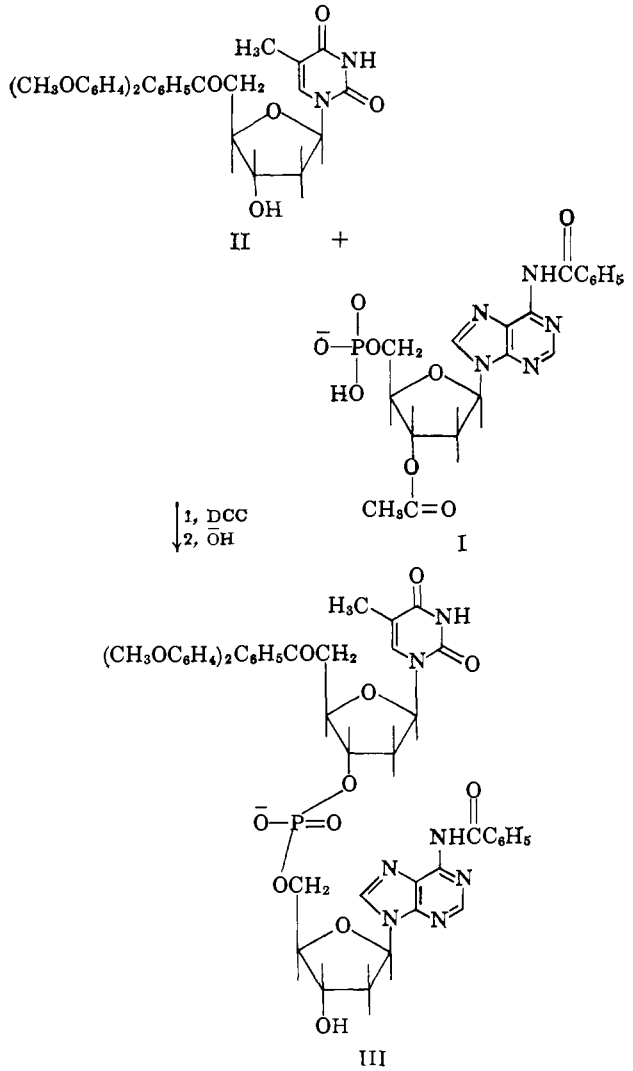


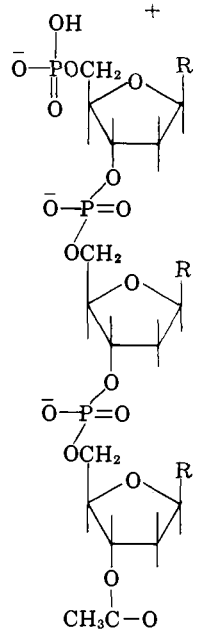
Fig. 1.—Chromatography of reaction products from condensation of the trinucleotide 3′-O-acetyl- (pTpTpT) and 5′-O-dimethoxytritylthymidylyl-(3′ → 5′)-N-benzoyladenosine; column size (50 × 1 cm.), DEAE-cellulose (carbonate); an elution with a linear gradient using 2 l. of water in the mixing vessel and 2 l. of 0.4 *M* triethylammonium bicarbonate in the reservoir; 20-ml. fractions were collected at 15-min. intervals. For contents of different peaks see text.

N-Benzoyl-3′-O-acetyldeoxyadenosine-5′ phosphate (I) was prepared by acetylation of the previously described N-benzoyldeoxyadenosine-5′ phosphate.[8] Condensation of this nucleotide with 5′-O-di-*p*-methoxytritylthymidine (II) in the presence of dicyclohexylcarbodiimide (DCC) was performed by the standard method.[2] Brief alkaline treatment caused the selective removal of the acetyl group and the product, 5′-O-dimethoxytritylthymidylyl-(3′ → 5′)-N-benzoyldeoxyadenosine (III), was purified by chromatography, the isolated yield (70%) being satisfactory.

For a number of reasons, it was decided to attempt the condensation of this protected derivative (III) with a suitably protected trinucleotide bearing a 5′-phosphomonoester group. The trinucleotide chosen was IV and it was prepared by acetylation of the 3′-hydroxyl group in the thymidine trinucleotide (pTpTpT).[9,10] The synthesis of the pentanucleotide VI which would thus result was expected to be instructive for further work and, moreover, the product containing a single purine unit in a specified place in a pyrimidine block was desired as a particularly suitable substrate in certain enzymic studies.[11]

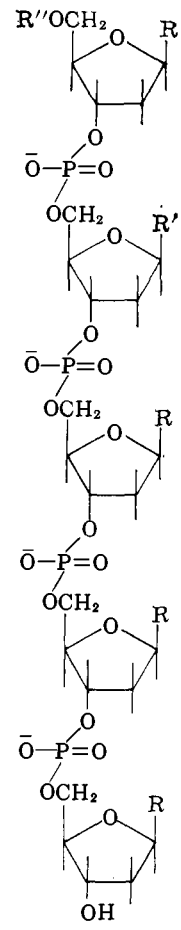


III
+



IV, R = thymine

1), DCC
2), NH_4OH
3), Chromatography



V, VI; R = thymine.
R = adenine.
V; R = dimethoxytrityl.
VI; R = H.

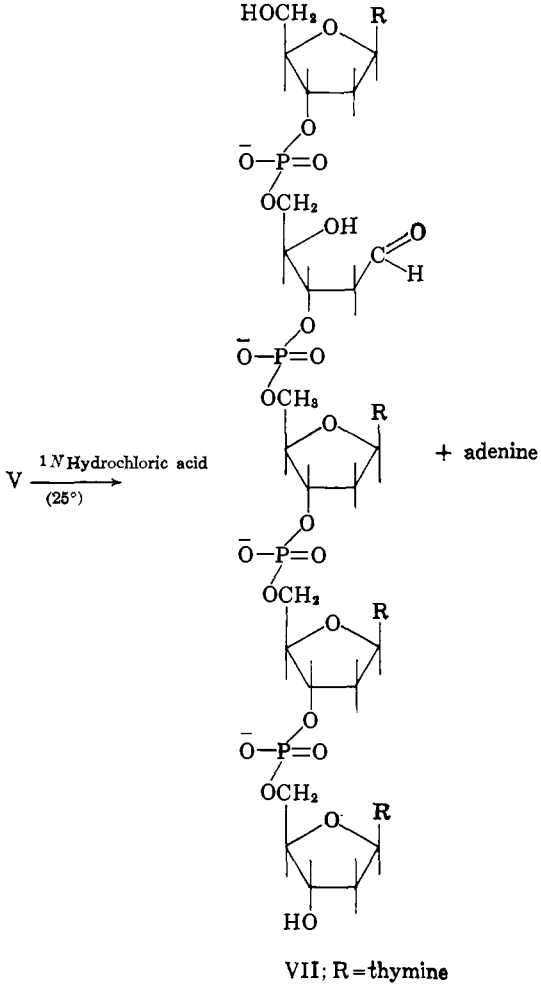
(8) R. K. Ralph and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 2926 (1961).

(9) Abbreviations used for polynucleotides are as described in ref. 4 and as adopted by *J. Biol. Chem.* (see Instructions for Authors in current issues of this journal).

(10) (a) G. M. Tener, H. G. Khorana, R. Markham, and E. H. Pol, *J. Am. Chem. Soc.*, **80**, 6224 (1958); (b) H. G. Khorana and J. P. Vizsolyi, *ibid.*, **83**, 675 (1961).

(11) For example, in the study of the preferential base specificity of the pancreatic deoxyribonuclease; *cf.* R. K. Ralph, R. A. Smith, and H. G. Khorana, *Biochem.*, **1**, 131 (1962).

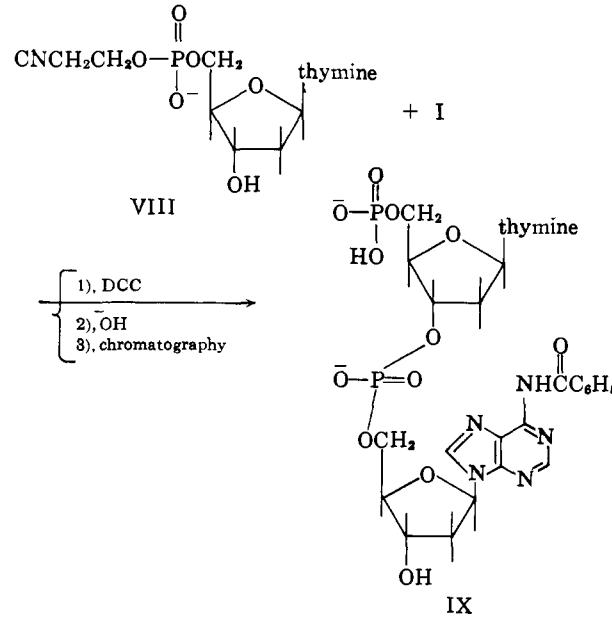
Because of the insolubility of the protected trinucleotide IV in dry pyridine, the reaction was carried out in a mixture of pyridine and dimethylformamide,[1,12] DCC being the condensing agent. After an ammoniacal treatment, the products were separated on a DEAE-cellulose column. The elution pattern is shown in Fig. 1. The detailed identification of the different peaks is given in the Experimental section. The dimethoxytritylpentanucleotide (V) and a portion of the same product having lost the dimethoxytrityl group (VI) were isolated, respectively, from the pooled fractions 85–90 and 67–80. The total yield of the isolated product, ignoring hypochromicity, was 12%. Both the protected pentanucleotide V and the product VI after selective removal of the dimethoxytrityl group were characterized by paper chromatography and paper electrophoresis and by the further selective degradation to adenine and, presumably, VII. The ratio of thymidine in VII to the adenine released (4.3:1) was close to theoretical. Enzymic experiments with this product will be published separately.



VII; R = thymine

Polynucleotides Containing Thymidine and Deoxyadenosine in Alternating Sequences.—The polymerization studies have so far been carried out only with the mononucleotides.[4,8,10,12,13] An attractive direction for further extension of these studies was the polymerization of preformed suitably protected di- and trinucleotides. The polymers thus obtained would clearly be important and of practical use in enzymic and physicochemical studies. The polymers chosen for the first study by chemical synthesis were those containing deoxyadenosine and thymidine in alternating sequence. Interest in studying polymers of this sequence but of relatively short size has been stimulated by the finding in certain species of crabs[14] of deoxyribonucleic acids containing predominantly the two above-mentioned nucleosides, thymidine and deoxyadenosine, in alternating sequence. Furthermore, *de novo* synthesis of deoxyadenylate–thymidylate copolymers with perfectly alternating sequence of the two nucleosides has been documented by Kornberg and co-workers in their studies with deoxyribonucleic acid polymerase.[15]

As a suitable starting material for polymerization the synthesis of the protected dinucleotide IX was undertaken. Thymidine-5′ β-cyanoethyl phosphate (VIII) was prepared in good yield from thymidine-5′ phosphate by reaction with DCC in the presence of an



excess of hydroacrylonitrile and pyridine; VIII is an example of a nucleotide bearing a free hydroxyl group but having the phosphomonoester group protected in the form of a diester by the alkali-labile cyanoethyl group.[16] The condensation of VIII with N-benzoyl-3′-O-acetyldeoxyadenosine-5′ phosphate (II) followed by a careful alkaline treatment to remove the 3′-O-acetyl and the cyanoethyl group[17] gave IX as the major new product. It was purified by column chromatography in the cold, there being detected no loss of the N-benzoyl group in the adenine moiety during any of the operations. The isolated yield of IX varied between 30 and 40% depending partly upon the efficiency of separation on the DEAE-cellulose column. The final product was homogeneous as determined by paper chromatography and by paper electrophoresis.

Described in the Experimental section is also the synthesis and characterization of the isomeric dinucleotide deoxyadenylyl-(3′ → 5′)-thymidine-3′ phosphate by condensation of 5′-O-dimethoxytrityldeoxyadenosine-3′ phosphate[2d] with thymidine-3′ β-cyanoethyl phos-

(12) R. K. Ralph, W. J. Connors, H. Schaller, and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 1983 (1963).

(13) H. G. Khorana, A. F. Turner, and J. P. Vizsolyi, *ibid.*, **83**, 686 (1961).

(14) N. Sueoka and T. Y. Cheng, *J. Mol. Biol.*, **4**, 161 (1962); *Proc. Natl. Acad. Sci. U. S.*, **48**, 1851 (1962); M. Smith, *Biochem. Biophys. Res. Commun.*, **10**, 67 (1963).

(15) H. K. Schachman, J. Adler, C. M. Radding, I. R. Lehman, and A. Kornberg, *J. Biol. Chem.*, **235**, 3242 (1960).

(16) *Cf.* the earlier work on the synthesis of 5′-O-tritylthymidylyl-(3′ → 5′)-thymidine-3′ phosphate by the condensation of 5′-O-tritylthymidine-3′ phosphate with thymidine-3′ β-cyanoethyl phosphate (G. Weimann and H. G. Khorana, ref. 2c).

(17) There is no danger of the N-benzoyl group coming off during this treatment (20 min. at 0° in 1 *N* sodium hydroxide), which removes the acetyl and the cyanoethyl groups.

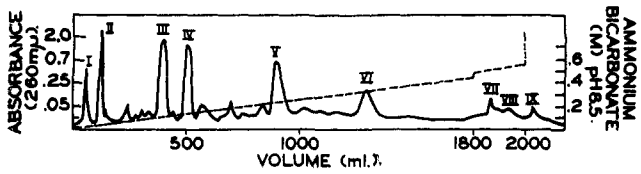
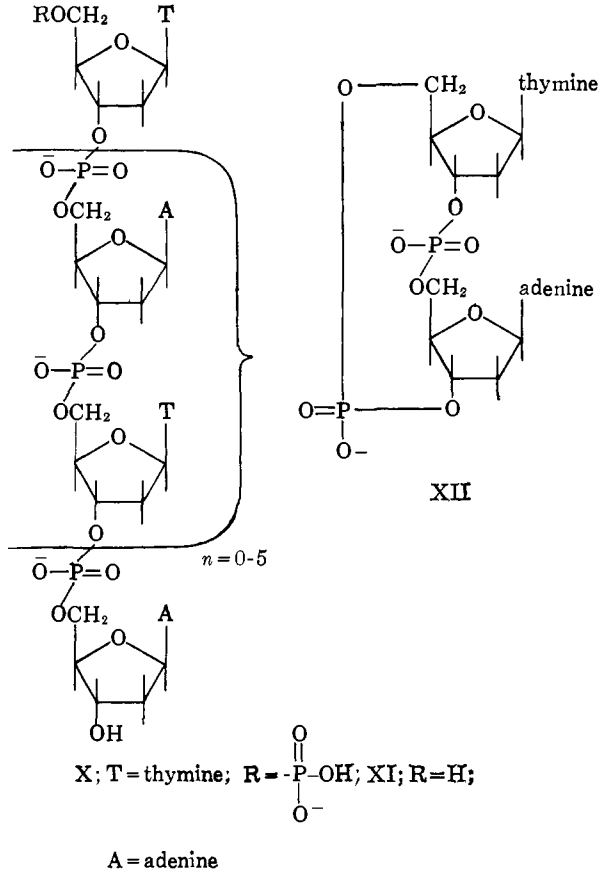


Fig. 2.—Chromatography of 1% of the polymeric mixture obtained by polymerization of the protected dinucleotide 5′-O-phosphorylthymidylyl-(3′ → 5′)-N-benzoyldeoxyadenosine followed by removal of the benzoyl group. A portion (about 96 optical density units at 260 mμ) was applied on top of a DEAE-cellulose (carbonate) column (25-ml. buret). A gradient of ammonium bicarbonate (1 l. of water in mixing vessel, 1 l. of 0.5 *M* salt in the reservoir) was applied; 20-ml. fractions were collected. Elution of ultraviolet-absorbing material was recorded by an automatic recorder (log scale). Peak I, pyridine; peak II, benzoic acid; peak III, cyclo-d-pTpA; peak IV, d-pTpA; peak V, d-pTpApTpA; peak VI, D-pTpApTpApTpA; peak VII, d-pTpApTpApTpApTpA; peak VIII, d-pTpApTpApTpApTpA-pTpA; peak IX, higher polynucleotides.

phate.[2c] The synthesis of suitably protected derivatives of this dinucleotide was undertaken at the start of the present work for their possible use in polymerization and stepwise synthesis. Further work with this and related compounds bearing the -3′ phosphate end groups was, however, not continued.

The polymerization of 5′-O-phosphorylthymidylyl-(3′ → 5′)-N-benzoyldeoxyadenosine (IX) was carried out using DCC by the standard method.[10b] In the work-up the treatment with acetic anhydride–pyridine was included so as to cleave the surviving pyrophosphate linkages in the products.[4,18] After an ammoniacal treatment to remove the N-benzoyl group, the products were separated on a DEAE-cellulose column. The elution pattern obtained is shown in Fig. 2 while the distribution of the nucleotidic material in different peaks as well as the identification of the products is shown in Table I. Characterization of the linear polynucleotides of the general structure X was accomplished by the general methods developed previously.[10,13] Thus their position of elution was the first indication of the chain length, the size identification being simplified by the fact that only the di-, tetra-, hexa-, and higher even-numbered polynucleotides were expected. The material in peak 3 (Fig. 2) was identified as d-pTpA and its position served as a reference standard for the subsequent peaks. The tetra- and hexanucleotides d-pTpApTpA and d-pTpApTpApTpA, respectively, were degraded by the bacterial alkaline phosphomonoesterase[10,13] to corresponding oligonucleotides lacking the terminal phosphomonoester group (XI). The latter were degraded by venom phosphodiesterase[10,19] and the molar proportions of the products confirmed the size of the original polynucleotides. Thus the dephosphorylated product d-TpApTpApTpA, from the hexanucleotide, when degraded by the venom phosphodiesterase gave thymidine, thymidine-5′ phosphate, and deoxyadenosine-5′ phosphate in the ratio 1:1.84:2.80, the theoretical ratio being 1:2:3.

The identification of the higher members, the octanucleotide and those purified[20] from the 2 *M* fraction (last line of Table I) followed accordingly from their



successively decreasing mobilities in solvent D. The R_f's of the purified polynucleotides bearing -5′ phosphate end groups and the corresponding series lacking the terminal phosphate group are listed in Table II.

The cyclic dinucleotide XII was present in peak 2 (Fig. 2). There was little difficulty in establishing its identity by the methods which are now standard. This intramolecular cyclization presents, as in the previous work, a seriously competing reaction. In fact, in one preliminary small scale experiment on the polymerization of IX, the cyclic dinucleotide XII accounted for more than 50% of the total nucleotidic material. It is hoped that, in the future, with further development of the polymerization techniques the cyclization reaction can be minimized.

While detailed physicochemical and enzymic studies with the above polynucleotides will be reported later, the following observations are worthy of mention here. A study of ultraviolet absorbtivity (260 mμ) as a function of temperature in the presence of 1 *M* sodium chloride (pH 7) showed that the above polynucleotides containing thymidine and deoxyadenosine possessed secondary structure. The temperature at which the increase in absorbtivity reached a plateau increased, as expected, with an increase in size. Thus, in the case of the octanucleotide d-TpApTpApTpApTpA, the increase in absorbtivity had leveled off at about 30°. The results, in fact, provided further confirmation of the size of the synthetic polynucleotides established above. Furthermore, in an experiment carried out in the laboratory of Dr. A. Kornberg, the dodecanucleotide d-T-(pApT)$_5$-pA and the fraction containing higher polynucleotides were shown to serve as templates for the DNA-polymerase-catalyzed synthesis of the larger thymidylic–deoxyadenylic copolymer.

General observations on the work reported in this paper and on the total approaches now available for

(18) H. G. Khorana, J. P. Vizsolyi, and R. K. Ralph, *J. Am. Chem. Soc.*, **84**, 414 (1962); H. G. Khorana and J. P. Vizsolyi, *ibid.*, **81**, 4660 (1959).

(19) W. E. Razzell and H. G. Khorana, *J. Biol. Chem.*, **234**, 2105 (1959).

(20) The total 2 *M* fraction (higher than the octanucleotide) was treated with alkaline phosphomonoesterase and the products separated by prolonged chromatography on a sheet of Whatman No. 40 paper in solvent D. After elution of the bands, their R_f's were compared side by side with the lower well characterized members of the series.

deoxyribopolynucleotide synthesis are presented in the following paper.

Experimental

General Methods.—The solvent systems for paper chromatography and other general methods were as described in the accompanying paper of Schaller, *et al.*[2d]

5'-O-Dimethoxytritylthymidylyl-(3' → 5')-N-benzoyldeoxyadenosine.—3'-O-Acetyl-N-benzoyldeoxyadenosine-5' phosphate was prepared by acetylation of N-benzoyldeoxyadenosine-5' phosphate. The N-benzoyl nucleotide (1 mmole) was treated with acetic anhydride (2 ml.) in 5 ml. of pyridine at room temperature in the dark for 6 hr. An excess of water was then added and the mixture evaporated at low temperature *in vacuo*. The addition of water and evaporation of the solution was repeated several times and finally an aqueous solution of the product was lyophilized. Paper chromatography in solvent C gave only a single spot with R_f 0.64 corresponding to 3'-O-acetyl-N-benzoyldeoxyadenosine-5' phosphate (N-benzoyldeoxyadenosine-5' phosphate, R_f 0.55).

Pyridinium 3'-O-acetyl-N-benzoyldeoxyadenosine-5' phosphate (0.7 mmole), as prepared above, and 5'-O-di-*p*-methoxytritylthymidine (925 mg., *ca* 1.5 mmoles) were allowed to react in anhydrous pyridine (2 ml.) with 1.5 g. (about 7.5 mmoles) of DCC at room temperature. The insoluble gummy material which separated at the start on addition of DCC had dissolved within 24 hr. and the solution was kept for a further period of 3 days. Water (5 ml.) was then added and the mixture kept at room temperature for 5 hr. Addition of some pyridine was necessary at this stage to keep the solution homogeneous, and the mixture was extracted with petroleum ether three times to remove the excess of DCC. Pyridine (25 ml.) followed by 2 *N* sodium hydroxide (10 ml.) was then added and the mixture shaken at room temperature for 10 min. An excess of pyridinium Dowex-50 ion exchange resin was added to remove the sodium ions and the total resin and insoluble material were removed by filtration. Paper chromatography in solvent C showed essentially two spots containing the di-*p*-methoxytrityl group, one with R_f 0.92 corresponding to di-*p*-methoxytritylthymidine and the second with R_f 0.84 being N-benzoyldeoxyadenylyl-(5' → 3')-5'-O-di-*p*-methoxytritylthymidine. The total solution obtained above was concentrated carefully at very low temperature (0–5°) and four-fifths of the total concentrated solution was applied to the top of a DEAE–cellulose column (35 × 4 cm. diam.) in the carbonate form. The washing and elution was carried out in a cold room at about 4°. The excess of di-*p*-methoxytritylthymidine was washed off with 1.5 l. of aqueous ethyl alcohol (20% ethyl alcohol by volume). Elution was then begun, using a linear gradient, with 2 l. of 20% aqueous ethyl alcohol in the mixing vessel and an equal volume of 0.15 *M* ammonium bicarbonate and then continued with 2 l. of 0.15 *M* salt in the mixing vessel and 2 l. of 0.2 *M* salt in the reservoir. Subsequently the column was eluted with more 0.2 *M* salt (2 l.), then 0.25 *M* salt (2 l.), and finally with 0.5 *M* salt (0.5 l.). Fractions of 16–17-ml. volume were collected every 10 min. Fractions 30–80 contained a minor product with λ_{max} at 286 mμ, which was not further investigated. The desired product appeared as a broad peak in fractions 81–622. All these fractions contained the same product as determined spectrophotometrically ($\epsilon_{280\ m\mu}/\epsilon_{260\ m\mu}$ = 1.25, λ_{max} 276 mμ, λ_{min} 254 mμ with inflection at 231 mμ) and by paper chromatography in solvent C. The pooled fractions were concentrated under the vacuum of an oil pump with frequent additions of pyridine. Ammonium bicarbonate which partly crystallized was removed by filtration, the solid being washed with pyridine. The residual salt was decomposed by addition of an excess of pyridinium Dowex-50 ion exchange resin, the complete removal of ammonium ions being ensured by final passage of the total solution through a column of freshly prepared pyridinium resin. The yield of isolated product recovered as pyridine salt was about 75% as based on the deoxyadenosine nucleotide.

Acetylation of Thymidine Trinucleotide.—The trinucleotide 5'-O-phosphorylthymidylyl-(3' → 5')-thymidylyl-(3' → 5')-thymidine (30 mg.),[10b] as the free acid, was suspended in 1 ml. of dry pyridine and acetic anhydride (0.1 ml., 1 mmole) was added. To the mixture was next added 0.5 ml. of freshly distilled dimethylformamide and the resulting clear solution was left at room temperature for 5 hr. Water (5 ml.) was then added and, after keeping the mixture overnight, it was evaporated. The addition of water and evaporation was repeated several times and finally the residue was lyophilized three times from water. The fine lyophilized powder gave a single spot in solvent C with R_f (0.62) distinctly higher than that of the starting material (0.59).

In an alternative general procedure for acetylation of the end 3'-hydroxyl group of thymidine oligonucleotides bearing -5' phosphate end groups at the other terminus, triethylammonium salts of the oligonucleotides were used. All members of the series up to the dodecanucleotide gave homogeneous solutions in anhydrous pyridine,[21] addition of dimethylformamide being unnecessary. The procedure for acetylation with acetic anhydride and subsequent work-up otherwise was as described above.

Synthesis of 5'-O-Di-*p*-methoxytritylthymidylyl-(3' → 5')-deoxyadenylyl-(3' → 5')-thymidylyl-(3' → 5')-thymidylyl-(3' → 5')-thymidine (V).—To a mixture of 5'-O-di-*p*-methoxytritylthymidylyl-(3' → 5')-N-benzoyldeoxyadenosine (III) (80 μmoles), the trinucleotide 5'-O-phosphorylthymidylyl-(3' → 5')-thymidylyl-(3' → 5')-3'-O-acetylthymidine (19 μmoles, 570 optical density units at 267 mμ), 150 mg. of dry pyridinium Dowex-50 ion exchange resin, dry pyridine (0.5 ml.), and dimethylformamide (0.2 ml.) was added 150 mg. of DCC and the mixture shaken at room temperature for 2 days. At this time, more DCC (100 mg.) was added and after shaking for 2 more days, water (5 ml.) was added and the mixture kept at room temperature for 8 hr. The mixture was then extracted with petroleum ether and the aqueous solution was lyophilized. The resulting yellow powder was treated with 15 ml. of concentrated ammonia for 24 hr. Since the mixture then was still inhomogeneous, ethyl alcohol (10 ml.) was added and the resulting clear solution shaken further for 3 days to remove the N-benzoyl group. Paper chromatography in solvent A at this stage showed two products containing di-*p*-methoxytrityl group: one corresponded to 5'-O-di-*p*-methoxytritylthymidylyl-(3' → 5')-deoxyadenosine (R_f 0.60, λ_{max} 263 mμ, λ_{min} 247 mμ) and the second, much less strong, with R_f 0.07, corresponded to the desired product. The resin was removed and the total filtrate and washings (with aqueous pyridine) were evaporated to a gum which as a solution in aqueous pyridine was applied to the top of a DEAE–cellulose (bicarbonate form) column (50 × 1 cm.). After a water wash (200 ml.), elution was carried out with a linear salt gradient, the mixing vessel containing 2 l. of water and the reservoir an equal volume of 0.4 *M* triethylammonium bicarbonate (pH 7.5). Fractions of 20-ml. volume were collected at 15-min. intervals. The elution pattern is shown in Fig. 1. Fractions 39–66 contained 5'-O-di-*p*-methoxytritylthymidylyl-(3' → 5')-deoxyadenosine. The contents and analysis of the subsequent peaks were:

Fractions 67–80.—The total material corresponded to 60 optical density units (267 mμ). When chromatographed in solvent A the material stayed at the origin, indicating the presence of the trinucleotide (pTpTpT) and/or the desired product d-TpApTpTpT and other compounds with multiple charges (the presence of deoxyadenosine-containing material was indicated by the result of 1 *N* hydrochloric acid treatment, which released a considerable amount of adenine). Chromatography on DEAE–cellulose paper (elution with 0.5 *N* triethylammonium bicarbonate) showed the presence of two main ultraviolet-absorbing materials and another weak spot (the slowest traveling). Of the main spots, the fastest corresponded in R_f to the trinucleotide pTpTpT, and the slower corresponded to TpApTpTpT. Paper electrophoresis at pH 2.7 (0.1 *M* formic acid) confirmed the above results. From spectrophotometric estimation after elution of the spots, the amount of TpApTpTpT present in fractions 67–80 was estimated to be 20% of the total ultraviolet-absorbing material present in this peak.

Fractions 85–96.—The total ultraviolet-absorbing material corresponded to 46 optical density units (267 mμ), the triethylammonium bicarbonate concentration at peak elution being about 0.18 *M*. Paper chromatography in solvent A showed the presence of 5'-O-di-*p*-methoxytrityl-containing pentanucleotide (V) (54% of total in the peak) and material staying close to the origin on the chromatogram. Evidently partial detritylation occurred during the work-up involving removal of the triethylammonium bicarbonate since the material traveling close to the origin, although not pure, contained the detritylated pentanucleotide TpApTpTpT. After purification of the latter by prolonged chromatography, its amount in this fraction was 12 optical density units (267 mμ).

Fractions 97–113.—This was the major peak containing thymidine residues only. The material in this peak was concluded to be the pyrophosphate formed by joining up of the 5'-phosphomonoester groups of the trinucleotide (pTpTpT) starting material. Thus, after treatment with an excess of acetic anhydride in pyridine for 3 days it gave the trinucleotide pTpTpT, as the sole product. The total optical density (266 mμ) recovered in this peak was 187 units.

Fractions 120–130 contained a small peak of thymidine-containing material (17 optical density units) which has not been identified.

Characterization of the Pentanucleotide TpApTpTpT (VI) and its Di-*p*-methoxytrityl Derivative V.—The di-*p*-methoxytrityl derivative V isolated from fractions 85–96 by prolonged paper chromatography in solvent A had R_f in this solvent system of 6 cm. relative to 1 cm. for the trinucleotide pTpTpT or the pentanucleotide TpApTpTpT. The di-*p*-methoxytrityl derivative had the following ultraviolet absorption characteristics: λ_{max}

(21) A. Falaschi, J. Adler, and H. G. Khorana, *J. Biol. Chem.*, **238**, 3080 (1963).

264 mμ at pH 7 and pH 12; λ_{min} at pH 7, 240 mμ, and at pH 12, 248 mμ.

An aliquot of the di-*p*-methoxytrityl derivative was kept in 1 *N* hydrochloric acid at 25° for 30 min. and the products were then chromatographed in solvent A. Two ultraviolet-absorbing spots were detected, one close to the origin and containing only thymidine residues (λ_{max} 266 mμ), presumably the compound VII and the second spot corresponding to adenine. The molar ratios of the two products after elution from the paper chromatogram with 0.1 *N* hydrochloric acid were determined to be 4.3:1, assuming ϵ_{max} for adenine at 262 mμ of 13.1×10^3 and that for thymidine of 9700 at 266 mμ. The selective removal of the di-*p*-methoxytrityl group from V was accomplished by treatment with 80% acetic acid for 15 min. at room temperature. During this treatment there was no evidence of release of any adenine. The R_f in solvent A (chromatography for 40 hr.) relative to pTpTpT (the trinucleotide) was 1. The electrophoretic mobility of the pentanucleotide TpApTpTpT (VI) at pH 2.7 relative to that of pTpTpT was 0.83.

The identification of the detritylated pentanucleotide TpApTpTpT present as a component of the fractions 67–80 and also in the slow traveling material in fractions 87–96 was also accomplished by the chromatographic and electrophoretic methods described above. The combined yield of the pentanucleotide (from different pooled fractions of Fig. 1) as based on the thymidine trinucleotide was 12%.

β-Cyanoethyl Thymidine-5′ Phosphate.—Pyridinium thymidine-5′ phosphate (1 mmole) was dissolved in a mixture of pyridine (5 ml.) and hydroacrylonitrile (2 ml.). The solution was treated with 2.06 g. of DCC for 4 days at room temperature. Paper electrophoresis showed at this stage two ultraviolet-absorbing products, one with zero mobility and the second major one with mobility expected of β-cyanoethyl thymidine-5′ phosphate. Water (20 ml.) was then added and, after keeping the mixture for 12 hr. at room temperature, DCC was extracted with petroleum ether and the aqueous solution filtered from the insoluble material (dicyclohexylurea and the adduct of hydroacrylonitrile and DCC). The clear solution was chromatographed on DEAE–cellulose column in the bicarbonate form in the cold at 4°. The neutral ultraviolet-absorbing material, as yet unidentified, was removed by a water wash and elution was then carried out with 0.1 *M* ammonium bicarbonate. In addition to the desired β-cyanoethyl thymidine-5′ phosphate, which was the major product (70%), there were present other minor ultraviolet-absorbing products, thymidine-5′ phosphate and polymeric compounds. β-Cyanoethyl thymidine-5′ phosphate was isolated by addition of an excess of Dowex-50 (H^+) resin to obtain the free acid, the total solution being passed finally through a short column of the same resin. The total acidic effluent was treated with some pyridine and the solution concentrated at reduced temperature. The product was homogeneous by paper chromatography (solvent A, R_f 0.34, that of β-cyanoethyl thymidine-3′ phosphate, 0.38) as well as on paper electrophoresis, the mobility being just like that of the previously synthesized isomeric β-cyanoethyl thymidine-3′ phosphate. After hydrolysis in alkali, it gave a single product identical with thymidine-5′ phosphate.

Condensation of 3′-O-Acetyl-N-benzoyldeoxyadenosine-5′ Phosphate with β-Cyanoethyl Thymidine-5′ Phosphate: Isolation of 5′-O-Phosphorylthymidylyl-(3′ → 5′)-N-benzoyldeoxyadenosine.—A mixture of pyridinium 3′-O-acetyl-N-benzoyldeoxyadenosine-5′ phosphate (0.59 mmole), β-cyanoethyl thymidine-5′ phosphate (0.88 mmole), and DCC (1.1 g.) was kept in anhydrous pyridine (1 ml.) at room temperature for 3 days. Water (10 ml.) was then added and after keeping the mixture overnight at room temperature, dicyclohexylurea was removed by filtration and washed with aqueous pyridine. The total filtrate was evaporated *in vacuo* to a gum which was taken up in 20 ml. of 1 *N* sodium hydroxide. The solution was kept at room temperature for 10 min. and the alkali was then neutralized by the addition of an excess of pyridinium Dowex-50 ion exchange resin. The latter was then removed by filtration and washed with aqueous pyridine. The total filtrate was concentrated to a small volume and then applied to the top of a DEAE–cellulose (bicarbonate form) column (35 × 4 cm.). Elution was carried out in the cold at 4°, first with water (500 ml.) and then with a salt gradient, the mixing vessel containing 3 l. of water and the reservoir an equal volume of 0.3 *M* triethylammonium bicarbonate (pH 7.5.). Fractions of 22 ml. at 15-min. intervals were collected. The benzoylated dinucleotide IX was eluted in fractions 200–230 at 0.18 *M* salt concentration, being immediately preceded by N-benzoyldeoxyadenosine-5′ phosphate. The yield as estimated spectrophotometrically was 31% (0.18 mmole) as based on 3′-O-acetyl-N-benzoyldeoxyadenosine-5′ phosphate. The dinucleotide showed λ_{max} (pH 7) at 277 mμ with a shoulder at 262 mμ and λ_{min} at 232 mμ.

It was isolated as the pyridine salt by treatment of the combined peak with an excess of pyridinium Dowex-50 resin and evaporation of the total solution in the presence of pyridine. It traveled as a single spot on paper electrophoresis at pH 7.1 (0.03 *M* phosphate buffer), with mobility 1.15 relative to N-benzoyldeoxyadenosine-5′ phosphate. After treatment with concentrated ammonium hydroxide at room temperature for 2 days, the mobility was equal to that of thymidine-5′ phosphate at pH 7.1, whereas at pH 2.7 (0.03 *M* ammonium citrate) it had mobility 0.86 relative to thymidine-5′ phosphate and like that of the separately characterized deoxyadenylyl-(3′ → 5′)-thymidine-3′ phosphate. The R_f of the N-benzoyl dinucleotide in solvent A was identical with that of thymidine-5′ phosphate.

Polymerization of 5′-O-Phosphorylthymidylyl-(3′ → 5′)-N-benzoyldeoxyadenosine (IX): The Isolation and Characterization of Polynucleotides Containing Alternating Thymidine and Deoxyadenosine Residues.—5′-O-Phosphorylthymidylyl-(3′ → 5′)-N-benzoyldeoxyadenosine (13,000 optical density units, 276 mμ, about 0.5 mmole) was rendered anhydrous with pyridine in a preweighed flask. The residual gum (730 mg.) was redissolved in dry pyridine (0.75 ml.) by shaking the sealed flask at room temperature for 3 hr.; DCC (420 mg., 2 mmoles) was added and the sealed reaction mixture shaken vigorously for about 0.5 hr. The flask was then set aside in the dark for 6 days at room temperature. The oil which separated immediately on addition of DCC went solid after about 3 hr. After 6 days, water (1 ml.) and pyridine (3 ml.) were added and the flask shaken until a homogenous mixture (except for the crystalline dicyclohexylurea) had resulted (4 hr.). Triethylamine (0.2 ml., 1.5 mmoles) was added and the solution made anhydrous by coevaporation with dry pyridine. The gum was finally dissolved in 15 ml. of pyridine, acetic anhydride (3 ml.) added, and the solution kept at room temperature for 4 days. Ethyl alcohol (5 ml.) was then added, the solution kept overnight at room temperature and then evaporated at low temperature to a small volume (1–2 ml.). Water (1 ml.) and ether (20 ml.) were added and after shaking, the separated aqueous layer treated with concentrated ammonia (10 ml.) for 2 days at room temperature. Subsequently, the ammoniacal solution was evaporated and the resulting aqueous solution filtered from the insoluble material. The resulting solution contained a total of 9600 optical density units at 260 mμ.

A small aliquot (1%) was applied on top of DEAE–cellulose (carbonate) column (30 × 0.8 cm.). Elution was carried out with a linear gradient of ammonium bicarbonate (0–0.5 *M* with 1 l. of water) in the mixing vessel and 1 l. of 0.5 *M* salt in the reservoir. The elution was followed by an automatic ultraviolet absorbance recorder (log scale). The pattern is shown in Fig. 2. The remainder was applied to the top of a DEAE–cellulose (carbonate) column (45 × 3.5 cm. diam.). Elution was carried out with a linear salt gradient: the mixing vessel contained 4 l. of water and the reservoir contained an equal volume of 0.5 *M* ammonium bicarbonate solution. (During the elution the pH of the salt solution rose from 7.7 to 8.6 due to the loss of carbon dioxide.) Fractions of 20-ml. volume at a flow rate of 2 ml./min. were collected. The major peaks obtained and their composition are given in Table I. The appropriately pooled fractions were evaporated and the salt removed by lyophilization.

TABLE I

CHROMATOGRAPHY OF POLYNUCLEOTIDES CONTAINING DEOXYADENOSINE AND THYMIDINE IN ALTERNATING SEQUENCES. DISTRIBUTION OF NUCLEOTIDIC MATERIAL IN DIFFERENT PEAKS

Peak no.	Fractions pooled	Total optical density (261 mμ)	% of total nucleotidic material	Identification remarks
1	58–85	160	2	Unidentified
2	167–186	2970	36.3	Cyclo-d-pTpA
3	191–210	1850	22.6	Dinucleotide, d-pTpA
4 + 5	243–286	400	4.9	Unidentified
6	288–321	875	10.7	d-pTpApTpA
7–9	322–391	600	7.3	Unidentified minor peaks
10	392–416	410	5	d-pTpApTpApTpA
11 + 12	417–458	250	3	Unidentified
13	459–487	165	2	d-pTpApTpApTpATpA
2 *M* eluate	499–512	91	1.1	Higher polynucleotides[a]

[a] Further purified by treatment with alkaline phosphomonoesterase followed by chromatography in solvent D. About 50% of this fraction was thus shown to be the decanucleotide d-pTpApTpApTpApTpApTpA and the remainder contained successively decreasing amounts of the higher members.

The residual powders were stored as solutions in water in a frozen state. Paper chromatography in solvent D (R_f's in Table II) showed the peaks up to the tetranucleotide d-pTpApTpA to be pure, but the higher homologs (the hexa-, octa-, and higher polynucleotides) to be contaminated with very small amounts (about 5%) of faster traveling products. Incubation of the major peaks with the bacterial phosphomonoesterase caused

conversion of the starting materials to faster traveling products, whose R_f's are also recorded in Table II. The contaminants in the larger oligonucleotides were also converted to products traveling proportionately higher, and for analysis by venom phosphodiesterase degradation the major bands obtained after dephosphorylation with the phosphomonoesterase were used.

TABLE II

R_f VALUES OF DIFFERENT POLYNUCLEOTIDES IN SOLVENT D ON WHATMAN PAPER 44

Compound	R_f
Polynucleotides bearing -5′ phosphate end groups	
pT (or d-pA)	0.55
d-pTpA	.49
Cyclo-d-pTpA	.58
d-pTpApTpA	.35
d-pTpApTpApTpA	.23[a]
d-pTpApTpApTpApTpA	.145[b]
Larger homologs	.03–0.10
Polynucleotides lacking the terminal -5′ phosphate groups	
d-TpApTpApTpA	0.36
d-TpApTpApTpApTpA	.24
d-TpApTpApTpApTpApTpA	.20
d-TpApTpApTpApTpApTpApTpA	.10
Higher than dodecanucleotide	0–0.06

[a] R_f of the faster traveling impurity in the original peak, 0.35. [b] R_f of the impurity in the original peak, 0.23.

Synthesis of 5′-O-Di-*p*-methoxytrityldeoxyadenylyl-(3′ → 5′)-thymidine-3′ Phosphate, and Deoxyadenylyl-(3′ → 5′)-thymidine-3′ Phosphate.—A dry pyridine (1 ml.) solution of 5′-O-di-*p*-methoxytrityldeoxyadenosine-3′ phosphate (0.075 mmole) was treated with DCC (103 mg., 0.5 mmole) for 1 hr. Separately an anhydrous pyridine (1 ml.) solution of β-cyanoethyl thymidine-3′ phosphate (0.1 mmole) was prepared and this solution was added to the first solution. The mixture was kept at room temperature for 4 days. Water (5 ml.) was then added and the reaction mixture left overnight; DCC was then extracted with petroleum ether (3 × 25 ml.) and the solution was then treated with 1 ml. of 1 *N* sodium hydroxide. The mixture was lyophilized. To the resulting powder a further amount (2 ml.) of 1 *N* sodium hydroxide was added and the solution heated at 100° for 10 min. The pH was brought to neutrality by addition of pyridinium Dowex-50 resin and the resin and dicyclohexylurea removed by filtration. Paper chromatography in solvent A showed the main product to be 5′-O-di-*p*-methoxytrityldeoxyadenylyl-(3′ → 5′)-thymidine-3′ phosphate (R_f 0.33), there being, in addition, four weak ultraviolet-absorbing spots. The mixture was applied to the top of a DEAE–cellulose (carbonate form) column (23 × 2.5 cm.) and elution was carried out with a linear gradient, there being 4 l. of water in the mixing vessel and an equal volume of 0.25 *M* triethylammonium bicarbonate in the reservoir; 16–18-ml. fractions were collected at 10-min. intervals. The desired product, 5′-O-di-*p*-methoxytrityldeoxyadenylyl-(3′ → 5′)-thymidine-3′ phosphate, was eluted in fractions 165–213. The combined fractions were evaporated *in vacuo* with all the precautions taken above for the preparation of the 5′-O-di-*p*-methoxytrityldeoxyadenosine-3′ phosphate. The total residue was passed through a column of ammonium Dowex-50 resin and the total effluent lyophilized. Detritylation occurred to the extent of 3–5% during lyophilization, but the product was homogeneous as shown by removal of the di-*p*-methoxytrityl group. Deoxyadenylyl-(3′ → 5′)-thymidine-3′ phosphate thus prepared was homogeneous by paper chromatography and on paper electrophoresis both in acidic and neutral pH range. Incubation of a sample with spleen phosphodiesterase under the standard conditions described previously caused complete degradation, deoxyadenosine-3′ phosphate and thymidine-3′ phosphate being formed in approximately equal amounts. The yield of the isolated dinucleotide was estimated spectrophotometrically to be 34%, using an extinction value at 263 mμ of 20,000.

[CONTRIBUTION FROM THE INSTITUTE FOR ENZYME RESEARCH, THE UNIVERSITY OF WISCONSIN, MADISON 6, WIS.]

Studies on Polynucleotides. XXVII.[1] The Stepwise Synthesis of Specific Deoxyribopolynucleotides (7).[2] The Synthesis of Polynucleotides Containing Deoxycytidine and Deoxyguanosine in Specific Sequences and of Homologous Deoxycytidine Polynucleotides Terminating in Thymidine[3]

BY H. SCHALLER AND H. G. KHORANA

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The following protected deoxyribodinucleotides were prepared: 5′-O-cyanoethylphosphoryl-N-anisoyldeoxycytidylyl-(3′ → 5′)-N-acetyldoxyadenosine, 5′-O-phosphorylthymidylyl-(3′ → 5′)-N-acetyldeoxyguanosine, the corresponding 3′-O-acetyl derivative, 5′-O-phosphoryl-N-acetyldeoxyguanylyl-(3′ → 5′)-N,3′-O-diacetyldeoxycytidine, and 5′-O-phosphorvl-N-anisoyldeoxycytidylyl-(3′ → 5′)-N-acetyldeoxyguanosine. The treatment of the last-mentioned protected dinucleotide with dicyclohexylcarbodiimide (DCC) in anhydrous pyridine–dimethylformamide followed by appropriate work-up gave homologous tetra-, hexa-, octa-, and a small amount of the decanucleotide containing deoxycytidine and deoxyguanosine nucleosides in alternating sequence. The pentanucleotide, deoxycytidylyl-(3′ → 5′)-deoxyguanylyl-(3′ → 5′)-deoxycytidylyl-(3′ → 5′)-deoxycytidylyl-(3′ → 5′)-deoxycytidine, was prepared by condensation of 5′-O-dimethoxytrityl-N-anisoyldeoxycytidylyl-(3′ → 5′)-N-acetyldeoxyguanosine with 5′-O-phosphoryl-N-acetyldeoxycytidylyl-(3′ → 5′)-N-acetyldeoxycytidylyl-(3′ → 5′)-N-3′-O-diacetyldeoxycytidine followed by removal of the protecting groups and ion-exchange chromatography. A new approach to the synthesis of homopolynucleotides containing a different nucleoside at one terminus was developed which involved the treatment of the total mixture obtained by polymerization of a protected mononucleotide with an excess of a suitably protected nucleoside in the presence of a condensing agent. Thymidylyl-(3′ → 5′)-deoxycytidylyl-(3′ → 5′)-deoxycytidylyl-(3′ → 5′)-deoxycytidine was also prepared by condensation of 5′-O-phosphoryl-N-anisoyldeoxycytidylyl-(3′ → 5′)-N-anisoyldeoxycytidylyl-(3′ → 5′)-N-anisoyl-3′-O-acetyldeoxycytidine with 5′-O-dimethoxytritylthymidine.

As part of a study of the synthesis of oligonucleotide chains by condensation of suitably protected di- and trinucleotides, the preceding paper described the synthesis of the pentanucleotide thymidylyl-(3′ → 5′)-deoxyadenylyl-(3′ → 5′)-thymidylyl-(3′ → 5′)-thymidylyl-(3′ → 5′)-thymidine and of polynucleotides containing thymidine and deoxyadenosine in alternating sequence.[1] The present paper deals, partly, with a parallel investigation of the synthesis of polynucleotides containing deoxycytidine and deoxyguanosine and, partly, with the evaluation of the available protecting groups in the general problems of polynucleotide synthesis. In addition, a new approach to the synthesis of homopolynucleotides containing a different nucleoside at one terminus is described. A brief report of a part of this work has already appeared.[4]

(1) Paper XXVI: G. Weimann, H. Schaller, and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 3835 (1963).

(2) Earlier papers in this series which deal with this topic: (a) P. T. Gilham and H. G. Khorana, *ibid.*, **80**, 6212 (1958); (b) *ibid.*, **81**, 4647 (1959); (c) G. Weimann and H. G. Khorana, *ibid.*, **84**, 419 (1962); (d) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *ibid.*, **85**, 3821 (1963); (e) H. Schaller and H. G. Khorana, *ibid.*, **85**, 3828 (1963); (f) ref. 1.

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