provoking misreading of the genetic message.[24,43] Similar antibiotics such as neomycin B have been shown to elicit messenger response from denatured DNA.[44] Ribopolynucleotides containing repeating dinucleotide sequences and certain single-stranded deoxyribopolynucleotides prepared by separation of the double-stranded DNA-like polymers have already permitted more precise studies of these processes.[45] The availability of a large selection of polynucleotides with repeating tri- and tetranucleotide sequences would permit an extension of these studies.

A series of accompanying papers describe the work on the synthesis of deoxyribopolynucleotides with repeating tri- and tetranucleotide sequences.[26-30] The synthetic deoxyribonucleotides have been used successfully in the synthesis of DNA polymerase catalyzed synthesis of DNA-like polymers containing the appropriate repeating sequences. The synthesis and characterization of the DNA-like polymers and their physicochemical properties are being reported elsewhere.[46-48] The DNA-like polymers have afforded, in turn, the expected sets of single-stranded ribopolynucleotides.[49] The use of the resulting ribopolynucleotides as messengers in the cell-free protein-synthesizing system have given new information, in particular on the codons whose assignments have hitherto been uncertain. As a consequence, essentially all of the genetic code is now established with certainty for the microorganism *E. coli* B.[50] Other biological studies made possible by the availability of the DNA-like polymers will be reported subsequently.

(43) J. Davies, W. Gilbert, and L. Gorini, *Proc. Natl. Acad. Sci. U. S.*, **51**, 883 (1964).
(44) B. J. McCarthy and J. J. Holland, *ibid.*, **54**, 880 (1965).
(45) A. R. Morgan, R. D. Wells, and H. G. Khorana, *J. Mol. Biol.*, in press.
(46) R. D. Wells, T. M. Jacob, S. A. Narang, and H. G. Khorana, *ibid.*, in press.
(47) R. D. Wells, H. Büchi, H. Kössel, E. Ohtsuka, and H. G. Khorana, *ibid.*, in press.
(48) R. D. Wells and J. Blair, *ibid.*, in press.
(49) H. Kössel A. R. Morgan, and H. G. Khorama, *ibid.*, in press.
(50) A. R. Morgan, R. D. Wells, and H. G. Khorana, *Proc. Natl. Acad. Sci. U. S.*, **56**, 1899 (1966).

Studies on Polynucleotides. LXII.[1] Deoxyribopolynucleotides Containing Repeating Trinucleotide Sequences (4).[2] Preparation of Suitably Protected Deoxyribotrinucleotides[3]

S. A. Narang, T. M. Jacob, and H. G. Khorana

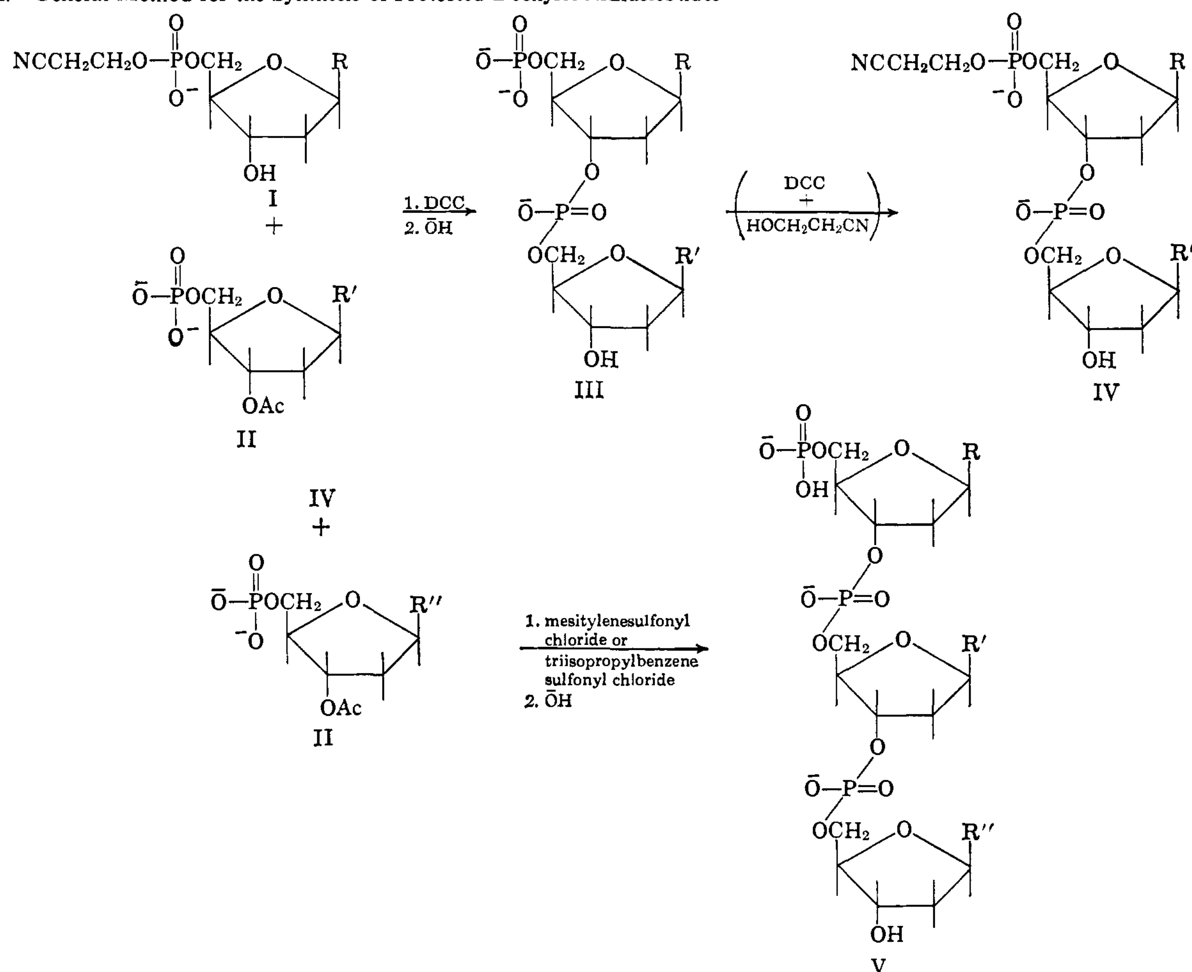
Contribution from the Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin 53706. *Received December 12, 1966*

Abstract: Suitably protected deoxyribotrinucleotides were required as starting materials for the preparation of deoxyribopolynucleotides containing repeating trinucleotide sequences. Using the stepwise method illustrated in Chart I, the syntheses of the following 13 protected deoxyribotrinucleotides[4] have been accomplished: d-pTpA^{Bz}pC^{An}, d-pTpA^{Bz}pG^{Ac}, d-pA^{Bz}pTpC^{An}, d-pA^{Bz}pTpG^{Ac}, d-pC^{An}pG^{Ac}pA^{Bz}, d-pC^{An}pG^{Ac}pT, d-pTpTpG^{Ac}, d-pA^{Bz}pA^{Bz}pC^{An}, d-pA^{Bz}pA^{Bz}pG^{Ac}, d-pC^{An}pC^{An}pA^{Bz}, d-pC^{An}pC^{An}pT, d-pG^{Bz}pG^{Bz}pA^{Bz}, and d-pG^{Bz}pG^{Bz}pT. Acetylation in acetic anhydride–pyridine gave the corresponding 3′-O-acetyl derivatives. All the trinucleotides were characterized to be pure with and without the protecting groups.

As discussed in the preceding introductory paper,[1] the synthesis of DNA-like polymers containing repeating trinucleotide sequences is of interest for further studies of the genetic code. It is also clear that such DNA-like polymers would be useful for further physicochemical studies of DNA. The maximum number of double-stranded DNA-like polymers with repeating trinucleotide sequences which can be formed from the four common deoxyribomononucleotides and which contain more than one base in each strand are ten. These are listed in Table I. Copying of the individual strands in these polymers by DNA-dependent RNA

(1) Preceding paper in this series: H. G. Khorana, H. Büchi, T. M. Jacob, H. Kössel, S. A. Narang, and E. Ohtsuka, *J. Am. Chem. Soc.*, **89**, 2154 (1967).
(2) Previous papers dealing with the preparation of deoxyribopolynucleotides with repeating trinucleotide sequences are: (a) T. M. Jacob and H. G. Khorana, *ibid.*, **87**, 2971 (1965); (b) S. A. Narang and H. G. Khorana, *ibid.*, **87**, 2981 (1965); (c) S. A. Narang, T. M. Jacob, and H. G. Khorana, *ibid.*, **87**, 2988 (1965).
(3) This work has been supported by grants from the National Science Foundation (Grant No. GB-3342), the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service (Grant No. CA-05178), and the Life Insurance Medical Research Fund (Grant No. G-62-54).
(4) For convenience and economy of space, abbreviations are used extensively for both the protected and unproctected series of compounds described in this and the accompanying papers. The basic system of abbreviations used for polynucleotides and their protected derivatives is as has been used in previous papers in this series and is in current use in *Biochemistry* and *The Journal of Biological Chemistry*. Thus the single letters A, T, C, and G represent the nucleosides of, respectively, adenine, thymine, cytosine, and guanine. The letter p to the left of the nucleoside initial indicates a 5′-phosphomonoester group and the same letter to the right indicates a 3′-phosphomonoester group. Thus, in going from the left to the right the polynucleotide chain is specified in the 3′→5′ direction. The protecting groups on the purine or pyrimidine rings are designated by two-letter abbreviations added as superscripts after the nucleoside initial: thus d-A^{Bz} for N-benzoyldeoxyadenosine, C^{An} for N-anisoylcytidine, G^{Ac} for N-acetylguanosine. The acetyl group at the 3′-hydroxyl group of a nucleoside is shown by -OAc added after the nucleoside initial. Thus, pT-OAc is the abbreviation of 3′-O-acetylthymidine 5′-phosphate. CE is the abbreviation for β-cyanoethyl: thus, d-CE-pG^{Ac} stands for N-acetyldeoxyguanosine 5′-β-cyanoethyl phosphate. Using this system, the abbreviation d-pC^{An}pG^{Ac}pA^{Bz}-OAc stands for the protected trinucleotide, 5′-O-phosphoryl-N-anisoyldeoxycytidylyl-(3′→5′)-N-acetyldeoxyguanylyl-(3′→5′)-3′-O-acetyl-N-benzoyldeoxyadenosine.

Chart I. General Method for the Synthesis of Protected Deoxyribotrinucleotides



I-V, R, R′, R″ = thymine or N-acetylguanine or N-benzoyladenine or N-anisoylcytosine

polymerase will afford 20 single-stranded ribopolynucleotides containing repeating trinucleotide sequences. These will contain all of the 60 possible trinucleotide codons (except for the four homotrinucleotides).

Table I. DNA-like Polymers with Repeating Trinucleotide Sequences[a] Derivable from Four Common Deoxyribomononucleotides

(1)	Poly d-TTC:GAA	(6)	Poly d-GCA:TGC
(2)	Poly d-TTG:CAA	(7)	Poly d-CCA:TGG
(3)	Poly d-TAC:GTA	(8)	Poly d-CCT:AGG
(4)	Poly d-ATC:GAT	(9)	Poly d-TAA:TTA
(5)	Poly d-CGA:TCG	(10)	Poly d-CCG:CGG

[a] Containing more than one nucleotide base in each strand. [b] All of the DNA-like polymers are written so that the colon separates the repeating trinucleotide units in the complementary strands. The sequence of the trinucleotide repeating unit in each strand is written so that antiparallel base pairing in the two complementary strands is evident.

The first step in the preparation of the DNA-like polymers is the chemical synthesis of short-chain deoxyribopolynucleotides containing repeating trinucleotide sequences. A set of two short deoxyribopolynucleotide chains, which are complementary to each other in the antiparallel Watson–Crick sense, are required for enzymatic synthesis of each double-stranded DNA-like polymer. It is the purpose of this and an accompanying paper to describe the chemical work on the synthesis of many complementary sets of short-chain deoxyribopolynucleotides. Several of these sets have been successfully used as templates for DNA polymerase, and the products of the enzymatic reaction have been characterized as high molecular weight DNA-like polymers.

The synthesis of deoxyribopolynucleotides containing repeating nucleotide sequences may, in principle, be approached either by stepwise procedures or by the polymerization of a suitably protected oligonucleotide unit. Previously described syntheses of deoxyribopolynucleotides containing repeating trinucleotide sequences were all accomplished by the successive additions of mononucleotide units to growing oligonucleotide chains.[2] Because of the advantages of rapidity and simplicity which the polymerization methods can offer, the latter methods were further investigated at the start of the present work. Marked improvements thus resulted, and the polymerization technique has now been used in the synthesis of repeating trinucleotide polymers. The present paper describes the preparation, in relatively large amounts, of suitably protected deoxyribotrinucleotides which were required as the starting materials for polymerization experiments. The polymerization of the protected trinucleotides and the isolation and characterizations of the resulting polynucleotides are described in the following paper.

The general method used for the preparation of the protected trinucleotides is shown in Chart I, the main steps used being as follows. (1) Condensation of two suitably protected mononucleotides (general structures

I and II) gave, after an alkaline treatment, a protected dinucleotide of general structure III. (2) The phosphomonoester group in the dinucleotides (III) was protected by esterification to form the cyanoethyl derivatives of type IV. Finally, repeat condensation of the latter with protected mononucleotides of type II followed by alkaline treatment gave the protected trinucleotides (general structure V).

β-Cyanoethyl esters of mononucleotides (general structure I) were prepared by the reaction of the N-protected deoxyribonucleoside 5′-phosphates with an excess of hydracrylonitrile in the presence of dicyclohexylcarbodiimide (DCC).[5-7] As noted previously, during this reaction some of the neutral phosphate esters containing two cyanoethyl groups are also obtained. These can be selectively hydrolyzed to the monocyanoethyl esters I by keeping them at slightly alkaline pH. The desired products (I) were isolated in 80–90% yield simply by precipitation from ether. Condensation of these protected derivatives with the appropriate 3′-O-acetyl N-protected nucleoside 5′-phosphates, all of which are available from previous work, in the presence of dicyclohexylcarbodiimide (DCC) or mesitylenesulfonyl chloride (MS) and subsequent purification by anion-exchange column chromatography gave the protected dinucleotides III. The isolated yields using approximately equivalent amounts of the two components were in the range of 60–70%. All the protected dinucleotide derivatives (III) were fully characterized and checked for homogeneity by paper chromatography and paper electrophoresis.

While most of the protected dinucleotides of type III were prepared as described above, an alternative method involved the polymerization of an N-protected deoxyribonucleoside 5′-phosphate in the presence of a substantial amount of the corresponding derivative carrying a 3′-O-acetyl group.[5] The major products thus obtained were the protected di- and trinucleotides, very small amounts of the larger oglionucleotides being present. This approach was used in the preparation of d-pA^{Bz}pA^{Bz}. The yield of this product was 34% and the higher homolog, d-pA^{Bz}pA^{Bz}pA^{Bz}, a potentially useful intermediate in synthetic work, was isolated in 16% yield.

Reaction of the protected dinucleotides III with an excess of hydracylonitrile in the presence of DCC gave the cyanoethyl derivatives (general structure IV). While the introduction of one cyanoethyl group at the 5′-phosphomonoester groups in III was invariably quantitative, there was frequent evidence of further cyanoethylation. This presumably involved partial esterification of the phosphodiester groups to form neutral esters. While the neutral esters could be converted by the usual treatment at slightly alkaline pH to the monocyanoethylated products IV, the alkaline treatment was, obviously, not necessary and, in most cases, the products of the cyanoethylation reaction were used directly after isolation for the next step.

In the synthesis of the protected trinucleotides (step IV → V) mesitylenesulfonyl chloride (MS) or triisopropylbenzenesulfonyl chloride (TPS) was used as the condensing agent. After an appropriate workup, including an alkaline treatment to remove the acetyl and cyanoethyl groups, the protected trinucleotides V were isolated by anion-exchange chromatography. The protecting groups used for the heterocyclic amino groups were stable throughout the duration of the various operations except for the acetyl group on the guanine ring, some loss (about 10%) of which was detected. The isolated yields of the protected trinucleotides as based on the protected dinucleotide starting materials (IV) were 50–80%, a 2–3 molar excess of the protected mononucleotide having been used.

The total sets of protected trinucleotides whose preparations are herein described are listed in Table II. It will be seen that, wherever possible, the syntheses were so planned that one protected dinucleotide served as an intermediate for the synthesis of both members of a set of the protected trinucleotide. For example, d-pTpA^{Bz}pC^{An} and d-pTpA^{Bz}pG^{Ac} both utilized the same protected dinucleotide, CE-pTpA^{Bz}. Synthetically, this principle lessened the number of preparations and, theoretically, it was justified because the resulting sets of protected trinucleotides would yield, on polymerization, deoxyribopolynucleotides which would be largely complementary to each other in the required antiparallel sense.

Table II. Sets of Protected Deoxyribotrinucleotides Synthesized

d-pTpA^{Bz}pC^{An}	d-pC^{An}pC^{An}pA^{Bz}
d-pTpA^{Bz}pG^{Ac}	d-pC^{An}pC^{An}pT
d-pA^{Bz}pTpC^{An}	d-pG^{Bz}pG^{Bz}pA^{Bz}
d-pA^{Bz}pTpG^{Ac}	d-pG^{Bz}pG^{Bz}pT
d-pC^{An}pG^{Ac}pT	d-pA^{Bz}pA^{Bz}pG^{Ac}
d-pC^{An}pG^{Ac}pA^{Bz}	
d-pA^{Bz}pA^{Bz}pC^{An}	
d-pTpTpG^{Ac}	

Characterization of all of the protected trinucleotides and the corresponding members obtained after removal of the protecting groups was accomplished by paper chromatography and the data are recorded in Table III. Further characterization of the unprotected trinucleotides was accomplished by enzymic removal of the phosphomonoester groups followed by paper chromatography (two solvents). Finally, the trinucleotides lacking phosphomonoester groups were characterized by degradation with spleen phosphodiesterase (Table IV). Degradation with this enzyme proceeded to completion, and the products (nucleosides and mononucleotides) were produced in the expected molar proportions.

Experimental Section

General Methods. Paper chromatography was carried out by the descending technique using mostly Whatman No. 40 or Whatman No. 1 paper. The solvent systems used were: solvent A, isopropyl alcohol–concentrated ammonia–water (7:1:2, v/v); solvent B, ethyl alcohol–1 *M* ammonium acetate, pH 7.5 (7:3, v/v); solvent C, isobutyric acid–concentrated ammonia–water, pH 3.7 (66:1:33, v/v); solvent D, *n*-propyl alcohol–concentrated ammonia–water (55:10:35, v/v).

Paper electrophoresis was performed in a high-voltage apparatus in which the paper was immersed in a high-boiling petroleum fraction (Varsol). The buffers used were: buffer I, potassium phosphate (0.03 *M*, pH 7.1), and buffer II, ammonium formate–formic acid (0.05 *M*, pH 2.7).

(5) H. Schaller and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 3841 (1963).
(6) G. Weimann, H. Schaller, and H. G. Khorana, *ibid.*, **85**, 3835 (1963).
(7) E. Ohtsuka, M. W. Moon, and H. G. Khorana, *ibid.*, **87**, 2956 (1965).

Table III. Paper Chromatography and Paper Electrophoresis of Di- and Trinucleotides and Their Derivatives[a]

Compd	Solvent B	Solvent D	Solvent C	Electrophoretic mobility, pH 7.1
pT	1.0	1.0	1.0	1.0
d-pTpA^{Bz}	1.05			
d-CE-pTpA^{Bz}	1.7			0.69
d-pTpA^{Bz}pC^{An}	0.7			
d-pTpA^{Bz}pC^{An}-OAc	0.92			
d-pTpApC		0.73	0.60	
d-TpApC		1.40	1.15	
d-pTpA^{Bz}pG^{Ac}	0.66			
d-pTpA^{Bz}pG^{Ac}-OAc	0.85			
d-pTpApG		0.61	0.70	
d-TpApG		1.20	1.30	
d-pA^{Bz}pT	1.07			
d-CE-pA^{Bz}pT	1.70			0.67
d-pA^{Bz}pTpC^{An}	0.72			
d-pA^{Bz}pTpC^{An}-OAc	0.90			
d-pApTpC		0.56	0.60	
d-ApTpC		1.30	1.0	
d-pA^{Bz}pTpG^{Ac}	0.70			
d-pA^{Bz}pTpG^{Ac}-OAc	0.90			
d-pApTpG		0.50	0.67	
d-ApTpG		1.05	1.30	
d-pC^{An}pG^{Ac}	0.90			
d-CE-pC^{An}pG^{Ac}	1.60			0.60
d-pC^{An}pG^{Ac}pA^{Bz}	0.80			
d-pC^{An}pG^{Ac}pA^{Bz}-OAc	1.0			
d-pCpGA		0.60	0.72	
d-CpGpA		1.20	1.30	
d-pC^{An}pG^{Ac}pT	0.71			
d-pC^{An}pG^{Ac}pT-OAc	0.92			
d-pCpGpT		0.50	0.60	
d-CpGpT		1.0	1.21	
d-pA^{Bz}pA^{Bz}	1.10			
d-CE-pA^{Bz}pA^{Bz}	1.60			0.50
d-pA^{Bz}pA^{Bz}pC^{An}	0.66			
d-pA^{Bz}pA^{Bz}pC^{An}-OAc	0.90			
d-pApApC		0.60	0.70	
d-ApApC		1.20		
d-pTpTpG^{Ac}	0.50			
d-pTpTpG^{Ac}-OAc	0.70			
d-pTpTpG		0.30	0.60	
d-TpTpG		1.0	1.20	
d-pA^{Bz}pA^{Bz}pG^{Ac}	0.9			
d-pA^{Bz}pA^{Bz}pG^{Ac}-OAc	0.95			
d-pApApG	0.11	0.47		
d-ApApG		0.95		
d-pC^{An}pC^{An}	1.05			
d-CE-pC^{An}pC^{An}	1.6			
d-pC^{An}pC^{An}pA^{Bz}	0.85			
d-pC^{An}pC^{An}pA^{Bz}-OAc	1.09			
d-pCpCpA	0.16	0.62		
d-CpCpA		1.2		
d-pC^{An}pC^{An}pT	0.61			
d-pC^{An}pC^{An}pT-OAc	0.7			
d-pCpCpT	0.19	0.63		
d-CpCpT	0.83	1.1	1.3	
d-pG^{Bz}pG^{Bz}	0.71			
d-CE-pG^{Bz}pG^{Bz}	1.43			
d-pG^{Bz}pG^{Bz}pA^{Bz}	0.67			
d-pG^{Bz}pG^{Bz}pA^{Bz}-OAc	0.81			
d-pGpGpA		0.38		
d-GpGpA		0.8		
d-pG^{Bz}pG^{Bz}pT	0.6			
d-pG^{Bz}pG^{Bz}pT-OAc	0.7			
d-pGpGpT		0.42		
d-GpGpT		0.9		

[a] R_f with respect to pT.

Table IV. Characterization of Products by Spleen Phosphodiesterase Degradation

Compd	OD_{260} units degraded	Nucleotides: nucleoside	Molar ratio of products Found	Theor
d-TpApC	2.0	Tp:d-Ap:d-C	1:1:0.91	1:1:1
d-ApApC	4.0	d-Ap:d-C	2:1	2:1
d-TpApG	4.0	Tp:d-Ap:d-G	1:1:0.92	1:1:1
d-ApTpC	3.5	d-Ap:Tp:d-C	1:1:0.94	1:1:1
d-ApTpG	3.0	d-Ap:Tp:d-G	1:1:0.9	1:1:1
d-CpGpA	3.0	d-Cp:d-Gp:d-A	1:1:0.91	1:1:1
d-CpGpT	3.2	d-Cp:d-Gp:T	0.93:1:1	1:1:1
d-TpTpG	5.0	Tp:d-G	2.1:1	2:1
d-ApApG	3.5	d-Ap:d-G	2:1	2:1
d-CpCpT	5.0	d-Cp:T	2.1:1	2:1
d-CpCpA	4.0	d-Cp:d-A	2:1	2:1

The ϵ_{max} values used for the protected mononucleotides are as follows: d-pC^{An}: 22,450 (302 mμ) and 18,000 (280 mμ); d-pA^{Bz}: 6100 (302 mμ), 18,300 (280 mμ), and 11,450 (260 mμ); d-pG^{Ac}: 16,700 (260 mμ) and 10,900 (280 mμ). The abbreviation OD_{280} refers to the extinction of the nucleotidic solution at neutral pH in 1 ml of solution using a 1-cm light-path quartz cell, the number in subscript being the wavelength used.

Pyridinium pT-OAc, d-pC^{An}-OAc, d-pG^{Ac}-OAc, d-pG^{Bz}-OAc, and d-pA^{Bz}-OAc were prepared as described previously[8] except for the ether precipitation step which was carried out as described earlier.[2a] Pyridinium CE-pT, d-CE-pC^{An}, d-CE-pG^{Ac}, and d-CE-pA^{Bz} were prepared as described elsewhere[5-7] except that the DEAE-cellulose column chromatography step was avoided. After the usual work-up the compounds were isolated by precipitation from ether as described under the cyanoethylation of protected dinucleotides.

General Method for Synthesis of Protected Dinucleotides Bearing a 5′-Phosphomonoester End Group. The pyridinium salts of β-cyanoethyl esters of protected mononucleotides and the appropriate 3′-O-acetyl N-protected nucleoside 5′-phosphate were dissolved in dry pyridine. Dry pyridinium Dowex-50W X2 ion-exchange resin was added, and the total mixture was rendered anhydrous by repeated evaporation of added pyridine. During the last evaporation a portion of dry pyridine was retained to serve as the medium of reaction. DCC (at least 5 equiv as based on the mononucleotide) was added, and the sealed reaction mixture was shaken in the dark for 3–4 days at room temperature. An equal volume of water was then added and the reaction mixture was filtered to remove dicyclohexylurea and the insoluble resin. The total filtrate and 50% aqueous pyridine wash were extracted twice with petroleum ether to remove unreacted DCC. The resulting clear solution was kept for 8–16 hr at room temperature and then treated with 2 *N* sodium hydroxide (over-all molarity of the final solution about 1 *N* with respect to sodium hydroxide for 20 min at 0°). The sodium ions were then removed by the addition of an excess of pyridinium Dowex 50 ion-exchange resin, and the total solution after removal of the resin (wash with 50% aqueous pyridine) was applied directly to a DEAE-cellulose (bicarbonate) column and chromatography was carried out at 4° using triethylammonium bicarbonate solution containing 10–25% alcohol for elution.

General Method for Cyanoethylation of the 5′-Phosphomonoester End Group in Protected Dinucleotides. A solution of pyridinium salt of protected dinucleotide and hydracrylonitrile (50 equiv as based on dinucleotidic component) in dry pyridine (5–15 ml) was shaken with DCC (10 equiv as based on dinucleotide) at room temperature for 1–3 days. Water (three times the volume of pyridine) was then added and the mixture was extracted with ether (three 50-ml portions. The resulting solution was kept for 8–16 hr at room temperature and then adjusted to pH 8.5 with 1 *M* triethylammonium bicarbonate, and the solution was allowed to stand for 4 hr at room temperature. Triethylammonium ions were removed by passing the solution through a column of pyridinium Dowex 50 ion-exchange resin, and the column washings were evaporated with frequent addition of pyridine. The syrupy residue obtained finally was rendered anhydrous by evaporation of added anhydrous pyri-

(8) (a) H. G. Khorana and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **83**, 675 (1961); (b) H. Schaller and H. G. Khorana, *ibid.*, **85**, 3828 (1963); H. G. Khorana, A. F. Turner, and J. P. Vizsolyi, *ibid.*, **83**, 686 (1961); (d) R. K. Ralph and H. G. Khorana, *ibid.*, **83**, 2926 (1961); (e) R. K. Ralph, W. J. Connors, H. Schaller, and H. G. Khorana, *ibid.*, **85**, 1983 (1963).

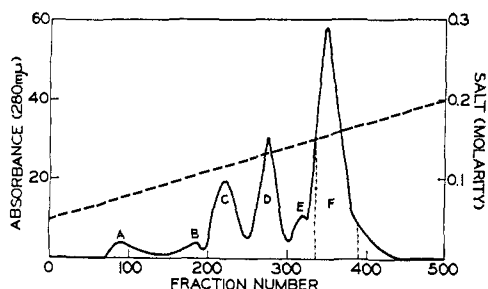


Figure 1. Chromatography of the reaction products in the preparation of dinucleotide d-pTpA^{Bz} on a DEAE-cellulose (carbonate) column. Peak F contains d-pTpA^{Bz}.

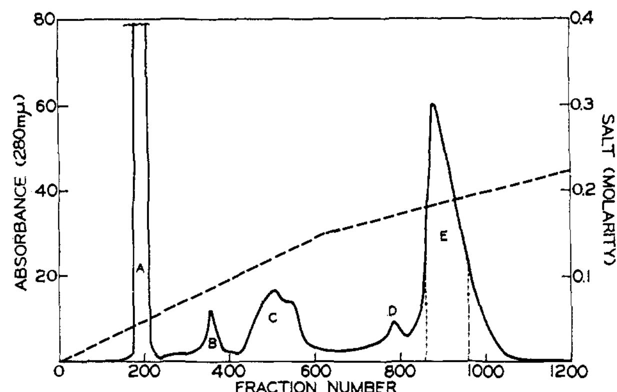


Figure 2. Chromatography of the reaction products in the preparation of dinucleotide d-pA^{Bz}pT on a DEAE-cellulose (carbonate) column. Peak E contains d-pA^{Bz}pT.

dine. The resulting solution was added dropwise to anhydrous ether (25–100-fold excess in volume). The β-cyanoethylated N-protected dinucleotides were separated as a fine white precipitate in most of the cases. These were collected by centrifugation and washed with fresh ether by suspension in the centrifuge tube.

In some cases, β-cyanoethylated N-protected dinucleotides separated as a gummy residue at the ether precipitation stage. The ethereal solution was centrifuged, and the gum was dissolved in anhydrous pyridine and reprecipitated in ether. The isolated yields of the desired products were 70–90%.

General Method for the Synthesis of Protected Trinucleotides Bearing a 5′-Phosphomonoester End Group. A mixture of pyridinium salts of β-cyanoethylated N-protected dinucleotide and the appropriate 3′-O-acetyl N-protected nucleoside 5′-phosphate was converted to *n*-trialkylammonium salt by treating its aqueous pyridine solution with a calculated amount of *n*-trialkylamine, *n*-trihexylamine, for example. The mixture was rendered anhydrous by repeated evaporation of added dry pyridine *in vacuo* until further addition formed a clear solution, and the condensation was carried out by the following general method.

To an anhydrous solution of *n*-trihexylammonium salt of β-cyanoethylated N-protected dinucleotide and 3′-O-acetyl N-protected mononucleoside 5′-phosphate (3–5 equiv as based on dinucleotide component) in dry pyridine was added mesitylenesulfonyl chloride (2.5 equiv as based on mononucleotide) in a drybox. The sealed reaction mixture was shaken for 3–4 hr at room temperature. An excess of water was then added under cooling, and the resulting solution after 1 hr was treated with 2 *N* sodium hydroxide sufficient to give an over-all molarity of 1 *N* for 20 min at 0°. An excess of pyridinium Dowex 50 ion-exchange resin was added to neutralize the alkali. The resin was removed by filtration and washed thoroughly to neutralize the alkali. The resin was removed by filtration and washed thoroughly with 20–50% aqueous pyridine. The total aqueous pyridine solution was applied directly to a DEAE-cellulose (bicarbonate) column and chromatography was carried out at 4° using triethylammonium bicarbonate solution containing 10–25% alcohol for elution.

General Method for Acetylation of the 3′-Hydroxyl End Group of Protected Trinucleotide. An anhydrous pyridine solution of protected trinucleotide was allowed to react with acetic anhydride (~10 equiv as based on trinucleotide component) for 6 hr. An equal volume of water was then added under cooling and the resulting solution was kept for 4 hr at room temperature. The solvent was removed by repeated evaporation of added pyridine *in vacuo*. The syrupy residue obtained finally was dissolved in dry pyridine and added dropwise to anhydrous ether (25–100-fold excess in volume). The precipitate was collected by centrifugation and washed with fresh ether by repeated suspension in the centrifuge tube. The yield of the acetylated product as determined spectrophotometrically was quantitative.

Isolation of Protected Di- and Trinucleotides. Following chromatography on DEAE-cellulose columns (bicarbonate) the appropriate fractions were pooled and evaporated under reduced pressure at temperatures below 10° in the presence of added pyridine. The syrupy residues obtained finally were rendered anhydrous by evaporation of added anhydrous pyridine. The resulting solutions were added dropwise to anhydrous ether (25–100-fold excess in volume). The protected nucleotidic materials separated as fine white precipitates. These were collected by centrifugation and washed with fresh ether by resuspension in the centrifuge tube.

Removal of the Protective Groups. The removal of the anisoyl, benzoyl, and acetyl groups from the amino groups of protected oligonucleotides was effected by treating the pyridine solution of the compound with an excess (two to three times the volume of pyridine) of concentrated ammonium hydroxide for 2–3 days at room temperature.

General Methods for Characterization of Di- and Trinucleotides. The formation of discrete peaks, well resolved from the starting components, and the expected extinction ratio at two wavelengths and its constancy in the column fractions corresponding to the desired product, indicated that the products isolated after each synthetic step were pure. After isolation procedure as described above, all members of di- and trinucleotides were characterized as pure by paper chromatography (a) of fully protected derivative (for example, d-pA^{Bz}pA^{Bz}, d-CE-pA^{Bz}pA^{Bz}, d-pA^{Bz}pA^{Bz}pC^{An}, and d-pA^{Bz}pA^{Bz}pC^{An}-OAc) in solvent B and paper electrophoresis (R_f values are given in Table III) and (b) of the products obtained after removal of the anisoyl, benzoyl, and acetyl groups from the amino groups of the oligonucleotides by ammoniacal treatment (*i.e.*, d-pApA, d-pApApC) (R_f values are given in Table III). Finally, all members of trinucleotides were checked for structure and purity by analysis of the products formed on degradation with spleen phosphodiesterase after removal of the 5′-phosphomonoester groups.[8] The results are given in Table IV. Thus, the degradation of d-TpApC gave, as expected, Tp:Ap:C in the ratio 1:1:1.

Synthesis of Dinucleotides. d-pTpA^{Bz}. An anhydrous pyridine solution (10 ml) of pyridinium d-CE-pT (2.5 mmoles), d-pA^{Bz}-OAc (2.5 mmoles), and DCC (3.2 g) was shaken in the presence of anhydrous pyridinium Dowex 50 ion-exchange resin (1.5 g) for 3 days at room temperature. After the usual work-up, the product was chromatographed on a DEAE-cellulose (carbonate) column (92 × 4.5 cm) at 4°. Elution was carried out using a linear gradient of triethylammonium bicarbonate (0.05–0.2 *M* in 10% ethyl alcohol, 4 l. each of the solution being in the mixing vessel and in the reservoir, respectively), and the elution pattern is shown in Figure 1. Fractions of about 20 ml were collected at a flow rate of 2 ml/min. Peak F contained pure protected dinucleotide (d-pTpA^{Bz}) having the spectral characteristics: λ_{max} 275 mμ; $\epsilon_{280}/\epsilon_{260}$, 1.29. The yield of the desired product was 40,000 OD_{280} units (67%).

d-pA^{Bz}pT. An anhydrous pyridine solution (20 ml) of pyridinium d-CE-pA^{Bz} (4.5 mmoles), d-pT-OAc (4.5 mmoles), and DCC (6.0 g) was shaken in the presence of anhydrous pyridinium Dowex 50 ion-exchange resin (2.0 g) for 3 days at room temperature. After the usual work-up, the product was chromatographed on a DEAE-cellulose (carbonate) column (104 × 5 cm) at 4°. Elution was carried out using a linear gradient of triethylammonium bicarbonate, 0–0.15 *M* in 10% ethyl alcohol, 3 l. each, followed by the next gradient, 0.15–0.25 *M* in 10% ethyl alcohol, 4 l. each, and the elution pattern is shown in Figure 2. Fractions of about 10 ml were collected at a flow rate of 1 ml/min. Peak E had a constant ratio of $\epsilon_{280}/\epsilon_{260}$ (1.15) throughout its fractions and contained pure desired product, d-pA^{Bz}pT. The yield of the pooled fractions excluding the edges was 66,500 OD_{280} units (65%), and the pooled fractions had the spectral characteristic: λ_{max} 275 mμ.

d-pC^{An}pG^{Ac}. An anhydrous pyridine solution (10 ml) of pyridinium d-CE-pC^{An} (2.5 mmoles), d-pG^{Ac}-OAc (2.5 mmoles), and DCC (3.2 g) was shaken in the presence of anhydrous pyridinium Dowex 50 ion-exchange resin (2.0 g) for 3 days at room temperature. After the usual work-up, the product was chromatographed on a

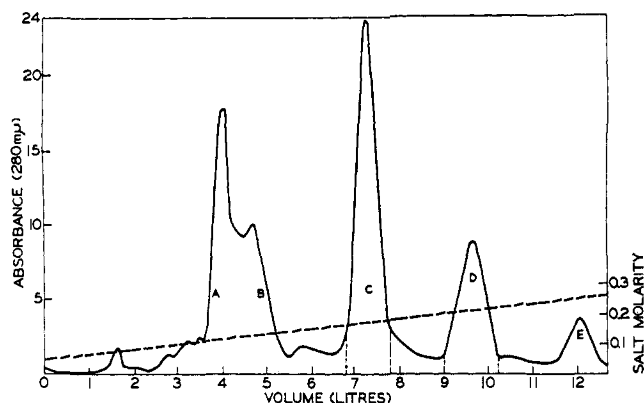


Figure 3. Chromatography of the reaction products in the preparation of dinucleotide d-$pA^{Bz}pA^{Bz}$ and trinucleotide d-$pA^{Bz}pA^{Bz}pA^{Bz}$ on a DEAE-cellulose (carbonate) column. Peak C contains d-$pA^{Bz}pA^{Bz}$ and peak D contains d-$pA^{Bz}pA^{Bz}pA^{Bz}$.

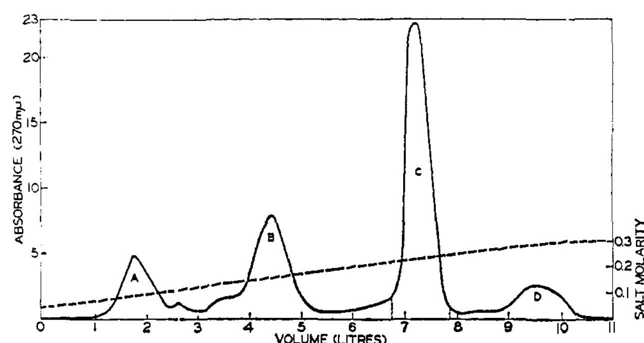


Figure 4. Chromatography of the reaction products in the preparation of dinucleotide d-$pC^{An}pC^{An}$ on a DEAE-cellulose (carbonate) column. Peak C contains d-$pC^{An}pC^{An}$.

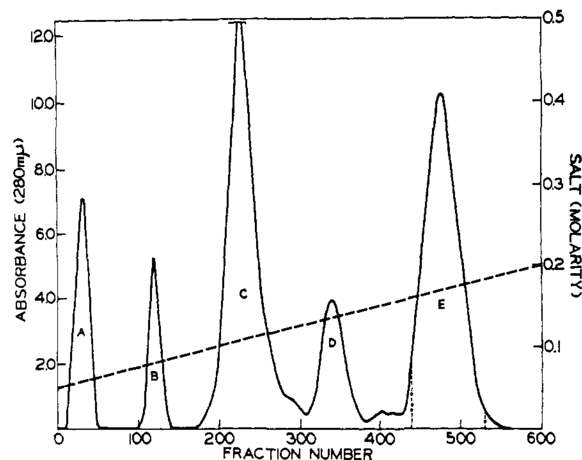


Figure 5. Chromatography of the reaction products in the preparation of trinucleotide d-$pTpA^{Bz}pC^{An}$ on a DEAE-cellulose (carbonate) column. Peak E contains d-$pTpA^{Bz}pC^{An}$.

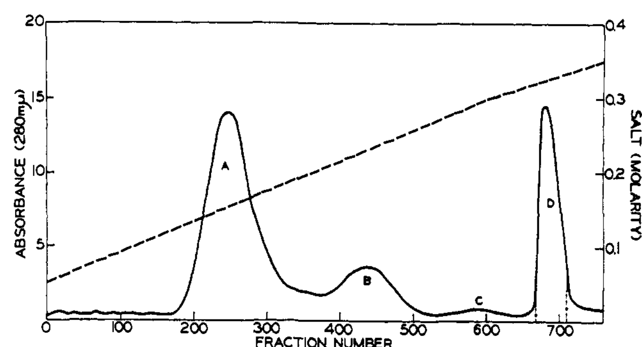


Figure 6. Chromatography of the reaction products in the prepara tion of trinucleotide d-$pTpA^{Bz}pG^{Ac}$ on a DEAE-cellulose (carbon ate) column. Peak D contains d-$pTpA^{Bz}pG^{Ac}$.

DEAE-cellulose (carbonate) column (90 × 4.5 cm) at 4°. Elution was carried out using a linear gradient of triethylammonium bicarbonate (0.05–0.25 *M* in 10% ethyl alcohol, 4 l. each of the solution being in the mixing vessel and in the reservoir, respectively). Fractions of about 20 ml were collected at a flow rate of 2 ml/min. The desired product, d-$pC^{An}pG^{Ac}$, appeared in fractions 330–400 having a constant $\epsilon_{280}/\epsilon_{302}$ (1.30). The yield of the pooled fractions was 56,000 OD_{280} units (85%), and the pooled fractions had the spectral characteristic: λ_{max} 286 mμ.

d-$pA^{Bz}pA^{Bz}$. An anhydrous pyridine solution (10 ml) of pyridinium d-pA^{Bz} (18,000 OD_{280} units), d-pA^{Bz}-OAc (27,000 OD_{280} units), and DCC (4.0 g) was shaken in the presence of anhydrous pyridinium Dowex 50 ion-exchange resin (2.0 g) for 5 days at room temperature. After the usual work-up, the product was chromatographed on a DEAE-cellulose (carbonate) column (92 × 4 cm) at 4°. After an initial wash to remove pyridine, elution was carried out using a linear salt gradient. The elution pattern is shown in Figure 3. Peak C (12,950 OD_{280} units) contained the desired dinucleotide, d-$pA^{Bz}pA^{Bz}$, and peak D (6090 OD_{280} units) contained the trinucleotide, d-$pA^{Bz}pA^{Bz}pA^{Bz}$.

d-$pC^{An}pC^{An}$. An anhydrous pyridine solution (10 ml) of pyridinium d-CE-pC^{An} (1 mmole) and d-pC^{An}-OAc (1.1 mmoles) was treated with mesitylenesulfonyl chloride (0.7 g, 3.2 mmoles) for 3 hr at room temperature. Water (2 ml) was then added under cooling. After 0.5 hr the solution was diluted to about 30 ml with water and treated with 2 *N* sodium hydroxide (30 ml) for 20 min at 0°. Excess pyridinium Dowex 50 ion-exchange resin was added to neutralize the alkali. After filtration and washings with aqueous pyridine, the total solution (500 ml) was applied directly to a DEAE-cellulose (carbonate) column (80 × 4.5 cm) at 4°. After washing off pyridine, elution was carried out using a linear salt gradient; the elution pattern is shown in Figure 4. Peak C contained the desired dinucleotide, d-$pC^{An}pC^{An}$ (21,360 OD_{302} units, 48%).

Synthesis of Trinucleotides. d-$pTpA^{Bz}pC^{An}$. To an anhydrous pyridine solution (5 ml) of tri-*n*-hexylammonium salts of d-CE-$pTpA$-^{Bz} (7000 OD_{280} units, 0.29 mmole) and d-pC^{Bz}-OAc (1.0 mmole) was added mesitylenesulfonyl chloride (500 mg). The sealed reaction mixture was shaken for 4 hr. After usual work-up, the product was chromatographed on a DEAE-cellulose (carbonate) column (45 × 4.5 cm) at 4°. Elution was carried out by using a linear gradient, 0.05–0.2 *M*, 4 l. each, of triethylammonium bicarbonate containing 10% ethyl alcohol, and the elution pattern is shown in Figure 5. Fractions of 15 ml were collected every 15 min. Peak E had a constant ratio of $\epsilon_{280}/\epsilon_{302}$ (1.50) and contained pure d-$pTpA^{Bz}pC^{An}$. The yield of this product was 6000 OD_{280} units (50%), and the product had the spectral characteristic: λ_{max} 280 mμ.

d-$pTpA^{Bz}pG^{Ac}$. To an anhydrous solution of tri-*n*-hexylammonium salts of d-CE-$pTpA^{Bz}$ (5000 OD_{280} units, 0.20 mmole) and d-pG^{Ac}-OAc (1.0 mmole) in pyridine (5 ml) was added mesitylenesulfonyl chloride (500 mg). The sealed reaction mixture which turned dark was kept for 4 hr at room temperature. After the usual work-up, the reaction mixture was chromatographed on a DEAE-cellulose (carbonate) column (45 × 4.5 cm) at 4°. Elution was carried out by using a linear gradient, 0.05–0.3 *M*, 4 l. each, of triethylammonium bicarbonate containing 10% ethyl alcohol, followed by the next gradient, 0.3–0.4 *M*, 2 l. each, of the same salt solution in 10% ethyl alcohol, fractions of about 12 ml being collected at intervals of 15 min. The elution pattern is shown in Figure 6. Peak D had a constant ratio of $\epsilon_{260}/\epsilon_{280}$ (1.07) throughout its fractions and contained pure d-$pTpA^{Bz}pG^{Ac}$. The yield of the pooled fractions, excluding the edges, was 3500 OD_{280} units (50%) and the pooled fractions had the spectral characteristic: λ_{max} 262 mμ.

d-$pA^{Bz}pTpC^{An}$. To an anhydrous solution of tri-*n*-hexylammonium salts of d-CE-$pA^{Bz}pT$ (20,000 OD_{280} units, 0.81 mmole) and d-pC^{An}-OAc (3 mmoles) in dry pyridine (7 ml) was added mesitylenesulfonyl chloride (1.65 g). The sealed reaction mixture was shaken for 4 hr at room temperature. After usual work-up, the reaction mixture was chromatographed on a DEAE-cellulose (carbonate) column (44 × 4.5 cm) at 4°. Elution was carried out by using a linear gradient, 0.05–0.15 *M*, 2 l. each, of triethylammo-

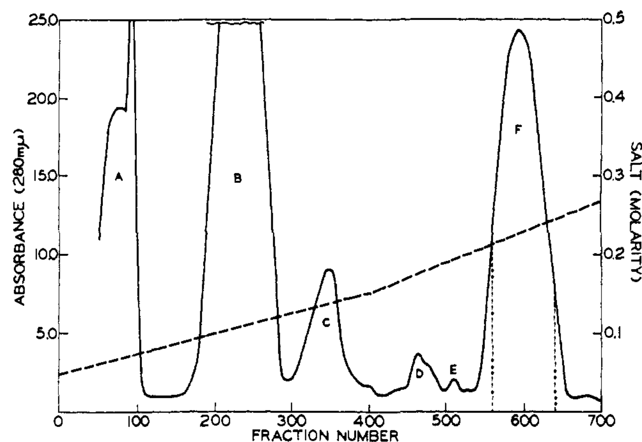


Figure 7. Chromatography of the reaction products in the preparation of trinucleotide d-pA^{Bz}pTpC^{An} on a DEAE-cellulose (carbonate) column. Peak F contains d-pA^{Bz}pTpC^{An}.

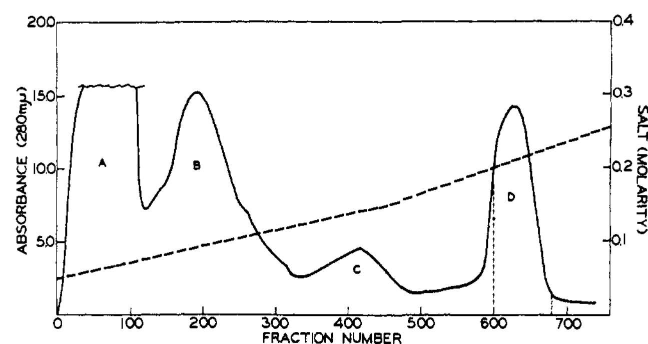


Figure 8. Chromatography of the reaction products in the preparation of trinucleotide d-pA^{Bz}pTpG^{Ac} on a DEAE-cellulose (carbonate) column. Peak D contains d-pA^{Bz}pTpC^{An}.

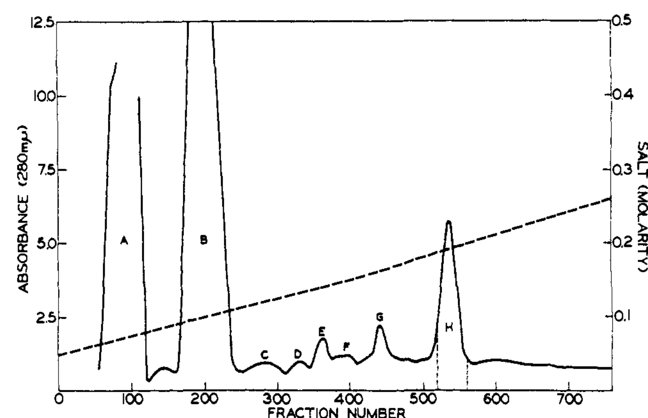


Figure 9. Chromatography of the reaction products in the preparation of trinucleotide d-pC^{An}pG^{Ac}pA^{Bz} on a DEAE-cellulose (carbonate) column. Peak H contains d-pC^{An}pG^{Ac}pA^{Bz}.

nium bicarbonate containing 10% ethyl alcohol, followed by the next gradient, 0.15–0.3 M, 2 l. each, of the same salt solution in 10% alcohol, fractions of about 10 ml being collected at intervals of 15 min. The elution pattern is shown in Figure 7. Peak F had a constant ratio of $\epsilon_{280}/\epsilon_{302}$ (1.45) and contained pure trinucleotide, d-pA^{Bz}-pTpC^{An}. The yield of the desired product was 26,000 OD$_{280}$ units (80%) and the product had the spectral characteristic: λ_{max} 280 mμ.

d-pA^{Bz}pTpG^{Ac}. To an anhydrous solution of tri-n-hexylammonium salts of d-CE-pA^{Bz}pT (10,000 OD$_{280}$ units, 0.4 mmole) and d-pG^{Ac}-OAc (2.0 mmoles) in dry pyridine (7 ml) was added mesitylenesulfonyl chloride (1.0 g). The sealed reaction mixture which turned dark was kept at room temperature for 4 hr. After

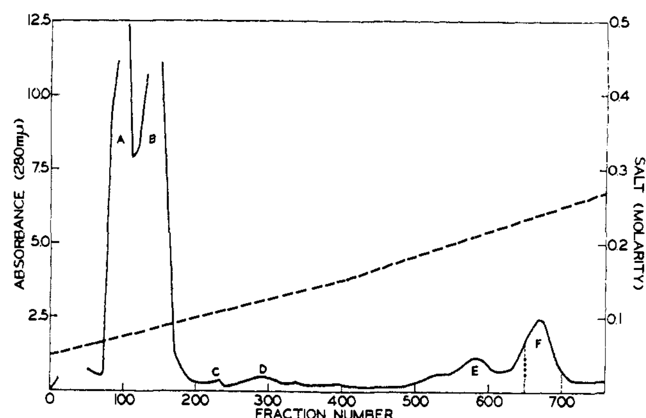


Figure 10. Chromatography of the reaction products in the preparation of trinucleotide d-pC^{An}pG^{Ac}pT on a DEAE-cellulose (carbonate) column. Peak F contains d-pC^{An}pG^{Ac}pT.

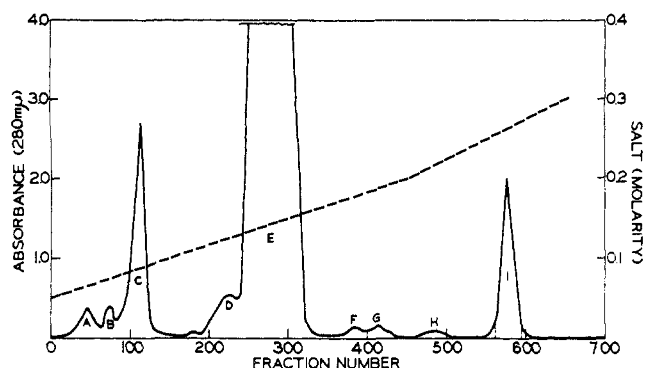


Figure 11. Chromatography of the reaction products in the preparation of trinucleotide d-pA^{Bz}pA^{Bz}pC^{An} on a DEAE-cellulose (carbonate) column. Peak I contains d-pA^{Bz}pA^{Bz}pC^{An}.

the usual work-up the reaction mixture was chromatographed on a DEAE-cellulose (carbonate) column (44 × 4.5 cm) at 4°. Elution was carried out by using a linear gradient, 0.05–0.15 M, 2 l. each, of triethylammonium bicarbonate containing 10% ethyl alcohol, followed by the next gradient, 0.15–0.3 M, 2 l. each, of the same salt solution in 10% ethyl alcohol, fractions of about 9 ml being collected at intervals of 15 min. The elution pattern is shown in Figure 8. Peak D had a constant ratio of $\epsilon_{260}/\epsilon_{280}$ (1.07) throughout its fraction and contained pure d-pA^{Bz}pTpG^{Ac}. The yield of the desired trinucleotide was 12,000 OD$_{280}$ units (80%), and the trinucleotide had the spectral characteristic: λ_{max} 262 mμ.

d-pC^{An}pG^{Ac}pA^{Bz}. To an anhydrous solution of tri-n-hexylammonium salt of d-CE-pC^{An}pG^{Ac} (3500 OD$_{280}$ units, 0.12 mmole) and d-pA^{Bz}-OAc (0.5 mmole) in dry pyridine (2.5 ml) was added mesitylenesulfonyl chloride (400 mg). The sealed reaction mixture was allowed to stand for 4 hr at room temperature. After the usual work-up, the reaction mixture was chromatographed on a DEAE-cellulose (carbonate) column (44 × 4.5 cm) at 4°. Elution was carried out by using a linear gradient, 0.05–0.15 M, 2 l. each, of triethylammonium bicarbonate containing 10% ethyl alcohol, followed by the next gradient, 0.15–0.3 M, 2 l. each, of the same salt solution in 10% ethyl alcohol, fractions of 10 ml being collected at intervals of 15 min. The elution pattern is shown in Figure 9. Peak H had a constant ratio of $\epsilon_{280}/_{302}$ (1.60) throughout its fractions and contained pure d-pC^{An}pG^{Ac}pA^{Bz}. The yield of the desired trinucleotide was 2800 OD$_{280}$ units (50%), and the trinucleotide had the spectral characteristic: λ_{max} 286 mμ.

d-pC^{An}pG^{Ac}pT. To an anhydrous solution of tri-n-hexylammonium salts of d-CE-pC^{An}pG^{Ac}(2000 OD$_{280}$ units, 0.071 mmole) and d-pT-OAc (0.5 mmole) in dry pyridine (2.5 ml) was added mesitylenesulfonyl chloride (400 mg). The sealed reaction mixture was allowed to stand for 4 hr at room temperature. After the usual work-up, the reaction mixture was chromatographed on a DEAE-cellulose (carbonate) column (44 × 4.5 cm) at 4°. Elution was carried out by using a linear gradient, 0.05–0.15 M, 2 l. each, of triethylammonium bicarbonate containing 10% ethyl alcohol,

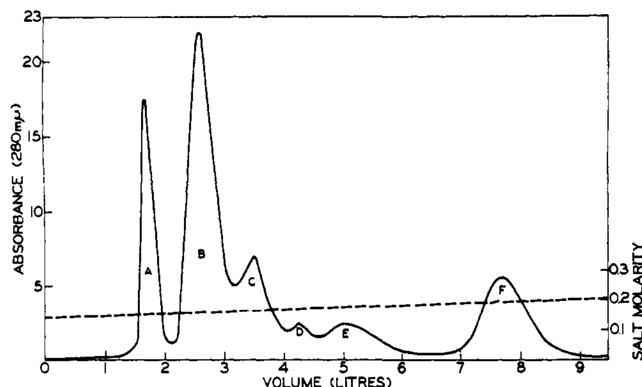


Figure 12. Chromatography of the reaction products in the preparation of trinucleotide d-pA^{Bz}pA^{Bz}pG^{Ac} on a DEAE-cellulose (carbonate) column. Peak F contains d-pA^{Bz}pA^{Bz}pG^{Ac}.

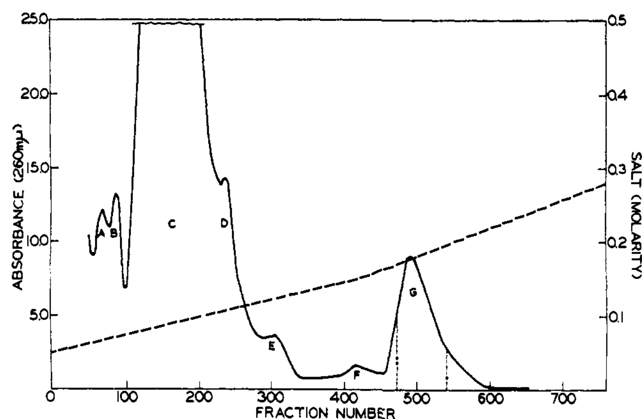


Figure 13. Chromatography of the reaction products in the preparation of trinucleotide d-pTpTpG^{Ac} on a DEAE-cellulose (carbonate) column. Peak G contains d-pTpTpG^{Ac}.

followed by the next gradient, 0.15–0.3 M, 2 l. each, of the same salt solution in 10% ethyl alcohol, fractions of 10 ml being collected at intervals of 15 min. The elution pattern is shown in Figure 10. Peak F had a constant ratio of $\epsilon_{280}/\epsilon_{302}$ (1.54) throughout its fractions and contained the desired trinucleotide, d-pC^{An}pG^{Ac}pT. The yield of the pooled fraction of peak F was 1250 OD_{280} units (50%), and the peak had the spectral characteristic: λ_{max} 277 mμ.

d-pA^{Bz}pA^{Bz}pC^{An}. To an anhydrous solution of tri-n-hexylammonium salts of d-CE-pA^{Bz}pA^{Bz} (1200 OD_{280} units, 0.033 mmole) and d-pC^{An}-OAc (0.5 mmole) in dry pyridine (4 ml) was added mesitylenesulfonyl chloride (210 mg). The sealed reaction mixture was shaken for 3 hr. After the usual work-up, the reaction mixture was chromatographed on a DEAE-cellulose (carbonate) column (72 × 2.2 cm) at 4°. Elution was carried out by using a linear gradient, 0.05–0.2 M, 2 l. each, of triethylammonium bicarbonate containing 10% ethyl alcohol, followed by the next gradient, 0.2–0.3 M, of the same salt solution in 20% ethyl alcohol, and fractions of 9 ml at 15-min intervals were collected. The elution pattern is shown in Figure 11. Peak I had a constant ratio of $\epsilon_{302}/\epsilon_{280}$ (0.68) throughout the fractions and contained the expected trinucleotide, d-pA^{Bz}pA^{Bz}pC^{An}. The yield was 1000 OD_{260} units (55%) and the trinucleotide had the spectral characteristic: λ_{max} 280 mμ.

d-pA^{Bz}pA^{Bz}pG^{Ac}. To an anhydrous solution of tri-n-hexylammonium salts of d-CE-pA^{Bz}pA^{Bz} (10,000 OD_{280} units, 0.27 mmole) and d-pG^{Ac}-OAc (2.0 mmoles) in dry pyridine (5 ml) was added mesitylenesulfonyl chloride (1.0 g), and the sealed reaction mixture was kept for 3 hr at room temperature. After the usual work-up, the reaction mixture was chromatographed on a DEAE-cellulose (carbonate) column (90 × 4 cm) at 4°. Elution was carried out by using a linear gradient, 0.15–0.25 M, of the triethylammonium bicarbonate containing 25% ethyl alcohol; the elution pattern is shown in Figure 12. Peak F contained the expected trinucleotide, d-pA^{Bz}pA^{Bz}pG^{Ac}. It had a constant ratio of $\epsilon_{280}/\epsilon_{260}$ (1.2) throughout the fractions. The yield of the desired product was 6395 OD_{280} units (49%). There was a trace impurity traveling slower on paper chromatograms, due to the partial deacylation of N-acetyldeoxyguanosine during column chromatography. On the removal of the protecting groups by treatment with concentrated ammonia (as described in General Methods) the product gave d-pApApG, which was homogeneous in solvents C and D.

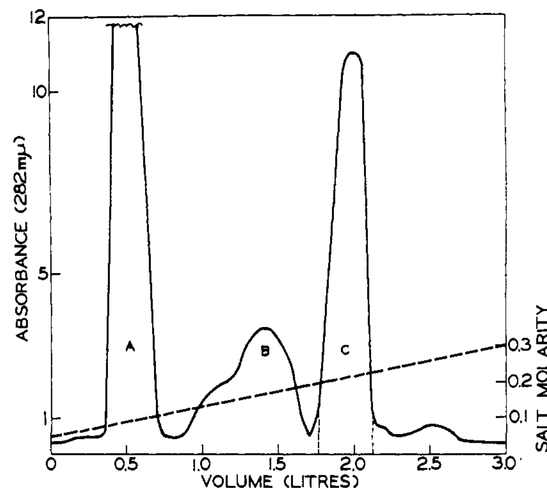


Figure 14. Chromatography of the reaction products in the preparation of trinucleotide d-pC^{An}pC^{An}pA^{Bz} on a DEAE-cellulose (carbonate) column. Peak C contains d-pC^{An}pC^{An}pA^{Bz}.

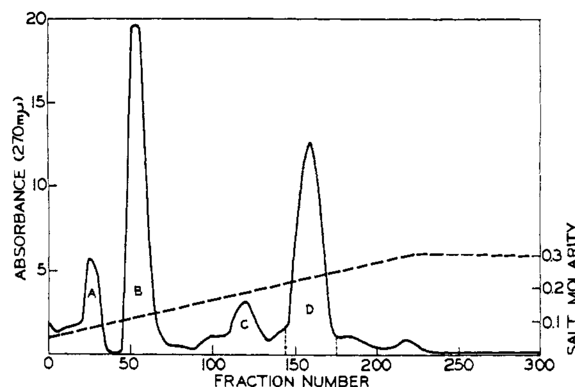


Figure 15. Chromatography of the reaction products in the preparation of trinucleotide d-pC^{An}pC^{An}pT on a DEAE-cellulose (carbonate) column. Peak D contains d-pC^{An}pC^{An}pT.

d-pTpTpG^{Ac}. To an anhydrous solution of tri-n-hexylammonium salts of d-CE-pTpT (10,000 OD_{267} units, 0.54 mmole) and d-pG^{Ac}-OAc (1.5 mmoles) was added mesitylenesulfonyl chloride (840 mg), and the sealed reaction mixture was kept for 4 hr at room temperature. After the usual work-up, the reaction product was chromatographed on a DEAE-cellulose (carbonate) column (44 × 4.5 cm) at 4°. Elution was carried out by using a linear gradient, 0.05–0.15 M, 2 l. each, of triethylammonium bicarbonate containing 10% ethyl alcohol, followed by the next gradient, 0.15–0.3 M, 2 l. each, of the same salt solution in 10% ethyl alcohol, and 9-ml fractions were collected at 15-min intervals. The elution pattern is shown in Figure 13. Fractions 475–540 of peak G had a constant ratio of $\epsilon_{270}/\epsilon_{260}$ (0.91) and contained the expected trinucleotide, d-pTpTpG^{Ac}, of spectral characteristic: λ_{max} 262 mμ. The yield of the pure protected trinucleotide was 10,000 OD_{260} units (55%).

Fractions 541–580 contained a mixture of d-pTpTpG^{Ac} and d-pTpTpG, as judged by paper chromatography in solvent B.

d-pC^{An}pC^{An}pA^{Bz}. To an anhydrous pyridine solution (2 ml) of tri-n-hexylammonium salts of d-CE-pC^{An}pC^{An} (3000 OD_{302} units, 0.065 mmole) and d-pA^{Bz}-OAc (0.5 mmole) was added mesitylenesulfonyl chloride (0.3 g), and the reaction mixture was kept at room temperature for 4 hr. After the usual work-up, the reaction mixture was chromatographed on a DEAE-cellulose (carbonate) column (90 × 2 cm) at 4°. Elution was carried out by using a linear gradient, 0.05–0.3 M, 1.5 l. each, of the triethylammonium bicarbonate containing 25% ethyl alcohol. The elution pattern is shown in Figure 14. Peak C had a constant ratio of $\epsilon_{282}/\epsilon_{302}$ (1.1) and contained the

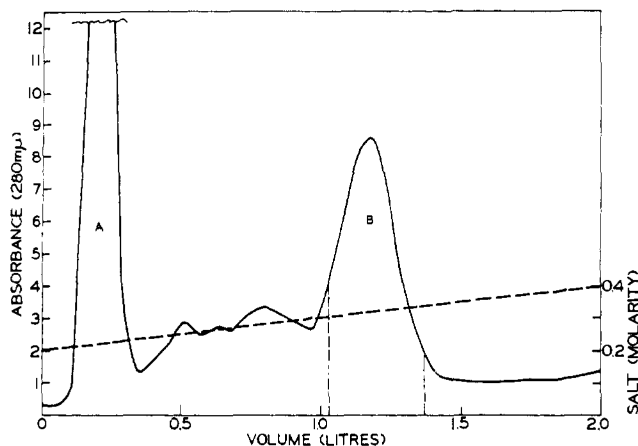


Figure 16. Chromatography of the reaction products in the preparation of trinucleotide d-pG^{Bz}pG^{Bz}pT on a DEAE-cellulose (carbonate) column. Peak B contains C-pG^{Bz}pG^{Bz}pT.

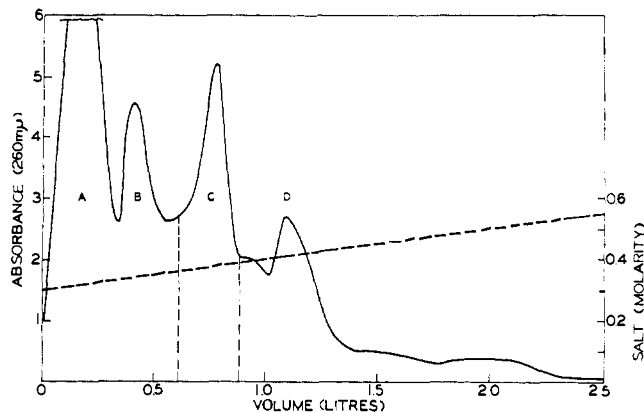


Figure 17. Chromatography of the reaction products in the preparation of d-pG^{Bz}pG^{Bz}pA^{Bz} on a DEAE-cellulose (carbonate) column. Peak C contains d-pG^{Bz}pG^{Bz}pA^{Bz}.

desired trinucleotide, d-pC^{An}pC^{An}pA^{Bz}. The yield of the expected product was 2100 OD$_{280}$ units (57%).

d-pC^{An}pC^{An}pT. To an anhydrous pyridine solution (2 ml) of tri-*n*-hexylammonium salts of d-CE-pC^{An}pC^{An} (6000 OD$_{302}$ units, 0.13 mmole) and d-pT-OAc (0.5 mmole) was added mesitylenesulfonyl chloride (220 mg), and the reaction mixture was kept at room temperature for 4 hr. After the usual work-up, the reaction mixture was chromatographed on a DEAE-cellulose (carbonate) column (90 × 2 cm) at 4°. Elution was carried out by using a linear gradient, 0.05–0.3 *M*, 1.5 l. each, of the triethylammonium bicarbonate containing 25% ethyl alcohol. The elution pattern is shown in Figure 15. Peak D had a constant ratio of $\epsilon_{302}/\epsilon_{270}$ (1.17) and contained the desired trinucleotide, d-pC^{An}pC^{An}pT. The yield of the expected product was 4014 OD$_{302}$ units (66%).

d-pG^{Bz}pG^{Bz}pT. To an anhydrous pyridine solution (2 ml) of tri-*n*-hexylammonium salts of d-CE-pG^{Bz}pG^{Bz}[9] (3000 OD$_{260}$ units) and pT-OAc (0.5 mmole) was added mesitylenesulfonyl chloride (1 mmole), and the reaction mixture was kept at room temperature for 4 hr. After the usual work-up, the reaction mixture was chromatographed on a DEAE-cellulose (carbonate) column (2 × 65 cms) at 4°. Elution was carried out using a linear gradient of triethylammonium bicarbonate containing 25% ethyl alcohol. The elution pattern is shown in Figure 16. Peak B had a ratio of $\epsilon_{260}/\epsilon_{290}$ of 1.57–1.6 at pH 8.5 and contained the desired trinucleotide, d-pG^{Bz}pG^{Bz}pT. The yield of the expected product was 1312 OD$_{260}$ units (35%).

d-pG^{Bz}pG^{Bz}pA^{Bz}. To an anhydrous pyridine solution (2 ml) of tri-*n*-hexylammonium salts of d-CE-pG^{Bz}pG^{Bz} (3000 OD$_{260}$ units) and d-pA^{Bz}-OAc (0.5 mmole) was added mesitylenesulfonyl chloride (1 mmole), and the reaction mixture was kept at room temperature for 4 hr. After the usual work-up, the reaction mixture was chromatographed on a DEAE-cellulose (carbonate) column (2 × 65 cm) at 4°. Elution was carried out using a linear gradient of triethylammonium bicarbonate containing 25% ethyl alcohol. The elution pattern is shown in Figure 17. Peak C had a ratio of $\epsilon_{260}/\epsilon_{290}$ of 0.94 at pH 8.5 and contained the desired trinucleotide, pG^{Bz}pG^{Bz}pA^{Bz}. The yield of the expected product was 2200 OD$_{260}$ units (55%).

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

(9) d-CE-pG^{Bz}pG^{Bz} was prepared from d-pG^{Bz}pG^{Bz} by the standard procedure using hydracrylonitrile and DCC. d-pG^{Bz}pG^{Bz} was prepared by the general method, using CE-pG^{Bz} and pG^{Bz}-OAc (unpublished work of Drs. Hans Weber and S. A. Narang in this laboratory).