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Synthesis of Oligodeoxyribonucleotide Ethyl Phosphotriesters and Their Specific Complex Formation with Transfer Ribonucleic Acid†

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ABSTRACT: Oligodeoxyribonucleotide ethyl phosphotriesters, d-Tp(Et)Gp(Et)G and d-Tp(Et)Tp(Et)Cp(Et)A, were synthesized by a stepwise, chemical procedure. These triesters are complementary, respectively, to 3′-CpCpA-OH (the 3′-amino acid accepting terminus) and to -UpGpApA- (the anticodon region) of phenylalanine tRNA from yeast and *Escherichia coli.* Tritium-labeled triesters were prepared by exchange of the H-8 protons of adenine and guanine in the oligomers in tritiated water. The association constants for binding of the triesters to their complementary regions on tRNA were measured by equilibrium dialysis and were compared with those of oligodeoxyribonucleotides and oligoribonucleotides of the same sequences. In 1 M NaCl–10 mM $MgCl_2$ at 0°, the association constants of the oligomers with both $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{coli}$ are very similar. The association constants of the ribooligonucleotides are 8 to 20 times larger than those of the corresponding deoxyribooligonucleotides, while the deoxyribooligonucleotide triesters exhibit binding constants slightly higher than those of the deoxyribooligonucleotides. These differences are discussed in terms of the differences in conformations of the various oligomers. At low salt concentration (0.1 M NaCl, 1 mM EDTA), the oligonucleotide triesters have the same binding constants as at high salt concentration, whereas the corresponding deoxyribo- and ribooligonucleotides show a four- to sixfold decrease in their binding constants. This reflects the removal of the charge repulsion between the neutral triesters and the tRNA. The binding of oligomers to modified $tRNA^{Phe}_{yeast}$ was also examined. Removal of the Y base decreased the binding of anticodon-complementary oligomers sixfold while removal of the 3′-CpA residues decreased the binding of the 3′-CpCpA-OH complementary oligomers 6- to 20-fold. This study provides the chemical and physicochemical basis for the investigation of the biochemical effects of these triesters on the aminoacylation of tRNA which is reported in the following paper (Barrett, J. C., Miller, P. S., and Ts'o, P. O. P. (1974), *Biochemistry 13,* 4897).

Oligodeoxyribonucleotide alkyl phosphotriesters are oligodeoxyribonucleotide analogs containing an alkylated 3′-5′ internucleotide phosphate linkage. In the preceding papers of this series (Miller *et al.,* 1971; DeBoer *et al.,* 1973; Kan *et al.,* 1973) we have shown that these oligodeoxyribonucleotide analogs have the following novel characteristics. (1) The triesters are uncharged at neutral pH. (2) Triesters form base-paired complexes with complementary polynucleotides. The complexes have a higher stability than similar complexes formed by the parent diester, presumably due to the removal of charge repulsion between the phosphate of polymer and the alkyl phosphotriester of the oligomer. (3) Trityl-containing derivatives of these compounds are very soluble in organic solvents, a feature which allowed us to investigate hydrogen-bonded, base-pairing interactions of these compounds in chloroform. (4) The methyl or ethyl groups of the dimeric phosphotriesters serve as reporters in proton magnetic resonance (pmr) studies on the conformation of the dimer, especially at the backbone region.

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(5) The solution conformations of dinucleoside alkyl phosphotriesters are similar to those of the parent diesters, although base-stacking interactions are slightly reduced. (6) The dinucleoside alkyl phosphotriesters are chemically stable in neutral solution at room temperature and are completely resistant to hydrolysis by exonuclease enzymes.

In this paper we describe the synthesis of d-Tp(Et)Gp(Et)G[1] and d-Tp(Et)Tp(Et)Cp(Et)A[1] and their tritium-labeled derivatives. The base sequences of these triesters are complementary to the 3′-CpCpA-OH, amino acid accepting terminus of most tRNAs, and the -UpGpApA-, anticodon region of phenylalanine transfer RNA from yeast and *Escherichia coli*. These triesters were found to form hydrogen-bonded complexes with their complementary region of these tRNAs at both high and low salt concentrations. The association constants of these interactions were determined by equilibrium dialysis experiments. The results of these binding studies are compared with the results of binding studies using the comparable oligoribo- and oligodeoxyribonucleotide diesters. In the following paper the effect of triester-tRNA complex formation on the aminoacylation of tRNA is described in detail (Barrett *et al.*, 1974).

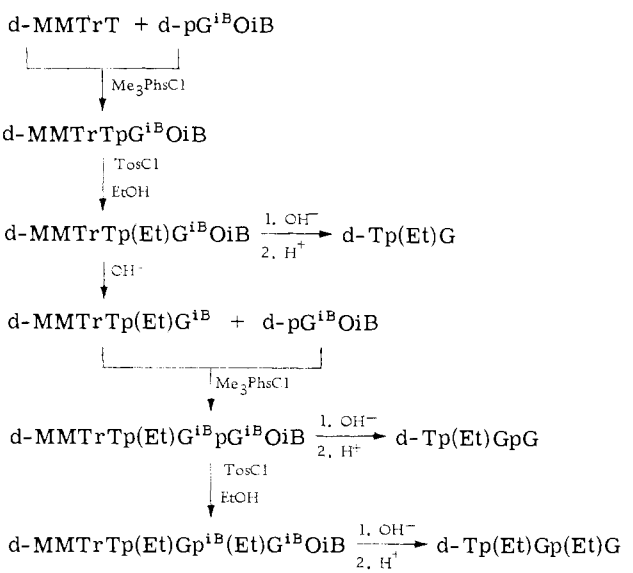
Results and Discussion

Syntheses and Stability of Oligonucleotide Ethyl Triesters. Oligonucleotide alkyl and aryl phosphotriester derivatives have been synthesized by a number of workers for use as intermediates in the syntheses of oligodeoxyribo- and oligoribonucleotides (Letsinger *et al.*, 1969; Eckstein and Rizk, 1967; Reese and Saffhill, 1968; Grams and Letsinger, 1970; van Boom *et al.*, 1971; Catlin and Cramer, 1973; Itakura *et al.*, 1973). In all cases an appropriately protected alkyl or aryl nucleoside phosphodiester was condensed with a suitably protected nucleoside or nucleoside 3′-phosphotriester to yield the triester.

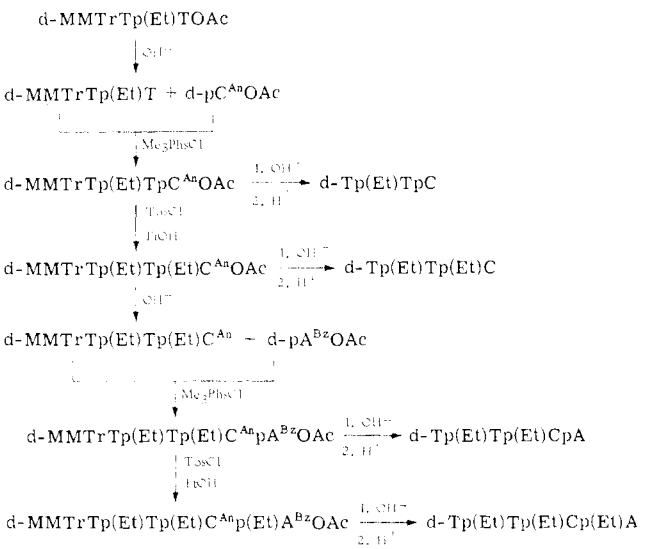
In our work we have explored an approach in which the triester is formed from a suitably protected oligonucleotide phosphodiester intermediate by direct alkylation of the phosphate group (Miller *et al.*, 1971). The alkylating agent in this reaction is ethanol together with *N,N*-dimethylformamide, 2,6-lutidine, and *p*-toluenesulfonyl chloride. The tosyl chloride or its complex formed by reaction with *N,N*-dimethylformamide, $(CH_3)_2N^+{=}CHOSO_2C_6H_4CH_3$-*p* ($Cl^-$) (Hall, 1956), activates the phosphate group for attack by the alcohol. Cramer and Winter (1961) reported similar formation of phosphotriesters from dinucleoside phosphomonochloridates in dimethylformamide.

Previously Holý and Scheit (1967) have reported alkylation of the phosphate group of d-TpT using diazomethane. An 8% yield of phosphotriester was obtained, but all the thymine bases were methylated. Successful alkylation of d-TpT using diazomethane has been reported by Scheit (1967), the phosphotriester being obtained in 80% yield. More recently Nagyvary and coworkers (1973) synthesized oligothymidylate methyl triesters by the reaction of oligothymidylate with methyl methanesulfonate as the alkylating agent. The reaction resulted in an 80% phosphotriester formation, accompanied by some methylation of the thymine base.

Scheme I: Synthesis of d-Tp(Et)Gp(Et)G



Scheme II: Synthesis of d-Tp(Et)Tp(Et)Cp(Et)A



The synthetic routes for the preparation of d-Tp(Et)Gp(Et)G and d-Tp(Et)Tp(Et)Cp(Et)A are shown in Schemes I and II. The basic scheme involves stepwise addition of protected nucleoside 5′-phosphates to a growing oligonucleotide chain followed by ethylation of the resulting phosphodiester linkage, to give oligonucleotide triesters of a defined sequence. Thus, in the case of d-Tp(Et)Gp(Et)G (Scheme I), the protected dinucleotide, d-MMTrTpG^{iB}OiB, was synthesized by condensation of the appropriately protected monomers and the product was isolated and purified by extraction procedures similar to those reported by Caruthers *et al.* (1972). The diester was then ethylated. In this particular reaction an equivalent of *N*-methylimidazole was added to the ethylation media. *N*-Methylimidazole has been shown to increase the amount of phosphotriester formation in the synthesis of oligothymidylate ethyl phosphotriesters (R. Pless, personal communication). However, a fluorescent side product which appeared to result from alkylation of the guanine ring was detected in this reaction. Therefore, addition of *N*-methylimidazole was avoided in further ethylation reactions involving gua-

[1] The system of abbreviations used is the same as that described by Schaller and Khorana (1963) and by van de Sande and Bilsker (1973). Np(Et)N indicates ethylation of the 3′-5′ internucleotide phosphate linkage. Abbreviations used are: TosCl, *p*-toluenesulfonyl chloride; Me_3PhsCl, trimethylbenzenesulfonyl chloride.

TABLE I: Spectral Characteristics of Oligonucleotides and Oligonucleotide Triesters in Water, pH 7.

	λ_{max} (nm)	λ_{min} (nm)	$\epsilon_{255}/\epsilon_{270}$	ϵ (λ_{max})
d-TpG[a]	255, 270 (s)	229	1.18	
d-TpGpG[b]	255, 270 (s)	230	1.24	32.5×10^3
d-Tp(Et)G	255, 270 (s)	228	1.17	
d-Tp(Et)GpG	255, 270 (s)	227	1.30	
d-Tp(Et)Gp(Et)G	255, 270 (s)	227	1.24	33.3×10^3
d-GpGpT[c]	255, 270 (s)	232	1.25	31.7×10^3
			$\epsilon_{260}/\epsilon_{280}$	
d-Tp(Et)T	267	236	1.53	
d-Tp(Et)TpC	267	237		
d-Tp(Et)Tp(Et)C	267	237	1.33	
d-Tp(Et)Tp(Et)CpA	264	234		
d-Tp(Et)Tp(Et)Cp(Et)A	263	234	1.77	35.8×10^3
d-TpTpCpA	263	235	1.80	34.2×10^3

[a] The spectral properties of this compound are similar to those of d-pTpG (Büchi and Khorana, 1972). [b] Similar spectral properties were observed by Caruthers *et al.* (1972). [c] The spectral properties of this compound are similar to those of d-TpGpG. Further characterization is given in the Experimental Section.

TABLE II: Paper Chromatography and Paper Electrophoresis of Oligonucleotides.

	$R_F{}^A$	$R_F{}^C$	$R_F{}^F$	$R_F{}^I$	R_m[a]
d-TpG[b]	0.24	0.42	0.51		0.39 (dpG)
d-Tp(Et)G	0.51	0.70	0.67	0.65	−0.21 (dpT)
d-Tp(Et)GpG	0.061	0.36	0.47		0.29 (dpG)
d-TpGpG[b]	0.037	0.22	0.32		0.70 (dpG)
d-Tp(Et)Gp(Et)G	0.29	0.63	0.60	0.45	−0.17 (dpT)
d-GpGpT[c]	0.033	0.18	0.34		0.66 (dpT)
d-Tp(Et)T				0.78	0.00 (dpT)
d-Tp(Et)TpC	0.23	0.45	0.56	0.39	0.45 (dpT)
d-Tp(Et)Tp(Et)C	0.34	0.67	0.66	0.43	0.10 (dpT)
d-Tp(Et)Tp(Et)CpA	0.16	0.40	0.56	0.27	0.23 (dpT)
d-Tp(Et)Tp(Et)Cp(Et)A	0.26	0.54	0.63	0.31	0.07 (dpT)
d-TpTpCpA[d]			0.37	0.07	0.57 (dpA)

[a] Mobility relative to the indicated nucleoside 5′-phosphate at pH 8.5. The negative sign indicates that the compound moved toward the cathode, probably due to solvent movement. [b] Similar chromatographic mobilities were observed by Ohtsuka *et al.* (1972) using solvent systems A and F, and by Caruthers *et al.* (1972) using solvent system F. [c] The chromatographic and electrophoretic mobilities of this compound are similar to those of d-TpGpG. Further characterization is given in the Experimental Section. [d] The chromatographic mobility of this compound was similar to that of d-TpTpApC (Ohtsuka and Khorana, 1967). Further characterization is given in the text.

nine-containing oligomers.

The 3′-*O*-isobutyryl protecting group was selectively removed by treatment with aqueous sodium hydroxide solution. The oligonucleotide chain was then extended by addition of d-pG^{iB}OiB. The resulting diester–triester intermediate, d-MMTrTp(Et)G^{iB}pG^{iB}OiB, was readily purified by silica gel column chromatography. Ethylation then gave the fully protected phosphotriester which was also purified by silica gel column chromatography. The protecting groups were removed by hydrolysis in concentrated ammonium hydroxide solution followed by treatment with 80% acetic acid. The resulting d-Tp(Et)Gp(Et)G was purified by preparative paper chromatography. An analogous stepwise synthetic procedure was followed in the preparation of d-Tp(Et)Tp(Et)Cp(Et)A (Scheme II). The resulting oligonucleotide triesters are obtained as a mixture of diasteroisomers (Miller *et al.*, 1971). No attempt was made to separate the diastereomers.

During the syntheses, protecting groups were removed from samples of the various protected triester intermediates and the products were further characterized. For example, d-MMTrTp(Et)G^{iB}OiB gave d-Tp(Et)G. This triester has ultraviolet (uv) spectral characteristics identical with those of d-TpG (Table I) and behaves as a neutral molecule on paper chromatography and paper electrophoresis (Table II). In addition, d-Tp(Et)G is resistant to hydrolysis by both spleen and snake venom phosphodiesterase enzymes. Removal of protecting groups from the triester–diester intermediates, d-MMTrTp(Et)G^{iB}pG^{iB}OiB and d-MMTrTp(Et)Tp(Et)C^{An}pA^{Bz}OAc, gave d-Tp(Et)GpG and d-Tp(Et)Tp(Et)CpA, respectively. These oligomers have uv spectral characteristics similar to those of the diesters d-TpGpG and d-TpTpCpA (Table I). Their paper electrophoretic mobilities indicated that these oligomers con-

TABLE III: Tritium-Labeled Oligodeoxyribonucleotides.

	cpm/A_{max} Unit	Sp Act. (Ci/mol)[a]	% Exchange
[^{3}H]d-Tp(Et)Gp(Et)G	1.14×10^6	45	52
[^{3}H]d-TpGpG	1.07×10^6	41	49
[^{3}H]d-Tp(Et)Tp(Et)Cp(Et)A	2.67×10^5	11	26
[^{3}H]d-TpTpCpA	1.86×10^5	7.6	18

[a] Counting efficiency, 38%.

tain only one charge (Table II). Treatment of d-Tp(Et)GpG with snake venom phosphodiesterase, an exonuclease which requires a free 3′-hydroxyl group and proceeds in a 3′ to 5′ direction, gave d-Tp(Et)G and d-pG as products. Similar treatment of d-Tp(Et)Tp(Et)CpA gave d-Tp(Et)Tp(Et)C and d-pA. Both oligomers were resistant to hydrolysis by spleen phosphodiesterase, an exonuclease which requires a free 5′-hydroxyl group and proceeds in a 5′ to 3′ direction.

In general the oligonucleotide ethyl phosphotriesters are stable to hydrolysis in neutral, aqueous solutions and have been stored for periods of months under these conditions. The ethyl phosphotriester linkage is stable in 0.1 M hydrochloric acid and in 0.1 M sodium hydroxide solutions for at least 24 hr at 28°. In addition, this triester linkage was found to be stable to hydrolysis in concentrated ammonium hydroxide solutions for at least 2 days and in 1 M sodium hydroxide for 10 min at room temperature. These various pH conditions are often encountered in deprotecting oligonucleotides during synthetic reactions.

While the triester linkage is resistant to snake venom and spleen phosphodiesterase, Dudman and Zerner (1973) recently reported a phosphotriesterase activity in mammalian sera. We found that oligonucleotide phosphotriesters are completely stable to hydrolysis when incubated in 10% fetal calf serum or 10% human cord serum at 37° for up to 139 hr. Under the same conditions the corresponding oligonucleotide diesters are hydrolyzed to their component monomers. The stability of the phosphotriesters under these conditions allows them to be used in biological studies on mammalian cells in culture.

Interaction of Oligonucleotide Triesters with tRNA. The interaction of the oligonucleotide triesters with $tRNA^{Phe}$ from yeast and *E. coli* was studied by equilibrium dialysis techniques. These studies required that the triesters contain a tritium label of relatively high specific activity. The triesters, d-Tp(Et)Gp(Et)G and d-Tp(Et)Tp(Et)Cp(Et)A, and the corresponding diesters were labeled by heating the oligomers in tritiated water at 90° to exchange the H-8 proton in the purine ring (Ts'o *et al.*, 1969). After removal of excess tritiated water by lyophilization, the labeled oligomers were purified by paper chromatography. As shown in Table III, this procedure gave oligomers of sufficiently high specific activity for use in the dialysis experiments.

The results of experiments measuring the interaction of the oligodeoxyribonucleotide triesters and their parent diesters with $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{coli}$ are presented in Table IV. These studies were carried out at 0° in a buffer containing 10 mM $MgCl_2$, 10 mM Tris (pH 7.5), and either 1 M NaCl or 0.1 M NaCl. The latter condition approximates the ionic strength conditions used in the *in vitro* aminoacylation of tRNA. The tRNA concentration was 40 μM. The association constants were determined directly by measurement of the ratio of tritium counts found on each side of the dialysis membrane as described by Uhlenbeck (1972). In the case of d-GpGpT, which did not contain a radioactive label, the binding constants to $tRNA^{Phe}$ were determined by competition with GpGpU (Uhlenbeck, 1972). Competition studies with GpGpU were also carried out for the oligomers d-Tp(Et)Gp(Et)G and d-TpGpG. The same results were obtained as those obtained by direct measurement.

For purposes of comparison, the ribooligonucleotides UpUpC, UpUpCpA, GpGpU, and UpGpG were also synthesized and their association constants to $tRNA^{Phe}_{yeast}$ were measured. Similar measurements on the binding of these oligoribonucleotides to $tRNA^{Phe}_{yeast}$ have been made independently by Pongs *et al.* (1973) and by Cameron and Uhlenbeck (1973). Their studies were carried out under essentially the same conditions of salt concentration, temperature, and tRNA concentration as the present investigation.

TABLE IV: Binding of Oligomers to $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{coli}$ at 0°.

	K (M^{-1})		
	$tRNA^{Phe}_{yeast}$; 1 M NaCl[a]	$tRNA^{Phe}_{coli}$; 1 M NaCl[a]	$tRNA^{Phe}_{coli}$; 0.1 M NaCl[a]
d-Tp(Et)Tp(Et)Cp(Et)A	3,000	3,600	2,400
d-TpTpCpA	2,200	2,400	1,300
d-Tp(Et)Gp(Et)G	2,400	1,700	2,000
d-TpGpG	600	1,100	500
d-GpGpT	12,900[b]	12,000[b]	11,200[b]
UpUpC	1,600 (2,000)[c]	2,000	2,300
UpUpCpA	48,000 (75,000)[c] (13,700)[d]	48,500	45,000
UpGpG	4,800 (4,500)[c]	7,300	4,900
GpGpU	224,000 (30,000)[c] (117,000)[d]	219,000	204,000

[a] The buffer also contained 10 mM $MgCl_2$–10 mM Tris (pH 7.5). [b] Measured by competition with GpGpU. [c] Pongs *et al.*, 1973. [d] Cameron and Uhlenbeck, 1973.

While the results are basically similar to each other there are some discrepancies in the association constants measured for UpUpCpA and GpGpU in the three studies. The different values obtained may reflect differences in the state of purity or the conformational state of the tRNAs used in these three studies.

The results in Table IV show that the oligodeoxyribonucleotide phosphotriesters form complexes with both $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{coli}$. Indeed the association constants observed for d-Tp(Et)Gp(Et)G and d-Tp(Et)Tp(Et)Cp(Et)A are slightly higher than those of the corresponding parent diesters, d-TpGpG and d-TpTpCpA. Thus, alkylation of the sugar–phosphate backbone does not interfere with binding of the triesters to the tRNA. These results are similar to those of our previous study on the 2U:1A complexes formed between poly(U) and phosphotriester derivatives of d-ApA (Miller *et al.*, 1971). Complexes of poly(U) with d-Ap(Et)A and d-Ap(Me)A have higher melting temperatures than the complex formed with d-ApA.

A significant difference is observed between the association constants for the oligoribonucleotides and the analogous oligodeoxyribonucleotides. The association constants of the oligoribonucleotides are eight to twenty times larger than those of the oligodeoxyribonucleotides. This difference may reflect the difference in conformations of the oligoribonucleotides *vs.* the conformations of the oligodeoxyribonucleotides. Our laboratory has previously shown (Kondo *et al.*, 1972) for the purine-containing dimers, d-ApA and ApA, that the extent of base–base overlap in the deoxydimer is much greater than that in the ribodimer. In addition, the extent of base–base overlap in a stacked dimer is much greater than that in a 10- to 11-fold helix. Thus in the present series, the deoxyoligomers must first reduce the extent of base–base overlap before they are in a suitable conformation to hydrogen bond with their complementary region of the tRNA. Compared to the corresponding ribooligomers, which have a lesser extent of base–base overlap in their stacking mode, the complexes formed with the deoxyoligomers would have a lower stability than the complexes formed with ribooligomers. A more rigorous discussion of this subject was presented in a recent paper concerning the effects of C-2′ substituents on polynucleotide conformation (Alderfer *et al.*, 1974). Therefore the association constant would also be expected to be lower for the oligodeoxyribonucleotide triester, since their conformations and extents of stacking are very similar to those of the corresponding oligodeoxyribonucleotide diesters (Miller *et al.*, 1971).

Table IV shows that the association constants for the various oligómers to both $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{coli}$ are similar. Since these oligomers serve as probes of the secondary structure of the anticodon and 3′-ApCpCpA-OH terminus of tRNAs in these binding studies (Uhlenbeck *et al.*, 1970), our results suggest that these regions in $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{coli}$ have very similar conformations.

The specificity of binding was investigated by studying the interaction of the triester oligomers with modified $tRNA^{Phe}_{yeast}$ and with crude $tRNA_{coli}$. The $tRNA^{Phe}_{yeast}$ was modified by treatment with dilute hydrochloric acid to remove the Y base from the anticodon loop (Thiebe and Zachau, 1970). The $tRNA^{Phe}_{yeast}$ was also modified by treatment with snake venom phosphodiesterase to remove the 3′-CpA residues from the amino acid accepting terminus (Simsek *et al.*, 1973). Recently Cameron and Uhlenbeck (1973) and Pongs and Reinwald (1973) have demonstrated that removal of the Y base from $tRNA^{Phe}_{yeast}$ causes a seven- to eightfold reduction in the association constant of UpUpCpA for the modified tRNA. As shown in Table V, we observe five- and sixfold reduction in the binding of d-Tp(Et)Tp(Et)Cp(Et)A and UpUpCpA, respectively, to $tRNA^{Phe}_{yeast}$ − Y. It is interesting to note that the binding of d-Tp(Et)Gp(Et)G and UpGpG to this tRNA was also decreased slightly.

The association of d-Tp(Et)Gp(Et)G and UpGpG with snake venom phosphodiesterase treated $tRNA^{Phe}_{yeast}$ was reduced 14- and 19-fold, respectively, when compared to binding with intact $tRNA^{Phe}_{yeast}$. The small amount of residual binding observed may be due to binding to some tRNA molecules from which only the terminal A residue has been removed. A reduction in the binding of d-Tp(Et)Tp(Et)Cp(Et)A and UpUpCpA to this modified tRNA was also observed.

The results of these experiments indicate that removal of the Y base not only disrupts binding of the oligomers to the anticodon region, but also affects binding at the more remote 3′ terminus. Similarly, removal of the 3′-CpA residues not only eliminates binding of the oligomers complementary to this region, but also reduces the binding constants of oligomers complementary to the anticodon region. This effect may result from a conformational change in the entire tRNA molecule induced by removal of the Y base or the 3′-CpA terminus.

The triester d-Tp(Et)Tp(Et)Cp(Et)A is complementary to the anticodon region of $tRNA^{Phe}$, while the triester d-Tp(Et)Gp(Et)G is complementary to the 3′-CpCpA terminus, a sequence common to all tRNAs. Therefore, the apparent association constants for binding of d-Tp(Et)Tp(Et)Cp(Et)A should be greatly reduced if a crude mixture of tRNAs is used instead of pure $tRNA^{Phe}$. However, the magnitude of binding of d-Tp(Et)Gp(Et)G to crude tRNA should be similar to the association constant for binding to

TABLE V: Binding of Oligomers to Modified tRNAs.[a]

	K (M^{-1})			K_{app} (M^{-1})
	$tRNA^{Phe}_{yeast}$	$tRNA^{Phe}_{yeast}$ − Y	$tRNA^{Phe}_{yeast}$ − CA	$tRNA_{coli}$[b]
d-Tp(Et)Tp(Et)Cp(Et)A	3,000	600	900	300
UpUpCpA	48,000	8200	29,100	1100
d-Tp(Et)Gp(Et)G	2,400	1200	170	2400
UpGpG	4,800	3400	250	9900

[a] 1 M NaCl, 10 mM $MgCl_2$, 10 mM Tris (pH 7.5); 0°; tRNA concentration 40 μM. [b] Unfractionated.

$tRNA^{Phe}$. The results of binding experiments using a mixture of crude tRNA from *E. coli* are shown in Table V. This mixture contains approximately 3.4% $tRNA^{Phe}$ as determined by aminoacylation with phenylalanine. The apparent binding constants of d-Tp(Et)Tp(Et)Cp(Et)A and UpUpCpA to crude $tRNA_{coli}$ are greatly reduced compared to their binding to $tRNA^{Phe}_{coli}$ (see Table IV). As predicted, the binding constants of d-Tp(Et)Gp(Et)G and UpGpG to the crude $tRNA_{coli}$ are almost the same as those obtained for binding to $tRNA^{Phe}_{coli}$ (Table IV). The above results (Table V) demonstrate that d-Tp(Et)Tp(Et)Cp(Et)A binds specifically with the anticodon region of $tRNA^{Phe}$ while d-Tp(Et)Gp(Et)G interacts specifically with the 3′-CpCpA terminus of all tRNA. This specificity of interaction is further demonstrated by the aminoacylation experiments described in the following paper (Barrett *et al.*, 1974).

The association constants for binding of the triesters d-Tp(Et)Gp(Et)G and d-Tp(Et)Tp(Et)Cp(Et)A to $tRNA^{Phe}_{yeast}$ show little or no change when the salt concentration is reduced from 1 M NaCl with Mg^{2+} to 0.1 M NaCl without Mg^{2+} (Table VI). In contrast, the association constants of the diesters, d-TpTpCpA, UpUpCpA, and UpGpG, are significantly reduced when the salt concentration is lowered. A similar effect on the magnitude of the association constant for binding of UpApCpA to $tRNA^{Tyr}_{coli}$ in 1 mM EDTA–10 mM Na_2HPO_4 at 0° was reported when the sodium chloride concentration was decreased from 1.5 to 0.2 M (Uhlenbeck, 1972). This decrease in binding is attributed to an increase in charge repulsion between the negatively charged sugar–phosphate backbone of the oligonucleotide diester and the tRNA as the ionic strength of the solution is lowered. Since the oligonucleotide triesters are neutral molecules, such charge repulsion is eliminated and changes in ionic strength do not significantly affect the binding constants.

The stability of the oligodeoxyribonucleotide triesters and their ability to form specific complexes with complementary regions of tRNA make these compounds attractive candidates as probes of the structure and function relationships of nucleic acid, both *in vitro* and in living cells. The effect of these triesters on the *in vitro* aminoacylation of tRNA is the subject of the following paper (Barrett *et al.*, 1974).

TABLE VI: Effect of Salt Concentration on the Binding of Oligomers to $tRNA^{Phe}_{yeast}$ at 0°.[a]

	K (M^{-1})	
	1 M NaCl–10 mM $MgCl_2$	0.1 M NaCl–1 mM EDTA
d-Tp(Et)Tp(Et)Cp(Et)A	3,000	3,400
d-Tp(Et)Gp(Et)G	2,400	2,500
d-TpTpCpA	2,200	0
UpUpCpA	48,000	14,800
UpGpG	4,800	800

[a] Each buffer contained 10 mM Tris (pH 7.5). The tRNA concentration was 40 μM.

Experimental Section

Chemical Synthesis of Oligodeoxyribonucleotides and Oligodeoxyribonucleotide Phosphotriesters

General. The deoxynucleosides and deoxynucleoside 5′-phosphates obtained commercially were checked for purity by paper chromatography. 5′-*O*-Mono-*p*-methoxytritylthymidine (Schaller *et al.*, 1965), 3′-*O*-acetylthymidine 5′-phosphate (Khorana and Vizsolyi, 1961), N^2,$O^{3'}$-diisobutyryldeoxyguanosine 5′-phosphate (Weber and Khorana, 1972), N^4-*p*-anisoyl-3′-*O*-acetyldeoxycytidine 5′-phosphate (Schaller and Khorana, 1963), and N^6-benzoyl-3′-*O*-acetyldeoxyadenosine 5′-phosphate (Weiman *et al.*, 1963) were prepared by methods described in the literature. 2,4,6-Trimethylbenzenesulfonyl chloride (Me_3PhsCl)[1] from Aldrich Chemical Co. and *p*-toluenesulfonyl chloride (TosCl)[1] from Eastman Chemical Co. were each recrystallized from pentane before use. Anhydrous pyridine and *N*,*N*-dimethylformamide were prepared according to Miller *et al.* (1971). Anhydrous ethanol was prepared by distillation from magnesium ethoxide (Shirley, 1951) onto molecular sieves (Linde Type 3A). 2,6-Lutidine was dried over molecular sieves.

Descending paper chromatography was performed on Whatman No. 3MM paper using the following solvent systems: solvent A, 2-propanol–concentrated ammonium hydroxide–water (7:1:2, v/v); solvent C, 1 M ammonium acetate–95% ethanol (3:7, v/v; pH 7.5); solvent F, 1-propanol–concentrated ammonium hydroxide–water (50:10:35, v/v); solvent I, 2-propanol–water (7:3, v/v). Paper electrophoresis was carried out on a Savant flat plate apparatus using 0.05 M triethylammonium bicarbonate as buffer (pH 8.5) at a voltage of 40 V/cm for 45 min. Ultraviolet spectra were recorded on a Cary 14 uv spectrophotometer. Unless otherwise noted, all reactions and operations were carried out at room temperature.

General Condensation Procedure. The protected nucleoside or the pyridinium salts of the oligonucleotide and protected nucleoside 5′-phosphate were dried by repeated evaporations with anhydrous pyridine. The resulting gum was dissolved in dry pyridine; the solution was cooled to 0°; mesitylenesulfonyl chloride was added and the reaction mixture was kept in the dark at room temperature. These operations were carried out in a dry nitrogen atmosphere. The reaction was terminated by addition of an equal volume of water at 0°, and, after storage (usually overnight) at room temperature, the reaction mixture was diluted with water and the products were purified by extraction and column chromatography. The isolated products were precipitated either from pyridine solution by dropwise addition to anhydrous ether for the diesters or from tetrahydrofuran solution by addition to hexane for the triesters. The precipitates were collected by centrifugation, thoroughly washed with solvent, and dried under vacuum to give amorphous, white powders. The spectral characteristics of the protected oligomers are given in Table VII.

General Ethylation Procedure. The protected oligomer diester was made anhydrous by repeated evaporation with anhydrous pyridine and the residue was dissolved in a solution containing *N*,*N*-dimethylformamide and 2,6-lutidine. Ethanol and *p*-toluenesulfonyl chloride (TosCl) were added at 0° and after 1 hr at room temperature an additional amount of ethanol and TosCl was added. These operations were carried out in a dry nitrogen atmosphere. The reaction was terminated after 2 hr total time by addition of water. The solvents were evaporated with frequent additions of 50% chloroform–methanol to aid in the removal of the dimethylformamide, and the residue was dissolved in chloroform. After extraction with water, the chloroform solution

TABLE VII: Spectral Characteristics of Protected Oligonucleotides and Triesters in Methanol.

	λ_{max} (nm)	λ_{min} (nm)	$\epsilon_{260}/\epsilon_{280}$
d-MMTrTpG^{iB}OiB	260, 230 (s), 277 (s)	234	1.44
d-MMTrTpG^{iB} [a]	260, 233 (s), 277 (s)	243	1.40
d-MMTrTp(Et)G^{iB}OiB	237, 263, 255 (s), 280 (s)	227, 243	1.38
d-MMTrTp(Et)G^{iB}	234, 262, 255 (s), 280 (s)	226, 243	1.34
d-MMTrTp(Et)G^{iB}pG^{iB}OiB	233, 258, 277 (s)	227, 238	1.41
d-MMTrTp(Et)G^{iB}p(Et)G^{iB}OiB	258, 237, 280 (s)	227	1.40
d-MMTrTp(Et)TOAc	267, 225 (s)	240	
d-MMTrTp(Et)TpC^{An}OAc	270, 305 (s)	242	1.00
d-MMTrTp(Et)Tp(Et)C^{An}OAc	270, 305 (s)	243	0.95
d-MMTrTp(Et)Tp(Et)C^{An}pA^{Bz}OAc	278, 315 (s)	245	
d-MMTrTp(Et)Tp(Et)C^{An}p(Et)A^{Bz}OAc [b]	278, 315 (s)	244	0.79

[a] Similar spectral properties were observed by Caruthers *et al.* (1972). [b] The spectral properties of this compound are similar to those of d-MMTrTpTpA^{Bz}pC^{An} (Ohtsuka and Khorana, 1967).

was dried over anhydrous sodium sulfate. The solution was then concentrated and the products were isolated by silica gel column chromatography. The products were precipitated from a tetrahydrofuran solution by addition to hexane. The spectral characteristics of the protected oligonucleotide triesters are presented in Table VII.

Removal of Protecting Groups. The 3′-*O*-protected oligomer was dissolved in 50% ethanol–pyridine and treated with an equal volume of 2 N sodium hydroxide for 7 min at −5° (3′-*O*-isobutyryl group) or 15 min at room temperature (3′-*O*-acetyl group). The reaction mixture was then neutralized with pyridinium Dowex 50X resin. The resin was filtered and thoroughly washed with 50% ethanol–pyridine and the combined filtrate and washings were evaporated. The products were isolated by precipitation.

For complete removal of protecting groups, the protected oligomer was first treated with 60% concentrated ammonium hydroxide in pyridine for 2–3 days. After evaporation of solvents the residue was treated with 80% acetic acid for 3–6 hr. The acetic acid was removed by evaporation. Spectral characteristics and mobilities on paper chromatography and paper electrophoresis of deblocked oligomers are presented in Tables I and II, respectively.

Hydrolysis with Snake Venom and Spleen Phosphodiesterase. The previously published procedure (Miller *et al.*, 1971) was followed.

Preparation of d-MMTrTpG^{iB}OiB and d-TpG. d-MMTrT (5.15 g, 10 mmol) and d-pG^{iB}OiB (6.25 g, 11 mmol) were allowed to react for 4 hr in 50 ml of pyridine containing Me_3PhsCl (5.26 g, 24 mmol). After overnight aqueous pyridine treatment, the reaction mixture dissolved in water (200 ml) was extracted with ether (2 × 600 ml). Additional water (700 ml) was added and the solution was extracted with 1-butanol (2 × 750 ml). The combined butanol extracts were evaporated and product, d-MMTrTpG^{iB}OiB, was precipitated from pyridine (120 ml) by addition to ether (2 l.): weight 9.4 g (88%); silica gel tlc, R_F (20% MeOH–$CHCl_3$) 0.34. The oligomer had the same spectral characteristics as those reported for d-MMTrTpG^{iB} (Caruthers *et al.*, 1972). Removal of the protecting groups from a 54-mg sample gave d-TpG, which had similar R_F values to d-TpG prepared previously by a different route (Ohtsuka *et al.*, 1972). The dimer was completely digested to d-T and d-pG by snake venom phosphodiesterase.

Preparation of d-TpGpG. d-MMTrTpG^{iB}OiB (198 mg, 0.186 mmol) was treated with 1 M NaOH in 4 ml of 50% pyridine–ethanol for 7 min at −5°. The product, d-MMTrTpG^{iB}, obtained after precipitation weighed 165 mg (89%) and had spectral properties similar to those of previously prepared material (Caruthers *et al.*, 1972): silica gel tlc, R_F (20% MeOH–$CHCl_3$) 0.04.

d-MMTrTpG^{iB} (155 mg, 0.156 mmol) and d-pG^{iB}OiB (226 mg, 0.4 mmol) were allowed to react for 4 hr in 1 ml of pyridine containing Me_3PhsCl (175 mg, 0.8 mmol) and then treated overnight with aqueous pyridine. The reaction mixture dissolved in water (14 ml) was extracted with 1-butanol (2 × 15 ml) and the combined butanol extracts were extracted with water (2 × 15 ml) and evaporated. The protecting groups were removed and the residue, after extraction with ether, was dissolved in 100 ml of 10% ethanol and applied to a DEAE-cellulose column in the bicarbonate form. The column was eluted with a linear gradient (0.0–0.25 M, 4 l. total) of triethylammonium bicarbonate in 10% ethanol. Fractions containing d-TpGpG were pooled and the product was isolated by evaporation and lyophilization: weight 112 mg (65%); chromatographic mobility identical with previously prepared d-TpGpG (Ohtsuka *et al.*, 1972). Hydrolysis with snake venom phosphodiesterase gave d-T and d-pG.

Preparation of d-MMTrTp(Et)G^{iB} and d-Tp(Et)G. d-MMTrTpG^{iB}OiBu (8.0 g, 7.52 mmol) was ethylated in *N,N*-dimethylformamide (75 ml), 2,6-lutidine (8.25 ml), and *N*-methylimidazole (6.0 ml) using two (18 ml) charges of ethanol and 2 (14.2 g) charges of TosCl. After work-up, the residue, dissolved in chloroform (750 ml), was extracted with water (750 ml). The product was isolated by silica gel chromatography (5 × 50 cm) using ethyl acetate and 5% methanol in tetrahydrofuran as solvents, followed by precipitation from hexane: weight 3.8 g (51%); silica gel tlc, R_F (10% MeOH–$CHCl_3$) 0.49, trace fluorescent contaminant R_F 0.52. The 3′-*O*-protecting group was removed by treatment with 1 M sodium hydroxide in 60 ml of 50% ethanol–pyridine for 7 min at −5°. After work-up, pure d-MMTrTp(Et)G^{iB} was isolated by silica gel chromatography (3.2 × 50 cm) using 5% methanol–chloroform and 20% methanol–chloroform as solvents: weight 3.0 g (74%); silica gel tlc, R_F (20% MeOH–$CHCl_3$) 0.47.

The protecting groups were removed from a 71-mg sample. The resulting d-Tp(Et)G was purified by paper chro-

matography using solvent I and was isolated by precipitation with ether, weight 24 mg (53%). The triester was resistant to hydrolysis by snake venom and spleen phosphodiesterase.

Preparation of d-MMTrTp(Et)G^{iB}pG^{iB}OiB and d-Tp(Et)GpG. d-MMTrTp(Et)G^{iB} (3.0 g, 3.18 mmol) and d-pG^{iB}OiB (3.3 g, 6.0 mmol) were allowed to react for 4 hr in pyridine (15 ml) containing Me_3PhsCl (2.63 g, 12 mmol). Following aqueous pyridine treatment, the aqueous solution (210 ml) was extracted with 1-butanol (2 × 240 ml). The combined butanol extracts were extracted with water (2 × 250 ml) and evaporated, and the residue was chromatographed on a silica gel column (3.5 × 50 cm). The column was eluted with 10% methanol in chloroform (2 l.) followed by 20% methanol in chloroform (3 l.). Fractions were obtained which contained the desired d-MMTrTp(Et)G^{iB}pG^{iB}OiB as well as some detriylated material, d-Tp(Et)G^{iB}pG^{iB}OiB. The total material from these fractions was treated with 428 mg (1.3 mmol) of monomethoxytrityl chloride in pyridine (3 ml) for 21 hr followed by 95% ethanol (2 ml) for 2 hr. The solvents were evaporated and the desired d-MMTrTp(Et)G^{iB}pG^{iB}OiB was isolated by precipitation with ether; weight 2.39 g (50%); silica gel tlc, R_F (20% MeOH-$CHCl_3$) 0.13; R_F (30% MeOH-$CHCl_3$) 0.47.

The protecting groups were removed from a 25-mg sample and the resulting d-Tp(Et)GpG was isolated after paper chromatography using solvent F. Incubation with snake venom phosphodiesterase (16 hr, 37°) gave d-Tp(Et)GpG, dpG, and d-Tp(Et)G. The oligomer was resistant to hydrolysis by spleen phosphodiesterase.

Preparation of d-MMTrTp(Et)G^{iB}p(Et)G^{iB}OiB and d-Tp(Et)Gp(Et)G. d-MMTrTp(Et)G^{iB}pG^{iB}OiB (580 mg, 0.39 mmol) was ethylated in *N,N*-dimethylformamide (8 ml) and 2,6-lutidine (4 ml) using 2 (4 ml) charges of ethanol and 2 (1.55 g, 8 mmol) charges of TosCl. After work-up, a chloroform (40 ml) solution of the residue was extracted with water (2 × 40 ml). The triester was purified by silica gel chromatography (2 × 30 cm) using chloroform (400 ml) and 10% methanol–chloroform (400 ml) as solvents. The resulting d-MMTrTp(Et)G^{iB}p(Et)G^{iB}OiB weighed 176 mg (31%); silica gel tlc, R_F (10% MeOH-$CHCl_3$) 0.29.

The protecting groups were removed from a 160-mg sample and the resulting d-Tp(Et)Gp(Et)G was purified by paper chromatography using solvent I; weight after precipitation with ether, 35 mg (33%). The triester was resistant to hydrolysis by snake venom phosphodiesterase.

Preparation of d-GpGpT. d-MMTrG^{iB}pG^{iB} (0.2 mmol; Agarwal *et al.*, 1972) was allowed to react with d-pTOAc (177 mg, 0.4 mmol) for 2.5 hr in pyridine (1 ml) containing Me_3PhsCl (175 mg, 0.8 mmol), followed by overnight aqueous pyridine treatment. The reaction mixture in 14 ml of water was extracted with 2 (15 ml) portions of 1-butanol, and the residue obtained after evaporation of the butanol was treated to remove protecting groups. Following extraction with ether, the residue was applied to a DEAE-cellulose column in the bicarbonate form and the column was eluted with a linear gradient of triethylammonium bicarbonate (0.0–0.50 M, 4 l. total). Fractions containing the desired d-GpGpT were pooled, evaporated, and lyophilized to give 14 mg (6%) of oligomer. Hydrolysis with snake venom phosphodiesterase gave d-G, dpG, and d-pT.

Preparation of d-MMTrTp(Et)TOAc. d-MMTrTpTOAc (3.58 g, 4.0 mmol, prepared by condensation of d-MMTrT with d-pTOAc) was ethylated in *N,N*-dimethylformamide (70 ml)–2,6-lutidine (35 ml) with 2 (35 ml) charges of ethanol and 2 (13.3 g, 70 mmol) charges of TosCl. Following work-up, a 200-ml chloroform solution of the residue was extracted with water (3 × 200 ml). The product was isolated by silica gel column chromatography (4 × 30 cm) using ether and tetrahydrofuran as solvents. The resulting d-MMTrTp(Et)TOAc was precipitated with hexane: weight 1.71 g (50%); silica gel tlc, R_F (EtOAc) 0.18; R_F (THF) 0.59. The material was identical with material previously prepared by a different synthetic route (DeBoer *et al.*, 1973). Removal of the protecting groups gave d-Tp(Et)T identical with an authentic sample (Miller *et al.*, 1971).

Preparation of d-MMTrTp(Et)TpC^{An}OAc and d-Tp(Et)TpC. d-MMTrTp(Et)T (1.89 mmol), prepared by hydrolysis of d-MMTrTp(Et)TOAc (1.71 g, 1.89 mmol) with 1 M NaOH in 10 ml of 50% pyridine, was allowed to react with d-pC^{An}OAc (1.13 g, 2.0 mmol) for 3 hr in 10 ml of pyridine containing Me_3PhsCl (880 mg, 4 mmol). Following aqueous pyridine treatment, the reaction mixture in 100 ml of water was extracted with 1-butanol (2 × 100 ml). The residue obtained after evaporation of the butanol was chromatographed on a silica gel column (2 × 40 cm) using 500 ml of ethyl acetate, 1 l. of tetrahydrofuran, and 20% methanol in chloroform, which eluted the product. The resulting d-MMTrTp(Et)TpC^{An}OAc, which was precipitated from pyridine by addition to ether, weighed 1.33 g (51%); silica gel tlc, R_F (THF) 0.07, R_F (20% MeOH-$CHCl_3$) 0.42. A sample of the material was treated to remove protecting groups and the resulting d-Tp(Et)TpC was purified by paper chromatography using solvent I. Hydrolysis with snake venom phosphodiesterase gave d-Tp(Et)T and d-pC. The trimer was resistant to hydrolysis by spleen phosphodiesterase.

Preparation of d-MMTrTp(Et)Tp(Et)C^{An}OAc and d-Tp(Et)Tp(Et)C. d-MMTrTp(Et)TpC^{An}OAc (1.23 g, 0.9 mmol) was ethylated in a solution of *N,N*-dimethylformamide (18 ml)–2,6-lutidine (9 ml) using 2 (9 ml) charges of ethanol and 2 (3.4 g, 18 mmol) charges of TosCl. After work-up, the residue in chloroform (50 ml) was extracted with water (2 × 50 ml) and dried over anhydrous sodium sulfate. Column chromatography on silica gel (2 × 35 cm) using ether (350 ml), tetrahydrofuran (500 ml), and 20% methanol in chloroform (150 ml) gave, after precipitation with hexane, d-MMTrTp(Et)Tp(Et)C^{An}OAc: 3.93 g (32%); silica gel tlc, R_F (THF) 0.45, R_F (20% MeOH-$CHCl_3$) 0.70.

The protecting groups were removed from a 20-mg sample and the resulting d-Tp(Et)Tp(Et)C was purified by paper chromatography using solvent I. The triester was resistant to hydrolysis by snake venom and spleen phosphodiesterase.

Preparation of d-MMTrTp(Et)Tp(Et)C^{An}pA^{Bz}OAc and d-Tp(Et)Tp(Et)CpA. The 3′-acetyl group was removed from d-MMTrTp(Et)Tp(Et)C^{An}OAc (366 mg, 0.27 mmol) by hydrolysis with 1 M NaOH in 50% pyridine for 15 min. Following work-up and isolation by precipitation from hexane, 350 mg (97%) of d-MMTrTp(Et)Tp(Et)C^{An} was obtained; silica gel tlc, R_F (THF) 0.33; R_F (20% MeOH-$CHCl_3$) 0.42. The triester was condensed with d-pA^{Bz}OAc (166 mg, 0.3 mmol) for 3.5 hr in pyridine (2 ml) containing Me_3PhsCl (132 mg, 0.6 mmol). Following aqueous pyridine treatment, the solution was diluted with water (50 ml) and the product was extracted into 1-butanol (3 × 50 ml). Col-

umn chromatography on silica gel (2 × 21 cm) using ether (150 ml), tetrahydrofuran (400 ml), and 20% methanol–chloroform (200 ml) gave d-MMTrTp(Et)Tp(Et)C^{An}p-A^{Bz}OAc (295 mg, 60%) after precipitation with hexane; silica gel tlc, R_F (THF) 0.03; R_F (20% MeOH–$CHCl_3$) 0.40.

The protecting groups were removed from a 10-mg sample and the resulting d-Tp(Et)Tp(Et)CpA was purified by paper chromatography using solvent I. Hydrolysis with snake venom phosphodiesterase gave d-Tp(Et)Tp(Et)C and d-pA. The tetramer was resistant to hydrolysis by spleen phosphodiesterase.

Preparation of d-MMTrTp(Et)Tp(Et)C^{An}p(Et)A^{An}OAc and d-Tp(Et)Tp(Et)Cp(Et)A. d-MMTrTp(Et)Tp(Et)C^{An}p-A^{Bz}OAc (285 mg, 0.155 mmol) was ethylated in a solution containing *N,N*-dimethylformamide (5 ml)–2,6-lutidine (2.5 ml) with 2 (2.5 ml) charges of ethanol and 2 (0.95 g, 5 mmol) charges of TosCl. After work-up, the residue in chloroform (100 ml) was extracted with water (2 × 100 ml) and chromatographed on a silica gel column (2 × 30 cm) using ethyl acetate (300 ml), tetrahydrofuran (700 ml), and 20% methanol–chloroform (200 ml). d-MMTrTp(Et)Tp-(Et)C^{An}p(Et)A^{Bz}OAc was isolated by precipitation into hexane: weight 182 mg (63%); silica gel tlc, R_F (THF) 0.39, R_F (20% MeOH–$CHCl_3$) 0.73.

The protecting groups were removed and the resulting d-Tp(Et)Tp(Et)Cp(Et)A was purified by paper chromatography using solvent I. The tetramer was completely resistant to hydrolysis by snake venom and spleen phosphodiesterase.

Preparation of d-TpTpCpA. d-DMTrTpT (100 mg, 0.1 mmol; Narang and Khorana, 1965) and d-pC^{Bz}pA^{Bz}OAc (55 mg, 0.25 mmol; prepared in a manner similar to that used for the preparation of d-pC^{An}pA^{Bz}OAc; Weber and Khorana, 1972) were condensed for 4 hr in 3 ml of pyridine containing Me_3PhsCl (66 mg, 0.3 mmol). After aqueous pyridine treatment the protecting groups were removed and the reaction mixture was chromatographed on a DEAE-cellulose column (2.5 × 40 cm) in the bicarbonate form using a linear gradient of triethylammonium bicarbonate (0.05–0.30 M, 4 l. total). Tetramer which was contaminated with a small amount of d-pCpA was isolated and incubated with 0.45 mg of alkaline phosphatase in 1 ml of 150 mM Tris (pH 8.2) for 4 hr at 37°. The digest, after extraction with chloroform–isoamyl alcohol (5:2, v/v), was chromatographed on a second DEAE-cellulose column (1.2 × 30 cm) using a linear triethylammonium bicarbonate gradient (0.0–0.25 M, 2 l. total). The desired tetramer, d-TpTpCpA (750 A_{260} units, 37%), was isolated and lyophilized. The oligomer was completely digested with snake venom phosphodiesterase to give d-T, d-pT, d-pC, and d-pA, and with spleen phosphodiesterase to give d-Tp, d-Cp, and d-A.

Equilibrium Dialysis Experiments

^{3}H-Labeled Oligodeoxyribonucleotides and Oligodeoxyribonucleotide Triesters. One to four milligrams of each of the oligomers was heated at 90° in a sealed tube with 100 μl of tritiated water (2.5 Ci/mmol). The adenine-containing oligomers were heated for 3 hr while the guanine-containing oligomers were heated for 5.5 hr. The oligomers were purified by paper chromatography using solvents C and I, after lyophilization from water several times to remove exchangeable tritium. The results of the exchange reactions are presented in Table III.

^{3}H-Labeled Ribooligonucleotides. ^{3}H-Labeled ribooligonucleotides (sp act. 0.35–3.2 Ci/mmol) were prepared using primer dependent polynucleotide phosphorylase as described by Uhlenbeck *et al.* (1970). After purification by paper chromatography, the oligomers were characterized by digestion with RNase T_1 or RNase A.

Purified tRNAs. tRNA$^{Phe}_{coli}$ was prepared by a three-step procedure: (1) fractionation of crude tRNA$_{coli}$ on a BD-cellulose column (Roy and Söll, 1968); (2) acylation of the resulting tRNA^{Phe} fraction and purification of Phe-tRNA^{Phe} on BD-cellulose (Gillam and Tener, 1971); (3) deacylation followed by reversed-phase chromatography of the tRNA^{Phe} on RPC 5 (Pearson *et al.*, 1971). The resulting tRNA^{Phe} had an amino acid acceptance activity of 1700 pmol of phenylalanine/A_{260} unit and was considered greater than 90% pure.

tRNA$^{Phe}_{yeast}$ which accepted 1600 pmol of phenylalanine/A_{260} unit was a generous gift from Professor Friederich Cramer and was considered greater than 90% pure. The extinction coefficients used were: tRNA$^{Phe}_{coli}$ ϵ_{258} 5.43 × 10^5, tRNA$^{Phe}_{yeast}$ ϵ_{258} 5.03 × 10^5 (Blum, 1971).

The Y base was removed from tRNA$^{Phe}_{yeast}$ using the procedure of Thiebe and Zachau (1970). After reaction, the reaction mixture was applied to a BD-cellulose column. The column was first eluted with 1.2 M NaCl–50 mM NaOAc (pH 5.0)–10 mM $MgCl_2$ which eluted tRNA^{Phe} minus Y base, and then with 1.2 M NaCl–50 mM NaOAc (pH 5.0)–10 mM $MgCl_2$ containing 15% ethanol, which eluted a small amount of unreacted tRNA$^{Phe}_{yeast}$. The 3′-CA terminus was removed from tRNA$^{Phe}_{yeast}$ following the method of Simsek *et al.* (1973). The reaction mixture was applied to a DEAE-cellulose column. The column was eluted with 0.3 M NaCl–50 mM Tris-HCl (pH 8.0) which removed the snake venom phosphodiesterase and mononucleotides. The column was further eluted with 1 M NaCl–50 mM Tris-HCl (pH 8.0) which removed the tRNA.

Dialysis Experiments. Dialysis experiments were carried out in 30- and 50-μl Plexiglass chambers separated by a dialysis membrane using the methods described by Uhlenbeck (1972). Precautions were taken to prevent contamination by ribonuclease. tRNA solutions (40 μM) were introduced into side A and oligomer solutions (0.1–46 μM, final concentration) into side B. After equilibration at 0° (from 4 to 14 days), two (10 μl) samples from each side were each diluted with 100 μl of water and counted in 10 ml of 4% Biosolv-toluene cocktail. For experiments where the oligomer concentration was greater than 0.5 μM, K was calculated using the equation: $K = (R - 1)/(\text{tRNA} - [O]b)$, where $[O]b = (\text{cpm A} - \text{cpm B})/(\text{cpm A} + \text{cpm B})[O]$ and [O] = the initial oligomer concentration (Högenauer, 1970). To ensure attainment of equilibrium, experiments were checked on successive days until no further change in K was observed.

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