(corresponding to that of an authentic sample of dinitrophenyl-*N*-methyl-γ-aminobutyric acid), and R_f 0.45 (corresponding to that of an authentic sample of dinitrophenyl-*N*-methyl-β-alanine (most abundant)), appeared. The yellow zone, R_f 0.45, was scraped off the plate, the DNP derivative eluted with ethanol, the solution evaporated, the residue dissolved in dilute hydrochloric acid, and the DNP derivative extracted into ether. The dried ether extract was evaporated and the residue sublimed at 160–170° and 10^{-3} mm, yielding dinitrophenyl-*N*-methyl-β-alanine (8 mg, 8%), mp 140–142° (lit.[55] mp 142–143°), mass spectrum *m/e* 269.

Radiogas Chromatography. A sample of the mixed bases dissolved in methanol (5 μl) was injected into a gas chromatograph (Varian Aerograph, 1840-1), equipped with a column (4 ft × 0.125 in. o.d., stainless steel) of 20% Carbowax 20M coated with 5% KOH on Chromosorb HMDS (mesh 60/70). Helium (40 ml/min) was passed through the column. The injector and detector were kept at 220°. The column was kept at 60° for the first 3 min after injection. The temperature was then raised at a rate of 6°/min to a maximum temperature of 220°. The effluent of the column was passed through a stream-splitter which channelled 10% of the effluent into a flame ionization detector and 90% through a heated (200°) tube into a radiogas chromatography counting system (Model 4998, Nuclear Chicago, connected to a Model 8731 single-channel rate meter, Nuclear Chicago). The counting system contained a model 461 gas flow detector (85 ml), which was kept at a temperature of 200°. The high voltage of the counting chamber with 4π geometry was set at 2000 to 2400 V. Before the effluent from the gas chromatograph had entered the counting chamber it was mixed with preheated butane gas, passing at a rate of 40 ml/min. The radioactivity was recorded on a radioactivity recorder (Model 8416, Nuclear Chicago). The time lag between mass recording and radioactivity recording depended on the gas flow rates and was 8 sec for the above rates. Column fractions emerging from the outlet of the counting chamber were trapped in methanol or methanolic hydrochloric acid. Radioactivity (or ^3H:^{14}C ratios) of the solutions containing the trapped column fractions was determined by liquid scintillation counting (Mark 1 liquid scintillation computer, Model 6860, Nuclear Chicago). For isolation of the labeled product, inactive carrier was added and the alkaloid purified by sublimation and recrystallization.

Acknowledgments. Thanks are due to Drs. L. Benoiton,[22] W. W. Chan,[47] and L. Maat.[50] This work was supported by a grant from the National Research Council of Canada. One of us (E. L.) thanks the Deutsche Forschungsgemeinschaft for an overseas fellowship.

Studies on Transfer Ribonucleic Acids and Related Compounds. VI.[1] Synthesis of Yeast Alanine Transfer Ribonucleic Acid 3′-Terminal Nonanucleotides and 5′-Terminal Hexanucleotides

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Contribution from the Faculty of Pharmaceutical Sciences, Osaka University, Toyonaka, Osaka, Japan. *Received December 11, 1972*

Abstract: The nonanucleotide, CpGpUpCpCpApCpCpA, was synthesized from the protected hexanucleotide, C^{Bz}(OBz)-p-C^{Bz}(OBz)-p-A^{Bz}(OBz)-p-C^{Bz}(OBz)-p-C^{Bz}(OBz)-p-$A^{Bz}2(OBz)_2$, in two different ways: (1) by condensation with the trinucleotide MMTr-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U(OBz)-p and (2) by two successive condensations involving the protected mononucleotide MMTr-U(OBz)-p and the dinucleotide MMTr-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p. In both condensations arylsulfonyl chloride was the activating reagent. The product was separated first by gel filtration on Sephadex LH-20 in the protected form, and the unprotected nonanucleotide was isolated by DEAE-cellulose chromatography in 7 *M* urea. The nonanucleotide was identified by tlc and base composition. The hexanucleotide GpGpGpCpGpU was synthesized by condensation of the protected ribodinucleotide GpGp and protected GpCpGpU using triisopropylbenzenesulfonyl chloride as the condensing reagent. The dinucleotide R-G^{iBu}(OiBu)-p-G^{iBu}(OiBu)-p was synthesized by polymerization of the mononucleotide, and the tetranucleotide G^{iBu}(OiBu)-p-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U(OBz) was prepared by stepwise condensation of mononucleotides. These synthetic oligonucleotides constitute double helical amino acid acceptor ends of the yeast alanine tRNA.

The synthesis of tRNA segments is important in its own right and may aid studies on the recognition of tRNA by enzymes.

Previously we have reported the synthesis of the hexanucleotide of the yeast alanine tRNA 3′ end by condensation of the trinucleotides.[2] The partially protected hexanucleotide bearing the free 5′-hydroxyl group (**1**)[3] (Chart I) was intended to be an intermediate for further elongation of the chain. The present paper describes the synthesis of the nonanucleotide having the sequence of the 3′ end of the yeast alanine tRNA (**3**) by condensation of this hexanucleotide **1** with properly protected mono- or oligonucleotides and also reports the synthesis of the ribooligonucleotide GpGpGpCpGpU (**9**) which corresponds to the 5′-terminal hexanucleotide of the same tRNA.

As shown in Chart II, the hexanucleotide **9** was synthesized by condensation of the dinucleotide R-G^{iBu}-(OiBu)-p-G^{iBu}(OiBu)-p (**7**) and the tetranucleotide G^{iBu}(OiBu)-p-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U$(OBz)_2$ (**8**). The homologous dinucleotide 7 was obtained by poly-

(1) Part V of this series: E. Ohtsuka, S. Morioka, and M. Ikehara, *J. Amer. Chem. Soc.*, **94**, 3229 (1972).

(2) E. Ohtsuka, M. Ubasawa, and M. Ikehara, *ibid.*, **93**, 2296 (1971).

(3) Abbreviations are as suggested by IUPAC–IUB combined commission, *J. Biol. Chem.*, **241**, 531 (1966). For the protected ribonucleotide, see ref 2.

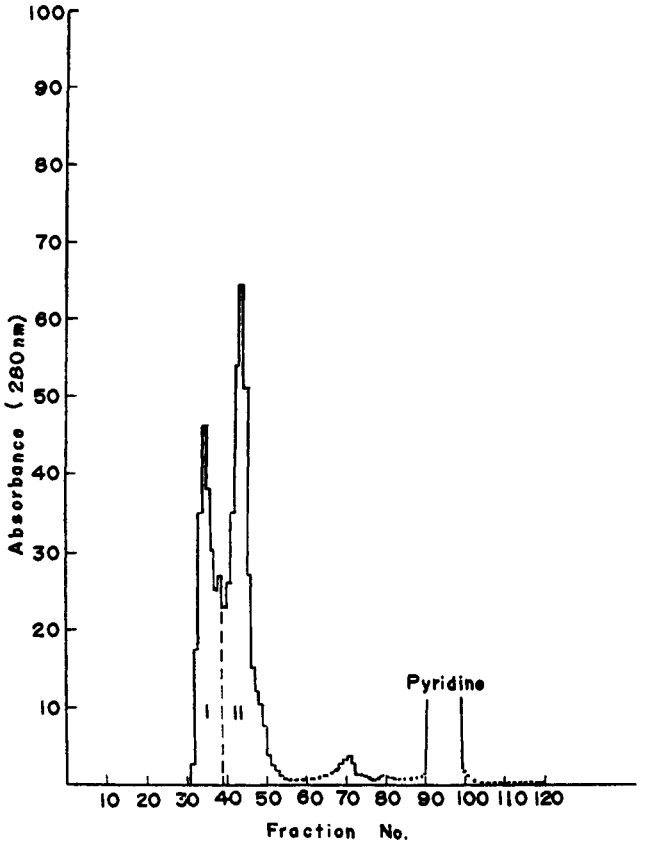
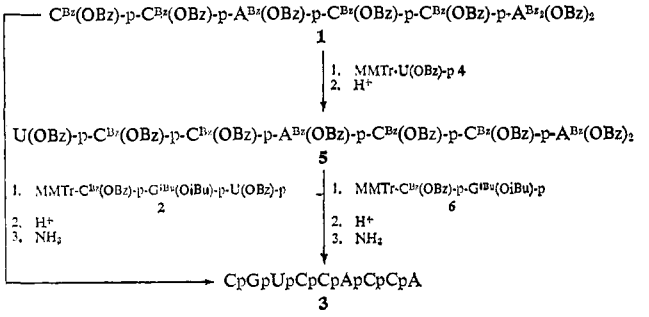


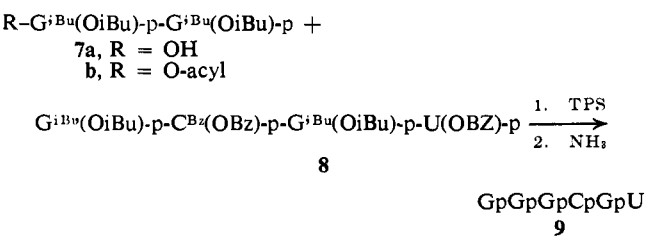
Figure 1. Gel filtration of the products obtained in the condensation of the hexanucleotide **1** with the trinucleotide **2** on a column (1.8 × 100 cm) of Sephadex LH-20 equilibrated with 0.1 *M* triethylammonium acetate in 90% DMF. Fractions of 2.2 ml were collected every 20 min. Peak I contained the nonanucleotide and peak II consisted mainly of the trinucleotide.

Chart I



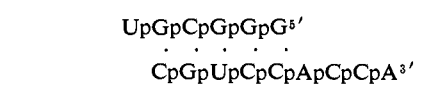
merization of the monocleotide, and the tetranucleotide **8** was synthesized by stepwise condensation of mononucleotides.

Chart II



The nonanucleotide **3** (lower strand in Chart III) forms five base pairs with the hexanucleotide **9** from the

Chart III



5′ end of the tRNA. The double strand shown in Chart III comprises part of the amino acid acceptor stem of the tRNA. Chambers and his coworkers reported that the combination of two enzymatically obtained fragments from the 3′ and 5′ ends of yeast alanine $tRNA_2$, which had chain lengths of 19 and 15, respectively, showed alanine acceptor activity.[4] The double helical oligonucleotide may possibly also be esterified with alanine in such a fragment charging experiment.

Synthesis of the Nonanucleotide by Condensation of the Hexanucleotide 1 with the Trinucleotide 2. The protected trinucleotide **2** used in the condensation reaction was synthesized using phosphoranisidate protection.[5] The same oligonucleotide with slightly different protecting groups has been synthesized by a different procedure.[6] The synthetic scheme is shown

Table I. Paper Chromatography and Electrophoresis

Compd	Paper chromatography —solvent— A[a]	B[b]	C[a]	Tlc solvent C[a]	Paper electrophoresis pH 2.7 R_{Up}	pH 7.5 R_{Gp}
Cp	2.22	0.16	1.44	1.80	0.00	0.97
Up	2.33	0.22	1.38		1.00	1.02
Gp	1.00	0.14	1.0	1.0	0.58	1.00
CpGp	0.45		0.75	0.60	0.51	1.00
CpGpUp			0.50	0.35	1.00	1.00
MMTr-CpGp	3.67	0.34				
MMTr-CpGpUp	3.22	0.32				
CpCpA		1.30			−0.38	0.67
CpCpApCpCpA			0.19	0.20	−0.15	0.87
UpCpCpApCpCpA				0.16		
CpGpUpCpCpApCpCpA				0.13	−0.14	0.88
GpGp	0.22		0.50			1.02
GpGpGp			0.23			1.02
GpGpGpGp			0			1.01
5′-C-pyridinium Gp	0.68					0.55
G cyclic p	3.7		1.65			0.55
GpG cyclic p	0.62					0.73
GpGpG cyclic p	0.18					0.76
GpGpGpG cyclic p			0			0.88
G	3.5	0.49	1.51			0
GpG			0.80			0.35
GpGpG			0.38			0.52
GpGpGpG			0			0.66
GpU	2.20		1.35			0.42
CpGpU	1.40		0.77			0.50
GpCpGpU			0.33			0.78
GpGpGpCpGpU			0.11			0.87
Gi-Bu(OiBu)-p		0.74				0.89
iBu-Gi-Bu(OiBu)-p		0.84				0.80

[a] R_{Gp} is given. [b] R_f is given.

in Chart IV. The starting mononucleotides **10** and **11** were condensed with dicyclohexylcarbodiimide (DCC) or triisopropylbenzenesulfonyl chloride (TPS) to give the dinucleotide **6**, the yield being 31 and 26%, respectively. With these different reagents the yield was the same. The product was isolated by TEAE-

(4) N. Imura, G. B. Weiss, and R. W. Chambers, *Nature (London)*, **222**, 1147 (1969).
(5) E. Ohtsuka, K. Murao, M. Ubasawa, and M. Ikehara, *J. Amer. Chem. Soc.*, **92**, 3441 (1970).
(6) E. Ohtsuka, M. Ubasawa, and M. Ikehara, *ibid.*, **92**, 3445 (1970).

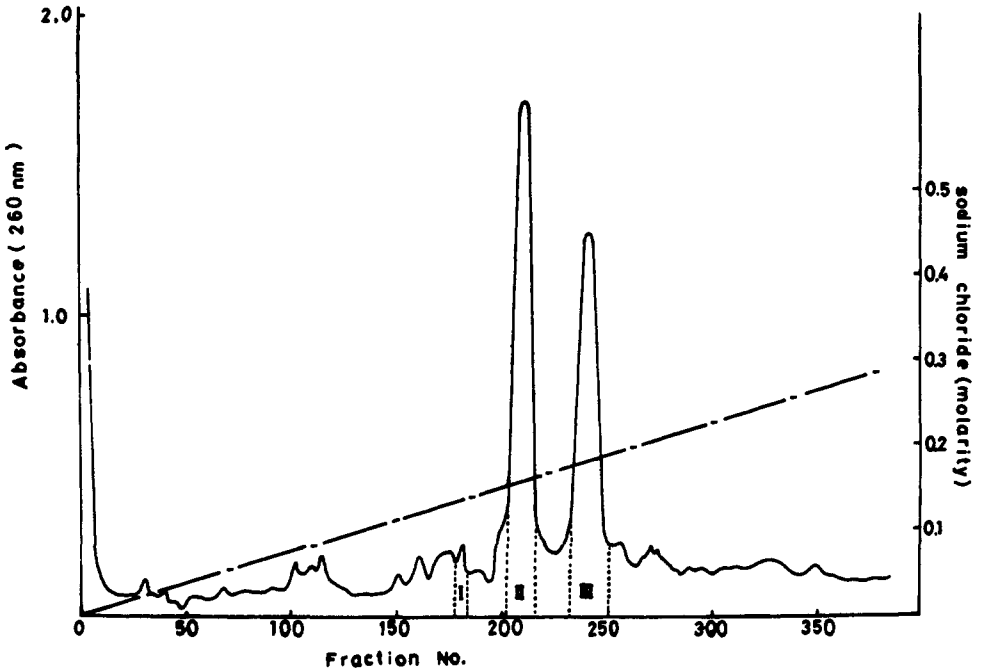
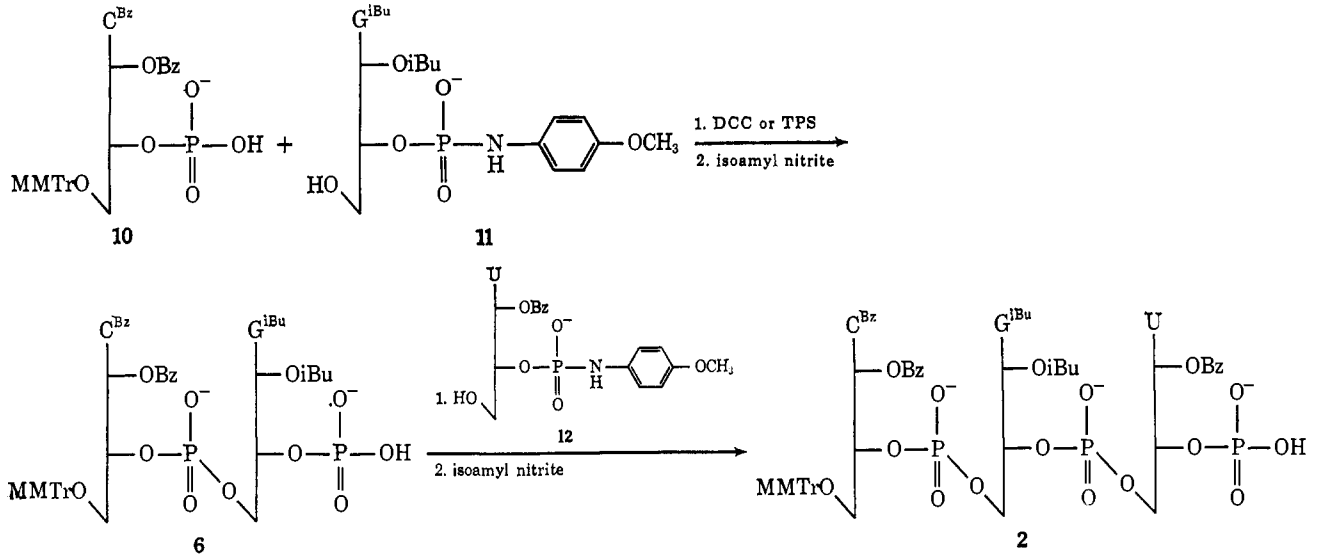


Figure 2. Chromatography of the unprotected products (peak I in Figure 1) obtained in the synthesis of the nonanucleotide on a column (0.7 × 100 cm) of DEAE-cellulose (Whatman DE-52) (chloride) equilibrated with 7 *M* urea in 0.02 *M* Tris-HCl (pH 7.5) at 50°; a linear gradient of sodium chloride produced by equilibration buffer (300 ml) and an equal volume of the same buffer containing 0.3 *M* sodium chloride. Fractions of 1.3 ml were collected every 30 min. Peak III (19 A_{260} units) contained essentially pure nonanucleotide CpGpUpCpCpApCpCpA and peak II (21 A_{260} units) contained the hexanucleotide CpCpApCpCpA contaminated with the trinucleotide.

Chart IV



cellulose chromatography after treatment with isoamyl nitrite to generate the phosphomonoesters. The R_f values of the protected and unprotected dimers are given in Table I. The dinucleotide was then condensed with protected Up (**12**) using DCC. The trinucleotide **2** was isolated by the similar method as the dimer. The yield was 21%. The unprotected trinucleotide CpGpUp was analyzed by hydrolysis with RNase M[7] to give Cp: Gp: Up in the correct ratio.

The hexanucleotide **1** was condensed with the trinucleotide **2** described above using TPS. The reaction mixture was first subjected to gel filtration on Sephadex LH-20 in 90% DMF containing 0.1 *M* triethylammonium acetate. The elution pattern is shown in Figure 1. Peak I contained the nonanucleotide and the starting materials as identified by tlc on cellulose plate[8] after removing protecting groups. Peak II contained the trinucleotide **2** and smaller compounds. Resolution in DMF seemed to be better than that in ethanol solution probably due to less interaction of aromatic moieties.[9] The material of peak I was detritylated, deacylated, and applied to a column of Sephadex G-50. The material of the first peak was further purified by DEAE-cellulose column chromatography in the presence of 7 *M* urea at 50°. The elution pattern is given in Figure 2. The material in the pooled fractions was desalted by gel filtration with

(7) M. Irie, *J. Biochem. (Tokyo)*, **62**, 509 (1967).

(8) (a) G. Katz and B. S. Dudock, *J. Biol. Chem.*, **244**, 3062 (1969); (b) F. Harada, F. Kimura, and S. Nishimura, *Biochim. Biophys. Acta*, **195**, 590 (1969).

(9) C. A. Steuli, *J. Chromatogr.*, **56**, 219 (1971).

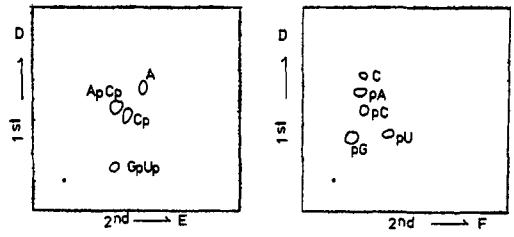


Figure 3. Two-dimensional tlc of the products of pancreatic RNase digestion (left) and those of venom phosphodiesterase digestion (right) of the nonanucleotide CpGpUpCpCpApCpCpA. Solvent systems used were as indicated.

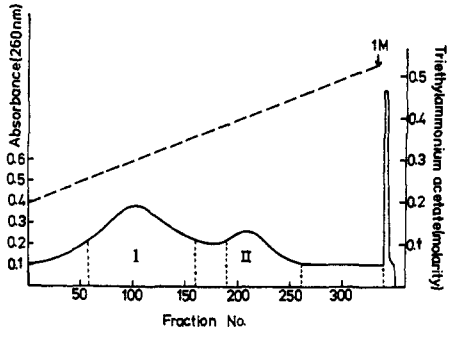


Figure 4. Chromatography of the heptanucleotide on a column (0.8 × 61 cm) of DEAE-cellulose (acetate) equilibrated with 0.005 *M* triethylammonium acetate in 60% ethanol. Elution was carried out using a linear gradient of triethylammonium acetate. The mixing chamber contained 0.25 *M* triethylammonium acetate in 60% ethanol (2 l.) and the reservior contained 0.55 *M* salt in 60% ethanol (2 l.). Peak II contained the protected heptanucleotide **5** (125 A_{260} units) and peak I contained the hexanucleotide **1** (290 A_{260} units).

Biogel P-2.[10] Peak III contained essentially pure nonanucleotide CpGpUpCpCpApCpCpA. The isolated yield was 8%. As shown in Figure 3, the product was characterized by two-dimensional tlc after enzymatic digestions. The base composition of the nonanucleotide was determined by high pressure liquid chromatography on a nucleic acid analyzer after enzymatic hydrolyses.

Synthesis of the Heptanucleotide 5 and Its Condensation with the Dinucleotide 6. A condensation using an excess of mononucleotide was tried to obtain a higher reaction yield. The hexanucleotide **1** was allowed to react with 20 equiv of the mononucleotide MMTr-U-(OBz)p (**4**) to yield the heptanucleotide **5**. Mesitylenesulfonyl chloride (MS) was used as the activating reagent. Although sulfonylation of the 5′-hydroxy group of uridine with MS was reported,[11] tosylation of mononucleotides was not found in pyridine solution.[12] The yield after two successive chromatographies on Sephadex LH-20 and DEAE-cellulose was 8%. The elution pattern of the ion-exchange-cellulose chromatography is shown in Figure 4. For the synthesis of the nonanucleotide, the heptanucleotide **5** was condensed with the dinucleotide **6** using MS. The reaction mixture was subjected to gel filtration on Sephadex LH-20. The material of the first part of the peak

(10) M. Uziel and W. E. Cohn, *Biochim. Biophys. Acta*, **103**, 539 (1965).
(11) R. Lohrmann and H. G. Khorana, *J. Amer. Chem. Soc.*, **88**, 829 (1966).
(12) M. Ikehara and S. Uesugi, *Tetrahedron*, **28**, 3687 (1972).

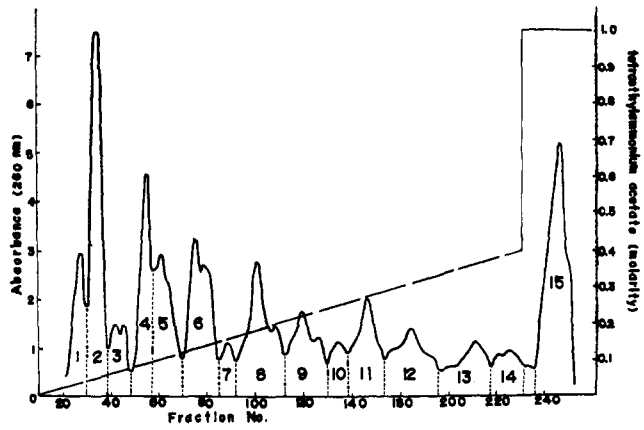


Figure 5. Chromatography of the products of polymerization of protected guanosine 3′-phosphate on a column (2.2 × 34 cm) of TEAE-cellulose (Serva) (acetate) equilibrated with 50% ethanol. Elution was carried out with a linear gradient of triethylammonium acetate. The mixing chamber contained 50% ethanol (2 l.) and the reservoir contained 0.4 *M* salt (0.4 *M* acetic acid and 0.2 *M* triethylamine). Fractions of 16 ml were collected every 20 min. Identification of peaks is shown in Table II.

was deprotected and chromatographed on DEAE-cellulose in 7 *M* urea. The elution was performed by the similar technique used in the condensation of the hexanucleotide and the trinucleotide. Oligonucleotides in the various peaks were identified by tlc and by base composition. The yield of the nonanucleotide was 5%.

Polymerization of the Protected Guanosine 3′-Phosphate and Preparation of Fully Acylated Dinucleotide 7b. The protected mononucleotide G^{iBu}(OiBu)-p (4 equiv) and the chain terminator iBuO-G^{iBu}(OiBu)-p (1 equiv) were polymerized by dicyclohexylcarbodiimide (DCC) in pyridine for 5 days. The reaction mixture was treated with acetic anhydride to cleave pyrophosphates.[13] The products with protecting groups were separated by ion-exchange chromatography on a TEAE-cellulose column. The elution pattern is shown in Figure 5. The identification of the material in the peak fractions is given in Table II. The linear oligonucleotides were identified by paper chromatography of the deacylated products before and after phosphatase treatment. Their R_f values are shown in Table I. The dephosphorylated homologs were hydrolyzed by RNase T_1 and the ratio of guanosine to guanosine 3′-phosphate was measured. The pure dinucleotide R-G^{iBu}-(OiBu)-p-G^{iBu}(OiBu)-p (**1**) eluted in peak 8. The trinucleotide R-G^{iBu}(OiBu)-p-G^{iBu}(OiBu)-p-G^{iBu}-(O-Bu)-p in peak 11 was contaminated with the trinucleotide having a 2′,3′-cyclic phosphate end group. The tetranucleotide GpGpGpGp was purified by paper electrophoresis at pH 7.5 before and after removal of the phosphomonoester end group. The 5′-hydroxyl group of the dinucleotide **7**, which was partially acylated, was acetylated before condensation with acetic anhydride in the presence of tetraethylammonium acetate.[14]

Synthesis of the Hexanucleotide 9 by Condensation of the Dinucleotide 7 and the Tetranucleotide 8. The steps to synthesize the tetranucleotide **8** are shown in Chart V. The principle of the synthesis was the same

(13) H. G. Khorana, J. P. Vizsolyi, and R. K. Ralph, *J. Amer. Chem. Soc.*, **84**, 414 (1962).
(14) D. H. Rammler, Y. Lapidot, and H. G. Khorana, *ibid.*, **85**, 1989 (1963).

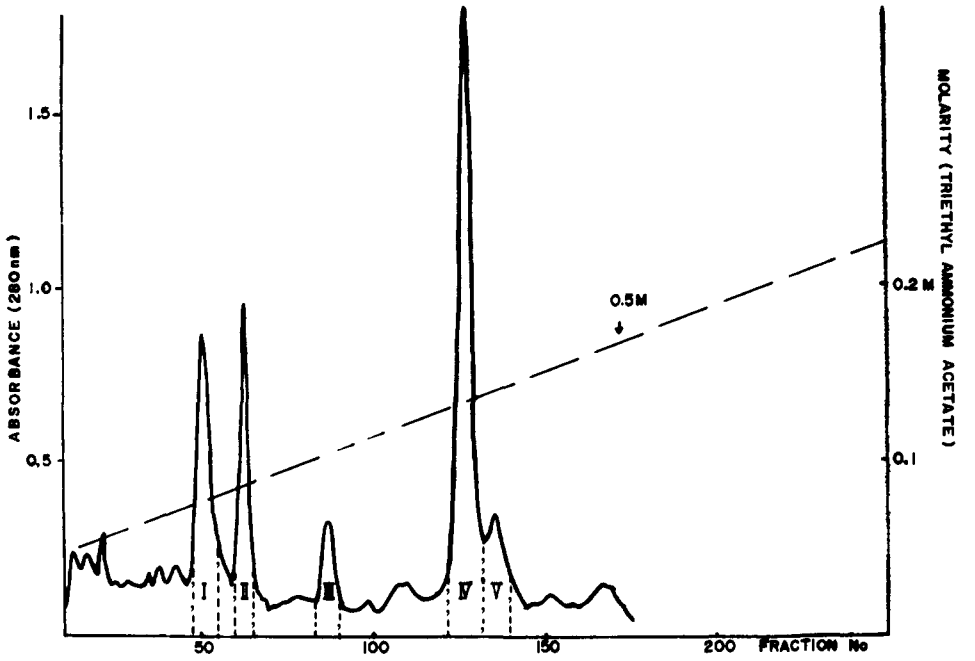


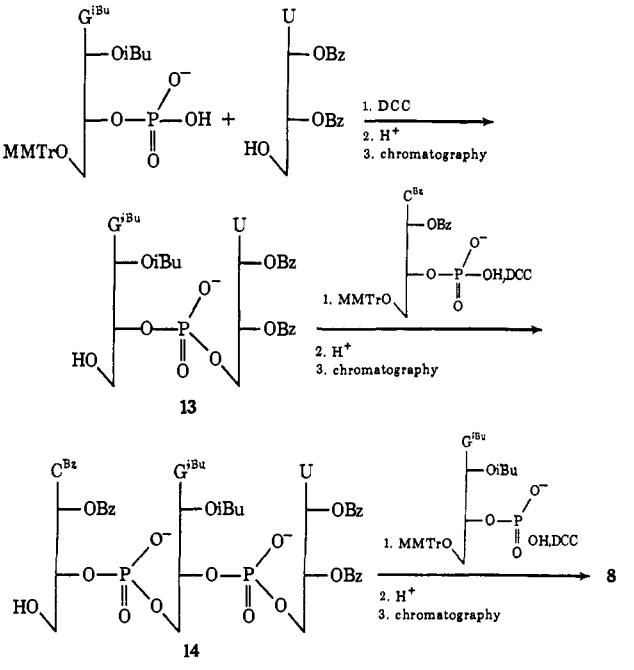
Figure 6. Chromatography of the products in the synthesis of the tetranucleotide **8** on a column (1.7 × 30 cm) of TEAE-cellulose (acetate) equilibrated with 0.02 *M* triethylammonium acetate in 70% ethanol. Elution was carried out using a linear gradient of triethylammonium acetate. The mixing chamber contained 0.05 *M* salt in 70% ethanol (2 l.) and the reservoir contained 0.25 *M* salt. Fractions of 16 ml were collected every 30 min. Identification of peaks is shown in Table III.

Table II. Identification of Peaks in Figure 5

Peak	Fractions pooled	Total A_{260} units	Identification
1	20–29	413	5′-C-pyridinium G^{iBu}(OiBu)-p cyclic mononucleotide
2	30–36	711	
3	37–47	256	N-Deacylated cyclic phosphates
4	48–56	462	G^{iBu}(OiBu)-p
5	57–69	692	Pyrophosphate of the mononucleotide
6	70–82	675	Cyclic dinucleotide
7	83–89	146	Unidentified
8	90–109	680	Linear dinucleotide
9	110–128	545	Cyclic trinucleotides
10	129–136	180	Unidentified
11	137–150	433	Linear trinucleotide (75%)
12	151–173	509	Cyclic tetranucleotides
13	174–194	366	Linear tetranucleotide
14	195–210	308	Unidentified
15	1 *M* fraction	1100	Higher oligomers

as described for that of the 3′-terminal trinucleotide of yeast alanine tRNA.[2] The dinucleoside monophosphate G^{iBu}(OiBu)-p-U$(OBz)_2$ (**13**) was synthesized by condensing 2′,3′-*O*-dibenzoyluridine with 5′-*O*-monomethoxytrityl-*N*,2′-*O*-diisobutyrylguanosine 3′-phosphate using DCC. After removal of the trityl group, the product (**13**) was isolated by extraction with butanol and precipitation into ether which separates the protected nucleoside. The isolated yield was 46%. For the synthesis of the trimer **14**, the dimer **13** was allowed to react with 5′-*O*-monomethoxytrityl-*N*,2′-*O*-dibenzoylcytidine 3′-phosphate using DCC as the condensing reagent, and the product was isolated by anion exchange chromatography on a TEAE-cellulose column. The yield was 26%. The tetranucleotide **8** was synthesized by condensing MMTr-G^{iBu}(OiBu)-p with the trinucleotide **14** using DCC. The reaction mixture was subjected to gel filtration

Chart V



on Sephadex LH-20. The first peak contained the tetranucleotide, the trinucleotide, and the mononucleotide as judged by ppc. The yield of the desired compound was 24% and it was 42% pure. In order to purify the compound completely, the mixture was treated with 80% acetic acid to remove the trityl group and chromatographed on a TEAE-cellulose column. The elution pattern and conditions are shown in Figure 6. The identification of some of the peaks is given in Table III. The isolated yield of the tetranucleotide (**2**) was 15%.

As shown in Chart II, the fully acylated dinucleotide **7** and the tetranucleotide bearing the free 5′-hydroxyl

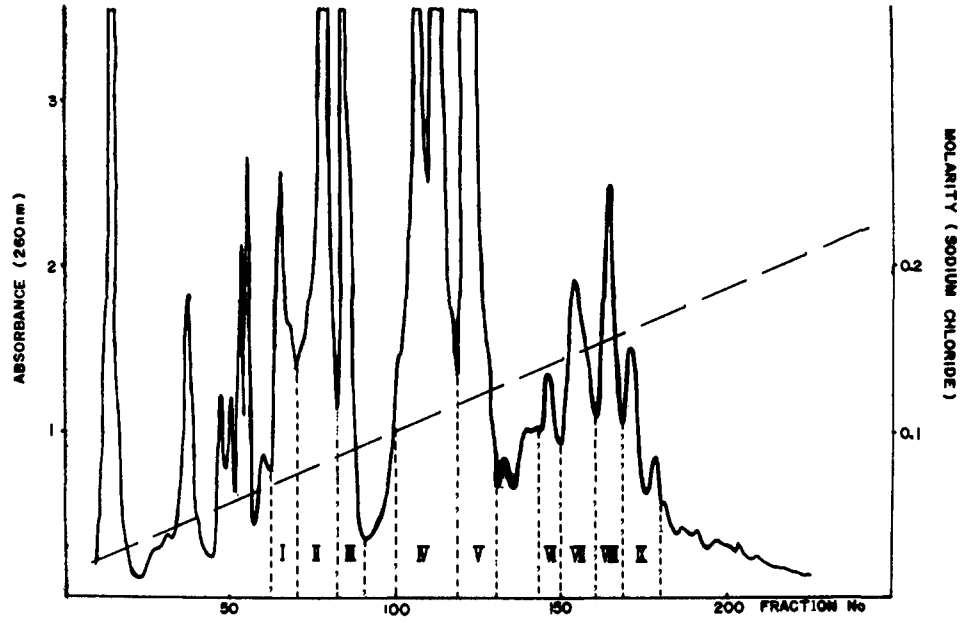


Figure 7. Chromatography of the products obtained in the synthesis of the hexanucleotide GpGpGpCpGpU on a column (0.7 × 90 cm) of DEAE-cellulose equilibrated with 7 *M* urea in 0.02 *M* Tris-HCl (pH 7.5). Elution was carried out using a linear gradient of sodium chloride. The mixing chamber contained 0.025 *M* sodium chloride in addition to the equilibration buffer (300 ml) and the reservoir contained 0.3 *M* salt. Fractions of 2.2 ml were collected every 40 min. Peak VIII contained the hexanucleotide GpGpGpCpGpU and peak V contained the dinucleotide GpGp.

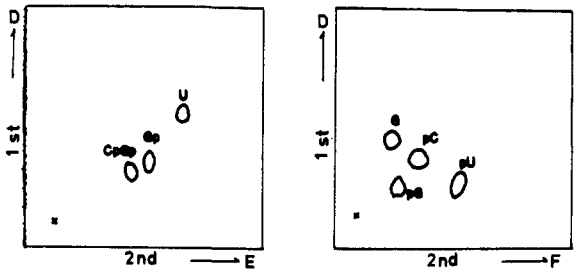


Figure 8. Two-dimensional tlc of the products of RNase St digestion (left) and those of venom phosphodiesterase digestion (right) of the hexanucleotide GpGpGpCpGpU. Solvent systems used were as indicated.

Table III. Identification of Peaks in Figure 6

Peak	Total A_{260} units	Identification
I	86	C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U(OBz)$_2$
II	61	Pyrophosphate of G^{iBu}(OiBu)-p
III	38	G^{iBu}(OiBu)-p-G^{iBu}(OiBu)-p
IV	292	G^{iBu}(OiBu)-p-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U(OBz)$_2$
V	63	Partially deacylated product

group (**8**) were condensed in pyridine using triisopropylbenzenesulfonyl chloride (TPS) as the activating reagent. The mixture was deacylated and subjected to DEAE-cellulose chromatography in 7 *M* urea. The elution pattern is shown in Figure 7. Peak VIII contained the hexanucleotide. The yield at this stage was 15% using a calculated extinction coefficient of 3.17×10^4 for GpGpG and 2.75×10^4 for CpGpU.[15] The hexanucleotide in peak VIII was rechromatographed on a DEAE-cellulose column. The product was characterized by base analysis after enzymatic hydrolysis in the nucleic acid analyzer. The digested products were also analyzed by two-dimensional thin layer chromatography[8] (Figure 8).

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General Comments

The ribononanucleotide having the sequence like the one found at the 3′ end of yeast alanine tRNA was synthesized by condensation of mono- or oligonucleotide blocks. This is the longest ribonucleotide obtained by chemical synthesis. Two approaches, condensing the hexanucleotide with either mono-, di-, or trinucleotides, gave almost the same yield. The rate of activation of ribodi- and ribotrinucleotides with arylsulfonyl chloride is not known. In the deoxyoligonucleotide field a systematic study on the condensation of thymidine oligonucleotides showed the dinucleotide reacted faster than the trinucleotide.[16] However, our results indicate no large difference in reactivity. Although other nucleotides containing heterocyclic amino groups have different reactivity or stability, it is plausible to think that smaller fragments can be activated more easily and usually in our reaction the concentration of the terminal phosphate is higher when smaller oligonucleotides are used. Though sulfonylation of oligonucleotides was not found so far, the main reason for low yields in condensations of mono- and dinucleotides appeared to be the degradative loss of oligonucleotides caused by the activating reagent. Relation between the reactivity of arylsulfonyl chlorides and cleavage of phosphodiester linkages has to be studied in a rather simple system. Block condensations with a protected ribodinucleotide U(OBz)-p-U(OBz)-p are in progress to investigate these questions.

Chemical polymerization of the ribomononucleotides Ap and Up has been performed by Khorana

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and his coworkers.[17,18] Although the chemically polymerized products were much shorter than those of enzymatic polymerization, the chemically polymerized oligonucleotides are useful for further synthesis since they possess N- and O-protecting groups. In the present paper guanosine 3′-phosphate was polymerized and one of the products, the protected dinucleotide **7**, was used in the block condensation reaction. To obtain homologous oligonucleotides polymerization does not require the protection of phosphomonoesters. The yield of a homolog, however, might be lower than those obtained by the stepwise condensation. The yield of the dinucleotide R-G^{iBu}(OiBu)-p-G^{iBu}(OiBu)-p (**7**) was 9% of the starting mononucleotide. It has been reported that the yields in condensations involving a guanosine phosphate are lower than those obtained with the other mononucleotides.[19] For example, the extent of polymerization of the guanine derivative[20] was also lower in deoxy series compared with that of thymine.[21]

The stepwise condensation to give the protected tetranucleotide **8** was carried out by the same method used for the synthesis of the trinucleotide. Principally, the hexanucleotide could be synthesized by this procedure. Repeated purification by ion exchange column chromatography in each step seems to be disadvantageous for the growing oligonucleotide. The low yield of the tetranucleotide **8** after DEAE-cellulose chromatography was caused by the poor recovery from the anion exchange cellulose. The final block condensation of the tetranucleotide **8** with the dinucleotide **7** yielded the hexanucleotide GpGpGpCpGpU. The pattern of the chromatography of the products on DEAE-cellulose in 7 *M* urea was rather complex. Higher temperature might have been needed to destack guanosine oligomers. It may be emphasized that these chemically synthesized nona- and hexanucleotides constitute the double helical amino acid acceptor stem of the yeast alanine tRNA. Physicochemical and biochemical properties of these ribooligonucleotides are under investigation.

Experimental Section

General Methods. Paper chromatography was performed by the descending technique in the following solvents: A, 2-propanol–concentrated ammonia–water (7:1:2, v/v); B, ethanol–1 *M* ammonium acetate, pH 7.5 (7:3, v/v); C, 1-propanol–concentrated ammonia–water (55:10:35, v/v). For two dimensional chromatography, a thin layer of cellulose (10 × 10 cm) was irrigated first with isobutyric acid–0.5 *M* ammonium hydroxide (5:3, v/v)[8a] (solvent D) and second with either *tert*-butyl alcohol–ammonium formate, pH 3.8 (1:1, v/v)[22] (solvent E) or 2-propanol–concentrated hydrochloric acid–water (70:15:15, v/v)[8a] (solvent F). Paper electrophoresis was performed at 700 V/40 cm using 0.05 *M* ammonium formate, pH 2.7, or at 900 V/40 cm using 0.05 *M* triethylammonium bicarbonate, pH 7.5.

Bacterial alkaline phosphatase and venom phosphodiesterase were purchased from Worthington Biochemical Corp. and RNase T_1 and pancreatic RNase were from Sankyo Co. Ltd. and Boehringer Biochemical Corp., respectively. RNase St was a generous gift from Dr. N. Yoshida of Shionogi Research Institute. RNase M was donated by Dr. M. Irie of Kyoto University and used for non-base-specific hydrolysis of oligonucleotides. For enzymic digests: oligonucleotides (*ca.* 2–3 A_{260}) were incubated at 37° for 6 hr with either bacterial alkaline phosphatase (4 μg) in 0.05 *M* ammonium bicarbonate (100 μl), or pancreatic, T_1 or St ribonucleases (10–50 μg) in 0.1 *M* ammonium acetate, pH 7.5 (100 μl), RNase M (20 μg) in 0.1 *M* ammonium acetate, pH 5.0 (100 μl), venom phosphodiesterase (100 μg) in 0.1 *M* ammonium carbonate, pH 9.2 (100 μl). The enzymatically digested products (0.1–0.2 A_{260}) were analyzed by high pressure anion exchange chromatography[23] using a Varian LCS 1000 apparatus (nucleic acid analyzer). Other general methods were as described in previous papers.[1,2]

Preparation of the Dinucleotide, MMTr-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p (6). (a) Using TPS. Pyridinium 5′-*O*-monomethoxytrityl-*N*,2′-*O*-dibenzoylcytidine 3′-phosphate (0.6 mmol) and pyridinium *N*,2′-*O*-diisobutyrylguanosine 3′-phosphoranisidate (9400 A_{260}, 0.52 mmol) were treated with TPS (3.6 mmol) in anhydrous pyridine (2 ml) at 25° for 12 hr. Water (20 ml) and tri-*n*-butylamine (1.7 ml) in pyridine (20 ml) were added in an ice bath. After 10 hr at 25° the solution was extracted with 1-butanol, and the washed butanol phase was evaporated with added pyridine. The anhydrous solution was added to ether–pentane (3:2) and the precipitate was treated with isoamyl nitrite (4 ml) in pyridine (2 ml) and acetic acid (2 ml) for 4 hr at 25°. Volatile materials were removed by evaporation and the residue was dissolved in a small amount of pyridine and 70% ethanol (200 ml). The solution was applied to a column (2.0 × 50 cm) of TEAE-cellulose (acetate) in 70% ethanol. The elution was performed by a linear gradient of triethylammonium acetate. The mixing chamber contained 70% ethanol (4 l.) and the reservoir contained an equal volume of 0.2 *M* salt (0.2 *M* acetic acid and 0.1 *M* triethylamine). Fractions (19 ml) were collected every 10 min. Fractions 14–21 contained G^{iBu}(OiBu)-p (2400 A_{260} units); 22–40, MMTr-C^{Bz}(OBz)-p (9000 A_{260} units); 86–100, MMTr-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p (4300 A_{260} units); 101–120, MMTr-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p contaminated with the partially deacylated dimer (3400 A_{260} units). Fractions 86–100 were combined and evaporated to a small volume and dissolved in butanol (20 ml), and the solution was washed with a small volume of water repeatedly. The washings had no absorption at 305 nm. The organic layer was made anhydrous by evaporation with pyridine and precipitated in ether–pentane (3:2). The precipitate was washed with the same solvent three times. The yield was 4260 A_{260}, 0.12 mmol (24%). An aliquot was treated with 80% acetic acid for 1 hr at 25° and with 15 *N* methanolic ammonia for 16 hr at 25°. The unprotected dinucleotide CpGp was homogeneous in ppc in solvent A (Table I) and was completely hydrolyzed by pancreatic RNase to yield Cp (0.16 A_{260}) and Gp (0.24 A_{260}) in a ratio of 1.0:1.0.

(b) Using DCC. Pyridinium 5′-*O*-monoethoxytrityl-*N*,2′-*O*-dibenzoylcytidine 3′-phosphate (0.3 mmol) and freshly precipitated pyridinium *N*,2′-diisobutyrylguanosine 3′-phosphate (3000 A_{260}, 0.20 mmol) were treated with DCC (3 mmol) in anhydrous pyridine (2 ml) in the presence of dry pyridinium Dowex 50-X2 (300 mg) for 5 days at 25°. Aqueous pyridine (50%, 20 ml) was added and DCC was extracted with *n*-pentane (10 ml) in two portions. After 12 hr at 25° the mixture was filtered and evaporated with added pyridine. The anhydrous residue was treated with isoamyl nitrite (2 ml) in pyridine (2 ml) and acetic acid (2 ml) at 25° for 4 hr. The reaction mixture was worked up as described in a and subjected to chromatography on a column (2.0 × 25 cm) of TEAE-cellulose (acetate) using the same conditions as in a. The product in the peak was homogeneous. After evaporation the protected dinucleotide **6** was isolated. The yield was 2430 A_{260} (0.065 mmol), 32%.

Preparation of the Trinucleotide, MMTr-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U(OBz)-p (2). Pyridinium dinucleotide **6** (4500 A_{305}, 0.3 mmol) and freshly reprecipitated pyridinium 2′-*O*-benzoyluridine 3′-phosphoranisidate (0.5 mmol) were treated with DCC (3.6 mmol) in pyridine (1.5 ml) in the presence of dry pyridinium Dowex 50-X2 (200 mg) at 25° for 6 days. Aqueous pyridine (50%, 20 ml) was added and DCC was extracted with *n*-pentane (20 ml, two portions). After 12 hr at 25° the mixture was filtered and the solution was evaporated with pyridine. The residue was dissolved in pyridine (5 ml) and precipitated in ether–pentane 3:2 (200 ml). The precipitate was treated with isoamyl nitrite (3.3 ml) in a mixture of pyridine (3.3 ml) and acetic acid (3.3 ml) at 25° for 4 hr. Volatile

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materials were evaporated and the residue was precipitated in ether–pentane (3:2) from its pyridine solution. The precipitate was dissolved in pyridine and 80% ethanol and applied to a column (2.8 × 58 cm) of TEAE-cellulose (acetate). The column was first eluted with a linear gradient of triethylammonium acetate in 80% ethanol (0.002–0.3 *M*), total volume being 6 l. and then using 0.3 *M* (1 l.) and 0.4 *M* (1 l.) of the same salt in 75% ethanol. Fractions of 19 ml were collected in 10 min. Fractions 291–343 contained the product **2** (3200 A_{260} units) and fractions 344–380 contained the product contaminated with the partially deacylated trinucleotide. The protected trinucleotide **2** was isolated as described in the synthesis of the dinucleotide **6**. The yield was 3150 A_{260} (67 μmol), 22%. The unprotected trinucleotide obtained from peak III was homogeneous in ppc in solvent C. About 3 A_{260} of CpGpUp was hydrolyzed with RNase M and subjected to ppc in solvent C. A spot which contained Cp and Up was transferred to paper electrophoresis at pH 2.7. The ratio of Cp:Up:Gp was found to be 1.0:1.0:1.0 as measured by absorption at 260 nm in 0.02 *N* HCl (Cp, 1.15 A_{280}; GP, 0.99 A_{260}; Up, 0.87 A_{260}).

Condensation of the Hexanucleotide 1 and the Trinucleotide 2. The hexanucleotide **1** (128 A_{305}, 2.7 μmol) and the trinucleotide **2** (540 A_{305}, 44 μmol) were treated with TPS (70 μmol) in pyridine (0.2 ml) at 20° for 3 hr. Aqueous pyridine (75%, 1 ml) was added in an ice bath and the solution was kept at 4° for 8 hr. 1-Butanol (2 ml) was added and the solution was washed with water (1 ml) in two portions. The organic layer was evaporated to a small volume and dissolved in 50% pyridine (2 ml) and 80% acetic acid (0.5 ml). The solution was kept at 4° for 24 hr and applied to a column (2 × 100 cm) of Sephadex LH-20 preequilibrated with 0.1 *M* triethylammonium acetate in 90% DMF. Peak I (in Figure 1) was evaporated and treated with 80% acetic acid at 25° for 1 hr. Acetic acid was evaporated and the residue was dissolved in 50% pyridine. The solution was evaporated with added water, and the residue was treated with 15 *N* methanolic ammonia for 16 hr at 25° and then dried with coevaporation of pyridine. The unprotected nucleotides were subjected to gel filtration on a column (2.0 × 80 cm) of Sephadex G-50 in 0.05 *M* triethylammonium bicarbonate (pH 7.5). Fractions of 2 ml were collected every hour. Fractions 52–74 (836 A_{280} units) contained the nonanucleotide and other oligonucleotides and fractions 75–83 (747 A_{280} units) contained materials smaller than the trinucleotide. The former fractions were chromatographed on DEAE-cellulose in 7 *M* urea. The pattern and the conditions of chromatography are shown in Figure 2. Peaks were desalted by gel filtration using a column (1.8 × 70 cm) of Biogel P-2 preequilibrated with chloroform saturated water adjusted to pH 7.5 with ammonia.[10] Peak III (19.0 A_{260}, 0.23 μmol) contained the nonanucleotide CpGpUpCpCpApCpCpA as identified by tlc on cellulose in solvent C and acidic electrophoresis. The base composition was assayed after RNase M and venom phosphodiesterase digestion using a nucleic acid analyzer. The ratio of Cp:Up:Ap:Gp was found to be 5.3:1.0:1.0:1.0 (theoretical, 5:1:1:1) and that of pC:pU:pA:pG was 4.0:1.0:2.3:0.73 (theoretical, 4:1:2:1). Spectral properties of the nonanucleotide CpGpUpCpCpApCpCpA are λ_{max} 261 nm, λ_{min} 232, $\epsilon_{280/260}$ 0.49 in 7 *M* urea, 0.02 *M* Tris–HCl (pH 7.5), and 0.17 *M* NaCl. The nonanucleotide was further characterized by two-dimensional tlc after pancreatic RNase and venom phosphodiesterase digestion as shown in Figure 3.

Synthesis of the Heptanucleotide 5. The triethylammonium salt of the hexanucleotide **1** (360 A_{305}, 7.6 μmol) and pyridinium MMTr-U(OBz)-p (160 μmol) were condensed with MS (53 mg, 0.24 mmol) in pyridine (0.3 ml) at 20° for 4 hr. Aqueous pyridine (50%, 10 ml) and tri-*n*-butylamine (47 μl) were added under cooling in an ice bath. The mixture was kept at 20° for 12 hr and concentrated with added pyridine to *ca.* 0.5 ml. The viscous solution was applied to a column (2 × 100 cm) of Sephadex LH-20 using the same condition as described in Figure 1. The first peak (984 A_{270} units) which contained mainly the hexa- and heptanucleotides was concentrated and the nucleotides were precipitated in ether–pentane 3:2 from their anhydrous pyridine solution. The precipitate was treated with 80% acetic acid for 1 hr and evaporated with water. The residue was dissolved in pyridine (1 ml) and 60% ethanol (100 ml) and applied to a column of DEAE-cellulose (acetate). The elution pattern and conditions are shown in Figure 4. Peak II contained essentially pure heptanucleotide **5**, 125 A_{260} (0.59 μmol), 8%. The product was characterized after removal of protecting groups by tlc in solvent C. Pancreatic RNase digestion gave Up, Cp, ApCp, and adenosine as examined by tlc in solvent D and solvent E (two-dimensional chromatography).

Condensation of the Heptanucleotide 5 and the Dinucleotide 6. The triethylammonium salt of the heptanucleotide **5** (27 A_{305}, 0.59 μmol) and the triethylammonium salt of the dinucleotide **6** (250 A_{305}, 25 μmol) were treated with MS (16.8 mg, 75 μmol) in pyridine (0.1 ml) at 20° for 4 hr. Aqueous pyridine (50%, 10 ml) was added and the solution was kept at 20° for 12 hr. The solution was evaporated with added pyridine and the residue was dissolved in pyridine (0.2 ml). The concentrated solution was applied to a column (2 × 100 cm) of Sephadex LH-20 equilibrated with 0.1 *M* triethylammonium acetate in 90% DMF. Fractions of 5 ml were collected every 20 min. Fractions 29–40 (276 A_{280}) were combined and treated with 80% acetic acid and then with 15 *N* methanolic ammonia as described in the condensation of the hexanucleotide **1** with the trinucleotide **2**. The deprotected product was chromatographed on a column (0.9 × 90 cm) of DEAE-cellulose (chloride) using the elution condition similar to Figure 2. The dinucleotide CpGp (45.5 A_{260}) was eluted at salt concentration of 0.145 *M*, the heptanucleotide (15.5 A_{260}) at 0.15 *M*, and the nonanucleotide (5.9 A_{260}) at 0.16 *M*. The nonanucleotide was contaminated with a faster traveling compound (R_{GP} 0.78) in tlc in solvent C and further purified by the same system. The yield was 2.3 A_{260}, 0.028 μmol, 5%. Base analysis using RNase M gave Cp:Up:Ap:Gp = 5.0:1.4:0.7:1.2 (theoretical, 5:1:1:1).

Polymerization of G^{iBu}(OiBu)-p and iBuO-G^{iBu}(OiBu)-p. Pyridinium *N*,2′-*O*-diisobutyrylguanosine 3′-phosphate[1] (1.0 mmol) and *N*,2′-*O*,5′-*O*-triisobutyrylguanosine 3′-phosphate (0.25 mmol) were passed through a small column of pyridinium Dowex 50-X2 in 50% aqueous pyridine. The nucleotides were precipitated from the anhydrous pyridine solution with ether–pentane 3:2. The precipitate and pyridinium Dowex 50-X2 (200 mg) were rendered anhydrous with added pyridine and treated with DCC (3.0 mmol) in pyridine (2 ml) for 7 days. Aqueous pyridine (50%, 2 ml) was added and the mixture was extracted with pentane. The aqueous pyridine solution was kept at 4° for 24 hr. One-half of the mixture was applied to a column (2.2 × 34 cm) of TEAE-cellulose equilibrated with 50% ethanol. The elution pattern and conditions are shown in Figure 5. The identification of some of the peaks is summarized in Table II. Unprotected linear oligonucleotides obtained from peaks 8, 11, and 12 were sensitive to bacterial alkaline phosphatase, and dephosphorylated products were hydrolyzed with RNase T_1 to yield guanosine 3′-phosphate and guanosine in the ratio of 1.0:1.0, 1.9:1.0, and 2.8:1.0, respectively. R_f values and electrophoretic mobilities of oligonucleotides with and without phosphate end groups are given in Table I. The protected dinucleotide 7 in peak 8 was extracted from the concentrated aqueous buffer with 1-butanol and the organic layer was washed with water. 1-Butanol was evaporated with added pyridine and the anhydrous pyridine solution was added to a mixture of ether–pentane (3:2) where the dinucleotide precipitated.

Acetylation of the 5′-Hydroxyl Group of the Dinucleotide 7. The dinucleotide 7 (640 A_{260}) obtained from peak 8 and tetraethylammonium acetate (0.5 mmol) were rendered anhydrous by coevaporation with added pyridine. The dry residue was evaporated with toluene three times and treated with acetic anhydride at 21° for 2 days. Acetic anhydride was evaporated and 50% aqueous pyridine was added at 0°. The mixture was passed through a column (2 × 15 cm) of pyridinium Dowex 50-X2 and kept at room temperature for 2 hr. The solution was evaporated at 10° and the residue was made anhydrous by coevaporation with added pyridine. The product was precipitated with 3:2 ether–pentane from its pyridine solution. The yield was 600 A_{260}.

G^{iBu}(OiBu)-p-U(OBz)$_2$ (13). Pyridinium MMTr-G^{iBu}(OiBu)-p (13,600 A_{280} units, 1.1 mmol) passed through a column of pyridinium Dowex 50-X2 was made anhydrous by coevaporation of pyridine and condensed with 2′,3′-*O*-dibenzoyluridine (452 mg, 1 mmol) using DCC (2.06 g, 10 mmol) in pyridine (5 ml) for 70 hr at 25°. Water (5 ml) was added and the mixture was extracted with *n*-pentane and kept at room temperature for 20 hr. The solution was filtered and evaporated to dryness. The residue was coevaporated with toluene and treated with 80% acetic acid (40 ml), at 28° for 1 hr. The solution was concentrated and acetic acid was removed by coevaporation with aqueous ethanol. The residue was dissolved in butanol (20 ml) and 10% aqueous pyridine (10 ml). The butanol phase was extracted with 10% aqueous pyridine until no G^{iBu}(OiBu)-p was detected by paper electrophoresis and made anhydrous by evaporation of added pyridine. The pyridine solution (5 ml) was precipitated with ether (200 ml) and the precipitated dinucleotide **13** was washed with ether three times. Unchanged starting material U(OBz)$_2$ was recovered in the supernatant. The yield was 12,100 A_{260}, 0.46 mmol, 46%.

C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U(OBz)$_2$ (14). Pyridinium G^{iBu}-

(OiBu)-p-U(OBz)$_2$ (6900 A_{260}, 0.26 mmol) was allowed to react with pyridinium MMTr-C^{Bz}(OBz)-p (1 mmol) and DCC (2 g) in pyridine (5 ml) for 72 hr. The reaction mixture was treated with aqueous pyridine and extracted with *n*-pentane. Cyclohexylurea was removed by filtration and the solution was evaporated with pyridine. The pyridine solution was precipitated with ether and the precipitate was washed with ether. The powder was treated with 80% acetic acid for 1 hr at 25°. Acetic acid was evaporated with added aqueous ethanol and the residue was dissolved in pyridine (5 ml) and 70% ethanol. The solution was applied to a column (3 × 45 cm) of TEAE-cellulose (acetate) preequilibrated with 70% ethanol. The trinucleotide **14** was eluted at salt concentration of about 0.11 *M*. The yield was 2690 A_{280} units, 0.06 mmol, 26%.

G^{iBu}(OiBu)-p-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U(OBz)$_2$ (8). The pyridinium salt of trinucleotide **14** (1260 A_{280} units, 0.03 mmol) was allowed to react with pyridinium MMTr-G^{iBu}(OiBu)-p (0.25 mmol) using DCC (2 mmol) in pyridine (1 ml) for 81 hr. Water (1 ml) was added and the mixture was kept at room temperature for 12 hr and concentrated to *ca.* 0.5 ml. The solution was applied to a column (1.5 × 25) of Sephadex LH-20 preequilibrated with 90% ethanol. The fractions of 2 ml were collected and fractions (51–57) which contained the product (1080 A_{280} units) were combined. The purity of the tetranucleotide was checked by ppc and found to be 42%. The yield was 24%. The tetranucleotide was further purified by ion-exchange chromatography on a TEAE-cellulose column (1.7 × 30 cm). The elution pattern is shown in Figure 6 and the identification of some of the peaks is given in Table III. Peak IV contained the tetranucleotide 292 A_{260} units (4.7 μmol), 15%.

Condensation of R-G^{iBu}(OiBu)-p-G^{iBu}(OiBu)-p (7b) with G^{iBu}-(OiBu)-p-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U(OBz)$_2$ (8). The tetranucleotide **8** (230 A_{260}, 3.7 μmol) and the dinucleotide 7 (600 A_{260}, 19 μmol) were treated with TPS (67 μmol) in pyridine (0.2 ml) at 22° for 6 hr. Aqueous pyridine (50%, 0.5 ml) and triethylamine (0.13 mmol) were added at 0° and the mixture was kept at room temperature for 16 hr. The solution was evaporated with pyridine and the dry residue was treated with 15 *M* methanolic ammonia (15 ml) for 18 hr at 26°. Volatile materials were evaporated and the residue was dissolved in 7 *M* urea (10 ml). The solution was adjusted to pH 7.5 with 2 *N* ammonium hydroxide and applied to a column (0.7 × 80 cm) of DEAE-cellulose equilibrated with 7 *M* urea and 0.02 *M* Tris–HCl, pH 7.5. The elution pattern and conditions are shown in Figure 7. Peak VIII contained the hexanucleotide, GpGpGpCpGpU (34 A_{260} units, 0.57 μmol). Fractions were combined and desalted by gel filtration[10] using a column (2 × 60 cm) of Biogel P-2 (100–200 mesh). The desalted oligonucleotide was subjected to paper electrophoresis and found to be homogeneous. In paper chromatography, however, two spots were detected besides the compound at the origin. An aliquot (8 A_{260} units) of the desalted product was subjected to rechromatography on a column (0.7 × 90 cm) of DEAE-cellulose equilibrated with 7 *M* urea and 0.02 *M* Tris–HCl. Elution was performed with a gradient of sodium chloride, 0.1 *M* (80 ml) in 7 *M* urea and 0.02 *M* Tris–HCl. Fractions of the main peak (2.7 A_{260} units) were desalted as described above. The hexanucleotide was characterized by two-dimensional tlc after RNase St[24] and venom phosphodiesterase digestion as shown in Figure 8. Base analysis by digestion with pancreatic RNase and RNase St gave Gp and Cp in a ratio of 4.3:1.0, theoretical being 4:1. Venom phosphodiesterase digestion gave the ratio pG:pU:pC = 2.8:1.0:1.0 (theoretical, 3:1:1).

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Ab Initio Calculations on Large Molecules Using Molecular Fragments. Polypeptides of Glycine[1a]

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Abstract: The *ab initio* SCF molecular fragment approach is applied to a study of mono-, di-, tri-, tetra-, and pentapeptides of glycine. Fully extended, anti-parallel-chain pleated sheet, parallel-chain pleated sheet, and α-helix conformations are studied. Trends in total energy, orbital energies, populations, and bond orders are discussed, and intramolecular hydrogen bonding is identified as an important stabilizing force in the α-helix conformation of the tetra- and pentapeptides of glycine.

The use of X-ray diffraction techniques[2] and other physical and chemical methods of analysis[3] has led to major advances in the knowledge of the geometric structure of polypeptides. However, even though such techniques may, in optimum cases, reveal the detailed coordinates of virtually every nonhydrogen nucleus in a polypeptide, the elucidation of the forces giving rise to these structures (*i.e.*, the reasons for the stability of such conformations) and how stable they will be relative to external perturbations are extremely difficult to extract. Consequently, there is a need for additional analytical techniques that do not need to introduce, *via ad hoc* postulates, the kinds of forces that may be acting, but rather, that will allow the nature of the interactions to be extracted from the results of studies utilizing the analytical techniques. Such a possibility exists in principle through the use of the techniques of molecular quantum mechanics. This work is a report of the application of an *ab initio* quantum mechanical technique to a study of polyglycine, and will show how the nature of the forces

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