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# Studies on Transfer Ribonucleic Acids and Related Compounds. XXI.[1] Synthesis and Properties of Guanine Rich Fragments from *E. coli* $tRNA_f^{Met}$ 5′-End

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A hexanucleotide GpGpGpUpGpG corresponding to bases 5—10 of the $tRNA_f^{Met}$ from *E. coli.* has been synthesized by stepwise addition of mononucleotides. The segment contained five guanine bases out of six nucleotides. We have improved the protection of the 2′,3′-hydroxyl groups of guanosine and guanosine 3′-phosphate by replacing to avoid undesirable deblocking during elongation of the chain. Condensation of 5′-O-monomethoxytrityl-2′-O-benzoyl-N-isobutyrylguanosine 3′-phosphate (9) with 2′,3′-O-dibenzoyl-N-isobutyrylguansoine (5) gave protected GpG in a yield of 62%. Subsequent addition of mononucleotides to the growing chain was performed after selective removal of the 5′-monomethoxytrityl group. Dicyclohexylcarbodiimide (DCC) was used as condensing reagent throughout the synthesis. The excess of mononucleotide and the yield in each addition reaction were as follows: UpGpG (protected) 2.2 fold, 28%, GpUpGpG (protected), 5 fold, 32%; GpGpUpGpG (protected) 10 fold, 35%; GpGpGpUpGpG, 10 fold, 12%. The protected intermediate oligomers were isolated by ion-exchange chromatography on TEAE-cellulose columns and identified by enzymatic hydrolyses after deblocking. The hexanucleotide was separated by gel filtration on Sephadex LH-20 and purified on DEAE-cellulose in the presence of 7 M urea at 55°. The circular dichroism spectra of these guanine rich ribooligomers have measured and a marked difference between the pentamer GpGpUpGpG and the hexamer GpGpGpUpGpG has been observed.

**Keywords**——phosphodiester synthesis; stepwise synthesis of ribooligonucleotides; guanine rich ribooligonucleotides; CD spectra of ribooligonucleotides; hypochromicity of ribooligonucleotides

For the synthesis of a tRNA molecule, chemical synthesis of ribooligonucleotides with defined sequences is the first step. We have been investigating synthesis of ribooligonucleotides by both blockwise[3] and stepwise[4] condensations, mainly using the diester method.[5] The triester approach has been reported by several investigators.[6] A combination of these methods has been claimed to have certain advantages[7] and we have found that the yield of the last condensation step in the synthesis of a trinucleoside diphosphate was higher in such a "combined method" synthesis than that in an analogous diester synthesis.[8,9] Recently we have been concentrating on the synthesis of $tRNA_f^{Met}$ fragments from *E. coli*. The 3′-terminal trimers CpCpA and CpApA have been synthesized[9] with partial 2′-O-(*o*-nitro-

1) Part XX: E. Ohtsuka, T. Tanaka, S. Tanaka, and M. Ikehara, *J. Am. Chem. Soc.*, **100**, 4580 (1978).
2) Location: *133–1 Yamadakami, Suita 565, Japan.*
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benzyl) protection in order to investigate enzymatic joining by T4 induced RNA ligase.[10] A preliminary paper on the synthesis of CpCpCpCpCpG and UpCpCpGpG has been presented.[4b] The 5'-terminal tetranucleotide CpGpCpGp has been synthesized using *p*-methylsulfoxylanilidate group for phosphate protection.[11] The present paper reports synthesis of a hexanucleotide GpGpGpUpGpG which has the sequence corresponding to bases 5—10 of $tRNA_f^{Met}$ from *E. coli*. Since the present hexamer contained five guanosines out of the six nucleotides, we employed the condensation of mononucleotide with dicyclohexylcarbodiimide (DCC) which should give less side reactions. Yields in condensations involving guanosine 3'-phosphate have been lower than those involving other nucleotides. One reason for the low yields was considered to be the relative instability of the 2'-O-isobutyryl group of guanosine 3'-phosphate presumably due to a neighboring effect of the phosphomonoester. In order to increase the stability of the 2'-O-acyl group we have benzoylated the 2'-hydroxyl function and 5'-O-monomethoxytrityl-2'-O-benzoyl-N-isobutyrylguanosine 3'-phosphate has been used in the present synthesis. The 2',3'-hydroxyl groups of the terminal guanosine were also protected by benzoyl groups.

Properties of the guanine rich oligomers have been studied by measuring their hypochromicities and circular dichroism (CD) spectra. A marked structural difference appeared between the pentamer and the hexamer.

**Protection of Gunanosine and Guanosine 3'-Phosphate**

A synthetic scheme for $ibG(Bz)_2$[12] (**5**) is shown in Chart 1. Guanosine was treated with isobutyryl chloride to give tetraisobutyryl-guanosine (**1**) and **2** was obtained by treating **1**

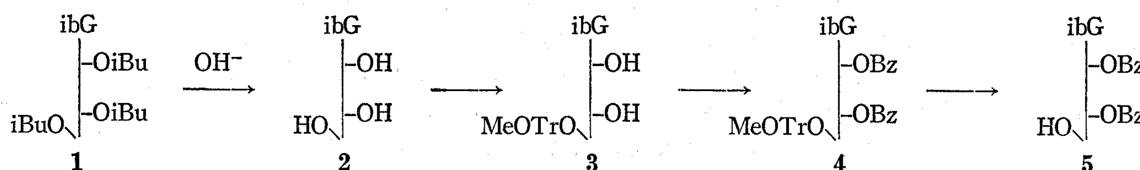


Chart 1

with 1 N sodium hydroxide at 0°. Treatment of **2** with monomethoxytrityl chloride yielded **3** and subsequent benzoylation was performed with benzoic anhydride at low temperature. When **3** was treated with benzoyl chloride, some *N*-benzoylation was observed. Demonomethoxytritylation of **4** with 80% acetic acid gave the product (**5**).

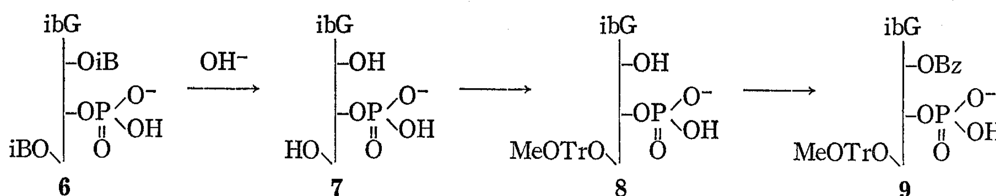


Chart 2

Protection of guanosine 3'phosphate was performed as shown in Chart 2. The triisobutyrylguanosine 3'-phosphate (**6**) was prepared by treatment of Gp (3') with isobutyric anhydride in the presence of isobutyrate anions.[13] **8** was synthesized by monomethoxytritylation of the *O*-deacylated compound **7**. Benzoylation of the 2'-hydroxyl group of **8**

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12) For IUPAC-IUB Commission recommended Biochemical Nomenclature see *J. Biol. Chem.*, **245**, 5171 (1970); *Proc. Nat. Acad. Sci. U.S.*, 74, 2222 (1977).
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with a limited amount of benzoic anhydride at low temperature in the presence of tetraethylammonium benzoate gave the fully protected product **9**.

**Synthesis of the Hexanucleotide (15)**

The hexanucleotide G–G–G–U–G–G (**15**) was synthesized according to a scheme shown in Chart 3. Protected guanosine (**5**) was condensed with the protected Gp (**9**) using DCC

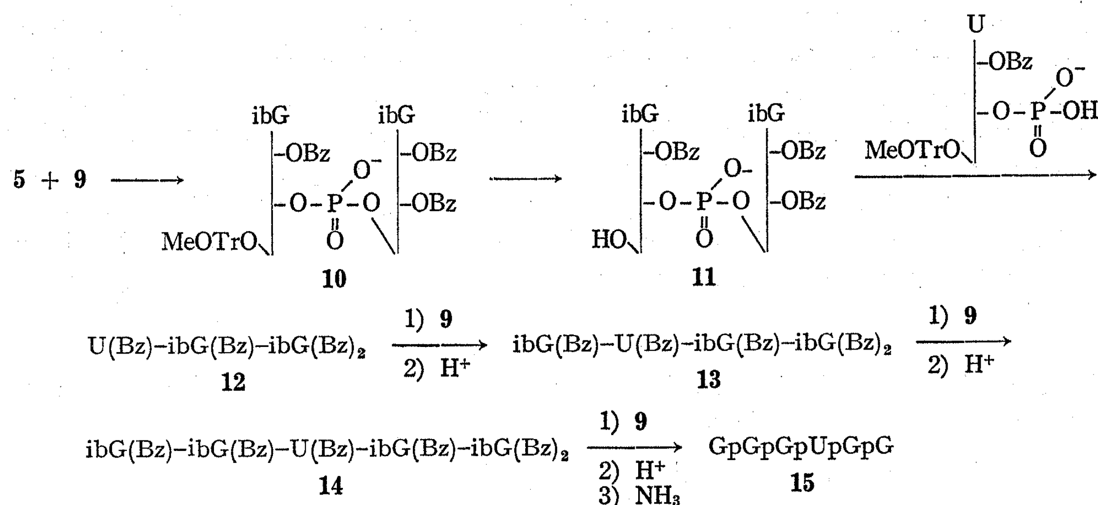


Chart 3

as the condensing reagent and the dimer **10** was isolated by precipitation from a mixture of ether and hexane. However, the product was contaminated with the starting materials and **10** was purified by chromatography on TEAE-cellulose column. The isolated yield of the monomethoxytritylated dimer **10** was 62%. After removal of the monomethoxytrityl group dinucleoside monophosphate (**11**) was allowed to react with a 2.2 fold excess of (MeOTr) U(Bz) p.[14] DCC was used throughout the series of syntheses. The trimer (**12**) was isolated by similar ion-exchange chromatography (Fig. 1) to that used for **10** and obtained in a yield of 28%. The tetramer (**13**) was synthesized by condensing **12** with a 5 fold excess of **9** in a yield of 32% (Fig. 2). To maintain a reasonable yield a large excess (10 fold) of the mononucleotide (**9**) was used in the next two reactions. In the synthesis of the pentamer (**14**) very litle of the unchangde tetramer (**13**) was recovered by TEAE-cellulose chromatography although the isolated yield of **14** was only 35%. Resolution of the oligonucleotides of this

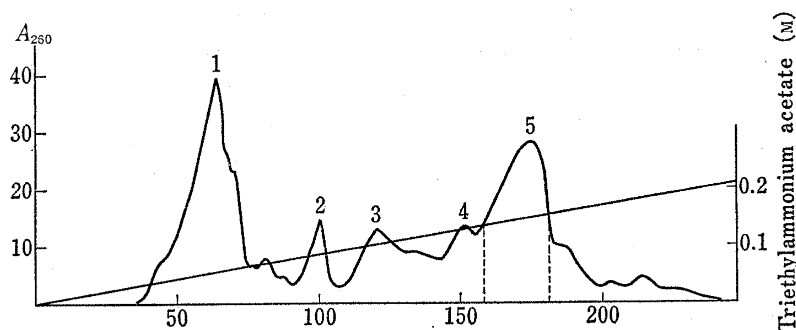


Fig. 1. Chromatography of the Mixture in the Synthesis of U(Bz)-ibG(Bz)-ibG(Bz$_2$) (**12**) on a Column (3.2×50 cm) of TEAE-Cellulose with a Gradient of 0 to 0.25 M Triethylammonium Acetate in 80% Ethanol (6 l)

Peak 1, U(Bz)>p and ibG(Bz)-ibG(Bz)$_2$; peak 2, U(Bz)p; Peak 5, the product.

14) This compound[5] contained the N-benzoylated derivative which had a slower mobility in electrophoresis.

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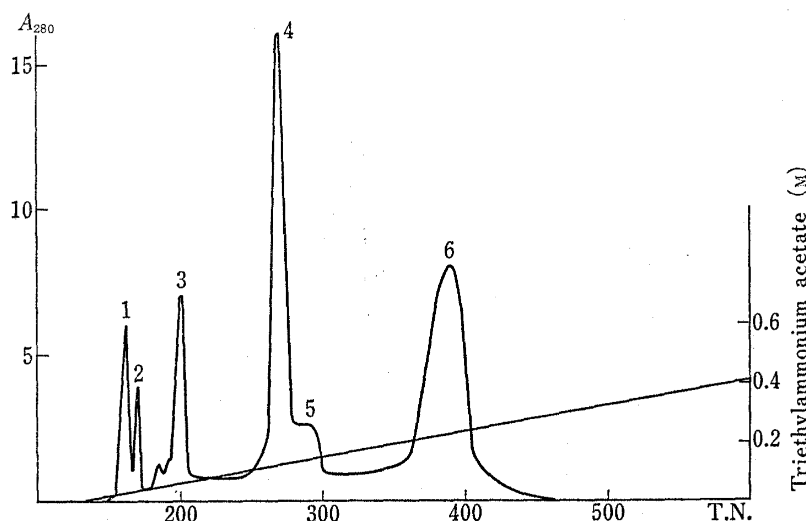


Fig. 2. Chromatography of the Mixture in the Synthesis of the Protected Tetranucleotide (**13**) on a Column (3.2×50 cm) of TEAE-Cellulose with a Gradient of 0 to 0.4 M Triethylammonium Acetate in 80% Ethanol (10 l)

Peak 3, ibG(Bz)p; peak 4, pyrophosphate of ibG(Bz)p; peak 5, (**12**); peak 6, the product (**13**).

size in this type of chromatography was not high. After the final condensation the mixture was first fractionated by gel filtration on Sephadex LH-20 (Fig. 3) following acid treatment. The fractions containing the hexamer (**15**) were deacylated and subjected to chromatography on DEAE-cellulose in the presence of 7 M urea[15] at 55°. This elevated temperature seemed to give a similar resolution as that obtained in a synthesis of guanine rich deoxyribohexanucleotide.[16] The elution profile is shown in Fig. 4. The last peak (**16**) contained almost pure product and peak 15 also contained the hexamer contaminated with a faster traveling compound in solvent B. The product GpGpGpUpGpG was desalted, isolated in a yield of 12%, and characterized by RNase M hydrolysis to give a correct nucleotide ratio. *Rf* values in paper chromatography and relative mobilities in paper electrophoresis of some of the protected and deprotected compounds are shown in Table I.

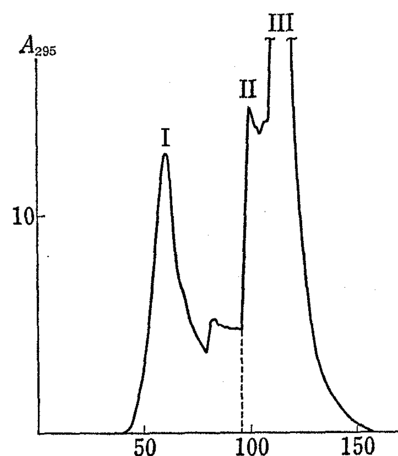


Fig. 3. Gel Filtration of the Mixture in the Synthesis of the Protected Hexanucleotide ibG(Bz)-ibG(Bz)-ibG(Bz)-U(Bz)-ibG(Bz)-ibG(Bz)$_2$ on a Column (2×74 cm) of Sephadex LH-20 in 95% Ethanol

Peak II and III contained mainly the mononucleotide and pyridine, respectively.

## Hypochromicities and CD Spectra of the Synthesized Oligonucleotides

Hypochromocities of the tri- to hexanucleotides were measured by enzymatic hydrolysis and were found not always to increase with the chain length. The results are summarized in Table II. UpGpG showed a larger hypochromicity than GpUpGpG. This may mean that the conformation of the tetramer is less ordered than that of the trimer. The CD spectra of these oligonucleotides are shown in Fig. 5 and Table II. The amplitude of the CD spectrum of UpGpG near 260 nm is larger than that of GpUpGpG although the peak of the tetramer

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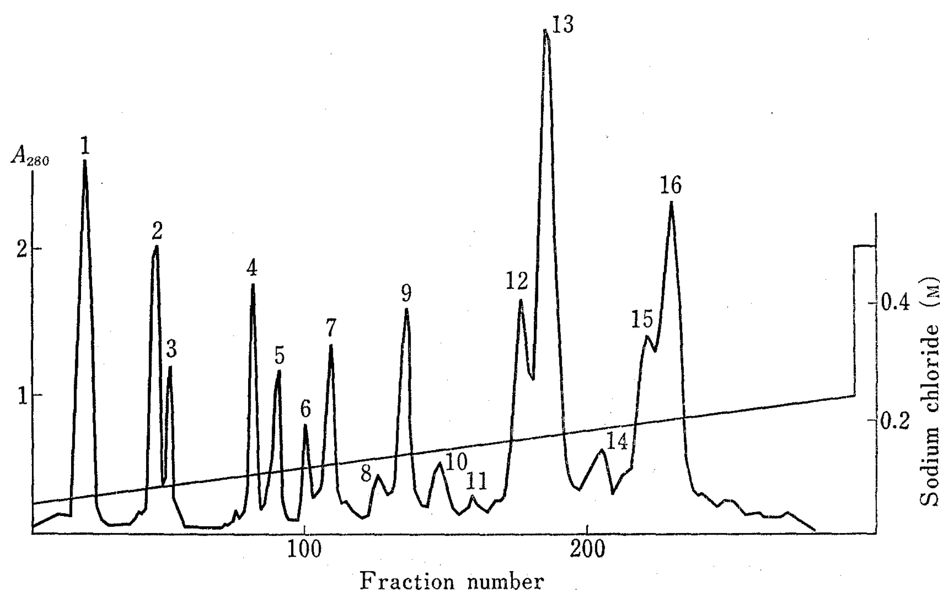


Fig. 4. Chromatography of the Hexanucleotide GpGpGpUpGpG on a Column (1.0×100 cm) of DEAE-Cellulose with a Gradient of 0.05M to 0.3M of Sodium Chloride (1 l) in 0.02 M Tris-HCl pH 7.5 and 7 M Urea at 55°

Fractions of 2.5 ml were collected every 20 min. Peak 16 contained the product.

Table I. Paper Chromatography and Electrophoresis

| Compound | *Rf* Solvent A | *Rf* Solvent B | *Rf* Solvent C | Relative mobility pH 7.5 |
|---|---|---|---|---|
| G | 2.2 | | 0.47 | 0 |
| Gp | 1 | 1 | 0.11 | 1 |
| G>p | 2.5 | | 0.44 | 0.6 |
| GpG | | | | 0.37 |
| UpGpG | 0.54 | | | 0.65 |
| GpUpGpG | 0.14 | | | 0.78 |
| GpGpUpGpG | 0.06 | 0.21 | | |
| GpGpGpUpGpG | | 0.08 | | |
| ibGp | | | 0.45 | 0.83 |
| ibG(Bz)p | | | 0.68 | 0.78 |
| (MeOTr)Gp | | | 0.47 | 0.71 |
| (MeOTr)ibGp | | | | 0.64 |
| (MeOTr)ibG(Bz)p | | | 0.85 | 0.56 |

Table II. Hypochromicity and CD Spectra of the Oligomers

| | Hypochromicity (%) | First extremam (nm) | $\times 10^{-4}$ | Crossover (nm) | Second extremam (nm) | $10\times^{-4}$ |
|---|---|---|---|---|---|---|
| UpGpG | 10.9 | 268 | 0.550 | 250 | 242 | −0.275 |
| GpUpGpG | 8.6 | 265 | 0.325 | 252 | 240 | −0.361 |
| GpGpUpGpG | 18.4 | 262 | 0.505 | 250 | 240 | −0.387 |
| GpGpGpUpGpG | 19.7 | 261.5 | 1.69 | 248 | 240 | −0.395 |

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is slightly blue-shifted. This is consistent with the unstacked nature of the tetramer observed in the hypochromicity measurements. However it is not clear whether or not the unstacking of GpUpGpG is due to the known relatively unstacked conformation of UpG.[17] Although the pentamer GpGpUpGpG showed a significant hypochromicity increase, the CD spectrum indicated only a slight blue-shift and the increase in the amplitude is small. However the CD spectra of the hexamer GpGpGpUpGpG showed a significant amplitude increase with a peak at 261 nm and a trough at 240 nm. The difference between the CD spectra of the pentamer and the hexamer may indicate that a marked conformational change occurrs between these sizes. On the other hand, it is possible that formation of gels[18] may become easier at certain chain lengths in these guanosine rich oligonucleotides.

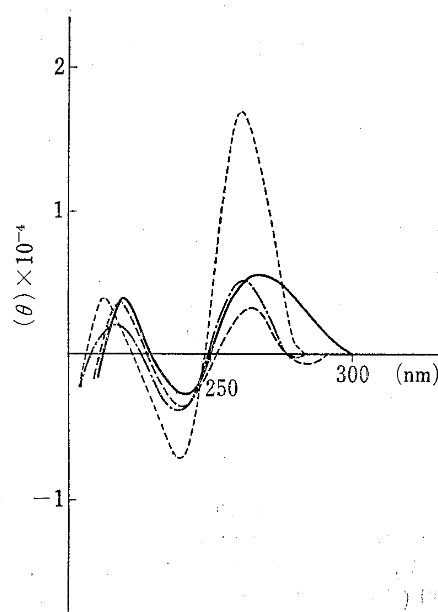


Fig. 5. CD Spectra of Oligonucleotides in 0.01 M Potassium Phosphate pH 7.0 and 0.1 M Potassium Fluoride at 25—28°

———, UpGpG.
———, GpUpGpG.
———, GpGpUpGpG.
-----------, GpGpGpUpGpG.

**Conclusions.**

Condensation of mononucleotides using DCC as the condensing reagent provided a straightforward method for the synthesis of a hexanucleotide. The present hexanucleotide contained five guanosines out of six nucleosides and was expected to be a less reactive sequence for chemical condensations. The yields of these oligonucleotides were not lower than those obtained in a previous synthesis of the pentamer UpApm$^2$GpCpC.[4] Stepwise synthesis of this type should be applicable to any hexanucleotide since the guanosine rich hexanucleotide was obtained in a reasonable yield. The use of the 2′-O-benzoylated derivative of guanosine 3′-phosphate improved the stability of protected oligonucleotides and prevented formation of complicated mixtures during purification of the intermediates. Further improvements in isolation methods for protected oligonucleotides seem necessary to shorten the time of syntheses. A combination of ion-exchange chromatography and gel filtration would be possible if acetic anhydride treatment were given after each condensation.[4b] In the present synthesis gel filtration on Sephadex LH-20 was used only at the last step and this facilitated the final purification by ion-exchange chromatography on DEAE-cellulose in 7 M urea.

The hexanucleotide GpGpGpUpGpG was phosphorylated with polynucleotide kinase[19] using [$\gamma$-$^{32}$P]ATP and joined to the previously synthesized CpGpCpG[11] by RNA ligase[20] in a yield of 25%. The guanosine rich hexanucleotide was thus proved to be a substrate for RNA ligase.

## Experimental

**General Methods**——Paper chromatography was performed by the descending technique using solvent systems: A, propane-1-ol–concentrated ammonia–water (6:1:3, v/v); B, propane-1-ol–concentrated ammonia–water (55:15:35, v/v); C, ethanol–1 M ammonium acetate (pH 7.5) (7:3, v/v): D, 0.1 M phosphate (pH, 6.8)–ammonium sulfate–propane-1-ol (100:60:2, v/w/v). Paper electrophoresis was performed using 0.05 M triethylammonium bicarbonate (pH 7.5) at 900 V/40 cm. Thin-layer chromatography (TLC) was performed

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on plates of silica gel (Merck HF 254) using mixtures of chloroform-methanol. All melting points were not corrected. *Rf* values and relative mobilities of compounds are shown in Table I. Other general methods for synthesis of oligonucleotides were as described previously.[3c)] Methods for optical measurements were essentially the same as described for the oligoadenylates.[21)] Hypochromicities of oligonucleotides were measured as described[21)] except that the amounts of oligonucleotides were reduced to *ca.* 0.2 $A_{260}$ and diluted to 1 ml for measurement of UV. For optical measurements the samples were heated at 60° for 1 hr and left at room temperature for 1 hr before measurements.

**ibG** (2)——Guanosine (1.7 g, 6 mmol) was dried at 100° for 5 hr *in vacuo* suspended in pyridine (60 ml) and treated with isobutyryl chloride (6 ml, *ca.* 60 ml) with stirring at 0°. After 1 hr TLC (20: 1) showed a new compound which had $\lambda_{max}$ 263 nm in 0.05 N NaOH. Ice-water (50 ml) was added and the nucleoside was extracted with chloroform (40 ml) 2 times. The organic layer was washed with 5% sodium bicarbonate, then with water and concentrated. The nucleoside was precipitates as syrup with hexane (300 ml) from its solution in pyridine (15 ml). A sample of 1 was recrystallized from ethanol-water. mp 81—82°. *Anal.* Calcd. for $C_{26}H_{37}N_5O_9$ (563.60): C, 55.40; H, 6.62; N, 12.43. Found: C, 55.25, H, 6.71; N, 12.35. UV: $\lambda_{max}$ 255, 260 ($\varepsilon$=15500), 283, $\lambda_{min}$ 228, 258, 272 in 95% ethanol; $\varepsilon$ 280/260=0.74. It was then dissolved in ethanol (55 ml), treated with 2 N NaOH (55 ml) at 0° for 5 min then at room temperature for 10 min and sodium ions were removed with Dowex 50×2 (pyridinium form, 150 ml). The mixture was poured onto a column containing 30 ml of the same resin and the resin was washed with water. The effluent and washings were concentrated and ibG recrystallized from water. The yield was 91%. mp 146—148°. *Anal.* Calcd. for $C_{14}H_{19}N_5O_6$ (353.33): C, 47.59; H, 5.42; N, 19.82. Found: C, 47.40; H, 5.62; N, 19.57. UV: $\lambda_{max}$ 259, 280 (sh), $\lambda_{min}$ 233 nm in 5% ethanol.

**(MeOTr)ibG** (3)——2 obtained as above was treated with monomethoxytrityl chloride (1.9 g, 6.1 mmol) at room temperature for 20 hr in pyridine/DMF (1: 1, 100 ml). After completion of the reaction ice-water (50 ml) was added and monomethoxytritanol was extracted with hexane (30 ml) 2 times. The nucleoside was extracted with chloroform, washed with water, concentrated with pyridine, crystallized by pouring its solution in pyridine to ethyl acetate (80 ml) and collected by filtration after having kept at 4° overnight. The overall yield from guanosine was 2.8 g (75%). The compound gave a positive test for *cis*-diol with the benzidine-periodate spray reagent.[22)] A sample for analysis was recrystallized from ethyl acetate/ethanol. mp 185—187°. *Anal.* Calcd. for $C_{34}H_{35}N_5O_7 \cdot H_2O$ (643.68): C, 63.44; H, 5.79; N, 10.88. Found: C, 63.57; H, 5.63; N, 10.56. UV: $\lambda_{max}$ 233.5, 257—261, 280 in 50% ethanol.

**ibG(BZ)$_2$** (5)——3 (1.0 g, 1.6 mmol) was dissolved in pyridine (10 ml) and benzoic anhydride (3.53 g, 15.6 mmol) was added at 0°. The mixture was kept at 4° for 4 days. The extent of the reaction was checked by TLC (20: 1). Aqueous pyridine (50%, 10 ml) was added at 0° and benzoic anhydride was removed by extraction with hexane (3 ml) 3 times. The nucleoside 4 was precipitated with hexane (300 ml) from its solution in pyridine (10 ml) and demonomethoxytritylated by treating with 80% acetic acid (50 ml) at room temperature for 4 hr. A sample of 4 for elemental analysis was recrystallized from ethanol-water. The melting point was 135—137° (resolidified) and 240° (dec.). *Anal.* Calcd. for $C_{48}H_{43}N_5O_9$ (833.87): C, 69.14; H, 5.20; N, 8.40. Found: C, 69.32; H, 5.34; N, 8.14. UV: $\lambda_{max}$, 233.5, 260(sh), 276 nm in 50% ethanol; $\lambda_{max}^{OH^-}$ 233, 260 (sh), 275 nm; $\lambda_{max}^{OH^-}$ 265 nm; $\varepsilon$230/260=1.55, $\varepsilon$280/260=0.73.

**ibGp** (7)——Disodium guanosine 3′-phosphate (5.33 mmol) was dissolved in 25% aqueous pyridine and passed through a column (2×10 cm) of Dowex 50×2 (pyridinium form). The column was washed with 25% aqueous pyridine and the eluent and washings were evaporated with tetraethylammonium isobutylate (pH 7.1, 53 mmol) and dried by evaporation of added pyridine (5 times) at 55°. A trace of pyridine was removed by evaporation of toluene (5 times) and the residue was treated with isobutylic anhydride (17 ml, 102 mmol) at 27° for 4 days. An aliquot was treated with 2 N NaOH at 0° for 5 min in aqueous pyridine to check the N-acylation by paper chromatography in solvent C and Gp(3′) obtained by subsequent methanolic ammonia treatment was run in solvent D to check the absence of Gp(2′). Cold aqueous pyridine (50%, 60 ml) was added to the reaction mixture and the solvent was applied to a column (130 ml) of Dowex 50×2 (pyridinium form). The column was washed with 50% aqueous pyridine (500 ml), then the eluent and washings were kept at room temperature for 15—20 hr to hydrolyze the isobutyryl phosphate and evaporated. The triisobutyryl derivative was precipitated with ether-hexane (3: 2, 1 l) from its solution in pyridine, then dissolved in 20% aqueous pyridine (45 ml), added to 2 N NaOH (45 ml) at 0° and kept at room temperature for 5 min. After neutralization with Dowex 50×2 (pyridinium form, 130 ml) at 0°, the suspension was poured into a column packed with 30 ml of the same kind of resin. The eluent and washings (20% pyridine) were concentrated by addition of pyridine and the product was precipitated with ether-hexane (3: 2, 1 l) from its solution in anhydrous DMF (30 ml). The yield was 78.9×10$^3$ $A_{260}$ units (4.73 mmol), 89%. *Rf* values are shown in Table I. UV: $\lambda_{max}^{H_2O}$ 257, 280, $\lambda_{max}^{OH^+}$ 262 nm.

**(MeOTr)ibG(Bz)p** (9)——Pyridinium ibGp (7) (4.73 mmol) was suspended in pyridine, dried azeotropically by evaporation of added pyridine and treated with monomethoxytrityl chloride (1.7 g, 5.5 mmol) for 12 hr

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with stirring. The extent of the reaction was checked by paper electrophoresis and the reaction was stopped by addition of ice water (30 ml). The solution was concentrated and the residue was mixed with water (150 ml). Monomethoxytritanol was removed with ethyl acetate (20 ml) 5 times and the product was extracted with *n*-butanol (30 ml) 5 times. The organic layer was washed with water (10 ml) 4 times, evaporated with pyridine and the product was precipitated with ether–hexane (3: 2, 500 ml) from its solution in pyridine (15 ml). The yield was $71.0\times10^3$ $A_{260}$ units (3.8 mmol), 80%. UV: $\lambda_{max}^{EtOH}$ 233, 256 and 280 nm. The 2′-hydroxyl group was benzoylated with benzoic anhydride (5.4 g, 23.9 mmol) in toluene (50 ml) after the nucleotide had been dried in the presence of tetraethylammonium benzoate (pH 7.1, 40 mmol) by evaporation of added pyridine 5 times and toluene 5 times. The mixture was kept at 4° for 18 hr and DMF was added to give a homogeneous solution. An aliquot was taken to check the extent of the reaction and the rest was kept at −20°. To stop the reaction 50% pyridine (30 ml) was added with cooling and the solution was concentrated to a gum. The residue was dissolved in 50% pyridine (70 ml), benzoic acid was removed with hexane (20 ml) 3 times and the benzoylated nucleotide was extracted with chloroform (30 ml) 3 times from the aqueous phase. The organic layer was washed with water (20 ml) 3 times and evaporated. The nucleotide was precipitated with ether–hexane (3: 2, 500 ml) from its solution in pyridine (15 ml), washed with hexane dried *in vacuo* and treated with acetic anhydride (30 ml) in pyridine (60 ml) at room temperature for 24 hr. Acetic anhydride was removed *in vacuo* and 50% aqueous pyridine (50 ml) was added to the residue. The solution was applied to a column (1.9×11 cm) of Dowex 50×2 (pyridinium form). The eluent and washings (50% aqueous pyridine, 150 ml) were kept at room temperature for 6 hr then evaporated with addition of pyridine. The product ($69.0\times10^3$ $A_{260}$, 3.5 mmol) was precipitated with ether–hexane (3: 2, 500 ml) from its solution in anhydrous pyridine (15 ml), washed with the same mixture 3 times and dried. The yield in the benzoylation was 92%.

**(MeTr)ibG(Bz)-ibG(Bz)$_2$ (10)**——The pyridinium salt of **9** (14200 $A_{280}$, 0.97 mmol) and the protected nucleoside **5** (1.26 mmol) were treated with DCC (9.7 mmol) in pyridine (25 ml) at 20° for 7 days in the presence of Dowex 50×2 (pyridinium form, 10 ml). Aqueous pyridine (50%, 30 ml) was added with cooling and DCC was removed with hexane. After the mixture had been kept at 4° overnight and filtered, the solution was rendered anhydrous by coevaporation of pyridine. The product (**10**) was precipitated with 3: 2 ether-hexane from its solution in pyridine to remove the nucleoside (**5**). However **10** was still contaminated with **5** and was further purified by a column (3.2×50 cm) of TEAE-cellulose (acetate form) in 80% ethanol. The product **10** was eluted at a salt concentration of around 0.1 M with a gradient of 0 to 0.2 M triethylammonium acetate in a total volume of 4 l of 80% ethanol. **10** was extracted with *n*-butanol (100 ml) from the concentrated syrup and the organic layer was washed with water (5 ml) 3 times. The yield was 22000 $A_{260}$ units, 0.60 mmol, 62%. An aliquot of **10** was deprotected and GpG (2 $A_{260}$) was hydrolyzed with RNase M which revealed no resistant material and the hydrolyzed products were analyzed by paper electrophoresis. The ratio of Gp (0.248 $A_{250}$) to G (0.248 $A_{250}$) was 1.00: 1.00.

**U(Bz)-ibG(Bz)-ibG(Bz)$_2$ (12)**——The dimer (**10**) was treated with 80% acetic acid and the demonomethoxytritylated compound (**11**) (22200 $A_{280}$, 1.0 mmol) was passed through a column (20 ml) of Dowex 50×2 (pyridinium form) together with (MeOTr)U(Bz)p (31000 $A_{260}$, 2.2 mmol) in 50% pyridine. The eluent and washings were rendered anhydrous by coevaporation of pyridine, treated with DCC (22 mmol) at room temperature for 8 days in pyridine (10 ml) in the presence of Dowex 50×2 (pyridinium form, 1 ml). Aqueous pyridine (50%, 30 ml) and hexane (15 ml) were added. The mixture was kept at 4° overnight, filtered, extracted with hexane, diluted with 80% ethanol (100 ml) and applied to a column (3.2×50 cm) of TEAE-cellulose (acetate form) in 80% ethanol (Fig. 1). The trimer was eluted at a salt concentration of 0.15 M with a gradient of 0 to 0.25 M triethylammonium acetate in 80% ethanol (6 l). It was extracted with *n*-butanol from the appropriate concentrated fractions, washed with water and precipitated with 3: 2 ether–hexane from its solution in pyridine. The yield was 28% (7360 $A_{280}$, 0.28 mmol). The product was treated with 80% acetic acid (30 ml) for 1.5 hr and complete demonomethoxytritylation was confirmed by TLC in 15: 1 chloroform–methanol. Volatile materials were removed in the presence of water and *n*-butanol. **12** was isolated by precipitation with ether–hexane (3: 2) from its solution in pyridine. An aliquot was deblocked by treatment with 15 N methanolic ammonia and UpGpG was separated by paper chromatography in solvent A. RNase M digestion gave G: Gp: Up in a ratio of 1.00: 1.01: 1.08. No resistant 2′—5′ linked material was detected.

**ibG(Bz)-U(Bz)-ibG(Bz)-ibG(Bz)$_2$ (13)**——The pyridinium salts of **12** (7360 $A_{280}$, 0.28 mmol) and (MeOTr)-ibG(Bz)p (12500 $A_{280}$, 1.09 mmol) were treated with DCC (12 mmol) in pyridine (3 ml) in the presence of Dowex 50×2 (pyridinium salt) at room temperature for 8 days. Aqueous pyridine (50%, 20 ml) was added and DCC was removed with hexane. The mixture was kept at 4° overnight, filtered, rendered anhydrous by evaporation with pyridine and the nucleotides were precipitated with ether–hexane. The dried powder was treated with 80% acetic acid (60 ml) for 2 hr and acetic acid was removed by evaporation with water. The residue was dissolved in pyridine (30 ml), diluted with 80% ethanol (500 ml) and applied to a column (3.2×50 cm) of TEAE-cellulose (Fig. 2). Elution was performed with a linear gradient of 0 to 0.4 M triethylammonium acetate in 80% ethanol (10 l). The tetramer (**13**) was eluted at a salt concentration 0.26 M, concentrated, extracted with *n*-butanol (100 ml) and the organic phase was washed with water (20 ml) 3 times. **13** was precipitated with 3: 2 ether–hexane. The yield was 3400 $A_{280}$, 0.089 mmol, 32%.

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**ibG(Bz)-ibG(Bz)-U(Bz)-ibG(Bz)-ibG(Bz)$_2$ (14)**——The pyridinium salt of **13** (1344 $A_{280}$, 0.032 mmol) and **9** (4921 $A_{280}$, 0.43 mmol) which had been passed through a column (100 ml) of Dowex 50×2 (pyridinium salt) were precipitated again with 3 : 2 ether–hexane. The mixture was combined with Dowex 50×2 (pyridinium form, 5 ml), rendered anhydrous by evaporation with pyridine 4 times and treated with DCC (4.3 mmol) in pyridine (3 ml) at room temperature for 8 days with stirring. An aliquot deblocked after 6 days showed a slower moving compound on paper chromatography in solvent A. Aqueous pyridine (50%, 20 ml) was added with cooling and DCC was removed with hexane. The mixture was kept at 4° overnight, filtered, precipitated with 3 : 2 ether–hexane from its solution in pyridine and demonomethoxytritylated by treatment with 80% acetic acid (60 ml) at room temperature for 2 hr. Complete demonomethoxytritylation was confirmed by TLC examination and acetic acid was removed by coevaporation with water. The residue was dissolved in a small amount of pyridine, diluted with 80% ethanol (500 ml) and applied to a column (2.5×46 cm) of TEAE-cellulose (acetate form). Elution was performed by a linear gradient of triethylammonium acetate (0 to 0.45 M) in 80% ethanol (12 l). Appropriate fractions were checked by paper chromatography after deblocking. Fractions containing the product were concentrated and desalted as described for the tetramer. The yield of **14** was 554 $A_{280}$, 0.011 mmol, 35%.

**GpGpGpUpGpG (15)**——The pyridinium salt of **14** (0.011 mmol) and **9** (0.14 mmol) were passed through a column of Dowex 50×2 (pyridinium form) then reprecipitated as described above. The mixture was treated with DCC (1.4 mmol) in pyridine (3 ml) with stirring at room temperature for 8 days. Aqueous pyridine (50%, 20 ml) was added and the reaction mixture was worked up as above. The monomethoxitrityl group was removed by treatment with 80% acetic acid (30 ml) and an aqueous pyridine treatment was performed at room temperature for 1 day after acetic acid had been removed. The aqueous pyridine solution was concentrated to 1 ml, applied to a column (2×74 cm) of Sephadex LH-20 equilibrated with 95% ethanol with the same solvent (Fig. 3). Fractions of 2 ml were collected every 30 min. Fractions 44 to 96 were combined, evaporated and the residue treated with 15 N methanolic ammonia for 24 hr. After removal of solvent, the residue (510 $A_{260}$) was dissolved in 7 M urea, and applied to a column (1.0×100 cm) of DEAE-cellulose (DE 52) equilibrated with 7 M urea in 0.02 M Tris–HCl pH 7.5, 0.05 M NaCl at 55°. Elution was performed with a linear gradient of sodium chloride (0.05 M to 0.3 M, 1 l). The elution pattern is shown in Fig. 4 and peak 16 contained the product (71.2 $A_{260}$, 1.3 μmol, 12%). Peak 15 also contained the hexamer contaminated with a faster travelling compound in solvent B. The product was desalted by adsorption onto a column (3 ml) of DEAE-cellulose (bicarbonate form) and elution was performed with 1 M triethylammonium bicarbonate after chloride anions had been eluted with 0.01 M triethylammonium bicarbonate. An aliquot (0.3 $A_{260}$) was hydrolyzed with RNase M (5 μg) and examined by high pressure liquid chromatography on a Varian nucleic acid analyzer. The ratio of Up to Gp was found to be 1.00 to 3.98.

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