Volume 7 Number 5 1979 Nucleic Acids Research

# Studies on transfer ribonucleic acids and related compounds. XXXII.[1] Synthesis of ribonucleotides corresponding to residues 1-5 and 6-10 of $tRNA^{fMet}$ from E.coli and their base conversion analogs

E.Ohtsuka, T.Tanaka and M.Ikehara

Faculty of Pharmaceutical Sciences, Osaka University, 133-1 Yamadakami Suita, Japan 565

Received 12 September 1979

## ABSTRACT

E.coli $tRNA^{fMet}$ fragments, C-G-C-G-Gp(bases 1-5), U-G-C-G-Gp(base 1 transition analog) pG-G-C-G-Gp(base 1 transversion analog) and pG-G-$s^4$U-G-Gp(bases 6-10) were synthesized by triester methods using 2'-O-(o-nitrobenzyl)nucleotides including a 3',5'-bisphosphorylated guanosine derivative. The $s^4$U containing pentanucleotide was derived from the pG-G-C-G-Gp by treatment with liquid hydrogen sulfide.

## INTRODUCTION

We have previously reported chemical synthesis of E.coli $tRNA^{fMet}$ [2] fragments by phosphodiester methods (bases 1-4,[3] 5-10,[4] 41-46,[5] 47-54,[5] 55-57,[5] 58-60,[6] 61-65,[7] 66-71,[7] 72-74,[8] 75-77,[8,9] or a phosphotriester method (bases 11-20,[10] 72-77,[1] and its analogs.[1] The phosphotriester approach using 2'-O-(o-nitrobenzylnucleoside) 3'-phosphorodianilidates[11] was also employed in the synthesis of a eukaryotic initiator tRNA loop IV heptanucleotide.[12] In the present paper we report a synthesis of 5'-pentanucleotides from E.coli $tRNA^{fMet}$, C-G-C-G-Gp (bases 1-5), U-G-C-G-Gp (base 1 transition analog), pG-G-C-G-Gp (base 1 transversion analog) and pG-G-$s^4$U-G-Gp (bases 6-10) using 2'-O-(o-nitrobenzyl nucleoside)-3'-p-chlorophenylphosphoranilidates.[10,13] Protected pGp was prepared for insertion of the 5'-phosphomonoester in the synthesis of the pentanucleotide, pG-G-C-G-Gp and the pentanucleotide was converted to the $s^4$U containing oligonucleotide by treatment with liquid hydrogen sulfide.[15]

### Synthesis of C-G-C-G-Gp (10) and U-G-C-G-Gp (11)

For the synthesis of pentanucleotides (8,9), a common intermediate tetranucleotide (5) was prepared by condensation of two

© Information Retrieval Limited 1 Falconberg Court London W1V 5FG England 

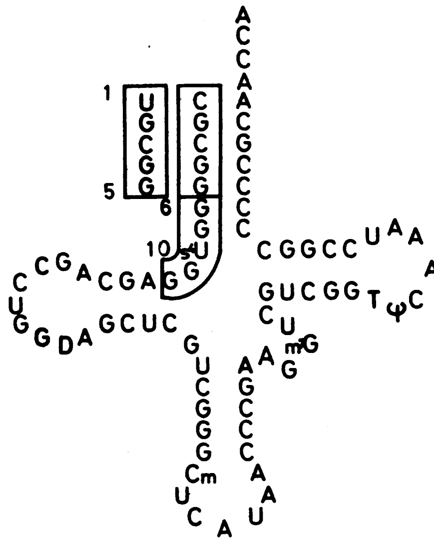

Figure 1. Pentanucleotides from E.coli $tRNA_f^{Met}$ and their base converted analogs.

dinucleotides (3,4) using TPS nitroimidazolide[16] (TPSNI) as shown in Fig. 2. The reaction conditions for synthesis of protected oligonucleotides are summarized in Table I. Protected GpCp (3) and GpGp (4) were obtained by condensation of mononucleotides as described previously.[10] The tetranucleotide (5) was condensed with two equivalents of (6) using TPSNI to give protected C-G-C-G-Gp (8). The product was isolated by chromatography on silica gel. Protected U-G-C-G-Gp (9) was obtained similarly in a yield of 60%. These protected pentanucleotides were deblocked by successive treatments with isoamylnitrite, concentrated ammonia, 80% acetic acid and UV irradiation. The completely deblocked pentanucleotides (10,11) were purified by ion-exchange chromatography on DEAE-cellulose in the presence of 7 M urea. The overall yield of 10 from 8 after all deblocking treatments and chromatography was 28%. The product was characterized by digestion with RNase M to yield Gp and Cp in the correct ratio. The sequence of 10 (C-G-C-C-Gp) was confirmed by two dimensional homochromatography after partial digestion of the 5'-phosphorylated pentanucleotide (Fig.3). The other pentanucleotide 11 was

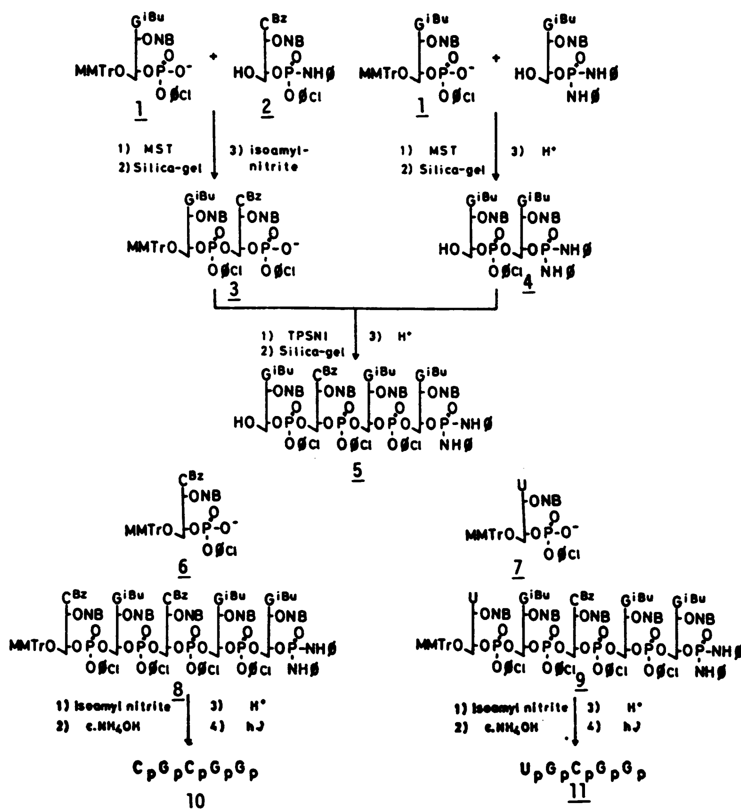


Figure 2.

isolated in a yield of 48% from 9 and characterized by similar methods.

Preparation of protected pGp (16) and synthesis of pG-G-C-G-Gp (18) and pG-G-$s^4$U-G-Gp (19) using the tetranucleotide intermediate (5)

We have previously introduced the 5'-phosphomonoester to a protected hexanucleotide by using dianilidophosphorochloridate.[5] However, guanosine containing oligonucleotides were also phosphorylated at guanine residues when the 5'-hydroxyl group of an oligomer was phosphorylated with dianilidophosphorochloridate. Furthermore, an acid treatment was then necessary. Yields in phosphorylations of larger molecules are generally rather low. As an alternative approach we prepared suitably protected pGp

Table I Reaction Conditions for Condensations

| 5'-Protected component amount (mmol) | 3'-Protected component amount (mmol) | Reagent amount (mmol) | Time (day) | Product amount (mmol) | Yield (%) |
|---|---|---|---|---|---|
| 1 (2.69) | 2 (1.94) | MST (8.09) | 1.5 | 3 (1.38) | 71 |
| 3 (0.83) | 4 (0.55) | TPSNI (1.66) | 1.5 | 5 (0.398) | 72 |
| 6 (0.10) | 5 (0.05) | TPSNI (0.20) | 1.5 | 7 (0.023) | 46 |
| 7 (0.10) | 5 (0.05) | TPSNI (0.15) | 1.5 | 8 (0.03) | 60 |
| 16 (0.20) | 5 (0.10) | TPSNI (0.30) | 2.5 | 17 (0.025) | 25 |

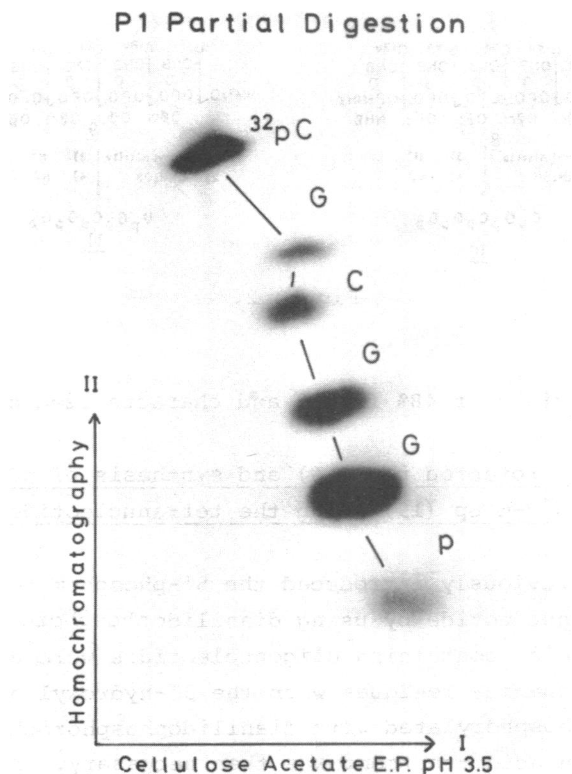


Figure 3. Mobility shift analysis of the pentanucleotide.

as the 5'-terminal unit for the synthesis of 5'-phosphorylated oligonucleotide. The reaction scheme is shown in Fig. 4. N,2', 3'-protected guanosine (13) was derived from 2'-O-(o-nitrobenzyl)-5'-O-monomethoxytrityl-N-isobutyrylguanosine (12) by acetylation and demonomethoxytritylation. Phosphorylation of 15 with dianilidophosphorochloridate yielded 14 after an acid treatment to remove the undesired phosphoryl group from the base. Strong alkaline treatment removed the 3'-acetyl group. 15 was obtained in an overall yield of 67% from 12 and characterized by dephosphorylation with venom 5'-nucleotidase. $H^1$ NMR data for 13-15 are shown in Fig. 5. Phosphorylation of 15 with p-chlorophenylphosphate and DCC gave the desired bis-phosphorylated guanosine derivative (16). The yield of 16 from 15 was quantitative.
The mononucleotide (16) was then condensed with the tetranucleotide block (5) to yield the protected pentanucleotide (17) using TPSNI as the activating reagent in a yield of 25%. 17 was purified by silica gel chramatography but it was contaminated with the starting material (5). The deblocked pentanucleotide (18) was isolated by chromatography on DEAE-cellulose in the presence of 7 M urea. The overall yield in deblocking (isoamylnitrite

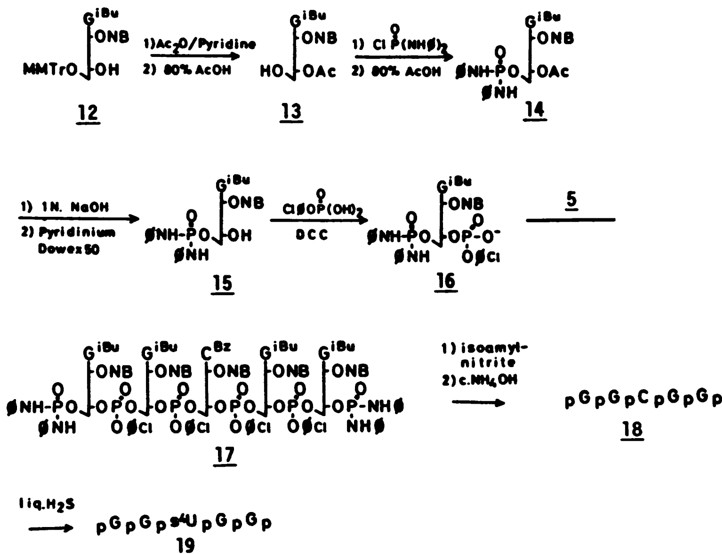


Figure 4.

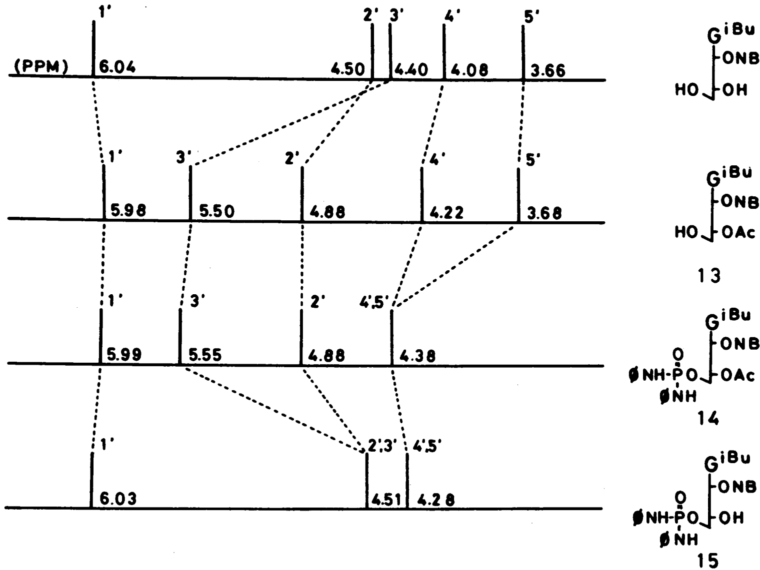


Figure 5. $C_{1'}$-H chemical shifts of guanosine derivatives.

treatment, ammonia treatment and UV irradiation and chromatography was 67%. pG-G-C-G-Gp (18) was characterized by digestion with RNase M to give pGp, Cp, Gp in the correct ratios. An aliquot was dephosphorylated with alkaline phosphatase and labeled with [$\gamma$-$^{32}$P]ATP and polynucleotide kinase. Partial nuclease P1 digestion of the labeled pentanucleotide showed the expected mobility shifts in two dimensional homochromatography.

pG-G-C-G-Gp (18) was then subjected to thiation for the synthesis of pG-G-s$^4$U-G-Gp (19). After treatment with liquid hydrogensulfide at 30° for 140 hr, the mixture was applied to a column of DEAE-cellulose. The elution profile and conditions are shown in Fig. 6. The thiolated pentamer[19] was separated from the C containing pentamer (18) with detection by measuring absorption at 330 nm. The product was characterized by RNase M digestion.

## EXPERIMENTAL

Paper chromatography was performed using solvent systems: A, propan-2-ol-c. ammonia-water (7:1:2, v/v); B, propan-1-ol-

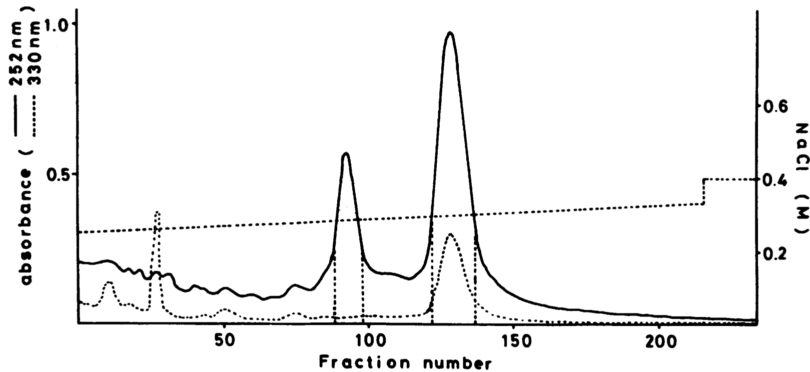


Figure 6. Chromatography of the hexanucleotide (19) on a column (1.0x90 cm) o DEAE-cellulose equilibrated with 7M urea-20 mM Tris-HCl (pH 8.0)-0.1 M NaCl. Elution was performed with a linear gradient of sodium chloride (0.25-0.4 M, total 1.2 L). Three ml fractions were collected every 22 min. The main peak contained the product pG-G-$s^4$U-G-Gp (19).

c. ammonia-water (55:10:35, v/v). Paper electrophoresis was performed using 0.05 M triethylammonium bicarbonate (pH 7.5) or 0.2 M morpholinium acetate (pH 3.5) at 900 V/40 cm. Rf values and relative mobilities of compounds are summarized in Table II. TLC was performed on plates of silica gel (Kieselgel $HF_{254}$, Merck) using a mixture of chloroform and methanol. For columns silica gel G (Merck, type 60) was used. Removal of the 5'-O-monomethoxytrityl, 2'-O-(o-nitrobenzyl), phosphoroanilidate and other general methods were as described previously.[12] Enzymatic hydrolyses were also described in Ref. 15.

Synthesis of 3.

The triethylammonium salts of 1 (2.69 mmol) and 2 (1.94 mmol) were condensed with MST (2.03 g, 8.08 mmol) as described previously and the fully protected dimer was isolated by chromatography on silica gel (220 g) using 50:1 chloroform-methanol in a yield of 71% (2.32 g, 1.38 mmol). UV: (95% EtOH) $\lambda$max 262 ($\varepsilon$=52900), $\lambda$min 243 nm ($\varepsilon$=38100). The fully protected dimer (2.32 g, 1.38 mmol) was dissolved in 1:1 pyridine-acetic acid (30 ml) and treated with isoamyl nitrite (6 ml) at 25-30°

Table II Paper Chromatography and Electrophoresis

| Compound | Rf solvent A | Rf solvent B | Relative mobility pH 7.5 |
|---|---|---|---|
| A | 0.68 | 0.67 | -0.27 |
| G | 0.40 | 0.51 | 0 |
| C | 0.57 | 0.51 | -0.19 |
| U | 0.56 | 0.58 | 0.04 |
| A>p | | | 0.41 |
| G>p | | | 0.61 |
| C>p | 0.36 | | 0.53 |
| U>p | 0.35 | | 0.73 |
| Ap | 0.26 | 0.44 | 0.91 |
| Gp | 0.13 | 0.33 | 1.00 |
| Cp | 0.22 | 0.43 | 0.99 |
| Up | 0.22 | 0.43 | 1.07 |
| G(nBzl)-G(nBzl)p | 0.15 | 0.44 | 0.94 |
| G-Gp | 0.07 | 0.17 | 1.01 |
| G(nBzl)-C(nBzl)p | 0.78 | | |
| G-C>p | 0.12 | | 0.71 |
| G-Cp | 0.07 | 0.26 | 1.00 |
| G(nBzl)-C(nBzl)-G(nBzl)-G(nBzl)p | | 0.32 | 0.88 |
| G-C-G-Gp | | 0.10 | 1.00 |
| U(nBzl)-G(nBzl)-C(nBzl)-G(nBzl)-G(nBzl)p | | 0.31 | |
| U-G-C-G-Gp | | 0.08 | 0.93 |
| C(nBzl)-G(nBzl)-C(nBzl)-G(nBzl)-G(nBzl)p | | 0.31 | |
| C-G-C-G-Gp | | 0.08 | 0.92 |
| pG(nBzl)-G(nBzl)-C(nBzl)-G(nBzl)-G(nBzl)p | | 0.10 | |
| pG-G-C-G-Gp | | 0.03 | 0.97 |

p = p-chlorochenyl phosphate

overnight. An additional (2 ml) isoamyl nitrite in 1:1 pyridine-acetic acid (10 ml) was added to complete the reaction. After one day trityl positive material was only detected at the origin

in TLC and the reaction mixture was treated with 0.1 M triethylammonium bicarbonate (pH 7.5, 60 ml) with cooling. The nucleotide was extracted with chloroform (30 ml) 3 portions, washed with water (30 ml) 3 portions, concentrated and precipitated with 1:1 ether-n-pentane (240 ml) from its solution in chloroform (20 ml). The yield was 99% (2.31 g, 71500 $A_{261.5}$, 1.36 mmol).

The tetranucleotide (5).

The triethylammonium salts of (3) (1.40 g, 43300 $A_{261.5}$, 0.83 mmol) and (4)[10] (765 mg, 0.55 mmol) were coevaporated with pyridine. The mixture was treated with TPSNI (630 mg, 1.66 mmol) at 25-30° for 1.5 days and checked by TLC (10:1). A new trityl positive spot (0.24) was detected and the starting material (4) disappeared. Water (5 ml) was added with cooling and the mixture was mixed with 0.1 M triethylammonium bicarbonate (pH 7.5) (40 ml). The nucleotide was extracted with chloroform (20 ml) 3 portions, washed with water (15 ml) 3 portions, coevaporated with pyridine, then with toluene. The residue was dissolved in chloroform (6 ml) and applied to a column of silica gel (90 g). The product was eluted with 35:1 chloroform-methanol and treated with 80% acetic acid (100 ml) at room temperature for 5 hr. The demonomethoxytritylation was confirmed by TLC (10:1) and the volatile materials were removed by evaporation. The residue was coevaporated with toluene, then with pyridine and precipitated with n-pentane (100 ml) from its solution in chloroform (6 ml). The yield was 72% (1074 mg, 0.398 mmol). An aliquot of the fully protected tetramer was deblocked by successive treatments with isoamyl nitrite, concentrated ammonia and 80% acetic acid. The o-nitrobenzylated tetranucleotide was isolated by paper electrophoresis. The o-nitrobenzyl groups were removed by irradiation with UV and the deblocked tetranucleotide was isolated by paper chromatography in solvent B. The base ratio was obtained by digestion of G-C-G-Gp with RNase M and measuring UV absorption in 0.01N HCl (1 ml): Gp, 0.537 $A_{256}$; Cp, 0.203 $A_{280}$; Gp:Cp=2.82:1.0.

2'-O-(o-Nitrobenzyl)-3'-O-acetyl-N-isobutyrylguanosine (13)

12 (1.49 g, 1.96 mmol) was treated with acetic anhydride (10 ml) in pyridine (20 ml) at 0° overnight and checked by TLC (15:1). A shift of Rf from 0.31 to 0.43 was observed. Water

(20 ml) was added with cooling and the nucleotide was extracted with chloroform (20 ml) 3 portions. The organic layer was washed with water (20 ml) 3 portions, concentrated with toluene, dissolved in chloroform (6 ml) and treated with 80% acetic acid at 25-33° for 6 hr. Demonomethoxytritylation was confirmed by TLC (15:1) and the product was precipitated with n-pentane (200 ml) from its solution in chloroform (10 ml) after successive evaporations with toluene and pyridine. The yield was 94% (979 mg, 1.84 mmol). Anal. Calcd. for $C_{23} H_{26} N_6 O_9$ $1/2H_2O$=539.56: C, 51.20, H, 5.05; N, 15.58. Found: C, 51.23; H, 4.75; N, 15.48. NMR (DMSO-$d_6$): 12.09 (brs, 1H, NH), 11.58 (brs, 1H, NH), 8.23 (S, 1H, $C_8$-H), 7.29-8.04 (m, 4H, Ar-H), 5.98 (d, 1H, $J_{1',2'}$=8 Hz, $C_{1'}$-H), 5.50 (d, 1H, $C_{3'}$-H), 5.28 (brs, 1H, $C_{5'}$-OH), 4.88 (m, 3H, Ar-$CH_2$, $C_{2'}$-H), 4.22 (m, 1H, $C_{4'}$-H), 3.68 (m, 2H, $C_{5'}$-H), 2.80 (m, 1H, $CH(CH_3)_2$), 2.12 (s, 3H, -($OCH_3$ ), 1.15 (d, 6H, $CH(CH_3)_2$).

2'-O-(o-Nitrobenzyl)-5'-O-dianilidophosphoryl-N-isobutyrylguanosine (15).

13 (797 mg, 1.5 mmol) was treated with dianilidophosphorochloridate (940 mg, 3.6 mmol) at 25-33° overnight and checked by TLC (10:1). The Rf(0.36) of 15 shifted to 0.43 and showed contamination with a base phosphorylated compound: $\lambda$ max (95% EtOH) 229, 260, 274, and $\lambda$ min 247.5 nm, $A_{229/260}$=2.48 and $A_{260/274}$=1.08. An aliquot was treated with 80% acetic acid for 3 hr to remove phosphorodianilidate from the base after which the starting material (Rf 0.36) was not detected. The reaction mixture was added to 5% potassium acetate (15 ml) with cooling and stirred at room temperature for 1 hr. The product (14) was extracted with chloroform (10 ml) 3 portions, washed with water (10 ml) 3 portions, concentrated with pyridine and with toluene. Some insoluble crystalline material in chloroform was removed by filtration and the chloroform solution was concentrated. The residue was treated with 80% acetic acid (80 ml) at 25-33° for 2 days until $A_{232/260.5}$=1.48 and $A_{260.5/274}$=1.27 were observed. Volatile materials were removed and 16 was coevaporated with toluene. Deacetylation of 16 was performed by treatment with 2N NaOH (8 ml) in pyridine (2 ml) and 95% ethanol (6 ml) at 0° for 10 min. Dowex 50X2 (pyridinium form)

(35 ml) was added at 0° and the mixture was pourred onto a column filled with the same resin (10 ml). The effluent and washings (150 ml, 50% pyridine) were concentrated, coevaporated with pyridine, with toluene, dissolved in chloroform (5 ml) and applied to a column of silicagel (60 g). Elution was performed with 20:1 chloroform-methanol and 17 was precipitated with n-pentane (120 ml) from its solution in chloroform (7 ml). The yield was 71% (771 mg, 1.07 mmol). Anal. Calcd. for $C_{33} H_{35} N_8 O_9 P$ 1/2 $H_2O$=727.75: C, 54.46; H, 5.00; N, 15.40. Found: C, 54.56; H, 4.80; N, 15.47. NMR(DMSO-$d_6$): 11.76 (brs, 2H, NH), 8.12 (s, 1H, $C_8$-H), 8.07 (d, 2H, $J_{H-p}$=6 Hz, N<u>H</u>Ar), 6.68-8.00 (m, 14H, Ar-H), 6.03 (d, 1H, $J_{1',-2'}$=6 Hz, $C_{1'}$-H), 5.55 (d, 1H, $C_{3'}$-OH), 4.79, 4.96 (ABq, 2H, $J_{gem}$=14.4 Hz, ArC<u>H</u>$_2$-), 4.51 (m, 2H, $C_{2'}$-H, $C_{3'}$-H), 4.28 (m, 3H, $C_{4'}$-H, $C_{5'}$-H), 2.76 (m, 1H, C<u>H</u>$(CH_3)_2$), 1.12 and 1.09 (d, 6H, $CH(CH_3)_2$). An aliquot was treated with isoamylnitrite and with conc. ammonia. 2'-O-(o-nitrobenzyl)guanosine 5'-phosphate showed Rf 0.24 in solvent A and was irradiated with UV light. pG($3A_{260}$) thus obtained (Rf, 0.09 in solvent A) was completely susceptible to crude snake venom 5'-nucleotidase and the product was identified as guanosine by paper chromatography, and UV absorption.

<u>Synthesis of 16.</u>

<u>15</u> (575 mg, 0.80 mmol) and p-chlorophenyl phosphate (500 mg, 2.4 mmol) were rendered anhydrous by evaporation of pyridine 3 times. The mixture was treated with DCC (1.19 g, 5.8 mmol) at 25-33° for 2 days. After the Rf has shifted from 0.28 to 0 in TLC (10:1), 50% pyridine (10 ml) and n-hexane (40 ml) were added to the mixture with cooling. The mixture was kept at room temperature overnight, decanted and the aqueous phase was filtered to remove dicyclohexylurea, DCC was removed with n-hexane (30 ml). The nucleotide (<u>16</u>) was extracted with chloroform (15 ml) 3 portions, washed with 0.1M triethylammonium bicarbonate (pH 7.5)(20 ml) 3 portions, concentrated with pyridine and precipitated with n-pentane (100 ml) from its solution in chloroform (5 ml). The yield was 99% (17400 $A_{261}$, 0.79 mmol).

<u>pG-G-C-G-Gp (18).</u>

<u>16</u> (228 mg, 4400 $A_{261}$, 0.2 mmol) and the tetramer (<u>5</u>) (270 mg, 0.1 mmol) were coevaporated with pyridine and treated with

TPSNI (114 mg, 0.3 mmol) at 25-33° for 36 hr. TLC (10:1) showed a spot (0.29) which was identical to 5. An aliquot was treated with isoamyl nitrite and with conc. ammonia. Paper chromatography at this stage showed two spots, $R_{Gp}$ 0.85 (o-nitrobenzylated tetramer) and 0.16. TPSNI (19 mg, 0.05 mmol) was added and the reaction was stopped after 24 hr by addition of water (2 ml) with cooling. Chloroform (20 ml) and 0.1M triethylammonium bicarbonate (pH 7.5, 40 ml) were added to the mixture. The oligonucleotides were extracted further from the aqueous layer with chloroform (15 ml) 2 portions, washed with water (15 ml), concentrated with pyridine, coevaporated with toluene and the residue in 2 ml of chloroform was applied to a column of silica gel (20 g). The product (17) was eluted with 25:1 chloroform-methanol, precipitated with n-pentane (25 ml) from its solution in chloroform (3 ml). The yield of 19 was 243 mg, 0.025 mmol (25 %), corrected for contaminating starting material (16, 63%). An aliquot of the product (17, 108 mg) was dissolved in 1:1 pyridine-acetic acid (15 ml) and treated with isoamyl nitrite (3.6 ml) at room temperature for 1 day. n-Butanol (10 ml) and 0.1 M triethylammonium bicarbonate (pH 7.5)(20 ml) were added with cooling. The product was further extracted from the aqueous phase with n-butanol (10 ml) 2 portions. The organic layer was concentrated and the residue was dissolved in pyridine (4 ml). The solution was treated with 28% aqueous ammonia (50 ml) at 50° for 5 hr, concentrated and the residue was dissolved in water (60 ml). The aqueous solution was washed with ether (20 ml) 3 portions and one half of the solution was concentrated. The residue was dissolved in water (210 ml), irradiated with UV, concentrated to a half volume, washed with ether (25 ml) 3 portions, concentrated and the residue was dissolved in 7M urea-20 mM Tris-HCl (pH 8.0)(30 ml). The solution was applied to a column (1.0x111 cm) of DEAE-cellulose ($Cl^-$) equilibrated with the same solution and elution was performed with a linear gradient of sodium chloride (0.2-0.5M, total 1.2L) at 50°. Three ml fractions were collected every 17 min. Fractions (133-150) were combined and desalted by absorption to DEAE-cellulose ($HCO_3^-$). The yield was 67% (184 $A_{252}$) assuming a 10% hypochromicity. The tetranucleotide G-C-G-Gp (260 $A_{253}$) was recovered from frac-

tions 75-87.

An aliquot (3 $A_{260}$) of 20 was digested with RNase M and nucleotides were separated by paper electrophoresis at pH 3.5: Cp, 0.355 $A_{279}$; Gp, 0.924 $A_{256}$; pGp, 0.363 $A_{256}$ in 0.01 NHCl (1 ml). The ratio was Cp:Gp:pGp=1.00:2.78:1.09. Phosphatase treatment of 20 yielded G-G-C-G-G which was separated by paper chromatography in solvent B and characterized by mobility shift analysis in two dimentional homochromatography.

pG-G-$s^4$U-G-Gp (19).

18 (100 $A_{254}$, 2.0 umol) in water (5 ml) was treated with liquid hydrogen sulfide (20 ml) prepared by absorbing in pyridine (5 ml)at -70°. The mixture was kept at 30° in a sealed stainless steal tube for 140 hr. The volatile materials were removed and the residue was dissolved in water. The filtered solution was concentrated, dissolved in 7M urea-20 mM, Tris-HCl (pH 8.0) 0.1M NaCl (20 ml), applied to a column of DEAE-cellulose. The elution profile and conditions are shown in Fig. 6. Fractions (123-137) were combined and desalted by absorption to DEAE-cellulose. The yield was 40%, 36 $A_{256}$, 0.8 μmol. UV: $\lambda$max ($H_2O$) 252, 274 (sh), 328, $\lambda$min ($H_2O$) 233, 302.5 nm, $A_{252/328}$=3.99. pG-G-C-G-Gp (13.2 $A_{254}$) was recovered from fractions (81-97). An aliquot (2 $A_{260}$) of 19 was digested with RNase M and nucleotides were separated by paper electrophoresis at pH 3.5: Gp, 0.414 $A_{252}$; pGp, 0.138 $A_{252}$; $s^4$U, 0.140 $A_{330}$ in water (1.5 ml). The ratio was Gp:pGp:$s^4$U=3.14:1.00:0.8.

REFERENCES


1. Part XXI of this series: Ohtsuka E., Tanaka T. and Ikehara M., Chem. Pharm. Bull. in press.
2. Dube, S.K., Marcker, K.A., Clark, B.F.C. and Corey. S. (1968) Nature, 218, 232-233
3. Ohtsuka, E., Miyake, T. and Ikehara, M. (1979) Chem. Pharm. Bull., 27, 341-345
4. Ohtsuka, E., Nakagawa, E., Tanaka, T., Markham, A.F. and Ikehara, M. (1978) Chem. Pharm. Bull., 26, 2998-3006
5. Ohtsuka, E., Tanaka, T., Miyake, T., Nakagawa, E., Antkowiak J., Wakabayashi, T., Taniyama, Y., Nishikawa, S., Uemura, H. and Ikehara, M. (1978)Nucleic Acids Res. Special Publication No. 5, s351-354
6. Uesugi, S., Tanaka, S., Ohtsuka, E. and Ikehara, M. (1978) Chem. Pharm. Bull., 26, 2396-2406
7. Markham, A.F., Miyake, T., Ohtsuka, E. and Ikehara, M. (1977) Heterocycles, 8, 229-235

8. Ohtsuka, E., Tanaka, S. and Ikehara, M. (1977) Chem. Pharm. Bull., 25, 949-959
9. Ohtsuka, E., Ubasawa, M. and Ikehara, M. (1971) J. Am. Chem. Soc., 93, 2296-2301
10. Ohtsuka, E., Tanaka, T. and Ikehara, M. (1979) J. Am. Chem. Soc. in press.
11. Ohtsuka, E., Tanaka, T., Tanaka, S. and Ikehara, M. (1978) J. Am. Chem. Soc., 100, 4580-4584
12. Ohtsuka, E., Tanaka, S. and Ikehara, M. (1978) J. Am. Chem. Soc., 100, 8210-8213
13. Ohtsuka, E., Tanaka, T., Wakabayashi, T., Taniyama, Y. and Ikehara, M., J. C. S. Chem. Comm. 824-825
14. Ueda, T., Imazawa, M. and Miura, K. (1971) Tetrahedron Lett. 2567-2570
15. Ohtsuka, E., Ubasawa, M., Morioka, S. and Ikehara, M. (1973) J. Am. Chem. Soc., 95, 4725-4733