# Studies on Transfer Ribonucleic Acids and Related Compounds. 23.[1] Synthesis of a Heptanucleotide Corresponding to a Eukaryotic Initiator tRNA Loop Sequence[2]

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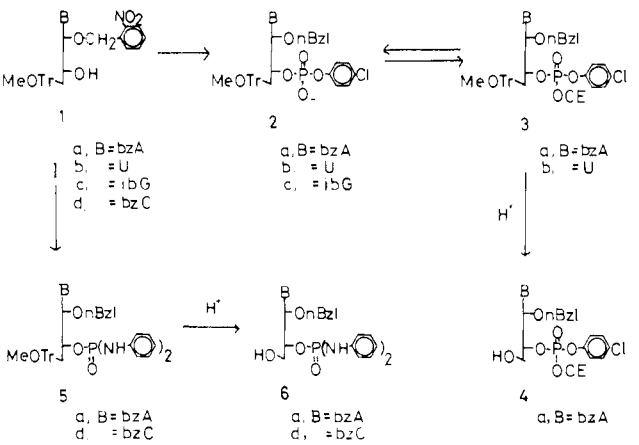
**Abstract:** A heptanucleotide A-U-C-G-A-A-Ap[3] corresponding to the bases (53–59) of yeast $tRNA_f^{Met}$ was synthesized by a block condensation. The 2′-hydroxyl groups were protected with the photolabile *o*-nitrobenzyl ether functionality. This allowed the use of alkaline-labile *p*-chlorophenyl for internucleotide phosphate protection and acid-labile monomethoxytrityl for the 5′-hydroxyl function. Two protected blocks with 3′-phosphorodianilidates were synthesized by the triester method using mesitylenesulfonyl triazolide as the activating reagent for phosphodiesters. The yields in condensations varied from 73 to 43%. The phosphorodianilidate of protected A-U-Cp (**9**) was removed by treatment with isoamyl nitrite and the trimer (**10**) was allowed to react with the 5′-demonomethoxytritylated tetranucleotide (**16**) using triisopropylbenzenesulfonyl chloride. The 2′-protected heptanucleotide (**17**) was isolated by chromatography on DEAE-Sephadex and irradiated with UV light of wavelength longer than 280 nm. After final purification by chromatography on DEAE-cellulose in the presence of 7 M urea the 3′-phosphorylated heptanucleotide (**18**) was obtained in an overall yield of 30% from **16**.

A photosensitive protecting group, *o*-nitrobenzyl ether has been proved to be a stable protecting group for the 2′-hydroxyl function of ribonucleosides during phosphorylation.[4,5] Removal of the *o*-nitrobenzyl ether from uridine,[5] cytidine,[6] adenosine,[6] *N*-isobutyrylguanosine,[7] U-A,[5] C-C-A,[6] C-Up,[4] G-Gp,[4] A-G-Cp,[4] and A-A-Ap[8] by UV irradiation showed no detectable photochemical side reactions. Ribooligonucleotide blocks with a 2′-*O*-(*o*-nitrobenzyl) nucleotide at the 3′ terminus have been synthesized previously.[4] In the present paper we report the syntheses of tri- and tetranucleotides composed of 2′-*O*-(*o*-nitrobenzyl) nucleotides by triester methods[9] and condensation of these by a diester method[10] to yield a heptanucleotide A-U-C-G-A-A-Ap (**18**) corresponding to the nascent loop sequence of the bases 53–59 of the yeast initiator methionine tRNA.[11] Other eukaryotic initiator tRNAs are known to have the same heptanucleotide in the same place[12] unlike the prokaryltic initiator tRNA[13] and most other tRNAs which have a common sequence (T-ψ-C) as a ribosomal binding site.[14] Synthesis of this oligonucleotide is of interest particularly for obtaining hybrid molecules containing prokaryotic and eukaryotic initiator tRNA sequences. Enzymatic joining of synthetic 3′ fragments from *E. coli* $tRNA_f^{Met}$ with T4 induced RNA ligase has yielded the 3′-heptadecanucleotide[2,15] and similar enzymatic joining would provide many larger ribooligonucleotides from chemically synthesized oligonucleotides. The present block condensation of the trinucleotide bearing the 3′-phosphomonoester with the 3′-dianilidated tetramer using 2,4,6-triisopropylbenzenesulfonyl chloride (TPS)[16] yielded a relatively large amount of the 3′-phosphorylated heptanucleotide (**18**) in good yield. The alkaline-stable *o*-nitrobenzyl ether at the 2′ position allowed the use of an alkaline-labile phenyl derivative for protection of the internucleotide linkage in the synthesis of the tri- and tetranucleotide blocks. Activation of diester phosphate was effected by mesitylenesulfonyl triazolide (MST).[17] The isolated yields of triesterified oligonucleotides containing 2′-*O*-(*o*-nitrobenzyl)nucleotides were encouragingly good.

**Synthesis of Protected Mononucleotides.** Mononucleotides suitable for the synthesis of fully protected oligonucleotides have been obtained as shown in Chart I. *p*-Chlorophenyl was used for protection of internucleotide linkages. The 3′ terminus of oligonucleotide blocks was protected either with ester (*p*-chlorophenyl and β-cyanoethyl) or dianilidate. The 3′-triesterified mononucleotide (**4a**) was used in the synthesis of the

Chart I




dimer (**11** in Chart II) which was elongated in the 3′ direction to yield the triesterified tetranucleotide (**15**). The 3′-dianilidates of nucleotides (**6**) were used in the synthesis of oligonucleotides with 3′-phosphomonoesters such as **10**. Diesterified mononucleotides (**2**) were key intermediates for these syntheses and these were prepared by two approaches. **2a,b** were synthesized via **3a,b**. The fully substituted compounds (**3a,b**) were obtained by treating **1a,b**[4] with *p*-chlorophenyl phosphate and TPS followed by addition of β-cyanoethanol, and then were isolated by silica gel chromatography as described for deoxynucleotides.[17] The isolated yields were between 40 and 50%, although TLC of the reaction mixtures showed almost quantitative conversion. The cyanoethyl group of **3a,b** was removed by treatment with triethylamine in pyridine[18] to yield **2a,b**. The guanosine derivative (**2c**) was prepared directly from **1c**[4] using *p*-chlorophenyl phosphate and DCC and then isolated by extraction with chloroform in a yield of 76%. This method is preferred to the other approach and may be applied to the other three nucleosides. The dianilidates **6a,b** were synthesized as described previously.[4]

**Synthesis of the Tri- and Tetranucleotide Blocks and Their Condensation.** The scheme for the synthesis of the heptanucleotides is shown in Chart II. The conditions for the synthesis of two oligonucleotide blocks **9** and **15** are summarized in Table I. MST[17] was used as the activating reagent of the diesterified phosphates. The dinucleotides with the 3′-phosphorodianilidate

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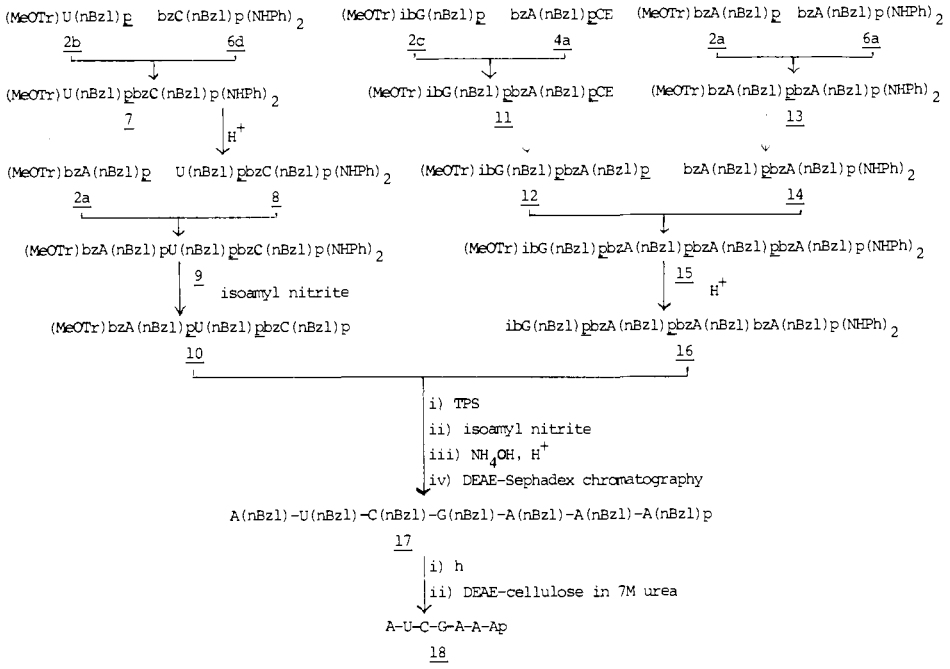
**Table I.** Reaction Conditions and the Yields by Triester Approach[a]

| 5-protected component | amount, mmol | 3-protected component | amount, mmol | MST, mmol | product yield, % (mg) |
|---|---|---|---|---|---|
| (MeOTr)U(nBzl)*p* | 1.25 | bzC(nBzl)p(NHPh)$_2$ | 1.50 | 3.75 | (MeOTr)U(nBzl)*p*bzC(nBzl)p(NHPh)$_2$ (**7**) 73% (1410) |
| (MeOTr)ibG(nBzl)*p* | 1.36 | bzA(nBzl)*p*CE | 1.12 | 4.00 | (MeOTr)ibG(nBzl)*p*bzA(nBzl)*p*CE (**11**) 43% (805) |
| (MeOTr)bzA(nBzl)*p* | 0.79 | bzA(nBzl)p(NHPh)$_2$ | 1.00 | 2.50 | (MeOTr)bzA(nBzl)*p*bzA(nBzl)p(NHPh)$_2$ (**13**) 67% (888) |
| (MeOTr)bzA(nBzl)*p* | 0.83 | U(nBzl)*p*bzCnBzlp-(NHPh)$_2$ | 0.70 | 3.36 | (MeOTr)bzA(nBzl)*p*U(nBzl)*p*bzC(nBzl)p(NHPh)$_2$ (**9**) 61% (946) |
| (MeOTr)ibG(nBzl)-*p*bzA(nBzl)*p* | 0.47 | bzA(nBzl)*p*bzA(nBzl)-p(NHPh)$_2$ | 0.31 | 1.87 | (MeOTr)ibG(nBzl)*p*bzA(nBzl)*p*bzA(nBzl)*p*bzA-(nBzl)p(NHPh)$_2$ (**15**) 68% (637) |

[a] *p*, *p*-chlorophenyl phosphate; p, phosphate; NHPh, anilido; CE, β-cyanoethyl; (MeOTr), 5′-*O*-monomethoxytrityl; (nBzl), 2′-*O*-(*o*-nitrobenzyl); bzA, $N^6$-benzoyladenosine; U, uridine; ibG, $N^2$-isobutyrylguanosine; bzC, $N^4$-benzoylcytidine; MST, mesitylenesulfonyl triazolide.

**Chart II**




**7** and **13** were obtained in high yields. The relatively low yield of the cyanoethylated dinucleotide **11** arose from poor recovery during chromatography on silica gel due to a loss of the cyanoethyl group. The yields of the trinucleotide (**9**) and the tetranucleotide (**15**) were not lower than those obtained in condensations of monomers. Increase in sizes did not seem to decrease the yields of these triesterified oligonucleotides. For the synthesis of the heptanucleotide (**18**), the trimer (**9**) was converted to the 3′-phosphomonoester (**10**) by treatment with isoamyl nitrite and the 5′-monomethoxytrityl group of the tetramer (**15**) was removed to yield **16**. **10** and **16** were then treated with TPS for 10 h. The heptanucleotide (**17**) was isolated after removal of the protecting groups except for the 2′-*O*-(*o*-nitrobenzyl) groups. Successive treatment with isoamyl nitrite, concentrated ammonia, and 80% acetic acid gave the partially protected heptamer (**17**). **17** was isolated by chromatography on DEAE-cellulose. The elution profile is shown in Figure 1 (supplementary material). The desalted product was irradiated with UV light to remove the 2′-*O*-(*o*-nitrobenzyl) groups and applied to a column of DEAE-cellulose in the presence of 7 M urea.[19] The elution profile and conditions are shown in Figure 2 (supplementary material). The heptanucleotide (**18**) was obtained as the major peak. The overall yield from **16** after deblocking and separation was 30%. The base ratio was analyzed by high-pressure anion-exchange chromatography after hydrolysis with RNase M and found to agree with the calculated value. $R_f$ values of deprotected and partially protected compounds are shown in Table II (supplementary material). The sequence was analyzed by two-dimensional homochromatography[20] after labeling and RNase P1 digestion[21] (Figure 3) (supplementary material).

## Conclusion

Two fully protected oligonucleotides (**9** and **15**) were synthesized using the 2′-*O*-(*o*-nitrobenzyl) nucleosides. All internucleotide linkages of these tri- and tetranucleotides were protected with the *p*-chlorophenyl group by condensation of the 3′-*p*-chlorophenyl nucleotides. MST activated these phosphodiesters almost quantitatively as measured by TLC of the reaction mixtures, although the reaction periods were rather long compared with those for the recently developed arenesulfonyl tetrazolide[22] or nitroimidazolide.[23] The isolated yield of the 3′-cyanoethylated dinucleotide (**11**) was not satisfactory in spite of the complete disappearance of the starting material (**4a**) by TLC. The dinucleotides with the 3′-phosphorodianilidate were obtained in satisfactory yields (61 and 67% after isolation) and other 3′-dianilidated oligonucleotides (**9, 15**) were also isolated in high yields. For the final block condensation the dianilidate of **9** was removed by treatment with isoamyl nitrite and the terminal monoesterified trimer (**10**) was condensed with the tetranucleotide (**16**) using TPS as the condensing reagent since MST did not activate **10** sufficiently. The protected heptamer having a phosphodiester linkage between C and G was not isolated and the protecting

groups, except for those on the 2′-hydroxyls, were removed before chromatography on DEAE-Sephadex. The partially protected product **17** was then subjected to photochemical removal of the 2′-*O*-(*o*-nitrobenzyl) group and the heptanucleotide (**18**) was purified by chromatography on a DEAE-cellulose column in 7 M urea. **17** was eluted at rather high ionic concentration (Figure 1) and the presence of some destacking reagent such as ethanol would seem to be needed in the chromatography. Although the overall yield of **18** from **16** after all deblocking and the separation was fairly high, it would be advantageous to obtain the completely protected heptamer if the chain were to be elongated further. One of the anilidates of **9** could be substituted by the *p*-chlorophenyl group for this purpose. Investigations along these lines are currently underway.

## Experimental Section

**General Methods.** Paper chromatography was performed by the descending technique using solvent systems A, 2-propanol-concentrated ammonia-water (7:1:2 v/v); B, 1-propanol-concentrated ammonia-water (55:10:35 v/v); C, ethanol-1 M ammonium acetate (pH 3.5) (1:1 v/v). Paper electrophoresis was performed uing 0.05 M triethylammonium bicarbonate (pH 7.5) or 0.2 M morpholinium acetate (pH 3.5) at 900 V/40 cm. Cellulose acetate (Selecta-Electrophoresis-Membrane Carl Schleicher and Schüll) was used for electrophoresis (pH 3.5) prior to homochromatography on precoated DEAE-cellulose sheets (Polygram CEL 300, DEAE/HR 2/15, Macherey-Nagel and Co.). Thin layer chromatography (TLC) was performed on plates of silica gel (Merck HF 254) using a mixture of chloroform-ethanol. For columns silica gel G (Merck, type 60) was used. For anion-exchange chromatography, DEAE-cellulose (DE-23, Whatman) and DEAE-Sephadex (Pharmacia) were used.

5′-Monomethoxytrityl groups were removed by treatment with 80% acetic acid until all monomethoxytrityl color behaved as the carbinol on TLC as detected by spraying with 30% sulfuric acid. The β-cyanoethyl groups were removed by treatment with triethylamine in pyridine.[18] *N*-Acyl groups and tertiary *p*-chlorophenyl groups were removed by treatment with 28% ammonia at 50% for 5 h. Removal of the *o*-nitrobenzyl groups was performed in aqueous solution by irradiation with UV light through a Pyrex filter (2 mm thick) over a Pyrex test tube (1 mm thick) for 2 h using an apparatus consisting of a 300-W high-pressure mercury lamp (Eikosha Co. Model PIH 300) and quartz water-circulating jacket.

For sequence analysis of **18** the oligonucleotide was phosphorylated with [$\gamma$-$^{32}$P]ATP using polynucleotide kinase and the 3′-phosphate was removed during kination.[24] The labeled compound was isolated by homochromatography using homomix V.[25] The nucleotide (1600 cpm) was digested with nuclease P1 (10-20 ng) in the presence of cold RNA (20 μg) in 40 mM ammonium acetate (pH 5.0, 10 μL) at 30 °C. Aliquots (2 μL) were taken at time intervals (2, 5, 10, 20, and 30 min) and added to 5 mM EDTA (10 μL), with inactivation of the enzyme at 100 °C for 2 min. The solution was lyophilized and the residue was dissolved in water (4 μL) and then subjected to two-dimensional homochromatography.[20]

RNase P1 was obtained from Yamasa Shoyu Co. and other enzymatic hydrolyses were as described previously.[26]

**2c.** (MeOTr)ibG(nBzl) (**1c**, 1.364 g, 1.79 mmol) was phosphorylated by condensation of *p*-chlorophenyl phosphate[27] (1.122 g, 5.37 mmol) with DCC (2.655 g, 12.89 mmol) at room temperature for 3 days. An aliquot was subjected to TLC to confirm the disappearance of the starting material. The reaction was stopped by addition of aqueous pyridine (50%, 20 mL) and the mixture kept for 2 days after DCC having been removed with pentane (20 mL) (two portions). The filtered solution was evaporated and the residue was dissolved in aqueous pyridine (50%, 40 mL). The product was extracted with chloroform, washed with 0.1 M triethylammonium bicarbonate (pH 7.5, 50 mL) (four portions), concentrated, dissolved in 50% aqueous pyridine, and passed through a column (20 mL) of Dowex 50 × 2 (pyridinium form). The eluent (31 200 $A_{260}$, 1.36 mmol, 76%) was used for the next reaction.

**3a.** (MeOTr)bzA(nBzl) (**1a**, 1.558 g, 2 mmol) and *p*-chlorophenyl phosphate (522 mg, 2.5 mmol) were rendered anhydrous by evaporation of pyridine, and then treated with TPS (1.51 g, 5 mmol) in pyridine (10 mL) for 48 h. TLC (15:1) showed complete phosphorylation ($R_f$, 0) and the nucleotide was triesterified with β-cyanoethanol (0.8 mL, 11 mmol) by further addition of TPS (2 mmol). After 24 h TLC showed only a faster traveling spot ($R_f$, 0.56). Aqueous pyridine (50%, 20 mL) was added and the mixture was kept at room temperature for 1 h. The product was extracted with chloroform, washed with 0.1 M triethylammonium bicarbonate (pH 7.5, 15 mL) three times, then washed with water (20 mL) and dried with sodium sulfate. Chloroform was evaporated and the residue was coevaporated with toluene three times. The pyridine-free residue was dissolved in chloroform and applied to a column (4 × 19 cm) of silica gel G (100 g). Elution was performed with 45:1 chloroform-ethanol. The product was identified by TLC, concentrated, and precipitated with 1:1 cyclohexane-pentane (200 mL) from its solution in 1:1 chloroform-benzene. The yield was 826 mg (0.81 mmol), 40%. Anal. Calcd for $C_{53}H_{45}O_{11}N_7ClP$ (1022.37): C, 62.26; H, 4.44; N, 9.59. Found: C, 61.78; H, 4.31; N, 9.68 Mp 82-85 °C. UV: in ethanol $\lambda_{max}$ 279 nm ($\epsilon$ 25.1 × $10^3$), $\lambda_{min}$ 255 (17.7 × $10^3$).

**3b.** (MeOTr)U(nBzl) (**1b**, 2.604 g, 4 mmol) was condensed with *p*-chlorophenyl phosphate (1.043 g, 5 mmol) using TPS (3.02, 10 mmol) in pyridine (20 mL). The reaction was checked by TLC after 48 h and found to be complete. The nucleotide was treated with β-cyanoethanol (1.36 mL, 20 mmol) and TPS (1.208 g, 4 mmol) for 23 h at room temperature. The triesterified product was detected by TLC ($R_f$, 0.50) and the mixture was treated with aqueous pyridine (50%, 40 mL) for 1 h. The product was extracted with chloroform (100 mL), washed with 0.1 M triethylammonium bicarbonate (pH 7.5, 30 mL) four times, then with water (40 mL), dried with sodium sulfate, concentrated, coevaporated with toluene three times, dissolved in chloroform, and applied to a column (6.5 × 16 cm) of silica gel G. Elution was performed with 50:1 chloroform-ethanol (1 L). The product was identified by TLC, collected, and precipitated with 1:6 ether-hexane (420 mL) from its solution in chloroform. The yield was 1.719 g (1.92 mmol), 48%. Anal. Calcd for $C_{45}H_{40}N_4O_{12}PCl{\cdot}H_2O$ (913.24): C, 59.17; H, 4.64; N, 6.14. Found: C, 58.83; H, 4.55; N, 6.37. Mp 60-65 °C (wet melt). UV: in methanol $\lambda_{max}$ 233 nm ($\epsilon$ 18.4 × $10^3$), 260 (14.2 × $10^3$), $\lambda_{min}$ 248 (13.5 × $10^4$).

**4a. 3a** (1.554 g, 1.52 mmol) was treated with 80% acetic acid (40 mL) in chloroform (3 mL) at 27 °C overnight. TLC showed two new spots ($R_f$, 0.41 and 0.35) and acetic acid was evaporated. The residue was coevaporated with toluene three times and applied to a column (2.9 × 15 cm) of silica gel (50 g). Elution was performed with 30:1 chloroform-ethanol and the isomers ($R_f$, 0.41, 331 mg, mp 73-75 °C; $R_f$ 0.35, 610 mg, mp 71-72 °C) were obtained in a combined yield of 83% (1.255 mmol). Anal. Calcd for $C_{33}H_{29}O_{10}N_7PCl$ (750.04); C, 52.84; H, 3.90; N, 13.07. Found (high $R_f$): C, 52.44; H, 3.80; N, 12.64. (Low $R_f$) C, 52.63; H, 3.74; N, 12.78.

**9. 2a** obtained from **3a** (849 mg, 0.827 mmol) by treatment with triethylamine as described for **2c** was combined with **8** (886 mg, 0.70 mmol), rendered anhydrous by coevaporation of pyridine four times, and treated with MST (633 mg, 2.52 mmol) at room temperature for 2 days. Additional MST (0.84 mmol) was added and the mixture was kept for a further 3 days. The reaction was stopped by treating with aqueous pyridine (50%, 40 mL) for 4 h and the product was extracted with chloroform. The organic layer was washed with 0.1 M triethylammonium bicarbonate (pH 7.5, 40 mL) (three portions) and water (60 mL), dried with sodium sulfate, and concentrated. The residue was coevaporated with toluene three times, dissolved in chloroform, and applied to a column (4 × 18 cm) of silica gel G (90 g). Elution was performed with 40:1 chloroform-ethanol (800 mL) and the product was precipitated with 5:1 hexane-ether (240 mL) from its solution in chloroform. The yield was 946 mg (0.427 mmol, 61%). An aliquot was deblocked and A-U-Cp was characterized by complete hydrolysis with RNase M. The ratio at pH 2 of Ap:Up:Cp was 1.00:1.18:1.105.

**10. 9** (288 mg, 0.13 mmol) was dissolved in 1:1 pyridine-acetic acid (5 mL) and treated with isoamyl nitrite (0.4 mL) overnight. TLC (15:1) showed the unchanged starting material ($R_f$, 0.42) and the mixture was treated further with isoamyl nitrite (0.8 mL) overnight. The completion of the reaction was confirmed by TLC ($R_f$, 0) and aqueous pyridine (50%, 15 mL) was added. The product was extracted with chloroform (50 mL), washed with water (80 mL), and concentrated to dryness. The residue was dissolved in 50% aqueous pyridine and passed through a column (5 mL) of Dowex 50 × 8 (pyridinium form). The eluent and washings (three bed volumes) were evaporated with added pyridine. The product was precipitated with 1:1 ether-pentane (150 mL) from its solution in pyridine-chloroform. The yield was 276 mg (99%) using a molecular weight of 2143.

**15. 11** (785 mg, 0.466 mmol) was treated with triethylamine (15 mL) and pyridine (15 mL) at room temperature overnight. The decyanoethylated dinucleotide (**12**) was coevaporated with pyridine twice and combined with **14** (the high $R_f$ isomer, 298 mg; the low $R_f$ isomer, 142 mg) (0.13 mmol). The mixture was coevaporated with pyridine three times and treated with MST (351 mg, 1.4 mmol) in pyridine (7 mL) for 3 days. The mixture was treated with additional MST (117 mg, 0.466 mmol) for a day, then 50% pyridine (40 mL) at room temperature for 3 h, and extracted with chloroform (100 mL). The chloroform layer was washed with 0.1 M triethylammonium bicarbonate (pH 7.5, 40 mL) (three portions) and water (60 mL), dried with sodium sulfate, concentrated, and coevaporated with toluene three times. The residue was dissolved in chloroform and applied to a column of silica gel G (60 g). The product was eluted with 28:1 chloroform-ethanol (700 mL), collected, and precipitated with 1:1 chloroform-pentane (200 mL) from its solution in 1:1 chloroform-benzene. The yield was 637 mg (0.210 mmol), 68%. An aliquot was deblocked by consecutive treatment with isoamyl nitrite, ammonia, and acetic acid as described for **7**. The partially deblocked tetranucleotide was irradiated with UV light for 2 h and G-A-A-Ap (3 $A_{260}$, eluted from a paper chromatogram in solvent B) was hydrolyzed by RNase M. The completely hydrolyzed products were separated in paper chromatography in solvent B and the ratio of Gp (0.28 $A_{max}$) to Ap (0.925 $A_{max}$) was 1:2.86.

**16. 15** (212 mg, 0.07 mmol) was dissolved in chloroform (2 mL) and treated with 80% acetic acid (10 mL) at 30 °C overnight. TLC showed complete demonomethoxytritylation. The volatile materials were removed by evaporation and the product was precipitated with a mixture of pentane (80 mL), cyclohexane (20 mL), and ether (20 mL) from its solution in chloroform–benzene. The yield was 190 mg (98%).

**The Heptanucleotide A-U-C-G-A-A-Ap (18).** **10** (215 mg, 0.1 mmol) and **16** (138 mg, 0.05 mmol) were rendered anhydrous by coevaporation of pyridine and treated with TPS (61 mg, 0.2 mmol) in pyridine (1 mL) at 20-21 °C for 10.5 h. After the reaction mixture had been kept at 4 °C for 15 h, aqueous pyridine (50%, 1 mL) was added and the solution was kept at room temperature for 2 days. One-half of this mixture was deblocked by the following treatments. The solution was concentrated with pyridine and the dried residue was dissolved in 1:1 pyridine-acetic acid (5 mL) and treated with isoamyl nitrite (1.2 mL) at 30 °C overnight. Aqueous pyridine (50%, 15 mL) was added and the nucleotides were extracted with chloroform (40 mL). The organic layer was washed with water (50 mL) and concentrated. The residue was dissolved in pyridine (3 mL) and treated with concentrated ammonia (40 mL) at 50 °C for 5 h. The volatile materials were removed and a trace of pyridine was removed by coevaporation with toluene. The residue was kept in 80% acetic acid at room temperature overnight. Demonomethoxytritylation was confirmed by TLC and acetic acid was removed by evaporation of added aqueous butanol. The residue was dissolved in 0.1 M triethylammonium bicarbonate (40 mL) and washed with ether (20 mL) (two portions). The aqueous solution was diluted with the same buffer (total 100 mL) and applied to a column (1.6 × 17 cm) of DEAE-Sephadex A-25 (bicarbonate). The column was washed with 0.1 M triethylammonium bicarbonate. The elution profile and conditions are shown in Figure 1 and identification of compounds obtained in peaks is shown in Table II. The partially protected heptanucleotide (**17**) was obtained in peak IVa,b and it was homogeneous in paper chromatography (Table II). An aliquot (193 $A_{260}$) of **17** was deblocked by photoirradiation in water (100 mL), lyophilized, and applied to a column of DEAE-cellulose. The elution conditions are shown in Figure 2. The heptanucleotide was obtained as a main peak (96 $A_{260}$). The product was characterized by paper chromatography and two-dimensional homochromatography (Figure 3). The base ratio was found to be Gp:Ap:Up:Cp = 1.00: 4.06:1.02:1.03 after digestion of **18** (1.6 $A_{260}$) with RNase M and analysis of an aliquot (10%) by high-pressure anion-exchange chromatography (Varian nuclei acid analyzer). The total amount calculated from the deblocked material was 1041 $A_{260}$ units (15 $\mu$mol). The overall yield was 30% from **16**.

**Acknowledgment.** The authors thank Dr. A. F. Markham for reading the manuscript.

**Supplementary Material Available:** Figures 1 (elution profile of **17**), 2 (elution profile of **18**), and 3 (homochromatography of *pA-U-C-G-A-A-A), and an $R_f$ table (4 pages). Ordering information is given on any current masthead page.

## References and Notes


(1) Part 22: E. Ohtsuka, S. Nishikawa, A. F. Markham, S. Tanaka, T. Miyake, T. Wakabayashi, M. Ikehara, and M. Sugiura, *Biochemistry*, in press.
(2) Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education of Japan.
(3) Abbreviations follow principally recommendations of the IUPAC–IUB Commission of Biochemical Nomenclature [*J. Biol. Chem.*, **245**, 5171 (1970); *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 2222 (1977)]. See also the footnote in Table I. Yeast $tRNA_f^{Met}$ refers to the initiation methionine transfer RNA from yeast.
(4) E. Ohtsuka, T. Tanaka, S. Tanaka, and M. Ikehara, *J. Am. Chem. Soc.*, **100**, 4580 (1978).
(5) E. Ohtsuka, S. Tanaka, and M. Ikehara, *Nucleic Acids Res.*, **1**, 1351 (1974).
(6) E. Ohtsuka, S. Tanaka, and M. Ikehara, *Chem. Pharm. Bull.*, **25**, 949 (1977).
(7) E. Ohtsuka, S. Tanaka, and M. Ikehara, *Synthesis*, 453 (1977).
(8) S. Uesugi, S. Tanaka, E. Ohtsuka, and M. Ikehara, *Chem. Pharm. Bull.*, **26**, 2396 (1978).
(9) R. L. Letsinger and K. K. Ogilvie, *J. Am. Chem. Soc.*, **91**, 3350 (1969).
(10) J. Hachmann and H. G. Khorana, *J. Am. Chem. Soc.*, **91**, 2749 (1969).
(11) M. Simsek and U. L. RajBhandary, *Biochem. Biophys. Res. Commun.*, **49**, 508 (1972).
(12) P. W. Piper and B. F. C. Clark, *Nature (London)*, **247**, 516 (1974); M. Simsek, U. L. RajBhandary, M. Boisnard, and G. Petrissant, *ibid.*, **247**, 518 (1974).
(13) S. K. Dube, K. A. Marcker, B. F. C. Clark, and S. Cory, *Nature (London)*, **218**, 232 (1968); *Eur. J. Biochem.*, **8**, 244 (1969); S. K. Dube and K. A. Marker, *ibid.*, **8**, 256 (1969).
(14) K. Nishikawa and S. Takemura, *J. Biochem. (Tokyo)*, **76**, 935 (1974).
(15) E. Ohtsuka, S. Nishikawa, M. Ikehara, and M. Sugiura, Proceedings of the 1977 Molecular Biology Meeting of Japan, p 48.
(16) R. Lohrmann and H. G. Khorana, *J. Am. Chem. Soc.*, **88**, 829 (1966).
(17) N. Katagiri, K. Itakura, and S. A. Narang, *J. Am. Chem. Soc.*, **97**, 7332 (1975).
(18) R. W. Adamiak, E. Biaja, K. Greskowiak, R. Kierzek, A. Kraszewski, W. T. Markiewicz, J. Stawinski, and M. Wiewiorowski, *Nucleic Acids Res.*, **4**, 2321 (1977).
(19) R. V. Tomlinson and G. M. Tener, *Biochemistry*, **2**, 697, 703 (1963).
(20) F. Sanger, J. E. Donelson, A. R. Coulson, H. Kössel, and D. Fischer, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 1209 (1973).
(21) M. Silberklang, A. M. Gillum, and U. L. RajBhandary, *Nucleic Acids Res.*, **4**, 4091 (1977).
(22) J. Stawinski, T. Hozumi, and S. A. Narang, *Can. J. Chem.*, **54**, 670 (1976).
(23) J. H. van Boom and P.M.J. Burgers, *Tetrahedron Lett.*, 4874 (1976).
(24) V. Cameron and O. C. Uhlenbeck, *Biochemistry*, **16**, 5120 (1977).
(25) E. Jay, R. Bambara, R. Padmanabhan, and R. Wu, *Nucleic Acids Res.*, **1**, 331 (1974).
(26) E. Ohtsuka, M. Ubasawa, S. Morioka, and M. Ikehara, *J. Am. Chem. Soc.*, **95**, 4725 (1973).
(27) G. R. Owne, C. B. Reese, C. J. Ransom, J. H. van Boom, and J.P.H. Herscheid, *Synthesis*, 704 (1974).