

[Chem. Pharm. Bull.
29(10)2799—2806(1981)]

Studies on Transfer Ribonucleic Acids and Related Compounds. XXXIX.¹⁾
Chemical Synthesis of Heptadeca- and Hexadecaribonucleotides
corresponding to the 3'-Terminus of the tRNA^{Met} of *E. coli*

EIKO OHTSUKA, KAZUO FUJIYAMA, TOSHIKI TANAKA, and MORIO IKEHARA

Faculty of Pharmaceutical Sciences, Osaka University 133-1 Yamadaoka, Suita, Osaka

(Received March 23, 1981)

A heptadecanucleotide corresponding to the 3'-end (bases 61—77) of the tRNA^{Met} of *E. coli* was synthesized by the phosphotriester method involving condensation of protected oligonucleotides with chain lengths of 3 to 6. 2'-O-(*o*-Nitrobenzyl) derivatives of N-acyl-5'-O-monomethoxytrityl nucleosides were used as the starting materials. To protect the 3'-end of the heptadecanucleotide with phosphorodiamidate during the synthesis, protected AACCAp having 3'-phosphorodiamidate was used as the starting nucleotide block. Condensation of protected CCCCGCp or successive condensations of protected CCCp and CGCp gave the protected undecamer in satisfactory yields. The final condensation of the undecamer to protected UCCGGCp yielded the protected heptadecamer, and the product was deprotected to give the 3'-phosphorylated heptadecanucleotide UCCGGCCCCCGAACCAp.

A hexadecanucleotide corresponding to the 3'-end of the same tRNA^{Met} was also synthesized by the phosphotriester method by condensation of the undecamer with protected CCGGCp. The hexadecamer was deblocked and purified by chromatography on ion-exchange and reverse phase supports. This deprotected hexadecanucleotide CCGGCCCGCAACCAp (corresponding to bases 62—77) can be joined to the previously synthesized heptamer corresponding to the eukaryotic loop VI sequences to construct a hybrid tRNA.

Keywords—tRNA fragments; phosphotriester synthesis; phosphotriester condensation; protected oligoribonucleotides; ion-exchange chromatography; reversed phase chromatography

The phosphotriester method, involving protections for internucleotidic phosphates with *p*-chlorophenyl groups, 2'-hydroxyl functions with *p*-nitrobenzyl groups, 5'-hydroxyl groups with monomethoxytrityl groups and heterocyclic amines with acyl groups, has been applied to the synthesis of the *E. coli* tRNA^{Met} fragments of chain lengths 5,²⁾ 6³⁾ and 10.⁴⁾ To extend this approach further to the synthesis of larger oligoribonucleotides, we have synthesized heptadeca- and hexadecanucleotides corresponding to the 3'-end of *E. coli* tRNA^{Met} as described in this paper (Fig. 1). Previously this heptadecanucleotide corresponding to bases 61—77 of the tRNA has been obtained by joining short fragments with RNA ligase.⁵⁾ Chemical synthesis of larger tRNA fragments such as quarter molecules would facilitate the synthesis of tRNAs and their analogs in large quantities for biochemical studies. The present hexadecanucleotides (bases 62—77 of the tRNA) would serve as a donor molecule for joining to a heptamer corresponding to the loop VI sequences of the eukaryotic initiator tRNA.⁶⁾

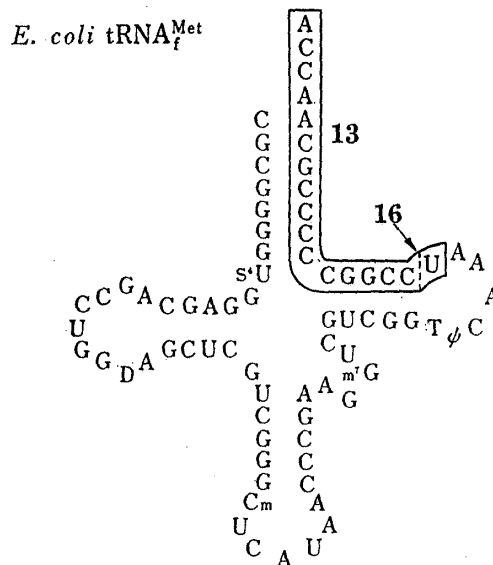


Fig. 1. The Location of the 3'-Terminal Heptadecamer (13) and Hexadecamer (16) in *E. coli* tRNA^{Met}

The synthetic scheme for the heptadecanucleotide and hexadecanucleotide is shown in Chart 1. The penta- and hexanucleotide blocks were prepared by condensation of protected di- or trinucleotides. The 3'-terminal pentanucleotide was protected with 3'-phosphorodanilidate.

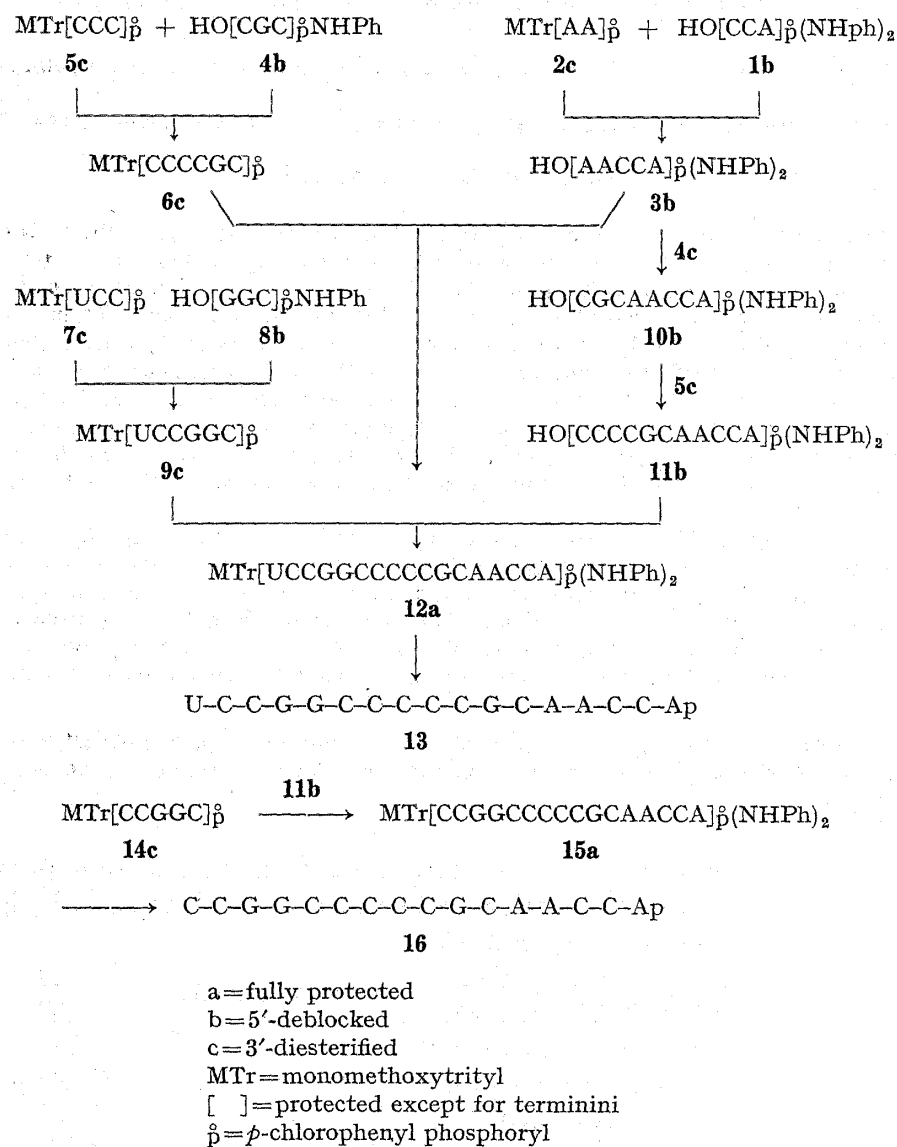


Chart 1

Preparation of Oligoribonucleotide Blocks

Oligoribonucleotides were synthesized by condensation of 5'-O-monomethoxytrityl-2'-O-(*o*-nitrobenzyl)nucleoside 3'-*p*-chlorophenylphosphates¹⁾ (3'-phosphodiester components) with 2'-O-(*o*-nitrobenzyl) nucleoside 3'-*p*-chlorophenyl phosphoranilidates²⁾ (5'-hydroxyl components) using mesitylenesulfonyl 1,2,4-triazolide⁷⁾ (MST) as the condensing reagent. The corresponding 3-nitrotriazolide⁸⁾ (MSNT) was also used. For the 3'-terminal block (1) 2'-O-(*o*-nitrobenzyl)-N-benzoyladenine 3'-phosphorodanilidate⁹⁾ was used as the starting material. The dinucleotides were elongated in the 5'-direction by removal of the 5'-monomethoxytrityl group followed by condensation with the phosphodiester. The scheme for the synthesis of protected CCAP (1) is shown in Chart 2. Conditions for the synthesis of 1b (5'-deblocked) are summarized in Table I together with those for other di- and trinucleotide blocks. Protected

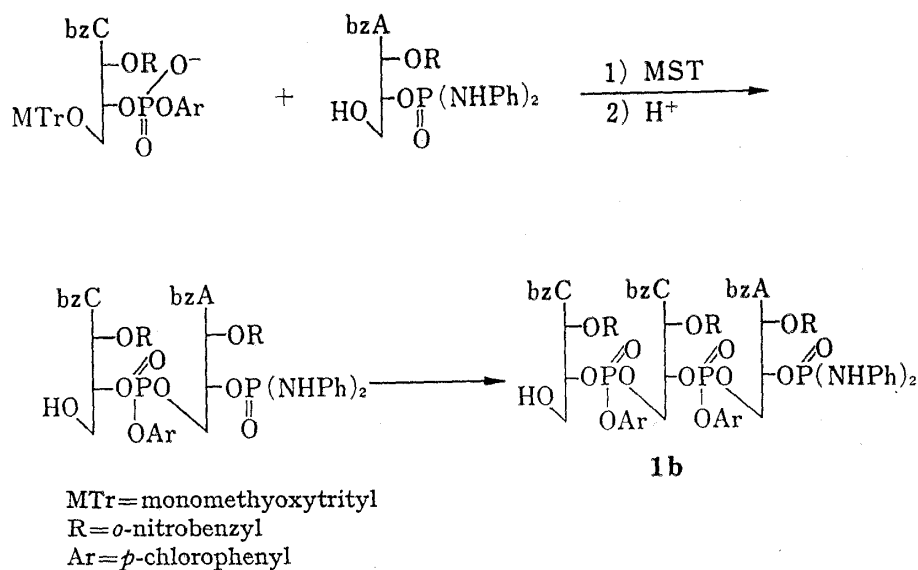


Chart 2

TABLE I. Reaction Conditions for the Synthesis of Di- and Trinucleotides

3'-Phosphodiester component (mmol)	5'-Hydroxyl component (mmol)	Product (mmol)	Yield %
(MeOTr)bzC(nBzl) ₂ 4.19	bzA(nBzl) ₂ (NHPh) ₂ 3.38	(nBzl)bzC ₂ bzA ₂ (NHPh) ₂ 2.56	78
(MeOTr)bzC(nBzl) ₂ 3.70	(nBzl)bzC ₂ bzA ₂ (NHPh) ₂ 2.64	(nBzl)bzC ₂ bzC ₂ bzA ₂ (NHPh) ₂ (1b) 1.76	67
(MeOTr)bzA(nBzl) ₂ 5.30	bzA(nBzl) ₂ (NHPh) 4.30	(nBzl)(MeOTr)bzA ₂ bzA ₂ (NHPh) (2) 3.34	78
(MeOTr)ibG(nBzl) ₂ 7.50	bzC(nBzl) ₂ (NHPh) 5.65	(nBzl)ibG ₂ bzC ₂ (NHPh) 4.06	72
(MeOTr)bzC(nBzl) ₂ 3.80	(nBzl)ibG ₂ bzC ₂ (NHPh) 2.90	(nBzl)(MeOTr)bzC ₂ ibG ₂ bzC ₂ (NHPh) (4a) 1.87	64
(MeOTr)ibG(nBzl) ₂ 1.55	(nBzl)ibG ₂ bzC ₂ (NHPh) 1.03	(nBzl)ibG ₂ ibG ₂ bzC ₂ (NHPh) (8b) 0.64	62
(MeOTr)bzC(nBzl) ₂ 5.30	bzC(nBzl) ₂ (NHPh) 4.30	(nBzl)bzC ₂ bzC ₂ (NHPh) 3.80	88
(MeOTr)bzC(nBzl) ₂ 3.10	(nBzl)bzC ₂ bzC ₂ (NHPh) 2.5	(nBzl)(MeOTr)bzC ₂ bzC ₂ bzC ₂ (NHPh) (5) 1.73	69
(MeOTr)U(nBzl) ₂ 1.71	(nBzl)bzC ₂ bzC ₂ (NHPh) 1.23	(nBzl)(MeOTr)U ₂ bzC ₂ bzC ₂ (NHPh) (7) 0.77	63

GCp was used as the common intermediate for the synthesis of protected CGCp (Chart 3) (**4**) and GGCp (**8**). Two condensing reagents, MST and MSNT, were compared in reactions for **8a**. The latter was found to require a much shorter time (30 min) and gave higher yields (*ca.* 90%) in the reaction of diesterified Gp. All condensations shown in Table I were performed with MST. The preparation of **1b** is described as a general method in "Experimental." **8a** was also isolated by silica gel chromatography and used for **14**. Derivatization of the fully protected trimer (**4a**) to **4b** (5'-deblocked) and **4c** (3'-deblocked) is shown in Chart 3.

Condensation of Oligonucleotides and Deblocking of the Protected Heptadeca- and Hexadecanucleotides (**12**, **15**)

As shown in Chart 1, the fully protected heptadecamer (**12a**) and hexadecamer (**15a**) were synthesized by using a protected undecamer (**11b**) as the common intermediate. **11b**

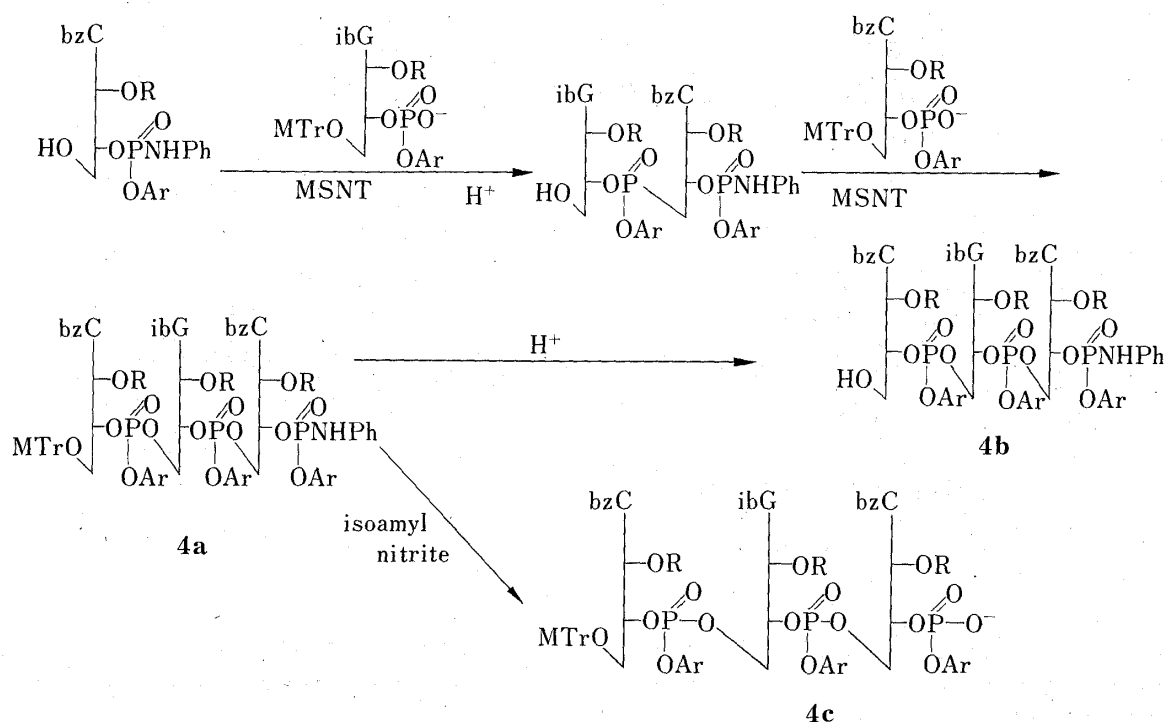


Chart 3

was prepared either by condensing trinucleotides **4c** and **5c** or a hexanucleotide **6c** with protected AACCAp (**3b**), which was obtained from **1b** and **2c**. Reaction conditions for these condensations are listed in Table II. Mesitylenesulfonyl nitroimidazolidine (MSNI)¹⁰⁾ and MSNT were used as condensing reagents. Fully protected oligonucleotides were isolated by chromatography on silica gel and the monomethoxytrityl group was removed for elongation of the chain in the 5'-direction (*e.g.* **3b**). The 3'-phosphorimidate of the fully protected oligomer (*e.g.* **4a**) was converted to the phosphate by treatment with isoamyl nitrite to give **4c**, which was isolated by extraction with organic solvents and then activated by treatment with the condensing reagent. The yields for condensation of trimers varied from 33 to 74% as summarized in Table II. Condensation of a larger block (hexamer, **6c**) with the pentamer (**3b**) gave the undecamer (**11a**) in a comparable yield, although separation of the product (**11a**) and the 5'-hydroxyl component (**3b**) was not complete. Contents of the product was estimated by thin layer chromatography (TLC) and the 5'-hydroxyl group of the starting material was blocked by acetylation of the mixture. The final condensations to give the heptadecamer (**12**) and hexadecamer (**15**) were performed with MSNI and MSNT, respectively. The products

TABLE II. Conditions for the Condensation of Oligonucleotides

3'-Phosphodiester component (mmol)	5'-Hydroxyl component (mmol)	Condensing reagent (mmol)	Reaction time (h)	Product (mmol)	Yield (%)
2c 1.69	1b 1.28	MSNI 2.64	36	3b 0.83	65
5c 0.89	4b 0.70	MSNI 1.77	24	6a 0.23	33
7c 0.71	8b 0.42	MSNI 1.41	20	9a 0.31	74
4c 0.64	3b 0.5	MSNI 1.46	36	10b 0.17	33
5c 0.2	10b 0.1	MSNI 0.30	16	11b 0.032	32
6c 0.15	3b 0.092	MSNI 0.30	24	11b 0.079	86
9c 0.061	11b 0.032	MSNI 0.122	36	12a 0.012	38
14c 0.044	11b 0.033	MSNT 0.176	1	15a 0.016	49

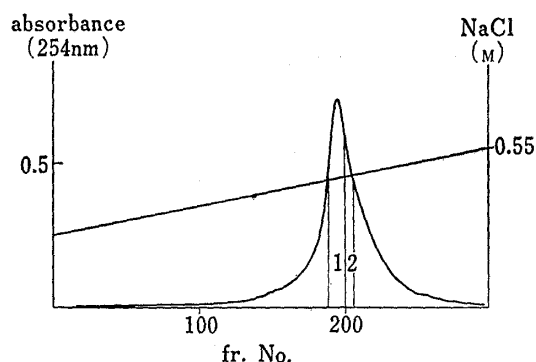


Fig. 2. Chromatography of the Hexadecamer (16) on a Column (0.9 x 64 cm) of DEAE-Sephadex A-25 in 7 M Urea and 0.02 M Tris-HCl (pH 7.5)

Elution was performed with a linear gradient of sodium chloride (0.25 M—0.55 M, total 900 ml). One 3 ml fraction was collected every minute.

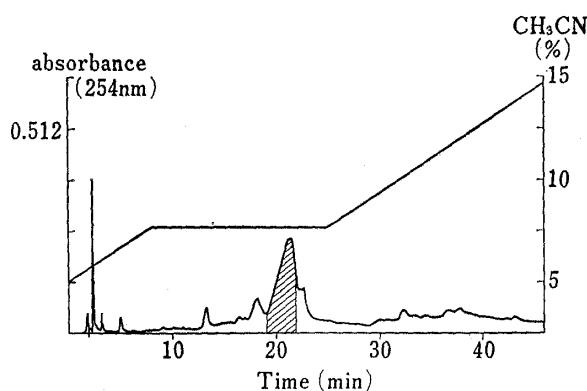


Fig. 3. HPLC of the Hexadecamer (16) obtained by Chromatography as Shown in Fig. 2 (peak 1) on a C_{18} Silica Column (Hypersil ODS, 5 μ) in 0.1 M Triethylammonium Acetate

Elution was performed with a linear gradient of acetonitrile. The flow rate was 2 ml/min.

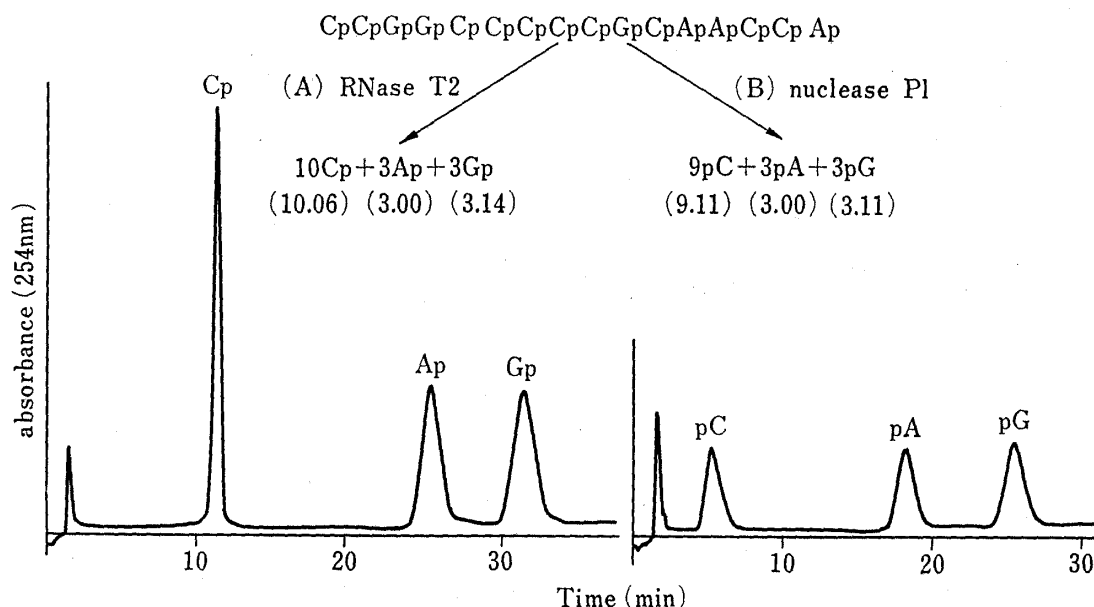


Fig. 4. Base Composition of the Hexadecamer (16)

a) **16** was digested with RNase T2 and applied to an anion-exchange high pressure liquid chromatograph (Varian LCS 1000). Elution was performed with a gradient of phosphate using 0.1 M potassium phosphate (pH 3.30) as a starting solution and 1 M potassium phosphate (pH 4.0) was added with a 13 min delay. b) **16** was digested with Nuclease P1 and analyzed as described in a) except that the gradient delay was 8 min.

were isolated by Silica gel chromatography and checked by TLC and reversed phase TLC (RTLC). The precipitated product was deblocked by successive treatment with isoamyl nitrite,²⁾ N^1,N^1,N^2,N^2 -tetramethylguanidinium syn-pyridine-2-carboxaldoximate,⁸⁾ concentrated ammonia at 50°C, 80% acetic acid and UV light with wavelength longer than 280 nm.⁶⁾ Oxamate treatment was reported to remove phenyl esters preferentially and to give higher overall yields in deblocking. However, it can be replaced by treatment with concentrated ammonia at room temperature for 48 h prior to the treatment at 50°C for removal of the N-acyl groups. The deblocked mixture was subjected to gel filtration on Sephadex G-50 and analyzed by high pressure liquid chromatography on a reversed phase support. The first part of the gel filtrate was found to contain the product and it was subjected to anion-exchange chromatography on DEAE-Sephadex in the presence of 7 M urea. Fig. 2 shows the conditions

and elution profile of the hexadecamer (16) from the ion-exchange column. Peaks 1 and 2 were analyzed by reverse phase chromatography and peak 1 was found to contain the product. The product was obtained by preparative chromatography on the same column (Fig. 3) and characterized by base analysis (Fig. 4) and mobility shift analysis. The heptadecanucleotide (13) was isolated similarly.

Experimental

Paper chromatography was performed by the descending technique using the following solvent systems: A, isopropanol–conc. ammonia–water (7:1:2, v/v); B, *n*-propanol–conc. ammonia (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. Gel electrophoresis on 10 or 20% acrylamide was as described previously.¹¹

TLC was performed on plates of silica gel (Kieselgel HF₂₅₄, Merck) using a mixture of chloroform and methanol. For RTLC, silanized silica gel and HPTLC (RP-8 or RP-18F₂₅₄) (Merck) were used with a mixture of acetone–water. For columns, Silica gel (Merck, G) and silanized silica gel (Merck, 70–230 mesh) were used unless otherwise specified with a mixture of chloroform–methanol.

HPLC was performed on an Altex 332 MP apparatus with a reversed phase column (alkylated silica, Hypersil ODS 5 μ , 4.6 $\times$ 250 nm).

A photochemical apparatus for UV irradiation and other general methods for the characterization of oligonucleotides were described previously.⁹

The Trinucleotide (1b)—2'-O-(*o*-Nitrobenzyl)-N-benzoyladenine 3'-phosphorodanilidate (2.49 g, 3.38 mmol) and the triethylammonium salt of 2'-O-(*o*-nitrobenzyl)-5'-O-monomethoxytrityl-N-benzoylcytidine 3'-*p*-chlorophenyl phosphate (4.19 g, 4.19 mmol) were dried by coevaporation with pyridine. The mixture was treated with MST (2.93 g, 11.67 mmol) for 44 h. The reaction was monitored by TLC (20:1) and RTLC (RP-2). Water (15 ml) was added with cooling and the product was extracted with chloroform (80 ml). The organic layer was washed three times with 0.1 M triethylammonium bicarbonate (80 ml), concentrated, and coevaporated with pyridine, then with toluene. The residue was dissolved in a small amount of chloroform and applied to a column of silica gel G (230 g). The product was eluted with chloroform–methanol (40:1). Impure fractions were rechromatographed on a Silica gel column (G, 45 g) using chloroform–methanol (50:1). The dimer was concentrated and treated with 80% acetic acid (206 ml) overnight. To complete demonomethoxytritylation, 80% acetic acid (55 ml) was added and the mixture was kept for 24 h. Volatile materials were removed by evaporation and the dinucleotide was precipitated with pentane from its solution in chloroform. The yield was 3.68 g, 2.65 mmol. Protected CAP (3.66 g, 2.64 mmol) was condensed with same mononucleotide (4 g, 4 mmol) using MST (2.59 g, 10.3 mmol) in pyridine (25 ml) as above and the product was extracted with chloroform similarly. The chloroform solution was concentrated and the residue was coevaporated with pyridine and toluene. Chromatography was performed with silica gel G (130 g) and the product was eluted with chloroform–methanol (60:1). Impure fractions were rechromatographed on Silica gel G (30 g). The trimer was demonomethoxytritylated by treatment with 80% acetic acid for 36 h and precipitated with pentane as described for the dimer. The yield was 3.6 g, 1.76 mmol, 67%. An aliquot (40 mg) was deblocked by treatment with isoamyl nitrite (1 ml) in pyridine–acetic acid (5:4, 4 ml), concentrated ammonia, 80% acetic acid and UV irradiation. Deblocked trinucleotide C–C–Ap (1 A₂₆₀) was isolated by paper chromatography in solvent B and digested with RNase A (20 μ g/ μ l, 3 μ l) in 0.05 M triethylammonium bicarbonate (0.1 ml) at 37°C for 6 h to yield Cp (0.548 A₂₆₀) and Ap (0.302 A₂₅₇) at pH 2 after separation by paper electrophoresis (pH 3.5). The ratio of Cp to Ap was 2.11 to 1.00.

The Fully Protected Pentanucleotide (14a)—The trimer prepared by successive condensation using MSNT (8b) (0.132 mmol) and triethylammonium (*n*Bzl) (MeOTr) bzC₂P₂C₂P₂ (0.2 mmol) were dried by evaporation with pyridine. The mixture was dissolved in pyridine (3 ml) treated with MSNT (0.5 mmol) and concentrated to ca. 1.3 ml. After 20 min the reaction was checked by TLC and found to be incomplete. The reaction was terminated after 50 min by treatment with 1 M triethylammonium bicarbonate (0.6 ml) and pyridine (4 ml) for 30 min. The product (14a) was extracted with ethyl acetate (50 ml), washed with 0.1 M triethylammonium bicarbonate, and concentrated with addition of pyridine and toluene. The residue was applied to a column of silica gel (13 g) and 14a was precipitated with pentane from its solution in chloroform. The yield was 0.374 g, 0.102 mmol.

Removal of the Anilidate to give the 3'-Diesterified Pentamer (14c)—14a (0.1 mmol) was dissolved in pyridine–acetic acid (5:4, 5.4 ml) and treated with isoamyl nitrite (0.9 ml) at room temperature for 21 h. Aqueous pyridine (50%, 50 ml) was added with cooling and the aqueous layer was washed with ether–pentane. The product 14c was extracted with ethyl acetate (50 ml), washed 5 times with 0.1 M triethylammonium bicarbonate (50 ml) and precipitated with ether–pentane (1:1) from its dry solution in chloroform. The yield was 0.338 g, 0.092 mmol (92%). A trace of 4a contaminated the product.

The Protected Octanucleotides (10b)—The trinucleotide (4c) (1.51 g, 0.64 mmol) and the pentanucleotide (3b) (1.7 g, 0.5 mmol) were treated with MSNI (2.8 g, 0.96 mmol) in pyridine (2.5 ml) at 31°C for 21 h.

Further reagent (0.15 g) was added after checking the reaction by TLC. The mixture was worked up as described for the synthesis of **14a** and applied to a column of silica gel (G, 50 g). Elution was performed with chloroform-methanol (45:1) and the mixture (35:1) containing 1% pyridine. Impure fractions were applied to a column (1.6 × 50 cm) of silanized Silica gel (60 g) and **10a** was eluted with 75% aqueous acetone. **10a** was then treated with 80% acetic acid (20 ml) at room temperature for 16 h and concentrated with added toluene. **10b** was precipitated with pentane from its solution in chloroform. The yield was 0.17 mmol.

The Undecanucleotide 11b—a) **10b** (0.538 mmol, 0.1 mmol) and **5c** (0.471 g, 0.2 mmol) were treated with MSNI (Table II). The mixture was worked up as described for **14a** and applied to a column of silica gel G (15 g). **11a** was eluted with chloroform-methanol (30:1) and the contaminating starting material (**10b**) was acetylated by treatment of the mixture with acetic acid (0.5 ml) in pyridine (4 ml) at room temperature. The mixture was treated with aqueous pyridine (50%, 10 ml) at room temperature for 30 min. **11a** was extracted with chloroform, detritylated by treatment with 80% acetic acid (70 ml) at 30°C for 17 h and precipitated with pentane (245 mg). **11b** was contaminated with *ca.* 10% of 5'-acetylated **10b**. The yield was *ca.* 0.032 mmol, 32%.

b) **6c** and **3b** were condensed under the conditions shown in Table II and worked up as described for **14b**. The mixture was dissolved in chloroform (5 ml) and applied to a column of Silica gel G (15 g). **11a** was eluted with chloroform-methanol (30:1) and treated with 80% acetic acid (80 ml) at room temperature overnight. **11b** was concentrated with added toluene and precipitated with pentane (60 ml) from its solution in chloroform (7 ml). The yield was 572 mg, 0.079 mmol (86%).

The Hexadecanucleotide (15a)—**14c** and **11b** were condensed with NSNT as shown in Table II. The reaction was checked by TLC and RTLC (RP-8), and **11b** was still detected after 20 min. After 1 h the reaction was terminated by addition of 1 M triethylammonium bicarbonate (0.2 ml) and pyridine (2 ml). The mixture was kept for 30 min and nucleotides were extracted with ethyl acetate. The organic phase was washed 3 times with 0.1 M triethylammonium bicarbonate (15 ml), and concentrated with pyridine and toluene. The residue was applied to a column of Silica gel (14 g). A part of **15a** was washed off with chloroform and the adsorbed material was eluted with 4% methanol and purified by passage through Silica gel (15 g). *R_f* in TLC (15:1) was 0.37, and that in RTLC (RP-8, 82% acetone) was 0.33. The yield was 174.7 mg, 0.016 mmol, 49% (crude).

Deblocking of 15a to give the Hexadecanucleotide (16)—**15a** (109 mg, 10 μmol) was treated with isoamyl nitrite (0.9 ml) in pyridine-acetic acid (5:4, 4.5 ml) at room temperature for 24 h and treated with aqueous pyridine (50%, 50 ml). The aqueous layer was washed twice with ether-pentane (1:1, 50 ml) and extracted with ethyl acetate (30 ml, 20 ml). The organic phase was washed 5 times with 0.1 M triethylammonium bicarbonate and concentrated with pyridine, then the residue was precipitated with ether-pentane (1:1) from its solution in chloroform (106.3 mg). The powder (37.9 mg, 3.42 μmol) was treated with 0.5 M pyridine aldoxime-tetramethylguanidine in dioxane-water (1:1) (5.1 ml) at room temperature for 3 days and concentrated to dryness. The residue was dissolved in aqueous pyridine (50%, 50 ml), washed with ethyl acetate and back-extracted with water. The aqueous phase was concentrated. The residue was dissolved in pyridine (7 ml) and treated with conc. ammonia (34 ml) at 60°C for 7 h. The volatile materials were removed by evaporation and the residue was dissolved in aqueous pyridine (50%, 10 ml). The solution was passed through a column of Dowex 50 × 2 (pyridinium form) (15 ml) and the column was washed with aqueous pyridine (50%, 60 ml). The combined solutions were concentrated and coevaporated with toluene, and the residue was treated with 80% acetic acid (50 ml) at 28°C overnight. Volatile materials were removed by evaporation, followed by coevaporation with toluene, and the residue was dissolved in 5% aqueous pyridine (40 ml). The solution was washed twice with ethyl acetate (50 ml) and concentrated to dryness. The residue was dissolved in water (50 ml), irradiated with UV light for 20 min, then washed with ethyl acetate (50 ml) and the aqueous phase was concentrated. This irradiation procedure was repeated and the aqueous solution (150 ml) was irradiated for 2 h. The total irradiation time was 2 h 40 min. The solution was then concentrated to *ca.* 50 ml, and washed twice with *n*-butanol (50 ml). The yield was 560 *A*₂₆₀. One-half of the solution was concentrated (*ca.* 0.2 ml) and applied to a column (0.7 × 147 cm) of Sephadex G-50. The first three peaks (53*A*₂₆₀, 55*A*₂₆₀, 28*A*₂₆₀) were analyzed by HPLC (Hypersil ODS) and the first peak was found to contain the hexadecamer (**16**). This part was subjected to ion-exchange chromatography on DEAE-Sephadex A-25 as shown in Fig. 2. Peaks 1 and 2 were desalted by dialysis and the product (**16**) in peak 1 was purified by HPLC (Fig. 3).

The Heptadecanucleotide (13)—The fully protected heptadecanucleotide (**12a**) was synthesized under the conditions shown in Table II, and an aliquot (48 mg, 4.19 μmol) was deblocked as described for **16**. The first peak (81.8*A*₂₆₀) in gel filtration (Sephadex G-50) was subjected to ion-exchange chromatography on DEAE-Sephadex A-25 (1.0 × 63 cm) using conditions similar to those in Fig. 2. The material in the main part of the peak (41.3 *A*₂₆₀) was desalted by gel filtration on Bio-gel P-2 and purified by HPLC on Hypersil ODS.

Acknowledgement This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan and by a grant from the Mitsubishi Foundation.

References and Notes

- 1) Part XXXVIII: E. Ohtsuka, T. Wakabayashi, and M. Ikehara, *Chem. Pharm. Bull.*, **29**, 759 (1981).
- 2) E. Ohtsuka, T. Tanaka, and M. Ikehara, *Nucleic Acids Res.*, **7**, 1283 (1979).
- 3) E. Ohtsuka, T. Tanaka, and M. Ikehara, *Chem. Pharm. Bull.*, **28**, 120 (1980).
- 4) E. Ohtsuka, T. Tanaka, and M. Ikehara, *J. Am. Chem. Soc.*, **101**, 6409 (1979).
- 5) E. Ohtsuka, S. Nishikawa, A.F. Markham, S. Tanaka, T. Miyake, T. Wakabayashi, M. Ikehara, and M. Sugihara, *Biochemistry*, **17**, 4894 (1978).
- 6) E. Ohtsuka, S. Tanaka, and M. Ikehara, *J. Am. Chem. Soc.*, **100**, 8210 (1978).
- 7) N. Katagiri, K. Itakura, and S.A. Narang, *J. Am. Chem. Soc.*, **97**, 7332 (1975).
- 8) C.B. Reese, R.C. Titmas, and L. Yau, *Tetrahedron Lett.*, **1978**, 2727.
- 9) E. Ohtsuka, T. Tanaka, S. Tanaka, and M. Ikehara, *J. Am. Chem. Soc.*, **100**, 4580 (1978).
- 10) J.H. van Boom and P.M.J. Burgers, *Tetrahedron Lett.* **1977**, 4875; G.R. Gough, C.K. Singleton, H.L. Weith, and P.T. Gilham, *Nucleic Acids Res.*, **6**, 1557 (1979).
- 11) E. Ohtsuka, S. Nishikawa, R. Fukumoto, H. Uemura, T. Tanaka, E. Nakagawa, T. Mitake, and M. Ikehara, *Eur. J. Biochem.*, **105**, 481 (1980).