respects with those of natural coriamyrtin.

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Registry No. (−)-**1**, 17617-45-7; (+)-**2**, 2571-86-0; (±)-**3**, 90742-33-9; (±)-**4**, 90122-93-3; (±)-**5a**, 90122-94-4; (±)-**5b**, 90122-95-5; (±)-**6a**, 90821-12-8; (±)-**6b**, 90821-13-9; (±)-**7**, 90821-14-0; (±)-**8**, 90123-00-5; (±)-**9**, 90123-01-6; (±)-**10**, 90192-36-2; (±)-**11**, 90742-34-0; (±)-**12**, 90123-03-8; (±)-**13**, 90123-05-0; (+)-**13**, 90192-37-3; (−)-**14**, 90123-06-1; (−)-**15a**, 90123-07-2; **15b**, 90192-38-4; (−)-**16a**, 90123-08-3; **16b** (isomer 1), 90821-87-7; **16b** (isomer 2), 90821-15-1; (−)-**17**, 90742-35-1; (−)-**18**, 90123-10-7; (−)-**19**, 20744-71-2; (−)-**20**, 90123-12-9; (−)-**21**, 90123-13-0; (−)-**22**, 90123-14-1; (−)-**23**, 90192-39-5; (±)-ii, 61242-43-1; (±)-iii, 90742-36-2.

Synthesis of a Dodecaribonucleotide, GUAUCAAUAAUG, by Use of "Fully" Protected Ribonucleotide Building Blocks

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Abstract: The fully protected ribonucleotide monomer units (**17**, **19**, **26**, and **32**) have been synthesized in excellent overall yields from unprotected ribonucleosides. Several carbamoyl groups were tested for protection of the guanosine base moiety. Finally, the diphenylcarbamoyl group was chosen and O^6-(diphenylcarbamoyl)-N^2-propionylguanosine was readily prepared in high yield and converted to the guanosine units **12** and **17**. The uridine unit **19** was prepared by the acylation of the previous unit **18** with anisoyl chloride in the presence of i-Pr_2EtN. In the case of the adenosine and cytidine units (**26** and **32**), the regioselective 2′-O-tetrahydropyranylation was involved in their syntheses. These "perfectly" protected monomer units have successfully been utilized in the synthesis of GUAUCAAUAAUG, a modified 5′-terminal structure, of brome mosic virus (BMV) mRNA no. 4 filament. The dodecamer chain was elongated by fragment condensation from the 3′–5′ direction. The yields of the oligomer blocks have proved to be dramatically high because no side reactions occurred during the condensation reactions. Indeed, the final coupling to give the target 12-mer was achieved in 91% yield. The deprotection of the fully protected in the usual manner gave GUAUCAAUAAUG in ca. 30% yield.

Current progress in molecular biology is due partly to the continuous development in the chemical synthesis of oligonucleotides.[1] In a recent study, we have faced the serious side reactions resulting from the reactive amide functions of nucleoside base residues. Similar observations have been reported in a number of laboratories.[2] This problem is more serious in the synthesis of oligoribonucleotides than that of oligodeoxyribonucleotides, because the condensation reaction requires longer periods of time owing to the steric effect of 2′-hydroxyl protecting groups. Several protecting groups have recently been proposed to overcome the inevitable side reactions.[3–6] In previous papers,[7,8] we have demonstrated the utility of the complete protection for the guanine[7a–c] and uracil[8] residues.

In this paper, we report a new strategy of introducing the protecting groups to the amide functions of the guanine and uracil residues and its application to the synthesis of GUAUCAAUAAUG, a modified 5′-terminal dodecaribonucleotide sequence of BMV mRNA filament,[9] no. 4, which has C in place of U at the fifth position from the 5′-terminus and is expected to bind more tightly to 18S rRNA than the original sequence (Figure 1).

Results and Discussion

We have recently described a general method for the synthesis of oligoribonucleotides by use of *S*,*S*-diphenyl *N*-(4-methoxytrityl)-2′-*O*-(tetrahydropyranyl)-5′-*O*-(4,4′-dimethoxytrityl)-ribonucleoside-3′-phosphorodithioates as the key intermediates.[10] Although a nonaribonucleotide, GpUpApUpUpApApUpAp, was successfully obtained by this method, we have encountered base modifications on the guanosine and uridine residues throughout

[†] The University of Tokyo.

[*] Tokyo Institute of Technology.

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Table II. Reactions of N^2-Protected 2′,3′,5′-Tri-O-acetylguanosine (**1a**,**b**) with Carbamoyl Chlorides (**5A–C**) in Pyridine in the Presence of Diisopropylethylamine

R	R^2 of R^2Cl	R^2Cl	equiv of **5**	equiv of i-Pr_2NEt	time, h	product	yield, %
Tr	$Me_2NC(O)$	**5A**	4	1.5	23	**2a**	92
	$Ph_2NC(O)$	**5B**	2	1.5	1	**3a**	96
	$Me_2NC(S)$	**5C**		no reaction[a]			
Bz	$Me_2NC(O)$	**5A**	6	2.0	20	**2b**	100
	$Ph_2N(C)$	**5B**	2	1.5	1	**3b**	98
	$Me_2NC(S)$	**5C**		no reaction[a]			

[a] The reagent **2c** was rapidly decomposed in pyridine so that no reaction occurred.

Table III. Stabilities of N^2-Protected 2′,3′,5′-Tri-O-acetyl-O^6-carbamoylguanosine Derivatives under Various Conditions[a,b]

compd	R^1	R^2	A	B	C	D	E	F
2a	Tr	$Me_2NC(O)$	95%[c]	100%[c]	stable		30%	50%
3a		$Ph_2NC(O)$		stable			60%	80%
2b	Bz	$Me_2NC(O)$	95%	50%	stable		90%	100%
3b		$Ph_2NC(O)$		stable			100%	100%
4b		$Me_2NC(O)$		stable			60%	80%

[a] (A) 0.5% TFA/$CHCl_3$, 0 °C, 2 h; (B) 80% AcOH, room temperature, 2 h; (C) 4 M $H_2PO_2HNEt_3$ (20 equiv) in py 40 °C, 2 h; (D) py–H_2O (2:1, v/v), room temperature, 1 day; (E) 0.2 M NaOH–dioxane (1:1, v/v), room temperature, 5 h; (F) concentrated NH_4OH–MeOH (1:1, v/v), room temperature, 1 day. [b] The figures shown in this table mean the percentage of removal of each carbamoyl group. [c] The trityl group was simultaneously removed.

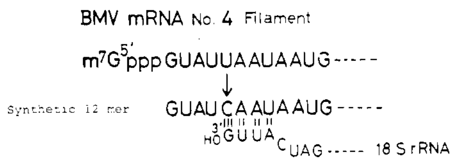


Figure 1. Sequence of the synthetic dodecaribonucleotide having a strong binding site to 18S rRNA.

the synthesis of the nonamer. Since we have aimed to synthesize chemically "capped" mRNAs where only acidic or neutral conditions were permitted for removal of protecting groups at the last stage because of the extreme instability[11] of 7-methylguanosine under basic conditions, the monoethoxytrityl (MMTr) group has been chosen as the acid-labile protecting group of the exo amino functions.[10]

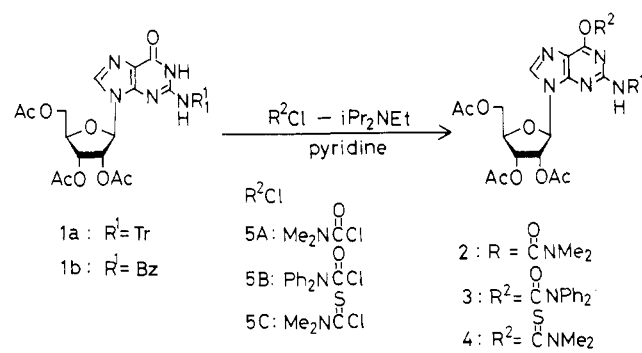
Introduction of the MMTr group on the base residues enhanced the lipophilicity of oligonucleotide building blocks and facilitated dramatically isolation of the coupling products. Additional protection for the guanosine and uridine base parts also plays the same role as the MMTr group as far as the liphilicity is concerned. If all the exo amino groups of C, A, and G in the oligomers are protected with the MMTr group, the final deprotection by using 0.01 M HCl may be inhibited owing to the heterogeneity in this medium. It seems desirable that at this stage one of three amino groups is protected with lipophilic protecting groups such as the MMTr or 4,4′-dimethoxytrityl (DMTr) group while the other two are masked with base-labile protecting groups that can be removed prior to the capping reaction.

On the basis of the above facts, in the present study we have chosen the following protecting groups of the four common nucleotide bases.

In the case of guanosine, the O^6-amido and N^2-amino groups were masked with the diphenylcarbamoyl and propionyl groups, respectively. We have briefly reported this new protection mode for the guanine moiety in the deoxy series.[7c]

At the beginning of this study, we have examined disubstituted carbamoyl groups as the suitable blockers for the amide function of the guanine residue. Three kinds of carbamoyl and thiocarbamoyl chlorides have been tested as the candidates and two

Scheme I



substrates (**1a**,**b**) were chosen. We first examined the reactions of **1a** and **1b** with **5A–C** in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in CH_2Cl_2–triethylamine. The conditions were known to be effective for introduction of various electrophiles on the O^6-carbonyl oxygen of the guanine residue.[12] These results are summarized in Table I (supplementary material). All the reactions were not completed even after prolonged periods of time. This was found to be due to the reverse reactions catalyzed by DMAP, which was ascertained by independent treatment of **2** with DMAP in the same medium. Nonetheless, the O^6-acylated products (**2**) were isolated in 44–70% yields (Scheme I). In the case of **4c**, the trityl group was lost during chromatographic purification. On the other hand, it was found that the use of trialkylamines in pyridine was much more effective. Finally, diisopropylethylamine was the reagent of choice, since this reagent resulted in rapid and irreversible O^6-acylation of **1**. The reactions of **1** with **5A** and **5B** in pyridine gave excellent yields of **2** and **3**, whereas the reagent **5C** was found to be unstable in pyridine and **4** was not obtained by this method. These results are summarized in Table II.

Next, we examined the stabilities of the three kinds of guanosine derivatives bearing carbamoyl groups under various conditions that would be used in oligoribonucleotide synthesis. These results are summarized in Table III. Among the three groups, the diphenylcarbamoyl and dimethyl(thiocarbamoyl) groups were found to possess the suitable properties for our purpose as shown in Table III. However, the dimethyl(thiocarbamoyl) group was introduced into the O^6 oxygen of the guanine moiety in only moderate yield by the undesirable DMAP-catalyzed reaction. Therefore, we finally chose the diphenylcarbamoyl group because

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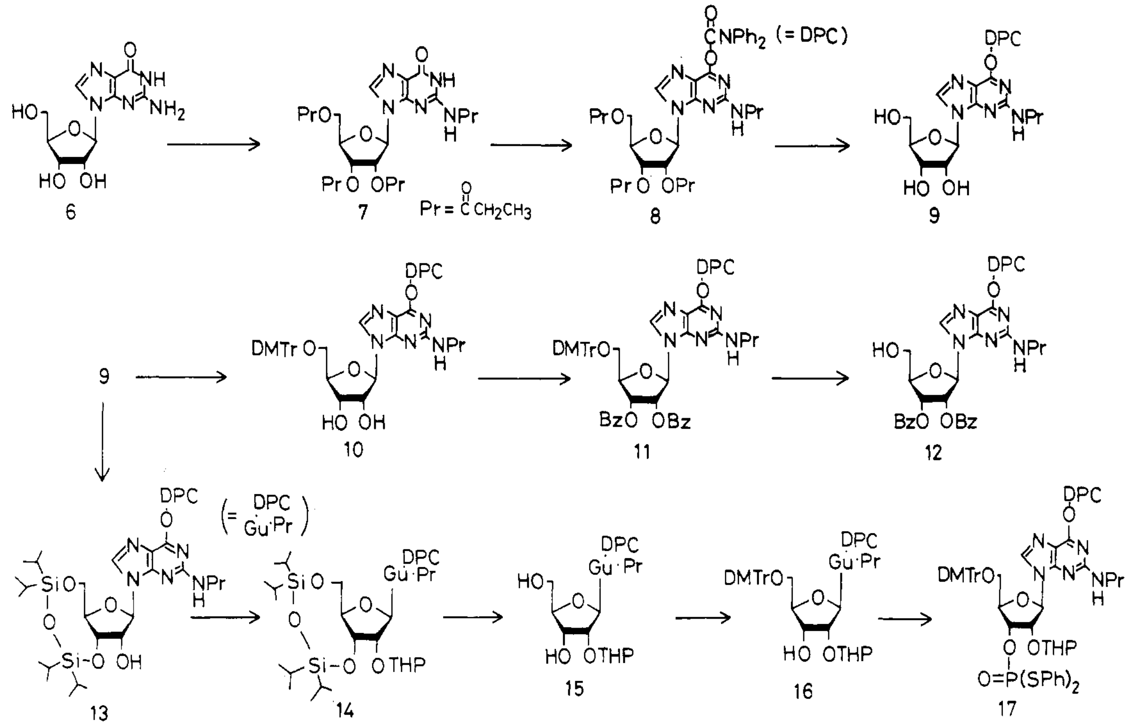
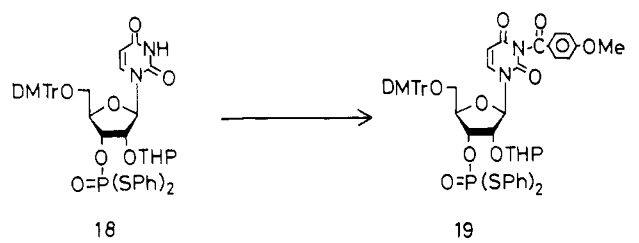
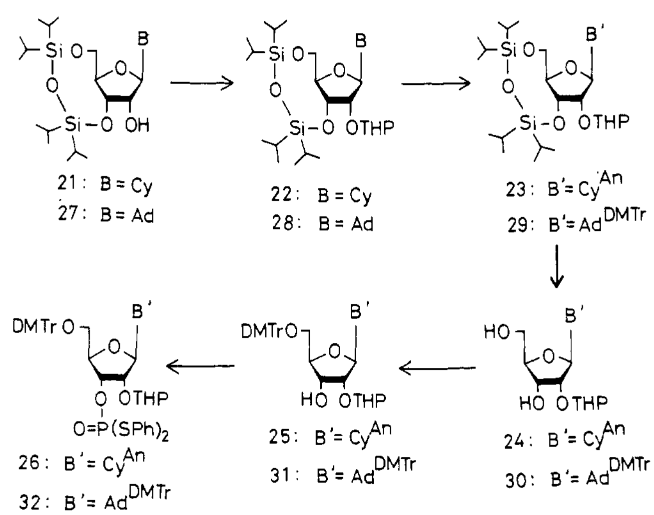
Scheme II



Scheme III



Scheme IV



its introduction was achieved in almost quantitative yield and its great liphophilicity is useful for chromatographic separation of the product like the DMTr group. An additional advantageous point of the DPC group is that its derivatives could be easily detected as distinct dark blue spots on TLC upon heating.

The N^3-imide group of the previous uridine unit **18** was protected with the anisoyl group, which can be removed by treatment with aqueous concentrated ammonia. The *exo*-amino group of cytidine was also protected with the conventional anisoyl group. Finally, we chosed the DMTr group[13] as the protecting group for the N^6-amino function of adenosine on the basis of the reason discussed in a later section.

We preserved the combination of the DMTr and tetrahydropyranyl groups as the blockers of the 5'- and 2'-OH groups, since the former can be discriminated from the latter in the deprotection process.[14] The outline for construction of the four common ribonucleotide units (**17**, **19**, **26**, and **32**) is depicted in Schemes II–IV.

There are two terminal guanosines in the base sequence of the target 12-mer. As the key intermediate in the oligoribonucleotide synthesis O^6-(diphenylcarbamoyl)-N^2-propionylguanosine (**9**) was prepared as crystals in 93% yield from guanosine in a manner similar to that described in the case of the deoxyguanosine derivative.[7c] The dimethoxytritylation of **9** followed by the benzoylation and the succesive detritylation afforded a dibenzoate (**12**) in an overall yield of 98%, which could be utilized as the 3'-terminal guanosine unit.

The guanosine unit **17** was synthesized also in an excellent overall yield of 73% through the four-step reactions from **9**.

Although introduction of the anisolyl group on the uracil base might be possible at the early stage, we did it at the final stage since the convenient synthesis of the previous unit **18** has been established in this laboratory.[10b] This approach gave **19** in high yield. Quite recently, Chattopadhyaya[6] reported the one-pot preparation of N^3-acylated uridines, aiming to use the acyl groups as the protecting groups of the uracil moiety. The cytidine unit **26** was obtained in an excellent overall yield of 79% via the five-step reactions from **21**. In this case, the tetrahydropyranyl (THP) group was introduced regioselectively into the 2'-OH prior to the anisoyl group. The 5'-*O*,N^6-bis(dimethoxytritylated)-adenosine unit **32** was prepared as shown in Scheme IV.

The regioselective 2'-O-tetrahydropyranylation can be performed with trifluoroacetic acid instead of *p*-toluenesulfonic acid that used in the previous preparation.[10b] In this condition, neither rearrangement nor cleavage of the 3',5'-cyclic silyl group has occurred.

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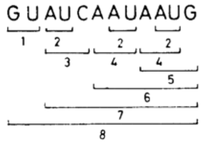
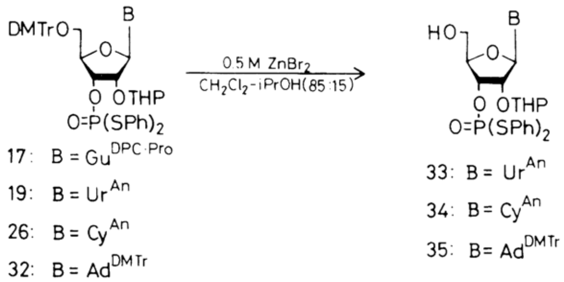


Figure 2. Synthetic strategy for GUAUCAAUAAUG.

Scheme V



All the reactions proceeded cleanly and gave both the intermediates and the units in high yields (see Experimental Section) All the synthetic approaches shown in Scheme II–IV have proved to be carried out readily with high reproducibility. One can prepare all the units in a large scale of 10 g within 1 month because all the reagents are now commercially available.[15]

The key step for the chain elongation is the selective removal of the DMTr group from each unit. Ito[16] has recently showed a modified procedure of Köster[17] for the deprotection[18] of the DMTr group using 1 M $ZnBr_2$ in CH_2Cl_2–*i*-PrOH (85:15, v/v). Since we have felt that there was a great difference in stability between the 5′- and N^6-DMTr groups on adenosine, the adenosine unit **32** was treated with $ZnBr_2$ under similar conditions. As a consequence, we found that the use of 0.5 M ZnBr was very effective for the selective cleavage of the 5′-O-(dimethoxytrityl) ether bond. The 5′-hydroxyl derivative **35** was obtained in 91% yield. This led us to examine the reactions of the other units, **17**, **19**, and **26**, with 0.5 M $ZnBr_2$ under the same conditions (Scheme V). It was found that the complete deprotection of the DMTr group from the units required much more time. Especially in the case of C, it took 19 h for completion. A similar observation has more recently been reported by Ohtsuka.[19]

In place of the DMTr group the MMTr group can apparently be used as the N^6-amino blocker of adenosine as reported before and the chain elongation is possible by use of 2% TFA in CH_2Cl_2.[10] In fact, we have succeeded in synthesizing another 12-mer using the MMTr group for protection of adenosine. This results will be reported soon elsewhere.

Nonetheless, the above-mentioned excellent selectivity led us to use DMTr as the masking group of adenosine N^6-amino function in this study and choose the synthetic strategy for the 12-mer as shown in Figure 2. Figure 2 shows that all the couplings were designed so that each fragment had a 5′-teminal A except for a GpU fragment.

The fully protected dimers GU and AU were prepared according to our original method using triethylammonium phosphinate[10,19,20] in pyridine for selective removal of the PhS group from G and A and 2% TFA in CH_2Cl_2 for removal of the DMTr group from U, respectively. The trimer blocks AUC and AAU were similarly synthesized.

(15) 1,1,3,3-Tetraisopropyl-1,3-dichlorosiloxane, DMTrCl, and MMTrCl are available from Yukigoseikagakuyakuhin Co. Ltd. (Tokyo). Et_4NBr and KF available from Wako Co. Ltd. PSS and other reagents were purchased from Tokyo Kasei Co. Ltd.

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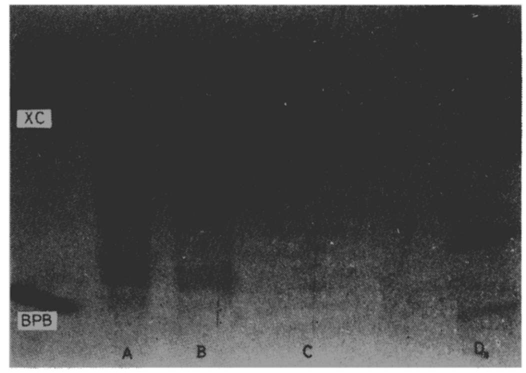

Figure 3. Electrophoresis of the synthetic 12-mer in 25% polyacrylamide gel in the presence of 7 M urea using 0.0089 M TBE buffer (pH 8.3) at 15 mA for 4 h. Lanes A and B, the mixture obtained after the full deprotection of the 12-mer; lane C, poly U ladder; lane D, authentic sample of UpUpUpU; XC, xylene cyanol; BPB, bromophenol blue.

The yields of these dimers and trimers were dramatically high except in the case of AUC. Since on treatment with the phosphinate an increase in the number of internucleotidic PhS groups caused significant partial deprotection of the internal PhS groups, the condensations were designed so that the phosphodiester components with the chain length of 2–3 were always employed. By the fragment condensations, 7-mer, 9-mer, and 12-mer could be synthesized likewise in excellent yields of 95%, 89%, and 91%, respectively. These conditions and results are listed in Table IV.

During these condensations, we did not observe any side reactions that hampered isolation of the product. The double protection of the remaining reactive guanine and uracil residues proved to be very useful. Moreover, the sulfonation of 5′-OH with condensing agents did not provide a severe problem in our case, since such a byproduct might be easily converted to a polar species upon hydrolysis after the reaction as reported previously.[21]

The "full" protection resulted in good recovery of nucleotide materials. The presence of the DPC, DMTr, and MMTr groups was very effective for elution of the products from silica gel column. In all cases elution was completed by use of CH_2Cl_2 containing only 1–3% MeOH. Thus, all the products, even 12-mer, were easily obtained in pure state as distinct single spots on TLC.

The full deprotection of the protected 12-mer was performed as follows: Treatment of the fully protected 12-mer with 1 M N^1,N^1,N^3,N^3-tetramethylguanidium 2-pyridine-*syncarboxaldoximate* (PAO)[22] in dioxane–H_2O (7:18 v/v) resulted in the simultaneous removal of all the internal PhS groups and the DPC groups. The partially deblocked material was further treated successively with concentrated ammonia at 60 °C for 3 h and with 0.01 M HCl in dioxane–H_2O (1:1, v/v) for 4 days. A part of the resulting mixture was separated by electrophoresis on 25% polyacrylamide gel to give an unprotected 12-mer as the main band (ca. 30%) as shown in Figure 3. Since the fully protected 12-mer did not contain any byproducts, and thereby the pattern of the gel electrophoresis was very clear, the isolation of an deblocked 12-mer could be done only by the electrophoresis without a multistep purification procedure. The full characterization of this band was done by the sequence analysis according to a modified procedure[23] of Maxam and Gilbert[24] after labeling of the 3′-terminal hydroxyl with 32pCp. This result is shown in Figure 4.

Experimental Section

^1H NMR spectra were recorded at 100 MHz on a JNM-PS-100 spectrometer. Melting points were taken on a Fisher-Johns melting point block and are uncorrected. Reagent-grade pyridine was distilled after being refluxed over *p*-toluenesulfonyl chloride for several hours, redistilled over calcium hydride after being refluxed for several hours, and stored

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over 3A molecular sieves. Methylene chloride was dried over P_4O_{10} overnight, decanted, distilled from potassium carbonate, and stored over 3A molecular sieves. Dioxane (1 L) was purified by passing nitrogen gas into the refluxing mixture that contained concentrated hydrochloric acid (13 mL) and water (10 mL) followed by neutralization with potassium hydroxide, extraction, and distillation over sodium wire. Column chromatography was performed by using silica gel C-200 purchased from Wako Co., Ltd., and a mini air pump for a goldfish basin was conveniently used to gain a medium pressure for rapid chromatographic separation. Elution was performed with CH_2Cl_2 or 1–3% MeOH containing CH_2Cl_2. In the case of the 3′-unprotected compounds and N[6]-(dimethoxytritylated)adenosine derivatives, 0.5–1% pyridine was added to the above solvent for elution to avoid their decomposition during chromatography. Thin-layer chromatography was performed on precoated TLC plates (silica gel 60 F-254 Merck, Art. No. 5715). The R_f values of the protected nucleoside derivatives were measured after development with the followng solvent systems: solvent A (CH_2Cl_2–MeOH, 9:1 v/v); solvent B (CH_2Cl_2–MeOH, 12:1, v/v); solvent C (CH_2Cl_2–MeOH, 20:1, v/v); solvent D (*i*-PrOH–concentrated ammonia–H_2O). Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology, at Nagatsuta. Analytically pure samples of nucleoside derivatives were obtained by reprecipitation from chloroform with hexane on a mixture of hexane–ether. Their melting points were not given here because they did not have clear melting points owing to diastereomeric mixture except for **9**, which was obtained as crystals. No attempts to crystallize the powdery materials from appropriate solvents have been made.

General Procedure for Reactions of N-Protected 2′,3′,5′-Tri-*O*-acetylguanosine 1 with Carbamoyl and Thiocarbamoyl Chlorides. To a solution of **1a** or **1b** in dry pyridine (10 mL/mmol) were added an appropriate reagent and diisopropylethylamine. After being stirred for the time listed in Table II, the mixture was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and evaporated under reduced pressure. The last traces of pyridine were removed by coevaporation with toluene. Chromatography gave the product listed in Table II.

2′,3′,5′-Tri-*O*-propionyl-*N*[2]-propionylguanosine (7). Guanosine (**6**) (1.42 g, 5 mmol) was suspended in dry pyridine (25 mL), and propionic anhydride (9.6 mL, 75 mmol) and DMAP (61 mg, 5 mmol) were added. The mixture was heated with stirring at 70 °C for 3 h. Then a 1 M Na_2CO_3 solution (74.5 mL) was added with external cooling until the evolution of CO_2 gas ceased. The aqueous solution was extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were combined, washed with 5% $NaHCO_3$ (2 × 30 mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was coevaporated several times with toluene to remove the last traces of pyridine and chromatographed on a column of silica gel to give **7** (2.44 g, 96%), R_f 0.46 (solvent A).

***O*[6]-(Diphenylcarbamoyl)-*N*[2]-propionylguanosine (9).** To a solution of **7** (2.44 g, 4.8 mmol) in dry pyridine (50 mL) were added diphenylcarbamoyl chloride (2.32 g, 10 mmol) and diisopropylethylamine (1.3 mL, 7.5 mmol). The mixture was stirred at room temperature for 1 h and then diluted with pyridine (10 mL) and EtOH (25 mL). To the solution cooled at 0 °C was added 2 M NaOH (25 mL), which was precooled. After 10 min, acetic acid (5 mL) was added to neutralize the solution. Extraction with CH_2Cl_2 followed by chromatography on silica gel afforded **9** (2.36 g, 91%): mp 140–142 °C (EtOH); R_f 0.40 (solvent A).

5′-*O*-(4,4′-Dimethoxytrityl)-*O*[6]-diphenylcarbamoyl-*N*[2]-propionylguanosine (10). Compound **9** (0.61 g, 1.14 mmol) was dried in the same manner as described before and dissolved in dry pyridine (10 mL). DMTrCl (0.46 g, 1.37 mmol) was added and the solution was kept for 25 min. Chromatography after the usual workup afforded **10** (0.94 g, 98%), R_f 0.47 (solvent B).

2′,3′-Di-*O*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-*O*[6]-(diphenylcarbamoyl)-*N*[2]-propionylguanosine (11). To a solution of **10** (0.50 g, 0.6 mmol) in pyridine (3 mL) was added benzoyl chloride (0.182 mL, 1.56 mmol). The solution was kept at room temperature for 1 h and then at 50 °C for 30 min. After water was added, the mixture was extracted with CH_2Cl_2. The usual workup gave **11** (0.63 g, 99%), R_f 0.46 (solvent C).

2′,3′-Di-*O*-benzoyl-*O*[6]-(diphenylcarbamoyl)-*N*[2]-propionylguanosine (12). To a solution of **11** (314 mg, 0.3 mmol) in dry $CHCl_3$ cooled at 0 °C was added 2% TFA in $CHCl_3$ (15 mL). After being kept at 0 °C for 3 min, the mixture was neutralized by addition of pyridine. Extraction with CH_2Cl_2 followed by chromatography gave **12** (232 mg, 99%), R_f 0.41 (solvent B).

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-*O*[6]-(diphenylcarbamoyl)-*N*[2]-propionylguanosine (13). To a solution of **9** (1.09 g, 2.04 mmol) in dry pyridine (20 mL) was added 1,1,3,3-tetraisopropyl-1,3-dichlorodisiloxane (0.66 mL, 2.24 mmol). After it was stirred for 2 h, the mixture was extracted with CH_2Cl_2. The usual workup followed by chromatography gave **13** (1.43 g, 90%), R_f 0.49 (solvent B).

2′-*O*-(Tetrahydropyran-2-yl)-*O*[6]-(diphenylcarbamoyl)-*N*[2]-propionylguanosine (15). To a solution of **13** (1.42 g, 1.84 mmol) in CH_2Cl_2 (20 mL) were added dihydropyran (3.36 mL, 36.8 mmol) and TFA (0.21 mL, 2.76 mmol). After being stirred for 11 h, the mixture was quenched by addition of pyridine and extracted with CH_2Cl_2–H_2O. The organic extracts were combined, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was dissolved in CH_3CN (55 mL).

To the solution were added KF (0.64 g, 11.0 mmol), Et_4NBr (2.31 g, 11.0 mmol), and water (0.55 mL). The resulting mixture was stirred vigorously at 50 °C for 45 min. The successive extractive workup and chromatography gave **15** (1.11 g, 97%), R_f 0.33, 0.36 (solvent B).

2′-*O*-(Tetrahydropyran-2-yl)-5′-*O*-(4,4′-dimethoxytrityl)-*O*[6]-(diphenylcarbamoyl)-*N*[2]-propionylguanosine (16). Compound **15** (1.11 g, 1.79 mmol) was rendered anhydrous by repeated coevaporations with dry pyridine and finally dissolved in dry pyridine (20 mL). The solution was treated with DMTrCl (0.72 g, 2.15 mmol) and stirred for 30 min. Then the mixture was quenched by addition of water and extracted with CH_2Cl_2. The usual workup involving dryness, filtration, evaporation, and chromatography gave **16** (1.56 g, 94%), R_f 0.52, 0.57 (solvent B).

***S*,*S*-Diphenyl 2′-*O*-(Tetrahydropyran-2-yl)-5′-*O*-(4,4′-dimethoxytrityl)-*O*[6]-(diphnylcarbamoyl)-*N*[2]-propionylguanosine-3′-phosphorodithioate (17).** PSS (1.28 g, 3.38 mmol) was rendered anhydrous be repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (7 mL). To the solution was added MDS (1.06 g, 3.38 mmol) and the mixture was stirred for 30 min. Then **16** (1.56 g, 1.69 mmol) was added and the mixture was stirred for 5 h. The extractive workup followed by chromatography using CH_2Cl_2–MeOH containing 1% pyridine as the solvent for elution gave **17** (1.81 g, 91%), R_f 0.65 (solvent B).

***S*,*S*-Diphenyl 2′-*O*-(Tetrahydropyran-2-yl)-5′-*O*-(4,4′-dimethoxytrityl)-*N*[3]-anisoyluridine-3′-phosphorodithioate (19).** Compound **18** (1.43 g, 1.6 mmol) was rendered anhydrous by repeated coevaporations with dry pyridine and then dissolved in dry pyridine (15 mL). To the mixture were added diisopropylethylamine (0.42 mL, 2.4 mmol) and anisoyl chloride (0.55 g, 3.2 mmol). The mixture was stirred for 14 h. Extraction with CH_2Cl_2 followed by chromatography gave **19** (1.53 g, 92%).

2′-*O*-(Tetrahydropyran-2-yl)-*N*[4]-anisoylcytidine (24). To a mixture of **21** (486 mg, 1 mmol) and dihydropyran (1.8 mL, 20 mmol) in dry dioxane (3 mL) was added TFA (155 L, 2 mmol). After being stirred for 11 h, the mixture was treated with pyridine and then evaporated under reduced pressure. The residue was coevaporated several times with ethanol to remove the last traces of dihydropyran and chromatographed on a column silica gel with CH_2Cl_2–MeOH to give **22**, R_f 0.43 (solvent A).

The roughly purified **22** was dissolved in dry pyridine (5 mL) and anisoyl chloride (0.44 g, 2.6 mmol) was added at 0 °C. After the solution was kept for 35 min, a 5% $NaHCO_3$ solution was added. The aqueous solution was extracted with CH_2Cl_2 (3 × 20 mL). The combined CH_2Cl_2 extracts were evaporated to dryness under reduced pressure. The residue containing **23** was dissolved in acetonitrile (30 mL), and Et_4NBr (1.26 g, 6 mmol), KF (349 mg, 6 mmol), and water (0.3 mL) were added. The resulting mixture was stirred vigorously at 65 °C for 30 min.

The precipitate was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed 3 times with water. The organic phase was evaporated and chromatorgraphed to give **24** (420 mg, 91%), R_f 0.45, 0.49 (solvent A).

2′-*O*-(Tetrahydropyran-2-yl)-5′-*O*-(4,4′-dimethoxytrityl)-*N*[4]-anisoylcytidine (25). Compound **24** (312 mg, 0.68 mmol), precoevaporated with dry pyridine, was dissolved in dry pyridine (1.4 mL) and mixed with DMTrCl (275 mg, 0.81 mmol). After it was stirred for 40 min, the mixture was extracted with CH_2Cl_2. The usual workup gave **25** (494 mg, 96%), R_f 0.50, 0.53 (solvent A).

***S*,*S*-Diphenyl 2′-*O*-(Tetrahydropyranyl)-5′-*O*-(4,4′-dimethoxytrityl)-*N*[4]-anisoylcytidine-3′-phosphorodithioate (26).** Cyclohexylammonium *S*,*S*-diphenyl phosphorodithioate (PSS) (852 mg, 2.24 mmol), precoevaporated with dry pyridine, was dissolved in dry pyridine (15 mL) and MDS (850 mg, 2.68 mmol) was added. After the solution was kept for 30 min, **25** (1.14 g, 1.49 mmol) was added and the mixture was stirred for 2 h. The usual workup followed by chromatography gave **26** (1.2 g, 78%), R_f 0.55 (solvent A).

2′-*O*-(Tetrahydropyran-2-yl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine (28). To a solution of **27** (2.55 g, 5 mmol) in dry dioxane (20 mL) were added dihydropyran (9.12 mL, 0.1 mmol) and TFA (0.56 mL, 7.5 mmol). After the mixture was stirred for 3 days, the same workup as described in the case of **14** gave **28** (2.2 g, 74%), R_f 0.66 (solvent A).

2′-*O*-(Tetrahydropyran-2-yl)-*N*[6]-(4,4′-dimethoxytrityl)adenosine (30). To a solution of **28** (1.78 g, 3 mmol) in dry CH_2Cl_2 (10 mL) were added

Table IV. Conditions and Results of Synthesis of Dodecaribonucleotide

fragment no.	3′-phospho component (equiv)	1.5 M (equiv)	Et_3N (equiv)	time, min	5′-hydroxyl component (mmol)	0.5 M $ZnBr_2$		coupling reaction			
						time, min	yield, %	MDS, equiv	NT, equiv	time, min	yield, %
1	G (1.2)	30	10	30	U (0.20)	4	88[a]	3	3	35	95
2	A (1.3)	30	10	60	U (0.55)	4	88[a]	3	3	30	95
3	AU (1.4)	30	10	90	C (0.050)	20	88[a]	3	3	90	61
4	A (1.3)	30	10	60	AU (0.167)	60	84	3	3	60	95
5	AAU (1.4)	30	10	60	G (0.037)	3	99	3	3	60	90
6	AAU (1.4)	30	10	60	AAUG (0.034)	40	78	3	3	120	95
7	AUC (1.4)	30	10	40	$(AAU)_2G$ (0.023)	90	84	3	3	120	89
8	GU (1.5)	30	10	40	$AUC(AAU)_2G$ (0.014)	120	69	4	4	120	91

[a] In this case 0.5% trifloroacetic acid in $CHCl_3$ at 0 °C was used for removal of the DMTr group. [b] PSA, phosphinic acid in dry pyridine; MDS, mesitylenedisulfonyl; NT, 3-nitro-1,2,4-triaxzole.

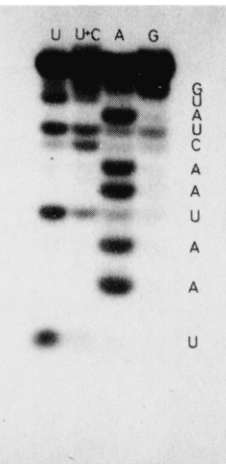

Figure 4. Sequence analysis of the synthetic 12-mer.

DMTrCl (2.03 g, 6 mmol), triethylamine (0.78 mL, 6 mmol), and DMAP (14.7 mg, 0.12 mmol). The solution was stirred for 2 h; the reaction was stopped by addition of ice. The usual extraction with CH_2Cl_2 followed by removal of the solvent under reduced pressure gave a gum, which was further treated with KF (1.01 g, 18 mmol) and Et_4NBr (3.78 g, 18 mmol) in CH_3CN (30 mL) at 50 °C for 70 min. The same workup as described in the case of **24** gave **30** (1.77 g, 90%), R_F 0.62, 0.76 (solvent A).

2′-*O*-(Tetrahydropyran-2-yl)-5′-*O*,*N*[6]-bis(4,4′-dimethoxytrityl)adenosine (31). Compound **30** (1.0 g, 1.53 mmol) was dried as described before and treated with DMTrCl (777 mg, 2.29 mL) in pyridine (5 mL) for 2.5 h. The usual workup gave **31** (1.33 g, 91%), R_f 0.81, 0.90 (solvent A). The ^1H NMR spectrum and elemental analysis indicated that this compound contained approximately one molecule of hexane used for reprecipitation.

***S*,*S*-Diphenyl 2′-*O*-(Tetrahydropyran-2-yl)-5′-*O*,*N*[6]-bis(4,4′-dimethoxytrityl)adenosine-3′-phosphorodithioate (32).** Compound **31** (956 mg, 1 mmol), coevaporated several times with dry pyridine, was mixed with a mixture of PSS (572 mg, 1.5 mmol) and MDS (634 mg, 2.0 mmol) in dry pyridine (5 mL) which been kept for 30 min. The resulting solution was stirred for 1.5 h and then worked up as described before. Chromatography gave 32 (1.16 g, 95%), R_f 0.98 (solvent A).

***S*,*S*-Diphenyl 2′-(Tetrahydropyran-2-yl)-*N*[6]-(4,4′-dimethoxytrityl)-adenosine-3′-phosphorodithioate (35).** For the synthesis of this compound see the general procedure, R_f 0.89 (solvent A).

General Procedure for Removal of the DMTr Group from Fully Protected Oligoribonucleotides. A fully protected oligoribonucleotide was dissolved at 0 °C in CH_2Cl_2–*i*-PrOH (85:15, v/v) containing 0.5 M $ZnBr_2$. The solution was kept at 0 °C for the time as listed in Table IV. Then water was added, and the mixture was extracted 3 times with CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was chromatographed with CH_2Cl_2–MeOH containing 0.1% pyridine to give the hydroxyl component as listed in Table IV.

General Procedure for Synthesis of Fully Protected Oligoribonucleotides. An appropriate fully protected oligoribonucleotide was dissolved in a 1.5 M solution of pyridinum phosphinate (30 equiv) in pyridine. To the solution was added triethylamine (10 equiv). The resulting mixture was warmed to 40 °C and kept for the time as listed in Table IV. Then pyridine–water (1:1, v/v) was added and the aqueous solution was washed 3 times with hexane–ether (2:1, v/v) to remove thiophenol and the unreacted starting material. The remaining aqueous layer was extracted 3 times with CH_2Cl_2, and the CH_2Cl_2 extracts were combined and washed with a 0.25 M triethylammonium bicarbonate solution. The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was mixed with a hydroxyl component and 3-nitro-1,2,4-triazole and rendered anhydrous by repeated coevaporation with dry pyridine. Finally, the mixture was dissolved in dry pyridine and MDS was added. The mixture was stirred at room temperature until the reaction was complete. Then pyridine–water (1:1, v/v) was added and the product was extracted 3 times with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed 3 times with a 0.25 M triethylammonium bicarbonate solution and then with water. The organic layer was dried over $MgSO_4$ and the solvent was removed by evaporation under reduced pressure. The residue was chromatographed with CH_2Cl_2–MeOH containing 0.1% pyridine to give the coupling product. The detailed conditions are summarized in Table IV.

Deprotection of the Fully Protected Dodecaribonucleotide. The dodecamer (9 mg, 1 μmol) was dissolved in a 1 M solution of N^1,N^1,N^3,N^3-tetramethylguanidium 2-pyridine-*syn*-carboxaldoximate in dioxane–water (7:1, v/v, 0.26 mL), and the solution was kept at room temperature for 3 days. Then the solution was passed slowly through a column of Dowex 50W × 2 (pyridinium salt, 3 mL), and the column was washed with pyridine–MeOH–water (3:1:1, v/v/v, 50 mL). The eluant and washings were combined and evaporated under reduced pressure. During this evaporation pyridine was added 3 times to avoid the partial loss of the tetrahydropyranyl and DMTr groups. The residue was dissolved in MeOH (5 mL) and concentrated ammonia (4.5 mL). The solution was sealed and kept at 60 °C for 3 h. The solution was evaporated under reduced pressure in the presence of pyridine. TLC analysis of the residue showed a distinct main spot with an R_f value of 0.62 (solvent D). The oligonucleotide containing the DMTr and THP groups was further dissolved in a 0.01 M solution of HCl in dioxane–water (1:1, v/v, 30 mL), and the solution was kept at room temperature for 4 days. Then a small amount of pyridine was added to neutralize the solution. The aqueous pyridine solution was washed with ether–ethyl acetate (3:1, v/v, 4 × 10 mL). Then a part of the aqueous solution was taken and analyzed by 25% polyacrylamide gel electrophoresis. This result is shown in Figure 3. The slowest moving main band (ca. 30%) was eluted, and its base sequence was performed by the standard method. This result is shown in Figure 4.

Registry No. 1a, 69471-51-8; **1b**, 66781-54-2; **2a**, 90742-07-7; **2b**, 90742-08-8; **3a**, 90742-09-9; **3b**, 90742-10-2; **4a** (detritiated), 90742-29-3; **4b**, 90742-11-3; **5A**, 79-44-7; **5B**, 83-01-2; **5C**, 16420-13-6; **6**, 118-00-3; **7**, 90742-12-4; **9**, 90742-13-5; **10**, 90742-14-6; **11**, 90742-15-7; **12**, 90742-16-8; **13**, 90742-17-9; **15**, 90742-18-0; **16**, 90742-19-1; **17**, 90762-77-9; **18**, 81244-07-7; **19**, 86964-78-5; **21**, 69304-42-3; **22**, 90742-20-4; **23**, 90742-21-5; **24**, 90742-22-6; **25**, 90742-23-7; **26**, 90335-48-1; **27**, 69304-45-6; **28**, 90742-24-8; **29**, 90742-25-9; **30**, 90742-26-0; **31**, 90742-27-1; **32**, 90335-47-0; **33**, 86964-81-0; **34**, 90742-28-2; **35**, 90335-50-5; GUAUCAAUAAUG, 90742-30-6; G (3′-phospho component), 117-68-0; A (3′-phospho component), 84-21-9; AU (3′-phospho component), 1985-21-3; AAU (3′-phospho component), 3079-92-3; AUC (3′-phospho component), 2769-96-2; GU (3′-phospho component), 2240-05-3; U, 58-96-8; C, 65-46-3; AU, 3051-84-1; AAUG, 67461-63-6; $(AAU)_2G$, 90742-31-7; $AUC(AAU)_2G$, 90742-32-8.

Supplementary Material Available: ^1H NMR and elemental analyses of **2a, 2b, 3a, 3b, 4b, 7, 9–13, 15–17, 22, 24–26, 28, 29, 31, 32,** and **35** and Table I of reaction data for the DMAP-catalyzed reaction of N^2-protected **1a,b** with **5A–C** (7 pages). Ordering information is given on any current masthead page.