Synthesis of Short Oligoribonucleotides Bearing a 3'- or 5'-Terminal Phosphate by use of 4,4',4''-Tris(4,5-dichlorophthalimido)trityl as a New 5'-Hydroxyl Protecting Group

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Abstract: Oligoribonucleotide synthesis by use of 4,4',4''-tris(4,5-dichlorophthalimido)trityl (CPTr) as a new 5'-hydroxyl protecting group is described. The reaction of N-protected 2'-*O*-(tetrahydropyran-2-yl) ribonucleosides with tris(4,5-dichlorophthalimido)trityl bromide (CPTrBr) in the presence of silver nitrate rapidly gave 5'-tritylated products **5** in high yields. Condensation of **5** with cyclohexylammonium *S,S*-diphenyl phosphorodithioate in the presence of mesitylenedisulfonyl chloride and tetrazole gave fully protected monomer units **6**. In this study, the lactone group of uridine and the amino group of cytidine were protected with the (pivaloyloxy)methyl and 4,4'-dimethoxytrityl groups. These new protecting groups were found to be stable upon deprotection of the CPTr group using hydrazine and removable under the usual basic and acidic conditions. Several oligoribonucleotide building blocks (**7** and **8**) were synthesized by selective removal of the CPTr or phenylthio group from **6** and utilized for synthesis of fully protected trimers (**17**, **18**, and **23**) containing a 3'- or 5'-terminal phosphate group and a tetramer (**25**). Removal of all protecting groups from the fully protected oligomers afforded AUAp, UUAp, pAUG, and CAUG. It was also found that the CPTr group could be removed by successive treatments with concentrated ammonia and with 0.01 M hydrochloric acid, which were the conditions used for the usual oligoribonucleotide synthesis. Several characteristic features of the CPTr group have also been described.

Recently, we have reported several methods for the synthesis of oligonucleotides employing appropriately protected *S,S*-diphenyl nucleoside 3'-phosphorodithioates and bifunctional condensing agents.[1-8] In the ribo series, we have succeeded in synthesizing a dodecaribonucleotide, GpUpApUpCpApApUpApApUpG, by the phosotriester method using the 4,4-dimethoxytrityl (DMTr) and tetrahydropyran-2-yl (THP) groups as the 5'- and 2'-OH protecting groups, respectively.[9] In our approach, the 5'-DMTr group was rather selectively removed from the fully protected monomer units and building blocks by acid treatment. However, partial loss of the THP group has been observed during each deprotection. Very careful handlings are required for the selective removal of the DMTr group. Especially, when DMTr and 4-monomethoxytrityl (MMTr) were employed as the exo amino protecting groups of adenosine, very restricted conditions are necessary[9] since they are also acid-labile groups and are more sensitive than the THP group. In fact, we have often obtained 5'-free oligonucleotide building blocks only in moderate yields (~70%) by acid treatment of the fully protected oligoribonucleotides. These facts suggest that an ideal 5'-OH protecting group should be investigated in more detail.

Letsinger[10] first reported that the benzoylpropionyl group can be used as a hydrazine-labile hydroxyl protecting group in nucleotide chemistry. Recently, van Boom[11] reported the use of hydrazine-labile levulinyl ester as a 5'-hydroxyl protecting group for oligonucleotide synthesis. The most severe problem in this synthetic strategy is that hydrazine reacts with pyrimidine nucleosides under certain conditions.[12] It is also known that *N*-acyl protecting groups on cytidine and adenosine are removed by the action of hydrazine.[13] The former problem is satisfactorily overcome by addition of acetic acid to a pyridine solution of hydrazine as reported by Letsinger.[14] The latter side reactions were avoided by shortening the reaction time for removal of the levulinyl group. However, the levulinyl group itself has inherent poor lipophilicity, which causes difficult extraction and low recoveries of materials from silica gel columns. In addition, its selective introduction on the 5'-OH has been achieved best in a sluggish condensation reaction of nucleosides with levulinic acid in the presence of condensing agents[11] which was not generally applicable to other 3',5'-free 2'-O-protected ribonucleosides. The levulinyl group is not sufficiently bulky to permit selective 5'-esterification. It is desirable that a protecting group should serve at the same time as a marker, like the DMTr group that appears as an orange spot upon heating if it is present on TLC.

In a previous paper,[15] we reported briefly on 4,4',4''-tris(4,5-dichlorophthalimido)trityl (CPTr) a new protecting group for primary hydroxyls. The CPTr group can be introduced onto the 5'-hydroxyls of nucleosides by the silver ion promoted reaction using the corresponding trityl bromide (CPTrBr) and removed by hydrazine treatment. In this paper, we wish to report the synthesis of several short oligoribonucleotides using the CPTr group as a promising 5'-hydroxyl protecting group in the phosphotriester approach.

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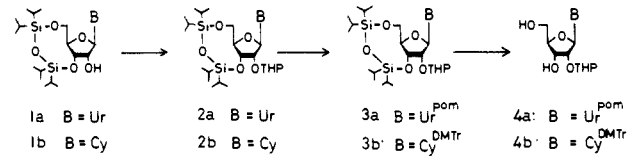
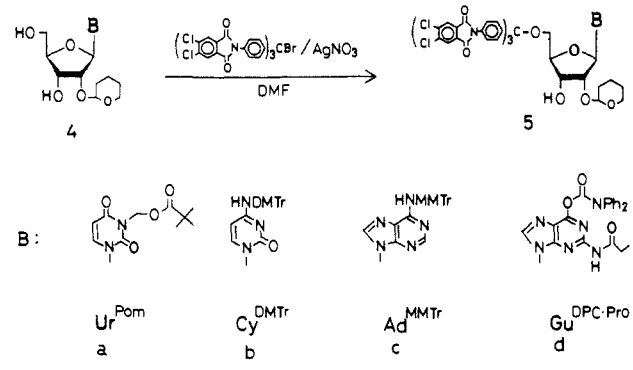
Scheme I



Scheme II



Results and Discussion

As mentioned above, protection of the amino groups of adenosine and cytidine with the usual acyl groups should be avoided in our approach that involves hydrazine treatment. Therefore, we have chosen a trityl-type protecting group for the above nucleoside bases. Although both DMTr and MMTr are available for adenosine, the former required inconvenient column chromatography in the presence of pyridine because of its lability on silica gel. Therefore, we chose the latter in this study. We have originally used MMTr for protection of the N^4-amino function of cytidine.[4] However, this group was later found to be extremely resistant to acids.[16] The preliminary experiments showed that the DMTr group is most suitable for this base and that the 4,4′,4″-trimethoxytrityl (TMTr) group can be also used but required chromatography in the presence of pyridine. The trityl-type blocking groups finally chosen were found to be stable in pyridine–acetic acid in various ratios from 3:2 to 1:3 regardless of the presence or absence of 1 M hydrazine. These conditions are employed for the selective deprotection of the CPTr group.

At the beginning of this study, we examined the 1,2-bis(isobutyryloxy)ethylene group[2] as a possible blocking group for the guanosine base. However, this group was very rapidly removed upon hydrazine treatment. As an alternative way, we finally chose a combination of diphenylcarbamoyl and propionyl as the O^6- and N^3-protecting groups, respectively.[6] Fortunately, this combination of protecting groups led to completely selective removal of the CPTr group. In the case of uridine, the N^3-anisoyl group was found to be somewhat labile and was partially lost during the hydrazinolysis. Although the loss of the anisoyl group is small at the monomer level, it seems difficult to apply this protecting group to the synthesis of longer oligoribonucleotides. Therefore, we have searched for more suitable masking groups for the uridine base. We first examined the *o*-methylbenzoyl group reported by Köster.[18] However, this group had a stability similar to that of the anisoyl group toward hydrazine. Finally, we found that the (pivaloyloxy)methyl (pom) group[19] had sufficient stability to base. The introduction of the pom group at the N^3-position was successfully achieved by alkylation of a protected uridine derivative (**2a**) with (pivaloyloxy)methyl chloride via metalation with sodium hydride in dry DMF (Scheme I).

Tritylation of 4 with CPTrBr. In the previous paper,[15] we showed that among several phthalimidotrityl groups studied the CPTr group was the most suitable for oligoribonucleotide synthesis and also described one example of the 5′-tritylation of **4a** with CPTrBr. The tritylation of **5a** required hindered bases such as 2,6-lutidine to neutralize nitric acid and avoid loss of the acid-sensitive 2′-*O*-THP group. (Scheme II).

Interestingly, we found that in the case of weakly basic ribonucleotide derivatives **4b–d** the tritylation proceeded selectively on the 5′-hydroxyls without any damage of the 2′-*O*-THP groups. These tritylations needed at least 2 equiv of CPTrBr because an unidentified slightly less polar byproduct was also formed. However, the byproduct and CPTrOH did not hamper isolation of the desired product since they were eluted readily in early fractions from a column. If 1.1 equiv of CPTrBr was used, the yield did not exceed 70%. The use of 2 equiv of CPTBr resulted in rapid and high-yield synthesis of **5a–d**. The reactions seemed to be finished within 15 min. These results are summarized in Table I. When the reaction time was prolonged to 2 h, rather complicated mixtures were obtained, suggesting 3′-tritylations and loss of the 2′-*O*-THP group.

Phosphorylation of **5** with *S,S*-diphenyl phosphorodithioate (PSS)[18] was carried out by use of mesitylenedisulfonyl chloride (MDS) in the presence of tetrazole in order to shorten the reaction time. Since all the remaining reaction sites were completely blocked by the pom and DPC groups, any side reactions associated with base modifications did not occur. A rapid and high-yield synthesis of the common ribonucleotide units **6a–d** was achieved as shown in Scheme III. By coaddition of tetrazole, the phosphorylations were completed in 15–30 min, whereas a much longer period of time (16 h) was needed in the absence of tetrazole as shown in Table II. The ^{1}H NMR spectra and elemental analyses

Table I. 5′-Selective Tritylation of **4** with CPTrBr

compd 4	B	CpTrBr–$AgNO_3$–lutidine, equiv	time, min	product	yield of **5**, %
a	Ur^{pom}	2:2:2	15	**5a**	84
b	Cy^{DMTr}	2:2	15	**5b**	86
c	Ad^{MMTr}	2:2	30	**5c**	80
d	$Gu^{DPC\text{-}pro}$	2:2	15	**5d**	96

Table II. Synthesis of the Fully Protected Ribonucleotide Monomer Units **6**

compd 5	B	PSS, equiv	MDS, equiv	tet, equiv	time, min	product	yield, %
a	UR^{pom}	1.5	2	4	10	**6a**	95
		1.2	3	4	20		90
b	Cy^{DMTr}	1.2	2	4	30	**6b**	88
c	Ad^{MMTr}	1.5	2		16 (h)	**6c**	95
		1.5	2	4	10		98
d	$Gu^{DPC\text{-}pro}$	1.5	2	2	45	**6d**	95

Scheme III

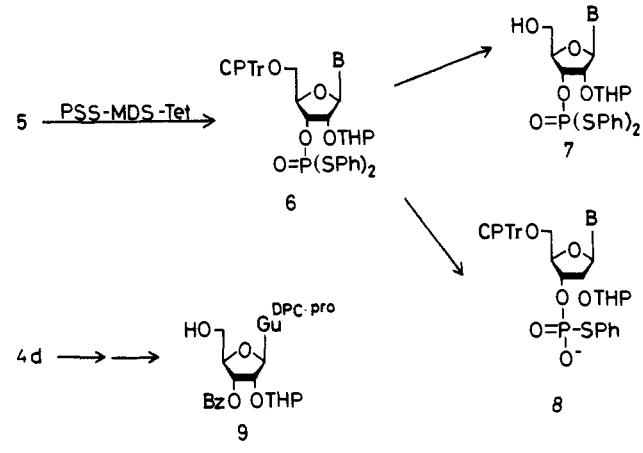


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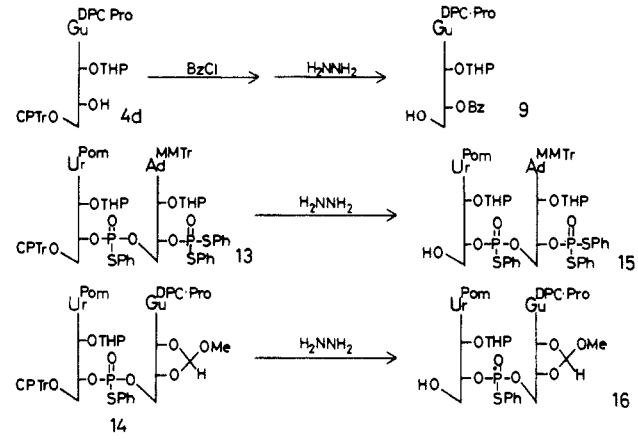
Table IV. Selective Removal of the CPTr Group from the Fully Protected Ribonucleotide Units **6a–d** and the Oligoribonucleotide Building Blocks **13**, **14**, **19**, and **20**

compd	1M H_2NNH_2 in pyridine–AcOH (3:1, v/v) time, min	quenching workup time, min	product	yield, %
6a	60		**7a**	91
6b	4 (h)		**7b**	70
6c	15		**7c**	89
6d	60		**7d**	80
6a	20	5	**7a**	92
6b	20	5	**7b**	96
6c	20	5	**7c**	95
6d	20	5	**7d**	93
13	20	5	**15**	86
14	20	5	**16**	88
19	20	5	**21**	83
20	20	5	**23**	82

Scheme IV



of compounds **3–9** are summarized in Table III of the supplementary material.

Selective Removal of the CPTr Group from the Fully Protected Ribonucleotide Units 6a–d. The bis(phenylthio)phosphoryl group was stable under the usual acidic conditions but yet was hydrolyzed promptly by treatment with a dilute alkali solution or concentrated ammonia with loss of one phenylthio group.[19] Since hydrazine was a weakly basic nucleophile, SN2 displacement of the phenylthio group with the hydrazino group may be expected to occur upon treatment of the units with hydrazine.

In order to see if the bis(phenylthio)phosphoryl (BPTP) group was stable under the conditions where the CPTr group was removed, *S*,*S*-diphenyl 5′-*O*-(dimethoxytrityl)thymidine 3′-phosphorodithioate was treated with a 1 M hydrazine solution in pyridine–acetic acid (3:1, v/v) at room temperature. On prolonged treatment the BPTP group was found to be gradually decomposed. About 5% of a base line material was formed after 5 h. From this result, the loss of the 3′-terminal phenylthio group due to the displacement is expected to be less than 1% under the conditions for removal of the CPTr group. The stability of the internucleotidic phenylthio group was also tested by use of *S*-phenyl dithymidine 3′, 5‴-phosphorothioate [Tp(SPh)T]. Consequently, the internal phenylthio group was stable under these conditions.

Treatment of the units **6** with hydrazine resulted in formation of **7**. The rate of dephthaloylation from the CPTr skeleton was very fast. The starting nucleotides disappeared within several minutes in all cases. The second step, i.e., the splitting of the resulting 4,4′,4″-triaminotrityl ether, may be the rate-determining step. After 20 min, TLC exhibited a major spot of **7** and a very minor spot of an unidentified intermediate in the case of **6a,b,d**. The latter appeared just below the former on TLC and turned reddish pink upon heating. This intermediate, however, was completely and rapidly converted to **7** by addition of acetic acid. In the case of **6c**, this intermediate was detected to a degree of ca. 10% but yet was converted easily to **7c** upon addition of acetic acid. Since the 5′-*O*-DMTr group of cytidine derivatives was relatively resistant to acids compared with that of the other nucleoside derivatives, the amounts of the intermediates seemed dependent on the kind of deoxyribonucleosides.

This quenching workup using acetic acid did not damage the other protecting groups (Table IV). Thus, compounds **7a–d** were obtained by chromatography in 92–96% yields. The above unidentified intermediates decomposed almost completely to **7** in the original media (pyridine–acetic acid, 3:1, v/v) when long reaction times (1–2 h) were employed. However, in this case, last traces of the intermediates still survived during silica gel column chromatography and could not be separated from the desired products. Therefore, the workup with acetic acid was very important for purification of **7**.

On the other hand, the CPTr group was found to be completely resistant to phosphinate reagents which were employed for selective removal of one phenylthio group from the BPTP group. Thus, treatment of **6a–d** with a 3.3 M phosphinate (PSA) solution in pyridine gave the diester units **8a–d** as triethylammonium salts in quantitative yields.

The 5′-*O*-DMTr group was relatively labile in the presence of 3′-phosphodiester residues having an ionic structure, so that the diester components having a 5′-DMTr group could not be stored for long periods of time, especially in the case of deoxyguanosine.[16] Contrary to these factors, the diester units **8a–d** can be stored in a refrigerator at 5 °C at least for 1 year or at room temperature for 5 months without any decomposition.

Synthesis of Oligoribonucleotide Building Blocks. Phosphodiester components **8** in situ generated by the phosphinate treatment were condensed with **7** by use of MDS and 3-nitro-1,2,4-triazole (NT). These conditions and results are shown in Table V.

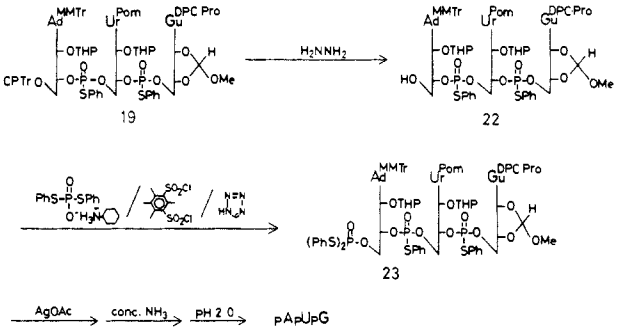
For the synthesis of trimer blocks, 5′-hydroxyl components **9**, **15**, and **16** have been synthesized by using the hydrazine reaction. The 3′-O-benzoylated guanosine derivative **9** was prepared in 96% yield by benzoylation of **6d** followed by in situ detritylation. Hydrazinolysis of **13** and **14** gave the 5′-hydroxyl components **15** and **16** in 86% and 88% yields, respectively. In these reactions, the acid-labile methoxymethylene group and base-labile functionalities such as the benzoyl, 3′-terminal BPTP, and internal

Table V. Conditions and Results of Synthesis of Fully Protected Oligoribonucleotides[a]

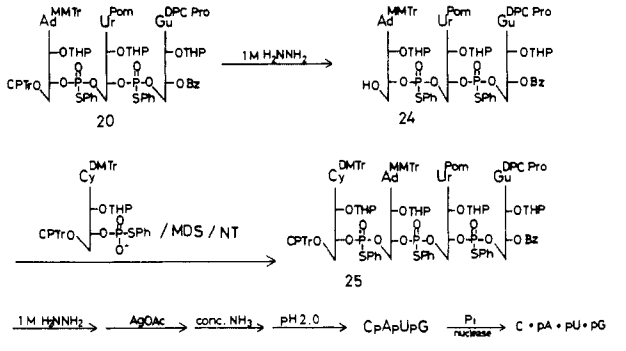
phosphodiester component, mmol			hydroxyl component, mmol		condensation condensing agent (3 equiv)		time, min	product		yield, %
CPTrAp	**6c**, 0.24	50	HOUp	**7**, 0.2	MDS	NT	45	CPTrAUp	**11**	89
CPTrGp	**6d**, 0.064	60	HOUp	**7**, 0.053	MDS	NT	45	CPTrGUp	**12**	82
CPTrUp	**6a**, 1.26	45	HOAp	**7**, 1.05	MDS	NT	15	CrTrUAp	**13**	84
CPTrUp	**6a**, 0.06	60	HOGmM	**10**, 0.05	MDS	NT	25	CPTrUGmM	**14**	80
GPTrUp	**6a**, 0.137	40 (40 °C)	HOUAp	**15**, 0.1	DDS	NT	40	CPTrUUAp	**17**	82
CPTrAp	**6c**, 0.195	50	HOAUp	**15**, 0.15	DDS	NT	60	CPTrAUAp	**18**	94
CPTrAp	**6c**, 0.0438	60	HOUGmM	**16**, 0.0337	DDS	NT	45	CPTrAUGmM	**19**	85
CPTrAUp	**11**, 0.12	45	HOGBz	**9**, 0.11	MDS	NT	40	CPTrAUGBz	**20**	93
CPTrCp	**6b**, 0.028	50	HOAUGBz	**24**, 0.023	MDS	NT	45	CPTrCAUGBz	**25**	81

[a] CPTrNp, fully protected; HONp, 5′-OH free; mM, methoxymethylene group; Bz, benzoyl group.

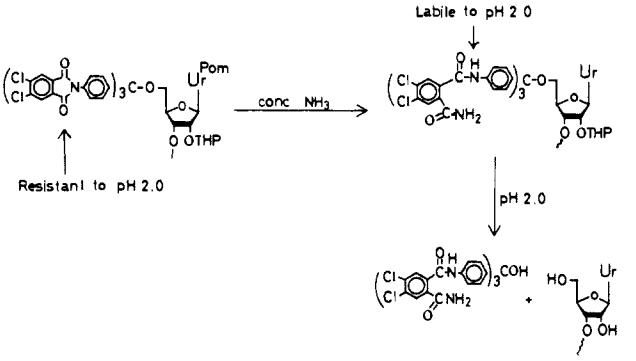
Scheme V



Scheme VI



Scheme VII



phenylthio groups were essentially stable (Scheme IV).

Fully protected trimer blocks were synthesized by condensation of these hydroxyl components with in situ generated phosphodiester components. In order to improve the coupling yields, we employed isodurenedisulfonyl chloride (DDS)[21] as a new bifunctional condensing agent which has recently proved more effective for internucleotidic bond formation than MDS.

Stepwise or block condensation by use of the four common monomer units **7a–d** provided oligoribonucleotides **17** and **18** bearing 3′-terminal phosphate as shown in Table V.

Next, we turned our interest to the synthesis of 5′-phosphorylated trinucleotides which would be used as key intermediates for the capping reaction. The fully protected trimer **20** was detritylated by the hydrazine reaction to give the hydroxyl component **22** in 90% yield. This compound was phosphorylated in the same manner as described in the synthesis of the units. The rapid phosphorylation was again attained, and the 5′-phosphorylated trimer **23** was obtained in 93% yield (Scheme V). Furthermore, a tetraribonucleoside triphosphate CpApUpG (**25**) containing all the common ribonucleosides was synthesized in high yield via compound **24** as shown in Scheme VI.

Deprotection. First, full deprotection of trimers **17** and **18** (see Table V) bearing a 3′-terminal phosphate has been examined. Aryloxy groups such as 4-chlorophenoxy on 3′-terminal phosphate residues could not be removed under mild conditions.[22] Their removal required the neighboring effect of the 2′-free hydroxyl capable of formation of a 3′-terminal 2′,3′-cyclic phosphate that upon acid hydrolysis was converted to a mixture of 2′- and 3′-phosphates. On the other hand, we have previously reported several procedures for deprotection of the phenylthio group from the 3′-terminal BPTP moiety in both deoxyribo and ribo series.[1-7] Especially, use of silver actetate in aqueous pyridine at an elevated temperature of 50 °C resulted in rapid dephenylthiolation of the fully protected oligoribonucleotides without 2′–3′ isomerization of the 3′-terminal phosphate group. Therefore, we employed this silver ion promoted dephenylthioylation at the first stage for the full deprotection of AUAp.[2,3,20] Moreover, we found that the CPTr group was simultaneously removed during the successive deprotection process for the base-labile pom group and the acid-labile MMTr and THP groups. The CPTr group was rather stable to 0.01 M HCl or 80% acetic acid as expected from its structure having three electron-withdrawing 4,5-dichlorophthalimido groups. However, once the CPTr group was exposed to concentrated ammonia, the phthalimido ring was rapidly opened to give a 4,4′,4″-tris(monoacylamino)trityl ether as shown in Scheme VII. This new ring-opened trityl group was found to be removed under the same conditions as used for removal of the THP group. Thus, the hydrazine reaction was not necessary at the final stage. The ring-opened trityl group was quite resistant to concentrated ammonia, probably because of the steric bulk of the acyl residue. During this two-step workup pararosaniline was not formed, although even a trace amount of this compound can be detected very easily because of its dye character. Thus, the successive treatments with silver acetate, concentrated ammonia, and 0.01 M HCl gave ApUpAp, which was separated by paper chromatography and finally purified by HPLC as shown in Figure 1. However, considerable loss of the trimer was observed during the isolation process, although the TLC pattern at every step was very clear, showing only a single spot. We feel that improved procedures should be studied for this problem. In a similar manner, UpUpAp was isolated in 32% overall yield. The purified trimer was completely degraded with nuclease P_1 and spleen phosphodiesterase to give A, pU, and pA and 2Ap and Up, respectively.

The 3′-terminal phosphate residue of 3′,5′-di-O-phosphorylated oligoribonucleotides is known to serve as a protecting group to avoid cyclization when they are employed as acceptors in RNA ligation between two different oligoribonucleotides. It should be noted that the phosphorothioate triester method enables us to synthesize directly 3′-masked oligoribonucleotides useful for the ligation. The trimer blocks having a 3′-terminal phosphate will be utilized for the stepwise synthesis of capped messenger ribonucleotides. The usual full deprotection of **23** gave 41% yield of pApUpG, which was consistent with an authentic sample synthesized previously.[23] In the full deprotection of **25**, the CPTr group was first removed, and the remaining protecting groups were stepwise eliminated in the usual way. Thus, CpApUpG, having no terminal phosphate, was isolated in 33% yield. The enzyme analysis of this tetramer with nuclease P_1 showed complete digestion to give C, pA, pU, and pG. This result indicated that 3′–5′ phosphoryl rearrangement did not occur when the 5′-free hydroxyl was present in the silver ion promoted hydrolysis. As described above, the CPTr group can be applied to the synthesis of several kinds of oligoribonucleotides, which will be useful for biological studies.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 100 MHz on a JEOL JNM PS-100 spectrometer using tetramethylsilane (Me_4Si) as an internal standard. UV spectra were obtained on a Hitachi 124 spectrophotometer. Paper chromatography was performed by use of a descending technique with Whatman 3 MM papers using solvent I (2-propanol–concentrated ammonia–water, 7:1:2, v/v/v) and solvent II (1-

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propanol–concentrated ammonia–water, 55:10:35, v/v/v). HPLC was performed on a Waters Model 440 apparatus by using the following conditions: method A, a linear gradient of 0–40% CH_3CN in 0.1 M NH_4OAc at 50 °C for 10 min; method B, a linear gradient of 0–50% CH_3CN in 0.1 M NH_4OAc at 50 °C for 30 min. Column chromatography was performed with silica gel C-200 purchased from Wako Co. Ltd., and a minipump for a goldfish basin was conveniently used to gain a medium pressure for rapid chromatographic separation. Thin-layer chromatography was performed on precoated TLC plates, Silica Gel 60 F-254 (Merck). The R_f values of the protected nucleoside derivatives were measured after development with CH_2Cl_2–MeOH (9:1, v/v) unless otherwise noted. Pyridine was distilled twice from *p*-toluenesulfonyl chloride and from calcium hydride and then stored over molecular sieves (3A). CH_2Cl_2 was dried over P_4O_{10} overnight, decanted, distilled from K_2CO_3, and stored over molecular sieves (3A). Spleen phosphodiesterase was purchased from Boehringer Co. Ltd. Nuclease P_1 was purchased from Yamasa Hoyu Co. Ltd. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology, at Nagatsuta.

4,4′,4″-Tris(4,5-dichlorophthalimido)tritanol (CPTrOH). To a solution of pararosaniline (76.3 g, 0.25 mol) in pyridine (800 mL) was added 4,5-dichlorophthalic anhydride (217 g, 1 mol). The mixture was stirred at 150 °C for 2 h, and then acetic anhydride (1.3 mL) was added. After it was kept at 150 °C for 20 min, the solution was cooled to room temperature and filtered. The filtrate was poured slowly with stirring into a mixture of pyridine–water (2:1, v/v, 1.2 L). The precipitate was collected by filtration and washed successively with pyridine–water (2:1, v/v, 600 mL) and with ether (1 L). Air-drying followed by drying over P_4O_{10} in a desiccator gave CPTrOH (205 g, 91%): mp > 270 °C; IR (KBr) 3098, 3040, 1780, 1720, 1510, 1380, 1310, 1220, 1140, 1120, 1090, 770, 740 cm^{-1}; ^1H NMR (C_5D_5N–$CDCl_3$, 2:1, v/v) δ 6.02 (br s, 1, OH), 7.41 (d, 6, J = 8.5 Hz, ArH), 7.61 (d, 6, J = 8.5 Hz, ArH), 7.86 (s, 6, ArH). Anal. Calcd for $C_{43}H_{19}N_3O_7Cl_6$: C, 57.24; H, 2.12; N, 4.66; Cl, 23.57. Found: C, 57.51; H, 2.00; N, 4.57; Cl, 23.32.

4,4′,4″-Tris(4,5-dichlorophthalimido)trityl Bromide (CPTrBr). CPTrOH (208 g, 0.23 mol) was supended in dry benzene (2.8 L), and acetyl bromide (128 g, 1.04 mol) was added. The heterogeneous solution was refluxed with vigorous stirring for 10 h until the granular CPTrOH had been completely converted into cottonlike CPTrBr. If stirring is not satisfactory, longer periods of time (>15 h) should be required. After the mixture was cooled to room temperature, the resulting precipitate was collected by filtration and washed with benzene (1 L) and then hexane (500 mL). Air-drying gave crude CPTrBr (218 g, 99%). The crude material was suspended in pyridine (600 mL) with stirring for 15 min, and then insoluble CPTrBr was collected by filtration and washed successively with pyridine (400 mL), benzene (200 mL), and hexane (500 mL). Air-drying followed by vacuum-drying over P_4O_{10} gave pure CPTrBr (198 g, 90%): mp > 270 °C; IR (KBr) 3030, 1780, 1720, 1510, 1384, 1315, 1224, 1142, 1120, 1105, 1093, 770, 760, 740 cm^{-1}. Anal. Calcd for $C_{43}H_{18}N_3O_6Cl_6Br$: C, 53.51; H, 1.88; N, 4.35; Cl_6Br, 30.32. Found: C, 53.68; H, 1.86; N, 4.31; Cl_6Br, 29.96.

2′-*O*-(Tetrahydropyran-2-yl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*N*³-((pivaloyloxy)methyl)uridine (3a). Compound **2a**[4b] (13.5 g, 23.7 mmol) was rendered anhydrous by coevaporation with dry DMF (2 × 20 mL) and finally dissolved in dry DMF (150 mL). Sodium hdyride (60%, 1.14 g, 28.4 mmol) was added, and the suspension was vigorously stirred for 4.5 h. Then, (pivaloyloxy)methyl chloride (4.27 g, 28.4 mmol) was added, and the mixture was continuously stirred. After 2 h, the mixture was partitioned between CH_2Cl_2–H_2O (500 mL). The CH_2Cl_2 extract was washed 5 times with H_2O (200 mL). Each aqueous layer was back-extracted with CH_2Cl_2 (100 mL). The two CH_2Cl_2 extracts, which were free of DMF, were combined, dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. After the residue was coevaporated 3 times with toluene, it was chromatographed on a silica gel column with hexane–CH_2Cl_2 (1:5–1:15, v/v) to give **3a** (9.95 g, 62%). The NMR spectra and elemental analysis of **3a**, 4a–**d**, 5a–**d**, and **6a–d** are summarized in Table III of the supplemental material.

2′-*O*-(Tetrahydropyran-2-yl)-*N*³-((pivaloyloxy)methyl)uridine (4a). A mixture of compound **4a** (7.88 g, 11.5 mmol), KF (3.87 g, 69 mmol), and Et_4NBr (14.5 g, 69 mmol) in CH_3CN (138 mL)–H_2O (3.45 mL) was heated at 50 °C with vigorous stirring. Then, extraction was performed with CH_2Cl_2–H_2O as in the case of extractive removal of DMF in the above experiment to remove completely the resulting ammonium salts. The combined CH_2Cl_2 extracts were dried over Na_2SO_4, filtered, and evaporated. The residue was chromatographed on a silica gel column with 1–2% MeOH-containing CH_2Cl_2 to give **4a** (5.52 g, 97%).

2′-*O*-(Tetrahydropyran-2-yl)-*N*⁴-(4,4′-dimethoxytrityl)cytidine (4b). To a solution of **2b** (4.86 g, 10 mmol) in dry CH_2Cl_2 (100 mL) were added 2,3-dihydropyran (18.3 mL, 200 mmol) and trifluoroacetic acid (1.54 mL, 20 mmol). After being stirred at room temperature for 22 h, the mixture was quenched by addition of 5% $NaHCO_3$. The organic phase was extracted, and the aqueous layer was further extracted with CH_2Cl_2 (2 × 100 mL). The combined CH_2Cl_2 extracts were dried over Na_2SO_4, evaporated under reduced pressure, and coevaporated with dry toluene. The residue was dissolved in dry CH_2Cl_2 (50 mL), and triethylamine (1.63 mL, 11.6 mmol) and 4,4′-dimethoxytrityl chloride (3.95 g, 11.6 mmol) were successively added. The mixture was stirred for 30 min and then quenched with 5% $NaHCO_3$. Extraction was performed with CH_2Cl_2 (3 × 100 mL). The CH_2Cl_2 extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved in acetonitrile (85.5 mL), and a mixture of KF (2.62 g, 45 mmol) and Et_4NBr (9.46 g, 45 mmol) was added. Finally, water (2.3 mL) was added, and the resulting mixture was vigorously stirred at 50 °C for 1 h. Then the mixture was diluted with CH_2Cl_2 (200 mL), and the CH_2Cl_2 solution was washed with water (5 × 150 mL) to remove the ammonium salt. Each washing was back-extracted with the same CH_2Cl_2 (100 mL) in another separatory funnel and discarded. The two CH_2Cl_2 extracts were combined, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was chromatographed on a column of silica gel (200 g) with 0.5–5% MeOH-containing CH_2Cl_2 to afford **4b** (4.69 g, 95%).

General Procedure for the Synthesis of 5. Compound **4** (1 mmol) was rendered anhydrous by coevaporations with dry DMF (2 × 10 mL) and finally dissolved in dry DMF (10 mL). In the case of **4a**, the coevaporation was done using dry toluene, and then lutidine (214 mg, 2 mmol) was added. To the DMF solution were added successively CPTrBr (1.91 g, 2 mmol) and $AgNO_3$ (339 mg, 2 mmol). After being stirred vigorously for 15 min (30 min for **4c**), the mixture was diluted with CH_2Cl_2 (20 mL), filtered, and extracted with CH_2Cl_2–H_2O as described in the case of the synthesis of **3a** for removal of DMF. The extracts were combined, dried over Na_2SO_4, filtered, and evaporated to dryness. After the residue was coevaporated with toluene (3 × 10 mL), it was dissolved in benzene (20 mL). After the mixture stood for 15 min, precipitated CPTrOH was filtered and washed with benzene. The filtrate and washing were combined and evaporated to dryness.

The residue was chromatographed on a silica gel column with hexane–CH_2Cl_2 to give **5**. In the case of **5a**, chromatography was performed using the above solvent system in the presence of 0.5% pyridine to avoid loss of the THP group.

General Procedure for the Synthesis of 6. A mixture of **5** (0.5 mmol), PSS (286 mg, 0.75 mmol), and tetrazole (140 mg, 2 mmol) was coevaporated 3 times with dry pyridine (5 mL) and finally dissolved in dry pyridine (5 mL). To the mixture was added MDS (317 mg, 1 mmol). After being stirred for the time listed in Table II, the solution was diluted with CH_2Cl_2 (20 mL) and then washed successively with H_2O (20 mL), 5% $NaHCO_3$ (2 × 20 mL), and H_2O (20 mL). Each aqueous layer was back-extracted with the same CH_2Cl_2 (15 mL). After the combined CH_2Cl_2 extracts were evaporated, the residue was chromatographed to give **6**.

General Procedure for the Synthesis of 7. Compound **6** (0.2 mmol) was dissolved in a solution of hydrazine monohydrate (300 mg, 6 mmol) in pyridine–acetic acid (3:1, v/v, 6 mL). The mixture was stirred at room temperature for 20 min, when 4,5-dichlorophthalhydrazide was precipitated. Then, acetic acid (12 mL) was added dropwise at 0 °C, and the resulting solution was stirred at room temperature for 5–10 min. Extractive workup using CH_2Cl_2–5% $NaHCO_3$ followed by chromatography afforded **7**.

2′-*O*-(Tetrahydropyran-2-yl)-3′-*O*-benzoyl-*N*²-propionyl-*O*⁶-(diphenylcarbamoyl)guanosine (9). Compound **5d** (308 mg, 0.2 mmol), which was dried by repeated coevaporations with dry pyridine, was allowed to react with benzoyl chloride (56 μL, 0.48 mmol) in dry pyridine (2 mL) for 5 h. Then, ice was added, extraction was performed with CH_2Cl_2–5% $NaHCO_3$ in the usual manner, and the CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and evaporated to dryness. The residue was treated with a solution of hydrazine monohydrate (600 mg, 12 mmol) in pyridine–acetic acid (3 L; 12 mL) for 20 min. A workup similar to that described in the above experiment gave **9** (135 mg, 93%).

General Procedure for the Fragment Condensation. An appropriate protected ribonucleotide unit or oligoribonucleotide block was dissolved in a mixture of 5 M pyridinium phosphinate (75 equiv)–triethylamine (2:1, v/v). After being stirred at room temperature for the time listed in Table V, the mixture was extracted with CH_2Cl_2 (3 × 20 mL) from its aqueous solution. The combined CH_2Cl_2 extracts were washed with 0.2 M triethylammonium bicarbonate (2 × 10 mL) and then with water. The organic extract was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was mixed with 3-nitro-1,2,4-triazole and a hydroxyl component, and the mixture was coevaporated several times with dry pyridine and finally dissolved in dry pyridine (1–5 mL/0.1 mmol of the hydroxyl component). The solution was stirred for the time listed in Table V. Then, the usual extractive workup followed by chromatog-

raphy gave an oligoribonucleotide building block.

Deprotection of the Fully Protected AUAp (18). Compound **18** (61 mg, 19.4 μmol) was dissolved in pyridine–water (2:1, v/v, 2.9 mL), and silver acetate (485 mg, 2.9 mmol) was added. After the mixture was stirred at 50 °C for 5 h, it was cooled and diluted with pyridine–water (2:1, v/v, 30 mL). The solution was treated with hydrogen sulfide gas at 0 °C until a clear supernatant had been obtained. The precipitate was removed by centrifugation, and the supernatant was passed through a column of Dowex 50 W × 2 (pyridinium form, 3 mL). The eluant was evaporated and coevaporated several times with pyridine to remove acetic acid. The residue was dissolved in pyridine (10 mL), and concentrated ammonia (50 mL) was added. After the solution was kept at room temperature for 18 h, it was evaporated under reduced pressure. The residue was coevaporated several times with water, and then dioxane (30 mL) was added. To the solution was added 0.02 M HCl (30 mL), and it was adjusted exactly to pH 2.0 by addition of 0.1 M HCl. After the mixture was stirred for 20 h, it was neutralized by addition of pyridine and extracted with CH_2Cl_2–H_2O. The aqueous layer was evaporated, and the residue was chromatographed on Whatman 3 MM papers with solvent to give AUAp (312 OD, 48%): UV λ_{max} 259. λ_{min} 229 nm; retention time, 11.9 min (method A).

Similarly, UUAp (168 OD, 32%) and pAUG (307 OD, 41%) nm, were obtained from **17** (17 μmol) and the fully protected pAUG (23 μmol), respectively. UUAp: λ_{max} 260 nm, λ_{min} 230 nm; retention time, 11.0 min (method A). pAUG: R_f 0.11 (solvent II, to pT); λ_{max} 257 nm, λ_{min} 228 nm; retention time, 5.6 min (method A).

Deprotection of the Fully Protected Tetramer 14. The fully protected tetramer **25** (18 mg, 5 μmol) was dissolved in a 1 M hydrazine solution in pyridine–acetic acid (3:1, v/v, 0.3 mL). After the mixture was stirred at room temperature for 20 min, the same workup as described in the synthesis of **7** was done. The mixture obtained after extraction was dissolved in pyridine–water (3:1, v/v, 0.6 mL), and silver acetate (125 mg, 0.75 mmol) was added. A workup similar to that described in the above experiment and the successive treatments with concentrated ammonia and 0.01 M HCl gave crude CAUG (100 OD, 47%). This crude tetramer was further purified by paper chromatography using concentrated ammonia–*n*-PrOH–H_2O (55:10:35, v/v/v) as the solvent for development. A single band at R_f 0.14 (to pU) was eluted (70 OD, 33%) similarly and analyzed by HPLC, which showed one peak: retention time, 11 min (method B).

Enzyme Assay. Each sample (10 OD) was incubated under the following conditions: spleen phosphodiesterase (10 μg, Boehringer), 0.01 M pyrophosphate buffer (pH 6.5, 100 μL), 0.05 M ammonium acetate (pH 6.5, 200 μL), 37 °C, 10 h; nuclease P_1 (10 μg, Yamasa), 0.02 M sodium acetate (pH 5.5, 100 μL), 37 °C, 4 h.

The degradation products were analyzed by paper electrophoresis or HPLC.

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Supplementary Material Available: Table III showing ^1H NMR spectral data and elemental analyses of compounds **3–9** and Figure 1 showing the HLPC profile of AUAp (5 pages). Ordering information is given on any current masthead page.

Total Synthesis of (+)-Dihydromevinolin

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Abstract: A total synthesis of (+)-dihydromevinolin (**2b**) is presented, as well as the preparation of three analogues (**62**, **63**, and **64**) for assessment of their biological activity. Base-promoted fragmentation of the tosylhydrazone (**20**) of key intermediate **6** affords acetylenic alcohol **23**. Partial hydrogenation and acetylation, followed by stereoselective epoxidation, provide oxirane **37**. Lewis acid mediated rearrangement followed by ozonolysis and epimerization furnishes keto aldehyde **49**, which contains the requisite stereochemistry at all six stereocenters of the octalin moiety. Selective reduction of the aldehyde affords keto alcohol **50**, which is elaborated to racemic diol **52** by the Shapiro olefin synthesis. Selective DCC-promoted esterification with (*R*)-(+)-*O*-methylmandelic acid provides diastereomers **53** and **54**, of which the desired isomer **54** is separated by fractional crystallization. Acylation and selective ester cleavage followed by Swern oxidation furnishes target intermediate **3b**. Coupling of **3b** with lactone synthon **4** affords enone **5b**, which is converted to ketone **58** by conjugate reduction. Acid-promoted lactonization of the major diol from hydride reduction of **58** furnishes **2b**, in 19 steps from pyranone **6**.

The mevinic acids compactin (**1a**),[1] mevinolin (**2a**),[2] dihydrocompactin (**1b**),[3] and dihydromevinolin (**2b**)[4] have attracted considerable synthetic attention[5-7] because of their significance as potent inhibitors of HMG CoA reductase, the enzyme that

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