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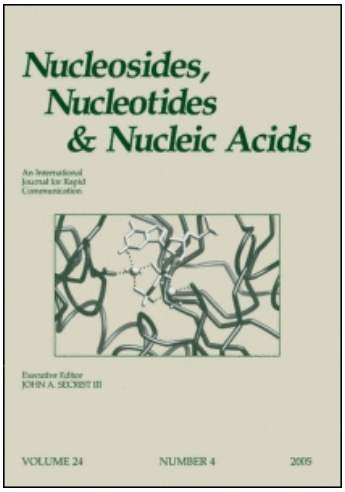

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Partial Protection of Carbohydrate Derivatives. Part 27.[1] Further Improvement in the Protecting Procedure for Oligonucleotide Synthesis in Terms of a Cellulose Acetate Derivative as a Polymer-Support

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PARTIAL PROTECTION OF CARBOHYDRATE DERIVATIVES. PART 27.[1] FURTHER IMPROVEMENT IN THE PROTECTING PROCEDURE FOR OLIGONUCLEOTIDE SYNTHESIS IN TERMS OF A CELLULOSE ACETATE DERIVATIVE AS A POLYMER-SUPPORT

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Abstract: Utilization of a (3-carboxy)propionyl spacer for the cellulose acetate polymer-support, a comparative study of 2-cyanoethyl and diphenylcarbamoyl protecting groups for the O^6-position of the guanosine unit, protecting groups for 1-β-D-ribofuranosylthymine (rT) and pseudouridine (Ψ) is described in connection with the syntheses of oligoribonucleotides, i.e., a tridecamer, ApAp-GpGpApApApApUpUpApUpG, and a dodecamer, UpCpCpGpGprTp-ΨpCpGpApUpU.

INTRODUCTION

A cellulose acetate derivative functionalized with a 4-(2-hydroxyethylsulfonyl)dihydrocinnamoyl spacer was reported as a novel polymer-support for the synthesis of both oligoribonucleotides and oligodeoxyribonucleotides.[1,2] The polymer-support is characterized by its unique solubility in organic solvents, i.e., being easily soluble in pyridine and chloroform,[3] but slightly soluble in

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The present paper is dedicated to the late Professor Tohru Ueda.

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ethanol. Such properties of the material made it possible to realize the idea originally developed by both Cramer et al.[4] and Khorana et al.[5] in terms of a non-crosslinking polystyrene bearing a monomethoxytrityl function for constructing oligodeoxyribonucleotides; the polystyrene derivative was potentially expected to make it possible to perform a deoxyribonucleotide unit introduction reaction in pyridine in a homogeneous state and to isolate the resulting polystyrene polymer-support by pouring the resulting reaction mixture into water and subsequent filtration.

Introduction of an appropriate protecting group onto the lactam-lactim systems involved in the nucleosides of guanine, uracil, and thymine is essential to prevent undesirable side reactions as indicated by Reese et al.[6] in oligonucleotide synthesis, in addition to an efficient and highly regioselective protecting procedure for the hydroxyl groups involved in the sugar moieties. Particularly, protection of the heterocyclic moieties of pseudouridine (Ψ) has not yet been established completely.[7-10]

Consequently, simplification of the spacer by the use of the (3-carboxy)propionyl function in place of that which we reported,[1,2] and protecting groups for the active hydrogens involved in the lactam-lactim tautomeric structure of the heterocyclic moieties of guanosine and Ψ were further undertaken to investigate; the results are reported in full herein.

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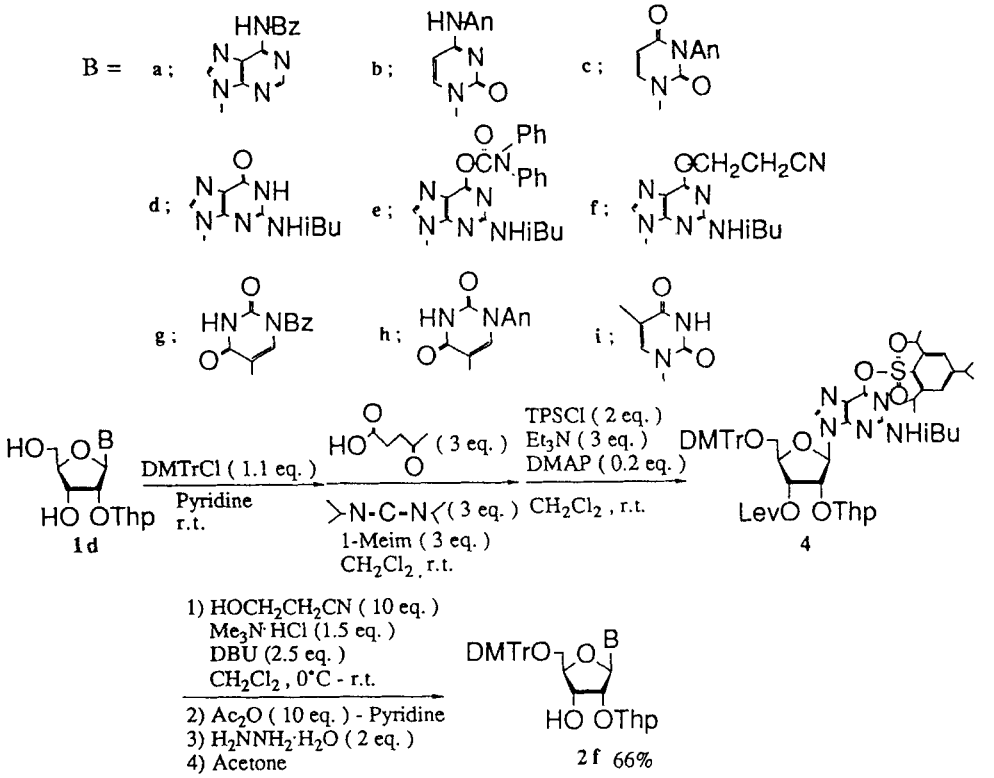
RESULTS AND DISCUSSION

Synthetic Intermediates for Oligoribonucleotides: N^6-Benzoyl-2'-*O*-(tetrahydropyran-2-yl)adenosine (**1a**) and N^4-anisoyl-2'-*O*-(tetrahydropyran-2-yl)cytidine (**1b**) were prepared according to the method we have reported.[11] N^3-Anisoyl-2'-*O*-(tetrahydropyran-2-yl)uridine (**1c**) and O^6-diphenylcarbamoyl-N^2-isobutyryl-2'-*O*-(tetrahydropyran-2-yl)guanosine (**1e**) were prepared as we separately reported.[12]

For comparing utility in oligonucleotide synthesis with **1e**, the corresponding O^6-(2-cyanoethyl) derivative (**1f**) was used. The introduction of the 2-cyanoethyl protecting group onto the O^6-position has been reported by Jones et al.,[13] although the yield was only 30 - 40%; they used trimethylamine (b.p. -3°C; 12 - 23 mol. equiv.) and 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) (1.5 mol. equiv.) for the reaction of O^6-(2,4,6-triisopropylbenzenesulfonate) (**4**) of 5'-*O*-dimethoxytrityl-N^2-isobutyryl-3'-*O*-levulinyl-2'-deoxyguanosine. We used solid trimethylamine hydrochloride (1.5 mol. equiv.), in place of the amine, in combination with DBU (2.5 mol. equiv.) for the preparation of **2f**; i.e., the treatment of **1d**, after introducing a dimethoxytrityl protecting group onto its 5'-position, levulinylation on its 3'-position and 2,4,6-triisopropylbenzene-



Scheme 1

sulfonylation at its O^6-position, with 2-cyanoethanol (10 mol. equiv.) in the presence of the reagents at 0°C - room temperature, followed by quenching of unchanged 2-cyanoethanol with acetic anhydride and by the delevulinylation with hydrazine, gave O^6-(2-cyanoethyl)-5'-*O*-dimethoxytrityl-N^2-isobutyryl-2'-*O*-(tetrahydropyran-2-yl)-guanosine (**2f**) in 66% overall yield based on **1d** (See Scheme 1).

Pseudouridine, Ψ, is known, under acidic conditions (e.g., *p*H 2.8, 80°C, 1.5 h) and under an alkaline condition (20% aqueous sodium hydroxide solution, 100°C, 15 min) to give a mixture of the corresponding four isomers.[14] Consequently, the N^1-position of Ψ should be protected on oligonucletide synthesis as has been reported by Ohtsuka et al.,[7] using a benzoyl group for protection in the synthesis of the trimer rTpΨpC through the phosphodiester approach, and Reese et al.,[9] using 4-bromobenzenesulfonyl group for that of the 3'-terminal 32-mer involved in Yeast $tRNA^{Ala}$ through a

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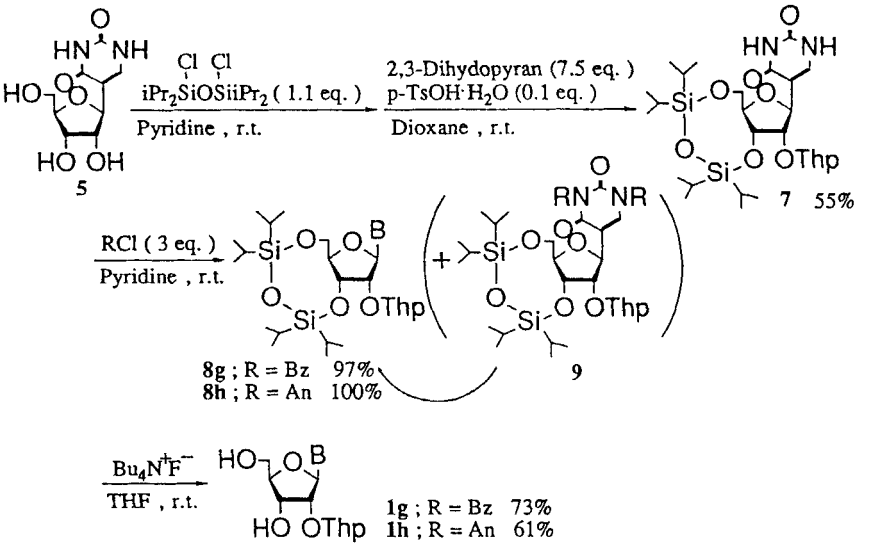

Scheme 2

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phosphotriester - liquid phase approach based upon the assumption that a benzoyl group would be much too sensitive to hydrolysis. Thus, a reinvestigation on the possibility of using a benzoyl group for the present purpose was undertaken because of its successful use reported by Ohtsuka et al.[7]

Different from ordinary ribonucleosides, Ψ was inappropriate as a substrate for the di-*O*-acylation reaction,[11] giving no significant regioselectivity between its 2' and 3' positions, probably due to the lowered electron-withdrawing effect of its heterocyclic moiety. Protection of both of the 3'- and 5'-hydroxyl groups was fulfilled through the 1,1,3,3-tetraisopropyl-1,3-disiloxan-1,3-diyl group[15] as usual, and the derivative was then subjected to (tetrahydropyran-2-yl)ation at 2'-position. The Ψ derivative was then subjected to acylation onto its heterocyclic moiety by the use of benzoyl and anisoyl chloride and, then, desilylated with tetrabutylammonium fluoride as usual. Both of the acylation reactions gave mixtures of N^1-acyl-2'-*O*-(tetrahydropyran-2-yl)-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxan-1,3-diyl)-pseudouridine [**8g** (acyl = benzoyl) and **8h** (acyl = anisoyl) and N^1,N^3-diacyl derivatives (**9**); the latter of which was easily hydrolyzed to give **8g** and/or **8h** on quenching the reaction with water or chromatographic separation of the mixture on silica gel.

Table 1 Stabilities of N^1-Acyl-2'-*O*-(tetrahydropyran-2-yl)pseudouridine Derivatives (**1g** and **1h**) under basic conditions

Compound	4 : 1 conc.NH_4OH-MeOH, r.t.		1 : 3 Et_3N-Pyridine r.t., 3h	1 : 2 H_2O-Pyridine r.t., 6h
	$t_{1/2}$[a]	$t_{100\%}$[b]		
1c	5min	30min	stable	stable
1g	1h	5h	stable	stable
1h	12h	24h	stable	stable

a ; 50% degradation of acyl group, b ; 100% degradation of acyl group

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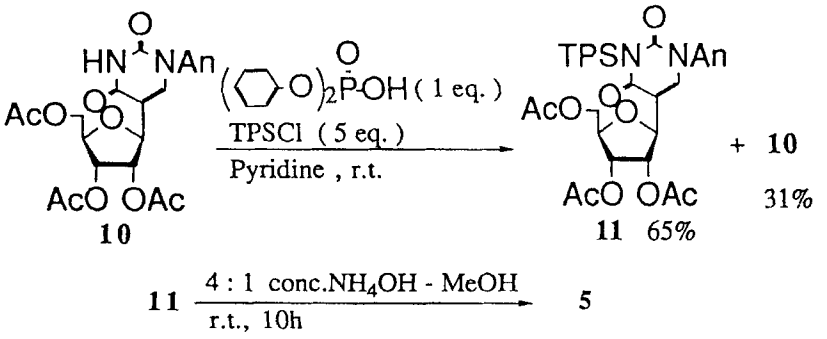
The stability of their N^1-acyl groups was examined under the basic conditions necessary for deprotection after the construction of an oligonucleotide backbone structure comparing with that of **1c**, monitoring the process in terms of t.l.c. All of the N^1-acyl groups were stable enough under the conditions of 1:3 triethylamine - pyridine at room temperature for 3 h[16] and of 1:2 water - pyridine at room temperature for 6 h. On the other hand, N^1-deacylation was induced under the conditions of 4:1 concentrated aqueous ammonia - methanol at room temperature; $t_{1/2}$ was 1 h for **1g**, 12 h for **1h**, and 5 min for **1c**, and $t_{100\%}$ was 5 h for **1g**, 24 h for **1h**, and 30 min for **1c**, respectively, as summarized in Table 1. It was thus confirmed that a benzoyl group was feasible for the protection of the N^1-position of Ψ as has been worked out by Ohtsuka et al.[7]

The stability of the tetrahydropyran-2-yl (THP) function involved in **1c** and **1h** was examined under the conditions for removal of the dimethoxytrityl group. Their treatment with 2% *p*-toluenesulfonic acid monohydrate in 7:3 chloroform - methanol at 0°C[7] proved that the THP group of **1h** was removed much faster than that of **1c**, i.e., the $t_{1/2}$ was 50 min and $t_{100\%}$ 90 min. These results were of great interest in comparison with those obtained in the highly regioselective 2',5'-di-O-acylation of ribonucleosides and in their efficient acyl migration from the 2' position to the 3' position on silica gel, which were accepted as arising from the electron-withdrawing effect of their heterocyclic moieties;[11] a higher electron density on the O^2-atom of **1h** might facilitate the protonation by a protic acid to bring about the rapid removal of its THP protecting group. The results obtained here led us to investigate the use of 2% *dichloroacetic acid in methylene chloride* at 0°C[18] in place of the

Table 2 Stabilities of N^1-Anisoyl-2'-*O*-(tetrahydropyran-2-yl)pseudouridine (1h) under the conditions for removal of dimethoxytrityl group

Compound	2% p-TsOH·H_2O / 7 : 3 $CHCl_3$-MeOH, 0°C		2% $Cl_2CHCOOH$ / CH_2Cl_2, 0°C
	$t_{1/2}$[a]	$t_{100\%}$[b]	$t_{1/2}$[a]
1c	2.5h	–	7 - 8h
1h	50min	4.5h	5 - 6h

a ; 50% degradation of the tetrahydropyran-2-yl group
b ; 100% degradation of the tetrahydropyran-2-yl group



Scheme 3

above conditions. These conditions showed a more favarable $t_{1/2}$ of 6 h for the removal of THP protection involved in **1h** as summarized in Table 2. Incidentally, Ψ was shown to be stable enough even on its treatment under acidic conditions at *p*H 2.0 (hydrochloric acid) for more than 48 h, which have been ordinarily used for the removal of the THP protecting group in oligoribonucleotide synthesis.[19]

The possibility of the introduction of the 2,4,6-triisopropyl-benezensulfonyl group onto the N^3 position of Ψ was next examined by the treatment of N^1-anisoyl-2',3',5'-tri-*O*-acetylpseudouridine (**10**), derived from Ψ, with diphenyl phosphate and 2,4,6-triiso-propylbenzenesulfonyl (TPS) chloride in pyridine for 2.5 h, which gave its N^3-TPS derivative (**11**)(65% yield) as expected and **10** (31% recovery yield).The TPS function introduced therein was proved to be easily hydro-lyzed to recover **10** on treatment with 4:1 concentrated aqueous ammonia - methanol (Scheme 3).

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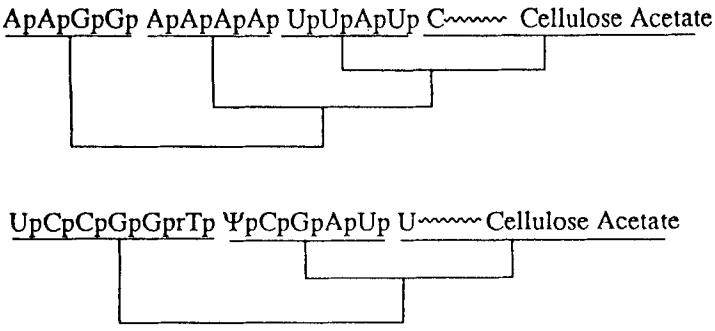

Scheme 4

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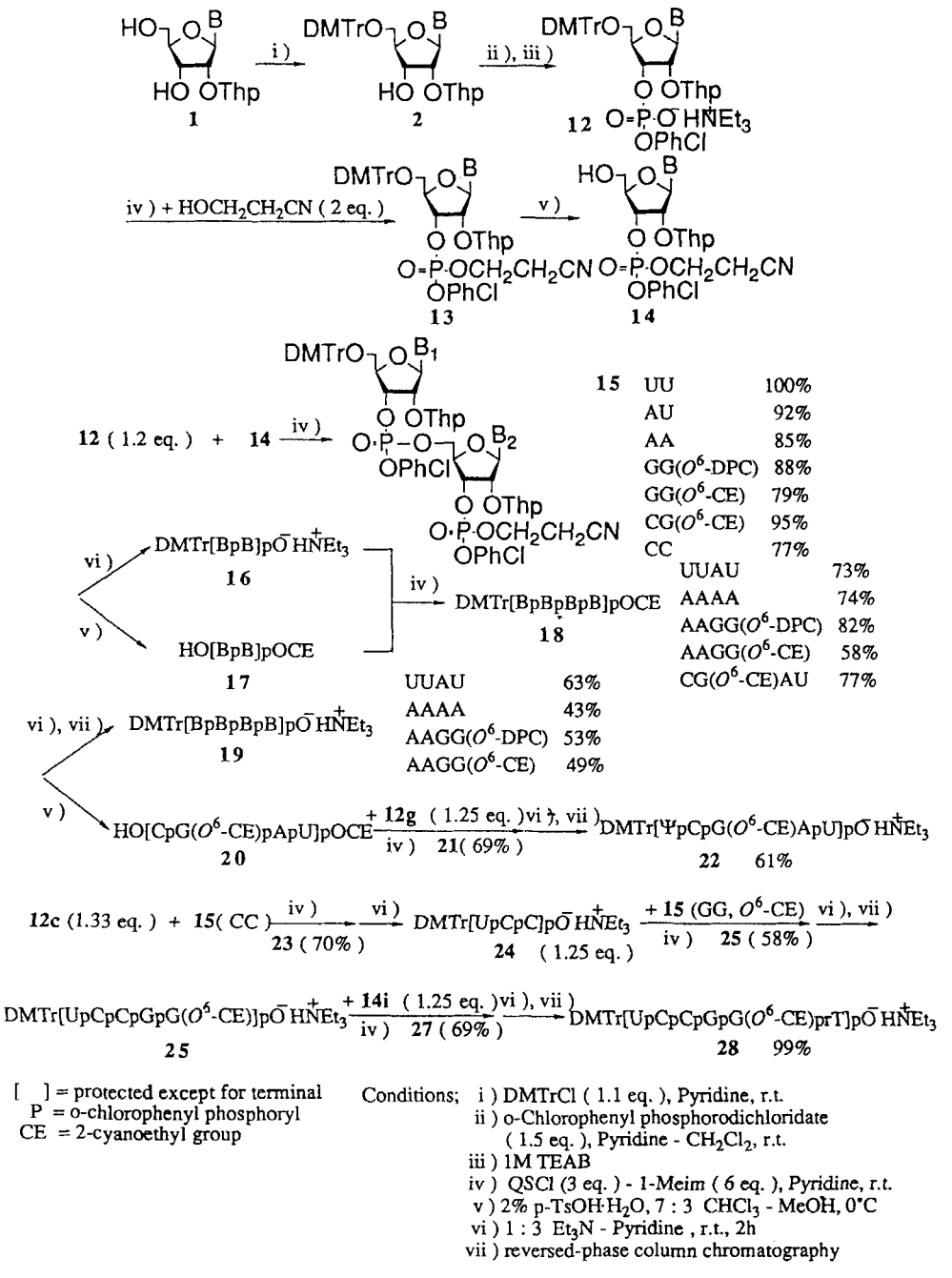
Consequently, it was concluded that the benzoyl group, which is more labile toward a nucleophile as compared with the anisoyl group, should be used for the N^1-position of Ψ, THP for the 2'-hydroxyl group, and the DMTr group for the 5'-hydroxyl group.

2'-*O*-(Tetrahydropyran-2-yl)-1-β-D-ribofuranosylthymidine (**1i**) was prepared by way of 1-[2-*O*-(tetrahydropyran-2-yl)-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxan-1,3-diyl)-β-D-ribofuranosyl]-thymine, followed by desilylation with tetrabutylammonium fluoride. In this case, protection of N^3-position was ignored based on the study by Reese et al.;[6] the active hydrogen in the heterocyclic moity of thymidine is not necessarily protected differently by from uridine, which is easily susceptible to side reactions during the oligonucleotide synthesis.

Synthesis of Oligoribonucleotides; Synthesis of two oligoribonucleotides, ApApGpGpApApApApUpUpApUpG (**35**) and UpCpCpGpGprTpΨpCpGpApUpU (**38**), was performed in terms of a liquid phase approach by the strategies shown in Scheme 4.

1) *Synthesis of Synthetic Oligomer Blocks*; The oligomer blocks were synthesized from a mononucleotide unit (**14**) bearing ordinary protecting groups, i.e., compounds **1** were subjected to a sequence of reactions of 5'-*O*-dimethoxytritylation,[20] and 3'-*O*-phosphorylation with 2-chlorophenyl phosphordichloridate,[1,21] to give ribonucleoside 3'-phosphodiester derivatives (**12**). Compounds **12** were further subjected to 2-cyanoethylation[16] with 2-cyanoethanol in the presence of 8-quinolinesulfonyl chloride (QSCl) and 1-methylimidazole (1-Meim),[22] to give **13** and removal of the 5'-*O*-dimethoxytrityl protection,[17,18] to give ribonucleoside 3'-phos-



Scheme 5

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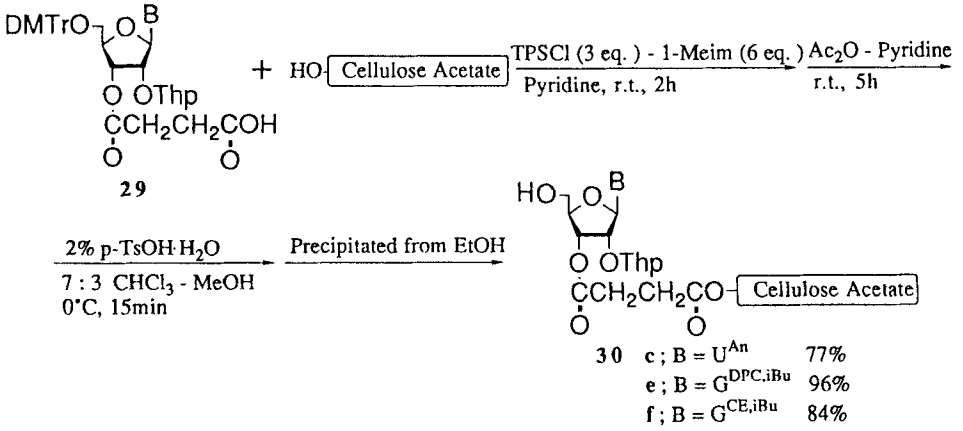

Scheme 6

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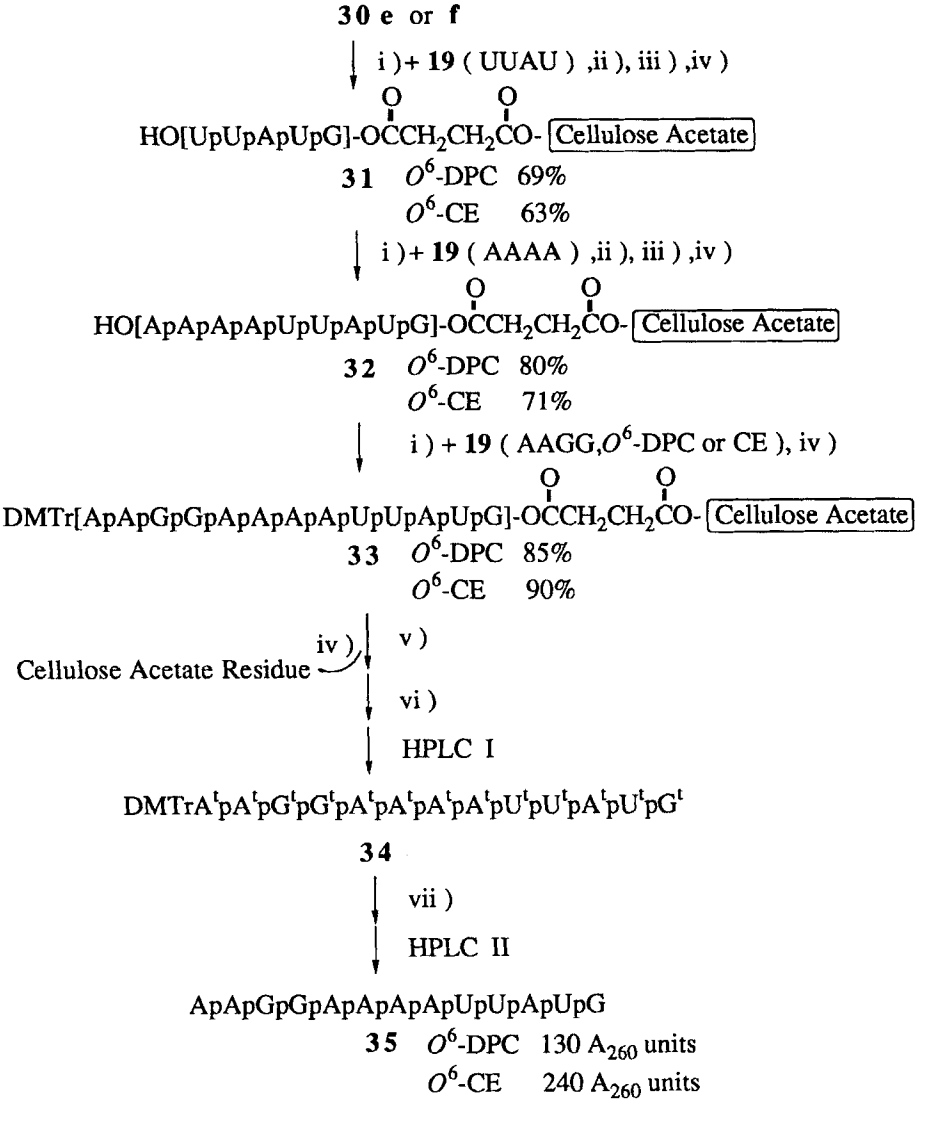
photriester derivatives (**14**), which compose the 3'-terminal structure in the oligoribonucleotide synthese. Incidentally, **12** and **13** were efficiently distributed between the aqueous pyridine layer and the diethyl ether layer, respectively, on shaking with aqueous pyridine - diethyl ether ; this procedure is practically convenient for purification of the resulting **13**.

Dimer blocks (**15**) were prepared by a coupling reaction of **12** and **14** in the presence of QSCl - 1-Meim[22] in pyridine. In this case, similarly, the distribution between aqueous pyridine and diethyl ether was successful in simplifying the purification of the resulting **15**. Compounds **15** were easily derived into **16** on removal of the 2-cyanoethyl protecting group by treatment with 1:3 triethylamine - pyridine,[16] and into **17** on removal of the 5'-*O*-dimethoxytrityl protecting group.[17,18] Tetramer blocks (**19**, UpUpApUp, ApApApAp, and ApApGpGp), a pentamer block (**22**, ΨpCpGpApUp), and a hexamer block (**28**, UpCpCpGpGprTp) were constructed in a similar manner as above described (See Scheme 5).

2) *3-(Carboxy)propionyl Function as a Novel Simplified Spacer for the Cellulose Acetate Polymer-support*: We reported the 4-{2-[3-(carboxy)propionyloxy]ethylsulfonyl}dihydrocinnamoyl function as a spacer for a cellulose acetate which made it possible to remove the objective oligonucleotide chain bearing original protecting groups on treating under mildly basic conditions.[1,2,3] Subsequently, simplification of the spacer was investigated by the use of the 3-(carboxy)-propionyl function as a novel spacer in place of the above complicated one; if the function would be practical, our approach to oligonucleotides synthesis would be accepted as a further more



Conditions ; i) TPSCl (3 eq.) - 1-Meim (6 eq.), Pyridine, r.t., 2h
ii) Ac_2O - Pyridine, r.t., 5h
iii) 2% p-TsOH·H_2O / 7 : 3 $CHCl_3$ - MeOH, 0°C, 15min
iv) Precipitated from EtOH
v) 0.5M TMG - PAO , 9 : 1 Pyridine - H_2O, r.t., 1 day
vi) conc.NH_4OH, 55°C, 6h
vii) HCl (pH 2), r.t., 2 days

Scheme 7

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significant procedure. A ribonucleoside 3'-(carboxy)propionate[1,23] (**29**) was thus introduced onto a free hydroxyl group involved in a cellulose acetate derivative, and the construction of an oligoribonucleotide chain was performed; the results thus obtained are described herein.

Condensation of the 3-(carboxy)propionate **29** with a cellulose acetate derivative (D.S. = 1.77)[24] through TPS chloride - 1-Meim,[25] followed by capping of the unchanged hydroxyl groups through acetylation, unmasking of its 5'-position, and sedimentation through pouring into ethanol, gave **30** as a powder in an excellent overall yield (77 - 96%) (Scheme 6).

3) *Synthesis of a Tridecamer, ApApGpGpApApApApUpUpApUpG* (**35**): Scheme 7 shows the flow-sheet for the synthesis of **35**, in which diphenylcarbamoyl and 2-cyanoethyl protecting groups were used for the protection of the O^6-position of guanosine unit in order to prove which one is better on the whole for the present case. The polymer-supports bearing the guanosine units **30e** and **30f** (6 mol. equiv.) were subjected to the coupling reaction with the fully protected tetramer block of $U^{An}U^{An}A^{Bz}U^{An}$ (**19**) bearing the triethylammonium 3'-(2-chlorophenyl)phosphate terminus (1 mol. equiv.) through TPS chloride - 1-Meim in pyridine, capping through acetylation with acetic anhydride, unmasking of 5'-position, and then sedimentation from ethanol, to give a cellulose derivative **31e** and **31f** bearing the pentamer blocks of $U^{An}U^{An}A^{Bz}U^{An}G^{DPC,iBu}$ (and -$G^{CE,iBu}$) (69% and 63% yields, respectively). Condensation reactions of **31** with the tetramer block of $A^{Bz}A^{Bz}A^{Bz}A^{Bz}$, followed by the same work-up as above gave **32e** and **32f** bearing the nonamer blocks of $A^{Bz}A^{Bz}A^{Bz}A^{Bz}U^{An}U^{An}A^{Bz}U^{An}G^{DPC,iBu}$ (and -$G^{CE,iBu}$) (80% and 71% yields, respectively). Finally, condensation reactions of the resultant **32** with the tetramer block of $A^{Bz}A^{Bz}G^{DPC,iBu}G^{DPC,iBu}$ (and -$G^{CE,iBu}$-$G^{CE,iBu}$), followed by the same work-up as above, gave the cellulose derivative **33e** and **33f** bearing the fully protected objective tridecamers (85% and 90% yields, respectively) (45% and 34% overall yields, respectively).

The objective tridecamer block, then, was removed from **33** by the sequence of treatments with tetramethylguanidium (*Z*)-pyridine-2-aldoximate (TMG - PAO), with concentrated aqueous ammonia, with hydrochloric acid (*p*H 2.0), and reversed-phase h.p.l.c., giving the tridecamers **35e** (130 A_{260} units; see Fig. 1 and 2 for its h.p.l.c. profiles) and **35f** (240 A_{260} units; see Fig. 3 and 4 for its h.p.l.c. profiles), whose electrophoretic patterns (Fig. 5) demonstrated their satisfactory purity for biological sciences. The electrophoretic experiments also showed the utility of 2-cyanoethyl protection as

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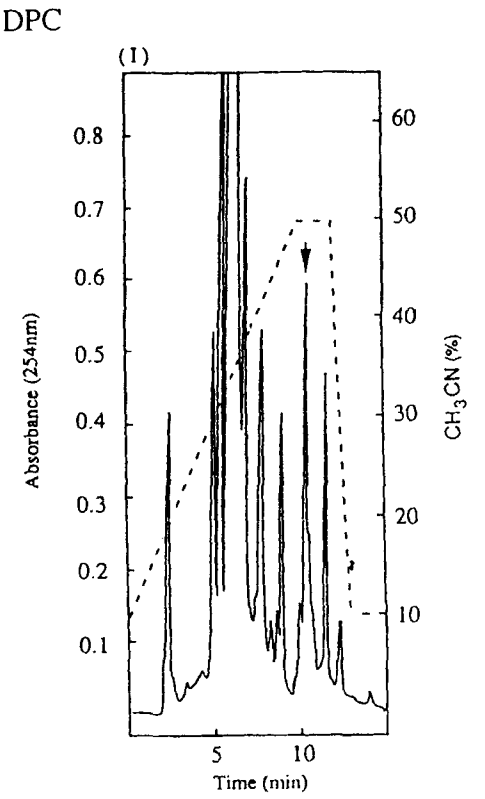

Fig. 1
Reversed-phase h.p.l.c. of the tridecamer bearing 2'-*O*-THP and 5'-terminal *O*-DMTr Groups

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superior to diphenylcarbamoyl protection for the O^6-position of the guanosine unit.

4)*Synthesis of a Dodecamer, UpCpCpGpGprTpΨpCpGpApUpU* (**38**): Based on the above results, synthesis of the title dodecamer was subsequently performed through the procedure described in the flow-sheet shown in Scheme 8. The polymer-support bearing the uridine unit **30c** (B = U^{An}; 6 mol. equiv.) was subjected to the coupling reaction with the pentamer block of $\Psi^{Bz}C^{An}G^{CE,iBu}A^{Bz}U^{An}$ (**22**) (1 mol. equiv.) through the reagent system described above and worked up in a similar manner to give the polymer-support bearing the hexamer block of $\Psi^{Bz}C^{An}G^{CE,iBu}A^{Bz}U^{An}U^{An}$ (**36**), to whose 5'-terminus the hexamer block of $U^{An}C^{An}C^{An}G^{CE,iBu}G^{CE,iBu}rT$ (**28**) was introduced similarly to give the fully protected objective dodecamer born on the polymer-support (**37**).

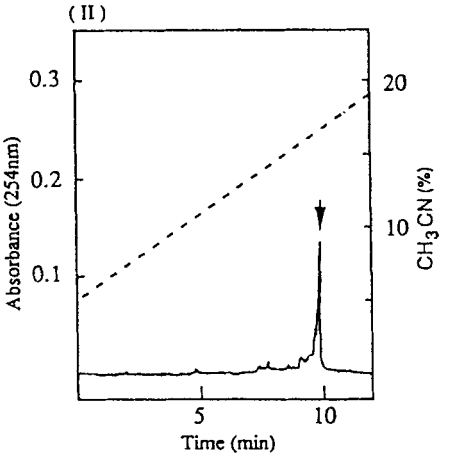

Fig. 2
Reversed-phase h.p.l.c. of the mixture obtained by removal of 2'-*O*-THP and 5'-terminal *O*-DMTr Groups

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The cellulose derivative **37** was then subjected to the processes for the removal and unmasking as described above in 3 to give the title dodecamer (**39**) (116 A_{260} units), whose purification was not complicated so far and would be properly accepted from reversed-phase h.p.l.c. profiles shown in Figures 6 and 7. The structure of **39** was further confirmed by consecutive enzymatic degradation reactions through snake venom phosphodiesterase and alkaline phosphatase; the result is shown in Figure 8, which shows reasonable mutual proportions of each ribonucleoside for the expected oligomer backbone structure.

The results described herein demonstrate the utility of the 3-(carboxy)propionyl spacer for the cellulose polymer-support and 2-cyanoethyl protection for the O^6-position of a guanosine unit for the synthesis of oligoribonucleosides.

EXPERIMENTAL

T.l.c. was conducted on Merck silica gel F_{254} by developing with 9:1 chloroform - methanol (Solvent A), 95:5 chloroform - methanol (Solvent B), and reversed-phase t.l.c. was on Merck silanized gel RP18 $F_{254}S$ with 6:4 acetone - water (Solvent C) or 7:3 acetone - water (Solvent D). Column chromatography was performed on silica gel (Wakogel C-300, purchased from Wako Pure Chemicals, Co. Ltd.),

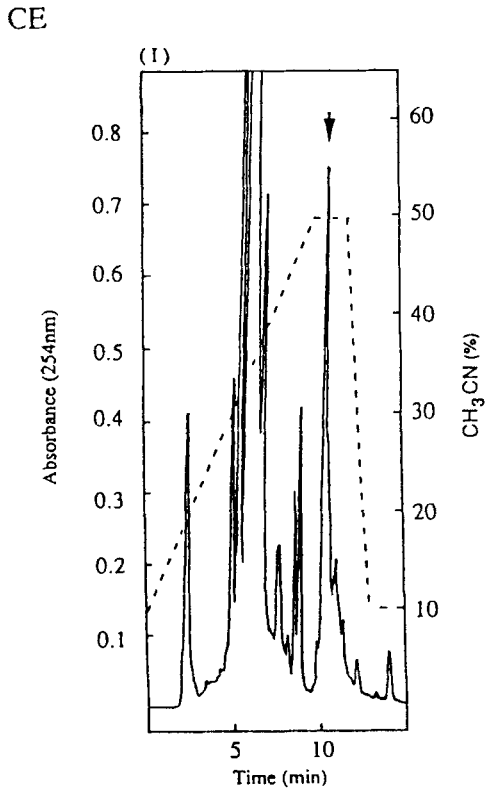

Fig. 3
Reversed-phase h.p.l.c. of the tridecamer bearing 2'-*O*-THP and 5'-terminal *O*-DMTr Groups

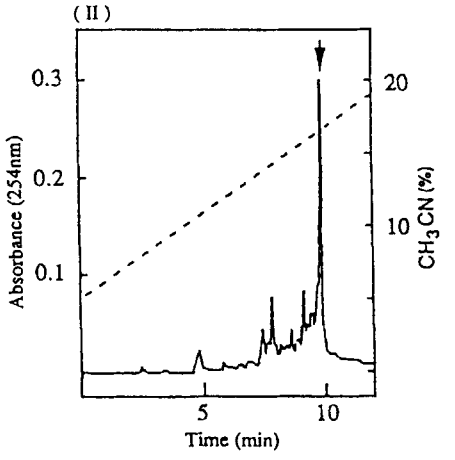

Fig. 4
Reversed-phase h.p.l.c. of the mixture obtained by removal of 2'-*O*-THP and 5'-terminal *O*-DMTr Groups

Conditions: column M&S PACK C18 (4.6 mm ID x 150 mm L); elution buffer CH_3CN - 0.1M TEAA (pH 7); flow rate 1 ml/min; detected by U.V. at 254 nm.

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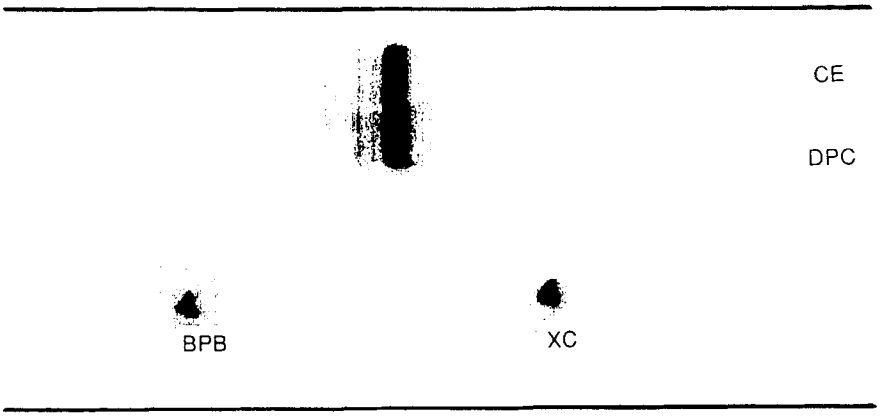

Fig. 5
Electrophoresis of Tridecamer (^{32}pApApGpGpApApApApUpUpApUpG) on 20% Polyacrylamide Gel Containing 7M Urea after 5'-O-Phosphorylation Using (γ-^{32}p)ATP and T4 Polynucleotide Kinase

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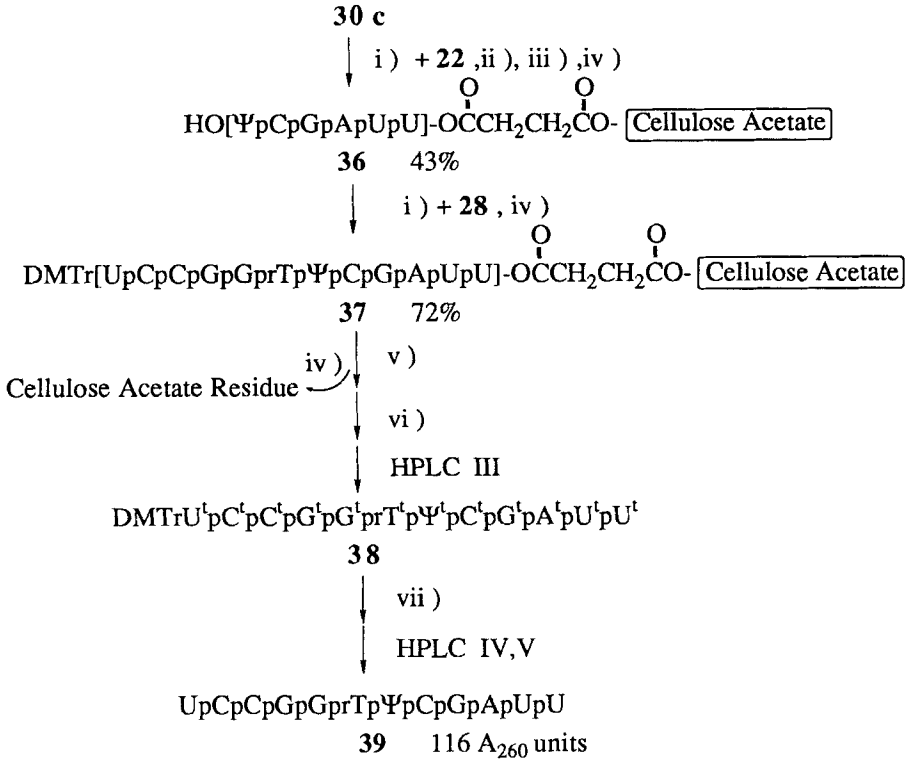
by the use of chloroform - methanol or methylene chloride - methanol, and reversed-phase column chromatography was on silanized silica gel (Kieselgel 60 silanisiert, 70 - 300 mesh, purchased from Merck), by the use of acetone - 0.05 M triethylammonium hydrogencarbonate (bicarbonate) (TEAB). High performance liquid chromatography (h.p.l.c.) was performed on M&S PACK C-18 (4.6 mm ID x 150 mm L) for purification of the tridecamer, or μBONDASHERE 5μ C-18 (3.9 mm ID x 150 mm L) for purification of the dodecamer, and on LiChrosorb RP18-5 (4.6 mm ID x 150 mm L) for analysis of digestion of the dodecamer with snake venom phosphodiesterase and alkalinephosphatase by the use of acetonitrile - 0.1 M triethylammonium acetate (TEAA; *p*H 7) as the eluant. ^{1}H-N.m.r. spectra were recorded on a Bruker AM 400 apparatus with tetramethylsilane (TMS) as the internal standard. Elemental analyses were achieved with a Perkin-Elmer 240-002 apparatus.

N^6-Benzoyl-2'-*O*-(tetrahydropyran-2-yl)adenosine (**1a**)[11] N^4-anisoyl-2'-*O*-(tetrahydropyran-2-yl)cytidine (**1b**),[11] N^3-anisoyl-2'-*O*-(tetrahydropyran-2-yl)uridine (**1c**),[12] N^2-isobutyryl-2'-*O*-(tetrahydropyran-2-yl)guanosine (**1d**),[11] and O^6-diphenylcarbamoyl-N^2-iaovutyryl-2'-*O*-(tetrahydropyran-2-yl)guanosine (**1e**)[12] were prepared according to the methods reported.



Conditions ; i) TPSCl (3 eq.) - 1-Meim (6 eq.), Pyridine, r.t., 2h
ii) Ac_2O - Pyridine, r.t., 5h
iii) 4% $Cl_2CHCOOH$ / CH_2Cl_2 , 0°C, 10min
iv) Precipitated from EtOH
v) 0.5M TMG - PAO , 9 : 1 Pyridine - H_2O , r.t., 1 day
vi) conc.NH_4OH, 55°C, 6h
vii) HCl (pH 2), r.t., 2 days

Scheme 8

O^6-*(2-Cyanoethyl)-5'*-O-*dimethoxytrityl*-N^2-*isobutyryl-2'*-O-*(tetrahydropyran-2-yl)guanosine* (**2f**)(See Scheme 1): The more polar diastereoisomer (with respect to the tetrahydropyran-2-yl function) of **1d** (1.3124 g, 3 mmol) was dissolved in pyidine, and the solution was evaporated in order to remove moisture azeotropically. The residue was dissolved in pyridine (15 mL), to which dimethoxytrityl chloride (DMTrCl)(1.0658 g, 3.15 mmol) was added and stirred for 1.5 h at room temperature. The mixture was quenched by the addition of water (2 mL) and extracted with

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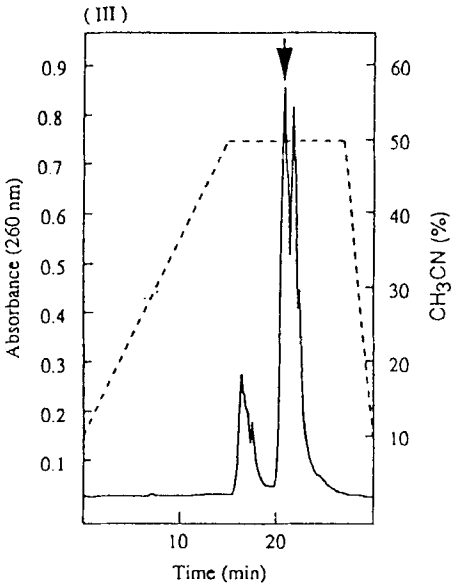

Fig. 6
Reversed-phase h.p.l.c. of the dodecamer bearing 2'-*O*-THP and 5'-terminal *O*-DMTr Groups

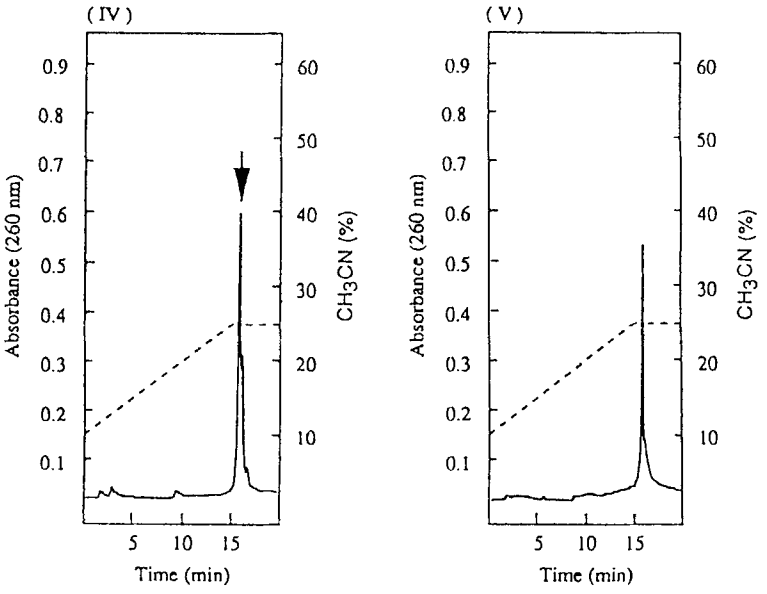

Fig. 7
Reversed-phase h.p.l.c. of the Completely Unmasked (IV) and Isolated Dodecamer (V)

Conditions: column μBONDASPHERE 5μ C18 (3.9 mm ID x 150 mm L);elution buffer CH_3CN - 0.1M TEAA (pH 7); flow rate 1 ml/min; detected by U.V. at 260 nm.

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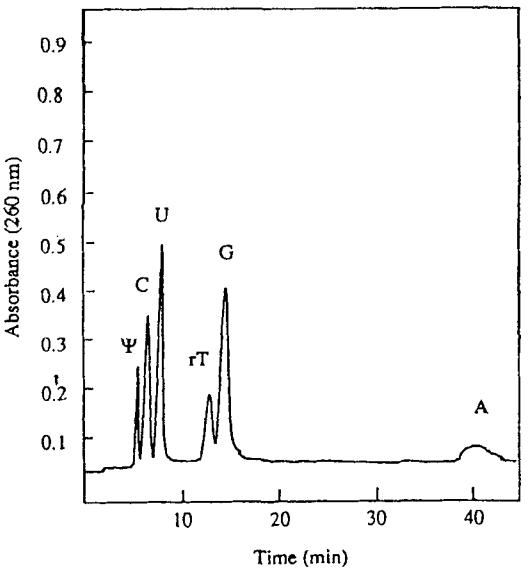

Fig. 8
Reversed-phase h.p.l.c. of Products Obtained by Digestion of the Completely Unmasked Dodecamer with Snake Venom Phosphodiesterase and Alkaline Phosphatase
Conditions: column LiChrosorb RP 18-5 (4.6 mm ID x 150 mm L); elution buffer 3% CH_3CN - 0.1M TEAA (pH 7); flow rate 0.5 ml/min; detected by U.V. at 260 nm.

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methylene chloride (50 mL). The extract was washed with water (50 mL x 2) and evaporated to give a residue, which was then subjected to azeotropic evaporation from pyridine similarly. The residue was dissolved in methylene chloride (15 mL), and to the resulting solution were added levulinic acid (0.92 mL, 9 mmol), *N,N'*-diisopropyl carbodiimide (DICI)(1.41 mL, 9 mmol), and 1-Meim (0.72 mL, 9 mmol) with stirring at room temperature. After 20 min, *N,N'*-diisopropyl urea precipitated out was filtered off, and washed with methylene chloride (50 mL). The filtrate and washings were combined and evaporated, after washing with water (50 mL x 2); the residue was then subjected to azeotropic evaporation from pyridine as above. The residue was dissolved in methylene chloride (15 mL), and the solution was treated with TPS chloride (1.8172 g, 6 mmol) in combination with triethylamine (1.25 mL, 9 mmol) and 4-(*N,N'*-dimethylamino)pyridine (DMAP) (0.0733 g, 0.6 mmol) at room temperature for 2 h with stirring. The resulting mixture was quenched with water (2 mL), and was extracted with methylene chloride (50 mL). The organic layer was then washed with water

(50 mL x 2) and evaporated. The residue was, after azeotropic evaporation from pyridine, dissolved in methylene chloride (7.5 mL). The solution was treated with 2-cyanoethanol (2.05 mL, 30 mmol) and trimethylamine hydrochloride (0.4301 g, 4.5 mmol), and, after cooling with ice, with DBU (0.67 mL, 4.5 mmol); formation of the trimethylammonio salt was confirmed by the change in t.l.c. in terms of the original spot corresponding to O^6-TPS (**4**) (Rf 0.66) to a new spot of the salt (Rf 0.09) by the use of Solvent B; after which was added DBU (0.34 mL, 2.25 mmol) to induce the 2-cyanoethylation; the spot with Rf 0.09 moved up to new one with Rf 0.27 (Solvent B). If an excess amount of DBU was used, β-elimination of the 2-cyanoethyl group was induced to bring about change of the spot with Rf 0.27 moving down to a new one with Rf 0.21 (Solvent B). For facilitating the purification of **2f**, the resulting mixture was treated with acetic anhydride (2.8 mL, 30 mmol) and pyridine (6 mL) which derived 2-cyanoethanol into its acetate. After stirring for 30 min, the solution was further treated with hydrazine hydrate (0.29 mL, 6 mmol) at room temperature for 10 min with stirring to remove the levulinyl protecting group, to which was added acetone (15 mL) in order to quench the reaction. After stirring for 20 min, the mixture was evaporated and the residue was dissolved in methylene chloride (50 mL). The extract was washed with water (50 mL x 2) and dried over anhydrous magnesium sulfate. The desiccant was filtered off, and the filtrate was subjected to chromatography on a column of silica gel to give **2f** (1.571 g, 1.98 mmol, 66% yield); ^{1}H-n.m.r. ($CDCl_3$ - TMS): δ 0.99 and 1.08 [6H, d x 2, J = 6.82 Hz, $CH(C\underline{H}_3)_2$], 1.30 - 1.78 (6H, m, $CC\underline{H}_2C$ x 3), 2.24 - 2.36 (1H, m, $C\underline{H}CMe_2$), 3.05 (2H, t, J = 6.54 Hz, $OC\underline{H}_2CH_2CN$), 3.17 - 3.50 (4H, m, H-5', 5", and $OC\underline{H}_2C$), 3.77 (3H, s, $OC\underline{H}_3$), 4.22 - 4.25 (1H, m, *H*-4'), 4.57 (1H, dd, $J_{2',3'}$ = 6.02 Hz, $J_{3',4'}$ = 4.05 Hz, *H*-3'), 4.76 - 4.78 (1H, m, $OC\underline{H}O$), 4.81 (2H, t, $OCH_2C\underline{H}_2CN$), 5.21 (1H, t, *H*-2'), 6.12 (1H, d, $J_{1',2'}$ = 6.02 Hz, *H*-1'), 6.78 (4H, d, J = 8.94 Hz, Ph-H), 7.17 - 7.48 (9H, m, Ph-H), 7.55 (1H, s, *H*-8), and 7.98 (1H, s, N^2-H).

Anal. Calcd for $C_{43}H_{48}N_6O_9$ H_2O: C, 63.69; H, 6.22; N, 10.36. Found: C, 63.81; H, 6.04; N, 10.48.

N^1-*Benzoyl-2'-O-(tetrahydropyran-2-yl)pseudouridine* (**1g**, R = Bz)(cf. Scheme 2): Compound Ψ (0.4644 g, 1.90 mmol) was, after drying through azeotropic evaporation from pyridine, treated with 1,1,3,3-tetraisopropyl-1,3-disiloxan-1,3-diyl dichloride (0.63 mL, 2.0 mmol) in pyridine (10 mL) at room temperature for 1 h with stirring. The reaction mixture was quenched by the successive addition of methylene chloride (4 mL) and then 1:1 pyridine - water (2.4 mL). The resulting mixture was extracted with methylene chloride (100 mL) and washed with water (50 mL x 2). The organic layer was,

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after drying over anhydrous magnesium sulfate, evaporated and chromatographed on a column of silica gel to give 3',5'-*O*-(1,-1,3,3-tetraisopropyl-1,3-disiloxan-1,3-diyl)pseudouridine (**6**) (0.7768 g, 1.63 mmol, 86% yield); ^{1}H-n.m.r. ($CDCl_3$ - TMS): δ 0.80 - 1.30 (28H, m, isopropyl protons x 4), 3.90 - 4.40 (6H, m, *H*-2', 3', 4', 5', 5", and 2'-O<u>H</u>), 4.76 - 4.8 (1H, m, *H*-1'), 7.58 (1H, d, $J_{6,N^1\text{-}H}$ = 5.71 Hz, *H*-6), 10.20 (1H, br.s., N^3-<u>H</u>), and 10.35 (1H, d, N^1-<u>H</u>). Compound **6** thus obtained was next dissolved in 1,4-dioxane (4 mL) and treated with dihydropyran (1.37 mL, 12.27 mmol) in the presence of *p*-toluenesulfonic acid monohydrate (0.0380 g, 0.16 mmol) at room temperature for 1 h with stirring. After quenching the reaction with aqueous saturated solution of sodium hydrogen carbonate, the mixture was extracted with methylene chloride (100 mL), and the solution was washed with water (50 mL x 2). The organic layer was, after drying over anhydrous magnesium sulfate, evaporated and chromatographed on a column of silica gel to give the 2'-*O*-(tetrahydropyran-2-yl) derivatives (**7**) (0.5992 g, 1.05 mmol, 64% yield). Both of the diasteromeric isomers obtained were, after azeotropic evaporation from pyridine, dissolved in pyridine (5 mL) and treated with benzoyl chloride (0.35 mL, 3.15 mmol) and triethylamine (0.70 mL, 3.15 mmol) at room temperature for 1 h with stirring. The mixture was, after quenching with water (1 mL), extracted with methylene chloride (100 mL) and the organic layer was washed with water (50 mL x 2). The layer was, after drying over anhydrous magnesium sulfate, subjected to the chromatographic purification on a column of silica gel to give the corresponding N^1-benzoyl derivatives (**8**, R = Bz) (0.6854 g, 1.02 mmol, 97% yield). The mixture of **8** thus obtained was dissolved in acetonitrile (5 mL) and then treated with 1 M tetrahydrofuran solution of tetrabutylammonium fluoride (3 mL) at room temperature for 2 h with stirring. The residue obtained by evaporation of the resulting mixture was dissolved in methylene chloride (100 mL), and the organic solution was washed with water (50 mL x 2). The solution was dried over anhydrous magnesium sulfate and evaporated. The residue was then subjected to chromatographic purification on a column of silica gel to give **1g** (R = Bz; less polar diastereoisomer = 0.2767 g, 0.27 mmol and more polar one = 0.4779 g, 0.47 mmol, totally 0.7546 g, 0.74 mmol, 39% overall yield based on Ψ); ^{1}H-m.n.r.($CDCl_3$ - TMS): δ for the less polar diastereoisomer 1.53 - 1.84 (6H, m, CC<u>H</u>$_2$C x 3), 3.45 - 3.85 (4H, m, *H*-5', 5", and OC<u>H</u>$_2$C), 4.02 - 4.08 (1H, m, *H*-4'), 4.15 - 4.19 (1H, m, *H*-3'), 4.35, (1H, dd, $J_{1',2'}$ = 6.30 Hz, $J_{2',3'}$ = 5.14 Hz, *H*-2'), 4.59,(1H, d, *H*-1'), 4.71 - 4.76 (1H, m, OC<u>H</u>O), and 7.50 - 7.95 (6H, m, Ph-<u>H</u> and *H*-6); δ for more polar one 1.52 - 1.84 (6H, m, CC<u>H</u>$_2$C x 3),

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3.46 - 3.87 (4H, m, H-5', 5", and OC$\underline{H}_2$-C), 4.00 - 4.02 (1H, m, *H*-4'), 4.24 (1H, t, *H*-3'), 4.49 (1H, dd, $J_{1',2'}$ = 6.35 Hz, $J_{2',3'}$ = 5.10 Hz, *H*-2'), 4.72 (1H, d, *H*-1'), 4.73 - 4.76 [1H, m, OC$\underline{H}$(O)C], and 7.50 - 7.98 (6H, m, Ph-$\underline{H}$ and *H*-6).

N^1-*Anisoyl-2'*-O-*(tetrahydropyran-2-yl)pseudouridine* (**1h**; R = An): Compound **7** (0.0540 g, 0.095 mmol) was, after azeotropic evaporation from pyridine, dissolved in pyridine (0.5 mL), and anisoyl chloride (0.0661 g, 0.39 mmol) and triethylamine (0.54 mL, 0.39 mmol) were added to the solution, which was then stirred for 1 h at room temperature. The reaction mixture was quenched with water (0.1 mL) by stirring for 30 min, which induced the removal of N^3-anisoyl group, and was subjected to extraction with methylene chloride (50 mL) and then to washing with water (30 mL x 2). The organic layer was, after drying over anhydrous magnesium sulfate, evaporated and the residue was subjected to chromatographic separation on a column of silica gel to give **8h** (R = An) in a quantitative yield. Compound **8h** was subsequently treated with 1 M tetrahydrofuran solution of tetrabutylammonium fluoride (0.3 mL) in a solution of acetonitrile (1 mL) at room temperature for 2 h with stirring. The resulting mixture was evaporated, and the residue was subjected to chromatographic separation on a column of silica gel to give **1h** (R = An; less polar diastereisomer = 0.0183 g, 0.040 mmol, 42% yield and more polar one = 0.0084 g, 0.018 mmol, 19% yield, totally 0.0267 g, 0.058 mmol, 61% yield); ^{1}H-n.m.r. ($CDCl_3$ - TMS): δ for the less polar diastereoisomer 1.28 - 1.86 (6H, m, CC$\underline{H}_2$C x 3), 3.53 - 4.10 (4H, m, H-5', 5", and OC$\underline{H}_2$C), 3.91 (3H, s, OC$\underline{H}_3$), 4.15 - 4.25 (1H, m, *H*-3'), 4.30 - 4.40 (1H, m, *H*-2'), 4.65 (1H, d, $J_{1',2'}$ = 6.10 Hz, *H*-1'), 4.67 - 4.77 [1H, m, OC$\underline{H}$(O)C], 6.99 (2H, d, J = 8.73 Hz, Ph-$\underline{H}$), 7.62 (1H, s, *H*-6), and 7.92 (2H, d, Ph-$\underline{H}$); δ for the more polar one 1.54 - 1.84 (6H, m, CC$\underline{H}_2$C x 3), 3.48 - 3.85 (4H, m, H-5', 5", and OC$\underline{H}_2$C), 3.90 (3H, s, OC$\underline{H}_3$), 4.01 - 4.03 (1H, m, *H*-4'), 4.24 (1H, t, *H*-3'), 4.48 (1H, dd, $J_{1',2'}$ = 6.17 Hz, $J_{2',3'}$ = 4.42 Hz, *H*-2'), 4.73 (1H, d, *H*-1'), 4.75 - 4.77 [1H, m, OC$\underline{H}$(O)C], 6.97 (2H, d, J = 8.73 Hz, Ph-$\underline{H}$), 7.53 (1H, s, *H*-6), and 7.90 (2H, d, Ph-$\underline{H}$).

Examination of the stability of the protecting groups for the heterocyclic moiety under basic conditions (See Table 1): 1) *4:1 28%-Ammonical water - methanol.* Each 1 mg of **1c**, **1g**, and **1h** was dissolved in the title mixture (1 mL), and monitored by t.l.c. 2) *1:3 Triethylamine - pyridine.* Each 1 mg of **1c**, **1g**, and**1h** was dissolved in the title mixture (1 mL), and allowed to stand at room temperature for 3 h. The resulting mixture was evaporated to dryness, and the residue was then dissolved in methylene chloride. The solution was subjected to monitoring by t.l.c. 3) *1:2 Water - pyridine.* Each of **1c**, **1g**, and **1h** was dissolved in the title mixture (1

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mL), and the solution was allowed to stand at room temperature for 6 h. The mixture was evaporated to dryness, and the residue was subjected to the t.l.c. examination similarly.

Examination of the stability of the protecting groups for the heterocylic moiety under acidic conditions (See Table 2): 1) *2% p-Toluenesulfonic acid solution in 7:3 chloroform - methanol.* Each 1 mg of **1c**, **1g**, and **1h** was dissolved in the title mixture, and monitored by t.l.c. 2) *2% Dichloroacetic acid solution in methylene chloride.* Each 1 mg of **1c**, **1g**, and **1h** was dissolved in the title mixture, and monitored by t.l.c.

Examination of the stability of Ψ under the acidic conditions for the removal of tetrahydropyran-2-yl protecting group at its 2'-position. Two mg of Ψ was dissolved in hydrochloric acid (*p*H 2.0), and monitored by h.p.l.c. No change was observed at all in its chromatographic behavior.

Examination of a Side Reaction onto N^3*-position of Ψ by the Use of* N^1*-Anisoyl-2',3',5'-tri-*O*-acetylpseudouridine* (**10**): *Synthesis of* **10**. Ψ (1.221 g, 5 mmol) was, after azeotropic evaporation from pyridine, dissolved in pyridine (25 mL), and acetic anhydride (4.7 mL, 50 mmol) was added to the solution at room temperature. The resulting mixture was, after 3 h, evaporated to dryness to give the tri-*O*-acetate quantitatively; ^{1}H-n.m.r. ($CDCl_3$ - CD_3OD - TMS): δ 2.07, 2.10, and 2.12 (9H, 3s, $C\underline{H}_3CO$ x 3), 4.23 - 4.44 (3H, m, H-4', 5', and 5"), 4.88 (1H, d, $J_{1',2'}$ = 4.6 Hz, *H*-1'), 5.30 (1H, t, *H*-3'), 5.42 (1H, dd, $J_{2',3'}$ = 5.80 Hz, *H*-2'), and 7.38 (1H, s, *H*-6). The tri-*O*-acetate (0.2222 g, 0.6 mmol) was, after azeotropic evaporation from pyridine, dissolved in pyridine (3 mL), and the solution was treated with anisoyl chloride (0.3067 g 1.8 mmol) in the presence of triethylamine (0.25 mL, 1.8 mmol) at room temperature. The resulting mixture was quenched with water (0.5 mL) with stirring, and extracted with chloroform (100 mL). The organic layer was, after drying over anhydrous magnesium sulfate, evaporated to dryness, and the residue was subjected to the chromatographic separation on a column of silica gel to give **10** (0.2972 g, 0.589 mmol, 95% yield); ^{1}H-n.m.r. ($CDCl_3$ - TMS): δ 1.98, 2.01, and 2.06 (9H, 3s, $C\underline{H}_3CO$ x 3), 3.88 (3H, s, $OC\underline{H}_3$), 4.17 - 4.40 (3H, m, H-4', 5', and 5"), 4.82 (1H, d, $J_{1',2'}$ = 4.6 Hz, *H*-1'), 5.25 (1H, t, $J_{2',3'} = J_{3',4'}$ = 5.80 Hz, *H*-3'), 5.32 - 5.36 (1H, m, *H*-2'), 6.97 (2H, d, J = 8.70 Hz, Ph-$\underline{H}$), 7.40 - 7.45 (1H, m, *H*-6), 7.89 (2H, d, Ph-$\underline{H}$), and 10.27 - 10.32 (1H, m, N^3-$\underline{H}$).

Examination of a side reaction for **10** *under the conditions for oligonucleotides synthesis*: Compound **10** (0.1010 g, 0.2 mmol) was, after azeotropic evaporation from pyridine, dissolved in pyridine (2 mL), and diphenylphosphate (0.05 g, 0.2 mmol) and TPS chloride (0.3029

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g, 1 mmol) were added to the solution. After stirring at room temperature for 2.5 h, the reaction mixture was quenched with water (0.5 mL), and extracted with methylene chloride (50 mL). The extract was washed with water (50 mL x 2) and dried over anhydrous magnesium sulfate. The desiccant was filtered off, and the filtrate was evaporated to dryness. The residue was chromatographed on a column of silica gel to give the corresponding TPS derivative **11** (0.1003 g, 65% yield) in addition to the recovery of **10** (0.0313 g, 31 % yield); ^{1}H-n.m.r. data ($CDCl_3$ - TMS) for **11**: δ 1.22 - 1.28 (18H, m, CH3 x 6), 2.06, 2.11, and 2.17 (9H, 3s, $C\underline{H}_3CO$ x 3), 2.87 - 2,96 (1H, m, $C\underline{H}Me_2$), 3.83 - 3.92 (2H, m, $C\underline{H}Me_2$ x 2), 3.85 (3H, s, $OC\underline{H}_3$), 4.25 4.48 (3H, m, *H*-4', 5', and 5"), 4.98 (1H, d, $J_{1',2'}$ = 4.5 Hz, *H*-1'), 5.35 (1H, t, $J_{2',3'} = J_{3',4'}$ = 5.4 Hz, *H*-3'), 5.39 (1H, dd, *H*-2'), 6.91 (2H, d, J = 8.70 Hz, Ph-H), 7.20 (2H, s, Ph-H), 7.80 (2H, d, Ph-H), and 8.37 (1H, s, *H*-6). Compound **11** was then treated with 4:1 concentrated aqueous ammonia - methanol at room temperature for 10 h in a flask with a tight stopper to give completely unmasked Ψ in a quantitative yield.

1-[2-O-(Tetrahydropyran-2-yl)-β-D-ribofuranosyl]thymine (**1i**): 1-β-D-Ribofuranosylthymine (0.3873 g, 1.5 mmol) was, after azeotropic evaporation from pyridine, dissolved in pyridine (7 mL), and 1,1,3,3-tetraisopropyl-1,3-disiloxan-1,3-diyl dichloride (0.50 mL, 1.58 mmol) were added to the solution. The mixture was, after stirring at room temperature for 1 h, mixed with methylene chloride (3 mL), and 1:1 pyridine - water (2.7 mL) was added to the solution, which was extracted with methylene chloride (50 mL), and the extract was washed with water (30 mL x 2). The organic layer was after drying over anhydrous magnesium sulfate, evaporated to dryness, and the residue was chromatographed on a column of silica gel to give the corresponding 3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxan-1,3-diyl) derivative (0.584 g, 1.17 mmol, 78% yield). This derivative was dissolved in dioxane (3 mL) and, after the addition of 2,3-dihydropyran (0.80 mL, 11.25 mmol) and *p*-toluenesulfonic acid monohydrate (0.0555 g, 0.375 mmol), stirred at room temperature for 1 h. The reaction mixture was quenched with saturated aqueous solution of sodium hydrogen carbonate (1 mL) and extracted with methylene chloride (50 mL). The extract was washed with water (30 mL x 2). The organic layer was, after drying over anhydrous magnesium sulfate, evaporated to dryness, and the residue was dissolved in acetonitrile (7.5 mL). The solution was treated with 1 M tetrahydrofuran solution of tetrabutylammonium fluoride (4.5 mL) and stirred for 1.5 h at room temperature. The mixture was evaporated to dryness, and the residue was dissolved in methylene

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chloride (50 mL). The organic layer was, after drying over anhydrous magnesium sulfate, evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel to give **1i** (less polar diastereoisomer = 0.173 g and more polar one = 0.185 g, totally 0.358 g, 1.05 mmol, 90% yield); ^{1}H-n.m.r. (CDCl3 - TMS): δ for less polar diastereoisomer 1.50 - 1.88 (6H, m, CC$\underline{H}_2$C x 3), 1.93 (3H, s, C$\underline{H}_3$), 3.37 - 3.57 (2H, m, OC$\underline{H}_2$C), 3.76 - 4.00 (2H, m, *H*-5' and 5"), 4.12 - 4.15 (1H, m, *H*-4'), 4.30 - 4.35 (1H, m, *H*-3'), 4.56 (1H, t, $J_{1',2'} = J_{2',3'} = 5.12$ Hz, *H*-2'), 4.60 - 4.62 [1H, m, OC$\underline{H}$(O)C], 5.61 (1H, d, *H*-1'), 7.36 (1H, s, *H*-6), and 8.75 (1H, br. s, N^1-$\underline{H}$); δ for more polar one 1.50 - 1.85 (6H, m, CC$\underline{H}_2$C x 3), 1.91 (3H, s, C$\underline{H}_3$), 3.10 - 3.50 (2H, m, OC$\underline{H}_2$C), 3.68 -3.81 (2H, m, *H*-5' and 5"), 3.90 - 3.96 (1H, m, *H*-4'), 4.07 - 4.09 (1H, m, *H*-3'), 4.39 - 4.43 (1H, m, *H*-2'), 4.67 - 4.70 [1H, m, OC$\underline{H}$(O)C], 5.64 (1H, d, $J_{1',2'} = 5.58$ Hz, *H*-1'), 7.26 (1H, s, *H*-6), and 8.67 (1H, br. s, N^1-$\underline{H}$).

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Oligoribonucleotides Synthesis

Syntheses of Oligomer-Blocks

Triethylammonium 5'-O-dimethoxytrityl-2'-O-(tetrahydropyran-2-yl)ribonucleosides 3'-(2-chlorophenyl)phosphates (**12**)(See Scheme 5): In a usual manner, **1** was derived into **2** by dimethoxytritylation at 5'-position,[20] and then subjected to 3'-phosphorylation, i.e., by the drop-by-drop addition of a methylene chloride solution of **2** into a pyridine solution of (2-chlorophenyl)phosphorodichloridate (1.5 mol. equiv.), followed by treatment with 1 M TEAB solution[21] to give **12**. Compound **12** was easily purified by distributing between 4:1 water - pyridine/diethyl ether; it was efficiently extracted into the layer of water - pyridine.

2'-O-(Tetrahydropyran-2-yl)ribonucleosides 3'-(2-chlorophenyl-2-cyanoethyl)phosphates (**14**)(See Scheme 5): Compound **12** was subjected to 2-cyanoethylation[16] by treatment with 2-cyanoethanol (2 mol. equiv.) in the presence of QS chloride (2.5 mol. equiv.) and 1-Meim (5 mol. equiv.) as usual[22] to give **13**, which was easily purified by distributing between 4:1 water - pyridine/diethyl ether; it was efficiently extracted into the layer of diethyl ether. The removal of the dimethoxytrityl group at the 5'-position by treating **13** as usual,[17,18] gave **14**.

Synthesis of a Tetramer Block ($U^{An}U^{An}A^{Bz}U^{An}$) Bearing the Phosphodiester Function on its 3'-Terminus (**19**): *Dimer-blocks of* ***15*** *($B_1 = B_2 = U^{An}$) and* ***15*** *($B_1 = A^{Bz}$, $B_2 = U^{An}$).* Compounds **12** (B = U^{An}) (2.5357 g, 2.4 mmol) and **14** (B = U^{An}) (1.4121 g, 2.0 mmol) were, after azeotropic evaporation from pyridine, dissolved in pyridine (10 mL), and QS chloride (1.3660 g, 6.0 mmol) and 1-Meim

(0.96 mL, 12 mmol) were added to the solution, which was then stirred at room temperature for 2 h. The intramolecular salt of QS precipitated and was filtered off, and the filtrate was treated with water (1 mL). The resulting mixture was distributed between 4:1 water -pyridine/diethyl ether, whose organic layer was, after drying over anhydrous magnesium sulfate, evaporated to give glassy **15** ($B_1 = B_2 = U^{An}$) (3.4233 g, quantitative yield). Separately, **12** ($B = A^{Bz}$) (1.2580 g, 1.2 mmol) and **14** ($B = U^{An}$) (0,7061 g, 1.0 mmol) were treated in pyridine (5 mL) with QS chloride (0.6830 g, 3.0 mmol) and 1-Meim (0.48 mL, 6 mmol) in the same way as above and the resulting mixture was worked up in the same way as above to give **15** ($B_1 = A^{Bz}$, $B_2 = U^{An}$) (1.4966 g, 0.915 mmol, 92% yield).

Synthesis of the Tetramer **19** *from* **15***s*: Compound **15** ($B_1 = B_2 = U^{An}$) (3.2868 g, 1 mmol) was subjected to removal of 2-cyanoethyl protecting group by treatment with 1:3 triethylamine - pyridine (20 mL) at room temperature for 2 h with stirring. The resulting mixture was evaporated to give the corresponding 3'-phosphodiester derivative (**16**, $B_1 = B_2 = U^{An}$) quantitatively. Separately, **15** ($B_1 = A^{Bz}$, $B_2 = U^{An}$) (1.6364 g, 1 mmol) was dissolved in 7:3 chloroform - methanol (10 mL), and, under cooling in an ice-bath, was added a solution of *p*-toluenesulfonic acid monohydrate (0.502 g) in 7:3 chloroform - methanol (10 mL) to the solution. After stirring for 15 min, the reaction mixture was treated with saturated aqueous solution of sodium hydrogen carbonate (30 mL), and was extracted with chloroform (50 mL). The extract was washed with water (20 mL x 2), and dried over anhydrous magnesium sulfate. The organic layer was, after filtering off the desiccant, evaporated to give the derivative with a free hydroxy group at the 5'-terminus **17** ($B_1 = A^{Bz}$, $B_2 = U^{An}$), which was used for the subsequent coupling reaction with **16** ($B_1 = B_2 = U^{An}$) without purification. Compounds **16** (2.0615 g, 1.22 mmol) and **17** were, after azeotropic evaporation from pyridine, treated with QS chloride (0.6659 g, 2.93 mmol) and 1-Meim (0.47 mL, 5.85 mmol) in pyridine (5 mL) at room temperature for 2 h with stirring.

The intramolecular salt of QS precipitated out was filtered off, the filtrate was treated with water (0.5 mL), and was, after the addition of 4:1 water - pyridine (25 mL), extracted with diethyl ether (30 mL x 3). The organic layer was, after drying over anhydrous magnesium sulfate, evaporated to give a mixture of DMTrityl alcohol and fully protected tetramer (**18**). The mixture was dissolved in 1:3 triethylamine - pyridine (7 mL) and the solution was stirred at room temperature for 2 h. The solution was evaporated to dryness and the residue was subjected to reversed-phase column chromatography to give **19** ($U^{An}U^{An}A^{Bz}U^{An}$) (1.3333 g, 0.45 mmol, 63% yield). Similarly, **19** ($A^{Bz}A^{Bz}A^{BZ}A^{Bz}$), **19**($A^{Bz}A^{Bz}G^{DPC,iBu}G^{DPC,iBu}$),**19** ($A^{Bz}A^{Bz}$-

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$G^{CE,iBu}G^{CE,iBu}$), **22** ($\Psi^{Bz}C^{An}G^{CE,iBu}A^{Bz}U^{An}$), and **28** ($U^{An}C^{An}C^{An}G^{CE,iBu}$-$G^{CE,iBu}rT$) were synthesized.

Acetylcellulose Derivative Bearing O^6*-Diphenylcarbamoyl-*N^2*-isobutyryl-2'-*O*-(tetrahydropyran-2-yl)Guanosine Unit with a Free Hydroxyl Group at the 5'-Position* (**30e**; B = $G^{DPC,iBu}$)[1,23](See Scheme 6): 3'-*O*-(3-Carboxy)propionyl-5'-*O*-dimethoxytrityl-O^6-diphenylcarbamoyl-2'-*O*-(tetrahydropyran-2-yl)guanosine **29e** (B = $G^{DPC,iBu}$) (0.3105 g, 0.3 mmol) and acetylcellulose (D.S. = 1.77) (0.2307 g) were, after azeotropic evaporation from pyridine, dissolved in pyridine (5 mL), and TPS chloride (0,3634 g, 1.2 mmol) and 1-Meim (0.19 mL. 2.4 mmol) were added to the solution, which was then stirred at room temperature for 6 h. The resulting solution was diluted with methylene chloride to a volume of 2 - 3 times, and poured slowly into ethanol (500 mL) under vigirous stirring. The sedimentation was induced to give a white powder of polymer-support loading the guanosine unit (0.5107 g, 0.3 mmol, quantitative yield). Dissolution of a small portion of the powder in 3:2 60% perchloric acid - ethanol solution, followed by u.v. determination in terms of λ_{max} 498 nm (ε 72,000) for dimethoxytrityl cation[26] gave the loaded amount of the guanosine unit (0.587 mmol/g). The powder was then dissolved in toluene (10 mL) and pyridine potentially included in it was azeotropically evaporated. The residue was dissolved in 7:3 chloroform - methanol (10 mL), and a solution of *p*-toluenesulfonic acid monohydrate (0.3954 g) in 7:3 chloroform - methanol (5 mL) was added to the solution under cooling in an ice-bath. After stirring for 15 min, the mixture was neutralized by the addition of pyridine (2 mL), and the resulting solution was added slowly into ethanol (500 mL) under vigorous stirring to give **30e** (B = $G^{DPC,iBu}$) (0.3993 g, 0.289 mmol, 96% yield).

Acetylcellulose Derivative Bearing O^6*-(2-Cyanoethyl)-*N^2*-isobutyryl-2'-*O*-(tetrahydropyran-2-yl)guanosine with a Free Hydroxyl Group at the 5'-Position* (**30f**; B = $G^{CE,iBu}$): Compound **29f** (B = $G^{CE,iBu}$)[1,23] (0.2679 g, 0.3 mmol) and acetylcellulose (D.S. = 1.77) (0.2326 g) were treated in the same way as above to give the title **30f** (0.3873 g, 0.252 mmol, 84% yield; loaded amount of the guanosine unit = 0.702 mmol/g).

Acetylcellulose Derivative Bearing N^3*-Anisoyl-2'-*O*-(tetrahydropyran-2-yl)uridine with a Free Hydroxy Group at the 5'-Position* (**30c**; B = U^{An}) (See Scheme 6): Compound **29c** (B = U^{An})[1,23] (0.2594 g, 0.3 mmol) and acetylcellulose (D.S. = 1.77) (0.2326 g) were treated in the same way as above to give the title **30c** (B = U^{An}) (0.3203 g, 0.198 mmol, 66% yield; loaded amount of the uridine unit = 0.617 mmol/g).

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Synthesis of Tridecamer (**33**) *Starting from* **30e** (B = $G^{DPC,iBu}$) (See Scheme 7): The tetramer **19** ($U^{An}U^{An}A^{Bz}U^{An}$) (0.1367 g, 0.046 mmol) and **30e** (B = $G^{DPC,iBu}$) (0.3990 g, 0.289 mmol) were, after azeotropic evaporation from pyridine, dissolved in pyridine (7 mL), and TPS chloride (0.0841 g, 0.278 mmol) and 1-Meim (0.044 mL, 0.556 mmol) ; the solution was stirred for 2 h at room temperature. The resulting solution was treated with acetic anhydride (4.5 mL) and stirred at room temperature for 6 h. The reaction mixture was evaporated to dryness and the residue was, after evaporation from toluene, dissolved in 7:3 chloroform - methanol (10 mL). A solution of *p*-toluenesulfonic acid (0.3954 g) in 7:3 chloroform - methanol (5 mL) was added under cooling in an ice-bath to the solution, which was stirred for 15 min. The solution was, after neutralization by the addition of pyridine (2 mL) and dilution with methylene chloride (15 mL), poured into ethanol (500 mL) under vigorous stirring to precipitate the polymer-support bearing the resulting pentamer (**31**) (0.4390 g, 0.031 mmol, 69% yield). The tetramer **19** ($A^{Bz}A^{Bz}A^{Bz}A^{Bz}$) (0.2848 g, 0.097 mmol) was similarly introduced onto **31** ($U^{An}U^{An}A^{Bz}U^{An}G^{DPC,iBu}$-cellulose acetate) (0.4390 g) by the sequence of procedures, i.e., the coupling reaction using TPS chloride (0.0882 g, 0.291 mmol) - 1-Meim (0.046 mL, 0.582 mmol) in pyridine (5 mL) for the introduction reaction, after azeotropic evaporation from pyridine, dilution with methylene chloride (15 mL), sedimentation from ethanol (500 mL), evaporation from toluene, removal of DMTr protection with *p*-toluenesulfonic acid (0.3954 g), and sedimentation from ethanol (500 mL), to give the polymer-support bearing the resulting nonamer (**32**) (0.4638 g, 0.0245 mmol, 80% yield). Finally, the introduction of the tetramer **19** ($A^{Bz}A^{Bz}G^{DPC,iBu}G^{DPC,iBu}$) (0.2377 g, 0.072 mmol) onto **32** (0.4639 g) was performed similarly by the sequence of procedures, i.e., the coupling reaction using TPS chloride (0.0658 g, 0.217 mmol) - 1-Meim (0.035 mL, 0.434 mmol) in pyridine (5 mL), dilution with methylene chloride (15 mL), and sedimentation from ethanol (500 mL), to give the polymer-support bearing the fully protected tridecamer (**33**) ($A^{Bz}A^{Bz}G^{DPC,iBu}G^{DPC,iBu}$-$A^{Bz}A^{Bz}A^{Bz}A^{Bz}U^{An}U^{An}A^{Bz}U^{An}G^{DPC,iBu}$-cellulose acetate) (0.4882 g, 0.0207 mmol, 85% yield).

Synthesis of Tridecamer (**33**) *Starting from* **30f** (B = $G^{CE,iBu}$) (See Scheme 7): The tetramer **19** ($U^{An}U^{An}A^{Bz}U^{An}$) (0.1290 g, 0.0437 mmol) was similarly introduced onto **30f** (B = $G^{CE,iBu}$) (0.3794 g, 0.266 mmol), after azeotropic evaporation from pyridine, by the sequence of procedures, i.e., the coupling reaction using TPS chloride (0.0794 g, 0.262 mmol) - 1-Meim (0.042 mL, 0.524 mmol) in pyridine (5 mL), acetylation with acetic anhydride (4.5 mL), removal of DMTr protection with *p*-toluenesulfonic acid (0.3954 g) in 7:3

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chloroform - methanol (5 mL), and sedimentation from ethanol, to give the polymer-support bearing the resulting pentamer (**31**) ($U^{An}U^{An}A^{Bz}U^{An}G^{CE,iBu}$-celloluose acetate) (0.4073 g, 0.027 mmol, 63% yield). The tetramer **19** ($A^{Bz}A^{Bz}A^{Bz}A^{Bz}$) (0.3012 g, 0.102 mmol) was further introduced onto **31** (0.5997 g, 0.0342 mmol) by the sequence of procedures, i.e., the coupling reaction using TPS chloride (0.0933 g, 0.308 mmol) - 1-Meim (0.049 mL, 0.515 mmol) in pyridine (5 mL), acetylation with acetic anhydride (4.5 mL), removal of DMTr protection with a solution of *p*-toluenesulfonic acid (0.3954 g) in 7:3 chloroform - methanol (5 mL), and sedimentation from ethanol (500 mL) after dilution with methylene chloride (15 mL), to give the polymer-support bearing the resulting nonamer (**32**) ($A^{Bz}A^{Bz}A^{Bz}A^{Bz}U^{An}U^{An}A^{Bz}U^{An}G^{CE,iBu}$-cellulose acetate) (0.4713 g, 0.0229 mmol, 71% yield). Finally, the tetramer **19** ($A^{Bz}A^{Bz}G^{CE,iBu}$-$G^{CE,iBu}$) was introduced onto **32** (0.4713 g) by the sequence of procedures, i.e, the coupling reaction using TPS chloride (0.1041 g, 0.344 mmol) - 1-Meim (0.055 mL, 0.688 mmol) in pyridine (5 mL), sedimentation from ethanol (500 mL) after dilution with methylene chloride (15 mL), to give the polymer-support bearing the resulting tridecamer (**33**) ($A^{Bz}A^{Bz}G^{CE,iBu}G^{CE,iBu}A^{Bz}A^{Bz}A^{Bz}A^{Bz}U^{An}U^{An}A^{Bz}U^{An}$-$G^{CE,iBu}$-cellulose acetate) (0.5603 g, 0.0205 mmol, 90 % yield).

Removal of the Tridecamer Block Fully Protected Except Its 3'-Terminus from the Polymer-supports (**33**) *and Their Complete Deprotection*: The polymer-supports, involving diphenylcarbamoyl protection for the O^6-position in the heterocyclic moiety of guanosine (24.00 mg, 1.02 μmol) and 2-cyanoethyl protection for that (26.97 mg, 0.99 μmol) (**33**s), were respectively dissolved in a 0.5 M solution of TMG - PAO in 9:1 pyridine - water (0.5 mL), and the solution was incubated at 37°C for 24 h. The resulting solution was treated with water (1 mL) and ethanol (30 mL) to precipitate the cellulose moiety of the polymer-support, which was then centrifuged (4000 r.p.m. at 0°C for 10 min) after cooling down to -20°C and leaving for 1 h at the temperature. The supernatant was evaporated and the residue was dissolved in 28% ammoniacal water (15 mL), and heated at 55°C for 6 h after being equiped with a tight stopper for the vessel. After cooling down, the resulting solution was evaporated and the residue was dissolved in water (0.5 mL). A 1/4-portion (0.125 mL) of the aqueous solution was then subjected to reversed-phase h.p.l.c. purification by the use of a column of M&S PACK C-18 (4.6 mm ID x 150 mm L) and 10% - 50% acetonitrile solution in 0.1 M aqueous TEAA solution (See Fig. 1 and 3). Fractions corresponding to the central portion of each of the main peaks were gathered to evaporate, and each of the residues was, after dissolving in water (1 mL),

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repeated by evaporated until the smell of triethylamine could not be detected. The residue was subsequently allowed to stand at room temperature for 2 days after dissolving in hydrochloric acid (*p*H 2.0, 5 mL). The resulting solution was neutralized with diluted ammoniacal water, and the aqueous layer was washed with ethyl acetate (20 mL). The aqueous layer was evaporated and the residue was again dissolved in water (0.3 mL); a portion of which (0.25 mL) was then subjected to reversed-phase h.p.l.c. by the use of a column of M&S PACK C-18 (4.6 mm ID x 150 mm L) and 5 - 19% acetonitrile solution in 0.1 M aqueous TEAA solution (See Fig. 2 and 4). Fractions corresponding to the central portion of each of the main peaks were gathered and lyophilized to give the objective tridecamer (**35**) (ApApGpGpApApApApUpUpApUpG); 1.60 A_{260} units starting from the oligomer unit involving guanosine with O^6-diphenylcarbamoyl protection, and 2.96 A_{260} units from that with O^6-(2-cyanoethyl) protection. Electrophoretic experiments were performed on 20% polyacryamide Gel containing 7 M urea, after incorporation of [^{32}P]-phosphate function onto its 5'-terminus by the use of (γ-^{32}P) ATP and T4 Polynucleotide Kinase as usual[27] (See Fig. 5).

Synthesis of Dodecamer (**37**) *Starting from* **30c** (B = U^{An}) (See Scheme 8): The pentamer **22** ($\Psi^{Bz}C^{An}G^{CE,iBu}A^{Bz}U^{An}$) (0.1186 g, 0.033 mmol) was similarly introduced onto **30c** (0.3203 g, 0.198 mmol), after azeotropic evaporation from pyridine, by the sequence of procedures, i.e., the coupling reaction by the use of TPS chloride (0.0498 g, 0.165 mmol) - 1-Meim (0.026 mL), 0.329 mmol) in pyridine (5 mL), acetylation with acetic anhdride (4.5 mL), removal of 5'-*O*-dimethoxytrityl protection with 4% dichloroacetic acid in methylene chloride (10 mL) at 0°C for 10 min, neutralization with pyridine (2 mL), and sedimentation from ethanol (500 mL), to give the polymer-support bearing the resultant hexamer (**36**) (0.2641 g, 0.0142 mmol, 43% yield). Subsequently, the hexamer **28** ($U^{An}C^{An}C^{An}G^{CE,iBu}G^{CE,iBu}rT$) (0.1798 g, 0.043 mmol), was introduced onto the polymer-support **36** (0.2641 g, 0.014 mmol), after azeotropic evaporation from pyridine, by the sequence of procedures, i.e., the coupling reaction with TPS chloride (0.0645 g, 0.213 mmol) - 1-Meim (0.034 mL, 0.426 mmol) in pyridine (5 mL), and sedimentation from ethanol (500 mL), to give the polymer-support bearing the objective dodecamer (**37**) ($U^{An}C^{An}C^{An}G^{CE,iBu}G^{CE,iBu}rT$-$\Psi^{Bz}C^{An}G^{CE,iBu}A^{Bz}U^{An}U^{An}$-cellulose acetate) (0.2460 g, 0.010 mmol, 72% yield).

Removal of the Dodecamer Fully Protected Except Its 3'-Terminus from the Polymer-suppport (**37**) *and Its Complete Deprotection*: The polymer-support (12.09 mg, 0.5 μmol) was treated

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in the same way as above by the sequence of procedures, i.e., removal of the dedecamer block and unmasking of 2-chlorophenyl protection for the phosphodiester function with 0.5 M TMG-PAO in 9:1 pyridine - water (0.2 mL), unmasking of other protecting groups labile under basic conditions with 28% ammoniacal water (15 mL), preliminary purification with a column of SEP-PAK (C-18), purification through the h.p.l.c. column (See Fig. 6), and unmasking of the tetrahydropyran-2-yl and DMTr protection through hydrochloric acid (*p*H 2.0) (5 mL) (See Fig. 7), to give **39** (5.76 A_{260} units).

Quantitative Analysis of the Dodecamer **39** *through the Consecutive Enzymatic Degratdation* (See Fig. 8): The dodecamer **39** (0.3 A_{260} units) was dissolved in 0.1 M Tris buffer solution (*p*H 7.0) (0.1 mL) and the solution was incubated at 37°C for 1 h after the addition of snake venom phosphodiesterase (3 μL), purchased from Behringer Mannheim. To the resulting solution, was further added alkaline phosphatase (2 μL), purchased from Behringer Mannheim, and the solution was allowed to stand for 12 h at same temperature. The solution was, subsequently, subjected to h.p.l.c. by the use of LiChrosorb RP 18-5 (4.6 mm ID x 150 mm L) and 2% acetonitrile solution in 0.1 M aqueous TEAA solution. The elution profile (Fig. 8) demonstrated all the peaks of ribonucleosides involved and their area-intensity proportion was as Ψ : C : U : rT : G : A = 0.98 : 3.20 : 3.06 : 1.20 : 3.00 : 0.92, which corresponded well to the theoretical proportion of 1 : 3 : 3 : 1 : 3 : 1.

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