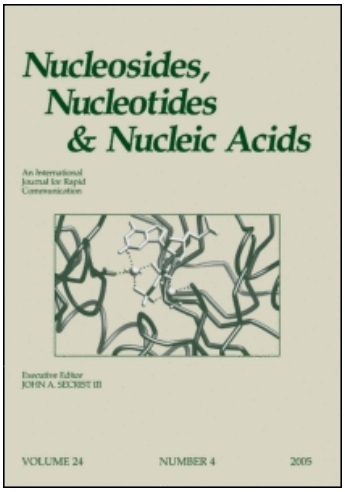

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Synthesis of an Oligonucleotide Bearing a Phosphate Function at the 5'-Terminus Using a Novel Protecting Group in Terms of a Cellulose Acetate Derivative as a Polymer-Support

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SYNTHESIS OF AN OLIGONUCLEOTIDE BEARING A PHOSPHATE FUNCTION AT THE 5'-TERMINUS USING A NOVEL PROTECTING GROUP IN TERMS OF A CELLULOSE ACETATE DERIVATIVE AS A POLYMER-SUPPORT[1]

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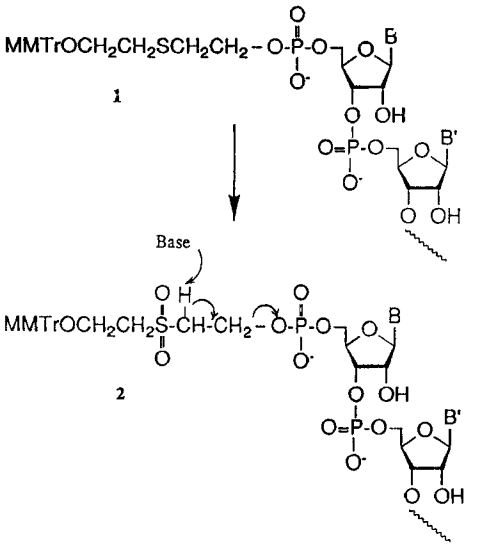
Abstract: Synthesis of the title oligonucleotide bearing a phosphate function at the 5'-terminus, i.e., pCpUpCpGpUpCpCpApCpCpA, by the use of the terminal cytidylic acid unit involving a novel protecting group of 2-[2-(monomethoxytrityloxy)ethylthio]ethyl group on its 5'-phosphoryl function in terms of a cellulose acetate polymer-support is described.

INTRODUCTION

An oligonucleotide bearing a phosphate function at the 5'-terminus is a potential substrate for constructing a longer chain DNA and RNA oligomer through ligation,[3] and is directly used for constructing a protein gene.[4] Moreover, it could be used for the synthesis of stable, non-radioactive probes, to which reporter groups, such as biotin,[5] a fluorescent moiety,[6] or a spin label,[7] were introduced. Therefore, the 5'-phosphorylated oligonucleotide could be acknowledged as an important tool for the studies in biosciences such as molecular biology, and applied fields like diagnostic medicines as well. Several methods for the synthesis of oligonucleotides bearing a phosphate function at their 5'-terminus have already been developed.[8,9] Agarwal et al.[9c] reported an efficient method for the synthesis of protected dinucleotides involving defined sequences with a 5'-phosphomonoester group by the use of 2-(4-tritylphenyl)thioethyl , a highly lipophilic protecting group, for 5'-phosphate of its terminal nucleotide. The 2-thioethyl function involved in the protecting group is characterized by its facile removal under basic conditions through β-elimination after oxidation with aqueous *N*-chlorosuccinimide to the corresponding sulfone.[9] On the other hand, synthetic oligonucleotides bearing lipophilic protecting groups, such as the trityl group, at their 5'-terminus are well known to

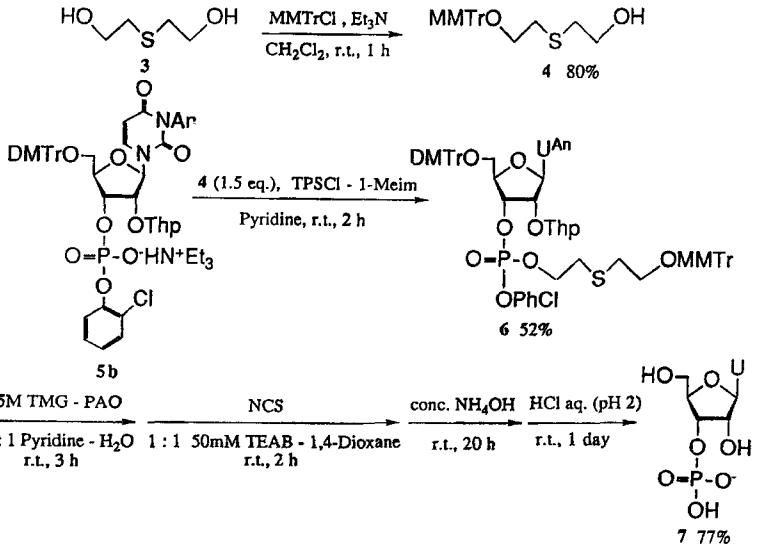


Scheme 1

be easier to purify by reversed-phase silica gel column chromatography.[8] Preparation of the protecting reagent, 2-(4-tritylphenyl)thioethanol, is, however, tedious to perform, including three or four steps of reactions involving an O - S rearrangement reaction at a high temperature, although the overall yield is good (71 - 81%). Therefore, we set out to establish an alternative protecting group for the 5′-phosphoryl function which is also proved to be efficient to perform the synthesis of an oligonucleotide. The potential of 2-[2-(monomethoxytrityloxy)ethylthio]ethyl group thus occurred to us since 2-[2-(monomethoxytrityloxy)ethylthio]ethanol is easily available from the coupling reaction of monomethoxytrityl chloride with 2,2′-thiodiethanol.

Incidentally, we have reported a cellulose acetate derivative functionalized with 4-(2-hydroxyethylsulfonyl)dihydrocinnamoyl spacer, which was chosen because of the utility of the 2-sulfonylethyl function for splitting-off an oligonucleotide 3′-phosphodiester derivative, as a novel polymer-support for the synthesis of both RNA and DNA oligomers.[2]

In this paper we describe a novel method for the synthesis of an oligonucleotide bearing a phosphate function at the 5'-terminus. We performed the synthesis of an RNA oligomer involved in the 3'-terminal structure of yeast $tRNA^{Ala}$, i.e., pCpUpCpGpUpCpCpApCpCpA, by the use of the cytidylic unit bearing a 2-[2-(monomethoxytrityloxy)ethylthio]ethyl protecting group on its 5'-phosphoryl function on a cellulose acetate polymer-support (See Scheme 1).



Scheme 2

RESULT AND DISCUSSION

Demonstration of the Feasibility of the Present Strategy by the Use of N^3-Anisoyl-5'-*O*-dimethoxytrityl-2'-*O*-(tetrahydropyra-2-yl)uridine 3'-[2-chlorophenyl-2-[2-(monomethoxytrityloxy)ethylthio]ethyl]phosphate (**6**)

The feasibility of the present strategy was examined by the use of 6, which was prepared by the sequence of reactions as shown in Scheme 2, as a model compound. Triethylammonium N^3-anisoyl-5'-*O*-dimethoxytrityl-2'-*O*-(tetrahydropyran-2-yl)uridine 3'-(2-chlorophenyl)phosphate (**5b**) was subjected to the coupling reaction with 2-[2-(monomethoxytrityloxy)ethylthio]ethanol (**4**), prepared from 2,2'-thiodiethanol and monomethoxytrityl chloride in the presence of triethylamine, in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) and 1-methylimidazole (1-Meim)[10] to give **6** (52% yield). Subsequently, **6** was treated with N^1,N^1,N^3,N^3-tetramethylguanidium (*E*)-pyridine-2-aldoximate (TMG - PAO) for 3 h at room temperature to remove the 2-chlorophenyl protecting group on the phosphate. The removal of 2-[2-(monomethoxytrityloxy)ethylthio]ethyl protecting group was effected by oxidation of the sulfide to the sulfone with *N*-chlorosuccinimide (NCS) in 1,4-dioxane - 50 mM triethylammonium hydrogencarbonate (TEAB) solution for 2 h at room temperature, followed by treatment with 28% ammoniacal water for 20 h at room temperature (β-elimination). The remaining acid-labile protecting groups were removed by treatment with hydrochloric acid (pH 2.0) for 1 day at room temperature. Finally, preparative paper chromatography on Whatman 3 MM paper gave uridine 3'-phosphate (**7**) in 77% yield.

Synthesis of Cytidylic Unit (**12**)

The key intermediate (**12**) for the synthesis of an oligonucleotide bearing a phosphate function at the 5'-terminus was prepared as shown in Scheme 3.

Cytidine 3'-phosphodiester derivative (**5a**) was subjected to a sequence of reactions of 2-cyanoethylation with 2-cyanoethanol in the presence of 8-quinolinesulfonyl chloride (QSCl) - 1-Meim,[11] removal of the 5'-*O*-dimethoxytrityl protecting group,[12] and 5'-*O*-phosphorylation with 2-chlorophenyl phosphorodichloridate,[2] to give **8** (67% overall yield). Compound **10** was further subjected to a coupling reaction with **4** in the presence of QSCl - 1-Meim, to give **11** (38% yield), and then removal of the 2-cyanoethyl protecting group by treatment with 1:3 triethylamine - pyridine,[13] to give **12** (quantitative yield).

Synthesis of an Undecamer (**31**)

Synthesis of the undecamer, pCpUpCpGpUpCpCpApCpCpAp (**31**), was performed by the strategy shown in Fig. 1.

1) Synthesis of Oligomer Blocks (**18** and **26**)

The hexamer block (**18**) was prepared as shown in Scheme 4. The dimer block (**15**) was prepared by a coupling reaction of **9b** and **12** in the presence of QSCl - 1-Meim in pyridine. Compounds **12** and **13** were found to be efficiently distributed between aqueous pyridine and diethyl ether layers, respectively, on shaking with aqueous pyridine - diethyl ether; this procedure is practically convenient for purification of the resulting **13** (86% yield). Compound **13** was easily derived into **15** on removal of the 2-cyanoethyl protecting group by treatment with 1:3 triethylamine - pyridine. Separately, the dimer block (**16**) was prepared by a coupling reaction of **5a** and **9d** as described above and removal of the 5'-*O*-dimethxytrityl protecting group by treatment with 2% dichloroacetic acid in methylene chloride.

The tetamer block (**18**) was prepared by a coupling reaction of **15** and **16** and removal of the 2-cyanoethyl protecting group.

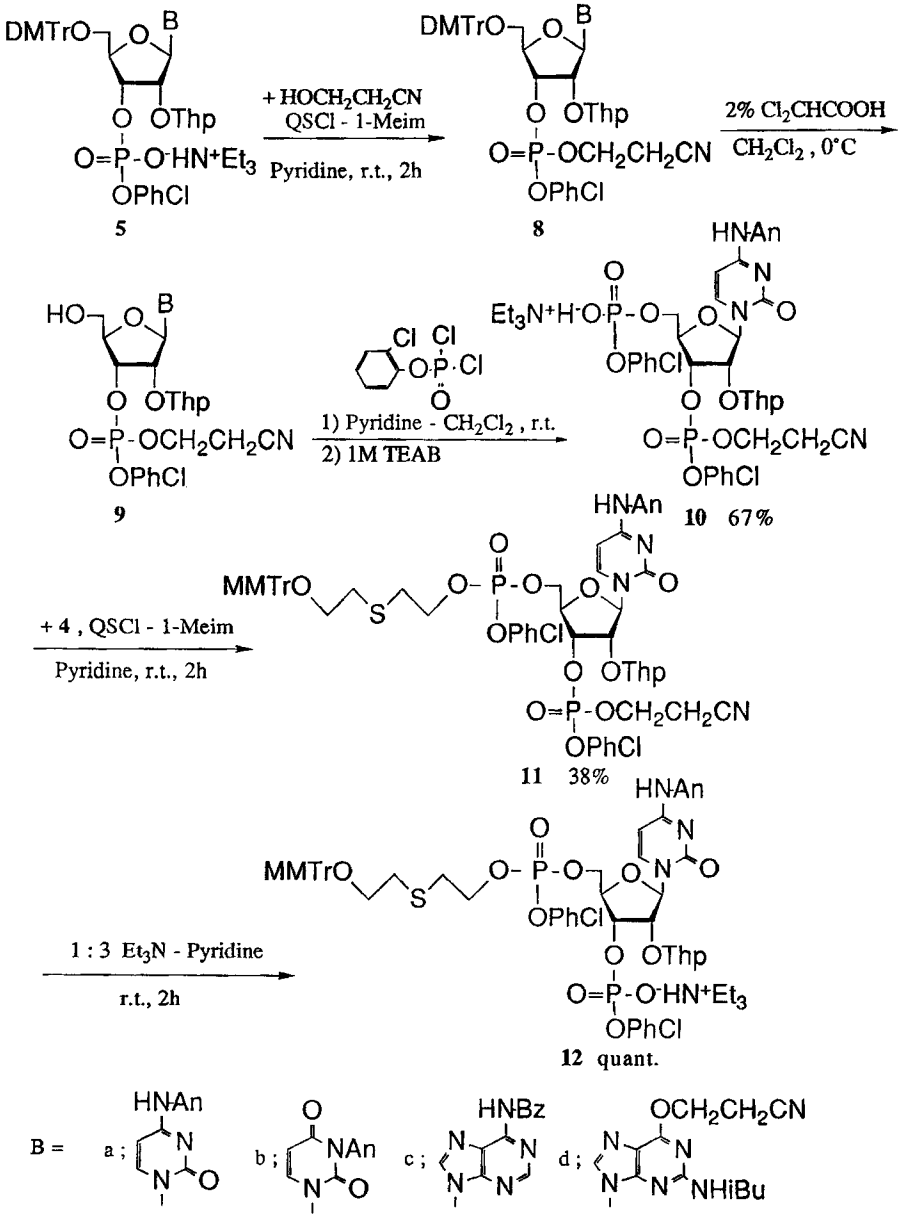
The hexamer block (**26**) was constructed as described above (See Scheme 5).

2) Synthesis of a Cellulose Acetate Polymer-support bearing the Adenosine Unit (**28**)

Condensation of 3'-(carboxy)propionate (**27**)[14] with a cellulose acetate derivative (D.S. = 1.77)[15] through TPSCl - 1-Meim, followed by capping of the unchanged hydroxyl groups through acetylation, unmasking of its 5'-position, and sedimentation from ethanol, gave a powder of **28** in 79% overall yield (Scheme 6).

3) Synthesis of the Undecamer Bearing a Phosphate Function at the 5'-Terminus (**31**)

Scheme 7 shows the flow-sheet for the synthesis of **31**. The polymer-support bearing the adenosine unit (**28**) was subjected to the coupling reaction with the hexamer block (**26**) through TPSCl - 1-Meim in pyridine, capping through acetylation with acetic



Scheme 3

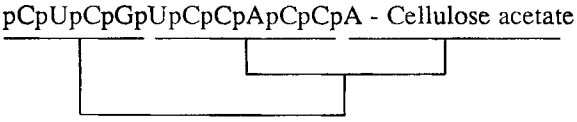


Fig.1

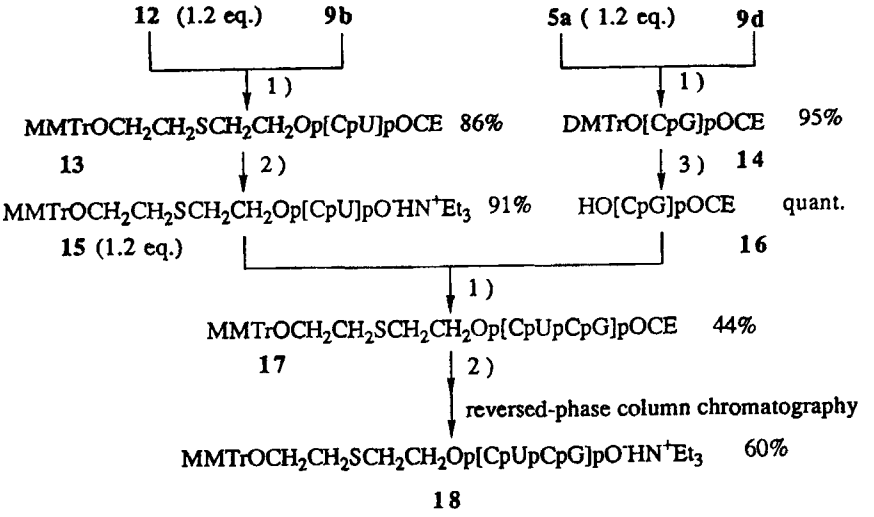
anhydride, unmasking of its 5'-position, and then sedimentation from ethanol, to give a *cellulose* derivative (**29**) (48% yield). **Condensation of the resultant 29 with the tetramer block (18), followed by the same work-up as above, gave the cellulose derivative (30)** bearing the fully protected objective undecamer (61% yield).

The splitting-off of the undecamer unit from **30** was fulfilled through the sequence of treatments with TMG - PAO, with concentrated aqueous ammonia, reversed-phase h.p.l.c., giving the undecamer bearing 2'-*O*-Thp and 5'-terminal *O*-protected phosphate groups (Fig. 2-I), a treatment with NCS,[9] with concentrated aqueous ammonia, with hydrochloric acid (pH 2.0), and reversed-phase h.p.l.c., giving the undecamer **31** [42 A_{260} units were obtained from **28** (7.38 μmol); see Fig. 2-II]. Complete degradation of **31** to each component of ribonucleosides 5'-phosphates was conducted by treatment with snake venom phosphodiesterase (Fig.3).

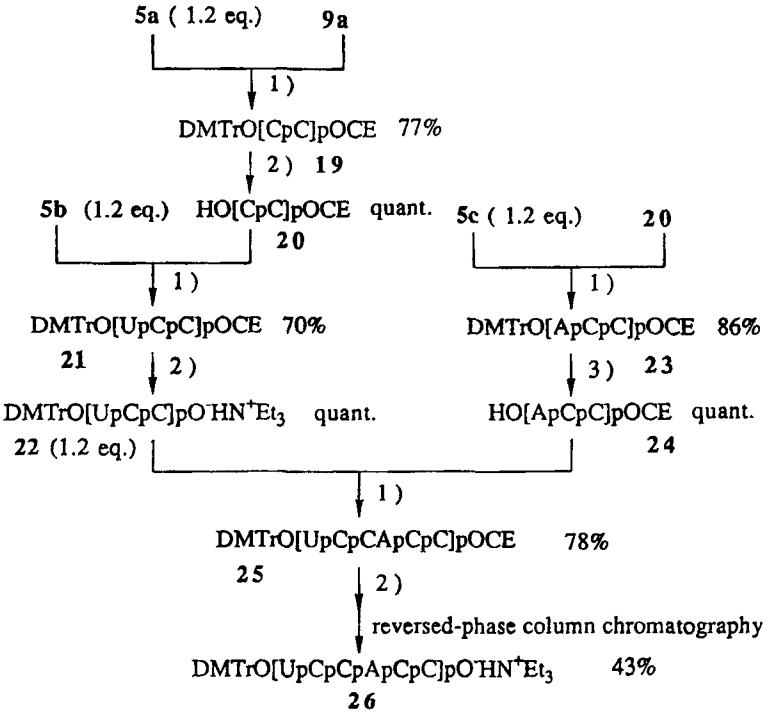
The results described herein demonstrate the utility of the 2-[2-(monomethoxytrityloxy)ethylthio]ethyl protecting group on the 5'-phosphoryl function for the synthesis of oligonucleotides bearing a phosphate function at the 5'-terminus.

EXPERIMENTAL

Column chromatography was performed on silica gel (Wakogel C-300, purchased from Wako Pure Chemicals, Co. Ltd.) 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 Inc.), by the use of acetone - 50 mM triethylammonium hydrogencarbonate (bicarbonate) (TEAB). Paper chromatography was performed using the ascending technique on Whatman 3 MM paper. The solvent system used was isopropyl alcohol - 28% ammoniacal water - Water (7: 1 : 2 v / v). High performance liquid chromatography (h.p.l.c.) was conducted on μBONDASPHERE 5μ C-18 (3.9 mm ID x 150 mm L) for purification of the undecamer and on LiChrosorb RP 18-5 (4.6 mm ID x 150 mm L) for analysis of digestion of the undecamer with snake venom phosphodiesterase by the use of acetonitrile - 0.1 M triethylammonium acetate (TEAA; pH 7.0) as the eluant. ^{1}H-N.m.r. spectra were recorded on a Brucker AM 400 apparatus with tetramethylsilane (TMS) as the internal standard. Elemental analyses were achieved with a Perkin-Elmer 240-002 apparatus.



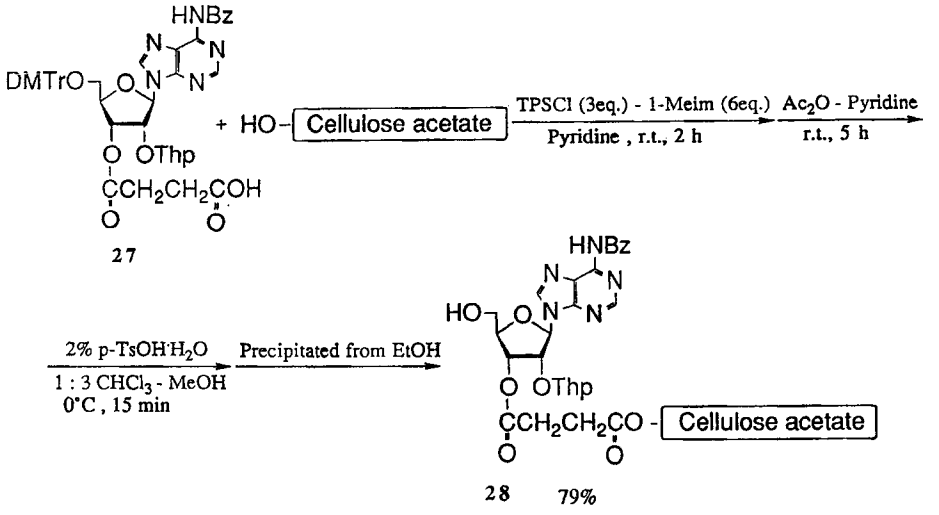
Scheme 4



Scheme 5

[ ] = protected except for terminal
p = 2-chlorophenyl phosphoryl
CE = 2-cyanoethyl group

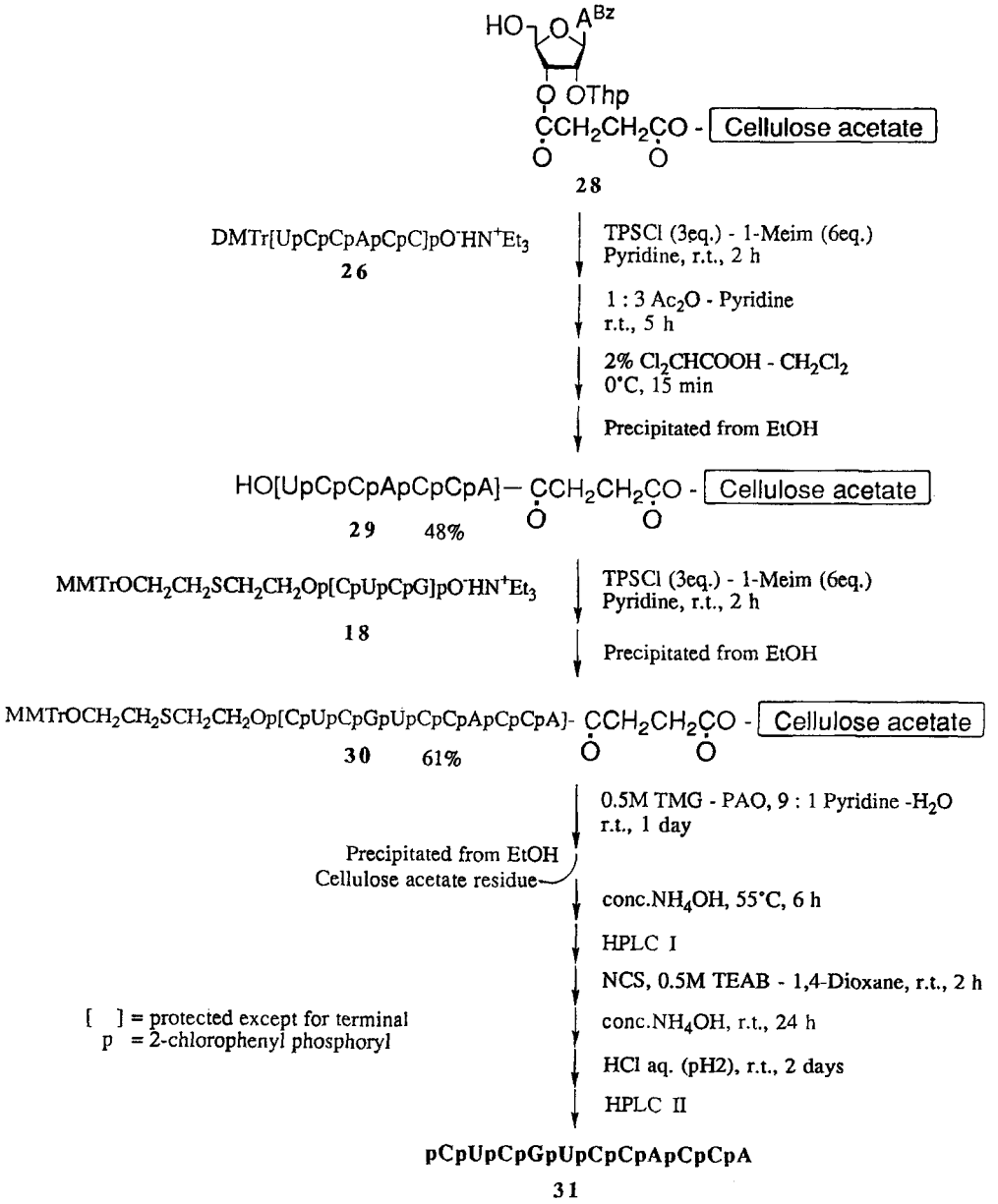
Conditions :
1) QSCl (3eq.) - 1-Meim (6eq.), Pyridine, r.t , 2 h
2) 1 : 3 Et_3N - Pyridine, r.t., 2 h
3) 2% $Cl_2CHCOOH$ - CH_2Cl_2, 0°C, 15 min



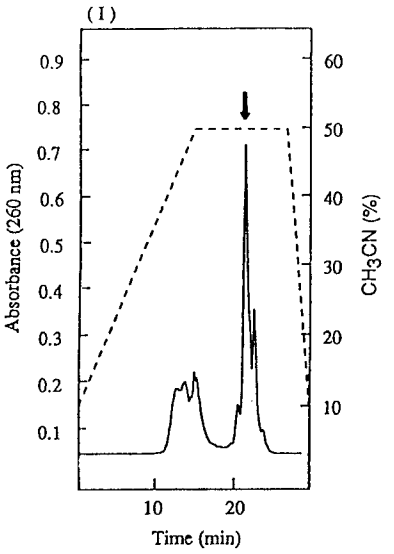
Scheme 6

2-[2-(Monomethoxytrityloxy)ethythio]ethanol (**4**): To a solution of 2,2'-thiodiethanol (4.5 mL, 75 mmol) and monomethoxytrityl chloride (4.632 g, 15 mmol) in dried methylene chloride (60 mL) was added triethylamine (2.1 mL, 15 mmol) dropwise during 5 min with stirring. After stirring for 1 h, the mixture was quenched with water (30 mL) and extracted with methylene chloride (100 mL). The organic layer was, after washing with water (100 mL x 2), dried over anhydrous magnesium sulfate, filtered, and evaporated. The residue was then subjected to chromatography on a column of silica gel with hexane - methylene chloride system as the eluant to give **4** (4.734 g, 80% yield); ^{1}H-n.m.r. ($CDCl_3$): δ 2.10 - 2.30 (m, 1H, OH), 2.63 (t, 2H, J = 5.7 Hz, CH_2-S), 2.70 (t, 2H, J = 6.5 Hz, CH_2-S), 3.28 (t, 2H, J = 6.5 Hz, CH_2), 3.60 (t, 2H, J = 5.7 Hz, CH_2-S), 3.75 (s, 3H, OCH_3), 6.83 (d, 2H, J = 9.0 Hz, Ph-H), and 7.03 - 7.80 (m, 12H, Ph-H).

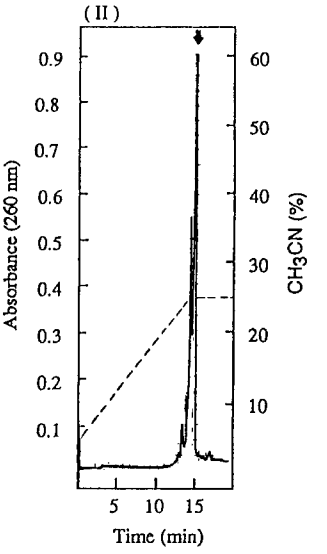
N^3-*Anisoyl-5'*-O-*dimethoxytrityl-2'*-O-*(tetrahydropyran-2-yl)uridine 3'-{2-chlorophenyl-2-[2-(monomethoxytrityloxy)ethylthio]ethyl}phosphate* (**6**): Compounds **4** (0.374 g, 0.75 mmol) and **5b** (0.528g, 0.5mmol) were, after azeotropic evaporation from pyridine, dissolved in dried pyridine (2.5mL), and TPSCl (0.303g, 1mmol) and 1-Meim (0.16 mL, 2 mmol) were added to the solution, which was then stirred at room temperature for 2 h. The reaction mixture was quenched with water (5mL) and diluted with methylene chloride (20 mL). The organic layer was, after washing with water (15



Scheme 7



Reversed-phase HPLC of the Undecamer bearing 2'-*O*-THP and 5'-terminal *O*-Protected Phosphate Groups



Reversed-phase HPLC of the Completely Unmasked Undecamer

Fig. 2 Conditions: column μBONDASPHERE 5μ C18(3.9mmID x 150mmL); elution buffer CH_3CN - 0.1M TEAA(pH7); flow rate 1ml/min; detected by U.V. at 260nm.

mL x 2), dried over anhydrous magnesium sulfate, filtered, and evaporated. The residue was then subjected to chromatography on a column of silica gel with methylene chloride - methanol system as the eluant to give **6** (0.346g, 52% yield); ^{1}H-n.m.r. ($CDCl_3$): δ 1.33 - 1.67 (m, 6H, CCH_2C x 3), 2.53 - 2.80 (m, 2H, CH_2 x 2), 3.23 (t, 12H, J = 6.0Hz, CH_2), 3.47 - 3.63 (m, 2H, OCH_2C), 3.77 (s, 12H, OCH_3 x 4), 3.80 - 3.87 (m, 2H, H-5' and 5"), 4.07 - 4.33 (m, 2H, $POCH_2$), 4.37 - 4.50 (m,1H, H-4'), 4.67 - 4.93 (m, 2H, OCH_2O and H-2'), 5.13 - 5.36 (m, 1H, H-3'), 5.37 (d, 1H, $J_{5,6}$ = 8.0Hz, H-5), 6.20 and 6.35 (d x 2, 1H, $J_{1',2'}$ = 6.0Hz and 7.5Hz, H-1'), 6.80 - 7.60 (m, 33H, Ph-H), 7.75 (d, 1H, H-6), and 7.95 (d, 2H, J = 9.0Hz, Ph-H).

Complete Deprotection of Compound **6**: Compound **6** (0.067 g, 50 μmol) was dissolved in a 0.5 M solution of TMG - PAO in 9:1 pyridine - water (5 mL), and the solution was incubated at room temperature for 3 h. The mixture was diluted with methylene chloride (25 mL) and washed with 0.2 M TEAB (25 mL x 2). The organic layer was evaporated. The residue was dissolved 1:2 pyridine - 0.2 M TEAB (50 mL) and washed with diethyl ether (25 mL x 2). The aqueous layer was evaporated. The residue was dissolved in 1:1 aqueous 50 mM TEAB - 1,4-dioxane solution (5 mL), and *N*-chlorosuccinimide (0.134 g, 1 mmol) was added to the solution. After stirring for 2 h

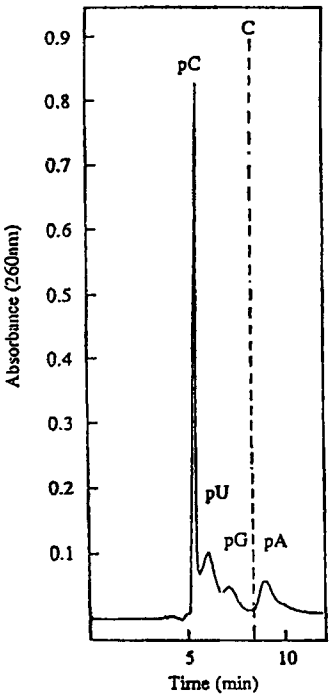


Fig. 3

Reversed-phase HPLC of Analysis of Products Obtained by Digestion of the Completely Unmasked Undecamer with Snake Venom Phosphodiesterase

Conditions:
column LiChrosorb RP18-5(4.6mmID x 150mmL);
elution buffer 1% CH_3CN - 0.1M TEAA(pH7);
flow rate 0.5ml/min; detected by U.V. at 260nm.

The dotted line shows the retention time of cytidine.

at room temperature, the mixture was evaporated. The residue was dissolved in 1:10 pyridine - 28% ammoniacal water (5.5 mL), stirred at room temperature for 20 h, and the solution was evaporated. The residue was subsequently allowed to stirred at room temperature for 1 day after dissolving in hydrochloric acid (pH 2.0) (5 mL). The resulting solution was neutralized with diluted ammoniacal water, diluted with water (10 mL), and washed with diethy ether (5 mL x 2). The aqueous layer was evaporated and the residue was again dissolved in water (5 mL). An aliquot (1 mL) of the solution was subjected to the chromatographic separation on Whatman 3 MM paper by the use of 7:1:2 isopropyl alcohol - 28% ammoniacal water - water solvent system. The chromatographic band corresponding to uridine 3'-phosphate (**7**) (Rf 0.12) was observed. Totally, **6** (10 μmol) afforded 76.1 A_{260} units of **7** (77% yield), which showed a u.v. spectrum involving λ_{max} at 262 nm and λ_{min} at 232 nm, and the yield was calculated in terms of the molar extinction of an authentic sample at 260 nm (ε 9,900).

2'-O-(Tetrahydropyran-2-yl)ribonucleosides 3'-(2-chlorophenyl-2-cyanoethyl)-phosphates (**9**) (See Scheme 3): Triethylammonium 5'-*O*-dimethoxytrityl-2'-*O*-(tetrahydropyran-2-yl)ribonucleosides 3'-(2-chlorophenyl)phosphates (**5**)[2] were subjected to 2-cyanoethylation by treatment with 2-cyanoethanol (2 mol. equiv.) in the presence of QSCl (2.5 mol. equiv.) and 1-Meim (5 mol. equiv.) as usual [11] to give **8**, which was

easily purified by distributing between 4:1 water - pyridine and diethyl ether ; it was efficiently extracted into the layer of diethyl ether. The removal of dimethoxytrityl group at 5'-position of **8** by treating as usual,[12] gave **9**.

Synthesis of Cytidylic Unit (**12**)(See Scheme 2): To a solution of (2-chlorophenyl)phosphorodichloridate (0.45 mL, 2.75 mmol) in dried pyridine (10 mL) was added a solution of **9a** (1.268 g, 1.83 mmol) in dried methylene chloride (10 mL) dropwise during 2 h with stirring. After stirring for 30 min, the mixture was quenched with 1 M TEAB solution (10 mL). The organic layer was evaporated and the residue was dissolved in 2:1 water - pyridine (100 mL). The solution was, after washing with diethyl ether (50 mL x 3), evaporated to give **10** (1.202 g, 67% yield); ^{1}H-n.m.r. ($CDCl_3$ - TMS): δ 1.30 (t, 9H, J = 7.32 Hz, $NCH_2C\underline{H}_3$ x 3), 1.40 - 1.85 (m, 6H, $C\underline{H}_2$ of Thp), 2.84 - 2.89 (m, 2H, $C\underline{H}_2CN$), 3.05 (q, 6H, $NC\underline{H}_2CH_3$ x 3), 3.28 - 3.52 (m, 2H, $OC\underline{H}_2$ of Thp), 3.88 (s, 3H, $OC\underline{H}_3$), 4.10 - 5.20 (m, 8H, H - 2', 3', 4', 5', 5", OC<u>H</u> of Thp and $POC\underline{H}_2$), 6.17 - 6.25 (m, 1H, H - 1'), 6.98 (d, 2H, J = 8.73 Hz, Ph-<u>H</u>), 7.12 - 7.86 (m, 10H, H - 5, 6 and Ph-<u>H</u>), and 8.62 - 8.66 (m, 1H, N^4-<u>H</u>).

Compounds **4** (1.436 g, 3.64 mmol) and **10** (1.192 g, 1.21 mmol) were, after azeotropic evaporation from pyridine, dissolved in dried pyridine (6 mL), and QSCl (0.828 g, 3.63 mmol) and 1-Meim (0.58 mL, 7.26 mmol) were added to the solution, which was then stirred at room temperature for 2 h. The intramolecular salt of QS precipitated out was filtered off, and the filtrate was treated with water (2 mL). The resulting mixture was distributed between 2:1 water - pyridine and diethyl ether, whose organic layer was, after dried over anhydrous magnesium sulfate, evaporated. The residue was then subjected to chromatography on a column of silica gel with methylene chloride - methanol system as the eluant to give **11** (0.588 g, 38% yield); ^{1}H-n.m.r. ($CDCl_3$ - TMS): δ 1.41 - 1.80 (m, 6H, $C\underline{H}_2$ of Thp), 2.67 - 2.71 (m, 2H, $C\underline{H}_2$), 2.74 - 2.82 (m, 4H, $C\underline{H}_2$ x 2), 3.24 - 3.28 (m, 2H, $C\underline{H}_2$), 3.37 - 3.62 (m, 2H, $OC\underline{H}_2$ of Thp), 3.78 (s, 3H, $OC\underline{H}_3$), 3.88 (s, 3H, $OC\underline{H}_3$), 4.27 - 4.72 (m, 7H, H - 2', 4', 5', 5", OC<u>H</u> of Thp and $POC\underline{H}_2$), 5.22 - 5.28 (m, 1H, H - 3'), 6.16 - 6.20 (m, 1H, H - 1'), 6.81 (d, 2H, J = 8.82 Hz, Ph-<u>H</u>), 6.97 (d, 2H, J = 8.62 Hz, Ph-<u>H</u>), 7.12 - 7.86 (m, 24H, H - 5, 6 and Ph-<u>H</u>), and 8.52 (brs, 1H, N^4-<u>H</u>).

Anal. Calcd for $C_{61}H_{62}N_4O_9P_2SCl_2$: C, 57.60; H, 4.92; N, 4.40. Found: C, 57.62; H, 4.91; N, 4.43.

Compound **11** (0.578 g, 0.46 mmol) was subjected to removal of the 2-cyanoethyl protecting group by treatment with 1:3 triethylamine - pyridine (5 mL) at room temperature for 2 h with stirring. The resulting mixture was evaporated to give the corresponding 3'-phosphodiester derivative (**12**) quantitatively; ^{1}H-n.m.r. ($CDCl_3$ - TMS): δ 1.27 (t, 9H, J = 7.32 Hz, $NCH_2C\underline{H}_3$ x 3), 1.40 - 1.71 (m, 6H, $C\underline{H}_2$ of Thp),

2.65 - 2.70 and 2.73 - 2.78 (m x 2, 4H, SC$\underline{H}_2$ x 2), 3.02 (q, 6H, NC$\underline{H}_2$CH$_3$ x 3), 3.22 - 3.32 (m, 2H, OC$\underline{H}_2$), 3.37 - 3.42 and 3.62 - 3.67 (m x 2, 2H, OC$\underline{H}_2$ of Thp), 3.78 (s, 3H, OC$\underline{H}_3$), 3.88 (s, 3H, OC$\underline{H}_3$), 4.26 (t, 2H, J = 7.43 Hz, POC$\underline{H}_2$), 4.36 - 4.60 and 4.85 - 4.94 (m x 2, 6H, H - 2', 3', 4', 5', 5", and OC$\underline{H}$ of Thp), 6.25 - 6.30 (m, 1H, H - 1'), 6.81 (d, 2H, J = 8.90 Hz, Ph-$\underline{H}$), 6.97 (d, 2H, J = 8.74 Hz, Ph-$\underline{H}$), 7.13 - 7.95 (m, 24H, H - 5, 6 and Ph-$\underline{H}$), and 8.58 - 8.67 (m, 1H, N^4-$\underline{H}$).

Synthesis of Oligomer Blocks(See Scheme 4):

Tetramer Block (**18**): Compounds **12** (0.849 g, 0.645 mmol) and **9b** (0.380 g, 0.538 mmol) were, after azeotropic evaporation from pyridine, dissolved in dried pyridine (6 mL), and QSCl (0.441 g, 1.94 mmol) and 1-Meim (0.31 mL, 0.387 mmol) were added to the solution, which was then stirred at room temperature for 2 h. The intramolecular salt of QS precipitated out was filtered off, and the filtrate was treated with water (1 mL). The resulting mixture was distributed between 2:1 water - pyridine and diethyl ether, whose organic layer was, after drying over anhydrous magnesium sulfate, evaporated The residue was then subjected to chromatgraphy on a column of silica gel with methylene chloride - methanol system as the eluant to give **13** (0.883 g, 86% yield).

Separately, **5a** (2.875 g, 2.76 mmol) and **9d** (1.690 g, 2.30 mmol) were with QSCl (1.885 g, 8.28 mmol) and 1-Meim (1.32 mL, 16.6 mmol) in pyridine (11.5 mL) in the same way as above to give **14** (3.652 g, 95% yield).

The dimer (**13**) (0.491 g, 0.26 mmol) was subjected to removal of the 2-cyano-ethyl protecting group by treatment with 1:3 triethylamine - pyridine (4 mL) at room temperature for 2 h with stirring. The resulting mixture was evaporated to give the corresponding 3'-phosphodiester derivative (**15**) quantitatively.

Separately, the dimer (**14**) was dissolved in methylene chloride (10 mL), and, under cooling in an ice-bath, was added 4% dichloroacetic acid in methylene chloride (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 hydroxyl group at the 5'-terminus. The residue was then subjected to chlomatographic separation on a column of silica gel to give **16** quantitatively.

Compounds **15** (0.426 g, 0.236 mmol) and **16** (0.268 g, 0.196 mmol) were, after azeotropic evaporation from pyridine, treated with QSCl (0.161 g, 0.71 mmol) and 1-Meim (0.11 mL, 1.41 mmol) in pyridine (3 mL) at room temperature for 2 h with stirring. The intramolecular salt of QS precipitated out was filtered off, and 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 dryness and the residue was subjected to chromatgraphic separation on a column of silica gel with methylene chloride - methanol system as the eluant to give **17** (0.280 g, 44% yield). The tetramer (**17**) was dissolved in 1:3 triethylamine - pyridine (1 mL) at room temperature and the solution was stirred at room temperature for 2 h. The resulting mixture was evaporated to dryness and the residue was subjected to reversed-phase column chromatography with acetone - aqueous 50 mM TEAB system as the eluant to give the tetramer 3'-phosphodiester derivative (**18**) (0.169 g, 60% yield).

Similarly, the hexamer block (**26**) was synthesized (See Scheme 5).

Acetylcellulose Derivarive Bearing N^6*-Benzoyl-2'-O-(tetrahydropyran-2-yl)-adenosine Unit with a Free Hydroxyl Group at the 5'-Position* (**28**)(See Sheme 6): N^6-Benzoyl-3'-*O*-(3-carboxy)propionyl-5'-*O*-dimethoxytrityl-2'-*O*-(tetrahydropyran-2-yl)adenosine (**27**) (0.858 g, 1.0 mmol) and acetylcellulose (D.S. = 1.77) (0.769 g) were, after azeotropic evaporation from pyridine, dissolved in pyridine (5 mL), and TPSCl (1.211 g, 4.0 mmol) and 1-Meim (0.64 mL, 8.0 mmol) were added to the solution, which was then stirred at room temperature for 2 h. The resulting solution was treated with acetic anhydride (2 mL) and stirred at room temperature for 6 h. The solution was diluted with methylene chloride (10 mL), and a small portion of the solution was poured slowly into ethanol (50 mL) under vigorous stirring. The sedimentation was induced to give a white powder of polymer-support loading the adenosine unit (7 mg). Dissolution 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[16] gave the loaded amount of the adenosine unit (0.511 mmol / g). The solution was evaporated and the residue was dissolved in toluene (10 mL). Pyridine potentially contained in it was azeotropically evaporated. The residue was dissolved in 7:3 chloroform - methanol (30 mL), to which a solution of *p*-toluenesulfonic acid monohydrate (1.186 g) in 7:3 chloroform - methanol (15 mL) was added under cooling in an ice-bath. After stirring for 15 min, the mixture was neutralized by the addition of pyridine (5 mL), and the resulting solution was added slowly into ethanol (500 mL) under vigorous stirring to give **28** (1.299 g, 0.785 mmol, 79% yield; loaded amount of the adenosine unit = 0.604 mmol / g).

Synthesis of the Undecamer (**30**)(See Sheme 6): The hexamer (**30**) (0.211 g, 0.05 mmol) and **28** (0.497 g, 0.30 mmol) were, after azeotropic evaporation from dried pyridine, dissolved in dried pyridine (7 mL), and TPSCl (0.0757 g, 0.25 mmol) and 1-Meim (0.04 mL, 0.50 mmol) were added to the solution, which was then stirred at room temperature for 2 h. The resulting solution was treated with acetic anhydride (1.5 mL)

and stirred at room temperature for 6 h. The solution was diluted with methylene chloride (10 mL), and poured slowly into ethanol (50 mL) under vigorous stirring. The sedimentation was induced to give a powder of the polymer-support loading the objective heptamer. The powder was dissolved in methylene chloride (10 mL), to which a solution of 4% dichloroacetic acid in methylene chloride (10 mL) was added 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 precipitate the polymer-support loading the resulting heptamer (**29**) (0.464 g, 24.2 μmol, 48% yield; loaded amount of the adenosine unit = 52.7 μmol / g). The tetramer (**18**) (0.119 g, 36.3 μmol) was similarly introduced onto **29** (0.232 g) by the sequence of procedures, i.e., the coupling reaction using TPSCl (0.0606 g, 0.182 mmol) - 1-Meim (0.032 mL, 0.363 mmol) in pyridine (2 mL) for introduction reaction, after azeotropic evaporation from dried pyridine, dilution with methylene chloride (10 mL), and sedimentation from ethanol (500 mL), to give the polymer-support loading the resulting undecamer (**30**) [0.221 g, 7.38 μmol, 61% 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} 470 nm (ε 56,000) for monomethoxytrityl cation[17] gave the loaded amount of the undecamer (33.4 μmol / g)].

Removal of the Undecamer Fully Protected Except Its 3'-Terminus from the Polymer-support (**30**) *and Its Complete Deprotection*(See Sheme 7): The polymer-support bearing the undecamer (**30**) (30.0 mg, 1.0 μmol) was 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 ethanol (30 mL) to precipitate the cellulose moiety of the resultant polymer-support, which was then centrifuged (4000 r.p.m. at -20°C for 30 min). The supernatant was evaporated and the residue was dissolved in 28% ammoniacal water (15 mL), which was then heated at 55°C for 6 h after being equipped with a tight stopper for the vessel. After cooling down, the resulting solution was evaporated and the residue was dissolved in aqueous 50 mM TEAB solution (20 mL), which was then poured onto SEP-PAK (C-18; Waters Co.) column [The column was treated in advance with 9:1 acetonitrile - water (10 mL) for 3 h, and washed with aqueous 50 mM TEAB solution]. Elution of the column was performed successively with 15% acetonitrile - aqueous 50 mM TEAB solution (30 mL) for the removal of TMG-PAO etc., and with 35% acetonitrile - aqueous 50 mM TEAB solution (20 mL), gave a fraction containing the objective undecamer, whose 5'-terminus phosphoryl function and 2'-hydroxy groups were protected with 2-[2-(monomethoxytrityloxy)ethylthio]ethyl and tetrahydropyran-2-yl groups, respectively. This fraction was then lyophilized, and the residue was dissolved in water (0.2 mL). An aliquot (77.6

μL) of the aqueous solution was then subjected to reversed-phase h.p.l.c. purification by the use of a column of μBONDASPHERE 5μ C-18 (3.9 mm ID x 150 mm L) and 10% - 50% acetonitrile solution in 0.1 M aqueous TEAA solution (Fig. 2-I). Fractions corresponding to the central portion of the main peaks were combined to be evaporated, and the residue was, after dissolving in water (1 mL), repeatedly evaporated until the smell of triethylamine could not be detected. The residue was dissolved in 1:1 aqueous 50 mM TEAB solution - 1,4-dioxane (0.1 mL) and *N*-chlorosuccinimide (3 mg) was added to the solution, which was then stirred for 2 h at room temperature. The resulting solution was treated with 28% ammoniacal water (1 mL) and stirred at room temperature for 20 h. The solution was evaporated after washing with ethyl acetate (5 mL x 2). The residue was subsequently allowed to stand at room temperature for 2 days after dissolving in hydrochloric acid (pH 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 (75 μL). An aliquot (30 μL) of the aqueus solution was then subjected to reversed-phase h.p.l.c. by the use of a column of μBONDASPHERE 5μ C-18 (3.9 mm ID x 150 mm L) and 5% - 25% acetonitrile solution in 0.1 M aqueous TEAA solution (See Fig. 2-II). Fractions corresponding to the central portion of the main peak were combined and lyophilized to give the objective undecamer (**31**) (0.884 A_{260} units).

Structure Confirmation of the Undecamer (**31**): The undecamer (**31**) (0.2 A_{260} units) was dissolved in 0.1 M Tris buffer solution (pH 7.0) (1 mL) and the solution was incubated at 37°C for 1 h after the addition of snake venom phosphodiesterase (3 μL), purchased from Beringer Mannheim Inc. The solution was subsequently subjected to h.p.l.c. by the use of LiChlosorb RP 18-5 (4.6 mm ID x 150 mm L) and 1% acetonitrile solution in 0.1 M aqueous TEAA solution. The elution profile (Fig. 3) demonstrated all the peaks of the ribonucleosides 5'-phosphates involved.

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